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Synthesis of Small Cyclic Peptides via Reverse Turn Induced Ring Closing Metathesis of Tripeptides

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Received January 15, 2002

Abstract: A reverse turn induced (γ/β-turn) cyclization of tripeptides **1** can be performed in a ring closing metathesis reaction with Grubbs' catalyst to the corresponding cyclic peptides **2**. These cyclic peptides may be useful probes as a conformationally constrained mimic of the bioactive conformation of structurally related HIV protease inhibitors.

The synthesis and screening of huge peptide libraries has led to the emergence of small peptides as important lead structures for the development[1] of potential therapeutic agents. It is known that the linear peptide fragments are flexible and exhibit numerous conformations in solution; however, if one can restrict the conformational freedom of these linear peptides by introducing[2] some constraints in the structure, it may render a biologically active peptide more potent, more specific, and orally active and this may give rise to species which are therapeutically useful. Thus, small cyclic peptides are of great interest for the elucidation of bioactive conformations due to their restricted conformational flexibility. In view of the importance of constrained conformations, there have been several attempts[3] to lock peptides into turn configurations and to synthesize molecules that might mimic a reverse turn present in proteins. Several types of turns are found and among them the type I and type II β-turns are most commonly observed in protein secondary structures. Therefore the understanding of the conformation of type I and II β-turn is very crucial to the development of inhibitors of HIV protease.[4]

In a previous communication[5] relating to the work on the search for HIV protease inhibitors based on pyrrolidine-containing α-hydroxy β-amino amide core structures, we have demonstrated that the later structural unit can be incorporated into a cyclic peptide obtainable by ring closing metathesis. Thus the presence of the known transition state analogue in the cyclic peptide may make them interesting as potential HIV protease inhibitors. It was reasoned that the tripeptides derived from sequence where L-proline is in the γ-turn forming $i+1$ position[6] would be an ideal precursor for such cyclizations. This assumption was vindicated as we had shown that the ring closing metathesis on a tripeptide such as *O*-allyl-Xaa-L-proline−β-phenylisoserine-*N*-allyl **1a** (Figure 1) led to cyclic peptide **2a**. We proposed that this cyclization was dictated by the presence of a γ-turn that was supported by the fact that tripeptides **1b**, consisting of L-proline residue in the $i+2$ position, did not yield any corresponding cyclic peptide on ring closing metathesis. Clearly the tripeptide **1b** lacks the intramolecular hydrogen bond and this led us to believe that the preorganization of such structures may be a necessary condition for ring closing metathesis. To assess the role of the intramolecular hydrogen bond in these cyclizations, we have further carried out studies on acyclic peptides capable of forming an intramolecular ten-membered hydrogen bond, i.e., a β-turn, and a detailed account of these findings is given below.

The design of these cyclic peptides is based upon the concept of mimicking the bioactive conformation by introducing constraints in the flexible molecules through their cyclization. We now show that tripeptides **3** derived from *O*-allyl-Xaa-L-proline-β-phenylisoserine-*N*-allyl or *O*-allyl-L-proline-Xaa-β-phenylisoserine-*N*-allyl derivatives are preorganized due to γ- or β-turn and can be cyclized by ring closing metathesis (Scheme 1 and 2) with Grubbs' catalyst.[3a] It is also demonstrated that irrespective of the position of L-proline in the tripeptide, the cyclization is facile only when a γ- or β-turn is present in such structures. The precursor peptides **3a**−**d** were prepared from *N*-cinnamoyl amino acids by conventional coupling, using mixed anhydride protocol followed by *O*- or *N*-allylation of the C-terminal end of the resulting peptides.

These tripeptides were found to exist as preorganized structures due to the presence of a γ- or β-turn formed by intramolecular hydrogen bonds. The presence of the intramolecular hydrogen bond was studied by ^{1}H NMR of the tripeptides **3a**−**d** in dilute $CDCl_3$ solution in the presence of different concentrations of DMSO-d_6. The δ_{NHa} and δ_{NHb} in **3a** and **3b** indicate (Table 1) that these amide protons appear at lower field as compared to δ_{NHa} for **3d**, suggesting a possible intramolecular hydrogen

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10.1021/jo0200320 CCC: $22.00
Published on Web 10/25/2002

