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Conformational Rearrangements by Water-Soluble Peptoid Foldamers

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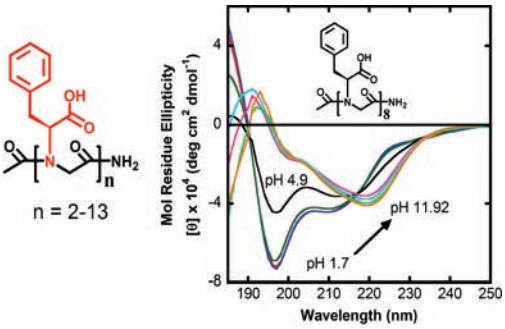
ABSTRACT



Peptoids are a family of N-substituted glycine oligomers that are capable of forming stable helical structures. We seek peptoid monomers that can establish a strong folding propensity in aqueous conditions. Here we utilize L-phenylalanine *tert*-butyl ester as a readily available reagent for the synthesis of (*S*)-*N*-(1-carboxy-2-phenylethyl)glycine oligomers. The products form stable secondary structures in aqueous solution in which the conformation is dramatically responsive to variations in pH and solvent composition.

Many biopolymers display the capability to interconvert between distinct folded states. Such conformational switching allows proteins to respond to their environment and to mediate signaling events.[1] Through conformational rearrangements, proteins can promote chemo-mechanical energy transduction or exhibit allosteric control of their activities. Extensive efforts have been made to design novel synthetic peptides and peptidomimetic compounds whose conformations are responsive to changes in pH, redox states, or solvent composition.[2]

Our investigations are focused on a class of peptidomimetics called peptoids, which comprise N-substituted glycine monomer units.[3] Peptoids are an example of oligomers that demonstrate a strong propensity to adopt well-ordered conformations. As such, they are among a family of compounds described as foldamers.[4] Peptoids are broadly resistant to proteolytic degradation, and a variety of bioactive peptoid compounds have been reported.[5] Peptoid sequences incorporating bulky chiral side chains, such as (*S*)-*N*-(1-phenylethyl)glycine (Figure 1), have the capacity to adopt a compact secondary structure resembling that of the polyproline I type helix.[6] However, helical oligopeptoids predominantly com-

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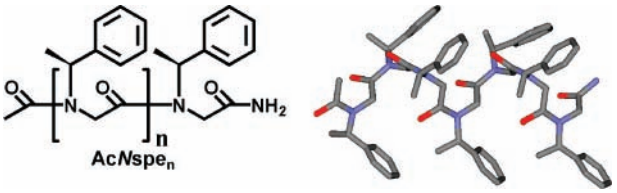
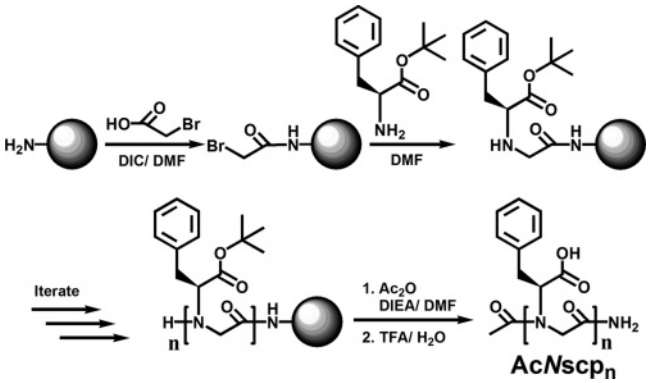


Figure 1. Model structure of previously studied helical peptoid oligomers containing bulky chiral (S)-*N*-(1-phenylethyl)glycine residues.

posed of (*S*)-*N*-(1-phenylethyl)glycine residues are generally insoluble in water. Efforts to enhance the solubility of structured peptoids have relied upon the additional presence of monomers bearing polar side chains.[5a,7] Because these oligomers rely on one set of side chains for structure formation and another set for water solubility, the diversity of structured sequences is limited. Our aim in this study is to demonstrate that α-amino acids can be used to afford water-soluble peptoid secondary structures and provide the constituents for conformational rearrangements that respond to environmental influences such as pH, solvent polarity, and ionic strength.

