JOC*Note*

Synthesis and Conformational Studies of Novel Cyclic Peptides Constrained into a 3_{10} Helical Structure by a Heterochiral D-Pro-L-Pro Dipeptide Template

I. Nageshwara Rao,[†] Anima Boruah,[†] S. Kiran Kumar,[‡] A. C. Kunwar,[‡] A. Sivalakshmi Devi,[†] K. Vyas,[†] Krishnan Ravikumar,[‡] and Javed Iqbal*[,†]

Dr. Reddy's Laboratories, Discovery Research, Bollaram Road, Miyapur, Hyderabad 500 050, India, and Indian Institute of Chemical Technology, Uppal Road, Tarnaka, Hyderabad 500 007, India

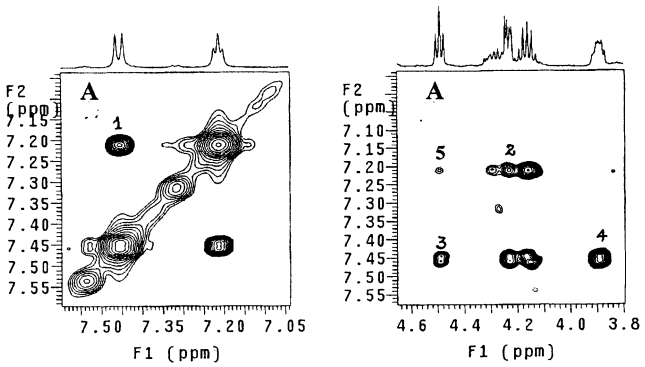
javediqbaldrf@hotmail.com

Received September 3, 2003

Abstract: An acyclic tripeptide based on a heterochiral D-*pro*-L-*pro* template shows a propensity to exist as a 3_{10} helical conformation and can be cyclized, via ring-closing metathesis, to the corresponding cyclic tetrapeptides without disrupting the helical conformations in $CDCl_3$ as well as in DMSO-d_6 solutions. The detailed conformational studies were carried out by using NMR spectroscopy, X-ray crystallography, molecular dynamic simulations, and circular dichroism spectroscopy.

Cyclic peptides containing heterochiral diproline[1] templates are an interesting class of protein loop mimetics, due to their strong tendency to nucleate the β-hairpin-inducing properties. A recent study has shown[2] that canonical conformation of CDR loops observed in high-resolution crystal structures of antibody fragments was accurately reproduced in cyclic peptides containing a D-*pro*-L-*pro* template. The eight-residue L3 loop from antibody HC19 attached to the D-*pro*-L-*pro* template was shown to adopt a backbone conformation very similar to that in the antibody. These studies suggest that β-hairpin mimetics based on cyclic peptides containing the D-*pro*-L-*pro* template might prove to be of general use as a starting point in protein ligand, vaccine, and receptor antagonist design. D-*Pro* and L-*pro* residues can restrict the backbone φ angle by $+60 \pm 20°$ and by $-60 \pm 20°$, respectively, and participate in the nucleation of β-turns in peptides. During the synthesis of cyclic peptides containing D-*pro*-L-*pro*, we observed that tripeptide **1** (Scheme 1), derived from this template and L-alanine, shows the presence of two intramolecular hydrogen bonds, which implies that these small peptides can exist as 3_{10} helical structures.[3] This observation led us to explore its cyclization using ring-closing metathesis (RCM) reaction.[4] A brief account of these findings is given below.

