Novabiochem®

innovations 3/07

Synthesis of the transmembrane region of bradykinin receptor

Transmembrane regions of receptor proteins represent some of the most challenging targets for peptide synthesis. This is because such sequences must be hydrophobic in order to be accommodated into the lipid bilayer of the cell wall and consequently tend to be composed of amino-acid residues that induce aggregation during solid phase assembly.

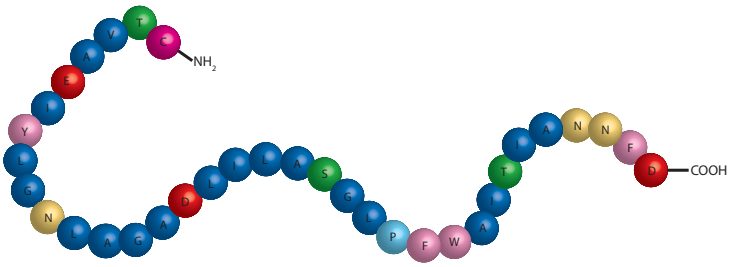

Fig. 1: Primary sequence of TM-34 [1].

This innovation details the synthesis of a peptide related to the rat bradykinin b2 receptor (TM-33). The preparation of the N-cysteinyl analog of this peptide (TM-34) (Figure 1) was first described by Oliveira, *et al.* [1]. It was chosen as a model to study the problems associated with the synthesis and purification of hydrophobic transmembrane sequences. Oliveira, *et al.* obtained their best results (12% yield) using Boc chemistry with 20% DMSO in NMP as reaction solvent. The synthesis using optimized Fmoc SPPS methods, however, was unsuccessful and did not result in the formation of any detectable product, despite the application of Fmoc-(Hmb) derivatives of Ala and Leu to prevent aggregation. Their failure to prepare this peptide and the even distribution of serine, threonine and glycine residues throughout the sequence make it an excellent test of our structure-breaking pseudoproline [2] and Dmb dipeptides [3, 4]. These derivatives were designed specifically for expediting the synthesis of difficult peptides. They work by exploiting the natural propensity of N-alkyl amino acids to disrupt the formation of the secondary structures during peptide assembly [5, 6]. Their use has been found to result in better and more predictable reaction kinetics, higher yields, purities, and solubilities of crude products [7 - 10].

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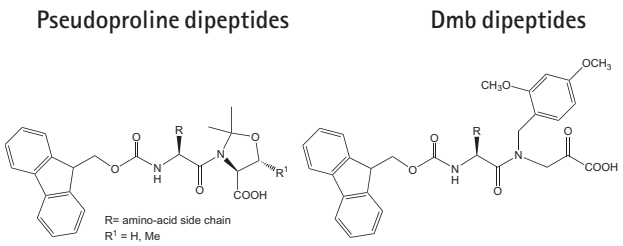
Using Dmb/pseudoproline dipeptides



Dmb and pseudoproline dipeptides consist of Fmoc-protected dipeptides in which the C-terminal residue is, respectively, a (Dmb)Gly or a dimethyloxazolidine derived from Ser/Thr. When coupled into a peptide chain, these derivatives temporarily introduce a structure-breaking imino acid which helps prevent aggregation during peptide assembly (Figure 2). During deprotection with TFA, the Dmb group and pseudoproline ring are cleaved and the native Gly and Ser/Thr residues are regenerated.

Dmb and pseudoproline dipeptides are extremely easy to use, as they can be introduced using any standard coupling method, substituting any Aaa-Gly or Aaa-Ser/Thr dipeptide, respectively, within the peptide chain. Novabiochem® Innovations 3/06 [4] and 1/07 [11] contain guidelines for selecting the optimum locations of Dmb and pseudoproline dipeptides.

