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Letters: 1/06

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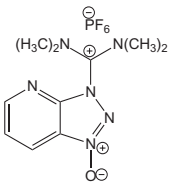
Product Focus: Reagents for peptide synthesis

NEW Coupling reagent

HATU



Features & Benefits

- Analog of HBTU that generates highly reactive OAt esters
- Promotes coupling with higher efficiency and less enantiomerization than HBTU or PyBOP®
- Preferred reagent for the acylation of N-alkyl and constrained amino acids
- Gives less epimerization during fragment condensation
- Excellent coupling reagent for mediating cyclization of peptides

Novabiochem® is very pleased to offer HATU [1], one of the most effective coupling reagents described to date. In the presence of base, HATU cleanly converts carboxylic acids to the corresponding OAt esters. These esters are far more reactive than the corresponding OBt esters towards aminolysis, due to OAt being a better leaving group than OBt and the active

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participation of the pyridine nitrogen in the amide bond forming reaction. This greater reactivity leads to higher coupling yields with less enantiomerization.

HATU has been found to be superior to conventional reagents at mediating the acylation of N-alkylated [2, 3] and conformationally constrained amino acids [4] and at promoting ring closure during the synthesis of cyclic peptides [5, 6, 7] and depsipeptides [8]. It is the reagent of choice for effecting the acylation of the secondary amino groups of resins like Biotin-PEG NovaTag™ resin or Weinreb resin [9]. Furthermore, the high reactivity of OAt esters makes HATU an excellent coupling reagent to use in the preparation of long [10] or difficult peptides [11].

When used in combination with the hindered base collidine, HATU has been found to be highly effective for promoting fragment condensation [12, 13] with low levels of epimerization. Similar benefits have also been observed during the coupling of optically labile chiral peptide nucleic acids [14].

HATU can be used exactly in the same manner as HBTU or PyBOP® (Method 1). Note: when using HATU, it is not standard practice to also add HOAt to the coupling mixture. Solutions of HATU are stable for a number of weeks, provided they are kept under nitrogen, and can be safely used on automated synthesizers, such as the ABi 433, for a considerable number of cycles without loss of coupling efficiency [15].

Method 1: HATU activation

1. Dissolve the Fmoc-amino acid (3 eq. relative to resin loading) and HATU (3 eq.) in the minimum volume of DMF.
2. Add DIPEA (6 eq.) to the amino-acid solution.
3. Stir the mixture and add immediately to resin.

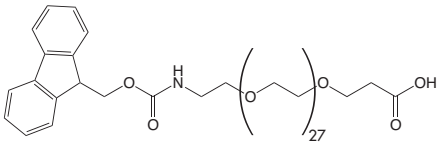
01-62-0041	HATU	5 g
NEW		25 g

Novabiochem®'s other coupling reagents

01-62-0001	BOP	5 g
		25 g
		100 g
01-62-0010	HBTU	5 g
		25 g
		100 g
01-62-0038	HCTU	5 g
		25 g
		100 g
01-62-0016	PyBOP®	5 g
		25 g
		100 g
01-62-0015	TBTU	5 g
		25 g
		100 g

NEW PEG building block

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



Features & Benefits

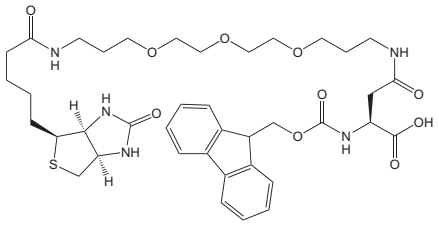
- Mono disperse amino-PEG-acid spacer containing 27 PEG units
- Introduced using standard activation methods
- Compatible with Fmoc SPPS
- Imparts solubility to end-product

Fmoc-NH-$(PEG)_{27}$-COOH is the latest addition to Novabiochem®'s range of PEG-based building blocks for solid phase peptide synthesis. It is prepared from highly purified monodisperse PEG to ensure homogeneous products free from contaminating oligomers. In contrast to similar linkers based on polydisperse PEG, this reagent gives products that are single chemical entities which can be characterized and purified using standard techniques. Fmoc-NH-$(PEG)_{27}$-COOH can be introduced using standard coupling methods, such as PyBOP® or HBTU, and is compatible with TFA cleavage protocols.

01-63-0150	Fmoc-NH-$(PEG)_{27}$-COOH (88 atoms)	500 mg
NEW		1 g

PEGylated biotinylation reagent

Fmoc-Asp(biotinyl-PEG)-OH



Features & Benefits

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

Fmoc-Asp(biotinyl-PEG)-OH is a new tool for the preparation of biotin-labeled peptides. Such peptides have many important applications in affinity purification [16], FRET-based flow cytometry [17], solid-phase immunoassays [18], and receptor localization [19] that exploit the high affinity of streptavidin and avidin for biotin. However, the solid phase synthesis of biotinylated peptides is often hampered by the poor solubility of many of the commonly used biotin derivatives, such as biotin-OSu or Fmoc-

Lys(biotin)-OH, which results in them having to be used in low concentrations with deleterious effects on coupling rates. Furthermore, the biotinylated products often exhibit low solubilities which can complicate subsequent biological testing.

