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Synthesis of a cyclic pseudo 3_{10} helical structure from a β-amino acid-L-proline derived tripeptide via a ring closing metathesis reaction

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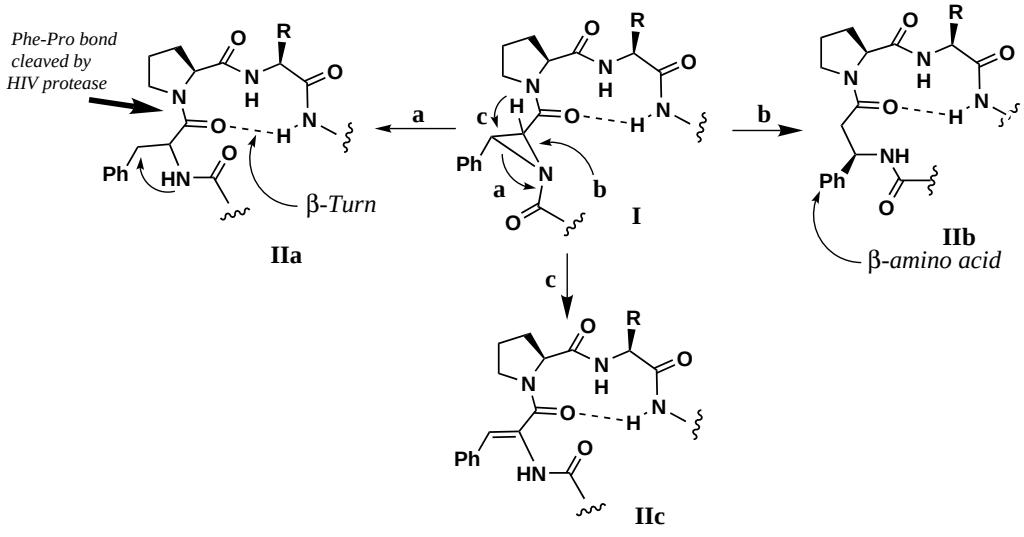
Abstract—The combination of homo-phenylglycine (Hpg) and proline leads to the formation of a β-turn mimic, which can be transformed into a cyclic peptide using a ring closing metathesis reaction. The presence of the pentenoyl and allyl groups at the terminus of the peptide leads to the concomitant formation of a linker surrogate fourth amino acid (6-amino-4-hexenoic acid; Aha) during the cyclization. The cyclic peptide is unique in having a pseudo 3_{10} helical structure.

One of the major drawbacks associated with small peptides as ideal drugs is their poor bioavailability due to rapid proteolytic degradation. Thus, the design and synthesis of small peptidomimetic[1] libraries with non-proteinogenic amino acids have gained tremendous importance in the field of bioorganic chemistry. β-Amino acids[2] are emerging as an attractive alternative to proteinogenic amino acids mainly due to their stability towards proteolytic degradation by endopeptidases. They are also attracting attention because of their abundant presence in various natural products and turn inducing properties in short chain peptides.



Scheme 1. Conformational mimics of Phe–Pro bond.

