Chapter II

Synthesis of Type VI β-turn Containing Cyclic Peptides using RCM
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2.1 Introduction

One of the defining characteristics of a living system is the ability of even the most intricate of its component molecular structures to self-assemble with precision and fidelity. Uncovering the mechanisms through which such processes take place is one of the grand challenges of modern science.\(^1\) The folding of proteins into their compact three-dimensional structures is the most fundamental and universal example of biological self-assembly; understanding this complex process will therefore provide a unique insight into the way in which evolutionary selection has influenced the properties of molecular systems for functional advantage. The wide variety of highly specific structures that result from protein folding and that bring key functional groups into close proximity has enabled living systems to develop astonishing diversity and selectivity in their underlying chemical processes.\(^2\) In addition to generating biological activity, folding is coupled to many other biological processes, including the trafficking of molecules to specific cellular locations and the regulation of cellular growth and differentiation. In addition, only correctly folded proteins have long-term stability in crowded biological environments and are able to interact selectively with their natural partners. It is therefore not surprising that the failure of proteins to fold correctly, or to remain correctly folded, is the origin of a wide variety of pathological conditions. The rational design of therapeutics based on peptide lead
structures requires detailed knowledge of their conformational requirements for biological activity. Hence, any attempt aimed at understanding the above mentioned biological mechanisms using synthetic small molecules would be a worthwhile exercise and in turn might lay foundation towards the identification of a lead molecule towards the amelioration of human sufferings.

One of the underlying mechanisms of the denaturation of proteins is the cis-trans isomerisation of the peptide bond, which participates in protein folding and unfolding as a rate-determining step. In proteins, the partial double bond character of the peptide bond results in two conformations depending upon the value of the dihedral angle $\omega$ [C$\alpha$(1)-C(1)-N(1')-C$\alpha$(1')]: cis and trans ($\omega$= 0 and 180$^\circ$, respectively)\(^3\), which are referred to as s-cis and s-trans respectively (figure-1).

Figure-1: s-trans and s-cis peptide bond.

In most peptide bonds, the s-trans conformer is greatly favored over the s-cis conformer, proline however, is unique as it is the only residue with an aliphatic ring that encompasses both the main and side-chains. On one hand, the proline ring serves to intrinsically restrict its $\phi$ dihedral angle; while on the other hand, the imidic bond formed with the preceding residue (Xaa-Pro) is readily subject to
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cis-trans isomerization (Figure-2). Additionally, the low activation barrier \(^4 (ca. 13 \text{ kcal/mol})\) for isomerization combined with the small free energy difference between the two Xaa-Pro peptide bond isomers provides a rationale for the putative role of proline in the limiting steps of protein folding pathway.\(^5\)

![Figure-2: cis/trans isomerization of peptidyl-prolyl bond.](image)

In 1998, M. S. Weiss et al.\(^6\) revisited the peptide bonds available from 571 protein crystal structures with resolution \(\leq 3.5 \text{ Å}\). Out of 1,53,209 peptide bonds available, 7,413 amides were found to have Xaa-Pro sequence. A total of 427 (0.28 \%) amide bonds were found to have cis amide geometry (defined as \(-45^\circ < \omega < 45^\circ\)). While 386 of the cis amides were observed in Xaa-Pro sequence, only 41 (0.028\%) contained a non Xaa-Pro sequence. Although, invariably the thermodynamically stable trans isomer predominates over the cis isomer in solution, non-covalent interactions such as hydrophobic, hydrophilic and hydrogen bonding interactions play critical role in dictating the population of the two isomers. It has been shown that a bulky side chain enhances the population of
cis isomer, example, when tyrosine residue precedes proline, a cis population of more than 90% was observed in a study involving several peptides (Figure 3).

Figure 3: Schematic representation of the nucleation of the cis amide bonds by various amino acids preceding proline.

Proline residues are usually encountered in loops or turn with the utmost preference at the $i+1$ position for type I or type II β-turn when the Xaa-Pro imide bond is trans ($\omega_i=180^\circ$) or at the $i+2$ position of turn type VI ($\omega_{i+1}=0^\circ$) in the cis form. Among the various sub types of β-turn present, the type VI β-turn is a unique secondary structure that features an amide cis-isomer N-terminal to a prolyl residue situated at the $i+2$ position of the peptide bond. Two classes of type VI β-turns have been identified based on the dihedral angles of central $i+1$, $i+2$ residues (figure-4).
In the type VIa β-turn, the proline ψ-dihedral angle is near 0° and an intramolecular hydrogen bond exists between the carbonyl oxygen of the \( i \) residue and amide hydrogen of the \( i+3 \) residue. The proline ψ-dihedral angle is situated around 150° in the type VIb geometry and cannot form an intramolecular H-bond. The reported values for central residue torsions for the two types of VI β-turns are shown in Table 1.

Table-1: \( \phi \), \( \psi \) and \( \omega \) backbone torsional angles for type VI β-turn

<table>
<thead>
<tr>
<th>β-turns</th>
<th>( \phi )</th>
<th>( \psi )</th>
<th>( \omega )</th>
<th>( \phi )</th>
<th>( \psi )</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIa</td>
<td>-60°</td>
<td>120°</td>
<td>0°</td>
<td>-90°</td>
<td>0°</td>
</tr>
<tr>
<td>VIb</td>
<td>-120°</td>
<td>120°</td>
<td>0°</td>
<td>-60°</td>
<td>0°</td>
</tr>
</tbody>
</table>

The type VI β-turns are located in many naturally occurring cyclic peptides possessing prolyl residues. For example, Evolidine \(^9\) (1), a cyclic heptapeptide of sequence c(Ser-Phe-Leu-Pro-Val-Asn-Leu) isolated from Evodia xanthoxyloides
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has been shown to have two β-turns, one of type I at Leu-Ser and one of type VI(a) which incorporates a cis peptide bond at Leu-Pro as shown in Figure-5. Aureobasidin E, a cyclic depsipeptide is also shown to adopt a cis-pro bond in solution. Recently, a novel cyclic hexapeptide, called segetalin A, isolated from the seeds of Vaccaria segetalis was found to have a potent estrogen-like activity. Its structure was investigated by both NMR and X-ray analysis which was found to possess two β-turn structures, one being type I and the other type VI. The cyclic dodecapeptide Cycloelonurinin, c(-Gly-Pro-Thr-Gln-Tyr-Pro-Pro-Tyr-Tyr-Thr-Pro-Ala-), isolated from the fruits of Leonurus heterophyllus, showed potent immunosuppressive effect on human peripheral blood lymphocytes. The backbone structure of cycloelonurinin consists of two β-turns, a β-turn type VI at Pro-Pro, and a β I turn at Pro-Ala.

