Novabiochem®
Peptide Synthesis

Anniversary Edition 2014/2015

30 Years of Innovation

MERCK MILLIPORE

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Nomenclature

In common with most peptide chemists, Novabiochem® uses a convention for naming amino acid derivatives which is clear and simple. This system is based on a general amino acid structure:

$$H - \underset{\underset{H}{|}}{N} - \underset{\underset{H}{\overset{\overset{R}{|}}{C}}}{} - \underset{\overset{\overset{O}{\|}}{C}}{} - OH$$
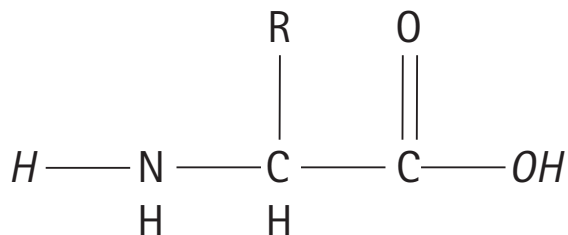
The italicized elements are those which are commonly replaced by protecting and / or activating groups, etc. The central portion of the structure is represented by the three letter amino acid code (Ala, Gly, Lys, etc.) and the L-configuration is assumed; D-amino acids are specified (see below). An amino substituent chemically replaces the proton on the a-nitrogen (represented by *H*), whilst a C-terminal substituent (e.g. an ester moiety) replaces the carboxylic hydroxyl (represented by the *OH*); in this nomenclature, the symbol for the amino substituent replaces the *H* and the symbol for the carboxyl substituent replaces the *OH*. This is illustrated by the following examples:

Conventional formula	Chemical name
H-Ala-OH	L-Alanine
H-D-Ala-OH	D-Alanine
Boc-Ala-OH	N-α-Boc-L-alanine
Boc-D-Ala-OH	N-α-Boc-D-alanine
H-Ala-OMe	L-Alanine methyl ester
Ac-Ala-OH	N-α-Acetyl-L-alanine

Side chain functional groups are handled in an analogous way, except that the symbol for the substituent is placed in brackets after the three letter code. This allows a very convenient way of distinguishing between the different esters of aspartic and glutamic acids and the N-α- and N-ε-substituted lysines.

We feel that this system is simpler and more concise than the use of the full chemical names. It also avoids confusion caused by use of different conventions for chemical names. The naming of amino acid derivatives is often not done in a rigorous and systematic way and there can often be several alternate names in common use. The chemical names for the α- and β-esters of aspartic acid differ only slightly, yet the compounds are quite different and this difference is made obvious by the convention we follow.

All catalog entries of amino acid derivatives (including amino acids attached to resins) show the main catalog description using the above convention. In addition, many entries also list alternate chemical names (these are also included in the alphabetical index).

How to use the catalog

Novabiochem products are categorized by application. For a list of the categories, please see the contents table beginning on page i. Alphabetical and categorical indexes are also provided at the back of the catalog to aid product location. Some of the important features of this catalog are illustrated by the typical product entries shown below.

For additional product information and updates, please visit our website at www.novabiochem.com or contact your local sales office.
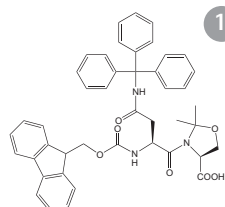
(1) 852185 **Fmoc-Asn(Trt)-Ser($\psi^{Me,Me}$pro)-OH** **(5)** 1 g   70.00

(2) NBC No.: 05-20-1010; CAS No.: 957780-59-5; $C_{44}H_{41}N_3O_7$; M.W.: 723.8   5 g   275.00

TLC: $CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 95.00%.

$CHCl_3$:MeOH:AcOH 32 % (15:4:1), purity: ≥ 95.00%.

HPLC: purity: ≥ 97.00%.

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(13)
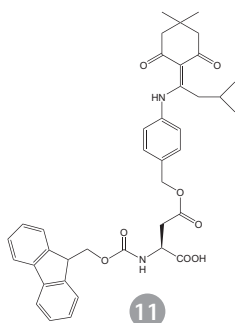
852078 **Fmoc-Asp(ODmab)-OH (3)**   1 g   160.00

(4) N-α-Fmoc-L-aspartic acid β-4-{N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-   5 g   640.00

methylbutyl]-amino } benzyl ester   25 g   2295.00

NBC No.: 04-12-1175; CAS No.: 269066-08-2; $C_{39}H_{42}N_2O_8$; M.W.: 666.7

(6) Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 98.00%.

(7) $CHCl_3$:MeOH:AcOH (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 96.00%.

Optical purity: ≥ 99.50% L-enantiomer.

(8) ⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

Quasi-orthogonally-protected Asp derivative.

For application information, please refer to entry for 852079.

ⓘ Ⓖ 4.13 **(12)**

(10) [1] W. C. Chan, et al. (1995) *J. Chem. Soc., Chem. Commun.*, 2209.

851072 **6-Carboxyfluorescein**   25 mg   28.00

6-FAM   100 mg   59.00
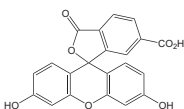
NBC No.: 01-63-0113; CAS No.: 3301-79-9; $C_{21}H_{12}O_7$; M.W.: 376.3

TLC: EtOH:MeOH:H_2O: DIPEA (60:30:10:3), purity: ≥ 95.00%.

HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light.

(9) This high purity fluorescent dye (λ_{ex}494 nm, λ_{em}518 nm) is supplied as a single isomer, which ensures production of labeled products of defined chemical structure, as well as greatly simplifying purification and characterization. FAM should be introduced as the last step in the synthesis to avoid formation of side-products, or protected by tritylation prior to acylation or hydrazine treatment [1].

ⓘ Ⓖ 5.12, Ⓜ 5.12

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1   Product number

2   Old Novabiochem product number

3   Product name

4   Alternate name.

5   Molecular formula and molecular weight

6   Solubility information

7   Important analytical data: purity by TLC and HPLC, optical purity and loading information of resins. More information is contained in the analytical data sheet that is supplied with each product.

8   Recommended storage conditions. All other products should be stored at ≤ +25°C.

9   General product information, including application information

10   Related literature references

11   Chemical structure

12   Page number of the appropriate cross-reference in Synthesis Notes:

Ⓖ   General information related to product;

Ⓜ   Methods related to use of product;

Ⓐ   Specific applications involving use of product.

13   Sizes available

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Abbreviations

A

Abu	2-Aminobutyric acid
γ–Abu	4-Aminobutytic acid
β–Ala	β-Alanine, 3-Aminopropionic acid
ε–Ahx	6-Aminohexanoic acid
Ac	Acetyl
ACE	Angiotensin I Converting Enzyme
ACE-Cl	α-Chloroethyl chloroformate
Acm	Acetamidomethyl
AcOH	Acetic acid
Ada	Adamantyl
ADMA	Dimethylarginine
Adoc	Adamantyloxycarbonyl
Aib	α-Aminoisobutyric acid
AIBN	Azoisobutyronitrile
Ala	Alanine
All	Allyl
Alloc	Allyloxycarbonyl
AM	Aminomethyl
AMC	Aminomethyl coumarin
Ams	Aminoserine
Ar	Aryl
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid

B

BAP	Borane/pyridine complex
BAL	Backbone amide linker
BBN	9-Borabicyclo[3.3.1]nonyl
BEMP	2-t-Butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-dia-zaphosphorine
BHA	Benzhydrylamine
BHT	2, 6-Di-t-butyl-4-methylphenol
Bn	Benzyl
Boc	tert. Butoxycarbonyl
Bom	Benzyloxymethyl
BOP	Benzotriazole-1-yl-oxy-tris(dimethylamino)-phosphoniumhexafluoro-phosphate
BSA	N,O-Bis(trimethylsilyl)acetamide
Bu	Butyl
Bum	tert. Butoxymethyl
BuOH	Butanol
Buthio	Butylthio
Bz	Benzoyl
Bzl	Benzyl

C

Cbz	Benzyloxycarbonyl
CDI	1,1'-Carbonyl-diimidazole
CDM	Counterion Distribution Monitoring

CHA

CHA	Cyclohexylamine (salt)
Cha	Cyclohexylalanine
cHx	Cyclohexyl
Cit	Citrulline
CSA	Camphor sulfonic acid
Cys	Cysteine
(Cys)$_2$	Cystine

D

Dab	Diaminobutyric acid
Dabcyl	4-(4-Dimethylaminophenylazo)benzoyl
Dansyl	5-Dimethylaminonaphthalene-1-sulfonyl
DCA	Dichloroacetic acid
DCC	N,N'-Dicyclohexylcarbodiimide
DCE	Dichloroethane
DCHA	Dicyclohexylamine (salt)
DCM	Dichloromethane
DCP	1,2-Dichloropropane
Dde	1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl
Ddz	α,α-Dimethyl-3,5-dimethoxy-benzyloxycarbonyl
DEA	Diethylamine (salt)
DEAD	Diethyl azodicaboxylate
DFPE	2-(3,5-Dimethoxy-4-formylphenoxy)ethyl
DFPEM	2-(3,5-Dimethoxy-4-formylphenoxy)ethoxy-methyl
Dhbt	3,4-Dihydro-4-oxobenzotriazin-3-yl
Dhc	S-[2,3-bis(palmitoyloxy)propyl]-cysteine
DHP	3,4-Dihydro-2H-pyran-2-ylmethoxy
DIBAL	Diisobutylaluminium hydride
DIC	N,N'-Diisopropylcarbodiimide
DIPCDI	N,N'-Diisopropylcarbodiimide
DIPEA	Diisopropylethylamine
DKP	Diketopiperazine
DMA	Dimethylacetamide
Dmab	4-{N-[1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)-3-methyl butyl]-amino} benzyl
DMAP	4-Dimethylaminopyridine
Dmb	Dimethoxybenzyl
DME	Dimethoxyethane
DMF	Dimethylformamide
DMS	Dimethylsulfide
DMSO	Dimethylsulfoxide
Dmt	4,4'-Dimethoxytrityl
Dnp	Dinitrophenyl
Dpa	N-3-(2,4-Dinitrophenyl)-L-2,3-diaminopropionyl
Dpr	Diaminopropionic acid
DSC	Di-(N-Succinimidyl)carbonate
DTNB	5,5'-Dithiobis (2-nitrobenzoic acid)
DVB	Divinylbenzene

E

EDANS	2-(5-sulfonaphth-1-ylamino)-ethylamino

EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride	
EDT	Ethanedithiol	
EEDQ	2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline	
EHL	Extremely high load	
El	Electrophile	
EMS	Ethylmethylsulfide	
ES-MS	Electrospray ionization mass spectroscopy	
Et	Ethyl	

F, G

FA	3-(2-Furylacryloyl)
FAM	Carboxyfluorescein
FDNP	2,4-Dinitro-5-fluorophenyl
Fmoc	9-Fluorenylmethoxycarbonyl
FMPB	4-(4-Formyl-3-methoxyphenoxy)butyryl
FMPE	2-(4-Formyl-3-methoxyphenoxy)ethyl
For	Formyl
Glc	Gluconyl
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine

H

Hal	Halogen
HATU	2-(1H-9-Azabenzotriazole-1-yl)-1,1,3,3-tetramethyl-aminium hexafluorophosphate
HBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexa-fluorophosphate
HCTU	2-(6-Chloro-1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyl-aminium hexafluorophosphate
HESM	Hydroxyethylsulfenylmethyl
HFIP	Hexafluoroisopropanol
His	Histidine
HL	High load
HM	Hydroxymethyl
Hmb	2-Hydroxy-4-methoxybenzyl
HMBA	4-Hydroxymethylbenzoic acid
HMP	4-Hydroxymethylphenoxy
HMPA	4-Hydroxymethylphenoxyacetic acid
HMPB	4-Hydroxymethyl-3-methoxyphenoxybutyric acid
HOAt	N-Hydroxy-9-azabenzotriazole
HOBt	N-Hydroxybenzotriazole
HONB	N-Hydroxy-5-norbornene-endo-2,3-dicarboximide
HOSu	N-Hydroxysuccinimide
HPLC	High performance liquid chromatography
Hse	Homoserine
Hyp	Hydroxyproline

I, J

IBX	Iodoxybenzoic acid
IEX	Ion exchange
Ile	Isoleucine
Im	Imidazole

iPr	Isopropyl
Isn	Isonipecotic acid
ivDde	1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl
JOE	Carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein

L

LDA	Lithium diisopropylamide
LHDMS	Lithium hexamethylsilazide
Leu	Leucine
LL	Low load
Lys	Lysine

M

MAP	Multiple antigenic peptide
MAPS	Multiple antigen peptide system
Mbh	4,4'-Dimethoxybenzyhydryl
MBHA	Methylbenzhydrylamine
Mca	(7-Methoxycoumarin-4-yl)acetyl
MCPBA	m-Chloroperbenzoic acid
MCR	Multiple component reaction
Me	Methyl
MeIm	N-Methylimidazole
MeO	Methoxy
MeOSu	Methoxysuccinyl
Met	Methionine
MMA	N-Methylmercaptoacetamide
Mmt	4-Methoxytrityl
MPAA	4-Mercaptophenylacetic acid
Mpr	3-Mercaptopropionic acid
MS	Mass spectrometry
MSNT	1-(Mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole
Mtr	4-Methoxy-2,3,6-trimethylbenzene-sulfonyl
Mts	Mesitylene-2-sulfonyl
Mtt	4-Methyltrityl

N

Nal	2-Naphthylalanine
NCS	N-Chlorosuccinimide
NHNH$_2$	Hydrazide
NHS	N-Hydroxysuccinimide
Nle	Norleucine
NMM	N-Methylmorpholine
NMO	N-Methylmorpholine oxide
NMP	N-Methylpyrrolidinone
NO$_2$	Nitro
Np	p-Nitrophenyl
Nps	o-Nitrophenylsulfenyl
Npys	3-Nitro-2-pyridinesulfenyl
Nva	Norvaline

O

o/n	Overnight
ODhbt	3-Hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine ester
OEt	Ethoxy

OMpe	3-Methylpent-3-yl
ONp	p-Nitrophenyl ester
OPfp	Pentafluorophenyl ester
Orn	Ornithine
OSu	N-Hydroxysuccinimide ester

P

PAL	5-(4'-Aminomethyl-3',5'-dimethoxyphenoxy)valeric acid
PAM	4-Hydroxymethylphenylacetamidomethyl
Pbf	2,2,4,6,7-Pentamethyldihydro-benzofuran-5-sulfonyl
Pd$_2$(dba)$_3$	Tris(dibenzylideneacetone)-dipalladium
PDC	Pyridinium dichromate
PdCl$_2$(dppf)	Bis[(2-diphenylphosphino)ferrocene]-dichloropalladium (II) complex
PEG	Polyethyleneglycol
PEGA	Polyethylene glycol- dimethylacrylamide co-polymer
Pen	Penicillamine
Pfp	Pentafluorophenyl
PG	Protecting group
Ph	Phenyl
Phe	Phenylalanine
Phg	Phenylglycine
PhiPr	Phenylisopropyl
Pht	Phthaloyl
Pmc	2,2,5,7,8-Pentamethylchroman-6-sulfonyl
pMeBzl	p-Methylbenzyl
pMeOBzl	p-Methoxybenzyl
pNA	p-Nitroanilide
PPOA	[4-Propionylphenoxy]-acetic acid
PPTS	Pyridinium p-toluenesulfonate
Pr	Propyl
Pro	Proline
PSA	Preformed symmetrical anhydride
PyBOP®	Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
PyBrOP®	Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate
PZ	4-Phenylazobenzyl-oxycarbonyl

R, S

Red-Al	Sodium bis(2-methoxyethoxy)aluminum hydride
Rh$_2$(pfbm)$_4$	Rhodium (II) perfluorobutyramide
SAMA	S-Acetylmercaptoacetic acid
Sar	Sarcosine
SBzl	Thiobenzyl
Sec	Selenocysteine
Ser	Serine
SPOS	Solid phase organic synthesis
SPPS	Solid phase peptide synthesis
Sta	Statine
Ste	Stearoyl
Su	Succinimide
Suc	Succinyl

T

Tacm	Trimethylacetamidomethyl
TAMRA	Carboxy-tetramethylrhodamin

TBTA	t-Butyl-2,2,2-trichloroacetimidate
TBAF	Tetrabutylammonium fluoride
TBDMS	tert.-Butyldimethylsilyl
TBP	Tributylphosphine
TBS	Tributylsilyl
TBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
tBu	tert. Butyl
tButhio	tert. Butylthio
TCEP	tris-(2-Carboxyethyl)phosphine
TEA	Triethylamine
TEMPO	2,2,6,6-Tetramethyl-1-piperidinyloxy free radical
Teoc	2,2,2-Trichloroetoxycarbonyl
TES	Triethylsilane
TET	Carboxy-2',4,7,7'-tetrachlorofluorescein
Tf	Trifluoromethanesulfonyl
Tfa	Trifluoroacetyl
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
TFE	Trifluoroethanol
TFMSA	Trifluoromethanesulfonic acid
TG	Tentagel
THF	Tetrahydrofuran
Thi	Thienylalanine
THP	Tetrahydropyranyl
Thr	Threonine
Tic	1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid
TIPS	Triisopropylsilyl
TIS	Triisopropylsilane
TMBS	Trimethyl-bromosilane
TMG	Tetramethylguanidine
Tmob	2,4,6-Trimethoxybenzyl
TMOF	Trimethylorthoformate
TMP	2,2,6,6-Tetramethylpiperidine
TMS	Trimethylsilyl
TMSBr	Trimethylsilyl bromide
TMSEt	2-Trimethylsilylethyl
TMSOTf	Trimethylsilyl triflate
TNBS	2,4,6-Trinitrobenzene sulfonic acid
TNTU	2-(5-Norbornene-2,3-dicarboximido)-1,1,3,3-tetramethyl-uronium tetrafluoroborate
Tos	Tosyl
Trp	Tryptophan
Trt	Trityl
Ts	Toluenesulfonyl
TSA	Toluenesulfonic acid
Tyr	Tyrosine

V, W, X, Z

Val	Valine
VHL	Very high load
WSC	Water soluble carbodiimide
Xan	Xanthyl
Z	Benzyloxycarbonyl

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€

Product No.	Product		Quantity	Price

Building blocks for solid phase synthesis

N-α-Fmoc protected amino acids

The Novabiochem® brand of Merck Millipore offers some of the highest quality Fmoc-amino acids available on the market. The vast majority of our 20 standard Fmoc-amino acids are specified ≥ 99.0% HPLC purity and ≥ 99.8% enantiomeric purity. We analyze for most of the by-products formed during the synthesis of these derivatives, such as β-alanine-related impurities, dipeptides and unprotected amino acids. We also specify a very low acetate and ethyl acetate content, to ensure formation of capped peptides is negligible.

The standard 20 Fmoc-amino acids Novabiochem® recommends for routine synthesis are given below:

Amino acid	Product No.	Page No.
Fmoc-Ala-OH	852003	2
Fmoc-Arg(Pbf)-OH	852067	9
Fmoc-Asn(Trt)-OH	852044	12
Fmoc-Asp(OtBu)-OH	852005	17
Fmoc-Cys(Trt)-OH	852008	24
Fmoc-Gln(Trt)-OH	852045	39
Fmoc-Glu(OtBu)-OH	852009	34
Fmoc-Gly-OH	852001	40
Fmoc-His(Trt)-OH	852032	44
Fmoc-Ile-OH	852010	46
Fmoc-Leu-OH	852011	47
Fmoc-Lys(Boc)-OH	852012	50
Fmoc-Met-OH	852002	60
Fmoc-Phe-OH	852016	65
Fmoc-Pro-OH	852017	69
Fmoc-Ser(tBu)-OH	852019	72
Fmoc-Thr(tBu)-OH	852000	78
Fmoc-Trp(Boc)-OH	852050	82
Fmoc-Tyr(tBu)-OH	852020	83
Fmoc-Val-OH	852021	87

⚠ Storage conditions   ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Alanine [Ala, A]
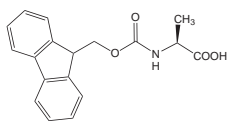
852003 **Fmoc–Ala–OH·H$_2$O** — 25 g — 25.00

N-α-Fmoc-L-alanine monohydrate — 100 g — 50.00

NBC No.: 04-12-1006; CAS No.: 35661-39-3; C$_{18}$H$_{17}$NO$_4$; M.W.: 311.3 — 250 g — 110.00

Solubility: 25 mmole in 50 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98%.

HPLC: purity: ≥ 99.0%.

Fmoc-β-Ala-OH ≤ 0.1%

Fmoc-β-Ala-Ala-OH ≤ 0.1%.

Fmoc-Ala-Ala-OH ≤ 0.1%.

Free amino acid: ≤ 0.2%.

EtOAc: ≤ 0.5%.

AcOH: ≤ 0.02%.

Optical purity: ≥ 99.8% L-enantiomer.
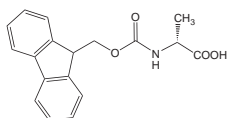
852142 **Fmoc–D–Ala–OH** — 5 g — 50.00

N-α-Fmoc-D-alanine — 25 g — 200.00

NBC No.: 04-13-1006; CAS No.: 79990-15-1; C$_{18}$H$_{17}$NO$_4$; M.W.: 311.3

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

Optical purity: ≥ 99.50% D-enantiomer.

852222 **Fmoc–Ala–OPfp** — 5 g — 82.00

N-α-Fmoc-L-alanine pentafluorophenyl ester

NBC No.: 04-12-1500; CAS No.: 86060-86-8; C$_{24}$H$_{16}$NO$_4$F$_5$; M.W.: 477.4

Solubility: 0.5 mmole in 3 ml DMF clearly soluble.

HPLC: purity: ≥ 98.00%.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

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Product No.	Product	Quantity	Price
852060	**Fmoc–(FmocHmb)Ala–OH**	1 g	295.00
	N-α-Fmoc-N-α-(2-Fmoc-oxy-4-methoxybenzyl)-L-alanine	5 g	990.00

NBC No.: 04-12-1127; CAS No.: 148515-85-9; $C_{41}H_{35}NO_{8}$; M.W.: 669.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Hmb protection of amide bonds has been shown to inhibit aggregation of "difficult" peptides, thereby leading to products of increased purity [1-6]. Retention of Hmb groups on the cleaved peptide can greatly improve the Solubility of protected peptide fragments [7-8] and otherwise intractable sequences [9-11]. Furthermore, using a Hmb-protected derivative for incorporation of the residue linked to the carboxyl group of Asp or Asn residues has been found to suppress formation of aspartimide and piperidide related by-products [12-14]. For a comparison of the efficiency of Hmb and pseudoprolines in preventing aggregation, see [15].

ⓘ Ⓖ 3.9, 3.18

[1]   T. Johnson, et al. (1993) *J. Chem. Soc., Chem. Commun.*, 369.
[2]   C. Hyde, et al. (1994) *Int. J. Peptide Protein Res.*, **43**, 431.
[3]   L. C. Packman, et al. (1994) *Pept. Res.*, **7**, 125.
[4]   T. Johnson, et al. (1994) *Tetrahedron Lett.*, **35**, 463.
[5]   R. G. Simmonds (1996) *Int. J. Peptide Protein Res.*, **47**, 36.
[6]   T. Johnson, et al. (1995) *Lett. Pept. Sci.*, **1**, 11.
[7]   M. Quibell, et al. (1995) *J. Am. Chem. Soc.*, **117**, 11656.
[8]   M. Quibell, et al. (1996) *J. Chem. Soc., Perkin Trans. 1*, 1227.
[9]   M. Quibell, et al. (1994) *Tetrahedron Lett.*, **35**, 2237.
[10] M. Quibell, et al. (1994) *J. Org. Chem.*, **59**, 1745.
[11] M. Quibell, et al. (1995) *J. Chem. Soc., Perkin Trans. 1*, 2019.
[12] M. Quibell, et al. (1994) *J. Chem. Soc., Chem. Commun.*, 2343.
[13] L. C. Packman (1995) *Tetrahedron Lett.*, **36**, 7523.
[14] J. Offer, et al. (1996) *J. Chem. Soc., Perkin Trans. 1*, 175.
[15] W. R. Sampson, et al. (1999) *J. Peptide Sci.*, **5**, 403.

| 852024 | **Fmoc–β–Ala–OH** | 5 g | 37.00 |
| | N-β-Fmoc-β-alanine | 25 g | 134.00 |

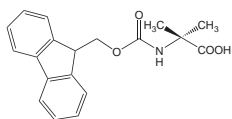NBC No.: 04-12-1044; CAS No.: 35737-10-1; $C_{18}H_{17}NO_{4}$; M.W.: 311.3

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
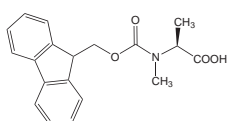
HPLC: purity: ≥ 98.00%.

| 852138 | **Fmoc–N–Me–Ala–OH** | 1 g | 72.00 |
| | N-α-Fmoc-N-α-methyl-L-alanine | 5 g | 280.00 |

NBC No.: 04-12-9027; CAS No.: 84000-07-7; $C_{19}H_{19}NO_{4}$; M.W.: 325.3

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH (85:10:5), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
852248	**Fmoc–D–N–Me–Ala–OH**	1 g	125.00
	N-α-Fmoc-N-α-methyl-D-alanine	5 g	499.00
	NBC No.: 04-13-9002; CAS No.: 138774-92-2; $C_{19}H_{19}NO_4$; M.W.: 325.3		

Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98.00%.
$CHCl_3$:MeOH:AcOH (85:10:5), purity: ≥ 98.00%.
HPLC: purity: ≥ 98.00%.
Optical purity: ≥ 99.50% D-enantiomer.

Product No.	Product	Quantity	Price
852321	**Fmoc–γ–azidohomoalanine**	100 mg	187.00
	CAS No.: 942518-20-9; $C_{19}H_{18}N_4O_4$; M.W.: 366.37	500 mg	749.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.
HPLC: purity: ≥ 95.00%.
⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

A useful tool for the synthesis of branched, side-chain modified and cyclic peptides by Fmoc SPPS. The side-chain azido group is completely stable to piperidine and TFA, but can be readily converted to an amine on the solid phase or in solution by reduction with thiols [1] or phosphines [2, 3].

ⓘ Ⓖ 4.12 Ⓜ 4.12

[1] M. Meldal, et al. (1997) *Tetrahedron Lett.*, **38**, 2531.
[2] J. T. Lundquist & J. C. Pelletier (2001) *Org. Lett.*, **3**, 781.
[3] N. Nepomniaschiy, et al. (2008) *Org. Lett.*, **10**, 5243.

Product No.	Product	Quantity	Price
852333	**(s)–N–Fmoc–α–4–pentenylalanine**	250 mg	437.00
	CAS No.: 288617-73-2; $C_{23}H_{25}NO_4$; M.W.: 379.5	1 g	1310.00

⚠ **Prolonged storage:** ≤ -20°C
Solubility: 1 mmole in 2 ml DMF clearly soluble.
HPLC: purity: ≥ 98.00%.

customer service: service@novabiochem.com   technical service: technical@novabiochem.com internet: novabiochem.com
Bulk quantities available, please enquire

€

Product No.	Product	Quantity	Price

p-Aminobenzoic acid [PABA]
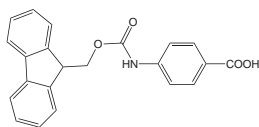
852219	**Fmoc–PABA–OH**	1 g	35.00
	N-α-Fmoc-p-aminobenzoic acid	5 g	133.00
	Fmoc-4-Abz-OH	25 g	466.00

NBC No.: 04-12-1211; CAS No.: 185116-43-2; $C_{22}H_{17}NO_4$; M.W.: 359.4

TLC: $CHCl_3$:EtOAc:AcOH (45:5:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Aminobutyric acid [Abu]
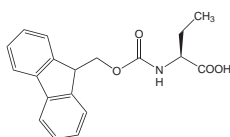
852048	**Fmoc–Abu–OH**	1 g	37.00
	N-α-Fmoc-L-α-aminobutyric acid	5 g	134.00
	Fmoc-2-aminobutanoic acid	25 g	515.00

NBC No.: 04-12-1099; CAS No.: 135112-27-5; $C_{19}H_{19}NO_4$; M.W.: 325.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% L-enantiomer.

852352	**(2S,3S)–Fmoc–Abu(3–N₃)–OH**	250 mg	75.00
	(2S,3S)-2-(Fmoc-amino)-3-azidobutanoic acid	1 g	225.00

CAS No.: 131669-42-6; $C_{19}H_{18}N_4O_4$; M.W.: 366.4

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; prevent exposure to light.

Solubility: 1 mmole in 2 ml DMF clearly soluble.

HPLC: purity: ≥ 98.00%.

A useful tool for the synthesis of branched, side-chain modified and cyclic peptides by Fmoc SPPS. The side-chain azido group is completely stable to piperidine and TFA, but can be readily converted to an amine on the solid phase or in solution by reduction with thiols [1] or phosphines [2, 3].

ⓘ Ⓖ 4.12 Ⓜ 4.12

[1] M. Meldal, et al. (1997) *Tetrahedron Lett.*, **38**, 2531.

[2] J. T. Lundquist & J. C. Pelletier (2001) *Org. Lett.*, **3**, 781.

[3] N. Nepomniaschiy, et al. (2008) *Org. Lett.*, **10**, 5243.

852043	**Fmoc–γ–Abu–OH**	1 g	26.00
	N-γ-Fmoc-γ-aminobutyric acid	5 g	104.00
	Fmoc-GABA-OH	25 g	416.00

NBC No.: 04-12-1088; CAS No.: 116821-47-7; $C_{19}H_{19}NO_4$; M.W.: 325.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 96.00%.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Aminocoumarin acetic acid [ACA]
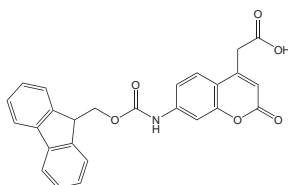
852309	**Fmoc–ACA–OH**	250 mg	94.00
	7-N-Fmoc-aminocoumarin-4-acetic acid	1 g	282.00

CAS No.: 378247-75-7; $C_{26}H_{19}NO_6$; M.W.: 441.4

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; prevent exposure to light.

solubility: 1 mmole in 2 ml DMF.

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 95.00%.

Fmoc-ACA-OH is a useful tool for the SPPS of AMC fluorogenic protease substrates. Fmoc-ACA-OH [1] must be first loaded onto a Wang-type resin. Any unreacted linker hydroxyls must be capped using acetic anhydride. Following Fmoc removal, the first amino acid should be coupled withy HATU/collidine [2]. Insertion of ACA into the middle of a peptide provides fluorogenic substrates for endopeptidases [3]. Treatment of the ACC peptide obtained after TFA cleavage with aqueous base causes facile decarboxylation and formation of the AMC peptide.

[1]   J. L. Harris, et al. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 7754.
[2]   D. J. Maly, et al. (2002) *J. Org. Chem.*, **67**, 910.
[3]   C.M. Salisbury, et.al. (2002), *J. Am. Chem. Soc.*, **124**, 14868.

Aminohexanoic acid [ε-Ahx]
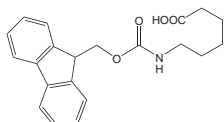
852053	**Fmoc–ε–Ahx–OH**	1 g	31.00
	N-ε-Fmoc-ε-aminocaproic acid	5 g	120.00
	Fmoc-6-aminohexanoic acid	25 g	411.00

NBC No.: 04-12-1111; CAS No.: 88574-06-5; $C_{21}H_{23}NO_4$; M.W.: 353.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Aminoisobutyric acid [Aib]

852049	**Fmoc–Aib–OH**	1 g	30.00
	N-α-Fmoc-α-aminoisobutyric acid	5 g	102.00
	N-Fmoc-C-α-methylalanine	25 g	404.00

NBC No.: 04-12-1100; CAS No.: 94744-50-0; $C_{19}H_{19}NO_4$; M.W.: 325.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

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Product No.	Product		Quantity	Price

Arginine [Arg, R]

For the routine synthesis of arginine-containing peptides by Fmoc SPPS, the use of Fmoc-Arg(Pbf)-OH is recommended. The Pbf group is the most acid-sensitive arginine protecting group in current use and gives rise to less sulfonated tryptophan by-products than Pmc or Mtr.
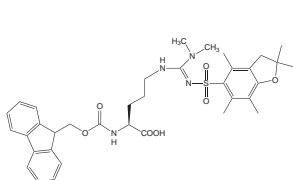
852107 **Fmoc-ADMA(Pbf)-OH**   1 g   315.00

N-α-Fmoc-N,N-ω-dimethyl-N-ω'-(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-L-arginine   5 g   1260.00

NBC No.: 04-12-1264; CAS No.: 11858411-84-2; $C_{36}H_{44}N_4O_7S$; M.W.: 676.8

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH:H$_2$O (90:10:0.5:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 96.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

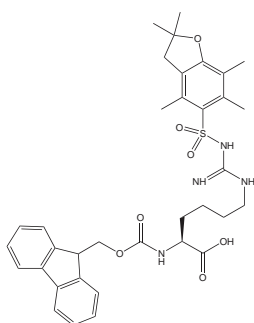A derivative for the introduction of asymmetric dimethyl-arginine during Fmoc SPPS. Coupling can be carried out using any standard activation method. Removal of the Pbf protecting group occurs during the course of the TFA-mediated cleavage reaction. Ref [1] contains methods and protocols for the synthesis of arrays of histone-related peptides containing methylated arginine and lysine-residues.

ⓘ Ⓖ 4.1

[1] S. Rothbart, et al. (2012) *Methods Enzymol.*, **512**, 107.
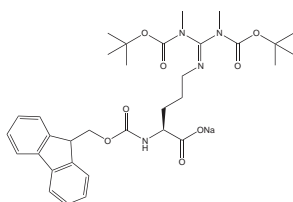
852310 **Fmoc-SDMA(Boc)$_2$-ONa**   1 g   350.00

N-α-Fmoc-N,N'-δ-dimethyl-N,N'-δ-di-t.-butoxycarbonyl-L-arginine sodium salt   5 g   1400.00

$C_{33}H_{43}N_4O_8Na$; M.W.: 646.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH:H$_2$O (90:10:0.5:1), purity: ≥ 90.00%.

HPLC: purity: ≥ 90.00%.

⚠ Prolonged storage: ≤ -20°C

A derivative for the introduction of symmetric dimethyl-arginine during Fmoc SPPS. Coupling can be carried out using any standard activation method. Removal of the Boc protecting groups occurs during the course of the TFA-mediated cleavage reaction. Ref [1] contains methods and protocols for the synthesis of arrays of histone-related peptides containing methylated arginine and lysine-residues.

[1] S. Rothbart, et al. (2012) *Methods Enzymol.*, **512**, 107.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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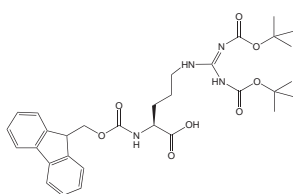
Product No.	Product	Quantity	Price
852101	**Fmoc–Arg(Boc)₂–OH**	1 g	198.00
	N-α-Fmoc-N-ω,N-ω'-bis-t-butoxycarbonyl-L-arginine	5 g	790.00

NBC No.: 04-12-1249; CAS No.: 143824-77-5; $C_{31}H_{40}N_4O_8$; M.W.: 596.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl₃:MeOH:AcOH (90:8:2), purity: ≥ 95.00%.

HPLC: purity: ≥ 91.00%.

Optical purity: ≥ 98.50% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

An excellent derivative for Fmoc SPPS of Arg-containing peptides [1]. Coupling of this derivative can be effected using standard activation methods, such as PyBOP® or TBTU, although longer reaction times may be necessary due to the bulkiness of the side-chain protection.

ⓘ Ⓖ 4.1

[1] A. S. Verdini, et al. (1992) *Tetrahedron Lett.*, **33**, 6541.

852105	**Fmoc–Arg(Me,Pbf)–OH**	1 g	440.00
	N-α-Fmoc-N-ω-methyl-N-ω'-(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-L-arginine	5 g	1760.00

NBC No.: 04-12-1261; CAS No.: 1135616-49-7; $C_{35}H_{42}N_4O_7S$; M.W.: 662.8

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl₃:EtOAc:AcOH (45:5:1), purity: ≥ 95.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

A derivative for the introduction of mono-methyl-arginine during Fmoc SPPS. Coupling can be carried out using any standard activation method. Removal of the Pbf protecting group occurs during the course of the TFA-mediated cleavage reaction. Ref [1] contains methods and protocols for the synthesis of arrays of histone-related peptides containing methylated arginine and lysine-residues.

ⓘ Ⓖ 4.1

[1] S. Rothbart, et al. (2012) *Methods Enzymol.*, **512**, 107.

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Product No.	Product	Quantity	Price

852067 **Fmoc-Arg(Pbf)-OH**

	5 g	65.00
	25 g	170.00
	100 g	510.00
	250 g	1125.00

N-α-Fmoc-N^G-(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-L-arginine
NBC No.: 04-12-1145; CAS No.: 154445-77-9; C$_{34}$H$_{40}$N$_4$O$_7$S; M.W.: 648.8
Solubility: 12.5 mmol in 25 ml DMF clearly soluble.
TLC: CHCl$_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98%.
CHCl$_3$:MeOH:AcOH:H$_2$O (85:13:0.5:1.5), purity: ≥ 98%.
HPLC: purity: ≥ 99.0%.
Fmoc-β-Ala-OH ≤ 0.1%.
Fmoc-β-Ala-Arg(Pbf)-OH ≤ 0.1%.
Fmoc-Arg(Pbf)-Arg(Pbf)-OH ≤ 0.1%.
Fmoc-Arg-OH ≤ 0.1%.
Free amino acid: ≤ 0.2%.
EtOAc: ≤ 0.5%.
AcOH: ≤ 0.02%.
Optical purity: ≥ 99.8% L-enantiomer.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
The standard derivative for the introduction of Arg in Fmoc SPPS [1, 2]. The Pbf side-chain protecting group is removed with TFA approximately 1-2 times faster than Pmc.
In the preparation of peptides containing both Arg and Trp, it is recommended that this derivative is used in conjunction with Fmoc-Trp(Boc)-OH (852050).
ⓘ Ⓖ 4.1
[1] L. A. Carpino, et al. (1993) *Tetrahedron Lett.*, **34**, 7829.
[2] C. G. Fields, et al. (1993) *Tetrahedron Lett.*, **34**, 6661.

852165 **Fmoc-D-Arg(Pbf)-OH**

| | 1 g | 65.00 |
| | 5 g | 260.00 |

N-α-Fmoc-N^G-(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-D-arginine
NBC No.: 04-13-1068; CAS No.: 187618-60-6; C$_{34}$H$_{40}$N$_4$O$_7$S; M.W.: 648.8
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: CHCl$_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.
CHCl$_3$:MeOH:AcOH:H$_2$O (85:13:0.5:1.5), purity: ≥ 98.00%.
HPLC: purity: ≥ 99.00%.
Optical purity: ≥ 99.50% D-enantiomer.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
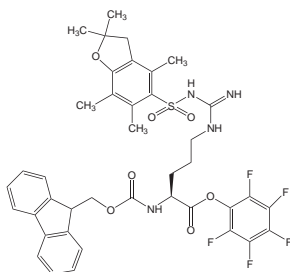
852134 **Fmoc-Arg(Pbf)-OPfp**

| | 5 g | 343.00 |

N-α-Fmoc-N^G-(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-L-arginine
pentafluorophenyl ester
NBC No.: 04-12-1544; CAS No.: 200132-16-7; C$_{40}$H$_{39}$N$_4$F$_5$O$_7$S; M.W.: 814.8
Solubility: 0.5 mmole in 3 ml DMF clearly soluble.
TLC: Toluene:Dioxane:AcOH (95:25:4), purity: ≥ %.
HPLC: purity: ≥ 90.00%.
Optical purity: ≥ 99.00% L-enantiomer.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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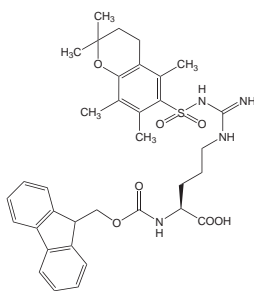
Product No.	Product	Quantity	Price
852034	**Fmoc-Arg(Pmc)-OH**	5 g	75.00
	N-α-Fmoc-N^G-(2,2,5,7,8-pentamethylchroman-6-sulfonyl)-L-arginine	25 g	289.00
	NBC No.: 04-12-1073; CAS No.: 119831-72-0; C$_{35}$H$_{42}$N$_4$O$_7$S; M.W.: 662.8	100 g	863.00

Solubility: 12.5 mmol in 25 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

CHCl$_3$:MeOH:AcOH:H$_2$O (85:13:0.5:1.5), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

An excellent derivative for the introduction of Arg in Fmoc SPPS. The Pmc group is removed by TFA in 1-3 hours [1-3].

For discussions on the side-reactions associated with the use of sulfonyl-based arginine protection, see [4-11].

In the synthesis of peptides containing both Arg and Trp, it is recommended that this derivative is used in conjunction with Fmoc-Trp(Boc)-OH (852050) [5, 6, 11].

ⓘ Ⓖ 4.1

[1] R. Ramage, et al. (1987) *Tetrahedron Lett.*, **28**, 2287.
[2] J. Green, et al. (1988) *Tetrahedron Lett.*, **29**, 4341.
[3] R. Ramage, et al. (1991) *Tetrahedron Lett.*, **47**, 6353.
[4] P. Sieber (1987) *Tetrahedron Lett.*, **28**, 1637.
[5] C. G. Fields, et al. (1993) *Tetrahedron Lett.*, **34**, 6661.
[6] H. Choi, et al. (1993) *Int. J. Peptide Protein Res.*, **42**, 58.
[7] A. Stierandova, et al. (1994) *Int. J. Peptide Protein Res.*, **43**, 31.
[8] P. M. Fischer, et al. (1992) *Int. J. Peptide Protein Res.*, **40**, 19.
[9] S. A. G. Beck, et al. (1991) *Int. J. Peptide Protein Res.*, **38**, 25.
[10] E. Jaeger, et al. (1993) *Biol. Chem. Hoppe-Seyler*, **374**, 349.
[11] P. White in "Peptides, Chemistry & Biology, Proc. 12th American Peptide Symposium", J. A. Smith & J. E. Rivier (Eds), ESCOM, Leiden, 1992, pp. 537.
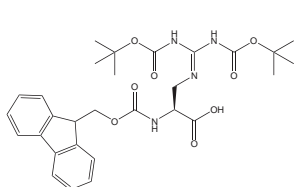
Product No.	Product	Quantity	Price
852336	**Fmoc-Agb(Boc)$_2$-OH**	1 g	203.00
	N-α-Fmoc-N,N'-γ-di-t.-butoxycarbonyl-L-diaminobutanoic acid	5 g	811.00

CAS No.: 313232-63-2; C$_{29}$H$_{36}$N$_4$O$_8$; M.W.: 568.3

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
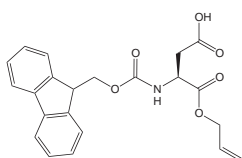
Fmoc-Agb(Boc)$_2$-OH is a useful tool for the introduction of shortened arginine analog, Agb, during Fmoc SPPS [1, 2]. It can be used in exactly the same manner as Fmoc-Arg(Pbf)-OH but coupling can be sluggish.

[1] R. Karstad, et. al. (2012) *J. Med. Chem.* **55**, 6294.
[2] L.Z. Yan, et. al. (2011), *J. Pept. Sci.* **17**, 383.

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Product No.	Product	Quantity	Price

852267   **Fmoc-hArg(Pbf)-OH**   1 g   73.00

N-α-Fmoc-N^G-(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-L-   5 g   291.00
homoarginine

CAS No.: 401915-53-5; C$_{35}$H$_{42}$N$_4$O$_7$S; M.W.: 662.8

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH:H$_2$O (85:13:0.5:1.5), purity: ≥ 97.00%.

HPLC: purity: ≥ 95.00%.

⚠ **Prolonged storage**: ≤ -20°C; keep cool and dry.

Fmoc-homoArg(Pbf)-OH is a useful tool for the introduction of homoarginine
during Fmoc SPPS. It can be used in exactly the same manner as Fmoc-Arg(Pbf)-
OH.

Asparagine [Asn, N]

852203   **Fmoc-Asn-OH**   5 g   21.00

N-α-Fmoc-L-asparagine   25 g   63.00
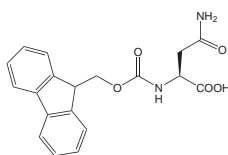
NBC No.: 04-12-1004; CAS No.: 71989-16-7; C$_{19}$H$_{18}$N$_2$O$_5$; M.W.: 354.4   100 g   182.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.

CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.50% L-enantiomer.

852236   **Fmoc-D-Asn-OH**   5 g   83.00

N-α-Fmoc-D-asparagine   25 g   333.00

NBC No.: 04-13-1042; CAS No.: 108321-39-7; C$_{19}$H$_{18}$N$_2$O$_5$; M.W.: 354.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.50% D-enantiomer.
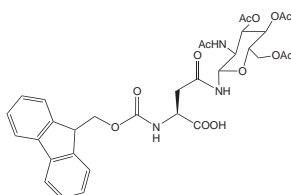
852135   **Fmoc-Asn(Ac$_3$AcNH-β-Glc)-OH**   100 mg   160.00

N-α-Fmoc-N-β-[3,4,6-tri-O-acetyl-2-(acetylamino)-deoxy-2-β-glucopyranosyl]-   500 mg   640.00
L-asparagine   1 g   1050.00

NBC No.: 04-12-8100; CAS No.: 131287-39-3; C$_{33}$H$_{37}$N$_3$O$_{13}$; M.W.: 683.7

TLC: CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 95.00%.

HPLC: purity: ≥ 95.00%.

⚠ **Prolonged storage**: ≤ -20°C; keep cool and dry.

An excellent derivative for the introduction of an Asn(AcNH-β-Glc) residue by
Fmoc SPPS [1-4].

ⓘ 🅐 3.44

[1] L. Otvos, et al. (1989) *Pept. Res.*, **2**, 362.

[2] M. Meldal, et al. (1990) *Tetrahedron Lett.*, **31**, 6987.

[3] L. Otvos, et al. (1990) *Tetrahedron Lett.*, **31**, 5889.

[4] J. Price, et. al., (2010) *J. Am. Chem. Soc.* **132**, 15359.

⚠ Storage conditions   🅖 Additional information   🅜 Method information   🅐 Application information

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Product No.	Product	Quantity	Price
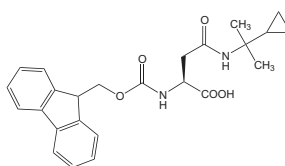
852119　**Fmoc-Asn(Dmcp)-OH**　　1 g　161.00

N-α-Fmoc-N-β-(1-cyclopropyl-1-methylethyl)-L-asparagine　　5 g　645.00

NBC No.: 04-12-1291; CAS No.: 172947-19-2; $C_{25}H_{28}N_2O_5$; M.W.: 436.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

A superior derivative to Fmoc-Asn(Trt)-OH for the synthesis of Asn-containing peptides by Fmoc SPPS. Fmoc-Asn(Dmcp)-OH is more soluble in DMF than Fmoc-Asn(Trt)-OH, thereby facilitating coupling reactions at higher concentration. Cleavage of the Dmcp group is rapid, even when the residue is located at the N-terminus of a peptide. Coupling of Dmcp-protected derivatives is faster than that of the corresponding hindered Trt derivatives. Dmcp-protected peptides have enhanced Solubility.

ⓘ Ⓖ 4.2

[1]　L. A. Carpino, et al. (1995) *J. Org. Chem.*, **60**, 7718.
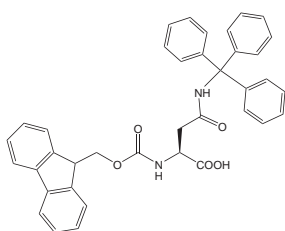
852044　**Fmoc-Asn(Trt)-OH**　　25 g　60.00

N-α-Fmoc-N-β-trityl-L-asparagine　　100 g　180.00

NBC No.: 04-12-1089; CAS No.: 132388-59-1; $C_{38}H_{32}N_2O_5$; M.W.: 596.7　250 g　395.00

Solubility: 25 mmole in 50 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98%.

HPLC: purity: ≥ 99.0%.

Fmoc-β-Ala-Asn(Trt)-OH ≤ 0.1%

Fmoc-Asn(Trt)-Asn(Trt)-OH ≤ 0.1%

Fmoc-Asn-OH ≤ 0.1%

Free amino acid: ≤ 0.2%

EtOAc: ≤ 0.5%

AcOH: ≤ 0.02%

Optical purity: ≥ 99.8% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

Fmoc-Asn(Trt)-OH has good Solubility properties in most organic solvents, and its use has been shown to result in significantly purer peptides than other derivatives used for the introduction of Asn [1, 2]. Coupling can be performed by standard procedures. The trityl group is normally removed by 95% TFA in 1-3 hours, with no alkylation of Trp residues. When Asn(Trt) is the N-terminal residue, the reaction time may need to be extended to ensure complete deprotection [3].

ⓘ Ⓖ 4.2

[1]　P. Sieber, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 1st International Symposium", R. Epton (Eds), SPCC UK Ltd., Birmingham, 1990, pp. 577.

[2]　P. Sieber, et al. (1991) *Tetrahedron Lett.*, **32**, 739.

[3]　M. Friede, et al. (1992) *Pept. Res.*, **5**, 145.

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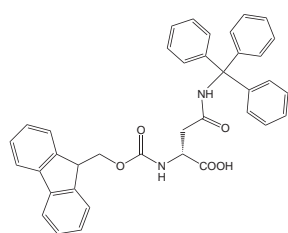
Product No.	Product	Quantity	Price
852159	**Fmoc-D-Asn(Trt)-OH**	1 g	50.00
	N-α-Fmoc-N-β-trityl-D-asparagine	5 g	200.00
	NBC No.: 04-13-1055; CAS No.: 180570-71-2; $C_{38}H_{32}N_2O_5$; M.W.: 596.7	25 g	800.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 99.00%.
Optical purity: ≥ 99.50% D-enantiomer.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
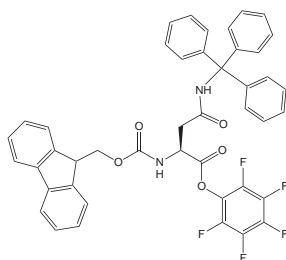
852132	**Fmoc-Asn(Trt)-OPfp**	5 g	185.00

N-α-Fmoc-N-β-trityl-L-asparagine pentafluorophenyl ester
NBC No.: 04-12-1538; CAS No.: 132388-64-8; $C_{44}H_{31}F_5N_2O_5$; M.W.: 762.7
Solubility: 0.5 mmole in 3 ml DMF clearly soluble.
TLC: CH$_3$CN:CHCl$_3$ (1 : 3), purity: ≥ %.
HPLC: purity: ≥ 97.00%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
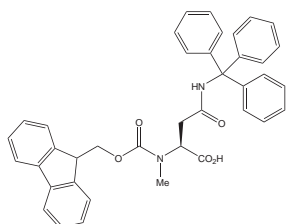
852353	**Fmoc-N-Me-Asn(Trt)-OH**	250 mg	78.00
NEW	N-α-Fmoc-N-α-methyl-N-β-trityl-L-asparagine	1 g	234.00

CAS No.: 941296-80-6; $C_{39}H_{34}N_2O_5$; M.W.: 610.7
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 97.00%.
HPLC: purity: ≥ 97.00%.
Optical purity: ≥ 98.00% L-enantiomer.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Aspartic acid [Asp, D]

Fmoc-Asp(OtBu)-OH is recommended for the routine preparation of aspartic acid-containing peptides. For the preparation of cyclic peptides and peptides containing side-chain modified Asp residues, derivatives such as Fmoc-Asp-OAll, Fmoc-Asp(OAll)-OH, Fmoc-Asp-ODmab or Fmoc-Asp(ODmab)-OH should be used since their respective carboxy-protecting groups can be removed selectively on the solid-phase.

It is important to be aware that peptide sequences containing the Asp-Gly motif are particularly prone to aspartimide formation. Fortunately, this side-reaction can be eliminated by using Fmoc-(FmocHmb)Gly-OH or Fmoc-(Dmb)Gly-OH to introduce the Gly residue. Alternatively, the dipeptide Fmoc-Asp(OtBu)-(Dmb)Gly-OH may be used.

For further information, please refer to the product entries.

852113	**Fmoc-Asp(biotinyl-PEG)-OH**	500 mg	296.00
	N-α-Fmoc-N-γ-(N-biotinyl-3-(2-(2-(3-aminopropyloxy)-ethoxy)-ethoxy)-propyl)-L-asparagine	1 g	530.00

NBC No.: 04-12-1279; $C_{39}H_{53}N_5O_{10}S$; M.W.: 783.9

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.

In contrast to Fmoc-Lys(biotin)-OH, this novel biotin-labeled amino acid has excellent Solubility in DMF and other solvents used in SPPS [1]. The PEG-spacer restricts hindrance between the peptide and avidin, leading to better biotin binding. Furthermore, the hydrophilic nature of the PEG minimizes non-specific interactions that can arise from the spacer group becoming buried in the hydrophobic pocket of proteins.

ⓘ Ⓖ 5.18

[1] B. Baumeister, et al. (2003) *Biopolymers*, 71, 339.

14

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€

Product No.	Product	Quantity	Price
852072	**Fmoc–Asp–OAll**	1 g	57.00
	N-α-Fmoc-L-aspartic acid α-allyl ester	5 g	224.00

NBC No.: 04-12-1157; CAS No.: 144120-53-6; $C_{22}H_{21}NO_6$; M.W.: 395.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

CHCl$_3$:MeOH:AcOH:H$_2$O (90:10:0.5:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% L-enantiomer.

Orthogonally-protected building block for the synthesis of head-to-tail cyclic peptides via side-chain anchoring of the Asp [1-5].

The α-allyl ester can be selectively removed in the presence of Fmoc- and tBu-based protecting groups by treatment with Pd(Ph$_3$P)$_4$/CHCl$_3$/AcOH/NMM [6], thereby facilitating the synthesis of branched esters, amides, lactones and lactams incorporating an aspartyl unit.

ⓘ Ⓖ 4.13, Ⓜ 4.14

[1] W. Bannwarth, et al. (1992) *Tetrahedron Lett.*, **33**, 4557.
[2] F. Albericio, et al. (1993) *Tetrahedron Lett.*, **34**, 1549.
[3] J. Eichler, et al. (1994) *Pept. Res.*, **7**, 300.
[4] J. Tulla-Puche & G. Barany (2004) *J. Org. Chem*, **69**, 4101
[5] S. H. Joo, et al. (2006) *J. Am. Chem. Soc.*, **128**, 13000.
[6] S. A. Kates, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 13th American Peptide Symposium", ESCOM, Leiden, 1994, pp. 113.

| 852122 | **Fmoc–Asp(OAll)–OH** | 5 g | 73.00 |
| | | 25 g | 281.00 |
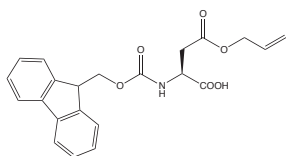
NBC No.: 04-12-1303; CAS No.: 146982-24-3; $C_{22}H_{21}NO_6$; M.W.: 395.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH:H$_2$O (90:10:0.5:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Orthogonally-protected building block for the synthesis of peptides modified at the Asp side chain, side-chain to side-chain cyclization and glycoprotein synthesis [1 - 4], thereby facilitating the synthesis of branched esters, amides, lactones and lactams incorporating an aspartyl unit. To overcome aspartimide formation, it is advisable to use a Hmb- or Dmb-protected derivative for the preceding amino acid.

ⓘ Ⓖ 4.13, Ⓜ 4.14

[1] T. Conroy, et. al. (2010) *Org. Biomol. Chem.*, **8**, 3723.
[2] P. Grieco, et. al. (2001) *J. Pept. Res.*, **57**, 250.
[3] P. Wang, et. al. (2012) *Angew. Chem., Int. Ed.*, **51**, 11576.
[4] V. M. Ullmann, et al. (2012). *Angew. Chem., Int. Ed.*, **51**, 11566.
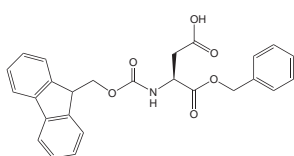
| 852220 | **Fmoc–Asp–OBzl** | 1 g | 71.00 |
| | N-α-Fmoc-L-aspartic acid α-benzyl ester | 5 g | 279.00 |

NBC No.: 04-12-1272; CAS No.: 86060-83-5; $C_{26}H_{23}NO_6$; M.W.: 445.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
852004	**Fmoc-Asp(OBzl)-OH**	5 g	83.00
	N-α-Fmoc-L-aspartic acid β-benzyl ester	25 g	333.00
	NBC No.: 04-12-1012; CAS No.: 86060-84-6; $C_{26}H_{23}NO_6$; M.W.: 445.5		
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (85:10:5), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 98.00%.		
	Optical purity: ≥ 99.50% L-enantiomer.		

852118	**Fmoc-Asp(EDANS)-OH**	500 mg	307.00
	NBC No.: 04-12-1288; CAS No.: 182253-73-2; $C_{31}H_{29}N_3O_8S$; M.W.: 603.64	1 g	567.00
	TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 95.00%.		
	Optical purity: ≥ 99.50% L-enantiomer.		

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

Fluorescence-labeled amino acid for preparing fluoresence-quenched peptide substrates. Most frequently used in conjunction with Dabcyl quenching group. For an application in high-throughput screening see [2].

ⓘ Ⓖ 5.12

[1] L. L. Maggiora, et al. (1992) *J. Med. Chem.*, **35**, 3727.

[2] M. Taliani, et. al., (1996) *Anal. Biochem.*, **240**, 60.

852037	**Fmoc-Asp-OtBu**	1 g	47.00
	N-α-Fmoc-L-aspartic acid α-t.-butyl ester	5 g	182.00
	NBC No.: 04-12-1080; CAS No.: 129460-09-9; $C_{23}H_{25}NO_6$; M.W.: 411.5		
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 98.00%.		
	Optical purity: ≥ 99.00% L-enantiomer.		

Selectively protected building block for library synthesis or the preparation of β-aspartyl peptides. After condensation of the side-chain carboxyl with an appropriate amine or alcohol, the α-amino and carboxyl functions can be selectively unmasked with 20% piperidine in DMF and 50% TFA in DCM respectively, facilitating the synthesis of branched esters and amides, and lactams and lactones containing the aspartyl unit.

852144	**Fmoc-D-Asp-OtBu**	1 g	161.00
	N-α-Fmoc-D-aspartic acid α-t.-butyl ester	5 g	645.00
	NBC No.: 04-13-1011; CAS No.: 134098-70-7; $C_{23}H_{25}NO_6$; M.W.: 411.5		
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 98.00%.		
	Optical purity: ≥ 99.00% D-enantiomer.		

| Product No.	Product	Quantity	Price €
852005	**Fmoc-Asp(OtBu)-OH** N-α-Fmoc-L-aspartic acid β-t.-butyl ester NBC No.: 04-12-1013; CAS No.: 71989-14-5; $C_{23}H_{25}NO_6$; M.W.: 411.5	25 g 100 g 250 g	60.00 180.00 395.00

Solubility: 25 mmole in 50 ml DMF clearly soluble.
TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.
CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98%.
HPLC: purity: ≥ 99.0%.
Fmoc-β-Ala-OH ≤ 0.1%.
Fmoc-β-Ala-Asp(OtBu)-OH ≤ 0.1%.
Fmoc-Asp(OtBu)-Asp(OtBu)-OH ≤ 0.1%.
Fmoc-Asp-OH ≤ 0.1%.
Free amino acid: ≤ 0.2%.
EtOAc: ≤ 0.5%.
AcOH: ≤ 0.02%.
Optical purity: ≥ 99.8% L-enantiomer.
ⓘ Ⓖ 4.3

| 852154 | **Fmoc-D-Asp(OtBu)-OH**
N-α-Fmoc-D-aspartic acid β-t.-butyl ester
NBC No.: 04-13-1050; CAS No.: 112883-39-3; $C_{23}H_{25}NO_6$; M.W.: 411.5 | 1 g
5 g
25 g | 44.00
172.00
688.00 |

Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 99.00%.
Optical purity: ≥ 99.50% D-enantiomer.

| 852125 | **Fmoc-Asp(OtBu)-OPfp**
N-α-Fmoc-L-aspartic acid β-t.-butyl ester pentafluorophenyl ester
NBC No.: 04-12-1502; CAS No.: 86061-01-0; $C_{29}H_{24}F_5NO_6$; M.W.: 577.5 | 5 g | 185.00 |

Solubility: 0.5 mmole in 3 ml DMF clearly soluble.
TLC: CH$_3$CN:CHCl$_3$ (1 : 3), purity: ≥ 2.00%.
HPLC: purity: ≥ 98.00%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

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Product No.	Product	Quantity	Price
852079	**Fmoc-Asp-ODmab**	1 g	198.00
	N-α-Fmoc-L-aspartic acid α-4-{N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino} benzyl ester	5 g	790.00

NBC No.: 04-12-1176; CAS No.: 17261-77-7; $C_{39}H_{42}N_2O_8$; M.W.: 666.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH:H$_2$O (90:10:0.5:1), purity: ≥ 97.00%.
CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

Quasi-orthogonally-protected Asp derivative.

The Dmab group can be removed selectively in the presence of tBu-based protecting groups by treatment with 2% hydrazine in DMF [1], making this derivative an extremely useful tool for the preparation of cyclic peptides by Fmoc SPPS or for library synthesis.

Occasionally sluggish cleavage of the aminobenzyl moiety is observed [2, 3]. In these instances, washing the support with 20% DIPEA in DMF/water (9:1) [2] or 5 mM NaOH in MeOH/H$_2$O [4] has been found to be efficacious.

For the on-resin synthesis of side-chain to side-chain lactam bridged peptides, the combination of Lys(ivDde) and Asp(ODmab) is particularly advantageous since both side-chains can be simultaneously unmasked in a single step.

For applications of this derivative in the synthesis of cyclic peptides, see references [5 - 7].

ⓘ Ⓖ 4.14, Ⓜ 4.10

[1] W. C. Chan, et al. (1995) *J. Chem. Soc., Chem. Commun.*, 2209.
[2] S. Künzel, et al. *Poster 17 presented at Solid Phase Synthesis & Combinatorial Libraries, Southampton, September 2001*; R.S. Harrison, et. al. (2010) *Proc. Natl. Acad. Sci. USA*, **107**, 11686.
[3] K. F. Medzihradszky, et al. (2002) *Lett. Pept. Sci.*, **8**, 1.
[4] T. Conroy, et. al. (2009) *Org. Biomol. Chem.*, **7**, 2255.
[5] M. Cudic, et al. in "Peptides 2000, Proc. 26th European Peptide Symposium", J. Martinez & J.-A. Fehrentz (Eds), Paris, Éditions EDK, 2001, pp. 203.
[6] M. Cudic, et al. (2000) *Tetrahedron Lett.*, **41**, 4527.
[7] J. P. Malkinson, et al. (2003) *Org. Lett.*, **5**, 5051.
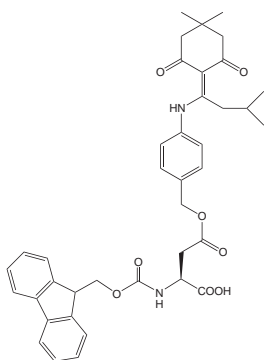
852078	**Fmoc-Asp(ODmab)-OH**	1 g	166.00
	N-α-Fmoc-L-aspartic acid β-4-{N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino} benzyl ester	5 g	666.00
		25 g	2387.00

NBC No.: 04-12-1175; CAS No.: 269066-08-2; $C_{39}H_{42}N_2O_8$; M.W.: 666.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH:H$_2$O (85:13:0.5:1.5), purity: ≥ 98.00%.
CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 96.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

Quasi-orthogonally-protected Asp derivative

For more information, see entry for 852079.

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Product No.	Product	Quantity	Price
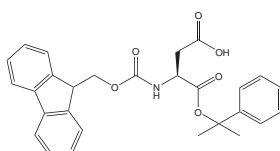
852335 Fmoc-Asp-O-2-PhiPr

N-α-Fmoc-L-aspartic acid α-2-phenylisopropyl ester

$C_{28}H_{27}NO_6$; M.W.: 473.5

1 g   280.00
5 g   666.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 96.00%.

HPLC: purity: ≥ 93.00%.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

Quasi-orthogonally-protected Asp derivative.

The 2-PhiPr group can be removed selectively in the presence of tBu-based protecting groups by treatment with 1% TFA in DCM [1], making this derivative an extremely useful tool for the preparation of cyclic peptides by Fmoc SPPS [2, 3, 4] or for library synthesis. For the on-resin synthesis of side-chain to side-chain lactam bridged peptides, the combination of Lys(Mtt) and Asp(O-2-PhiPr) is particularly advantageous since both side-chains can be simultaneously unmasked in a single step.

[1] C. Yue, et al. (1993) *Tetrahedron Lett.*, **34**, 323.
[2] F. Dick, et al. in "Peptides 1996, Proc. of 24th European Peptide Symposium", R. Ramage & R. Epton (Eds), Mayflower Scientific Ltd., Birmingham, 1998, pp. 339.
[3] J. W. Taylor, et al., Poster 185 presented at the American Peptide Symposium, San Diego, 2001.
[4] S. Booth, et al. (1998) *J. Chem. Soc., Perkin Trans*, 1467.
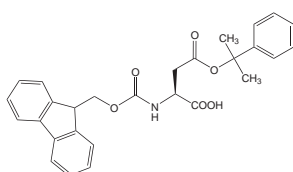
852086 Fmoc-Asp(O-2-PhiPr)-OH

N-α-Fmoc-L-aspartic acid β-2-phenylisopropyl ester

NBC No.: 04-12-1200; CAS No.: 200336-86-3; $C_{28}H_{27}NO_6$; M.W.: 473.5

1 g   135.00
5 g   562.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 97.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

Quasi-orthogonally-protected Asp derivative.

The 2-PhiPr group can be removed selectively in the presence of tBu-based protecting groups by treatment with 1% TFA in DCM [1], making this derivative an extremely useful tool for the preparation of cyclic peptides by Fmoc SPPS [2 -4] or for library synthesis. For the on-resin synthesis of side-chain to side-chain lactam bridged peptides, the combination of Lys(Mtt) and Asp(O-2-PhiPr) is particularly advantageous since both side-chains can be simultaneously unmasked in a single step.

This derivative is reported to be advantageous for on-resin glycosylation of the aspartic acid side-chain since the phenylisopropyl protecting group efficiently prevents aspartimide formation during Fmoc SPPS [5].

ⓘ Ⓖ 4.15

[1] C. Yue, et al. (1993) *Tetrahedron Lett.*, **34**, 323.
[2] F. Dick, et al. in "Peptides 1996, Proc. of 24th European Peptide Symposium", R. Ramage & R. Epton (Eds), Mayflower Scientific Ltd., Birmingham, 1998, pp. 339.
[3] R.S. Harrison, et. al. (2010) *Proc. Natl. Acad. Sci. USA*, **107**, 11686.
[4] L. Charoenchai, et. al. (2008) *J. Med. Chem.*, **51**, 4385.]
[5] R. Chen & T.J. Tolbert (2010) *J. Am. Chem. Soc.*, **132**, 3211.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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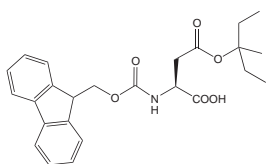
Product No.	Product	Quantity	Price
852104	**Fmoc-Asp(OMpe)-OH**	1 g	210.00
	N-α-Fmoc-(O-3-methyl-pent-3-yl)aspartic acid	5 g	840.00

NBC No.: 04-12-1259; CAS No.: 180675-08-5; $C_{25}H_{29}NO_6$; M.W.: 439.50

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:EtOAc:AcOH (45:5:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

An excellent derivative for minimizing aspartimide formation during Fmoc SPPS of Asp-containing peptides [1]. The bulky OMpe protecting group offers considerably more protection against the formation of aspartimide-related by-products than the commonly used OtBu group [1, 2].

ⓘ Ⓖ 3.23

[1] A. Karlström (1996) *Tetrahedron Lett,*, **37**, 4243.
[2] M. Mergler (2003) *J. Pept. Sci.*, **9**, 518.
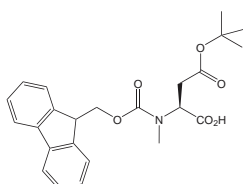
| 852329 | **Fmoc-N-Me-Asp(OtBu)-OH** | 250 mg | 78.00 |
| | N-α-Fmoc-N-α-methyl-L-aspartic acid β-tert butyl ester | 1 g | 234.00 |

CAS No.: 152548-66-8; $C_{24}H_{27}NO_6$; M.W.: 425.5

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 98.00% L-enantiomer.

Butylglycine
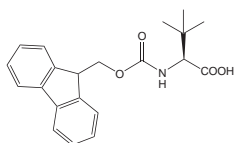
| 852027 | **Fmoc-α-t.-butylglycine** | 1 g | 124.00 |
| | N-α-Fmoc-L-α-t.-butyl-glycine | 5 g | 467.00 |

Fmoc-Tle-OH

NBC No.: 04-12-1054; CAS No.: 132684-60-7; $C_{21}H_{23}NO_4$; M.W.: 353.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

Optical purity: ≥ 99.50% L-enantiomer.
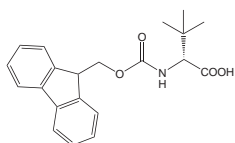
| 852235 | **Fmoc-D-α-t.-butylglycine** | 1 g | 239.00 |
| | N-α-Fmoc-D-α-t.-butyl-glycine | 5 g | 931.00 |

N-α-Fmoc-D-t-leucine

NBC No.: 04-13-1041; CAS No.: 198543-64-5; $C_{21}H_{23}NO_4$; M.W.: 353.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% D-enantiomer.

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Product No.	Product	Quantity	Price

Citrulline [Cit]
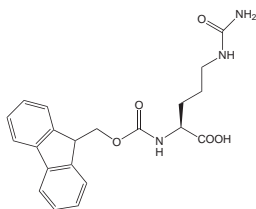
852025	**Fmoc-Cit-OH**	1 g	20.00
	N-α-Fmoc-L-2-amino-5-ureido-n-valeric acid	5 g	78.00
	NBC No.: 04-12-1052; CAS No.: 133174-15-9; $C_{21}H_{23}N_3O_5$; M.W.: 397.4	25 g	312.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

Optical purity: ≥ 99.50% L-enantiomer.

Cyclohexylalanine [Cha]
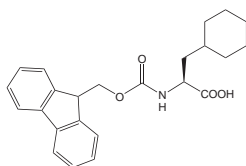
852038	**Fmoc-Cha-OH**	1 g	47.00
	N-α-Fmoc-β-cyclohexyl-L-alanine	5 g	186.00
	NBC No.: 04-12-1081; CAS No.: 135673-97-1; $C_{24}H_{27}NO_4$; M.W.: 393.5	25 g	723.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.
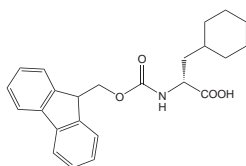
852162	**Fmoc-D-Cha-OH**	1 g	68.00
	N-α-Fmoc-β-cyclohexyl-D-alanine	5 g	270.00
	NBC No.: 04-13-1058; CAS No.: 144701-25-7; $C_{24}H_{27}NO_4$; M.W.: 393.5	25 g	1030.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% D-enantiomer.

N – α – FMOC PROTECTED AMINO ACIDS

⚠ Storage conditions　　Ⓖ Additional information　　Ⓜ Method information　　Ⓐ Application information

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Product No.	Product	Quantity	Price

Cysteine [Cys, C]

For the routine preparation of peptides containing cysteine and cystine, the simplest approach is to use Fmoc-Cys(Trt)-OH for introduction of the Cys residue. The Trt group is removed by TFA during the course of the normal deprotection-cleavage reaction to give the reduced sulfhydryl form of the peptide, which can either be used directly or subsequently oxidized to the corresponding cystinyl peptide.
Derivatives such as Cys(tButhio) and Cys(Mmt) allow selective unmasking of a Cys-thiol group on the polymer-support, whilst acid-stable Cys(Acm) enables cystinyl peptides to be formed directly in solution by iodine or thallium (III) oxidation.

For further information on selecting a cysteine derivative, please refer to the product entries.

852006 **Fmoc–Cys(Acm)–OH**

	5 g	44.00
	25 g	155.00
	100 g	458.00
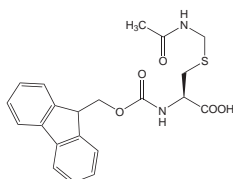
N-α-Fmoc-S-acetamidomethyl-L-cysteine
NBC No.: 04-12-1014; CAS No.: 86060-81-3; $C_{21}H_{22}N_2O_5S$; M.W.: 414.5
Solubility: 25 mmole in 50 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (85:10:5), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 99.00%.
Optical purity: ≥ 99.50% L-enantiomer.
The side-chain Acm group is stable to TFA, but can be removed with Hg(II) [1] or Ag(I) [2] to give cysteinyl peptides, or oxidatively with Tl(III) [3] or I_2 [4] to generate cystinyl peptides.
ⓘ Ⓖ 3.32, 4.3, Ⓜ 3.33, 3.35

[1] D. F. Veber, et al. (1972) *J. Am. Chem. Soc.*, **94**, 5456.
[2] M. Yoshida, et al. (1990) *Chem. Pharm. Bull.*, **38**, 273.
[3] N. Fujii, et al. (1987) *Chem. Pharm. Bull.*, **36**, 2339.
[4] B. Kamber, et al. (1980) *Helv. Chim. Acta*, **63**, 899.

852158 **Fmoc–D–Cys(Acm)–OH**

	1 g	63.00
	5 g	248.00

N-α-Fmoc-S-acetamidomethyl-D-cysteine
NBC No.: 04-13-1054; CAS No.: 168300-88-7; $C_{21}H_{22}N_2O_5S$; M.W.: 414.5
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (85:10:5), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 97.00%.
Optical purity: ≥ 99.50% D-enantiomer.

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Product No.	Product	Quantity	Price
852007	**Fmoc-Cys(tBu)–OH**	5 g	49.00
	N-α-Fmoc-S-t.-butyl-L-cysteine	25 g	206.00
	NBC No.: 04-12-1016; CAS No.: 67436-13-9; $C_{22}H_{25}NO_4S$; M.W.: 399.5	100 g	619.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 98.00%.
Optical purity: ≥ 99.50% L-enantiomer.
ⓘ Ⓖ 3.20, 4.4, Ⓜ 3.22
[1] S. N. McCurdy (1989) *Pept. Res.*, **2**, 147.

852022	**Fmoc-Cys(tButhio)–OH**	5 g	113.00
	N-α-Fmoc-S-t.-butylthio-L-cysteine	25 g	453.00
	NBC No.: 04-12-1041; CAS No.: 73724-43-3; $C_{22}H_{25}NO_4S_2$; M.W.: 431.6		

Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (85:10:5), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 97.00%.
Optical purity: ≥ 99.50% L-enantiomer.
⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.
The t-Buthio group can be selectively removed using thiols [1] or tributylphosphine[2, 3]. This protecting group is stable to TFA if thiol scavengers are not used.
ⓘ Ⓖ 3.32, 4.4, 4.12, Ⓜ 3.34
[1] U. Weber & P. Hartter (1970) *Hoppe-Seyler's Z. Physiol. Chem.*, **351**, 1384.
[2] U. T. Ruegg & H. G. Gattner (1975) *Hoppe-Seyler's Z. Physiol. Chem.*, **356**, 1527.
[3] N. J. C. Beekman, et al. (1997) *J. Peptide Res.*, **50**, 357.

852373	**Fmoc-Cys(STmp)–OH**	1 g	200.00
NEW	N-α-Fmoc-S-2,4,6-trimethoxyphenylthio-L-cysteine	5 g	800.00
	$C_{27}H_{27}NO_7S_2$; M.W.: 541.1		

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
HPLC: purity: ≥ 97.00%.
EtOAc: HS-GC ≤ 0.50%
AcOH: IC ≤ 0.02%
Optical purity: ≥ 99.50% L-enantiomer.
Fmoc-Cys(STmp)-OH [1] is a novel new tool for the regioselective synthesis of multiple disulfide bridged peptides. The STmp group is stable to piperidine but is extremely easy to remove by mild thiolysis. Albericio [2] has reported removing four STmp groups on the solid phase with only three 5 minute treatments of 0.1 M N-methylmorpholine (NMM) in DMF containing 5% DTT.
ⓘ Ⓖ 3.32, 4.4, 4.12, Ⓜ 3.34
[1] T. M. Postma, et al. (2012) *Org. Lett.*, **14**, 5468.
[2] T. M. Postma & F. Albericio (2013) *Org. Lett.*, **15**, 616.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

852031 — Fmoc-Cys(Mmt)-OH

		Quantity	Price
		1 g	52.00
		5 g	192.00
		25 g	723.00

N-α-Fmoc-S-p-methoxytrityl-L-cysteine

NBC No.: 04-12-1061; CAS No.: 177582-21-7; $C_{38}H_{33}NO_5S$; M.W.: 615.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 99.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 99.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

The Mmt group can be selectively deprotected on the solid phase with 1% TFA in DCM containing 5% TIS [1 - 4]. Reference 4 describes a novel method for on-resin disulfide bond formation which uses Cys(Mmt) in combination with Cys(tBuS).

ⓘ Ⓖ 3.34, 4.3, Ⓜ 3.34

[1] K. Barlos, et al. in "Peptides 1992, Proc. 22nd European Peptide Symposium", C. H. Schneider & A. N. Eberle (Eds), ESCOM, Leiden, 1993, pp. 283.
[2] C. Kellenberger, et al. (1995) *Pept. Res.*, **8**, 321.
[3] K. Barlos, et al. (1996) *Int. J. Peptide Protein Res.*, **47**, 148.
[4] A. K. Galande, et al. (2005) *J. Comb. Chem.*, **7**, 174.

852008 — Fmoc-Cys(Trt)-OH

		Quantity	Price
		25 g	60.00
		100 g	180.00
		250 g	395.00

N-α-Fmoc-S-trityl-L-cysteine

NBC No.: 04-12-1018; CAS No.: 103213-32-7; $C_{37}H_{31}NO_4S$; M.W.: 585.7

Solubility: 25 mmole in 50 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98%.

HPLC: purity: ≥ 99.0%.

Fmoc-β-Ala-OH ≤ 0.1%.

Fmoc-β-Ala-Cys(Trt)-OH ≤ 0.1%.

Fmoc-Cys(Trt)-Cys(Trt)-OH ≤ 0.1%.

Fmoc-Cys-OH ≤ 0.1%.

Free amino acid: ≤ 0.2%.

EtOAc: ≤ 0.50%.

AcOH: ≤ 0.02%.

Optical purity: ≥ 99.8% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

The standard derivative for Fmoc SPPS of peptides containing Cys [1]. The Trt group is removed with 95% TFA containing 1-5% TIS. Ideally, this derivative should be introduced using the symmetrical anhydride or DIPCDI/HOBt activation [2,3] to minimize racemization. If activation with uronium or phosphonium reagents, such as HBTU or PyBOP®, is to be employed, it is strongly recommended that collidine is used as the base [4], as this has been shown to significantly reduce loss of optical integrity during coupling.

ⓘ Ⓖ 3.33, 4.3

[1] S. N. McCurdy (1989) *Pept. Res.*, **2**, 147.
[2] T. Kaiser, et al. (1996) *Tetrahedron Lett.*, **37**, 1187.
[3] Y. X. Han, et al. (1997) *J. Org. Chem.*, **62**, 4307.
[4] Y. N. Angell (2002) *J. Peptide Res.*, **5**, 292.

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Product No.	Product	Quantity	Price
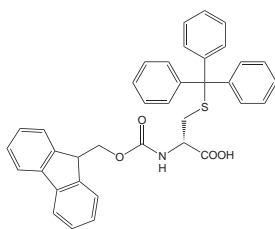
852143 **Fmoc–D–Cys(Trt)–OH**   1 g   35.00

N-α-Fmoc-S-trityl-D-cysteine   5 g   140.00

NBC No.: 04-13-1010; CAS No.: 167015-11-4; $C_{37}H_{31}NO_4S$; M.W.: 585.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

Optical purity: ≥ 99.50% D-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.
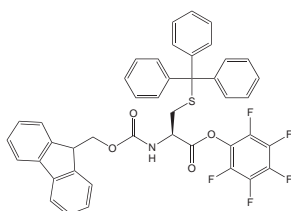
852126 **Fmoc–Cys(Trt)–OPfp**   5 g   185.00

N-α-Fmoc-S-trityl-L-cysteine pentafluorophenyl ester

NBC No.: 04-12-1504; CAS No.: 115520-21-3; $C_{43}H_{30}F_5NO_4S$; M.W.: 751.8

Solubility: 0.5 mmole in 3 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$ (1 : 3), purity: ≥ %.

HPLC: purity: ≥ 97.00%.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

An excellent derivative for the introduction of Cys(Trt) without racemization [1,2].

ⓘ Ⓖ 3.3, 4.4

[1] T. Kaiser, et al. (1996) *Tetrahedron Lett.*, **37**, 1187.

[2] Y. X. Han, et al. (1997) *J. Org. Chem.*, **62**, 4307.
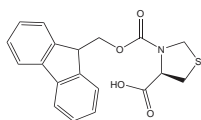
852338 **Fmoc–Thz–OH**   1 g   21.00

Fmoc-thiaproline   5 g   83.00

Fmoc-thiazolidine-4-carboxylic acid   25 g   333.00

CAS No.: 133054-21-4; $C_{19}H_{17}NO_4S$; M.W.: 355.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry.

A building block for the intoduction of thiaproline (Thz) during Fmoc SPPS. Thz has been employed as a masked cysteine residue to prevent self-ligation during native chemical ligation reactions (NCL) of peptide thioesters bearing an N-terminal Cys residue [6]. Once ligation is complete, the N-terminal Cys residue of the resultant peptide is unmasked by ring opening the Thz residue by treatment with methoxyamine, thereby enabling its subsequent ligation to another peptide thioester fragment.

[1] M. Villain, et al. in "Peptides: The wave of the future, Proc. Second Int.& 17th American Peptide Symposium", M. Lebl & R A. Houghten (Eds), American peptide Society, 2001, pp. 107.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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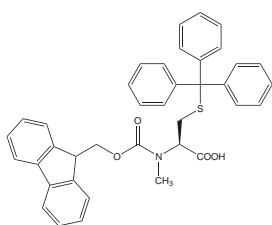
Product No.	Product	Quantity	Price
852348	**Fmoc-N-Me-Cys(Trt)-OH**	250 mg	166.00
	N-α-Fmoc-N-α-methyl-S-trityl-L-cysteine	1 g	510.00

CAS No.: 944797-51-7; $C_{38}H_{33}NO_4S$; M.W.: 599.7

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

Solubility: 1 mmole in 2 ml DMF clearly soluble.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.50% L-enantiomer.
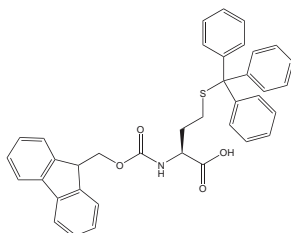
852266	**Fmoc-hCys(Trt)-OH**	1 g	203.00
	Fmoc-S-trityl-L-homocysteine	5 g	806.00

(S)-2-(Fmoc-amino)-4-tritylsulfanyl-butyric acid

CAS No.: 167015-23-8; $C_{38}H_{33}NO_4S$; M.W.: 599.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 97.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 98.00% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
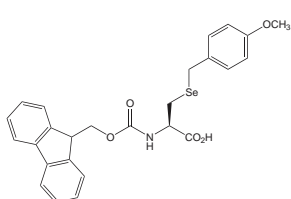
852346	**Fmoc-Sec(pMeOBzl)-OH**	250 mg	281.00
	Fmoc-Sec(Mob)-OH	1 g	842.00

Fmoc-S-4-methoxybenzyl selenocysteine

CAS No.: 150308-80-8; $C_{26}H_{25}NO_5Se$; M.W.: 510.4

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

Building block for the introduction of selenocysteine during Fmoc SPPS [1-3]. Selenocysteine is regarded as the 21st amino acid of the genetic code (4). Due to its unique redox properties selenocysteine containing peptides have been used in protein folding studies (5, 6) and native chemical ligation-deselenization methods (7). Selenocysteine derivatives can undergo racemization during coupling and can form dehydroalanine and β-piperidinylalanine containing side products during subsequent chain elongation [3]. Therefore, activation methods, such as HBTU or PyBOP which involve the addition of a tertiary, base should be avoided for addtion of the Sec and all subsequent residues.

Cleavage and side-chain deprotection of Sec-containing peptides can be effected using TFA-m-cresol-thioanisole-EDT-water (80:5:5:5:5)[2] or TFA-water-DCM-TIS (89:5:5:1) at 4 °C [3]. As the Se-pMeOBzl bond is stable to TFA, these methods result in formation of the corresponding Sev(pMeOBzl) peptide.

Peptides containing two Sec(pMeOBzl) residues can be oxidized directly to the corresponding selenocystinyl peptides by treatment with 5-10% DMSO [1 - 3] or DTNP in TFA [8].

[1] T. Koide, et al. (1993) *Chem. Pharm. Bull.*, **41**, 502.

[2] T. Koide, et al. (1993) *Chem. Pharm. Bull.*, **41**, 1596.

[3] D. Besse & L. Moroder (1997) *J. Peptide Sci.*, **3**, 442.

[4] A. Bock, et. al. (1991) *Mol. Microbiol.*, **5**, 515.

[5] S. Pegoraro, et. al. (1999) *Protein Sci.*, **8**, 1605.

[6] M. Muttenthaler & P.F. Alewood (2008) *J. Pept. Sci.*, **14**, 1223.

[7] P.E. Dawson (2011) *Isr. J. Chem.*, **51**, 862.

[8] A.L. Schroll, et. al. (2012) *J. Pept. Sci.*, **18**, 155.

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Product No.	Product	Quantity	Price

Diaminobutanoic acid [Dab]
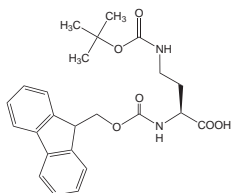
852074 **Fmoc-Dab(Boc)-OH** — 1 g 102.00 / 5 g 408.00

N-α-Fmoc-N-γ-t.-Boc-L-diaminobutanoic acid

NBC No.: 04-12-1160; CAS No.: 125238-99-5; $C_{24}H_{28}N_2O_6$; M.W.: 440.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: nBuOH:AcOH:H₂O (7:1:1), purity: ≥ 98.00%.

nBuOH:AcOH:H₂O:Pyridine (15:3:12:10), purity: ≥ 98.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.00% L-enantiomer.
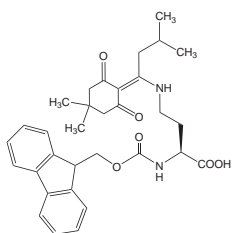
852084 **Fmoc-Dab(ivDde)-OH** — 1 g 156.00 / 5 g 624.00

N-α-Fmoc-N-γ-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl-L-diaminobutanoic acid

NBC No.: 04-12-1196; CAS No.: 607366-21-2; $C_{32}H_{38}N_2O_6$; M.W.: 546.6

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl₃:MeOH:AcOH:H₂O (90:10:0.5:1), purity: ≥ 98.00%.

CHCl₃:MeOH:AcOH:H₂O (85:13:0.5:1.5), purity: ≥ 98.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

This orthogonally-protected diaminobutyric acid derivative is based on the hindered Dde variant ivDde. The side-chain ivDde group is considerably more stable to piperidine than Dde and is less prone to migrate from protected to unprotected side-chains [1] and from side-chain to α-amino group [2]. When removing ivDde in the presence of allyl-based protecting groups, allyl alcohol should be included in the deprotection solution to prevent reduction of the allyl group [3].

ⓘ Ⓖ 4.9, Ⓜ 4.10

[1] S. R. Chhabra, et al. (1998) *Tetrahedron Lett.*, **39**, 1603.

[2] R. Wilhelm, personal communication.

[3] B. Rohwedder, et al. (1998) *Tetrahedron Lett.*, **39**, 1175.

[4] S. K. Sharma, et al. (1999) *J. Peptide Res.*, **53**, 501.
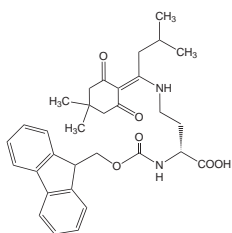
852167 **Fmoc-D-Dab(ivDde)-OH** — 1 g 322.00 / 5 g 1248.00

N-α-Fmoc-N-γ-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl-D-diaminobutanoic acid

NBC No.: 04-13-1075; CAS No.: 872169-32-9; $C_{32}H_{38}N_2O_6$; M.W.: 546.6

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl₃:MeOH:AcOH:H₂O (90:10:0.5:1), purity: ≥ 98.00%.

CHCl₃:MeOH:AcOH:H₂O (85:13:0.5:1.5), purity: ≥ 98.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.00% D-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

⚠ Storage conditions　　Ⓖ Additional information　　Ⓜ Method information　　Ⓐ Application information

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Product No.	Product	Quantity	Price
852092	**Fmoc-Dab(Mtt)-OH**	1 g	250.00
	N-α-Fmoc-N-γ-4-methyltrityl-L-diaminobutanoic acid	5 g	998.00

NBC No.: 04-12-1210; CAS No.: 851392-68-2; $C_{39}H_{36}N_2O_4$; M.W.: 596.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 95.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

The side-chain Mtt group can be selectively removed with 1% TFA in DCM [1, 2, 3] or DCM/HFIP/TFE/TES (6.5:2:1:0.5) [4], making this an excellent derivative for the synthesis of branched peptides and peptides modified at the Dab side-chain [4, 5], and for the construction of templates and multifunctionalized resins for combinatorial synthesis.

ⓘ Ⓖ 4.10, Ⓜ 4.12

[1] K. Barlos, et al., C. H. Schneider & A. N. Eberle (Eds), ESCOM, Leiden, 1993, pp. 283.
[2] A. Aletras, et al. (1995) *Int. J. Peptide Protein Res.*, **45**, 488.
[3] L. Bourel, et al. (2000) *J. Peptide Sci.*, **6**, 264.
[4] K. Barlos, personal communication.
[5] P. Hoogerhout, et al. (1999) *J. Peptide Res.*, **54**, 436.
[6] C. Park & K. Burgess (2001) *J. Comb. Chem.*, **3**, 257.

Diaminopropionic acid [Dpr]

852087	**Fmoc-Dpr(Boc)-OH**	1 g	120.00
	N-α-Fmoc-N-β-t.-Boc-L-diaminopropionic acid	5 g	359.00

NBC No.: 04-12-1201; CAS No.: 162558-25-0; $C_{23}H_{26}N_2O_6$; M.W.: 426.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: nBuOH:AcOH:H$_2$O (7:1:1), purity: ≥ 97.00%.

nBuOH:AcOH:H$_2$O:Pyridine (15:3:12:10), purity: ≥ 97.00%.

HPLC: purity: ≥ 96.00%.

Optical purity: ≥ 99.50% L-enantiomer.

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Product No.	Product	Quantity	Price
852083	**Fmoc-Dpr(ivDde)-OH**	1 g	156.00
	N-α-Fmoc-N-β-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl-L-diaminopropionic acid	5 g	624.00

NBC No.: 04-12-1195; CAS No.: 607366-20-1; $C_{31}H_{36}N_2O_6$; M.W.: 532.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98.00%.
$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

This orthogonally-protected diaminopropionic acid derivative is based on the hindered Dde variant ivDde. The side-chain ivDde group is considerably more stable to piperidine than Dde and is less prone to migrate from protected to unprotected side-chains [1]. However, migration from side-chain to the unprotected α-amino group of Dpr is unavoidable [2]. This side-reaction can, however, be minimized by the appropriate choice of coupling method for the subsequent residue, see [3].

When removing ivDde in the presence of allyl-based protecting groups, allyl alcohol should be included in the deprotection solution to prevent reduction of the allyl group [4].

ⓘ Ⓖ 4.9, Ⓜ 4.10

[1] S. R. Chhabra, et al. (1998) *Tetrahedron Lett.*, **39**, 1603.
[2] R. Wilhelm, et al., Poster 34 presented at the 16th American Peptide Symposium, Minneapolis, 1999.
[3] J. Beythien & P. Schneeberger, in "Peptides 2000, Proc. 26th European Peptide Symposium", EDK, Paris, 2001, pp. 361.
[4] B. Rohwedder, et al. (1998) *Tetrahedron Lett.*, **39**, 1175.
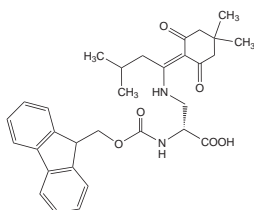
852243	**Fmoc-D-Dpr(ivDde)-OH**	1 g	322.00
	N-α-Fmoc-N-β-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl-D-diaminopropionic acid	5 g	1290.00

NBC No.: 04-13-1074; CAS No.: 1228900-15-9; $C_{31}H_{36}N_2O_6$; M.W.: 532.5

TLC: $CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98.00%.
$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.00% D-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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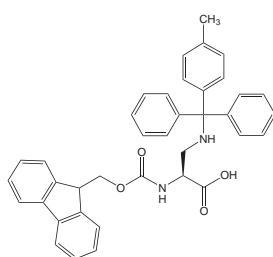
Product No.	Product	Quantity	Price
852089	**Fmoc-Dpr(Mtt)-OH**	1 g	250.00
	N-α-Fmoc-N-β-4-methyltrityl-L-diaminopropionic acid	5 g	998.00

NBC No.: 04-12-1204; CAS No.: 654670-89-0; $C_{38}H_{34}N_2O_4$; M.W.: 582.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH:H$_2$O (90:10:0.5:1), purity: ≥ 95.00%.

HPLC: purity: ≥ 95.00%.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

The side-chain Mtt group can be selectively removed with 1% TFA in DCM [1, 2, 3] or DCM/HFIP/TFE/TES (6.5:2:1:0.5) [4], making this an excellent derivative for the synthesis of branched peptides and peptides modified at the Dpr side-chain [4, 5], and for the construction of templates and multifunctionalized resins for combinatorial synthesis[6].

ⓘ Ⓖ 4.9, 4.10, Ⓜ 4.12

[1] K. Barlos, et al., C. H. Schneider & A. N. Eberle (Eds), ESCOM, Leiden, 1993, pp. 283.
[2] A. Aletras, et al. (1995) *Int. J. Peptide Protein Res.*, **45**, 488.
[3] L. Bourel, et al. (2000) *J. Peptide Sci.*, **6**, 264.
[4] K. Barlos, personal communication.
[5] P. Hoogerhout, et al. (1999) *J. Peptide Res.*, **54**, 436.
[6] C. Park & K. Burgess (2001) *J. Comb. Chem.*, **3**, 257.

Dihydroxyphenylalanine [DOPA]
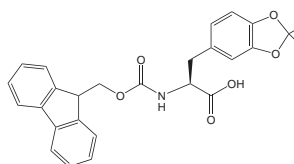
852093	**Fmoc-DOPA(acetonide)-OH**	1 g	551.00

NBC No.: 04-12-1212; CAS No.: 852288-18-7; $C_{27}H_{25}NO_6$; M.W.: 459.5

TLC: CHCl$_3$:MeOH:AcOH:H$_2$O (90:10:0.5:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

A useful building block for the synthesis of DOPA-containing peptides by Fmoc SPPS. Cleavage with TFA mixtures containing water results in deprotection of the DOPA side chain.

The redox-induced chemical crosslinking of the catechol moiety has been used to investigate peptide-protein interactions [1 - 2] and to synthesize polymer-coated metal nanoparticles [3].

[1] L. Burdine, et. al. (2004) *J. Am. Chem.*, **126**, 11442.
[2] B. Liu, et. al. (2007) *J. Am. Chem.*, **129**, 12348.
[3] K. C. L. Black, et. al. (2011) *Chem. Mat.*, **23**, 1130.

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Product No.	Product	Quantity	Price

Glutamic acid [Glu, E]

Fmoc-Glu(OtBu)-OH is recommended for the routine preparation of glutamic acid-containing peptides. For the preparation of cyclic peptides and peptides containing side-chain modified Glu residues, derivatives such as Fmoc-Glu-OAll, Fmoc-Glu(OAll)-OH, Fmoc-Glu(O-2-PhiPr)-OH, Fmoc-Glu-ODmab, and Fmoc-Glu(ODmab)-OH should be used since their respective carboxy-protecting groups can be removed selectively on the solid-phase.

For further information, please refer to the product entries.

852102	Fmoc-Glu(biotinyl-PEG)-OH	500 mg	296.00
		1 g	530.00

N-α-Fmoc-N-γ-(N-biotinyl-3-(2-(2-(3-aminopropyloxy)-ethoxy)-ethoxy)-propyl)-L-glutamine

NBC No.: 04-12-1250; CAS No.: 817169-73-6; $C_{40}H_{55}N_5O_{10}S$; M.W.: 798.0

Solubility: 0.2 mmol in 1 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ **Prolonged storage**: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.

In contrast to Fmoc-Lys(biotin)-OH, this novel biotin-labeled amino acid has excellent Solubility in DMF and other solvents used in SPPS [1]. The PEG-spacer restricts hindrance between the peptide and avidin, leading to better biotin binding. Furthermore, the hydrophilic nature of the PEG minimizes non-specific interactions that can arise from the spacer group becoming buried in the hydrophobic pocket of proteins.

For recent applications, please see [2 - 5].

ⓘ Ⓖ 5.18

[1]   B. Baumeister, et al. (2003) *Biopolymers*, **71**, 339.
[2]   X. Zhou et al., (2004) *J. Am. Chem. Soc.*, **126**, 15656.
[3]   B. F. Gilmore, et al. (2006) *Biochem. Biophys. Res. Commun,*, **347**, 373.
[4]   C. T. Archer, et al. (2005) *Mol. BioSyst.*, **1**, 366.
[5]   N. Srinivas, et. al. (2010) *Science*, **327**, 1010.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
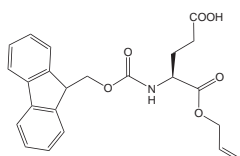
852073   **Fmoc-Glu-OAll**   1 g   57.00

N-α-Fmoc-L-glutamic acid α-allyl ester   5 g   224.00

NBC No.: 04-12-1158; CAS No.: 144120-54-7; $C_{23}H_{23}NO_6$; M.W.: 409.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

Orthogonally-protected building block for the synthesis of head-to-tail cyclic peptides [1, 2, 3] or for C-terminal modifications via side-chain anchoring [4, 5]. The α-allyl ester can be selectively removed in the presence of Fmoc- and tBu-based protecting groups by treatment with $Pd(Ph_3P)_4$/ $CHCl_3$/AcOH/NMM [6], thereby facilitating the synthesis of branched esters and amides, and lactones and lactams incorporating a glutamyl unit.

ⓘ Ⓖ 4.13, Ⓜ 4.14

[1] W. Bannwarth, et al. (1992) *Tetrahedron Lett.,* **33**, 4557.
[2] F. Albericio, et al. (1993) *Tetrahedron Lett.,* **34**, 1549.
[3] Z. Hayouka, et. al. (2010) *Bioorg. Med. Chem.,* **18**, 8388
[4] S. Ficht, et. al. (2008) *Chem. Eur. J.,* **13**, 3620.
[5] T. Gemma, et. al., (2011) *Org. Lett.,* **13**, 4770.
[6] S. A. Kates, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 13th American Peptide Symposium", ESCOM, Leiden, 1994, pp. 113.
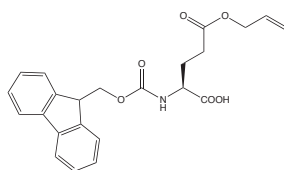
852123   **Fmoc-Glu(OAll)-OH**   5 g   73.00

NBC No.: 04-12-1304; CAS No.: 133464-46-7; $C_{23}H_{23}NO_6$; M.W.: 409.43   25 g   281.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 97.00%.

Orthogonally-protected building block for the synthesis of peptides modified at the Glu side chain. The α-allyl ester can be selectively removed in the presence of Fmoc- and tBu-based protecting groups by treatment with $Pd(Ph_3P)_4$/ $CHCl_3$/AcOH/NMM, thereby facilitating the synthesis of branched esters and amides, and lactones and lactams incorporating a glutamyl unit.

ⓘ Ⓖ 4.13, Ⓜ 4.14

852098   **Fmoc-Glu(EDANS)-OH**   500 mg   307.00

NBC No.: 04-12-1238; CAS No.: 193475-66-0; $C_{32}H_{31}N_3O_8S$; M.W.: 617.7   1 g   567.00

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 95.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

Fluorescence-labeled amino acid for preparing fluorescence-quenched peptide substrates [1]. Most frequently used in conjunction with Dabcyl quenching group.

ⓘ Ⓖ 5.12

[1] J. W. Drijfhout, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 14th American Peptide Symposium", P. T. P. Kaumaya & R. S. Hodges (Eds), Kingswinford, Mayflower Scientific Ltd., 1996, pp. 129.

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Product No.	Product	Quantity	Price

852221   **Fmoc–Glu–OBzl**   1 g   71.00

N-α-Fmoc-L-glutamic acid α-benzyl ester   5 g   279.00

NBC No.: 04-12-1273; CAS No.: 122350-52-1; $C_{27}H_{25}NO_6$; M.W.: 459.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 97.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.00% L-enantiomer.

852204   **Fmoc–Glu(OBzl)–OH**   5 g   38.00

N-α-Fmoc-L-glutamic acid γ-benzyl ester   25 g   146.00

NBC No.: 04-12-1019; CAS No.: 123639-61-2; $C_{27}H_{25}NO_6$; M.W.: 459.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

852127   **Fmoc–Glu(OtBu)–OPfp**   5 g   185.00

N-α-Fmoc-L-glutamic acid γ-t.-butyl ester pentafluorophenyl ester

NBC No.: 04-12-1506; CAS No.: 86061-04-3; $C_{30}H_{26}F_5NO_6$; M.W.: 591.6

Solubility: 0.5 mmole in 3 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$ (1 : 3), purity: ≥ %.

HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

852035   **Fmoc–Glu–OtBu**   1 g   60.00

N-α-Fmoc-L-glutamic acid α-t.-butyl ester   5 g   229.00

NBC No.: 04-12-1075; CAS No.: 84793-07-7; $C_{24}H_{27}NO_6$; M.W.: 425.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

Optical purity: ≥ 99.50% L-enantiomer.

Selectively protected building block for library synthesis or the preparation of γ-glutamyl peptides. After condensation of the side-chain carboxy with an appropriate amine or alcohol, the α-amino and carboxy functions can be selectively unmasked with 20% piperidine in DMF and 50% TFA in DCM respectively, facilitating the synthesis of branched esters, amides, lactams and lactones containing the glutamyl unit.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
852009	**Fmoc-Glu(OtBu)-OH**	25 g	60.00
	N-α-Fmoc-L-glutamic acid γ-t.-butyl ester	100 g	180.00
	NBC No.: 04-12-1020; CAS No.: 71989-18-9; $C_{24}H_{27}NO_6$; M.W.: 425.5	250 g	395.00

Solubility: 25 mmole in 50 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98%.

$CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.

HPLC: purity: ≥ 99.0%.

Fmoc-β-Ala-OH ≤ 0.1%.

Fmoc-β-Ala-Glu(OtBu)-OH ≤ 0.1%.

Fmoc-Glu(OtBu)-Glu(OtBu)-OH ≤ 0.1%.

Fmoc-Glu-OH ≤ 0.1%.

Free amino acid: ≤ 0.2%.

EtOAc: ≤ 0.5%.

AcOH: ≤ 0.02%.

Optical purity: ≥ 99.8% L-enantiomer.

Product No.	Product	Quantity	Price
852155	**Fmoc-D-Glu(OtBu)-OH**	1 g	44.00
	N-α-Fmoc-D-glutamic acid γ-t.-butyl ester	5 g	172.00
	NBC No.: 04-13-1051; CAS No.: 104091-08-9; $C_{24}H_{27}NO_6$; M.W.: 425.5	25 g	688.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% D-enantiomer.

customer service: service@novabiochem.com   technical service: technical@novabiochem.com internet: novabiochem.com
Bulk quantities available, please enquire

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Product No.	Product	Quantity	Price
852077	**Fmoc-Glu-ODmab**	1 g	187.00
	N-α-Fmoc-L-glutamic acid α-4-{N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino} benzyl ester	5 g	749.00

NBC No.: 04-12-1174; CAS No.: 172611-75-5; $C_{40}H_{44}N_2O_8$; M.W.: 680.8

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

Quasi-orthogonally-protected Glu derivative.

The Dmab group can be removed selectively in the presence of tBu-based protecting groups by treatment with 2% hydrazine in DMF [1], making this derivative an extremely useful tool for the preparation of cyclic peptides by Fmoc SPPS or for library synthesis. For the on-resin synthesis of side-chain to side-chain lactam bridged peptides, the combination of Lys(ivDde) and Glu(ODmab) is particularly advantageous since both side-chains can be simultaneously unmasked in a single step.

Occasionally sluggish cleavage of the aminobenzyl moiety is observed [2, 3]. In these instances, washing the support with 20% DIPEA in DMF/water (9:1) [2] or 5 mM NaOH in MeOH/H$_2$O [4] has been found to be efficacious.

ⓘ Ⓖ 4.14, Ⓜ 4.10 Ⓐ 4.16

[1] W. C. Chan, et al. (1995) *J. Chem. Soc., Chem. Commun.*, 2209.

[2] S. Künzel, et al. *Poster 17 presented at Solid Phase Synthesis & Combinatorial Libraries, Southampton, September 2001*; R.S. Harrison, et. al. (2010) *Proc. Natl. Acad. Sci. USA*, **107**, 11686.

[3] K. F. Medzihradszky, et al. (2002) *Lett. Pept. Sci.*, **8**, 1.

[4] T. Conroy, et. al. (2009) *Org. Biomol. Chem.*, **7**, 2255.

852247	**Fmoc-D-Glu-ODmab**	1 g	364.00
	N-α-Fmoc-D-glutamic acid α-4-{N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino} benzyl ester	5 g	1451.00

NBC No.: 04-13-1083; CAS No.: 874486-65-4; $C_{40}H_{44}N_2O_8$; M.W.: 680.8

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 97.00%.
CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 98.00% D-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

See the product entry for 852077 for further details.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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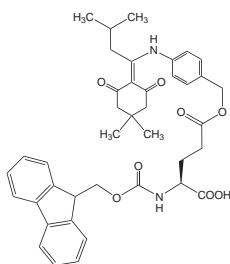
Product No.	Product	Quantity	Price
852076	**Fmoc-Glu(ODmab)-OH**	1 g	166.00
		5 g	666.00
		25 g	2387.00

N-α-Fmoc-L-glutamic acid γ-4-{N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino} benzyl ester

NBC No.: 04-12-1173; CAS No.: 268730-86-5; $C_{40}H_{44}N_2O_8$; M.W.: 680.8

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

CHCl$_3$:MeOH:AcOH:H$_2$O (90:10:0.5:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 96.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

The Dmab group can be removed selectively in the presence of tBu-based protecting groups by treatment with 2% hydrazine in DMF [1], making this derivative an extremely useful tool for the preparation of cyclic peptides by Fmoc SPPS or for library synthesis. For the on-resin synthesis of side-chain to side-chain lactam bridged peptides, the combination of Lys(ivDde) and Glu(ODmab) is particularly advantageous since both side-chains can be simultaneously unmasked in a single step.

Occasionally sluggish cleavage of the aminobenzyl moiety is observed [2, 3]. In these instances, washing the support with 20% DIPEA in DMF/water (9:1) [2] or 5 mM NaOH in MeOH/H$_2$O [4] has been found to be efficacious.

ⓘ Ⓖ 4.14, Ⓜ 4.10 Ⓐ 4.16

[1] W. C. Chan, et al. (1995) *J. Chem. Soc., Chem. Commun.*, 2209.

[2] S. Künzel, et al.*Poster 17 presented at Solid Phase Synthesis & Combinatorial Libraries, Southampton, September 2001*; R.S. Harrison, et. al. (2010) *Proc. Natl. Acad. Sci. USA*, **107**, 11686.

[3] K. F. Medzihradszky, et al. (2002) *Lett. Pept. Sci.*, **8**, 1.

[4] T. Conroy, et. al. (2009) *Org. Biomol. Chem.*, **7**, 2255.

852117	**Fmoc-Glu-O-2-PhiPr**	1 g	260.00

N-α-Fmoc-L-glutamic acid α-2-phenylisopropyl ester

NBC No.: 04-12-1284; CAS No.: 207305-97-3; $C_{29}H_{29}NO_6$; M.W.: 487.6

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:EtOAc:AcOH (45:5:1), purity: ≥ 97.00%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

An excellent tool for the synthesis of head-to-tail cyclic peptides by Fmoc SPPS [1]. The 2-PhiPr group can be selectively removed on the resin with 1% TFA in DCM in the presence of the standard t-butyl-based side-chain protecting groups.

ⓘ Ⓖ 4.14, Ⓜ 4.12

[1] F. Dick, et al. in "Peptides 1996, Proc. of 24th European Peptide Symposium", R. Ramage & R. Epton (Eds), Mayflower Scientific Ltd., Birmingham, 1998, pp. 339.

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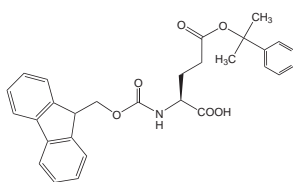
Product No.	Product	Quantity	Price
852085	**Fmoc-Glu(O-2-PhiPr)-OH**	1 g	135.00
	N-α-Fmoc-L-glutamic acid γ-2-phenylisopropyl ester	5 g	562.00

NBC No.: 04-12-1199; CAS No.: 200616-39-3; $C_{29}H_{29}NO_6$; M.W.: 487.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 97.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen; hygroscopic.

Quasi-orthogonally-protected Glu derivative.

The 2-PhiPr group can be removed selectively in the presence of tBu-based protecting groups by treatment with 1% TFA in DCM [1], making this derivative an extremely useful tool for the preparation of cyclic peptides by Fmoc SPPS [2] or for library synthesis. For the on-resin synthesis of side-chain to side-chain lactam bridged peptides, the combination of Lys(Mtt) and Glu(O-2-PhiPr) is particularly advantageous since both side-chains can be simultaneously unmasked in a single step.

This derivative has also been used for the on-resin synthesis of peptide-viologen conjugates [3] and employed in the synthesis of peptide thioesters with self-purification effect for the selective on-resin deprotection of Glu [4].

ⓘ Ⓖ 4.14, Ⓜ 4.12

[1] C. Yue, et al. (1993) *Tetrahedron Lett.*, **34**, 323.
[2] F. Dick, et al. in "Peptides 1996, Proc. of 24th European Peptide Symposium", R. Ramage & R. Epton (Eds), Mayflower Scientific Ltd., Birmingham, 1998, pp. 339.
[3] J. J. Reczek, et. al. (2010) *J. Org. Chem.*, **75**, 2111.
[4] F. Mende, et. al. (2010) *J. Am. Chem. Soc.*, **132**, 11110.
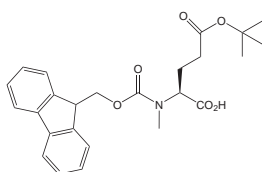
Product No.	Product	Quantity	Price
852330	**Fmoc-N-Me-Glu(OtBu)-OH**	250 mg	78.00
	N-α-Fmoc-N-α-methyl-Lglutamic acid γ-tert butyl ester	1 g	234.00

$C_{25}H_{29}NO_6$; M.W.: 439.5

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
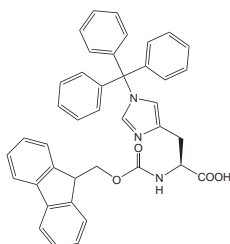
Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% L-enantiomer.

N-α-FMOC PROTECTED AMINO ACIDS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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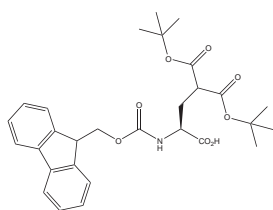
Product No.	Product	Quantity	Price
852345	**Fmoc-Gla(OtBu)₂-OH**	100 mg	150.00
NEW	Fmoc-γ-carboxy-Glu(OtBu)₂-OH	500 mg	600.00

CAS No.: 111662-64-7; $C_{29}H_{35}NO_8$; M.W.: 525.6

⚠ Prolonged storage: +2 to +8°C

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 95.00%.

Fmoc-Gla(OtBu)₂-OH is a building block for the introduction of γ-carboxyglutamic acid (Gla). γ-Carboxylation of glutamic acid is a rare post-translational modification that occurs in blood coagulation factors and in some snake and cone snail venoms [1].

[1] P. K. Bandyopadhyay (2002) *Proc. Natl. Acad. Sci. USA*, **99**, 1264.
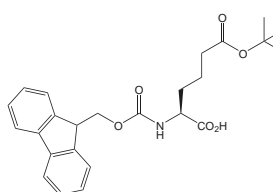
852308	**Fmoc-hGlu(OtBu)-OH**	250 mg	75.00
NEW	Fmoc-L-α-homoglutamic acid 6-t-butyl ester	1 g	200.00
	(S)-2-(Fmoc-amino)adipic acid 6-t-butyl ester	5 g	800.00

CAS No.: 159751-47-0; $C_{25}H_{29}NO_6$; M.W.: 439.5

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

A useful homolog of glutamic aicd for the study of protein-protein interactions, tumor targeting, and SAR studies [1, 2].

[1] R. Carpenter, et al. (2007) *J. Med. Chem.*, **23**, 5863.

[2] P. Li, et al. (2006) *Nat. Chem. Biol.*, **7**, 381.

Glutamine [Gln, Q]
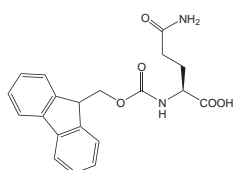
852205	**Fmoc-Gln-OH**	5 g	21.00
	N-α-Fmoc-L-glutamine	25 g	63.00
	NBC No.: 04-12-1021; CAS No.: 71989-20-3; $C_{20}H_{20}N_2O_5$; M.W.: 368.4	100 g	182.00

Solubility: 1 mmole in 2ml DMF with 1 equivalent of DIPEA.

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

Optical purity: ≥ 99.50% L-enantiomer.
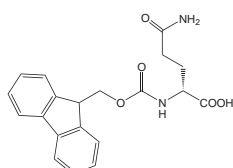
852237	**Fmoc-D-Gln-OH**	1 g	52.00
	N-α-Fmoc-D-glutamine	5 g	208.00
	NBC No.: 04-13-1043; CAS No.: 112898-00-7; $C_{20}H_{20}N_2O_5$; M.W.: 368.4	25 g	827.00

Solubility: 1 mmole in 2ml DMF with 1 equivalent DIPEA.

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.50% D-enantiomer.

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Product No.	Product	Quantity	Price
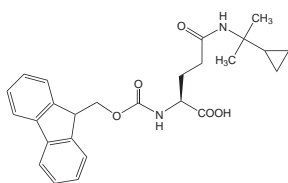
852120   **Fmoc-Gln(Dmcp)-OH**

N-α-Fmoc-N-γ-(1-cyclopropyl-1-methylethyl)-L-glutamine

NBC No.: 04-12-1292; CAS No.: 172947-20-5; $C_{26}H_{30}N_2O_5$; M.W.: 450.53

| | 1 g | 161.00 |
| | 5 g | 645.00 |

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

A superior derivative to Fmoc-Gln(Trt)-OH for the synthesis of Gln-containing peptides by Fmoc SPPS. Fmoc-Gln(Dmcp)-OH is more soluble in DMF than Fmoc-Gln(Trt)-OH, thereby facilitating coupling reactions at higher concentration. Cleavage of the Dmcp group is rapid, even when the residue is located at the N-terminus of a peptide. Coupling of Dmcp-protected derivatives is faster than that of the corresponding hindered Trt derivatives. Dmcp-protected peptides have enhanced Solubility.

These advantageous properties Fmoc-Gln(Dmcp)-OH have been exploited in the synthesis of peptide segments [2] and glycoprotein hormones [3].

ⓘ Ⓖ 4.2

[1] L. A. Carpino, et al. (1995) *J. Org. Chem.,* **60**, 7718.

[2] C. Heinlein, et. al. (2011) *Angew. Chem., Int. Ed.,* **50**, 6406.

[3] B. Aussedat, et. al. (2012) *J. Am. Chem. Soc.,* **134**, 3532.
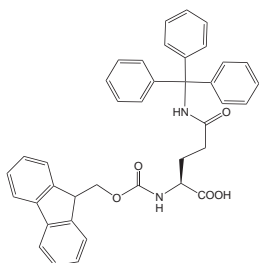
852045   **Fmoc-Gln(Trt)-OH**

N-α-Fmoc-N-γ-trityl-L-glutamine

NBC No.: 04-12-1090; CAS No.: 132327-80-1; $C_{39}H_{34}N_2O_5$; M.W.: 610.7

	25 g	60.00
	100 g	180.00
	250 g	395.00

Solubility: 25 mmole in 50 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98%.

HPLC: purity: ≥ 99.0%.

Fmoc-β-Ala-OH ≤ 0.1%.

Fmoc-β-Ala-Gln(Trt)-OH ≤ 0.1%.

Fmoc-Gln(Trt)-Gln(Trt)-OH ≤ 0.1%.

Fmoc-Gln-OH ≤ 0.1%.

Free amino acid: ≤ 0.2%.

EtOAc: ≤ 0.5%.

AcOH: ≤ 0.02%.

Optical purity: ≥ 99.8% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

Fmoc-Gln(Trt)-OH has good Solubility properties in most organic solvents, and its use has been shown to result in significantly purer peptides than other derivatives used for the introduction of Gln [1]. Coupling can be performed by standard procedures. The trityl-protecting group is normally removed by 95% TFA in 1-3 hours, with no alkylation of Trp.

ⓘ Ⓖ 4.2

[1] P. Sieber, et al. (1991) *Tetrahedron Lett.,* **32**, 739.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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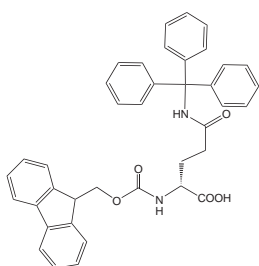
Product No.	Product	Quantity	Price
852160	**Fmoc-D-Gln(Trt)-OH**	1 g	50.00
	N-α-Fmoc-N-γ-trityl-D-glutamine	5 g	200.00
	NBC No.: 04-13-1056; CAS No.: 200623-62-7; $C_{39}H_{34}N_2O_5$; M.W.: 610.7	25 g	800.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

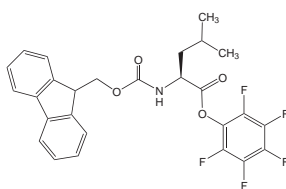CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% D-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

852133	**Fmoc-Gln(Trt)-OPfp**	5 g	185.00

N-α-Fmoc-N-γ-trityl-L-glutamine pentafluorophenyl ester

NBC No.: 04-12-1539; CAS No.: 132388-65-9; $C_{45}H_{33}F_5N_2O_5$; M.W.: 776.8

Solubility: 0.5 mmole in 3 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$ (1 : 3), purity: ≥ %.

HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

Glycine [Gly, G]
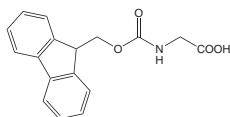
852001	**Fmoc-Gly-OH**	25 g	25.00
	N-α-Fmoc-glycine	100 g	50.00
	NBC No.: 04-12-1001; CAS No.: 29022-11-5; $C_{17}H_{15}NO_4$; M.W.: 297.3	250 g	110.00

Solubility: 25 mmole in 50 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98%.

HPLC: purity: ≥ 99.0%.

Fmoc-β-Ala-OH ≤ 0.1%.

Fmoc-β-Ala-Gly-OH ≤ 0.1%.

Fmoc-Gly-Gly-OH ≤ 0.1%.

Free amino acid: ≤ 0.2%.

EtOAc: ≤ 0.5%.

AcOH: ≤ 0.02%.
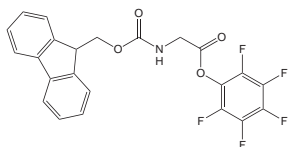
852128	**Fmoc-Gly-OPfp**	5 g	82.00

N-α-Fmoc-glycine pentafluorophenyl ester

NBC No.: 04-12-1507; CAS No.: 86060-85-7; $C_{23}H_{14}NO_4F_5$; M.W.: 463.4

Solubility: 0.5 mmole in 3 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$ (1 : 3), purity: ≥ %.

HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

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Product No.	Product	Quantity	Price

852110   **Fmoc–(Dmb)Gly-OH**

N-α-Fmoc-N-α-(2, 4-dimethoxybenzyl)-glycine

NBC No.: 04-12-1268; CAS No.: 166881-42-1; $C_{26}H_{25}NO_6$; M.W.: 447.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

Fmoc-(Dmb)Gly-OH is an excellent reagent for enhancing synthetic efficiency of glycine-containing peptides.

Like the analogous Hmb derivative Fmoc-(FmocHmb)Gly-OH, the use of this derivative prevents aggregation during chain assemby, thereby leading to faster and more predictable acylation and deprotection reactions. Furthermore, it can prevent aspartimide formation when used to introduce a Gly immediately before an Asp residue and help promote cyclization of Gly-containing peptides [1]. This derivative has been used in the synthesis of insulin-like peptide 5 [2] and 101 residues of d2 domain VEGF receptor [3].

ⓘ Ⓖ 3.9, 3.18

[1] M. El Haddadi, et al. (2000) *J. Pept. Sci.*, **6**, 560.
[2] M. A. Hossain, et. al. (2008) *ChemBioChem*, **9**, 1816.
[3] V. Goncalves, et al. (2009) *J. Pept. Sci.*, **15**, 417.

| | | 1 g | 95.00 |
| | | 5 g | 350.00 |

852064   **Fmoc–(FmocHmb)Gly-OH**

N-α-Fmoc-N-α-(2-Fmoc-oxy-4-methoxybenzyl)-glycine

NBC No.: 04-12-1135; CAS No.: 148515-84-8; $C_{40}H_{33}NO_8$; M.W.: 655.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry.

Hmb protection of amide bonds has been shown to inhibit aggregation of "difficult" peptides, thereby leading to products of increased purity [1-6]. Retention of Hmb groups on the cleaved peptide can greatly improve the Solubility of protected peptide fragments [7, 8] and otherwise intractable sequences [9-11]. Furthermore, using a Hmb-protected derivative for incorporation of the residue linked to the carboxyl group of Asp or Asn residues has been found to suppress formation of aspartimide and piperidide related by-products [12-14]. For a comparison of the efficiency of Hmb and pseudoprolines in preventing aggregation, see [15].

ⓘ Ⓖ 3.9, 3.18

[1] T. Johnson, et al. (1993) *J. Chem. Soc., Chem. Commun.*, 369.
[2] C. Hyde, et al. (1994) *Int. J. Peptide Protein Res.*, **43**, 431.
[3] L. C. Packman, et al. (1994) *Pept. Res.*, **7**, 125.
[4] T. Johnson, et al. (1994) *Tetrahedron Lett.*, **35**, 463.
[5] R. G. Simmonds (1996) *Int. J. Peptide Protein Res.*, **47**, 36.
[6] T. Johnson, et al. (1995) *Lett. Pept. Sci.*, **1**, 11.
[7] M. Quibell, et al. (1995) *J. Am. Chem. Soc.*, **117**, 11656.
[8] M. Quibell, et al. (1996) *J. Chem. Soc., Perkin Trans. 1*, 1227.
[9] M. Quibell, et al. (1994) *Tetrahedron Lett.*, **35**, 2237.
[10] M. Quibell, et al. (1994) *J. Org. Chem.*, **59**, 1745.
[11] M. Quibell, et al. (1995) *J. Chem. Soc., Perkin Trans. 1*, 2019.
[12] M. Quibell, et al. (1994) *J. Chem. Soc., Chem. Commun.*, 2343.
[13] L. C. Packman (1995) *Tetrahedron Lett.*, **36**, 7523.
[14] J. Offer, et al. (1996) *J. Chem. Soc., Perkin Trans. 1*, 175.
[15] W. R. Sampson, et al. (1999) *J. Peptide Sci.*, **5**, 403.

| | | 1 g | 295.00 |
| | | 5 g | 990.00 |

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
852324	**Fmoc-bishomopropargylglycine**	100 mg	187.00
	CAS No.: 1097192-05-6; $C_{22}H_{21}NO_4$; M.W.: 363.41	500 mg	749.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.
HPLC: purity: ≥ 95.00%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
Building block for the introduction by Fmoc SPPS of an amino acid bearing an alkyne side chain. Such derivatives are useful tools for the synthesis of cyclic peptides by an alkyne-alkyne Glaser coupling, involving $Cu(OAc)_2$/pyridine and heating to 60 °C using microwaves.. Any other use of the reagent may be covered by third party patents and it is the customers' obligation to check this if they have the intention to use them for other purposes beside the recommended use.

852323	**Fmoc-homopropargylglycine**	100 mg	187.00
	CAS No.: 942518-21-0; $C_{21}H_{19}NO_4$; M.W.: 349.38	500 mg	749.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.
HPLC: purity: ≥ 97.00%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
Building block for the introduction by Fmoc SPPS of an amino acid bearing an alkyne side chain. Such derivatives are useful tools for the synthesis of cyclic peptides by an alkyne-alkyne Glaser coupling, involving $Cu(OAc)_2$/pyridine and heating to 60 °C using microwaves.. Any other use of the reagent may be covered by third party patents and it is the customers' obligation to check this if they have the intention to use them for other purposes beside the recommended use.

852360	**Fmoc-propargylglycine**	1 g	170.00
	N-Fluorenemethoxycarbonyl-L-propargyl glycine	5 g	690.00
	CAS No.: 198561-07-8; $C_{20}H_{17}NO_4$; M.W.: 335.3		

Solubility: 1 mmole in 2 ml DMF clearly soluble.
HPLC: purity: ≥ 98.00%.
Optical purity: ≥ 99.50% L-enantiomer.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
Building block for the introduction by Fmoc SPPS of an amino acid bearing an alkyne side chain. Such derivatives are useful tools for the synthesis of cyclic peptides by an alkyne-alkyne Glaser coupling, involving $Cu(OAc)_2$/pyridine and heating to 60 °C using microwaves.. Any other use of the reagent may be covered by third party patents and it is the customers' obligation to check this if they have the intention to use them for other purposes beside the recommended use.

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Product No.	Product	Quantity	Price

Histidine [His, H]

In routine synthesis Fmoc-His(Trt)-OH is normally used for introduction of His residues. This approach can, however, lead to extensive racemization, particularly when coupling is sluggish. The use of Fmoc-His(Clt)-OH is recommended for such cases. For further information, please refer to the product entries.
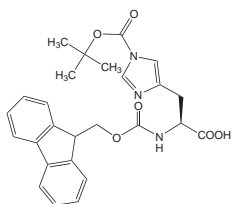
852052   **Fmoc-His(Boc)-OH · CHA**   5 g   98.00

N-α-Fmoc-N-im-t.-Boc-L-histidine cyclohexylammonium salt   25 g   390.00

NBC No.: 04-12-1110; CAS No.: 210820-99-8; $C_{26}H_{27}N_3O_6 \cdot C_6H_{13}N$; M.W.: 477.5 · 99.2

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 97.00%.

$CHCl_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 97.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

The N-im-Boc group is unstable to prolonged treatment with piperidine [1]; the utility of this derivative is therefore limited to the preparation of short sequences by Fmoc SPPS. This derivative is supplied as a CHA-salt. The specific method for the conversion to the free acid is given on page 4.6.

ⓘ Ⓖ 4.6

[1] P. Sieber, et al. (1987) *Tetrahedron Lett.,* **28**, 6031.

852371   **Fmoc-His(Clt)-OH**   5 g   80.00

NEW
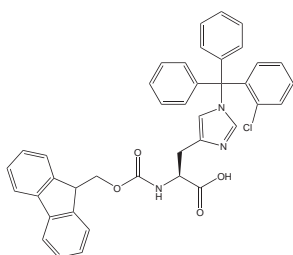
N-α-Fmoc-N-im-2-chlorotrityl-L-histidine   25 g   240.00

$C_{40}H_{32}ClN_3O_4$; M.W.: 654.2

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

ⓘ Ⓖ 4.6

A useful derivative for convergent synthesis of peptide due to the enhanced stability of Clt group compared to Trt group to mildy acidic reagents. Fmoc-His-(Clt)-OH gives approximately a third less racemization when compared to Fmoc-His(Trt)-OH during slow coupling reactions.

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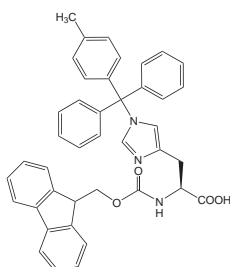
Product No.	Product	Quantity	Price
852211	**Fmoc-His(Mtt)-OH**	1 g	21.00
	N-α-Fmoc-N-im-methyltrityl-L-histidine	5 g	77.00

NBC No.: 04-12-1108; CAS No.: 133367-34-7; $C_{41}H_{35}N_3O_4$; M.W.: 633.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

The Mtt group can be removed from the side-chain of histidine by treatment with dilute TFA [1].

ⓘ G 4.5

[1] K. Barlos, et al. (1991) *Tetrahedron Lett.*, **32**, 475.

852032	**Fmoc-His(Trt)-OH**	25 g	60.00
	N-α-Fmoc-N-im-trityl-L-histidine	100 g	180.00
	NBC No.: 04-12-1065; CAS No.: 109425-51-6; $C_{40}H_{33}N_3O_4$; M.W.: 619.7	250 g	395.00

Solubility: 25 mmole in 50 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98%.

HPLC: purity: ≥ 99.0%.

Fmoc-β-Ala-OH ≤ 0.1%.

Fmoc-β-Ala-His(Trt)-OH ≤ 0.1%.

Fmoc-His(Trt)-His(Trt)-OH ≤ 0.1%.

Fmoc-His-OH ≤ 0.1%.

Free amino acid: ≤ 0.2%.

EtOAc: ≤ 0.5%.

AcOH: ≤ 0.02%.

Optical purity: ≥ 99.8% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

ⓘ G 4.5

[1] P. Sieber, et al. (1987) *Tetrahedron Lett.*, **28**, 6031.
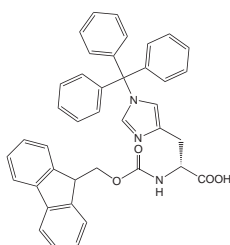
852161	**Fmoc-D-His(Trt)-OH**	1 g	44.00
	N-α-Fmoc-N-im-trityl-D-histidine	5 g	172.00

NBC No.: 04-13-1057; CAS No.: 135610-90-1; $C_{40}H_{33}N_3O_4$; M.W.: 619.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% D-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

customer service: service@novabiochem.com   technical service: technical@novabiochem.com internet: novabiochem.com
Bulk quantities available, please enquire

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Product No.	Product	Quantity	Price
852258	**Fmoc-His(1-Me)-OH**	250 mg	270.00
	Fmoc-1-Methyl-L-Histidine	1 g	1030.00
	Fmoc-His(τ-Me)-OH		
	CAS No.: 202920-22-7; $C_{22}H_{21}N_3O_4$; M.W.: 391.4		
	HPLC: purity: ≥ 98.00%.		
	Building block for the incorporation of 1-methylhistidine by Fmoc SPPS.		

852286	**Fmoc-His(3-Me)-OH**	250 mg	104.00
	Fmoc-3-Methyl-L-Histidine	1 g	411.00
	Fmoc-His(ϖ-Me)-OH		
	CAS No.: 252049-16-4; $C_{22}H_{21}N_3O_4$; M.W.: 391.4		
	Solubility: 0.5 mmole in 4 ml NMP + 500 µl DIPEA.		
	HPLC: purity: ≥ 98.00%.		
	Building block for the incorporation of 1-methylhistidine by Fmoc SPPS.		

852354	**Fmoc-N-Me-His(Trt)-OH**	250 mg	166.00
NEW	N-α-Fmoc-N-α-methyl-im-trityl-L-histidine	1 g	499.00
	CAS No.: 121870-61-3; $C_{41}H_{35}N_3O_4$; M.W.: 633.7		
	HPLC: purity: ≥ 97.00%.		
	Product has poor Solubility but does dissolve upon activation in NMP with HBTU/DIPEA.		

Hydroxyproline [Hyp]

852033	**Fmoc-Hyp-OH**	5 g	62.00
	N-α-Fmoc-L-trans-4-hydroxyproline	25 g	245.00
	NBC No.: 04-12-1068; CAS No.: 88050-17-3; $C_{20}H_{19}NO_5$; M.W.: 353.4		
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 98.00%.		
	Optical purity: ≥ 99.50% L-enantiomer.		

852036	**Fmoc-Hyp(tBu)-OH**	1 g	47.00
	N-α-Fmoc-O-t.-butyl-L-trans-4-hydroxyproline	5 g	182.00
	NBC No.: 04-12-1078; CAS No.: 122996-47-8; $C_{24}H_{27}NO_5$; M.W.: 409.5	25 g	619.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 98.00%.		
	Optical purity: ≥ 99.00% L-enantiomer.		
	⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen; hygroscopic.		

⚠ Storage conditions　　ⓖ Additional information　　Ⓜ Method information　　Ⓐ Application information

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Product No.	Product	Quantity	Price

Isoleucine [Ile, I]

852010 **Fmoc-Ile-OH** | | 25 g | 25.00 |

N-α-Fmoc-L-isoleucine | 100 g | 50.00

NBC No.: 04-12-1024; CAS No.: 71989-23-6; $C_{21}H_{23}NO_4$; M.W.: 353.4 | 250 g | 110.00

Solubility: 25 mmole in 50 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98%.

HPLC: purity: ≥ 99.0%.

Fmoc-β-Ala-OH ≤ 0.1%.

Fmoc-β-Ala-Ile-OH ≤ 0.1%.

Fmoc-Ile-Ile-OH ≤ 0.1%.

Fmoc-Leu-OH ≤ 0.1%.

Free amino acid: ≤ 0.2%.

EtOAc: ≤ 0.5%.

AcOH: ≤ 0.02%.

Optical purity: ≥ 99.7% L-enantiomer.

852374 **Fmoc-D-Ile-OH** ▬NEW▬ | 1 g | 210.00

N-α-Fmoc-D-Isoleucine

$C_{21}H_{23}NO_4$; M.W.: 353.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 98.00% L-enantiomer.
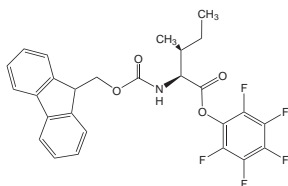
852223 **Fmoc-Ile-OPfp** | 5 g | 82.00

N-α-Fmoc-L-isoleucine pentafluorophenyl ester

NBC No.: 04-12-1509; CAS No.: 86060-89-1; $C_{27}H_{22}NO_4F_5$; M.W.: 519.5

Solubility: 0.5 mmole in 3 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$ (1 : 3), purity: ≥ 5.00%.

HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
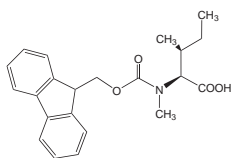
852231 **Fmoc-N-Me-Ile-OH** | 1 g | 72.00

N-α-Fmoc-N-α-methyl-L-isoleucine | 5 g | 280.00

NBC No.: 04-12-9030; CAS No.: 138775-22-1; $C_{22}H_{25}NO_4$; M.W.: 367.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 97.00%.

$CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.50% L-enantiomer.

customer service: service@novabiochem.com   technical service: technical@novabiochem.com internet: novabiochem.com
Bulk quantities available, please enquire

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Product No.	Product	Quantity	Price

Isonipecotic acid [Isn]
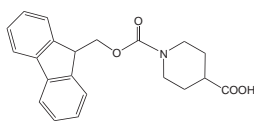
852215 **Fmoc-Isn-OH**

N-α-Fmoc-piperidine-4-carboxylic acid

Fmoc-Inp-OH

NBC No.: 04-12-1183; CAS No.: 148928-15-8; $C_{21}H_{21}NO_4$; M.W.: 351.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

		1 g	20.00
		5 g	80.00
		25 g	316.00

Leucine [Leu, L]

852011 **Fmoc-Leu-OH**

N-α-Fmoc-L-leucine

NBC No.: 04-12-1025; CAS No.: 35661-60-0; $C_{21}H_{23}NO_4$; M.W.: 353.4

Solubility: 25 mmole in 50 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98%.

HPLC: purity: ≥ 99.0%.

Fmoc-β-Ala-OH ≤ 0.1%.

Fmoc-β-Ala-Leu-OH ≤ 0.1%.

Fmoc-Leu-Leu-OH ≤ 0.1%.

Fmoc-Ile-OH ≤ 0.1%.

Free amino acid: ≤ 0.2%.

EtOAc: ≤ 0.5%.

AcOH: ≤ 0.02%.

Optical purity: ≥ 99.8% L-enantiomer.

		25 g	25.00
		100 g	50.00
		250 g	110.00
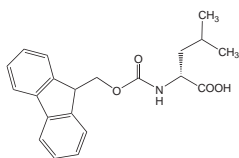
852145 **Fmoc-D-Leu-OH**

N-α-Fmoc-D-leucine

NBC No.: 04-13-1025; CAS No.: 114360-54-2; $C_{21}H_{23}NO_4$; M.W.: 353.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

Optical purity: ≥ 99.50% D-enantiomer.

| | | 5 g | 65.00 |
| | | 25 g | 260.00 |

852224 **Fmoc-Leu-OPfp**

N-α-Fmoc-L-leucine pentafluorophenyl ester

NBC No.: 04-12-1510; CAS No.: 86060-88-0; $C_{27}H_{22}NO_4F_5$; M.W.: 519.5

Solubility: 0.5 mmole in 3 ml DMF clearly soluble.

HPLC: purity: ≥ 97.50%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

| | | 5 g | 82.00 |

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
852061	**Fmoc-(FmocHmb)Leu-OH**	1 g	295.00
	N-α-Fmoc-N-α-(2-Fmoc-oxy-4-methoxybenzyl)-L-leucine	5 g	990.00

NBC No.: 04-12-1129; CAS No.: 148515-87-1; $C_{44}H_{41}NO_8$; M.W.: 711.8

Solubility: 1 mmole in 2 ml DCM.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95.00%.

HPLC: purity: ≥ 96.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Hmb protection of amide bonds has been shown to inhibit aggregation of "difficult" peptides, thereby leading to products of increased purity [1-6]. Retention of Hmb groups on the cleaved peptide can greatly improve the Solubility of protected peptide fragments [7, 8] and otherwise intractable sequences [9-11]. Furthermore, using a Hmb-protected derivative for incorporation of the residue linked to the carboxy group of Asp or Asn residues has been found to suppress formation of aspartimide and piperidide related by-products [12-14]. For a comparison of the efficiency of Hmb and pseudoprolines in preventing aggregation, see [15].

ⓘ Ⓖ 3.9, 3.18

[1] T. Johnson, et al. (1993) *J. Chem. Soc., Chem. Commun.*, 369.
[2] C. Hyde, et al. (1994) *Int. J. Peptide Protein Res.*, **43**, 431.
[3] L. C. Packman, et al. (1994) *Pept. Res.*, **7**, 125.
[4] T. Johnson, et al. (1994) *Tetrahedron Lett.*, **35**, 463.
[5] R. G. Simmonds (1996) *Int. J. Peptide Protein Res.*, **47**, 36.
[6] T. Johnson, et al. (1995) *Lett. Pept. Sci.*, 369.
[7] M. Quibell, et al. (1995) *J. Am. Chem. Soc.*, **117**, 11656.
[8] M. Quibell, et al. (1996) *J. Chem. Soc., Perkin Trans. 1*, 1227.
[9] M. Quibell, et al. (1994) *Tetrahedron Lett.*, **35**, 2237.
[10] M. Quibell, et al. (1994) *J. Org. Chem.*, **59**, 1745.
[11] M. Quibell, et al. (1995) *J. Chem. Soc., Perkin Trans. 1*, 2019.
[12] M. Quibell, et al. (1994) *J. Chem. Soc., Chem. Commun.*, 2343.
[13] L. C. Packman (1995) *Tetrahedron Lett.*, **36**, 7523.
[14] J. Offer, et al. (1996) *J. Chem. Soc., Perkin Trans. 1*, 175.
[15] W. R. Sampson, et al. (1999) *J. Peptide Sci.*, **5**, 403.
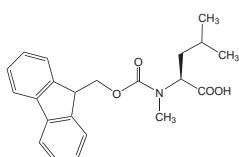
| 852139 | **Fmoc-N-Me-Leu-OH** | 1 g | 72.00 |
| | N-α-Fmoc-N-α-methyl-L-leucine | 5 g | 280.00 |

NBC No.: 04-12-9031; CAS No.: 103478-62-2; $C_{22}H_{25}NO_4$; M.W.: 367.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.
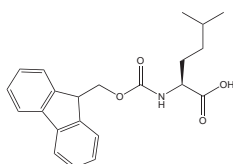
| 852327 | **Fmoc-hLeu-OH** | 1 g | 125.00 |
| | *NEW* Fmoc-homo-L-leucine | 5 g | 499.00 |

CAS No.: 180414-94-2; $C_{22}H_{25}NO_4$; M.W.: 367.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

Optical purity: ≥ 99.00% L-enantiomer.

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Product No.	Product		Quantity	Price

Lysine [Lys, K]

Fmoc–Lys(Boc)–OH is recommended for the routine preparation of lysine-containing peptides. For the preparation of cyclic peptides and peptides containing side-chain modified Lys residues, derivatives such as Fmoc–Lys(Alloc)–OH, Fmoc–Lys(Mtt)–OH, Fmoc–Lys(ivDde)–OH should be used since their respective side-chain protecting groups can be removed selectively on the solid-phase.

For further information, please refer to the product entries below.
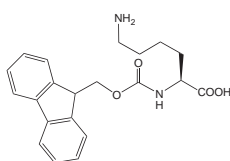
852023	**Fmoc–Lys–OH**	1 g	21.00
	N-α-Fmoc-L-lysine	5 g	63.00
	NBC No.: 04-12-1042; CAS No.: 105047-48-8; $C_{21}H_{24}N_2O_4$; M.W.: 368.4	25 g	182.00

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: $\geq$ 98.00%.
EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: $\geq$ 98.00%.
HPLC: purity: $\geq$ 97.00%.
Optical purity: $\geq$ 99.00% L-enantiomer.
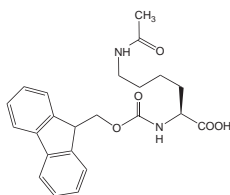
852042	**Fmoc–Lys(Ac)–OH**	1 g	45.00
	N-α-Fmoc-N-ε-acetyl-L-lysine	5 g	180.00
	NBC No.: 04-12-1086; CAS No.: 159766-56-0; $C_{23}H_{26}N_2O_5$; M.W.: 410.5	25 g	720.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: $\geq$ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: $\geq$ 98.00%.
HPLC: purity: $\geq$ 97.00%.
Optical purity: $\geq$ 99.50% L-enantiomer.

The acetylation of lysine is a post-translational modification of histone proteins. Fmoc–Lys(Ac)–OH is therefore a useful derivative to examine epigenetic regulation in histone deacetylase inhibitors [1 - 2] and in SAR studies [3].

[1] B. Heltweg, et. al., *J. Med. Chem.* (2004), 47, 5235.
[2] C.A. Olsen & M.R. Ghadiri (2009) *J. Med. Chem.*, **52**, 7836.
[3] R.M. Hughes & M.L. Waters (2006) *J. Am. Chem. Soc.*, **128**, 13586.
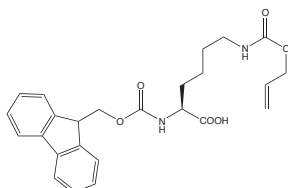
852124	**Fmoc–Lys(Alloc)–OH**	5 g	90.00
	NBC No.: 04-12-1305; CAS No.: 146982-27-6; $C_{25}H_{28}N_2O_6$; M.W.: 452.5	25 g	343.00
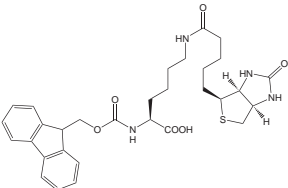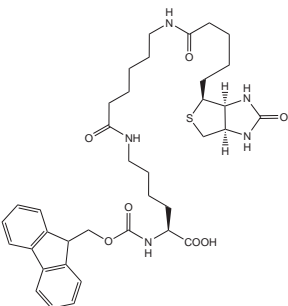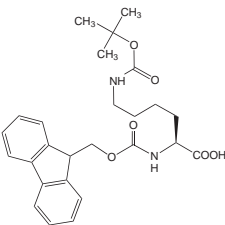
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: $\geq$ 98.00%.
HPLC: purity: $\geq$ 98.00%.
Orthogonally-protected building block for the synthesis of branched and cyclic peptides and peptides modified at the Lys side chain [1, 2]. The Alloc group can be selectively removed in the presence of standard Fmoc- and t-butyl-based protecting groups by treatment with Pd(Ph$_3$P)$_4$/$CHCl_3$/AcOH/NMM.

ⓘ Ⓖ 4.12, Ⓜ 4.14

[1] S. A. Kates, et. al. (1993) *Anal. Biochem.*, **212**, 303.
[2] J. Buchardt, et. al. (2000) *J. Comb. Chem.*, **2**, 624.

€

Product No.	Product	Quantity	Price
852097	**Fmoc-Lys(biotin)-OH**	500 mg	203.00
	N-α-Fmoc-N-ε-biotinyl-L-lysine	1 g	395.00

NBC No.: 04-12-1237; CAS No.: 146987-10-2; $C_{31}H_{38}N_4O_6S$; M.W.: 594.7

Solubility: 0.1 mmole in 1 ml NMP.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 95.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.50% L-enantiomer.

A modified lysine derivative for the preparation of biotin-labeled peptides by Fmoc SPPS.

ⓘ Ⓖ 5.18

852100	**Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH**	500 mg	172.00
	N-α-Fmoc-N-ε-(biotinylcaproyl)-L-lysine	1 g	307.00

NBC No.: 04-12-1243; CAS No.: 160158-05-4; $C_{37}H_{49}N_5O_7S$; M.W.: 707.9

Solubility: 100mg in 10 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 96.00%.

⚠ Prolonged storage: +15 to +25°C; keep cool and dry; keep open bottle under nitrogen.

A modified lysine derivative for the preparation of biotin-labeled peptides by Fmoc SPPS, in which the biotin is separated from the lysine side-chain by a 6 C-atom spacer.

ⓘ Ⓖ 5.18

852012	**Fmoc-Lys(Boc)-OH**	25 g	60.00
	N-α-Fmoc-N-ε-t.-Boc-L-lysine	100 g	180.00
	NBC No.: 04-12-1026; CAS No.: 71989-26-9; $C_{26}H_{32}N_2O_6$; M.W.: 468.5	250 g	395.00

Solubility: 25 mmole in 50 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98%.

HPLC: purity: ≥ 99.0%.

Fmoc-β-Ala-OH ≤ 0.1%.

Fmoc-β-Ala-Lys(Boc)-OH ≤ 0.1%.

Fmoc-Lys(Boc)-Lys(Boc)-OH ≤ 0.1%.

Fmoc-Lys-OH ≤ 0.1%.

Free amino acid: ≤ 0.2%.

EtOAc: ≤ 0.5%.

AcOH: ≤ 0.02%.

Optical purity: ≥ 99.8% L-enantiomer.

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Product No.	Product	Quantity	Price
852146	**Fmoc–D–Lys(Boc)–OH**	1 g	35.00
	N-α-Fmoc-N-ε-t.-Boc-D-lysine	5 g	140.00

NBC No.: 04-13-1026; CAS No.: 92122-45-7; $C_{26}H_{32}N_2O_6$; M.W.: 468.5
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 98.00%.
Optical purity: ≥ 99.50% D-enantiomer.

| 852129 | **Fmoc–Lys(Boc)–OPfp** | 5 g | 161.00 |

N-α-Fmoc-N-ε-t.-Boc-L-lysine pentafluorophenyl ester
NBC No.: 04-12-1511; CAS No.: 86060-98-2; $C_{32}H_{31}F_5N_2O_6$; M.W.: 634.6
Solubility: 0.5 mmole in 3 ml DMF clearly soluble.
TLC: CH_3CN:$CHCl_3$ (1 : 3), purity: ≥ %.
HPLC: purity: ≥ 98.00%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

| 852096 | **Fmoc–Lys(Dabcyl)–OH** | 500 mg | 338.00 |
| | | 1 g | 614.00 |

NBC No.: 04-12-1236; CAS No.: 146998-27-8; $C_{36}H_{37}N_5O_5$; M.W.: 619.7
TLC: $CHCl_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 97.00%.
HPLC: purity: ≥ 96.00%.
Optical purity: ≥ 99.00% L-enantiomer.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light.
A modified lysine derivative for the preparation of chromogenically-labeled peptides by Fmoc chemistry.
The Dabcyl group quenches the fluorescence of EDANS, Mca, TET, JOE, FAM fluorophores, making it an extremely useful tool for the synthesis of fluorescence-quenched peptide substrates [1 , 2].
ⓘ Ⓖ 5.11
[1] M. Taliani, et. al. (1996) *Anal. Biochem.*, **240**, 60.
[2] O. Seitz & O. Kohler (2001) *Chem. Eur. J.*, **7**, 3911.

| 852099 | **Fmoc–Lys(Dnp)–OH** | 500 mg | 270.00 |
| | | 1 g | 458.00 |

N-α-Fmoc-N-ε-2,4-dinitrophenyl-L-lysine
NBC No.: 04-12-1239; CAS No.: 148083-64-1; $C_{27}H_{26}N_4O_8$; M.W.: 534.5
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 97.00%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
A modified lysine derivative for the preparation of chromogenically-labeled peptides by Fmoc chemistry.
The Dnp group is the preferred quencher for use in conjunction with the Mca fluorophore, making it an extremely useful tool for the synthesis of fluorescence-quenched peptide substrates.
ⓘ Ⓖ 5.12

N–α–FMOC PROTECTED AMINO ACIDS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
852057	**Fmoc-Lys(Dde)-OH**	1 g	140.00
	N-α-Fmoc-N-ε-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-L-lysine	5 g	562.00

NBC No.: 04-12-1121; CAS No.: 150629-67-7; $C_{31}H_{36}N_2O_6$; M.W.: 532.6

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH:H$_2$O (85:13:0.5:1.5), purity: ≥ 98.00%.

CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

Quasi-orthogonally-protected Lys derivative. The Fmoc group can be removed selectively by treatment with piperidine; the Dde group is cleaved with 2% hydrazine in DMF [1]. When removing Dde in the presence of allyl based protecting groups, allyl alcohol should be included in the deprotection solution to prevent reduction of the allyl group [2].

Lys(Dde) has been employed in the following applications: synthesis of branched peptides [1] and di-epitopic peptides [3]; preparation of MAP core molecules and lipo-MAPs [4, 5]; construction of cyclic peptides [6], TASP molecules [7], templates for combinatorial chemistry [8] and synthetic proteins [9]; preparation of peptides modified at the lysine side-chain [10-14].

It has been reported that Dde can migrate from the side-chain of Lys to the unprotected side-chain of another Lys residue [15], and from the β-amino group to the α-amino group of Dpr [16]. In the former instance, this problem can be overcome by using Fmoc-Lys(ivDde)-OH (852082) or using DBU/DMF (2:98) for Fmoc group removal [15, 17].

Full orthogonality of Dde with Fmoc has recently been demonstrated when hydroxylamine is used for Dde removal [18].

For recent applications of Lys(Dde) see [19 - 21].

ⓘ Ⓖ 4.9, Ⓜ 4.10

[1] B. W. Bycroft, et al. (1993) *J. Chem. Soc., Chem. Commun.*, 778.
[2] B. Rohwedder, et al. (1998) *Tetrahedron Lett.*, **39**, 1175.
[3] N. Ahlborg (1995) *J. Immun. Meth.*, **179**, 269.
[4] B. W. Bycroft, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 13th American peptide Symposium", R. S. Hodges & J. A. Smith (Eds), ESCOM, Leiden, 1994, pp. 727.
[5] J. Mack, et al. (2001) *J. Peptide Sci.*, **7**, 338.
[6] G. B. Bloomberg, et al. (1993) *Tetrahedron Lett.*, **34**, 4709.
[7] P. Dumy, et al. (1995) *Tetrahedron Lett.*, **36**, 1255.
[8] J. Eichler, et al. (1994) *Pept. Res.*, **7**, 300.
[9] C. G. Fields, et al. (1993) *Biopolymers*, **33**, 1695.
[10] H. F. Brugghe, et al. (1994) *Int. J. Peptide Protein Res.*, **43**, 166.
[11] P. Hoogerhout, et al. (1995) *Infection & Immunity*, **63**, 3473.
[12] D. Lelievre, et al. (1995) *Tetrahedron Lett.*, **36**, 9317.
[13] P. Hoogerhout, et al. (1999) *J. Peptide Res.*, **54**, 436.
[14] P.J. Conolly, et al. (2000) *Tetrahedron Lett.*, **41**, 5187.
[15] K. Augustyns, et al. (1998) *J. Peptide Res.*, **51**, 127.
[16] A. Srinivasan, et al., Poster 111 presented at the 15th American Peptide Symposium, Nashville, 1997.
[17] S. Peluso, et al. (1999) *J. Org. Chem.*, **64**, 7114.
[18] J. J. Diaz-Mochón, et al. (2004) *Org. Lett.*, **6**, 1127.
[19] N. Nasongkla, et. al. (2004) *Angew. Chem. Int. Ed.*, **43**, 6323.
[20] A.A. Aimetti, et. al. (2010) *Chem. Commun.*, 4061.
[21] D. Maxwell, et. al. (2009) *Bioconjugate Chem.*, **20**, 702.

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Product No.	Product	Quantity	Price

852147 **Fmoc–D–Lys(Dde)–OH** — 1 g — 192.00 / 5 g — 770.00

N-α-Fmoc-N-ε-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-D-lysine

NBC No.: 04-13-1028; CAS No.: 333973-51-6; $C_{31}H_{36}N_2O_6$; M.W.: 532.6

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH:H$_2$O (85:13:0.5:1.5), purity: ≥ 98.00%.

CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% D-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

For applications information, please refer to entry for 04-12-1121.

854000 **Dde–Lys(Fmoc)–OH** — 1 g — 177.00 / 5 g — 707.00

N-α-1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-N-ε-Fmoc-L-lysine

NBC No.: 04-12-5201; CAS No.: 156648-40-7; $C_{31}H_{36}N_2O_6$; M.W.: 532.6

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH:H$_2$O (85:13:0.5:1.5), purity: ≥ 97.00%.

CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 96.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

Quasi-orthogonally-protected Lys derivative. The Fmoc group can be removed selectively by treatment with piperidine; the Dde group is cleaved with 2% hydrazine in DMF [1]. When removing Dde in the presence of allyl-based protecting groups, allyl alcohol should be included in the deprotection solution to prevent reduction of the allyl group [2].

Also available Fmoc-Lys(Dde)-OH 852057.

This derivative has been employed in Fmoc SPPS to facilitate the introduction of biotin to the side-chain of lysine [3].

ⓘ Ⓖ 4.9, Ⓜ 4.10

[1] B. W. Bycroft, et al. (1993) *J. Chem. Soc., Chem. Commun.*, 778.

[2] B. Rohwedder, et al. (1998) *Tetrahedron Lett.*, **39**, 1175.

[3] J. Mack, et al. (1999) *Lett. Pept. Sci.*, **6**, 135.

852041 **Fmoc–Lys(Fmoc)–OH** — 5 g — 62.00 / 25 g — 244.00 / 100 g — 619.00

N-α,ε-di-Fmoc-L-lysine

NBC No.: 04-12-1085; CAS No.: 78081-87-5; $C_{36}H_{34}N_2O_6$; M.W.: 590.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 97.00%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% L-enantiomer.

Useful derivative for the synthesis of branched peptides [1].

[1] D. N. Posnett, et al. (1988) *J. Biol. Chem.*, **263**, 1719.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
852082	**Fmoc-Lys(ivDde)–OH**	1 g	140.00
	N-α-Fmoc-N-ε-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl-L-lysine	5 g	560.00

NBC No.: 04-12-1193; CAS No.: 204777-78-6; $C_{34}H_{42}N_2O_6$; M.W.: 574.6

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 97.00%.

$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 99.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

This orthogonally-protected lysine derivative is based on the hindered Dde variant ivDde. It has very similar chemical properties to Fmoc-Lys(Dde)–OH, except that the side-chain ivDde group is considerably more stable to piperidine than Dde, and is less prone to migrate from protected to unprotected lysine side-chains [1]. When removing ivDde in the presence of allyl based protecting groups, allyl alcohol should be included in the deprotection solution to prevent reduction of the allyl group [2]. Recent applications of this derivative include the synthesis of multifunctional probes [3] and ubiquinated peptides [4].

ⓘ Ⓖ 4.9, Ⓜ 4.10

[1] S. R. Chhabra, et al. (1998) *Tetrahedron Lett.,* **39**, 1603.

[2] B. Rohwedder, et al. (1998) *Tetrahedron Lett.,* **39**, 1175.

[3] E. Garanger, et. al. (2008) *Chem. Commun.,* 4792.

[4] K. S. A. Kumar, et. al. (2011) *Bioconjugate Chem.,* **22**, 137.
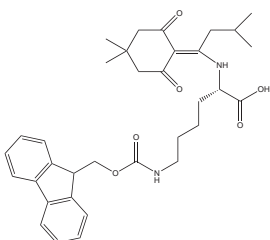
| 852370 | **ivDde–Lys(Fmoc)–OH** | 1 g | 170.00 |
| NEW | N-α-1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)-3methylbutyl-N-ε-Fmoc-L-lysine | 5 g | 680.00 |

$C_{34}H_{42}N_2O_6$; M.W.: 574.6

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 97.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

The novel derivatives enables side-chain modification during chain extension, thereby avoiding the difficulties of slow selective side-chain deprotection on the full length peptide.

ⓘ Ⓖ 4.9, Ⓜ 4.10
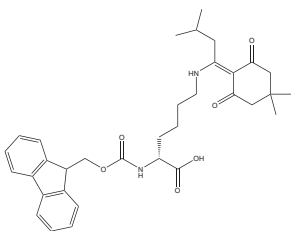
| 852369 | **Fmoc-D-Lys(ivDde)–OH** | 1 g | 145.00 |
| NEW | N-α-Fmoc-N-ε-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3methylbutyl-D-lysine | 5 g | 580.00 |

CAS No.: 1272755-33-5; $C_{34}H_{42}N_2O_6$; M.W.: 576.6

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

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Product No.	Product	Quantity	Price

852095 **Fmoc–Lys(Mca)–OH**

500 mg 270.00
1 g 411.00
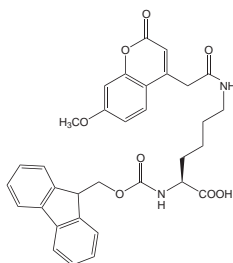
N-α-Fmoc-N-ε-7-methoxycoumarin-4-acetyl-L-lysine

NBC No.: 04-12-1233; CAS No.: 386213-32-7; $C_{33}H_{32}N_2O_8$; M.W.: 584.6

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 97.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; protect from light.

A modified lysine derivative for the preparation of fluorogenically-labeled peptides by Fmoc chemistry [1].

The Mca group fluoresces at 405 nm when stimulated at 340 nm, and is most commonly used in conjunction with Dabcyl and 2,4-dinitrophenyl quenching groups.

Recently, Fmoc-Lys(Mca)-OH has been used to prepare lanthanide-based luminescent proteins by native chemical ligation [2]. Lys(Mca) was found to be stable to HF cleavage conditions used to synthesize the thioester fragments needed for ligation.

ⓘ Ⓖ 5.11

[1] J. L. Lauer-Fields, et al. (2001) *Biochemistry*, **40**, 5795.

[1] C. F. W. Becker, et al. (2004) *Bioconjugate Chem.*, **15**, 1118.

852106 **Fmoc–Lys(Me,Boc)–OH**

500 mg 300.00
1 g 600.00
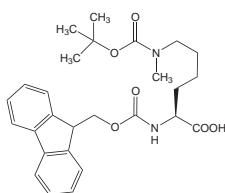
N-α-Fmoc-N-ε-methyl-N-ε-t.-Boc-L-lysine

NBC No.: 04-12-1263; CAS No.: 951695-85-5; $C_{27}H_{34}N_2O_6$; M.W.: 482.6

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 97.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen; hygroscopic.

A novel derivative for the Fmoc SPPS of peptides containing monomethyl-lysine, which are useful tools for the studying of the role of histone lysine methylation in gene expression [1, 2].

Coupling can be carried out using any standard activation method. Removal of the Boc protecting group occurs during the course of the TFA-mediated cleavage reaction. Ref [3] contains methods and protocols for the synthesis of arrays of histone-related peptides containing methylated arginine and lysine-residues.

[1] G. Egger, et. al. (2004) Nature, 429, 457.

[2] A. Shilatifard (2006) Ann. Rev. Biochem., 75, 243.

[3] S. Rothbart, et al. (2012) *Methods Enzymol.*, **512**, 107.

852376 **Fmoc–Lys(Boc,iPr)–OH**

1 g 350.00
5 g 1400.00

NEW
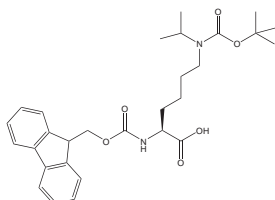
N-α-Fmoc-N-ε-isopropyl-N-ε-t.-Boc-L-lysine

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: +2 to +8°C

Fmoc-Lys(iPr,Boc)-OH is used for incorporation of N-d-isopropyl-lysine (Lys(iPr)) during Fmoc SPPS. Lys(iPr) has been utilized in the GnRH antagonist Degarelix and the LHRH antagonist Antide.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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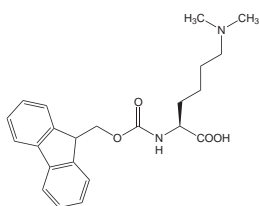
Product No.	Product	Quantity	Price
852111	**Fmoc–Lys(Me)₂–OH · HCl**	500 mg	165.00
	N-α-Fmoc-N-ε-dimethyl-N-L-lysine · hydrochloride	1 g	295.00
	NBC No.: 04-12-1269; CAS No.: 252045-10-8; $C_{23}H_{26}N_2O_4$ · HCl; M.W.: 396.5 · 36.5	5 g	1190.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl₃:MeOH:AcOH 32% (5:3:1), purity: ≥ 95.00%.

HPLC: purity: ≥ 95.00%.

AcOH: ≤ 1.0%

Optical purity: ≥ 99.50% L-enantiomer.

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry; keep open bottle under nitrogen; hygroscopic.

A novel derivative for the Fmoc SPPS of peptides containing dimethyl-lysine, which are useful tools for the studying of the role of histone lysine methylation in gene expression [1, 2].

Coupling can be carried out using any standard activation method. Removal of the Boc protecting group occurs during the course of the TFA-mediated cleavage reaction. Ref [3] contains methods and protocols for the synthesis of arrays of histone-related peptides containing methylated arginine and lysine-residues.

[1] G. Egger, et. al. (2004) Nature, 429, 457.
[2] A. Shilatifard (2006) Ann. Rev. Biochem., 75, 243.
[3] S. Rothbart, et al. (2012) *Methods Enzymol.*, **512**, 107.
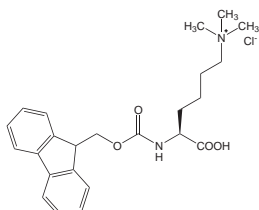
Product No.	Product	Quantity	Price
852112	**Fmoc–Lys(Me₃Cl)–OH**	500 mg	425.00
	N-α-Fmoc-N-ε-trimethyl-N-L-lysine · chloride	1 g	685.00
	NBC No.: 04-12-1270; CAS No.: 201004-29-7; $C_{24}H_{31}N_2O_4$ · Cl; M.W.: 411.5 · 35.5	5 g	2200.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl₃:MeOH:AcOH 32% (5:3:1), purity: ≥ 95.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.

A novel derivative for the Fmoc SPPS of peptides containing trimethyl-lysine, which are useful tools for the studying of the role of histone lysine methylation in gene expression [1, 2].

Coupling can be carried out using any standard activation method. Removal of the Boc protecting group occurs during the course of the TFA-mediated cleavage reaction. Ref [3] contains methods and protocols for the synthesis of arrays of histone-related peptides containing methylated arginine and lysine-residues.

[1] G. Egger, et. al. (2004) Nature, 429, 457.
[2] A. Shilatifard (2006) Ann. Rev. Biochem., 75, 243.
[3] S. Rothbart, et al. (2012) *Methods Enzymol.*, **512**, 107.

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Product No.	Product	Quantity	Price
852094	**Fmoc-Lys(Mmt)-OH**	1 g	70.00
	N-α-Fmoc-N-ε-4-methoxytrityl-L-lysine	5 g	280.00

NBC No.: 04-12-1232; CAS No.: 159857-60-0; $C_{41}H_{40}N_2O_5$; M.W.: 640.8

TLC: $CHCl_3$:MeOH (9:1), purity: ≥ 95.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ **Prolonged storage:** +2 to +8°C; keep dry.

An excellent derivative for the synthesis of branched peptides and peptides modified at the lysine side-chain [1], and for the construction of templates and multifunctionalized resins for combinatorial synthesis.

The side-chain Mmt group can be selectively removed in the same manner as Mtt with 1% TFA in DCM [2, 3, 4] or DCM/HFIP/TFE/TES (6.5:2:1:0.5) [5]. Alternatively, it can be removed under milder conditions with AcOH/TFE/DCM (1:2:7) [1], leaving Mtt intact.

ⓘ Ⓖ 4.9, Ⓜ 4.12

[1] S. Matysiak, et al. (1998) *Tetrahedron Lett.*, **39**, 1733.
[2] K. Barlos, et al., C. H. Schneider & A. N. Eberle (Eds), ESCOM, Leiden, 1993, pp. 283.
[3] A. Aletras, et al. (1995) *Int. J. Peptide Protein Res.*, **45**, 488.
[4] L. Bourel, et al. (2000) *J. Peptide Sci.*, **6**, 264.
[5] K. Barlos, personal communication.
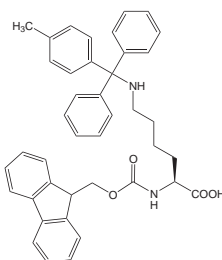
852065	**Fmoc-Lys(Mtt)-OH**	1 g	42.00
	N-α-Fmoc-N-ε-4-methyltrityl-L-lysine	5 g	155.00
		25 g	541.00

NBC No.: 04-12-1137; CAS No.: 167393-62-6; $C_{41}H_{40}N_2O_4$; M.W.: 624.8

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry.

The side-chain Mtt group can be selectively removed with 1% TFA in DCM [1, 2, 3] or DCM/HFIP/TFE/TES (6.5:2:1:0.5) [4], making this an excellent derivative for the synthesis of branched peptides, peptides modified at the lysine side-chain [5,6], for the construction of templates and multifunctionalized resins for combinatorial synthesis.

ⓘ Ⓖ 4.10, Ⓜ 4.12, Ⓐ 4.12

[1] K. Barlos, et al., C. H. Schneider & A. N. Eberle (Eds), ESCOM, Leiden, 1993, pp. 283.
[2] A. Aletras, et al. (1995) *Int. J. Peptide Protein Res.*, **45**, 488.
[3] L. Bourel, et al. (2000) *J. Peptide Sci.*, **6**, 264.
[4] K. Barlos, personal communication.
[5] P. Hoogerhout, et al. (1999) *J. Peptide Res.*, **54**, 436.
[6] C. Park & K. Burgess (2001) *J. Comb. Chem.*, **3**, 257.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
852326	**Fmoc-ε-azidonorleucine**	250 mg	130.00
	CAS No.: 159610-89-6; $C_{21}H_{22}N_4O_4$; M.W.: 394.42	1 g	450.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

A useful tool for the synthesis of branched, side-chain modified and cyclic peptides by Fmoc SPPS. The side-chain azido group is completely stable to piperidine and TFA, but can be readily converted to an amine on the solid phase or in solution by reduction with thiols [1] or phosphines [2, 3].

ⓘ Ⓖ 4.12, Ⓜ 4.12

[1] M. Meldal, et al. (1997) *Tetrahedron Lett.*, **38**, 2531.
[2] J. T. Lundquist & J. C. Pelletier (2001) *Org. Lett.*, **3**, 781.
[3] N. Nepomniaschiy, et al. (2008) *Org. Lett.*, **10**, 5243.

852013	**Fmoc-Lys(Z)-OH**	5 g	62.00
	N-α-Fmoc-N-ε-benzyloxycarbonyl-L-lysine	25 g	244.00

NBC No.: 04-12-1027; CAS No.: 86060-82-4; $C_{29}H_{30}N_2O_6$; M.W.: 502.6

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.
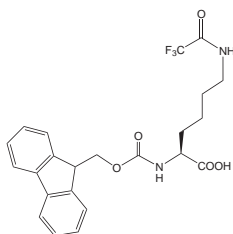
852040	**Fmoc-Lys(Tfa)-OH**	5 g	98.00
	N-α-Fmoc-N-ε-trifluoroacetyl-L-lysine	25 g	390.00

NBC No.: 04-12-1083; CAS No.: 76265-69-5; $C_{23}H_{23}N_2O_5F_3$; M.W.: 464.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 97.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.50% L-enantiomer.

The trifluoroacetyl group can be selectively removed by aqueous basic hydrolysis [1,2].

[1] E. Atherton, et al. (1980) *J. Chem. Soc., Chem. Commun.*, 970.
[2] D. A. Stetsenko & M. J. Gait (2001) *Bioconjugate Chem.*, **12**, 576.

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Product No.	Product	Quantity	Price

852068   **Fmoc-(FmocHmb)Lys(Boc)-OH**   1 g   295.00

N-α-Fmoc-N-α-(2-Fmoc-oxy-4-methoxybenzyl)-N-ε-t.-butoxycarbonyl-L-lysine   5 g   990.00

NBC No.: 04-12-1148; CAS No.: 166881-56-7; $C_{49}H_{50}N_2O_{10}$; M.W.: 826.9

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 98.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry.

Hmb protection of amide bonds has been shown to inhibit aggregation of "difficult" peptides, thereby leading to products of increased purity [1-6]. Retention of Hmb groups on the cleaved peptide can greatly improve the Solubility of protected peptide fragments [7, 8] and otherwise intractable sequences [9-11]. Furthermore, using a Hmb-protected derivative for incorporation of the residue linked to the carboxy group of Asp or Asn residues has been found to suppress formation of aspartimide and piperidide related by-products [12-14]. For a comparison of the efficiency of Hmb and pseudoprolines in preventing aggregation, see [15].

ⓘ Ⓖ 3.9, 3.18

[1] T. Johnson, et al. (1993) *J. Chem. Soc., Chem. Commun.*, 369.
[2] C. Hyde, et al. (1994) *Int. J. Peptide Protein Res.*, **43**, 431.
[3] L. C. Packman, et al. (1994) *Pept. Res.*, **7**, 125.
[4] T. Johnson, et al. (1994) *Tetrahedron Lett.*, **35**, 463.
[5] R. G. Simmonds (1996) *Int. J. Peptide Protein Res.*, **47**, 36.
[6] T. Johnson, et al. (1995) *Lett. Pept. Sci.*, **1**, 11.
[7] M. Quibell, et al. (1995) *J. Am. Chem. Soc.*, **117**, 11656.
[8] M. Quibell, et al. (1996) *J. Chem. Soc., Perkin Trans. 1*, 1227.
[9] M. Quibell, et al. (1994) *Tetrahedron Lett.*, **35**, 2237.
[10] M. Quibell, et al. (1994) *J. Org. Chem.*, **59**, 1745.
[11] M. Quibell, et al. (1995) *J. Chem. Soc., Perkin Trans. 1*, 2019.
[12] M. Quibell, et al. (1994) *J. Chem. Soc., Chem. Commun.*, 2343.
[13] L. C. Packman (1995) *Tetrahedron Lett.*, **36**, 7523.
[14] J. Offer, et al. (1996) *J. Chem. Soc., Perkin Trans. 1*, 175.
[15] W. R. Sampson, et al. (1999) *J. Peptide Sci.*, **5**, 403.

852305   **Fmoc-Aea-OH**   100 mg   135.00

Fmoc-allysine ethylene acetal   500 mg   541.00

CAS No.: 1234692-73-9; $C_{23}H_{25}NO_6$; M.W.: 411.45

Solubility: 1 mmole in 2 ml DMF clearly soluble.

HPLC: purity: ≥ 98.00%.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

Useful derivative for introducing a side chain aldehyde functionality.

852361   **Fmoc-N-Me-Lys(Boc)-OH**   250 mg   166.00

NEW

N-α-Fmoc-N-α-methyl-N-ε-t.-Boc-L-lysine   1 g   499.00

CAS No.: 197632-76-1; $C_{27}H_{34}N_2O_6$; M.W.: 482.57

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 96.00%.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
852375	**Fmoc-hLys(Boc)-OH**	250 mg	180.00
NEW	N-α-Fmoc-N-ω-t.-Boc-L-homolysine	1 g	540.00
	C27H34N2O6; M.W.: 482.6		

⚠ Prolonged storage: +2 to +8°C

This homolog of lysine has been used to modulate ion-pairing interactions between Lys and enzyme carboxylate groups in SAR studies [1].

[1]   K. J. Kennedy, et al. (1997) *Bioorg. Med. Chem. Lett.*, 7, 1937.

Methionine [Met, M]
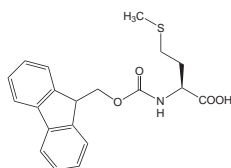
852002	**Fmoc-Met-OH**	25 g	25.00
	N-α-Fmoc-L-methionine	100 g	50.00
	NBC No.: 04-12-1003; CAS No.: 71989-28-1; $C_{20}H_{21}NO_4S$; M.W.: 371.5	250 g	110.00

Solubility: 25 mmole in 50 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98%.
HPLC: purity: ≥ 99.0%.
Fmoc-β-Ala-OH ≤ 0.1%.
Fmoc-β-Ala-Met-OH ≤ 0.1%.
Fmoc-Met-Met-OH ≤ 0.1%.
Free amino acid: ≤ 0.2%.
EtOAc: ≤ 0.5%.
AcOH: ≤ 0.02%.
Optical purity: ≥ 99.8% L-enantiomer.
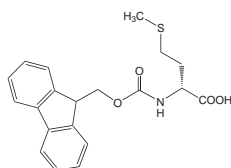
852140	**Fmoc-D-Met-OH**	5 g	65.00
	N-α-Fmoc-D-methionine	25 g	260.00
	NBC No.: 04-13-1003; CAS No.: 112883-40-6; $C_{20}H_{21}NO_4S$; M.W.: 371.5		

Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 99.00%.
Optical purity: ≥ 99.00% D-enantiomer.
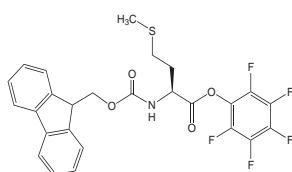
852225	**Fmoc-Met-OPfp**	5 g	82.00
	N-α-Fmoc-L-methionine pentafluorophenyl ester		
	NBC No.: 04-12-1512; CAS No.: 86060-94-8; $C_{26}H_{20}NO_4SF_5$; M.W.: 537.5		

Solubility: 0.5 mmole in 3 ml DMF clearly soluble.
TLC: CH_3CN:$CHCl_3$ (1 : 3), purity: ≥ 2.00%.
HPLC: purity: ≥ 97.00%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

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Product No.	Product	Quantity	Price
852054	**Fmoc-Met(O)-OH**	1 g	25.00
	N-α-Fmoc-L-methionine-DL-sulfoxide	5 g	99.00

NBC No.: 04-12-1112; CAS No.: 76265-70-8; $C_{20}H_{21}NO_5S$; M.W.: 387.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% L-enantiomer.

Product No.	Product	Quantity	Price
852212	**Fmoc-Met(O₂)-OH**	1 g	52.00
	N-α-Fmoc-L-methionine-sulfone	5 g	206.00

NBC No.: 04-12-1113; CAS No.: 163437-14-7; $C_{20}H_{21}NO_6S$; M.W.: 403.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

Optical purity: ≥ 99.00% L-enantiomer.

Product No.	Product	Quantity	Price
852362	**Fmoc-N-Me-Met-OH**	1 g	72.00
	NEW	5 g	280.00

N-α-Fmoc-N-α-methyl-L-methionine

$C_{21}H_{23}NO_4S$; M.W.: 385.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Product No.	Product	Quantity	Price
852372	**Fmoc-selenomethionine-OH**	1 g	300.00
	NEW		

N-Fmoc-L-amino-4-(methylselanyl)butanoic acid

CAS No.: 1217852-49-7; $C_{20}H_{21}NO_4Se$; M.W.: 418.4

⚠ Prolonged storage: +2 to +8°C; keep cool and dry and under argon.

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 96.00%.

Optical purity: ≥ 99.50% L-enantiomer.

Fmoc-selenomethionine can be introduced under standard conditions. Any selenoxide formed during synthesis can be easily reduced back to selenide by treatment with β-mercaptoethanol. Introduction of selenium can help facilitate solid phase and solution structural determination and the study of peptide-protein interactions [1].

[1] L. Moroder (2005) *J. Pept.Sci.*, 11, 187.

⚠ Storage conditions　　Ⓖ Additional information　　Ⓜ Method information　　Ⓐ Application information

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Product No.	Product	Quantity	Price

Norleucine [Nle]

852014   **Fmoc-Nle-OH**

N-α-Fmoc-L-norleucine

NBC No.: 04-12-1028; CAS No.: 77284-32-3; $C_{21}H_{23}NO_4$; M.W.: 353.4

Solubility: 25 mmole in 50 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

HPLC: Fmoc-β-Ala-Nle-OH , ≤ 0.10%

Optical purity: ≥ 99.50% L-enantiomer.

| | | 5 g | 62.00 |
| | | 25 g | 244.00 |

852163   **Fmoc-D-Nle-OH**

N-α-Fmoc-D-norleucine

NBC No.: 04-13-1059; CAS No.: 112883-41-7; $C_{21}H_{23}NO_4$; M.W.: 353.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% D-enantiomer.

| | | 5 g | 125.00 |
| | | 25 g | 489.00 |

Norvaline [Nva]

852047   **Fmoc-Nva-OH**

N-α-Fmoc-L-norvaline

NBC No.: 04-12-1097; CAS No.: 135112-28-6; $C_{20}H_{21}NO_4$; M.W.: 339.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% L-enantiomer.

| | | 5 g | 62.00 |
| | | 25 g | 245.00 |

852239   **Fmoc-D-Nva-OH**

N-α-Fmoc-D-norvaline

NBC No.: 04-13-1060; CAS No.: 144701-24-6; $C_{20}H_{21}NO_4$; M.W.: 339.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% D-enantiomer.

| | | 5 g | 83.00 |
| | | 25 g | 333.00 |

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Product No.	Product	Quantity	Price

Ornithine [Orn]

Fmoc-Orn(Boc)-OH is recommended for the routine preparation of ornithine-containing peptides. For the preparation of cyclic peptides and peptides containing side-chain modified Orn residues, Fmoc-Orn(Mtt)-OH or Fmoc-Orn(ivDde)-OH should be used since Mtt and ivDde groups can be removed selectively on the solid-phase.

For further information, please refer to the product entries.
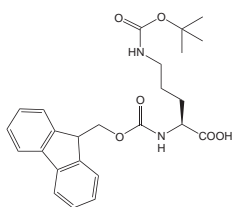
852015	**Fmoc-Orn(Boc)-OH**	5 g	72.00
	N-α-Fmoc-N-δ-t.-Boc-L-ornithine	25 g	286.00

NBC No.: 04-12-1029; CAS No.: 109425-55-0; $C_{25}H_{30}N_2O_6$; M.W.: 454.5
Solubility: 25 mmole in 50 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 98.00%.
Optical purity: ≥ 99.50% L-enantiomer.
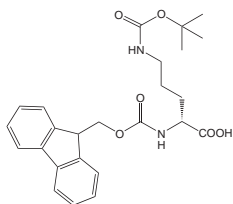
852141	**Fmoc-D-Orn(Boc)-OH**	1 g	63.00
	N-α-Fmoc-N-δ-t.-Boc-D-ornithine	5 g	250.00
		25 g	998.00

NBC No.: 04-13-1005; CAS No.: 118476-89-4; $C_{25}H_{30}N_2O_6$; M.W.: 454.5
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 98.00%.
Optical purity: ≥ 99.50% D-enantiomer.
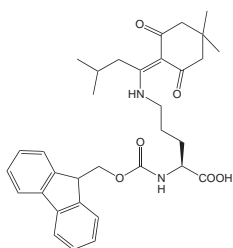
852088	**Fmoc-Orn(ivDde)-OH**	1 g	187.00
	N-α-Fmoc-N-δ-1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl-L-ornithine	5 g	749.00
		25 g	2964.00

NBC No.: 04-12-1203; CAS No.: 1198321-33-3; $C_{33}H_{40}N_2O_6$; M.W.: 560.7
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 98.00%.
Optical purity: ≥ 99.00% L-enantiomer.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

This orthogonally-protected ornithine derivative is based on the hindered Dde variant ivDde. The side-chain ivDde group is considerably more stable to piperidine than Dde and is less prone to migrate from protected to unprotected side-chains [1]. When removing ivDde in the presence of allyl-based protecting groups, allyl alcohol should be included in the deprotection solution to prevent reduction of the allyl group [2].
See references [3, 4] for applications.

ⓘ Ⓖ 4.9, Ⓜ 4.10

[1] S. R. Chhabra, et al. (1998) *Tetrahedron Lett.*, **39**, 1603.
[2] B. Rohwedder, et al. (1998) *Tetrahedron Lett.*, **39**, 1175.
[3] V. Wittmann & S. Seeberger (2000) *Angew. Chem. Int. Ed. Engl.*, **39**, 4348.
[4] S. M. Dankwardt, et al. (2001) *Bioorg. Med. Chem. Lett.*, **11**, 2085.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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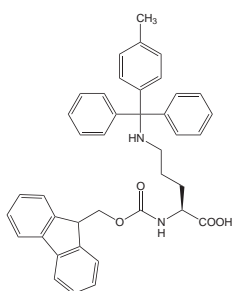
Product No.	Product	Quantity	Price
852075	**Fmoc-Orn(Mtt)-OH**	1 g	63.00
	N-α-Fmoc-N-δ-4-methyltrityl-L-ornithine	5 g	250.00
	NBC No.: 04-12-1168; CAS No.: 343770-23-0; $C_{40}H_{38}N_2O_4$; M.W.: 610.8	25 g	998.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

The side-chain Mtt group can be selectively removed with 1% TFA in DCM [1-3] or DCM/HFIP/TFE/TES (6.5:2:1:0.5) [4], making this an excellent derivative for the synthesis of branched peptides, peptides modified at the ornithine side-chain [4,5], for the construction of templates and multifunctionalized resins for combinatorial synthesis [6].

ⓘ Ⓖ 4.10, Ⓜ 4.12

[1] K. Barlos, et al., C. H. Schneider & A. N. Eberle (Eds), ESCOM, Leiden, 1993, pp. 283.
[2] A. Aletras, et al. (1995) *Int. J. Peptide Protein Res.*, **45**, 488.
[3] L. Bourel, et al. (2000) *J. Peptide Sci.*, **6**, 264.
[4] K. Barlos, personal communication.
[5] P. Hoogerhout, et al. (1999) *J. Peptide Res.*, **54**, 436.
[6] C. Park & K. Burgess (2001) *J. Comb. Chem.*, **3**, 257.
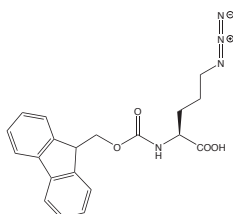
852322	**Fmoc-δ-azidonorvaline**	100 mg	187.00
	CAS No.: 1097192-04-5; $C_{20}H20_{16}N_4O_4$; M.W.: 380.40	500 mg	749.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

HPLC: purity: ≥ 95.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

A useful tool for the synthesis of branched, side-chain modified and cyclic peptides by Fmoc SPPS. The side-chain azido group is completely stable to piperidine and TFA, but can be readily converted to an amine on the solid phase or in solution by reduction with thiols [1] or phosphines [2, 3].

ⓘ Ⓖ 4.12, Ⓜ 4.12

[1] M. Meldal, et al. (1997) *Tetrahedron Lett.*, **38**, 2531.
[2] J. T. Lundquist & J. C. Pelletier (2001) *Org. Lett.*, **3**, 781.
[3] N. Nepomniaschiy, et al. (2008) *Org. Lett.*, **10**, 5243.

Penicillamine [Pen]
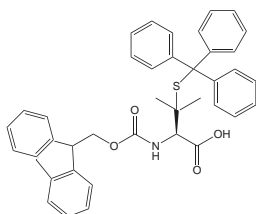
852339	**Fmoc-Pen(Trt)-OH**	1 g	99.00
	Fmoc-α, α-dimethyl-Cys(Trt)-OH	5 g	395.00

Fmoc-S-trityl-L-penicillamine

CAS No.: 201531-88-6; $C_{39}H_{35}NO_4S$; M.W.: 613.8

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

This derivative has been used for the preparation of constrained cyclic peptides [1] and for the native chemical ligation-desulfurization at valine [2] employing standard Fmoc SPPS.

[1] S. Jackson , et. al. (1994) *J. Am. Chem. Soc.*, **116**, 3220.
[2] C. Haase, et. al. (2008) *Angew. Chem. Int.Ed.*, **47**, 6807.

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Product No.	Product	Quantity	Price

Phenylalanine [Phe, F]
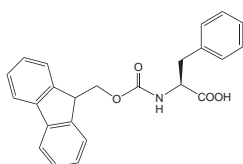
852016 **Fmoc-Phe-OH** — 25 g — 25.00

N-α-Fmoc-L-phenylalanine — 100 g — 50.00

NBC No.: 04-12-1030; CAS No.: 35661-40-6; $C_{24}H_{21}NO_4$; M.W.: 387.4 — 250 g — 110.00

Solubility: 25 mmole in 50 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98%.

HPLC: purity: ≥ 99.0%.

Fmoc-β-Ala-OH ≤ 0.1%.

Fmoc-β-Ala-Phe-OH ≤ 0.1%.

Fmoc-Phe-Phe-OH ≤ 0.1%.

Free amino acid: ≤ 0.2%.

EtOAc: ≤ 0.5%.

AcOH: ≤ 0.02%.

Optical purity: ≥ 99.8% L-enantiomer.
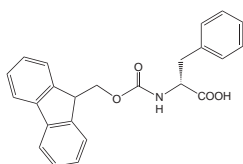
852148 **Fmoc-D-Phe-OH** — 5 g — 50.00

N-α-Fmoc-D-phenylalanine — 25 g — 200.00

NBC No.: 04-13-1030; CAS No.: 86123-10-6; $C_{24}H_{21}NO_4$; M.W.: 387.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

Optical purity: ≥ 99.50% D-enantiomer.

852226 **Fmoc-Phe-OPfp** — 5 g — 82.00

N-α-Fmoc-L-phenylalanine pentafluorophenyl ester

NBC No.: 04-12-1514; CAS No.: 86060-92-6; $C_{30}H_{20}NO_4F_5$; M.W.: 553.5

Solubility: 0.5 mmole in 3 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$ (1 : 3), purity: ≥ 2.00%.

HPLC: purity: ≥ 98.00%.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

⚠ Storage conditions Ⓖ Additional information Ⓜ Method information Ⓐ Application information

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Product No.	Product	Quantity	Price
852081	**Fmoc-(FmocHmb)Phe-OH**	1 g	295.00
	N-α-Fmoc-N-α-(2-Fmoc-oxy-4-methoxybenzyl)-L-phenylalanine	5 g	990.00

NBC No.: 04-12-1187; CAS No.: 148515-88-2; $C_{47}H_{39}NO_8$; M.W.: 745.8

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH:H$_2$O (90:10:0.5:1), purity: ≥ 95.00%.

CHCl$_3$:MeOH:AcOH:H$_2$O (85:13:0.5:1.5), purity: ≥ 95.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Hmb protection of amide bonds has been shown to inhibit aggregation of "difficult" peptides, thereby leading to products of increased purity [1-6]. Retention of Hmb groups on the cleaved peptide can greatly improve the Solubility of protected peptide fragments [7, 8] and otherwise intractable sequences [9-11]. Furthermore, using a Hmb-protected derivative for incorporation of the residue linked to the carboxyl group of Asp or Asn residues has been found to suppress formation of aspartimide and piperidide related by-products [12-14]. For a comparison of the efficiency of Hmb and pseudoprolines in preventing aggregation, see [15].

ⓘ Ⓖ 3.9, 3.18

[1] T. Johnson, et al. (1993) *J. Chem. Soc., Chem. Commun.*, 369.
[2] C. Hyde, et al. (1994) *Int. J. Peptide Protein Res.*, **43**, 431.
[3] L. C. Packman, et al. (1994) *Pept. Res.*, **7**, 125.
[4] T. Johnson, et al. (1994) *Tetrahedron Lett.*, **35**, 463.
[5] R. G. Simmonds (1996) *Int. J. Peptide Protein Res.*, **47**, 36.
[6] T. Johnson, et al. (1995) *Lett. Pept. Sci.*, **1**, 11.
[7] M. Quibell, et al. (1995) *J. Am. Chem. Soc.*, **117**, 11656.
[8] M. Quibell, et al. (1996) *J. Chem. Soc., Perkin Trans. 1*, 1227.
[9] M. Quibell, et al. (1994) *Tetrahedron Lett.*, **35**, 2237.
[10] M. Quibell, et al. (1994) *J. Org. Chem.*, **59**, 1745.
[11] M. Quibell, et al. (1995) *J. Chem. Soc., Perkin Trans. 1*, 2019.
[12] M. Quibell, et al. (1994) *J. Chem. Soc., Chem. Commun.*, 2343.
[13] L. C. Packman (1995) *Tetrahedron Lett.*, **36**, 7523.
[14] J. Offer, et al. (1996) *J. Chem. Soc., Perkin Trans. 1*, 175.
[15] W. R. Sampson, et al. (1999) *J. Peptide Sci.*, **5**, 403.

852287	**Fmoc-p-Bz-Phe-OH**	1 g	114.00
	(S)-3-(4-Benzoyl-phenyl)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-propionic acid	5 g	458.00

4-Benzoyl-N-[(9H-fluoren-9-ylmethoxy)carbonyl]phenylalanine

CAS No.: 117666-96-3; $C_{31}H_{25}NO_5$; M.W.: 269.3

Solubility: 1 mmole in 2 ml DMF clearly soluble.

HPLC: purity: ≥ 98.00%.
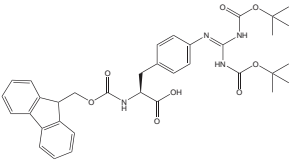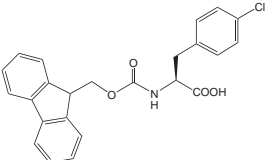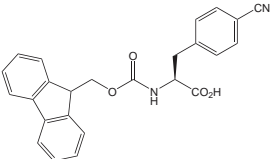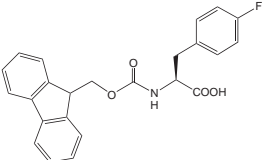
Fmoc-p-Bz-Phe-OH-OH is a useful tool for preparing photoactivatable peptide-based affinity probes [1]. On photolysis at 366 nm, Benzoylphenylalanine (Bpa) generates a biradical that has a preference for insertion into C-H bonds, particularly those of Leu, Val and Met side chains.

The derivative can be introduced using standard coupling method such as PyBOP and is stable to conditions used in peptide chain extension. For the cleavage of Bpa containing peptide from the resin, the use of thiols and silanes should be avoided as dithioketal formation and reduction, respectively, have been observed.

[1] K. T. O'Neil, et al. (1989) *J. Biol. Chem.*, **264**.

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Product No.	Product	Quantity	Price
852337	**Fmoc-Phe(bis-Boc-4-guanidino)-OH**	1 g	182.00
	CAS No.: 187283-25-6; $C_{35}H_{40}N_4O_8$; M.W.: 644.7	5 g	728.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

HPLC: purity: ≥ 93.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

A novel arginine mimetic.

