Novabiochem®

Letters: 04/04

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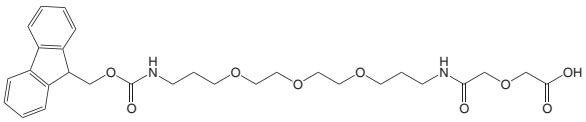
Product Focus: PEG reagents

Bifunctional amino-PEG-acid spacers

NEW **Fmoc–NH–$(PEG)_2$–COOH (20 atoms)**



Features & Benefits

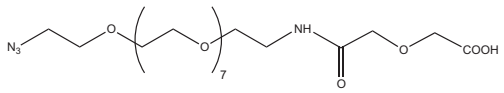
- 20 atom (~ 20 Å) amino-PEG-acid spacers
- Introduced using standard activation methods
- Compatible with Fmoc SPPS
- Imparts solubility to end-product

Fmoc-NH-$(PEG)_2$-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, and is compatible with standard TFA cleavage protocols.

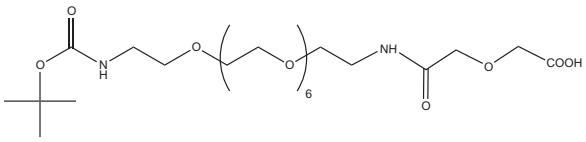
Such derivatives have numerous applications in peptide and protein chemistry. For example, they can be used as flexible spacers to link peptides together, to separate peptides from reporter groups such as fluorophores and biotin, or to increase peptide solubility in both aqueous and organic solvents [1-3]. When employed to link biotin or fluorophores, the PEG spacer can also improve biological activity by reducing the steric hindrance that results from the introduction of the reporter group.

MERCK

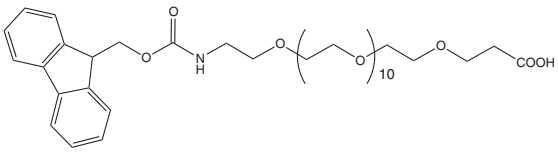
O-(2-Azidoethyl)-O'-(N-diglycolyl-2-aminoethyl)-hepta-ethyleneglycol



O-(N-Boc-2-aminoethyl)-O'-(N-diglycolyl-2-aminoethyl)-hexaethyleneglycol



O-(N-Fmoc-2-aminoethyl)-O'-(2-carboxyethyl)-undeca-ethyleneglycol



For applications requiring spacers of longer chain length, Novabiochem offers O-(2-azidoethyl)-O'-(N-diglycolyl-2-aminoethyl)-heptaethyleneglycol, O-(N-Boc-2-aminoethyl)-O'-(N-diglycolyl-2-aminoethyl)-hexaethyleneglycol, and O-(N-Fmoc-2-aminoethyl)-O'-(2-carboxyethyl)-undecaethyleneglycol. These derivatives are prepared from highly purified monodisperse PEG to ensure homogeneous products free from contaminating oligomers. Unlike similar PEG linkers based on polydisperse PEGs, products prepared using these reagents will be single chemical entities and can therefore be characterized and purified using standard techniques.

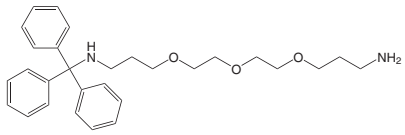
High molecular weight PEGs

Derivatization of therapeutic proteins with high molecular PEG is frequently used to improve their resistance to proteolysis, increase plasma half-life, and reduce their immunogenicity [4]. In recent years, there has been a move away from using polydisperse PEG chains, with their inherent problems with characterization and reproducibility, towards using chains of defined composition [5]. Using Novabiochem's amino-PEG-acid building blocks, such high molecular weight PEGs of defined chain length can be assembled quickly and efficiently by step-wise solid phase synthesis.

