Oligomers of *cis*-β-norbornene amino acid: Formation of β-strand mimetics†

Srivari Chandrasekhar,*[a] Bathini Nagendra Babu,[a] Anabathula Prabhakar,[b] Ambadi Sudhakar,[a] Marepally Srinivasa Reddy,[a] Marelli Udaya Kiran[b] and Bharatam Jagadeesh*[b]

Received (in Cambridge, UK) 4th January 2006, Accepted 14th February 2006
First published as an Advance Article on the web 2nd March 2006
DOI: 10.1039/b518420g

The oligomers of constrained *cis-exo-β*-norbornene amino acid were synthesised and characterised by extensive NMR, CD, IR and MD studies. The results showed the formation of both right and left handed consecutive 6-membered hydrogen-bonded strands for [2*S*,3*R*] and [2*R*,3*S*] enantiomers, respectively.

Analogous to the proteins that consist of α-peptides, certain β-peptide oligomers, exhibit an impressive range of structural diversity such as helices, strands, sheets and turns.[1] It has been evident from earlier studies by several researchers, including our group, that the type of secondary structural conformation can be controlled by a choice of the amino acid residue and its stereochemistry.[2,3] The fundamental structural element in proteins is the β-strand, which is known to be conformationally suitable for specific recognition by biomolecular receptors such as proteolytic enzymes, major histocompatibility complex (MHC) proteins and transferases.[3] In the light of these findings, synthesis of β-strand mimetics consisting of unnatural constrained β-amino acid residues would be of considerble interest in foldamer chemistry. However in a recent review, Loughlin *et al.*,[4] have reported that there are relatively few studies on isolated β-strand mimetics compared to those on β-sheets (formed upon pairing of strands), suggesting an immense need and scope for generic rigid scaffolds that exhibit strand-forming propensities.

The present work focuses in this direction and reports the design, synthesis and characterization of stereo-specific oligomers of bicyclic *exo-cis-β*-amino acids derived from norbornadiene.

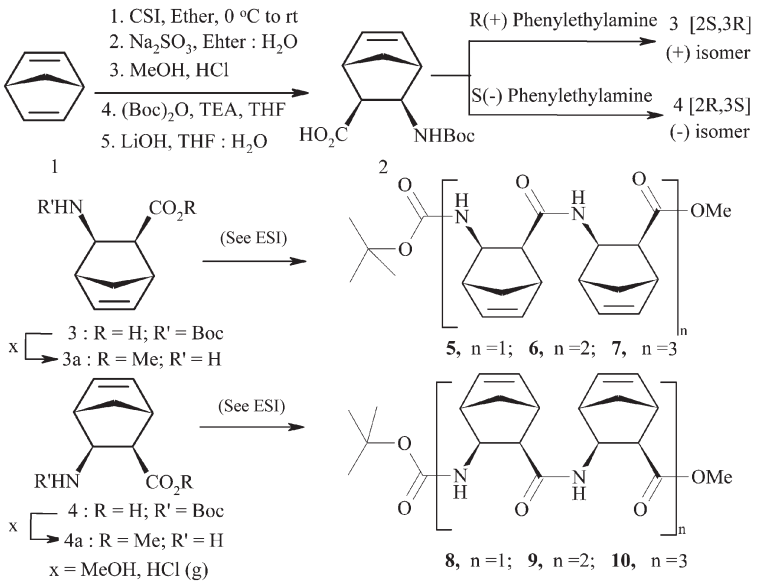
Tuning the conformational space of the β-residues, represented by the dihedral angles Φ, θ and Ψ (convention of Balaram),[5] provides a variety of folding possibilities. Recently we have shown that the homo and hetero oligomers based on bicyclic *cis*-FSAA residues with *gauche*-conformation around "θ", form short and stable right-handed 14-helices.[3] In pursuance of conformationally rigid motifs that promote strand-like structures upon oligomerization, we have carried out preliminary molecular mechanics calculations on various stereo isomers of norbornadiene derived amino acid oligomers. The norbornene amino acid, particularly the *endo*-residue, has been exploited by a few groups as turn inducing monomer in a hetero peptide chain.[2f,2g,6] However, oligomerisation of this monomer (*exo* in the present context) has never been explored. Herein, to the best of our knowledge, we report for the first time the oligomerisation of *cis-exo*-norborn-5-ene amino acid residues.

