Peptoid Oligomers with α-Chiral, Aromatic Side Chains: Sequence Requirements for the Formation of Stable Peptoid Helices

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Abstract: The achiral backbone of oligo-*N*-substituted glycines or "peptoids" lacks hydrogen-bond donors, effectively preventing formation of the regular, intrachain hydrogen bonds that stabilize peptide α-helical structures. Yet, when peptoids are *N*-substituted with α-chiral, aromatic side chains, oligomers with as few as five residues form stable, chiral, polyproline-like helices in either organic or aqueous solution. The adoption of chiral secondary structure in peptoid oligomers is primarily driven by the steric influence of these bulky, chiral side chains. Interestingly, peptoid helices of this class exhibit intense circular dichroism (CD) spectra that closely resemble those of peptide α-helices. Here, we have taken advantage of this distinctive spectroscopic signature to investigate sequence-related factors that favor and disfavor stable formation of peptoid helices of this class, through a comparison of more than 30 different heterooligomers with mixed chiral and achiral side chains. For this family of peptoids, we observe that a composition of at least 50% α-chiral, aromatic residues is necessary for the formation of stable helical structure in hexameric sequences. Moreover, both CD and $^{1}H-^{13}C$ HSQC NMR studies reveal that these short peptoid helices are stabilized by the placement of an α-chiral, aromatic residue on the carboxy terminus. Additional stabilization can be provided by the presence of an "aromatic face" on the helix, which can be patterned by positioning aromatic residues with three-fold periodicity in the sequence. Extending heterooligomer chain length beyond 12−15 residues minimizes the impact of the placement, but not the percentage, of α-chiral aromatic side chains on overall helical stability. In light of these new data, we discuss implications for the design of helical, biomimetic peptoids based on this structural motif.

Introduction

Taking inspiration from proteins and nucleic acids, researchers in organic and medicinal chemistry are working to design biomimetic oligomer systems that capture the fundamental molecular features of these natural, structured polymer systems. Toward this end, a number of groups have ventured into bioinspired molecular design to invent novel, sequence-specific oligomers such as peptoids,[1,2] vinylogous polypeptides,[3] peptide nucleic acids,[4] oligoureas,[5] oligopyrrolinones,[6] β-peptides[7,8] and γ-peptides,[9,10] oligo(phenylene ethynylenes),[11] and aedamers.[12] Relying upon these and other nonnatural oligomer chemistries, researchers are striving to develop new polymer systems that simultaneously offer biomimicry, biostability, biocompatibility, and bioactivity and that can be synthesized with sequence specificity and chain length specificity to fold into tailored three-dimensional structures. Successful research in this area promises to open new frontiers in the engineering of novel therapeutic agents and biomimetic materials that will be useful and safe for in vivo applications.[13,14] A challenging aspect of this field is that for each new family of self-ordering, sequence-specific oligomers, new rules for how chain sequence relates to folded structure will have to be determined to enable *de novo* design.

"Peptoids" (poly-*N*-substituted glycines) are a diverse family of nonnatural oligomers whose close similarity to natural polypeptides and ease of synthesis offer particular advantages for the design of biomimetic materials. Peptoids are based on

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Table 1. N-Substituted Glycine Side Chains

H-[N(R)-CH2-C(=O)]n-NH2	*N*-substituted glycine oligomer, or *polypeptoid*
R = Side chain	Designator
(R)-CH(CH3)(C6H5)	*N*rpe = (R)-*N*-(1-phenylethyl)glycine
CH2(C6H5)	*N*pm = *N*-(1-phenylmethyl)glycine
CH2CH2OCH3	*N*me = *N*-(methoxyethyl)glycine
CH2(C6H11)	*N*chm = *N*-(cyclohexylmethyl)glycine

an achiral backbone, with their side chains appended to the amide nitrogen rather than to the α-carbon (see top of Table 1). As potential mimics of bioactive polypeptides, peptoids offer the advantages of protease-resistance,[15] biocompatibility,[16] and facile incorporation of diverse side chain chemistries through a "submonomer" synthetic methodology.[2] In addition, protocols for polypeptoid synthesis have been optimized to a high degree, enabling their sequence-specific, automated, solid-phase synthesis with very high monomer coupling efficiencies, such that it is a relatively simple, quick, and inexpensive matter to create chains as long as 50 residues.[17,18]

In previous studies, a variety of peptoid oligomers with α-chiral, aromatic side chains were reported to exhibit intense circular dichroism (CD) revealing the presence of regularly repeating, chiral secondary structure.[17] Depending upon their chemical composition and solubility, peptoids of this class exhibit this α-helix-like CD spectrum in both organic and aqueous solution. However, all peptoid oligomers thus far reported to exhibit this "biomimetic" CD signature have had periodic sequences composed of at least two-thirds α-chiral side chains, with at least one-third of these being α-chiral, *aromatic* (phenylethyl) side chains (Table 1).[17] Belying their α-helix-like CD spectra, this class of peptoids has been both predicted and observed to adopt a polyproline type I-like conformation.[17,19] Although it is clear that the chiral information necessary to direct the formation of peptoid secondary structure is transmitted from the chiral side chains, the sequence-related "rules" for forming stable, chiral helices have been yet to be deduced.

