letters 2.Vol.13

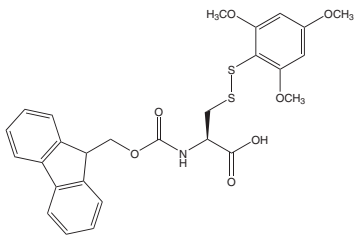
Novabiochem®
NEW • NEW • NEW

- Orthogonally protected cysteine derivative
- γ-Carboxyglutamic acid
- Selenomethionine
- Sulfophenylalanine
- N-ε-isopropyl-lysine
- High-purity 20 and 30 kDa MPEGs

NEW Derivatives for Fmoc SPPS

NEW • Orthogonally protected cysteine derivative

Fmoc-Cys(STmp)-OH



Features & Benefits

- Ideal tool for the synthesis peptides containing multiple disulfide bridges
- Cys(STmp) is orthogonal to Cys(Mmt) and Cys(Trt)
- STmp group is stable to piperidine but removed in 5 min upon treatment with mercaptoethanol or DTT, enabling selective disulfide bridge formation on the solid phase

The unequivocal synthesis of peptides containing multiple disulfide bridges involves step-wise formation of each individual disulfide bond. This approach necessitates the use of pairs of orthogonally protected Cys residues, which can be sequentially deprotected and oxidized without effecting the other cysteine and cystine residues [1]. However, there is a lack of orthogonal protecting group combinations for cysteine protection, with many of the available protecting groups, such as 4-methoxytrityl (Mmt), trityl (Trt), and t-butyl, requiring selective removal by graduated acidolysis. One of the few that is truly orthogonal is the t-butylsulfenyl (tButhio) group [2]. It is stable to acid and piperidine, making it compatible with Fmoc SPPS, but is cleaved by reduction with thiol or phosphines. Unfortunately removal of this group is very sluggish (4 - 24h) [3], which significantly limits its utility in routine synthesis. Frequently incomplete deprotection [4] or desulfurization [5] during the extended exposure to reducing agents is observed.

The novel derivative Fmoc-Cys(STmp)-OH developed by Albericio [3] overcomes these limitations. Like tButhio, the 2,4,6-trimethoxyphenylsulfenyl (STmp) group is stable to piperidine but, in contrast, is extremely easily removed by mild thiolysis. 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% dithiothreitol (DTT).

In the synthesis of peptides containing multiple disulfide bridges by an orthogonal protecting group strategy, deprotection of the Cys(STmp) residues must be done first since the conditions required for this step will cause reduction of any disulfide bridges already present in the peptide. This is best carried out before the peptide is cleaved from the resin as STmp is slightly labile to TFA. The pseudodilution effect can then be exploited during on-resin oxidation, or alternatively the peptide can be cleaved from the resin and cyclized in dilute solution.

Albericio and coworkers have recently described a synthesis of SI conotoxin by sequential disulfide bond formation on the solid phase utilizing a combination of STmp and Mmt cysteinyl protection [6]. Removal of STmp from the side-chains of Cys and Cys with DTT followed by mild oxidation with N-chlorosuccinimide (NCS) in DMF afforded the first disulfide bridge. The second bridge was introduced by treatment with 2% TFA in DCM to remove the Mmt protection from Cys and Cys and subsequent oxidation with NCS. Interestingly, oxidation with NCS is so rapid that no scrambling of the existing disulfide bond was observed following removal of the Mmt protection. NCS has been recently described as a novel reagent for on-resin disulfide bond formation. This reagent effects complete oxidation in minutes, without affecting Trp residues. Some oxidation of Met is observed but this can be kept to a minimum by using the reagent in slight excess.

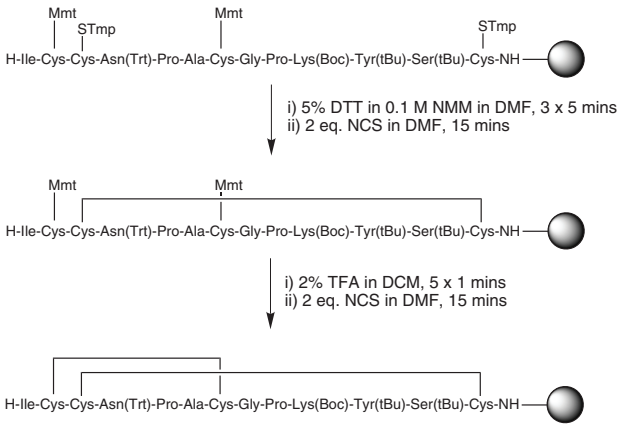
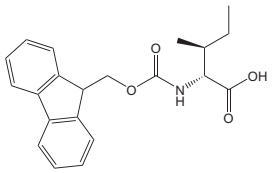


Figure 1: Synthesis of SI conotoxin [6].

