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A facile conversion of a 3_{10} helical structure to a cyclic β-turn mimic in dehydrophenylalanine-derived small peptides through ring-closing metathesis

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Abstract—Dehydrophenylalanine-derived small peptides can be preorganized in a 3_{10} helical structure which is transformed into a β-turn mimic during a ring-closing metathesis cyclization. © 2002 Elsevier Science Ltd. All rights reserved.

Recent developments in the domain of drug-discovery have focused attention on the synthesis of small-constrained mimics of bioactive conformations of potent therapeutic molecules.[1] It is very important for a peptide molecule to retain its conformational features in vivo to bind strongly to the target. Thus elements of constraint (local or global) in a molecule can lock it into a particular conformation, which may mimic the exact bioactive conformation. Local constraints in terms of side-chain modifications[2] in the amino acids, incorporation of protein secondary structures like turns, helices, etc., into the molecule and cyclization[3] as part of global constraints are commonly practiced.

These molecules can also be very useful as pharmaceutical probes towards various proteases. The dehydrophenylalanine (Δ*Phe*) residue as a constrained phenylalanine mimic has gained much importance in particular because of its turn inducing as well as helix-forming propensity.[4] It was also observed that the Δ*Phe* residue when present as part of a peptide renders stability towards proteolytic degradation. The sp^2-C_α in the dehydro residue results in specific ϕ and ψ angles which facilitate β-turn formation capability of this residue. In an ongoing project in our laboratory[5] on the development of potent HIV-protease inhibitors,[6] we developed a strategy to synthesize Δ*Phe*-derived small cyclic β-turn mimics. Recently,[7] we have shown that in a small peptide the presence of a Δ*Phe* residue at the C-terminal (**I**) or N-terminal (**II**) of L-proline can constrain the molecule to adopt a β-turn (Fig. 1). We have shown that such preorganization into a β-turn in the

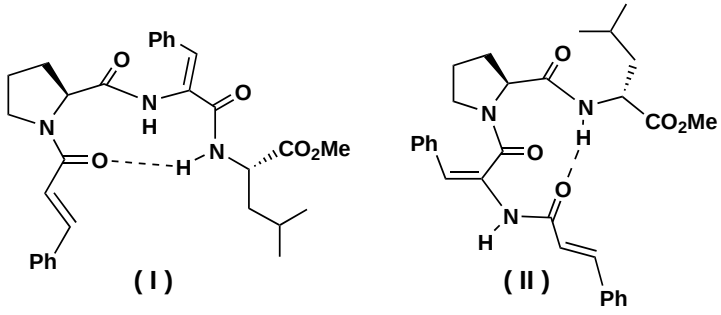

Figure 1. Dehydrophenylalanine-derived β-turn mimics.