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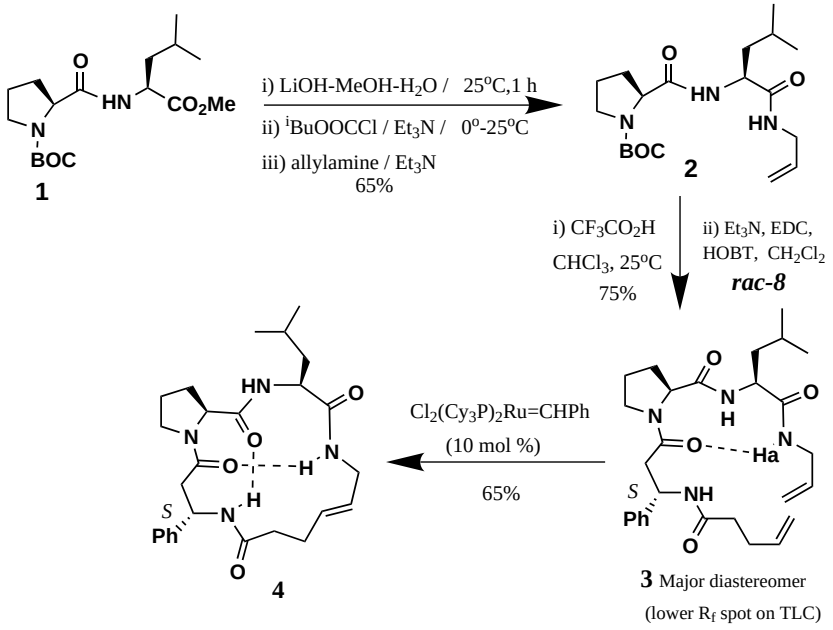
In an ongoing project in our laboratory[3] on the discovery of new chemical entities inhibiting HIV-I protease,[4] we have undertaken the synthesis of small cyclic peptides based upon the structural mimicry of the 'Phe-Pro' bond present in the 'gag-pol' polyprotein. It is known that HIV protease is very specific cleaving the peptide bond between phenylalanine and proline. So the design of a molecule, mimicking the stereoelectronic environment of this scissile bond may lead to potent inhibitors based on structural mimicry. In an attempt to access a conformational mimic of the 'Phe–Pro' bond, we initiated a program where aziridine peptide (**I**) was conceptualized to function as the surrogate of the 'bioactive' conformation during the cleavage by protease (Scheme 1). Conceivably, the breaking of the aziridine bond '**a**' may lead to a β-turn[5] mimic (**IIa**) of the natural *Phe–Pro* analogue whereas that of '**b**' may afford a homo-phenylglycine (*Hpg*)-L-proline containing β-turn mimic (**IIb**). On the other hand, the removal of the aziridine α-proton may lead to the dehydrophenylalanine-L-proline derived β-turn mimic (**IIc**). Recently, Sung et al.[6] have shown that short peptides with an induced β-turn can inhibit HIV replication.

We reasoned here that the synthesis of cyclic β-turn mimics possessing a β-amino acid may lead to the development of small potent HIV-protease inhibitors characterized by high selectivity, bioavailability and resistance to proteolytic degradation. Thus, with appropriate terminal double bonds, these acyclic β-turn containing structures can also be cyclized using ring closing metathesis (RCM) leading to cyclic β-turn mimics. In this paper, we report the synthesis of a cyclic β-turn mimic derived from a *Hpg*-L-*proline* derived tripeptide through the RCM protocol.[7] It is noteworthy that such cyclization leads to the concomitant formation of 6-amino-hex-4-enoic acid (*Aha*) as the surrogate for the fourth amino acid in **4**.

The β-amino acid required for the synthesis of the acyclic peptide precursor was synthesized by a multicomponent coupling procedure previously developed in our laboratory[8] from benzaldehyde, ethyl acetoacetate and acetyl chloride using catalytic anhydrous cobalt(II) chloride in acetonitrile. On the other hand *N*-Boc-L-Pro-L-LeuOMe **1** was hydrolyzed (LiOH–MeOH) and the resulting carboxylic acid was coupled with allylamine (^{i}BuOCOCl–Et_3N) to give *N*-Boc-L-Pro-L-Leu allyl amide **2** in 65% yield (Scheme 2). The peptide **2** was treated with CF_3CO_2H and the resulting product was then coupled with the racemic acylated β-amino acid (***rac*-8**) by a standard amide (Et_3N-HOBT-EDC) coupling procedure[9] to afford the tripeptide **3** as the major diastereomer (65%, lower R_f value in TLC). The other diastereomer corresponding to **3** was obtained as the minor component (35%) and had a higher R_f value on TLC. The presence of an unequal mixture of **3** may be due to the difference in reactivity of the diastereomers during the amide coupling reaction. Deuterium exchange studies[10] on compound **3** suggested no deuterium exchange for the allyl-NH_a over a period of 5 hours on addition of CD_3OD in $CDCl_3$ solvent. This observation strongly supports the existence of an intramolecular hydrogen bond indicating that the molecule may be preorganized by a β-turn formed between the β-Phe carbonyl and the NH_a of the allyl amide. A similar β-turn has been observed in small peptides having L-*proline* in the i+1 or i+2 positions.[11] We demonstrated earlier that the tripeptides similar to **3** (with double bonds at both termini) generally preorganize themselves leading to a β-turn and have a strong propensity to undergo cyclization when subjected to a RCM reaction.[3] In view of these observations we subjected **3** to RCM reaction conditions (0.01 M solution in CH_2Cl_2) in the presence of $Cl_2[(Cy)_3P]_2Ru{=}CHPh$ (10 mol%) to give the corresponding cyclic peptide **4** mainly as the *E*-isomer in good yield (Scheme 2). It is interest-



Scheme 2. Synthesis of cyclic β-turn mimic **4** by RCM.

