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# Convenient Solution-Phase Synthesis and Conformational Studies of Novel Linear and Cyclic α,β-Alternating Peptoids

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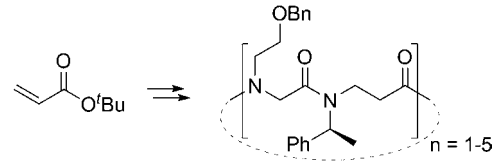
## ABSTRACT




**The synthesis of a novel family of peptidomimetics composed of linear and cyclic α,β-alternating peptoids is described. Oligomers consisting of up to six peptoid residues ($n = 1-3$) were synthesized on large scale with use of an efficient iterative solution-phase method and longer oligomers ($n = 4, 5$) were obtained by the coupling of appropriately protected shorter oligomers. Preliminary conformational studies of these hybrid peptoids are reported.**

Oligomers of *N*-substituted glycines, termed peptoids,[1] were created in the early 1990s in the search for novel peptidomimetics that met several criteria such as achirality of the backbone, resistance to proteases, potential for diversity, and straightforward synthesis amenable to automation.[2,3] The first synthesis of β-peptoids (oligomeric *N*-substituted β-alanines) then followed in the late 1990s.[4] Peptoids differ from peptides in that the backbone side chains are "moved" to the adjacent amide nitrogen atoms (Figure 1). Peptoid oligomers can be synthesized via a "monomer method" much like standard peptide synthesis with suitably protected, presynthesized building blocks.[2] However, the simplicity of

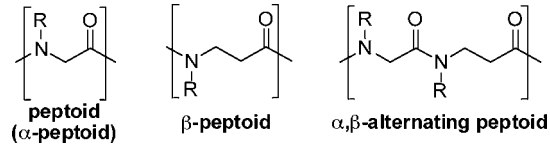



**Figure 1.** Peptoid architectures.

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(1) For clarity, oligomers of *N*-substituted glycines will be referred to as α-peptoids herein.

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the peptoid backbone also allows a unique "submonomer method" where the peptoid residues are created directly on the growing peptoid chain in an iterative manner.[4,5] The absence of backbone chirality and amide protons deprives peptoids of features that are believed to be crucial for formation of secondary structures in peptides. However, in the late 1990s it was discovered that α-peptoids containing α-chiral side chains were capable of forming stable helical conformations.[6] Evidence of the same phenomenon in analogous *β*-peptoids has recently been presented,[7] although one study showed inconclusive results.[8] The presence of secondary structures in α-peptide/*β*-peptoid chimeras has likewise been described.[9]

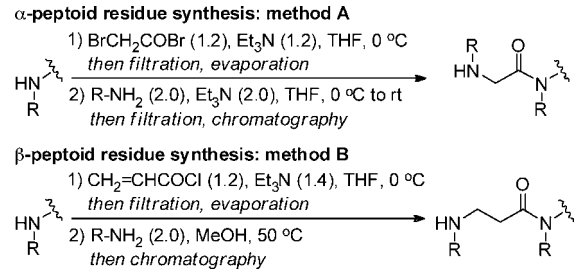
A plethora of interesting biological applications of peptoids has already been demonstrated.[3] In our opinion, the above-mentioned characteristics of peptoids also make them highly suited for use as scaffolds for multivalent ligand display. We have thus recently published the first macrocyclization study of achiral *β*-peptoids which were subsequently functionalized using click-chemistry.[10]

In an effort to develop novel peptoid-based ligand presentation platforms and explore the effects of combined α- and *β*-peptoid residues on the secondary structures of peptoids we decided to synthesize and study the conformational preferences of novel linear and cyclic α,*β*-alternating peptoids (Figure 1). Furthermore, recent advances have revealed intriguing properties concerning the related hybrid α,*β*-peptides.[11] Herein we present our results concerning the synthesis and preliminary conformational studies of the first family of these hybrid peptoids.

The formation of a secondary structure in peptoids is promoted when at least half of the side chains are α-chiral and the *C*-terminal peptoid residue has an α-chiral side chain.[12] The novel peptoids herein were therefore conceived with (*S*)-α-methylbenzyl side chains at all the *β*-peptoid residues while all the α-peptoid residues carry 2-(benzyloxy)ethyl side chains (see Schemes 2−4). The latter side chains can be cleanly debenzylated to unveil free alcohols ready for subsequent functionalization.

