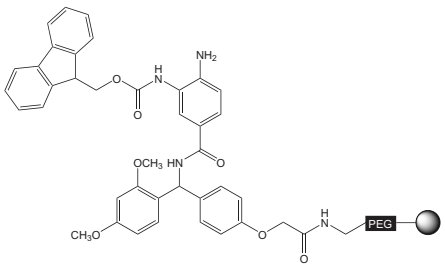
Novabiochem® Letters: 2/11

NEW Resin for the synthesis of peptide thioesters

Dawson Dbz NovaSyn®TGR resin



Features & Benefits

- Novel safety catch resin for synthesis of thioesters by Fmoc SPPS
- Activated peptide cleaved with TFA, allowing easier monitoring of activation process compared to sulfamylbutyryl resins
- Thioester generated in situ from activated peptide in native chemical ligation reaction

The synthesis of thioesters by Fmoc methods can not be effected directly by solid phase synthesis, owing to the instability of thioesters to piperidine. They are generally prepared using a safety catch approach on a sulfamylbutyryl resin by displacement of the peptide fragment from the activated resin with a thiol [1]. However, this approach suffers from poor yields and difficulties associated with loading of the linker that have led to the development of a number of alternative strategies [2]. One of the most promising of these is the

Inside this issue

Merck Millipore is a division of MERCK

method of Dawson, which is based on a diaminobenzoyl (Dbz) linker attached to Rink Amide AM resin (Figure 1) [3]. Peptide synthesis is performed on one of the linker amino groups. Prior to release of the peptide from the support, the linker is converted by treatment with *p*-nitrophenyl chloroformate to the corresponding imidazolinone (Nbz) (Method 1). Cleavage with TFA releases the unprotected peptide bearing at its C-terminus the mildly activating Nbz group. This moiety can be displaced with a thiol to give a thioester either prior to, or *in situ* during, native chemical ligation reactions.

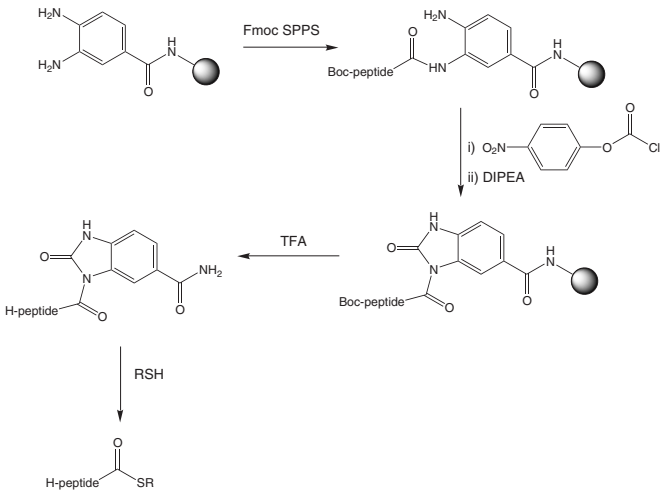


Fig. 1: Synthesis of peptide thioesters using Dawson Dbz AM resin.

The Novabiochem® brand is pleased to expand our range of Dbz resins with the introduction of Dawson Dbz NovaSyn®TGR resin. Based on the PEG-PS-based NovaSyn®TGR resin, this resin is suitable for the synthesis by both batch and continuous flow Fmoc SPPS. In our hands, this resin gives cleaner products after cyclization with *p*-nitrophenyl chloroformate than the more densely loaded Dawson Dbz AM resin, presumably due to less cross-linking between Dbz moieties.

Mild acylation methods should be employed for chain extension to avoid formation of branched peptides by unwanted acylation of the unprotected Dbz amino functionality. Generally, coupling with HBTU/HOBt/DIPEA or DIPCI/HOBt works well. Branching due to double addition of Gly has been observed, particularly where it is located close to the C-terminus of the peptide. In such cases, Fmoc-Gly-OPfp/HOBt should be used for introduction of this residue. Recently, the Dbz linker has been successfully used to prepare a number of peptides and small proteins [4].

Method 1: Thioester ligation with Dawson Dbz NovaSyn®TGR resin

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. Add Fmoc-Aaa-OH (0.6 mmole), HCTU (0.6 mmole) and DIPEA (0.9 mmole). For Ile, Val, Thr, Pro, Arg, use HATU in place of HCTU and double couple. For Gly, use Fmoc-Gly-OPfp (0.4 mmole) and HOBt (0.4 mmol) to avoid double addition of Gly.
3. Agitate gently for 1 h.
4. Check loading using Method 3-6, p. 3.6 of the 2010/2011 Novabiochem catalog.

Synthesis & Activation

1. Following SPPS under normal conditions, the N-terminal residue is introduced using a Boc-amino acid and then the resin is washed with DMF and DCM.
2. Add *p*-nitrophenyl chloroformate (0.5 mmole) in DCM and leave to gently agitate under N_2 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.

