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Microwaves enhance cyclisation of tetrapeptides

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ABSTRACT

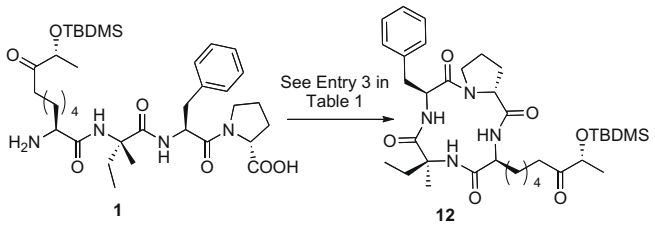
Head-to-tail cyclisation tetrapeptides can be improved using microwave dielectric heating. Cyclisation occurs rapidly at millimolar dilution giving the product in higher purity and yields if compared with standard conditions. The reaction can be applied to sequences containing at least one D-amino acid.

The application of microwave dielectric heating to solid phase peptide synthesis is a well established procedure that gives better yields and purity if compared with standard room temperature methods.[1] The most remarkable achievement is the chance to prepare very long peptides in short time minimizing elongation issues such as incomplete peptide coupling and formation of stable β-sheets which tend to aggregate and prevent further acylation.[2] Several cyclopeptides, important molecules with interesting biological activities, have also been prepared under microwave dielectric heating through side chain-to-tail (or head-to-tail) or disulfide bridge cyclisations on solid support.[3] Moreover, microwave-assisted nucleophilic aromatic substitution or Heck reactions have also been used to close the peptide cycle on the resin.[4]

However, the backbone (head-to-tail) cyclisation of linear peptides in solution is the more extensively employed approach for the synthesis of cyclopeptides. All coupling agents are suitable for this cyclisation, although the reaction proceeds more slowly when compared to normal peptide couplings. Undesired by products are oligomers derived by intermolecular cyclodimerization. This problem can be reduced carrying out the cyclisation in solution under high dilution.[5] While head-to-tail cyclisations of hexapeptides occur without particular sequence-specific problems, tetra- and pentapeptides can be assembled only when preorganization of the linear precursor is possible and tripeptides do not cyclize to the nine-membered ring with few exceptions.[6] Peptide hesitancy to cyclise is attributed to the predominantly trans-configured peptide bonds favouring an extended conformation of the precursor. Since heating a solution containing a linear peptide would accelerate the trans–cis-equilibration,[7] we decided to investigate the influence of microwave dielectric heating on the head-to-tail cyclisation of different tetrapeptides (see Scheme 1).

Being interested in the preparation of cyclopeptide analogues of the potent histone deacetylase (HDAC) inhibitor FR235222,[8] we synthesized the simplified linear tetrapeptide H-Ahoda-Iva-Phe-(D)-Pro-OH (**1** in Table 1) following a microwave assisted solid phase synthesis Fmoc protocol.[9] The reference peptide **12** was prepared under standard conditions using HATU (3.5 equiv) and DIEA (4 equiv) in DCM/DMF at 8×10^{-5} M concentration.[10] Stirring the reaction mixture at 4 °C for 1 h and at rt for 4 h gave 55% conversion of **1** into **12**. HPLC purification from higher molecular weight oligomers (dimers and trimers) gave cyclopeptide **12** in 25% yield (entry 1 in Table 1).

The reduction of the solvent amount required for an efficient cyclisation was an additional achievement of this study. Submitting 5 mg of peptide **1** to HATU (3.5 equiv) and DIEA (4 equiv) in 5 mL of DMF (1.8×10^{-3} M) to microwave dielectric heating (25 W) at 75 °C for 10 min, the cyclopeptide **12** was obtained as the main product of the reaction (much less dimers or trimers



Scheme 1. Microwave-assisted cyclisation of tetrapeptides.

