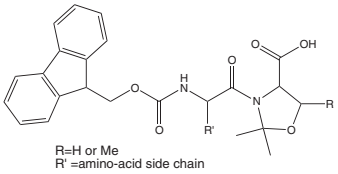
Novabiochem®

innovations 1/04

Guidelines for the use of Pseudoproline Dipeptides

Fmoc-Aaa-Ser/Thr($\Psi^{Me,Me}$pro)-OH



Mutter's pseudoproline dipeptides (PPs) [1, 2] are undoubtably the most powerful tools described to date for enhancing synthetic efficiency in Fmoc SPPS [3 - 7]. The routine use of pseudoprolines has been found to:

- help avoid costly and unnecessary repeat syntheses of failed sequences;
- increase purity of crude products;
- simplify HPLC purification;
- increase yield of crude and purified products;
- enable synthesis to be carried out on a lower scale.

Use pseudoprolines and enjoy the benefits at no extra cost.

'Using one pseudoproline in the synthesis of a 20mer biotinylated peptide, purified yields went from 11 to 27 mg and purity from 88 to 100%. Pseudoprolines are the most powerful tool I've ever been given to improve the quality of my synthetic peptides.'

Charlie Seiler, Protein Core Laboratory Manager, Utah State University.

How do they work?

Pseudoprolines consist of a dipeptide in which the Ser or Thr residue has been reversibly protected as proline-like TFA-labile oxazolidine (Figure 1). The insertion of a pseudoproline into a sequence disrupts the formation of the secondary structures thought responsible for problems during peptide assembly, leading to better and more predictable acylation and deprotection kinetics. The most dramatic results are seen in the preparation of highly aggregated sequences, where 10-fold increases in product yield have been achieved from insertion of a single pseudoproline [3]. However, the enhanced and more uniform reaction rates also benefit routine synthesis, providing improved yields, purities and solubilities of crude products, and easier HPLC purification with higher product return.

For longer peptides, the incorporation of several pseudoprolines at regular intervals throughout the sequence has been found to be particularly effective [5].

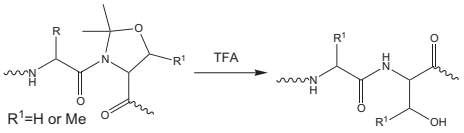


Fig. 1: Conversion of a pseudoproline residue to a Ser or Thr residue.

Guidelines for use of pseudoproline dipeptides

Pseudoprolines are extremely simple to use. They are introduced into the peptide sequence using standard coupling methods, substituting any Aaa-Ser or Aaa-Thr dipeptide. Experience has led to the development of the following empirical guidelines for their use:

- Optimal results are obtained if the PPs are spaced 5-6 residues apart throughout the sequence.
- The optimum separation between a PP and a Pro residue is 5-6 amino acid residues.
- The minimum separation between a PP and another PP or Pro residue is 2 residues.
- Aim to insert a PP before regions of hydrophobic residues.

These recommendations have been vindicated in a recent publication, which describes the synthesis of a peptide related to Pro-neuropeptide Y that could only be achieved through the use of two pseudoproline dipeptides separated by 6 residues [6]. This work is reproduced below by kind permission of Wiley-VCH and Prof. Beck-Sickinger.

Synthesis of Pro-neuropeptide Y related peptide [6]

H-Cys-Pro-Glu-Thr-Leu-Ile-Ser-Asp-Leu-Leu-Met-Arg-Glu-Ser-Thr-Glu-Asn-Val-Pro-Arg-Thr-Arg-Leu-Glu-Asp-Pro-Ala-Lys-Trp-OH

Initial analysis of the peptide sequence using Peptide Companion Software indicated that the synthesis would involve coupling reactions of easy to moderate difficulty. Automated synthesis under standard Fmoc SPPS conditions yielded a highly heterogeneous product (Figure 2a) containing numerous truncated sequences. Attempts to improve the synthesis by varying solvents (DMF/DCM, DCM, DMSO), coupling reagents (TBTU, HATU) or solid support (PEG-PS, pyridine-co-Wang) had little effect. Repeating the synthesis with substitution of Ser-Thr by Ser-Thr($\Psi^{Me,Me}$pro) (Figure 2c) led to improved efficiency in the middle of the assembly, but the major product obtained was missing the N-terminal Cys residue. Substitution of Ile-Ser by Ile-Ser($\Psi^{Me,Me}$pro) on the other hand, led to a considerable improvement in the coupling of Cys but to a product containing numerous deletion and truncated peptides (Figure 2b). When the synthesis was repeated using both pseudoproline dipeptides, the desired product was obtained in a purity of 60 - 70% (Figure 2d), which could be readily purified by HPLC to a purity of >95%.

