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Application of Microwave Irradiation to the Synthesis of 14-Helical β-Peptides

Justin K. Murray and Samuel H. Gellman*

Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706

gellman@chem.wisc.edu

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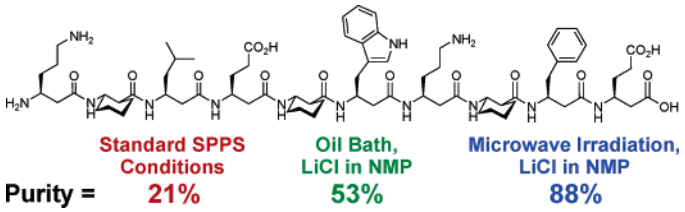
ABSTRACT



We have evaluated the effects of microwave irradiation on the solid-phase synthesis of β-peptides. Sequences designed to adopt the 14-helix, especially those containing the structure-promoting residue *trans*-2-aminocyclohexanecarboxylic acid (ACHC), suffer from poor synthetic efficiency under standard SPPS conditions. A comparison of microwave and conventional heating shows that both provide excellent synthetic results for shorter sequences; however, we identify a clear benefit from microwave irradiation for longer β-peptides.

Microwave irradiation has been applied to a large and expanding range of chemical transformations in recent years, and this method of energy transfer to a reaction mixture can provide impressive enhancements in product yield, selectivity, and/or reaction rate.[1] However, there have been only a handful of reports on the application of microwave methods to solid-phase peptide synthesis (SPPS), and as discussed below, these reports all have limitations.[2] This paucity of attention presumably results from the fact that most peptide sequences, particularly those comprised of conventional α-amino acid residues, are readily available via standard methods.[3] We have been motivated to explore microwave effects on carboxamide bond-forming reactions by our need to streamline the synthesis of β-amino acid oligomers (β-peptides). Interest in these unnatural oligomers is growing because they can adopt a wide variety of discrete secondary structures; the predictable relationship between β-amino acid sequence and folding opens the prospect of endowing β-peptides with useful functions.[4]

The most widely studied β-peptide secondary structure is the 14-helix, which is defined by 14-membered ring N−H_i→O═C_{i+2} hydrogen bonds between backbone amide groups.[4] Seebach et al. discovered that β-peptides composed exclusively of β^3-residues (Figure 1) can form the 14-helix,[5] and we have shown that use of β-amino acids with a six-membered ring constraint, such as *trans*-2-aminocyclohexane carboxylic acid (ACHC), leads to a dramatic enhancement in 14-helix stability relative to β^3-amino acids.[6] Combining constrained and β^3-residues allows one to prepare β-peptides that display specific constellations of diverse side chains on a stable three-dimensional scaffold. A variety of applications of 14-helical β-peptides have been reported.[7] For many such applications it would be desirable to prepare and screen large β-peptide libraries. Such libraries are most useful if the compounds are generated with sufficient purity for direct

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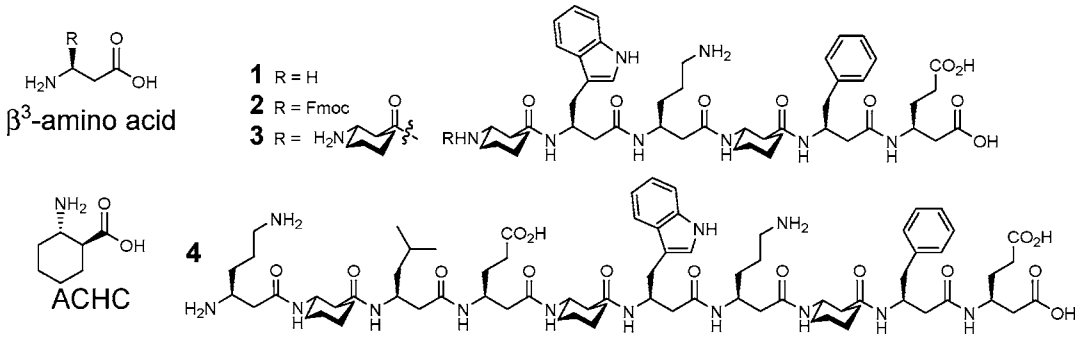

Figure 1. β-Amino acids and 14-helical β-peptides for synthetic optimization.

evaluation. However, standard SPPS protocols are not efficient enough to support a library approach.