According to a general synthetic plan, dipeptide **4** was hydrolyzed (LiOH) and subsequently coupled with allylamine by a mixed anhydride protocol ($ClCO_2Bu^i/Et_3N$) to afford the dipeptide **5** (Scheme 1). The removal of Boc (TFA) from **5** followed by the coupling with Boc-D-*pro* (EDC/HOBt) afforded the tripeptide **6**, which was transformed to **1** in 51% yield by Boc removal (TFA) followed by mixed anhydride coupling ($ClCO_2Bu^i/Et_3N$) with pentenoyl chloride. The ^1H NMR investigations on **1** have been carried out in both polar (DMSO-d_6) and nonpolar ($CDCl_3$) solvents. In $CDCl_3$ solution, the tripeptide **1** shows the presence of a single rotamer. The cross-peaks in the NOESY spectrum between *ala* NH/D-*pro* $C_\alpha H$ ($i/i + 2$), allyl NH/L-*pro* $C_\alpha H$ ($i/i + 2$), *ala* NH/L-*pro* $C_\delta H$, and *ala* NH/allyl NH, as well as participation of both amide protons in hydrogen bonding, which show very small shifts of <0.1 ppm for the amide chemical shifts during the solvent titration (Figure 1a), confirm the presence of two successive β-turns, nucleating a minimal 3_{10} helix. Thus, in the above two β-turns, the one that involves pentenoyl-D-*pro*-L-*pro*-*ala* is a type II′ and the second one with D-*pro*-L-*pro*-*ala*-allyl amide sequence is a type I β-turn. In DMSO-d_6 solution, peptide **1** shows the presence of three isomers in a ratio of 8:1:1. The major isomer having all *trans*-amide bonds, which were assigned by the presence of cross-peaks in the ROESY/NOESY spectrum (Figure 1c) between D-*pro* $C_\alpha H$/L-*pro* $C_\delta H$ and $COCH_2$/D-*pro* $C_\delta H$, was studied in detail. The participation of allyl NH and *ala* NH in hydrogen bonding is confirmed by the small magnitude of their temperature coefficients ($\Delta\delta/\Delta T$) of -1.7 and -1.9 ppb/K, respectively. The presence of these hydrogen bonds, as well as very similar cross-peaks (*ala* NH/D-*pro* $C_\alpha H$ ($i/i + 2$), allyl NH/L-*pro* $C_\alpha H$ ($i/i + 2$), *ala* NH/L-*pro* $C_\delta H$, and *ala* NH/allyl NH) in the ROESY/NOESY spectrum, shows that the peptide has an identical structure in both solvents. In addition to the above NOEs, a weak NOE between allyl NH/D-pro CαH ($i/i + 3$) is also observed (Spectrum A). Thus, the resultant structure resembles a minimal stable 3_{10} helical conformation in solution. One of the lowest energy structures obtained in the MD calculations shows incipient 3_{10} helical features very clearly in Figure 1b.



SPECTRUM A: Expanded region of NOESY spectrum of **1** in DMSO-d_6 shows some of the diagnostic NOE cross peaks. Ala NH/Allyl NH (1), Allyl NH/L-Pro $C_\alpha H$ (2), Ala NH/D-Pro $C_\alpha H$ (3), Ala NH/L-Pro $C_\delta H$ (4), Allyl NH/D-Pro $C_\alpha H$ (5).

A successful olefin metathesis[5] involving the two termini in **1** to cyclize the molecule would be an added proof for this structural preorganization, which in turn would ensure the possible existence of the β-turns that induces

* Corresponding author. J.I. is a Distinguished Research Scientist at Discovery Research.
[†] Discovery Research.
[‡] Indian Institute of Chemical Technology.

(1) (a) Bean, J. W.; Kopple, K. D.; Peishoff, C. E. *J. Am. Chem. Soc.* **1992**, *114*, 5328−5334. (b) Nair, C. M.; Vijayan, M.; Venkatachalapathi, Y. V.; Balaram, P. *J. Chem. Soc., Chem. Commun.* **1979**, 1183−1184. (c) Chalmers, D. K.; Marshall, G. R. *J. Am. Chem. Soc.* **1995**, *117*, 5927−5937. (d) Spath, J.; Stuart, F.; Jiang, L.; Robinson, J. A. *Helv. Chim. Acta* **1998**, *81*, 1726−1738.
(2) Favre, M.; Moehle, K.; Jiang, L.; Pfeiffer, B.; Robinson, J. A. *J. Am. Chem. Soc.* **1999**, *121*, 2679−2685.

