Novel helical foldamers: organized heterogeneous backbone folding in 1 : 1 α/nucleoside-derived-β-amino acid sequences†‡

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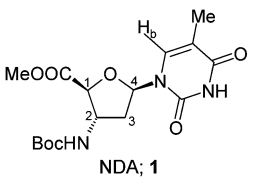
Received 3rd June 2010, Accepted 29th July 2010
DOI: 10.1039/c0cc01724h

Secondary structural conformation of hybrid oligo-peptides comprised of 1 : 1 alternating Nucleoside Derived β-Amino acid (NDA) and L-amino acid residues has been reported. The studies reveal that the NDA residues organize the heterogeneous backbone featuring the surface properties of both nucleic acids and peptides, to adopt a novel 11/8-helical fold.

Hybrid peptides[1,2] comprised of natural α and unnatural cyclic β-amino acids, particularly in 1 : 1, 1 : 2 and 2 : 1 ratios have emerged as new generation foldamers.[3–7] Based on the choice of β-amino acid monomer and α/β ratio, the relative positions or orientations of the α-amino acid side-chains can be controlled so as to access higher order folds[8,9] and specific molecular recognitions, inluding protein inhibition.[10–13] These reports have highlighted the scope for developing new α/β-hybrid templates and the importance of obtaining information about their conformational preferences. The present work focuses in this direction.

Diederichsen's research group has shown residue based conformational control in hetero-oligomers comprised of cyclic-*trans*-β-ACHC (14-helix promoting motif) and linear β^3-amino acids with nucleobase side-chains. These oligomers preferentially adopt 14-helix[14] and form self-assembled complexes through inter-helical complementary base-pairing.[15] Recently, our group[16] and Threlfall *et al.*[17] have independently reported Nucleoside Derived β-Amino acid (NDA), **1**, which is a novel cyclic-*trans*-β-amino acid with nucleobase as a side-chain. In a logical extension, developing hybrid peptide oligomers based on a α/NDA combination, where NDA provides the conformational control, would enhance the realm of the function oriented molecules as they carry the surface properties of natural peptides as well as nucleic acids. Gogoi and co-workers have shown that the 1 : 1 α/NDA peptides exhibit binding affinity for DNA/RNA strands.[18] However, it is important to know the conformational preferences and spatial organization of this novel class of molecules for subsequent design and development, which hitherto have not been explored. Herein we report the formation of 11/8 helical folding in short oligomers **2–5** (trimer to hexamer) comprised of 1 : 1 alternating NDA and L-amino acid monomers. Essentially in these heterogeneous backbone structures, the 4-atom achiral phosphodiester linker in a nucleic acid chain is replaced by a 5-atom linker consisting of a chiral amino acid, flanked by two peptide bonds.

The compounds **2–5** (Fig. 1) have been synthesized from NDA (**1**) and L-amino acids (Ala, Phe and Val) by using standard coupling protocols and have been characterized by using extensive NMR studies, restrained MD simulations and DFT calculations.‡ ^{1}H chemical shift assignment has been carried out by using DQF-COSY, TOCSY and ROESY experimental data. NH proton chemical shifts are found to be nearly invariant with the increase of sample concentration varied up to 10 mM,‡ suggesting the absence of inter-molecular aggregation. Sample concetration of ~5 mM has been used for NMR studies of compounds **2–5**. Trimer **2** has been analyzed in pure $CDCl_3$ solvent at 300 K. Complete exchange of thymidine base NHs and Boc-NH with residual water present in $CDCl_3$ has suggested that they are freely accessible by the solvent and not participating in any hydrogen bonding. The dispersion and down field shifts of the 2NH and 3NH is consistent with their involvement in hydrogen bonding. $^3J_{H1,H2}$ coupling constants of 0–2 Hz for NDA residues in **2** indicate dihedral angles θ of ~90°–110°. Detailed analysis of ROESY spectra of **2** has shown a long range ^iNH_n–$^{(i-2)}H2_n$ (i = 3) ROE cross-peak, which is characteristic of a turn that favours a 11-membered hydrogen bonding between ^iNH_n–$^{(i-3)}CO_\alpha$ (Fig. 2a).‡ The subscripts 'n' and 'α' represent NDA and L-amino acid/Boc residues, respectively, and the superscript 'i' represents the residue number. This folding is further strongly supported by unambiguous ^iNH_n–$^{(i-2)}H1_n$, ^iH1_n–$^{(i-2)}H2_n$ and ^iH4_n–$^{(i-2)}H2_n$ (i = 3) ROEs (for detailed ROESY spectra, please see ESI‡). Only one such 11-membered H-bonding is possible in **2** involving 3NH–(Boc)CO. The DMSO-titration studies‡ have suggested that in addition to the 3NH, the 2NH also participates in



