Chapter IV

Synthesis and Conformation of Δ-Phe derived Small Cyclic Peptides via RCM
4.1 Introduction

Dehydro (or $\alpha$,$\beta$-unsaturated) amino acids have been frequently found in naturally occurring peptides of microbial origin$^1$ and in some proteins, e.g., histidine ammonia lyase from bacterial and mammalian sources and phenylalanine ammonia lyase from plants.$^2$ They are also constituents of a separate class of polycyclic peptide antibiotics, lantibiotics,$^3$ such as nisin, epidermin, and subtilin.$^4$ Nisin and subtilin both contain dehydroalanine ($\Delta$Ala) and dehydrobutyrine ($\Delta$Abu). A recent study suggests that nisin, well known as a food preservative, latches to a molecule known as lipid-II on bacterial cell membrane and kills the hosts by punching a hole in the membrane.$^5$ Dehydroleucine ($\Delta$Leu) and dehydrophenylalanine ($\Delta$Phe) are present in albonoursin,$^6$ while dehydrovaline ($\Delta$Val) is found in penicillin$^7$ and cephalosporin (antibacterials$^{11}$). Dehydrotryptophan ($\Delta$Trp) is contained in neochinulins$^8$ (growth inhibitor) and dehydrophenylalanine is found in tentoxin$^9$ (phytotoxic). In some cases, dehydroalanine residues form a part of the active site in the protein and lack of these residues result in certain diseases.$^{10}$ The presence of dehydroresidues in peptides confer altered bioactivity as well as increased resistance to enzymatic degradation.$^{11}$ Dehydroresidues have been introduced in several bioactive sequences in order to obtain highly active agonist and antagonist analogs, and this modification has become one of the most promising methods to study structure–
function relationships in biologically active peptides. Based on this precedence, we recognized dehydroamino acid (Dhaa) residues as one of the important conformational constraint in the design of cyclic peptides because the presence of a sp$^2$ hybridized carbon atom in the backbone, the altered electronic distribution (conjugation) caused by the $\alpha$, $\beta$ $\pi$-system, and the change in the side-chain rotamer populations all contribute significantly to the conformation of the peptide backbone and may in turn increase peptide-receptor affinity by reducing the entropic costs of binding.
4.2 Present Study

In continuation with our studies on synthesizing cyclic peptides containing unnatural amino acids-proline residues\(^{13}\) and based on the specificity of HIV protease towards cleaving the Phe-Pro bond,\(^{14}\) we reasoned that incorporation of dehydrophenylalanine (\(\Delta\text{Phe}\)), a constrained phenylalanine mimic in the place of Phe in A may possess high stereo electronic complementarity resulting in better recognition by the protease (Figure-1).

![Figure-1](image)

Figure-1: Schematic representation of \(\Delta\text{Phe}\) containing cyclic peptide

It is also noteworthy that the presence of two constrained amino acids alpha to each other (namely D-proline and constrained phenylalanine) may lead to either a \(\beta\)-strand conformation or a turn conformation thereby rendering an additional element of recognition for the protease active site. We believed that the configuration of the double bond in the dehydro residue (\(\Delta\text{Phe}\) in this case) may control the conformations of proximal amino acid residues and will afford
information on conformations acceptable to the bioreceptor and hence, it is important that we be able to incorporate both double bond isomers into cyclic peptides. Therefore, we became interested to synthesize ΔPhe-Pro containing cyclic peptides.  

4.2.1 Synthetic approaches to dehydroamino acids:

There is extensive literature on the preparation of α, β-dehydroamino acid derivatives and some of the frequently used methods are depicted in Scheme-1.

