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Expedient Synthesis and Design Strategies for New Peptoid Construction

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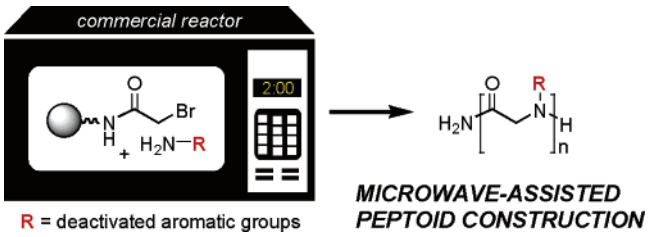
ABSTRACT



A range of peptoids can be prepared efficiently using microwave-assisted solid-phase chemistry in a commercial reactor. This method is most effective for the installation of electronically deactivated benzylic amines. The systematic incorporation of these amines into peptoids can deliver oligomers capable of displaying unique and stable structural motifs—microwave-assisted solid-phase synthesis will enable their future study and application.

Oligomers of *N*-substituted glycine, or peptoids, have emerged as promising new tools to probe macromolecular folding and function.[1,2] Further, as they exhibit enhanced stability toward proteolysis relative to natural α-peptides,[3] peptoids have found direct application in the design of new biological probes[4,5] and in drug discovery.[6] Despite these advances, a general set of rules to predict or design peptoid secondary structure a priori is only now being delineated.[7] New methods to construct and perturb peptoid architectures are required to further elucidate these rules. We hypothesize that noncovalent aromatic interactions could direct peptoid folding along new and biologically relevant avenues.[8] Further, the strategic incorporation of aromatic substituents with differing electronic properties into peptoids would permit the first systematic study of the role of $\pi-\pi$ stacking in peptoid folding. Here, we report a general method for the synthesis of this new peptoid class that is enabled by microwave-assisted solid-phase reactions. We also introduce a streamlined procedure for the room-temperature synthesis of peptoids. Both methods are significant because they provide products rapidly (on the order of minutes) and in sufficient purity for immediate screening applications.

Peptoids are routinely synthesized via the solid-phase submonomer method developed by Zuckermann et al. (shown in Scheme 1).[9] This method consists of iterative acylation and amination reactions and is attractive because of its

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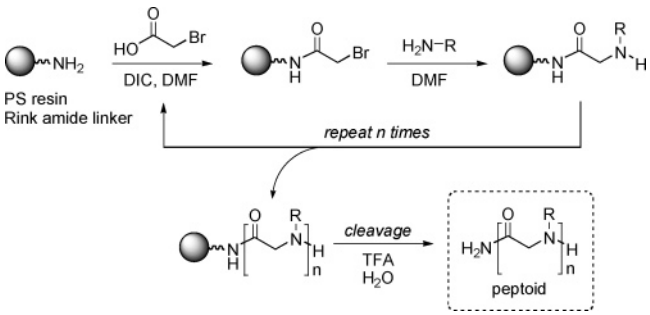
Scheme 1. Schematic of the Solid-Phase Submonomer Peptoid Synthesis Method[a]



[a] PS resin = polystyrene beads. DIC = *N*,*N*′-diisopropylcarbodiimide. DMF = dimethylformamide. TFA = trifluoroacetic acid. For Rink linker, see ref 9.

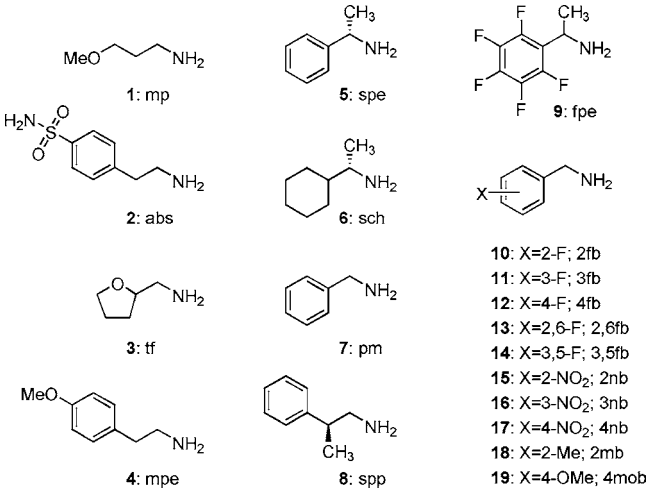


Figure 1. Amines studied in MW-assisted peptoid synthesis.