Figure-5: Naturally occurring cis-proline containing peptides.
Apart from proline itself, a variety of its derivatives are found in nature. Dehydrogenated, mono- and polysubstituted Pro derivatives have been found (Figure-6).

Figure-6: Naturally occurring proline derivatives

In many cases, the proline derivatives themselves, or peptides containing them, display antibiotic, neurotoxic or anti-tumor activity. The most common proline derivative is (4R)-hydroxyproline 3, which is a major component of collagen and was first discovered in gelatin hydrolysates in 1902. Collagen is an abundant triple-helical structure protein. In collagen, free hydroxyproline is not incorporated directly, instead proline is converted to hydroxyproline after its incorporation the peptide chain. (3S)-hydroxyproline 4 was first isolated from hydrolysates of Mediterranean sponge and was later found in human urine resulting from collagen metabolism. Moreover, many members of the class of the actinomycin antibiotics also contain (4R)-hydroxyproline as well as 4-
ketoproline 6,19 3-hydroxy-5-methylproline 520 or 5-methylproline 2.21 Both diastereomers of 3-methyl proline 822 and 923 are known both as part of cyclic peptides and even cis 3,4-methano-L-proline 1024 has been isolated. Another noteworthy member of the proline derivatives is the kainic acid 7,25 (a conformationally restricted analog of glutamic acid) which is a neurotoxin and exhibits its neurotransmitting effect through glutamate receptors.

In particular, the cis-proline conformation has been known to play an important role in the folding and activity of proteins. An example, in which a cis-proline is important for the folding of enzymes, is the class of the glutathione S-transferases.26 In this class of enzymes, a cis-proline unit mediates a sharp turn between an α-helical part of the protein and a β-strand, essential for its conformational stability and activity. Bovine prothrombin27 is another example, in which the change from a trans-proline to a cis-proline is a switch for the activity of the protein. Based on Pro to Ala mutation studies inside the loop of the Bowmann Birk Inhibitor 29 (BBI), (a family of serine protease inhibitor that contains a canonical disulfide-linked nine-residue loop), it was established that Pro is not essential for the interaction with the protease, however, it stabilizes the peptide in a biologically active cis-conformation. Biochemical assays and structural studies on Morphiceptin 30 (Tyr-Pro-Phe-Pro-NH₂), an opioid peptide that exhibited cis-trans isomerization around the Tyr-Pro peptide bond suggested
that a cis conformation around the Tyr-Pro bond is required for the biological activity of morphiceptin and related analogues. A cis conformation between two hydroxy-proline residues at positions 7 and 8 was also required for muscle-selective µ conotoxins GIIB\textsuperscript{31} for blocking voltage sensitive sodium channels.

The functional relevance of the proline cis/trans isomerization is supported by the existence of special enzymes called peptidyl-prolyl isomerases (PPIases) such as cyclophilins, FKBP’s and paruvilin family of PPIases both in vitro and in vivo.\textsuperscript{32}

For example, the phosphorylation-dependent PPIase pin1 is suggested to regulate mitosis via cis-trans isomerization of phospho Ser-Pro amide bonds in a variety of cell cycle proteins,\textsuperscript{33} particularly Cdc25 phosphatase,\textsuperscript{34} a key regulator of the Cdc2/cyclinB complex in mitosis.\textsuperscript{35} Cyclophilin HCyp 18 another PPIase, is known to take part at several steps of the HIV-1 viral lifecycle. In particular, hCyp-18 interacts with a loop located in the N-terminal part of the CA domain of the Gag polyprotein, the precursor of the nucleocapsid, capsid and matrix proteins. Even though the exact role of hCyp-18 has not been solved, viruses depleted in cyclophilin are not infectious anymore. Therefore, intense efforts are on to evaluate the role of hCyp 18 as a novel target for the development of anti-AIDS drugs. It is interesting to note that X-ray structures of human CypA (cyclophilin A) complexes with Xaa-Pro peptides showed a preference for cis-pro amides\textsuperscript{37}.
Based on the critical role of X-Pro amide bond geometry in biological processes, attempts to understand the relationship between X-Pro amide isomer geometry and protein bioactivity have led to many strategies for preparing conformationally rigid isosteres to mimic type VI $\beta$-turns of X-Pro amide bonds by stabilizing the $cis$-amide bond through different mechanisms. Efforts to mimic X-Pro dipeptide residues in peptides have focused on the geometry of the backbone, the hydrogen bond acceptor properties of the amide carbonyl as well as the shape, function and geometry of the amino acid side chains which are given below under different headings.

