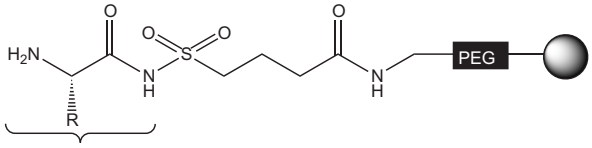
Novabiochem®

Letters: 02/04

Contents

Product Focus: Products for peptide ligation

Pre-loaded sulfamylbutyryl resins



Ala, Gln(Trt), Gly

Features & Benefits

- High and reproducible substitution
- Better quality end-products
- Assurance that the resin is loaded before starting synthesis
- No need for difficult off-instrument chemistry

In contrast to the Boc strategy, the production of thioesters by the Fmoc method can not be effected directly using solid phase synthesis, owing to the instability of thioesters to piperidine. They are instead prepared by thiolytic cleavage from a sulfamylbutyryl resin (Figure 1) [1- 5]. One of the principle difficulties of using this approach is the initial attachment of the first amino acid onto the sulfamylbutyryl linker. Various methods have been described, such as the use of PyBOP in $CHCl_3$ [6] and DIPCDI/N-methylimidazole [7], but none is ideal. Yields are highly variable, problems can occur with overacylation of the sulfamyl group, and the substitution of the support must be determined before starting peptide synthesis.

MERCK

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 and 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. Novabiochem offers resins loaded with Gly, Ala and Gln(Trt); additional products will be added in the near future.

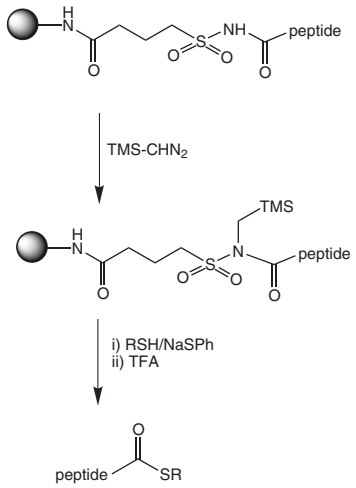


Fig. 1: Synthesis of peptide thioesters using sulfamylbutyryl resin.

Method 1: Thioester synthesis 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

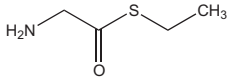
1. Pre-swell activated resin in DMF for 1 h before use.
2. Add ethyl-3-mercaptopropionate (50 eq.) and sodium thiophenoxide (0.5 eq.), cap the syringe and agitate the mixture gently for 24 h.
3. Remove the resin by filtration and wash it three times with DMF.
4. Combine the filtrates and evaporate to 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 dropwise 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.

The supports can be used directly in automated peptide synthesis without modification of existing protocols. Following chain assembly, the linker is most efficiently activated for cleavage by treatment with TMS-CHN_2 [2]. The resulting N-alkyl-N-acylsulfonamide is cleaved by treatment with ethyl mercaptopropionate/sodium thiophenoxide (Method 1). The use of 2M LiBr in THF as the cleavage solvent has been shown to lead to greatly improved yields of peptide thioester [8]. The resulting protected peptide thioester is finally treated with TFA containing the appropriate scavengers to give the deprotected peptide ready for ligation.

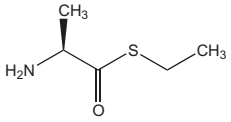
04-12-3715	H-Ala-Sulfamylbutyryl NovaSyn® TG resin	1 g
NEW		5 g
04-12-3717	H-Gln(Trt)-Sulfamylbutyryl NovaSyn® TG resin	1 g
NEW		5 g
04-12-3714	H-Gly-Sulfamylbutyryl NovaSyn® TG resin	1 g
NEW		5 g

Amino acid thioesters

H-Gly-SEt·HCl



H-Ala-SEt·HCl



Features & Benefits

- Ideal for synthesis of peptide thioesters
- No need for thiolytic cleavage
- Higher yields than thiolytic cleavage

Amino acid thioesters are useful intermediates for the synthesis of peptide thioesters (Figure 2). They can be employed as nucleophiles to effect cleavage from sulfamylbutyrl resins and hydrazinobenzoyl resins [9]. Yields are often higher than with similar approaches involving direct thiolysis, such as that described in Figure 1, as cleavage involves attack by an amine rather than thiol.

Amino acid thioesters have also been used to prepare peptide thioesters on solid phase by the BAL approach [10].

