Self-assembly of cyclic homo- and hetero-β-peptides with *cis*- furanoid sugar amino acid and β-hGly as building blocks†‡

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The design, synthesis and characterization of a new class of peptide nanotubes, self-assembled from cyclic homo- and hetero-β-peptides based on *cis*-furanoid sugar amino acid and β-hGly residues are described; these results represent the expansion of the conformational pool of *cis* β-sugar amino acids in the design of peptide nanotubes.

The self-assembly of peptide building blocks into nanotubular objects mediated by noncovalent interactions has become an important area of research in supramolecular chemistry,[1] due to their tremendous potential in biomedical and material sciences.[2] The seminal works of the groups of Ghadiri[3] and Seebach[4] have stimulated interest in design and synthesis of a expanding class of motifs which form predictable peptide nanotubes (PNTs) whose surfaces can be functionalized.[5–12] Homo- and hetero-oligomers based on constrained β-amino acid residues fold into well-defined secondary structures.[13,14] Sugar amino acids (SAAs), which are basically hybrids of carbohydrates and amino acids, have attracted special attention as they serve as rigid scaffolds for building novel secondary structures known as foldamers.[15] Recently Fujimura *et al.* have reported the columnar assembly of cyclic peptides based on six-membered *trans* β-SAAs.[16]

We have reported that short homo-oligomers composed of *cis*-β-furanoid sugar amino acids (FSAA) adopt a robust right-handed 14-helix that exhibit β-type torsion angles.[17a] Subsequently, we have also shown that hetero-oligomers built from FSAA and flexible β-hGly also adopt a stable 14-helix thereby revealing the conformational control of the FSAA.[17b] These results have highlighted that amide groups are oriented along the helix axis and three β-residues, Boc-hGly-FSAA-hGly are sufficient to nucleate the 14-helix. A logical extension of these architectures would be the design of peptidic nano-rings based on β-SAAs and their self-assembly into "nanotubes". In this communication, we present the design, synthesis and characterization of a new class of tubular structures based on cyclic homo-β-tripeptide **1** that contains repeating units of FSAA and the cyclic hetero-β-tetrapeptide **2** that contains alternating FSAA and β-hGly residues. Molecular modeling studies are carried out on **1** and **2**, using a conformational space search method CONFLEX.[18] The results suggest that the backbones of cyclic β-peptides **1** and **2** adopt flat-ring shaped conformation with C_3 and C_2 symmetry, respectively, consisting of oppositely faced NH and C=O groups lying perpendicular to the ring. The dihedral angles φ, θ and ψ (convention of Balaram[19]) were also found to be in the range usually observed for the β-sheet region of the Ramachndran map. This molecular arrangement is expected to favor self-assembly by intermolecular hydrogen bonding that leads to the formation of tubular structures. Fig. 1 schematically outlines our approach.

The cyclic peptides **1** and **2** were synthesized starting from the corresponding monomers 3-azido-3-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranoic acid and β-alanine derivative using reported procedures (ESI‡). The purified peptides **1** (11.1 mg) and **2** (4.0 mg) were dissolved separately in a mixture of $CDCl_3$–CCl_4 in 2 : 3 ratio (1 ml). The solution turned cloudy and this was used for further analysis by nuclear magnetic resonance spectroscopy (NMR), Fourier transform infrared spectroscopy (FT-IR), electrospray ionization mass spectrometry (ESI-MS), transmission electron microscopy (TEM), scanning electron microscopy (SEM) and differential interference contrast (DIC) microscopy. ^{1}H NMR spectroscopic studies were also carried out in DMSO-d_6 and $CDCl_3$ solutions to obtain detailed information on the structures of **1** and **2**.

In the highly polar solvent DMSO, these cyclic peptides do not form intermolecular hydrogen bonding and the NMR spectra correspond to only isolated monomeric units. In DMSO, the NMR spectra (ESI‡) showed sharp and well-resolved signals, which are consistent with the predominant conformations of C_3 and C_2 point-group symmetries for **1** and **2** (Fig. 2(a)), respectively. The observed coupling constant $J_{NH,\beta H}$ is >8.5 Hz for FSAA, a value typical for the peptides that adopt an all-*trans* conformation with a flat ring-shaped backbone.[3] From the parameterized Karplus equation,[20] the corresponding ϕ angles were calculated

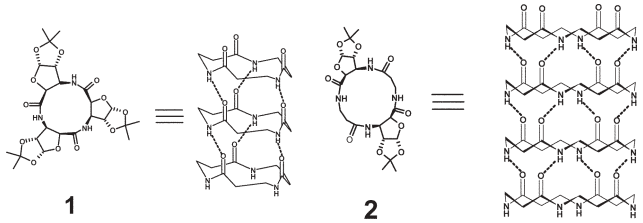

Fig. 1 Schematic representation of **1** and **2** and their supramolecular stacking mediated by intermolecular hydrogen bonding, shown by dotted lines. For the sake of clarity only the backbone is shown.

