Novabiochem®

innovations 3/06

Synthesis of neurotoxic prion peptide (106 - 126)

Spongiform encephalopathies such as bovine spongiform encephalopathy and Creutzfeldt-Jakob disease are caused by prion protein (PrP) [1]. The peptide comprising residues 106-126 of human PrP has been found to be highly amyloidogenic and toxic to neurons [2].

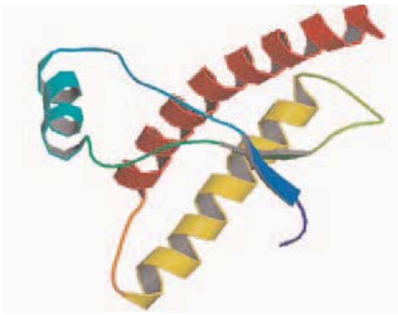

Fig. 1: Schematic representation of PrP [3].

The SPPS of PrP (106-126) has been described by Jobling, *et al.* [4]. In their hands, the synthesis of this peptide using standard Fmoc-protected amino acids was unsuccessful, with chain extension terminating at Ala^{117}. Incorporation of (Hmb)Gly residues at positions 114 and 119 resulted in a marked improvement in synthetic efficiency and afforded the full length peptide in a yield of 7.3%. However, the target peptide could only be obtained in good yield and purity by using Boc-protected amino acids with *in situ* neutralization coupling protocols.

The difficulties in the synthesis of PrP (106-126) were ascribed to the inherent propensity of this peptide to aggregate, particularly in the region of the AGAAAAGA sequence. The presence of glycine residues on either side of the problematic region make this sequence an excellent test of Novabiochem's structure-breaking Dmb dipeptides. These derivatives were designed specifically for expediting the synthesis of such hydrophobic glycine-containing peptides. They work in an identical manner to pseudoproline dipeptides by exploiting the natural propensity of N-alkyl amino acids to disrupt the formation of the secondary structures during peptide assembly. Their use results in better and more predictable reaction kinetics, and higher yields, purities, and solubilities of crude products.

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Using Dmb dipeptides

The use of Dmb dipeptides is very straight forward (Figure 2); simply substitute a Gly reside together with the preceding amino acid residue in the peptide sequence with the appropriate dipeptide. They can be introduced using any standard coupling methods, and the native sequence is regenerated on TFA-mediated cleavage and deprotection. For best results, it is recommended to follow the guidelines below.

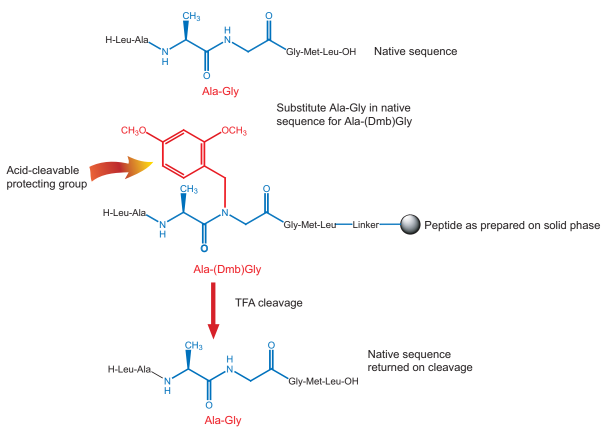

Fig. 2: Principles of using Dmb dipeptides.

General guidelines for the use of Dmb dipeptides

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

Synthesis of PrP (106-126)

In view of the difficulties experienced by Jobling, *et al.* [4] in the Fmoc SPPS of PrP (106-126), we decided to prepare this peptide using three Dmb dipeptides at the positions marked in Figure 3. This approach would introduce structure-breaking (Dmb)Gly residues before Ala^{120} and Ala^{117}, regions of the sequence which were found to be particularly problematic, and would therefore maximize the chances of a successful synthesis. The peptide was prepared using Fmoc-Gly-Wang resin (0.1 mmol, 0. 71 mmol/g) on an ABi 433A peptide synthesizer using standard FastMoc protocols with feedback conductivity monitoring and a 30 minutes coupling time. The Dmb dipeptides were introduced using a 3-fold excess of reagents instead of the standard 10-fold excess which was used for all other amino acids. At residue Ala^{115}, the synthesis was paused and a portion of resin was removed. A small amount of this resin was treated with TFA/water/TIPS (95:2.5:2.5) for 3 hours. The cleaved product was analyzed by LC-MS and found to be essentially homogeneous. The assembly was then continued on the bulk of the resin to give the full length peptide. Treatment of the resin with TFA as previously described afforded the crude PrP in excellent purity, and MS analysis of the product showed no evidence of deletion or truncated peptides (Figure 4a, b).

The synthesis of PrP (106-126) was then repeated using standard Fmoc-amino acid building blocks. A sample of resin was taken at Ala^{115} and peptide was cleaved and analyzed by LC-MS. The crude peptide obtained was of excellent quality, which was rather surprising in view of the difficulties reported by Jobling, *et al.* The synthesis was continued and the full length PrP was cleavage as described previously. Characterization of this material by LC-MS indicated that the product contains less than 48% of the target peptide, together with significant amounts of the des-Asn^{108} and des-(Lys^{106}, Asn^{108}) deletion peptides, and smaller amounts of other peptides missing residues from the N-terminal sequence (Figure 4c, d). These results indicate that there are no issues with aggregation and failed coupling reactions during the assembly of residues 117-120 of PrP. Instead, it rather appears that the difficulties occur predominantly during the introduction of the N-terminal residues, and these can be effectively overcome by the use of Fmoc-Ala-(Dmb)Gly-OH for the incorporation of Ala-Gly residues.