Table 1. ^{1}H chemical shifts (δ in ppm), coupling constants (J in Hz) of **4** in $CDCl_3$ at 500 MHz

Protons	Hpg	Pro	Leu	Aha
NH	7.79 (d, $J_{NH-\beta H}=8.4$)	–	5.70 (d, $J_{NH-\alpha H}=9.8$)	7.15 (dd, $J_{NH-\alpha H}=2.5$, $J_{NH-\alpha' H}=7.3$)
CαH	3.00 (t, $J_{\beta H-\alpha H}=3.8$, $J_{\alpha H-\alpha' H}=15.4$)	4.15 (dd, $J_{\alpha H-\beta H}=5.3$, $J_{\alpha H-\beta' H}=8.6$)	4.49 (dt, $J_{\alpha H-\beta H}=3.5$, $J_{\alpha H-\beta' H}=9.8$)	4.10 (dt, $J=7.3$, 14.8)
Cα′H	2.83 (dd, $J_{\beta H-\alpha' H}=3.8$)	–	–	3.62 (ddd, $J=2.5$, 6.6, 14.8)
CβH	5.52 (dt, $J_{NH-\beta H}=8.4$)	2.17 (m)	2.00 (m)	–
CβH	–	1.94 (m)	1.55 (m)	–
CγH	–	1.77 (m)	1.55 (m)	–
Cγ′H	–	1.87 (m)	–	–
CδH	–	3.56 (dt, $J=6.0$, 9.5)	0.93 (d, $J_{\gamma H-CH_3}=6.4$)	–
Cδ′H	–	3.17 (dt, $J=7.4$, 9.5)	0.90 (d, $J_{\gamma H-CH_3}=6.4$)	–

Others: 5.75 (dt, $J=5.7$, 15.4, *olefin*-C*H*), 5.70 (dt, $J=6.4$, 15.4, *olefin*-C*H*), 2.43–2.49 (m, 4H, $2CH_2$), 7.22–7.40 (phenyls)

ing to note that **4** is a 17-membered macrocyclic peptide with one β-amino acid and the cyclization results in the synthesis of an unsaturated amino acid spacer, *Aha*. As described below, the cyclic peptide exhibits a unique pseudo 3_{10} helical structure as revealed by NMR studies.

The data from the solution ^{1}H NMR (500 MHz) spectra of compound **4** are presented in Table 1. Solvent titration studies showed that the variation for the amide chemical shift for the *Hpg* (<0.31 ppm) and the *Aha* (<0.07 ppm) residues is very small when up to 33% v/v DMSO-d_6 was added to the chloroform solution, which indicates their participation in intramolecular hydrogen bonding. The appearance of the *Hpg* NH_f (7.79 ppm) as well as the *Aha* NH_c (7.15 ppm) at low field in the proton spectrum further confirms the presence of H-bonding. In $CDCl_3$ solution, only one isomer with a *trans* amide bond preceding the proline was observed. The observation of a NOE cross peak between *Pro*$_\delta$ H_d-*Hpg* $C_\alpha H_e$ in the NOESY spectrum strongly supports the presence of the *trans* imide bond geometry in **4**. Restricted conformational mobility at the proline backbone ($\phi=-60$) may result in reverse turns. Usually proline takes an i+1 position in such β-turns, however, the presence of NOE cross peaks between *Aha* NH_c–*Leu* NH_b, *Aha* NH_c–*Pro* $C_\alpha H_a$, *Aha* NH_c–*Leu* $C_\alpha H_g$ and *Leu* NH_b–*Pro* $C_\alpha H_a$ in the NOESY spectrum coupled with the *Aha* NH_c hydrogen bonding implies the presence of a β-turn involving the *Pro–Leu* residues as shown in Fig. 1. The observation of a strong NOE cross peak of *Leu* NH_b–*Pro*$C_\alpha H_a$ compared with *Leu* NH_b–*Leu* $C_\alpha H_g$ in the NOESY spectrum, indicates that it is a type-II β-turn.[12] Such a turn brings the proline carbonyl and the *Hpg* NH_f in proximity which results in the subsequent formation of a hydrogen bond between them leading to a 9-membered turn structure. The ^{1}H NMR studies clearly support the presence of two consecutive 9- and 10-membered turns, which suggests that the cyclic peptide **4** is organized in a pseudo 3_{10} helical structure.