Product No.	Product	Quantity	Price
852210	**Fmoc-Phe(4-Cl)-OH**	1 g	77.00
	N-α-Fmoc-4-chloro-L-phenylalanine	5 g	308.00

NBC No.: 04-12-1096; CAS No.: 175453-08-4; $C_{24}H_{20}NO_4Cl$; M.W.: 421.9

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

Product No.	Product	Quantity	Price
852341	**Fmoc-Phe(4-CN)-OH**	1 g	229.00
	Fmoc-4-cyanophenylalanine	5 g	915.00

CAS No.: 173963-93-4; $C_{25}H_{20}N_2O_4$; M.W.: 412.4

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 97.00%.

Starting amino acid for the preparation of 4-(tetrazol-5-yl-)-phenylalanine.

[1] J. S. McMurray, et al. (2000) *Tetrahedron Lett.*, **41**, 6555.

Product No.	Product	Quantity	Price
852214	**Fmoc-Phe(4-F)-OH**	1 g	77.00
	N-α-Fmoc-4-fluoro-L-phenylalanine	5 g	308.00

NBC No.: 04-12-1122; CAS No.: 169243-86-1; $C_{24}H_{20}NO_4F$; M.W.: 405.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% L-enantiomer.

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Product No.	Product	Quantity	Price
852288	**Fmoc-Phe(CF$_2$PO$_3$H)-OH**	100 mg	380.00
	Fmoc-F$_2$Pmp-OH	250 mg	760.00

Fmoc-L-p-(phosphono-difluoromethyl)phenylalanine

CAS No.: 160751-44-0; C$_{25}$H$_{22}$NO$_7$PF$_2$; M.W.: 517.4

HPLC: purity: ≥ 90.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Building block [1] for the introduction by Fmoc SPPS of the hydrolytically stable phosphotyrosine analog phosphonodifluoromethylphenylalanine (F$_2$Pmp) [2]. The pKa of F$_2$Pmp more closely resembles that of a phosphate group making it better analog than phosphonomethylphenylalanine.

ⓘ Ⓖ 3.41, Ⓜ 3.39, Ⓐ 3.41

[1] M. F. Gordeev, et al. (1994) *Tetrahedron Lett.*, **35**, 7585.
[2] T. R. Burke, Jr. (2006) *Curr. Top. Med. Chem.*, **6**, 1465.

Product No.	Product	Quantity	Price
852029	**Fmoc-Phe(4-NO$_2$)-OH**	1 g	32.00
	N-α-Fmoc-4-nitro-L-phenylalanine	5 g	125.00
		25 g	494.00

NBC No.: 04-12-1059; CAS No.: 95753-55-2; C$_{24}$H$_{20}$N$_2$O$_6$; M.W.: 432.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.00% L-enantiomer.
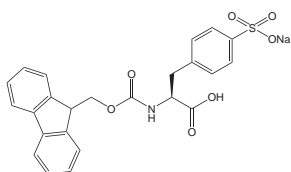
Product No.	Product	Quantity	Price
852378	**Fmoc-Phe(SO$_3$Na)-OH**	1 g	200.00
	NEW N-α-Fmoc-4-sulfophenylalanine sodium salt	5 g	800.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 97.00%.

HPLC: purity: ≥ 97.00%.

Free amino acid: ≤ 0.2%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry and under argon; hygroscopic.

Introduction of 4-sulfo-phenylalanine is an effective strategy for increasing Solubility of a peptide. This amino acid has also been employed in analogs of angiotensin 2 [1], phytosulfokine [2], and cyclolinopeptide A [3]. Its incorporation during solid phase synthesis using Fmoc-Phe(SO$_3$Na)-OH is best carried out with base-mediated coupling methods such as PyBOP® or HBTU.

[1] E. Escher, et al. (1983) , **66**, 1355.
[2] A. Bahyrycz, et al. (2004) , **10**, 462.
[3] Y. Fujiwara, et al. (1994) , **42**, 724.
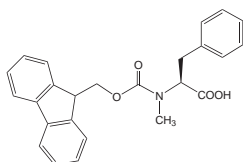
Product No.	Product	Quantity	Price
852137	**Fmoc-N-Me-Phe-OH**	1 g	72.00
	N-α-Fmoc-N-α-methyl-L-phenylalanine	5 g	280.00

NBC No.: 04-12-9025; CAS No.: 77128-73-5; C$_{25}$H$_{23}$NO$_4$; M.W.: 401.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CHCl$_3$:MeOH:AcOH:H$_2$O (90:10:0.5:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% L-enantiomer.

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Product No.	Product	Quantity	Price
852328	**Fmoc–hPhe–OH**	1 g	52.00
	Fmoc-homo-L-phenylalanine	5 g	203.00
	CAS No.: 132684-59-4; $C_{25}H_{23}NO_4$; M.W.: 401.5		
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: $\geq$ 98.00%.		
	HPLC: purity: $\geq$ 97.00%.		

Phenylglycine [Phg]

852213	**Fmoc–Phg–OH**	1 g	37.00
	N-α-Fmoc-L-phenylglycine	5 g	142.00
	NBC No.: 04-12-1117; CAS No.: 102410-65-1; $C_{23}H_{19}NO_4$; M.W.: 373.4	25 g	566.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: $\geq$ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: $\geq$ 98.00%.		
	HPLC: purity: $\geq$ 98.00%.		
	Optical purity: $\geq$ 99.00% L-enantiomer.		

852232	**Fmoc–D–Phg–OH**	1 g	27.00
	N-α-Fmoc-D-phenylglycine	5 g	108.00
	NBC No.: 04-13-1004; CAS No.: 111524-95-9; $C_{23}H_{19}NO_4$; M.W.: 373.4	25 g	433.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: $\geq$ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: $\geq$ 98.00%.		
	HPLC: purity: $\geq$ 98.00%.		
	Optical purity: $\geq$ 99.50% D-enantiomer.		

Proline [Pro, P]
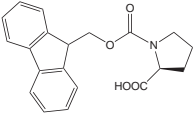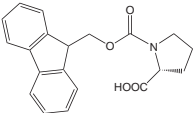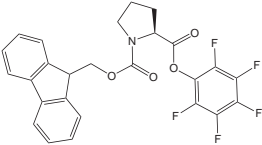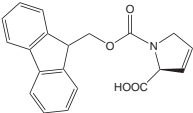
852351	**cis-Fmoc-Pro(4-N₃)-OH**	250 mg	65.00
	Fmoc-(2S, 4S)-4-azidoproline	1 g	195.00
	CAS No.: 263847-08-1; $C_{20}H_{18}N_4O_4$; M.W.: 378.4		

⚠ Prolonged storage: $\leq$ -20°C; keep cool and dry; prevent exposure to light.
Solubility: 1 mmole in 2 ml DMF clearly soluble.
HPLC: purity: $\geq$ 98.00%.
A useful tool for the synthesis of branched, side-chain modified and cyclic peptides by Fmoc SPPS. The side-chain azido group is completely stable to piperidine and TFA, but can be readily converted to an amine on the solid phase or in solution by reduction with thiols [1] or phosphines [2, 3].

ⓘ Ⓖ 4.12, Ⓜ 4.12

[1] M. Meldal, et al. (1997) *Tetrahedron Lett.*, **38**, 2531.
[2] J. T. Lundquist & J. C. Pelletier (2001) *Org. Lett.*, **3**, 781.
[3] N. Nepomniaschiy, et al. (2008) *Org. Lett.*, **10**, 5243.

N–α–FMOC PROTECTED AMINO ACIDS

⚠ Storage conditions Ⓖ Additional information Ⓜ Method information Ⓐ Application information

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Product No.	Product	Quantity	Price
852017	**Fmoc–Pro–OH**	25 g	25.00
	N-α-Fmoc-L-proline	100 g	50.00
	NBC No.: 04-12-1031; CAS No.: 71989-31-6; $C_{20}H_{19}NO_4$; M.W.: 337.4	250 g	110.00
	Solubility: 25 mmole in 50 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.		
	HPLC: purity: ≥ 99.0%.		
	HPLC: Fmoc-β-Ala-OH ≤ 0.1%.		
	HPLC: Fmoc-β-Ala-Pro-OH ≤ 0.1%.		
	HPLC: Fmoc-Pro-Pro-OH ≤ 0.1%.		
	Free amino acid: ≤ 0.2%.		
	EtOAc: ≤ 0.5%.		
	AcOH: ≤ 0.02%.		
	Optical purity: ≥ 99.8% L-enantiomer.		
852149	**Fmoc–D–Pro–OH**	1 g	23.00
	N-α-Fmoc-D-proline	5 g	69.00
	NBC No.: 04-13-1031; CAS No.: 101555-62-8; $C_{20}H_{19}NO_4$; M.W.: 337.4	25 g	352.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 99.00%.		
	Optical purity: ≥ 99.50% D-enantiomer.		
852227	**Fmoc–Pro–OPfp**	5 g	82.00
	N-α-Fmoc-L-proline pentafluorophenyl ester		
	NBC No.: 04-12-1515; CAS No.: 86060-90-4; $C_{26}H_{18}NO_4F_5$; M.W.: 503.5		
	Solubility: 0.5 mmole in 3 ml DMF clearly soluble.		
	TLC: CH_3CN:$CHCl_3$ (1 : 3), purity: ≥ 2.00%.		
	HPLC: purity: ≥ 98.00%.		
	⚠ Prolonged storage: ≤ -20°C; keep cool and dry.		
852208	**Fmoc–3,4–dehydro–Pro–OH**	1 g	634.00
	N-α-Fmoc-3,4-dehydro-L-proline		
	NBC No.: 04-12-1051; CAS No.: 135837-63-7; $C_{20}H_{17}NO_4$; M.W.: 335.4		
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 98.00%.		
	Optical purity: ≥ 97.00% L-enantiomer.		
	⚠ Prolonged storage: ≤ -20°C; keep cool and dry.		

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Product No.	Product	Quantity	Price

Sarcosine [Sar]
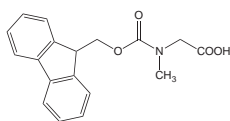
| 852055 | **Fmoc-Sar-OH** | 5 g | 50.00 |
| | N-α-Fmoc-sarcosine | 25 g | 176.00 |

Fmoc-N-Me-Gly-OH

NBC No.: 04-12-1114; CAS No.: 77128-70-2; $C_{18}H_{17}NO_4$; M.W.: 311.3

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Serine [Ser, S]

Fmoc-Ser(tBu)-OH is recommended for the routine preparation of serine-containing peptides. For the preparation of peptides containing side-chain modified Ser residues, Fmoc-Ser(Trt)-OH should be used since the Trt group can be removed selectively on the solid-phase.

For further information, please refer to the product entries.
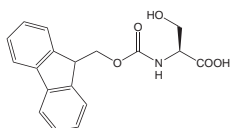
| 852028 | **Fmoc-Ser-OH** | 5 g | 62.00 |
| | N-α-Fmoc-L-serine | 25 g | 176.00 |

NBC No.: 04-12-1057; CAS No.: 73724-45-5; $C_{18}H_{17}NO_5$; M.W.: 327.3

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

Useful derivative for the synthesis of phosphoserine peptides by the global phosphorylation methodology.
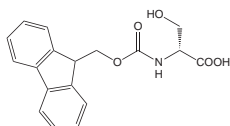
| 852233 | **Fmoc-D-Ser-OH** | 1 g | 43.00 |
| | N-α-Fmoc-D-serine | 5 g | 171.00 |

NBC No.: 04-13-1033; CAS No.: 116861-26-8; $C_{18}H_{17}NO_5$; M.W.: 327.3

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% D-enantiomer.

⚠ Storage conditions   ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
852136	**Fmoc-Ser(Ac₃AcNH-α-Gal)-OH**	100 mg	975.00

852136 **Fmoc-Ser(Ac$_3$AcNH-α-Gal)-OH**

N-α-Fmoc-O-(2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-galactopyranosyl)-L-serine

NBC No.: 04-12-8103; CAS No.: 120173-57-1; C$_{32}$H$_{36}$N$_2$O$_{13}$; M.W.: 656.6

HPLC: purity: ≥ 95.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

The O-glycosidic linkage and the O-acetyl protection are stable to both piperidine and TFA [1], making this building block completely compatible with standard protocols in Fmoc solid phase peptide synthesis. Removal of the acetyl protecting groups is best carried out by treatment of the peptidyl resin with methanolic ammonia prior to cleavage.

ⓘ Ⓖ 3.44

[1] J. Kihlberg in "Fmoc solid phase peptide synthesis: a practical approach", W.C. Chan & P.D. White (Eds.), Oxford University Press, Oxford, 2000, pp. 195.

852349 **Fmoc-Ser(Ac$_3$AcNH-β-Glc)-OH** 25 mg 300.00
100 mg 900.00

N-α-Fmoc-O-(2-Acetamido-2-deoxy-tri-O-acetyl-β-D-glucopyranosyl)-L-serine

CAS No.: 160067-63-0; C$_{32}$H$_{36}$N$_2$O$_{13}$; M.W.: 656.7

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

HPLC: purity: ≥ 97.00%.

for more iformation, please see the entry for 852136.

852019 **Fmoc-Ser(tBu)-OH** 25 g 60.00
100 g 180.00

N-α-Fmoc-O-t.-butyl-L-serine

NBC No.: 04-12-1033; CAS No.: 71989-33-8; C$_{22}$H$_{25}$NO$_5$; M.W.: 383.4 250 g 395.00

Solubility: 25 mmole in 50 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98%.

HPLC: purity: ≥ 99.0%.

Fmoc-β-Ala-OH ≤ 0.1%

Fmoc-β-Ala-Ser(tBu)-OH ≤ 0.1%

Fmoc-Ser(tBu)-Ser(tBu)-OH ≤ 0.1%

Fmoc-Ser-OH ≤ 0.1%

Free amino acid: ≤ 0.2%.

EtOAc: ≤ 0.5%

AcOH: ≤ 0.02%

Optical purity: ≥ 99.8% L-enantiomer.

852365 **Fmoc-Ser(tBu)-OPfp** 5 g 200.00
NEW

N-α-Fmoc-O-t.-butyl-L-serine pentafluorophenyl ester

CAS No.: 105751-13-1; C$_{28}$H$_{24}$F$_5$NO$_5$; M.W.: 549.5

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

Solubility: 1 mmol in 6 ml DMF clearly soluble.

TLC: CH$_3$CN:CHCl$_3$ (1 : 3), purity: ≥ 98.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.00% L-enantiomer.

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Product No.	Product	Quantity	Price
852156	**Fmoc–D–Ser(tBu)–OH**	1 g	44.00
	N-α-Fmoc-O-t.-butyl-D-serine	5 g	172.00
	NBC No.: 04-13-1052; CAS No.: 128107-47-1; $C_{22}H_{25}NO_5$; M.W.: 383.4	25 g	688.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 99.00%.
Optical purity: ≥ 99.50% D-enantiomer.

852069	**Fmoc–Ser(PO(OBzl)OH)–OH**	1 g	157.00
	N-α-Fmoc-O-benzyl-L-phosphoserine	5 g	628.00
	NBC No.: 04-12-1154; CAS No.: 158171-14-3; $C_{25}H_{24}NO_8P$; M.W.: 497.4		

Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 97.00%.
HPLC: purity: ≥ 95.0%.
EtOAc: ≤ 0.5%.
AcOH: ≤ 0.1%.
Optical purity: ≥ 99.0% L-enantiomer.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

An excellent building block for the preparation of phosphoserine-containing peptides [1]. This derivative can be introduced using standard activation methods, such as PyBOP® and TBTU. The monoprotected phosphoserine residue once incorporated is stable to piperidine. Using this reagent, even peptides containing multiple phosphorylation sites can be prepared efficiently by standard Fmoc SPPS methods [2].

Applications of this derivative include the preparation of phospholamban [3], a 52 residue peptide containing both phosphoserine and phosphothreonine, and human salivary statherin, a 42 residue phosphoserine peptide [4]; for other examples see references [5-8].

Recently, β-piperidinylalanine formation has been shown to occur during Fmoc deprotection of N-terminal Ser(PO(OBzl)OH), particularly under microwave conditions. This side reaction can be eliminated by using cyclohexylamine or DBU just for this Fmoc deprotection step [9].

ⓘ Ⓖ 3.38, 3.39, Ⓜ 3.39

[1] T. Wakamiya, et al. (1994) *Chem. Lett.*, 1099.
[2] P. White & J. Beythien in "Innovations & Perspectives in Solid Phase Synthesis and Combinatorial Libraries, 4th International Symposium", Mayflower Scientific Ltd., Birmingham, 1996, pp. 557.
[3] H. Schmid, et al., Poster 423 presented at the 15th American Peptide Symposium, Nashville, 1997.
[4] T. L. Gururaja & M. J. Levine (1996) *Pept. Res.*, **9**, 283.
[5] T. Vorherr, et al. (1995) *Bioorg. Med. Chem. Lett.*, **5**, 2661.
[6] G. Shapiro, et al. (1996) *Bioorg. Med. Chem. Lett.*, **6**, 409.
[7] M. John, et al. (1996) *Pept. Res.*, **9**, 71.
[8] K. Teruya, et al. (2004) *J. Pept. Sci.*, **10**, 479.
[9] T. J. Attard, et al. (2009) *Int. J. Pept. Res. Ther.*, **15**, 69.

⚠ Storage conditions　　Ⓖ Additional information　　Ⓜ Method information　　Ⓐ Application information

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Product No.	Product	Quantity	Price
852244	**Fmoc–D–Ser(PO(OBzl)OH)–OH**	1 g	325.00
	N-α-Fmoc-O-benzyl-D-phosphoserine	5 g	1200.00

NBC No.: 04-13-1078; CAS No.: 1212481-01-0; $C_{25}H_{24}NO_8P$; M.W.: 497.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 95.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

See entry for 852069.

852046	**Fmoc–Ser(Trt)–OH**	5 g	63.00
	N-α-Fmoc-O-trityl-L-serine	25 g	229.00
		100 g	707.00

NBC No.: 04-12-1092; CAS No.: 111061-56-4; $C_{37}H_{31}NO_5$; M.W.: 569.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

The side-chain Trt group can be selectively removed with 1% TFA in DCM
containing 5% TIS [1, 2] or 20% dichloroacetic acid in DCM [3], enabling the side-
chain hydroxyl group to be selectively modified whilst the derivative is attached
to the solid support. This is an excellent derivative for the synthesis of
phosphoserine containing peptides and peptides modified at the serine side-chain
[1]. The use of trityl protected amino acids was shown to result in purer products
than when standard t-Bu protected amino acids were utilized [2, 4].

ⓘ Ⓖ 4.13, Ⓜ 4.12

[1] K. Barlos, et al. (1991) *Tetrahedron Lett.*, **32**, 471.

[2] K. Barlos, et al. (1998) *J. Peptide Res.*, **51**, 194.

[3] M. P. Coba, et al. (2003) *J. Peptide Res.*, **61**, 17.

[4] X.-H. Tong, et al. in "Peptide Revolution: Genomics, Proteomics & Therapeutics, , Proc.
18th American Peptide Symposium", M. Chrev & T. K. Sawyer (Eds), Cardiff, American
Peptide Society, 2003, pp. 21.
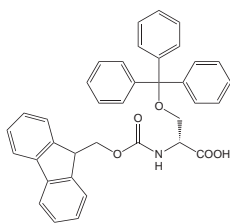
852166	**Fmoc–D–Ser(Trt)–OH**	1 g	47.00
	N-α-Fmoc-O-trityl-D-serine	5 g	188.00
		25 g	752.00

NBC No.: 04-13-1072; CAS No.: 212688-51-2; $C_{37}H_{31}NO_5$; M.W.: 569.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% D-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

For applications information, please refer to entry for 852046.

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Product No.	Product	Quantity	Price
852289	**Fmoc-N-Me-Ser(tBu)-OH**	250 mg	78.00
	Fmoc-N-α-methyl-O-t-butyl-L-serine	1 g	234.00
	CAS No.: 197632-77-2; $C_{23}H_{27}NO_5$; M.W.: 397.5	5 g	936.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.
HPLC: purity: ≥ 95.00%.
Optical purity: ≥ 98.50% L-enantiomer.

852059	**Fmoc-Hse(Trt)-OH**	1 g	96.00
	N-α-Fmoc-O-trityl-L-homoserine	5 g	381.00

NBC No.: 04-12-1126; CAS No.: 111061-55-3; $C_{38}H_{33}NO_5$; M.W.: 583.7
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: CHCl₃:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH₃CN:CHCl₃:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 98.00%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
The Trt group can be removed with 1% TFA in DCM containing 5% TIS, enabling the side-chain hydroxyl group to be selectively modified whilst the derivative is attached to the solid support. This derivative has been employed to prepare oligonucleotide conjugates [1].

ⓘ Ⓖ 4.13, Ⓜ 4.12

[1] D. A. Stetsenko & M. J. Gait (2001) *Bioconjugate Chem.*, **12**, 576.

Statine & derivatives [Sta]

852026	**Fmoc-Sta-OH**	1 g	723.00
	N-Fmoc-L-statine		

N-Fmoc-(3S,4S)-4-amino-3-hydroxy-6-methylheptanoic acid
NBC No.: 04-12-1053; CAS No.: 158257-40-0; $C_{23}H_{27}NO_5$; M.W.: 397.5
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: CH₃CN:CHCl₃:AcOH (8:1:1), purity: ≥ 98.00%.
CHCl₃:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
HPLC: purity: ≥ 97.00%.
Optical purity: ≥ 99.00% L-enantiomer.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Tetrahydroisoquinoline-3-carboxylic acids [Tic]
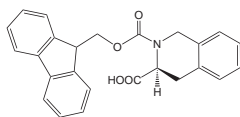
852062   **Fmoc-Tic-OH**   1 g   99.00

N-1-Fmoc-L-1,2,3,4,-tetrahydro-isoquinoline-3-carboxylic acid   5 g   395.00

NBC No.: 04-12-1130; CAS No.: 136030-33-6; $C_{25}H_{21}NO_4$; M.W.: 399.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

[1] M. Vincent, et al. (1982) *Tetrahedron Lett.*, **16**, 1677.

[2] P. L. Julian, et al. (1984) *J. Am. Chem. Soc.*, **79**, 180.

[3] W. A. Kazmierski, et al. (1988) *Tetrahedron Lett.*, **44**, 697.

852240   **Fmoc-D-Tic-OH**   1 g   156.00

N-1-Fmoc-D-1,2,3,4,-tetrahydro-isoquinoline-3-carboxylic acid   5 g   619.00

NBC No.: 04-13-1065; CAS No.: 130309-33-0; $C_{25}H_{21}NO_4$; M.W.: 399.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% D-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

[1] M. Vincent, et al. (1982) *Tetrahedron Lett.*, **16**, 1677.

[2] P. L. Julian, et al. (1984) *J. Am. Chem. Soc.*, **79**, 180.

[3] W. A. Kazmierski, et al. (1988) *Tetrahedron Lett.*, **44**, 697.

Thienylalanine [Thi]
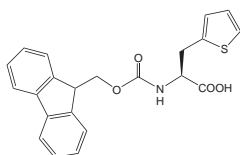
852039   **Fmoc-Thi-OH**   1 g   99.00

N-α-Fmoc-β-thienyl-L-alanine   5 g   395.00

NBC No.: 04-12-1082; CAS No.: 130309-35-2; $C_{22}H_{19}NO_4S$; M.W.: 393.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

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Product No.	Product	Quantity	Price
852238	**Fmoc–D–Thi–OH**	1 g	113.00
	N-α-Fmoc-β-thienyl-D-alanine	5 g	447.00

NBC No.: 04-13-1049; CAS No.: 201532-42-5; $C_{22}H_{19}NO_4S$; M.W.: 393.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CHCl$_3$:MeOH:AcOH:H$_2$O (85:13:0.5:1.5), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% D-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

Threonine [Thr, T]

Fmoc-Thr(tBu)-OH is recommended for the routine preparation of threonine-containing peptides. For the preparation of peptides containing side-chain modified Thr residues, Fmoc-Thr(Trt)-OH should be used since the Trt group can be removed selectively on the solid-phase.

For further information, please refer to the product entries.

Product No.	Product	Quantity	Price
852030	**Fmoc–Thr–OH**	5 g	62.00
	N-α-Fmoc-L-threonine	25 g	176.00

NBC No.: 04-12-1060; CAS No.: 73731-37-0; $C_{19}H_{19}NO_5$; M.W.: 341.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

Useful derivative for the synthesis of phosphothreonine peptides by the global phosphorylation methodology.

[1] P. M. Fischer, et al. (1991) *Int. J. Peptide Protein Res.*, **38**, 491.

Product No.	Product	Quantity	Price
852234	**Fmoc–D–Thr–OH**	1 g	83.00
	N-α-Fmoc-D-threonine / (2R,3S)	5 g	333.00

NBC No.: 04-13-1034; CAS No.: 118609-38-4; $C_{19}H_{19}NO_5$; M.W.: 341.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

CHCl$_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% D-enantiomer.

N-α-FMOC PROTECTED AMINO ACIDS

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Product No.	Product	Quantity	Price
852229	**Fmoc-Thr(Ac₃AcNH-α-Gal)-OH**	100 mg	975.00

N-α-Fmoc-O-(2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-galactopyranosyl)-L-threonine

NBC No.: 04-12-8104; CAS No.: 116783-35-8; $C_{33}H_{38}N_2O_{13}$; M.W.: 670.7

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 95.00%.

HPLC: purity: ≥ 95.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

The O-glycosidic linkage and the O-acetyl protection are stable to both piperidine and TFA [1], making this building block completely compatible with standard protocols in Fmoc solid phase peptide synthesis. Removal of the acetyl protecting groups is best carried out by treatment of the peptidyl resin with methanolic ammonia prior to cleavage.

ⓘ Ⓖ 3.44

[1]   J. Kihlberg in "Fmoc solid phase peptide synthesis: a practical approach", W.C. Chan & P.D. White (Eds.), Oxford University Press, Oxford, 2000, pp. 195.

| 852350 | **Fmoc-Thr(Ac₃AcNH-β-Glc)-OH** | 25 mg | 300.00 |
| | | 100 mg | 920.00 |

N-α-Fmoc-O-(2-Acetamido-2-deoxy-tri-O-acetyl-β-D-glucopyranosyl)-L-threonine

CAS No.: 160168-40-1; $C_{33}H_{38}N_2O_{13}$; M.W.: 670.7

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

HPLC: purity: ≥ 95.00%.

For further information, please see the entry for 852229.
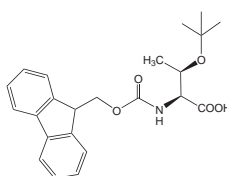
852000	**Fmoc-Thr(tBu)-OH**	25 g	60.00
		100 g	180.00
		250 g	395.00

N-α-Fmoc-O-t.-butyl-L-threonine

NBC No.: 04-12-1000; CAS No.: 71989-35-0; $C_{23}H_{27}NO_5$; M.W.: 397.5

Solubility: 25 mmole in 50 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98%.

HPLC: purity: ≥ 99.0%.

Fmoc-β-Ala-OH ≤ 0.1%.

Fmoc-β-Ala-Thr(tBu)-OH ≤ 0.1%.

Fmoc-Thr(tBu)-Thr(tBu)-OH ≤ 0.1%.

Fmoc-Thr-OH ≤ 0.1%.

Free amino acid: ≤ 0.2%.

EtOAc: ≤ 0.5%.

AcOH: ≤ 0.02%.

Optical purity: ≥ 99.7% L-enantiomer.

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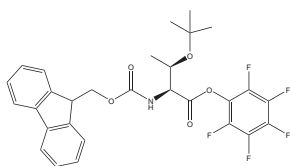
Product No.	Product	Quantity	Price
852366	**Fmoc–Thr(tBu)–OPfp** NEW	5 g	200.00

N-α-Fmoc-O-t.-butyl-L-threonine pentafluorophenyl ester
CAS No.: 117088-31-0; $C_{29}H_{26}F_5NO_5$; M.W.: 563.5
⚠ Prolonged storage: ≤ -20°C
Solubility: 1 mmol in 6 ml DMF clearly soluble.
TLC: CH_3CN:$CHCl_3$ (1 : 3), purity: ≥ %.
HPLC: purity: ≥ 98.00%.
Optical purity: ≥ 98.00% L-enantiomer.
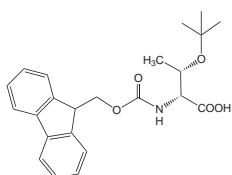
852157	**Fmoc–D–Thr(tBu)–OH**	1 g	44.00
		5 g	172.00
		25 g	688.00

N-α-Fmoc-O-t.-butyl-D-threonine / (2R,3S)
NBC No.: 04-13-1053; CAS No.: 138797-71-4; $C_{23}H_{27}NO_5$; M.W.: 397.5
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 99.00%.
Optical purity: ≥ 99.50% D-enantiomer.
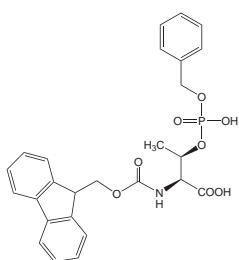
| 852070 | **Fmoc–Thr(PO(OBzl)OH)–OH** | 1 g | 170.00 |
| | | 5 g | 680.00 |

N-α-Fmoc-O-benzyl-L-phosphothreonine
NBC No.: 04-12-1155; CAS No.: 175291-56-2; $C_{26}H_{26}NO_8P$; M.W.: 511.5
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 98.00%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
An excellent building block for the preparation of phosphothreonine-containing peptides [1, 2]. This derivative can be introduced using standard activation methods, such as PyBOP® and TBTU. The monoprotected phosphothreonine residue once incorporated is stable to piperidine. Using this reagent, even peptides containing multiple phosphorylation sites can be prepared efficiently by standard Fmoc SPPS methods [1].
A paper describes the use of this derivative in the preparation of phospholamban [3], a 52 residue peptide containing both phosphoserine and phosphothreonine.
ⓘ Ⓖ 3.39, Ⓜ 3.39, Ⓐ 3.40

[1] P. White & J. Beythien in "Innovations & Perspectives in Solid Phase Synthesis and Combinatorial Libraries, 4th International Symposium", Mayflower Scientific Ltd., Birmingham, 1996, pp. 557.
[2] T. Vorherr, et al. (1995) *Bioorg. Med. Chem. Lett.*, **5**, 2661.
[3] H. Schmid, et al., Poster 423 presented at the 15th American Peptide Symposium, Nashville, 1997.

N-α-FMOC PROTECTED AMINO ACIDS

⚠ Storage conditions　　Ⓖ Additional information　　Ⓜ Method information　　Ⓐ Application information

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Product No.	Product	Quantity	Price
852245	**Fmoc-D-Thr(PO(OBzl)OH)-OH**	1 g	325.00
	N-α-Fmoc-O-benzyl-D-phosphothreonine	5 g	1200.00
	NBC No.: 04-13-1079; CAS No.: 937171-63-6; $C_{26}H_{26}NO_8P$; M.W.: 511.5		

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 95.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

See entry for 852070.

852066	**Fmoc-Thr(Trt)-OH**	5 g	102.00
	N-α-Fmoc-O-trityl-L-threonine	25 g	408.00
	NBC No.: 04-12-1141; CAS No.: 133180-01-5; $C_{38}H_{33}NO_5$; M.W.: 583.7	100 g	1030.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

The side-chain Trt group can be selectively removed with 1% TFA in DCM containing 5% TIS, enabling the side-chain hydroxyl group to be selectively modified whilst the derivative is attached to the solid support. This is an excellent derivative for the synthesis of phosphothreonine containing peptides and peptides modified at the threonine side-chain [1]. The use of trityl protected amino acids was shown to result in purer products than when standard t-Bu protected amino acids were utilized [2].

ⓘ Ⓖ 4.12, Ⓜ 4.13

[1] K. Barlos, et al. (1991) *Tetrahedron Lett.*, **32**, 471.

[2] K. Barlos, et al. (1998) *J. Peptide Res.*, **51**, 194.

852241	**Fmoc-D-Thr(Trt)-OH**	1 g	57.00
	N-α-Fmoc-O-trityl-D-threonine / (2R,3S)	5 g	229.00
	NBC No.: 04-13-1070; CAS No.: 682800-84-6; $C_{38}H_{33}NO_5$; M.W.: 583.7		

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.00% D-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

For application information, please refer to entry for 852066.

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Product No.	Product	Quantity	Price
852331	**Fmoc–N–Me–Thr(tBu)–OH**	250 mg	78.00
	N-α-Fmoc-N-α-methyl-O-tert-butyl-L-threonine	1 g	234.00
	CAS No.: 117106-20-4 ; $C_{24}H_{29}NO_5$; M.W.: 411.50		
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (85:10:5), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 95.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
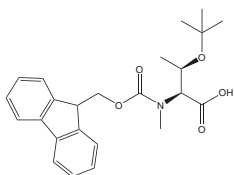
Tryptophan [Trp, W]

Fmoc–Trp(Boc)–OH is recommended for the routine preparation of tryptophan-containing peptides. The use of this derivative minimizes most of the side-reactions associated with the TFA-mediated cleavage of Trp-containing peptides.

For further information, please refer to the product entries.

852207	**Fmoc–Trp–OH**	5 g	11.00
	N-α-Fmoc-L-tryptophan	25 g	34.00
	NBC No.: 04-12-1035; CAS No.: 35737-15-6; $C_{26}H_{22}N_2O_4$; M.W.: 426.5	100 g	101.00
	Solubility: 25 mmole in 50 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 98.00%.		
	Optical purity: ≥ 99.00% L-enantiomer.		
	ⓘ Ⓖ 4.6		

852150	**Fmoc–D–Trp–OH**	5 g	94.00
	N-α-Fmoc-D-tryptophan	25 g	374.00
	NBC No.: 04-13-1035; CAS No.: 86123-11-7; $C_{26}H_{22}N_2O_4$; M.W.: 426.5		
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 98.00%.		
	Optical purity: ≥ 99.50% D-enantiomer.		

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
852050	**Fmoc-Trp(Boc)-OH**	25 g	125.00
	N-α-Fmoc-N-in-t.-Boc-L-tryptophan	100 g	375.00
	NBC No.: 04-12-1103; CAS No.: 143824-78-6; $C_{31}H_{30}N_2O_6$; M.W.: 526.6	250 g	825.00

Solubility: 25 mmole in 50 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98%.

HPLC: purity: ≥ 97.5%.

Fmoc-β-Ala-OH ≤ 0.3%.

Fmoc-β-Ala-Trp(Boc)-OH ≤ 0.1%.

Fmoc-Trp(Boc)-Trp(Boc)-OH ≤ 0.1%.

Fmoc-Trp-OH ≤ 1.0%.

Free amino acid: ≤ 0.2%.

EtOAc: ≤ 0.5%.

AcOH: ≤ 0.02%.

Optical purity: ≥ 99.7% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

The use of this N-in-Boc protected derivative overcomes most of the problems associated with the preparation of Trp containing-peptides by Fmoc SPPS [1]. Cleavage with TFA generates an N-in-carboxy indole which protects the Trp from alkylation [1, 2, 3] and sulfonation [1, 4, 5, 6, 7]. The N-in-carboxy group is removed under aqueous conditions during normal work-up of the peptide.

ⓘ Ⓖ 4.6, Ⓜ, Ⓐ 4.7

[1] P. White in "Peptides, Chemistry & Biology, Proc. 12th American Peptide Symposium", J. A. Smith & J. E. Rivier (Eds), ESCOM, Leiden, 1992, pp. 537.

[2] B. Riniker, et al. (1993) *Tetrahedron*, **49**, 9307.

[3] T. Johnson, et al. (1993) *J. Chem. Soc., Chem. Commun.*, 369.

[4] H. Choi, et al. (1993) *Int. J. Peptide Protein Res.*, **42**, 58.

[5] C. G. Fields, et al. (1993) *Tetrahedron Lett.*, **34**, 6661.

[6] T. Lescrinier, et al. (1995) *Lett. Pept. Sci.*, **2**, 225.

[7] M. Noda & M. Kiffe (1997) *J. Peptide Res.*, **50**, 329.
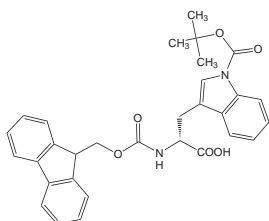
852164	**Fmoc-D-Trp(Boc)-OH**	1 g	65.00
	N-α-Fmoc-N-in-t.-Boc-D-tryptophan	5 g	260.00

NBC No.: 04-13-1062; CAS No.: 163619-04-3; $C_{31}H_{30}N_2O_6$; M.W.: 526.6

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% D-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
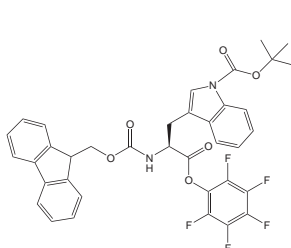
852131	**Fmoc-Trp(Boc)-OPfp**	5 g	310.00

N-α-Fmoc-N-in-t.-Boc-L-tryptophan pentafluorophenyl ester

NBC No.: 04-12-1535; CAS No.: 181311-44-4; $C_{37}H_{29}F_5N_2O_6$; M.W.: 692.7

Solubility: 0.5 mmole in 3 ml DMF clearly soluble.

TLC: CH$_3$CN:CHCl$_3$ (1 : 3), purity: ≥ %.

HPLC: purity: ≥ 95.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

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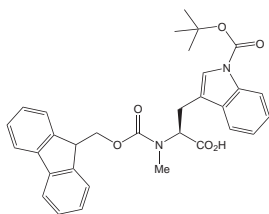
Product No.	Product	Quantity	Price
852344	**Fmoc-N-Me-Trp(Boc)-OH**	250 mg	166.00
	N-α-(9-Fluorenylmethoxycarbonyl)-N-α-methyl-N-in-t-butoxycarbonyl-L-tryptophan	1 g	499.00
	CAS No.: 197632-75-0; $C_{32}H_{32}N_2O_6$; M.W.: 540.6		
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 97.00%.		
	HPLC: purity: ≥ 97.00%.		

Tyrosine [Tyr, Y]

Fmoc-Tyr(tBu)-OH is recommended for the routine preparation of tyrosine-containing peptides. For the preparation of peptides containing side-chain modified Tyr residues, Fmoc-Tyr(2-ClTrt)-OH should be used since the 2-ClTrt group can be removed selectively on the solid-phase.

For further information, please refer to the product entries below.
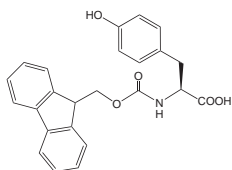
Product No.	Product	Quantity	Price
852051	**Fmoc-Tyr-OH**	1 g	51.00
	N-α-Fmoc-L-tyrosine	5 g	204.00
	NBC No.: 04-12-1105; CAS No.: 92954-90-0; $C_{24}H_{21}NO_5$; M.W.: 403.4		
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 96.00%.		
	$CHCl_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 96.00%.		
	HPLC: purity: ≥ 95.00%.		
	Optical purity: ≥ 99.50% L-enantiomer.		

A useful derivative for the synthesis of phosphotyrosine peptides by the global phosphorylation methodology [1].

[1] E. A. Kitas, et al. (1991) *Helv. Chim. Acta*, **74**, 1314.
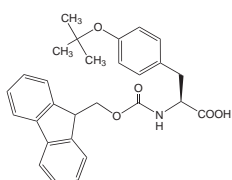
Product No.	Product	Quantity	Price
852020	**Fmoc-Tyr(tBu)-OH**	25 g	60.00
	N-α-Fmoc-O-t.-butyl-L-tyrosine	100 g	180.00
	NBC No.: 04-12-1037; CAS No.: 71989-38-3; $C_{28}H_{29}NO_5$; M.W.: 459.6	250 g	395.00
	Solubility: 25 mmole in 50 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98%.		
	HPLC: purity: ≥ 99.0%.		
	Fmoc-β-Ala-OH ≤ 0.1%.		
	Fmoc-β-Ala-Tyr(tBu)-OH ≤ 0.1%.		
	Fmoc-Tyr(tBu)-Tyr(tBu)-OH ≤ 0.1%.		
	Fmoc-Tyr-OH ≤ 0.1%.		
	Free amino acid: ≤ 0.2%.		
	EtOAc: ≤ 0.5%.		
	AcOH: ≤ 0.02%.		
	Optical purity: ≥ 99.8% L-enantiomer.		

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Product No.	Product	Quantity	Price
852151	**Fmoc-D-Tyr(tBu)-OH**	1 g	50.00
	N-α-Fmoc-O-t.-butyl-D-tyrosine	5 g	200.00
	NBC No.: 04-13-1037; CAS No.: 118488-18-9; $C_{28}H_{29}NO_5$; M.W.: 459.6	25 g	800.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 98.00%.
Optical purity: ≥ 99.50% D-enantiomer.

852130	**Fmoc-Tyr(tBu)-OPfp**	5 g	185.00
	N-α-Fmoc-O-t.-butyl-L-tyrosine pentafluorophenyl ester		
	NBC No.: 04-12-1519; CAS No.: 86060-93-7; $C_{34}H_{28}F_5NO_5$; M.W.: 625.6		

Solubility: 0.5 mmole in 3 ml DMF clearly soluble.
TLC: CH$_3$CN:CHCl$_3$ (1 : 3), purity: ≥ %.
HPLC: purity: ≥ 98.00%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

852080	**Fmoc-Tyr(2-ClTrt)-OH**	5 g	120.00
	N-α-Fmoc-O-2-chlorotrityl-L-tyrosine	25 g	478.00
	NBC No.: 04-12-1184; CAS No.: 350241-80-4; $C_{43}H_{34}NO_5Cl$; M.W.: 680.2	100 g	1342.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 99.00%.
Optical purity: ≥ 99.50% L-enantiomer.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
The side-chain ClTrt group can be removed with 1% TFA/ 5% TIS in DCM, enabling the side-chain hydroxyl group to be selectively modified whilst the derivative is attached to the solid support.
ⓘ Ⓖ 4.13, Ⓜ 4.12
[1] K. Barlos, et al. (1998) *J. Peptide Res.*, **51**, 194.

852056	**Fmoc-Tyr(Me)-OH**	1 g	120.00
	N-α-Fmoc-O-methyl-L-tyrosine	5 g	359.00
	NBC No.: 04-12-1118; CAS No.: 77128-72-4; $C_{25}H_{23}NO_5$; M.W.: 417.5		

Solubility: 1 mmole in 2 ml DMF clearly soluble.
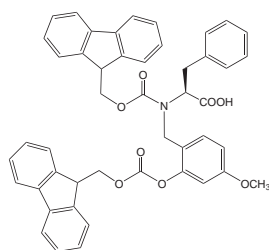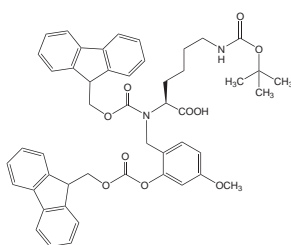TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 99.00%.
Optical purity: ≥ 99.50% L-enantiomer.

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Product No.	Product	Quantity	Price

852058   **Fmoc–Tyr(PO₃H₂)–OH**

1 g   120.00
5 g   478.00

N-α-Fmoc-O-phospho-L-tyrosine

NBC No.: 04-12-1125; CAS No.: 147762-53-6; $C_{24}H_{22}NO_8P$; M.W.: 483.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl₃:MeOH:AcOH 32% (5:3:1), purity: ≥ 95.00%.

EtOAc:nBuOH:AcOH:H₂O (2:1:1:1), purity: ≥ 95.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ **Prolonged storage**: ≤ -20°C; keep cool and dry.

A useful derivative for the synthesis of phosphotyrosine peptides [1-7]. Pyrophosphate formation has been noted in peptides containing adjacent Tyr(PO₃H₂) residues [4, 5]. An evaluation of the optimal coupling conditions for introduction of this residue has been made [6].

ⓘ Ⓖ 3.40, Ⓜ 3.39

[1]   E. A. Ottinger, et al. (1993) *Biochemistry*, **32**, 4354.
[2]   E. A. Ottinger, et al. (1995) *Int. J. Peptide Protein Res.*, **46**, 346.
[3]   F. Anjuere, et al. (1995) *Anal. Biochem.*, **229**, 61.
[4]   C. Garcia-Echeverria (1995) *Lett. Pept. Sci.*, **2**, 93.
[5]   R. M. Valerio, et al. (1995) *Lett. Pept. Sci.*, **2**, 33.
[6]   C. Garcia-Echeverria (1996) *Lett. Pept. Sci.*, **2**, 369.
[7]   L. F. Bonewald, et al. (1999) *J. Peptide Res.*, **53**, 161.

852071   **Fmoc–Tyr(PO(OBzl)OH)–OH**

1 g   185.00
5 g   750.00
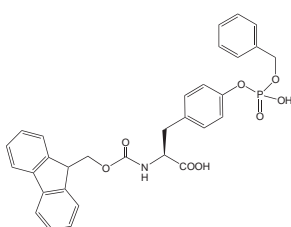
N-α-Fmoc-O-benzyl-L-phosphotyrosine

NBC No.: 04-12-1156; CAS No.: 191348-16-0; $C_{31}H_{28}NO_8P$; M.W.: 573.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl₃:MeOH:AcOH 32% (5:3:1), purity: ≥ 95.00%.

HPLC: purity: ≥ 95.0%.

EtOAc: ≤ 0.5%.

AcOH: ≤ 0.1%.

Optical purity: ≥ 98.5% L-enantiomer.

⚠ **Prolonged storage**: ≤ -20°C; keep cool and dry.

An excellent building block for the preparation of phosphotyrosine-containing peptides [1]. This derivative can be introduced using standard activation methods, such as PyBOP® and TBTU. Using this reagent, even peptides containing multiple phosphorylation sites have been prepared efficiently by standard Fmoc SPPS methods [1].

ⓘ Ⓖ 3.39, Ⓜ 3.39, Ⓐ 3.39

[1]   P. White & J. Beythien in "Innovations & Perspectives in Solid Phase Synthesis and Combinatorial Libraries, 4th International Symposium", Mayflower Scientific Ltd., Birmingham, 1996, pp. 557.

852246   **Fmoc–D–Tyr(PO(OBzl)OH)–OH**

1 g   325.00
5 g   1200.00

N-α-Fmoc-O-benzyl-D-phosphotyrosine

NBC No.: 04-13-1080; $C_{31}H_{28}NO_8P$; M.W.: 573.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl₃:MeOH:AcOH 32% (5:3:1), purity: ≥ 95.00%.

HPLC: purity: ≥ 90.00%.

⚠ **Prolonged storage**: ≤ -20°C; keep cool and dry.

See entry for 852071.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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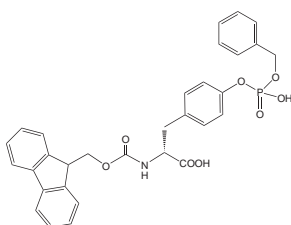
Product No.	Product	Quantity	Price
852090	**Fmoc–Tyr(PO(NMe$_2$)$_2$)–OH**	1 g	172.00
		5 g	686.00

N-α-Fmoc-O-(bis-dimethylamino-phosphono)-L-tyrosine

NBC No.: 04-12-1205; CAS No.: 172611-23-3; $C_{28}H_{32}N_3O_6P$; M.W.: 537.6

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 96.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

Fmoc-Tyr(PO(NMe$_2$)$_2$)-OH is a useful derivative for the synthesis of phosphotyrosine-containing peptides by Fmoc SPPS [1]. As the side-chain phosphate group is fully protected, this derivative is compatible with all coupling methods.

Regeneration of phosphotyrosine from the phosphodiamidate is effected by acid catalyzed hydrolysis using 90% aq. TFA.

ⓘ Ⓖ 3.40, Ⓐ 3.40

[1] H. G. Chao, et al. (1995) *J. Org. Chem.*, **60**, 7710.

852103	**Fmoc–Tyr(SO$_3$ · NnBu$_4$)–OH**	1 g	177.00
		5 g	707.00

N-α-Fmoc-O-sulfo-L-tyrosine tetrabutylammonium salt

NBC No.: 04-12-1251; CAS No.: 279668-32-5; $C_{40}H_{56}N_2O_8S$; M.W.: 724.9

Solubility: 1 mmole in 2 ml DMF clearly soluble.

HPLC: purity: ≥ 95.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep opened bottle under nitrogen; hygroscopic.

A useful derivative for the introduction of tyrosine sulfate residues in Fmoc SPPS [1]. The amino acid should be coupled using TBTU. To minimize loss of the sulfate group, the TFA cleavage reaction should be done at 0 °C [2].

[1] M. Ueki, et al. (2001) *Bioorg. Chem. Lett.*, **9**, 477.

[2] K. Kitagawa, et al. (2001) *J. Org. Chem.*, **66**, 1.

852347	**Fmoc–Tyr(SO$_3$nP)–OH** NEW	1 g	185.00
		5 g	750.00
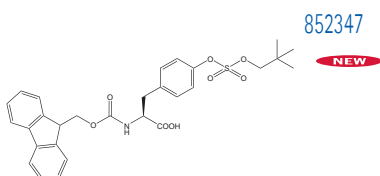
N-Fmoc-O-(2,2 dimethylpropylsulfo)-L-tyrosine

CAS No.: 878408-63-0; $C_{29}H_{31}NO_8S$; M.W.: 553.6

Solubility: 1 mmole in 4 ml DMF clearly soluble.

TLC: nBuOH:AcOH:H$_2$O (2:1:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

ⓘ Ⓖ 3.45, Ⓜ 3.45, Ⓐ 3.46

A novel derivative for the synthesis of sulfotyrosine-containing peptides by Fmoc SPPS. The sulfate neopentyl group is stable to TFA, and thus protects the sulfotyrosine residue from degradation during the cleavage reaction, but is easily cleaved post-cleavage by treatment of the peptide with aqueous ammonium acetate [1].

[1] L. S. Simpson & T. S. Widlanski (2006) *J. Am. Chem. Soc.*, **128**, 1605.

[2] L. S. Simpson, et al. (2009) *Chemistry & Biology*, **16**, 153.

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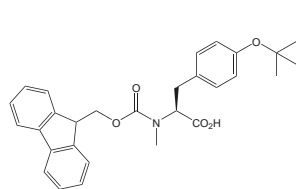
Product No.	Product	Quantity	Price
852332	**Fmoc–N–Me–Tyr(tBu)–OH**	250 mg	78.00
		1 g	234.00

N-α-Fmoc-N-α-methyl-O-tert-butyl-L-tyrosine
CAS No.: 133373-24-7 ; $C_{29}H_{31}NO_5$; M.W.: 473.6
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (85:10:5), purity: ≥ 98.00%.
HPLC: purity: ≥ 98.00%.
Optical purity: ≥ 99.50% L-enantiomer.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Valine [Val, V]

852021	**Fmoc–Val–OH**	25 g	25.00
		100 g	50.00
		250 g	110.00
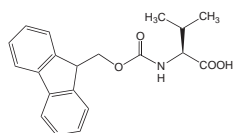
N-α-Fmoc-L-valine
NBC No.: 04-12-1039; CAS No.: 68858-20-8; $C_{20}H_{21}NO_4$; M.W.: 339.4
Solubility: 25 mmole in 50 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98%.
HPLC: purity: ≥ 99.0%.
Fmoc-β-Ala-OH ≤ 0.1%.
Fmoc-β-Ala-Val-OH ≤ 0.1%.
Fmoc-Val-Val-OH ≤ 0.1%.
Free amino acid: ≤ 0.2%.
EtOAc: ≤ 0.5%.
AcOH: ≤ 0.02%.
Optical purity: ≥ 99.8% L-enantiomer.
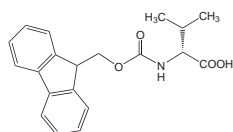
852152	**Fmoc–D–Val–OH**	5 g	50.00
		25 g	200.00

N-α-Fmoc-D-valine
NBC No.: 04-13-1039; CAS No.: 84624-17-9; $C_{20}H_{21}NO_4$; M.W.: 339.4
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 99.00%.
Optical purity: ≥ 99.50% D-enantiomer.
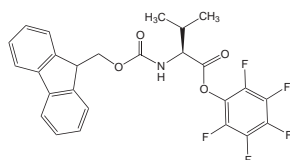
852228	**Fmoc–Val–OPfp**	5 g	82.00

N-α-Fmoc-L-valine pentafluorophenyl ester
NBC No.: 04-12-1520; CAS No.: 86060-87-9; $C_{26}H_{20}NO_4F_5$; M.W.: 505.5
Solubility: 0.5 mmole in 3 ml DMF clearly soluble.
TLC: CH_3CN:$CHCl_3$ (1 : 3), purity: ≥ 2.00%.
HPLC: purity: ≥ 98.00%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

⚠ Storage conditions　Ⓖ Additional information　Ⓜ Method information　Ⓐ Application information

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Product No.	Product	Quantity	Price
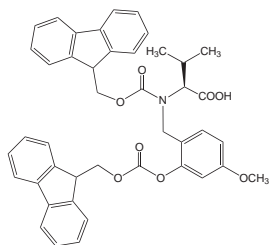
852063   **Fmoc-(FmocHmb)Val-OH**   1 g   295.00

N-α-Fmoc-N-α-(2-Fmoc-oxy-4-methoxybenzyl)-L-valine   5 g   990.00

NBC No.: 04-12-1134; CAS No.: 148515-86-0; C$_{43}$H$_{39}$NO$_8$; M.W.: 697.3

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 95.00%.

CHCl$_3$:MeOH:AcOH:H$_2$O (85:13:0.5:1.5), purity: ≥ 95.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry.

Hmb protection of amide bonds has been shown to inhibit aggregation of "difficult" peptides, thereby leading to products of increased purity [1-6]. Retention of Hmb groups on the cleaved peptide can greatly improve the Solubility of protected peptide fragments [7, 8] and otherwise intractable sequences [9-11]. Furthermore, using a Hmb protected derivative for incorporation of the residue linked to the carboxyl group of Asp or Asn residues has been found to suppress formation of aspartimide and piperidide related by-products [12-14]. For a comparison of the efficiency of Hmb and pseudoprolines in preventing aggregation, see [15].

ⓘ Ⓖ 3.9, 3.18

[1]   T. Johnson, et al. (1993) *J. Chem. Soc., Chem. Commun.*, 369.
[2]   C. Hyde, et al. (1994) *Int. J. Peptide Protein Res.*, **43**, 431.
[3]   L. C. Packman, et al. (1994) *Pept. Res.*, **7**, 125.
[4]   T. Johnson, et al. (1994) *Tetrahedron Lett.*, **35**, 463.
[5]   R. G. Simmonds (1996) *Int. J. Peptide Protein Res.*, **47**, 36.
[6]   T. Johnson, et al. (1995) *Lett. Pept. Sci.*, **1**, 11.
[7]   M. Quibell, et al. (1995) *J. Am. Chem. Soc.*, **117**, 11656.
[8]   M. Quibell, et al. (1996) *J. Chem. Soc., Perkin Trans. 1*, 1227.
[9]   M. Quibell, et al. (1994) *Tetrahedron Lett.*, **35**, 2237.
[10] M. Quibell, et al. (1994) *J. Org. Chem.*, **59**, 1745.
[11] M. Quibell, et al. (1995) *J. Chem. Soc., Perkin Trans. 1*, 2019.
[12] M. Quibell, et al. (1994) *J. Chem. Soc., Chem. Commun.*, 2343.
[13] L. C. Packman (1995) *Tetrahedron Lett.*, **36**, 7523.
[14] J. Offer, et al. (1996) *J. Chem. Soc., Perkin Trans. 1*, 175.
[15] W. R. Sampson, et al. (1999) *J. Peptide Sci.*, **5**, 403.

852230   **Fmoc-N-Me-Val-OH**   1 g   72.00

N-α-Fmoc-N-α-methy-L-valine   5 g   280.00

NBC No.: 04-12-9026; CAS No.: 84000-11-3; C$_{21}$H$_{23}$NO$_4$; M.W.: 353.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CHCl$_3$:MeOH:AcOH:H$_2$O (90:10:0.5:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

Optical purity: ≥ 99.50% L-enantiomer.

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| Product No. | Product | Quantity | Price |

Pseudoproline (oxazolidine) dipeptides

Mutter's pseudoproline dipeptides [1, 2] are undoubtedly the most powerful tools described to date for enhancing synthetic efficiency in Fmoc SPPS. They have proven particularly effective in the synthesis of intractable peptides [3 - 6], long peptides/small proteins [7 -15], and cyclic peptides [16, 17], enabling in many cases the production of peptides that otherwise could not be made. The insertion of a pseudop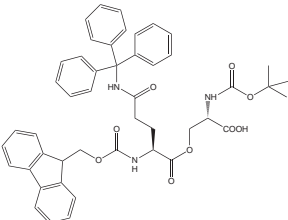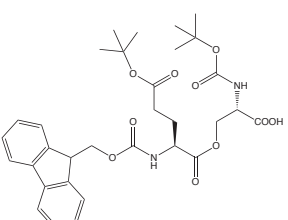roline into a sequence disrupts the formation of the secondary structures thought responsible for problems during peptide assembly, leading to better and more predictable acylation and deprotection kinetics, improved yields, purities and solubilities of crude products, and easier HPLC purification with higher product return. Since the pseudoproline unit is stable to AcOH/TFE/DCM, peptides prepared on 2–chlorotrityl and NovaSyn® TGT resins can be isolated with the pseudoproline moiety still in place [2]; this can be particularly advantageous when preparing peptides for use in fragment condensation reactions, as peptides containing pseudoproline residues often exhibit markedly improved solubility properties. Furthermore, the confirmation that peptides containing a C-terminal pseudoproline residue can be coupled without risk of epimerization has been reported [18].

Insertion of pseudoproline close to vulnerable Asp(OAll) residues has been recently shown to significantly reduce aspartimide during the synthesis of glycopeptides by Landsbury aspartylation [19, 20].

⚠ Prolonged storage: +2 to +8 °C; keep cool and dry.

ⓘ Ⓖ 3.9, 3.10, Ⓜ 3.10

[1]   M. Mutter, et al. (1995) *Pept. Res.*, **8**, 145.
[2]   T. Haack & M. Mutter (1992) *Tetrahedron Lett.*, **33**, 1589.
[3]   P. White in "Peptides 1998, Proc. 25th European Peptide Symposium", S. Bajusz & F. Hudecz (Eds), Budapest, Akadémiai Kiadó, 1999, pp. 120.
[4]   W. R. Sampson, et al. (1999) *J. Peptide Sci.*, **5**, 403.
[5]   K. Page, et al. (2007) *J. Pept. Sci.*, **13**, 833.
[6]   M. Pudelko, et al. (2007) *J. Pept. Sci.*, **13**, 354.
[7]   P. White, et al. (2004) *J. Pept. Sci.*, **10**, 18.
[8]   F. García-Martín, et al. (2006) *Bipolymers*, **84**, 566.
[9]   S. Abu-Baker & G. A. Lorigan (2006) *Biochemistry*, **45**, 13312.
[10] F. Shabanpoor, et al. (2007) *J. Pept. Sci.*, **13**, 113.
[11] V. Goncalves, et al. (2009) *J. Pept. Sci.*, **15**, 422.
[12] F. El Oualid, et al. (2010) *Angew. Chem. Int. Ed.*, **49**, 10149.
[13] S. N. Bavikar, et al. (2012) *Angew. Chem. Int. Ed.*, **51**, 758.
[14] P. Nagorny, et al. (2012) Angew. Chem. Int. *Ed.*, **51**, 975.
[15] C. Yves-Marie, et al. (2010) *J. Pept. Sci.*, **16**, 98.
[16] N. Schmiedeberg & H. Kessler (2002) *Org. Lett.*, **4**, 59.
[17] B. van Lierop, et al. (2010) *Int. J. Pept. Res. Ther.*, **16**, 133.
[18] C. Heinlein, et al. (2011) *Angew. Chem. Int. Ed.*, **50**, 6406.
[19] P. Wang, et al. (2012) *Angew. Chem. Int Ed.*, **51**, 11571.
[20] V. Ullmann (2012) *Angew. Chem. Int. Ed.*, **51**, 11566.
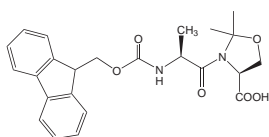
852175   **Fmoc-Ala-Ser(ψ^{Me,Me}pro)–OH**   1 g   50.00
NBC No.: 05-20-1000; CAS No.: 252554-78-2; $C_{24}H_{26}N_2O_6$; M.W.: 438.4   5 g   200.00
TLC: CHCl₃MeOH:AcOH:H₂O (90:10:0.5:1), purity: ≥ 97%.
CHCl₃:MeOH:AcOH 32% (15:4:1), purity: ≥ 97%.
HPLC: purity: ≥ 97.0%.
single impurities ≤ 1.0%.
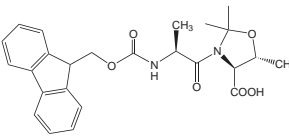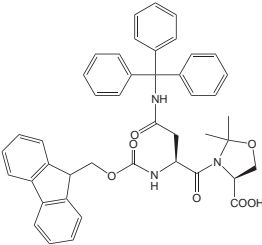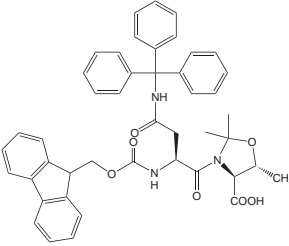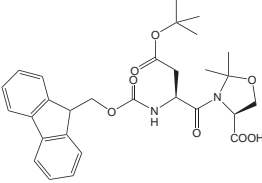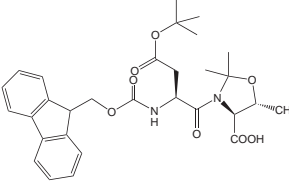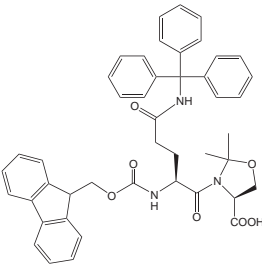
⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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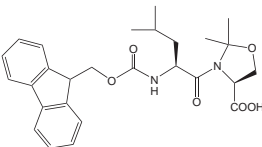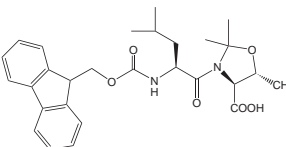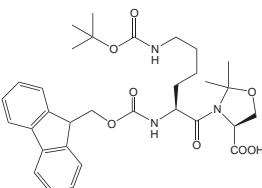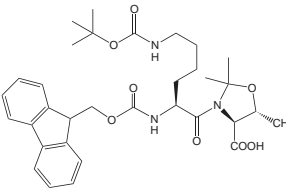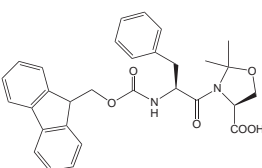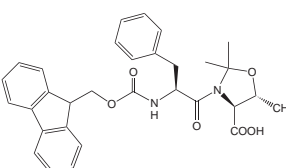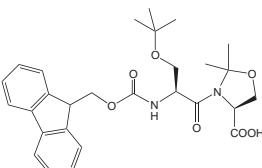
Product No.	Product	Quantity	Price

852180   Fmoc-Ala-Thr($\psi^{Me,Me}$pro)-OH   1 g   50.00   5 g   200.00

NBC No.: 05-20-1005; CAS No.: 252554-79-3; $C_{25}H_{28}N_2O_6$; M.W.: 452.5

TLC: $CHCl_3MeOH:AcOH:H_2O$ (90:10:0.5:1), purity: ≥ 97%.

$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97%.

HPLC: purity: ≥ 97.0%.

single impurities ≤ 1.0%.

852185   Fmoc-Asn(Trt)-Ser($\psi^{Me,Me}$pro)-OH   1 g   60.00   5 g   240.00

NBC No.: 05-20-1010; CAS No.: 957780-59-5; $C_{44}H_{41}N_3O_7$; M.W.: 723.8

TLC: $CHCl_3MeOH:AcOH:H_2O$ (90:10:0.5:1), purity: ≥ 95%.

$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 95%.

HPLC: purity: ≥ 97.0%.

852183   Fmoc-Asn(Trt)-Thr($\psi^{Me,Me}$pro)-OH   1 g   60.00   5 g   240.00

NBC No.: 05-20-1008; CAS No.: 957780-59-5; $C_{45}H_{43}N_3O_7$; M.W.: 737.8

TLC: $CHCl_3MeOH:AcOH:H_2O$ (85:13:0.5:1.5), purity: ≥ 97%.

$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97%.

HPLC: purity: ≥ 97.0%.

single impurities ≤ 1.0%.

852186   Fmoc-Asp(OtBu)-Ser($\psi^{Me,Me}$pro)-OH   1 g   60.00   5 g   240.00

NBC No.: 05-20-1011; CAS No.: 955048-92-7; $C_{29}H_{34}N_2O_8$; M.W.: 538.2

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97%.

$CHCl_3MeOH:AcOH:H_2O$ (90:10:0.5:1), purity: ≥ 97%.

HPLC: purity: ≥ 97.0%.

single impurities ≤ 1.0%.

852199   Fmoc-Asp(OtBu)-Thr($\psi^{Me,Me}$pro)-OH   1 g   60.00   5 g   240.00

NBC No.: 05-20-1126; CAS No.: 920519-32-0; $C_{30}H_{36}N_2O_8$; M.W.: 552.2

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 95%.

HPLC: purity: ≥ 97.0%.

single impurities ≤ 1.0%.

852190   Fmoc-Gln(Trt)-Ser($\psi^{Me,Me}$pro)-OH   1 g   60.00   5 g   240.00

NBC No.: 05-20-1115; $C_{45}H_{43}N_3O_7$; M.W.: 737.8

TLC: $CHCl_3MeOH:AcOH:H_2O$ (90:10:0.5:1), purity: ≥ 95%.

$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 95%.

HPLC: purity: ≥ 97.0%.

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Product No.	Product	Quantity	Price
852198	**Fmoc-Gln(Trt)-Thr($\psi^{Me,Me}$pro)-OH** NBC No.: 05-20-1125; $C_{46}H_{45}N_3O_7$; M.W.: 751.8 TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 97%. $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97%. HPLC: purity: ≥ 97.0%. single impurities ≤ 1.0%.	1 g 5 g	60.00 240.00
852177	**Fmoc-Glu(OtBu)-Ser($\psi^{Me,Me}$pro)-OH** NBC No.: 05-20-1002; CAS No.: 909115-33-9; $C_{30}H_{36}N_2O_8$; M.W.: 552.6 TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 97%. $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97%. HPLC: purity: ≥ 97.0%.	1 g 5 g	60.00 240.00
852196	**Fmoc-Glu(OtBu)-Thr($\psi^{Me,Me}$pro)-OH** NBC No.: 05-20-1122; CAS No.: 957780-56-2; $C_{31}H_{38}N_2O_8$; M.W.: 566.6 TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 97%. HPLC: purity: ≥ 97.0%. single impurities ≤ 1.5%.	1 g 5 g	60.00 240.00
852200	**Fmoc-Gly-Ser($\psi^{Me,Me}$pro)-OH** NBC No.: 05-20-1127; $C_{23}H_{24}N_2O_6$; M.W.: 424.4 TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97%. HPLC: purity: ≥ 97.0%. single impurities ≤ 1.0%.	1 g 5 g	50.00 200.00
852197	**Fmoc-Gly-Thr($\psi^{Me,Me}$pro)-OH** NBC No.: 05-20-1124; $C_{24}H_{26}N_2O_6$; M.W.: 438.4 TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 97%. $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97%. HPLC: purity: ≥ 97.0%.	1 g 5 g	50.00 200.00
852194	**Fmoc-Ile-Ser($\psi^{Me,Me}$pro)-OH** NBC No.: 05-20-1119; CAS No.: 1147996-34-6; $C_{27}H_{32}N_2O_6$; M.W.: 480.6 TLC: $CHCl_3$MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 97%. $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97%. HPLC: purity: ≥ 96.0%.	1 g 5 g	50.00 200.00
852193	**Fmoc-Ile-Thr($\psi^{Me,Me}$pro)-OH** NBC No.: 05-20-1118; CAS No.: 957780-52-8; $C_{28}H_{34}N_2O_6$; M.W.: 494.6 TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 97%. HPLC: purity: ≥ 97.0%. single impurities ≤ 1.0%.	1 g 5 g	50.00 200.00

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⚠ Storage conditions   **G** Additional information   **M** Method information   **A** Application information

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Product No.	Product	Quantity	Price
852179	Fmoc-Leu-Ser($\psi^{Me,Me}$pro)-OH	1 g	50.00
	NBC No.: 05-20-1004; CAS No.: 339531-50-9; $C_{27}H_{32}N_2O_6$; M.W.: 480.5	5 g	200.00
	TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 97%.		
	$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97%.		
	HPLC: purity: ≥ 97.0%.		
	single impurities ≤ 1.2%.		
852184	Fmoc-Leu-Thr($\psi^{Me,Me}$pro)-OH	1 g	50.00
	NBC No.: 05-20-1009; CAS No.: 955048-89-2; $C_{28}H_{34}N_2O_6$; M.W.: 494.5	5 g	200.00
	TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97%.		
	$CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 97%.		
	HPLC: purity: ≥ 97.0%.		
	single impurities ≤ 2.0%.		
852178	Fmoc-Lys(Boc)-Ser($\psi^{Me,Me}$pro)-OH	1 g	60.00
	NBC No.: 05-20-1003; CAS No.: 957780-54-0; $C_{32}H_{41}N_3O_8$; M.W.: 595.7	5 g	240.00
	TLC: $CHCl_3$MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 97%.		
	HPLC: purity: ≥ 97.0%.		
852191	Fmoc-Lys(Boc)-Thr($\psi^{Me,Me}$pro)-OH	1 g	60.00
	NBC No.: 05-20-1116; CAS No.: 911838-56-7; $C_{33}H_{43}N_3O_8$; M.W.: 609.7	5 g	240.00
	TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 97%.		
	HPLC: purity: ≥ 97.0%.		
	single impurities ≤ 1.0%.		
852195	Fmoc-Phe-Ser($\psi^{Me,Me}$pro)-OH	1 g	50.00
	NBC No.: 05-20-1121; CAS No.: 878797-01-4; $C_{30}H_{30}N_2O_6$; M.W.: 514.6	5 g	200.00
	TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 97%.		
	$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97.00%.		
	HPLC: purity: ≥ 97.0%.		
	single impurities ≤ 1.0%.		
852201	Fmoc-Phe-Thr($\psi^{Me,Me}$pro)-OH	1 g	50.00
	NBC No.: 05-20-1128; CAS No.: 1196703-48-6; $C_{31}H_{32}N_2O_6$; M.W.: 528.6	5 g	200.00
	TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 97%.		
	HPLC: purity: ≥ 97.0%.		
852187	Fmoc-Ser(tBu)-Ser($\psi^{Me,Me}$pro)-OH	1 g	60.00
	NBC No.: 05-20-1012; CAS No.: 1000164-43-1; $C_{28}H_{34}N_2O_7$; M.W.: 510.6	5 g	240.00
	TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 97%.		
	$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97%.		
	HPLC: purity: ≥ 97.0%.		
	single impurities ≤ 1.2%.		

Product No.	Product	Quantity	Price

852192 **Fmoc-Ser(tBu)-Thr($\psi^{Me,Me}$pro)-OH**

NBC No.: 05-20-1117; $C_{29}H_{36}N_2O_7$; M.W.: 524.6

TLC: $CHCl_3MeOH:AcOH:H_2O$ (90:10:0.5:1), purity: ≥ 95%.

HPLC: purity: ≥ 92.0%.

1 g 60.00
5 g 240.00

852202 **Fmoc-Trp(Boc)-Ser($\psi^{Me,Me}$pro)-OH**

NBC No.: 05-20-1130; $C_{37}H_{39}N_3O_8$; M.W.: 653.7

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97%.

$CHCl_3MeOH:AcOH:H_2O$ (90:10:0.5:1), purity: ≥ 97%.

HPLC: purity: ≥ 97.0%.

single impurities ≤ 1.0%.

1 g 60.00
5 g 240.00

852188 **Fmoc-Trp(Boc)-Thr($\psi^{Me,Me}$pro)-OH**

NBC No.: 05-20-1013; CAS No.: 936707-21-0; $C_{38}H_{41}N_3O_8$; M.W.: 667.8

TLC: $CHCl_3MeOH:AcOH:H_2O$ (85:13:0.5:1.5), purity: ≥ 97%.

$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97%.

HPLC: purity: ≥ 92.0%.

1 g 60.00
5 g 240.00

852189 **Fmoc-Tyr(tBu)-Ser($\psi^{Me,Me}$pro)-OH**

NBC No.: 05-20-1014; CAS No.: 878797-09-2; $C_{34}H_{38}N_2O_7$; M.W.: 586.7

TLC: $CHCl_3MeOH:AcOH:H_2O$ (90:10:0.5:1), purity: ≥ 95%.

$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 95%.

HPLC: purity: ≥ 97.0%.

1 g 60.00
5 g 240.00

852182 **Fmoc-Tyr(tBu)-Thr($\psi^{Me,Me}$pro)-OH**

NBC No.: 05-20-1007; CAS No.: 920519-31-9; $C_{35}H_{40}N_2O_7$; M.W.: 600.7

TLC: $CHCl_3MeOH:AcOH:H_2O$ (90:10:0.5:1), purity: ≥ 97%.

$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97%.

HPLC: purity: ≥ 97.0%.

single impurities ≤ 1.0%.

1 g 60.00
5 g 240.00

852176 **Fmoc-Val-Ser($\psi^{Me,Me}$pro)-OH**

NBC No.: 05-20-1001; CAS No.: 186023-49-4; $C_{26}H_{30}N_2O_6$; M.W.: 466.5

TLC: $CHCl_3MeOH:AcOH:H_2O$ (90:10:0.5:1), purity: ≥ 97%.

$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97%.

HPLC: purity: ≥ 97.0%.

single impurities ≤ 1.0%.

1 g 50.00
5 g 200.00

852181 **Fmoc-Val-Thr($\psi^{Me,Me}$pro)-OH**

NBC No.: 05-20-1006; CAS No.: 168216-05-5; $C_{27}H_{32}N_2O_6$; M.W.: 480.5

TLC: $CHCl_3MeOH:AcOH:H_2O$ (90:10:0.5:1), purity: ≥ 97%.

$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97%.

HPLC: purity: ≥ 97.0%.

1 g 50.00
5 g 200.00

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Dmb & Hmb amino acids and dipeptides

Hmb-amino acids

Incorporation of an Hmb protected amino acid into a peptide chain represents one of the most efficient ways of overcoming the effects of aggregation during peptide chain assembly [1 - 6]. The effects are long-range, with onset of aggregation often being postponed for as many as six residues.

Incorporation of the Hmb derivative can be effected using normal methods of amide-bond formation. Coupling of the following residue is best achieved using the appropriate symmetrical anhydride in DCM.

The Hmb group is removed during the course of the standard TFA-mediated cleavage and deprotection reaction. For applications, see references [7 - 18].

ⓘ 3.9, 3.18

[1] T. Johnson, et al. (1993) *J. Chem. Soc., Chem. Commun.*, 369.
[2] C. Hyde, et al. (1994) *Int. J. Peptide Protein Res.*, **43**, 431.
[3] L. C. Packman, et al. (1994) *Pept. Res.*, **7**, 125.
[4] T. Johnson, et al. (1994) *Tetrahedron Lett.*, **35**, 463.
[5] R. G. Simmonds (1996) *Int. J. Peptide Protein Res.*, **47**, 36.
[6] T. Johnson, et al. (1995) *Lett. Pept. Sci.*, **1**, 11.
[7] M. Quibell, et al. (1995) *J. Am. Chem. Soc.*, **117**, 11656.
[8] M. Quibell, et al. (1996) *J. Chem. Soc., Perkin Trans. 1*, 1227.
[9] M. Quibell, et al. (1994) *Tetrahedron Lett.*, **35**, 2237.
[10] M. Quibell, et al. (1994) *J. Org. Chem.*, **59**, 1745.
[11] M. Quibell, et al. (1995) *J. Chem. Soc., Perkin Trans. 1*, 2019.
[12] M. Quibell, et al. (1994) *J. Chem. Soc., Chem. Commun.*, 2343.
[13] L. C. Packman (1995) *Tetrahedron Lett.*, **36**, 7523.
[14] J. Offer, et al. (1996) *J. Chem. Soc., Perkin Trans. 1*, 175.
[15] M. Quibell & T. Johnson in "Fmoc solid phase peptide synthesis - a practical approach", W. C. Chan & P. D. White (Eds), Oxford, Oxford University Press, 2000, pp. 115.
[16] A. B. Clippingdale, et al. (1999) *J. Peptide Res.*, **53**, 665.
[17] W. R. Sampson, et al. (1999) *J. Peptide Sci.*, **5**, 403.
[18] M. N. Mathieu, et al. (2001) *J. Peptide Res.*, **57**, 374.

852060	**Fmoc-(FmocHmb)Ala-OH**	1 g	295.00
	N-α-Fmoc-N-α-(2-Fmoc-oxy-4-methoxybenzyl)-L-alanine	5 g	990.00

NBC No.: 04-12-1127; CAS No.: 148515-85-9; $C_{41}H_{35}NO_8$; M.W.: 669.7

solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98%.

$CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98%.

HPLC: purity: ≥ 95.0%.

Optical purity: ≥ 99.5% L-enantiomer.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

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Product No.	Product	Quantity	Price

852064   **Fmoc-(FmocHmb)Gly-OH**   1 g   295.00

N-α-Fmoc-N-α-(2-Fmoc-oxy-4-methoxybenzyl)-glycine   5 g   990.00

NBC No.: 04-12-1135; CAS No.: 148515-84-8; $C_{40}H_{33}NO_8$; M.W.: 655.7

solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3MeOH:AcOH:H_2O$ (90:10:0.5:1), purity: ≥ 98%.

$CHCl_3MeOH:AcOH:H_2O$ (85:13:0.5:1.5), purity: ≥ 98%.

HPLC: purity: ≥ 98.0%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

852061   **Fmoc-(FmocHmb)Leu-OH**   1 g   295.00

N-α-Fmoc-N-α-(2-Fmoc-oxy-4-methoxybenzyl)-L-leucine   5 g   990.00

NBC No.: 04-12-1129; CAS No.: 148515-87-1; $C_{44}H_{41}NO_8$; M.W.: 711.8

solubility: 1 mmole in 2 ml DCM.

TLC: $CHCl_3:MeOH:AcOH$ (90:8:2), purity: ≥ 95%.

HPLC: purity: ≥ 96.0%.

Optical purity: ≥ 99.5% L-enantiomer.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

852068   **Fmoc-(FmocHmb)Lys(Boc)-OH**   1 g   295.00

N-α-Fmoc-N-α-(2-Fmoc-oxy-4-methoxybenzyl)-N-ε-t.-butoxycarbonyl-L-   5 g   990.00

lysine

NBC No.: 04-12-1148; CAS No.: 166881-56-7; $C_{49}H_{50}N_2O_{10}$; M.W.: 826.9

solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3MeOH:AcOH:H_2O$ (90:10:0.5:1), purity: ≥ 98%.

$CHCl_3MeOH:AcOH:H_2O$ (85:13:0.5:1.5), purity: ≥ 98%.

HPLC: purity: ≥ 97.0%.

Optical purity: ≥ 99.5% L-enantiomer.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

852081   **Fmoc-(FmocHmb)Phe-OH**   1 g   295.00

N-α-Fmoc-N-α-(2-Fmoc-oxy-4-methoxybenzyl)-L-phenylalanine   5 g   990.00

NBC No.: 04-12-1187; CAS No.: 148515-88-2; $C_{47}H_{39}NO_8$; M.W.: 745.8

solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3MeOH:AcOH:H_2O$ (90:10:0.5:1), purity: ≥ 95%.

$CHCl_3MeOH:AcOH:H_2O$ (85:13:0.5:1.5), purity: ≥ 95%.

HPLC: purity: ≥ 97.0%.

Optical purity: ≥ 99.0% L-enantiomer.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

DMB & HMB AMINO ACIDS & DIPEPTIDES

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
852063	**Fmoc-(FmocHmb)Val-OH**	1 g	295.00
	N-α-Fmoc-N-α-(2-Fmoc-oxy-4-methoxybenzyl)-L-valine	5 g	990.00
	NBC No.: 04-12-1134; CAS No.: 148515-86-0; $C_{43}H_{39}NO_8$; M.W.: 697.3		

solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 95%.

$CHCl_3$MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 95%.

HPLC: purity: ≥ 95.0%.

Optical purity: ≥ 99.5% L-enantiomer.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

customer service: service@novabiochem.com   technical service: technical@novabiochem.com internet: novabiochem.com
Bulk quantities available, please enquire

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Product No.	Product	Quantity	Price

Dmb-amino acids
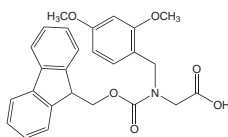
852110	**Fmoc–(Dmb)Gly–OH**	1 g	95.00
	N-α-Fmoc-N-α-(2, 4-dimethoxybenzyl)-glycine	5 g	350.00

NBC No.: 04-12-1268; CAS No.: 166881-42-1; $C_{26}H_{25}NO_6$; M.W.: 447.5

solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3MeOH:AcOH:H_2O$ (85:13:0.5:1.5), purity: ≥ 98%.

HPLC: purity: ≥ 99.0%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

An excellent alternative to Fmoc-(FmocHmb)Gly-OH for those situations where the unprotected hydroxyl of the Hmb group can cause problems, such as in post-synthetic phosphorylation [1] and depsipeptide synthesis.

Furthermore, it can prevent aspartimide formation when used to introduce a Gly immediately before an Asp residue and help promote cyclization of Gly-containing peptides [2]. This derivative has been used in the synthesis of insulin-like peptide 5 [3] and 101 residues of d2 domain VEGF receptor [4].

ⓘ Ⓖ 3.18

[1] T. Johnson, et al. (1996) *J. Chem. Soc., Perkin Trans. 1*, 719.
[2] M. El Haddadi, et al. (2000) *J. Pept. Sci.*, **6**, 560.
[3] M. A. Hossain, et. al. (2008) *ChemBioChem*, **9**, 1816.
[4] V. Goncalves, et al. (2009) *J. Pept. Sci.*, **15**, 417.

DMB & HMB AMINO ACIDS & DIPEPTIDES

⚠ Storage conditions　　　Ⓖ Additional information　　　Ⓜ Method information　　　Ⓐ Application information

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Product No.	Product	Quantity	Price

Dmb-dipeptides

Dmb dipeptides are extremely powerful tools for enhancing synthetic efficiency in Fmoc SPPS of intractable peptides, long peptides and cyclic peptides [1 - 4]. They work in exactly the same way as pseudoproline dipeptides by exploiting the "proline effect" to disrupt the formation of the secondary structures during peptide assembly. The results are better and more predictable acylation and deprotection kinetics, enhanced reaction rates, and improved yields, purities and solubilities of crude products. In addition, Asp-(Dmb)Gly provides complete protection against aspartimide formation during Fmoc SPPS [1].

Unlike Hmb dipeptides, these derivatives cannot form lactones and so can be introduced using standard coupling methods like PyBOP®/DIPEA or DIPCDI/HOBt.

(i) 3.14

[1]   V. Cardona, et al. (2008) *Int. J. Pept. Res. Ther.*, **14**, 285.
[2]   V. Goncalves, et al. (2009) *J. Pept. Sci.*, **15**, 417.
[3]   F. El Qualid, et al. (2010) *Angew. Chem. Int. Ed.*, **49**, 10149.
[4]   A. De Jong, et al. (2012) *ChemBioChem*, **13**, 2251.
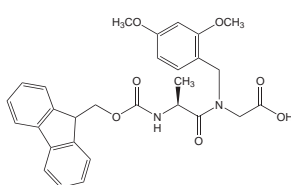
852108   **Fmoc-Ala-(Dmb)Gly-OH**   1 g   165.00

N-α-Fmoc-L-alanyl-N-α-(2, 4-dimethoxybenzyl)-glycine   5 g   680.00

NBC No.: 04-12-1265; CAS No.: 1188402-17-6; $C_{29}H_{30}N_2O_7$; M.W.: 518.6

solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 97%.

HPLC: purity: ≥ 97.0%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
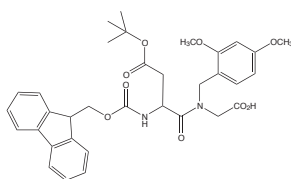
852115   **Fmoc-Asp(OtBu)-(Dmb)Gly-OH**   1 g   165.00

N-α-Fmoc-β-O-t.-butyl-L-aspartyl-N-α-(2,4-dimethoxybenzyl)-glycine   5 g   680.00

NBC No.: 04-12-1282; CAS No.: 900152-72-9; $C_{34}H_{38}N_2O_9$; M.W.: 618.7

solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98%.

HPLC: purity: ≥ 98.0%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Fmoc-Asp(OtBu)-(Dmb)Gly-OH offers the complete protection against aspartimide formation during Fmoc SPPS [1], in addition to the aggregation disrupting effects of Dmb-dipeptides. The inclusion of Asp-(Dmb)Gly has facilitated the synthesis of a number of long and complex peptides [2 -6].

(i) 3.14,, 3.23, 3.15

[2]   F. El Qualid, et al. (2010) *Angew. Chem. Int. Ed.*, **49**, 10149.
[3]   A. De Jong, et al. (2012) *ChemBioChem*, **13**, 2251.
[4]   M. Haj-Yahya, et al. (2012) *Angew. Chem., Int Ed.*, **51**, 11535.
[5]   V. R. Pattabiraman, et al. (2012) *Angew. Chem. Int. Ed.*, **51**, 5114.
[6]   C. Chamorro, et al. (2009) *Chem. Commun.*, 821.

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Product No.	Product	Quantity	Price

852109 Fmoc-Gly-(Dmb)Gly-OH

N-α-Fmoc-L-glycyl-N-α-(2, 4-dimethoxybenzyl)-glycine

NBC No.: 04-12-1266; CAS No.: 848861-65-4; $C_{28}H_{28}N_2O_7$; M.W.: 504.53

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98%.

HPLC: purity: ≥ 95.0%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

The use of this derivative was found to be essential for the synthesis of peptides related to nucleolin [1].

Fmoc-Aaa-(Dmb)Gly-OH dipeptides offer the same benefits as pseudoproline dipeptides but for peptide sequences containing Gly. They are extremely easy to use. Standard coupling methods like PyBOP®/DIPEA or DIPCDI/HOBt can be used for their introduction. Removal of the Dmb group and regeneration of the glycine residue occurs during the course of standard TFA-mediated cleavage reaction.

[1] S. Zahariev, et al. (2005) *J. Pept. Sci.*, 11, 17.

| | | 1 g | 165.00 |
| | | 5 g | 680.00 |

852114 Fmoc-Ile-(Dmb)Gly-OH

N-α-Fmoc-L-Isoleucyl-N-α-(2,4-dimethoxybenzyl)-glycine

NBC No.: 04-12-1280; $C_{32}H_{36}N_2O_7$; M.W.: 560.6

solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98%.

HPLC: purity: ≥ 97.5%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

| | | 1 g | 165.00 |
| | | 5 g | 680.00 |

852121 Fmoc-Leu-(Dmb)Gly-OH

N-α-Fmoc-L-leucyl-N-α-(2,4-dimethoxybenzyl)-glycine

NBC No.: 04-12-1294; $C_{32}H_{36}N_2O_7$; M.W.: 560.6

solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98%.

HPLC: purity: ≥ 97.5%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

| | | 1 g | 165.00 |
| | | 5 g | 680.00 |

852116 Fmoc-Val-(Dmb)Gly-OH

N-α-Fmoc-L-valyl-N-α-(2,4-dimethoxybenzyl)-glycine

NBC No.: 04-12-1283; $C_{30}H_{32}N_2O_7$; M.W.: 532.58

solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98%.

HPLC: purity: ≥ 97.0%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

| | | 1 g | 165.00 |
| | | 5 g | 680.00 |

DMB & HMB AMINO ACIDS & DIPEPTIDES

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Isoacyl dipeptides

Isoacyl dipeptides are remarkable new tools for enhancing synthetic efficiency in Fmoc SPPS that consist of a Boc-protected serine or threonine derivative in which the β-hydroxyl group is acylated by an Fmoc-amino acid [1 - 3]. They offer the same benefits as pseudoproline dipeptides [4 - 8], but with the added advantage that the depsipeptides obtained directly from the TFA cleavage reaction are often markedly more soluble than the native target sequence. This property allows insoluble aggregated sequences such as β-amyloid to be purified in the soluble isoacyl form prior to conversion to the insoluble native form [7].

Isoacyl dipeptides can also be used to prevent epimerization during fragment coupling [9, 11] and cyclization [12, 13].

Isoacyl dipeptides are best coupled using DIPCDI/HOBt in DCM [10, 11]. Applications of isoacyl dipeptides have been recently reviewed [14].

Ⓖ 3.19, Ⓜ 3.20

[1] Y. Sohma, et al. (2006) *Tetrahedron Lett.*, **47**, 3013.
[2] I. Coin, et al. (2006) *J. Org. Chem.*, **71**, 6171.
[3] T. Yoshiya, et al. (2007) *Org. Biomol. Chem.*, **5**, 1720.
[4] Y. Sohma, et al. (2004) *Biopolymers*, **76**, 344.
[5] M. Mutter, et al. (2004) *Angew. Chem. Int. Ed.*, **43**, 4172.
[6] L. A. Carpino, et al. (2004) *Tetrahedron Lett.*, **45**, 7519.
[7] Y. Sohma, et al. (2005) *J. Pept. Sci.*, **11**, 441.
[8] Y. Sohma & Y. Kiso (2006) *ChemBioChem*, **7**, 1549.
[9] Y. Yoshiya, et al. (2006) *Tetrahedron Lett.*, **47**, 7905.
[10] I. Coin, et al. (2008) *J. Pept. Sci.*, **14**, 299.
[11] J. Lécaillon, et al. (2008) *Tetrahedron Lett.*, **49**, 4674.
[12] T. Yoshiya, et al. (2010) *J. Pept. Sci.*, **16**, 437.
[13] A. Taniguchi, et al. (2007) *J. Pept. Sci.*, **13**, 868.
[14] I. Coin (2010) *J. Pept. Sci.*, **16**, 223.
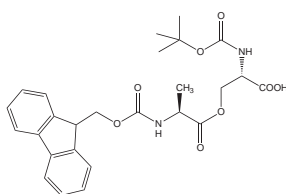
852174 **Boc-Ser(Fmoc-Ala)–OH** 1 g 98.00
NBC No.: 05-20-0015; CAS No.: 944283-07-2; $C_{26}H_{30}N_2O_8$; M.W.: 498.5 5 g 390.00
Solubility: 0.5 mmol in 1 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%.
HPLC: purity: ≥ 95.0%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
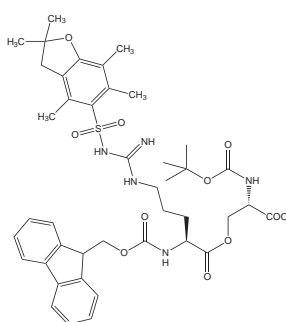
852249 **Boc-Ser(Fmoc-Arg(Pbf))–OH** 1 g 98.00
NBC No.: 05-20-0016; CAS No.: 944283-22-1; $C_{42}H_{53}N_5O_{11}S$; M.W.: 836.0 5 g 390.00
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%.
HPLC: purity: ≥ 95.0%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
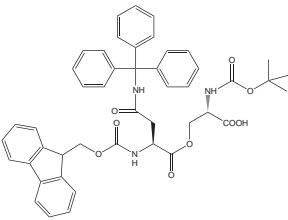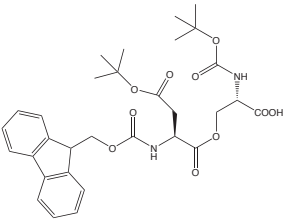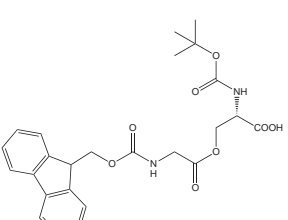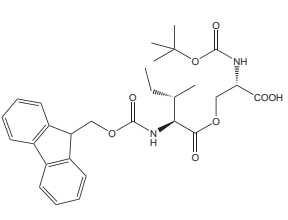
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Product No.	Product	Quantity	Price

852257   Boc-Ser(Fmoc-Asn(Trt))-OH   1 g   98.00
   5 g   390.00

NBC No.: 05-20-0022; CAS No.: 944283-17-4; $C_{46}H_{45}N_3O_9$; M.W.: 783.9

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%.

HPLC: purity: ≥ 95.0%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

852298   Boc-Ser(Fmoc-Asp(OtBu)-OH   1 g   98.00
   5 g   390.00

CAS No.: 944283-16-3; $C_{31}H_{38}N_2O_{10}$; M.W.: 598.6

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%.

HPLC: purity: ≥ 95.0%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

852256   Boc-Ser(Fmoc-Gln(Trt))-OH   1 g   98.00
   5 g   390.00

NBC No.: 05-20-0023; CAS No.: 944283-19-6; $C_{47}H_{47}N_3O_9$; M.W.: 797.9

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%.

HPLC: purity: ≥ 95.0%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

852295   Boc-Ser(Fmoc-Glu(OtBu)-OH   1 g   98.00
   5 g   390.00

CAS No.: 944283-18-5; $C_{32}H_{40}N_2O_{10}$; M.W.: 612.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%.

HPLC: purity: ≥ 95.0%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

852168   Boc-Ser(Fmoc-Gly)-OH   1 g   98.00
   5 g   390.00

NBC No.: 05-20-0009; CAS No.: 944283-06-1; $C_{25}H_{28}N_2O_8$; M.W.: 484.5

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%.

HPLC: purity: ≥ 95.0%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

852250   Boc-Ser-(Fmoc-Ile)-OH   1 g   98.00
   5 g   390.00

NBC No.: 05-20-0017; CAS No.: 944283-10-7; $C_{29}H_{36}N_2O_8$; M.W.: 540.6

Solubility: 0.5 mmole in 1 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%.

HPLC: purity: ≥ 95.0%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

ISOACYL DIPEPTIDES

⚠ Storage conditions   **G** Additional information   **M** Method information   **A** Application information

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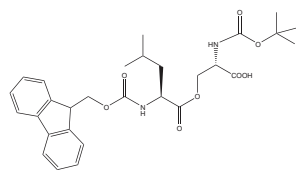
Product No.	Product	Quantity	Price
852262	**Boc-Ser(Fmoc-Leu)-OH**	1 g	98.00
	CAS No.: 944283-09-4; $C_{29}H_{36}N_2O_8S$; M.W.: 540.6	5 g	390.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%.		
	HPLC: purity: ≥ 95.0%.		
	⚠ Prolonged storage: ≤ -20°C; keep cool and dry.		
852293	**Boc-Ser(Fmoc-Met)-OH**	1 g	98.00
	CAS No.: 944283-14-1; $C_{28}H_{34}N_2O_8S$; M.W.: 558.6	5 g	390.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%.		
	HPLC: purity: ≥ 95.0%.		
	⚠ Prolonged storage: ≤-20°C; keep cool and dry.		
852169	**Boc-Ser(Fmoc-Phe)-OH**	1 g	98.00
	NBC No.: 05-20-0010; $C_{32}H_{34}N_2O_8$; M.W.: 574.62	5 g	390.00
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%.		
	HPLC: purity: ≥ 95.0%.		
	⚠ Prolonged storage: ≤ -20°C; keep cool and dry.		
852172	**Boc-Ser(Fmoc-Ser(tBu))-OH**	1 g	98.00
	NBC No.: 05-20-0013; CAS No.: 944283-11-8; $C_{30}H_{38}N_2O_9$; M.W.: 570.66	5 g	390.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 97%.		
	HPLC: purity: ≥ 98.0%.		
	⚠ Prolonged storage: ≤ -20°C; keep cool and dry.		
852173	**Boc-Ser(Fmoc-Thr(tBu))-OH**	1 g	98.00
	NBC No.: 05-20-0014; CAS No.: 944283-12-9; $C_{31}H_{40}N_2O_9$; M.W.: 584.66	5 g	390.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 97%.		
	HPLC: purity: ≥ 97.0%.		
	⚠ Prolonged storage: ≤ -20°C; keep cool and dry.		
852290	**Boc-Ser(Fmoc-Val)-OH**	1 g	98.00
	CAS No.: 944283-08-3; $C_{28}H_{34}N_2O_8$; M.W.: 526.6	5 g	390.00
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%.		
	HPLC: purity: ≥ 95.0%.		
	⚠ Prolonged storage: ≤ -20°C; keep cool and dry.		
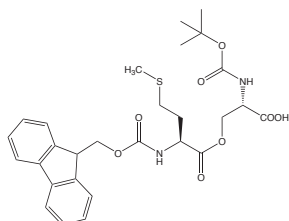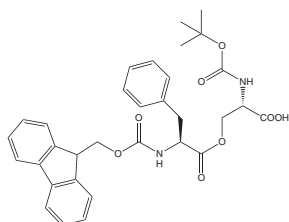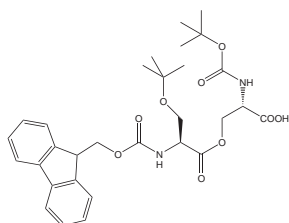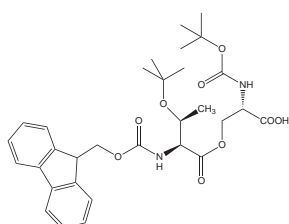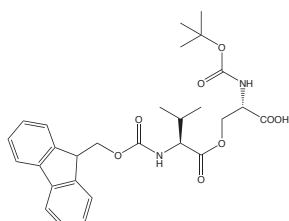
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Product No.	Product	Quantity	Price

852170 **Boc-Thr(Fmoc-Ala)-OH**
NBC No.: 05-20-0011; $C_{27}H_{32}N_2O_8$; M.W.: 512.55
TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 95%.
HPLC: purity: ≥ 95.0%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

1 g 98.00
5 g 390.00

852297 **Boc-Thr(Fmoc-Asp(OtBu))-OH**
$C_{32}H_{40}N_2O_{10}$; M.W.: 612.7
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%.
HPLC: purity: ≥ 95.0%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

1 g 98.00
5 g 390.00

852296 **Boc-Thr(Fmoc-Glu(OtBu)-OH**
$C_{33}H_{42}N_2O_{10}$; M.W.: 626.7
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%.
HPLC: purity: ≥ 95.0%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

1 g 98.00
5 g 390.00

852294 **Boc-Thr(Fmoc-Arg(Pbf))-OH**
$C_{43}H_{55}N_5O_{11}S$; M.W.: 850.0
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%.
HPLC: purity: ≥ 95.0%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

1 g 98.00
5 g 390.00

852171 **Boc-Thr(Fmoc-Gly)-OH**
NBC No.: 05-20-0012; CAS No.: 944283-25-4; $C_{26}H_{30}N_2O_8$; M.W.: 498.5
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 95%.
HPLC: purity: ≥ 95.0%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

1 g 98.00
5 g 390.00

852252 **Boc-Thr(Fmoc-Ile))-OH**
NBC No.: 05-20-0019; $C_{30}H_{38}N_2O_8$; M.W.: 554.6
Solubility: 0.5 mmole in 1 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%.
HPLC: purity: ≥ 95.0%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

1 g 98.00
5 g 390.00

ISOACYL DIPEPTIDES

⚠ Storage conditions   🄶 Additional information   🄼 Method information   🄐 Application information

ISOACYL DIPEPTIDES
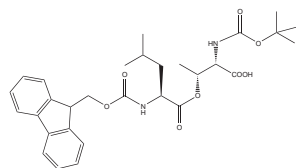
			€
Product No.	Product	Quantity	Price
852263	**Boc–Thr(Fmoc–Leu)–OH** CAS No.: 944283-26-5; $C_{30}H_{38}N_2O_8S$; M.W.: 554.2 Solubility: 0.5 mmole in 1 ml DMF clearly soluble. TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%. HPLC: purity: ≥ 95.0%. ⚠ Prolonged storage: ≤ -20°C; keep cool and dry.	1 g 5 g	98.00 390.00
852292	**Boc–Thr(Fmoc–Met)–OH** $C_{29}H_{36}N_2O_8S$; M.W.: 572.7 TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%. HPLC: purity: ≥ 95.0%. ⚠ Prolonged storage: ≤ -20°C; keep cool and dry.	1 g 5 g	98.00 390.00
852299	**Boc–Thr(Fmoc–Thr(tBu))–OH** CAS No.: 944283-29-8; $C_{32}H_{42}N_2O_9$; M.W.: 598.6 TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%. HPLC: purity: ≥ 95.0%. ⚠ Prolonged storage: ≤ -20°C; keep cool and dry.	1 g 5 g	98.00 390.00
852253	**Boc–Thr(Fmoc–Val))–OH** NBC No.: 05-20-0018; CAS No.: 887707-95-1; $C_{29}H_{36}N_2O_8$; M.W.: 540.6 Solubility: 0.5 mmole in 1 ml DMF clearly soluble. TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 95%. HPLC: purity: ≥ 95.0%. ⚠ Prolonged storage: ≤ -20°C; keep cool and dry.	1 g 5 g	98.00 390.00
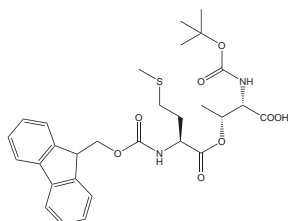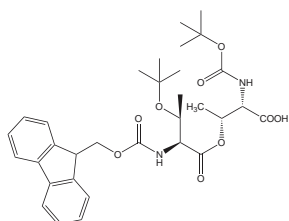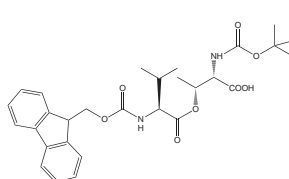
Product No.	Product	Quantity	Price

Derivatives for phosphopeptide synthesis

N-α-Fmoc protected phosphoamino acids

These building blocks allow the direct incorporation of phosphorylated amino acids during Fmoc SPPS.

These monobenzyl diesters are stable to piperidine and do not undergo dehydroamino acid formation during Fmoc group removal [1 - 3], except in the case of the serine derivative which has been seen to eliminate under heating [4]. Using these reagents, even peptides containing multiple phosphorylation sites can be prepared efficiently by standard Fmoc SPPS methods [2]. Coupling of these derivatives is best performed using TBTU/DIPEA activation [5], although more recent results indicate the EEDQ may also be very effective [11]. Cleavage of the benzyl ester occurs during the course of the standard TFA-mediated deprotection reaction.

Applications of these derivatives include the preparation of phospholamban [6], a 52 residue peptide containing both phosphoserine and phosphothreonine, and human salivary statherin, a 42 residue phosphoserine peptide [7]; for other examples see references [8 - 10].

ⓘ Ⓖ 3.38

[1]   T. Wakamiya, et al. (1994) *Chem. Lett.*, 1099.
[2]   P. White & J. Beythien in "Innovations & Perspectives in Solid Phase Synthesis and Combinatorial Libraries, 4th International Symposium", Mayflower Scientific Ltd., Birmingham, 1996, pp. 557.
[3]   T. Vorherr, et al. (1995) *Bioorg. Med. Chem. Lett.*, **5**, 2661.
[4]   T. J. Attard, et al. (2009) *Int. J. Pept. Res. Ther.*, **15**, 69; M. Self, et al. (2013) *Pept. Sci.*, 125.
[5]   J. Perich, et al. (1999) *Lett. Pept. Sci.*, **6**, 91.
[6]   H. Schmid, et al., Poster 423 presented at the 15th American Peptide Symposium, Nashville, 1997.
[7]   T. L Gururaja & M. J. Levine (1996) *Pept. Res.*, **9**, 283.
[8]   G. Shapiro, et al. (1996) *Bioorg. Med. Chem. Lett.*, **6**, 409.
[9]   M. John, et al. (1996) *Pept. Res.*, **9**, 71.
[10] M. Brandt, et al. (2006) *ChemBioChem*, **7**, 623.
[11] V. Tapia, et al. (2008) *J. Pept. Sci.*, **14**, 1309.

852069	Fmoc–Ser(PO(OBzl)OH)–OH	1 g	157.00
	N-α-Fmoc-O-benzyl-L-phosphoserine	5 g	628.00

NBC No.: 04-12-1154; CAS No.: 158171-14-3; $C_{25}H_{24}NO_8P$; M.W.: 497.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 97%.

HPLC: purity: ≥ 95.0%.

EtOAc: ≤ 0.5%

AcOH: ≤ 0.1%

Optical purity: ≥ 99.0% L-enantiomer.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

An excellent building block for the preparation of phosphoserine-containing peptides.

ⓘ Ⓖ 3.38, Ⓜ 3.39

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Product No.	Product	Quantity	Price

852244   Fmoc–D–Ser(PO(OBzl)OH)–OH   1 g   325.00

N-α-Fmoc-O-benzyl-D-phosphoserine   5 g   1200.00

NBC No.: 04-13-1078; CAS No.: 1212481-01-0; $C_{25}H_{24}NO_8P$; M.W.: 497.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 97%.

HPLC: purity: ≥ 95.0%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

See entry for 852069.

852070   Fmoc–Thr(PO(OBzl)OH)–OH   1 g   170.00

N-α-Fmoc-O-benzyl-L-phosphothreonine   5 g   680.00

NBC No.: 04-12-1155; CAS No.: 175291-56-2; $C_{26}H_{26}NO_8P$; M.W.: 511.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98%.

HPLC: purity: ≥ 98.0%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

An excellent building block for the preparation of phosphothreonine-containing

peptides.

ⓘ Ⓖ 3.38, Ⓜ 3.39, Ⓐ 3.40

852245   Fmoc–D–Thr(PO(OBzl)OH)–OH   1 g   325.00

N-α-Fmoc-O-benzyl-D-phosphothreonine   5 g   1200.00

NBC No.: 04-13-1079; CAS No.: 937171-63-6; $C_{26}H_{26}NO_8P$; M.W.: 511.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 97%.

HPLC: purity: ≥ 95.0%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

See entry for 852070.

852071   Fmoc–Tyr(PO(OBzl)OH)–OH   1 g   185.00

N-α-Fmoc-O-benzyl-L-phosphotyrosine   5 g   750.00

NBC No.: 04-12-1156; CAS No.: 191348-16-0; $C_{31}H_{28}NO_8P$; M.W.: 573.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 95%.

HPLC: purity: ≥ 95.0%.

EtOAc: ≤ 0.5.%

AcOH: ≤ 0.1%

Optical purity: ≥ 98.5% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

An excellent building block for the preparation of phosphotyrosine-containing

peptides.

ⓘ Ⓖ 3.38, Ⓜ 3.39, Ⓐ 3.39

Product No.	Product	Quantity	Price

€

852246 **Fmoc–D–Tyr(PO(OBzl)OH)–OH** — 1 g — 325.00 / 5 g — 1200.00

N-α-Fmoc-O-benzyl-D-phosphotyrosine

NBC No.: 04-13-1080; $C_{31}H_{28}NO_8P$; M.W.: 573.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 95%.

HPLC: purity: ≥ 90.0%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

See entry for 852071.

852058 **Fmoc–Tyr(PO_3H_2)–OH** — 1 g — 120.00 / 5 g — 478.00

N-α-Fmoc-O-phospho-L-tyrosine

NBC No.: 04-12-1125; CAS No.: 147762-53-6; $C_{24}H_{22}NO_8P$; M.W.: 483.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 95%.

EtOH:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 95%.

HPLC: purity: ≥ 95.0%.

Optical purity: ≥ 99.5% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

A useful derivative for the synthesis of phosphotyrosine peptides [1-3]. Pyrophosphate formation has been noted in peptides containing adjacent $Tyr(PO_3H_2)$ residues [4, 5]. An evaluation of the optimal coupling conditions for introduction of this residue has been made [6].

ⓘ Ⓖ 3.40, Ⓜ 3.39

[1] E. A. Ottinger, et al. (1993) *Biochemistry*, **32**, 4354.
[2] E. A. Ottinger, et al. (1995) *Int. J. Peptide Protein Res.*, **46**, 346.
[3] F. Anjuere, et al. (1995) *Anal. Biochem.*, **229**, 61.
[4] C. Garcia-Echeverria (1995) *Lett. Pept. Sci.*, **2**, 93.
[5] R. M. Valerio, et al. (1995) *Lett. Pept. Sci.*, **2**, 33.
[6] C. Garcia-Echeverria (1996) *Lett. Pept. Sci.*, **2**, 369.

852090 **Fmoc–Tyr(PO(NMe_2)_2)–OH** — 1 g — 172.00 / 5 g — 686.00

N-α-Fmoc-O-(bis-dimethylamino-phosphono)-L-tyrosine

NBC No.: 04-12-1205; CAS No.: 172611-23-3; $C_{28}H_{32}N_3O_6P$; M.W.: 537.6

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98%.

HPLC: purity: ≥ 96.0%.

Optical purity: ≥ 99.5% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

Fmoc-Tyr(PO(NMe_2)_2-OH is a useful derivative for the synthesis of phosphotyrosine-containing peptides by Fmoc SPPS [1]. As the side-chain phosphate group is fully protected, this derivative is compatible with all coupling methods.

Regeneration of phosphotyrosine from the phosphodiamidate is effected by acid catalyzed hydrolysis using 90% aq. TFA.

ⓘ Ⓖ 3.40, Ⓐ 3.40

[1] H. G. Chao, et al. (1995) *J. Org. Chem.*, **60**, 7710.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
852288	**Fmoc-Phe(CF$_2$PO$_3$H)-OH**	100 mg	380.00
	Fmoc-F$_2$Pmp-OH	250 mg	760.00

Fmoc-L-p-(phosphono-difluoromethyl)phenylalanine

CAS No.: 160751-44-0; C$_{25}$H$_{22}$NO$_7$PF$_2$; M.W.: 517.4

HPLC: purity: ≥ 90.0%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Building block [1] for the introduction by Fmoc SPPS of the hydrolytically stable phosphotyrosine analog phosphonodifluoromethylphenylalanine (F$_2$Pmp) [2]. The pKa of F$_2$Pmp more closely resembles that of a phosphate group making it better analog than phosphonomethylphenylalanine.

ⓘ Ⓖ 3.41, Ⓜ 3.39, Ⓐ 3.41

[1] M. F. Gordeev, et al. (1994) *Tetrahedron Lett.,* **35**, 7585.

[2] T. R. Burke, Jr. (2006) *Curr. Top. Med. Chem.,* **6**, 1465.

Phosphoramidites

These reagents are valuable tools for the preparation of phosphopeptides by post-synthetic phosphorylation of unprotected hydroxyl groups on the solid support. This method has the advantage over direct incorporation of pre-formed phosphoamino acid building blocks of furnishing both phosphorylated and unphosphorylated peptides in a single synthesis.

Phosphorylation is performed in two stages. Firstly, the peptidyl resin, containing an unprotected serine or threonine residue at the phosphorylation site, is reacted with an excess of phosphoramidite and tetrazole in DMF to generate the corresponding phosphite. Secondly, this intermediate is oxidized with t-butyl hydroperoxide to the appropriate serine or threonine phosphotriester. The phosphate protecting groups are removed during the course of the standard TFA-mediated deprotection reaction.

ⓘ Ⓖ 3.41, Ⓜ 3.42
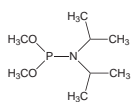
Product No.	Product	Quantity	Price
851047	**Dimethyl-N,N-diisopropylphosphoramidite**	500 mg	99.00
		1 g	177.00

NBC No.: 01-60-0030; CAS No.: 122194-07-4; C$_8$H$_{20}$NO$_2$P; M.W.: 193.2

GC: purity: ≥ 95.0%.

⚠ Prolonged storage: ≤-20°C; keep cool and dry; keep open bottle under nitrogen.

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Product No.	Product	Quantity	Price

N-α-Boc protected amino acids

Alanine [Ala, A]

	853001	**Boc-Ala-OH**	5 g	11.00
		N-α-t.-Boc-L-alanine	25 g	31.00
		NBC No.: 04-12-0002; CAS No.: 15761-38-3; $C_8H_{15}NO_4$; M.W.: 189.2	100 g	63.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (85:10:5), purity: ≥ 98.00%.
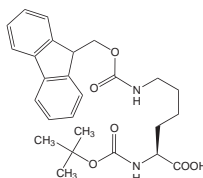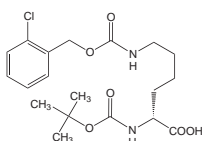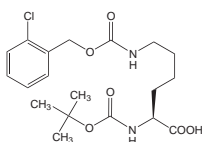CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

	853087	**Boc-D-Ala-OH**	5 g	26.00
		N-α-t.-Boc-D-alanine	25 g	104.00
		NBC No.: 04-13-0002; CAS No.: 7764-95-6; $C_8H_{15}NO_4$; M.W.: 189.2	100 g	312.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (85:10:5), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

	853057	**Boc-β-Ala-OH**	5 g	15.00
		N-β-t.-Boc-β-alanine	25 g	54.00
		NBC No.: 04-12-0100; CAS No.: 3303-84-2; $C_8H_{15}NO_4$; M.W.: 189.2	100 g	162.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

	853082	**Boc-N-Me-Ala-OH**	1 g	67.00
		N-α-t.-Boc-N-α-methyl-L-alanine	5 g	266.00
		NBC No.: 04-12-9002; CAS No.: 16948-16-6; $C_9H_{17}NO_4$; M.W.: 203.2		

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

Aminobutyric acid [Abu]

	853058	**Boc-Abu-OH**	1 g	26.00
		N-α-t.-Boc-L-α-aminobutyric acid	5 g	104.00
		t.-Boc-L-2-aminobutanoic acid	25 g	416.00
		NBC No.: 04-12-0101; CAS No.: 34306-42-8; $C_9H_{17}NO_4$; M.W.: 203.2		

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.
$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

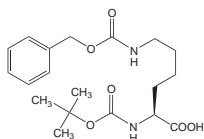⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
853064	**Boc-γ-Abu-OH**	5 g	52.00
	t.-Boc-4-aminobutanoic acid	25 g	208.00
	t.-Boc-GABA		
	NBC No.: 04-12-0116; CAS No.: 57294-38-9; $C_9H_{17}NO_4$; M.W.: 203.2		
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		

Aminohexanoic acid [ε-Ahx]
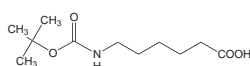
853059	**Boc-ε-Ahx-OH**	5 g	60.00
	N-ε-t.-Boc-ε-aminocaproic acid	25 g	237.00
	t.-Boc-6-aminohexanoic acid		
	NBC No.: 04-12-0102; CAS No.: 6404-29-1; $C_{11}H_{21}NO_4$; M.W.: 231.3		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		

Aminoisobutyric acid [Aib]
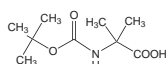
853077	**Boc-Aib-OH**	5 g	23.00
	N-α-t.-Boc-α-aminoisobutyric acid	25 g	92.00
	N-α-t.-Boc-α-methylalanine		
	NBC No.: 04-12-0203; CAS No.: 30992-29-1; $C_9H_{17}NO_4$; M.W.: 203.2		
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		

Arginine [Arg, R]
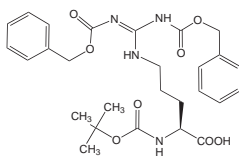
853036	**Boc-Arg(di-Z)-OH**	5 g	130.00
	N-α-Boc-N^G,N^G-bis-CBZ-L-arginine	25 g	520.00
	NBC No.: 04-12-0220; CAS No.: 51219-19-3; $C_{27}H_{34}N_4O_8$; M.W.: 542.6		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 98.00%.		
	⚠ Prolonged storage: ≤ -20°C; keep cool and dry.		
	ⓘ Ⓖ 4.1		

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Product No.	Product	Quantity	Price
853063	**Boc-Arg(Mtr)-OH**	5 g	152.00
	N-α-t.-Boc-N^G-(4-Methoxy-2,3,6 trimethylbenzenesulfonyl)-L-arginine	25 g	607.00
	NBC No.: 04-12-0113; CAS No.: 102185-38-6; $C_{21}H_{34}N_4O_7S$; M.W.: 486.6		
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 98.00%.		
	⚠ Prolonged storage: ≤ -20°C; keep cool and dry.		

[1] E. Atherton, et al. (1983) *J. Chem. Soc., Chem. Commun.*, 1060.
[2] E. Atherton, et al. (1985) *J. Chem. Soc., Perkin Trans. 1*, 2065.
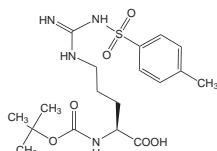
853013	**Boc-Arg(Tos)-OH**	5 g	31.00
	N-α-t.-Boc-N^G-tosyl-L-arginine	25 g	94.00
	NBC No.: 04-12-0036; CAS No.: 13836-37-8; $C_{18}H_{28}N_4O_6S$; M.W.: 428.5	100 g	281.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 99.00%.		
	⚠ Prolonged storage: ≤ -20°C; keep cool and dry.		
	ⓘ Ⓖ 4.1		
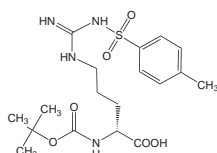
853037	**Boc-D-Arg(Tos)-OH**	1 g	26.00
	N-α-t.-Boc-N^G-tosyl-D-arginine	5 g	104.00
	NBC No.: 04-13-0044; CAS No.: 61315-61-5; $C_{18}H_{28}N_4O_6S$; M.W.: 428.5	25 g	416.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: ≤ -20°C; keep cool and dry.		

Asparagine [Asn, N]
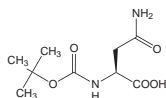
853039	**Boc-Asn-OH**	5 g	11.00
	N-α-t.-Boc-L-asparagine	25 g	31.00
	NBC No.: 04-12-0008; CAS No.: 7536-55-2; $C_9H_{16}N_2O_5$; M.W.: 232.2	100 g	89.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	ⓘ Ⓖ 4.2		
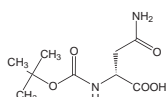
853088	**Boc-D-Asn-OH**	1 g	37.00
	N-α-t.-Boc-D-asparagine	5 g	140.00
	NBC No.: 04-13-0005; CAS No.: 75647-01-7; $C_9H_{16}N_2O_5$; M.W.: 232.2	25 g	697.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		

N-α-BOC PROTECTED AMINO ACIDS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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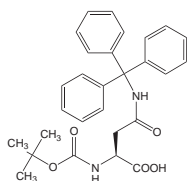
Product No.	Product	Quantity	Price
853074	**Boc-Asn(Trt)-OH**	5 g	135.00
	N-α-t.-Boc-β-trityl-L-asparagine	25 g	541.00
	NBC No.: 04-12-0187; CAS No.: 132388-68-2; $C_{28}H_{30}N_2O_5$; M.W.: 474.6		

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

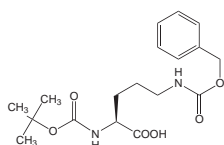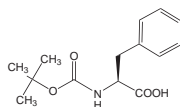CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

A trityl-protected derivative which increases the Solubility of Asn during the coupling step in SPPS [1]. Its use gives higher yields and lower by-product formation. During the following coupling cycles the temporary side-chain protection is removed.

[1] P. Sieber, et al. (1991) *Tetrahedron Lett.*, **32**, 739.
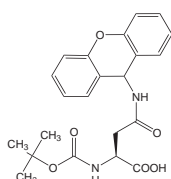
Product No.	Product	Quantity	Price
853007	**Boc-Asn(Xan)-OH**	5 g	42.00
	N-α-t.-Boc-N-β-xanthyl-L-asparagine	25 g	135.00
	NBC No.: 04-12-0022; CAS No.: 65420-40-8; $C_{22}H_{24}N_2O_6$; M.W.: 412.4	100 g	411.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

ⓘ Ⓖ 4.2

Aspartic acid [Asp, D]
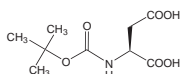
Product No.	Product	Quantity	Price
853070	**Boc-Asp-OH**	5 g	21.00
	N-α-t.-Boc-L-aspartic acid	25 g	81.00
	NBC No.: 04-12-0129; CAS No.: 13726-67-5; $C_9H_{15}NO_6$; M.W.: 233.2	100 g	243.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.
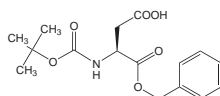
Product No.	Product	Quantity	Price
853014	**Boc-Asp-OBzl**	5 g	47.00
	N-α-t.-Boc-L-aspartic acid α-benzyl ester	25 g	187.00
	NBC No.: 04-12-0038; CAS No.: 30925-18-9; $C_{16}H_{21}NO_6$; M.W.: 323.4		

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

Useful derivative for the synthesis of β-aspartyl peptides.
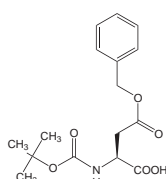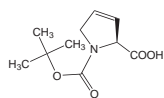
Product No.	Product	Quantity	Price
853045	**Boc-Asp(OBzl)-OH**	5 g	24.00
	N-α-t.-Boc-L-aspartic acid β-benzyl ester	25 g	72.00
	NBC No.: 04-12-0028; CAS No.: 7536-58-5; $C_{16}H_{21}NO_6$; M.W.: 323.4	100 g	215.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

ⓘ Ⓖ 4.3

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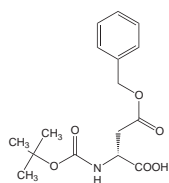
Product No.	Product	Quantity	Price
853105	**Boc-D-Asp(OBzl)-OH**	1 g	33.00
	N-α-t.-Boc-D-aspartic acid β-benzyl ester	5 g	133.00
	NBC No.: 04-13-0051; CAS No.: 51186-58-4; $C_{16}H_{21}NO_6$; M.W.: 323.4	25 g	532.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.
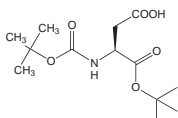
853032	**Boc-Asp-OtBu**	1 g	33.00
	N-α-t.-Boc-L-aspartic acid α-t.-butyl ester	5 g	130.00
	NBC No.: 04-12-0132; CAS No.: 34582-32-6; $C_{13}H_{23}NO_6$; M.W.: 289.4	25 g	515.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
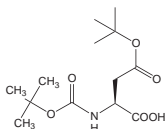
853048	**Boc-Asp(OtBu)-OH · DCHA**	5 g	73.00
	N-α-t.-Boc-L-aspartic acid β-t.-butyl ester dicyclohexylammonium salt	25 g	291.00

NBC No.: 04-12-0040; CAS No.: 1913-12-8; $C_{13}H_{23}NO_6$ · $C_{12}H_{23}N$; M.W.: 289.3 · 181.3

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

This derivative is supplied as a DCHA-salt since the free acid is not crystalline.
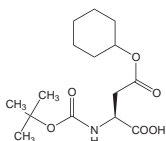
853030	**Boc-Asp(OcHx)-OH**	5 g	31.00
	N-α-t.-Boc-L-aspartic acid β-cyclohexyl ester	25 g	94.00
	NBC No.: 04-12-0123; CAS No.: 73821-95-1; $C_{15}H_{25}NO_6$; M.W.: 315.4	100 g	281.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

Minimizes aspartimide formation, particularly in problematic sequences such as Asp-Gly [1-3].

ⓘ Ⓖ 4.3

[1] J. P. Tam, et al. (1979) *Tetrahedron Lett.*, **42**, 4033.

[2] E. Nicolas, et al. (1989) *Tetrahedron Lett.*, **30**, 497.

[3] B. Penke, et al. in "Peptides 1988, Proc. 20th European Peptide Symposium", G. Jung & E. Bayer (Eds), Walter de Gruyter, Berlin, 1989, pp. 67.

⚠ Storage conditions　　Ⓖ Additional information　　Ⓜ Method information　　Ⓐ Application information

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Product No.	Product	Quantity	Price

Butylglycine
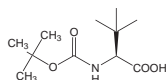
853068   **Boc‑L‑α‑t‑butylglycine**

N‑α‑t.‑Boc‑L‑α‑t.‑butyl‑glycine

N‑α‑t.‑Boc‑L‑t‑leucine

NBC No.: 04‑12‑0126; CAS No.: 62965‑35‑9; $C_{11}H_{21}NO_4$; M.W.: 231.3

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

	1 g	130.00
	5 g	520.00

Cyclohexylalanine [Cha]
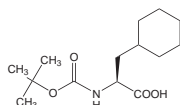
853072   **Boc‑Cha‑OH · DCHA**

N‑α‑t.‑Boc‑β‑cyclohexyl‑L‑alanine dicyclohexylammonium salt

NBC No.: 04‑12‑0169; CAS No.: 37736‑82‑6; $C_{14}H_{25}NO_4 \cdot C_{12}H_{23}N$; M.W.: 271.4 · 181.3

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

This derivative is supplied as a DCHA‑salt since the free acid is not crystalline.

	1 g	45.00
	5 g	179.00
	25 g	716.00

Cysteine [Cys, C]
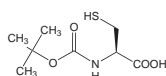
853031   **Boc‑Cys‑OH (cryst.)**

N‑α‑t.‑Boc‑L‑cysteine

NBC No.: 04‑12‑0125; CAS No.: 20887‑95‑0; $C_8H_{15}NO_4S$; M.W.: 221.3

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ ‑20°C; keep cool and dry; keep open bottle under nitrogen.

[1] R. H. Buck, et al. (1984) *J. Chromatogr.*, **315**, 279.
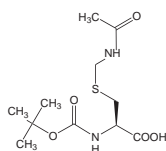
	1 g	41.00
	5 g	162.00
	25 g	649.00

853049   **Boc‑Cys(Acm)‑OH**

N‑α‑t.‑Boc‑S‑acetamidomethyl‑L‑cysteine

NBC No.: 04‑12‑0042; CAS No.: 19746‑37‑3; $C_{11}H_{20}N_2O_5S$; M.W.: 292.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

ⓘ Ⓖ 3.33, 4.4, Ⓜ 3.33, 3.35, 3.36

	5 g	37.00
	25 g	120.00
	100 g	348.00

Product No.	Product	Quantity	Price €
853109	**Boc-D-Cys(Acm)-OH**	1 g	63.00
	N-α-t.-Boc-S-acetamidomethyl-D-cysteine	5 g	203.00
	NBC No.: 04-13-0059; CAS No.: 138775-00-5; $C_{11}H_{20}N_2O_5S$; M.W.: 292.4	25 g	822.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
853046	**Boc-Cys(Bzl)-OH**	5 g	24.00
	N-α-t.-Boc-S-benzyl-L-cysteine	25 g	106.00
	NBC No.: 04-12-0030; CAS No.: 5068-28-0; $C_{15}H_{21}NO_4S$; M.W.: 311.4	100 g	318.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
853050	**Boc-Cys(4-MeOBzl)-OH**	5 g	24.00
	N-α-t.-Boc-S-p-methoxybenzyl-L-cysteine	25 g	72.00
	NBC No.: 04-12-0045; CAS No.: 18942-46-6; $C_{16}H_{23}NO_5S$; M.W.: 341.4	100 g	215.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	ⓘ Ⓖ 4.4		
	[1] O. Nishimura, et al. (1978) *Chem. Pharm. Bull.*, **26**, 1576.		
853033	**Boc-Cys(4-MeBzl)-OH**	5 g	24.00
	N-α-t.-Boc-S-p-methylbenzyl-L-cysteine	25 g	72.00
	NBC No.: 04-12-0134; CAS No.: 61925-77-7; $C_{16}H_{23}NO_4S$; M.W.: 325.4	100 g	215.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 98.50%.		
	ⓘ Ⓖ 4.4		
853005	**Boc-Cys(Trt)-OH**	5 g	47.00
	N-α-t.-Boc-S-trityl-L-cysteine	25 g	187.00
	NBC No.: 04-12-0020; CAS No.: 21947-98-8; $C_{27}H_{29}NO_4S$; M.W.: 463.6	100 g	562.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 99.00%.		
	⚠ Prolonged storage: ≤ -20°C; keep cool and dry.		
	ⓘ Ⓖ 3.33, 4.4		
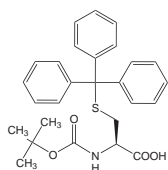
⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information
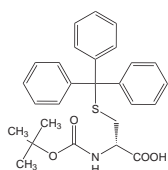
Product No.	Product	Quantity	Price €
853115	**Boc-D-Cys(Trt)-OH**	1 g	67.00
	N-α-t.-Boc-S-trityl-D-cysteine	5 g	266.00
	NBC No.: 04-13-0081; CAS No.: 87494-13-1; $C_{27}H_{29}NO_4S$; M.W.: 463.6	25 g	1035.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CH_3CN:CHCl_3:AcOH$ (8:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

Glutamic acid [Glu, E]
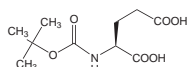
853066	**Boc-Glu-OH**	5 g	21.00
	N-α-t.-Boc-L-glutamic acid	25 g	81.00
	NBC No.: 04-12-0119; CAS No.: 2419-94-5; $C_{10}H_{17}NO_6$; M.W.: 247.3	100 g	243.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3:MeOH:AcOH$ (77.5:15:7.5), purity: ≥ 98.00%.

$CH_3CN:CHCl_3:AcOH$ (8:1:1), purity: ≥ 98.00%.
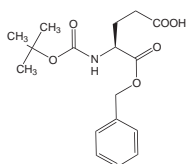
853015	**Boc-Glu-OBzl (cryst.)**	5 g	33.00
	N-α-t.-Boc-L-glutamic acid α-benzyl ester	25 g	133.00
	NBC No.: 04-12-0048; CAS No.: 30924-93-7; $C_{17}H_{23}NO_6$; M.W.: 337.4		

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3:MeOH$ (7:3), purity: ≥ 98.00%.

Useful derivative for the synthesis of γ-glutamyl peptides.
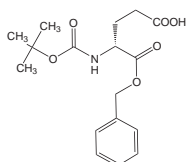
853113	**Boc-D-Glu-OBzl**	1 g	47.00
	N-α-t.-Boc-D-glutamic acid α-benzyl ester	5 g	180.00
	NBC No.: 04-13-0072; CAS No.: 34404-30-3; $C_{17}H_{23}NO_6$; M.W.: 337.4	25 g	720.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3:MeOH$ (7:3), purity: ≥ 98.00%.

$CH_3CN:CHCl_3:AcOH$ (8:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
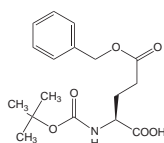
853010	**Boc-Glu(OBzl)-OH (cryst.)**	5 g	24.00
	N-α-t.-Boc-L-glutamic acid γ-benzyl ester	25 g	96.00
	NBC No.: 04-12-0025; CAS No.: 13574-13-5; $C_{17}H_{23}NO_6$; M.W.: 337.4	100 g	239.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3:MeOH:AcOH$ (90:8:2), purity: ≥ 98.00%.
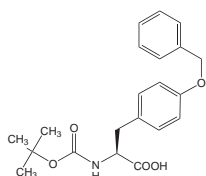
$CH_3CN:CHCl_3:AcOH$ (8:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

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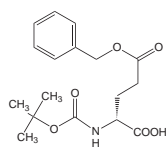
Product No.	Product	Quantity	Price

853089   **Boc-D-Glu(OBzl)-OH**

N-α-t.-Boc-D-glutamic acid γ-benzyl ester

NBC No.: 04-13-0009; CAS No.: 35793-73-8; $C_{17}H_{23}NO_6$; M.W.: 337.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
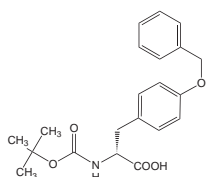
HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

| | 5 g | 67.00 |
| | 25 g | 266.00 |

853028   **Boc-Glu-OtBu**

N-α-t.-Boc-L-glutamic acid α-t.-butyl ester

NBC No.: 04-12-0111; CAS No.: 24277-39-2; $C_{14}H_{25}NO_6$; M.W.: 303.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

	1 g	33.00
	5 g	130.00
	25 g	515.00
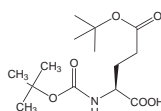
853052   **Boc-Glu(OtBu)-OH**

N-α-t.-Boc-L-glutamic acid γ-t.-butyl ester

NBC No.: 04-12-0051; CAS No.: 13726-84-6; $C_{14}H_{25}NO_6$; M.W.: 303.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

| | 5 g | 55.00 |
| | 25 g | 220.00 |
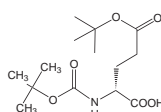
853114   **Boc-D-Glu(OtBu)-OH**

N-α-t.-Boc-D-glutamic acid γ-t.-butyl ester

NBC No.: 04-13-0079; CAS No.: 104719-63-3; $C_{14}H_{25}NO_6$; M.W.: 303.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

| | 5 g | 78.00 |
| | 25 g | 312.00 |
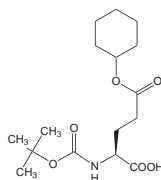
853029   **Boc-Glu(OcHx)-OH**

N-α-t.-Boc-L-glutamic acid γ-cyclohexyl ester

NBC No.: 04-12-0121; CAS No.: 73821-97-3; $C_{16}H_{27}NO_6$; M.W.: 329.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH:H_2O (70:42:0.5:10), purity: ≥ 98.00%.

This side-chain protecting group for glutamic acid is used to minimize side

reactions during acidic and basic treatments [1].

ⓘ Ⓖ 4.3

[1] J. P. Tam, et al. (1979) *Tetrahedron Lett.*, **42**, 4033.

	5 g	42.00
	25 g	94.00
	100 g	281.00

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Glutamine [Gln, Q]
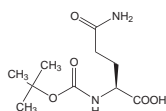
853040	**Boc-Gln-OH**	5 g	11.00
	N-α-t.-Boc-L-glutamine	25 g	31.00
	NBC No.: 04-12-0009; CAS No.: 13726-85-7; $C_{10}H_{18}N_2O_5$; M.W.: 246.3	100 g	89.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

853100	**Boc-D-Gln-OH**	5 g	99.00
	N-α-t.-Boc-D-glutamine	25 g	395.00
	NBC No.: 04-13-0037; CAS No.: 61348-28-5; $C_{10}H_{18}N_2O_5$; M.W.: 246.3		

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
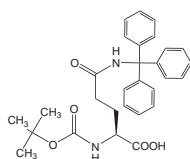
853075	**Boc-Gln(Trt)-OH**	5 g	135.00
	N-α-t.-Boc-γ-trityl-L-glutamine	25 g	541.00
	NBC No.: 04-12-0188; CAS No.: 132388-69-3; $C_{29}H_{32}N_2O_5$; M.W.: 488.6		

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
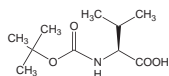
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

A trityl-protected derivative which increases the Solubility of glutamine during the coupling step in SPPS [1]. Its use gives higher yields and lower by-product formation. During the following coupling cycles the temporary side-chain protection is removed.

[1] P. Sieber, et al. (1991) *Tetrahedron Lett.*, **32**, 739.
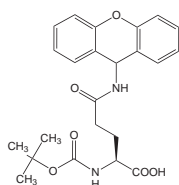
853016	**Boc-Gln(Xan)-OH**	5 g	37.00
	N-α-t.-Boc-γ-xanthyl-L-glutamine	25 g	135.00
	NBC No.: 04-12-0054; CAS No.: 55260-24-7; $C_{23}H_{26}N_2O_6$; M.W.: 426.5	100 g	411.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

Optical purity: ≥ 99.50% L-enantiomer.

ⓘ Ⓖ 4.2

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Product No.	Product	Quantity	Price

Glycine [Gly, G]
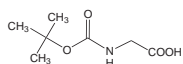
853000	**Boc–Gly–OH**	5 g	11.00
	N-α-t.-Boc-glycine	25 g	31.00
	NBC No.: 04-12-0001; CAS No.: 4530-20-5; $C_7H_{13}NO_4$; M.W.: 175.2	100 g	63.00
	Solubility: 1 mmole in 2 ml DCM.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		

Histidine [His, H]
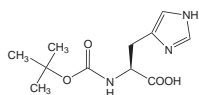
853044	**Boc–His–OH**	5 g	26.00
	N-α-t.-Boc-L-histidine	25 g	104.00
	NBC No.: 04-12-0019; CAS No.: 17791-52-5; $C_{11}H_{17}N_3O_4$; M.W.: 255.3	100 g	312.00
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
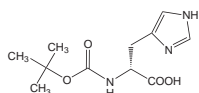
853090	**Boc–D–His–OH**	1 g	40.00
	N-α-t.-Boc-D-histidine	5 g	155.00
	NBC No.: 04-13-0014; CAS No.: 50654-94-9; $C_{11}H_{17}N_3O_4$; M.W.: 255.3	25 g	620.00
	TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
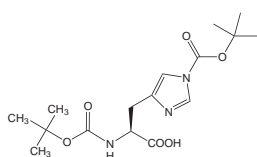
853067	**Boc–His(Boc)–OH · DCHA**	5 g	106.00
	N-α-N-im-di-t.-Boc-L-histidine dicyclohexylammonium salt	25 g	424.00
	NBC No.: 04-12-0124; CAS No.: 31687-58-8; $C_{16}H_{25}N_3O_6 \cdot C_{12}H_{23}N$; M.W.: 355.4 · 181.3		
	TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

A protected histidine derivative for only the critical coupling step. After coupling the two Boc-groups are cleaved simultaneously by TFA.

ⓘ Ⓜ 4.6

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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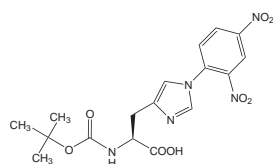
Product No.	Product	Quantity	Price
853008	**Boc-His(Dnp)–OH · isopropanol**	5 g	37.00
	N-α-t.-Boc-N-im-dinitrophenyl-L-histidine isopropanol	25 g	135.00
	NBC No.: 04-12-0023; CAS No.: 25024-53-7; $C_{17}H_{19}N_5O_8$ · C_3H_8O; M.W.: 421.4 · 60.1	100 g	395.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

The Dnp group is stable to HF and TFMSA. Normally removed with thiophenol prior to HF treatment.

ⓘ Ⓖ 4.5, Ⓜ 3.25
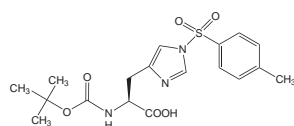
Product No.	Product	Quantity	Price
853041	**Boc-His(Tos)–OH**	5 g	33.00
	N-α-t.-Boc-N-im-tosyl-L-histidine	25 g	133.00
	NBC No.: 04-12-0012; CAS No.: 35899-43-5; $C_{18}H_{23}N_3O_6S$; M.W.: 409.5	100 g	399.00

Solubility: 50 mg in ml EtOH.

TLC: $CHCl_3$:MeOH:AcOH (85:10:5), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

ⓘ Ⓖ 4.5

[1] T. Fujii, et al. (1974) *Bull. Chem. Soc. Jpn.*, **47**, 3146.
[2] J. M. v. d. Eijk, et al. (1980) *J. Org. Chem.*, **45**, 547.
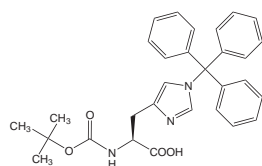
Product No.	Product	Quantity	Price
853034	**Boc-His(Trt)–OH**	1 g	60.00
	N-α-t.-Boc-N-im-trityl-L-histidine	5 g	237.00
	NBC No.: 04-12-0160; CAS No.: 32926-43-5; $C_{30}H_{31}N_3O_4$; M.W.: 497.6	25 g	948.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

Optical purity: ≥ 99.0% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen; hygroscopic.

A protected histidine derivative for only the critical coupling step. After coupling the Boc- and Trt-groups are cleaved simultaneously by TFA.

Hydroxyproline [Hyp]
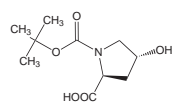
Product No.	Product	Quantity	Price
853026	**Boc-Hyp–OH (cryst.)**	5 g	52.00
	N-α-t.-Boc-L-trans-4-hydroxyproline	25 g	208.00
	NBC No.: 04-12-0104; CAS No.: 13726-69-7; $C_{10}H_{17}NO_5$; M.W.: 231.3	100 g	624.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 97.00%.

$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97.00%.

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Product No.	Product	Quantity	Price
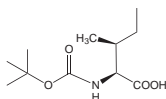
Isoleucine [Ile, I]

853047	**Boc–Ile–OH · 0.5 H$_2$O**	5 g	11.00
	N-α-t.-Boc-L-isoleucine hemihydrate	25 g	31.00
	NBC No.: 04-12-0032; CAS No.: 13139-16-7; C$_{11}$H$_{21}$NO$_4$ · 0.5 H$_2$O; M.W.: 231.3 · 9.0	100 g	89.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

Leucine [Leu, L]
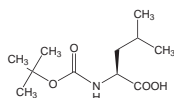
853002	**Boc–Leu–OH · H$_2$O**	5 g	11.00
	N-α-t.-Boc-L-leucine hydrate	25 g	31.00
	NBC No.: 04-12-0005; CAS No.: 13139-15-6; C$_{11}$H$_{21}$NO$_4$ · H$_2$O; M.W.: 231.3 · 18.0	100 g	89.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
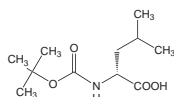
853092	**Boc–D–Leu–OH · H$_2$O**	5 g	42.00
	N-α-t.-Boc-D-leucine hydrate	25 g	166.00
	NBC No.: 04-13-0018; CAS No.: 16937-99-8; C$_{11}$H$_{21}$NO$_4$ · H$_2$O; M.W.: 231.3 · 18.0		

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.
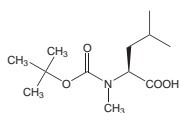
853083	**Boc–N–Me–Leu–OH**	1 g	67.00
	N-α-t.-Boc-N-α-methyl-L-leucine	5 g	266.00
	NBC No.: 04-12-9007; CAS No.: 53363-89-6; C$_{12}$H$_{23}$NO$_4$; M.W.: 245.3		

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CHCl$_3$:MeOH:AcOH:H$_2$O (90:10:0.5:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Lysine [Lys, K]
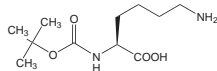
853017	**Boc–Lys–OH**	5 g	78.00
	N-α-t.-Boc-L-lysine	25 g	312.00
	NBC No.: 04-12-0062; CAS No.: 13734-28-6; C$_{11}$H$_{22}$N$_2$O$_4$; M.W.: 246.3		

TLC: EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.

CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

N–α–BOC PROTECTED AMINO ACIDS

⚠ Storage conditions   ⑤ Additional information   ⑩ Method information   ⓐ Application information

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Product No.	Product	Quantity	Price
853076	**Boc-Lys(Ac)-OH**	1 g	52.00
	N-α-t.-Boc-N-ε-acetyl-L-lysine	5 g	208.00
	NBC No.: 04-12-0191; CAS No.: 6404-26-8; $C_{13}H_{24}N_2O_5$; M.W.: 288.4		
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
853053	**Boc-Lys(Boc)-OH · DCHA**	5 g	21.00
	N-α,ε-di-t.-Boc-L-lysine dicyclohexylammonium salt	25 g	81.00
	NBC No.: 04-12-0063; CAS No.: 15098-69-8; $C_{16}H_{30}N_2O_6$ · $C_{12}H_{23}N$; M.W.: 346.4 · 181.3		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	This derivative is supplied as a DCHA-salt since the free acid is not crystalline.		
	ⓘ Ⓜ 4.5		
853018	**Boc-Lys(2-Cl-Z)-OH (cryst.)**	5 g	37.00
	N-α-t.-Boc-N-ε-2-chloro-CBZ-L-lysine	25 g	109.00
	NBC No.: 04-12-0068; CAS No.: 54613-99-9; $C_{19}H_{27}N_2O_6Cl$; M.W.: 414.9	100 g	328.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
	The Cl-Z group is more stable to TFA than the Z group [1, 2], so this derivative is preferred to Boc-Lys(Z)-OH in the synthesis of longer peptides.		
	[1] K. Noda, et al. (1969) *Bull. Chem. Soc. Jpn.*, **43**, 1883.		
	[2] B. W. Erickson, et al. (1973) *J. Am. Chem. Soc.*, **95**, 3757.		
853103	**Boc-D-Lys(2-Cl-Z)-OH (cryst.)**	1 g	42.00
	N-α-t.-Boc-N-ε-2-chloro-CBZ-D-lysine	5 g	166.00
	NBC No.: 04-13-0046; CAS No.: 57096-11-4; $C_{19}H_{27}N_2O_6Cl$; M.W.: 414.9	25 g	666.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
853019	**Boc-Lys(Fmoc)-OH**	1 g	42.00
	N-α-t.-Boc-N-ε-Fmoc-L-lysine	5 g	166.00
	NBC No.: 04-12-0069; CAS No.: 84624-27-1; $C_{26}H_{32}N_2O_6$; M.W.: 468.6	25 g	666.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		

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Product No.	Product	Quantity	Price

**853081** **Boc-Lys(Me)₂-OH**

| | 1 g | 260.00 |
| | 5 g | 1035.00 |

NBC No.: 04-12-0244; CAS No.: 65671-53-6; $C_{13}H_{26}N_2O_4$; M.W.: 274.4

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.

Boc-Lys(Me)₂OH [1] is a building block for the introduction of the naturally

occurring proteinogenic amino acid N^ϵ,N^ϵ-dimethyl-L-lysine [2].

[1]   F. M. F. Chen & N. L. Benoiton (1978) *Can. J. Biochem.*, **56**, 150.

[2]   W. K. Paik & S. Kim (1971) *Science*, **174**, 114.

853012 **Boc-Lys(Z)-OH (cryst.)**

	5 g	24.00
	25 g	96.00
	100 g	287.00

N-α-t.-Boc-N-ε-benzyloxycarbonyl-L-lysine

NBC No.: 04-12-0031; CAS No.: 2389-45-9; $C_{19}H_{28}N_2O_6$; M.W.: 380.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

Methionine [Met, M]

853054 **Boc-Met-OH**

	5 g	11.00
	25 g	31.00
	100 g	94.00

N-α-t.-Boc-L-methionine

NBC No.: 04-12-0071; CAS No.: 2488-15-5; $C_{10}H_{19}NO_4S$; M.W.: 249.3

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

ⓘ Ⓖ 4.6

853093 **Boc-D-Met-OH**

| | 5 g | 52.00 |
| | 25 g | 208.00 |

N-α-t.-Boc-D-methionine

NBC No.: 04-13-0021; CAS No.: 5241-66-7; $C_{10}H_{19}NO_4S$; M.W.: 249.3

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

853035 **Boc-Met(O)-OH**

	1 g	16.00
	5 g	47.00
	25 g	140.00

N-α-t.-Boc-L-methionine-DL-sulfoxide

NBC No.: 04-12-0167; CAS No.: 34805-21-5; $C_{10}H_{19}NO_5S$; M.W.: 265.3

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

ⓘ Ⓖ 4.6

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Norleucine [Nle]

853060	**Boc-Nle-OH · DCHA**	1 g	26.00
	N-α-t.-Boc-L-norleucine dicyclohexylammonium salt	5 g	104.00
	NBC No.: 04-12-0106; CAS No.: 21947-32-0; $C_{11}H_{21}NO_4 \cdot C_{12}H_{23}N$; M.W.: 231.3 · 181.3	25 g	416.00

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

This derivative is supplied as a DCHA-salt since the free acid is not crystalline.

ⓘ Ⓜ 4.5

Norvaline [Nva]
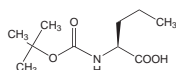
853027	**Boc-Nva-OH**	1 g	21.00
	N-α-t.-Boc-L-norvaline	5 g	78.00
	NBC No.: 04-12-0107; CAS No.: 53308-95-5; $C_{10}H_{19}NO_4$; M.W.: 217.3	25 g	255.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Ornithine [Orn]

853025	**Boc-Orn(Z)-OH**	5 g	41.00
	N-α-t.-Boc-N-δ-benzyloxycarbonyl-L-ornithine	25 g	162.00
	NBC No.: 04-12-0099; CAS No.: 2480-93-5; $C_{18}H_{26}N_2O_6$; M.W.: 366.4		

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

Phenylalanine [Phe, F]

853006	**Boc-Phe-OH**	5 g	11.00
	N-α-t.-Boc-L-phenylalanine	25 g	31.00
	NBC No.: 04-12-0021; CAS No.: 13734-34-4; $C_{14}H_{19}NO_4$; M.W.: 265.3	100 g	63.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

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Product No.	Product	Quantity	Price
853094	**Boc–D–Phe–OH**	5 g	33.00
	N-α-t.-Boc-D-phenylalanine	25 g	133.00
	NBC No.: 04-13-0024; CAS No.: 18942-49-9; $C_{14}H_{19}NO_4$; M.W.: 265.3	100 g	399.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 98.00%.
Optical purity: ≥ 99.50% D-enantiomer.

853084	**Boc–N–Me–Phe–OH · DCHA**	1 g	67.00
	N-α-t.-Boc-N-α-methyl-L-phenylalanine dicyclohexylammonium salt	5 g	266.00
	NBC No.: 04-12-9010; CAS No.: 40163-88-0; $C_{15}H_{21}NO_4 \cdot C_{12}H_{23}N$; M.W.: 279.3 · 181.3		

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CHCl$_3$:MeOH:AcOH:H$_2$O (90:10:0.5:1), purity: ≥ 98.00%.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
This derivative is supplied as a DCHA-salt since the free acid is not crystalline.
ⓘ Ⓖ 4.5

Phenylglycine [Phg]
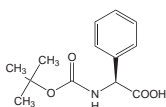
853061	**Boc–Phg–OH**	1 g	42.00
	N-α-t.-Boc-L-phenylglycine	5 g	166.00
	NBC No.: 04-12-0108; CAS No.: 2900-27-8; $C_{13}H_{17}NO_4$; M.W.: 251.3	25 g	666.00

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

853101	**Boc–D–Phg–OH**	1 g	42.00
	N-α-t.-Boc-D-phenylglycine	5 g	166.00
	NBC No.: 04-13-0039; CAS No.: 33125-05-2; $C_{13}H_{17}NO_4$; M.W.: 251.3	25 g	666.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

Proline [Pro, P]

853003	**Boc–Pro–OH**	5 g	11.00
	N-α-t.-Boc-L-proline	25 g	31.00
	NBC No.: 04-12-0010; CAS No.: 15761-39-4; $C_{10}H_{17}NO_4$; M.W.: 215.3	100 g	63.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

⚠ Storage conditions　　Ⓖ Additional information　　Ⓜ Method information　　Ⓐ Application information

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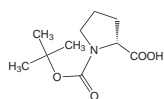
Product No.	Product	Quantity	Price
853095	**Boc-D-Pro-OH**	1 g	26.00
	N-α-t.-Boc-D-proline	5 g	104.00
	NBC No.: 04-13-0027; CAS No.: 37784-17-1; $C_{10}H_{17}NO_4$; M.W.: 215.3	25 g	416.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
853069	**Boc-3,4-dehydro-Pro-OH**	1 g	255.00
	N-α-t.-Boc-3,4-dehydro-L-proline		
	NBC No.: 04-12-0128; CAS No.: 51154-06-4; $C_{10}H_{15}NO_4$; M.W.: 213.2		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 97.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 97.00%.		
	Useful amino acid for incorporation into biologically active peptides, e.g. oxytocin, bradykinin, and ACE-inhibitors.		

Sarcosine [Sar]
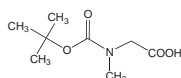
Product No.	Product	Quantity	Price
853062	**Boc-Sar-OH**	5 g	17.00
	N-α-t.-Boc-sarcosine	25 g	67.00
	NBC No.: 04-12-0109; CAS No.: 13734-36-6; $C_8H_{15}NO_4$; M.W.: 189.2	100 g	200.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		

Serine [Ser, S]
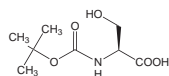
Product No.	Product	Quantity	Price
853020	**Boc-Ser-OH**	5 g	21.00
	N-α-t.-Boc-L-serine	25 g	78.00
	NBC No.: 04-12-0078; CAS No.: 3262-72-4; $C_8H_{15}NO_5$; M.W.: 205.2	100 g	255.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	Optical purity: ≥ 99.00% L-enantiomer.		
853096	**Boc-D-Ser-OH**	1 g	21.00
	N-α-t.-Boc-D-serine	5 g	83.00
	NBC No.: 04-13-0028; CAS No.: 6368-20-3; $C_8H_{15}NO_5$; M.W.: 205.2	25 g	312.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.		
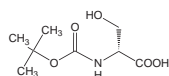
customer service: service@novabiochem.com   technical service: technical@novabiochem.com internet: novabiochem.com
Bulk quantities available, please enquire

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Product No.	Product	Quantity	Price
853009	**Boc-Ser(Bzl)–OH**	5 g	31.00
	N-α-t.-Boc-O-benzyl-L-serine	25 g	94.00
	NBC No.: 04-12-0024; CAS No.: 23680-31-1; $C_{15}H_{21}NO_5$; M.W.: 295.3	100 g	281.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
853111	**Boc-D-Ser(Bzl)–OH**	1 g	47.00
	N-α-t.-Boc-O-benzyl-D-serine	5 g	140.00
	NBC No.: 04-13-0065; CAS No.: 47173-80-8; $C_{15}H_{21}NO_5$; M.W.: 295.3	25 g	562.00
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
853021	**Boc-Ser(tBu)–OH · DCHA**	1 g	33.00
	N-α-t.-Boc-O-t.-butyl-L-serine	5 g	133.00
	NBC No.: 04-12-0081; CAS No.: 18942-50-2; $C_{12}H_{23}NO_5$ · $C_{12}H_{23}N$; M.W.: 261.3 · 181.3	25 g	532.00
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	This derivative is supplied as a DCHA-salt since the free acid is not crystalline.		
	ⓘ Ⓜ 4.5		
853073	**Boc-Ser(Me)–OH**	1 g	203.00
	N-α-t.-Boc-O-methyl-L-serine	5 g	811.00
	NBC No.: 04-12-0186; CAS No.: 51293-47-1; $C_9H_{17}NO_5$; M.W.: 219.2		
	Solubility: 1 mmole in 2 ml DMF clearly soluble.		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	Replacement for Methionine.		

Tetrahydroisoquinoline-3-carboxylic acids [Tic]

Product No.	Product	Quantity	Price
853079	**Boc-Tic-OH**	1 g	138.00
	N-1-t.-Boc-L-1,2,3,4,-tetrahydro-isoquinoline-3-carboxylic acid	5 g	553.00
	NBC No.: 04-12-0214; CAS No.: 78879-20-6; $C_{15}H_{19}NO_4$; M.W.: 277.3		
	TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.		

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

N-α-BOC PROTECTED AMINO ACIDS

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Product No.	Product	Quantity	Price
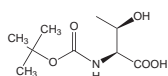
Threonine [Thr, T]

853065 **Boc–Thr–OH**

N-α-t.-Boc-L-threonine

NBC No.: 04-12-0118; CAS No.: 2592-18-9; $C_9H_{17}NO_5$; M.W.: 219.2

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

Optical purity: ≥ 99.00% L-enantiomer.

5 g	26.00
25 g	104.00
100 g	312.00
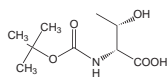
853102 **Boc–D–Thr–OH**

N-α-t.-Boc-D-threonine / (2R,3S)

NBC No.: 04-13-0041; CAS No.: 55674-67-4; $C_9H_{17}NO_5$; M.W.: 219.2

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

1 g	21.00
5 g	83.00
25 g	312.00
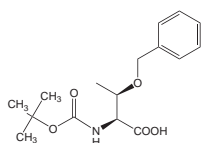
853004 **Boc–Thr(Bzl)–OH**

N-α-t.-Boc-O-benzyl-L-threonine

NBC No.: 04-12-0015; CAS No.: 15260-10-3; $C_{16}H_{23}NO_5$; M.W.: 309.4

Solubility: 1 mmole in 2 ml DCM.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

5 g	31.00
25 g	109.00
100 g	328.00
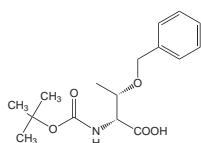
853112 **Boc–D–Thr(Bzl)–OH**

N-α-t.-Boc-O-benzyl-D-threonine / (2R,3S)

NBC No.: 04-13-0066; CAS No.: 69355-99-3; $C_{16}H_{23}NO_5$; M.W.: 309.4

Solubility: 1 mmole in 2 ml DCM.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

1 g	42.00
5 g	166.00
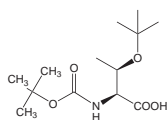
853055 **Boc–Thr(tBu)–OH**

N-α-t.-Boc-O-t.-butyl-L-threonine

NBC No.: 04-12-0084; CAS No.: 13734-40-2; $C_{13}H_{25}NO_5$; M.W.: 275.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

1 g	33.00
5 g	130.00
25 g	515.00

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Product No.	Product	Quantity	Price

Tryptophan [Trp, W]
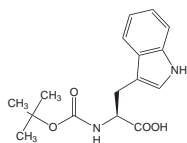
853038 **Boc-Trp-OH**

N-α-t.-Boc-L-tryptophan

NBC No.: 04-12-0006; CAS No.: 13139-14-5; $C_{16}H_{20}N_2O_4$; M.W.: 304.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

ⓘ Ⓖ 4.6

5 g	16.00
25 g	57.00
100 g	166.00
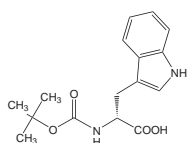
853086 **Boc-D-Trp-OH**

N-α-t.-Boc-D-tryptophan

NBC No.: 04-13-0001; CAS No.: 5241-64-5; $C_{16}H_{20}N_2O_4$; M.W.: 304.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

5 g	33.00
25 g	133.00
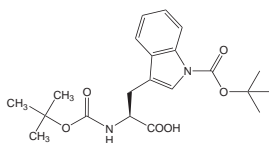
853078 **Boc-Trp(Boc)-OH**

N-α-t.-Boc-N-in-t.-Boc-L-trypophan

NBC No.: 04-12-0205; CAS No.: 144599-95-1; $C_{21}H_{28}N_2O_6$; M.W.: 404.5

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

N-in-Boc protection limits reattachment and leads to higher cleavage yields of C-terminal Trp-containing peptides.

ⓘ Ⓖ 4.6

5 g	138.00
25 g	553.00

853022 **Boc-Trp(For)-OH**

N-α-t.-Boc-N-in-formyl-L-tryptophan

NBC No.: 04-12-0087; CAS No.: 47355-10-2; $C_{17}H_{20}N_2O_5$; M.W.: 332.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

The formyl group is stable to standard high HF cleavage but can be removed by HF containing thiophenol [1-3]. The formyl group can also be removed with piperidine in DMF prior to HF cleavage.

ⓘ Ⓖ 4.6, Ⓜ 3.25

[1]   M. Ohno, et al. (1973) *Bull. Chem. Soc. Jpn.*, **46**, 3280.

[2]   W. F. Heath, et al. (1982) *J. Chem. Soc., Chem. Commun.*, 896.

[3]   G. R. Matsueda (1982) *Int. J. Peptide Protein Res.*, **20**, 26.

5 g	42.00
25 g	135.00
100 g	520.00

N-α-BOC PROTECTED AMINO ACIDS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Tyrosine [Tyr, Y]
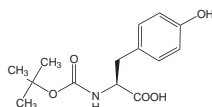
853043	**Boc-Tyr-OH**	5 g 26.00
	N-α-t.-Boc-L-tyrosine	25 g 104.00
	NBC No.: 04-12-0016; CAS No.: 3978-80-1; $C_{14}H_{19}NO_5$; M.W.: 281.3	100 g 312.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
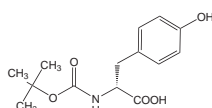
853099	**Boc-D-Tyr-OH**	1 g 30.00
	N-α-t.-Boc-D-tyrosine	5 g 121.00
	NBC No.: 04-13-0033; CAS No.: 70642-86-3; $C_{14}H_{19}NO_5$; M.W.: 281.3	25 g 483.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

853056	**Boc-Tyr(Bzl)-OH**	5 g 21.00
	N-α-t.-Boc-O-benzyl-L-tyrosine	25 g 81.00
	NBC No.: 04-12-0090; CAS No.: 2130-96-3; $C_{21}H_{25}NO_5$; M.W.: 371.4	100 g 243.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

853097	**Boc-D-Tyr(Bzl)-OH**	1 g 31.00
	N-α-t.-Boc-O-benzyl-D-tyrosine	5 g 125.00
	NBC No.: 04-13-0030; CAS No.: 63769-58-4; $C_{21}H_{25}NO_5$; M.W.: 371.4	25 g 499.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

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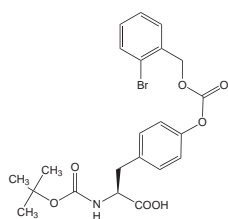
Product No.	Product	Quantity	Price
853024	**Boc-Tyr(2-Br-Z)-OH**	5 g	37.00
	N-α-t.-Boc-O-2-bromobenzyloxycarbonyl-L-tyrosine	25 g	109.00
	NBC No.: 04-12-0094; CAS No.: 47689-67-8; $C_{22}H_{24}NO_7Br$; M.W.: 494.3	100 g	328.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
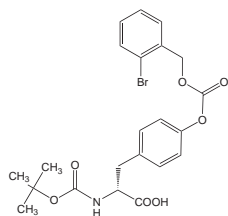
Product No.	Product	Quantity	Price
853104	**Boc-D-Tyr(2-Br-Z)-OH**	1 g	52.00
	N-α-t.-Boc-O-2-bromobenzyloxycarbonyl-D-tyrosine	5 g	208.00
	NBC No.: 04-13-0050; CAS No.: 81189-61-9; $C_{22}H_{24}NO_7Br$; M.W.: 494.3		

Solubility: 2 mmole in 2 ml DCM.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
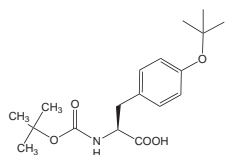
Product No.	Product	Quantity	Price
853023	**Boc-Tyr(tBu)-OH**	1 g	33.00
	N-α-t.-Boc-O-t.-butyl-L-tyrosine	5 g	133.00
	NBC No.: 04-12-0093; CAS No.: 47375-34-8; $C_{18}H_{27}NO_5$; M.W.: 337.4	25 g	532.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
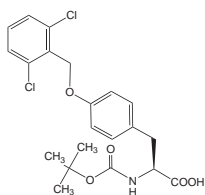
Product No.	Product	Quantity	Price
853042	**Boc-Tyr(2,6-di-Cl-Bzl)-OH**	5 g	52.00
	N-α-t.-Boc-O-2,6-dichlorobenzyl-L-tyrosine	25 g	208.00
	NBC No.: 04-12-0014; CAS No.: 40298-71-3; $C_{21}H_{23}NO_5Cl_2$; M.W.: 440.3	100 g	624.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
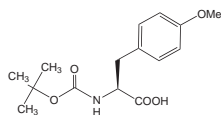
Product No.	Product	Quantity	Price
853071	**Boc-Tyr(Me)-OH**	1 g	52.00
	N-α-t.-Boc-O-methyl-L-tyrosine	5 g	208.00
	N-α-t.-Boc-p-methoxy-L-phenylalanine		
	NBC No.: 04-12-0165; CAS No.: 53267-93-9; $C_{15}H_{21}NO_5$; M.W.: 295.4		

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH (85:10:5), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

N-α-BOC PROTECTED AMINO ACIDS

⚠ Storage conditions　　ⓖ Additional information　　ⓜ Method information　　ⓐ Application information

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Product No.	Product	Quantity	Price

Valine [Val, V]

853011 **Boc-Val-OH**
N-α-t.-Boc-L-valine
NBC No.: 04-12-0029; CAS No.: 13734-41-3; $C_{10}H_{19}NO_4$; M.W.: 217.3
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

5 g	11.00
25 g	31.00
100 g	63.00
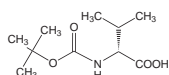
853098 **Boc-D-Val-OH**
N-α-t.-Boc-D-valine
NBC No.: 04-13-0031; CAS No.: 22838-58-0; $C_{10}H_{19}NO_4$; M.W.: 217.3
Solubility: 1 mmole in 2 ml DMF clearly soluble.
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

5 g	47.00
25 g	140.00
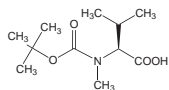
853085 **Boc-N-Me-Val-OH**
N-α-t.-Boc-N-α-methyl-L-valine
NBC No.: 04-12-9013; CAS No.: 45170-31-8; $C_{11}H_{21}NO_4$; M.W.: 231.3
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
$CHCl_3$:MeOH (9:1), purity: ≥ 98.00%.

1 g	67.00
5 g	266.00

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Product No.	Product	Quantity	Price

Other amino acid derivatives

Alanine [Ala, A]

	854084	**H–Ala–NH$_2$ · HCl**	1 g	24.00
		L-Alanine amide hydrochloride	5 g	96.00
		NBC No.: 04-12-5043; CAS No.: 33208-99-0; C$_3$H$_8$N$_2$O · HCl; M.W.: 88.1 · 36.5	25 g	383.00
		TLC: EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.		
		nBuOH:AcOH:H$_2$O (2:1:1), purity: ≥ 98.00%.		

	854165	**H–D–Ala–NH$_2$ · HCl**	1 g	47.00
		D-Alanine amide hydrochloride	5 g	187.00
		NBC No.: 04-13-5004; CAS No.: 71810-97-4; C$_3$H$_8$N$_2$O · HCl; M.W.: 88.1 · 36.5	25 g	749.00
		TLC: CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
		EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.		
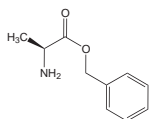
	854086	**H–Ala–OBzl · p-tosylate**	5 g	24.00
		L-Alanine benzyl ester tosylate	25 g	96.00
		NBC No.: 04-12-5045; CAS No.: 42854-62-6; C$_{10}$H$_{13}$NO$_2$ · C$_7$H$_8$O$_3$S; M.W.: 179.2 · 172.2		
		TLC: EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.		
		CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
		Optical purity: ≥ 99.00% L-enantiomer.		
		⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
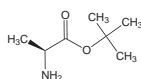
	854072	**H–Ala–OtBu · HCl**	5 g	83.00
		L-Alanine t.-butyl ester hydrochloride	25 g	333.00
		NBC No.: 04-12-5019; CAS No.: 13404-22-3; C$_7$H$_{15}$NO$_2$ · HCl; M.W.: 145.2 · 36.5		
		TLC: EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.		
		EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.		
		⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		

	854080	**H–Ala–OMe · HCl**	5 g	19.00
		L-Alanine methyl ester hydrochloride	25 g	75.00
		NBC No.: 04-12-5029; CAS No.: 2491-20-5; C$_4$H$_9$NO$_2$ · HCl; M.W.: 103.1 · 36.5		
		TLC: CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 97.00%.		
		EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 97.00%.		
		⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		

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Product No.	Product	Quantity	Price
854168	**H–D-Ala-OMe · HCl**	5 g	93.00
	D-Alanine methyl ester hydrochloride	25 g	370.00
	NBC No.: 04-13-5007; CAS No.: 14316-06-4; $C_4H_9NO_2$ · HCl; M.W.: 103.1 · 36.5		
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
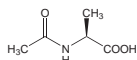
854149	**Ac-Ala-OH**	1 g	24.00
	N-α-Acetyl-L-alanine	5 g	96.00
	NBC No.: 04-12-8000; CAS No.: 97-69-8; $C_5H_9NO_3$; M.W.: 131.1	25 g	383.00
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
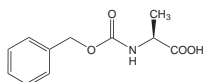
854025	**Z-Ala-OH**	25 g	37.00
	N-α-CBZ-L-alanine	100 g	109.00
	NBC No.: 04-12-0510; CAS No.: 1142-20-7; $C_{11}H_{13}NO_4$; M.W.: 223.2		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
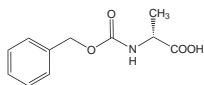
854152	**Z-D-Ala-OH**	5 g	56.00
	N-α-CBZ-D-alanine	25 g	225.00
	NBC No.: 04-13-0500; CAS No.: 26607-51-2; $C_{11}H_{13}NO_4$; M.W.: 223.2	100 g	674.00
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
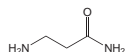
854085	**H–β-Ala-NH$_2$ · HCl**	1 g	24.00
	β-Alanine amide hydrochloride	5 g	96.00
	NBC No.: 04-12-5044; CAS No.: 64017-81-8; $C_3H_8N_2O$ · HCl; M.W.: 88.1 · 36.5	25 g	383.00
	TLC: nBuOH:AcOH:H_2O (2:1:1), purity: ≥ 98.00%.		
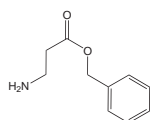
854127	**H–β-Ala-OBzl · p-tosylate**	5 g	24.00
	β-Alanine benzyl ester tosylate	25 g	96.00
	NBC No.: 04-12-5217; CAS No.: 27019-47-2; $C_{10}H_{13}NO_2$ · $C_7H_8O_3S$; M.W.: 179.2 · 172.2		
	TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		

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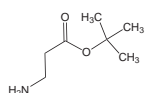
Product No.	Product	Quantity	Price

854128 **H–β–Ala–OtBu · HCl** 5 g 83.00

β-Alanine t.-butyl ester hydrochloride 25 g 333.00

NBC No.: 04-12-5218; CAS No.: 58620-93-2; $C_7H_{15}NO_2$ · HCl; M.W.: 145.2 · 36.5

TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
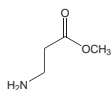
854087 **H–β–Ala–OMe · HCl** 5 g 19.00

β-Alanine methyl ester hydrochloride 25 g 75.00

NBC No.: 04-12-5047; CAS No.: 3196-73-4; $C_4H_9NO_2$ · HCl; M.W.: 103.1 · 36.5

TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
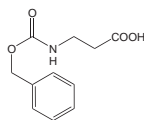
854034 **Z–β–Ala–OH** 25 g 56.00

N-β-CBZ-β-alanine 100 g 168.00

NBC No.: 04-12-0532; CAS No.: 2304-94-2; $C_{11}H_{13}NO_4$; M.W.: 223.2

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

Aminohexanoic acid [ε-Ahx]
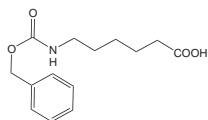
854035 **Z–ε–Ahx–OH** 5 g 22.00

N-ε-CBZ-ε-aminocaproic acid 25 g 88.00

Z-6-Aminohexanoic acid 100 g 262.00

NBC No.: 04-12-0534; CAS No.: 1947-00-8; $C_{14}H_{19}NO_4$; M.W.: 265.3

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

OTHER AMINO ACIDS

⚠ Storage conditions   ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Arginine [Arg, R]
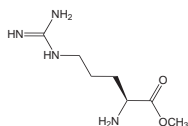
854088 **H–Arg–OMe · 2HCl** 5 g 28.00
L-Arginine methyl ester dihydrochloride 25 g 112.00
NBC No.: 04-12-5058; CAS No.: 26340-89-6; $C_7H_{16}N_4O_2 \cdot$ 2HCl; M.W.: 188.2 · 73.0
TLC: nBuOH:AcOH:H_2O (2:1:1), purity: ≥ 98.00%.
EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.
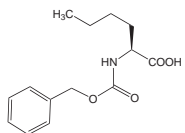⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
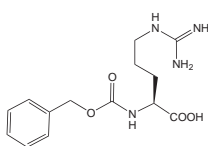
854022 **Z–Arg–OH** 25 g 37.00
N-α-CBZ-L-arginine 100 g 109.00
NBC No.: 04-12-0506; CAS No.: 1234-35-1; $C_{14}H_{20}N_4O_4$; M.W.: 308.3
TLC: CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 97.00%.
nBuOH:AcOH:H_2O (2:1:1), purity: ≥ 97.00%.
Optical purity: ≥ 99.50% L-enantiomer.

Asparagine [Asn, N]
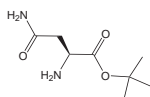
854103 **H–Asn–OtBu · HCl** 1 g 73.00
L-Asparagine t.-butyl ester hydrochloride 5 g 291.00
NBC No.: 04-12-5110; CAS No.: 63094-81-5; $C_8H_{16}N_2O_3 \cdot$ HCl; M.W.: 188.2 · 36.5
TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.
CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
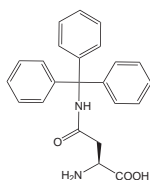
854143 **H–Asn(Trt)–OH** 5 g 208.00
N-β-Trityl-L-asparagine 25 g 832.00
NBC No.: 04-12-5268; CAS No.: 132388-58-0; $C_{23}H_{22}N_2O_3$; M.W.: 374.4
TLC: CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.
EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.
Optical purity: ≥ 99.00% L-enantiomer.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
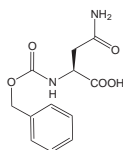
854029 **Z–Asn–OH** 25 g 56.00
N-α-CBZ-L-asparagine 100 g 168.00
NBC No.: 04-12-0521; CAS No.: 2304-96-3; $C_{12}H_{14}N_2O_5$; M.W.: 266.3
TLC: CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.
CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.
HPLC: purity: ≥ 98.00%.

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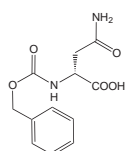
Product No.	Product	Quantity	Price
854154	**Z–D–Asn–OH**	5 g	93.00
	N-α-CBZ-D-asparagine	25 g	370.00
	NBC No.: 04-13-0509; CAS No.: 4474-86-6; $C_{12}H_{14}N_2O_5$; M.W.: 266.3	100 g	988.00
	TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
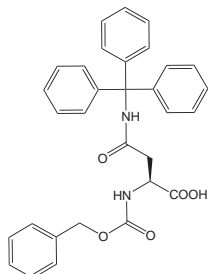
Product No.	Product	Quantity	Price
854058	**Z–Asn(Trt)–OH**	1 g	37.00
	N-α-CBZ-β-trityl-L-asparagine	5 g	146.00
	NBC No.: 04-12-0626; CAS No.: 132388-57-9; $C_{31}H_{28}N_2O_5$; M.W.: 508.6	25 g	582.00
	TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		

⚠ Prolonged storage: +15 to +25°C; keep cool and dry.

[1] P. Sieber, et al. (1991) *Tetrahedron Lett.*, **32**, 739.

Aspartic acid [Asp, D]
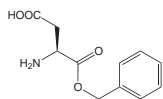
Product No.	Product	Quantity	Price
854089	**H–Asp–OBzl**	5 g	109.00
	L-Aspartic acid α-benzyl ester	25 g	437.00
	NBC No.: 04-12-5063; CAS No.: 7362-93-8; $C_{11}H_{13}NO_4$; M.W.: 223.2		
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 98.00%.		
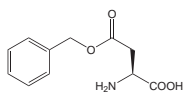
Product No.	Product	Quantity	Price
854090	**H–Asp(OBzl)–OH**	5 g	47.00
	L-Aspartic acid β-benzyl ester	25 g	187.00
	NBC No.: 04-12-5064; CAS No.: 2177-63-1; $C_{11}H_{13}NO_4$; M.W.: 223.2		
	TLC: nBuOH:AcOH:H_2O (2:1:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
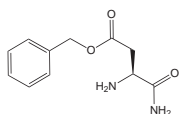
Product No.	Product	Quantity	Price
854132	**H–Asp(OBzl)–NH$_2$ · HCl**	1 g	78.00
	L-Aspartamide β-benzyl ester hydrochloride	5 g	307.00
	NBC No.: 04-12-5230; CAS No.: 19918-68-8; $C_{11}H_{14}N_2O_3$ · HCl; M.W.: 222.3 · 36.5		
	TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
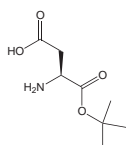
Product No.	Product	Quantity	Price
854126	**H–Asp–OtBu**	1 g	47.00
	L-Aspartic acid α-t.-butyl ester	5 g	187.00
	NBC No.: 04-12-5209; CAS No.: 4125-93-3; $C_8H_{15}NO_4$; M.W.: 189.2	25 g	749.00
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

OTHER AMINO ACIDS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
854061	**H–Asp(OtBu)–OH**	5 g	148.00
	L-Aspartic acid β-t.-butyl ester	25 g	591.00
	NBC No.: 04-12-5000; CAS No.: 3057-74-7; $C_8H_{15}NO_4$; M.W.: 189.2		
	TLC: nBuOH:AcOH:H_2O (2:1:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
854091	**H–Asp(OtBu)–OtBu · HCl**	5 g	109.00
	L-Aspartic acid β-t.-butyl α-t.-butyl ester hydrochloride	25 g	437.00
	NBC No.: 04-12-5066; CAS No.: 1791-13-5; $C_{12}H_{23}NO_4$ · HCl; M.W.: 245.3 · 36.5		
	TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
854092	**H–Asp(OtBu)–OMe · HCl**	1 g	109.00
	L-Aspartic acid β-t.-butyl α-methyl ester hydrochloride	5 g	437.00
	NBC No.: 04-12-5067; CAS No.: 2673-19-0; $C_9H_{17}NO_4$ · HCl; M.W.: 203.2 · 36.5		
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	EtOAc:Pyridine:AcOH:H_2O (60:10:3:4), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
854036	**Z–Asp–OH**	25 g	57.00
	N-α-CBZ-L-aspartic acid	100 g	172.00
	NBC No.: 04-12-0541; CAS No.: 1152-61-0; $C_{12}H_{13}NO_6$; M.W.: 267.2		
	TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
854053	**Z–Asp–OBzl**	5 g	73.00
	N-α-CBZ-L-aspartic acid α-benzyl ester	25 g	291.00
	NBC No.: 04-12-0618; CAS No.: 4779-31-1; $C_{19}H_{19}NO_6$; M.W.: 357.4		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
854037	**Z–Asp(OBzl)–OH**	5 g	73.00
	N-α-CBZ-L-aspartic acid β-benzyl ester	25 g	291.00
	NBC No.: 04-12-0542; CAS No.: 3479-47-8; $C_{19}H_{19}NO_6$; M.W.: 357.4		
	TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	[1] S. Iguchi, et al. (1987) *Int. J. Peptide Protein Res.*, **30**, 695.		

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Product No.	Product	Quantity	Price
854032	**Z–Asp(OtBu)–OH · H$_2$O**	5 g	73.00
	N-α-CBZ-L-aspartic acid β-t.-butyl ester hydrate	25 g	291.00
	NBC No.: 04-12-0529; CAS No.: 5545-52-8; C$_{16}$H$_{21}$NO$_6$ · H$_2$O; M.W.: 323.3 · 18.0	100 g	874.00

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
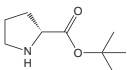
CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.

Product No.	Product	Quantity	Price
854164	**Z–D–Asp(OtBu)–OH · H$_2$O**	1 g	56.00
	N-α-CBZ-D-aspartic acid β-t.-butyl ester hydrate	5 g	225.00
	NBC No.: 04-13-0590; CAS No.: 71449-08-6; C$_{16}$H$_{21}$NO$_6$ · H$_2$O; M.W.: 323.3 · 18.0	25 g	899.00

TLC: CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

CHCl$_3$:MeOH:AcOH (85:10:5), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen; hygroscopic.

## Cyclohexylalanine [Cha]

Product No.	Product	Quantity	Price
854016	**H–Cha–OH · HCl**	1 g	41.00
	L-2-Amino-3-cyclohexyl-propionic acid hydrochloride	5 g	162.00
	β-Cyclohexyl-L-alanine hydrochloride	25 g	649.00
	NBC No.: 04-11-0077; CAS No.: 25528-71-6; C$_9$H$_{17}$NO$_2$ · HCl; M.W.: 171.2 · 36.5		

TLC: nBuOH:AcOH:H$_2$O (2:1:1), purity: ≥ 98.00%.

EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.

Product No.	Product	Quantity	Price
854137	**H–Cha–OMe · HCl**	1 g	56.00
	L-2-Amino-3-cyclohexyl-propionic acid methyl ester hydrochloride	5 g	225.00
	β-Cyclohexyl-L-alanine methyl ester hydrochloride	25 g	899.00
	NBC No.: 04-12-5256; CAS No.: 17193-39-4; C$_{10}$H$_{19}$NO$_2$ · HCl; M.W.: 185.2 · 36.5		

TLC: EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.

CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

[1] G. M. Bonora, et al. (1977) *Gazz. Chim. Ital.*, **107**, 195.

[2] P. F. Schuda, et al. (1988) *J. Org. Chem.*, **53**, 873.

Cysteine [Cys, C]

Product No.	Product	Quantity	Price
854003	**H–D–Cys–OH · HCl · H$_2$O**	5 g	28.00
	D-Cysteine hydrochloride hydrate	25 g	112.00
	NBC No.: 04-10-0057; CAS No.: 32443-99-5; C$_3$H$_7$NO$_2$S · HCl · H$_2$O; M.W.: 121.2 · 36.5 · 18.0	100 g	337.00

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

Product No.	Product	Quantity	€ Price
854094	**H–Cys(Bzl)–OMe · HCl**	5 g	47.00
	S-Benzyl-L-cysteine methyl ester hydrochloride	25 g	187.00
	NBC No.: 04-12-5070; CAS No.: 16741-80-3; $C_{11}H_{15}NO_2S$ · HCl; M.W.: 225.3 · 36.5		
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
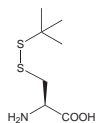
Product No.	Product	Quantity	€ Price
854125	**H–Cys(tButhio)–OH**	5 g	109.00
	S-t.-Butylthio-L-cysteine	25 g	437.00
	NBC No.: 04-12-5204; CAS No.: 30044-51-0; $C_7H_{15}NO_2S_2$; M.W.: 209.3		
	solubility: 2 mmole in 3 ml 1N HCl/40°C.		
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 99.00%.		
	nBuOH:AcOH:H_2O (2:1:1), purity: ≥ 99.00%.		
	Optical purity: ≥ 99.00% L-enantiomer.		
	⚠ Prolonged storage: ≤ -20°C; keep cool and dry.		
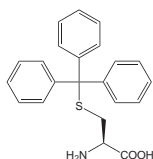
Product No.	Product	Quantity	€ Price
854093	**H–Cys(Trt)–OH**	5 g	47.00
	S-Trityl-L-cysteine	25 g	187.00
	NBC No.: 04-12-5069; CAS No.: 2799-07-7; $C_{22}H_{21}NO_2S$; M.W.: 363.5		
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: ≤ -20°C; keep cool and dry.		

Diaminopropionic acid [Dpr]

Product No.	Product	Quantity	€ Price
854146	**Trt–Dpr(Fmoc)–OH**	1 g	260.00
	N-α-Trityl-N-ε-Fmoc-L-diaminopropionic acid	5 g	1274.00
	NBC No.: 04-12-5312; CAS No.: 1263046-25-8; $C_{37}H_{32}N_2O_4$; M.W.: 568.7		
	TLC: $CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 95.00%.		
	⚠ Prolonged storage: ≤ -20°C		

Trt-Dpr(Fmoc)-OH is an excellent tool for the synthesis of branched, cyclic side-chain modified peptides containing diaminopropionic acid.

The Trt group can be removed using 1% TFA in DCM, allowing selective unmasking of the Dpr α-amine on the solid phase in the presence of the standard t-butyl-based protecting groups. The use of Trt-Dpr(Fmoc)-OH overcomes the problems of scrambling associated with Dde-Dpr(Fmoc)-OH, since unlike Dde, the Trt group does not migrate from the α-amino to β-amino functionality during Fmoc deprotection.

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Product No.	Product	Quantity	Price

Glutamic acid [Glu, E]

854095   **H–Glu–OBzl**   5 g   148.00
L-Glutamic acid α-benzyl ester   25 g   591.00
L-α-Benzyl glutamate
NBC No.: 04-12-5073; CAS No.: 13030-09-6; $C_{12}H_{15}NO_4$; M.W.: 237.3
TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.
CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.
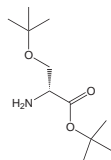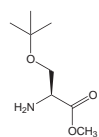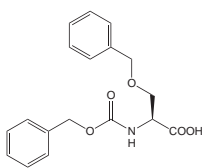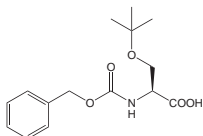⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

854082   **H–Glu(OBzl)–OH**   5 g   47.00
L-Glutamic acid γ-benzyl ester   25 g   187.00
L-γ-Benzyl glutamate
NBC No.: 04-12-5034; CAS No.: 1676-73-9; $C_{12}H_{15}NO_4$; M.W.: 237.3
TLC: CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.
EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

854062   **H–Glu(OtBu)–OH**   5 g   148.00
L-Glutamic acid γ-t.-butyl ester   25 g   591.00
L-γ-t-Butyl glutamate
NBC No.: 04-12-5001; CAS No.: 2419-56-9; $C_9H_{17}NO_4$; M.W.: 203.2
TLC: nBuOH:AcOH:H_2O (2:1:1), purity: ≥ 98.00%.
EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

854096   **H–Glu(OtBu)–OtBu · HCl**   5 g   109.00
L-Glutamic acid di-t.-butyl ester hydrochloride   25 g   437.00
NBC No.: 04-12-5075; CAS No.: 32677-01-3; $C_{13}H_{25}NO_4$ · HCl; M.W.: 259.4 · 36.5
TLC: CHCl$_3$:MeOH:AcOH (85:10:5), purity: ≥ 98.00%.
CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

854097   **H–Glu(OtBu)–OMe · HCl**   1 g   93.00
L-Glutamic acid γ-t.-butyl ester α-methyl ester hydrochloride   5 g   370.00
NBC No.: 04-12-5076; CAS No.: 6234-01-1; $C_{10}H_{19}NO_4$ · HCl; M.W.: 217.3 · 36.5
TLC: CHCl$_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 98.00%.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information
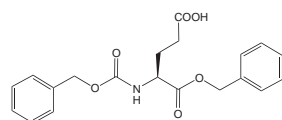
Product No.	Product	Quantity	€ Price
854059	**Z–Glu–OBzl** N-α-CBZ-L-glutamic acid α-benzyl ester NBC No.: 04-12-0628; CAS No.: 3705-42-8; $C_{20}H_{21}NO_6$; M.W.: 371.4 TLC: CHCl$_3$:MeOH (7:3), purity: ≥ 98.00%. CHCl$_3$:MeOH:AcOH:H$_2$O (70:42:0.5:10), purity: ≥ 98.00%.	5 g 25 g	73.00 291.00
854054	**Z–Glu(OBzl)–OH** N-α-CBZ-L-glutamic acid γ-benzyl ester NBC No.: 04-12-0621; CAS No.: 5680-86-4; $C_{20}H_{21}NO_6$; M.W.: 371.4 TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%. CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.	1 g 5 g 25 g	23.00 92.00 366.00
854038	**Z–Glu–OtBu · DCHA** N-α-CBZ-L-glutamic acid α-t.-butyl ester dicyclohexylammonium salt NBC No.: 04-12-0549; CAS No.: 34897-61-5; $C_{17}H_{23}NO_6 \cdot C_{12}H_{23}N$; M.W.: 337.4 · 181.3 TLC: CHCl$_3$:MeOH:AcOH:H$_2$O (90:10:0.5:1), purity: ≥ 98.00%. CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%. This derivative is supplied as a DCHA-salt since the free acid is not crystalline.	5 g 25 g	135.00 541.00
854039	**Z–Glu(OtBu)–OH** N-α-CBZ-L-glutamic acid γ-t.-butyl ester NBC No.: 04-12-0550; CAS No.: 3886-08-6; $C_{17}H_{23}NO_6$; M.W.: 337.4 TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%. CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.	5 g 25 g 100 g	65.00 258.00 754.00
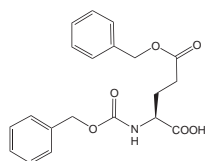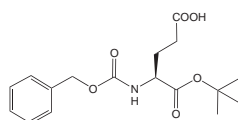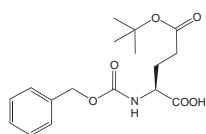
Glutamine [Gln, Q]
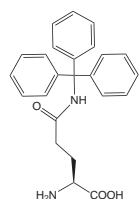
Product No.	Product	Quantity	€ Price
854144	**H–Gln(Trt)–OH** N-γ-Trityl-L-glutamine NBC No.: 04-12-5269; CAS No.: 102747-84-2; $C_{24}H_{24}N_2O_3$; M.W.: 388.5 TLC: EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 97.00%. CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 97.00%. Optical purity: ≥ 99.50% L-enantiomer. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	5 g 25 g	182.00 728.00
854057	**Z–Gln(Trt)–OH** N-α-CBZ-γ-trityl-L-glutamine NBC No.: 04-12-0625; CAS No.: 132388-60-4; $C_{32}H_{30}N_2O_5$; M.W.: 522.6 TLC: CHCl$_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%. CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%. [1] P. Sieber, et al. (1991) *Tetrahedron Lett.*, **32**, 739.	1 g 5 g 25 g	37.00 146.00 582.00
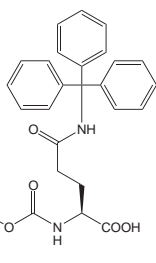
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Product No.	Product	Quantity	Price

Glycine [Gly, G]
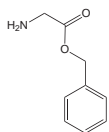
854099   **H–Gly–OBzl · p-tosylate**   5 g   24.00

Glycine benzyl ester tosylate   25 g   96.00

NBC No.: 04-12-5087; CAS No.: 1738-76-7; $C_9H_{11}NO_2 \cdot C_7H_8O_3S$; M.W.: 165.2 · 172.2

TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
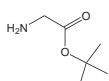
854129   **H–Gly–OtBu · AcOH**   5 g   93.00

Glycine t.-butyl ester acetate   25 g   370.00

NBC No.: 04-12-5224; CAS No.: 38024-18-9; $C_6H_{13}NO_2 \cdot CH_3COOH$; M.W.: 131.2 · 60.1

TLC: nBuOH:AcOH:H_2O (2:1:1), purity: ≥ 98.00%.

EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
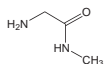
**854098**   **H–Gly–NHMe · HCl**   1 g   73.00

Glycine-N-methylamide hydrochloride   5 g   291.00

NBC No.: 04-12-5086; CAS No.: 49755-94-4; $C_3H_8N_2O \cdot HCl$; M.W.: 88.1 · 36.5

TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
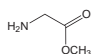
854066   **H–Gly–OMe · HCl**   25 g   19.00

Glycine methyl ester hydrochloride   100 g   54.00

Methyl glycinate hydrochloride

NBC No.: 04-12-5007; CAS No.: 5680-79-5; $C_3H_7NO_2 \cdot HCl$; M.W.: 89.1 · 36.5

TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
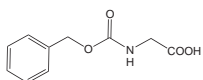
854024   **Z–Gly–OH**   25 g   37.00

N-α-CBZ-glycine   100 g   109.00

NBC No.: 04-12-0509; CAS No.: 1138-80-3; $C_{10}H_{11}NO_4$; M.W.: 209.2

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.

