Formation of left-handed helices in hybrid peptide oligomers with *cis* β-sugar amino acid and L-Ala as building blocks†‡

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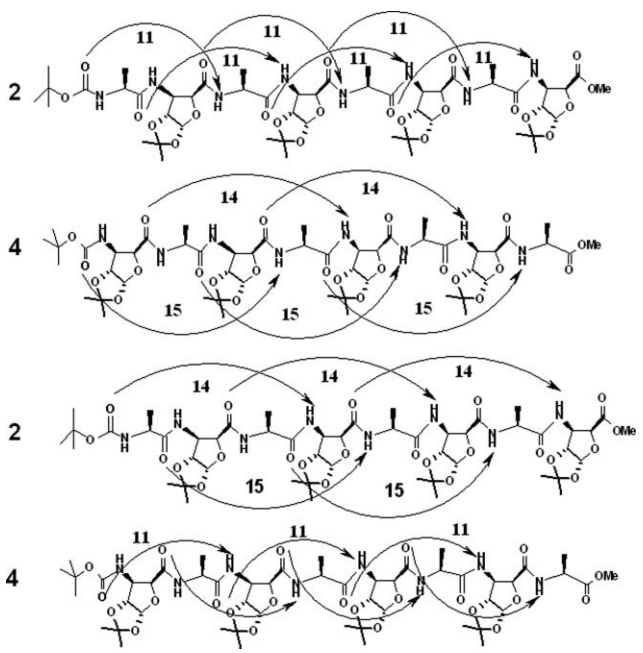
Received (in Cambridge, UK) 22nd August 2006, Accepted 10th November 2006
First published as an Advance Article on the web 7th December 2006
DOI: 10.1039/b612058j

Residue based control of specific helical folding is explored in hybrid peptide oligomers consisting of alternating L-Ala and *cis*-β-furanoid sugar amino acid (FSAA) residues as building blocks; two series of these hybrid oligomers are designed, synthesized and extensively characterized by using NMR, CD, FT-IR and MD simulation studies; results show the co-existence of left-handed 11- and 14/15-helical conformations in these short oligomers of Boc-(α/β) and Boc-(β/α) series.

Design, synthesis and characterization of unnatural peptide oligomers, particularly with β-amino acids, that adopt well-defined secondary structures, 'foldamers' has become an active area of research over the past decade.[1,2] Efforts have been made to gain insight into the folding mechanism of natural biopolymers and to construct novel peptidomimetics that are tolerant to enzymatic degradation. Recently, new classes of hybrid helical foldamers consisting of both natural and unnatural amino acids have gained attention.[3–8] Among them, oligomers with a combination of natural amino acids and cyclic β-amino acids have offered a unique advantage as the rigid cyclic β-residues preorganize the conformational space, while the readily available natural residues allow to design functional foldamers.[1,6,9] Our previous reports have shown that homooligomers of β-amino acids with a five-membered ring, *cis*-β-furanoid sugar amino acids (FSAA),[10] as well as heterooligomers consisting of FSAA and flexible β-Ala at alternate positions,[11] form robust right-handed 14-helices in solution, with a nucleation of helical folding in the trimer, Boc-Ala-FSAA-Ala. Hoffman's research group has predicted that the helical periodicity in hybrid peptides originates at the level of dimer units (αβ).[12] The choice of *cis*-β-FSAA over other β-amino acid residues in the α/β hybrids,[5–8] is expected to exhibit a varied folding propensity and our preliminary molecular mechanics calculations support this possibility.

In the light of earlier reports as well as our own findings, the present work aims at understanding the mechanism of residue based conformational preferences[13] and the role of the N-terminus residue[10,11,14] in the formation of *short* hybrid helices. Here we report a new class of distinctively folded oligopeptides obtained by combining L-Ala and *cis*-β-FSAA residues in two sequentially alternating patterns: Boc-$(\alpha\beta)_2$ (**1**), Boc-$(\alpha\beta)_4$ (**2**), Boc-$(\beta\alpha)_2$ (**3**) and Boc-$(\beta\alpha)_4$ (**4**), The present results show that these peptides adopt 11- and 14/15-helical conformations, that co-exist. It can be highlighted that the present hybrid combination offers left-handed helical folding in contrast to right-handed helices reported earlier. As biomolecules occasionally adopt left-handed helical folds for specific functions, the present work sheds some light on their structural aspects. The heterooligomers **1–4** (Scheme 1) were prepared by conventional coupling methods (see ESI‡).[10,11] They were characterized by using NMR, IR, CD and MD studies.