The synthesis of both the possible *cis* enantiomers *viz. cis-exo*-**3** [2*S*,3*R*] and **4** [2*R*,3*S*] for oligomerization and their further characterization has been carried out by following the classical resolution technique of the (±)*N*-Boc amino acid **2**. This has been obtained in multi gram quantities from norbornadiene **1** on [2 + 2] cycloaddition with chlorosulfonyl isocyanate followed by reduction with Na_2SO_3 and esterification which furnished the amino acid hydrochloride as the methyl ester. Boc protection (TEA, Boc_2O) and ester hydrolysis has yielded the (±)acid **2**, which is ready for resolution.[7] The commercially available phenyl ethylamine (both *R* and *S* isomers) turned out to be the best source of chirality for resolution (Scheme 1).† The oligomerization of the resolved [2*S*,3*R*] and [2*R*,3*S*] enantiomers has been achieved independently following the conventional peptidation as described previously,[3] which is expected to show opposite sense handedness. These peptides were characterised in detail by using NMR, IR, CD, and MD techniques.

Information on the preferred conformation of the homo-oligomers in solution was obtained in structure-supporting solvents ($CDCl_3$ and DMSO-d_6) by 1D and 2D NMR techniques. The chemical shifts of the NH's remained constant when diluted from 10 mM to ~0.5 mM suggesting the absence of aggregation. Large values (>8.3 Hz) of $^3J_{NH-C\beta H}$ (ϕ = ≈ −135°) in **5–10** corresponded to an antiperiplanar arrangement between these protons and also indicated the presence of a secondary structure in solution.[3] In all these peptides the observed coupling constant $^3J_{C\alpha H-C\beta H}$ (>8 Hz) clearly demonstrated the presence of predominantly *cis*-conformation around Cα–Cβ(θ) ≈ ±10° for each residue, which promotes strand-like conformation. It may be noted that the "ϕ" in the present case is comparable to that observed earlier for 14-helix,[3] whereas the "θ" deviates from the *gauche* conformation. The ROESY data of **5–10** revealed several medium and long–range backbone NOE's between NH_i–$C\beta H_{i+1}$, and NH_i–$C\beta H_{i-1}$ (Fig. 1), which are distinctive for the strand-like structures.[8] For dimer **5** the only possible NOE's are NH_1–$C\beta H_2$ and NH_2–$C\beta H_1$, which were clearly observed. For tetramer **6**, all the possible six NH_i–$C\beta H_{i+1}$ and NH_i–$C\beta H_{i-1}$ cross peaks (Fig. 1) were observed, which confirm the strand-like secondary

[a]*Division of Organic Chemical Sciences, Hyderabad, India. E-mail: srivaric@iict.res.in*
[b]*Center for Nuclear Magnetic Resonance, Indian Institute of Chemical Technology, Hyderabad, India. E-mail: bj@iict.res.in; Fax: 91-40-27160512; Tel: 91-40-27193128*

† Electronic supplementary information (ESI) available: Experimental details, FTIR spectra, Newman projections for the two enantiomers of norbene, ^{1}H NMR, ^{13}C NMR and TOCSY NMR spectra, tables of chemical shifts and coupling constants, solvent titration studies, ROESY expansion results, molecular dynamics calculations, dynamic pictures of **6**, **7**, **9** and **10**. See DOI: 10.1039/b518420g



