Novabiochem®

innovations 5/05

Expediting convergent synthesis with pseudoproline dipeptides

Convergent synthesis, rather than step-wise solid phase assembly, is the favored strategy for large scale GMP production of peptides [1], as the process is easier to control, validate and document, and the purity of intermediates can be more easily characterized. The method involves the systematic joining together of fully protected fragments that have been previously prepared either by solution or solid phase methods (Figure 1).

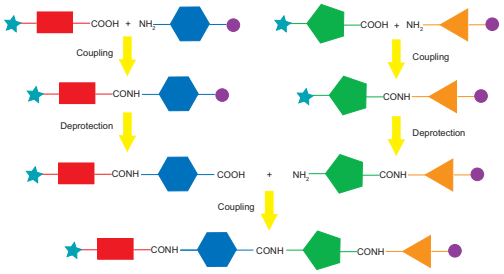


Fig. 1: Convergent synthesis.

The most efficient approach is to synthesize the component fragments by solid phase methods and then couple them together in solution to give the target peptide [2]. Indeed, this is the strategy used on multi-ton scale synthesis of the 36 residue HIV-fusion inhibitor Fuzeon. Crucial to the success of this approach is the capability to prepare all protected fragments as pure as possible since their poor solubility can make them almost impossible to purify by normal or reverse phase chromatographic methods. If the peptide contains serine or threonine residues, one way to ensure the production of the highest quality fragments is to employ pseudoproline dipeptides as doing so can markedly improve synthetic efficiency [3]. Furthermore, since the pseudoproline oxazolidine ring is stable to 1% TFA in DCM and HFIP/TFE/DCM, peptides can be cleaved from 2-chlorotrityl chloride or Sieber amide resin with the pseudoproline residue intact. Such peptides often have enhanced solubility, enabling reactions to be carried out at higher concentrations, with associated improvements in coupling rates and yields of products.

The benefits of using pseudoproline dipeptides are illustrated overleaf in the multigram convergent synthesis of the 37 residue peptide CGRP.

MERCK

"In our hands, pseudoproline derivatives have proven very effective - particularly in the synthesis of peptides with difficult and long sequences. Using pseudoprolines, we saved time and money for repeat synthesis of failed sequences. We now routinely use pseudoproline derivatives for our peptide synthesis. I would highly recommend using them for peptide synthesis in the manufacturing industry as well. I am glad Novabiochem took the lead in manufacturing pseudoprolines in bulk".

Ved Srivastava, Amylin Pharmaceuticals Inc, San Diego, 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!"

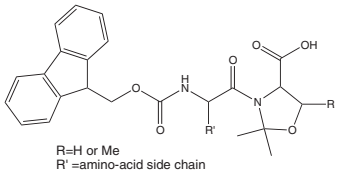
Yingwei He, Protein Chemistry Dept., Abgent, San Diego, CA.

"Biomol started incorporating pseudoproline derivatives into its everyday schedules for routine peptide synthesis some eight years ago. Over the intervening years, the use of these reagents on a routine basis has led to a dramatic reduction in the necessity for repeat synthesis. When coupled with an undoubted improvement in the yield and purity of crude peptides obtained, this has meant considerable financial savings in terms of both synthesis and purification costs. We are firmly of the opinion that the benefits of incorporation of pseudoproline analogs into peptide synthesis protocols is fully justifiable on both scientific and commercial grounds and is to be recommended on a routine basis."

Paul Sheppard, Biomol International Lp, Exeter, UK.

Synthetic Strategy

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



The primary sequence of CGRP is shown in Figure 2. It was decided to prepare the peptide by condensing two fragments. The N-fragment was selected to have an achiral glycine residue at its C-terminus to avoid any issues with epimerization during fragment coupling. The preparation of the fully protected C-fragment was first carried out on Sieber amide resin (0.52 mmole/g) using 4-fold excesses of standard Fmoc-amino acids activated with PyBOP®/NMM. Treatment with 1% TFA in DCM afforded the protected peptide in poor yield (50%) and low purity (Figure 4a). The synthesis was therefore repeated under identical conditions except that dipeptides Leu^{16}Ser^{17} and Gly^{33}Ser^{34} were introduced using Fmoc-Leu-Ser($\Psi^{Me,Me}$pro)-OH and Fmoc-Gly-Ser($\Psi^{Me,Me}$pro), respectively. This process provided the product in excellent yield (85%) and in high purity (Figure 4b).

The synthesis of the N-terminal fragment was then undertaken on Fmoc-Gly-HMPB resin (0.55 mmole/g) in a similar manner, except that Boc-Ala-OH was used for coupling of the final residue. The dipeptide Val^{8}-Thr^{9} was introduced using Fmoc-Val-Thr($\Psi^{Me,Me}$pro). Treatment with 1% TFA in DCM gave the desired fragment in 67% yield and in good purity (Figure 4c). This material was cyclized by treatment with iodine in MeOH to give the required disulfide bridged protected N-fragment (Figure 4d).

