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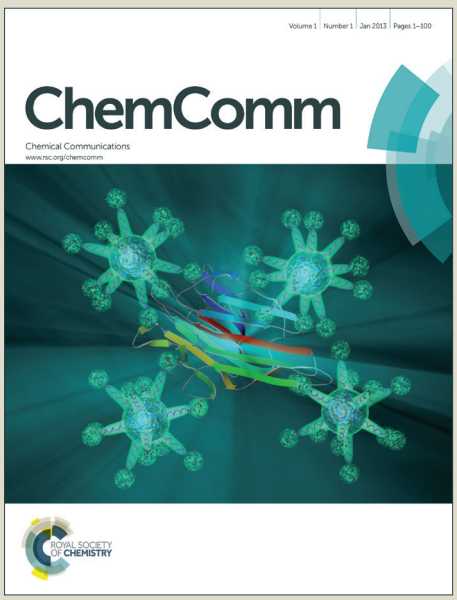

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ARTICLE TYPE

Peptoid Helicity Modulation: Precise Control of Peptoid Secondary Structures via Position-Specific Placement of Chiral Monomers†

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The degree of peptoid helicity can be effectively modulated by position-specific incorporation of α-chiral aromatic monomers. In this study, we report the structural role of each monomer position collected from 30 comprehensive model peptoid oligomers demonstrating a meticulous manner to fine-tune peptoid secondary structures.

Inspired by exquisite natural systems, scientists have sought to generate protein-like structures and functions from non-natural heteropolymers.[1] A prerequisite for successful mimicry would be to understand how a heteropolymer sequence determines its folded structure and consequently function.

Peptoids are a class of bioinspired heteropolymers based on oligo-*N*-substituted glycine backbones, which are often used as a versatile platform to mimic the functions of natural peptides and proteins.[2] A central theme in the peptoid research community has been discovering the factors that influence three-dimensional (3-D) peptoid conformation.[3-7] Unlike natural peptides, each side chain of peptoid monomers is appended to the amide nitrogen rather than to the α-carbon, eliminating the possibility of backbone hydrogen-bonding driven secondary structure formation. However, peptoids fully modified with bulky α-chiral side chains have been demonstrated to adopt stable and well-defined 'fully helical' folds in solution by mainly employing local steric and electronic interactions.[3] Thereafter, the dependence of such a peptoid helical conformation on oligomer sequence,[4] chain length,[5] monomer identity,[6] and solvent composition[7] has been elucidated. Based on these information, a variety of functional peptoid foldamers have been successfully developed including antimicrobial peptoids,[8] pulmonary surfactant protein mimics,[9] asymmetric catalysts,[10] zinc binding peptoids,[11] antifreeze protein mimics,[12] and artificial light-harvesting complexes.[13]

To further emulate the elegant 3-D structures of natural proteins and expand the functional applications of peptoids, it is desirable to access more precisely controlled peptoid conformations (or partially unfolded peptoids) and thereby to understand further the underlying principles of sequence-structure relationship in peptoid foldamers. While early peptoid research mainly focused on the construction of stable helical peptoid structures, a wide range of other conformational possibilities between fully helical and non-helical still remains unexplored. The conformational space is exploited by natural proteins extensively, and the partially unfolded structure plays pivotal roles in signaling and regulatory pathways.[18] In this study, we employed an approach inspired by 'sergeant-and-soldier effect' in the seminal polyisocyanate research[14] and investigated the effect of position-specific placement of α-chiral monomer(s) on the 3-D peptoid conformation.

Our hypothesis is that there exist multiple specific positions in a peptoid sequence where structure-inducing α-chiral aromatic monomers (i.e., sergeants) can effectively regulate the conformations of a multitude of achiral monomers (i.e., soldiers) in the same chain; therefore, the extent of peptoid helicity can be effectively manipulated by regio-specific incorporation of a few structure-inducing monomers. Such a fine adjustability of peptoid helicity serves as a rational design strategy to generate moderately helical peptoids that fill the gap between fully helical and non-helical structures (Fig. 1(A)) and provide an insight into fine-tuning peptoid functions.