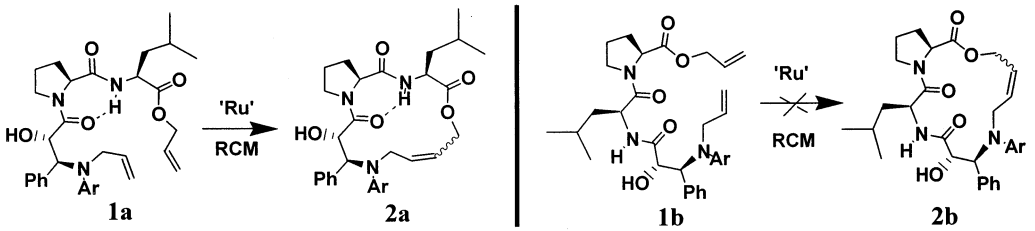
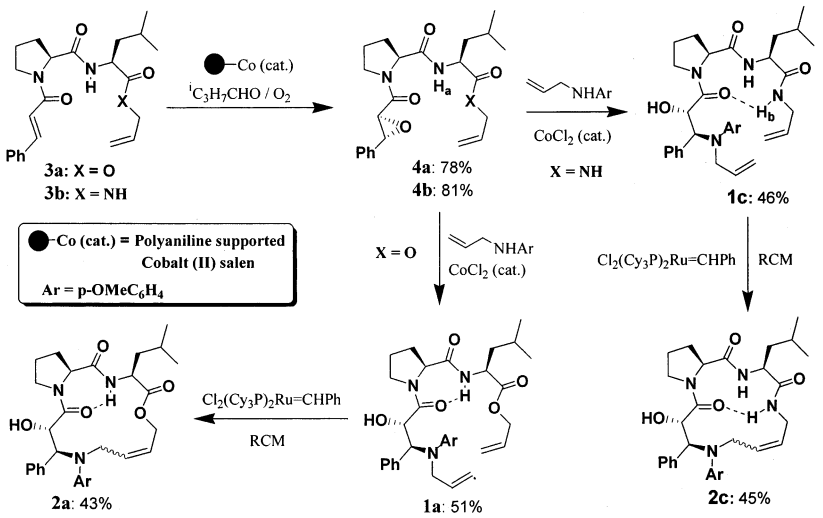
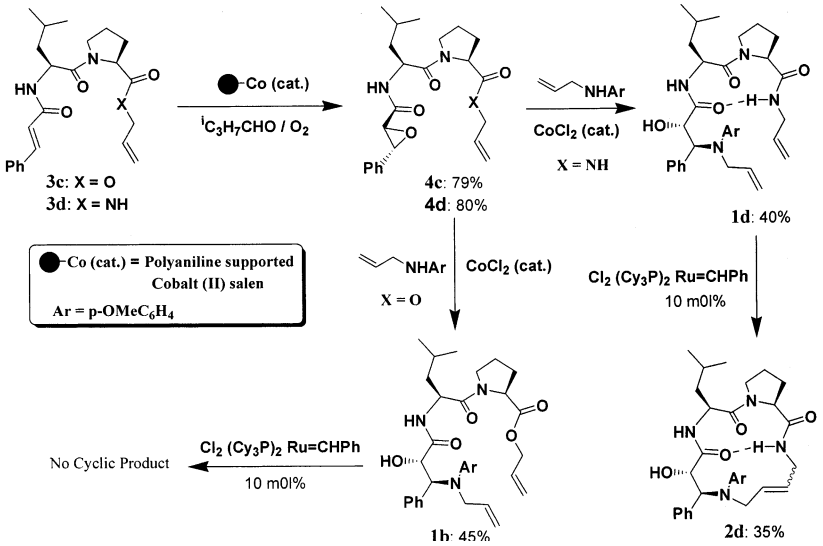


FIGURE 1.

SCHEME 1



SCHEME 2



bond. On the other hand, the δ_{NHa} values in **3c** and **3d** suggest that these amide protons are not involved in hydrogen bonding whereas the δ_{NHb} chemical shifts are supportive of an intramolecular hydrogen bond. Also the value in variable-temperature ^{1}H NMR suggests that peptide **3c** lacks the intramolecular hydrogen bonding while for **3a**, **3b**, and **3d** it falls in a range indicative of the presence of a β- or γ-turn. The FT-IR spectra (Table 1) of peptides **3a**−**d** (3250−3350 cm^{-1}) also reveal that **3a**, **3b**, and **3d** possess an intramolecular hydrogen bond whereas **3c** (∼3550 cm^{-1}) is devoid of it. The ^{1}H NMR in $CDCl_3$ solution showed that **3a** exists predominantly (80%) as the trans rotamer. The leu NH_a in **3a** appeared at lower field (compared to NH_a in **3b**) with a δ value of 7.67 ppm, indicating its possible participation in H-bonding. Additional support for such a H-bond came from solvent titration studies where addition of up to 33% DMSO (v/v) in $CDCl_3$ shifted the resonance signal only by 0.4 ppm. The most likely donor/acceptor in **3a** would be NH_a and the carbonyl of the cinnamoyl group. A strong pro$_\alpha$H$_c$-leu NH_a NOESY peak in addition to the above-mentioned H-bonding implies the propensity of a γ-turn in the molecule (Table 1). The presence of a β-turn in **3b** is quite evident from variable-temperature experiment