OTHER AMINO ACIDS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

Product No.	Product	Quantity	Price €
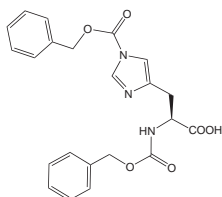
Histidine [His, H]

854040   **Z–His(Z)–OH**
N-α-N-im-di-CBZ-L-histidine
NBC No.: 04-12-0557; CAS No.: 35016-67-2; $C_{22}H_{21}N_3O_6$; M.W.: 423.4
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
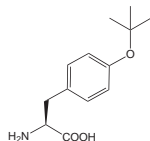CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

	5 g	28.00
	25 g	112.00
	100 g	337.00
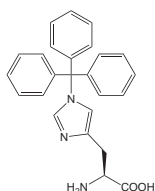
854135   **H–His(Trt)–OH**
N-im-Trityl-L-histidine
NBC No.: 04-12-5241; CAS No.: 35146-32-8; $C_{25}H_{23}N_3O_2$; M.W.: 397.5
TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.
$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

| | 5 g | 93.00 |
| | 25 g | 370.00 |
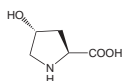
Hydroxyproline [Hyp]

854001   **H–Hyp–OH**
L-4-trans-Hydroxyproline
(2S, 4R)-4-hydroxy-pyrrolidine-2-carboxylic acid
NBC No.: 04-10-0020; CAS No.: 51-35-4; $C_5H_9NO_3$; M.W.: 131.1
Optical purity: ≥ 99.50% L-enantiomer.

| | 25 g | 47.00 |
| | 100 g | 140.00 |
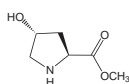
854100   **H–Hyp–OMe · HCl**
L-4-trans-Hydroxyproline methyl ester hydrochloride
NBC No.: 04-12-5096; CAS No.: 40216-83-9; $C_6H_{11}NO_3$ · HCl; M.W.: 145.2 · 36.5
TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.
$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen; hygroscopic.

| | 5 g | 37.00 |
| | 25 g | 146.00 |
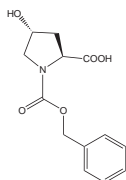
854041   **Z–Hyp–OH**
N-α-CBZ-L-4-trans-hydroxyproline
NBC No.: 04-12-0558; CAS No.: 13504-85-3; $C_{13}H_{15}NO_5$; M.W.: 265.3
TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.
CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

	5 g	47.00
	25 g	187.00
	100 g	562.00

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Product No.	Product	Quantity	Price
854056	**Z–Hyp(tBu)–OH**	1 g	37.00
	N-α-CBZ-O-t.-butyl-L-4-trans-hydroxyproline	5 g	146.00
	NBC No.: 04-12-0624; CAS No.: 85201-91-8; $C_{17}H_{23}NO_5$; M.W.: 321.4	25 g	582.00
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		

Isoleucine [Ile, I]

Product No.	Product	Quantity	Price
854142	**H–Ile–OtBu · HCl**	5 g	73.00
	L-Isoleucine t.-butyl ester hydrochloride	25 g	291.00
	NBC No.: 04-12-5267; CAS No.: 69320-89-4; $C_{10}H_{21}NO_2$ · HCl; M.W.: 187.3 · 36.5		
	TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
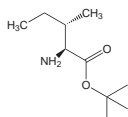
Product No.	Product	Quantity	Price
854079	**H–Ile–OMe · HCl**	5 g	28.00
	L-Isoleucine methyl ester hydrochloride	25 g	112.00
	NBC No.: 04-12-5028; CAS No.: 18598-74-8; $C_7H_{15}NO_2$ · HCl; M.W.: 145.2 · 36.5		
	TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
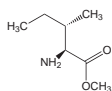
| Product No.	Product	Quantity	Price
854030	**Z–Ile–OH (oil)**	25 g	37.00
	N-α-CBZ-L-isoleucine	100 g	109.00
	NBC No.: 04-12-0522; CAS No.: 3160-59-6; $C_{14}H_{19}NO_4$; M.W.: 265.3		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		

Leucine [Leu, L]
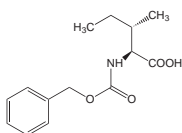
Product No.	Product	Quantity	Price
854101	**H–Leu–OBzl · p-tosylate**	5 g	19.00
	L-Leucine benzyl ester tosylate	25 g	75.00
	NBC No.: 04-12-5107; CAS No.: 1738-77-8; $C_{13}H_{19}NO_2$ · $C_7H_8O_3S$; M.W.: 221.3 · 172.2		
	TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.		
	Optical purity: ≥ 99.50% L-enantiomer.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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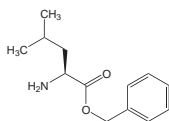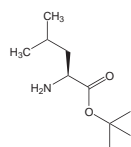
Product No.	Product	Quantity	Price
854102	**H–Leu–OtBu · HCl**	5 g	73.00
	L-Leucine t.-butyl ester hydrochloride	25 g	291.00
	NBC No.: 04-12-5108; CAS No.: 2748-02-9; $C_{10}H_{21}NO_2$ · HCl; M.W.: 187.3 · 36.5		
	TLC: CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
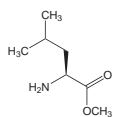
854071	**H–Leu–OMe · HCl**	5 g	14.00
	L-Leucine methyl ester hydrochloride	25 g	54.00
	NBC No.: 04-12-5018; CAS No.: 7517-19-3; $C_7H_{15}NO_2$ · HCl; M.W.: 145.2 · 36.5		
	TLC: CHCl$_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
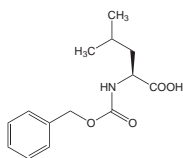
854019	**Z–Leu–OH (oil)**	25 g	37.00
	N-α-CBZ-L-leucine	100 g	109.00
	NBC No.: 04-12-0501; CAS No.: 2018-66-8; $C_{14}H_{19}NO_4$; M.W.: 265.3		
	TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.		

Lysine [Lys, K]
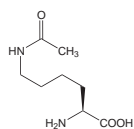
854140	**H–Lys(Ac)–OH**	5 g	73.00
	N-ε-Acetyl-L-lysine	25 g	291.00
	NBC No.: 04-12-5261; CAS No.: 692-04-6; $C_8H_{16}N_2O_3$; M.W.: 188.2		
	TLC: nBuOH:AcOH:H$_2$O (2:1:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.		
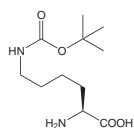
854104	**H–Lys(Boc)–OH**	5 g	148.00
	N-ε-t.-Boc-L-lysine	25 g	591.00
	NBC No.: 04-12-5117; CAS No.: 2418-95-3; $C_{11}H_{22}N_2O_4$; M.W.: 246.3		
	TLC: CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.		
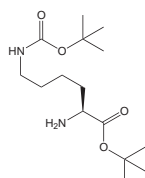
854105	**H–Lys(Boc)–OtBu · HCl**	1 g	56.00
	N-ε-t.-Boc-L-lysine t.-butyl ester hydrochloride	5 g	225.00
	NBC No.: 04-12-5118; CAS No.: 13288-57-8; $C_{15}H_{30}N_2O_4$ · HCl; M.W.: 302.4 · 36.5	25 g	899.00
	TLC: EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.		
	CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		

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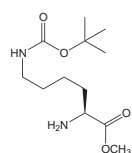
Product No.	Product	Quantity	Price
854106	**H–Lys(Boc)–OMe · HCl**	1 g	47.00
	N-ε-t.-Boc-L-lysine methyl ester hydrochloride	5 g	187.00
	NBC No.: 04-12-5119; CAS No.: 2389-48-2; $C_{12}H_{24}N_2O_4$ · HCl; M.W.: 260.3 · 36.5	25 g	749.00
	TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
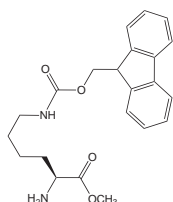
854121	**H–Lys(Fmoc)–OMe · HCl**	1 g	37.00
	N-ε-Fmoc-L-lysine methyl ester hydrochloride	5 g	146.00
	NBC No.: 04-12-5193; CAS No.: 201009-98-5; $C_{22}H_{26}N_2O_4$ · HCl; M.W.: 382.5 · 36.5		
	TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
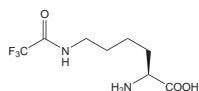
854136	**H–Lys(Tfa)–OH**	5 g	56.00
	N-ε-Trifluoroacetyl-L-lysine	25 g	225.00
	NBC No.: 04-12-5245; CAS No.: 10009-20-8; $C_8H_{13}N_2O_3F_3$; M.W.: 242.2		
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	nBuOH:AcOH:H_2O (2:1:1), purity: ≥ 98.00%.		
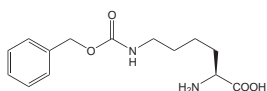
854075	**H–Lys(Z)–OH**	5 g	19.00
	N-ε-CBZ-L-lysine	25 g	75.00
	NBC No.: 04-12-5022; CAS No.: 1155-64-2; $C_{14}H_{20}N_2O_4$; M.W.: 280.3		
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	nBuOH:AcOH:H_2O (2:1:1), purity: ≥ 98.00%.		
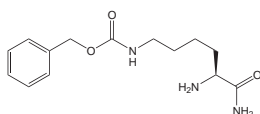
854133	**H–Lys(Z)–NH$_2$ · HCl**	1 g	83.00
	N-ε-CBZ-L-lysine amide hydrochloride	5 g	333.00
	NBC No.: 04-12-5234; CAS No.: 58117-53-6; $C_{14}H_{21}N_3O_3$ · HCl; M.W.: 279.3 · 36.5		
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
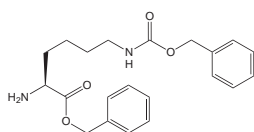
854107	**H–Lys(Z)–OBzl · HCl**	5 g	73.00
	N-ε-CBZ-L-lysine benzyl ester hydrochloride	25 g	291.00
	NBC No.: 04-12-5121; CAS No.: 6366-70-7; $C_{21}H_{26}N_2O_4$ · HCl; M.W.: 370.5 · 36.5		
	TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		

OTHER AMINO ACIDS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
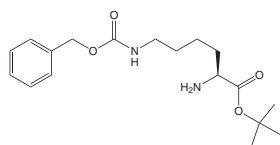
854108   **H-Lys(Z)-OtBu · HCl**   5 g   148.00

N-ε-CBZ-L-lysine t.-butyl ester hydrochloride   25 g   591.00

NBC No.: 04-12-5122; CAS No.: 5978-22-3; $C_{18}H_{28}N_2O_4 \cdot$ HCl; M.W.: 336.4 · 36.5

TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
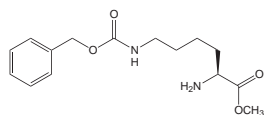
854077   **H-Lys(Z)-OMe · HCl**   5 g   56.00

N-ε-CBZ-L-lysine methyl ester hydrochloride   25 g   225.00

NBC No.: 04-12-5024; CAS No.: 27894-50-4; $C_{15}H_{22}N_2O_4 \cdot$ HCl; M.W.: 294.4 · 36.5

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

854000   **Dde-Lys(Fmoc)-OH**   1 g   177.00

N-α-1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-N-ε-Fmoc-L-lysine   5 g   707.00

NBC No.: 04-12-5201; CAS No.: 156648-40-7; $C_{31}H_{36}N_2O_6$; M.W.: 532.6

solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 97.00%.

$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 96.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

Quasi-orthogonally-protected Lys derivative. The Fmoc group can be removed
selectively by treatment with piperidine; the Dde group is cleaved with 2%
hydrazine in DMF [1]. When removing Dde in the presence of allyl-based
protecting groups, allyl alcohol should be included in the deprotection solution
to prevent reduction of the allyl group [2].
Also available Fmoc-Lys(Dde)-OH 852057.
This derivative has been employed in Fmoc SPPS to facilitate the introduction of
biotin to the side-chain of lysine [3].

ⓘ Ⓖ 4.9, Ⓜ 4.10

[1]   B. W. Bycroft, et al. (1993) *J. Chem. Soc., Chem. Commun.*, 778.

[2]   B. Rohwedder, et al. (1998) *Tetrahedron Lett.*, **39**, 1175.

[3]   J. Mack, et al. (1999) *Lett. Pept. Sci.*, **6**, 135.

854042   **Z-Lys-OH**   5 g   56.00

N-α-CBZ-L-lysine   25 g   225.00

NBC No.: 04-12-0562; CAS No.: 2212-75-1; $C_{14}H_{20}N_2O_4$; M.W.: 280.3   100 g   674.00

TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 97.00%.

$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 97.00%.

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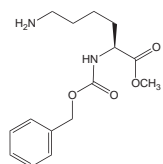
Product No.	Product	Quantity	Price
854052	**Z–Lys–OMe · HCl**	1 g	83.00
	N-α-CBZ-L-lysine methyl ester hydrochloride	5 g	333.00
	NBC No.: 04-12-0607; CAS No.: 26348-68-5; $C_{15}H_{22}N_2O_4$ · HCl; M.W.: 294.4 · 36.4		
	TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 97.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
854031	**Z–Lys(Boc)–OH (cryst.)**	5 g	73.00
	N-α-CBZ-N-ε-t.-Boc-L-lysine	25 g	291.00
	NBC No.: 04-12-0524; CAS No.: 2389-60-8; $C_{19}H_{28}N_2O_6$; M.W.: 380.4	100 g	874.00
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 99.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 99.00%.		
854161	**Z–D–Lys(Boc)–OH (cryst.)**	1 g	65.00
	N-α-CBZ-N-ε-t.-Boc-D-lysine	5 g	258.00
	NBC No.: 04-13-0527; CAS No.: 66845-42-9; $C_{19}H_{28}N_2O_6$; M.W.: 380.4		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
854043	**Z–Lys(Z)–OH**	25 g	83.00
	N-α-N-ε-di-CBZ-L-lysine	100 g	250.00
	NBC No.: 04-12-0565; CAS No.: 405-39-0; $C_{22}H_{26}N_2O_6$; M.W.: 414.5		
	TLC: $CHCl_3$:MeOH:AcOH (85:10:5), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
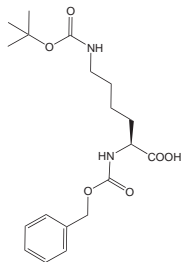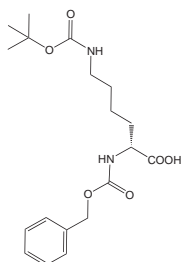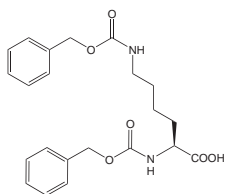
Methionine [Met, M]
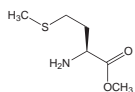
Product No.	Product	Quantity	Price
854076	**H–Met–OMe · HCl**	5 g	31.00
	L-Methionine methyl ester hydrochloride	25 g	130.00
	NBC No.: 04-12-5023; CAS No.: 2491-18-1; $C_6H_{13}NO_2S$ · HCl; M.W.: 163.2 · 36.5		
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
854172	**H–D–Met–OMe · HCl**	5 g	56.00
	D-Methionine methyl ester hydrochloride	25 g	225.00
	NBC No.: 04-13-5026; CAS No.: 69630-60-0; $C_6H_{13}NO_2S$ · HCl; M.W.: 163.2 · 36.5		
	TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
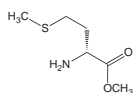
⚠ Storage conditions　　Ⓖ Additional information　　Ⓜ Method information　　Ⓐ Application information
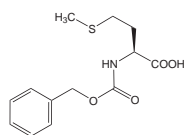
Product No.	Product	Quantity	€ Price
854044	**Z–Met–OH** N-α-CBZ-L-methionine NBC No.: 04-12-0567; CAS No.: 1152-62-1; $C_{13}H_{17}NO_4S$; M.W.: 283.4 TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%. CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.	25 g 100 g	73.00 218.00
854156	**Z–D–Met–OH** N-α-CBZ-D-methionine NBC No.: 04-13-0515; CAS No.: 28862-80-8; $C_{13}H_{13}NO_4S$; M.W.: 283.4 TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%. $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.	5 g 25 g 100 g	73.00 291.00 874.00
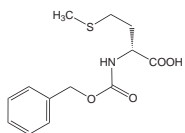
Norleucine [Nle]

Product No.	Product	Quantity	€ Price
854045	**Z–Nle–OH** N-α-CBZ-L-norleucine NBC No.: 04-12-0568; CAS No.: 65081-86-9; $C_{14}H_{19}NO_4$; M.W.: 265.3 TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%. CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.	5 g 25 g	93.00 370.00

Ornithine [Orn]
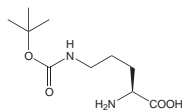
Product No.	Product	Quantity	€ Price
854145	**H–Orn(Boc)–OH** N-δ-t.-Boc-L-ornithine NBC No.: 04-12-5283; CAS No.: 13650-49-2; $C_{10}H_{20}N_2O_4$; M.W.: 232.3 TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%. EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.	5 g 25 g	109.00 437.00
854027	**Z–Orn–OH** N-α-CBZ-L-ornithine NBC No.: 04-12-0514; CAS No.: 2640-58-6; $C_{13}H_{18}N_2O_4$; M.W.: 266.3 TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%. $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.	5 g 25 g	148.00 591.00
854051	**Z–Orn(Boc)–OH (cryst.)** N-α-CBZ-N-δ-t.-Boc-L-ornithine NBC No.: 04-12-0600; CAS No.: 7733-29-1; $C_{18}H_{26}N_2O_6$; M.W.: 366.4 TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%. CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.	5 g 25 g	109.00 437.00
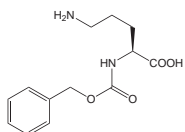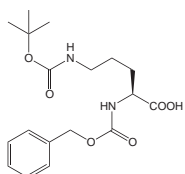
customer service: service@novabiochem.com   technical service: technical@novabiochem.com internet: novabiochem.com
Bulk quantities available, please enquire

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Product No.	Product	Quantity	Price

Phenylalanine [Phe, F]
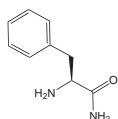
854110	H–Phe–NH$_2$ · HCl	5 g	56.00
	L-Phenylalanine amide hydrochloride	25 g	225.00
	NBC No.: 04-12-5143; CAS No.: 65864-22-4; C$_9$H$_{12}$N$_2$O · HCl; M.W.: 164.2 · 36.5		
	TLC: CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.		
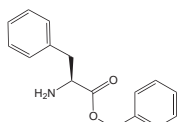
854111	H–Phe–OBzl · HCl	5 g	37.00
	L-Phenylalanine benzyl ester hydrochloride	25 g	146.00
	NBC No.: 04-12-5144; CAS No.: 2462-32-0; C$_{16}$H$_{17}$NO$_2$ · HCl; M.W.: 255.3 · 36.5		
	TLC: CHCl$_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.		
	Optical purity: ≥ 99.50% L-enantiomer.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
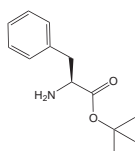
854112	H–Phe–OtBu · HCl	5 g	73.00
	L-Phenylalanine t.-butyl ester hydrochloride	25 g	291.00
	NBC No.: 04-12-5145; CAS No.: 15100-75-1; C$_{13}$H$_{19}$NO$_2$ · HCl; M.W.: 221.3 · 36.5		
	TLC: CHCl$_3$:MeOH:AcOH:H$_2$O (70:42:0.5:10), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
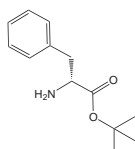
854174	H–D–Phe–OtBu · HCl	5 g	161.00
	D-Phenylalanine t.-butyl ester hydrochloride	25 g	614.00
	NBC No.: 04-13-5034; CAS No.: 3403-25-6; C$_{13}$H$_{19}$NO$_2$ · HCl; M.W.: 221.3 · 36.5		
	TLC: CHCl$_3$:MeOH:AcOH:H$_2$O (90:10:0.5:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
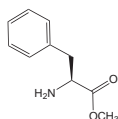
854064	H–Phe–OMe · HCl	5 g	19.00
	L-Phenylalanine methyl ester hydrochloride	25 g	75.00
	NBC No.: 04-12-5004; CAS No.: 7524-50-7; C$_{10}$H$_{13}$NO$_2$ · HCl; M.W.: 179.2 · 36.5		
	TLC: CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
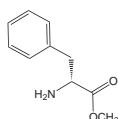
854175	H–D–Phe–OMe · HCl	5 g	56.00
	D-Phenylalanine methyl ester hydrochloride	25 g	225.00
	NBC No.: 04-13-5035; CAS No.: 13033-84-6; C$_{10}$H$_{13}$NO$_2$ · HCl; M.W.: 179.2 · 36.5		
	TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		

OTHER AMINO ACIDS

⚠ Storage conditions   ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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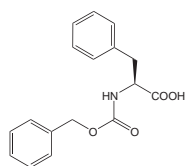
Product No.	Product	Quantity	Price
854018	**Z–Phe–OH**	25 g	56.00
	N-α-CBZ-L-phenylalanine	100 g	168.00
	NBC No.: 04-12-0500; CAS No.: 1161-13-3; $C_{17}H_{17}NO_4$; M.W.: 299.3		
	TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
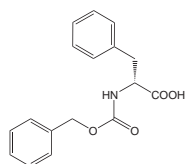
854157	**Z–D–Phe–OH**	5 g	56.00
	N-α-CBZ-D-phenylalanine	25 g	225.00
	NBC No.: 04-13-0516; CAS No.: 2448-45-5; $C_{17}H_{17}NO_4$; M.W.: 299.3	100 g	674.00
	TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.		

Phenylglycine [Phg]
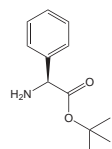
854113	**H–Phg–OtBu · HCl**	1 g	65.00
	L-Phenylglycine t.-butyl ester hydrochloride	5 g	258.00
	NBC No.: 04-12-5147; CAS No.: 161879-12-5; $C_{12}H_{17}NO_2$ · HCl; M.W.: 207.3 · 36.5		
	TLC: CHCl$_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
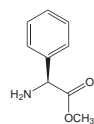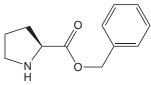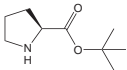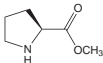
854114	**H–Phg–OMe · HCl**	1 g	42.00
	L-Phenylglycine methyl ester hydrochloride	5 g	161.00
	NBC No.: 04-12-5148; CAS No.: 15028-39-4; $C_9H_{11}NO_2$ · HCl; M.W.: 165.2 · 36.5	25 g	614.00
	TLC: nBuOH:AcOH:H$_2$O (2:1:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
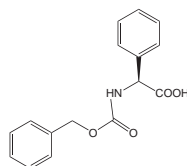
854046	**Z–Phg–OH**	1 g	37.00
	N-α-CBZ-L-phenylglycine	5 g	146.00
	NBC No.: 04-12-0575; CAS No.: 53990-33-3; $C_{16}H_{15}NO_4$; M.W.: 285.3	25 g	582.00
	TLC: CHCl$_3$:MeOH:AcOH (85:10:5), purity: ≥ 98.00%.		
	CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.		

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Product No.	Product	Quantity	Price

Proline [Pro, P]

854115   **H–Pro–NH$_2$**
5 g   93.00
25 g   370.00

L-Proline amide

NBC No.: 04-12-5153; CAS No.: 7531-52-4; C$_5$H$_{10}$N$_2$O; M.W.: 114.1

TLC: nBuOH:AcOH:H$_2$O (2:1:1), purity: ≥ 98.00%.

CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen; hygroscopic.

854116   **H–Pro–OBzl · HCl**
5 g   56.00
25 g   225.00

L-Proline benzyl ester hydrochloride

NBC No.: 04-12-5154; CAS No.: 16652-71-4; C$_{12}$H$_{15}$NO$_2$ · HCl; M.W.: 205.3 · 36.5

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

854117   **H–Pro–OtBu · HCl**
5 g   83.00
25 g   333.00

L-Proline t.-butyl ester hydrochloride

NBC No.: 04-12-5155; CAS No.: 5497-76-7; C$_9$H$_{17}$NO$_2$ · HCl; M.W.: 171.2 · 36.5

TLC: nBuOH:AcOH:H$_2$O (2:1:1), purity: ≥ 98.00%.

EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen; hygroscopic.

854177   **H–D–Pro–OtBu · HCl**
1 g   93.00
5 g   370.00

D-Proline t.-butyl ester hydrochloride

NBC No.: 04-13-5042; CAS No.: 184719-80-0; C$_9$H$_{17}$NO$_2$ · HCl; M.W.: 171.2 · 36.5

TLC: nBuOH:AcOH:H$_2$O (2:1:1), purity: ≥ 98.00%.

EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen; hygroscopic.

854065   **H–Pro–OMe · HCl (cryst.)**
5 g   27.00
25 g   108.00

L-Proline methyl ester hydrochloride

NBC No.: 04-12-5005; CAS No.: 2133-40-6; C$_6$H$_{11}$NO$_2$ · HCl; M.W.: 129.2 · 36.5

TLC: CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

nBuOH:AcOH:H$_2$O (2:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.

OTHER AMINO ACIDS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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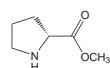
Product No.	Product	Quantity	Price
854178	**H–D–Pro–OMe · HCl** D-Proline methyl ester hydrochloride NBC No.: 04-13-5043; CAS No.: 65365-28-8; $C_6H_{11}NO_2$ · HCl; M.W.: 129.2 · 36.5 TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%. nBuOH:AcOH:H_2O (2:1:1), purity: ≥ 98.00%. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.	1 g 5 g	73.00 291.00
854026	**Z–Pro–OH** N-α-CBZ-L-proline NBC No.: 04-12-0513; CAS No.: 1148-11-4; $C_{13}H_{15}NO_4$; M.W.: 249.3 TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%. CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.	25 g 100 g	56.00 168.00
854158	**Z–D–Pro–OH** N-α-CBZ-D-proline NBC No.: 04-13-0518; CAS No.: 6404-31-5; $C_{13}H_{15}NO_4$; M.W.: 249.3 TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%. CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.	1 g 5 g 25 g	37.00 146.00 582.00
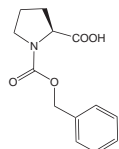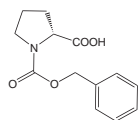
Pyroglutamic acid [Pyr]
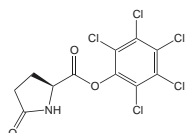
854193	**Pyr–OPcp** L-Pyroglutamatic acid pentachlorophenyl ester Pentachlorophenyl pyroglutamate CAS No.: 28990-85-4; $C_{11}H_6Cl_5NO_3$; M.W.: 377.4 TLC: Toluene:dioxane:AcOH (95:25:4), purity: < 2% DBU ⚠ Prolonged storage: ≤ -20°C; keep cool and dry. Useful derivative for introduction of Pyr without risk of formation Pyr oligomers.	1 g 5 g	52.00 156.00

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Product No.	Product	Quantity	Price

Serine [Ser, S]
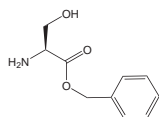
854109	**H–Ser–OBzl · HCl** L-Serine benzyl ester NBC No.: 04-12-5123; CAS No.: 60022-62-0; $C_{10}H_{13}NO_3$ · HCl; M.W.: 195.2 · 36.5 TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.	5 g 25 g	65.00 258.00
854067	**H–Ser–OMe · HCl** L-Serine methyl ester hydrochloride NBC No.: 04-12-5009; CAS No.: 5680-80-8; $C_4H_9NO_3$ · HCl; M.W.: 119.1 · 36.5 TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%. $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	5 g 25 g	24.00 96.00
854179	**H–D–Ser–OMe · HCl** D-Serine methyl ester hydrochloride NBC No.: 04-13-5044; CAS No.: 5874-57-7; $C_4H_9NO_3$ · HCl; M.W.: 119.1 · 36.5 TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%. $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	5 g 25 g	109.00 437.00
854070	**H–Ser(Bzl)–OH** O-Benzyl-L-serine NBC No.: 04-12-5014; CAS No.: 4726-96-9; $C_{10}H_{13}NO_3$; M.W.: 195.2 TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%. nBuOH:AcOH:H_2O (2:1:1), purity: ≥ 98.00%.	5 g 25 g	68.00 270.00
854063	**H–Ser(tBu)–OH** O-t.-Butyl-L-serine NBC No.: 04-12-5002; CAS No.: 18822-58-7; $C_7H_{15}NO_3$; M.W.: 161.2 TLC: nBuOH:AcOH:H_2O (2:1:1), purity: ≥ 98.00%. EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.	5 g 25 g	148.00 591.00
854181	**H–D–Ser(tBu)–OtBu · HCl** O-t.-Butyl-D-serine t.-butyl ester hydrochloride NBC No.: 04-13-5046; CAS No.: 179559-35-4; $C_{11}H_{23}NO_3$ · HCl; M.W.: 217.3 · 36.5 TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%. $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	93.00 370.00
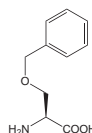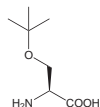
OTHER AMINO ACIDS

⚠ Storage conditions   **G** Additional information   **M** Method information   **A** Application information

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Product No.	Product	Quantity	Price
854073	**H–Ser(tBu)–OMe · HCl**	5 g	109.00
	O-t.-Butyl-L-serine methyl ester hydrochloride	25 g	437.00
	NBC No.: 04-12-5020; CAS No.: 17114-97-5; $C_8H_{17}NO_3$ · HCl; M.W.: 175.2 · 36.5		
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
854182	**H–D–Ser(tBu)–OMe · HCl**	1 g	93.00
	O-t.-Butyl-D-serine methyl ester hydrochloride	5 g	370.00
	NBC No.: 04-13-5047; CAS No.: 78537-14-1; $C_8H_{17}NO_3$ · HCl; M.W.: 175.2 · 36.5		
	TLC: CHCl$_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
854047	**Z–Ser–OH**	25 g	73.00
	N-α-CBZ-L-serine	100 g	218.00
	NBC No.: 04-12-0582; CAS No.: 1145-80-8; $C_{11}H_{13}NO_5$; M.W.: 239.2		
	TLC: CHCl$_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
854159	**Z–D–Ser–OH**	5 g	93.00
	N-α-CBZ-D-serine	25 g	370.00
	NBC No.: 04-13-0520; CAS No.: 6081-61-4; $C_{11}H_{13}NO_5$; M.W.: 239.2	100 g	988.00
	TLC: CHCl$_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.		
	CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
854048	**Z–Ser(Bzl)–OH**	5 g	93.00
	N-α-CBZ-O-benzyl-L-serine	25 g	370.00
	NBC No.: 04-12-0584; CAS No.: 20806-43-3; $C_{18}H_{19}NO_5$; M.W.: 329.4		
	TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH$_3$CN:CHCl$_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
854033	**Z–Ser(tBu)–OH**	5 g	73.00
	N-α-CBZ-O-t.-butyl-L-serine	25 g	291.00
	NBC No.: 04-12-0530; CAS No.: 1676-75-1; $C_{15}H_{21}NO_5$; M.W.: 295.3	100 g	874.00
	TLC: CHCl$_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 98.00%.		
	CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.		
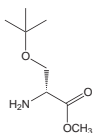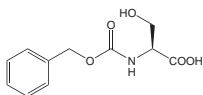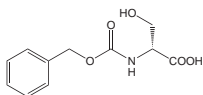
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Product No.	Product	Quantity	Price

Threonine [Thr, T]

854074	**H–Thr–OMe · HCl**	5 g	56.00
	L-Threonine methyl ester hydrochloride	25 g	225.00

NBC No.: 04-12-5021; CAS No.: 39994-75-7; $C_5H_{11}NO_3$ · HCl; M.W.: 133.1 · 36.5

TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen; hygroscopic.
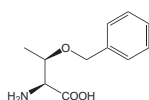
854141	**H–Thr(Bzl)–OH**	5 g	83.00
	O-Benzyl-L-threonine	25 g	333.00

NBC No.: 04-12-5262; CAS No.: 4378-10-3; $C_{11}H_{15}NO_3$; M.W.: 209.2

TLC: nBuOH:AcOH:H_2O (2:1:1), purity: ≥ 98.00%.

EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.
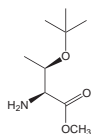
854119	**H–Thr(tBu)–OMe · HCl**	5 g	192.00
	O-t.-Butyl-L-threonine methyl ester hydrochoride	25 g	905.00

NBC No.: 04-12-5176; CAS No.: 71989-43-0; $C_9H_{19}NO_3$ · HCl; M.W.: 189.3 · 36.5

TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
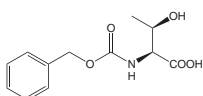
854049	**Z–Thr–OH**	25 g	56.00
	N-α-CBZ-L-threonine	100 g	168.00

NBC No.: 04-12-0586; CAS No.: 19728-63-3; $C_{12}H_{15}NO_5$; M.W.: 253.3

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

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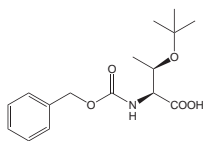
Product No.	Product	Quantity	Price
854020	**Z–Thr(tBu)–OH · DCHA**	5 g	73.00
	N-α-CBZ-O-t.-butyl-L-threonine dicyclohexylammonium salt	25 g	291.00
	NBC No.: 04-12-0502; CAS No.: 16966-07-7; $C_{16}H_{23}NO_5 \cdot C_{12}H_{23}N$; M.W.: 309.4 ·	100 g	874.00
	181.3		
	TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		
	This derivative is supplied as a DCHA–salt since the free acid is not crystalline.		

Tryptophan [Trp, W]
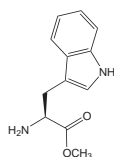
854081	**H–Trp–OMe · HCl**	5 g	28.00
	L-Tryptophan methyl ester hydrochloride	25 g	112.00
	NBC No.: 04-12-5031; CAS No.: 7524-52-9; $C_{12}H_{14}N_2O_2 \cdot$ HCl; M.W.: 218.3 · 36.5		
	TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
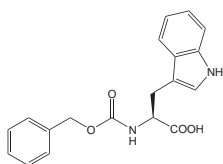
854050	**Z–Trp–OH**	25 g	56.00
	N-α-CBZ-L-tryptophan	100 g	168.00
	NBC No.: 04-12-0591; CAS No.: 7432-21-5; $C_{19}H_{18}N_2O_4$; M.W.: 338.4		
	TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.		

Tyrosine [Tyr, Y]
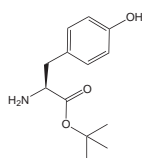
854078	**H–Tyr–OtBu**	5 g	73.00
	L-Tyrosine t.-butyl ester	25 g	291.00
	NBC No.: 04-12-5026; CAS No.: 16874-12-7; $C_{13}H_{19}NO_3$; M.W.: 237.3		
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		
	$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
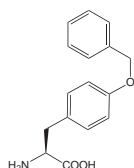
854069	**H–Tyr(Bzl)–OH**	5 g	47.00
	O-Benzyl-L-tyrosine	25 g	187.00
	4-Benzyloxy-L-phenylalanine		
	NBC No.: 04-12-5013; CAS No.: 16652-64-5; $C_{16}H_{17}NO_3$; M.W.: 271.3		
	TLC: nBuOH:AcOH:H_2O (2:1:1), purity: ≥ 98.00%.		
	EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.		

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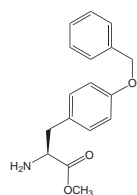
Product No.	Product	Quantity	Price
854122	**H–Tyr(Bzl)–OMe · HCl**	5 g	65.00
	O-Benzyl-L-tyrosine methyl ester hydrochloride	25 g	258.00
	NBC No.: 04-12-5198; CAS No.: 34805-17-9; $C_{17}H_{19}NO_3 \cdot$ HCl; M.W.: 285.3 · 36.5		
	TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: $\geq$ 98.00%.		
	CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: $\geq$ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
854068	**H–Tyr(tBu)–OH**	5 g	148.00
	O-t.-Butyl-L-tyrosine	25 g	591.00
	NBC No.: 04-12-5012; CAS No.: 18822-59-8; $C_{13}H_{19}NO_3$; M.W.: 237.3		
	TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: $\geq$ 98.00%.		
854123	**H–Tyr(tBu)–OtBu · HCl**	1 g	47.00
	O-t.-Butyl-L-tyrosine t.-butyl ester hydrochloride	5 g	187.00
	NBC No.: 04-12-5199; CAS No.: 17083-23-7; $C_{17}H_{27}NO_3 \cdot$ HCl; M.W.: 329.8	25 g	749.00
	TLC: $CHCl_3$:MeOH:AcOH:H_2O (90:10:0.5:1), purity: $\geq$ 95.00%.		
	$CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: $\geq$ 95.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
854124	**H–Tyr(tBu)–OMe · HCl**	5 g	73.00
	O-t.-Butyl-L-tyrosine methyl ester hydrochloride	25 g	291.00
	NBC No.: 04-12-5200; CAS No.: 51482-39-4; $C_{14}H_{21}NO_3 \cdot$ HCl; M.W.: 251.3 · 36.5		
	TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: $\geq$ 98.00%.		
	nBuOH:AcOH:H_2O (2:1:1), purity: $\geq$ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
854021	**Z–Tyr–OH**	25 g	73.00
	N-α-CBZ-L-tyrosine	100 g	218.00
	NBC No.: 04-12-0505; CAS No.: 1164-16-5; $C_{17}H_{17}NO_5$; M.W.: 315.3		
	TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: $\geq$ 98.00%.		
	$CHCl_3$:MeOH:AcOH (90:8:2), purity: $\geq$ 98.00%.		
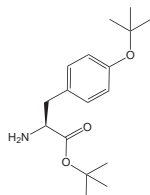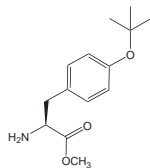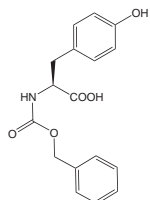
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Product No.	Product	Quantity	Price

Valine [Val, V]
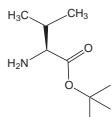
	854134	**H–Val–OtBu · HCl**	5 g	73.00
		L-Valine t.-butyl ester hydrochloride	25 g	291.00

NBC No.: 04-12-5238; CAS No.: 13518-40-6; $C_9H_{19}NO_2$ · HCl; M.W.: 173.3 · 36.5

TLC: EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
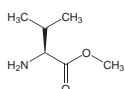
	854118	**H–Val–OMe · HCl**	5 g	19.00
		L-Valine methyl ester hydrochloride	25 g	75.00

NBC No.: 04-12-5156; CAS No.: 6306-52-1; $C_6H_{13}NO_2$ · HCl; M.W.: 131.2 · 36.5

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

EtOAc:nBuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
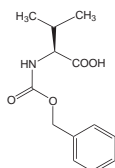
	854023	**Z–Val–OH**	25 g	56.00
		N-α-CBZ-L-valine	100 g	168.00

NBC No.: 04-12-0507; CAS No.: 1149-26-4; $C_{13}H_{17}NO_4$; M.W.: 251.3

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.
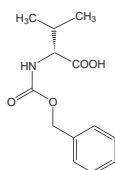
	854160	**Z–D–Val–OH**	5 g	66.00
		N-α-CBZ-D-valine	25 g	264.00
			100 g	785.00

NBC No.: 04-13-0523; CAS No.: 15099-82-8; $C_{13}H_{17}NO_4$; M.W.: 251.3

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

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Bulk quantities available, please enquire

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Product No.	Product	Quantity	Price

Mono-protected diamines

Fmoc-protected diamines
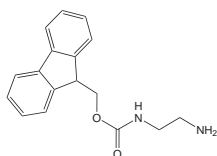
851064 **mono–Fmoc ethylene diamine hydrochloride** 1 g 72.00

Fmoc-NH(CH$_2$)$_2$NH$_2$ · HCl 5 g 287.00

NBC No.: 01-63-0064; CAS No.: 166410-32-8; C$_{17}$H$_{18}$N$_2$O$_2$ · HCl; M.W.: 282.3 · 36.5 25 g 1142.00

TLC: CHCl$_3$:MeOH:AcOH 32 % (15:4:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
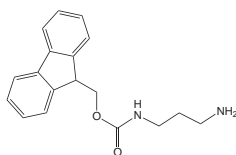
851065 **mono–Fmoc 1,3-diaminopropane hydrochloride** 1 g 72.00

Fmoc-NH(CH$_2$)$_3$NH$_2$ · HCl 5 g 287.00

NBC No.: 01-63-0067; CAS No.: 166410-34-0; C$_{18}$H$_{20}$N$_2$O$_2$ · HCl; M.W.: 296.4 · 36.5 25 g 1142.00

TLC: CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
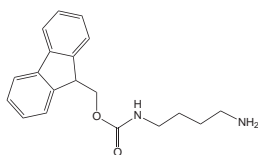
851063 **mono–Fmoc 1,4-diaminobutane hydrochloride** 1 g 72.00

Fmoc-NH(CH$_2$)$_4$NH$_2$ · HCl 5 g 287.00

NBC No.: 01-63-0055; CAS No.: 117048-49-4; C$_{19}$H$_{22}$N$_2$O$_2$ · HCl; M.W.: 310.4 · 36.5 25 g 1300.00

TLC: CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
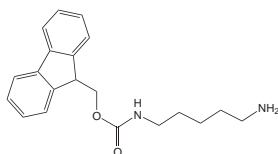
851068 **mono–Fmoc 1,5-diaminopentane hydrochloride** 1 g 72.00

Fmoc-NH(CH$_2$)$_5$NH$_2$ · HCl 5 g 287.00

NBC No.: 01-63-0075; CAS No.: 118119-32-7; C$_{20}$H$_{24}$N$_2$O$_2$ · HCl; M.W.: 324.4 · 36.5 25 g 1142.00

TLC: CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
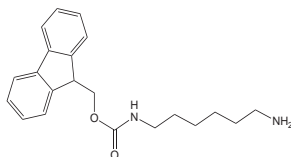
851066 **mono–Fmoc 1,6-diaminohexane hydrochloride** 1 g 72.00

Fmoc-NH(CH$_2$)$_6$NH$_2$ · HCl 5 g 287.00

NBC No.: 01-63-0070; CAS No.: 166410-37-3; C$_{21}$H$_{26}$N$_2$O$_2$ · HCl; M.W.: 338.4 · 36.5 25 g 1142.00

TLC: CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Boc-protected diamines
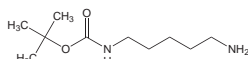
851067 **mono–t-Butoxycarbonyl 1,5-diaminopentane** 1 g 52.00

toluenesulfonic acid salt 5 g 208.00

Boc-NH(CH$_2$)$_5$NH$_2$ · nTosOH 25 g 811.00

NBC No.: 01-63-0073; CAS No.: 51644-96-3; C$_{10}$H$_{22}$N$_2$O$_2$ · C$_7$H$_8$O$_3$S; M.W.: 202.3 · 172.2

TLC: EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 98.00%.

CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

PROTECTED DIAMINES

⚠ Storage conditions   ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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| Product No. | Product | Quantity | Price |

PEGylation reagents

Monodisperse

Novabiochem® offers a range of bifunctional PEG reagents of different lengths with many useful applications in peptide and protein chemistry. For example, they can be used as solubilizing spacer groups to link peptide subunits or protein domains, to separate reporter groups, such as a fluorescent label or biotin, from the peptide chain, and to increase the solubility of otherwise intractable sequences.

Our derivatives are either single chemical entities, or in the case of longer chains, prepared from highly purified PEG, to ensure products of maximum homogeneity, free from contaminating oligomers.

In the naming convention adopted by Novabiochem, the number of atoms introduced by the spacer is given after the name and the number of PEG units by a subscript. Other components such as glycolic acid and aminopropyl groups are not included in the trivial name; only terminal functionalities are given.
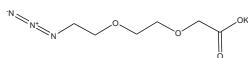
851205	**N$_3$–PEG–COOK (8 atoms)**	250 mg	104.00
	2-[2-(2-Azidoethoxy)ethoxy]-acetic acid potassium salt	1 g	312.00

C$_6$H$_{10}$KN$_3$O$_4$; M.W.: 227.3

HPLC: purity: ≥ 94.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; prevent exposure to light.

In this derivative the azido group acts as an orthogonally masked amine; it is stable to both TFA and piperidine but is easily converted to an amine by treatment with phosphines or thiols.
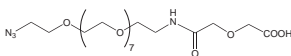
| | | | |
| 851021 | **N$_3$–PEG$_7$–COOH (33 atoms)** | 1 g | 482.00 |

O-(2-Azidoethyl)-O'-(N-diglycolyl-2-aminoethyl)-heptaethyleneglycol

NBC No.: 01-63-0103; C$_{22}$H$_{42}$N$_4$O$_{12}$; M.W.: 554.6

TLC: EtOAc:n-BuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 97.00%.

CHCl$_3$:MeOH:AcOH 32 % (5:3:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 97.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

This building block is prepared from highly purified PEG to ensure homogeneous products free from contaminating PEG oligomers. In this derivative the azido group acts as an orthogonally masked amine; it is stable to both TFA and piperidine but is easily converted to an amine by treatment with phosphines or thiols.

For a similar spacer, see reference [1].This material is now only available as an oil.

[1] J. Mack, et al. (1999) *J. Peptide Sci.*, **6**, 135.

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Product No.	Product	Quantity	Price
851039	**Boc–NH–PEG–COOH · DCHA (9 atoms)** Boc-8-amino-3,6-dioxaoctanoic acid · DCHA NBC No.: 01-63-0205; CAS No.: 560088-79-1; $C_{11}H_{21}NO_6 \cdot C_{12}H_{23}N$; M.W.: 444.6 TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 99.00%. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g	312.00
851040	**Boc–NH–PEG₃–COOH (16 atoms)** Boc-15-amino-4,7,10,13-tetraoxapentadecanoic acid NBC No.: 01-63-0206; CAS No.: 756525-91-4; $C_{16}H_{31}NO_8$; M.W.: 365.4 TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g	369.00
851041	**Boc–NH–PEG₅–COOH (22 atoms)** Boc-21-amino-4,7,10,13,16,19-hexaoxaheneicosanoic acid NBC No.: 01-63-0207; CAS No.: 882847-13-4; $C_{20}H_{39}NO_{10}$; M.W.: 453.5 TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%. ⚠ Prolonged storage: +2 to +8°C	1 g	510.00
851020	**Boc–NH–PEG₆–COOH (30 atoms)** O-(N-Boc-2-aminoethyl)-O'-(N-diglycolyl-2-aminoethyl)-hexaethyleneglycol NBC No.: 01-63-0102; $C_{25}H_{48}N_2O_{13}$; M.W.: 584.7 TLC: EtOAc:n-BuOH:AcOH:H_2O (2:1:1:1), purity: ≥ 95.00%. HPLC: purity: ≥ 80.00%. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry. This building block is prepared from highly purified PEG to ensure homogeneous products free from contaminating PEG oligomers. It can be introduced using standard coupling methods, such as PyBOP® or TBTU. Removal of the Boc group can be effected with TFA. The use of HF should be avoided as this reagent can promote breakdown of the PEG.	1 g	610.00
851083	**Boc–NH–PEG₂₇–COOH (88 atoms)** NBC No.: 01-63-0151; $C_{64}H_{127}NO_{32}$; M.W.: 1422.7 TLC: $CHCl_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 98.00%. HPLC: purity: ≥ 95.00%. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry. This building block is prepared from highly purified PEG to ensure homogeneous products free from contaminating PEG oligomers.	1 g	988.00
851037	**Fmoc–NH–PEG–COOH (9 atoms)** Fmoc-8-amino-3,6-dioxaoctanoic acid NBC No.: 01-63-0203; CAS No.: 166108-71-0; $C_{21}H_{23}NO_6$; M.W.: 385.4 TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%. HPLC: purity: ≥ 99.00%. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g	312.00

⚠ Storage conditions   🄖 Additional information   🄜 Method information   🄐 Application information

	Product No.	Product	Quantity	Price €

851024 **Fmoc–NH–PEG$_{11}$–COOH (40 atoms)**   1 g   920.00

O-(N-Fmoc-2-aminoethyl)-O'-(2-carboxyethyl)-undecaethyleneglycol
NBC No.: 01-63-0109; CAS No.: 756526-01-9; C$_{42}$H$_{65}$NO$_{16}$; M.W.: 839.9
TLC: EtOH:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 95.00%.
HPLC: purity: ≥ 95.00%.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
This building block is prepared from highly purified PEG to ensure homogeneous products free from contaminating PEG oligomers. It can be introduced using standard coupling methods, such as PyBOP® or TBTU, and is compatible with standard TFA cleavage protocols.

851034 **Fmoc–NH–PEG$_2$–COOH (13 atoms)**   1 g   348.00

Fmoc-12-amino-4,7,10-trioxadodecanoic acid
NBC No.: 01-63-0198; CAS No.: 867062-95-1; C$_{24}$H$_{29}$NO$_7$; M.W.: 443.5
TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
HPLC: purity: ≥ 99.00%.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

851031 **Fmoc–NH–PEG$_2$–COOH (20 atoms)**   1 g   369.00

O-(N-Fmoc-3-aminopropyl)-O'-(N-diglycolyl-3-aminopropyl)-diethyleneglycol
NBC No.: 01-63-0141; CAS No.: 946585-44-9; C$_{29}$H$_{38}$N$_2$O$_9$; M.W.: 558.62
TLC: EtOAc:nBuOH:AcOH:H$_2$O (2:1:1:1), purity: ≥ 92.00%.
HPLC: purity: ≥ 92.00%.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
Fmoc-NH-(PEG)$_2$-COOH is a bifunctional 20 atom PEG spacer. It can be introduced using standard coupling methods, such as PyBOP®, HBTU or TBTU, and is compatible with standard TFA cleavage protocols.
[1] B. Baumeister, et al. (2003) *Biopolymers*, **71**, 339.
[2] V. Kumar & J. Aldrich (2003) *Org. Lett.*, **5**, 613.
[3] E. Agner, et al. in "Peptides 2000, Proc. 26th European Peptide Symposium", Paris, J. Martinez & J.-A- Fehrentz, 2001, pp. 1011.

851035 **Fmoc–NH–PEG$_3$–COOH (16 atom)**   1 g   369.00

Fmoc-15-amino-4,7,10,13-tetraoxapentadecanoic acid
NBC No.: 01-63-0199; CAS No.: 557756-85-1; C$_{26}$H$_{33}$NO$_8$; M.W.: 487.5
TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
HPLC: purity: ≥ 98.00%.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

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Product No.	Product	Quantity	Price
851036	**Fmoc–NH–PEG$_4$–COOH (19 atoms)** Fmoc-18-amino-4,7,10,13,16-pentaoxaoctadecanoic acid NBC No.: 01-63-0200; CAS No.: 882847-32-7; C$_{28}$H$_{37}$NO$_9$; M.W.: 531.6 TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 97.00%. HPLC: purity: ≥ 97.00%. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g	426.00
851038	**Fmoc–NH–PEG$_5$–COOH (22 atoms)** Fmoc-21-amino-4,7,10,13,16,19-hexaoxaheneicosanoic acid NBC No.: 01-63-0204; CAS No.: 882847-34-9; C$_{30}$H$_{41}$NO$_{10}$; M.W.: 575.7 TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 95.00%. HPLC: purity: ≥ 93.00%. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g	510.00
851033	**Fmoc–NH–PEG$_{27}$–COOH (88 atoms)** NBC No.: 01-63-0150; C$_{74}$H$_{129}$NO$_{32}$; M.W.: 1544.8 TLC: CHCl$_3$:MeOH:AcOH:H$_2$O (85:13:0.5:1.5), purity: ≥ 95.00%. HPLC: purity: ≥ 95.00%. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry. This building block is prepared from highly purified PEG to ensure homogeneous products free from contaminating PEG oligomers. It can be introduced using standard coupling methods, such as PyBOP® or TBTU, and is compatible with standard TFA cleavage protocols.	1 g	1035.00
851032	**Trt–NH–PEG$_2$–NH$_2$ (15 atoms)** O-(N-Trt-3-aminopropyl)-O'-(3-aminpropyl)-diethyleneglycol NBC No.: 01-63-0143; CAS No.: 927888-44-6; C$_{29}$H$_{38}$N$_2$O$_3$; M.W.: 462.62 TLC: CHCl$_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 97.00%. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen. This mono-protected diamino functionalized PEG is a useful building block for the introduction of a PEG spacer into molecules containing a carboxylic acid functionality. Removal of the Trt group can be effected using 1-5% TFA in combination with 2-3% TES in DCM.	1 g	112.00

PEGYLATION REAGENTS

⚠ Storage conditions   ⑥ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Polydisperse

Modification of peptide and protein-based drugs with 20 and 30 kD polyethylene glycol chains is used to improve stability and pharmokinetics by protecting them from proteolysis, inhibiting aggregation, and reducing their immunogenicity [1]. Novabiochem is therefore pleased to offer Merck Eprova®'s MPEG derivatives. These MPEGs are manufactured to high standards and are specified for the modification of protein APIs, making them some of the highest quality PEGs available for the research market.

ⓘ Ⓖ 5.19

[1]   G. Pasut, et al (2004) *Expert Opin. Ther. Patents*, 14, 859.
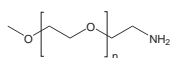
851216	**MPEG–20kDa ethylamine**	1 g	200.00

ω-Methoxy-α-aminoethyl polyethylene glycol 20 kDa

⚠ Prolonged storage: ≤-20°C; keep cool and dry.
HPLC: purity: ≥ 95.00%.
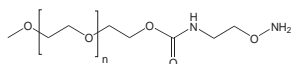
851217	**MPEG–20kDa oxyamine**	1 g	250.00

ω-Methoxy-α-2-aminoxyethyl carbamate polyethylene glycol 20 kDa

⚠ Prolonged storage: ≤-20°C; keep cool and dry and under argon.
HPLC: purity: ≥ 95.00%.
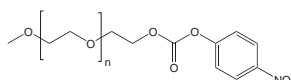
851218	**MPEG–20kDa pNPC**	1 g	200.00

ω-Methoxy-α-propionic acid polyethylene glycol 20 kDa p-nitrophenyl ester

⚠ Prolonged storage: ≤-20°C; keep cool and dry and under argon.
HPLC: purity: ≥ 95.00%.
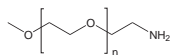
851219	**MPEG–30kDa ethylamine**	1 g	200.00

ω-Methoxy-α-aminoethyl polyethylene glycol 30 kDa

⚠ Prolonged storage: ≤-20°C; keep cool and dry and under argon.
HPLC: purity: ≥ 95.00%.
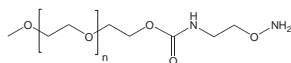
851220	**MPEG–30kDa oxyamine**	1 g	290.00

ω-Methoxy-α-2-aminooxyethyl carbamate polyethylene glycol 30 kDa

⚠ Prolonged storage: ≤-20°C; keep cool and dry and under argon.
HPLC: purity: ≥ 95.00%.

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Product No.	Product	Quantity	Price

Labeling reagents & resins

Chromogenic reagents & resins
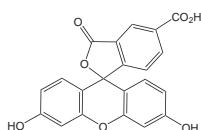
851025 **5-Carboxyfluorescein**   25 mg   25.00
5-FAM   100 mg   75.00
NBC No.: 01-63-0112; CAS No.: 76823-03-5; $C_{21}H_{12}O_7$; M.W.: 376.3   1 g   550.00
TLC: EtOAc:MeOH:H_2O:DIPEA (60:30:10:3), purity: $\geq$ 98.00%.
HPLC: purity: $\geq$ 99.00%.
⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry; protect from light.
This high purity fluorescent dye (λ_{ex}494 nm, λ_{em}518 nm) is supplied as a single isomer, which ensures production of labeled products of defined chemical structure, as well as greatly simplifying purification and characterization. FAM should be introduced as the last step in the synthesis to avoid formation of side-products, or be protected by tritylation prior to acylation or hydrazine treatment [1].
ⓘ Ⓖ 5.15, Ⓜ 5.15
[1] R. Fischer, et al. (2003) *Bioconjugate Chem.*, **14**, 653.
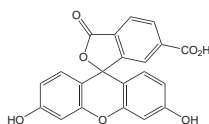
851072 **6-Carboxyfluorescein**   25 mg   29.00
6-FAM   100 mg   62.00
NBC No.: 01-63-0113; CAS No.: 3301-79-9; $C_{21}H_{12}O_7$; M.W.: 376.3
TLC: EtOAc:MeOH:H_2O:DIPEA (60:30:10:3), purity: $\geq$ 95.00%.
HPLC: purity: $\geq$ 98.00%.
⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry; protect from light.
This high purity fluorescent dye (λ_{ex}494 nm, λ_{em}518 nm) is supplied as a single isomer, which ensures production of labeled products of defined chemical structure, as well as greatly simplifying purification and characterization. FAM should be introduced as the last step in the synthesis to avoid formation of side-products, or protected by tritylation prior to acylation or hydrazine treatment [1].
ⓘ Ⓖ 5.15, Ⓜ 5.15
[1] R. Fischer, et al. (2003) *Bioconjugate Chem.*, **14**, 653.

851082 **5(6)-Carboxyfluorescein**   1 g   31.00
NBC No.: 01-63-0149; CAS No.: 72088-94-9; $C_{21}H_{12}O_7$; M.W.: 376.3   5 g   125.00
TLC: EtOAc:MeOH:H_2O:DIPEA (60:30:10:3), purity: $\geq$ 97.00%.
HPLC: purity: $\geq$ 96.00%.
⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry; protect from light.
Novabiochem®'s 5(6)-carboxyfluorescein (FAM; λ_{ex}494 nm, λ_{em} 518 nm) is a useful tool for preparing fluorescently-labeled peptides. It is supplied as a mixture of 5-and 6-isomers, making it cost effective for those applications which do not require a single isomer dye.
ⓘ Ⓖ 5.15, Ⓜ 5.15

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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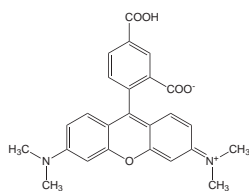
Product No.	Product	Quantity	Price
851026	**5-Carboxytetramethylrhodamine**	10 mg	105.00
	5-TAMRA	50 mg	420.00

NBC No.: 01-63-0114; CAS No.: 91809-66-4; $C_{25}H_{22}N_2O_5$; M.W.: 430.5
TLC: EtOAc:MeOH:H$_2$O:DIPEA (60:30:10:3), purity: ≥ 97.00%.
HPLC: purity: ≥ 96.00%.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light.
This fluorescent dye (λ_{ex}555 nm, λ_{em}580 nm) is supplied as a single isomer, which ensures production of labeled products of defined chemical structure, as well as greatly simplifying purification and characterization.
ⓘ Ⓖ 5.15, Ⓜ 5.15
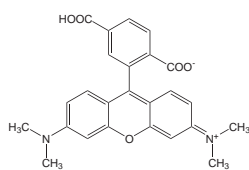
851073	**6-Carboxytetramethylrhodamine**	10 mg	109.00
	6-TAMRA	50 mg	437.00

NBC No.: 01-63-0115; CAS No.: 91809-67-5; $C_{25}H_{22}N_2O_5$; M.W.: 430.5
TLC: EtOAc:MeOH:H$_2$O:DIPEA (60:30:10:3), purity: ≥ 97.00%.
HPLC: purity: ≥ 96.00%.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light.
This high purity fluorescent dye (λ_{ex}555 nm, λ_{em}580 nm) is supplied as a single isomer, which ensures production of labeled products of defined chemical structure, as well as greatly simplifying purification and characterization.
ⓘ Ⓖ 5.15, Ⓜ 5.15
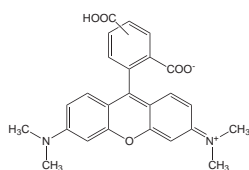
851030	**5(6)-Carboxytetramethylrhodamine**	100 mg	68.00
	5(6)-TAMRA	500 mg	270.00

NBC No.: 01-63-0134; CAS No.: 98181-63-6; $C_{25}H_{22}N_2O_5$; M.W.: 430.5
TLC: CHCl$_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 97.00%.
HPLC: purity: ≥ 95.00%.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light.
Novabiochem®'s 5(6)-carboxytetramethylrhodamine (λ_{ex}555 nm, λ_{em} 580 nm) is a useful tool for preparing fluorescently-labeled peptides. It is supplied as a mixture of 5-and 6-isomers, making it cost effective for those applications which do not require a single isomer dye.
ⓘ Ⓖ 5.15, Ⓜ 5.15
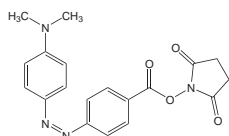
851022	**Dabcyl-OSu**	1 g	243.00

N-(4-[4'-(Dimethylamino)phenylazo]benzoyloxy)succinimide
NBC No.: 01-63-0105; CAS No.: 146998-31-4; $C_{19}H_{18}N_4O_4$; M.W.: 366.4
TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
HPLC: purity: ≥ 98.00%.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.
Pre-activated derivative for the introduction of the Dabcyl group during SPPS. The Dabcyl group quenches the fluorescence of EDANS, Mca, TET, JOE, FAM fluorophores, making it an extremely useful tool for the synthesis of fluorescence-quenched peptide substrates.
ⓘ Ⓖ 5.11

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Product No.	Product	Quantity	Price

856081 · **Fmoc–Arg(bis–Boc–resin)–AMC** · 500 mg · 840.00

NBC No.: 04-12-3912

Loading: 0.20 - 0.30 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; protect from light.

A novel resin for the preparation of peptide substrates based on 3-amino-7-methylcoumarin (AMC) by Fmoc SPPS [1]. Following Fmoc removal with 20% piperidine or 2-3% DBU in DMF, peptide assembly can be effected using standard coupling methods. Treatment with 50%TFA in DCM for 2-3h releases the peptide-AMC directly from the solid phase.

ⓘ Ⓖ 5.10, Ⓐ 5.11

[1] J. Beythien et al. (2006) *Tetrahedron Lett.*, **47**, 3009.

852118 · **Fmoc–Asp(EDANS)–OH** · 500 mg · 307.00 · 1 g · 567.00

NBC No.: 04-12-1288; CAS No.: 182253-73-2; $C_{31}H_{29}N_3O_8S$; M.W.: 603.64

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

Fluorescence-labeled amino acid for preparing fluorescence-quenched peptide substrates [1]. Most frequently used in conjunction with Dabcyl quenching group.

ⓘ Ⓖ 5.12

[1] L. L. Maggiora, et al. (1992) *J. Med. Chem.*, **35**, 3727.

856146 · **Fmoc–Asp(Wang–resin)–AMC** · 500 mg · 295.00 · 1 g · 495.00

NBC No.: 04-12-3915

Loading: 0.40 - 1.00 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light.

A novel resin for the preparation of peptide substrates based on 3-amino-7-methylcoumarin (AMC) by Fmoc SPPS. Following Fmoc removal with 20% piperidine, peptide assembly can be effected using standard coupling methods. Treatment with 95% TFA releases the peptide-AMC directly from the solid phase.

ⓘ Ⓖ 5.10, Ⓐ 5.11

852098 · **Fmoc–Glu(EDANS)–OH** · 500 mg · 307.00 · 1 g · 567.00

NBC No.: 04-12-1238; CAS No.: 193475-66-0; $C_{32}H_{31}N_3O_8S$; M.W.: 617.7

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 95.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

Fluorescence-labeled amino acid for preparing fluorescence-quenched peptide substrates [1]. Most frequently used in conjunction with Dabcyl quenching group.

ⓘ Ⓖ 5.12

[1] J. W. Drijfhout, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 14th American Peptide Symposium", P. T. P. Kaumaya & R. S. Hodges (Eds), Kingswinford, Mayflower Scientific Ltd., 1996, pp. 129.

CHROMOGENIC REAGENTS & RESINS

⚠ Storage conditions Ⓖ Additional information Ⓜ Method information Ⓐ Application information

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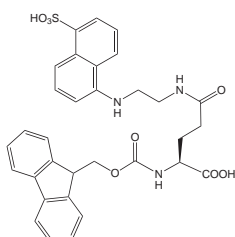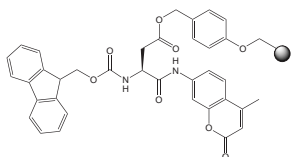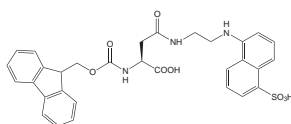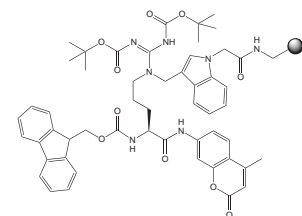
Product No.	Product	Quantity	Price
856147	**Fmoc-Lys(carbamate Wang resin)-AMC**	500 mg	295.00
	NBC No.: 04-12-3917	1 g	495.00

Loading: 0.40 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light.

A novel resin for the preparation of peptide substrates based on 3-amino-7-methylcoumarin (AMC) by Fmoc SPPS. Following Fmoc removal with 20% piperidine, peptide assembly can be effected using standard coupling methods. Treatment with 95% TFA releases the peptide-AMC directly from the solid phase.

ⓘ Ⓖ 5.10, Ⓐ 5.11

852096	**Fmoc-Lys(Dabcyl)-OH**	500 mg	338.00
	NBC No.: 04-12-1236; CAS No.: 146998-27-8; $C_{36}H_{37}N_5O_5$; M.W.: 619.7	1 g	614.00

TLC: $CHCl_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 97.00%.

HPLC: purity: ≥ 96.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light.

A modified lysine derivative for the preparation of chromogenically-labeled peptides by Fmoc.

The Dabcyl group quenches the fluorescence of EDANS, Mca, TET, JOE, FAM fluorophores, making it an extremely useful tool for the synthesis of fluorescence-quenched peptide substrates.

ⓘ Ⓖ 5.11

852099	**Fmoc-Lys(Dnp)-OH**	500 mg	270.00
	N-α-Fmoc-N-ε-2,4-dinitrophenyl-L-lysine	1 g	458.00

NBC No.: 04-12-1239; CAS No.: 148083-64-1; $C_{27}H_{26}N_4O_8$; M.W.: 534.5

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 97.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

A modified lysine derivative for the preparation of chromogenically-labeled peptides by Fmoc.

The Dnp group is the preferred quencher for use in conjunction with the Mca fluorophore, making it an extremely useful tool for the synthesis of fluorescence-quenched peptide substrates.

ⓘ Ⓖ 5.12

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Product No.	Product	Quantity	Price

852095   **Fmoc–Lys(Mca)–OH**   500 mg   270.00

N-α-Fmoc-N-ε-7-methoxycoumarin-4-acetyl-L-lysine   1 g   411.00

NBC No.: 04-12-1233; CAS No.: 386213-32-7; $C_{33}H_{32}N_2O_8$; M.W.: 584.6

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 97.00%.

HPLC: purity: ≥ 97.00%.

Optical purity: ≥ 99.00% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; protect from light.

A modified lysine derivative for the preparation of fluorogenically-labeled

peptides by Fmoc chemistry [1].

The Mca group fluoresces at 405 nm when stimulated at 340 nm, and is most

commonly used in conjunction with Dabcyl and 2,4-dinitrophenyl quenching

groups.

ⓘ Ⓖ 5.11

[1] J. L. Lauer-Fields, et al. (2001) *Biochemistry*, **40**, 5795.

851071   **Mca–OH**   1 g   78.00

7-Methoxycoumarin-4-acetic acid   5 g   312.00

NBC No.: 01-63-0111; CAS No.: 629395-72-2; $C_{12}H_{10}O_5$; M.W.: 234.2

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 95.00%.

HPLC: purity: ≥ 95.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light.

7-Methoxycoumarin (Mca) fluoresces at 405 nm when stimulated at 340 nm, and

is most commonly used in conjunction with 2,4-dinitrophenyl quenching group.

ⓘ Ⓖ 5.11

CHROMOGENIC REAGENTS & RESINS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

NovaTag™ resins

Novabiochem's NovaTag™ resins are novel supports for the preparation of C-terminally-modified peptides. Pre-loaded resins are available which on cleavage directly provide peptides containing a range of fluorophores (Mca, EDANS) and quencher groups (Dnp, Dabcyl) for FRET applications, or affinity labels (biotin, biotin-PEG, hydroxylamine) for bioconjugation and surface immobilization.

ⓘ Ⓖ 5.9
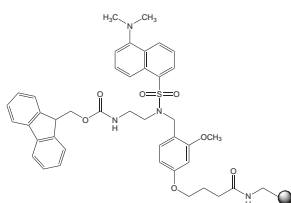
855050	**Dansyl NovaTag™ resin**	100 mg	40.00
	N-Dansyl-N'-Fmoc-ethylenediamine MPB-AM resin	500 mg	160.00

NBC No.: 04-12-3900

Loading: 0.30 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

⚠ Prolonged storage: ≤-20°C; keep cool and dry; protect from light.

Dansyl NovaTag™ resin is an ideal tool for the synthesis of Dansyl-labeled peptides. The resin pre-loaded with the Dansyl chromophore can be used direcly in Fmoc SPPS. Following peptide assembly under standard Fmoc SPPS conditions, cleavage with TFA affords the Dansyl-labeled peptide. This resin has been recently used to dansyl-labeled amyloid-RGD peptides [1] and FRET probes for mercury binding protein MerP [2].

ⓘ Ⓖ 5.14, Ⓐ 5.14

[1]   S. L. Gras, et al. (2008) *Biomaterials*, **29**, 1553.
[2]   B. R. White, et al. (2008) *Analyst,*, **133**, 65.
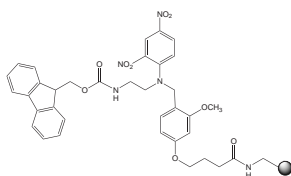
855053	**Dnp NovaTag™ resin**	100 mg	40.00
	N-Dnp-N'-Fmoc-ethylenediamine MPB-AM resin	500 mg	160.00

NBC No.: 04-12-3903

Loading: 0.30 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; protect from light.

Dnp NovaTag™ resin is an ideal tool for the synthesis of FRET peptide substrates based on the 7-methoxycoumarin (Mca) and 2,4-dintrophenyl (Dnp) fluorophore/quench pair. The resin pre-loaded with the Dnp quencher group can be used direcly in Fmoc SPPS. Following peptide assembly, the Mca group is coupled to the N-terminal N-amino group using Mca-OSu, or introduced to a side-chain of Lys using Fmoc-Lys(Mca)-OH, to give after TFA cleavage the desired FRET peptide substrate.

ⓘ Ⓖ 5.12

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Product No.	Product	Quantity	Price
855054	EDANS NovaTag™ resin	100 mg	40.00
	EDANS-MPB-AM resin	500 mg	160.00

NBC No.: 04-12-3904

Loading: 0.30 - 0.60 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin.

⚠ **Prolonged storage**: ≤ -20°C; keep cool and dry; protect from light.

The EDANS/dabcyl fluorophore-quencher pair is one of the most commonly-used for FRET peptides, owing to excellent spectral overlap between the emission spectrum of EDANS and absorbance spectrum of dabcyl [1]. The introduction of the EDANS moiety is traditionally achieved either by coupling of a peptide fragment to EDANS in solution [1, 2] or through the use of a pre-formed derivative, such as Fmoc-Glu(EDANS)-OH [3]. Novabiochem's EDANS resin enables for the first time the direct synthesis of C-terminally EDANS-labeled peptides by solid phase synthesis [4]. Loading of the first residue requires acylation of the resin-bound secondary amine and is, therefore, best carried out using HATU activation. Chain extension and cleavage can then be effected using standard methods.

EDANS NovaTag™ resin has been recently employed to prepare fluorescently labeled aminoalkane diphenyl phosphonate affinity probes for chymotrypsin- and elastase-like serine proteases [5].

ⓘ Ⓖ 5.13, Ⓐ 5.13, 5.14

[1] G. T. Wang, et al. (1990) *Tetrahedron Lett.*, **31**, 6493.
[2] C. Garcia-Echeverria & D. H. Rich (1992) *Febs Lett.*, **297**, 100.
[3] J. W. Drijfhout, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 14th American Peptide Symposium", P. T. P. Kaumaya & R. S. Hodges (Eds), Kingswinford, Mayflower Scientific Ltd., 1996, pp. 129.
[4] J. Beythien & P. White (2005) *Tetrahedron Lett.*, **46**, 101.
[5] B. F. Gilmore, et al. (2009) *Bioconjugate Chem.*, **20**, 2098.
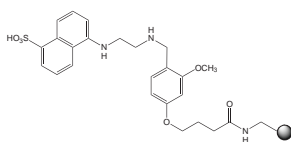
855052	Mca NovaTag™ resin	100 mg	40.00
	N-Mca-N'-Fmoc-ethylenediamine MPB-AM resin	500 mg	160.00

NBC No.: 04-12-3902

Loading: 0.20 - 0.50 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

⚠ **Prolonged storage**: ≤-20°C; keep cool and dry; protect from light.

Mca NovaTag™ resin is an ideal tool for the synthesis of FRET peptide substrates based on the 7-methoxycoumarin (Mca) and 2,4-dinitrophenyl (Dnp) fluorophore/quench pair. The resin pre-loaded with the Mca fluorophore can be used directly in Fmoc SPPS. Following peptide assembly, the Dnp group can be introduced using Fmoc-Lys(Dnp)-OH, to give after TFA cleavage the desired FRET peptide substrate.

ⓘ Ⓖ 5.11, Ⓐ 5.12

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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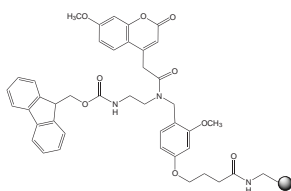
Product No.	Product	Quantity	Price

Universal resins

When preparing labeled peptides it is not always apparent at the outset which is the optimum combination of fluorophore and quencher or biotin-derivative of a given application. In addition, certain chromophores, such as FAM and TAMRA, are not totally stable to the conditions employed in Fmoc SPPS. For these reasons, Novabiochem® has developed linkers which facilitate the synthesis of peptides bearing any number of different acyl moieties at N- and C-termini from a single solid phase synthesis. After loading of the first amino acid to the resin-bound secondary amine, chain extension is carried out under standard Fmoc methods. Following synthesis, the resin can be partitioned and each aliquot end-capped with the appropriate carboxyl-functionalized label. The pendant protected amine is then deprotected (Mmt: using 1M HOBt in TFE/DCM; azide: by reduction with DDT) and the C-terminal label introduced to each resin aliquot. Thus, from a single synthesis any number of label variations for a given sequence can be prepared.

ⓘ ⒢ 5.9

| 855143 | **Azido-NovaTag™ resin** | 500 mg | 205.00 |
| | ⚠ Prolonged storage: ≤ -20°C; keep cool and dry; prevent exposure to light. | 1 g | 380.00 |

Loading: 0.30 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

The azido group acts as an orthogonally masked amine; it is stable to both TFA and piperidine but is easily converted to an amine by reduction with phosphines or thiols.
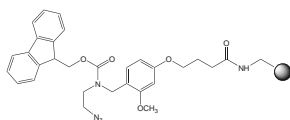
| 855057 | **Universal NovaTag™ resin** | 500 mg | 180.00 |
| | N-Fmoc-N'-Mmt-ethylenediamine-MPB-AM resin | 1 g | 335.00 |

NBC No.: 04-12-3910

Loading: 0.30 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

This resin has been recently used to prepare C-terminally modified peptide aldehydes and ketones for ligation [1, 2].

ⓘ ⒢ 5.9, Ⓐ 5.10

[1]   P. Marceau, et al. (2005) *Bioorg. Med. Chem. Lett.*, **15**, 5442.
[2]   C. Bure, et al. (2012) *J. Pept. Sci.*, **18**, 147.

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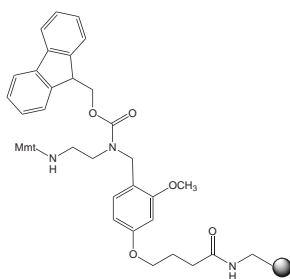
Product No.	Product	Quantity	Price
855058	**Universal PEG NovaTag™ resin**	500 mg	205.00
	N-Fmoc-N'-Mmt-PEG-diamine-MPB-AM resin	1 g	380.00

NBC No.: 04-12-3911

Loading: 0.20 - 0.50 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry.

This resin has recently been used to prepare Shc and Src homology domain binding peptoid-peptide hybrids [1] and dimeric SMAC PEG-linked peptides [2].

ⓘ Ⓖ 5.9

[1] W. J. Choi (2009) *J. Med. Chem.*, **52**, 1612.
[2] Z. Gao, et al. (2007) *J. Biol. Chem.*, **282**, 30718.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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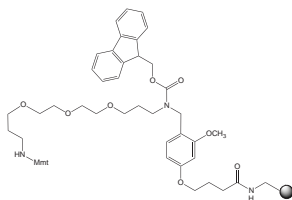
Product No.	Product	Quantity	Price

Biotinylation reagents
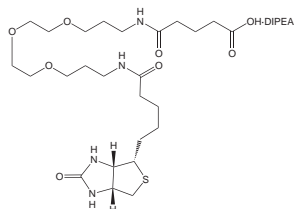
851029 **N–Biotinyl–NH–PEG$_2$–COOH · DIPEA (20 atoms)**   500 mg   268.00

O-(N-Biotinyl-3-aminopropyl)-O'-(N-glutaryl-3-aminopropyl)-diethyleneglycol · DIPEA   1 g   443.00

NBC No.: 01-63-0133; C$_{25}$H$_{44}$N$_4$O$_8$S · C$_8$H$_{19}$N; M.W.: 560.7 · 129.2

Solubility: 50 mg in 1 ml NMP.

TLC: CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 95.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.

The use of this novel biotin derivative incorporates a hydrophilic 20 atom PEG spacer between the peptide and biotin. This not only greatly improves the solubility of the resultant peptide, but reduces steric hindrance between the peptide and biotin, leading to better avidin binding and higher biological activity. The biotin-PEG derivative can be easily incorporated using any standard coupling method as the final step in SPPS, immediately prior to cleavage of the peptide from the resin.

ⓘ Ⓖ 5.18
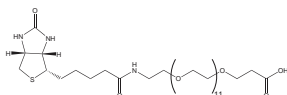
852340 **N–Biotinyl–NH–PEG$_{11}$–COOH (40 atoms)**   250 mg   333.00

O-[2-(Biotinylamino)ethyl]-O'-(2-carboxyethyl)undecaethylene glycol   1 g   998.00

C$_{37}$H$_{69}$N$_3$O$_{16}$S; M.W.: 844.0

TLC: CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

The use of this novel biotin derivative incorporates a hydrophilic 40 atom PEG spacer between the peptide and biotin. This not only greatly improves the solubility of the resultant peptide, but reduces steric hindrance between the peptide and biotin, leading to better avidin binding and higher biological activity. The biotin-PEG derivative can be easily incorporated using any standard coupling method as the final step in SPPS, immediately prior to cleavage of the peptide from the resin.

ⓘ Ⓖ 5.18

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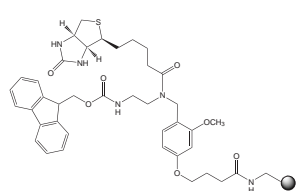
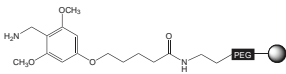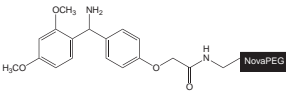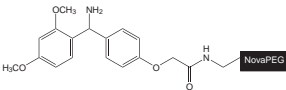| Product No.	Product	Quantity	Price
855051	**Biotin NovaTag™ resin**	500 mg	205.00
	N-Biotin-N'-Fmoc-ethylenediamine MPB-AM resin	1 g	390.00

NBC No.: 04-12-3901

Loading: 0.35 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

Biotin NovaTag™ resin is an ideal tool for the synthesis of peptides labeled iwth biotin. As the biotin moiety is incorporated into the linker, its use eliminates the problems associated with biotin-labeling of peptides, namely poor solubility and sluggish coupling kinetics of biotin and its derivatives. Following peptide assembly under standard Fmoc SPPS conditions, cleavage with TFA affords the peptide labeled with biotin. For a recent applications see [1, 2].

[1] M. Carraz, et al. (2009) *Chemisty & Biology*, **16**, 702.
[2] S. Millward, et al. (2007) *Chem. Biol.*, **2**, 625.

ⓘ Ⓖ 5.15, Ⓐ 5.16
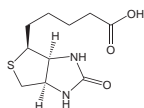
851209	**D(+)–Biotin**	1 g	30.00
NEW	D-Biotin	5 g	125.00
	Vitamin H	25 g	480.00

CAS No.: 58-85-5; $C_{10}H_{16}N_2O_3S$; M.W.: 244.31

⚠ Prolonged storage: +2 to +8°C
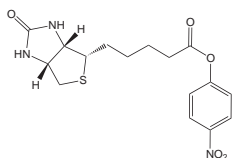
| 851027 | **Biotin–ONp** | 1 g | 166.00 |

Biotin p-nitrophenyl ester

5-(2-Oxo-hexahydro-thieno[3,4-d]imidazol-6-yl)-pentanoic acid p-nitrophenyl ester

NBC No.: 01-63-0116; CAS No.: 33755-53-2; $C_{16}H_{19}N_3O_5S$; M.W.: 365.4

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

Pre-activated reagent for the introduction of biotin by solid phase synthesis. More soluble and more reactive than 851023 [1]. This reagents has recently been found to be effective in SPOT synthesis of biotinylated peptides [2].

[1] B. Baumeister, et al.*Int. J. Pept. Res. Ther.*, **11**, 139.
[2] D. Winkler & P. McGeer (2008) *Proteomics*, **8**, 961.

ⓘ Ⓖ 5.18,
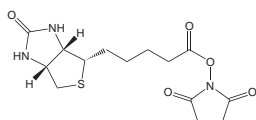
| 851023 | **Biotin–OSu** | 1 g | 183.00 |

N-(Biotinyloxy)succinimide

NBC No.: 01-63-0106; CAS No.: 35013-72-0; $C_{14}H_{19}N_3O_5S$; M.W.: 341.4

solubility: 50 mg in 1 ml NMP.

TLC: $CHCl_3$:MeOH:AcOH (85:10:5), purity: ≥ 97.00%.

HPLC: purity: ≥ 97.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

Pre-activated reagent for the introduction of biotin by solid phase synthesis.

ⓘ Ⓖ 5.18

BIOTINYLATION REAGENTS

⚠ Storage conditions	Ⓖ Additional information	Ⓜ Method information	Ⓐ Application information

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Product No.	Product	Quantity	Price
855055	**Biotin–PEG NovaTag™ resin**	500 mg	260.00
	N-Biotin-N'-Fmoc-PEG-diamine-MPB-AM resin	1 g	500.00

NBC No.: 04-12-3908

Loading: 0.20 - 0.50 mmole/g resin; as photometric determination of the Fmoc-chromophore liberated upon treatment with piperidine/DMF.

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

Biotin NovaTag™ resin is an ideal tool for the synthesis of peptides labeled iwth biotin. As the biotin moiety is incorporated into the linker, its use eliminates the problems associated with biotin-labeling of peptides, namely poor solubility and sluggish coupling kinetics of biotin and its derivatives.

Attachment of the first amino acid should be effected using HATU/DIPEA. Following peptide assembly under standard Fmoc SPPS conditions, cleavage with TFA affords the peptide labeled with biotin.

Biotin-PEG NovaTag™ resin incorporates a PEG spacer into the peptide, which leads to products having better solubilities compared to those prepared using standard biotin derivatives [1]. Furthermore, the PEG spacer reduces steric hindrance between the peptide and avidin, leading to better binding of biotin. For recent applications, see [2, 3].

ⓘ Ⓖ 5.15, Ⓐ 5.16

[1] B. Baumeister, et al. (2003) *Biopolymers*, **71**, 339.
[2] J. Parisot, et al. (2009) *J. Sep. Sci.*, **32**, 1613.
[3] S. Shiryaev, et al. (2007) *Biochem. J.*, **401**, 743.
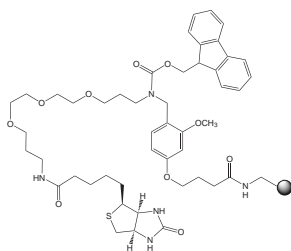
855145	**Fmoc–PEG Biotin NovaTag™ resin**	500 mg	285.00
		1 g	550.00

⚠ **Prolonged storage:** ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

Loading: 0.20 - 0.50 mmole/g resin; as photometric determination of the Fmoc-chromophore liberated upon treatment with piperidine/DMF.

Fmoc-PEG Biotin NovaTag™ resin offers all the benefits as biotin-PEG NovaTag™ resin (855055) in the synthesis of biotinylated peptides, with the additional advantage that the C-terminal amino acid can be coupled to this resin using any coupling methods.
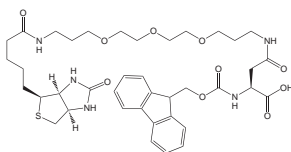
ⓘ Ⓖ 5.15

customer service: service@novabiochem.com   technical service: technical@novabiochem.com internet: novabiochem.com
Bulk quantities available, please enquire

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Product No.	Product	Quantity	Price
852113	**Fmoc–Asp(biotinyl–PEG)–OH**	500 mg	296.00
		1 g	530.00

N-α-Fmoc-N-γ-(N-biotinyl-3-(2-(2-(3-aminopropyloxy)-ethoxy)-ethoxy)-propyl)-L-asparagine

NBC No.: 04-12-1279; $C_{39}H_{53}N_5O_{10}S$; M.W.: 783.9

Solubility: 1 mmole in 2 ml DMF.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.

In contrast to Fmoc-Lys(biotin)-OH, this novel biotin-labeled amino acid has excellent solubility in DMF and other solvents used in SPPS [1]. The PEG-spacer restricts hindrance between the peptide and avidin, leading to better biotin binding. Furthermore, the hydrophilic nature of the PEG minimizes non-specific interactions that can arise from the spacer group becoming buried in the hydrophobic pocket of proteins.

ⓘ Ⓖ 5.18

[1] B. Baumeister, et al. (2003) *Biopolymers*, **71**, 339.

852102	**Fmoc–Glu(biotinyl–PEG)–OH**	500 mg	296.00
		1 g	530.00

N-α-Fmoc-N-γ-(N-biotinyl-3-(2-(2-(3-aminopropyloxy)-ethoxy)-ethoxy)-propyl)-L-glutamine

NBC No.: 04-12-1250; CAS No.: 817169-73-6; $C_{40}H_{55}N_5O_{10}S$; M.W.: 798.0

Solubility: 0.2 mmol in 1 ml DMF.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.

In contrast to Fmoc-Lys(biotin)-OH, this novel biotin-labeled amino acid has excellent solubility in DMF and other solvents used in SPPS [1]. The PEG-spacer restricts hindrance between the peptide and avidin, leading to better biotin binding. Furthermore, the hydrophilic nature of the PEG minimizes non-specific interactions that can arise from the spacer group becoming buried in the hydrophobic pocket of proteins.

For recent applications, please see [2 - 4].

ⓘ Ⓖ 5.18

[1] B. Baumeister, et al. (2003) *Biopolymers*, **71**, 339.
[2] X. Zhou et al., (2004) *J. Am. Chem. Soc.*, **126**, 15656.
[3] B. F. Gilmore, et al. (2006) *Biochem. Biophys. Res. Commun,*, **347**, 373.
[4] C. T. Archer, et al. (2005) *Mol. BioSyst.*, **1**, 366.

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Product No.	Product	Quantity	Price
852097	**Fmoc–Lys(biotin)–OH**	500 mg	203.00
	N-α-Fmoc-N-ε-biotinyl-L-lysine	1 g	395.00

NBC No.: 04-12-1237; CAS No.: 146987-10-2; $C_{31}H_{38}N_4O_6S$; M.W.: 594.7

Solubility: 0.1 mmole in 1 ml NMP.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 95.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.50% L-enantiomer.

A modified lysine derivative for the preparation of biotin-labeled peptides by Fmoc SPPS.

ⓘ Ⓖ 5.18

852100	**Fmoc–Lys(biotinyl-ε-aminocaproyl)–OH**	500 mg	172.00
	N-α-Fmoc-N-ε-(biotinylcaproyl)-L-lysine	1 g	307.00

NBC No.: 04-12-1243; CAS No.: 160158-05-4; $C_{37}H_{49}N_5O_7S$; M.W.: 707.9

Solubility: 100mg in 10 ml DMF.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 96.00%.

⚠ Prolonged storage: +15 to +25°C; keep cool and dry; keep open bottle under nitrogen.

A modified lysine derivative for the preparation of biotin-labeled peptides by Fmoc SPPS, in which the biotin is separated from the lysine side-chain by a 6-atom spacer.

ⓘ Ⓖ 5.18

856193	**Fmoc–Lys(biotinyl-ε-aminocaproyl)–NovaSyn® TG^R A resin**	250 mg	250.00
		1 g	475.00

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Product No.	Product	Quantity	Price

Other labeling reagents

Chelators
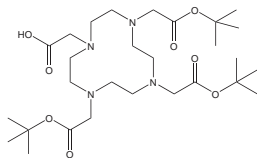
851200 **DOTA tris-t-Bu ester**   100 mg   187.00

Tri-tert-butyl 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate   250 mg   406.00

CAS No.: 137076-54; $C_{28}H_{52}N_4O_8$; M.W.: 572.7

Assay: purity: ≥ 95.00%.

⚠ Prolonged storage: +2 to +8°C

The most convenient reagent for the labeling of a peptide with DOTA [1]. This reagent has good solubility in DMF and DCM, and its free carboxyl group can be easily activated using uronium or phosphonium coupling reagents, enabling it to be attached to peptide amino groups. Removal of the t-butyl ester groups from the DOTA occurs during the course of the TFA-mediated cleavage reaction. The progress of the reaction should be monitored as the cleavage of these esters is slow, owing to the proximity of the basic ring nitrogens.

[1] L. M. De León-Rodríguez & Z. Kovacs (2008) *Bioconjugate Chem*, **19**, 391.

Spin-labeling reagents
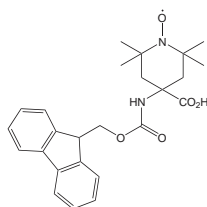
852342 **Fmoc-TOAC-OH**   100 mg   135.00

2,2,6,6-Tetramethylpiperidine-N-oxyl-4-(9-fluorenylmethyloxycarbonyl-amino)-4-carboxylic acid   500 mg   541.00

CAS No.: 93372-25-9; $C_{25}H_{29}N_2O_5$; M.W.: 437.5

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; prevent exposure to light.

HPLC: purity: ≥ 98.00%.

Fmoc-TOAC-OH is a useful tool for the incorporation of the ESR spin-label TOAC into peptide sequences [1]. Incorporation of this derivative and the following residue is best achieved using HATU activation. TFA/water/TIS should be used for cleavage of TOAC-containing peptides. The use of EDT should be avoided as it can cause permanent reduction of the nitroxide radical [2]. Following cleavage, the TOAC peptide should be treated with aqueous ammonia in air to regenerate the nitroxide from the hydroxylamine that is generated by the TFA treatment.

[1] R. Marchetto, et at. (1993) *J. Am. Chem. Soc.*, **115**, 11042.
[2] L. Martin, et al. (2001) *J. Pept. Res.*, **58**, 424.

Photo-cross linking reagents

851093 **TDBA**   25 mg   208.00
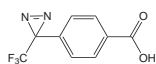
4-(3-(Trifluoromethyl)-3H-diazirin-3-yl)benzoic acid   100 mg   624.00

CAS No.: 85559-46-2; $C_9H_5F_3N_2O_2$; M.W.: 230.15   250 mg   1248.00

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; prevent exposure to light.

A highly sensitive photocross-linker activated at 300 nm for the preparation of photoactivatable biological probes [1].

[1] M.Nassal, et al. (1983) *Liebigs Ann. Chem.,,* 1510.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Chemoselective purification reagents
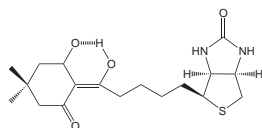
851069	**2-Biotinyldimedone**	1 g	146.00
	NBC No.: 01-63-0108; CAS No.: 194038-08-9; $C_{18}H_{26}N_2O_4S$; M.W.: 366.5	5 g	582.00

TLC: CHCl$_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.
HPLC: purity: ≥ 99.00%.
A reagent for the reversible labeling of peptides with biotin [1]. Labeling of the N-terminal amine of resin-bound peptides is carried out by incubating overnight with excess reagent in DMF. The biotinylated peptide is obtained following TFA cleavage. Removal of the biotin label is effected by treatment with 5% aqueous hydrazine.

ⓘ Ⓖ 5.19

[1] B. Kellam, et al. (1997) *Tetrahedron Lett.*, **38**, 5391.
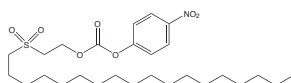
851092	**4-Nitrophenyl-2-(octadecylsulfonyl)ethylcarbonate**	250 mg	99.00
	C$_{18}$ Tag	1 g	374.00

C$_{25}$H$_{45}$O$_7$NS; M.W.: 503.7
Solubility: 0.5 mmol in 2 ml DCM.
TLC: EtOAc:Hexane (4:5), purity: ≥ 97.00%.
⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry.
4-Nitrophenyl-2-(octadecylsulfonyl)ethylcarbonate is lipophilic peptide purification tag [1]. By capping unreacted sites during synthesis, the N-terminal amino group of the desired peptide can be labeled with this tag. The lipophilic nature of the tag dramatically changes the HPLC characteristics of the product enabling it to be easily separated form capped by-products. Cleavage of the tag can be effected by treatment with 5-10% NH$_4$OH in TFE. For a detailed discussion on the use of lipophilic purification tags see [2].

ⓘ Ⓖ 5.19, 5.21

[1] C. Garcia-Echeverria (1995) *J. Chem. Soc., Chem. Commun.*, 779.
[2] P. Mascagni in "Fmoc solid phase peptide synthesis - a practical approach", W. C. Chan & P. D. White (Eds), Oxford, Oxford University Press, 2000, pp. 243.
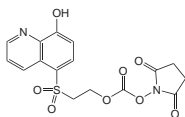
851208	**IMAC Tag**	250 mg	85.00
NEW	N-(2-(8-hydroxyquinolin-5-ylsulfonyl)ethoxycarbonyloxy)succinimide	1 g	325.00

C$_{16}$H$_{14}$N$_2$O$_6$S; M.W.: 394.4
⚠ **Prolonged storage:** ≤-20°C; keep cool and dry.
HPLC: purity: ≥ 98.00%.
Novabiochem®'s new IMAC-based purification Tag provides an simple alternative to the use of RP-HPLC for the purification of long peptides. The method is extremely easy-to-use, gives higher recoveries than RP-HPLC, and because it has a selectivity orthogonal to RP-HPLC, it is more effective at removing closely eluting impurites.

ⓘ Ⓖ 5.19, 5.20, Ⓐ 5.20

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| Product No. | Product | Quantity | Price |

Conjugation & ligation reagents

Novabiochem® provides reagents and amino–acid building blocks that enable incorporation of thiol, hydroxylamine and aldehyde functionalities for oxime [1 – 3], thiazolidine [4], and thioether [5.] conjugation of peptides to biomolecules. Resins for native thiol ligation and synthesis of peptide thioesters are found in the Resins for solid phase peptide synthesis section.

ⓘ Ⓖ 5.1

[1]   K. Rose, et al. (1994) *J. Am. Chem. Soc.*, **116**, 30.
[2]   J. Shao & J. P. Tam (1995) *J. Am. Chem. Soc.*, **117**, 3893.
[3]   F. Wahl & M. Mutter (1996) *Tetrahedron Lett.*, **37**, 6861.
[4]   C. F. Liu & J. P. Tam (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 6584.
[5]   J. W. Drijfhout & P. Hoogerhout in "Fmoc solid phase peptide synthesis - a practical approach", W. C. Chan & P. D. White (Eds), Oxford, Oxford University Press, 2000, pp. 229.

Building blocks
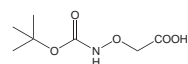
851017 **Boc-amino-oxyacetic acid** — 1 g 49.00 / 5 g 196.00
Boc-NH-O-CH$_2$COOH
Boc-Aoa-OH
NBC No.: 01-63-0060; CAS No.: 429890-85-5; C$_7$H$_{13}$NO$_5$; M.W.: 191.2
TLC: CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.
This reagent can be used to introduce a hydroxylamine functionality to N-terminal or side-chain amino groups. The use of this reagent may lead to double acylation of the hydroxylamine nitrogen. Hydroxylamine-labeled peptides prepared in this manner can be ligated in aqueous solution at pH 3.5 to aldehyde-containing peptides via oxime formation.
ⓘ Ⓖ 5.3
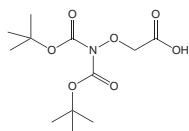
851028 **Bis-Boc-amino-oxyacetic acid** — 1 g 98.00 / 5 g 390.00
Boc$_2$-Aoa-OH
NBC No.: 01-63-0129; CAS No.: 293302-31-5; C$_{12}$H$_{21}$NO$_7$ · H$_2$O; M.W.: 291.3 · 18.0
TLC: CHCl$_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97.00%.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
This reagent can be used to introduce a hydroxylamine functionality to N-terminal or side-chain amino groups. Hydroxylamine-labeled peptides prepared in this manner can be ligated in aqueous solution at pH 3.5 to aldehyde-containing peptides via oxime formation.
This bis-protected derivative eliminates oligomer formation which can occur with mono-protected hydroxylamine reagents as a result of double acylation.
Coupling of this derivative should be done using an OSu ester.
ⓘ Ⓖ 5.3

[1]   J. C. Spetzler & T. Hoeg-Jensen (2001) *J. Peptide Sci.*, **7**, 537.

⚠ Storage conditions Ⓖ Additional information Ⓜ Method information Ⓐ Application information

CONJUGATION & LIGATION REAGENTS

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Product No.	Product	Quantity	Price
852305	**Fmoc–Aea–OH**	100 mg	135.00
	Fmoc-allysine ethylene acetal	500 mg	541.00

CAS No.: 1234692-73-9; $C_{23}H_{25}NO_6$; M.W.: 411.45

Solubility: 1 mmole in 2 ml DMF.

HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: ≤-20°C; keep cool and dry.

Product No.	Product	Quantity	Price
852216	**Fmoc–Dpr(Boc-Aoa)–OH**	1 g	114.00
	N-α-Fmoc-N-β-(N-t.-Boc-amino-oxyacetyl)-L-diaminopropionic acid	5 g	458.00
		25 g	1820.00

NBC No.: 04-12-1185; CAS No.: 600153-12-6; $C_{25}H_{29}N_3O_8$; M.W.: 499.5

Solubility: 1 mmole in 2 ml DMF.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 96.00%.

Optical purity: ≥ 99.50% L-enantiomer.

An excellent derivative for the chemoselective ligation of unprotected peptides in aqueous media [1] via oxime formation. This derivative introduces a hydroxylamine functionality which can couple with aldehyde groups present in another peptide unit.

ⓘ Ⓖ 5.3

[1]   F. Wahl & M. Mutter (1996) *Tetrahedron Lett.*, **37**, 6861.

Product No.	Product	Quantity	Price
851016	**SAMA–OPfp**	1 g	51.00
	S-Acetylthioglycolic acid pentafluorophenyl ester	5 g	205.00

NBC No.: 01-63-0041; CAS No.: 129815-48-1; $C_{10}H_5F_5O_3S$; M.W.: 300.1

TLC: Toluene:Dioxane:AcOH (95:25:4), purity: ≥ 98.00%.

⚠ Prolonged storage: ≤-20°C; keep cool and dry.

This reagent provides an effective means of linking synthetic peptide antigens to MAP core peptides or carrier proteins for the purpose of raising antibodies. Using this technique many of the problems associated with the analysis and purification of MAPs are avoided, since the linear peptide antigen can be fully characterized before conjugation to the preformed lysine tree.

ⓘ Ⓖ 5.7, Ⓜ 5.7

[1]   J. W. Drijfhout, et al. (1990) *Anal. Biochem.*, **187**, 349.

[2]   J. W. Drijfhout (1991) *Int. J. Peptide Protein Res.*, **37**, 27.

[3]   H. F. Brugghe, et al. (1994) *Int. J. Peptide Protein Res.*, **43**, 166.

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Product No.	Product	Quantity	Price
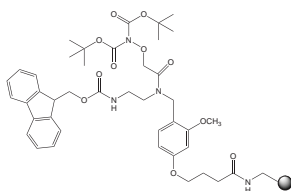
NovaTag™ resins

855056   **Hydroxylamine NovaTag™ resin**   500 mg   205.00

N-Boc$_2$-Aoa-N'-Fmoc-ethylenediamine MPB-AM resin   1 g   390.00

NBC No.: 04-12-3909

Loading: 0.20 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene-1 % DVB), 100 -200 mesh.

⚠ **Prolonged storage:** ≤-20°C; keep cool and dry.

Hydroxylamine NovaTag™ resin is an ideal tool for the synthesis of peptides labeled at the C-terminus with an hydroxylamine group. Such peptides readily undergo oxime formation in aqueous media with aldehyde-modified proteins or peptides, MAP core peptides, or surfaces. For the synthesis of MAPs, this approach avoids many of the problems associated with their purification and analysis, since the linear peptide antigen can be fully characterized prior to conjugation with the pre-formed lysine tree.

ⓘ   Ⓖ 5.6

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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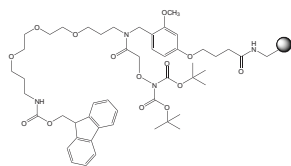
Product No.	Product	Quantity	Price
855144	**Hydroxylamine PEG NovaTag™ resin**	500 mg	260.00
	⚠ Prolonged storage: ≤-20°C; keep cool and dry.	1 g	500.00

Loading: 0.20 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

Hydroxylamine PEG NovaTag™ resin is an ideal tool for the synthesis of peptides labeled at the C-terminus with an hydroxylamine group. Such peptides readily undergo oxime formation in aqueous media with aldehyde-modified proteins or peptides, MAP core peptides, or surfaces. For the synthesis of MAPs, this approach avoids many of the problems associated with their purification and analysis, since the linear peptide antigen can be fully characterized prior to conjugation with the pre-formed lysine tree.

ⓘ Ⓖ 5.6

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Resins for solid phase peptide synthesis

Base resins

Novabiochem® offers a range of amino–functionalized base resins based on polystyrene, PEG–polystyrene (NovaSyn® TG), PEG (NovaPEG), PEG-PDA (PEGA) and polylysine (SpheriTide™). To aid selection, the properties and applications of these resins are given below.

In order to be used in routine peptide synthesis, the resins must be first derivatized with the appropriate TFA-cleavable carboxy-functionalized linker. Peptides prepared directly on these resins can not be cleaved off and are permanently anchored to the support. Such peptide-resins, if based on water compatible and biomolecule permeable resins such as PEGA, can be used for bead-based assays or affinity purification.

ⓘ Ⓖ 2.1

	Composition	Bead size (μm)	Loading	DMF	H$_2$O	DCM	Application	Comments
Polystyrene	Styrene cross-linked with divinylbenzene	75 - 150	0.5 -1.0	3	0	7	Routine and large scale synthesis	Most cost-effective but can fail on synthesis of difficult or long sequences
NovaSyn® TG	PEG grafted on polystyrene	90	0.2 – 0.3	5	4	5	Research scale medium to long peptides	Pressure resistant, thus ideal for continuous flow
NovaSyn® TG^R	PEG grafted on polystyrene	90	0.2 – 0.3	5	4	5	Research scale medium to long peptides	Special formulation of NovaSyn TG resin which gives even better results for long peptides . Works particularly well under microwave heating
PEGA	Polyacrylamide-PEG copolymer	150 - 300	0.2 – 0.4	11	16	13	On-bead enzyme assays	Internal bead space accessible to many proteins
NovaGel™ (Champion)	PEG grafted on polystyrene	75 - 150	0.6 – 0.8	7	n/d	n/d	Synthesis of medium length peptides	High-loading and high PEG content make it ideal for preparing medium-length peptides in quantity.
NovaPEG (ChemMatrix)	Polyethene cross-linked with PEG	75 - 100	0.4 – 0.6	8	11	13	Long or difficult peptides	Quality of long peptides excellent but yields often low
SpheriTide™	Polylysine cross-linked with sebacic acid	250 - 350	2.7 – 3.3	5	7	2	Large scale synthesis	Very high loading to swell ratio, thus saves on reagent and solvent usage

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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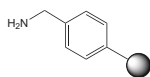
Product No.	Product	Quantity	Price
855115	**Aminomethylated polystyrene LL (100–200 mesh)**	5 g	43.00
	AM resin LL (100-200 mesh)	25 g	172.00
	NBC No.: 01-64-0447	100 g	516.00

Loading: 0.30 - 0.50 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The polymer matrix is copoly (styrene-1% DVB). Novabiochem®'s AM resin is prepared according to optimized protocols by direct aminomethylation of polystyrene [1]. This process gives a highly homogeneous and chemically defined support free from potentially reactive chloromethyl groups. This resin can be easily acylated with any appropriate carboxylic acid-containing linker using standard methods of amide bond formation to furnish supports for both Boc and Fmoc batch SPPS and SPOS.

(i) (G) 2.21

[1] A. R. Mitchell, et al. (1978) *J. Org. Chem.*, **30**, 2845.
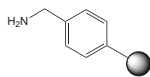
Product No.	Product	Quantity	Price
855020	**Aminomethylated polystyrene HL (100–200 mesh)**	5 g	43.00
	AM resin HL (100-200 mesh)	25 g	172.00
	NBC No.: 01-64-0143	100 g	516.00

Loading: 0.50 - 1.50 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The polymer matrix is copoly (styrene-1% DVB).

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Product No.	Product	Quantity	Price
855015	**Amino PEGA resin**	1 g	85.00
	NBC No.: 01-64-0100	5 g	340.00

Loading: 0.30 - 0.50 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin.

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry.

PEGA resins consist of dimethyl acrylamide and mono-2-acrylamidoprop-1-yl[2-aminoprop-1-yl] polyethylene glycol cross-linked with bis 2-acrylamidoprop-1-yl polyethyleneglycol. These supports swell extensively in a wide range of solvents and are permeable to macromolecules up to 35 kD, making them ideal for the preparation of combinatorial libraries, affinity purification and on-resin enzyme assays. For applications of these resins in SPPS and SPOS see [1-18].

Note: PEGA resins are supplied swollen in ethanol, with 1g dry resin corresponding to approximately 15 mL of swollen resin. As the beads of PEGA resins become very sticky and easily damaged when shrunk or dried, they are best handled in a swollen state. To use, the appropriate amount of swollen resin should be weighed into a reaction vessel and any residual ethanol removed by copious washings with the appropriate solvent or solvent mixture. After peptide assembly, the resin should be washed with DCM and transferred to the cleavage vessel. Excess DCM can be removed under vacuum, and the shrunk resin treated with the TFA cocktail. The characteristics of the swollen resin, its robustness and high permeability enables its use in both batch and continuous flow synthesis apparatus.

ⓘ Ⓖ 2.3

[1] M. Meldal, et al. (1992) *Tetrahedron Lett.,* **33**, 3077.
[1] M. Meldal, et al. (1992) *Tetrahedron Lett.,* **33**, 3077.
[2] F. I. Auzanneau, et al. (1994) *J. Peptide Sci.,* **1**, 31.
[3] M. Meldal in "Peptides 1992, Proc. 22nd European Peptide Symposium", C. H. Schneider & A. N. Eberle (Eds), ESCOM, Leiden, 1993, pp. 61.
[4] M. Meldal, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 3rd International Symposium", R. Epton (Eds), Mayflower Worldwide, Birmingham, 1994, pp. 259.
[5] M. Meldal, et al. (1993) *Int. J. Peptide Protein Res.,* **41**, 250.
[6] M. Meldal, et al. (1994) *J. Chem. Soc., Chem. Commun.,* 1849.
[7] M. Meldal, et al. (1994) *Proc. Natl. Acad. Sci. USA,* **91**, 3314.
[8] M. Renil, et al. (1998) *J. Peptide Sci.,* **4**, 195.
[9] J. C. Spetzler, et al. (1998) *J. Peptide Sci.,* **4**, 128.
[10] M. Meldal, et al. (1998) *J. Peptide Sci.,* **4**, 83.
[11] J. A. Camarero, et al. (1998) *J. Peptide Res.,* **51**, 303.
[12] J. A. Camarero, et al. (2000) *Lett. Pept. Sci.,* **7**, 17.
[13] O. Melnyk, et al. (2001) *J. Org. Chem.,* **66**, 4153.
[14] J. Buchardt, et al. (2000) *J. Comb. Chem.,* **2**, 624.
[15] J. F. Tolberg, et al. (2002) *J. Org. Chem.,* **67**, 4143.
[16] J. Bondebjerg, et al. (2002) *J. Am. Chem. Soc.,* **124**, 11046.
[17] J. Tulla-Puche, et al. (2004) *J. Org. Chem.,* **69**, 4101.
[18] S. Aggarwal, et al. (2005) *Biomaterials,* **26**, 6077.

H₂N [PEGA]

RESINS FOR SOLID PHASE PEPTIDE SYNTHESIS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
855084	**Aminomethyl NovaGel™**	1 g	43.00
	NBC No.: 01-64-0283	5 g	172.00
	Loading: 0.60 - 0.90 mmole/g resin; as determined from the substitution of the	25 g	688.00

Loading: 0.60 - 0.90 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Aminomethyl NovaGel™ is a PEG-PS resin which has been designed to meet the requirements of organic chemists for resins of high substitution coupled with broad solvent compatibility. This support has a nominal loading of 0.7 mmole/g – almost twice that of comparable resins – but still retains most of the swelling characteristics of conventional PEG-PS supports. It is prepared from a special high-swell version of AM resin by partial derivatization with methyl-PEG-p-nitrophenylcarbonate [1]. Resin functionality is provided by residual aminomethyl groups.

The urethane linkage between the PEG and the base resin is stable to strongly acidic, basic and reducing conditions, ensuring minimal loss of PEG chains during synthesis. Furthermore, because the linker is not attached to the end of the PEG chains, if leaching of PEG does occur, the product is not contaminated with U.V. absorbing linker-PEG fragments which can complicate the nmr spectra and HPLC profiles of products obtained with other PEG-PS supports.

ⓘ ⓖ 2.3

[1] J. H. Adams, et al. (1998) *J. Org. Chem.*, **63**, 3706.

Product No.	Product	Quantity	Price
855126	**NovaPEG amino resin**	1 g	65.00
	NBC No.: 01-64-0485	5 g	258.00
		25 g	995.00

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Loading: 0.45 - 1.00 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin.

A remarkable totally PEG-based resin for solid phase peptide synthesis. The support has excellent swelling properties in a wide range of solvents, including water, MeCN, MeOH, DCM, DMF, THF, and toluene. In comparative studies this resin was found to give better results than polystyrene-based supports in the synthesis of hydrophobic peptides. The high hydrophilicity was also found to benefit on-resin immunoassays with one-bead-one-peptide libraries [1].

ⓘ ⓖ 2.4

[1] F. Garcia-Martin, et al. (2006) *J. Comb. Chem.*, **8**, 213.

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Product No.	Product	Quantity	Price
855007	**NovaSyn® TG amino resin (90 μm)**	1 g	65.00
	NBC No.: 01-64-0043	5 g	260.00
	Loading: 0.20 - 0.30 mmole/g resin; as determined from the substitution of the	25 g	1040.00

Fmoc-Leu loaded resin.

NovaSyn® TG resin is a composite of polyethylene glycol and a low-cross linked polystyrene gel-type resin [1-3]. The 90 μm beads have a narrow size distribution, excellent pressure stability and swelling properties, and high diffusion rates, making them ideally suited for both batch or continuous flow peptide synthesis. Functionalized with an amino group, the resin can be readily derivatized with any carboxylic acid-containing linker using standard methods of amide bond formation. Furthermore, the excellent swelling properties in water of this support make it well suited to the preparation of peptide libraries.

ⓘ Ⓖ 2.3

[1] W. Rapp, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 1st International Symposium", R. Epton (Eds), SPCC UK Ltd., Birmingham, 1990, pp. 205.

[2] L. Zhang, et al. in "Peptides 1990, Proc. 21st European Peptide Symposium", E. Giralt & D. Andreu (Eds), ESCOM, Leiden, 1990, pp. 196.

[3] E. Bayer (1991) *Angew. Chem. Int. Ed. Engl.*, **30**, 113.

855014	**NovaSyn® TG amino resin (130 μm)**	1 g	50.00
	NBC No.: 01-64-0094	5 g	200.00
	Loading: 0.20 - 0.30 mmole/g resin; as determined from the substitution of the	25 g	800.00

Fmoc-Leu loaded resin.

NovaSyn® TG amino resin is a composite of low cross-linked polystyrene and 3000-4000 M.W. polyethylene glycol [1-3], in which the PEG chains have been terminally functionalized with an amino group. The 130 μm beads have a narrow size distribution, high diffusion rates and excellent swelling across a wide range of solvents from toluene to water, making them ideally suited to the synthesis of serial and parallel libraries by organic solid phase synthesis.

This resin can be easily converted into other supports suitable for use in solid phase synthesis by acylation with the appropriate carboxylic acid-containing linker..

ⓘ Ⓖ 2.3

[1] W. Rapp, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 1st International Symposium", R. Epton (Eds), SPCC UK Ltd., Birmingham, 1990, pp. 205.

[2] L. Zhang, et al. in "Peptides 1990, Proc. 21st European Peptide Symposium", E. Giralt & D. Andreu (Eds), ESCOM, Leiden, 1990, pp. 196.

[3] E. Bayer (1991) *Angew. Chem. Int. Ed. Engl.*, **30**, 113.

855073	**NovaSyn® TG amino resin HL**	1 g	50.00
	NBC No.: 01-64-0144	5 g	200.00
	Loading: 0.30 - 0.60 mmole/g resin; as determined from the substitution of the	25 g	800.00

Fmoc-Leu loaded resin. This resin is a composite of low-crossed linked polystyrene and 3000 - 4000 MW polyethyleneglycol, which has been terminally amino functionalised 110 μm beads.

For applications information, please refer to the entry for 855014.

ⓘ Ⓖ 2.3

⚠ Storage conditions　　Ⓖ Additional information　　Ⓜ Method information　　Ⓐ Application information

€

Product No.	Product	Quantity	Price

Resins for Fmoc SPPS of peptide acids

Resins supplied by Novabiochem® for the synthesis of peptide acids are functionalized with either the acid labile hydroxymethylphenoxyacetic acid linker (NovaSyn® TGA) or an equivalent hydroxymethylphenoxymethyl handle directly linked to a polystyrene base matrix (Wang resin). Attachment of the first residue to these supports can be achieved either by DMAP catalyzed esterification with the appropriate symmetrical anhydride, by the 2,6-dichlorobenzoyl chloride technique of Sieber [1], or by using MSNT/MeIm activation [2 - 4]. Detachment of the peptide with concurrent side-chain deprotection can be effected by treatment with 95% TFA.

ⓘ Ⓖ 2.6, 2.32, Ⓜ 3.6, 3.29

[1] P. Sieber (1987) *Tetrahedron Lett.*, **28**, 6147.
[2] B. Blankemeyer-Menge, et al. (1990) *Tetrahedron Lett.*, **31**, 1701.
[3] J. Nielsen (1996) *Tetrahedron Lett.*, **37**, 8439.
[4] E. Harth-Fritschy & D. Cantacuzène (1997) *J. Peptide Res.*, **50**, 415.
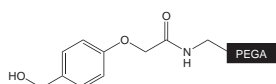
855066	**HMPA–PEGA resin**	1 g	140.00
	4-Hydroxymethylphenoxyacetyl PEGA resin	5 g	560.00

NBC No.: 01-64-0102

Loading: 0.20 - 0.40 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The resin is sold swollen in ethanol.

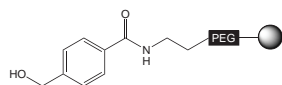⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

An excellent acid-labile resin for the continuous flow and batch Fmoc SPPS of peptide acids. It is derived from amino PEGA resin by derivatization with the TFA-labile 4-hydroxymethylphenoxyacetic acid linker. For further information, please refer to the product entry for amino PEGA resin (855015).

Note: PEGA resins are supplied swollen in ethanol, with 1g dry resin corresponding to approximately 15 ml of swollen resin. As the beads of PEGA resins become very sticky and easily damaged when shrunk or dried, they are best handled in a swollen state. To use, the appropriate amount of swollen resin should be weighed into a reaction vessel and any residual ethanol removed by copious washings with the appropriate solvent or solvent mixture. After peptide assembly, the resin should be washed with DCM and transferred to the cleavage vessel. Excess DCM can be removed under vacuum, and the shrunk resin treated with the TFA cocktail. The characteristics of the swollen resin, its robustness and high permeability enables its use in both batch and continuous flow synthesis apparatus.

ⓘ Ⓖ 2.3, 2.6, 2.32, Ⓜ 3.6, 3.29

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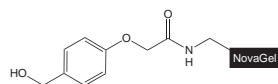
Product No.	Product	Quantity	Price
855085	**HMPA–NovaGel™**	1 g	50.00
	4-Hydroxymethylphenoxyacetyl NovaGel™	5 g	200.00
	NBC No.: 01-64-0284	25 g	800.00

Loading: 0.50 - 0.80 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin.

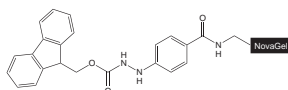⚠ **Prolonged storage**: +2 to +8°C; keep cool and dry.

This resin is prepared by derivatization of aminomethyl NovaGel™ with the TFA-labile 4-hydroxymethylphenoxyacetic acid linker. NovaGel™ resins combine the high functionality of polystyrene resins with the excellent swelling properties of PEG-PS type supports [1].

ⓘ Ⓖ 2.3, 2.6, 2.32, Ⓜ 3.6, 3.29

[1] J. H. Adams, et al. (1998) *J. Org. Chem.*, **63**, 3706.
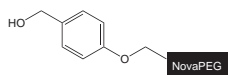
Product No.	Product	Quantity	Price
855122	**NovaPEG Wang resin**	1 g	70.00
	NBC No.: 01-64-0474	5 g	275.00
		25 g	995.00

Loading: 0.40 - 0.80 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin.
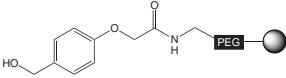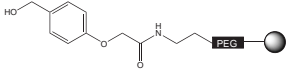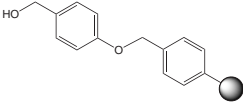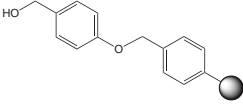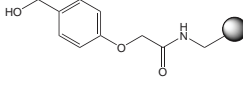
⚠ **Prolonged storage**: +2 to +8°C; keep cool and dry.

An excellent support for the synthesis of peptide acid. The support has excellent swelling properties in a wide range of solvents, including water, MeCN, MeOH, DCM, DMF, THF, and toluene. In comparative studies this resin was found to give better results than polystyrene-based supports in the synthesis of hydrophobic peptides. The high hydrophilicity was also found to benefit on-resin immunoassays with one-bead-one-peptide libraries [1].

ⓘ Ⓖ 2.4, 2.6, 2.32, Ⓜ 3.6, 3.29

[1] F. Garcia-Martin, et al. (2006) *J. Comb. Chem.*, **8**, 213.

RESINS FOR SOLID PHASE PEPTIDE SYNTHESIS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
855005	**NovaSyn® TGA resin (90 μm)**	1 g	49.00
	NBC No.: 01-64-0040	5 g	196.00
	Loading: 0.20 - 0.30 mmole/g resin; as determined from the substitution of the	25 g	784.00
	Fmoc-Leu loaded resin.		
	NovaSyn® TGA is an excellent resin for both the continuous flow and batch Fmoc		
	SPPS of peptide acids.		
	It is derived from 90 μm NovaSyn® TG amino resin by derivatization with the TFA		
	labile 4-hydroxymethylphenoxyacetic acid linker.		
	ⓘ Ⓖ 2.3, 2.6, 2.32, Ⓜ 3.6, 3.29		
855127	**NovaSyn® TGR A resin**	1 g	55.00
	Loading: 0.15 - 0.23 mmole/g resin; as determined from the substitution of the	5 g	200.00
	Fmoc-Leu loaded resin.		
	Special version NovaSyn® TGA resin (855005) with increased swelling properties		
	that is designed for the synthesis of long peptides (>50 residues). In comparative		
	tests, this resin was found to be superior to ChemMatrix and standard Tentagel		
	resins [1].		
	ⓘ Ⓖ 2.3, 2.6, 2.32, Ⓜ 3.6, 3.29		
	[1] K. K. Sorensen, et al. (2012) *J. Pept. Sc.*, **18**, S1 P201.		
855002	**Wang resin (100-200 mesh)**	5 g	35.00
	p-Benzyloxybenzyl alcohol resin (100-200 mesh)	25 g	140.00
	HMP resin (100-200 mesh)	100 g	420.00
	NBC No.: 01-64-0014		
	Loading: 0.50 - 1.30 mmole/g resin; as determined from the substitution of the		
	Fmoc-Leu loaded resin. The polymer matrix is copoly (styrene-1% DVB).		
	The standard resin for the Fmoc batch SPPS of peptide acids [1, 2].		
	ⓘ Ⓖ 2.6, 2.32, Ⓜ 3.6, 3.29		
855121	**Wang resin LL (100-200 mesh)**	5 g	35.00
	p-Benzyloxybenzyl alcohol resin (100-200 mesh) LL	25 g	140.00
	NBC No.: 01-64-0471	100 g	420.00
	Loading: 0.30 - 0.50 mmole/g resin; as determined from the substitution of the		
	Fmoc-Leu loaded resin. The polymer matrix is copoly (styrene-1% DVB).		
	A special version of Wang resin with low substitution. Ideal for use in the		
	synthesis of long and difficult peptides.		
	ⓘ Ⓖ 2.6, 2.32, Ⓜ 3.6, 3.29		
855150	**HMPA SpheriTide resin**	1 g	54.00
	Loading: 1.30 - 1.80 mmole/g resin; as determined from the substitution of the	5 g	216.00
	Fmoc-Leu loaded resin.	25 g	864.00
	A ultra-load support for the synthesis of peptide acids by both batch-wise and		
	continuous flow SPPS. The base matrix is based on cross-linked poly-ε-lysine,		
	which has been shown to produce peptides of excellent quality even at high		
	substitutions.		
	ⓘ Ⓖ 2.4, 2.6, 2.32, Ⓜ 3.6, 3.29		

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Product No.	Product	Quantity	Price

Resins for Fmoc SPPS of peptide amides

Resins supplied by Novabiochem® for the synthesis of peptide amides are functionalized with either the acid-labile modified Rink amide linker (Rink AM resin, Rink MBHA resin, and NovaSyn®TGR) or an equivalent handle directly linked to a polystyrene base matrix (Rink amide resin), or the PAL linker (Fmoc-PAL-AM resins and PAL NovaPEG resin). Because attachment of the peptide is made via an amide bond, coupling of the first amino acid can be achieved using normal methods of amide bond formation. Since DMAP is not required for this step, there is no risk of enantiomerization or dipeptide formation. Detachment of peptide amides from these supports can be effected in a single step by treatment with 95% TFA.

ⓘ Ⓖ 2.6 Ⓜ 3.7, 3.29

[1]   H. Rink (1987) *Tetrahedron Lett.*, **28**, 3787.
[2]   M. S. Bernatowicz, et al. (1989) *Tetrahedron Lett.*, **30**, 4645.
[3]   S. C. Story, et al. (1992) *Int. J. Peptide Protein Res.*, **39**, 87.
[4]   F. Albericio, et al. (1990) *J. Org. Chem.*, **55**, 3730.
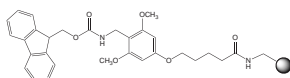
855133	**Fmoc-PAL-AM resin**	1 g	60.00
	Loading: 0.50 - 0.80 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene-1% DVB), 100 - 200 mesh.	5 g	240.00

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Fmoc-PAL-AM resin consists of Barany's aminomethyl-dimethoxyphenoxyvaleric acid linker [1] attached to aminomethylated polystyrene. The amino group of this linker can be easily acylated under standard coupling conditions. Following peptide assembly, treatment with 95% TFA containing scavengers releases the desired peptide amide. Studies have shown the acid sensitivity of this linker to be around twice that of the Rink amide linker [2]. There is some evidence to suggest that PAL resins give greater yields in microwave assisted synthesis.

ⓘ Ⓖ 2.6, 2.23 Ⓜ 3.7, 3.29

[1]   F. Albericio & G. Barany (1987) *Int. J. Peptide Protein Res.*, **30**, 206.
[2]   M. S. Bernatowicz, et al. (1989) *Tetrahedron Lett.*, **30**, 4645.
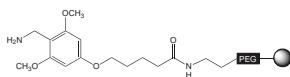
855136	**PAL-NovaPEG resin**	1 g	150.00
	Loading: 0.30 - 0.70 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.	5 g	600.00

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

PAL-NovaPEG resin consists of Barany's aminomethyl-dimethoxyphenoxyvaleric acid linker [1] attached to NovaPEG amino resin. The amino group of this linker can be easily acylated under standard coupling conditions. Following peptide assembly, treatment with 95% TFA containing scavengers releases the desired peptide amide. Studies have shown the acid sensitivity of this linker to be around twice that of the Rink amide linker [2].

ⓘ Ⓖ 2.4, 2.6, 2.23 Ⓜ 3.7, 3.29

[1]   F. Albericio & G. Barany (1987) *Int. J. Peptide Protein Res.*, **30**, 206.
[2]   M. S. Bernatowicz, et al. (1989) *Tetrahedron Lett.*, **30**, 4645.

RESINS FOR SOLID PHASE PEPTIDE SYNTHESIS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

Product No.	Product	Quantity	€ Price

855137   **PAL NovaSyn® TG resin**   1 g   80.00

Loading: 0.20 - 0.30 mmole/g resin; as determined from the substitution of the   5 g   320.00
Fmoc-Leu loaded resin.

⚠ **Prolonged storage**: +2 to +8°C; keep cool and dry.

PAL-NovaSyn® TG resin consists of Barany's aminomethyl-dimethoxyphenoxyvaleric acid linker [1] attached to NovaSyn® TG resin. The amino group of this linker can be easily acylated under standard coupling conditions. Following peptide assembly, treatment with 95% TFA containing scavengers releases the desired peptide amide. Studies have shown the acid sensitivity of this linker to be around twice that of the Rink amide linker [2]. There is some evidence to suggest that PAL resins give greater yields in microwave assisted synthesis.

ⓘ Ⓖ 2.3, 2.6, 2.22 Ⓜ 3.7, 3.29

[1] F. Albericio & G. Barany (1987) *Int. J. Peptide Protein Res.*, **30**, 206.
[2] M. S. Bernatowicz, et al. (1989) *Tetrahedron Lett.*, **30**, 4645.

855047   **NovaPEG Rink Amide resin**   1 g   70.00

NBC No.: 01-64-0473   5 g   275.00

Loading: 0.30 - 0.60 mmole/g resin; as determined from the substitution of the   25 g   995.00
Fmoc-Leu loaded resin.

⚠ **Prolonged storage**: +2 to +8°C; keep cool and dry.

An excellent acid-labile resin for the continuous flow and batch Fmoc SPPS of peptide amides. This resin is prepared by derivatization of NovaPEG amino resin with the modified Rink linker. The support has excellent swelling properties in a wide range of solvents, including water, MeCN, MeOH, DCM, DMF, THF, and toluene. In comparative studies this resin was found to give better results than polystyrene-based supports in the synthesis of hydrophobic peptides. The high hydrophilicity was also found to benefit on-resin immunoassays with one-bead-one-peptide libraries [1].

ⓘ Ⓖ 2.4, 2.6, 2.22 Ⓜ 3.7, 3.29

[1] F. Albericio, et al. *Poster presented at 18th American Peptide Symposium, San Diego, June 2005.*

855125   **NovaPEG Rink Amide resin LL**   1 g   70.00

NBC No.: 01-64-0483   5 g   275.00

Loading: 0.15 - 0.25 mmole/g resin; as determined from the substitution of the
Fmoc-Leu loaded resin.

⚠ **Prolonged storage**: +2 to +8°C; keep cool and dry.

See the entry of 855047

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Product No.	Product	Quantity	Price
855009	**NovaSyn® TGR resin**	1 g	80.00
	NBC No.: 01-64-0060	5 g	320.00
	Loading: 0.20 - 0.30 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin..	25 g	1280.00
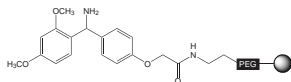
⚠ **Prolonged storage**: +2 to +8°C; keep cool and dry.

An excellent acid labile support for Fmoc continuous flow and batch synthesis of peptide amides. This resin is prepared from 90 μm NovaSyn® TG amino resin by derivatization with the modified Rink linker. It is supplied without Fmoc protection of the linker benzhydrylamine group as this has been found to improve stability. Cleavage is effected with 95% TFA.

ⓘ Ⓖ 2.3, 2.6, 2.22 Ⓜ 3.7, 3.29

855128	**NovaSyn® TGR R resin**	1 g	55.00
	Loading: 0.15 - 0.23 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin.	5 g	220.00
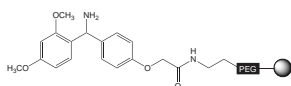
Special version of NovaSyn® TGR resin (855009) with increased swelling properties that is designed for the synthesis of long peptides (>50 residues). In comparative tests, this resin was found to be superior to Chemmatrix and standard Tentagel resins [1].

ⓘ Ⓖ 2.3, 2.6, 2.22 Ⓜ 3.7, 3.29

[1] K. K. Sorensen, et al. (2012) *J. Pept. Sc.*, **18**, S1 P201.

855134	**Ramage Amide AM resin**	1 g	80.00
	2-{[(R,S)-5-(9-Fluorenylmethyloxycarbonyl-amino)-10,11-dihydro-5H-dibenzo[a,d]cycloheptene-2-yl]oxy}acetyl-AM resin	5 g	320.00
	Loading: 0.40 - 0.80 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene-1% DVB), 100 - 200 mesh.		
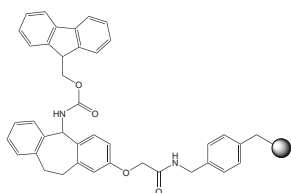
⚠ **Prolonged storage**: +2 to +8°C; keep cool and dry.

Ramage Amide AM resin comprises Ramage's dibenzocycloheptadiene linker [1] attached to aminomethylated polystyrene. Following Fmoc removal, the resin can be acylated under standard conditions and used in Fmoc SPPS. The linker is considerably more acid sensitive than the Rink amide or PAL linkers. This enables peptide amides to be released from the resin with 3% TFA in DCM and thus makes it a useful tool for the synthesis of acid sensitive peptides or protected peptide fragments.

ⓘ Ⓖ 2.3, 2.6, 2.23 Ⓜ 3.7, 3.29

[1] R. Ramage, et al. (1993) *Tetrahedron Lett.*, **34**, 6599.

RESINS FOR SOLID PHASE PEPTIDE SYNTHESIS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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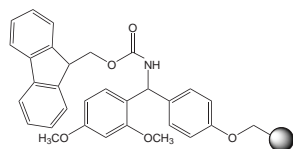
Product No.	Product	Quantity	Price
855001	**Rink Amide resin (100–200 mesh)**	1 g	28.00
	4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy resin	5 g	110.00
	NBC No.: 01-64-0013	25 g	330.00

Loading: 0.40 - 0.80 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene-1% DVB).

An excellent support for the Fmoc SPPS of peptide amides.

This resin is more acid sensitive than Rink Amide AM and Rink Amide MBHA resins. Cleavage with high concentrations of TFA can lead to the breakdown of the linker, with the concomitant formation of by-products that can not be removed by simple washes. These problems appear to be minimized through the use of low TFA concentrations or by the addition of trialkylsilanes to the cleavage mixture.

ⓘ Ⓖ 2.6, 2.22 Ⓜ 3.7, 3.29
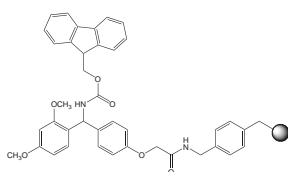
Product No.	Product	Quantity	Price
855130	**Rink Amide AM resin (100–200 mesh)**	1 g	28.00
	4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamido-aminomethyl resin	5 g	110.00
		25 g	330.00

Loading: 0.40 - 0.80 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene-1% DVB).

This support comprises the modified Rink amide linker attached to aminomethylpolystyrene, and is an ideal tool for the Fmoc SPPS of peptide amides. Cleavage from this resin can be effected by a single step treatment with 95% TFA, providing peptide amides in high yields and purities.

ⓘ Ⓖ 2.6, 2.22 Ⓜ 3.7, 3.29
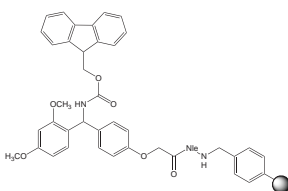
Product No.	Product	Quantity	Price
855004	**Rink Amide AM resin (200–400 mesh)**	1 g	28.00
	4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamido-norleucylaminomethyl resin	5 g	110.00
	NBC No.: 01-64-0038	25 g	330.00

Loading: 0.40 - 0.80 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene-1% DVB).

This support comprises the modified Rink amide linker attached via norleucine to aminomethylpolystyrene, and is an ideal tool for the Fmoc SPPS of peptide amides. Cleavage from this resin can be effected by a single step treatment with 95% TFA, providing peptide amides in high yields and purities.

ⓘ Ⓖ 2.6, 2.22 Ⓜ 3.7, 3.29

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Product No.	Product	Quantity	Price
855120	**Rink Amide AM resin LL (100–200 mesh)** 4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamido- norleucylaminomethyl resin NBC No.: 01-64-0470	1 g 5 g 25 g	28.00 110.00 330.00

Loading: 0.20 - 0.40 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene-1% DVB).
This support is comprised of the modified Rink amide linker attached to aminomethylpolystyrene.
A special version of Rink Amide AM resin with low substitution. Ideal for use in the synthesis of long and difficult peptides.

ⓘ Ⓖ 2.6, 2.22 Ⓜ 3.7, 3.29

Product No.	Product	Quantity	Price
855003	**Rink Amide MBHA resin (100–200 mesh)** 4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamido-norleucyl- MBHA resin (100-200 mesh) NBC No.: 01-64-0037	1 g 5 g 25 g	28.00 110.00 330.00

Loading: 0.40 - 0.90 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene-1% DVB).
This support comprises the modified Rink amide linker attached via norleucine to MBHA resin, and is an ideal tool for the Fmoc SPPS of peptide amides. Cleavage from this resin can be effected by a single step treatment with 95% TFA, providing peptide amides in high yields and purities.

ⓘ Ⓖ 2.6, 2.22 Ⓜ 3.7, 3.29

Product No.	Product	Quantity	Price
855045	**Rink Amide MBHA resin LL (100–200 mesh)** 4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamido norleucyl- MBHA resin (100-200 mesh) NBC No.: 01-64-0467	1 g 5 g 25 g	28.00 110.00 330.00

Loading: 0.30 - 0.40 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene-1 % DVB).
A special version of Rink Amide MBHA resin with low substitution. Ideal for use in the synthesis of long and difficult peptides.

ⓘ Ⓖ 2.6, 2.22 Ⓜ 3.7, 3.29

Product No.	Product	Quantity	Price
855119	**Rink Amide resin HL (100–200 mesh)** 4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy resin (100-200 mesh) NBC No.: 01-64-0464	1 g 5 g 25 g	28.00 110.00 330.00

Loading: 0.70 - 1.00 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene-1% DVB), 100 - 200 mesh.
A special high load version of Rink Amide resin.

ⓘ Ⓖ 2.6, 2.22 Ⓜ 3.7, 3.29

RESINS FOR SOLID PHASE PEPTIDE SYNTHESIS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

855031   **Rink Amide NovaGel™**

NBC No.: 01-64-0286

Loading: 0.50 - 0.70 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The PEG-PS resin is prepared from aminomethyl copoly(styrene-1 % DVB).

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

This resin is prepared by derivatization of aminomethyl NovaGel™ with the modified Rink linker. NovaGel resins combine many of the excellent properties of PEG-PS-based resins with the high loading efficiency of conventional polystyrene-based supports. In a comparison with Rink amide resin, this resin was found to give superior results in the synthesis of peptides up to 17 amino acids in length [1]. The use of this support in the preparation of oligonucleotide-peptide conjugates has been described [2].

ⓘ Ⓖ 2.3, 2.6, 2.22 Ⓜ 3.7, 3.29

[1]   R. Pipkorn & P. White, Novabiochem Innovations 3/99.

[2]   D. A. Stetsenko & M. J. Gait (2000) *J. Org. Chem.*, **65**, 4900.
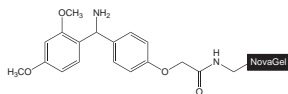
		1 g	65.00
		5 g	260.00
		25 g	990.00

855016   **Rink Amide PEGA resin**

NBC No.: 01-64-0101

Loading: 0.20 - 0.50 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The resin is sold swollen ethanol.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

An excellent acid-labile support for the solid phase synthesis of peptide amides. This resin is prepared by derivatization of amino PEGA resin with the modified Rink linker. It is supplied without Fmoc protection of the linker benzhydrylamine group as this has been found to improve stability. Cleavage is effected with 95% TFA. PEGA-based resins are compatible with aqueous and alcoholic solvents, and are permeable to macromolecules up to 35 kD.

For further information, please refer to the product entry for amino PEGA resin (855015).

Note: PEGA resins are supplied swollen in ethanol, with 1g dry resin corresponding to approximately 15 mL of swollen resin. As the beads of PEGA resins become very sticky and easily damaged when shrunk or dried, they are best handled in a swollen state. To use, the appropriate amount of swollen resin should be weighed into a reaction vessel and any residual ethanol removed by copious washings with the appropriate solvent or solvent mixture. After peptide assembly, the resin should be washed with DCM and transferred to the cleavage vessel. Excess DCM can be removed under vacuum, and the shrunk resin treated with the TFA cocktail. The characteristics of the swollen resin, its robustness and high permeability enables its use in both batch and continuous flow synthesis apparatus.

ⓘ Ⓖ 2.3, 2.6, 2.22 Ⓜ 3.7, 3.29

| | | 1 g | 120.00 |
| | | 5 g | 480.00 |

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Product No.	Product	Quantity	Price
855118	**Knorr Amide MBHA resin**	5 g	35.00
	4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamido-MBHA resin	25 g	140.00
	NBC No.: 01-64-0459	100 g	420.00

Loading: 0.60 - 1.00 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene-1% DVB), 100 - 200 mesh.
This support comprises of the modified Rink amide linker attached to MBHA resin [1].

(i) G 2.6, 2.23 M 3.7, 3.29
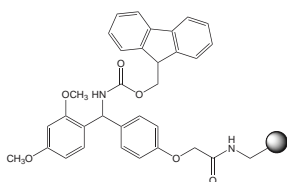
855149	**Rink Amide SpheriTide resin**	1 g	70.00
		5 g	275.00
		25 g	995.00

Loading: 1.0 - 1.30 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.
A ultra-load support for the synthesis of peptide amides by both batch-wise and continuous flow SPPS. The base matrix is based on cross-linked poly-ε-lysine, which has been shown to produce peptides of excellent quality even at high substitutions.

(i) G 2.4, 2.6, 2.22 M 3.7, 3.29

RESINS FOR SOLID PHASE PEPTIDE SYNTHESIS

⚠ Storage conditions   G Additional information   M Method information   A Application information

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Product No.	Product		Quantity	Price

Resins for Fmoc SPPS of peptide N-alkyl amides

These resins are novel tools for the synthesis of N–methyl and N–ethyl peptides amides by Fmoc SPPS. TFA cleavage of peptide prepared on these resins provided the peptide *N*–alkylamide directly without the need for further synthetic manipulation.

855102 **Ethyl Indole AM resin**

1 g   67.00
5 g   268.00
25 g   995.00
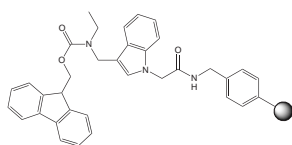
NBC No.: 01-64-0398

Loading: 0.70 - 1.00 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene-1% DVB), 100 - 200 mesh.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

[3-((Ethyl-Fmoc-amino)-methyl)indol-1-yl]acetyl AM resin is an extremely useful support for the production of peptide N-ethylamides, N-ethyl substituted carboxamides and sulfonamides [1]. After first removing the Fmoc group, loading of the resin with Fmoc-amino acids can be effected using HATU/DIPEA. Cleavage with TFA directly provides the product N-ethylamide.

ⓘ Ⓖ 2.30, Ⓜ 3.7, 3.29, Ⓐ 2.30

[1]   K. G. Estep, et al. (1998) *J. Org. Chem.*, **63**, 5300.

855116 **Methyl Indole AM resin**

1 g   67.00
5 g   268.00
25 g   995.00
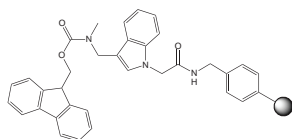
NBC No.: 01-64-0451

Loading: 0.60 - 1.00 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene-1 % DVB), 100 - 200 mesh.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

[3-((Methyl-Fmoc-amino)-methyl)indol-1-yl]acetyl AM resin is an extremely useful support for the production of N-methyl substituted carboxamides and sulfonamides, as well as peptide N-methylamides [1]. After first removing the Fmoc group, loading of the resin with Fmoc-amino acids can be effected using HATU/DIPEA. Cleavage with TFA directly provides the product N-methylamide.

ⓘ Ⓖ 2.30, Ⓜ 3.7, 3.29, Ⓐ 2.30

[1]   K. G. Estep, et al. (1998) *J. Org. Chem.*, **63**, 5300.

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Product No.	Product	Quantity	Price

Resins for Fmoc SPPS of protected peptide fragments

Novabiochem® supplies a complete range of resins for the production of protected peptide acid and amide fragments by both Fmoc batch and continuous flow methodologies. Cleavage can be achieved by mild acidolysis to afford the protected peptide fragments with minimal loss of side-chain protecting groups.

ⓘ
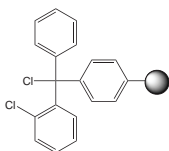
855017 **2-Chlorotrityl chloride resin (100-200 mesh), 1% DVB**

1 g	29.00
5 g	75.00
25 g	295.00

NBC No.: 01-64-0114

Loading: 1.00 - 1.80 mmole/g resin; as determined from the substitution of the Fmoc-Ala-Leu loaded resin. The polymer matrix is copoly (styrene-1 % DVB), 100 - 200 mesh.

⚠ Prolonged storage: +15 to +25°C; bottle must always be kept tightly closed, and if kept cold, it must be allowed to reach room temperature before opening; use only with dried solvents in dry glassware and apparatus; keep open bottle under nitrogen.

An extremely acid-labile resin for preparing peptides and partially protected peptide fragments by the Fmoc strategy [1-7].

This support is ideal for use in the preparation of peptides containing C-terminal Cys, His, Met, Tyr and Pro residues: the method employed for attachment of the first residue does not involve activation of the incoming amino acid derivative, and is thus free from enantiomerization [6]; the bulky trityl cation generated on the resin during the cleavage reaction reacts reversibly, if at all, with the side-chains of those residues, such as Trp, Cys and Met, that are prone to reattachment to benzyl-based linkers; the bulk of the trityl cation also prevents diketopiperazine formation in dipeptides containing proline [1, 8, 9].

Cleavage for protected peptides from this matrix can be effected by treatment with AcOH/TFE/DCM [1-6], 0.5% TFA or HFIP [10]. Fully deprotected peptides can also be obtained by cleaving with 95% TFA in the usual manner.

ⓘ Ⓖ 2.6, 2.18, Ⓜ 3.6, 3.30

[1] K. Barlos, et al. (1989) *Tetrahedron Lett.*, **30**, 3947.
[2] K. Barlos, et al. (1989) *Tetrahedron Lett.*, **30**, 3943.
[3] K. Barlos, et al. (1991) *Angew. Chem.*, **103**, 572.
[4] K. Barlos, et al. (1991) *Int. J. Peptide Protein Res.*, **37**, 513.
[5] K. Barlos, et al. (1991) *Int. J. Peptide Protein Res.*, **38**, 562.
[6] K. Barlos, et al. (1991) *Int. J. Peptide Protein Res.*, **38**, 555.
[7] P. Athanassopoulos, et al. (1995) *Tetrahedron Lett.*, **36**, 5645.
[8] K. Barlos, et al. (1993) *Ann. Chem.*, 215.
[9] R. Steinauer, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 3rd International Symposium", R. Epton (Eds), Mayflower Worldwide Ltd., Birmingham, 1993, pp. 689.
[10] R. Bollhagen, et al. (1994) *J. Chem. Soc., Chem. Commun.*, 2559.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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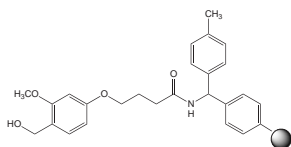
Product No.	Product	Quantity	Price
855061	**HMPB–MBHA resin**	1 g	50.00
	4-Hydroxymethyl-3-methoxyphenoxybutyric acid MBHA resin	5 g	200.00

NBC No.: 01-64-0036

Loading: 0.40 - 1.00 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The polymer matrix is copoly (styrene - 1% DVB), 100 - 200 mesh.

This support consists of MBHA resin functionalized with Riniker's hyperacid-labile 4-hydroxymethyl-3-methoxyphenoxybutyric acid linker [1].

This hyperacid-sensitive resin is a useful tool for the Fmoc SPPS of protected peptide acid fragments.

Attachment of carboxylic acids is normally achieved by DMAP catalyzed esterification with the appropriate symmetrical anhydride, or by using 2,6-dichlorobenzoyl chloride/pyridine [2] or MSNT/MeIm activation [3, 4, 5]. Detachment of these functionalities can be effected by treatment with 1-5% TFA in DCM.

ⓘ Ⓖ 2.6, 2.34, Ⓜ 3.6, 3.30

[1] A. Flörsheimer, et al. in "Peptides 1990, Proc. 21st European Peptide Symposium", E. Giralt & D. Andreu (Eds), ESCOM, Leiden, 1991, pp. 131.
[2] P. Sieber (1987) *Tetrahedron Lett.,* **28**, 6147.
[3] B. Blankemeyer-Menge, et al. (1990) *Tetrahedron Lett.,* **31**, 1701.
[4] J. Nielsen (1996) *Tetrahedron Lett.,* **31**, 1710.
[5] E. Harth-Fritschy & D. Cantacuzène (1997) *J. Peptide Res.,* **50**, 415.
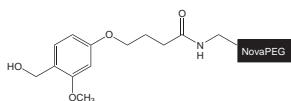
855124	**NovaPEG HMPB resin**	1 g	70.00
	NBC No.: 01-64-0478	5 g	275.00

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Loading: 0.50 - 0.80 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin.

This support consists of NovaPEG resin functionalized with Riniker's hyperacid-labile 4-hydroxymethyl-3-methoxyphenoxybutyric acid linker (see entry for 01-64-0033 for applications information).

NovaPEG resins have excellent swelling properties in a wide range of solvents, including water, MeCN, MeOH, DCM, DMF, THF, and toluene and exhibit exceptional chemical stability to acids, bases and, organometallic reagents.

ⓘ Ⓖ 2.3, 2.6, 2.34, Ⓜ 3.6, 3.30

[1] F. Garcia-Martin, et al. (2006) *J. Comb. Chem.,* **8**, 213.

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Product No.	Product	Quantity	Price

855010   **NovaSyn® TGT alcohol resin**

     NBC No.: 01-64-0066

		1 g	73.00
		5 g	292.00
		25 g	1168.00

Loading: 0.15 - 0.30 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

NovaSyn® TGT alcohol resin is derived from TG amino resin by acylation with Bayer's 4-carboxytrityl linker [1]. Before use the resin must be converted to the chloride form by heating with AcCl [2, 3] or $SOCl_2$ [4, 5] in toluene. The unstable trityl chloride resin should then be used immediately.

Attachment of α-amino acids is free from enantiomerization, making this support ideal for the immobilization of sensitive residues such as Cys and His.

Iodine oxidation of peptides containing Cys(Trt) occurs with release of the peptide from the resin.

This support is also ideally suited to the synthesis of prolyl peptides as the bulk of the trityl handle prohibits diketopiperazine formation [6].

Cleavage of protected peptide acids from this matrix can be effected by treatment with AcOH/MeOH/DCM [1], 0.5% TFA or HFIP [7].

ⓘ Ⓖ 2.3, 2.6, 2.19, Ⓜ 2.19, 3.6, 3.30

[1] E. Bayer, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 13th American Peptide Symposium", R. S. Hodges & J. A. Smith (Eds), ESCOM, Leiden, 1994, pp. 156.
[2] J. M. J. Frechet, et al. (1975) *Tetrahedron Lett.*, 3055.
[3] T. M. Fyles, et al. (1976) *Can. J. Chem.*, **54**, 935.
[4] A. v. Vliet, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 2nd International Symposium", R. Epton (Eds), Intercept UK Ltd., Andover, 1992, pp. 475.
[5] A. v. Vliet, et al. in "Peptides 1992, Proc. 22nd European Peptide Symposium", C. H. Schneider & A. N. Eberle (Eds), ESCOM, Leiden, 1993, pp. 279.
[6] G. Grübler, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 3rd International Symposium", R. Epton (Eds), Mayflower Worldwide Ltd., Birmingham, 1994, pp. 517.
[7] R. Bollhagen, et al. (1994) *J. Chem. Soc., Chem. Commun.*, 2559.
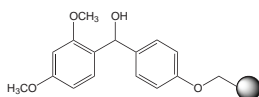
855060   **Rink Acid resin (100–200 mesh)**

     4-(2',4'-Dimethoxyphenyl-hydroxymethyl)-phenoxy resin

     NBC No.: 01-64-0012

		1 g	35.00
		5 g	140.00
		25 g	560.00

Loading: 0.35 - 0.80 mmole/g resin; as determined from the substition of the Fmoc-NH_2 loaded resin. The polymer matrix is copoly (styrene - 1% DVB), 100 - 200 mesh.

A super acid-labile support for the Fmoc batch SPPS of protected peptide fragments [1]. Products can be released from this support using as little as 10% AcOH in DCM, providing the protected fragments without premature loss of side-chain protecting groups in high yields and purities. However, due to extreme acid sensitivity of this resin, coupling reactions should be carried out under basic conditions to prevent loss of peptide chains during chain extension. Immer, et al. [2] have shown that PyBOP® can be used successfully with this resin, giving very short coupling times and excellent yields.

ⓘ Ⓖ 2.6, 2.34, Ⓜ 3.6, 3.30

[1] H. Rink (1987) *Tetrahedron Lett.*, **28**, 3787.
[2] H. U. Immer, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 11th American Peptide Symposium", J. E. Rivier & G. R. Marshall (Eds), ESCOM, Leiden, 1990, pp. 1054.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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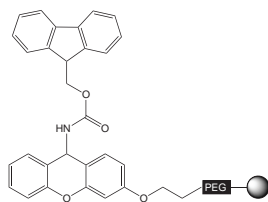
Product No.	Product	Quantity	Price
855013	**NovaSyn® TG Sieber resin**	1 g	130.00
	9-Fmoc-amino-xanthen-3-yloxy TG resin	5 g	520.00
	NBC No.: 01-64-0092	25 g	1990.00

Loading: 0.10 - 0.25 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

⚠ **Prolonged storage**: +2 to +8°C; keep cool and dry.

NovaSyn® TG Sieber resin consists of 130 μm NovaSyn® TG bromo resin derivatized with Sieber's xanhydrylamine linker [1, 2]. This resin is ideal for the Fmoc SPPS of partially protected peptide amide fragments. Cleavage from this support is effected by treatment with 1% TFA in DCM, conditions that leave most standard side-chain protecting groups intact.

This resin can be reductively alkylated and used to prepare secondary carboxamides [3]. It can also be employed to produce protected peptide fragments in which the C-terminal carboxylic acid group is blocked as a hydroxymethylphenoxy-β-alaninamide ester [4].

ⓘ Ⓖ 2.6, 2.24, Ⓜ 3.7, 3.30

[1] P. Sieber (1987) *Tetrahedron Lett.*, **28**, 2107.
[2] C. Somlai, et al. in "Peptides 1992, Proc. 22nd European Peptide Symposium", C. H. Schneider & A. N. Eberle (Eds), ESCOM, Leiden, 1993, pp. 198.
[3] W. C. Chan, et al. (1995) *J. Chem. Soc., Chem. Commun.*, 1475.
[4] W. C. Chan, et al. (1995) *J. Chem. Soc., Chem. Commun.*, 589.
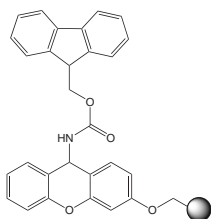
Product No.	Product	Quantity	Price
855008	**Sieber Amide resin**	1 g	80.00
	9-Fmoc-amino-xanthen-3-yloxy-Merrifield resin	5 g	320.00
	NBC No.: 01-64-0059	25 g	1280.00

Loading: 0.40 - 0.80 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene - 1% DVB), 100 - 200 mesh.

⚠ **Prolonged storage**: +2 to +8°C; keep cool and dry.

A hyper acid-labile linker for the Fmoc SPPS of protected peptide amides via mild 1% TFA cleavage [1, 2]. The resin can be readily reductively alkylated to provide a support suited to the synthesis of secondary carboxamides [3].

This resin has also been employed to produce protected peptide fragments in which the C-terminal carboxylic acid group is blocked as a hydroxymethylphenoxy-β-alaninamide ester [4].

ⓘ Ⓖ 2.6, 2.24, Ⓜ 3.7, 3.30

[1] P. Sieber (1987) *Tetrahedron Lett.*, **28**, 2107.
[2] C. Somlai, et al. in "Peptides 1992, Proc. 22nd European Peptide Symposium", C. H. Schneider & A. N. Eberle (Eds), 1993, pp. 198.
[3] W. C. Chan, et al. (1995) *J. Chem. Soc., Chem. Commun.*, 1475.
[4] W. C. Chan, et al. (1995) *J. Chem. Soc., Chem. Commun.*, 589.

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Product No.	Product	Quantity	Price

Resins for Fmoc SPPS of carboxy-modified peptides

Formyl resins

These supports are useful tools for the production of C-terminal peptide N-alkylcarboxamides [1, 2, 3], esters [1, 2, 3], p-nitroanilides [4], and aldehydes [5]. Reductive amination with excess amine or amino acid alkyl ester in TMOF/DCE and NaBH(OAc)$_3$ [2] or AcOH/DMF and NaCNBH$_3$ [1] converts the formyl group quantitatively to the appropriate secondary amine [1]. This resin can be loaded with the appropriate protected amino acid using HATU/DIPEA activation. In the case of the resin pre-loaded with amino acid alkyl esters it is necessary, in order to avoid diketopiperazine formation, to employ Ddz/Trt-protected amino acids [1, 3], or an Fmoc-protected dipeptide [2], for the subsequent coupling reaction. Cleavage for the desired carboxy-modified peptide is effected with 95% TFA under standard conditions.

ⓘ Ⓖ 2.38, Ⓜ 2.40

[1] K. J. Jensen, et al. (1998) *J. Am. Chem. Soc.*, **120**, 5441.
[2] B. Dörner & P. White in "Peptides 1998, Proc. 25th European Peptide Symposium", S. Bajusz & F. Hudecz (Eds), Budapest, Akadémiai Kiadó, 1999, pp. 90.
[3] J. Alsina, et al. (1999) *Chem. Eur. J.*, **5**, 2787.
[4] J. Alsina, et al. (1999) *J. Org. Chem.*, **64**, 8761.
[5] J. Tulla-Puche & G. Barany (2004) *J. Org. Chem.*, **69**, 4101.
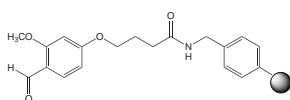
855028	**FMPB AM resin**	1 g	50.00
	4-(4-Formyl-3-methoxyphenoxy)butyryl AM resin	5 g	200.00
	NBC No.: 01-64-0209	25 g	800.00

Loading: 0.80 - 1.10 mmole/g resin; as determined by elemental analysis of sulfur after derivatization with tosylhydrazide. The polymer matrix is copoly (styrene - 1% DVB), 100 - 200 mesh.

⚠ Prolonged storage: +15 to +25°C; keep cool and dry; keep open bottle under nitrogen.

ⓘ Ⓖ 2.38, Ⓜ 2.40, 3.7, 3.29 Ⓐ 2.40
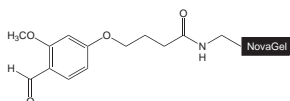
855087	**FMPB NovaGel™ HL**	1 g	72.00
	4-(4-Formyl-3-methoxyphenoxy)butyryl NovaGel™ HL	5 g	288.00
	NBC No.: 01-64-0287	25 g	1152.00

Loading: 0.30 - 0.70 mmole/g resin; as determined by elemental analysis of sulfur after derivatization with tosylhydrazide. The PEG-PS resin is prepared from aminoethyl copoly (styrene- 1% DVB), 100 - 200 mesh.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.
This product is prepared from aminomethyl NovaGel™ by derivatization with the 4-(4-formyl-3-methoxyphenoxy)butanoic acid. For further information on NovaGel™ supports, please refer to the product entry for 855084.

ⓘ Ⓖ 2.38, Ⓜ 2.40, 3.7, 3.29 Ⓐ 2.40

⚠ Storage conditions Ⓖ Additional information Ⓜ Method information Ⓐ Application information

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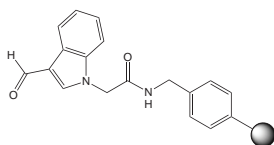
Product No.	Product	Quantity	Price
855098	**(3-Formylindolyl)acetamidomethyl polystyrene**	1 g	50.00
	NBC No.: 01-64-0361	5 g	200.00
	Loading: 0.70 - 1.20 mmole/g resin; as determined by elemental analysis of sulfur	25 g	800.00

after coupling with tosylhydrazide. The polymer matrix is copoly (styrene-1%
DVB), 100 - 200 mesh.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under
nitrogen.

Versatile indole-based linker for the synthesis of secondary amides, carbamates,
ureas [1-4] and aminopurines [5]. In a study comparing the cleavage rates of
secondary amides, ureas, carbamates and sulfonamides from indole,
dialkoxybenzaldehyde and Rink amide resins, the indole resin was found to be the
most acid-labile [6].

Reductive amination with excess of amine and NaBH(OAc)$_3$ in DCE/TMOF [see
page], NaBH$_3$CN in THF/TMOF/AcOH, or NaBH$_4$-Ti(OiPr)$_4$ in THF [2], converts the
formyl group to the appropriate secondary amine. Cleavage can be effected with
2-5 % TFA in DCM, depending on the nature of the product being released from
the resin.

ⓘ **G** 2.38, **M** 2.40, 3.7, 3.29 **A** 2.41

[1] K. G. Estep, et al. (1998) *J. Org. Chem.*, **63**, 5300.
[2] S. Bhattacharyya, et al. (2003) *Tetrahedron Lett.*, **44**, 6099.
[3] N. Hone, et al. (2003) *Tetrahedron Lett.*, **44**, 8169.
[4] L. Sereni, et al. (2004) *Tetrahedron*, **60**, 8561.
[5] P. H. Dorff and R. S. Garigipati (2001) *Tetrahedron Lett.*, **42**, 2771.
[6] B. Yan, et al. (2000) *J. Comb. Chem.*, **2**, 66.

€
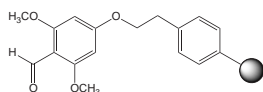
Product No.	Product	Quantity	Price
855035	**DFPE polystyrene**	1 g	80.00
	2-(3,5-Dimethoxy-4-formylphenoxy)ethyl polystyrene	5 g	320.00
	NBC No.: 01-64-0360	25 g	1280.00

Loading: 0.50 - 1.00 mmole/g resin; as determined by elemental analysis of sulfur after derivatization with tosylhydrazide. The polymer matrix is copoly (styrene-1 % DVB), 100 - 200 mesh.

⚠ **Prolonged storage**: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.

This resin is prepared under conditions optimized to minimize styrene formation during attachment of the linker to the functionalized ethylpolystyrene support. This high substitution, coupled with the lack of amide bonds and potentially acid-sensitive benzylic linkages, makes this support the preferred choice of SPOS of secondary sulfonamides and carboxamides. Applications of FMPE include aminofurazanopyrazines [1] and peptide agonists for human orphan receptor BRS-3 [2].

ⓘ Ⓖ 2.38, Ⓜ 2.40, 3.7, 3.29 Ⓐ 2.40

[1] B. Dörner & P. White in "Peptides 1998, Proc. 25th European Peptide Symposium", S. Bajusz & F. Hudecz (Eds), Budapest, Akadémiai Kiadó, 1999, pp. 90.
[2] M. Grimstrup & F. Zaragoza (2002) *Eur. J. Org. Chem.*, 2953.
[3] K. J. Jensen, et al. (1998) *J. Am. Chem. Soc.*, **120**, 5441.
[4] M. del Fresno, et al. (1998) *Tetrahedron Lett.*, **39**, 2639.
[5] N. S. Gray, et al. (1997) *Tetrahedron Lett.*, **38**, 1161.
[6] J. Alsina, et al. (1999) *Chem. Eur. J.*, **5**, 2787.
[7] J. F. Tolborg & K. J. Jensen (2000) *Chem. Commun.*, 147.
[8] A. E. Fenwick, et al. (2001) *Bioorg. Med. Chem. Lett.*, **11**, 195.
[9] J. Giovannoni, et al. (2001) *Tetrahedron Lett.*, **42**, 5389.
[10] J. T. Bork, et al. (2003) *Org. Lett.*, **5**, 117.
[11] C. P. Holmes, Paper ORGN 383 presented at the 213th National Meeting of the American Chemical Society, San Francisco, 1997.
[12] C. Pégurier, et al. (2000) *Bioorg. Med. Chem.*, **8**, 163.
[13] T. F. Herpin, et al. (2000) *J. Comb. Chem.*, **2**, 513.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

HMBA resins

These supports consist of amino functionalized resins which have been derivatized with Sheppard's base labile 4–hydroxymethyl benzoic acid linker [1]. This is perhaps one of the most versatile linkers used in Fmoc SPPS as, by varying the nature of the nucleophile employed for cleavage, a wide variety of C–terminal modified peptide fragments can be obtained from the one support: primary amides [2, 3, 4, 5], secondary amides [2], hydrazides [3, 4, 5], alcohols [3, 4], methyl esters [2 – 7]. Attachment of the first amino acid to these resins can be achieved either by DMAP catalyzed esterification with the appropriate symmetrical anhydride, or by using 2,6–dichlorobenzoyl chloride/pyridine [8] or MSNT/MeIm [9, 10, 11] activation methods.

ⓘ 🄶 2.6, 🄼 3.6, 3.30

[1] R. C. Sheppard, et al. (1982) *Int. J. Peptide Protein Res.*, **20**, 451.
[2] E. Atherton & R. C. Sheppard in "Solid phase peptide synthesis; a practical approach", IRL Press, Oxford, 1989, pp. 152.
[3] J. M. Stewart & J. D. Young in "Solid phase peptide synthesis, 2nd Ed.", Pierce Chemical Company, Rockford, 1984, pp. 91.
[4] *Novabiochem Technical Notes 1/94.*
[5] D. Wellings in "Fmoc solid phase peptide synthesis: a practical approach", W. C. Chan & P. D. White (Eds), Oxford, Oxford University Press, 2000, pp. 146.
[6] S. M. Hutchins & K. T. Chapman (1996) *Tetrahedron Lett.*, **37**, 4869.
[7] Y. Cheng & K. T. Chapman (1997) *Tetrahedron Lett.*, **38**, 1497.
[8] P. Sieber (1987) *Tetrahedron Lett.*, **28**, 6147.
[9] B. Blankemeyer-Menge, et al. (1990) *Tetrahedron Lett.*, **31**, 1701.
[10] J. Nielsen (1996) *Tetrahedron Lett.*, **37**, 1710.
[11] E. Harth-Fritschy & D. Cantacuzène (1997) *J. Peptide Res.*, **50**, 415.
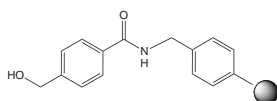
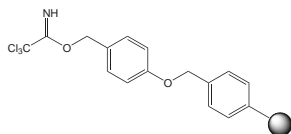|			
855018	**HMBA–AM resin**	1 g	50.00
	4-Hydroxymethylbenzoic acid AM resin	5 g	200.00
	NBC No.: 01-64-0122	25 g	800.00

Loading: 0.70 - 1.20 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The polymer matrix is copoly (styrene - 1% DVB), 200 - 400 mesh.

This support consists of aminomethyl polystyrene resin functionalized with Sheppard's base-labile 4-hydroxymethylbenzoic acid linker [1].

ⓘ 🄶 2.6, 🄼 3.6, 3.30

[1] R. C. Sheppard, et al. (1982) *Int. J. Peptide Protein Res.*, **20**, 451.

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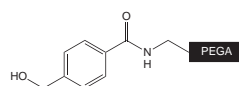
Product No.	Product	Quantity	Price
855070	**HMBA–PEGA resin**	1 g	142.00
	4-Hydroxymethylbenzoic acid PEGA resin	5 g	568.00

NBC No.: 01-64-0113

Loading: 0.10 - 0.40 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The resin is sold swollen in ethanol.

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry.

This support consists of amino PEGA resin derivatized with Sheppard's base-labile 4-hydroxymethylbenzoic acid linker [1].

For further information, please refer to the product entry for amino PEGA resin (855015). **Note:** PEGA resins are supplied swollen in ethanol, with 1g dry resin corresponding to approximately 15 mL of swollen resin. As the beads of PEGA resins become very sticky and easily damaged when shrunk or dried, they are best handled in a swollen state. To use, the appropriate amount of swollen resin should be weighed into a reaction vessel and any residual ethanol removed by copious washings with the appropriate solvent or solvent mixture. After peptide assembly, the resin should be washed with DCM and transferred to the cleavage vessel. Excess DCM can be removed under vacuum, and the shrunk resin treated with the TFA cocktail.

The characteristics of the swollen resin, its robustness and high permeability enables its use in both batch and continuous flow synthesis apparatus.

ⓘ Ⓖ 2.3, 2.6, Ⓜ 3.6, 3.30

[1] R. C. Sheppard, et al. (1982) *Int. J. Peptide Protein Res.,* **20**, 451.

855062	**NovaSyn® TG HMBA resin**	1 g	80.00
	NBC No.: 01-64-0091	5 g	320.00
		25 g	1280.00

Loading: 0.20 - 0.30 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin.

This support consists of NovaSyn® TG amino resin derivatized with Sheppard's base-labile 4-hydroxymethylbenzoic acid linker [1].

ⓘ Ⓖ 2.3, 2.6, Ⓜ 3.6, 3.30

[1] R. C. Sheppard, et al. (1982) *Int. J. Peptide Protein Res.,* **20**, 451.
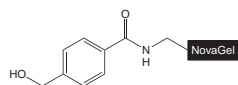
855086	**HMBA–NovaGel™**	1 g	60.00
	4-Hydroxymethylbenzoic acid NovaGel™	5 g	240.00
	NBC No.: 01-64-0285	25 g	960.00

Loading: 0.50 - 0.80 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin.

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry.

This resin is prepared by derivatization of aminomethyl NovaGel™ with the base-labile 4-hydroxymethylbenzoic acid linker. NovaGel™ resins combine the high functionality of polystyrene resins with the excellent swelling properties of PEG-PS type supports [1].

ⓘ Ⓖ 2.3, 2.6, Ⓜ 3.6, 3.30

[1] J. H. Adams, et al. (1998) *J. Org. Chem.,* **63**, 3706.

RESINS FOR SOLID PHASE PEPTIDE SYNTHESIS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Hydrazinobenzoyl resins

855037   **4–Fmoc–hydrazinobenzoyl AM NovaGel™**   1 g   60.00
NBC No.: 01-64-0377   5 g   240.00
Loading: 0.40 - 0.80 mmole/g resin; by photometric determination of the Fmoc-   25 g   940.00
chromophore liberated upon treatment with DBU/DMF. The PEG-PS resin is
prepared from aminomethyl copoly (styrene -1% DVB), 100 - 200 mesh.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

This safety-catch resin is a versatile tool for the production of C-terminal peptide
acids [1], esters [1, 2] and amides [1], p-nitroanilides [3], and thioesters [4].
Following removal of the Fmoc group with piperidine, the resin can be acylated
with the first residue under standard conditions. Acyl hydrazines derived from this
resin are stable to TFA and piperidine, thereby providing the capability of removing
protecting groups sensitive to these reagents prior to release of the target from
the support. Oxidation with copper (II) acetate or NBS converts the hydrazine to a
highly reactive diazene, which in the presence of water, amine, alcohol or thiol,
decomposes to give the corresponding carboxylic acid, amide or ester. N-terminal
or side-chain amino groups of the immobilized peptide can be used as the
nucleophilic component, in which case the product is the appropriate cyclic
peptide [5].

A similar support based on a sulfonylphenylhydrazine linker has also been
employed in peptide synthesis [3]. For a review on the applications of this resin,
see [6].

ⓘ Ⓖ 2.29, Ⓜ 3.7, 2.29

[1] C. R. Millington, et al. (1998) *Tetrahedron Lett.*, **39**, 7201.
[2] C. Peters & H. Waldmann (2003) *J. Org. Chem.*, **68**, 6053.
[3] Y. Kwon, et al. (2004) *Org. Lett.*, **6**, 3801.
[4] J. A. Camarero, et al. (2004) *J. Org. Chem.*, **69**, 4145.
[5] C. Rosenbaum & H. Waldmann (2001) *Tetrahedron Lett.*, **42**, 5677.
[6] Y.-H. Woo, et al. (2007) *Int. J. Pept. Res. Ther.*, **13**, 181.

€

Product No.	Product	Quantity	Price

Amino aldehydes attached to H-Thr-Gly-NovaSyn® TG resin

These resins are a useful tool for the synthesis of peptide aldehydes [1]. Following chain extension using standard protocols, side-chain protecting groups can be removed with anhydrous TFA, prior to cleavage of the fully deprotected peptide aldehyde with MeCN/water/TFA.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

ⓘ Ⓖ 3.47, Ⓜ 3.48 Ⓐ 3.48

[1] N. J. Ede, et al. (2000) *J. Peptide Sci.*, **6**, 11.
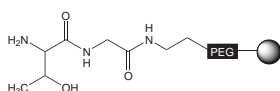
855049	**H–Thr-Gly-NovaSyn® TG resin**	1 g	100.00
	NBC No.: 04-12-3710	5 g	400.00

Loading: 0.15 - 0.30 mmole/g resin; as determined from the substitution of the Fmoc- oxybenzaldehyde loaded resin. The base resin is amino PEG-PS-copolymer (90 μm).

This resin is a useful tool for the synthesis of peptide aldehydes [1]. Attachment of pre-formed amino acid aldehydes to this resin is effected by heating in 1% DIPEA in MeOH at 60 °C. Following chain extension using standard protocols, side-chain protecting groups can be removed with anhydrous TFA, prior to cleavage of the fully deprotected peptide aldehyde with MeCN/water/TFA.

An analogous resin has been recently used to capture and purify peptide aldehydes generated by oxidation of peptide alcohols with IBX-polystyrene [2]. Novabiochem's pre-loaded amino aldehyde resins can be found on page x.

[1] N. J. Ede, et al. (2000) *J. Peptide Sci.*, **6**, 11.
[2] G. Sorg, et al. (2005) *J. Peptide Sci.*, **11**, 142.
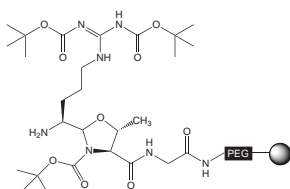
856073	**H–Arg(Boc)₂–H NovaSyn® TG resin**	1 g	200.00
	NBC No.: 04-12-3723	5 g	800.00

Loading: 0.15 - 0.25 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. Base resin is amino PEG-PS-polymer (90μm).
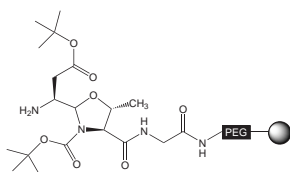
856072	**H–Asp(OtBu)–H NovaSyn® TG resin**	1 g	175.00
	NBC No.: 04-12-3722	5 g	700.00

Loading: 0.15 - 0.25 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 μm).

856071	**H–Leu-H NovaSyn® TG resin**	1 g	175.00
	NBC No.: 04-12-3720	5 g	700.00

Loading: 0.15 - 0.25 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 μm).

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
856144	**H–Phe–H NovaSyn® TG resin**	1 g	170.00
	NBC No.: 04-12-3721	5 g	680.00
	Loading: 0.15 - 0.25 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS copolymer (90μm).		
856145	**H–Val–H NovaSyn® TG resin**	1 g	250.00
	NBC No.: 04-12-3725	5 g	1250.00
	Loading: 0.15 - 0.25 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 μm).		
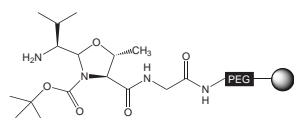
Peptide aldehydes & ketones
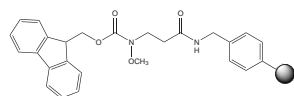
855074	**Weinreb AM resin**	1 g	41.00
	N-Fmoc- N-methoxy-β-alanine AM resin	5 g	164.00
	NBC No.: 01-64-0153	25 g	656.00

Loading: 0.40 - 1.00 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene - 1% DVB), 100 - 200 mesh..

This support is useful for the production of peptide aldehydes and other carboxaldehydes. Following removal of the Fmoc group with 20% piperidine in DMF, acylation of the resin-bound methoxylamine is best effected using DIPCDI/HOAt or HATU/DIPEA activation. This results in formation of a supported Weinreb-type amide which can be either reduced to an aldehyde with $LiAlH_4$ [1, 2] or cleaved with Grignard reagents to give ketones [2, 3].

ⓘ Ⓖ 2.28, Ⓜ 3.7, 2.29

[1] J. A. Fehrentz, et al. (1995) *Tetrahedron Lett.*, **36**, 7871.
[2] T. Q. Dinh & R. W. Armstrong (1996) *Tetrahedron Lett.*, **37**, 1161.
[3] C. M. Tice, et al. (2002) *Tetrahedron Lett.*, **43**, 7491.

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Product No.	Product	Quantity	Price

Hydroxamic acids
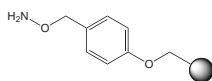
855117 **Hydroxylamine Wang resin**

Amino-oxy Wang resin

NBC No.: 01-64-0454

Loading: 1.5 - 2.50 mmole/g resin; as determined by elemental analysis of nitrogen. The polymer matrix is copoly (styrene - 1% DVB), 100 - 200 mesh.

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry.

An excellent support for the preparation of peptide hydroxamic acids [1]. Acylation of the resin has been carried out using TBTU/DIPEA activation [1]. Alkylation of the resin-bound nitrogen has been effected using TMOF/DCE/AcOH for imine formation, followed by reduction with BH_3-pyridine [2], by base-mediated alkylation of the N–Boc-protected hydroxylamine, followed by Boc removal with 20% TFA in DCM [3], or by Michael-addition [4]. Cleavage of hydroxamates from the resin has been effected with TFA/DCM/water (70:28:2) [1] or TFA/DCM (1:1) [2]. Hydroxylamine Wang resin has also been utilized to prepare tertiary amines by base-mediated elimination of the quaternized polymer-bound hydroxylamine [3, 5].

ⓘ Ⓖ 2.30

[1] C. D. Floyd, et al. (1996) *Tetrahedron Lett.*, **37**, 8045.

[2] D. E. Robinson & M. W. Holladay (2000) *Org. Lett.*, **2**, 2777.

[3] P. Blaney, et al. (2000) *Tetrahedron Lett.*, **41**, 6635.

[4] A. Volonterio, et al. (2002) *Eur. J. Org. Chem.*, 428.

[5] P. Blaney, et al. (2000) *Tetrahedron Lett.*, **41**, 6639.

	Quantity	Price
	5 g	110.00
	25 g	440.00
	100 g	1320.00

RESINS FOR SOLID PHASE PEPTIDE SYNTHESIS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Resins for native chemical ligation by Fmoc SPPS

Dawson resins

Dbz resins are novel suports for the synthesis of peptide thioesters by Fmoc SPPS [1]. After removal of the Fmoc group, the resin is acylated with the first amino acid (see Method 5.3 page 5.2 for details) then synthesis is carried out using HBTU/HOBt activation. To avoid branching, especially in case of Fmoc-Gly-OH couplings, protection of the second amino group with allyloxycarbonyl (Alloc) is recommended [2]. Following chain assembly the resin is activated by treatment with p-nitrophenyl chloroformate. Treatment with TFA liberates the fully deprotected peptide benzimidazolinone which can be converted to a thioester with aryl thiol or used directly in native chemical ligation and cyclization reactions. For recent applications see [3-6].

ⓘ Ⓖ 5.4, Ⓜ 5.5

[1]   J. B. Blanco-Canosa (2008) *Angew. Chem. Int. Ed.*, **47**, 6851.
[2]   S. K. Mahto, et al. (2011) *ChemBioChem* , **12**, 2488.
[3]   Z. Harpaz, et al. (2010) *ChemBioChem*, **11**, 1232.
[4]   B. L. Pentelute, et al. (2010) *Chem. Biol.*, **5**, 359.
[5]   T. K. Tiefenbrunn, et al. (2010) *Pept. Sci.*, **94**, 405.
[6]   S. Gunasekera, et al. (2013) *Int. J. Pept. Res. Ther.*, **19**, 43.
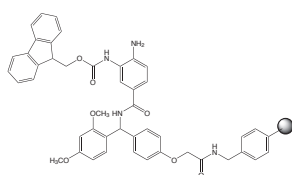
855131	**Dawson Dbz Rink AM resin (100 – 200 mesh)**	1 g	60.00
	3-(Fmoc-amino)-4-aminobenzoyl Rink Amide AM resin (100 - 200 mesh)	5 g	240.00
	Loading: 0.40 - 0.80 mmole/g resin; as photometric determination of the Fmoc-chromophore liberated upon treatment with piperidine/DMF.	25 g	940.00
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		

855142	**Dawson Dbz NovaSyn® TGR resin**	1 g	80.00
	Loading: 0.10 - 0.30 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.	5 g	320.00
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		

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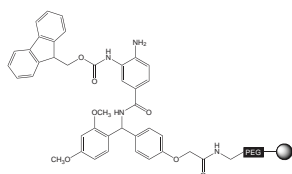
Product No.	Product	Quantity	Price

SEA resins

SEA are useful tools for the synthesis for peptides for used in native chemical ligation reactions (NCL) [1-3]. Loading of the resin with the *C*-terminal amino acid is best done using HATU activation. On cleavage, a peptide is produced bearing a bis(2-sulfanylethyl)amide (SEA) on the *C*-terminus of the peptide. To stabilize the peptide and to simplify HPLC in acidic buffers, the SEA peptide should be converted to the disulfide form by air or iodine oxidation. In the presence of TCEP, cyclic SEA peptides undergo rapid NCL or can be converted to thioesters. SEA disulfide peptides do not undergo ligation in the absence of reducing reagents. This has been exploited to perform one-pot three segment ligations [4].

ⓘ Ⓖ 5.5

[1]   N. Ollivier, et al. (2010) *Org. Lett.*, **12**, 5238.
[2]   J. Dheur, et al. (2011) *J. Org. Chem.*, **76**, 3194.
[3]   J. Dheur, et al. (2011) *Org. Lett.*, **13**, 1560.
[4]   N. Ollivier, et al. (2012) *Angew. Chem. Int. Ed.*, **51**, 209.
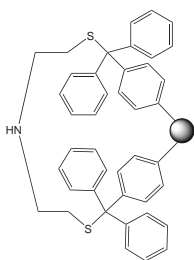
855152 **NEW**	**SEA-PS resin** bis(2-Sulfanylethyl)aminotrityl polystyrene Loading: 0.10 - 0.16 mmole/g resin; as photometric determination of the Fmoc-chromophore liberated upon treatment with piperidine/DMF. The polymer matrix is: copoly (styrene-1 % DVB), 200-400 mesh.	1 g 5 g	180.00 580.00

RESINS FOR SOLID PHASE PEPTIDE SYNTHESIS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Sulfamylbutyryl resins & linkers

Sulfamylbutyryl resins facilitate the preparation by Fmoc SPPS of the *C*-terminal peptide thioesters required for native thiol ligation.

The resin-bound sulfamyl group is acylated by carboxylic acids activated with PyBOP® and DIPEA in CHCl$_3$ at –20 °C [1] or DIPCDI and N-methylimidazole in DCM [2]. It can be sometimes problematic to obtain acceptable loadings by these methods, and so it is for this reason that Novabiochem® now offers a number of pre-loaded sulfamylbutyryl resins.

The resin-bound acylsulfonamide is completely stable to basic or strongly nucleophilic reagents, making it compatible with Fmoc SPPS.

Following completion of the synthesis, the sulfonamide can be activated by methylation with TMS-CHN$_2$, and then cleaved with ethyl mercaptopropionate in the presence of sodium thiophenolate, to yield the appropriate thioester [3]. Activation can be performed with iodoacetonitrile/DIPEA and cleavage by benzylmercaptan [4]. Following removal of side-chain protecting groups with TFA, this material can be used for native thiol ligation. Alternatively, if a sulfamylbutyryl Rink amide resin is used, treatment with TFA generated a peptide N-methylsulfonamide which can be used directly in the NCL reaction without need for prior formation of the thioester. Furthermore, this method enables the progress of the synthesis and the extent of methylation to be checked by performing test cleavages during the synthesis [5].

For further examples of the use of sulfamyl resins in the preparation of thioesters, see [6 - 10]. The use of this type of linker has been reviewed [11, 12].

ⓘ Ⓖ 5.1, Ⓜ 5.2 Ⓐ 5.3, 5.4

[1]   B. J. Backes & J. A.Ellman (1999) *J. Org. Chem.*, **64**, 2322.
[2]   Novabiochem Innovations 4/99.
[3]   R. Ingenito, et al. (1999) *J. Am. Chem. Soc.*, **121**, 11369.
[4]   Y. Shin, et al. (1999) *J. Am. Chem. Soc.*, **121**, 11684.
[5]   F. Burlina, et al. (2012) *Chem. Commun.*, 2579.
[6]   S. Biancalana, et al. (2001) *Lett. Pept. Sci.*, **7**, 291.
[7]   D. Fattori, et al. (2002) Bioorg. Med. Chem. Lett., 12, 1143.
[8]   P. Buczek, et al. (2004) Biopolymers, 80, 50.
[9]   K. Teruya, et al. (2004) J. Pept. Sci., 10, 479.
[10] D. Macmillan, et al. (2002) Org. Lett., 4, 1467.
[11] P. Heidler & A. Link (2005) Biorg. Med. Chem., 13, 585.
[12] J. C. M. Monbaliu & A. R. Katritzky (2012) *Chem. Commun.*, 11601.
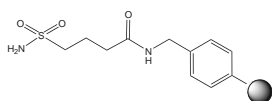
855021	**4-Sulfamylbutyryl AM resin**	1 g	65.00
	NBC No.: 01-64-0152	5 g	260.00
	Loading: 0.60 - 1.20 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The polymer matrix is copoly (styrene-1 % DVB), 200 -400 mesh.	25 g	990.00
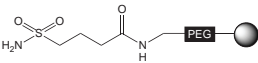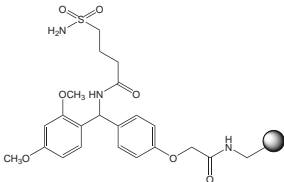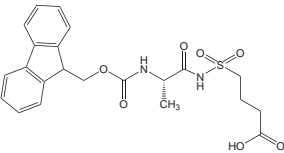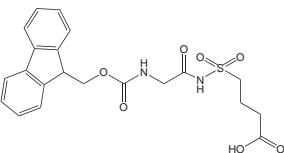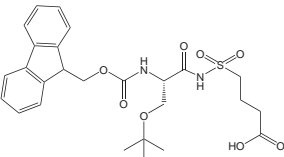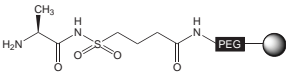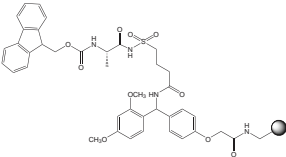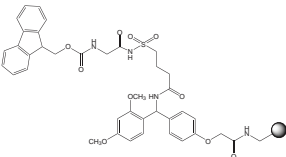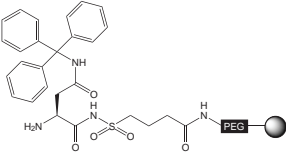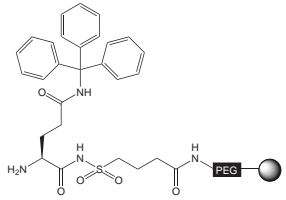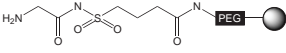
⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
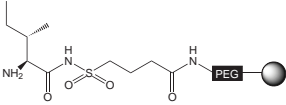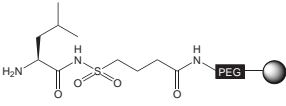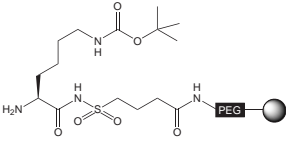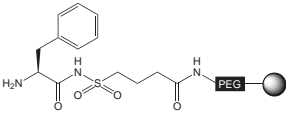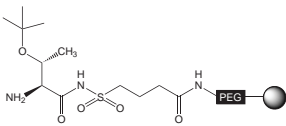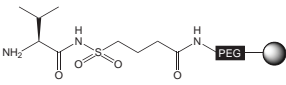
Product No.	Product	Quantity	Price
855044	**4–Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 01-64-0458 **Loading**: 0.15 - 0.30 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is PEG-PS-copolymer (90 µm), functionalized with an amino group. ⚠ **Prolonged storage**: +2 to +8°C; keep cool and dry.	1 g 5 g	100.00 400.00
855147	**4–Sulfamylbutyryl Rink Amide AM resin** CAS No.: 878408-63-0; $C_{29}H_{31}NO_8S$; M.W.: 553.6 **Loading**: 0.60 - 1.20 mmole/g resin; as determined by elemental analysis of sulfur. ⚠ **Prolonged storage**: +2 to +8°C; keep cool and dry. TFA cleavage, following activation of the linker by methylation, liberates a peptidyl N-methyl-sulfonamide which can be used directly in native chemical ligation reagents without the need for prior conversion to the thioester [1]. ⓘ [1] F. Burlina, et al. (2012) *Chem. Commun.*, 2579.	1 g 5 g	90.00 360.00
851213	**Fmoc-Ala-sulfamylbutyryl linker** **TLC**: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%. **HPLC**: purity: ≥ 97.00%. **Optical purity**: ≥ 99.50% L-enantiomer. ⚠ **Prolonged storage**: +2 to +8°C; keep cool and dry. ⓘ Ⓐ 5.3, 5.4	1 g	290.00
851214	**Fmoc-Gly-sulfamylbutyryl linker** **TLC**: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%. **HPLC**: purity: ≥ 97.00%. **Optical purity**: ≥ 99.50% L-enantiomer. ⚠ **Prolonged storage**: +2 to +8°C; keep cool and dry. ⓘ Ⓐ 5.3, 5.4	1 g	290.00
851215	**Fmoc-Ser(tBu)-sulfamylbutyryl linker** **TLC**: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%. **HPLC**: purity: ≥ 97.00%. **Optical purity**: ≥ 99.50% L-enantiomer. ⓘ Ⓐ 5.3, 5.4	1 g	290.00

RESINS FOR SOLID PHASE PEPTIDE SYNTHESIS

⚠ Storage conditions Ⓖ Additional information Ⓜ Method information Ⓐ Application information

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Product No.	Product	Quantity	Price
856069	**H–Ala–Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 04-12-3715 Loading: 0.18 - 0.25 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 µm). ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	234.00 926.00
856191	**Fmoc–Ala–4–Sulfamylbutyryl Rink Amide AM resin** Loading: 0.20 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry. TFA cleavage, following activation of the linker by methylation, liberates a peptidyl N-methyl-sulfonamide which can be used directly in native chemical ligation reagents without the need for prior conversion to the thioester [1]. ⓘ Ⓖ 2.22 [1] F. Burlina, et al. (2012) *Chem. Commun.*, 2579.	1 g 5 g	200.00 800.00
856192	**Fmoc–Gly–4–Sulfamylbutyryl Rink Amide AM resin** Loading: 0.20 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry. TFA cleavage, following activation of the linker by methylation, liberates a peptidyl N-methyl-sulfonamide which can be used directly in native chemical ligation reagents without the need for prior conversion to the thioester [1]. ⚠ Prolonged storage: keep cool and dry. ⓘ Ⓐ 5.3, 5.4 [1] F. Burlina, et al. (2012) *Chem. Commun.*, 2579.	1 g 5 g	200.00 800.00
856078	**H–Asn(Trt)–Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 04-12-3730 Loading: 0.15 - 0.25 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 µm). ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	200.00 800.00
856070	**H–Gln(Trt)–Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 04-12-3717 Loading: 0.15 - 0.30 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 µm). ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	234.00 926.00
856068	**H–Gly–Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 04-12-3714 Loading: 0.18 - 0.25 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 µm). ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	234.00 926.00

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Product No.	Product	Quantity	Price
856076	**H–Ile–Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 04-12-3727 Loading: 0.20 - 0.30 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 µm). ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	200.00 800.00
856077	**H–Leu–Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 04-12-3728 Loading: 0.15 - 0.25 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 µm). ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	200.00 800.00
856074	**H–Lys(Boc)–Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 04-12-3724 Loading: 0.15 - 0.30 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 µm). ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	200.00 800.00
856079	**H–Phe–Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 04-12-3731 Loading: 0.18 - 0.25 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. Base resin is amino PEG-PS-polymer (90µm). ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	200.00 800.00
856080	**H–Thr(tBu)–Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 04-12-3732 Loading: 0.20 - 0.30 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. Base resin is amino PEG-PS-polymer (90µm). ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	200.00 800.00
856075	**H–Val–Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 04-12-3726 Loading: 0.15 - 0.25 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 µm). ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	200.00 800.00

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Resins for Boc SPPS of peptide acids
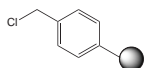
855059 **Merrield resin LL (100-200 mesh)**

Chloromethylpolystyrene-divinylbenzene (100-200 mesh)

NBC No.: 01-64-0008

	Quantity	Price
	5 g	17.00
	25 g	68.00
	100 g	200.00

Loading: 0.40 - 1.00 mmole/g resin; as determined by elemental analysis for sulfur after derivatization with KSAc. The polymer matrix is copoly (styrene-1% DVB), 100 -200 mesh (75 - 150 μm).

Merrifield resin was the standard support for the synthesis of peptide acids by Boc SPPS [1, 2]. In peptide chemistry, it is now really only used in the synthesis of small to medium sized peptides, as the benzylic ester resin linkage is not completely stable towards repetitive treatment with TFA.

Attachment of the C-terminal residue is generally achieved by heating the resin in DMF with the appropriate amino acid cesium salt in the presence of KI [3]; although, Me_4N salts [4], sodium salts [5] in THF with Bu_4NF catalysis [6] and zinc salts in EtOH have also been used [7].

Cleavage is normally effected by treatment of resin with HF or TFMSA, or by hydrogenolysis [8]. Alcohols can be released using reducing agents such as DIBAL [6] or $LiBH_4$ [9]. Methyl esters can be produced by transesterification with methoxide [10].

ⓘ Ⓖ 2.10, 2.14, Ⓜ 2.14, 3.26

[1] M. Bodanszky, et al. in "Peptide Synthesis", E. Gross & J. Meienhofer (Eds), Academic Press, Y. Wiley, New York, 1976.
[2] G. Barany, et al. (1980) *Peptides*, **2**, 1.
[3] B. F. Gisin (1973) *Helv. Chim. Acta*, **56**, 1476.
[4] A. Loffet (1971) *Int. J. Protein Res.*, **3**, 297.
[5] S.-S. Wang (1975) *J. Org. Chem.*, **40**, 1235.
[6] M. J. Kurth, et al. (1994) *J. Org. Chem.*, **59**, 5862.
[7] M. V. Anuradha, et al. (1995) *Tetrahedron*, **51**, 5671.
[8] J. M. Schlatter, et al. (1977) *Tetrahedron Lett.*, 2851.
[9] J. M. Stewart, et al. (1981) , U.S. Patent 4,254,023.
[10] R. Frenette, et al. (1994) *Proc. Natl. Acad. Sci. USA*, **90**, 6906.

855011 **Merrield resin HL (100-200 mesh)**

Chloromethylpolystyrene-divinylbenzene (100-200 mesh)

NBC No.: 01-64-0070

	Quantity	Price
	5 g	22.00
	25 g	88.00
	100 g	264.00

Loading: 1.0 - 1.60 mmole/g resin; as determined by elemental analysis for sulfur after derivatization with KSAc. The polymer matrix is copoly (styrene-1% DVB), 100 -200 mesh.

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Product No.	Product	Quantity	Price

Resins for Boc SPPS of peptide amides

MBHA resins are particularly recommended for the synthesis of peptide amides by Boc chemistry. These supports are more acid sensitive than BHA resins, and thus allow release of the product to be effected with HF or TFMSA under less drastic conditions.

ⓘ Ⓖ 2.10, 2.21, Ⓜ 3.7, 3.26
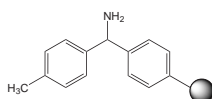
855000	**MBHA resin LL (100–200 mesh) · HCl**	5 g	50.00
	4-Methylbenzhydrylamine resin LL (100-200 mesh) hydrochloride salt	25 g	200.00
	NBC No.: 01-64-0006	100 g	600.00

Loading: 0.60 - 0.80 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin.

Standard resin for the synthesis of peptide amides by Boc SPPS [1, 2]. Carboxylic acids or amino acids can be attached using standard methods of amide bond formation. Cleavage of the carboxamides from this resin can be effected by treatment with HF or TFMSA.

[1] G. R. Matsueda, et al. (1981) *Peptides*, **2**, 45.

[2] K. Channabasavaiah, et al. (1981) *Biochem. Biophys. Res. Commun.*, **86**, 45.
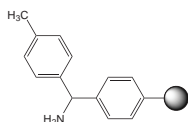
855006	**MBHA resin HL (100–200 mesh) · HCl**	5 g	50.00
	4-Methylbenzhydrylamine resin HL (100-200 mesh) hydrochloride salt	25 g	200.00
	NBC No.: 01-64-0042	100 g	600.00

Loading: 0.80 - 1.40 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The polymer matrix is copoly (styrene-1% DVB), 100 - 200 mesh.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Resins for Boc SPPS of carboxy-modified peptides
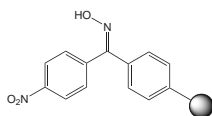
	855089	**Oxime resin LL (100–200 mesh)**	1 g	60.00
		NBC No.: 01-64-0293	5 g	240.00

Loading: 0.50 - 1.00 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The polymer matrix is copoly (styrene - 1% DVB) 100 - 200 mesh.

