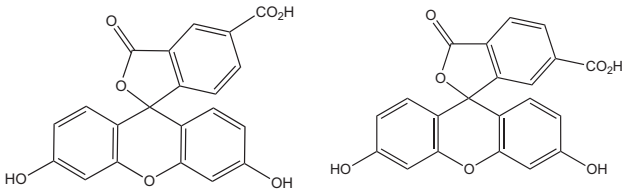
Advancing your life science discoveries™

novabiochem letter 1/03

Contents

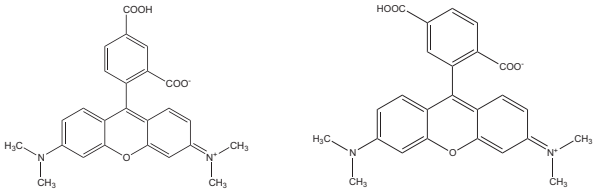
NEW Fluorescent dyes

5-Carboxyfluorescein/6-Carboxyfluorescein



5-Carboxytetramethylrhodamine/

6-Carboxytetramethylrhodamine



These high purity fluorescent dyes are useful tools for preparing fluorescently-labeled peptides and fluorescence-quenched peptide substrates. Novabiochem's carboxyfluorescein (FAM; λ_{ex} 494 nm; λ_{em} 518 nm) and carboxytetramethyl-rhodamine (TAMRA; λ_{ex} 555 nm; λ_{em} 580 nm) are supplied as single isomers, ensuring labeled products of defined chemical structure, as well as greatly

assisting product purification and characterization.
The dyes are most conveniently introduced during solid phase synthesis by coupling to N-terminal or side-chain amino groups. 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. FAM should be introduced as the last step in the synthesis to avoid the formation of esters with activated amino acids or side reactions during hydrazine treatment [1].
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.

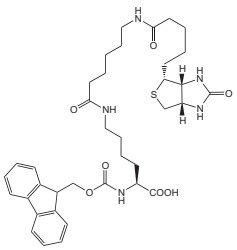
01-63-0112	**5-Carboxyfluorescein**	25 mg
NEW		100 mg
01-63-0113	**6-Carboxyfluorescein**	25 mg
NEW		100 mg
01-63-0114	**5-Carboxytetramethylrhodamine**	10 mg
NEW		50 mg
01-63-0115	**6-Carboxytetramethylrhodamine**	10 mg
NEW		50 mg

Novabiochem's other chromogenic derivatives

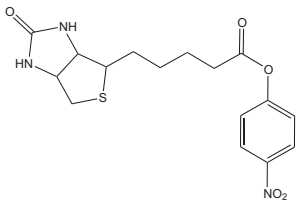
01-63-0105	**Dabcyl-OSu**	1 g
04-12-1238	**Fmoc-Glu(EDANS)-OH**	0.5 g
		1 g
04-12-1236	**Fmoc-Lys(Dabcyl)-OH**	0.5 g
		1 g
04-12-1239	**Fmoc-Lys(Dnp)-OH**	0.5 g
		1 g
04-12-1233	**Fmoc-Lys(Mca)-OH**	0.5 g
		1 g

NEW Biotinylating reagents

Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH



Biotin-ONp

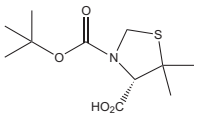


Biotin-labeled peptides have many important applications in immunology and histochemistry, such as affinity purification [2] and FRET-based flow cytometry [3], solid-phase immunoassays [4], and receptor localization [5], that exploit the high affinity of streptavidin and avidin for biotin.
N-terminal labeling of peptides with biotin is most easily performed as the last cycle in solid phase synthesis, using biotin-OSu or biotin-ONp in DMF or NMP containing one equivalent of HOBt. The use of biotin-ONp is preferred owing to its superior solubility and coupling kinetics. The coupling of biotin derivatives is often sluggish and so completeness of reaction should be monitored using TNBS or ninhydrin. If a spacer unit is required between the peptide and the biotin, the simplest approach is to introduce an ϵ-Ahx residue at the N-terminus of the peptide before addition of the biotin.
Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH facilitates the introduction of biotin incorporating an ϵ-Ahx spacer at a precise location within the peptide chain. It can be introduced using PyBOP®/DIPEA or TBTU/DIPEA activation; however, due to its poor solubility in DMF, the reaction should be performed in NMP.

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

NEW Unusual amino acid

Boc-D-DMTA

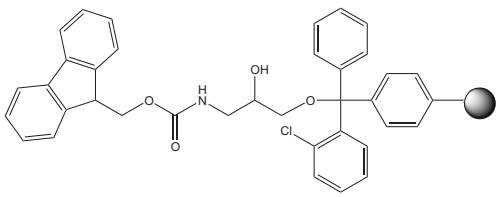


Novabiochem is pleased to introduce the following Boc protected heterocyclic amino acid derivative.