10.1021/jo030282w CCC: $27.50
Published on Web 02/26/2004

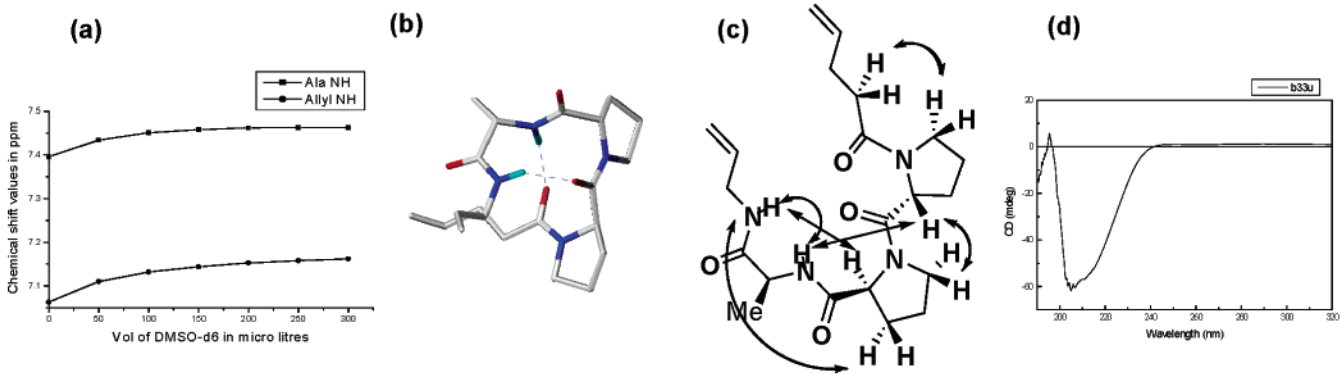
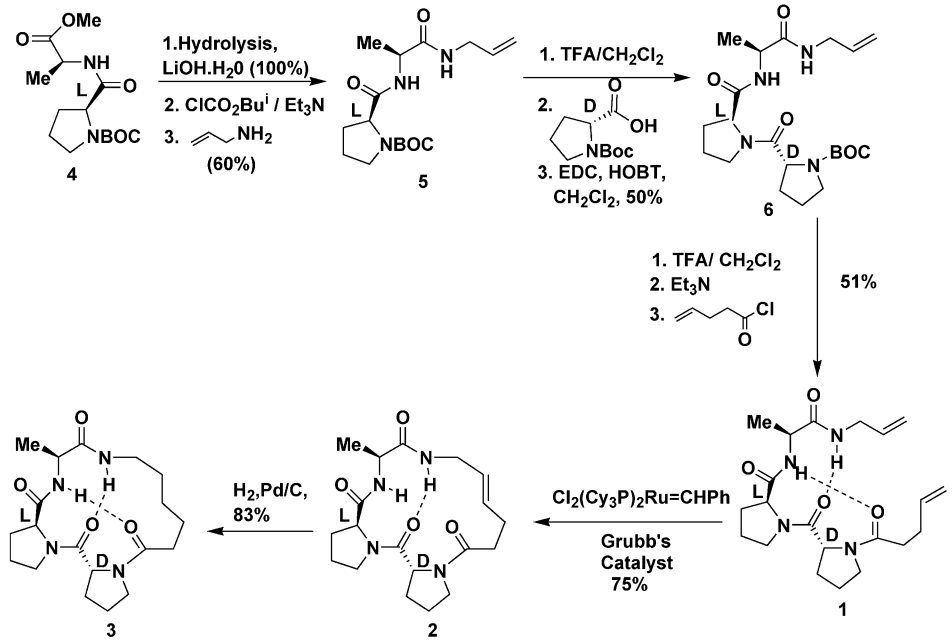


FIGURE 1. (a) NMR titration plot for hydrogen bonding, (b) MD simulated structure, (c) NOEs observed, and (d) CD spectrum of **1**.