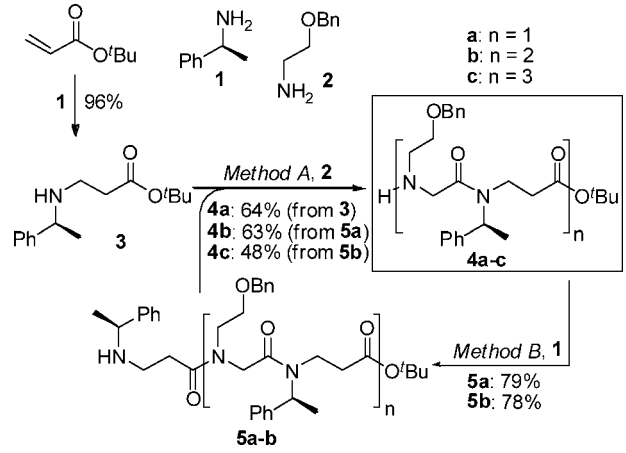
For our purposes, the development of a convenient solution-phase approach using the iterative "submonomer method" seemed most rational (large scale synthesis of relatively short peptoids). Furthermore, we envisaged obtaining longer peptoids by the coupling of suitably protected

**Scheme 1.** Submonomer Solution-Phase Synthesis of α- and *β*-Peptoid Residues[a]

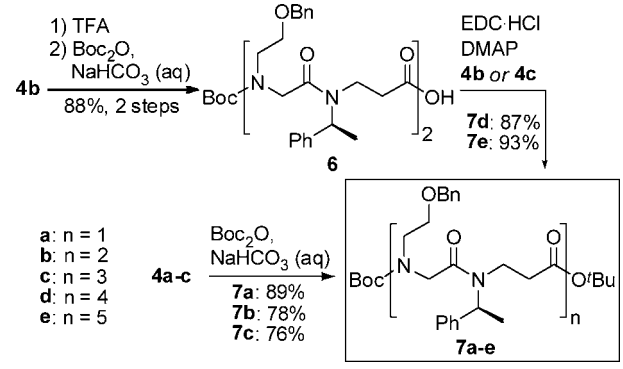



[a] The numbers in parentheses are equivalents.

**Scheme 2.** Iterative Solution-Phase Synthesis of α,*β*-Alternating Peptoids (HPLC purity ≥96%)




**Scheme 3.** Coupling and Protection of Peptoid Oligomers (HPLC purity ≥96%)




shorter oligomers. Only a handful of examples of submonomer solution-phase syntheses of peptoid oligomers have been demonstrated.[10,13] This may be due to the need for purifica-

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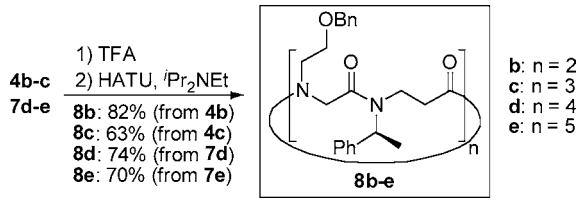
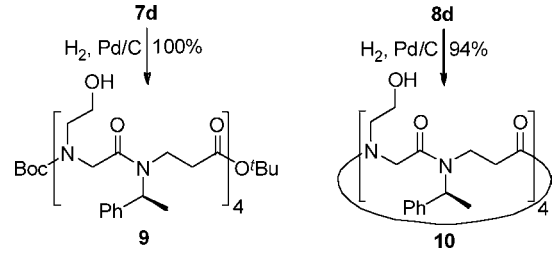
**Scheme 4.** Macrocyclization (HPLC purity ≥96%)




**Scheme 5.** Debenzylation (HPLC purity ≥90%)



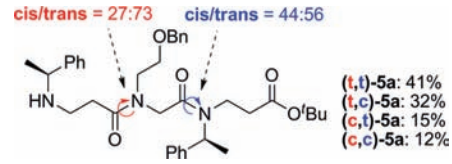




**Figure 2.** Cis/trans ratios of the two amide bonds of peptoid **5a** determined using NOESY correlations in $CDCl_3$.

tion operations such as washings and/or column chromatography after each step.