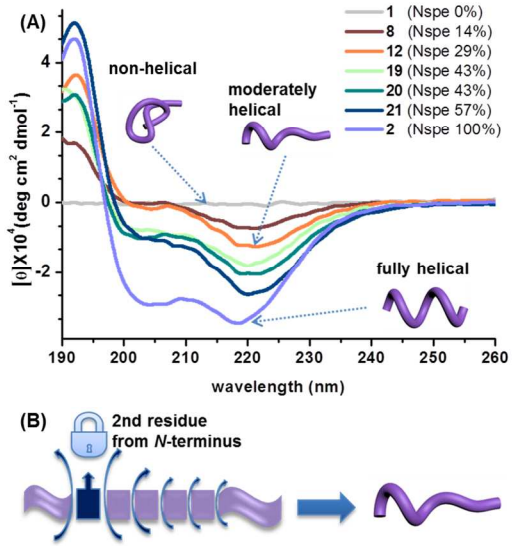
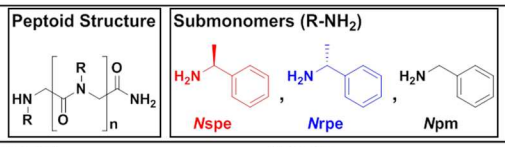


Fig. 1 (A) CD plots of representative peptoid heptamers showing helicity modulation. The term '*fully helical*' was used to indicate the highest helicity reached by a heptameric peptoid composed of *N*spe and *N*pm monomers, as a comparative term in conjunction with *non-helical* and *moderately helical*. (B) Schematics of effective control over peptoid folding via position-specific placement of an α-chiral monomer.

Initially, we posed three major questions as follows: (1) Do there exist 'lynchpin' monomer positions that predominantly

regulate the overall peptoid structure? Conversely, do there exist trivial positions that minimally affect the given peptoid structure?; (2) If two α-chiral aromatic monomers are incorporated, is the level of each contribution simply additive or synergistic?; and (3) If two α-chiral aromatic monomers with opposite senses (i.e., monomer with (*R*) or (*S*) chirality) are incorporated into the same sequence, which one will govern the overall handedness? To address the aforementioned questions, we chose a family of peptoid heptamers composed of an α-chiral aromatic monomer (*N*spe or (*S*)-(–)-1-phenylethylamine) as a structure-inducer and achiral monomers (*N*pm or benzylamine) as structural followers. Firstly, peptoid heptamers **1-9** were synthesized using microwave-assisted[15] solid-phase peptoid submonomer synthesis methods[16] varying the position of the α-chiral aromatic monomer from C-terminus (Table 1). Peptoids **1** and **2** were prepared as non-helical and fully helical sequence controls, respectively.[4a] Peptoids **3-9** were designed to evaluate the contribution of a single *N*spe to the helical fold at a series of positions from C-terminus. The degree of helical fold was measured by circular dichroism (CD) intensity at 220 nm, which has been assigned as a relevant signal to the helical conformation of *N*spe-based peptoids.[5, 6j, 7]

Table 1 Sequences of the peptoids synthesized herein.



