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Tandem Incorporation of Enantiomeric Residues Engenders Discrete Peptoid Structures

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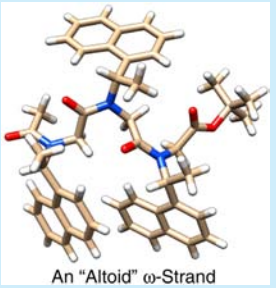
Supporting Information

ABSTRACT: A new peptoid design strategy entailing the concurrent inclusion of enantiomeric side chains enabled the construction of several new structural motifs, including a newly characterized "ω-strand". This new architectural technique significantly expands peptoid structural and functional space and can potentially be applied to other foldameric systems.



Foldameric architecture has generated numerous important advances across a wide range of fields, including the development of new therapeutic compounds, polymeric materials, molecular machines, and biomimetic molecules capable of metal sequestration, sensitive biomolecule detection, and chemical catalysis.[1,2] Among the many well-developed classes of peptidomimetic foldamers, peptoids (*N*-alkylglycine oligomers) exhibit exceptional functional versatility, due in no small part to the powerful "submonomer synthesis" methodology that enables rapid construction of highly diverse peptoid libraries.[3] This structural diversity of peptoid primary structures has in turn provided access to many secondary structures, including helices,[4] ribbons,[5] and loops,[6] that endow peptoids with a variety of useful functions. Several facile design strategies for accessing these structures rely upon choosing side chains that control backbone tertiary amide *cis*−*trans* rotamerism, and thus the backbone ω-dihedral angle, on a per residue basis.[7] However, aside from cyclization tactics,[8] few avenues exist for rationally controlling the other dihedral angles within peptoids; as a result, biomolecular motifs such as β-hairpins and β-sheets, as well as many other potentially new peptoid structures, remain largely inaccessible.[9]

Herein we report that peptoids comprising enantiomeric side chains can adopt structures with high conformational homogeneity in both the solution and the solid phases. Inspired by the catalytic potency of peptides containing both D- and L-residues,[10] we sought to determine the structural potential of analogously designed peptoids. To date, several studies have shown that mixing side chain stereoconfigurations within a peptoid *decreases* its conformational stability and homogeneity, likely due to the suppression of folding cooperativity.[4a,11] We reasoned that 1-naphthylethyl (1*npe*) groups in particular might stabilize new peptoid structures in the absence of cooperative folding due to their extended reach, which could facilitate contact with distant atoms and thereby restrict not only the ω dihedral angle of the nearby amide but also the φ, ψ, and nearby side chain dihedral angles as well. This design scheme allowed us to realize several new and unique peptoid structures with high conformational homogeneity, including a "ω-strand" motif[12] for which we were able to obtain atomic resolution X-ray crystal structures. These previously inaccessible secondary structures constitute an entirely new class of discretely folded peptoids with potential applications in materials design, biochemical investigation, and catalysis.

At the outset of our studies we synthesized and examined peptoids of various lengths containing 1-naphthylethyl side chains with alternating stereoconfigurations and capped with acetyl and *tert*-butyl ester moieties at their *N*- and *C*-termini, respectively (Figure 1; Table 1). Since 1*npe* groups greatly increase the *cis*−*trans* ratios of tertiary amides in the peptoid backbone,[7c−e] we hypothesized that their incorporation would enhance peptoid conformational homogeneity in the absence of typical cooperative folding effects that depend upon a consistent stereoconfiguration throughout the peptoid. In order to evaluate this hypothesis, we obtained COSY, NOESY, and HSQC NMR spectra for each compound in $CDCl_3$ and used them both to determine the overall *cis*−*trans* ratios ($K_{cis/trans}$) of the backbone tertiary amides and assess overall conformational homogeneity. We observed that dimer **1** exhibited a high $K_{cis/trans}$ comparable to that previously reported for the stereogenically consistent stereoisomer Ac-(*s*1*npe*)$_2$-O*t*Bu.[7d] Moreover, the NMR spectra of **1** did not exhibit evidence of significant conformational heterogeneity that might have resulted from weakened folding cooperativity (see the SI). Trimer **2** likewise gave primarily a single set of peaks in its