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Here, we have taken advantage of the distinctive CD signature of peptoid helices to systematically investigate a large series of oligomers comprised of mixed α-chiral aromatic, achiral aromatic, and achiral aliphatic side chains in a variety of sequence arrangements to determine sequence effects on the stability of helical structure. Through this study, we have succeeded in deducing some of the primary sequence criteria for the design of stable, biomimetic peptoid helices that incorporate achiral as well as chiral side chains. Fundamental studies of the sequence requirements for formation of stable secondary structure in nonnatural oligomers will offer insight into important design criteria for novel biomaterials and therapeutic agents based on biomimetic, structured oligomers and polymers.

Materials and Methods

Peptoid Synthesis, Purification, and Characterization. Peptoid oligomers were synthesized and purified as previously described;[2,17,20] their general structure is shown at the top of Table 1. For peptoid oligomers **11** and **12** only, synthesis was performed using an alternative solid-phase resin[21] which, upon cleavage, yields an oligomer with a *des*-carboxamidomethyl C terminus. (*R*)-*N*-(1-phenylethyl)glycine (*N*rpe), *N*-(1-phenylmethyl)glycine (*N*pm), *N*-(cyclohexylmethyl)glycine (*N*chm), and *N*-(methoxyethyl)glycine (*N*me) monomers were made from the amines (*R*)-1-phenylethylamine, phenylmethylamine, cyclohexylmethylamine, and methoxyethylamine, respectively, all obtained from Aldrich Chemical Co. (Milwaukee, WI) at a purity >99%. Structures of the peptoid side chains derived from these amine submonomers are shown in Table 1. Electrospray mass spectrometry of synthesis products to confirm correct molar mass and high purity was performed at Northwestern University's Analytical Services Laboratory. CD studies were performed as described previously.[20]

NMR Data Acquisition. Peptoid samples were dissolved in acetonitrile-d_3 at concentrations of ~2 mg/mL. All spectra were acquired on a Varian Inova 600 at 25 °C. Natural-abundance 2D heteronuclear single quantum coherence spectroscopy (HSQC) experiments were collected with a spectral width of 6000 Hz in the ^{1}H dimension and 21117 Hz in the ^{13}C dimension. The numbers of t_1 increments, transients, and t_2 complex data points are 512, 32, and 512, respectively. Data processing was carried out with FELIX97 (Molecular Simulations). HSQC spectra were processed as phase-sensitive in both dimensions, and were zero-filled to give a final data matrix of 1024 × 1024 points.

Results and Discussion

Synthesis and Characterization of Peptoid Oligomers. We have created a series of 30 different peptoid oligomers of varying length and sequence, based on different arrangements of monomers carrying the side chains shown in Table 1. *N*-substituted glycine oligomers comprising 6−24 monomers were synthesized in good yield and crude purity, estimated by analytical reversed-phase HPLC to range from 32 to 91% depending upon oligomer length and composition. Compounds were purified to >97% purity by preparative reversed-phase HPLC before being analyzed by CD. Purities and molar masses of HPLC-purified samples were confirmed by analytical reversed-phase HPLC and electrospray mass spectroscopy. In all cases, masses were consistent with expected values (Table 2).

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Table 2. Peptoid Oligomer Structures