Peptoid oligomers can be synthesized via efficient solid-phase "submonomer chemistry" methods (Scheme 1) to

Scheme 1. Solid-Phase Submonomer Synthesis of Ac*N*scp$_n$: DIC, Diisopropylcarbodiimide; DMF, *N*,*N*-Dimethylformamide; DIEA, Diisopropylethylamine; TFA, Trifluoroacetic Acid



incorporate specific sequences of chemically diverse N-substituted glycine monomer units.[8] This approach iterates sequential steps of bromoacetylation and nucleophilic displacement to construct each monomer unit. The side chain moieties are introduced upon the displacement of bromide by diverse primary amine submonomer reagents. In this study, we evaluate α-amino acids as readily accessible chiral reagents for the solid-phase synthesis of peptoid oligomers. We use L-phenylalanine *tert*-butyl ester as a submonomer reagent for synthesis of a family of (*S*)-*N*-(1-carboxy-2-phenylethyl)glycine (*N*scp) oligomers (Scheme 1). The carboxy phenylethyl side chains are anticipated to provide both water solubility and structure-inducing elements. These side chains endow the peptoid with a chiral center and steric bulk—two characteristics that have been described as important contributors in directing stable secondary structure formation.[5,9] Many α-amino acids, including phenylalanine, provide both these structural elements and additionally offer carboxylic acid as an ionizable functional group. Foldamer structures that incorporate ionizable groups may display sensitivity toward pH conditions and act as elements that trigger conformational rearrangements driven by electrostatic interactions.

Oligopeptoids used in this study were synthesized on Rink amide resin following the previously reported solid-phase peptoid synthesis protocol (Scheme 1) with adjustments in reaction time and washing conditions.[4,6]

The oligomers were cleaved from the resin with 95% TFA/H_2O. All compounds were purified to >95% homogeneity by reversed phase HPLC (Figure S1). Molecular weights were confirmed by LC-MS and were uniformly in agreement with expected values (Table S1).

The ability of the *N*scp monomers to direct the formation of stable secondary structure was initially evaluated by CD analysis of a series of N-acetylated *N*scp homo-oligomers (Ac*N*scp$_n$) ranging in length from a dimer to a 13-mer (Scheme 1 and Figure 2). The magnitude of change in CD intensity (per mole residue; in 5 mM phosphate and 5 mM citric acid buffer, pH 2/40% acetonitrile) was substantial between dimer to 7-mer. Little to no change in CD signature was observed between 7-mer to 13-mer. Similar length-dependent spectroscopic features have been considered to be a hallmark for the presence of stable secondary structures in synthetic foldamer systems.[10] These results indicate that *N*scp homo-oligomers are capable of adopting stable secondary structures even in the absence of other structure-inducing residues.

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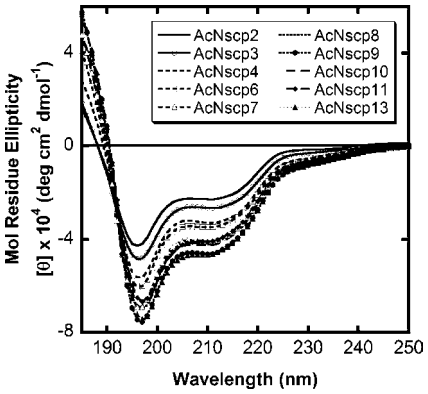

Figure 2. Length-dependent CD spectra of Ac*N*scp$_n$ (0.1 mg/mL; dimer to 13-mer) in 5 mM sodium phosphate and 5 mM citric acid buffer (pH 2)/40% acetonitrile at 25 °C.