ⓘ Ⓖ 2.10, 2.36, Ⓜ 3.6, 3.30
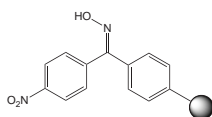
	855090	**Oxime resin HL (100–200 mesh)**	1 g	75.00
		NBC No.: 01-64-0294	5 g	300.00

Loading: 1.00 - 1.50 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The polymer matrix is copoly (styrene - 1% DVB) 100 - 200 mesh.

ⓘ Ⓖ 2.10, 2.36, Ⓜ 3.6, 3.30

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Resins for immunology

855129	NovaSyn® TG PAP resin	1 g	76.00

Loading: 0.20 - 0.30 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin.

⚠ **Prolonged storage**: +2 to +8°C; keep cool and dry.

NovaSyn® TG PAP resin contains an acid cleavable linkage between the polystyrene matrix and the PEG chain. Treatement of peptides prepared on this resin with TFA/thioanisole (95:5) for 12 hours or TMSBr/TFA/thioanisole (1:94:5) for 60 minutes releases a fully deprotected peptide possessing a PEG-chain attached to the C-terminus [1]. Such peptide-PEG conjugates can be used directly as immunogens, without the need for a carrier protein. The peptide-PEG conjugates are also highly soluble and provide an effective solution to insoluble peptides.

[1] W. Rapp in "Combinatorial Peptide and Nonpeptide Libraries", G. Jung (Eds), Vch, 1996, pp. 425.

(price 5 g: 303.00)

Multiple antigen peptide systems (MAPS)

The MAP system represents an approach for anti-peptide antibody elicitation. Originally developed by Dr. James Tam, MAPs are comprised of small immunogenically inert, two-fold bifurcating, polylysine cores onto which peptide antigens can be synthesized. The resulting macromolecule possesses a high molar ratio of peptide antigen to core molecule and is capable of eliciting a strong antibody response, with often significantly higher titers being obtained for a MAP than the monomeric counterpart attached to a carrier protein.

As the leading supplier of resins for MAP synthesis, Novabiochem® offers a range of 8-branch (octavalent) and 4-branch (tetravalent) MAP core molecules attached to several resins for the production if MAPs by both Boc and Fmoc strategies.

ⓘ 2.10

856083	Fmoc$_4$-Lys$_2$-Lys-β-Ala-Wang resin	250 mg	185.00
	NBC No.: 05-24-0152	1 g	555.00

Loading: 0.10 - 0.30 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene-1 % DVB) 100 - 200 mesh.

RESINS FOR SOLID PHASE PEPTIDE SYNTHESIS

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⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
856082	**Fmoc$_8$-Lys$_4$-Lys$_2$-Lys-β-Ala-Wang resin** NBC No.: 05-24-0150 **Loading**: 0.20 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene-1 % DVB) 200 - 400 mesh.	250 mg 1 g	250.00 745.00
856165	**Fmoc$_8$-Lys$_4$-Lys$_2$-Lys-Cys(Acm)-β-Ala-Wang resin** NBC No.: 05-24-0151 **Loading**: 0.20 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene -1% DVB), 200 - 400 mesh.	250 mg 1 g	280.00 840.00

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Product No.	Product	Quantity	Price

Resins for solid phase organic synthesis

Alkenyl based resins
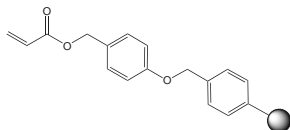
855101 **REM resin (50-100 mesh)**

Acryloyl Wang resin

NBC No.: 01-64-0393

1 g	50.00
5 g	200.00
25 g	800.00

Loading: 0.80 - 1.20 mmole/g resin; as determined by elemental analysis of chlorine after derivatization with 4-chloro-benzylamine. The polymer matrix is copoly(styrene-1% DVB), 50 - 100 mesh.

Novabiochem®'s 50-100 mesh REM resin [1] is an exceptionally high quality resin, designed specially for use in IRORI Kans and other parallel synthesis applications that require supports with reproducible substitution and swelling properties containing minimal leachable impurities.

This material is derived from poly(chloromethylstyrene-styrene-divinylbenzene) manufactured to ISO 9001 standards. Unlike other Merrifield supports, which are prepared by chloromethylation of polystyrene, this matrix is prepared by co-polymerization of chloromethylstyrene, styrene and divinylbenzene. Since the amount of chloromethylstyrene added to the polymerization reaction can be precisely controlled, this leads to a highly defined resin with little batch to batch variation in functionality. The swelling characteristics of such resins are also more reproducible since the process of manufacture does not introduce additional cross-linking, as is the case with resins produced by chloromethylation.

For applications of REM resin in SPOS see references [2-4].

ⓘ Ⓖ 2.44, Ⓜ 2.45

[1]   A. Brown, et al. (1997) *J. Am. Chem. Soc.*, **119**, 3288.
[2]   J. Cottney, et al. (1999) *Bioorg. Med. Chem. Lett.*, **9**, 1323.
[3]   A. Born, et al. (2001) *Bioorg. Med. Chem. Lett.*, **11**, 2351.
[4]   S. Caix-Haumesser, et al. (2001) *Tetrahedron Lett.*, **42**, 3721.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Br, Cl functionalized resins
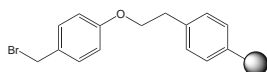
855104 **2-(4-Bromomethylphenoxy)ethyl polystyrene HL**
NBC No.: 01-64-0400
Loading: 0.80 - 1.20 mmole/g resin; as determined by elemental analysis of bromine. The polymer matrix is copoly(styrene - 1% DVB), 100 - 200 mesh.

1 g	40.00
5 g	160.00
25 g	640.00

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

This versatile support is an excellent tool for the solid phase immobilization of carboxylic acids, amines and alcohols. Acids are attached by treating the resin with the appropriate DIPEA salt in DMF in the presence of CsI [1,2]. Reaction with aryl and alkyl amines provides resin-bound secondary amines. These can be readily acylated to give sulfonamides or anilides that can be cleaved with 95% TFA. Since this resin does not contain a benzylic ether linkage, it gives cleaner products than similar supports derived from Wang resin, free from impurities arising from breakdown of the linker. For examples using a similar resin, please see [1-4].

ⓘ Ⓖ 2.15, Ⓦ 2.16, 2.45

[1]   J. W. Corbett, et al. (1997) *Bioorg. Med. Chem. Lett.*, **7**, 1371.
[2]   G. A. Morales, et al. (1998) *J. Org. Chem.*, **63**, 1172.
[3]   K. Ngu & D. V. Patel (1997) *Tetrahedron Lett.*, **38**, 973.
[4]   B. Raju & T. P. Kogan (1997) *Tetrahedron Lett.*, **38**, 4965.

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Product No.	Product	Quantity	Price
855012	**Trityl chloride resin**	1 g	50.00
	NBC No.: 01-64-0074	5 g	200.00
	Loading: 1.1 - 1.70 mmole/g resin; as determined from the substitution of the	25 g	800.00

Loading: 1.1 - 1.70 mmole/g resin; as determined from the substitution of the Fmoc-Ala-Leu loaded resin. The polymer matrix is copoly(styrene- 1% DVB), 200 - 400 mesh.

⚠ **Prolonged storage**: +15 to +25°C; bottle must always be kept tightly closed, and if kept cold, it must be allowed to reach room temperature before opening; use only with dried solvents in dry glassware and apparatus; keep open bottle under nitrogen.

An acid-labile resin for the solid phase immobilization of alcohols [1-5], amines [4, 6-12], sugars [13], O- and N-linked hydroxylamines [14, 15], imidazoles [16] and carboxylic acids [16]. Release of these functionalities is generally achieved using 1-5% TFA in DCM containing 5% TIS. Amines can also be cleaved with 30% HFIP in DCM [4]. This resin, although very acid sensitive, can also be used in peptide synthesis [17].

ⓘ Ⓖ 2.16, Ⓜ 2.17, 2.45

[1]   C. C. Leznoff (1978) *Acc. Chem. Res.*, **11**, 327.
[2]   C. Chen, et al. (1994) *J. Am. Chem. Soc.*, **116**, 2661.
[3]   Z. Li & A. Ganesan (1998) *Syn. Lett.*, 405.
[4]   P. Athanassopoulos, et al., Poster 316 presented at the 24th European Peptide Symposium, Edinburgh, 1996.
[5]   K. Barlos, et al. (1988) *Liebigs Ann. Chem.*, **1079**.
[6]   K. Barlos, et al. in "Peptides 1992, Proc. 22nd European Peptide Symposium", C. H. Schneider & A. N. Eberle (Eds), ESCOM, Leiden, 1993, pp. 281.
[7]   M. Schuster, et al. (1996) *Angew. Chem. Int. Ed. Engl.*, **35**, 1979.
[8]   P. Garibay, et al. (1998) *Tetrahedron Lett.*, **39**, 2207.
[9]   Y. Guan, et al. (2000) *J. Comb. Chem.*, **2**, 297.
[10] S. Manku, et al. (2001) *J. Org. Chem.*, **66**, 874.
[11] D. Jönsson, et al. (2001) *Tetrahedron Lett.*, **42**, 6953.
[12] C. A. Olsen, et al. (2003) *Org. Lett.*, **5**, 4183.
[13] F. Peri, et al. (2000) *Tetrahedron Lett.*, **41**, 8587.
[14] U. Bauer, et al. (1997) *Tetrahedron Lett.*, **38**, 7233.
[15] O. Kinzel, et al. (2003) *J. Peptide Sci.*, **9**, 375.
[16] D. P. Matthews, et al. (2000) *J Comb. Chem.*, **2**, 19.
[17] K. Barlos, et al. (1989) *Tetrahedron Lett.*, **30**, 3943.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
855063	**NovaSyn® TG bromo resin**	1 g	50.00
	NBC No.: 01-64-0095	5 g	200.00
	Loading: 0.20 - 0.40 mmole/g resin; as determined by elemental analysis for sulfur after derivatization with KSAc.	25 g	800.00

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

NovaSyn® TG bromo resin is a composite of low cross-linked polystyrene and 3000-4000 M.W. polyethylene glycol [1-3], in which the ends of the PEG chains have been bromo functionalized. The 130 μm beads have a narrow size distribution, high diffusion rates and excellent swelling properties across a wide range of solvents from toluene to water, making them ideally suited to the synthesis of serial and parallel libraries by organic solid phase synthesis. This support may be used for the immobilization of alcohols, carboxylic acids, phenols and thiols, or as base support for other linkers [4, 5]. Loading of this support can be carried out using the same methods as those previously described for Merrifield resin.

ⓘ Ⓖ 2.19

[1] W. Rapp, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 1st International Symposium", R. Epton (Eds), SPCC UK Ltd., Birmingham, 1990, pp. 205.
[2] L. Zhang, et al. in "Peptides 1990, Proc. 21st European Peptide Symposium", E. Giralt & D. Andreu (Eds), ESCOM, Leiden, 1990, pp. 196.
[3] E. Bayer (1991) *Angew. Chem. Int. Ed. Engl.*, **30**, 113.
[4] K. H. Gordon & S. Balasubramanian (2001) *Org. Lett.*, **3**, 53.
[5] D. Tumelty, et al. (2001) *Org. Lett.*, **3**, 83.

Carbonate resins

Product No.	Product	Quantity	Price
855072	**p–Nitrophenyl carbonate Merrifield resin**	1 g	60.00
	NBC No.: 01-64-0131	5 g	240.00
	Loading: 0.80 - 1.40 mmole/g resin; as photometric determination of p-nitrophenyl released upon treatment with piperidine. The polymer matrix is copoly (styrene - 1 % DVB), 100 - 200 mesh.	25 g	960.00

⚠ Prolonged storage: ≤ -20°C; bottle must always be kept tightly closed when cold and must be allowed to reach room temperature before opening; use only with dried solvents in dry glassware and apparatus.

Resin-bound p-nitrophenyl carbonate esters react readily with amines to provide the corresponding resin-bound carbamate. Leznoff [1] was the first to demonstrate the utility of such resins in solid phase synthesis with the preparation of mono-acylated diamines from symmetrical diamines. Dressman, et al. [2] have used carbamate linked resin-bound amino acid amides to make hydantoins via a base-mediated cyclization/cleavage strategy; a similar approach has also been used to prepare quinazolidine-2,4-diones [3]. Gordon, et al. [4] have used p-nitrophenyl carbonate Merrifield resin to immobilize a ketone linker through a phenyl carbonate linkage.

Loading of this resin can be quantified by measuring the release of p-nitrophenolate [5].

ⓘ Ⓖ 2.42, Ⓜ 2.44, 2.45

[1] D. M. Dixit, et al. (1978) *Israel J. Chem.*, **17**, 248.
[2] B. A. Dressman, et al. (1996) *Tetrahedron Lett.*, **37**, 937.
[3] L. Gouilleux, et al. (1996) *Tetrahedron Lett.*, **37**, 7031.
[4] K. Gordon, et al. (2000) *Tetrahedron Lett.*, **41**, 8621.
[5] A. Paio, et al. (2003) *Tetrahedron Lett.*, **44**, 1867.

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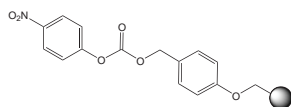
Product No.	Product	Quantity	Price
855019	**p-Nitrophenyl carbonate Wang resin**	1 g	60.00
	NBC No.: 01-64-0123	5 g	240.00
	Loading: 0.60 - 1.20 mmole/g resin; by photometric determination of	25 g	960.00

Loading: 0.60 - 1.20 mmole/g resin; by photometric determination of p-nitrophenol released upon treatment with piperidine/DMF. The polymer matrix is copoly(styrene - 1 % DVB), 100 - 200 mesh.

⚠ Prolonged storage: ≤ -20°C; bottle must always be kept tightly closed when cold and must be allowed to reach room temperature before opening; use only with dried solvents in dry glassware and apparatus.

Resin-bound p-nitrophenyl carbonate esters react readily with amines to provide the corresponding resin-bound carbamate. Leznoff [1] was the first to demonstrate the utility of such resins in solid phase synthesis with the preparation of mono-acylated diamines from symmetrical diamines. Similarly, Dressman, et al. [2] have used carbamate linked resin-bound amino acid amides to make hydantoins via a base-mediated cyclization/cleavage strategy.

Amines linked to p-nitrophenyl carbonate Wang resin possess similar chemical properties to methoxybenzyloxycarbonyl protected amines. The resin-bound carbamate is, therefore, cleaved with TFA or hydrogenolysis to afford the free amine [3]. Alternatively, cleavage by reduction with lithium aluminium hydride can be used to generate N-methylamines [4], and Raju & Kogan [5] have utilized this resin to prepare sulfonamides by acylation of immobilized arylamines and cleavage with LiOH or NaOMe. An analogous p-nitrophenyl carbonate resin prepared from NovaSyn® TGA resin was employed as a support for the solid phase immobilization of amidines [6]. This resin was also used to synthesize polyamines [7, 8], and to immobilize indoles [9] and guanidinylating reagents [10].

Loading of this resin can be quantified by measuring the release of p-nitrophenolate [11].

ⓘ Ⓖ 2.42, Ⓜ 2.44, 2.45

[1] D. M. Dixit, et al. (1978) *Israel J. Chem.*, **17**, 248.
[2] B. A. Dressman, et al. (1996) *Tetrahedron Lett.*, **37**, 937.
[3] J. R. Hauske, et al. (1995) *Tetrahedron Lett.*, **36**, 1589.
[4] C. Y. Ho & M. J. Kukla (1997) *Tetrahedron Lett.*, **38**, 2799.
[5] B. Raju & T. P. Kogan (1997) *Tetrahedron Lett.*, **38**, 3373.
[5] A. K. Ghosh, et al. (2001) *J. Org. Chem.*, **66**, 2161.
[6] R. Mohan, et al. (1998) *Bioorg. Med. Chem. Lett.*, **8**, 1877.
[7] S. Tomasi, et al. (1998) *Bioorg. Med. Chem. Lett.*, **8**, 635.
[8] N. D. Hone & L. J. Payne (2000) *Tetrahedron Lett.*, **41**, 6149.
[9] A. L. Smith, et al. (2000) *Bioorg. Med. Chem. Lett.*, **10**, 2693.
[10] A. K. Ghosh, et al. (2001) *J. Org. Chem.*, **66**, 2161.
[11] A. Paio, et al. (2003) *Tetrahedron Lett.*, **44**, 1867.

RESINS FOR SOLID PHASE ORGANIC SYNTHESIS

⚠ Storage conditions Ⓖ Additional information Ⓜ Method information Ⓐ Application information

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Product No.	Product	Quantity	Price

CHO functionalized resins
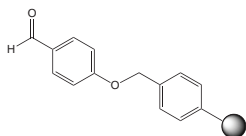
855026 **4–Benzyloxybenzaldehyde polystyrene HL**

Aldehyde Wang HL resin

NBC No.: 01-64-0182

5 g	60.00
25 g	240.00
100 g	720.00

Loading: 2.00 - 3.50 mmole/g resin; as determined by elemental analysis of sulfur after derivatization with tosylhydrazide. The polymer matrix is copoly(styrene - 2 % DVB), 200 -400 mesh.

⚠ **Prolonged storage**: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.

This support is an excellent tool for the production of sulfonamides and anilides by SPOS [1]. Amines have been released from this resin with DDQ [2] and tertiary amides by acylation of resin-bound secondary amines with acid chlorides [3].

ⓘ Ⓖ 2.38

[1] B. Raju & T. P. Kogan (1997) *Tetrahedron Lett.*, **38**, 4965.

[2] S. Kobayashi & Y. Aoki (1998) *Tetrahedron Lett.*, **39**, 7345.

[3] M. W. Miller, et al. (1998) *Tetrahedron Lett.*, **39**, 3429.

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Product No.	Product	Quantity	Price

CO$_2$H functionalized resins
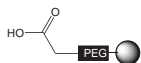
855065	**NovaSyn® TG carboxy resin**	1 g	75.00
	NBC No.: 01-64-0097	5 g	300.00
	Loading: 0.30 - 0.60 mmole/g resin; as determined by elemental analysis of	25 g	1180.00

nitrogen after derivatization with methylamine. 130 µm beads.

NovaSyn®TG carboxy resin is a composite of low cross-linked polystyrene and 3000-4000 M.W. polyethylene glycol, in which the ends of the PEG chains have been functionalized with carboxyl groups. The 130 µm beads have a narrow size distribution, high diffusion rates and excellent swelling properties across a wide range of solvents from toluene to water [1-3], making them ideally suited to the synthesis of serial and parallel libraries by organic solid phase synthesis.

This support can be used to immobilize alcohols through formation of an ester with the resin-bound carboxy group. Cleavage can be effected either by saponification, hydrazinolysis or reduction with LiBH$_4$.

In studies on the synthesis of furans via intramolecular radical cyclization, NovaSyn®TG carboxy resin was found to give superior results to carboxy polystyrene [4]. This observation was attributed to the notion that, in the PEG-based support, the radical intermediates would be prevented, by virtue of being tethered to the ends of PEG chains, from approaching and thus being quenched by the benzylic protons of the polystyrene backbone.

ⓘ Ⓖ 2.37, Ⓜ 2.37

[1] W. Rapp, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 1st International Symposium", R. Epton (Eds), SPCC UK Ltd., Birmingham, 1990, pp. 205.

[2] L. Zhang, et al. in "Peptides 1990, Proc. 21st European Peptide Symposium", E. Giralt & D. Andreu (Eds), ESCOM, Leiden, 1990, pp. 196.

[3] E. Bayer (1991) *Angew. Chem. Int. Ed. Engl.*, **30**, 113.

[4] A. Routledge, et al. (1997) *Synlett*, 61.

⚠ Storage conditions Ⓖ Additional information Ⓜ Method information Ⓐ Application information

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Product No.	Product	Quantity	Price

Enol functionalized resins
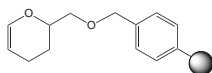
855079   **DHP HM resin (100-200 mesh)**

3,4-Dihydro-2H-pyran-2-yl-methoxymethyl polystyrene (100-200 mesh)

Ellman's dihydropyran resin

NBC No.: 01-64-0192

	1 g	72.00
	5 g	288.00
	25 g	1152.00

Loading: 0.70 - 1.20 mmole/g resin; as determined from the substitution of the Fmoc-Gly-ol loaded resin. The polymer matrix is copoly(styrene-1 % DVB), 100 - 200 mesh.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

A highly versatile support for the solid phase immobilization of primary, secondary alcohols [1-11], phenols [12], purines [13], and indoles [14]. Derivatization is effected by treating the support in DCM with an excess of alcohol in the presence of a catalytic amount of pyridinium p-toluenesulfonate. A similar DHP-based support has also been used for the immobilization of tetrazoles [15].

Release of the product alcohol from the support has been carried out by treating with 95% TFA/5% water [1] and TFA/DCM/EtOH [2, 12]. However, the use of TFA can lead, in some cases, to trifluoroacetate formation. Methods which eliminate this potential side reaction include PPTS/BuOH/DCE [3], TosOH in DCM and TosOH in DCE/BuOH [16].

ⓘ   **G** 2.40, **M** 2.42, **A** 2.42

[1]   L. A. Thompson & J. A. Ellman (1994) *Tetrahedron Lett.*, **35**, 9333.

[2]   O. B. Wallace (1997) *Tetrahedron Lett.*, **38**, 4939.

[3]   G. Liu & J. A. Ellman (1995) *J. Org. Chem.*, **60**, 7712.

[4]   E. K. Kick & J. A. Ellman (1995) *J. Med. Chem.*, **38**, 1427.

[5]   J. S. Koh & J. A. Ellman (1996) *J. Org. Chem.*, **61**, 4494.

[6]   J. Cossy, et al. (2000) *Synlett*, **3**, 409.

[7]   M. Ramaseshan, et al. (2000) *J. Comb. Chem.*, **2**, 615.

[8]   M. Ramaseshan, et al. (2000) *Tetrahedron Lett.*, **41**, 4743.

[9]   A. Bianco, et al. (2000) *J. Org. Chem.*, **65**, 2179.

[10] M. Steger, et al. (2001) *Bioorg. Med. Chem. Lett.*, **11**, 2537.

[11] A. Dahlgren, et al. (2003) *Bioorg. Med.Chem. Lett.*, **11**, 827.

[12] W. H. Pearson & R. B. Clark (1997) *Tetrahedron Lett.*, **38**, 7669.

[13] D. A. Nugiel, et al. (1997) *J. Org. Chem.*, **62**, 201.

[14] A. L. Smith, et al. (1998) *Tetrahedron Lett.*, **39**, 8317.

[15] S.-E. Yoo, et al. (1997) *Tetrahedron Lett.*, **38**, 1203.

[16] M. R. Tremblay, et al. (1999) *Bioorg. Med. Chem. Lett.*, **9**, 2827.

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Product No.	Product	Quantity	Price

OH functionalized resins

Wang-type resins

Hydroxymethylphenoxy (Wang-type, HMP) resins are the standard supports for the solid phase immobilization of acids [1,2] and phenols for SPOS [3-5]. Attachment of carboxylic acids is achieved by either DMAP catalyzed esterification with the appropriate symmetrical anhydride, or by using 2,6-dichlorobenzoyl chloride/ pyridine [6] or MSNT/MeIm activation [7, 8]. Phenols are best immobilized using the Mitsunobu reaction [3, 4, 5].

An alternative strategy that avoids basic conditions involves conversion of Wang-type resins to the corresponding trichloroacetimidate. Such resins can be loaded using Lewis acid catalysis with alcohols [9, 10], carboxylic, phosphonic and sulfonic acids [11], thiols and thioacids [11], and phenols [12].

For the immobilization of amines, Wang-type resins can be readily converted into solid phase equivalents of standard urethane-based protecting groups by reaction with phosgene or activated carbonates, such as carbonyl diimidazole or bis(p-nitrophenyl)-carbonate [13].

Detachment of acids and phenols [5,14] can be effected by treatment with 15 - 95% TFA.

HMP resins supplied by Novabiochem® are prepared either by coupling hydroxymethylphenoxyacetic acid linker to the appropriate amino-functionalized resin (NovaSyn® TGA, HMPA-PEGA resin, HMPA-NovaGel™) or by linking of an equivalent hydroxymethylphenoxymethyl handle directly to a polystyrene base matrix (Wang resin). These resins are generally regarded as more robust than Wang resin, particularly under acidic conditions, as they do not contain a potentially acid sensitive benzylic ether linkage.

ⓘ Ⓖ 2 32. Ⓜ 3.6, 2.45
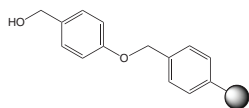
855075	**Wang resin VHL (100-200 mesh)**	5 g	85.00
	p-Benzyloxybenzyl alcohol resin VHL (100-200 mesh)	25 g	340.00
	HMP resin VHL (100-200 mesh)	100 g	980.00

NBC No.: 01-64-0174

Loading: 1.60 - 3.00 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The polymer matrix is copoly(styrene-1 % DVB), 100 - 200 mesh.

For an application of very-high load Wang resin in SPOS see reference [1].

ⓘ Ⓖ 2 32. 2.33, Ⓜ 3.6, 2.45

[1]   M. G. Valverde, et al. (2001) *Synlett*, **6**, 741.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
855094	**Trichloroacetimidate Wang resin**	1 g	24.00
	NBC No.: 01-64-0314	5 g	96.00
	Loading: 0.60 - 1.20 mmole/g resin; as determined by elemental analysis after	25 g	384.00

derivatization with thioacetic acid. The polymer matrix is copoly(styrene-1 %
DVB), 100 - 200 mesh.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

A highly versatile reagent for the solid phase immobilization of alcohols [1 - 3];
carboxylic, phosphonic and sulfonic acids [4]; thiols and thioacids [4]; and
phenols [5]. In contrast to the analogous bromide resin which is loaded under
basic conditions, derivatization of this support is achieved under acidic
conditions, making it compatible with base-sensitive compounds such as Fmoc-
amino alcohols.

ⓘ Ⓖ 2.34,

[1]   S. Hanessian & X. Fang (1998) *Tetrahedron Lett.*, **39**, 733.
[2]   A. Bianco, et al. (2000) *J. Comb. Chem.*, **2**, 681.
[3]   A. Kamal, et al. (2002) *Tetrahedron Lett.*, **43**, 2103.
[4]   C. W. Phoon, et al. (1998) *Tetrahedron Lett.*, **39**, 7959.
[5]   A. Kamal, et al. (2001) *Tetrahedron Lett.*, **42**, 6969.

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Product No.	Product	Quantity	Price

Rink acid resins
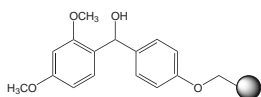
855060 **Rink Acid resin (100–200 mesh)**

4-(2',4'-Dimethoxyphenyl-hydroxymethyl)-phenoxy resin

NBC No.: 01-64-0012

1 g	35.00
5 g	140.00
25 g	560.00

Loading: 0.35 - 0.80 mmole/g resin; as determined from the substition of the Fmoc-NH$_2$ loaded resin). The polymer matrix is copoly(styrene - 1 % DVB) 100 -200 mesh.

Rink acid resin is a super acid-labile support for the solid phase immobilization of carboxylic acids [1]. Cleavage can be effected with as little as 10% AcOH in DCM, providing highly acid-sensitive products in high yields and purities. However, care must be taken to prevent product loss during synthetic manipulations [2], owing to the extreme acid sensitivity of this support. Treatment with HCl in THF [3] or Ph$_3$PCl$_2$ [4] has been shown to efficiently convert this resin to the corresponding benzhydryl chloride, to which can be coupled a wide range of functional groups: hydroxylamines [3], alcohols, amines, acids and thiols [4].

Rink acid resin has also been converted to a trifluoroacetate with TFA and used in a similar manner to immobilize amines, thiols, alcohols, and phenols [5]. In a more detailed study, the same authors found 1 M trifluoroacetic anhydride in 2,6-lutidine to give superior results with less degradation of the linker [6, 7]. Rink resin trifluoroacetate has also been used to prepare purines [8, 9].

Cleavage of amines and alcohols from this support has been carried out with either 5% TFA in DCM [4] or 20% TFA in DCM [5]; thiols were released with either 5% TFA in DCM [4] or 95% aq. TFA [5].

ⓘ Ⓖ 2.34, Ⓜ 3.6, 2.45

[1] H. Rink (1987) *Tetrahedron Lett.*, **28**, 3787.

[2] H. U. Immer, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 11th American Peptide Symposium", J. E. Rivier & G. R. Marshall (Eds), ESCOM, Leiden, 1990, pp. 1054.

[3] S. L. Mellor & W. C. Chan (1997) *J. Chem. Soc., Chem. Commun.*, 2005.

[4] R. S. Garigipati (1997) *Tetrahedron Lett.*, **38**, 6807.

[5] W.K.-D. Brill in "First Conference on Synthetic Organic Chemistry", www.mdpi.org/ecsoc, 1997.

[6] W.K.-D. Brill (1998) *Syn. Lett.*, 906.

[7] R. A. Tommesi, et al. (1998) *Tetrahedron Lett.*, **39**, 5477.

[8] W.K.-D. Brill (2001) *Syn. Lett.*, 1097.

[9] W.K.-D. Brill & C. Riva-Toniolo (2001) *Tetrahedron Lett.*, **42**, 65`5.

RESINS FOR SOLID PHASE ORGANIC SYNTHESIS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Other hydroxy resins

855064   **NovaSyn® TG hydroxy resin**   1 g   50.00

NBC No.: 01-64-0096   5 g   200.00

Loading: 0.20 - 0.30 mmole/g resin; as determined from the substitution of the   25 g   800.00
Fmoc-Leu loaded resin. 130 µm beads.

NovaSyn®TG hydroxy resin is a composite of low cross-linked polystyrene and 3000-4000 M.W. polyethylene glycol, in which the ends of the PEG chains have been functionalized with hydroxyl groups. The 130 µm beads have a narrow size distribution, high diffusion rates and excellent swelling across a wide range of solvents from toluene to water [1-3], making them ideally suited to the synthesis of serial and parallel libraries by organic solid phase synthesis.

This support has been used for the solid phase immobilization of carboxylic acids [4-9], with cleavage by saponification with dilute aq. NaOH.

ⓘ Ⓖ 2.32, Ⓜ 3.6, 2.45

[1]   W. Rapp, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 1st International Symposium", R. Epton (Eds), SPCC UK Ltd., Birmingham, 1990, pp. 205.
[1]   W. Rapp, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 1st International Symposium", R. Epton (Eds), SPCC UK Ltd., Birmingham, 1990, pp. 205.
[2]   L. Zhang, et al. in "Peptides 1990, Proc. 21st European Peptide Symposium", E. Giralt & D. Andreu (Eds), ESCOM, Leiden, 1990, pp. 196.
[3]   E. Bayer (1991) *Angew. Chem. Int. Ed. Engl.*, **30**, 113.
[4]   M. Hiroshige, et al. (1995) *Tetrahedron Lett.*, **36**, 4567.
[5]   M. C. Fagnola, et al. (1997) *Tetrahedron Lett.*, **38**, 2307.
[6]   D. Fancelli, et al. (1997) *Tetrahedron Lett.*, **38**, 2311.
[7]   M. Patek, et al. (1995) *Tetrahedron Lett.*, **36**, 2227.
[8]   V. Krchnak, et al. (1995) *Tetrahedron Lett.*, **36**, 6193.
[9]   A. K. Szardenings & T. S. Burkoth (1997) *Tetrahedron*, **53**, 6573.

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Product No.	Product	Quantity	Price
855068	**Hydroxymethyl polystyrene (100–200 mesh), 1% DVB**	5 g	43.00
		25 g	172.00
	Polystyrene-CH$_2$OH (100-200 mesh)	100 g	516.00

NBC No.: 01-64-0110

Loading: 0.60 - 1.60 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The polymer matrix is copoly (styrene - 1% DVB) 100 -200 mesh.

This resin is ideal for the solid phase immobilization of carboxylic acids. Less cross-linking is observed than with Merrifield resin during the attachment of diacids [1]. Reaction with a suitable phosgene equivalent converts this resin to a support suitable for the immobilization of amines [2].

Release of carboxylic acids from this support is normally effected by treatment of the resin with HF or TFMSA, or by hydrogenolysis [3]. Alcohols can be liberated by reduction with DIBAL [4] or LiBH$_4$ [5]. Methyl esters can be produced by transesterification with MeONa [6] or Ti(OEt)$_4$/C$_2$H$_5$CO$_2$Me [7]. Carboxamides are also accessible via Lewis acid catalyzed aminolysis [8].

ⓘ Ⓜ 3.6, 2.45

[1] J. M. Goldwasser, et al. (1978) *Can. J. Chem.*, **56**, 1562.
[2] D. J. Burdick, et al. (1993) *Tetrahedron Lett.*, **34**, 2589.
[3] J. M. Schlatter, et al. (1977) *Tetrahedron Lett.*, 2851.
[4] M. J. Kurth, et al. (1994) *J. Org. Chem.*, **59**, 5862.
[5] J. M. Stewart & J. D. Young in "Solid Phase Peptide Synthesis, 2nd Ed.", Rockford, Illinois, Pierce Chemical Company, 1984, pp. 92.
[6] R. Frenette, et al. (1994) *Tetrahedron Lett.*, **35**, 9177.
[7] L. T. Tietze & A. Steinmetz (1996) *Angew. Chem. Int. Ed. Engl.*, **35**, 651.
[8] D. R. Barn, et al. (1996) *Tetrahedron Lett.*, **37**, 3213.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

SH functionalized resins
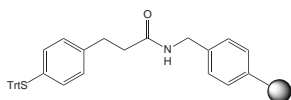
855091   **3–[4–(Tritylmercapto)phenyl]propionyl AM resin**   5 g   152.00
NBC No.: 01-64-0301   25 g   608.00

Loading: 0.70 - 1.00 mmole/g resin; as determined by elemental analysis of sulfur. The polymer matrix is copoly(styrene - 1 % DVB) 100 - 200 mesh.

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.

The versatile resin can be used not only as a scavenger for electrophilic reagents but also as support for SPOC. A number of thiol-based resins were compared with respect to their efficacies as S_NAR-labile linkers in the synthesis of aminopyridazines. This support was found to give the best results. Its use facilitated quantitative cleavage of thioether immobilized pyridazines with amines, without the need for prior oxidation [1, 2].

This support has also been used to prepare polymer-supported NHS by reaction with N-hydroxymaleimide [3].

ⓘ Ⓖ 2.42

[1]   I. Parrot, et al., Poster presented at Combinatorial Chemistry Meeting, Rennes, 1999.
[2]   I. Parrot, et al. (1999) *Tetrahedron Lett.*, **40**, 7975.
[3]   M. Katoh & M. Sodeoka (1999) *Biorg. Med. Chem. Lett.*, **9**, *881*.

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Product No.	Product	Quantity	Price

Pre-loaded resins

N-α-Fmoc protected amino acids attached to Wang resin
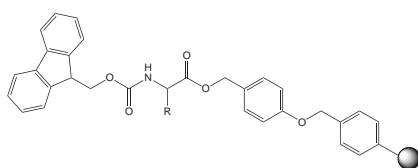
Wang resins are the standard supports for the preparation of peptide acids by the Fmoc batch solid phase synthesis strategy [1, 2]. The resins consist of 1% cross-linked polystyrene beads functionalized with the TFA labile p-benzyloxybenzyl alcohol handle.

Novabiochem®s Wang resins pre-loaded with Fmoc-L-amino acids are characterized for D-amino acid and dipeptide content, to ensure they produce products free from by-products arising from the resin-loading processes. Furthermore, the consistency and quality of the base resin from which these resins are made is confirmed by performance of a test peptide synthesis.

Loading: 0.40 - 1.00 mmole/g resin, as determined photometrically from the amount of Fmoc chromophore liberated upon treatment of the resin with DBU/DMF. The polymer matrix is copoly(styrene-1 % DVB), 100 - 200 mesh.

ⓘ Ⓜ 2.6, 3.29

[1]   S.-W. Wang (1976) *J. Org. Chem.*, **41**, 3258.
[2]   G. Lu, et al. (1981) *J. Org. Chem.*, **46**, 3433.

High-load

856100	**Fmoc-β-Ala-Wang resin (100-200 mesh)**	1 g	60.00
	NBC No.: 04-12-2042	5 g	240.00
856001	**Fmoc-Ala-Wang resin (100-200 mesh)**	1 g	30.00
	NBC No.: 04-12-2043	5 g	80.00
856150	**Fmoc-D-Ala-Wang resin (100-200 mesh)**	1 g	70.00
	NBC No.: 04-13-2002	5 g	275.00
856002	**Fmoc-Arg(Pbf)-Wang resin (100-200 mesh)**	1 g	80.00
	NBC No.: 04-12-2044	5 g	290.00
856164	**Fmoc-D-Arg(Pbf)-Wang resin (100-200 mesh)**	1 g	85.00
	NBC No.: 04-13-2027	5 g	340.00

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Product No.	Product	Quantity	Price
856003	**Fmoc-Arg(Pmc)-Wang resin (100-200 mesh)** NBC No.: 04-12-2045	1 g 5 g	80.00 290.00
856004	**Fmoc-Asn(Trt)-Wang resin (100-200 mesh)** NBC No.: 04-12-2046	1 g 5 g	60.00 210.00
856155	**Fmoc-D-Asn(Trt)-Wang resin (100-200 mesh)** NBC No.: 04-13-2013 L	1 g 5 g	80.00 320.00
856005	**Fmoc-Asp(OtBu)-Wang resin (100-200 mesh)** NBC No.: 04-12-2047	1 g 5 g	50.00 150.00
856159	**Fmoc-D-Asp(OtBu)-Wang resin (100-200 mesh)** NBC No.: 04-13-2017	1 g 5 g	80.00 320.00
856023	**Fmoc-Asp(Wang resin)-OAll (100-200 mesh)** NBC No.: 04-12-2072 A useful support for the synthesis of cyclic peptides [1]. ⓘ Ⓖ 4.13, Ⓜ 4.14 [1] F. Albericio, et al. (1993) *Tetrahedron Lett.*, **34**, 1549.	1 g 5 g	89.00 345.00
856146	**Fmoc-Asp(Wang-resin)-AMC (100-200 mesh)** NBC No.: 04-12-3915 ⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light. A novel resin for the preparation of peptide substrates based on 3-amino-7-methylcoumarin (AMC) by Fmoc SPPS. Following Fmoc removal with 20% piperidine, peptide assembly can be effected using standard coupling methods. Treatment with 95% TFA releases the peptide-AMC directly from the solid phase.	500 mg 1 g	295.00 495.00
856101	**Fmoc-Cys(Acm)-Wang resin (100-200 mesh)** NBC No.: 04-12-2048	1 g 5 g	60.00 240.00
856157	**Fmoc-D-Cys(Acm)-Wang resin (100-200 mesh)** NBC No.: 04-13-2015	1 g 5 g	80.00 320.00
856102	**Fmoc-Cys(tButhio)-Wang resin (100-200 mesh)** NBC No.: 04-12-2049	1 g 5 g	80.00 320.00

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Product No.	Product	Quantity	Price
856006	**Fmoc-Cys(Trt)-Wang resin (100-200 mesh)** NBC No.: 04-12-2050	1 g 5 g	60.00 210.00
856007	**Fmoc-Gln(Trt)-Wang resin (100 –200 mesh)** NBC No.: 04-12-2051	1 g 5 g	60.00 210.00
856156	**Fmoc-D-Gln(Trt)-Wang resin (100-200 mesh)** NBC No.: 04-13-2014	1 g 5 g	80.00 320.00
856008	**Fmoc-Glu(OtBu)-Wang resin (100-200 mesh)** NBC No.: 04-12-2052	1 g 5 g	50.00 150.00
856158	**Fmoc-D-Glu(OtBu)-Wang resin (100-200 mesh)** NBC No.: 04-13-2016	1 g 5 g	80.00 320.00
856024	**Fmoc-Glu(Wang resin)-OAll (100-200 mesh)** NBC No.: 04-12-2178 A useful support for the preparation of cyclic peptides by SPPS [1]. [1] F. Albericio, et al. (1993) *Tetrahedron Lett.*, **34**, 1549.	1 g 5 g	89.00 345.00
856022	**Fmoc-Glu(Wang resin)-ODmab (100-200 mesh)** NBC No.: 04-12-2071 ⚠ Prolonged storage: +2 to +8°C; keep cool and dry. A useful support for the synthesis of cyclic peptides [1]. ⓘ G 4.14, M 4.10, A 4.16 [1] W. C. Chan, et al. (1995) *J. Chem. Soc., Chem. Commun.*, 2209.	1 g 5 g	190.00 950.00
856009	**Fmoc-Gly-Wang resin (100-200 mesh)** NBC No.: 04-12-2053	1 g 5 g	30.00 80.00
856010	**Fmoc-His(Trt)-Wang resin (100-200 mesh)** NBC No.: 04-12-2054 This product will contain significant amounts of Fmoc-D-His(Trt)-Wang resin. For applications where an enantiomerization-free product is required, Novabiochem® recommends the use of 856056.	1 g 5 g	60.00 210.00
856011	**Fmoc-Ile-Wang resin (100-200 mesh)** NBC No.: 04-12-2055	1 g 5 g	30.00 80.00

⚠ Storage conditions   G Additional information   M Method information   A Application information

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Product No.	Product	Quantity	Price
856012	Fmoc-Leu-Wang resin (100-200 mesh) NBC No.: 04-12-2056	1 g 5 g	30.00 80.00
856148	Fmoc-D-Leu-Wang resin (100-200 mesh) NBC No.: 04-13-2000	1 g 5 g	70.00 275.00
856013	Fmoc-Lys(Boc)-Wang resin (100-200 mesh) NBC No.: 04-12-2057	1 g 5 g	50.00 150.00
856153	Fmoc-D-Lys(Boc)-Wang resin (100-200 mesh) NBC No.: 04-13-2011	1 g 5 g	80.00 320.00
856147	Fmoc-Lys(carbamate Wang resin)-AMC (100-200 mesh) NBC No.: 04-12-3917 ⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light. A novel resin for the preparation of peptide substrates based on 3-amino-7-methylcoumarin (AMC) by Fmoc SPPS. Following Fmoc removal with 20% piperidine, peptide assembly can be effected using standard coupling methods. Treatment with 95% TFA releases the peptide-AMC directly from the solid phase.	500 mg 1 g	295.00 495.00
856186	Fmoc-Lys(ivDde)-Wang resin (100-200 mesh)	1 g 5 g	89.00 345.00
856021	Fmoc-Lys(Mtt)-Wang resin (100-200 mesh) NBC No.: 04-12-2067	1 g 5 g	80.00 290.00
856014	Fmoc-Met-Wang resin (100-200 mesh) NBC No.: 04-12-2058	1 g 5 g	30.00 80.00
856151	Fmoc-D-Met-Wang resin (100-200 mesh) NBC No.: 04-13-2004	1 g 5 g	70.00 275.00
856015	Fmoc-Phe-Wang resin (100-200 mesh) NBC No.: 04-12-2060	1 g 5 g	30.00 80.00
856149	Fmoc-D-Phe-Wang resin NBC No.: 04-13-2001	1 g 5 g	70.00 275.00

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Product No.	Product	Quantity	Price
856016	Fmoc-Ser(tBu)-Wang resin (100-200 mesh) NBC No.: 04-12-2062	1 g 5 g	50.00 150.00
856161	Fmoc-D-Ser(tBu)-Wang resin (100-200 mesh) NBC No.: 04-13-2019	1 g 5 g	80.00 320.00
856017	Fmoc-Thr(tBu)-Wang resin (100-200 mesh) NBC No.: 04-12-2063	1 g 5 g	50.00 150.00
856160	Fmoc-D-Thr(tBu)-Wang resin (100-200 mesh) NBC No.: 04-13-2018	1 g 5 g	80.00 320.00
856018	Fmoc-Trp(Boc)-Wang resin (100-200 mesh) NBC No.: 04-12-2064 N-in-Boc-protection limits reattachment and so leads to higher cleavage yields of C-terminal Trp-containing peptides.	1 g 5 g	80.00 290.00
856163	Fmoc-D-Trp(Boc)-Wang resin (100-200 mesh) NBC No.: 04-13-2026	1 g 5 g	85.00 340.00
856019	Fmoc-Tyr(tBu)-Wang resin (100-200 mesh) NBC No.: 04-12-2065	1 g 5 g	50.00 150.00
856154	Fmoc-D-Tyr(tBu)-Wang resin (100-200 mesh) NBC No.: 04-13-2012	1 g 5 g	80.00 320.00
856020	Fmoc-Val-Wang resin (100-200 mesh) NBC No.: 04-12-2066	1 g 5 g	30.00 80.00
856152	Fmoc-D-Val-Wang resin (100-200 mesh) NBC No.: 04-13-2007	1 g 5 g	70.00 275.00

PRE-LOADED RESINS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Low-load
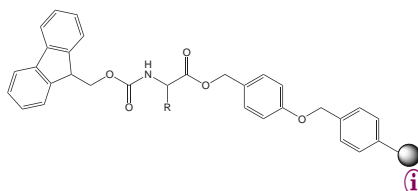
These special low pre-loaded Wang resins are the ideal supports for the preparation of long or difficult peptides by batch-wise Fmoc SPPS. The resins consist of 1% cross-linked polystyrene beads functionalized with the TFA labile p-benzyloxybenzyl alcohol handle.

Loading: 0.20 - 0.40 mmole/g resin, as determined photometrically from the amount of Fmoc chromophore liberated upon treatment of the resin with DBU/DMF. The polymer matrix is copoly(styrene - 1 % DVB), 100 - 200 mesh.

ⓘ Ⓜ 2.6, 3.29

856104	**Fmoc-Ala-Wang resin LL (100–200 mesh)** NBC No.: 04-12-2074	1 g 5 g	30.00 80.00
856105	**Fmoc-Arg(Pbf)-Wang resin LL (100–200 mesh)** NBC No.: 04-12-2075	1 g 5 g	80.00 290.00
856106	**Fmoc-Asn(Trt)-Wang resin LL (100–200 mesh)** NBC No.: 04-12-2076	1 g 5 g	60.00 230.00
856103	**Fmoc-Asp(OtBu)-Wang resin LL (100–200 mesh)** NBC No.: 04-12-2073	1 g 5 g	50.00 150.00
856121	**Fmoc-Asp(Wang resin LL)-OAll (100–200 mesh)** NBC No.: 04-12-2092 Useful resin for preparing head-to-tail cyclic peptides. Allyl group can be removed on the solid phase in the presence of standard t-butyl-based protecting groups by Pd-mediated allyl transfer. ⓘ Ⓖ 4.13, Ⓜ 4.14	1 g 5 g	89.00 345.00
856123	**Fmoc-Asp(Wang resin LL)-ODmab (100–200 mesh)** NBC No.: 04-12-2094 ⚠ Prolonged storage: +2 to +8°C; keep cool and dry. Useful resin for preparing head-to-tail cyclic peptides. Dmab group can be removed on the solid phase in the presence of standard t-butyl-based protecting groups by treatment with 2% hydrazine in DMF [1]. ⓘ Ⓖ 4.14, Ⓜ 4.10, Ⓐ 4.16 [1] W. C. Chan, et al. (1995) *J. Chem. Soc., Chem. Commun.*, 2209.	1 g 5 g	105.00 420.00
856107	**Fmoc-Cys(Trt)-Wang resin LL (100–200 mesh)** NBC No.: 04-12-2077	1 g 5 g	60.00 210.00

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Product No.	Product	Quantity	Price
856108	**Fmoc-Gln(Trt)-Wang resin LL (100–200 mesh)** NBC No.: 04-12-2078	1 g 5 g	60.00 230.00
856109	**Fmoc-Glu(OtBu)-Wang resin LL (100–200 mesh)** NBC No.: 04-12-2079	1 g 5 g	50.00 150.00
856122	**Fmoc-Glu(Wang resin LL)-OAll (100–200 mesh)** NBC No.: 04-12-2093 Useful resin for preparing head-to-tail cyclic peptides. Allyl group can be removed on the solid phase in the presence of standard t-butyl-based protecting groups by Pd-mediated allyl transfer.	1 g 5 g	89.00 345.00
856124	**Fmoc-Glu(Wang resin LL)-ODmab (100–200 mesh)** NBC No.: 04-12-2095 ⚠ Prolonged storage: +2 to +8°C; keep cool and dry. Useful resin for preparing head-to-tail cyclic peptides. Dmab group can be removed on the solid phase in the presence of standard t-butyl-based protecting groups by treatment with 2% hydrazine in DMF [1]. ⓘ 🅖 4.14, Ⓜ 4.10, 🅐 4.16 [1]　W. C. Chan, et al. (1995) *J. Chem. Soc., Chem. Commun.*, 2209.	1 g 5 g	105.00 420.00
856110	**Fmoc-Gly-Wang resin LL (100–200 mesh)** NBC No.: 04-12-2080	1 g 5 g	30.00 80.00
856111	**Fmoc-Ile-Wang resin LL (100–200 mesh)** NBC No.: 04-12-2081	1 g 5 g	30.00 80.00
856112	**Fmoc-Leu-Wang resin LL (100–200 mesh)** NBC No.: 04-12-2082	1 g 5 g	30.00 80.00
856113	**Fmoc-Lys(Boc)-Wang resin LL (100–200 mesh)** NBC No.: 04-12-2083	1 g 5 g	50.00 150.00

PRE-LOADED RESINS

⚠ Storage conditions　🅖 Additional information　Ⓜ Method information　🅐 Application information

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Product No.	Product	Quantity	Price
856114	Fmoc-Met-Wang resin LL (100-200 mesh) NBC No.: 04-12-2084	1 g 5 g	30.00 80.00
856115	Fmoc-Phe-Wang resin LL (100-200 mesh) NBC No.: 04-12-2085	1 g 5 g	30.00 80.00
856116	Fmoc-Ser(tBu)-Wang resin LL (100-200 mesh) NBC No.: 04-12-2086	1 g 5 g	50.00 150.00
856117	Fmoc-Thr(tBu)-Wang resin LL (100-200 mesh) NBC No.: 04-12-2087	1 g 5 g	50.00 150.00
856118	Fmoc-Trp(Boc)-Wang resin LL (100-200 mesh) NBC No.: 04-12-2088	1 g 5 g	80.00 290.00
856119	Fmoc-Tyr(tBu)-Wang resin LL (100-200 mesh) NBC No.: 04-12-2089	1 g 5 g	50.00 150.00
856120	Fmoc-Val-Wang resin LL (100-200 mesh) NBC No.: 04-12-2090	1 g 5 g	30.00 80.00

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Product No.	Product		Quantity	Price

N-α-Fmoc protected amino acids attached to NovaSyn® TGA resin
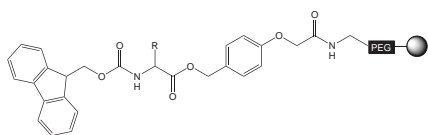
NovaSyn® TGA resins are based on Tentagel [1 – 3], a composite of polyethylene oxide grafted on to a low cross-linked polystyrene gel-type matrix, which has been amino functionalized and derivatized with the TFA-labile 4-hydroxymethylphenoxyacetic acid linker (HMPA) [4]. The 90 µm beads have a narrow size distribution, excellent pressure stability and swelling properties, and high diffusion rates, making them ideally suited for both batch or continuous flow peptide synthesis. The resin swells in a wide range of solvents, enabling coupling reactions to be carried out under a variety of conditions.

Loading: 0.10 - 0.30 mmole/g resin, as determined photometrically from the amount of Fmoc chromophore liberated upon treatment of the resin with DBU/DMF. 90 µm beads.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

ⓘ G 2.3, .2.6, M 3.29

[1] W. Rapp, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 1st International Symposium", R. Epton (Eds), SPCC UK Ltd., Birmingham, 1990, pp. 205.

[2] L. Zhang, et al. in "Peptides 1990, Proc. 21st European Peptide Symposium", E. Giralt and D. Andreu (Eds), ESCOM, Leiden, 1990, pp. 196.

[3] E. Bayer (1991) Angew. Chem., 103, 117.

[4] R. C. Sheppard, et al. (1982) Int. J. Peptide Protein Res., 20, 451.

856026	Fmoc–Ala–NovaSyn® TGA		1 g	75.00
	NBC No.: 04-12-2650		5 g	300.00
856042	Fmoc–Arg(Pbf)–NovaSyn® TGA		1 g	90.00
	NBC No.: 04-12-2678		5 g	360.00
856039	Fmoc–Asn(Trt)–NovaSyn® TGA		1 g	90.00
	NBC No.: 04-12-2672		5 g	360.00
856027	Fmoc–Asp(OtBu)–NovaSyn® TGA		1 g	90.00
	NBC No.: 04-12-2653		5 g	360.00
856025	Fmoc–Asp(NovaSyn® TGA) –OAll		1 g	127.00
	NBC No.: 04-12-2179		5 g	510.00

856025 Useful resin for preparing head-to-tail cyclic peptides.

ⓘ G 4.13, M 4.14

856028	Fmoc–Cys(Trt)–NovaSyn® TGA		1 g	90.00
	NBC No.: 04-12-2656		5 g	360.00

⚠ Storage conditions   G Additional information   M Method information   A Application information

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Product No.	Product	Quantity	Price
856040	Fmoc-Gln(Trt)-NovaSyn® TGA NBC No.: 04-12-2674	1 g 5 g	90.00 360.00
856029	Fmoc-Glu(OtBu)-NovaSyn® TGA NBC No.: 04-12-2657	1 g 5 g	90.00 360.00
856043	Fmoc-Glu(NovaSyn® TGA) -OAll NBC No.: 04-12-2681	1 g 5 g	125.00 500.00
856030	Fmoc-Ile-NovaSyn® TGA NBC No.: 04-12-2660	1 g 5 g	75.00 300.00
856031	Fmoc-Leu-NovaSyn® TGA NBC No.: 04-12-2661	1 g 5 g	75.00 300.00
856032	Fmoc-Lys(Boc)-NovaSyn® TGA NBC No.: 04-12-2662	1 g 5 g	90.00 360.00
856193	Fmoc-Lys(biotinyl-ε-aminocaproyl)-NovaSyn® TG^R A resin NEW	250 mg 1 g	250.00 475.00
856033	Fmoc-Met-NovaSyn® TGA NBC No.: 04-12-2663	1 g 5 g	75.00 300.00
856034	Fmoc-Phe-NovaSyn® TGA NBC No.: 04-12-2665	1 g 5 g	75.00 300.00
856035	Fmoc-Ser(tBu)-NovaSyn® TGA NBC No.: 04-12-2667	1 g 5 g	90.00 360.00

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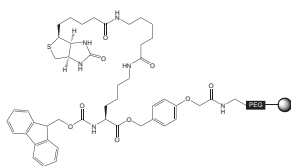
Product No.	Product	Quantity	Price
856036	Fmoc–Thr(tBu)–NovaSyn® TGA NBC No.: 04-12-2668	1 g 5 g	90.00 360.00
856041	Fmoc–Trp(Boc)–NovaSyn® TGA NBC No.: 04-12-2675 N-in-Boc-protection limits reattachment and so leads to higher cleavage yield of C-terminal Trp-containing peptides.	1 g 5 g	90.00 360.00
856037	Fmoc–Tyr(tBu)–NovaSyn® TGA NBC No.: 04-12-2670	1 g 5 g	90.00 360.00
856038	Fmoc–Val–NovaSyn® TGA NBC No.: 04-12-2671	1 g 5 g	75.00 300.00

⚠ Storage conditions　　🄖 Additional information　　🅜 Method information　　🅐 Application information

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Product No.	Product	Quantity	Price

N-α-Fmoc protected amino acids attached to NovaSyn® TGT resin
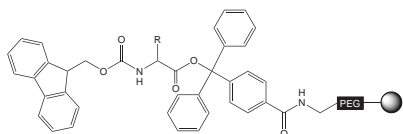
NovaSyn® TGT resins consist of NovaSyn® TG resin derivatized with Bayer's extremely acid-sensitive 4-carboxytrityl linker [1]. The 90 μm beads have a narrow size distribution, excellent pressure stability and swelling properties, and high diffusion rates, making them ideally suited for both batch or continuous flow peptide synthesis.

Cleavage can be effected by treatment with AcOH/TFE/DCM (2:2:6), 0.5% TFA in DCM, or 30% HFIP in DCM, to yield protected peptide acid fragments without any loss of sensitive side-chain protecting groups.

Because enantiomerization can not occur during attachment of the first residue to TGT resin, peptides containing C-terminal Cys and His residues can be produced free from contamination by diastereomeric side-products. This support is also particularly suited to the synthesis of prolyl peptides as the bulk of the trityl handle prohibits diketopiperazine formation [2].

Loading: 0.10 - 0.30 mmole/g resin, as determined photometrically from the amount of Fmoc chromophore liberated upon treatment of the resin with DBU/DMF. 90 μm beads.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry

ⓘ Ⓖ 2.3, 2.6, Ⓜ 3.30

[1] E. Bayer, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 13th American Peptide Symposium", R. S. Hodges & J. A. Smith (Eds), ESCOM, Leiden, 1994, pp. 156.

[2] G. Grübler, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 3rd International Symposium", R. Epton (Eds), Mayflower Worldwide, Birmingham, 1994, pp. 517.

Product No.	Product	Quantity	Price
856125	Fmoc-Ala–NovaSyn® TGT	1 g	85.00
	NBC No.: 04-12-2700	5 g	340.00
856052	Fmoc-Arg(Pbf)–NovaSyn® TGT	1 g	105.00
	NBC No.: 04-12-2727	5 g	420.00
856126	Fmoc-Asn(Trt)–NovaSyn® TGT	1 g	105.00
	NBC No.: 04-12-2703	5 g	420.00
856127	Fmoc-Asp(OtBu)–NovaSyn® TGT	1 g	105.00
	NBC No.: 04-12-2704	5 g	420.00
856044	Fmoc-Cys(Trt)–NovaSyn® TGT	1 g	105.00
	NBC No.: 04-12-2705	5 g	420.00
856128	Fmoc-Gln(Trt)–NovaSyn® TGT	1 g	105.00
	NBC No.: 04-12-2708	5 g	420.00

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Product No.	Product	Quantity	Price
856129	Fmoc-Glu(OtBu)-NovaSyn® TGT NBC No.: 04-12-2710	1 g 5 g	105.00 420.00
856045	Fmoc-Gly-NovaSyn® TGT NBC No.: 04-12-2711	1 g 5 g	85.00 340.00
856046	Fmoc-His(Trt)-NovaSyn® TGT NBC No.: 04-12-2712 An excellent resin for the Fmoc continuous flow synthesis of peptides containing C-terminal His free from enantiomerization.	1 g 5 g	105.00 420.00
856130	Fmoc-Ile-NovaSyn® TGT NBC No.: 04-12-2713	1 g 5 g	85.00 340.00
856047	Fmoc-Leu-NovaSyn® TGT NBC No.: 04-12-2714	1 g 5 g	85.00 340.00
856048	Fmoc-Lys(Boc)-NovaSyn® TGT NBC No.: 04-12-2715	1 g 5 g	105.00 420.00
856131	Fmoc-Met-NovaSyn® TGT NBC No.: 04-12-2716	1 g 5 g	85.00 340.00
856132	Fmoc-Phe-NovaSyn® TGT NBC No.: 04-12-2718	1 g 5 g	85.00 340.00
856049	Fmoc-Pro-NovaSyn® TGT NBC No.: 04-12-2719 An excellent resin for the Fmoc continuous flow synthesis of peptides containing C-terminal Pro without risk of diketopiperazine formation. [1] G. Grübler, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 3rd International Symposium", R. Epton (Eds), Mayflower Worldwide Ltd., Birmingham, 1994, pp. 517.	1 g 5 g	85.00 340.00
856133	Fmoc-Ser(tBu)-NovaSyn® TGT NBC No.: 04-12-2720	1 g 5 g	105.00 420.00

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
856050	Fmoc-Thr(tBu)-NovaSyn® TGT NBC No.: 04-12-2721	1 g 5 g	105.00 420.00
856190	Fmoc-Trp(Boc)-NovaSyn® TGT resin	1 g 5 g	108.00 429.00
856135	Fmoc-Tyr(tBu)-NovaSyn® TGT NBC No.: 04-12-2723	1 g 5 g	105.00 420.00
856051	Fmoc-Val-NovaSyn® TGT NBC No.: 04-12-2724	1 g 5 g	85.00 340.00

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Product No.	Product	Quantity	Price

Amino acids attached to HMPB NovaPEG (ChemMatrix) resin
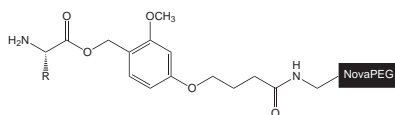
Novabiochem®'s preloaded HMPB NovaPEG (ChemMatrix) resins are excellent acid-labile resins for the continuous flow and batch Fmoc SPPS of peptide acids. These resins are prepared by derivatization of NovaPEG amino resin with mild-acid labile HMPB linker. Peptide acids can be cleaved using 95% TFA whereas treatment with 1% TFA in DCM prvodes protected peptide fragments.

These remarkable PEG-based resins have excellent swelling properties in a wide range of solvents, including water, MeCN, MeOH, DCM, DMF, THF, and toluene. In comparative studies this resin was found to give better results than polystyrene-based supports in the synthesis of hydrophobic peptides. The high hydrophilicity was also found to benefit on-resin immunoassays with one-bead-one-peptide libraries [1].

Loading: 0.40 - 0.80 mmole/g resin, as determined photometrically from the amount of Fmoc chromophore liberated upon treatment of the Fmoc-Leu loaded resin with DBU/DMF.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry

ⓘ Ⓖ 2.4, 2.6, Ⓜ 3.29

[1] F. Garcia-Martin, et al. (2006) *J. Comb. Chem.*, **8**, 213.

856166	**H-Ala-HMPB NovaPEG resin**	1 g	98.00
	NBC No.: 04-12-2212	5 g	385.00
856167	**H-Arg(Pbf)-HMPB NovaPEG resin**	1 g	150.00
	NBC No.: 04-12-2213	5 g	595.00
	Loading: 0.20 - 0.60 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin.		
856168	**H-Asn(Trt)-HMPB NovaPEG resin**	1 g	130.00
	NBC No.: 04-12-2214	5 g	520.00
856169	**H-Asp(OtBu)-HMPB NovaPEG resin**	1 g	130.00
	NBC No.: 04-12-2215	5 g	520.00
856170	**H-Cys(Trt)-HMPB NovaPEG resin**	1 g	130.00
	NBC No.: 04-12-2216	5 g	520.00
856171	**H-Gln(Trt)-HMPB NovaPEG resin**	1 g	130.00
	NBC No.: 04-12-2217	5 g	520.00
856172	**H-Glu(OtBu)-HMPB NovaPEG resin**	1 g	130.00
	NBC No.: 04-12-2218	5 g	520.00

Product No.	Product	Quantity	€ Price
856173	H–Gly–HMPB NovaPEG resin NBC No.: 04-12-2219	1 g 5 g	98.00 385.00
856174	H–His(Trt)–HMPB NovaPEG resin NBC No.: 04-12-2220	1 g 5 g	130.00 520.00
856175	H–Ile–HMPB NovaPEG resin NBC No.: 04-12-2221	1 g 5 g	98.00 385.00
856176	H–Leu–HMPB NovaPEG resin NBC No.: 04-12-2222	1 g 5 g	98.00 385.00
856177	H–Lys(Boc)–HMPB NovaPEG resin NBC No.: 04-12-2223	1 g 5 g	130.00 520.00
856178	H–Met–HMPB NovaPEG resin NBC No.: 04-12-2224	1 g 5 g	98.00 385.00
856179	H–Phe–HMPB NovaPEG resin NBC No.: 04-12-2225	1 g 5 g	98.00 385.00
856181	H–Ser(tBu)–HMPB NovaPEG resin NBC No.: 04-12-2227	1 g 5 g	130.00 520.00
856182	H–Thr(tBu)–HMPB NovaPEG resin NBC No.: 04-12-2228	1 g 5 g	130.00 520.00
856183	H–Trp(Boc)–HMPB NovaPEG resin NBC No.: 04-12-2229	1 g 5 g	150.00 595.00
856184	H–Tyr(tBu)–HMPB NovaPEG resin NBC No.: 04-12-2230	1 g 5 g	130.00 520.00
856185	H–Val–HMPB NovaPEG resin NBC No.: 04-12-2231	1 g 5 g	98.00 385.00

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Product No.	Product	Quantity	Price

Amino acids attached to Trt NovaPEG (ChemMatrix) resin
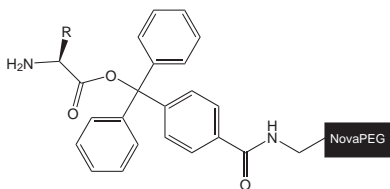
Novabiochem®'s preloaded HMPB NovaPEG (ChemMatrix) resins are excellent acid-labile resins for the continuous flow and batch Fmoc SPPS of peptide acids. This resin is prepared by derivatization of NovaPEG amino resin with mild-acid labile Trt linker. Peptide acids can be cleaved using 95% TFA whereas treatment with 1% TFA in DCM prvodes protected peptide fragments.

The remarkable totally PEG-based resin has excellent swelling properties in a wide range of solvents, including water, MeCN, MeOH, DCM, DMF, THF, and toluene. In comparative studies this resin was found to give better results than polystyrene-based supports in the synthesis of hydrophobic peptides. The high hydrophilicity was also found to benefit on-resin immunoassays with one-bead-one-peptide libraries [1].

Trt NovaPEG resins are ideal for the synthesis of peptides containing C-terminal Cys, His and Pro residues as Trt linker overcomes the problems of racemization and diketopiperazine formation associated with these amino acids.

Loading: 0.20 - 0.40 mmole/g resin, as determined photometrically from the amount of Fmoc chromophore liberated upon treatment of the Fmoc-Leu loaded resin with DBU/DMF.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry

ⓘ Ⓖ 2.4, 2.6, Ⓜ 3.30

[1] F. Garcia-Martin, et al. (2006) *J. Comb. Chem.*, **8**, 213.

856187	H–Cys(Trt)–Trityl–NovaPEG	1 g	150.00
		5 g	815.00
856188	H–His(Trt)–Trityl–NovaPEG	1 g	150.00
		5 g	600.00
856189	H–Pro–Trityl–NovaPEG	1 g	135.00
		5 g	540.00

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Amino acids attached to 2-chlorotrityl resin
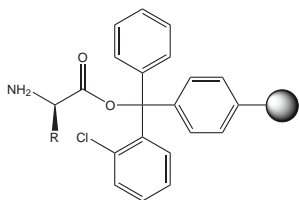
2-Chlorotrityl resins are excellent supports for the preparation of partially protected and fully deprotected peptide fragments by Fmoc batch SPPS [1-7].
Cleavage can be effected by treatment with AcOH/TFE/DCM (2:2:6), 0.5% TFA in DCM or 30% HFIP in DCM, to yield protected peptide acid fragments without any loss of sensitive side-chain protecting groups.
Because enantiomerization does not occur during the loading of 2-chlorotrityl resin, peptides containing C-terminal Cys and His residues can be produced free from contamination by diastereomeric side-products [3]. These supports are useful tools for the synthesis of prolyl peptides as the bulk of the trityl handle prevents diketopiperazine formation [1,7].

Loading: 0.30 - 0.90 mmole/g resin, as determined from the Fmoc-Leu loaded resin. The polymer matrix is copoly(styrene - 1% DVB), 200 - 400 mesh.

⚠ Prolonged storage: +2 to +8 °C; keep cool and dry.

ⓘ Ⓖ 2.6, Ⓜ 3.30

[1]   K. Barlos, et al. (1989) *Tetrahedron Lett.*, **30**, 3947.
[2]   K. Barlos, et al. (1989) *Tetrahedron Lett.*, **30**, 3943.
[3]   K. Barlos, et al. (1991) *Angew. Chem.*, **103**, 572.
[4]   K. Barlos, et al. (1991) *Int. J. Peptide Protein Res.*, **37**, 513.
[5]   K. Barlos, et al. (1991) *Int. J. Peptide Protein Res.*, **38**, 562.
[6]   K. Barlos, et al. (1991) *Int. J. Peptide Protein Res.*, **38**, 555.
[7]   K. Barlos, et al. (1993) *Ann. Chem.*, 215.

Product No.	Product	Quantity	Price
856055	**H–Ala–2–ClTrt resin** NBC No.: 04-12-2803	1 g 5 g	40.00 80.00
856142	**H–β–Ala–2–ClTrt resin** NBC No.: 04-12-2821	1 g 5 g	40.00 80.00
856067	**H–Arg(Pbf)–2–ClTrt resin** NBC No.: 04-12-2823	1 g 5 g	60.00 120.00
856195	**H–Asn(Trt)–2–ClTrt resin** NEW	1 g 5 g	60.00 120.00
856065	**H–Asp(OtBu)–2–ClTrt resin** NBC No.: 04-12-2815	1 g 5 g	60.00 120.00
856061	**H–Cys(Trt)–2–ClTrt resin** NBC No.: 04-12-2811	1 g 5 g	60.00 120.00

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Product No.	Product	Quantity	Price
856194 NEW	H-Gln(Trt)-2-ClTrt resin	1 g 5 g	60.00 120.00
856063	H-Glu(OtBu)-2-ClTrt resin NBC No.: 04-12-2813	1 g 5 g	60.00 120.00
856053	H-Gly-2-ClTrt resin NBC No.: 04-12-2800	1 g 5 g	40.00 80.00
856056	H-His(Trt)-2-ClTrt resin NBC No.: 04-12-2804 An excellent resin for the synthesis of peptides containing C-terminal His without risk of enantiomerization.	1 g 5 g	60.00 120.00
856136	H-Ile-2-ClTrt resin NBC No.: 04-12-2801	1 g 5 g	60.00 120.00
856060	H-Leu-2-ClTrt resin NBC No.: 04-12-2810	1 g 5 g	40.00 80.00
856054	H-Lys(Boc)-2-ClTrt resin NBC No.: 04-12-2802	1 g 5 g	60.00 120.00
856138	H-Met-2-ClTrt resin NBC No.: 04-12-2816	1 g 5 g	60.00 120.00
856059	H-Phe-2-ClTrt resin NBC No.: 04-12-2809	1 g 5 g	60.00 120.00
856057	H-Pro-2-ClTrt resin NBC No.: 04-12-2805 An excellent resin for the synthesis of peptides containing C-terminal Pro without risk of diketopiperazine formation.	1 g 5 g	40.00 80.00
856064	H-Ser(tBu)-2-ClTrt resin NBC No.: 04-12-2814	1 g 5 g	60.00 120.00

PRE-LOADED RESINS

⚠ Storage conditions   ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
856062	H-Thr(tBu)-2-ClTrt resin NBC No.: 04-12-2812	1 g 5 g	60.00 120.00
856141	H-Trp(Boc)-2-ClTrt resin NBC No.: 04-12-2819	1 g 5 g	60.00 120.00
856066	H-Tyr(tBu)-2-ClTrt resin NBC No.: 04-12-2820	1 g 5 g	60.00 120.00
856058	H-Val-2-ClTrt resin NBC No.: 04-12-2808	1 g 5 g	40.00 80.00

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Product No.	Product	Quantity	Price

Amino alcohols attached to 2-chlorotrityl resin

Novabiochem®'s amino alcohol supports are specially prepared to ensure that only O-linked products are obtained during resin derivatization.

Cleavage can normally be effected using 5% TFA in DCM containing 5% TIS, or with 30% HFIP in DCM [1].

These resins are useful tools for the synthesis of peptide alcohols [2].

Loading: 0.40 - 0.90 mmole/g, as determined from the substitution of the Fmoc-Leu loaded resin. The polymer matrix is copoly(styrene - 1 % DVB), 200 - 400 mesh.

⚠ Prolonged storage: +2 to +8 °C; keep cool and dry.

ⓘ Ⓖ 2.6

[1] P. Athanassopoulos, et al., Poster 316 presented at the 24th European Peptide Symposium, Edinburgh, 1996 (not published in proceedings).

[2] H. H. Nguyen, et al., Poster 130 presented at the 17th American Peptide Symposium, San Diego, 2001.
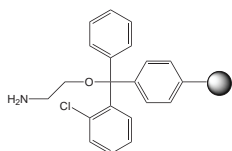
856088	**Glycinol 2-chlorotrityl resin**	1 g	78.00
	NBC No.: 01-64-0087	5 g	310.00
		25 g	1227.00
856098	**O-t.-Butylthreoninol 2-chlorotrityl resin**	1 g	78.00
	NBC No.: 01-64-0353	5 g	310.00
	C-terminal amino alcohol of Octreotide.	25 g	1227.00
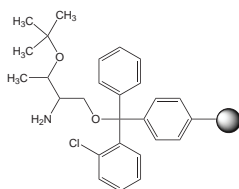
⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product		Quantity	Price

Diamines attached to trityl resin

Novabiochem®'s polyamine supports are specially prepared to ensure the minimum of trityl polyamine cross-linking. The bulky trityl linker offers complete protection to the resin linked secondary amino functionality, thereby leaving the pendant primary amino group free to participate as a nucleophile in further reactions.

Cleavage can normally be effected using 1 – 5% TFA in DCM containing 5% TIS, or with 30% HFIP in DCM [1]. For examples of this approach see [2 – 7].

⚠ Prolonged storage: +2 to +8 °C; keep cool and dry.

ⓘ ⑥ 2.25,

[1] P. Athanassopoulos, et al., Poster 316 presented at the 24th European Peptide Symposium, Edinburgh, 1996 (not published in proceedings).

[2] K. Barlos, et al. (1988) *Liebigs Ann. Chem.*, 1079.

[3] A. v. Vliet, et al. in "Peptides 1994, Proc. 23rd European Peptide Symposium", H. Maia (Eds), ESCOM, Leiden, 1995, pp. 267.

[4] I. A. Nash, et al. (1996) *Tetrahedron Lett.*, **37**, 2625.

[5] S. Manku, et al. (2001) *J. Org. Chem.*, **66**, 874.

[6] A. P. Tamiz, et al. (2000) *Bioorg. Med. Chem. Lett.*, **10**, 2741.

[7] T. Kan, et al. (2002) *Synlett*, **8**, 1338.

856093	**Bis-(2-aminoethyl)-ether trityl resin**	1 g	114.00
	NBC No.: 01-64-0141	5 g	458.00
	Loading: 0.30 - 1.00 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The polymer matrix is copoly(styrene-1% DVB), 200 - 400 mesh.	25 g	1830.00

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

856097	**O-Bis-(aminoethyl)ethylene glycol trityl resin**	1 g	78.00
	NBC No.: 01-64-0235	5 g	310.00
	Loading: 0.30 - 1.00 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The polymer matrix is copoly(styrene-1 % DVB), 200 - 400 mesh.	25 g	1227.00

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.
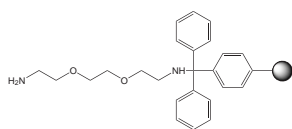
856085	**1,4-Diaminobutane trityl resin**	1 g	78.00
	NBC No.: 01-64-0082	5 g	310.00
	Loading: 0.20 - 1.00 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The polymer matrix is copoly(styrene-1 % DVB), 200 - 400 mesh.	25 g	1227.00

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

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Product No.	Product	Quantity	Price
856084	**1,2-Diaminoethane trityl resin**	1 g	78.00
	NBC No.: 01-64-0081	5 g	310.00
	Loading: 0.30 - 1.25 mmole/g resin; as determined from the substitution of the	25 g	1227.00
	Fmoc-Leu loaded resin. The polymer matrix is copoly(styrene-1 % DVB), 200 - 400		
	mesh.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
	For applications of ethylenediamine trityl resins, see [1, 2].		
	[1] A. Srinivasan & M. A. Schmidt, Poster 110 presented at the 15th American Peptide symposium, Nashville, 1997.		
	[2] E. Suarez, et al. in "Poster 107, presented at the 4th Forum on Peptides & Proteins, Montpellier, 1997".		
856086	**1,6-Diaminohexane trityl resin**	1 g	78.00
	NBC No.: 01-64-0083	5 g	310.00
	Loading: 0.30 - 1.00 mmole/g resin; as determined from the substitution of the	25 g	1227.00
	Fmoc-Leu loaded resin. The polymer matrix is copoly(styrene-1% DVB) 200 -400		
	mesh.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
856090	**1,5-Diaminopentane trityl resin**	1 g	78.00
	NBC No.: 01-64-0133	5 g	310.00
	Loading: 0.25 - 1.00 mmole/g resin; as determined from the substitution of the	25 g	1227.00
	Fmoc-Leu loaded resin. The polymer matrix is copoly(styrene-1 % DVB), 200 - 400		
	mesh.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
	This resin could have important applications in the preparation of Nephilatoxin analogs.		
856089	**1,3-Diaminopropane trityl resin**	1 g	78.00
	NBC No.: 01-64-0132	5 g	310.00
	Loading: 0.30 - 1.00 mmole/g resin; as determined from the substitution of the	25 g	1227.00
	Fmoc-Leu loaded resin. The polymer matrix is copoly(styrene-1 % DVB), 200 - 400		
	mesh.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
	For examples of the use of this resin in polyamine synthesis see references [1, 2].		
	[1] F. Wang, et al. (2000) *Org. Lett.*, **2**, 1581.		
	[2] S. Manku, et al. (2001) *J. Org. Chem.*, **66**, 874.		

PRE-LOADED RESINS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price

Amino thiols attached to 2-chlorotrityl resin
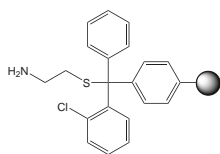
856000	**Cysteamine 2-chlorotrityl resin**	1 g	70.00
	NBC No.: 01-64-0107	5 g	280.00
	Loading: 1.00 - 2.00 mmole/g resin; as determined from the substitution of the	25 g	995.00

Fmoc-Leu loaded resin. The polymer matrix is copoly(styrene-1% DVB), 200 - 400 mesh.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

An acid-labile resin for the preparation of N-acyl or N-alkyl cysteamines. The free amino functionality of the resin-bound cysteamine can be readily acylated or reductively alkylated using standard procedures. Cleavage can be effected with electrophilic oxidants such as I_2 or Tl^{3+} to produce a dimeric disulfide bridged product, or with 50-100% TFA to give the monomeric sulfhydryl product. Peptidylaminoethylthiols produced in this manner have been used to prepare PEG-conjugates by chemoselective ligation to pegylated maleimide [1]. This method is particularly useful for forming intramolecular disulfide bridges in molecules containing two thiol groups where one is protected with Acm.

For a similar application, see [2, 3, 4].

[1] J. Zhang, et al. Poster 162 presented at the 16 American Peptide Symposium, Minneapolis, 1999.

[2] A. v. Vliet, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 2nd International Symposium", R. Epton (Eds), Intercept UK Ltd., Andover, 1992, pp. 475.

[3] A. v. Vliet, et al. in "Peptides 1992, Proc. 22nd European Peptide Symposium", C. H. Schneider & A. N. Eberle (Eds), ESCOM, Leiden, 1993, pp. 279.

[4] A. v. Vliet, et al. in "Peptides, Chemistry, Structure, & Biology, Proc. 13th American Peptide Symposium", R. S. Hodges & J. A. Smith (Eds), ESCOM, Leiden, 1994, pp. 151.

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Product No.	Product	Quantity	Price

Amino thiols attached to 4-methoxytrityl resin

These acid-labile resins are ideal for the preparation of N–acyl or N–alkyl aminoalkylthiols. The free amino functionality of the resin bound aminothiol can be readily acylated using standard methods of amide bond formation, or reductively alkylated. Cleavage can be effected with 1% TFA in DCM containing 5% triethylsilane [1].

Disulfides can also be generated using electrophilic oxidants such as I_2 or Tl^{3+}.

⚠ **Prolonged storage:** +2 to +8 °C; keep cool and dry.

ⓘ Ⓖ 2.6

[1] S. Mourtas, et al. (2003) *Tetrahedron Lett.*, **44**, 179.

856095	**4-Aminobutanethiol 4-methoxytrityl resin**	1 g	114.00
	NBC No.: 01-64-0228	5 g	458.00
	Loading: 0.50 - 1.00 mmole/g resin; as determined from the substitution of the	25 g	1830.00
	Fmoc-Leu loaded resin. The polymer matrix is copoly(styrene-1 % DVB), 200 - 400 mesh.		

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry.

856087	**Cysteamine 4-methoxytrityl resin**	1 g	114.00
	NBC No.: 01-64-0086	5 g	458.00
	Loading: 0.20 - 1.30 mmole/g resin; as determined from the substitution of the	25 g	1830.00
	Fmoc-Leu loaded resin. The polymer matrix is copoly(styrene-1 % DVB), 200 - 400 mesh.		

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry.

PRE-LOADED RESINS

⚠ Storage conditions	Ⓖ Additional information	Ⓜ Method information	Ⓐ Application information

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Product No.	Product	Quantity	Price

Acids attached to resins
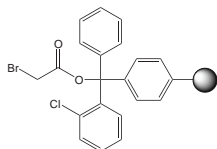
856096   **Bromoacetic acid 2-chlorotrityl resin**

NBC No.: 01-64-0233

Loading: 0.90 - 1.40 mmole/g resin; as determined by elemental analysis of bromine. The polymer matrix is copoly(styrene-1 % DVB), 200 - 400 mesh.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

An acid-labile resin for the preparation of substituted acetic acids. Release of the product can be effected with 1-5% TFA in DCM containing 5% TIS or with 30% HFIP in DCM.

This resin may have similar applications to bromoacetic acid attached to Wang resin, which has been utilized in the traceless synthesis of cyclopropanecarboxylates [1] and tricyclic isoxazolines [2].

[1] N. H. Vo, et al. (1997) *Tetrahedron Lett.,* **38**, 7951.

[2] A. J. Bicknell, et al. (1998) *Tetrahedron Lett.,* **39**, 5869.

1 g	78.00
5 g	310.00
25 g	1227.00

customer service: service@novabiochem.com   technical service: technical@novabiochem.com internet: novabiochem.com
Bulk quantities available, please enquire

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Coupling reagents

Novabiochem® offers one of the most extensive ranges of high-quality coupling reagents for *in situ* activation on the market. However, with such a plethora of reagents available, the choice of the optimum coupling reagent for a particular application is not always straightforward. Condensation reagents vary in terms of coupling efficiency, stability, solubility, or reactivity of active species. To simplify your reagent selection, the following table summarizes the properties and advantages of the most commonly used coupling reagents.

Coupling reagent	Solubility (M)	Stability in DMF closed vial	Reactivity of active species	Comments
BOP	>1.5	n/a	4	Water soluble by-products make it useful for solution phase synthesis.
COMU	1.5	Low	2	High reactivity but very limited solution stability. Causes guanidylation.
DEPBT	n/a	n/a	4	Excellent reagent for coupling for protected fragments with low racemization.
HATU	0.45	excellent	1	Gold standard for hindered couplings, but expensive compared to other reagents.
HBTU	0.5	Excellent	4	Excellent reagent for routine synthesis. May cause guanidinylation.
HCTU	0.75	Excellent	3	More reactive and expensive than HBTU. May cause guanidinylation.
PyBOP	>1.5	Moderate. Solutions need making fresh daily	4	Excellent reagent for routine synthesis. Ideal of in situ activation as it does not cause guanidinylation. Clean.
PyAOP	>1.5	Moderate. Solutions need making fresh daily	1	Excellent reagent for hindered couplings and peptide cyclization. Ideal of in situ activation as it does not cause guanidinylation. Clean.
PyOxim	>1.5	Moderate. Solutions need making fresh daily	2	Similar advantages to PyBOP but generates a more reactive active species. Clean.
DIPCDI/ Oxyma	n/a	n/a	5	Slow activation at rt. Works well in microwave machines.

COUPLING REAGENTS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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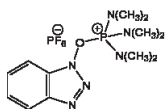
Product No.	Product	Quantity	Price
851004	**BOP**	25 g	66.00
	Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphoniumhexafluorophosphate	100 g	220.00

Castro's Reagent

NBC No.: 01-62-0001; CAS No.: 56602-33-6; $C_{12}H_{22}N_6OP_2F_6$; M.W.: 442.3

Solubility: 0.6 mmole in 1 ml DMF clearly soluble.

HPLC: purity: $\geq$ 99.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Peptide coupling reagent which suppresses enantiomerization [1-15]. Reduction of carboxylic acids activated with BOP provides a facile route to alcohols [16]. Novabiochem® recommends the replacement of BOP by PyBOP®(for PyBOP® please see 01-62-0016). The manufacture of BOP, as well as its utilization, involves the use or the formation of hexamethylphosphotriamide, the carcinogenicity and respiratory toxicity of which has been the subject of numerous reports.

ⓘ Ⓖ 3.3

[1]   B. Castro, et al. (1975) *Tetrahedron Lett.*, **16**, 1219.
[2]   B. Castro, et al. (1976) *Synthesis*, 751.
[3]   B. Castro, et al. (1977) *Synthesis*, 413.
[4]   B. Castro, et al. (1977) *J. Chem. Res. (S)*, 182.
[5]   P. Rivaille, et al. (1980) *Tetrahedron*, **36**, 3413.
[6]   D. L. Nguyen, et al. (1985) *J. Chem. Soc., Perkin Trans. 1*, 1025.
[7]   D. L. Nguyen, et al. (1987) *J. Chem. Soc., Perkin Trans. 1*, 1915.
[8]   A. Fournier, et al. (1988) *Int. J. Peptide Protein Res.*, **31**, 86 and 231.
[9]   J.-P. Briand, et al. (1989) *Pept. Res.*, **2**, 381.
[10] A. Fournier, et al. (1989) *Int. J. Peptide Protein Res.*, **33**, 133.
[11] D. L. Nguyen, et al. (1989) *Biochem. Biophys. Res. Commun.*, **162**, 1425.
[12] W. K. Rule, et al. in "Peptides 1988, Proc. 20th European Peptide Symposium", G. Jung & E. Bayer (Eds), Walter de Gruyter, Berlin, 1989, pp. 238.
[13] M. Forest, et al. (1990) *Int. J. Peptide Protein Res.*, **35**, 89.
[14] R. Seyer, et al. (1990) *Int. J. Peptide Protein Res.*, **35**, 465.
[15] H. Gausepohl, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 2nd International Symposium", R. Epton (Eds), Intercept UK Ltd., Andover, 1993, pp. 387.
[16] R. P. McGeary (1998) *Tetrahedron Lett.*, **39**, 3319.
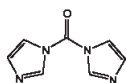
Product No.	Product	Quantity	Price
851054	**CDI**	25 g	125.00
	1,1'-Carbonyl-diimidazole	100 g	374.00

NBC No.: 01-62-0002; CAS No.: 530-62-I; $C_7H_6N_4O$; M.W.: 162.2

Solubility: 0.6 mmole in 2 ml DMF clearly soluble.

⚠ Prolonged storage: ≤-20°C; keep cool and dry.

A useful coupling reagent for amidations, esterifications and thioesterifications [1-4].

[1]   H. A. Staab (1957) *Ann. Chem.*, **609**, 75.
[2]   R. Paul, et al. (1960) *J. Am. Chem. Soc.*, **82**, 4596.
[3]   R. Paul, et al. (1962) *J. Org. Chem.*, **27**, 2094.
[4]   T. Kamijo, et al. (1984) *Chem. Pharm. Bull.*, **32**, 5044.

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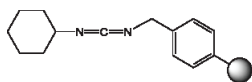
Product No.	Product	Quantity	Price
855029	**N–Cyclohexylcarbodiimide, N'–methyl polystyrene**	5 g	115.00
	NBC No.: 01-64-0211	25 g	460.00
	HPLC: purity: ≥ 1.30%.	100 g	1380.00

Loading: - 1.30 mmole/g resin; as determined by HPLC, after reaction of Z-Phe-OH with cyclohexylamine. The polymer matrix is copoly(styrene-2 % DVB), 200-400 mesh.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep opened bottle under nitrogen; hygroscopic.

N-Cyclohexylcarbodiimide,N'-methyl polystyrene [1] is an ideal tool for mediating solution phase coupling reactions. Typically, the amine and carboxylic acid are treated with a 2-3 fold excess of resin in DCM or DMF [2, 3]; a slight excess of carboxyl component over amine component is often used to help drive the reaction to completion. As excess acid and urea by-products remain attached to the resin at the end of the reaction, the product can normally be isolated pure by filtration and evaporation of the reaction mixture. Reactions can be accelerated by the addition of auxiliary nucleophiles such as HOBt; these can be removed using polyamine- or TBD-polystyrene. This resin has also been used in conjunction with DMAP to mediate ester formation [4].

[1] G.M Weinshenker, et al. (1988) *Org. Synth.*, **Coll. Vol. VI**, 951.
[2] Y. Guan, et al. (2000) *J. Comb. Chem.*, **2**, 297.
[3] K. Senton, et al. (2003) *J. Comb. Chem.*, **5**, 336.
[4] M. Cano, et al. (2002) *J. Org. Chem.*, **67**, 129.
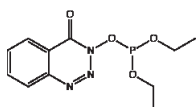
851091	**DEPBT**	25 g	166.00
	3-(Diethoxy-phosphoryloxy)-1,2,3-benzo[d]triazin-4(3H)-one	100 g	499.00

Diethyl-(4-oxo-3H-1,2,3-benzotriazin-3-yl)phosphate
CAS No.: 165534-43-0; $C_{11}H_{14}N_3O_5P$; M.W.: 299.2
HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

DEPBT is an in situ coupling reagent that generates racemization resistant Dbht esters [1]. DEPBT has been shown to be highly effective for cyclolactamization of peptides.

[1] Y.–H. Ye, et al. (2002) *Pept. Sci.*, **80**, 172.

COUPLING REAGENTS

⚠ Storage conditions　　Ⓖ Additional information　　Ⓜ Method information　　Ⓐ Application information

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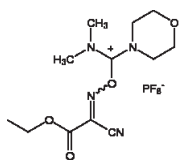
Product No.	Product	Quantity	Price
851085	**COMU**	25 g	125.00
	1-[(1-(Cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylamino-morpholino)]	100 g	375.00
	uranium hexafluorophosphate		
	CAS No.: 1075198-30-9; $C_{12}H_{12}F_6N_4O_4P$; M.W.: 428.1		

Solubility: 10% in acetonitrile clearly soluble.

HPLC: purity: ≥ 99.00%.

⚠ Prolonged storage: ≤-20°C; keep cool and dry.

COMU is a novel reagent which mediates coupling reactions with efficiencies comparable to HATU [1, 2].

It has higher solubility than HATU and HBTU. Furthermore, it is not explosive under normal operating conditions and is less likely to exhibit allergenicity compared to other coupling reagents. Recently, the application of COMU in the preparation of esters has been demonstrated [3].

ⓘ Ⓖ 3.3

[1] A. El-Faham, et al. (2009) *Chem. Eur. J.*, **15**, 9404.
[2] A. El-Faham & F. Albericio (2010) *J. Pept. Sci.*, **16**, 6.
[3] Jean-d'Amour, et al. (2011) *Org. Lett.*, **13**, 2988.
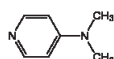
851055	**DMAP**	25 g	31.00
	4-Dimethylaminopyridine	100 g	94.00
	NBC No.: 01-62-0004; CAS No.: 1122-58-3; $C_7H_{10}N_2$; M.W.: 122.2		

Solubility: 1 mmole in 2 ml DMF clearly soluble.

GC: purity: ≥ 98.00%.

Catalyst for esterification and amidation reagents [1-3].

ⓘ Ⓖ 3.6, Ⓜ 3.8

[1] S. S. Wang, et al. (1981) *Int. J. Peptide Protein Res.*, **18**, 459.
[2] J.-P. Gamet, et al. (1984) *Tetrahedron*, **40**, 1995.
[3] K. Takeda, et al. (1991) *Synthesis*, **1991**, 689.
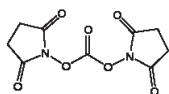
851005	**DSC**	25 g	151.00
	N,N'-Disuccinimidyl carbonate	100 g	454.00
	Di-(N-Succinimidyl)carbonate		
	NBC No.: 01-62-0006; CAS No.: 74124-79-1; $C_9H_8N_2O_7$; M.W.: 256.2		

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

A convenient reagent for the synthesis of N-succinimidyl esters of N-protected amino acids [1-4].

[1] H. Ogura, et al. (1979) *Tetrahedron Lett.*, **20**, 4745.
[2] H. Ogura, et al. (1981) *Tetrahedron Lett.*, **22**, 4817.
[3] K. Takeda, et al. (1982) *Synth. Commun.*, **12**, 213.
[4] K. Takeda, et al. (1983) *Tetrahedron Lett.*, **24**, 4569.
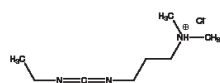
851007	**EDC · HCl**	25 g	100.00
	1-Ethyl-3-(3'-dimethylaminopropyl)carbodiimide · HCl; WSC · HCl		
	NBC No.: 01-62-0011; CAS No.: 25952-53-8; $C_8H_{17}N_3$ · HCl; M.W.: 155.2 · 36.5		

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.

A carbodiimide coupling reagent that generates a urea by-product which can be easily removed from the reaction media by extraction with water [1-3].

ⓘ Ⓖ 3.1

[1] K. D. Kopple, et al. (1962) *J. Am. Chem. Soc.*, **84**, 4457.
[2] J. C. Sheehan, et al. (1965) *J. Am. Chem. Soc.*, **87**, 2492.
[3] J. C. Sheehan, et al. (1961) *J. Org. Chem.*, **26**, 2525

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Product No.	Product	Quantity	Price

851013   **HATU**   25 g   300.00
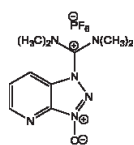
NBC No.: 01-62-0041; CAS No.: 148893-10-1; $C_{10}H_{15}N_6OPF_F$; M.W.: 380.3   100 g   600.00

Solubility: 1 mmole in 2 ml DMF clearly soluble.

⚠ **Prolonged storage:** +2 to +8°C

A highly efficient coupling reagent for solid and solution phase peptide synthesis. In comparative studies HATU has been found to give better coupling yields with less enantiomerization that HBTU, TBTU or PyBOP® [1-3]. It is particularly effective at coupling to N-alkyl amines where other coupling reagents give poor yields and is the preferred reagent for loading resins bearing secondary amino groups.

ⓘ Ⓖ 3.3

[1] L. A. Carpino (1993) *J. Am. Chem. Soc.*, **115**, 4397.
[2] F. Albericio, et al. (1998) *J. Org. Chem.*, **63**, 9678.
[3] J. A. Carpino, et al., in "Innovation & Perspectives in Solid Phase Synthesis, 3rd International Symposium", R. Epton (Ed.), Mayflower Worldwide Ltd., 1994, pp. 95.
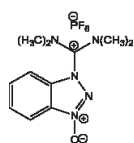
851006   **HBTU**   25 g   40.00

2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate   100 g   120.00
NBC No.: 01-62-0010; CAS No.: 94790-37-1; $C_{11}H_{16}N_5OPF_6$; M.W.: 379.3

Solubility: 1 mmole in 2 ml DMF clearly soluble.

HPLC: purity: ≥ 99.00%.

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry.

TBTU and HBTU are two of the most popular in situ activation reagents used in solid phase and solution phase peptide synthesis [1-9]. These reagents offer reactivity similar to symmetrical anhydrides and BOP [1]. Couplings proceed smoothly and rates can even be enhanced by the addition of HOBt [2, 4, 5, 6]. In addition to having high reactivity, TBTU and HBTU have also been shown to limit enantiomerization during fragment condensation and during DMAP catalyzed esterification of arginine derivatives [3]. Comparative experiments between HBTU and TBTU have shown that the counter ion has no influence on coupling rates or levels of enantiomerization [1].

ⓘ Ⓖ 3.3

[1] R. Knorr, et al. (1989) *Tetrahedron Lett.*, **30**, 1927.
[2] M. S. Bernatowicz, et al. (1989) *Tetrahedron Lett.*, **30**, 4645.
[3] D. Ambrosius, et al. (1989) *Biol. Chem. Hoppe-Seyler*, **370**, 217.
[4] C. G. Fields, et al. (1991) *Pept. Res.*, **4**, 95.
[5] A. G. Beck-Sickinger, et al. (1991) *Pept. Res.*, **4**, 88.
[6] G. E. Reid, et al. (1992) *Anal. Biochem.*, **200**, 301.
[7] G. B. Fields, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 1st International Symposium", R. Epton (Eds), SPCC UK Ltd., Birmingham, 1990, pp. 241.
[8] P. A. Baybayan, et al. in "Peptides, Chemistry & Biology, Proc. 12th American Peptide Symposium", J. A. Smith & J. E. Rivier (Eds), ESCOM, Leiden, 1992, pp. 566.
[9] J. J. Dudash, et al. (1993) *Synth. Commun.*, **23**, 349.

COUPLING REAGENTS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
851012	**HCTU**	25 g	82.00
	2-(6-Chloro-1-H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate	100 g	245.00

NBC No.: 01-62-0038; CAS No.: 330645-87-9; $C_{11}H_{15}N_5OPSF_6Cl$; M.W.: 413.7

Solubility: 1 mmole in 2 ml DMF clearly soluble.

HPLC: purity: ≥ 99.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light.

HCTU [1] is a novel aminium-based coupling reagent, analogous to HBTU, which in comparative studies was found to give superiort results to TBTU in the synthesis of difficult peptides [2], in hindered couplings [3], and cyclizations [4].

ⓘ Ⓖ 3.3

[1]   O. Marder, et al. (2002) *Chimica Oggi*, 37.

[2]   G. Sabatino, et al. in "Peptides 2002, Proceedings of the 27th European Peptide Symposium", E. Benedetti & C. Pedone (Eds), Naples, Edizioni Ziino, 2002, pp. 272.

[3]   M. Gude & S. Barthélémy in "Peptides 2002, Proceedings of the 27th European Peptide Symposium", E. Benedetti & C. Pedone (Eds), Naples, Edizioni Ziino, 2002, pp. 122.

[4]   G. Sabatino, et al. in "Peptide Revolution: Genomics, Proteomics & Therapeutics, , Proc. 18th American Peptide Symposium", M. Chrev & T. K. Sawyer (Eds), Cardiff, American Peptide Society, 2003, pp. 49.

Product No.	Product	Quantity	Price
851056	**HOSu**	25 g	19.00
	N-Hydroxysuccinimide	100 g	56.00
	NHS		

NBC No.: 01-62-0009; CAS No.: 6066-82-6; $C_4H_5NO_3$; M.W.: 115.1

Solubility: 1 mmole in 2 ml DMF clearly soluble.

TLC: $CHCl_3$:MeOH:AcOH 32 % (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Reagent used for the synthesis of water-soluble active esters and as an additive to suppress enantiomerization during carbodiimide condensations [1, 2].

[1]   G. W. Anderson, et al. (1964) *J. Am. Chem. Soc.*, **86**, 1839.

[2]   F. Weygand, et al. (1966) *Z. Naturforsch.*, **21**, 426.

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Product No.	Product	Quantity	Price
855046	**IIDQ–polystyrene**	5 g	190.00
	NBC No.: 01-64-0469	25 g	760.00

HPLC: purity: ≥ 1.5 - 1.9%.

Loading: 1.50 - 1.90 mmole/g resin; as determined by HPLC, after reaction of 3-phenylpropionic acid with cyclohexylamine. The polymer matrix is copoly(styrene-1 % DVB), 200-400 mesh.

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.

IIDQ-polystyrene (IIDQ-PS) [1] is a polymer-supported version of the IIDQ coupling reagent [2]. IIDQ has many advantages over conventional carbodiimide- or uronium-based reagents: no preactivation step is required, and acid, amine and coupling reagent can be added in any order; in contrast to uronium-based reagents like HBTU, it cannot form guanidinium by-products; and it is totally stable to base.

The treatment of a carboxylic acid with IIDQ-PS in DCM or MeCN rapidly generates in situ the corresponding isobutoxycarbonyl mixed anhydride [3]. Attack by nucleophiles preferentially takes place at the less hindered and more electrophilic carbonyl of the carboxylic acid moiety, releasing only volatile carbon dioxide and isobutanol as by-products. If reaction is carried out in the presence of an amine, amide bond formation occurs concurrently with generation of the anhydride. Alternatively, addition of $NaBH_4$ or polymer-supported borohydride to the anhydride will lead directly to the corresponding alcohol.

IIDQ-PS appears to be particularly effective for mediating the acylation for anilines, and has also been found to couple peptide fragments without epimerization. In a comparative study, IIDQ-PS was found to give higher yields and greater purities than HATU, EDC-PS or DCC-PS [4]. Occasionally, with some secondary amines the formation of isobutyl carbamate by-products has been observed, resulting from attack by the amine at the carbonyl group.

[1]   E. Valeur, et al. (2005) *Chem. Commun.*, 1164.
[2]   Y. Kiso, et al. (1973) *Chem. Pharm. Bull.*, **21**, 2507.
[3]   J. R. Vaughan (1951) *J. Am. Chem. Soc.*, **73**, 3547.
[4]   E. Valeur & M. Bradley, unpublished results.

COUPLING REAGENTS

⚠ Storage conditions   ⑤ Additional information   Ⓜ Method information   Ⓐ Application information

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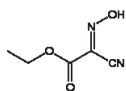
Product No.	Product	Quantity	Price
851086	**Oxyma Pure**	25 g	25.00
	Ethyl cyano(hydroxyimino)acetate	100 g	75.00

CAS No.: 3849-21-6; $C_5H_6N_2O_3$; M.W.: 142.1

HPLC: purity: ≥ 99.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Oxyma Pure is a non-explosive alternative to HOBt. When used in place of HOBt in carbodiimide-mediated coupling reactions it provides products in higher yields with less racemization [1].

ⓘ Ⓖ 3.1

[1] R. Subirós-Funosas, et al. (2009) *Chem. Eur. J.*, **15**, 9394.
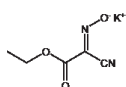
| 851212 | **K-Oxyma Pure** | 25 g | 25.00 |
| NEW | (Z)-ethyl 2-cyano-3-hydroxyacrylate potassium salt | 100 g | 75.00 |

$C_6H_6NO_3K$; M.W.: 179.2

HPLC: purity: ≥ 99.00%.

⚠ Prolonged storage: + 2 to + 8°C; keep cool and dry.

This salt version of Oxyma Pure has improved solubility in organic and aqueous solvents compared to Oxyma Pure and does not cause premature cleavage of peptides from trityl-based resins [1].

ⓘ Ⓖ 3.2

[1] P. Cherkupally, et al. (2013) *Eur. J. Org. Chem.*, 6372.
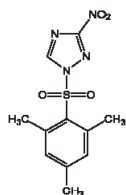
851011	**MSNT**	1 g	47.00
	1-(Mesitylene-2-sulfonyl)-3-nitro-1H-1,2,4-triazole	5 g	186.00
	NBC No.: 01-62-0021; CAS No.: 74257-00-4; $C_{11}H_{12}N_4O_4S$; M.W.: 296.3	25 g	742.00

HPLC: purity: ≥ 99.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

This versatile reagent was originally used as a coupling reagent in phosphotriester oligonucleotide synthesis [1-3]. In recent years, it has been increasingly used for the esterification of Fmoc-amino acids onto solid phase synthesis supports [4-12]. It has proved particularly effective in difficult cases involving unreactive linkers [8, 9] or enantiomerization prone amino acids [7]. In an investigation [6] into ester formation, in which the alcohol or carboxylic acid component was immobilized on a solid support, MSNT was found to give the best results of all the reagents tested (DCC, DCC/HOBt, DCC/DMAP, Ph₃P/DEAD).

ⓘ Ⓖ 3.6, Ⓜ 3.6

[1] C. B. Reese, et al. (1978) *Tetrahedron Lett.*, **19**, 2727.
[2] N. Balgobin, et al. (1981) *Tetrahedron Lett.*, **22**, 1915.
[3] W. Bannwarth (1987) *Chimia*, **41**, 302.
[4] R. Frank, et al. (1988) *Tetrahedron*, **44**, 6031.
[5] B. Blankemeyer-Menge, et al. (1990) *Tetrahedron Lett.*, **31**, 1701.
[6] J. Nielsen (1996) *Tetrahedron Lett.*, **37**, 8439.
[7] E. Harth-Fritschy & D. Cantacuzène (1997) *J. Peptide Res.*, **50**, 415.
[8] M. Renil, et al. (1998) *J. Peptide Sci.*, **4**, 195.
[9] M. Meldal, et al. (1998) *J. Peptide Sci.*, **4**, 83.
[10] A. Graven, et al. (2000) *J. Chem. Soc., Perkin Trans. 1*, 955.
[11] K. S. Kumar, et al. (2001) *J. Peptide Res.*, **57**, 140.
[12] H. Emtenäs, et al. (2002) *J. Comb. Chem.*, **4**, 630.

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Product No.	Product	Quantity	Price
851221	**PyAOP**	25 g	125.00
	CAS No.: 156311-83-0; $C_{17}H_{27}F_6N_7OP_2$; M.W.: 521.4	100 g	375.00

NEW

Solubility: 1 mmole in 2 ml DMF clearly soluble.

HPLC: purity: ≥ 99.00%.

⚠ Prolonged storage: ≤-20°C; keep cool and dry.

PyAOP is a extremely effective coupling which combines the high reactivity of HATU with the lack of side reactions of PyBOP® [1]. It is one of the best coupling reagents for use in cyclization reactions, as it mediates amide bond formation with low racemization and does not cause guanidinylation of free-amino groups.

ⓘ Ⓖ 3.3

[1]   F. Albericio, et al. (1997) *Tetrahedron Lett.*, **38**, 4853.

851009	**PyBOP®**	25 g	82.00
	Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate	100 g	245.00

NBC No.: 01-62-0016; CAS No.: 128625-52-5; $C_{18}H_{28}N_6OP_2F_6$; M.W.: 520.3

Solubility: 1 mmole in 2 ml DMF clearly soluble.

HPLC: purity: ≥ 99.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; hygroscopic; keep open bottle under nitrogen.

The standard in situ coupling reagent for solid phase peptide synthesis [1-5]. This product can replace BOP in all applications without loss of performance. Unlike uronium-based reagents, PyBOP® does not give rise to guanidinated by-products during cyclization and fragment condensation reactions.

ⓘ Ⓖ 3.3

[1]   J. Martinez, et al. (1985) *J. Med. Chem.*, **28**, 1874.
[2]   J. Coste, et al. (1990) *Tetrahedron Lett.*, **31**, 205.
[3]   G. B. Fields, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 1st International Symposium", R. Epton (Eds), SPCC UK Ltd., Birmingham, 1990, pp. 241.
[4]   T. Høeg-Jensen, et al. (1991) *Tetrahedron Lett.*, **32**, 7617.
[5]   R. von Eggelkraut-Gottanka, et al. (2003) *Tetrahedron Lett.*, **44**, 3551.

COUPLING REAGENTS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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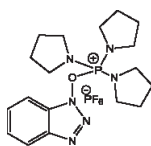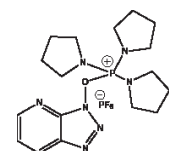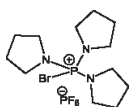
Product No.	Product	Quantity	Price
851010	**PyBroP®**	25 g	230.00
	Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate	100 g	680.00

NBC No.: 01-62-0017; CAS No.: 132705-51-2; $C_{12}H_{24}N_3P_2BrF_6$; M.W.: 466.2

Solubility: 1 mmole in 2 ml DMF clearly soluble.

HPLC: purity: $\geq$ 98.00%.

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry.

A superior reagent for rapid and effective coupling of N-methyl amino acids with low enantiomerization and for other critical applications [1-8].

The coupling of N-methyl amino acids is difficult, and usually only gives low to moderate yields of products which are often contaminated with unwanted diastereomers. Many coupling reagents are ineffective or not practical to use: BOP and HOBt are often useless; BOP-Cl, pivaloyl chloride and Dpp-Cl require long reaction times and preactivation at 0°C.

For coupling α-aminoisobutyric acid (Aib), PyBroP® is most effective when used with dimethylaminopyridine (DMAP) [3].

ⓘ Ⓖ 3.3

[1] J. Coste, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 11th American Peptide Symposium", J. E. Rivier & G. R. Marshall (Eds), ESCOM, Leiden, 1990, pp. 900.
[2] J. Coste, et al. (1990) *Tetrahedron Lett.*, **31**, 669.
[3] J. Coste, et al. in "Peptides 1990, Proc. 21st European Peptide Symposium", E. Giralt & D. Andreu (Eds), ESCOM, Leiden, 1990, pp. 76.
[4] J. Coste, et al. (1991) *Tetrahedron Lett.*, **32**, 1967.
[5] P. W. Baures, et al. (1997) *J. Peptide Res.*, **50**, 1.
[6] T. Doi, et al. (1999) *Synlett*, **11**, 1751.
[7] A. M. Boldi, et al. (2001) *J. Comb. Chem.*, **3**, 367.
[8] S. Gazal, et al. (2001) *J. Peptide Res.*, **58**, 527.
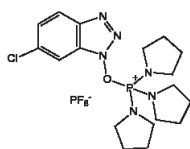
Product No.	Product	Quantity	Price
851087	**PyClocK**	25 g	102.00
	6-Chloro-benzotriazole-1-yloxy-tris-pyrrolidinophosphonium hexafluorophosphate	100 g	300.00

CAS No.: 893413-42-8; $C_{18}H_{27}ClF_6N_6OP_2$; M.W.: 554.1

Solubility: 5% in NMP clearly soluble.

HPLC: purity: $\geq$ 98.00%.

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry.

PyClocK is the 6-chloro analog of PyBOP. Reaction of protected amino acids with PyClocK in the presence of base generates the corresponding 6-chloro-1-benzotriazolyl (Cl-OBt) ester. These active esters are considerably more reactive than those produced when using PyBOP or HBTU, owing to 6-chloro-1-hydroxybenzotriazole (Cl-HOBt) being more acidic than HOBt.

PyClocK is an excellent coupling reagent for situations where carboxyl activation may be sluggish, such as cyclizations and fragment condensations, as unlike imminium-based reagents such as HBTU and HATU excess PyClocK cannot cause end-capping of peptide chains.

ⓘ Ⓖ 3.3

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Product No.	Product	Quantity	Price
851095	**PyOxim**	25 g	100.00
	1-Cyano-2-ethoxy-2-oxoethylideneaminooxy-tris-pyrrolidino-phosphonium hexafluorophosphate	100 g	360.00

$C_{17}H_{29}F_6N_5O_3P_2$; M.W.: 527.4

Solubility: 1 mmole in 2 ml DMF clearly soluble.

HPLC: purity: ≥ 99.00%.

⚠ Prolonged storage: ≤-20°C; keep cool and dry.

PyOxim [1] is a novel reagent which mediates coupling reactions with efficiencies superior to HATU and PyBOP and comparable to COMU. It is has excellent solubility in DMF and is stable in solution under an inert atmosphere for two days. Unlike HATU and HBTU, it cannot cause chain terminating side reactions and is, therefore, ideal for the synthesis of cyclic peptides. Furthermore, it is not explosive under normal operating conditions and is less likely to exhibit allergenicity compared to other coupling reagents.

ⓘ Ⓖ 3.3

[1] R. Subiros-Funosas, et al. (2010) *Org. Biomol. Chem.*, **8**, 3665.

851008	**TBTU**	25 g	40.00
	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate	100 g	120.00

NBC No.: 01-62-0015; CAS No.: 125700-67-6; $C_{11}H_{16}N_5OBF_4$; M.W.: 321.1

Solubility: 1 mmole in 2 ml DMF clearly soluble.

HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: ≤-20°C; keep cool and dry; protect from light.

See entry for HBTU (851006). TBTU has been employed to generate 1,2,4-oxadiazoles from carboxylic acids [1].

ⓘ Ⓖ 3.23

[1] R. F. Poulain, et al. (2001) *Tetrahedron Lett.*, **42**, 1495.

851090	**TFFH**	1 g	37.00
	Tetramethylfluoroformamidinium hexafluorophosphate	5 g	130.00
	Fluoro-N,N,N',N'-bis(tetramethylene)formamidinium hexafluorophosphate	25 g	390.00

HPLC: purity: ≥ 99.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen; hygroscopic.

TFFH is an in situ coupling reagents which smoothly converts protected amino acids to the corresponding acid fluoride in the presence of base [1]. Acid fluorides have proven are highly effective at coupling hindered amino acids such as α, α-disubstituted amino acids [2].

[1] L. A. Carpino, et al. (1995) *J. Am. Chem. Soc.*, **117**, 5401.

[1] S. A. Triolo, et al. in "Peptides 1996, Proc. of 24th European Peptide Symposium", R. Ramage & R. Epton (Eds), Mayflower Scientific Ltd., Birmingham, 1998, pp. 839.

COUPLING REAGENTS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

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Product No.	Product	Quantity	Price
851088	**TOTU**	25 g	102.00
	O-[(Ethoxycarbonyl)cyanomethylenamino]-N,N,N',N'-tetramethyluronium tetrafluoroborate	100 g	300.00
	CAS No.: 136849-72-4; $C_{10}H_{17}BF_4N_4O_3$; M.W.: 328.1		

Solubility: 1 mmole in 2 ml DMF clearly soluble.

HPLC: purity: ≥ 99.00%.

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry.

TOTU is an excellent alternative to HOBt-based coupling reagents such as HBTU and TBTU. It has high reactivity and is likely to have low allergenicity and low explosivity. The by-products of using this coupling reagent are water-soluble, making it an ideal choice for solution phase coupling reactions.

[1] G. Breipohl & W. Koenig, US patent 5166394.

ⓘ Ⓖ 3.3
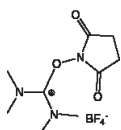
851206	**TSTU**	25 g	104.00
	O-[N-Succinimidyl)-1,1,3,3-tetramethyluronium tetrafluoroborate		
	CAS No.: 105832-38-0; $C_9H_{16}BF_4N_3O_3$; M.W.: 301.1		

⚠ **Prolonged storage:** +2 to +8°C; keep cool and dry.

HPLC: purity: ≥ 99.00%.

Useful reagent for the conversion of carboxylic acids to water soluble OSu esters [1, 2]. Ideal for the conversion of dyes and PEG building blocks to the corresponding active esters for coupling in aqueous media.

[1] W. Bannwarth, et al. (1988) *Helv. Chim. Acta*, **71**, 2085.

[2] R. Knorr, et al. (1989) *Tetrahedron Lett.*, **30**, 1927.

€

Product No.	Product	Quantity	Price

Linkers, protecting groups & other reagents

Linkers for solid phase synthesis
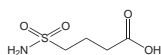
851002 **3-Carboxypropanesulfonamide** — 1 g — 54.00

4-Sulfamoyl-butyric acid — 5 g — 214.00

NBC No.: 01-60-0051; CAS No.: 175476-52-5; $C_4H_9NO_4S$; M.W.: 167.2 — 25 g — 861.00

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +15 to +25°C; keep cool and dry.

[1] G. W. Kenner, et al. (1971) *J. Chem. Soc., Chem. Commun.*, 636.
[2] B. J. Backes, et al. (1996) *J. Am. Chem. Soc.*, **118**, 3055 .
[3] R. Ingenito, et al. (1999) *J. Am. Chem. Soc.*, **121**, 11369.
[4] Y. Shin, et al. (1999) *J. Am. Chem. Soc.*, **121**, 11684.
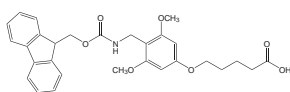
855140 **Fmoc-PAL-linker** — 1 g — 40.00

NEW

TLC: CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%. — 5 g — 160.00

HPLC: purity: ≥ 95.00%.

Fmoc-PAL-linker is an Fmoc-protected version of Barany's aminomethyl dimethoxyphenoxyvaleric acid linker [1]. It can be attached to any suitable aminofunctionalized resin to produce a support for the synthesis of peptide amides by Fmoc SPPS. Studies have shown the acid sensitivity of this linker to be around twice that of the Rink amide linker [1].

[1] F. Albericio & G. Barany (1987) Int. J. Peptide Protein Res., 30, 206.
[2] M. S. Bernatowicz, et al. (1989) Tetrahedron Lett., 30, 4645.
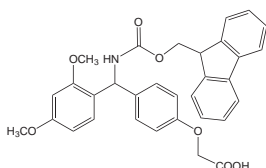
851001 **Fmoc-Rink linker** — 1 g — 29.00

p-{(R,S)-α-[1-(9H-Fluoren-9-yl)-methoxyformamido]-2,4-dimethoxybenzyl}- — 5 g — 114.00

phenoxyacetic acid — 25 g — 406.00

NBC No.: 01-60-0025; CAS No.: 145069-65-3; $C_{32}H_{29}NO_7$; M.W.: 539.6

TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

A TFA-labile trialkoxybenzhydryl-type linker ideally suited for the preparation of peptide amides using Fmoc-strategy [1].

[1] M. S. Bernatowicz, et al. (1989) *Tetrahedron Lett.*, **30**, 4645.

⚠ Storage conditions Ⓖ Additional information Ⓜ Method information Ⓐ Application information

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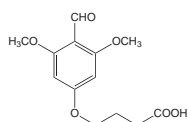
Product No.	Product	Quantity	Price
851003	**4-(4-Formyl-3,5-dimethoxyphenoxy)butyric acid**	1 g	57.00
	BAL-linker	5 g	230.00
	NBC No.: 01-60-0086; $C_{13}H_{16}O_6$; M.W.: 268.3	25 g	921.00

TLC: $CHCl_3MeOH:AcOH:H_2O$ (90:10:0.5:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

BAL-linker. Very acid sensitive linker for the preparation of carboxamides, carbamates and C-terminally modified peptides [1-4]

[1] K. J. Jensen, et al. in "Peptides: Chemistry, Structure and Biology: Proc. 14th American Peptide Symposium", P. T. P. Kaumaya & R. S. Hodges (Eds), Mayflower Scientific Ltd., 1996, pp. 30.
[2] K. J. Jensen, et al. (1998) *J. Am. Chem. Soc.*, **120**, 5441.
[3] J. Alsina, et al. (1999) *Chem. Eur. J.*, **5**, 2787.
[4] D. Fernández-Forner, et al. (2002) *Tetrahedron Lett.*, **43**, 3543.
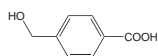
851042	**HMBA**	5 g	94.00
	4-Hydroxymethylbenzoic acid	25 g	374.00
	NBC No.: 01-60-0007; CAS No.: 3006-96-0; $C_8H_8O_3$; M.W.: 152.2		

TLC: $CHCl_3:MeOH:AcOH$ (90:8:2), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Base-labile, TFA-stable linker for Fmoc SPPS. Peptide release can be effected with a variety of nucleophiles to generate peptides with various C-terminal carboxy modifications.
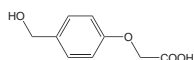
851000	**HMPA**	5 g	99.00
	4-Hydroxymethylphenoxyacetic acid	25 g	393.00
	NBC No.: 01-60-0017; CAS No.: 68858-21-9; $C_9H_{10}O_4$; M.W.: 182.2		

TLC: $CH_3CN:CHCl_3:AcOH$ (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Standard TFA-labile linker for the preparation of peptide acids by Fmoc SPPS.
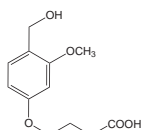
851046	**HMPB**	1 g	52.00
	4-(4-Hydroxymethyl-3-methoxyphenoxy)-butyric acid	5 g	208.00
	NBC No.: 01-60-0029; CAS No.: 136849-75-7; $C_{12}H_{16}O_5$; M.W.: 240.3	25 g	832.00

TLC: $CHCl_3MeOH:AcOH:H_2O$ (90:10:0.5:1), purity: ≥ 99.00%.

$CH_3CN:CHCl_3:AcOH$ (8:1:1), purity: ≥ 99.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

A 1% TFA-labile dialkoxybenzyl linker for the preparation of protected peptides by the Fmoc strategy [1]. This linker gives purer peptides compared to Sheppard's original 4-hydroxymethyl-3-methoxyphenoxyacetic acid due to its increased acid sensitivity.

[1] A. Flörsheimer, et al. in "Peptides 1990, Proc. 21st European Peptide Symposium", E. Giralt & D. Andreu (Eds), ESCOM, Leiden, 1991, pp. 131.

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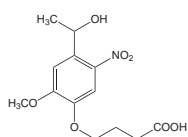
Product No.	Product	Quantity	Price
851049	**Hydroxyethyl photolinker**	1 g	185.00
	4-[4-(1-Hydroxyethyl)-2-methoxy-5-nitrophenoxy]butanoic acid	5 g	740.00

NBC No.: 01-60-0043; CAS No.: 175281-76-2; $C_{13}H_{17}NO_7$; M.W.: 299.3

HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: +15 to +25°C; keep cool and dry; protect from light.

A photocleavable linker for the preparation of carboxylic acids.

[1] C. P. Holmes, et al. (1995) *J. Org. Chem.*, **60**, 2318.

Reagents for the introduction of protecting groups

Product No.	Product	Quantity	Price
851210 NEW	**1-Acetylimidazole**	25 g	30.00
	CAS No.: 2466-76-4; $C_5H_6N_2O$; M.W.: 110.1	100 g	90.00

Assay: purity: ≥ 97.50%.

solubility: 1 mmole in 2 ml DMF.

Reagent for acetylation of amines under mild conditions.

851061	**2-Chlorotrityl chloride**	25 g	114.00

NBC No.: 01-63-0045; CAS No.: 42074-68-0; $C_{19}H_{14}Cl_2$; M.W.: 313.2

TLC: EtOAc:Hexane (1:4), purity: ≥ 98.00%.

$CHCl_3$: EtOAc (9:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +15 to +25°C; bottle must always be kept tightly closed when cold and must be allowed to reach room temperature before opening; use only with dried solvents in dry glassware and apparatus.

Hyperacid-labile ester protecting group [1]. Introduced under extremely mild conditions by reacting together the acid, trityl chloride and DIPEA in DCM. Ideal for the protection of the C-terminal carboxylic acid group of C-terminal protected peptide fragments, monoprotection of diacids and as a general carboxylic acid protecting group in organic synthesis.

[1] P. Athanassopoulos, et al. (1995) *Tetrahedron Lett.*, **36**, 5645.
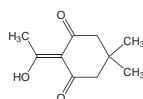
851015	**Dde-OH**	5 g	122.00
	2-Acetyldimedone	25 g	488.00
	NBC No.: 01-63-0033; CAS No.: 94142-97-9; $C_{10}H_{14}O_3$; M.W.: 182.2	100 g	1464.00

TLC: EtOAc:Hexane (1:4), purity: ≥ 98.00%.

CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Reagent for the introduction of the Dde protecting group [1-4].

[1] B. W. Bycroft, et al. (1993) *J. Chem. Soc., Chem. Commun.*, 778.

[2] I. A. Nash, et al. (1996) *Tetrahedron Lett.*, **37**, 2625.

[3] F. Berst, et al. (2000) *Tetrahedron Lett.*, **41**, 6649.

[4] A. N. Acharaya, et al. (2001) *J. Comb. Chem.*, **3**, 612.

⚠ Storage conditions Ⓖ Additional information Ⓜ Method information Ⓐ Application information
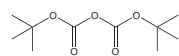
Product No.	Product	Quantity	€ Price
852261	**DiBoc**	25 g	18.00
	Di-tert-butyl dicarbonate	100 g	52.00
	Di-tert-butyl pyrocarbonate		
	CAS No.: 24424-99-5; $C_{10}H_{18}O_5$; M.W.: 218.3		
	GC: purity: ≥ 98.00%.		
	Reagent for introduction of the Boc protecting group .		
851062	**Dmab-OH**	1 g	38.00
	4-{N-[1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino}	5 g	150.00
	benzyl alcohol	25 g	599.00
	NBC No.: 01-63-0048; CAS No.: 172611-73-3; $C_{20}H_{27}NO_3$; M.W.: 329.4		
	TLC: CHCl$_3$:MeOH (9:1), purity: ≥ 98.00%.		
	CHCl$_3$MeOH:AcOH:H$_2$O (90:10:0.5:1), purity: ≥ 98.00%.		
	HPLC: purity: ≥ 98.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
	Reagent for the introduction of Dmab carboxylic acid protecting group. Dmab esters are stable to TFA and piperidine, but are cleaved under mild conditions by treatment with 2% hydrazine in DMF.		
852259	**Fmoc–Cl**	25 g	42.00
	(9-Fluorenylmethyl) chloroformate	100 g	125.00
	Chloroformic acid 9-fluorenmethyl ester		
	CAS No.: 28920-43-6; $C_{15}H_{11}ClO_2$; M.W.: 258.7		
	HPLC: purity: ≥ 98.00%.		
	Reagent for introduction of the Fmoc protecting group [1].		
	[1] L.A.Carpino et al. (1987) *Acc. Chem. Res.*, **20**, 401.		
851014	**Fmoc–OSu**	5 g	23.00
	N-(9-Fluorenylmethoxycarbonyloxy)succinimide	25 g	89.00
	9-Fluorenylmethyl-succinimidyl carbonate	100 g	260.00
	NBC No.: 01-63-0001; CAS No.: 82911-69-1; $C_{19}H_{15}NO_5$; M.W.: 337.3		
	solubility: 1 mmole in 2 ml DMF.		
	HPLC: purity: ≥ 99.00%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
	Reagent for the preparation of pure Fmoc-amino acids that are free from contamination by Fmoc-dipeptides [1-3].		
	[1] A. Paquet (1982) *Can. J. Chem.*, **60**, 976.		
	[2] L. Lapatsanis, et al. (1983) *Synthesis*, 671.		
	[3] G. F. Sigler, et al. (1983) *Biopolymers*, **22**, 2157.		
851094	**Fmoc–oxyma**	5 g	40.00
	$C_{20}H_{16}N_2O_5$; M.W.: 364.4	25 g	160.00
	HPLC: purity: ≥ 99.50%.		
	⚠ Prolonged storage: +2 to +8°C; keep cool and dry.		
	A novel derivative for introduction of Fmoc protecting group to hindered amino acids with negligible formation of β-alanyl related impurities [1].		
	[1] S. N. Khattab, et al. (2010) *Eur. J. Org. Chem.*, 3275.		
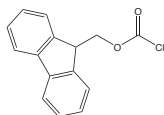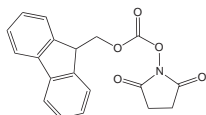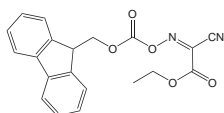
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€
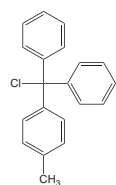
Product No.	Product	Quantity	Price
851060	**4–Methyltrityl chloride**	25 g	125.00

NBC No.: 01-63-0044; CAS No.: 23429-44-9; $C_{20}H_{17}Cl$; M.W.: 292.8

TLC: EtOAc:Hexane (1:4), purity: ≥ 95.00%.

$CHCl_3$: EtOAc (9:1), purity: ≥ 95.00%.

⚠ Prolonged storage: ≤-20°C; bottle must always be kept tightly closed when cold and must be allowed to reach room temperature before opening; use only with dried solvents in dry glassware and apparatus.

Hyperacid-labile protecting group. Introduced under extremely mild conditions by treating the amine or thiol containing compound with trityl chloride and DIPEA in DCM.
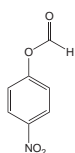
851201	**4–Nitrophenyl formate**	1 g	10.00
		5 g	26.00
		25 g	104.00

CAS No.: 1865-01-6; $C_7H_5NO_4$; M.W.: 167.1

GC: purity: ≥ 89.00%.

⚠ Prolonged storage: ≤-20°C; keep cool and dry.

Reagent for introduction of N-terminal formyl group.
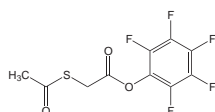
851016	**SAMA-OPfp**	1 g	51.00
		5 g	205.00

S-Acetylthioglycolic acid pentafluorophenyl ester

NBC No.: 01-63-0041; CAS No.: 129815-48-1; $C_{10}H_5F_5O_3S$; M.W.: 300.1

TLC: Toluene:Dioxane:AcOH (95:25:4), purity: ≥ 98.00%.

⚠ Prolonged storage: ≤-20°C; keep cool and dry.

This reagent [1] provides an effective means of linking synthetic peptide antigens to MAP core peptides [2] or carrier proteins for the purpose of raising antibodies [3]. Using this technique many of the problems associated with the analysis and purification of MAPs are avoided, since the linear peptide antigen can be fully characterized before conjugation to the preformed lysine tree.

ⓘ Ⓖ 5.7, Ⓜ 5.7

[1]   J. W. Drijfhout, et al. (1990) *Anal. Biochem.*, **187**, 349.

[2]   J. W. Drijfhout (1991) *Int. J. Peptide Protein Res.*, **37**, 27.

[3]   H. F. Brugghe, et al. (1994) *Int. J. Peptide Protein Res.*, **43**, 166.
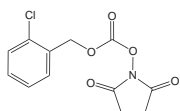
851057	**Z(2-Cl)-OSu**	5 g	25.00
		25 g	100.00
		100 g	300.00

N-(2-Chlorobenzyloxycarbonyloxy)succinimide

NBC No.: 01-63-0003; CAS No.: 65853-65-8; $C_{12}H_{10}ClNO_5$; M.W.: 283.7

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

A reagent for the mild introduction of the Z(2-Cl)-amino protecting group.

LINKERS PROTECTING GROUPS & OTHER REAGENTS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

€

Product No.	Product	Quantity	Price

Other reagents
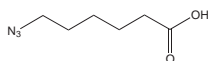
851097 | **ε-Azidocaproic acid** | 1 g | 130.00
5 g | 520.00

CAS No.: 79598-53-1; $C_6H_{11}N_3O_2$; M.W.: 157.2

GC: purity: ≥ 98.00%.

⚠ Prolonged storage: ≤-20°C; keep cool and dry.
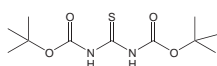
851080 | **Bis-Boc-thiourea** | 1 g | 78.00
5 g | 307.00

NBC No.: 01-63-0135; CAS No.: 145013-05-4; $C_{11}H_{20}N_2O_4S$; M.W.: 276.4

TLC: EtOAc:Hexane (1:4), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

Reagent for the guanidation of amines.

851018 | **Fmoc-NCS** | 5 g | 152.00
25 g | 610.00
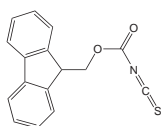
Fmoc-isothiocyanate

NBC No.: 01-63-0086; CAS No.: 199915-38-3; $C_{16}H_{11}NO_2S$; M.W.: 281.3

HPLC: purity: ≥ 94.00%.

⚠ Prolonged storage: ≤-20°C; keep cool and dry; keep open bottle under nitrogen.

This reagent reacts with amines to generate an Fmoc-protected thiourea [1-5]. Removal of the Fmoc group with 20% piperidine in DMF, followed by alkylation with iodomethane, results in formation of a S-methyl thiourea that can be reacted with amines to give the corresponding guanidine [1].

[1]   P. C. Kearney, et al. (1998) *Tetrahedron Lett.*, **39**, 2663.
[2]   P. C. Kearney, et al. (1998) *J. Org. Chem.*, **63**, 196.
[3]   M. Fu, et al. (1999) *Org. Lett.*, **1**, 1351.
[4]   A. Gopalsamy & H. Yang (2001) *J. Comb. Chem.*, **3**, 278.
[5]   M. Grimstrup & F. Zaragoza (2002) *Eur. J. Org. Chem.*, 2953.

855043 | **IBX polystyrene** | 1 g | 70.00
5 g | 280.00
25 g | 1120.00
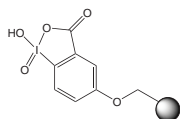
NBC No.: 01-64-0445

Loading: 0.90 - 1.50 mmole/g resin; as determined by elemental analysis of iodine). The polymer matrix is copoly (styrene - 1% DVB) 200 -400 mesh.

0.90 - 1.50 mmole/g resin; oxidation of cinnamyl alcohol to cinnamaldehyde (2 eq. IBX PS). The polymer matrix is copoly (styrene - 1% DVB) 200 -400 mesh.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

This polymer-bound version of IBX cleanly converts primary and secondary alcohols to the corresponding aldehydes and ketones [1], 2. It is particularly useful for the synthesis of peptide aldehydes as it oxidises peptide alcohols efficiently and spent resin can be simply removed by filtration.

[1]   G. Sorg, et al. (2001) *Angew. Chem. Int. Ed.*, **40**, 4395.
[2]   G. Sorg, et al. (2005) *J. Pept. Sci.*, **11**, 147.

Solvents and cleavage reagents

High purity and quality are major factors for successful syntheses. Merck offers a complete range of solvents and reagents tailor made for the needs of peptide chemistry.

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Synthesis solvents & reagents
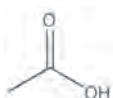
818755   **Acetic acid 99–100% (for synthesis)**
Acetic acid glacial, Methane carboxylic acid, Methylformic acid
CAS No.: 64-19-7; $C_2H_4O_2$; M.W.: 60.1
Assay: purity: ≥ 99.00%.
Density: 1.05 g/ml
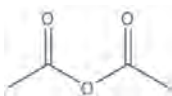
822278   **Acetic anhydride (for synthesis)**
CAS No.: 108-24-7; $C_4H_6O_3$; M.W.: 102.1
GC: purity: ≥ 98.00%.
Density: 1.08 g/ml
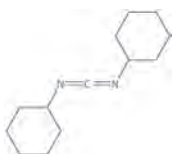
802954   **N,N'–Dicyclohexylcarbodiimide (for synthesis)**
DCC, DCCI
CAS No.: 538-75-0; $C_{13}H_{22}N_2$; M.W.: 206.3
GC: purity: ≥ 99.00%.
Density: 0.95 g/ml
Classic carbodiimide coupling reagent producing insoluble DCU.
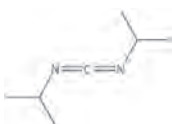
803649   **N,N'–Diisopropylcarbodiimide (for synthesis)**
DIC, DIPCDI
CAS No.: 693-13-0; $C_7H_{14}N_2$; M.W.: 126.2
GC: purity: ≥ 99.00%.
Density: 0.81 g/ml
Classic carbodiimide coupling reagent for use in SPPS as produces soluble urea.

SOLVENTS & CLEAVAGE REAGENTS

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

Product No.	Product	Quantity	Price
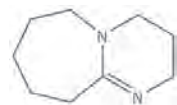
803282

1,8-Diazabicyclo[5.4.0]undec-7-ene (for synthesis)

Octahydropyrimido(1,2:a)azepine, DBU

CAS No.: 6674-22-2; $C_9H_{16}N_2$; M.W.: 152.2

GC: purity: ≥ 98.00%.

Density: 1.02 g/ml

DBU/piperidine/DMF 1:1:48 is a highly effective reagent for removal of Fmoc group from hindered and aggregated peptides.

106051

Dichloromethane (dried (max. 0,004% H2O) SeccoSolv®)

Methylene chloride, Chloromethylene

CAS No.: 75-09-2; CH_2Cl_2; M.W.: 85.0

GC: purity: ≥ 99.90%.

Acidity: ≤ 0.0005 meq/g

Alkalinity: ≤ 0.0002 meq/g

Density: 1.33 g/ml
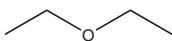
100923

Diethyl ether (Emplura®)

CAS No.: 60-29-7; $C_4H_{10}O$; M.W.: 74.1

GC: purity: ≥ 99.00%.

Density: 0.71 g/ml
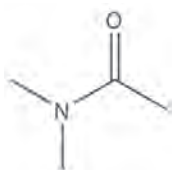
803235

N,N-Dimethylacetamide (for synthesis)

Acetic acid dimethylamide

CAS No.: 127-19-5; C_4H_9NO; M.W.: 87.1

GC: purity: ≥ 99.00%.

Density: 0.94 g/ml
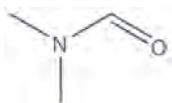
100397

N,N-Dimethylformamide for peptide synthesis

DMF

CAS No.: 68-12-2; C_3H_7NO; M.W.: 73.1

GC: purity: ≥ 99.00%.

Amine: ≤ 10 ppm

Acidity: ≤ 0.0005 meq/g

Alkalinity: ≤ 0.0002 meq/g

Density: 0.94 g/ml
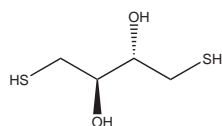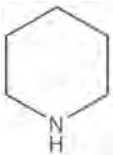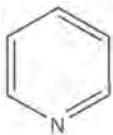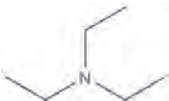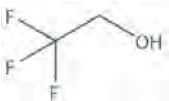
124511

1,4-Dithioerthyritol

DTE

Cleland's reagent

CAS No.: 6892-68-8; $C_4H_{10}N_2S_2$; M.W.: 154.2

Product No.	Product	Quantity	Price

111474

1,4-Dithiothreitol

DTT

Cleland's reagent

CAS No.: 6892-68-8; $C_4H_{10}N_2S_2$; M.W.: 154.2

800894

N-Ethyldiisopropylamine (for synthesis)

N,N-Diisopropylethylamine, DIPEA

CAS No.: 7087-68-5; $C_8H_{19}N$; M.W.: 129.3

GC: purity: ≥ 98.00%.

Density: 0.76 g/ml

Standard hindered tertiary amine base used with HBTU or PyBOP activation.

804515

1,1,1,3,3,3-Hexafluoro-2-propanol (for synthesis)

1,1,1,3,3,3-Hexafluoroisopropyl alcohol, Hexafluoroisopropanol, HFIP

CAS No.: 920-66-1; $C_3H_2F_6O$; M.W.: 168.0

GC: purity: ≥ 99.00%.

Density: 1.62 g/ml

Excellent solvent for hydrophobic peptides and protected peptide fragments. 30% HFIP in DCM is also useful for cleaving protected peptide fragments from 2-chlorotrityl resin.

804608

Hydrazinium hydroxide (for synthesis)

Hydrazine hydrate

CAS No.: 7803-57-8; H_6N_2O; M.W.: 50.1

Assay: purity: ≥ 99.00%.

Density: 1.03 g/ml

2% Hydrazine hydrate in DMF removes ivDde or Dde amino-protecting groups.

805894

4-Methylmorpholine (for synthesis)

NMM, N-Methylmorpholine

CAS No.: 109-02-4; $C_5H_{11}NO$; M.W.: 101.2

GC: purity: ≥ 98.00%.

Density: 0.92 g/ml

100574

1-Methyl-2-pyrrolidone (for peptide synthesis)

N-Methyl-2-pyrrolidinone, NMP

CAS No.: 872-50-4; C_5H_9NO; M.W.: 99.1

Density: 1.03 g/ml

SOLVENTS & CLEAVAGE REAGENTS

⚠ Storage conditions   **G** Additional information   **M** Method information   **A** Application information

Product No.	Product	Quantity	Price

822299

Piperidine (for synthesis)

Hexahydropyridine, Pentamethyleneimine
CAS No.: 110-89-4; $C_5H_{11}N$; M.W.: 85.2
GC: purity: ≥ 99.00%.
Density: 0.86 g/ml
Standard reagent for removal of Fmoc groups.

107462

Pyridine (for synthesis)

CAS No.: 110-86-1; C_5H_5N; M.W.: 79.1
GC: purity: ≥ 99.00%.
Density: 0.98 g/ml

808352

Triethylamine (for synthesis)

N,N-Diethylethanamine, TEA
CAS No.: 121-44-8; $C_6H_{15}N$; M.W.: 101.2
GC: purity: ≥ 99.00%.
Density: 0.73 g/ml

808259

2,2,2-Trifluoroethanol (for synthesis)

ß,ß,ß-Trifluoroethyl alcohol, TFE
CAS No.: 75-89-8; $C_2H_3F_3O$; M.W.: 100.0
GC: purity: ≥ 99.00%.
Density: 1.38 g/ml
Excellent solvent for protected peptide fragments. 20% TFE in DCM can also be
used to cleave protected peptide fragments from 2-chlorotrityl resin.

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Product No.	Product	Quantity	Price

Purification solvents
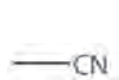
	100030	**Acetonitrile (gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur)**	

Methyl cyanide

CAS No.: 75-05-8; C_2H_3N; M.W.: 41.1

GC: purity: ≥ 99.90%.

Density: 0.79 g/ml
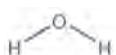
	115333	**Water (for chromatography LiChrosolv®)**	

CAS No.: 231-791-2; H_2O; M.W.: 18.0

Density: 1.00 g/ml

Cleavage reagents
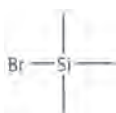
	814324	**Bromotrimethylsilane (for synthesis)**	

Trimethylbromosilane, Trimethylsilyl bromide, TMSBr

CAS No.: 2857-97-8; C_3H_9BrSi; M.W.: 153.1

Assay: purity: ≥ 98.00%.

Density: 1.17 g/ml

1 M TMSBr in TFA effectively cleaves Mtr protecting groups from Arg without modification of Trp residues,

	800795	**1,2-Ethanedithiol (for synthesis)**	

1,2-Dimercaptoethane, Dithioglycol, Dithioethylene glycol, Ethylene dimercaptan, EDT

CAS No.: 540-63-6; $C_2H_6S_2$; M.W.: 94.2

GC: purity: ≥ 99.00%.

Density: 1.12 g/ml

Highly effective scavenger for protection of Cys and Met residues.
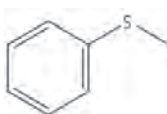
	820825	**Methyl phenyl sulfide (for synthesis)**	

Thioanisole

CAS No.: 100-58-5; C_7H_8S; M.W.: 124.2

GC: purity: ≥ 99.00%.

Density: 1.05 g/ml

Soft nucleophile catalyst for accelerating removal of protecting groups during TFMSA and TFA-mediated cleavage reactions.

⚠ Storage conditions   Ⓖ Additional information   Ⓜ Method information   Ⓐ Application information

Product No.	Product	Quantity	Price
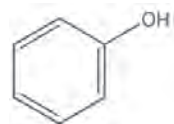
822296 — **Phenol (for synthesis)**
CAS No.: 108-95-2; C_6H_6O; M.W.: 94.1
GC: purity: $\geq$ 99.00%.
Density: 1.06 g/ml
Scavenger for protection of Tyr residues.
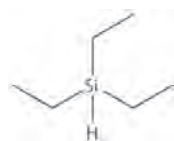
818806 — **Triethylsilane (for synthesis)**
TES
CAS No.: 617-86-7; $C_6H_{16}Si$; M.W.: 116.3
GC: purity: $\geq$ 99.00%.
Density: 0.73 g/ml
Highly effective scavenger for Trt and tBu cations. May cause reduction of Trp residues.
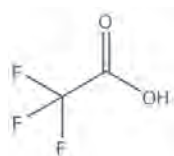
808260 — **Trifluoroacetic acid (for synthesis)**
TFA
CAS No.: 76-05-1; $C_2HF_3O_2$; M.W.: 114.0
Assay: purity: $\geq$ 99.00%.
Density: 1.48 g/ml
Highly quality TFA for cleavage of peptide prepared by Fmoc SPPS.
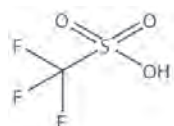
821166 — **Trifluoromethanesulfonic acid (for synthesis)**
TFMSA
CAS No.: 1493-13-6; CHF_3O_3S; M.W.: 150.1
Assay: purity: $\geq$ 98.00%.
Density: 1.71 g/ml
TFSMA is a strong acid replacement for HF in Boc SPPS.
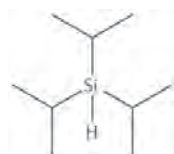
841359 — **Triisopropylsilane (for synthesis)**
TIPS
CAS No.: 6485-79-6; $C_9H_{22}Si$; M.W.: 158.4
GC: purity: $\geq$ 98.00%.
Density: 0.77 g/ml
Highly effective scavenger for Trt and tBu cations.
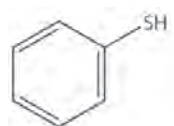
808159 — **Thiophenol (for synthesis)**
Phenyl mercaptan, Mercaptobenzene
CAS No.: 108-98-5; C_6H_6S; M.W.: 110.2
GC: purity: $\geq$ 99.00%.
Density: 1.08 g/ml

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Synthesis Notes

edited by P. White

1: Introduction and background

1.1   Solid phase peptide synthesis

Solid phase peptide synthesis (SPPS) is based on sequential addition of α-amino and side-chain protected amino acid residues to an insoluble polymeric support (Figure 1-1). The acid-labile Boc group or base-labile Fmoc-group is used for N-α-protection. After removal of this protecting group, the next protected amino acid is added using either a coupling reagent or pre-activated protected amino acid derivative. The resulting peptide is attached to the resin, *via* a linker, through its C-terminus and may be cleaved to yield a peptide acid or amide, depending on the linking agent used. Side-chain protecting groups are often chosen so as to be cleaved simultaneously with detachment of the peptide from the resin.

Cleavage of the Boc protecting group is achieved by trifluoroacetic acid (TFA) and the Fmoc protecting group by piperidine. Final cleavage of the peptidyl resin and side-chain deprotection requires strong acid, such as hydrogen fluoride (HF) or trifluoromethanesulfonic acid (TFMSA), in the case of Boc chemistry, and TFA in Fmoc chemistry. Dichloromethane (DCM) and N,N-dimethylformamide (DMF) are the primary solvents used for resin deprotection, coupling and washing.

Peptide synthesis can be carried out in a batch-wise or continuous flow manner. In the former technique, the peptidyl resin is contained in a filter reaction vessel and reagents added and removed under manual or computer control. In the continuous flow method, the resin is contained in a column through which reagents and solvents are pumped continuously, again under manual or automatic control. A range of manual, semi-automatic or automatic synthesizers are commercially available for both batch-wise or continuous flow methods [see 1 for an overview]. Only the Fmoc strategy is fully compatible with the continuous flow method, which, depending on the instrument used, allows for real-time spectrophotometric monitoring of the progress of coupling and deprotection [2, 3].

For general accounts of solid phase methods, the following reviews are recommended [4 - 14].
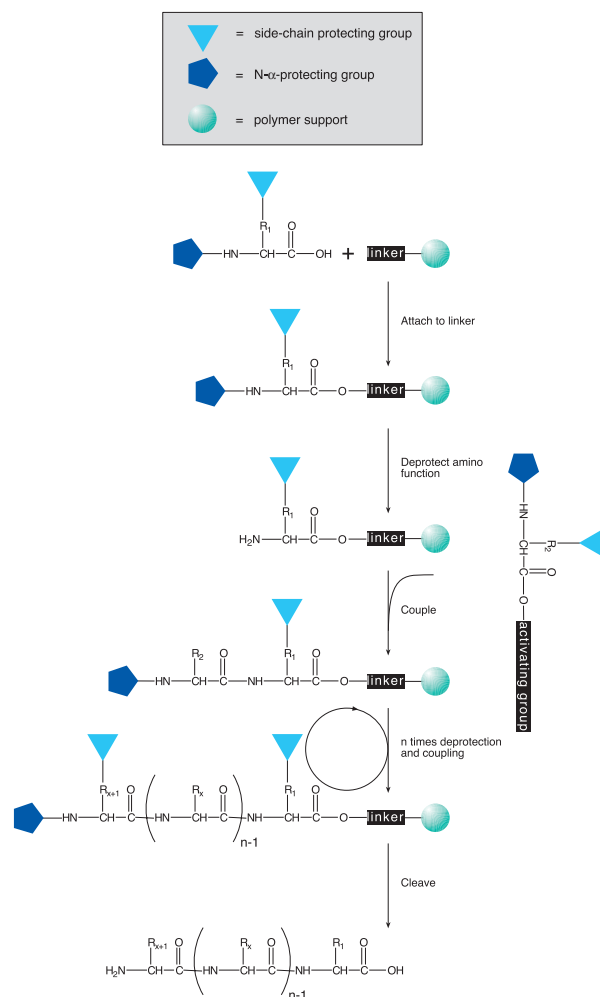
Fig. 1-1: General scheme of SPPS.

1.2   Fmoc and Boc compared

The development of Fmoc SPPS arose out of concern that repetitive TFA acidolysis in Boc-group deprotection could lead to alteration of sensitive peptide bonds as well as acid catalyzed side reactions. In Fmoc synthesis, the growing peptide is subjected to mild base treatment using piperidine during Fmoc-group deprotection and TFA is required only for the final cleavage and deprotection of peptidyl resin (Figure 1-2). By contrast, cleavage and deprotection in Boc strategy requires the use of dangerous HF and expensive laboratory apparatus which is not always readily available to many researchers.

Fmoc

Boc
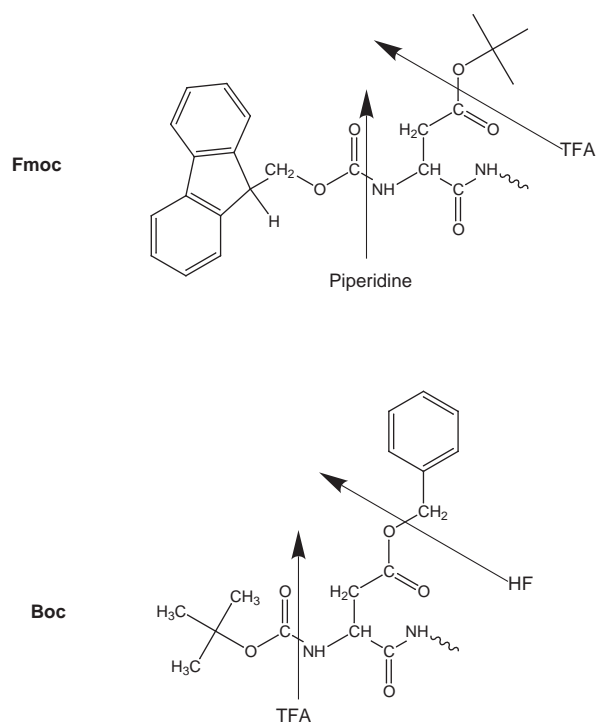
Fig. 1-2: Protecting group strategies in SPPS.

The value and usefulness of Boc chemistry can be easily found in most SPPS textbooks and papers, and any comparison done here is not intended to disclaim the merits of Boc strategy in SPPS.

An unprecedented survey paper by the Association of Biomolecular Research Facilities (ABRF) involving 40 of its member laboratories describes the synthesis, using Boc or Fmoc chemistry, of a peptide containing sixteen amino acids [9]. This test peptide has potential sites for post-synthesis modification and multiple sites for problematic or slow couplings. The members were asked to provide a crude peptide for analysis by different methods. The results very much proved the superiority of the Fmoc strategy. Over 33% of the crude cleavage

products made using Boc chemistry did not contain any of the desired peptide and over 44% of the Boc derived peptides were unable to achieve greater than 25% purity. In contrast, 31% of the samples made using the Fmoc procedure had over 75% of the desired compound.

The purity of the best peptides made by Boc chemistry was comparable with that of the best made by Fmoc chemistry. This suggests that, in skilled and experienced hands, either method can give good results. However, the results do indicate that Fmoc-based synthesis in the hands of the "average user" can be far more accessible and more likely to provide the best avenue for routine synthesis of peptides. Readers are encouraged to read the review of Fields, *et al.* [8] for more specific information on Fmoc chemistry. Figure 1-3 shows the protecting group and cleavage strategy for Fmoc chemistry.

References

1. L. E. Cammish & S. A. Kates in "Fmoc solid phase peptides sythesis: a practical approach", W. C. Chan & P. D. White (Eds.), Oxford University Press, Oxford, 2000, pp. 277.
2. S.A. Salisbury, et al. (1990) *J.Chem. Soc., Chem. Commun.*, 538.
3. S.C. Young, et al. (1990) *Biochem. Soc. Trans.*, **18**, 131.
4. J. Stewart & J. Young, "Solid phase peptide synthesis", Pierce Chemical Company, Rockford, 1984.
5. E. Atherton & R. C. Sheppard, "Solid phase peptide synthesis: a practical approach", IRL Press, Oxford, 1989.
6. "Methods in molecular biology, Vol. 35: Peptide synthesis protocols", M.W. Pennington & B.M. Dunn (Eds.), Humana Press, Totowa, 1994, pp. 91.
7. "Synthetic peptides", G. Grant (Ed.), W.H. Freeman & Co., New York, 1992.
8. G.B. Fields, et al. (1990) *Int. J. Peptide Protein Res.*, **35**, 161.
9. A. J. Smith, et al. in "Techniques in protein chemistry III", R. Angeletti (Ed.), Academic Press, Orlando, 1992, pp. 219.
10. "Method in enzymology: Vol. 289", G. B. Fields (Eds.), Academic Press, New York, 1997.
11. W. C. Chan & P. D. White, "Fmoc solid phase peptide synthesis: a practical approach", Oxford University Press, Oxford, 2000.
12. "Chemical appraches to the synthesis of peptides and proteins", P. Lloyd-Williams & F. Albericio (Eds.), CRC Press, New York, 1997.
13. N. L. Benoiton, "Chemistry of Peptide Synthesis", CRC Press, New York, 2006.
14. "Methods in molecular biology, Vol. 298: Peptide synthesis and applications", J. Howl (Ed.), Humana Press, Totwa, 2005.

Fig. 1-3: General protecting group and cleavage strategy for Fmoc chemistry.
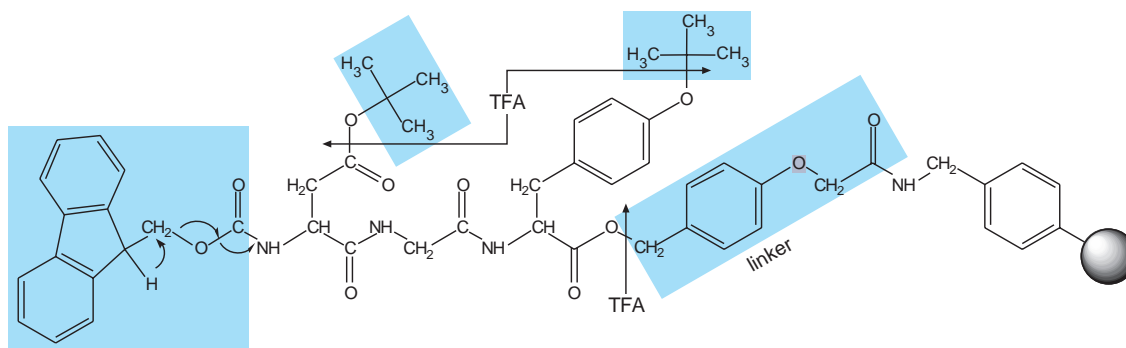
2: Resins for solid phase synthesis

2.1   Resins for peptide synthesis

Novabiochem® has one of the most extensive ranges of polymer-supports for solid phase peptide synthesis. They range from high-loaded, low swelling for the large scale production of relatively short peptides to high-swelling, low-loaded for the synthesis of long or difficult sequences.

For peptide synthesis, the use of small particle-sized resins of low cross-linking is favored. Such resins allow for rapid diffusion of reagents inside the beads and their swelling enables them to better accommodate the bulk of the growing peptide chain. The most commonly used resins are based on 1% divinylbenzene-crosslinked polystyrene. These are relatively low-cost, easy-to-handle, and have high substitution. They are normally employed for batch-wise synthesis, with gas-bubbling, shaking, paddle stirring, or vessel inversion used for mixing. However, they can also be used in continuous flow synthesis, provided a low flow-rate is used or the resin is co-packed with glass beads, although for this application NovaSyn® TG, NovaGel™, NovaPEG, or PEGA composite resins are preferred. Here, the solvation of the PEG confers pressure stability on the polymer, making them suitable for use in pumped-flow systems.

For the synthesis of long peptides and sequences prone to aggregation, it is generally regarded that better results are obtained if more polar supports based on PEG or polyacrylamide are used instead of polystyrene. A wide variety are available ranging from PEG-polystyrene composites (NovaSyn® TG, NovaSyn® TG^R and NovaGel™), PEG-cross-linked

polyethylene (NovaPEG), or PEG-acrylamide (PEGA). In a recent comparative study [1], NovaSyn® TG^R was found to perform the best of resins tested in conventional and microwave synthesis (Table 2-1).

For large scale production, a very high load polar support based on cross-linked polylysine (SpheriTide®) has been developed. The base poymer has a loading of 3 mmol/g, swells well in solvents ranging from DMF to water, and offers a high substitution to bed volume ratio.

A guide to the selection of supports is given in Table 2-2. More detailed information on each polymer is provided in the following sections.

Table 2-1. Comparison of NovaSyn TG, NovaPEG and Polystyrene resins.

Resin	Conditions	LysM1 54mer	GRP 31mer	JR 10mer
NovaSyn TG^R	MW 75 °C 10 min	68	71	34
NovaSyn TG	MW 75 °C 10 min	62	67	28
NovaPEG	MW 75 °C 10 min	58	65	28
Polystyrene	MW 75°C 10 min	37	47	15
NovaSyn TG^R	RT 2h	33	26	20
NovaSyn TG	RT 2h	28	24	17
NovaPEG	RT 2h	27	23	10
Polystyrene	RT 2h	2	16	2

Table 2-2. Properties of base matrices for SPPS.

	Composition	Bead size (μm)/ mesh	Loading	DMF	H$_2$O	DCM	Application	Comments
Polystyrene	Styrene cross-linked with divinylbenzene	75 - 150/ 100 - 200	0.5 -1.0	3	0	7	Routine and large scale synthesis	Most cost-effective but can fail on synthesis of difficult or long sequences
NovaSyn® TG	PEG grafted on polystyrene	90/160	0.2 – 0.3	5	4	5	Research scale medium to long peptides	Pressure resistant, thus ideal for continuous flow
NovaSyn® TG^R	PEG grafted on polystyrene	90/160	0.2 – 0.3	5	4	5	Research scale medium to long peptides	Special formulation of NovaSyn TG resin which gives even better results for long peptides . Works particularly well under microwave heating
PEGA	Polyacrylamide-PEG copolymer	150 - 300/ 100 - 300	0.2 – 0.4	11	16	13	On-bead enzyme assays	Internal bead space accessible to many proteins
NovaGel™ (Champion)	PEG grafted on polystyrene	75 - 150/ 100 - 200	0.6 – 0.8	7	n/d	n/d	Synthesis of medium length peptides	High-loading and high PEG content make it ideal for preparing medium-length peptides in quantity.
NovaPEG (ChemMatrix)	Polyethene cross-linked with PEG	75 - 100/ 100 - 200	0.4 – 0.6	8	11	13	Long or difficult peptides	Quality of long peptides excellent but yields often low
SpheriTide	Polylysine cross-linked with sebacic acid	250 - 350/ 45 - 60	2.7 – 3.3	5	7	2	Large scale synthesis	Very high loading to swell ratio, thus saves on reagent and solvent usage

Table 2-3. Properties of base matrices for SPOC.

Resin	Beads/g	Loading	Swelling					
		pmol/bead	DMF	THF	MeOH	Tol.	H_2O	DCM
Polystyrene								
(200-400 mesh)	$4-10 \times 10^6$	40-320	3	8	2	7	-	7
Polystyrene								
(100-200 mesh)	$0.6-2 \times 10^6$	320-2560	3	8	2	7	-	7
TG (90μm)	2.9×10^6	80-100	5	6	4	5	4	5
TG (130μm)	9×10^5	280-330	5	6	4	5	4	5
PEGA	$1-1.5 \times 10^5$	n/d	11	13	13	12	16	13
NovaGel	n/d	n/d	7	7.5	4	n/d	n/d	n/d
NovaPEG	n/d	n/d	8	n/d	9	n/d	11	13

2.2 Resins for solid phase organic synthesis

Solid phase synthesis of small organic molecules has several advantages over solution methods:

a) reactions can be driven to completion through the use of excess reagents;
b) excess reagents and soluble by-products can be simply removed by resin washing;
c) physical losses are minimal since the product remains attached to the polymer throughout synthesis;
d) the process is highly amenable to automation;
e) pseudo-dilution phenomena can be exploited for cyclization and mono-derivatization of bifunctional molecules;
f) reactions which exhibit poor chemoselectivity can often be directed by attachment of the appropriate component to the solid support to give only the desired product;
g) resin-bound toxic or hazardous compounds can be handled safely without risk to users or the environment;
h) single resin beads can function as "micro-reactors", thus enabling large numbers of compounds to be prepared simultaneously by parallel or serial combinatorial methods.

Novabiochem® synthesis supports are based on the matrices given in Table 2-3.

For routine solid phase organic synthesis polystyrene, NovaSyn® TG and NovaGel™ resins are particularly recommended. These supports are chemically robust and are compatible with a wide range of reaction conditions. A cross-section of reagents and conditions that have been employed with these supports is given below:

Acids: HF, TFMSA, 6 M HCl, TFA, TSA, HBr/TFA, TMSBr/TFA, TMSOTf/TFA, $POCl_3$

Lewis acids: $BF_3 \cdot Et_2O$, $AlCl_3$, $ZnBr_2$, $Ti(OEt)_4$, Me_3Al, $Sc(OTf)_3$, $Yb(OTf)_3$
Bases: DIPEA, NaOMe, NaOtBu, $KOC(C_2H_5)_3$, BuLi, LDA, TMG
Reducing agents: H_2/Pd, $NaBH_4$, $LiAlH_4$, $LiBH_4$, DIBAL, $NaCNBH_3$, Red-Al, $BH_3 \cdot THF$, $BH_3 \cdot$pyridine, $SnCl_4$
Oxidizing agents: MCPBA, $pyrSO_3$/DMSO, PDC, $Pb(OAc)_4$, CAN, $VOCl_3$, $K_2Cr_2O_7$
Organometallics: RMgX, Pd complexes, PhMnI, Ph_2Cd, RLi
Temperature: -78 – +200 °C

As can be seen from the above, with the exception of powerful oxidizing agents and strongly electrophilic reagents, most classes of reagents are represented.

Polystyrene-based resins have the longest track record of use in solid phase organic synthesis. 1% or 2% Divinylbenzene cross-linked polymers are normally employed; the latter are favored for reactions at elevated temperatures or for those involving organometallic reagents. These supports have a significantly higher loading capacity than NovaSyn® TG resins, but they do not swell in polar solvents such as water and methanol.

The use of microwave heating to accelerate solid phase reactions has recently attracted considerable interest [2]. The method is particularly useful in high throughput synthesis, where large numbers of small scale reactions need to be driven to completion as quickly and efficiently as possible.

The base resin matrices appear to be compatible with this technique as no visible changes in morphology are being observed even after heating to 300 °C [3]. These findings are supported by differential scanning calorimetric measurements on Merrifield resin which have shown the resin to be completely stable up to 200 °C [4].

2.3   Solid supports

2.3.1   NovaSyn® TG resins
NovaSyn® TG and NovaSyn® TG^R resins [5] are based on a composite of low cross-linked hydroxyethylpolystyrene and polyethylene glycol, which has been terminally functionalized. NovaSyn® TG resin is available as 90 µm and 130 µm beads. The smaller bead size resin is generally preferred for peptide synthesis and peptide libraries; 1 g contains almost a sufficient number of beads to represent a pentameric peptide library. The higher capacity of the large beads make the 130 µm resin ideal for the production of non-peptidic libraries.

These resins are suitable for both continuous flow and batch peptide synthesis. The NovaSyn® TG^R resins have been specifically designed for the synthesis of long and challenging sequences. They have excellent physical stability in flow systems, are resistant to abrasion and mechanical pressure, and offer improved chemical efficiency. High flow rates have even been reported to increase the acylation and deprotection rates of NovaSyn® TG resins [5b]. NovaSyn® TG resins are also recommended for use with the one-bead-one-compound approach to chemical libraries. The beads have a narrow size distribution and swell in water, facilitating biological assays in aqueous systems. The resins swell in a wide range of solvents from toluene to water, and the environment provided by the PEG is thought to closely resemble that found in THF.

Contrary to popular belief, the PEG is not anchored in NovaSyn® TG resins to the polystyrene backbone *via* an acid sensitive benzylic ether, but rather *via* an acid stable ethyl ether (Figure 2-1). Furthermore, the phenomenon of PEG leakage that many report as being associated with the use of these resins is not due to any inherent acid instability, but arises through formation of PEG peroxides by the actions of oxygen and light during long-term storage. This degradation is an intrinsic property of all PEG resins. Unfavorable comparisons made between NovaSyn® TG and other similar PEG-based supports were almost certainly a reflection on differences in age and storage histories of the samples tested and not due to any fundamental differences in chemical stability.
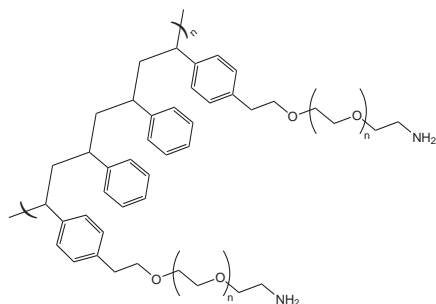
Fig. 2-1: Structure of NovaSyn® TG resin.

2.3.2   NovaGel™ resins
NovaGel™, also know as Champion resin, is a type of PEG-PS resin that has been designed to meet the requirements of chemists for resins of high substitution with broad solvent compatibility. It is prepared from a special high-swelling version of aminomethylated resin by partial derivatization with methyl-PEG$_{2000}$-p-nitro-phenylcarbonate [6, 7]. This produces a resin containing approximately 48% PEG, with a substitution

of 0.7 mmole/g, which is almost twice that of conventional PEG-PS supports. It swells in solvents of such widely different polarities as THF, DMF, acetonitrile and methanol, making it an excellent support for both peptide and organic synthesis (Table 2-3).

The urethane linkage between the PEG and the base resin is stable to piperidine and TFA, ensuring minimal loss of PEG chains during synthesis. However, if leaching of PEG does occur, this does not result in loss of substitution, as can be the case with other PEG-PS-based resins, because the linker is not attached to the end of the PEG chains.

Since their introduction, NovaGel™ resins have proven to be useful tools for solid phase organic synthesis, with applications in multicomponent condensations, Suzuki cross-coupling and reductive amination reactions [8, 9]. In a study [10], Rink amide NovaGel™ resin was found to give superior yields and higher enantiomeric excesses than other PEG-based resins in asymmetric Pd-catalyzed allylic substitutions.

The combination of high substitution and excellent swelling characteristics also makes NovaGel™-based supports ideal for use in solid phase peptide synthesis, as was recently demonstrated in the comparative synthesis of cyclic peptides *via* S$_N$Ar and S$_N$2 ring closure [11].
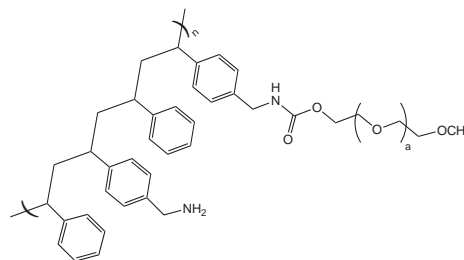
Fig. 2-2: Structure of NovaGel™ resin.

In an independent study, Rink Amide NovaGel™ was found to give superior results to conventional polystyrene-based Rink Amide resin in the synthesis of small linear peptides [12]. Peptide-templated oligosaccharide synthesis on NovaGel™ resin has also been described [13].

2.3.3   PEGA resins
PEGA resins are hydrophilic polymers that were originally developed for batch and continuous flow peptide synthesis [14] but which are also used in SPOS. They consist of 2-acrylamidoprop-1-yl-(2-aminoprop-1-yl) polyethylene glycol$_{800}$ and dimethylacylamide cross-linked with bis 2-acrylamidoprop-1-yl polyethylene glycol$_{800}$ (Figure 2-3). These supports swell extensively in a wide range of solvents, including water, DMF, DCM, THF and MeOH, and are freely permeable to macromolecules up to 35kD, making them ideally suited for the preparation of peptide libraries, affinity purification, and on-resin enzyme assays. These properties have been exploited in the on-resin enzymatic synthesis of glycopeptides [15]; for determining the inhibitors of subtilisin Carlsberg [16], Cruzipain [17], cysteine proteases [18], and matrix metalloproteinases [19]; and in studies on protein disulfide isomerases, using fluorescence-quenched libraries [20].

Smith & Bradley [21] compared solution and solid phase polyamine

libraries in an assay against trypanathione reductase. The library on PEGA resin performed as well as that in solution, whilst the library on TG resin failed to work.

PEGA resin has been employed in conjunction with a tartaric acid-based linker to prepare, *via* periodate oxidation, peptide α-oxoaldehydes [22, 23]. PEGA resins have been employed to prepare cyclic peptides by on-resin native chemical ligation [24] and in SPOS to produce triazoles by Cu-catalyzed 1,3-addition of alkynes to azides [25].

The water compatibility of PEGA resins has been exploited in supported Pd [26] and Ru [27] catalysts for asymmetric Wacker-type cyclization and metathesis in aqueous media.
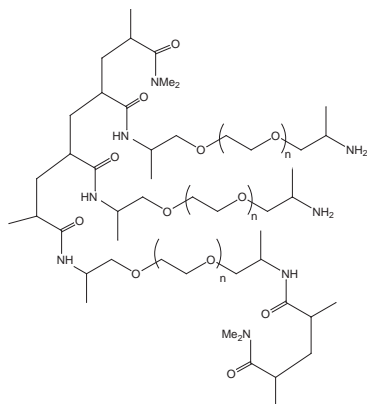
Fig. 2-3: Structure of PEGA resin.

2.3.4 NovaPEG resins

NovaPEG resins are identical to ChemMatrix resins and are excellent solid supports for solid phase peptide and organic synthesis [28]. Unlike other PEG-based polymer supports such as NovaSyn® TG and PEGA resins, which contain either polystyrene or polyacrylamide backbones, NovaPEG resin contains only PEG units (Figure 2-4). This unique composition confers excellent swelling and mechanical properties on the polymer. The resin beads have similar swelling properties to PEGA resins (Figure 2-6), but unlike PEGA resins are free flowing beads in the dry state, making them much easier to handle. Furthermore, in contrast to polystyrene and other commonly used supports, NovaPEG resin appears not to suffer from osmotic shock when solvent is exchanged from hydrophobic to hydrophilic solvents. NovaPEG resin also has excellent chemical stability, particularly towards strong acids and bases.
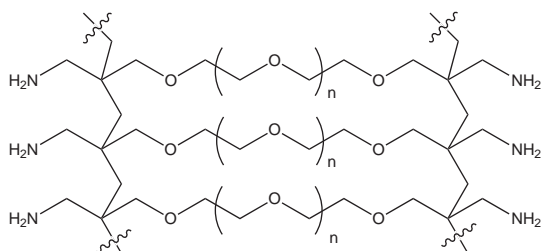
Fig. 2-4: Structure of NovaPEG resin.

The amphiphilic nature of this resin makes it an excellent support for the synthesis of difficult, aggregated peptides and of long peptides and small proteins. In a reported synthesis of Bacuma [28], a 38-residue potential

synthetic vaccine, the use of polystyrene-based resin gave an extremely heterogeneous product, whereas NovaPEG Rink Amide resin afforded the target in excellent purity and yield. More remarkable is the result obtained from the synthesis of β-amyloid (1-42) [28]. This sequence is notoriously difficult to prepare owing to its propensity to aggregate. Numerous strategies have been advocated for its synthesis, including the use of DBU, Hmb-dipeptides, and O-N intramolecular acyl migration. Using NovaPEG resin, this extremely problematic peptide was obtained in a crude purity of 91% using standard Fmoc SPPS methods. NovaPEG resin also gave a spectacular result in the synthesis of HIV-protease, affording an almost homogeneous product after 78 amino acid additions (Figure 2-5) [29].

Application 2-1: Comparison of NovaPEG and polystyrene resins in the synthesis of HIV-1 protease (1-78).
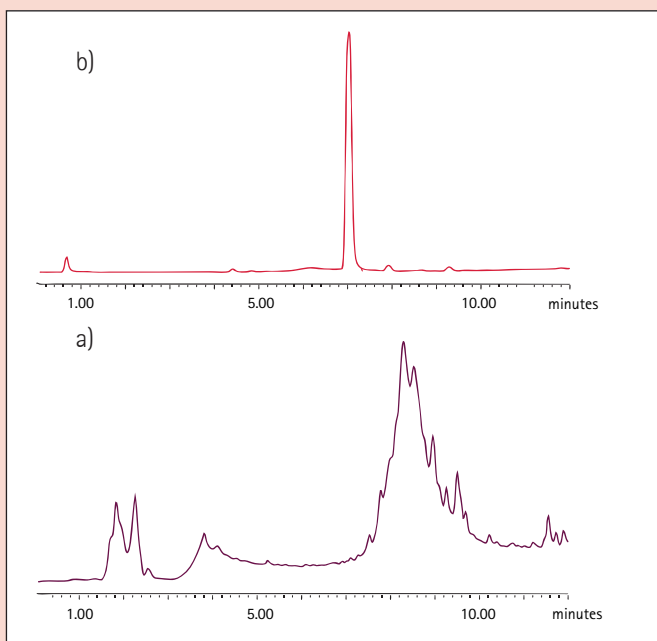
Fig. 2-5: HPLC profiles of crude HIV-1 protease prepared on a) NovaPEG resin by Fmoc SPPS and b) PAM resin by Boc SPPS.

Remarkable synergies have been observed when they are used in combination with pseudoproline dipeptides. For example, the syntheses of the chemokines Rantes [30] (Figure 2-6) and CCL4-L1 [31] could only be achieved by employing both NovaPEG resins and pseudoproline dipeptides.

2.3.5 SpheriTide resins

SpheriTide resin is a novel hydrophilic, high-load support for both research and large-scale production of peptides that consists of poly-ε-lysine cross-linked with sebacic acid. In contrast to other supports that are manufactured from products derived from fossil fuels, both these starting materials are obtained from renewable biological sources. Poly-ε-lysine is a naturally occurring short-chain polyamide consisting of 25-35 lysine residues linked through their α-carboxyl and ε-amino groups that is produced by bacterial fermentation for use as a food preservative, whereas sebacic acid is made from castor oil for use in the textile industry. SpheriTide is thus one of the first sustainable supports especially designed for peptide synthesis - it is also biodegradable as the polyamide backbone is susceptible to proteolysis.

Application 2-2: Comparison of NovaPEG and polystyrene resins in the synthesis of Rantes
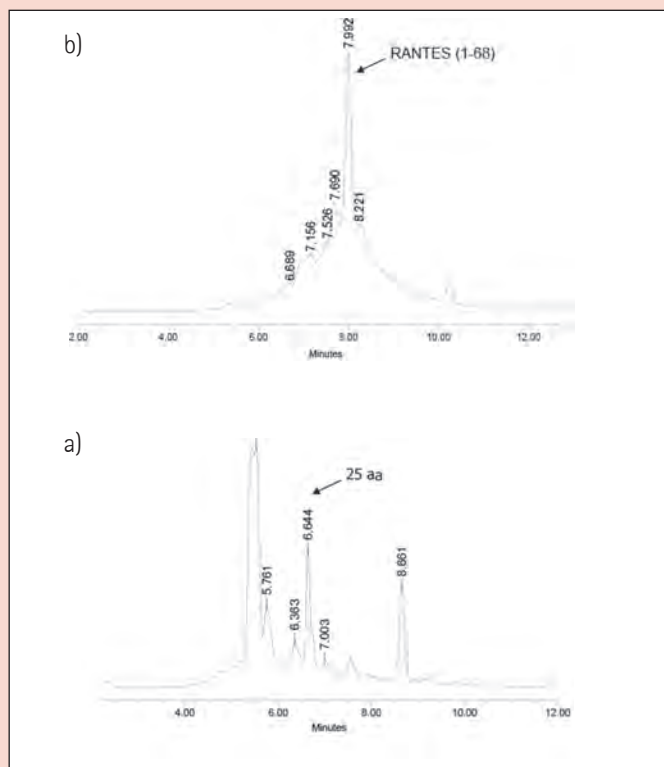
Fig. 2-6 HPLC profiles of crude Rantes prepared on a) NovaPEG resin and b) Wang resin (synthesis failed by 25 residues).

Because SpheriTide resin is simply a polymer of lysine, very high levels of functionality can be obtained without compromising its swelling and usability in peptide synthesis. Typically, the unloaded polymer has 3 mmol/g of reactive amino groups, which is approximately 2 times that of hydrophobic polystyrene resins and 3-10 times that of other comparable hydrophilic supports such as NovaPEG, PEGA and NovaSyn® TG. Due to addition of the mass of linker, loadings of HMPA SpheriTide resin and Rink Amide SpheriTide® resin drop to 1.8 - 2.2 mmol/g and 1.0 - 1.3 mmol/g, respectively, which is still significantly higher than Wang and Rink Amide AM resins. This high level of substitution means bed-volumes can be kept to a minimum (Figure 2-8). This results in lower solvent consumption and allows the use of lower excesses of activated amino acids since more concentrated solutions can be employed.
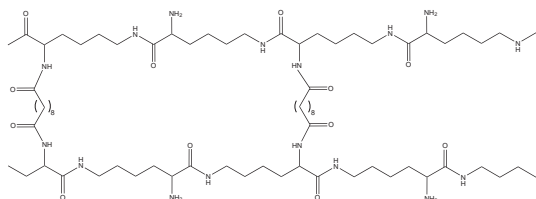
Fig. 2-7: Structure of SpheriTide resin.

The uniform spacing of functional groups in SpheriTide® resin is unique and not chemically feasible in traditional polymers formed by free-radical polymerization. In resins made by radical polymerization and post-synthesis functionalization, the cross-linking and functionalization is not uniform throughout the polymer but tends to cluster. This leads to batch-to-batch irreproducibilty due to inconsistent levels of cross-linking, and introduces

pockets of hindrance which ultimately leads to problems with formation of deletion and truncation sequences.

The polar backbone of the polymer allows the resin to swell in a wide range of solvents, including water, DMF, DCM and MeOH (Table 2-2). However, it is important to note that because of the exceptional high loading of the polymer, the swelling properties of derivatized SpheriTide® resins will be largely determined by the nature of the attached peptide or linker.

References
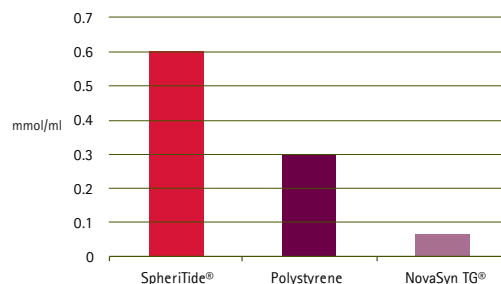
Fig. 2-8: Loading per ml in DMF for SpheriTide®, polystyrene and NovaSyn® TG resins.

1. K. K. Sorensen, et al. (2012) *J. Pept. Sc.*, **18**, S1 P201.
2. a) C. O. Kappe (2002) *Curr. Opin. Chem. Biol.*, **6**, 314; b) A. Lew, et al. (2002) *J. Comb. Chem.*, **4**, 95.
3. Novabiochem Innovations 4/03.
4. R. Perez, et al. (2003) *J. Comb. Chem.*, **4**, 501.
5. a) W. Rapp et al. in "Peptides 1988, Proc. 20st European Peptide Symposium", G. Jung & E. Bayer (Eds.), Walter de Gruyter, Berlin, 1989, pp. 199; b) W. Rapp, et al. in "Innovation and Perspectives in Solid Phase Synthesis", R.Epton (Ed.), SPCC (UK) Ltd, Birmingham, 1990, pp. 205; c) L. Zhang et al. in "Peptides 1990, Proc. 21st European Peptide Symposium", E. Giralt & D. Andreu (Eds), ESCOM, Leiden, 1990, pp. 196; d) E. Bayer (1991) *Angew. Chem. Int. Ed. Engl.*, **30**, 113; e) E. Bayer et al. in "Peptides Structure and Function", V.J. Hubry & D.M. Rich (Eds.), Pierce Chemical Company, Rockford, 1983, pp. 87; f) W. Rapp and E. Bayer in "Polyethyleneglycol chemistry, Biochemical and Biomedical Applications", J.Milton-Harris (Ed.), Plenum Press, 1992, pp. 325; g) W. Rapp, et al. in "Peptides 1994, Proc. 23rd European Peptide Symposium", H. Maia (Ed.), ESCOM, Leiden, 1995, pp. 87.
6. J. H. Adams, et al. (1998) *J. Org. Chem.*, **63**, 3706.
7. D. Hudson, et al. (1999) in "Peptides 1998: Proceedings of the 25th European Peptide Symposium", S. Bajusz & F. Hudecz, Akadémiai Kiadó, Budapest, pp. 40.
8. P. White, Novabiochem Innovations 1/98.
9. J. H. Adams, et al., Poster presented at CHI meeting, High-throughput Organic Synthesis, San Diego, 1999.
10. S. R. Gilbertson & S. Yamada (2004) *Tetrahedron Lett.*, **45**, 3917.
11. Y. Feng & K. Burgess (2000) *Biotechnology & Bioengineering*, **71**, 3.
12. R. Pipkorn & P. White, Novabiochem Innovations 3/99.
13. D. R. Greenwell, et al. (2002) *Angew. Chem.*, **114**, 1263.
14. M. Meldal (1992) *Tetrahedron Lett.*, **33**, 3077.
15. M. Meldal et al. (1994) *J. Chem. Soc., Chem. Commun.*, 1849.
16. M. Meldal et al. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 3314.
17. M. Meldal, et al. (1998) *J. Peptide Sci.*, **4**, 83.
18. P. M. St. Hilaire, et al. (1999) *J. Comb. Chem.*, **1**, 509.
19. J. Buchardt, et al. (2000) *J. Comb. Chem.*, **2**, 624.
20. J. C. Spetzler, et al. (1998) *J. Peptide Sci.*, **4**, 128.
21. H. K. Smith & M. Bradley (1999) *J. Comb. Chem.*, **1**, 326.
22. S. Fruchart, et al. (1999) *Tetrahedron Lett.*, **40**, 6225.
23. O. Melnyk, et al. (2001) *J. Org. Chem.*, **66**, 4153,
24. J. Tulla-Puche & G. Barany (2004) *J. Org. Chem.*, **69**, 4101.
25. C. W. Tornøe, et al. (2002) *J. Org. Chem.*, **67**, 3057.
26. H. Hocke & Y. Uozumi (2002) Synlett, 2049.
27. S. J. Connon & S. Blechert (2002) *Bioorg. Med. Chem. Lett.*, **12**, 1873.
28. F. Garcia-Martin, et al. (2006) *J. Comb. Chem.*, **8**, 213.
29. S. Frutos, et al. (2007) *Int. J. Pept. Res. Ther.*, **13**, 221.
30. F. Garcia-Martin, et al. (2006) *Biopolymers*, **84**, 566.
31. B. G. de la Torre, et al. (2007) *Int. J. Pept. Res. Ther.*, **13**, 265.

2.4   Linkers for SPPS

2.4.1   Linkers for Fmoc SPPS

Novabiochem® has one of the most extensive ranges of linkers and derivatized resins for Fmoc and Boc solid phase peptide synthesis. The properties of these resins are summarized in Tables 2-4 and 2-5, together with cross-references to the appropriate loading and cleavage protocols. For more detailed information, see the section related to the resin of interest later in this chapter.

Peptide acids

For the synthesis of peptide acids, benzyl alcohol-type linkers such as Wang or HMPA are used. Special consideration must be given to peptides containing Cys, His, Gly, Pro, Met and Trp residues at the C-terminus. Esterification of Cys and His to these linkers is always problematic, as these amino acids are extremely prone to enantiomerize under the rather forcing conditions used. For preparing such peptides, resins derivatized with trityl-type linkers, such as NovaSyn® TGT resin, NovaPEG Trt or 2-chlorotrityl resin, should be used because loading these supports does not necessitate activation of the Fmoc-amino acid carboxyl group and so is completely free from racemization and dipeptide formation (see sections 2.4.5 & 2.4.6 in this chapter). Trityl-based resins are also strongly recommended for the preparation of peptides containing C-terminal Gly, Pro, Met and Trp residues. Glycine- and proline-containing dipeptides attached to benzyl alcohol-based linkers are prone to undergo diketopiperazine formation during Fmoc deprotection, with loss of peptide chains from the support, whereas Met and Trp residues can be alkylated by the cations generated at the linker during the cleavage reaction, resulting in reattachment of the peptide to the support. In both instances, these side reactions are reduced, if not eliminated, with trityl resins, owing to the bulk of the linker.

Novabiochem® also offers the convenience of pre-loaded Wang and NovaSyn® TGA resins. The bulk resin used to prepare our pre-loaded Wang resins is synthesis tested, to ensure consistent and reliable performance in synthesis. The major impurties arising from loading of the amino acid, such as dipeptide content and racemization, are also determined.

Protected peptide acids

Resins derivatized with trityl-type linkers, such as NovaSyn® TGT resin, NovaPEG Trt or 2-chlorotrityl resin, provide the simplest approach to the synthesis of protected peptide acids. Treatment with 20% TFE in DCM (Method 3-31, p. 3.30) releases fully protected peptide acids, with negiglible loss of side-chain protecting groups provided Fmoc-His(Clt)-OH is employed for introduction of His residues. After evaporation of the solvent, the product is isolated by precipitation with water.

Novabiochem® provides NovaSyn® TGT resin, NovaPEG Trt and 2-chlorotrityl resins pre-loaded with the first amino acid residue.

Peptide amides

Resins based on the Fmoc-Rink Amide linker, such as Rink Amide AM or Rink Amide MBHA, are recommended for routine synthesis of peptide amides. Loading of the C-terminal residue can be effected using any standard coupling method. Peptide amides are released by treatment with 95% TFA cleavage cocktails.

Protected peptide amides

Sieber Amide resin offers the most practical route to protected peptide amides. The C-terminal amino acid is loaded on the resin using standard coupling methods. Treatment with 1% TFA in DCM releases the protected peptide amide (Method 3-30, p. 3.30).

Peptide N–alkyamides

The simplest approach involves reductive amination of the appropriate primary amine to FIA AM resin (Method 2-15, p. 2,30), to generate a resin-bound secondary amine. Acylation of this group with the C-terminal residue of the peptide, chain extension, and TFA cleavage, affords the peptide N-alkylamide. For peptide N-methyl- and N-ethylamide, FIA-AM resins are available pre-derivatized with methylamine and ethylamine.

Peptide alcohols

Novabiochem® offers 2-chlorotrityl resin pre-loaded with glycinol and theoninol. These resins enable the direct synthesis of peptide alcohols containing these residues at the C-terminus. For peptides containing other C-terminal amino alcohols, the use of resins derivatized with the HMBA linker is recommended. Peptides attached to this linker are cleaved by reduction with $NaBH_4$ as peptide alcohols.

Peptide aldehydes

Novabiochem® offers a number of aldehydes derived from amino acids pre-loaded on Thr-Gly-NovaSyn® resin. Following side-chain deprotection with TFA, peptide aldehydes are released by treatment with acetic acid/water/DCM/MeOH (page 3.47). Peptide aldehydes may also by prepared by reductive cleavage from Weinreb AM resin. For larger scale synthesis, oxidation of protected peptide alcohols with IBX or Dess-Martin reagent in solution provides the most effective method
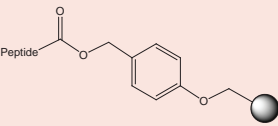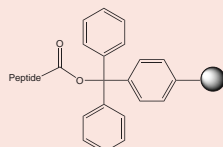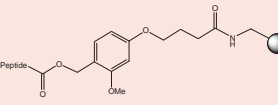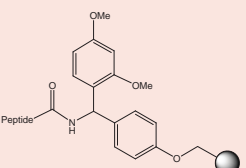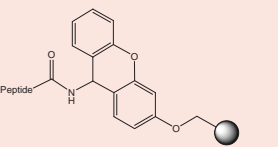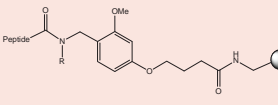
Peptide esters

Small scale synthesis of peptide methyl esters can be achieved by methanolysis of peptides attached to the HMBA linker (Method 3-35, page 3.31). However, the yields are often poor and the products contaminated with peptide acid. One very effective scaleable method involves the reaction of the protected peptide acid with TMS diazomethane in MeOH. For synthesis of other esters, use of the BAL approach involving anchoring of a pre-formed amino-acid ester to a formyl resin is recommended.

Peptide thioesters

Small scale synthesis of peptide thioesters can be achieved by thiolysis from sulfamylbutyryl and Dbz linkers as described in chapter 5, section 5.1.1, page 5.1. For larger quantities coupling of a protected peptide acid to a pre-formed amino acid thioester, followed by side-chain deprotection is recommended.

Table 2-4: Resins for Fmoc SPPS.

	Resin	Cleavage	Loading protocol	Cleavage protocol
Peptide acid				
	Wang resin NovaSyn® TGA resin HMPA-PEGA resin HMPA-NovaGel™ HMPA SpheriTide resin NovaPEG Wang resin (4-Bromomethyl)phenoxy-methyl polystyrene	95 % TFA	3-8, 3-9 (p. 3.6)	3-26 (p. 3.29)
Protected peptide acid				
	2-Chlorotrityl resin NovaSyn® TGT alcohol resin	1% TFA in DCM, AcOH/DCM/TFE, 20% TFE in DCM	2-3 (p. 2.17)	3-30, 3-31 (p. 3.30)
	HMPB-MBHA resin NovaPEG HMPB resin	1% TFA in DCM	3-8, 3-9 (p. 3.6)	3-31 (p. 3.30)
Peptide carboxamide				
	NovaSyn® TGR resin Fmoc-PAL AM resin PAL NovaPEG/NovaSyn® TG resin Ramage Amide AM resin Rink Amide resin Rink Amide AM/MBHA resin Rink Amide NovaGel™/ PEGA resin Rink Amide SpheriTide resin NovaPEG Rink Amide resin	95 % TFA	3-11 (p. 3.7)	3-26 (p. 3.29)
Protected peptide carboxamide				
	Sieber Amide resin NovaSyn® TG Sieber resin	1% TFA in DCM	3-11 (p. 3.7)	3-31 (p. 3.30)
Peptide N–alkylamide				
	FMPB AM resin FMPB NovaGel™	95% TFA	2-15 (p. 2.40)	3-26 (p. 3.29)
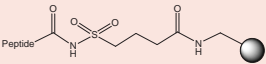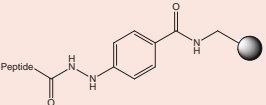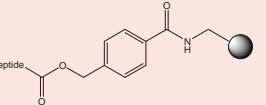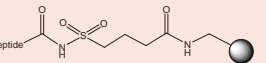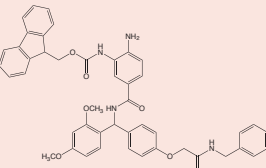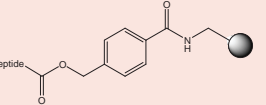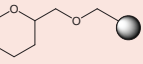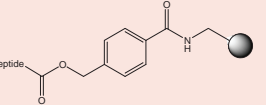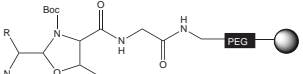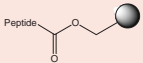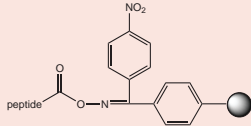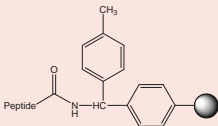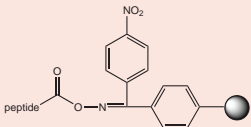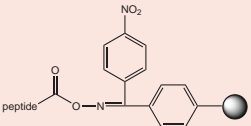
	Resin	Cleavage	Loading protocol	Cleavage protocol
	4-Sulfamylbutyryl AM resin	i) ICH$_2$CN or TMSCHN$_2$ ii) RNH$_2$	2–6 (p. 2.27)	2–7 (p. 2.27)

Peptide ester

	Resin	Cleavage	Loading protocol	Cleavage protocol
	4-Fmoc-hydrazinobenzoyl AM No-vaGel™	Cu(OAc)$_2$, ROH, pyridine	3–11 (p. 3.7)	2–9 (p. 2.29)
	HMBA-AM resin, HMBA-NovaGel™, HMBA-PEGA resin	ROH/DIPEA/DMF	3–8, 3–9 (p. 3.6)	3–35 (p. 3.31)

Peptide thioester

	Resin	Cleavage	Loading protocol	Cleavage protocol
	4-Sulfamylbutyryl AM resin, and others	i) TMSCHN$_2$ ii) RSH	2–6 (p. 2.27)	5–1 (p. 5.2)
	Dawson Dbz AM resin	i) p-NO$_2$PhOCOCl ii) DIPEA iii) TFA	5–2 (p. 5.5)	5–2 (p. 5.5)

Peptide hydrazide

	Resin	Cleavage	Loading protocol	Cleavage protocol
	HMBA-AM resin, HMBA-NovaGel™ HMBA-PEGA resin	NH$_2$NH$_2$/DMF	3–8, 3–9 (p. 3.6)	3–33 (p. 3.31)

Peptide alcohols

	Resin	Cleavage	Loading protocol	Cleavage protocol
	DHP HM	95% TFA	2–16 (p. 2.42)	3–26 (p. 3.29)
	HMBA-AM resin, HMBA-NovaGel™, HMBA-PEGA resin	NaBH$_4$/EtOH	3–8, 3–9 (p. 3.6))	3–36 (p. 3.31)

Peptide aldehydes

	Resin	Cleavage	Loading protocol	Cleavage protocol
	H-Aaa-H NovaSyn® TG resin	AcOH/water/DCM/MeOH (10:5:63:21)		Appl. 3–13 (p. 3.48)

Table 2–5: Resins for Boc SPPS.

