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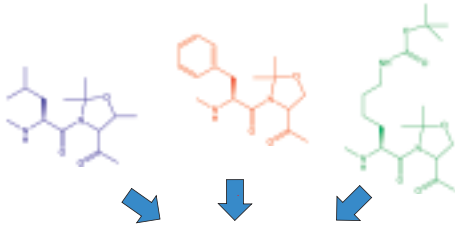
innovations 4/04

Exploring strategies for peptide synthesis

To obtain the best possible yields and purities of peptides by solid phase synthesis, it is essential to achieve high coupling efficiency at every cycle. One of the factors most thought to influence coupling yield is the choice of carboxyl activation. Modern coupling methods based on phosphonium or aminium compounds, such as PyBOP® or TBTU, provide rapid and efficient generation of OBt esters with the minimum of side-reactions. For difficult couplings, reagents like HATU and HCTU are particularly effective as they produce the more reactive OAt and OCt esters. Another important factor is the nature of the solid support. Polar resins based on PEG and PEG-polystyrene have been particularly advocated for challenging sequences as their use is regarded to lead to better solvation of the growing peptide chain. A further approach that has proved extremely effective in the synthesis of Ser- and Thr-containing peptides has been the use of Mutter's dimethyloxazolidine (pseudoproline) dipeptides [1, 2], which have been employed to great effect to prepare long peptides/small proteins [3], cyclic peptides [4], and intractable sequences [5].

To explore how such factors influence the outcome of Fmoc SPPS, the model peptide **1** was prepared under 12 different sets of conditions, varying the coupling method, solid support and position of the pseudoproline dipeptide. In this particular sequence, only the use of a pseudoproline dipeptide had a marked influence on the purity of the final product. The exact position of the pseudoproline dipeptide had little effect on the outcome of the synthesis, provided it was no more than six residues before the onset of coupling and deprotection difficulties.



H-Val-Thr-Arg-Tyr-Leu-Thr-Phe-Ser-Asn-Lys-Ser-Val-Leu-Gln-X **1** X=NH_2, OH

*Fig. 1: Sequence of peptide **1**, highlighting possible points for pseudoproline dipeptide insertion.*

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Synthesis of peptide **1**

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.

Synthesis without pseudoproline dipeptides

Experiments 1, 2, 3, 9

- The synthesis of the peptide on Wang and NovaSyn® TGA resins gave very poor results irrespective of the coupling method used (Figure 2, Table 1).
- The use of HATU (experiment 9, Fig. 2d) 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 Fig. 2a.

Synthesis with pseudoproline dipeptides

Experiments 4-8, 10-12

- Good to excellent purities were obtained for all syntheses, regardless of support or coupling method used.
- Position of the pseudoproline dipeptide had no influence on peptide purity (Figure 2e, g, h). All sites were within 6 residues of the region of difficulty.
- Inexpensive DIPCDI/HOBt coupling in conjunction with a single pseudoproline dipeptide substitution was far more effective than using HATU without the substitution.
- 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.

