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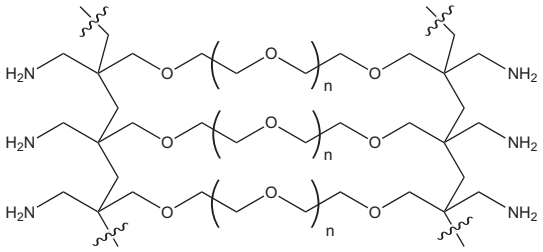
Contents

Product Focus: New resins for peptide synthesis

NEW PEG-based resins for smart peptide synthesis

NovaPEG



Features & Benefits

- Comprise 100% cross-linked PEG
- Contain no polystyrene or polyacrylamide backbone
- Superior results for difficult or long peptide sequences
- Higher loading than NovaSyn® TG or PEGA resins

Novabiochem®'s NovaPEG resins are novel solid phase supports for solid phase peptide and organic synthesis [1]. 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 [2] (Figure 1), but unlike PEGA resins are free flowing beads in the dry state, making them much easier to handle. Furthermore, in contrast to polystyrene and other commonly used supports, NovaPEG resin

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appears not to suffer from osmotic shock when solvent is exchanged from hydrophobic to hydrophilic solvents [1]. NovaPEG resin also has excellent chemical stability, particularly towards strong acids and bases.

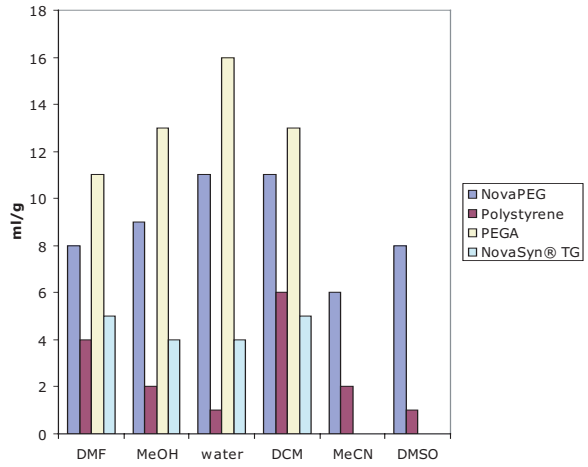
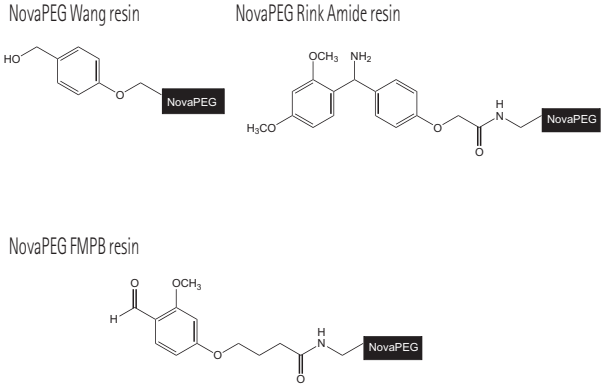


Fig. 1: Swelling properties of NovaPEG and other resins.

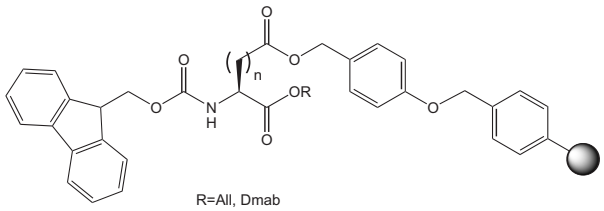
The hydrophilic nature of these resins makes them excellent supports for the synthesis of difficult, aggregated peptides and of long peptides and small proteins. In a reported synthesis of Bacuma [1], a 38-residue potential synthetic vaccine, the use of polystyrene-based supported gave an extremely heterogeneous product, whereas NovaPEG Rink Amide resin afforded the target in excellent purity and yield. More remarkable is the result obtained from the synthesis of β-amyloid (1-42) [1]. This sequence is notoriously difficult to prepare owing to its propensity to aggregate. Numerous strategies have been advocated for its synthesis, including the use of DBU, Hmb-dipeptides, and O-N intramolecular acyl migration. Using NovaPEG resin, this extremely problematic peptide was obtained in a crude purity of 91% using standard Fmoc SPPS methods.

