

Novabiochem[®]

innovations 4/05

New tools for SPPS of labeled peptides

NovaTag[™] resins

Novabiochem's NovaTag[™] resins are unique tools designed to streamline the Fmoc SPPS of chromogenically labeled peptides [1]. Each resin contains a standard TFA-cleavable linker which has been modified to incorporate a diamine spacer that is either orthogonally protected or pre-derivatized with a chromogenic label (Fig. 1). This arrangement allows C-terminally labeled peptides to be prepared quickly and efficiently with the minimum number of synthetic steps.

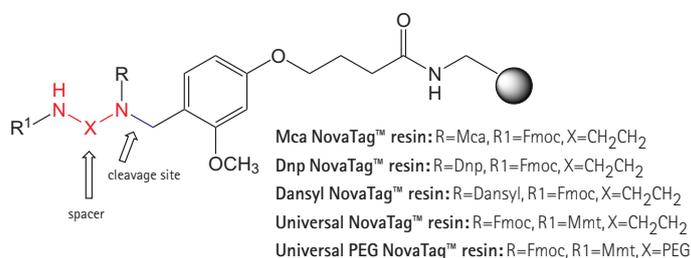


Fig. 1: NovaTag[™] resins.

Pre-loaded resins are available which provide directly on TFA cleavage peptides labeled with either dansyl (Dansyl NovaTag[™] resin), 4-methoxycoumarinacetyl (Mca NovaTag[™] resin), or dinitrophenyl (Dnp NovaTag[™] resin) fluorophores. As the label forms an integral part of the resin linker, no additional steps are required for label introduction, and the presence of the label in every peptide molecule is assured from the outset. These supports are ideal for the synthesis of peptides containing multiple labels, such as FRET peptides and labeled biotin conjugates, as the additional fluorophore or biotin label can simply be added to the N-terminus of the peptide chain prior to cleavage.

For situations where it is not always apparent at the outset which is the optimum label or combination of labels for a given application, Novabiochem offers the Universal NovaTag[™] resins. These supports facilitate the synthesis of peptides bearing any number of different acyl moieties at N- and C-termini from a single solid phase synthesis. Universal resins are also useful for preparing labeled peptides containing fluorophores that are not compatible with Fmoc SPPS protocols, such as TAMRA and FAM, since they allow the labels to be easily introduced as the final step before cleavage. Universal PEG NovaTag[™] resin is particularly efficacious for applications where a hydrophilic spacer is required between the peptide and the label and where the peptide solubility is an issue.

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Features and benefits of NovaTag™ resins

Features & Benefits

Pre-loaded NovaTag™ resins	<p>No additional step required for introduction of label</p> <p>Avoids use of poorly soluble and slow to couple labeling reagents</p> <p>Presence of label in every peptide chain assured</p> <p>Ideal for synthesis of large arrays of labeled peptides</p> <p>Avoids introduction of unnatural labeled amino acid into peptide sequence</p> <p>N-terminal amine left free for introduction of other labels</p>
Universal NovaTag™ resins	Ideal for optimization of label, as any number of label variations can be prepared from a single solid phase synthesis
Universal PEG NovaTag™ resin	PEG spacer enhances solubility of labeled peptide

Spectral properties of pre-loaded NovaTag™ resins

The most important application of pre-loaded NovaTag™ resins is in the synthesis of FRET enzyme substrates (Figure 2). The spectral properties of available dyes and their FRET partners are summarized in Table 1.

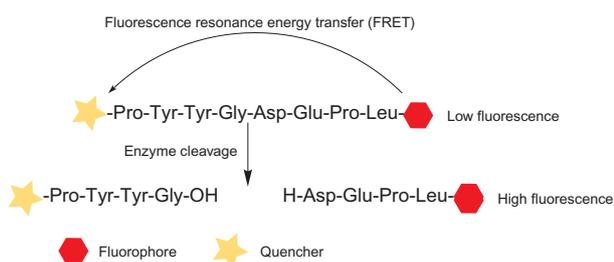


Fig. 2: FRET-based substrates.

Table 1: Spectral properties of dyes used in NovaTag™ resins and their complements.