Information on the preferred conformation of the hybrid oligomers is obtained in structure-supporting solvents ($CDCl_3$, DMSO-d_6 and CD_3OH) by 1D and 2D NMR techniques (see ESI‡). The chemical shifts of amide protons remain constant as the sample concentration is diluted from 10 mM to ~0.5 mM, suggesting the absence of aggregation. Large values (7.0–9.4 Hz) of $^3J_{NH-C\beta H}$ and $^3J_{NH-C\alpha H}$ of *cis*-β-sugar ring and L-Ala, respectively, in **1–4** correspond to an antiperiplanar arrangement between these protons. In all these peptides the observed coupling constant $^3J_{C\alpha H-C\beta H}$ for FSAA (<5 Hz) clearly demonstrates the presence of predominantly *gauche* conformation around Cα–Cβ(θ)[10,11] for



Scheme 1 Possible 11-, 14- and 15-membered hydrogen bonding patterns in hybrid peptides **2** and **4**.

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† IICT communication: no. 050710.
‡ Electronic supplementary information (ESI) available: Synthesis, experimental details and analysis. See DOI: 10.1039/b612058j

each residue and also indicates the presence of a secondary structure in solution.

Gellman and coworkers[5,6] have shown in their theoretical and experimental studies that hybrid oligomers consisting of β-cyclic and α-amino acid residues can adopt only 11, 14/15 and 18 helical foldings. Molecular mechanics calculations were carried out on model helices of **1–4** for three different backbone conformations, adopting 11-, 14/15- and 18-membered C=O–H–N hydrogen bonds, respectively, with N→C directionality. These structures were fine-tuned by constraining the dihedral angles derived from NMR data (see ESI‡). These studies have suggested that the formation of 18-helix is ruled out due to unfavourable inter-residue distances, while the 11- and 14/15-helical foldings are simultaneously possible (Fig. 1). The possible inter-residue backbone NOEs derived from these structures are (see ESI‡): Rβ-$H^{\beta}(i)$/Rβ-NH (i + 2), Rβ- $H^{\alpha}(i)$/Rβ-NH(i + 2), Rα-$H^{\alpha}(i)$/Rα-NH (i + 2), Rβ-$H^{\beta}(i)$/Rα-NH (i + 3), Rβ-$H^{\beta}(i)$/Rα-$H^{\alpha}(i$ + 3), Rα-$H^{\alpha}(i)$/Rβ-NH(i + 3), Rα-$H^{\alpha}(i)$/Rβ-$H^{\alpha}(i$ + 3), Rβ-$H^{\beta}(i)$/Rβ-NH (i + 4), Rα-$H^{\alpha}(i)$/Rα-$H^{\alpha}(i$ + 4), where Rα and Rβ represent α and β residues, respectively. In fact, these NOE connectivities match with those reported for 11 and 14/15 helices composed of α/β hybrids.[5,6,8] The CD studies carried for **1–4** suggest that these oligomers adopt left-handed helical foldings (see ESI‡).

The ^{1}H NMR spectra of peptides **1–4** displayed dispersion of NH protons and downfield shift >7 ppm in $CDCl_3$, which suggests their involvement in hydrogen bonding (see ESI‡). Solvent (DMSO-d_6) titration studies have shown a nominal change (0.2 to 0.58 ppm) in the chemical shift values of NH protons that are participating in intramolecular H-bonding (see ESI‡).[15] The ROESY-^{1}H NMR spectra of **1–4** in $CDCl_3$ exhibited a complete series of inter-residue NH–NH sequential NOE cross-peaks, which are characteristic of a helical structure.[16] These findings are further substantiated by the clear observation of other inter-residue NOEs, *viz*., 3NH–2Hβ, $2H^{\beta}$–$4H^{\delta}$, $1H^{\beta}$–$3H^{\alpha}$, $1H^{\beta}$–$4H^{\gamma}$ for **1**, and 3NH–$1H^{\beta}$ NOE for **3**, which are characteristic for a helical structure (see ESI‡). The other expected NOE cross peaks 4NH–1Hβ, 4NH–1Hα, are overlapped in **3**. On the other hand, a detailed elucidation of secondary structures of octamers **2** and **4** in $CDCl_3$ is hampered due to poor solubility and overlapped Hα and Hβ resonances. Because of these reasons, we have explored the formation of helical structures in DMSO-d_6 for **1–4** and the corresponding structural elucidations are discussed below.

In DMSO, the observed sequential NH–NH NOE cross-peaks (Fig. 2 and 3) for **1–4** indicate that the helical foldings are also sustained in the polar solvent. In **1**, the distinctive inter-residue NOEs, $C^{\alpha}H(1)$–NH(3), $4H^{\delta}$–$1H^{\beta}$, $4H^{\gamma}$–$1H^{\beta}$, 2NH–$1H^{\beta}$, 4NH–$3H^{\beta}$, $3H^{\beta}$–$1H^{\alpha}$, $3H^{\alpha}$–$1H^{\beta}$, CH_3–$2H^{\beta}$, OCH_3–$2H^{\alpha}$, are clearly observed (see ESI‡), confirming the helical structure in DMSO-d_6.

