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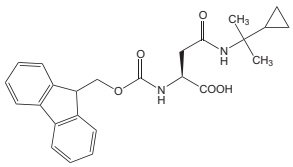
Letters: 3/06

Contents

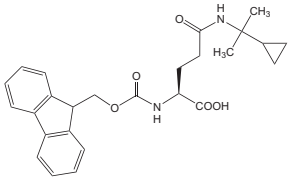
Product Focus: New reagents for peptide synthesis

NEW Asn and Gln derivatives for Fmoc SPPS

Fmoc-Asn(Dmcp)-OH



Fmoc-Gln(Dmcp)-OH



Features & Benefits

- Dmcp provides complete protection to side-chain carboxamide
- Dmcp is more easily cleaved than Trt group
- Faster coupling than Trt-protected derivatives
- Higher solubility than Trt-protected derivatives

Since the introduction of Fmoc-Asn(Trt)-OH and Fmoc-Gln(Trt)-OH by Sieber [1], they have become the standard derivatives for the routine synthesis of asparagine- and glutamine-containing peptides by Fmoc SPPS. These derivatives, however, do have certain limitations. Firstly, cleavage of the N-Trt group can be sluggish, particularly in the case of N-terminal Asn residues which can require over 4 hours for complete Trt removal with 95% TFA [2]. Secondly, these derivatives have low solubility in DMF compared to other Fmoc-protected amino acids. Thirdly, protected peptides containing Asn(Trt) and Gln(Trt) often have poor solubility. Finally, Asn(Trt) in particular

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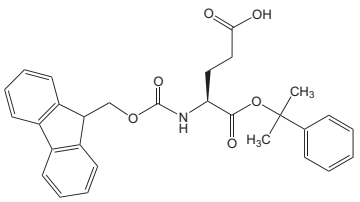
is notorious for inducing aggregation during solid phase synthesis.

Novabiochem® is, therefore, pleased to introduce L. A. Carpino's N-dimethylcyclopropylmethyl (Dmcp) derivatives of Fmoc-protected asparagine and glutamine [3]. Cleavage of the Dmcp group from the side-chains of Asn and Gln is rapid, even when the residue is located at the N-terminus of a peptide. Coupling of Dmcp-protected derivatives appears to be faster than that of the corresponding hindered Trt derivatives: for example, coupling of Fmoc-Asn(Trt)-OH with TFFH in the synthesis of ACP gave sigificant quantities of des-Asn peptide, whereas with Fmoc-Asn(Dmcp)-OH none of this by-product was generated [3]. Furthermore, protected peptides containing Asn(Dmcp) and Gln(Dmcp) residues appear to have enhanced solubility. This observation would indicate that peptides containing these residues would be less prone to aggregation during SPPS. Finally, Fmoc-Asn(Dmcp)-OH and Fmoc-Gln(Dmcp)-OH are also more soluble in DMF than Fmoc-Asn(Trt)-OH and Fmoc-Gln(Trt)-OH, thereby facilitating coupling reactions at higher concentration.

04-12-1291	Fmoc-Asn(Dmcp)-OH	1 g
NEW		5 g
04-12-1292	Fmoc-Gln(Dmcp)-OH	1 g
NEW		5 g

NEW orthogonally-protected Glu derivative for Fmoc SPPS

Fmoc-Glu-O-2-PhiPr



Features & Benefits

- 2-PhiPr group is removed with 1% TFA in DCM
- Ideal tool for on-resin synthesis of head-to-tail cyclic peptides

Fmoc-Glu-O-2-PhiPr is an excellent tool for the synthesis of head-to-tail cyclic peptides by Fmoc SPPS, as the O-2-PhiPr group is stable to bases but can be selectively cleaved on-resin with 1% TFA in DCM, in the presence of standard tBu-based protecting groups [4]. Typically, the amino acid is anchored *via* the side-chain γ-carboxyl group to Wang or Rink amide-type resins. Following chain extension, the α-carboxyl is unmasked by treatment with 1% TFA in DCM, and on-resin cyclization can be carried out (Figure 1).

