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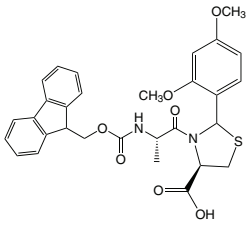
Over 30 Years of Innovation

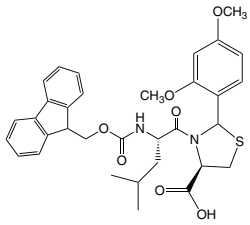
Extending pseudoprolines beyond Ser and Thr

Introducing Fmoc-Xaa-Cys($\psi^{Dmp,H}$pro)-OH dipeptides

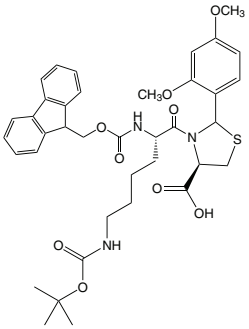
Fmoc-Ala-Cys($\psi^{Dmp,H}$pro)-OH



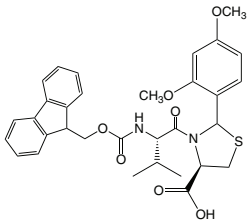
Fmoc-Leu-Cys($\psi^{Dmp,H}$pro)-OH



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



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



Mutter's pseudoproline dipeptides [1] are powerful tools for enhancing synthetic efficiency in Fmoc SPPS. 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. They have proved particularly effective in the synthesis of intractable peptides [2 - 5], long peptides/small proteins [6 - 13], and cyclic peptides [14, 15], enabling in many cases the production of peptides that otherwise could not be made. Pseudoproline derivatives can be derived from Ser, Thr or Cys, however, until now only those based on Ser and Thr have been commerically available. Novabiochem's new cysteine-based pseudoproline dipeptides expand the scope of the structure breaking building blocks available for Fmoc SPPS.

Cys-based pseudoproline dipeptides are used in exactly the same manner as those derived from Ser or Thr. 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 1). The thiazolidine ring is labile to TFA, so the native sequence cysteinyl-containg peptide is regenerated on cleavage and deprotection.

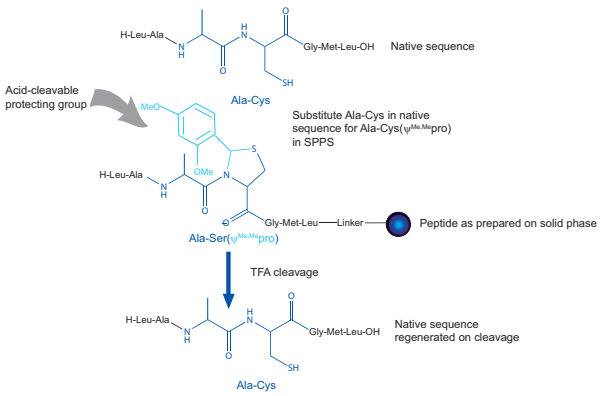


Fig. 1: Principles of using cysteinyl pseudoproline dipeptides.

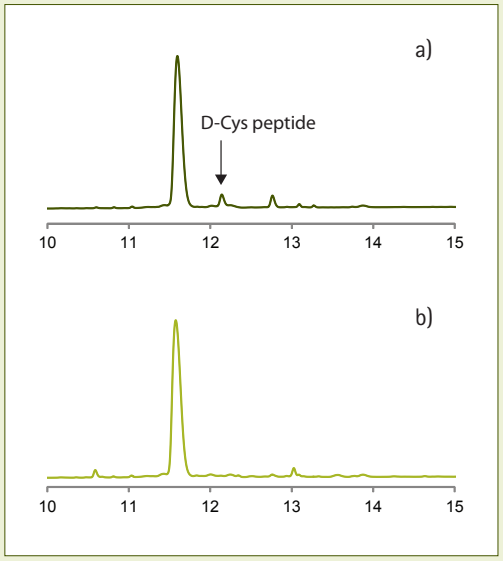


Fig. 2: HPLC profile of crude H-Lys-Cys-Phe-Pro-Glu-Tyr-Thr-Pro-Asn-Phe-OH prepared with TBTU/DIPEA activation a) using Fmoc-Cys(Trt)-OH and b) using Fmoc-Lys(Boc)-Cys($\psi^{Dmp,H}$pro)-OH.

