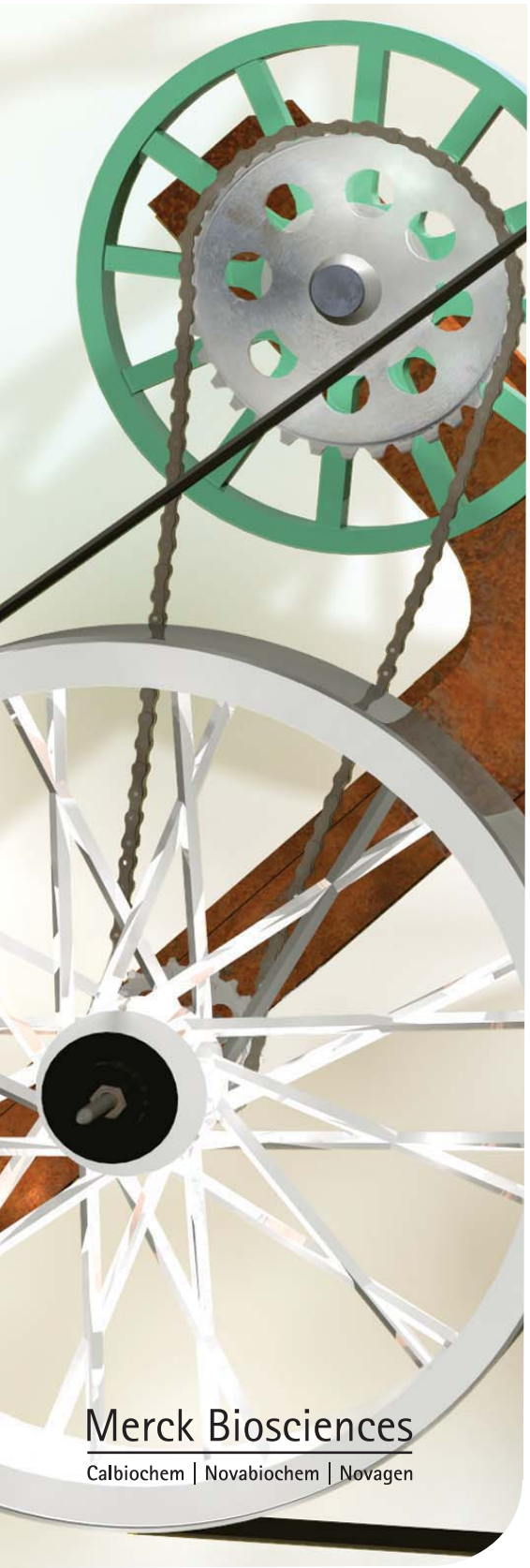


Novabiochem®

innovations 3/04

Synthesis design using pseudo-proline dipeptides

The routine use of pseudoproline (oxazolidine) dipeptides [1, 2] in the Fmoc SPPS of Ser- and Thr-containing peptides leads to remarkable improvements in quality and yield of crude products and helps avoid unnecessary repeat synthesis of failed sequences. Pseudoproline dipeptides have proven particularly effective in the synthesis of intractable peptides [3 - 6], long peptides/small proteins [7 - 9], and cyclic peptides [10], enabling in many cases the production of peptides that otherwise could not be made.
These dipeptides are extremely easy to use: simply substitute a Ser or Thr residue together with the preceding amino acid residue in the peptide sequence with the appropriate pseudoproline dipeptide (Figure 1). The native sequence is regenerated on cleavage and deprotection. To achieve the maximum benefits, it is important to follow the guidelines set-out below.
An example of synthesis planning is shown in Figure 2.

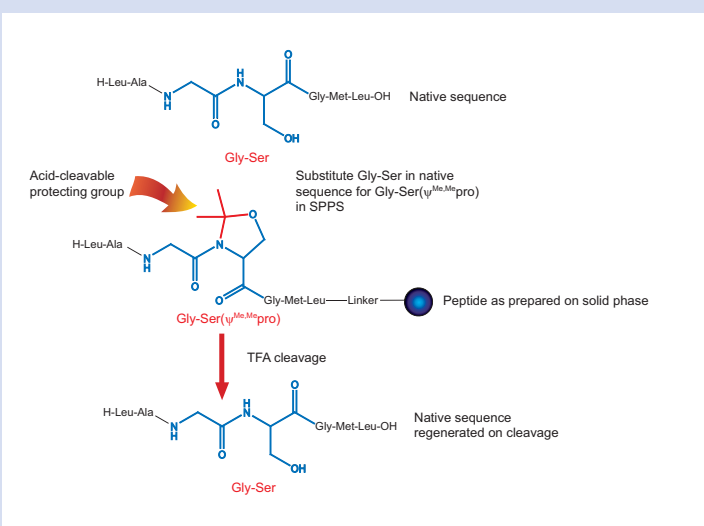


Fig. 1: Relationship between peptide sequence and pseudoproline dipeptide.