peptoid	monomer sequence (amino-to-carboxy)	molar mass calculated: found	purity[a] %	% chiral residues	% aromatic residues
1	$(N\text{pm})_6$	900.2:900.5	87	0	100
2	$(N\text{pm}_5N\text{rpe})$	914.3:914.6	87	17	100
3	$(N\text{pm}_4N\text{rpe}_2)$	928.3:928.6	72	33	100
4	$(N\text{pm}_3N\text{rpe}_3)$	942.3:942.5	72	50	100
5	$(N\text{pm}_2N\text{rpe}_4)$	956.3:956.7	82	67	100
6	$(N\text{rpe})_6$	984.4:984.5	74	100	100
7	$(N\text{pm}N\text{rpe}N\text{pm}_2N\text{rpe}_2)$	942.3:942.7	87	50	100
8	$(N\text{rpe}_2N\text{pm}_2N\text{rpe}N\text{pm})$	942.3:942.0	87	50	100
9	$(N\text{rpe}N\text{pm}_3N\text{rpe}_2)$	942.3:942.7	87	50	100
10	$(N\text{rpe}_2N\text{pm}_3N\text{rpe})$	942.3:942.7	71	50	100
11	$(N\text{pm}N\text{rpe}N\text{pm}_2N\text{rpe}$-*N*H-1-(*R*)-phenylethyl)	885.2:885.6	91	50	100
12	$(N\text{rpe}_2N\text{pm}_2N\text{rpe}$-*N*H-benzyl)	885.2:885.6	85	50	100
13	$(N\text{rpe}N\text{pm}_2N\text{rpe}_3)$	956.3:956.7	82	67	100
14	$(N\text{rpe}_3N\text{pm}_2N\text{rpe})$	956.3:956.7	82	67	100
15	$(N\text{me}N\text{rpe})_6$	1675.2:1675.0	68	50	50
16	$(N\text{pm}N\text{rpe})_6$	1867.6:1867.8	73	50	100
17	$(N\text{me}N\text{rpe})_3$	846.1:846.4	77	50	50
18	$(N\text{pm}N\text{rpe})_3$	942.2:942.7	86	50	100
19	$(N\text{rpe}N\text{me})_3$	846.1:846.5	91	50	50
20	$(N\text{rpe}N\text{me})_6$	1675.2:1675.0	68	50	50
21	$(N\text{rpe}N\text{pm})_3$	942.2:942.9	89	50	100
22	$(N\text{rpe}N\text{pm})_6$	1867.6:1867.8	66	50	100
23	$(N\text{me}N\text{rpe}N\text{me}_2N\text{rpe}_2)$	846.1:846.8	70	50	50
24	$(N\text{rpe}_2N\text{me}_2N\text{rpe}N\text{me})$	846.1:846.8	75	50	50
25	$(N\text{rpe}N\text{me}_2N\text{rpe}_3)$	892.2:892.8	90	67	67
26	$(N\text{rpe}_3N\text{me}_2N\text{rpe})$	892.2:892.8	86	67	67
27	$(N\text{me}N\text{rpe}N\text{me}_2N\text{rpe}_2)_2$	1675.2:1676.1	54	50	50
28	$(N\text{me}N\text{rpe}N\text{me}_2N\text{rpe}_2)_3$	2504.2:2502.7	40	50	50
29	$(N\text{me}N\text{rpe}N\text{me}_2N\text{rpe}_2)_4$	3333.3:3331.4	32	50	50
30	$(N\text{chm}N\text{rpe})_6$	1903.7:1904.1	72	50	50

[a] As estimated by analytical reversed-phase HPLC of crude product. All compounds purified to >97% homogeneity before analysis by CD.

Since we wished to study and compare the degree of helicity of a large number of different peptoid sequences composed of the same or similar side chains, CD was the most useful tool to use. Since the amide bond chromophore is acutely sensitive to the structural, solvation, and electronic environment in which it lies, CD spectroscopy can allow rapid assessment and classification of secondary structure in folded polypeptides. However, prior to using this tool for structural classification of other types of oligomers such as peptoids, it is necessary that distinct CD spectral line shapes are connected, through structural studies, with a particular class of secondary structure. For oligopeptoids with α-chiral, aromatic side chains such as *N*rpe shown in Table 1, the correlation of intense CD spectra with the existence of helical, polyproline-like secondary structure in solution has been supported by both 2D-NMR structural determination and molecular modeling.[17,19,22] In particular, structural data for a peptoid with five para-substituted (*S*)-*N*-(1-phenylethyl)glycine residues has indicated that the major family of conformers in methanol solution are right-handed helices with *cis*-amide bonds, having a periodicity of approximately three residues per turn and a pitch of ∼6 Å.[22] The predilection of this oligomer to form a helical structure fitting this polyproline-like description was also predicted by molecular mechanical modeling of an *N*spe octamer.[19]

As for other classes of nonnatural oligomers under investigation for their ability to fold into biomimetic secondary structures,[13,14,23] our CD results indicate that a wide variety of peptoid sequences ranging from 6 to 24 residues in length adopt repeating secondary structures in organic solution. Importantly, we have also identified a significant number of peptoid sequences comprising high percentages of α-chiral, aromatic side chains that do *not* form stable structures at a hexamer length, exhibiting weak and featureless CD spectra. Our experimental deduction of the sequence-related factors that lead to this *lack* of structure formation allows us to suggest some general rules for the stabilization of short peptoid helices.

Peptoid Heterooligomers: The Sequence Dependence of Helical Stability. Given that the ordered, helical peptoids previously reported[17,20] were composed of at least two-thirds α-chiral side chains, with at least one-third of these being α-chiral aromatic (1-phenylethyl) side chains,[17] a question arises as to how one goes about creating chiral, biomimetic peptoid helices that also include other, chemically diverse, achiral side chains. To answer this question it is necessary to establish sequence requirements for stable peptoid helix formation, in terms of the composition and placement of chiral and achiral monomers.