Scheme-1: Synthetic approach to dehydro amino acid derivatives

The Erlenmeyer synthesis via azlactone (method A), condensation of aldehydes with phosphorylglycine esters 6 (method B) are the most frequently used. The main limitation of approach (A) lies in the harsh reaction conditions, which limits its use to aromatic aldehydes devoid of acid sensitive groups. In
addition, substrates with carbamate protecting groups cannot be prepared (method A). The use of phosphorylglycine esters 6 is instead more versatile and the synthesis is milder. Both β-alkenyl (10) and β-aryl (13) substituted α-dehydroamino acids derivatives can be conveniently obtained through Suzuki coupling (Scheme-2, method A). Nevertheless, the preparation of the starting methyl β-bromo-(acetamido)acrylate 9 is quite laborious and low yielding.

Scheme-2: Synthetic approach to dehydro amino acid derivatives

The other common procedures for the preparation of dehydroamino acids are by the β-elimination of α-amino acid derived alcohols or halides. Recently, a similar but modified protocol for the synthesis of ΔAla and ΔAbu (Z-selective) in excellent yields was reported by Chandrasekaran et al. Their method is based on the anti-selective β-elimination of O-Cbz and O-Eoc derivatives of serine and threonine, using K₂CO₃ in DMF (Scheme-3).
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Scheme-3: Synthetic approach to ‘Z’ selective ΔAla and ΔAbu dehydro amino acid derivatives

\[
\begin{align*}
\text{R} & = \text{H or CH}_3, \ P^1 \text{ and } P^2 = \text{Protecting groups; } R^1 = \text{ethyl or benzyl} \\
\text{K}_2\text{CO}_3 & \quad 65 \degree \text{C} \\
\text{DMF} & \\
\end{align*}
\]

On the other hand, Li et al.\textsuperscript{21} have reported a new facile and highly stereoselective protocol toward α,β-dehydroamino acid derivatives by using the aminohalogenation reaction of α,β-unsaturated esters and ketones followed by treatment with specific bases (Scheme-4)

Scheme-4: Synthetic approach to ‘Z’ selective dehydroamino acid derivatives

\[
\begin{align*}
\text{R} & = \text{Alkyl, Aryl or Alkoxy} \\
\text{Ar} & \quad \text{O} \\
& + \quad 4-\text{TsNCl}_2 \\
& \quad \text{1. CuOTf (10 mol %)} \quad \text{ACN, rt} \\
& \quad \text{2. Base} \\
\end{align*}
\]

Nakamura et al.\textsuperscript{22} has reported the selective synthesis of Z- and E-ΔAbu (18a and 18b) from L-Threonine (14) and L-allo-threonine (15) as starting materials through selenation and oxidative elimination processes with a selenyl linker (scheme-5). The usefulness of this linker for solid-phase synthesis of dehydropeptides has been demonstrated.\textsuperscript{23}
An expedient stereo selective rearrangement of the acyl aziridines to the corresponding ∆² Phe was developed in our group²⁴ (scheme-6). N-cinnamoyl proline was treated with a diastereomeric mixture of 3-phenyl-aziridine-2-carboxylic acid ethyl ester under kinetic resolution conditions to obtain peptide 19 in diastereomerically pure form which was converted to compound-20 via novel rearrangement of acyl aziridine mediated by iodotrimethylsilane in the presence of NEt₃. The geometry of the double bond was assigned as ‘Z’ based on nOe studies. This methodology was applied for the synthesis of Pro-∆² Phe containing cyclic peptides using RCM.
Scheme-6: Synthetic approach to ‘Z’-ΔPhe containing peptides

Rich et al.\textsuperscript{25} during their synthesis of cyclic tetrapeptide Tentoxin converted benzylthio-DL-phenylalanine in 21 to diastereomeric sulfoxides 22 (using sodium periodate oxidation) followed by thermal elimination to generate 23. The required geometrical isomer (Δ’Phe) was separated and used for the synthesis of Tenotoxin (Scheme-7).

