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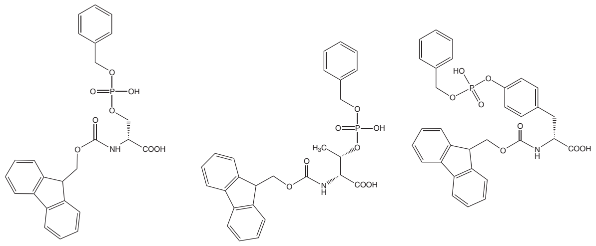
Letters: 03/04

Contents

Product Focus: Amino acids for Fmoc SPPS

NEW Derivatives for the introduction of D-phosphoamino acids

Fmoc-D-Aaa(PO(OBzl)OH)-OH; Aaa=Ser, Thr, Tyr



Features & Benefits

- Direct synthesis of phosphopeptides by automated methods
- Introduced using HBTU/DIPEA activation methods
- Compatible with Fmoc SPPS
- No need for difficult post-synthetic phosphorylation

Since their commercial introduction by Novabiochem in 1994, Fmoc-Ser(PO(OBzl)OH)-OH [1], Fmoc-Thr(PO(OBzl)OH)-OH [2, 3], and Fmoc-Tyr(PO(OBzl)OH)-OH [2, 3] have become

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the reagents of choice for the synthesis of phosphopeptides by Fmoc SPPS. The use of these building blocks enables phosphoamino acids to be directly incorporated into a sequence during solid phase synthesis, thereby eliminating the need for complex post-synthetic phosphorylation.

Coupling of these derivatives is best achieved using TBTU or HBTU activation, as described in Method 1, with a 3 -fold excess of base relative to amino acid derivative [4]. With the hindered Thr derivative, the coupling can be sluggish, particularly to β-branched amino acids, so an extended reaction time may be required. Furthermore, the coupling of consecutive phospho residues is also difficult. When synthesizing peptides containing several phospho residues, the excess of activated amino acid may need to be increased to compensate for loss of reagent that results from acylation of the piperidine associated with each acidic phosphate group.

The benzyl side-chain protecting group is normally removed in 3 hours during the course of the standard 95% TFA cleavage reaction. For peptides containing several phospho residues, a longer cleavage time may be required.

Method 1: TBTU activation of Fmoc-Aaa(PO(OBzl)OH)-OH

1. Dissolve Fmoc-Aaa(PO(OBzl)OH)-OH (5 eq.[a]), TBTU/HBTU (5 eq.[a]) and HOBt (5 eq.[a]) in the minimum volume of DMF.
2. Add DIPEA (15 eq.[a]) to mixture, mix and add immediately to Fmoc-deblocked peptide resin.
3. Allow to couple for 1 - 2h.
4. Check completeness of reaction with the Kaiser or TNBS test. Wash resin and repeat reaction if necessary.

[a] Relative to resin substitution.

Method 2: Cleavage and side-chain deprotection of phosphopeptides prepared by Fmoc SPPS

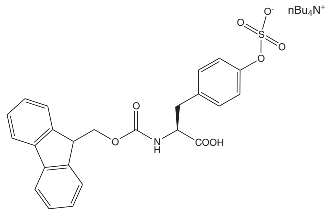
1. Place dry resin in a flask and add TFA solution containing appropriate scavengers[b] (10 - 25 ml/g). Stopper flask and leave to stand at rt for 3 h with occasional swirling.
2. Remove the resin by filtration under reduced pressure. Wash the resin twice with TFA. Combine filtrates and add dropwise 8 - 10 volumes of cold ether.
3. Isolate peptide by filtration or centrifugation.

[b] See Novabiochem 2004/5 catalog, page 3.16 for appropriate selection of scavengers.