01-63-0141	Fmoc-NH-$(PEG)_2$-COOH (20 atoms)	1 g
NEW		
01-63-0103	O-(2-Azidoethyl)-O'-(N-diglycolyl-2-aminoethyl)-heptaethyleneglycol	1 g
01-63-0102	O-(N-Boc-2-aminoethyl)-O'-(N-diglycolyl-2-aminoethyl)-hexaethyleneglycol	1 g
01-63-0109	O-(N-Fmoc-2-aminoethyl)-O'-(2-carboxy-ethyl)-undecaethyleneglycol	1 g

NEW Protected PEG diamine

Trt-NH-$(PEG)_2$-NH_2



Features & Benefits

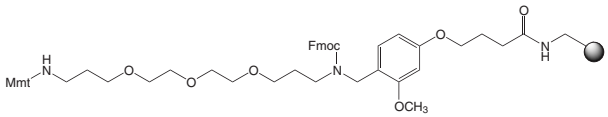
- Mono-protected diamine eliminates cross-linking
- Trt group removed with 1-5 % TFA in DCM
- Compatible with standard Fmoc peptide synthesis protocols

This mono-protected diamino-functionalized PEG is a useful building block for the introduction of a PEG spacer into molecules containing a carboxylic acid functionality. Removal of the Trt group can be effected using 1-5 % TFA in DCM (2004/5 Catalog, Method 4-4, page 4.14).

01-63-0143	Trt-NH-$(PEG)_2$-NH_2	1 g
NEW		

PEGylation resin

Universal PEG NovaTag™ resin



Features & Benefits

- Provides peptides with a C-terminal PEG spacer
- PEG spacer improves solubility of peptides
- Facilitates introduction of different groups at N- and C-termini of peptides
- Mmt group removed with HOBt/TFE/DCM
- Compatible with standard Fmoc peptide synthesis protocols

For the introduction of a PEG spacer at the C-terminus of peptides, Novabiochem offers Universal PEG NovaTag™ resin [6]. This unique product consists of a bifunctional PEG chain built into a standard acid-cleavage linker.

This resin is particularly useful for preparing peptides labeled with fluorophores, as the PEG greatly enhances product solubility. Moreover, the use of orthogonal protecting groups allows, from a single solid phase assembly, the preparation of peptides bearing different acyl moieties at their N- and C-termini (Figure 1). This can be particularly advantageous when preparing FRET peptides since it is not always apparent at the outset which is the optimum combination of fluorophore and quencher for a given application. In addition, this approach allows chromophores, such as FAM and TAMRA, which are not totally stable to the conditions employed in Fmoc SPPS [6], to be introduced in the final step.

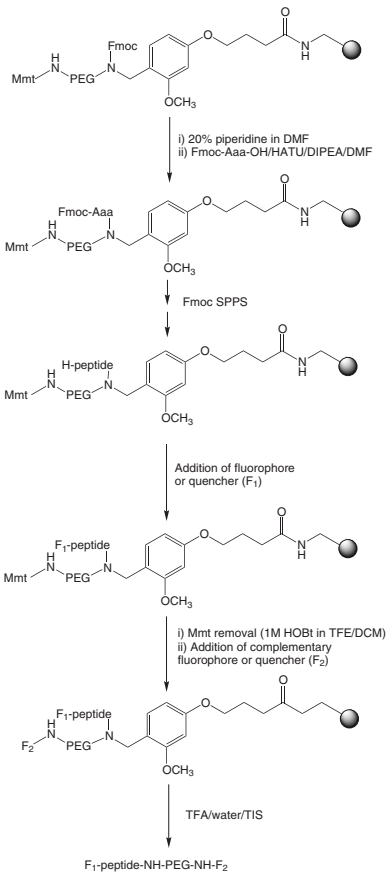


Fig. 1: Synthesis using Universal PEG NovaTag™ resin.

After loading of the first amino acid to the resin-bound secondary amine using HATU, chain extension is carried out under standard Fmoc methods. Following synthesis, the resin can be partitioned and each aliquot end-capped with the appropriate carboxyl-functionalized label. The Mmt group is then removed using HOBt/TFE/DCM (Method 1), and the C-terminal label introduced to each resin aliquot. Thus, from a single synthesis any number of label variations for a given sequence can be prepared.

