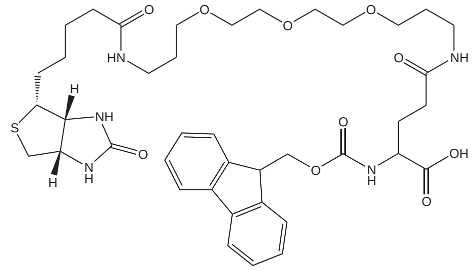
novabiochem letter 2/03

Contents

NEW Solubilizing biotinylating reagent

Fmoc-Glu(biotinyl-PEG)-OH



Biotin-labeled peptides have many important applications in immunology and histochemistry, such as affinity purification [1], FRET-based flow cytometry [2], solid-phase immunoassays [3], and receptor localization [4], that exploit the high affinity of streptavidin and avidin for biotin.

The solid phase synthesis of biotinylated peptides is often hampered by the poor solubility of many of the commonly used biotin derivatives, such as biotin-OSu or Fmoc-Lys(biotin)-OH. This means that they have to be used in low concentrations, which can have deleterious effects on coupling rates. Furthermore, the biotinylated products often exhibit low solubilities, which can complicate subsequent biological testing.

Fmoc-Glu(biotinyl-PEG)-OH should help alleviate many of these difficulties. The introduction of the PEG spacer between the amino acid and the biotin confers on the derivative excellent solubility in

DMF. Peptides incorporating this amino acid are expected to have improved solubilities. The PEG spacer should reduce steric hindrance between the peptide and avidin, leading to better biotin binding. Furthermore, the hydrophilic nature of the PEG should prevent the spacer group from becoming buried in the hydrophobic pocket of target proteins.

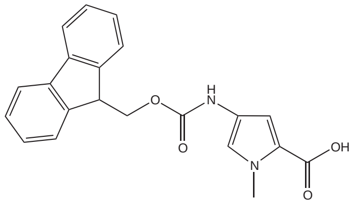
04-12-1250	**Fmoc-Glu(biotinyl-PEG)-OH**	0.5 g
NEW		1 g

Novabiochem's other biotinylating reagents

04-12-1243	**Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH**	0.5 g 1 g
04-12-1237	**Fmoc-Lys(biotin)-OH**	0.5 g 1 g
01-63-0116	**Biotin-ONp**	1 g
01-63-0106	**Biotin-OSu**	1 g

NEW Building blocks

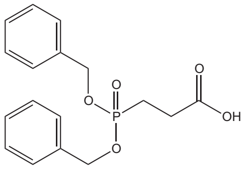
Fmoc-4-amino-1-methylpyrrole-2-carboxylic acid



This novel building block is designed for the Fmoc SPPS of polypyrroleamides and their peptide conjugates [5, 6]. Such compounds have attracted considerable interest in recent years, owing to their ability to modulate gene expression through highly selective binding to specific DNA sequences [5 - 9]. Coupling of the derivative can be effected using standard Fmoc activation methods, such as PyBOP or TBTU, although longer reaction times are required than with standard amino acids [5]. The quantitative Fmoc test should be used to monitor the coupling to aminopyrrolecarboxylic acid residues as the ninhydrin test is not compatible with the aromatic pyrrole amine [6].

04-12-1240	**Fmoc-4-amino-1-methylpyrrole-2-carboxylic acid**	1 g
NEW		

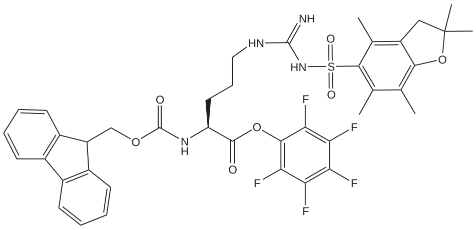
Dibenzyl-phosphonopropionic acid



This novel derivative can be introduced using standard coupling methods, such as PyBOP or DIPCDI/HOBt activation. Removal of the benzyl protecting groups can be effected by treatment with TFA or by hydrogenolysis.