Scheme-7: Brief synthetic approach to Tenotoxin
4.3 Results and discussion

Schmidt’s\textsuperscript{26} protocol emanating from glyoxalic acid looked more attractive and convenient to incorporate both isomers of ΔPhe into the designed cyclic peptides. According to this, coupling of glyoxylic acid monohydrate with benzyl carbamate in diethyl ether yielded adduct \textbf{25} which was transformed to methyl ester \textbf{25a}. Chlorination of the secondary alcohol followed by Arbuzov reaction\textsuperscript{27} with trimethylphosphite yielded glycyl phosphite \textbf{26} in good yields. The swapping of protecting group in \textbf{26} with ‘Boc’ was essential at this stage so as to enable selective deprotection of amine without affecting the double bond in ΔPhe at a later stage (scheme-8).

Scheme-8: \textit{Preparation of N-Boc-ΔPhe from benzyl carbamate}

\textbf{Reagents and conditions: (a) i. Glyoxylic acid. Monohydrate, Ether, rt, 12h, ii. MeOH, H_2SO_4 61.4 \% (b) i. POCl_3, Toluene, 70 °C, 18 h, ii. PO(Et)_3, 70 °C, 2 h, 61 \% (c) 10 \% Pd/C, MeOH, 40 psi, 4h, rt (d) (Boc)_2O, CH_2Cl_2, rt, 12h, 49 \% (e) KOtBu, Dry THF, -60 °C-rt, 2.5 h, 67 \%}
This was achieved by reacting 26 initially with 10 % Pd/C in MeOH and protecting the resultant amine with ‘Boc’ to yield compound-28 which was transformed to a mixture of E:Z dehydro amino acids (29) using Horner-Emmons reaction. The isomers 29a and 29b were confirmed based on the $^1$H NMR values reported in the literature.26 These residues where then coupled with dipeptide 30a (obtained by deprotecting ‘Boc’ group in 30 with TFA/NEt$_3$) as shown in scheme-9 using standard mixed anhydride protocol (ClCO$_2$Bu/NEt$_3$) to yield corresponding tripeptides 31 and 32 respectively.

Scheme-9: Preparation of ΔPhe containing acyclic tripeptides 33 and 34

At this stage, we attempted to deprotect the ‘Boc’ in 31 and 32 to install the pentenoyl group required for RCM reaction. However, acid mediated deprotection proved futile, leading to decomposition of the starting material under the reaction
conditions. Alternatively, after unmasking the ‘Cbz’ the resulting amine 27 was coupled with 4-pentenoic acid using (ClCO$_2$Bu/NEt$_3$) to yield compound 35 which upon Horner-Emmons reaction with benzaldehyde yielded a mixture of geometrical isomers in good yields. The isomers were separated using column chromatography and hydrolysis using LiOH yielded ‘$E$’ and ‘$Z$’ N-pentenoyl dehydrophenylalanine 37a and 37b respectively as shown in scheme-10.

Scheme-10: Preparation of “$E$” and ‘$Z$’ N-pentenoyl dehydrophenylalanine

\[ \begin{align*}
  \text{Reagents and conditions:} & \quad (a) \text{ 4-pentenoic acid, ClCO}_2\text{Bu, NEt}_3, \text{CH}_2\text{Cl}_2, rt, 12h, 40 \% \quad (b) \text{ KOtBu, Dry CH}_2\text{Cl}_2, -60^\circ\text{C}-rt, 2.5 h, 86 \% \quad (c) \text{ LiOH, MeOH:H}_2\text{O,rt, 4h, 71.4 \% for 37a and 66 \% for 37b.}
\end{align*} \]

With both geometrical isomers of $\Delta$Phe in hand, these residues were coupled (ClCO$_2$Bu/NEt$_3$) independently with dipeptide Pro-Leu allylamide (30a) to yield corresponding tripeptides in good yields (scheme-11).
Scheme-11: Preparation of acyclic tripeptides 38 and 39 from dipeptide 30a

Reagents and conditions: (a) ClCO₂iBu, NEt₃, dry CH₂Cl₂, 0-rt, 12-14 h, 30 % for 38 and 42 % for 39.