	Resin	Cleavage	Loading protocol	Cleavage protocol
Peptide acid				
	Merrifield resin	HF, TFMSA	2-1 (p. 2.11)	3-20 (p. 3.26)
Protected peptide acid				
	Oxime resin	NaOH/dioxane	3-8, 3-9 (p. 3.6))	3-34 (p. 3.31)
Peptide carboxamide				
	MBHA resin	HF, TFMSA	2-5 (p. 2.20)	3-32 (p. 3.31)
Peptide ester				
	Oxime resin	MeOH/DMF/TEA	3-8, 3-9 (p. 3.6))	3-35 (p. 3.31)
Peptide hydrazide				
	Oxime resin	NH_2NH_2/DMF	3-8, 3-9 (p. 3.6))	3-34 (p. 3.31)

2.4.2 Resins for Boc SPPS

The properties of Novabiochem®'s resins for Boc solid phase peptide synthesis are summarized in Table 2-5, together with cross-references to the appropriate loading and cleavage protocols. For more detailed information, see the section related to the resin of interest later in this chapter.

2.4.3 Multiple antigenic peptides (MAPs)

The MAP system represents an approach to anti-peptide antibody elicitation. The system is based on a small immunogenically inert core molecule of radially branching lysine dendrites (see Figure 2-9) onto which a number of peptide antigens are anchored. The result is a large macromolecule with a unique three-dimensional configuration which has a high molar ratio of peptide antigen to core molecule and does not require the use of a carrier protein to elicit an antibody response. The subject has been reviewed [1].

In immunological studies, MAPs have been shown to be valuable in experimental vaccine development [2-6]. The antigenic peptide-dense MAP greatly increases coating efficiency on solid surfaces and enhances detection sensitivity for solid phase immunoassays [2, 7] and provides enhanced reproducibility not possible with linear, monomeric peptide antigens.

The inert MAP core is attached to a solid phase peptide synthesis support and the desired peptide antigens are synthesized directly on the branched-lysine core. After the synthesis is complete the MAP macromolecule is cleaved from the support using standard techniques. Because the lysine core of a MAP is small compared with the peptide antigen, the concentration of antigen is at a maximum. The result is a highly immunogenic MAP which exhibits significantly higher titers than its monomeric counterpart attached to a carrier protein [2, 3, 8].

Novabiochem offers 4-MAP and 8-MAP core molecules for use in Fmoc-SPPS. Unlike regular peptide synthesis, there is no need for purification of the crude cleavage products other than desalting using a Sephadex column [9]. In cases where tryptophan and/or arginine residues are present, the scavengers used during HF cleavage and side-chain deprotection cycle adhere tightly to the MAP macromolecule and must be removed by dialysis before desalting [2, 3, 5, 6, 9].

It is strongly recommended that users monitor the acylation reaction or perform double couplings to ensure complete coupling. Incomplete acylations can lead to sub-populations of MAP antigens with deletion sequences.
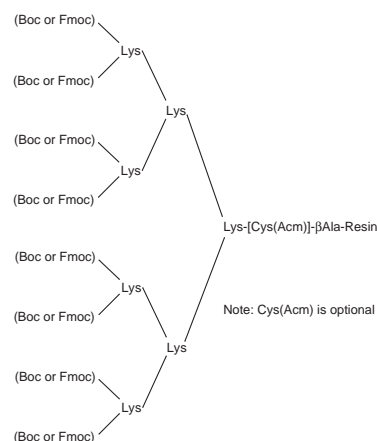
Fig. 2-9: General structure of an 8-branch MAP core.

Di-epitope MAPs

The system also offers the flexibility of synthesizing di-epitopes for vaccine development [10] and multiple immunoassays. Di-epitope MAPs can be synthesized by coupling two heterologous MAPs through disulfide linkages *via* a Cys residue in the core [4, 5, 6, 10]. Alternatively, the di-epitopes can be synthesized on alternating branches of the terminal lysine groups using Boc- and Fmoc-strategies. T-cell and B-cell epitopes can also be combined sequentially within a single linear sequence. The introduction of the Dde type-protecting groups for lysine has made possible the synthesis of di-epitopic MAPs using Fmoc chemistry [11].

4-Branch MAP cores

Novabiochem® offers 4-branch MAP cores which give superior results for 10-15 residue epitopes compared to normal 8-branch (octavalent) MAPs. For example, Tam & Lu [6] have shown that, for certain sequences of the malaria circumsporozoite protein, 4-branch MAPs give higher antibody titres than 8-branch. Furthermore, Francis, *et al.* [12], working with foot-and-mouth disease virus, found 8-branch MAPs gave slightly higher titres than 4-branch MAPs, although the 8-branch MAPs produced antibodies with reduced recognition for *N*-terminal residues.

Good results have been obtained using MAPs consisting of linear sequences incorporating both B-cell and T-cell epitopes; recent examples include the P. falciparum circumsporozoite protein [13] and the HIV-1 coat protein [14]. The work with HIV-1 has been further developed by incorporating a tripalmitoyl-amino acid (Pam_3Cys) into the MAP, which removes the need for Freund's complete adjuvant [11, 15].

NovaSyn® TG PAP resin

An alternative to MAPS resins is NovaSyn® PAP resin. This resin consists of a PEG-polystyrene composite in which the linkage between the PEG and polystyrene is labile to TFA/thioanisole (95:5) or TMSBr/TFA/thioanisole (1:94:5). Cleavage with these reagents releases the fully deprotected peptide possessing a C-terminal PEG resin that can be used directly as an immunogen without the need for a carrier protein [16].

Related products		
856082	$Fmoc_8-Lys_4-Lys_2-Lys-\beta Ala$-Wang resin	p. 226
856165	$Fmoc_8-Lys_4-Lys_2-Lys-Cys(Acm)-\beta Ala$-Wang resin	p. 226
856083	$Fmoc_4-Lys_2-Lys-\beta Ala$-Wang resin	p. 225

References

1. P. Veprek & J. Jezek (1999) *J. Peptide Sci.,* **5**, 5; *ibid* **5**, 203.
2. J.P. Tam in "Vaccines 90. Modern approaches to new vaccines including prevention of AIDS", R. A. Lerner, et al. (Eds.), Cold Spring Harbor Lab., New York, 1990, pp. 21.
3. J.P. Tam (1988) *Proc. Natl. Acad. Sci. USA,* **85**, 5409.
4. Y.A. Lu & J.P. Tam in "Peptides: Chemistry, Structure & Biology: Proc. 11th American Peptide Symp., J.E. Rivier & G.R. Marshall (Eds.), ESCOM, 1990, pp. 724.
5. J.P. Tam, et al. (1990) *J. Exp. Med.,* **171**, 299.
6. J.P. Tam & Y.-A. Lu (1989) *Proc. Natl. Acad. Sci. USA,* **86**, 9084.
7. J.P. Tam & F. Zavala (1989) *J. Immun. Methods,* **124**, 53.
8. G.W. McLean, et al. (1991) *J. Immunol. Methods,* **137**, 149.
9. A. Pessi, et al. (1990) *J. Chem. Soc., Chem. Commun.,* 8.
10. R. Ramasamy (1991) *Pept. Res.,* **4**, 210.
11. W.C. Chan, et al. in "Peptides; Chemistry, Structure & Biology, Proc. 13th American Peptide Symposium, R.A. Hodges & J. A. Smith (Eds.), ESCOM, Leiden, 1994, pp. 727.
12. M.J. Francis, et al. (1991) *Immunology* **73**, 249.
13. D.Y. Munesinghe, et al. (1991) *Eur. J. Immunol.,* **21**, 3015.
14. B. Nardelli, et al. (1992) *J. Immunol.,* **148**, 914.
15. J.-P. Defoort, et al. (1992) *Proc. Natl Acad. Sci. USA,* **89**, 3879.
16. W. Rapp in "Combinatorial peptide and non-peptide libraries", G. Jung (Ed.), Vch, 1996, pp. 425.

2.5   Resins for SPOS

Novabiochem® has one of the most extensive ranges of linkers and derivatized resins for solid phase organic synthesis. The loading and cleavage profiles of many of these are given in Table 2-6. For comprehensive reviews on linkers see [40-43].

References

1. J. M. Schlatter, et al. (1977) *Tetrahedron Lett.,* 2851.
2. J. M. Stewart & J. D. Young in "Solid Phase Peptide Synthesis, 2nd Ed.", Pierce Chemical Company, Rockford, 1984, pp. 92.
3. M. J. Kurth, et al. (1994) *J. Org. Chem.,* **59**, 5862.
4. a) R. Frenette, et al. (1994) *Tetrahedron Lett.,* **35**, 9177; b) S. Marquais & M. Arlt (1996) *Tetrahedron Lett.,* **37**, 5491.
5. L. F. Tietze & A. Steinmetz (1996) *Angew. Chem. Int. Ed. Engl.,* **35**, 651.
6. S. Chamoin, et al. (1998) *Tetrahedron Lett.,* **39**, 4175.
7. D. Stones, et al. (1998) *Tetrahedron Lett.,* **39**, 4875.
8. R. L. E. Furlán, et al. (1998) *Tetrahedron,* **54**, 13023.
9. J. W. Corbett, et al. (1997) *Bioorg. Med. Chem. Lett.,* **7**, 1371.
10. S. Hanessian & F. Xie (1998) *Tetrahedron Lett.,* **39**, 733.
11. K. Ngu, et al. (1997) *Tetrahedron Lett.,* **38**, 973.
12. M. W. Miller, et al. (1998) *Tetrahedron Lett.,* **39**, 3429.
13. S. Kobayashi & Y. Aoki (1998) *Tetrahedron Lett.,* **39**, 7345.
14. D. S. Brown, et al. (1998) *Tetrahedron Lett.,* **39**, 8533.
15. W. Brill, et al. (1998) *Synlett,* 906.
16. D. R. Barn, et al. (1996) *Tetrahedron Lett.,* **37**, 3213.
17. S. V. Ley, et al. (1995) *Synlett,* 1017.
18. G. J. Kuster & H. W. Scheeren (1998) *Tetrahedron Lett.,* **39**, 3613.
19. H. Rink (1987) *Tetrahedron Lett.,* **28**, 3787.
20. C. P. Holmes, et al. (1995) *J. Org. Chem.,* **60**, 2318.
21. E. Atherton et al. in "Solid phase peptide synthesis; a practical approach", IRL Press, 1989, pp. 152.
22. a) W. F. DeGrado, et al. (1980) *J. Org. Chem.,* **45**, 1295; b) W. F. DeGrado, et al. (1982) *J. Org. Chem.,* **47**, 3258.
23. M. A. Scialdone (1996) *Tetrahedron Lett.,* **37**, 8141.
24. P. Sieber (1987) *Tetrahedron Lett.,* **28**, 2107.
25. J.-A. Fehrentz, et al. (1995) *Tetrahedron Lett.,* **36**, 7871.
26. T. Q. Dinh & R. W. Armstrong (1996) *Tetrahedron Lett.,* **37**, 1161.
27. B. J. Backes & J. A. Ellman (1994) *J. Am. Chem. Soc.,* **116**, 11171.
28. a). B. J. Backes, et al. (1996) *J. Am. Chem. Soc.,* **118**, 3055; b). R. Ingenito, et al. (1999) *J. Am. Chem. Soc.,* **121**, 11369.
29. a) E. E. Swayze (1997) *Tetrahedron Lett.,* **38**, 8464; b) D. Sarantakis & J. J. Bicksler (1997) *Tetrahedron Lett.,* **38**, 7325; c) A. M. Fivush & T.M. Willson (1997) *Tetrahedron Lett.,* **38**, 7151; d) M. T. Bilodeau & A. M. Cunningham (1998) *J. Org. Chem.,* **63**, 2800; e) P. T. Kearney, et al. (1998) *J. Org. Chem.,* **63**, 196; f) K. G. Estep, et al. (1998) *J. Org. Chem.,* **63**, 5300.
30. J. S. Panek & B. Zin (1996) *Tetrahedron Lett.,* **37**, 8151.
31. H. V. Meyers, et al. (1995) *Mol. Div.,* **1**, 13.
32. T. M. Fyles, et al. (1978) *Can. J. Chem.,* **56**, 1031.
33. L. A. Thompson, et al. (1994) *Tetrahedron Lett.,* **35**, 9333.
34. O. B. Wallace (1997) *Tetrahedron Lett.,* **38**, 4939.
35. W. H. Pearson & R. B. Clark (1998) *Tetrahedron Lett.,* 39, 7669.
36. D. A. Nugiel, et al. (1997) *J. Org. Chem.,* **62**, 201.
37. A. L. Smith, et al. (1998) *Tetrahedron Lett.,* **39**, 8317.
38. I. W. James (1999) *Tetrahedron,* **55**, 4855.
39. K. Gordon & S. Balasubramanian (1999) *J. Chem. Tech. Biotech.,* **74**, 835.
40. F. Guillier, et al. (2000) *Chem. Rev.,* **100**, 2091.
41. P. Blaney, et al. (2002) *Chem. Rev.,* **102**, 2607.
42. N.-H. Nam, et al. (2003) *J. Comb. Chem.,* **5**, 479.
43. "The power of functional resins in organic synthesis", J. Tulla-Puche & F. Albericio (Eds.), Wiley-Vch, Weinheim, 2008, pp. 383-610.

Table 2-6: Resins for SPOS.

Linker Type	Resin	Attached group	Leaving group	Cleavage reagent
Halomethyl resins	Merrifield	RCO₂H	RCO₂H	TFMSA, HF, H₂/Pd [1]
		ROH	ROH	TFMSA, HF, H₂/Pd
		RCO₂H	RCH₂OH	LiBH₄ [2]; DIBAL [3]
		RCO₂H	RCO₂Me	MeONa [4]
		RCO₂H	RCO₂Me	C₂H₅CO₂Me, Ti(OEt)₄ [5]
		RCO₂H	RCO₂H	LiOH/H₂O/MeOH/THF [6]
		RCO₂H	RCO₂H	SnCl₄ [7]; Me₃SnOH [8]
	4-(Bromomethyl)phenoxy ethyl polystyrene	RCO₂H	RCO₂H	TFA [9]
		ROH	ROH	TFA [10]
		RNH₂	RNHSO₂R¹	i) R¹SO₂Cl ii) TFA [11]
		R₂NH	R¹CONR₂	R¹COCl [12]
		RNH₂	RNH₂	DDQ [13]
		RNH₂	RNHCOR¹	i) R¹COX ii) TFA [14]
	Rink acid chloride/ trifluoroacetate	RNH₂	RNH₂	20% TFA in DCM [15]
		ROH	ROH	20% TFA in DCM [15]
		RSH	RSH	95% TFA/5% H₂O [15]
Hydroxy resins	Wang, NovaSyn® TGA	RCO₂H	RCO₂H	TFA
		ROH	ROH	TFA [10]
		RCO₂H	RCONR¹R²	R¹R²NH, AlCl₃, DCM [16]
		RCO₂H	RCH₂OH	DIBAL [17]
		RCO₂H	RCO₂Me	TEA/MeOH/KCN/benzene [18]
	HMPB AM	RCO₂H	RCO₂H	1%–5% TFA in DCM
	Rink acid	RCO₂H	RCO₂H	10% AcOH in DCM [19]
		ROH	ROH	5% TFA in DCM
	Hydroxyethyl-Photolinker	RCO₂H	RCO₂H	hν [20]
	HMBA–AM	RCO₂H	RCO₂H	NaOH aq. [21]
			RCONH₂	NH₃/MeOH [22]
			RCONHR¹	R¹NH₂
			RCH₂OH	NaBH₄/EtOH
			RCO₂Me	MeOH/TEA
			RCONHNH₂	NH₂NH₂/DMF
	Oxime	R₂NCO	R¹NHCONHR²	R¹NH₂ [23]
Amino resins	Rink Amide, NovaSyn® TGR, Rink Amide MBHA	RCO₂H	RCONHR¹	TFA [19]
	Sieber Amide	RCO₂H	RCONHR¹	1% TFA in DCM [24]
	Weinreb AM	RCO₂H	RCHO	LiAlH₄, DIBAL [25]
		RCO₂H	RCOR¹	R¹MgX [26]
	4-Sulfamylbutyryl AM,	RCO₂H	RCONHR¹	i) CH₂N₂, ii) R¹NH₂ [27]
				i) I–CH₂CN, ii) R¹NH₂ [28a]
			RCOSR¹	i) TMS–CHN₂, ii) R¹SH [28b]

Linker Type	Method of Attachment	Resin	Attached group	Leaving group	Cleavage reagent
Formyl resins		Formylalkoxy phenoxy resins	RNH₂	RNHSO₂R¹	i) R¹SO₂Cl ii) TFA [29]
		(3-Formylindolyl) acetamidomethyl polystyrene [29f]	R₂NH	R¹CONR₂	i) R¹COCl ii) TFA [12]
Trityl resins		Trityl chloride	RNH₂	RNH₂	1 – 5% TFA in DCM or 30% HFIP in DCM
		2-Chlorotrityl chloride, NovaSyn ®TGT	ROH, RNH₂, RCONHNH₂, RCO₂H	ROH, RNH₂ RCONHNH₂, RCO₂H	1 – 5% TFA in DCM 5 – 20% TFA in DCM 1 – 5% TFA in DCM or 30% HFIP(TFE) in DCM
			RSH	RSH	> 50% TFA in DCM
Carboxy resins		NovaSyn ® TG carboxy	ROH	ROH	K₂CO₃, 1:2 MeOH/ THF [30, 31] MeONa/MeOH (nBu)₄NOH/THF [32] NaOMe, 4:1 THF/MeOH [31]
DHP resin		3,4-Dihydro-2H-pyran-2-methoxymethyl polystyrene	ROH	ROH	TFA/H₂O [33]
			PhOH	PhOH	TFA/DCM/EtOH [34]
			Purine	Purine	TFA/DCM/MeOH [35] 20% TFA in DCM [36]
			Indole	Indole	10% TFA in DCM [37]
					HSiCl₃ [41]

2.6 Chloro, bromo functionalized resins

2.6.1 Merrifield resin
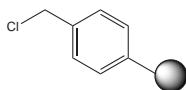
Merrifield resins have been used for more than thirty years for the synthesis of peptides and are the standard supports for the SPPS of small or medium sized peptides using the Boc strategy. The resin consists of 1% divinylbenzene cross-linked polystyrene beads that have been functionalized with chloromethyl groups.

For peptide synthesis, 1% cross-linked 100-200 and 200-400 mesh resins are usually used. However, the limited stability of the peptide-resin ester bond to repetitive treatments with TFA means that, in practice, its use is now restricted to the production of small to medium sized peptides. For the synthesis of longer peptides, the use of PAM resin is favored. Merrifield resin can be loaded with Boc-amino acids as described in Method 2-1, or can be purchased pre-loaded with the *C*-terminal amino acid.

Attachment of Boc amino acids and carboxylic acids is generally achieved by heating the resin, in DMF, with the appropriate carboxylic acid cesium salt in the presence of KI [1] or dibenzo-18-crown-6 [2], although Me_4N salts [3], sodium salts [4] in THF with Bu_4NF catalysis [5], and zinc salts in EtOH have also been used [6]. Release of the peptide or carboxylic acid is normally effected by treatment of resin with HF or TFMSA (Methods 3-20 & 3-21, page 3.26). In the case of peptides, this results in concurrent removal of benzyl-based side-chain protecting groups. Cleavage can also be effected by hydrogenolysis [7], or by hydrolysis with LiOH/water/ MeOH/ THF [8] (Method 2-20, page 2.46), or Me_3SnOH in refluxing DCE [9]. Alcohols can be liberated by reduction with DIBAL [5] or $LiBH_4$ [10] (Method 2-22, page 2.46). Methyl esters can be produced by transesterification with MeOH/TEA/DMF, MeONa [11, 12](Method 2-21, page 2.46), or $Ti(OEt)_4/C_2H_5CO_2Me$ [13]. Carboxamides are also accessible *via* Lewis acid catalyzed aminolysis [14].

Method 2-1: Attachment of acids/phenols to Merrifield resin and bromomethyl resins using cesium salts

1. Dissolve acid/phenol in EtOH (2 ml/mmole) and add water (0.5 ml/mmole). Adjust pH to 7 with 2M aq. Cs_2CO_3. Evaporate solution to dryness. Add dioxane and evaporate to dryness. Repeat evaporation with dioxane.
2. Pre-swell Merrifield resin in DCM for 1 h and then wash with DMF. Add Cs salt (1.2 eq.) in DMF to the resin and heat at 50 °C o/n. The reaction may be catalyzed by the addition of KI (0.1 eq.). At the end of this time, wash the resin with 3x DMF, 3x DMF/water (1:1), 3x DMF, 3x DCM, 3x MeOH. Dry *in vacuo* over KOH.

The completeness of the reaction can be checked by treating resin with a solution of 4-(4-nitrobenzyl)pyridine in DMF/DCM. A pink to violet color indicates the presence of unreacted halomethyl groups [15].

Alcohols can be coupled to chloromethyl-polystyrene by heating the resin in DMF or THF with the corresponding potassium or sodium alkoxide, which is normally generated *in situ* using an appropriate metal hydride

[16-19]. The addition of a phase transfer catalyst such as 18-crown-6 is often useful [1, 18]. Similar methods can be used for the immobilization of phenols, thiols and amines, although with amines no additional base is required. Phenols can also be attached to Merrifield resin by means of the corresponding cesium salt [20]. Release of the product alcohol or phenol can normally be effected by acidolysis or hydrogenolysis, using comparable methods to those described above for the acid-loaded resin or by cleavage with a Lewis acid, such as $SnCl_4$ [19]. Tertiary alcohols can be prepared by cleavage of resin-bound esters with Grignard reagents [21].
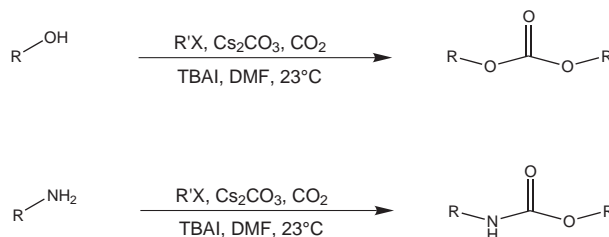
Fig. 2-10: Anchoring of alcohols and amines *via* carbonate and carbamate formation.

Salvatore, *et al* [22] have introduced a useful strategy for anchoring alcohols and amines to Merrifield resin involving formation of the corresponding carbonate and carbamate, respectively, by the reaction of the resin with alcohol or amine in the presence of $CsCO_3$, CO_2 and TBAI, see Figure 2-10.

For secondary amines, Conti, *et al.* [23] have devised the cleavage strategy shown in Figure 2-11. In a similar manner, polymer-supported quaternary amines derived from Merrifield resin can be cleaved by heating with morpholine to provide tertiary amines [24], see Figure 2-12.
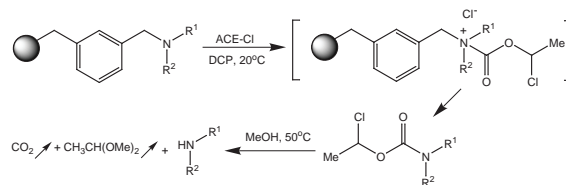
Fig. 2-11: Release of secondary amines from Merrifield resin.
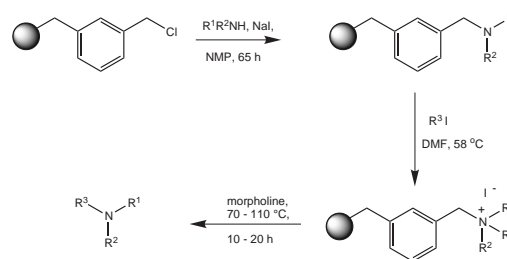
Fig. 2-12: Release of tertiary amines from Merrifield resin.

Hennequin & Piva-Le Branc [25] have found that mercaptoquinazolines immobilized on Merrifield resin could be displaced by nucleophiles, without prior conversion to the sulfone, see Figure 2-13.
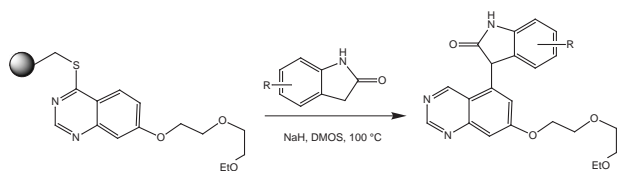
Fig. 2-13: Direct cleavage of mercaptoquinazolines from Merrifield resin.

After derivatization of the resin, unreacted chloromethyl functionalities can be removed by radical hydrogenolysis using Bu_3SnH [16, 26]. It is particularly important to do this prior to carrying out alkylation reactions.

Merrifield resin can be readily modified to provide a number of different resins suitable for SPOC. Hydroxymethyl polystyrene can be prepared by hydrazinolysis of acetyl- [27] or Boc-Gly Merrifield resin [28]. Oxidation with $DMSO/NaHCO_3$ converts Merrifield resin to the corresponding formyl support [29]; further oxidation with MCPBA or dichromate leads to formation of carboxypolystyrene.

A polymer-bound thiouronium salt, prepared from Merrifield resin by the reaction with thiourea, has been exploited in the combinatorial synthesis of pyrimidines [30] (Figure 2-14).
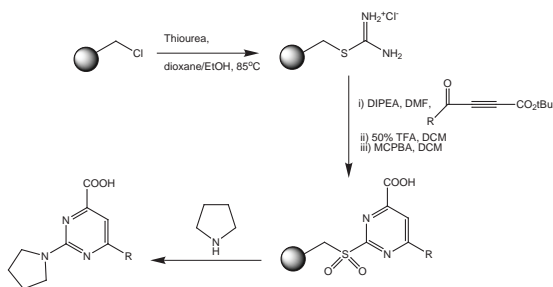
Fig. 2-14: Pyrimidine synthesis using isothiouronium resin.

Merrifield resin has also been used in a similar manner to produce piperazinomethyl polystyrene [31] and polystyrene methylenetriphenyl-phosphonium chloride [32], which have been utilized in the SPOS of α,β-unsaturated ketones (see Figure 2-15) and dihydropyrans (see Figure 2-16), respectively. The analogous phosphonium bromide has been used to prepare peptide aldehydes [33].
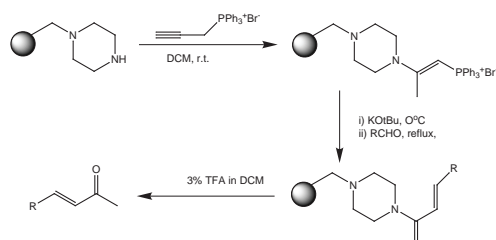
Fig. 2-15: Solid phase unsaturated ketone synthesis.
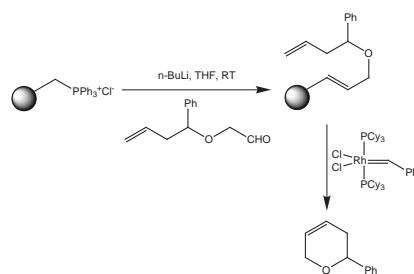
Fig. 2-16: Preparation of a dihydropyran *via* metathesis.

2.6.2 2–(4-Bromomethylphenoxy)ethyl polystyrene
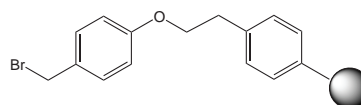
(4-Bromomethylphenoxy)ethyl polystyrene is a variant of 4-bromomethylphenoxymethyl polystyrene, in which the TFA-labile benzylic ether linker of the Wang linker has been replaced by a much more stable ethyl ether. The use of an ethylpolystyrene base resin eliminates the formation of hydroxybenzyl alcohol modified peptides which can arise with standard Wang resins.

Bromomethylphenoxy-type resins are extremely versatile tools for peptide and solid phase organic synthesis. The reaction of Fmoc-amino acid DIPEA salts in DMF containing CsI provides a mild, racemization-free method of loading Wang resin [34, 35] (Method 2-2). Phenols can be loaded using Method 2-1. Amination with both alkyl and aryl amines proceeds with high efficiency, leading to resin-bound secondary amines in yields of between typically 92-100%; the lowest value was recorded for electron deficient *p*-nitroaniline, which could be attached in a 78% yield [36]. This approach provides an excellent alternative to reductive alkylation methods for preparing secondary amides and sulfonamides by solid phase synthesis (see section 2.10, page 2.37), particularly in cases where racemization can occur during immobilization of the amine to the support. Thiols can be attached using DBU in toluene [37]. The completeness of these reactions can be checked using the NBP test described in Method 2-1 [18]. In the case of sulfonamides and anilides, cleavage can be effected under mild conditions using 95% TFA [38, 39], see Figure 2-17.

Primary amines and alcohols attached to (4-bromomethylphenoxy) type resins can be released by oxidative cleavage using DDQ [40], whereas secondary amines can be cleaved with ACE-Cl (Figure 2-11) [41], or as tertiary amides by treatment with acid chlorides (Figure 2-18) [42].
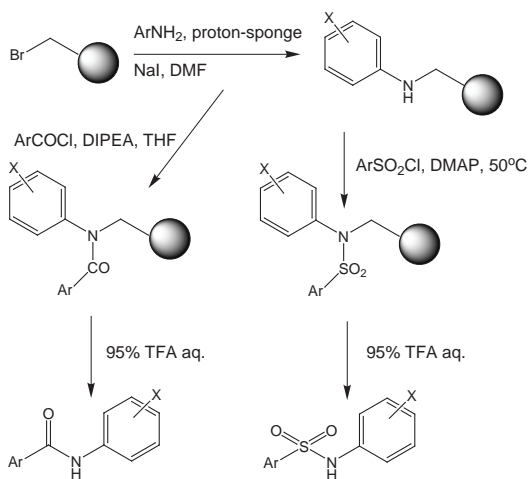
Fig. 2-17: SPOS of anilides.
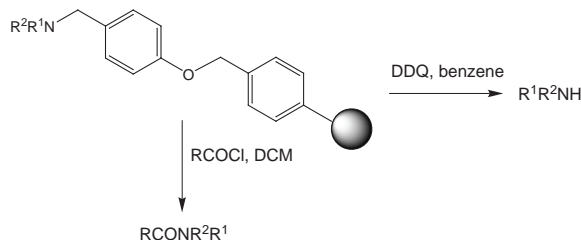
Fig. 2-18: Acylative and oxidative cleavage from methylphenoxy resins.

Method 2-2: Loading of bromomethylphenoxy resins

NOTE: it is important to dry all solvents and glassware before use.

Attachment of carboxylic acids
1. Pre-swell the resin for 1 h in DCM, then wash with DMF.
2. Dissolve the carboxylic acid (3 eq.), DIPEA (3 eq.) and CsI (0.3 eq.) in the minimum volume of DMF.
3. Add this to the resin and stir gently o/n at rt. At the end of this time, wash the resin with 3x DMF, 3x DCM, 3x hexane. Dry *in vacuo* over KOH.

Attachment of amines
1. Pre-swell the resin for 1 h in DCM, then wash with DMF.
2. Dissolve the amine (10 eq.) in DMF and add this to the resin. Leave to stir o/n at rt.
3. Wash the resin with 3x DMF, 3x DCM, 3x hexane. Dry *in vacuo* over KOH.
4. The loading can be determined in the case of primary amines by coupling an Fmoc amino acid to the resin with DIPCDI / HOAt and measuring the Fmoc content using the Fmoc U.V. assay.

Attachment of alcohols/phenols
1. Pre-swell the resin for 1 h in DCM, then wash with DMF.
2. Dissolve the alcohol (3 eq.) in the minimum volume of DMF. Add carefully NaH (3 eq., washed free of petroleum oil).
3. Add this slurry to the resin, together with KI (0.3 eq.) and stir gently o/n at 60 °C. At the end of this time, wash the resin with 3x DMF, 3x DCM, 3x hexane. Dry *in vacuo* over KOH.

Attachment of thiols
1. Pre-swell the resin for 1 h in toluene
2. Dissolve the thiol (10 eq.) and DBU (4 eq.) in the minimum volume of toluene. Add mixture to resin.
3. Stir gently o/n at rt. At the end of this time, wash the resin with 3x toluene, 3x DCM, 3x hexane. Dry *in vacuo* over KOH.

Related products

855104	2-(4-Bromomethylphenoxy)ethyl polystyrene HL	p. 228

2.6.3 Trityl resins

The two key steps in SPOC are the initial loading of the starting material and the final cleavage of synthesized product from the insoluble polymer support. In these respects, the standard matrices traditionally used in peptide synthesis such as Merrifield and Wang resin have limitations. These supports are not suitable for the immobilization of imino, guanidino, carboxamido and thiol functionalities, as the resulting products can not be cleaved without employing drastic measures. Attachment of hydroxyl functionalities is difficult, and the yields are often poor. Furthermore, cleavage of carboxyl or hydroxyl functionalities from these supports requires treatment with strong acids such as HF or TFA.

These problems can be overcome through the use of trityl-based resins. Almost any nucleophilic functionality can be linked to these resins under extremely mild conditions (Method 2-3). Bernhardt, *et al.* [43] have demonstrated that most of the side-chains of the trifunctional amino acids can be attached to 2-chlorotrityl resin, albeit with varying degrees of efficiency. Furthermore, symmetrical bifunctional compounds, such as diamines, diols, diphenols and diacids, are in effect monoprotected by this process, allowing one end of the molecule to be selectively modified.

Cleavage of products from these supports takes place under very mild conditions, owing to the high stability of trityl cations. Furthermore, trityl cations are extremely poor electrophiles and as such do not undergo alkylation side-reactions.

The properties of Novabiochem® trityl resins and other trityl resins are summarized in Table 2-7. It is important to note that in addition to being acid sensitive, some loaded-trityl resins are also sensitive to heat. Therefore, these supports should not be subjected to excessive heating as this may give rise to some loss of product.

2.6.4 Trityl chloride resin
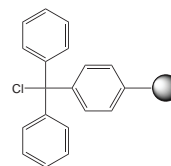
Trityl chloride resin has been used for many years for the solid phase immobilization of alcohols: notably by Leznoff in the 70s for preparation of insect sex attractants [44-46] (Figure 2-19) and phthalocyanines [47]; by Chen, *et al.* in their combinatorial synthesis of β-mercaptoketones [48, 49]; by Gennari, *et al.* in the synthesis of polyketide libraries [50]; and by De Luca, *et al.* [51] to immobilize alkynyl alcohols as precursors to isoxazoles.

Fig. 2-19: SPOS of oak leaf roller sex attractant.

Cleavage of alcohols from this resin has been effected with HCl in dioxane [44-46], TFA in DCM (Method 2-19, page 2.46), PTSA in THF/MeOH [50] and HCO$_2$H/THF [49]. In the case of TFA, formation of trifluoroacetates can be a problem, although these may be hydrolyzed by treatment with sodium carbonate. It is interesting to note that cleavage of allyl alcohols with HBr/AcOH results in release of the product as the bromide [52].

Trityl chloride resin is also very useful for the immobilization of amines (Method 2-3, page 2.17), since detachment of simple alkyl amines can be effected under extremely mild conditions, using either 30% HFIP in DCM or 1% TFA in DCM [53]. Manku, *et al.* [54] have exploited these properties to prepare polyamines *via* on-resin borane reduction of amides (Figure 2-20).

In compounds containing both amino and hydroxyl groups, the polymer-bound trityl chloride reacts preferentially with the amino group; this observation has been used to prepare indolizidine and quinolizidines [55], and libraries based on 3,4-diaminocyclopentanol (Figure 2-21) [56].

Method 2-3: Loading of trityl resins

NOTE: it is important to dry all solvents and glassware before use.

Attachment of carboxylic acids

1. Dissolve the carboxylic acid (0.6-1.2 eq. relative to the resin for 2-ClTrityl resin and 2 eq. for NovaSyn® TGT chloride resin) and DIPEA (4 eq. relative to carboxylic acid) in dry DCM (approx. 10 ml per gram of resin) containing, if necessary, a small amount of dry DMF (just enough to facilitate dissolution of the acid). For pseudoproline dipeptides add 3ml of NMP/gram of resin.
2. Add this to the resin and stir for 30-120 min. For pseudoproline dipeptides leave to react o/n. At the end of this time, wash the resin with 3x DCM/MeOH/DIPEA (17:2:1), 3x DCM; 2x DMF, 2x DCM. Dry *in vacuo* over KOH.

Fmoc-amino acids are best dried before use by repeated evaporation from dioxane; determine loading using Method 3-6, page 3.6.

Attachment of imidazoles

1. Dissolve the imidazole (2 eq.) and DIPEA (4 eq.) in dry DCM (approx. 10 ml per gram of resin).
2.. Add this to the resin and stir for 120 min. At the end of this time, wash the resin with 3x DCM/MeOH/DIPEA (17:2:1), 3x DCM; 2x DMF, 2x DCM. Dry *in vacuo* over KOH.

Attachment of amines

1. Dissolve the amine (2-4 eq.) in dry THF (approx. 10 ml per gram of resin). Normally with aliphatic amines no additional base is required.
2. Add this to the resin and stir for 120 min. At the end of this time, wash the resin with 3x DCM/MeOH/DIPEA (17:2:1), 3x DCM; 2x DMF, 2x DCM. Dry *in vacuo* over KOH.

Attachment of alcohols and phenols

1. Dissolve the alcohol or phenol (2 eq.) and pyridine (4 eq.) in THF (approx. 10 ml per gram of resin).
2. Add this to the resin and heat at 60 °C with stirring for 2-6 h. At the end of this time, wash the resin with 3x DCM/MeOH/DIPEA (17:2:1), 3x DCM; 2x DMF, 2x DCM. Dry *in vacuo* over KOH.

Table 2-7: Properties of trityl resins.

Resin / Function	R-CO-OH (A)	(C)	R-CH₂OH (A)	(C)	R₂CH-OH (A)	(C)	phenol-OH (A)	(C)	FmocNH-OH (A)	(C)
Clt resin	V	H	F	H	S	H	F	H	F	H
Trt Resin	V	H	F	H	S	E	F	H	F	H
Mtt Resin	V	E	F	H	S	E	F	E	F	H
Mmt Resin	V	E	F	E	S	E	F	E	F	E

Resin / Function	NH₂–NH₂ (A)	(C)	Ph–NH₂ (A)	(C)	Me₃SiO–NH₂ (A)	(C)	guanidine (A)	(C)	H₂N–NH₂ (A)	(C)
Clt resin	F	M	F	M	F	M	F	L	F	H
Trt Resin	F	H	F	M	F	H	F	L	F	H
Mtt Resin	F	E	F	H	F	E	F	L	F	H
Mmt Resin	F	E	F	H	F	E	F	M	F	E

Resin / Function	Alkyl–NH₂ (A)	(C)	imidazole (A)	(C)	R–SH (A)	(C)	Me₃SiO–NH₂ (A)	(C)	H₂N–CHR–CO₂R (A)	(C)
Clt resin	F	H	F	L	V	L	F	M	F	H
Trt Resin	F	H	F	M	V	L	F	H	F	H
Mtt Resin	F	E	F	H	V	M	F	E	F	E
Mmt Resin	F	E	F	H	V	H	F	E	F	E

A = ease of attachment
V = fast
F = moderate
S = slow

C = acid sensitivity of resin-function bond
L = >50% TFA in DCM
M = >5% TFA in DCM
H = <5% TFA in DCM
E = extreme sensitivity (generally too labile for use)

Trityl chloride resin has also been used in the same manner as 2-chlorotrityl chloride resin to immobilize protected hydroxylamine in the synthesis of hydroxamic acids [57], and more recently, to anchor tetrazoles, in the preparation of a small library of alkyl tetrazole derivatives [58] (Figure 2-22).
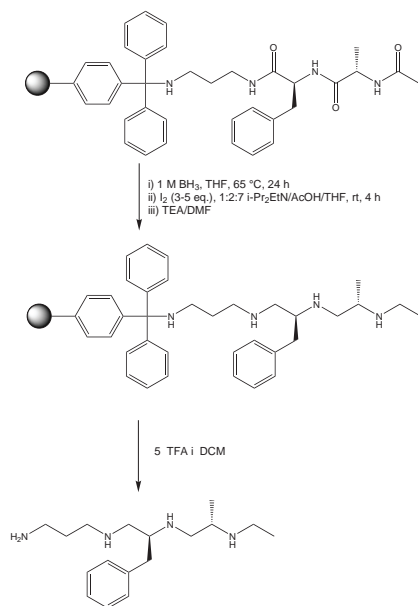
Fig. 2-20: Synthesis of polyamines by on-resin borane reduction.
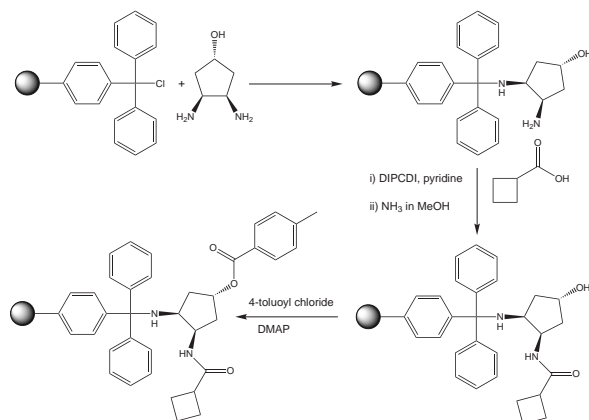
Fig. 2-21: Diaminocyclopentanol libraries [56].
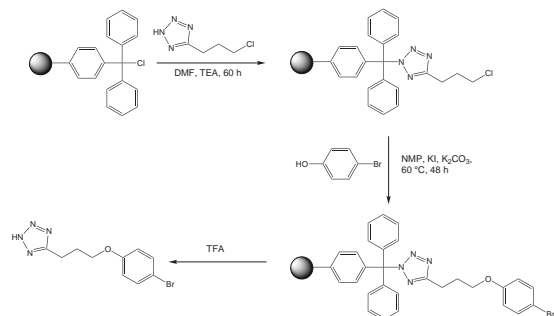
Fig. 2-22: Synthesis of tetrazole derivatives using trityl chloride resin.

2.6.5 2–Chlorotrityl chloride resin
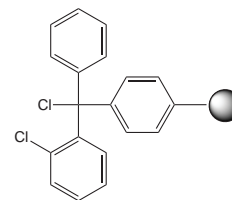
Despite its slow initial acceptance by peptide chemists, 2-chlorotrityl chloride polystyrene has proved to be one of the most significant developments in linker technology [59-64]. Unlike conventional benzyl alcohol-based supports, such as Wang resin, attachment of α-amino acids to 2-chlorotrityl is free from racemization [64] (Method 2-3, page 2.17), making it ideal for the immobilization of sensitive residues such as Cys and His. It is particularly useful in the synthesis of C-terminal prolyl peptides where the bulk of the trityl linker helps to prevent diketopiperazine formation [59, 65, 66]. The use of this resin also minimizes many other side reactions associated with benzyl-based supports, such as racemization of C-terminal Cys residues during chain extension and reattachment of Met and Trp residues during TFA-mediated cleavage and side-chain deprotection. Cleavage of peptide acids, and other carboxylic acids, from 2-chlorotrityl chloride resin can be effected by treatment with AcOH/TFE/DCM [59-64], 1% TFA in DCM (Method 3-30, p. 3.30), 20% HFIP in DCM [67] or 20% TFE in DCM (Method 3-31, p. 3.30).

This resin can also be used for anchoring imidazoles [68], alcohols [53, 69, 70], phenols [53, 71-74], amines [54, 75-80], and hydroxylamine [81, 82] (Method 2-3, page 2.17). Release of these functionalities is generally achieved using 1-50% TFA in DCM containing 1% TIS (Method 2-19, page 2.46).

Nash and co-workers [76] used this support to prepare philanthotoxin-343, an analog of the polyamine-based wasp toxin philanthotoxin-433. In this work, they exploited the "fish-hook" principle [83] and the ability of Dde to react specifically with primary amines, to prepare on the solid phase the key selectively protected spermine (**1**, Figure 2-23), from which philanthotoxin-343 could be synthesized in four steps. Furthermore, the fish-hook principle was used to prepare libraries of monoacyl diamines [77] and to immobilize piperazine for use as the amine component in solid phase Mannich reactions (Figure 2-24) [78]. Youngman & Dax [79] have utilized a similarly immobilized propargylamine as the acidic component in solid phase Mannich reactions, and Hoekstra, *et al.* [80] immobilized piperidines to prepare a range of nipecotamide-based GPIIb/IIIa antagonists. Another SPOS application of 2-chlorotrityl resin is the synthesis of 4-substituted imidazoles using polymer-supported Grignard reagents [68] (Figure 2-25).
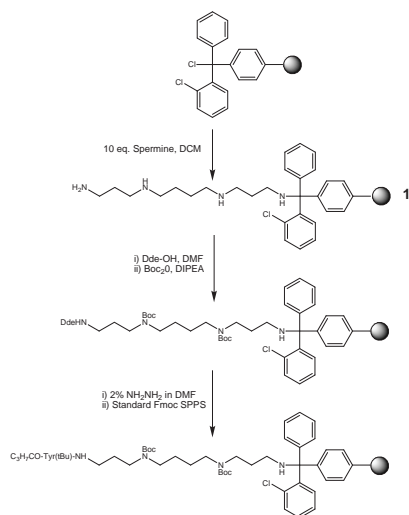
Fig. 2-23: SPOS of Philanthotoxin-343.
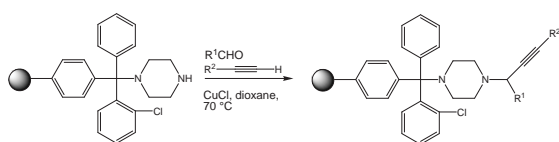
Fig. 2-24: Solid phase Mannich reaction utilizing a resin-bound piperazine.
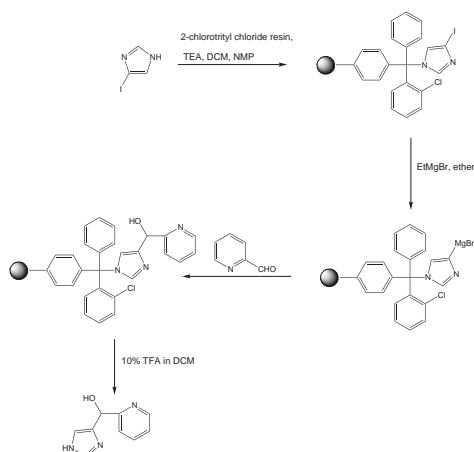
Fig. 2-25: SPOS of substituted imidazoles.

2.6.6 NovaSyn® TGT alcohol resin
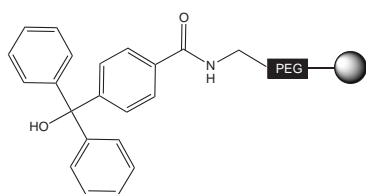
NovaSyn® TGT alcohol resin is derived from NovaSyn® TG amino resin by acylation with Bayer's 4-carboxytrityl linker [84]. The chemical properties of this resin are almost identical to those of 2-chlorotrityl resin, but it additionally offers the advantages of the highly polar PEG-PS base matrix.

In common with 2-chlorotrityl resin, this support protects C-terminal Cys against racemization and helps minimize diketopiperazine formation in peptides containing proline [85].

Before use this resin must first be converted to the chloride form by heating with AcCl [86] or $SOCl_2$ [87, 88] in toluene (Method 2-4). The unstable trityl chloride resin should then be used immediately. NovaSyn® TGT alcohol resin has been employed to prepare a range of immobilized amino alcohols and diamines for use as building blocks in the solid phase synthesis of a library of perhydro-1,4-diazepine-2,5-diones. The final resin cleavage step was effected using TFA vapor [89].

Method 2-4: Chloridation of NovaSyn® Trityl resins

NOTE: it is important to dry all solvents and glassware before use.
1. Place NovaSyn® TGT alcohol resin in a sintered glass funnel and wash the resin consecutively with DMF (2x), dry DCM (3x) and dry toluene (3x).
2. Drain off excess toluene from the resin and transfer damp material to a round bottom flask equipped with a reflux condenser.
3. Add sufficient toluene to cover resin, then add freshly distilled AcCl (1 ml/g of resin). Heat at 60-70 °C for 3 h.
4. Slurry mixture to a sintered glass funnel. Wash resin with dry toluene (3x) and dry DCM (3x).
5. Drain excess solvent from resin and use immediately.

Related products
855010   NovaSyn® TGT alcohol resin   p. 205

2.6.7 NovaSyn® TG bromo resin

NovaSyn® TG bromo resin [90] can be used for the immobilization of alcohols, carboxylic acids, phenols and thiols. Loading of this support can be carried out using the same methods as those previously described for Merrifield resin. This support has been used as the basis of a benzoxyaniline linker, which is cleaved by aqueous ceric ammonium nitrate [91], and a thioether-based safety catch linker, which is activated towards elimination by oxidation with aqueous oxone [92].

Related products
855063   NovaSyn® TG bromo resin   p. 230

References
1. B. F. Gisin (1973) *Helv. Chim. Acta*, **56**, 1476.
2. S. Sharma, et al. (1997) *Bioorg. Med. Chem. Lett.*, **7**, 2077.
3. A. Loffet (1971) *Int. J. Protein Res.*, **3**, 297.
4. S. S. Wang (1975) *J. Org. Chem.*, **40**, 1235.
5. M. J. Kurth, et al. (1994) *J. Org. Chem.*, **59**, 5862.
6. M. V. Anuradha, et al. (1995) *Tetrahedron*, **51**, 5671.
7. J. M. Schlatter, et al. (1977) *Tetrahedron Lett.*, 2851.
8. S. Chamoin, et al. (1998) *Tetrahedron Lett.*, **39**, 4175.
9. R. L. E. Furlán, et al. (1998) *Tetrahedron*, **54**, 13023.
10. J. M. Stewart & J. D. Young in "Solid Phase Peptide Synthesis, 2nd Ed.", Pierce Chemical Company, Rockford, 1984, pp. 92.
11. R. Frenette, et al. (1994) *Tetrahedron Lett.*, **35**, 9177.
12. S. K. Kang, et al. (1998) *Tetrahedron Lett.*, **39**, 3011.
13. L. F. Tietze & A. Steinmetz (1996) *Angew. Chem. Int. Ed. Engl.*, **35**, 651.
14. D. R. Barn, et al. (1996) *Tetrahedron Lett.*, **37**, 3213.
15. F. Galindo, et al. (2004) *J. Comb. Chem.*, **6**, 859.
16. C. C. Leznoff, et al. (1973) *Can. J. Chem.*, **51**, 3756.
17. C. R. McArthur, et al. (1982) *Can. J. Chem.*, **60**, 1836.
18. H.-S. Moon, et al. (1994) *Tetrahedron Lett.*, **35**, 8915.

19. D. Stones, et al. (1998) *Tetrahedron Lett.,* **39**, 4875.
20. J. Beythien, Personal communication.
21. S. Chandrasekhar, et al. (2000) *J. Comb. Chem.,* **2**, 246.
22. R. Salvatore, et al. (2002) *Tetrahedron Lett.,* **58**, 3329.
23. P. Conti, et al. (1997) *Tetrahedron Lett.,* **38**, 2915.
24. J. Cai & B. Wathey (2001) *Tetrahedron Lett.,* **42**, 1383.
25. F. Hennequin & S. Piva-Le Blanc (1999) *Tetrahedron Lett.,* **40**, 3881.
26. P. M. Worster, et al. (1979) *Angew. Chem. Int. Ed. Engl.,* **18**, 221.
27. J. M. Stewart, et al. in "Solid Phase Peptide Synthesis", (Eds.), Pierce Chemical Company, Rockford, 1984, pp. 54.
28. D. J. Burdick, et al. (1993) *Tetrahedron Lett.,* **34**, 2589.
29. J. T. Ayres, et al. (1965) *J. Polym. Sci., Polym. Lett. Ed.,* **3**, 505.
30. D. Obrecht, et al. (1997) *Helv. Chim. Acta,* **80**, 65.
31. N. W. Hird, et al. (1997) *Tetrahedron Lett.,* **38**, 7111.
32. A. D. Piscopio, et al. (1997) *Tetrahedron Lett.,* **38**, 7143.
33. B. J. Hall & J. D. Sutherland (1998) *Tetrahedron Lett.,* **39**, 6593.
34. J. W. Corbett, et al. (1997) *Bioorg. Med. Chem. Lett.,* **7**, 1371.
35. G. A. Morales, et al. (1998) *J. Org. Chem.,* **63**, 1172.
36. K. Ngu & D. V. Patel (1997) *Tetrahedron Lett.,* **38**, 973.
37. T. Zoller, et al. (2000) *Tetrahedron Lett.,* **41**, 9989.
38. B. Raju & T. P. Kogan (1997) *Tetrahedron Lett.,* **38**, 4965.
39. P. White, unpublished results.
40. S. Kobayashi & Y. Aoki (1998) *Tetrahedron Lett.,* **39**, 7345.
41. X.-Y. Xiao, et al. (2002) *J. Comb. Chem.,* **4**, 536.
42. M. W. Miller, et al. (1998) *Tetrahedron Lett.,* **39**, 3429.
43. A. Bernhardt, et al. (1997) *J. Peptide Res.,* **50**, 143.
44. T. M. Fyles, et al. (1978) *Can. J. Chem.,* **56**, 1031.
45. C. C. Leznoff, et al. (1982) *Tetrahedron Lett.,* **23**, 3023.
46. C. C. Leznoff, et al. (1977) *Can. J. Chem.,* **55**, 1143.
47. C. C. Leznoff, et al. (1976) *J. Chem. Soc., Chem. Commun.,* 251.
48. C. Chen, et al. (1994) *J. Am. Chem. Soc.,* **116**, 2661.
49. C. Chen, et al. (1997) *Tetrahedron,* **53**, 6595.
50. C. Gennari, et al. (1998) *Tetrahedron,* **54**, 14999.
51. L. De Luca, et al. (2001) *J. Org. Chem.,* **66**, 6823.
52. J. M. Frechet & L. J. Nuyens (1976) *Can. J. Chem.,* **54**, 926.
53. P. Athanassopoulos, et al., Poster P316, 24th European Peptide Symposium, Edinburgh, 1996 (not published in the conference proceedings).
54. S. Manku, et al. (2001) *J. Org. Chem.,* **66**, 874.
55. A. Barco, et al. (2000) *J. Comb. Chem.,* **2**, 337.
56. Y. Guan, et al. (2000) *J. Comb. Chem.,* **2**, 297.
57. U. Bauer, et al. (1997) *Tetrahedron Lett.,* **38**, 7233.
58. D. P. Matthews, et al. (2000) *J Comb. Chem.,* **2**, 19.
59. K. Barlos, et al. (1989) *Tetrahedron Lett.,* **30**, 3947.
60. K. Barlos, et al. (1989) *Tetrahedron Lett.,* **30**, 3943.
61. K. Barlos, et al. (1991) *Angew. Chem. Int. Ed. Engl.,* **30**, 590.
62. K. Barlos, et al. (1991) *Int. J. Peptide Protein Res.,* **37**, 513.
63. K. Barlos, et al. (1991) *Int. J. Peptide Protein Res.,* **38**, 562.
64. K. Barlos, et al. (1991) *Int. J. Peptide Protein Res.,* **38**, 555.
65. K. Barlos, et al. (1993) *Ann. Chem.,* 215.
66. R. Steinauer, et al. in "Innovations & Perspectives in Solid Phase Synthesis", R. Epton (Ed.), Mayflower Scientific Ltd., Birmingham, 1994, pp. 689.
67. R. Bollhagen, et al. (1994) *J. Chem. Soc., Chem. Commun.,* 2559.
68. E. Gelens, et al. (2000) *Bioorg. Med. Chem. Lett.,* **10**, 1935.
69. H. Wenschuh, et al. (1995) *J. Org. Chem.,* **60**, 405.
70. C. L. Lee & M. M. Sim (2000) *Tetrahedron Lett.,* **41**, 5729.
71. Z. Zhu & B. Mckittrick (1998) *Tetrahedron Lett.,* **39**, 7479.
72. U. Heinelt, et al. (2001) *Bioorg. Med. Chem. Lett.,* **11**, 227.
73. K. J. Elgie, et al. (2000) *Tetrahedron Lett.,* **41**, 2753.
74. S. Batra, et al. (2000) *Tetrahedron Lett.,* **41**, 5971.
75. M. Cardno, et al. (1995) *J. Chem. Soc., Chem. Commun.,* 2163.
76. I. A. Nash, et al. (1996) *Tetrahedron Lett.,* **37**, 2625.
77. J. Perumattam, et al. (1998) *Mol. Div.,* **3**, 121.
78. J. J. McNally, et al. (1998) *Tetrahedron Lett.,* **39**, 967.
79. M. A. Youngman & S. L. Dax (1997) *Tetrahedron Lett.,* **38**, 6347.
80. a) W. J. Hoekstra, et al. (1996) *Bioorg. Med. Chem. Lett.,* **6**, 2371; b) W. J. Hoekstra, et al. (1997) *Tetrahedron Lett.,* **38**, 2629.
81. S. L. Mellor, et al. (1997) *Tetrahedron Lett.,* **38**, 3311.
82. M. M. Meloni & M. Taddei (2001) *Org. Lett.,* **3**, 337,
83. C. C. Leznoff (1974) *Chem. Soc. Rev.,* **3**, 65.
84. E. Bayer, et al. in "Peptides; Chemistry, Structure & Biology, Proc. 13th American Peptide Symposium", R. S. Hodges & J. A. Smith (Eds.), ESCOM, Leiden, 1994, pp. 156.

85. G. Grübler, et al. in "Innovations & Perspectives in Soli Phase Synthesis, 2nd International Symposium", R. Epton (Ed.), Intercept Ltd., Andover, 1992, pp. 475.
86. T. M. Fyles & C. C. Leznoff (1976) *Can. J. Chem.,* **54**, 935.
87. A. van Vliet, et al. in "Peptides 1992, Proc. 22nd European Peptide Symposium", C. H. Schneider, A. N. Eberle (Eds.), ESCOM, Leiden, 1993, pp. 279.
88. A. van Vliet, et al. in "Peptides; Chemistry, Structure & Biology, Proc. 13th American Peptide Symposium", R. S. Hodges & J. A. Smith (Eds.), ESCOM, Leiden, 1994, pp. 151.
89. V. Krchňák, et al. (1997) *Tetrahedron Lett.,* **38**, 7299.
90. a) W. Rapp, et al. in "Peptides 1988; Proc. 20st European Peptide Symposium", G. Jung & E. Bayer (Eds.), Walter de Gruyter, Berlin, 1989, pp. 199; b) W. Rapp, et al. in "Innovation and Perspectives in Solid Phase Synthesis; 1st International Symposium", R. Epton (Ed.), SPCC (UK) Ltd, Birmingham, 1990, pp. 205; c) L. Zhang, et al. in "Peptides 1990; Proc. 21st European Peptide Symposium", E. Giralt & D. Andreu (Eds.), ESCOM, Leiden, 1990, pp. 196; d) E. Bayer (1991) *Angew.Chem. Int. Ed. Engl.,* **30**, 113.
91. K. H. Gordon & S. Balasubramanian (2001) *Org. Lett.,* **3**, 53.
92. D. Tumelty, et al. (2001) *Org. Lett.,* **3**, 83.

2.7   Amino, hydrazino functionalized resins

The applications of Novabiochem®'s amino functionalized resins are given in Table 2-6.

Attachment of amino acid derivatives and other carboxylic acids to linkers containing primary amino groups can normally be effected using standard methods of amide bond formation. Hydroxylamine, Weinreb amide, and resins functionalized with secondary amines are much more difficult to load; for these the use of Oxyma Pure/DIPCDI or HATU/DIPEA activation is required.

Method 2-5: Attachment of carboxylic acids to amino resins

1. Pre-swell the resin with DCM (polystyrene-based resins) or DMF (PEG-PS or PEGA resin) for 1 h. Wash resin thoroughly with DMF.
2. If the resin is Fmoc protected, treat with 20% piperidine in DMF for 20 min, then wash resin thoroughly with DMF.

Rink Amide, Sieber Amide, MBHA

3. Dissolve amino acid derivative or carboxylic acid (5 eq.) and Oxyma Pure in DMF. Add DIPCDI (5 eq.) and leave to stand for 10 min. Add mixture to resin. Leave for 1-6 h.

N-Alkylamino resins

4. Dissolve amino acid derivative or carboxylic acid (5 eq.) and HATU in DMF. Add DIPEA (10 eq.) and add immediately to resin. Leave for 6 h.
5. Remove a small quantity of resin and test this for the presence of unreacted amines using the TNBS test (primary amine resins) or chloranil test (secondary amine resins) as described in Methods 3-8 and 3-9, page 3.6. If the result is positive, wash the resin with DMF and repeat coupling. Continue this procedure until a negative result is obtained.

2.7.1 AM Polystyrene
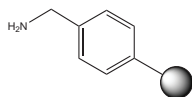
AM polystyrene can be easily acylated with any carboxylic acid-containing linker using standard methods of amide bond formation, to furnish supports for solid phase peptide and organic synthesis. Amides prepared on this resin are stable to acid but can be cleaved by hydrogenolysis.

Related products		
855115	Aminomethylated polystyrene LL (100-200 mesh)	p. 188
855020	Aminomethylated polystyrene HL (100-200 mesh)	p. 188

2.7.2 NovaSyn® TG amino resin

NovaSyn® TG amino resin [1] is a composite of low cross-linked polystyrene and 3000-4000 M.W. polyethylene glycol, which has been terminally amino functionalized. The 90 or 130 µm beads have a narrow size distribution, high diffusion rates, and excellent swelling properties in solvents from toluene to water, making them ideally suited to the synthesis of serial and parallel libraries by organic solid phase synthesis. Resins are available with loadings of 0.2-0.3 and 0.3-0.6 mmole/g.

Gowravaram and Gallop [2] have utilized NovaSyn® TG amino resin as a tether for isomünchnones, which were generated by rhodium catalyzed decomposition of resin-bound diazoimides, in their elegant traceless linker approach to furans. These highly reactive intermediates readily add to electron deficient acetylenes, to form bicyclic intermediates that undergo cycloreversion to furans with concomitant cleavage from the support (Figure 2-26).
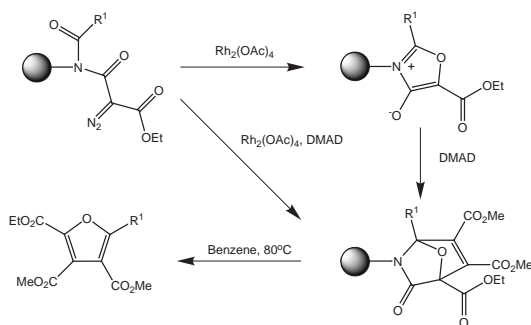
Fig. 2-26: SPOS of furans from resin-bound isomünchnones.

Related products		
855007	NovaSyn® TG amino resin (90 µm)	p. 191
855014	NovaSyn® TG amino resin (130 µm)	p. 191
855073	NovaSyn® TG amino resin HL	p. 191

2.7.3 MBHA polystyrene
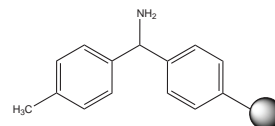
MBHA resin is based on 100-200 mesh, 1% DVB cross-linked polystyrene functionalized with a 4-methylphenyl-aminomethyl group. These resins are the most widely used resins for synthesis of peptide amides by the Boc strategy [3]. Amino acids or carboxylic acids can be attached to the amino group of the resin using standard methods of amide bond formation. Cleavage of the peptide amides and other carboxamides from this resin can be achieved by treatment with HF, TFMSA or HBF$_4$ (section 3.7, page 3.24). In Fmoc SPPS, this resin has been used in conjunction with a low-high acid cleavage: where side-chain protecting groups were first removed using reagent K prior to cleavage from the resin with TFMSA [4].

The excellent swelling properties of this resin make it an ideal matrix onto which to attach TFA-labile linkers for combinatorial synthesis, although care must be taken to avoid strongly acidic conditions during synthesis as this may result in some leaching of the linker . MBHA resin has been extensively utilized by Houghten and coworkers in their libraries-from-libraries strategy to prepare heterocycles. Examples of this approach include imidazolidinones (Figure 2-27) [5], bicyclic guanidines (Figure 2-28) [6], and bis-piperazines [7]. In these examples, amines rather than the usual amides were cleaved. A further example of such "traceless synthesis" using MBHA resin has been described by Krchňák, et al. [8] (Figure 2-29).
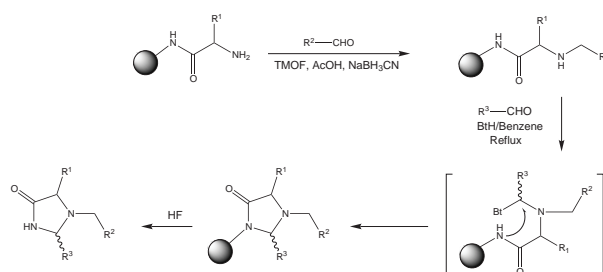
Fig. 2-27: SPOS of imidazolidinones.
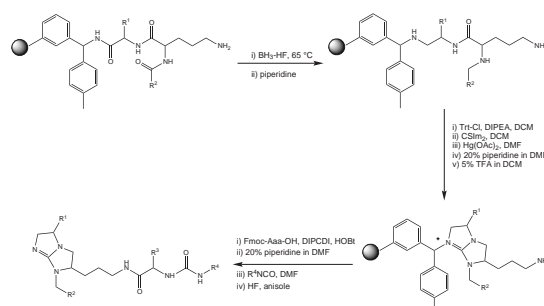
Fig. 2-28: SPOS of urea-linked bicyclic guanidines.
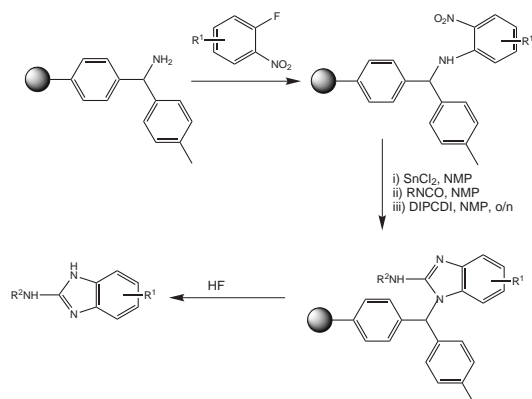
Fig. 2-29: SPOS of benzimidazoles.

Related products		
855000	MBHA resin LL (100-200 mesh) · HCl	p. 223
855006	MBHA resin HL (100-200 mesh) · HCl	p. 223

2.7.4 Rink Amide resins

Novabiochem® offers a wide range of polystyrene, TG, PEGA and NovaGel™-based supports derivatized with the Fmoc-Rink Amide linker for the preparation of peptide amides by Fmoc SPPS [9]. The linker amino function is easily acylated using standard methods of amide formation (Method 2-5, page 2.20). Cleavage of peptide amides and other carboxamides from these supports is usually effected with 95% TFA (Methods 3-26 & 2-19, pages 3.29 & 2.46).

For rout ine synthesis of peptide amides, resins such as Rink Amide AM and Rink Amide MBHA are recommended as these produce less linker by-products during TFA-cleavage than Rink Amide resin.
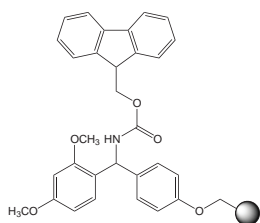
Rink Amide resin

Rink Amide resin is more acid sensitive than Rink Amide AM and Rink Amide MBHA resins because the benzyhydrylamine linker is joined to the support through a benzylic ether bond rather than through an electron-withdrawing acetamido spacer. Lacking any amide groups, Rink Amide resin, therefore, has a broader range of chemical compatibility than other Rink Amide resins, particularly towards strong reducing agents. Breakdown of the linker during cleavage with high concentrations of TFA can, however, occur, leading to the formation of highly colored by-products. Fortunately, these problems can be minimized through the use of low concentrations of TFA, or by the addition of trialkylsilanes to the cleavage mixture.

Resin-bound imines generated from Rink Amide resin have been employed on a number of occasions as intermediates in synthesis. Katritzky and co-workers [10] reacted resin-bound imines with Grignard and organolithium reagents to afford, after TFA cleavage, primary amines. Reduction with NaCNBH$_3$ in THF/AcOH/water was used by Brown & Nuss

[11] to produce resin-bound secondary amines from which were generated secondary amides following acylation and TFA cleavage. Rink Amide resin has also been employed as an ammonia equivalent in Ugi MCR reactions [12, 13]. In each of these examples the resin functions as a traceless linker (Figure 30).
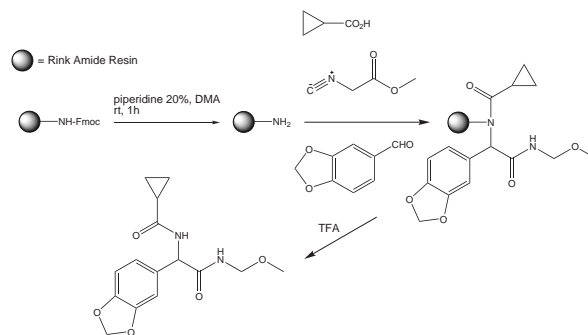
Fig. 2-30: Use of Rink Amide resin as traceless linker.

Treatment of Rink Amide-derivatized crowns with 70% TFA in DCM has been used to generate polymer-bound cations, which were then employed to trap amines and alcohols in good yields [14].

Rink Amide AM, Rink/Knorr Amide MBHA, NovaSyn® TGR, NovaPEG Rink Amide & Rink Amide PEGA/NovaGel™ resins

Rink Amide MBHA/Rink Amide AM
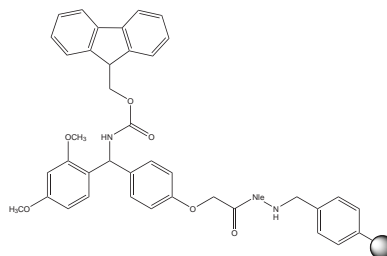
Rink Amide AM and Rink Amide MBHA resins consist of aminomethyl (100-200 mesh, 1% DVB) and 4-methylbenzhydrylamine (100-200 mesh, 1% DVB) polystyrene respectively, derivatized sequentially with norleucine and the Fmoc Rink Amide linker. This modified form of the Rink Amide linker, which incorporates an acetic acid spacer, is not degraded by TFA, and is therefore compatible with the standard 95% TFA cleavage reaction.

Rink Amide SpheriTide
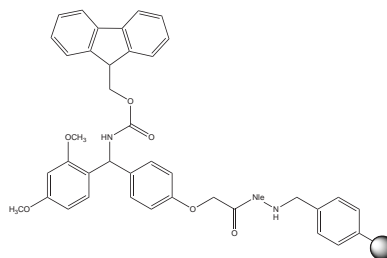
Rink Amide SpheriTide is based on a novel ultra-high load cross-linked polylysine resins (see section 2.3.5) that has been derivatized with Fmoc protected modified Rink Amide linker. These resins have nominal loadings

of 1.2 mmol/g and a very high loading to swell volume ratio, making them perfect for large scale production of peptide amides by Fmoc SPPS.

NovaSyn® TGR/NovaPEG Rink Amide/Rink Amide PEGA
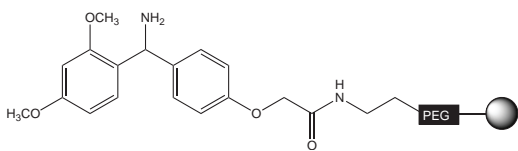
NovaSyn® TGR, NovaSyn® TGR R, NovaPEG Rink Amide, Rink Amide PEGA, NovaPEG Rink Amide resin, and Rink Amide NovaGel™ are based on the same linker but attached directly to TG amino, NovaPEG, PEGA and aminomethyl NovaGel™ resins, respectively. They are supplied with a free amino group as this has been found to increase the stability of these products. This also means that the resin can be used directly without the need for a preliminary deprotection cycle.

NovaSyn® TGR was employed by Virgilio & Ellman [15] in the SPOS of β-turn mimics. This work could only be completed using TG resin as polystyrene-based supports were not swollen by the aqueous solvent mixtures required to effect reduction of the intermediate resin-bound disulfide.

In a SPOS of propargylamines by a combination of Sonogashira & Mannich reactions, NovaSyn® TGR was found to give better results than Rink Amide resin (Figure 2-31) [16].
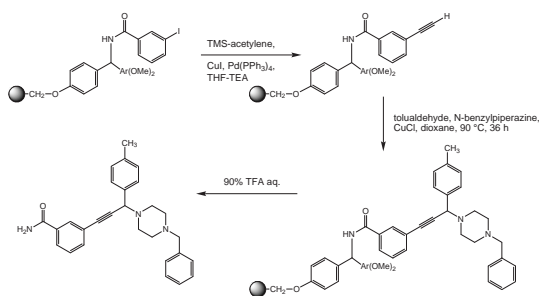
Fig. 2-31 SPOS of propargylamines.

Knorr Amide MBHA
This version of Rink amide resin has a higher substitution than Rink Amide MBHA as it does not contain the norleucine spacer.

2.7.5 PAL resins

Fmoc–PAL AM resin

PAL–NovaPEG resin
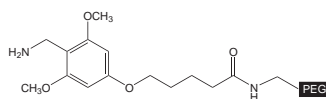
PAL NovaSyn® TG resin
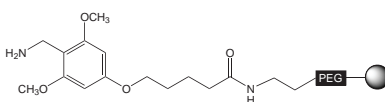
PAL resins are excellent supports for the synthesis of peptide amides by Fmoc SPPS. They consist of Barany's aminomethyl-dimethoxyphenoxy-valeric acid linker [17] attached to aminomethylated polystyrene, NovaSyn® TG or NovaPEG resin. The amino group of this linker can be easily acylated under standard coupling conditions. Following peptide assembly, treatment with 95% TFA containing scavengers releases the desired peptide amide. Studies have shown the acid sensitivity of this linker to be around twice that of the Rink amide linker [18]. There is some evidence to suggest that PAL resins give greater yields in microwave assisted synthesis.

2.7.6 Ramage Amide AM resin
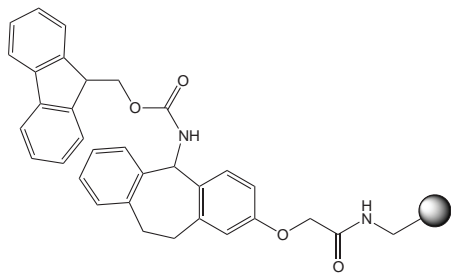
The Ramage linker [19] is considerably more acid sensitive than the Rink amide or PAL linkers. This enables peptide amides to be released from the resin with 3% TFA in DCM and thus makes it a useful tool for the synthesis of acid sensitive peptides or protected peptide fragments.

Related products		
855134	Ramage Amide AM resin	p. 197

2.7.7 Sieber Amide resins
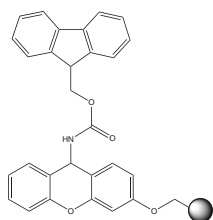
Sieber Amide resin [20] and NovaSyn® TG Sieber Amide resin are the preferred supports for the preparation of protected peptide amide fragments and acid-labile carboxamides as product release can be realized by mild acidolysis with 1% TFA in DCM (Method 3-30, page 3.30). The amino group of Sieber-type resins is less hindered than that of Rink Amide resins, making it better suited to applications sensitive to steric factors. Thus, Sieber Amide resin was found to give better results than Rink Amide resin in the preparation of peptide C-terminal secondary amides *via* reductive alkylation and acylation [21, 22].

Applications of Sieber Amide resin include the solid phase synthesis of tetrahydropyridones (Figure 2-32) [23], *via* aza-annulation of enamines, and furan-based libraries derived from a hydroxymethylfurfural template [24].
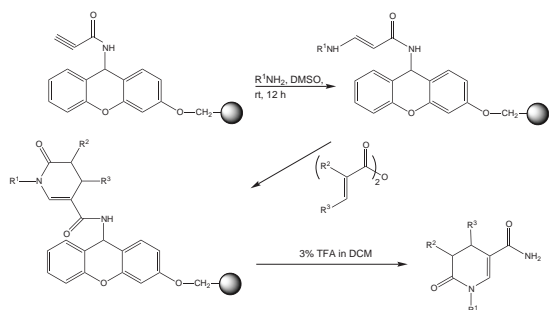
Fig. 2-32: Synthesis of SPOS of tetrahydropyridones on Sieber Amide resin.

Sieber Amide resin has been employed to produce protected peptides in which the C-terminal carboxyl group is blocked as an acid-sensitive hydroxymethylphenoxyacetyl-β-alaninamide ester (Figure 2-33) [25], which are useful building blocks for segment condensation reactions.
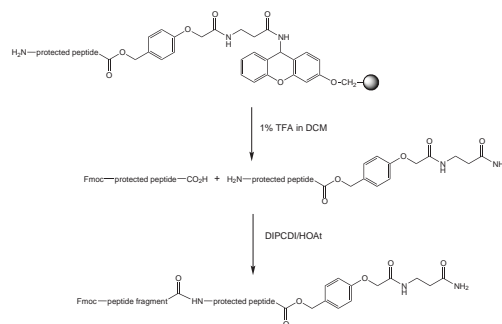
Fig. 2-33: Synthesis of carboxy-blocked peptide fragments using a multidetachable linker approach based on Sieber Amide resin.

Brill, *et al.* [26] have effected transamination of trifluoroacetylated Sieber Amide resin with benzylamine in good yield (Figure 2-34). This approach offers considerable potential for the immobilization of amines.

Another novel application of Sieber Amide resin is in the synthesis of nitriles. Hone, *et al.* [27] have obtained these valuable derivatives in excellent yields and purities by treating carboxylic acids attached to Sieber Amide resin with TFAA/pyridine or trichloroacetyl chloride/TEA.
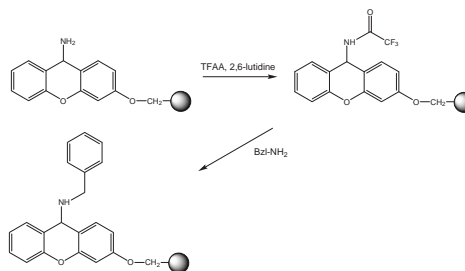
Fig. 2-34: Transamination of Sieber Amide resin.

Related products		
855008	Sieber Amide resin	p. 206
855013	NovaSyn® TG Sieber resin	p. 206

2.7.8 Amino trityl resins
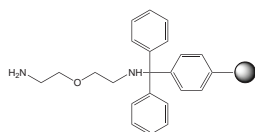
Bis-(2-aminoethyl)-ether trityl resin
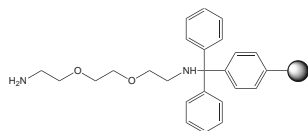
O-Bis-(aminoethyl)ethylene glycol trityl resin
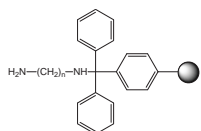
Diaminoalkyl trityl resins; n = 2 – 6

Novabiochem®'s diamine functionalized trityl resins are excellent tools for preparing peptides or small organic molecules containing structural elements derived from asymmetrically substituted diamines. These supports are prepared from trityl chloride resin under carefully controlled conditions to ensure the minimum of trityl-diamine cross-linking.

The bulky trityl linker provides excellent protection to the resin-linked amino functionality, particularly against basic and highly nucleophilic reagents, but allows cleavage of the final product from the resin to be effected under very mild conditions using 1-5% TFA in DCM or 30% HFIP in DCM. The resin is supplied in the free amino form, ready for immediate use.

One important application of diamine resins is the preparation of polyamine conjugates, as these naturally occurring derivatives of diamines and polyamines are of considerable interest owing to their varied biological activities [28]. One example of such an approach is the SPOS of philanthotoxin-433, the neuroactive constituent of the venom of *Philanthus triangulum*, which was prepared from diaminobutyl trityl resin *via* sequential Fukuyama/Mitsunobu alkylation reactions (Figure 2-35) [29].

Related products

856093	Bis-(2-aminoethyl)-ether trityl resin	p. 262
856097	O-Bis-(aminoethyl)ethylene glycol trityl resin	p. 262
856085	1,4-Diaminobutane trityl resin	p. 262
856084	1,2-Diaminoethane trityl resin	p. 263
856086	1,6-Diaminohexane trityl resin	p. 263
856090	1,5-Diaminopentane trityl resin	p. 263
856089	1,3-Diaminopropane trityl resin	p. 263
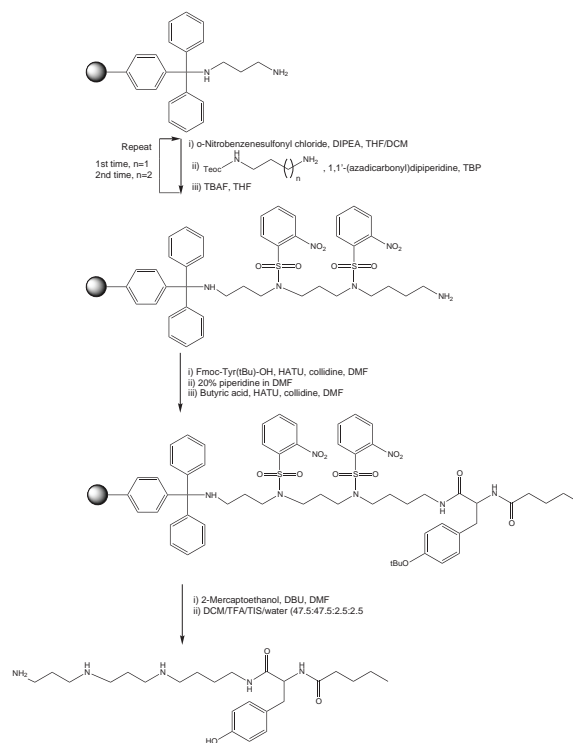
Fig. 2-35: SPOS of philanthotoxin-433.

Manku, *et al.* [30] have described a solid phase route to chiral polyamines *via* borane reduction of peptide-polyamine conjugates prepared on diaminopropyl trityl resin. Key to the success of this approach was the development of an extremely mild procedure for the destruction of the polymer-bound borane-amine complexes without premature release of the polyamine from the highly acid-sensitive trityl support (Figure 2-36).
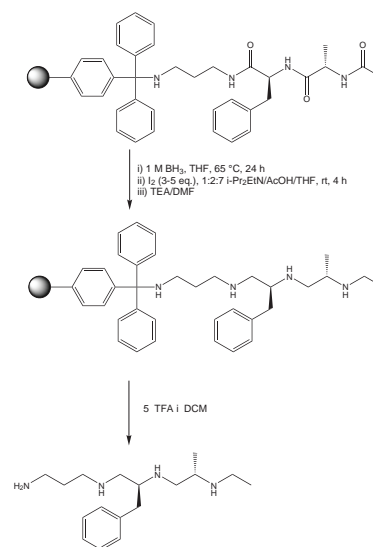
Fig. 2-36: SPOS of chiral polyamines.

Aminothiol 4-methoxytrityl and 2-chlorotrityl resins

Cysteamine 2-chlorotrityl resin

Aminothiol 4-methoxytrityl resin; n=2, 4

These are useful supports for the synthesis of *N*-acyl or *N*-alkyl aminothiols and *N*, *S*-heterocycles. The free amino functionality of the resin-bound cysteamine can be readily reductively alkylated or acylated using standard methods. Cleavage from the 4-methoxytrityl support can be effected with 3% TFA in DCM containing TIS (Method 2-19, page 2.46).

Disulfides can also be generated using electrophilic oxidants such as I_2 or Tl^{3+}. This method is particularly useful for forming intramolecular disulfide bridges in molecules containing two thiol groups where one is protected with Acm.

Mourtas, *et al.* [31] have described a simple route to alkyl- or aminoalkylbenzo-thiazoles, involving acylation of 2-aminobenzenethiol 4-methoxytrityl resin with acids or amino acids, followed by treatment with 1.5 % TFA in DCM and cyclization in MeOH or DMF (see Figure 2-37).
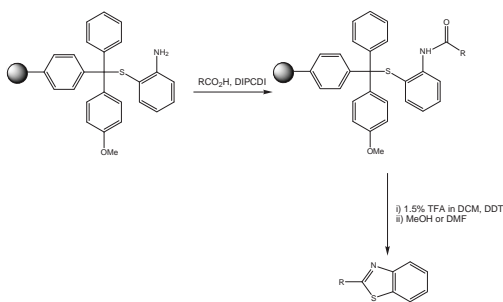
Fig. 2-37: Synthesis of 2-aminomethylbenzothiazoles.

Related products		
856000	Cysteamine 2-chlorotrityl resin	p. 264
856095	4-Aminobutanethiol 4-methoxytrityl resin	p. 265
856087	Cysteamine 4-methoxytrityl resin	p. 265

2.7.9 Sulfamyl-based resins
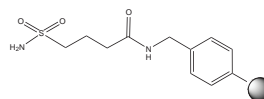
4-Sulfamylbutyryl AM resin   4-Sulfamylbutyryl NovaSyn® TG

4-Sulfamylbutyryl based-resins are highly versatile tools for the preparation of *N*-alkyl peptide amides, peptide thioesters, ureas, and other carboxamides by solid phase synthesis [32-35]. For a review on this topic, please see [36].

Acylation of these resin-bound sulfonamides is best achieved with carboxylic acids activated with PyBOP® and DIPEA in $CHCl_3$ at -20°C [33] or with DIPCDI/*N*-methylimidazole (see Table 2-8). In the case of PyBOP® activation, the loading efficiencies are reported to vary from >95% for Cys, Met and His to 44% for Pro, the worst case. Extent of racemization for the loading of Fmoc-Phe and Fmoc-Leu by these methods are 0.5% and 0.3%, respectively. However, in practice the loading obtained by these methods can be highly variable, and problems can occur with over acylation of the linker. Furthermore, the substitution of the support must be determined before starting peptide synthesis.

For these reasons, Novabiochem® has introduced a range of pre-loaded sulfamylbutyryl NovaSyn® TG resins. Here, coupling of the first amino acid to the sulfamyl linker is carried out in solution prior to attachment of the purified, fully characterized Fmoc-amino acid linker to amino NovaSyn® TG. This produces high-quality supports of defined substitution, free from by-products arising from overacylation. The supports can be used directly in automated peptide synthesis without modification of existing protocols.

The polymer-supported acylsulfonamides are stable to strongly nucleophilic and basic reagents. Once the synthesis is complete, they can be activated for nucleophilic displacement by treating with diazomethane, trimethylsilyldiazomethane or iodoacetonitrile. This results in formation of a *N*-alkyl-*N*-acylsulfonamide which can be cleaved with nucleophiles, such as primary and secondary amines, thiols, aryl amines, amino acid esters, alcohols and hydroxides, to provide compounds possessing a wide range of carboxyl group modifications (Figure 2-38). Cyclic products are generated when displacement is effected by intramolecular attack, a strategy which has been utilized in the preparation of cyclic peptides [37, 38]. Acylation of the sulfonamide with isocyanates yields supported ureas, which following activation can be cleaved with amines to yield asymmetric ureas (Figure 2-39) [39].

The cyanomethyl-activated supports are thought to be equivalent to pentafluorophenyl esters in respect of their reactivity towards nucleophiles. The *N*-methylated materials are much less reactive, requiring the use of excess nucleophile and elevated temperatures, but are much easier to generate. No methylation of Met and Cys residues has been observed during the activation step. However, methylation of the phosphate group mono-benzyl protected phosphoamino acids has been observed. Fortunately, this can be reversed by treatment with TMSBr/TFA..

Cyanomethylation does appear to be incompatible with His(Trt) and Met; however, no problems were encountered with His(Boc). Care should also be exercised when cleaving with hydroxide as it can give rise to considerable racemization of the C-terminal amino acid. The use of 4-sulfamylbutyryl resins is preferred with electron-withdrawing carboxylic acids, owing to the greater nucleophilicity of the corresponding acylsulfonamide. They have also proved particularly suited to the synthesis of peptide thioesters for use in native chemical ligation (see Chapter 5, page 5.1) [34, 35].

Recently, a variation of the sulfamylbutyryl resin has been introduced where the linker is attached to the acid-labile Rink Amide AM resin [40]. This enables the fully unprotected peptide *N*-methylsulfonamide to be cleaved from the resin, facilitating monitoring of the methylation reaction by LC-MS. More importantly, the peptide *N*-methylsulfonamide can be used directly in native chemical ligation (NCL) reactions, avoiding the need for prior conversion to the thioester (see chapter 5, page 5.2) (Figure 2-39). Furthermore, this gives much improves yields because direct displacement of a protected peptide thioester by treatment with thiols is notoriously inefficient.

Sulfamyl resins have been used to prepare a library of acylaminodeoxyadenosines [41], fluorogenic peptide substrates [42, 43], and peptide vinyl sulfones and epoxyketones [44].
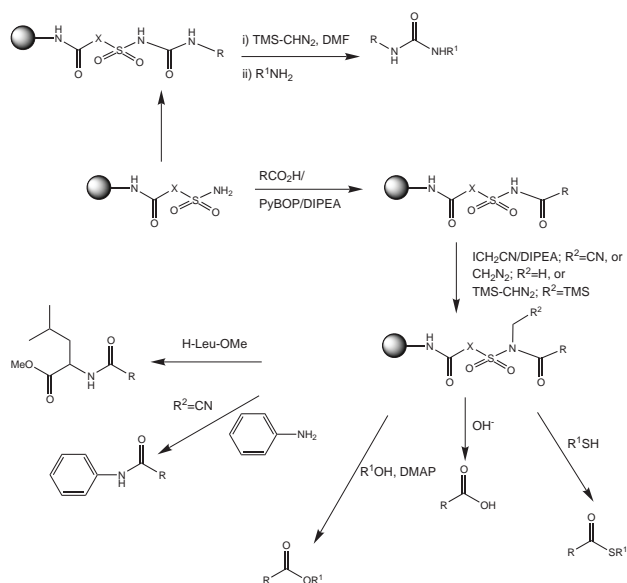
Fig. 2-38: Applications of 4-sulfamyl resins
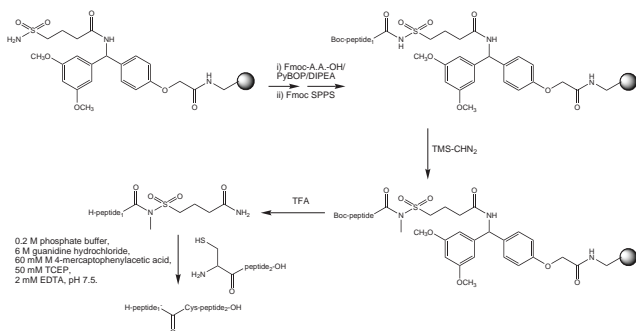
Fig. 2-39: NCL with peptide *N*-methylsulfonamides.

Table 2-8: Coupling of Fmoc-Aaa-OH to 4-sulfamylbutyryl AM resin.

Coupling reagent	Base	Amino acid	Solvent	% Fmoc loading
PyBOP®(4)	DIPEA (8)	Leu (4)	DMF[a]	55
PyBOP®(3)	DIPEA (5)	Phe (3)[g]	CHCl3[d]	89[f]
BOP (4)	DIPEA (4)	Leu (4)	DMF[a]	36
DIPCDI (2)	1-MeIm (2)	Leu (2)	DCM/DMF 4:1[a]	45
DIPCDI (4)	1-MeIm (4)	Leu (4)[e]	DCM/DMF 4:1[a]	100
DIPCDI (4)	1-MeIm (4)	Leu (4)	THF/DMF 4:1[a]	57
TFFH (6)	DIPEA (6)	Leu (6)	DMF[b]	36
MSNT (2)	1-MeIm (2)	Leu (2)	DCM[c]	5

Reaction time: [a] 18 h; [b] 1 h; [c] 0.5 h; [d] 8 h. [e] Enantiomerization ~ 0.3 %. [f] Ref [33]. [g] Enantiomerization <0.5 %. Excess of reagent given in parentheses.

Method 2-6: Loading of sulfamyl resins

DIPCDI method
1. Pre-swell resin (1 mmole) in DCM for 1 h before use.
2. Dissolve amino acid derivative or carboxylic acid (4 mmole) and 1-MeIm (4 mmole) in DCM/DMF (4:1). Add DIPCDI (4 mmole), mix and add to resin. Leave for stand with gentle agitation for 18 h. Wash resin with DMF, DCM, MeOH and dry.

PyBOP® method
1. Pre-swell resin (1 mmole) in CHCl3 for 1 h before use.
2. Dissolve amino acid derivative or carboxylic acid (4 mmole) and DIPEA (8 mmole) in CHCl3. Add to resin.
3. Cool mixture to –20 °C. Add PyBOP® (4 mmole) and leave for stand with gentle agitation for 8 h at –20 °C. Wash resin with DCM, DMF, DCM, MeOH and dry.

Method 2-7: Activation of acylsulfamyl resins

To prevent side-reactions with certain amino acid side-chain functionalities, it is advisable to leave all protecting groups in place until after release of the peptide from the resin.

ICH2CN method
1. Pre-swell resin (0.1 mmole) in DCM for 1 h before use. Wash the resin with NMP.
2. Dissolve ICH2CN (180 µl, 2.5 mmole) and DIPEA (172 µl, 1 mmole) in NMP (4ml) and filter through a plug of basic alumina.
3. Add mixture to resin and agitate gently for 18 h. Wash resin with NMP, THF and use immediately, or wash with NMP, THF, DCM and dry.

TMS-CHN2 method
1. Pre-swell resin (0.1 mmole) in DCM for 1 h before use.
2. Add 1 M TMS-CHN2 in hexane/DCM (1:1) to cover.
3. Agitate gently for 2 h. Wash resin with THF and use immediately, or wash with THF, then DCM and dry.

Cleavage with amines
1. Pre-swell cyanomethylated resin in THF or DMF for 1 h before use.
2. Add excess amine (1.5-20 eq.) in THF. Leave for 4 h-18 h.
3. Remove the resin by filtration and wash it three times with THF or DMF.
4. Combine the filtrates and evaporate to dryness on a rotary evaporator. Depending on the nature of the product, wash with ether or water.

Examples of the use of 4–Sulfamylbutyryl AM resin

The utility of sulfamylbutyryl AM resin is demonstrated in the synthesis of Boc-Gly-Gly-Leu-NHCH$_2$CH$_2$CH$_3$ and Boc-Gly-Gly-Leu-OMe (Application 2-3).

Application 2-3: Preparation of Boc-Gly-Gly-Leu-NHCH$_2$CH$_2$CH$_3$ and Boc-Gly-Gly-Leu-OMe

H-Gly-Gly-Leu was assembled automatically using a NovaSyn® Crystal peptide synthesizer on Fmoc-Leu-4-Sulfamylbutyryl AM resin (254 mg), which was prepared by treating the base resin with Fmoc-Leu-OH (4 eq.), PyBOP® (4 eq.) and DIPEA (8 eq.) in DMF for 18 h. All acylation reactions were carried out for 1 h, using Fmoc-amino acids activated with 1 eq. of PyBOP® in the presence of 2 eq. of DIPEA and 1 eq. of HOBt. After the final Fmoc deprotection reaction, the *N*-terminal Gly residue was capped by treating the resin with Boc$_2$O in DMF for 1 h. This resin was transferred into a manual peptide synthesis reaction vessel, washed with dry DMF, and allowed to react o/n under nitrogen with iodoacetonitrile (290 µl, 4 mmole) and DIPEA (139 µl, 0.8 mmole). The resin was then washed with DMF and THF, and divided into two roughly equal portions.

Cleavage with propylamine

Half of the cyanomethylated resin was treated with propylamine (214 µl, 2.6 mmole) in THF at rt for 4 h. The resin was removed by filtration and washed with THF. Evaporation of the combined filtrates provided the crude peptide propylamide. This material was analyzed by HPLC (Figure 2-40) and ES-MS (Figure 2-41) [expected M+H$^+$ 387.5, found 387.4].
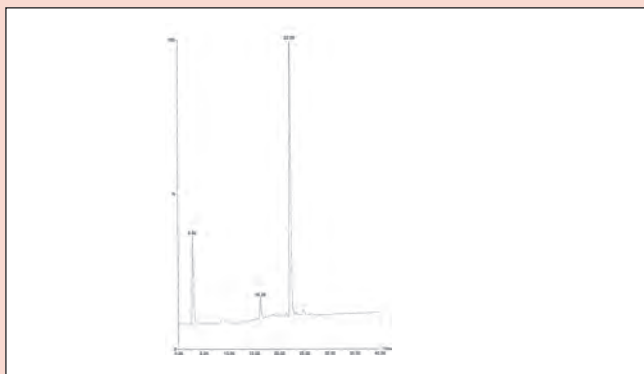
Fig. 2-40: HPLC elution profile of crude Boc-Gly-Gly-Leu-NHCH$_2$CH$_2$CH$_3$.
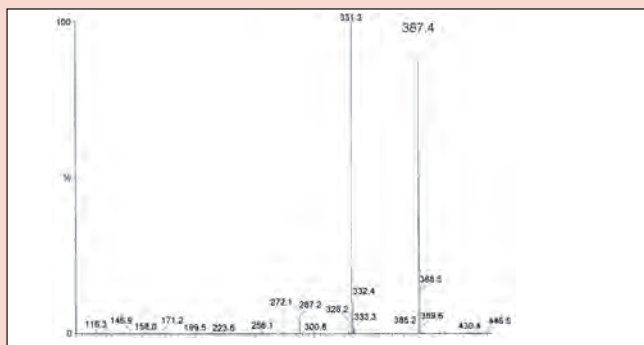
Fig. 2-41: ES-MS of major product from propylamine cleavage.

Cleavage with methanol

The remaining half of resin was treated o/n with MeOH (100 µl) and DMAP (9 mg) in THF. After this time, the resin was removed by filtration and washed with THF. The filtrates were combined and evaporated to dryness. The residue was then washed with water and freeze dried to provide 13 mg of the peptide methyl ester. This material was analyzed by HPLC (Figure 2-42) and ES-MS (Figure 2-43) [expected M+H$^+$ 360.4, found 360.2].
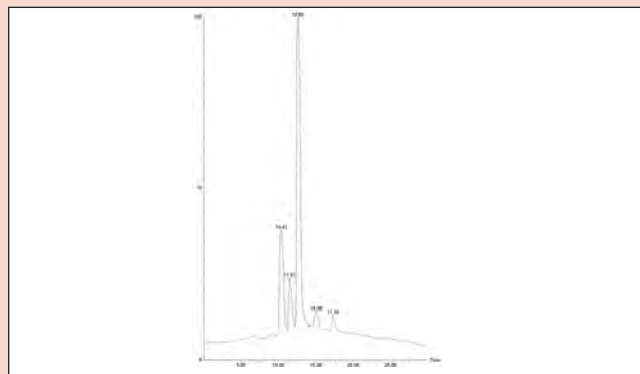
Fig. 2-42: HPLC elution profile of crude product from methanol cleavage: a) Boc-Gly-Gly-Leu-OMe; b) Boc-Gly-Gly-Leu-OH.
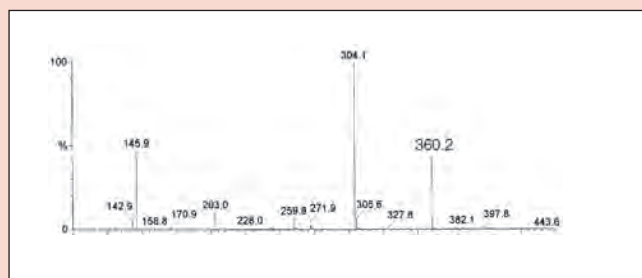
Fig. 2-43: ES-MS of major product from methanol cleavage.

2.7.10 Weinreb AM resin
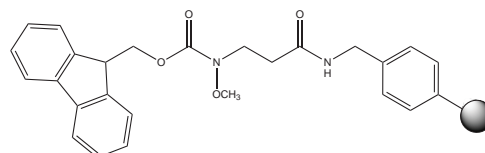
The reduction of *N*-methoxy-*N*-methylamides (Weinreb amides) with LiAlH$_4$ or DIBAL is a well-established strategy for the production of peptide and α-amino-aldehydes [45]. Fehrentz and co-workers [46, 47] have adapted this methodology for use in SPOS, through the simple device of replacing the *N*-methyl group of a Weinreb amide with a linker that enables the methoxyamine to be attached to a solid support.

Due to the hindered nature of the resin-bound secondary amine, HOAt/DIPCDI or HATU/DIPEA should be used for the addition of the first residue (Method 2-5, page 2.20). The resulting support is stable to the conditions of Fmoc and Boc SPPS. After reduction with LiAlH$_4$, Fehrentz, *et al.* reported obtaining peptide aldehydes in yields of 30-40%.

In a parallel study, Dinh & Armstrong [48] have prepared ketones by cleaving resin-bound Weinreb amides with Grignard reagents. The yields reported vary from 16-78%, depending on the nature of the Grignard reagent and the ligand on the resin. The best results were generally obtained using methyl-magnesium chloride.

O'Donnell, *et al.* [49] prepared libraries of unnatural amino acid aldehyde and ketone derivatives using Weinreb amide resin in conjunction with DIBAL-H reduction and Grignard reagent displacement, respectively (Figure 2-44). The Grignard displacement approach was also used by Tice, *et al.* [50] to prepare acylamino-disubstituted ketones.
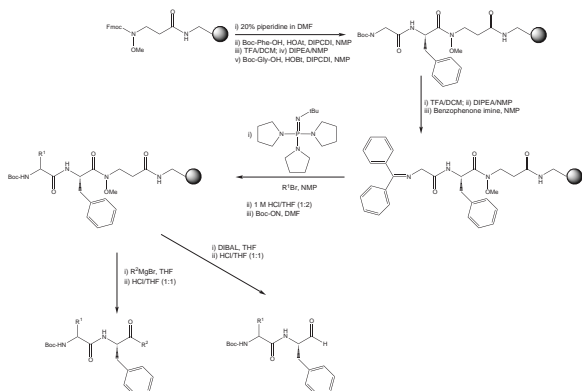
Fig. 2-44: SPOS of unnatural amino acid aldehydes and ketones.

Method 2-8: Reductive cleavage of Weinreb amides

To prevent side-reactions with certain amino acid side-chain functionalities, it is normally advisable to leave all protecting groups in place; the exception to this is when the peptide contains Asp and Glu since even the t-Bu esters of these residues are reduced by LiAlH$_4$. With peptides, the *N*-terminal amino group should be blocked with a Boc group; the Fmoc group is not stable under these conditions .

1. Pre-swell resin (0.1 mmole) in dry THF for 1 h before use, in a round-bottomed flask equipped with a magnetic stirrer.
2. Flush the flask with argon, then seal and place in an ice bath.
3. Add 1 M LiAlH$_4$ in THF (0.5 ml, 0.5 mmole[a]) using a syringe. Stir gently for 40 min.
4. Dilute the mixture with THF and add saturated KHSO$_4$ (0.5 ml) and K, Na tartrate (0.3 ml). Gently agitate mixture for 30 min and allow to warm to rt.
5. Remove resin by filtration and wash it with DCM.
6. Dry combined organic filtrates with anhydrous MgSO$_4$ and evaporate to dryness.

[a]For long peptides the quantity of LiAlH$_4$ may need to be increased; conversely, for small organic molecules this amount may be considerably reduced.

Related products

855074	Weinreb AM resin	p. 214

2.7.11 Fmoc–4–hydrazinobenzoyl NovaGel™ resin
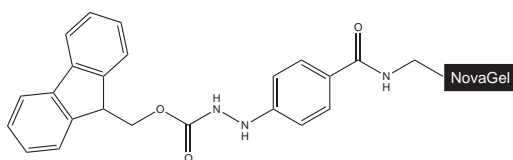
Fmoc–4–hydrazinobenzoyl NovaGel™

Aryl diazenes, produced by oxidation of aryl hydrazines, readily decompose under mild conditions to give arenes and nitrogen [51]. This process has been exploited by Millington, *et al.* [52] as the basis of a novel safety-catch linker, *N*–Fmoc-4-hydrazinobenzoic acid (Figure 2-45), which is supplied by Novabiochem® attached to NovaGel™ resin.
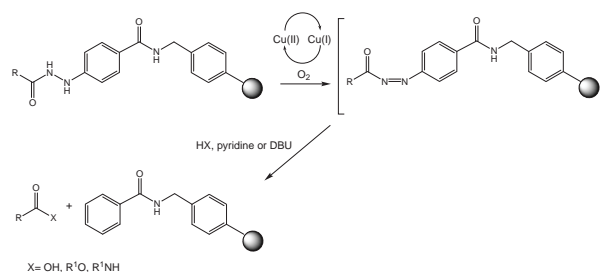
Fig. 2-45: Applications of Fmoc-4-hydrazinobenzoyl resins.

Following removal of the Fmoc group, the resin-bound hydrazino group can be readily acylated using standard coupling methods. The support is stable to piperidine and TFA, facilitating the synthesis of both protected and unprotected peptide fragments.

Cleavage can be effected under mild oxidative conditions by treatment with air and an appropriate nucleophile, in the presence of copper (II) acetate and pyridine (or DBU), to provide products containing a range of carboxy modifications such as acids, esters and amides. In cases where the nucleophilic component poorly solvates the resin, a co-solvent such as THF or DMF should be included in the reaction mixture.

Alternatively, the reaction can be carried out in two stages by first generating the diazene by oxidation with NBS followed by cleavage with nucleophile once excess oxidant is removed. This method has the advantage of avoiding copper contamination of the product. In the synthesis of esters of primary and secondary alcohols, Peters & Waldmann [53] found NBS activation to give the highest yield. Camarero and coworkers also used this method to prepare peptide p-nitroanilides [54] and peptide thioesters [55] by employing p-nitroaniline and amino acid thioesters as nucleophiles.

This resin has also been used to prepare cyclic peptides *via* a cyclative cleavage strategy involving intramolecular attack of the *N*-terminal amino group on the diazene [56].

The use of Fmoc-hydrazinobenzoyl resins has recently been reviewed [57].

Method 2-9: Oxidative cleavage of hydrazinobenzoyl resins

To give amides using Cu(II) oxidation [53]
1. Suspend the resin in neat amine
2. Add Cu(OAc)$_2$ (0.5 eq.) and bubble air vigorously through the resin for 4 h.
3. Remove resin by filtration and wash it with DCM.
4. Evaporate combined organic filtrates to dryness. Redissolve in DCM and wash with 1 M KHSO$_4$, water, and sat. NaCl.
5. Dry organic layer over Na$_2$SO$_4$ and evaporate to dryness.

To give ester or amine using NBS [54]
1. Suspend the resin in dry DCM
2. Add NBS (2 eq.) and dry pyridine (2 eq.). Agitate gently for 5 min.
3. Remove resin by filtration and wash it with dry DCM and dry THF.
4. Resuspend resin in dry DCM and add alcohol or amine (5 eq.) and gently agitate for 4 h.
5. Remove resin by filtration and wash with DCM.
6. Evaporate combined filtrates to dryness.

Related products

855037	4-Fmoc-hydrazinobenzoyl AM NovaGel™	p. 212

2.7.12 Alkyl aminomethylindole resins
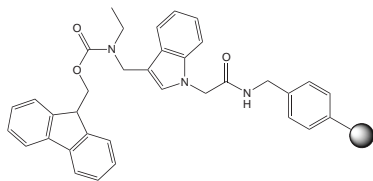
Ethyl Indole AM resin
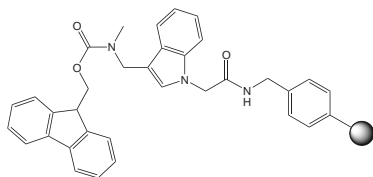
Methyl Indole AM resin

Ethyl Indole AM resin and Methyl Indole AM resin are useful supports for the production of *N*-ethyl and *N*-methyl substituted peptide amides. After first removing the Fmoc group, loading of the resin with Fmoc-amino acids can be effected using HATU/DIPEA as described in Method 2-5, page 2.20. Cleavage with TFA directly provides the product *N*-ethyl- or *N*-methylamide.

Related products		
855102	Ethyl Indole AM resin	p. 202
855116	Methyl Indole AM resin	p. 202

The use of this resin is exemplified below:

Application 2-4: Synthesis of *N*-phenyl-*N*-ethylurea

Ethyl Indole AM resin (0.1 g, 0.085 mmole) was swollen in DCM in 15 min. This resin was washed with DMF and then treated with 20 % piperidine in DMF (2 x 5 min) to remove the Fmoc group. It was washed again with DMF and suspended in DMF containing *N*-phenylisocyanate (46 µl, 0.425 mmole). This mixture was gently agitated at rt overnight under N$_2$. After this time, the resin was washed with DMF, DCM and MeOH and dried. Treatment of the resin with 2% TFA in DCM (3 x 3 min) gave the desired product in 95 % yield. This material was found to be pure by ^{1}H nmr and gave the expected result on ES-MS [expected M+H$^+$ 165, found 165].

Application 2-5: Synthesis of *N*-ethyl-4-methoxy-benzenesulfonamide

N-Ethyl-4-methoxy-benzenesulfonamide was prepared as described above using 4-methoxybenzenesulfonyl chloride (88 mg, 0.425 mmole) and DIPEA (73 µl, 0.425 mmole). Following TFA cleavage, the product was obtained in quantitative yield, and was found to be pure by ^{1}H nmr and gave the expected result on ES-MS [expected M+H$^+$ 216, found 216].

2.7.13 Hydroxylamine Wang resin
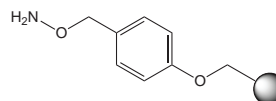
Hydroxylamine Wang resin is an excellent support for the SPPS of peptide hydroxamic acids [58]. It is compatible with the standard conditions employed in Fmoc SPPS and acylation of the hydroxylamine amino group is effected using TBTU/DIPEA activation.

Cleavage of hydroxamic acids has been effected by treatment with TFA/TIPS/DCM or TFA/anisole [59]. The use of water as a scavenger should be avoided as this has been reported to promote hydrolysis of the hydroxamic acid. Reductive cleavage with SmI$_2$ results in N-O bond cleavage and release of the appropriate amide [60].

Reaction of the resin with aldehydes and ketones in TMOF/THF, followed by reduction with BH$_3$·pyridine/dichloroacetic acid/DCM gives the corresponding supported *N*-alkyl hydroxylamine [61] (Figure 2-46). Acylation of this material, followed by TFA cleavage, affords the *N*-alkyl hydroxamic acid.
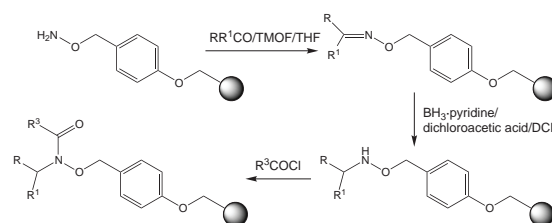
Fig. 2-46: Synthesis of *N*-alkylhydroxamates with hydroxylamine Wang resin.

N-Benzylhydroxylamine Wang resin has been used to prepare supported Weinreb amides, which were cleaved by reduction with LiAlH$_4$ to provide the corresponding aldehydes in moderate yields (Figure 2-47) [62].
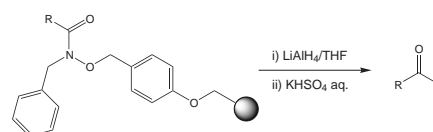
Fig. 2-47: Synthesis of aldehydes with *N*-benzylhydroxylamine Wang resin.

Related products		
855117	Hydroxylamine Wang resin	p. 215

2.7.14 Dawson Dbz AM resin (100 – 200 mesh)/Dawson Dbz NovaSyn® TGR resin
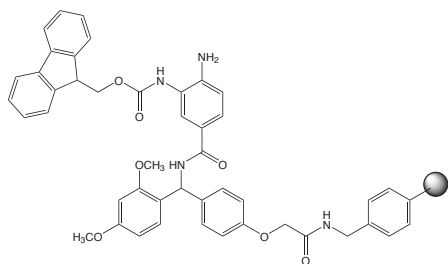
Dawson Dbz AM resin (100 - 200 mesh) and Dawson Dbz NovaSyn® TGR resins are novel supports for the synthesis of peptide thioesters by Fmoc SPPS. After removal of the Fmoc group with 20% piperidine in DMF, one of the anilino amino groups can be acylated with the *C*-terminal amino acid [63] using standard coupling methods. Following assembly of the desired peptide, the linker is activated by treatment with *p*-nitrophenyl chloroformate to generate the resin-bound peptidyl benzimidazolinone. TFA cleavage releases the benzimidazolinone which can be converted to a thioester by treatment with a thiol or used directly in native chemical ligation (Figure 2-48). For further information on the use of these resins see section 5.1, page 5.2.
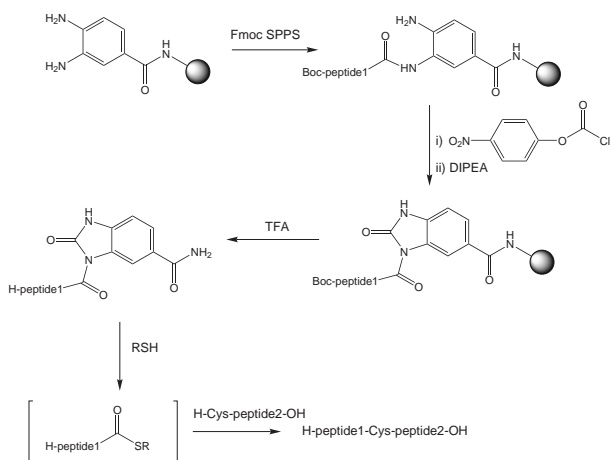
Fig. 2-48: Synthesis of thioesters using Dawson Dbz AM resin.

Related products

855131	Dawson Dbz AM resin	p. 216
855142	Dawson Dbz NovaSyn® TGR resin	p. 216

References

1. a) W. Rapp, et al. in "Peptides 1988; Proc. 20st European Peptide Symposium", G. Jung & E. Bayer (Eds.), Walter de Gruyter, Berlin, 1989, pp. 199; b) W. Rapp, et al. in "Innovation and Perspectives in Solid Phase Synthesis; 1st International Symposium", R. Epton (Ed.), SPCC (UK) Ltd, Birmingham, 1990, pp. 205; c) L. Zhang, et al. in "Peptides 1990; Proc. 21st European Peptide Symposium", E. Giralt & D. Andreu (Eds.), ESCOM, Leiden, 1990, pp. 196; d) E. Bayer (1991) *Angew. Chem. Int. Ed. Engl.*, **30**, 113.
2. M. Gowravaram & M. Gallop (1997) *Tetrahedron Lett.,* **38**, 6973.
3. G. R. Matsueda, et al. (1981) *Peptides,* **2**, 45.
4. P. E. Thompson, et al. (1995) *Int. J. Peptide Protein Res.,* **46**, 174.
5. M. Rinnová, et al. (2001) *J. Comb. Chem.,* **4**, 209.
6. A. N. Acharya, et al. (2001) *J. Comb. Chem.,* **3**, 189.
7. A. Nefzi, et al. (2001) *J. Comb. Chem.,* **3**, 68.

8. V. Krchnák, et al. (2001) *Tetrahedron Lett.,* **42**, 1627.
9. H. Rink (1987) *Tetrahedron Lett.,* **28**, 3787.
10. A. R. Katritzky, et al. (1997) *Tetrahedron Lett.,* **38**, 7011.
11. E. G. Brown & J. M. Nuss (1997) *Tetrahedron Lett.,* **38**, 8457.
12. P. A. Tempest, et al. (1996) *Angew. Chem. Int. Ed. Engl.,* **35**, 640.
13. S. W. Kim, et al. (1998) *Tetrahedron Lett.,* **39**, 6993.
14. R. A. Tommasi, et al. (1998) *Tetrahedron Lett.,* **39**, 5477.
15. A. A. Virgilio, et al. (1994) *J. Am. Chem. Soc.,* **116**, 11580.
16. A. Dyatkin & R. A. Rivero (1998) *Tetrahedron Lett.,* **39**, 3647.
17. F. Albericio & G. Barany (1987) *Int. J. Peptide Protein Res.,* **30**, *206*
18. M. S. Bernatowicz, et al. (1989) *Tetrahedron Lett.,* **30**, 4645.
19. R. Ramage, et al. (1993) *Tetrahedron Lett.,* **34**, 6599.
20. P. Sieber (1987) *Tetrahedron Lett.,* **28**, 2107.
21. W. C. Chan, et al. (1995) *J. Chem. Soc., Chem. Commun.,* 1475.
22. S. Mellor, et al. in "Fmoc solid phase peptide synthesis: a practical approach", W. C. Chan & P. D. White, Oxford University Press, Oxford, 2000, pp. 137.
23. K. Paulvannan & T. Chen (2000) *J. Org. Chem.,* **65**, 6160.
24. P. Gupta, et al. (2002) *Tetrahedron,* **58**, 10469.
25. W. C. Chan, et al. (1995) *J. Chem. Soc., Chem. Commun.,* 589.
26. W. K.-D. Brill, et al. (1998) *Synlett,* 906.
27. N. D. Hone, et al. (2001) *Tetrahedron Lett.,* **42**, 1115.
28. I. R. Marsh, et al. (1996) *Mol. Div.,* **2**, 165.
29. K. Strømgaard, et al. (2001) *Synthesis,* **6**, 877.
30. S. Manku, et al. (2001) *J. Org. Chem.,* **66**, 874.
31. S. Mourtas, et al. (2001) *Tetrahedron Lett.,* **42**, 2201.
32. a) B. J. Backes, et al. (1994) *J. Am. Chem. Soc.,* **116**, 11171; b) B. J. Backes, et al. (1996) *J. Am. Chem. Soc.,* **118**, 3055.
33. B. J. Backes & J. A. Ellman (1999) *J. Org. Chem.,* **64**, 2322.
34. Y. Shin, et al. (1999) *J. Am. Chem. Soc.,* **121**, 11684.
35. R. Ingenito, et al. (1999) *J. Am. Chem. Soc.,* **121**, 11369.
36. P. Heidler & A. Link (2005) *Bioorg. Med. Chem.,* **13**, 583.
37. L. Yang & G. Morriello (1999) *Tetrahedron Lett.,* **40**, 8197.
38. P. C. de Visser. et al. (2003) *J. Peptide Res.,* **61**, 298.
39. D. Fattori, et al. (2003) *Tetrahedron Lett.,* **44**, 811.
40. F. Burlina, et al. (2011), paper submitted for publication.
41. A. Link, et al. (1998) *Tetrahedron Lett.,* **39**, 5175.
42. B. J. Backes, et al. (2000) *Nature Biotech.,* **18**, 187.
43. J. E. Sheppeck, et al. (2000) *Bioorg. Med. Chem. Lett.,* **10**, 2639.
44. H. S. Overkleeft, et al. (2000) *Tetrahedron Lett.,* **41**, 6005.
45. S. Nahm & S. M. Weinreb (1981) *Tetrahedron Lett.,* **22**, 3815.
46. J. A. Fehrentz, et al. (1995) *Tetrahedron Lett.,* **36**, 7871.
47. S. Mellor, et al. in "Fmoc solid phase peptide synthesis: a practical approach", W. C. Chan & P. D. White (Eds.), Oxford University Press, 2000, pp. 153.
48. T. Q. Dinh & R. W. Armstrong (1996) *Tetrahedron Lett.,* **37**, 1161.
49. M. J. O'Donnell, et al. (2000) *J. Comb. Chem.,* **2**, 172.
50. C. M. Tice, et al. (2002) *Tetrahedron Lett.,* **43**, 7491.
51. B. T. Newbold in "The Chemistry of the hydrazo, azo and azoxy group" Part 1, S. Patai (Ed.), J. Wiley & Sons, Chichester, 1975, pp. 542.
52. C. R. Millington, et al. (1998) *Tetrahedron Lett.,* **39**, 7201.
53. C. Peters & H. Waldmann (2003) *J. Org. Chem.,* **68**, 6053.
54. Y. Kwon, et al. (2004) *Org. Lett.,* **6**, 3801.
55. J. A. Camarero, et al. (2004) *J. Org. Chem.,* **69**, 4145.
56. C. Rosenbaum & H. Waldmann (2001) *Tetrahedron Lett.,* **42**, 5677.
57. Y.-H. Woo, et al. (2007) *Int. J. Peptide Res. Therapeutics,* **13**, 181.
58. C. Floyd, et al. (1996) *Tetrahedron Lett.,* **37**, 8045.
59. L. S. Richter & M. C. Desai (1997) *Tetrahedron Lett.,* **38**, 321.
60. R. M. Myers, et al. (2000) *Org. Lett.,* **2**, 1349.
61. D. E. Robinson & M. W. Holladay (2000) *Org. Lett.,* **2**, 2777.
62. J. M. Salvino, et al. (1999) *J. Org. Chem.,* **64**, 1823.
63. J. P. Blanco-Canosa & P. E. Dawson (2008) *Angew. Chem. Int. Ed.,* **47**, 6851.

2.8 Hydroxy functionalized resins

Novabiochem® offers an extensive range of hydroxy functionalized supports, encompassing a wide spectrum of chemical properties from hyper acid to base labile, for the the synthesis of peptide acids, carboxyl-modified peptides and small organic molecules.

Attachment of amino acids to hydroxy functionalized supports is normally achieved by DMAP catalyzed esterification with the appropriate symmetrical anhydride (see Method 3-8, page 3.6), and by using MSNT / MeIm [1] (Method 3-9, page 3.6) or 2,6-dichlorobenzoyl chloride [2] activation. The MSNT method is the method of choice in difficult circumstances, such as loading of HMBA resins or when attaching racemization prone amino acid derivatives. Alcohols and phenols have normally been immobilized to hydroxymethyl functionalized supports using the Mitsunobu reaction [3-7].

Simple, but sensitive color tests have been developed for detecting the presence of residual hydroxyl groups on solid supports (Method 2-10) [8, 9]. These tests should prove very useful for checking the completeness of loading of hydroxy functionalized resins.

Karoyan, *et al.* [10] have loaded Wang resin by transesterification of an ethyl ester with the resin lithium salt generated by pre-treatment of the polymer with LDA. Hanessian & Huynh [11] have converted Wang resin, by reaction with TOPCAT, to the corresponding 2-pyridylthio-carbonate; this intermediate reacted rapidly with alcohols in the presence of AgOTf to produce the polymer-bound ether. Reaction with phosgene or activated carbonates, such as carbonyl diimidazole or bis(*p*-nitrophenyl)-carbonate, converts these supports into solid phase equivalents of standard urethane-based amine protecting groups [12].

The applications of Novabiochem's hydroxy functionalized resins are given in Table 2-6, page 2.12.

Method 2-10: Colorimetric tests for resin-bound hydroxyl groups

Alizarin-cyanuric chloride test [8]
1. Place a few swollen resin beads in a test tube and wash several times with DMF.
2. Add DMF (3 ml) containing NMM (1 ml) and cyanuric chloride (5 mg).
3. Heat the tube at 70 °C for 20 min.
4. Remove the solution and rinse the resin beads several times with DMF.
5. Add DMF (3 ml) containing Alizarin R (5 mg) and NMM (1 ml).
6. After 5 min, remove the solution and wash resin beads several times with fresh DMF until the solution is clear.
7. The presence of unreacted hydroxyl groups is indicated by red-colored beads.

Note: This method will also detect 1° and 2° amines and thiols.

Methyl red-diphenyldichlorosilane test [9]
1. Place a few resin beads (~5 mg) in a test tube with 10% TEA in dry DCM (200 µl).
2. Add diphenyldichlorosilane (100 µl) and leave to stand for 10 min.
3. Isolate resin by filtration and wash twice with 10% TEA in DCM.
4. Suspend resin in DMF (300 µl) containing 0.75% (w/v) of methyl red.
5. After 10 min, remove the solution and wash resin beads several times with fresh DMF and DCM until the solution is colorless.
6. The presence of unreacted hydroxyl groups is indicated by orange/red-colored beads.

Note: This method will also detect phenols.

2.8.1 NovaSyn® TG hydroxy resin

NovaSyn® TG hydroxy resin consists of a 130 µm beaded PEG-polystyrene graft polymer, in which the ends of the PEG chains are hydroxy functionalized [13].

This support has been employed on a number of occasions to immobilize carboxylic acids: Hiroshige, *et al.* [14] have anchored iodobenzoic acid in a model study on solid phase Heck reactions; Pàtek, *et al.* [15] constructed substituted thiazolidines on the Fmoc-Cys(Trt) derivatized support (Figure 2-49); and Krchnák, *et al.* [5] attached Ac-Tyr and examined solid phase Mitsunobu ether formation. In all three instances, the products were released from the resin by saponification with dilute aq. NaOH.
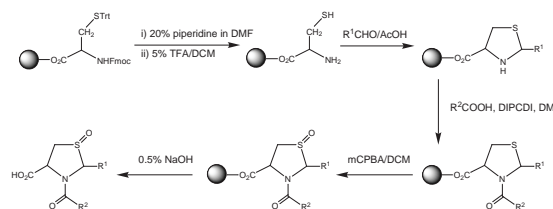
Fig. 2-49: SPOS of thiazolidines.

NovaSyn® TG hydroxy resin has also been used in the cyclization-cleavage strategy adopted by Szardenings and Burkoth [16] to prepare diketopiperazine and diketomorpholine derivatives. A similar hydroxy functionalized PEG-PS support was utilized to prepare hydroxamic acids by cleavage for a resin-bound ester with hydroxylamine [17].

Exposure of NovaSyn® TG hydroxy resin to phosgene in toluene generates a resin-bound chloroformate, which has been used as a linker for amines, and for the formation of solid phase immobilized *N*-carboxyanhydrides [12].

Related products

855064	NovaSyn® TG hydroxy resin	p. 238

2.8.2 Hydroxymethylphenyl functionalized resins

Novabiochem® offers a wide range of polystyrene and PEG-PS supports derivatized with hydroxymethylphenoxy-based linkers for the solid phase synthesis of peptide acids by Fmoc SPPS. These supports are also extremely useful in SPOS for the immobilization of carboxylic acids, alcohols and phenols. Release of these functionalities is usually effected by mild acidolysis with TFA (Method 2-19, page 2.46). Cleavage of esters attached to these resins has also been effected by Lewis acid catalyzed aminolysis [18], DIBAL reduction (Method 2-22, page 2.46) and transesterification (Method 2-21, page 2.46), to give the amides, alcohols and methyl esters, respectively.
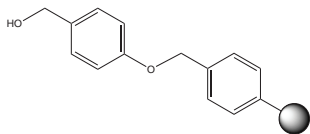
Wang resin

Wang resin is the standard support for Fmoc SPPS by the batch-wise method. These resins consist of chloromethylpolystyrene (100-200 mesh, 1% DVB) modified with 4-hydroxybenzyl alcohol.

Wang resins are available underivatized or pre-loaded with the first amino acid. Novabiochem®'s standard Fmoc-amino acid Wang resins are some of the most highly specified on the market. The quality of the base resin is checked by performance of a test synthesis. The pre-loaded resins are checked for any racemization or dipeptide that may have occured during attachment of the amino-acid. For the synthesis of peptides containing Cys or Pro as the *C*-terminal residue, the use of trityl-based resins is recommended. The bulky trityl linker helps minimize racemization of Cys during piperidine treatments and inhibits diketopiperazine formation with peptides containing *C*-terminal Pro.

Notable applications of Wang resin in SPOS include fumiquinazoline alkaloids *via* a cyclative cleavage strategy (Figure 2-50) [21]; dihydropyrans by Eu(fod)$_3$ catalyzed cycloaddition (Figure 2-51) [22]; benzofurans through titanocene-mediated alkylidenation (Figure 2-52) [23]; drimane-type sesquiterpenes *via* sequential Michaelis-Arbuzov, Horner-Wadsworth-Emmons and Baylis-Hillman reactions (Figure 2-53) [24]; and the synthesis of isoxazolines (Figure 2-54) [25].
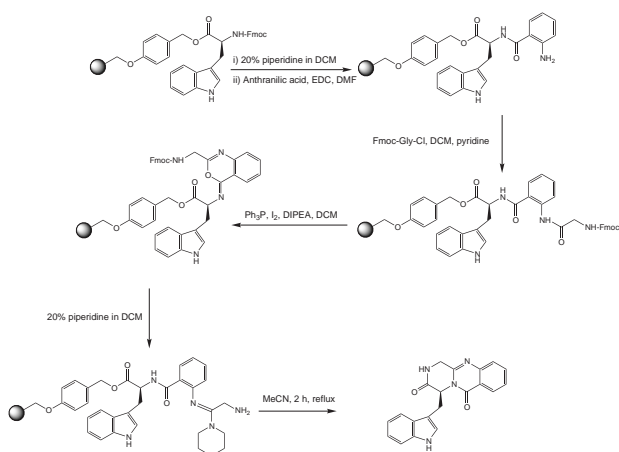
Fig. 2-50: SPOS of fumiquinazoline alkaloids.
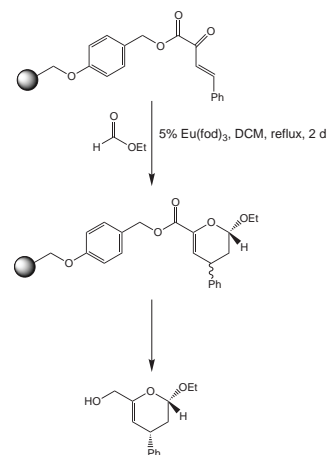
Fig. 2-51: Eu(fod)$_3$ catalyzed synthesis of dihydropyrans.
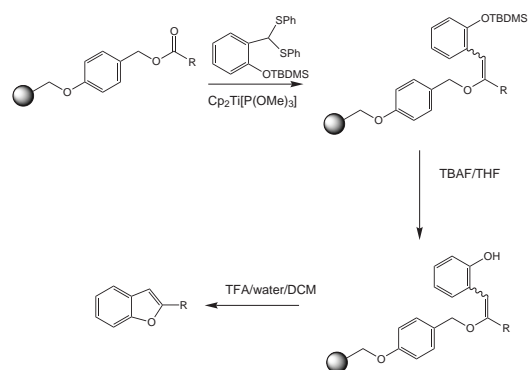
Fig. 2-52 SPOS of benzofurans *via* titanocene-mediated alkylidenation.
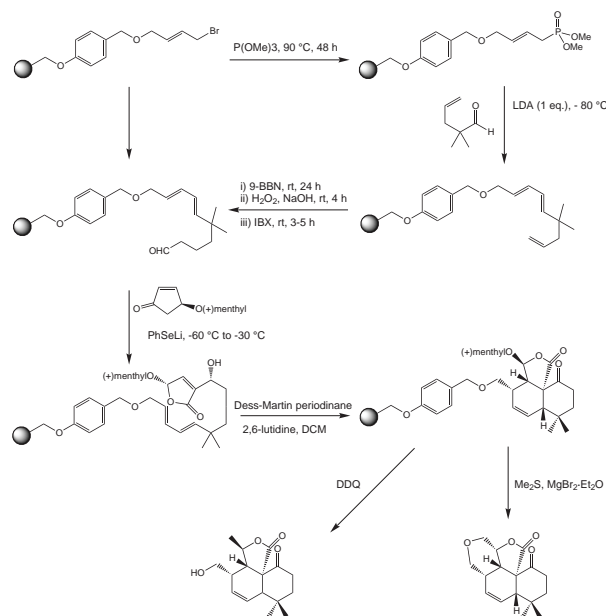
Fig. 2-53: SPOS of Mniopetal-related sesquiterpenes.

2.33
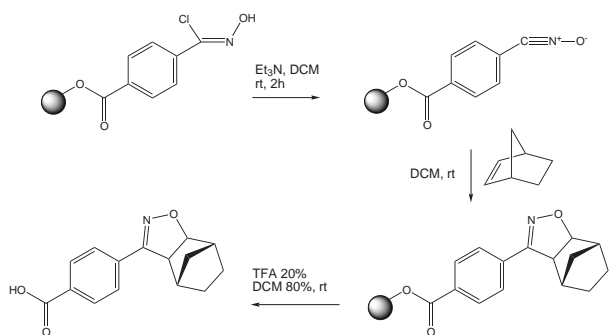
Fig. 2-54: Solid phase synthesis of isoxazolines.

Trichloroacetimidate Wang resin
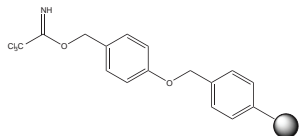
This activated version of Wang resin is a highly versatile support for the solid phase immobilization of alcohols [26]; carboxylic, phosphonic and sulfonic acids; thiols and thioacids [27] (Figure 2-55). In contrast to the analogous bromide resin, which is loaded under basic conditions, derivatization of this support is achieved under acidic conditions, making it compatible with base-sensitive compounds such as Fmoc-amino alcohols.
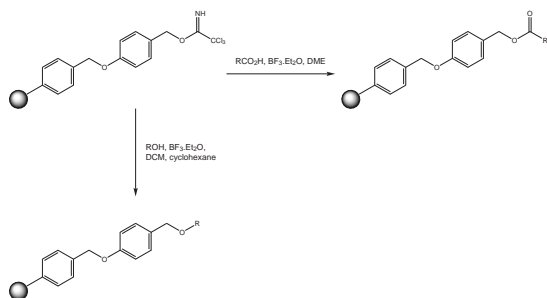
Fig. 2-55: Loading of trichloroacetimidate Wang resin.

NovaSyn® TG**R**A, NovaPEG Wang, HMPA–PEGA and HMPA NovaGel™ & SpheriTide resins
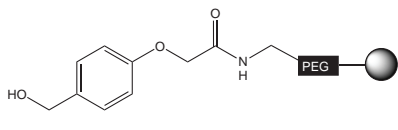
NovaSyn® TGA, HMPA-PEGA, NovaPEG Wang, HMPA-NovaGel™ and HMPA SpheriTide resins are based on 90 μm TG, amino PEGA and aminomethyl NovaGel™ and SpheriTide resins respectively, derivatized with hydroxymethylphenoxy-acetic acid. These resins are ideal for the synthesis of peptide acids by Fmoc continuous-flow solid phase peptide

synthesis. NovaSyn® TGA resin is available pre-loaded with Fmoc-amino acids. Cleavage of peptides from this linker is effected by treatment with 95% TFA containing scavengers.

HMPA SpheriTide resin is particularly useful for large scale synthesis, owing to its exceptionally high capaicity (~2.3 mmol/g) and high loading/swelling volume ratio.

HMPB–MBHA and NovaPEG HMPB resins
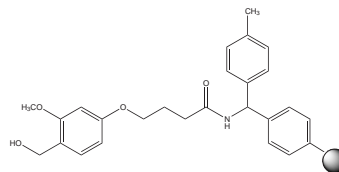
HMPB–MBHA resin

This support consists of 4-methylbenzhydrylamine resin functionalized with Riniker's hyperacid-labile 4-hydroxymethyl-3-methoxyphenoxy-butyric acid linker [28].

The acid-lability of this linker is intermediate between those of Sheppard's 4-hydroxymethyl-3-methoxyphenoxyacetic linker [29] and the very acid-labile 2-chlorotrityl or trialkoxybenzyhydryl-based resins [30]. Fully protected peptide fragments can be released as free acids in very good purity by treatment with 1% TFA in DCM, conditions which leave the side-chain protecting groups normally employed in Fmoc SPPS intact. (Method 3-30, page 3.30)

These hyperacid-labile resins can also be used for the solid phase immobilization of carboxylic acids, alcohols and phenols. Detachment of these functionalities can be effected by treatment with 1-5% TFA (Method 3-30, page 3.30).

Rink Acid resin
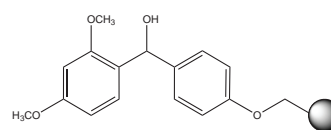
This super acid-labile support was originally introduced for the preparation of protected peptide fragments [30]. Cleavage is effected with AcOH in DCM, providing highly acid-labile peptides in high yield and purity. This degree of acid-lability does mean that there is some risk of

premature cleavage of product from the resin during coupling. Immer, *et al.* [31] have overcome this problem through the use of PyBOP® activation.

The conversion of this support to the corresponding benzhydryl chloride [32-34] or trifluoroacetate [35, 37] has been described. These supports have many useful applications in SPOS for the immobilization of a wide range of nucleophiles, such as alcohols [32, 35], phenols [32, 35], thiols [32, 35], purines [36, 37], amines [32, 34, 35] and hydroxylamines [33]. The products derived from this reaction are considerably more stable than the corresponding esters, requiring 5-95% TFA in DCM or DCE to effect release from the resin, with thiols being the most difficult functionality to remove (Figure 2-56).
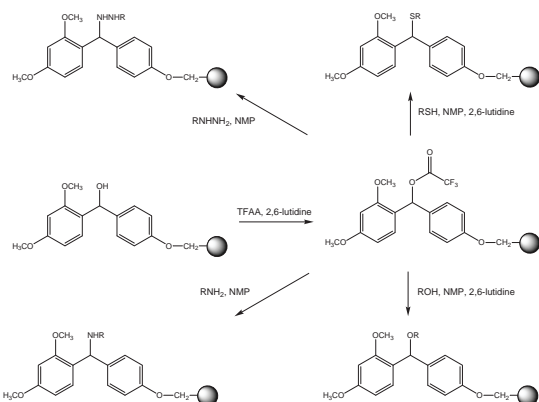
Fig. 2-56: Applications of activated Rink resin.

Edvinsson, *et al.* have utilized this resin to immobilize anilines in the synthesis of diamides as bone resorption inhibitors (Figure 2-57) [34].
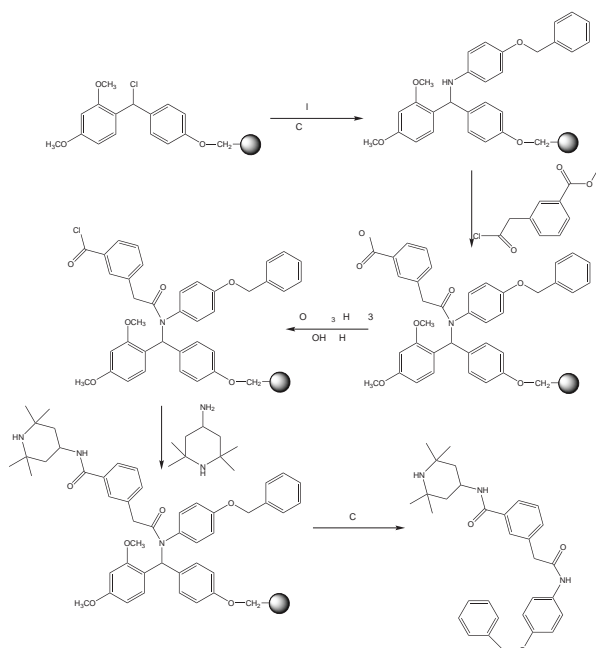
Fig. 2-57: SPOS of diamides using Rink chloride resin.

NovaSyn® TG HMBA, HMBA–AM, HMBA–PEGA and HMBA NovaGel™ resins
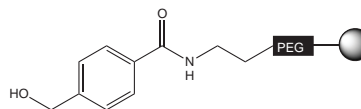
Hydroxymethylbenzoic acid [HMBA] is perhaps the most versatile linker for peptide synthesis and for anchoring carboxylic functionalities [38]. The resin ester bond is stable to acid, but can be cleaved with a variety of nucleophiles to generate products in which the carboxyl group carries a range of modifications [39-41], see Table 2-6, page 2.12 In cases involving intramolecular attack of an internal nucleophile, cyclization occurs with concurrent release of the product from the resin e.g. diketopiperazine from dipeptides. The use of this linker in the synthesis of peptide amides, hydrazides, alcohols, and methyl esters is detailed in section 3.5.6, page 3.28. The stability of the peptide ester linkage to acid allows removal of all acid sensitive side-chain protecting groups prior to cleavage from the resin. These resins are therefore ideal for the synthesis of large arrays of peptide acids since, following cleavage with alkali, the peptides are presented in aqueous saline solution ready for immediate screening.

HMBA-PEGA resin has been used extensively by Meldal's group because of the compatibility of the PEGA resin to the conditions cleavage with 0.1 M NaOH [42-44].

Hutchins & Chapman [45] have utilized HMBA-AM for solid-phase Fischer indole synthesis (Figure 2-58). The products were released as methyl esters by cleavage with methanol/triethylamine, despite the support's limited swelling in this solvent mixture.
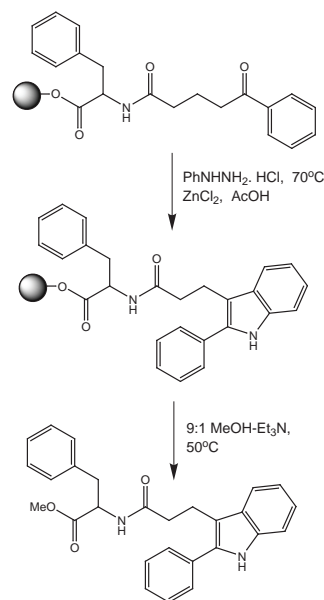
Fig. 2-58: Solid phase Fischer indole synthesis.

Blackburn & Guan [46] have used HMBA-AM resin to prepare 3-aminoimidazopyridines using a 3-component condensation. In this example, the product methyl esters were released using sodium methoxide in MeOH/THF (Figure 2-59).

Fig. 2-59: SPOS of aminoimidazolpyridines.

Meldal and co-workers have employed HMBA-PEGA resin to prepare triazoles *via* Cu-catalyzed cycloadditions of polymer-supported alkynes to azide [44] (Figure 2-60).

Fig. 2-60: SPOS of triazoles on HMBA-PEGA resin.

Related products

855018	HMBA-AM resin	p. 210
855086	HMBA-NovaGel™	p. 211
855070	HMBA-PEGA resin	p. 211
855062	NovaSyn® TG HMBA resin	p. 211

2.8.3 Oxime resin

Oxime resin is the standard support for the synthesis of *C*-terminally modified peptide fragments by Boc SPPS [47]. It consists of an oxime functional group (*p*-nitrobenzophenone oxime) attached to polystyrene-1% divinylbenzene.

Attachment of amino acids and other carboxylic acids to this resin is normally performed using DCC, followed by acetylation of unreacted oxime groups.

Peptides can be displaced from the support by various nucleophiles:: hydrazine gives peptide hydrazides; amino acid or peptide esters [47, 48] provide a convenient route for the assembly of analogs differing at the *C*-terminus. (The addition of a catalytic amount of cyanide has been found to accelerate the aminolysis [49]); alcohols or water in the presence of DBU produce peptide esters or free acids [50]; trimethylsilyldisulfide and TBAF give the thioacid [51]; hydroxylamine affords the corresponding hydroxamate [52, 53].

Peptide chain elongation is performed using standard protocols, although the limited stability of the oxime ester linkage to TFA can lead to premature release of protected peptide from the resin. To minimize side reactions, it is therefore recommended that TFA concentrations should be reduced to 25% and the exposure to TFA be limited by keeping the peptide fragment length to less than 10 residues. It is also advisable to end-cap by acetylation following each coupling. Results have been published on segment-condensation [54-56] and synthesis of cyclic peptides [57-59] and farnesylated peptides [60].

Related products

855089	Oxime resin LL (100-200 mesh)	p. 224
855090	Oxime resin HL (100-200 mesh)	p. 224

References

1. B. Blankemeyer-Menge, et al. (1990) *Tetrahedron Lett.*, **31**, 1701.
2. P. Sieber (1987) *Tetrahedron Lett.*, **28**, 6147.
3. T. A. Rano, et al. (1995) *Tetrahedron Lett.*, **36**, 3789.
4. L. S. Richter, et al. (1994) *Tetrahedron Lett.*, **35**, 4705.
5. V. Krchn?ák, et al. (1995) *Tetrahedron Lett.*, **36**, 6193.
6. R. M. Valerio, et al. (1996) *Tetrahedron Lett.*, **37**, 3019.
7. V. Krchn?ák, et al. in "Peptides: Chemistry, Structure and Biology, Proc. 14th American Peptide Symposium*", P. T. P. Kaumaya & R. S. Hodges (Eds.), Mayflower Scientific Ltd., Birmingham, 1996, pp. 307.
8. M. E. Attardi & M. Taddei (2001) *Tetrahedron Lett.*, **42**, 2927.
9. B. A. Burkett, et al. (2001) *Tetrahedron Lett.*, **42**, 5773.
10. P. Karoyan, et al. (1999) *Tetrahedron Lett.*, **40**, 71.
11. S. Hanessian & H. K. Huynh (1999) *Tetrahedron Lett.*, 40, 671.
12. J. R. Hauske, et al. (1995) *Tetrahedron Lett.*, **36**, 1589.
13. a) W. Rapp, et al. in "Peptides 1988; Proc. 20st European Peptide Symposium", G. Jung & E. Bayer (Eds.), Walter de Gruyter, Berlin, 1989, pp. 199; b) W. Rapp, et al. in "Innovation and Perspectives in Solid Phase Synthesis; 1st International Symposium", R. Epton (Ed.), SPCC (UK) Ltd, Birmingham, 1990, pp. 205; c) L. Zhang, et al. in "Peptides 1990; Proc. 21st European Peptide Symposium", E. Giralt & D. Andreu (Eds.), ESCOM, Leiden, 1990, pp. 196; d) E. Bayer (1991) *Angew.Chem. Int. Ed. Engl.*, **30**, 113.
14. M. Hiroshige, et al. (1995) *Tetrahedron Lett.*, **36**, 4567.
15. M. Pàtek, et al. (1995) *Tetrahedron Lett.*, **36**, 2227.
16. A. Szardenings, et al. (1997) *Tetrahedron*, **53**, 6573.
17. S. Dankwardt (1998) *Synlett*, 761.
18. D. R. Barn, et al. (1996) *Tetrahedron Lett.*, **37**, 3213.
19. G. Lu, et al. (1981) *J. Org. Chem.*, **46**, 3433.
20. S. S. Wang (1973) *J. Am. Chem. Soc.*, **95**, 1328.
21. H. Wang & A. Ganesan (2000) *J. Comb. Chem.*, **2**, 186.
22. S. Leconte, et al. (2000) *Eur. J. Org. Chem.*, 639.
23. E. J. Guthrie, et al. (2000) *Tetrahedron Lett.*, **41**, 4987.
24. U. Reiser & J. Jauch (2001) *Synlett*, **1**, 90.
25. G. Faita, et al. (2002) *Eur. J. Org. Chem.*, 1175.
26. S. Hanessian & F. Xie (1998) *Tetrahedron Lett.*, **39**, 733.
27. C. W. Phoon, et al. (1998) *Tetrahedron Lett.*, **39**, 7959.
28. A. Flörsheimer, et al. in "Peptides 1990, Proc. 21st European Peptide Symposium", E. Giralt & D. Andreu (Eds.), ESCOM, Leiden, 1990, pp. 131.

29. R. C. Sheppard, et al. (1982) *J. Chem. Soc., Chem. Commun.*, 587.
30. H. Rink (1987) *Tetrahedron Lett.*, **28**, 3787.
31. H. U. Immer, et al. in "Peptides, Chemistry, Structure & Biology" Proc. 11th American Peptide Symposium, J. E. Rivier & G. R. Marshall (Eds.), ESCOM, Leiden, 1990, pp. 1054.
32. R. S. Garigipati (1997) *Tetrahedron Lett.,* **38**, 6807.
33. S. L. Mellor, et al. (1997) *Chem. Commun.,* 2005.
34. K. M. Edvinsson, et al. (2000) *Bioorg. Med. Chem. Lett.,* **10**, 503.
35. W. K.-D. Brill, et al. (1998) *Synlett.*, 906.
36. W. K.-D. Brill & C. Riva-Toniolo (2001) *Tetrahedron Lett.,* **42**, 6515.
37. D. Beer, et al. (2002) *Bioorg. Med. Chem. Lett.,* **12**, 1973.
38. R. C. Sheppard & B. J. Williams (1982) *Int. J. Peptide Protein Res.,* **20**, 451.
39. E. Atherton & R. C. Sheppard in "Solid phase peptide synthesis; a practical approach", IRL Press, Oxford, 1989, pp. 152.
40. S. Mellor, et al. in "Fmoc solid phase peptide synthesis: a practical approach", W. C. Chan & P. D. White (Eds.), Oxford University Press, Oxford, 2000, pp. 141.
41. M. Munns & P. White, Unpublished results.
42. M. Renil, et al. (1998) *J. Pep. Sci.,* **4**, 195.
43. P. M. St. Hilaire, et al. (1999) *J. Comb. Chem.,* **1**, 509.
44. C. W. Tornøe, et al. (2002) *J. Org. Chem.,* **67**, 3057.
45. M. Hutchins & K. T. Chapman (1996) *Tetrahedron Lett.,* **37**, 4869.
46. C. Blackburn & B. Guan (2000) *Tetrahedron Lett.,* **41**, 1495.
47. W. F. DeGrado, et al. (1980) *J. Org. Chem.,* **45**, 1295.
48. W. F. DeGrado, et al. (1982) *J. Org. Chem.,* **47**, 3258.
49. B. H. Lee, et al. (2002) *Bioorg. Med. Chem. Lett.,* **12**, 353.
50. A. Pichette, et al. (1997) *Tetrahedron Lett.,* **38**, 1279.
51. A. W. Schwacher & T. Maynard (1993) *Tetrahedron Lett.,* **34**, 1269.
52. A. Golebiowski & S. Klopfenstein (1998) *Tetrahedron Lett.,* **39**, 3397.
53. E. Thouin & W. D. Lubell (2000) *Tetrahedron Lett.,* **41**, 457.
54. E. T. Kaiser, et al. (1989) *Science*, **243**, 187.
55. G. Oesapay (1990) *J. Am. Chem. Soc.,* **112**, 6046.
56. T. Sasaki, et al. *J. Org. Chem.,* **56**, 3159.
57. G. Oesapay, et al. (1990) *Tetrahedron Lett.,* **31**, 6121.
58. N. Nishino, et al. (1992) *J. Chem. Soc., Chem. Commun.,* 180.
59. S. R. Gilbertson & P. Lan (2002) *Tetrahedron Lett.,* **43**, 6961.
60. E. K. Dolence, et al. (2000) *J. Comb. Chem.,* **2**, 522.

2.9   Carboxy functionalized resins

NovaSyn® TG carboxy resin

NovaSyn® TG carboxy resin. can be used to immobilize alcohols through formation of an ester with the resin-bound carboxy group. Cleavage can be effected either by saponification [1] or transesterification [2-4] (Table 2-6, page 2.12).

In studies on the synthesis of furans *via* intramolecular radical cyclization, NovaSyn® TG carboxy resin was found to give superior results to carboxy polystyrene (Figure 2-61) [10]. This observation was attributed to the idea that the radical intermediates, by virtue of being tethered to the end of PEG chains, would be prevented from being quenched by the benzylic protons of the polystyrene backbone.

Fig. 2-61: Solid phase synthesis of dihydrobenzofurans.

Method 2-11: Conversion to acid chloride

1. Place the resin (1 g) in a clean, dry flask, and add sufficient toluene to just cover and allow to swell for 30 min. Add extra solvent if necessary just to cover the resin.
2. Add oxalyl chloride or thionyl chloride (5.0 eq. relative to resin substitution).
3. Fit flask with a reflux condenser and heat at 60 °C for 1-2 h.
4. Wash resin with dry toluene and DCM.

Method 2-12: Attachment of an alcohol

1. Suspend the acid chloride resin in DCM.
2. Add alcohol (2.0 eq.), DIPEA (2.0 eq.) and DMAP (0.1 eq.) dissolved in DCM. Leave o/n.
3. Wash resin with DCM, DCM/MeOH (2:1), MeOH. Dry resin.

Method 2-13: Colorimetric test for presence of COOH groups

1. Suspend a few beads of resin in 0.25% malachite green oxalate in EtOH.
2. Add 1 drop of TEA and leave to stand for 2 min.
3. Isolate beads by filtration and wash with EtOH. A positive test is indicated by green beads.

Related products

855065   NovaSyn® TG carboxy resin   p. 233

References

1. C. C. Leznoff, et al. (1977) *Can. J. Chem.,* **55**, 3351.
2. H. J. Meyers, et al. (1995) *Mol. Div.,* **1**, 13.
3. J. S. Panek & B. Zhu (1996) *Tetrahedron Lett.,* **37**, 8151.
4. R. C. D. Brown, et al. (2000) *Tetrahedron Lett.,* **41**, 3247.
5. B. A. Lorsbach, et al. (1998) *J. Org. Chem.,* **63**, 2244.
6. J. Cossy, et al. (2001) *Synlett*, **5**, 629.
7. K.-I. Washizuka, et al. (2000) Tetrahedron Lett., 41, 691.
8. S. Kobayashi, et al. (1999) *Tetrahedron Lett.,* 40, 1341.
9. L. J. Wilson (2001) *Org. Lett.,* **3**, 585.
10. A. Routledge, et al. (1997) Synlett, 61.
11. S. Porto, et al. (2003) *Org. Lett.,* **5**, 2979.

2.10 Aldehyde functionalized resins

Novabiochem® offers one of the most comprehensive selections of aldehyde-based resins for solid phase peptide and organic synthesis. These supports have numerous applications ranging from the synthesis of carboxy-modified peptides to nitrogen-containing heterocycles.

A useful and sensitive colorimetric test for monitoring aldehydes on solid phase has been described by Vazquez & Albericio (Method 2-14) [1].

2.10.1 Benzyloxybenzaldehyde polystyrene

This versatile resin has been used on a number of occasions in SPOS: by Reggelin & Brenig [2] in their efforts to develop polyketide libraries *via* polymer supported aldol reactions using chiral boron enolates (Figure 2-62); and by Wang & Wilson [3] to prepare 2,3-dihydro-4-pyridones *via* the Lewis acid catalyzed reaction of Danishefsky's dione with resin-bound imines. Kobayashi & Akiyama [4] have used this support to prepare isoxazolines *via* 3 + 2 addition of polymer-supported nitrones with alkenes. This strategy utilized an oxidative cleavage with DDQ to liberate the target isoxazoline (Figure 2-63).

Treatment with TMOF and catalytic TsOH converts this resin to the corresponding dimethyl acetal, which has been used by Furman, *et al.* [5] to prepare oxacephams in a strategy involving a cyclative-cleavage step (Figure 2-64).
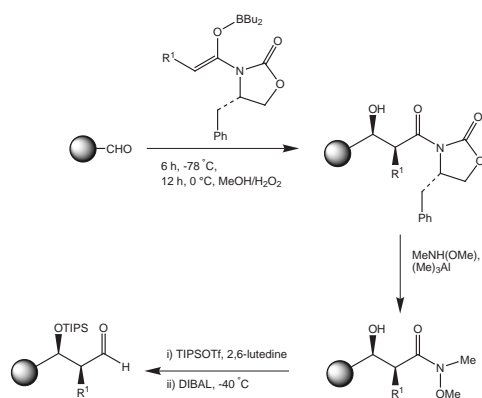
Fig. 2-62: Aldol synthesis on benzyloxybenzaldehyde polystyrene.
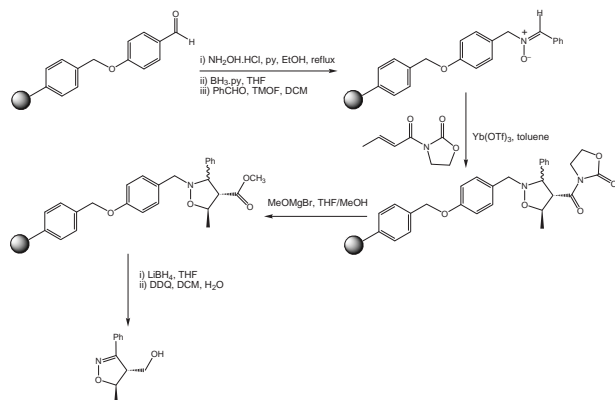
Fig. 2-63: SPOS of isoxazolines on benzyloxybenzaldehyde polystyrene.
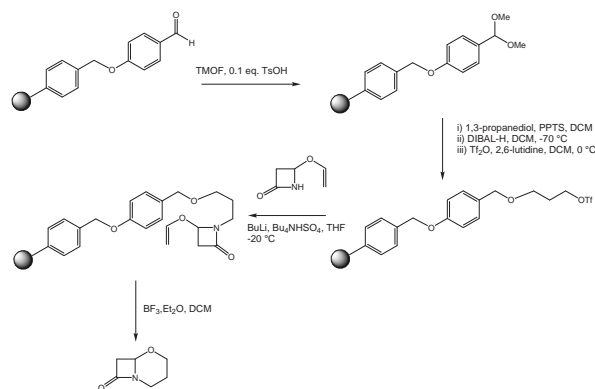
Fig. 2-64: SPOS of oxacephams.

2.10.2 FMPB AM, FMPB NovaGel™, DFPE, FIA AM resins
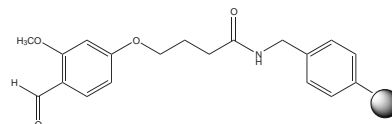
FMPB AM resin
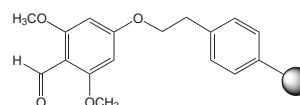
DFPE polystyrene
[2–(3,5–Dimethoxy–4–formylphenoxy)ethyl polystyrene]
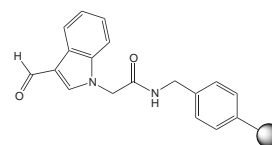
(3–Formylindolyl)acetamidomethyl polystyrene (FIA AM resin)

Alkoxybenzaldehyde-based and formyl indoles linkers are versatile tools for the synthesis peptide *N*-alkylamides and sulfonamides (Figure 2-65). The first linkers described for this purpose were based on trialkoxy systems related to the amide-releasing PAL resin [6-10]. More recently, linkers based on simpler and more accessible dialkoxybenzaldehydes [11-14] and formylindoles [15] have been exploited.

Novabiochem® offers both dialkoxy- and trialkoxybenzaldehyde-based and formyl indole linkers on a wide range of polymer-supports. Initial functionalization of these supports normally involves conversion of the resin-bound formyl group to the corresponding secondary amine, using excess amine in the presence of either $NaBH(OAc)_3$ in trimethylortho-formate (TMOF)/DCE or $NaBH_3CN$ in 1% AcOH in DMF, as described in Method 2-15, page 2.40. Acylation of the resultant supported amine with Fmoc-amino acids, carboxylic acids or sulfonic acids yields carboxamides or sulfonamides that can be later cleaved with TFA, with the exact concentration required depending on the nature of the linker and amide being released.

Fig. 2-65: Applications of formyl resins.

Fig. 2-66: Backbone-amide linker approach to carboxy-modified peptides.

The most important uses of formyl resins is the synthesis of carboxy-modified peptides as, depending on the nature of the amine initially anchored to the solid phase, these supports can be used to prepare esters [6-8, 14], *N*-alkylamides [6-8, 14, 18], alcohols [6-8, 14], aldehydes [6-8, 18], p-nitroanilides [19] and even peptide thioesters [19] (Figure 2-66). It is important to note that when amino acid esters are used as the first building block, DKP formation can occur following Fmoc removal at the dipeptide stage. This can be circumvented through use of *N*-Trt or Ddz protection on the second amino acid [7], or by incorporation of a dipeptide [14]. The syntheses of peptide *N*-alkylamides and a peptide ester are exemplified in Applications 2-6 to 2-10, respectively.

References

1. J. Vázquez & F. Albericio (2001) Tetrahedron Lett., 42, 6691.
2. M. Reggelin & V. Brenig (1996) Tetrahedron Lett., 37, 6851.
3. Y. Wang & S. R. Wilson (1997) Tetrahedron Lett., 38, 4021.
4. S. Kobayashi & R. Akiyama (1998) Tetrahedron Lett., 39, 9211.
5. B. Furman, et al. (1999) Tetrahedron Lett., 40, 5909.
6. K. J. Jensen, et al. in "Peptides: Chemistry, Structure and Biology: Proc. 14th American Peptide Symposium", P. T. P. Kaumaya & R. S. Hodges (Eds.), Mayflower Scientific Ltd., 1996, pp. 30.
7. K. J. Jensen, et al. (1998) J. Am. Chem. Soc., 120, 5441.
8. J. Alsina, et al. (1999) Chem. Eur. J., 5, 2787.
9. C. P. Holmes, Presented at the 213th National Meeting of the American Chemical Society, San Francisco, CA, April 1997, paper ORGN 383.
10. a) C. G. Boojamra, et al. (1995) J. Org. Chem., 60, 5742; b) C. G. Boojamra, et al. (1997) J. Org. Chem., 62, 1240.
11. A. M. Fivush & T. M. Willson (1997) Tetrahedron Lett., 38, 7151.
12. E. E. Swayze (1997) Tetrahedron Lett., 38, 8465.
13. D. Sarantakis & J. J. Bicksler (1997) Tetrahedron Lett., 38, 7325.
14. B. Dörner & P. White in "Peptides 1998, Proc. 25th European Peptide Symposium", S. Bajusz & F. Hudecz (Eds.), Budapest, Akadémiai Kiadó, 1999, pp. 90.
15. K. G. Estep, et al. (1998) J. Org. Chem., 63, 5300.
16. B. Yan, et al. (1998) J. Comb. Chem., 1, 58.
17. S. Bhattacharyya, et al. (2003) Tetrahedron Lett., 44, 6099.
18. J. C. Kappel & G. Barany (2005) J. Pept. Sci., 11, 525.
19. J. Alsina, et al. (1999) J. Org. Chem., 64, 8761.

Trialkoxybenzyl-based and formyl indole linkers are the most acid labile. In a study, which compared the cleavage rates of secondary amides, ureas, carbamates and sulfonamides from formylindole, dialkoxybenzaldehyde and Rink Amide resins, the formyl indole resin was found to be the most acid-labile [16]. Using 95% and 50% TFA, respectively, even anilines and guanidines could be released from this support, without the presence of an activating capping group. Another more recent study has shown that sulfonamides and ureas can be cleaved with as little as 1% TFA in DCM [17].

Application 2-6: Synthesis of Tos-Ala-NHCH₂CH(CH₃)₂

FMPB AM resin (0.26 g, 0.13 mmole) was swollen in DCE (2 ml) and TMOF (2 ml). Isobutylamine (199 µl, 2 mmole) and NaBH(OAc)$_3$ (424 mg, 2 mmole) were added, and the mixture left to stir at rt o/n under N$_2$, after which time the resin was washed with DMF, 10% DIPEA in DMF and DMF. The amine resin was then acylated with a mixture of Fmoc-Ala-OH (311 mg, 1 mmole), HOAt (136 mg, 1 mmole) and DIPCDI (157 µl, 1 mmole) in DMF for 5 h. The yield after two steps, as determined by Fmoc content, was quantitative. The Fmoc group was removed using 20% piperidine in DMF, and the resin was treated with Tos-Cl (191 mg, 1 mmole) and DIPEA (348 µl, 2 mmole) in DCM for 2 h. Treatment of this resin with 25% TFA in DCM for 1 h afforded, following evaporation and precipitation, the desired product in 80% overall yield. This material was analyzed by HPLC (Figure. 2-67) and ES-MS [expected M+H$^+$ 299.4, found 299.3].
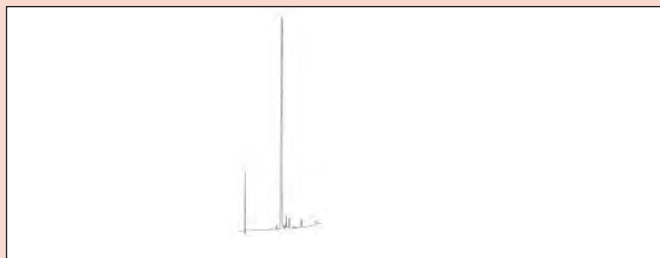
Fig. 2-67: HPLC elution profile of crude carboxamide.

Application 2-7: Synthesis of H-Tyr-Asp-Val-Thr-Ser-Pro-Phe-Ser-Ile-Ala-NHiPr on FMPE resin

FMPE resin (0.2 g, 0.11 mmole) was reductively aminated with isopropylamine (94 µl, 1.1 mmole) and NaBH(OAc)$_3$ (233 mg, 1.1 mmole) in TMOF/DCE (1:2), as described in Method 2-17, and loaded with Fmoc-Ala-OH, using DIPEA/HATU activation, in quantitative yield for two steps. Chain extension was carried out using standard Fmoc protocols using PyBOP®/HOBt/DIPEA activation. Treatment of the peptidyl resin with TFA/TIS/water (95:2.5:2.5) for 3 h afforded the desired product in 76% overall yield. This material was analyzed by HPLC (Figure 2-68) and ES-MS [expected M+H$^+$ 1140.5, found 1141.8].
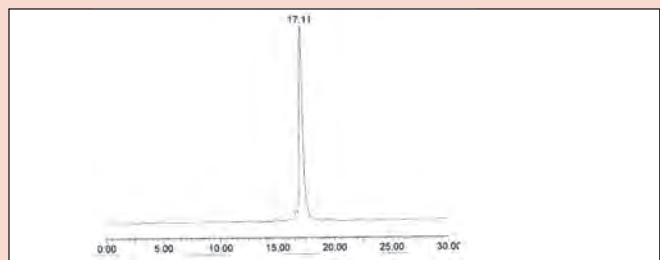
Fig. 2-68: HPLC elution profile of crude peptide.

Application 2-8: Synthesis of H-Tyr-Asp-Val-Thr-Ser-Pro-Phe-Ser-Ile-Ala-NHiPr on DFPEM resin

DFPEM resin was reductively aminated with isopropylamine, as described in Application 2-7, and loaded with Fmoc-Ala-OH, using DIPEA/HATU activation, in 72% yield for two steps. Chain extension was carried out using standard Fmoc protocols using PyBOP/HOBt/DIPEA activation. Treatment of the peptidyl resin with TFA/TIS/water (95:2.5:2.5) for 3 h afforded the desired product in 62% overall yield. This material was analyzed by HPLC (Figure 2-69) and ES-MS [expected M+H$^+$ 1140.5, found 1140.5].
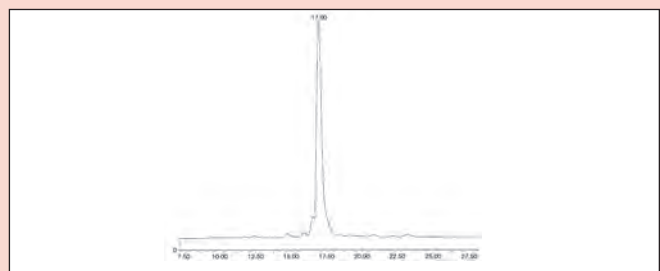
Fig. 2-69: HPLC elution profile of crude carboxamide.

Application 2-9: Synthesis of H-Tyr-Glu-Pro-Phe-Leu-Lys-Ala-Thr-Gly-OMe

FMPB AM resin (0.3 g, 0.23 mmole) was swollen in DMF (4 ml) and TMOF (2 ml). H-Gly-OMe·HCl (0.3 g, 2.4 mmole), DIPEA (377 µl, 2.4 mmole) and NaBH(OAc)$_3$ (493 mg, 2.3 mmole) were added, and the mixture left to stir at rt o/n under N$_2$, after which time the resin was washed with DMF, 10% DIPEA in DMF and DMF. The amine resin was then acylated in DMF for 18 h with Fmoc-Ala-Thr($\chi^{Me,Me}_{pro}$)-OH (452 mg, 1.1 mmole) activated with HATU (445 mg, 1.1 mmole) and DIPEA (360 µl, 2.3 mmole). The yield after two steps, as determined by the Fmoc loading, was 44%. Chain extension was carried out using standard Fmoc protocols with PyBOP/HOBt/DIPEA activation. Treatment of the peptidyl resin with TFA/TIS/water (95:2.5:2.5) for 3 h gave the desired product in 70% yield, based Fmoc-Ala loaded resin. This material was analyzed by HPLC (Figure 2-70) and ES-MS [expected M+H$^+$ 1040.2, found 1039.6].
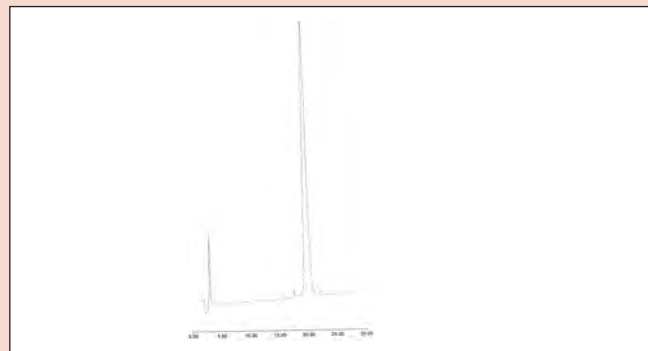
Fig. 2-70: HPLC elution profile of crude peptide ester.

Method 2-15: Reductive amination of formyl resins

TMOF/DCE method
1. Pre-swell the resin in DCE for 1 h and drain off excess solvent.
2. Dissolve the amine (10 eq.) in DCE/TMOF (2:1). Add to resin.
3. Add NaBH(OAc)$_3$ (10 eq.) and additional DCE/TMOF to keep the mixture mobile. Stir the mixture o/n at rt.
4. Wash the resin with DMF, 10% DIPEA in DMF, DMF, DCM and dry.

DMF/NaBH$_3$CN method
1. Pre-swell the resin in DMF for 1 h.
2. Dissolve the amine (10 eq.) in 1% AcOH in DMF. Add to resin.
3. Add NaBH$_3$CN (10 eq.) and additional DMF to keep the mixture mobile. Stir the mixture o/n at rt.
4. Wash the resin with DMF, 10% DIPEA in DMF, DMF, DCM and dry.

Acylation of aminated resins
Aminated resins derived from methoxyformyl supports can be loaded satisfactorily as described in Method 2-5, page 2.20. For resins derived from dimethoxyformyl resins the same protocol can be applied except DCM/DMF (9:1) should be employed as the solvent.

2.11 Enol functionalized resins

2.11.1 DHP HM resins
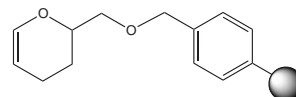
Novabiochem®'s DHP HM resin consists of Ellman's 3,4-dihydro-2*H*-pyran-2-yl-methanol linker [1] attached to 100-200 mesh chloromethyl polystyrene, and is an ideal support for the reversible solid phase immobilization of primary and secondary alcohols [1-9], phenols [10], indoles [11] and purines [12]. A similar support has also been used to immobilize tetrazoles [13].

In contrast to trityl-based supports, where the use of prolonged reaction

times and elevated temperatures are often required to achieve satisfactory loadings, derivatization of DHP HM resin is relatively straightforward, with even secondary alcohols being loaded without difficulty. Typically, this process involves treating the resin in DCE with an excess of alcohol, phenol or indole in the presence of pyridinium *p*-toluenesulfonate (PPTS); full experimental details are given in Method 2-16.

Resin-bound THP ethers generated by this process are stable to basic and strongly nucleophilic reagents, but are easily cleaved by treating with 95% TFA/5% water [2], TFA/DCM/EtOH [1]. However, the use of TFA can lead, in some cases, to trifluoroacetate formation. Methods which eliminate this potential side reaction include PPTS/BuOH/DCE [3], PPTS/DCE/PrOH [4] (Figures 2-73), PPTS/DCE/MeOH [5] (Figure 2-74), and TosOH in DCM [14]. Indoles and purines have been cleaved with 10% and 20% TFA in DCM, respectively.

An example of the use of this resin in the preparation of a peptide alcohol is given in Application 2-12.
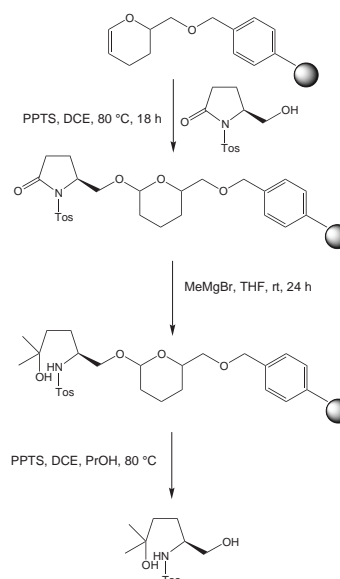
Fig. 2-73: Synthesis of amino alcohols using DHP HM resin.
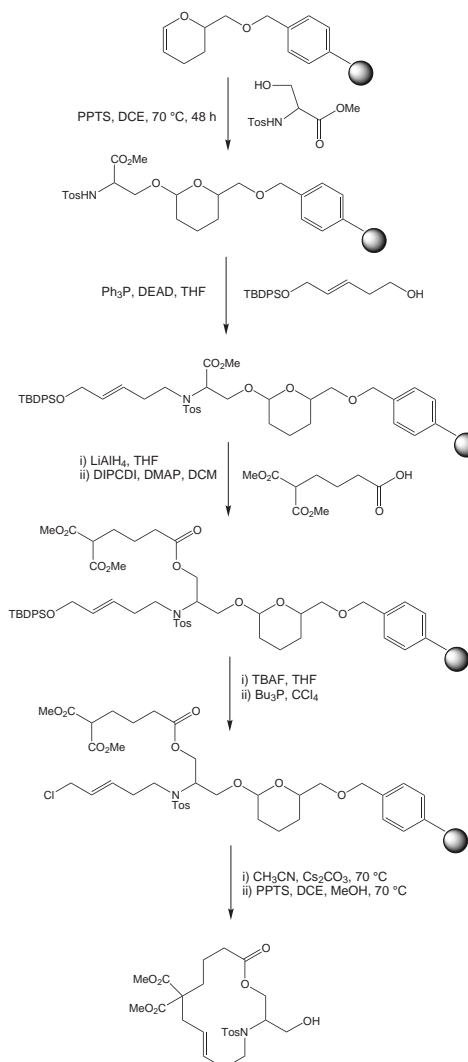
Fig. 2-74: SPOS of macroheterocycles using DHP HM resin.

Application 2-12: Preparation of a peptide alcohol using DHP HM resin

H-Val-Asp(OtBu)-Tyr(tBu)-Ser(tBu)-Phe-Thr(tBu)-Glu(OtBu)-Leu-Gly-ol-DHP HM resin was assembled using a NovaSyn® Crystal peptide synthesizer on Fmoc-Gly-ol-DHP HM resin (0.75 mmole/g, 0.1 g), which had been previously prepared according to Method 2-18. All acylation reactions were carried out for 1 h using Fmoc-amino acids activated with 1 eq. of PyBOP® in the presence 2 eq. of DIPEA and 1 eq. of HOBt.

Treatment of the peptidyl resin with TFA/TIS/H$_2$O (95:5:5) for 2 h provided the crude peptide alcohol in a yield of 58 mg (76%). This material was analyzed by RP-HPLC (Figure 2-75) and ES-MS (Figure 2-76) [expected M+H$^+$ 1016.1, found 1016.7].
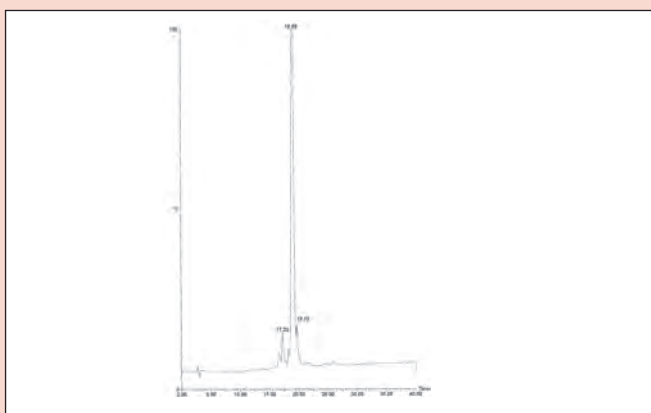
Fig. 2-75: HPLC elution profile of crude peptide alcohol.
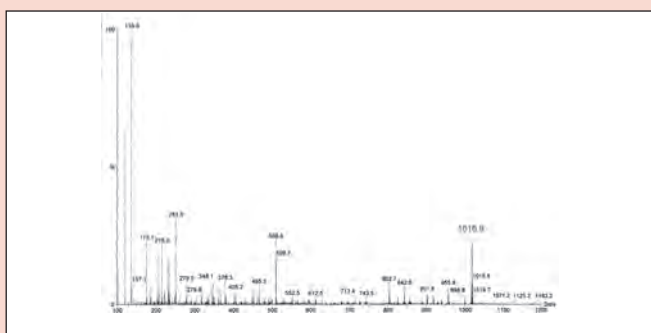
Fig. 2-76: ES-MS of crude peptide alcohol.

References

1. L. A. Thompson, et al. (1994) *Tetrahedron Lett.*, 35, 9333.
2. O. B. Wallace (1997) *Tetrahedron Lett.*, 38, 4939.
3. G. Liu & J. A. Ellman (1995) *J. Org. Chem.*, 60, 7712.
4. J. Cossy, et al. (2000) *Synlett*, 3, 409.
5. M. Ramaseshan, et al. (2000) *J. Comb. Chem.*, 2, 615.
6. M. Ramaseshan, et al. (2000) *Tetrahedron Lett.*, 41, 4743.
7. B. Meseguer, et al. (2000) *Chem. Eur. J.*, 6, 3943.
8. A. Bianco, et al. (2000) *J. Org. Chem.*, 65, 2179.
9. J. Zhou, et al. (1999) *Tetrahedron Lett.*, 40, 2729.
10. W. H. Pearson, et al. (1997) *Tetrahedron Lett.*, 38, 7669.

11. A. L. Smith, et al. (1998) *Tetrahedron Lett.*, 39, 8317.
12. D. Nugiel, et al. (1997) *J. Org. Chem.*, 62, 201.
13. S.-e. Yoo, et al. (1997) *Tetrahedron Lett.*, 38, 1203.
14. J. Beythien, personal communucation.

2.12 Thiol functionalized resins

2.12.1 3–[4–(Tritylmercapto)phenyl]–propionyl AM resin

This highly reactive resin-bound thiophenol can be used not only as a scavenger for electrophilic reagents but also as support for SPOS. In a study, in which a number of thiol-based resins were compared with respect to their efficacies as S$_N$Ar-labile linkers in the synthesis of aminopyridazines [1], this support was found to give the best results. Its use facilitated quantitative cleavage of thioether immobilized pyridazines with amines, without the need for prior oxidation (Figure 2-77. This support has also been used to prepare polymer-supported NHS by reaction with *N*-hydroxymaleimide [2].
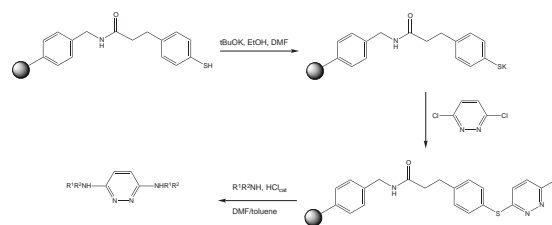
Fig. 2-77: SPOS of pyridazines.

Before use, the trityl group must be removed by treatment of the resin with 50% TFA in DCM as described in Method 2.19, page 2.46.

References

1. I. Parrot, et al. (1999) *Tetrahedron Lett.*, **40**, 7975.
2. M. Katoh & M. Sodeoka (1999) *Bioorg. Med. Chem. Lett.*, **9**, 881.

2.13 Carbonate resins
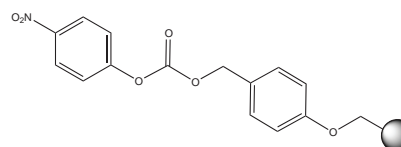
p–Nitrophenyl carbonate Wang resin

Resin-bound *p*-nitrophenyl carbonate esters react readily with amines to provide the corresponding resin-bound carbamate. Dixit & Leznoff [1] were the first to demonstrate the utility of such resins in solid phase synthesis with the preparation of monoamides from symmetrical diamines. Dressman, *et al.* [2] have subsequently used carbamate linked resin-bound amino acid amides to make hydantoins *via* a base mediated cyclization / cleavage strategy; a similar strategy was also employed by

Gouilleux and co-workers [3] to prepare quinazoline-2,4-diones. More recently, *p*-nitrophenyl carbonate Wang resin has been used to prepare homoallylic amines *via* *N*-acyliminium ion reactions (Figure 2-78) [4], guanidines using immobilized pyrazolecarboamidine [5], carbocyclic nucleosides through Pd(0) mediated allylic substitution (Figure 2-79) [6], neuroimmunophilin ligands derived from proline [7], and benzimidazolones by cyclative cleavage [8].

Smith, *et al.* [9] have loaded Wang carbonate with indoles under strongly basic conditions, and used such compounds to prepare novel h5-HT$_{2A}$ antagonists (Figure 2-80). To avoid reattachment of the indole to the resin during TFA cleavage, product release was effected by either nucleophilic displacement with pyrrolidine or by hydrolytic cleavage with AcOH.

Ho & Kukla [10] have utilized carbamates derived Wang resin as latent methylamines (see Figure 2-81). Conversely, Salvino, *et al.* [11] have exploited the relative stability of carbamates to borane to prepare piperazines by the on-resin borane reduction of acylpiperazines immobilized on carbonate Wang resin (Figure 2-82).

The enhanced stability and greater susceptability to nucleophiles of carbamates derived from Merrifield nitrophenyl carbonate resin have been exploited by His, *et al.* [12] to prepare amides *via* on-resin Beckman rearrangment of ketoxime carbonates (Figure 2-83) and by Park & Cox [13] to synthesize triazolidinediones *via* cyclative cleavage of immobilized semicarbazides (Figure 2-84).
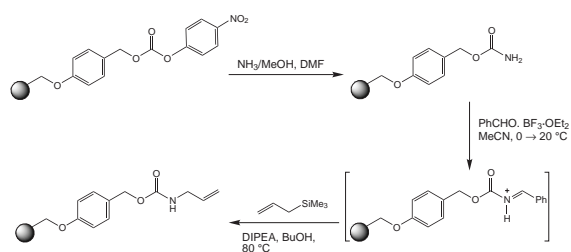
Fig. 2-78: Synthesis of homoallylic amines *via* N-acyliminium ion reactions.
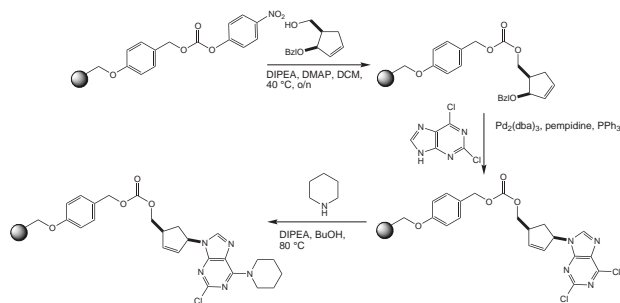
Fig. 2-79: Preparation of carbocyclic nucleosides.
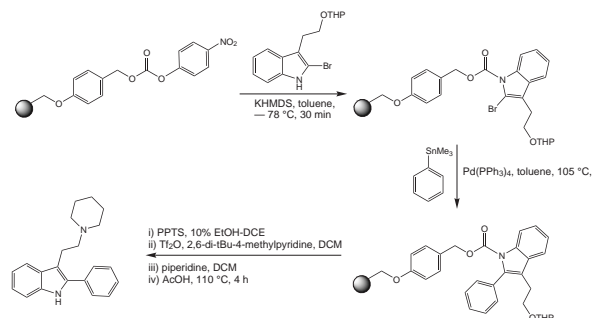
Fig. 2-80: Synthesis of h5-HT$_{2A}$ receptor antagonists.
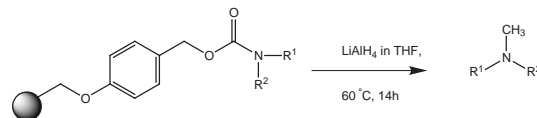
Fig. 2-81: Preparation of methylamines from supported carbamates.
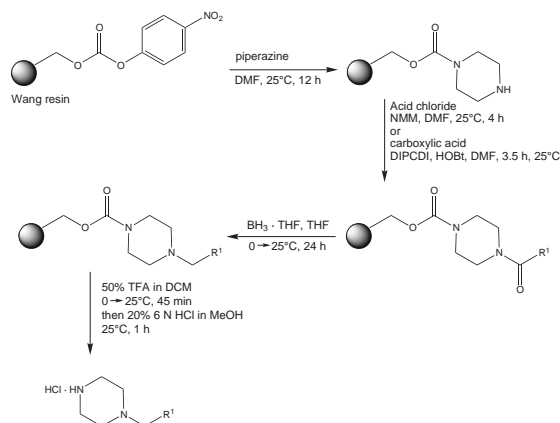
Fig. 2-82: Preparation of alkylpiperazines from supported carbamates..
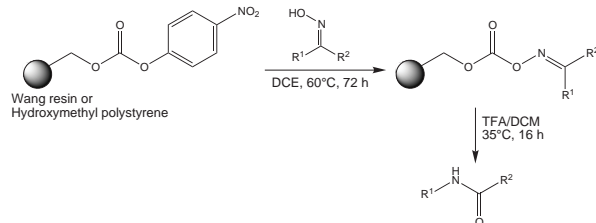
Fig. 2-83: Preparation of amides by on-resin Beckmann rearrangement.
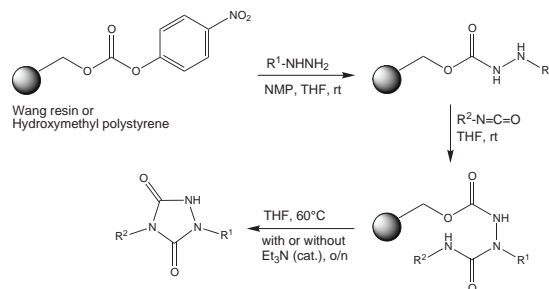
Fig. 2-84: Preparation of triazolidinediones by cyclative cleavage.

Amines linked to Wang and Merrifield *p*-nitrophenyl carbonate resins possess similar chemical properties to methoxybenzyloxycarbonyl and Z-protected amines respectively. In the case of *p*-nitrophenyl carbonate Wang resin, the resin-bound carbamate can be cleaved with TFA (Method 2-19,

page 2.46) or by hydrogenolysis to afford the free amine [14]. TFA/DMS (4:1) has been used to cleave Merrifield *p*-nitrophenyl carbonate resin [15]. Amides can be obtained by Lewis acid catalyzed cleavage [16]. The degree of loading of this resin can be determined using Method 2-17 [17].

Method 2-17: Determination of the loading of carbonate resins

1. Take approximately 1.5 µmole of p-nitrocarbonate starting resin and suspend in 20% piperidine in DMF (1 ml) for 1 h. Take an identical quantity of amino-loaded resin and treat with piperidine in DMF in the same way.
2. Take 20 µl of each solution and dilute to 25 ml with DMF.
3. Determine optical density of both solutions at 434 nm, measured against a reagent blank.
4. Calculate the loading using the following formula:
   Loading (mmole/g)=(Abs$_{starting\ resin}$-Abs$_{loaded\ resin}$) x 1250/(32,800 x weight of resin in gram)

Related products

855072	p-Nitrophenyl carbonate Merrifield resin	p. 230
855019	p-Nitrophenyl carbonate Wang resin	p. 231

References

1. D. M. Dixit & C. C. Leznoff. (1978) *Israel J. Chem.*, **17**, 248.
2. B. A. Dressman, et al. (1996) *Tetrahedron Lett.*, **37**, 937.
3. L. Gouilleux, et al. (1996) *Tetrahedron Lett.*, **37**, 7031.
4. W. J. N. Meester, et al. (1999) *Tetrahedron Lett.*, **40**, 1601.
5. A. K. Ghosh, et al. (2001) *J. Org. Chem.*, **66**, 2161.
6. M. T. Crimmins & W. J. Zuercher (2000) *Org. Lett.*, **2**, 1065.
7. M. Rabinowitz, et al. (2000) *Bioorg. Med. Chem. Lett.*, **10**, 1007.
8. C.-C. Wang & W.R. Li (2004) *J. Comb. Chem.*, **6**, 899.
9. A. L. Smith, et al. (2000) *Bioorg. Med. Chem. Lett.*, **10**, 2693.
10. C. Y. Ho & M. J. Kukla (1997) *Tetrahedron Lett.*, **38**, 2799.
11. J. M. Salvino, et al. (2003) *J. Comb. Chem.*, **5**, 260.
12. S. His, et al. (2003) *Tetrahedron Lett.*, **44**, 8581.
13. K.-H. Park & L. J. Cox (2002) *Tetrahedron Lett.*, **43**, 3899.
14. J. R. Hauske, et al. (1995) *Tetrahedron Lett.*, **36**, 1589.
15. L. Yang (2000) *Tetrahedron Lett.*, **41**, 6981.
16. W.-R. Li, et al. (2000) *Tetrahedron Lett.*, **41**, 6619.
17. A. Paio, et al. (2003) *Tetahedron Lett.*, **44**, 1867.

2.14 Alkenylcarbonyl resins

2.14.1 REM resin
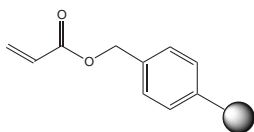
REM resin [1] is an excellent tools for the SPOS of tertiary amines. Conjugate addition of primary or secondary amines results in formation of the corresponding resin-bound secondary or tertiary amine. Following quaternization with the appropriate alkyl halide, treatment with DIPEA causes the target tertiary amine to be eliminated from the resin (Figure 2-85), in the process regenerating the starting support which can then be reused. DMSO appears to be the optimum solvent for quarternization [2].

The cleavage reaction has also been effected in a heterogeneous fashion using a weakly basic ion-exchange resin or Rink Amide resin [3], solid-supported piperazine [4], and a soluble polymer-bound tertiary amine [5]. Alhambra, *et al.* [6] have reported on the use of TBD-methyl polystyrene, which was found to give superior results owing to its greater basicity. Morphy, *et al.* have described a one-pot elimination-transesterification approach using perfluorosolvents to accelerate resin cleavage [7].

New methods of product release from REM resins have been described: Kondo, *et al.* [8] have prepared heteroaromatic systems *via* photoinduced cyclorelease (Figure 2-86); Sammelson & Kurth [9] have generated hydroxylamines *via* Cope elimination following oxidation of the polymer-bound tertiary amine with MCPBA (Figure 2-87); Hanessian & Bayrakdarian [10] have synthesized substituted pyrrolidines by a cyclative-cleavage strategy (Figure 2-88).