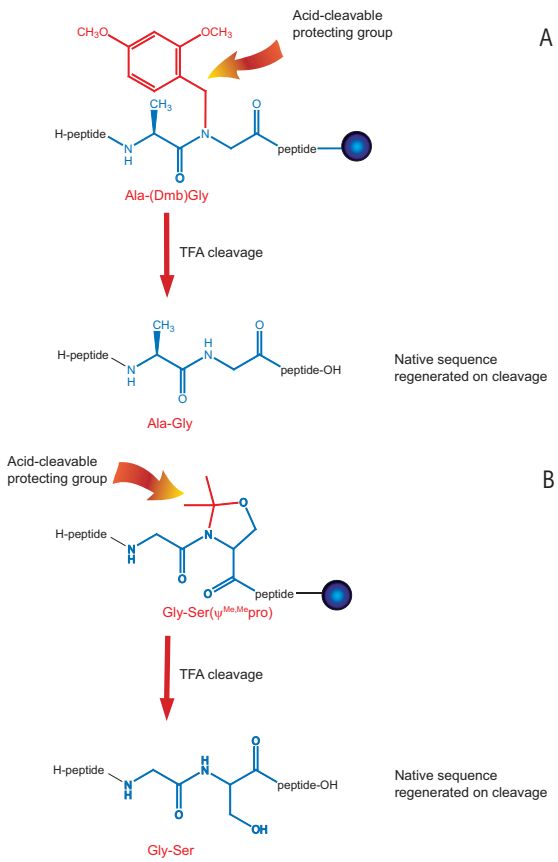


Fig. 2: TFA cleavage of A) Dmb and B) pseudoproline dipeptides.

Synthesis of TM-33

For our investigations it was decided to study the synthesis of a version of TM-34 which omits the N-terminal Cys residue, to avoid complications due to dimerization via disulfide bond formation (TM-33, Figure 3).

TM-33 was first assembled on Fmoc-Asp(OtBu)-Wang resin using a Protein Technologies Symphony peptide synthesizer. Couplings were carried out for 1 h using 10-fold excesses of standard Fmoc-amino acid building blocks activated with HCTU/NMM. Fmoc groups were removed by two treatments with 20% piperidine in DMF (1 x 10 min, 1 x 20 min). Global deprotection and cleavage of the peptide from the resin was effected by treatment with TFA/water/TIS (95:2.5:2.5) for 3 hours, and the product isolated by precipitation with ether. This highly insoluble material was dissolved in neat TFA, which was then diluted with MeCN/water, and analyzed by HPLC (Figure 4a) and MALDI-TOF. The product was found to be highly heterogeneous and not to contain any major component. These results confirm the findings of Oliveira, *et al.* [1] that TM-34 is a peptide difficult to be synthesized.

The synthesis of TM-33 was then undertaken using Novabiochem® secondary structure-breaking dipeptides. Ile^{26}-Thr^{27}/Ala^{18}-Ser^{19} and Ala^{11}-Gly^{12} were introduced using the Fmoc-Ile-Thr($\Psi^{Me,Me}$pro)-OH/Fmoc-Ala-Ser($\Psi^{Me,Me}$pro)-OH and Fmoc-Ala-(Dmb)Gly-OH (Figure 3), respectively according to our usage guidelines [4, 11]. Based on the findings of Oliveira, *et al.* [1], it was anticipated that insertion of structure-breaking dipeptides at these locations would result in marked improvements in synthetic efficiency.

Peptide assembly was performed on an ABi 433A peptide synthesizer using Fmoc-Asp(OtBu)-Wang resin with 1 h couplings of 10-fold excesses of Fmoc-amino acids and dipeptides activated with HCTU/DIPEA. Fmoc groups were removed by three treatments of 3 minutes with 20% piperidine in DMF. Samples of resin were taken at Leu^{21} (TM-13) and Leu^{15} (TM-19) and the peptides were cleaved with TFA/water/TIS as described previously and analyzed by HPLC and MALDI-TOF. In both cases the crude peptides obtained were of excellent quality (Figure 5), indicating aggregation had been prevented during peptide assembly. The synthesis was continued and the full length TM-33 was cleaved as described previously. The highly insoluble product was dissolved in TFA/MeCN/water as before and analyzed by HPLC. The HPLC profile of this material consisted of a very late eluting single peak with a long tailing edge (Figure 4b). MALDI-TOF analysis of samples taken across the peak indicated the peak represented the desired TM-33 (Figure 4c). The tailing of the peak is almost certainly caused by the hydrophobic nature of the peptide.

Fig. 3: Primary sequence of peptides prepared in this study. Sites of pseudoproline substitution are marked in red. Site of Dmb dipeptide substitution is marked in blue.

H-Leu-Pro-Phe-Trp-Ala- Ile-Thr-Ile-Ala-Asn-Asn-Phe-Asp-OH **TM-13**

H-Leu-Ile-Leu- Ala-Ser-Gly-Leu-Pro-Phe-Trp-Ala- Ile-Thr-Ile-Ala-Asn-Asn-Phe-Asp-OH **TM-19**

H-Thr-Val-Ala-Glu-Ile-Tyr-Leu-Gly-Asn-Leu^{10}- Ala-Gly-Ala-Asp-Leu-Ile-Leu- Ala-Ser-Gly^{20}-Leu-Pro-Phe-Trp-Ala-Ile-Thr-Ile-Ala-Asn^{30}-Asn-Phe-Asp-OH **TM-33**