Compounds	Sequence
1	H–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm–NH_2
2	H–***N*spe**–***N*spe**–***N*spe**–***N*spe**–***N*spe**–***N*spe**–***N*spe**–NH_2
3	H–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm–***N*spe**–NH_2
4	H–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm–***N*spe**–*N*pm–NH_2
5	H–*N*pm–*N*pm–*N*pm–*N*pm–***N*spe**–*N*pm–*N*pm–NH_2
6	H–*N*pm–*N*pm–*N*pm–***N*spe**–*N*pm–*N*pm–*N*pm–NH_2
7	H–*N*pm–*N*pm–***N*spe**–*N*pm–*N*pm–*N*pm–*N*pm–NH_2
8	H–*N*pm–***N*spe**–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm–NH_2
9	H–***N*spe**–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm–NH_2
10	H–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm–***N*spe**–***N*spe**–NH_2
11	H–*N*pm–*N*pm–*N*pm–*N*pm–***N*spe**–***N*spe**–*N*pm–NH_2
12	H–*N*pm–***N*spe**–***N*spe**–*N*pm–*N*pm–*N*pm–*N*pm–NH_2
13	H–*N*pm–***N*spe**–*N*pm–*N*pm–*N*pm–*N*pm–***N*spe**–NH_2
14	H–*N*pm–***N*rpe**–*N*pm–*N*pm–*N*pm–*N*pm–***N*rpe**–NH_2
15	H–*N*pm–***N*rpe**–*N*pm–*N*pm–*N*pm–*N*pm–***N*spe**–NH_2
16	H–*N*pm–***N*spe**–*N*pm–*N*pm–*N*pm–*N*pm–***N*rpe**–NH_2
17	H–***N*spe**–*N*pm–*N*pm–***N*spe**–*N*pm–***N*spe**–*N*pm–NH_2
18	H–*N*pm–***N*spe**–***N*spe**–*N*pm–***N*spe**–*N*pm–*N*pm–NH_2
19	H–*N*pm–***N*spe**–*N*pm–*N*pm–*N*pm–***N*spe**–***N*spe**–NH_2
20	H–*N*pm–***N*spe**–***N*spe**–*N*pm–*N*pm–*N*pm–***N*spe**–NH_2
21	H–*N*pm–***N*spe**–***N*spe**–*N*pm–*N*pm–***N*spe**–***N*spe**–NH_2
22	H–*N*pm–*N*pm–*N*pm–*N*pm–***N*spe**–*N*pm–*N*pm–*N*pm–*N*pm–NH_2
23	H–*N*pm–(*N*pm)$_3$–*N*pm–***N*spe**–*N*pm–*N*pm–(*N*pm)$_3$–NH_2
24	H–*N*pm–(*N*pm)$_3$–*N*pm–*N*pm–***N*spe**–*N*pm–(*N*pm)$_3$–*N*pm–*N*pm–NH_2
25	H–*N*pm–(*N*pm)$_3$–*N*pm–*N*pm–***N*spe**–*N*pm–(*N*pm)$_3$–*N*pm–NH_2
26	H–*N*pm–(*N*pm)$_3$–*N*pm–***N*spe**–*N*pm–*N*pm–(*N*pm)$_3$–*N*pm–NH_2
27	H–*N*pm–(*N*pm)$_3$–*N*pm–***N*spe**–***N*spe**–*N*pm–(*N*pm)$_3$–*N*pm–NH_2
28	H–*N*pm–***N*spe**–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm-NH_2
29	H–*N*pm–***N*spe**–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm-NH_2
30	H–*N*pm–***N*spe**–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm–*N*pm-NH_2

Incorporation of a single *N*spe monomer at different positions showed distinct CD spectra (Fig. 2(A) and 2(B)). The CD spectra of peptoids **5**, **7** and **8** indicate a typical signature of a right-handed polyproline type-I (PPI) peptoid helix, with two CD minima at 202 and 220 nm (Fig. 2(A)). Among these three peptoids, the placement of *N*spe at the second position from N-terminus (peptoid **8**) provided the largest CD signal at 220 nm. The degree of helical fold gradually decreased as the *N*spe position shifted from the second (**8**) to the third (**7**) and then to the fifth (**5**) positions from N-terminus. On the other hand, peptoids **6** and **9**, where *N*spe was located at the middle of the heptamer sequence and at the first from N-terminus, respectively, showed CD spectra similar to non-helical peptoid **1** with virtually flat ellipticity over 190 to 260 nm region (Fig. 2(B)). Peptoid **4** revealed the moderate signal at the short-wavelength (~195 nm) $\pi \rightarrow \pi^*$ transition region, however, no apparent CD signal was observed at longer wavelength (Fig. 2(B)). Notably, peptoid **3** with *N*spe at the first position from C-terminus showed a helix-like CD signature with strong $\pi \rightarrow \pi^*$ transition at 198 nm (Fig. 2(A)). This position was previously pointed out by Barron and coworkers as crucial for stabilizing helical conformation of peptoid oligomers.[4a] Based on the CD spectra of peptoids **3-9**, peptoids **10-21** were synthesized to investigate the cooperative effects of multiple α-chiral monomer positions on regulating the resultant heptamer conformation.