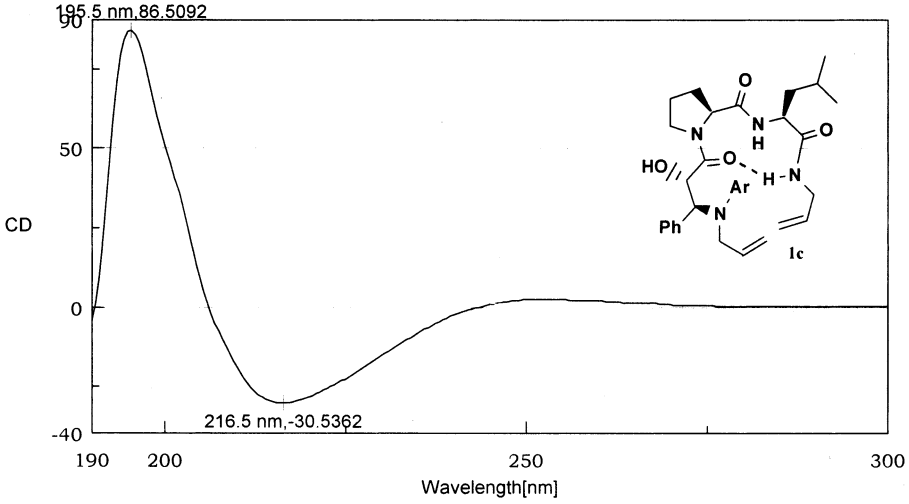
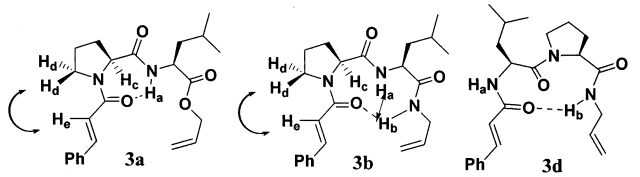


FIGURE 2. CD spectra of **1c** in acetonitrile.

TABLE 1. Amide Chemical Shifts (ppm) in $CDCl_3$, FT-IR (cm^{-1}, CH_2Cl_2), and NOE's in 3a, 3b, and 3d



compd	δ_{NH_a}	δ_{NH_b}	Ir−H_a	Ir−H_b
3a	7.67		3324	
3b	7.78	7.98	3265	3305
3d	6.34	7.56	3506	3354

where the low magnitude of the chemical shift/temperature coefficient ($\Delta\delta/\Delta T$) for the amide proton (NH_b) is an indicator of its participation in H-bonding. Expectedly the ($\Delta\delta/\Delta T$) values for the cis isomer of **3b** have a large magnitude (($\Delta\delta/\Delta T = -6$ ppb/°C) for both the NH's while it is moderately small for the trans isomer ($\Delta\delta/\Delta T = -2.4$ ppb/°C for allyl NH_b and −4.5 ppb/°C for leu NH_a). This suggests that a sizable fraction of the trans isomer has allyl NH_b participating in H-bonding. The presence of such a H-bond coupled with the existence of NOESY peaks between leu NH_a-allyl NH_b and strong Pro $C_\alpha H_c$-leu NH_a suggests the preference of a β-turn around pro-leu in **3b** (Table 1). Interestingly, the ^{1}H NMR of **3d** in $CDCl_3$ showed the presence of a mixture of conformations and it was not possible to correlate any NOESY peaks from the spectrum. However, the solvent titration studies upon addition of up to 33% DMSO (v/v) in $CDCl_3$ showed the shift of NH_b resonance signal only by 0.3 ppm, indicating the presence of an intramolecular hydrogen bond in **3d**.

The tripeptide derivatives **1** were prepared by our polyaniline supported cobalt catalyzed[7] aerobic epoxidation and its opening protocol as described earlier (Scheme 1). The peptides **3a** and **3b** were subjected to aerobic epoxidation in the presence of 2-methylpropanal and a catalytic amount of polyaniline supported cobalt(II) salen to yield the corresponding epoxides **4a** and **4b**, respectively, obtained after purification by chromatography as a single diastereomer in good yields. The absolute stereochemistry (2*R*,3*S*) for these epoxides was assigned based on the correlation studies as described in an earlier[5a] communication. The opening of epoxides **4a** and **4b** was achieved with *N*-allyl anisidine in the presence of a catalytic amount of cobalt(II) chloride to afford the corresponding tripeptide derivatives **1a** and **1c**, respectively, mainly as the anti diastereomer in 55−60% yields. We have shown[7b,c] earlier that the cobalt-catalyzed opening of cinnamoyl epoxide takes place by an S_N2 pathway leading to the anti diastereomer as the predominant product.