Well resolved diagnostic signals in the ¹H NMR and molecular ion in the mass spectrum (spectrum 1 and 2, page# 275, spectrum 3 and 4, page# 276) confirmed the formation of product 38 and 39 in good yields. Low field appearance of Leu NH and allylic NH in CDCl₃ solution suggested their participation in intramolecular H-bonding which was confirmed solvent titration studies. The evaluation of the NOESY spectrum of these peptides reveals a number of critical NOEs. In particular, the NOESY cross peaks Leu NH↔Aha NH, Leu NH↔Pro CδH, and Aha NH↔Pro CαH confirmed the presence of a trans imide bond preceding proline residue with a 10-membered hydrogen bonding network.
involving Aha NH ← ∆-Phe CO. Another 10-membered H-bonding network was evident between Leu NH ← Pentenoyl C=O indicating a 3₁₀ helical conformation with two intra molecular H-bonds (Figure-2).

Figure-2: Diagnostic NOEs observed in 38 and 39

Recent studies from our laboratory have shown that preorganized peptides folded into a 3₁₀ helical/ β-turn structures undergo facile RCM reaction. It was gratifying but not surprising to observe peptides 38 and 39 undergo smooth cyclization in a stereospecific manner to afford E-isomer exclusively in excellent yields when subjected to ring closing metathesis reaction using 10 mol % of Grubb’s first generation catalyst in good yields (scheme-11). It is noteworthy that in the process of cyclization a new unnatural ω-amino acid was created and the resulting cyclic peptides now contain 2-natural and 2-unnatural amino acids. Being a cyclic peptide with ∆-Phe-Pro linkage, 40 and 41 can potentially belong to a new class of structural analogues of HIV protease inhibitors.
Scheme-12: Synthesis of cyclic peptides 40 and 41 from acyclic tripeptides 38 and 39

The solution conformational analysis of cyclic peptides based on the diagnostic nOe’s between Leu NH ↔ Aha NH, Leu NH ↔ Pro CδH, and Aha NH ↔ Pro CαH in the NOESY spectrum, and down field shift of Leu NH and Aha NH in the NMR spectrum (spectrum, page# 277 and spectrum, page# 279), suggested no significant conformational change when compared to their linear counterparts.

Figure-3: Diagnostic NOEs observed in 40 and 41
4.4 Conclusion

In conclusion it is demonstrated that acyclic and cyclic peptides containing both geometrical isomers of dehydrophenylalanine at N-terminal to proline (Δ-Phe-Pro) invoke $3_{10}$ helical structures in solution. It is noteworthy that these peptides have two natural and two unnatural amino acid residues and we expect such peptides to be proteolytically stable and may possess high stereo electronic complementarity resulting in better recognition by the HIV protease.
4.5 Experimental

Preparation of Methyl 2-pent-4-enamido-3-phenylacrylate (36a and 36b):

To an cold (-15°C) stirred solution 35 (3 g, 9.77 mmol) in dry THF was added NaH (0.58 g, 14.65 mmol) portion wise over a period of 10 min. After 30 min at the same temperature was added freshly distilled benzaldehyde in 10 ml of dry THF and stirred at RT for 12h. THF was evaporated completely and the residue was purified using EtOAc-hexane system to afford 1.1 g of ‘Z’ and 1.1 g of ‘E’ isomer in 86 % yield.

IR (Neat): 3287, 2925, 2854, 1731, 1664, 1631 cm⁻¹, ¹H NMR (400 MHz, CDCl₃) of 36a: 7.96 (s, 1H), 7.53-7.49 (m, 1H), 7.37-7.22 (m, 5H), 5.92-5.82 (m, 1H), 5.15-5.04 (m, 2H), 3.63 (s, 3H), 2.49-2.42 (m, 4H), Mass (CI method): 260 (M+H, 100). IR (Neat): 3267, 2927, 1716 cm⁻¹, ¹H NMR (400 MHz, CDCl₃) of 36b: 7.44 (bs, 1H), 7.38-7.32 (m, 5H), 6.96 (bs, 1H), 5.85 (bs, 1H), 5.12-5.03 (m, 2H), 3.85 (s, 3H), 2.44 (bs, 4H), Mass (CI method): 260 (M+H, 100).

