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Synthesis of cyclic β-turn mimics from L-Pro-Phe/Phe-L-Pro derived di- and tripeptides via ring closing metathesis: the role of chirality of the Phe residue during cyclization

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Abstract—Pent-4-enoyl-L-Pro-Phe-N-allyl/pent-4-enoyl-Phe-L-Pro-N-allyl and pent-4-enoyl-L-Phe-Pro-Gly-N-allyl/pent-4-enoyl-Gly-Pro-Phe-N-allyl amide derived di- and tripeptides can be cyclized leading to β-turn mimics via ring closing metathesis using Grubbs' catalyst. The chirality of the Phe residue in these di- and tripeptides controls the cyclization during ring closing metathesis. The presence of pent-4-enoyl and allyl groups at the termini of these peptides leads to the concomitant formation of the amino acid, 4,5-dehydro-6-aminocaproic acid, as a linker, during cyclization.

One of the major challenges in the domain of rational drug design is to understand the bound conformation of bioactive peptides.[1] Generally, peptides are difficult to develop as drugs and this limitation has necessitated the use of peptidomimetics as potent therapeutic agents in recent years. In addition to circumventing the problems of poor bioavailability and proteolytic degradation, peptidomimetics are also designed to mimic the 'bioactive conformation' which enhances the affinity of such structures to the target. Peptidomimetics[2] based on β-turns[3] are attractive mimics of the 'bioactive conformations' because numerous peptides elicit a biological response via such a conformation. Earlier work on β-turn mimicry has resulted in the synthesis of rigid heterocyclic units that are incorporated into a polypeptide chain in place of the central dipeptide of the reported turn. Aside from this, several other protocols have been developed which involve mimicry based on constraining the dipeptide in question with a linker consisting of heteroatoms or aromatic groups in a carbon chain.

In an ongoing program in our laboratory on the discovery of small molecule ligands inhibiting HIV-I protease,[4] we have undertaken the synthesis of cyclic peptides based upon the dipeptides derived from L-*proline* and L-/D-*phenylalanine*. The choice of this dipeptide is dictated by the fact that HIV protease is very specific in cleaving the peptide bond between these two residues (Fig. 1) and the synthesis of the cyclic β-turn mimics derived from *Pro–Phe* may lead to the development of small inhibitors characterized by high affinity, bioavailability and resistance to proteolytic degradation. In this paper, we report the synthesis of cyclic peptides derived from *Pro–Phe* by ring closing metathesis (RCM). It is noteworthy that RCM cyclization leads to the formation of 4,5-dehydro-6-aminocaproic acid as a surrogate for the third/fourth amino acid.

In order to probe the conformational features of such cyclizations we needed to find out the positional requirements of L-*proline* in these peptides. Thus, dipeptides derived from *Pro-XAA* or *XAA-Pro* would

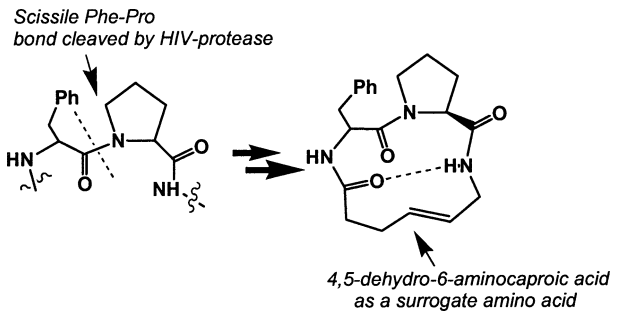

Figure 1.

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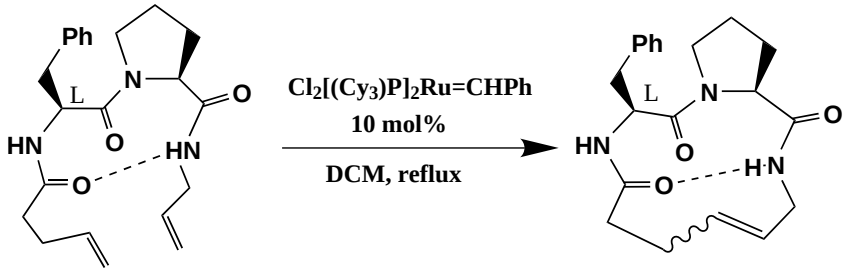
provide an interesting study on their folding properties. In this regard we have chosen *phenylalanine* as an amino acid and synthesized pent-4-enoyl-L-*Phe*-L-*Pro*-*N*-allyl amide **1a** (Scheme 1) by the usual amide coupling procedures.[5] We have used *N*-pent-4-enoyl and *N*-allyl amides at the two ends of these peptides so that RCM[6] involving these terminal double bonds would result in formation of 4,5-dehydro-6-aminocaproic acid as linker.