compatibility with automated and combinatorial synthetic procedures.[4] However, this method also presents challenges, in particular the slow reaction rates, which can require up to 3-h reaction times per monomer unit at 25 °C; this is a common drawback of solid-phase reactions.[10] This problem is exacerbated in both the synthesis of longer peptoid sequences (>8 residues) and in the incorporation of amine building blocks with low intrinsic reactivities, frequently resulting in the isolation of peptoids in low yield and purity over extended reaction times (>36 h). Moreover, lower coupling efficiencies create the need for an additional purification step after cleavage from the solid support.

We are interested in the systematic incorporation of electronically deactivated, and thus less reactive, benzylic amine building blocks into peptoids to study their effects on peptoid structure. Therefore, we required a submonomer synthesis protocol that addressed the aforementioned challenges. The application of microwave (MW) irradiation to solid-phase reactions has emerged as a powerful strategy to both accelerate reaction rates and deliver marked enhancements in product purity.[11] Recently, Kodadek and co-workers demonstrated that MW heating can reduce peptoid synthesis times dramatically (from 3 h to 1 min per monomer synthesis step) and give higher product purities (60−90% for nonamers)[12] relative to the standard room-temperature protocol.[9] As such, we reasoned that this MW protocol would be useful for the efficient installation of less reactive amines into peptoids.

We first aimed to transfer the MW synthesis method of Kodadek and co-workers, developed in a domestic MW oven,[12] to a commercial MW reactor, as the latter systems provide consistently safer and more reproducible MW methodology.[11,13] We evaluated four peptoid homononamers consisting of amines **1**−**4** (Figure 1, three of which (**1**−**3**) also were studied by Kodadek), using identical reaction conditions[12] to facilitate a direct comparison of the two methods. Inputting the domestic MW oven wattages (100 W) and reaction times (30 s per submonomer synthesis step) on a commercial MW reactor,[14] however, gave peptoid products with irreproducible purities that were lower (by 10−50%) relative to Kodadek's work (data not shown).[12,15] Such inconsistent results are not unexpected for MW-assisted reactions performed using only power control.[11,13] Surprisingly, we also observed high and reproducible peptoid purities (ca. 83−87%) for homononamers of amines **1**−**4** in room-temperature control experiments where *no MW irradiation* was applied over similar reaction times. These control experiments revealed serendipitously that for unhindered primary amines (e.g., **1**−**4**), neither MW heating nor the standard 3-h room-temperature reaction time[9] is required to deliver high-purity peptoids. These abbreviated peptoid synthesis conditions allow for monomer construction to proceed over just 1 min and should be of high utility to the peptoid community.[15]

We next evaluated this short room-temperature method alongside MW-assisted methods for the installation of both electronically deactivated and chiral, structurally relevant amines into peptoids. We chose a pentamer of (*S*)-1-phenylethylamine (**5**, spe) units as a test case, as spe (**5**) is known to enforce well-defined helical structure[7] and is of interest for our evaluation of $\pi-\pi$ interactions in peptoids (see below). Here, the short room-temperature synthesis protocol gave a reduced purity for $(N\text{spe})_5$ (71%, Table 1) relative to those observed for nonamers of primary amines **1**−**4**.[16] This result prompted our further evaluation of MW-

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(13) Commercial MW reactors allow for real-time temperature monitoring and variable power control during chemical reactions.
(14) All MW-assisted reactions were performed in a Milestone Ethos Microsynth multimodal microwave reactor.
(15) See the Supporting Information for full details of room-temperature and MW-assisted synthesis, characterization, and purification methods.