Novabiochem presently offers aminomethyl, Rink Amide, FMPB and Wang NovaPEG resins. Resins with other linkers will be offered in the near future.



01-64-0472	NovaPEG amino resin hydrochloride	1 g 5 g 25 g
01-64-0474	NovaPEG Wang resin	1 g 5 g 25 g
01-64-0473	NovaPEG Rink Amide resin	1 g 5 g 25 g
01-64-0477 *NEW*	NovaPEG FMPB resin	1 g 5 g 25 g

NEW Resins for the synthesis of cyclic peptides



Features & Benefits

- Allow on-resin synthesis of cyclic peptides
- Ideal for high-throughput synthesis of libraries of cyclic peptides

The synthesis and biological properties of cyclic peptides is of enormous interest. Introduction of conformational restraint through head-to-tail cyclization is a standard strategy in medicinal chemistry for increasing the receptor affinity and selectivity of peptide ligands. Furthermore, cyclization has often been employed as a means of prolonging the duration of action of peptide hormones, since in general cyclic peptides are more stable to proteolysis than their linear counterparts [3]. Cyclic peptides are also used as synthetic immunogens [4], as by restricting conformational flexibility, the peptide is thought to adopt a conformation which more closely mimics that of the epitope as presented on the surface of the native protein.

The on-resin cyclization is the simplest approach for the preparation of head-to-tail cyclic peptides. The strategy involves anchoring to a solid phase an Asp or Glu residue bearing orthogonal α-carboxyl protection *via* the side chain β- or γ-carboxyl group, respectively [5-8] (Figure 2). The linear peptide is assembled on the α-amino group of the Asp or Glu residue. The orthogonal carboxyl protecting group is then removed and a lactam bridge is formed between the N-terminal amine and C-terminal carboxyl group, before cleavage of the desired cyclic peptide from the solid phase. This approach can give superior results compared to cyclization in solution due to resin induced pseudo-dilution effects. The synthesis of cyclic peptides has been reviewed [9].

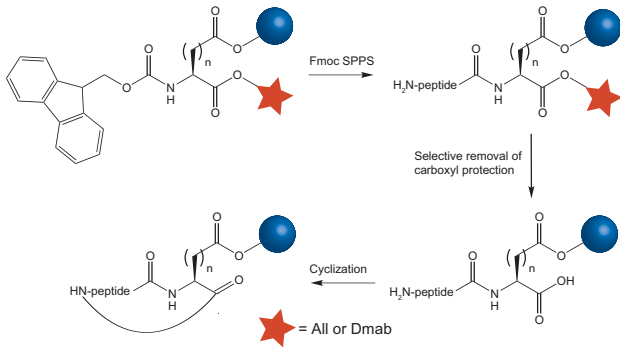


Fig. 2: Synthesis of cyclic peptides via side-chain anchoring of Asp or Glu.

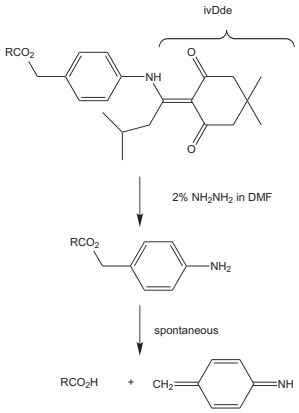


Fig. 3: Selective cleavage of Dmab esters.

To facilitate the synthesis of cyclic peptides, Novabiochem offers Wang LL and NovaSyn® TGA resins pre-loaded with Fmoc-Asp/Glu-OAll and Fmoc-Asp/Glu-ODmab residues. Low loaded Wang and NovaSyn® TG were used as base polymers as resins with high substitution have been found to give poor results [5] in on-resin cyclization reactions. The tentacle nature of NovaSyn® TG resins is also thought to help minimize inter-chain oligomerization.