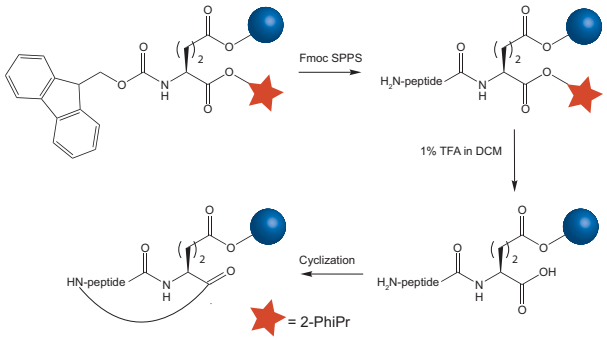


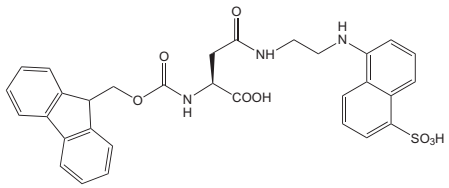
Fig. 1: Synthesis of cyclic peptides via side-chain anchoring of Glu-O-2-PhiPr.

For side-chain to tail lactam-bridged peptides, the combination of Lys(Mtt) and Glu-O-2-PhiPr is particularly advantageous since both side-chain protecting groups can be removed in a single step.

04-12-1284	Fmoc-Glu-O-2-PhiPr	1 g
NEW		
04-12-1199	Fmoc-Glu(O-2-PhiPr)-OH	1 g
		5 g

NEW reagent for the synthesis of labeled peptides

Fmoc-Asp(EDANS)-OH



Features & Benefits

- Direct synthesis of peptide fluorescent substrate containing EDANS
- Compatible with Fmoc SPPS
- TFA cleavage provides labeled peptide directly

The EDANS/Dabcyl fluorophore-quencher pair is one of the most commonly used for FRET applications, owing to excellent spectral overlap between the emission spectrum of EDANS (λ_{ex} 341 nm, λ_{em} 471 nm) and absorbance spectrum of Dabcyl (λ_{max} 453 nm) [5, 6] (Figure 2). Quenching of the fluorescence of EDANS by Dabcyl is consequently highly efficient, with up to 40-fold enhancements in fluorescence having been observed upon proteolysis of Dabcyl/EDANS-labeled peptides [5].

To incorporate EDANS within the peptide chain, the simplest approach is to use Fmoc-Asp(EDANS)-OH or Fmoc-Glu(EDANS)-OH during peptide assembly [7, 8]. Introduction

of the analogous Glu derivative during SPPS has been achieved using PyBOP®/DIPEA activation in conjunction with an extended coupling time [8]. Powerful acylating reagents such as PyBrOP® should be avoided as their use can lead to acylation of the naphthylamine nitrogen.

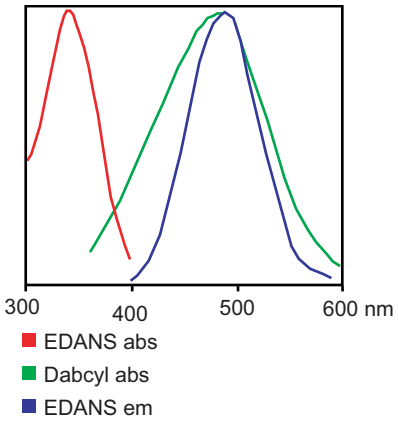
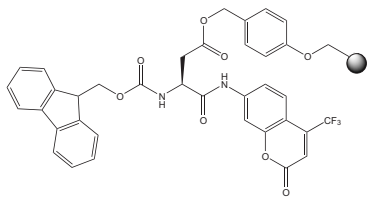


Fig. 2: Absorbance and emission spectra of Dabcyl and EDANS [7].

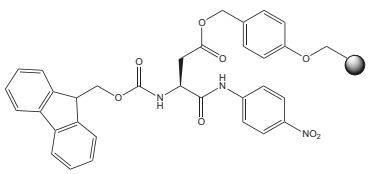
04-12-1288	Fmoc-Asp(EDANS)-OH	500 mg
NEW		1 g
04-12-1291	Fmoc-Glu(EDANS)-OH	500 mg
		1 g

NEW resins for the synthesis of P1-labeled protease substrates

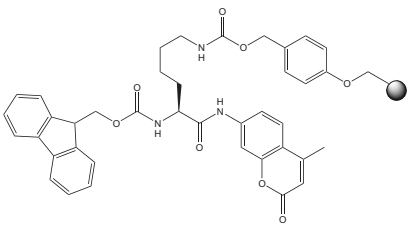
Fmoc-Asp(Wang resin)-AFC



Fmoc-Asp(Wang resin)-pNA



Fmoc-Lys(carbamate Wang resin)-AMC



Features & Benefits

- Direct synthesis of peptide fluorescent substrates containing C-terminal Asp-pNA, Asp-AFC or Lys-AMC
- Compatible with Fmoc SPPS
- TFA cleavage provides labeled peptide directly
- Ideal for peptide substrate optimization and enzyme profiling

P1-labeled peptide substrates are the most commonly used tools for studying protease activity and specificity [9]. They comprise a peptide in which the carboxyl group of the P1 residue of the protease substrate recognition sequence forms an amide bond with a dye. When proteolysis of this amide bond occurs, the dye is released enabling the process to be detected by changes in optical density or fluorescence (Figure 3).