**By disulfide bonds:**

Cyclocystine and cyclolanthione derivatives have been examined as amide $cis$-isomer mimics that replicate the backbone geometry of X-Pro residues. Brady et al.\textsuperscript{38} during their study in testing and refining the receptor bound conformation of the small-ring somatostatin analog c(Pro\textsuperscript{6}-Phe\textsuperscript{7}-D-Trp\textsuperscript{8}-Lys\textsuperscript{9}-Thr\textsuperscript{10}-Phe\textsuperscript{11}) investigated structures constrained within bicyclic systems. Incorporation of an 8-membered $\text{Cys-Cys}$- unit in place of the Phe\textsuperscript{11}-Pro\textsuperscript{6} segment showed retention of high potency, in confirmation of cyclocystine as a good mimic for $cis$ amide (Figure-7).
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By incorporation of heterocycles and related isosteres:

Rigid sp² hybridized amide isomer surrogates that forfeit the potential hydrogen acceptor properties of the carbonyl group, have been generated using different heterocycles, for example, Marshall et al. reported the novel synthesis of 1,5-disubstituted tetrazole dipeptide analogues 12 which were shown to be the conformational mimics of the cis amide bond. Following this, proline was replaced with these surrogates and incorporated into bradikynin, somatostatin and TRH analogues for evaluating their biological activities. On the other hand, Hruby et al. with an aim of generating potent peptidomimetics incorporated the same 1,5-disubstituted tetrazole into CCK-B receptor ligands and Leucine enkephalin. In a closely related study, Takeya et al. reported the synthesis of 1,2,4-triazole 12 as a cis-amide bond surrogate via an easily accessible thiono peptide (Figure-8).
Figure-8: *cis-amide bond surrogates*

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11 synthesized conformationally rigid (Z)-alkene isosteres of Ala-cis-Pro and Ser-cis-Pro in a suitably protected form for incorporation into peptidomimetics. The key steps in their synthesis of Boc-Ala-ψ[(z)CH=C]-Pro-OH and Boc-Ser-ψ[(z)CH=C]-Pro-OH were stereoselective reduction of 14 to the (S,S)-alcohol 16, and Still-Wittig rearrangement to (Z)-alkene 18 (scheme-1).

Scheme-1 Synthetic approach to (Z)-alkene isosteres

Reagents and conditions: (a) i. cyclopentenyl lithium, sec-BuLi, THF, -40 °C (b). LAH, THF, (c). (i) Bu3SnCH2I, KH 18-crown-6, THF (ii) n-BuLi, THF, -78 °C (d) i. Formic acid, 20 % Pd(OH)2/C, MeOH, ii. Boc2O, CH2Cl2, iii. Jones reagent iv. Na/NH3, -33 °C.
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Based on this approach, inhibitors of Cyclophilin and Pin1 were designed and synthesized. The central Ala-cis-Pro core of the substrate succ-AAPF-pNA was replaced with a (Z)-alkene isostere 19 and was shown to inhibit the PPIase activity with an IC$_{50}$ value of 6.5± 0.5 µM. On the other hand, the central PhosphoSer-Pro core of the Pin1 substrate was replaced by cis and trans amide isosteres 20 and 21 peptidomimetics. The protease coupled Pin1 assay showed that the cis isostere ($K_i$=1.74±0.08 µM) to be 23 times more potent than its trans counterpart ($K_i$= 40±2 µM) in inhibiting the Pin 1 PPIase activity (Figure-9).

![Inhibitors of Cyclophilin and Pin1](image)

Figure-9: Inhibitors of Cyclophilin and Pin1

In another study to access a rigid mimic that possesses the conformational characteristics and side chain functional groups of the central two residues of the type VI β-turn, Geramanas et al. conceived that connection of alpha carbon atoms with a two atom covalent bridge would result in a conformationally stable bicyclic structure as shown in Figure-10.
An enantioselective approach for their synthesis was followed by the incorporation of L-amino acids at amino and carboxy terminus to bicyclic lactams resulting in the formation of stable H-bond between $i$ and $i+3$ residues typical of a type VI $\beta$-turn and an antiparallel $\beta$-ladder formation (Scheme-2).

A modified approach to overcome the low yielding, nonselective multi-step synthetic sequence during the synthesis of bicyclic lactams described above was
reported off late by Peter Gmeiner and co-workers. The synthesis of enantio pure compound involving Seebech’s self production of chiral methodology and ring closing metathesis strategy gave access to the lactam-bridged type VI a β-turn mimetic as shown in the Scheme-3.

Scheme-3 RCM approach to type VI a β-turn mimetic

Alternatively, based on the unique role of proline residues in cis-trans isomerization and its high frequency at the central residue of β-turns, several strategies have been employed to stabilize the cis-amide bond through directly modifying proline residues. For example, the steric interactions of methyl prolines have been employed to augment the population of the X-Pro amide cis-isomer. A
single methyl substituent at the proline 5-position was shown to have a subtle influence on the X-Pro amide isomer equilibrium of \(N\text{-}(acetyl)\) proline \(N^\prime\)-methylamide. This effect was contingent on the relative stereochemistry of the 5-methylproline. These results prompted Lubell \textit{et al}.\textsuperscript{45} to use bulkier substituents at 5-position in order to prepare X-Pro analogues with greater \textit{cis}-isomer population. Enantiopure 5-\textit{t}butyl prolines have been synthesized from glutamic acid via an acylation/diastereoselective reductive amination sequence (Scheme-4).

\textbf{Scheme-4} \textit{Synthetic approach to enantiopure 5 \textit{t}butyl prolines}

To support the importance of a particular conformation for bioactivity, Lubell and co-workers\textsuperscript{46} designed and synthesized three oxytocin analogues by substituting (2\textit{S}, 5\textit{R})-5-\textit{t}butyl proline for proline in the native peptide, the potent agonist [Mpa\textsuperscript{1}]oxytocin and the potent antagonist[dPen\textsuperscript{1}]-oxytocin (Figure-11). Their studies have led to the development of two new partial agonists and a novel
inhibitor of oxytocin action on the uterus, thereby providing additional evidence to the hypothesis that the prolyl amide *cis*-isomer may favor antagonism and the *trans* isomer is necessary for agonist activity.

Manfred Mutter introduced pseudoprolines (ψPro) as synthetic proline analogues readily obtained by cyclocondensation of the amino acids cysteine, threonine, or serine with aldehydes or ketones.\(^47\) In particular, they introduced a 2,2-dimethylated thiazolidine ψ-Pro derivative into an eleven residue cyclic loop structure with a sequence based on HIV-1MN V3 variant and containing the tetrapeptide motif Gly-Xaa-Gly-Arg to understand the underlying mechanism of conformational change during HIV life cycle.\(^48\) Their study offered interesting perspectives for applying the ψ-Proline concept as a diagnostic tool for the detection of conformational changes during biological processes.
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2.2 Present study

The choice of synthesizing conformationally locked \textit{cis}-peptidyl-prolyl bond such peptides stems from the role of PPIases in critical biological processes (e.g., in HIV and cancer). Hence, we believed that such peptides in the form of cyclic peptides without modifying proline may help in understanding protein-protein interactions thereby paving the way for the development of lead molecules. With this vision in mind, in continuation with our study on Pro containing peptides,\textsuperscript{49} we have explored the syntheses of cyclic tripeptides containing Xaa-Pro-Yaa segment with proline at \textit{i+2} position (scheme-5), which is a prerequisite for the nucleation of type VI $\beta$-turn. In the subsequent paragraphs our efforts towards the generation of a library of \textit{cis}-proline containing compounds is discussed.

