Novabiochem®

Amide-protected building blocks for smart peptide synthesis

M. Petretta[1], S. Barthélémy[1], B. Dörner[1], J. Keyte[2], and P. D. White[3]

[1]Novabiochem, Merck Biosciences AG, Weidenmattweg 4, CH-4448 Läufelfingen, Switzerland

[2]School of Biomedical Sciences, University of Nottingham, Notts, NG7 2UH, U.K.

[3]Novabiochem, Merck Biosciences Ltd., Beeston, Notts, NG9 2JR, U.K.

Introduction

Amide-protected building blocks, such as those shown in Figure 1, can be used to improve the efficiency of Fmoc solid phase peptide synthesis of aggregation-prone sequences.

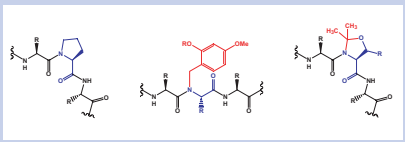

Figure 1: Structure breaking building blocks – proline, Hmb/Dmb amide protection and pseudoproline.

The use of pseudoproline dipeptides [1], in particular, has been found to result in remarkable improvements in the purities and yields of cyclic [2], long [3] and difficult peptides [4]. Here, we examine the application of these building blocks in convergent synthesis, and the use of novel Dmb dipeptides in the synthesis of a difficult sequence.

Results and discussion

Convergent synthesis

Minimising the risk of racemisation of the C-terminal amino acid of the carboxylic component during fragment condensation is of paramount concern. Peptide fragments containing serine and threonine residues at their C-terminus are known to undergo extensive epimerisation upon carboxyl activation. In order to determine whether protection of these residues as a pseudoproline would protect against loss of chiral integrity, we prepared a model tripeptide (Figure 2), and the extent of epimerisation was assessed by HPLC (Table 1, Figure 3).

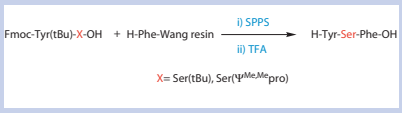

Figure 2: Synthesis of model tripeptide.

Results

- Serine residue introduced using standard tBu protection was nearly totally racemised.
- No detectable epimerisation was observed with the peptide prepared using a pseudoproline dipeptide.
- Use of pseudoproline protection for Ser or Thr doubles the number of sites in any given peptide available for epimerisation-free fragment condensation: Gly, Pro, Ser and Thr.

Table 1: Studies comparing the racemisation of C-terminal Ser residue when protected as a pseudoproline or tBu ether during the coupling to H-Phe-Wang resin. Peptide 1: H-Tyr(tBu)-Ser(tBu)-Phe-OH; Peptide 2: H-Tyr(tBu)-D-Ser(tBu)-Phe-OH; Peptide 3: H-Tyr(tBu)-Ser(ΨMe,Mepro)-Phe-OH.

Experiment	Peptide	Conditions	Solvent	% L-Ser	% D-Ser
1	1	PyBOP/DIPEA	DMF	65	35
2	2	PyBOP/DIPEA	DMF	36	64
3	3	PyBOP/DIPEA	DMF	100	0
4	1	PyBOP/collidine	DMF	68	32
5	1	HATU/DIPEA	DMF	70	30
6	1	HCTU/DIPEA	DMF	60	40

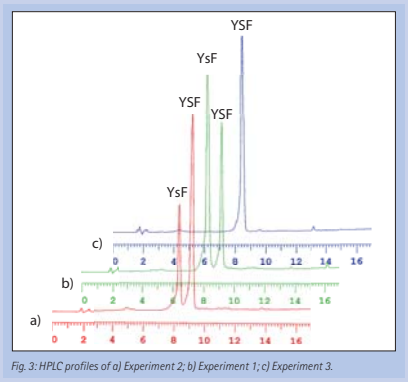

Fig. 3: HPLC profiles of a) Experiment 2; b) Experiment 1; c) Experiment 3.

Synthesis of Fibrinogen A related peptide

To exemplify the use of pseudoproline dipeptides in convergent synthesis, a fibrinogen A related peptide was prepared by a 2 fragment condensation approach, utilising a pseudoproline residue as the C-terminal residue in the N-terminal fragment (Figure 4).

2-Chlorotityl resin was selected as the support for the synthesis of the N-terminal fragment, as cleavage of protected peptides can be achieved without affecting the pseudoproline ring and the bulky trityl linker prevents diketopiperazine formation, which can be a problem with C-terminal proline on other linkers.

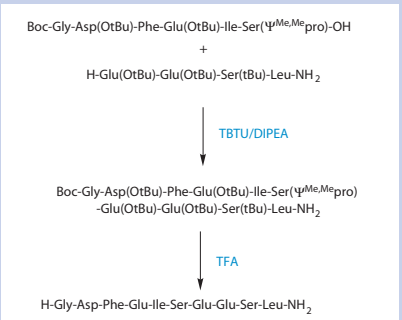

Fig. 4: Synthesis of Fibrinogen A related peptide.

