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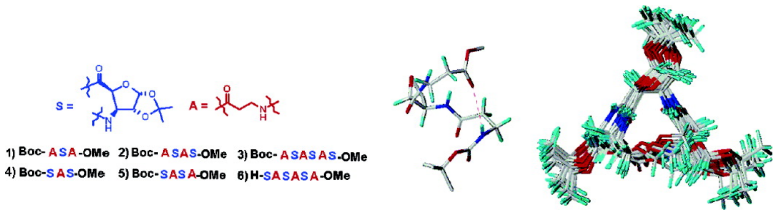

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Expanding the Conformational Pool of *cis*-β-Sugar Amino Acid: Accommodation of β-hGly Motif in Robust 14-Helix[||]

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Complex biological functions of biopolymers are due to their inherent property to adopt specific folded conformations, which brings about the appropriate spatial arrangement of the functional groups. β-Peptides have become the subject of active research since they show distinct folding patterns, including helices, sheets, and turns analogous to those of α-peptides.[1−4] These experimental studies together with the theoretical studies by several groups[5,6] have now made it possible for the synthesis of new β-amino acid monomers with predictable and well-defined secondary templates.

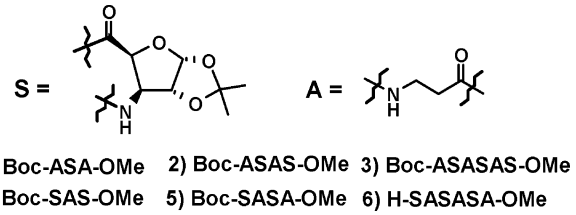
The sugar β-amino acids (SAAs) can be considered as a bridge between carbohydrates and proteins.[7] Our laboratory has initiated research activity on the helix-forming tendency of C-linked SAAs.[8] Our previous work has shown that short oligomers built from a *cis*-furanoid sugar amino acid (*cis*-FSAA) can have a high propensity to form a stable right-handed 14-helix (characterized by a 14-membered hydrogen bond ring $NH_i \rightarrow CO_{i+3}$).[9]

Molecular mechanics calculations carried out in our laboratory using a conformational space search method[10] for the *trans*-FSAA monomer have shown that the angle N−Cβ−Cα−C(=O), θ,[11] between the two vicinal substituents exists in the range of 90−140°, which is in agreement with the theoretically observed angle obtained for *trans*-ACPC.[1] However, for the *cis*-FSAA, θ is restrained in the gauche position and adopts values of −40°, which is in agreement with the value observed in a *cis*-ACPC (θ = ±30°).[12] Due to the pronounced gauche conformer of the *cis*-FSAA motif, the angle θ in β-hGly preferably adopts a conformation (∼60°) usually seen in β^3-substituted amino acids.[13] Kessler's research group[7] has reported that a mixed oligomer containing *trans*-SAA and β-hGly generates a mixed 12/10 helix. Fulop et al. have recently shown that the 14-helical propensity increases with the chain length, and that 10-helical conformation could coexist at shorter chains consisting of three or four residues.[14]

These observations have motivated the synthesis and structural characterization of mixed peptides **1**−**6** (Scheme 1) composed of alternating conformationally rigid *cis*-FSAA and β-hGly motifs, which should preferentially form single well-folded secondary structures. The monomer **S** was synthesized as described previously,[9] and the monomer **A** was obtained either by esterification or by Boc-protection of β-alanine amino acid. The heterooligomers **1**−**6** were synthesized using standard coupling protocols (EDCI/HOBt).[15] The synthesized peptides were characterized by routine spectral analysis.

Structural investigations of the peptides **1**−**6** were carried out by using CD in methanol. The CD spectra of **1**−**3** (Figure 1a) and **6** (Figure 1b) exhibit a distinct secondary structural pattern with a minimum zero crossing and a maximum around 198, 208, and 219 nm, respectively, the characteristic signatures that represent a right-handed 14-helix.[16] Observation of this CD pattern for Boc-ASA trimer **1** also is rather interesting (Figure 1a). On the other hand, its SAS analogue **4** did not exhibit any characteristic secondary structure, whereas the tetramer **5** exhibited predominantly a negative *cotton* effect with a very weak positive signal, a feature that represents a 10-helix.[17] These observations indicate that the Boc-SA series seem to require a minimum of four residues to nucleate the helical conformation and more than five residues for a stabilization of the 14-helix. These studies warrant a detailed inspection of the helix-forming propensities when the choice of N-terminus residue is altered in the short oligomers.

Scheme 1



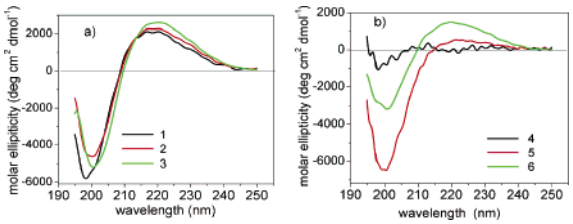


Figure 1. Normalized CD spectra of **1**−**3** (a) and **4**−**6** (b) recorded in MeOH solution.

