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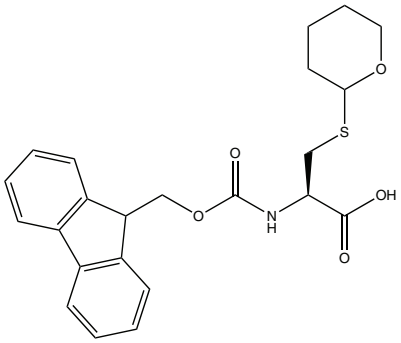
Novabiochem®
Over 30 Years of Innovation

Focus on cysteine

NEW • Fmoc-Cys(Thp)-OH

New Derivative for introduction of cysteine in Fmoc SPPS



Features & Benefits

- Couples with lower racemisation than other Fmoc-cysteine derivatives
- Lower racemisation and β-piperidinylalanine formation for C-terminal Cys residues on Wang type resins
- Thp group removed with 95% TFA

The synthesis of cysteine containing peptides by Fmoc SPPS is hampered by a number of well documented side reactions: cysteine residues anchored to Wang-type resins are prone to β-elimination and conversion to piperidinylalanine; loss of chiral integrity can occur during base-mediated coupling; incomplete removal of Trt protecting groups is frequently encountered during TFA cleavage [1].

Fmoc-Cys(Thp)-OH is a new building block for Fmoc SPPS, in which the sulfhydryl group is protected with the acid-cleavable tetrahydropyranyl (Thp) group [2]. The use of Fmoc-Cys(Thp)-OH has been shown to give superior results to the corresponding S-Trt, S-Dpm, S-Acm, and S-StBu derivatives. Significantly lower racemisation and β-piperidinylalanine formation was observed for C-terminal cysteine residues attached to Wang resins during prolonged piperidine treatments. Racemisation during DIPCDI/Oxyma Pure coupling of Fmoc-Cys(Thp)-OH was only 0.74% compared with Fmoc-Cys(Trt)-OH (3.3%) and Fmoc-Cys(Dpm)-OH (6.8%). Complete removal of Thp group was effected by treatment with TFA/water/TIS 95:2.5:2.5 in 2 hours. The Thp group is, however, stable to 1% TFA in DCM, facilitating the synthesis of protected peptide fragments on hyper-acid labile resins such as 2-chlorotrityl or HMPB resins. Initial evidence suggests S-Thp peptides have enhanced solubility compared to those protected with Trt.

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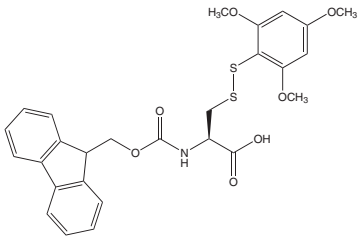
Fmoc-Cys(STmp)-OH



Features & Benefits

- Ideal tool for the synthesis of peptides containing multiple disulfide bridges
- Cys(STmp) is orthogonal to Cys(Mmt) and Cys(Trt)
- STmp group is stable to piperidine but removed in 5 min upon treatment with mercaptoethanol or DTT, enabling selective disulfide bridge formation on the solid phase

The unequivocal synthesis of peptides containing multiple disulfide bridges involves step-wise formation of each individual disulfide bond. This approach necessitates the use of pairs of orthogonally protected Cys residues, which can be sequentially deprotected and oxidized without effecting the other cysteine and cystine residues [1]. However, there is a lack of genuine orthogonal protecting group combinations for cysteine protection, with many of the available protecting groups, such as 4-methoxytrityl (Mmt), benzhydryl (Dpm), and t-butyl, requiring selective removal by graduated acidolysis. One of the few groups that is truly orthogonal is the t-butylsulfenyl (tButhio) group [3]. It is stable to acid and piperidine, making it compatible with Fmoc SPPS, but is cleaved by reduction with thiol or phosphines. Unfortunately removal of this group is very sluggish (4 - 24h) [4], which significantly limits its utility in routine synthesis. Frequently incomplete deprotection [5] or desulfurization [6] during the extended exposure to reducing agents is observed.