For **2**, out of the three possible backbone CαH(1)–NH(3), CαH(3)–NH(5), CαH(5)–NH(7) NOEs, the former two are clearly observed while the latter is ambiguous. In addition, inter-residue NOEs between 6NH–$5H^{\beta}$, 4NH–$3H^{\beta}$, 2NH–$1H^{\beta}$, OCH_3–$6H^{\beta}$, OCH_3–$7H^{\beta}$, OCH_3–$8H^{\alpha}$ are also clearly observed, thereby supporting the derived structure. The other expected NOEs, $4H^{\delta}$–$1H^{\beta}$, $4H^{\gamma}$–$1H^{\beta}$, $1H^{\beta}$–3NH and $3H^{\beta}$–$6H^{\gamma}$ are ambiguous because of overlap. For **3**, along with sequential NH–NH NOEs,

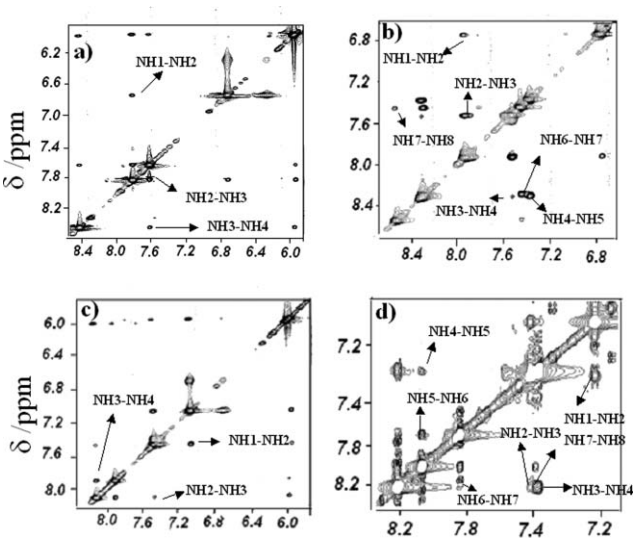

Fig. 2 Sequential NH–NH NOEs (a)–(d) for **1–4**, respectively; NH5–NH6 cross-peaks are overlapped in **2**.

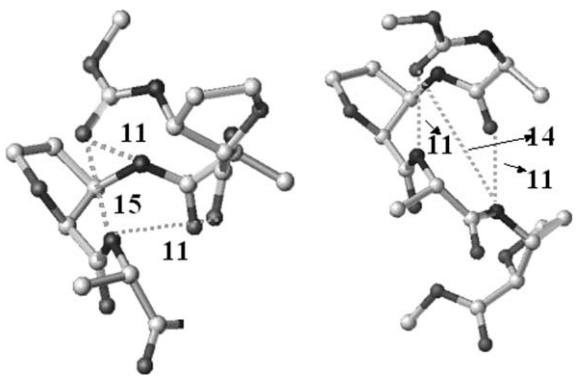

Fig. 1 Energy minimized structures of **1** and **3**, showing the possible 11-, 14- and 15-membered H-bonded turns.

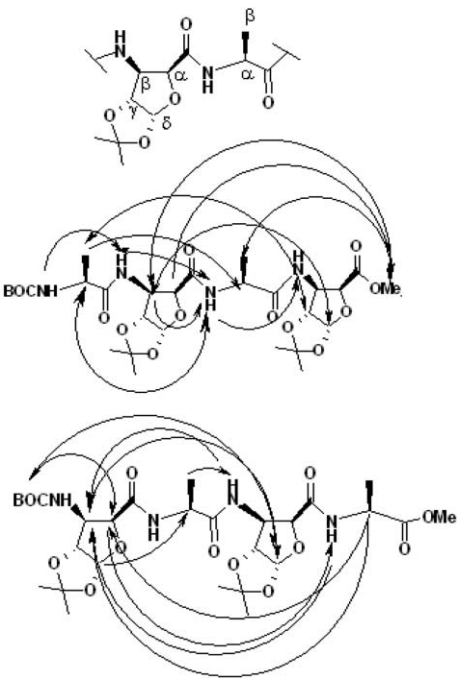

Fig. 3 Sequential NH–NH NOE cross-peak connectivities for **1–4**.

