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Letters 2 Vol. 15

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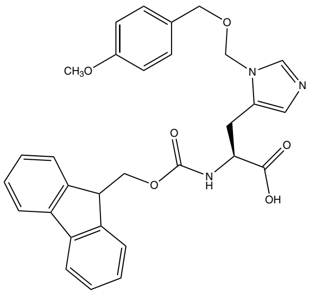
New Building blocks for Fmoc SPPS

NEW • Histidine derivative for minimization of racemization

Fmoc-His(MBom)-OH



Features & Benefits

- N$^{\pi}$-MBom group provides excellent protection against racemization during coupling at room and elevated temperatures
- MBom group is cleaved with TFA

Histidine is notoriously prone to racemization during carboxyl activation due to the propensity of the imidazole π-nitrogen to promote deprotonation of the amino acid α-hydrogen [1 - 4]. In the standard derivative used for the introduction of His residues, Fmoc-His(Trt)-OH, the protecting group resides on the τ-nitrogen and so provides little protection from this side reaction. Significant racemization during coupling has been observed when based-mediated activation and pre-activation is used [6], amide bond formation is slow [5], and at elevated temperatures [6]. In contrast histidine derivatives bearing protection on the π-nitrogen are highly resistant to loss of chiral integrity. Jones and coworkers were the first to describe the use of N$^{\pi}$-imidazole protected derivatives: Boc-His(Bom)-OH [7] and Fmoc-His(Bum)-OH [8] based on the HF-labile benzyloxymethyl (Bom) and TFA labile t-butoxymethyl (Bum) groups, respectively. Others also described N-1-adamantyloxymethyl protection [9] .

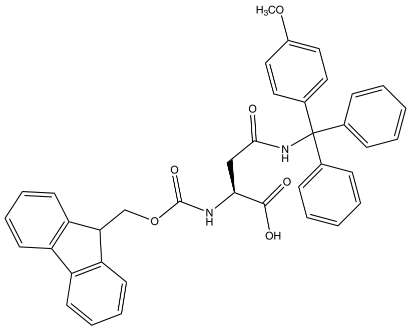
The Novabiochem brand is therefore very pleased to introduce Fmoc-His(MBom)-OH, in which the imidazole π-nitrogen is protected by the TFA-labile p-methoxybenzyloxy (MBom) group [10]. In model studies [6] which compared the loss of chiral integrity of Fmoc-His(Trt)-OH and Fmoc-His(MBom)-OH during HCTU/6-Cl-HOBt/DIPEA (4/4/8) activation, the use of this new derivative resulted in only 0.3% D-His whereas with Fmoc-His(Trt)-OH 7.8 % D-His formation was observed. Under microwave heating at 80°C, N^{τ}-Trt protection resulted in 16.6% D-His whereas the N^{π}-MBom group gave only 0.8%.

MBom groups release upon treatment with TFA formaldehyde and p-methoxybenzyl cations that have the potential to modify Cys and His residues. Reaction of formaldehyde with N-terminal Cys or Trp residues results in formation of a thiazolidine [11] or a tetrahydroxy-β-carboline [12], respectively. This side reaction can be prevented by addition of 5 eq. of methoxyamine hydrochloride to the TFA cleavage cocktail. Alkylation of Trp by p-methoxybenzyl cations can be eliminated by the use of Fmoc-Trp(Boc)-OH for introduction of the Trp residue. Cysteine alkylation can be ameliorated by addition of thiols to the cleavage cocktail.

Cat.No.	Product	Contents
852412	Fmoc-His(MBom)-OH	1 g
NEW		5 g
852052	Fmoc-His(Boc)-OH · CHA	5 g
		25 g
852371	Fmoc-His(Clt)-OH	5 g
		25 g
852402	Fmoc-His(Clt)-OPfp	5 g
852211	Fmoc-His(Mtt)-OH	1 g
		5 g
852032	Fmoc-His(Trt)-OH	25 g
		100 g

NEW • Asparagine derivative

Fmoc-Asn(Mmt)-OH



Features & Benefits

- Mmt group is removed with TFA approximately 3 times faster than Trt
- Eliminates problems with incomplete side-chain deprotection of N-terminal Asn residues
- May improve solubility of protected peptide
- May help reduce aggregation during peptide assembly

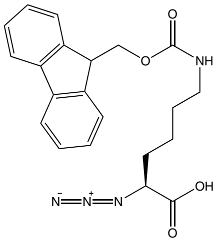
Fmoc-Asn(Trt)-OH, which is currently used for the incorporation of Asn during Fmoc SPPS, is not ideal because cleavage of the side chain N-Trt group is often sluggish. Incomplete Trt removal has been observed with peptides containing reduced amide bonds [13], multiple Asn(Trt) residues and N-terminal Asn(Trt) residues [14]. To overcome this problem, we have introduced Fmoc-Asn(Mmt)-OH.