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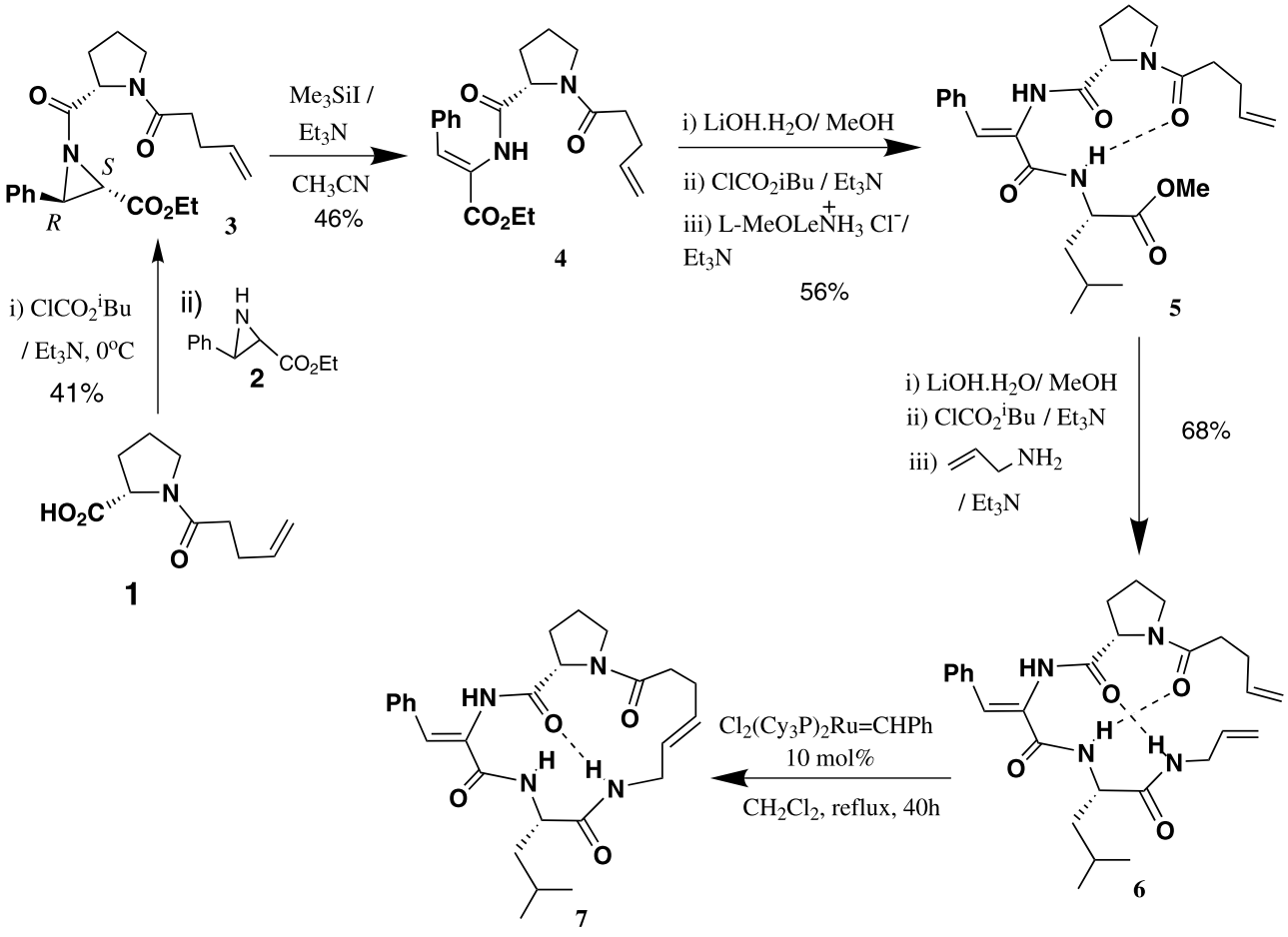
molecule can bring the terminal olefin bonds in sufficiently close proximity to undergo a facile ring-closing metathesis (RCM)[5] cyclization. In this communication we report the synthesis of a Δ*Phe*-containing small cyclic β-turn mimic[8] through a RCM reaction.

We synthesized tripeptide **6** having two terminal olefinic bonds at the C- and N-termini with the aim of cyclizing them by a RCM reaction. Accordingly, *N*-pent-4-enoyl-L-proline **1** was used to resolve the racemic aziridine **2** as described earlier[7] to get the optically pure dipeptide **3** in 41% yield (Scheme 1). We have recently proved the stereochemistry of the stereogenic centers in the aziridine to be 2*S*,3*R*.[7] The compound **3** was then subjected to a stereoselective conversion to the corresponding Δ*Phe*-containing peptide **4** using our novel Me_3SiI/Et_3N mediated methodology.[7] Subsequently, the peptide **4** was hydrolyzed (LiOH–MeOH/H_2O) and extended at its C-terminus with L-LeuOMe ($ClCO_2Bu^i/Et_3N$)[9] to afford the corresponding peptide **5**. An alkaline hydrolysis (LiOH–MeOH/H_2O) of **5** and subsequent coupling with allylamine ($ClCO_2Bu^i/Et_3N$) afforded the peptide **6** in an overall yield of 68% (Scheme 1). The solution ^1H NMR data of compound **6** is presented in Table 1. The presence of two intramolecular hydrogen bonds[10] was revealed by sol-



Scheme 1. Synthesis of cyclic peptide **7** as a β-turn mimic.