SCHEME 1. Synthesis of d-*pro*-L-*pro*-Derived Peptides



this organization. The circular dichroism (CD) studies in methanol showing an intense band with minima at 205 nm and a shoulder at 215 nm clearly suggests that peptide **1** is folded in a helical conformation (Figure 1d). To our gratification, when the tripeptide **1** was subjected to RCM conditions, it indeed underwent a smooth cyclization to afford the corresponding cyclic peptide **2** exclusively as the *E*-geometrical isomer in good yields (Scheme 1).

In the process of cyclization of **1**, one new unnatural amino acid (6-aminohex-4-enoic acid; Δ*aha*) is created, and **2** can be considered as a cyclic tetrapeptide with two natural (*pro* and *ala*) and two unnatural (D-*pro* and Δ*aha*) residues. The ^{1}H NMR of **2** was investigated in both solvents. In $CDCl_3$ solution, **2** showed the presence of two isomers in a ratio of 4:1. The major isomer corresponding to all *trans*-amide bonds was studied in detail. The solvent titration studies on **2** showed that chemical shifts of both the amide protons, *ala* NH and Δ*aha* NH, changed only by 0.38 and 0.13 ppm, respectively, when subjected to NMR titration by adding up to 300 μL of DMSO-d_6 to 600 μL of $CDCl_3$ solutions, confirming their participation in hydrogen bonding (Figure 2a). In addition to these hydrogen bonds, Δ*aha* NH/L-*pro* C_αH, *ala* NH/D-*pro* C_αH, Δ*aha* NH/D-*pro* C_αH, *ala* NH/L-*pro* C_δH, and Δ*aha* NH/*ala* NH cross-peaks in the NOESY spectrum imply that **2** has two successive β-turns about Δ*aha*-D-*pro*-L-*pro*-*ala* and D-*pro*-L-*pro*-*ala*-Δ*aha* residues, respectively (Figure 2c).

In DMSO-d_6, peptide **2** has three isomers in a ratio of 8:3:2, but the detailed structural information was obtained only on the major isomer, which has all *trans*-amide bonds. $\Delta\delta/\Delta T$ of −1.9 and −6.5 ppb/K for Δ*aha* NH and *ala*-NH, respectively, indicate that only the former participates in hydrogen bonding. The presence of two

(3) (a) Patel, H. C.; Singh, T. P.; Chauhan, V. S.; Kaur, P. *Biopolymer* **1990**, *29*, 509. (b) Ciajolo, M. R.; Tuzi, A.; Pratesi, C. R.; Fissi, A.; Pieroni, O. *Biopolymer* **1992**, *32*, 727. (c) Rajashankar, K. R.; Ramakumar, S.; Chauhan, V. S. *J. Am. Chem. Soc.* **1992**. *114*, 9225.

(4) For ring-closing metathesis, see: (a) Phillips, A. J.; Abell, A. J, *Aldrichimica Acta* **1999**, *32*, 75−90. (b) Schuster, M.; Blechert, S. *Angew. Chem., Int. Ed.* **1997**, *36*, 2036−2056 and references cited therein. (c) Furstner, A. *Angew. Chem., Int. Ed.* **2000**, *39*, 3012−3043 and references cited therein. (d) Miller, S. C.; Blackwell, H. E.; Grubbs, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 9606−961.

(5) *For a recent study from our group on cyclic peptides, see*: (a) Banerji, B.; Mallesham, B.; Kiran kumar, S.; Kunwar, A. C.; Iqbal, J. *Tetrahedron Lett.* **2002**, *43*, 6479. (b) Banerji. B.; Bhattacharya, M.; Madhu, B. R.; Das, S. K.; Iqbal, J. *Tetrahedron Lett.* **2002**, *43*, 6473. (c) Saha, B.; Das, D.; Banerji, B.; Iqbal, J. *Tetrahedron Lett.* **2002**, *43*, 6467. (d) Sastry, T. V. R. S.; Banerji, B.; Kirankumar, S.; Kunwar, A. C.; Das, J.; Nandy, J. P.; Iqbal, J. *Tetrahedron Lett.* **2002**, *43*, 7621.