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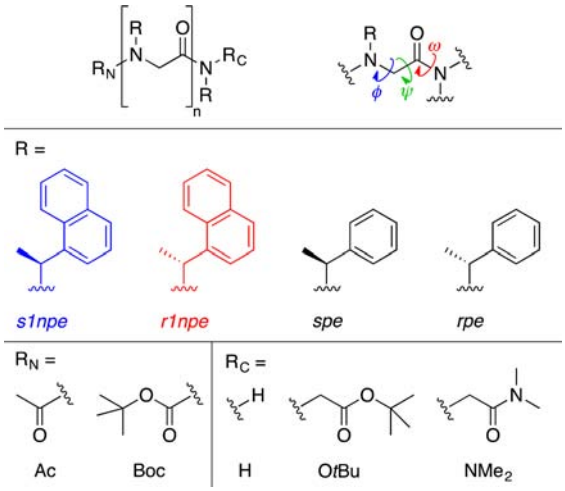

Figure 1. General structure of a peptoid oligomer and structures of the side chains (R), *N*-terminal capping groups (R_N), and *C*-terminal capping groups (R_C) examined.

Table 1. Peptoid Structures and Overall *Cis*/*Trans* Ratios

entry	monomer sequence	$K_{cis/trans}$[a]
1	Ac-*r1npe*-*s1npe*-O*t*Bu	7.9
2	Ac-*s1npe*-*r1npe*-*s1npe*-O*t*Bu	4.2
3	Ac-*r1npe*-*s1npe*-*r1npe*-*s1npe*-O*t*Bu	4.9
4	Ac-*s1npe*-*r1npe*-*s1npe*-*r1npe*-*s1npe*-O*t*Bu	2.2
5	Ac-*s1npe*-*r1npe*-*s1npe*-NMe_2	5.0
6	Boc-*s1npe*-*r1npe*-*s1npe*-NMe_2	>15
7	Ac-*r1npe*-*s1npe*-H	12
8	Ac-*s1npe*-*r1npe*-*s1npe*-H	7.9
9	Ac-*r1npe*-*s1npe*-*r1npe*-*s1npe*-H	5.1
10	Ac-*s1npe*-*r1npe*-*s1npe*-*r1npe*-*s1npe*-H	5.1
11	Ac-*spe*-*rpe*-*spe*-H	1.1
12	Ac-*r1npe*-*r1npe*-*s1npe*-*s1npe*-H	4.7
13	Boc-*r1npe*-*r1npe*-*s1npe*-*s1npe*-H	1.1
14	Boc-*s1npe*-*r1npe*-*s1npe*-*r1npe*-*s1npe*-H	1.5

[a]Determined by integrating ^{1}H NMR spectra of ~25 mM solutions in $CDCl_3$ at 24 °C.

NMR spectra, while the tetramer **3** and pentamer **4** exhibited progressively larger numbers of peaks, with the latter also displaying a markedly lower $K_{cis/trans}$. Suspecting that the *C*-terminal ester might engender strong $n \rightarrow \pi^*$ interactions that could stabilize *trans*-amides in a relatively nonpolar solvent such as $CDCl_3$,[7b,13] the spectra of **2** in CD_3CN were also obtained; the higher $K_{cis/trans}$ (5.5 vs 4.2) observed in CD_3CN buttressed this suspicion. We thus synthesized peptoid trimers **5** and **6**, in which the *C*-terminal ester was replaced with a dimethylamide that would be less susceptible to $n \rightarrow \pi^*$ interactions.[7c] As expected, **5** in $CDCl_3$ reproduced the higher $K_{cis/trans}$ observed for **2** in CD_3CN. Intriguingly, replacing the *N*-terminal acetyl group of **5** with a Boc group in **6** dramatically increased $K_{cis/trans}$ relative to **5** and reduced the number of conformations seen in the NMR spectra.