04-12-5319	H-Ala-SEt·HCl	1 g
NEW		5 g
04-12-5318	H-Gly-SEt·HCl	1 g
NEW		5 g

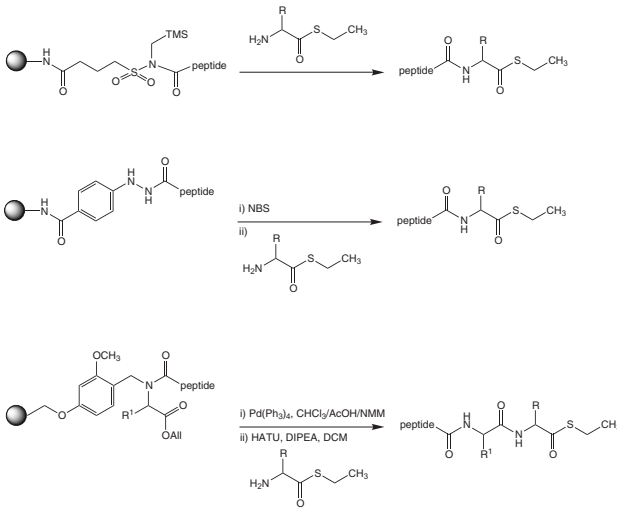
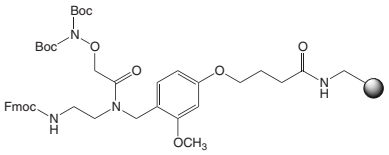


Fig. 2: Synthesis of peptide thioesters using amino acid thioesters.

Hydroxylamine NovaTag™ resin



Features & Benefits

- Direct synthesis of hydroxylamine-labeled peptides
- Compatible with standard Fmoc protocols
- Ideal for synthesis of ligands for peptide arrays

Hydroxylamine NovaTag™ resin is an excellent tool for the synthesis of C-terminally hydroxylamine-modified peptides. Such peptides readily undergo oxime ligation in aqueous media with biomolecules or surfaces modified with aldehyde groups. Typical applications include peptide-MAPs; branched and cyclic peptides; peptide-protein, peptide-DNA, glycan-peptide conjugates; and peptide arrays (Figure 3).
Following removal of the Fmoc group with piperidine in DMF, addition of the first residue to the primary amine can be accomplished using standard acylation methods such as PyBOP or TBTU. Cleavage with 95% TFA furnishes the fully deprotected hydroxylamine-labeled peptide.

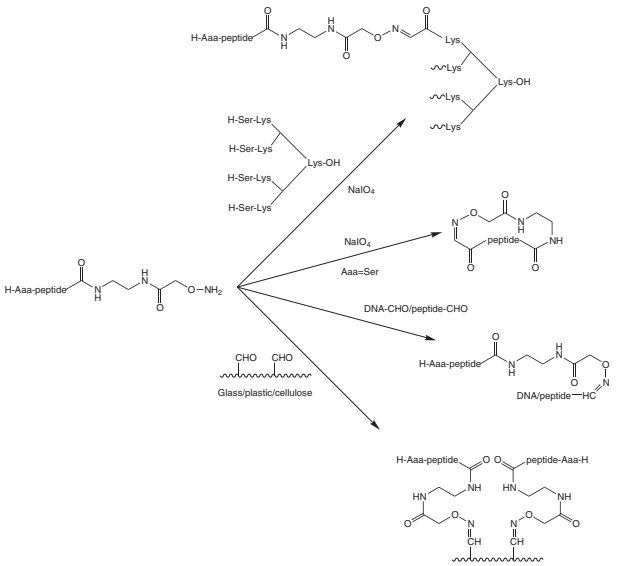
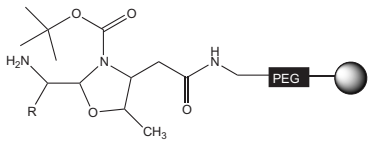


Fig. 3: Applications of peptide hydroxylamines. Note: in the examples given Ser is used as aldehyde precursor.

04-12-3909	**Hydroxylamine NovaTag™ resin**	0.5 g
NEW	Patent pending	1 g

NEW Pre-loaded resins for the synthesis of aldehydes



Features & Benefits

- Peptide aldehydes without complex off-instrument chemistry
- Compatible with standard Fmoc protocols
- Ideal for high-throughput synthesis of peptide aldehyde arrays

Peptide aldehydes are potent inhibitors of serine, aspartyl and cysteinyl proteases and are valuable intermediates for the preparation of reduced amide-bond peptidomimetics. Peptides containing C-terminal aspartinal, leucinal and phenylalaninal are of particular interest as they are active against therapeutically important targets such as caspases [11] or the chymotrypsin-like activity of the proteasome [12].
The classical methods for preparing peptide aldehydes, such as oxidation of a peptide alcohol [13], reduction of a peptide Weinreb amide [14, 15] or step-wise or fragment synthesis using a protected pre-formed aldehyde [16 - 20], can be very laborious and time-consuming. Novabiochem's pre-loaded aldehyde resins, which are based on the work of Ede, et al. [21], offer a simple and convenient alternative. The resins are loaded in the automated synthesizer, and peptide synthesis is carried out using standard instrument protocols. Cleavage from the resin and side-chain deprotection is carried out in two stages. Firstly, side-chain protecting groups are first removed with anhydrous TFA. For $Arg(Boc)_2$, a cleavage time of at least 2 hours should be used to ensure complete removal of both Boc groups. Secondly, final cleavage is carried out by treatment with either 0.1% TFA in MeCN:water (60:40 v/v) at 60 °C for 1 h [21] or AcOH/water/DCM/MeOH (10:5:63:21) for 3 times 30 min [22]. This two stage approach enables all by-products from