Boc-NDA-Ala-NDA-OMe; **2**
Boc-Phe-NDA-Ala-NDA-OMe; **3**
Boc-NDA-Ala-NDA-Phe-OMe; **4**
Boc-NDA-Ala-NDA-Phe-NDA-Val-OMe; **5**

Fig. 1 A schematic representation of Nucleoside Derived Amino acid, NDA and the hetero-oligomers synthesized from NDA and L-amino acids.

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† The work has been dedicated to Dr J. S. Yadav on his 60th birthday.
‡ Electronic supplementary information (ESI) available: Synthesis, NMR Experimental, MD and DFT data. See DOI: 10.1039/c0cc01724h

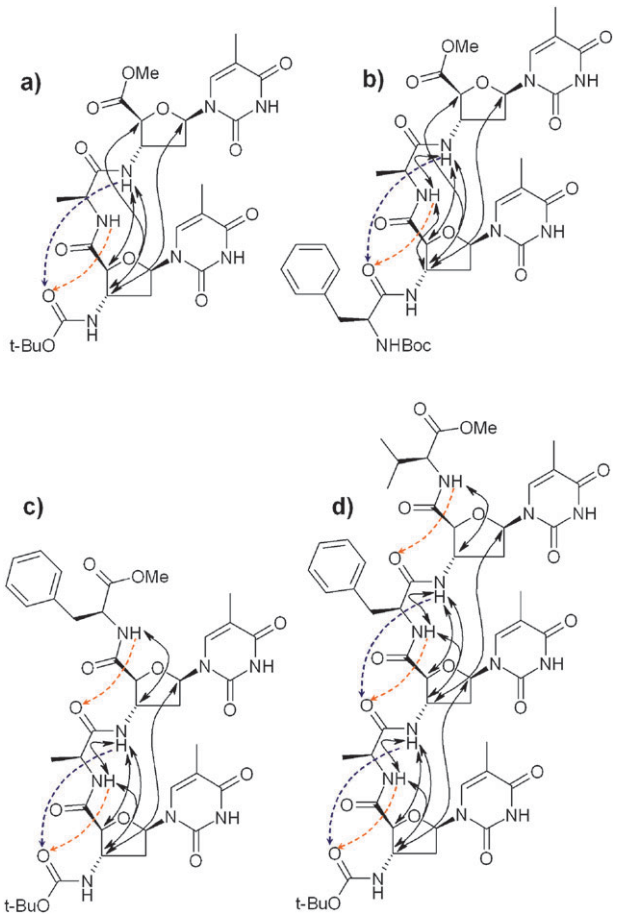

Fig. 2 Schematic representations of observed ROEs (black solid arrows) that characterize the turns with 11/8 hydrogen bonding for **2** (a), **3** (b), **4** (c) and **5** (d). The 11-member and 8-member hydrogen bonds are shown in dotted blue and orange lines, respectively.

hydrogen bonding of moderate strength, which is likely an 8-membered hydrogen bonding with the same (Boc)CO. As shown later, the ROE and $^{3}J_{H}$ restrained MD simulations‡ have also supported the formation of 11/8-helical fold, possibly with a rapid inter-conversion of 11 and 8-membered hydrogen bonding on the time-scales of NMR.

