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Synthesis of a Novel *cis*-Proline-Derived Cyclic Type VI β-Turn Mimic via Ring-Closing Metathesis†

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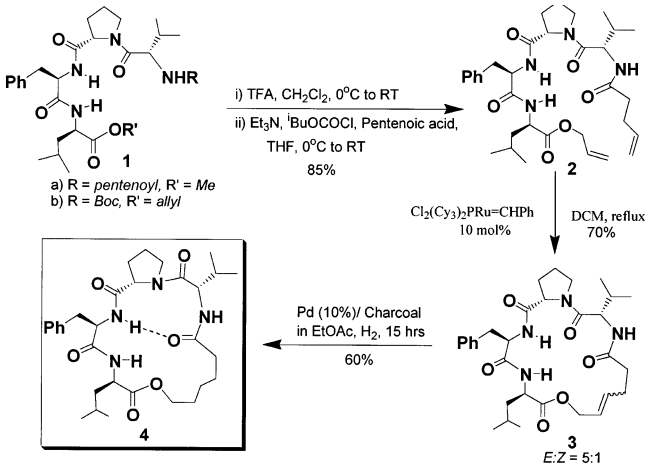
Abstract: A *cis*-proline derived cyclic mimic of a type VI β-turn is synthesized via a ring-closing metathesis reaction. The solution NMR conformational study indicates that the major conformer of the cyclic peptide adopts a type VIa β-turn in $CDCl_3$ and a type VIb β-turn in DMSO-d_6.

Proline is the only cyclic proteinogenic amino acid that plays a significant role in the structural and conformational properties of peptides and proteins.[1] It has the unique ability to undergo cis−trans isomerization about the imide bond linking proline and the preceding amino acid residue. Despite its rare occurrence, the *cis*-proline−imide bond is present in various biologically important molecules.[2] It is also known that the *cis*-proline residue alters the backbone chain direction through a type VI β-turn and is often observed to terminate α-helices.[3] The type VI β-turn is a relatively less common protein secondary structure involving a *cis* imide bond N-terminal to the L-proline residue situated at the $i + 2$ position.[4] This particular motif plays a significant role in protein folding and has a profound influence on the recognition process involving protein−ligand interaction.[55] Aromatic amino acids preceding the proline residue have been found to stabilize the *cis*-proline imide bond conformations.[6]

In an ongoing study[7] in our laboratory on the development of the small cyclic peptides as protease inhibitors,[8] we have observed that a tetrapeptide, *N*-cinnamoyl-val-pro-phe-leu-methyl ester **1a** (Scheme 1), exists in a 3_{10} helical structure.[9] To synthesize the L-proline-derived cyclic 3_{10} helical structure via ring-closing metathesis,[10] we have installed a pentenoyl group at the N-terminus of **1a** and an allyl ester moiety in the C-terminus to get the precursor. The synthesis of the peptide **2** was achieved as shown in the Scheme 1. *O*-allyl-*N*−Boc-val-pro-phe-leu, **1b**, was synthesized by usual amide coupling protocols. It was then treated with trifluoroacetic acid (TFA) and finally coupled with pentenoic acid by mixed anhydride reaction (^{i}BuOCOCl/Et_3N) to get the acyclic precursor **2** in 85% yield.

SCHEME 1. Synthesis of *cis*-Proline-Derived Cyclic Type VI β-Turn Mimic 4



The peptide **2** afforded the corresponding cyclic peptide **3** as a mixture of *E*/*Z* isomers (5:1) in good yield (70%) when subjected to RCM using Grubbs' "Ru"-catalyst. Detailed solution ^{1}H NMR, molecular dynamics (MD), and circular dichroism (CD) studies show an interesting conformational behavior for these peptides (Figure 1). It is noteworthy that unlike the acyclic peptide **1a**,[11] having

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(11) The peptide **1a** showed a 3_{10} helical conformation (unpublished work).

