

# Novel Peptoid Building Blocks: Synthesis of Functionalized Aromatic Helix-Inducing Submonomers

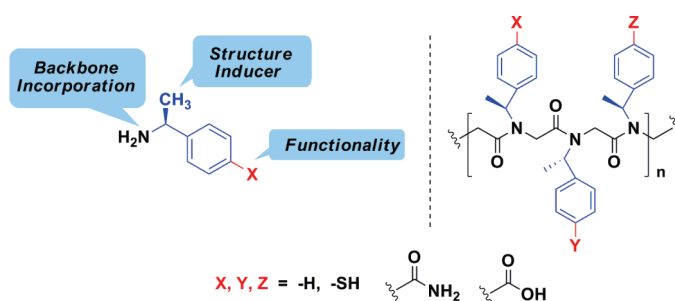
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## ABSTRACT



Peptoids, oligo-*N*-substituted glycines, can fold into well-defined helical secondary structures. The design and synthesis of new peptoid building blocks that are capable of both (a) inducing a helical secondary structure and (b) decorating the helices with chemical functionalities are reported. Peptoid heptamers containing carboxamide, carboxylic acid or thiol functionalities were synthesized, and the resulting peptoids were shown to form stable helices. A thiol-containing peptoid readily formed the homodisulfide, providing a convenient route to prepare peptoid helix homodimers.

One of the hallmarks of protein structure is precise specificity of heteropolymer sequence. A set of chemically diverse amino acid building blocks comprise the sequence and encode basic information; then, the sequence undergoes folding to obtain a distinct structure and a function.<sup>1</sup> For decades, scientists have attempted to mimic the exquisite natural system and to generate protein-like structures and functions from non-natural heteropolymers.<sup>2</sup> To that end, a prerequisite would be to have equivalents of amino acids that have a secondary structure-inducing element as well as a variety of chemical functional groups.

Peptoids are a class of biomimetic polymers based on oligo-*N*-substituted glycine backbones, designed to mimic peptides and proteins.<sup>3</sup> Peptoids can be efficiently synthesized with precisely defined sequences and chain lengths (up to ~50 monomers)<sup>4</sup> employing solid-phase submonomer synthesis protocol (Figure 2a).<sup>5</sup> Peptoids can form well-defined three-dimensional folds in solution: (1) peptoid oligomers with  $\alpha$ -chiral side chains were shown to adopt helical

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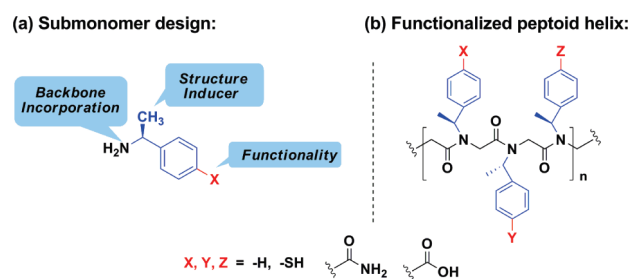
structures;<sup>6</sup> (2) a threaded loop structure was formed by intramolecular hydrogen bonds in peptoid nonamers;<sup>7</sup> and (3) head-to-tail macrocyclizations provided conformationally restricted cyclic peptoids.<sup>8</sup>

Understanding the factors that influence peptoid conformation has been a central theme of peptoid research. Previously, we elucidated the dependence of oligomer sequence,<sup>9</sup> chain length,<sup>10</sup> and solvent composition<sup>7,11</sup> on the formation of the helical or threaded loop conformations. More recently, Blackwell and co-workers demonstrated the ability to control peptoid secondary structure using electron-deficient submonomers such as (*S*)-1-(pentafluorophenyl)ethylamine<sup>12</sup> or (*S*)-1-(2-nitro-phenyl)ethylamine.<sup>13</sup> In particular, they provided strong evidence that an electronic  $n \rightarrow \pi^*$  interaction at the monomer level was essential to the backbone cis/trans isomerism<sup>14</sup> and, potentially, to the global peptoid conformation. The role of monomer units on peptoid folding was also investigated by Kirshenbaum et al. They introduced novel submonomers such as (1) (*S*)-*N*-(1-carboxy-2-phenylethyl)-glycine<sup>15</sup> that provided pH dependent conformational change in peptoid secondary structure and (2) *N*-aryl glycines<sup>16</sup> that could control the backbone cis/trans isomerism.