The cysteine pseudoprolines can be used in combination with standard pseudoprolines and Dmb-dipeptides. Positioning of these structure-breaking derivatives approximately 6 residues apart in the peptide sequence at regular intervals has proven to be an extremely effective approach for the synthesis of long and amyloidogenic peptides.

Prevention of epimerization during coupling

Trityl-protected cysteine is known to undergo racemization during coupling, particularly if base-mediated activated methods are used. Cysteine-derived pseudoprolines, in constrast, have excellent chiral stability, as illustrated by the results shown in Figure 2. H-Lys-Cys-Phe-Pro-Glu-Tyr-Thr-Pro-Asn-Phe-OH (EGF (36-45)) prepared with TBTU/DIPEA activation using Fmoc-Cys(Trt)-OH contained 3.7% D-Cys Table 1, **A**), whereas using Fmoc-Lys(Boc)-Ser($\psi^{Dmp,H}$pro)-OH only 0.4% D-Cys ((Table 1, **B**) was generated.

Overcoming aggregation

The ability of pseudoprolines-derived from Ser and Thr to disrupt aggregation during peptide assembly is well demonstrated. It is thought that the dimethyloxazolidine ring of the pseudoproline imposes a kink on the peptide chain due to it favouring a cis-amide bond conformation. Pseudoprolines derived from cysteine and dimethoxybenzaldehyde are know to be less effective at promoting a cis-amide conformation and, therefore, might be expected to be less efficient at preventing aggregation. To determine if this is indeed the case, analogs of the difficult peptide influenza virus hemagglutinin were prepared using either Fmoc-Ser(tBu)-OH, Fmoc-Ala-Ser($\psi^{Me,Me}$pro)-OH or Fmoc-Ala-Cys($\psi^{Dmp,H}$pro)-OH. Figure 3 shows the HPLC profiles of the crude peptides obtained from these syntheses. As expected, the peptide prepared using Fmoc-Ser(tBu)-OH

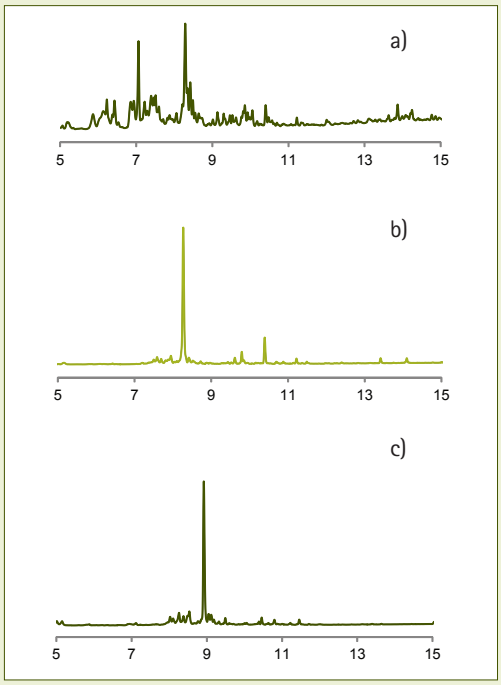


Fig. 3: HPLC profiles of crude H-Met-Glu-Asp-Ser-Thr-Tyr-Tyr-Lys-Ala-Ser-Lys-Gly-Cys-NH_2 prepared with a) Fmoc-Ser(tBu)-OH and b) Fmoc-Ala-Ser($\psi^{Me,Me}$pro)-OH, and c) crude H-Met-Glu-Asp-Ser-Thr-Tyr-Tyr-Lys-Ala-Cys-Lys-Gly-Cys-NH_2 prepared with Fmoc-Ala-Cys($\psi^{Dmb,H}$pro)-OH.

was highly heterogeneous. In constrast, the purities of the analogs prepared using both pseudoproline building blocks were excellent, indicating the Cys-derived pseudoproline dipeptides to be equally as effective as those derived from Ser or Thr at inhibiting aggregation (Figure 3).