Given both the known flexibility of peptoid *C*-termini[11b] and their complicating influences on the structural studies above, we proceeded to construct a series of peptoids analogous to **1**–**4**, but without the *C*-terminal CH_2CO_2tBu fragment (**7**–**10**). This series exhibited both a higher $K_{cis/trans}$ compared to **1**–**4** and less evidence of minor conformers in the NMR spectra. In order to establish that 1*npe* residues play a critical role in enhancing the conformational homogeneity of these systems, we also examined **11** – an analog of **8** comprised of 1-phenylethyl (*rpe* and *spe*) residues that constitute a very well studied class of structured peptoids.[6,11a,b] The striking loss of overall preference for either the *cis*- or *trans*-rotamer and concomitant increase in the number of observable conformations compared to **8** (Figure 2) strongly suggest that the phenylethyl residues alone are insufficient to promote structure; this observation is presumably due to the abrogation of cooperative folding that previously was suggested to arise from maintaining a consistent stereogenicity among these side chains. Indeed, this finding is consistent with other studies of peptoids containing both *rpe* and *spe* residues.[4a,11b]

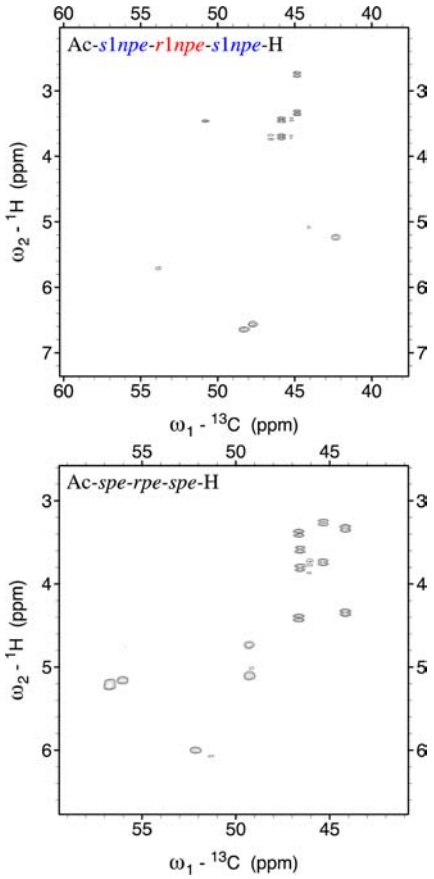

Figure 2. The number of peaks in the backbone methylene and side chain methine regions of the HSQC spectra of **8** (top) and **11** (bottom) indicate significantly greater conformational homogeneity in the former.

For further verification of our hypotheses regarding the structural potential of peptoids containing both *s1npe* and *r1npe* residues, we obtained X-ray crystal structures of trimers **2** and **8** (Figure 3). Consistent with the high $K_{cis/trans}$ values measured for these compounds, both structures exhibit only *cis*-amides in their backbones. Moreover, inspection of the structures reveals what we have dubbed a "*ω*-strand" motif. This *cis*-amide "zig-zag" motif resembles the analogous *trans*-amide Σ-strand secondary structure that pervades peptoid nanosheets.[12] Indeed, computational analysis of Σ-strand-forming peptoids suggested that structures similar to *ω*-strands are conformationally accessible.[12] We observed that the NOESY spectra of **2**

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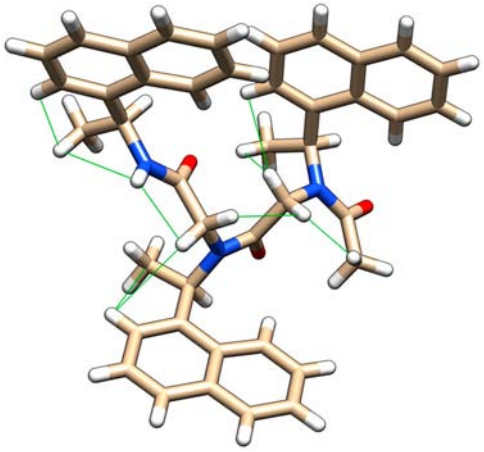

Figure 3. X-ray crystallographic structure of **8**, with relevant nOes indicated in green.