Fluorophore	Quencher	λ_{\max} (nm)	λ_{em} (nm)
Dansyl	Dabsyl	335	526
Trp	Dansyl	295	353
Mca	Dnp	328	393

Using NovaTag™ resins

Using Dansyl, Mca and Dnp NovaTag™ resins for the synthesis of labeled peptides is very easy. The resins can simply be loaded onto an automated peptide synthesizer and treated like an Fmoc-protected Rink amide resin. Once the Fmoc group is removed with piperidine/DMF, peptide assembly can be carried out using standard Fmoc SPPS protocols. When making FRET peptides, the complementary dye can be added to the N-terminal amino acid immediately prior to cleavage of the peptide from the resin with standard TFA cocktails. The use of Mca NovaTag™ resin is illustrated in Application 1.

With the Universal NovaTag™ resins the procedure is the same, except that the first residue should be coupled using HATU. Following chain extension and end-capping of the peptide chain with either the desired label or a Boc group, the pendant Mmt group is removed using HOBt/TFE/DCM, and the C-terminal label introduced. The use of Universal NovaTag™ resin in the synthesis of a calpain substrate is illustrated in Application 2.

Applications

Synthesis of Glu-C substrate

Application 1: Synthesis of endopeptidase Glu-C substrate

Mca NovaTag™ resin (155 mg, 0.045 mmole) was swollen in DMF and the Fmoc group removed with 20% piperidine. Peptide assembly was carried out using 30 min couplings of Fmoc-amino acids (6 eq.) activated with PyBOP® (6 eq.) in the presence of DIPEA (12 eq.). The peptide was cleaved from the resin using 95:2.5:2.5 TFA/water/TIS for 3 h and purified directly by HPLC. The purified peptide gave the HPLC profile shown in Figure 3 and was characterized by ES-MS [expected $M+H^+$ 1142.4, found 1142.4].

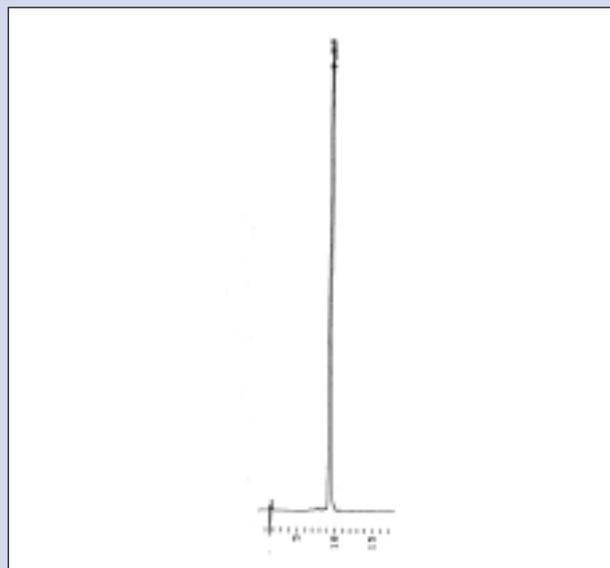


Fig. 3: HPLC elution profile of purified H-Lys(Dnp)-Leu-Glu-Val-Asp-Gly-Trp-NHCH₂CH₂NH-Mca prepared with Mca NovaTag™ resin.

Synthesis of calpain substrate

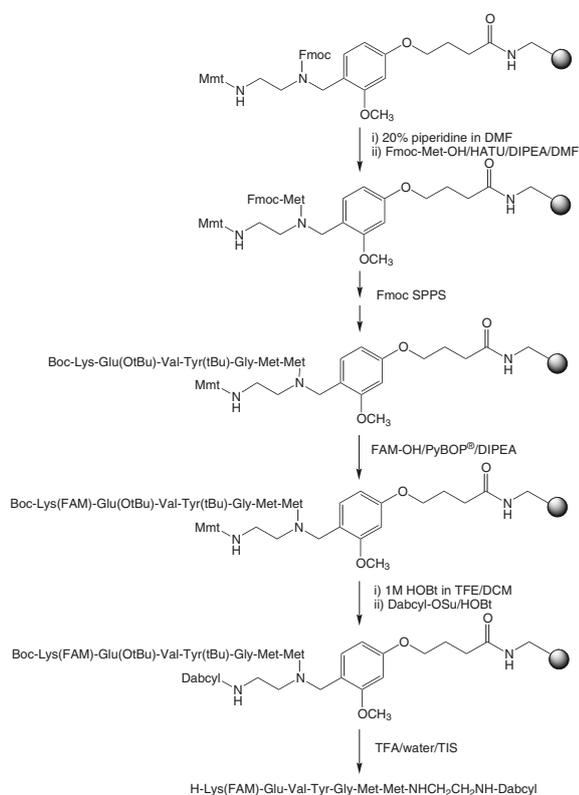


Fig. 4: Synthesis of calpain substrate using Universal NovaTag™ resin.