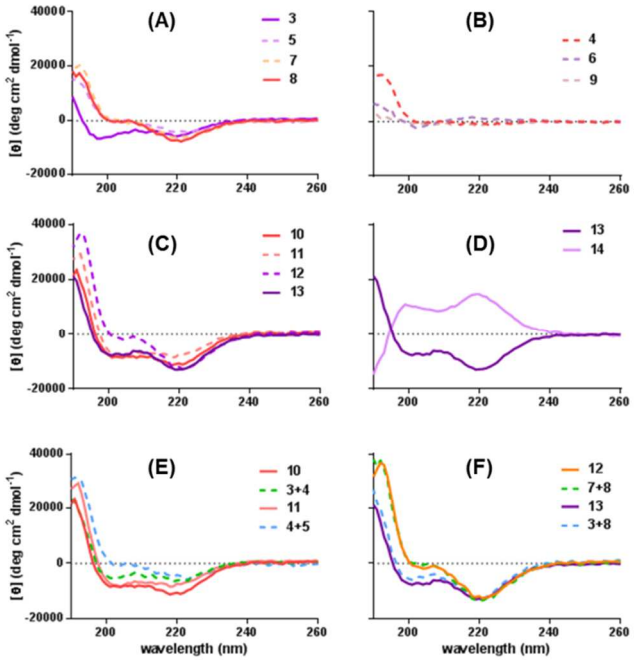


Fig. 2 CD spectra of peptoid heptamers **3-14** (50 μM in acetonitrile) were recorded as per-residue molar ellipticity, or [θ]. Data were acquired at 20ºC. Each spectral plot shows distinct effects of position-specific incorporation of a single *N*spe (A, B) and two *N*spe's (C). Additional plots are displayed for demonstration of helicity inversion (D), synergistic (E), and additive contribution (F) with two *N*spe monomers incorporated.

Firstly, peptoids **10-13**, which contains two *N*spe monomers at the helix-inducing positions, were synthesized. As shown in Fig. 2(C), peptoids **10-13** showed characteristic CD spectra of a peptoid helix. Moderate but well-established helical fold was observed in peptoids **10**, **12**, and **13**, confirming the importance of the second position from N-terminus and the first position from C-terminus. Peptoid **11**, however, showed relatively weaker degree of helicity probably due to the incorporation of *N*spe monomers at the less critical positions. It is also noteworthy that the level of contribution of the two α-chiral aromatic side chains

was either synergistic (**10** and **11**, Fig. 2(E)) or additive (**12** and **13**, Fig. 2(F)); for instance, the CD intensity of **13** was similar to the sum of those of **3** and **8** (additive, Fig. 2(F)) while **10** showed stronger intensity at 198 nm and at 220 nm compared to the added intensity of **3** and **4** (synergistic, Fig. 2(E)). When two identical monomers with the opposite chirality (i.e., *N*rpe) were incorporated at the helix-inducing sites, the opposite CD signature showing helicity inversion was observed (Fig. 2(D)).[3a, 5] Moreover, when both *N*spe and *N*rpe were incorporated into the same chain, uncharacteristic CD spectra were obtained, indicating the counteractive effect of two opposite chirality (**15** and **16**, see Supporting Information, Fig. S6).[17]

Next, a series of peptoid heptamers **17**-**20** with three *N*spe monomers at different positions were synthesized. Based on the two chiral monomer results, peptoids **17** and **20** were synthesized to have three *N*spe monomers at minimally influential positions (i.e., the first, fourth, and sixth positions from N-terminus) and at most influential positions (i.e., the second, third, and seventh positions from N-terminus), respectively. As expected, the CD spectra of **17** and **20** showed a dramatic contrast (Fig. 3(A)): Peptoid **20** showed a typical helix CD signature, whereas peptoid **17** exhibited an almost non-helical CD signature despite the same percentage of helix-inducing chiral monomer contents (*N*spe 43%). Therefore, we confirmed that the abovementioned uninfluential positions show almost-zero synergetic contribution to helix formation even with three chiral monomers present.

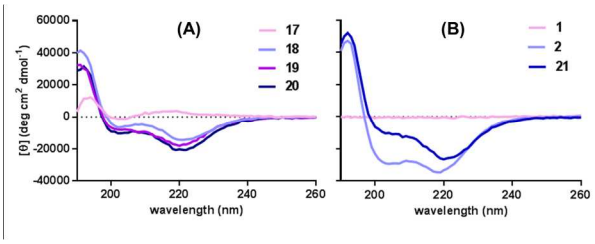


Fig. 3 CD spectra of peptoid heptamers (50 μM in acetonitrile) with (A) three (**17**-**20**) and (B) four chiral monomers (**21**). In (B), CD spectra of **1** and **2** were drawn for comparison.

