Novabiochem®

Letters: 01/05

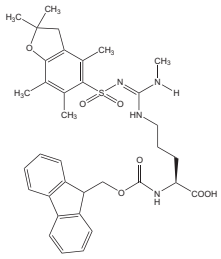
Contents

NEW Derivatives for the introduction of methylated Arg and Lys 1
NEW Derivative for the minimization of aspartimide formation 2
NEW Technical resources on Novabiochem.com 3
NEW Resins for the synthesis of peptide thioesters 3

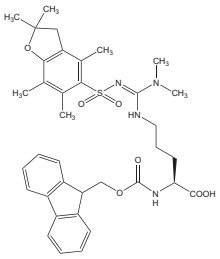
Product Focus: New products for Fmoc SPPS

NEW Derivatives for the introduction of methylated Arg and Lys

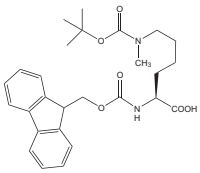
Fmoc-Arg(Me,Pbf)-OH



Fmoc-ADMA(Pbf)-OH



Fmoc-Lys(Me,Boc)-OH



Features & Benefits

- Direct synthesis of methylated Arg and Lys containing peptides by automated methods
- Introduced using PyBOP®/DIPEA activation methods
- Compatible with Fmoc SPPS
- Unspecific post-synthetic methylation can be avoided

Post-translational methylation of arginine and lysine residues is emerging as an important control mechanism for the regulation of protein expression in eukaryotes [1, 2]. Most of the possible methylated derivatives have been found to occur *in vivo*, including mono-, di- and trimethylated lysine, and mono- and dimethylarginine, both asymmetric and symmetric. Whilst

the exact roles of such modifications are currently poorly understood, they are thought to be involved in cell proliferation, signal transduction, protein transport and transcription activation [1 - 3].

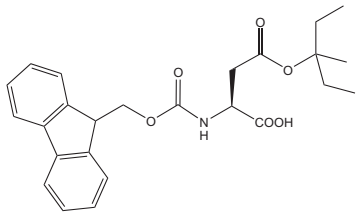
Until recently, work in this field has been hampered by lack of efficient methods for synthesis of peptides containing these amino acids [4], and it is for this reason that Novabiochem® has introduced a range of N-methylated amino acids bearing standard Fmoc-compatible protecting groups.

For the incorporation of the most common arginine modifications, monomethyl arginine and asymmetric dimethylarginine (ADMA), Novabiochem offers Fmoc-Arg(Me,Pbf)-OH and Fmoc-ADMA(Pbf)-OH. These derivatives can be coupled using standard activation methods, and the Pbf group is cleaved with standard TFA cleavage cocktails. Fmoc-Lys(Me,Boc)-OH is available for the introduction of monomethyl lysine and is also fully compatible with Fmoc SPPS protocols. Other methylated amino acid derivatives will be introduced in the near future.

04-12-1264	Fmoc-ADMA(Pbf)-OH	1 g
NEW		5 g
04-12-1261	Fmoc-Arg(Me,Pbf)-OH	1 g
NEW		
04-12-1263	Fmoc-Lys(Me,Boc)-OH	500 mg
NEW		1 g

NEW Derivative for the minimization of aspartimide formation

Fmoc-Asp(OMpe)-OH



Features & Benefits

- Significantly reduces aspartimide formation compared to tBu derivative
- Less dehydration and piperidide by-products
- Introduced using PyBOP® or TBTU/DIPEA activation methods
- Mpe group removed with 95% TFA

Fmoc-Asp(OMpe)-OH is an excellent derivative for minimizing aspartimide formation during Fmoc SPPS [5]. Aspartimides are formed by a ring-closure between the nitrogen of the α-carboxy amide bond and the β-carboxy side-chain, with loss of the ester protecting group (Figure 1) [6, 7]. Such aspartimides are very susceptible to base-catalyzed epimerization [8] and readily undergo ring-opening reactions, which lead to the formation of a complex mixture of epimeric α, β-aspartyl peptides and α, β-piperidides [9 - 11].