The novel derivative Fmoc-Cys(STmp)-OH developed by Albericio, et al. [4] overcomes these limitations. Like tButhio, the 2,4,6-trimethoxyphenylsulfenyl (STmp) group is stable to piperidine but, in contrast, is extremely easily removed by mild thiolysis. Albericio reported removing four STmp groups on the solid phase with only three 5 minute treatments of 0.1 M N-methylmorpholine (NMM) in DMF containing 5% dithiothreitol (DTT).

In the synthesis of peptides containing multiple disulfide bridges by an orthogonal protecting group strategy, deprotection of the Cys(STmp) residues must be done first since the conditions required for this step will cause reduction of any disulfide bridges already present in the peptide. This is best carried out before the peptide is cleaved from the resin as STmp is slightly labile to TFA. The pseudodilution effect can then be exploited during on-resin oxidation, or alternatively the peptide can be cleaved from the resin and cyclized in dilute solution.

Albericio and coworkers have recently described a synthesis of SI conotoxin by sequential disulfide bond formation on the solid phase utilizing a combination of STmp and Mmt cysteinyl protection [7]. Removal of STmp from the side-chains of Cys and Cys with DTT followed by mild oxidation with N-chlorosuccinimide (NCS) in DMF afforded the first disulfide bridge. The second bridge was introduced by treatment with 2% TFA in DCM to remove the Mmt protection from Cys and Cys and subsequent oxidation with NCS. Interestingly, oxidation with NCS is so rapid that no scrambling of the existing disulfide bond was observed following removal of the Mmt protection. NCS has been recently described as a novel reagent for on-resin disulfide bond formation. This reagent effects complete oxidation in minutes, without affecting Trp residues. Some oxidation of Met is observed but this can be kept to a minimum by using the reagent in slight excess.

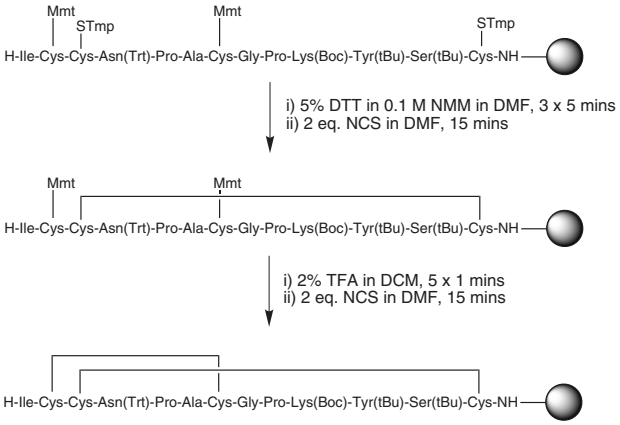
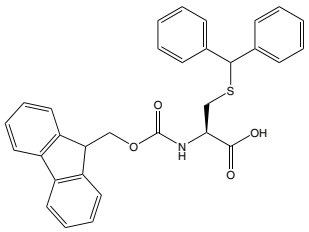


Figure 1: Synthesis of SI conotoxin [7].

Fmoc-Cys(Dpm)-OH



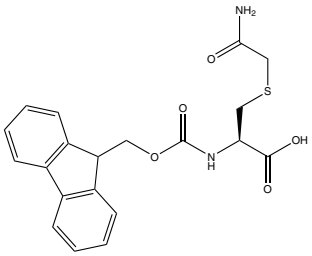
Features & Benefits

- 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 valuable alternative to Fmoc-Cys(Trt)-OH for introduction of Cys residues during Fmoc SPPS [8]. The regioselective synthesis of cyclic peptides containing two disulfide bridges can be readily achieved using a combination of Dpm and Mmt sulfhydryl protecting groups. S-Dpm protection is stable to 1 - 3% TFA, in contrast to S-Trt which is slowly cleaved, but is removed with 95% TFA. These properties enable 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 effects formation of the second disulfide bridge in one step.