Peptide Synthesis

N-terminal fragment

Resin: Fmoc-Ile-Ser(ψMe,Mepro)-2-Cl-Trt resin 0.26 mmol/g

Coupling: PyBOP®/DIPEA 1 h

Cleavage: 0.5% TFA in DCM (HPLC analysis Fig. 5a)

C-terminal fragment

Resin: Sieber amide resin 0.55 mmol/g

Coupling: PyBOP®/DIPEA 1 h

Cleavage: 1 % TFA in DCM (HPLC analysis Fig. 5a)

Fragment condensation

TBTU (1.2 eq)/DIPEA (4 eq)/DMF, 4 h (Fig. 5a)

Side-chain deprotection: TFA/TIS/water 95:2.5:2.5 3 h. (HPLC analysis Fig. 5b)

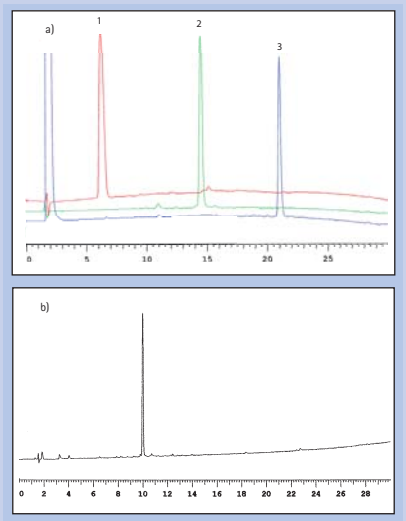

Fig. 5: a) HPLC profile of 1) C-terminal fragment; 2) N-terminal fragment; 3) full length protected peptide; b) HPLC profile of crude product after TFA cleavage.

Dmb dipeptides

In another aspect of our ongoing program to develop structure-breaking amide-protected building blocks, we have prepared a number of Dmb-protected dipeptides. These derivatives can be used in a similar manner to pseudoproline dipeptides and offer the same enhancements in synthetic efficiency but for peptides containing Gly residues.

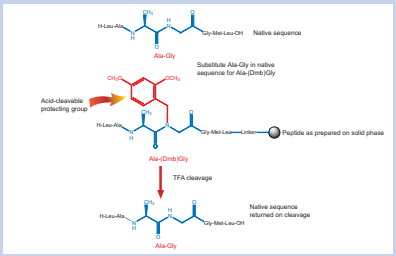

Fig. 6: Principles of using Dmb dipeptides.

To demonstrate the utility of these derivatives, the Fmoc synthesis of the amyloidogenic Neurotoxin prion peptide (PrP) 106-126 [5] was performed using standard derivatives and with Dmb-dipeptides introduced at the position indicated in Figure 7.

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. 7: Primary sequence of PrP (106-126). The residues highlighted were introduced using Dmb dipeptides.

Peptide Synthesis

Resin: Fmoc-Gly-Wang resin (0.1 mmol, 0. 71 mmol/g)

Coupling: FastMoc Protocols on ABi 433A. 30 min 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.

Cleavage: 95:2.5:2.5 TFA/TIS/water 3 h

Results

- Material prepared using standard building blocks contained less than 48% of the target peptide.
- Peptide prepared using Dmb dipeptides was over 90% pure by HPLC.

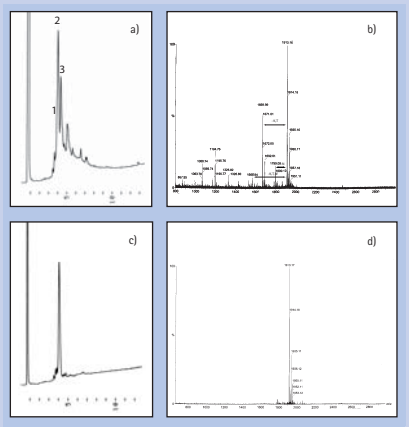

Fig. 8: a) HPLC profile and b) MALDI-TOF of crude PrP (106-126) prepared using standard Fmoc-amino acid derivatives. Peak 1: des-Asn108 PrP (106-126); Peak 2: PrP (106-126); Peak 3: des-(Lys106, Asn108) PrP (107-126); c) HPLC profile and d) MALDI-TOF of crude PrP (106-126) prepared using 3 Dmb dipeptides.

Conclusions

- Protecting C-terminal Ser or Thr residues as pseudoprolines protects against epimerisation during fragment condensation.
- Dmb dipeptides are effective tools for enhancing the synthetic efficiency of hydrophobic peptides.

References

1. Wöhr T., et al. *J. Am. Chem. Soc.* **118**, 9218 (1996).
2. Schmiedeberg N. & Kessler H. *Org. Lett.* **4**, 59 (2002).
3. White P. D., et al. *J. Pept. Sci.* **10**, 18 (2004).
4. Abedini A. & Raleigh D. P., *Org. Lett.* **7**, 693 (2005).
5. Jobling M.F., et al. *Lett. Pept. Sci.* **6**, 129 (1999).

Merck Biosciences

Calbiochem | Novabiochem | Novagen

MERCK