Cat.No.	Product	Contents	Price EUR
852373	Fmoc-Cys(STmp)-OH	1 g	200.00
NEW		5 g	800.00
Other protected cysteine derivatives			
852006	Fmoc-Cys(Acm)-OH	5 g	42.00
		25 g	149.00
		100 g	440.00
852007	Fmoc-Cys(tBu)-OH	5 g	47.00
		25 g	198.00
		100 g	595.00
852022	Fmoc-Cys(tButhio)-OH	5 g	109.00
		25 g	436.00
852031	Fmoc-Cys(Mmt)-OH	1 g	50.00
		5 g	185.00
		25 g	695.00
852008	Fmoc-Cys(Trt)-OH	25 g	60.00
		100 g	180.00
		250 g	395.00

• Other new Fmoc-amino acid derivatives

Fmoc-D-Ile-OH

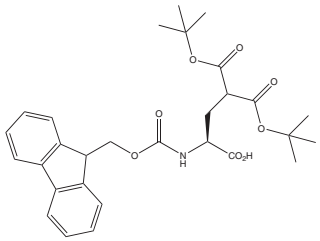


The introduction of Fmoc-D-Ile-OH completes our range of Fmoc-protected D-isomers of the 20 proteinogenic amino acids.

Cat.No.	Product	Contents	Price EUR
852374	Fmoc-D-Ile-OH	1 g	250.00
NEW			
Other Fmoc-protected D-amino acids			
852142	Fmoc-D-Ala-OH	5 g	50.00
		25 g	200.00
852165	Fmoc-D-Arg(Pbf)-OH	1 g	65.00
		5 g	260.00
852159	Fmoc-D-Asn(Trt)-OH	1 g	50.00
		5 g	200.00
		25 g	800.00
852154	Fmoc-D-Asp(OtBu)-OH	1 g	44.00
		5 g	172.00
		25 g	688.00
852143	Fmoc-D-Cys(Trt)-OH	1 g	35.00
		5 g	140.00
852155	Fmoc-D-Glu(OtBu)-OH	1 g	44.00
		5 g	172.00
		25 g	688.00
852160	Fmoc-D-Gln(Trt)-OH	1 g	50.00
		5 g	200.00
		25 g	800.00
852161	Fmoc-D-His(Trt)-OH	1 g	44.00
		5 g	172.00
852145	Fmoc-D-Leu-OH	5 g	65.00
		25 g	260.00
852146	Fmoc-D-Lys(Boc)-OH	1 g	35.00
		5 g	140.00
852140	Fmoc-D-Met-OH	5 g	65.00
		25 g	260.00
852148	Fmoc-D-Phe-OH	5 g	50.00
		25 g	200.00
852149	Fmoc-D-Pro-OH	1 g	23.00
		5 g	69.00
		25 g	352.00

852156	Fmoc-D-Ser(tBu)-OH	1 g	44.00
		5 g	172.00
		25 g	688.00
852157	Fmoc-D-Thr(tBu)-OH	1 g	44.00
		5 g	172.00
		25 g	688.00
852164	Fmoc-D-Trp(Boc)-OH	1 g	65.00
		5 g	260.00
852151	Fmoc-D-Tyr(tBu)-OH	1 g	50.00
		5 g	200.00
		25 g	800.00
852152	Fmoc-D-Val-OH	5 g	50.00
		25 g	200.00

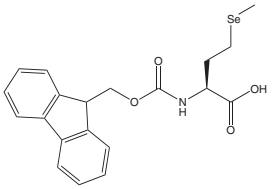
Fmoc-Gla(OtBu)$_2$-OH



Fmoc-Gla(OtBu)$_2$-OH is a building block for the introduction of γ-carboxy-glutamic 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 [7]. Carboxylation of glutamic acid is mediated by γ-glutamyl carboxylase in the presence of vitamin K1. Addition of the carboxyl group facilitates chelation of calcium ions, which is thought to be the trigger for biological activity of most Gla-containing peptides and proteins.

Cat.No.	Product	Contents	Price EUR
852345	Fmoc-Gla(OtBu)$_2$-OH	100 mg	150.00
NEW		500 mg	600.00

Fmoc-selenomethionine-OH

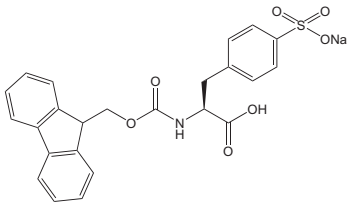


Selenomethionine is an analog of methionine in which the sulfur atom is replaced by selenium. Substitution of methionine by selenomethionine has little influence on biological activity but the introduction of a selenium atom into a peptide can help facilitate structural and biological studies. The presence of selenium enables multiwavelength anomalous diffraction (MAD) to be used to overcome the phase problem in X-ray crystallography and can greatly simplify the solution of three-dimensional structures [8]. The nucleus of selenium-77 has a spin 1/2 which allows ^{77}Se NMR spectroscopy for probing solution structures. Furthermore, selenomethionine is very effective at quenching Trp fluorescence, making selenomethionine-containing peptides useful tools for studying peptide-protein interactions.