The first of the two steps of the submonomer methodology is an acylation reaction of the *N*-terminus of the growing peptoid chain (Scheme 1). We found that the subtle change of using THF as a solvent (instead of the commonly used $CH_2Cl_2$) in the presence of $Et_3N$ would cause the ammonium salts formed to precipitate allowing for easy removal of these by filtration.[14] The acylated intermediates were obtained in high yield after ensuing evaporation and were sufficiently pure for direct use in the second step. Thus, in our optimized method, an α-peptoid residue (Scheme 1, method A) is formed first by *N*-terminus acylation with bromoacetyl bromide in THF in the presence of $Et_3N$. After filtration and evaporation, the crude bromoacetyl intermediate is then reacted with the chosen primary amine in THF in the presence of $Et_3N$. After filtration, which is facilitated by the formation of $Et_3N$-ammonium salts, the pure product with a newly formed α-peptoid residue is then isolated by flash chromatography. Similarly, a *β*-peptoid residue (Scheme 1, method B) is formed first by *N*-terminus acylation with acryloyl chloride in THF in the presence of $Et_3N$. After filtration and evaporation, the intermediate acrylamide is then subjected to aza-Michael addition of the chosen primary amine in MeOH.[10] The pure product with a newly formed *β*-peptoid residue is then isolated by flash chromatography. Overall, our method allows for submonomer solution-phase synthesis of α- and *β*-peptoid residues where the need for purification operations is greatly reduced to a few filtrations and a single flash chromatography purification for each synthesized residue. The method is furthermore well suited for multigram synthesis.

Synthesis of the family of α,*β*-alternating peptoids started with aza-Michael addition of (*S*)-α-methylbenzyl amine **1** to *tert*-butyl acrylate giving **3** in 96% yield (Scheme 2). The alternating α- and *β*-peptoid residues, carrying 2-(benzyloxy)ethyl and (*S*)-α-methylbenzyl side chains, respectively, were then created directly on the growing peptoid chain by applying the methods outlined above (Scheme 2). The peptoids were isolated in high purity, and good yields (63−64% for α-peptoid residues and 78−79% for *β*-peptoid residues) were observed up until creation of the sixth peptoid residue (α-peptoid residue in **4c**, 48%). This decrease in yield when approaching longer peptoids is in accordance with our previous findings.[10] However, the longer, fully protected oligomers **7d** and **7e** were obtained in excellent yields (87−93%) and high purity by the coupling of peptoids **4b** and **4c** with acid **6**, using EDC·HCl in the presence of DMAP (Scheme 3). To create a coherent family of linear, fully protected α,*β*-alternating peptoids for conformational analysis, **4a**−**c** were *N*-Boc protected to provide **7a**−**c** in 76−89% yield.

Peptoids are known to undergo highly efficient head-to-tail macrocyclization.[10,15] Thus, a subfamily of cyclic α,*β*-alternating peptoids **8b**−**e** was obtained from **4b**−**c** and **7d**−**e** after termini deprotection with TFA followed by cyclization, using HATU/$^i$Pr$_2$NEt (Scheme 4).[10] The macrocycles **8b**−**e** were obtained in high purity and good overall yields (63−82%, two steps).

Side chains with free alcohols ready for subsequent functionalization can easily be obtained by cleavage of the benzyloxy groups. Thus, the linear peptoid **7d** and the cyclic peptoid **8d** were debenzylated in excellent yields (94−100%) at 1 atm of $H_2$ in the presence of Pd/C to give the desired products **9** and **10** (Scheme 5).

Compared to peptides, both cis and trans conformations of the backbone amide bonds of peptoids can be significantly populated.[16] With use of NOESY correlations, cis/trans ratios of 27:73 and 44:56 were determined for the *N*-2-(benzyloxy)ethyl and *N*-(*S*)-α-methylbenzyl amides, respectively, in linear peptoid **5a** (Figure 2). Overall, average cis/trans ratios of the *N*-(*S*)-α-methylbenzyl amides of 45:55 ± 5 were observed in all linear and cyclic α,*β*-peptoids and the largest proportion of cis conformation was found in the longest chains (see the SI).