In designing peptoid architectures based on a helical motif, we cannot rely upon hydrogen bonding to provide structural stabilization. Yet, fortunately, the absence of hydrogen-bond donors in the peptide backbone does not preclude the formation of stable secondary structure, as also exemplified by the formation of polyproline helices[24] and by helices of β-peptides having homoproline residues.[25,26] In the absence of backbone hydrogen bonding, steric, hydrophobic, van der Waals, and electrostatic forces can be exploited to provide stabilization of secondary structure. On the basis of a consideration of peptoid chemical structure as well as experimental evidence gathered

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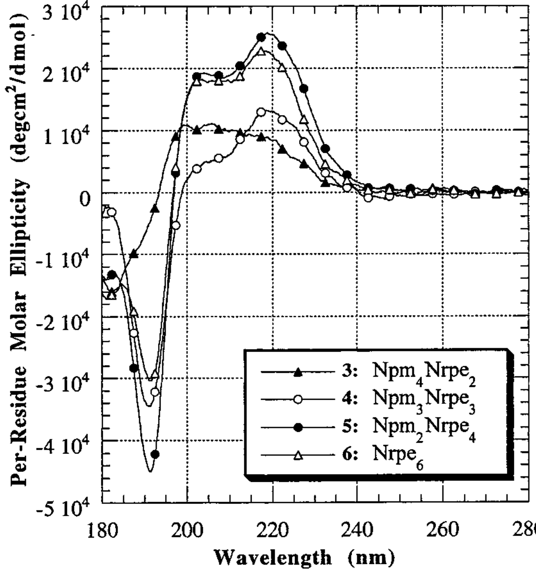

Figure 1. CD spectra of *N*rpe oligopeptoids with varying numbers of *C*-terminal, chiral residues, including oligomers **3**, **4**, **5**, and **6** (Table 2). For all samples, peptoid concentration was ~60 μM in acetonitrile, and spectra were acquired at room temperature.

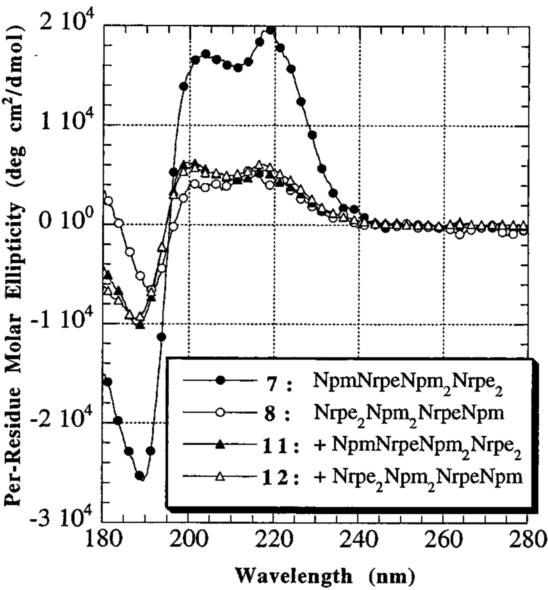

Figure 2. Comparison of the CD spectra of peptoid/retropeptoid pairs (a) **7** and **8**, (b) **11** and **12**, which have the same sequences as **7** and **8** (respectively), with a *des*-carboxamidomethyl C terminus. Peptoid concentration was ~60 μM in acetonitrile, and spectra were acquired at room temperature.

from previous work, both steric and electrostatic interactions are hypothesized to play a significant role in the stabilization of peptoid secondary structure.[22] Our present results with heterooligomers are fully consistent with this hypothesis.

First, we systematically varied the percentage of α-chiral aromatic and achiral aromatic side chains in a set of peptoid hexamers. As discussed in previous work, an *N*-(*R*)-1-phenylethylglycine (*N*rpe) hexamer exhibits an intense, helical CD spectrum (Figure 1) that is quite stable to elevated temperature.[20] Hence, in our first set of peptoids comprising mixed α-chiral and achiral side chains, the chiral monomer was chosen to be *N*rpe, while the achiral monomer was phenylmethyl (*N*pm). In this set of hexamers, we "scanned" through the sequence, replacing *N*rpe residues with different numbers of achiral *N*pm residues, counting in from the individual hexamer termini. Sets of peptoids and retropeptoids (hexamers with the reversed sequence), for a total of 10 molecules, were synthesized and analyzed. The "peptoid" set is exemplified by hexamers **1**−**6** in Table 2, in which achiral *N*pm residues are located on the carboxy terminus; the retropeptoid set is not shown, as its analysis led us to the same conclusions as our comparison of oligomers **1**−**6**.

As mentioned previously, peptoids comprising 100% achiral, aromatic *N*pm side chains display no net CD.[20] In studying the CD spectra of heterooligomers **2**−**5**, we found that hexamers with just one or two terminal *N*rpe residues likewise show virtually no helical structure by CD (e.g., compound **3** in Figure 1). However, peptoid hexamers that comprise one-half α-chiral, aromatic side chains (**4**) display a nascent chiral helical CD signal about half the strength of that observed for the *N*rpe hexamer. Similar CD results, showing that a certain percentage of chiral substituents is required to cooperatively drive an achiral polymer backbone into a chiral, helical conformation, have also been observed for polyisocyanates.[27,28] Interestingly, we find that peptoid oligomer **5** composed of just two-thirds α-chiral, aromatic substituents gives an equivalent and even somewhat *stronger* helical CD spectrum than the *N*rpe hexamer.

