Communication

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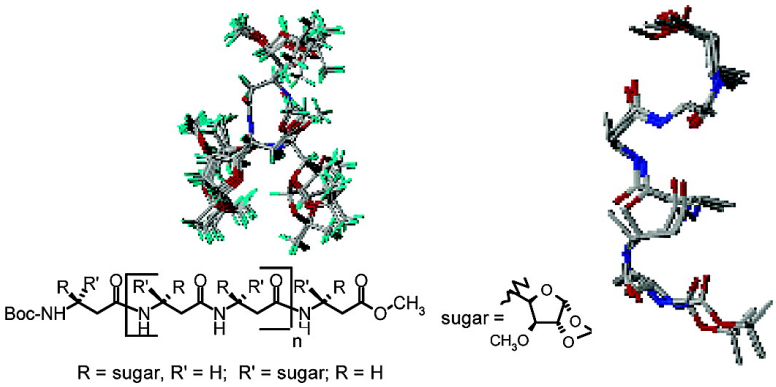

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Robust Mixed 10/12 Helices Promoted by "Alternating Chirality" in a New Family of C-Linked Carbo-β-peptides†

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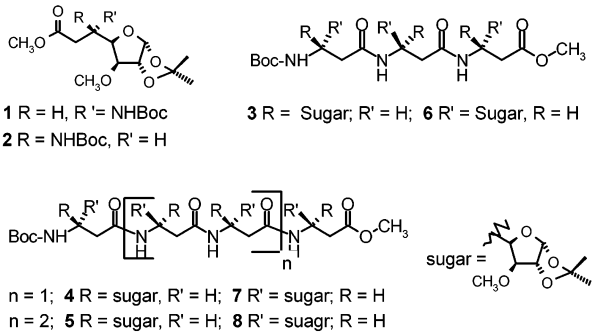
β-Peptides,[1] the oligomers of unnatural β-amino acids, adopt a variety of novel secondary structures. Seebach and Gellman have demonstrated that stable 14-, 12-, and mixed 10/12-helices[2−4] are formed in short (hexa- and tetra-) peptides, from unconstrained acyclic- and constrained cyclic β-amino acids, respectively. Seebach's hexapeptide[4] with alternating β^2- and β^3-substituted residues and Kessler's[5] hetero-oligomer with sugar amino acid-β-alanine, gave novel 12/10/12··· helices. Unlike a wide variety of sugar amino acids,[6] having amine and acid functionalities on sugar scaffold, we[7] and Palomo et al.[8] have designed a new class of C-linked carbohydrate β^3-amino acids, wherein the carbohydrate moieties[9,10] adorn the side chains. This communication reports the design, synthesis, and structural studies of a new class of C-linked carbo-β-peptides with novel helical structures (10/12 and 12/10 helices) in short peptides, using the above monomers.

Our unique design in the present investigation uses "alternating chirality", as predicted by Wu et al.,[11] of the "epimeric" C-linked carbo-β^3-amino acids **1** and **2** as the controlling point in defining stable helices in the C-linked carbo-β-peptides **3**−**8**. The requisite monomer **1** ("S" at amine center)[7] and its epimer **2** ("R" at amine center) were prepared and used in the synthesis of **3**−**8** (Scheme 1), which possess remarkable solubility in organic solvents, by conventional procedures (EDCI, HOBt). Peptides **3**−**5** were prepared starting from **2**, while **6**−**8** were prepared starting from **1**.

Scheme 1



The CD spectra of **3**−**5**, **7**, and **8** in 100 uM solutions in methanol showed characteristics of a mixed helix with maxima at about 203 nm without an isodichroic point. Aggregation for these peptides was ruled out on the basis of the CD and NMR studies at various concentrations for some of the compounds.