Scheme 1

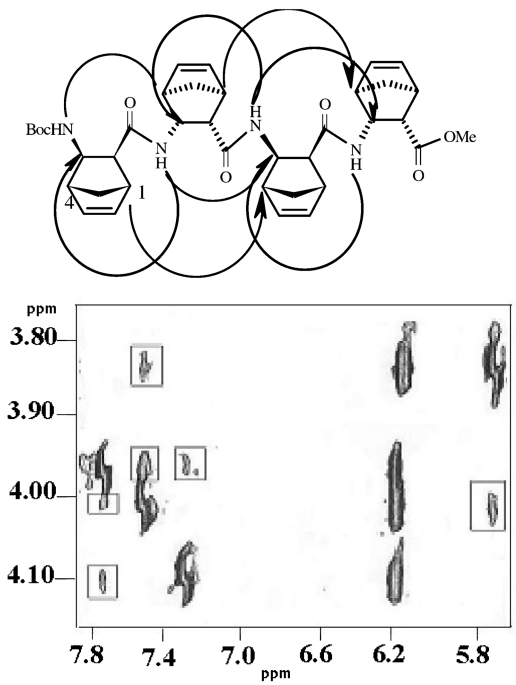


Fig. 1 ROESY expansion for **6** showing the six backbone NH_i–$C\beta H_{i+1}$ and NH_i–$C\beta H_{i-1}$ NOE's.

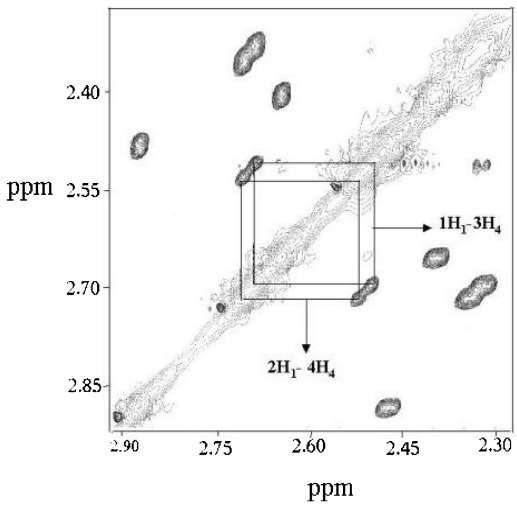


Fig. 2 ROESY expansion for **6** showing the two possible interresidue H_1–H_4 NOE's.

structure. The observed strong intensity NH–CβH NOE's within the residue, and medium intensity NOE's between consecutive modules $C\beta H_{i-1}$–NH_i, could be translated into distance intervals of 2.0–3.0 Å and 3.0–5.0 Å, respectively. Furthermore, the inter-residue NOE's between H_1–H_4 also support the derived structure. For tetramer **6**, two inter-residue H_1–H_4 NOE's are possible, *viz.*, between the first to third residue (2.69–2.50 ppm) and the second to fourth residue (2.71–2.52 ppm), which are unambiguosly assigned as shown in Fig. 2. In fact, intra-residue H_1–H_4 NOE's are also possible. However, such intra-residue NOE's are expected to be weak as the corresponding distance is about 4.3 Å, which has been estimated from the minimum-energy structures. Hence the observed strong NOE cross-peaks are predominantly due to the proximities of inter-residue H_1–H_4 protons that belong to the alternate residues, and the corresponding average distance is estimated to be about 2.65 Å. Despite some unacceptable overlaps of cross-peaks for hexamer **7**, this peptide has also exhibited similar conformational behaviour.† The intensity ratio (R) of cross-peaks NH_i–$C\alpha H_{i-1}$ to NH_i–$C\beta H_i$ can also yield clues about the possible secondary structure. The estimated R for the compounds **5–10**, is ~5.2, which is in agreement with the predominance of the six-strand conformation.[8]

The presence of specific intramolecular H-bonds in peptides **5–10** have been obtained by using the solvent dependence of the amide NH chemical shifts in $CDCl_3$–DMSO mixtures.†[9] From the observed NOE's and the couplings, it is suggested that the backbone of **5–10** forms a strand-like structure that is stabilized by the intra-residue six-memberd H-bonds. The stability of the