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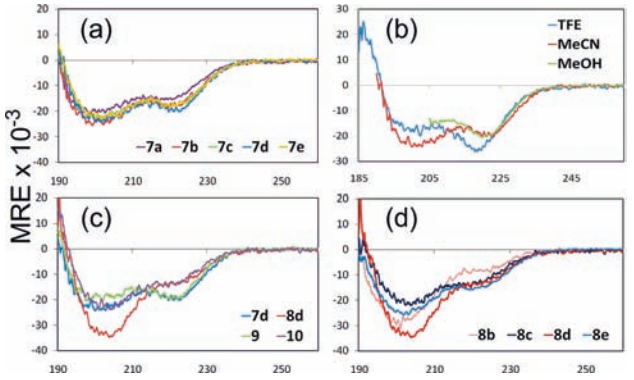


**Figure 3.** CD spectra: (a) linear peptoids **7a**−**e** in MeCN; (b) linear peptoid **7d** in different solvents; (c) comparison of benzylated and debenzylated peptoids in MeCN; (d) cyclic peptoids **8b**−**e** in MeCN. All spectra were recorded at 20 °C in the solvent stated with known concentrations in the 2.0−15.0 $\mu$M range. MRE = mean residue ellipticity (in deg·cm$^2$/dmol).

The linear and cyclic peptoids were studied by circular dichroism (CD) to establish if they adopted ordered conformations and also to explore the effect of both primary structure and solvent upon conformational preference.

The CD spectra of linear peptoids **7b**−**e** in MeCN (Figure 3a) show the same features (negative maxima at ∼202 and ∼222 nm and zero crossing at ∼190 nm) and mean residue ellipticity (MRE) intensities regardless of chain length. However, the dipeptoid **7a** has a slightly decreased MRE. Although there is no dramatic change in the CD spectrum of **7a** versus **7b**−**e**, the observed difference in MRE could indicate that when going from dipeptoid **7a** to tetrapeptoid **7b** there is an increase in ordered structure. This could indicate the presence of a very subtle cooperative effect but it is evident that the conformation(s) adopted are stabilized by local interactions which are typical for non-hydrogen bonded conformation(s).[3c] The spectral features and intensity of **7a**−**e** are consistent with those reported for $\beta$-peptoids with (*S*)-α-methylbenzyl side chains.[8] The intensity is also consistent with ordered conformations of α-peptide/$\beta$-peptoid chimeras although the spectral features are different.[9] Perhaps most significantly, the MRE of **7a**−**e** are consistent with an ordered conformation, e.g., adopted by a $\beta$-peptoid pentamer in TFE and MeOH (∼15 × 10$^3$), which is in contrast to that observed in MeCN (∼3 × 10$^3$), which indicated collapse of a given secondary structure.[7b]

The effect of different solvents upon **7d** was explored and spectral changes are observed (Figure 3b). The spectral features in trifluoroethanol (TFE) for **7d** are reminiscent of those reported for helical α-peptoids (in MeCN).[12] Debenzylation of **7d** (**9**) caused no significant change in the spectrum, which indicates that the benzyl group on the ethoxy side chain is not critical for stabilization of an ordered conformation (Figure 3c and the SI). Therefore, the conformation(s) adopted by the linear peptoids **7a**−**e** and **9** are attributable to ordered conformation(s) which can be perturbed by solvent. In contrast to this, a comparison of the CD spectra of linear peptoid **7b** with its counterparts with different *C*- and *N*-termini (**4b** and **6**) in MeCN demonstrated that simple structural changes have a significant effect on the observed CD spectrum (see the SI).

The CD spectra of cyclic peptoids **8c** and **8e** exhibit a different trace from **8b** and **8d** (Figure 3d). This difference can be attributed to odd and even numbers of the α,$\beta$-dipeptoid repeating unit. Debenzylation of **8d** (**10**) caused a more pronounced reduction (∼32%) in ellipticity at ∼200 nm than debenzylation of the linear counterpart **7d** (∼22%) (Figure 3c). This may be the result of a larger contribution from the benzyl chromophores to the conformation(s) of the cyclic peptoids. Furthermore, this contribution is greatest for even numbers of the α,$\beta$-dipeptoid repeating unit and with increased ring size (given the increase in MRE for **8d** vs. **8b**). Like their linear counterparts, no significant solvent effect was observed for **8d** and **10** (see the SI).

In summary, we have developed efficient and convenient solution-phase methods for large scale synthesis of a family of novel linear and cyclic α,$\beta$-alternating peptoids intended for use as scaffolds for multivalent ligand display. Linear and cyclic α,$\beta$-peptoids can adopt more than one ordered conformation, which appears to be stabilized by local interactions and can be altered by solvent environment. The extension of this novel peptoid family and detailed conformational studies of this new architecture will be reported shortly.

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**Supporting Information Available:** Full experimental procedures, characterization data, and NMR spectra of all new compounds, and further detailed CD information. This material is available free of charge via the Internet at http://pubs.acs.org.

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