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 [9] have been reported that compared the 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.

Fmoc-Cys(methylcarboxamide)-OH



Features & Benefits

- Tool for direct introduction of Cys(methylcarboxamide) by Fmoc SPPS.
- Compatible with piperidine and TFA treatments.

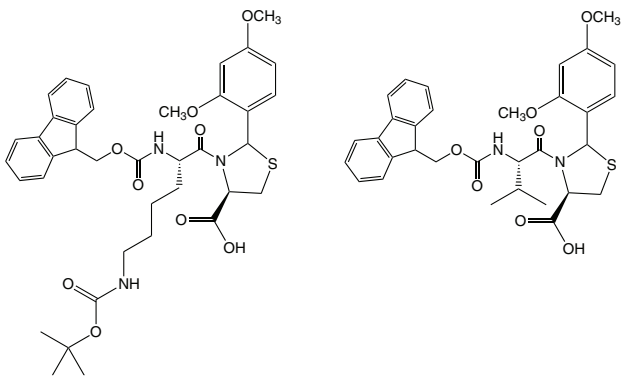
During the standard procedure for protein sequencing, cysteine residues are usually derivatized with iodoacetamide [10] prior to enzymatic digestion and identification of the peptide fragments by MS analysis. Protein quantitation is achieved by spiking the digest with a known quantity of one of the expected tryptic fragments, that has been prepared using heavy atom amino-acid building blocks, and comparing the relative abundance of the natural and heavy peptides in the MS [11]. The Fmoc SPPS of heavy atom internal standards containing Cys(methylcarboxamide) involves on-resin selective deprotection of Cys(Mmt) and S-alkylation with iodoacetamide. However, this approach can be problematic as cleavage of Mmt groups is often accompanied by loss of other protecting groups and the reaction of the released sulfhydryls with iodoacetamide does not always proceed to completion.

Fmoc-Cys(methylcarboxamide)-OH is a new building block which enables Cys(methylcarboxamide)-containing peptides to be prepared directly by Fmoc SPPS, without the need for post-assembly manipulations. It can be introduced using activation methods and the S-methylcarboxamide group is stable to TFA, making it compatible with standard Fmoc SPPS protocols.

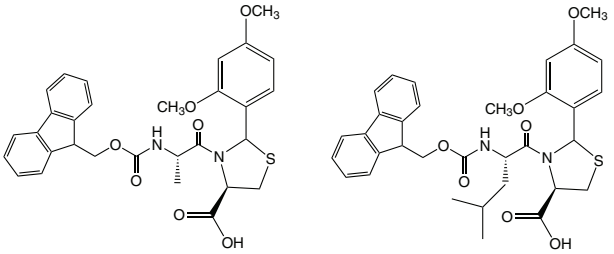
Cat.No.	Product	Contents
852419	Fmoc-Cys(Thp)-OH	1 g
NEW		5 g
852373	Fmoc-Cys(STmp)-OH	1 g
		5 g
852413	Fmoc-Cys(methylcarboxamide)-OH	5 g
		25 g
852417	Fmoc-Cys(Dpm)-OH	5 g
		25 g

NEW • Cysteine pseudoproline dipeptides

Fmoc-Lys(Boc)-Cys($\psi^{Dmp,H}$pro)-OH Fmoc-Val-Cys($\psi^{Dmp,H}$pro)-OH



Fmoc-Ala-Cys($\psi^{Dmp,H}$pro)-OH Fmoc-Leu-Cys($\psi^{Dmp,H}$pro)-OH



Features & Benefits

- Pseudoproline dipeptides for cysteine-containing peptides
- Prevents aggregation and helps helps improve yield and purity of desired peptide
- Couples without epimerization of cysteine residue

Pseudoproline dipeptides [12] are extremely powerful tools for enhancing synthetic efficiency in Fmoc SPPS. They work by mimicking the natural propensity of proline [13] to disrupt the formation of the secondary structures during peptide assembly. Their use leads to better and more predictable acylation and deprotection kinetics, which results in higher purities and solubilities of crude products, easier HPLC purification and improved yields, with less need to repeat failed syntheses. Traditionally, pseudoproline dipeptides are based on oxazolidines derived from Ser or Thr residues. Novabiochem now offers cysteine-based pseudoproline dipeptides in which the cysteine residue is protected as a thiazolidine by reaction with dimethoxybenzaldehyde.