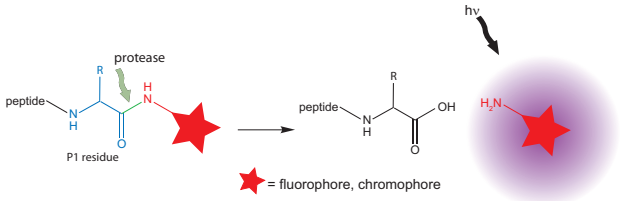


Fig. 3: Principle of P1-labeled fluorogenic substrates.

Fmoc-Asp(Wang resin)-pNA, Fmoc-Asp(Wang resin)-AFC and Fmoc-Lys(carbamate Wang resin)-AMC are the latest editions to Novabiochem's range of pre-loaded resins for the synthesis of P1-labeled peptides. These resins are ideal tools for the production of enzyme substrate libraries for protease profiling as their use allows aspartyl p-nitroanilides and 7-amido-4-trifluoromethylcoumarins, and lysyl 7-amido-4-methylcoumarins, respectively, to be prepared directly by Fmoc SPPS. They are extremely simple to use as they are fully compatible with standard Fmoc SPPS protocols. The Fmoc group is removed with 20% piperidine in DMF under standard conditions, and the free amine group can be acylated with PyBOP® or TBTU. Following peptide assembly, cleavage with 95% TFA releases the labeled peptide directly from the solid support without any additional steps.

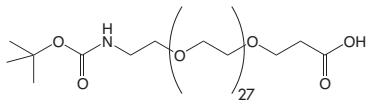
Table 1: Optical properties of dyes used in P1-labeled substrates.

Dye	$\lambda_{max/ex}$ (nm)	ε ($M^{-1}cm^{-1}$)	λ_{em} (nm)
pNA	405	10,500	
AMC	342	16,000 (354 nm)	441
AFC	400	12,600 (380 nm)	505

04-12-3918	Fmoc-Asp(Wang resin)-AFC	500 mg
NEW		1 g
04-12-3919	Fmoc-Asp(Wang resin)-pNA	500 mg
NEW		1 g
04-12-3917	Fmoc-Lys(carbamate Wang resin)-AMC	500 mg
NEW		1 g
04-12-3912	Fmoc-Arg(bis-Boc-resin)-AMC	500 mg
04-12-3915	Fmoc-Asp(Wang resin)-AMC	500 m
		1 g

NEW Bifunctional amino-PEG-acid spacer

Boc-NH-$(PEG)_{27}$-COOH (88 atoms)



Features & Benefits

- 88 atom amino-PEG-acid spacer
- Introduced using standard activation methods
- Imparts solubility to end-product
- Yields homogeneous product

Boc-NH-$(PEG)_{27}$-COOH is the latest addition to Novabiochem's range of PEG-based building blocks for solid phase peptide synthesis. It can be introduced using standard coupling methods, such as PyBOP® or TBTU.

This derivative is prepared from highly purified monodisperse PEG to ensure an homogeneous product free from contaminating oligomers. Unlike similar PEG linkers based on polydisperse PEGs, products prepared using this reagent will be a single chemical entity and can therefore be characterized and purified using standard techniques.

01-63-0151	Boc-NH-$(PEG)_{27}$-COOH (88 atoms)	500 mg
NEW		1 g
01-63-0141	Fmoc-NH-$(PEG)_2$-COOH (20 atoms)	500 mg
		1 g
		5 g
01-63-0103	N_3-$(PEG)_7$-COOH (33 atoms)	500 mg
		1 g
01-63-0102	Boc-NH-$(PEG)_6$-COOH (30 atoms)	500 mg
		1 g
01-63-0109	Fmoc-NH-$(PEG)_{11}$-COOH (40 atoms)	500 mg
		1 g
01-63-0150	Fmoc-NH-$(PEG)_{27}$-COOH (88 atoms)	500 mg
		1 g

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

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