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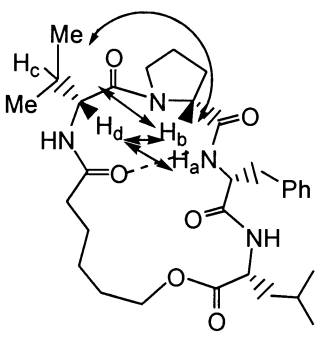
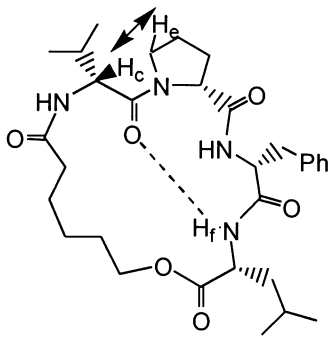
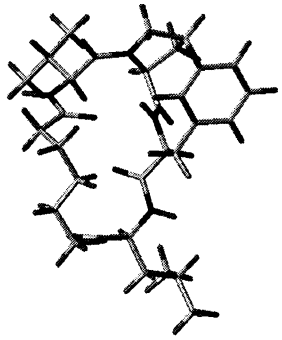


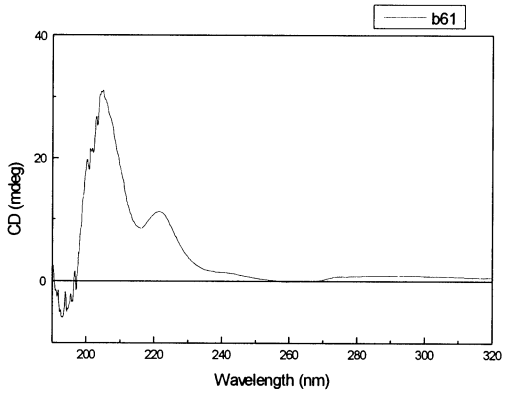
(a) *Cis*-4



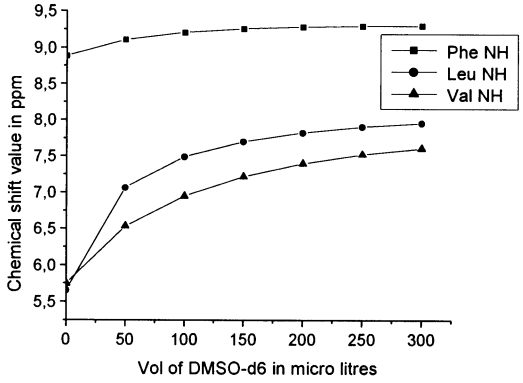
(b) *Trans*-4



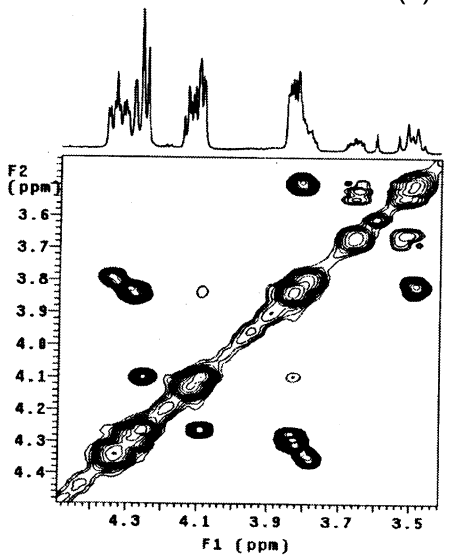
(c) *MD picture of Cis*-4



(d) *CD spectra of* 4



(e) ^{1}*H NMR titration on Cis*-4



(f) *ROSEY spectra of Cis*-4

FIGURE 1. (a, b) Different NOEs observed in **4**, (c) Molecular Dynamics simulation picture of *cis*-**4**, (d) circular dichroism spectra, (e) ^{1}HNMR titration curve, and (f) ROSEY for *cis*-**4**.

a 3_{10} helical organization, **2** does not possess any organized structure. Similarly, the corresponding cyclic peptide **3** did not show the presence of any predominantly organized structure as the solution ^{1}H NMR indicated a mixture of species without any preferred conformation. To facilitate the formation of an intramolecular hydrogen bond in **3**, we removed the constraint of the double bond in the linker by subjecting it to catalytic hydrogenation (Pd/C, H_2), leading to a relatively more flexible cyclic peptide **4**. It exhibited interesting conformational properties which are described below.