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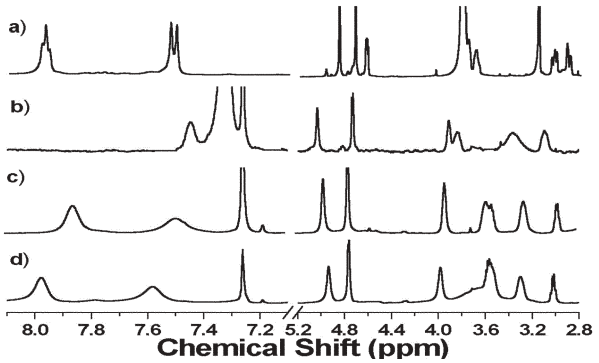

Fig. 2 Selected region of ^{1}H NMR spectrum of **2** (a) in DMSO, (b) 0.5 mM, (c) 10 mM and (d) 20 mM are in 2 : 3 $CDCl_3$–CCl_4.

to be 120–140°. The observed $J_{C\alpha H,C\beta H}$ coupling constants, <4 Hz for the FSAA and β-hGly residues and an additional $J_{C\alpha H,C\beta H}$ coupling of 8.7 Hz for β-hGly, have clearly demonstrated the presence of a *gauche* conformation around Cα–Cβ ($\theta \approx 60°$) in **1** and **2**. Based on these findings and molecular mechanics calculations, a most plausible conformation is with all C=O and NH groups lying opposite sides of the ring. The measured *J* values for **1** and **2** are nearly identical to those observed in the 14-helix, in which the C=O and N–H groups orient along the helix axis. These findings suggest that the cyclic β-peptides **1** and **2** are conceptually related to the 14-helical conformation obtained for the linear analogues of **1** and **2** studied previously.[17]

In the non-polar solvent 2 : 3 $CDCl_3$–CCl_4, the NH resonances displayed downfield shifts upon increasing the concentration (Fig. 2). These features are characteristic of intermolecular hydrogen bonding.[10,12] The self-assembly in peptides **1** and **2** was also evident by ESI-MS studied over the range up to 1700 Da (ESI‡). For **1**, monomeric $[\mathbf{1} + H]^+$, $[\mathbf{1} + Na]^+$, dimeric $[\mathbf{1}\cdot\mathbf{1} + Na + 1]^+$ and trimeric $[\mathbf{1}\cdot\mathbf{1}\cdot\mathbf{1} + Na + 1]^+$ were observed at 556, 578, 1134 and 1689 Da, respectively. For **2** monomeric $[\mathbf{2} + H]^+$, $[\mathbf{2} + Na]^+$ and dimeric $[\mathbf{2}\cdot\mathbf{2} + Na + 1]^+$ were observed at 513, 535, 1048 Da, respectively.

FT-IR studies of films deposited on KBr pellets as well as in solution have furnished substantial evidence for the self-assembly of peptides by β-sheet like hydrogen bonding (ESI‡). The observed N–H stretching frequencies of 3272 and 3283 cm^{-1} for **1** and **2**, respectively indicate tight intermolecular hydrogen bonding (ESI‡).[3] Following Krimm's correlation[21] for estimating hydrogen bond distances, the observed N–H stretching frequencies suggest a distance of about 4.7 Å between the stacked peptide rings. The amide-I frequencies offer probable conformational features of the backbone. The observed amide-IA bands for **1** and **2** at 1636 and 1639 cm^{-1}, respectively and the amide-II bands at 1550 cm^{-1}, are characteristic for nanotubes formed from β-sheet-like networks.[5a] The amide-IB bands have appeared at 1658 and 1672 cm^{-1} for **1** and **2**, respectively. The absence of the characteristic amide-IB band (1680–1695 cm^{-1}) corresponding to the antiparallel β-sheets[5b] in **1** and **2**, suggests tightly hydrogen bonded parallel β-sheet structures in these compounds.

The morphologies of the self-assembled peptides **1** and **2** were analyzed by transmission electron microscopy (TEM), scanning electron microscopy (SEM) (Fig. 3) and differential interference contrast (DIC) microscopy (ESI‡). The typical SEM and TEM images of **1** and **2** show rod-like assemblies whose diameters are in

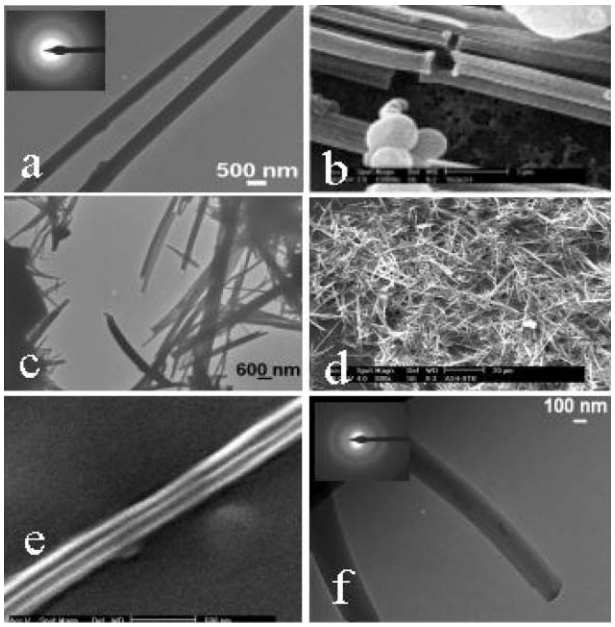

Fig. 3 TEM images of the rod-like assemblies of **1** (a) and **2** (c) and (f). The SEM images are of **1** (b), **1** (e) and **2** (d). The insets of (a) and (f) show the corresponding selected area electron diffraction (SAED) pattern recorded from the rod–like assemblies.