Aspartimide formation particularly affects peptides containing Asp-Aaa, where Aaa is Gly, Asp, Arg, or Asn [10 - 12]. The problem is most serious when Aaa=Gly and has been estimated to occur to the extent of approximately 0.5% per Fmoc deprotection cycle [13]. The impact is most apparent in sequences containing more than one site of potential aspartimide formation and during the synthesis of long peptides, since the degree of aspartimide formation is dependent on the total exposure time to piperidine. It is also exacerbated by the use of DBU.

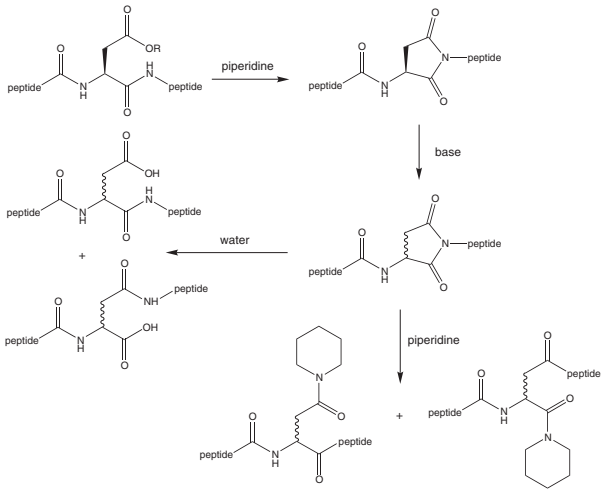


Fig. 1: Mechanism for aspartimide-related by-product formation.

Only masking of the amide bond between the Asp and the following residue offers complete protection against aspartimide formation [13, 14]. For the most problematic Asp-Gly sequence, Novabiochem offers Fmoc-Asp(OtBu)-(Hmb)Gly-OH [15] (see Novabiochem Catalog 2004/5, page 3.10) where the backbone amide is reversibly blocked by an Hmb group. Unfortunately, this solution is not appropriate to other sequences, as such dipeptides will undergo enantiomerization during coupling. In such cases, Novabiochem recommends Fmoc-Asp(OMpe)-OH instead of Fmoc-Asp(OtBu)-OH for the introduction of Asp. By substituting OtBu for the more bulky OMpe group in the synthesis of a model Asp-Gly-containing peptide, aspartimide related by-products were reduced from 58% to 25% following treatment with 20% piperidine for 20 h (equivalent to 120 coupling cycles) [5]. The benefits of using OMpe are even more pronounced when DBU is employed for Fmoc removal. In model studies, a peptide containing Asp(X)-Asp(OtBu) when treated with DBU/piperidine/DMF (1:20:79) for 225 min gave only 9% product when X=OtBu compared to 34% when X=OMpe (see Table 1) [16].

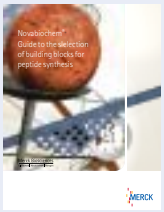
04-12-1259	Fmoc-Asp(OMpe)-OH	1 g
NEW		5 g
04-12-1235	Fmoc-Asp(OtBu)-(Hmb)Gly-OH	1 g
		5 g

Xaa	Yaa	Base	% Product	% Aspartimide by-products
Asp(OtBu)	Asp(OtBu)	piperidine	90	nd
Asp(OMpe)	Asp(OtBu)	piperidine	94	nd
Asp(OtBu)	Asp(OtBu)	DBU	9	44
Asp(OMpe)	Asp(OtBu)	DBU	34	25
Asp(OtBu)	Asn(Mtt)	piperidine	80	18
Asp(OMpe)	Asn(Mtt)	piperidine	92	6
Asp(OtBu)	Asn(Mtt)	DBU	1	81
Asp(OMpe)	Asn(Mtt)	DBU	26	62

Table 1: Effects of base and Asp side-chain protection on aspartimide formation during synthesis of H-Val-Lys-Xaa-Yaa-Tyr-Ile-OH. All Fmoc deprotection reactions were conducted for 225 min [16].