Utilizing the relationship between peptoid monomer sequence and adopted secondary structure, functional peptoid foldamers have been developed including antimicrobial peptoids,<sup>17</sup> pulmonary surfactant protein mimics,<sup>18</sup> asymmetric catalysts,<sup>19</sup> and zinc binding peptoids.<sup>20</sup> These studies demonstrate the importance of (1) access to chemically

diverse monomer units and (2) precise control of secondary structures to expand applications of peptoid helices.

To form secondary and tertiary structures, natural proteins utilize noncovalent interactions (i.e., hydrophobic, electrostatic, and hydrogen bonds) and covalent bonds (i.e., disulfides). Inspired by the natural system, our goal is to build higher-order structures by modulating monomer functionality and sequence.<sup>2,21</sup> In this study, we present the synthesis of several novel peptoid submonomers that are capable of displaying functional groups as well as inducing a helical conformation. A sophisticated decoration of the peptoid helix can promote interactions between helices and can therefore be used to create novel peptoid-based ordered constructs.



**Figure 1.** Peptoid submonomer design.

As shown in Figure 1, three new peptoid submonomers were designed.<sup>22</sup> These new submonomers are derivatives of (*S*)-1-phenylethylamine, or *N*spe, which can be readily incorporated into a peptoid and induces a stable helical fold.<sup>6a</sup> Functionalized peptoids containing the new submonomers can form noncovalent bonds (i.e., hydrogen bonds) as well as covalent bonds (i.e., disulfide bonds, metal–ligand interactions). In addition, they can modulate the hydrophobicity and water solubility<sup>11,15</sup> of the peptoid helix.

Thiol submonomer **6** was synthesized as shown in Scheme 1. For the asymmetric synthesis of the  $\alpha$ -branched amine, we employed Ellman's *N*-*tert*-butanesulfinyl imine as a key intermediate.<sup>23</sup> A condensation of commercially available (+)-*tert*-butanesulfinamide (**1**) and 4-(methylthio)benzaldehyde with  $\text{Ti}(\text{OEt})_4$  as a Lewis acid catalyst<sup>24</sup> provided sulfinimine **2** in a 94% yield. *S*-Methyl thioether protecting group was well-tolerated in the Grignard reaction conditions, and the addition of methylmagnesium bromide to sulfinimine

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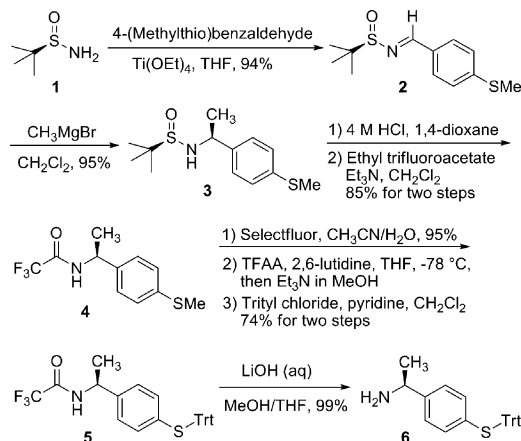
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2 proceeded efficiently. After recrystallization, sulfonamide 3 was isolated in a 95% yield, and the crystal was confirmed as a single diastereomer by <sup>1</sup>H NMR.

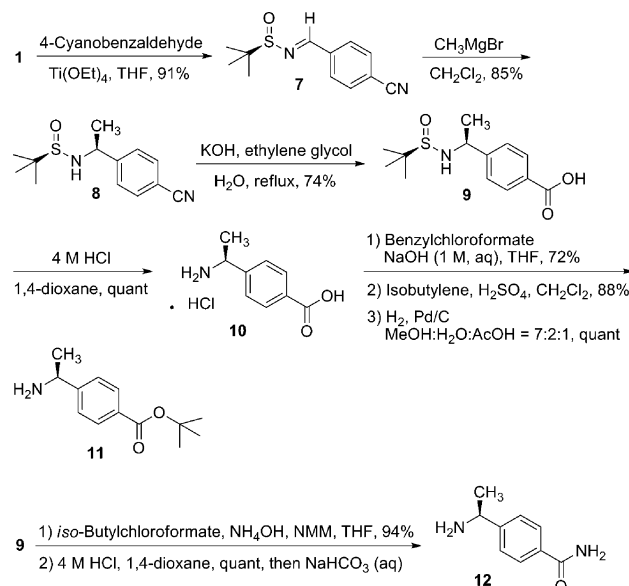
**Scheme 1.** Synthesis of **6** (*N*spe-thiol)