Table 1. Crude Purities (%) for Homopentamers of Amines **5**−**19**

amine	peptoid	MW conditions[a,b]	25 °C conditions[a,c]
5	(*N*spe)$_5$	82	71
6	(*N*sch)$_5$	89	81
7	(*N*pm)$_5$	84	86
8	(*N*spp)$_5$	93	92
9	(*N*fpe)$_5$	56	22
10	(*N*2fb)$_5$	87	69
11	(*N*3fb)$_5$	82	76
12	(*N*4fb)$_5$	80	75
13	(*N*2,6fb)$_5$	83	76
14	(*N*3,5fb)$_5$	80	69
15	(*N*2nb)$_5$	80	42
16	(*N*3nb)$_5$	71	47
17	(*N*4nb)$_5$	62	50
18	(*N*2mb)$_5$	88	88
19	(*N*4mob)$_5$	89	88

[a] Peptoid synthesized on 50 mg of Rink amide resin (loading ca. 0.7 mmol/g). Purities based on integration of LC spectra with UV detection at 220 nm; error = ±2%. [b] Acylation: MW 25 s at 35 °C. Amination: MW 90 s at 95 °C. [c] Acylation 25 s at 25 °C. Amination 90 s at 25 °C.[15]

assisted synthesis methods in a commercial reactor.[14] Careful analysis of reaction conditions, including solvents, reagent equivalents, time, power, and temperature, revealed MW-assisted reaction conditions that delivered improved peptoid purity for (*N*spe)$_5$ (82%) with reaction times of only 2 min per monomer unit. We found that a mild acylation reaction (2 M bromoacetic acid and 2 M DIC in DMF, 30 s, 35 °C) and a higher temperature amination reaction (1 M amine in DMF, 90 s, 95 °C) were required for efficient sub-monomer synthesis. This MW-assisted peptoid synthesis methodology is the first developed in a commercial MW reactor and was used throughout the remainder of our work.

Our results with spe (**5**) stimulated an examination of amine electronic and steric effects in MW-assisted peptoid synthesis. We synthesized a series of peptoid homopentamers composed of amines **6**−**19** (Figure 1) for direct comparison to (*N*spe)$_5$ (Table 1). (*S*)-1-cyclohexylethylamine (sch, **6**), which is sterically similar to spe and also known to promote peptoid helix formation,[17] showed ca. 10% purity enhancement using MW heating relative to the 25 °C control (89% vs 81%). However, decreasing the steric hindrance of the amine by either removing the α-methyl substituent, as in benzylamine (**7**), or moving the methyl group to the *β*-position, as in (*S*)-2-phenylpropan-1-amine (spp, **8**), revealed similar peptoid purities using either synthesis protocol. These data indicate that MW heating provides a modest advantage for the incorporation of this pair of structurally relevant[7,17] α-branched primary amines (**5**−**6**) in peptoid synthesis.

We observed a marked improvement in purity using our MW-assisted protocol relative to the 25 °C control for the novel perfluorinated analogue of spe (**5**), 1-(pentafluorophenyl)ethylamine (fpe, **9**) (Table 1).[18,19] Encouraged by this result, we systemically probed a series of benzylamines with varying electron-withdrawing substituents (amines **10**−**17**, Table 1). For these electronically deactivated amines, MW-assisted heating consistently gave higher purities relative to 25 °C control experiments. This effect was most pronounced for amines with one ortho substituent (e.g., **10**, **13**, and **15**) and nitro groups, with *o*-nitrobenzylamine (2nb, **15**) displaying a nearly 2-fold enhancement in purity (80% vs 42%) using our MW method. To determine whether this result was due to sterics or electronics, we examined *o*-methylbenzylamine (2mb, **18**) and detected no change in product purity using MW irradiation. A similar result was observed for *p*-methoxybenzylamine (4mob, **19**), indicating that electron-donating groups do not couple synergistically with MW heating to enhance product purity. Collectively, these data suggest that our MW-assisted peptoid synthesis protocol is especially useful for the synthesis of peptoids from benzylic amines with electron-withdrawing substituents.[20] The higher polarity of these amines could be one reason for their enhanced reactivity using MW-assisted methods relative to less polar amines;[21] comparison of our results to those obtained using conventional heating methods is ongoing.[22]

