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Pseudoproline dipeptides in Fmoc-solid phase peptide synthesis

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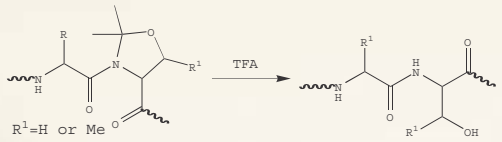
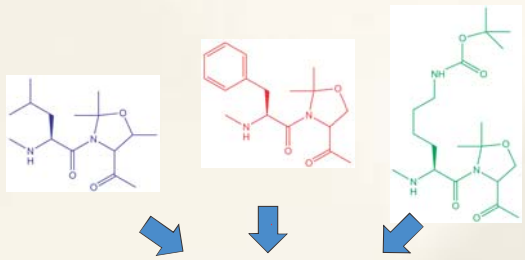
Introduction

Pseudoproline dipeptides were introduced by Mutter, et al. [1-2] as reversible proline mimetics for modulation of protein structure. The proline-like moiety is generated by formation of an oxazolidine ring between the α-amino and the side chain hydroxy groups of Ser or Thr with an aldehyde or ketone. Such pseudoproline dipeptides are highly effective at preventing aggregation during solid phase synthesis as they disrupt the formation of β-sheets and helical conformations. The effects can be long range, with the outset of aggregation often being postponed for as many as six residues, or eliminated altogether. They have been used with great effect to prepare long peptides / small proteins [3,4], cyclic peptides [5], or to disrupt structure as synthetic proline analogues [6]. The incorporation of pseudoproline dipeptides has been found to lead to overall improvements in acylation and deprotection kinetics, resulting in better yield, purity and solubility of crude products and easier HPLC purification with higher amounts of isolated products.



In this poster we explore the factors influencing the synthesis of a model difficult peptide **1** (Scheme1). We compare the effectiveness of using pseudoproline dipeptides versus costly but efficient coupling methods such as HATU and examine how the location of the pseudoproline within the peptide sequence and the nature of the solid support influence peptide purity.



Scheme 1: Peptide 1 was prepared by Fmoc SPPS applying 3 different pseudoproline dipeptides as outlined. During final TFA treatment the oxazolidine is cleaved resulting in the native peptide sequence.

Results & Discussion

Peptide 1 was prepared by Fmoc SPPS on a Rainin Symphony automated synthesizer under 12 different sets of reaction conditions as summarized in Table 1. In all cases, cleavage of the peptides from the solid support with concommitant side-chain deprotection was effected by treatment with TFA / water / triisopropylsilane (95:2.5:2.5) for 2 h.

Table 1: Reaction Conditions.

Experiment	Resin	Coupling reagent (3.3 eq.)	Coupling time (min)	Pseudoproline dipeptide	Coupling time for pseudoproline (min)
1	Wang	PyBOP/HOBt/DIPEA (1:1:1.5)	30	No	N/A
2	Wang	HCTU/DIPEA (1:1.5)	30	No	N/A
3	Fmoc-Gln(Trt)-TGA	HCTU/DIPEA (1:1.5)	30	No	N/A
4	Wang	HCTU/DIPEA (1:1.5)	30	F^7S^8	60
5	Wang	HCTU/DIPEA (1:1.5)	30	F^7S^8	30
6	Wang	HCTU/DIPEA (1:1.5)	30	$K^{10}S^{11}$	60
7	Wang	HCTU/DIPEA (1:1.5)	30	L^5F^6	60
8	Wang	DIC/HOBt (1:1)	60	F^7S^8	60
9	Wang	HATU/DIPEA (1:1.5)	30	No	N/A
10	2ClTrt	HCTU/DIPEA (1:1.5)	30	F^7S^8	N/A
11	Sieber amide	HCTU/DIPEA (1:1.5)	30	F^7S^8	N/A
12	Rink amide MBHA	HCTU/DIPEA (1:1.5)	30	F^7S^8	60

Synthesis without pseudoproline dipeptides (Experiments 1, 2, 3, 9)

The synthesis of the peptide on Wang (0.57 mmol / g) and NovaSyn® TGA (0.19 mmol / g) resins gave very poor results irrespective of the coupling method used (Figure 1a-d, Table 1). The use of HATU (experiment 9, Figure 1d) appeared to offer no benefit over less expensive coupling reagents. LC-ES analysis of the crude peptide obtained from experiment 2 indicated that problems occur after introduction of Leu-5. The compound with an elution time of 18 min is Fmoc-Leu-Thr-Phe-Ser-Asn-Lys-Ser-Val-Leu-Gln-OH. The other major peaks represent peptides arising from single and multiple deletions of residues Val-1, Thr-2, Arg-3 and Tyr-4, as indicated in Figure 1a.