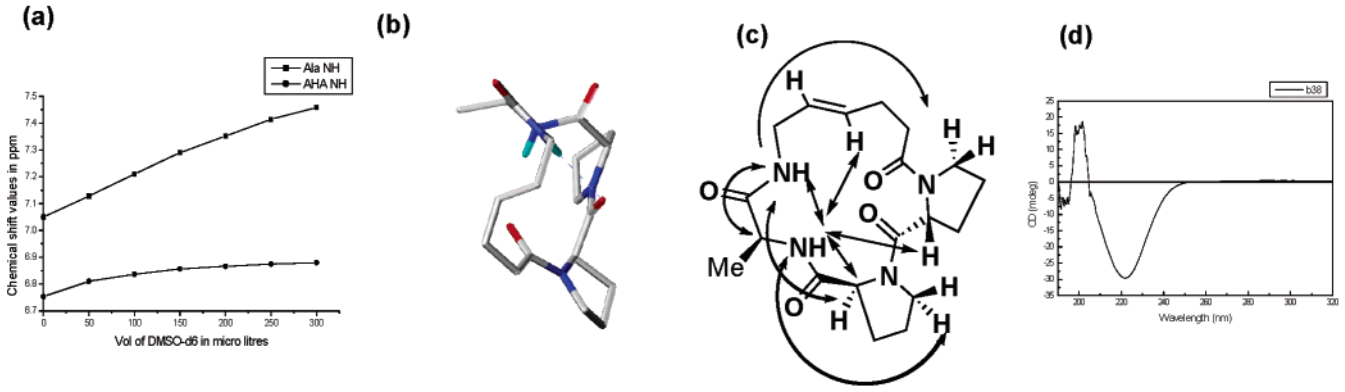


FIGURE 2. (a) NMR titration plot for hydrogen bonding, (b) MD simulated structure, (c) NOEs observed, and (d) CD spectrum of **2**.

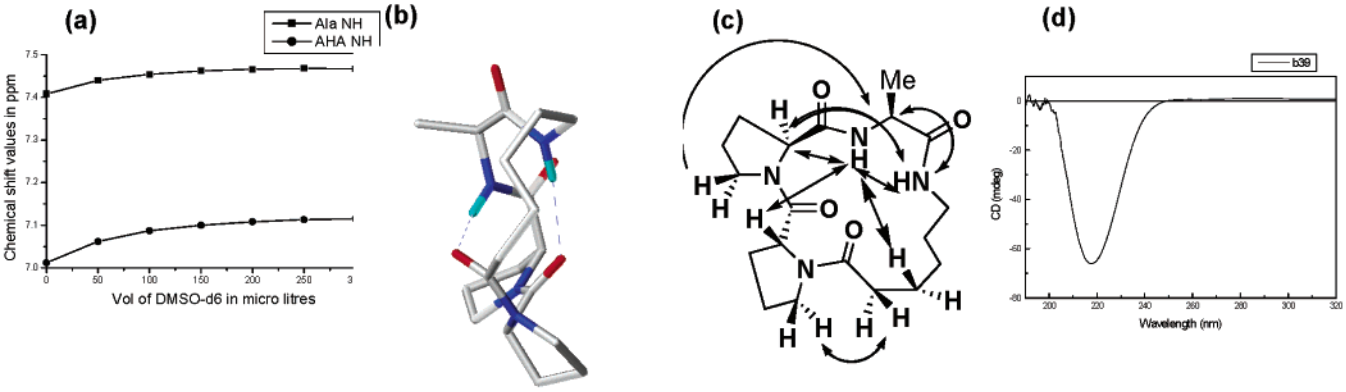


FIGURE 3. (a) NMR titration plot for hydrogen bonding, (b) MD simulated structure, (c) NOEs observed, and (d) CD spectrum of **3**.