The intramolecular hydrogen bonding properties of **1a** were studied by its solution NMR, which showed it to be organized in a 10-membered β-turn fashion. In order to prove this we carried out a deuterium exchange study[7] on **1a** using CD_3OD in $CDCl_3$ which showed that no deuterium exchange took place for the bonded amide proton ($allyl_{\delta NH}=7.04$ ppm; IR: 3360 cm^{-1}) even after 5 hours. So having established the presence of the β-turn in **1a**, we subjected it to RCM using Grubbs' ruthenium catalyst.[8] It was gratifying to find that this peptide cyclized smoothly to the corresponding cyclic peptide **2** in good yield (Scheme 1). It is also interesting to note that the β-turn, which was present in the acyclic precursor **1a**, was retained ($allyl_{\delta NH}=7.26$ ppm; IR: 3300 cm^{-1}) in the cyclic peptide **2**. The β-turn in **1a** and **2** is also evident from the CD spectra[9] (acetonitrile), which shows an intense maximum at 195.5 nm and a minimum near 216.5 nm for the former whereas in the latter case a maximum at 201.5 nm and a minimum around 232 nm was observed (Chart 1). These values suggest that peptides **1a** and **2** both exist in a type II β-turn which agrees with a similar observation by Aube and co-workers.[9c] We were also interested in investigating the role of chirality in this peptide and thus synthesized the corresponding acyclic peptide with inverted configuration at the i+1 residue. The corresponding peptide, pent-4-enoyl-D-*Phe*-L-*Pro*-*N*-allyl amide **1b** was synthesized and subjected to RCM using Grubbs' catalyst. No cyclization took place, thereby indicating the role of ϕ and ψ angles in bringing the terminal double bonds into close proximity.

Interestingly, **1b** did not show the presence of any intramolecular hydrogen bonding ($allyl_{\delta NH}=6.62$ ppm; FTIR: 3297 cm^{-1}) when it was subjected to ^1H NMR deuterium exchange studies ($CD_3OD/CDCl_3$).

We also wanted to probe the influence of the chirality of the amino acid residue at the C-terminus of L-*proline* and accordingly synthesized pent-4-enoyl-L-*Pro*-L-*Phe*-*N*-allyl amide **3a**. The solution NMR study of the peptide **3a** indicated the absence of any intramolecular hydrogen bonding ($allyl_{\delta NH}=6.61$ ppm; IR: 3290 cm^{-1}), suggesting that here the two terminal olefinic bonds may not come in close proximity. Indeed, it was found that no corresponding cyclic peptide was obtained on subjecting **3a** to RCM conditions.[10]



1a (L-Phe-L-Pro; β-*turn*) **2** (68%; *E*:*Z* = 4:1)

Scheme 1.