The p-methoxytrityl group (Mmt) is significantly more acid labile than the Trt group being cleaved approximately 3 times faster than Trt. Furthermore, Mmt is more hydrophilic than the Trt so its use should enhance the solubility of protected peptides and possibly help reduce aggregation during SPPS. Finally, Fmoc-Asn(Mmt)-OH is more soluble in DMF than Fmoc-Asn(Trt)-OH, thereby facilitating coupling reactions at higher concentration.

Cat.No.	Product	Contents
852411	Fmoc-Asn(Mmt)-OH	1 g
NEW		5 g
		25 g
852119	Fmoc-Asn(Dmcp)-OH	1 g
		5 g
852044	Fmoc-Asn(Trt)-OH	25 g
		100 g
852132	Fmoc-Asn(Trt)-OPfp	5 g

NEW • Azido derivative

N_3-Lys(Boc)-OH



Features & Benefits

- Azido group orthogonal to standard protecting groups
- Azido group transformed to amine under mild reductive conditions
- Building blocks compatible with standard Fmoc SPPS methods

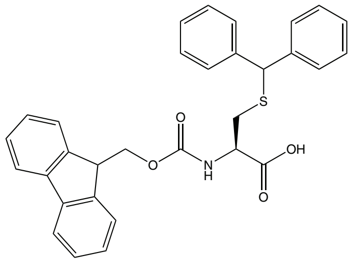
The Novabiochem® brand has one of the largest collections of orthogonally- and selectively-protected amino acid derivatives available. These reagents provide the capability to selectively unmask a single functionality of a peptide without affecting others, thus enabling the facile synthesis of cyclic, branched and side-chain modified peptides.

For the incorporation of differentially-protected amino groups within a peptide sequence, we offer derivatives bearing protecting groups cleavable with reagents ranging from mild acids, Pd(0), alpha-effect nucleophiles, and phosphines. N_3-Lys(Boc)-OH is our latest building blocks for Fmoc SPPS in which the α-amino group is unmasked by reduction. This derivative can be coupled using standard activation methods. The azido group is stable to both TFA and piperidine, so it can be used in both solid phase and solution phase synthetic strategies. Thiols should be omitted from the TFA cleavage mixture as their use can lead to reduction of the azido group [15]. Liberation of the amine group from the azide has been accomplished by reduction with phosphines [16, 17], thiols [18] or Zn/AcOH [19].

Cat.No.	Product	Contents
852415 NEW	N_3-Lys(Fmoc)-OH	1 g 5 g
	Other orthogonally azido acids	
851097	ε-Azidocaproic acid	1 g 5 g
852321	Fmoc-L-γ-azidohomoalanine	100 mg 500 mg
852322	Fmoc-L-δ-azidonorvaline	100 mg 500 mg
852326	Fmoc-L-ε-azidonorleucine	250 mg 1 g
852352	(2S,3S)-Fmoc-Abu(3-N_3)-OH	250 mg 1 g
852351	cis-Fmoc-Pro(4-N_3)-OH	250 mg 1 g

NEW • Cysteine derivative for regioselective disulfide bond formation

Fmoc-Cys(Dpm)-OH



Features & Benefits

- Dpm group is more stable than Trt group
- Dpm is completely stable to 1 - 3% TFA in DCM but completely removed with 95% TFA
- Couples with reduced racemization compared with Fmoc-Cys(Trt)-OH

Fmoc-Cys(Dpm)-OH is a new tool for the regioselective synthesis of disulfide bridged peptides [20]. It should be particularly useful when paired with Fmoc-Cys(Mmt)-OH for the selective formation of two disulfide bonds. In contrast to S-Trt, which is slowly cleaved by treatment 1 - 3% TFA, S-Dpm is completely stable. This property enables S-Mmt groups to be removed with dilute TFA on the solid phase without loss of S-Dpm groups. The free sulfhydryls can then be oxidized to form the first disulfide bridge. Subsequent treatment with a TFA/DMSO/anisole cocktail cleaves the peptide from the resin, removes the S-Dpm groups and effect formation of the second disulfide bridge in one step (Figure 1).