04-19-0127	**Dibenzyl-phosphonopropionic acid**	1 g
NEW		

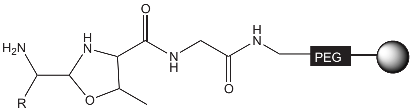
Fmoc-Arg(Pbf)-OPfp



Novabiochem is pleased to offer the Pfp active ester of this popular arginine derivative [10].

04-12-1544	**Fmoc-Arg(Pbf)-OPfp**	1 g
NEW		5 g

NEW Resins for the 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. Peptides containing C-terminal aspartinal or leucinal and phenylalaninal are particularly interesting as they are active against therapeutically important targets such as caspases [11] or the chymotrypsin-like activity of the proteasome [12].

One of the simplest approaches to the solid phase synthesis of peptide aldehydes involves immobilization of a pre-formed Fmoc-amino aldehyde to an amino alcohol linker *via* oxazolidine formation [13]. The linker is most conveniently supplied by an N-terminal Thr residue attached *via* an acid-stable bond to a water compatible resin, i.e. H-Thr-Gly-NovaSyn TG resin.

Novabiochem presently offers this support pre-loaded with aldehydes of Asp, Leu and Phe. Others will be added to the range in the near futures. These resins are ideal for use in library synthesis, making them perfect tools for the identification of optimized protease inhibitors.

The resin-bound oxazolidine is stable to base and so is compatible with Fmoc protocols. There is, however, the possibility to acylate the nitrogen of the oxazolidine during chain extension, but it is relatively unreactive and no acylation has been reported with DIPCDI/HOBt activation.

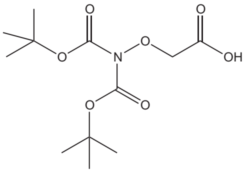
Cleavage from the resin and side-chain deprotection is carried out in two stages. Side-chain protecting groups are first removed with anhydrous TFA, prior to final cleavage with 0.1% TFA in MeCN:water (60:40 v/v) at 60 °C for 1 h. This approach enables all by-products from side-chain deprotection to be removed from the peptide aldehyde by washing before the product is released into aqueous solution.

04-12-3712	**H-Asp(OtBu)-methyloxazolidine**	1 g
NEW	**NovaSyn TG resin**	5 g

04-12-3719	H-Leu-methyloxazolidine NovaSyn TG resin	1 g
NEW		5 g
04-12-3713	H-Phe-methyloxazolidine NovaSyn TG resin	1 g
NEW		5 g

NEW Derivatives for chemoselective ligation

Bis-Boc-amino-oxyacetic acid



The chemoselective ligation of unprotected peptide fragments in aqueous media *via* oxime or hydrazone formation is a powerful and versatile method for the production of MAPs [14, 15], cyclic peptides [16 - 19], lipopeptides [20 - 23], peptide-DNA [24], peptide-glycopeptide conjugates [25 - 27], and Lys-dendrimers [28] (Figure 1).

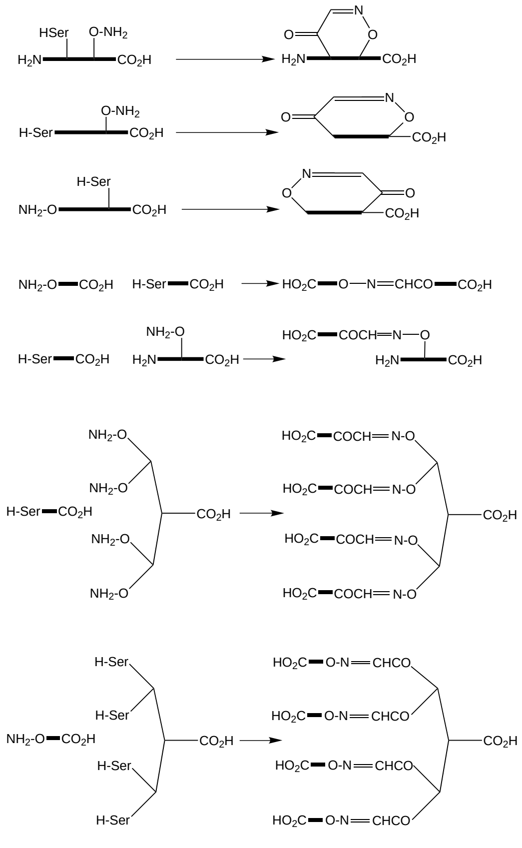


Fig. 1: Oxime and hydrazone ligation (X=O, NH).