Synthesis with pseudoproline dipeptides (Experiments 4-8, 10-12)

Good to excellent purities were obtained for all syntheses, regardless of the position of the pseudoproline dipeptide (Figure 1e-f). This result was not unexpected as all pseudoproline substitution sites were within 6 residues of the Leu-5.

Reducing the coupling time used for introduction of the pseudoproline from 60 to 30 min (experiment 5) reduced peptide purity, indicating that a minimum coupling time of 1 h is required for pseudoproline dipeptides.

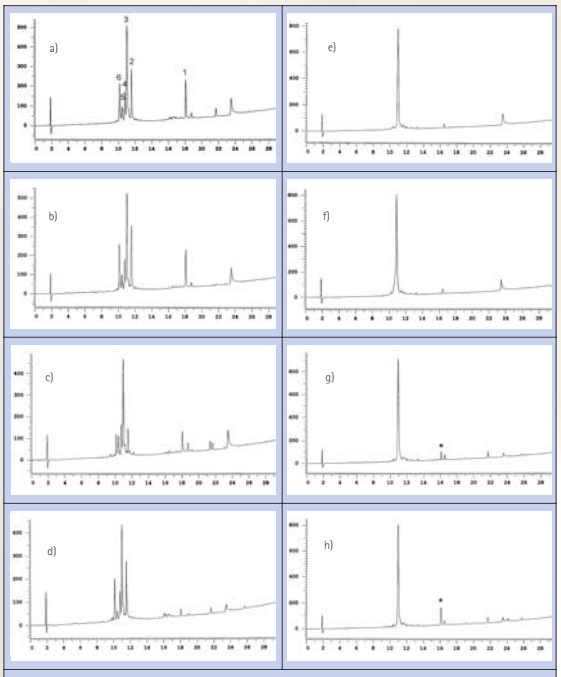


Figure 1:
Crude HPLC profiles of peptides obtained from a) experiment 1, b) experiment 2, c) experiment 3, d) experiment 9, e) experiment 4, f) experiment 5, g)experiment 6, h) experiment 7. * Partially protected peptide. Peak 1: Fmoc-Leu-Thr-Phe-Ser-Asn-Lys-Ser-Val-Leu-Gln-OH; Peak 2: H-Val-Thr-Tyr-Leu-Thr-Phe-Ser-Asn-Lys-Ser-Val-Leu-Gln-OH + H-Val-Tyr-Leu-Thr-Phe-Ser-Asn-Lys-Ser-Val-Leu-Gln-OH; Peak 3: H-Val-Thr-Arg-Tyr-Leu-Thr-Phe-Ser-Asn-Lys-Ser-Val-Leu-Gln-OH; Peak 4: H-Tyr-Leu-Thr-Phe-Ser-Asn-Lys-Ser-Val-Leu-Gln-OH; Peak 5: H-Val-Leu-Thr-Phe-Ser-Asn-Lys-Ser-Val-Leu-Gln-OH; Peak 6: H-Leu-Thr-Phe-Ser-Asn-Lys-Ser-Val-Leu-Gln-OH. HPLC conditions: Nucleosil C18 300-5 mm; 5-97% B in 30 min at 30°C, 0.8 ml / min; A: 0.1% TFA in water; B: 0.1 TFA in acetonitrile.

Conclusion

- These results confirm that insertion of a single pseudoproline is sufficient to prevent aggregation for up to 6 amino acid additions
- Incorporation of a pseudoproline dipeptide is a more effective strategy for overcoming difficulties in peptide assembly than the use of powerful coupling methods such as HATU

References

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