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Table 1
Head-to tail microwave-assisted cyclisation of linear tetrapeptides

En.	Linear peptide	Reaction conditions	Cyclopeptide	Yield[a]
1	H-Ahoda(OTBDMS)-Iva-Phe-(D)-Pro-OH **1**	DCM/DMF 1/1 (8×10^{-5} M) HATU (3.5 equiv), DIEA (4 equiv) 4 °C for 1 h, then rt for 4 h	*cyclo*(Ahoda(OTBDMS)-Iva-Phe-(D)-Pro-) (**12**)	25%
2	H-Ahoda(OTBDMS)-Iva-Phe-(D)-Pro-OH **1**	DMF, (1.8×10^{-3} M), HATU (3.5 equiv), DIEA (4 equiv), microwave, 10 min, 75 °C, 25 W	*cyclo*(Ahoda(OTBDMS)-Iva-Phe-(D)-Pro-) **12**	45%
3	H-Ahoda(OTBDMS)-Iva-Phe-(D)-Pro-OH **1**	CH_2Cl_2, (4×10^{-3} M), HATU (3.5 equiv), DIEA (4 equiv), microwave, 10 min, 75 °C, 25 W	*cyclo*(Ahoda(OTBDMS)-Iva-Phe-(D)-Pro-) **12**	43%
4	H-Ahoda(OTBDMS)-Iva-Phe-(D)-Pro-OH **1**	CH_2Cl_2, (4×10^{-3} M), HATU (3.5 equiv), DIEA (4 equiv), 4 °C for 1 h, then rt for 6 h	*cyclo*(Ahoda(OTBDMS)-Iva-Phe-(D)-Pro-) **12**	22%[b]
5	H-Ahoda(OTBDMS)-Iva-Phe-(D)-Pip-OH **2**	CH_2Cl_2, (4×10^{-3} M), HATU (1.5 equiv), DIEA (2 equiv), microwave, 2×10 min, 75 °C, 25 W	*cyclo*(Ahoda(OTBDMS)-Iva-Phe-(D)-Pip-) **13**	45%
6	H-Ahoda(OTBDMS)-Trp-Trp-(D)-Pro-OH **3**	CH_2Cl_2, (4×10^{-3} M), HATU (1.5 equiv), DIEA (2 equiv), microwave, 2×10 min, 75 °C, 25 W	*cyclo*(Ahoda(OTBDMS)-Trp-Trp-(D)-Pro-) **14**	43%
7	H-Ahoda(OTBDMS)-Pro-Phe-(D)-Pro-OH **4**	CH_2Cl_2, (4×10^{-3} M), HATU (1.5 equiv), DIEA (2 equiv), microwave, 2×10 min, 75 °C, 25 W	*cyclo*(Ahoda(OTBDMS)-Pro-Phe-(D)-Pro-) **15**	38%
8	H-Ahoda(OTBDMS)-Phe-Phe-(D)-Pro-OH **5**	CH_2Cl_2, (4×10^{-3} M), HATU (1.5 equiv), DIEA (2 equiv), microwave, 2×10 min, 75 °C, 25 W	*cyclo*(Ahoda(OTBDMS)-Phe-Phe-(D)-Pro-) **16**	35%
9	H-Ahoda(OTBDMS)-Iva-Phe-(D)-Trp-OH **6**	CH_2Cl_2, (4×10^{-3} M), HATU (1.5 equiv), DIEA (2 equiv), microwave, 2×10 min, 75 °C, 25 W	*cyclo*(Ahoda(OTBDMS)-Iva-Phe-(D)-Trp-) **17**	46%
10	H-Ahoda(OTBDMS)-Trp-Phe-(D)-Pro-OH **7**	CH_2Cl_2, (4×10^{-3} M), HATU (1.5 equiv), DIEA (2 equiv), microwave, 2×10 min, 75 °C, 25 W	*cyclo*(Ahoda(OTBDMS)-Trp-Phe-(D)-Pro-) **18**	48%
11	H-Ala-Val-Phe-(D)-Pro-OH **8**	CH_2Cl_2/DMF 1/1 (4×10^{-3} M), HATU (1.5 equiv), DIEA (2 equiv), microwave, 2×10 min, 75 °C, 25 W	*cyclo*(Ala-Val-Phe-(D)-Pro-) **19**	39%
12	H-Ala-Pro-Phe-(D)-Val-OH **9**	CH_2Cl_2/DMF 1/1 (4×10^{-3} M), HATU (1.5 equiv), DIEA (2 equiv), microwave, 2×10 min, 75 °C, 25 W	*cyclo*(Ala-Pro-Phe-(D)-Val-) **20**	40%
13	H-Ala-Val-Phe-(D)-Trp-OH **10**	DMF (10^{-5} M), HATU (3.5 equiv), DIEA (4 equiv), microwave, 2×10 min, 75 °C, 25 W	*cyclo*(Ala-Val-Phe-Pro-) **21**	36%
14	H-Ala-Val-Phe-Pro-OH **11**	CH_2Cl_2/DMF 1/1 (4×10^{-3} M), HATU (3.5 equiv), DIEA (4 equiv), microwave, 2×10 min, 75 °C, 25 W	*cyclo*(Ala-Val-Phe-Trp-) **22**	0