successive β-turns in **2** is again supported by ROESY data. The MD calculations also confirm the existence of hydrogen bonding between Δ*aha* NH and C=O (D-*pro*) (Figure 2b). *It is worth mentioning that a β-turn need not have a C=O (i)−NH (i + 3) hydrogen bond*.[7] In both solvents, the medium/weak cross-peak intensities of sequential (L-*pro*C_αH/*ala* NH) compared to self-cross-peaks (*ala* NH/*ala* C_αH) in the NOESY/ROESY spectrum suggest that the second turn, which involves L-*pro*-*ala*, is a type I β-turn, while the first one involving D-*pro*-L-*pro* is a type II′ β-turn without forming intramolecular hydrogen bonding. However, the cyclization of the peptide results in distortion of the dihedral angles in **2**, which differ considerably from that of **1**. The circular dichroism studies on **2** in methanol also support a helical structure as evidenced by the appearance of minima at 222 nm (Figure 2d).

The cyclic peptide **2** was hydrogenated catalytically (H_2/Pd) to give the corresponding saturated cyclic peptide **3** containing a 6-aminohexanoic acid (*aha*) residue. Peptide **3**, a 16-membered macrocyclic peptide containing two natural and two unnatural amino acids, was extensively characterized by solution NMR as well as X-ray crystallographic data.[8] An ORTEP diagram of **3** and a crystal packing structure are presented in Figures 4 and 5, respectively. The conformation of the five-membered ring in D-proline is best explained as being half-chair (asymmetric parameter $\Delta C2 = 6.7$), and the five-membered ring in L-proline is assumed to be an envelope (asymmetric parameter $\Delta Cs = 6.5$) from observed endocyclic torsion angles.

The model with C1, C6, and C11 with absolute configurations *R*, *S*, and *S*, respectively, has been refined as known from the synthetic procedure. The geometry of the α-carbon of D-*pro* (C1) to the next α-carbon, i.e., C18 of *aha*, is trans (C1−N1−C19−C18 = −176.6), and the geometry of the α-carbon of L-*pro* (C6) to the next α-carbon, i.e., C1 of D-*pro*, is trans (C6−N2−C5−C1 = −172.4). The cyclic peptide possesses two successive β-turn hydrogen bonds between *aha* C=O(*i*) to ala NH(*i* + 3) and D-pro C=O(*i*) to *aha* NH(*i* + 3). According to the nomenclature of β-turns, the interatomic distances between Cα(*i*) to Cα(*i* + 3) should be less than 7 Å. In the present case, the distance in the first β-turn [C(19)=O···HN(3)] Cα(*i*) to Cα(*i* + 3) is found to be 5.186 Å, and for the second β-turn [C(5)=O···HN(4)] Cα(*i*) to Cα(*i* + 3) is found to be 5.581 Å. The first β-turn (D-*pro*-L-*pro*-L-*ala*-*aha*) belongs to type I, while the second β-turn (*aha*-

(6) For a review on the use of CD in study of protein structure and function, see: Kelly, S. M.; Price, N. C. *Curr. Protein Pept. Sci.* **2000**, *1*, 349−384.

(7) (a) Hutchinson, E. G.; Thornton, J. M. *Protein Sci.* **1994**, *3*, 2207. (b) Kessler, H.; Matter, H.; Gemmecker, G.; Kottenhahn, M.; Bats, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 4805−4818.