"We have had great success using the pseudoproline derivatives for the synthesis of difficult sequences. For example, we used these reagents to aid formation of side-chain lactam-bridged peptides that were otherwise unobtainable. I would highly recommend these reagents for your difficult syntheses."

Clifford Quan, Genentech, Inc., South San Francisco, CA.

"Pseudoproline dipeptides have greatly increased our success rate for synthesizing both long and difficult peptides. If we are able to integrate pseudoprolines into our syntheses, we can easily machine-synthesize peptides up to 80 amino acids in length. Routine use of pseudoprolines in our peptide syntheses has considerably increased the yield and purity, as well as decreased the number of failed syntheses. They are wonderful products!"

Yingwei He, Protein Chemistry Dept., Abgent, San Diego, CA.

"Really the most powerful tool I've ever been given to improve the quality of peptides... The results are absolutely phenomenal."

Charlie Seiler, Protein Core Service Laboratory, Utah State University, UT.

'Using pseudoproline derivatives in several instances has improved the purities of crude product by more than 30%. In some cases, without the use of pseudoprolines we were unable to synthesize hydrophobic fragments within the sequence. The use of pseudoprolines allows us to make peptides with less deletions, thereby increasing crude purities and allowing the sequences to be purified more easily."

Tim Weeden, New England Peptide, Inc., Gardner MA.

General guidelines for the use of pseudoproline dipeptides

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

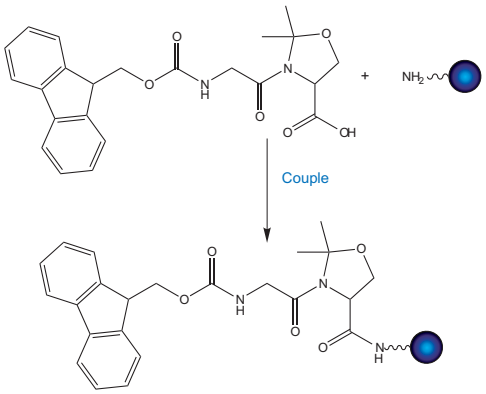
Fig: 2: Designing a synthesis with pseudoproline dipeptides.

[a]Aggregation does not normally occur until after 6 residues, so insertion of pseudoproline dipeptide at C-terminus is not necessary.

[b]Insertion too near a Pro or another pseudoproline dipeptide should be avoided.

[c]Insertion at N-terminus is not necessary.

Protocols for the use of pseudoproline dipeptides

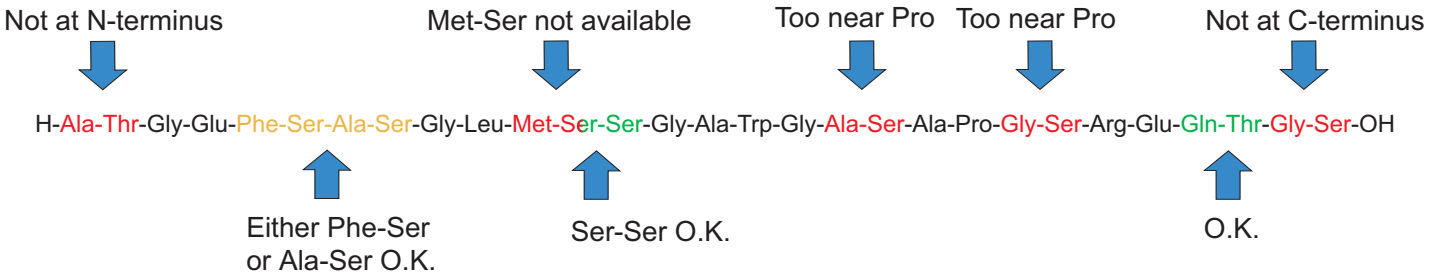


Pseudoproline dipeptides can be introduced in the same manner as other amino acid derivatives. Pseudoproline dipeptide have been coupled using phosphonium and aminium activation reagents, such as PyBOP® [1], HBTU [4], TBTU [3], and HATU [7] activation methods, as well as with carbodiimide-mediated coupling strategies, such as DIPCDI/HOBt [3, 5] (Method 1).