[a] Yields of isolated product.
[b] Linear peptide **1** was present in the crude in about 50% (HPLC analysis).

present in the crude comparing with rt reaction). After medium pressure chromatography on a reverse phase cartridge, cyclopeptide **12** was isolated in 45% yield (entry 2 in Table 1).

Moreover, the cyclisation was repeated in dichloromethane, still at 10^{-3} M concentration obtaining a promising HPLC/ESMS layout (Fig 1). Cyclopeptide **12** was isolated in 43% yield after chromatography (entry 3 in Table 1) allowing us to reduce the potential inconvenience associated with the use of large amounts of DMF and to cut down on HATU and DIEA up to 1.5 and 2 equiv, respectively.[11] However, these seem to be the best conditions to run this kind of cyclisation since further attempts to reduce the amount of solvent employed gave worse results in terms of crude composition and cyclopeptide yields. In order to give credit to microwaves for this result, compound **1** was stirred at room temperature for 6 h under the same conditions giving a mixture of starting material, cyclopeptide **12** and several oligomers (entry 4 in Table 1). When the cyclisation was carried out at 75 °C under conventional heating (sealed tube, 4×10^{-3} M concentration) a strong influence of the solvent was observed. In CH_2Cl_2 (or $CHCl_3$), compound **12** was formed in very small amount, while in DMF a result comparable to microwave irradiation was obtained after 3 h of heating (36%, isolated yields of **12**).[12]

Using the millimolar as the limit concentration, we repeated the microwave cyclisation on tetrapeptides **2–7**[13] obtaining always cyclic compounds **13–18** in high purity and yields. Moreover, other peptides without the Ahoda amino acid (**8–10**), were submitted to the microwave assisted head-to-tail cyclisation conditions, giving the desired cycles **19–21** in acceptable yields (entries 11–13 in

