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Microwave-Assisted Solid-Phase Synthesis of Peptoids

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ABSTRACT

Microwave irradiation reduces the reaction time for the solid-phase synthesis of peptoids. Under these conditions, coupling of each residue requires only 1 min. The purity and yields of peptoids synthesized in this way are as good as or better than those achieved using standard methods.

The relatively new science of proteomics has created an unprecedented need for large numbers of protein-binding molecules. For example, the construction of protein-detecting microarrays will require the isolation of large numbers of protein ligands for use as immobilized capture agents. Synthetic molecules may play a decisive role in this regard if molecules with sufficient affinity and specificity can be mined from combinatorial libraries rapidly.[1] Libraries of peptide-like compounds are attractive sources of binding agents for proteomics applications. Unlike native peptides, peptidomimetic compounds are immune to proteases and other modifying enzymes. The synthesis of oligomeric combinatorial libraries of peptidomimetics is usually more straightforward than the creation of large libraries of more "drug-like" molecules. Furthermore, many of the most important proteomics applications of protein ligands do not require the same properties important for pharmaceutical applications, such as cell permeability. Many classes of peptidomimetic compounds have been reported.[2] For a variety of reasons, we have focused on peptoids, developed by Zuckerman[3] and colleagues, for further development. Peptoids are oligo(*N*-alkyl) glycines that differ from peptides in that the side chain is connected to the amide nitrogen rather than the α carbon atom (Figure 1). Peptoids have been shown to be capable of acting as protein ligands, in some cases exhibiting high affinity.[4]

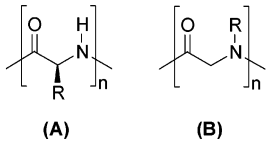

Figure 1. Representation of a native peptide (A) and a peptoid (B).

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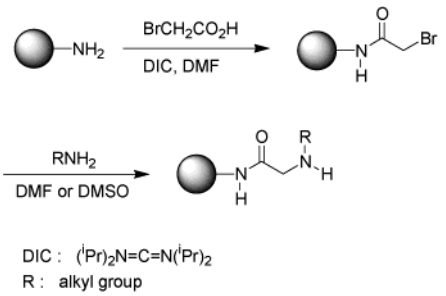
From the standpoint of library synthesis,[5] perhaps the most attractive feature of peptoids is that they can be synthesized via a "sub-monomer" route.[6] This method (Scheme 1)

Scheme 1



consists of (a) an acylation step performed by the addition of bromoacetic acid and *N*,*N*′-diisopropyl carbodiimide (DIC) and (b) a nucleophilic displacement of bromide with a primary amine. Thus, peptoid libraries can be made by split and pool solid-phase synthesis using primary amines as the diversity-generating elements. The commercial availability of hundreds of structurally diverse primary amines means that large, chemically diverse peptoid libraries can be made easily and cheaply, often without the need for protecting groups (depending on the nucleophilicity of other functional groups in the amine molecule). As will be reported elsewhere, we have recently completed the synthesis of several large (>300,000 compounds) peptoid libraries and have successfully screened them for protein-binding molecules.

In a high-throughput effort such as this, the time required for the synthesis of the library and for resynthesis of the "hits" obtained in screening experiments becomes an important issue. Using standard literature protocols for peptoid synthesis, the coupling time per residue (including acylation and nucleophilic displacement) at room temperature varies between 2.5 and 3 h.[7] Increasing the temperature to 35 °C reduces this time to approximately 80 min,[8] but this still

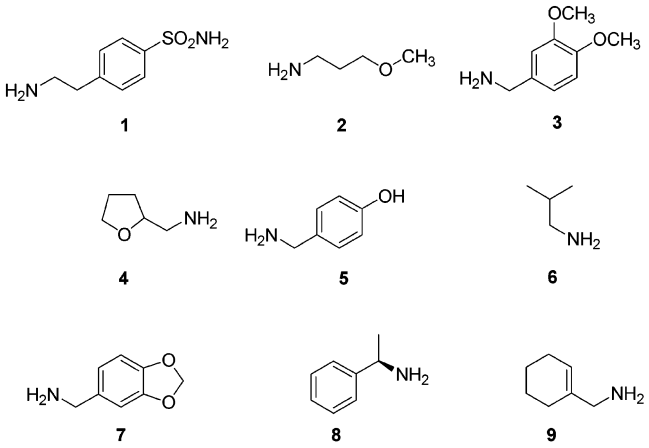


Figure 2. Amines used to synthesize the peptoids.

means that the synthesis of a 10mer peptoid requires about a day. It would be advantageous to increase the speed of this process and thus relieve this potential bottleneck. It is known that microwave irradiation accelerates the rate of many chemical reactions,[9] including the solid-phase synthesis of peptides.[10] This prompted us to study the possibility of using microwave irradiation to accelerate peptoid synthesis.

Several amines (Figure 2) were used to construct various 9-residue peptoids (Table 1) that included homo-oligomers

Table 1. Sequences of Peptoids Prepared in This Study[11]

peptoid	sequence[a]	molecular weight (g/mol)	theoretical yield (mg)[b]
10	$(1)_9$	2179.3	79.5
11	$(2)_9$	1179.1	43.0
12	$(3)_9$	1881.9	68.7
13	$(4)_9$	1287.3	46.9
14	5-8-6-3-2-1-9-7-4	1542.6	56.3
15	$(2)_{20}$	2599.4	94.8

[a] The C terminus (to the right of the sequence) is an amide, and the N terminus was not capped. [b] Peptoids synthesized on 50 mg of Rink resin (substitution, 0.73 mmol/g).