Preparation of (E)-2-pent-4-enamido-3-phenylacrylic acid (37a):

To a stirred solution of compound-36a (1g, 3.85 mmol) in ml of MeOH (16 ml) was added an aqueous solution of LiOH (80 mg, 4.22 mmol) in 4 ml of water and stirred for a period of 6h. Methanol was evaporated completely and the residue
was diluted with water. The aqueous layer was cooled and acidified using 2N HCl to yield a white solid which was filtered off and dried (695 mg, 71.4%).

\[
\text{IR (KBr): 3301, 1670, 1650 cm}^{-1}, \quad \text{\textsuperscript{1}H NMR (400 MHz, CDCl}_3) \text{ of 25 a: } \delta \text{ 7.53 (s, 1H), 7.51 (bs, 2H), 7.46-7.36 (m, 3H), 7.07 (bs, 1H), 5.85 (m, 1H), 5.13-5.03 (m, 2H), 2.46-2.26 (m, 4H), Mass (CI method): 246 (M+H, 100).}
\]

**Preparation of (Z)-2-pent-4-enamido-3-phenylacrylic acid (37b):**

To a stirred solution of compound-36b (1.1 g, 4.24 mmol) in 16 ml of MeOH was added a solution of LiOH (196 mg, 4.66 mmol) in 4 ml of water and stirred for a period of 6h. Methanol was evaporated completely and the residue was diluted with water, acidified using 2N HCl to yield a white solid which was filtered off and dried (690 mg, 66%).

\[
\text{IR (KBr): 3310, 1679, 1640 cm}^{-1}, \quad \text{\textsuperscript{1}H NMR (400 MHz, CDCl}_3) \text{ of 25 b: } \delta \text{ 8.15 (s, 1H), 7.57 (bs, 1H), 7.33-7.26 (m, 5H), 5.89-5.82 (m, 1H), 5.13-5.04 (m, 2H), 2.46-2.42 (m, 4H), Mass (CI method): 246 (100).}
\]

**Preparation of N-Boc-ΔZ-Phe-Pro-Leu allylamide (31):**

A. To an ice cold stirred solution of compound-30 (1.0 g, 2.72 mmol) in dry CH\textsubscript{2}Cl\textsubscript{2} was added trifluoroacetic acid (TFA) (10 eq) at 0°C under argon.
atmosphere and stirred at the same temperature for 3h. Solvent was evaporated to afford the TFA salt as a pale yellow gum, which was neutralized with NEt₃ at 0°C to obtain the free amine.

B. To an ice cold stirred solution of \(N\)-Boc-Z-\(\Delta\)-Phe-OH 37b (716 mg, 2.72 mmol) and HOBT (440 mg, 3.26 mmol) in dry CH₂Cl₂ was added a solution of Pro-Leu allylamine (obtained in part A) (1 equivalent) in dry CH₂Cl₂. EDC.HCl (780 mg, 4.08 mmol) was added portion wise to the reaction mixture at 0°C and then stirred at room temperature for a period of 12 h. The reaction mixture was diluted with CH₂Cl₂ and washed with water, brine, dried over Na₂SO₄ and evaporated to afford crude product which was purified using 100-200 mesh silica and MeOH-CHCl₃ as the eluent to afford the title compound (1.10 g, 79.13 %).