14-Helical β-peptides can be prepared via manual or automated methods originally developed for α-peptide synthesis, but efficiency is greatly diminished for these β-peptides relative to typical α-peptides. Seebach et al. have reported profound difficulties in the preparation of relatively short sequences composed exclusively of β^3-residues, with problems in both N-deprotection of the 9-fluorenylmethoxycarbonyl (Fmoc) group and amide bond formation steps, usually starting with the sixth residue from the C-terminus.[8] For example, their optimized stepwise SPPS conditions provided a nona-β-peptide in only 12% purity.[8c] We have observed even more severe problems with analogous sequences that contain mixtures of β^3-residues and ACHC residues.[7c]

We contemplated the application of microwave methodology to improve the synthesis of β-peptides, but we were surprised to learn how little precedent was available for microwave-enhanced peptide coupling reactions. The first report of microwave-assisted solid-phase synthesis of an α-peptide was performed in a domestic microwave oven, making the conditions difficult to reproduce.[2a] A more recent report describes the synthesis of only very short α-peptides (≤ three residues) using conditions that we quickly found to be counterproductive (≥110 °C, closed vessel).[2b] In both cases, no comparison was made between conventional heating and microwave irradiation; i.e., it was not clear from the available literature whether microwave irradiation provides any advantage relative to more traditional methods of reaction rate enhancement for SPPS. Recently, an automated microwave peptide synthesizer became available, though neither reaction conditions nor synthetic results have been published.[2c]

Hexamer **1** (Figure 1) was selected as our initial target for synthetic optimization because it contains a variety of side chain functionalities and the minimum proportion of ACHC residues necessary for high 14-helicity,[6e] and it is just long enough to present a synthetic challenge.[8] Fmoc-(*S*)-β^3-homoglutamic acid[8a] was anchored to polystyrene Wang resin.[8c,9,10] The manual SPPS of β-peptide **1** was performed under standard conditions, with double coupling and double deprotection of the N-terminal ACHC residue (i.e., ACHC1, in standard peptide numbering).[11] Although the penta-β-peptide precursor was >95% pure, hexamer **1** produced in this way was only 55% pure (Figures 2 and 3A). The two major impurities were the unreacted pentamer (33%) and the Fmoc-protected hexamer **2** (8%). Use of a microwave reactor[12] again gave the pentamer in high purity, but now β-peptide **1** was provided in 80% purity with only 5% of the ACHC1-deletion impurity and complete elimination of the Fmoc-protected β-peptide **2** (Figure 2). This dramatic improvement led us to extend our effort to deca-β-peptide **4**. Under standard conditions, the synthesis of **4** is very inefficient (21% product purity; Figures 4 and 5A). Use of microwave irradiation for all reactions gave only 57% purity (data not shown), indicating the challenge of coupling the final five residues in **4**.

We returned our focus to the N-terminal ACHC residue of β-peptide **1**, reasoning that further optimization of this difficult coupling reaction would lead to improvements that could be applied to the preparation of longer targets such as **4**. Altering microwave parameters did not provide any benefit: increasing power, time of irradiation or temperature led to an increase in ACHC-addition impurity **3**, which presumably results from premature Fmoc-deprotection during the double coupling of ACHC1. We then screened ca. 80 combinations of coupling reagents, additives, bases, and

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(11) See Figure 2 for reaction conditions or the Supporting Information for full experimental details.