Ac*N*scp$_8$ was chosen for more thorough conformational analysis. CD studies (Figure 3a) indicate that Ac*N*scp$_8$ adopts different secondary structures in aqueous and organic conditions. Under neutral pH aqueous condition (5 mM sodium phosphate buffer, pH 7), Ac*N*scp$_8$ exhibits an ellipticity maximum at ca. 190 nm and two minima at ca. 198 and 218 nm. In organic solvent (100% acetonitrile), the CD signature is significantly altered. The CD band intensity at ca. 198 nm is increased by almost 7-fold, and the higher wavelength band is also enhanced. The ratio of these two CD bands is altered from 0.6 (198:218 nm) in aqueous conditions to 1.8 (196:210 nm) for the sample in acetonitrile. The overall magnitude of the CD ellipticity observed in organic solvent ($\theta_{198\ \mathrm{nm}} = -93\,000$ deg cm^2 dmol^{-1}) is among the most intense observed in this family of peptidomimetics.

The results seen in Figure 3a were further analyzed by adding acetonitrile to buffered aqueous sample (59 mM of Ac*N*scp$_8$ in 5 mM sodium phosphate buffer, pH 7). The results (Figure 3b) show a solvent-dependent alteration of the CD spectra with an isodichroic point at ca. 200 nm, indicative of a transition between two defined conformational states. It should be noted that no CD spectral variations are observed when acetonitrile was titrated to an aqueous sample at pH 2 (Figure S2), which suggests that carboxylate anions are required and play a dominant role in the observed conformational rearrangements.

In order to evaluate the electrostatic influence of carboxylate anions on the secondary structure, CD measurements were made at varying ionic strengths at a fixed acetonitrile/H_2O composition and pH. The data (Figure 3c) show that screening charge−charge repulsive interactions leads to enhanced CD signal intensity as the concentration of NaCl is increased. The observed CD signal transition in this experiment resembles the solvent-dependent CD transition (Figure 3b) between 10 and 30% acetonitrile/H_2O at pH 7, suggesting that these processes represent similar conformational transitions. The results provide additional evidence that electrostatic interactions in Ac*N*scp$_8$ play a major role in promoting conformational rearrangements.

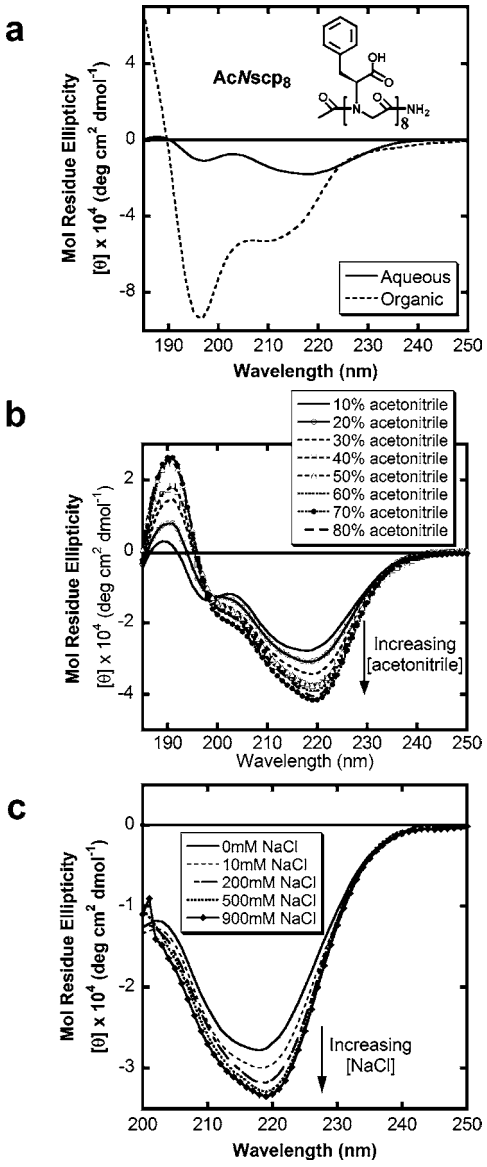

Figure 3. Circular dichroism spectra of (a) Ac*N*scp$_8$ in aqueous (5 mM sodium phosphate buffer, pH 7) and organic solvent (100% acetonitrile) at 25 °C. (b) Ac*N*scp$_8$ in 5 mM sodium phosphate buffer, pH 7, at 25 °C and various % acetonitrile compositions. (c) Ac*N*scp$_8$ in 5 mM sodium phosphate buffer, pH 7/10% acetonitrile at 25 °C and various NaCl concentrations.