IR (KBr): 3320, 2954, 1698, 1665, 1659, 1612 cm⁻¹, (400 MHz, CDCl₃): δ 7.57 (d, \(J = 9.1\) Hz, 1H), 7.45-7.41 (m, 2H), 7.35-7.26 (m, 4H), 6.66 (s, 1H), 6.10 (s, 1H), 5.87-5.78 (m, 1H), 5.21-5.02 (m, 2H), 4.62-4.56 (m, 2H), 3.94-3.87 (m, 1H), 3.78-3.66 (m, 3H), 2.38-2.31 (m, 1H), 2.22-2.15 (m, 1H), 2.03-1.94 (m, 2H), 1.73-1.66 (m, 3H), 1.45 (s, 9H), 0.95 (d, \(J = 6.4\) Hz, 3H), 0.92 (d, \(J = 6.3\) Hz, 3H), Mass (CI method): 513 ((M+H)⁺, 100).
Preparation of \(N\text{-Boc-}^{\Delta}\text{Phe-Pro-Leu allylamide (32)}\):

A. To an ice cold stirred solution of compound-30 (300 mg, 0.81 mmol) in dry CH\(_2\)Cl\(_2\) was added trifluoroacetic acid (10 eq) at 0 °C under argon atmosphere and stirred at the same temperature for 3h. Solvent was evaporated to afford the TFA salt as a pale yellow gum, which was neutralized with NEt\(_3\) at 0 °C to obtain the free amine.

B. To an ice cold stirred solution of \(N\text{-Boc-}E\text{-}^{\Delta}\text{Phe-OH 37a}\) and HOBt (125 mg, 0.81 mmol) in dry CH\(_2\)Cl\(_2\) was added a solution of Pro-Leu allylamide (obtained in part A) (1 equivalent) in dry CH\(_2\)Cl\(_2\). EDC.HCl (200 mg, 1.017 mmol) was added portion wise to the reaction mixture at 0°C and then stirred at room temperature for a period of 12 h. The reaction mixture was diluted with CH\(_2\)Cl\(_2\) and washed with water, brine. The organic layer was dried over sodium sulfate and evaporated to afford crude product which was purified using 100-200 mesh silica and MeOH-CHCl\(_3\) as the eluent to afford the title compound (139 mg, 42 %).

IR (KBr): 3330, 2967, 1667, 1520 cm\(^{-1}\), \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\ 7.53\) (d, \(J = 8.1\) Hz, 1H), 7.43-7.21 (m, 6H), 6.65 (s, 1H), 6.17 (s, 1H), 5.91-5.81 (m, 1H), 5.24-5.07 (m, 2H), 4.59-4.53 (m, 1H), 4.38-4.35 (m, 1H), 3.94-3.85 (m, 1H), 3.80-3.73 (m, 1H), 3.60-3.56 (m, 1H), 2.89-2.82 (m, 1H), 2.08-2.0 (m, 1H), 1.99-
1.93 (m, 1H), 1.79-1.70 (m, 5H), 1.40 (s, 9H), 0.88 (m, 6H), Mass (Cl method): 513 ((M+H)$^+$, 100).

**Preparation of N-Pentenoyl-$\Delta^E$-Phe-Pro-Leu-allylamine (38):**

A. To an ice cold stirred solution of compound-30 (300 mg, 0.817 mmol) in dry CH$_2$Cl$_2$ was added trifluoroacetic acid (10 eq) at 0 °C under argon atmosphere and stirred at the same temperature for 3h. Solvent was evaporated to afford the TFA salt as a pale yellow gum, which was neutralized with NEt$_3$ at 0°C to obtain the free amine (181 mg) in 83 % yield.

B. To an ice cold stirred solution of N-Pentenoyl-$\Delta^E$-Phe-OH (37a) (181 mg, 0.677 mmol) and HOBt (125 mg, 0.813 mmol) in dry CH$_2$Cl$_2$ was added a solution of Pro-Leu allylamide (obtained in part A) (1equivalent) in dry CH$_2$Cl$_2$. EDC.HCl (200 mg, 1.017 mmol) was added portion wise to the reaction mixture at 0 °C and then stirred at room temperature for a period of 12h. The reaction mixture was diluted with CH$_2$Cl$_2$ and washed with water, brine, dried over Na$_2$SO$_4$ and evaporated to afford crude product which was purified using 100-200 mesh silica gel and MeOH-CHCl$_3$ as the eluent to afford the title compound (140 mg, 42 %) as a hygroscopic solid.