On-resin removal of the allyl group is best achieved using the method of Kates et al. [6] as described in Method 1. It is important to note that the allyl group is not compatible with the conditions employed for the removal of the Dmab or ivDde group [10]. It is thought that the presence of a small amount of diazine in hydrazine causes reduction of the double bond in the allyl group. Fortunately, this side reaction can be easily overcome by the addition of allyl alcohol to the hydrazine reagent.

Method 1: Removal of allyl protecting groups

IMPORTANT: This reaction is air-sensitive and all manipulations should be carried out under Ar.

1. Weigh the peptidyl resin into a test tube and dry at 40°C under high vacuum. Seal the tube with a rubber septum. Flush the vessel with a stream of Ar delivered via a needle inserted through the septum.
2. Weigh $Pd(PPh_3)_4$ (3 eq.) into a dry test tube, add $CHCl_3$-AcOH-N-methylmorpholine (37:2:1) (15 ml/g of resin), dissolve catalyst by bubbling a stream of Ar through the solution, and seal the tube with a rubber septum.
3. Transfer this mixture using an Ar flushed gas-tight syringe to the tube containing the resin. Leave to stand for 2 h with occasional gentle agitation.
4. Transfer the resin to a sintered glass funnel and wash consecutively with 0.5% DIPEA in DMF and sodium diethyldithiocarbamate (0.5% w/w) in DMF to remove the catalyst.

NOTE: If the N-terminal Fmoc group is removed after cleavage of the allyl ester or if a carbodiimide is to be used to effect cyclization, then the resin should also be washed with HOBt/DMF.

Selective on-resin deprotection of Dmab is effected by treatment with 2% hydrazine in DMF [11]. Removal of Dmab involves a two step process: treatment with hydrazine initially removes the N-ivDde group; this is then followed by collapse of the resultant *p*-amino benzyl ester, with concurrent release of the carboxylic acid (Figure 3).

The deprotection reaction can either be carried out in a batch-wise or continuous flow manner. In the latter case, the reaction can be monitored spectrophotometrically at 290 nm by following release of the indazole and then iminomethine by-products. Sluggish cleavage of the aminobenzyl moiety has been occasionally observed [12], and appears to be very sequence dependent. In these instances washing the support with 2mM HCl aq. in dioxan [13] has been found to be efficacious. It is important to note that as hydrazine will remove Fmoc, assembly of the peptide backbone must be completed prior to deprotection of the Dmab side chain. The N-terminus of the peptide should be protected with Boc. This can be achieved either by direct incorporation of the N-terminal residue as a Boc protected amino acid or acylation of the free N-terminal amino group with Boc_2O.

Fmoc-Asp-ODmab has been employed to prepare a cyclic analog of pyrrhocoricin [14], a 29-mer head-to-tail cyclic peptide [15], and chlorofusin peptide [16].

Method 2: Selective removal of Dmab 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.

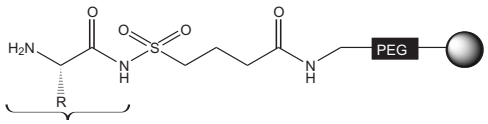
Continuous flow

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.

04-12-2179	Fmoc-Asp(NovaSyn TGA)-OAll	1 g
		5 g
04-12-2092	Fmoc-Asp(Wang resin LL)-OAll	1 g
NEW		5 g
04-12-2094	Fmoc-Asp(Wang resin LL)-ODmab	1 g
NEW		5 g
04-12-2681	Fmoc-Glu(NovaSyn TGA)-OAll	1 g
NEW		5 g
04-12-2093	Fmoc-Glu(Wang resin LL)-OAll	1 g
NEW		5 g
04-12-2095	Fmoc-Glu(Wang resin LL)-ODmab	1 g
NEW		5 g

NEW Resin for making peptide thioesters

H-Phe-Sulfamylbutyryl NovaSyn® TG resin



Ala, Asn(Trt), Gln(Trt), Gly, Ile, Leu, Lys(Boc), Phe, 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-Phe-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.

04-12-3731	H-Phe-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
		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
04-12-3726	H-Val-Sulfamylbutyryl NovaSyn® TG resin	1 g
		5 g

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