Prevention of alkylation during cleavage

Ring opening of Cys($\psi^{Dmb,H}$pro) residues with TFA releases reactive dimethoxybenzalehyde. Cleavage of the EGF model peptide with the standard TFA/TIPS/water cocktail gave two major by-products: dimeric peptide derived from dimethoxybenzaldehyde and an dimethoxybenzaldehyde adduct. The addition of EDT to the cocktail eliminated both by-products and led to a clean product. Omission of TIPS from the cocktail afforded products containing free dimethoxybenzaldehyde. Therefore, peptides containing Cys(Dmb,Hpro) residues should be cleaved with TFA/TIPS/water/EDT (Table 1).

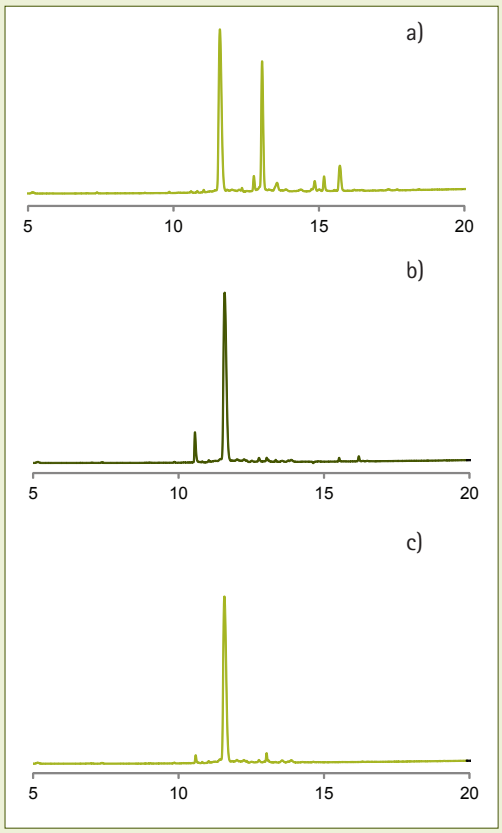


Fig. 4-: HPLC profiles of crude H-Lys(Boc)-Cys($\psi^{Dmb,H}$pro)-Phe-Pro-Glu(OtBu)-Tyr(tBu)-Thr(tBu)-Pro-Asn(Trt)-Phe-Wang cleaved with a) TFA/TIPS/water (95:2.5/2.5), b) TFA/EDT/water (95:2.5/2.5) and c) TFA/TIPS/water/EDT (92.5:2.5/2.5/2.5).

EGF	Peptide purity [% area]	% D-Cys
A	81	3.9
B, TFA/H_2O/TIPS	47	0.4
B, TFA/H_2O/EDT	76	0.4
B, TFA/H_2O/TIPS/EDT	81	0.4

Table 1: Purity and D-Cys content of EGF (36-45) prepared with Fmoc-Cys(Trt)-OH **A** and Fmoc-Lys(Boc)-Cys($\psi^{Dmp,H}$pro)-OH **B**.

Conclusion

Fmoc-Xaa-Cys($\psi^{Dmp,H}$pro)-OH pseudoproline dipeptides are excellent tools for the synthesis of cysteine-containing peptides. In constrast to Fmoc-Cys(Trt)-OH, they can be coupled without epimerization under basic conditions. Cys($\psi^{Dmb,H}$pro) residues appear to be equally effective as Ser/Thr(Me,Mepro) residues in disrupting aggregation during peptide assembly. To avoid by-product formation during cleavage from the resin and side-chain deprotection, TFA cocktails incorporating EDT and TIPS should be used.

Ordering Information

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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For more information please contact:

Merck KGaA
64271 Darmstadt, Germany
E-mail: contact@merckgroup.com
www.merckmillipore.com/peptides

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