Two different tetramers: **3** and **4**, with L-amino acid (Phe) and NDA at N-terminus, respectively, were characterized further. NMR spectroscopic studies of **3** and **4** have been carried out in $CDCl_3$ and a mixture $CDCl_3$ (500 mL)/DMSO (10 mL), respectively. The 2NH resonance in **3** is very broad and is not useful for accurate estimates of ROEs. Nevertheless, **3** and **4** have exhibited characteristic $^{i}NH_{n}{-}^{(i-2)}H2_{n}$, $^{i}NH_{n}{-}^{(i-2)}H1_{n}$ and $^{i}H4_{n}{-}^{(i-2)}H2_{n}$ long range ROE patterns‡ (from fourth NDA residue to the second NDA residue and from third NDA residue to the first NDA residue, respectively, Fig. 2b and c) similar to that observed in trimer **2**. These findings are consistent with the formation of 11-membered $^{i}NH_{n}{-}^{(i-3)}CO_{\alpha}$ hydrogen bonded turns involving 4NH and (Phe)CO in **3**; and 3NH and (Boc)CO in **4**. Furthermore, the $^{i}NH_{\alpha}{-}^{(i+1)}NH_{n}$, $^{i}NH_{\alpha}{-}^{(i-1)}H1_{n}$ and $^{i}NH_{\alpha}{-}^{(i-1)}H2_{n}$ ROEs observed for the 3NH (Ala) in **3** and 2NH (Ala) in **4** (Fig. 2b and c)‡ support a closer proximity between $^{i}NH_{\alpha}$ and $^{(i-2)}CO_{\alpha}$, which implicates this carbonyl oxygen in an

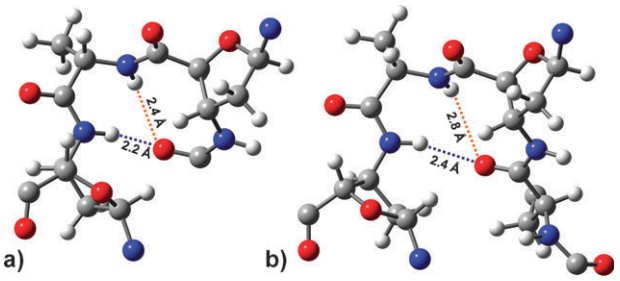

Fig. 3 Minimum energy structures obtained from DFT calculations on (a) trimer **2** and (b) tetramer **3** show the possible 11/8 hydrogen bonded turns. The 11-member and 8-member hydrogen bonds are shown in dotted blue and orange lines, respectively.

additional 8-membered H-bonding with $^{i}NH_{\alpha}$, leading to $[^{i}NH_{n}{-}^{(i-3)}CO_{\alpha}]/[^{i}NH_{\alpha}{-}^{(i-2)}CO_{\alpha}]$ 11/8 H-bonds in equilibrium. In **4**, the later two ROEs are found for 4NH also, which, however, support only a 4NH–2CO 8-membered H-bonded turn as the chain length of the tetramer limits any further possibility of a 11-membered H-bond for 2CO. The $^{i}H4_{n}{-}^{(i-2)}H2_{n}$ and $^{i}H1_{n}{-}^{(i-2)}H2_{n}$ ROEs observed for the oligomers **2–4** further portray the overlying arrangement of consecutive NDA residues. No ROEs are observed that support the geometrical positioning of $^{i}NH_{\alpha}$'s near $^{(i-3)}CO_{\beta}$ to favor only a single 11-helix in these oligomers. As suggested by the DMSO titration studies of **3** and **4**,‡ the relatively lower strength of the 8-membered H-bonds in the 11/8 helical fold may be attributed to the possible non-linearity of the H-bonds.‡ Similar observations are noted for bifurcated H-bonds in other peptides.[19]

To ascertain the folding propensities and hydrogen bond modes in these hybrid peptides, we have carried out DFT calculations for trimer **2** and tetramer **3**, at B3LYP/6-31G* level by using Gaussian 09.[20] The minimum energies of the system were obtained by harmonic frequency calculations. The optimization initially was carried out in vacuum and then optimized in solvent by using PCM (Polarizable Continuum Model). The lowest energy structures (Fig. 3) have shown a helical turn with 11/8 hydrogen bonding involving $^{i}NH_{n}{-}^{(i-3)}CO_{\alpha}$ and $^{i}NH_{\alpha}{-}^{(i-2)}CO_{\alpha}$, respectively. The structures did not violate the observed ROEs and are consistent with the solution state NMR structures.