REM-type resins have also been exploited in the following syntheses: analogs of the delta opioid ligand SNC-80 [11]; tropane derivatives by 1,3-dipolar cycloaddition [12]; Zatebradine analogs [13]; tetrahydrocarbolines [15]; dimethyltyptamines [16].
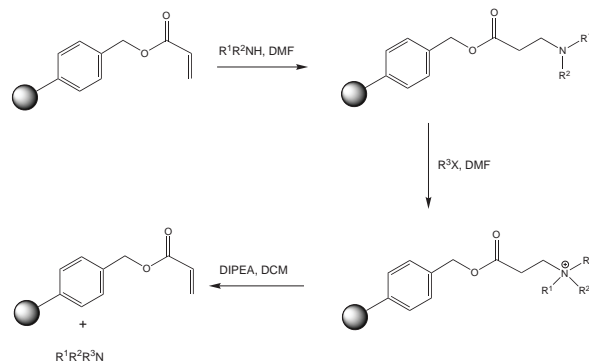
Fig. 2-85: Uses of alkenylcarbonyl/sulfonyl resins.
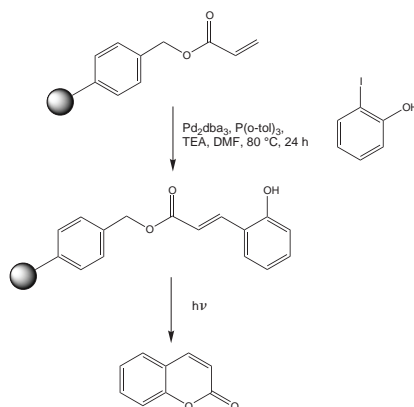
Fig. 2-86: Photoinduced cyclorelease from REM resin.
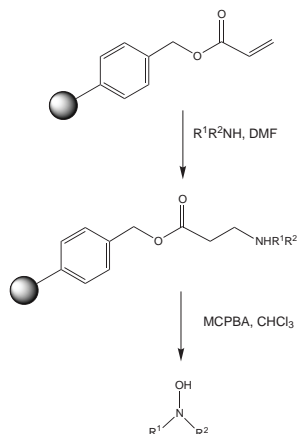
Fig. 2-87: Oxidation–Cope elimination from REM resin.
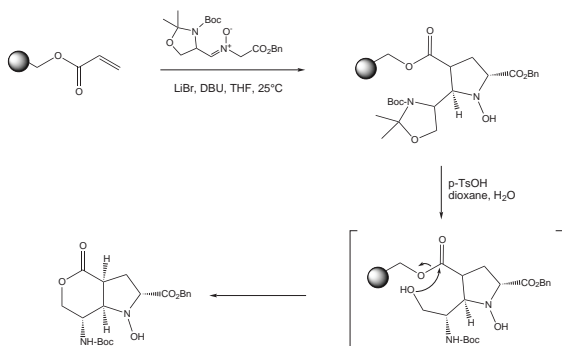
Fig. 2-88: Cyclative cleavage of pyrrolidines from REM resin.

Method 2-18: Use of REM resin

Loading
1. Pre-swell REM resin in DCM for 1 h. Wash resin with DMF.
2. Add amine (10 eq.) and agitate gently at rt for 18 h.
3. Wash resin with DMF and DCM.

Cleavage
1. Pre-swell resin in DCM for 1 h. Wash resin with DMF.
2. Add alkyl bromide (10 eq.) and agitate gently at rt for 18 h.
3. Wash resin with DMF and DCM.
4. Suspend resin in DCM, add DIPEA (2 eq.) and agitate gently at rt for 18 h; or
4. Suspend resin in THF, add TBD-methyl polystyrene (3 eq.) and agitate gently at rt for 24 h.
5. Remove the resin by filtration. Wash with DCM.
6. Evaporate combined filtrates to dryness.

Related products
855101	REM resin (50-100 mesh)	p. 227

References
1. A. Brown, et al. (1997) *J. Am. Chem. Soc.,* **119**, 3288.
2. K. S. Cameron, et al. (2002) *J. Comb. Chem.,* **4**, 199.
4. X. Ouyang, et al. (1998) *J. Org. Chem.,* **63**, 1027.
5. Y. Yamamoto, et al. (1999) *Chem. Lett.,* 103.
6. P. H. Toy, et al. (2000) *Org. Lett.,* **2**, 2205.
7. C. Alhambra, et al. (2001) *Tetrahedron Lett.,* **42**, 6675.
8. J. R. Morphy, et al. (2002) *Tetrahedron Lett.,* **43**, 6413.
9. Y. Kondo, et al. (2000) *J. Comb. Chem.,* **2**, 232.
10. R. E. Sammelson & M. J. Kurth (2001) *Tetrahedron Lett.,* **42**, 3419.
11. S. Hanessian & M. Bayrakdarian (2002) *Tetrahedron Lett.,* **43**, 9441.
12. J. Cottney, et al. (1999) *Bioorg. Med. Chem. Lett.,* **9**, 1323.
13. S. Caix-Haumesser, et al. (2001) *Tetrahedron Lett.,* **42**, 3721.
14. A. Bom, et al. (2001) *Bioorg. Med. Chem. Lett.,* **11**, 2351.
15. R. V. Connors, et al. (2002) *Tetrahedron Lett.,* **43**, 6661.
16. T. Y. H. Wu & P. G. Schultz (2002) *Org. Lett.,* **4**, 4033.

2.15 Basic resin handling procedures

It is important to ensure that all solid phase supports are fully swollen before use. This is particularly important for polystyrene resins as their swelling properties vary widely from solvent to solvent. For such resins, it is simplest to swell the resin first in good swelling solvent, such as DCM or THF, for a minimum of 1 hour, before exchanging this for the reaction solvent. With Fmoc-protected amino-functionalized polystyrene resins, it is often best to remove this first Fmoc group with 20% piperidine in DCM.

With PEGA and PEG-PS-based resins, such as TG and NovaGel™ resins, it is possible to swell the resin directly in the reaction solvent. However, before exchanging solvents of widely differing polarities or those that are immiscible, it is best to wash the resin with solvents of intermediate polarity, changing the polarity gradually from one extreme to the other. For example, to exchange DCM for water, one could wash consecutively with THF, MeOH, MeOH/water, water. The same consideration applies to polystyrene resins, although to a lesser extent because of the more limited range of solvents in which these resins swell. However, with large bead-sized polystyrene resins it is important to use a solvent gradient, when exchanging from a good swelling to a poor swelling solvent, to avoid the risk of the beads bursting through osmotic shock.

2.15.1 TFA cleavage procedures
The vast majority of the resins described previously have linkers or handles which release the product upon treatment with acid. The strength of the acid required to effect cleavage depends both on the nature of the linker and the leaving group. In general, the more electron-rich the linker, the easier it is to cleave the product. The ease of release of common functionalities approximately follows the order: $RCO_2H > ArOH > AlkOH > ArNH_2 \sim RCONHR > AlkNH_2 > RSH \sim RCONH_2$. With very electron-rich linkers, such as those of the trialkoxybenzhydryl and trityl type, all the functional groups listed will be released with TFA. Conversely, with relatively electron-poor linkers, such as hydroxymethylpolystyrene, even the most easily released functionality, CO_2H, may require the use of the extremely strong acid HF.

The most frequently used combinations of immobilized functional group and linker are those that are cleaved with TFA; these are given in Table 2-6 on page 2-12. The standard method of TFA cleavage involves treatment with 25-50% TFA in DCM. In some instances lower amounts of TFA can be used, but unless there is a requirement to leave certain TFA-labile protecting groups in place, this is not generally practised. When the cleavage reaction is reversible, such as when detaching thiol groups from trityl resins, it is advisable to add a scavenger to the cleavage mixture. The most frequently used scavengers are water and triisopropylsilane. The latter is particularly useful since it can react under acidic conditions with the cation formed at the linker, converting it irreversibly to the corresponding hydrocarbon. It is also important to employ a scavenger when the product contains nucleophilic groups which remain reactive under acidic conditions, as alkylation of these by the linker-cation can result in reattachment of the target to the solid phase. The

problematic functionalities in this regard are pyrroles, indoles, phenols, and thioethers.

Detachment of alcohols with TFA can lead to O-trifluoro-acetylation. In most cases this problem can be overcome either by hydrolyzing the acetate with aq. sodium acetate or by using HCl in dioxane to effect cleavage.

Method 2-19: TFA cleavage

1. Suspend the resin in TFA/DCM (1:1)[a] for 2 h.
2. Remove resin by filtration and wash with DCM.
3. Combine filtrates and evaporate to dryness.

[a]For trityl resins lower concentrations may be used. For the release of thiol, alcohol and acid groups, the addition of 1% TIS is efficacious.

2.15.2 Nucleophilic cleavage of solid–phase esters

Nucleophilic cleavage of polymer-supported esters is an attractive synthetic strategy since, depending on the nature of the nucleophile employed, a wide variety of carboxylic acid derivatives can be prepared from just one resin. Treatment with hydroxide gives the carboxylate, methoxide the methyl ester and a primary amine the *N*-alkylcarboxamide.

The ease of this reaction depends obviously on the nature of the nucleophile and on the electronic properties of the linker. Esters derived from linkers bearing electron-withdrawing groups are particularly susceptible: for example, hydroxymethylbenzoyl, oxime and bromoacetyl. However, even esters derived from Wang-type resins can be cleaved under forcing conditions.

The methods given below work for the resins listed above. One important major consideration is the nature of the support as aqueous and methanolic reaction mixtures are not compatible with polystyrene supports, although in practice poor swelling can be generally overcome by inclusion of a good solvent, such as dioxane or THF, into the reaction mixture.

Method 2-20: Hydroxide cleavage [1]

1. Pre-swell the resin in THF for 1 h (12 ml/g).
2. Add LiOH (5 eq.) dissolved in MeOH:water (2:1) (8 ml/g).
3. Leave to stand for 1-2h (HMBA resins, oxime resin, bromoacetamidomethyl polystyrene) at rt. For Merrifield resin, reflux mixture o/n.
4. Acidify to pH 7.0 with 1 M HCl. Remove resin by filtration and wash resin with THF.
5. Combine filtrates and evaporate.
6. Redissolve residue in EtOAc and wash organic layer with water, sat. NaCl. Dry organic layer over $MgSO_4$ and evaporate to dryness.

Method 2-21: Cleavage to give methyl ester

Methoxide method [2]

1. Pre-swell the resin in THF for 1 h.
2. Add NaOMe (2 eq.) in minimum volume of MeOH, and reflux for 3-5 h.
3. Remove the resin by filtration and wash with THF, DCM.
3. Acidify to pH 7.0 with 1 M HCl.
6. Remove water with anhydrous $MgSO_4$, filter and evaporate to dryness.

Methanol/TEA method

1. Pre-swell the resin in THF for 1 h.
2. Suspend the resin in TEA / MeOH / THF (1:5:5) and heat at 50 °C for 3-18 h. The addition of KCN is known to catalyze the reaction [3].
3. Remove the resin by filtration and wash with THF, DCM.
4. Evaporate combined filtrates to dryness.

2.15.3 Reductive cleavage to give alcohols

Cleavage of polymer bound esters derived from benzyl alcohol-based linkers by reduction is a useful strategy for the preparation of alcohols from carboxylic acids. Reduction has been carried out using $LiBH_4$ [4] or DIBAL-H [5]. Activated systems such as those derived from hydroxymethylbenzoic acid can even be cleaved with $NaBH_4$ (Method 3-36, page 3.30).

Method 2-22: Reductive cleavage

DIBAL-H reduction

1. Suspend the resin (0.1 mmole) in dry toluene, and leave to swell for 1 h under N_2. Cool to 0 °C.
2. Add 1 M DIBAL-H in toluene (0.5 mmole, 0.5 ml) and leave mixture to stir under N_2 for 18 h.
3. Dilute the mixture with THF and add saturated $KHSO_4$ (0.5 ml) and K, Na tartrate (0.3 ml). Gently agitate mixture for 30 min.
4. Remove resin by filtration and wash it with DCM.
5. Dry combined organic filtrates with anhydrous $MgSO_4$, filter and evaporate to dryness.

$LiBH_4$ reduction

1. Pre-swell the resin (0.1 mmole) in dry THF for 1 h under N_2.
2. Add $LiBH_4$ (0.5 mmole) portion-wise and leave mixture to stir under N_2 for 1 h.
3. Dilute the mixture with THF and add saturated $KHSO_4$ (0.5 ml) and K, Na tartrate (0.3 ml). Gently agitate mixture for 30 min.
4. Remove resin by filtration and wash it with DCM.
5. Dry combined organic filtrates with anhydrous $MgSO_4$, filter and evaporate to dryness.

References

1. S. Chamoin, et al. (1998) *Tetrahedron Lett.*, **39**, 4175.
2. L. F. Tietze, et al. (1996) *Synlett*, 1043.
3. G. J. Kuster & H. W. Scheeren (1998) *Tetrahedron Lett.*, **39**, 3613.
4. J. M. Stewart & J. D. Young in "Solid Phase Peptide Synthesis, 2nd Ed.", Pierce Chemical Company, Rockford, 1984, pp. 92.
5. M. J. Kurth, et al. (1994) *J. Org. Chem.*, **59**, 5862.

3: Peptide synthesis protocols

For detailed protocols on most aspects of Fmoc solid phase synthesis, the reader is referred to W. C. Chan & P. D. White, "Fmoc solid phase peptide synthesis: a practical approach", Oxford University Press, Oxford, 2000.

3.1   Coupling methods

Efficient and unambiguous peptide-bond formation requires chemical activation of the carboxyl component of the N-α-protected amino acid. The activating group or reaction must be chosen carefully to achieve a very high coupling efficiency and at the same time avoid potential side reactions.

There are basically four major types of coupling techniques currently employed for step-wise introduction of N-α-protected amino acids in solid phase synthesis: carbodiimide; symmetrical anhydride; active ester; and coupling reagent. For a recent review on coupling methods, see [1].

3.1.1   Carbodiimides
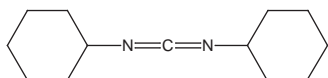
Carbodiimides have been some of the most popular *in situ* activating reagents in peptide synthesis. Dicyclohexylcarbodiimide (DCC), first described in the 1950s [2], remains one of the most popular, particularly for solution phase synthesis, owing to the insolubility of the by-product dicyclohexylurea (DCU) which can be removed by filtration. However, DCU can be difficult to completely remove as it is partially soluble in most organic solvents. For this reason, EDC (1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride), which forms a water-souble urea that is easily removed by extraction, is now preferred for solution synthesis [3].

For solid phase synthesis, carbodiimides, such as diisopropylcarbodiimide (DIPCDI) [4, 5], *t*-butylmethylcarbodiimide [6, 7] and *t*-butylethylcarbodiimide [6], which form DMF soluble ureas are preferred.

OBt and Oxyma Pure esters

Activation with carbodiimides is fast in DCM but sluggish in DMF. The highly reactive O-acylurea intermediate that is initially formed can undergo side-reactions such as the dehydration of Asn and Gln residues, racemization and N-acylurea or oxazolone formation. For this reason, auxillary nucleophiles such as HOBt (1-hydroxybenzotriazole) [8], HOOBt (1-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine) [9] or HOSu (N-hydroxysuccinimide) [10] are commonly employed in conjunction with carbodiimides to capture the O-acylurea and convert it to a less-reactive and more selective active ester.

Of these, HOBt is one of the most widely used, owing to the excellent reactivity and chiral stability of benzotriazolyl (Bt) esters of amino acids and peptides. However, recently HOBt monohydrate, the standard form of this reagent, was reclassified by the UN as a class 1c explosive. This measure, unfortunately, means that we can no longer ship the product by air or sea and has the effect of making land shipment prohibitively expensive.

The lack of availability of HOBt has caused enormous difficulties to peptide chemists and has led to a search for a non-explosive alternative to HOBt. One candidate, ethyl 2-cyano-2-(hydroxyimino)acetate, also known as Oxyma Pure, shows particular promise. Subirós-Funosas, *et al.* [11] have shown this reagent to be more effective and give lower racemization than HOBt in carbodiimide-mediated coupling reactions.

Oxyma Pure was first identified as a potential coupling additive in the 1970s [12, 13]. It has a pK_a of 4.60, the same as HOBt, but lacks the potentially explosive triazole structure of HOBt and analogous compounds such as HOAt [14] and Cl-HOBt [15] (Table 3-1).

Table 3-1: pK_a of Oxyma Pure and benzotriazole-based coupling reagents.

Compound	pKa
HOAt	3.28
HOBt	4.6
6-Cl-HOBt	3.35
Oxyma	4.60

Like HOBt, Oxyma Pure reacts with protected amino acids or peptides in the presence of carbodiimides to form active esters capable of acylating amines (Figure 3-1) (Method 3-1).

> **Method 3-1: Oxyma ester formation**
> 1. Dissolve the Fmoc amino acid (5 eq. relative to resin loading) and Oxyma (5 eq.) in DCM/DMF (1:1).
> 2. Add DIPCDI (5 eq. relative to resin loading) to the amino acid solution.
> 3. Stir the mixture for 10 min at rt, keeping the reaction mixture free of moisture with a calcium chloride drying tube.
> 4. Add the solution to the resin.
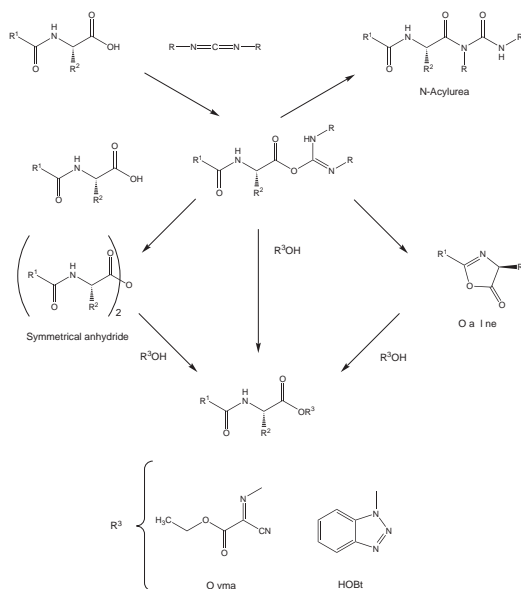
Fig. 3-1: Possible reaction pathways for carbodiimide activation in presence of Oxyma Pure or HOBt.

Racemization

Subirós-Funosas, et al. [11] selected the coupling of the notoriously racemization-prone Z-Phg-OH to H-Pro-NH$_2$, the fragment condensation of Z-Phe-Val-OH to H-Pro-NH$_2$ and the solid phase synthesis of H-Gly-Cys-Phe-NH$_2$ as model systems to compare Oxyma Pure against HOBt and HOAt. In these tests (Tables 3-2 - 3-4), Oxyma Pure performed extremely effectively giving levels of racemization comparable to HOAt and better than HOBt. Moreover, the yields of products were higher for Oxyma Pure than both HOBt and HOAt.

Table 3-2: Product composition obtained from the coupling of Z-Phg-OH to H-Pro-NH$_2$.

Coupling method	Yield	DL (%)
HOAt/DIPCDI	81	3.3
HOBt/DIPCDI	82	9.3
Oxyma/DIPCDI	90	1.0
Oxyma/DIPCDI	88	1.1

Table 3-3: Product composition obtained from the coupling of Z-Phe-Val-OH to H-Pro-NH$_2$.

Coupling method	Yield	DL (%)
HOAt/DIPCDI	86	2.1
HOBt/DIPCDI	79	8.9
Oxyma/DIPCDI	90	3.8

Table 3-4: Product composition obtained from the solid phase synthesis of H-Gly-Cys-Phe-NH$_2$.

Coupling method	Yield	DL (%)
HOAt/DIPCDI	88	0.1
HOBt/DIPCDI	84	0.2
Oxyma/DIPCDI	91	0.1

As a further test, we undertook the step-wise solid phase syntheses of the ABRF test peptide on a PTI Symphony using HOBt/DIPCDI and Oxyma Pure/DIPCDI activation. The resulting peptidyl resins were subjected to total acid hydrolysis in deuterium chloride, and the extent of racemization of certain amino acids was determined by chiral-GC/MS. In this case, the results given by HOBt and Oxyma Pure were comparable, with the exception of that for histidine (Table 3-5). This result may be a reflection of the slower formation of the Oxyma Pure active ester noted by Subirós-Funosas, *et al* [11].

Table 3-5: Racemization of key residues during SPPS of ABRF peptide using DIPCDI/HOBt and DIPCDI/Oxyma coupling.

Amino acid	HOBt (%D)	Oxyma (%D)
Arg	0.1	0.1
Asx	0.1	0.1
Cys	0.3	0.3
His	0.4	0.8

Coupling efficiency

Subirós-Funosas, et al. [11] also compared the coupling efficiencies obtained with Oxyma Pure, HOBt and HOAt in solid phase synthesis of enkephalin analogs containing MeGly, MeAla and Aib residues in place of Gly residues (Tables 3-6 - 3-8). In all cases, Oxyma Pure/DIPCDI activation gave consistently better results than HOBt/DIPCDI and in some cases as good or if not better results than HOAt/DIPCDI.

Table 3-6: Product composition obtained from synthesis of H-Tyr-MeGly-MeGly-Phe-Leu-NH$_2$ with 5 min coupling times.

Coupling	Yield	des-MeGly (%)	des-Tyr (%)
HOAt/DIPCDI	95	1.4	3.2
HOBt/DIPCDI	85	7.5	6.6
Oxyma/DIPCDI	91	3.8	4.2

Table 3-7: Product composition obtained from synthesis of H-Tyr-MeAla-MeAla-Phe-Leu-NH$_2$ with 30 min coupling times.

Coupling	Yield	des-MeAla (%)	des-Tyr(%)
HOAt/DIPCDI	74	23.2	3.1
HOBt/DIPCDI	46	38.1	15.2
Oxyma/DIPCDI	79	16.2	4.0

Table 3-8: Product composition obtained from synthesis of H-Tyr-Aib-Aib-Phe-Leu-NH$_2$ with 30 min coupling times.

Coupling	Yield	des-Aib (%)	des-Tyr(%)
HOAt/DIPCDI	11.3	86.3	1.8
HOBt/DIPCDI	3.0	91.0	5.1
Oxyma/DIPCDI	28.0	70.5	0.4

SPPS using Oxyma Pure

In practice, Oxyma Pure can be used in an identical manner to HOBt in carbodiimide mediated couplings. Oxyma Pure can be dissolved in DMF and used as a solution on automated synthesizers in place of the standard HOBt/DMF solution. The instrument can be programmed to deliver Oxyma Pure/DMF and DIPCDI in either DMF or DCM to the amino acid derivative, and mixture allowed to preactivate for 2 - 10 minutes before the activated amino acid solution is transferred to the reaction vessel containing the resin.

Recently, the potassium salt of Oxyma Pure has been introduce (K-Oxyma Pure). This material has higher solubility than Oxyma Pure in organic and aqueous solvents and does not promote premature cleavage of peptides from highly-acid labile trityl-based resins [16].

3.1.2 Preformed symmetrical anhydrides

Preformed symmetrical anhydrides (PSA) [17, 18] have been used by many research groups, mainly in Boc chemistry, because of their high reactivity. They are generated *in situ* using two equivalents of protected amino acid and one equivalent of DCC in DCM. The urea formed is removed by filtration and the couplings then proceed in DMF.

The symmetrical anhydride of Boc-Arg(Tos)-OH should not be used for coupling because it has been found to cause an undesirable insertion reaction. Fmoc-amino acid PSAs have been well studied [19]. Some Fmoc

amino acids, such as Gly, Ala, Nle, Cys(Acm), Gln(Mbh), Asn(Mbh) among others, are not readily soluble in DCM [21] and require significant amounts of DMF for solubilization, but DMF slows down the rate of activation.

Another drawback is that the preparation and use of PSA is quite wasteful, since two equivalents of protected amino acids are required to form one equivalent of activated species and the latter has to be used in excess. It is also recommended that pre-formed symmetrical anhydrides should be made fresh prior to use.

Method 3-2: Symmetrical anhydride formation

1. Dissolve the Fmoc amino acid (10 eq. relative to resin loading) in dry DCM. One or two drops of DMF may be needed to aid complete dissolution.
2. Add a solution of diisopropylcarbodiimide (5 eq. relative to resin loading) in dry DCM to the amino acid solution.
3. Stir the mixture for 20 min at 0°C, keeping the reaction mixture free of moisture with a calcium chloride drying tube.
4. Remove the DCM by evaporation under reduced pressure using a rotary evaporator.
5. Dissolve the residue in the minimum of DMF and add the solution to the resin.

3.1.3 Active esters

OPfp esters
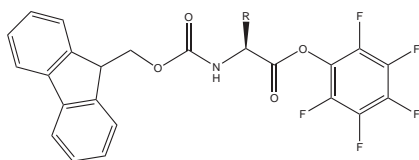
Extensive studies have been carried out on the use of Fmoc amino acid activated esters. [22]. However, of these only pentafluorophenyl (OPfp) esters are still in widespread use today.

In many ways pentafluorophenyl (OPfp) esters [23] of Fmoc-protected amino acids are the ideal building blocks for Fmoc SPPS [24, 25]. To use, simply dissolve in DMF in the presence of an equivalent of HOBt or Oxyma Pure and add directly to the resin to initiate coupling. As there is no added base in the reaction, epimerization during peptide bond formation is almost negligible. For introduction of Cys, the use of Fmoc-Cys(Trt)-OPfp is particularly recommended, as activation methods that use base, such as HBTU/DIPEA, have been reported to cause as much as racemization of cysteine [26].

OPfp ester cause little or no side reactions and their solutions in DMF have good stability. OPfp are, therefore, ideal for situations where a long, slow coupling is required. In many cases cleaner peptides can be obtained than when faster more highly activated coupling methods are used. Furthermore, the reactions of OPfps are very selective towards amines, so their use does not cause acylation of unprotected hydroxyl groups, ideal for synthesising peptides involving post-synthetic modification of Ser or Thr residues.

Addition of bromophenol blue to OPfp mediated coupling reactions allows real-time monitoring of amide bond formation [27]. The dye forms a blue ion-pair with the basic free amines on the solid support. As the reaction proceeds, the color fades to pale yellow as the dye is released into the acidic coupling medium. This property makes OPfp esters ideal for SPOT synthesis, enabling simultaneous monitoring of all coupling reactions on the array.

Method 3-3: Use of OPfp esters

1. Dissolve the Fmoc amino acid pentafluorophenyl ester (5 eq. relative to resin loading) and HOBt (5 eq.) in DMF.
2. Add the solution to the resin.

3.1.4 Coupling reagents

In situ activating reagents are widely accepted because they are easy to use, they give fast reactions, even between sterically hindered amino acids, and their use is generally free of side reactions. Most are based on phosphonium or aminium (formerly known as uronium) salts which, in the presence of a tertiary base, can smoothly convert protected amino acids to a variety of activated species. The most commonly employed, BOP, PyBOP, HBTU and TBTU, generate OBt esters, and these have found wide application in routine SPPS and solution synthesis for difficult couplings. BOP should be handled with great care as the by-product formed during activation is highly carcinogenic. BOP can be substituted by PyBOP without loss of performance.

Care should be taken when using aminium-based activation reagents not to use an excess relative to the carboxylic acid component as this can lead to capping of the amino terminus through guanidine formation [28]. This side reaction is commonly encountered during on-resin cyclization reactions. Phosphonium-based activating reagents, with the exception of PyBrOP® when used in DMF, do not give rise to such by-products [29].
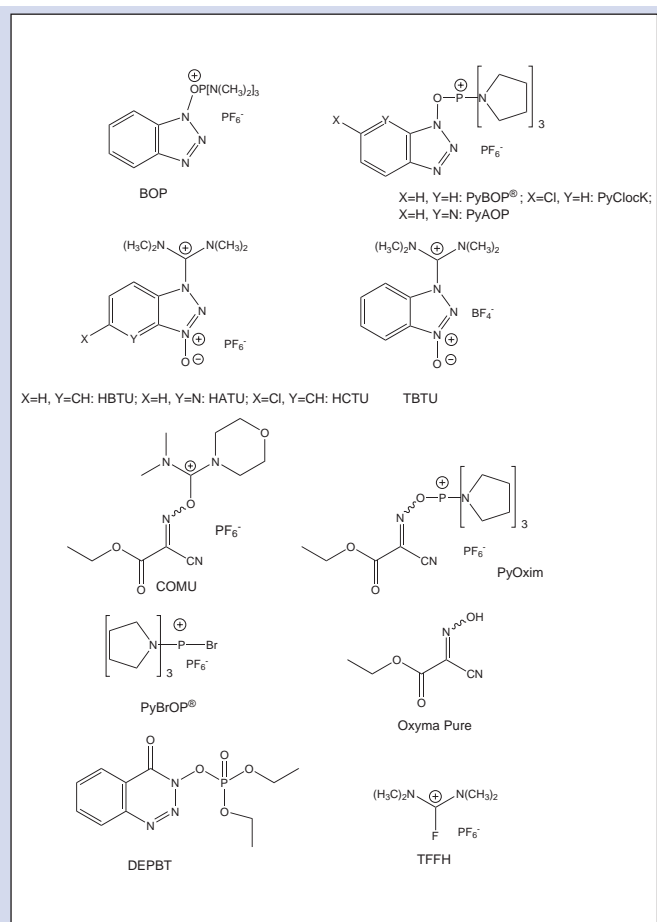
Table 3-9: Coupling reagents.

Coupling reagents are also available which generate esters that are more reactive than OBt. Four such reagents are HATU, PyAOP [30, 31], and HCTU [32], PyClocK, which in the presence of base convert carboxylic acids to the corresponding OAt and O-6-ClBt esters respectively. Such esters are more reactive than their OBt counterparts owing to the lower pKa of HOAt and HO-6-ClBt compared to HOBt. Furthermore, HOAt has the added benefit of the pyridine nitrogen which provides anchiomeric assistance to the coupling reaction [30]. HATU and PyAOP are the most efficient coupling reagent of this series. However, owing to their cost, their use is generally recommended for difficult and hindered couplings and the synthesis of long peptides.

Table 3-10: Product composition obtained from the solid phase synthesis of H-Tyr-MeLeu-MeLeu-Phe-Leu-NH$_2$ using various coupling reagents. The last tow residues were coupled for only 5 minutes to exaggerate differences in efficiencies between reagents.

% Compostion of products by HPLC				
Coupling reagents	MeLFL	YMeLFL	MeLMeLFL	YMeLMeLFL
PyBOP	60	31	5	4
PyOxim	10	45	5	40
HBTU	57	31	8	4
HCTU	31	35	9	25
HATU	0	16	7	77
TOTU	5	58	2	35
COMU	9	47	4	40
HDMC	24	46	7	21

Recently, coupling reagents based on the Oxyma Pure leaving group have been introduced, the most useful of which are COMU [33] and PyOxim [34]. However, oxyma-based coupling reagents generate esters as almost as reactive (Table 3-10) [35] as HATU but are much less expensive. Furthermore, Oxyma-based coupling reagents are not-based on potentially explosive triazole reagents. COMU is an excellent reagent for solution phase synthesis as the by-products of using it are water soluble and easily removed. COMU is not suitable for use with synthesizers that require pre-prepared solutions of activator as its solution stabililty is poor (Table 3-11). PyOxim on the otherhand has solution stability comparable to PyBOP, mediates couplings with high efficiency and gives a 1.5 M solution in DMF, compared to 0.5 M for HATU and HBTU (Table 3-12). The latter has important practical implications, it enables reactions to be performed at higher concentrations with concomitant improvements in efficiency. Epimerization during coupling of fragments also appears to be less when using COMU or PyOxim than with HBTU or HATU.

Table 3-11: Closed vialstability in d7-DMF as determined by ^{1}H nmr.

Coupling reagent	2 days	7 days	14 days
HDMC	99	99	98
COMU	67	46	36
PyOxim	90	88	86
TOTU	89	85	74
PyBOP	88	85	81
HATU	99	99	98

COMU and PyOxim are used in exactly the same way as PyBOP®, HBTU or HATU (Method 3-4), but instead of generating a benzotriazolyl ester it forms the corresponding ester of Oxyma Pure. In situations where racemization is an issue, COMU can be used with as little as one equivalent of base since the morpholino oxygen acts as an integral base.

Table 3-12: Solubility of couplng reagents in DMF.

Coupling reagent	Molarity
HATU	0.45
HBTU	0.5
HCTU	0.8
HDMC	0.75
COMU	1.5
TOTU	0.8
PyBOP	1.5
PyOxim	1.5

The coupling of N-methyl amino acids can be a difficult and slow process. PyBrOP [36] is reported to allow efficient, fast and racemization-free couplings. HATU is also very effective at coupling these derivatives.

Method 3-4: COMU/PyBOP/PyAOP/PyOxim/HATU/HBTU/HCTU activation
1. Dissolve the Fmoc amino acid (5 eq. relative to resin loading), COMU, PyBOP, HATU, HCTU or HBTU (4.5 eq.) in DMF.
2. Add DIPEA (10 eq. relative to resin loading) to the amino acid solution.
3. Stir the mixture and add the solution immediately to the resin.

3.1.5 Resin tests
A number of qualitative color tests are available to comfirm the completion of a coupling reaction by detection of residual resin amino groups. The most widely used was devised by Kaiser [37] (Method 3-5). The test is simple, quick and highly senstive. However, it should be noted that some deprotected amino acids do not show the expected dark blue color typical of free primary amino groups [38] (e.g. serine, asparagine, aspartic acid). Occasionally false negative tests are observed, particularly with strongly aggregated sequences. False positives can also occur owing to the partial heat senstitvity of Fmoc and some trityl-based protecting groups, so excess

heating of the test solution should be avoided. Other methods such as picric acid monitoring [39] or mass spectrophotometry [38] and the TNBS test [40] (Method 3-6) are also available. The TNBS test is particularly useful in Fmoc SPPS, as it is conducted at room temperature and so does not give false positives with Fmoc-amino acids and Lys(Mtt) and Lys(Mmt).

The Kaiser and TNBS tests do not work for secondary amino acids like proline. For these, the chloranil test is recommended [41] (Method 3-7).

Blackburn has described a method for the detection of resin-bound primary and secondary amines [42], treating resins with 2,3-dichloro-5-nitro-1,4-naphthoquinone in DMF or DCM in the presence of 2,6-di-tert-butylpyridine. The presence of primary or secondary amines is indicated by the beads turning bright orange/red. If the resin contains an acid-labile linker, the products of the reaction can be cleaved off and quantified by measuring the optical density of the solution.

Method 3-5: Kaiser test

Prepare the following solutions:
1. Dissolve 5 g of ninhydrin in 100 ml ethanol.
2. Dissolve 80 g of liquified phenol in 20 ml of ethanol.
3. Add 2 ml of a 0.001 M aqueous solution of potassium cyanide to 98 ml pyridine.
4. Sample a few resin beads and wash several times with ethanol.
5. Transfer to a small glass tube and add 2 drops of each of the solutions above.
6. Mix well and heat to 120°C for 4-6 min. A positive test is indicated by blue resin beads.

Method 3-6: TNBS test

Prepare following solutions:
1. 10% DIPEA in DMF.
2. 1% 2,4,6-trinitrobenzenesulfonic acid (TNBS) in DMF.
3. Sample a few resin beads and wash several times with DMF.
4. Suspend the beads in DMF and add 1 drop of each solution and leave for 5 min.
5. Wash the beads with DMF to remove the red solution. A positive test is indicated by red beads.

Method 3-7: Chloranil test

1. To 1-5 mg of resin add one drop of 2% acetaldehyde in DMF followed by one drop of 2% p-chloranil in DMF.
2. Allow to stand at rt for 5 min. Blue-stained resin beads indicate the presence of secondary amines.

Related products

851004	BOP	p. 268
851085	COMU	p. 270
851091	DEPBT	p. 269
851055	DMAP	p. 270
851007	EDC · HCl	p. 270
851013	HATU	p. 271
851006	HBTU	p. 271
851012	HCTU	p. 272
851011	MSNT	p. 274
851086	Oxyma Pure	p. 274
851212	K-Oxyma Pure	p. 274
851221	PyAOP	p. 275
851009	PyBOP	p. 275
851010	PyBroP	p. 276
851087	PyClocK	p. 276
851095	PyOxim	p. 277
851008	TBTU	p. 277

References

1. E. Valeur & M. Bradley (2009) *Chem. Soc. Rev.*, **38**, 606; A. El-Faham & F. Albericio (2011) *Chem. Rev.*, **111**, 6557.
2. J.C. Sheehan & G.P. Hess (1955) *J. Am. Chem. Soc.*, **77**, 1067.
3. K. D. Kopple, et al. (1962) *J. Am. Chem. Soc.*, **84**, 4457.
4. D. Sarantakis, et al. (1976) *Biochem. Biophys. Res. Commun.*, **73**, 336.
5. D. Hudson, et al. in "Peptide Chemistry", Y. Kiso (Ed.) Protein Research Foundation, Osaka, 1986, pp. 4113.
6. J. Izdebski & J. Pelka in "Peptides 1984", U. Ragnarsson (Ed.) 1985, pp. 113.
7. J. Izdebski, et al. (1989) *Int. J. Peptide Protein Res.*, **33**, 77.
8. W. König & R. Geiger (1970) *Chem. Ber.*, **103**, 788.
9. W. König & R. Geiger (1970) *Chem. Ber.*, **103**, 2024.
10. E. Wünsch & F. Drees (1966) *Chem. Ber.*, **99**, 110.
11. R. Subirós-Funosas, et al. (2009) *Chem. Eur. J.*, **15**, 9394.
12. M. Itoh (1973) *Bull. Chem. Soc. Jpn*, **46**, 2219.
13. J. Izdebski (2007) *Pol. J. Chem.*, **53**, 1049.
14. L. A. Carpino (1993) *J. Am. Chem. Soc.*, **115**, 4397.
15. O. Marder & F. Albericio (2003) *Chimica Oggi*, **21**, 6.
16. P. Cherkupally, et al. (2013) *Eur. J. Org. Chem.*, 6372.
17. F. M. Chen, et al. (1978) *Synthesis*, 928.
18. E. P. Heimer, et al. (1981) *Int. J. Pept. Protein Res.*, **18**, 237.
19. E. Brown, et al. (1983) *J. Chem. Soc., Perkin Trans*, **1**, 75.
21. M. Carlquist (1987) *Acta Chem. Scand.*, **41**, 494.
22. G. B. Fields & R. L. Noble (1990) *Int. J. Pept. Protein Res.*, **35**, 161.
23. L. Kisfaludy & I. Schön (1983) *Synthesis*, 325.
24. E. Atherton, et al. (1988) *J. Chem. Soc., Perkin Trans. 1*, **1988**, 2887.
25. A. Dryland & R.C. Sheppard (1988) *Tetrahedron*, **44**, 859.
26. Y. X. Han (1997) *J. Org. Chem.*, **62**, 4307.
27. V. Krchňák, et al. (1988) *Collect. Czech. Chem. Commun.* **53**, 2542.
28. H. Gausepohl, et al. in "Peptides, Chemistry & Biology, Proc. 12th American Peptide Symposium", J. A. Smith & J. E. Rivier (Eds.), ESCOM, Leiden, 1992, pp. 523.
29. F. Albericio, et al. (1997) *Tetrahedron Lett.*, **38**, 4853.
30. L. A. Carpino (1993) *J. Am. Chem. Soc.*, **115**, 4397.
31. F. Albericio, et al. (1998) *J. Org. Chem.*, **63**, 9678.
32. O. Marder, et al. (2002) *Chimica Oggi*, **20**, 37.
33. A. El-Fahem, et al. (2009) *Chem. Eur. J.*, **15**, 9404.
34. R. Subirós-Funosas, et al. (2010) *Org. Biomol. Chem.*, **8**, 3665.
35. R. Behrendt, et al., Poster 83, Presented at the 31st European peptide Symposium, Copenhagen, Sept 2010
36. J. Coste, et al. (1990) *Tetrahedron Lett.*, **31**, 669.
37. E. Kaiser, et al. (1970) *Anal. Biochem.*, **34**, 595.
38. J. D. Fontenot, et al. (1991) *Pept. Res.*, **4**, 19.
39. A. Oded & R.A. Houghten (1990) *Pept. Res.*, **3**, 42.
40. W.S. Hancock & J.E. Battersby (1976) *Anal. Biochem.*, **71**, 261.
41. T. Vojkovsky (1995) *Pept. Res.*, **8**, 236.
42. C. Blackburn (2005) *Tetrahedron Lett.*, **46**, 1405.

3.2 Fmoc resin loading protocols

A wide range of linkers is available for use with the Fmoc strategy, facilitating the synthesis of a large variety of peptide types, e.g. peptide acids, amides, protected fragments, etc, and these are discussed in detail in section 2.4, page 2.6.

Linkers generally fall into two categories, depending on whether the C-terminal residue is anchored *via* an ester bond or an amide bond. In the classic supports, such as Wang resin and those like NovaSyn TGA based on the hydroxymethylphenoxyacetyl linker (HMPA), used to prepare peptides bearing a C-terminal carboxylic acid group, the first amino acid is generally anchored *via* a benzyl ester bond. Loading of these is often difficult process which, unless carried out under controlled conditions, can lead to a low substitution, racemization or dipeptide formation. Whereas, the resins used to peptide bearing a C-terminal carboxamide group, such as Rink Amide resin and Sieber Amide resin, anchoring of the first amino acid is made *via* an amide bond. Here loading of these supports is straightforward and can be generally achieved using standard coupling methods.

3.2.1 Resins with ester linkages

One of the simplest methods for esterification to benzyl alcohol-based linkers is to use the symmetrical anhydride of the protected amino acid in the presence of a catalytic amount of *p*-dimethylaminopyridine (DMAP) (Method 3-8). Due to the basic character of this material, racemization and dipeptide formation can be expected, the amount depending on the quantity of DMAP used, the length of the reaction and the nature of the amino acid. Cysteine and histidine are particularly prone to racemization and should not be loaded by this method. Futhermore, benzyl alcohol-based resins are not recommended for making peptides containing C-terminal proline residues due to problems with diketopiperazine formation. For these amino acids trityl-based resins are recommended.

Method 3-8: Attachment to benzyl alcohol resins using symmetrical anhydride

1. Place the resin (1 g) in a clean, dry flask, and add sufficient DMF to just cover and allow to swell for 30 min. Add extra DMF if necessary just to cover the resin.
2. Dissolve the Fmoc amino acid (10 eq. relative to resin loading) in dry DCM. One or two drops of DMF may be needed to aid complete dissolution.
3. Add a solution of diisopropylcarbodiimide (5 eq. relative to resin loading) in dry DCM to the amino acid solution.
4. Stir the mixture for 20 min at 0 °C, keeping the reaction mixture free of moisture with a calcium chloride drying tube.
5. Remove the DCM by evaporation under reduced pressure using a rotary evaporator.
6. Dissolve the residue in the minimum of DMF and add the solution to the resin prepared in step 1.
7. Dissolve DMAP (0.1 eq. relative to resin loading) in DMF and add this solution to the resin/amino acid mixture. Stopper the flask and allow the mixture to stand at rt for 1 h with occasional swirling.
8. Remove a small sample of resin (20 mg) and wash, dry and estimate the level of first residue attachment using the procedure described in Method 3-11, page 3.6. If the value obtained is less than 70% the first residue attachment procedure should be repeated.

Note: This method is not suitable for His or Cys.

A good alternative to the symmetrical anhydride method is the MSNT method (Method 3-9) [1, 2]. It is the method of choice in difficult circumstances, such as loading of hydroxymethylbenzoic acid (HMBA) resins or when attaching racemization prone amino acid derivatives [3, 4].

Method 3-9: Attachment to benzyl alcohol resins using MSNT/MeIm

1. Place the resin in a dry reaction vessel. Swell and wash with DCM, add sufficient DCM to cover resin and flush vessel with nitrogen.
2. Weigh the appropriate Fmoc amino acid (5 eq.) into a dry round bottom flask. Add dry DCM to dissolve the amino acid derivative (approximately 3 ml/mmole); one or two drops of THF can be added to aid dissolution.
3. Add N-methylimidazole (3.75 eq.) followed by MSNT (5eq.). Flush flask with nitrogen and seal. Stir the mixture until the MSNT has dissolved.
4. Using a syringe, transfer the amino acid solution to the vessel containing the resin.
5. Allow the mixture to stand at rt for 1 h, with gentle agitation.
6. Wash with DCM (5 times) and DMF (5 times).
7. Remove a small sample of resin (20 mg) and wash, dry and estimate the level of first residue attachment using the procedure described in Method 3-12.
8. If the value obtained is less than 70% the first residue attachment procedure should be repeated.

In contrast to benzyl alcohol-based supports, attachment of amino acids to trityl-based resins, such as 2-chlorotrityl or NovaSyn® TGT resins, is free from racemization [5], making them ideal for the immobilization of sensitive residues such as Cys and His. The resin also protects Cys from racemization during chain extension. They are particularly useful in the synthesis of C-terminal prolyl peptides as the bulk of the trityl linker helps to prevent diketopiperazine formation [6 - 8]. When loading 2-chlorotrityl chloride resin, it is important to ensure that all amino-acid derivatives, glassware and solvent are thoroughly dried before use. NovaSyn® TGT alcohol resins must be converted to the chloride form before attachment of the amino acid.

Method 3-10: Loading of Fmoc-amino acids to trityl chloride resins

NOTE: it is important to dry all solvents and glassware before use.

1. Dissolve the carboxylic acid (0.6-1.2 eq. relative to the resin for 2-chlorotrityl chloride resin and 2 eq. for NovaSyn® TGT chloride resin) and DIPEA (4 eq. relative to carboxylic acid) in dry DCM (approx. 10 ml per gram of resin) containing, if necessary, a small amount of dry DMF (just enough to facilitate dissolution of the acid). For pseudoproline dipeptides add 3ml of NMP/gram of resin.
2. Add this to the resin and stir for 30-120 min. For pseudoproline dipeptides leave to react o/n. At the end of this time, wash the resin with 3x DCM/MeOH/DIPEA (17:2:1), 3x DCM; 2x DMF, 2x DCM. Dry in vacuo over KOH.

Fmoc-amino acids are best dried before use by repeated evaporation from dioxane; determine loading using Method 3-12.

3.2.2 Resins with amide linkages

Attachment of Fmoc-amino acid derivatives to linkers containing primary amino groups, such as Rink Amide, Sieber Amide, PAL etc., can normally be effected using standard methods of amide bond formation. Hydroxylamine, Weinreb amide, and resins functionalized with secondary amines are much more difficult to load; for these the use of HOAt/DIPCDI or HATU/DIPEA activation is required. Dawson and sulfamylbutyryl resins require special procedures for loading and these are described elsewhere

Method 3-11: Loading of Fmoc-amino acids to amino resins

1. Pre-swell the resin with DCM (polystyrene-based resins) or DMF (PEG-PS or PEGA resin) for 1 h. Wash resin thoroughly with DMF.
2. If the resin is Fmoc protected, treat with 20% piperidine in DMF for 20 min, then wash resin thoroughly with DMF.

Rink Amide, Sieber Amide, PAL

3. Dissolve Fmoc-amino acid derivative (5 eq.) and PyBOP in DMF. Add DIPEA (10 eq.), mix and add immediately to resin. Leave for 1 h.

N-Alkyl amino resins (Biotin PEG NovaTag resin, N-Ethyl Indole AM resin)

3. Dissolve Fmoc-amino acid derivative (5 eq.) and HATU in DMF. Add DIPEA (10 eq.), mix and add immediately to resin. Leave for 6 h.
4. Remove a small quantity of resin and test this for the presence of unreacted amines using the TNBS test (primary amine resins) or chloranil test (secondary amine resins) as described in Methods 3-6 and 3-7. If the result is positive, wash the resin with DMF and repeat coupling. Continue this procedure until a negative result is obtained.

3.2.3 Fmoc loading test

For estimating the loading of resins derivatized with Fmoc-amino acids, the simplest approach involves cleaving the Fmoc group with DBU and measuring the solution concentration of the liberated dibenzofluvene by U.V. spectroscopy.

Method 3-12: Estimation of level of first residue attachment [9]

1. Take 3 x 10 mm matched silica UV cells.
2. Weigh dry Fmoc amino acid-resin (approx. 5 μmole with respect to Fmoc) into a 10 ml graduated flask. Add 2 ml of 2% DBU in DMF. Agitate gently for 30 min. Dilute solution to 10 ml with MeCN. Take 2 ml of this solution and dilute to 25 ml in a graduated flask.
3. Prepare a reference solution as in step 2, but without addition of the resin.
4. Fill two cuvettes with 3 ml of test solution and one cuvette with 3 ml of reference solution.
NOTE: Do not cross-contaminate the solutions. Allow the resin to settle to the bottom of the cells.
5. Place the cells in a spectrophotometer and record optical density at 304 nm.
6. Obtain an estimate of first residue attachment from equation below
Fmoc loading: mmole/g $=(Abs_{sample}-Abs_{ref})$ X 16.4/mg of resin).

References

1. R. Frank, et al. (1988) *Tetrahedron*, **44**, 6031.
2. B. Blankemeyer-Menge, et al. (1990) *Tetrahedron Lett.*, **31**, 1701.
3. E. Harth-Fritschy & D. Cantacuzène (1997) *J. Pept. Res.*, **50**, 415.
4. M. Renil, et al. (1998) *J. Pept. Sci.*, **4**, 195.
5. K. Barlos, et al. (1991) *Int. J. Peptide Protein Res.*, **37**, 513.
6. K. Barlos, et al. (1989) *Tetrahedron Lett.*, **30**, 3947.
7. K. Barlos, et al. (1993) *Ann. Chem.*, 215.
8. R. Steinauer, et al. in "Innovations & Perspectives in Solid Phase Synthesis", R. Epton (Ed.), Mayflower Scientific Ltd., Birmingham, 1994, pp. 689.
9. M. Gude, et al. (2003) *Lett. Pept. Sci.*, **9**, 203.

3.3 Temporary protecting group removal

To obtain high purity products by solid phase synthesis, not only is it essential that coupling reactions proceed to completion but the same also goes for the removal of temporary protecting groups such as Boc and Fmoc.

In Boc SPPS, removal of the *N*-terminal Boc group is normally achieved by treatment with TFA in DCM or neat TFA. Due to the excellent solvation properties of TFA for protected peptides, this reaction is invariably complete. This forms the basis of the Boc *in situ* neutralization methodology, [1] in which peptide aggregation is prevented by maintaining the peptide protonated, following Boc removal by flow-washes with TFA, until coupling.

With the Fmoc methodology, 20% piperidine in DMF is usually used for removal of the Fmoc temporary protecting group. However, in many countries access to piperidine is regulated as it is an intermediate in the synthesis of phencyclidine. In such instances, 4-methylpiperidine should be considered as an alternative as studies have shown it to be as effective as piperidine [2].

Whilst in most cases the deprotection schemes are effective, it has been shown that for long or aggregated peptides incomplete Fmoc-deprotection can occur even in the presence of high concentrations of piperidine [3]. In these cases, it is advisable to increase the time required for deprotection or to use a stronger base such as 1,8-diazabicyclo-[5.4.0] undec-7-ene (DBU) [4]. This tertiary base appears to be a very good alternative to piperidine, since it causes more rapid deprotection, less racemization of resin-bound C-terminal Cys(Trt) and reduces the extent of broadening of UV Fmoc-deprotection peaks occasionally observed when piperidine is employed. In batch synthesis, it is advisable when using DBU to also add 2% piperidine to the deprotection mixture in order to scavenge the dibenzofulvene produced on Fmoc removal, and thus prevent alkylation of resin amino groups. However, it should be noted that the DBU promotes aspartimide formation, so should not be used for sequences containing aspartic acid.

Base-promoted aspartimide formation is an issue particular to Fmoc SPPS (see section 3.6, page 3.23). The addition of weak acids such as HOBt or dinitrophenol to the deprotection mixture has been found to suppress this side reaction [5]. The use of 1 M Oxyma Pure in 20% piperidine has recently been shown to be particularly effective in this regard [6].

Method 3-13: Fmoc removal

1. Treat the Fmoc-protected peptidyl resin with piperidine/DMF (2:8) or piperidine/DBU/DMF (2:2:96) for 3 min.
2. Drain resin and repeat treatment 3 or 4 times
3. Wash resin with DMF.

References

1. M. Schnölzer, et al. (1992). *Int. J. Pept. Protein Res.*, **40**: 180.
2. J. Hachmann & M. Lebl (2006) *J. Comb. Chem*, **8**, 149.
3. J.D. Fontenot, et al. (1991) *Pept. Res.*, **4**, 19.
4. J.D. Wade, et al. (1991) *Pept. Res.*, **4**, 194.
5. J. Lauer, et al. (1994) *Lett. Pept. Sci.*, **1**, 197.
6. R. Subirós-Funosas,, et al. (2012) *Biopolymers*, **98**, 89.

3.4 Manual peptide synthesis

A simple, manual system can be assembled by any well-equipped laboratory. A suitable peptide vessel is shown in Figure 3-2; these can be constructed from a sintered glass funnel by a skilled glass-blower, or can be purchased from several laboratory supply houses.

For best results, we recommend using pre-loaded Wang or 2-chlorotrityl resins for production of peptide acids and Rink Amide MBHA resin for the preparation of peptide amides.

3.4.1 Use of PyBOP®, PyAOP, TBTU, HATU, HBTU and HCTU coupling reagents

PyBOP®, PyOxim, TBTU, HATU, HCTU, and HBTU are so similar in their chemical properties that they can be used interchangeably.

Only purified or freshly distilled DMF stored over activated 4Å molecular sieves should be used. If solutions are to be kept for extended periods (up to 6 weeks), DMA should be used as it is much more stable than DMF; DMA does not decompose as readily to give carbon monoxide and deleterious amines. Note: Solutions of PyBOP® are not stable for more than about 48 hours.

While these coupling reagents have been used successfully with as little as a 1.5-fold excess of Fmoc-amino acid (compared to resin substitution), we recommend the use of a 2- or 2.5-fold excess of Fmoc-amino acid, making it less likely that double coupling will be needed. Details of the elongation cycle (Fmoc removal followed by addition of the next Fmoc-amino acid) are given in Method 3-14.

Table 3-13: Materials for manual Fmoc synthesis.

> Resin (substitution about 0.5 mmole/g)
> Fmoc-amino acids
> 20% Piperidine in DMF (for Fmoc-deprotection)
> 0.5 M PyBOP® in DMF (for coupling)
> DIPEA (for coupling or to neutralize)
> Peptide vessel with 3-way stopcock (see Figure 3-2)
> Nitrogen gas, suction flask

Fig. 3-2: Vessel for manual SPPS.

Method 3-14: Elongation cycle for peptide synthesis

1. Place an Fmoc-amino acid loaded resin or Rink Amide MBHA resin in peptide flask set up for N_2 bubbling and suction.
2. Add piperidine/DMF solution (10 ml/g resin) and mix by bubbling for 5 min. Repeat. Drain by applying vacuum.
3. Wash 3 x DMF. The Kaiser test should be positive; if not repeat deprotection. Wash 3 x DMF.
4. Dissolve 2 eq. of Fmoc-amino acid, PyBOP® and HOBt in DMF (5 ml/g resin),. Add 4 eq. of DIPEA and add mixture to resin.
5. Bubble gently for 10-60 min until a negative Kaiser test on a small sample of resin is obtained. Wash 3 x DMF.
6. Repeat steps to 2 to 5 until required peptide is assembled.
7. After the final coupling wash 3 x DMF, 3 x DCM, 3 x MeOH. Dry under high vacuum with P_2O_5 or KOH.

3.5 Improving synthetic efficiency in SPPS

3.5.1 Introduction

The 3D structure, and consequently bioactivity, of individual proteins is largely determined by the primary sequence of its component amino acids. This inherent propensity of peptide chains to form ordered structures is the principle cause of the highly sequence specific variability in synthetic efficiency encountered during peptide assembly. As the peptide is elongated, it can form secondary structures that cause peptide chain aggregation, resulting in lower reaction rates. The effects can range from just a subtle slowing down to a complete failure of both deprotection and acylation reactions [1]. In such extreme cases, the peptide has effectively become insoluble and is no longer available for reaction.

The onset of severe aggregation in batch synthesis is generally indicated by shrinking of the resin matrix, whereas in continuous flow synthesis, it is detected by flattening and broadening of the deprotection profile. In such circumstances usual coupling tests with ninhydrin or TNBS are often no longer reliable and can give false negative results.

The ease of assembly of a given sequence is generally hard to predict and is one of the factors that makes peptide synthesis interesting and challenging, although peptides containing stretches of contiguous hydrophobic amino acids like Ala, Val, Ile as well as those containing amino acids which can form intra-chain hydrogen bonds, such as Gln, Ser and Thr, are frequently difficult to make. For this reason, it is generally better to adopt from the outset synthetic strategies that mitigate the effects of structure formation, rather than trying to second guess which peptides may be problematic and wasting time and resources repeating failed syntheses. For peptide sequences longer than 20 amino acids it is therefore strongly recommended to monitor the peptide assembly by small TFA cleavages (see chapter 3.8 , page 3.27).

The majority of approaches developed to ameliorate this issue attempt to improve the solvation of the peptide-resin complex. These include using dipolar aprotic solvents, such as DMF, DMSO or NMP [2], chaotopic salts [3], special solvent cocktails like "Magic Mixture" [4], or PEG-based polar resins like NovaSyn® TG [5], NovaPEG or PEGA [6] (Table 3-14). The degree of cross-linking of the polystyrene resin with DVB can also play a key role and should not be higher than 1%, otherwise proper swelling can be inhibited [7]. Resin functionalization is another important factor, with high loading resins exacerbating the effects of aggregation, and it is for this reason that the Novabiochem® brand offers special low-loading versions of the most popular supports used for Fmoc SPPS. The choice of side-chain protecting group can also influence peptide-solvation. Substitution of Ser(tBu) or Thr(tBu) with the Trt derivatives [8], or Lys(Boc) with Lys(Tfa) [9] can have a beneficial effect. However, the most universally effective way of improving synthetic efficiency is to reversibly protect the amide backbone of key residues through the introduction of secondary amino acid surrogates

For a detailed discussion on how to identify and overcome the effects of aggregation, the reader is referred to the excellent article by Quibell & Johnson [10].

Table 3-14: Overcoming difficult sequences.

Resins
Use resins with good swelling properties, like the NovaPEG, PEGA and NovaSyn® TG family [5], and resins with low substitution for batch synthesis.

Solvents
Use DMF, DMA, NMP or 25% DMSO in DMF instead of DCM.

Time
Use longer coupling time and/or double coupling.

Chaotropic salts
Wash resin with solutions of chaotropic salts like 0.8 M NaClO$_4$ or LiCl or 4 M KSCN in DMF before coupling or add them to the coupling mixture [6].

Coupling reagents
Use different activation methods like PyBOP®, PyBOP®/HOBt, HBTU, TBTU, PyBroP®, and HATU and allow longer reaction times.

Protect the peptide bond using secondary amino acid surrogates
Incorporate a Dmb- or Hmb-protected derivative or pseudoproline dipeptide for every sixth residue, if possible.

"Magic Mixture"
Use a mixture of DCM/DMF/NMP (1:1:1) with 1% Triton X100 and 2 M ethylenecarbonate at 55°C as the solvent system for acylation and 20% piperidine in DCM/DMF/NMP (1:1:1) with 1% Triton X100 for Fmoc-cleavage [4].

3.5.2 Secondary amino acid surrogates

Secondary amino acid surrogates are analogs of proline or N-alkylamino acids that are derived from standard primary amino acids by reversible protection of the backbone amide bond. They work by mimicking the natural propensity of proline and N-alkyl amino acids [11, 12] to disrupt the formation of the secondary structures during peptide assembly. Their use leads to better and more predictable acylation and deprotection kinetics, which results in higher purities and solubilities of crude products, easier HPLC purification and improved yields, with less need to repeat failed syntheses. They have proved particularly effective in the synthesis of intractable peptides [13-16], long peptides/small proteins [17 - 26], and cyclic peptides [26, 27], enabling in many cases the production of peptides that otherwise could not be made.

Novabiochem at present supplies three kinds of secondary amino acid surrogates: pseudoproline dipeptides [28]; Dmb dipeptides [29]; Dmb/Hmb amino acids [30] (Figure 3-3). All work on the same principle by temporarily introducing a structure breaking iminoacid into the peptide sequence. The effects can be long range, with formation of structures often being postponed for as many as six residues, or eliminated altogether. Following peptide assembly, treatment with TFA cleaves the amide bond protecting group regenerating the native sequence containing the primary amino acid from which the secondary amino acid surrogate was derived.
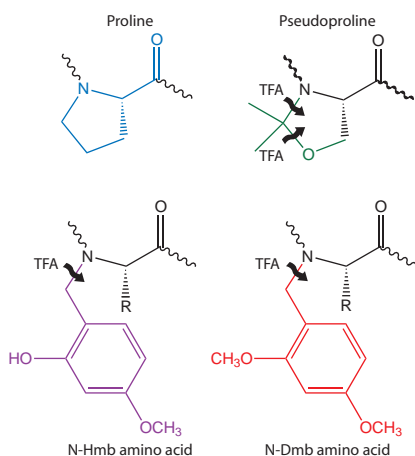
Fig. 3-3: Secondary structure disrupting N–alkyl amino acids.

Fig. 3-5: Relationship between peptide sequence and pseudoproline dipeptide.

General guidelines for the use of secondary amino acid surrogates (Figure 3–4)

- Optimal results are obtained if the imino acid surrogates are spaced 5-6 residues apart throughout the sequence.

- The optimum separation between an imino acid surrogate and a Pro residue is 5-6 amino acid residues.

- The minimum separation between an imino acid surrogate and another imino acid surrogate or Pro residue is 2 residues.

- Aim to insert an imino acid surrogate before regions of hydrophobic residues.

3.5.3 Pseudoproline dipeptides

Mutter's pseudoproline dipeptides [28] are undoubtedly the most effective and simplest to use secondary amino acid surrogates, but are only appropriate for use in Ser- or Thr-containing sequences. They consist of a dipeptide in which the Ser or Thr residue has been reversibly protected as a proline-like TFA-labile oxazolidine. The reason the pseudoproline residue is introduced as a dipeptide is because it avoids the need to acylate the hindered oxazolidine nitrogen. This has the added advantage of extending the peptide chain by two residues in one step.

These dipeptides are extremely easy to use: simply substitute a Ser or Thr residue together with the preceding amino acid residue in the peptide sequence with the appropriate pseudoproline dipeptide (Figure 3-5). The native sequence is regenerated on cleavage and deprotection. To achieve the maximum benefits, it is important to follow the guidelines set-out above.

Positioning of pseudoproline residues at regular intervals has proven to be an extremely effective approach for the synthesis of long and amyloidogenic peptides. This approach has been exemplified by the synthesis of a 95 residue peptide in remarkable purity through the expeditious use of 7 pseudoproline dipeptides [17] in the synthesis of 101mer related to d2 domain of VEGF receptor [21], and parallel production of ubiquitin analogs [22].
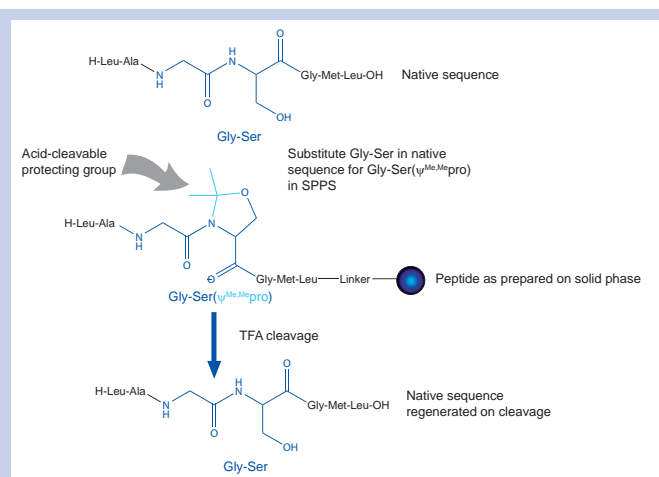
Protocols for the use of pseudoproline dipeptides

Method 3-15a: Manual coupling of pseudoproline & Dmb dipeptides

Phosphonium/aminium activation
1. Dissolve derivative (5 eq.) and coupling reagent (PyBOP®, TBTU, HBTU, HCTU, or HATU, 5 eq.) in minimum volume of DMF or NMP.
2. Add DIPEA (10 eq.) and mix thoroughly.
3. Add immediately to Fmoc-deprotected peptide resin, and agitate for 1-2 h. Check completion of coupling by the TNBS test. If reaction is not complete, extent coupling time or repeat reaction using fresh reagents.

DIPCDI/HOBt activation
1. Dissolve derivative (3 eq.) and HOBt (3 eq.) in minimum volume of DMF/DCM (2:1).
2. Add DIPCDI (3 eq.) and mix thoroughly.
3. Leave to activate for 10 minutes and then add to Fmoc-deprotected peptide resin, and agitate for 1-2 h. Check completion of coupling by the TNBS test. If reaction is not complete, extend coupling time or repeat reaction using fresh reagents.

Method 3-15b: Automated coupling of pseudoproline dipeptides

Instruments using dry Fmoc-amino acids in cartridges (ABi 433, Pioneer, Millipore 9050)
1. Pack empty vials or cartridges with the amount of pseudoproline or Dmb dipeptide appropriate to the instrument protocols (i.e. 1 mmole for ABi 433 and 0.8 mmole for Pioneer).
2. Program instrument to couple dipeptide as Ser, Thr or Gly residue (1 h coupling, HBTU, HATU activation). Omit amino acid cycle for next amino acid.
3. Smaller excesses of dipeptides can be used, but the delivery volumes of coupling and base reagents will need to be modified in the coupling protocols to maintain the correct ratio of dipeptide/coupling reagent/base (1:1:2). Alternatively, the instrument can be programmed to pause after the wash step following Fmoc removal, and the dipeptide coupled manually by adding a solution of activated derivative to the reaction vessel.

Instruments which needle dispense solutions of reagents from vials (ACT 396, Zinsser 350)
1. Dissolve derivative in DMF or NMP to exactly the same concentration as standard Fmoc-amino acid derivatives in reagent vial.
2. Place vial containing dipeptide in an appropriate position of autosampler rack.
3. Program instrument to couple dipeptide as Ser, Thr or Gly residue (1 h coupling, HBTU, HATU activation). Omit amino acid cycle for next amino acid.

Instruments which use stock solutions of Fmoc-amino acids with dedicated solvent lines (Protein Technologies Symphony)
Whilst it is perfectly feasible to use pre-dissolved solutions of pseudoproline dipeptides on instruments such as the Symphony, this can be quite wasteful as reagent solvent lines would need to be primed with dipeptide solution before use. For such instruments, the cycle should be programmed to pause after the wash step following Fmoc removal and the "add" amino acid and coupling reagent steps in the cycle replaced by a "none" reagent step. The dipeptide can then be coupled manually by adding a solution of activated derivative to the reaction vessel.

Not at N-terminus   Met-Ser not available   Too near Pro   Too near Pro   Not at C-terminus

H-Ala-Thr-Gly-Glu-Phe-Ser-Ala-Ser-Gly-Leu-Met-Ser-Ser-Gly-Ala-Trp-Gly-Ala-Ser-Ala-Pro-Gly-Ser-Arg-Glu-Gln-Thr-Gly-Ser-OH

Either Phe-Ser   Ser-Ser O.K.   O.K.
or Ala-Ser O.K.
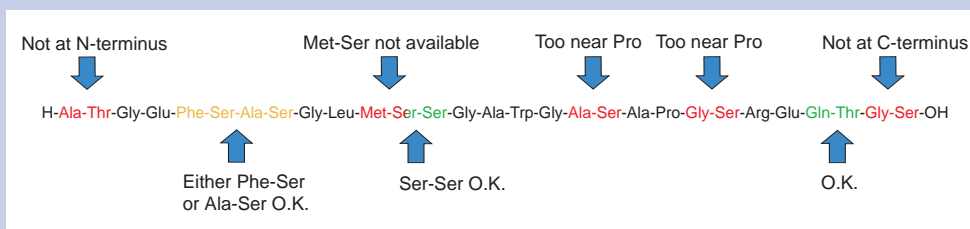
Pseudoproline dipeptides can be introduced in the same manner as other amino acid derivatives. Pseudoproline dipeptide have been coupled using phosphonium and aminium activation reagents, such as PyBOP® [28], HBTU [31], TBTU [13], and HATU [17] activation methods, as well as with carbodiimide-mediated coupling strategies, such as DIPCDI/HOBt [13, 15] (Method 3-15a).

On automated instruments, the simplest approach is to use the same amount of pseudoproline dipeptide as any other amino acid, since this avoids having to reprogram coupling cycles or make any manual intervention to the synthesis. Program the instrument to add a Ser or Thr residue, and remember to omit from the synthesis program the cycle for the next amino acid as this will be introduced as part of the pseudoproline dipeptide.

Using 5-fold excess of phosphonium- or aminium-activated pseudoproline dipeptide to resin functionality, coupling reactions are generally complete in 1 h. Lower excesses of reagent can also be used, but it then becomes advisable to check the completeness of reactions using an amine test, such as the Kaiser or TNBS test (Method 3-15b).

Regeneration of serine or threonine from the pseudoproline occurs during the course of the TFA cleavage reaction using standard cleavage cocktails, such as TFA/water/TIS (95:2.5:2.5), and is generally complete in 3h.

Examples of the use of pseudoproline dipeptides

Intractable sequences

Aggregation during peptide assembly is generally characterized by shrinkage of the resin, accompanied by incomplete coupling and deprotection reactions. Figure 3-6a is typical for the HPLC elution profile obtained for such an aggregated sequence. In this instance, incomplete Fmoc removal at residue Leu5 led to the formation of numerous truncated and deleted sequences. Resynthesis of peptide 1 with substitution of residues 7 and 8 with Fmoc-Phe-Ser($\psi^{Me,Me}$pro)-OH resulted in dramatic improvements in yield and purity.

Amyloidogenic sequences

Human islet amyloid polypeptide (IAPP), hAmylin, is a mono-cyclic, C-terminally amidated, 37-residue peptide hormone (Figure 3-7) [32]. Amylin is also thought to play a role in the pathology of type-II diabetes mellitus, as it is the major constituent of plaque deposits found in the islets of type-II diabetic patients [33], and Amylin fibrils are toxic to insulin-producing cells [34].

The Fmoc SPPS of hAmylin$_{1-37}$ has been recently described by Abedini & Raleigh [35]. As expected, the inherent propensity of amylins to aggregate was found to make the synthesis of this peptide extremely problematic. Highly heterogeneous products were obtained even when double couplings of standard Fmoc-amino acid derivatives were

employed at every cycle. However, it was found that by simply making three pseudoproline dipeptide substitutions, hAmylin$_{1-37}$ could be obtained in excellent purity. This work is reproduced here by kind permission of Prof. Raleigh and the American Chemical Society.
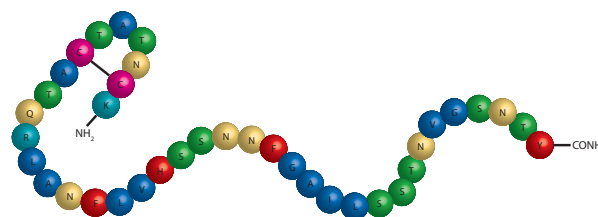
Fig. 3-7: Primary sequence of hAmylin$_{1-37}$.

Application 3-1: Synthesis of H-Val-Thr-Arg-Tyr-Leu-Thr-Phe-Ser-Asn-Lys-Ser-Val-Leu-Gln-OH (1)

The peptides were assembled automatically using a PTI Symphony peptide synthesizer on Fmoc-Gln(Trt) Wang resin. All acylation reactions were carried out using a 3.3-fold excess of Fmoc-amino acid activated with 1 eq. of HCTU in the presence of DIPEA. A coupling time of 30 min was used throughout. Cleavage and side-chain deprotection was effected by treatment of the peptidyl resins with TFA/ TIS /water 95:2.5:2.5 for 3 h.
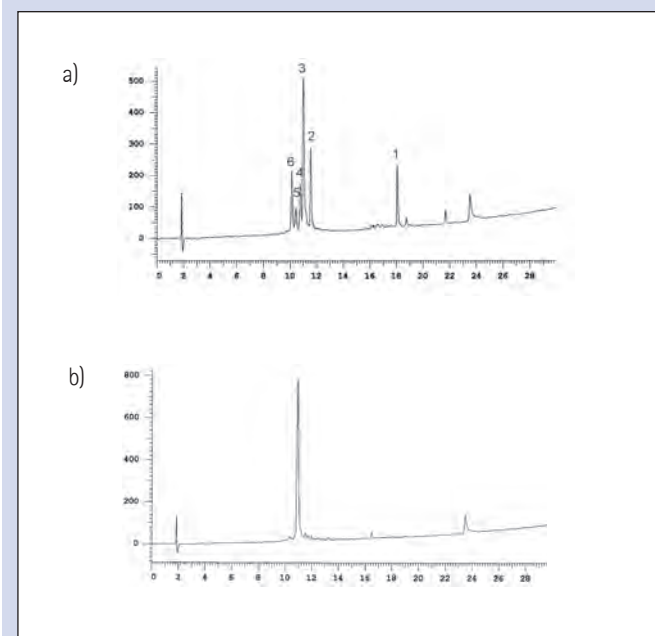
Figure 3-6. HPLC profiles of crude peptide 1 obtained under standard conditions (a) and using Fmoc-Phe-Ser($\psi^{Me,Me}$pro)-OH (b). Peak 1: Fmoc-LTFSNKSVLQ-OH; 2: H-VTYLTFSNKSVLQ-OH + H-VYLTFSNKSVLQ-OH; 3: H-VTRYLTFSNKSVLQ-OH; 4: H-YLTFSNKSVLQ-OH; 5: H-VLTFSNKSVLQ-OH; 6: H-LTFSNKSVLQ–OH.

The synthesis of amylin was optimized using the highly aggregation prone 8-37 fragment as a model system. Three different strategies were investigated:

1) Double couple all β-branched amino acid residues and those that immediately follow;

2) Double couple all amino acid residues;

3) Substitute dipeptides A^8T, $S^{19}S$, $L^{27}S$ for the corresponding pseudoproline dipeptides. Double couple all β-branched residues, pseudoproline dipeptides and residues following either of these.

All the syntheses were carried out on an ABi 433A peptide synthesizer using HBTU activation. For strategy 3, the dipeptides A^8T, $S^{19}S$, $L^{27}S$ were substituted with pseudoproline dipeptides, in accordance with the above guidelines.

Strategy 1 totally failed to produce any of the target peptide, instead producing a mixture of truncated peptides indicative of extensive aggregation during peptide assembly (Figure 3-8A). The material obtained from strategy 2 was also highly heterogeneous (Figure 3-8B). Some of the desired peptide was produced but this co-eluted with two deletion products and could not be easily isolated. The synthesis using pseudoproline dipeptides on the other hand afforded the desired peptides in a purity of greater than 90% (Figure 3-8C). The synthesis was then continued to produce full-length linear hAmylin1-37. Treatment of the peptidyl resin with TFA/anisole/thioanisole/ethanedithiol (90:3.33:3.33:3.33) afforded crude material of excellent quality as shown in Figure 3-9. Characterization of this material by ES-MS confirmed the major component to be the desired peptide.
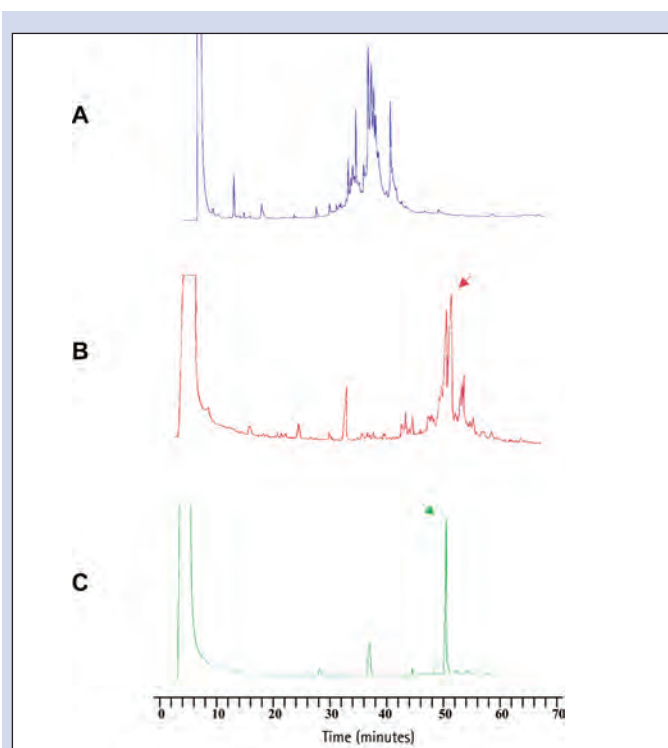
Fig. 3-9: HPLC profile of crude linear hAmylin1-37 obtained using the pseudoproline dipeptide strategy 3. Arrow indicates elution position of the desired product [35].

Phosphopeptides

H-Glu-Asn-Ser-Thr-Tyr(PO$_3$H$_2$)-Tyr(PO$_3$H$_2$)-Lys-**Ala–Ser**-Lys-Gly-Lys-Leu-Cys-NH$_2$

Substitution of Ser, Thr or Tyr by the corresponding phosphoamino acid residue often can have a marked effect on product purity. Figure 3-10 shows the HPLC profile of the above peptide which contains 2 phosphotyrosine residues. Repeating synthesis and substituting residues 8 and 9 for Fmoc-Ala-Ser($\psi^{Me,Me}$pro)-OH resulted in a remarkable improvement in purity and 10-fold increase in yield.
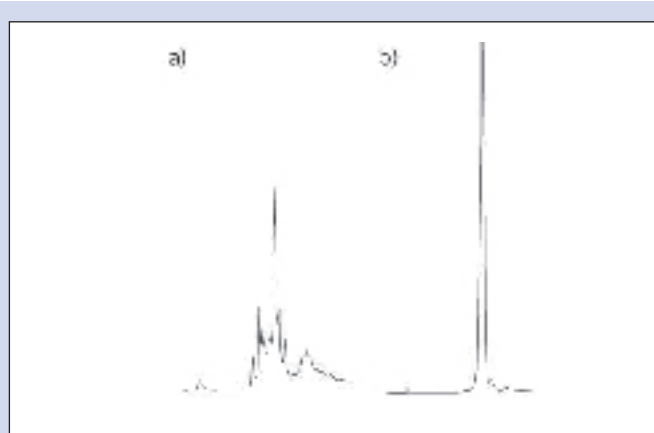
Fig. 3-10: HPLC profiles of crude phosphopeptide obtained under standard conditions (a) and using Fmoc-Ala-Ser($\psi^{Me,Me}$pro)-OH (b).

Long peptides

LSQVKGFVRKNGVNEAKIDEIKNDN VQ**DT**AEQKVQ LLRNWHQKVQ LLRN-WHQLHG KKEAY**DT**LIK DLKKANL**ST**L AEKI**QT**IILK DIT**S**DSE**NS**N EHKL**T–S**EKDL (**2**)

The enhanced and more uniform reaction rates typically observed when using pseudoproline dipeptides particularly benefit the synthesis of long peptides [17]. Incorporation of pseudoprolines at regular intervals throughout the peptide has been found particularly effective. Figure 3-11b) shows the HPLC profile of crude peptide **2** (95mer) prepared using 8 pseudoproline dipeptides (bolded residues). The same sequence prepared without using pseudoprolines gave the HPLC profile shown in Fig 3-11a after just 50 cycle.

Fig. 3-8: HPLC profiles of crude Ac-hAmylin$_{8-37}$ obtained using A) double coupling of all β-branched residues and those residues which directly follow; B) double coupling of all residues; C) double coupling of all pseudoproline, β-branched residues and those which directly follow either of these [35].

Fig. 3-11: HPLC profiles of crude peptide 3 obtained under standard conditions after 50 cycles (a) and (b) using pseudoproline dipeptides after 95 cycles.

Cyclic peptides

Pseudoproline dipeptides are also extremely useful tools for assisting the cyclization of peptides [27,36]. Due to the propensity of pseudoproline residues to adopt a cis-conformation, substitution of Ser or Thr by a pseudoproline has the effect of bringing the ends of the chain together, promoting cyclization and reducing oligomerization and cyclodimer formation. Table 3-15 illustrates the synthesis of a cyclohexamer that could only be achieved through the use of 3 pseudoproline dipeptides.

Table 3-15: Preparation of cyclo(Val-Thr-Val-Thr-Val-Thr) using standard building blocks or pseudoproline dipeptides [36].

Sequence	Yield(%)	Epimerization
H-Val-Thr(TBS)-Val-Thr(TBS)-Val-Thr(TBS)-OH	–	–
H-Val-Thr($\psi^{Me,Me}$pro)-Val-Thr(TBS)-Val-Thr(TBS)-OH	5	Yes
H-Val-Thr($\psi^{Me,Me}$pro)-Val-Thr($\psi^{Me,Me}$pro)-Val-Thr(TBS)-OH	40	Yes
H-Val-Thr($\psi^{Me,Me}$pro)-Val-Thr(TBS)-Val-Thr(TBS)-OH	10	Yes
H-Val-Thr($\psi^{Me,Me}$pro)-Val-Thr($\psi^{Me,Me}$pro)-Val-Thr($\psi^{Me,Me}$pro)-OH	90	No

Racemization–free fragment condensation

Whilst in normal step-wise synthesis, racemization is generally not considered an issue, in fragment condensation the prevention of epimerization is of paramount concern. This is because, in contrast to urethane-protected amino acids, peptides easily form chirally labile oxazolones upon C-terminal carboxyl activation that participate in amide bond formation. The normal strategy for overcoming this problem is to design syntheses in such a way that, wherever possible, N-terminal fragments are selected which contain either a C-terminal Gly or Pro residue, as the former is achiral and the latter does not readily form

oxazolones. Peptides containing C-terminal pseudoproline residues can also be coupled without stereomutation [37], and since pseudoproline residues are simply masked serine or threonine, this effectively doubles the number of sites in any given peptide available for epimerization-free fragment condensation.

The remarkable resistance of C-terminal pseudoproline residues to racemization during peptide bond formation is demonstrated in Table 3-16. Coupling of Fmoc-Tyr(tBu)-Ser(tBu)-OH (Experiment 1) or Fmoc-Tyr(tBu)-D-Ser(tBu)-OH (Experiment 2) to H-Phe-Wang resin using PyBOP® activation resulted in nearly complete racemization of the serine residue, whereas the peptide prepared using a pseudoproline dipeptide (Experiment 3) exhibited no detectable epimer formation. A typical result using PyBOP® activation is shown in Figure 3-12.

Table 3-16: Studies comparing the racemization of C-terminal Ser residue when protected as a pseudoproline or t-Bu ether during synthesis of YSF model peptides.

Experiment	Coupling Method	Solvent	% L–Ser	% D–Ser
1	PyBOP®/DIPEA	DMF	65	35
2	PyBOP®/DIPEA	DMF	36	64
3	PyBOP®/DIPEA	DMF	100	0
4	PyBOP®/collidine	DMF	68	32
5	HATU/DIPEA	DMF	70	30
6	HCTU/DIPEA	DMF	60	40
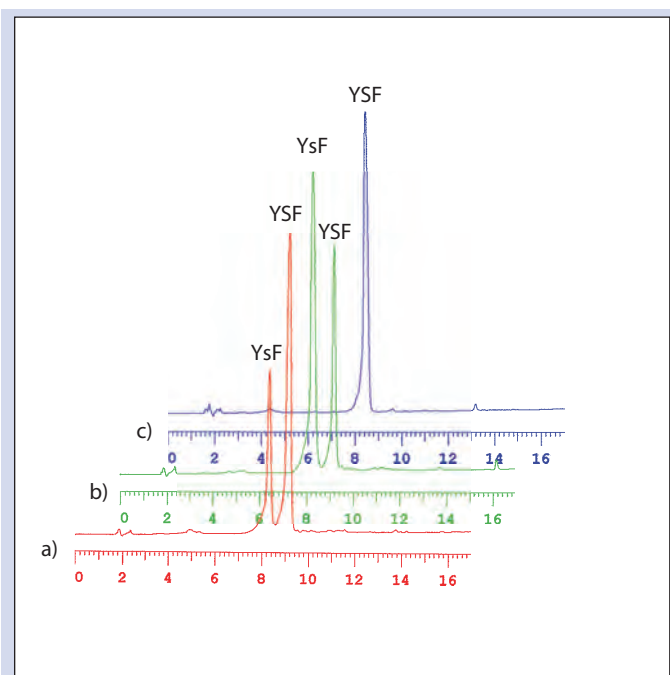
Fig. 3-12: HPLC profiles of a) Experiment 1; b) Experiment 2; c) Experiment 3.

The optimal support for the preparation of protected fragments containing C-terminal pseudoproline dipeptides is 2-chlorotrityl chloride resin. It has a high substitution, is loaded under conditions which do not require carboxyl activation, and peptides can be released under conditions which do not affect the pseudoproline ring. Furthermore, the bulky 2-chlorotrityl group minimizes diketopiperazine formation during deprotection of the C-terminal pseudoproline dipeptide. Interestingly, if the 4-carboxytrityl linker isused instead of the 2-chlorotrityl extensive DKP is observed [38].

Attachment of the appropriate pseudoproline dipeptide to this resin is carried out using Method 3-10, page 3.6. Cleavage of fully protected peptides can be effected by treatment with 0.5% TFA in DCM according to Method 3-25, page 3.28.

The use of pseudoproline dipeptides in the convergent synthesis is demonstated below by the synthesis of a fibrinopeptide A related sequence.

Application 3-3: Synthesis of H-Gly-Asp-Phe-Glu-Ile-Ser-Glu-Glu-Ser-Leu-NH$_2$

Boc-Gly-Asp(OtBu)-Phe-Glu(OtBu)-Ile-Ser($\Psi^{Me,Me}$pro)-OH + H-Glu(OtBu)-Glu(OtBu)-Ser(tBu)-Leu-NH$_2$

TBTU/DIPEA

Boc-Gly-Asp(OtBu)-Phe-Glu(OtBu)-Ile-Ser($\Psi^{Me,Me}$pro)-Glu(OtBu)-Glu(OtBu)-Ser(tBu)-Leu-NH$_2$

TFA

H-Gly-Asp-Phe-Glu-Ile-Ser-Glu-Glu-Ser-Leu-NH$_2$
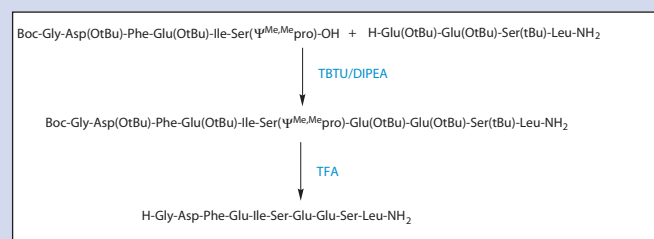
2-Chlorotrityl chloride resin was loaded with Fmoc-Ile-Ser($\varphi^{Me,Me}$pro)-OH as described in Method 3-10, page 3.6, to give a resin with a substitution of 0.26 mmole/g, as determined by the Fmoc UV assay. Boc-Gly-Asp(OtBu)-Phe-Glu(OtBu)-Ile-Ser($\varphi^{Me,Me}$pro)-2-ClTrt resin was assembled on this support by standard Fmoc chemistry, using 2-fold excesses of Fmoc-amino acids activated with PyBOP® (1 eq.) and DIPEA (4 eq.), and DBU/piperidine/DMF (2:2:96) for Fmoc removal. A coupling time of 60 min was used throughout. Cleavage of the protected peptide from the resin was carried out by treatment of the peptidyl resin with 0.5 % TFA in DCM, as described in Method 3-30 on p. 3.30, to yield the product with the HPLC profile shown in Figure 3-13.
The C-terminal component, H-Glu(OtBu)-Glu(OtBu)-Ser(tBu)-Leu-NH$_2$, was prepared on Sieber amide resin in a similar manner except 1 % TFA in DCM was used to effect cleavage. The HPLC profile of the crude product is shown in Figure 3-13.
The two fragments were dissolved in DMF and coupled using TBTU (1.2 eq) and DIPEA (4 eq). The reaction was complete after 4 h as determined by HPLC, so the solvent was evaporated, and the protected peptide precipitated with water. The product was isolated by filtration, washed with water and dried in vacuo over P$_2$O$_5$. The peptide was then treated with TFA/water/TIS (95:2.5:2.5) for 3 h. After this time, the TFA was removed by evaporation, and the product precipitated with ether. The crude decapeptide was characterized by HPLC (Figure 3-14) and LC-MS.
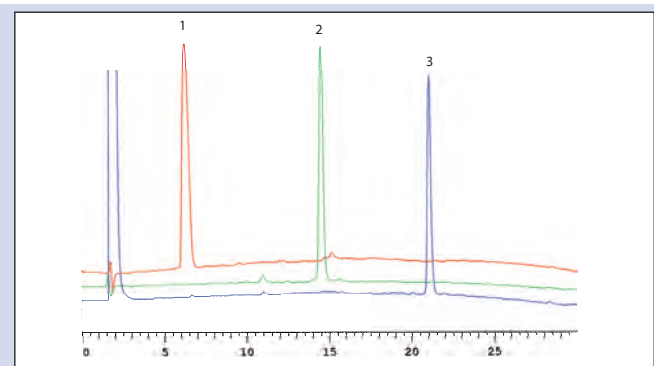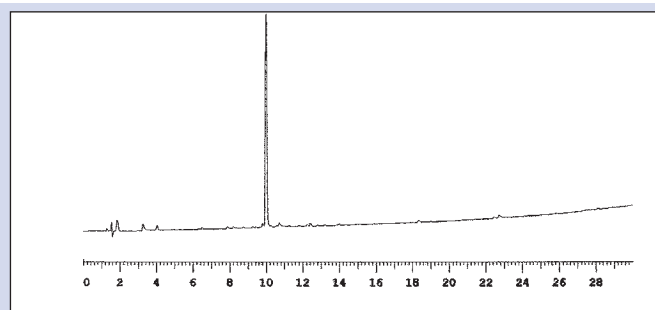
Fig. 3-14: HPLC profile of crude product after TFA cleavage.

Related products

852175	Fmoc-Ala-Ser($\psi^{Me,Me}$pro)-OH	p. 89
852180	Fmoc-Ala-Thr($\psi^{Me,Me}$pro)-OH	p. 90
852185	Fmoc-Asn(Trt)-Ser($\psi^{Me,Me}$pro)-OH	p. 90
852183	Fmoc-Asn(Trt)-Thr($\psi^{Me,Me}$pro)-OH	p. 90
852186	Fmoc-Asp(OtBu)-Ser($\psi^{Me,Me}$pro)-OH	p. 90
852199	Fmoc-Asp(OtBu)-Thr($\psi^{Me,Me}$pro)-OH	p. 90
852190	Fmoc-Gln(Trt)-Ser($\psi^{Me,Me}$pro)-OH	p. 90
852198	Fmoc-Gln(Trt))-Thr($\psi^{Me,Me}$pro)-OH	p. 91
852177	Fmoc-Glu(OtBu)-Ser($\psi^{Me,Me}$pro)-OH	p. 91
852196	Fmoc-Glu(OtBu)-Thr($\psi^{Me,Me}$pro)-OH	p. 91
852200	Fmoc-Gly-Ser($\psi^{Me,Me}$pro)-OH	p. 91
852197	Fmoc-Gly-Thr($\psi^{Me,Me}$pro)-OH	p. 91
852194	Fmoc-Ile-Ser($\psi^{Me,Me}$pro)-OH	p. 91
852193	Fmoc-Ile-Thr($\psi^{Me,Me}$pro)-OH	p. 91
852179	Fmoc-Leu-Ser($\psi^{Me,Me}$pro)-OH	p. 92
852184	Fmoc-Leu-Thr($\psi^{Me,Me}$pro)-OH	p. 92
852178	Fmoc-Lys(Boc)-Ser($\psi^{Me,Me}$pro)-OH	p. 92
852191	Fmoc-Lys(Boc)-Thr($\psi^{Me,Me}$pro)-OH	p. 92
852195	Fmoc-Phe-Ser($\psi^{Me,Me}$pro)-OH	p. 92
852201	Fmoc-Phe-Thr($\psi^{Me,Me}$pro)-OH	p. 92
852187	Fmoc-Ser(tBu)-Ser($\psi^{Me,Me}$pro)-OH	p. 92
852192	Fmoc-Ser(tBu)-Thr($\psi^{Me,Me}$pro)-OH	p. 93
852202	Fmoc-Trp(Boc)-Ser($\psi^{Me,Me}$pro)-OH	p. 93
852188	Fmoc-Trp(Boc)-Thr($\psi^{Me,Me}$pro)-OH	p. 93
852189	Fmoc-Tyr(tBu)-Ser($\psi^{Me,Me}$pro)OH	p. 93
852182	Fmoc-Tyr(tBu)-Thr($\psi^{Me,Me}$pro)OH	p. 93
852176	Fmoc-Val-Ser($\psi^{Me,Me}$pro)-OH	p. 93
852181	Fmoc-Val-Thr($\psi^{Me,Me}$pro)OH	p. 93

3.5.4 Dmb dipeptides

Dmb dipeptides work in exactly the same way and offer many of the same benefits as pseudoproline dipeptides but for Gly-containing sequences i.e. faster and more predictable acylation reactions, higher yields and purities of crude products, and less failed syntheses. Like pseudoproline dipeptides, insertion of a Dmb dipeptide introduces two amino acids at once and avoids the difficult acylation of the (Dmb)Gly residue.

Using them is very straightforward, one simply substitutes a Gly residue together with the preceding amino acid residue in the peptide sequence with the appropriate dipeptide (Figure 3-15). The native sequence is regenerated on TFA-mediated cleavage and deprotection.

For best results when using Dmb dipeptides, it is best to follow the guidelines set out on page 3.10. Dmb dipeptides can be introduced using any standard coupling method.

Fig. 3-13: HPLC profile of 1) C-terminal fragment; 2) N-terminal fragment; 3) full length protected peptide.
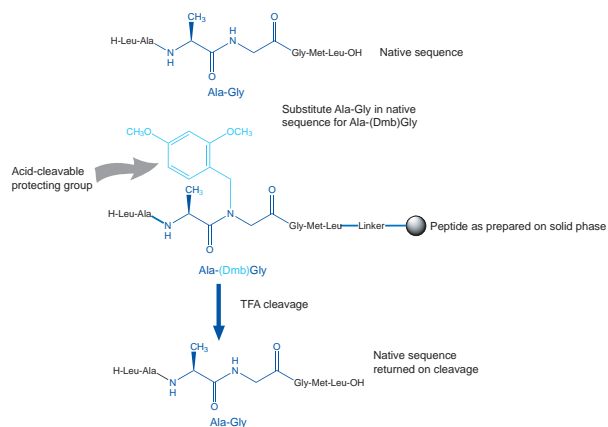
Fig. 3-15: Principles of using Dmb dipeptides.

Fmoc-Gly-(Dmb)Gly-OH is particularly useful for improving the synthesis of peptides containing the commonly occurring Gly-Gly motif. The production of such peptides is often problematic, owing to their propensity to aggregate and the difficulties in separating closely eluting desGly by-products. Insertion of Gly-(Dmb)Gly into a peptide sequence has the same benefits on synthetic efficiency as a pseudoproline dipeptide, preventing aggregation and improving acylation and deprotection kinetics. The use of this derivative was found to be essential for the synthesis of peptides related to nucleolin [29].

The Ala-Gly dipeptide sequence is frequently found in hydrophobic transmembrane and amyloidogenic peptides. Therefore, Fmoc-Ala-(Dmb)Gly-OH should prove to be a useful tools for the synthesis of such peptides.

For peptides containing the Asp-Gly motif, Fmoc-Asp(OtBu)-(Dmb)Gly-OH is highly recommended as its use for introduction of the Asp-Gly dipeptide completely prevents aspartimide formation, see section 3.6, page 3.23 for further details.

Synthesis of neurotoxic prion peptide (106 – 126)
H-Lys-Thr-Asn-Met-Lys-His-Met-Ala-Gly-Ala-Ala-Ala-Ala-Gly-Ala-Val-Val-Gly-Gly-Leu-Gly-OH

Spongiform encephalopathies such as bovine spongiform encephalopathy and Creutzfeldt-Jakob disease are caused by prion protein (PrP). The peptide comprising residues 106-126 of human PrP has been found to be highly amyloidogenic and toxic to neurons [39].

The SPPS of PrP (106-126) has been described by Jobling, et al. [40]. In their hands, the synthesis of this peptide using standard Fmoc-protected amino acids was unsuccessful, with chain extension terminating at Ala117. Incorporation of (Hmb)Gly residues at positions 114 and 119 resulted in a marked improvement in synthetic efficiency and afforded the full length peptide in a yield of 7.3%. However, the target peptide could only be obtained in good yield and purity by using Boc-protected amino acids with *in situ* neutralization coupling protocols.

The difficulties in the synthesis of PrP (106-126) were ascribed to the inherent propensity of this peptide to aggregate, particularly in the region of the AGAAAAGA sequence. The presence of glycine residues on either side of the problematic region therefore made this sequence an excellent test of Novabiochem's structure-breaking Dmb dipeptides.

In view of the difficulties experienced by Jobling, et al. [40] in the Fmoc

SPPS of PrP (106-126), this peptide was prepared using three Dmb dipeptides at the positions marked in blue. This approach would introduce structure-breaking (Dmb)Gly residues before Ala120 and Ala117, regions of the sequence which were found to be particularly problematic, and would therefore maximize the chances of a successful synthesis. The peptide was prepared using Fmoc-Gly-Wang resin (0.1 mmole, 0. 71 mmole/g) on an ABi 433A peptide synthesizer using standard FastMoc protocols with feedback conductivity monitoring and a 30 minutes coupling time. The Dmb dipeptides were introduced using a 3-fold excess of reagents instead of the standard 10-fold excess which was used for all other amino acids. At residue Ala115, the synthesis was paused and a portion of resin was removed. A small amount of this resin was treated with TFA/water/TIPS (95:2.5:2.5) for 3 hours. The cleaved product was analyzed by LC-MS and found to be essentially homogeneous. The assembly was then continued on the bulk of the resin to give the full length peptide. Treatment of the resin with TFA as previously described afforded the crude PrP in excellent purity, and MS analysis of the product showed no evidence of deletion or truncated peptides (Figure 3-16a, b).

The synthesis of PrP (106-126) was then repeated using standard Fmoc-amino acid building blocks. A sample of resin was taken at Ala115 and peptide was cleaved and analyzed by LC-MS. The crude peptide obtained was of excellent quality, which was rather surprising in view of the difficulties reported by Jobling, et al. The synthesis was continued and the full length PrP was cleaved as described previously. Characterization of this material by LC-MS indicated that the product contains less than 48% of the target peptide, together with significant amounts of the des-Asn108 and des-(Lys106, Asn108) deletion peptides, and smaller amounts of other peptides missing residues from the N-terminal sequence (Figure 3-16c, d). These results indicate that there are no issues with aggregation and failed coupling reactions during the assembly of residues 117-120 of PrP. Instead, it rather appears that the difficulties occur predominantly during the introduction of the N-terminal residues, and these can be effectively overcome by the use of Fmoc-Ala-(Dmb)Gly-OH for the incorporation of Ala-Gly residues.

Dmb-derivatives when used in combination with pseudoproline dipeptides enable insertion of structure breaking derivatives at regular intervals throughtout a peptide sequence. This strategy has recently facilitated the synthesis of the transmembrane region of the bradykin receptor [41], 101 residues of the d2 domain of VEGF receptor 1 [21], and ubitiquin and related analogs [22].

Synthesis of transmembrane region of bradykin receptor [41]
H-Thr-Val-Ala-Glu-Ile-Tyr-Leu-Gly-Asn-Leu- Ala-Gly-Ala-Asp-Leu-Ile-Leu- Ala-Ser-Gly-Leu-Pro-Phe-Trp-Ala-Ile-Thr-Ile-Ala-Asn-Asn-Phe-Asp-OH (TM-33)

TM-33 was assembled on Fmoc-Asp(OtBu)-Wang resin using a Protein Technologies Symphony peptide synthesizer. Couplings were carried out for 1 h using 10-fold excesses of standard Fmoc-amino acid building blocks activated with HCTU/NMM. Fmoc groups were removed by two treatments with 20% piperidine in DMF (1 x 10 min, 1 x 20 min). The residues in blue and gray were introduced with pseudoproline- and Dmb-dipeptides, respectively. This highly insoluble material was dissolved in neat TFA, which was then diluted with MeCN/water, and analyzed by HPLC and MALDI-TOF. The product made without using structure breaking derivatives was found to be highly heterogeneous (Figure 3-17a) and not to contain any major component, whereas that prepared using these derivatives was of excellent quality (Figure 3-17b). These results confirm the findings of Oliveira, et al. [42] that this is a peptide difficult to be synthezised.
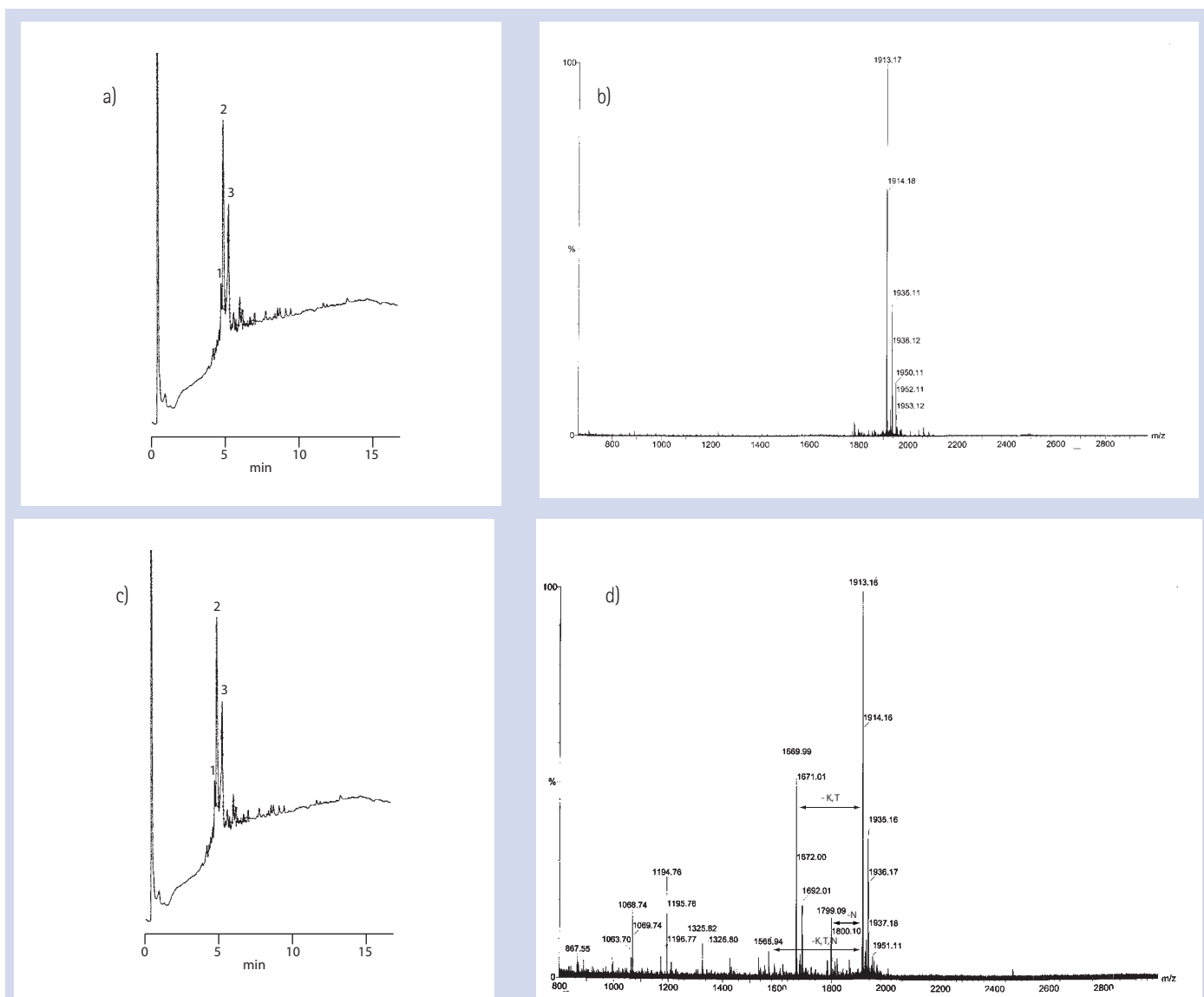
Fig. 3-16: a) HPLC profile and b) MALDI-TOF of crude Prp (106-126) prepared using 3 Dmb dipeptides. c) HPLC profile and d) MALDI-TOF of crude PrP (106-126) prepared using standard Fmoc-amino acid derivatives. Peak 1: des-Asn[108] PrP (106-126); Peak 2: PrP (106-126); Peak 3: des-(Lys[106], Asn[108]) PrP (107-126).

Synthesis of d2 domain of VEGF receptor [21]

H-**Ser–Asp–Thr–Gly–Arg–Pro–Phe–Val–Glu–Met–Tyr–Ser–Glu–Ile–Pro–Glu–Ile–Ile–His–Met–Thr–Glu–**Gly-Arg-Glu-Leu-Val-Ile-Pro-Cys-Arg-Val-Thr-**Ser**-Pro-**Asn–Ile–Thr**-Val-Thr-Leu-Lys-Lys-**Phe**-Pro-Leu-Asp-Thr-Leu-Ile-**Pro–Asp**-Gly-Lys-**Arg–Ile–**Ile-**Trp**-Asp-Ser-**Arg**-Lys-Gly-Phe-Ile-Ile-Ser-Asn-Ala-Thr-Tyr-**Lys–Glu–Ile**-Gly-Leu-Leu-Thr-Cys-Glu-Ala-Thr-Val-Asn-Gly-His-Leu-Tyr-Lys-Thr-Asn-Tyr-Leu-Thr-His-Arg-Gln-Thr-Asn-Thr-Ile-OH.

Peptide synthesis was carried out using an ABI 433A peptide synthesizer on NovaSyn TGA resin (0.23 mmol/g). Couplings were performed using 4-fold excess of Fmoc-amino acids activated with HBTU/HOBt/DIPEA (1:1:2) for 1 hour. A 5 minute capping step was performed after each coupling reaction using 0.5 M acetic anhydride : 0.125 M DIPEA : 0.015 M HOBt in NMP. Fmoc removal was effected by treating the resin with 20% piperidine in NMP for 15 minutes. The residues above in bold were double coupled, whereas those in blue and gray were introduced with pseudoproline dipeptides and Fmoc-(Dmb)Gly-OH, respectively. The HPLC profiles of the crude products in shown in Figure 3-18. Following purification by RP-HPLC, re-folding and oxidation, and filtration through a 10 kD cut-off Amicon centrifugation tube, the folded protein domain was obtained in good purity (Figure 3-19 and Figure 3-20).
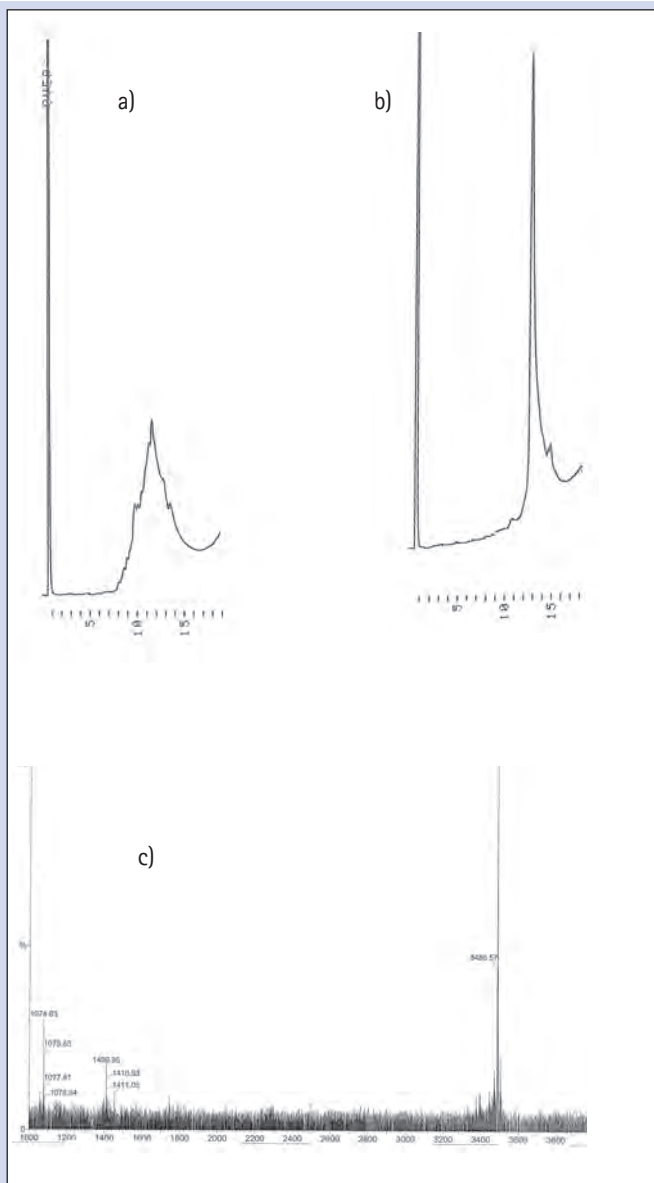
Fig. 3-17: a) HPLC elution of crude TM-33 prepared with standard amino acid derivatives. b) HPLC elution profile of crude TM-33 prepared using Dmb and pseudoproline dipeptides. c) MALDI-TOF spectrum of total crude TM-33 prepared with Dmb and pseudoproline dipeptides. Expected M+Na$^+$ 3487.9; found 3488.6.

Related products
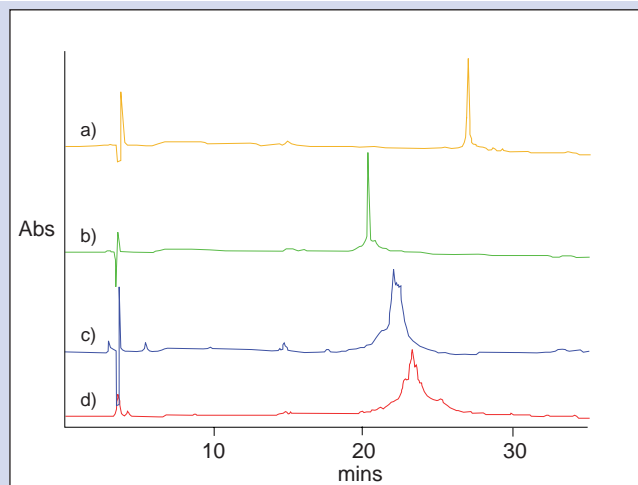
Fig. 3-18: HPLC elution profiles of a) 29mer, b) 49mer, c) 79mer and d) 101mer.
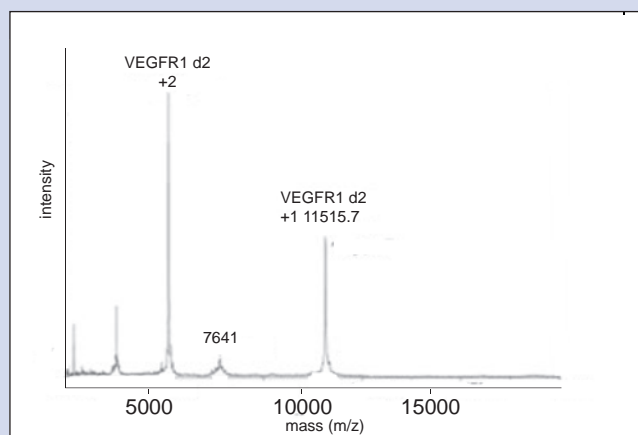
Fig. 3-19: MALDI-TOF spectrum of reduced VEGFR1 d2 after first HPLC purification.
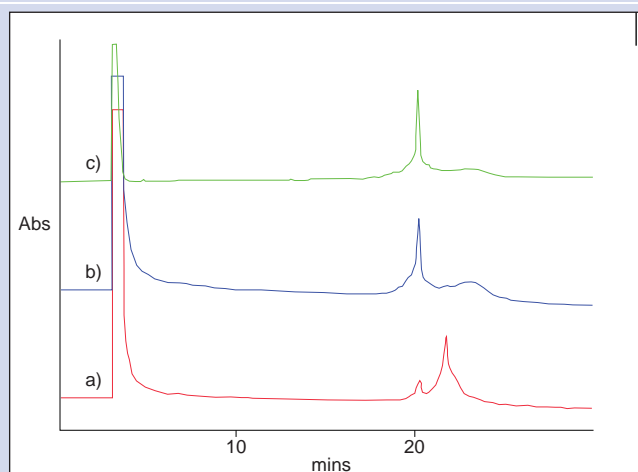
Fig. 3-20: Refolding and purification of VEGFR1 d2. HPLC elution profiles of recovered after a) 0 h and b) 18 h of air oxidation, and c) dialysis and ultrafiltration.

3.5.5 Dmb/Hmb amino acids

Unlike the previously described pseudoproline and Dmb dipeptides, these derivatives are used to introduce backbone-amide protected residues individually rather than as dipeptides. However, once incorporated into a peptide they work in exactly the same way as pseudoproline and Dmb dipeptides by disrupting secondary structure formation, to give the same enhancements in synthetic efficiency.

By using such amino acid derivatives, one is not limited to introduction of backbone amide protection at either a Gly, Ser or Thr residue, as is the case with the dipeptide derivatives. However, the drawback to this approach is that it now becomes necessary to couple onto the hindered secondary amine of the Hmb or Dmb residue.

Sheppard and colleagues designed their Hmb derivatives to mitigate against this problem [14, 30, 43]. Addition of the amino acid immediately following the Hmb-protected proceeds initially *via* internal base-catalyzed capture of the acyl component to give the phenyl ester which then slowly undergoes intramolecular O→N acyl transfer to afford the desired tertiary amide (Figure 3-21). Acylation of (Hmb)Gly is generally straightforward and works well for most amino acids with most coupling methods. For other Hmb residues, Sheppard and colleagues found that the use of PSAs in DCM or Fmoc N-carboxyanhydrides gave the best results. Other authors have also found TBTU/HOBt/DIPEA [44] activation and amino acid fluorides [45] to be effective. In practice, as the side-chain becomes more bulky, coupling gets more difficult and the steric hindrance of the incoming amino acid becomes an increasingly important factor. Using acid fluorides, the most difficult case, the addition of Fmoc-Val to (Hmb)Val, could be effected in a yield of 95% by carrying out the coupling overnight in toluene at 80°C. Despite these limitations, Hmb derivatives have proven to be remarkably effective in a wide range of difficult and aggregated peptides [43].
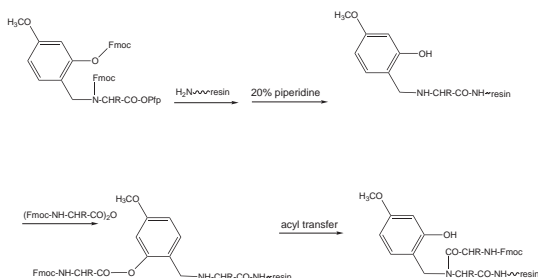
Fig. 3-21: Mechanism of acylation of Hmb-protected residues.

(Hmb)Gly and (Dmb)Gly have particular applications in the synthesis of hydrophobic amyloid and transmembrane peptides. These sequences are extremely difficult to prepare using conventional methods, and are generally not amenable to pseudoproline substitution as they rarely contain multiple serine or threonine residues. Glycine, however does occur frequently in such peptides, often adjacent to hydrophobic residues such as Ile, Val, and Ala. Therefore, substitution of Gly by (Dmb)Gly or (Hmb)Gly should prove to be a highly effective method of overcoming these problems. Indeed, using six substitutions of the analogous (Tmob) Gly derivative, Bayer and colleagues were able to prepare a 64-residue transmembrane peptide in remarkable purity [46].

The coupling of Fmoc-(Dmb)Gly-OH can be achieved using standard methods such as PyBOP®/DIPEA. Following Fmoc removal with piperidine/ DMF, the glycine secondary amine can be acylated with Fmoc-amino acids by a single coupling with PyBrOP® or HATU, or by using pre-formed amino acid fluorides [46]. (Dmb)Gly is to be preferred over (Hmb)Gly in applications requiring post-synthesis acylation or phosphorylation as the Dmb group lacks potentially reactive hydroxyl functionality.

Deprotection

Dmb and Hmb groups are removed under the normal conditions required for the final cleavage and deprotection of the peptide. However, we recommend the addition of approx. 2% TIS to the cleavage mixture.

NOTE: the cleavage products of Dmb and Hmb can cause side-chain modification of unprotected Trp. We, therefore, strongly recommend the use of Fmoc-Trp(Boc) in all syntheses where these groups are used.

Solubilization of aggregated sequences

Acylation of the Hmb group with acetic anhydride in the presence of DIPEA greatly increases its acid stability, enabling peptides retaining Ac-Hmb protection to be generated directly on cleavage with TFA under normal conditions [47]. Backbone protected peptides exhibit markedly improved solubility properties, facilitating purification by HPLC of otherwise intractable sequences. The free peptide may be regenerated, after deacetylation with 20% piperidine in DMF, by cleavage with TFA (Figure 3-22.

i) Ac$_2$O (10 eq.); ii) 95% TFA; iii) Ac$_2$O (10 eq.), DIPEA (10 eq.); iv) 20% piperidine/DMF; v) Boc$_2$O (10 eq.)
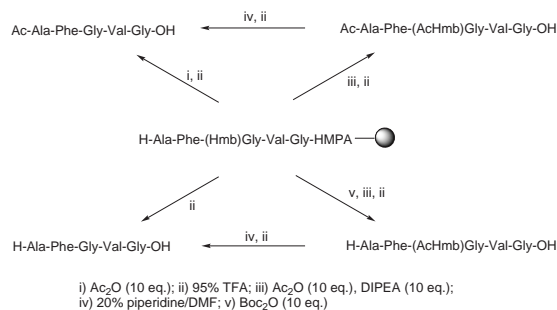
Fig. 3-22: Synthesis of Ac-Hmb protected peptides.

Elimination of aspartimide formation

Using a Dmb/Hmb protected derivative for the incorporation of the residue linked to the carboxy group of Asp or Asn residues has been found to totally remove the risk of formation of aspartimide and piperidide by-products [48]. For a further discussion on aspartimide formation and the use of Fmoc-Asp(OtBu)-(Dmb)Gly-OH, please see this chapter, section 3.6.

Fragment condensation

The solubility of protected peptide fragments can be greatly increased through the use of Hmb amide backbone protection, enabling reactions to be carried out at higher concentrations than otherwise possible, with associated improvements in coupling rates and yields of product [49].

Peptide Cyclization

Dmb and Hmb-protected residues have been found to be excellent tools for improving yields of cyclic peptides. Introduction of a turn-inducing Hmb or Dmb-protected residue promotes cyclization by helping the ends of the peptide chain together, resulting in better yields of cyclic monomer with less oligomer and cyclic dimer formation [50].

3.5.6 Isoacyl dipeptides
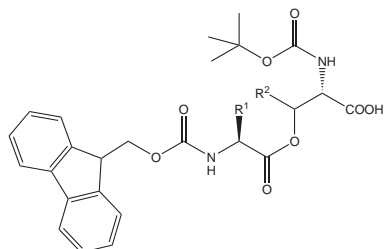
Isoacyl dipeptides are remarkable new tools for enhancing synthetic efficiency in Fmoc SPPS that consist of a Boc-protected serine or threonine derivative in which the β-hydroxyl group is acylated by an Fmoc-amino acid [51, 52]. They perform the same role, and are used in exactly the same manner, as pseudoproline dipeptides. Substitution of Aaa-Ser or Aaa-Thr in a peptide sequence with an isoacyl dipeptide results in the formation of a depsipeptide analog of the native sequence in which the amide bond between Aaa and Ser or Thr is replaced by an ester linkage (Figure 3-22A). This modification results in a marked change in the conformation of the peptide chain which leads to disruption of aggregation in much the same way as would insertion of a pseudoproline or *N*-Dmb/Hmb-residue [53-58].

The real benefits of using isoacyl dipeptides become apparent once the peptide is released from the solid phase. In contrast to pseudoproline dipeptides, the product cleaved when using isoacyl dipeptides is the depsipeptide and not the native peptide sequence (Figure 3-23B). Such depsipeptide analogs of aggregation prone peptides have been found to be more soluble and consequently more easily purified than the highly structured native peptide [53-58]. For example, isoacyl β-amyloid (1-42) has a solubility of 15 mg/ml in water, whereas for the natural peptide it is only 0.14 mg/ml [56]. Once the depsipeptide form is purified, it can be easily converted to the native form by adjusting the pH to 7.4 when spontaneous O- to N-acyl migration occurs, with formation of an amide bond between the Ser or Thr residue and the next amino acid (Figure 3-23C). This pH controlled switching of the peptide conformation can be very advantageous in the study of amyloidogenic peptides, where the initial aggregation state of the peptide to be used in the assay is often unknown. For instance, a non-aggregated depsipeptide can be introduced into a bioassay and the native peptide formed *in situ* under physiological conditions, enabling activity of the native sequence in a non-aggregated state to be determined [57, 59].

Coupling of isoacyl dipeptides

Activation of isoacyl dipeptides with base-mediated coupling methods such as HBTU/DIPEA has be shown to cause β-elimination of the Fmoc-amino acid from the serine or threonine side chain [60]. This can lead to the formation of peptides omitting serine/threonine or by-products derived from dehydroresidues. Coupling under non-basic conditions using HOBt/DIPCDI (Method 3-16) appears to eliminate this problem [60, 61].
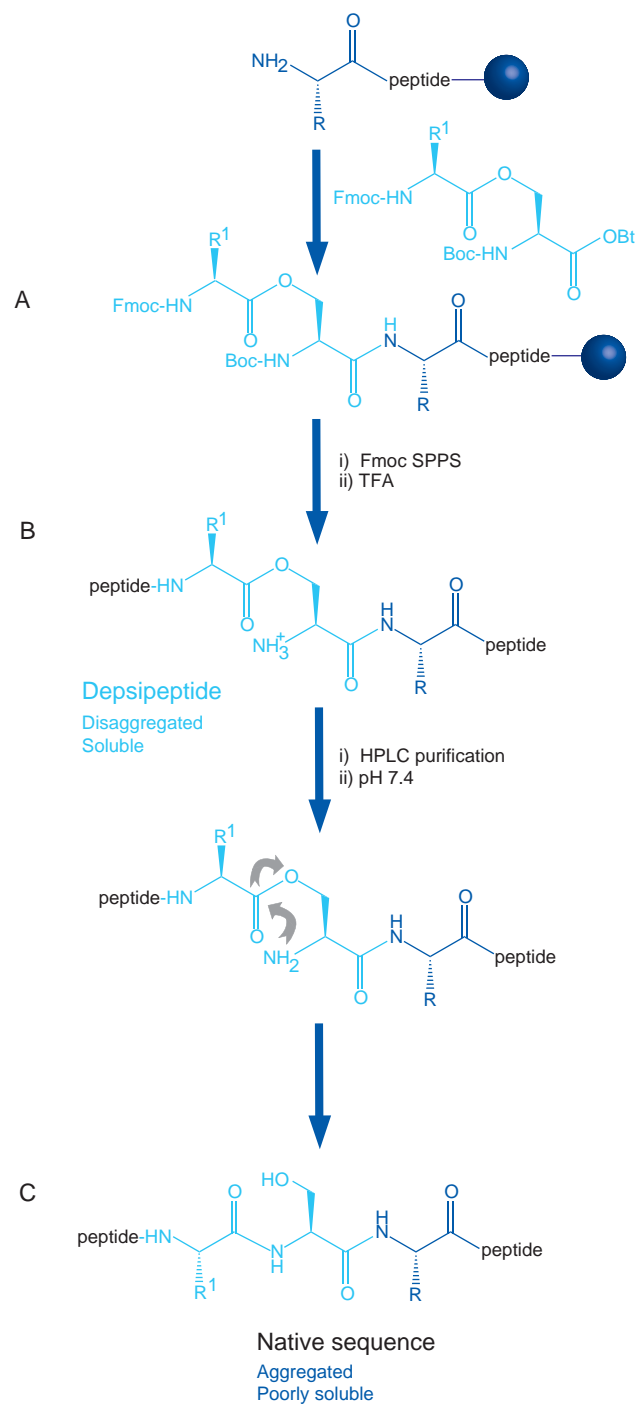
Fig. 3-23: Principles of peptide synthesis with isoacyl dipeptides, illustrated with Ser. A = leaving group. R and R^1 = amino-acid side chains.

Method 3-16: Coupling of isoacyl dipeptides

1. Dissolve the isoacyl dipeptide (4 eq.[a]) and HOBt (4 eq.[a]) in DCM.
2. Add DIPCDI (4.4 eq.[a]) and agitate for 10 min.
3. Add solution to peptidyl resin.

[a]relative to resin loading

DKP formation

Occasionally sequence dependent cleavage of the ester bond has been observed. This presumably arises from diketopiperazine formation during the removal of Fmoc from the residue following introduction of the isoacyl dipeptide, and is particularly problematic when the non-hindered residues are present in the ester-linked dipeptide. Beyermann and colleagues eliminated this problem by employing Bsmoc-protected amino acids for introduction of the residue immediately following the isoacyl dipeptide [62]. More recently, T. Yoshiya, et al. have shown that this side reaction can be suppressed by using 1-methylpyrrolidine/ hexamethyl-eneimine/HOBt in NMP/DMSO [63] for Fmoc removal. The use of this mixture should only be necessary for removing the Fmoc from the residue following the isoacyl dipeptide.

O– to N–Shift

Conversion of the depsipeptide to the native sequence can normally be achieved by dissolving the depsipeptide in pH 7 - 8 buffer (Method 3-17). This reaction is facile and is usually complete in a few minutes. The process can be monitored by RP-HPLC; the native peptide is invariably more strongly retained on the column than the depsipeptide.

Method 3-17: O to N migration

1. Dissolve depsipeptide in PBS (pH 7.4) or 0.05 M sodium bicarbonate. Monitor reaction by HPLC until complete.
2. Desalt peptide by gel filtration, solid phase extraction or HPLC.

Difficult peptides

Isoacyl dipeptides and pseudoproline dipeptides have been found to perform equally well in expediting the synthesis of aggregated sequences, as illustrated by the examples in Figures 3-24 and 3-25
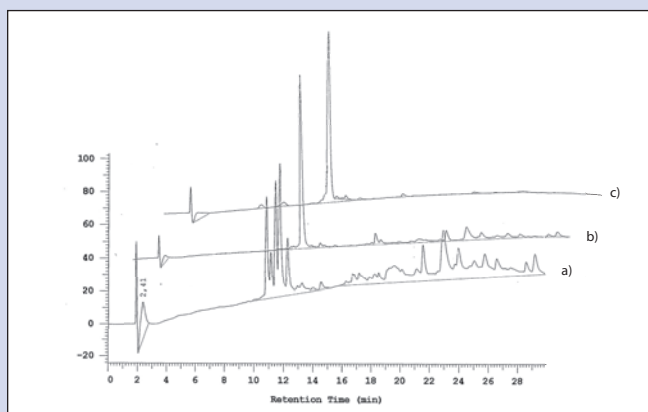
Fig. 3-24: HPLC profiles of H-Val-Thr-Arg-Tyr-Leu-Thr-Phe-Ser-Asn-Lys-Ser-Val-Leu-Gln-OH prepared a) with Fmoc-amino acid derivatives; b) FS pseudoproline dipeptide; c) FS isoacyl dipeptide.
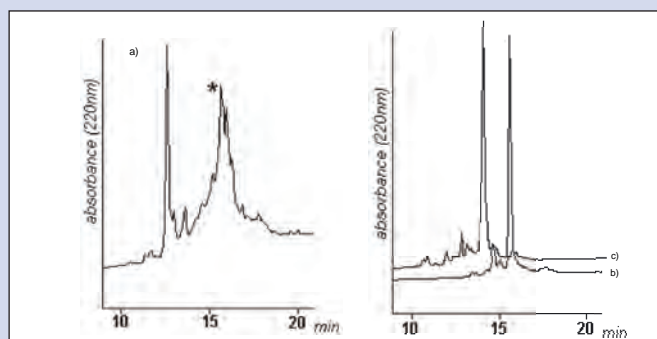
Fig. 3-25: HPLC profile of N(15)-FBP28WW (H-Gly-Ala-Thr-Ala-Val-Ser-Glu-Trp-Thr-Glu-Tyr-Lys-Thr-Ala-Asn-Gly-Lys-Thr-Tyr-Tyr-Tyr-Asn-Asn-Arg-Thr-Leu-Glu-Ser-Thr- Trp-Glu-Lys-Pro-Gln-Glu-Leu-Lys-NH₂) prepared using a) Fmoc amino acid building blocks, b) pseudoproline dipeptides, and c) isoacyl dipeptides.

Synthesis of β–amyloid with isoacyl dipeptides

β-Amyloid (1-42) is a notoriously difficult peptide to synthesize. Not only does it undergo serious aggregation on the solid phase during assembly, but it also aggregates in solution, making analysis and purification of the peptide extremely difficult.

26-O-Isoacyl-β-amyloid (1-42) was assembled on an ABi 433A peptide synthesizer using FastFmoc cycles. HCTU was used instead of using HBTU/HOBt as the activator and all Fmoc deprotection reactions were performed 3 times by treatment with 20% piperidine in NMP for 3 minutes. Val-36 and Gly-37 were introduced at the same time using the Dmb dipeptide Fmoc-Val-(Dmb)Gly-OH. This measure was introduced to help effect assembly of the hydrophobic C-terminal region of this peptide. The isoacyl dipeptide Boc-Ser(Fmoc-Gly)-OH was activated using HOBt/DIPCDI in DCM/DMF (3:1) and used to concurrently introduce Gly-25 and Ser-26. Kiso [53], as Carpino [54], and Mutter [55] have independently found insertion of an isoacyl dipeptide at this position to be efficacious. This measure allowed isoacyl-β-amyloid to be prepared in moderate purity without obvious problems with aggregation during chain assembly (Figure 3-26).

A sample of this material was purified on the Supelco column and gave the HPLC profile and MS spectrum shown in Figures 2-27 and 2-28, respectively. The purified peptide was converted to native β-amlyoid by dissolution in 0.1 M ammonium bicarbonate. The rearrangement appears to be complete after 1 h. The HPLC profile of a co-injection of 26-O-isoacyl-β-amyloid and β-amyloid is shown in Figure 3-29.
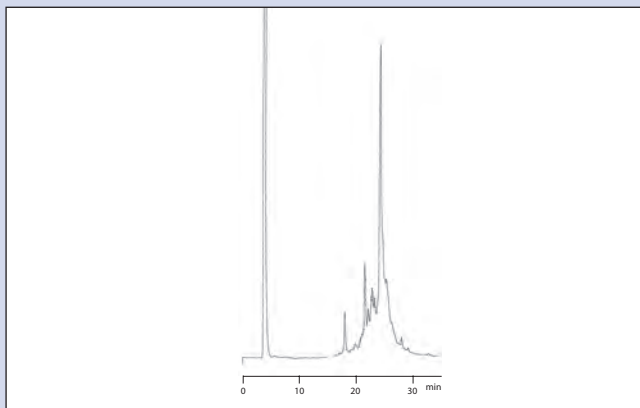
Fig. 3-26: HPLC profile of crude 26-O-isoacyl-β-amyloid. Column: Supelco 300 Å 2.4 mm. Buffer A: 0.1% TFA aq. Buffer B: MeCN/water/TFA (70:30:0.1). Gradient: 30% B for 5 min then 30 - 100% in 35 min. Flow rate: 0.2 ml/min.
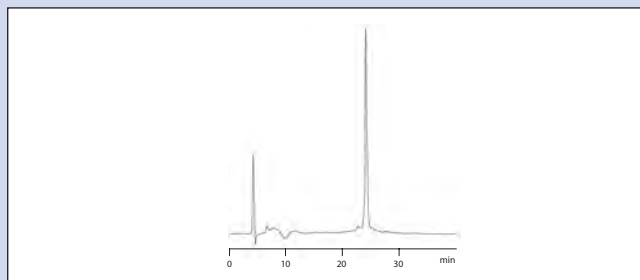
Fig. 3-27: HPLC profiles of purified 26-O-isoacyl-β-amyloid. HPLC conditions as Figure 2-22.
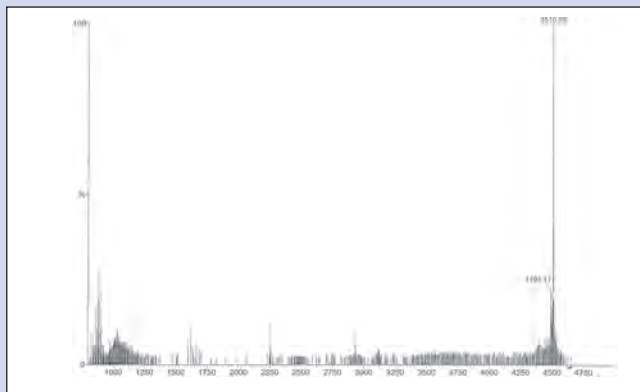
Fig. 3-28: MALDI-TOF spectrum of purified 26-O-isoacyl-β-amyloid.
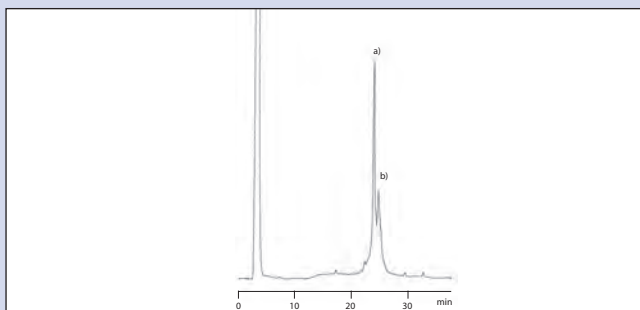
Fig. 3-29: HPLC profile of a co-injection of a) 26-O-isoacyl-β-amyloid and b) β-amyloid. HPLC conditions as Figure 2-22.

Fragment condensation

By employing an isoacyl dipeptide as the *C*-terminal dipeptide within the sequence of the carboxyl component, epimerization during fragment coupling can be avoided [60, 64]. This is because the amine group of the Ser/Thr is protected as a urethane and therefore can not easily become involved in oxazolone formation (Figure 3-29). This approach effectively doubles the number of sites available in a given peptide sequence for epimerization-free fragment condensation from Gly and Pro to now include Ser and Thr. Furthermore, protected depsipeptides are more soluble and couple significantly faster than the corresponding native sequences.

Activation of the depsipeptide fragment is best achieved using DIPCDI/HOBt in DCM, to suppress β-elimination of the ester containing Ser or Thr residue. This approach has recently been used to prepare humanin in excellent purity [63].
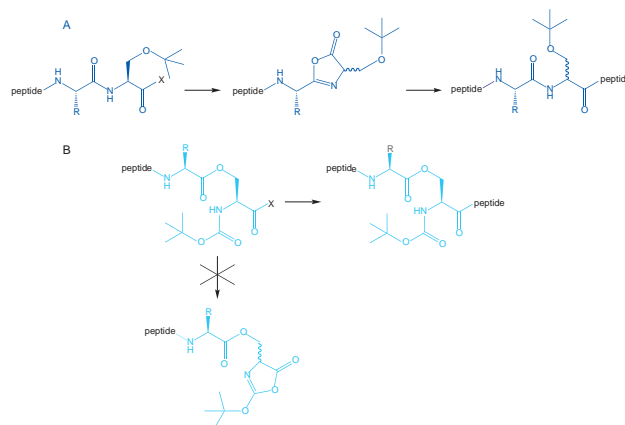
Fig. 3-30: A: Epimerization during fragment condensation via oxazolone formation; B: Epimerization-free fragment coupling using isoacyl peptide.
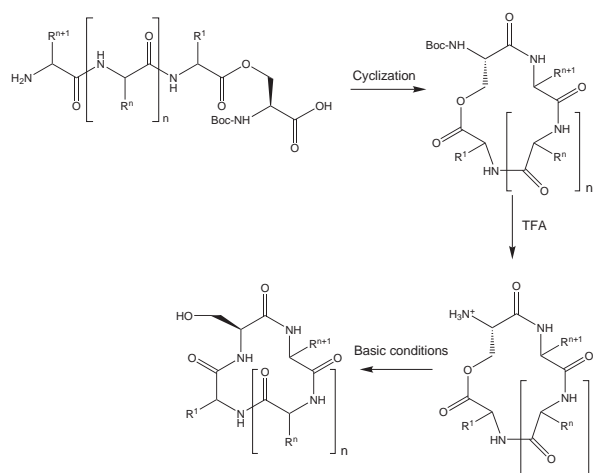
Fig. 3-31: Synthesis of cyclic peptides via depsipeptides.

Cyclizations

Protected peptides incorporating an isoacyl dipeptide at the *C*-terminus are excellent intermediates for the preparation of cyclic peptides, as they couple rapidly and undergo carboxyl activation without the risk of epimerization. Cyclic depsipeptides produced in the manner will rearrange to the native sequence upon dissolution in pH 7.4 buffer or piperidine in DMF (Figure 3-31). This approach afforded all L-amino acid cyclic pentapeptides that are extremely difficult to access by conventional methods [65].

Related products

852174	Boc-Ser(Fmoc-Ala)-OH	p. 100
852249	Boc-Ser(Fmoc-Arg(Pbf))-OH	p. 100
852257	Boc-Ser(Fmoc-Asn(Trt))-OH	p. 101
852298	Boc-Ser(Fmoc-Asp(OtBu))-OH	p. 101
852256	Boc-Ser(Fmoc-Gln(Trt))-OH	p. 101
852295	Boc-Ser(Fmoc-Glu(OtBu))-OH	p. 101
852257	Boc-Ser(Fmoc-Gly)-OH	p. 101
852298	Boc-Ser(Fmoc-Ile)-OH	p. 101
852293	Boc-Ser(Fmoc-Met)-OH	p. 102
852169	Boc-Ser(Fmoc-Phe)-OH	p. 102
852172	Boc-Ser(Fmoc-Ser(tBu))-OH	p. 102
852173	Boc-Ser(Fmoc-Thr(tBu))-OH	p. 102
852290	Boc-Ser(Fmoc-Val)-OH	p. 102
852170	Boc-Thr(Fmoc-Ala)-OH	p. 103
852294	Boc-Thr(Fmoc-Arg(Pbf))-OH	p. 103
852297	Boc-Ser(Fmoc-Asp(OtBu))-OH	p. 103
852296	Boc-Ser(Fmoc-Glu(OtBu))-OH	p. 103
852171	Boc-Thr(Fmoc-Gly)-OH	p. 103
852252	Boc-Thr(Fmoc-Ile)-OH	p. 103
852292	Boc-Thr(Fmoc-Met)-OH	p. 104
852299	Boc-Thr(Fmoc-Thr(tBu))-OH	p. 104
852253	Boc-Thr(Fmoc-Val)-OH	p. 104

References

1. S.B. Kent, et al. in "Innovations and Perspectives in Solid Phase Synthesis", R. Epton (Ed.), Intercept Ltd., Andover, 1992, pp. 1.
2. E. Oliveira, et al. in "Peptides, Chemisty, Structure & Biology: Proc. 12th American Peptide Symposium" J. Smith & J. Rivier, (Eds.), ESCOM, Leiden, 1992, pp. 569.
3. J.M. Stewart & W.A. Klis in "Innovations and Perspectives in Solid Phase Synthesis", R. Epton (Ed.), SPCC (UK) Ltd., Birmingham, 1990, pp. 1.3.
4. L. Zhang, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 3rd International Symposium", R. Epton (Ed.), Mayflower Scientific Ltd., Birmingham, 1994, pp. 711.
5. E. Bayer (1991) *Angew. Chem. Int. Engl. Ed.,* **30**, 113.
6. M. Meldal (1992) *Tetrahedron Lett.,* **33**, 3077.
7. K.C. Pugh, et al. (1992) *Int. J. Peptide Protein Res.,* **40**, 208.
8. K. Barlos, et al. (1998) *J. Peptide Res.,* **51**, 194.
9. E. Atherton, et al. (1980) *J. Chem. Soc., Chem. Commun.,* 970.
10. M. Quibell & T. Johnson in "Fmoc solid phase peptide synthesis: a practical approach", Oxford University Press, Oxford, 2000, pp. 115.
11. a) C. Toniolo, et al. (1981) *Makromol. Chem.,* **182**, 1997; b) C. Toniolo, et al. (1981) *Makromol. Chem.,* **182**, 2007.
12. J. Bedford, et al. (1992) *Int. J. Peptide Protein Res.,* **40**, 300.
13. P. White, et al. in "Peptides 1998, Proc. of 25th European Peptide Symposium", Budapest, Akadémiai Kiadó, 1998, pp. 120.
14. C. Hyde, et al. (1994) *Int. J. Peptide Protein Res.,* **43**, 431.
15. R. von Eggelkraut-Gottanka, et al. (2003) *ChemBioChem,* **4**, 425.
16. F. Shabanpoor, et al. (2007) *J. Pept. Sci.,* **13**, 113.
17. a) P. White, et al. (2004) *J. Pept. Sci.,* **10**, 18; b) P. White, et al. (2003) *Biopolymers,* **71**, 338.
18. F. García-Martín, et al. (2006) *Bipolymers,* **84**, 566.
19. S. Abu-Baker & G. A. Lorigan (2006) *Biochemistry,* **45**, 13312.
20. F. Shabanpoor, et al. (2007) *J. Pept. Sci.,* **13**, 113.
21. V. Goncalves, et al. (2009) *J. Pept. Sci.,* **15**, 422.
22. F. El Oualid, et al. (2010) *Angew. Chem. Int. Ed.,* **49**, 10149.
23. S. N. Bavikar, et al. (2012) *Angew. Chem. Int. Ed.,* **51**, 758.
24. P. Nagorny, et al. (2012) Angew. Chem. Int. *Ed.,* **51**, 975.
25. C. Yves-Marie, et al. (2010) *J. Pept. Sci.,* **16**, 98.
26. A. Ehrlich, et al. (1996) *J. Org. Chem.,* **61**, 8831.
27. N. Schmiedeberg & H. Kessler (2002) *Org. Lett.,* **4**, 59.
28. a) T. Haack & M. Mutter (1992) *Tetrahedron Lett.,* **33**, 1589; b) M. Mutter, et al. (1995) *Pept. Res.,* **8**, 145.
29. S. Zahariev, et al. (2005) *J. Pept. Sci.,* **11**, 17.
30. T. Johnson (1995) *J. Pept. Sci.,* **1**, 11.
31. W. R. Sampson, et al. (1999) *J. Pept. Sci.,* **5**, 403.
32. P. Westermark, et al. (1987) *Proc. Natl. Acad. Sci. USA,* **84**, 3881.
33. G. J. S. Cooper, et al. (1987) *Proc. Natl. Acad. Sci. USA,* **84**, 8628.
34. A. Lorenzo, et al. (1994) *Nature,* **368**, 756.
35. A. Abedini & D. P. Raleigh (2005) *Org. Lett.,* **7**, 693.
36. D. Skropeta, et al. (2004) *J. Org. Chem.,* **69**, 8804.
37. T. Wöhr & M. Mutter (1996) *J. Am. Chem. Soc.,* **118**, 9218.
38. C. Heinlein, et al. (2011) *Angew. Chem. Int. Ed.,* **50**, 6406..
39. F. Tagliavini, et al. (1993) *Proc. Natl. Acad. Sci. USA,* **90**, 9678.
40. M. F. Jobling, et al. (1999) *Lett. Pept. Sci.,* **6**, 129.
41. V. Cardona, et al. (2008) *Int. J. Pept. Res. Ther.,* **14**, 285.
42. E. Oliveira, et al. (1997) *J. Pept. Res.,* **49**, 300.
43. T. Johnson, et al. (1993) *J. Chem. Soc., Chem. Commun.,* 369; b) T. Johnson & M. Quibell (1994) *Tetrahedron Lett.,* **35**, 463; c) L.C. Packman, et al. (1994) *Pept. Res.,* **7**, 125; d) R. Simmonds (1996) *Int. J. Peptide Protein Res.,* **47**, 36.
44. W. Zeng, et al. (1997) *J. Pept. Res.,* **49**, 273.
45. J. Bacardit, et al. Poster 190 presented at the 15th American Peptide Symposium, Nashville, 1995.
46. K. Jauch, et al. in "Peptides 1996: Proc. 24th European Peptide Symposium", R. Ramage and R. Epton (Eds), Mayflower Scientific Ltd., 1996, pp 497.
47. a) M. Quibell, et al. (1994) *Tetrahedron Lett.,* **35**, 2237; b) M. Quibell, et al. (1994) *J. Org. Chem.,* **59**, 1745.
48. M. Quibell, et al. (1994) *J. Chem. Soc., Chem. Commun.,* 2343; b) L.C. Packman (1995) *Tetrahedron Lett.,* **36**, 7523; c) J. Offer, et al. (1996) *J. Chem. Soc., Perkin Trans. 1,* 175.
49. a) M. Quibell, et al. (1995) *J. Am. Chem. Soc.,* **117**, 11656; b) M. Quibell, et al. (1996) *J. Chem. Soc., Perkin Trans. 1,* 1227.
50. A. Ehrlich, et al. (1996) *J. Org. Chem.,* **61**, 8831.
51. a) Y. Sohma, et al. (2006) *Tetrahedron Lett.,* **47**, 3013; b) T. Yoshiya, et al. (2007) *Org. Biomol. Chem.,* **5**, 1720.
52. I. Coin, et al. (2006) *J. Org. Chem.,* **71**, 6171.
53. Y. Sohma, et al. (2004) *Biopolymers,* **76**, 344.
54. M. Mutter, et al. (2004) *Angew. Chem. Int. Ed.,* **43**, 4172.
55. L. A. Carpino, et al. (2004) *Tetrahedron Lett.,* **45**, 7519.
56. Y. Sohma, et al. (2005) *J. Pept. Sci.,* **11**, 441.
57. Y. Sohma & Y. Kiso (2006) *ChemBioChem,* **7**, 1549.
58. Y. Sohma, et al. (2007) *Pept. Sci.,* **88**, 253.
59. S. Dos Santos, et al. (2005) *J. Am. Chem. Soc.,* **127**, 11888.
60. I. Coin, et al. (2008) *J. Pept. Sci.,* **14**, 299.
61. A. Taniguchi, et al. (2007) *J. Pept. Sci.,* **13**, 868.
62. I. Coin, et al. (2007) *Nature Protocols,* **2**, 3247.
63. T. Yoshiya, et al. (2009) *Org. Biomol. Chem.,* **7**, 2871.
64. T. Yoshiya, et al. (2006) *Tetrahedron Lett.,* **47**, 7905.
65. J. Lécaillon, et al. (2008) *Tetrahedron Lett.,* **49**, 4674.

3.6 Aspartimide formation

3.6.1 Fmoc–Asp(OtBu)–(Dmb)Gly–OH
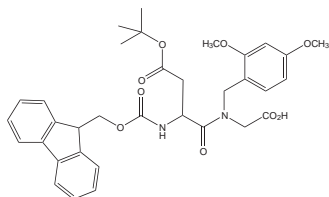
The most frequently encountered side reaction affecting Asp residues during solid phase synthesis is aspartimide formation, resulting from a ring-closure between the nitrogen of the α-carboxy amide bond and the β-carboxy side-chain, with loss of the ester protecting group (Figure 3-32) [1, 2]. It is a particularly serious problem in Fmoc SPPS as cyclization is promoted by strong bases such as piperidine and DBU used to effect Fmoc group removal [3-6].

Aspartimides are very susceptible to base-catalyzed epimerization [7] and readily undergo ring-opening reactions, leading to the formation of a variety of by-products. Attack by water yields predominantly the β-aspartyl peptide, the presence of which can often be overlooked as it has an identical mass to the desired α-isomer and frequently co-elutes on HPLC. Ring-opening by piperidine gives a mixture of α- and β-piperidides, which are characterized in MS as peaks at 67u greater than that of the expected peptide.
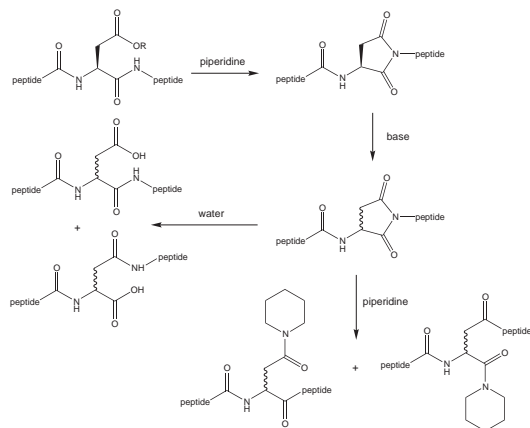
Fig. 3-32: Mechanism for aspartimide-related by-product formation.

The Asp-Gly sequence is particularly prone to aspartimide formation, which is estimated to occur to the extent of approximately 0.5% per Fmoc deprotection cycle [8]. The problem is, therefore, most serious in sequences containing more than one site of potential aspartimide formation and in long peptides, as the degree of aspartimide formation is dependent on the total exposure time to piperidine. It is also exacerbated by the use of DBU as this base has been shown to more effectively promote aspartimide formation than piperidine.

Of the various approaches that have been advocated to overcome this problem, such as the addition of dinitrophenol or HOBt to the piperidine solution [6], only masking of the Asp-Gly amide bond with Hmb offers complete protection [8, 9]. However, the acylation of Hmb derivatives can be problematic, and the reaction is difficult to follow and often requires nonstandard acylation conditions. The solution is to use Fmoc-Asp(OtBu)-(Dmb)Gly-OH [10]. Unlike the previously reported Fmoc-Asp(OtBu)-(Hmb)Gly-OH [11], whose coupling can be a little sluggish due

to lactone formation between the phenolic hydroxyl and carboxyl group, this derivative can be introduced using standard coupling methods, extending the peptide chain by two residues in one step. Its use has also the additional advantage of overcoming aggregation during chain extension.

The benefit of using Fmoc-Asp(OtBu)-(Dmb)Gly-OH is exemplified through the synthesis of the following peptide.

Application 3-4: Synthesis of H-Val-Lys-Asp-Gly-Tyr-Leu-NH₂

H-Val-Lys(Boc)-Asp(OtBu)-Gly-Tyr(tBu)-Leu-Rink Amide MBHA resin was prepared manually. All acylation reactions were carried out using a 2-fold excess of Fmoc-amino acid activated with 1 eq. of TBTU in the presence of 2 eq. of DIPEA. A coupling time of 30 min was used throughout. Fmoc removal was effected with 20% piperidine in NMP (2 x 15 min). The synthesis of H-Val-Lys(Boc)-Asp(OtBu)-(Dmb)Gly-Tyr(tBu)-Leu-Rink Amide MBHA resin was also carried out in an identical manner except Fmoc-Asp(OtBu)-(Dmb)Gly-OH was used to introduce the Asp-Gly dipeptide. The peptidyl resins were treated with TFA/ TIS /water 95:2.5:2.5 (5 ml) for 3 h, after which time the peptides were isolated in the usual manner by evaporation and ether precipitation.
The product obtained using standard conditions was characterized by LC/ESI-MS (Figure 3-33) to contain approximately 14% aspartimide, whereas the product from the synthesis using Fmoc-Asp(OtBu)-(Dmb)Gly-OH was essentially free of these impurities.
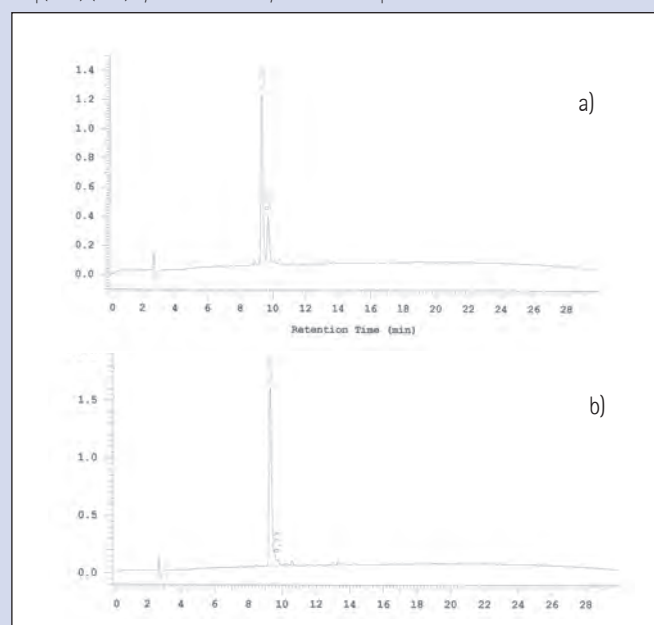
Fig. 3-33: a) HPLC profile of crude H-Val-Lys-Asp-Gly-Tyr-Leu-NH₂ prepared using standard amino acid building blocks; b) peptide prepared using Fmoc-Asp(OtBu)-(Dmb)Gly-OH [10].

Related products
852115   Fmoc-Asp(OtBu)-(Dmb)Gly-OH   p. 98

3.6.2 Fmoc–Asp(OMpe)–OH
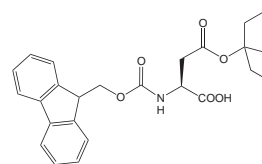
For other Asp-XXX, where XXX is not Gly, the use of Fmoc-Asp(OMpe)-OH instead of Fmoc-Asp(OtBu)-OH is strongly recommended [12]. By substituting OtBu for the more bulky OMpe group in the synthesis of a model Asp-Gly-containing peptide, aspartimide related by-products were reduced from 58% to 25% following treatment with 20% piperidine for

20h (equivalent to 120 coupling cycles) [12]. The benefits of using OMpe are even more pronounced when DBU is employed for Fmoc removal. In model studies, a peptide containing Asp(X)-Asp(OtBu) when treated with DBU/piperidine/DMF (1:20:79) for 225 min gave only 9% product when X=OtBu compared to 34% when X=OMpe (see Table 3-17) [13].

Table 3-17: Effects of base and Asp side-chain protection on aspartimide formation during synthesis of H-Val-Lys-Xaa-Yaa-Tyr-Ile-OH. All Fmoc deprotection reactions were conducted for 225 min [13].

Xaa	Yaa	Base	% Product	% Aspartimide by–products
Asp(OtBu)	Asp(OtBu)	piperidine	90	nd
Asp(OMpe)	Asp(OtBu)	piperidine	94	nd
Asp(OtBu)	Asp(OtBu)	DBU	9	44
Asp(OMpe)	Asp(OtBu)	DBU	34	25
Asp(OtBu)	Asn(Mtt)	piperidine	80	18
Asp(OMpe)	Asn(Mtt)	piperidine	92	6
Asp(OtBu)	Asn(Mtt)	DBU	1	81
Asp(OMpe)	Asn(Mtt)	DBU	26	62

3.6.3 Oxyma Pure
Subirós-Funosas, *et al.* [14] have recently demonstrated that the addition of 1 M Oxyma Pure to 20% piperidine in DMF reduced the levels of aspartimide-related impurities in a Asp(OtBu)-Gly-containing model peptide from 44% to 15%. Therefore, for less aspartimide prone sequences, the addition of Oxyma Pure to the Fmoc removal solution should prove a very useful strategy for ameloriating this side reaction.

3.6.4 Pseudoproline dipeptide
Recently, insertion of a pseudoproline dipeptide immediately before an extremely vulnerable Asp(OAll) residue has been shown to reduce aspartimide to acceptable levels [15, 16]. This strategy should prove extremely useful for the synthesis of *N*-glycopeptides *via* Landsbury aspartylation since Asn-Xxx-Ser/Thr is the census sequence for protein *N*-gycosylation.

References
1. G. Barany & R. B. Merrifield in "The peptides, Vol. 2", E. Gross & J. Meienhofer (Eds), Academic Press, New York, 1979, pp. 1-234.
2. M. Bodansky & J. Martinez (1978) in "The peptides, Vol. 5", E. Gross & J. Meienhofer (Eds), Academic Press, New York, 1983, pp. 111-216.
3. E. Nicolás, et al. (1989) *Tetrahedron Lett.*, **30**, 497.
4. R.Dölling, et al. (1994) *J. Chem. Soc., Chem. Commun.*, 853
5. Y. Yang, et al. (1994) *Tetrahedron Lett.*, **35**, 9689.
6. J. Lauer, et al. (1994) *Lett. Pept. Sci.*, **1**, 197.
7. I. Schön, et al. (1991) *J. Chem. Soc., Chem. Commun.*, 3213.
8. M. Quibell, et al. (1994) *J. Chem. Soc., Chem. Commun.*, 2343
9. L.C. Packman (1995) *Tetrahedron Lett.*, **36**, 7523.
10. V. Cardona, et al. (2008) *Int. J. Pept. Res. Ther.*, **14**, 285.
11. M. Mergler, et al. (2003) *J. Pept. Sci.*, **9**, 36.
12. A. Karlström & A. Undén (1996) *Tetrahedron Lett.*, **37**, 4243.
13. M. Mergler, et al. (2003) *J. Pept. Sci.*, **9**, 518.
14. R. Subirós-Funosa, et al. (2011) *Biopolymers*, **98**, 89.
15. P. Wang, et al. (2012) *Angew. Chem. Int Ed.*, **51**, 11571.
16. V. Ullmann (2012) *Angew. Chem. Int. Ed.*, **51**, 11566.

3.7 Boc resin cleavage and deprotection

The most popular reagent for cleavage of peptides from Boc-based resins is anhydrous HF. Of all the cleavage procedures HF appears to be the most versatile and least harmful to a wide variety of peptides synthesized on Boc-based resins. The major drawback of this procedure remains its highly toxic and reactive nature which necessitates the use of expensive HF-resistant fume hoods and cleavage apparatus.

Other strong acids such as TFMSA and trimethylsilyl trifluoromethanesulfonate (TMSOTf) can be used as alternatives to HF for cleavage from MBHA resins. Although less reactive than HF, it should be noted, however, that both TFMSA and TMSOTf are extremely corrosive and great care must be taken when using either.

In addition to acid cleavage, several resin types such as oxime can be cleaved using a variety of different methods to yield peptide hydrazides and analogs of protected fragments. For the purposes of this discussion we will concentrate on the most common acid cleavage methods currently in use. Please refer to the literature cited with each resin for details on cleavage and deprotection specific for that resin type.

Table 3-18: Boc-compatible amino acid derivatives.

HF compatible

Arg(Mts)	Arg(NO₂)	Arg(Tos)	Asp(OBzl)	
Asp(OcHx)	Cys(Acm)[1]	Cys(pMeBzl)	Cys(pMeOBzl)	Glu(OBzl)
Glu(OcHx)	His(Bom)	His(Dnp)		
His(Tos)	His(Z)	Lys(2-Cl-Z)	Ser(Bzl)	Thr(Bzl)
Trp(For)[2]	Tyr(2-Br-Z)			

TMSOTf compatible

Asp(OcHp)	Asp(OBzl)	Arg(Mts)	Cys(Acm)[1]	Glu(OBzl)
Glu(OcHp)	His(Bom)	Lys(2-Cl-Z)	Met(O)[3]	Ser(Bzl)
Thr(Bzl)	Tyr(2-Br-Z)			
Tyr(Bzl)	Tyr(di-Cl-Bzl)	Trp(For)	Trp(Mts)	

TFMSA compatible

Arg(Mts)	Asp(OBzl)	Cys(Acm)1	Cys(pMeOBzl)	Glu(OBzl)
His(Bom)	His(Dnp)	His(Tos)	His(Z)	Lys(2-Cl-Z)
Cl-Z)	Met(O)	Ser(Bzl)	Thr(Bzl)	Trp(For)[2]
Tyr(2-Br-Z)				

HBr compatible

Arg(Mts)	Asp(OBzl)	Cys(Acm)[1]	Cys(pMeBzl)	Glu(OBzl)
His(Bom)	His(Dnp)	His(Tos)		
His(Z)	Lys(2-Cl-Z)	Met(O)	Ser(Bzl)	
Thr(Bzl)	Trp(For)[2]	Tyr(2-Br-Z)		

1. Cys(Acm) is cleaved with cyclization by iodine or without cyclization by mercury (II) acetate.
2. Trp(For) should be deprotected using the low-high HF method. Alternatively, it should be treated with 10% piperidine in DMF (Method 3-19) before treatment with strong acid.
3. Met(O) is not quantitatively reduced by TMSOTf; Low-High HF or TFMSA are preferred.

3.7.1 Preparing the resin for cleavage

Proper preparation of the peptidyl resin prior to cleavage is important in preventing side reactions and incomplete cleavage and deprotection of the peptide. The choice of cleavage and deprotection method is dependent not only on the resin used, but also on the sequence and choice of amino acid side-chain protection. Before beginning your synthesis, please ensure that the resin and side-chain protection used is compatible with the cleavage method. All resins should be properly washed and dried before cleavage.

Removal of the Dnp protecting group of His

If your peptide contains His(Dnp), the histidine must be deprotected before removal of the N-terminal Boc-group [1]. For specific details on the removal of the Dnp group of histidine and alternative histidine derivatives, please see Method 3-18 below.

> #### Method 3-18: Removal of Dnp protecting groups
>
> 1. Swell the resin in the minimum volume of DMF.
> 2. Treat with a 20-fold molar excess of thiophenol for 1-2 h at rt (this reaction can be left o/n).
> 3. Transfer the resin to a sintered glass funnel and wash sequentially with DMF, methanol and diethyl ether before treating with liquid HF or TFMSA.

Removal of the N–Terminal Boc–group

Before HF cleavage can be performed the N-terminal Boc-group must be removed using TFA. This not only prevents possible *t*-butylation of susceptible residues during HF cleavage, but also removes any Boc-amino acid held on the resin by ion exchange [2]. Removal of the N-terminal Boc-group is not required for TFMSA cleavage. Check with the instruction manual of your synthesizer; many synthesizers automatically program the removal of the N-terminal Boc-group as a last step in the synthesis.

Manual removal of the N-terminal Boc-group can be accomplished by placing the resin in a round bottom flask and washing with 50% (v/v) TFA/DCM for 15 minutes at room temperature with constant mixing.

After removal of the N-terminal Boc-group the peptide resin can then be transferred to a sintered glass funnel and washed twice (5 volumes) with DCM, twice with MeOH and a final couple of washes with DCM. Avoid excessive sucking of air as moisture in the air will absorb onto the cold resin and may be difficult to remove. Moisture can be very harmful in HF cleavage [2].

After washing, the peptide resin should be dried under high vacuum for 4 hours, or preferably overnight over KOH or P_2O_5.

If you are using TFMSA cleavage without removal of the N-terminal Boc-group use the same washing and drying steps as above.

Deformylation of Trp(For)–containing peptide resins

The formyl group is stable to acid cleavage reagents and can be removed prior to cleavage using standard HF protocols (Method 3-19). Although alkylation of the unprotected indole ring of tryptophan by *t*-butyl carbonium ions is a problem during cleavage with HF, scavengers such as indole [2] have been used to successfully protect Trp-containing peptides from alkylation. However, indole can also undergo acid catalyzed dimerization with tryptophan and irreversibly modify the indole ring [3].

> #### Method 3-19: Piperidine deformylation of Trp(For)-containing peptide resins
>
> 1. Place piperidine: DMF 1:10 (v/v) in a round bottom flask and cool with an ice bath to 0°C.
> 2. Add the peptide resin (1 g per 10 ml) and stir for 2 h maintaining the temperature at 0°C.
> 3. Filter the resin twice with DMF (5 volumes), twice with DCM and twice with MeOH.
> 4. Dry under high vacuum as above for at least 4 h prior to cleavage using HF.

Alternatively, the formyl-group may be removed by thiolytic cleavage using the "low-high" protocol (HF [4] or TFMSA [5]) with DMS, *p*-thiocresol or thiophenol. Under this protocol the low cleavage conditions remove most of the benzyl groups prior to the thiolytic cleavage of the Trp(For) under the high conditions. Removal of the *t*-butyl carbonium ions during the low cleavage greatly minimizes alkylation of the indole ring of tryptophan during the high procedure [4].

Another option, although possibly less desirable, involves the removal of the formyl group after high HF cleavage using aqueous base such as 0.03 M hydroxylamine at pH 9.0 for two hours [6].

3.7.2 HF cleavage

CAUTION: *Anhydrous HF is an extremely toxic, corrosive and volatile (b.p.19°C) liquid. All procedures requiring HF must be performed in HF-resistant apparatus in a fume hood equipped with a scrubber. DO NOT USE GLASS APPARATUS as HF will dissolve glass in an extremely rapid and exothermic reaction.*

Proper eye protection, face shield, rubber apron and rubber gloves are mandatory when using HF. Do not take chances. Follow local, state/ provincial and federal safety and environmental codes and regulations. Breathing HF can cause death.

For an excellent description of HF apparatuses and their use, please refer to Solid Phase Peptide Synthesis by Stewart & Young [2]. Always follow the information provided by the manufacturer of the HF cleavage apparatus you are using.

Standard HF cleavage

HF cleavage is generally performed at temperatures of 0-5°C for a period 30-60 minutes. Peptides containing Arg(Tos) may require longer cleavage times. Problems may be encountered if the peptide contains one or a combination of the problem residues such as Trp, Met, Asp, Glu, Cys and Tyr. Several strategies for dealing with these potential problems can be found in Synthesis Note, section 4.1, page 4.1.

Both time and temperature play a key role in minimizing possible side reactions during cleavage and deprotection. Peptide resins containing Asp/Glu (OBzl) or Asp/Glu (OcHx) should be cleaved at 5°C or lower to reduce aspartimide formation [7] and anisylation of the Glu respectively [8]. Lowering the temperature affects the rate of removal of the side-chain protecting groups. Cleavage of peptide resins containing Arg(Tos), Cys(pMeBzl) and Lys(2-Cl-Z) at temperatures lower than 5°C can be very slow and impractical. Peptide resins with Arg(Tos) could require cleavage times up to 2 hours at 5°C. When His(Dnp) and/or Trp(For) is used, pre-cleavage treatment is required.

Scavengers play a key role in reducing the possibility of side reactions. Anisole remains one of the most widely used scavengers for HF cleavage and prevents alkylation of tryptophan by t-butyl and benzyl cations. In

combination with DMS and p-thiocresol, anisole will prevent alkylation of Met and Cys [9]. Avoid the use of thioanisole if your peptide contains tryptophan. Thioanisole cation adducts can alkylate the nitrogen of the indole ring of Trp.

Method 3-20: Standard HF cleavage

0.2 mmole scale

1. Place the peptide resin, a Teflon-coated stirring bar and the scavenger mixture into the reaction vessel. For peptides containing Cys most researchers use HF/anisole/DMS/p-thiocresol (10:1:1:0.2). For other peptides not containing Cys use HF/DMS/anisole (10:1:1).
2. Screw the cap on the reaction vessel and cool in a dry ice methanol bath for at least 5 min before proceeding with the cleavage.
3. Distil 10 ml of HF into the flask following the manufacturer's instruction (maintaining the temperature between -5°C and 0°C). For peptides containing Arg(Tos) the reaction will require up to 2 h.
4. At the end of the reaction time, evaporate the HF and DMS under a stream of N_2.
5. Extract the resin with TFA to remove the peptide from the resin matrix.
6. Remove the resin by filtration under reduced pressure. Wash the resin twice with clean TFA. Combine filtrates, and add (drop-wise) an 8-10 fold volume of cold ether. Sometimes it is necessary to evaporate most of the TFA to achieve a good precipitation of the crude peptide. The ether can be cooled with ice to further assist precipitation.
7. Isolate the peptide as described in Method 3-37.

3.7.3 Low–high HF procedure

The low-high procedure of Tam, *et. al.* [4] uses low concentrations of HF in a large amount of scavenger such as DMS (1:3 by volume). Under low conditions the cleavage mechanism changes from the usual S_N1 (where carbonium and nitronium ions are produced) to S_N2. This procedure prevents alkylation of tyrosine by benzyl and *t*-butyl cations, formation of succinimide peptides from Asp-Gly sequences and acylation of scavenger molecules by glutamyl side chains. At low concentrations of HF and in the presence of DMS, Met(O) will be reduced to Met.

If the low procedure is followed by a standard HF cleavage, Arg(Tos) and Arg(NO$_2$) will be deprotected. The low procedure alone may not be sufficient to cleave peptides from the BHA resins. The major drawbacks of this procedure are the additional time required for cleavage, the use of large quantities of DMS and the formation of thiol scavenger adducts which have very strong and offensive odors.

Merrifield resins

For Merrifield-based resins, Tam, *et al.* [4] recommends cleavage using HF/DMS/p-cresol, 25:65:10, v/v at 0°C for 2 hours. If the peptide contains Arg(Tos) or other functionalities stable to low HF they recommend first cleaving with HF/DMS/p-cresol 25:65:10, v/v for 2 hours at 0°C, followed by the high procedure, which involves evaporating all the HF and DMS *in vacuo* at 0°C and then recharging the vessel with anhydrous HF and cleaving for 30-60 minutes at 0°C to 10°C depending on the sequence. If Trp(For) is present in the peptide, the formyl group can be removed under low HF by replacing the *p*-cresol with *p*-thiocresol or thiophenol [4].

MBHA resins

For peptides synthesized on MBHA resins Tam, *et al.* [4] recommends treatment under the low conditions for 1-2 hours to deprotect the side chains. After deprotection, evaporate the HF and DMS and wash the resin with DCM or EtOAc to remove sulfonium salts, dimethylsulfoxide and thiol derivatives and suction dry. The peptide can then be cleaved using HF/*p*-cresol 9:1(v/v) for 30-60 minutes at 0°C which will remove all remaining protecting groups and cleave the peptide from the resin.

3.7.4 TFMSA cleavage

CAUTION:*TFMSA is an extremely strong acid; proper eye protection, face shield, rubber apron and rubber gloves are mandatory. Follow local, state/provincial and federal safety and environmental codes and regulations. Always use in an efficient fume hood. Vapor is harmful if inhaled.*

TFMSA is an alternative to HF cleavage. The main advantage of this procedure is that it can be performed using standard laboratory glassware. Unlike HF, TFMSA is not volatile and is therefore difficult to remove by evaporation [2]. The peptide must be precipitated from solution using a dry solvent such as ethyl ether.

TFMSA-cleaved peptides are susceptible to salt and scavenger association. The precipitated peptides [9] should be neutralized and the salts removed either by ion exchange or by using Sephadex columns before further purification.

Similar to HF, TFMSA can be used either with a standard protocol or a "low-high" protocol depending on the sequence. Please note that this reagent will not deprotect Arg(NO$_2$) or Arg(Tos) groups, if present [10]. His(Dnp) requires pre-cleavage treatment.

Standard TFMSA cleavage

MBHA resins will require cleavage times between 90 minutes and two hours at room temperature (Method 3-21).

Asp(OcHx) and Cys(MeBzl) groups are not efficiently removed by this procedure. Peptide resins containing Trp(For) should be cleaved using the low-high procedure with EDT (Method 3-22).

Method 3-21: Standard TFMSA cleavage

250 mg scale

1. Place 250 mg dried resin in a round bottom flask with a stirring bar. Add 750 µl of thioanisole/EDT (2:1).
2. Chill the round bottom flask in an ice bath and add 5 ml of TFA and stir for 5-10 min.
3. Add (slowly) 500 µl of TFMSA drop-wise with vigorous stirring to dissipate the heat generated.
4. Allow the reaction to continue at rt for the desired length of time.
5. Remove the resin by filtration under reduced pressure. Wash the resin twice with clean TFA. Combine filtrates, and add (drop-wise) an 8-10 fold volume of cold ether.
6. Isolate the peptide as described in Method 3-37.

TFMSA low–high cleavage

The low-high procedure initially uses low concentration of TFMSA in a large volume of the scavenger DMS. Under low conditions the cleavage mechanism changes from S_N1 (where carbonium and nitronium ions are produced) to S_N2. If your peptides contain Met, use the Met(O) derivative for your synthesis. Under low conditions in the presence of DMS the Met(O) will be reduced to Met [11]. The addition of EDT to the cleavage cocktail under the high conditions will deprotect Trp(For) *via* thiolytic cleavage [5]. EDT will also minimize the formation of dimers via disulfide bridges when the peptide contains Cys residues.

In the low phase of the low-high cleavage protocol it is important to maintain low temperatures of 0°C to 5°C during the entire reaction.

Method 3-22: Low-high TFMSA cleavage [9]

Low cleavage

1. Place the peptide resin (250 mg) in a round bottom flask with a stirring bar and cool in an ice bath to between 5°C and 0°C.
2. Add 250 µl of m-cresol, 750 µl DMS, and 1.25 ml of TFA, add 250 µl of TFMSA drop-wise to the reaction mixture with stirring to dissipate any heat produced.
3. For peptides containing Trp(For) add 50 µl of EDT to the cleavage cocktail.
4. Allow the mixture to react for 3 h, keeping the temperature between 0°C and 5°C.
5. Transfer the contents of the flask to a medium sintered glass funnel and wash the resin with several volumes of Et₂O and suction dry.
6. Dry the resin under high vacuum for a minimum of 4 h over P₂O₅ or KOH.

High cleavage

1. Place the dried resin from above in a round bottom flask with a stirring bar and add 250 µl of thioanisole and 75 µl of EDT and stir for 5-10 min.
2. Cool to 5°C to 0°C using an ice bath and add 2.5 ml of TFA. Mix 5-10 min followed by addition of 250 µl of TFMSA drop-wise with mixing to dissipate heat.
3. Remove the flask from the ice bath and allow to react at rt for 30 min with PAM resins. MBHA resins require 90-120 min for complete cleavage.
4. Remove the resin by filtration under reduced pressure. Wash the resin twice with TFA. Combine filtrates and add (drop-wise) an 8-10 fold volume of cold ether.
5. Isolate the peptide as described in Method 3-37.

3.7.5 TMSOTf Cleavage

CAUTION: *TMSOTf is an extremely corrosive and flammable liquid. Proper eye protection, face shield, rubber apron, and rubber gloves are mandatory. Follow local, state/provincial and federal safety and environmental codes and regulations. Always use an efficient fume hood. The vapor is harmful if inhaled.*

TMSOTf represents an alternative to HF and TFMSA cleavage. Yajima, *et al.* [12] reported that TMSOTf cleavage produces fewer side reactions and yields a product which is less hygroscopic than peptide products from TFMSA cleavage. This procedure cleaves most of the side-chain protecting groups found in Boc synthesis. Peptides containing Cys(Bzl), Cys(Acm) and Arg(NO₂) should not be deprotected using TMSOTf since these side-chain protecting groups are stable to TMSOTf.

Peptides containing Arg(Tos) will require longer cleavage times. Unlike TFMSA, the Met(O) will not be quantitatively reduced to Met and therefore an alternative method for the reduction of Met(O) to Met is necessary [13], section 3.7.6, page 3.34 Peptides containing Trp(For) require the presence of EDT in the cleavage cocktail in order to fully remove the formyl group.

Method 3-23: TMSOTf cleavage

1. Place 1 g of dried resin in a round bottom flask containing a stirring bar.
2. Cool the flask to 0°C using an ice bath and add the cooled cleavage mixture to the flask (1.95 ml TMSOTf, 6.90 ml of TFA, 1.2 ml m-cresol). Maintain the temperature at 0°C for 2h with constant mixing to ensure complete deprotection.
3. Remove the resin by filtration under reduced pressure. Wash the resin twice with clean TFA. Combine filtrates, and add (drop-wise) an 8-10 fold volume of cold ether.
4. Isolate the peptide as described in Method 3-32.

A two-step hard acid deprotection/cleavage procedure for solid phase peptide synthesis involving TMSBr/TFA followed by TMSOTf/TFA has been reported by Nomizu, *et al.* [14]. Its usefulness is demonstrated by comparison with other deprotection methods in the synthesis of two different test peptides. Readers are encouraged to refer to this paper for further details.

3.7.6 Hydrogen bromide cleavage

CAUTION: *HBr in acids such as TFA, pivalic acid, isobutyric acid, isovaleric acid and acetic acid are extremely strong acids. Proper eye protection, face shield, rubber apron, and rubber gloves are mandatory. Follow local, state/provincial and federal safety and environmental codes and regulations. Always use an efficient fume hood. The vapor is harmful if inhaled.*

This procedure [15] is used for cleaving peptides from MBHA-resin. The advantages of this method are its use of commercially available 30% HBr in acetic acid (about 5 M), the ability to use standard laboratory glassware and the potential for easy scale up. The cleavage mixture should contain pentamethylbenzene which accelerates the cleavage and the acidolytic removal of the protecting groups. Experiments also demonstrated that Met(O) is quantitatively reduced to Met during the HBr procedure but dimethylsulfide has to be used instead of thioanisole to prevent formation of S-methylmethionyl peptides.

Standard HBr/acetic acid cleavage

The reaction time for MBHA-resin is typically between 60 and 90 minutes at room temperature. Boc and formyl protecting groups should be removed prior to the cleavage procedure. The procedure is compatible with Asp(OBzl), Glu(OBzl) and Lys(ClZ) protection (Method 3-24). Please note that the peptide resin should be washed and well dried before cleavage.

Method 3-24: HBr cleavage

250 mg scale

1. Place 250 mg dried resin in a round bottom flask with a magnetic stirring bar and mix with 500 mg pentamethylbenzene, 600 µl thioanisole and 10 ml TFA.
2. To this suspension add 400 µl 30% HBr/acetic acid (5 M) and stir for 60-90 min.
3. Remove the resin by filtration and evaporate the filtrate under reduced pressure at 30°C after completion of the reaction.
4. Triturate the peptide with 50 ml dry ether and isolate as described in Method 3-32.

References

1. J.M. Stewart, et al. in "Progress in Peptide Research", S. Lande (Ed.), 1972, pp. 52.
2. J.M. Stewart & J.D. Young, "Solid Phase Peptide Synthesis", Pierce Chemical Company, Rockford, 1984.
3. Y. Omon, et al. (1976) *Chem. Lett.*, **1976**, 85.
4. J.P. Tam, et al. (1983) *J. Am. Chem. Soc.*, **105**, 6442.
5. N. Fujii, et al. (1987) *Chem. Pharm. Bull.*, **35**, 3447.
6. R.B. Merrifield, et al. (1982) *Biochemistry*, **21**, 5020.
7. J.P. Tam, et al. (1988) *Pept. Res.*, **1**, 6.
8. S. Sano & S. Kawanishi (1975) *J. Am. Chem. Soc.*, **97**, 3480.
9. Introduction to Cleavage Techniques, Applied Biosystems, Inc., Foster City, 1990.
10. H. Yajima, et al. (1974) *J. Chem. Soc., Chem. Commun.*, 108.
11. J.P. Tam, et al. (1982) *Tetrahedron Lett.*, **23**, 2939.
12. H. Yajima, et al. (1988) *Tetrahedron*, **44**, 805.
13. R.A. Houghten & C.H. Li (1979) *Anal. Biochem.*, **98**, 36.
14. M. Nomizu, et al. (1991) *Int. J. Peptide Protein Res.*, **37**, 145.
15. S.S. Wang, et al. (1992) *Int. J. Peptide Protein Res.*, **40**, 344.

3.8 Fmoc resin cleavage and deprotection

Having successfully synthesized a protected peptide, one is confronted with a difficult task of having to simultaneously detach the peptide from the resin support and remove all the side-chain protecting groups of the amino acid residues to yield the desired peptide. In Fmoc SPPS, this step is normally carried out by treating the peptidyl resin with TFA. During this process, highly reactive cationic species are generated from the protecting groups [1] and the handles on the resin [2], and these can, unless trapped, react with, and hence modify, those residues which contain nucleophilic functional groups: Trp, Met, Tyr and Cys. To prevent this, various nucleophilic reagents (known as scavengers) are added to the TFA to quench these ions.

A number of universal cleavage mixtures have been advocated, the most popular of which is Reagent K (TFA/water/phenol/thioanisole/ EDT [82.5:5:5:5:2.5]). However, with recent advances in protecting group and linker technology, particularly the introduction of Fmoc-Trp(Boc) and Fmoc-Arg(Pmc/Pbf) derivatives, such complex mixtures containing toxic and malodorous reagents are no longer necessary, except in exceptional circumstances.

Most problems can be ameliorated by the appropriate choice of protected amino acid derivative and resin (Table 3-19). If these recommendations are followed, the use of TFA/TIS/water (95:2.5:2.5) will suffice for most sequences. There are, of course, sequences, especially those which contain cysteine and numerous *t*-butyl protected residues, for which this mixture does not give satisfactory results; in these cases, the addition of EDT to the mixture or the use of reagent K is recommended. Nevertheless, as a general, non-malodorous cleavage cocktail, this mixture has proved remarkably effective.

For those who do not wish to use the recommendations given in Table 3-19, the flow-chart shown in Figure 3-33 will aid in the selection of the most appropriate mixture.

3.8.1 Removal of N–terminal Fmoc group
Before acid cleavage of the peptidyl resin can be performed, the N-terminal Fmoc group must be removed using piperidine. Check with the instruction manual of your synthesizer; many synthesizers will automatically program the removal of the N-terminal Fmoc-group as a last step in the synthesis.

3.8.2 Preparing peptide resin for cleavage
The peptide resin should be thoroughly washed, especially when DMF is used during synthesis as it is nonvolatile and residual basic DMF can have a marked inhibitory effect on TFA-acidolysis. For PEG and polyacrylamide-based supports, washing with a mildly acidic reagent, such as acetic acid which does not cause release of the peptide, is desirable since these types of resin have a tendency to hold onto DMF [3]. Thorough washing and drying must be effected before cleavage (Method 3-25).

Note: Acetic acid should not be used for washing of extremely acid-labile Rink acid, TGT or 2-chlorotrityl resins.

Method 3-25: Preparing peptide resin for cleavage
1. Place the peptide resin in a sintered glass funnel and apply some suction.
2. Wash with DMF, acetic acid, then with DCM several times. Wash further with MeOH (polystyrene) or ether (polyacrylamide) to shrink the resin.
3. Remove the peptide resin and dry under high vacuum for 4 h, or preferably o/n, over KOH.

3.8.3 TFA cleavage and deprotection
Optimum cleavage conditions are very much dependent on the individual amino acid residues present, their number and sequence, the side-chain protecting groups, and the type of linker attached to the resin.

Due to the variability in the behaviour of different peptidyl resins, it is recommended that a preliminary small scale cleavage of peptide resin using 20-50 mg sample is carried out to determine the optimum cleavage conditions, such as the choice of scavenger(s) and length of reaction.

Table 3-19: Recommended protecting group and resin strategy.

Fmoc amino acid derivatives

Arg(Pmc)/Arg(Pbf)	Asp(OtBu)/(OMpe)	Asn(Trt)	Cys(Acm)	Cys(Mmt)
Cys(tBu)	Cys(Trt)	Glu(OtBu)	Gln(Trt)	His(Clt)
His(Trt)	Lys(Boc)	Lys(ivDde)	Lys(Mmt)	Ser(tBu)
Ser(Trt)	Thr(tBu)	Thr(Trt)	Tyr(tBu)	Tyr(2-
ClTrt)	Trp(Boc)			

Resins

2-Chlorotrityl chloride resin for C-terminal Cys, Met, Trp, Tyr

This will enable the extent of cleavage (e.g. by quantitative analysis of the reference amino acid attached to the linker, where appropriate) and the quality of the crude cleaved peptide (by HPLC and amino acid analysis) to be determined. For the majority of peptides, provided the recommendations given in Table 3-19 are followed, cleavage can be effected with TFA/TIS/water (95:2.5:2.5). In cases where problems do occur, the use of Reagent K, or the addition of EDT to the above mixture, will generally provide a satisfactory solution.

In the case of the Rink Amide resin, the phenyl benzyl ether bond, which links the handle to the resin, is acid sensitive and can be broken, especially when product release is sluggish during the cleavage reaction, resulting in colored by-products which are not easily removed from the product by simple washes. This can be avoided using the 2-step procedure outlined in Method 3-27, or better by using silane scavengers. These steps are not necessary with resins incorporating the more stable modified Rink linker, such as the Rink Amide AM, Rink Amide MBHA resin, and NovaSyn® TGR resins.

Methionine, cysteine and tryptophan are extremely susceptible to alkylation by cations produced during the cleavage process. Reaction of tryptophan, methionine or cysteine with *t*-butyl cations results in modification of the product peptide; reaction with the linker cation gives irreversible reattachment of the peptide to the resin [3]. With methionine, further reaction can occur giving rise to homoserine and fragmentation of the peptide chain. By adding scavengers to the cleavage mixture, these side reactions can be largely suppressed. One exception is sulfonation of tryptophan by the products formed on cleavage of Mtr, Pmc and Pbf protected arginine residues [4]. Fortunately, this side reaction can be eliminated by using Fmoc-Trp(Boc) [5-8]. This derivative also suppresses reattachment of *C*-terminal Trp residues to the cation generated at the resin linker. Sulfonyl-based protecting groups have also been shown to be associated with the formation of *N*-sulfonated Arg [9] and *O*-sulfonated Ser and Thr [10].

The most commonly used scavenger is EDT. Not only is it an extremely good scavenger for *t*-butyl cations, but it assists in the removal of the trityl protecting group from cysteine and is particularly effective in preventing acid catalyzed oxidation of tryptophan residues.

Suppression of acid-catalyzed Met oxidation can be effected by including ethylmethyl sulfide (EMS), EDT or thioanisole into the scavenger mixture; although methionine sulfoxide formation can be minimized by carrying out the cleavage reaction under nitrogen, ensuring only peroxide-free ether is used for product precipitation, and that all solvents are thoroughly degassed before use. Thioanisole is also known to accelerate Arg(Mtr/Pmc/Pbf) removal in TFA; however, it is advisable to exercise care when using this reagent as there is evidence to suggest that it can cause partial removal of Acm, tButhio or tBu protecting groups from Cys residues [11].

Fig. 3-33: Flow-chart for selecting cleavage cocktail for Fmoc SPPS.

Phenol is thought to offer some protection to Tyr and Trp residues [1]. Trialkylsilanes, such as TIS and TES, have been shown to be effective, non-odorous substitutes for EDT [12], particularly for peptides containing Arg(Pmc) and Trp(Boc) [5, 8]. These reagents are also very efficient at quenching highly stabilized cations liberated on cleavage of Trt [12], Tmob [13] and the Rink Amide linker, and therefore their use is strongly recommended when these moieties are present.

When several of the less acid-labile protecting groups are present in a peptide or the peptide is long and therefore contains numerous protecting groups, cleavage time usually needs to be extended significantly. The Mtr group is less acid-labile than Pmc or Pbf groups, and its complete removal can take as long as 24 hours. In such cases where Trp is present with several Mtr protecting groups, it is extremely useful to be able to optimize the cleavage conditions by monitoring removal of this protecting group by HPLC. A compromise needs to be

made between partially tryptophan-modified peptide and incomplete deprotection of Arg(Mtr). Therefore, with peptides containing Trp, the use of Trp(Boc)-derivatives is strongly recommended to avoid modification of the tryptophan side chain.

With long peptides, it can be necessary to use an extended cleavage time to completely remove all side-chain protection. If complete deportection is not achieved in 6 hours, the peptide should be precipitated with ether, and the cleavage repeated with fresh reagents. Test cleavages should be performed to find the optimum cleavage regime. Incomplete side-chain deprotection is often overlooked as the cause for failure in the synthesis of long peptides.

Problems have been observed with sluggish deprotection of *N*-terminal Asn(Trt) residues. These can easily be overcome by extending the cleavage time to 4 hours or using Asn(Dmcp) in place of Asn(Trt)..

Method 3-26: General TFA cleavage

CAUTION: TFA is an extremely corrosive liquid; great care must be taken when using this reagent. Proper eye protection, lab coat, and gloves are mandatory. Follow local, state/provincial and federal safety regulations. Use in an efficient fume hood.

1. Place dry resin in a flask and add TFA solution containing appropriate scavengers (10-25 ml/g resin, Figure 3-33). NOTE: a calculation should be made to ensure sufficient scavenger is present for the quality of peptide and protecting groups present. Stopper the flask and leave to stand at rt with occasional swirling. Reaction time depends upon the sequence (see "Monitoring the cleavage reaction", below).
2. Remove the resin by filtration under reduced pressure. Wash the resin twice with TFA. Combine filtrates, and add (drop-wise) an 8-10 fold volume of cold ether. Sometimes it is necessary to evaporate most of the TFA to achieve a good precipitation of the crude peptide. The ether can be cooled with ice to further assist precipitation.
3. Isolate the peptide as described in Method 3-37.

Method 3-27: Two-stage procedure for detachment/deprotection of Rink amide resin

1. Slurry the resin in 10% TFA in DCM and pour into a glass funnel with a fine sinter.
2. Allow the solvent to percolate slowly through the resin bed. Wash the resin with 5% TFA, allowing it to pass through the resin bed slowly. The detachment is an acid-catalyzed equilibrium, so it is important to continually remove the detached peptide by using this flow method. Carrying out the reaction in a flask will not achieve complete detachment. Yields can be improved by the addition of 1-5% TIS to the cleavage mixture.
3. Remove the excess TFA/DCM under reduced pressure and complete the deprotection with 95% TFA plus scavengers, according to the amino acid composition (see Figure 3-37).

Monitoring the cleavage reaction

The presence of Mtr protected arginine in a peptide necessitates protracted reaction times varying from 3 to 6 hours depending upon the choice of scavengers used (Figure 3-33). Multiple arginine residues can require an extension of reaction times up to 24 hours. In such cases it is extremely useful to be able to optimize the cleavage conditions by monitoring removal of this protecting group by HPLC.

3.8.4 General protocols involving strong acids

As an alternative to TFA, for rapid deprotection of less acid-labile side-chain protecting groups such as Arg(Mtr/Pmc/Pbf), Asn(Mbh) and Gln(Mbh), stronger acids can be used with appropriate scavengers with no report of side reactions.

HBF$_4$ in TFA

Several papers [14, 15, 16] have described the use of HBF$_4$ (1 M) in TFA as an alternative to TFA cleavage and deprotection. This reagent completely removes the tBu protecting-group from Ser, Thr, Tyr, Asp and Glu residues, Boc from Lysine, and the Mbh from Gln and Asn.

Both Cys(pMeOBzl) and Cys(tBu) residues are deprotected by this procedure. However, Cys(pMeBzl) deprotection is incomplete and Cys(Acm) remains intact even after the addition of thioanisole.

The cleavage of Wang and Rink Amide resins is complete within 30-60 minutes at 4°C, while MBHA resin requires more than 90 minutes of cleavage at 25°C.

The main advantage of this method over TFA alone is that cleavage with HBF$_4$ in TFA cleaves the Mtr group of arginine in under an hour, thus greatly reducing the risk of Trp alkylation. EDT should be used as a second scavenger when Trp residues are present.

Method 3-28: Cleavage and deprotection of peptide resins using HBF$_4$ in TFA

CAUTION: HBF$_4$ is an extremely strong acid; proper eye protection, face shield, rubber apron and rubber gloves are mandatory. Follow local, state/provincial and federal safety and environmental codes and regulations. Always use in an efficient fume hood. Vapor is harmful if inhaled.

1. Add 10 mmole of HBF$_4$-diethylether complex to 10 mmole thioanisole dissolved in TFA and adjust the total volume to 10 ml.
2. Treat the peptide resin (10–25 ml/g) using the above reagent in the presence of m-cresol and EDT at 0°C for 60 min. For MBHA resins cleave for 90 min at 25°C.
3. Remove the resin by filtration under reduced pressure. Wash the resin twice with clean TFA. Combine filtrates, and add (drop-wise) an 8-10 fold volume of cold ether.
4. Isolate the peptide as described in Method 3-37.

Resin cleavage using trimethylsilyl bromide [17]

The long cleavage times often found necessary for the complete deprotection of peptide containing multiple Arg(Mtr) residues can lead to serious degradation in product quality. In particular, prolonged exposure of tryptophan-containing peptides to EDT in TFA can lead to modification of tryptophan residues due to dithioketal formation. Cleavage with trimethylsilyl bromide (TMSBr) eliminates these problems since this reagent has been shown to cleanly deprotect up to 4 Arg(Mtr) residues in 15 minutes. Furthermore, this method has been found to completely suppress formation of sulfonation by-products, even when unprotected tryptophan is used [18].

Method 3-29: Cleavage with TMSBr

1. Add TMSBr (1.32 ml) to a solution of EDT (0.50 ml), m-cresol (0.1 ml) and thioanisole (1.17 ml) in TFA (7.5 ml) cooled to 0°C. Add the peptide resin (200 mg) and allow the mixture to stand for 15 min under a blanket of N$_2$ at 0°C.
2. Remove the resin by filtration under reduced pressure. Wash the resin twice with clean TFA. Combine filtrates, and add (drop-wise) an 8-10 fold volume of cold ether. Sometimes it is necessary to evaporate most of the TFA to achieve a good precipitation of the crude peptide. The ether can be cooled with ice to further assist precipitation.
3. Isolate the peptide as described in Method 3-37.
NOTE: Occasionally an additional treatment of the peptide with ammonium fluoride is required to reverse any silylation which may have occurred.

3.8.5 Cleavage from very acid–sensitive resins

The Rink Acid resin [19], 2-chlorotrityl [20], HMPB [21], NovaSyn TGT [22] and Sieber resins [23] contain highly acid-sensitive linkers and are suitable for the synthesis of protected peptides.

HMPB resins & Sieber amide resins

Fully protected peptide acids can be generated from the HMPB linker [21] and protected amides from the Sieber amide resin [23]. However, careful experimentation is essential if premature loss of side-chain protecting groups is to be avoided. Repetitive treatment of peptidyl resin with a solution of 1% solution of TFA in dichloromethane in tandem with minimum reaction times will give the best results.