Scheme-5: \textit{RCM} approach to cyclic peptides
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2.3 Results and Discussions

Previous studies in our group have shown that acyclic peptide $N$-pentenoyl-Gly-Pro-Gly allylamide 31 failed to undergo RCM reaction which was attributed to the flexibility in terms of $\phi$ and $\psi$ angles of Gly residues due to the achiral nature by virtue of which the double bonds move far apart eluding the possibility of an RCM reaction (Scheme-6). Therefore, it was imperative for us to know the side chain requirements (amino acid residues) at $N$-terminal (Xaa) and $C$-terminal (Yaa) positions of proline in order to undergo ring closing metathesis reaction.

Scheme-6: RCM of acyclic peptide 31

As a first step towards the synthesis of cis-proline containing cyclic peptides, we intended to introduce chiral amino acid in the form of L- and D-Phenylalanine in place of Gly residue in first at C-terminal to proline. The peptides $N$-pentenoyl-Gly-Pro-Phe allylamide(33) and $N$-pentenoyl-Gly-Pro-D-Phe allylamide(34) were synthesized using solution phase peptide coupling protocols based on protection-deprotection strategy as outlined in scheme-7.
According to this, the syntheses commenced with the conversion of both N-Boc-L-Phe 33 and D-Phe 34 to the corresponding allylamides 35 & 36 employing a mixed anhydride protocol using CICO₂Bu, NEt₃ and allylamine in excellent yields. The protecting groups in 35 & 36 were unmasked using TFA to obtain the amine.TFA salt, which was neutralized with NEt₃ to afford the corresponding free amine required for peptide coupling. Subsequently it was coupled with N-Boc Proline to yield the dipeptides N-Boc-Pro-L-Phe allylamide 37 and N-Boc-Pro-D-Phe allylamide 38 respectively in good yields. Deprotection and subsequent coupling with N-Boc Glycine using EDC.HCl-HOBt in dry CH₂Cl₂ yielded the tripeptides N-Boc-Gly-Pro-L-Phe allylamide 39 and N-Boc-Gly-Pro-D-Phe 40.
allylamide 40 respectively. The pentenoyl segment required for the RCM reaction was installed by coupling 4-pentenoic acid with ‘Boc’ deprotected peptides 39 and 40 using (ClCO₂Bu/NEt₃) to obtain 41 and 42 in good yields. Before proceeding for the RCM reaction, we studied the conformation of these peptides to ascertain the presence of intramolecular H-bonding. It was interesting to note that a down field shift of allylic NH (6.98 ppm) in tripeptide 41 suggested the presence of a 10-membered intramolecular H-bonding around Pro-Phe residues which was confirmed by diagnostic NOE cross peak Gly CαH ↔ Pro CδH and solvent titration studies. On the other hand, relatively upfield shift of allylic NH (6.31 ppm) in 42 and the lack of well defined NOEs suggested the absence of H-bonding thereby hinting a possible extended conformation in this molecule. We subjected these peptides to intramolecular ring closing metathesis reaction using 10 mol% Grubb’s first generation catalyst in dry CH₂Cl₂ and found that, the acyclic peptide which was organized by a β-turn underwent smooth RCM reaction to give the cyclic peptide 43, while peptide 42 did not undergo RCM reaction clearly indicating the influence of chirality at C-terminal to proline in dictating the outcome of cyclization reaction (Scheme-8).
Scheme-8: RCM of acyclic peptides 41 and 42

Reagents and conditions: (a) 1st generation Grubb's catalyst, Dry CH₂Cl₂, (b) H₂ (g), 10 % Pd/C, MeOH, 54 % over two steps.

The conformation of cyclic peptide 43 in CDCl₃ (spectrum 1A, page #132) showed the presence of a trans amide bond preceding Pro based on the diagnostic NOE between Gly CαH↔ProCδH (Figure-12). The cross peaks between AhaNH↔PheNH, PheNH↔ProCαH, PheNH↔GlyCαH (spectrum 4, page #134) coupled with the down field shift of the allylic NH and Phe NH in the ¹H NMR spectrum confirmed the presence of a β-turn around Pro-Phe residues.

To further understand the role of d-Phe at C-terminal to Pro in dictating the outcome of RCM reaction, we introduced a chiral amino acid Leucine at N-terminal to Pro and subsequently synthesized acyclic peptide N-Pentenoyl-Leu-Pro-d-Phe.
allylamide by transforming dipeptide 38 to acyclic peptide 46 via tripeptide N-Boc-Leu-Pro-D-Phe allylamide (45) following iterative protection-deprotection strategy as shown in scheme-9. It is noteworthy that RCM reaction using 1st and 2nd generation Grubb’s catalyst (20 mol %) failed to cyclize in refluxing DCM and DME thereby establishing that D-amino acid at C-terminal to Pro is not suitable for undergoing RCM reactions.

Scheme-9: Preparation of acyclic tripeptide 46 from dipeptide 38

With this encouraging result our next endeavor was to explore the role of chirality at N-terminal to proline and subsequently synthesized two acyclic peptides 51 and 52 following scheme-10. Accordingly, the free amine obtained after the deprotection of dipeptide 48 was coupled with N-Boc-L-Phe and N-Boc-D-Phe to obtain the corresponding tripeptides 49 and 50 respectively in good yields.
Deprotection followed by coupling with 4-pentenoic acid (ClCO$_2$Bu/NEt$_3$) afforded the acyclic peptides 51 & 52.