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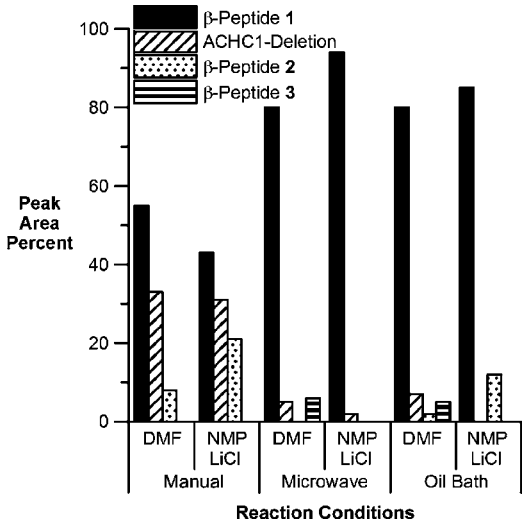

Figure 2. Amount of β-peptide **1** and major impurities (peak area percent, from analytical reversed-phase (RP) HPLC monitored via UV absorbance at 220 nm) resulting from different synthetic conditions. All coupling and deprotection reactions in the synthesis of the hexamer were conducted under the given reaction condition, i.e., manual, microwave, or oil bath, as described below. The given solvent refers only to the coupling of ACHC1; all other coupling reactions were performed in DMF. ACHC1 was double coupled and double deprotected in all cases. Manual: 15 min deprotection, 1.5 h coupling, rt; Microwave: 4 min deprotection at 60 °C; all couplings were 6 min at 50 °C in DMF, except for 6 min at 45 °C in 0.8 M LiCl in NMP for ACHC1 where noted; oil bath: 15 min deprotection, 1.5 h coupling, 60 °C.

solvents with microwave irradiation.[13] None of these variations completely eliminated the ACHC1-deletion impurity, which suggests that the difficult coupling cannot be improved by increasing the reactivity of the coupling reagent.

Difficulties arising at intermediate lengths in the SPPS of α-peptides have been attributed to aggregation and/or folding of resin-bound intermediates,[14] and salt additives have been reported to alleviate such problems.[15] We observed that the deletion impurity was completely eliminated by double

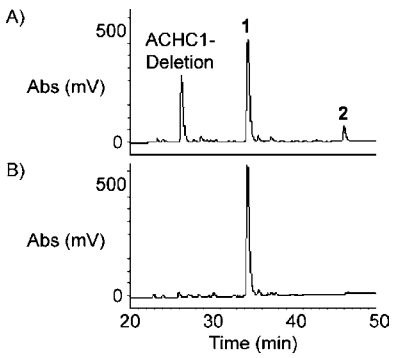

Figure 3. HPLC chromatograms (UV absorbance at 220 nm) of β-peptide **1** prepared under reactions conditions described in Figure 2: (A) Manual-DMF; (B) Microwave-NMP/LiCl.

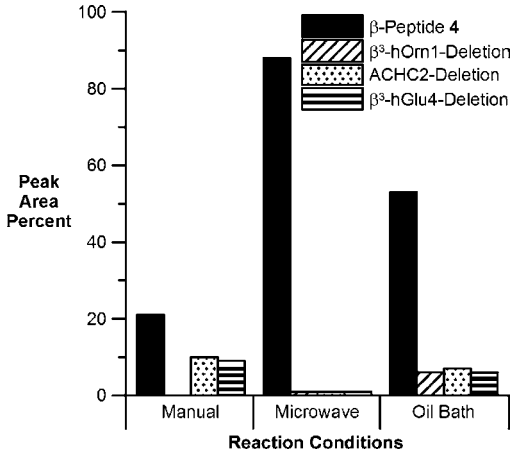

Figure 4. Amount of β-peptide **4** and major impurities resulting from different synthetic conditions. All coupling and deprotection reactions in the synthesis of the decamer were conducted under the given reaction conditions, i.e., manual, microwave, or oil bath, as described in Figure 2. ACHC2 and ACHC5 were double coupled and double deprotected in all cases. ACHC2 and ACHC5 were coupled in 0.8 M LiCl in NMP in the microwave and oil bath syntheses. All other coupling reactions were performed in DMF (β^3-hOrn = β^3-(*S*)-homoornithine; β^3-hGlu = β^3-(*S*)-homoglutamic acid.)

coupling the N-terminal ACHC residue of **1** in a 0.8 M solution of LiCl in DMF with microwave irradiation (data not shown). This improvement, however, was accompanied by a substantial increase in the amount of ACHC-addition impurity, **3**. Further optimization was achieved by switching the solvent from DMF to 1-methyl-2-pyrrolidinone (NMP) for the double coupling of ACHC1, producing **1** in 94% purity (Figures 2 and 3B) and 81% yield.[16] Application of these conditions to the couplings of ACHC2 and ACHC5 in deca-β-peptide **4** provided the desired product in 88% purity and 65% yield (Figure 4).[16] The HPLC comparison of crude products from the standard and microwave-enhanced solid-phase syntheses of **4** (Figure 5) reveal the extent of improvement in the latter case.