The tripeptides **1a** and **1c** also showed the presence of intramolecular hydrogen bonding as indicated by the appearance of a low-field NMR signal at 7.43 ($J = 8$ Hz) for **1a** and 7.16 ($J = 8.2$ Hz) for **1c**. Also the support for the intramolecular hydrogen bond came from solvent titration studies where addition of up to 33% DMSO (v/v) in $CDCl_3$ shifted the resonance signal NH_a in **1a** by 0.35 ppm and that of NH_b in **1c** by 0.4 ppm. A small shift in amide (NH_b) signal in **1c** is indicative of a hydrogen-bonded/solvent-shielded proton like those involved in C^{10} β-turn conformations. The CD spectra[8] of **1c** also indicated the presence of a β-turn as it most closely resembled a type II β-turn with a maxima at 195.5 nm and a minima at 216.5 nm (Figure 2). The presence of an intramolecular hydrogen bond in **1a** and **1c** suggests that these molecules are preorganized due to the presence of a γ-turn (i.e., **1a**) and a β-turn (i.e., **1c**) which may facilitate the cyclization of these acyclic peptides via ring closing metathesis with Grubbs' catalyst.[3a] This indeed was found to be the case, as subjecting **1a** and **1c** to heating in the presence of 10 mol % of ruthenium alkylidene (Grubbs' catalyst) in dichloromethane (0.6 mM) for 12−30 h yielded the cyclized peptides **2a** and **2c**, respectively, in 40−45% yields after column chroma-

(7) For the synthesis and reaction of this catalyst see: (a) Das, B. C.; Iqbal, J. *Tetrahedron Lett*. **1997**, *38*, 2903. (b) Punniyamurthy, T.; Iqbal, J.; *Tetrahedron Lett*. **1997**, *38*, 4463. (c) De, A.; Basak, P.; Iqbal, J. *Tetrahedron Lett*. **1997**, *38*, 8383.

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tography. It is noteworthy though not particularly surprising that the tripeptide **1c** underwent smooth ring closing metathesis in a relatively shorter period (12 h) as compared with the reaction time taken (30 h) by **1a** for similar cyclization. *This difference in the reaction time suggests that the cyclization of* **1c** *is facile due to the presence of a β-turn, which appears to preorganize these structures much more efficiently than the γ-turn present in* **1a**. These cyclic peptides were obtained as a mixture of *E:Z* (4:1) isomers as indicated by the ^{1}H NMR. The reaction mixture also consisted of some minor oligomeric products and ~20% of the tripeptides **1** were recovered unchanged. The ^{1}H NMR titration with DMSO-d_6 (δ 7.45 ppm, J = 8.23 Hz) of the cyclic peptides **2c** revealed the presence[2] of the intramolecular hydrogen bonding, which clearly suggests that a β-turn is also present in the cyclic form and might have been responsible for the cyclization.

That the presence of a γ-turn/β-turn may be responsible for such cyclization is evident from the ring closing studies on the acyclic tripeptides **1b** and **1d** containing L-proline at the i+2 position of the residue (Scheme 2). The tripeptides **1b** and **1d** were synthesized by the protocol as described earlier and accordingly **3c**,**d** were transformed to the corresponding *N*-cinnamoyl epoxides **4c**,**d** which were subjected to cobalt(II) chloride catalyzed opening by *N*-allyl anisidine to afford the tripeptides **1b** and **1d**, respectively (Scheme 2). The tripeptides **1b** and **1d** were subjected (0.6 mM in dichloromethane) to ring closing metathesis with Grubbs' catalyst and as expected the corresponding cyclic peptide from **1b** was not observed, instead the reaction mixture consisted of intractable oligomeric material apart from the unreacted **1b** (20−30%), whereas **1d** afforded the corresponding cyclic peptide **2d** in 35% yield after column chromatography. The cyclic peptide **2d** was obtained as a mixture of *E:Z* (4:1) isomers as indicated by NMR. The reaction mixture also consisted of some minor oligomeric products and ~20% of the tripeptide **1d** was recovered unchanged. This study clearly suggests that absence of a γ-turn in **1b** will not render these tripeptides preorganized for cyclization under ring closing metathesis conditions. On the contrary the tripeptide containing an allyl amide **1d** underwent ring closing metathesis leading to the synthesis of the cyclic peptide in moderate yields. The cyclic peptide **2d** showed the presence of the intramolecular hydrogen bond in ^{1}H NMR and FT-IR. A careful analysis of the ^{1}H NMR of **2d** revealed the presence of two intramolecular hydrogen bonded species which were found to be in equilibrium. The equilibrium ratio was altered upon addition of DMSO-d_6, indicating that these hydrogen bonded species rapidly equilibrate between different bonded and nonbonded structures. These observations also reflect the moderate yield of the cyclic peptide **2d** as the presence of the different bonded and nonbonded species in **1d** is likely to yield products arising due to intra- as well as intermolecular reactions during ring closing metathesis with Grubbs' catalyst.