The two fragments were dissolved in DMF and coupled using TBTU (1.2 eq), HOBt (2 eq.), and DIPEA (2 eq). The reaction was complete after 4 h as determined by HPLC, so the solvent was evaporated, and the protected peptide precipitated with water. The product was isolated by filtration, washed with water and dried in vacuo over P_2O_5. The peptide was then treated with TFA/water/TIS (95:2.5:2.5) for 3 h. After this time, the TFA was removed by evaporation and the product precipitated with ether. The crude decapeptide was obtained in 98% yield with excellent purity (Figure 4e).

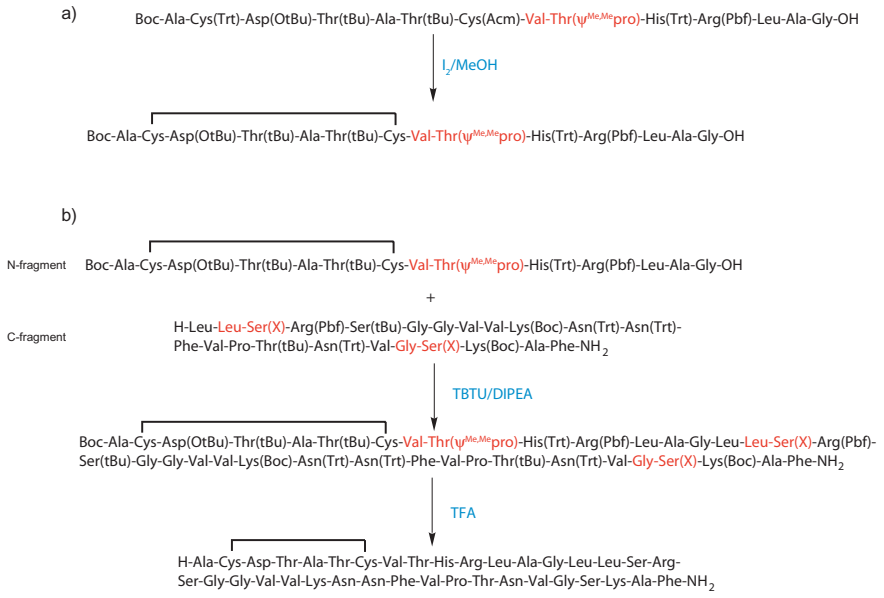
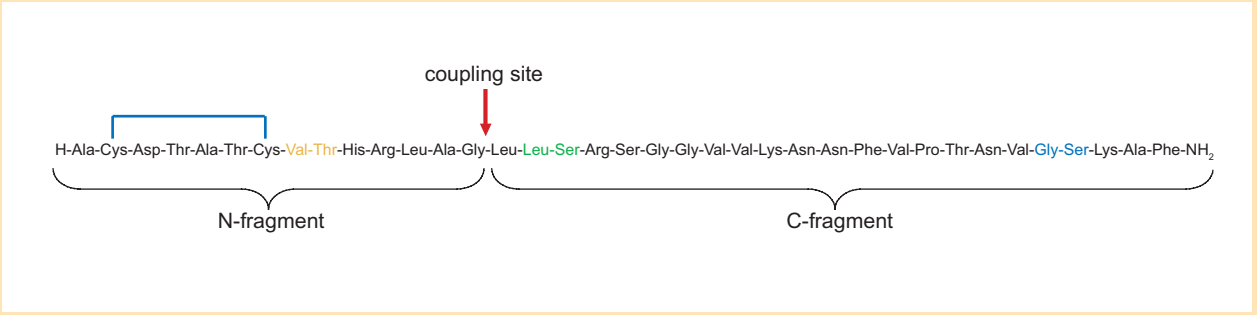


Fig. 3: Strategy for the synthesis of CGRP. x=OtBu or $\Psi^{Me,Me}$pro. a) Iodine oxidation of fully protected N-fragment produced using 2-chlorotrityl chloride resin; b) coupling with C-fragment produced on Sieber amide resin to produce full length protected peptide, followed by side-chain deprotection with TFA.

Fig. 2: Primary sequence of CGRP. Divide point for fragment synthesis is indicated.