IR (KBr):3315, 2956, 1651, 1520 cm$^{-1}$, (400 MHz, CDCl$_3$): $\delta$ 7.66 (d, $J = 8.6$ Hz, 1H), 7.44-7.33 (m, 6H), 7.15 (bt, 1H), 6.21 (s, 1H), 5.84-5.77 (m, 2H), 5.18-5.01
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(\text{m}, 4\text{H}), 4.58-4.49 (\text{m}, 2\text{H}), 3.86-3.84 (\text{m}, 3\text{H}), 3.82-3.81 (\text{m}, 1\text{H}), 2.41-2.36 (\text{m}, 6\text{H}), 1.89-1.86 (\text{m}, 5\text{H}), 0.97 (d, J = 6.8 \text{ Hz}, 3\text{H}), 0.92 (d, J = 6.7 \text{ Hz}, 3\text{H}), \text{Mass (CI method)}: 495 ((\text{M+H})^+, 100).

**Preparation of $N$-Pentenoyl-$\Delta^2$-Phe-Pro-Leu-allylamine (39):**

A. To an ice cold stirred solution of compound-30 (250 mg, 0.68mmol) in dry CH$_2$Cl$_2$ was added trifluoroacetic acid (10 eq) at 0°C under argon atmosphere and stirred at the same temperature for 3h. Solvent was evaporated to afford the TFA salt as a pale yellow gum, which was neutralized with NEt$_3$ at 0°C to obtain the free amine in quantitative yield.

B. To an ice cold stirred solution of $N$-Pentenoyl-$\Delta^2$ Phe-OH (166 mg, 0.677 mmol) (37b) and HOBt (125 mg, 0.81 mmol) in dry CH$_2$Cl$_2$ was added a solution of Pro-Leu allylamide (obtained in part A) (1eq) in dry CH$_2$Cl$_2$. EDC.HCl (200 mg, 1.01 mmol) was added portion wise to the reaction mixture at 0 °C and then stirred at room temperature for a period of 12h. The reaction mixture was diluted with CH$_2$Cl$_2$ and washed with water, brine. The organic layer was dried over sodium sulfate and evaporated to afford crude product which was purified using 100-200 mesh silica and MeOH-CHCl$_3$ as the eluent to afford the title compound (100 mg, 30 %) yield.
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IR (KBr): 3319, 2960, 1659, 1520 cm⁻¹, ¹H NMR (400 MHz, CDCl₃): δ 7.68 (d, J = 8.8 Hz, 1H), 7.52 (s, 1H), 7.45-7.27 (m, 5H), 7.20 (t, J = 5.3 Hz, 1H), 6.22 (s, 1H), 5.86-5.77 (m, 2H), 5.18-5.00 (m, 4H), 4.57-4.54 (m, 1H), 4.51-4.45 (m, 1H), 3.88-3.71 (m, 3H), 3.69-3.62 (m, 1H), 2.44-2.24 (m, 6H), 1.98-1.76 (m, 5H), 0.98 (d, J = 6.3 Hz, 3H), 0.86 (d, J = 6.3 Hz, 3H), Mass (CI method): 495 ((M+H)⁺, 100)

Preparation of cyclo(Δ²-Phe-Pro-Leu-aha) (40): 430

To a stirred solution of Grubb’s ruthenium catalyst (10 mol%) in dry CH₂Cl₂ (in high dilution) under nitrogen was added a solution of compound-38 (132 mg, 0.26 mmol) slowly over a period of 10 min and the mixture refluxed for 7 h. The reaction was exposed to air and purified by column using 100-200 mesh silica gel and EtOAc:hexane as eluent to afford the title product (70 mg, 56 %) yield as a brown solid.