Table 1. ^1H chemical shifts (δ in ppm), coupling constants (J in Hz) of **6** in $CDCl_3$ at 500 MHz

Protons	Pro	Δ*Phe*	Leu	Allyl
NH	–	7.62 (s)	7.17 (d, $J_{NH-\alpha H}=8.2$)	7.08 (t, $J=5.5$)
CαH	4.44 (t, $J=6.8$)	–	4.59 (ddd, $J_{\alpha H-\beta H}=4.3$, $J_{\alpha H-\beta' H}=10.4$)	3.90 (m)
CβH	2.22 (m)	7.40 (s)	1.93 (m)	5.87 (tdd, $J=5.3$, 10.4, 17.1)
Cβ′H	2.22 (m)		1.70 (m)	–
CγH	2.07 (m)	–	1.68 (m)	5.22 (qd, $J=1.6$, 17.2)
Cγ″H	2.07 (m)	–	–	5.09 (qd, $J=1.6$, 10.4)
CδH	3.57 (dt, $J=7.2$, 9.8)	–	0.96 (d, $J=6.3$)	–
	3.62 (ddd, $J=5.6$, 7.2, 9.8)			
Cδ′H	–	–	0.95 (d, $J=6.3$)	–

Others: 2.43–2.37 (m, 4H, $2CH_2$), 5.79 (tdd, $J=6.2$, 10.3, 17.2 Hz), 4.99 (qd, $J=1.5$, 17.2), 4.92 (qd, $J=1.5$, 10.3), 7.32–7.42 (m, 5H)

vent titration studies. Titrating with DMSO-d_6 in $CDCl_3$ showed that the variation of the chemical shift of the allyl-NH_b (<0.36 ppm) and Leu-NH_a (<0.70 ppm) is very small when 33% v/v DMSO-d_6 was added to the $CDCl_3$ solution. The appearance of the Leu NH_a (7.17 ppm) as well as allyl-NH_b (7.08 ppm) at low field in the proton spectrum confirms their participation in H-bonding. The ^1H NMR of **6** in $CDCl_3$ solution showed only one isomer with a *trans* imide bond preceding the proline residue, as shown by the cross peak between the Proδ-H_d/pentenoyl-H_c in the NOESY spectrum. The appearances of NOE cross peaks between H_e/H_a, H_a/H_j and H_e/H_j strongly indicates a ten-membered hydrogen bonding involving the leucine NH_a and the pentenoyl carbonyl in **6** (Fig. 2). The presence of strong NOE peaks between H_a/H_b, H_b/H_j and H_i/H_b along with the observation of the allyl-NH_b being hydrogen bonded (DMSO-d_6 study), confirms the presence of a second β-turn in **6** (Fig. 2). Also the strong ROE peak between the Δ*Phe*-β-H_f/leu NH_a confirms the *Z*-geometry of the double bond.