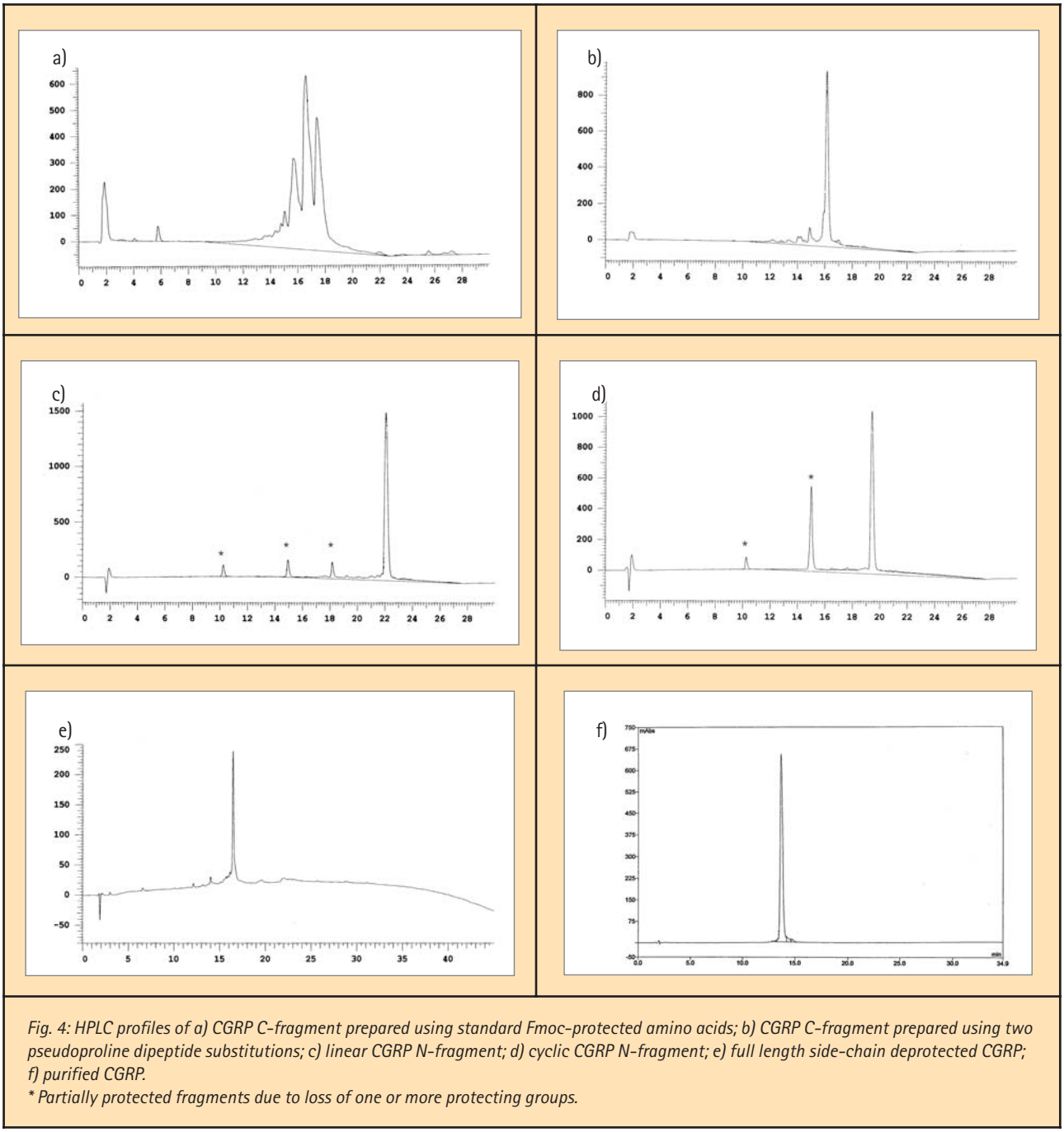


Fig. 4: HPLC profiles of a) CGRP C-fragment prepared using standard Fmoc-protected amino acids; b) CGRP C-fragment prepared using two pseudoproline dipeptide substitutions; c) linear CGRP N-fragment; d) cyclic CGRP N-fragment; e) full length side-chain deprotected CGRP; f) purified CGRP.
** Partially protected fragments due to loss of one or more protecting groups.*

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

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2. K. Barlos & D. Gatos in "Fmoc solid phase peptides sythesis: a practical approach", W. C. Chan & P. D. White (Eds.), Oxford University Press, Oxford, 2000, pp. 215.
3. a) T. Wöhr, et al. (1996) *J. Am. Chem. Soc.*, **118**, 9218; b) W. R. Sampson, et al. (1999) *J. Pept. Sci.*, **5**, 403; c) R. von Eggelkraut-Gottanka, et al. (2003) *ChemBioChem*, **4**, 425; d) M. Keller & A. D. Miller (2001) *Bioorg. Med. Chem. Lett.*, **11**, 857; e) P. White, et al. (2004) *J. Pept. Sci.*, **10**, 18.

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