Usually the hydroxylamine or hydrazine functionality is introduced using a mono-protected carboxylic acid derivative such as Boc-amino-oxyacetic acid or Boc-hydrazinoacetic acid, or Fmoc-Dpr(Boc-Aoa)-OH, Fmoc-Ams(Boc)-OH and Boc-Ams(Fmoc)-OH for ligation through amino acid side chains. However, it has been found that use of a single protecting group for hydrazine or hydroxylamine functionalities does not provide complete protection, and this can lead to formation of oligomers arising from double acylation [20, 29].

Such problems can be avoided using bis-Boc-amino-oxyacetic acid (Boc$_2$-Aoa-OH) as the nucleophilicity of the hydroxylamine functionality is completely masked by the two Boc groups [20]. This compound can be incorporated into peptides using HOBt/DIPCDI. Following cleavage and side-chain deprotection, a peptide is produced bearing a hydroxylamine moiety.

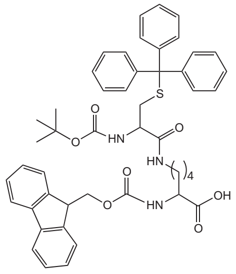
At pH ~5 in aqueous media, hydroxylamine and hydrazine-labeled peptides ligate with peptides, or other entities, possessing an aldehyde functionality. The reaction is extremely selective and is compatible with all standard amino-acid residues, with the exception of N-terminal cysteine which can undergo thiazolidine formation.

Peptides bearing aldehyde groups are usually generated *in situ* by periodate oxidation of a precursor peptide containing a 1,2-diol or 1,2-aminoalcohol group. For peptides containing an N-terminal aldehyde group, the most convenient precursor is the corresponding N-terminal serinyl peptide [28, 29]. Side-chain aldehyde groups are generated by oxidation of the peptide containing a Dpr(Ser) residue, which is introduced using Fmoc-Dpr(Boc-Ser)-OH [14]. Peptides containing C-terminal aldehyde functionalities can be made from peptide dihydroxypropylamides prepared from aminopropanediol 2-chlorotrityl resin [15]. 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.

The types of peptide conjugates that can be prepared by oxime/hydrazone ligation are summarized in Figure 1. By incorporating the hydroxylamine/hydrazine and aldehyde component into the same sequence, side-chain to side-chain cyclized peptides are produced [14, 16]; the oxime or hydrazone 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.

01-63-0129	Bis-Boc-amino-oxyacetic acid	1 g
NEW		5 g

Fmoc-Lys(Boc-Cys(Trt))-OH



Native thiol ligation is perhaps the most powerful method for peptide-peptide and peptide-protein ligation as it provides molecules containing an amide bond at the site of connection. The approach normally involves coupling together in aqueous media a peptide thioester with a peptide bearing an N-terminal Cys residue [30] (Figure 2). However, there are instances, particularly where the N-terminus of a peptide is required for biological activity, when it is not appropriate to ligate via an N-terminal Cys residue. In such cases, the use of Fmoc-Lys(Boc-Cys(Trt))-OH provides the solution [31]. This novel building block is introduced using standard Fmoc coupling methods and following cleavage and side-chain deprotection furnishes peptides bearing a pendant Cys residue attached to the side-chain of lysine. This Cys residue readily undergoes native thiol ligation with peptide or protein thioesters to produce branched molecules. Peptides containing multiple Lys(Cys) residues can be utilized as scaffolds for preparing polyvalent systems containing multiple copies of peptides or proteins.