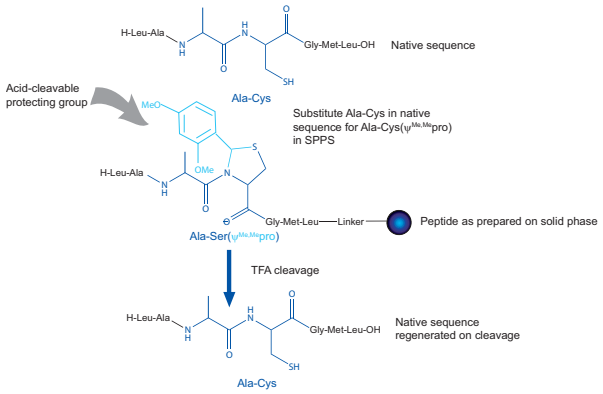


Fig. 2: Principles of using cysteinyl pseudoproline dipeptides.

These dipeptides are used in exactly the same manner as standard pseudoproline dipeptides. They can be coupled using any standard coupling method, such as PyBOP/DIPEA or DIPCDI/Oxyma Pure, substituting a Cys residue together with the preceding amino acid residue in the peptide sequence with the appropriate pseudoproline dipeptide (Figure 2). The thiazolidine ring is labile to TFA, so the native sequence cysteinyl-containg peptide is regenerated on cleavage and deprotection.

Cat.No.	Product	Contents
852381	Fmoc-Ala-Cys($\psi^{Dmp,H}$pro)-OH	1 g
NEW		5 g
852382	Fmoc-Leu-Cys($\psi^{Dmp,H}$pro)-OH	1 g
NEW		5 g
852383	Fmoc-Val-Cys($\psi^{Dmp,H}$pro)-OH	1 g
NEW		5 g
852384	Fmoc-Lys(Boc)-Cys($\psi^{Dmp,H}$pro)-OH	1 g
NEW		5 g

852175	Fmoc-Ala-Ser($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852180	Fmoc-Ala-Thr($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852185	Fmoc-Asn(Trt)-Ser($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852183	Fmoc-Asn(Trt)-Thr($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852186	Fmoc-Asp(OtBu)-Ser($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852199	Fmoc-Asp(OtBu)-Thr($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852190	Fmoc-Gln(Trt)-Ser($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852198	Fmoc-Gln(Trt)-Thr($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852177	Fmoc-Glu(OtBu)-Ser($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852196	Fmoc-Glu(OtBu)-Thr($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852200	Fmoc-Gly-Ser($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852197	Fmoc-Gly-Thr($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852194	Fmoc-Ile-Ser($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852193	Fmoc-Ile-Thr($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852179	Fmoc-Leu-Ser($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852184	Fmoc-Leu-Thr($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852178	Fmoc-Lys(Boc)-Ser($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852191	Fmoc-Lys(Boc)-Thr($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852195	Fmoc-Phe-Ser($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852201	Fmoc-Phe-Thr($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852187	Fmoc-Ser(tBu)-Ser($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852192	Fmoc-Ser(tBu)-Thr($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852202	Fmoc-Trp(Boc)-Ser($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852188	Fmoc-Trp(Boc)-Thr($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852189	Fmoc-Tyr(tBu)-Ser($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852182	Fmoc-Tyr(tBu)-Thr($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852176	Fmoc-Val-Ser($\psi^{Me,Me}$pro)-OH	1 g
		5 g
852181	Fmoc-Val-Thr($\psi^{Me,Me}$pro)-OH	1 g
		5 g

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