**Scheme-10: Preparation of acyclic tripeptides 51 and 52 from peptide 47**

The solution conformation of these peptides was studied in CDCl$_3$. The downfield appearance of the allylic NH in the NMR spectrum (Allylic NH = 7.41 ppm) and the indicative NOEs between d-Phe CαH↔Pro CδH, GlyNH↔Pro CαH, GlyNH↔allylNH in 52 suggested the existence of a β-turn around Pro-Gly residues. Conversely, the lack of diagnostic NOEs and upfield shift of Allylic NH
(6.52 ppm) in 51 indicated an extended conformation. It is noteworthy, though not surprising, that the peptide 52 preorganized by a β-turn underwent facile RCM in good yields and was converted to cyclic peptide 54 in fairly good yields while 51 did not (Scheme-11).

Scheme-11: *RCM of acyclic tripeptides* 51 and 52

![Scheme-11: RCM of acyclic tripeptides 51 and 52](image)

The conformational signatures in 54 based on the Solvent titration study, TOCSY and NOESY spectrum confirmed the presence of a 3_10 helical structure, similar to that observed in cyclic peptide 43 as shown in the Figure-12. We concluded that with Gly residue at C-terminal to Pro, it requires a D-amino acid at N-terminal to Pro for successful RCM reaction.
Before concluding our study about the side chain requirements needed for successful RCM reactions, we probed the influence of L-amino acid at C-terminal to Pro in the sequence peptide N-Pentenoyl-D-Phe-Pro-Leu allylamide (57). Accordingly, its synthesis was accomplished by coupling Pro-Leu allylamide (obtained after deprotecting the ‘Boc’ group in 55) with N-Boc-D-Phe to afford N-Boc-D-Phe-Pro-Leu allylamide 56 (scheme-12). This was transformed to 57 by treating with TFA followed by coupling with 4-pentenoic acid (CICO₂Bu/NEt₃).

Scheme-12: Preparation of acyclic tripeptide 57 from dipeptide 55

Reagents and conditions: (a) i. TFA, CH₂Cl₂, 0 ℃, 3h ii. N-Boc D-Phe, EDC, HCl, HOBT, Dry CH₂Cl₂, 0 ℃-rt, 81 % (b) i. TFA, CH₂Cl₂, 0 ℃, 3h ii. 4-pentenoic acid, CICO₂Bu, NEt₃, CH₂Cl₂ 0 ℃-rt, 70 %
Conformation of 57:

Acyclic peptide 57 yielded well-resolved $^1$H NMR spectra in both CDCl$_3$ and DMSO$_d_6$. Sequential resonance assignments were achieved using a combination of TOCSY and NOESY spectra. The solvent sensitivity of NH chemical shifts Leu NH (7.03 ppm) and allylic NH (6.92 ppm) was probed by addition of varying concentrations of the strongly hydrogen-bonding solvent DMSO in which Leu NH and Allylic NH changed by only nominal amount indicating their participation in H-bonding. The long distance NOE between D-Phe $\text{C}\alpha\text{H}/\text{Leu NH}$ suggested a $\beta$-turn about D-Phe-Pro residues. On the other hand, the D-Phe $\text{C}\alpha\text{H}/\text{Pro C}\delta\text{H}$ NOE together with the involvement of allylic NH in H-bonding supports the presence of $\text{trans}$-amide bond preceding proline with another $\beta$-turn around Pro-Leu residues as shown in Figure-13.

![Figure-13: Diagnostic NOEs in Pentenoyl-D-Phe-Pro-Leu allylamide (57)](image URL)
Crystals required for the X-ray studies of 57 were grown from MeOH-EtoAc-hexane system as colorless blocks in orthorhombic P2₁2₁2₁ space group (Figure-14).

Figure-14: ORTEP plot of Pentenoyl-d-Phe-Pro-Leu allylamide (57)

An intramolecular H-bond (10-membered) between the carbonyl oxygen of D-Phe(O2) and Allylic NH(N4-H) was evident from the interatomic distance of 2.05 Å. The dihedral angles around Pro-Leu residues resembled those of the \(i+1\) and \(i+2\) residues of a type I β-turn.

**Table-I \(\phi, \psi\) angles around Pro-Leu residues** (Standard values in brackets)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Dihedral angles between atoms</th>
<th>(\phi(i+1)) (\psi(i+1))</th>
<th>Dihedral angles between atoms</th>
<th>(\phi(i+2)) (\psi(i+2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C14-N2-C18-C19</td>
<td>-66.1(-60)</td>
<td>C19-N3-C20-C21</td>
<td>-110.0(-90)</td>
</tr>
<tr>
<td>2</td>
<td>N2-C18-C19-N3</td>
<td>-11(-30)</td>
<td>N3-C20-C21-N4</td>
<td>20.0 (0)</td>
</tr>
</tbody>
</table>
Another intramolecular H-bond (10-membered) between the carbonyl oxygen of pentenoyl(O1) and Leu amide proton(N3-H) was apparent from the interatomic distance of 2.39 Å. The dihedral angles around D-Phe-Pro residues resembled those of the $i+1$ and $i+2$ residues of a type II' β-turn thereby establishing $3_{10}$ helical conformation for 57.

**Table-II φ, ψ angles around D-Phe-Pro residues** (Standard values in brackets)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Dihedral angles between atoms</th>
<th>φ(i+1)</th>
<th>ψ(i+1)</th>
<th>Dihedral angles between atoms</th>
<th>φ(i+2)</th>
<th>ψ(i+2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C5-N1-C6-C14</td>
<td>59(60)</td>
<td>-127.22(-120)</td>
<td>C14-N2-C18-C19</td>
<td>-66.1(-80)</td>
<td>20 (0)</td>
</tr>
<tr>
<td>2</td>
<td>N1-C6-C14-N2</td>
<td></td>
<td></td>
<td>N2-C18-C19-N7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As expected, based on previous results, the resulting acyclic peptide underwent smooth cyclization with 10 mol% Grubb’s catalyst in dry CH$_2$Cl$_2$ to afford the cyclic peptide 58 in excellent yields ($E:Z=5:1$). The double bond was reduced using 10% Pd/C in methanol to afford the corresponding saturated cyclic peptide 59 in almost quantitative yields (scheme-13).