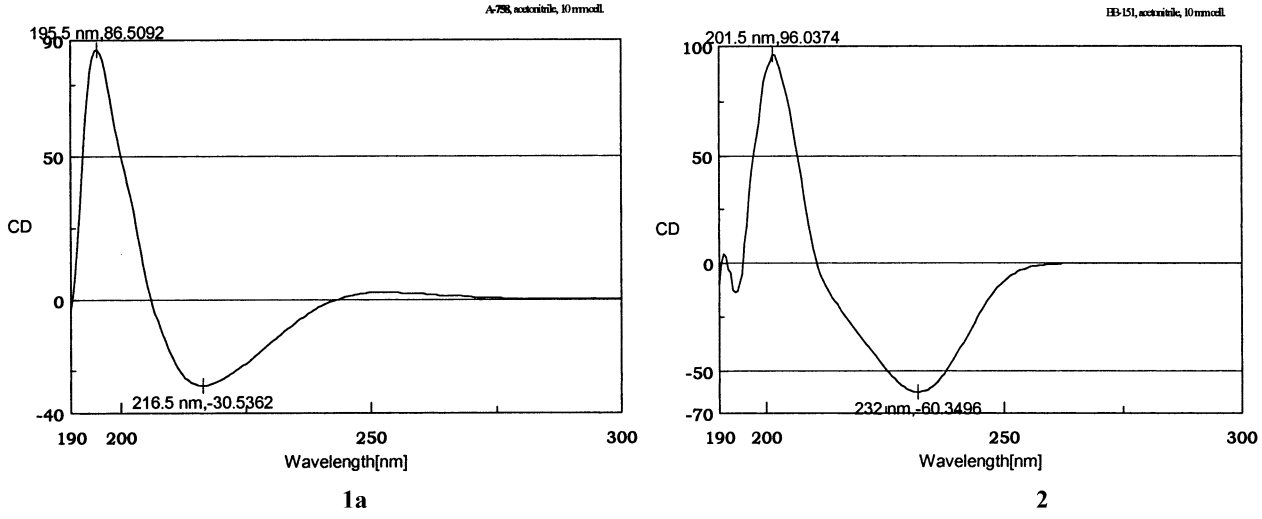
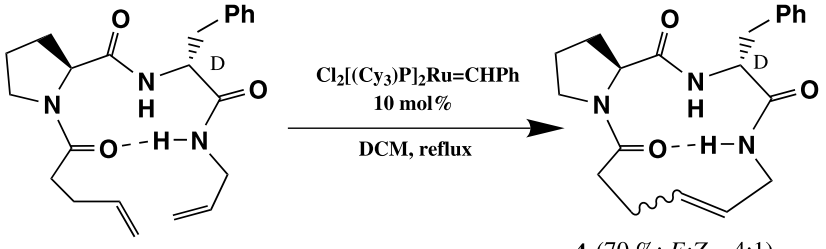


Chart 1. CD spectra of acyclic (**1a**) and cyclic (**2**) peptides.

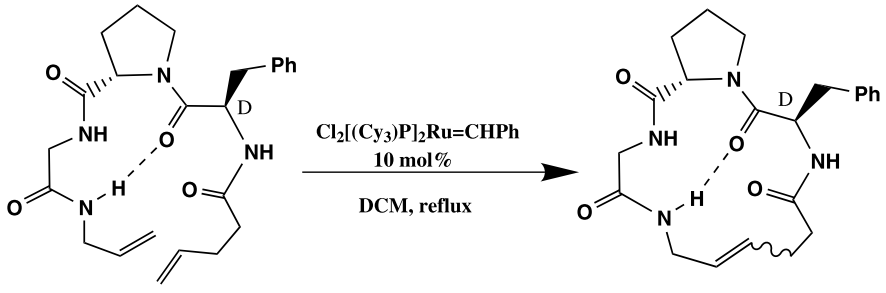
This clearly indicates that the configuration at the C-terminal residue may be influencing the formation of the β-turn. So in order to probe this, the configuration of the amino acid residue at the i+2 position was inverted and the corresponding dipeptide, pent-4-enoyl-L-*Pro*-D-*Phe*-*N*-allyl amide **3b** was synthesized and subjected to solution NMR studies, which indicated the presence of intramolecular hydrogen bonding ($allyl_{\delta NH}$ = 7.32 ppm; IR: 3306 cm^{-1}), suggesting that the molecule is organized in a β-turn fashion. Subjection of peptide **3b** to RCM conditions produced the corresponding cyclic peptide **4** in good yield (Scheme 2) as a mixture of *E* and *Z* isomers (4:1). A deuterium exchange study on **4** also revealed the existence of strong intramolecular hydrogen bonding ($allyl_{\delta NH}$ = 7.91 ppm; IR: 3305 cm^{-1}), suggesting the molecule to be organized in a β-turn. In order to further probe the role of configuration of the amino acids linked to L-*proline*, we also studied the β-turn formation and RCM by introducing *glycine* as a third amino acid in peptides **1** and **3**. It is generally known that *Pro–Gly* residues in proteins are turn inducers and thus introduction of a *Gly* residue at either end of L-*proline* in **1** or **3** should add an additional element of constraint which may help in β-turn formation in the resulting tripeptides **5** and **7** (Schemes 3 and 4). Accordingly, tripeptides **5** and **7** were synthesized and their solution conformations were studied by ^1H NMR in CD_3OD and $CDCl_3$. It was interesting to note that the tripeptide pent-4-enoyl-D-*Phe*-L-*Pro*-*Gly*-*N*-allyl amide **5a** showed the presence of an intramolecular hydrogen bond ($allyl_{\delta NH}$ = 7.41 ppm; IR: 3305 cm^{-1}) suggesting the presence of a β-turn conformation. This tripeptide underwent smooth cyclization to give **6** (*E*:*Z* = 3:1). The cyclic peptide **6** also showed the presence of an intramolecular hydrogen bond ($allyl_{\delta NH}$ = 7.16 ppm; IR: 3323 cm^{-1}).