Application 2: Synthesis of calpain substrate using Universal NovaTag™ resin

Fmoc-Met-OH (6 eq.) was coupled to Universal NovaTag™ resin (155 mg, 0.045 mmole) using HATU (6 eq.) and DIPEA (12 eq.) in DMF for 1 h. The chain was extended using Fmoc-amino acids (6 eq.) (with the exception Boc-Lys(Fmoc) which was used for addition of Lys) activated with PyBOP® (6 eq.) and DIPEA (12 eq.) in DMF for 30 min. Following Fmoc deprotection of the Lys side chain, the resin was treated with FAM (3 eq.), PyBOP® (3 eq.) and DIPEA (6 eq.) in DMF for 1 h. The resin was treated twice with 0.6 M HOBT in DCM/TFE (1:1) for 1 h to remove the Mmt group, and then reacted with DabcyI-Osu (2 eq.) and HOBT (0.06 eq.) in DMF o/n. Labeled peptide was cleaved from the resin using TFA/water/phenol/EDT/thioanisole (82.5:5:5:2.5:5) for 3 h and then purified directly by HPLC. The purified peptide was analyzed by HPLC (Figure 5) and characterized by ES-MS [expected $M+H^+$ 1508.5, found 1507.7].

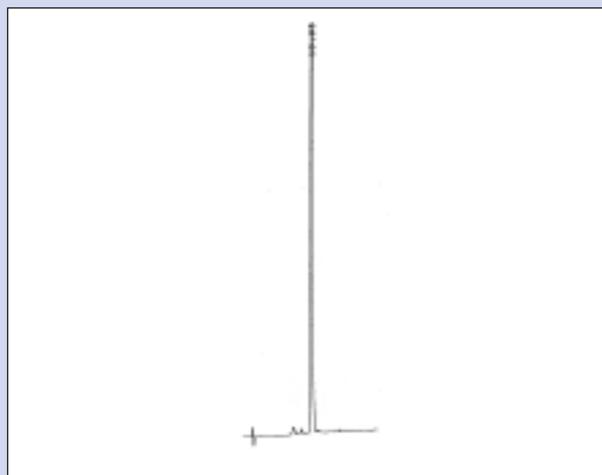


Fig. 5: HPLC elution profile of crude H-Lys(FAM)-Glu-Val-Tyr-Gly-Met-Met-NHCH₂CH₂NH-DabcyI prepared with Universal NovaTag™ resin.

Enzyme assays

Endopeptidase Glu-C and calpain substrates were evaluated in FRET-based enzyme assays. Lineweaver-Burk plots for the assays are shown in Figure 6. The K_m for the calpain substrate was found to be 2.9 μM , which compares favorably with the value of 4.5 μM obtained for H-Lys(FAM)-Glu-Val-Tyr-Gly-Met-Met-Lys(DabcyI)-OH [2].

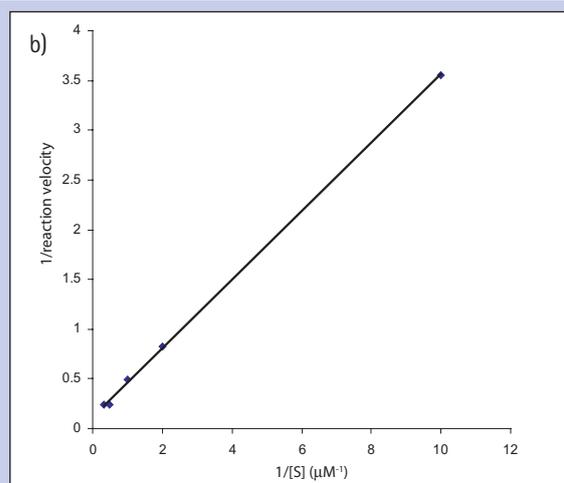
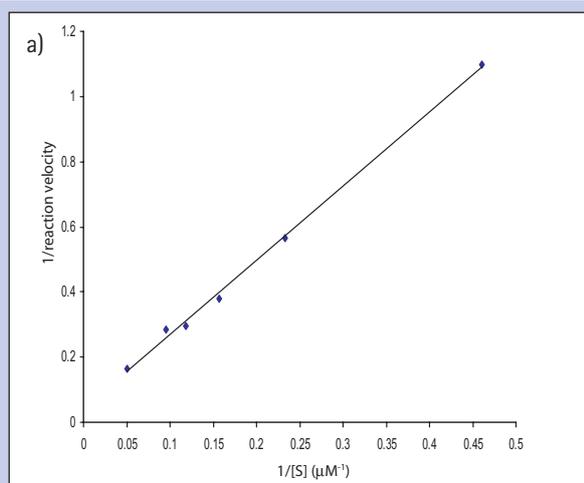


