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Letters: 4/06

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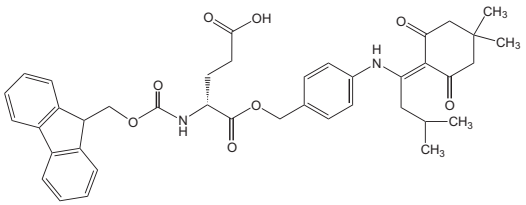
Product Focus: Reagents for the synthesis of cyclic and modified peptides

NEW Orthogonally-protected Glu derivative for Fmoc SPPS

Fmoc-D-Glu-ODmab



Features & Benefits

- Dmab group is removed with 2% hydrazine in DMF
- Excellent tool for on-resin synthesis of head-to-tail cyclic peptides

Fmoc-D-Glu-ODmab is the latest addition to our range of hydrazine-labile orthogonally-protected amino acids. These reagents were developed in collaboration with Prof. Bycroft and Dr. Chan at the University of Nottingham as tools to facilitate the Fmoc SPPS of side-chain modified and cyclic peptides. The range includes α- and side-chain Dmab esters of Asp and Glu together with the complementary ivDde-protected derivatives of Dab, Dpr, Lys and Orn.

Dmab [1] and ivDde [2] protecting groups have unique properties which make them ideal for use in Fmoc SPPS. Firstly, they are stable to piperidine in DMF but removed by treatment with 2% hydrazine in DMF (Method 1), enabling side-

Method 1: Selective removal of Dmab and ivDde with 2% hydrazine in DMF

Batch

1. Place the peptidyl-resin in a flask and treat with 2% hydrazine monohydrate in DMF (25 ml/g). Stopper the flask and leave to stand at rt for 3 min.
2. Filter the resin and repeat the hydrazine treatment four more times. Wash the partially protected resin with DMF.

Continuous flow

1. Flow 2% hydrazine monohydrate in DMF at 3 ml/min through the peptidyl resin packed in a 1 cm diameter reaction column. Deprotection can be followed by monitoring spectrophotometrically at 290 nm the absorbance of the column eluant using a 0.1 mm path-length cell.
2. When the reaction is complete, as indicated by return of the absorbance to its original value, flush the column with DMF.

functionalities protected by Dmab or ivDde to be selectively unmasked on the solid phase without affecting the standard t-butyl-based protecting groups. Secondly, the deprotection reaction generates a UV active indazole by-product (Figure 1) whose release can be followed spectrophotometrically to monitor the progress of the deprotection reaction. In a batch-wise reaction, the extent of the reaction can be determined by sampling the resin filtrate after each addition of deprotection solution and measuring the optical density of the solution at 290 nm. With continuous flow synthesizers, monitoring is carried out by pumping the column eluant through a UV flow-cell.

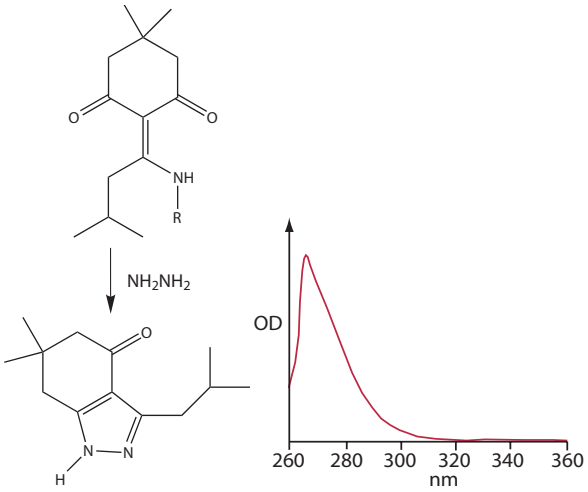


Fig. 1: Formation of indazole from ivDde and Dmab. Inset: UV spectrum of the indazole.

The cleavage of Dmab differs from that of ivDde in that it is a two step process (Figure 2). Firstly, hydrazine initially removes the N-ivDde group, then in a second step, the resultant *p*-amino benzyl ester undergoes a 1,6-elimination with concurrent release of the carboxylic acid. Sluggish cleavage of the aminobenzyl moiety has been occasionally observed [3-5], and appears to be very sequence dependent. In these instances washing the support with 20% DIPEA in DMF/water (90:10) [3] or 2 mM HCl in dioxan [6] has been found to be efficacious.

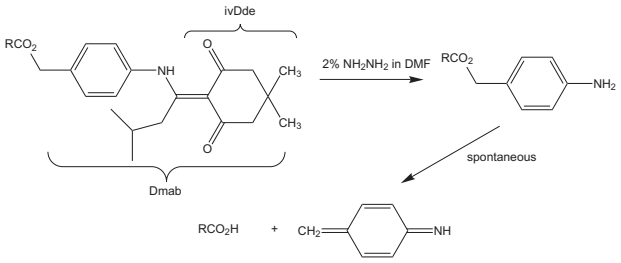
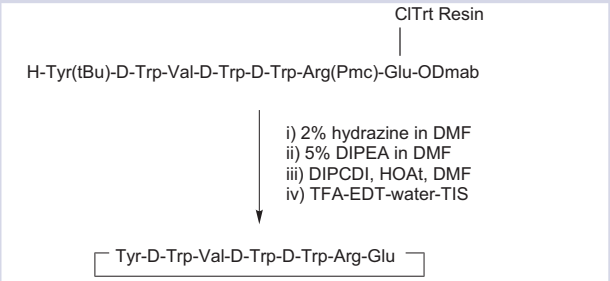


Fig. 2: Removal of Dmab.

