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β-Strand mimetics: formation of bend-strands in oligomers of enantiomeric β-amino acids

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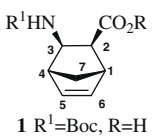
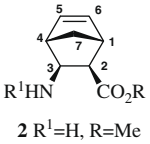
ABSTRACT

Oligomers comprising of enantiomeric *cis-oxo*-β-norbornene amino acid [2*R*,3*S*] and [2*S*,3*R*] residues at alternate positions were synthesized and characterized by extensive NMR and MD studies, which showed robust bend-strand secondary structures.

β-Strands, which are fundamental secondary structural elements in proteins, usually pair up to form β-sheet structures via inter-strand hydrogen bonding. However, isolated β-strands are identified to be conformationally suitable for certain crucial biomolecular recognitions: proteolytic enzyme inhibitors,[1] immune defence protein binding peptides[2] and SRC kinases.[3] Due to their favourable geometry, which exposes functional side-chains effectively, synthetic mimetics of such strand or ribbon structures have immense potential in the design of therapeutic agents and drug delivery systems,[4] and the present work is focused in this direction. In connection with the above, unnatural peptides, derived from β-amino acids,[5a] have special appeal as they adopt predictable and well-defined secondary structures ('foldamers') similar to natural biomolecules and exhibit a better resistance to enzymatic degradation.[5b] The pioneering studies by Gellman,[6] Seebach[7] and subsequently by other research groups,[8] including ours,[9,10] have shown that in β-peptides, the dihedral angle HCα–CβH (θ) exerts significant control over the backbone folding and distinct secondary structures can be accessed, depending upon the choice of substitution pattern at the Cα and Cβ positions[11] and the geometry (cis vs trans) around the Cα–Cβ bond.[9,10] However, although Hofmann and co-workers[12] have predicted β-peptidic strand structures theoretically, relatively few experimental studies have been reported so far. The research groups of Balaram and co-workers[13] and Fulop and co-workers[14] have reported the switching of helical backbone folding to an extended conformation, ribbon or strand structure, respectively, by substituting enantiomerically opposite residues in short homo γ- and β-peptides. In the light of these findings and earlier theoretical predictions,[12] it is interesting to study the effects of such mutations in homo β-peptidic strand structures as well to look for the possible reorganization of the backbone, which may have practical relevance. Earlier, we reported that the oligomers of homo-chiral *cis-exo*-β-norborn-5-ene amino acid residues exhibit saw-tooth type 6-strands (6-membered intra-residue hydrogen bonding).[10] Adopting the above strategy, we herein report the oligomers of dipeptide repeats comprising oppositely handed 6-strand-promoting building blocks, **1** [2*S*,3*R*] (+) and **2** [2*R*,3*S*] (−), which form bend-strand structures.



1 R^1=Boc, R=H



2 R^1=H, R=Me

The N and C-protected forms of the two enantiomers **1** and **2** were synthesized as described earlier[10] and were coupled by using the well-established HOBt–EDCI protocol to generate the protected dimer 3 in good yield (80%) (Scheme 1). The required acid functionality in the dimer was installed by treating 3 with LiOH in 90% yield.

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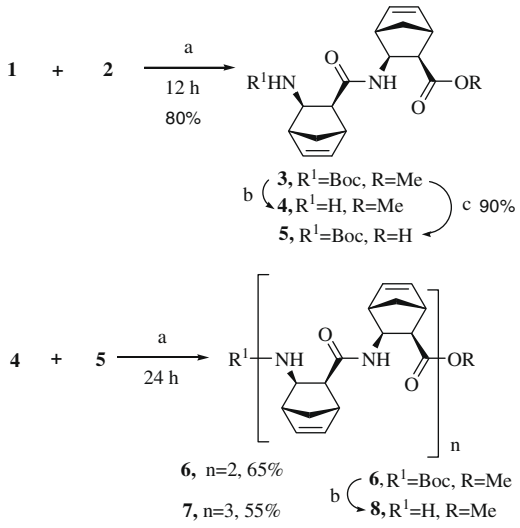

Scheme 1. Reagents and conditions: (a) HOBt, EDCI, DIPEA, DCM, rt; (b) TFA, CH_2Cl_2, 0 °C, 1 h; (c) LiOH, THF:H_2O (3:1), 3 h.