**Scheme-13 Preparation of cyclic peptide 59 from acyclic peptide 57**

Reagents and conditions: (a) Grubb’s catalyst, Dry CH$_2$Cl$_2$, reflux, 24 h, 64 % (b) H$_2$, 10% Pd/C, MeOH, 88.7 %
**Conformation of 59:**

At the outset, the down field appearance of Aha NH at 6.92 ppm and Leu NH at 7.03 ppm relative to d-Phe NH (at 6.07 ppm) in the $^1$H NMR spectrum (spectrum 5A, page# 135), suggested the participation of both amide protons in intramolecular H-bonding leading to the formation of a $3_{10}$ helix. Solvent titration studies (in CDCl$_3$) and variable temperature experiments (in DMSO-$d_6$) confirmed that Leu NH and Aha NH take part in intra molecular H-bonds. The nOe cross peaks, Leu NH$\leftrightarrow$Aha NH, Leu NH$\leftrightarrow$Pro C$\delta$H, Leu NH$\leftrightarrow$dPhe C$\alpha$H, Aha NH$\leftrightarrow$Pro C$\alpha$H (spectrum 8, Page # 136) in the NOESY spectrum as well as involvement of Leu NH and Aha NH in H-bonding are in agreement with two successive $\beta$-turns (Figure 15).

![NMR titration plot and NOE correlations observed in CDCl$_3$ & DMSO-$d_6$ of peptide 59](image)

**Figure-15** NMR titration plot and NOE correlations observed in CDCl$_3$ & DMSO-$d_6$ of peptide 59
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The conformation of 59 was further established by X-ray studies. Crystals of cyclo(d-Phe-Pro-Phe-Aha) required for X-ray studies were grown from a mixture of MeOH-EtoAc-Hexane system in P2₁₂₁₂₁ space group (Figure-16).

![ORTEP of cyclo(D-Phe-Pro-Leu-Aha) 59 (H atoms are not shown for clarity).](image)

An intramolecular H-bond (10-membered) between the amide carbonyl oxygen(O1) of Aha residue (Amino hexanoic acid) and Leu amide proton(N4-H) was apparent from the interatomic distance of 2.29 Å in the structure. The dihedral angle around d-Phe-Pro residue was in agreement with the standard values of a type II’ β-turn (Table III).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Dihedral angles between atoms</th>
<th>φ(i+1)</th>
<th>ψ(i+1)</th>
<th>Dihedral angles between atoms</th>
<th>φ(i+2)</th>
<th>ψ(i+2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C6-N2-C7-C15</td>
<td>48.87(60)</td>
<td></td>
<td>C15-N3-C16-C20</td>
<td>-69.5(-80)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>N2-C17-C15-N3</td>
<td>-131.47(-120)</td>
<td></td>
<td>N3-C16-C20-N4</td>
<td>-10.9 (0)</td>
<td></td>
</tr>
</tbody>
</table>
Another intramolecular H-bond (10-membered) between the amide carbonyl oxygen of d-Phe(O2) and Aha amide proton(N1-H) was obvious from the interatomic distance of 2.26 Å in the structure. The dihedral angle around Pro-Leu residue is in consonance with the \(i+1\) and \(i+2\) residues of a type I \(\beta\)-turn.

**Table IV \(\phi, \psi\) angles around Pro-Leu residues** (Standard values in brackets)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Dihedral angles between atoms</th>
<th>(\phi(i+1))</th>
<th>(\psi(i+1))</th>
<th>Dihedral angles between atoms</th>
<th>(\phi(i+2))</th>
<th>(\psi(i+2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C15-N3-C16-C20</td>
<td>-69.5(-60)</td>
<td>-10.9(-30)</td>
<td>C20-N4-C21-C26</td>
<td>-101.1(-90)</td>
<td>-10.2 (0)</td>
</tr>
<tr>
<td>2</td>
<td>N3-C16-C20-N4</td>
<td>-101.1(-90)</td>
<td>-10.2 (0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The molecules are connected by NH of d-Phe and the C=O of Leu with strong intermolecular N-H...O bonds in a helical fashion as shown in the packing diagram along a-axis (Figure-17).

![Figure-17](image)

**Figure-17** Two intramolecular N–H···O H bonds in cyclic peptide 59 and one intermolecular H bond between such rings.

These studies helped us to conclude that (a) d-amino acid at C-terminal to Pro is not apt for RCM reactions as exemplified by peptides 41 and 42. (b) Chirality in
the form of D-amino acid is apt at N-terminal to proline for RCM reactions as exemplified by peptides 50 and 57 (Scheme-14).

**Scheme-14 summary of side chain requirements**

<table>
<thead>
<tr>
<th>Structure</th>
<th>R=Ph, 41</th>
<th>R_1=H, 42 (isopropyl 55), does not undergo RCM</th>
<th>R_1=H (50) or isobutyl (57) undergoes RCM</th>
</tr>
</thead>
</table>

To summarize

Although we identified the side chain requirements at N- and C-terminal to proline needed for RCM reactions, we ended up with all these peptides preferring a *trans* geometry preceding Pro residue. However, the single crystal structure of 59 provided key insights for our synthesis of *cis*-Pro containing peptides based on the following observations. We envisaged that if one mentally visualizes L-Phe in place of D-Phe in the crystal structure of 59, the benzyl group will now be occupying a position which hydrogen occupies in 59. This would be an unfavorable situation as it would experience severe steric interactions with the proline ring (Figure-18).
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Therefore, we expected one of the two things i.e. the amide bond between Pro and Phe in the corresponding acyclic peptide \(N\)-pentenoyl-L-Phe-Pro-Leu allylamide during RCM may either try to orient itself in such a way to compensate the steric interactions between the aromatic ring and the proline moiety or may not undergo an RCM reaction. To validate our assumption we synthesized acyclic peptide \(N\)pentenoyl-L-Phe-Pro-Leu allylamide (Scheme-15). The synthesis commenced with the coupling of Leucine allylamide with \(N\)-Boc-Pro using ClO\(_4\)Bu/NEt\(_3\) to yield 53 in excellent yields. Its deprotection and subsequent coupling with \(N\)-Boc-L-Phe (EDC.HCl/HOBt) afforded the tripeptide which was further transformed (by coupling with 4-pentenoic acid) to yield tripeptide 61 with olefinic linkers needed for RCM reaction.
Scheme 15: Preparation of cyclic peptide 63 from dipeptide 53