On the other hand, the tripeptide pent-4-enoyl-L-*Phe*-L-*Pro*-*Gly*-*N*-allyl amide **5b** did not show the presence of an intramolecular hydrogen bond ($allyl_{\delta NH}$ = 6.52 ppm) and also did not undergo cyclization under RCM conditions. These observations are quite interesting as



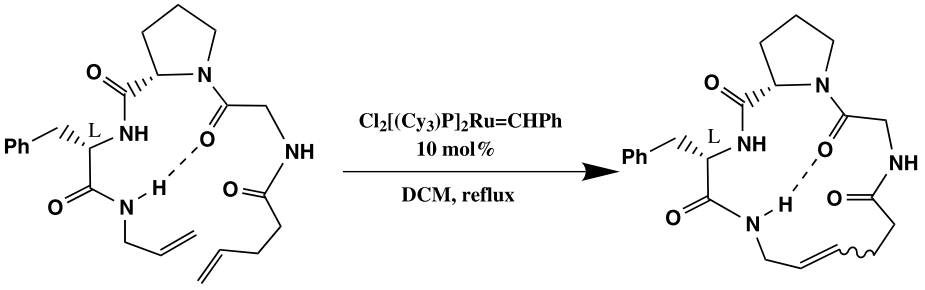
3b (L-Pro-D-Phe; β-*turn*) **4** (70 %; *E*:*Z* = 4:1)

Scheme 2.



5a (D-Phe-L-Pro-Gly; β-*turn*) **6** (62 %; *E*:*Z* = 3:1)

Scheme 3.



7a (Gly-L-Pro-L-Phe; β-*turn*) **8** (75 %; *E*:*Z* = 5:1)

Scheme 4.

insertion of a *Gly* residue at the C-terminal of L-proline changes the hydrogen atom donor–acceptor partners. Also, it requires the opposite configuration of the Phe-residue at the *N*-terminal of L-proline for successful RCM cyclization as compared to peptide **1** (Scheme 1).

It is noteworthy, though not surprising, that the tripeptide pent-4-enoyl-*Gly*-L-*Pro*-L-*Phe*-*N*-allyl amide **7a** showed the presence of intramolecular hydrogen bonding ($allyl_{\delta NH}=6.98$ ppm; IR: 3308 cm^{-1}) and underwent cyclization to give **8** ($E:Z=5:1$) on treatment with Grubbs' catalyst (Scheme 4). The presence of intramolecular hydrogen bonding ($allyl_{\delta NH}=6.85$ ppm; IR: 3364 cm^{-1}) was also observed in **8** when subjected to solution ^1H NMR studies in CD_3OD–$CDCl_3$. The tripeptide pent-4-enoyl-*Gly*-L-*Pro*-D-*Phe*-*N*-allylamide **7b** did not show any intramolecular hydrogen bonding ($allyl_{\delta NH}=6.31$ ppm) and also did not undergo cyclization to any appreciable extent when subjected to RCM. Thus, addition of a *Gly* residue in dipeptides **1** and **3** changes the course of RCM in tripeptides **5** and **7** because the hydrogen bond donor/acceptor partners (*Phe or Gly* CO/*6-aminohex-4-enoate* NH) in these are different from those present in **2** and **4** where the intramolecular hydrogen bond is formed by the *6-aminohex-4-enoate* residue. The presence of a β-turn in cyclic peptides **6** and **8** was also confirmed by NOE studies (Fig. 2). Thus the NOEs between H_a/H_b, H_a/H_c, H_a/H_j, H_d/H_i and H_j/H_k clearly support the intramolecular hydrogen bond formed between the D-*Phe* carbonyl and H_a in **6** and between the *Gly* carbonyl and H_a in **8**.