The second dimer, having a free amino functionality, was obtained from protected dimer 3 by treatment with trifluoroacetic acid. The two dimers 4 and 5 were coupled under the same set of coupling conditions to afford the tetramer 6 in 65% yield. The protected tetramer 6 was treated with trifluoroacetic acid to give tetramer amine 8. The dimer acid 5 and tetramer amine 8 were also coupled to afford hexamer 7 in 55% yield. The purity (98%) of these peptides was confirmed by HPLC.[15] The peptides were characterized in detail by mass and NMR spectroscopy and by MD simulation studies.

NMR studies of tetramer **6** and hexamer **7** have been carried out in polar (DMSO-d_6) and weakly polar ($CDCl_3$) solvents at 303 K on a 600 MHz spectrometer. Very similar structural information was obtained in both the solvents, but for the weak hydrogen bonding of terminal residues in DMSO. The complete resonance assignments were accomplished using a combination of 1D, DQF-COSY, TOCSY and ROESY data. The proton resonances of H2 and H3 are assigned through their COSY cross-peak and the H3 resonances could be easily verified as they showed very specific COSY cross-peaks with the corresponding vicinal NHs. Though the H1 and H4 resonances showed weak COSY cross-peaks with the vicinal H2 and H3 protons, respectively (due to the nearly perpendicular dihedral angle between H1–C1–C2–H2 and H4–C4–C3–H3 in the norbornene ring), they could be clearly assigned from their TOCSY correlations with the respective adjacent protons in each residue. They were further confirmed by the NOE intensity relationships between the H1–H2, H3–H4 and H4–NH proton sets of individual residues. In $CDCl_3$, the ^{1}H NMR spectra of both **6** and **7** showed a clear dispersion of all the NH resonances (δ_{NH}) over 6.5–8.2 ppm, indicating their possible involvement in hydrogen bonding and the presence of a secondary structure. No considerable change in the NH chemical shielding values over a concentration range, 10–0.5 mM, demonstrated the absence of aggregation. In order to assess the strength of the hydrogen bonding, changes in the NH proton chemical shifts ($\Delta\delta_{NH}$) with respect to sequential addition of aliquots of DMSO-d_6 (up to 33% v/v) to a solution of **6** in $CDCl_3$ were recorded. The results showed (Table 1) $\Delta\delta_{NH} < 0.1$ ppm for the first three ($i = 1$–3) NHs and $\Delta\delta_{NH} \sim 0.8$ ppm for the C-terminus NH ($i = 4$), establishing that the former are not solvent accessible and are strongly hydrogen bonded while the $NH_{i=4}$ is weakly hydrogen bonded, which could be due to the ester-protected terminal carbonyl. Furthermore, in polar DMSO, the measured temperature gradient values (Table 1), $\Delta\delta_{NH}/\Delta T$ (over a temperature range 298–343 K, with 5 K temperature step) for **6,** show that the first

Table 1
Tabulated values of $\Delta\delta_{NH}$ and $\Delta\delta_{NH}/\Delta T$ for **6**

NH	$\Delta\delta_{NH}$ measured by $CDCl_3$/DMSO-d_6 titration (ppm)	$\Delta\delta_{NH}/\Delta T$ in DMSO-d_6 (ppb/K)
NH_1	−0.03	−2.4
NH_2	−0.04	−0.8
NH_3	−0.09	−0.8
NH_4	0.78	−6.8