The absolute stereochemistry of the stereogenic center in the β-amino acid in **3** and **4** was proved by chemical correlation studies starting from L-*phenylglycine*. The corresponding diazoketone **6** was made from **5** by base hydrolysis (LiOH–THF–H_2O) followed by a mixed anhydride protocol using isobutyl chloroformate and diazomethane in dichloromethane solvent. A general Arndt–Eistert homologation[13] on **6** using Ag(OAc)/Et_3N gave the β-amino ester **7** which was hydrolyzed (LiOH–THF–H_2O) to afford the corresponding β-amino acid **8** without any racemization. Coupling of the pure acid **8** with L-*proline*-L-*leucine* allyl amide by the EDC-HOBT method yielded the pure diastereomer **3**.

HPLC studies and optical rotation indicated that the absolute configuration of the β-amino acid residue in the compound obtained by homologation ($[\alpha]_D$ −12.2) as described in Scheme 3 is identical to the diastereomer **3** ($[\alpha]_D$ −16.6) obtained by the procedure described in Scheme 2. Therefore the major diastereomer, **3** was assigned the '*S*' absolute stereochemistry at the asymmetric center in the β-amino acid residue. Similarly the '*R*' assignment was made for the minor diastereomer (higher R_f value in TLC) and the corresponding cyclic peptide (i.e. **4**) also.

In conclusion, this paper describes the synthesis of a novel β-and ω-aminoacid containing cyclic peptide having two consecutive intramolecular 9- and 10-membered hydrogen bonds. This is the first example of a small cyclic peptide having a pseudo 3_{10} helical structure.

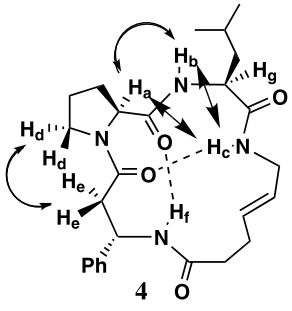
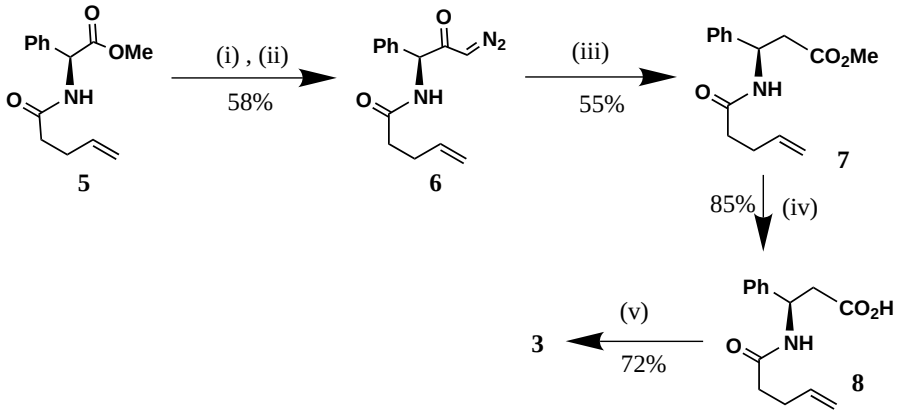


Figure 1. Important NOE interactions and hydrogen bonding pattern of compound **4**.



Scheme 3. Assignment of the absolute configuration of the β-amino acid residue in **4**. (i) LiOH, THF:H_2O, rt; (ii) isobutyl chloroformate, Et_3N, CH_2N_2 (excess) in CH_2Cl_2; (iii) Ag(OAc), Et_3N, MeOH, rt; (iv) LiOH, THF:H_2O, (v) Et_3N, EDC, HOBT, *N*-allyl-L-leucine-L-proline: TFA, Et_3N, CH_2Cl_2.

Acknowledgements

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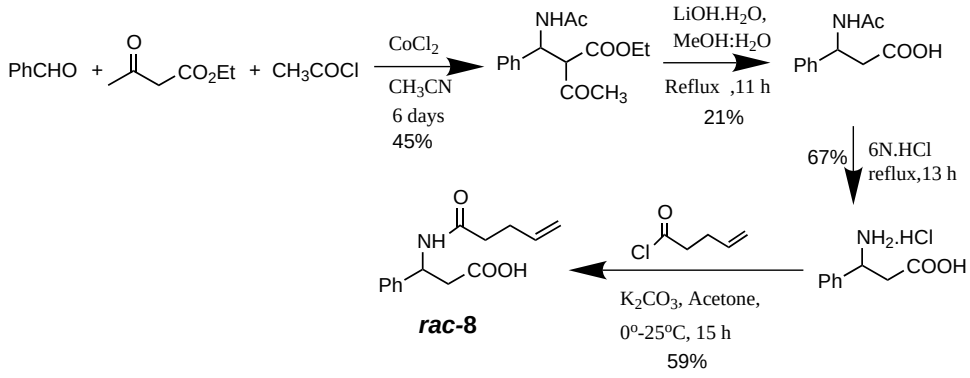
8. (a) Prabhakaran, E. N.; Iqbal, J. *J. Org. Chem*. **1999**, *64*, 3339–3341; (b) The β-amino acid (***rac*-8**) was synthesized in the following manner:



9. ***Standard procedure for amide coupling***: To an ice-cold stirred solution of the acid (***rac*-8**) (247 mg, 1 equiv., 1 mmol) in dry dichloromethane (5 mL) was added EDC (191 mg, 1 equiv., 1 mmol) followed by HOBT (135 mg, 1 equiv., 1 mmol). The resulting mixture was stirred vigorously for 30 min and then proline–leucine allyl amide (267 mg, 1 equiv., 1 mmol) was added to it followed by 1 equiv. of triethylamine and the mixture stirred for 5 h, then washed thoroughly with saturated citric acid solution and water (10 mL×3). Drying and concentration in vacuo yielded the crude peptide as a diastereomeric mixture (3:2). Preparative HPLC (MeOH:water:acetonitrile) afforded the desired amide **3** in 75% yield as a white solid. ***General procedure for RCM***: To a stirred solution of Grubbs' ruthenium catalyst (10 mol%) in dry dichloromethane (60 mL) under nitrogen was added the di-allylated peptide **3** (1 mmol) dissolved in dry dichloromethane (40 mL) slowly over a period of 30 min and the mixture refluxed. After 12 h, further catalyst (10 mol%) was added to the reaction mixture and the refluxing continued for another 12 h. After this time, the reaction was exposed to air and directly subjected to column chromatography (silica gel; $CHCl_3$:CH_3OH) to afford the corresponding cyclic product **4** mainly as the *E* isomer in 65% yield. **Spectral data for selected compounds**: ***rac*-8** IR (neat): 3298, 3069, 1718, 1641, 1545, 1386 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 7.28 (m, 5H), 6.68 (bs, 1H), 5.85–5.63 (m, 1H), 5.44–5.4 (m, 1H), 5.03 (d, 1H, $J=14$ Hz), 4.97 (d, 1H, $J=10$ Hz), 2.89 (d, 1H, $J=6$ Hz); 2.86 (d, 1H, $J=8$ Hz); 2.30 (bs, 4H); MS *m*/*z* (*iso*-butane): 248 (*M*+1, 100%), 204, 164, 149, 106, 100; **3**: $[\alpha]_D$ −16.6; IR ($CHCl_3$): 3304, 3067, 1728, 1652, 1544 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 7.41–7.17 (m, 7H), 6.84 (bs, 1H), 5.88–5.66 (m, 2H), 5.40–5.24 (m, 1H), 5.13–4.95 (m, 4H), 4.54–4.34 (m, 2H), 3.85 (m, 2H), 3.58 (m, 2H), 3.08–2.93 (m, 2H), 2.34 (bs, 4H), 2.10–1.40 (m, 7H), 0.95 (d, 3H, $J=6$ Hz), 0.89 (d, 3H, $J=6$ Hz); CI MS *m*/*z* (*iso*-butane): 497 (*M*+1), 440, 412, 327, 268, 230 (100%), 181, 131, 106. **4**: IR ($CHCl_3$): 3318, 1645, 1517, 1449 cm^{-1}; MS *m*/*z* (*iso*-butane): 491 $(M+Na)^+$, 469 (*M*+1); **7**: $[\alpha]_D$ +22.8; IR ($CHCl_3$): 3291, 3065, 1740, 1648, 1544 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 7.36 (m, 5H), 6.65 (d, 1H, $J=8$ Hz), 5.88–5.71 (m, 1H), 5.48–5.38 (m, 1H), 5.06 (d, 1H, $J=16$ Hz), 4.99 (d, 1H, $J=9$ Hz), 3.60 (s, 3H), 2.93 (dd, 1H, $J=6$ Hz, $J=5.8$ Hz), 2.80 (dd, 1H, $J=6$ Hz, $J=5.8$ Hz), 2.4–2.2 (bs, 4H); CI MS *m*/*z* (*iso*-butane): 262 (*M*+1, 100%), 230, 178, 100.

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