In conclusion, the studies described here indicate that the position of L-*Pro* in a L-*Pro*-*Phe* dipeptide and the configuration of the *Phe* residue either at the C- or N-terminus control the formation of a β-turn. The acyclic peptides where L-*Pro* is in the i+2 position requires an L-*Phe* residue in the i+1 position for β-turn formation. However, when L-*Pro* is present in the i+1 position it is the D-*Phe* residue at i+2 that dictates the formation of the β-turn. It is also noteworthy that tripeptides **5** and **7** obtained by insertion of a *Gly* residue at C- or N-termini in dipeptides **1** and **3**, respectively, behave oppositely to the latter peptides during RCM reactions. The acyclic peptides, which are pre-organized in a β-turn conformation, undergo facile cyclization leading to the corresponding cyclic peptide as mimics of a β-turn. Therefore, RCM reactions can act as a probe for the β-turn conformation in small peptides. We are further pursuing studies to understand the role of absolute configuration in RCM of larger peptides

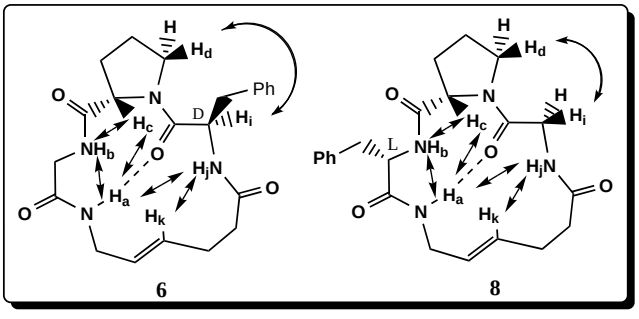


Figure 2. NOEs observed in cyclic peptides **6** and **8**.

Acknowledgements

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5. **General procedure of amide coupling**: To a stirred solution of pent-4-enoyl-XAA acid in THF at 0°C was added triethylamine (1 equiv.), followed by isobutyl chloroformate (1 equiv.) and the mixture vigorously stirred for 5 min and then finally to this was added XA′A′-allyl amide

followed by triethylamine (1 equiv.). The reaction mixture was stirred for 5 h at rt and after that the solvent was removed, the residue taken up in ethyl acetate, washed with sodium bicarbonate and saturated citric acid solution and finally with brine. Drying over sodium sulfate, then concentration in vacuo yielded the crude peptide, which was subjected to column chromatography (silica gel, EtOAc:hexane) to afford the desired peptide in good yields.

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10. **General procedure for RCM**: To a stirred solution of Grubb's ruthenium catalyst (10 mol%) in dry dichloromethane (in high dilution) under nitrogen was added the di-olefin dissolved in dry dichloromethane slowly over a period of 30 min and the mixture refluxed for 12 h after which a further portion of catalyst (10 mol%) was added to the reaction mixture and refluxing continued. Finally, after 28–30 h the reaction was exposed to air and directly subjected to column chromatography (silica gel, EtOAc:hexane) to afford the corresponding cyclic product as a mixture of *E* and *Z* isomers in 50–60% yield. ***Spectral data of some compounds*: 2**: CIMS (m/z): 356 (M+H)$^+$, 100%; ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 7.26 (m, 6H), 6.09 (d, 1H, $J=12$ Hz), 5.47–5.45 (m, 0.4H), 5.40–5.05 (m, 1.6H), 4.58 (d, 1H, $J=6.8$ Hz), 4.07–3.71 (m, 3H), 3.24–2.87 (m, 4H), 2.62–1.86 (m, 8H); FT-IR ($CHCl_3$): 3300, 2927, 1656, 1534, 1448, 1159 cm^{-1}; **4**: $[\alpha]_D$ −9.42 (*c* 0.35, MeOH); CIMS (m/z): 356 (M+H)$^+$, 100%; ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 7.91 (d, 1H, $J=8.8$ Hz), 7.25 (m, 5H), 6.48 (bs, 1H), 5.48–5.46 (m, 0.4H), 5.42–5.0 (m, 1.6H), 4.59–4.29 (m, 2H), 3.85–3.79 (m, 2H), 3.54–3.39 (m, 2H), 3.29–3.18 (m, 2H), 2.25–1.91 (m, 8H); FT-IR ($CHCl_3$): 3305, 2928, 2855, 1645, 1530, 1445, 1383 cm^{-1}; **6**: $[\alpha]_D$ +25.00 (*c* 0.1, MeOH); CIMS (m/z): 413 (M+H)$^+$; ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 1.98–2.67 (m, 8H), 3.15–2.86 (m, 3H), 3.57–3.48 (m, 2H), 3.82–3.74 (m, 2H), 4.13–3.96 (m, 3H), 4.89 (s, 1H), 5.64–5.58 (m, 0.5H), 5.56–5.42 (m, 1.5H), 6.61 (m, 1H), 6.74 (s, 1H), 7.16–7.23 (m, 5H); FT-IR: (cm^{-1}): 3322, 1639, 1542, 1449.