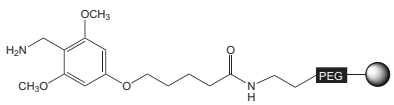
Ligation of unprotected peptide fragments

1. Dissolve peptidyl 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 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.

855142	Dawson Dbz NovaSyn® TGR resin	1 g
NEW		5 g
855131	Dawson Dbz AM resin	1 g
		5 g
		25 g

NEW Resins for Fmoc SPPS of peptide amides

PAL NovaPEG resin



Features & Benefits

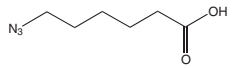
- Resin is loaded with C-terminal residue using standard coupling methods
- Treatment with 95% TFA releases peptide amides
- Gives higher yields in microwave-assisted SPPS than Rink amide-based resins

PAL resins are excellent supports for the synthesis of peptide amides by Fmoc SPPS. They consist of Barany's aminomethyldimethoxyphenoxy-valeric acid linker [5] attached to aminomethylated polystyrene or 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 [6]. There is some evidence to suggest that PAL resins give greater yields in microwave assisted synthesis.

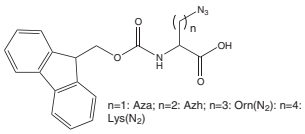
855136	PAL-NovaPEG resin	1 g
NEW		5 g
855133	Fmoc-PAL AM resin	1 g
		5 g
855137	PAL NovaSyn® TG resin	1 g
		5 g

NEW Orthogonally protected Fmoc amino acid building blocks

ε-Azidocaproic acid



Fmoc-L-β-azidoalanine/Fmoc-γ-azidohomoalanine/ Fmoc-L-δ-azidonovaline/Fmoc-ε-azidonorleucine



n=1: Aza; n=2: Azh; n=3: Orn(N_2): n=4: Lys(N_2)

Features & Benefits

- Azido group orthogonal to standard protecting groups
- Azido group transformed to amine under mild conditions
- Building blocks compatible with standard Fmoc SPPS methods

The Novabiochem® brand has one of the largest collections of orthogonally- and selectively-protected amino acid derivatives available. These reagents provide the capability to selectively unmask a single functionality of a peptide without affecting others, thus enabling the facile synthesis of cyclic, branched and side-chain modified peptides.

For the incorporation of differentially-protected amino groups within a peptide sequence, we offer derivatives bearing protecting groups cleavable with reagents ranging from mild acids, Pd(0) and alpha-effect nucleophiles, such as hydrazine and hydroxylamine (Table 1). With the introduction of our latest building blocks for Fmoc SPPS, ε-azidocaproic acid, Fmoc-L-β-azidoalanine, Fmoc-L-γ-azidohomoalanine, Fmoc-L-δ-azidonorvaline and Fmoc-L-ε-azidonorleucine, the range of available deprotection chemistries has now been expanded to include reduction. These derivatives can be coupled using standard activation methods, and as the azido group is stable to both TFA and piperidine, they can be used in both solid phase and solution phase synthetic strategies. However, it should be noted that the use of thiols in the TFA cleavage mixture should be avoided as this has been shown to lead to azide reduction [7]. Liberation of the amine group from the azide has been accomplished by reduction with phosphines [8, 9], thiols [10] or Zn/AcOH [11].

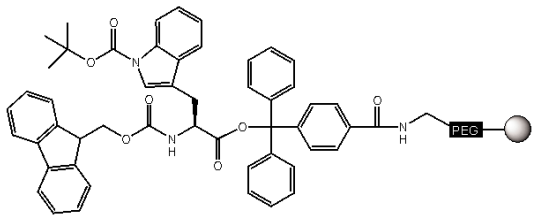
851097	ε-Azidocaproic acid	1 g
NEW		5 g
852320	Fmoc-L-β-azidoalanine	100 mg
NEW		500 mg
852321	Fmoc-L-γ-azidohomoalanine	100 mg
NEW		500 mg
852322	Fmoc-L-δ-azidonorvaline	100 mg
NEW		500 mg
852326	Fmoc-L-ε-azidonorleucine	250 mg
NEW		1 g
852084	Fmoc-Dab(ivDde)-OH	1 g
		5 g
852092	Fmoc-Dab(Mtt)-OH	1 g
		5 g
852083	Fmoc-Dpr(ivDde)-OH	1 g
		5 g
852089	Fmoc-Dpr(Mtt)-OH	1 g
		5 g
852124	Fmoc-Lys(Alloc)-OH	1 g
		5 g
852082	Fmoc-Lys(ivDde)-OH	1 g
		5 g
852094	Fmoc-Lys(Mmt)-OH	1 g
		5 g
852065	Fmoc-Lys(Mtt)-OH	1 g
		5 g
		25 g

Table 1: Selectively cleavable amino protecting groups.