There are a few considerations to be taken into account when using Dmab and ivDde protected amino acids. As hydrazine will remove Fmoc, assembly of the peptide backbone must be completed prior to deprotection of the Dmab or ivDde side chain. Peptides with N-terminal Glu(0Dmab) residues should not be left to stand with the α-amino functionality unprotected as this can lead to pyroglutamate formation. Finally, in common with Asp(OBzl) [7] and Asp(OAll) [8], Asp(ODmab) residues can undergo aspartimide formation. Fortunately, this can be prevented by using the Hmb-derivative for introduction of the previous residue.
The most important application of Glu and Asp Dmab esters is the on-resin synthesis of cyclic peptides, which is illustrated by the example below.

Synthesis of cyclo(Tyr-D-Trp-Val-D-Trp-D-Trp-Arg-Glu)



Removal of Dmab was effected by flowing 2% hydrazine monohydrate in DMF through the resin bed, until no further release of indazole by-product could be detected by spectrophotometric monitoring at 290 nm. The resin bound hydrazine salt of glutamic acid was then converted to a DIPEA salt by washing the remainder of the resin with 5% DIPEA in DMF. On-resin cyclization was achieved by treatment of the resin with DIPCDI (1.1 eq.) and HOAt (1.1 eq.) for 18 h. Cleavage was carried out by treatment of the peptidyl resin with TFA/TIS/water/EDT (90:1:5:4) for 2h. The crude peptide was analyzed by HPLC (Figure 3) and characterized by PD-MS [expected $M+H^+$ 1107.2, found 1107.7].

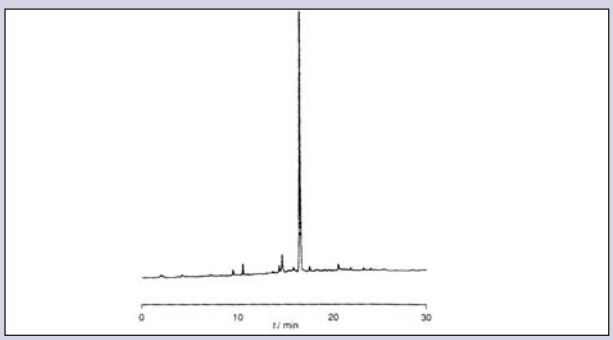
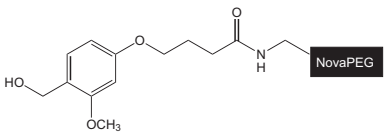


Fig. 3: HPLC elution profile of crude cyclo(Tyr-D-Trp-Val-D-Trp-D-Trp-Arg-Glu).

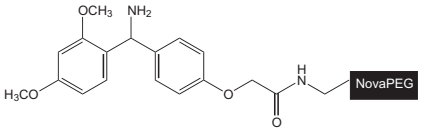
04-13-1083	Fmoc-D-Glu-ODmab	1 g
NEW		5 g
04-12-1176	Fmoc-Asp-ODmab	1 g
		5 g
04-12-1175	Fmoc-Asp(ODmab)-OH	1 g
		5 g
04-12-1174	Fmoc-Glu-ODmab	1 g
		5 g
04-12-1173	Fmoc-Glu(ODmab)-OH	1 g
		5 g
04-12-2094	Fmoc-Asp(Wang LL)-ODmab	1 g
NEW		5 g
04-12-2095	Fmoc-Glu(Wang LL)-ODmab	1 g
NEW		5 g
04-12-2071	Fmoc-Glu(Wang resin)-ODmab (100-200 mesh)	1 g
		5 g
04-12-1195	Fmoc-Dpr(ivDde)-OH	1 g
		5 g
04-13-1074	Fmoc-D-Dpr(ivDde)-OH	1 g
		5 g
04-12-1196	Fmoc-Dab(ivDde)-OH	1 g
		5 g
04-13-1075	Fmoc-D-Dab(ivDde)-OH	1 g
		5 g
04-12-1193	Fmoc-Lys(ivDde)-OH	1 g
		5 g
04-12-1203	Fmoc-Orn(ivDde)-OH	1 g
		5 g

NEW PEG-based resins for smart peptide synthesis

NovaPEG HMPB resin



NovaPEG Rink Amide LL

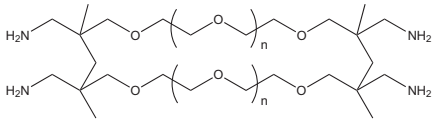


Features & Benefits

- Consist of 100% cross-linked PEG
- Contain no polystyrene or polyacrylamide backbone
- Superior results for difficult or long peptide sequences
- Reduced loading of NovaPEG Rink Amide resin LL

NovaPEG Rink Amide resin LL and NovaPEG HMPB resin are the latest additions to our range of NovaPEG resins [9]. NovaPEG Rink Amide resin LL is a special low load version of NovaPEG Rink Amide resin that is ideal for the synthesis of long and difficult peptides. NovaPEG HMPB resin comprises amino NovaPEG resin derivatized with Riniker's 1% TFA labile HMPB linker [10] and is an excellent support for the synthesis of protected peptide fragments.