The molecular dynamics simulation studies[9] on **1c** and **2c** also supported the presence of an intramolecular hydrogen bond leading to the β-turn. The bond distance of 2.1 Å clearly indicates that **1c** has the propensity to exist in the form of a β-turn (Figure 3). It is also evident from these structures that the terminal double bonds in

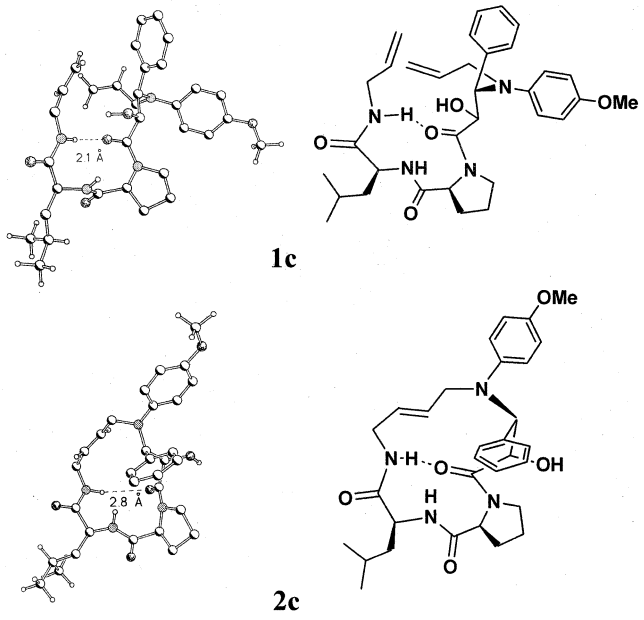

FIGURE 3. Simulated low-energy conformer for **1c** and **2c**.

1c are in close proximity for cyclization during ring closing metathesis.

In conclusion, we have synthesized cyclic peptides consisting of Xaa-L-proline-β-phenylisoserine tripeptides from the acyclic precursors having an olefinic group at both ends via ring closing metathesis using Grubbs' catalyst. These cyclizations are controlled by the presence of a γ-turn/β-turn in the acyclic precursor and the cyclic peptides thus obtained may be a useful probe for understanding the role of constrained structures in the search for a bioactive conformation of species related to HIV protease inhibitors.

Acknowledgment. We thank DST, New Delhi and Dr. Reddy's Laboratories Ltd., Hyderabad for the financial support of this work. We are grateful to Dr. Ram Thaimattam for helping us on molecular dynamics simulation studies.

Supporting Information Available: Spectroscopic and analytical data for **1a**, **1b**, **1c**, **1d**, **2a**, **2c, 2d**, ^{1}H NMR titration spectra for **1c** and **2c**, along with the NOESY spectra of **3a** and **3b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Molecular dynamics (MD) calculations for compounds **1c** and **2c** were carried out with the Cerius^2 program on a Silicon graphics Indigo^2 workstation. Charges were calculated by using the charge-equilibration method. The CFF9 force field with default parameters was used throughout the simulations. To understand the conformational freedom, simulated annealing molecular dynamics calculations were carried out. The temperature was varied between 300 and 1200 K in steps of 50 K for 100/150 cycles. The molecules were allowed to equilibrate for 0.5 ps for every change in temperature. Minimizations were done first with steepest descent, followed by conjugate gradient methods for a maximum of 1000 iterations each or a root-mean-square deviation of 0.01 kcal/mol, whichever was earlier. The energy-minimized structures were then subjected to MD simulations. Various conformers obtained in each MD run were minimized by using the above-mentioned minimization protocol. The energy difference between the lowest energy conformer and the next higher energy conformer was 5.6 kcal/mol for **1c** and 8.35 kcal/mol for **2c**.