three NHs are involved in hydrogen bonding (<−4.0 ppb/K), a trend that was observed in $CDCl_3$, whereas NH_4 is not hydrogen bonded. The weak hydrogen bonding of the C-terminal NH_4 observed in $CDCl_3$ seems to be consistent with this observation. Similar results were exhibited by hexamer **7** as well. For all the residues of **6** and **7**, the observed couplings $^3J_{NH-C\beta H} \approx 7.3$–8 Hz (CβH═H3) suggest a small deviation from the anti-periplanar orientation between these protons and $^3J_{C\alpha H-C\beta H} \approx 8.3$ Hz (corresponds to $\theta \sim 5°$) (CαH═H2), clearly demonstrates a *cis*-configuration around Cα–Cβ for each residue, consistent with that observed for the parent monomer residue. These findings suggest a uniform backbone conformation and a highly restricted rotation around the Cα–Cβ bond. The preorganized conformational space of **1** and **2** with low values of θ cannot generate a pitch for helical folding, but is suitable for a strand or ribbon structure. For these *cis-exo*-β-norbornene residues, this conformation favours intra-residue 6-membered hydrogen bonding.[10] For **6** and **7**, an explicit analysis of the ^{1}H–^{1}H ROESY in DMSO-d_6 has shown periodic NH_i–$H3_{i+1}$, NH_i–$H4_{i+1}$, NH_i–$H1_{i-1}$ and NH_i–$H2_{i-1}$ backbone NOE cross-peaks (Figs. 1 and 2), which can be assigned to a predominantly populated strand-like secondary structure.[10,14,16] The clear presence of two of three possible NOEs: $H3_i$–$H2_{i-1}$ for $i = 3$ and 4 (for $i = 2$ the NOE is ambiguous due to an overlap) and $H3_i$–$H2_{i-2}$ and the absence of $H1_i$–$H4_{i+2}$ NOEs corroborate the above findings and further suggest that the backbones adopt a curvature in contrast to the linear 6-strands,[10] leading to a bend-strand[8d] conformation. It appears that the conformational restriction of the alternate (+−) residues necessitates that the backbones adopt a constant curvature. The earlier reported X-ray studies on γ-peptidic (+−) oligomers support this possibility.[13] Analysis of the hexamer **7** has re-established these findings and indicated the propagation of the bend-strand secondary structure in this higher oligomer. The ratio (R) of NOE intensities, NH_i–$H2_{i-1}$/NH_i–$H3_i$, is a sensitive tool in distinguishing linear strand ($R = \sim 5.5$) and helical structures ($R = \sim 0.5$).[14] The estimated R values for tetramer **6** and hexamer **7** are 4.6 and 4.0, respectively. The structural stability of **6** and **7** has been tested through NH/ND exchange in the presence of methanol as solvent (200 μL CD_3OH + 300 μL of CD_3OD) at 298 K. The results showed some loss in the NH signal intensities immediately after adding the solvent,

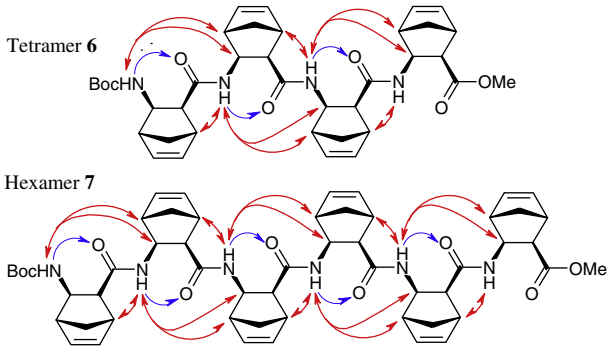

Figure 1. Schematic representation of selected NOEs (solid curves) and hydrogen bonding (dashed curves) for **6** and **7**.

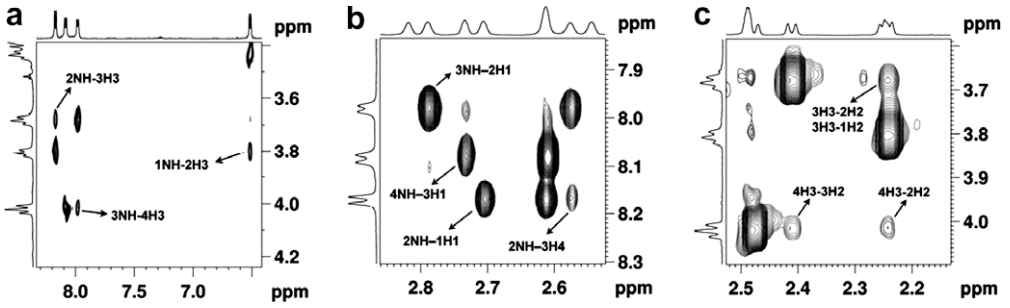

Figure 2. Expanded ^{1}H–^{1}H ROESY plots of tetramer **6** representing the observed characteristic NOEs, NH_i $H3_{i+1}$ (a), NH_i $H1_{i-1}$ (b) and $H3_i$ $H2_{i-1}$, $H3_i$ $H2_{i-2}$ (c).

but led to a prolonged appearance of all the NH resonances with about equal intensity even after three days. In contrast to the DMSO titration and temperature gradient studies, these findings indicate that all the NHs exhibit almost similar shielding from the solvent accessibility, and thereby confirm that these strand structures are robust and are stabilized by intramolecular hydrogen bonding in methanol.

These findings are further substantiated by FT-IR studies. These data exhibited predominant bands around 3300 cm^{-1} (NH) and 1646 cm^{-1} (C═O), which are characteristic of intramolecular hydrogen bonded strand structures,[10] and are consistent with our NMR findings.