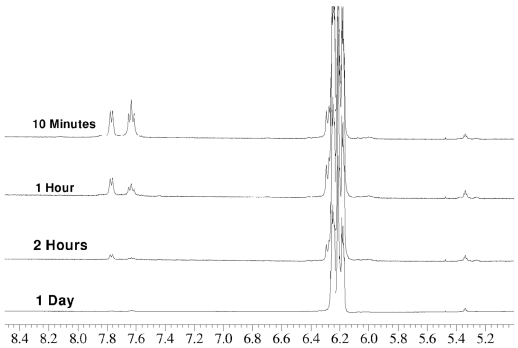


Fig. 3 ^{1}H NMR NH/ND exchange studies of **6** in methanol-d_4.

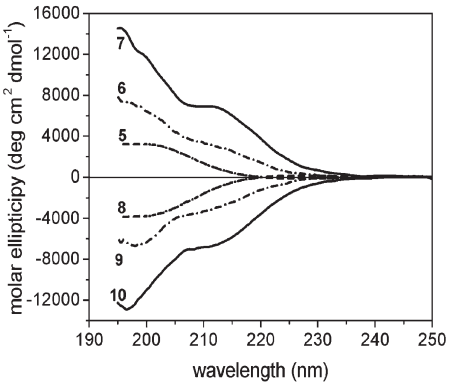


Fig. 4 Circular dichroism spectra of **5–10** in methanol showing the handedness of oligomers.

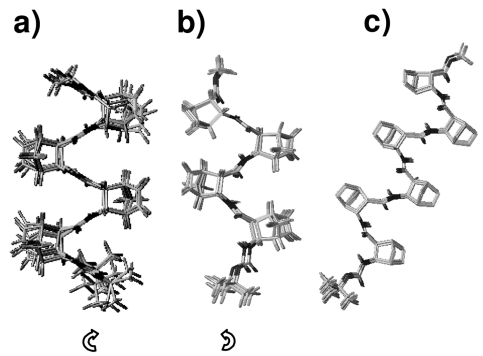


Fig. 5 Superposition of the lowest energy structures of (a) left handed (top view) and (b) right handed (top view) strands of **6** and **9**, respectively; (c) is the side view of **7**.

structure is further checked in CD_3OD. The shielding of the hydrogen bonded NH-protons has been assessed by ^{1}H NMR NH/ND exchange measurements. The prolonged NH/ND exchange beyond 24 h is indicative of a stable conformation and intra-residue NH–CO hydrogen bonding in this solvent (Fig. 3). The CD spectra that were recorded in CH_3OH at a concentration of 0.5 mM have also shown features corresponding to strand or sheet-like structure and further suggest right and left handed conformations for **5–7** and **8–10**, respectively (Fig. 4).

Furthermore, these observations are substantiated by FT-IR studies (in $CHCl_3$) exhibiting characteristic NH-stretching ($\sim$3300 cm^{-1}) and amide-1 ($\sim$1653 cm^{-1}) bands that correspond to the hydrogen bonded peptide residues.†[10]

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 following the Insight II(97.0)/Discover1 program.† The superposition of several energy-minimized structures have shown a well-defined backbone that corresponds to a strand conformation (Fig. 5). For better clarity, the coloured version of Fig. 5 is given in the supporting information.†

In summary, we have synthesized and carried out detailed characterization of β-strand mimetics derived from the oligomerization of *exo*-norborn-5-ene amino acid residues. Further applications of these peptides in chemical biology programmes is currently underway.

AP, BNB, MSR, AS and MUK are thankful to CSIR, New Delhi for fellowship. SC thanks DST for funding the project.