Typical deprotection of an *S*-methyl group requires two step reactions: first, oxidation of the thioether to a sulfoxide; second, Pummerer rearrangement of the sulfoxide and subsequent methanolysis to a thiol.<sup>25</sup> Initially, we focused on carrying out the *S*-methyl deprotection on solid-phase. Oxidation to sulfoxide proceeded smoothly, but we found the following Pummerer rearrangement gave inconsistent results. Therefore, we decided to switch the *S*-methyl to an acid-labile *S*-trityl protecting group. First, *tert*-butanesulfinyl group was removed by HCl in 1,4-dioxane, and trifluoroacetamide protecting group was introduced to provide **4**. *S*-Methyl group was then removed by oxidation,<sup>26</sup> Pummerer rearrangement,<sup>27</sup> and methanolysis in a good yield to provide an aromatic thiol. Without isolating the aromatic thiol, we proceeded the thiol protection reaction using trityl chloride and pyridine to provide **5**; a basic hydrolysis of **5** removed trifluoroacetamide protecting group, and free amine **6** was obtained in a quantitative yield. Notably the synthesis of **6** did not require a single chromatographic purification; all the intermediates could be easily recrystallized, and a gram scale synthesis of **6** was efficiently accomplished.

Scheme 2 depicts the synthesis of *N*spe-acid (**11**) and *N*spe-amide (**12**). The two submonomers can be accessed from common intermediate **9**. Aromatic nitrile **8** was prepared employing a similar strategy as the one in Scheme 1. Unlike thioether **3**, aromatic nitrile **8** was an oily residue and not recrystallizable. The diastereomeric impurity was identified by <sup>1</sup>H NMR (*dr* = 86:14). Although the diastereomers were separable by chromatography, we went on to

**Scheme 2.** Synthesis of **11** (*N*spe-acid) and **12** (*N*spe-amide)



the next hydrolysis without purification of **8**. Several conditions were tried for the hydrolysis of aromatic nitrile **8** to acid **9**. The best result was obtained when the alkaline hydrolysis was performed in refluxing water/ethylene glycol mixture.<sup>28</sup> Pleasantly, acid **9** could be recrystallized, yielding the desired diastereomeric isomer in a 74% yield. Amino acid **10** was obtained after deprotection of *tert*-butanesulfinyl group. Subsequent Cbz protection, *tert*-butyl ester formation, and Cbz deprotection<sup>29</sup> provided **11**. Carboxamide **12** was prepared by an amidation of **9** followed by the deprotection of *tert*-butanesulfinyl group. We found the synthetic route to be highly reproducible. We prepared key intermediate **9** in a multigram scale, and **11** and **12** were synthesized in a gram scale.

Submonomers **6** (*N*spe-thiol), **11** (*N*spe-acid), and **12** (*N*spe-amide) were then employed to synthesize peptoid oligomers (**14**–**20**, Figure 2). Nonfunctionalized peptoid heptamer **13** was synthesized as a control. Peptoid **14** contains one amide in the middle; **15** has two amides on the same face of the helix (three residues per turn), while **16** has two amides placed on different faces. **17** has four amides, two of which are facing the same side of the helix. **18** has amides on all three faces. Interestingly, peptoid **18** was soluble in water (~2 mg/mL). Peptoid **19** is an acid equivalent of peptoid **15**. Peptoid **20** contains one thiol in the middle, and *N*-terminus was acetylated.