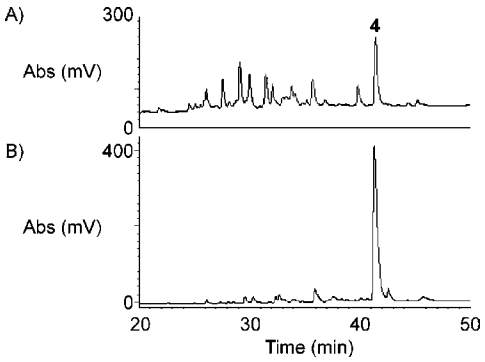

Figure 5. HPLC chromatograms (UV absorbance at 220 nm) of β-peptide **4** prepared under reaction conditions described in Figure 4: (A) Manual; (B) Microwave.

Is microwave irradiation necessary for the results outlined above, or would conventional heating offer similar benefits?[14] For the synthesis of hexamer **1**, we found that conventional heating (oil bath) to 60 °C for 90 min per coupling step and 15 min per Fmoc removal step provided product purities comparable to those obtained upon microwave irradiation for 6 min per coupling step and 4 min per Fmoc removal step. (Conventional heating for shorter periods led to diminished synthetic efficacy, data not shown.) When the final ACHC coupling reactions were carried out in NMP containing LiCl, the oil bath approach provided **1** in 85% purity, which nearly matches the results from microwave irradiation;[17] thus, in this case, the principal advantage of microwave irradiation is diminished reaction time. The heat and LiCl work synergistically, because use of LiCl/NMP for the double ACHC1 coupling at room temperature provides **1** in only 43% purity (Figure 2).

A clear benefit from microwave irradiation became evident, however, in the synthesis of decamer **4**. Oil bath synthesis provided this β-peptide in only 53% purity (Figure 4), substantially lower than the 88% purity achieved with microwave irradiation.[18] We speculate that the microwave advantage for **4** reflects the increasing difficulty of couplings and deprotections after the fifth residue, which may arise from aggregation and/or folding during growth of the protected β-peptide chain.[8c,14] Synthesis of other β-peptides (data not shown) indicates that these conditions are of general utility; these methods may prove useful for difficult α-peptide sequences as well.

Through judicious choice of test cases and careful comparisons among reaction conditions, we have shown that the difficulty of synthesizing 14-helical β-peptides can be alleviated by using microwave irradiation. Further, we have identified a synergy between microwave irradiation and the use of a salt additive. It is conceivable that the salt does double duty under microwave conditions, disrupting the folding and/or aggregation of resin-bound intermediates, as proposed for conventional α-peptide synthesis,[15] and increasing the efficiency of microwave energy transfer, as proposed for microwave enhancements of other reaction types.[19] For short β-peptides, the advantage of microwave irradiation relative to conventional heating is modest, principally an economy in reaction time (>10-fold). For longer β-peptides, however, microwave irradiation displays a clear superiority relative to conventional heating. The results described here should provide a foundation for rapid synthesis of high-purity β-peptide libraries, via both parallel and combinatorial methods, that can be used to discover β-peptides with useful biological or chemical properties.

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Supporting Information Available: Experimental procedures and characterization of **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) (a) Coupling reagents: HBTU, HATU, PyBOP, BOP, PyBrOP, DIC, and DCC. (b) Additives: HOBt, HOAt, and DMAP. (c) Bases: *i*Pr_2EtN, NMM, and collidine. (d) Solvents: DMF, NMP, CH_2Cl_2, DMSO, DMPU, THF, and mixtures thereof.

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