We evaluated our MW methodology through the construction of two model heteropeptoids consisting of differing arrangements of spe (**5**) and 2nb (**15**) units (nonamers **20** and **21**, Figure 2a). Our MW-assisted synthesis protocol yielded **20** and **21** in good purities (69% and 68%, respectively) in a total 18 min reaction time (Figure 2c). This represents a substantial improvement over room-temperature synthesis methods (by ca. 20%)[15] and underscores the value of MW-assisted reactions for the synthesis of peptoids containing electronically deactivated aromatic side chains.

We predicted that the alternating pattern of aromatic side chains with contrasting electronic properties in heteropeptoid **20** would allow for enhanced $\pi-\pi$ stacking interactions that could influence its propensity to adopt a helical conformation.[7,8] A schematic of possible amide side-chain stacking in **20** in a helical conformation is shown in Figure 2b. To probe this effect, we compared the circular dichroism (CD) spectrum of **20** with the CD spectrum of homononamer (*N*spe)$_9$ **22** (Figure 2a).[7b,23] Interestingly, the CD spectrum of the alternating nonamer **20** shows the diagnostic peaks (i.e., at 192, 202, and 218 nm) of the peptoid-type helix[7,17]

(16) The major impurities observed in peptoid syntheses were either terminal bromides or oligomers with one or more monomer deletions (as determined by LC−MS analyses).

(17) Wu, C. W.; Kirshenbaum, K.; Sanborn, T. J.; Patch, J. A.; Huang, K.; Dill, K. A.; Zuckermann, R. N.; Barron, A. E. *J. Am. Chem. Soc.* **2003**, *125*, 13525−13530.

(18) A report of our syntheses of (±)-1-(pentafluorophenyl)ethylamine (fpe, **9**) and (*S*)-1-(pentafluorophenyl)ethylamine is forthcoming.

(19) A single solution of fpe (**9**) was used to construct the (*N*fpe)$_5$ peptoids in order to conserve this valuable amine. We found that 10 couplings could be performed by recycling the same 2 mL of 1 M amine solution before product purity was affected (by >5%).

(20) Aniline was incorporated into peptoids more efficiently using the MW method relative to the 25 °C control, albeit with reduced purities overall (30% vs 11% for a homopentamer); see the Supporting Information.

(21) The heating profile of a compound under MW irradiation is directly dependent on its dielectric constant. See ref 11a,c.

(22) These control studies are complicated by our inability to attain the rapid heating profile achieved in the MW reactor for the amination step (25−95 °C in 90 s) using conventional heating methods (e.g., in an oil or sand bath).

(23) Homononamer (*N*spe)$_9$ **22** was synthesized using the MW-assisted method described herein. Peptoids were purified to ≥95% homogeneity by reverse-phase HPLC prior to CD analysis.