In order to further examine the influence of ionizable groups on the secondary structure, the CD spectra of Ac*N*scp$_8$ were evaluated under varying pH conditions (Figure 4). The results show that Ac*N*scp$_8$ undergoes dramatic pH-dependent conformational rearrangements in which two major sets of CD signals are observed. At pH values below 4, Ac*N*scp$_8$ generates intense CD signatures that resemble the CD characteristics seen in 100% acetonitrile (Figure 3a). At pH values greater than 6, the CD signal intensity of Ac*N*scp$_8$ decreases dramatically. The CD spectra seen at this pH range show a maximum at ca. 193 nm and a minimum at ca. 220 nm. The midpoint of the CD transition is observed around pH 4.9, near the pK_a value of carboxylic acids. This suggests

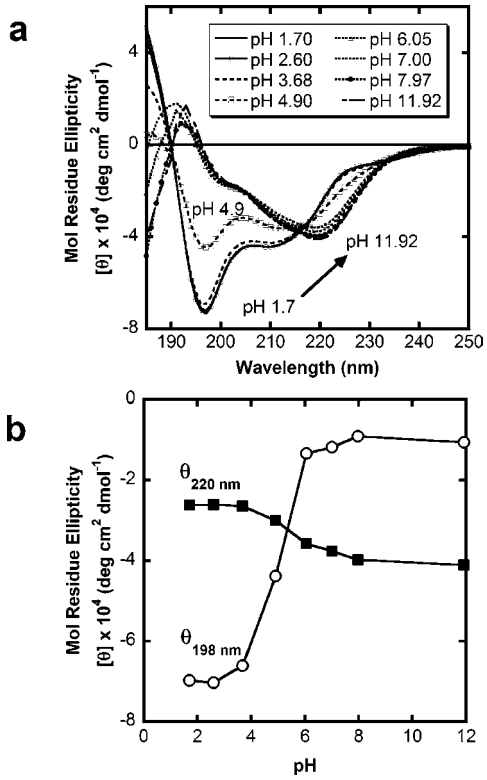

Figure 4. (a) Circular dichroism spectra of Ac*N*scp$_8$ in 5 mM sodium phosphate and 5 mM citric acid buffer/40% acetonitrile at 25 °C and various pH conditions. (b) Per-residue molar ellipticity, θ, versus pH for Ac*N*scp$_8$ in 5 mM sodium phosphate and 5 mM citric acid buffer/40% acetonitrile at 25 °C. Solid squares, $\theta_{220\ nm}$; open circles, $\theta_{198\ nm}$.

that the changes in the CD signals seen in Figure 4a are representative of conformational changes triggered by variations in the ionization state of carboxylic acid side chains. At high pH, Ac*N*scp$_8$ displays multiple carboxylate anions, in which charge−charge repulsive forces are expected to generate more extended secondary structure, especially at low ionic strength and high solvent polarity. At acidic pH conditions, the side chains are neutralized, alleviating electrostatic interactions, thereby allowing more compact secondary structure. In addition, these variations in charge states may also influence the overall conformation by modulating the backbone carbonyl to side chain dipole interactions.[11]

In conclusion, we demonstrate how suitably modified amino acids can readily be used as convenient synthons that allow facile access to a broad range of biochemically relevant side chains. These side chains direct the formation of stable secondary structures and provide pH sensitivity that can trigger conformational rearrangements. Spectroscopic studies will be employed to elucidate the structural features of this new class of peptidomimetic conformational rearrangements. We seek to exploit these initial findings to enable the regulation of functional synthetic foldamers.[12]

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Supporting Information Available: Full experimental procedures and additional spectroscopic data are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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