IR (Neat): 3329, 2956, 1651, 1518 cm⁻¹, ¹H NMR (400 MHz, CDCl₃): δ 7.44-7.41 (m, 4H), 7.36-7.32 (m, 2H), 7.15 (d, J = 9.7 Hz, 1H), 6.98 (d, J = 9.4 Hz, 1H), 6.15 (s, 1H), 5.85-5.78 (m, 1H), 5.67-5.60 (m, 1H), 4.66-4.60 (m, 1H), 4.55-4.53 (m, 1H), 4.33-4.26 (m, 1H), 4.01-3.96 (m, 1H), 3.71-3.64 (m, 1H), 3.10-3.06 (m, 1H), 2.55-2.52 (m, 1H), 2.43-2.39 (m, 1H), 2.30-2.23 (m, 4H), 2.05-1.91 (m, 3H), 1.68-1.56 (m, 2H), 0.98-0.95 (m, 6H), ¹³CNMR (100 MHz, CDCl₃): δ 172.1,
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171.2, 166.9, 133.1, 131.4, 130.2, 129.5, 129.1, 128.6, 128.4, 119.12, 61.4, 51.22, 50.37, 39.46, 38.65, 37.08, 30.2, 28.7, 25.3, 24.3, 23.5, 20.8, Mass (CI method): 467 ((M+H)^+ 100)

**Preparation of cyclo(Δ^2-Phe-Pro-Leu-Aha)(41):**

To a stirred solution of Grubb’s ruthenium catalyst (10 mol%) in dry CH₂Cl₂ (in high dilution) under nitrogen was added a solution of compound 39 (200 mg, 0.40 mmol) slowly over a period of 10 min and the mixture refluxed for 7 h while monitoring the reaction by TLC. The reaction was exposed to air and directly subjected to column chromatography (silica gel, EtOAc:hexane) to afford the product (E-isomer) (143 mg, 76%) yield.

IR (Neat): 3330, 2956, 1651, 1626, 1518 cm⁻¹, ^1^H NMR (400 MHz, CDCl₃): δ 7.45-7.41 (m, 2H), 7.39-7.30 (m, 3H), 7.27 (s, 1H), 7.14 (d, J = 9.7 Hz, 1H), 6.98 (d, J = 9.4 Hz, 1H), 6.16 (s, 1H), 5.86-5.80 (m, 1H), 5.68-5.60 (m, 1H), 4.68-4.62 (m, 1H), 4.56-4.54 (m, 1H), 4.35-4.27 (m, 1H), 4.01-3.99 (m, 1H), 3.72-3.65 (m, 1H), 3.12-3.07 (m, 1H), 2.54-2.29 (m, 2H), 2.22-2.04 (m, 4H), 2.03-1.91 (m, 2H), 1.68-1.57 (m, 3H), 0.97 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.5 Hz, 3H), ^13^C NMR (100 MHz, CDCl₃): δ 171.8 171.2, 171.1, 166.7, 133.1, 131.1, 130.5, 129.6,
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129.2, 128.6, 128.4, 118.8, 61.5, 51.2, 50.3, 39.5, 38.6, 37.1, 30.3, 28.8, 25.4,
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4.7 Spectral Data

Spectrum 1: $^1$H NMR spectrum of peptide 38 in CDCl$_3$

Spectrum 2: Mass spectrum of peptide 38
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Spectrum 3: $^1$H NMR of peptide in 39 CDCl$_3$

Spectrum 4: Mass spectrum of peptide 39
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Spectrum 5: $^1$H NMR spectrum of cyclic peptide 40 in CDCl$_3$

Spectrum 6: Mass spectrum of cyclic peptide 40
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Spectrum 7: $^{13}$C NMR spectrum of cyclic peptide 40 in CDCl$_3$
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Spectrum 8: $^1$H NMR spectrum of cyclic peptide 41 in CDCl$_3$

![NMR Spectrum Image]

Spectrum 9: Mass spectrum of cyclic peptide 41

![Mass Spectrum Image]
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Spectrum 10: $^{13}$C NMR spectrum of cyclic peptide 41 in CDCl$_3$