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Published on Web 10/05/2004

Formation of a Stable 14-Helix in Short Oligomers of Furanoid *cis*-β-Sugar-Amino Acid[||]

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The design and synthesis of new molecular architectures with predictable, well-defined secondary templates is an important area of research. β-Peptides have recently emerged as key leads in the design of such structures because they display an impressive range of structural diversity, including helices, sheets, and turns.[1] The interest in unnatural biopolymers is attracting increasing attention due to the new biocompatible materials that can be made from them. The applications include, among others, self-assembling complexes and lead candidates in drug discovery programs. Research groups of Seebach[2] and Gellman[3] have shown in their pioneering contributions that the oligomers using different side chains at the β^2 or β^3 positions offer the opportunity for the rational design of different types of helical conformations. The conformational space of β-peptides was extensively studied to understand the design principles of the secondary structures.[4]

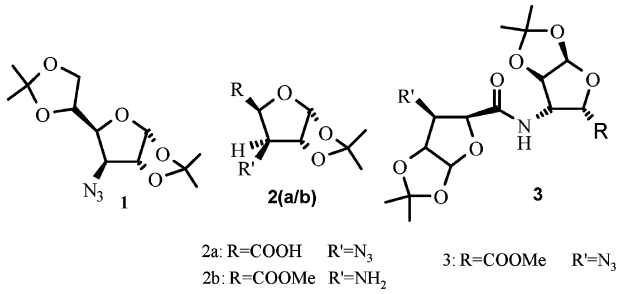
The ongoing research activity in our laboratory focuses on the conformational control over the helix type and symmetry.[5] Gellman's research group[3b] has shown that by incorporating the Cα and Cβ bond into a trans cycloalkane based β-amino acid and by varying the ring size the helix type can be controlled. Five- and six-membered rings stabilize the 12- and 14-helix, respectively.

Kessler's research group[6] has shown that a mixed oligomer containing a furanoid (ribofuranoic acid) *trans*-sugar amino acids (SAA) and a β-Ala generated a mixed 12/10-helix. Here, we show that a choice of xylofuranoic acid over a ribofuranoic acid in a *cis*-SAA can induce the formation of a stable 14-helix in a homooligomer. The molecular mechanics calculations carried out by us on a ribofuranoic acid and a xylofuranoic acid have shown that the angle N−Cβ−Cα−C(=O), designated[7] as θ, takes a value either ∼90° (in a 12-helix) or ∼60° (in a 14-helix). The calculations have encouraged the synthesis and structural characterization of *cis*-SAA homooligomers.

The monomers **2a** and **2b** were synthesized[8] from known azido sugar derivative[9] **1** (Scheme 1) which were subsequently used to prepare dimer **3**, tetramer **4**, hexamer **5**, and octamer **6** (Figure 2) by standard coupling protocol using EDCI and HOBt reagents.[10,11] In the present scheme the azido group was retained until the end of oligomer synthesis, and then it was converted to the NH−Boc.

The circular dichroism (CD) spectroscopy of β-amino acids provides characteristic signatures of helical conformation of various peptides. The CD spectra for **4**−**6** in 200 μM solutions in methanol presented in Figure 1 suggest the adoption of a distinctive secondary structure. Tetramer **4** displays a minimum, zero crossing and a maximum at 198, 209, and 218 nm, respectively, corresponding to the formation of a right-handed 14-helix.[12]

Scheme 1



The increased molecular ellipticity per residue in the CD spectra of **5** and **6** confirm the stabilization of and increase in the population of the 14-helix with the increasing length of the peptide.

It would be instructive to obtain structural insight into the new amino acid in particular regarding the helix-forming nature of the homooligomer. Accordingly, we have studied the conformations of **4**−**6** by NMR investigations, which were supplemented by molecular mechanics and restrained molecular dynamics calculations.

NMR studies were carried out in $CDCl_3$ solution, and the signal assignments were established by two-dimensional DQF-COSY, TOCSY, and ROESY experiments. The dispersion of the chemical shifts of the amide protons indicates the presence of a secondary structure, which increases from 1.06 to 1.33 ppm with increasing number of residues in **4**−**6**. For all these peptides $^3J_{C\alpha H-C\beta H}$ was observed to be <5 Hz which clearly demonstrated the presence of predominantly a single conformation around Cα−Cβ (θ) ≈ 60° for each residue, a prerequisite for a helix[3a]. Furthermore, the calculations of sugar-ring pucker using the refined Karplus equation[13] has resulted in $P \approx 180°$ and $\phi_m \approx 55°$ for all the residues. The ϕ_m value also agrees with the requirement of a 14-helix.

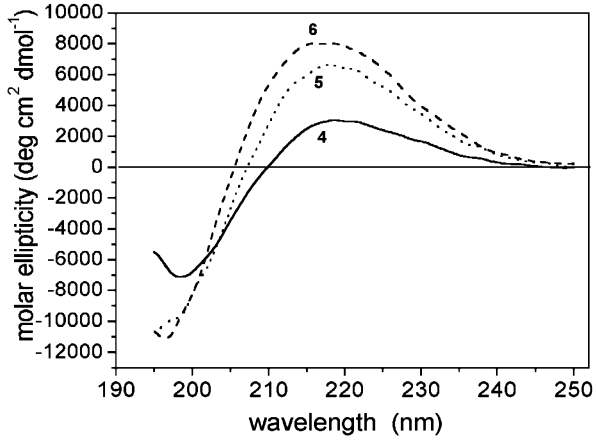


Figure 1. CD spectra of **4**−**6** normalized for amide chromophores.