The hexamer **5**, due to its polar nature, is insoluble in $CDCl_3$ and the NMR spectroscopic studies had to be carried out in a mixture of $CDCl_3$ (500 μL) and DMSO (90 μL). The ^{1}H resonances are relatively less resolved, however, adequate spatial information is obtained that can be attributed to a specific folding. The peptide **5** has exhibited similar ROE pattern‡ (Fig. 2d) as observed for the oligomers **2–4**: $^{i}NH_{n}{-}^{(i-2)}H2_{n}$ (two out of the two possible ROEs), $^{i}H4_{n}{-}^{(i-2)}H2_{n}$ (one out of two), $^{i}NH_{\alpha}{-}^{(i+1)}NH_{n}$ (two out of two), and $^{i}NH_{\alpha}{-}^{(i-1)}H2_{n}$ (two out of two) that strongly support the propagation of 11/8-helix in this higher oligomer as well. The ROEs that support other helical conformations are not observed. It is interesting to note that the conformational preference in these oligomers is unique and differs from that of other known 1 : 1 α/β hybrid peptides (11 and 14/15-helical folds),[4a,5] which are based on cyclo alkane or furanoid sugar β-amino acids.

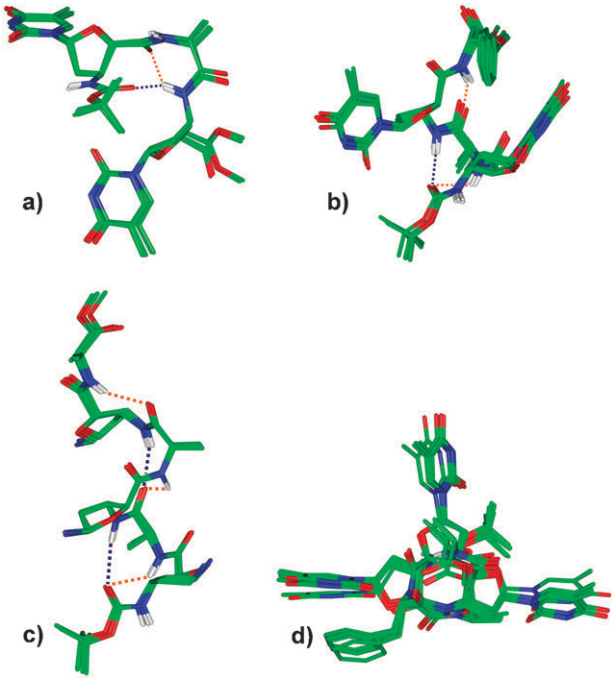

Fig. 4 Superimposed minimum energy structures from MD studies showing side views for (a) trimer **2**; (b) tetramer **4** and (c) hexamer **5** with the 11-member and 8-member hydrogen bonds shown in dotted blue and orange lines, respectively; (for **5** side-chains are removed for the sake of clarity). (d) Top view of **5** showing the radial disposition of nucleobases along the helical axis.

Molecular dynamics studies‡ for **2–5** have been carried out on Insight II Discover platform by using the distances derived from ROESY cross-peak intensities (normalised with respect to the ROE intensity of geminal protons present in NDAs or Phe as 1.8 Å reference) and dihedral angles derived from $^{3}J_{H}$ coupling constants, as the restraints. The minimum energy structures obtained from the simulations have shown a good convergence to 11/8 helical folds (Fig. 4), with a non-linear (90°–130°) and relatively longer H-bond distance (2.5–2.8 Å) for 8-membered H-bond.‡ However, the H-bond distances are observed to be ~2.4 Å for 11-membered and isolated C-terminal 8-membered H-bonds. The representative dihedral angles for NDA residues are (ϕ, θ, ψ) = (−80°, +120°, −90°) and for L-amino acids (ϕ, ψ) = (−65°, −50°).

In summary, the present study reveals that the heterogeneous backbone structures comprised of 1 : 1 NDA and L-amino acids preferentially adopt a right-handed helical fold with rapidly interconverting periodic 11/8-hydrogen bonded rings. The study signifies that the NDA organized spatial arrangement of the heterogeneous backbone and side-chains differs from that of other α/β peptides. These studies offer input for subsequent design and control of the backbone folding with functional traits of both the biomolecules: proteins and nucleic acids. Further studies are in progress in this direction.

NK, MUK and GPKR thank CSIR, New Delhi for research fellowship.

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