Reagents and conditions: (a) i. TFA, CH₂Cl₂, ii. N-Boc Phe, EDC.HCl, HOBT, CH₂Cl₂, 0 °C-rt, 59 %
(b) i. TFA, CH₂Cl₂, ii. 4-pentenoic acid, ClCO₂-iBu, NEt₃, CH₂Cl₂, 0 °C-rt, 53 %
(c) 10 mol % Grubbs catalyst, Dry CH₂Cl₂, reflux, 24 h, 80 %
(d) 10 % Pd/C, H₂ (g), quantitative

It is noteworthy that the acyclic peptide 61 is similar to 57 except for the change in stereochemistry at the phenylalanine center. The ¹H NMR of acyclic peptide 61 showed the presence of two rotamers in the ratio of 7:3 in CDCl₃. The major isomer showed the presence of a trans-imide bond preceding Pro which was supported by the NOE between Phe CαH↔Pro CδH in the NOESY spectrum. Although, participation of amide protons in H-bonding was ruled out by solvent titration studies, interestingly, the acyclic peptide underwent smooth RCM to yield the 16-mer macrocycle in very good yields as a mixture of E:Z isomers (4:1
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by TLC). The double bond was reduced using 10% Pd/C in quantitative yields to afford cyclic peptide 63. The diagnostic signals in the $^1$H NMR confirmed the presence of the product (spectrum 9, page # 138).

**Conformation of 63:**

The Phe C$\alpha$H→Pro C$\alpha$H correlation in the NOESY spectrum (spectrum 10, page # 139) provided unequivocal evidence of *cis* amide bond preceding proline. Further the $^{13}$C chemical shift difference between C$\beta$ and C$\gamma$ ($\Delta$δ$^{\beta\gamma}$ = 9.24 ppm using HSQC spectrum 12 on page # 140) was used as a diagnostic tool for the confirmation of *cis*-amide bond (The C$\gamma$-endo (DOWN) pucker is preferable for the *cis*-amide bond and the C$\gamma$-exo (UP) is preferable for *trans*-amide bond). It was observed during solvent titration studies that Leu NH shifted only by 0.62 ppm when 33% v/v DMSO-$d_6$ was added to chloroform solution (Figure-19), which implied that Leu NH is participating in intramolecular H-bonding. In addition, the observation of NOEs Phe C$\alpha$H→Pro C$\alpha$H, Leu NH→Phe C$\alpha$H, Leu NH→Pro C$\alpha$H and Leu NH→Aha NH (spectrum 10, page # 139) strongly supported the existence of hydrogen bond between Leu NH - Aha CO, which nucleates a type VIa β-turn around Phe-Pro residues.
Figure 19: Titration plot and diagnostic NOE correlations observed in CDCl$_3$ and DMSO-$d_6$ for 63

The unprecedented up field shift of the Pro C$_\alpha$H (3.45 ppm), Pro C$_\beta'$H (1.21 ppm) signals were attributed to the ring current effect of phenyl ring. The NOE cross peaks Phe ortho-H$\leftrightarrow$Pro C$_\alpha$H, Phe ortho-H$\leftrightarrow$Pro C$_\delta'$H provide strong evidence for the aromatic ring and proline ring CH--$\pi$ interaction, attributing to the stabilization of the structure. In polar media (DMSO-$d_6$ solution), the moderate values of the temperature coefficients of Leu NH (-3.1 ppb/$^\circ$K) and Aha NH (-3.4 ppb/$^\circ$K) indicated that these amides participated in intramolecular hydrogen bonding. The observation of NOE correlations Phe C$_\alpha$H$\leftrightarrow$Pro C$_\alpha$H,
Leu NH↔Phe CαH and Leu NH↔Pro CδH in the NOESY spectrum, coupled with Leu NH hydrogen bonding implied a type VIa β-turn about Phe-Pro residues. Additionally, the NOEs Aha NH↔Leu NH, Aha NH↔Phe CαH, and Aha NH hydrogen bonding, suggests the presence of Leu NH-Aha C=O and Aha NH-Aha C=O H-bonds, which corresponds to a three centre hydrogen bonding network between them.

The presence of the cis-Pro amide bond in 63 was further supported by X-ray studies. Crystallization from EtOAc-MeOH-n-hexane or aqueous MeCN afforded diffraction quality single crystals for X-ray diffraction and refinement in $P2_12_12_1$ space group showed that the asymmetric unit contains one tetrapeptide and three water molecules. Interestingly, the solid state conformation was not in agreement with the solution conformation (in terms of intramolecular H-bonding) which was attributed to the presence of three water molecules in the crystal lattice of the peptide (Figure-20). Of the four amide groups, two C=Os point above the molecular rim and two below, while two NHs point upwards and one downward. All donor/ acceptor groups are aligned roughly perpendicular to the cyclic peptide rim. There are three water molecules in a sandwich of cyclic peptides connected via network of N–H···Ow, Ow–H···Ow, Ow–H···O=C hydrogen bonds.
The molecular conformation of cyclo(Phe-Pro-Leu-Aha) has a type VIa2 β-turn around Phe-Pro residues with C–H···π interaction of 2.70 Å, 154.2º (C11–H11···π, π = C4–C9 ring centroid). The carbonyl group at Phe-Pro residue is in cis conformation (ω = 4.17º, ω = 0 for the standard cis-Pro amide bond). The φ, ψ angles of the central i+1 (L-Phe), i+2 (L-Pro) residues (Table V) are consistent with a type VI a2 β-turn around Phe–Pro residues. The Cγ-down puckering of the Pro residue is in consonance with the observed cis-Pro bond and the C–H···π interaction in folded L-Phe conformation.