The ^1H NMR studies thus clearly support the presence of two consecutive β-turns, which suggests that the acyclic peptide **6** is organized as a 3_{10} helical structure. Molecular dynamics simulation studies on **6** also show the presence of a 3_{10} helical structure in this peptide[11] (Fig. 2). In a recent study from our laboratory[12] we have shown that peptides folded in a 3_{10} helical structure undergo facile RCM cyclization leading to the corresponding cyclic 3_{10} helical structure. In order to probe this we have subjected peptide **6** to RCM conditions. To our gratification, when the tripeptide **6** was subjected to Ru-carbene (Grubbs' catalyst) catalyzed RCM reactions,[3,13] it indeed underwent a smooth cyclization to afford the corresponding cyclic peptide **7** as an *E*-isomer in good yields (Scheme 1). In the process of cyclization of **6**, one new unnatural ω-amino acid, 6-aminohex-4-enoic acid (*Aha*) has been created and **7** can be considered as a cyclic tetrapeptide with two natural (Pro and Leu) and two unnatural (Δ*Phe* and *Aha*) residues arranged in an alternating manner. Also, being a cyclic peptide with a Pro-Δ*Phe* linkage, **7** can potentially belong to a new class of structural analogues of HIV protease inhibitors. The solution ^1H NMR study on the cyclic peptide **7** revealed interesting conformational properties. It was observed that after cyclization the 3_{10} helical structure in peptide **6** has been transformed into a single cyclic β-turn mimic **7**. An almost equal abundance of both *cis* and *trans* conformational isomers are observed in the DMSO-d_6 medium. The low temperature coefficient ($\Delta\delta/\Delta T$) for the *Aha*-NH_b chemical shifts (−2.4 ppb/K for the *trans*-**7** and −1.8 ppb/K for *cis*-**7**) indicates its participation in intramolecular hydrogen bonding. The ROE cross peaks between H_a/H_b, H_b/H_i, H_a/H_e confirms the presence of β-turn involving the Δ*Phe*/leu residue. The energy minimized structures for both *cis*-**7** and *trans*-**7** show the presence of intramolecular hydrogen bonding involving the proline carbonyl and the *Aha*-NH_b (Fig. 2).

To ensure the role of the Δ*Phe* in the cyclization of **6**, we synthesized two analogous peptides **8** and **9** by standard amide coupling procedures[9] replacing the Δ*Phe* with L-Phe and D-Phe (Fig. 3), respectively, whose solution NMR did not show any well defined structure. When subjected to RCM condition, peptide **8** did not undergo any cyclization thereby rendering a strong support to our hypothesis.

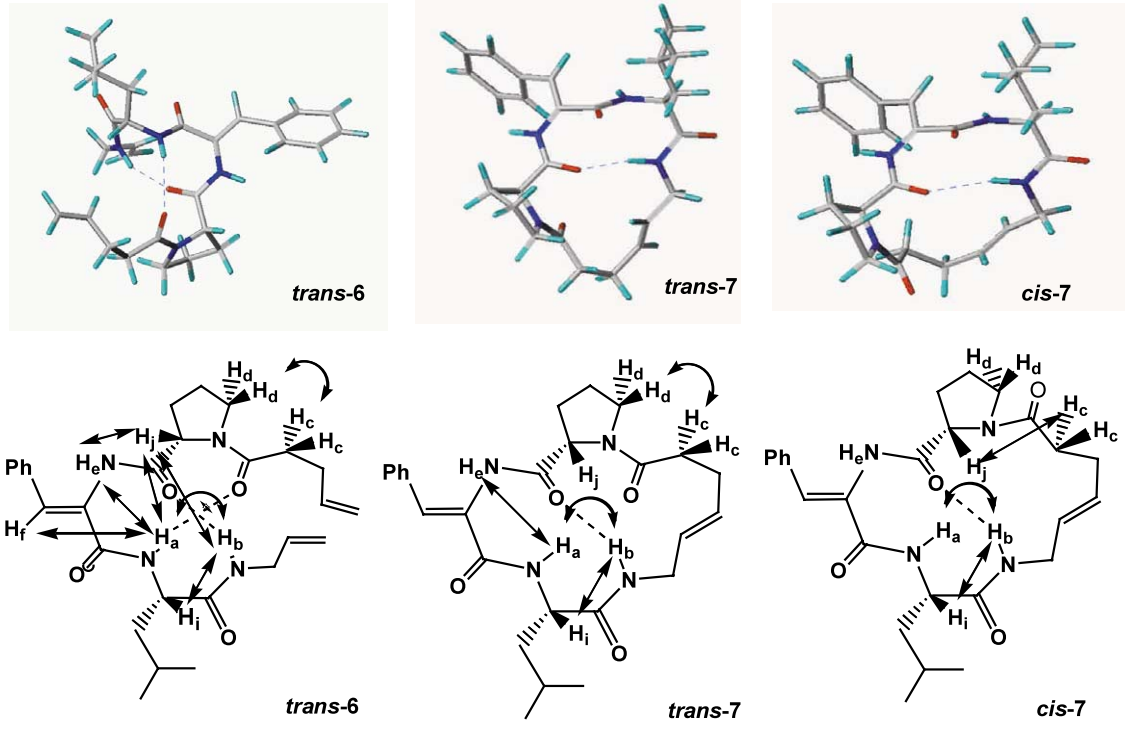


Figure 2. Energy minimized structures and different NOEs observed in *trans*-**6**, *cis*-**7** and *trans*-**7**.