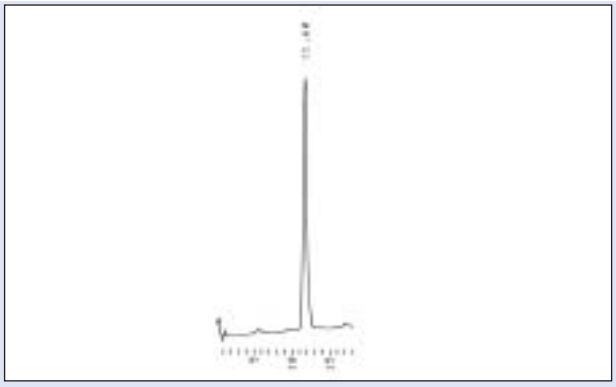
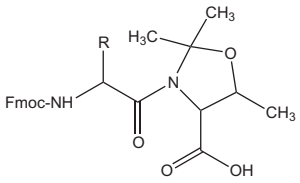


Fig. 4: HPLC elution profile of crude Fmoc-Lys(Fmoc)-Leu-Phe-H prepared with H-Phe-H aldehyde resin. (Conditions: Merck Chromolith Speed Rod; Gradient: 35%-97% B in 15 min, 2 ml/min; A: 0.1 TFA aq.; B: 0.1% TFA in acetonitrile.

side-chain deprotection to be removed by washing before the product is released into aqueous solution.
Novabiochem presently offers this support pre-loaded with aldehydes of Arg, Asp, Leu and Phe. Others will be added to the range in the near future. These resins are ideal for the synthesis of libraries for protease inhibitor optimization.

04-12-3723	H-Arg(Boc)$_2$-H NovaSyn® TG resin	1 g
NEW		5 g
04-12-3722	H-Asp(OtBu)-H NovaSyn® TG resin	1 g
NEW		5 g
04-12-3720	H-Leu-H NovaSyn® TG resin	1 g
NEW		5 g
04-12-3721	H-Phe-H NovaSyn® TG resin	1 g
NEW		5 g

NEW Pseudoproline dipeptides



Pseudoproline dipeptides are excellent tools for preventing aggregation during the assembly of peptides by the Fmoc methodology [23]. Not only does their use aid in the synthesis of difficult sequences but it also appears to offer remarkable benefits in the synthesis of long and complex peptides [24].

05-20-1008	Fmoc-Asn(Trt)-Thr($\psi^{Me,Me}$pro)-OH	1 g
NEW		5 g
05-20-1013	Fmoc-Trp(Boc)-Thr($\psi^{Me,Me}$pro)-OH	1 g
NEW		5 g

References

1. Y. Shin, et al. (1999) *J. Am. Chem. Soc*., **121**, 11684.
2. R. Ingenito, et al. (1999) *J. Am. Chem. Soc*., **121**, 11369.
3. S. Biancalana, et al. (2001) *Lett. Pept. Sci*., **7**, 291.
4. S. F. Escher, et al. (2001) *Lett. Pept. Sci*., **8**, 349.
5. D. Fattori, et al. (2002) *Bioorg. Med. Chem. Lett*., **12**, 1143.
6. B. J. Backes & J. A. Ellman (1999) *J. Org. Chem*., **64**, 2322.
7. J. Beythien, personal communication.
8. R. Quaderer & D. Hilvert (2001) *Org. Lett*., **3**, 3181.
9. J. A. Camarero, et al. (2003) *Biopolymers*, **71**, 363 (P274).
10. J. Alsina, et al. (1999) *J. Org. Chem*., **64**, 8761.
11. Z. Han, et al. (1997) *J. Biol. Chem*., **272**, 13432.
12. G. Fenteany & S. L. Schreiber (1996) *Chemistry & Biology*, **3**, 905.
13. J.-T. Woo, et al. (1995) *Bioorg. Med. Chem. Lett*., **5**, 1501.
14. G. Guichard, et al. (1993) *Pept. Res*., **6**, 121.
15. J.-A. Fehrentz, et al. (1995) *Tetrahedron Lett*., **36**, 7871.
16. R. M. McConnell, et al. (1993) *J. Med. Chem*., **36**, 1084.
17. K. T. Chapman (1992) *Bioorg. Med. Chem. Lett*., **2**, 613.
18. A. M. Murphy, et al. (1992) *J. Am. Chem. Soc*., **114**, 3156.
19. C. Pothion, et al. (1997) *Tetrahedron Lett*., **38**, 7749.
20. F. Guillaumie, et al. (2000) *Tetrahedron Lett*., **41**, 6131.
21. N. J. Ede, et al. (2000) *J. Pept. Sci*., **6**, 11.
22. S. Barthélémy, et al. Poster presented at abc Technologies 2004, Basel.
23. M. Mutter, et al. (1995) *Pept. Res*., **8**, 145.
24. P. White, et al. (2004) *J. Peptide Sci*., **10**, 18.

Merck Biosciences AG · Switzerland
Weidenmattweg 4
4448 Läufelfingen
Phone +41 (62) 285 2525
Fax +41 (62) 285 2520

www.novabiochem.com