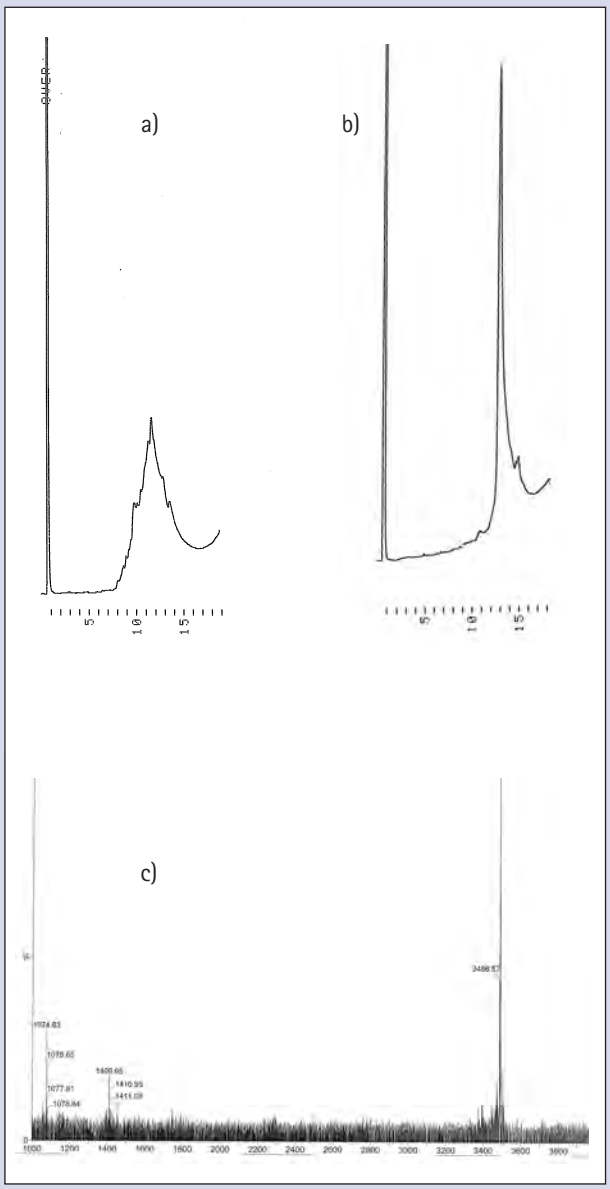


Fig. 4: a) HPLC elution of crude TM-33 prepared with standard amino acid derivatives. b) HPLC elution profile of crude TM-33 prepared using Dmb and pseudoproline dipeptides. c) MALDI-TOF spectrum of total crude TM-33 prepared with Dmb and pseudoproline dipeptides. Expected M+Na⁺ 3487.9; found 3488.6.

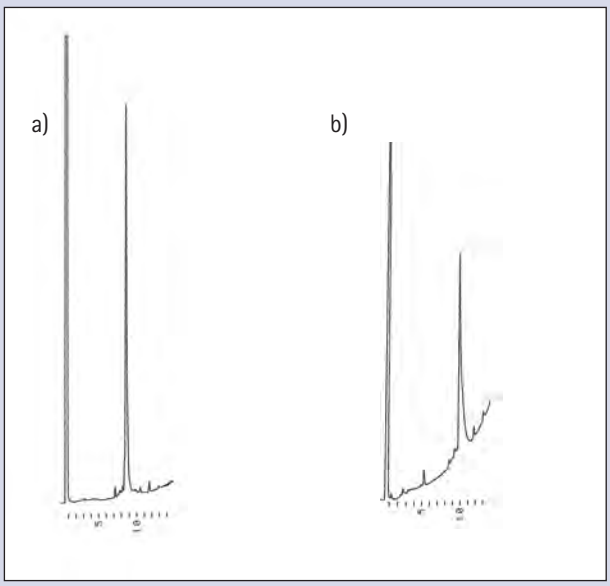


Fig. 5: HPLC elution profiles of a) crude TM-13 and b) crude TM-19 using pseudoproline dipeptides.

References

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Ordering information

Dmb dipeptides

04-12-1265	Fmoc-Ala-(Dmb)Gly-OH	1 g
04-12-1282	Fmoc-Asp(OtBu)-(Dmb)Gly-OH	1 g
04-12-1266	Fmoc-Gly-(Dmb)Gly-OH	1 g
04-12-1280	Fmoc-Ile-(Dmb)Gly-OH	1 g
04-12-1294	Fmoc-Leu-(Dmb)Gly-OH	1 g
04-12-1283	Fmoc-Val-(Dmb)Gly-OH	1 g

Pseudoproline dipeptides

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

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