Ideally, the cleavage should be carried out in a sealable sintered glass funnel to prevent evaporation of the highly volatile DCM, and the filtration should be carried out by applying nitrogen pressure rather than by the use of a vacuum.

If the peptide contains Met or Trp, 1% EDT should be added to the cleavage mixture to prevent reattachment of the peptide. If the peptide contains a C-terminal Trp residue, the use of Trp(Boc) is strongly recommended [24].

In this batch-wise procedure, the acid strength increases step-wise, as determined by the amount of TFA-buffering groups present. The maximal concentration of peptide may be contained in the first or in one of the later washes, depending on the buffering capacity of the amide bonds and other functional groups present. The side-chain protecting groups of the *t*-butyl type as well as Trt (on Asn and Gln) remain completely intact during this process [21].

Method 3-30: Cleavage with dilute TFA

1. Pre-swell the dry resin (1 g) with DCM in a sealable sintered glass funnel and remove excess DCM.
2. Add 1% TFA in dry DCM (10 ml), seal funnel and shake for 2 min. Filter solution by applying nitrogen pressure into a flask containing 10% pyridine in methanol (2 ml).
3. Repeat step 2 up to 10 times, wash the residual protected peptide from the resin with 3 x 30 ml DCM, 3 x 30 ml MeOH, 2 x 30 ml DCM, 3 x 30 ml MeOH, and check filtrates by TLC or HPLC.
4. Combine filtrates which contain product and evaporate under reduced pressure to 5% of the volume. Add water (40 ml) to the residue and cool mixture with ice to aid precipitation of the product.
5. Isolate product by filtration through a sintered glass funnel. Wash product three times with fresh water. Dry sample in a desiccator under vacuum over KOH, and later over P$_2$O$_5$.

2–Chlorotrityl, NovaSyn® TGT and NovaPEG Trt resins

2-Chlorotrityl [20], NovaSyn® TGT [22], and NovaPEG Trt resins can be cleaved with 1% TFA, as described above, or under milder conditions with AcOH or TFE [25] to produce protected peptides. When preparing protected peptides, the use of Fmoc-His(Clt)-OH is particularly recommended for introduction of histidine. This helps avoids partial side-chain deprotection of histidine which can occur when His(Trt) is used.

Method 3-31: Cleavage with TFE/DCM

1. Treat the peptidyl resin at rt with TFE/DCM (2:8) for 3 x1 h.
2. After the appropriate time, remove resin by filtration, wash 3 times with cleavage mixture.
3. Evaporate solution to dryness and precipitate protected peptide with ether.
The detached, fully protected peptide will be very hydrophobic and may require to be extracted further from the resin with DMF, DMSO. The completeness of the cleavage can be checked by TLC of the filtrates. Purify by low pressure chromatography on silica gel, HPLC on phenyl silica or by recrystallization.

3.8.6 Peptides attached by the HMBA and oxime linker

For the synthesis of peptide carboxamides, the HMBA linker has been largely superseded by those of the Rink Amide type. Nevertheless, this linker is still one of the most flexible of the peptide-resin linkers. Peptides

can be released from linker with a number of different nucleophiles to yield peptides with a variety of functional groups at the C-terminus [3, 27-30] (Table 3-20). These protocols are also compatible with the oxime linker used in Boc SPPS.

Table 3-20: Products from cleavage of HMBA linker.

Reagent	Product
NH_3/MeOH	Peptide carboxamide; Method 3-32
NH_2NH_2	Peptide hydrazide; Method 3-33
aq.NaOH	Peptide acid; Method 3-34
MeOH/DIPEA	Peptide methyl ester; Method 3-35
$NaBH_4$/EtOH	Peptide alcohol; Method 3-36

Before cleaving the peptide from HMBA derivatized resins, it is important that the side-chain protecting groups are removed, especially if the peptide contains Asp or Glu. This is achieved by treating the peptidyl resin with 95% aq. TFA. If the peptide contains Arg(Mtr), the Arg residue is best deprotected after cleavage of the peptide from the resin in an additional step by treatment with reagent K.

Method 3-32: Methanolic ammonia cleavage to give peptide amides [3, 26]

1. Place dry resin in a flask and add 95% aqueous TFA (20 ml/g). Stopper the flask and leave to stand at rt for 1 h with occasional swirling.
2. Isolate the resin by filtration under reduced pressure and wash with TFA. Discard the filtrate. Wash the resin with DCM, 10% DIPEA in DCM, DCM. Dry the resin under vacuum over P_2O_5 o/n.
3. Place the dried resin in a clean, dry pressure vessel and sufficient DMF to swell the resin, followed by a solution of dry methanol saturated with ammonia at 0°C (20 ml/g).
4. Seal the flask and let it warm to rt. Leave to stand for 18 h and then cool the flask to 0°C again. Carefully open the cooled vessel and filter the resin through a sintered glass funnel.
5. Wash the resin first with methanol, and then with TFA into a separate flask to remove any methanol insoluble peptide.
6. Evaporate the filtrates separately to dryness on a rotary evaporator. Precipitate peptide with ether and isolate by filtration.

NOTE: If the peptide resin is not thoroughly dried prior to this cleavage procedure, peptide acid may be obtained as a by-product.

Method 3-33: Cleavage with hydrazine to give the C-terminal hydrazide [27]

If required, the side-chain protecting groups should be first removed following Method 3-32, steps 1 & 2.

1. Suspend the peptide resin in DMF and add a solution of 5% hydrazine hydrate in DMF (20 ml/g). Leave to stand for 1 h at rt.
2. Filter the resin through a sintered glass funnel and wash the resin first with DMF, and then with TFA into a separate flask to remove any DMF insoluble peptide.
3. Evaporate the filtrates separately to dryness on a rotary evaporator. Precipitate peptide with ether and isolate by filtration.

Method 3-34: Cleavage with alkali to give the free acid [3]

The side-chain protecting groups should be first removed using Method 3-32, steps 1 & 2.

1. Pre-swell the resin in dioxane.
2. Cool 0.1 M NaOH/ dioxane (1:3, 20 ml/g) to 0°C in an ice/water bath. Add the peptide resin and leave to stand for 15 min at rt.
2. Filter the resin using a glass sintered funnel into a flask containing 0.1 M HCl (5 ml/g). This flask should be cooled in an ice bath to prevent warming as the base solution is neutralized.
3. Wash the resin with water and adjust the pH of the filtrate to 7.0. Lyophilize the filtrate and remove the sodium chloride by gel-filtration.

Method 3-35: Cleavage with methanol/DIPEA to give the methyl ester [27]

The side-chain protecting groups should be first removed using Method 3-32, steps 1 & 2.

1. Place the resin in a clean flask and add sufficient DMF to swell the resin. Cleave o/n with DIPEA/MeOH/DMF (1:5:5, 50 ml/g).
2. Wash the resin first with MeOH/DMF and then with TFA into a separate flask to remove any methanol insoluble peptide.
3. Evaporate the filtrates separately to dryness on a rotary evaporator. Precipitate peptide with ether and isolate by filtration.

If the yields are low, repeat the reaction with fresh reagents at 50°C.

Method 3-36: Cleavage with borohydride to give the peptide alcohol [28]

This method is only applicable to TG and PEGA-type resins.
The side-chain protecting groups should be first removed using Method 3-32, steps 1 & 2.

1. Treat the peptidyl resin (1.0 g) with 50% aq. EtOH, and allow to drain. Add $NaBH_4$ (126 mg) in 3 ml 50% aq. EtOH and gently agitate for 4 h.
2. Remove the resin by filtration and wash with 50% aq. EtOH (40 ml) to give a solution of peptide at pH~9.

3.8.7 Peptides attached to sulfamylbutyryl, DHP, Weinreb and hydrazinobenzoyl resins

Cleavage of peptides from these supports is dealt with elsewhere:

Sulfamylbutyryl resins	Method 2-7, page 2.27
DHP resins	Method 2-19, page 2.46
Weinreb resin	Method 2-8, page 2.29
Hydrazinobenzoyl resins	Method 2-9, page 2.29

References

1. D.S. King, et al. (1990) *Int. J. Peptide Protein Res.*, **36**, 255.
2. F. Albericio, et al. (1990) *J. Org. Chem.*, **55**, 3730.
3. E. Atherton & R.C. Sheppard, "Solid phase peptide synthesis: a practical approach", 1989, IRL Press, Oxford.
4. a) P. Sieber (1987) *Tetrahedron Lett.*, **28**, 1637; b) B. Riniker & A. Hartmann in "Peptides, Chemistry, Structure & Biology, Proc. 11th American Peptide Symposium", J.E. Rivier & G.R. Marshall (Eds.), ESCOM, Leiden, pp. 950.
5. a) P. White in "Peptides, Chemistry, Structure & Biology, Proc. 12th American Peptide Symposium", J.A. Smith & J.E. Rivier (Eds.), ESCOM, Leiden, 1992, pp. 537;b) P. White, Novabiochem Satellite Meeting, Solid Phase Synthesis, 2nd International Meeting, Canterbury, 1991.
6. H. Choi & J.V. Aldrich (1993) *Int. J. Peptide Protein Res.*, **42**, 58.
7. C.G. Fields & G.B. Fields in "Innovations & Perspectives in Solid Phase Synthesis, 3rd International Symposium", R. Epton (Ed.), Mayflower Worldwide Ltd., Birmingham, 1994, pp. 251.
8. C.G.Fields & G.B.Fields (1993) *Tetrahedron. Lett.*, **34**, 6661.
9. A.G. Beck-Sickinger, et al. (1991) *Int. J. Peptide Protein Res.*, **38**, 25.
10. E. Jaeger, et al. (1993) *Biological Chemistry Hoppe Seyler*, **374**, 349.
11. E. Atherton, et al. (1985) *J. Chem., Perkin Trans I*, 2065.
12. D.A. Pearson, et al. (1989) *Tetrahedron Lett.*, **30**, 2739.
13. N.A. Solé & G. Barany (1992) *J. Org. Chem.*, **57**, 5399.
14. Y. Kiso, et al. (1989) *Chem. Pharm. Bull.*, **37**, 3432.
15. K. Akaji, et al. (1990) *J. Chem. Soc., Chem. Commun.*, 288.
16. M. Yoshida, et al. (1990) *Chem. Pharm. Bull.*, **38**, 1551.
17. S. Guo, et al. (1988) *Chem. Pharm. Bull.*, **36**, 4989; H. Yajima, et al. (1988) *Tetrahedron Lett.*, **44**, 805.
18. A. Penrose & P. White, Poster 487 presented at the 19th Meeting of the Federation of European Biochemical Societies, Rome, 1989.
19. a) H. Rink (1987) *Tetrahedron Lett.* **28**, 3787;b) H. Rink & B. Ernst in "Peptides 1990, Proc. 21st European Peptide Symposium", E. Giralt & D. Andreu (Eds.), ESCOM, Leiden, 1991, pp. 419.
20. a) K.Barlos, et al. (1989) *Tetrahedron Lett.*, **30**, 3943; b) K.Barlos, et al. (1989) *Tetrahedron Lett.*, **30**, 3947; K.Barlos, et al. (1991) *Int. J. Peptide Protein Res.*, **37**, 513.

21. A. Flörsheimer & B. Riniker in "Peptides 1990, Proc. 21st European Peptide Symposium", E. Girald & D. Andreu (Eds.), ESCOM, Leiden, 1991, pp. 131.

22. E.Bayer, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 13th American Peptide Symposium:, R.S. Hodges & J.A. Smith (Eds.), ESCOM, Leiden, 1994, pp. 157.

23. P. Sieber (1987) *Tetrahedron Lett.*, **28**, 2107.

24. B. Kamber & B. Riniker in "Peptides, Chemistry & Biology, Proc. 12th American Peptide Symposium", J.A. Smith & J.E. Rivier (Eds.), ESCOM, Leiden, 1992, pp. 525.

25. K. Barlos & D. Gatos in "Fmoc solid phase peptide synthesis: a practical approach", W. C. Chan & P. D. White (Eds.), Oxford University Press, Oxford, 2000, pp. 218.

26. J. M. Stewart & J. D. Young in "Solid phase peptide synthesis, 2nd edition", Pierce Chemical Company, Rockford, pp. 91.

27. S. Mellor, et al. in "Fmoc solid phase peptide synthesis: a practical approach", Oxford University Press, Oxford, 2000, pp. 147-148.

28. M.Munns & P.White, unpublished results.

3.9   Post-cleavage work-up

DO NOT DISCARD resin support or ether until peptide analysis is complete. Both can be stored under nitrogen or argon at 4°C to prevent oxidation.

3.9.1 Ether precipitation

Most cleavage protocols involve precipitation of the crude cleaved peptide using cold ethyl ether. The following are general procedures for post cleavage work-up.

Method 3-37: Post-cleavage work-up

Peptide isolation and work-up can be achieved by ether precipitation (1) or centrifugation (2). For water soluble peptides, the method in steps 3-6 can be used.

1. **Precipitation**: Filter the precipitated peptide through hardened filter paper in a Hirsch funnel under a light vacuum. Wash the precipitate further with cold ether, dissolve the peptide in a suitable aqueous buffer and lyophilize.
2. **Centrifugation**: Add a small volume of *t*-butyl methyl ether to the residue and triturate thoroughly until a free suspension is obtained. Transfer the suspension to a clean centrifuge tube, seal and centrifuge. It is essential that a spark-free centrifuge is used for this process. Carefully decant the ether from the tube. Repeat the ether wash as necessary. Dissolve the residual solid in a suitable aqueous buffer and lyophilize.
3. **Water-soluble peptides**: After precipitation, add water to the residue and transfer mixture to a separating funnel. A little AcOH may be necessary to aid dissolution.
4. Shake the stoppered funnel well. Release the stopper and allow the two layers to separate by standing. Isolate the lower (aqueous) layer.
5. Add more water to the funnel and repeat step 4 three times. Remove the upper (ethereal) layer and store in a clean flask. Return the combined aqueous extracts to the separating funnel.
6. Add a small amount of fresh diethyl ether and repeat step 4 two or three times, each time removing the ethereal layer and returning the aqueous layer to the separating funnel. Collect the aqueous layer in a clean flask and lyophilize.

In the methods described above, the yield of peptide can often be increased if the TFA is first removed using a rotary evaporator (equipped with a CO_2/acetone cold finger, oil pump and acid trap) prior to the ether precipitation step. In most cases, after adding the ether, the peptide will adhere to the sides of the reaction flask, enabling the scavengers to be quickly and easily removed by repeated ether washing. Note: cleavage mixtures containing TFMSA and HBF_4 should not be evaporated to dryness.

Since peptides prepared using the low-high HF cleavage method may contain water soluble sulfonium derivatives, it is advisable to remove these immediately prior to lyophilization as, under neutral or slightly basic conditions, they may cause alkylation of methionine and cysteine residues.

3.10 Protocols for the Fmoc SPPS of cysteine & methionine-containing peptides

3.10.1 Introduction

Peptides containing Cys can exist in either reduced (sulfhydryl) or oxidized inter/intra chain disulfide bonded forms. The synthesis of peptides containing Cys, therefore, presents special challenges to the peptide chemist. Careful planning of the synthetic strategy is essential, if the peptide possessing the correct structure is to be obtained in good yield. Choosing the most appropriate sulfhydryl protecting group is of paramount importance as a "bad" choice can lead to insurmountable difficulties during synthesis or subsequent disulfide bond formation.

It is important to remember that the procedures described here are only a starting point and cannot be guaranteed to work in every case. Problems are sequence dependent and considerable optimization of reaction conditions is often required. If difficulties do arise, our technical service will be glad to assist you whenever possible. For discussions on the management of cysteine-containing peptide, together with detailed practical protocols, see [1a] and the regioselective versus random approach for disulfide bond formation, see [1b, c].

3.10.2 Cysteinyl protection

A wide variety of cysteinyl protecting groups are available for use in Fmoc SPPS. The choice depends on the nature of the desired peptide and synthetic strategic. A summary of thiol protecting groups commonly used in Fmoc SPPS is given in Table 3-21.

For routine synthesis of cysteinyl peptide containing free thiol groups, the trityl group is particularly recommended, as it is labile to TFA and is therefore removed during the normal cleavage procedure. The peptide is initially obtained in the reduced monomeric form but, if required, can be readily converted to a dimeric or cyclic disulfide bonded form by oxidation.

For resin selective modification of thiol groups or on-resin disulfide formation, STmp and Mmt are the most useful as they can be removed under conditions orthogonal to standard side-chain protecting groups employed in Fmoc SPPS.

The selection of protecting groups for selective disulfide is less straightforward and finding the optimal combination may take extensive experimentation. Detailed discussion on this issue is provided in section 3.10.7.

3.10.3 Fmoc SPPS using protected cysteine derivatives.

In contrast to other Fmoc-protected amino acids, Fmoc-protected Cys derivatives can undergo significant racemization during standard coupling reactions. The problem is particularly acute when base-mediated methods like HBTU/DIPEA are used for carboxyl activation. Microwave heating and pre-activation exacerbate the problem. Fortunately, racemization is negligible if coupling is performed under acidic/neutral conditions using preformed symmetrical anhydrides [2] or OPfp esters [3] or with DIPCDI/HOBt or DIPCDI/Oxyma activation (Method 3-1).

The synthesis of a peptide acid containing a *C*-terminal cysteine residue require special consideration as extensive epimerization [4] and β-piperidinylalanine formation [5] can occur during chain extension. These side-reactions are most problematic where the cysteine residue is anchored to a Wang-type resin. Fortunately, the use of

Table 3-21: Cysteine thiol protection.

Protecting group	Cleavage reagent	Comments
Acm	Hg^{2+}, Ag^+, I2, Tl^{3+}, RSCl, $PhSOPh$-CH_3SiCl_3	Stable to TFA. Enables peptide to be purified in a protected form prior to liberation of the easily oxidizable thiol groups. Removal of Acm and simultaneous disulfide bond formation can be carried out by treatment with I_2 or Tl^3
tBu	Hg(II), HF(20 ºC), TFA/DMSO, $PhSOPh$-CH_3SiCl_3	Stable to TFA and iodine oxidation. Treatment with $MeSiCl_3$/PhSOPh removes t-Bu and cyclizes in one step without scrambling existing disulphide bonds. Treatment with DPDS in acid leads to direct formation of Cys(Pys) in the presence of existing disulfide bridges and Cys(Acm).
Trt	Hg^{2+}, Ag^+, I_2, Tl^{3+}, TFA, I_2, Tl^{3+}	Most useful derivative for routine use in Fmoc SPPS as it generates the sulfhydryl peptide directly from the TFA cleavage reaction.
StBu	RSH, R_3P	Stable to TFA providing thiol scavengers are not used. Has been used in combination with Acm for selective formation of two disulfide bonds. However, removal is sluggish and STmp is preferred for on-resin deprotection.
STmp	RSH, R_3P	Stable to TFA providing thiol scavengers are not used. Has been used in combination with Mmt for selective on-resin formation of two disulfide bonds.
Mmt	2% TFA in DCM, Hg^{2+}, Ag^+, I_2, Tl^{3+},	Can be selectively removed whilst the peptide remains attached to the solid phase. Ideal for on-resin disulfide bond formation or modification of Cys side-chain.
Npys	RSH, R_3P	Stable to TFA providing thiol scavengers are not used. Activates thiol groups towards disulfide bond formation. Useful for the selective preparation of mixed disulfides.

trityl-type resins [6] like 2-chlorotrityl resin, NovaSyn TGT, NovaPEG Trityl resins appear to reduce these issues to acceptable levels and are strongly recommended for the synthesis of peptide acids containing *C*-terminal cysteine.

3.10.4 Synthesis of cysteinyl peptides

Cys(Trt)

For the synthesis of peptide containing free sulfhydryl groups the use of Fmoc-Cys(Trt)-OH is the simplest approach. The trityl group is labile to TFA and is therefore removed during the normal course of the cleavage reaction.

Due to the high stability of the trityl cation and the strongly nucleophilic nature of the thiol group, this reaction is reversible and so special attention needs to be given to the cleavage conditions to ensure complete deprotection. Problems with incomplete deprotection of Cys(Trt) residues can normally be overcome by employing cleavage cocktails which contain TIS [7]. This reagent is extremely effective at quenching the trityl cation, converting it irreversibly to triphenylmethane. It may be substituted by triethylsilane, although care must be taken with peptides containing unprotected tryptophan as silanes are known to cause reduction of indoles.

With peptides containing multiple Cys(Trt) residues, best results are obtained if the peptide is precipitated directly into diethyl ether from the TFA cleavage mixture. Addition of 2.5 % ethanedithiol to the cleavage cocktail helps ensure the peptide is maintained in the reduced state and minimizes side-products due to alkylation of this cysteine thiol group. It is essential to use sufficient cleavage cocktail for the amount of peptide cleaved. Generally 30 ml per 0.5 mmole is sufficient. (approx 16 eq. of EDT/Cys residue) However, if the peptide contains multiple Cys residues and many t-butyl protecting groups, the concentration of EDT or volume of cocktail used needs to be increased to ensure effective scavenging.

Cys(Acm) and Cys(tBu)

Acm and tBu protection can also be used to prepare cysteinyl peptide. However, this approach is no longer in general use.

The Acm and *t*-butyl groups are stable to the conditions required for the removal of all other side-chain protecting groups. Therefore, intermediate purification of the peptide is possible prior to cleavage of these cysteine protecting groups. Removal of these protecting groups can be achieved by treatment with mercury (II) acetate or silver trifluoro-methanesulfonate. In the latter case, treating the silver salt of the cysteinyl peptide with aq. HCl-DMSO leads to direct disulfide bond formation [8].

CAUTION: Mercury and silver salts are toxic and corrosive; great care must be taken when using these reagents. Proper eye protection, lab. coat and gloves are mandatory. Follow local, state/ provincial and federal safety regulations. Use in an efficient fume cupboard.

Method 3-38: Deprotection of Acm protected peptides with Hg(II) [9]

For convenience, these reactions may be carried out in a centrifuge tube.
1. Dissolve the (Acm) peptide in 10% aq. AcOH (5-10 mg/ml) and adjust the pH of the solution, very carefully, to 4.0 with glacial AcOH.
2. Add mercury (II) acetate (10 eq./Acm) and readjust the pH to 4.0 with AcOH or aq. NH_3. Stir the mixture gently at r.t. under a blanket of N_2.
3. Add β-mercaptoethanol (20 eq./Acm) and leave the mixture to stand for 5 h.
4. Remove the precipitate by centrifugation and desalt the supernatant by HPLC.

Cys(t–butylthio) and Cys(STmp)

Insertion of Cys(tButhio) [12] or Cys(STmp) [13] residues into a sequence allow selective deprotection of the thiol group on the solid phase, enabling either modification of Cys residues or on-resin disulfide bridge formation.

The t-butylthio group is stable to TFA, providing thiols are not used as scavengers in the cleavage reaction. It is removed by reduction with either thiols [12] or trialkylphosphines [14, 15]. Recently, Góngora-Benítez, et al. [16] demonstrated the effectiveness of 20% β-mercaptoethanol, 0.1 M NMM in DMF for removing tbutylthio on solid phase, where β-mercaptoethanol alone or phosphines were unsuccessful.

However in practice, it often proves extremely difficult to remove the tButhio group on the solid support. For this reason, Albericio has recently introduced the STmp group [13]. The STmp group appears to be extremely easily removed by mild thiolysis, as Albericio has reported removing four STmp groups on the solid phase with only three 5 minute treatments of 0.1 M N-methylmorpholine (NMM) in DMF containing 5% mercaptoethanol.

Cys(Mmt)

On-resin deprotection of Cys(Mmt) residues with 2% TFA in DCM can be effected in a batch-wise or continuous flow manner. In the latter case, the reaction can be monitored spectrophotometrically by following release of the trityl cation at 460 nm. For batch synthesis, a trityl scavenger such as TIS or TES can be added to the reaction to enhance cleavage. However, the color indication provided by the trityl cation will be lost.

Ideally, the reaction should be carried out in a sealed sintered glass funnel to prevent evaporation of the highly volatile DCM, and the filtration reaction should be carried out by applying N_2 pressure rather than by use of a vacuum.

In practice, complete removal of the Mmt group can be difficult to achieve, particularly if the Mmt is situated towards to the C-terminus of the peptide. Extended 2% TFA treatment may lead to loss of protecting groups from amino acid side chains.

3.10.5 Handling of sulfhydryl peptides

Sulfhydryl peptides are readily oxidized by atmospheric oxygen, so they should be freeze dried immediately after cleavage and stored dry under argon. To minimize premature oxidation, cysteine-containing peptides should be handled in acidic (0.1% TFA) degassed buffers. For analysis, HPLC buffers should also be degassed by He sparging. Peptides containing multiple Cys residues may require reduction following cleavage.

3.10.6 Disulfide bond formation by oxidation of cysteinyl peptides

Random oxidation of sulfhydryl peptides

The simplest method for the formation one or more disulfide bridges is by random oxidation of the free sulfhydryl peptide. In this case of peptides containing more than two Cys residues, the composition of the products formed may vary depending on whether the oxidation is done under thermodynamic or kinetic control.

Thermodynamic control

Of these techniques, the easiest procedure to perform is the air oxidation which gives the thermodyamincally most stable product. The procedure involves simply exposing an aqueous solution of peptide to the atmosphere in a volatile buffer and then isolating the peptide by lyophilization. Small monocyclic peptides can be readily prepared by carrying out the air oxidation immediately after TFA cleavage of Cys(Trt) without purification of the intermediate sulfhydryl peptide (Method 3-43). The disadvantage of this procedure is that reactions can sometimes be very slow. Activated charcoal has also been shown to effectively catalyze this process [17].

Oxidation with a mixture of cysteine and cystine or reduced and oxidized glutathione is useful for oxidation of peptides containing multiple disulfide bridges. The presence of excess reduced component leads to the slow rearrangement of the intermediate bridged peptides to the most thermodyamically stable isomer.

Kinetic oxidation

Various oxidants have been used for rapid formation of disulfide bridges to produce the kinetically most favored product. These include potassium ferricyanide [18], iodine [19], DMSO [20, 21], N-chlorosuccinimide (NCS) [22], and DPDS (2,2'-dithiopyridine) [23].

Oxidation with iodine is extremely rapid and can be accomplished by dropping a solution of iodine into the rapidly stirred peptide solution until the solution is premanantly colored yellow.

DMSO oxidation is very mild and works at pHs from 3 to 8. No side-reactions have been reported involving sensitive residues (Met, Trp, Tyr).

NCS in DMF has been used for oxidation of sulfhydryls on the solid phase, with as little as 2 equivalents affecting oxidation in 15 mins [22]. This reagent can cause oxidation of methionine.

3.10.7 Disulfide bond formation by oxidation of protected cysteinyl peptides

Disulfide bond formation by iodine oxidation

Treatment of peptides containing Cys(Acm)/Cys(Trt) residues with iodine results in simultaneous removal of the sulfhydryl protecting groups and disulfide bond formation. Peptides containing a single Cys residue are converted to the symmetrical dimer, whilst those containing multiple residues are converted to mixtures of cyclic monomer and polymers, depending on the solvent and concentration. The rate of reaction is very solvent dependent, allowing a degree of selectivity between Cys(Trt) and Cys(Acm); in dipolar solvents, such as aq. MeOH and aq. AcOH, both Cys(Trt) and Cys(Acm) react rapidly, whereas in non-polar solvents, such as DCM and $CHCl_3$, the oxidation of Cys(Acm) is extremely sluggish [24].

The most common application of this method is the conversion of side-chain deprotected peptides containing two Cys(Acm) residues into the cyclic disulfide bridged monomer; the use of trityl protection is not appropriate as this group will be removed during TFA cleavage. The reaction is normally carried out in high dilution in mixtures of aq. MeOH or aq. AcOH, with the exact choice of solvent being dependent on the sequence. Reactions are fastest in aq. MeOH; but for peptides containing Tyr, His and Trp, aq. AcOH is to be preferred as its use limits iodination of these sensitive residues.

For the iodine oxidation of protected peptide fragments in solution, it is recommended that of the two Cys residues to be joined together in the disulfide bridge, one should be protected with Acm and the other with Trt. This measure limits polymer formation and allows the use of non-polar solvent mixtures such as $TFE/CHCl_3$ which favor dissolution of the protected fragment. Iodine oxidation of peptides containing a Cys residue with a free Nα-amino function has been noted to be extremely sluggish, possibly a result of the positive charge on the amino group inhibiting formation of the sulfonium intermediate.

Migration of Acm from cysteine to the side chains of Gln [25a] and Ser/Thr [25b] residues has been reported during iodine and thallium (III) oxidation reactions.

Thallium trifluoroacetate oxidation

Like iodine, $TI(CF_3CO_2)_3$ converts peptides containing multiple Cys(Acm) residues to the corresponding disulfide bridged cystine peptides [28]. In solution, this reaction has generally been carried out in TFA, as this is an excellent solvent for both free and protected peptides. For direct disulfide bond formation on the solid phase, DMF has been employed as the solvent, allowing good resin swelling without premature cleavage of peptide chains. It is important to note that Met and Trp must be protected during $TI(CF_3CO_2)_3$ treatment to avoid oxidation of these sensitive residues; Met(O) and Trp(Mts), and Trp(Boc) have been employed in conjunction with t-Boc [28] and Fmoc [29, 30] strategies, respectively.

CAUTION: *Thallium salts are highly toxic and corrosive; great care must be taken when using these reagents. Proper eye protection, lab. coat and gloves are mandatory. Follow local, state/ provincial and federal safety regulations. Use in an efficient fume cupboard.*

Method 3-50: Solution phase oxidation with TI(III) [28]

1. Dissolve the peptide in TFA (1-10 mg/ml) with anisole (10 µl/mg), and cool in an ice bath
2. Add $TI(CF_3CO_2)_3$ (1.2 eq.) and allow to react for 5-18 h.
3. Remove TFA by evaporation in vacuo and precipitate peptide with ether. Centrifuge and decant ether, removing excess thallium reagent.
4. Add fresh ether, shake tube to disperse peptide and centrifuge. Decant ether and repeat washing process three times.

Method 3-51: Solid phase oxidation with TI(III) [29, 30]

1. Suspend peptidyl resin in DMF/anisole (19:1) and add $TI(CF_3CO_2)_3$ (1.2 eq. relative to peptide)
2. Leave to stand at 0°C for 80 min, and wash resin with DMF
3. Cleave the peptide from the resin as described in Method 3-26, page 3.29. Thiol scavengers should not be used as these reagents will cause reduction of the disulfide bond. In most cases, EDT can be substituted with TIS.

Regioselective disulfide bond formation

Synthesis of multiple disulfide containing peptides by selective bridge formation is not a task to be undertaken lightly. In the synthesis of peptides containing multiple disulfide bonds the best results are often obtained by random oxidation, as the desired biologically active isomer is generally the most thermodynamically stable. Methods that work for one peptide do not necessarily work for another. Sometimes two bridges can be formed without issues, but the formation of the third can then prove intractable. Furthermore, the order in which the bridges are formed is often crucial. Selective formation of the first bridge can provide a template for the correct formation of other bridges by random oxidation. A considerable time investment is required with no guarantee of success.

For the selective formation of two disulfide bonds, combinations of either Trt and Acm, or STmp and Acm protection can be used: the first disulfide bond is formed after selective removal of Trt or STmp protection; generation of the second disulfide bond is then carried out in a single step by treatment of the Acm protected peptide with iodine or thallium trifluoroacetate. Alternatively, with Trt and Acm, a one-pot reaction can be performed in solution, where the first disulfide is formed by the fast oxidation of disulfhydryl peptide with a stoichiometric amount of iodine, followed by the addition of excess iodine and water to effect oxidation of the Acm-protected thiols (Method 3-50).

The combination of STmp and Mmt facilitate selective formation of two bridges on the solid phase [22]. STmp groups are removed by treatment with mercaptoenthanol (Method 3-41) and the first bridge formed by oxidation with NCS (Methd 3-48). Removal of Mmt with 2% TFA in DCM (Method 3-42), followed by oxidation again with NCS furnishes the second bridge.

Table 3-22: Protecting group combinations for slective synthesis of multiple disulfide bonds.

First	Second	Third	Comments
Trt	Acm		1: bridge by air oxidation or stoichiometric I2 oxidation. 2: by excess I_2 oxidation.
tBu	MeBzl		Temperature controlled oxidation with DMSO/TFA
STmp	Mmt		On-resin formation of both bridges with NCS oxidation
STmp	Acm		1: On-resin with NCS oxidation. 2: with solid or solution phase I_2 oxidation.
STmp	Mmt	Acm	1 & 2: On-resin formation of both bridges with NCS oxidation. 3: I_2 oxidation in solution.
STmp	Mmt	tBu	1 & 2: On-resin formation of both bridges with NCS oxidation. 3: DMSO/TFA or $MeSiCl_3$/Ph_2SO in solution
Trt	Acm	tBu	1: bridge by air oxidation or stoichiometric I2 oxidation. 2: by excess I_2 oxidation. 3: TFA/DMSO or $MeSiCl_3$/Ph_2SO.
Trt	tBu	MeBzl	1: bridge by air oxidation or stoichiometric I_2 oxidation. 2 & 3: by temperature controlled oxidation with TFA/DMSO

t-Butyl protection, in conjunction with one step cleavage and cyclization with $MeSiCl_3$/Ph_2SO, has been used to introduce a third disulfide bridge, leading to the selective synthesis of ϖ-conotoxin and insulin [31]. In a similar manner, a combination of tBu and MeBzl cysteine protection has been employed in a regioselective one-pot formation of the two disulfide bonds of α-conotoxin SI [32]. The first disulfide bond was formed by cleavage of the tBu groups and simultaneous oxidation with TFA/DMSO/anisole at room temperature. Subsequent heating to 70°C resulted in cleavage of the MeBzl groups and formation of the second disulfide bridge. For other examples of the application of this approach in the synthesis of multi-bridged peptides see references [33-36]. These methods may cause oxidation of Met and Trp residues.

Method 3-52: $MeSiCl_3$/Ph_2SO oxidation [31]

1. Dissolve Cys-tBu peptide in TFA (1 - 10 mg/ml)
2. Add Ph_2SO (10 eq.), CH_3SiCl_3 (100-250 eq.) and anisole (100 eq.)
3. Allow reaction to proceed for 10-30 min at 25°C.
4. Quench reaction with NH_4F (300 eq.) and precipitate peptide by addtion of a large volume of cold diethyl ether and isolate by centrifugation.

Method 3-53: TFA/DMSO oxidation [32]

1. Dissolve peptide in TFA/DMSO/anisole (97.9/2/0.1) (2 mg/ml)
2. Stir at rt for 40 mins. Add an additional aliquot of TFA/DMSO/anisole (40 µl/mg peptide)
3. Heat at 70°C for 3 h. Precipitate peptide with diethyl ether and isolate by centrifugation.

Asymmetric disulfides

S-Npys protected peptides react rapidly with thiols over a wide range of pH to form mixed disulfides [37], making this protecting group particularly useful for preparation of peptide-protein conjugates or peptides with two different chains bonded by a disulfide bridge [38-41]. However, owing to the instability of the Npys group to piperidine when using Fmoc chemistry, the Cys(Npys) residue is usually introduced at the *N*-terminus of the peptide using Boc-Cys(Npys)-OH. Alternatively, it has been found that the Npys group can be added post-synthetically to any Trt protected Cys residue by adding 5 equivalents of 2,2'-dithio-bis-(5-nitropyridine) to the standard TFA/TIS/water (95:2.5:2.5) cleavage mixture [42].

Direct conversion of Cys(tBu) to Cys(Pys) in the presence of Cys(Acm) and a disulfide bridge has been achieved by treatment with DPDS/thioanisole/TFA/TFMSA [43]. This method was used to provide activated relaxin A chain, a key intermediate for the synthesis of the insulin like hormone relaxin [43].

Method 3-54: tBu to Pys conversion [43]

1. Dissolve Cys-tBu peptide and DPDS (4 eq.) in TFA and thioanisole (9:1 v/v) (50 mg/ ml).
2. The mixture was chilled on ice after which a similar volume of TFMSA in TFA (1:4 v/v) was added and the reaction continued for 45 min at 0°C.
3. Precipitate the Cys(Pys) peptide with cold diethyl ether and isolate by centrifugation. Wash pellet 4 times with fresh ether to remove excess DPDS.

3.10.8 Reduction of disulfide bonds

Reduction with DTT

Since its introduction by W.W. Cleland [44], DTT (Calbiochem cat. no. 233153) has become the standard reagent for the reduction of cystinyl peptides. It has little odor, is highly water soluble, and reduces disulfides quantitatively in aqueous media at pH 8.

Method 3-55: Reduction with DTT

1. Dissolve peptide in 0.1 M ammonium bicarbonate (1-3 mg/ml).
2. Add DTT (5 -fold excess relative to thiol groups).
3. Blanket mixture with N_2 and leave to stand for 6 h.
4. Acidify mixture with AcOH and lyophilize.
5. Remove DTT by gel-filtration.

Reduction with TCEP

TCEP is a water-soluble phosphine that reduces disulfides over a wide range of pH (1.5 -9.0) [45]. Unlike, thiol-based reducing agents, TCEP is not sensitive to oxygen, so no special precautions are necessary to exclude air. The reaction is irreversible and kinetically controlled, unlike the reaction with DTT which is thermodynamically controlled [42]. The use of TCEP is ideal for the reduction of peptides that are not soluble at pH 8 required for reduction with DTT. It should be noted that prolonged contact of peptide with excess TCEP can cause desulfurization of cysteine residues.

Method 3-56: Reduction with TCEP

1. Dissolve peptide in any suitable aqueous/organic solvent mixture (1-3 mg/ml, pH).
2. Add TCEP (5-fold excess relative to disulfide).
3. Gently agitate solution for 1 h.
4. Purify reduced peptide by HPLC or gel-filtration.

3.10.9 Ellman test [46, 47]

5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) [Calbiochem cat. no. 322123], reacts quantitatively with aliphatic sulfhydryl groups to generate a yellow anion. This reaction can be used to follow the progress of air oxidation reactions. Samples from the reaction mixture are taken at suitable intervals and the thiol content tested.

Method 3-57: Ellman test

1. Dissolve DTNB (40 mg) in 0.1 M sodium phosphate buffer, pH 8 (10 ml).
2. Take a sample from the reaction mixture and dilute with buffer to give 3 ml solution containing 0.1-0.2 µmole of peptide. Add DTNB reagent (0.1 ml) to the peptide solution and leave the mixture to stand for 15 min. Measure the absorbance at 410 nm using a 1 cm cell.
3. Prepare a reference solution by adding DTNB reagent (0.1 ml) to buffer (3 ml) and measure the absorbance.
4. Calculate the concentration of sulfhydryl groups using the following equation; $[SH] = [A410(sample)-A410(reference)]/13650$.

3.10.10 Reduction of methionine sulfoxide

Methionine sulfoxide, Met(O), can be present in peptides by unintentional oxidation during chain assembly, cleavage by strong acid or prolonged storage and handling, or intentionally by coupling sulfoxide protected methionine in order to prevent alkylation during synthesis.

Reduction with N–methylmercaptoacetamide (MMA) [48, 49]

Method 3-58: MMA reduction

1. Dissolve the peptide in 10% acetic acid to a concentration of 1-10 mg/ml.
2. Add MMA (10 eq.) to the solution and incubate the solution under N_2 at 35-40°C for 12-36 h.
3. Lyophilize after completion of the reaction, and isolate peptide by HPLC.
Monitoring by HPLC is recommended: the reduced peptide will have a longer retention time in HPLC due to a higher hydrophobicity.

Reduction with NH_4I/DMS [50, 51]

NH_4I/DMS reduces methionine sulfoxide without destruction of existing disulfide bridged cystine residues.

Method 3-59: Reduction with NH_4I/DMS

1. Dissolve peptide in TFA (10 mg/ml) and cool to 0°C.
2. Add NH_4I (20 eq.) and DMS (20 eq.), and leave to stand for 30 min.
3. Add 4 volumes of water and CCl_4. Wash aqueous layer with CCl_4 (3 x)
4. Evaporate and isolate peptide by HPLC.

Related products

853049	Boc-Cys(Acm)-OH	p. 114
853050	Boc-Cys(4-MeOBzl)-OH	p. 115
853033	Boc-Cys(4-MeBzl)-OH	p. 115
853005	Boc-Cys(Trt)-OH	p. 115
852006	Fmoc-Cys(Acm)-OH	p. 22
852007	Fmoc-Cys(tBu)-OH	p. 23
852022	Fmoc-Cys(tButhio)-OH	p. 23
852373	Fmoc-Cys(STmp)-OH	p. 23
852031	Fmoc-Cys(Mmt)-OH	p. 24
852008	Fmoc-Cys(Trt)-OH	p. 24

References

1. F. Albericio, et al. in "Fmoc solid phase peptide synthesis: a practical approach", W. C. Chan & P. D. White (Eds.), Oxford University Press, Oxford, 2000, pp. 77.
2. T. Kaiser, et al. (1996) *Tetrahedron Lett.*, **37**, 1187.
3. Y. X. Han, et al. (1997) *J. Org. Chem.*, **62**, 4307; Y. M. Angell, et al. (2002) *J. Peptide Res.*, **60**, 292.
4. J. Lukszo, et al. (1996) *Lett. Pept. Sci.*, **3**, 157.
5. E. Atherton, et al. in "Peptides 1990, Proc. 21st European Peptide Symposium, E. Giralt & D. Andreu (Eds), 1991, Escom, Leiden, pp. 243.
6. Y. Fujiwara, et al. (1994) *Chem. Pharm. Bull.*, **42**, 724.
7. D. A. Pearson, et al. (1989) *Tetrahedron Lett.*, **30**, 2739.
8. H. Tamamura, et al. (1995) *Int. J. Pept. Protein Res.*, **45**, 312.
9. D. F. Veber, et al. (1972) *J. Am. Chem. Soc.*, **94**, 5456.
10. O. Nishimura, et al. (1978) *Chem. Pharm. Bull.* **26(5)**, 1576.

11. F. Albericio, et al. in "Fmoc solid phase peptide synthesis: a practical approach", W. C. Chan & P. D. White (Eds.), Oxford University Press, Oxford, 2000, pp. 104.

12. T. M. Postma, et al. (2012) *Org. Lett.*, **14**, 5468.

13. U. Weber & P. Hartter (1970) *Hoppe-Seyler's Z. Physiol. Chem.*, **351**, 1384.

14. U. T. Ruegg & H. G. Gattner (1975) *Hoppe-Seyler's Z.Physiol.Chem.*, **356**, 1527.

15. N. J. C. M. Beekman, et al. (1997) *J. Pept. Res.*, **50**, 357.

16. M. Góngora-Benítez, et al. (2011)*Biopolymers*, **96**, 69.

17. R. Volkmer-Engert (1998) *J. Pept. Res.*, **51**, 365.

18. S. N. McCurdy (1989) *Pept. Res.*, **2**, 147.

19. M. Pohl, et al. (1993) *Int. J. Peptide Protein Res.*, **41**, 362.

20. A. Otaka, et al.(1991) *Tetrahedron Lett.*, **32**, 1223.

21. J. P.Tam, et al. (1991) *J. Am. Chem. Soc.*, **113**, 6657.

22. T. M. Postma & F. Albericio (2013) *Org. Lett.*, **15**, 616.

23. K. Maruyama, et al. (1999) *Peptides*, **20**, 881.

24. B. Kamber, et al. (1980) *Helv. Chim. Acta*, **63**, 899.

25. a) H. Lamtanh, et al. (1993) *Int. J. Peptide Protein Res.*, **41**, 85; b) H. Lamthanh, et al. (1995) *Pept. Res.*, **8**, 316.

26. S. Zhang, S., et al. (2008) *Int. J. Pept. Res. Ther.*, **14**, 301.

27. R. Söll & A. G. Beck-Sickinger (2000) *J. Pept. Sci.* **6**, 387.

28. N. Fujii, et al. (1987) *Chem. Pharm. Bull.*, **36**, 2339.

29. M. C. Munson, et al. (1993) *J. Am. Chem. Soc.*, **115**, 10203.

30. F. Albericio, et al. (1991) *Int. J. Peptide Protein Res.*, **37**, 4024.

31. K. Akaji et al (1993) *J. Am. Chem. Soc.*, **115**, 11384.

32. A. Cuthbertson & B. Indrevoll (2000) *Tetrahedron Lett.*, **41**, 3661.

33. E. Atherton, et al. (1985), *J. Chem. Soc., Perkin Trans 1*, 2065;

34. a) Y. Yang, et al. (1994) *Protein Sci.*, **3**, 1267; b) K. Akaji et al (1993) *J. Am.Chem. Soc.*, **115**, 11384; c) T. J. Zamborelli, et al. (2000) *J. Pept. Res.*, **55**, 359.

35. H.G. Ihlenfeldt, et al. in "Peptides 1994, Proc. 23rd European Peptide Symposium", H. Maia (Ed.), ESCOM, Leiden, 1994, pp. 369.

36. J. S. Nielsen, et al. (2004) *J. Pept. Sci.*, **10**, 249.

37. R. Matsueda & K. Aiba (1978) *Chem. Lett.*, 951.

38. J. W. Drijfhout, et al. (1988) *Int. J. Peptide Protein Res.*, **29**, 161.

39. F. Albericio, et al. (1987) *Int. J. Peptide Protein Res.*, **34**, 124.

40. O. Ploux, et al. (1987) *Int. J. Peptide Protein Res.*, **29**, 162.

41. R.G. Simmonds, et al. (1994) *Int. J. Peptide Protein Res.*, **43**, 363.

42. A. K. Ghosh & E. Fan (2000) *Tetrahedron Lett.*, **41**, 165

43. R. A. D. Bathgate, et al. (2006) *Biochemistry*, **45**, 1043.

44. W. W. Cleland (1964) *Biochemistry*, **3**, 480.

45. J. A. Burns, et al. (1991) *J. Org. Chem.* **56**, 2648.

46. G. L. Ellman (1959) *Arch. Biochem. Biophys.*, **82**, 70.

47. G. L. Ellman, et al. (1961) *Biochem. Pharmacol.*, **7**, 68.

48. R. A. Houghten & C. H. Li (1978) *Int. J. Peptide Protein Res.*, **11**, 345.

49. R. A. Houghten & C. H. Li (1979) *Anal. Biochem.*, **98**, 36.

50. H. Yajima, et al. (1988) *Tetrahedron*, **44**, 819.

51. E. Nicolás, et al. in "Innovation and Perspectives in Solid Phase Synthesis, 3rd International Symposium", R. Epton (Ed.), Mayflower Worldwide Ltd., Birmingham, 1994, pp. 619.

3.11. Synthesis of phosphopeptides

There are currently two strategies for the synthesis of phosphopeptides: the building block approach using preformed protected phosphoamino acids and the global phosphorylation method which involves post-synthetic phosphorylation of unprotected hydroxyl groups on the solid support. Each approach has its own merits, and this discussion is intended to help you make an informed choice as to the best strategy to use for a particular application. The procedures and applications given are a compilation of methods developed at Novabiochem and tested literature procedures. For a review, see J. S. McMurray, *et al.* (2001) *Biopolymers*, 60, 3.

3.11.1 Building block approach

The building block approach is, in principal, the more straightforward method for preparing phosphopeptides since it involves only incorporation of a suitably N-α-protected phosphoamino acid into the appropriate point of the peptide chain. For the incorporation of phosphotyrosine in Fmoc SPPS, methyl [1], benzyl [2, 3], *t*-butyl [4, 5], and alkylamide [6, 7] protected derivatives have been used. Unfortunately, many of these protecting groups have limitations. Benzyl and methyl phosphates are subject to mono-dealkylation by piperidine during the deprotection step. Although this side reaction does not compromise peptide purity, it can be overcome by using the non-nucleophilic base DBU for deprotection [2], albeit at the risk of α,β rearrangement of aspartyl residues [2b]. Deprotection of methyl phosphates can not be achieved under the normal conditions of Fmoc synthesis with TFA, but requires the use of strong acids such as TFMSA or TMSBr. The TFA labile *t*-butyl derivative is stable under conditions of synthesis, but undergoes autocatalytic degradation on storage and consequently must be prepared immediately before use.

In 1994, Ottinger, *et al.* [8] demonstrated the utility of Fmoc-Tyr(PO$_3$H$_2$)-OH for the introduction of phosphotyrosine. This derivative, despite having no phosphate protection, facilitates the synthesis of small to medium phosphopeptides in good yield and purity, although pyrophosphate formation is known to be a problem in peptides containing adjacent Tyr(PO$_3$H$_2$) residues [9, 10]. More recently, White & Beythien [11] have introduced the monoprotected derivative, Fmoc-Tyr(PO(OBzl)OH)-OH, which, like Fmoc-Tyr(PO$_3$Bzl$_2$)-OH, has excellent reactivity and solubility properties.

It was once believed that phosphoserine was incompatible with the Fmoc solid phase methodology as treatment with piperidine was reported to lead to β-elimination with loss of the phosphate group and formation of the corresponding dehydroalaninyl peptide (Figure 3-34) [12]. This is indeed the case when the serine phosphate is bis-protected. However, Wakamiya, *et al* [13], in 1994, showed that if a mono-protected derivative, such as Fmoc-Ser(PO(OBzl)OH)-OH, is used then the formation of dehydroalanine can be almost totally suppressed. (This stability is attributed to ionization of the phosphate groups inhibiting elimination under basic conditions.) The efficacy of this compound and that of Fmoc-Thr(PO(OBzl)OH)-OH [11, 14] in the synthesis of a range of peptides containing one, two and three phosphorylation sites has since been demonstrated [11, 15], and such derivatives have now become the reagents of choice for the Fmoc SPPS of phosphopeptides [16-22].
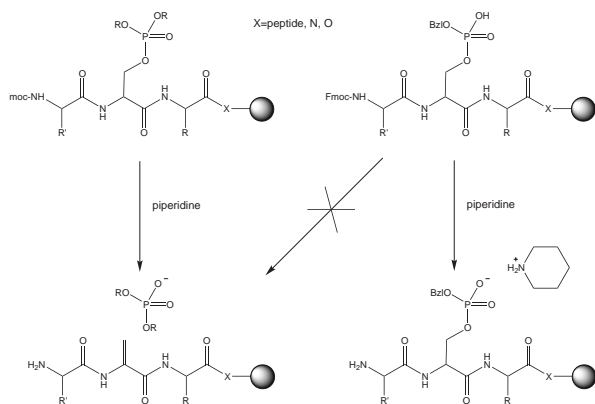
Fig. 3-34. Stability of phosphoamino acid residues to piperidine.

3.11.2 Introduction of building blocks
Fmoc–Ser(PO(OBzl)OH)–OH/Fmoc–Thr(PO(OBzl)OH)–OH/Fmoc–Tyr(PO(OBzl)OH)–OH

As the side-chain phosphate group is only partially protected in these derivatives, special consideration must be given to the conditions employed for their introduction. Perich, *et al.* [20] have found uronium-based coupling reagents in conjunction with HOBt(or HOAt) and DIPEA to be the most effective coupling reagents; poor incorporations were observed using PyBOP® (or BOP) and DIPCDI, which they ascribed to involvement of the phosphate hydroxyl in the activation process.

White [15] has subsequently shown that by employing a 3-fold excess of DIPEA in the standard TBTU/HOBt/DIPEA coupling regime advocated by Perich, *et al.* (Method 3-61) the yield for the sterically challenging coupling of Fmoc-Thr(PO(OBzl)OH)-OH with H-Val-resin could be increased from 80% to 100%; under standard conditions the quantity of base used was thought insufficient for efficient activation as 1 eq. will be consumed by the acidic partially protected phosphate group.

Pascal, *et al.* [23] have observed that acylation of the piperidine associated with partially protected phosphate groups results in a reduction in the excess of activated amino acid and tertiary amine present in the coupling reaction. Whilst this does not pose a significant problem when the peptide contains only one phosphoamino acid, it could lead to serious difficulties with incomplete coupling reactions with peptides containing numerous phosphorylated residues. Fortunately, this problem can be overcome by simply increasing the excess of coupling reagents used or by exchanging the piperidine counterion for a tertiary amine, as described in Method 3-62.

The benzyl side-chain protecting groups are normally removed in 1-2 hours during the course of the standard 95% TFA cleavage reaction. Recently, Attard, et al. [24] reported that N-terminal Ser(PO(OBzl)OH) residues can undergo β-elimination during piperidine-mediated Fmoc removal under microwave conditons. This problem could be eliminated by replacing piperidine with cyclohexylamine just for this step.

Application 3-5: Synthesis of H-Gly-Asp-Phe-Glu-Ile-Pro-Glu-Glu-Tyr(PO₃H₂)-Leu-NH₂ using Fmoc-Tyr(PO(OBzl)OH)-OH

H-Gly-Asp(OtBu)-Phe-Glu(OtBu)-Ile-Pro-Glu(OtBu)-Glu(OtBu)-Tyr(PO(OBzl)OH)-Leu-NovaSyn® TGR resin was prepared automatically using a Shimadzu PSSM8 on 75 mg of NovaSyn® TGR resin (0.2 meq./g). All acylation reactions were carried out using a 10-fold excess of Fmoc-amino acid activated with TBTU (1 eq.) in the presence of HOBt (1 eq.) and DIPEA (2 eq.). A coupling time of 60 min was used throughout. Cleavage of the peptide from the resin and side-chain deprotection was carried out by treatment of the peptidyl resin with TFA/ TIS/water (95:2.5:2.5) for 2.5 h. The crude peptide was analyzed by HPLC (Figure 3-35) and PD-MS (Figure 3-36) [expected M+H+ 1291.3, found 1290.4].
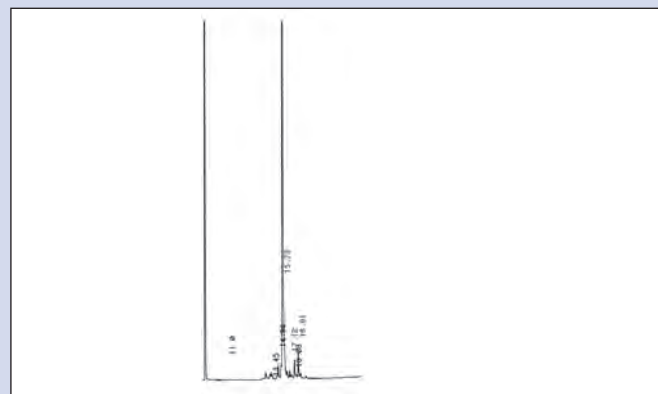
Fig. 3-35: HPLC of total crude peptide.
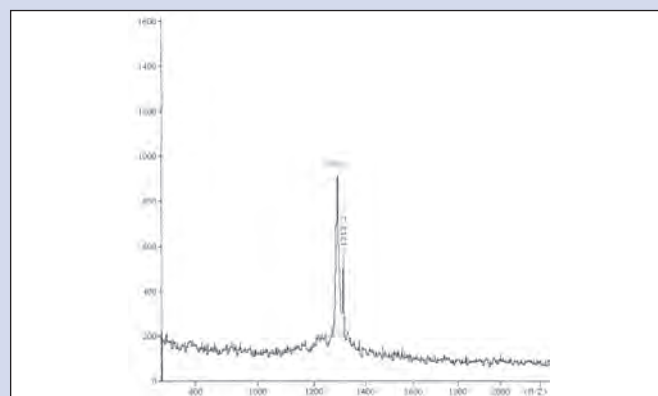
Fig. 3-36: PDMS of isolated major peak.

Application 3-6: Synthesis of H-Gly-Phe-Glu-Thr(PO₃H₂)-Val-Pro-Glu-Thr(PO₃H₂)-Gly-NH₂ using Fmoc-Thr(PO(OBzl)OH)-OH

H-Gly-Phe-Glu(OtBu)-Thr(PO(OBzl)OH)-Val-Pro-Glu(OtBu)-Thr(PO(OBzl)OH)-Gly-NovaSyn® TGR resin was prepared automatically using a NovaSyn® Crystal peptide synthesizer on NovaSyn® TGR resin. All acylation reactions were carried out using a 5-fold excess of Fmoc-amino acid activated with 1 eq. of PyBOP® in the presence of 1 eq. of HOBt and 2 eq. of DIPEA, with the exception of Fmoc-Thr(PO(OBzl)OH)-OH which was introduced using Method 3-47. A coupling time of 1 h was used throughout. The peptidyl resin was treated with TFA/TIS/water (95:2.5:2.5) (5 ml) for 3 h, and then the peptide was isolated in the usual manner by evaporation and ether precipitation. The crude peptide was analyzed by HPLC (Figure 3-37) and ES-MS [expected M+H⁺ 1095.4, found 1095.3]; minor peaks a and c were identified by MS as the des-Val and benzyl adduct respectively.
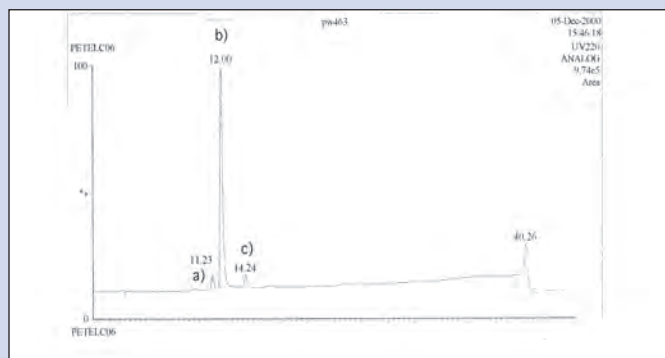
Fig. 3-37: HPLC elution of total crude peptide.

Fmoc-Tyr(PO₃H₂)-OH

This derivatives should be introduced using Method 3-61. In peptides containing multiple phosphotyrosine residues, piperidine counterion exchange should be carried out as described in Method 3-62.

It is important to note that coupling of this derivative and the following residue can be sluggish. Furthermore, in peptides containing adjacent Tyr(PO₃H₂) pyrophosphate formation can occur.

Fmoc-Tyr(PO(NMe₂)₂)-OH

In contrast to Fmoc-Tyr(PO₃H₂)-OH and Fmoc-Tyr(PO(OBzl)OH)-OH, which have potentially reactive acid functionalities in their side-chains, the phosphate group in **Fmoc-Tyr(PO(NMe₂)₂)-OH** [7] is fully protected. This confers not only improved solubility properties on the compound but, more importantly, eliminates a number of problems associated with the use of partially-protected phosphoamino acids, namely pyrophosphate formation [9,10], incompatibility with PyBOP® and carbodiimide coupling reagents [20], and quenching of activated amino acid derivatives through reaction with resin-bound piperidinium phosphate salts [23].

Thus, Fmoc-Tyr(PO(NMe₂)₂)-OH can be introduced using any of the standard coupling methods, such as PyBOP®/DIPEA, TBTU/DIPEA and DIPCDI/HOBt, and in peptides containing multiple phosphotyosine residues, it is not necessary to increase the excess of reagents to compensate for the consumption of activated amino acid derivatives through piperidide formation.

Regeneration of phosphotyrosine from the phosphodiamidate is effected by acid catalyzed hydrolysis [7]. Detachment of the peptide from the resin and deprotection of side-chains is first carried out in the usual manner with TFA containing the appropriate scavengers. 10 % by volume of water is then added to the cleavage solution and the mixture left to stand overnight, during which time hydrolysis of the phosphodiamidate takes place.

The reaction can be monitored by HPLC by following the disappearance of the more hydrophobic phosphodiamidate and phosphoamidates, as illustrated in Application 3-7. Since the reaction does not liberate any reactive cationic species, there is no risk of formation of alkylated by-products, as can be the case with Fmoc-Tyr(PO(OBzl)OH)-OH.

Application 3-7: Synthesis of H-Gly-Asp-Phe-Glu-Ile-Pro-Glu-Glu-Tyr(PO₃H₂)-Leu-NH₂ using Fmoc-Tyr(PO(NMe₂)₂)-OH

H-Gly-Asp(OtBu)-Phe-Glu(OtBu)-Ile-Pro-Glu(OtBu)-Glu(OtBu)-Tyr(PO(NMe2)2)-Leu-Rink Amide MBHA resin was prepared automatically using a NovaSyn® Crystal peptide synthesizer on Rink Amide MBHA resin. All acylation reactions were carried out using a 5-fold excess of Fmoc-amino acid activated with 1 eq. of PyBOP® in the presence of 1 eq. of HOBt and 2 eq. of DIPEA. A coupling time of 1 h was used throughout. The peptidyl resin was treated with TFA/TIS/water (95:2.5:2.5) (5 ml) for 3 h, after which time a sample was removed for HPLC analysis (Figure 3-38). Water (0.5 ml) was then added and the mixture was left to stand for a further 3 h when another sample was removed for analysis (Figure 3-39). After an additional 16 h, the peptide was isolated in the usual manner by evaporation and ether precipitation. The crude peptide was analyzed by HPLC (Figure 3-40) and ES-MS [expected M+H⁺ 1291.1, found 1290.3].
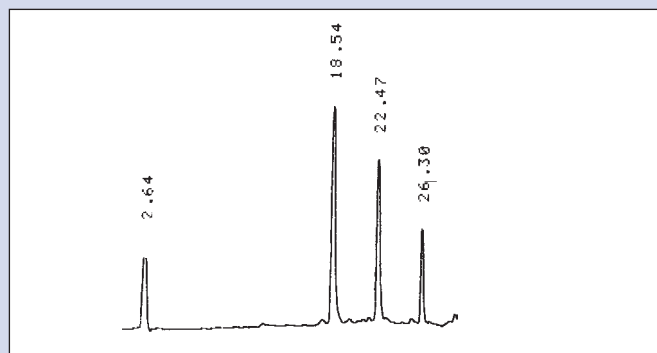
Fig. 3-38: HPLC elution profile of cleavage reaction after 3 h.
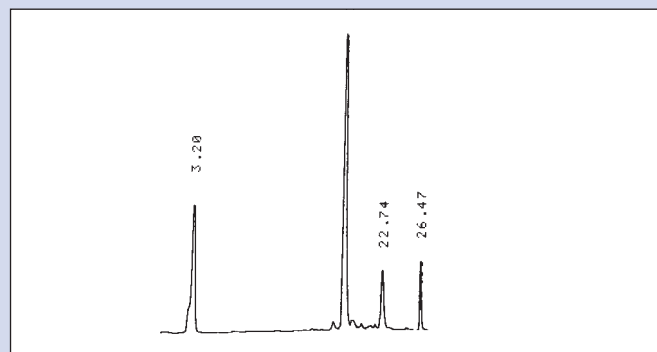
Fig. 3-39: HPLC elution profile of cleavage reaction after 6 h.
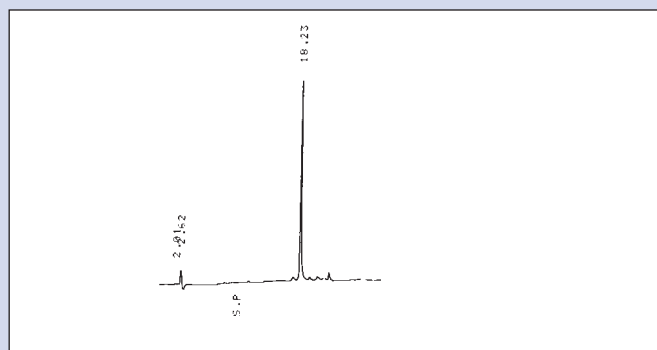
Fig. 3-40: HPLC elution profile of cleavage reaction after 22 h.

Fmoc–Phe(CF$_2$PO$_3$H$_2$)–OH
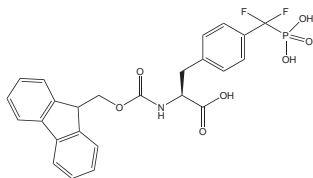
Fmoc-Phe(CF$_2$PO$_3$H$_2$)-OH (Fmoc-F$_2$Pmp-OH) was developed by Burke and coworkers [25a] to overcome the limitations of the original phosphoTyr isostere phosphonophenylalanine (Pmp) [25b]. The former isostere, whilst overcoming the inherent instability of the nature Tyr phosphate moiety, appears to be a poor substitute for pTyr, as substitution of pTyr by Pmp often leads to a significant reduction in biological activity [26]. It was postulated this effect was due to the lack of the H-bond acceptor phenyl oxygen and incomplete ionization of the phosphonic acid at neutral pH (Pmp, pKa2 7.72 vs pTyr, pKa2 6.22). F$_2$Pmp on the otherhand has a pKa2 of 5.71 and is therefore fully ionized at neutral pH, and the methylene fluorine atoms can undergo H-bonding. Peptides substituted with F$_2$Pmp exhibit higher binding affinities to SH2 domains than Pmp analogs [27]. 1000-Fold enhancements in affinities of F$_2$Pmp-containing peptide compared with those containing Pmp have been observed in assays against PTPs [28].

Fmoc-F$_2$Pmp-OH is the preferred derivative for the introduction of F$_2$Pmp residues as its use avoids the harsh conditions required to remove the ethyl protecting group usually employed for phosphonate protection [29]. However, lack of phosphate protection does result in sluggish coupling of Fmoc-F$_2$Pmp-OH and the subsequent amino acid derivative. Overnight coupling with HATU and 3 equivalents of DIPEA appears to be effective (Application 3-8).

Application 3-8: Synthesis of H-Trp-Ser-Lys-Asp-Thr-Ser-Phe(CF$_2$PO$_3$H$_2$)-Ala-OH using Fmoc-Phe(CF$_2$PO$_3$H$_2$)-OH

H-Trp(Boc)-Ser(tBu)-Lys(Boc)-Asp(OtBu)-Thr(tBu)-Ser(tBu)-Phe(CF$_2$PO$_3$H$_2$)-Ala-Wang resin was prepared automatically using an ABI 433 peptide synthesizer on Rink Amide MBHA resin. Phe(CF$_2$PO$_3$H$_2$) was introduced by treatment of the peptidyl resin with Fmoc-Phe(CF$_2$PO$_3$H$_2$)-OH/HATU/DIPEA 3:3:9 for 18 h. All other acylation reactions were carried out using a 5-fold excess of Fmoc-amino acids activated with 1 eq. of HBTU in the presence of 1 eq. of HOBt and 2 eq. of DIPEA for 1 h. The product was isolated by treating the peptidyl resin with TFA/TIS/water (95:2.5:2.5) (5 ml) for 3 h. The crude peptide was analyzed by HPLC (Figure 3-41) and ES-MS [expected M+H$^+$ 1071.4, found 1071.5].
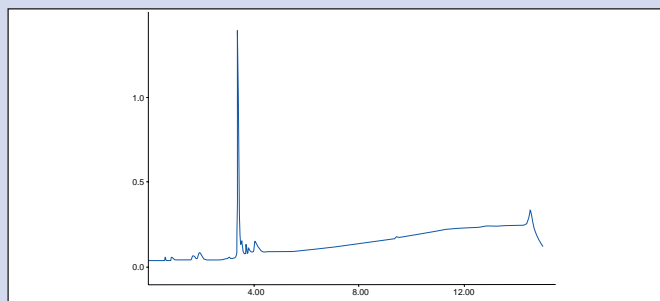
Fig. 3-41: HPLC elution profile and ESI-MS of crude phosphonopeptide.

Related products

852069	Fmoc-Ser(PO(OBzl)OH)-OH	p. 105
852244	Fmoc-D-Ser(PO(OBzl)OH)-OH	p. 106
852070	Fmoc-Thr(PO(OBzl)OH)-OH	p. 106
852245	Fmoc-D-Thr(PO(OBzl)OH)-OH	p. 106
852071	Fmoc-Tyr(PO(OBzl)OH)-OH	p. 106
852246	Fmoc-D-Tyr(PO(OBzl)OH)-OH	p. 107
852058	Fmoc-Tyr(PO$_3$H$_2$)-OH	p. 107
852090	Fmoc-Tyr(PO(NMe$_2$)$_2$)-OH	p. 107
852288	Fmoc-Phe(CF$_2$PO$_3$H$_2$)-OH	p. 108

3.11.3 Global phosphorylation

Global phosphorylation involves post-synthetic phosphorylation of unprotected hydroxyl groups on the solid support [2, 3, 30, 31] or in solution [32]. Of the two approaches, it is the more generally applicable, being suited to the synthesis of peptides containing phosphorylated Ser, Thr and Tyr residues by both Boc and Fmoc methodologies, and offers the additional advantage of furnishing both phosphorylated and unphosphorylated peptides from the same synthesis. A general outline of this method is shown in Figure 3-42.

The residue to be phosphorylated is generally incorporated without side-chain protection, although a selectively protected derivative such as Fmoc-Ser(Trt)-OH [33] can also be used. A variety of activation methods, including Pfp and Dhbt esters [3, 31, 34], TBTU [2] and HBTU [35], have been used for subsequent chain elongation without apparent unwanted acylation of the unprotected hydroxy side chain, however acylation of Ser by PyBOP® activated Fmoc-Arg(Pmc) has been reported [36]. The N-terminal residue is generally protected with Boc, either by direct incorporation of an N-α-Boc amino acid or by acylation of the unprotected N-terminus with Boc$_2$O.

Functionalization of the free resin bound hydroxyl has been carried out using either phosphochloridates [31, 37], H-phosphonates [38] and phosphoramidites [30]. The most frequently used reagents are dimethyl [39] , di-*t*-butyl [12, 30, 40], di-*p*-chlorobenzyl [30, 41] and dibenzyl-N,N-dialkyl phosphoramidites [2, 3, 42]. The dimethyl and dichlorobenzyl reagents are particularly suited to Boc synthesis.

The benzyl and *t*-butyl derivatives are compatible with Fmoc strategy because their respective protecting groups are removed during the course of the normal TFA cleavage reaction. However, best results are obtained using the benzyl protected amidite as the use of the *t*-butyl amidite can give rise to *H*-phosphonate by-products [8, 43]. Formation of this side-product is thought to arise through premature loss of one *t*-butyl group from the phosphite intermediate [44].

The introduction of diallyl-N,N-diisopropylphosphoramidite [45] adds a further degree of flexibility to the method since allyl is truly orthogonal to both Fmoc and Boc strategies. Mild and effective removal is achieved by either hydro-stannolysis or treatment with tetrakis-(triphenylphosphine) Pd(0).

For oxidation of the intermediate phosphite ester, *m*-chloroperbenzoic acid [35], aq. iodine [2] and *t*-butyl hydroperoxide [34, 35, 46] have been used. It was originally believed that this approach was not compatible with peptides containing the oxidizable residues, Met , Trp and Cys. However, if anhydrous *t*-butyl hydroperoxide is used, phosphopeptides containing these sensitive residues can be prepared without significant oxidation.

Oxidation can also be effected using elemental sulfur in CS$_2$ [47], tetraethylthiuram disulfide in CH$_3$CN [47], dibenzoyl tetrasulfide [48] and DTS [49] to yield the corresponding thiophosphorylated peptide. Phosphitylation should be effected with the benzyl protected amidite since S-*t*-butylation has been observed during cleavage when the *t*-butyl amidite was used for introduction of the phosphite moiety [48]. Furthermore, care must be taken to avoid prolonged exposure of thiophosphoryltyrosine-containing peptides to acidic conditions as the thiophosphate group is rapidly hydrolyzed at acidic pH [47, 48].

H-Ala-Gly-Arg(Pbf)-Th r(tBu)-Val-Ser-Gly-Tyr(tBu)-Gly-O-Resin

N-Cap with Boc$_2$O   Boc$_2$O

Boc-Ala-Gly-Arg(Pbf)-Thr(tBu)-Val-Ser-Gly-Tyr(tBu)-Gly-O-Resin

Phosphitylation   (BzlO)$_2$PN(i-Pr)$_2$/tetrazole

P(OBzl)$_2$

Boc-Ala-Gly-Arg(Pbf)-Thr(tBu)-Val-Ser-Gly-Tyr(tBu)-Gly-O-Resin

Oxidation   tBuOOH

$\overset{O}{\parallel}$P(OBzl)$_2$

Boc-Ala-Gly-Arg(Pbf)-Thr(tBu)-Val-Ser-Gly-Tyr(tBu)-Gly-O-Resin

Cleavage   TFA

O
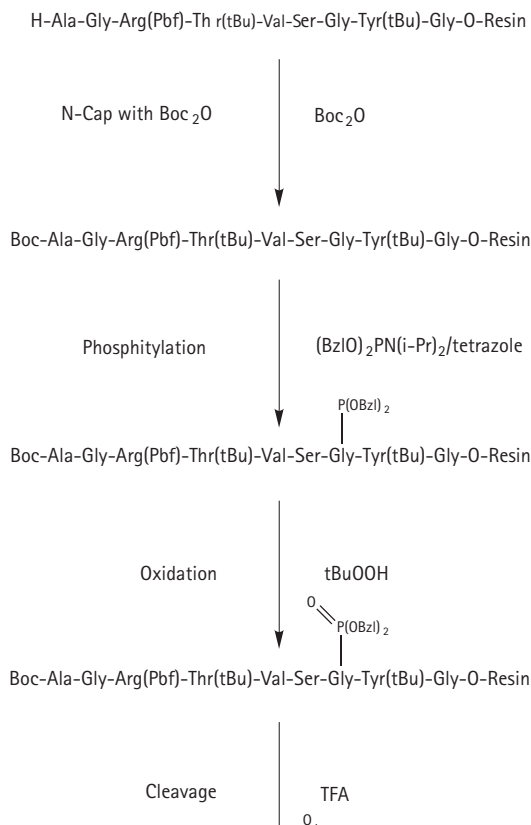
Fig. 3-42: Global phosphorylation.

General protocol for post-synthetic phosphorylation

Dibenzyl-N,N-diisopropylphosphoramidite is suitable for post-synthetic ("global") phosphorylation of peptides prepared using the Fmoc strategy as the benzyl protecting groups can be cleaved with TFA under standard conditions. The dibenzyl derivative is less hindered than the corresponding di-*t*-butyl derivative. This makes it more suitable for use in situations where the phosphorylation site is flanked by residues with bulky protecting groups (e.g. Pmc or Trt). Furthermore, in contrast to the *t*-butyl analog, the use of this reagent does not appear to be associated with the formation of *H*-phosphonate by-products.

Ser or Thr can be incorporated without side-chain protection, allowing for post-synthetic phosphorylation. However, there is an increasing risk of side reactions with every coupling cycle after incorporation of the unprotected Ser or Thr. Therefore, in longer peptides, or where there may be some other risk of unwanted side reaction, protection of the Ser or Thr side chain is recommended. Trt offers the ideal protection in these circumstances as it can be selectively removed from Ser or Thr under conditions where the peptide remains attached to the resin and all other side-chain protecting groups remain intact.

Method 3-62: General protocol for phosphorylation

IMPORTANT: Phosphoramidites are moisture and air sensitive, and so all manipulations of these reagents must be carried out under an atmosphere of dry nitrogen or argon.

1. Place 0.07 mmole of peptidyl resin in a suitable reaction vessel and dry o/n at 40°C under a good vacuum. Dry a 5 ml, gas-tight syringe, equipped with a needle, at 120°C for 1 h, and allow to cool in a desiccator.
2. Seal reaction vessel with a rubber septum. Flush the vessel with a stream of Ar delivered via a needle inserted through the septum . Flush the syringe with argon.
3. Insert needle of Ar line and the syringe needle into the septum of the DMF bottle and fill the syringe with 5 ml of solvent. Remove syringe and Ar line.
4. Remove the metal insert from the top of a tetrazole vial (350 mg, 5 mmole, Sigma). Insert syringe needle into the vial septum. Slowly add DMF to vial, allowing the displaced argon to fill the syringe between additions. Remove syringe; shake vial until contents dissolved.
5. Insert needle of Ar line and syringe needle into tetrazole vial septum; fill syringe with 3.6 ml of tetrazole solution. Remove syringe and Ar line.
6. Remove the metal insert from the top of a vial containing dibenzyl-N,N-diisopropylphosphoramidite (690 mg, 1.44 mmole) and insert syringe needle through septum. Add tetrazole solution to vial as described in step 4. Shake vial to mix contents.
7. Insert needle of Ar line and syringe into amidite vial septum. Fill syringe with amidite/tetrazole solution, and transfer to the reaction vessel using technique described in step 4. Gently agitate reaction vessel for 1 h.
8. Remove septum from reaction vessel and slurry resin onto a sintered glass funnel, quickly wash the resin three times with DMF, then immediately transfer resin to a clean, dry flask. Add enough dry DMF to cover resin and then add 3 M t-butyl hydroperoxide in isooctane (2.5 ml). Gently agitate flask for 30 min.
9. Remove septum, slurry resin into a clean sintered glass funnel, and wash with DMF, MeOH and ether. Dry resin and cleave in the normal manner.

Related products

Application 3-9: Synthesis of H-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Ser(PO$_3$H$_2$)-Leu-NH$_2$

Boc-Gly-Asp(OtBu)-Phe-Glu(OtBu)-Glu(OtBu)-Ile-Pro-Glu(OtBu)-Glu(OtBu)-Ser(Trt)-Leu-Rink Amide MBHA resin was prepared on an ABi 431A instrument using normal FastMoc chemistry. The N-terminal Gly was introduced as the Boc derivative. The Trt group was removed from Ser by treatment with 1% TFA/5% TES in DCM (3 x 3 min).

Phosphorylation of the resin was carried out as follows:

The peptidyl resin (0.1 g) was weighed into a test tube and dried at 40°C under high vacuum o/n. The test tube was sealed with a rubber septum and flushed with a stream of dry argon delivered via a needle.

Dry DMF (3.5 ml) was added to an ampoule of DNA grade tetrazole (500mg, 7.1 mmole), using a dry, argon flushed, gas tight syringe, equipped with a needle. 2.5 ml of this solution was syringe transferred to a vial containing dibenzyl-N,N-diisopropylphosphoramidite (586 mg, 2 mmole), and the resulting mixture added, again using a syringe, to the reaction vessel containing the peptidyl resin. Extra dry DMF was then added to ensure complete coverage of the resin, and the mixture gently agitated for 1 h. After this time, the resin was filtered off on a sintered glass funnel, washed with DMF, and treated for 30 min with 5.5 M t-butyl hydroperoxide in nonane (1 ml, 5 mmole), diluted with a small volume of DMF. The resin was washed and dried in the normal manner.

The phosphorylated peptide was cleaved from the resin using TFA /water/TIS (95:2.5:2.5) for 2 h. The crude peptide was analyzed by HPLC (Figure 3-43) and ESI (Figure 3-44) [expected M+H$^+$ 1366.3, found 1367.0].

Fig. 3-43: HPLC of crude phosphoserine peptide.
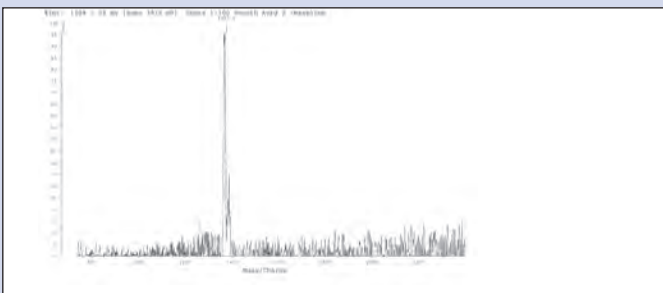
Fig. 3-44: MaldiTof-MS of crude phosphoserine peptide; the minor peak is attributed to M+K$^+$.

References

1. E. A. Kitas, et al. (1989) *Tetrahedron Lett.*, **30**, 6229.
2. a) E. A. Kitas, et al. (1991) *J. Chem. Soc., Chem. Commun.*, 338; b) E. A. Kitas, et al. (1991) *Helv. Chim. Acta*, **74**, 1314.
3. D. W. Andrews, et al. (1991) *Int. J. Peptide Protein Res.*, **38**, 469.
4. J. W. Perich & E. C. Reynolds (1991) *Int. J. Peptide Protein Res.*, **37**, 572.
5. J. W. Perich, et al. (1994) *Int. J. Peptide Protein Res.*, **43**, 39.
6. M. Ueki, et al. (1996) *Tetrahedron Lett.*, **37**, 4953.
7. H. G. Chao, et al. (1995) *J. Org. Chem.*, **60**, 7710.
8. E.A Ottinger, et al. (1993) *Biochemistry* **32**, 4354.
9. C. Garcia Echeverria (1995) *Lett. Pept. Sci.*, **2**, 93.
10. E. A. Ottinger, et al. (1996) *Pept. Res.*, **9**, 223.
11. P. White & J. Beythien in "Innovations & Perspectives in Solid Phase Synthesis & Combinatorial Libraries, 4th International Symposium", R. Epton (Ed.), Mayflower Scientific Ltd., Birmingham, 1996, pp. 557.
12. J. M. Lacombe, et al. (1990) *Int. J. Peptide Protein Res.*, **36**, 275.
13. T. Wakamiya, et al. (1994) *Chem. Lett.*,1099.
14. T. Vorherr, et al. (1995) *Bioorg. Med. Chem. Lett.*, **5**, 2661.
15. P. White, Poster 159 presented at the 17th American Peptide Symposium, San Diego, June 2001.
16. T. L. Gururaja & M. J. Levine (1996) *Pept. Res.*, **9**, 283.
17. G. Shapiro, et al. (1996) *Bioorg. Med. Chem. Lett.*, **6**, 409.
18. M. John, et al. (1996) *Pept. Res.*, **9**, 71.
19. T. Wakamiya, et al. (1997) *Bioorg. Med. Chem.*,**5**, 135.
20. J. Perich, et al. (1999) *Lett. Pept. Sci.*, **6**, 91.
21. N. L. Daly, et al. (2000) *Biochemistry*, **39**, 9039.
22. A. Ishida, et al. (2001) *J. Biochem.*, **129**, 745.
23. R. Pascal, et al. in "Peptides 2000, Proc. 26th European Peptide Symposium", J. Martinez & J.-A. Fehrentz (Eds), Éditions EDK, Paris, 2001, pp. 263.
24. T. Attard, et al. (2009) I*nt. J. Pept. Res. Ther.*, **15**, 69.
25. a) T. R. Burke, Jr. (2006) *Curr. Top. Med. Chem.*, **6**, 1465; b) I. Marseigne, et al. (1988) *J. Org. Chem.*, **53**, 3621.
26. S. M. Domchek, et al. (1992) *Biochemistry.*, **31**, 9865.
27. T. R. Burke, Jr., et al. (1994) *Biochemistry.* **33**, 6490.
28. T. R. Burke, Jr., et al. (1994) *Biochem. Biophys. Res. Commun.*, **204**, 129.
29. M. F. Gordeev, et al. (1994) *Tetrahedron Lett.*, **35**, 7585.
30. J. W. Perich & R. B. Johns (1988) *Tetrahedron Lett.*, **29**, 2369.
31. L. Otvos Jr., et al. (1989) *Int. J. Peptide Protein Res.*, **34**, 129.
32. J. W. Perich & R. B. Johns (1988) *Aust. J. Chem.*, **43**, 1623.
33. N. Pullen, et al. (1993) *Biochemistry*, **32**, 3958.
34. H. B. A. de Bont, et al. (1990) *Tetrahedron Lett.*, **31**, 2497.
35. J. W. Perich, et al. (1991) *Tetrahedron Lett.*, **32**, 4033.
36. M. Yon, et al. in "Innovations and Perspectives in Solid Phase Synthesis, 1993: Biological and Biomedical Applications", R. Epton (Ed.), Mayflower Worldwide Ltd., Birmingham, 1994, pp. 707.
37. P. Homozdiari & D. Gani (1996) *Tetrahedron Lett.*, **37**, 8227.
38. E. Larsson & B. Lüning (1994) *Tetrahedron Lett.*, **35**, 2737.
39. J. W. Perich & R. B. Johns (1989) *Synthesis*, 701.
40. J. W. Perich & R. B. Johns (1988) *Synthesis*, 142.
41. H. B. A. deBont, et al. (1990) *Recl. Trav. Chim. Pays-Bas*, **109**, 27.
42. W. Bannwarth & A. Trzeciak (1987) *Helv. Chim. Acta*, **70**, 175.
43. Q. Xu, et al. (1997) *Lett. Pept. Sci.*, **3**, 333.
44. J. W. Perich (1998) *Lett. Pept. Sci.*, **5**, 49.
45. W. Bannwarth & E. Küng (1989) *Tetrahedron Lett.*, 32, 4219.
46. G. Staerkaer, et al. (1991) *Tetrahedron Lett.*, **32**, 5389.
47. E. Kitas, et al. (1994) *Int. J. Peptide Protein Res.*, 43, 146.
48. W. Tegge (1994) *Int. J. Peptide Protein Res.*, 43, 448.
49. Q. Xu, et al. in "Peptides: Chemistry, Structure & Biology", P. T. P. Kaumaya & R. P. Hodges (Eds.), Mayflower Scientific Ltd., Birmingham, 1996, pp. 123.

3.12 Glycopeptide synthesis

3.12.1 Fmoc–Ser/Thr(Ac$_3$AcNH–α–Gal)–OH

One of the most important classes of *O*-linked glycosides is based on 2-acetamido-2-deoxy-α-D-galactopyranosyl (AcNH-α-Gal) attached to serine or threonine. Such O-glycosides are found in a wide range of proteins, such as mucin secreted from epithelial cells, the tumor-associated Tn-antigen, and gp120 from HIV [1]. Thus, synthetic O-linked glycopeptides are important tools for elucidating the role of O-linked glycosides and for drug discovery and design.

Fmoc-Ser(Ac$_3$AcNH-α-Gal)-OH and Fmoc-Thr(Ac$_3$AcNH-α-Gal)-OH are excellent tools for the introduction of AcNH-α-Gal modified serine and threonine residues as the *O*-glycosidic linkage and the *O*-acetyl protection in these building blocks are stable to both piperidine and TFA [2].

3.12.2 Fmoc–Asn(Ac$_3$AcNH–β–Glc)–OH

In contrast with the *O*-linked glycosides, which are structurally diverse, the *N*-linked glycosides all contain a mannotriosido-di-*N*-acetyl-chitobiose core linked via a $\beta(1\rightarrow N^\beta)$ linkage to Asn. Access to partial *N*-linked glycopeptide structures, containing some or all of this common core, will facilitate the elucidation of the role of *N*-linked glycosides through the study of model systems.

Novabiochem offers a glycopeptide precursor for the simplest of these model systems, in which a single *N*-acetylglucosamine moiety is attached to the side chain of Asn. The material is provided with acetyl protection of the carbohydrate hydroxyls. The *N*-glycosidic linkage and the acetyl protection are stable to both piperidine and TFA [3-7] making it possible to use this derivative with standard protocols in solid phase peptide synthesis.

3.12.3 Fmoc–Ser/Thr(Ac$_3$AcNH–β–Glc)–OH

Modification of serine or threonine residues with 2-acetamido-2-deoxy-β-D-glucopyranosyl (AcNH-β-Glc) is more akin to phosphorylation than other protein glycosylations [8]. The addition and removal of *O*-AcNH-β-Glc is a dynamic process controled by a transferase, UDPGlcNAc polypeptide transferase (OGT), and removed by a β-*N*-acetylglucos-aminidase. Only a single sugar is added the carbohydrate is not further extended. *O*-AcNH-β-Glc glycosylation processes are thought to be involved in transcription, signal transduction, apoptosis, and glucose homeostasis. Fmoc-Ser(Ac$_3$AcNH-β-Glc)-OH and Fmoc-Thr(Ac$_3$AcNH-β-Glc)-OH are excellent tools for the introduction of AcNH-β-Glc modified serine and threonine residues as the *O*-glycosidic linkage and the *O*-acetyl protection in these building blocks are stable to both piperidine and TFA.

3.12.3 Glycopeptide synthesis

All N- and O-linked oligosaccharide amino acid derivatives offered by Novabiochem are compatible with standard protocols in Fmoc solid phase peptide synthesis. Owing to the high cost of these reagents, their introduction is best carried out manually using small reagent excesses and the coupling checked using the TNBS or Kaiser. Any standard coupling method can be used for this purpose.

Following chain assembly, removal of the acetyl protecting groups can be carried out by treatment of the peptidyl resin with methanolic ammonia [2] or sodium methoxide/DMF/methanol [9], or treatment of the cleaved peptide with catalytic sodium methoxide in methanol [5].

Application 3-10 illustrates the synthesis of an Asn(Ac$_3$AcNH-β-Glc) containing glycopeptide.

Application 3-10: Synthesis of H-Ala-Gly-Gly-Asp-Tyr-Asn(Ac$_3$AcNH-β-Glc)-Ala-OH

The peptide was assembled on an ABI431A instrument, using standard FastMoc cycles with pre-loaded Fmoc-Ala-Wang resin (0.1 mmole scale). Whilst the standard FastMoc cycle uses a 10-fold excess of Fmoc-amino acid, this was reduced to a 2-fold excess of the Fmoc-glycoamino acid in order to conserve material. Because of the lower excess, the coupling time was extended to 2 h and checked for completeness by the Kaiser test .

The peptide was cleaved and deprotected using TFA/water/TIS (95:2.5:2.5) for 2 h. The crude, O-acetylated peptide was analyzed by HPLC (Figure 3-45) and PD-MS, expected 996.9, found 996.9 (Figure 3-46).

The peptide was suspended in MeOH and treated with sodium methoxide until the apparent pH of the mixture reached 12.5 when tested with damp pH paper. The removal of acetyl groups was monitored by HPLC. When judged to be complete by this criterion, the reaction was halted by adding glacial AcOH drop-wise to neutralize (again, judged by damp pH paper). The crude product was purified by HPLC, isolated and lyophilized.
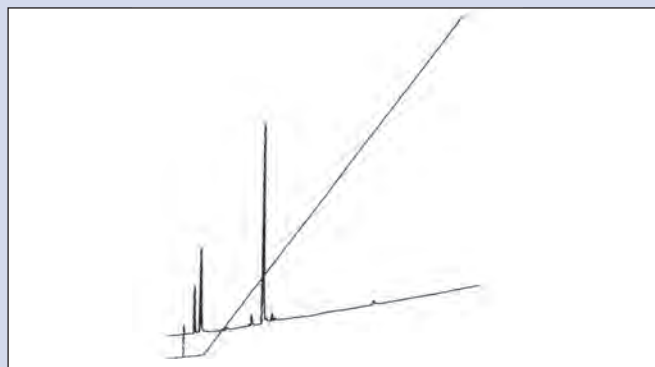
Fig. 3-45: HPLC of crude O-acetylated glycopeptide.
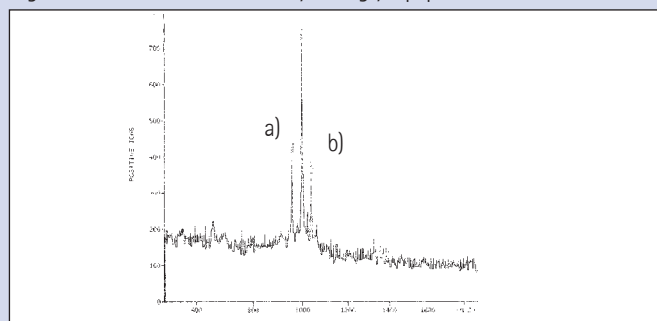
Fig. 3-46: PD-MS of O-acetylated glycopeptide; a) is due to de-O-acetylation which occurs during analysis, b) represents M+K$^+$.

Related products

References

1. N. Sharon & H. Lis in "Glycosciences: status & perspectives", H.-J. Gabius & S. Gabius (Eds.), Chapman & Hall, Weinheim, 1997, pp. 133.
2. J. Kihlberg in "Fmoc solid phase peptide synthesis: a practical approach", W. C. Chan & P. D. White (Eds.), Oxford University Press, Oxford, 2000, pp. 195.
3. L. Otvos, et al. (1989) *Pept. Res.*, **2**, 362.
4. L. Otvos, et al. (1990) *Tetrahedron Lett.*, **31**, 5889.
5. M. Meldal & K. Bock (1990) *Tetrahedron Lett.*, **31**, 6987.
6. J. Kihlberg & T. Vuljanic (1993) *Tetrahedron Lett.*, **34**, 6135.
7. M. Meldal, et a.l (1994) *Int. J. Peptide Protein Res.*, **43**, 529.
8. M. C. Galan, et al. (2011) *Org. Biomol. Chem.*, **9**, 3598.
9. M. Liu, et al. (2005) *Carbohydrate Res.*, **340**, 2111.

3.13 Synthesis of sulfotyrosine peptides

3.13.1 Introduction

It is believed that up to 1% of all protein tyrosine residues in eukaryotes may be sulfated [1]. However, the biological role of tyrosine sulfation is poorly understood compared to that of other post-translational modifications like phosphorylation. Sulfation is thought to be involved in the modulation of the extracellular protein-protein interactions of secreted and transmembrane proteins. It is also an essential requirement for maintaining the biological activity of a number of peptide hormones such as gastrin II, cholecystokinin, and caerulein.

One of the principal hurdles to studying tyrosine sulfation is the difficulty in obtaining site-specifically sulfated peptides for use as bioloigical probes or antigens for raising antibodies, as until recently the chemical synthesis of sulfotyrosine-containing peptides has been far from routine. This is because tyrosine sulfate esters are rapidly degraded in acid and fragment during mass spectrometry, making their synthesis and characterisation highly problematic. The issue of desulfation has been addressed by employing low temperature TFA cleavage [2] or introducing the sulfate group post cleavage [reviewed in 1]. However, the latter is technically difficult and not compatible with all amino acid side chains and the former is only partially effective. Furthermore, both approaches need careful attention to experimental conditions and are not amenable to high-throughput synthesis.

Recently, a number of research groups have found that protecting the sulfate stabilizes it during the TFA cleavage, enabling standard reaction conditions to be used without significant loss of the sulfate. The use of three protecting groups have been examined in detail: trichloroethyl (TCE) [3, 4], dichlorovinyl (DCV) [4] and neopentyl (nP) [5, 6]. Of these nP protection appears to offer particular promise as the group is stable to piperidine and TFA, but can be removed post-cleavage with either sodium azide or ammonium acetate [6].

3.13.2 Fmoc–Tyr(SO₃nP)–OH
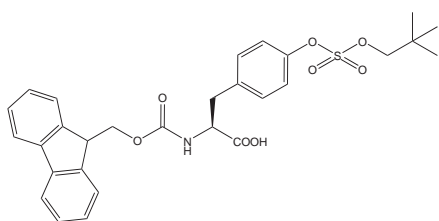
Assembly

In contrast to the traditional derivatives for introduction of sulfoTyr, such as Fmoc-Tyr(SO₃·NnBu₄)-OH and Fmoc-Tyr(SO₃Na)-OH, which require the use of HBTU/DIPEA activation for their introduction, the fully side-chain protected derivative, Fmoc-Tyr(SO₃nP)-OH, can be coupled using any standard coupling method. Furthermore, Fmoc-Tyr(SO₃nP)-OH also has excellent solubility in DMF or NMP, faciliting its use in automated synthesizers without modification of existing protocols.

Cleavage

Whilst peptides containing Tyr(SO₃nP) can be cleaved from the resin with TFA-based cleavage cocktails, extended cleavage times should be avoided as some loss of the nP group can occur during prolonged contact with TFA. Ideally, the progress of the cleavage reaction should be followed by

sampling of the reaction and analysis by HPLC. Cleavage times of 1.5 hours generally result in minimal loss of the nP group. Peptides containing Tyr(SO₃nP) are more strongly retained on HPLC columns than the corresponding Tyr and Tyr(SO₃⁻) peptides. They can also be detected by positive mode ES-MS and MALDI-MS.

Deprotection of SO₃nP

nP sulfate esters are stable to piperidine but are readily cleaved using small powerful nucleophiles such as azide or cyanide. For peptides the reaction is most conveniently conducted in DMSO or DMF at 50 °C. Overnight treatment is usually sufficient to effect complete reaction. Following reaction, removal of excess azide can be effected by SPE or HPLC, using non-acidic buffers. For some peptides, the nP group can be removed by simply dissolving the peptide in 2 M ammonium acetate and warming the mixture at 37 °C overnight.

Method 3-63: Cleavage of nP group

Azide method
1. Dissolve the nP protected sulfoTyr peptide in minimum volume of DMSO.
2. Add NaN₃ (10 eq.) relative to peptide. Incubate peptide at 50 °C overnight.
3. Dilute reaction mixture with three volumes of water and apply to a conditioned SPE cartridge.
4. Elute cartridge with 5 column volumes of water, and then elute peptide from the cartridge with MeCN/water 6:4 and lyophilize peptide containing eluates.

Ammonium acetate method
1. Dissolve the nP protected sulfoTyr peptide in minimum volume of 2 M ammonium acetate. MeCN may be added to aid dissolution of the peptide.
2. Incubate peptide at 37 °C overnight.
3. Apply mixture to an HPLC column and elute peptide with a gradient formed between 0.1 M ammonium acetate and MeCN/0.1 M ammonium acetate (7:3).
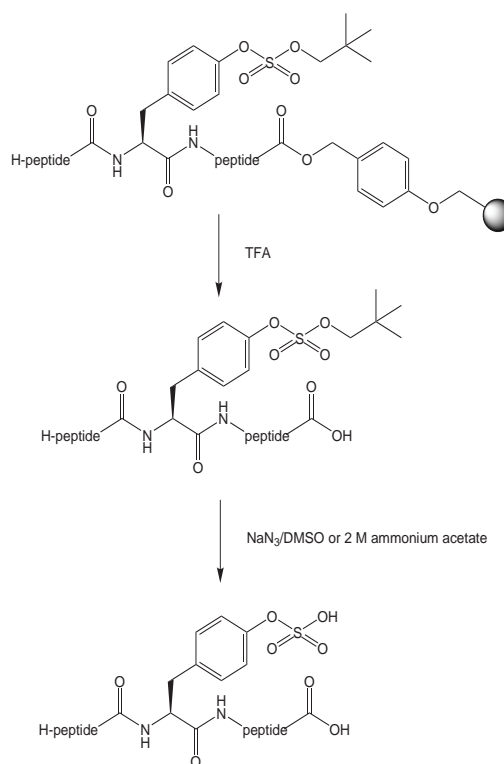
Fig. 3-47: Synthesis of Tyr(SO₃⁻) peptides using Fmoc-Tyr(SO₃nP)-OH

Purification and mass spectrometry

For RP-HPLC purification of sulfotyrosine, buffers containing ammonium acetate at pH 7 should be used, as some loss of the sulfate group may occur if standard TFA-containing buffers are used. A gradient formed from 0.1 M ammonium acetate and acetonitrile/0.1 M ammonium acetate (7:3) works well. Repeated lyophilization of the product fractions will be required to fully remove ammonium acetate.

Characterization of sulfopeptides by mass spectrometry is best performed using negative ion mode. With MALDI in positive ion mode, only the desulfated peptide is usually detected. In the case of ES ionization, the target peptide together with the desulfated peptide is generally detected.

Example syntheses

The utility of Fmoc-Tyr(SO$_3$nP)-OH was exemplified through the synthesis of two model peptides. In the case of the first example, the nP group was not removed by treatment with ammonium acetate but was easily removed with azide.

Application 3-11: Synthesis of H-Tyr(SO$_3^-$)-Glu-Phe-Lys-Lys-Ala-NH$_2$

H-Tyr(SO$_3$nP)-Glu(OtBu)-Phe-Lys(Boc)-Lys(Boc)-Ala was assembled using an ABI 433 automated synthesizer on Rink Amide MBHA resin using 10-fold excesses of Fmoc-amino acids activated with HCTU/DIPEA. A coupling time of 60 min was used throughout. Fmoc removal was effected by three 5 min treatments of 20% piperidine in DMF. The resin was treated with TFA/TIS/water(95:2.5:2.5) for 1.5 h and the purity of the isolated product checked by RP-HPLC (Figure 3-48).

A small sample of H-Tyr(SO$_3$nP)-Glu-Phe-Lys-Lys-Ala-NH$_2$ was dissolved in DMSO containing 10 eq. NaN$_3$. The mixture was heated overnight at 50 °C. HPLC analysis (Figure 3-49) revealed the removal of the nP group to be almost complete. The reaction mixture was applied to a HPLC column and eluted with a gradient of 0.1 M ammonium acetate/acetonitrile, to afford the desired peptide, ES-MS (M+H$^+$)found 978.6; expected 978.4.
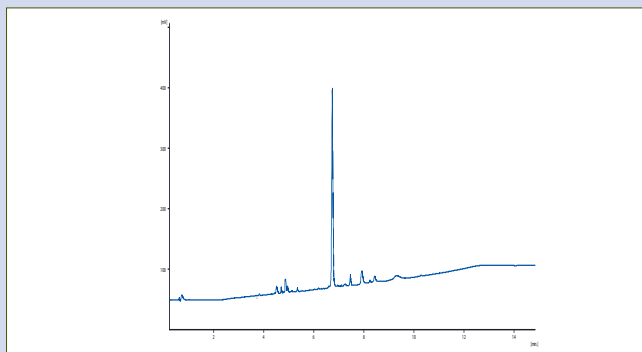
Fig. 3-48: HPLC profile of crude H-Tyr(SO$_3$nP)-Glu-Phe-Lys-Lys-Ala-NH$_2$.
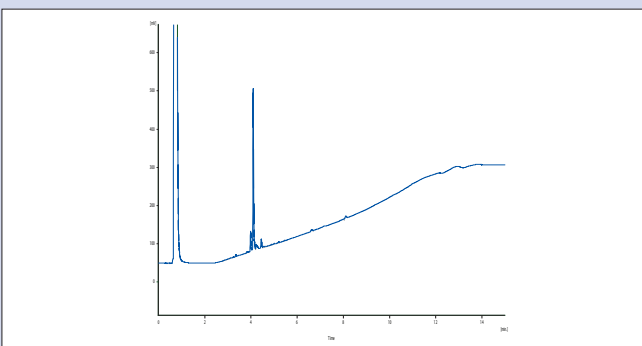
Fig. 3-49: HPLC profile of crude H-Tyr(SO$_3^-$)-Glu-Phe-Lys-Lys-Ala-NH$_2$.

Application 3-12: Synthesis of H-Tyr-Glu-Tyr(SO$_3^-$)Leu-Asp-Tyr-Asp-Phe-NH$_2$

H-Tyr(tBu)-Glu(OtBu)-Tyr(SO$_3$nP)-Leu-Asp(OtBu)-Tyr(tBu)-Asp(OtBu)-Phe was assembled using an ABI 433 automated synthesizer on Rink Amide SpheriTide resin using 10-fold excesses of Fmoc-amino acids activated with HCTU/DIPEA. A coupling time of 60 min was used throughout. Fmoc removal was effected by three 5 min treatments of 20% piperidine in DMF. The resin was treated with TFA/TIS/water(95:2.5:2.5) for 1.5 h and the purity of the isolated product checked by HPLC (Figure 3-50) and characterized by ES-MS (M+H$^+$) found 1276.2; expected 1276.4.

A small sample of H-Tyr-Glu-Tyr(SO$_3$nP)-Leu-Asp-Tyr-Asp-Phe-NH$_2$ was dissolved in 2 M ammonium acetate/MeCN (2:1) The mixture was incubated overnight at 37 °C. HPLC analysis (Figure 3-51) revealed the removal of the nP group to be almost complete. The reaction mixture was applied to a HPLC column and eluted with a gradient of 0.1 M ammonium acetate/acetonitrile, to afford the desired peptide, ES-MS (M+Na$^+$)found 1227.6; expected 1227.4.
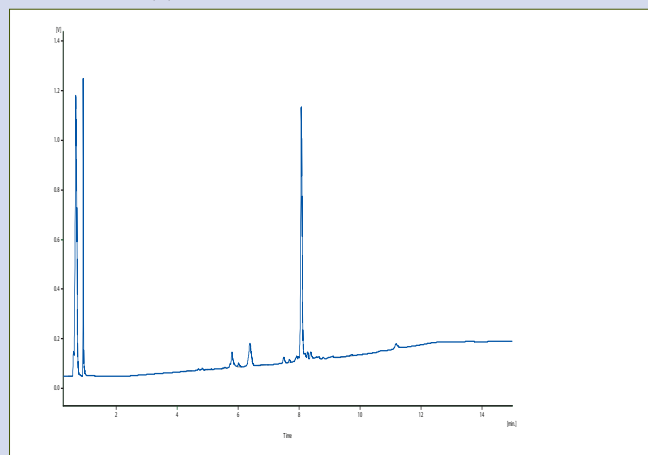
Fig. 3-50: HPLC profile of crude H-Tyr-Glu-Tyr(SO$_3$nP)-Leu-Asp-Tyr-Asp-Phe-NH$_2$.
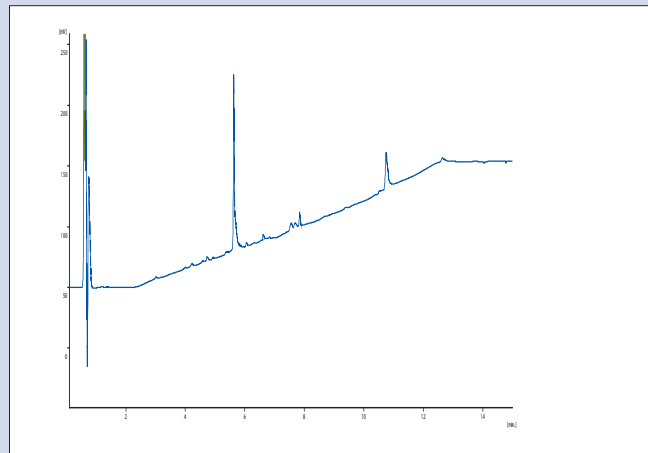
Fig. 3-51: HPLC profile of crude Fig. 4: HPLC profile of crude H-Tyr-Glu-Tyr(SO$_3^-$)-Leu-Asp-Tyr-Asp-Phe-NH$_2$.

Related products

| 852347 | Fmoc-Tyr(SO$_3$nP)-OH | p. 86 |

3.14 Synthesis of peptide aldehydes

Peptide aldehydes are potent inhibitors of serine, aspartyl and cysteinyl proteases and are valuable intermediates for the preparation of reduced amide-bond peptidomimetics and transition-state isosteres.

There are three principle approaches for their preparation: firstly, oxidation of an appropriate peptide alcohol [1] or 1,2-diol [2]; secondly, reduction of a peptide carboxylic acid derivative, such as a Weinreb ester [3, 4]; finally, step-wise or fragment synthesis using a masked pre-formed aldehyde [5-10]. The last forms the basis of one of the simplest and most effective methods, which involves the solid-phase immobilization of an amino aldehyde by formation of an oxazolidine between a pre-formed Fmoc-amino aldehyde and H-Thr-Gly-NovaSyn® TG resin (Figure 3-52) [10].
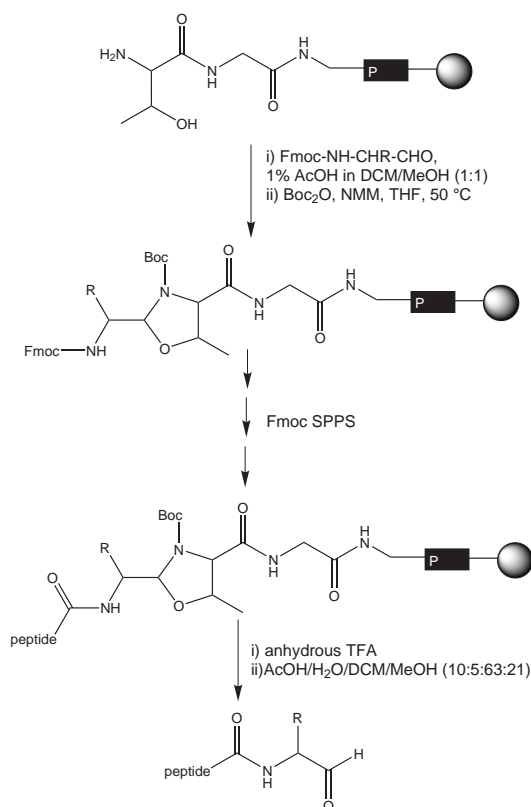
Fig. 3-52: Preparation of peptide aldehydes using H-Thr-Gly-NovaSyn® TG resin.

After loading the resin, the oxazolidine nitrogen should be blocked by treatment with Boc-anhydride. The resultant acyloxazolidine is stable to base and is compatible with Fmoc protocols.

Cleavage from the resin and side-chain deprotection is carried out in two stages. Firstly, side-chain protecting groups are removed with anhydrous TFA. Secondly, the peptide aldehyde is cleaved from the resin with AcOH/water/DCM/MeOH (10:5:63:21) [11].

Novabiochem® presently offers H-Thr-Gly-NovaSyn® TG resin pre-loaded with aldehydes of Arg, Asp, Leu, Phe, and Val.

If the appropriate pre-loaded resin is not available, the required Fmoc-amino aldehyde intermediate can be prepared by oxidation of the corresponding amino alcohol using Dess-Martin's periodinane [11]. A very

promising variation of this method, particularly for small scale production of Fmoc-amino aldehydes, involves the use of IBX-polystyrene (Method 3-63, Figure 3-53) [12]. This reagent cleanly converts protected amino and peptide alcohols to aldehydes, and because it is attached to an insoluble polymer, it can be simply removed at the end of the reaction by filtration. Amino aldehydes produced in this way can be loaded onto H-Thr-Gly NovaSyn® TG resin and then used for standard peptide chemistry. Since oxazolidine formation is completely selective for aldehydes, even mixtures of amino alcohol and aldehyde obtained from incomplete oxidation reactions can be used to load the resin.
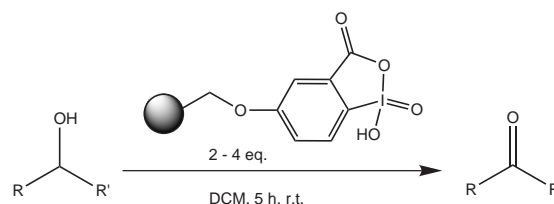
Fig. 3-53: Oxidation of alcohols with IBX-PS.

3.14.1 Preparation of aldehydes using IBX-PS

Oxidation of Fmoc–Ile–ol and Boc–D–Phe–Cys(Trt)–Phe–D–Trp–Lys(Boc)–Thr(tBu)–Cys(Acm)–Thr(tBu)-ol

To exemplify the use of IBX-PS in the preparation of peptide and amino aldehydes, Fmoc-Ile-ol and a crude fully protected octreotide (prepared by NaBH$_4$ cleavage from HMBA-AM resin) were oxidized as described in Method 3-64. In both cases, conversion to the aldehyde was complete in 24h (Figure 3-54).

> ### Method 3-64: Oxidation with IBX-PS
> 1. Dissolve protected peptide or amino alcohol (1 eq.) in DCM. Add IBX-PS (3 eq.).
> 2. Gently agitate mixture o/n.
> 3. Remove resin by filtration, wash with DCM and evaporate filtrates to dryness.

3.14.2 Solid phase synthesis of peptide aldehydes

The synthesis of peptide aldehydes using H-Thr-Gly-NovaSyn® TG resin (Figure 3-55) is illustrated in Application 3-13. The resin can be loaded with an Fmoc-protected amino aldehyde as described in Method 3-65, or alternatively, for peptides containing a C-terminal aspartal, argininal, leucinal, phenylalaninal, or valinal residue, pre-loaded resins are available.
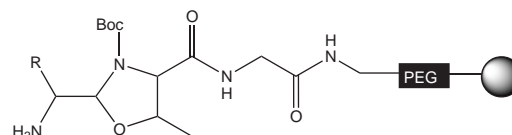
Fig. 3-55: Amino aldehydes attached to H-Thr-Gly-NovaSyn® TG resin.
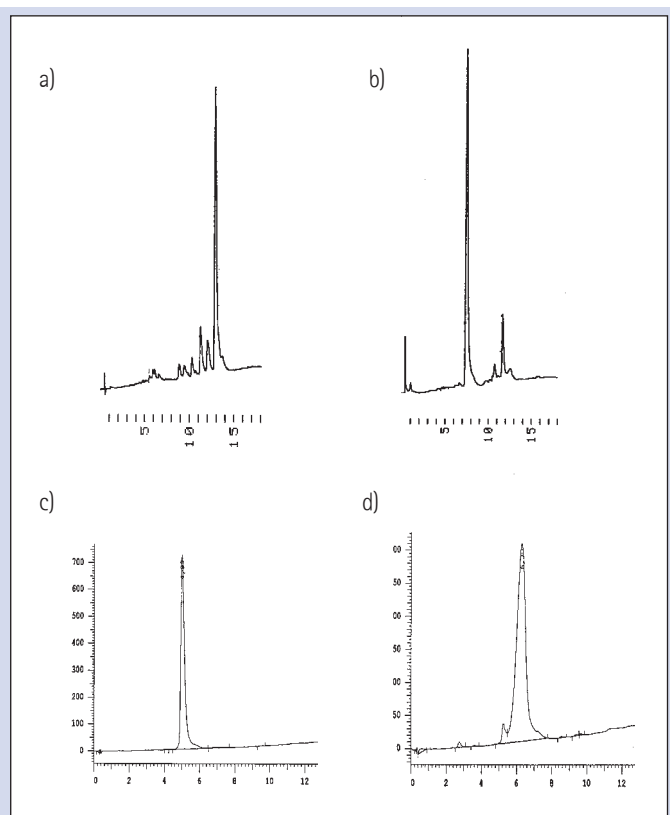
Fig. 3-54: HPLC elution profile of a) crude protected octreotide; b) crude protected octreotide aldehyde; c) Fmoc-Ile-ol; d) Fmoc-Ile-H.

Method 3-65: Loading of H-Thr-Gly-NovaSyn® TG resin

1. Suspend H-Thr-Gly-NovaSyn® TG resin in 1% AcOH in MeOH/DCM (1:1) containing Fmoc-amino aldehyde (5 eq. relative to resin substitution) in DCM.
2. Gently agitate mixture at rt for 4 h and monitor by TNBS test.
3. Remove resin by filtration, wash with DCM, DMF, and THF.
4. Treat resin with Boc$_2$O (5 eq.) and NMM (5eq.) in THF at 50°C for 3 h to cap oxazolidine nitrogen.
5. Remove resin by filtration and wash with THF, DCM, and DMF

Related products

855049	H-Thr-Gly-NovaSyn® TG resin	p. 213
856073	H-Arg(Boc)$_2$-H NovaSyn® TG resin	p. 213
856072	H-Asp(OtBu)-H NovaSyn® TG resin	p. 213
856071	H-Leu-H NovaSyn® TG resin	p. 213
856144	H-Phe-H NovaSyn® TG resin	p. 214
856145	H-Val-H NovaSyn® TG resin	p. 214
855043	IBX polystyrene	p. 284

References

1. J.-T. Woo, et al. (1995) *Bioorg. Med. Chem. Lett.*, **5**, 1501.
2. L. Zhang, et al. (1998) *Proc. Natl. Acad. Sci. USA*, **95**, 9184.
3. G. Guichard, et al. (1993) *Pept. Res.*, **6**, 121.
4. J. A. Fehrentz, et al (1995) *Tetrahedron Lett.*, **36**, 7871.
5. R. M. McConnell, et al. (1993) *J. Med. Chem.*, **36**, 1084.
6. K. T. Chapman (1992) *Bioorg. Med. Chem. Lett.*, **2**, 613.
7. A. M. Murphy, et al. (1992) *J. Am. Chem. Soc.*, **114**, 3156.
8. C. Pothion, et al. (1997) *Tetrahedron Lett.*, **38**, 7749.
9. F. Guillaumie, et al. (2000) *Tetrahedron Lett.*, **41**, 6131.
10. N. J. Ede, et al. (2000) *J. Pept. Sci.*, **6**, 11.
11. S. Barthélémy, et al. poster presented at "abc Technologies 2004", Basel, Switzerland
12. G. Sorg, et al. (2001) *Angew. Chem. Int. Ed.*, **40**, 4395.

Synthesis of H–Ala–Arg–Gly–Leu–Pro–Tyr–Ala–Glu–Leu–H

Application 3-13: Synthesis of H-Ala-Arg-Gly-Leu-Pro-Tyr-Ala-Glu-Leu-H

H-Leu-H NovaSyn® TG resin (1 g, 0.23 mmole) was suspended in DMF and left to swell for 30 min. Chain extension was then carried out for 1 h using a 3-fold excess of Fmoc-amino acid activated with PyBOP® (3 eq.) in the presence of HOBt (0.5 eq.) and DIPEA (6 eq.), with the exception of the N-terminal residue (Ala), which was introduced using a Boc-protected derivative. Fmoc group removal was effected with DBU/piperidine/DMF (2:2:96). After assembly of the target sequence, the resin was washed with DMF, i-PrOH, THF, and MeOH and dried o/n. The side-chain protecting groups were removed by treatment with 100% TFA (2 x10 min). The resin was then washed with DCM and the product was cleaved from the resin by three treatments with AcOH/water/DCM/MeOH (10:5:63:21) for 30 min, to afford the product in 38% yield with the HPLC profile shown in Figure 3-56a. Peak* was identified by ESMS to be the dimethyl acetal. ESMS expected (M+H$^+$)1044.56, found 1045.59.
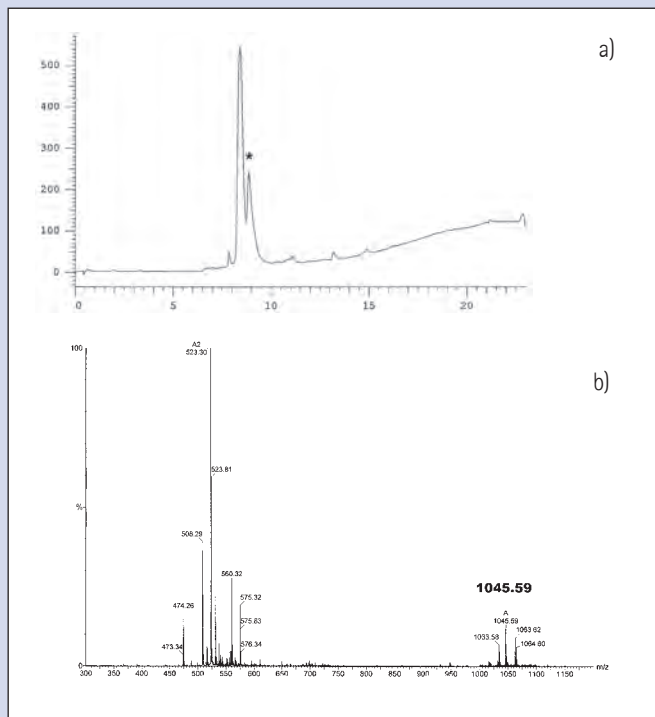
Figure 3-56: a) HPLC elution profile of crude H-Ala-Arg-Gly-Leu-Pro-Tyr-Ala-Glu-Leu-H; b) ES mass spectrum of crude H-Ala-Arg-Gly-Pro-Tyr-Ala-Glu-Leu-H.

4: Building blocks for solid phase synthesis

4.1   Choosing amino acid derivatives

Choosing the "best" amino acid derivative is one of the most important and sometimes difficult aspects of peptide synthesis. Often compromises must be made in yield, purity, or both when choosing the most appropriate derivatives for a particular synthesis. We can not overemphasize the importance of taking the time to plan a synthesis carefully. Inappropriate choice of amino acid derivatives can lead to insurmountable problems later in the synthesis or during purification.

Not all amino acids require side-chain protection. Those which do can undergo irreversible side reactions, either during the synthesis or during cleavage, if the protecting group is not compatible, or if an inappropriate cleavage method is used. The choice of side-chain protection is not only dependent on the chemistry used, but also on the coupling and cleavage methods employed, solubility of the derivative in question, and the sequence of the peptide being synthesized. Because of the sheer number of different amino acid derivatives available, it would be impossible to list all their properties and applications here. For that reason, this section will concentrate only on the "problem species". All the information provided is a starting point and is not meant to be an extensive review of the literature. We encourage you to read the literature cited and the reviews by Stewart and Young [1], Barany, *et al.* [2], Fields & Noble [3], Atherton & Sheppard [4] and Chan & White [5].

Table 4-1 shows Novabiochem®'s recommendation for Boc-amino acids, whilst Table 4-2 shows our recommended Fmoc-amino acids. In both cases, only the tri-functional amino acids are listed.

4.1.1   Arginine
The tri-functional guanidino side-chain group of arginine is strongly nucleophilic and as such is easily acylated during SPPS if not protected. Ideally all three side-chain nitrogen atoms should be blocked; however, in practice, the majority of protecting groups only block the ϖ-nitrogen.

These groups can be divided into several classes [6, 7]: nitro, urethane (acyl), arenesulfonyl and trityl. The NO_2 group protects the ϖ-nitrogen of arginine and is normally removed using H_2/Pd [8]. Unfortunately this group is prone to several side reactions during acylation and cleavage [9]. Arg(NO_2) is available in both N-α-Boc and N-α-Fmoc protected forms. For urethane protection of the Fmoc-Arg side chain, blocking of a single ϖ-nitrogen with Boc [10] or δ- and ϖ-nitrogens with Adoc groups [11] has been investigated. However, the protection offered by these derivatives does not appear to be sufficient to mask the nucleophilicity of the guanidino function, leading to substantial ornithine formation through acylation of the unprotected ϖ-nitrogen during coupling and subsequent intramolecular decomposition during deprotection [12, 13]. This problem has been overcome with the introduction by Verdini [14] of

a derivative in which both ϖ-nitrogens have been protected by Boc, but this compound is hindered and often requires extended coupling times to ensure complete reaction.

In contrast to urethane, a single arene sulfonyl-based arginine protecting group appears to offer complete blocking of the guanidino side chain and this class includes the most commonly used groups: Tos, Mts, Mtr, Pmc and Pbf. Arg(Tos) prepared from tosyl chloride is widely used in Boc SPPS. This group is extremely stable, and may only be removed by high HF/anisole [15] or Na/NH$_3$ [16]. Peptides containing this group normally require extended cleavage times which can have a deleterious impact on sensitive residues.

In order to design more acid-labile protecting groups, the effects of adding electron-donating substituents to the phenyl ring of the benzenesulfonyl group have been studied [17, 18], leading to the development of the 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr) group. Mtr protection has been extensively used in Fmoc synthesis, despite the fact that Mtr removal often requires several hours of treatment with TFA/thioanisole [19] and can lead to modification of Trp residues. Several different cleavage mixtures have been developed to overcome these problems, and the best of these, especially for the cleavage of peptides containing multiple Arg and Trp residues, is 1 M TMSBr in TFA [20]. This reagent has been shown to remove up to four Mtr groups in 15 minutes without modification of Trp residues [21].

To overcome the obvious limitations of the Mtr group, Ramage [22, 23] undertook a thorough examination of the mechanistic factors controlling lability of arene sulfonyl protecting groups. These studies led to the development of the Pmc group. It has comparable acid sensitivity to *t*-butyl, making it ideally suited to the preparation of peptides containing multiple arginine residues, but like Mtr, it also causes sulfonation of Trp residues during TFA mediated cleavage [24]. This side reaction does appear to be less serious with Pmc; presumably the rapid cleavage of this protecting group limits the opportunity for intramolecular transfer of the Pmc group from Arg to Trp. This problem can be eliminated by the use of Fmoc-Trp(Boc)-OH for the introduction of Trp residues [25].

Table 4-1: Recommended protected derivatives for Boc synthesis.

Boc-Arg(Tos)-OH[1]	Boc-Asp(OBzl)-OH
Boc-Asp(OcHx)-OH[2]	Boc-Cys(4-MeBzl)-OH[3]
Boc-Cys(Acm)-OH[4]	Boc-Glu(OBzl)-OH
Boc-His(Dnp)-OH[5]	Boc-Lys(2-Cl-Z)-OH
Boc-Ser(Bzl)-OH	Boc-Thr(Bzl)-OH
Boc-Trp(For)-OH[6]	Boc-Tyr(2-Br-Z)-OH

1   Recommended for long peptides.
2   Recommended for protection of Asp in sequences prone to aspartimide formation, Asp-Gly, Asp-Ser, Asp-Asn. Requires high HF for removal.
3   Generates a cysteinyl peptide on HF treatment.
4   Stable to HF. Enables peptide to be purified prior to liberation of air sensitive thiol groups.
5   Dnp group should be removed prior to liberation of N-terminal or side-chain amino functions. Cleaved by treatment with thiophenol.
6   Removed during low-high HF cleavage if thiophenol is used. Can be removed prior to cleavage by treatment of the peptidyl resin with 10% piperidine in DMF, or after high HF cleavage by treatment with 30 mM hydroxylamine.

During their work on the development of the Pmc group, Ramage and co-workers [26] had originally discounted dihydrobenzofuran derivatives in favor of those based on chromans, as X-ray crystallographic data indicated the latter to have a more favorable oxygen lone pair-aromatic π-system orbital overlap. This assumption has been challenged by Carpino, *et al.* [27], who found Pbf, the dihydrofuran analog of Pmc, to possess superior deprotection kinetics. The rate of removal of Pbf during TFA cleavage appears to be 1.2 - 1.4 times faster than that of Pmc. More importantly, Pbf has been shown to give rise to lower levels of Trp sulfonation than Pmc, particularly when used in combination with Trp(Boc) and silane scavengers [28]. Pbf can also be used for side-chain protection of monomethyl and asymmetric dimethyl-arginine (ADMA) in Fmoc SPPS. Using Fmoc-Arg(Me,Pbf)-OH and Fmoc-ADMA(Pbf)-OH, peptides containing monomethyl and asymmetric dimethyl-arginine can be prepared without modification of standard protocols. For symmetric dimethyl-arginine (SDMA) di-Boc protection is used.

O-Sulfonation of Ser and Thr residues has been noted during the deprotection of sulfonyl-based protecting groups, such as Mtr and Pmc [29]. This side reaction was suppressed by the addition of thiocresol to the cleavage cocktail.

The Trt group is well known in peptide chemistry for its acid-labile properties and was investigated for Fmoc-Arg protection [30]. However, Fmoc-Arg(Trt) is not readily soluble in DCM/DMF [3] and is therefore not normally used.

Bernatowicz and Matsueda have introduced a novel approach to the introduction of arginine involving the guanylation of ornithine side chains using 1-guanylpyrazole [31].

Related products

853036	Boc-Arg(di-Z)-OH	p. 110
853013	Boc-Arg(Tos)-OH	p. 111
853037	Boc-D-Arg(Tos)-OH	p. 111
852107	Fmoc-ADMA(Pbf)-OH	p. 7
852310	Fmoc-SDMA(Boc)$_2$-ONa	p. 7
852101	Fmoc-Arg(Boc)$_2$-OH	p. 8
852105	Fmoc-Arg(Me,Pbf)-OH	p. 8
852067	Fmoc-Arg(Pbf)-OH	p. 9
852165	Fmoc-D-Arg(Pbf)-OH	p. 9
852034	Fmoc-Arg(Pmc)-OH	p. 10

4.1.2 Asparagine and glutamine

Asparagine and Glutamine derivatives can be incorporated without side-chain protection. However, they are known to undergo several side reactions, of which the most important is nitrile formation *via* dehydration of the carboxamide side chain during acylation [32 - 34]. This side reaction has been reported to occur with a variety of activating reagents, including carbodiimides [32, 34], BOP [33, 34], PyBOP® [35] and HBTU [35], but fortunately this can be minimized by the addition of HOBt to the coupling reaction. Acylation with activated esters results in minimal dehydration of both Asn and Gln [33, 36, 37, 38].

Blocking the free amide side chain also eliminates dehydration, and a variety of groups have been exploited for this purpose, including Dmcp, Mbh, Xan, Tmob and Trt. In Fmoc synthesis, the trityl protected derivatives Fmoc-Asn(Trt)-OH and Fmoc-Gln(Trt)-OH are the most widely used [39, 40]. These compounds are more soluble in DMF and DMA than their Mbh or Tmob counterparts [39] and the trityl group, once liberated by TFA (usually 90 - 95% TFA with water and scavengers) does not alkylate Trp or other sensitive side chains. Coupling protocols using DIC/HOBt, PyBOP®, TBTU or HBTU have given excellent results, even in peptides containing multiple Asn or Gln derivatives. Trt protection does, however, have certain limitations. Firstly, cleavage of the N-Trt group can be sluggish, particularly in the case of N-terminal Asn residues which can require over 4 hours for complete Trt removal with 95% TFA [41]. Secondly, these derivatives have low solubility in DMF compared to other Fmoc-protected amino acids. Thirdly, protected peptides containing Asn(Trt) and Gln(Trt) often have poor solubility. Finally, Asn(Trt) in particular is notorious for inducing aggregation during solid phase synthesis. Carpino's N-dimethylcyclo-propylmethyl (Dmcp) derivatives of Fmoc-protected asparagine and glutamine [42] solve these issues. Cleavage of the Dmcp group from the side-chains of Asn and Gln is rapid, even when the residue is located at the N-terminus of a peptide. Coupling of Dmcp-protected derivatives appears to be faster than that of the corresponding hindered Trt derivatives: for example, coupling of Fmoc-Asn(Trt)-OH with TFFH in the synthesis of ACP gave significant quantities of des-Asn peptide, whereas with Fmoc-Asn(Dmcp)-OH none of this by-product was generated [42]. Furthermore, protected peptides containing Asn(Dmcp) and Gln(Dmcp) residues appear to have enhanced solubility. This observation would indicate that peptides containing these residues would be less prone to aggregation during SPPS. Finally, Fmoc-Asn(Dmcp)-OH and Fmoc-Gln(Dmcp)-OH are also more soluble in DMF than Fmoc-Asn(Trt)-OH and Fmoc-Gln(Trt)-OH, thereby facilitating coupling reactions at higher concentration.

Intramolecular cyclization of Gln to pyroglutamate is an important cause of chain termination. This side reaction is particularly problematic in the Boc strategy as it is catalyzed by weak acids such as Boc-amino acids during coupling and TFA after deprotection [1]. Fortunately, this problem can be minimized by using high concentrations of TFA for Boc removal

Table 4-2: Recommended protected derivatives for Fmoc synthesis.

Fmoc-Arg(Pbf)-OH	Fmoc-Asn(Dmcp)-OH[1]
Fmoc-Asn(Trt)-OH	Fmoc-Asp(OtBu)-OH
Fmoc-Asp(OMpe)-OH[2]	Fmoc-Cys(Trt)-OH[3]
Fmoc-Cys(Acm)-OH[4]	Fmoc-Cys(STmp)-OH[5]
Fmoc-Gln(Trt)-OH	Fmoc-Glu(OtBu)-OH
Fmoc-His(Clt)-OH[6]	Fmoc-His(Trt)-OH
Fmoc-Lys(Boc)-OH	Fmoc-Ser(tBu)-OH
Fmoc-Thr(tBu)-OH	Fmoc-Tyr(tBu)-OH
Fmoc-Trp(Boc)-OH	

1 Recommended for N-terminal Asn residues.
2 Recommended for peptides containing Asp-Xaa, where Xaa=Asp, Asn, Cys, Gly, Thr.
3 Recommended for the routine preparation of cysteinyl peptides.
4 Stable to TFA. Can be removed with I$_2$ to form cyclic disulfide peptides in a single step. Has been used in combination with Trt to prepare peptides containing multiple disulfide bonds.
5 Removed with thiols or tributylphosphine. Can be removed whilst the peptide is still attached to the resin to allow on resin disulfide bond formation. Not compatible with cleavage mixtures containing thiol scavengers.
6 Reduced enantiomerization compared to His(Trt). Ideal if coupling is slow or hindered.

and by separately activating the incoming amino acid [43]. Side-chain protection of Gln residues also prevents pyroglutamate formation, and Boc-Gln(Xan)-OH can be used to this effect. Base catalyzed pyroglutamate formation in Fmoc chemistry is extremely low [3].

4.1.3 Aspartic and glutamic acids

The most common side reaction of Asp residues is cyclization to form aspartimide, with concomitant epimerization, and the subsequent reopening of the ring to yield undesirable β-aspartyl peptides [44-46]. In Fmoc chemistry, it was originally believed this could be overcome by the use of Asp(OtBu) [47] and Asp(O-1-Ada) [48] derivatives. However, a number of authors have reported observing aspartimide and piperidide formation [49 - 54] and the problem is clearly more widespread than originally thought. Dölling, *et al.* [49] detected piperidides and aspartimides in peptides containing the Asp(OX)-Asn(Trt) sequence [X=tBu, Ada]. In a more systematic study Lauer, *et al.* [50] found sequences of the type Asp(OtBu)-X, where X is Gly, Thr(tBu), Cys(Acm), Asn(Trt) and Asp(OtBu) to be most problematic. The addition of either HOBt or 2,4-dinitrophenol to the piperidine reagent was shown to suppress these side reactions. More recently, have shown the addtion of 1 M Oxyma Pure to be particularly effective in this regard [51]. However, ultimately total elimination of the problem in difficult cases can only be achieved by employing amide protected derivatives for the introduction of the residue preceding the Asp [53] or Fmoc-Asp(OtBu)-(Dmb)Gly-OH (see section 3.6, page 3.23).

Karlström and Undén [54] have introduced the extremely hindered Mpe (β-3-methylpent-3-yl) protecting group in an attempt to overcome this problem. This group was shown to drastically reduce aspartimide formation compared to tBu in model systems, and the routine use of Fmoc-Asp(OMpe)-OH is strongly recommended. For further information, please see section 3.6, page 3.23.

In Boc chemistry, Asp(OcHx) provides protection against succinimide formation in sensitive sequences, such as Asp-Gly and Asp-Ser [55, 56]. Temperature and time play an important role in suppressing aspartimide formation when using strong acids. Please refer to the section on cleavage and deprotection for more details.

Like Asp, Glu can also undergo cyclization, leading to the subsequent formation of γ-Glu peptides. Although not as serious a problem as with Asp, Glu is normally protected using the same side-chain protecting groups as Asp. Glu dehydration, followed by anisylation of the resulting acylium ion can occur during cleavage with HF. Both succinimide formation (Asp) and acylation of scavenger molecules by the glutamyl side chains can be prevented by using the low-high cleavage procedure of Tam, *et al.* [57].

4.1.4 Cysteine

The synthesis of peptides containing Cys residues is often complicated because some peptide products require the Cys residue to be in the free sulfhydryl form while others require inter- or intramolecular disulfide bonds. For a discussion on the management of cysteine containing peptides, the reader is referred to the excellent article by Albericio, *et al.* [58] which contains many detailed protocols and section 3.10, page 3.32.

There are currently a number of different groups which can effectively protect cysteine. In Fmoc chemistry, these have included Trt, Mmt, Acm, tBu, tButhio, and STmp, of which the most commonly used is Trt. This group generates the free thiol upon deprotection with TFA and is particularly useful for the preparation of peptide antigens which require conjugation of the peptide to carrier proteins. Table 4-3 summarizes the deprotection methods for the most commonly used groups.

Unlike Trt and Mmt, the Acm, tBu, tButhio and STmp protecting groups require additional procedures for deprotection after assembly of the peptide. When used in combination, these derivatives enable differential deprotection of Cys residues in polycysteinyl peptides, thus permitting selective formation of disulfide bridges [59]. The *t*-butyl protecting group of cysteine is labile to TFMSA [60, 61], TMSBr/thioanisole [62] and HF at 20°C [58], and like the Acm group, it can be deprotected using mercury (II) acetate [60, 61, 63, 64] (Method 3-39, page 3.34). Cys(Acm), Cys(Mmt) and Cys(Trt) residues can be oxidized directly to cystine (disulfide) using iodine or thallium (III) trifluoroacetate [58]; Trp and Met should be protected to prevent side-reactions. The rates of iodine oxidation of Cys(Acm) and Cys(Trt) are strongly solvent dependent, and this fact can be utilized synthetically for the selective conversion of Cys(Trt) residues to disulfide bridges in the presence of Cys(Acm) groups [65]. Iodine oxidation can cause the formation of Trp-2-thioesters and Met sulfoxides [66] and iodination of Tyr residues [67]. However, these side reactions can be minimized through the appropriate choice of reaction conditions [66, 68]. In addition, iodine and thallium oxidation of Cys(Acm) has recently been found to cause migration of the Acm group to the side chains of Ser, Thr or Gln residues [67-70].

Cys(tBu), Cys(MeBzl) and Cys(MeOBzl) residues may be directly converted to cystine by treatment with MeSiCl$_3$/Ph$_2$SO/TFA [58, 71]. This method provides an extra level of selectivity, and has been used to prepare regioselectively peptides containing up to three disulfide bridges [71]. Similarly, Cys(tBu), Cys(MeBzl) residues may be converted to cystine by TFA/DMSO/anisole [72]. At room temperature only Cys(tBu) is affected; Cys(MeBzl) requires heating to 70°C to react. This selectively has been exploited to achieve in one-pot the regioselective formation of two disulfide bridges [73].

Table 4-3: Deprotection methods for cysteine. Key: + cleaved; – not cleaved; n not tested. [1]Reaction should be conducted in TFA. [2]Product of the reaction is cystine. [3]1–3%TFA.

Protecting group	Reagent						
	TFA	TFMSA	Hg^{2+}	Ag$^+$	I$_2$	Tl^{3+}	RSH
tBu	–	+	+[1]	–	–	n	–
Trt	+	+	+	+	+[2]	+[2]	–
Mmt	+[3]	+	+	+	+[2]	+[2]	–
StBu	–	–	n	n	n	n	+
Acm	–	–	+	+	+[2]	+[2]	–
STmp	–/+	+	n	n	n	n	+

Yoshida, et al. have reported the use of silver tetrafluoroborate (AgBF$_4$) in TFA for the deprotection of Tacm and Acm protecting groups of cysteine [74]. In an improved approach, Tamamura, et al. instead used silver trifluoromethanesulfonate; treatment of the product generated in this reaction with DMSO-aq. HCl results in removal of the silver ions as AgCl and disulfide bond formation (Method 3-36, page 3.32) [75].

Disulfide bond formation from free sulfhydryls can be performed using a number of different methods. The two most popular are air oxidation [1, 58, 61] or oxidation using potassium ferricyanide (K$_3$Fe(CN)$_6$) [58, 61, 76]. Both procedures use a dilute solution of peptide in water to prevent the formation of aggregates and polymers. The potassium ferricyanide method is faster than air oxidation which may require several days. In both cases disulfide bond formation can be monitored by HPLC. Another mild method has been described which makes use of DMSO in TFA [58, 72, 77] or aqueous buffered solutions [78] for disulfide bond formation. No side reactions were observed with amino acids such as Met, Tyr, Trp with aqueous DMSO [78], but DMSO in TFA should be avoided with Trp and Met.

In Boc chemistry, the pMeOBzl, pMeBzl, and Acm groups are often employed. Cys(Acm) residues are resistant to HF cleavage and require additional methods for their deprotection.

Another approach is to perform the cyclization while the protected peptide is still anchored to polymeric supports used for solid phase synthesis [58]. Varying results were obtained for Boc- and Fmoc-strategies using either thallium (III) trifluoroacetate in TFA or iodine for

the cyclization step [79] with Acm protection. Cys(tButhio) and Cys(STmp) can be deprotected on the solid phase by reduction with thiols (e.g. β-mercaptoethanol [76])(Method 3-41, page 3.34) or tributylphosphine [59], whereas Mmt can be selectively removed on the solid phase with 1-3% TFA in DCM (Method 3-42 page 3.34). The combination of STmp and Mmt have been used to form two disulfide bridges on the solid phase [80]. The method involved first removing the STmp group with mercaptoethanol, and then conversion of the liberated thiols to a disulfide with N-chlorosuccinimide (NCS). Treatment with 2% TFA in DCM cleaved the Mmt groups and the second disulfide formed with oxidation again with NCS.

Hondal and colleagues [81] have shown that DTNB in TFA can cleave MeOBzl, tBu, StBu, and Acm from side chain of cysteine to form the corresponding Cys(Npys) derivative. In the case of StBu and Acm, the reaction requires the addition of thioanisole. A similar approach was adopted by Wade and co-workers [82] in the synthesis of relaxin. Here, the A chain containing one disulfide bridge, a Cys(tBu) and Cys(Acm) residues, was converted by treatment with TFA/TFMSA/DPDS to the activated disulfide bridged Cys(Pys), Cys(Acm) peptide, which could then be selectively linked via another disulfide bridge to the B-chain.

Considerable enantiomerization has been noted during the introduction of Fmoc-Cys(Trt)-OH using base mediated in situ activation with reagents such as TBTU [83 - 85]. Coupling under neutral conditions using preformed symmetrical anhydrides [83], OPfp esters [84], DIPCDI/HOBt [85]; or the use of HBTU or PyBOP®/HOBt in DMF/DCM without preactivation [84, 85] appears to minimize this problem. In automated synthesis where preactivation is often unavoidable, substitution of DIPEA for TMP has been shown to reduce enantiomerization to within acceptable levels (1.1%) [85]. Enantiomerization can also occur during coupling of the first residue and during chain extension. When Cys is the C-terminal residue of peptide acids, the use of 2-ClTrt resin is recommended, since the loading of this support does not require carboxy activation and is consequently free from enantiomerization. The trityl resin also protects the Cys residue from enantiomerization during piperidine-mediated Fmoc removal [86]. The use of Wang-type resins for the anchoring of Cys is not recommended.

4.1.5 Histidine

Histidine is one of the most problematic amino acids in peptide synthesis, by both Boc and Fmoc strategies. There are two main problems associated with the use of histidine: acylation and enantiomerization.

Acylation

Acylation of unprotected histidine during subsequent coupling cycles occurs when temporary protection of histidine is used (e.g. Boc-His(Boc)), or when the protecting group is not completely stable to the conditions of subsequent coupling or deprotection (e.g. Boc-His(Tos)). It is a relatively minor problem as the products are normally removed during either the deprotection step of each cycle or the final deprotection of the peptide, although there can be wastage of reagents as a result of this side reaction. However, the imidazole must remain protected for use in segment condensation. The unprotected imidazole group can also catalyze peptide formation at free hydroxyl groups on the resin. This emphasizes the importance of capping the derivatized resin.

Enantiomerization

This is the most serious problem during coupling of histidine due to the reactivity of the imidazole nucleus. It has been shown that enantiomerization can be suppressed by blocking the π-nitrogen [87] (Figure 4-1). However, most protecting groups for histidine actually block the τ-nitrogen. The fact that these suppress enantiomerization, to a greater or lesser extent, is due to electronic effects reducing the basicity of the π-nitrogen (steric effects may also play a part).
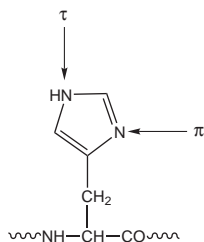
Fig. 4-1: Histidine: imidazole nitrogens.

The various histidine derivatives in current use (mostly offered by Novabiochem®) are described overleaf.

Boc–His(Tos)–OH

Boc-His(Tos)-OH is probably the most popular of the Boc derivatives due to its solubility and the ease with which the tosyl group can be removed by the normal deprotection conditions (liquid HF or TFMSA) [88]. It does, however, have a number of disadvantages. The N-im-tosyl group on histidine is susceptible to cleavage by HOBt. This can cause problems in subsequent coupling cycles if DCC/HOBt or the PyBOP® reagent are used, leading to chain termination by tosylation of the amino terminus. This only has to happen to the extent of a few percent each cycle to cause appreciable reduction in yield in a long peptide. For this reason, many people prefer to remove the tosyl group with 1 M HOBt in DCM after the coupling reaction. Another serious side reaction with Boc-His(Tos)-OH has been reported, when it is used in conjunction with HOBt and acetic anhydride capping [89].

Boc-His(Tos)-OH is not stable for long periods at room temperature. For this reason, the material must be stored in the freezer as soon as it is received. Boc-His(Tos)-OH does not couple satisfactorily in some sequences. In these cases, Boc-His(Dnp)-OH has been found to work.

Boc–His(Dnp)–OH

The advantage of Dnp for protection of histidine is that it is stable to almost all coupling conditions and, indeed, may succeed in difficult couplings where Boc-His(Tos)-OH has failed. However, its main drawback is the fact that it is also stable to liquid HF and TFMSA, so a separate deprotection step is necessary at the end of the synthesis (Method 3-18, page 3.26). Another disadvantage of Dnp is that it leaves the peptide bright yellow after deprotection. The color (due to Dnp-thiophenol) can be removed by gel filtration on Sephadex G-25 in 10-30% acetic acid. This simple purification step is well worth incorporating routinely in the work-up of all peptides, as it removes scavengers and nonvolatile products of the deprotection reaction before proceeding to further purification by ion exchange or HPLC.

Boc–His(Boc)–OH•DCHA

This derivative provides protection for only the critical coupling step as both Boc groups are removed simultaneously during deprotection [90]. This leads to acylation of the imidazole at every coupling and, although the adduct is removed by TFA at each cycle, this is wasteful of reagents.

Fmoc–His(Trt)–OH

Although the Trt group blocks the τ-nitrogen rather than the π-nitrogen, it has been shown to be effective at suppressing enantiomerization [91], except in those instances where coupling is sluggish due to steric hindrance or aggregation [92]. In the coupling of His to Pro with TBTU/DIPEA 4.5% enantiomerization was observed [93], which could be reduced to >0.8% with DEPBT and 2.8% with DCC/HOBt activation respectively [94]. The Trt group is stable to the conditions of coupling and deprotection, but is readily removed by 95% TFA for 1 hour at 20°C. This derivative couples well as the symmetrical anhydride, OPfp ester or using the PyBOP® reagent or TBTU/HBTU. Enantiomerization has been observed when using Fmoc-His(Trt)-OH for attachment to Wang resin. Therefore, it is better to use the 2-chlorotrityl resin as attachment of Fmoc-His(Trt)-OH to this resin is without risk of enantiomerization. See section 2.4, page 2.6 for more details.

Fmoc–His(Clt)–OH

The use of Fmoc-His(Trt)-OH for the production of protected peptide fragments can result in the formation of a mixture of fully and partially protected peptides because loss of the τ-trityl group can occur during the HFIP/DCM or 1% TFA in DCM treatments used to release products from 2-chlorotrityl or HMPB-AM resins. In constrast, the τ-Clt group in Fmoc-His(Clt)-OH is completely stable to these mild acids, enabling the preparation of protected peptides free from partially deprotected by-products.

Furthermore, in model studies we have found Fmoc-His(Clt)-OH to give about a third less enantiomerization than Fmoc-His(Trt)-OH.

Fmoc–His(Boc)–OH•CHA

Like Fmoc-His(Trt)-OH, the Boc-group protects the τ-nitrogen. However, the smaller bulk of the Boc group compared to Trt, coupled with the greater stability of Trt towards nucleophiles, suggests that Boc will be less effective than Trt in suppressing enantiomerization [19]. Several side reactions leading to a C-terminal truncated sequence and the formation of succinimide from Asp-His were encountered when using the above-mentioned derivative on a continuous flow instrument during the synthesis of an N-acetylated undecapeptide [95].

4.1.6 Methionine

The principal side reaction with methionine is the acid catalyzed oxidation of Met to Met(O) during cleavage and deprotection [96, 97]. In Fmoc chemistry Met is introduced without side-chain protection. The formation of Met(O) is prevented by using ethylmethylsulfide [98] or thioanisole [99] during cleavage and deprotection.

In Boc chemistry, methionine can be introduced in its unprotected form. However, spontaneous oxidation can occur during cleavage with strong acids. Alternatively methionine can be introduced as Met(O) during the synthesis and then reduced to Met using the low-high HF (or TFMSA) cleavage procedure outlined in Method 3-17, page 3.25.

Alkylation of methionine during HF cleavage can be prevented by using DMS in combination with p-thiocresol or anisole [100]. Met(O) can also be reduced to Met after cleavage using N-methylmercaptoacetamide (MMA)[101-103], ammonium iodide/DMS [104], ammonium fluoride/2-mercaptoethanol [105], TiCl$_4$ [106] or TMSBr/EDT [58, 107]. Reduction with ammonium iodide/DMS has been shown not to affect disulfide bonds [108].

4.1.7 Tryptophan

There are two main side reactions which occur with tryptophan residues: oxidation of Trp during synthesis and alkylation of the indole ring by carbonium ions generated during cleavage [1]. In Boc chemistry, the Trp(For) derivative has been used to overcome both of these problems [1]. The formyl group is stable to acid cleavage reagents and can be deprotected either before normal HF cleavage or by thiolytic deprotection using the low-high HF or TFMSA procedure [109]. These procedures are provided in Method 3-19, page 3.25, and Method 3-22, page 3.27. Avoid the use of thioanisole during HF cleavage if your peptide contains tryptophan. In Fmoc chemistry, the Trp has been incorporated without side-chain protection. Problems due to t-butylation were normally controlled using scavenging mixtures containing EDT [110-112]. However, sulfonation by the by-products from the deprotection of Mtr [113], Pmc [24] and Pbf [28] protected Arg residues, and reattachment of the peptide to the resin support *via* the side chain of a C-terminal Trp residue [114] can only be reduced, but not eliminated, by varying the scavenger mixtures. These problems were resolved with the introduction by Novabiochem®'s own research group of Fmoc-Trp(Boc)-OH [25]. This derivative has proven to be extremely effective in preventing these side reactions, and is now firmly established in the repertoire of standard reagents for Fmoc SPPS.

Mechanism of Boc removal

During normal deprotection with TFA the t-butyl moiety is removed, leaving the indole protected with an N-carboxy group [115], thereby preventing alkylation, peptide re-attachment or sulfonation. Tryptophan is subsequently regenerated during the course of the normal lyophilization procedure, since this carbamic acid intermediate readily decarboxylates in acidic aqueous media (Figure 4-2).
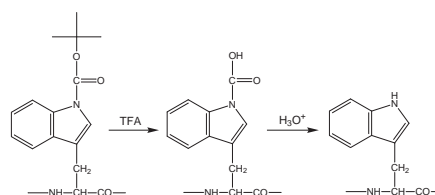
Fig. 4-2: Mechanism of Boc removal.

Cleavage of Trp and Arg–containing peptides

N^{in}-Boc protection prevents sulfonation of Trp, even with sequences containing multiple Arg residues [116] (Application 4-1). It is especially effective when used in conjunction with thiol-containing scavenger mixtures such as Reagent K [117], but also works well with silanes [28,116]. The efficacy of using Trp(Boc) in Fmoc synthesis has now been confirmed by a number of independent studies [28, 118, 119]. The combination of Trp(Boc) and Arg(Pbf) seems to be optimal [28].

Application 4-1: Synthesis of peptides containing multiple Arg(Pmc) residues with Trp(Boc)

The peptides shown in Figure 4-3 were prepared automatically on a NovaSyn® Crystal using either N^{in}-Boc protected or unprotected tryptophan. Coupling reactions were carried out using a mixture of Fmoc amino acid/HOBt/PyBOP® (1:1:1) activated with DIPEA (2 eq.) or Fmoc amino acid pentafluorophenyl esters. Peptidyl resins were cleaved by treatment with TFA/thioanisole/phenol/EDT/water (87.5:5:2.5:2.5:2.5).

Ac-Trp(X)-Arg(Pmc)-Arg(Pmc)-Arg(Pmc)-Arg(Pmc)-Val-KA
1:Product
2:Sulfonated by-products

H-Ile-Asn(Trt)-Ser(tBu)-Arg(Pmc)-Trp(X)-Trp(X)-Cys(Acm)-Asn(Trt)-Asp(OtBu)-Gly-Arg(Pmc)-Thr(But)-Pro-Gly-Ser(tBu)-Arg(Pmc)-Asn(Trt)-Leu-Ala-Asn(Trt)-Ile-Pro-Cys(Acm)-Ser(tBu)-Ala-Leu-KR
1:Product
2:Sulfonated by-products
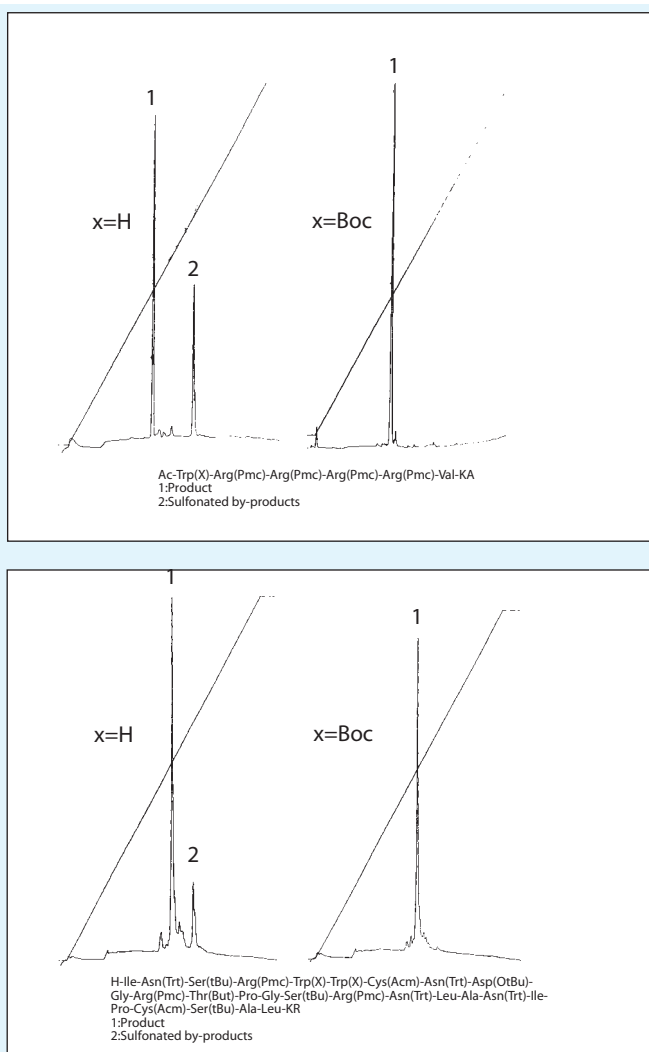
Fig. 4-3: HPLC profiles of total crude products obtained from syntheses of model peptides containing Trp and Arg residues.

Reduction of Trp by silanes

Trialkylsilanes are very effective scavengers, especially for those highly stabilized cations which are not irreversibly scavenged by thiols, such as trityl [120], Tmob [121] and the Rink linker. However, these reagents can cause reduction of tryptophan residues [122], converting the side-chain indole to an indoline. This effect is greatly reduced with Trp(Boc), as demonstrated in Application 4-2.

Peptide re-attachment

This can be a particular problem for peptides containing C-terminal Trp, giving severely reduced yields in some cases. Trp(Boc) is effective in limiting this side reaction.

Application 4-2: Cleavage with silanes

The peptide was prepared automatically on a NovaSyn® Crystal using either N^{in}-Boc protected or unprotected tryptophan. Coupling reactions were carried out using a mixture of Fmoc amino acid/HOBt/PyBOP® (1:1:1) activated with DIPEA (2 eq.). Peptidyl resins were cleaved by treatment with TFA/TES/water (90:5:5). The HPLC elution profile of the crude product is shown in Figure 4-4.

Ac-Trp(X)-Arg(Pmc)-Arg(Pmc)-Arg(Pmc)-Arg(Pmc)-Val-KA
1:Reduced by-products
2:Product
3:Sulphonated by-products
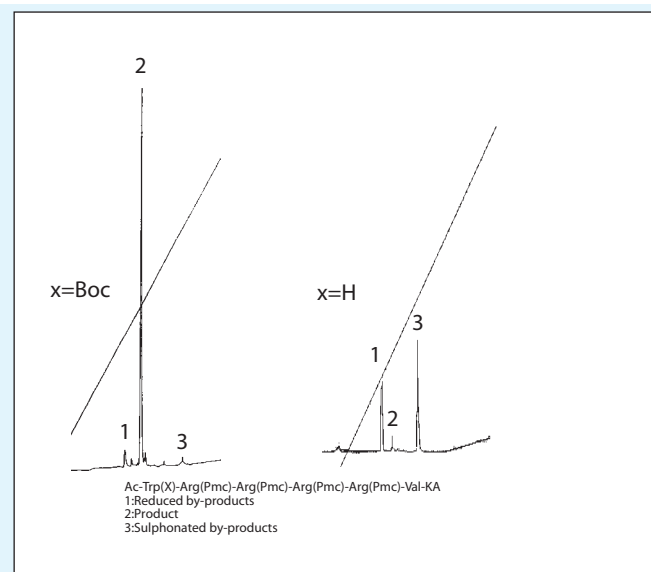
Fig. 4-4: Cleavage of a model peptide with TFA mixtures containing silanes.

Related products

853038	Boc-Trp-OH	p. 129
853086	Boc-D-Trp-OH	p. 129
853078	Boc-Trp(Boc)-OH	p. 129
853022	Boc-Trp(For)-OH	p. 129
853108	Boc-D-Trp(For)-OH	p. 130
852207	Fmoc-Trp-OH	p. 81
852150	Fmoc-D-Trp-OH	p. 81
852050	Fmoc-Trp(Boc)-OH	p. 82
852164	Fmoc-D-Trp(Boc)-OH	p. 82
852131	Fmoc-Trp(Boc)-OPfp	p. 82

References

1. J.M. Stewart & J.D. Young, "Solid phase peptide synthesis", Pierce Chemical Company, Rockford, 1984.
2. G. Barany, et al. (1987) *Int. J. Peptide Protein Res.,* **30**, 705.
3. G. Fields & R.L. Noble (1990) *Int. J. Peptide Protein Res.,* **35**, 161.
4. E. Atherton & R.C. Sheppard, "Solid phase peptide synthesis: a practical approach", IRL Press, Oxford, 1989.
5. W. C. Chan & P. D. White, "Fmoc solid phase peptide synthesis: a practical approach", Oxford University Press, Oxford, 2000.
6. J. Meienhofer in "Chemistry & Biochemistry Amino Acids", G.C. Barrett (Ed.), Chapman & Hall, London, 1985, pp. 297.
7. B. Rzesotarska, E. Masukiewicz (1988) *Org. Prep. Proc. Int.,* 427.
8. T. Copeland & S. Oroszlan (1988) *Gen. Anal. Techn.,* **5**, 109.
9. E. Wünsch in "Houben-Weyls Methoden der Organischen Chemie"; E. Müller (Ed.), Vol. 15, Parts 1 & 2, Thieme, Stuttgart, 1974.
10. F.C. Gronvald, et al. in "Peptides 1980", K. Brunfeldt (Ed.), 1981, pp. 111.
11. E. Atherton, et al. (1985) *J. Chem. Soc. Perkin Trans. I,* 2065.
12. H. Rink, et al. (1984) *Tetrahedron Lett.,* **25**, 621.
13. I. L. Rodionov, et al. in "Innovations & Perspectives in Solid Phase Synthesis, 3rd International Symposium", R. Epton (Ed.), Mayflower Worldwide Ltd., Birmingham, 1994, pp 669.
14. A.S. Verdini, et al. (1992) *Tetrahedron Lett.,* **33**, 6541.
15. R.H. Mazur (1968) *Experimentia,* **24**, 661.
16. Y. Kiso, et al. (1980) *J. Chem. Soc., Chem. Commun.,* 1063.
17. M. Fujino, et al. (1981) *Chem. Pharm. Bull.,* **29**, 2825.

18. J. Yajima, et al. (1978) *Chem. Pharm. Bull.,* **26**, 3752.

19. E. Atherton, et al. in "Peptides 1984", U. Ragnarsson (Ed.), 1985, pp. 153.

20. a) N.Fujii,et al. (1987) *Chem. Pharm. Bull.,* **35**, 3880; b) L.Guo, et al. (1988) *Chem. Pharm. Bull.,* **36**, 4989.

21. A.Penrose & P.D.White, Poster 487 presented at the 19th Meeting of the Federation of European Biochemical Societies, Rome, 1989.

22. R. Ramage & J. Green (1987) *Tetrahedron Lett.,* **28**, 2287.

23. J Green, et al. (1988) *Tetrahedron Lett.,* **29**, 4341.

24. B. Riniker & A. Hartmann in "Peptides, Chemistry, Structure & Biology, Proc. 11th American Peptide Symposium", J.E. Rivier & G.R. Marshall (Eds.), Escom, Leiden, 1990, pp. 950.

25. P. White in "Peptides, Chemistry, Structure & Biology, Proc. 12th American Peptide Symposium", J.A. Smith & J.E. Rivier (Eds.) ESCOM, Leiden, 1992, pp 537.

26. R. Ramage, et al. (1991) *Tetrahedron,* **47**, 6353.

27. L.A. Carpino, et al. (1993) *Tetrahedron Lett.,* **34**, 7829.

28. C.G.Fields & G.B.Fields (1993) *Tetrahedron. Lett.,* **34**, 6661.

29. E. Jaeger, et al. (1993) *Biol. Chem. Hoppe Seyler,* **374**, 349.

30. V. Caciagli & A. S. Verdini in "Peptide Chemistry 1987", T. Shiba & S. Sakakibara (Eds.), Protein Research Foundation, Osaka, 1988, pp. 283.

31. M. Bernatowicz & G. Matsueda in "Peptides, Chemistry, Structure & Biology, Proc. 13th American Peptide Symposium", R.S. Hodges & J.A. Smith (Eds.), ESCOM, Leiden, 1994, pp 107.

32. D.T. Gish, et al. (1971) *J. Am. Chem. Soc.,* **80**, 2558.

33. H. Gausepohl, et al. in "Peptides 1988, Proc. 20th European Peptide Symposium", G. Jung & E. Bayer (Eds.), Walter de Gruyter, Berlin, 1989, pp. 241.

34. H. Gausepohl, et al. (1989) *Int. J. Peptide Protein Res.,* **34**, 287.

35. P. Rovero, et al. (1993) T*etrahedron. Lett.,* 34, 2199.

36. G. Barany & R.B. Merrifield in "Peptides 2", E. Gross & J. Meienhofer (Eds.); Academic Press, New York, 1980, pp. 1.

37. S. Mojsov, et al. (1980) *J. Org. Chem.,* **45**, 555.

38. M. Bodanszky & V. du Vigneaud (1959) *J. Am. Chem. Soc.,* **81**, 5688.

39. P. Sieber & B. Riniker in "Innovations & Perspectives in Solid Phase Synthesis, 1st International Symposium", R. Epton (Ed.), SPCC (UK) Ltd., Birmingham, 1990, pp 577.

40. P.Sieber & B.Riniker (1991) *Tetrahedron. Lett.,* **32**, 739.

41. M. Friede, et al. (1992) *Pept. Res.,* **5**, 145.

42. L. A. Carpino, et al. (1995) *J. Org. Chem.,* **60**, 7718.

43. R.D.Dimarchi, et al. (1982) *Int. J. Peptide Protein Res.,* **19**, 88.

44. J.P. Tam, et al. (1988) *Pept. Res.,* **1**, 6.

45. E. Nicolás, et al. (1989) *Tetrahedron Lett.,* **30**, 497.

46. R. Subiros-Funosas, et al. (2011) *Tetrahedron,* **67**, 8595.

47. C.D. Chang, et al. (1980) *Int. J. Peptide Protein Res.,* **15**, 59.

48. Y. Okada, et al. (1987) *J. Chem. Soc., Chem. Commun.,* 1532.

49. R.Dölling, et al. (1994) *J. Chem. Soc., Chem. Commun.,* 853

50. J. Lauer, et al. (1994) *Lett. Pept. Sci.,* **1**, 197.

51. R. Subirós-Funosa, et al. (2011) *Biopolymer*s, **98**, 89.

52. Y. Yang, et al. (1994) *Tetrahedron Lett.,* **35**, 9689.

53. a) M. Quibell, et al. (1994) *J. Chem. Soc., Chem. Commun.,* 2343;b) L.C. Packman (1995) *Tetrahedron Lett.,* **36**, 7523.

54. A. Karlström & A. Undén (1996) *Tetrahedron Lett.,* **37**, 4243.

55. J. P. Tam, et al. (1979) *Tetrahedron Lett.,* **42**, 4033.

56. J.M. Stewart, et al. (1979) *J. Med. Chem.,* **45**, 555.

57. J.P. Tam, et al. (1977) *J. Am. Chem. Soc.,* **99**, 7363.

58. F. Albericio, et al. in "Fmoc solid phase peptide synthesis", W. C. Chan & P. D. White (Eds.), Oxford University Press, Oxford, 2000, pp. 77, and references therein.

59. E. Atherton, et al. (1985) *J. Chem. Soc., Perkin Trans. I,* 2065.

60. S.N. McCurdy, Applied Biosystems User Bulletin, Peptide Synthesizer; Applied Biosystems, Foster City, 1989, 25, 42

61. S.N. McCurdy (1989) *Pept. Res.,* **2**, 147.

62. H. Wang, et al. (2000) *Synth. Commun.,* **30**, 727.

63. A. Chimiak in "Peptides 1962", G.T. Young (Ed.), 1963, pp.37.

64. H.C. Beyerman in "Peptides 1962", G.T. Young (Ed.), 1963, pp. 53.

65. B. Kamber, et al. (1980) *Helv. Chim. Acta,* **63**, 899.

66. P. Sieber, et al. (1977) *Helv. Chim. Acta,* **60**, 27.

67. H. Lamthanh, et al. (1993) *Int. J. Peptide Protein Res.,* **41**, 85.

68. R. G. Hiskey in "The Peptides, Synthesis, Function & Biology, Vol 3", E. Gross & J. Meienhofer (Eds), Academic Press, New York, 1981, pp. 157.

69. H. Lamthanh, et al. (1995) *Pept. Res.,* **8**, 316.

70. J. Jezek, et al. in "Peptides 1994, Proc. 23rd European Peptide Symposium", H. Maia (Ed.), ESCOM, Leiden, 1994, pp 250.

71. K. Akaji, et al. (1993) *J. Am. Chem. Soc.,* **115**, 11384.

72. A. Otaka, et al. (1991) *Tetrahedron Lett.,* **32**, 1223.

73. A. Cuthbertson & B. Indrevoll (2000) Tetrahedron Lett., 41, 3661.

74. M. Yoshida, et al. (1990) *Chem. Pharm. Bull.,* **38**, 273.

75. H. Tamamura, et al. (1998) *Chem. Commun.,* 151.

76. R. Eritja, et al. (1987) *Tetrahedron Lett.,* **43**, 2675.

77. J. C. Spetzler, et al. (1997) *Lett. Pept. Sci.,* **3**, 327.

78. J.P. Tam, et al. (1991) *J. Am. Chem. Soc.,* **113**, 6657.

79. F. Albericio, et al. (1991) *Int. J. Peptide Protein Res.,* **37**, 402.

80. T. M. Postma, et al. (2012) *Org. Lett.,* **14**, 5468; T. M. Postma & F. Albericio (2013) *Org. Lett.,* **15**, 616.

81. K. M. Harris, et al. (2007) *J. Pept. Sci.,* **13**, 81.

82. R. A. D. Bathgate, et al. (2006) *Biochemistry,* **45**, 1043.

83. T. Kaiser, et al. (1996) *Tetrahedron Lett.,* **37**, 1187.

84. Y. X. Han, et al. (1997) *J. Org. Chem.,* **62**, 4307.

85. Y. M. Angell, et al. (2002) J. Peptide Res., **5**, 239.

86. Y. Fujiwara, et al. (1994) *Chem. Pharm. Bull.,* **42**, 724.

87. a) J.H. Jones & W.I. Ramage (1978) *J. Chem. Soc., Chem. Commun.,* 472; b) J.H. Jones & A.V. Stachulski (1979) *J. Chem. Soc., Perkin Trans. 1,* 2261.

88. T. Fuji & S. Sakakibara (1974) *Bull. Chem. Soc. Jpn.,* **47**, 3146.

89. T. Ishiguro & C. Eguchi (1989) *Chem. Pharm. Bull.,* **37**, 506.

90. E. Schnabel, et al. (1968) *Ann. Chem.,* **716**, 175.

91. P. Sieber & B. Riniker (1987) *Tetrahedron Lett.,* **28**, 6031.

92. S. J. Harding, et al. in "Peptides 1994, Proc. 24th European Peptide Symposium", H. Maia (Ed.), ESCOM, Leiden, 1995, pp. 189.

93. M. Mergler, et al. (2001) *J. Pept. Sci.,* **7**, 502.

94. M. Mergler, et al. , in "Innovations & Perspectives in Solid Phase Synthesis & Combinatorial Libraries,", R. Epton (Ed.), Mayflower Wordwide, Kingswinford, 2002, pp. 235.

95. A. Pessi, et al. (1992) *Int. J. Peptide Protein Res.,* **39**, 58.

96. K. Hofmann, et al. (1965) *J. Am. Chem. Soc.,* **87**, 945.

97. K. Norris, et al. (1971) *Acta. Chem. Scand.,* **25**, 945.

98. S. Guttmann & R.A. Boissonnas (1959) *Helv. Chim. Acta,* **42**, 1257.

99. H. Yajima, et al. (1980) *Chem. Pharm. Bull.,* **28**, 1214.

100. J. Blake & C.H. Li (1968) *J. Am. Chem. Soc.,* **90**, 5882.

101. R.A. Houghten & C.H. Li in "Peptides, Chemistry, Structure & Biology, Proc. 5th American Peptide Symposium", M. Goodman & J. Meienhofer (Eds.), Pierce Chemical Company, Rockford, 1977, pp 458.

102. R.A. Houghten & C.H. Li (1978) *Int. J. Peptide Protein Res.,* **11**, 345.

103. R.A. Houghten & C.H. Li (1979) *Anal. Biochem.,* **98**, 36.

104. a) C. Garcia-Echeverria, et al. (1989) *Tetrahedron Lett.,* **30**, 2441; b) H. Huang & D. L. Rabenstein (1999) *J. Pept. Res.,* **53**, 548.

105. L. Guo, et al. (1988) *Chem. Pharm. Bull.,* **36**, 4989.

106. M.W. Pennington & M.E. Byrnes (1995) *Pept. Res.,* 39.

107. W. Beck & G. Jung (1994) *Lett. Pept. Sci.,* **1**, 31.

108. E.Nicolas, et al. in "Innovations & Perspectives in Solid Phase Synthesis, 3rd International Symposium", R. Epton (Ed.), Mayflower Worldwide Ltd., Birmingham, 1994, pp 619.

109. J.P. Tam, et al. (1983) *J. Am. Chem. Soc.,* **105**, 6443.

110. A. Dryland & R.C. Sheppard (1986) *J. Chem. Soc., Perkin Trans. 1,* 125.

111. C.-D. Chang, et al. (1980) *Int. J. Peptide Protein Res.,* **15**, 485.

112. A.N. Eberle, et al. (1986) *J. Chem. Soc., Perkin Trans. 1,* 361.

113. P. Sieber (1987) *Tetrahedron Lett.,* **28**, 1637.

114. a) E. Atherton, et al. (1988) *Tetrahedron* **44**, 843; b) J.D. Wade, et al. in "Solid phase Synthesis, 1st International Symposium", R. Epton (Ed.), SPCC (UK) Ltd., Birmingham, 1990, pp 585.

115. H. Franzen, et al. (1984) *J. Chem. Soc., Chem. Commun.,* 1699.

116. a) P. White, Novabiochem Satellite Meeting, Solid phase Synthesis, 2nd International Symposium, Canterbury, 1991; b) P. White, Novabiochem Innovation Sheet 2/93.

117. D.S. King, et al. (1990) *Int. J. Peptide Protein Res.,* **36**, 255.

118. H. Choi & J.V. Aldrich (1993) *Int. J. Peptide Protein Res.,* **42**, 58.

119. C.G. Fields & G.B. Fields in "Innovations & Perspectives in Solid Phase Synthesis, 3rd International Symposium", R. Epton (Ed.), Mayflower Worldwide Ltd., Birmingham, 1994, pp 251.

120. D. A. Pearson, et al. (1989) *Tetrahedron Lett.,* **30**, 2739.

121. N.A. Solé & G. Barany (1992) *J. Org. Chem.,* **57**, 5399.

122. B.Kamber & B.Riniker in "Peptides, Chemistry & Biology, Proc. 12th American Peptide Symposium", J.A.Smith & J.E.Rivier (Eds.) , ESCOM, Leiden, 1992, pp 525.

4.2 Selectively protected amino acid building blocks

Novabiochem® has one of the largest collections of orthogonally and quasi-orthogonally protected tri-functional amino acids. These derivatives have many interesting applications in peptide and combinatorial chemistry, such as synthesis of cyclic [1] and branched peptides [2] and the preparation of peptides carrying side-chain modifications [3]; the construction of orthogonal multi-release resins for solution screening of one bead-one compound libraries [4, 5]; the preparation of dendritic or cyclic scaffolds for ligand [6, 7] or protein motif presentation [8, 9]; libraries of cyclic glycopeptides [10]; and synthesis of β-turn mimetics [11]. The properties of Novabiochem®'s selectively protected amino acids are given in Table 4-4, page 4-11.

4.2.1 Diaminopropionic/butanoic acid, ornithine and lysine derivatives
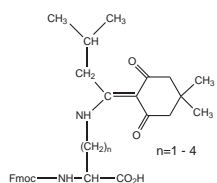
Fmoc–Dpr(ivDde)–OH, Fmoc–Dab(ivDde)–OH, Fmoc–Orn(ivDde)–OH, Fmoc–Lys(ivDde)–OH, ivDde–Lys(Fmoc)–OH
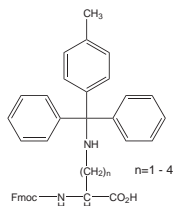
Fmoc–Dpr(Mtt)–OH, Fmoc–Dab(Mtt)–OH, Fmoc–Orn(Mtt)–OH, Fmoc–Lys(Mtt)–OH
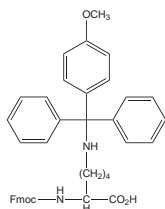
Fmoc–Lys(Mmt)–OH

The side chains of lysine, ornithine, diaminopropionic acid (Dpr) and diaminobutanoic acid (Dab) are very useful for the introduction of a range of modifications, e.g. biotin, fluorescent labels, etc., as well as for the synthesis of branched and cyclic peptides.

Symmetrically branched peptides or MAP core molecules can be produced using the symmetrically protected Fmoc-Lys(Fmoc)-OH, in which both the α- and ε-amino groups are simultaneously deprotected with 20% piperidine [12].

However, for specific modification of side-chain amino groups, the use of selective, or ideally orthogonal, protecting group strategies are required. For such applications Novabiochem is able to offer the following derivatives.

Dde/ivDde

Since the introduction of the Dde [2] and ivDde [13] amino-protecting groups in 1993 and 1998, respectively, the Fmoc/xDde strategy has become the standard approach for the synthesis of branched, cyclic and side-chain modified peptides by Fmoc SPPS, with over 200 publications citing the use of these protecting groups [14].

Their utility stems from the fact that Dde and ivDde-protected primary amines are stable to 20% piperidine and TFA but are cleaved with 2% hydrazine in DMF. Thus, amino groups protected by these groups can be selectively unmasked on the solid phase without affecting the side-chain protecting groups of other residues, facilitating subsequent site-specific modification. Furthermore, the reaction can be monitored by spectrophotometry since the indazole cleavage product absorbs strongly at 290 nm (Figure 1).

6,6-Dimethyl-3-(2-methylpropyl)-4-oxo-4,
5,6,7-tetrahydro-1H-indazole
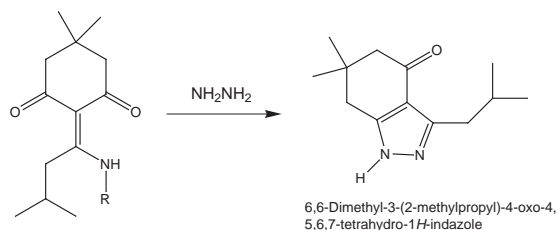
Fig. 4–5: Removal of ivDde.

Examples of the use of the Fmoc/Dde strategy include: branched [2] and di-epitopic peptides [15]; MAP core molecules and lipo-MAPs [16 -18; cyclic peptides [1, 19]; TASP molecules [8]; templates for combinatorial chemistry [6, 7]; synthetic proteins [9]; fluorescently-labeled peptides [3]; modified Lys and Dpr derivatives [20, 21]. the synthesis of multi-functional probes [22]; and ubiquinated peptides [23].

The choice between Dde or ivDde depends on the application. Dde is easier to remove than ivDde but is much less robust. Dde has been observed to undergo migration during piperidine mediated deprotection of N-ε-Fmoc-Lys and N-terminal Dpr residues, leading to scrambling of its position within the peptide chain; and partial loss has been noted during the synthesis of long sequences [24]. The more hindered ivDde on the otherhand does not undergo leaching or side-chain migration to any significant extent, except in the special case of Dpr [25] (see Method 4-3), but can occasionally prove extremely hard to remove, particularly if located at the C-terminus of a peptide or in an aggregated region of the sequence.

Fmoc derivatives of lysine are available in which the α- and ε-amino groups are protected with Dde and ivDde: Fmoc-Lys(Dde)-OH, Dde-Lys(Fmoc)-OH, Fmoc-Lys(ivDde)-OH; and ivDde-Lys(Fmoc)-OH. For Dpr and Dab, only side-chain ivDde-protected derivatives are available: Fmoc-Dpr(ivDde)-OH and Fmoc-Dab(ivDde)-OH.

As hydrazine can also remove Fmoc groups as well as Dde and ivDde groups, when preparing cyclic or side-chain modified peptides by the Fmoc/Dde strategy, assembly of the peptide backbone is generally completed prior to deprotection of the Dde/ivDde groups. The *N*-terminus of the peptide should be protected with Boc, which can be achieved either by direct incorporation of the *N*-terminal residue as a Boc protected amino acid or acylation of the free *N*-terminal amino group with Boc$_2$O.

Removal of Dde or ivDde is achieved by treating the resin with 2% hydrazine in DMF, although in cases where ivDde removal has proved difficult, solutions of as much as 10% hydrazine have been employed. The process can be followed spectrophotometrically at the same wavelength used for monitoring removal of Fmoc, since the reaction-product of the Dde or ivDde group with hydrazine is a chromophoric indazole derivative (Figure 4-5). Typical traces obtained for Dde removal on the NovaSyn® Crystal and Millipore 9050 peptide synthesizers are shown in Figure 4-6; ivDde gives similar results. Dde and ivDde also stable to the normal reagents employed for Boc cleavage (TFA or 50% TFA in DCM) and to DBU at the normal concentrations (approx. 2%) used for Fmoc removal.

As previously mentioned, if the ivDde group is close to the *C*-terminus of the peptide or the peptide has aggregated, removal ivDde can be very sluggish and often incomplete. The problem can be avoided for peptides containing side-chain modified lysine by using ivDde-Lys(Fmoc)-OH instead of Fmoc-Lys(ivDde)-OH for incorporation of those residues. The use of the former allows side-chain modification of lysine to be accomplished during chain extension. Following incorporation of ivDde-Lys(Fmoc)-OH into the peptide chain, the side-chain Fmoc group can be removed with piperidine, the side-chain amino group reacted with the desired carboxylic acid-functionalized moiety, before removal of the ivDde with hydrazine, and chain extension in the usual manner.

Recently, complete orthogonality of Dde with Fmoc has been demonstrated if hydroxylamine hydrochloride/imidazole (1.3:1) in NMP instead of hydrazine in DMF is used for Dde removal [26].

Method 4-2: Selective removal of Dde/ivDde with 2% hydrazine in DMF

Batch
1. Place the peptidyl-resin in a flask and treat with 2% hydrazine monohydrate in DMF (25 ml/g). Stopper the flask and leave to stand at rt for 3 min.
2. Filter the resin and repeat the hydrazine treatment two more times. Wash the partially protected resin with DMF.

Continuous flow
1. Flow 2% hydrazine monohydrate in DMF at 3 ml/min through the peptidyl resin packed in a 1 cm diameter reaction column. Deprotection can be followed by monitoring spectrophotometrically at 290 nm the absorbance of the column eluant using a 0.1 mm path length cell.
2. When the reaction is complete, as indicated by return of the absorbance to its original value, flush the column with DMF.

Method 4-3: Special procedures for chain extension following introduction of Fmoc-Dpr(ivDde)-OH

Fmoc removal
1. Treat the Fmoc-Dpr(ivDde)-peptidyl resin with morpholine-DMF (1:1) (2 x 15 min).
2. Quickly wash resin with DMF.

Addition of next amino acid
1. Treat resin with Fmoc-A.A.-OH (1.5 eq.) and DIPEA (3 eq.) in DMF at-20°C.
2. Add PyBOP (1.6 eq.) to the slurry and allow to warm to rt.

In the worst case (Fmoc-Val), migration observed when using this procedure is <10%.

Millipore 9050   NovaSyn Crystal
360 nm   300 nm

Typical deprotection profiles for Fmoc and Dde obtained from NovaSyn Crystal and Millipore peptide synthesisers
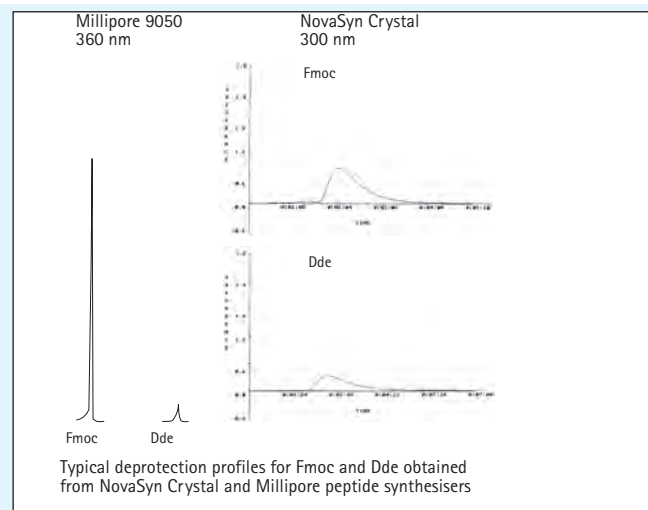
Fig. 4-6: Spectroscopic monitoring of the removal of Dde based protecting groups.

Mtt

The Mtt group can be removed from the side chain of lysine or ornithine using 1% TFA in DCM (Method 4-4) [27, 28] or with DCM/HFIP/TFE/TES (6.5:2:1:0.5) (Method 4-5) [29], allowing selective removal in the presence of other side-chain protecting groups which require up to 95% TFA for removal. When using TFA, addition of 1-5% TIS or MeOH is recommended to quench the trityl cations released. Preliminary results indicate MeOH scavenging prevents loss of t-butyl groups and premature cleavage from Rink Amide resins [30] (Method 4-4b).

The application of Fmoc-Lys(Mtt)-OH is illustrated by the synthesis of a branched peptide in which the sequence Ile→Leu was assembled on an amide resin (Application 4-3). The *N*-terminal Ile residue was introduced as the Boc-amino acid. The Mtt group was removed from the ε-amino group of the lysine residue with 1% TFA (Method 4-4a). The Gly→Glu branch was then assembled on the lysine side chain using normal Fmoc chemistry. The peptide was cleaved with 95% TFA, removing all other side-chain protecting groups, as well as the N-α-Boc group from the Ile.

Applications of Mtt derivatives include the use of Lys(Mtt) in conjunction with Lys(Dde) to prepare fluorescence-quenched substrates [3], and of Lys(Mtt) and Orn(Mtt) in the preparation of peptidomimetic macrocycles [11, 31].

Table 4–4: Protecting groups.

Name	Structure	Removed by	Stable to
O-All/Alloc	$H_2C=C-CH_2-$	3 eq. Pd(Ph$_3$P)$_4$ in CHCl$_3$/AcOH/NMM (37:2:1)	TFA, piperidine, hydrazine
Azido		3 eq. Me$_3$P in dioxane/water	TFA, piperidine
O-tBu		95% TFA	1% TFA, piperidine, Pd(O), hydrazine
STmp		β-Mercaptoethanol, 0.1 NMM in DMF	TFA(partial), piperidine
N-xDde		2% hydrazine in DMF or 20% hydroxylamine/ 15% imidazole in NMP/DCM (5:1)	TFA, piperidine, Pd(0), DBU
O-Dmab		2% hydrazine in DMF	TFA, piperidine, Pd(0), DBU
N-Fmoc		20% piperidine in DMF	TFA, Pd(0)
S-Mmt / N-Mmt		1% TFA in DCM containing 1-5% TIS / AcOH/TFE/DCM (1:2:7)	Pd(0), hydrazine, piperidine
N-Mtt		1% TFA in DCM containing 1-5% TIS	Pd(0), hydrazine, piperidine
O-2-(PhiPr)		2% TFA in DCM containing 1-5% TIS	Pd(0), piperidine
O-Trt		1% TFA in DCM containing 1-5% TIS	Pd(0), hydrazine, piperidine
O-2-ClTrt		1% TFA in DCM containing 1-5% TIS	Pd(0), hydrazine, piperidine
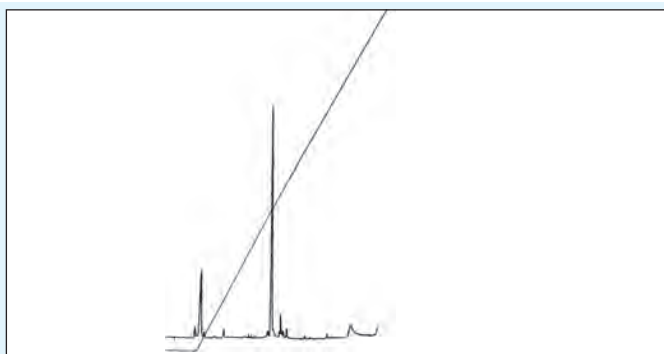
Fig. 4-7: HPLC of crude branched peptide.
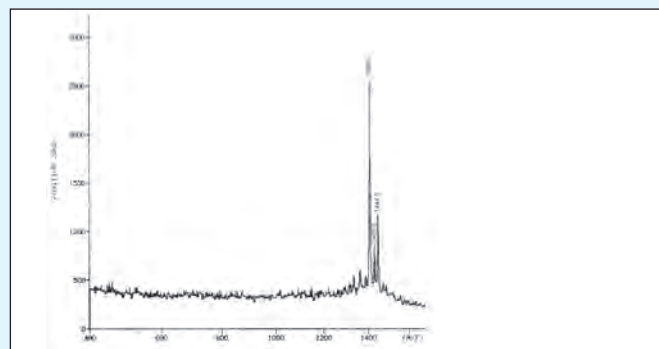
Fig. 4-8: PD-MS of crude product; peaks at 1427.5 and 1444.5 are due to M+Na+ and M+K+, respectively.

Application 4-3: Synthesis of H-Ile-Pro-Glu-Lys(H-Gly-Asp-Phe-Glu-Glu)-Glu-Thr-Leu-NH$_2$

Boc-Ile-Pro-Glu(OtBu)-Lys(Mtt)-Glu(OtBu)-Thr(tBu)-Leu-NovaSyn® KR was prepared using PyBOP®/HOBt chemistry on a NovaSyn® Crystal fully automatic, continuous flow synthesizer. Lys(Mtt) was deprotected by flowing 1% TFA in DCM through the reaction column and monitoring the removal of Mtt spectrophotometrically at 470 nm.

After neutralization of the TFA salt with piperidine, the synthesis continued in the usual way on the side chain of lysine.

The peptide was cleaved using TFA/water/TIS (95:2.5:2.5) for 3 h This treatment also removed all side-chain protecting groups as well as the N-α-Boc group on Ile.

The crude product was analyzed by HPLC (Figure 4-7) and PD-MS, expected 1406.5, found 1405.7 (Figure 4-8).

Method 4-4: Removal of trityl groups on solid phase

Batch-wise method
1. Pre-swell the dry resin (1 g) with DCM in a sintered glass funnel (of a type with a tap and stopper). Remove excess DCM.
2. Add 94:1:5 DCM/TFA/TIS (10 ml), seal funnel and shake for 2 min. Remove solvent by applying N$_2$ pressure.
3. Repeat step 2 three times.
4. Wash resin with DCM and dry under vacuum.

Flow method
1. Pre-swell resin (1 g) with DCM and pack into reaction column.
2. Pump 1% TFA in DCM (2 ml/min) through resin. The reaction can be followed by measuring the absorbance of the column eluant using a 0.1 mm flow cell at 460 nm[a].
3. Once reaction is finished, as indicated by the absorbance returning to baseline, flush column with DCM.

[a]If the peptide contains other trityl-based protecting groups, the level will not return to baseline owing to slow leaching of Trt groups [27].

Method 4-5: Removal of Mtt from Lys on solid phase

a) DCM/HFIP/TFE/TES [29]
1. Add DCM/HFIP/TFE/TES (6.5:2:1:0.5) (20 ml/g of resin) to peptidyl resin.
2. Leave mixture to stand with gentle agitation for 1 h.
3. Remove a small sample of resin and wash with DCM. Add 1% TFA in DCM to resin sample. If an orange color is formed instantly then leave the reaction for a further hour.
4. Once the trityl test is negative, wash the resin with DMF, 10% DIPEA in DMF, DMF and use in next step of synthesis.

b) TFA/DCM/MeOH [28]
1. Add TFA/DCM/MeOH (1:98:1) (15 mg/ml) to peptidyl resin. Drain after 1 min.
2. Add fresh TFA/DCM/MeOH (1:98:1) (15 mg/ml) to peptidyl resin and leave for 16 h.
3. Wash the resin with DMF, 10% DIPEA in DMF, DMF and use in next step of synthesis.

Mmt

The Mmt group is considerably easier to remove than Mtt. It is cleaved rapidly from the side-chain of lysine using the same methods that were described for Mtt but can be even removed using AcOH/TFE/DCM (1:2:7) [32] or HOBt in DCM/TFE (Method 5-4). The use of Fmoc-Lys(Mmt)-OH is ideal for those cases where removal of Mtt is problematic. Owing to the extreme acid sensitivity of the Mmt group, coupling of Fmoc-Lys(Mmt)-OH is best carried out using PyBOP®/DIPEA or other base-mediated coupling methods.

Alloc

The Alloc group is stable to treatment with piperidine and TFA, but can be easily removed under mild conditions by Pd(0) catalyzed allyl transfer [33]. A variety of methods have been used for this step, but perhaps the most useful is that of Kates, *et al.* utilizing Pd(Ph$_3$P)$_4$/CHCl$_3$/HOAc/NMM [34] (Method 4-7). The Alloc group is not compatible with the conditions employed for removal of ivDde [35]. It is thought that the presence of a small amount of diazine in hydrazine causes reduction of the double bond in the allyl group. Fortunately, this side reaction can be easily overcome by the addition of allyl alcohol to the hydrazine reagent.

Azido

Azides can be reduced under mild conditions with thiols or phosphines to the corresponding amines. Therefore, Fmoc-protected amino acids bearing side-chain azido functionalities are useful tools for preparing branched and side-chain modified peptides. The azido group is stable to coupling conditions and to treatment with piperidine. It is also stable to TFA cleavage conditions provided thiols are omitted from the cocktail [36]. Reduction of the azide on the solid phase selectively unmasks the side-chain amino group without affecting any other amino-acid residues [37, 38].

Method 4-6: Reduction of azido group on solid phase

1. Wash resin with three times with dioxane and dioxane/water (4:1).
2. Drain resin and add 1 M Me$_3$P in toluene (6 eq.) in dioxane/water (4:1). Gently agitate for 30 mins.
3. Remove a small sample of resin and wash with dioxane then DCM. Add 95% TFA to leave to cleave for 1.5 h. Analyze by HPLC to check completeness of reduction of azido group.

4.2.2   Hse, Ser, Thr, Tyr and Cys derivatives
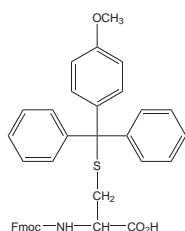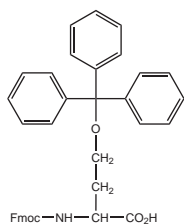
Fmoc-Cys(Mmt)-OH   **Fmoc-Hse(Trt)-OH**
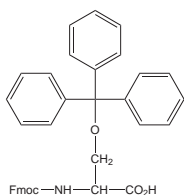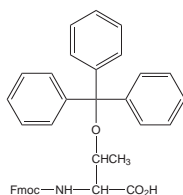
Fmoc-Ser(Trt)-OH   **Fmoc-Thr(Trt)-OH**
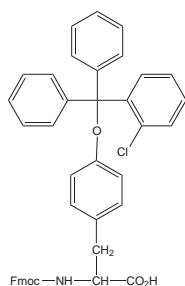
Fmoc-Tyr(2-ClTrt)-OH

2-ClTrt/Trt/Mmt

2-ClTrt, Trt and Mmt groups can be removed of the side chains of Tyr, Hse/Ser/Thr [39] and Cys [40] using only 1% TFA in DCM, leaving all other protecting groups intact (Method 4-4). This allows the selective

deprotection of a single residue for subsequent side-chain modification. In products with multiple Ser, Thr or Tyr residues, selectivity is possible by incorporating all other such residues, with the exception of the residue to be modified, as the *t*-butyl ether. Trityl group removal is an equilibrium process, so must be performed using silanes to scavenge the trityl cations, or in a continuous flow mode to drive the equilibrium. For Ser(Trt), Coba, *et al.* [41] have described the use of 20% dichloroacetic acid in DCM for 10 min for Trt removal. This procedure may also have wider application for the deprotection of other trityl-protected amino acids.

