The use of microwave irradiation in peptide chemistry

The introduction of high-throughput biological screening and the accelerated discovery of new biological targets has increased the demand on synthetic chemists to produce new compounds for testing. One response to this demand has been the development of new techniques to greatly increase the speed and efficiency of compound synthesis. In the case of peptides combinatorial libraries containing large numbers of individual components have afforded high-affinity ligands and potent inhibitors to a variety of targets. Recently there has been growing interest in applying microwave heating for many chemical applications requiring energy input1-3. Although microwave techniques have been used in organic chemistry since 1984, publications reporting this nonconventional heating method have had an exceptional increasing especially in latest 10 years. In this paper, we will review the most interesting examples of microwave-promoted reactions in peptides and peptidomimetics synthesis.

SOLID-PHASE PEPTIDE SYNTHESIS UNDER MICROWAVE IRRADIATION

The first example of microwave application in peptide synthesis has been reported in 1992 by Yu et al.4. In this paper a significant improvement of the coupling efficiency (2-4-fold), especially in side-chain-hindered amino acids, in solid-phase peptide synthesis, was described. The authors synthesized three fragments of acyl carrier protein (Acp) by stepwise coupling using pre-formed active ester in DMF and microwave irradiation. In each step the peptide bound formation was completed within 4 min. Using microwave irradiation no detectable racemization was observed and a consistent reduced reaction time was obtained. First studies on peptide synthesis has been performed employed a domestic microwave oven that did not allow accurate temperature measurements and, therefore, it may prove difficult to reproduce the results obtained. However, today this inconvenience have been overcame by availability of dedicated synthesizer suitable for peptide and organic chemistry.

An improvement on peptide synthesis has been reported in 2002 where the authors performed a rapid solid-phase peptide synthesis by microwave-assisted using sterically hindered Fmoc-amino acids. By this study optimized condition has been found to obtain di- and tripeptides rapidly (1.5-20 min) and without racemization, in presence of a variety of coupling reagents5. The studies were conducted in a Smith Synthesizer (Figure 1) with a single mode microwave cavity producing continuous irradiation with monitoring of temperature, pressure and irradiation power, making the procedure highly reproducible. However, this synthesizer has been designed specially for microwave-organic synthesis (MOS). Recently, microwave application in solid-phase peptide synthesis has been performed in a new automated and dedicated synthesizer, Odyssey System (CEM) (Figure 2) that allows to carry on the entire solid-phase peptide synthesis process including the deprotection, coupling, and cleavage reaction6,7. In an optimized study the complete cycle, as well as final peptide cleavage, is performed in ten minutes.

The system used a single mode cavity and the coupling efficiency was investigated using PyBOP and HBTU. By this dedicate system has been possible to synthesize a variety of peptide sequences8. Again, it was demonstrated that microwave irradiation can help in peptide synthesis to drive difficult reactions to completion much faster comparing to conventional methodology9. Microwave application has been also applied in difficult coupling of a-aminobutyric acid (Aib) in the synthesis of several dipeptides using PyBOP/HOBt and HBTU/HOBt as coupling reagents. The study demonstrated that this approach allows to obtain the desired compounds in higher yields and in shorter reaction time when compared with to conventional heating10. Microwave irradiation has proved to be useful in increasing the efficiency of deprotection step of amino acid.
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Although these protecting groups have today limited application, the methods proposed are particularly suitable for the synthesis of short peptides and can also be carried out on appropriate molecular supports.

SYNTHESIS OF PEPTIDOMIMETICS

Microwave-assisted

Microwave-irradiation methodology has having particular attention in peptidomimetic chemistry. It is known that peptidomimetic compounds are immune to proteases and other modifying enzymes and the synthesis of oligomeric combinatorial libraries of peptidomimetics is usually more straightforward than the creation of large libraries at molecules. In particular the peptoids developed by Zuckerman, are oligo(N-alkyl) glycines that differ from peptides in that the side chain is connected to the amide nitrogen rather than the R carbon atom (Figure 3). Peptoids have resulted to be interesting as ligands of important target. Recently a small library of these interesting building blocks has been prepared on Rink MBHA amide resin using microwave irradiation. To perform this library several amines were used to construct various 9-residue peptoids that included homo-oligomers and a hetero-oligomer (Scheme 1).