In our investigation of peptoids and retropeptoids that have mixed α-chiral and achiral aromatic side chains, we have observed α-helix-like CD spectra of widely varying magnitudes. To study effects of the *placement* of α-chiral, aromatic side chains on the amino versus the carboxamide terminus of peptoid oligomers, we synthesized a series of analogous peptoids and retropeptoids with mixed chiral and achiral aromatic side chains, in different sequence arrangements (compounds **7**−**14**, Table 2). All oligomers were composed of at least one-half α-chiral side chains, to maximize the probability of helix formation. We find that for chains with identical monomer compositions, differences in sequence can have a dramatic effect on helical structure. The CD spectra of peptoid and retropeptoid hexamers **7** and **8** (Figure 2) provide a good example of this. The intensity of ellipticity on a per-residue molar basis is about 400% greater for peptoid **7**, in which an α-chiral, aromatic residue is placed on the carboxy terminus rather than on the amino terminus. This striking "end effect" is also evident, in a very similar manner, for peptoid/retropeptoid pair **9** and **10**, where peptoid **9** with two α-chiral substituents on its carboxy terminus exhibits significantly stronger CD than retropeptoid **10** with a single α-chiral substituent on its carboxy terminus (data not shown). These CD results suggest that the carboxy terminus of the peptoid helix is less structurally stable than the amino terminus (i.e., that the peptoid helix is more easily "frayed" by the presence of a mobile, achiral side chain in a carboxy-terminal position). Hence, in short peptoid oligomers comprising both chiral and achiral side chains, the carboxy terminus may require the stabilizing influence of an α-chiral, aromatic side chain to induce and stabilize secondary structure. This finding is consistent with the 2D-NMR and molecular modeling studies of Armand et al.,[19,22] in which the carboxamide terminus was observed to have increased conformational freedom relative to that of the amino terminus.We suspected that the lack of conformational stability at the carboxy terminus of the peptoid results from the inherent flexibility of the carboxamide tail. To confirm this hypothesis, we synthesized two *des*-carboxamidomethyl peptoids (compounds **11** and **12** in Table 2). These compounds are direct analogues of compounds **7** and **8**,

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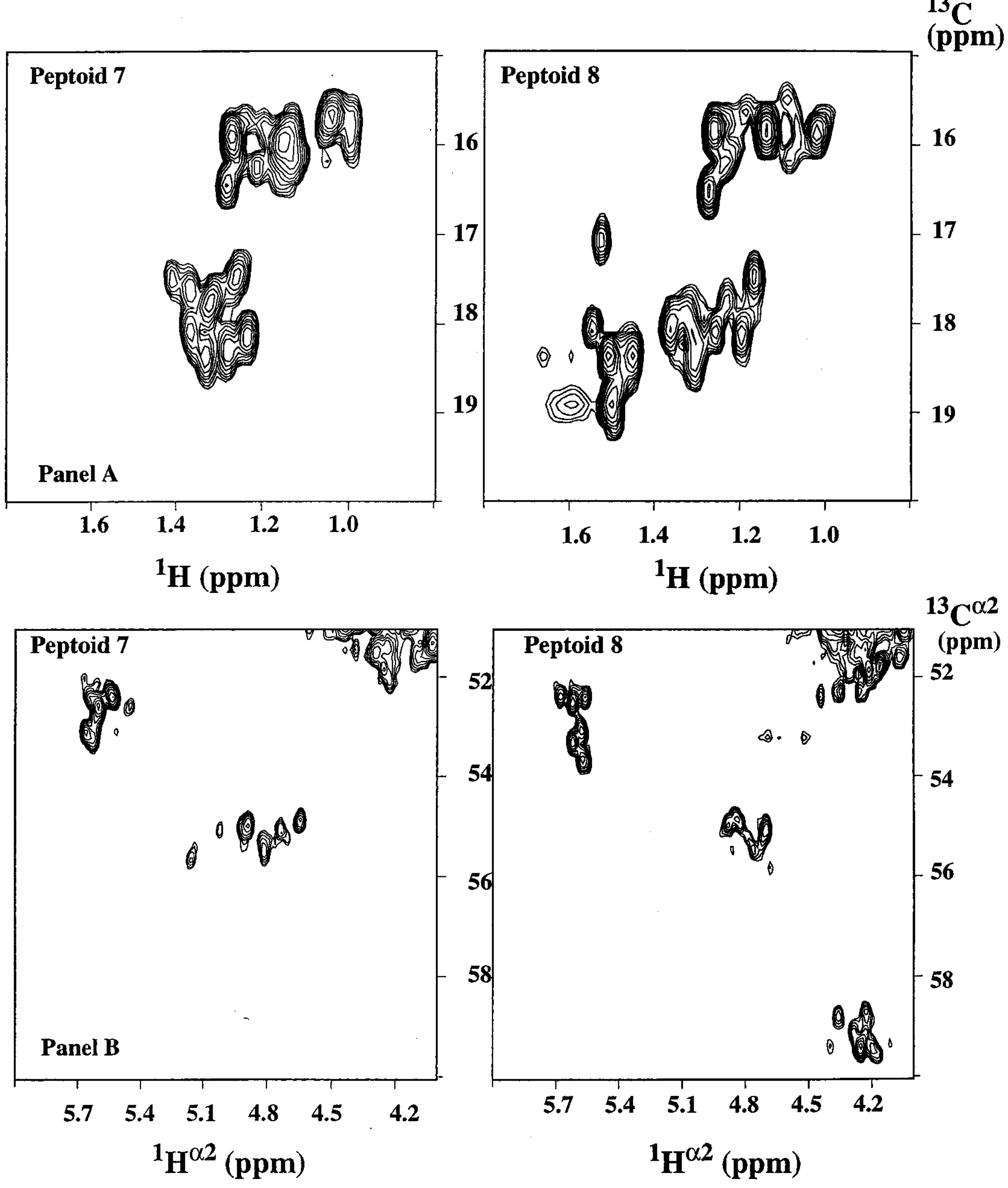