04-13-0083	**Boc-D-DMTA**	1 g
NEW		
04-12-0246	**Boc-L-DMTA**	1 g

NEW Resins for SPOS of peptide aldehydes

Fmoc-1-amino-2,3-propanediol-2'-chlorotrityl resin



Fmoc-1-amino-2,3-propanediol-2'-chlorotrityl resin is a novel support for the preparation of peptides containing a C-terminal glycinal residue [6]. Following removal of the Fmoc group with piperidine/DMF, the resin can be acylated with Fmoc-amino acids under standard coupling conditions. Upon cleavage and side-

chain deprotection with standard TFA cocktails, a peptide bearing a C-terminal diol functionality is obtained. Oxidation with aqueous sodium periodate converts this moiety to the corresponding glycinal residue. These aldehydes readily undergo chemoselective ligation in aqueous media with unprotected peptides containing hydrazine or hydroxylamine groups. This approach provides a powerful and versatile method for the production of MAPs [7, 8], cyclic peptides [9 - 12], lipopeptides [13 - 16], peptide-DNA [17], peptide-glycopeptide conjugates [18 - 20], and Lys-dendrimers [21] (Figure 1). If the oxidation is performed in the presence of the hydroxylamine or hydrazine component, oxime or hydrazone formation occurs concurrently with liberation of the aldehyde.

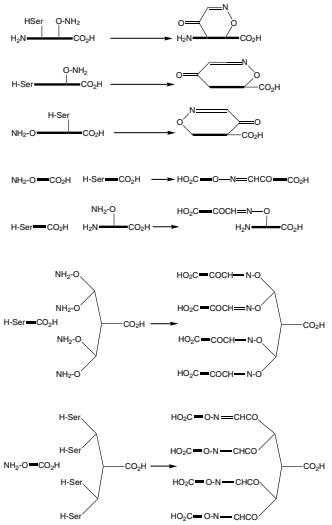
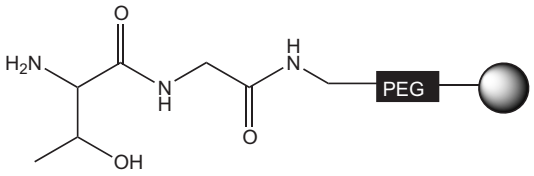


Fig. 1: Hydrazone/oxime ligation.

01-64-0450 **Fmoc-1-amino-2,3-propane-diol-2'-chlorotrityl resin** 1 g
NEW

H-Thr-Gly-NovaSyn TG resin

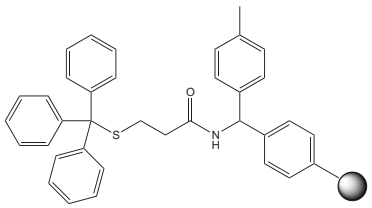


Peptide aldehydes are potent inhibitors of serine, aspartyl and cysteinyl proteases and are valuable intermediates for the preparation of reduced amide-bond peptidomimetics.
One of the simplest approaches to the solid phase synthesis of peptide aldehydes involves immobilization of a pre-formed Fmoc-amino aldehyde to an amino alcohol linker *via* oxazolidine formation [22]. 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.
Attachment of aldehydes to this resin is effected by heating the aldehyde in 1% DIPEA in methanol at 60˚C. The aldehyde is normally prepared by reduction of the corresponding Fmoc amino acid Weinreb amide with $LiAlH_4$. Since the reaction between the linker and aldehyde is highly selective, the aldehyde does not need to be extensively purified prior to attachment to the support.
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). 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.

04-12-3710 **H-Thr-Gly-NovaSyn TG resin** 1 g
NEW 5 g

NEW Resins for the synthesis of peptide thioesters

3-S-Tritylmercaptopropionyl MBHA resin



Native thiol ligation is a highly effective method for preparing large peptides and small proteins. The approach involves coupling together in aqueous media a peptide thioester with a peptide bearing an N-terminal Cys residue, to provide a product containing an amide bond at the site of ligation [23] (Figure 2).

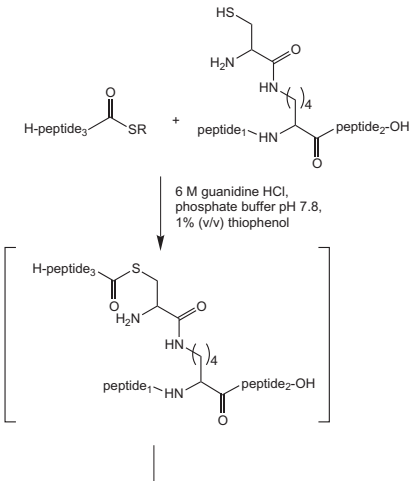


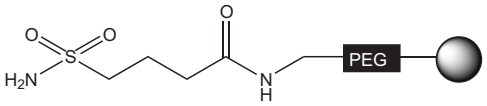
Fig. 2: Native thiol ligation

The synthesis of peptide thioesters is most simply carried out using Boc chemistry on the acid-labile Trt-mercapto-propionyl-MBHA resin [24]. The Trt group is removed with TFA/DCM. Following

washing and neutralization with DIPEA, the resin can be loaded with the C-terminal residue using DIPCDI/HOBt activation. Unreacted thiol sites should then be capped with AcCl/DIPEA in DCM. After chain extension using standard Boc protocols, HF cleavage provides the side-chain deprotected peptide thioester.