Notes and references

1 (*a*) S. H. Gellman, *Acc. Chem. Res.*, 1998, **31**, 173–180; (*b*) D. Seebach, A. K. Beck and D. J. Bierbaum, *Chem. Biodiversity*, 2004, **1**, 1111–1239 and reference cited therein; (*c*) D. Seebach, M. Overhand, F. N. M. Kuhnle, B. Martinoni, L. Oberer, U. Hommel and H. Widmer, *Helv. Chim. Acta*, 1996, **79**, 913–941; (*d*) R. P. Cheng, S. H. Gellman and W. F. DeGrado, *Chem. Rev.*, 2001, **101**, 3219–3232.

2 (*a*) D. H. Appella, L. A. Christianson, D. A. Klein, D. R. Powell, X. Huang, J. J. Barchi, Jr. and S. H. Gellman, *Nature*, 1997, **387**, 381; (*b*) D. W. Timothy, J. M. Claridge, A. M. Goodman, A. Donald, F. B. Sarah, T. Claude, P. W. Mark and G. W. J. Fleet, *Tetrahedron Lett.*, 2001, **42**, 4251–4255; (*c*) A. Hetenyi, I. M. Mandity, T. A. Martinek, G. K. Toth and F. Fulop, *J. Am. Chem. Soc.*, 2005, **127**, 547–553; (*d*) G. V. M. Sharma, P. Nagendar, P. Jayaprakash, P. R. Krishna, K. V. S. Ramakrishna and A. C. Kunwar, *Angew. Chem., Int. Ed.*, 2005, **117**, 6028–6032; (*e*) Christian P. R. Hackenberger, Ingo Schiffers, Jan Runsink and Carsten Bolm, *J. Org. Chem.*, 2004, **69**, 739–743; (*f*) I. G. Jones, W. Jones and M. North, *J. Org. Chem.*, 1998, **63**, 1505–1513; (*g*) D. E. Hibbs, M. B. Hursthouse, I. G. Jones, W. Jones, K. M. A. Malik and M. North, *J. Org. Chem.*, 1998, **63**, 1496–1504.

3 (*a*) S. Chandrasekhar, M. S. Reddy, B. Jagadeesh, A. Prabhakar, M. H. V. R. Rao and B. Jagannadh, *J. Am. Chem. Soc.*, 2004, **126**, 13586–13587; (*b*) S. Chandrasekhar, M. S. Reddy, B. N. Babu, B. Jagadeesh, A. Prabhakar and B. Jagannadh, *J. Am. Chem. Soc.*, 2005, **127**, 9664–9665.

4 W. A. Loughlin, J. D. A. Tyndall, M. P. Glenn and D. P. Fairlie, *Chem. Rev.*, 2004, **104**, 6085–6117 and references cited therein.

5 J. Venkatraman, S. C. Shankaramma and P. Balaram, *Chem. Rev.*, 2001, **101**, 3131–3152.

6 (*a*) D. Ranganathan, V. Haridas, S. Kurur, A. Thomas, K. P. Madhusudanan, R. Nagaraj, A. C. Kunwar, A. V. S. Sarma and I. L. Karle, *J. Am. Chem. Soc.*, 1998, **120**, 8448–8460; (*b*) R. J. Doerksen, B. Chen, J. Yuan, J. D. Winkler and M. L. Klein, *Chem. Commun.*, 2003, 2534–2535.

7 G. Stajer, L. Mod, A. E. Szabo, F. Fullop, G. Bernath and P. Sohar, *Tetrahedron*, 1984, **40**, 2385–2393.

8 T. A. Martinek, G. K. Toth, E. Vass, M. Hollosi and F. Fulop, *Angew. Chem., Int. Ed.*, 2002, **41**, 1718–1721.

9 The solvent titration was carried out by sequentially adding up to 33% DMSO-d_6 to $CDCl_3$ solution of the peptide.

10 L. K. Isabella, L. Judith, A. Flippen, R. Gurunath and P. Balaram, *Protein Sci.*, 1994, **3**, 1547–1555.