Figure 3. $^{1}H-^{13}C$ HSQC spectra of the peptoid/retropeptoid pair **7** and **8**. (Panel A) Chiral methyl protons. (Panel B) Tertiary substituted methyne protons. Peptoid concentration was ~2 mM in deuterated acetonitrile, and spectra were acquired at 25 °C.

respectively, and their CD spectra are compared in Figure 2. In the absence of the carboxamide tail, superimposable CD spectra are obtained for peptoid **11** and retropeptoid **12** (Figure 2). Interestingly, the absence of the terminal carboxamide moiety also results in a much weaker CD spectrum for **11** as compared to that for **7**. Hence, it appears that loss of the carboxamide tail increases configurational freedom of the backbone, reducing helicity for short oligomers. We conclude that the carboxamide moiety plays some role in the stabilization of short peptoid helices, and that placement of one or more α-chiral monomers on the carboxy end of the peptoid can disfavor the fraying of short peptoid helices. This effect is expected to be more pronounced at short chain lengths such as those we have examined here, given the apparently cooperative nature of peptoid helix formation.[20] A different mode of terminal stabilization has been observed for isolated peptide α-helices, where removal of charge from the carboxy terminus can result in overall reduction of helical content.[29] Furthermore, it has been shown that the orientation of the terminal side chain relative to the backbone can have a significant effect on peptide secondary structure, as exemplified by peptide substance P, for which the choice of terminal amino acids can affect the degree of helix formation.[30] To gain additional insight into differences we observe between the CD spectra of **7** and **8**, 2D-NMR experiments, specifically $^{1}H-^{13}C$ HSQC, were conducted. As shown in Figure 3 and in Supporting Information for this article, analysis of the $^{1}H-^{13}C$ HSQC spectra of **7** and **8** reveal distinct differences in the chemical shift distribution of the carbon-proton cross-peaks for these peptoid oligomers. Figure 3 shows $^{1}H-^{13}C$ HSQC spectral regions corresponding to the chiral methyl (Panel A) and tertiary substituted carbon methyne (Panel B) groups of peptoid chains **7** and **8**. Close comparison of these spectral regions reveals that the chemical shift distribution of

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peaks corresponding to the chiral methyl protons of **7** is significantly narrower than that for retropeptoid **8**. The increased degeneracy of these peaks in the 2D-NMR spectrum of **7** indicates that down the peptoid chain, chiral methyl groups reside in a more similar chemical environment than in the conformer formed by retropeptoid **8**. Consistent with this finding, $^{1}H-^{13}C$ HSQC spectra of side-chain methyne protons reveal the presence of an additional set of cross-peaks for **8** in the proton chemical shift range of 4.0−4.5 ppm and the carbon chemical shift range of 58−60 ppm (Figure 3, Panel B). We performed volume integration of the peaks shown in Panel B, and the data suggest that peptoid **7** has two major families of conformers, in a ratio of approximately 2:1, that are dominated by *cis* and *trans* backbone amide rotamers, respectively. On the other hand, peptoid **8** has three different families of rotamers, which are populated in a ratio of 1:1:1 (*cis* amide-dominated: *trans* amide-dominated:other). The appearance of this additional group of NMR cross-peaks reflects the existence of another family of conformers for **8**, most likely having mixed *cis* and *trans* amide bonds or an intermediate configuration for some bonds, which are not accessed by peptoid **7**.

Taken together, the HSQC results shown in Panels A and B suggest that the range of chain configurations adopted by peptoid **7** is more limited, and these conformations are more stable, than those accessed by the retropeptoid **8**. This is consistent with the CD results shown in Figure 2, in which **7** exhibits a much more intense helical spectrum. The greater degree of conformational disorder and the reduced helicity of retropeptoid **8** most likely arise from the conformational flexibility of a carboxy terminus which is not "pinned down" with a chiral *N*rpe group. These and other results obtained in the course of this study indicate that for the formation of stable helices in short peptoid oligomers, the C terminus must be stabilized by substitution with a chiral, bulky group.