The ^{1}H NMR spectrum of **3** showed wide dispersion of amide (2.82 ppm) as well as the CαH chemical shifts (about 0.69 ppm) in $CDCl_3$. The low-field shifts for NH(2) and NH(3) at 7.19 and 8.08 ppm, respectively, indicate their involvement in hydrogen bonds in the tripeptide. Solvent titration studies[12] confirmed their participation in H-bonding as they showed <0.2 ppm change in chemical shifts. $^3J_{C\alpha H-C\beta H}$ >10 Hz and < 5 Hz very clearly demonstrated the presence of predominantly a single conformation around Cα−Cβ (θ). Considering individual $^3J_{C\alpha H-C\beta H}$ and various NH(i+1)/CαH(i) and CαH(i)/CβH(i) nOes, a value of $\theta \approx 60°$ for each residue, a prerequisite for a helix, was confirmed.[1e] Further, the distinctive signature of 12/10 helix was observed in the ROESY spectrum, wherein the strong intense backbone nOes CβH(1)/NH(3) and CβH(1)/CαH (*pro-R*)(3) qualify a 12-membered H bond involving Boc CO−NH(3) (Figure 1). The presence of weak NH(2)/NH(3) nOes justified a 10-membered H bond between NH(2)−CO(3). The helical structure was also supported by large $^3J_{NH-C\beta H}$ (>9 Hz), which corresponds to ϕ values for the 12/10 helix. Some variation in the conformations about CβH−Cβ-C4−C4H (χ1) was indicated from the $^3J_{C\beta H-C4H}$ of 7.3, 9.8, and 9.7 Hz for residues 1−3, respectively. For the first residue, existence of averaging over several conformations, as a result of fraying, may lead to smaller values of $^3J_{C\beta H-C4H}$, while the second and third residues exist predominantly in a single conformation. For these residues $\chi 1 \approx 180°$. The observation of a 12/10 helix in a tripeptide is unprecedented.

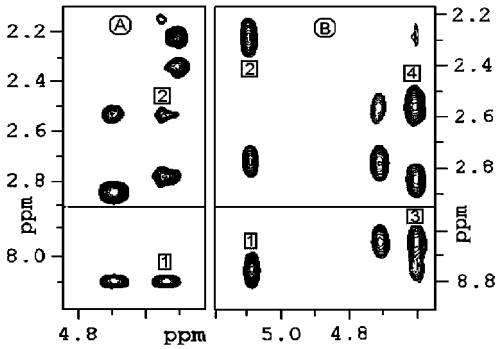


Figure 1. (A) ROESY spectrum of **3**. The nOes CβH(1)/NH(3) and CβH(1)/CαH *(pro-R)*(3) are marked as 1 and 2, respectively. (B) ROESY spectrum of **8**. The nOes CβH(2)/NH(4), CβH(2)/CαH *(pro-R)*(4), CβH(4)/NH(6), and CβH(4)/CαH *(pro-R)*(6) are marked as 1, 2, 3, and 4, respectively.

The exceptional stability and organization observed in **3** was further confirmed in the tetra- and hexapeptides **4** and **5**. Presence of the unique nOe and the couplings, as discussed for **3**, reaffirmed the 12/10 helical structure of **4**, while $^3J_{C\alpha H-C\beta H}$ values indicate that θ for all four residues is about 60°. The nonparticipation of the first and the fourth residue in H-bonding results in the fraying of the structure at the C terminus, which is reflected in somewhat larger values of $^3J_{C\alpha H-C\beta H}$. In the hexapeptide **5** the presence of a 12/10/12/10 helix was evident as all the characteristics discussed above propagate with the chain length. The increased molecular ellipticity per residue in the CD spectra of **3**−**5** confirm the stabilization of the helix with the increasing length of the peptide.

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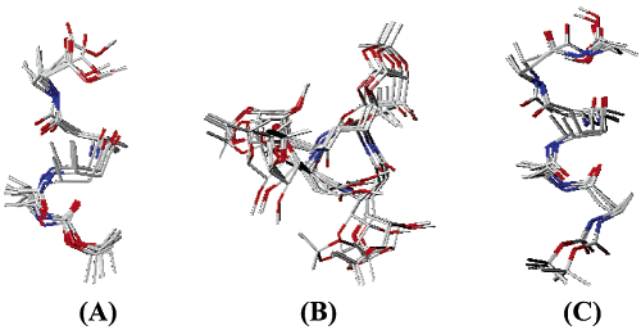

Figure 2. MD structures. (A) Side view of **4**, (B) top view of **4** from C terminus, and (C) side view of **8**.

