Novabiochem®

Comparative study of conventional and microwave assisted synthesis

P.White[1], J. Collins[2], and Z. Cox[2]
[1]Merck Biosciences Ltd. Novabiochem, Padge Road, Beeston, NG9 2JR, United Kingdom
[2]CEM Corp., Peptide Synthesis Division, Matthews, NC-28106, USA

Introduction

Microwave irradiation is increasingly being used to accelerate the rate of reactions between soluble and polymer-bound reactants [1]. The application of microwave heating to solid-phase peptide synthesis is particularly advantageous as the acceleration of coupling and deprotection reactions should lead to shorter cycle times, higher repetitive yields, and ultimately purer peptides.

In this poster, we compare the synthesis of a difficult peptide carried out under both conventional ambient and microwave conditions. Synthesis of this peptide with standard amino acid derivatives is known to lead to a complex mixture of truncated peptides. In previous studies it was found that satisfactory results could only be obtained by the substitution of a serine or threonine and its preceding residue with a pseudoproline dipeptide [2]. We were therefore very interested to explore how microwave heating would influence the assembly of this peptide, and in particular to see if the combination of microwaves and pseudoproline substituent would prove synergistic.

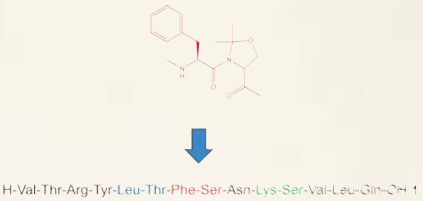

Fig. 1: Primary sequence of model peptide **1**. Site of pseudoproline dipeptide substitution marked in red.

Results & Discussion

Peptide **1** was prepared initially by Fmoc SPPS on Fmoc-Gln(Trt)-Wang resin (0.57 mmol/g) using either a Rainin Symphony™- or a CEM Liberty™-synthesizer under the conditions set out in Table 1. On the Liberty both acylation and deprotection reactions were accelerated by microwave heating (Table 2). 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). The reaction was carried out for 2 h under ambient conditions on the Symphony and 18 min on Liberty with microwave heating (Table 2) in DMF.

Table 1: Reaction Conditions

Experiment	Instrument	Coupling reagent (3.3 eq.)	Coupling time (min)	Double couple Arg	Pseudoproline dipeptide	Fmoc deprotection time (min)	Microwave
1	Symphony	PyBOP/DIPEA (1:1.5)	30	No	No	2 x 3.5	No
2	Symphony	HCTU/DIPEA (1:1.5)	30	No	F^7S^8	2 x 3.5	No
3	Liberty	HBTU/HOBt/DIPEA (1:1:1)	5	No	No	2 x 3.5	No
4	Liberty	HBTU/HOBt/DIPEA (1:1:1)	5	No	No	1	Yes
5	Liberty	HBTU/HOBt/DIPEA (1:1:1)	5	No	F^7S^8	1	Yes
6	Liberty	HBTU/HOBt/DIPEA (1:1:1)	5	Yes	No	1, 3	Yes
7	Liberty	HBTU/HOBt/DIPEA (1:1:1)	5	Yes	F^7S^8	1, 3	Yes

Table 2: Microwave Conditions

Microwave assisted reaction	Power (W)	Time (min)	Temperature (°C)
Deprotection	1) 25 2)25	1) 1 2) 3	70 70
Acylation	25	5	75
Cleavage	25	18	40

Ambient (Experiments 1, 3)

The synthesis of peptide **1** under standard conditions (without a pseudoproline substitution or microwave heating) gave very poor results irrespective of which instrument was used (Figure 3a and 3c, Table 1, experiments 1, 3). LC-ES analysis of the crude peptide obtained from experiment 1 indicated that problems occur after introduction of Leu-5. The compound with an elution time of 18 min (Figure 3a, peak 1) 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.

In experiment 2, pseudoproline dipeptide substitution of residues F^7S^8 led to a dramatic increase in synthetic efficiency and the desired product being obtained in excellent yield (Figure 3b). A single pseudoproline dipeptide substitution in this case is evidently sufficient to totally overcome aggregation during chain assembly.

Microwave heating (Experiments 4 -7)

The synthesis using microwave heating (experiment 4) did not give the desired peptide as the major product, instead giving a 3:1 mixture of des-Arg peptide **1** and peptide **1** itself (Figure 3d). In the case of the synthesis using a combination of both microwave heating and pseudoproline dipeptide substitution, the situation was reversed and a mixture of des-Arg-peptide 1 and peptide 1 in a ratio of 1:3 was obtained (Figure 3e).