04-12-3911	**Universal PEG NovaTag™ resin**	0.5 g 1 g

Method 1: Loading Biotin-PEG NovaTag™ resin

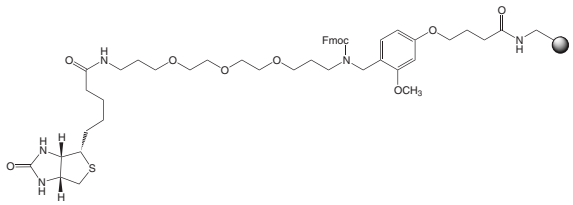
1. Suspend resin in DMF and leave to swell for 30 min. The Fmoc group should be removed at this stage with 20% piperidine in DMF.
2. Dissolve Fmoc-amino acid (2.5 eq.) and HATU (2.5 eq.) in minimum volume of DMF and add to resin. Add DIPEA (5 eq.) and mix. Leave the mixture to stand for 2 h with gentle agitation. A sample of resin can be removed and the loading determined using the Fmoc UV assay [see 2004/5 Catalog, Method 3-6, page 3.4]. Repeat the coupling with fresh reagents if necessary.
3. The resin is removed by filtration, washed with DMF and used immediately in synthesis, or washed further with DCM and then MeOH, dried and stored for later use.

Method 2: Removal of Mmt group

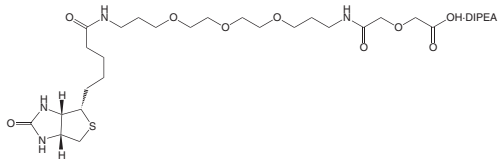
1. Add 1 M HOBt in DCM/TFE (1:1) to resin swollen in DCM.
2. Gently agitate for 1h; solution goes dark red.
3. The solvent is removed by filtration, and steps 1 & 2 are repeated.
4. The resin is removed by filtration, washed with DMF and used immediately in synthesis, or washed further with DCM and then MeOH, dried and stored for later use.

Solubilizing biotinylation reagents

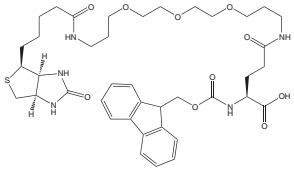
Biotin-PEG NovaTag™ resin



N-Biotinyl-NH-$(PEG)_2$-COOH•DIPEA (20 atoms)



Fmoc-Glu(biotinyl-PEG)-OH



Features & Benefits

- Improves solubility of biotinylated peptide
- Reduces hindrance between biotin and peptide
- Compatible with standard Fmoc peptide synthesis protocols

Biotin-labeled peptides have many important applications in biochemistry, such as affinity purification [7] and FRET-based flow cytometry [8], solid-phase immunoassays [9], and receptor localization [10], that exploit the high affinity of streptavidin and avidin for biotin. However, biotinylated peptides often have poor solubility, which can cause problems with dissolution for bioassay and product purification.

One effective way to ameliorate this problem is to incorporate a PEG-based spacer between the peptide and biotin. This not only greatly improves the solubility of the resultant peptide, but reduces steric hindrance between the peptide and biotin, leading to better avidin binding and higher biological activity. Incorporation of the biotin-PEG label is best carried out during solid phase synthesis of the peptide ligand, with the optimum location for the biotin label dependent on the nature of the application. Novabiochem therefore offers a range of resins and reagents for introduction of the biotin-PEG label to the N- or C-terminus of a peptide, or to an amino-acid side chain.

The simplest approach to obtain biotin-PEG modified peptides is to use Novabiochem's Biotin-PEG NovaTag™ resin [1]. As it is supplied pre-loaded with biotin, the need to carry out a separate biotinylation step is thus eliminated, making it

particularly convenient for the synthesis of peptide arrays. Loading of the first residue to Biotin-PEG NovaTag™resin requires acylation of the resin-bound secondary amine and is best carried out using HATU activation. Chain extension and cleavage can then be effected using standard methods.

For addition of biotin-PEG to the N-terminus of a resin - bound peptide, Novabiochem offers N-Biotinyl-NH-$(PEG)_2$-COOH, whereas for introduction of a biotin-PEG moiety into the peptide-chain, the pre-formed building block Fmoc-Glu(biotinyl-PEG)-OH can be used. Both derivatives can be introduced using standard coupling methods, such as PyBOP® or TBTU, and have much better solubility in DMF and NMP than comparable non-PEG-containing derivatives, such as biotin-Ahx and biocytin.

04-12-3908	Biotin-PEG NovaTag™ resin	0.5 g 1 g
01-63-0133 ***NEW***	Biotinyl-NH-$(PEG)_2$-COOH•DIPEA (20 atoms)	0.5 g 1 g
04-12-1250	Fmoc-Glu(biotinyl-PEG)-OH	0.5 g 1 g

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