05-20-1119	**Fmoc-Ile-Ser($\Psi^{Me,Me}$pro)-OH**	1 g 5 g
05-20-1117	**Fmoc-Ser(tBu)-Thr($\Psi^{Me,Me}$pro)-OH**	1 g 5 g

For details of Novabiochem's 24 other pseudoproline dipeptides visit our website at www.novabiochem.com.

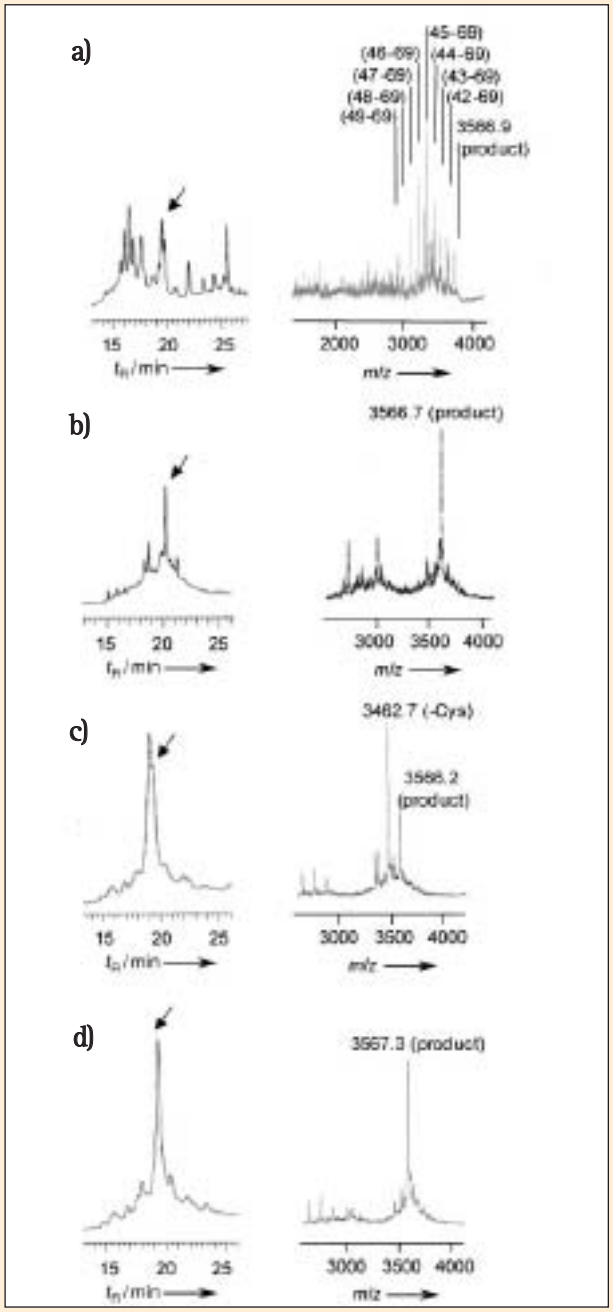


Fig. 2: HPLC elution profiles and MALDI-MS spectra obtained from synthesis of neuropeptide Y-related peptide [8]. Product a) obtained using standard Fmoc SPPS conditions; b) obtained by substitution of positions 46 & 47 with Ile-Ser($\Psi^{Me,Me}$pro); c) obtained by substitution of positions 54 & 55 with Ser-Thr($\Psi^{Me,Me}$pro); d) obtained by substitution of both positions. Arrow indicates product.

References

1. M. Mutter, et al. (1995) *Pept. Res.*, **8**, 145.
2. T. Haack & M. Mutter (1992) *Tetrahedron Lett.*, **33**, 1589.
3. P. White, et al. in "Peptides 1998, Proc. of 25th European Peptide Symposium", Budapest, Akadémiai Kiadó, 1998, pp. 120.
4. W. R. Sampson, et al. (1999) *J. Pept. Sci.*, **5**, 403.
5. P. White, et al. (2004) *J. Pept. Sci.*, **10**, 18.
6. R. von Eggelkraut-Gottanka, et al. (2003) *ChemBioChem*, **4**, 425.
7. M. Keller & A D. Miller (2001) *Bioorg. Med. Chem. Lett.*, **11**, 857.
8. Data reproduced from ref. [6] with the kind permission of Wiley-VCH.

Merck Biosciences AG · Switzerland
Weidenmattweg 4
4448 Läufelfingen
Phone +41 (62) 285 2525
Fax +41 (62) 285 2520

www.novabiochem.com