A further advantage of S-Dpm protection over S-Trt is that it confers greater protection to the cysteine from loss of chiral integrity during carboxyl activation. Model studies [21] have been reported that compare extent of racemization during the coupling of Fmoc-Cys(Trt)-OH or Fmoc-Cys(Dpm)-OH with HCTU/6-Cl-HOBt/DIEA (4/4/8) activation. With Fmoc-Cys(Trt)-OH, D-Cys formation was 8.0%, 10.9% and 26.6% at 25 °C, 80 °C and 90 °C, respectively, whereas, Fmoc-Cys(Dpm)-OH gave only 1.2%, 3.0% and 4.5% D-Cys.

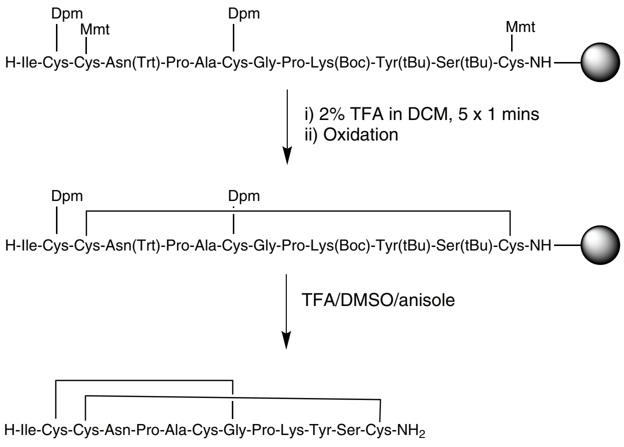
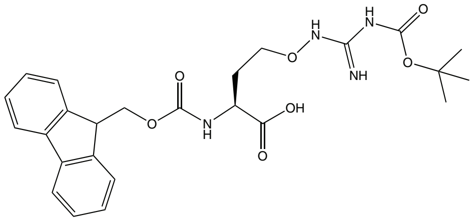


Figure 1: Putative synthesis of SI conotoxin using a combination of Mmt and Dpm protecting groups.

Cat.No.	Product	Contents
852417 NEW	Fmoc-Cys(Dpm)-OH	5 g 25 g
	Other cysteine derivatives	
852006	Fmoc-Cys(Acm)-OH	5 g 25 g 100 g
852007	Fmoc-Cys(tBu)-OH	5 g 25 g 100 g
852022	Fmoc-Cys(tButhio)-OH	5 g 25 g
852373	Fmoc-Cys(STmp)-OH	1 g 5 g
852031	Fmoc-Cys(Mmt)-OH	1 g 5 g 25 g
852008	Fmoc-Cys(Trt)-OH	25 g 100 g
852338	Fmoc-Thz-OH	1 g 5 g 25 g

NEW • Arginine analog

Fmoc-Cav(Boc)-OH



Features & Benefits

- Building block for introduction of cavanavine during Fmoc SPPS
- Cavanavine is an arginine analog in which the δ-methylene is substituted for oxygen.

Arginine is unique amongst proteogenic amino acids in that the basic side-chain guanidino group remains positively charged across all pHs found in biological systems. Arginine residues often play pivotal roles in mediating receptor interactions, protein-protein recognition and maintaining protein structure through salt bridge formation. Furthermore, many therapeutically relevant proteases display a preference for arginine residues in their endogenous substrates. Consequently, arginine mimetics are important tools for studying the role of arginine in biological functions and for developing enzyme inhibitors as potential therapeutics.

Fmoc-Cav(Boc)-OH is a new tool for the incorporation of cavanavine during Fmoc SPPS. Canavanine is a structural arginine analog in which the δ-methylene group is substituted for oxygen. This derivative is compatible with standard Fmoc SPPS methods. Removal of the side-chain Boc groups is effected during the course of the TFA cleavage reaction.

Cat.No.	Product	Contents
852414 NEW	Fmoc-Cav(Boc)-OH	250 mg 1 g 5 g
	Other arginine analogs	
852107	Fmoc-ADMA(Pdf)-OH	1 g 5 g
852310	Fmoc-SDMA(Boc)$_2$-OH	1 g 5 g
852336	Fmoc-Agb(Boc)2-OH	1 g 5 g
852267	Fmoc-hArg(Pdf)-OH	1 g 5 g

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