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[||] IICT Communication No: 040516.

10.1021/ja0467667 CCC: $27.50

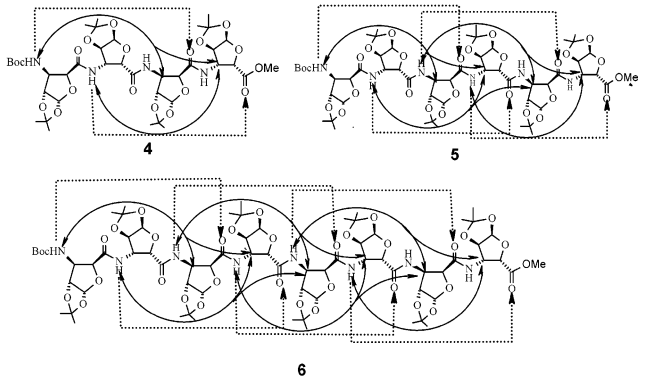

Figure 2. Schematic view of the hydrogen bonding (dashed arrows) and NOEs (dark arrows) of NH_i- -$C_\beta H_{i+2}$ and NH_i- -$C_\beta H_{i+3}$ that characterize the 14-helix.

Large values (8.0−10.8 Hz) of $^3J_{NH-C\beta H}$ in **4**−**6** correspond to an antiperiplanar arrangement between these protons and also indicates the presence of a secondary structure in solution. NOESY data of **4**−**6** revealed several medium and long-range backbone NOEs between $NH_i \rightarrow C_\beta H_{i+2}$ and $NH_i \rightarrow C_\beta H_{i+3}$ (shown in Figure 2), which are distinctive for the 14-helix. For the tetramer **4**, the two possible NOE signals between $NH_i \rightarrow C_\beta H_{i+2}$ are well resolved, while the assignment of $NH_i \rightarrow C_\beta H_{i+3}$ ($i = 1$), NOE signal is obscured due to resonance overlap. Nevertheless, despite the overlap of several resonances, the characteristic NOEs that represent a 14-helix are more pronounced for hexamer **5** and octamer **6**. In the case of **5**, all four expected $NH_i \rightarrow C_\beta H_{i+2}$ NOEs are observed and two out of three $NH_i \rightarrow C_\beta H_{i+3}$ NOEs are assigned without ambiguity. Similarly four out of six $NH_i \rightarrow C_\beta H_{i+2}$ and three out of five $NH_i \rightarrow C_\beta H_{i+3}$ NOEs are clearly distinguished for **6**. Furthermore, formation of 14-membered $NH_i \rightarrow CO_{i+3}$ hydrogen bonds in all the peptides has been confirmed by individual titration studies.[14] Two, four, and six hydrogen bonds are formed in **4**, **5**, and **6**, respectively, which are shown schematically in Figure 2. For all the peptides studied the hydrogen bonds of the 14-helix begin from the first residue. The exceptional stability and organization of the 14-helix observed in tetramer **4** are more pronounced in the hexamer **5** and octamer **6**.

The restrained MD calculations[11] for **4**−**6** very clearly bring out the salient features. The distance restraints were obtained from the ROESY spectra by using the volume integrals and two-spin approximation. Figure 3 depicts the superimposition of the 10 lowest-energy structures of the peptides **4**−**6**. They are representative of the ordered structures in solution. The NMR structures of **4**−**6** show the 14-helix with the pitch of ∼5 Å and three residues per turn. Fraying is seen at the C-terminus end of **4**−**6** consistent with the NMR experiment (decrease in the value of $^3J_{C\alpha H-C\beta H}$).

In summary, this study shows that the furanoid *cis*-*β*-sugar amino acid oligomer adopts in solution a well-defined right-handed 14-helix. Functionalization of the conformationally rigid oligomers with

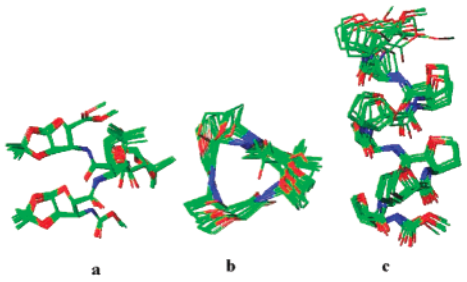

Figure 3. NMR structures of the *cis*-SAA peptides **4**−**6** as a bundle of the 10 lowest-energy structures calculated from restrained MD simulations: (a) tetramer **4**, side view; (b) hexamer **5**, top view from C-terminus; and (c) octamer **6**, side view. For the sake of clarity acetanilide groups in **5** and **6** are not shown.

defined medicinal properties makes these molecules useful in pharmaceutical applications. Work is in progress in this direction.

Acknowledgment. M.S.R. is thankful to CSIR, New Delhi; A.P. and M.H.V.R.R. acknowledge IICT for financial support. This work is dedicated to the memory of Dr. A. K. Singh.

Supporting Information Available: Synthesis, NMR, and distance constraints used for the MD calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0467667