*Table 1: Summary of experiments carried out on peptide **1**.*

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

Summary

- These results confirm that insertion of a single pseudoproline dipeptide 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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2. M. Mutter et al. (1995) *Pept. Res.* **8**, 145.
3. P. White et al. (2004) *J. Pept. Sci.* **10**, 18.
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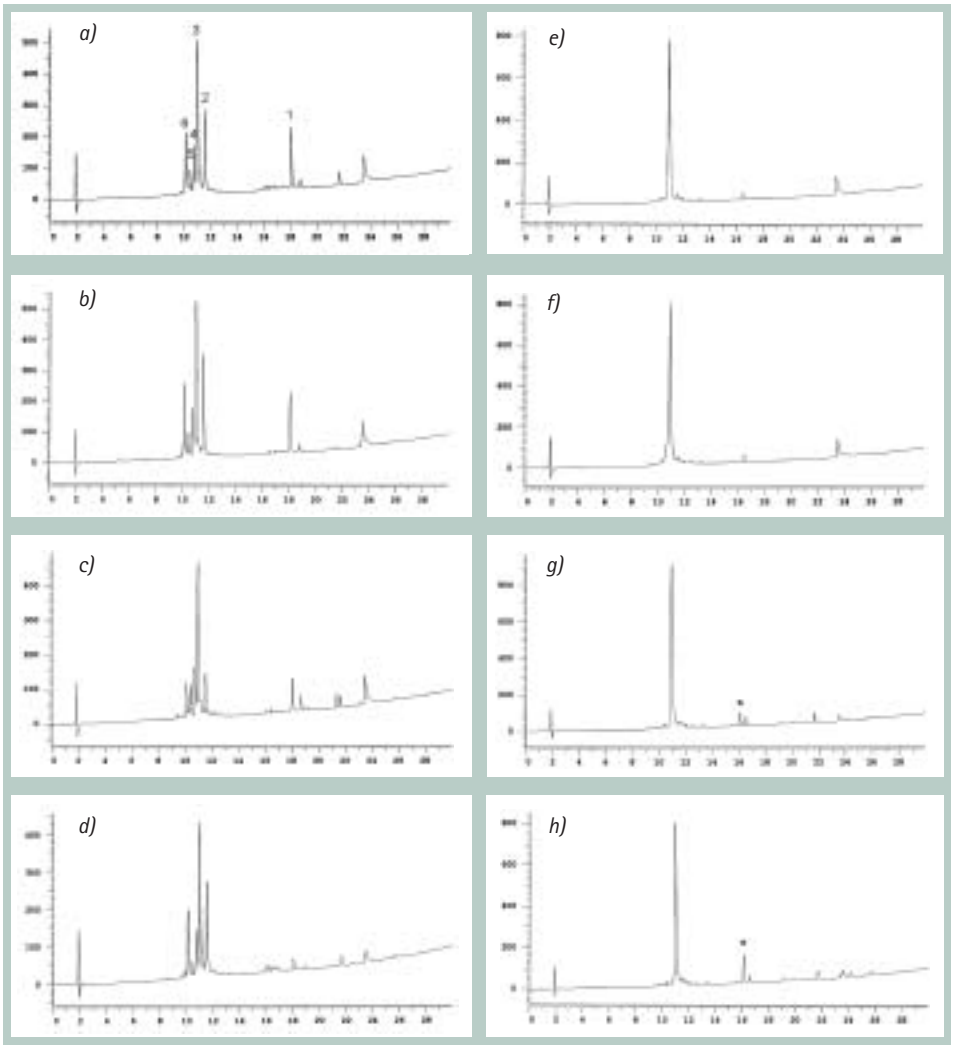

*Fig. 2: Crude HPLC profiles of peptides obtained from a) experiment 1, b) experiment 2, c) experiment 3, d) experiment 9, e) experiment 4, f) experiment 5, g) experiment 6, h) experiment 7. *Partially protected peptide. Peak 1: Fmoc-Leu-Thr-Phe-Ser-Asn-Lys-Ser-Val-Leu-Gln-OH; Peak 2: H-Val-Thr-Tyr-Leu-Thr-Phe-Ser-Asn-Lys-Ser-Val-Leu-Gln-OH + 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.*

"We have had great success using the pseudoproline derivatives for the synthesis of difficult sequences. For example, we used these reagents to aid formation of side-chain lactam-bridged peptides that were otherwise unobtainable. I would highly recommend these reagents for your difficult syntheses."

Clifford Quan, Genentech, Inc., South San Francisco, CA.

"Pseudoproline dipeptides have greatly increased our success rate for synthesizing both long and difficult peptides. If we are able to integrate pseudoprolines into our syntheses, we can easily machine-synthesize peptides up to 80 amino acids in length. Routine use of pseudoprolines in our peptide syntheses has considerably increased the yield and purity, as well as decreased the number of failed syntheses. They are wonderful products!"

Yingwei He, Protein Chemistry Dept., Abgent, San Diego, CA.

"Biomol started incorporating pseudoproline derivatives into its everyday schedules for routine peptide synthesis some eight years ago. Over the intervening years, the use of these reagents on a routine basis has led to a dramatic reduction in the necessity for repeat synthesis. When coupled with an undoubted improvement in the yield and purity of crude peptides obtained, this has meant considerable financial savings in terms of both synthesis and purification costs. We are firmly of the opinion that the benefits of incorporation of pseudoproline analogs into peptide synthesis protocols is fully justifiable on both scientific and commercial grounds and is to be recommended on a routine basis."

Paul Sheppard, Biomol International Lp, Exeter, UK.

Ordering information

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05-20-1011	Fmoc-Asp(OtBu)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
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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
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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
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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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