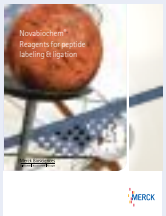
New technical resources on Novabiochem.com

Guide to the selection of building blocks for solid phase peptide synthesis



This valuable resource offers detailed practical advice, exemplified by numerous protocols and real-life examples, to aid selection of the optimum combination of amino acid derivatives for SPPS.

Reagents for labeling & ligation



This brochure features Novabiochem's extensive range of reagents for fluorescent- and biotin-labeling and biomolecule conjugation.

Ever needed to search for a Novabiochem product by chemical structure?

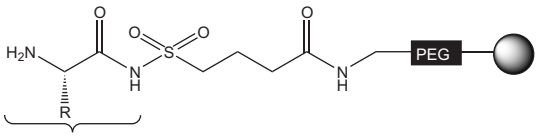
Fully searchable SD file, Chemfinder and ISISbase databases of the complete Novabiochem product line are now available online for download.

Pseudoproline dipeptide resource center

Learn more about pseudoproline dipeptides and their applications. Download Novabiochem literature or access published articles. View some of the remarkable results obtained by customers using pseudoproline dipeptides.

NEW Resins for the synthesis of peptide thioesters

Pre-loaded sulfamylbutyryl resins



Ala, Gln(Trt), Gly, Ile, Leu, 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

Peptide thioesters required for native chemical ligation are usually prepared using the Fmoc strategy by thiolytic cleavage from a sulfamylbutyryl resin (Figure 2) [17- 21]. One of the principal difficulties of using this approach is the initial attachment of the first amino acid onto the sulfamylbutyryl linker: yields are highly variable; problems can occur with over acylation of the sulfamyl group; the substitution of the support must be determined before starting peptide synthesis. For these reasons, Novabiochem has introduced a range of pre-loaded sulfamylbutyryl NovaSyn® TG resins. Here, 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.

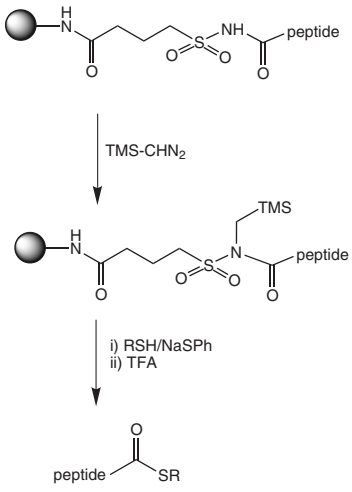


Fig. 2: Synthesis of peptide thioesters using sulfamylbutyryl resin.

The supports can be used directly in automated peptide synthesis without modification of existing protocols. Following chain assembly, the linker is most efficiently activated for cleavage by treatment with TMS-CHN_2 [18]. The resulting N-alkyl-N-acylsulfonamide is cleaved by treatment with ethyl mercaptopropionate/sodium thiophenoxide (Novabiochem Catalog 2004/5, page 5.2, Method 5-1). The use of 2M LiBr in THF as the solvent for the cleavage reaction has been shown to lead to greatly improved yields of peptide thioester [22]. The resulting protected peptide thioester is finally treated with TFA containing the appropriate scavengers to give the deprotected peptide ready for ligation.

04-12-3728	H-Leu-Sulfamylbutyryl NovaSyn® TG resin	1 g
NEW		5 g
04-12-3727	H-Ile-Sulfamylbutyryl NovaSyn® TG resin	1 g
NEW		5 g
04-12-3726	H-Val-Sulfamylbutyryl NovaSyn® TG resin	1 g
NEW		5 g
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

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