(peptoids **10**−**13**) and a hetero-oligomer (peptoid **14**). A 20-residue homo-oligomer was also prepared (peptoid **15**). All of these peptoids were synthesized on Rink MBHA amide resin that allows for the TFA-mediated cleavage of the peptoids from the resin and subsequent analytical assays by means of reversed-phase HPLC and MALDI-MS. For comparison, the peptoids shown in Table 1 were prepared using standard conditions (both room temperature and 37 °C) as well as the microwave-based protocol described below. The analytical information is presented in Table 2. In all cases, stock solutions of bromoacetic acid in DMF and DIC in DMF were employed at a concentration of 2 M. Solutions of the amines in DMF or DMSO were employed at a concentration of 1 M.

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(11) **General Procedure.** In a typical experiment, the Rink resin (50 mg, substitution of 0.73 mmol/g) was placed in a standard glass peptide synthesis vessel, swollen in DMF, and deprotected with 20% piperidine in DMF for 20 min at room temperature. After washing with DMF, 2 M solutions of bromoacetic acid and DIC (1 mL each) were added to the vessel and placed inside a beaker in the center of a Whirlpool 1000-W microwave oven (model MT1130SG). The vessel was heated at a power setting of 10% for 15 s. Then the vessel was gently shaken for several seconds, and the microwave process was repeated one more time. After washing with DMF, a 1 M solution of the amine (2 mL) was added, the mixure was heated at a power setting of 10% for 15 s and shaken, and the microwave process was repeated one more time. Then the beads were washed with DMF and prepared for the formation of the next residue. See Supporting Information for more details.

Table 2. Crude Product Characteristics Based on the Dry Peptoid[a]

	microwave[b]		37 °C[c]		room temp[d]	
peptoid	yield (%)	purity (%)	yield (%)	purity (%)	yield (%)	purity (%)
10	70	90	70	70	70	25
11	88	90	90	80	80	70
12	75	60	80	63	60	45
13	65	75	65	65	81	50
14	85	80	88	80	60	70
15	85	80	*e*	*e*	*e*	*e*

[a] Peptoids were synthesized on 50 mg of Rink resin (substitution, 0.73 mmol/g). [b] 15 s for acylation (2 times) and 15 s for amine displacement (2 times). [c] 45 min for acylation and 1 h for amine displacement). [d] 30 min for acylation (2 times) and 2 h for amine displacement. [e] Not tested.

The microwave experiments were performed in a 1000-W commercial microwave oven with the power set at 10%. Several 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. Because we had no means of stirring the reaction inside the microwave, the solution was irradiated for 15 s, stirred by gentle manual agitation, and then irradiated for another 15 s. Under these conditions, the temperature of the DMF or DMSO solutions did not exceed 35 °C, as determined using a thermometer after the second 15-s irradiation. More sophisticated in situ measurements of temperature during irradiation have not been tested. Indeed, we do not wish to make any conclusive statements regarding the effect of microwaves versus local heating on the coupling reactions but merely wish to report the empirical observation that these reaction conditions support rapid and efficient peptoid synthesis.

The yields and purities of the peptoids obtained using the microwave-accelerated protocol were comparable with those obtained at 37 °C (Table 2 and Figure 3). Both methods provided better quality peptoids than did room-temperature couplings. However, all the methods provided a major product with identical retention times and masses (see Supporting Information). The HPLC profiles of all of the peptoids obtained under microwave conditions and at 37 °C were practically identical. The main impurities consisted of shorter residues (8-mers, 7-mers, etc.) and, also present in some experiments, the intermediates where a hydroxyl group replaced the bromine and terminated the chain elongation. This can be explained on the basis of the presence of traces of water in the solvents and the relatively inefficient acylation of the α-hydroxyacids.

As a result, a fast and efficient way to synthesize peptoids using a conventional microwave has been developed. Of course, the overall process still requires time for washing steps, bead swelling, etc., all of which are unaffected by this microwave-assisted protocol. Nonetheless, this large acceleration of the chemical steps translates into a significant overall time saving. For example, a 9-residue peptoid can be made on preswollen beads in about 3 h using the microwave protocol described here, compared to 20−32 h using the standard protocol (depending on temperature). The 20-residue peptoid was made on preswollen beads in only 7 h using the microwave-accelerated chemistry.

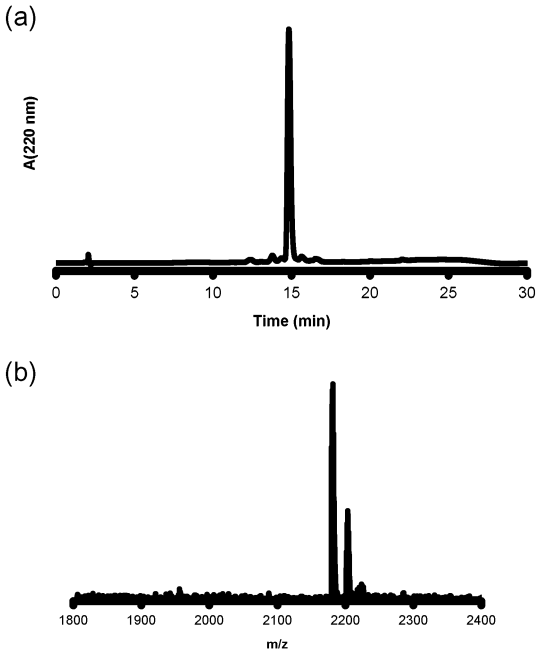

Figure 3. (a) HPLC trace of a crude peptoid **10** prepared under microwave conditions. (b) MALDI-MS of the main peak.

Given the fact that the overall yields and purities of these peptoids were similar to or better than those of the peptoids obtained at 37 °C, the microwave-assisted protocol should be of great utility in the high-throughput synthesis of peptoids and peptoid libraries.

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Supporting Information Available: Experimental conditions, including detailed protocols, and HPLC and MALDI-MS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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