Unlike those of **3**, NMR and CD spectra of tripeptide **6** showed no helical structure. However, NMR spectra of tetrapeptide **7** very clearly indicated the presence of a stable secondary structure with wide dispersion of amide (2.27 ppm) and CαH chemical shifts (0.70 ppm). The participation of residues 1, 3, and 4 in H bonds was supported by the solvent titration studies as well as the low-field chemical shift (NH(1) = 5.99, NH(3) = 7.12, and NH(4) = 8.23 ppm). ROESY experiments further showed strong CβH(2)/NH(4) and CβH(2)/CαH (*pro-R*)(4) and weak NH(1)/NH(2) and NH(3)/NH(4) nOe peaks. Thus a 10-membered H bond forms at the beginning of the sequence, between NH(1)−CO(2), compared to **3**, resulting in a 10/12/10 helix in **7**, which is observed for the first time. All the couplings and other nOes support formation of a stable and well-defined helix. In **7**, for the second residue, the $^3J_{C\beta H-C4H}$ coupling is only 6.0 Hz, much smaller than the corresponding coupling in other residues. This implies averaging over conformations with predominance of $\chi 1 = -60°$, as supported by nOes between C3H(2)/CαH (*pro-R*)(2) and C3H(2)/CαH (*pro-S*)(2). Similarly in **8**, excluding the second residue, all other amides participate in H-bonding, displaying characteristic nOes (Figure 1) and couplings for a 10/12/10/12/10 helix. These results show that in general the amides of the R residue take part in 12-membered H bonds, while those for the S residue participate in 10-membered H bonds.

The couplings, $^3J_{C1H-C2H} \approx 4$ Hz, $^3J_{C2H-C3H} \approx 0$ Hz, and $^3J_{C3H-C4H} \approx 3$ Hz, for the sugar rings correspond to a sugar pucker of $^3T_2$. Strong nOes between Me (*pro-R*)/C1H and Me (*pro-R*)/C2H as well as weak Me (*pro-S*)/C4H nOes further show the envelope conformation of the isopropylidine ring. These observations are also in confirmity with the structure of the sugar unit in other molecules with C−C linkages.[13]

The restrained MD calculations[14] for **3**−**5**, **7**, and **8** very clearly bring out the salient features of the mixed helices. The distance restraints were obtained from the ROESY spectra by using the volume integrals and two-spin approximation. Figure 2A depicts the superimposition of 20 lowest-energy structures of **4** (sugars are replaced with methyl groups, and protons have been removed for clarity) with maximum nOe violation of 0.11 Å and with average pair wise heavy atom and backbone RMSD values of 0.62 and 0.42 Å, respectively. Figure 2B shows the top view of the structures for **4** from the C-terminal end. For **8**, superimposition of 20 minimum energy structures is shown in Figure 2C (sugars are replaced with methyl groups, and protons have been removed for clarity). The calculations converge well with maximum violation of 0.37 Å and average pair wise heavy atom and backbone RMSD of 0.89 and 0.76 Å, respectively. Analogous behavior was observed for **3**, **5**, and **7**. Apart from the first residue in **3**−**5** and the second residue in **7** and **8**, the C−O bond of the methoxy group in the R residue points along the helix axis toward the C-terminal, while in S residue they point perpendicular to the helix axis.

NMR studies on **3**−**5**, **7**, and **8** in DMSO-d_6 showed that they still retain predominantly mixed helical structures. They depict a considerable amount of weakening of the hydrogen bonds, and averaging of couplings implies contribution from other disordered structures. These results are in conformity with theoretical studies.[11,15,16]

Thus, the present study reports the design and synthesis of a new class of C-linked carbo-β-peptides, whose uniqueness lies in that (a) the monomers are of a new class of C-linked carbo-β^3-amino acids, wherein carbohydrate moieties adorn the side chains, (b) alternating chirality of the epimeric monomers is used to define the helical structures with robust 10/12··· and 12/10··· helices in short (tri- and tetra-) peptides, and (c) the novel 10/12/10··· helical pattern is reported for the first time. An important consequence of having carbohydrate moieties in this new family of peptides is their availability as carbohydrate recognition sites and the hydrophilic free hydroxy groups permitting additional interactions in the design of water-soluble peptides with well-defined secondary structures.

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

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