(8) Suitable crystals for X-ray diffraction were obtained from slow evaporation of a mixture of ethyl acetate and hexane solution. The peptide crystallizes as a monohydrate in acentric space group $P2_1$ in a monoclinic system. Both hydrogens of the lattice water participate in hydrogen bonding. Hydrogen H32 of O5 is involved in hydrogen bonding with carbonyl oxygen O3 of alanine, whereas the hydrogen H33 of O5 is involved in the hydrogen bonding with carbonyl oxygen O2 of L-proline related by a 2-fold screw leading to formation of a molecular chain along the "b" axis. The C_γ atom (C8) of L-proline is disordered in two positions with occupancies of 0.8 and 0.2. Crystallographic data (CIF) can be found in Supporting Information.

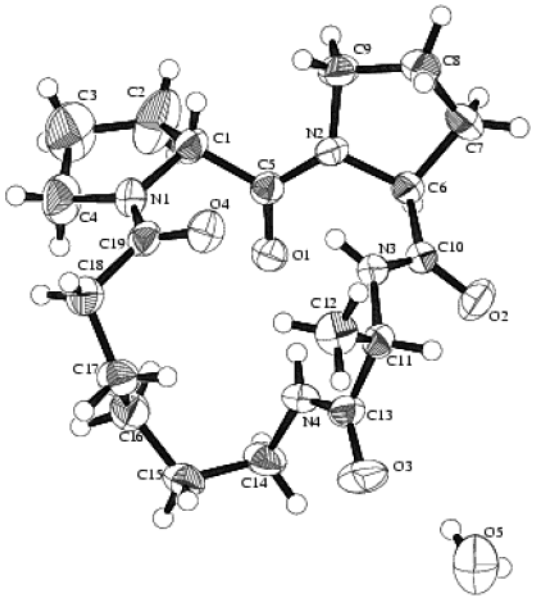


FIGURE 4. ORTEP view of **3**.

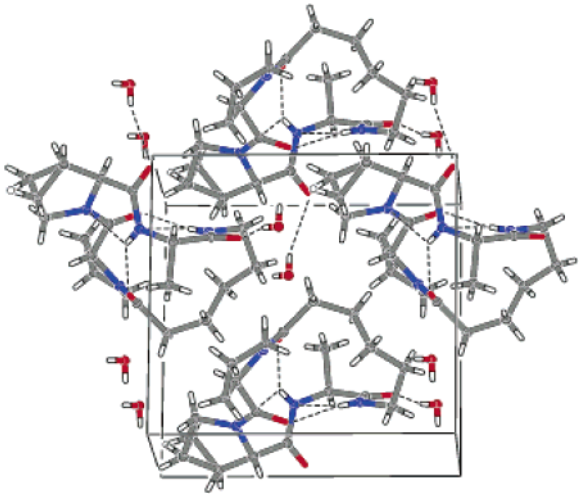
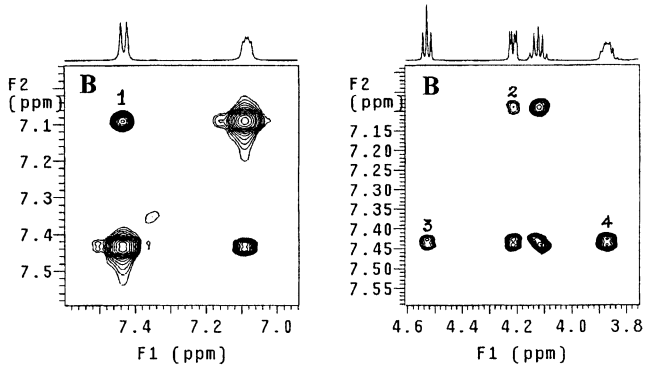


FIGURE 5. Crystal Packing Structure of **3**.