Selenomethionine can be introduced in peptides using Fmoc-selenoMet-OH under standard conditions. Any selenoxide formed during synthesis can be easily reduced back to selenide by treatment with β-mercaptoethanol [8].

Cat.No.	Product	Contents	Price EUR
852372	Fmoc-L-selenomethionine-OH	1 g	300.00
NEW			
Other selenoamino acids			
852346	Fmoc-Sec(pMeOBzl)-OH	250 mg	270.00
		1 g	810.00

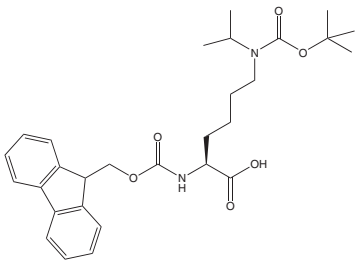
Fmoc-Phe(SO$_3$Na)-OH



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 [9], phytosulfokine [10], and cyclolinopeptide A [11]. 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.

Cat.No.	Product	Contents	Price EUR
852378	Fmoc-Phe(SO$_3$Na)-OH	1 g	200.00
NEW		5 g	800.00
Other sulfoamino acids			
852347	Fmoc-Tyr(SO$_3$nP)-OH	1 g	185.00
		5 g	750.00
852103	Fmoc-Tyr(SO$_3$ · NnBu$_4$)-OH	1 g	170.00
		5 g	680.00

Fmoc-Lys(iPr,Boc)-OH

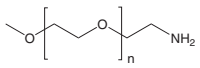


Fmoc-Lys(iPr,Boc)-OH is used for incorporation of N-δ-isopropyl-lysine (Lys(iPr)) during Fmoc SPPS. Lys(iPr) has been utilized in the GnRH antagonist Degarelix and the LHRH antagonist Antide.

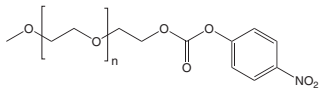
Cat.No.	Product	Contents	Price EUR
852376	Fmoc-Lys(iPr,Boc)-OH	1 g	350.00
NEW		5 g	1400.00
Other monoalkylated lysine derivatives			
852106	Fmoc-Lys(Me,Boc)-OH	500 mg	490.00
		1 g	780.00

NEW High purity PEG derivatives

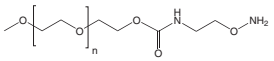
MPEG-ethylamine



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 [12].

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.

Compounds are available in cGMP quality upon request.

Cat.No.	Product	Contents	Price EUR
851216	MPEG-20kDa ethylamine	1 g	200.00
NEW			
851217	MPEG-20kDa oxyamine	1 g	250.00
NEW			
851218	MPEG-20kDa pNPC	1 g	200.00
NEW			
851219	MPEG-30kDa ethylamine	1 g	200.00
NEW			
851220	MPEG-30kDa oxyamine	1 g	290.00
NEW			

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. U. Weber & P. Hartter (1970) *Hoppe-Seyler's Z. Physiol. Chem.*, **351,** 1384.
3. T. M. Postma, et al. (2012) *Org. Lett.*, **14**, 5468.
4. B. Dennis & E. Triflieff (2000) *J. Pept. Sci.*, **6**, 372.
5. D. T. S. Rijkers, et al. (2005) *Tetrahedron Lett.* **46**, 3341.
6. T. M. Postma & F. Albericio (2013) *Org. Lett.*, **15**, 616.
7. P. K. Bandyopadhyay (2002) *Proc. Nat. Acad. Sci. USA*, **99**, 1264.
8. L. Moroder (2005) *J. Pept.Sci.* **11**, 187.
9. E. Escher, et al. (1983) *Helv. Chim. Acta 66*, 1355.
10. A. Bahyrycz, et al. (2004) *J. Pept. Sci.* **10**, 462.
11. Y. Fujiwara, et al. (1994) *Chem. Pharm. Bull.*, **42**, 724.
12. G. Pasut, et al. (2004) *Expert Opin. Ther. Patents*, **14**, 859.

For more information please contact our local offices:

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Germany: 0800 6931 000
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Spain: 00800 1166 8811
Switzerland: 00800 1166 8811
United Kingdom: 0800 622935

For other countries across Europe, please call:
+44 (0) 115 943 0840

For Technical Service contact:
technical@novabiochem.com

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