NMR spectroscopic studies were undertaken to obtain more detailed information on the secondary structures of **1**−**6** in $CDCl_3$ solution. Two-dimensional NMR signal assignments were established using DQF−COSY, TOCSY, and ROESY experiments. The large dispersions of chemical shifts in amide H-atoms indicate the presence of a secondary structure. The data showed an increase in the dispersion of the amide chemical shifts from 1.8 to 2.4 ppm for **1**−**3** and **5**−**6**, with increase in the chain length. In all of these peptides, the observed coupling constant $^3J_{C\alpha H-C\beta H}$ for SAA (<5 Hz) and the two possible couplings for β-hGly (>7.5 Hz and <5 Hz) clearly demonstrate the presence of predominantly a single conformation around Cα−Cβ (θ) ≈ 60° for each residue, a prerequisite for a helix.

Compounds **1**−**3** and **5**−**6** revealed a majority of the possible medium and long-range backbone NOEs between $NH_i \rightarrow C_\beta H_{i+2}$, $NH_i \rightarrow C_\beta H_{i+3}$ and $C_\alpha H_i \rightarrow C_\beta H_{i+3}$, which have been assigned unambiguously and are summarized in Figure 2. However, the remaining few NOEs could not be assigned due to signal overlap.[18]

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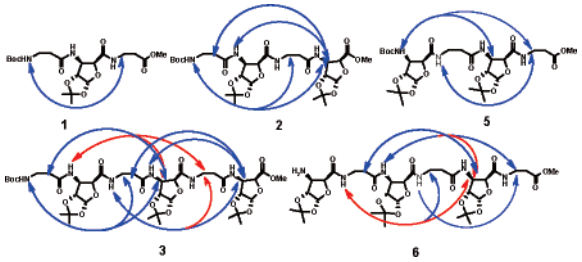

Figure 2. The blue arrowheads represent the observed $NH_i - C_\beta H_{i+2}$, $NH_i - C_\beta H_{i+3}$, and $C_\alpha H_i - C_\beta H_{i+3}$ NOEs for **1**−**3** and **5**−**6**. The overlapped NH−C_βH NOEs are shown by red arrows.

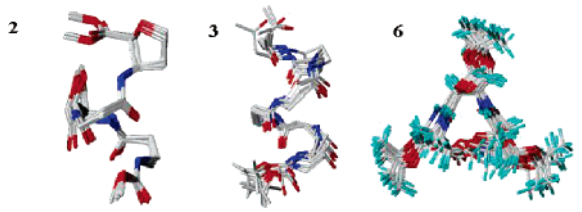

Figure 3. Right-handed 14-helical solution structures of **2**, **3** (side view), and **6** with acetonide groups (top view) obtained from restrained MD simulations.

For the trimer **1**, the only possible inter-residue NOE is between $NH_i \rightarrow C_\beta H_{i+2}$, which has been observed with some overlap. This NOE is characteristic of a 10-helical motif, but the corresponding CD spectrum does not reflect the feature (single *cotton* effect) corresponding to a 10-helix.[14,17] Seebach et al.[17] have shown that the observed *cotton* effect could be attributed to 14-membered ring, instead of a helix. In **2** and **5**, the two possible NOEs between $NH_i \rightarrow C_\beta H_{i+2}$ and one $NH_i \rightarrow C_\beta H_{i+3}$ ($i = 1$) are clearly assigned, and an additional NOE between $C_\alpha H_i - C_b H_{i+3}$ ($i = 1$) is also observed for **2**. These results are suggestive of 14-helical conformation. However, in the case of **5**, the possibility of 10-helical conformation being in equilibrium with a 14-helix[17] cannot be ruled out, as evidenced by the CD spectrum with predominant negative cotton effect. In **3** and **6**, despite the overlap of a few resonances, the 14-helical conformation has been proved unambiguously with the observation of characteristic NOEs.[16]

Formation of $NH_i \rightarrow CO_{i+3}$ hydrogen bonds in **1**−**3** and **5**−**6** peptides has been confirmed by individual titration studies.[19] The chemical shifts of the amide protons involved in hydrogen bonding were almost invariant, indicating strong hydrogen bonding. For all of the peptides studied, the inter-residue hydrogen bonding begins from the NH of the first residue. However, the peptide **4** did not exhibit any characteristic NOE or hydrogen bond to represent a well-defined secondary structure. Furthermore, these observations were substantiated by FT-IR results exhibiting characteristic NH stretching ($\sim$3300 cm^{-1}) and amide-1 ($\sim$1650 cm^{-1}) bands, and prolonged NH/ND exchange (in CD_3OD) investigations, thereby confirming the strength of the inter-residue NH−CO hydrogen bonding and conformational stability.[18]

The distance restraints obtained from the ROESY experiments and the torsion angle restraints derived from the coupling constants were used in restrained molecular dynamics (MD) simulations using a simulated annealing protocol.[20] Figure 3 shows superimposed structures obtained from 10 low-energy conformers for tetramer **2** and hexamers **3** and **6**.

In summary, our structural data indicate that the mixed peptide heterooligomers comprising alternating *cis*-FSAA and β-h Gly **1**−**3** and **5**−**6** form robust right-handed helical secondary structures in solution. The design was based on the premise that the "adaptable rigidifying element" of the five-membered ring of the *cis*-FSAA motif can only attain gauche conformation about N−Cβ−Cα−C(=O), and other conformations are inaccessible. The conformational control that is exerted by the motif can be used to maintain the overall folded conformation and also to modulate the conformational preferences of adjacent residues. Studies have shown that conformationally rigid modified peptides with appropriate functional groups and 14-helical fold have medicinal applications, such as disruption of protein−protein interactions.[21] Work is in progress in this direction.

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Supporting Information Available: Synthesis, CD spectra, FT-IR, NMR analysis, distance constraints used, and Cartesian coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.

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