Detailed solution ^{1}H NMR studies on peptide **4** revealed the presence of a type VI β-turn structure involving a *cis*-proline−imide bond geometry in it. In both $CDCl_3$ and DMSO-d_6 solvent, the major conformer of **4** (*cis*-**4**) exhibited a β-turn involving a *cis*-proline imide

geometry (cis/trans: 63:37 in $CDCl_3$ and 77:23 in DMSO-d_6). The NOE cross-peaks in $CDCl_3$ between *pro* $C_\alpha H_b$/*val* $C_\alpha H_d$ confirms the existence of the cis conformation in the ROSEY spectra (Figure 1f).

The downfield chemical shift of the *phe* NH_a (δ 8.90 ppm) in *cis*-**4** indicated its participation in the intramolecular hydrogen bonding. This was further supported by a small variation of *phe* NH_a chemical shift during the solvent titration studies while 33% v/v of DMSO-d_6 was added to the $CDCl_3$ solution (Figure 1e). The presence of NOE cross-peaks between *val* $C_\alpha H_d$/*pro* $C_\alpha H_b$, *val* CH_3/*pro* $C_\alpha H_b$, *phe* NH_a/*val* $C_\alpha H_d$, and *phe* NH_a/*pro* $C_\alpha H_b$ coupled with the *phe* NH_a hydrogen bond implies that it is a type-VIa β turn around *val*-*pro* residues in *cis*-**4**. The $J_{NH-C\alpha H} = 3.6$ Hz for the valine residue corresponds to an φ angle of about $-60°$ which further supports the turn structure. The other structure of **4** (*trans*-**4**) has a *trans*-proline imide bond which was characterized on the basis of NOE cross-peak between *pro* $C_\delta H_e$/*val* $C_\alpha H_c$. The appearance of *leu* NH_f at downfield (δ 7.99 ppm) indicates its participation in H-bonding ($\Delta\delta/\Delta T = -2.4$ ppb/K), which may nucleate a 10-membered β-turn structure in *trans*-**4** (Figure 1b). In DMSO-d_6, the major conformation (*cis*-**4**) was characterized to have a cis imide bond preceding the proline residue by the observation of an NOE cross-peak between *pro* $C_\alpha H_b$/*val* $C_\alpha H_d$ in the ROESY spectrum. However, the large values of all the amide proton's temperature coefficients ($\Delta\delta/\Delta T = -5.9-5.5$ ppb/K) indicate the absence of any intramolecular hydrogen bonding. The appearance of ROE cross-peaks between *val* $C_\alpha H_d$/*pro* $C_\alpha H_b$, *val* CH_3/*pro* $C_\alpha H_b$, *val* $C_\beta H_c$/*pro* $C_\alpha H_b$, and *phe* NH_a/*val* $C_\alpha H_d$ as well as the $J_{NH-C\alpha H} = 2.5$ Hz for the *valine* residue suggests presence of a β-turn about the *val*-*pro* residues in *cis*-**4**. However, the nonparticipation of *phe* NH_a in intramolecular hydrogen bonding indicates the presence of a type VIb β-turn structure in DMSO-d_6. It is known that the type VIb β-turn structure does not contain any intramolecular hydrogen bond.[12] The MD simulation studies on *cis*-**4** also showed the presence of cis imide bond across the *pro*-*phe* residue (Figure 1c). The CD spectra of the peptide **4** also indicate the presence of a type VI β-turn structure (Figure 1d). Similar curve shape for type VI β-turn was also reported[12c] recently by Lubell and Halab. All these data unambiguously support the presence of a cis-proline imide bond and the type VI β-turn structure for the major conformer of peptide **4** in $CDCl_3$ and DMSO-d_6 medium.

In conclusion, this paper describes the synthesis of a novel *cis*-proline derived cyclic tetrapeptide having a unique type VI β-turn structure. The nature of the turn is influenced by the solvent as the solution ^{1}H NMR conformational study indicates that the major conformer of the cyclic peptide adopts a type VIa β-turn in $CDCl_3$ and a type VIb β-turn in relatively more polar DMSO-d_6 solution. The presence of *cis*-*pro* imide geometry is quite unique and the conformationally constrained small cyclic peptides having a cis imide bond are valuable tools for probing the protein−ligand interactions.

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Supporting Information Available: Spectral data (NMR, mass, IR) and experimental procedures for the synthesis of compounds **2**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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