$1H^{\alpha}$–4NH. 2NH–$1H^{\beta}$, 3NH–$2H^{\beta}$, $2H^{\beta}$–1NH, 1Hβ–$3H^{\delta}$ NOEs are also clearly observed, which confirm the helical structure.

In octamer **4**, out of three possible backbone NOEs, 4NH–$1H^{\alpha}$, 6NH–$3H^{\alpha}$ and 8NH–$5H^{\alpha}$, the latter has been observed clearly whereas the former two are observed with some overlap. Out of three other expected NOEs, $1H^{\beta}$–$3H^{\delta}$, $3H^{\beta}$–$5H^{\delta}$ and $5H^{\beta}$–$7H^{\delta}$, the former is clearly observed.

As described above, in principle, these oligomers can exhibit both 11 and 14/15 helical conformations simultaneously and the observed NOEs are also consistent with this. However, it would be interesting to evaluate whether these two series, Boc-(α/β) and Boc-(β/α) adopt any preferential conformation over the other, particularly in tetramers **1** and **3**, as it was the case with the Boc-FSAA-βAla series.[11] In the case of **1**, two 11-helical turns involving third and fourth NH groups, and one 14-helical turn that involves the fourth NH group, are possible (Fig. 1). The relatively strong temperature coefficient for the third NH (-1.6 ppb K^{-1}) indicates that probably the 11-helical turn is predominant over the 14-helical turn in **1**. The observed sequential NH–NH and $1H^{\alpha}$–3NH NOE also supports this possibility. On the other hand, in **3**, two 11-helical turns involving third and fourth NHs and one 15-helical turn involving fourth NH, are possible. The observed temperature coefficient for the fourth NH (-4.0 ppb K^{-1}) is relatively stronger than that of the third NH (-6.8 ppb K^{-1}), which allowed us to speculate that the 15-helical turn could be predominant in **3**. The subtle conformational differences in these two tetramers could be due to the relatively rigid Boc-FSAA being the first residue in **3**. Such differences were found to be more significant for tri- and tetrapeptide oligomers of FSAA and β-Ala residues.[11] However, for octamers, it was not possible to distinguish any preferred conformation among the allowed 15/14/15/14/15 (for **2**), 14/15/14/15 (for **4**) and 11 helices. These observations are also consistent with the previous studies.[6]

The solution IR spectra (at 2 mM concentration in chloroform) of **1–4** exhibit characteristic NH-stretching ($\sim$3300 cm^{-1}) and amide-1 ($\sim$1670 cm^{-1}) bands thereby confirming the inter-residue NH–CO hydrogen bonding (see ESI‡). The observed increase in the intensity of characteristic peaks with the chain length, suggest a progressive increase in the strength of the H-bonding[11] Furthermore, it has been observed that the NH/ND exchange is not rapid in CD_3OD solvent, thereby suggesting a prolonged stability of secondary structures of **1–4** in this solvent (see ESI‡).

The distance constraints obtained from the ROESY experiments and the torsion angle restraints derived from the measured coupling constants, are used in molecular dynamics (MD) simulations by using Sybyl software. The superposition of several energy-minimized structures (see ESI‡) have shown a well-defined backbone that corresponds to left-handed helical conformations (Fig. 4).

In summary, the present work describes that the hybrid tetramers and octamers consisting of alternating *cis*-β-FSAA and L-Ala monomers adopt left-handed 11- and 14/15-helical conformations that co-exist in these oligomers. In tetramers, the two conformational preferences of the backbone seem to be modulated by altering the N-terminus residue. By replacing β-Ala with L-Ala in right handed 14-helical foldamers reported earlier, the handedness of the helix in the present series has switched from right to left, which is an interesting aspect in designing functional foldamers. Further studies on understanding the mechanism for specific folding preferences are underway.

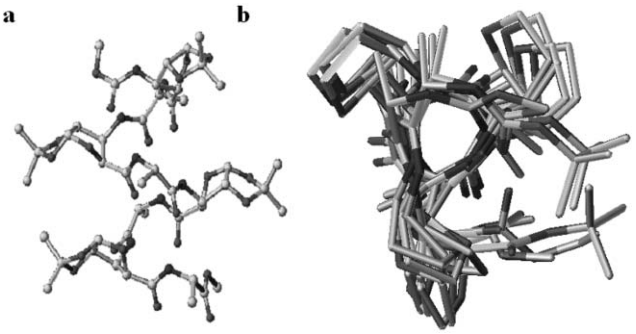

Fig. 4 MD simulated left-handed helical structures of octamers **4** (a, side view) and **2** (b, top view). For the sake of clarity only the average structure is shown for the side view (a).

A. P., G. D. S, G. C. S. and M. S. R are thankful to CSIR, India for fellowships.

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