Fig. 3: Primary sequence of PrP (106-126). The residues highlighted in red were introduced using Dmb dipeptides.

H-Lys^{106}-Thr-Asn-Met-Lys-His-Met-Ala-Gly-Ala^{115}-Ala-Ala-Ala-Gly-Ala-Val-Val-Gly-Gly-Leu-Gly^{126}-OH

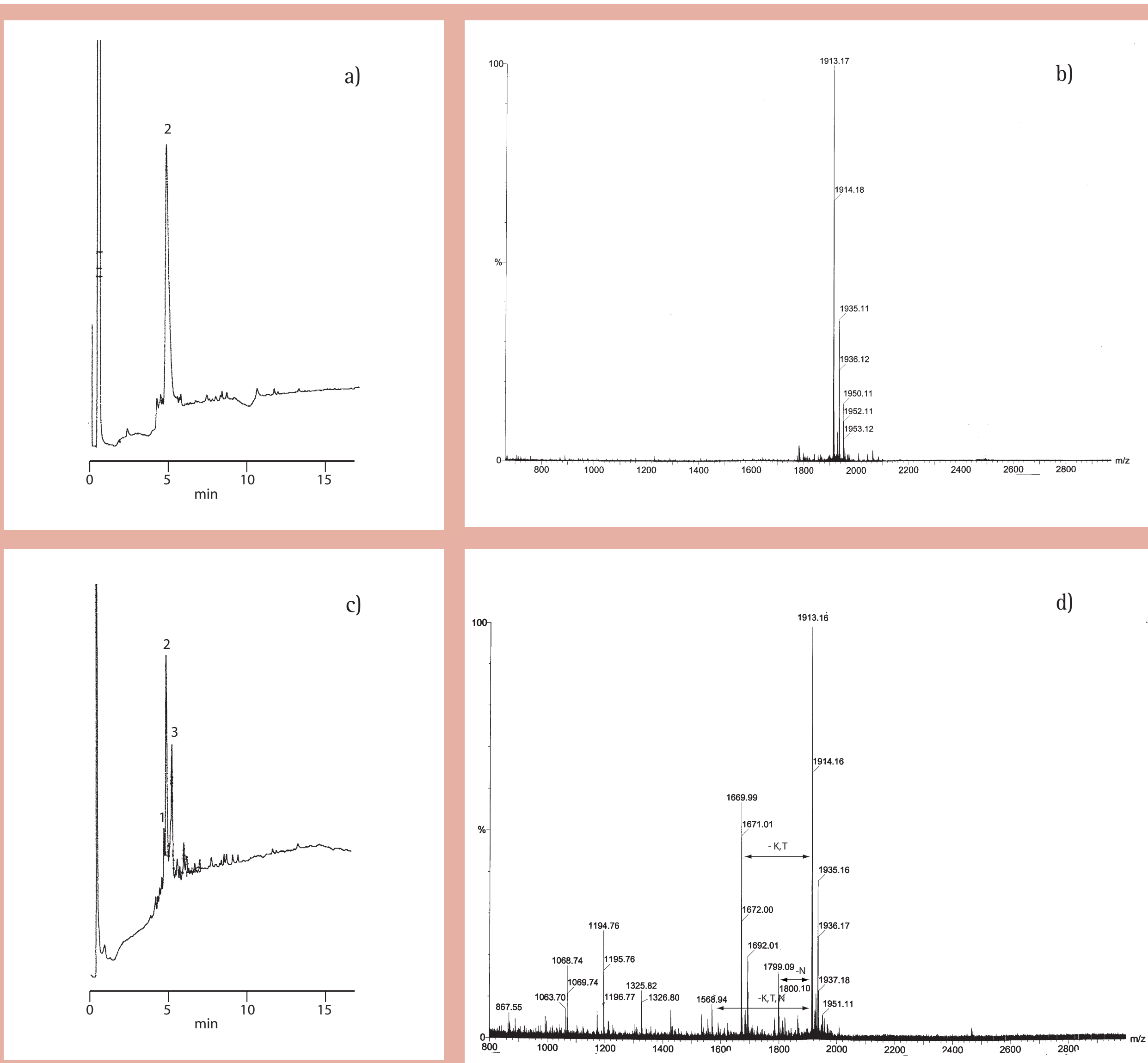

Fig. 4: a) HPLC profile and b) MALDI-TOF of crude Prp (106-126) prepared using 3 Dmb dipeptides. c) HPLC profile and d) MALDI-TOF of crude PrP (106-126) prepared using standard Fmoc-amino acid derivatives. Peak 1: des-Asn^{108} PrP (106-126); Peak 2: PrP (106-126); Peak 3: des-(Lys^{106}, Asn^{108}) PrP (107-126).

Ordering information

04-12-1265	Fmoc-Ala-(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

Novabiochem's other structure disrupting dipeptides

04-12-1235	Fmoc-Asp(OtBu)-(Hmb)Gly-OH	1 g 5 g
04-12-1253	Fmoc-Gly-(Hmb)Gly-OH	1 g 5 g
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. F. Tagliavini, et al. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 9678.
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4. M. F. Jobling, et al. (1999) *Lett. Pept. Sci.*, **6**, 129.

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