The most common application of the Ser/Thr/Tyr derivatives is in the preparation of phosphopeptides by the post-synthetic method using a phosphoramidite reagent (section 3.11.3, page 3.41).

In a comparative study, purer products were obtained using side-chain trityl protected amino acids than with standard *t*-butyl protected amino acids [42].

tButhio/STmp
The tButhio/STmp can be removed from the side chain by reduction with thiols [43, 44] (Method 3-41, page 3.34).

4.2.3   Asp and Glu derivatives
Orthogonally and quasi-orthogonally protected Asp and Glu derivatives have numerous applications in peptide synthesis and combinatorial chemistry. α-Esters are particularly useful for the preparation of head-to-tail cyclic peptides by on-resin cyclization, whereas selectively protected β- and γ- esters of Asp and Glu can be utilized in the synthesis of glycopeptides and side chain-to-side chain or head-to-side chain lactam bridged peptides [33, 34, 45-48]. Similar strategies can also be applied to the construction of cyclic library templates [6, 7].

Allyl
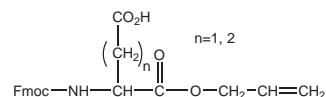
Fmoc-Asp/Glu-OAll/Fmoc-Asp/Glu(OAll)-OH

Allyl esters are stable to treatment with piperidine and TFA but can be easily removed under mild conditions by Pd(0) catalyzed allyl transfer [33], as previously described for the Alloc group (Method 4-7).

The allyl group is not compatible with the conditions employed for removal of ivDde [35]. It is thought that the presence of a small amount of diazine in hydrazine causes reduction of the double bond in the

allyl group. Fortunately, this side reaction can be easily overcome by the addition of allyl alcohol to the hydrazine reagent.

Dmab

Fmoc–Asp–ODmab, Fmoc–Glu–ODmab
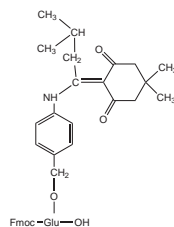
Fmoc–Asp(ODmab)–OH, Fmoc–Glu(ODmab)–OH

Dmab esters were developed in collaboration between Prof. B. W. Bycroft and Dr. W. Chan, of the University of Nottingham, and Novabiochem®, to provide a complement to Dde amino protection. The design of the Dmab blocking group is based on the safety-catch principle and the known propensity of p-aminobenzyl esters to undergo 1,6-elimination [49, 50]. The safety catch is provided by the ivDde group, which protects the amino function of the unstable p-aminobenzyl ester during synthesis.

Deprotection

Dmab protection is quasi-orthogonal to the Fmoc/tBu strategy, since Dmab esters are stable to 20% piperidine in DMF and to TFA, but are quantitatively cleaved with 2% hydrazine in DMF within minutes. Removal of Dmab involves a two step process: treatment with hydrazine initially removes the N-ivDde group; this is then followed by collapse of the resultant p-amino benzyl ester, with concurrent release of the carboxylic acid (Figure 4-9).

The deprotection reaction can either be carried out in a batch-wise or continuous flow manner. In the latter case, the reaction can be monitored spectrophotometrically at 290 nm by following release of the indazole by-product. Sluggish cleavage of the aminobenzyl moiety has been occasionally observed [51-54], and appears to be very sequence dependent. In these instances washing the support with 20% DIPEA in DMF/water (90:10) [51] or 5 mM sodium hydroxide in methanol [54] has been found to be efficacious. As hydrazine will remove Fmoc, assembly of the peptide backbone must be completed prior to deprotection of the Dmab side chain. The N-terminus of the peptide should be protected with Boc. This can be achieved either by direct incorporation of the N-terminal residue as a Boc protected amino acid or acylation of the free N-terminal amino group with Boc$_2$O.

Fmoc-Asp-ODmab has been employed to prepare a cyclic analog of pyrrhocoricin [55], a 29-mer head-to-tail cyclic peptide [56], and chlorofusin peptide [57].
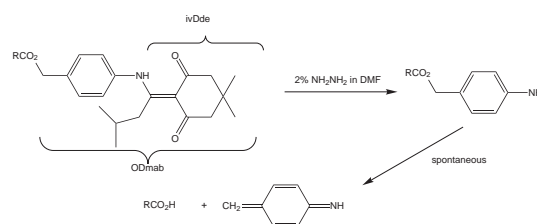
Fig. 4-9: Removal of Dmab.

2–PhiPr

Fmoc-Glu(O-2-PhiPr)-OH   Fmoc-Asp(O-2-PhiPr)-OH

The 2-phenylisopropyl group (2-PhiPr) [58] can be removed from the side chain of Asp and Glu using 1% TFA as in the protocol given in Method 4-4. The use of this protecting group in combination with Mtt on Lys or Orn provides an excellent strategy for the synthesis of side chain-to-side chain lactam bridged peptides. In contrast to Dmab and All, the 2-PhiPr group offers significant protection against aspartimide formation, which makes Fmoc-Asp(O-2-PhiPr) the derivative of choice for the synthesis of cyclic peptides that are prone to this side reaction.

Cyclic peptide synthesis

In recent years the synthesis and biological properties of cyclic peptides has attracted considerable interest. Introduction of conformational restraint through head-to-tail cyclization has become a standard strategy in medicinal chemistry for increasing the receptor affinity and selectivity of peptide ligands. Furthermore, cyclization has often been employed as a means of prolonging the duration of action of peptide hormones, since

in general cyclic peptides are more stable to proteolysis than their linear counterparts [59]. Cyclic peptides are also used as synthetic immunogens [60], as by restricting conformational flexibility, the peptide is thought to adopt a conformation which more closely mimics that of the epitope as presented on the surface of the native protein.

There are two approaches currently available for the preparation of head-to-tail cyclic peptides. The first involves cyclization of a protected fragment in solution [61], and the second, on-resin cyclization of a peptide attached *via* the side chain of an Asp or Glu residue [45-48, 49, 62]. The solid phase technique is the more straightforward, as excess reagents and by-products can be easily removed by washing of the resin. In addition, this method often gives superior results due to resin induced pseudo-dilution effects. The synthesis of cyclic peptides has been recently reviewed [63].

Peptide assembly
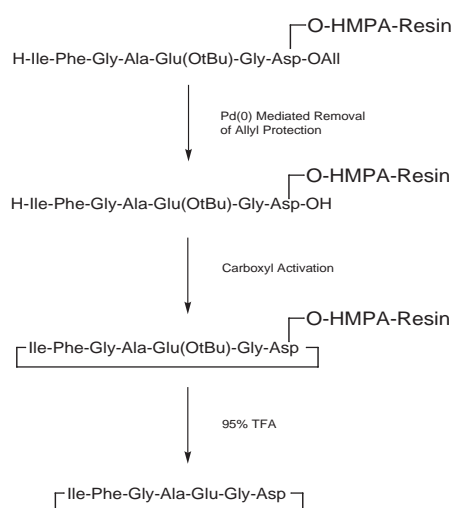
Fig. 4-10: Head-to-tail cyclization using α-allyl esters.

Figure 4-10 shows the synthesis of cyclic peptide using Fmoc amino acid allyl esters.

For the production of head-to-tail cyclic peptides, the C-terminal amino acid, either Fmoc-Asp-OX or Fmoc-Glu-OX, is attached to the resin via its side chain. If an Asn or Gln residue is required then an amide resin, such as NovaSyn® TGR, should be employed. Chain elongation can be carried out using standard Fmoc SPPS procedures.

Problems of aspartimide or piperidide formation can occur with both Dmab and allyl [64-66] esters of aspartic acid, involving the nitrogen of the preceding residue or linker. In the former case, this side reaction can be overcome by employing a N-Hmb protected derivative for introduction of the preceding residue [64, 66].

The yield and purity of peptides obtained by this approach are heavily influenced by the choice of solid support, with higher loading resins giving the poorest results [33]. PEG-PS composite resins, such as NovaSyn® TGA and TGR, are particularly recommended as these supports swell well in the chlorinated solvents used for allyl deprotection and the tentacle nature of the resin matrix helps minimize inter-chain oligomerization.

Cyclization

Cyclization occurs upon activation of the free resin-bound carboxy group with coupling reagents such as TBTU, PyBOP® or DIPCDI/Oxyma Pure. The reaction normally takes between 2-5 hours, and can be monitored using TNBS or ninhydrin.
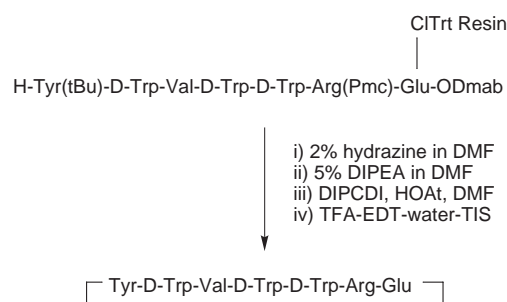
Fig. 4-11: Synthesis of cyclo(Tyr-D-Trp-Val-D-Trp-D-Trp-Arg-Glu) using a Dmab ester.

NOTE: An excess of uronium coupling reagents (i.e. TBTU, HBTU) should be avoided as this can result in capping of resin amino groups. Furthermore, in common with other fragment condensation reactions, epimerization may accompany activation of the carboxy-terminal residue. The extent of this side reaction will vary depending on the solvent, coupling reagent used and peptide sequence. Some experimentation may be required to optimize reaction conditions. The use of Oxyma Pure/ DIPCDI activation, for this step is particularly recommended.

The strategy for using Dmab is exemplified through synthesis of a cyclic peptide (Application 4-4).

Related products

852072	Fmoc-Asp-OAll	p. 15
852122	Fmoc-Asp(OAll)-OH	p. 15
856023	Fmoc-Asp(Wang resin)-OAll	p. 242
856121	Fmoc-Asp(Wang resin LL)-OAll	p. 246
852079	Fmoc-Asp-ODmab	p. 18
852078	Fmoc-Asp(ODmab)-OH	p. 18
856123	Fmoc-Asp(Wang resin LL)-ODmab	p. 246
852086	Fmoc-Asp(O-2-PhiPr)-OH	p. 19
852335	Fmoc-Asp-O-2-PhiPr	p. 19
852073	Fmoc-Glu-OAll	p. 32
852123	Fmoc-Glu(OAll)-OH	p. 32
856024	Fmoc-Glu(Wang resin)-OAll	p. 243
856022	Fmoc-Glu(Wang resin)-ODmab	p. 243
852077	Fmoc-Glu-ODmab	p. 35
852076	Fmoc-Glu(ODmab)-OH	p. 36
852117	Fmoc-Glu-O-2-PhiPr	p. 36
852085	Fmoc-Glu(O-2-PhiPr)-OH	p. 37

References

1. G.B. Bloomberg, et al. (1993) *Tetrahedron Lett.*, **34**, 4709.
2. B. W. Bycroft, et al.(1993) *J. Chem. Soc., Chem. Commun.*, 778.
3. P. Hoogerhout, et al. (1999) *J. Pept. Res.*, **54**, 436.
4. M. Lebl, et al. (1993) *Int. J. Peptide Protein Res.*, 41, 201.
5. J. M. Kerr, et al. (1993) *J. Am. Chem. Soc.*, **115**, 2529.
6. J. Eichler, et al. (1994) *Pept. Res.*, **7**, 300.
7. J. Eichler, et al. in "Innovation & Perspectives in Solid Phase Synthesis, 4th International Symposium", R. Epton (Ed.), Mayflower Scientific Ltd., Birmingham, 1996, pp. 201.
8. P. Dumy, et al. in "Peptides 1994, Proc. 23rd European Peptide Symposium", H. Maia (Ed.), ESCOM, Leiden, 1995, pp. 307.
9. C. G. Fields, et al. (1993) *Biopolymers*, 33, 1695.
10. V. Wittmann & S. Seeberger (2000) *Angew. Chem. Int. Ed.*, **39**, 4348.
11. a) C. Park & K. Burgess (2001) J. Comb. Chem., 3, 257; b) Y. Feng & K. Burgess (1999) *Chem. Eur. J.*, **5**, 3261.

12. D.N. Posnett, et al. (1988) *J. Biol. Chem.*, **263**, 1719.

13. S. R. Chhabra, et al. (1998) *Tetrahedron Lett.*, **39**, 1603.

14. http://scholar.google.co.uk/scholar?cites=7545760316968945982.; http://scholar.google.co.uk/scholar?cites=16073846467466700087

15. N. Ahlborg (1995) *J. Immun. Meth.*, **179**, 269.

16. B. W. Bycroft, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 13th American Peptide Symposium", R.S. Hodges & J.A. Smith (Eds.), ESCOM, Leiden, 1994, pp. 727.

17. D. Lelièvre, et al. (1995) *Tetrahedron Lett.*, **36**, 9317.

18. J. Mack, et al (2001) *J. Pept. Sci.*, **7**, 338.

19. S. K. Sharma, et al. (1999) *J. Pept. Res.*, **53**, 501.

20. P. J. Connolly, et al. (2000) *Tetrahedron Lett.*, **41**, 5187.

21. A. Gopalsamy, et al. (2000) *Bioorg. Med. Chem. Lett.*, **10**, 1715.

22. E. Garanger, et. al. (2008) *Chem. Commun.*, 4792.

23. K. S. A. Kumar, et. al. (2011) *Bioconjugate Chem.*, **22**, 137.

24. K. Augustyns, et al. (1998) *J. Pept. Res.*, **51**, 127.

25. R. Wilhelm, et al. in "Peptides for the New Millennium, Proc. 16th American Peptide Symposium", G. B. Fields, J. P. Tam & G. Barany (Eds), Kluwer Academic Publishers, Dordrecht, pp. 58.

26. J. J. Diaz-Mochon, et al. (2004) *Org. Lett.*, **6**, 1127.

27. A. Aletras, et al. (1995) *Int. J. Peptide Protein Res.*, **45**, 488.

28. Z Flegelova, et al. in "Proc. 32nd European Peptide Symposium", Athens, 2012, poster 43.

29. K. Barlos, Personal communication.

30. L. Bourel, et al. (2000) *J. Pept. Sci.*, **6**, 264.

31. Y. Feng & K. Burgess (1999) *Chem. Eur. J.*, **5**, 3261.

32. S. Matysiak, et al. (1998) *Tetrahedron Lett.*, **39**, 1733.

33. F. Albericio, et al (1993) *Tetrahedron Lett.*, **34**, 1549.

34. S. A. Kates, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 13th American Peptide Symposium", R. S. Hodges & J. A. Smith (Eds.), ESCOM, Leiden, 1994, pp. 113.

35. B. Rohwedder, et al. (1998) *Tetrahedron Lett.*, **39**, 1175.

36. P. E. Schneggenburger, et al. (2010) *J. Pept. Sci.*, **16**, 10.

37. M. Meldal, et al. (1997) *Tetrahedron Lett.*, **38**, 2531.

38. J. T. Lundquist & J. C. Pelletier (2001) *Org. Lett.*, **3**, 781.

39. K. Barlos, et al. (1991) *Tetrahedron Lett.*, **32**, 471.

40. K. Barlos, et al. in "Peptides 1992, Proc. 22nd European Peptide Symposium", C. H. Schneider & A. N. Eberle (Eds.), ESCOM, Leiden, 1993, pp. 283.

41. M. P. Coba, et al. (2003) *J. Peptide Res.*, **61**, 17.

42. K. Barlos, et al. (1998) *J. Pept. Res.*, **51**, 194.

43. U. Weber & P. Hartter (1970) *Hoppe-Seyler's Z. Physiol. Chem.*, **351**, 1384.

44. T. M. Postma, et al. (2012) *Org. Lett.*, **14**, 5468.

45. F. Albericio et al. in "Peptides 1992, Proc. 22nd European Peptide Symposium", C. H. Schneider & A. N. Eberle (Eds.), ESCOM, Leiden, 1993, pp. 191.

46. W. Bannwarth, et al. (1992) *Tetrahedron Lett.*, **33**, 4557.

47. N. A. Solé, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 13th American Peptide Symposium", R. S. Hodges & J. A. Smith (Eds.), ESCOM, Leiden, 1994, pp. 93.

48. C. Flouzat, et al. (1997) *Tetrahedron Lett.*, **38**, 1191.

49. W. C. Chan, et al. (1995) *J. Chem. Soc., Chem. Commun.*, 2209.

50. D. J. Evans, et al. Poster 122 presented at the 24th European Peptide Symposium, Edinburgh, 1996.

51. M. Valldosera, et al. (2008) *Int. J. Pept. Res. Ther.*, **14**, 273.

52. K. F. Medzihradszky, et al. (2002) *Lett. Pept. Sci.*, **8**, 1.

53. T. Johnson, et al. (2000) *J. Chem. Soc., Perkin Trans. 1*, 2811.

54. T. Conroy, et al. (2009) *Org. Biomol. Chem.*, **7**, 2255.

55. M. Cudic, et al. in Peptides 2000, Proc. 26th European Peptide Symposium", J. Martinez & J.-A. Fehrentz (Eds), Éditions EDK, Paris, 2001, pp. 203.

56. M. Cudic, et al. (2000) *Tetrahedron Lett.*, **41**, 4527.

57. J. P. Malkinson, et al. (2003) *Org. Lett.*, **5**, 5051.

58. F. Dick, et al. in "Peptides 1996, Proc. of 24th European Peptide Symposium", R. Ramage & R. Epton (Eds.), Mayflower Scientific Ltd., Birmingham, 1998, pp. 339.

59. a) A. S. Dutta (1989) *Chem. Br.*, **25**, 159. ;
    b) K. D. Kopple (1972) *J. Pharm. Sci.*, **61**, 1345.

60. H. F. Brugge, et al. (1994) *Int. J. Peptide Protein Res.*, **43**, 166.

61. a) N. Izumiya, et al. (1981) Biopolymers, 20, 1785. ;b) S. F. Brady, et al. in "Peptides, Structure & Function", V. J. Hruby & D. H. Rich (Eds.), Pierce Chemical Company, Rockford, 1983, pp. 127.

62. a) P. Rovero, et al. (1991) *Tetrahedron Lett.*, **32**, 2639. ;b) J. S. McMurray, et al. (1994) *Pept. Res.*, **7**, 195.

63. J. S. Davies (2003) *J. Pept. Sci.*, **9**, 471.

64. J. Offer, et al. (1996) *J. Chem. Soc., Perkin Trans. I*, 175.

65. D. Delforge, et al. (1996) *Lett. Pept. Sci.*, **3**, 89.

66. M. Quibell, et al. (1994) *J. Chem. Soc., Chem. Commun.*, 2343.

Application 4-4: Synthesis of cyclo(Tyr-D-Trp-Val-D-Trp-D-Trp-Arg-Glu)

H-Tyr(tBu)-D-Trp-Val-D-Trp-D-Trp-Arg(Pmc)-Glu(O-2-ClTrt resin)-ODmab was prepared automatically using a Perspective Millipore 9050 peptide synthesizer on 2-ClTrt resin (Figure 4-11). All acylation reactions were carried out using Fmoc-amino acids activated with HBTU (1 eq.) in the presence of DIPEA (2 eq.) and HOBt (1 eq.). Removal of Dmab was effected by flowing 2% hydrazine monohydrate in DMF through the resin bed, until no further release of indazole by-product could be detected by spectrophotometric monitoring at 290 nm. To assess the purity of the intermediate peptide prior to cyclization, a small sample of peptidyl resin was treated with 1% TFA in DCM and the partially protected peptide so released was analyzed by HPLC (Figure 4-12). The resin bound hydrazine salt of glutamic acid was then converted to a DIPEA salt by washing the remainder of the resin with 5% DIPEA in DMF. On-resin cyclization was achieved by treatment of the resin with DIPCDI (1.1 eq.) and HOAt (1.1 eq.) for 18 h. Cleavage was carried out by treatment of the peptidyl resin with TFA/TIS/water/EDT (90:1:5:4) for 2h. The crude peptide was analyzed by HPLC (Figure 4-13) and PD-MS (Figure 4-14) [expected M+H⁺ 1107.2, found 1107.7].

Fig. 4-13: HPLC elution profile of crude H-Tyr(tBu)-D-Trp-Val-D-Trp-D-Trp-Arg(Pmc)-Glu-OH.
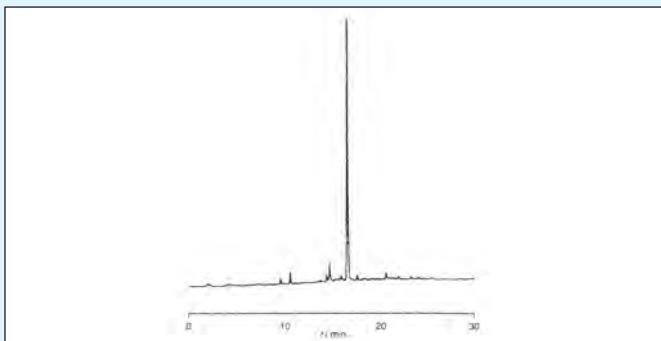
Fig. 4-14: HPLC elution profile of crude cyclo(Tyr-D-Trp-Val-D-Trp-D-Trp-Arg-Glu).
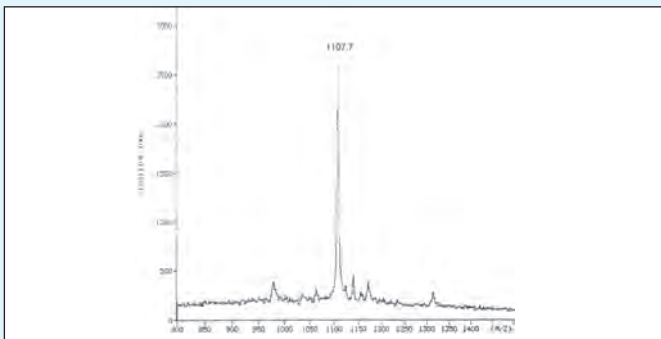
Fig. 4-15: PD-MS of total crude peptide.

5: Reagents for peptide ligation & labeling

5.1 Chemoselective ligation

In spite of the numerous methodological advances made over the last two decades, the preparation of large peptides and proteins by step-wise solid phase synthesis still remains problematic. This is principally due to difficulties in separating the target molecule from the melange of closely related truncated and deletion products which arise during the synthetic process. In addition, the purified products, despite giving the appearance of being homogeneous by HPLC analysis, are often heterogeneous, being contaminated with numerous co-eluting sequences which, because they are individually only present in small amounts, escape detection by mass spectrometry.

These limitations have led a number of different research groups to develop methods based on the conjugation of unprotected peptide segments in aqueous media *via* formation of oxime [1, 2], hydrazone [3], and thiazolidine [4] linkages, utilizing the selectivity of the reaction of aldehydes with hydroxylamines, hydrazines and aminothiols in the presence of protonated amino functions (see Figure 5-1), triazole formation by azide-alkyne addition [5] or by formation of an amide bond, through the intermediacy of a thioester [6-8] or thiazolidine linkage [9]. The subject has been reviewed by Tam, et al. [10, 11] (the latter paper contains detailed laboratory protocols), Dawson & Kent [12, 13] and Casi & Hilvert [14], and more recently Dirksen & Dawson [15].

Ligation strategies have obvious benefits: by coupling together small to medium sized peptides, which can be produced routinely to a high level of homogeneity, the task of product purification can be greatly simplified; and by using unprotected peptides, the problems of poor fragment solubility normally associated with fragment condensation methods are eliminated.
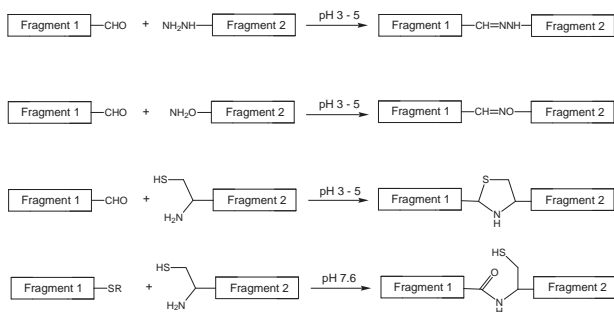
Fig. 5-1: Approaches to chemoselective ligation.

5.1.1 Native thiol ligation

Native thiol ligation is perhaps the most powerful ligation procedure since it provides the product containing an amide bond at the site of connection (Figure 5-2) [6, 7]. This approach generally requires the amino fragment to have a Cys residue at its *N*-terminus or acyl-transfer auxilaries [16] and the C-terminal carboxy group of the carboxy fragment to be present as a thioester. The required thioesters can be prepared either by chemical synthesis (see below), or from larger fragments by splicing from an expressed extein-intein construct [17].
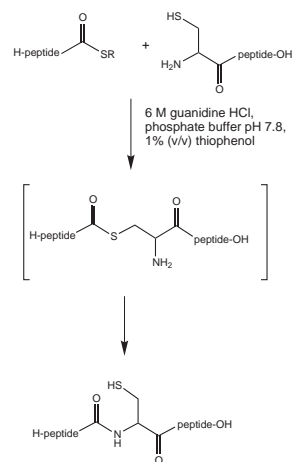
Fig. 5-2: Native chemical ligation.

5.1.2 Synthesis of thioesters by Fmoc SPPS

The synthesis of thioesters by Fmoc methods can not be reliably effected directly by solid phase synthesis, owing to the facile cleavage of thioesters by piperidine. To overcome this limitation, a number of innovative strategies have been devised, of which the three most frequently employed are solution synthesis of thioester, use of safety-catch linker and N-to-S acyl migration.

Solution synthesis of thioester

Peptide thioesters can be prepared by coupling of protected peptides to thiols [18] or amino-acid thioesters. Generally, the latter approach is preferred as the reaction is more facile and leads to less epimerization of the *C*-terminal amino acid of the peptide fragment. Recently, Danishefsky and coworkers [19] used peptide thioesters prepared in this way as building blocks for the synthesis of glycosylated EPO by native chemical ligation. Here they prepared fully protected peptide fragments on 2-chlorotrityl resin, incorporating pseudoproline and Dmb-dipeptides to aid solubility and synthesis of the fragments. These fragments were coupled in solution to amino-acid thioesters by Sakabara's method [20] with water-soluble carbodiimide and HOOBt. Treatment of the resultant peptide with TFA yielded the desired peptide-thioester.

Safety–catch approach

Synthesis of peptide thioesters by the safety-catch approach involves the use of a specially designed linker that, after peptide chain extension, can be chemically activated towards thiolysis. The two most frequently used are the sulfamylbutryryl (Ellman) and diaminobenzoyl (Dbz, Dawson) linkers, and these are available from Novabiochem attached to variety of different resins.

Sulfonamide method

This approach, first described by Pessi and coworkers [21] and then later by others [22 - 24], involves displacement of the peptide fragment with a thiol from an alkylated sulfamylbutyryl resin (Figure 5-3). Following chain assembly the resin is activated by alkylation of the sulfonamide nitrogen, usually treatment with iodoacetonitrile or TMS-CHN$_2$. Activation with iodoacetonitrile produces a more reactive intermediate, whereas with TMS-CHN$_2$ the actual process of activation is more efficient. Activation methods have been reviewed in ref. [25]. The resulting N-alkyl-N-acylsulfonamide is then cleaved by treatment with either benzylmercaptan [21] or ethyl mercaptopropionate /thiophenol [22]. However, the combination of activation with TMS-CHN$_2$ and displacement with ethyl mercaptopropionate/thiophenol appears to be optimal (Method 5-1). The use of 2M LiBr in THF as the cleavage solvent has been shown to lead to greatly improved yields of peptide thioester [26].

The resulting protected peptide thioester is then treated with TFA and the appropriate scavengers to give the deprotected peptide ready for ligation. The process of ligation is described in detail in ref. [10].
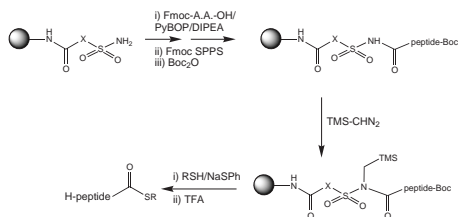
Fig. 5-3: Synthesis of peptide thioesters using sulfamylbutyryl resin.

Method 5-1: Thioester ligation with sulfamyl resins

Activation of acylsulfamyl resins
1. Pre-swell the resin (0.1 mmole) in dry THF in a 10 ml polypropylene syringe fitted with a 20 μm polyethylene filter.
2. Add 5 ml of 1 M TMS-CHN$_2$ in dry hexane/THF (1:1). Cap the syringe.
3. Agitate gently for 2 h. Wash resin with THF and use immediately, or wash with THF, then DCM and dry in vacuo.

Cleavage of thioester
1. Pre-swell methylated resin in DMF for 1 h before use.
2. Add ethyl-3-mercaptopropionate (50 eq.) and sodium thiophenoxide (0.5 eq.), cap the syringe and agitate the mixture gently for 24 h.
3. Remove the resin by filtration and wash it three times with DMF.
4. Combine the filtrates and evaporate to dryness on a rotary evaporator. Triturate the product with ether.
5. Treat the residue with TFA/water/TIS /phenol (88:5:2:5) for 2 h at rt.
6. Add the cleavage solution drop-wise to 10 volumes of cold methyl t-butyl ether (MTBE), and isolate the product by filtration or centrifugation using standard methods. Purify product by RP-HPLC.

Ligation of unprotected peptide fragments
1. Dissolve peptide thioester (1 eq.) and N-terminal-Cys peptide (1 eq.) in a screw-cap tube containing degassed 0.1 M sodium phosphate buffer, pH 7.8. If necessary, the solution can also contain guanidine hydrochloride (up to 6 M) to add dissolution of the peptide components. The final concentration of the peptides should be 2-5 mM.
2. Add thiophenol (1% by volume of total solution), flush with nitrogen, recap tube, and agitate mixture vigorously. The progress of the reaction can be monitored by HPLC. The reaction is typically over in 5-16 h.
3. Acidify the reaction with TFA (0.1% by volume of solution), lyophilize and purify by standard procedures.

Novabiochem offers the sulfamylbutyryl linker attached to AM resin and NovaSyn® TG resin. Loading of these resins with Fmoc-amino acids is best achieved PyBOP® and DIPEA in CHCl$_3$ at -20°C [27] or with DIPCDI/N-methylimidazole. In the case of PyBOP® activation, the loading efficiencies are reported to vary from >95% for Cys, Met and His to 44% for Pro, the worst case. Extent of racemizationization for the loading of Fmoc-Phe and Fmoc-Leu by these methods are 0.5% and 0.3%, respectively. Novabiochem® also offers a range of pre-loaded sulfamylbutyryl NovaSyn® TG resins. Here, coupling of the first amino acid to the sulfamyl linker is carried out in solution prior to attachment of the purified, fully characterized Fmoc-amino acid linker to amino NovaSyn® TG. This produces high-quality supports of defined substitution, free from by-products arising from overacylation. The supports can be used directly in automated peptide synthesis without modification of existing protocols.

The sulfonamide method, whilst popular, has been plagued by notoriously low yields. These originate from three sources:

1) incomplete acylation of the resin-bound sulfonamide with the C-terminal residue;

2) incomplete alkylation of the sulfonamide, which is usually performed blind and not optimized;

3) incomplete thiolysis, due to the inherent low reactivity of the activated sulfonamide towards thiols and poor solvation of the resin-bound protected peptide.

Recently, a novel dual linker strategy has been developed [28] that involves anchoring of the sulfamylbutyryl linker to a standard acid-labile resin. This approach appears to overcome all the limitations of the sulfonamide method and provides a simple and robust strategy for Fmoc SPPS-based NCL (Figure 5-4):

The sulfamylbutyryl linker is pre-loaded with the C-terminal residue prior to its attachment to the solid phase, thereby overcoming issues with incomplete loading, racemizationization and double addition that can occur during on-resin linker functionalization;

By using Rink amide or Sieber amide resin as the solid support for the pre-loaded linker, the N-peptidyl-N-methylsulfonamide can be released from the resin by TFA treatment, enabling the progress of the synthesis and extent of methylation to be easily checked by LC-MS;

Moreover and most importantly, the intermediate N-peptidyl-N-methylsulfonamide can be cleaved from the support and be used as a surrogate thioester directly in NCL reactions, without the need for prior conversion to the thioester.

For this method, Novabiochem offers sulfamylbutyryl linkers pre-loaded with Fmoc-amino acids, which can be attached to any TFA-labile resins, sulfamylbutyryl Rink Amide resins, and Fmoc-amino acid sulfamylbutyryl Rink Amide resins.
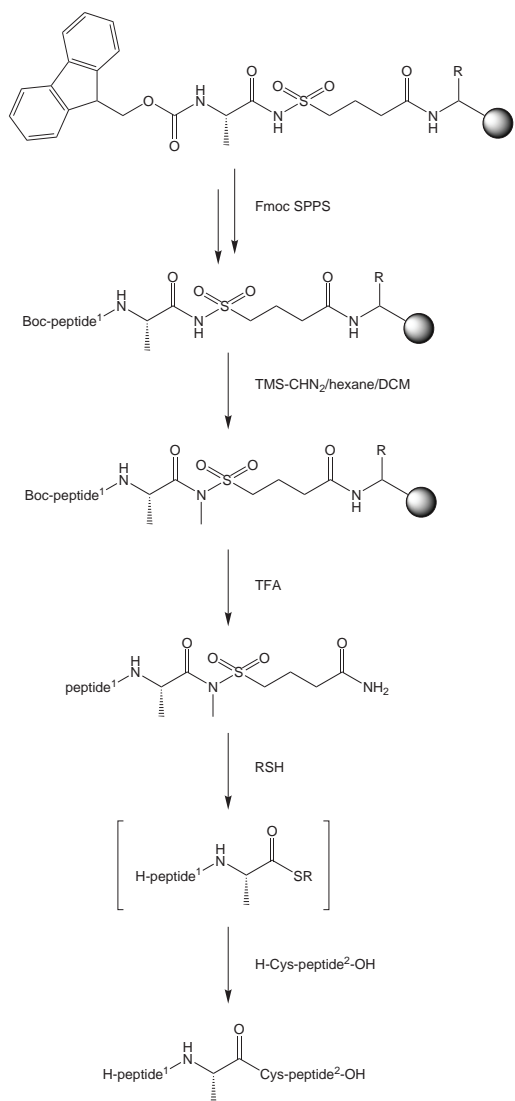
Fig. 5-4: NCL using sulfamylbutyryl Rink amide resins.

Synthesis of BPTI using sulfamyl method

The double linker strategy is exemplified in the synthesis of BPTI, a 58 residue, small protein containing a full range of side chain functional groups. The strategy of Lu and co-workers [29] was selected for the preparation of BPTI, involving the ligation of two fragments: BPTI(1-37) and BPTI (38-58) (Figure 5-5).

H-RPDFCLEPPYTGPCKARIIRYFYNAKAGLCQTFVYGG-N(Me)SO$_2$CH$_2$CH$_2$CH$_2$CONH$_2$ **BPTI(1-37)**

+

H-CRAKRNNFKSAEDCMRTCGGA-OH **BPTI(38-58)**

0.2 M sodium phosphate buffer,
1 M guanidine.HCl,
2 mM EDTA, 50 mM TCEP,
60 mM MPAA, pH 7.5

H-RPDFCLEPPYTGPCKARIIRYFYNAKAGLCQ
TFVYGGCRAKRNNFKSAEDCMRTCGGA-OH
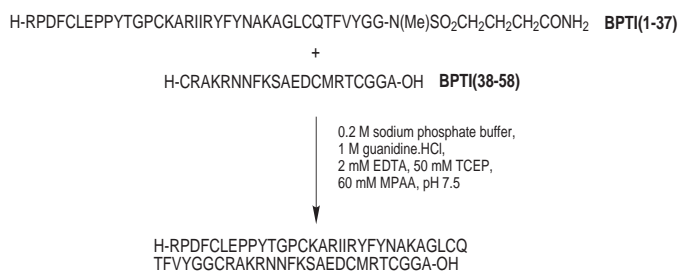
Fig. 5-5: Synthesis of BPTI.

BPTI(38-58) was assembled on Fmoc-Ala-Wang resin using standard HOBt/DIPCI activation (Application 1).
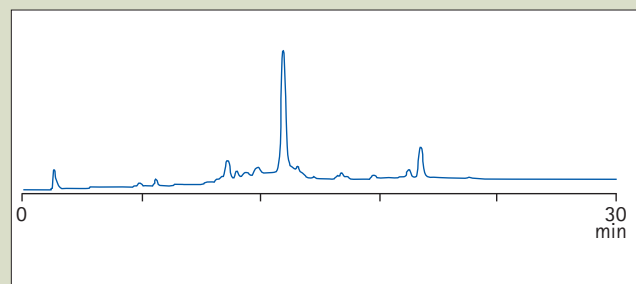
BPTI(1-37) was assembled in a similar manner on Fmoc-Gly-sulfamylbutyryl Sieber Amide resin. Methylation of the linker was effected by treatment with 2M TMS-CHN$_2$ in hexane/DCM (1:1) for 18 hours. TFA cleavage afforded the deprotected *N*-BPTI(1-37)-*N*-methylsulfonamide. Following purification by RP-HPLC, the two fragments were ligated (Fig. 2) using low millimolar fragment concentration. The reaction was almost complete in 8 h, a time comparable to that observed by Lu and co-workers [29] with classical NCL using a preformed thioester.
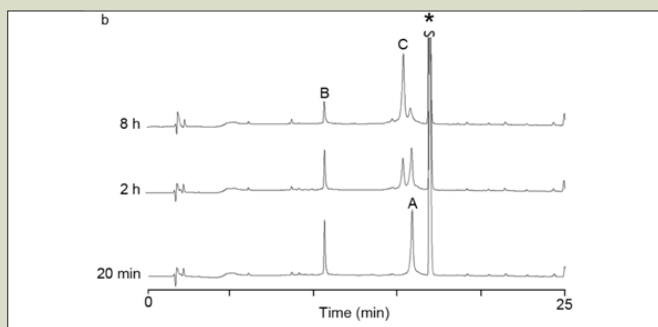
Dbz method
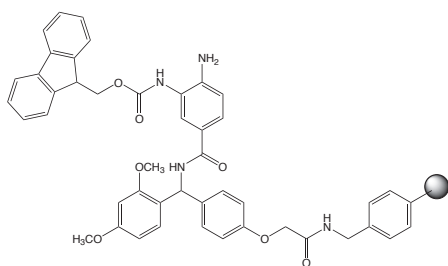
This strategy utilizes a 3,4-diaminobenzoic acid (Dbz) attached *via* its carboxyl group to a TFA-cleavable amino functionalized resin [30, 31]. Peptide chain extension is performed on one of the anilino groups, followed by formation of an imidazolidinone (Nbz) with p-nitrophenyl chloroformate, and cleavage form the resin with TFA. The peptide-Nbz is used directly in chemical ligation reactions to generate in situ the desired peptide thioester (Figure 5-9).
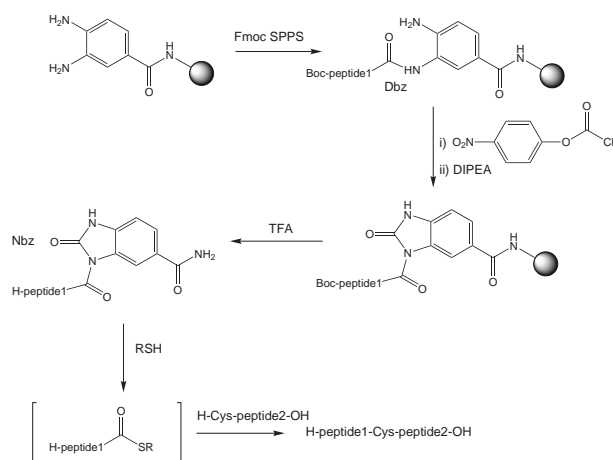
Fig. 5-9: Synthesis of peptide thioesters using Dawson Dbz AM resin.

Success in using the Dbz strategy depends on being able to fully acylate only one of the two linker amines with the *C*-terminal amino acid residue and to avoid acylation of the unprotected amine during chain extension. Incomplete acylation leads to formation of *C*-terminally truncated peptides as new chains are propagated by acylation of any unreacted amines during subsequent coupling cycles. Whereas, overacylation results to formation of branched peptides with chains growing off both linker amines. Therefore, the selection of acylation method of attachment of the *C*-terminal residue and subsequent couplings is critical if good results are to be obtained (Method 5-2).

Particularly problematic is the coupling of glycine residues, especially if they occur close to the *C*-terminus of the peptide. This reactive and unhindered amino acid can couple to the free Dbz-amino group if uronium or phosphonium activation is used. In our hands best results are obtained if glycine residues are introduced using Fmoc-Gly-OPfp/HOBt. This precaution may be unnecessary once the peptide is extended beyond 10 residues, as hindrance should reduce the reactivity of unprotected Dbz amine.

Blocking of the *N*-terminal amino group, prior to activation of the linker with *p*-nitrophenyl chloroformate, is essential. The easiest and simplest way is to use a Boc-amino acid to introduce the final residue. If *N*-terminal capping is necessary, it must be done using very mild reagents if blocking of the second amine is to be avoided. Recently, Dawson and coworkers [31c] found *N*-terminal acylation could be performed successfully using *N,N*-diacetylaminoquinazolinone. Boc-N$_3$ should be suitable for introduction of an *N*-terminal Boc group.

Recently, the use of Alloc protection for blocking the second amino group has been advocated as a way to avoid all issues with branching and truncation [32] (Figure 5-10). Dbz resins as supplied contain mostly 3-Fmoc-Dbz, with small amounts of 4-Fmoc-Dbz and bis-Fmoc-Dbz. Capping the resin with Alloc-Cl prior to removal of the Fmoc group will thus reduce the maximum potential for branching or truncation to 6%. For hindered amino acids, it has been found necessary to load the resin prior to capping with Alloc. The Alloc group must be cleaved off with Pd(0) before conversion to the Nbz form.
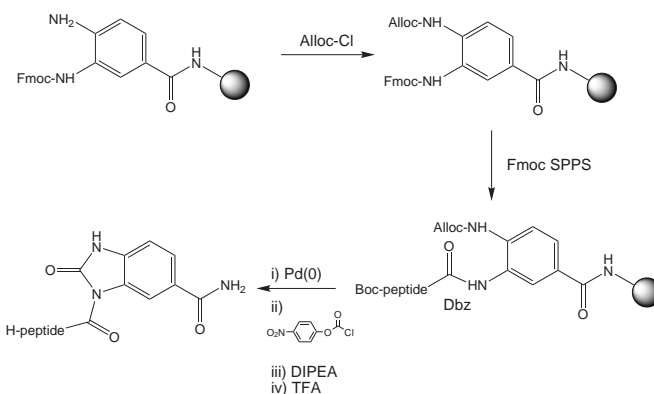
Fig. 5-10: Alloc protection strategy [32].

Loading of the C–terminal residue

Loading of the *C*-terminal residue is best achieved using Method 5-2a. With synthesizers that use dry reagents, such as the ABI 443A, loading of the first residue can be automated by using a mixture of Fmoc-amino acid and the appropriate coupling reagent packed in a cartridge or vial, with activation effected by addition of DIPEA in DMF. For subsequent amino acids, the standard pre-dissolved activator can be used. Some optimization of the loading reaction may be required to maximize loading and minimize branching. Treatment of a small sample of loaded resin with TFA and analysis by HPLC of the cleaved product can be helpful in monitoring this process.

Chain extension

In general, strong activators like HATU or HCTU should be avoided as their use can lead to branching. In our hands, HBTU/HOBt appears to work well for coupling of all residues except Gly, where the use of the pre-formed OPfp in conjunction with HOBt gives minimal branching.

Conversion to Nbz

Activation of the linker by conversion to the Nbz is achieved according to Method 5-2b. The reaction is usually quantitative. With high loaded resins like Dawson Dbz AM resins, some cross-linking of Dbz moieties can occur. In our experience, much cleaner results are obtained with low-loaded resins like Dawson Dbz NovaSyn® TGR resin. Treatment of the Nbz resin with TFA releases the fully deprotected peptide-Nbz, which can be used directly in the NCL reaction. The Nbz peptide is obtained as a mixture of regioisomers.

Method 5-2: Thioester ligation with Dawson Dbz AM resin

a) Loading
1. Pre-swell the resin (0.1 mmole) in DCM for 60 mins and wash with DMF. Remove Fmoc group with 20% piperidine in DMF and wash with DMF.
2. Ile, Val, Thr, Pro, Arg: Add Fmoc-Aaa-OH (0.6 mmole), HATU (0.6 mmole) and DIPEA (0.9 mmole). Agitate gently for 1 h. Wash resin with DMF and repeat coupling.
   Gly: Add Fmoc-Gly-OPfp (0.6 mmole) and HOBt (0.6 mmole). Agitate gently for 1 h.
   Other amino acids: Add Fmoc-Aaa-OH (0.6 mmole), HCTU (0.6 mmole) and DIPEA (0.9 mmole). Agitate gently for 1 h. .
3. Check loading using Method 3-11, p. 3.7. Alternatively, wash a sample of resin with DCM and treat with 95% TFA aq. for 30 min. Anaylze cleaved product by HPLC.

b)Synthesis & Activation
1. Extend peptide chain using HBTU/HOBt/DIPEA activation, except for Gly which should be introduced using Fmoc-Gly-OPfp/HOBt. The N-terminal residue must be introduced using a Boc-amino acid. Wash the resin with DMF and DCM.
2. Add p-nitrophenyl chloroformate (0.5 mmole) in DCM and leave to gently agitate under N2 for 1 h. Wash resin with DCM and add 0.5 M DIPEA in DMF (10 ml) and leave for 30 min. Wash resin with DMF and DCM.
3. Cleave peptide with TFA/water/TIS 95:2.5:2.5 for 3 h.

Ligation of unprotected peptide fragments
1. Dissolve purified peptide-Nbz (1 eq.) and N-terminal-Cys peptide (1.5 eq.) in a screw-cap tube containing degassed ligation buffer (0.2 M phosphate buffer, 6 M guanidine hydrochloride, 0.2 M 4-mercaptophenylacetic acid, 0.02M TCEP, pH 7.0). The final concentration of the peptides should be approximately 2 mM.
2. Monitor the progress of the reaction by HPLC.
3. Acidify the reaction with TFA (0.1% by volume of solution), lyophilize and purify by standard procedures.

N to S Acyl migration/thiol capture

Peptides containing cysteine residues are in equilibrium between the amide and thiodepsipeptide forms. The position of the equilibrium between peptide and thiodepsipeptide is dependent on pH. At neutral pH, the amide form of peptide is strongly favored, but under acidic conditions, the equilibrium is shifted towards the thioester. If an excess of external thiol is present, the thiodepsipeptide can be cleaved to give a peptide thioester (Figure 5-11). This principle forms the basis of a number of methods for peptide thioester synthesis. In these, *N*-alkylation of the key amide bond, covalent capture of the thiodepsipeptide amine, and enhancement of the local thiol concentration have all been employed to move the position of the equilibrium to favor the thiodepsipeptide, and hence formation of the peptide thioester.
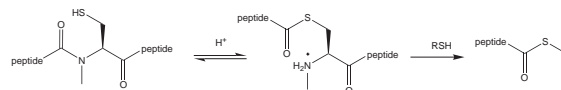
Fig. 5-11: N to S acyl migration and thiol capture.

One simple and effective approach to NCL based on N to S acyl migration/thiol capture is known as SEA ligation [33 - 35]. Here, peptides are prepared bearing a bis(2-sulfanylethyl)amide (SEA) on their *C*-terminus. These peptide at neutral pH exist as a mixture of amide and thioester forms. In presence of added thiol, the SEA group is cleaved to give the peptide thioester, which can be isolated or used *in situ* for NCL (Figure 5-12).

For the synthesis of peptide-SEAs, Novabiochem provides SEA-polystyrene resin. Loading of the resin with the *C*-terminal residue is best done using Fmoc-amino acid coupled activated with HATU. After peptide assembly, the peptide-SEA is cleaved from the resin using standard TFA cocktails. To stabilize the peptide and to simplify HPLC analysis and purification in acidic buffers, the SEA peptide should be converted to the disulfide form by air or iodine oxidation (Figure 5-12). In the presence of a reducing agent such as TCEP, peptide-SEA disulfides undergo rapid NCL or can be converted to thioesters. In the absence of a reducing reagent, peptide-SEA disulfides do not undergo ligation, which has been exploited to perform one-pot three segment ligations [36].
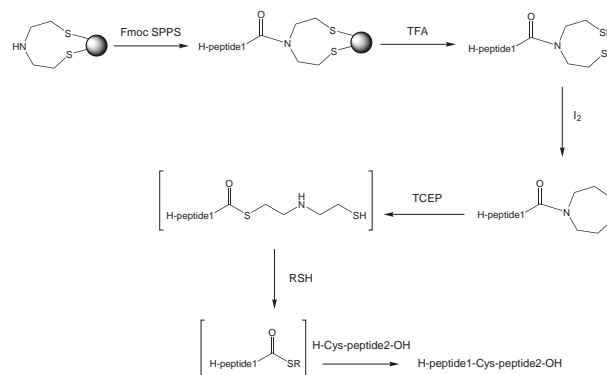
Fig. 5-12: SEA strategy [33–35].

5.1.3 Oxime ligation

Oxime ligation is a powerful and versatile method for the production of MAPs [1, 2], cyclic peptides [37-40], and peptide-glycopeptide conjugates [41]. At pH ~5 in aqueous media, hydroxylamine-labeled peptides ligate with peptides, lipids, sugars, or other entities possessing an aldehyde functionality. The reaction is extremely selective and is compatible with all the functional groups present in natural amino acids, with the exception of N-terminal cysteine which can undergo thiazolidine formation.

The types of peptide conjugates that can be prepared by oxime ligation are summarized in Figure 5-13. By incorporating the hydroxylamine and aldehyde component into the same sequence, side-chain to side-chain cyclized peptides are produced [38-42]; the oxime serves in this instance as a kinetically and metabolically stable analog of a cysteine bridge. Peptides conjugated in a side-chain to head manner can be prepared by incorporation of aminoserine into the sequence and utilizing an N-terminal serine residue as the precursor to the glyoxylic acid moiety. MAPs can also be produced in an analogous fashion.

Hydroxylamine–functionalized building blocks

Fmoc-Dpr(Boc-Aoa)-OH

Boc-Aoa-OH
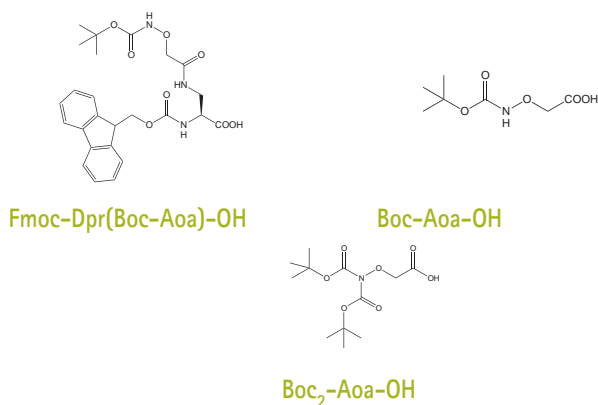
Boc$_2$–Aoa–OH

For introduction of the hydroxylamine label, Novabiochem® provides Fmoc-Dpr(Boc-Aoa)-OH [40], as well as protected amino-oxy carboxylic acids Boc-Aoa-OH and Boc$_2$-Aoa-OH. Following cleavage and side-chain deprotection, peptides are produced bearing either a pendant or an N-terminal hydroxylamine moiety.

Incorporation of mono-protected hydroxylamine compounds like Boc-Aoa-OH is best achieved using HOBt/DIPCDI or HATU/collidine in order to minimize problems with double acylation of the hydroxylamine nitrogen [42]. Such problems can be avoided using Boc$_2$-Aoa-OH where the nucleophilicity of the hydroxylamine functionality is completely masked.

Due to the high reactivity of Aoa-labeled peptides, care must be taken to avoid formation of formaldehyde, acetaldehyde and acetone adducts from the corresponding aldehydes and ketones present in the ambient environment. Addition of excess Boc-Aoa-OH to the cleavage mixture as a carbonyl scavenger has been found to be highly effective at protecting the Aoa moiety prior to HPLC [43].
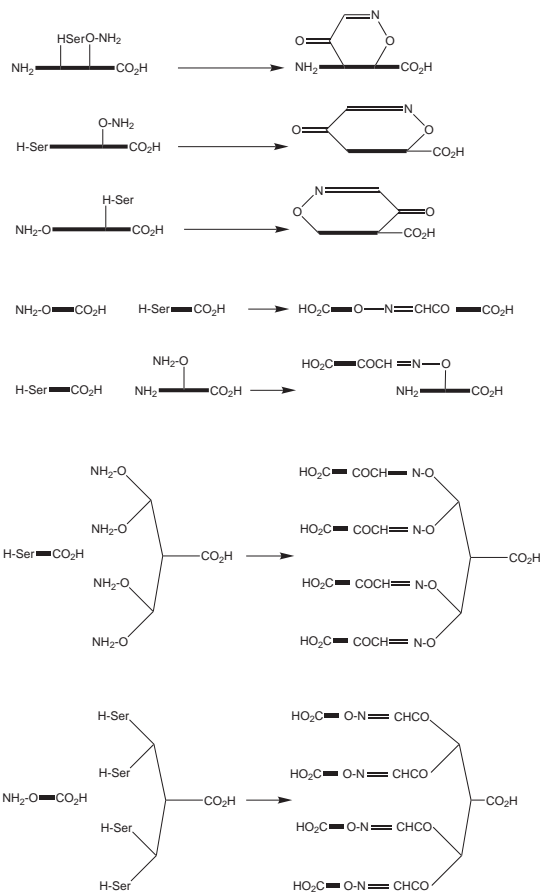
Fig. 5-13: Oxime ligation.

Hydroxylamine NovaTag™ resin/Hydroxylamine PEG NovaTag™ resin
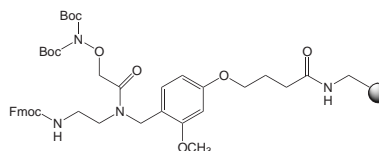
For the production of peptides which can undergo oxime ligation at their C-terminus, Novabiochem® has developed Hydroxylamine NovaTag™ and Hydroxylamine PEG NovaTag™ resins. The Fmoc group is preferably removed using 2% DBU in DMF. Peptide chain elongation can be achieved using standard acylation methods. Cleavage with 95% TFA furnishes the fully deprotected hydroxylamine-labeled peptide.

Reagent for synthesis of aldehyde components
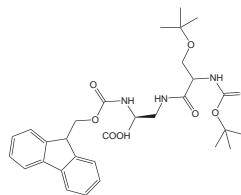
Fmoc–Dpr(Boc-Ser(tBu))-OH

Peptides bearing aldehyde groups are usually generated *in situ* by periodate oxidation of a precursor peptide containing a 1,2-aminoalcohol group (Figure 5-14). If the oxidation reaction is carried out in the

presence of the hydroxylamine component, cis-oxime formation with coupling of the peptide fragments occurs concurrently. For peptides containing an *N*-terminal aldehyde group, the most convenient precursor is the corresponding N-terminal serinyl peptide [44, 45]. Side-chain aldehyde groups are generated by oxidation of the peptide containing a Dpr(Ser) residue, which is introduced using Fmoc-Dpr(Boc-Ser(tBu))-OH [37].
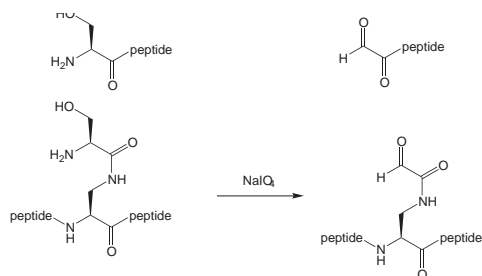
Fig. 5-14: Periodate oxidation route to N-terminal and side-chain aldehyde groups.

5.1.4 SAMA-OPfp
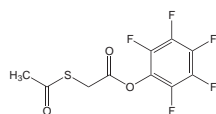
This reagent [46] provides an effective means of linking synthetic peptide antigens to MAP core peptides [47] or carrier proteins for the purpose of raising antibodies [48] (Figure 5-8). Using this technique many of the problems associated with the analysis and purification of MAPs are avoided, since the linear peptide antigen can be fully characterized before conjugation to the preformed lysine tree. For protocols describing the use of SAMA derivatives in the preparation of peptide-protein conjugates and MAPs, see [49]. The derivative is supplied in the form of a Pfp ester, so labeling of an N-terminal amino group, or a lysine side chain, with SAMA is just a matter of treating the peptidyl resin with the reagent in DMF in the presence of HOBt. The S-acetyl group is stable to TFA and remains intact during cleavage of the peptide from the resin. Conjugation is carried out by incubating the SAMA labeled peptide with the bromoacetylated MAP, or protein, in the presence of hydroxylamine. For further details see Method 5-3.
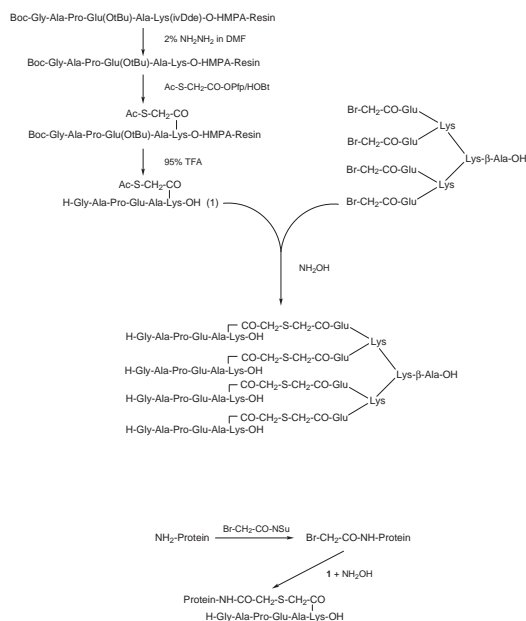
Fig. 5-15: Applications of SAMA.

Method 5-3: Use of SAMA

Derivatization with SAMA

1. Assemble the peptide sequence; if the SAMA moiety is to be attached via a lysine side chain then incorporate that residue using Fmoc-Lys(ivDde)-OH and introduce a Boc-amino acid at the N-terminus.
2. Deprotect either the N-terminal amino function or lysine side chain, depending on where the SAMA moiety is to be linked, with 20% piperidine in DMF or 2% hydrazine in DMF, respectively. Wash the resin with DMF.
3. Dissolve SAMA-OPfp (5 eq.) and HOBt (5 eq.) in DMF and add to the resin. Leave to stand with gentle agitation for 1 h. Check the completeness of the reaction with ninhydrin. Repeat the SAMA addition if necessary.

Bromoacetylation

MAP resin

1. Prepare the MAP resin as normal. After completion of the lysine scaffold, introduce a Glu residue; this will greatly improve the solubility of the MAP core.
2. Deprotect this residue with 20% piperidine, and wash the resin with DMF. Dissolve N-succinimidyl bromoacetate (5 eq.) and HOBt (5 eq.) in DMF, and add the solution to the resin. Leave to stand with gentle agitation for 1 h. Check the completeness of the reaction with ninhydrin. Repeat the N-succinimidyl bromoacetate addition if necessary.

Carrier Protein

1. Dissolve the carrier protein (BSA, KLH or ovalbumin; see CALBIOCHEM catalog) in 100 mM phosphate buffer/100 mM NaCl, pH 7.4.
2. Add N-succinimidyl bromoacetate (10 mg/ml) in DMF (final molar ratio of protein to N-succinimidyl bromoacetate of 1:40). Stir for 2 h at rt.
3. Isolate the protein by gel-filtration on a Sephadex G-25 column.

Conjugation

1. Dissolve the SAMA labeled peptide in 2.5% sodium dodecyl sulfate (Calbiochem cat. no. 428015).
2. Add a solution of bromoacetylated carrier protein (1.1 eq. relative to bromoacetyl functions) in 0.1 M sodium phosphate/5 mM EDTA (Calbiochem cat. no. 34103) at pH 6, followed by 2 M NH$_2$OH (50 eq.) dissolved in the same buffer.
3. Stir at rt for 2 d, and then add 2-aminoethanethiol (6 eq.) in 0.1 M sodium phosphate, pH 6. After a further 16 h, purify the conjugate by gel-filtration on a Sephadex G-25 column.

Related products

References

1. K. Rose. (1994) *J. Am. Chem. Soc.*, **116**, 30.
2. J. Shao & J. P. Tam (1995) *J. Am. Chem. Soc.*, **117**, 3893
3. I. Fisch, et al. (1992) *Bioconjugate Chem.*, **3**, 147.
4. C.-F. Liu & J. P. Tam (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 6584.
5. a) H. C. Kolb, et al. (2001) *Angew. Chem. Int. Ed.*, **40**, 2004; b) C. W. Tornøe & M. Meldal in "Peptides 2001, Proc. 17th Amercian Peptide Symposium", 2001, pp. 263; c) C. W. Tornøe, et al. (2002) *J. Org. Chem.*, **67**, 3057; d) N. N. Rostovtsev, et al. (2002) *Angew. Chem. Int. Ed.*, **41**, 2596.
6. P. E. Dawson, et al. (1994) *Science*, **266**, 776.
7. J. P. Tam, et al. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 12485.
8. a) E. Saxon, et al. (2000) *Org. Lett.*, **2**, 2141; b) B. L. Nilsson, et al. (2001) *Org. Lett.*, **3**, 9.
9. C. F. Liu & J. P. Tam (1994) *J. Am. Chem. Soc.*, **116**, 4149.
10. J. P. Tam, et al. (2000) *Biopolymers*, **51**, 311.
11. J.P. Tam & Y. - A. Lu in "Fmoc solid phase peptide synthesis: a practical approach", W. C. Chan & P. D. White (Eds.), Oxford University Press, Oxford, 2000, pp. 243.
12. P. E. Dawson & S. B. H. Kent (2000) *Ann. Rev. Biochem.*, **69**, 925.
13. S. Kent (2003) *J. Peptide Sci.*, **9**, 574
14. G. Casi & D. Hilvert (2003) *Curr. Opin. Struct. Biol.*, **13**, 589.
15. A. Dirksen & P. E. Dawson (2008) *Curr. Opin. Chem. Biol.*, **18**, 760.
16. J. Offer(2010) *Biopolymers*, **94**, 530.
17. T. W. Muir (2003) *Ann. Rev. Biochem.*, **72**, 249.
18. R. von Eggelkraut-Gottanka, et. al. (2003) *Tetrahedron Lett.*, **44**, 3551.
18. P.S. Wang, et al. (2012). *Angew. Chem.* **124**, 11744; P. Wang ,et al. (2013) *Science*, **342**, 1357.
20. S. Sakakibara (1995) *Biopolymers*, **37**, 17.
21. R. Ingenito, et al. (1999) *J. Am. Chem. Soc.*, **121**, 11369.
22. Y. Shin, et al. (1999) *J. Am. Chem. Soc.*, **121**, 11684.
23. S. Biancalana, et al. (2001) *Lett. Pept. Sci.*, **7**, 291.
24. S. F. Escher, et al. (2001) *Lett. Pept. Sci.*, **8**, 349.
25. P. Heidler & A. Link (2005) *Bioorg. Med. Chem.*, **13**, 585.
26. R. Quaderer & D. Hilvert (2001) *Org. Lett.*, **3**, 3181.
27. B. J. Backes & J. A. Ellman (1999). *J. Org. Chem.*, **64**, 2322.
28. F. Burlina, et al. (2012) *Chem. Commun.*, 2579.
29. W. Lu, et al. (1998) *FEBS Lett*, **429**, 31.
30. J. B. Blanco-Canosa & P. E. Dawson (2008) *Angew. Chem. Int. Ed.*, **47**, 6851.
31. a) Z. Harpaz, et al. (2010) *ChemBioChem*, **11**, 1232; b) B. L. Pentelute, et al. (2010) *ACS Chem. Biol.*, **5**, 359; c) T. K. Tiefenbrunn, et al. (2010) *Pept. Sci.*, **94**, 405.
32. S. K. Mahto, et al. (2011) *ChemBioChem* **12**, 2488.
33. N. Ollivier, et al. (2010) *Org. Lett.*, **12**, 5238.
34. J. Dheur, et al. (2011) *J. Org. Chem.*, **76**, 3194.
35. J. Dheur, et al. (2011) *Org. Lett.*, **13**, 1560.
36. N. Ollivier, et al. (2012) *Angew. Chem. Int. Ed.*, **51**, 209.
37. D. A. Alves, et al. (2003) *J. Peptide Sci.*, **9**, 221.
38. F. Wahl & M. Mutter (1996) *Tetrahedron Lett.*, **37**, 6861.
39. T. D. Pallin & J. P. Tam (1995) *Chem. Commun.*, 2021.
40. J. C. Spetzler & T. Hoeg-Jensen (2001) *J. Pept. Sci.*, **7**, 537.
41. J. C. Spetzler & T. Hoeg-Jensen (1999) *J. Pept. Sci.*, **5**, 582.
42. I. P. Decostaire, et al. (2006) *Tetrahedron Lett.*, 47, 7057.
43. G. Mezö, et al. (2011) *J. Pept. Sci.*, **17**, 39.
44. R. Clamp & L. Hough (1965) *Biochem. J.*, **94**, 17.
45. H. B. F. Dixon & R. Fields in "Methods in Enzymology", C. H. W. Hirs & S. N. Timasheff (Eds), Academic Press, New York, 1972, Vol. 25, pp. 409.
46. J. W. Drijfhout, et al. (1990) *Anal. Biochem.*, **187**, 349.
47. J. W. Drijfhout & W. Bloemhoff (1991) *Int. J. Peptide Protein Res.*, **37**, 27.
48. H. F. Brugghe, et al. (1994) *Int. J. Peptide Protein Res.*, **43**, 166.
49. J. W. Drijfhout & P. Hoogerhout in "Fmoc solid phase peptide synthesis: a practical approach", W. C. Chan & P. D. White (Eds.), Oxford University Press, Oxford, 2000, pp. 229.

5.2   Peptide-labeling

Fluorescent- and biotin-labeled peptides are invaluable tools for biochemistry, having numerous applications in enzymology, protein chemistry, immunology and histochemistry. Novabiochem® offers one of the most extensive ranges of labeling reagents for the synthesis of such peptides, including the unique NovaTag™ resins for the production of C-terminally-labeled peptides.

5.2.1 Chromogen–labeling

The spectral properties of Novabiochem's chromogenic and fluorogenic derivatives are summarized in Table 5-1.

One of the most important applications of such reagents is in the synthesis of fluorescence-quenched enzyme substrates. These are highly sensitive tools for probing protease specificity and activity, particularly for endoproteases where conventional carboxy-terminally labeled substrates are not always appropriate. Such substrates typically contain a fluorophore and a quencher group attached to either side of the cleavage site. In the intact molecule, the natural fluorescence of the fluorophore is suppressed by the proximity of the quencher through a process called fluorescence resonance energy transfer (FRET). Upon cleavage of the substrate by a protease, the quencher and fluorophore become separated, leading to an increase in fluorescence, which can then be detected spectrophotometrically (Figure 5-16). Sensitivity is determined primarily by the distance between fluorophore and quencher, which should be in the range 10-100 Å, and the extent of overlap between the absorbance spectrum of the quencher and the emission spectrum of the fluorophore. The recommended fluorophore-quencher pairs are listed in Table 5-1.

Table 5-1: Spectral properties of Novabiochem dyes.

Chromophore/ Fluorophore	I_{max} (nm)	I_{em} (nm)	Quencher
AMC	342	441	-
Dabcyl	453	-	-
Dansyl	335	526	Dabsyl
Dnp	348	-	-
EDANS	341	471	Dabcyl
Mca	328	393	Dnp
FAM	494	518	Dabcyl
TAMRA	555	580	-

Fluorescence resonance energy transfer (FRET)

-Pro-Tyr-Tyr-Gly-Asp-Glu-Pro-Leu-   Low fluorescence

Enzyme cleavage

-Pro-Tyr-Tyr-Gly-OH   H-Asp-Glu-Pro-Leu-   High fluorescence

Fluorophore   Quencher
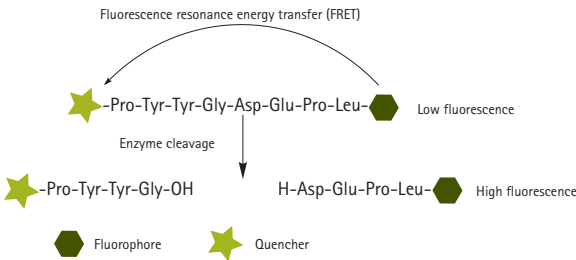
Fig. 5-16: Fluorescence-quenched peptide substrate.

5.2.2 NovaTag™ resins

Fig. 5-17: NovaTag™ resins.

Mca NovaTag™ resin: R=Mca, R^1=Fmoc, X=CH$_2$CH$_2$
Dnp NovaTag™ resin: R=Dnp, R^1=Fmoc, X=CH$_2$CH$_2$
Dansyl NovaTag™ resin: R=Dansyl, R^1=Fmoc, X=CH$_2$CH$_2$
EDANS NovaTag™ resin: R=H, R^1=5-sulfonaphthyl, X=CH$_2$CH$_2$
Universal NovaTag™ resin: R=Fmoc, R^1=Mmt, X=CH$_2$CH$_2$
Universal PEG NovaTag™ resin: R=Fmoc, R^1=Mmt, X=PEG
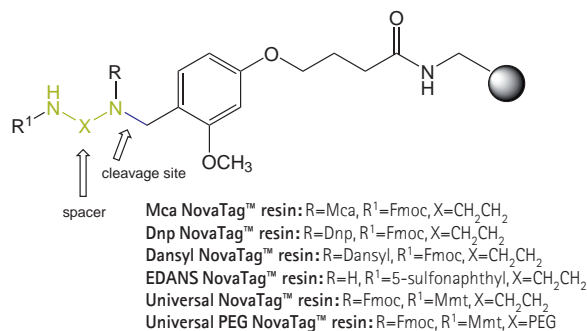
Labels are most frequently incorporated at the N-terminus of the peptide during solid phase synthesis as this is synthetically very straightforward using carboxylic acid derivatives of labels such as biotin-OSu, TAMRA, etc. For many applications, however, it is advantageous to place the label at the C-terminus, particularly if the N-terminus is required for biological activity, or if the peptide contains more than one label.

Furthermore, for FRET-based enzyme substrates, it is often preferable to place the fluorophore/quencher pair at the N- and C-termini, rather than on amino-acid side chains as this avoids modification of the native peptide sequence.

Novabiochem®'s NovaTag™ resins are unique tools designed to streamline the Fmoc SPPS of chromogen and biotin labeled peptides [1]. Each resin contains a standard TFA-cleavable linker which has been modified to incorporate a diamine spacer that is either orthogonally protected or pre-derivatized with a chromogenic label (Fig. 5-9). This arrangement allows C-terminally labeled peptides to be prepared quickly and efficiently with the minimum number of synthetic steps. Pre-loaded resins are available which on cleavage directly provide peptides containing fluorophores (Dansyl, Mca, EDANS) and quencher groups (Dnp) for FRET applications, or affinity labels (biotin, biotin-PEG, hydroxylamine) for bioconjugation and surface immobilization.

The pre-loaded resins are easy to use, and can be generally employed in automated instrumentation without modification of existing Fmoc synthesis protocols. The only exceptions are the EDANS NovaTag™ and Universal NovaTag™ resins where attachment of the first residue must be carried out under modified conditions as this involves acylation of a secondary amine (Method 3-10, page 3.7). Following chain extension and cleavage with TFA in the usual manner, products are obtained containing the appropriate fluorophore or biotin at the C-terminus.

The use of pre-loaded resins avoids the inherent problems of poor solubility and inefficient coupling, as well as the additional steps and costs, that are associated with the introduction of biotin and certain fluorophores in solid phase synthesis. The approach is particularly suited to the production of peptides for high-throughput screening, as it removes the difficulty of ensuring complete label incorporation for all molecules in the array. NovaTag™ resins incorporating different labels and spacer groups can also be custom manufactured.

Universal PEG NovaTag™ resin
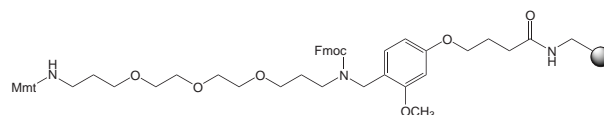
For situations where it is not always apparent at the outset which is the optimum label or combination of labels for a given application, Novabiochem® offers the Universal NovaTag™ resins. These supports facilitate the synthesis of peptides bearing any number of different acyl moieties at N- and C-termini from a single solid phase synthesis (Figure 5-18). Universal resins are also useful for preparing labeled peptides containing fluorophores that are not compatible with Fmoc SPPS protocols, such as TAMRA and FAM [2], since they allow the labels to be easily introduced as the final step before cleavage. Universal PEG NovaTag™ resin is particularly efficacious for applications where a hydrophilic spacer is required between the peptide and the label and where the peptide solubility is an issue.
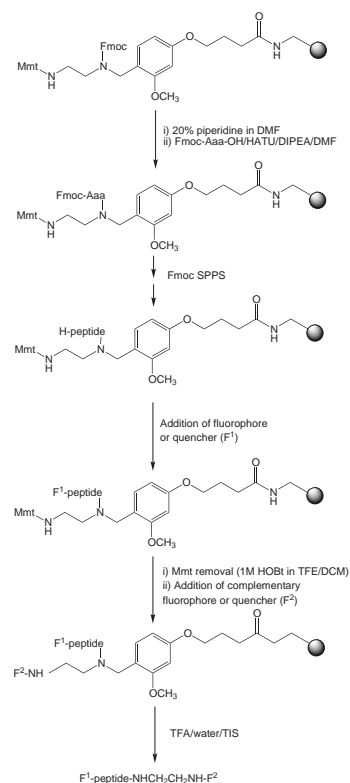
Fig. 5-18: Synthesis using Universal NovaTag™ resins.

After loading of the first amino acid to the resin-bound secondary amine, chain extension is carried out under standard Fmoc methods. Following synthesis, the resin can be partitioned and each aliquot end-capped with the appropriate carboxyl-functionalized label. The pendant Mmt group is then removed using HOBt/TFE/DCM (Method 5-4) and the C-terminal label introduced to each resin aliquot. Thus, from a single synthesis any number of label variations for a given sequence can be prepared.

Universal PEG NovaTag™ resin has been used to prepare fluorescently-labled Shc Src homology 2 domain-binding peptides linked to cell

penetrating sequences via a PEG spacer [3] and dimeric SMAC PEG-linked peptides [4]. Universal NovaTag™ resin has been utilized to prepare peptides *C*-terminally modified to ketone for ligation [5].

Application 5-4: Synthesis of Dansyl-Pro-Leu-Gly-Leu-NHCH₂CH₂NH-Dabcyl using Universal NovaTag™ resin

Fmoc-Leu-OH (3 eq.) was coupled to Universal NovaTag™ resin (0.4 mmole) using PyBrOP® (3 eq.) and DIPEA (10 eq.) in DMF for 18 h. The chain was extended by addition of Gly, Leu and Pro using Fmoc-amino acids (3 eq.) activated with PyBOP®(3 eq.), DIPEA (10 eq.) and HOBt (1 eq.) in DMF for 35 min. Following Fmoc deprotection of Pro, the resin was treated with Dansyl chloride (1.5 eq.) and DIPEA (10 eq.) in THF for 1 h. The Mmt group was then removed as described in Method 5-4, and the resin was treated with Dabcyl-OSu (2 eq.) and HOBt (0.06 eq.) in DMF for 18 h. Labeled peptide was cleaved from the resin using TFA/DCM/water (50:50:1) for 2 h. The crude peptide was analyzed by HPLC (Figure 5-19).
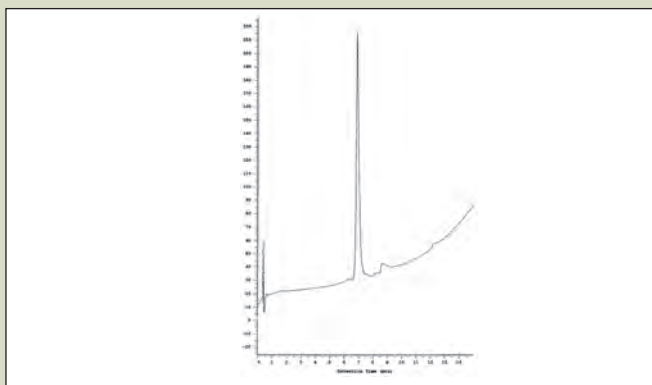
Fig. 5-19: HPLC elution profile of crude Dansyl-Pro-Leu-Gly-Leu-NHCH₂CH₂NH-Dabcyl with Universal NovaTag™ resin.

5.2.3 Reagents for introduction of optical labels

AMC

Fmoc-Arg(bis-Boc-resin)-AMC

Fmoc-Asp(Wang resin)-AMC

Fmoc-Lys(carbamate Wang resin)-AMC
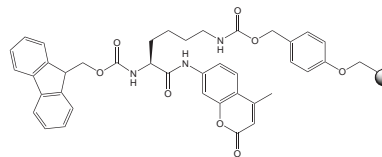
Enzyme substrates based on the 7-amino-4-methylcoumarin (AMC) fluorophore are very popular tools for studying protease activity and specificity [6]. In such substrates, the AMC is typically linked to the peptide through formation of an amide bond between the coumarin amine and the carboxyl group of the C-terminal amino-acid residue (Figure 5-20). Proteolysis of this amide bond liberates free AMC, resulting in a large increase in fluorescence that can be detected at 441 nm upon excitation at 342 nm.
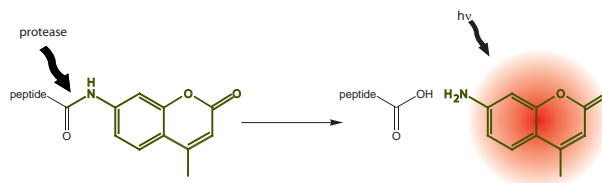
Fig. 5-20 Principle of AMC-labeled fluorogenic substrates.

The synthesis of peptide-AMC derivatives is particularly problematic owing to the poor nucleophilicity of the AMC amine group. The usual strategy involves first formation of the AMC derivative of the C-terminal amino-acid residue followed by fragment condensation or stepwise elongation. This approach is obviously not amenable to solid phase methods and cannot be applied to the production of enzyme substrate libraries for protease profiling. To overcome these limitations, Novabiochem® has introduced a range of resins pre-loaded with amino acid-AMC derivatives: Fmoc-Asp(Wang resin)-AMC; Fmoc-Arg(bis-Boc-resin)-AMC[7]; Fmoc-Lys(carbamate Wang resin)-AMC. Arginine, aspartic acid and lysine were selected as these amino acids occur at the P1 position of endogenous substrates for a number of important proteases.

These resins are extremely simple to use and are fully compatible with standard Fmoc SPPS protocols. In the case of Fmoc-Arg(bis-Boc-resin)-AMC, the use of 2% DBU in DMF for Fmoc removal gives remarkably better results. The free amine group can be acylated with Fmoc amino acids activated with PyBOP® or TBTU. Following peptide assembly, cleavage with 95% TFA releases the peptide-AMC directly from the solid support without any additional steps.

The use of these new resins is illustrated in the examples given in Figures 5-21 and 5-22.
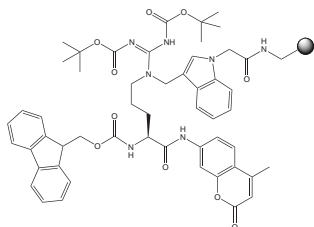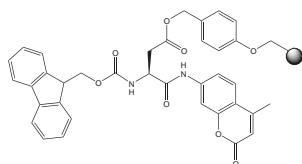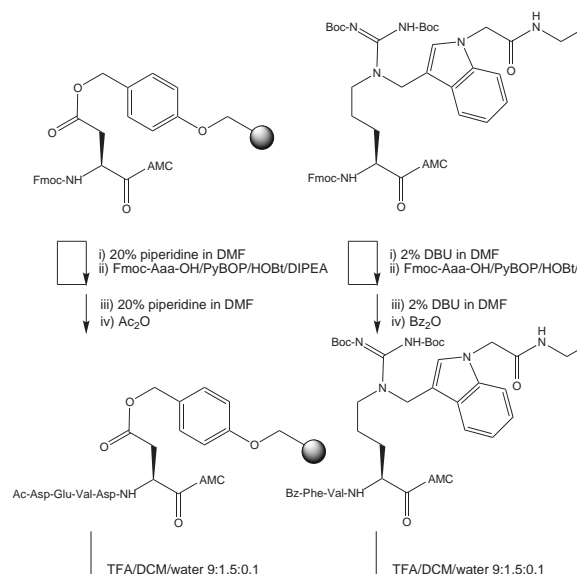
Fig. 5-21: Synthesis of Ac-Asp-Glu-Val-Asp-AMC using Fmoc-Asp(Wang resin)-AMC and Bz-Phe-Val-Arg-AMC using Fmoc-Arg(bis-Boc-resin)-AMC.
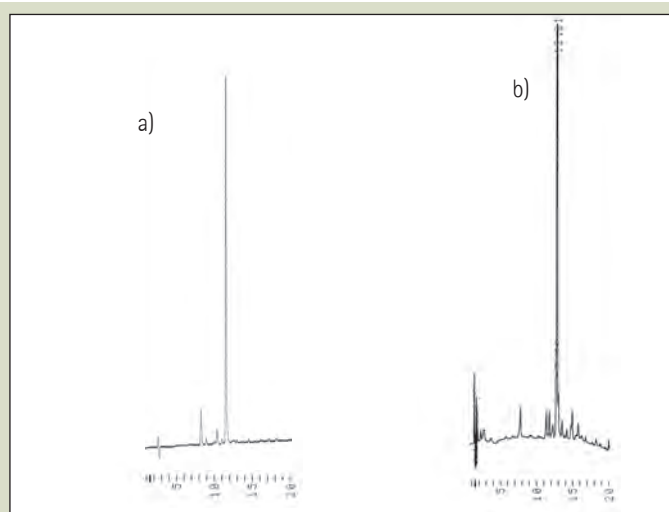
Fig. 5-22: HPLC elution profiles of a) crude Ac-Asp-Glu-Val-Asp-AMC prepared with Fmoc-Asp(Wang resin)-AMC b) crude Bz-Phe-Val-Arg-AMC prepared with Fmoc-Arg(bis-Boc-resin)-AMC.

7-Methoxycoumarin (Mca)

Fmoc-Lys(Mca)-OH
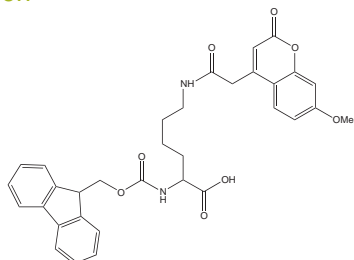
Mca-OH
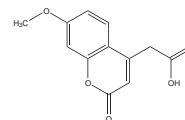
Mca NovaTag™ resin
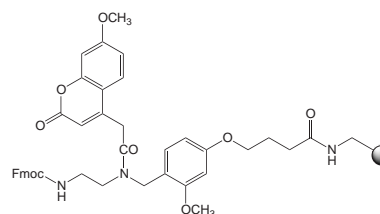
The 7-methoxycoumarin group (Mca) fluoresces at 393 nm when stimulated at 328 nm, and is most commonly used in conjunction with 2,4-dinitrophenyl (Dnp) quenching groups [8]. It can be coupled on solid phase to free amines using Mca-OSu, Mca-OH, or incorporated during chain extension using a pre-formed building block such as Fmoc-Lys(Mca)-OH [9]. Fmoc-Lys(Mca)-OH and Mca-OH can be coupled using any standard coupling method, such as PyBOP®/DIPEA and DIPCDI/HOBt, whereas the preactivated derivative, Mca-OSu, couples best in DMSO or NMP in the presence of HOBt. The coumarin moiety is stable to the standard conditions employed in Fmoc SPPS.

Mca NovaTag™ resin provides peptides C-terminally modified with the Mca fluorophore attached via an ethylene diamine spacer [1]. Following removal of the Fmoc group, the resin-bound primary amine can be loaded with the first amino acid residue using standard activation methods, such as PyBOP, HOBt/DIPCDI. After peptide assembly, treatment with 95% TFA cleaves the Mca peptide directly from the resin. The use of this resin is illustrated in Application 5-5.

Dabcyl
Fmoc-Lys(Dabcyl)-OH
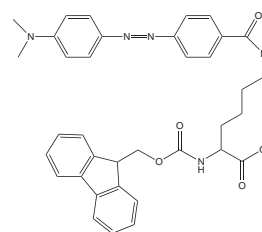
Dabcyl-OSu
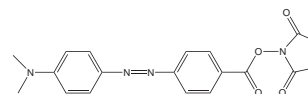
Dabcyl is one of the most frequently utilized quenching groups because of its lack of innate fluorescence and spectral overlap (λ_{max} 453 nm) with a number of commonly-used fluorophores, such as EDANS, Mca, TET, JOE, FAM. In the preparation of fluorescence-quenched peptide substrates, it is most frequently used in conjunction with EDANS as this pairing is particularly efficacious owing to their excellent spectral overlap [10, 11].

Application 5-5: Synthesis of endopeptidase Glu-C substrate

Mca NovaTag™ resin (155 mg, 0.045 mmole) was swollen in DMF and the Fmoc group removed with 20% piperidine. Peptide assembly was carried out using 30 min couplings of Fmoc-amino acids (6 eq.) activated with PyBOP® (6 eq.) in the presence of DIPEA (12 eq.). The peptide was cleaved from the resin using 95:2.5:2.5 TFA/water/TIS for 3 h and purified directly by HPLC. The purified peptide gave the HPLC profile shown in Figure 5-23 and was characterized by ES-MS [expected M+H⁺ 1142.4, found 1142.4].
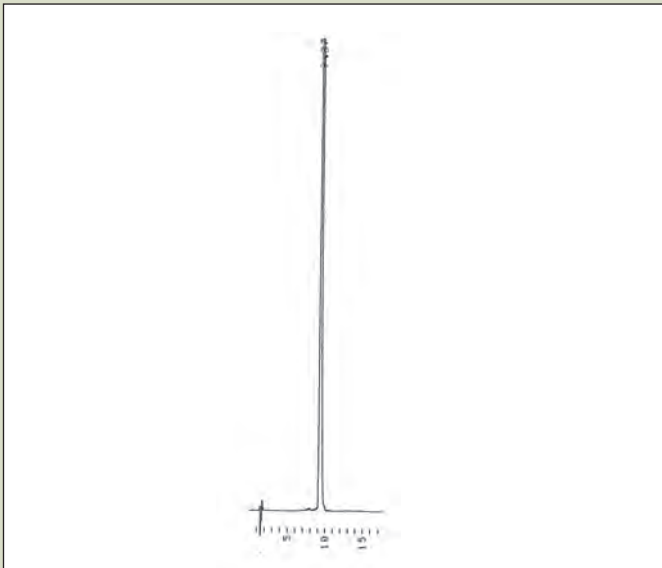
Fig. 5-23: HPLC elution profile of purified H-Lys(Dnp)-Leu-Glu-Val-Asp-Gly-Trp-NHCH₂CH₂NH-Mca prepared with Mca NovaTag™ resin.

The Dabcyl group is most conveniently introduced during solid phase synthesis of the substrate. Addition to the N-terminal amino group is best achieved using Dabcyl-OSu in DMSO or NMP in the presence of HOBt. When the Dabcyl group is to be located in the peptide chain, the simplest approach is to introduce Lys(Dabcyl) at the desired position using Fmoc-Lys(Dabcyl)-OH activated with PyBOP®/DIPEA.

2,4–Dinitrophenyl (Dnp)
Fmoc-Lys(Dnp)-OH
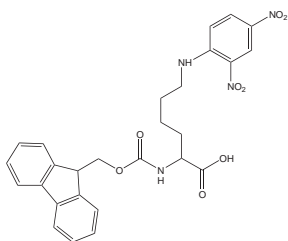
The Dnp group (λ_{max} 348 nm) is the preferred quenching group for the Mca fluorophore [8]. It is most easily incorporated into a peptide as Fmoc-Lys(Dnp)-OH, which can be coupled using any standard activation method.

Dnp NovaTag™ resin
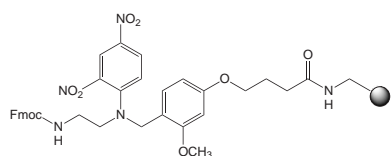
This resin is ideal for the assembly of labeled peptides incorporating a C-terminal Dnp group [1]. Following removal of the Fmoc group, the resin-bound primary amine can be loaded with the first amino acid residue using standard activation methods, such as PyBOP®, HOBt/DIPCDI. After peptide assembly, treatment with 95% TFA cleaves the Dnp peptide directly from the resin. This resin is particularly advantageous for the synthesis of FRET peptides, since the presence of the quencher in every peptide chain minimizes the levels of fluorescent unquenched by-products and so reduces background fluorescence of the product FRET substrates.

EDANS

Fmoc–Asp(EDANS)–OH
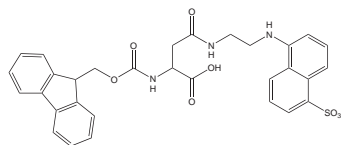
Fmoc-Glu(EDANS)-OH
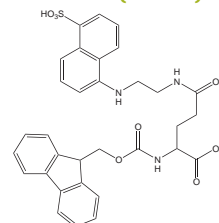
The EDANS/Dabcyl fluorophore-quencher pair is one of the most commonly-used for FRET applications, owing to excellent spectral overlap between the emission spectrum of EDANS (λ_{ex} 341 nm, λ_{em} 471 nm) and absorbance spectrum of Dabcyl (λ_{max} 453 nm) [10, 11] (Figure 5-24). Quenching of the fluorescence of EDANS by Dabcyl is consequently highly efficient, with up to 40-fold enhancements in fluorescence having been observed upon proteolysis of Dabcyl/EDANS-labeled peptides [10].
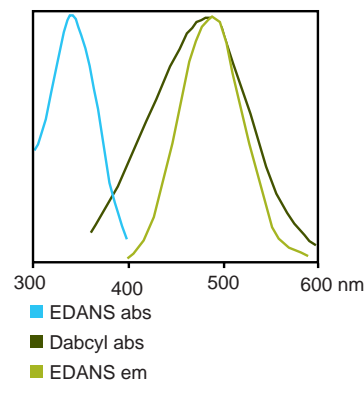
Fig. 5-24: Absorbance and emission spectra of Dabcyl and EDANS [8].

To incorporate EDANS within the peptide chain, the simplest approach is to use either Fmoc-Asp(EDANS)-OH or Fmoc-Glu(EDANS)-OH during peptide assembly [12, 13]. Introduction of these derivatives during SPPS can be achieved using PyBOP®/DIPEA activation in conjunction with an extended coupling time [13]. Powerful acylating reagents such as PyBrOP should be avoided as their use may lead to acylation of the naphthylamine nitrogen.

EDANS NovaTag™ resin
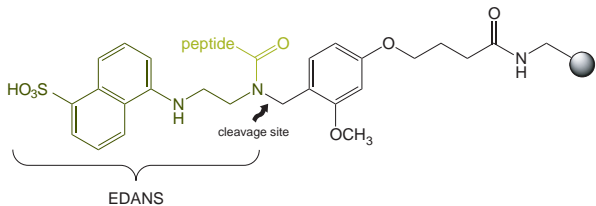
Fig. 5-25: Loaded EDANS NovaTag™ resin showing point of attachment of peptide and site of cleavage.

Traditionally, the introduction of the EDANS moiety at the C-terminus of a peptide is achieved by coupling of a peptide fragment to EDANS in solution [14]. Novabiochem®'s EDANS NovaTag ™ resin enables for the first time the direct synthesis of C-terminally EDANS-labeled peptides by solid phase synthesis [1b] (Figure 5-25). The use of EDANS NovaTag™ resin in the solid phase synthesis of FRET peptides is illustrated by the examples given in Applications 5-3 and 5-4. Since the EDANS fluorophore is built into the linker, it becomes linked to the C-terminus of the peptide when the first amino acid is attached to the resin. The Dabcyl quencher group can be introduced to the N-terminus using Dabcyl-OSu in DMSO or DMF in the presence of HOBt. If the Dabcyl group is to be located in the peptide chain, the simplest approach is to introduce Lys(Dabcyl) at the desired position using Fmoc-Lys(Dabcyl)-OH activated with PyBOP®/DIPEA.

The first and most important step in using EDANS NovaTag™ resin is attachment of the first amino acid residue. As this process involves acylation of a resin-bound secondary amine, it is best carried out using HATU activation (Method 5-5). Pfp esters in the presence of collidine can also be employed, although longer acylation times may be required. It is important that this reaction is carried out to completion, as any unreacted amino groups left on the resin may react in subsequent couplings and lead to the formation of truncated sequences. Following loading, the substitution of the resin should be checked with the Fmoc UV assay, and if necessary, the loading reaction repeated using fresh reagents. Once loaded with the first amino acid, peptide synthesis can be carried out under standard conditions. The use of PyBroP® should be avoided as this can lead to double acylation. Cleavage from the resin can be effected using standard TFA cocktails; however, due to the proximity of the naphthylamine nitrogen to the cleavage site of the linker, product release can sometimes be sluggish. The reaction can be accelerated, if necessary, by the addition of a few drops of TMSBr to the standard TFA cocktail provided water is omitted.

EDANS NovaTag™ resin has been recently employed to prepare fluorescently labeled aminoalkane diphenyl phosphonate affinity probes for chymotrypsin- and elastase-like serine proteases [15].

Application 5-6: Synthesis of Dabcyl-Asp-Glu-Val-Asp-Ala-Arg-EDANS using EDANS NovaTag™ resin
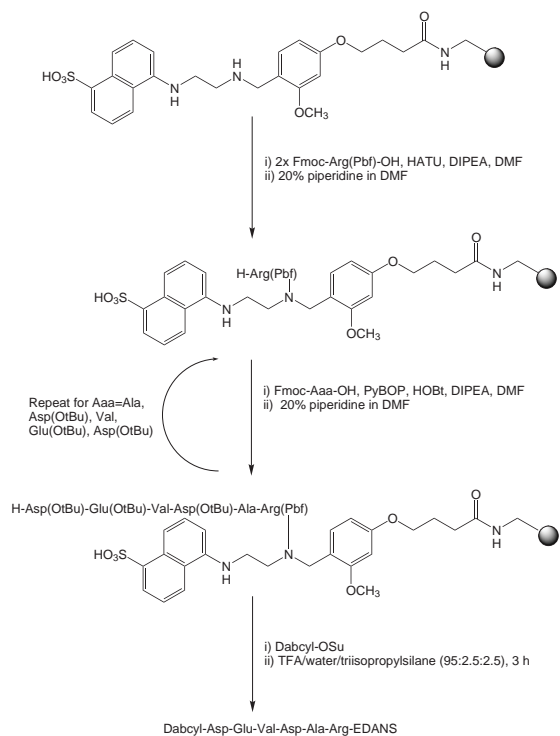
EDANS NovaTag™ resin (188 mg, 0.1 mmole) was loaded with Fmoc-Arg(Pbf)-OH as described in Method 5-5. Using this resin, H-Asp(OtBu)-Glu(OtBu)-Val-Asp(OtBu)-Ala-Arg(Pbf)-EDANS NovaTag™ resin was prepared automatically on a NovaSyn Crystal peptide synthesizer. All acylation reactions were carried out for 1 h using Fmoc-amino acids (5 eq.) activated with PyBOP® (5 eq.) in the presence of DIPEA (10 eq.) and HOBt (1 eq.). Dabcyl was introduced to the N-terminus using Dabcyl-OSu (2.5 eq) dissolved in DMSO. The labeled peptide was cleaved from the resin using TFA /TIS/water (95:2.5:2.5) for 3 h and was obtained after ether precipitation in a yield of 68 mg (76%). The crude peptide was analyzed by HPLC (Figure 5-26) and ES-MS [expected M+H+ 1230, found 1230].
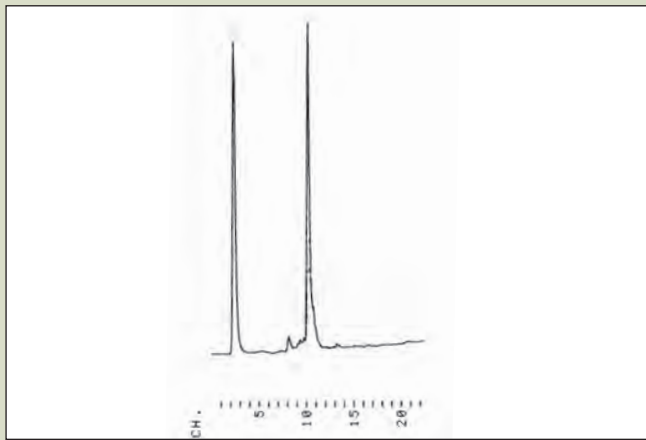
Fig. 5-26: HPLC elution profile of crude Dabcyl-Asp-Glu-Val-Asp-Ala-Arg-EDANS prepared with EDANS NovaTag™ resin .

Application 5-7: Synthesis of Dabcyl-Pro-Tyr-Tyr-Gly-Asp-Glu-Pro-Leu-EDANS using EDANS NovaTag™ resin

i) Fmoc-Leu-OH, HATU, DIPEA, DMF
ii) 4% DBU in DMF

Repeat for Aaa=Pro, Glu(OtBu), Asp(OtBu), Gly, Tyr(tBu), Tyr(tBu), Pro

i) Fmoc-Aaa-OH, PyBOP, HOBt, DIPEA, DMF
ii) 4% DBU in DMF

H-Pro-Tyr(tBu)-Tyr(tBu)-Gly-Asp(OtBu)-Glu(OtBu)-Pro-Leu

i) Dabcyl-OSu
ii) 10% TMSBr in TFA, 1 h

Dabcyl-Pro-Tyr-Tyr-Gly-Asp-Glu-Pro-Leu-EDANS
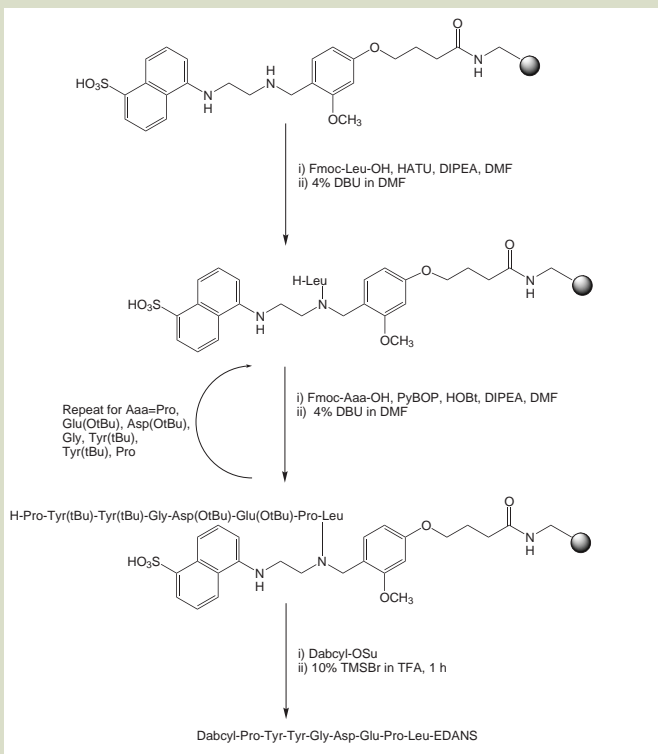
EDANS NovaTag™ resin (300 mg, 0.16 mmole) was loaded with Fmoc-Leu-OH as described in Method 5-5. Using this resin, H-Pro-Tyr(tBu)-Tyr(tBu)-Gly-Asp(OtBu)-Glu(OtBu)-Pro-Leu-EDANS NovaTag™ resin was prepared manually. All acylation reactions were carried out for 1 h using Fmoc-amino acids (2 eq.) activated with PyBOP® (2 eq.) in the presence of DIPEA (5.5 eq.) and HOBt (1.3 eq.). Removal of Fmoc was effected by treatment with 4% DBU in DMF. Dabcyl was introduced to the N-terminus using Dabcyl-OSu (120 mg, 0.32 mmole) with HOBt (30 mg, 0.2 mmole) and collidine (500 µl) dissolved in DMF. The labeled peptide was cleaved from the resin using 10% TMSBr in TFA for 1 h and was obtained after ether precipitation in a yield of 68 mg (63%). The crude peptide was analyzed by HPLC (Figure 5-27) and ES-MS [expected M+H+ 1453, found 1453].
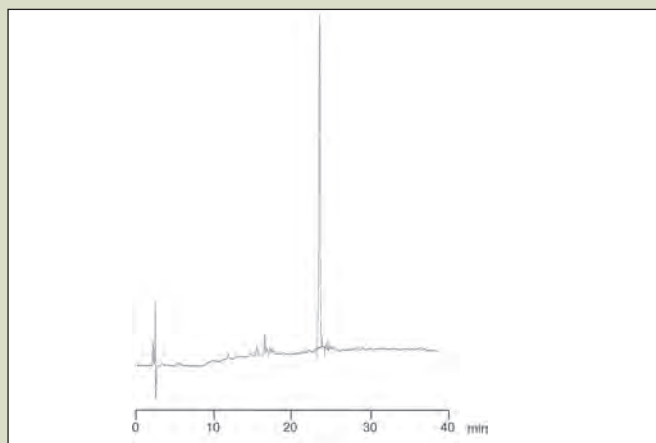
Fig. 5-27: HPLC elution profile of crude Dabcyl-Pro-Tyr-Tyr-Gly-Asp-Glu-Pro-Leu-EDANS prepared with EDANS NovaTag™ resin .

Method 5-5: Loading EDANS and Biotin-PEG NovaTag™ resins

1. Suspend resin in DMF and leave to swell for 30 min. (In the case of Biotin-PEG NovaTag™ resin the Fmoc group should be removed at this stage with 20% piperidine in DMF.)
2. Dissolve Fmoc-amino acid (2.5 eq.) and HATU (2.5 eq.) in minimum volume of DMF and add to resin. Add DIPEA (5 eq.) and mix.
3. The mixture is left to stand for 2 h with gentle agitation. A sample of resin can be removed and the loading determined using the Fmoc UV assay [see Method 3-11. page 3.7]. Repeat the coupling with fresh reagents if necessary.
4. The resin is removed by filtration, washed with DMF and used immediately in synthesis, or washed further with DCM and then MeOH, dried and stored for later use.

Dansyl NovaTag™ resin
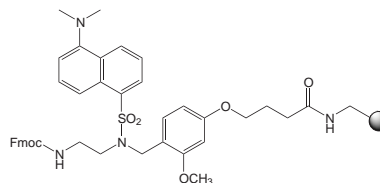
This resin facilitates the direct synthesis of peptides C-terminally labeled with the Dansyl group (λ_{ex} 335 nm, λ_{em} 526 nm). Following removal of the Fmoc group, the resin-bound primary amine can be loaded with the first amino acid residue using standard activation methods, such as PyBOP®, HOBt/DIPCDI. After peptide assembly, treatment with 95% TFA cleaves the Dansyl peptide directly from the resin (Application 5-5).

Dansyl NovaTag™ resin has been recently employed to prepare FRET probes for mercury binding protein MerP [16] and functionalized amyloid fibrils [17].

Application 5-8: Synthesis of H-Asp-Glu-Val-Asp-Ala-Arg-NHCH$_2$CH$_2$NH-Dansyl using Dansyl NovaTag™ resin

H-Asp(OtBu)-Glu(OtBu)-Val-Asp(OtBu)-Ala-Arg(Pbf)-Dansyl NovaTag™ resin was prepared automatically on a NovaSyn Crystal peptide synthesizer using Dansyl NovaTag™ resin (263 mg, 0.1 mmole). All acylation reactions were carried out for 1 h using Fmoc-amino acids (5 eq,) activated with PyBOP® (5 eq.) in the presence of DIPEA (10 eq.) and HOBt (5 eq.). The labeled peptide was cleaved from the resin using TFA /TIS/water (95:2.5:2.5) for 2.5 h. The crude peptide was analyzed by HPLC (Figure 5-28) [expected M+H+ 979, found 979].
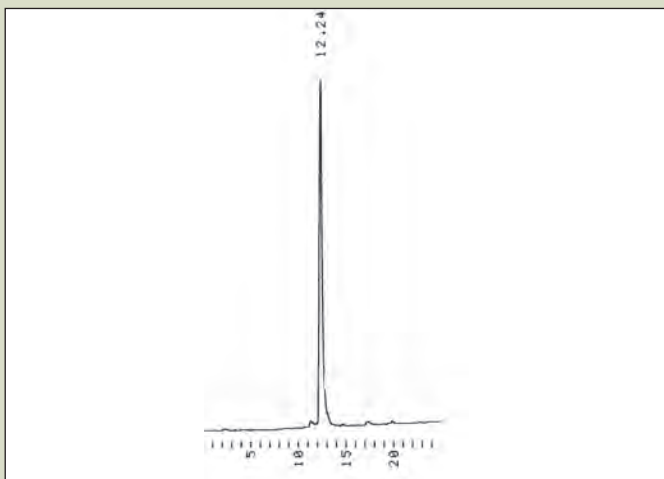
Fig. 5-28: HPLC elution profile of crude H-Asp-Glu-Val-Asp-Ala-Arg-Dansyl prepared with Dansyl NovaTag™ resin [1].

5–Carboxyfluorescein/6–Carboxyfluorescein

5–Carboxytetramethylrhodamine/6–Carboxytetramethylrhodamine
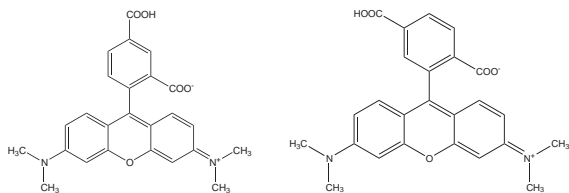
Novabiochem® supplies carboxyfluorescein (FAM; λ_{ex} 494 nm, λ_{em} 518 nm) and carboxytetramethylrhodamine (TAMRA, λ_{ex} 555 nm; λ_{em} 580 nm) as single isomers, ensuring labeled products of defined chemical structure, as well as greatly assisting product purification and characterization. However, for those applications which do not require a single isomer dye, 5,6-carboxyfluorescein is also available as the cost effective option.

The dyes are most conveniently introduced during solid phase synthesis by coupling to *N*-terminal or side-chain amino groups using HOBt or HOAt/DIPCDI in DMF (Method 5-6). When one of the dyes is to be located on a side-chain amino group, the simplest approach is to incorporate an orthogonally-protected derivative, such as Lys(Mtt) or Lys(ivDde), that can be later selectively deprotected on the resin immediately prior to coupling of the dye. After addition of FAM and subsequent amino acids, the resin should be washed with 20% piperidine in DMF to remove phenyl esters formed by acylation of the FAM phenolic hydroxyls. Treatment of FAM peptides with hydrazine can result in hydrazone formation. Esters and hydrazone formation can both be avoided if, following the introduction of FAM and the subsequent piperidine treatment, the phenolic hydroxyls are blocked by tritylation with Trt-Cl and DIPEA in DCM [2].

When used together in the same peptide, fluorescence resonance energy transfer (FRET) between FAM and TAMRA results in quenching of the fluorescence of both dyes, making them excellent reagents for FRET applications [18].

Method 5-6: Coupling of carboxyl-functionalized dyes

1. Dissolve dye (1.5 eq.) in minimum DMF with HOBt or HOAt (1.5 eq.). If necessary, add DMSO to aid dissolution.
2. Add DIPCDI (1.7 eq.) and stir mixture and leave to stand for 10 min.
3. Add mixture of drained peptidyl resin and agitate gently for 2 h. Check for free amino groups using the Kaiser test. Note: the TNBS test does not work for beads loaded with colored dyes. If the reaction is not complete, leave o/n and then recheck. If after this time it is still not complete, wash resin and repeat with fresh reagents.

5.2.4 Biotinylating reagents

Biotin-labeled peptides have many important applications in immunology and histochemistry, such as affinity purification [19] and FRET-based flow cytometry [20], solid-phase immunoassays [21], and receptor localization [22], that exploit the high affinity of streptavidin and avidin for biotin.

Incorporation of the biotin label is best carried out during solid phase synthesis of the peptide ligand, with the optimum location for the biotin label dependent on the nature of the application.

Biotin NovaTag™ resin
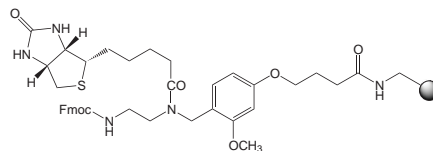
Biotin–PEG NovaTag™ resin
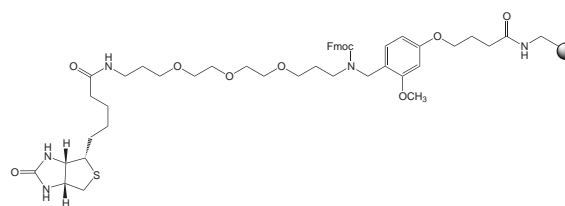
Fmoc–PEG Biotin NovaTag™ resin
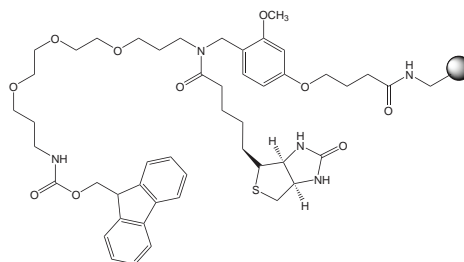
The biotin label is most frequently located directly on the N-terminal group of the peptide, often without any regard to how this may affect peptide-target interactions, biotin-avidin binding, and the solubility properties of the resultant peptide. In many instances the products are poorly soluble, and have little biological activity and poor affinity for biotin. Problems can also arise during the synthesis of such N-terminally biotinylated peptides due to the poor solubility and reactivity of many of the reagents used for biotin introduction.

Novabiochem®'s biotin-loaded NovaTag™ resins provide a simple and elegant solution to these problems [23-27]. Using these resins, biotinylated peptides are obtained directly following TFA cleavage, without the need for any additional biotinylation steps. Resins incorporate either an ethylenediamine or a 15 atom PEG spacer between the peptide and biotin to reduce steric hindrance.

The use of Biotin-PEG NovaTag™ resin or Fmoc-PEG Biotin NovaTag™ resins are particularly advantageous because not only does the

hydrophilic PEG chain confer better solubility to the peptide biotin conjugate, but its extended conformation leads to better avidin binding which can dramatically improve assay sensitivity as demonstrated in Figure 5-23. As the biotin is an integral part of the linker, its presence in every peptide chain is assured from the outset.

Using Novabiochem®'s NovaTag™ resins for biotinylated peptide synthesis is extremely easy. Biotin NovaTag™ resin and Fmoc-PEG Biotin NovaTag™ resin can be used directly in an automated synthesizer in the same manner as Rink amide resin. The Fmoc group is removed with 20% piperidine and the peptide assembled on the support using standard protocols. With Biotin-PEG NovaTag™ resin, the procedure is the same except that the first residue should be coupled using HATU as described in Method 5-5, since this reaction involves acylation of a less reactive secondary amine. Cleavage from the resin can be effected using standard TFA cocktails, providing the C-terminally labeled biotinylated peptide.

The use of Biotin NovaTag™ and Biotin-PEG NovaTag™ resins is illustrated in Applications 5-9 & 5-10.

Application 5-9: Synthesis of H-KKKKXXLLDXXXXXXXXXXMKDEE-NH-PEG-NH-biotin (23mer) [28]

Biotin-PEG NovaTag™ resin (345 mg, 0.145 mmole) was swollen in DMF and the Fmoc group removed with 20% piperidine. Peptide assembly was carried out on a Protein Technologies, Inc. Symphony peptide synthesizer using 30 min couplings of Fmoc-amino acids (3 eq.) activated with HCTU (3 eq.) in the presence of NMM (5 eq.). The biotinylated peptide was cleaved from the resin using Reagent K for 2.5 h. The crude peptide gave the HPLC profile shown in Figure 5-29. The minor component eluting ahead of the main product, is the corresponding methionine sulfoxide peptide.
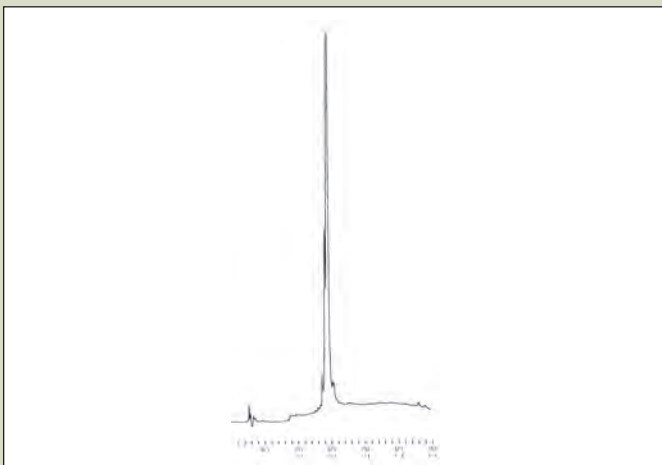
Fig. 5-29: HPLC elution profile of crude H-KKKKXXLLDXXXXXXXXXX-MKDEE-NH-PEG-NH-biotin prepared with Biotin-PEG NovaTag™ resin.

Application 5-10: Synthesis of H-KKKKXXLLDXXXXXXXXXXMKDEE-NHCH$_2$CH$_2$NH-biotin (23mer) [28]

Biotin NovaTag™ resin (354 mg, 0.145 mmole) was swollen in DMF and the Fmoc group removed with 20% piperidine. Peptide assembly was carried out on a Protein Technologies, Inc. Symphony peptide synthesizer using 30 min couplings of Fmoc-amino acids (3 eq.) activated with HCTU (3 eq.) in the presence of NMM (5 eq.). The biotinylated peptide was cleaved from the resin using Reagent K for 2.5 h. The crude peptide gave the HPLC profile shown in Figure 5-30. The minor component eluting ahead of the main product, is the corresponding methionine sulfoxide peptide.
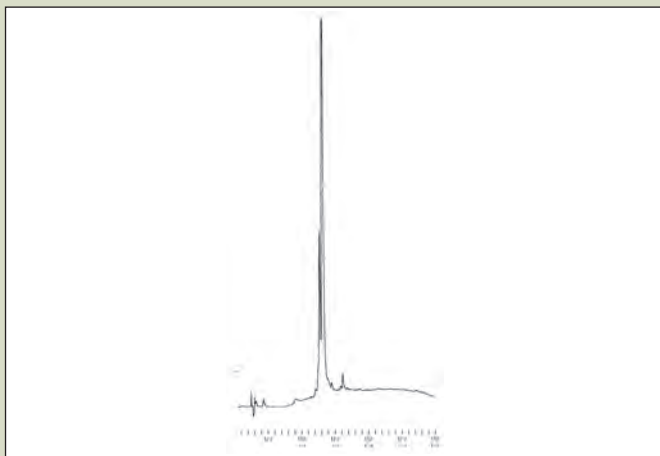
Fig. 5-30: HPLC elution profile of crude H-KKKKXXLLDXXXXXXXXXXM-KDEE-NHCH$_2$CH$_2$NH-biotin prepared with Biotin NovaTag™ resin.

Biotinylated peptide design

When designing biotinylated peptides for use in assays, two of the most important considerations are the position of the biotin moiety and the nature of the spacer group between the peptide and biotin. This is because these can profoundly effect the strength of peptide-protein and biotin-avidin interactions and consequently the sensitivity of the assay. The importance of correct peptide presentation is illustrated in the following examples taken from developmental work on protein-binding and kinase assays carried out at Merck Pharma KGaA [29].

AlphaScreen™ protein–binding assay

The peptide-protein binding assay was conducted using the AlphaScreen™ technology as shown in Figure 5-31. *N*- and *C*-terminally biotin-labeled versions of the native peptide ligand immobilized on streptavidin-coated donor beads were screened against acceptor beads loaded with target protein. Only the peptide which was *C*-terminally labeled with PEG-biotin had acceptable solubility in the test buffer and showed significant levels of protein binding (Figure 5-32).
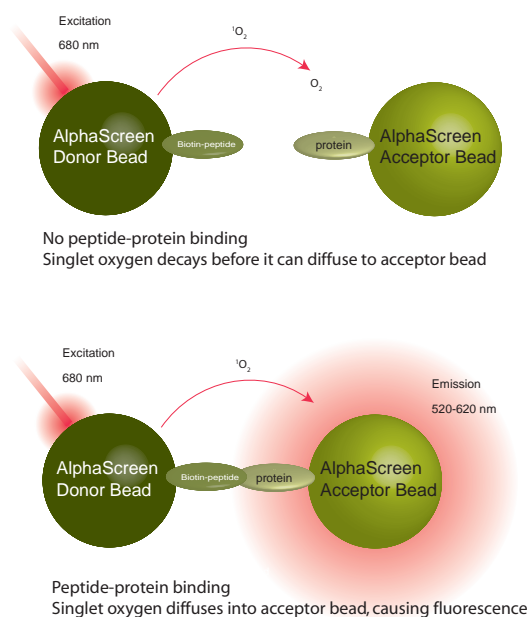
Fig. 5-31: Principles of the protein-peptide binding AlphaScreen™ assay.
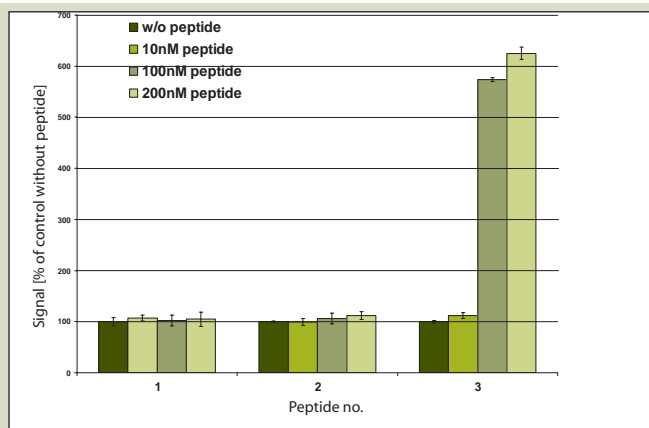
Fig. 5-32: AlphaScreen protein-binding assay. Peptide 1: N-biotin-XXXXX-NH$_2$; peptide 2: H-XXXXX-NHCH$_2$CH$_2$NH-biotin; peptide 3: H-XXXXX-NH-PEG-NH-biotin [28].

Kinase bind assay

N- and C-terminally biotin-labeled versions of a kinase substrate were evaluated in the assay shown in Figure 5-33. Peptides that were C-terminally labeled with biotin were found to give better reponses than those that were labeled on the N-terminus, whilst inclusion of a PEG spacer between the peptide and biotin appeared to have little effect (Figure 5-34).
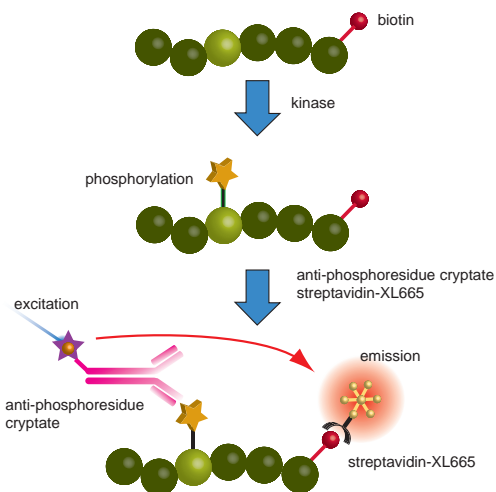
Fig. 5-33: Principles of the kinase assay.
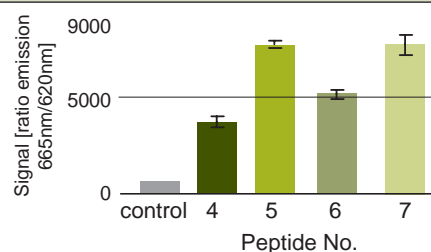
Fig. 5-34: Kinase assay. Peptide 4: biotin-KKKKXXLLDXXXXXXXXXXMKDEE-NH$_2$; peptide 5: H-KKKKXXLLDXXXXXXXXXXMKDEE-NHCH$_2$CH$_2$NH-biotin; peptide 6; biotin-NH-PEG-KKKKXXLLDXXXXXXXXXXMKDEE-NH$_2$; peptide 7: H-KKKKXXLLDXXXXXXXXXXMKDEE-NH-PEG-NH-biotin [28].

Biotin-OSu
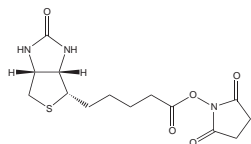
Biotin-ONp
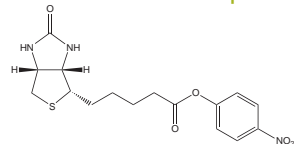
Fmoc-Lys(biotin)-OH
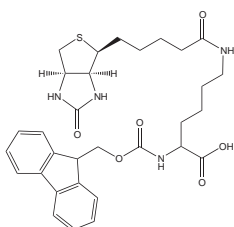
Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH
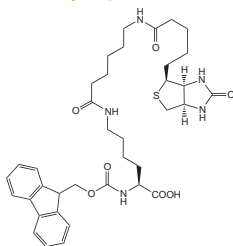
Fmoc-Asp(biotinyl-PEG)-OH
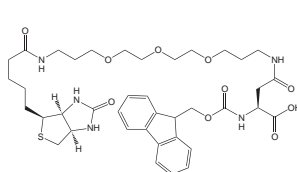
Fmoc-Glu(biotinyl-PEG)-OH
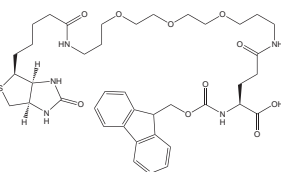
N-Biotinyl-NH-PEG₂-COOH
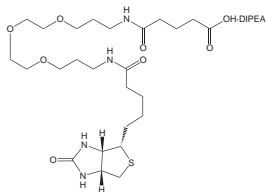
N-Biotinyl-NH-PEG₁₁-COOH
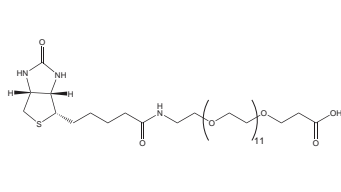
For coupling of biotin to amines on the solid phase the use of Biotin-ONp is strongly recommended [30]. As can be seen from Table 5-2 and Figure 5-35, the solubility of this reagent in DMF or NMP is much greater than that of Biotin-OSu, and it couples with amines much more rapidly: typically in 40 min as opposed to 12 h for Biotin-OSu.

Alternatively, preformed derivatives such as Fmoc-Lys(biotin)-OH, Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH, Fmoc-Asp(biotinyl-PEG)-OH and Fmoc-Glu(biotinyl-PEG)-OH can be used to introduce biotin at a precise location within a peptide chain. The use of Fmoc-Glu(biotinyl-PEG)-OH is particularly recommended. In contrast to the other derivatives, it possesses good solubility in DMF (Fmoc-Glu(biotinyl-PEG)-OH, 0.5 mmole/ml; Fmoc-Lys(biotin)-OH, <0.05 mole/ml; Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH, <0.05 mmole/ml) and the presence of the PEG chain improves the solubility of the product biotinylated peptide [1b] and helps reduce steric hindrance between peptide and biotin, leading to better avidin binding. It is recommended that NMP or NMP/DMSO be used as the solvents for Fmoc-Lys(biotin)-OH and Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH. For convenience, Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH pre-loaded onto NovaSyn ® TG^R A resin is available.

N-Biotinyl-NH-PEG₂-COOH and N-Biotinyl-NH-PEG₁₁-COOH offer the same benefits as Fmoc-Glu(biotinyl-PEG)-OH and are ideal for incorporation of biotin-PEG at the N-terminus of a peptide. The long 40 atom spacer in the latter has an marked affect on peptide solubility compared to that of Glu(biotinyl-PEG) or N-biotinyl-NH-PEG₂.

Table 5-2: Solubilities of Novabiochem®'s biotin derivatives.

Compound	Solubility (mmole/ml)		
	DCM	DMF	NMP
Biotin-OSu	>0.02	0.1	0.15
Biotin-ONp	0.09	0.5	0.5
Fmoc-Lys(biotin)-OH	<0.025	<0.05	0.25
Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH	<0.025	0.05	0.5
Fmoc-Glu(biotinyl-PEG)-OH	<0.025	0.5	0.5
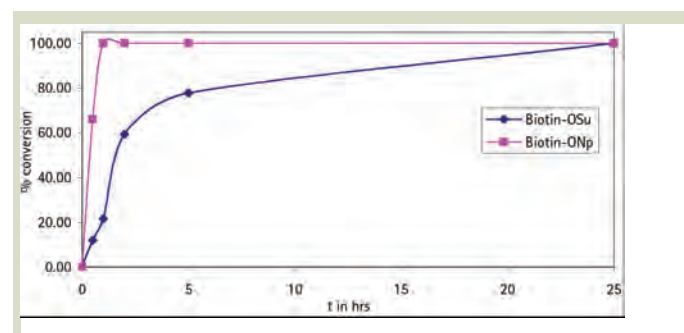
Fig. 5-35: Coupling rate of Biotin-OSu and Biotin-ONp to H-Asp(OtBu)-Glu(OtBu)-Val-Glu(OtBu)-Wang resin.

Polydisperse PEGs

MPEG–ethylamine
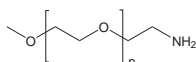
MPEG–pNPC
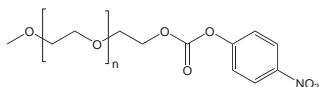
MPEG–oxyamine
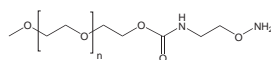
Modification of peptide and protein-based drugs with 20 and 30 kDa polyethylene glycol chains is used to improve stability and pharmokinetics by protecting them from proteolysis, inhibiting aggregation, and reducing their immunogenicity [31].

Novabiochem is therefore pleased to offer Merck Eprova®'s MPEG derivatives. These MPEGs are manufactured to high standards and are specified for the modification of protein APIs, making them some of the highest quality PEGs available for the research market. For the synthesis of these reagents, MPEG with a polydispersity of < 1.03 is used.

MPEG-pNPC reagents will couple to any primary amines without any further activation. For optimal yields of desired carbamate, the pH needs to be maintained at 7.0. The reaction is best performed on purified peptides since the PEGylation leads to an hydrophobic shift on RP-HPLC and to significant peak broadening.

Conjugation of MPEG-oxyamine to aldehyde-modified peptides works best in aqueous buffers like HEPES at a pH of 2-4.

Compounds are available in cGMP quality upon request.

References

1. a) J. Beythien & P. White (2003) *Biopolymers*, **71**, 362; b) B. Baumeister, et al. (2003) *Biopolymers*, **71**, 339; c) J. Beythien & P. White (2005) *Tetrahedron Lett.*, **46**, 101.
2. R. Fischer, et al. (2003) *Bioconjugate Chem.*, **14**, 653.
3. Z. Gao, et al. (2007) *J. Biol. Chem.*, **282**, 30718.
4. W. J. Choi, et al. (2009) *J. Med. Chem.*, **52**, 1612.
5. P. Marceau, et al. (2005) *Bioorg. Med. Chem. Lett.*, **15**, 5442.
6. D. J. Maly, et al. (2002) *Chembiochem*, **3**, 16.
7. J. Beythien et al. (2006) *Tetrahedron Lett.*, **47**, 3009.
8. C. G. Knight, et al. (1992) *FEBS Lett.*, **296**, 263.
9. J. L. Lauer-Fields, et al. (2001) *Biochemistry*, **40**, 5795.
10. G. T. Wang, et al. (1990) *Tetrahedron Lett.*, **31**, 6493.
11. E. D. Matayoshi, et al. (1990) *Science*, **247**, 954.
12. L. L. Maggiora, et al. (1992) *J. Med. Chem.*, **35**, 3727.
13. J. W. Drijfhout, et al. in "Peptides: Chemistry, Structure & Biology: Proc. 14th American Peptide Symposium", P. T. P. Kaumaya & R. S. Hodges (Eds), Mayflower Scientific Ltd., England, 1996, pp. 129.
14. C. Garcia-Echeverria & D. H. Rich (1992) *Febs Lett.*, **297**, 100.
15. B. F. Gilmore, et al. (2009) *Bioconjugate Chem.*, **20**, 2098.
16. B. R. White, et al. (2008) *Analyst*, 133, 65.
17. S. L. Gras, et al. (2008) *Biomaterials*, 29, 1553.
18. P. Hoogehout, et al. (1999) *J. Peptide Res.*, **54**, 436.
19. K. Hofmann & Y. Kiso (1976) *Proc. Natl. Acad. Sci. USA*, **73**, 3516.
20. T. Buranda, et al. (1999) *Cytometry*, **37**, 21.
21. I. Sélo, et al. (1996) *J. Immunol. Methods*, **199**, 127.
22. J. Howl, et al. (1993) *Eur. J. Biochem.*, **213**, 711.
23. V. Kumar & J. Aldrich (2003) *Org. Lett.*, **5**, 613.
24. M. Carraz, et al. (2009) *Chemistry & Biology*, 16, 709.
25. J. Parisot, et al. (2009) *J. Sep. Sci.*, **32**, 1613.
26. S. A. Shiryaevs, et al. (2007) *Biochem. J.*, **401**, 743.
27. S. W. Millward, et al. (2007) *Chem. Biol.*, **2**, 625.
28. Sequences proprietary.
29. O. Pöschke, unpublished results.
30. B. Baumeister, et al. (2005) *Int. J. Pept. Res. Ther.*, **11**, 139.
31. G. Pasut, et al. (2004) *Expert Opin. Ther. Patents,* **14**, 859.

Related products

851209	D(+)-Biotin	p. 177
851027	Biotin-ONp	p. 177
851023	Biotin-OSu	p. 177
855051	Biotin NovaTag™ resin	p. 177
855055	Biotin-PEG NovaTag™ resin	p. 178
855145	Fmoc-PEG Biotin NovaTag™ resin	p. 178
851029	N-Biotinyl-NH-PEG$_2$-COOH	p. 176
852340	N-Biotinyl-NH-PEG$_{11}$-COOH	p. 176
851025	5-Carboxyfluorescein	p. 167
851072	6-Carboxyfluorescein	p. 167
851082	5(6)-Carboxyfluorescein	p. 167
851026	5-Carboxy-tetramethylrhodamine	p. 168
851073	6-Carboxy-tetramethylrhodamine	p. 168
851030	5(6)-Carboxy-tetramethylrhodamine	p. 168
851022	Dabcyl-OSu	p. 168
855050	Dansyl NovaTag™ resin	p. 172
855053	Dnp NovaTag™ resin	p. 172
855054	EDANS NovaTag™ resin	p. 173
856081	Fmoc-Arg(bis-Boc-resin)-AMC	p. 169
856146	Fmoc-Asp(Wang-resin)-AMC	p. 169
856147	Fmoc-Lys(carbamate Wang-resin)-AMC	p. 170
852113	Fmoc-Asp(biotinyl-PEG)-OH	p. 179
852102	Fmoc-Glu(biotinyl-PEG)-OH	p. 179
852118	Fmoc-Asp(EDANS)-OH	p. 169
852098	Fmoc-Glu(EDANS)-OH	p. 169
852097	Fmoc-Lys(biotin)-OH	p. 180
852100	Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH	p. 180
856193	Fmoc-Lys(biotinyl-ε-aminocaproyl)-NovaSyn ® TG^R A resin	p. 180
852096	Fmoc-Lys(Dabcyl)-OH	p. 170
852099	Fmoc-Lys(Dnp)-OH	p. 170
852095	Fmoc-Lys(Mca)-OH	p. 171
855052	Mca NovaTag™ resin	p. 173
851071	Mca-OH	p. 171
851216	MPEG-20kDa ethylamine	p. 166
851217	MPEG-20kDa oxyamine	p. 166
851218	MPEG-20kDa pNPC	p. 166
851219	MPEG-30kDa ethylamine	p. 166
851220	MPEG-30kDa oxyamine	p. 166
855057	Universal NovaTag™ resin	p. 174
855058	Universal PEG NovaTag™ resin	p. 175

5.3   Chemoselective purification tags

5.3.2   IMAC Tag

5.3.1   Introduction

Whilst RP-HPLC is an extremely powerful tool for the purification of small to medium sized peptides, for long peptides the technique lacks the resolution necessary to separate the target molecule from the melange of closely related deletion and truncation products that arise during synthesis. In addition, the purified products, despite giving the appearance of being homogeneous by HPLC analysis, are often microheterogeneous, being contaminated with numerous co-eluting sequences which, because they are individually only present in small amounts, escape detection by mass spectrometry.

One solution is to utilize a combination of chemoselective purification tags [1] and standard RP-HPLC. In the former, unreacted amino groups are capped after each coupling step, converting deletion sequences to shorter truncation sequences. Prior to cleavage of the peptide from the resin, the *N*-terminal amino functionality of the full length peptide is labeled with an affinity tag which permits selective separation of the tagged target peptide from these truncation sequences. Following affinity purification, the tag is cleaved and the desired peptide isolated. The method is especially effective at removing impurities that are closely eluting or hidden under the isolated product peak. Furthermore, removal of these ion-suppressing smaller impurities can greatly enhance the signal of the target peptide in ES-MS. Final polishing by HPLC, removes modified and partially-protected by-products.

Novabiochem® offers three chemoselective purification tags: IMAC Tag, C18 Tag (p-nitrophenyl-2-(octadecylsulfonyl)ethyl carbonate), and 2-biotinyldimedone.
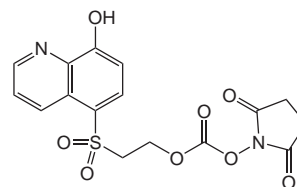
The mechanism of purification using the IMAC Tag [2] is analogous to HisTag affinity purification, the traditional method in use for isolation and purification of recombinant proteins. The IMAC purification method is extremely easy-to-use, gives higher recoveries than RP-HPLC, and is more effective at removing closely eluting impurites. Furthermore, as the purification is an on-off process, it can be readily automated using standard HPLC or FPLC instrumentation.
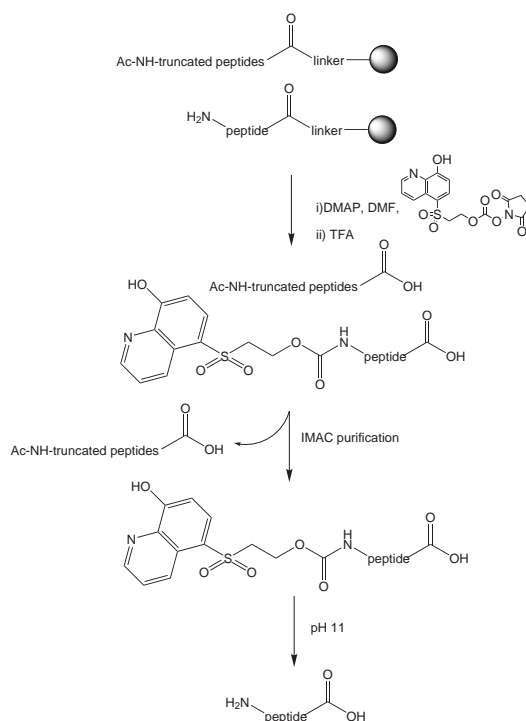
Fig. 5-36: IMAC purification of peptides.

Fig. 5-37: A typical IMAC purification trace obtained using a GE Akta Explorer purification system. Untagged truncates elute during the load and wash step and tagged full length product remains bound to the column until the elute step.
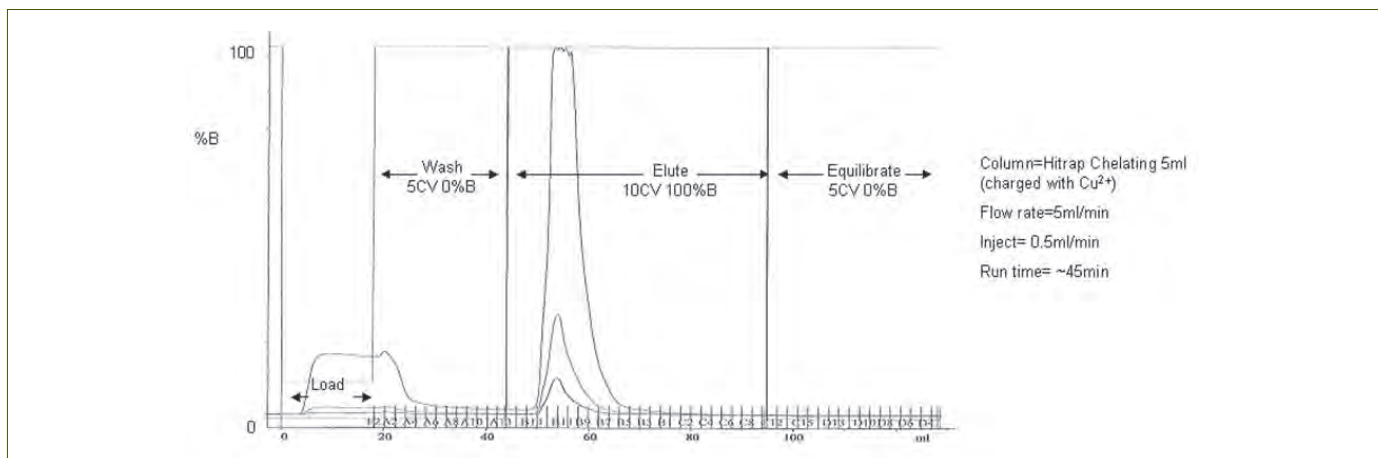
The peptide is synthesized using standard methods. After each coupling unreacted amino groups are capped using Ac$_2$O or Z(Cl)-OSu. Once the final Fmoc group is removed, the IMAC tag is attached to the peptide *via* an ONp carbonate (Figure 5-36). Peptides are then cleaved from the resin under standard conditions and purified using a column functionalised with iminodiacetic acid (IDA) loaded with Cu^{2+} ions. Tagged peptide is bound to the column at pH 6.5 – 8.0 and truncated sequences are washed away before the tagged peptide is eluted from the column by adjusting the pH to 3.5 (Figure 5-37). Buffers incorporating sodium phosphate, sodium chloride and urea are used to ensure maximum solubility of substrates.

Once the purified tagged peptide is eluted from the column, the TAG can be removed by simply raising the pH to 11.0 using 2 M NaOH for a short period. The liberated TAG and purified peptide can then be separated using the same IMAC column or an HPLC column for combined isolation and de-salting. As reducing agents are not compatible with IDA, any cysteines present in peptides must be protected during the IMAC step. This can be done simply and reversibly using for example StBu protection.

Protocols

The procedure of attachment of the IMAC and cleavage of tagged peptide from the resin are shown in Method 5-7.

Method 5-7: Formation of IMAC-tagged peptide

Attachment of IMAC tag
1. Dissolve Tag-OSu in the minimum volume of DMF and add to pre-swollen resin. Coupling has been shown to be effective using as little as 1 eq Tag-OSu with respect to the initial resin loading.
2. Agitate the mixture for one hour, add a catalytic amount of DMAP and agitate for a further hour.
3. Wash the resin with DMF, DCM and diethyl ether before drying under vacuum.

Cleavage of tagged peptide from the resin
1. Cleave non-cysteine containing peptides from the solid phase with concomitant side-chain deprotection by treatment with 90%TFA v/v, 5% H$_2$O v/v, 2.5% TIS v/v, 2.5% EDT v/v for 4.5 hours.
2. Cleave peptide-resins containing Cys(StBu) by treatment with 90% TFA v/v, 5% H$_2$O v/v, 5% TIS v/v for 4.5 hours.
3) Work up cleavage reactions in the standard manner by precipitation.

The IMAC purification using a copper (II) loaded IDA column is given in method 5-8. Columns such as HiTrap Chelating HP worked well for this application.

Method 5-8: Purification of tagged peptide

Buffer preparation
1. Binding buffer for the IMAC chromatography may consist of 20 mM sodium phosphate, 2-8 M urea (depending on peptide solubility) and 0.5 M NaCl. A pH range of 6.5-8.5 can be used.
2) Elution Buffer should have a pH of 3.5. A suggested buffer within this range is citric acid-sodium phosphate system. Elution buffer should also contain Urea (2-8 M) and NaCl (0.5 M).

IMAC purification
1. Charge column with 0.5 column volumes (CV) of 0.1 M CuSO4 in H2O.
2. Wash column with 2 CV H$_2$O, 7.5 CV elution buffer, 10 CV binding buffer.
3. Solubilise crude tagged peptide in binding buffer.
4. Load sample onto IMAC column.
5. Wash any unbound material from the column with 10 CV binding buffer.
6. Elute tagged peptide from column using 20 CV elution buffer.

Method 5-9: Removal of tag
1. Combine fractions containing purified tagged product. Adjust to pH 11 with 2 M NaOH. If product contains Cys(StBu) add TCEP up to a concentration of 5 mM to remove S-tBu protecting groups simultaneously.
2. Agitate reaction for 1-2 hours at room temperature and then adjust pH to ~ 4 with 2 M HCl.
3. Separate liberated tag and purified peptide using a second IMAC step or carry out RP-HPLC to combine isolation with desalting.

Example purifications

Application 5-11: Purification of Gly-GLP (2-36)

Gly-GLP (2-36) was also tagged and purified using IMAC methodology. The crude peptide was shown to contain several impurities identified as capped truncates formed during SPPS (Figure 4). A recovery of 22% pure peptide (89% purity) was obtained after IMAC purification and TAG cleavage. For comparison, purification using HPLC gave pure peptide (81%) with a lower recovery of 5%.
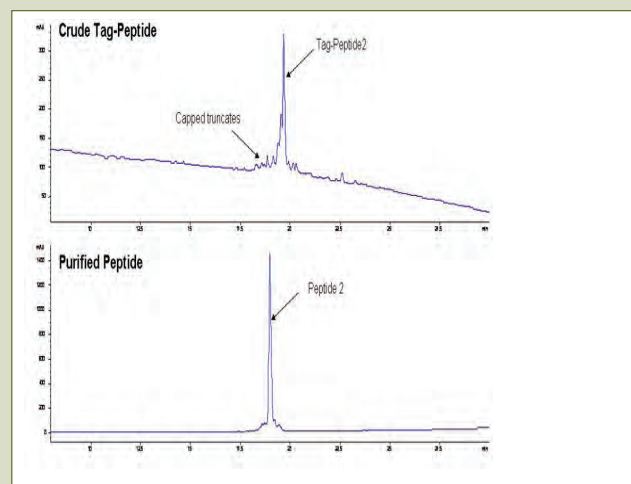
Fig. 5-38: HPLC profiles of Gly-GLP 2-36 before and after IMAC purification.

Application 5-12: Purification of Ubiquitin

Ubiquitin was also tagged and purified using IMAC methodology. The crude peptide was shown to contain several impurities identified as capped truncates formed during SPPS (Scheme 4). A recovery of 62% pure peptide (89% purity) was obtained after IMAC purification and TAG cleavage. For comparison, purification using HPLC gave pure peptide (82%) with a lower recovery of 21%..
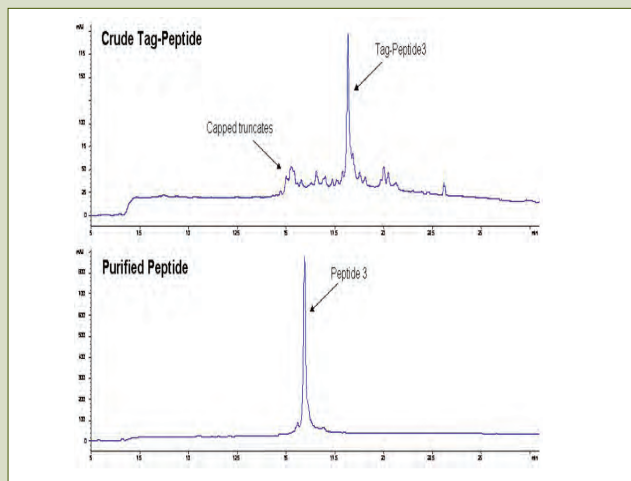
Fig. 5-39: HPLC profiles of Ubiquitin before and after IMAC purification.

5.3.3 C18–Tag
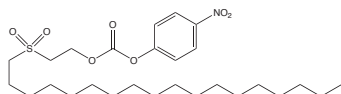
This lipophilic tag allows separation of the tagged peptide by HPLC. Typically, tagged peptides elute 5 - 10 minutes later than the capped by-products. Removal of the tag is effected by treatment with 5% aq. ammonia [3] or by Method 5-9. This tag is useful for the purification of hydrophilic peptides.

5.3.4 2–Biotinyldimedone
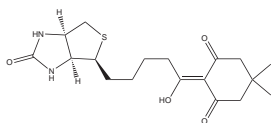
2-Biotinyldimedone tag allows product isolation by biotin-avidin affinity chromatography [4]. Without any pre-activation, it reacts with free primary amino groups to give derivatives that are stable to both acid and base conditions employed in Fmoc SPPS. Labeling of the *N*-terminal amine of resin-bound peptides is simply carried out by incubating overnight the peptidyl resin with a fourfold excess of the reagent in DMF. The biotinylated peptide is obtained following the standard TFA cleavage-deprotection procedure. Following cleavage, this biotin-labeled peptide can be bound to an avidin-coated support, allowing capped fragments to be simply washed away. The purified product can then be eluted with aqueous hydrazine, leaving the used tag attached to the support.

References

1. P. Mascagni in "Fmoc solid phase peptide synthesis - a practical approach", W. C. Chan & P. D. White (Eds), Oxford University Press, 2000, pp. 243.
2. PCT Application No.: PCT/GB2011/000363
3. C. Garcia-Echeverria (1995) *J. Chem. Soc., Chem. Commun.*, 779.
4. B. Kellam, et al. (1997) *Tetrahedron Lett.*, **38**, 5391.

Related products

851069	2-Biotinyldimedone	p. 182
851208	IMAC Tag	p. 182
851092	4-Nitrophenyl-2-(octadecylsulfonyl)ethylcarbonate	p. 182

6: Peptide analysis & purification

6.1   Analysis of crude peptides

Analysis of peptide purity of synthetic peptides is carried out almost without exception by reverse-phase HPLC. This techique provides excellent resolution for small to medium sized peptides of moderate hydrophilicity, but suffers from poor retention for highly polar peptides, and poor recoveries for hydrophobic peptides. In such cases, HILIC (Hydrophilic Liquid Interaction Chromatography) should be considered, as separation is achieved by a normal phase mechanism. Merck offers a broad selection of HPLC columns for reversed phase separations and also for HILIC.

6.1.1   Reversed phase HPLC

Depending on the peptide's size and hydrophobicity, packings of C_{18}, C_8, C_4 are recommended. Peptides above 30 residues are best separated on 300 Å wide-pore silica. A water/MeCN, water/MeOH or water/isopropanol gradient with acidic ion pairing reagents like TFA, heptafluorobutyric acid (HFBA), phosphoric acid or triethylammonium phosphate (TEAP) pH 2.25 are generally used. Isopropanol is favored for hydrophobic and protected peptide fragments, but is more viscous than MeCN or MeOH. The TEAP system normally gives better resolution but requires desalting with reverse-phase chromatography afterwards if used preparatively. Ideally, peptides should also be analysed using neutral buffers such as ammonium acetate at pH 7.00. Monitoring is normally performed using a wavelength of 210-220 nm. However, if the peptide contains the aromatic residues Phe, Tyr, or Trp, monitoring can be performed at 240-280 nm. Monolithic silica columns are particularly recommended as they provide very rapid analysis, making them ideal for high-throughput applications and real-time reaction monitoring.

Particulate standard HPLC columns

Reversed phase particulate silica LiChrospher and Purospher STAR columns are commonly used for separations of peptides. Reversed phase LiChrospher columns are being offered in pore sizes of 100 Å or 300 Å. Long peptides and small proteins should be separated with LiChrospher 300Å. For short peptides LiChrospher 100 Å is being recommended. Purospher STAR columns with pore diameter of 120 Å are the method of choice for short basic peptides, because the Purospher STAR sorbent is based on a metal-free silica backbone, thus will enable peak-tailing-free separations of basic peptides.

LiChrospher WP
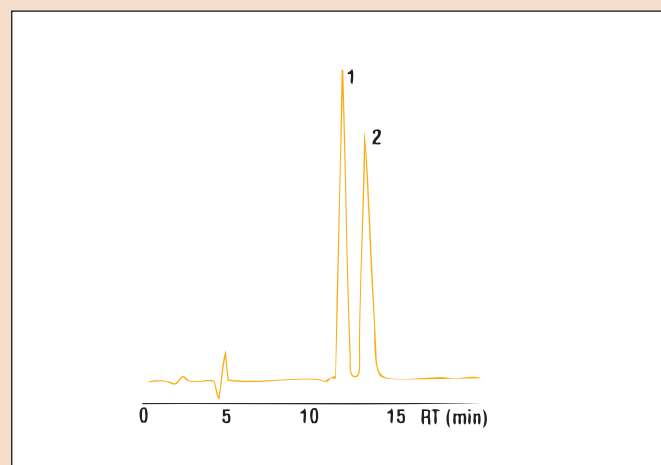
Fig. 6-1 HPLC profiles of 1) h-angiotensin II and 2) h-angiotensin I. Column: LiChroCART® 250-4 LiChrospher® WP 300 RP-18, 5 μm; buffer A: 0.1% TFA; buffer B: 0.1% TFA in MeCN; gradient: 20 - 60% B in 20 min; flow rate: 0.6 ml/min; detection: 214 nm.

Purospher STAR, 3μm

Reversed phase LiChrospher and Purospher STAR columns are available in RP-8 or RP-18 modifications and are offered in analytical or semi-preparative column dimensions.
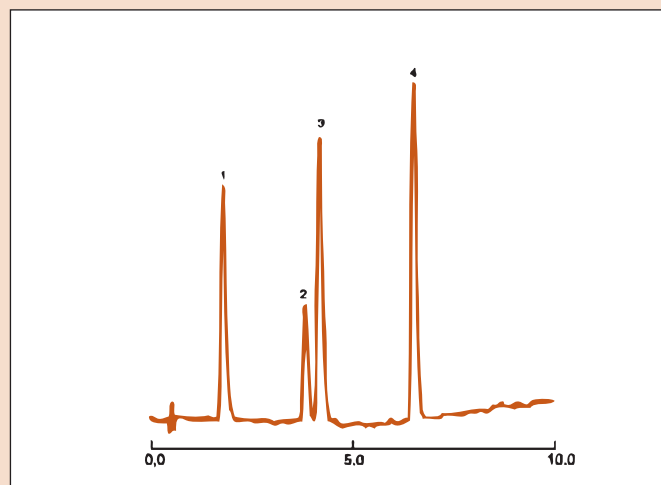
Fig. 6-2 HPLC profiles of 1) H–Ala-Tyr-OH, 2) H-Tyr-Tyr-OH, 3) H-Gly-Phe-Gly-OH and 4) H-Leu-Tyr-OH. Column: LiChroCART® 55-4 Purospher® STAR RP-18 endcapped, 3 μm; buffer A: 0.1% TFA; buffer B: 0.1% TFA in MeCN; gradient: 0 - 80% B in 10 min; flow rate: 1.0 ml/min; detection: 254 nm.

LiChrospher Ordering information

HPLC columns

Column Type	Sorbent	Cat.No.	Pieces
Hibar® 150-4.6	LiChrospher® RP-18e, 5µm	1.50549.0001	1
Hibar® 150-4.6	LiChrospher® RP-8e, 5µm	1.50582.0001	1
Hibar® 250-4.6	LiChrospher® RP-18e, 5µm	1.50550.0001	1
Hibar® 250-4.6	LiChrospher® RP-8e, 5µm	1.50583.0001	1

HPLC cartridges
(to be mounted with the cartridge holder manu-CART®, Cat. No.1.51486.0001 for 4.6mm i.d. and 1.51419.0001 for 10 mm i.d.)

Column Type	Sorbent	Cat.No.	Pieces
LiChroCART® 150-4.6	LiChrospher® RP-18e, 5µm	1.50604.0001	1
LiChroCART® 150-4.6	LiChrospher® RP-8e, 5µm	1.50638.0001	1
LiChroCART® 250-4.6	LiChrospher® RP-18e, 5µm	1.50605.0001	1
LiChroCART® 250-4.6	LiChrospher® RP-8e, 5µm	1.50638.0001	1
LiChroCART® 250-10	LiChrospher® RP-18e, 5µm	1.50858.0001	1
LiChroCART® 250-4	LiChrospher® WP RP-18, 5µm	1.50137.0001	1

Guard columns and holders
(to be connected with the cartridge holder manu-CART®, Cat. No.1.51486.0001 to LiChroCART® cartridges and with the holder 1.51487.0001 to Hibar® columns)

Column Type	Sorbent	Cat.No.	Pieces
LiChroCART® 4-4	LiChrospher® RP-18, 5µm	1.50957.0001	10
LiChroCART® 4-4	LiChrospher® RP-8, 5µm	1.50956.0001	10
"manu-CART® for LiChroCART® Cartridges"		1.51486.0001	1
Holder for Hibar® columns		1.51487.0001	1

Purospher STAR ordering information

HPLC columns

Column Type	Sorbent	Cat.No.	Pieces
Hibar® 150-4.6	Purospher® STAR RP-18e, 5µm	1.51455.0001	1
Hibar® 150-4.6	Purospher® STAR RP-8e, 5µm	1.51453.0001	1
Hibar® 250-4.6	Purospher® STAR RP-18e, 5µm	1.51454.0001	1
Hibar® 250-4.6	Purospher® STAR RP-8e, 5µm	1.50583.0001	1

HPLC cartridges
(to be mounted with the cartridge holder manu-CART®, Cat. No.1.51486.0001 for 4.6mm i.d.and 1.51419.0001 for 10 mm i.d.)

Column Type	Sorbent	Cat.No.	Pieces
LiChroCART® 150-4.6	Purospher® STAR RP-18e, 5µm	1.50358.0001	1
LiChroCART® 150-4.6	Purospher® STAR RP-8e, 5µm	1.50638.0001	1
LiChroCART® 250-4.6	Purospher® STAR RP-8e, 5µm	1.50359.0001	1
LiChroCART® 250-4.6	Purospher® STAR RP-18e, 5µm	1.50638.0001	1
LiChroCART® 250-10	Purospher® STAR RP-18e, 5µm	1.50257.0001	1

Guard columns and holders
(to be connected with the cartridge holder manu-CART®, Cat. No.1.51486.0001 to LiChroCART® cartridges and with the holder 1.51487.0001 to Hibar® columns)

Column Type	Sorbent	Cat.No.	Pieces
one package (pcs)"			
LiChroCART® 4-4	Purospher® STAR RP-18, 5µm	1.50250.0001	10
LiChroCART® 4-4	Purospher® STAR RP-8, 5µm	1.50270.0001	10
"manu-CART® for LiChroCART® Cartridges"		1.51486.0001	1
Holder for Hibar® columns		1.51487.0001	1

Particulate fast HPLC Columns

Reversed phase particulate silica Purospher STAR columns for UHPC are suitable for fast separations of short peptides. These columns are available in RP-8 and RP-18 modifications in analytical column dimensions.

Purospher STAR UHPLC ordering information

HPLC columns

Column Type	Sorbent	Cat.No.	Pieces
Hibar® HR 30-2.1	Purospher® STAR RP-18e, 2µm	1.50645.0001	1
Hibar® HR 30-2.1	Purospher® STAR RP-18e, 3µm	1.50650.0001	1
Hibar® HR 50-2.1	Purospher® STAR RP-18e, 2µm	1.50646.0001	1
Hibar® HR 50-2.1	Purospher® STAR RP-18e, 3µm	1.50651.0001	1

Monolithic fast HPLC Columns

Reversed phase monolithic silica Chromolith columns provide excellent separations of short peptides in a fraction of time that a particulate standard column will take, because they are made from highly porous monolithic silica rods with a bimodal pore structure. This pore structure provides a unique combination of macropores and mesopores. The macropores (average size: 2µm) allow a rapid flow of the mobile phase at very low pressure. The mesopores (average size: 130 Å) are responsible for the surface area, enabling the adsorption / desorption of peptides, thus being responsible for the high performance separation process.

Benefits of Chromolith HPLC columns at a glance:

- Separations are at least twice as fast with half the back pressure compared to 5µm particulate columns.

- Higher sample throughput compared to particulate columns – separations up to 9 times faster if required.

- Fast column re-equilibration between analyses.

- Significantly increased column life time compared to particulate columns.

- Reduced need for sample preparation as monolithic columns are very resistant to blocking, compared to particulate columns.

- Higher separation efficiency (by column coupling compared to particulate columns.

Reversed phase Chromolith columns are available in RP-8 or RP-18 modifications and are being offered in analytical or semi-preparative column dimensions.

FAS Death Domain related petides

Fig. 6-3: HPLC profiles of a) 30 residue and b) 46 residue FAS Death Domain related peptide. Column: Merck Chromolith SpeedRod; buffer A: 0.1% TFA; buffer B: MeCN/water/TFA 90:10:0.1; gradient: 5 - 100% B in 6 min; flow rate: 5.0 ml/min; detection: 220 nm.

6.1.2   Normal phase HPLC

HILIC (Hydrophilic Liquid Interaction Chromatography) is a technique for separation of hydrophilic and hydrophobic peptides. The elution order in HILIC is the opposite of that in reversed phase HPLC and retention increases with hydrophilicity and charge of peptide. This enables straightforward separation of hyphophilic peptides that otherwise elute in the void volume of reversed phase columns or hydrophobic peptides than stick too tightly to reverse phase silica.

SeQuant ZIC–HILIC columns

SeQuant ZIC-HILIC silica and polymeric columns can be used to separate short peptides with high selectivity and reproducibility. The ZIC-HILIC sorbents have a bonded stationary phase consisting of a highly polar, permanent zwitterion. Separation efficiency is favored by the 1:1 zwitterionic charge balance, which makes the ZIC-HILIC columns overall neutral, with weak, but important ionic interactions.

The robust bonded hydrophilic phase on ZIC-HILIC ensures a stable environment for the HILIC partitioning process during peptide retention. The compatibility of ZIC-HILIC with a range of different buffers, organic solvents and temperatures makes it straightforward to develop robust isocratic and gradient methods for MS, ELSD, UV detection of peptides. The ZIC-HILIC robustness and excellent batch-to-batch reproducibility ensures method scalability all the way from nano and capillary to semi-preparative size.

SeQuant ZIC-HILIC columns are based either on silica sorbents or polymeric sorbents. The polymer-based ZIC-pHILIC columns offer an extended pH stability range compared to the silica based ZIC HILIC columns (pH 2-10, compared to 2-7.5). This introduces a unique opportunity to change the selectivity by altering the ionization of functional groups on a peptide. The ZIC-pHILIC sorbent has the same bonded permanent zwitterion group as the silica based ZIC-HILIC column.
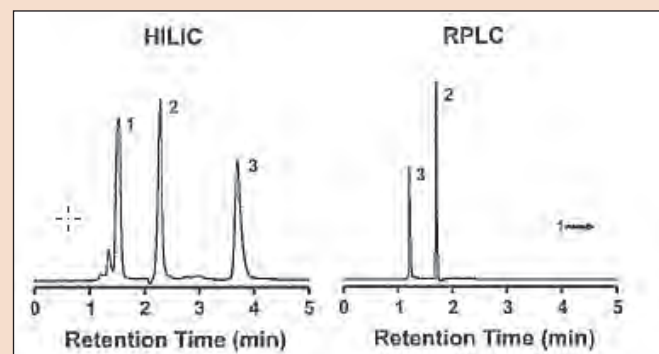
Fig. 6-4: HPLC profiles of 1) H-Phe-Gly-Gly-Phe-OH, 2) H-Leu-Gly-Gly-OH, and 3) H-Gly-Gly-Gly-OH . HILIC: 60:40 MeCN/10 mM ammonium acetate; RP-HPLC: 95:5 10 mM ammonium acetate/MeCN.

6.2 Purification of synthetic peptides

Purification is generally the final step in the production of a synthetic peptide. One or two different procedures are usually necessary. The peptide should be desalted. The likely contaminants such as scavengers, truncated/capped sequences or small molecules can be removed by gel-filtration chromatography. Semi-preparative or preparative HPLC is then necessary to achieve purities of >95%. Depending on the peptide's size and hydrophobicity, packings of C_{18}, C_8, C_4 or diphenyl are recommended. A water/MeCN, water/MeOH or water/isopropanol gradient with acidic ion pairing reagents like TFA, heptafluorobutyric acid (HFBA), phosphoric acid, or buffers like ammonium acetate or triethylammonium phosphate (TEAP) pH 2.25 or 7.00 should be used. Long peptides or peptides with a number of similarly charged groups are best separated on ion-exchange columns.

Solubility problems can be encountered, due to such factors as hydrophobic residues, aggregation, disulfide formation between Cys side chains, secondary structure formation and high content of acidic residues, etc.

Basic peptides dissolve in aqueous acetic acid, up to 10%. For acidic peptides dilute ammonia or basic buffers such as ammonium bicarbonate can be used. Keep in mind that a pH higher than 8 will damage silica-based reverse-phase columns.

Although DMF and DMSO are highly absorbing at wavelengths betweeb 210-220 nm, they can be used in small amounts. Dissolve the peptide in DMSO or DMF and dilute with the chosen buffer. The DMSO or DMF will elute at a low retention time on reverse-phase chromatography, and would usually not interfere with the peptide peak.
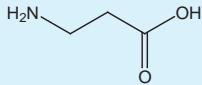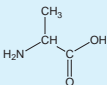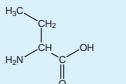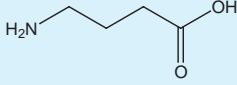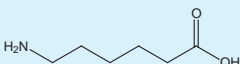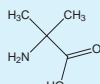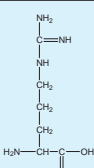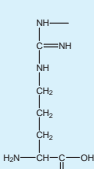
Some peptides will dissolve in salt solutions such as urea or guanidinium hydrochloride. In other instances, low concentrations of propanol have been used.

It is strongly recommended to keep the pure, lyophilized peptides frozen at -20°C under nitrogen. Peptides should not contain residual acid, because the latter can slowly react with unprotected side chains, N-terminal Gln or hydrolyse acid-labile bonds such as Asp-Pro, even at low temperatures. Peptides can be re-lyophilized or suspended in water and dried again to remove all residual acid. However, there is always the possibility that some loss of biological activity may occur upon re-lyophilization.
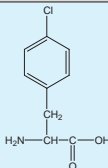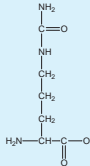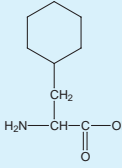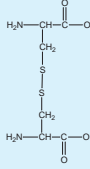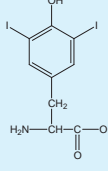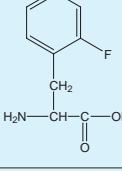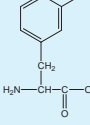
System code	Solvent	Composition
0000	Diisopropylether	
0001	Diethylether	
0002	EtOAc	
0003	Toluene : Dioxane : AcOH	95 : 25 : 4
0004	CH_3CN : $CHCl_3$	1 : 3
0005	Toluene : AcOH	10 : 1
0006	Toluene : EtOH	9 : 1
0007	$CHCl_3$: AcOH	95 : 5
0008	$CHCl_3$: MeOH : Benzene : H_2O	8 : 8 : 8 : 1
0009	$CHCl_3$: MeOH	9 : 1
0010	$CHCl_3$: TFE : Propionic acid : H_2O	100 : 20 : 17 : 3
0014	Isopropanol : NH_4OH conc.	1 : 1
0015	2-Butanol : 3% NH_4OH	1 : 1
0016	2-Butanol : Pyridine : H_2O	7 : 7 : 6
0017	MeOH : $CHCl_3$: 17% NH_4OH	2 : 2 : 1
002A	nBuOH : AcOH : H_2O : EtOAc	3 : 1 : 1 : 5
005A	Toluene : AcOH	17 : 3
007A	$CHCl_3$: AcOH	195 : 5
009A	$CHCl_3$: MeOH	4 : 1
009B	$CHCl_3$: MeOH	7 : 3
009C	$CHCl_3$: MeOH	29 : 1
0100	EtOAc : Pyridine : AcOH : H_2O	60 : 20 : 6 : 11
0110	EtOAc : nBuOH : AcOH : H_2O	2 : 1 : 1 : 1
011A	$CHCl_3$: MeOH : AcOH	90 : 8 : 2
011B	$CHCl_3$: MeOH : AcOH	85 : 10 : 5
011C	$CHCl_3$: MeOH : AcOH	77.5 : 15 : 7.5
011D	$CHCl_3$: MeOH : AcOH	7 : 1 : 2
0120	EtOAc : Formic acid 90% : H_2O	7 : 2 : 1
012A	EtOAc : Hexane	1 : 4
012B	EtOAc : Hexane	4 : 5
012C	EtOAc : Hexane	1 : 3
012D	EtOAc : Hexane	3 : 2
013A	$CHCl_3$: EtOAc	1 : 1
013B	$CHCl_3$: EtOAc	9 : 1
014A	EtOH : 17% NH_4OH	4 : 1
0150	$CHCl_3$: MeOH : H_2O	12 : 6 : 1
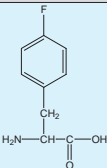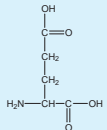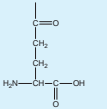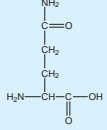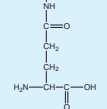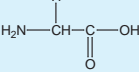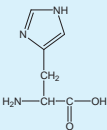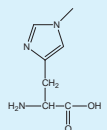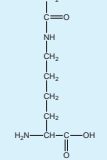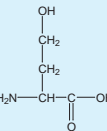
Tlc system used for analysis

System code	Solvent	Composition
0157	$CHCl_3$: MeOH : AcOH : H_2O	70 : 42 : 0.5 : 10
015A	2-Butanol : 3% NH_4OH	50 : 22
0160	$CHCl_3$: MeOH : Formic acid 10%	65 : 15 : 1
018A	$CHCl_3$: EtOAc : AcOH	25 : 25 : 2
018B	$CHCl_3$: EtOAc : AcOH	45 : 5 : 1
0211	Hexane : EtOAc : AcOH	20 : 10 : 1
0711	nBuOH : AcOH : H_2O	7 : 1 : 1
0811	CH_3CN : $CHCl_3$: AcOH	8 : 1 : 1
100A	EtOAc : Pyridine : AcOH : H_2O	60 : 10 : 3 : 4
100B	EtOAc : Pyridine : AcOH : H_2O	40 : 20 : 6 : 11
100C	EtOAc : Pyridine : AcOH : H_2O	6 : 5 : 1 : 3
100D	EtOAc : Pyridine : AcOH : H_2O	6 : 4 : 1 : 3
100E	EtOAc : Pyridine : AcOH : H_2O	5 : 5 : 1 : 3
100X	EtOAc : Pyridine : AcOH : H_2O	80 : 20 : 5 : 10
110A	EtOAc : nBuOH : AcOH : H_2O	1 : 1 : 1 : 1
157A	$CHCl_3$: MeOH : AcOH : H_2O	90 : 10 : 0.5 : 1
157B	$CHCl_3$: MeOH : AcOH : H_2O	85 : 13 : 0.5 : 1.5
BAPW	nBuOH : AcOH : H_2O : Pyridine	4 : 1 : 2 : 1
BAW0	nBuOH : AcOH : H_2O	2 : 1 : 1
BAW1	nBuOH : AcOH : H_2O	4 : 1 : 1
BAW2	nBuOH : AcOH : H_2O	10 : 2 : 3
BAW3	nBuOH : AcOH : H_2O	12 : 3 : 5
BAW4	nBuOH : AcOH : H_2O	3 : 1 : 1
BAWP	nBuOH : AcOH : H_2O : Pyridine	15 : 3 : 12 : 10
BPAW	nBuOH : AcOH : H_2O : Pyridine	9 : 2 : 4 : 3
CMA1	$CHCl_3$: MeOH : AcOH 32%	5 : 3 : 1
CMA2	$CHCl_3$: MeOH : AcOH 32%	15 : 4 : 1
MWA1	MeOH : H_2O : CH_3CN	5 : 5 : 3
OCRB	Benzene : Dioxane : AcOH	95 : 25 : 4
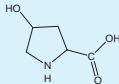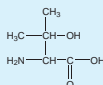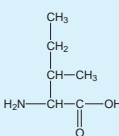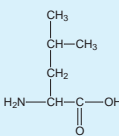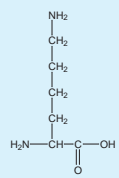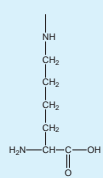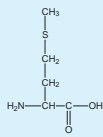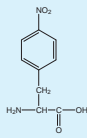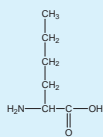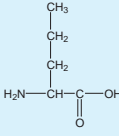
	Amino acid		Amino acid		Residue ($-H_2O$)	
Name	Structure	Symbol	Formula	Mol. Wt.	Formula	Residue Wt.
β-Alanine		βAla	$C_3H_7NO_2$	89.095	C_3H_5NO	71.079
Alanine		Ala	$C_3H_7NO_2$	89.095	C_3H_5NO	71.079
2-Aminobutyric Acid		Abu	$C_4H_9NO_2$	103.122	C_4H_7NO	85.106
4-Aminobutyric Acid		γAbu	$C_4H_9NO_2$	103.122	C_4H_7NO	85.106
6-Aminocaproic Acid		εAhx	$C_6H_{13}NO_2$	131.176	$C_6H_{11}NO$	113.161
α-Aminoisobutyric Acid		Aib	$C_4H_9NO_2$	103.122	C_4H_7NO	85.106
α-Aminosuberic Acid		Asu	$C_8H_{15}NO_4$	279.329	$C_8H_{13}NO_3$	261.314
Arginine		Arg	$C_6H_{14}N_4O_2$	174.204	$C_6H_{12}N_4O$	156.189
Arginine		Arg	$C_6H_{13}N_4O_2$	173.196	$C_6H_{11}N_4O$	155.181
Asparagine		Asn	$C_4H_8N_2O_3$	132.120	$C_4H_6N_2O_2$	114.105
Asparagine		Asn	$C_4H_7N_2O_3$	131.112	$C_4H_5N_2O_2$	113.097
Aspartic acid		Asp	$C_4H_7NO_4$	133.105	$C_4H_5NO_3$	115.089
Aspartic Acid		Asp	$C_4H_6NO_3$	116.097	$C_4H_4NO_2$	98.082
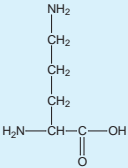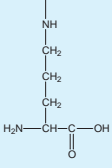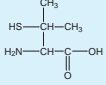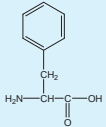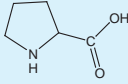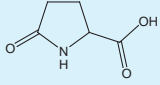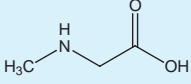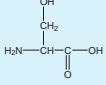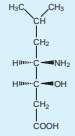
Amino acid molecular and residue masses

Name	Structure	Symbol	Amino acid Formula	Amino acid Mol. Wt.	Residue (−H_2O) Formula	Residue (−H_2O) Residue Wt.
4-Chlorophenylalanine		Phe(4-Cl)	$C_9H_{10}ClNO_2$	199.639	C_9H_8ClNO	181.624
Citrulline		Cit	$C_6H_{13}N_3O_3$	175.189	$C_6H_{11}N_3O_2$	157.173
ß-Cyclohexylalanine		Cha	$C_9H_{17}NO_2$	171.241	$C_9H_{15}NO$	153.226
Cysteine		Cys	$C_3H_7NO_2S$	121.159	C_3H_5NOS	103.145
Cysteine		Cys	$C_3H_6NO_2S$	120.151	C_3H_4NOS	102.135
Cystine		(Cys)$_2$	$C_6H_{12}N_2O_4S_2$	240.302	$C_6H_8N_2O_2S_2$	204.271
3,4-Dehydroproline		D-Pro	$C_5H_7NO_2$	113.118	C_5H_5NO	95.103
3,5-Diiodotyrosine		Tyr(3,5-di-I)	$C_9H_9I_2NO_3$	432.986	$C_9H_7I_2NO_2$	414.971
2-Fluorophenylalanine		Phe(2-F)	$C_9H_{10}FNO_2$	183.184	C_9H_8FNO	165.169
3-Fluorophenylalanine		Phe(3-F)	$C_9H_{10}FNO_2$	183.184	C_9H_8FNO	165.169

Name	Amino acid		Amino acid		Residue (–H_2O)	
	Structure	Symbol	Formula	Mol. Wt.	Formula	Residue Wt.
4-Fluorophenylalanine		Phe(4-F)	$C_9H_{10}FNO_2$	183.184	C_9H_8FNO	165.169
Glutamic Acid		Glu	$C_5H_9NO_4$	147.132	$C_5H_7NO_3$	129.116
Glutamic Acid		Glu	$C_5H_8NO_3$	130.124	$C_5H_6NO_2$	112.109
Glutamine		Gln	$C_5H_{10}N_2O_3$	146.147	$C_5H_8N_2O_2$	128.132
Glutamine		Gln	$C_5H_9N_2O_3$	145.139	$C_5H_7N_2O_2$	127.124
Glycine		Gly	$C_2H_5NO_2$	75.068	C_2H_3NO	57.052
Histidine		His	$C_6H_9N_3O_2$	155.158	$C_6H_7N_3O$	137.142
Histidine		His	$C_6H_8N_3O_2$	154.150	$C_6H_6N_3O$	136.134
Homocitrulline		Hci	$C_7H_{15}N_3O_3$	189.216	$C_7H_{13}N_3O_2$	171.201
Homoserine		hSer	$C_4H_9NO_3$	119.122	$C_4H_7NO_2$	101.107

Name	Amino acid Structure	Symbol	Amino acid Formula	Mol. Wt.	Residue ($-H_2O$) Formula	Residue Wt.
Hydroxyproline		Hyp	$C_5H_9NO_3$	131.132	$C_5H_7NO_2$	113.117
β-Hydroxyvaline		Val(βOH)	$C_5H_{11}NO_3$	133.148	$C_5H_9NO_2$	115.133
Isoleucine		Ile	$C_6H_{13}NO_2$	131.176	$C_6H_{11}NO$	113.161
Leucine		Leu	$C_6H_{13}NO_2$	131.176	$C_6H_{11}NO$	113.161
Lysine		Lys	$C_6H_{14}N_2O_2$	146.191	$C_6H_{12}N_2O$	128.175
Lysine		Lys	$C_6H_{13}N_2O_2$	145.183	$C_6H_{11}N_2O$	127.167
Methionine		Met	$C_5H_{11}NO_2S$	149.213	C_5H_9NOS	131.198
4-Nitrophenylalanine		Phe(4-NO₂)	$C_9H_{10}N_2O_4$	210.191	$C_9H_8N_2O_3$	192.176
Norleucine		Nle	$C_6H_{13}NO_2$	131.176	$C_6H_{11}NO$	113.161
Norvaline		Nva	$C_5H_{11}NO_2$	117.149	C_5H_9NO	99.134
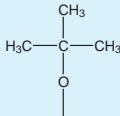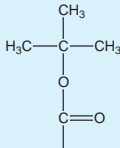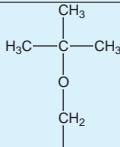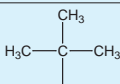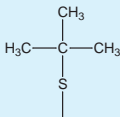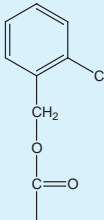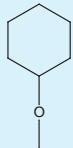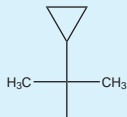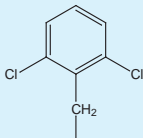
Name	Structure	Symbol	Amino acid Formula	Mol. Wt.	Residue (–H$_2$O) Formula	Residue Wt.
Ornithine		Orn	C$_5$H$_{12}$N$_2$O$_2$	132.164	C$_5$H$_{10}$N$_2$O	114.148
Ornithine		Orn	C$_5$H$_{11}$N$_2$O$_2$	131.156	C$_5$H$_9$N$_2$O	113.140
Penicillamine		Pen	C$_5$H$_{11}$NO$_2$S	149.213	C$_5$H$_9$NOS	131.198
Phenylalanine		Phe	C$_9$H$_{11}$NO$_2$	165.194	C$_9$H$_9$NO	147.178
Phenylglycine		Phg	C$_8$H$_9$NO$_2$	151.167	C$_8$H$_7$NO	133.152
Proline		Pro	C$_5$H$_9$NO$_2$	115.133	C$_5$H$_7$NO	97.118
Pyroglutamine		Pyr,Glp	C$_5$H$_7$NO$_3$	129.117	C$_5$H$_5$NO$_2$	111.101
Sarcosine		Sar	C$_3$H$_7$NO$_2$	89.095	C$_3$H$_5$NO	71.079
Serine		Ser	C$_3$H$_7$NO$_3$	105.094	C$_3$H$_5$NO$_2$	87.079
Serine		Ser	C$_3$H$_6$NO$_3$	104.086	C$_3$H$_4$NO$_2$	86.071
Statine		Sta	C$_8$H$_{17}$NO$_3$	175.229	C$_8$H$_{15}$NO$_2$	157.214
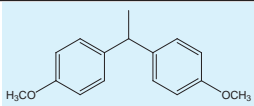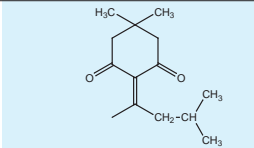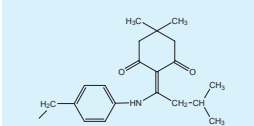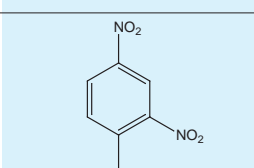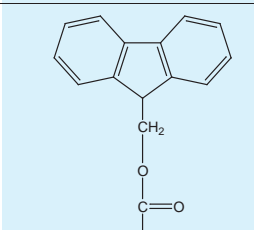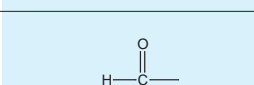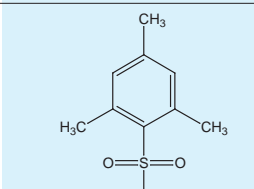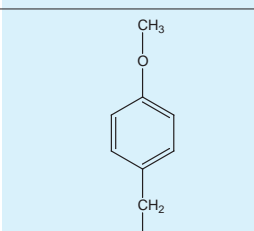
Name	Structure	Symbol	Amino acid Formula	Amino acid Mol. Wt.	Residue (–H_2O) Formula	Residue (–H_2O) Residue Wt.
β-(2-Thienyl)alanine		Thi	$C_7H_9NO_2S$	171.220	C_7H_7NOS	153.205
Threonine		Thr	$C_4H_9NO_3$	119.121	$C_4H_7NO_2$	101.106
Threonine		Thr	$C_4H_8NO_3$	118.113	$C_4H_6NO_2$	100.098
Threonine		Thr	$C_4H_8NO_3$	118.113	$C_4H_6NO_2$	100.098
Tryptophan		Trp	$C_{11}H_{12}N_2O_2$	204.230	$C_{11}H_{10}N_2O$	186.215
Tryptophan		Trp	$C_{11}H_{11}N_2O_2$	203.223	$C_{11}H_9N_2O$	185.207
Tyrosine		Tyr	$C_9H_{11}NO_3$	181.193	$C_9H_9NO_2$	163.178
Tyrosine		Tyr	$C_9H_{10}NO_3$	180.185	$C_9H_8NO_2$	162.170
Valine		Val	$C_5H_{11}NO_2$	117.149	C_5H_9NO	99.134
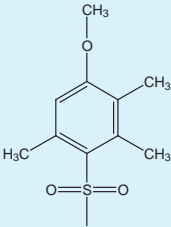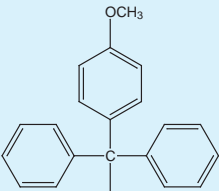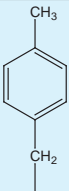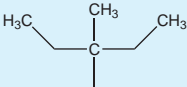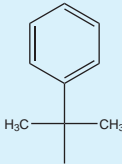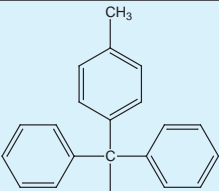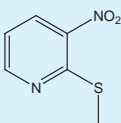
Name	Structure	Symbol	Formula	Residue Wt.
Acetamidomethyl		Acm	C_3H_6NO	72.087
Acetyl		Ac	C_2H_3O	43.046
Adamantyloxy		AdaO	$C_{10}H_{13}O$	149.214
Benzoyl		Bz	C_7H_5O	105.117
Benzyl		Bzl	C_7H_7	91.134
Benzyloxy		BzlO	C_7H_7O	107.125
Benzyloxycarbonyl		Z	$C_8H_7O_2$	135.144
Benzyloxymethyl		Bom	C_8H_9O	121.16
2-Bromobenzyloxycarbonyl		2-Br-Z	$C_8H_6BrO_2$	214.045

Name	Structure	Symbol	Formula	Residue Wt.
t-Butoxy		tBuO	C_4H_9O	73.116
t-Butoxycarbonyl		Boc	$C_5H_9O_2$	101.126
t-Butoxymethyl		Bum	$C_5H_{11}O$	87.143
t-Butyl		tBu	C_4H_9	57.117
t-Butylthio		tButhio	C_4H_9S	89.181
2-Chlorobenzyloxycarbonyl		2-Cl-Z	$C_8H_6ClO_2$	169.589
Cyclohexyloxy		cHxO	$C_6H_{11}O$	99.154
1-Cyclopropyl-1-methyl-ethyl		Dmcp	C_6H_{11}	83.15
2,6-Dichlorobenzyl		2,6-Di-Cl-Bzl	$C_7H_5Cl_2$	160.024

Name	Structure	Symbol	Formula	Residue Wt.
4,4'-Dimethoxybenzhydryl		Mbh	$C_{15}H_{15}O_2$	227.286
1-(4,4-Dimethyl-2,6-dioxo-cyclohexylidene)3-methylbutyl		ivDde	$C_{13}H_{19}O_2$	207.295
4{N-[1-(4,4-Dimethyl-2,6-dioxo-cyclohexylidene)-3-methylbutyl]-amino}benzyloxy		ODmab	$C_{20}H_{26}NO_3$	328.425
2,4-Dinitrophenyl		Dnp	$C_6H_3N_2O_4$	167.102
Fluorenylmethoxycarbonyl		Fmoc	$C_{15}H_{11}O_2$	223.254
Formyl		For	CHO	29.018
Mesitylene-2-sulfonyl		Mts	$C_9H_{11}O_2S$	183.251
4-Methoxybenzyl		MeOBzl	C_8H_9O	121.160

Name	Structure	Symbol	Formula	Residue Wt.
4-Methoxy-2,3,6-trimethyl-benzenesulfonyl		Mtr	$C_{10}H_{13}OS$	213.278
4-Methoxytrityl		Mmt	$C_{20}H_{17}O$	273.357
4-Methylbenzyl		MeBzl	C_8H_9	105.161
3-Methylpent-3-yl		Mpe	C_6H_{13}	85.168
1-Methyl-1-phenyl-ethyl		PhiPr	C_9H_{11}	119.18
4-Methyltrityl		Mtt	$C_{20}H_{17}$	257.358
3-Nitro-2-pyridinesulfenyl		Npys	$C_5H_3N_2O_2S$	155.156

Name	Structure	Symbol	Formula	Residue Wt.
2,2,4,6,7-Pentamethyl-dihydrobenzofurane-5-sulfonyl		Pbf	$C_{13}H_{17}O_3S$	253.338
2,2,5,7,8-Pentamethyl-chromane-6-sulfonyl		Pmc	$C_{14}H_{19}O_3S$	267.369
Tosyl		Tos	$C_7H_7O_2S$	155.197
Trifluoroacetyl		Tfa	C_2F_3O	97.017
Trimethylacetamidomethyl		Tacm	$C_6H_{12}NO$	114.169
Trimethoxyphenylthio		STmp	$C_9H_{11}O_3S$	199.250
Trityl		Trt	$C_{19}H_{15}$	243.331
Xanthyl		Xan	$C_{13}H_9O$	181.216

Molecular weights and melting point data for commonly used Boc and Fmoc amino acids

Product name	Catalog number	M.W.	Mp(°C)	Side-chain deprotection
Boc–amino acids				
Boc-Ala-OH	853001	189.2	78–85	
Boc-ßAla-OH	853057	189.2	74–75	
Boc-Arg(Tos)-OH	853013	428.5	85–95	HF(long), Na/NH$_3$
Boc-Asn-OH	853039	232.2	178–185	
Boc-Asn(Trt)-OH	853074	474.6	200–210	TFA
Boc-Asn(Xan)-OH	853007	412.5	183–198	TFA
Boc-Asp-OBzl	853014	323.4	95–100	HF, TFMSA, TMSOTf, H$_2$/Pd/C
Boc-Asp(OBzl)-OH	853045	323.4	95–105	HF, TFMSA, TMSOTf, H$_2$/Pd/C
Boc-Asp(OcHx)-OH	853030	315.4	88–95	HF,TMSOTf
Boc-Cha-OH DCHA	853072	452.7	165–170	
Boc-Cys(Acm)-OH	853049	292.4	110–118	I$_2$, Hg^{2+},Tl^{3+}
Boc-Cys(pMeBzl)-OH	853033	325.4	75–88	HF, TFMSA
Boc-Cys(pMeOBzl)-OH	853050	341.4	65–75	HF, TFMSA
Boc-Cys(Trt)-OH	853005	463.6	122–132	TFA
Boc-Gln-OH	853040	246.3	112–120	
Boc-Gln(Xan)-OH	853016	426.5	160–185	TFA
Boc-Glu-OBzl	853015	337.4	90–98	HF, TFMSA
Boc-Glu(OBzl)-OH	853010	337.4	63–69	HF, TFMSA, TMSOTf, H$_2$/Pd/C
Boc-Glu(OcHx)-OH	853029	329.4	57–60	HF, TMSOTf
Boc-Gly-OH	853000	175.2	80–88	
Boc-His(Dnp)-OH·IPA	853008	481.5	105–110	Thiophenol
Boc-His(Tos)-OH	853041	409.5	130–140 (dec)	HF, TFMSA
Boc-His(Trt)-OH	853034	497.6	123-133	TFA
Boc-Hyp-OH	853026	231.3	119–125	
Boc-Ile-OH·1/2H$_2$O	853047	240.3	50–60	
Boc-Leu-OH H$_2$O	853002	249.3	74–82	
Boc-Lys(2-Cl-Z)-OH	853018	414.9	63–75	HF, TFMSA, TMSOTf, H$_2$/Pd/C
Boc-Lys(Z)-OH	853012	380.4	75–85	HF, TFMSA, TMSOTf, H$_2$/Pd/C
Boc-Lys(Fmoc)-OH	853019	468.6	75–85	20% Piperidine in DMF
Boc-Met-OH	853054	249.3	47–51	
Boc-Nle-OH·DCHA	853060	412.6		
Boc-Nva-OH	853027	217.3	Oil	
Boc-Orn(Z)-OH	853025	366.4	85–90	HF, TFMSA, TMSOTf, H$_2$/Pd/C
Boc-Phe-OH	853006	265.3	80–88	

Product name	Catalog number	M.W.	Mp(°C)	Side-chain deprotection
Boc-Pro-OH	853003	215.3	130-138	
Boc-Ser(Bzl)-OH	853009	295.3	60-70	HF, TFMSA, TMSOTf, H_2/Pd/C
Boc-Thr(Bzl)-OH	853004	309.4	102-116	HF, TFMSA, TMSOTf, H_2/Pd/C
Boc-Trp-OH	853038	304.4	135-145	
Boc-Trp(For)-OH	853022	332.4	115-130 (dec)	10% Piperidine in DMF
Boc-Tyr(Bzl)-OH	853056	371.4	104-110	HF, TFMSA, TMSOTf, H_2/Pd/C
Boc-Tyr(2-Br-Z)-OH	853024	494.3	90-108	HF, TFMSA, TMSOTf, H_2/Pd/C
Boc-Tyr(2,6-di-Cl-Bzl)-OH	853024	440.3	100-110	HF, TFMSA, TMSOTf, H_2/Pd/C
Boc-Val-OH	853011	217.3	75-82	

Fmoc-amino acids

Product name	Catalog number	M.W.	Mp(°C)	Side-chain deprotection
Fmoc-Ala-OH	852003	311.3	140-150	
Fmoc-ßAla-OH	852024	311.3	143-147	
Fmoc-Arg(Pbf)-OH	852067	648.8	60-125 (dec)	TFA
Fmoc-Arg(Pmc)-OH	852034	662.8	70-120 (dec)	TFA
Fmoc-Asn-OH	852203	354.4	175-190	
Fmoc-Asn(Trt)-OH	852044	596.7	209-220	TFA
Fmoc-Asp-OtBu	852037	411.5	90-98	TFA
Fmoc-Asp(OtBu)-OH	852005	411.5	143-150	TFA
Fmoc-Cha-OH	8520381	393.5	125-130	
Fmoc-Cys(Acm)-OH	852006	414.5	145-153	Hg^{2+}, Ag^+, I_2, Tl^{3+}
Fmoc-Cys(tBu)-OH	852007	399.5	133-136	Hg^{2+}
Fmoc-Cys(tButhio)-OH	852022	431.6	69-79	Bu_3P, RSH
Fmoc-Cys(STmp)-OH	852373	541.1	nd	RSH, R_3P
Fmoc-Cys(Trt)-OH	852008	585.7	164-175	TFA
Fmoc-Gln-OH	852205	368.4	220-225	
Fmoc-Gln(Trt)-OH	852045	610.7	110-125 (dec)	TFA
Fmoc-Glu-OtBu	852035	425.5	105-110	TFA
Fmoc-Glu(OtBu)-OH	852009	425.5	80-95	TFA
Fmoc-Gly-OH	852001	297.3	168-175	
Fmoc-His(Trt)-OH	852032	619.7	130-145 (dec)	TFA
Fmoc-Hyp-OH	852033	353.4	189-193	
Fmoc-Hyp(tBu)-OH	852036	457.2	60-70	TFA
Fmoc-Ile-OH	852010	353.4	143-145	
Fmoc-Leu-OH	852011	353.4	145-153	
Fmoc-Lys(Boc)-OH	852012	468.5	123-130	TFA

Molecular weights and melting point data for commonly used Boc and Fmoc amino acids

Product name	Catalog number	M.W.	Mp(°C)	Side-chain deprotection
Fmoc-Lys(ivDde)-OH	852082	574.6		1–2% Hydrazine in DMF
ivDde-Lys(Fmoc)-OH	852370	574.6		1–2% Hydrazine in DMF
Dde-Lys(Fmoc)-OH	854000	532.6	>70 (dec)	1–2% Hydrazine in DMF
Fmoc-Lys(Fmoc)-OH	852041	590.7	170-180	20% Piperidine in DMF
Fmoc-Lys(Tfa)-OH	852040	464.4	150-160	aq. Piperidine
Fmoc-Met-OH	852002	371.5	130-140	
Fmoc-Nle-OH	852014	353.4	135-142	
Fmoc-Nva-OH	852047	339.4	143-150	
Fmoc-Orn(Boc)-OH	852015	454.5	100-115	TFA
Fmoc-Phe-OH	852016	387.4	180-188	
Fmoc-Pro-OH	852017	337.4	100-110	
Fmoc-Ser(tBu)-OH	852019	383.4	123-130	TFA
Fmoc-Ser(Trt)-OH	852046	569.7	200-210	1% TFA in DCM
Fmoc-Sta-OH	852026	397.5	92-94	
Fmoc-Thi-OH	852039	393.4	170-180	
Fmoc-Thr(tBu)-OH	852000	397.5	125-135	TFA
Fmoc-Trp-OH	852207	426.5	175-185	
Fmoc-Trp(Boc)-OH	852050	526.6	>70 (dec)	TFA
Fmoc-Tyr(tBu)-OH	852020	459.6	143-155	TFA
Fmoc-Val-OH	852021	339.4	140-145	

ALPHABETICAL INDEX

Micron (μm)	Mesh
297	50
250	60
210	70
177	80
149	100
125	120
105	140
88	170
74	200
63	230
53	270
44	325
37	400

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