Protecting group	Cleaved on solid phase by:	Stable to:
N-Mtt	1% TFA in DCM; DCM/HFIP/TFE/TES (6.5:2:1:0.5)	piperidine, hydrazine, phosphines,
N-Mmt	1% TFA in DCM; DCM/HFIP/TFE/TES (6.5:2:1:0.5)	piperidine, hydrazine, phosphines,
N-Alloc	3 eq. in $Pd(Ph_3P)_4$ in $CHCl_3$/AcOH/NMM (37:2:1)	piperidine, hydrazine, TFA, phosphines
N-ivDde	2% NH_2NH_2 in DMF	piperidine, TFA, Pd(0), phosphines
N_3	6 eq. Me_3P in aq. dioxane	piperidine, TFA

NEW Pre-loaded NovaSyn®TG resin

Fmoc-Trp(Boc)-NovaSyn®TGT



Fmoc-Trp(Boc)-NovaSyn®TGT resin is the latest addition to our range of pre-loaded NovaSyn TGT resins. These supports are ideal tools for the synthesis of both protected (20% TFE in DCM cleavage) and unprotected peptide acids (95% TFA cleavage) by batch or continuous flow Fmoc SPPS. Fmoc-Pro-NovaSyn®TGT resin and Fmoc-Cys(Trt)-NovaSyn®TGT resin are recommended for the production of peptides containing C-terminal Pro and Cys residues, respectively, as the trityl-based linker protects against diketopiperazine formation and racemization.

856190	Fmoc-Trp(Boc)-NovaSyn®TGT resin	1 g
NEW		5 g

Other NovaSyn®TGT resins

856125	Fmoc-Ala-NovaSyn®TGT resin	1 g
		5 g
856052	Fmoc-Arg(Pbf)-NovaSyn®TGT resin	1 g
		5 g
856126	Fmoc-Asn(Trt)-NovaSyn® TGT resin	1 g
		5 g
856127	Fmoc-Asp(OtBu)-NovaSyn®TGT resin	1 g
		5 g
856044	Fmoc-Cys(Trt)-NovaSyn®TGT resin	1 g
		5 g
856128	Fmoc-Gln(Trt)-NovaSyn®TGT resin	1 g
		5 g
856129	Fmoc-Glu(OtBu)-NovaSyn®TGT resin	1 g
		5 g
856045	Fmoc-Gly-NovaSyn®TGT resin	1 g
		5 g
856046	Fmoc-His(Trt)-NovaSyn®TGT resin	1 g
		5 g
856130	Fmoc-Ile-NovaSyn®TGT resin	1 g
		5 g
856047	Fmoc-Leu-NovaSyn®TGT resin	1 g
		5 g
856048	Fmoc-Lys(Boc)-NovaSyn®TGT resin	1 g
		5 g
856131	Fmoc-Met-NovaSyn®TGT resin	1 g
		5 g
856132	Fmoc-Phe-NovaSyn®TGT resin	1 g
		5 g
856049	Fmoc-Pro-NovaSyn®TGT resin	1 g
		5 g
856133	Fmoc-Ser(tBu)-NovaSyn®TGT resin	1 g
		5 g
856050	Fmoc-Thr(tBu)-NovaSyn®TGT resin	1 g
		5 g
856134	Fmoc-Trp-NovaSyn®TGT resin	1 g
		5 g
856135	Fmoc-Tyr(tBu)-NovaSyn®TGT resin	1 g
		5 g
856051	Fmoc-Val-NovaSyn®TGT resin	1 g
		5 g

References

1. a) R. Ingenito, et al. (1999) *J. Am. Chem. Soc.*, **121**, 11369; b) Y. Shin, et al. (1999) *J. Am. Chem. Soc.*, **121**, 11684.
2. F. Mende & O. Sietz (2011) *Angew. Chem. Int. Ed.*, **50**, 1232.
3. J. B. Blanco-Canosa & P. E. Dawson (2008) *Angew. Chem. Int. Ed.*, **47**, 6851.
4. 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.
5. F. Albericio & G. Barany (1987) *Int. J. Peptide Protein Res.*, **30**, 206
6. M. S. Bernatowicz, et al. (1989) *Tetrahedron Lett.*, **30**, 4645.
7. P. E. Schneggenburger, et al. (2010) *J. Pept. Sci.*, **16**, 10.
8. J. T. Lundquist & J. C. Pelletier (2001) *Org. Lett.*, **3**, 781.
9. N. Nepomniaschiy, et al. (2008) *Org. Lett.*, **10**, 5243.
10. M. Meldal, et al. (1997) *Tetrahedron Lett.*, **38**, 2531.
11. H. Katayama, et al. (2008) *Tetrahedron Lett.*, **49**, 5492.