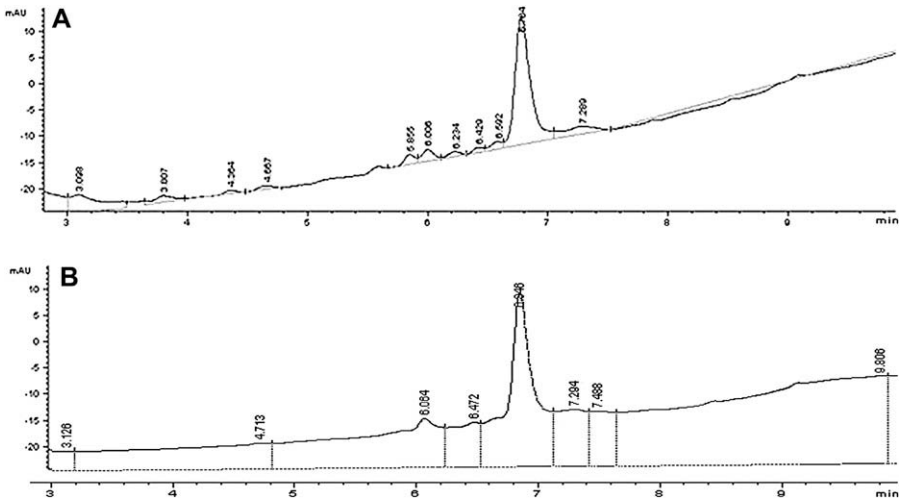

Figure 1. Comparison between HPLC/ESMS profiles of the crude reaction mixture: (A) standard high dilution conditions (entry 1 in Table 1); (B) microwave dielectric heating.

Table 1). In some cases the solvent was changed from CH_2Cl_2 to DMF (or a mixture CH_2Cl_2/DMF), as the starting peptide was not soluble in CH_2Cl_2 alone at millimolar concentration. The only limitation was (as expected) the presence of one D-amino acid in the sequence. In fact, all L-series amino acid sequence, such as tetrapeptide **11,** did not cyclise under described conditions (entry 14 in Table 1). Analogously no results were obtained even at higher dilution.

In summary we found that it is possible to enhance the head-to-tail cyclisation of tetra- (and higher oligo-) peptides under controlled microwave dielectric heating shortening the time required for the reaction, decreasing the amount of solvent and streamlining the procedure for work-up and isolation.

Acknowledgments

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9. The synthesis was performed using an automated microwave peptide synthesizer (Liberty from CEM Corporation). The 2-chlorotrityl resin was loaded with the first amino acid in DMF with a double coupling protocol at 23 W and 75 °C. Fmoc deprotection and HBTU/HOBT mediated couplings with the other amino acids were both carried out in 5 min at 23 W and 75 °C. The last amino acid introduced was *N*-FmocAhoda(OTBDMS) (Ahoda: 2-amino-9-hydroxy-8-oxodecanoic acid). The resin was removed from the microwave synthesizer and the tetrapeptide was cleaved from the support using AcOH/TFE/DCM for 3 h at rt.
10. Cyclic tetrapeptides are sufficiently small to be considered 'druglike' and have been used in the search for novel bioactive molecules. Unfortunately their use is limited by the short supply of the natural ones and by the difficulties in synthesizing them: Norgren, A. S.; Büttner, F.; Prabpai, S.; Kongsaeree, P.; Arvidsson, P. R. *J. Org. Chem.* **2006**, *71*, 6814; Cavelier-Frontin, F.; Pèpe, G.; Verducci, J.; Siri, D.; Jacquier, R. *J. Am. Chem. Soc.* **1992**, *114*, 8885.
11. The crude product obtained form resin cleavage was dissolved in dry CH_2Cl_2 (4×10^{-3} M) and to this solution, cooled to 0 °C, HATU (1.5 equiv) and DIEA (2 equiv) were added. The vial was inserted in the cavity of a Discover synthesizer (CEM Corporation) and heated at 75 °C (25 W power, max internal pressure 100 psi) for two cycles of 10 min each (with a no irradiation interval of 2 min). The solvent was evaporated and the product isolated by preparative HPLC (Column Phenomenex C18, flow 1 mL/min, 40 °C, Solvent A: water with 0.1% TFA. Solvent B: MeCN. Gradien from 95/5 A/B to 0/100 A/B in 10 min).
12. For a critical comparison between microwaves and conventional heating technologies see: Bacsa, B.; Horváti, K.; Bõsze, S.; Andreae, F.; Kappe, C. O. *J. Org. Chem.* **2008**, *73*, 7532–7541.
13. All linear peptides were prepared using the automated microwave peptide synthesizer.