Fig. 6: a) Plot of inverse of reaction velocity against inverse of Glu-C substrate concentration. Assay conditions: 100 mM Tris, pH 7.4, 25 °C; endoprotease Glu-C (0.34 μM) detection: $\lambda_{\text{ex}} = 328 \text{ nm}$; $\lambda_{\text{em}} = 393 \text{ nm}$; b) Plot of inverse of reaction velocity against inverse of calpain substrate concentration. Assay conditions: 55 mM HEPES, pH 7.5, 2.5 mM CaCl₂, 5 mM DTE, 25 °C, calpain 1 (0.13 μM); detection: $\lambda_{\text{ex}} = 494 \text{ nm}$; $\lambda_{\text{em}} = 518 \text{ nm}$.

Ordering information

01-63-0112	5-Carboxyfluorescein	25 mg 100 mg
01-63-0113	6-Carboxyfluorescein	25 mg 100 mg
04-12-3900	Dansyl NovaTag™ resin	100 mg 500 mg
04-12-3903	Dnp NovaTag™ resin	100 mg 500 mg
04-12-3902	Mca NovaTag™ resin	100 mg 500 mg
04-12-3910	Universal NovaTag™ resin	500 mg 1 g
04-12-3911	Universal PEG NovaTag™ resin	500 mg 1 g
01-63-0105	Dabcyl-OSu	1 g
Other NovaTag™ resins		
04-12-3901	Biotin NovaTag™ resin	500 mg 1 g
04-12-3908	Biotin-PEG NovaTag™ resin	500 mg 1 g
04-12-3904	EDANS NovaTag™ resin	100 mg 500 mg
AMC pre-loaded resins		
04-12-3912	Fmoc-Arg(bis-Boc-resin)-AMC	500 mg
NEW		
04-12-2177	Fmoc-Asp(Wang resin)-AMC	500 mg 1 g
NEW		

Novabiochem's other chromogenic derivatives

01-63-0147	5(6)-Carboxyfluorescein diisobutyrate	100 mg 500 mg
NEW		
01-63-0114	5-Carboxy-tetramethylrhodamine	10 mg 50 mg
01-63-0115	6-Carboxy-tetramethylrhodamine	10 mg 50 mg
01-63-0134	5(6)-Carboxy-tetramethylrhodamine	100 mg 500 mg
01-63-0138	EVOblue™ 10	0.5 mg 1 mg
NEW		
01-63-0139	EVOblue™ 30	0.5 mg 1 mg
NEW		
01-63-0140	EVOblue™ 30-OSu	0.5 mg 1 mg
NEW		
04-12-1238	Fmoc-Glu(EDANS)-OH	500 mg 1 g
04-12-1236	Fmoc-Lys(Dabcyl)-OH	500 mg 1 g
04-12-1239	Fmoc-Lys(Dnp)-OH	500 mg 1 g
04-12-1233	Fmoc-Lys(Mca)-OH	500 mg 1 g
01-63-0111	Mca-OH	1 g 5 g
01-63-0110	Mca-OSu	1 g

References

1. J. Beythien & P. D. White (2003) *Biopolymers*, 71, 362: Poster 270; Downloadable at: www.emdbiosciences.com/SharedImages/novabiochem/APSPoster3.pdf
2. S. Mittoo, et al. (2003) *Anal. Biochem.*, 319, 234.

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