01-64-0449	**3-S-Tritylmercaptopropionyl**	1 g
NEW	MBHA resin	

4-Sulfamylbutyryl NovaSyn TG resin



The production of thioesters by Fmoc SPPS can not be achieved directly, owing to the instability of thioesters to piperidine. One of the most effective Fmoc compatible methods described to date involves thiolytic cleavage from the sulfamylbutyryl safety-catch linker [25, 26], as described in Figure 3.

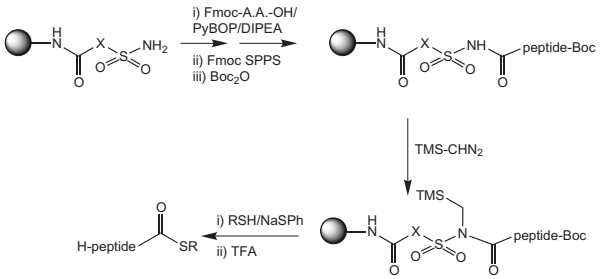


Fig. 3: Preparation of thioesters using sulfamylbutyryl resins.

Displacement from the activated linker can also be performed with other nucleophiles, such as primary and secondary amines, anilines, and hydroxide, to afford products possessing a wide range of carboxyl group modifications [27]. Even cyclic products can be obtained if the displacement is intramolecular [28].

01-64-0458	**4-Sulfamylbutyryl**	1 g
NEW	NovaSyn TG resin	5 g

References

1. R. Fischer, et al., Poster A82 presented at 27th European Peptide Symposium, Sorrento, 2002.
2. Anon. (1992) *Pinnacles*, **2**, 3.
3. T. Buranda, et al. (1999) *Cytometry*, **37**, 21.
4. I. Sélo, et al. (1996) *J. Immunol. Methods*, **199**, 127.
5. J. Howl, et al. (1993) *Eur. J. Biochem.*, **213**, 711.
6. L. Zhang, et al. (1998) *Proc. Natl. Acad. Sci. USA*, **95**, 9184.
7. K. Rose. (1994) *J. Am. Chem. Soc.*, **116**, 30.
8. J. Shao & J. P. Tam (1995) *J. Am. Chem. Soc.*, **117**, 3893
9. F. Wahl & M. Mutter (1996) *Tetrahedron Lett.*, **37**, 6861.
10. P. Botti, et al. (1996) *J. Am. Chem. Soc.*, **118**, 10018.
11. T. D. Pallin & J. P. Tam (1996) *Chem. Commun.*, 1345.
12. J. C. Spetzler & T. Hoeg-Jensen (2001) *J. Pept. Sci.*, **7**, 537.
13. D. Bonnet, et al. (2000) *Tetrahedron Lett.*, **41**, 45.
14. D. Bonnet, et al. (2000) *Tetrahedron Lett.*, **41**, 10003.
15. D. Bonnet, et al. (2001) *J. Org. Chem.*, **66**, 443.
16. L. Bourel-Bonnet, et al. (2001) *Tetrahedron Lett.*, **42**, 6851.
17. N. Ollivier, et al. (2002) *Tetrahedron Lett.*, **43**, 997.
18. C. Grandjean, et al. (2001) *Chem. Eur. J.*, **7**, 230.
19. C. Grandjean, et al. (2000) *Angew. Chem.*, **112**, 1110.
20. J. C. Spetzler & T. Hoeg-Jensen (1999) *J. Pept. Sci.*, **5**, 582.
21. O. Melnyk, et al. (2001) *J. Org. Chem.*, **66**, 4153.
22. N. J. Ede. et al. (2000) *J. Pept. Sci.*, **6**, 11.
23. P. E. Dawson, et al. (1994) *Science*, *266*, 776.
24. J. P. Tam, et al. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 12485.
25. Y. Shin, et al. (1999) *J. Am. Chem. Soc.*, **121**, 11684.
26. R. Ingenito, et al. (1999) *J. Am. Chem. Soc.*, **121**, 11369.
27. a) B. J. Backes, et al. (1996) *J. Am. Chem. Soc.*, **118**, 3055; b) B. J. Backes & J. A. Ellman (1999) J. *Org. Chem.*, **64**, 2322.
28. L. Yang & G. Morriello (1999) *Tetrahedron Lett.*, **40**, 8197.

.

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

www.novabiochem.com