The use of Fmoc-Asp(biotinyl-PEG)-OH helps alleviate many of these difficulties. The introduction of the PEG spacer between the amino acid and the biotin confers excellent solubility in DMF on the derivative. Peptides incorporating this amino acid are also expected to have improved solubilities. The PEG spacer also reduces steric hindrance between the peptide and avidin, which can lead to better biotin binding. Furthermore, the hydrophilic nature of the PEG should prevent the spacer group from becoming buried in the hydrophobic pocket of target proteins.

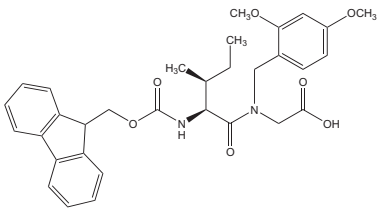
04-12-1279	Fmoc-Asp(biotinyl-PEG)-OH	500 mg
NEW		1 g

Novabiochem's other PEGylated biotinylation reagents

04-12-3908	Biotin-PEG NovaTag™ resin	500 mg
		1 g
01-63-0133	N-Biotinyl-NH-$(PEG)_2$-COOH•DIPEA (20 atoms)	500 mg
		1 g
04-12-1250	Fmoc-Glu(biotinyl-PEG)-OH	500 mg
		1 g

NEW Derivative for enhancing peptide synthesis

Fmoc-Ile-(Dmb)Gly-OH



Features & Benefits

- "Pseudoproline effect" for Ile-Gly-containing sequences
- Compatible with standard Fmoc protocols
- Dmb group removed during TFA cleavage
- Cannot form cyclic lactones

Novabiochem is pleased to introduce Fmoc-Ile-(Dmb)Gly-OH, the latest product in our new range of Dmb dipeptides. These novel derivatives offer the same benefits as pseudoproline dipeptides but for peptide sequences containing Gly. They work in exactly the same way as pseudoproline dipeptides by exploiting the natural propensity of N-alkyl amino acids [20, 21] (Figure 1) to disrupt the formation of the secondary structures during peptide assembly. The results are better and more predictable acylation and deprotection kinetics, enhanced reaction rates, and improved yields, purities and solubilities of crude products. The use of Fmoc-Ile-(Dmb)Gly-OH should prove to be particularly beneficial in the synthesis of β-amyloid peptides since they contain an Ile-Gly motif within the highly aggregation-prone C-terminal sequence.

Dmb dipeptides are extremely easy to use: simply substitute a Gly residue together with the preceding Ala, Ile, or Gly residue in the peptide sequence with the appropriate Dmb dipeptide. They are fully compatible with standard coupling methods such as PyBOP® or HBTU, since unlike analogous Hmb-based derivatives, they cannot form cyclic lactones [22]. Removal of the Dmb group and regeneration of the glycine residue occurs during the course of the standard TFA-mediated cleavage reaction. It is important to note, however, that the Dmb cation produced during this process is a very powerful alkylating agent and can cause side-chain modification of unprotected tryptophan residues. Therefore, the use of Fmoc-Trp(Boc) is strongly recommended in such cases.

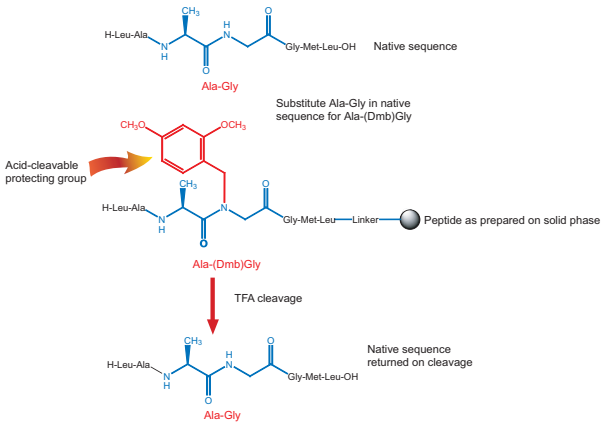


Fig. 1: Principles of using Dmb dipeptides.

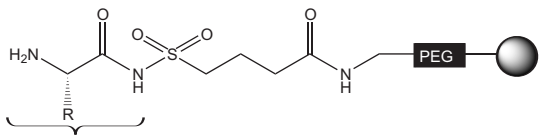
04-12-1280	Fmoc-Ile-(Dmb)Gly-OH	1 g
NEW		

Novabiochem's other Dmb dipeptides

04-12-1265	Fmoc-Ala-(Dmb)Gly-OH	1 g
NEW		
04-12-1266	Fmoc-Gly-(Dmb)Gly-OH	1 g
NEW		

NEW Resin for making peptide thioesters

H-Asn(Trt)-Sulfamylbutyryl NovaSyn® TG resin



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

04-12-3730	H-Asn(Trt)-Sulfamylbutyryl NovaSyn® TG resin	1 g
NEW		5 g

Novabiochem's other pre-loaded sulfamylbutyryl resins

04-12-3715	H-Ala-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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