04-13-1078	**Fmoc-D-Ser(PO(OBzl)OH)-OH**	1 g
NEW		5 g
04-13-1079	**Fmoc-D-Thr(PO(OBzl)OH)-OH**	1 g
NEW		5 g
04-13-1080	**Fmoc-D-Tyr(PO(OBzl)OH)-OH**	1 g
NEW		5 g

NEW Derivative for introduction of sulfotyrosine

Fmoc-Tyr(SO$_3$·NnBu$_4$)-OH



Features & Benefits

- Direct synthesis of sulfopeptides by automated methods
- No need for complex off-instrument chemistry
- Compatible with standard Fmoc peptide assembly protocols
- Introduced using HBTU/DIPEA activation methods
- Increased acid-stability compared with sodium or barium salts
- Cleave with 90% TFA at 0 °C to minimize desulfation

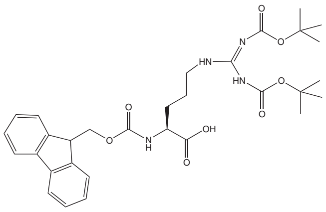
Sulfation of tyrosine is an important post-translational modification [5] which is involved in protein-protein recognition [6] and is found in a number of biologically-active peptides such as gastrin II, cholecystokinin, and caerulein [7].

For the Fmoc SPPS of sulfotyrosine-containing peptides, Novabiochem has introduced Fmoc-Tyr(SO$_3$·NnBu$_4$)-OH. In contrast to other commercially available building blocks for the introduction of this amino acid, which use a metal ion as the sulfate counterion, Novabiochem's derivative employs tetrabutylammonium as this is thought to reduce desulfation during TFA cleavage [8, 9]. Fmoc-Tyr(SO$_3$·NnBu$_4$)-OH is best coupled using TBTU/DIPEA activation. Cleavage from the resin should be carried out using 90% TFA at 0 °C. Using this approach, desulfation can be reduced to less than 15% [7].

04-12-1251	**Fmoc-Tyr(SO$_3$·NnBu$_4$)-OH**	1 g
NEW		5 g

NEW Derivative for introduction of arginine

Fmoc-Arg(Boc)$_2$-OH



Features & Benefits

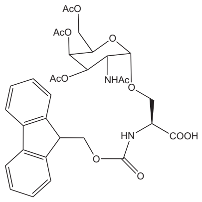
- No sulfonated modifications of Trp or Tyr
- Compatible with standard Fmoc protocols

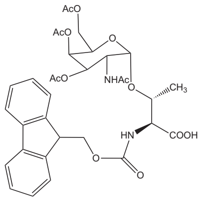
Fmoc-Arg(Boc)$_2$-OH is an excellent derivative for the Fmoc SPPS of arginine-containing peptides [10]. Unlike other arginine derivatives such as Arg(Pmc) or Arg(Pbf), deprotection of the side-chain of Arg(Boc)$_2$ does not release by-products that can cause sulfonation of Trp or Tyr residues [11, 12]. Fmoc-Arg(Boc)$_2$-OH can be coupled using standard activation methods such as PyBOP or TBTU, although longer reaction times compared with Arg(Pbf) may be required due to the bulkiness of the side-chain protecting groups.

04-12-1249	**Fmoc-Arg(Boc)$_2$-OH**	1 g
NEW		5 g

NEW Glycosylated amino acid derivatives

Fmoc-Ser(Ac$_3$AcNH-α-Gal)-OH **Fmoc-Thr(Ac$_3$AcNH-α-Gal)-OH**





Features & Benefits

- Direct synthesis of glycopeptides using automated methods
- Compatible with standard Fmoc protocols

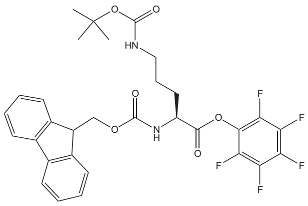
In contrast with N-linked glycosides, which all contain a mannotriosido-di-N-acetyl-chitobiose core linked via a β(1→Nβ) linkage to Asn, O-linked glycosides are structurally diverse. One of the most important classes of O-linked glycosides are based on 2-acetamido-2-deoxy-α-D-galactopyranosyl (AcNH-α-Gal) attached to serine or threonine. Such O-glycosides are found in a wide range of proteins, such as mucin secreted from epithelial cells, the tumor-associated Tn-antigen, and gp120 from HIV [13]. Thus, synthetic O-linked glycopeptides are important tools for elucidating of the role of O-linked glycosides and for drug discovery and design.