The minimum energy structures derived from NOE-restrained MD calculations (Insight-II, Discoverer) for **6** and **7** following a simulated annealing protocol are found to be in excellent agreement with those discussed above. Initially, the molecule was heated to 500 K and gradually cooled to 303 K with a 1 ns time period. At 303 K, a prolonged dynamics (5 ns) was run. By collecting a snapshot for every 50000 history files, 100 structures were obtained, which were energy-minimized using the conjugate method. Among these structures the lowest energy structure was subjected to restrained (distances and torsion angles) MD studies. The NOE cross-peak intensities for **6** and **7** are converted into distances (Table 2) by normalizing with respect to the averaged NOE intensity between the geminal protons present on carbon C7 of the norbornene residues. During the MD trajectory over a period of 1 ns, 100 structures were collected by taking a snapshot for every 10 ps. The resulting ensemble of structures exhibit a strand-like backbone with constant curvature with their adjacent carbonyls oriented in opposite directions (alternate dipole moment), resembling natural non-polar β-strands.[14] However, the present strands are further stabilized by intra-residue 6-membered hydrogen bonding, leading to robust structures. The dihedral angles $|\phi|$, $|\theta|$ and $|\Psi|$ measured from these structures are 160°, 5° and 150°, respectively. Superposition of these energy-minimized structures showed a good convergence (Fig. 3), suggesting a predominantly single conformation for **6** and **7**. Such a curved backbone should allow its functional side-chains to be fanned out for specific accessibility.[17]

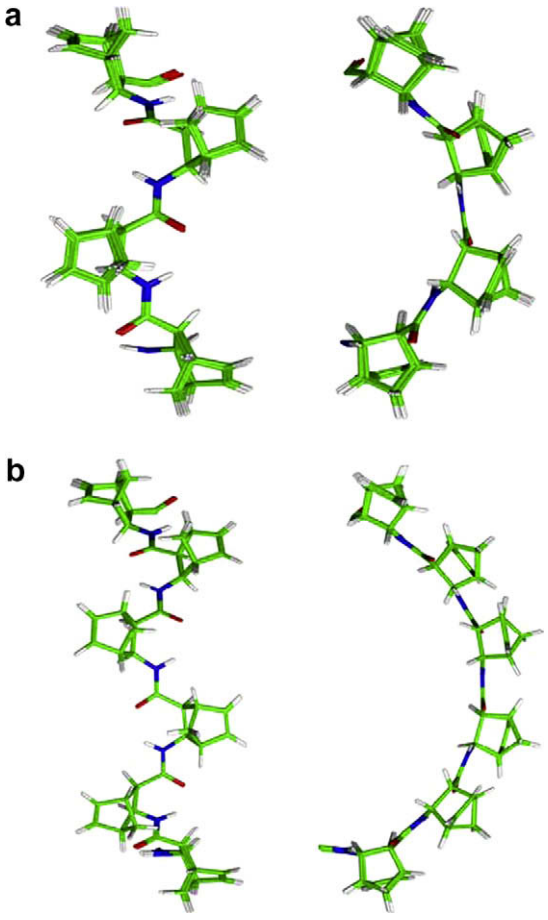

Figure 3. Superposition of 15 energy minimized structures showing top and side views for **6** (a), and **7** (b). Protecting groups are not shown for the sake of clarity.

Table 2
List of distance constraints used in MD simulations for tetramer **6**

NOEs	Distance (Å)	NOEs	Distance (Å)
4NH↔4H7′	2.6–3.2	2NH↔2H7′	2.7–3.2
4NH↔4H4	2.9–3.5	2NH↔2H4	2.9–3.5
4NH↔3H1	3.2–3.9	2NH↔1H1	3.0–3.7
4NH↔3H2	2.2–2.7	2NH↔1H2	2.2–2.7
3NH↔3H7′	2.7–3.3	2NH↔3H3	3.7–4.5
3NH↔3H4	3.1–3.8	1NH↔1H7′	2.5–3.0
3NH↔3H2	3.3–4.1	1NH↔1H4	2.9–3.5
3NH↔2H1	2.7–3.3	1NH↔1H2	3.5–4.2
3NH↔2H2	2.3–2.8	1NH↔2H3	3.6–4.4
3NH↔4H3	3.9–4.8	1NH↔2H4	3.9–4.8

In summary, we have exploited the concept of opposite chirality in dipeptide repeats of strand-forming (2*S*,3*R*) and (2*R*,3*S*)-*cis*-*exo*-β-norbornene amino acids. NMR and MD studies have convincingly shown that the backbone of the mixed enantiomers adopts a bend-strand structure with intra-residue 6-membered hydrogen bonding.

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