range of 100–500 nm and their lengths are over several microns. The surface of these assemblies appears smooth and exhibit uniformity all through the length. These observations indicate that the rods are made up of hundreds of tightly packed PNTs aligned in one direction. The selected area electron diffraction (SAED) patterns of **1** and **2** indicate that these self-assembled structures possess microcrystallinity (see insets to Fig. 3(a) and (f)). Though the actual mechanism is not known, Dory and co-workers have proposed a "hierarchical" process[11b] that involves an assembly of individual PNTs and over several generations to finally form "needle-shaped crystals". The forces associated with this process is speculated to be due to inter-tube interactions, which are non-covalent in nature, such as hydrophobic, van der Waals, and electrostatic interactions involving dipoles.

The relative orientation of the peptide rings in the peptide tube has been studied for **2** by using NMR spectroscopy. The hybrid cyclic peptide **2** has two faces, with NH and C=O groups of the sugar amino acid (FSAA face) and NH and C=O groups of the β-hGly amino acid (β-hGly face). The peptide rings can, in principle, vertically stack by hydrogen bonding either with the faces of FSAA-FSAA aligned (inter-subunit homo-stacking) or with the faces of FSAA-βhGly aligned (inter-subunit hetero-stacking). In order to elucidate the probable alignment, NOE studies were carried out for the monomer unit of **2** in DMSO, and for the corresponding aggregated units in a $CDCl_3$–CCl_4 mixture. These studies infer that the self-assembly favours FSAA-FSAA and the βhGly-βhGly inter-subunit homo-stacking as shown in Fig. 4 (see ESI‡ for further details).

In order to explore the other conformations in the cyclic peptide stacking, we have collected the ^{1}H NMR spectrum at a lower temperature, 253 K. The NH proton resonances have marginally shifted downfield, suggesting further strengthening of the hydrogen bonding. However, the data did not exhibit any additional sets of

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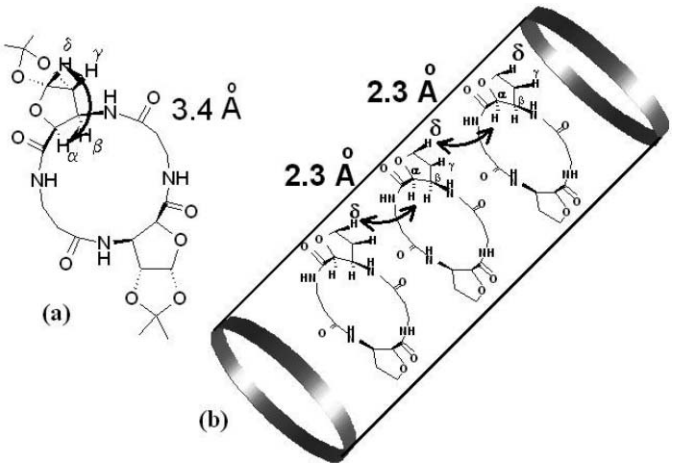

Fig. 4 (a) Schematic diagram of the observed NOE connectivities between Hα–Hδ protons in an isolated monomer unit of **2**; (b) Cartoon representation showing a closer proximity (~2.3 Å) of inter Hα–Hδ protons of the vertically stacked sugar rings in the tubular assembly, compared to that in the isolated unit. Acetonides are not shown for the sake of clarity (see ESI‡ for further details).

signals, thereby suggesting no observable inequivalence on the time-scales of NMR. This is expected because of the highly rigid nature of the *cis*-FSAA motif,[17] which promotes a uniform C_2 conformation of the planar β-sheet.

In summary, we have reported the design, synthesis and characterization of new class of cyclic β-peptide tubular assemblies consisting of *cis*-β-furanoid sugar amino acids and β-hGly residues on the backbone. NMR, ESI-MS, FT-IR, DIC, TEM and SEM techniques have been employed. The results have shown that the cyclic tripeptide **1** and tetrapeptide **2** adopt a parallel β-sheet arrangement with inter-subunit homostacking, linked by three and four hydrogen bonds, respectively. These results revealed that FSAA-based short oligomers that exhibit a 14-helix can be cyclized to yield flat nano-rings that are suitable for intermolecular hydrogen bonding. TEM and SEM images showed 'needle-shaped' assemblies, which are microcrystalline in nature. Further pursuits are to fabricate functionalized supramolecular assemblies by introduction of a wide variety of side chains. Work is in progress to this end.

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