TABLE 1. Torsion Angles (*i* + 1, *i* + 2) of STD *β*-Turns and Observed *β*-Turns

β-turn	*φ*	*ψ*	*φ*	*ψ*
STD type II′ *β*-turn	60	−120	−80	0
observed type II′ *β*-turn	52.1	−138.5	−68.5	−13.4
STD type I *β*-turn	−60	−30	−90	0
observed type I *β*-turn	−68.5	−13.4	−74.4	−17.4

D-*pro*-L-*pro*-L-*ala*) is defined as type II′ as evidenced by the relevant torsion angles and comparison with those reported (as shown in Table 1). Interestingly, these two *β*-turns are successive and hence the peptide **3** has a propensity to exist in a 3_{10} helical structure. Indeed, there is a 3_{10} helical structure, as indicated by the relevant torsion angles ($\varphi = -68.5$ and $\psi = -13.4$ for the L-proline residue, and $\varphi = -74.4$ and $\psi = -17.4$ for the L-alanine residue), which compares reasonably with those proposed by Pauling for the 3_{10} helix ($\varphi = -74$, $\psi = -4$). In solution conformational studies, the ^{1}H NMR of **3** in $CDCl_3$ showed the presence of a single species. The *ala* NH and *aha* NH chemical shifts changed only by 0.06 and 0.10 ppm, respectively, when 300 μL of DMSO-d_6 was added to 600 μL of $CDCl_3$ solutions, confirming their participation in hydrogen bonding (Figure 3a). In addition to these hydrogen bonds, the presence of *ala* NH/D-*pro* $C_\alpha H$, *aha* NH/L-*pro* $C_\alpha H$, *ala* NH/L-*pro* $C_\delta H$, and *aha* NH/*ala* NH cross-peaks in the ROESY spectrum implies the presence of two successive *β*-turns about *aha*-D-*pro*-L-*pro*-*ala* and D-*pro*-L-*pro*-*ala*-*aha* residues, respectively (Figure 3c).



Spectrum B: Expanded region of NOESY spectrum of **3** in DMSO-d_6 shows some of the diagnostic NOE cross peaks. Ala NH/Aha NH (1), Aha NH/L-Pro $C_\alpha H$ (2), Ala NH/D-Pro $C_\alpha H$ (3), Ala NH/L-Pro $C_\delta H$ (4).

The studies in DMSO-d_6 show the presence of two isomers in a ratio of 99:1. The major isomer has all *trans*-amide bonds and a structure that is similar to that in $CDCl_3$. The appearance of cross-peaks in the ROESY spectrum between *ala* NH/D-*pro* $C_\alpha H$, *aha* NH/L-*pro* $C_\alpha H$, *ala* NH/L-*pro* $C_\delta H$, and *ala* NH/*aha* NH (Spectrum B) and $\Delta\delta/\Delta T$ of −3.4 and −3.1 ppb/K for *aha* NH and *ala* NH, respectively, support the presence of two successive *β*-turns. The appearance of *aha* NH as "*dd*" implies that the molecule contains a single predominant conformation in solution. The MD calculations show that the structure of **3** has two successive *β*-turns (Figure 3b). It is also noteworthy that like **2**, the first turn in **3** and also the first turn in **1** are type II′ and the second one is a type I *β*-turn. The appearance of minima at 220 nm in the CD spectra of **3** confirms its helical structure (Figure 3d).

In conclusion, we have demonstrated for the first time that tripeptides based on a heterochiral D-*pro*-L-*pro* template show a propensity to exist in a 3_{10} helical structure in nonpolar and polar solvents. The helical structure in X-ray diffraction and solution is also retained in the corresponding cyclic peptide obtained by RCM reaction. We are currently pursuing the synthesis and conformational properties of other cyclic peptides based on a D-*pro*-L-*pro* template as potent vaccines.

Acknowledgment. We thank Dr. Reddy's Laboratories, Hyderabad, India, for financial support of this work. We are grateful to Dr. K. N. Ganesh, NCL, Pune, India, for helping us in CD studies.

Supporting Information Available: Full experimental details and spectral data (NMR, mass, IR) of compounds **1**−**3** and a CIF file are included. This material is available free of charge via the Internet at http://pubs.acs.org.

JO030282W