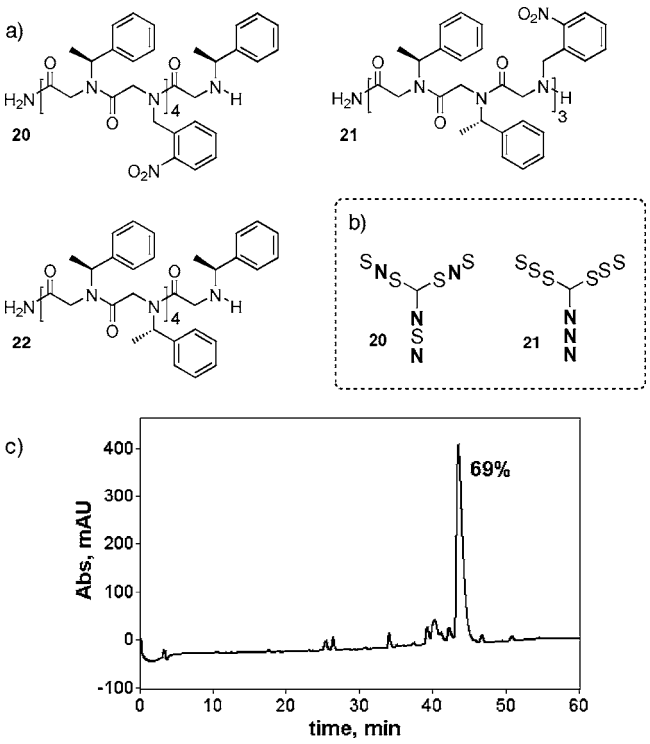

Figure 2. (a) Structures of peptoid heterooligomers **20** and **21** and homooligomer **22**. (b) Schematic views down the long axis of a three-residue per turn peptoid helix[7] showing possible amide side-chain stacking for **20** and **21** (S = spe, **N** = 2nb). (c) Representative crude LC trace for heteropeptoid **20** prepared using the MW-assisted synthesis protocol described herein.[14,15]

and is qualitatively different from that observed for (*N*spe)$_9$ **22** (Figure 3). Barron and co-workers have postulated that the anomalous CD spectrum observed for nonamer **22** is due to the existence of conformers containing at least one *trans*-amide bond that disrupts an otherwise all *cis*-amide, helical peptoid backbone.[7b] The CD spectrum of heterononamer **20**, however, more closely resembles that of *N*rpe octamer and decamer peptoids (as the mirror image), which are both believed to adopt more stable helical conformations relative to **22**.[7b] For further comparison, we examined the CD spectrum of hetero-peptoid **21** (Figure 2a), which displays 2nb side chains every third residue and cannot adopt an alternating pattern of electrostatically differing aromatic side-chains in a helical conformation (Figure 2b).[24] Peptoid **21** gave a weaker CD signature relative to **20** (Figure 3; e.g., *2-fold* less intense at 202 nm), which strengthens our hypothesis that side-chain electrostatic $\pi-\pi$ stacking interactions can stabilize peptoid conformations and direct their folding patterns. Ongoing studies are focused on further evaluating this effect.

(24) Peptoid **21** could also experience electrostatically destabilizing side-chain interactions in a helical conformation (Figure 2b).

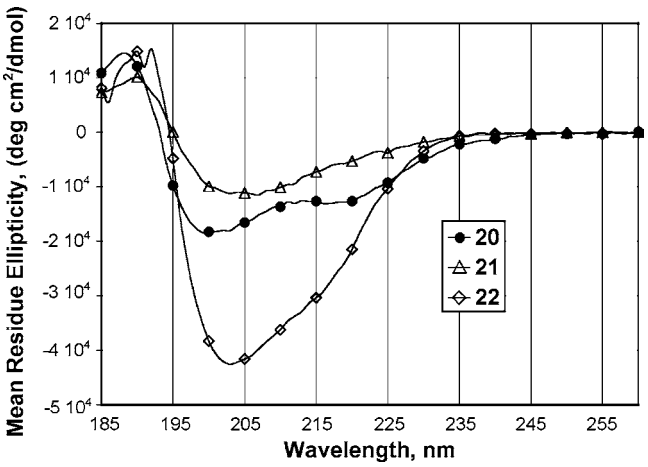

Figure 3. Comparison of CD spectra for peptoids **20**−**22**. Peptoid concentration was 60 μM in acetonitrile, and CD spectra were obtained at room temperature.[23]

In summary, we have developed an efficient method for MW-assisted solid-phase peptoid synthesis that is most effective for the installation of electronically deactivated benzyl amide side chains. Preliminary structural analyses reveal that the systematic incorporation of this side-chain class into peptoids can deliver oligomers with well-defined conformations. We also have shown that, in the absence of sterically or electronically deactivated amines, peptoids can be generated at room temperature with dramatically reduced reaction times and in sufficient purity for immediate screening applications. These methods will accelerate peptoid synthesis and significantly enable the construction of new peptoid architectures.

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Supporting Information Available: Full details of microwave-assisted synthesis, characterization, and purification procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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