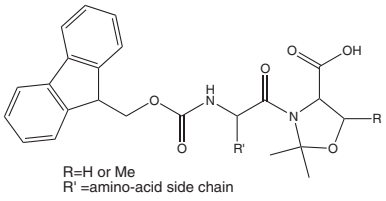
novabiochem
innovations 2/03

Synthesis of long peptides using pseudoproline dipeptides

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



R=H or Me
R' =amino-acid side chain

Mutter's pseudoproline dipeptides are highly effective tools for expediting the synthesis of difficult peptides by Fmoc solid phase methods [1, 2].
The majority of published papers which relate to pseudoprolines describe their use in overcoming problems in short highly aggregated sequences [3, 4]. However, the real benefits of employing pseudoprolines are really only realized when they are used routinely. Apart from the obvious advantage of avoiding costly repeat syntheses of failed sequences, their incorporation leads to overall improvements in acylation and deprotection kinetics, resulting in improved yields, purities and solubilities of crude products and easier HPLC purification with higher product return.
This is most easily demonstrated through the synthesis of long peptides, where incremental increases in reaction efficiencies can have remarkable influence on overall product purities, as illustrated by the examples given overleaf.

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
05-20-1011	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
05-20-1014	Fmoc-Tyr(tBu)-Ser($\Psi^{Me,Me}$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

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Applications

Synthesis of 80 residue FAS-related peptide

The synthesis of the following peptide was carried out with insertion of pseudoproline dipeptides at the points indicated in bold. A parallel synthesis performed using standard amino acid building blocks was terminated at 50 cycles as the desired peptide could not be detected in the crude product. These results are published in full in ref. [5].

AKIDEIKNDN VQ***DT***AEQKVQ LLRNWHQKVQ LLRNWHQLHG
KKEAY***DT***LIK DLKKANL***ST***L AEKI***QT***ILK DI***TS***DSE***NS***N EHKL***TS***EKDL **2**

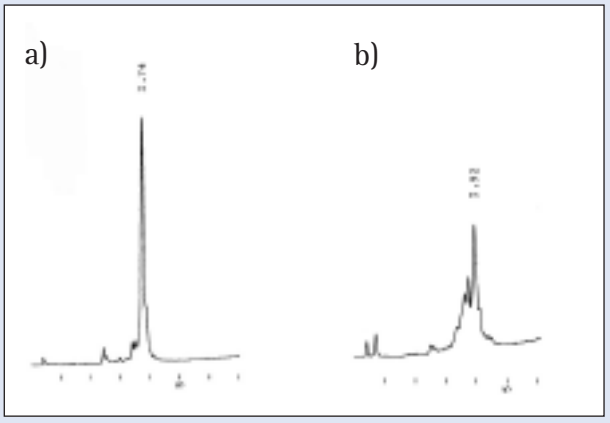

Figure 1: HPLC profiles of a) crude 80mer prepared using pseudoproline dipeptides; b) crude peptide after 50 cycles prepared using standard amino acid building blocks.

Application 1: Synthesis of FAS-death domain related peptides

The peptides were assembled automatically using a ABi 431A peptide synthesizer on Rink amide MBHA resin, which had been pre-loaded with a mixture of Boc-Leu-OH/Fmoc-Leu-OH (2:1) to give a resin with an Fmoc loading of 0.2 mmol/g. All acylation reactions were carried out using a 10-fold excess of Fmoc-amino acid activated with 1 eq. of HATU® in the presence of DIPEA. A coupling time of 60 min was used throughout. Cleavage and side-chain deprotection was effected by treatment of the peptidyl resins with TFA/ TIS /water 95:2.5:2.5 (5 ml) for 3 h. The peptides were isolated in the usual manner by evaporation and ether precipitation. The products were characterized by HPLC and ES-MS.

Synthesis of Domain 1 peptide [6]

The synthesis of the following peptide was carried out without and with insertion of a pseudoproline dipeptide at the point indicated. The peptide prepared without use of the pseudoproline dipeptide was contaminated with considerable amounts of a truncation sequence arising from incomplete Fmoc removal at cycle 29.
GRTCPKPDDL PFSTVVPLKT FYEPGEEITY SCKPGY***VS***RG GMRKFICPLT GLWPINTLKCTPRV

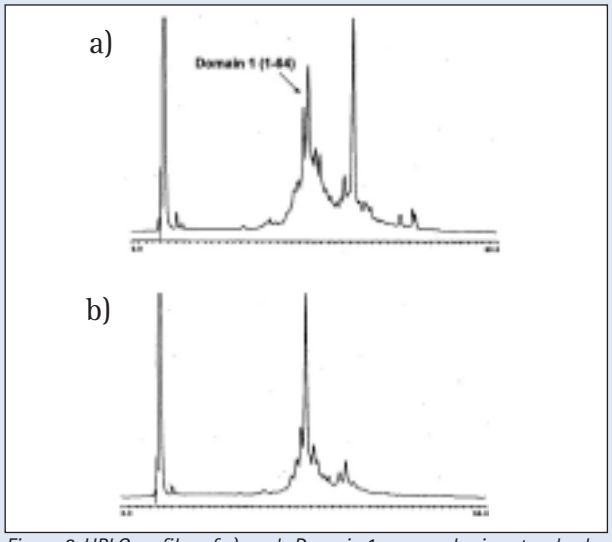

Figure 2: HPLC profiles of a) crude Domain 1 prepared using standard amino acid building blocks; b) crude Domain 1 prepared using a single pseudoproline dipeptide.

Application 2: Synthesis of Domain 1 peptide

The peptides were assembled automatically using a ABi 433A peptide synthesizer on Fmoc-Val-Wang resin. All acylation reactions were carried out using a 8-fold excess of Fmoc-amino acid activated with 1 eq. of HATU® in the presence of DIPEA. A coupling time of 60 min was used throughout. Cleavage and side-chain deprotection was effected by treatment of the peptidyl resins with TFA/ TIS /water 95:2.5:2.5 (5 ml) for 3 h. The peptides were isolated in the usual manner by evaporation and ether precipitation. The products were characterized by HPLC and ES-MS.

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. (2003) *J. Pept. Sci.*, in press.
6. Results kindly provided by Sharon Walker, La Jolla Pharmaceutical Company. Present address: Dept. of Chemistry, Massachusetts Institute of Technology.

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