In addition to this "end effect," we find that certain types of sequence patterning within the body of the peptoid chain exert helix-stabilizing influences. For example, a large difference in the helicities of peptoid/retropeptoid pair **13** and **14**, both of which have 67% α-chiral, aromatic residues and *N*rpe-stabilized C termini, initially took us by surprise. Peptoid **14** shows greater helicity than retropeptoid **13** as indicated by stronger and more well-defined CD peaks. (See Supporting Information for a comparison of the CD spectra and an explanatory helical wheel diagram.) Upon examination of the two sequences, noting that there are three residues per turn in this class of peptoid helix,[19,22] we reasoned that a helix based on retropeptoid **14** has an α-chiral, "aromatic face" of phenylethyl groups (i.e., a plane of aromatic rings) running down the longitudinal axis of the helix, that involves the carboxy-terminal residue. The α-chiral, aromatic face in peptoid **13** is associated instead with the *amino*-terminal residue, which is less likely to fray in these short peptoids. Hence, one explanation of the greater helicity of **14** is that interactions between α-chiral, aromatic side chains that are "stacked" in the peptoid helix serve to "pin down" the carboxy terminus, increasing the stability of the folded structure.

To provide additional insight into the apparent importance of aromatic interactions in peptoids, we investigated a series of heterooligomeric hexamers designed to have varying *numbers* of aromatic faces (see Supporting Information). We find that the presence of achiral, aromatic *N*pm groups in a three-fold periodic pattern with *N*rpe residues can also provide helix stabilization. In general, peptoids having three aromatic faces including both chiral and achiral side chains (**7**, **14**) display stronger CD spectra than analogous peptoids with the same

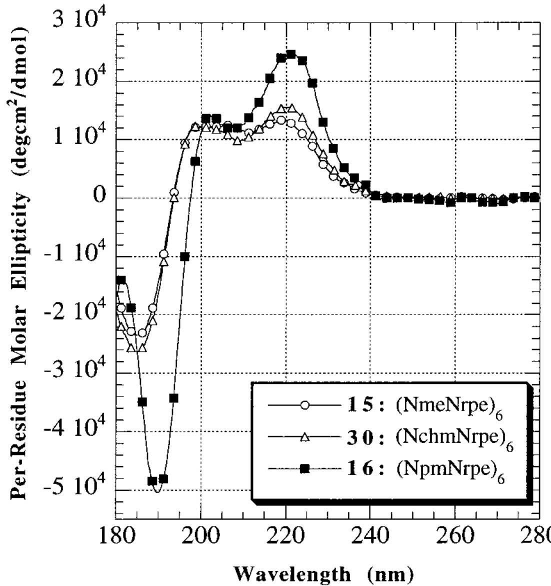

Figure 4. Comparison of the CD spectra of analogous peptoid dodecamers **15**, **16**, and **30**. While they have the same overall percentage of chiral residues and the same C termini, inclusion of *N*me or *N*chm as an achiral residue, as compared to *N*pm, dramatically reduces the overall helicity.

percentage of chiral and achiral side chains in their sequence but just one aromatic face (**23**, **26**).

Effects of Including Aromatic versus Aliphatic Achiral Side Chains in Model Peptoids. To determine the importance of bulky, aromatic side chains as helix stabilizers, the achiral, aromatic side chain *N*-phenylmethylglycine (*N*pm) was replaced in short model peptoids with the achiral, *aliphatic* side chain, *N*-methoxyethylglycine (*N*me). We find that substitution of *N*me for *N*pm in a series analogous to $(NpmNrpe)_{n=3,6}$ has a dramatic effect on the degree of overall helicity. CD spectra show that peptoid dodecamer **15**, in which achiral side chains are aliphatic and flexible, exhibits relatively weak CD in comparison to peptoid **16** in which the achiral *N*pm side chains are aromatic and bulky (Figure 4). In a very similar manner, the CD spectrum of *N*me-containing hexamer **17** shows a less well-defined CD than *N*pm-containing peptoid **18** (see Supporting Information), and both of these hexamers show weaker CD spectra than their respective dodecamers, **15** and **16**. Also, for both of these pairs of peptoids, we observe blue-shifting of CD peaks for the *N*me-containing hexamer relative to the *N*pm-containing hexamer. The importance of side-chain identity is also evidenced in the comparable retropeptoid series, where hexamer **19** and dodecamer **20**, both with 50% aliphatic, achiral side chains, exhibit much weaker CD than the analogous peptoids **21** and **22**, respectively, for which the achiral side chains are all aromatic (data not shown). These results again indicate that the presence of one or more aromatic faces on a peptoid helix can play an important role in structural stabilization. Analogously, the ability of the bulky aromatic ring of phenylalanine to restrict side-chain conformations has been seen to influence helix formation in peptides.[31−33] We had some concern that the flexibility and hydrophilicity of the *N*me side chain may also play a role in destabilizing the peptoid helix, in addition to its nonaromatic

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nature; therefore, we also substituted the more hydrophobic and sterically hindered *N*-cyclohexylmethyl (*N*chm) monomer for *N*pm in a peptoid analogous to $(N\text{pm}N\text{rpe})_6$, creating dodecamer **30**. We observe that the CD spectrum of **30**, in which achiral side chains are aliphatic but not hydrophilic, likewise exhibits a weaker, blue-shifted CD spectrum in comparison to that of peptoid **16**, further suggesting the importance of aromatic interactions in stabilizing peptoid helices. Moreover, the spectra of oligomers **15** and **30** are quite similar.