Isolated thiol peptoid **20** readily formed peptoid disulfide by air oxidation in methanol over a prolonged time (i.e., 2–3 days). The homodimeric peptoid helix **21** (see Supporting

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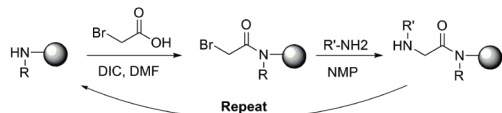
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(a) Submonomer synthesis scheme



(b) Functionalized peptoid helices

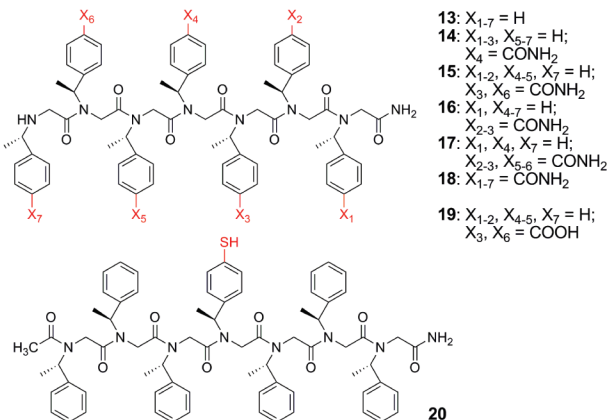


Figure 2. Synthesis of functionalized peptoid heptamers.

Information S20–S21) was purified by preparative HPLC and characterized by MALDI-TOF mass spectrometry ( $[\text{M} + \text{Na}]^+ = 2458.4$ ). A number of natural helices function as sulfhydryl-dependent homodimers.<sup>30</sup> Hence, our method to synthesize homodimeric peptoid helices will be a useful tool to study protein mimicry.

The maintenance of helical folds was demonstrated by circular dichroism (CD) spectroscopy for peptoid heptamers **13–16** and **19** and **20**, and peptoid homodimer **21** (Figure 3).<sup>31</sup> The amide functionalized peptoids exhibited decreased spectral intensity compared to nonfunctionalized peptoid **13** (Figure 3A).<sup>6a</sup> CD spectra of **15** and **19** were almost identical indicating that the influences of amides and acids on the helical integrity were similar. Thiol peptoid **20** and peptoid homodimer **21** also exhibited identical CD spectra, which suggests the dimer formation did not affect the secondary structure of each helix. *N*-Termini of **20** and **21** are acetylated, and direct comparison with other peptoid heptamers was not attempted.

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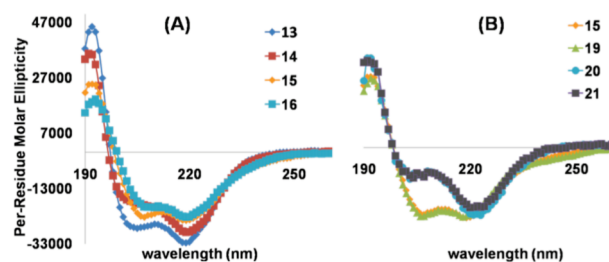


Figure 3. CD spectra of peptoids in acetonitrile (**13**, **14**, **15**, **16**, and **19**: 50  $\mu\text{M}$ ; **20** and **21**: 46  $\mu\text{M}$ ) were recorded as per-residue molar ellipticity ( $\text{deg cm}^2/\text{dmol}$ ). Data were acquired at room temperature. (A) Comparison of amide functionalized peptoids **14–16** and control peptoid **13**. (B) Comparison of amide peptoid **15** and acid peptoid **19**. Also the effect of peptoid homodimerization via disulfide (**20** and **21**).

In summary, we described the efficient synthesis of new peptoid submonomers that can incorporate into peptoids, induce helical conformations, and present a variety of functional groups on the helix. Our synthetic strategy can be applied to the synthesis of various functionalized aromatic helix-inducing peptoid submonomers. The functionalized peptoids showed helical conformations, and we demonstrated the ability to create homodimeric peptoid helices by using thiol submonomer **6**. The new submonomers should provide versatility to peptoid helices, and the functionalized peptoid helices should find numerous applications in the field of biomedicine and nanoscience.

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**Supporting Information Available:** Detailed procedures for the synthesis, characterization, and purification of all new compounds and peptoids. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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