and **8** are wholly consistent with the adoption of a ω-strand structure in solution (Figure 3). While the NMR spectra of **2** show two principal conformations in $CDCl_3$ (likely due to the presence of *trans*-amide rotamers arising from $n \rightarrow \pi^*$ interactions in $CDCl_3$, as discussed above), the spectra of **2** in CD_3CN manifest a single principal set of peaks and nOes indicative of the ω-strand structure. Notably, the backbone methylene units of proximal residues exhibit a unique pattern of strong pro-*R*-to-pro-*S* proton correlations that serve as a hallmark of a ω-strand. Intriguingly, the NMR spectra of **8** in $CDCl_3$ exhibit similar peaks and nOes to those of **2**, except for those associated with the *C*-terminal residue, for which the side chain methyl peak is shifted significantly upfield. However, this anomalous peak is greatly reduced in intensity in the spectra of **8** dissolved in 1:1 $CD_3CN/CDCl_3$ (**8** was insoluble in CD_3CN alone); instead, the spectra of **8** again strongly correlate with those observed in the solid-state ω-strand structure. Overall, these solvent-dependent effects suggest that ω-strands generated using 1*npe* residues can exhibit flexibility in certain environments and need not be dictated entirely by steric factors. Remarkably, while the amphiphilic "block-charge" architecture of peptoid Σ-strands is thought to require oligomers of at least 12 residues in length to form stable structures,[12] our new design principle entailing the incorporation of alternating *s*1*npe* and *r*1*npe* residues generates stable ω-strands using as few as three residues and thus represents a powerful, atom-economical approach to constructing such motifs. Moreover, this approach enables rational design of small peptoids containing *both* ω-strand and other canonical peptoid secondary structures, greatly expanding the structural scope of peptoids in general.

In addition to ω-strands, the admixture of *s*1*npe* and *r*1*npe* residues within peptoids can generate other rotamerically homogeneous secondary structures. Interestingly, the choices of the *C*- and *N*-termini, along with peptoid length, can dramatically affect the secondary structures of this class of peptoids. The NMR spectra of tetramer **9** reveal that, while the ω-strand appears to remain the primary conformation in $CDCl_3$, a second distinct conformation is also significantly populated; this secondary conformation becomes even more pronounced in the spectra of pentamer **10**. Altering the sequence of residues in tetramer **9** gives tetramer **12**, which, while giving a $K_{cis/trans}$ comparable to that of **9**, displays less conformational homogeneity (see the SI). However, exchanging the *N*-terminal acetyl group of **12** for a Boc group (**13**) reduces $K_{cis/trans}$ to near unity while concomitantly enhancing conformational homogeneity. Likewise, the NMR spectra of **14**, which is identical to **10** aside from the Boc group at its *N*-terminus, revealed a rotamerically homogeneous structure in $CDCl_3$ consisting of three *cis*-amides and two *trans*-amides. Elucidation of the structures of **14** and related compounds is ongoing in our laboratory and will be reported in due course. We speculate that the exquisite sensitivity of these structures to the terminal capping groups may arise from the notably different steric profiles of the latter. If this hypothesis is correct, strategic incorporation of less sterically demanding side chains alongside bulky *s*1*npe* and *r*1*npe* residues may enable the construction of intriguing new structures.

In summary, we have developed a new design strategy for the production of structured biomimetic foldamers that entails incorporation of enantiomeric residues within peptoid oligomers. This strategy preserves the power and versatility of the peptoid submonomer synthesis method by relying solely upon side chain and terminal capping group selection to produce well-folded peptoids. The application of this strategy enabled the first reported syntheses of peptoid ω-strands and facilitated their analysis at atomic resolution; moreover, other structures with high conformational homogeneity (e.g., **13** and **14**) were also realized. Since careful application of this design principle can generate structures that are largely unaffected by solvation (e.g., **8**), we suggest that appendage of charged residues could afford water-soluble ω-strands. Additionally, ω-strands could serve as frameworks for the development of new catalysts of chemical reactions in a range of solvents[2c] – an area of active interest in our laboratory. We anticipate that further exploration of the structural tolerances of the compounds described above will reveal additional design parameters that could be adjusted to expand the repertoire of both rationally and combinatorially accessible peptoid structures. Ultimately, the significant expansion of structural space described herein will broaden the already tremendous diversity of peptoid-derived biological probes, materials, and functional foldamers in general.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01283.

CIF file for compound **2** (CIF)
CIF file for compound **8** (CIF)
Characterization, NMR, and X-ray diffraction data (PDF)

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Notes

The authors declare no competing financial interest.

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