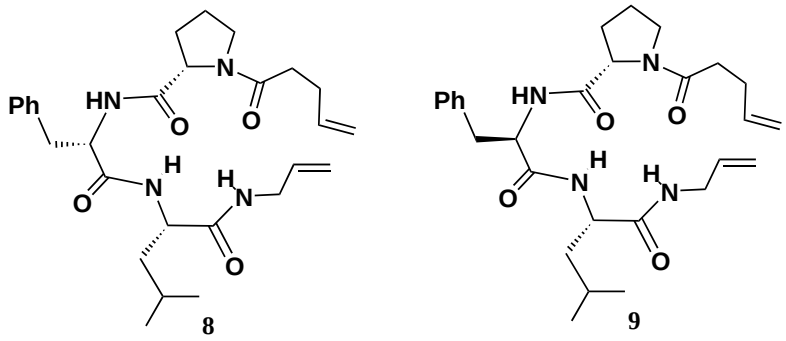


Figure 3. The dehydrophenylalanine residue has been replaced by L-Phe and D-Phe in **8** and **9** respectively.

On the other hand peptide **9** was cyclized in a very poor yield (5–10%). These results clearly indicate that the close proximity of the terminal olefins, a prerequisite for RCM cyclization, is missing in peptides **8** and **9**. Furthermore absence of the β-turn in **8** and **9**, also supports the role of the Δ*Phe* induced β-turn preorganization of **6** leading to the proximity of terminal alkenes for a successful RCM reaction.

In conclusion, we have demonstrated that the Δ*Phe* residue present in peptide **6** can invoke a 3_{10} helical structure which changes into a simple β-turn structure after RCM cyclization. We are currently pursuing the cyclization studies on the related helical structures.

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9. **Standard amide coupling procedure**: To an ice-cold stirred solution of *N*-pentenoyl proline acid (1 equiv.) in dry dichloromethane (5 mL) was added triethylamine (1 equiv.) followed by isobutyl chloroformate (1 equiv.). The resulting mixture was stirred vigorously for 5 min and then XAA-leucine allyl amide (1 equiv.) was added followed by 1 equiv. of triethylamine. It was stirred for 5 h. After that the reaction mixture was washed thoroughly

with saturated sodium bicarbonate solution, followed by citric acid solution and water (3×10 mL). Drying and concentration in the vacuum yielded the crude peptide which on column chromatography (silica gel, EtOAc:hexane) afforded the desired peptide in good yield. **Spectral data of some other compounds**. Compound **6**: MS m/z: 495 $(M+1)^+$, 438, 407, 350, 325, 180, 172. Compound **8**: ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.30–7.14 (m, 5H), 6.98 (d, $J=7.81$ Hz, 1H), 6.79 (bs, 1H), 6.28 (d, $J=7.2$ Hz, 1H), 5.90–5.74 (m, 1H), 5.23–4.99 (m, 4H), 4.59–4.53 (m, 1H), 4.36–4.26 (m, 1H), 4.23–3.85 (m, 2H), 3.48–3.32 (m, 2H), 3.24–3.15 (m, 1H), 2.97 (dd, $J=5.8$ Hz, 13.8 Hz, 1H), 2.4–1.86 (m, 8H), 1.8–1.61 (m, 2H), 1.38–1.29 (m, 1H), 0.96 (d, $J=6.2$ Hz, 3H), 0.90 (d, $J=6.2$ Hz, 3H); MS (CI) m/z: 497 $(M+1)^+$, 440, 412, 384, 327, 229, 180. Compound **9**: ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.36–7.10 (m, 5H), 6.99 (d, $J=8.3$ Hz, 1H), 6.77 (bs, 2H), 5.89–5.75 (m, 2H), 5.21–4.97 (m, 2H), 4.57–4.38 (m, 2H), 4.2 (bs, 1H), 3.84 (bs, 2H), 3.70–3.28 (m, 2H), 3.21–3.18 (m, 2H), 2.37 (s, 4H), 2.2–2.15 (m, 11H), 0.88–0.85 (m, 6H); MS (CI) m/z: 497 $(M+1)^+$, 440, 412, 327, 180.