NovaPEG resin



NovaPEG resins are novel solid phase supports for solid phase peptide and organic synthesis [9]. Unlike other PEG-based polymer supports, such as NovaSyn® TG and PEGA resins, which contain either polystyrene or polyacrylamide backbones, NovaPEG resin contains only PEG units. This unique composition confers excellent swelling and mechanical properties on the polymer. The resin beads have similar swelling properties to PEGA resins (Figure 4), but unlike PEGA resins are free flowing beads in the dry state, making them much easier to handle.

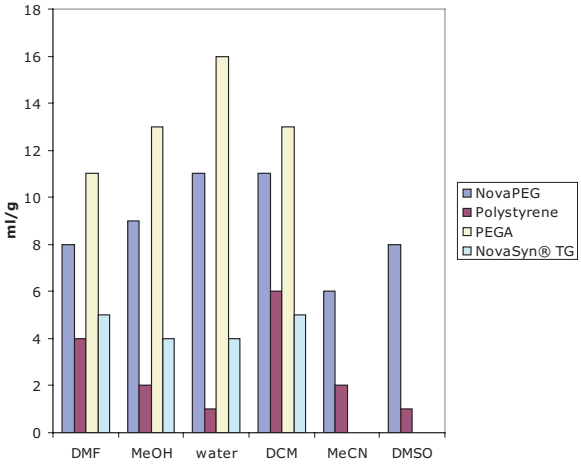


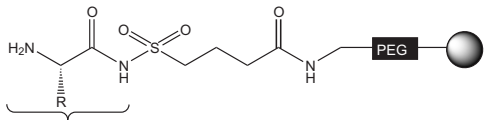
Fig. 4: Swelling properties of NovaPEG and other resins.

The amphilic nature of these resins makes them excellent supports for the synthesis of difficult, aggregated peptides and of long peptides and small proteins [9]. Remarkable synergies have been observed when they are used in combination with pseudoproline dipeptides. For example, the recently reported syntheses of the chemokines Rantes [11] and CCL4-L1 [12] could only be achieved by employing both NovaPEG resins and pseudoproline dipeptides.

01-64-0483	NovaPEG Rink Amide resin LL	1 g
NEW		5 g
01-64-0473	NovaPEG Rink Amide resin	1 g
		5 g
01-64-0478	NovaPEG HMPB resin	1 g
NEW		5 g
01-64-0472	NovaPEG amino resin	1 g
		5 g
01-64-0474	NovaPEG Wang resin	1 g
		5 g
01-64-0477	NovaPEG FMPB resin	1 g
NEW		5 g

NEW Resin for making peptide thioesters

H-Thr(tBu)-Sulfamylbutyryl NovaSyn® TG resin



Ala, Asn(Trt), Gln(Trt), Gly, Ile, Leu, Lys(Boc), Phe, Thr(tBu), Val

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

H-Thr(tBu)-Sulfamylbutyryl NovaSyn® TG resin is the latest addition to our range of pre-loaded sulfamylbutyryl resins. With all these supports, coupling of the first amino acid to the sulfamyl linker is carried out in solution prior to attachment of the purified, 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.

04-12-3732	H-Thr(tBu)-Sulfamylbutyryl NovaSyn® TG resin	1 g
NEW		5 g
04-12-3715	H-Ala-Sulfamylbutyryl NovaSyn® TG resin	1 g
		5 g
04-12-3730	H-Asn(Trt)-Sulfamylbutyryl NovaSyn® TG resin	1 g
NEW		5 g
04-12-3717	H-Gln(Trt)-Sulfamylbutyryl NovaSyn® TG resin	1 g
		5 g
04-12-3714	H-Gly-Sulfamylbutyryl NovaSyn® TG resin	1 g
		5 g
04-12-3727	H-Ile-Sulfamylbutyryl NovaSyn® TG resin	1 g
		5 g
04-12-3728	H-Leu-Sulfamylbutyryl NovaSyn® TG resin	1 g
		5 g
04-12-3724	H-Lys(Boc)-Sulfamylbutyryl NovaSyn® TG resin	1 g
		5 g
04-12-3731	H-Phe-Sulfamylbutyryl NovaSyn® TG resin	1 g
NEW		5 g
04-12-3726	H-Val-Sulfamylbutyryl NovaSyn® TG resin	1 g
		5 g

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