The strong CD of this family of peptoids does not simply correlate with the presence of aromatic side chains, for even peptoids with 100% aromatic side chains can show very minimal CD (e.g., **2**, **3**, **7**). Our results suggest that steric or electronic interactions, occurring either between ordered aromatic side chains or between backbone carbonyls and the π-electron clouds of the aromatic rings, lead to the formation of a distinct helical structure that in turn gives rise to the CD *signature* of these ordered peptoid helices. The substitution of *N*me or *N*chm for *N*pm at a given position can destabilize a peptoid helix in two ways: (1) by removing stabilizing hydrophobic or electrostatic aromatic–aromatic and aromatic-carbonyl interactions and (2) by reducing the steric driving force for helical ordering at that position. There may be some role as well for aromatic stacking of the side chain phenyl rings; we hope to determine the structure of a longer, more stable peptoid helix to ascertain the relative positioning of these phenyl rings. Secondary structure stabilization by aromatic stacking has been observed in other systems, where alternating pyridine–pyrimidine heterocycles resulted in spontaneous formation of helical structures.[34,35] Influence of aromatic–aromatic interactions has also been observed in phenylene ethynylene oligomers.[36,37] Importantly, the design rules that we have observed in the course of this study for stable peptoid helix formation—a required composition of at least one-half α-chiral monomers, placement of one or more chiral residues on the carboxy terminus to prevent helix fraying, and sequence designs that maximize the number of stabilizing "aromatic faces"—appear to be less critical for longer peptoids. In particular, when chain length is increased beyond 12–15 residues, we find that a stable peptoid helix can be attained even for a sequence that is quite unstable at the hexamer length. For example, although the CD spectrum of the hexamer **23** is quite weak and blue-shifted, the analogous 12mer **27**, 18mer **28**, and 24mer **29** exhibit helical spectra that are increasingly more intense and well-developed in shape (Figure 5). In other words, peptoid sequences that are unstable at shorter lengths, providing only a hint of helical CD, can be transformed into very stable helices simply by extending the chain, presumably because peptoid helix stabilization is provided by cooperative interactions that propagate down the ordered chain.

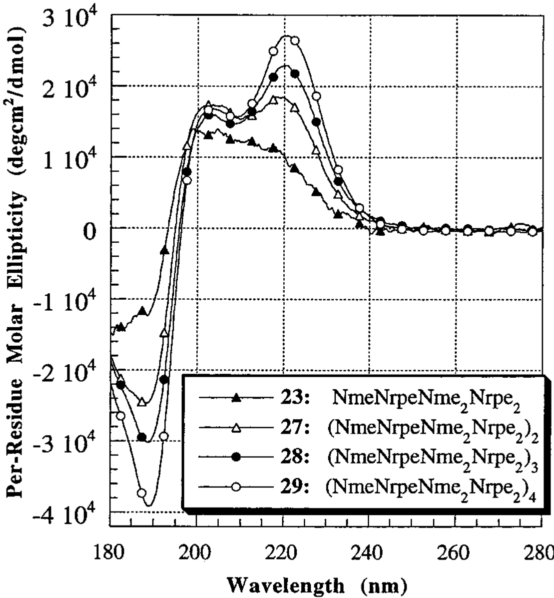

Figure 5. Comparison of the CD spectra of a hexamer, dodecamer, octadecamer, and 24mer consisting of one, two, three, and four repeats of hexapeptoid **23**. Overall helicity is seen to increase markedly with chain length.

Conclusions

This detailed, comparative study of more than 30 different peptoid oligomers has yielded a better understanding of the design criteria for formation of chiral secondary structure. We find that short, heterooligomeric peptoids that include achiral side chains are typically stable if (1) at least 50% of the constituent monomers are α-chiral and aromatic, (2) an α-chiral, aromatic monomer is placed on the carboxy terminus, and (3) sequences are designed periodically to maximize the number of "aromatic faces" on the peptoid helix. Extending chain length beyond 12–15 residues reduces the impact of these specific, sequence-dependent factors on helix stabilization. Through further 2D-NMR as well as crystallographic studies, we are presently working to obtain a more complete understanding of the helical structures adopted by peptoid chains that include aromatic and aliphatic α-chiral side chains. Such insight will inform the design of helical peptoids with application as biomimetic oligomers. Organosoluble helices and amphipathic helices may find useful application in hydrophobic biological environments or at interfaces, for example if they are designed to specifically interact with or to selectively disrupt phospholipid monolayers and bilayers.

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Supporting Information Available: CD and HSQC spectra of a number of different peptoid oligomers (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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