These results indicate that in general microwave heating is effective in accelerating acylation and deprotection reactions and overcoming aggregation in this difficult sequence, but for arginine in particular there appear to be difficulties.

The formation of γ-lactams during the carboxy-activation of arginine derivatives is well documented. We, therefore, speculated that under microwave heating this side reaction may compete against amide-bond formation, particularly if the coupling is difficult as is the case in our test peptide. This notion is borne out by the observation that in the synthesis using a pseudoproline dipeptide, where aggregation is suppressed, levels of arginine incorporation were much higher compared to the synthesis using standard Fmoc amino-acid derivatives.

To test this theory, a sample of Fmoc-Arg(Pbf)-OH was activated with HBTU/HOBt/DIPEA and subjected to microwave heating for 4:30 min. LC-MS analysis indicated approximately 80% conversion to the γ-lactam under these conditions (Figure 3h). In order to determine whether it would be possible to compensate for lactam formation by double coupling of arginine, experiments 4 & 5 were repeated under the same conditions except the arginine coupling was performed twice with fresh reagents (experiments 6 & 7). Following cleavage from the resin and side-chain deprotection, crude products were obtained that gave the elution profiles shown in Figures 3f & 3g. From these results, it can be seen that with double coupling the levels of arginine incorporation significantly improved, but in neither case was the reaction complete. The best result was obtained for the synthesis using a combination of microwaves and pseudoproline dipeptide where the amounts of des-Arg peptide was reduced to less than 5 %.

Further studies are now ongoing to optimize arginine coupling for microwave heating.

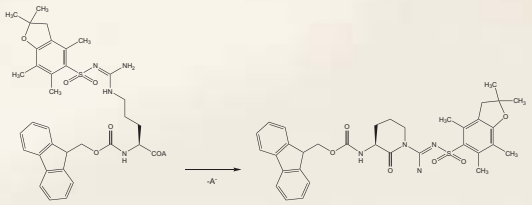

Fig. 2: Formation of γ-lactam during carboxy-activation of Fmoc-Arg(Pbf)-OH.

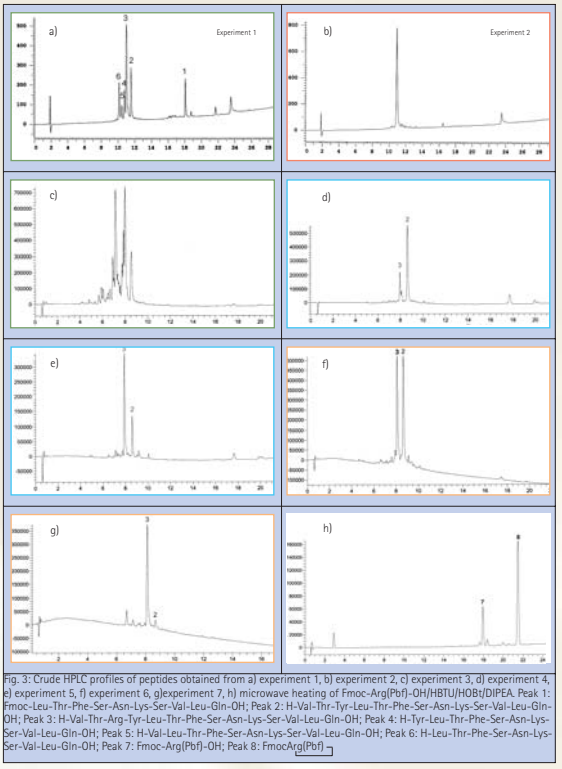

Fig. 3: Crude HPLC profiles of peptides obtained from a) experiment 1, b) experiment 2, c) experiment 3, d) experiment 4, e) experiment 5, f) experiment 6, g)experiment 7, h) microwave heating of Fmoc-Arg(Pbf)-OH/HBTU/HOBt/DIPEA. Peak 1: Fmoc-Leu-Thr-Phe-Ser-Asn-Lys-Ser-Val-Leu-Gln-OH; Peak 2: H-Val-Thr-Tyr-Leu-Thr-Phe-Ser-Asn-Lys-Ser-Val-Leu-Gln-OH; 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; Peak 7: Fmoc-Arg(Pbf)-OH; Peak 8: FmocArg(Pbf)

Conclusion

- Microwave heating significantly reduced coupling times compared to ambient coupling.
- Best results were obtained using pseudoproline dipeptide substitution, regardless of heating method.
- Microwave heating appears to accelerate γ-lactam formation during arginine coupling.

References

[1] M. Larhed & A. Hallberg (2001) *Drug Discovery Today*, **6**, 406.
[2] Novabiochem Innovations 4/04.

EMD Biosciences
Calbiochem | Novabiochem | Novagen

EMD