In this study the microwave experiments were performed in a commercial microwave oven and the tests showed that a reaction time of 30-40 s for each acylation and amine displacement step was sufficient to produce 9-residue peptoids with good yields and purities. The yields and purities of the peptoids obtained using the microwave-accelerated protocol were comparable with those obtained at 37 °C but in a significant reduced time. For example, a 9-residue peptoid can be made in 3 h using the microwave with the protocol described by authors, compared to 20-32 h of the standard protocol. Tandem combination of solid-phase peptide synthesis and microwave-assisted reaction has been used to perform macrocyclization reactions to prepare peptidomimetics of the type showed in Figure 4.

These kinds of macrocyclic in which the cystine bridge has been replaced, have been the subject of considerable study. These novel macrocycles have often improved pharmacokinetic and conformational properties. Peptide synthesis was performed on ACT 348 °C synthesizer and alter the capping with fluorobenzoic acid the macrocyclization step was performed on in a Milestone Ethos CombChem microwave synthesizer (Figure 5). Macrocyclization was performed at 450 W, 50 °C, in DMF for 10 min. These conditions were suitable to provide more than 70% conversion of all linear peptides in cyclic peptide without decomposition of desired compounds.

In our search of new Urotensin-II analogues we have reported a high-speed, one-pot protocol for the generation of cyclic peptides with a thiosulphide bridge from readily available building blocks precursors. Our methodology involves the use of iodide derivatives of serine for the synthesis of cyclic peptide and subsequently to carry on the

Figure 3. Native peptide (A) and Peptoid (B)

Scheme 1. Synthesis of Peptoids by microwave irradiation

Figure 4. Macrocyclization under microwave irradiation

Figure 5. CombChem Microwave Synthesizer (Mileston)

Scheme 2. Urotensin-II analogues synthesized under microwave irradiation
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thioalkylation reaction by microwave irradiation at 450 W, 50 °C, in DMF and DIEA for 10 min. Final one-step cyclization was achieved after removing of the Fmoc and OFm protecting groups and using HBTU/HOBt as coupling reagent (Scheme 2). The final products were isolated with good level of purity following cleavage from the resin using a mixture of TFA/TES/H2O (95: 2.5: 2.5). To demonstrate the real advantage of associating the microwave-assisted reaction with the solid-phase synthesis in peptide thioalkylation step, we compared the results obtained with to conventional thermal heating. Results demonstrated the undoubted advantages in yield and reaction time with microwave heating, being the reaction time reduced to 10 min from 8-12 h. Several others examples of syntheses in which microwave irradiation combined with peptide chemistry afford to peptidomimetics as important building blocks are reported in literature. Thus, the preparation of Fmoc-protected isocyanates which can be used directly or via carbamates to prepare urea peptides is an interesting and useful application. These building blocks can be prepared easily starting from powdered Fmoc-amino acid azides, upon microwave irradiation, in almost quantitative yields without any side product (Scheme 3). Finally, as latest example to take in consideration, the assistance of microwave irradiation has been also applied in the synthesis of new 3,6-disubstituted-1,4-diazepan-2,5-diones that can employed as new scaffolds for combinatorial chemistry and as conformational constrains for short peptides (Scheme 4). The examples here reported confirms that microwave irradiation combined with the peptide synthesis or solid-phase peptide chemistry represents a powerful technique for accelerating the synthesis of peptides and peptidomimetics in a combinatorial chemistry context.

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I apologize to the many authors whose work was not cited, in an attempt to limit the reference list. However, several cited reviews contain more exhaustive coverage of specific areas of research on microwave-assisted in organic synthesis.

REFERENCES


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Scheme 3. Synthesis of Fmoc-protected isocyanates

Scheme 4. Synthesis of 3,6-disubstituted-1,4-diazepan-2,5-diones