On automated instruments, the simplest approach is to use the same amount of pseudoproline dipeptide as any other amino acid, since this avoids having to reprogram coupling cycles or make any manual intervention to the synthesis. Program the instrument to add a Ser or Thr residue, and remember to omit from the synthesis program the cycle for the next amino acid as this will be introduced as part of the pseudoproline dipeptide.

Using 5-fold excess of phosphonium- or aminium-activated pseudoproline dipeptide to resin functionality, coupling reactions are generally complete in 1 h. Lower excesses of reagent can also be used, but it then becomes advisable to check the completeness of reactions using an amine test, such as the Kaiser or TNBS test (Method 2).

Regeneration of serine or threonine from the pseudoproline occurs during the course of the TFA cleavage reaction using standard cleavage cocktails, such as TFA/water/TIS (95:2.5:2.5), and is generally complete in 3 h.



Method 1: Manual coupling of pseudoproline dipeptides

Phosphonium/aminium activation

1. Dissolve pseudoproline dipeptide (5 eq.) and coupling reagent (PyBOP, TBTU, HBTU, HCTU, or HATU, 5 eq.) in minimum volume of DMF or NMP.
2. Add DIPEA (10 eq.) and mix thoroughly.
3. Add immediately to Fmoc-deprotected peptide resin, and agitate for 1-2 h. Check completion of coupling by the TNBS test. If reaction is not complete, extent coupling time or repeat reaction using fresh reagents.

DIPCDI/HOBt activation

1. Dissolve pseudoproline dipeptide (3 eq.) and HOBt (3 eq.) in minimum volume of DMF/DCM (2:1).
2. Add DIPCDI (3 eq.) and mix thoroughly.
3. Leave to activate for 10 minutes and then add to Fmoc-deprotected peptide resin, and agitate for 1-2 h. Check completion of coupling by the TNBS test. If reaction is not complete, extend coupling time or repeat reaction using fresh reagents.

Method 2: Automated coupling of pseudoproline dipeptides

Instruments using dry Fmoc-amino acids in cartridges (ABi 433, Pioneer, Millipore 9050)

1. Pack empty vials or cartridges with the amount of pseudoproline dipeptide appropriate to the instrument protocols (i.e. 1 mmole for ABi 433 and 0.8 mmole for Pioneer).
2. Program instrument to couple pseudoproline dipeptide as Ser or Thr residue (1 h coupling, HBTU, HATU activation). Omit amino acid cycle for next amino acid.
3. Smaller excesses of pseudoproline dipeptides can be used, but the delivery volumes of coupling and base reagents will need to be modified in the coupling protocols to maintain the correct ratio of dipeptide/coupling reagent/base (1:1:2). Alternatively, the instrument can be programmed to pause after the wash step following Fmoc removal, and the dipeptide coupled manually by adding a solution of activated derivative to the reaction vessel.

Instruments which needle dispense solutions of reagents from vials (ACT 396, Zinsser 350)

1. Dissolve pseudoproline dipeptides in DMF or NMP to exactly the same concentration as standard Fmoc-amino acid derivatives in reagent vial.
2. Place vial containing pseudoproline in an appropriate position of autosampler rack.
3. Program instrument to couple pseudoproline dipeptide as Ser or Thr residue (1 h coupling, HBTU, HATU activation). Omit amino acid cycle for next amino acid.

Instruments which use stock solutions of Fmoc-amino acids with dedicated solvent lines (Protein Technologies Symphony)

Whilst it is perfectly feasible to use pre-dissolved solutions of pseudoproline dipeptides on instruments such as the Symphony, this can be quite wasteful as reagent solvent lines would need to be primed with pseudoproline dipeptide solution before use. For such instruments , the cycle should be programmed to pause after the wash step following Fmoc removal and the "add" amino acid and coupling reagent steps in the cycle replaced by a "none" reagent step. The dipeptide can then be coupled manually by adding a solution of activated derivative to the reaction vessel.