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11. (a) Molecular mechanics/dynamics calculations were carried out using Sybyl 6.8 programme on a Silicon Graphics O2 workstation. The tripos force field, with default parameters were used throughout the simulations. In order to mimic DMSO solvent a dielectric constant of 47 Debye was used in all minimizations as well as MD runs. Minimizations were done first with steepest decent, followed by conjugate gradient methods for a maximum of 2000 iterations each or RMS deviation of 0.005 kcal/mol, whichever was earlier. The energy-minimized structures were then subjected to MD. A number of inter atomic distances and torsional angle constraints obtained from NMR data were used. Distance constraints with a force constant of 15 kcal/Å were applied in the form of flat bottom potential well with a common lower bound of 2.0 Å and the upper bound of 2.8, 3.5, 4.0 and 4.5 Å, in accordance with the NOE intensities. Force constants of 30 and 5 kcal/Å were employed for H-bond distance and dihedral angle constraints, respectively. The energy-minimized structures were subjected to constrained MD simulations for duration of 120 ps (20 cycles each of 6 ps period, of the Simulated Annealing protocol). The atomic velocities were applied following Boltzmann distribution about the center of mass, to obtain a starting temperature of 700 K. After simulating for 1.5 ps at high temperature, the system temperature was reduced stepwise over a 4.5 ps period to reach a final temperature of 300 K. Resulting structures were sampled for each and every cycle, leading to an ensemble of total 20 structures. The samples were minimized using the above-mentioned protocol, and one of the lowest energy conformations of *trans*-**7**, *cis*-**7** and *trans*-**6** is shown in Fig. 2; (b) Kessler, H.; Griesinger, C.; Lautz, J.; Muller, A.; VanGunsteren, W. F.; Berendsen, H. J. C. *J. Am. Chem. Soc.* **1988**, *110*, 3393–3396.
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13. **General procedure for ring-closure metathesis**: To a stirring solution of ruthenium methylidene catalyst (Grubbs catalyst) (10 mol%) in dichloromethane (60 mL) under nitrogen, the diene **6** (1 mmol) dissolved in dichloromethane (40 mL) was added and the mixture was refluxed for 10–12 h. At this stage an additional ruthenium methylidine catalyst (10 mol%) was added and the mixture was further refluxed for 8 h. The solvent was evaporated to yield a residue which was chromatographed over silica gel (EtOAc: hexane) to afford **7** (55%) as a gum. ^{1}H NMR (500 MHz, DMSO-d_6) *trans*-**7**: ^{1}H NMR (200 MHz, $CDCl_3$): δ 10.09 (s, 1H), 8.62 (d, $J=7.8$ Hz, 1H), 7.58 (dd, $J=2.0$, 7.9 Hz, 1H), 7.52–7.28 (m, 5H), 6.30 (s, 1H), 5.46 (m, 1H), 5.27 (m, 1H), 4.65 (dd, $J=3.5$, 8.2 Hz), 4.05 (m, 1H), 4.02 (m, 1H), 3.56 (m, 2H), 3.23 (m, 1H), 2.55 (m, 1H), 2.33 (m, 1H), 2.19 (m, 2H), 2.14 (m, 2H), 1.89 (m, 2H), 1.74 (m, 1H), 1.67 (m, 1H), 1.57 (m, 1H), 0.91 (d, $J=6.4$ Hz, 3H), 0.85 (d, $J=6.4$ Hz); MS (CI) m/z: 468 (M+1, 41%)$^+$, 467 (M^+, 100%), 379, 339, 322, 297, 281, 223, 197, 180.