Slightly weaker negative Cotton effect was observed with peptoids **18** and **19** which have one *N*spe site variation from **20** (Fig. 3(A)). When four *N*spe's were positioned at the most influential sites (peptoid **21** in Fig. 3(B)), even larger magnitude of the ellipticity at 220 nm was observed. The percentage of *N*spe was calculated as 57% in the heptamer (i.e., four out of seven), but the CD intensity of **21** at 220 nm was obtained as 76% of the intensity of the fully helical peptoid **2** (Fig. 4). Interestingly, as the number of incorporated *N*spe's are increased (**13** < **20** < **21** < **2**), we observed more pronounced synergistic effects among the influential sites (Fig. 4 and Fig. S8, see Supporting Information).

Noticeable observations with peptoid heptamers are as follows: (1) the second position from N-terminus was most influential in helical structure formations; and (2) the first position from N-terminus and the middle position in the oligomer were minimally effective in the secondary structure formation. To confirm the validity of these arguments in the even longer oligomer sequences, peptoids **22**-**30** were synthesized. Peptoid heptamer, octamer, and decamer (**8**, **28**, and **30**, respectively), which all have one *N*spe at the *influential* second position from N-terminus, clearly exhibited helical signature as denoted by almost identical CD features of these oligomers (Fig. S7(A), see Supporting Information). However, nonamer (**29**) showed an unusually enhanced negative CD signal around 203 nm, reminiscent of that of a threaded loop conformation of the *N*spe nonamer.[7] In the case of longer peptoid sequences with a single *N*spe incorporated at the *uninfluential* middle position, undecamer (**23**) and tridecamer (**24**) showed almost non-helical CD signatures (Fig. S7(B)).

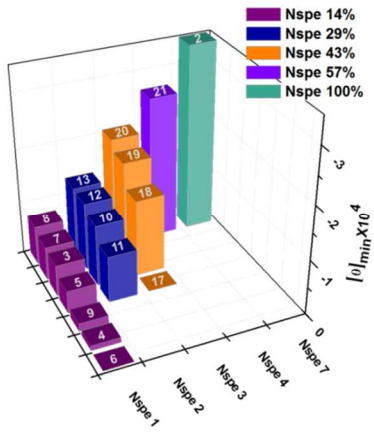


Fig. 4 Comparison of the magnitude of the ellipticity measured by CD at 220 nm for selected peptoids studied herein.

In the context of the questions we posed at the beginning, the abovementioned results suggest the following: Even a single α-chiral aromatic monomer at one of the influential sites of oligopeptoids provided a moderately helical signature in CD spectra. The particular role of this specific position is conserved in the longer peptoid oligomers. When multiple numbers of helix-inducing monomers are incorporated into these influential positions, the cooperative structure-inducing effect is more pronounced as exemplified by peptoids **13**, **20**, and **21** in striking contrast to **17** (Fig. 4). In other words, the position-specific placement as well as the numerical percentage of α-chiral aromatic monomers[4a] in a given peptoid chain is crucial in terms of determining the 3-D conformation of a peptoid. It is noteworthy that the less influential positions can be used as the sites to introduce various functional groups appended to achiral monomers without hampering overall structural integrity of peptoids.

In summary, we investigated the effect of position-specific placement of α-chiral aromatic monomer(s) on the peptoid secondary structure. The structural role of each position in a peptoid heptamer is now well understood; in particular, the existence of lynchpin-like structure-inducing positions and virtually uninfluential positions in an oligopeptoid sequence was confirmed. The judicious placement of α-chiral aromatic monomers can effectively modulate overall peptoid helicity, and the helicity modulation can be used as a way to fine-tune peptoid functions. Both theoretical modeling and structural analysis, which can provide a deeper understanding on our experimental observations, are currently underway, and we anticipate this study can offer a useful insight toward a rational design of various functional peptoid oligomers.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Detailed procedures for the synthesis, characterization, and purification of all new compounds. See DOI: 10.1039/b000000x/

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