To facilitate the synthesis of such peptides, Novabiochem has introduced Fmoc-Ser(Ac$_3$AcNH-α-Gal)-OH and Fmoc-Thr(Ac$_3$AcNH-α-Gal)-OH. The O-glycosidic linkage and the O-acetyl protection in these building blocks are stable to both piperidine and TFA [14], making them completely compatible with standard protocols in Fmoc solid phase peptide synthesis. Removal of the acetyl protecting groups is best carried out by treatment of the peptidyl resin with methanolic ammonia prior to cleavage.

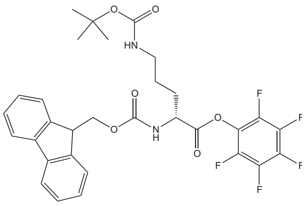
04-12-8103	Fmoc-Ser(Ac$_3$AcNH-α-Gal)-OH	100 mg
NEW		
04-12-8104	Fmoc-Thr(Ac$_3$AcNH-α-Gal)-OH	100 mg
NEW		

NEW Fmoc-amino acid pentafluorophenyl esters

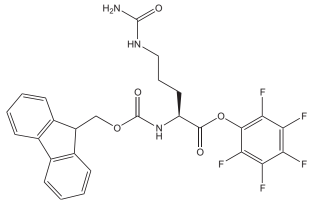
Fmoc-Orn(Boc)-OPfp



Fmoc-D-Orn(Boc)-OPfp



Fmoc-Cit-OPfp

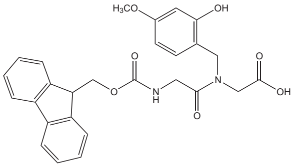


Novabiochem is pleased to offer the pentafluorophenyl esters of Fmoc-protected L- and D-ornithine, and Fmoc-citrulline.

04-12-1545	Fmoc-Cit-OPfp	1 g
NEW		5 g
04-12-1546	Fmoc-Orn(Boc)-OPfp	1 g
NEW		5 g
04-13-1507	Fmoc-D-Orn(Boc)-OPfp	1 g
NEW		5 g

NEW Derivative for enhancing peptide synthesis

Fmoc-Gly-(Hmb)Gly-OH



Features & Benefits

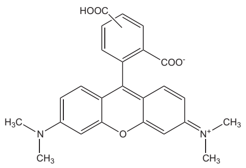
- "Pseudoproline effect" for Gly-Gly-containing sequences
- Eliminates Gly deletions in Gly-Gly repeats
- Compatible with standard Fmoc protocols
- Hmb group removed during TFA cleavage

Fmoc-Gly-(Hmb)Gly-OH is a novel building block for improving the synthesis of peptides containing the commonly occurring Gly-Gly motif. The production of such peptides is often problematic, owing to their propensity to aggregate and the difficulties in separating closely eluting desGly by-products. Insertion of Gly-(Hmb)Gly into a peptide sequence has the same benefits on synthetic efficiency as a pseudoproline dipeptide, preventing aggregation and improving acylation and deprotection kinetics. The use of the analogous Fmoc-Gly-(Dmb)Gly-OH was found to be essential for the synthesis of peptides related to nucleolin [15].

04-12-1253	Fmoc-Gly-(Hmb)Gly-OH	1 g
NEW		5 g

New Fluorescent dye for peptide-labeling

5(6)-Carboxytetramethylrhodamine



This high purity fluorescent dye is a useful tool for preparing fluorescently-labeled peptides and fluorescence-quenched peptide substrates. Novabiochem's 5(6)-carboxytetra-methylrhodamine (TAMRA; λ_{ex} 555 nm; λ_{em} 580 nm) is supplied as a mixture of 5- and 6-isomers, making it cost effective for those applications which do not require a single isomer dye.

5(6)-TAMRA is conveniently introduced during solid phase synthesis by coupling to N-terminal or side-chain amino groups. When it is to be located on a side-chain amino group, the simplest approach is to incorporate an orthogonally-protected derivative, such as Lys(Mtt) or Lys(ivDde), which can be later selectively deprotected on the resin immediately prior to coupling of the dye.

01-63-0134	**5(6)-Carboxytetramethylrhodamine**	100 mg
NEW		500 mg
Novabiochem's single isomer dyes		
01-63-0114	**5-Carboxytetramethylrhodamine**	10 mg
		50 mg
01-63-0115	**6-Carboxytetramethylrhodamine**	10 mg
		50 mg

28th European Peptide Symposium

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