04-12-1248	**Fmoc-Lys(Boc-Cys(Trt))-OH**	1 g
NEW	Patent pending. Sold under license from Adprotech.	5 g

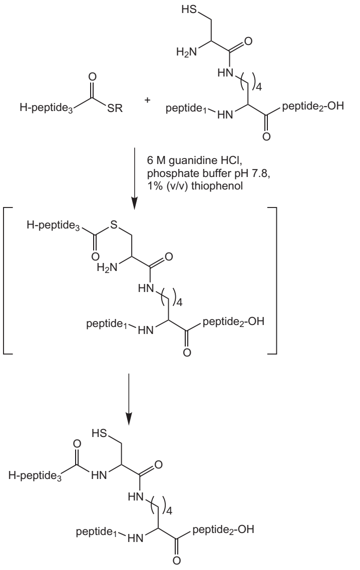


Fig. 2: Native thiol ligation

References

1. Anon. (1992) *Pinnacles*, **2**, 3.
2. T. Buranda, et al. (1999) *Cytometry*, **37**, 21.
3. I. Sélo, et al. (1996) *J. Immunol. Methods*, **199**, 127.
4. J. Howl, et al. (1993) *Eur. J. Biochem.*, **213**, 711.
5. E. Vázquez, et al. (1999) *Tetrahedron Lett.*, **40**, 3621.
6. A. L. Satz & T. C. Bruice (2001) *J. Am. Chem. Soc.*, **123**, 2469.
7. L. A. Dickinson, et al. (1999) *J. Biol. Chem.*, **274**, 12765.
8. A. K. Mapp, et al. (2000) *Proc. Natl. Acad. Sci.*, **97**, 3930.
9. N. R. Wurtz, et al. (2001) *Org. Lett.*, **3**, 1201.
10. L. A. Carpino, et al. (1993) *Tetrahedron Lett.*, **34**, 7829.
11. Z. Han, et al. (1997) *J. Biol. Chem.*, **272**, 13432.
12. G. Fenteany & S. L. Schreiber (1996) *Chemistry & Biology*, **3**, 905.
13. N. J. Ede. et al. (2000) *J. Pept. Sci.*, **6**, 11.
14. L. Zhang, et al. (1998) *Proc. Natl. Acad. Sci. USA*, **95**, 9184.
15. K. Rose. (1994) *J. Am. Chem. Soc.*, **116**, 30.
16. J. Shao & J. P. Tam (1995) *J. Am. Chem. Soc.*, **117**, 3893.
17. F. Wahl & M. Mutter (1996) *Tetrahedron Lett.*, **37**, 6861.
18. P. Botti, et al. (1996) *J. Am. Chem. Soc.*, **118**, 10018.
19. T. D. Pallin & J. P. Tam (1996) *Chem. Commun.*, 1345.
20. J. C. Spetzler & T. Hoeg-Jensen (2001) *J. Pept. Sci.*, **7**, 537.
21. D. Bonnet, et al. (2000) *Tetrahedron Lett.*, **41**, 45.
22. D. Bonnet, et al. (2000) *Tetrahedron Lett.*, **41**, 10003.
23. D. Bonnet, et al. (2001) *J. Org. Chem.*, **66**, 443.
24. L. Bourel-Bonnet, et al. (2001) *Tetrahedron Lett.*, **42**, 6851.
25. N. Ollivier, et al. (2002) *Tetrahedron Lett.*, **43**, 997.
26. C. Grandjean, et al. (2001) *Chem. Eur. J.*, **7**, 230.
27. C. Grandjean, et al. (2000) *Angew. Chem.*, **112**, 1110.
28. J. C. Spetzler & T. Hoeg-Jensen (1999) *J. Pept. Sci.*, **5**, 582.
29. O. Melnyk, et al. (2001) *J. Org. Chem.*, **66**, 4153.
30. P. E. Dawson, et al. (1994) *Science*, **266**, 776.
31. D. A. Alves, et al. (2003) *J. Pept. Sci.*, **9**, 221.

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