Synthesis of p62 UBA and CGRP using pseudoproline dipeptides

Figures 3 and 4 show the results obtained using pseudoproline dipeptides in the synthesis of CGRP (37 residues) and p62 (50 residues), respectively. The data were kindly provided by Cliff Rush, BioMol International Lp, Exeter, UK.

Application 1: Synthesis of CGRP and p62

CGRP (H-Ser-Cys-Asn-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Val-Val-Lys-Asp-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Glu-Ala-Phe-NH_2) and p62 (H-Pro-Pro-Glu-Ala-Asp-Pro-Arg-Leu-Ile-Glu-Ser-Leu-Ser-Gln-Met-Leu-Ser-Met-Gly-Phe-Ser-Asp-Glu-Gly-Gly-Trp-Leu-Thr-Arg-Leu-Leu-Gln-Thr-Lys-Asn-Tyr-Asp-Ile-Gly-Ala-Ala-Leu-Asp-Thr-Ile-Gln-Tyr-Ser-Lys-His-OH) were both prepared by Fmoc SPPS methods. The pseudoproline dipeptides, marked in red, were introduced using Method 1 with DIPCDI/HOBt activation. The peptides were cleaved from the supports by treatment with TFA/water/TIS (95:2.5:2.5) for 3 h.

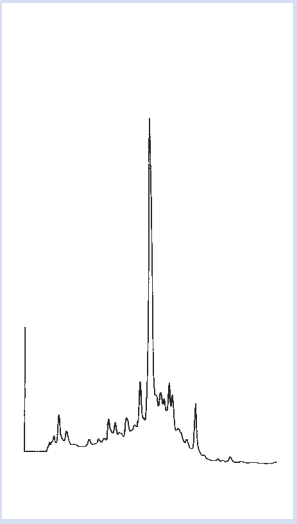


Fig. 3: HPLC profile of total crude CGRP.

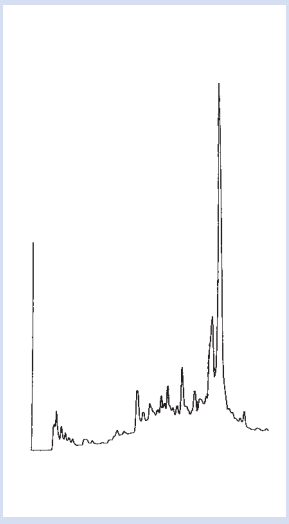


Fig. 4: HPLC profile of total crude p62.

Ordering information

05-20-1000	Fmoc-Ala-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1005	Fmoc-Ala-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1010	Fmoc-Asn(Trt)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1008	Fmoc-Asn(Trt)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1011	Fmoc-Asp(OtBu)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1126	Fmoc-Asp(OtBu)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1115	Fmoc-Gln(Trt)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1125	Fmoc-Gln(Trt)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1002	Fmoc-Glu(OtBu)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1122	Fmoc-Glu(OtBu)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1127	Fmoc-Gly-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1124	Fmoc-Gly-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1119	Fmoc-Ile-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1118	Fmoc-Ile-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1004	Fmoc-Leu-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1009	Fmoc-Leu-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1003	Fmoc-Lys(Boc)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1116	Fmoc-Lys(Boc)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1121	Fmoc-Phe-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1128	Fmoc-Phe-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1012	Fmoc-Ser(tBu)-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
05-20-1130	Fmoc-Trp(Boc)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1013	Fmoc-Trp(Boc)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1014	Fmoc-Tyr(tBu)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1007	Fmoc-Tyr(tBu)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1001	Fmoc-Val-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1006	Fmoc-Val-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g

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. R. von Eggelkraut-Gottanka, et al. (2003) *ChemBioChem*, **4**, 425.
6. M. Keller & A. D. Miller (2001) *Bioorg. Med. Chem. Lett.*, **11**, 857.
7. P. White, et al. (2004) *J. Pept. Sci.*, **10**, 18.
8. P. White, et al. (2003) *Biopolymers*, **71**, 338. P156.
9. Novabiochem Innovations 2/03
10. N. Schmiedeberg & H. Kessler (2002) *Org. Lett.*, **4**, 59.

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

www.novabiochem.com

NVI304-EUR