Table V φ,ψ angles of i+1 and i+2 residues

<table>
<thead>
<tr>
<th>S.No</th>
<th>Dihedral angles between atoms</th>
<th>φ(i+1) ψ(i+1)</th>
<th>Dihedral angles between atoms</th>
<th>φ(i+2) ψ(i+2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C1–N1–C2–C10 N1–C2–C10–N2</td>
<td>-169.3(-120)</td>
<td>C10–N2–C11–C15</td>
<td>-75.2(-60)</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>135.62(120)</td>
<td>N2–C11–C15–N3</td>
<td>-19.3 (0)</td>
</tr>
</tbody>
</table>
The hydrophobic interior of 6.5 x 4.5 Å cavity is empty and there are no intramolecular N–H···O=C H bonds. Tetrapeptide–water clusters are aligned parallel and stacked to make organic tubes along the b-axis (Figure 21).

Figure-21: Water mediated crystal structure of the cyclic peptide. Trifurcated acceptor of Pro C=O from Phe NH, O6 water and Aha CH donors. Side chains are omitted for clarity

The Pro C=O participates in an acceptor-trifurcated H bond motif from NH of Phe, OH of O6 water, and CH of Aha donors. Leu C=O accepts H bonds from O6 and phenyl CH in bifurcated mode. O5 and O7 water molecules participate in the tubular assembly via O_w–H···O=C and N–H···O_w H bonds with Aha moiety. The cylindrical architecture is visualized in Figure 22 wherein peptide rings stack with ~40% offset to make a columnar structure. Adjacent cylindrical columns are separated by weak C–H···O and van der Waals interactions.
Water inclusion frequency as a function of polar groups and hydrogen bond motifs of water in organic hydrates were analyzed in the Cambridge Structural Database. We analyzed the CSD for cyclic peptides having a tubular architecture by visualization of 58 X-ray crystal structures from a sub-database of 210 cyclic peptide hydrates. Peptide rings are connected via water molecules in 21 structures (peptide···water···peptide), via water and inter-peptide (peptide···peptide) H bonds in 28 structures, and inter peptide H bonds only in 9 structures (Table-VI, page# 93). Cyclic peptide trihydrate has strong peptide···water H bonds and a weak N1–H1···O3 inter-peptide H bond. This inspiring result prompted us to substantiate the effect of chiral amino acid at C-terminal to Proline. Consequently, we planned to substitute L-Ala in place of Leu in cyclo(Phe-Pro-Leu-Aha) as shown in scheme-16.

Figure-22: Stacking of adjacent cyclic peptides with incomplete offset generates a curving tubular architecture. Water molecules are not shown for clarity.
Accordingly our synthesis instigated with deprotection and coupling of N-Boc Ala allylamide, with N-Boc Pro to yield dipeptide 65. The amine after deprotection of 65 with TFA was coupled with N-Boc Phe (EDC.HCl/HOBt) to afford tripeptide in good yields. The presence of amide proton signals, relevant α-protons of amino acid residues in the $^1$H NMR spectrum and molecular ion in the mass spectrum
confirmed the product. This was transformed to 67 by deprotection (TFA) and amide bond formation (4-pentenoic acid).

**Conformation of 67 and 69:**

The $^1$H NMR spectrum of 67 showed the presence of two rotamers in the ratio of 3:1 in CDCl$_3$. The complete spin system of the major isomer was assigned using a TOCSY spectrum based on which the distribution of NH signals and the lack of well defined NOEs suggested the absence of intramolecular H-bonding. However, RCM reaction using Grubb’s catalyst (10 mol %) in dry CH$_2$Cl$_2$ yielded the cyclized product 68 in good yields as a mixture of E:Z isomers which was transformed to cyclic peptide 69 using 10% Pd/C in quantitative yield. The NOE cross correlation Phe C$_\alpha$H$\leftrightarrow$Pro C$_\alpha$H (spectrum 16, page # 142) and the large difference between the $^{13}$C chemical shift of C$_\beta$ and C$_\gamma$ of proline ring (9.22 ppm) using HSQC spectrum corroborated with *cis* amide bond preceding proline (spectrum 14 page #141). In both the solvents the NOEs Ala NH$\leftrightarrow$Phe C$_\alpha$H, Ala NH$\leftrightarrow$Pro C$_\delta$(Pro-S)H and Ala NH$\leftrightarrow$Pro C$_\gamma$(Pro-S)H in the NOESY spectrum (page # 142) coupled with H-bonding of Ala NH, supported for the existence of a type-VIa $\beta$-turn around Phe-Pro residues (Figure-23). The up field shift of proline C$_\alpha$H (3.42 ppm), C$_\beta$(Pro-S)H (1.22 ppm), Pro C$_\delta$(Pro-R)H (3.38 ppm) and NOE peaks between aromatic ortho H with proline C$_\alpha$H, C$_\beta$(Pro-S)H and C$_\delta$(Pro-R)H was due to the influence Phe aromatic ring indicating a strong CH$^-$-$\pi$ interaction.
between the two rings. In DMSO-$d_6$ the NOE Aha NH$\leftrightarrow$Phe C$\alpha$H suggested Aha NH H-bond between Aha NH$\leftrightarrow$Aha CO resulting in a 9-membered ring.

Figure-23: Solvent titration plot and diagnostic NOEs of cyclo(Phe-Pro-Ala-Aha)$_{69}$ in CDCl$_3$ and DMSO$_{d_6}$

Both the cis-Pro containing peptides studied till this point possessed L-Phe at N-terminal to Pro residue which is known to augment cis-Pro population. Our study of cyclic peptides synthesized by sequence mutation of peptide 63 with different amino acids namely Ala, Val and Leu respectively in place to Phe (Scheme-17) was crucial to generalize the chirality based nucleation of cis-Pro amide bond formation in cyclic peptides.
Scheme-17: Preparation of Cyclic Peptides 73, 77 and 81 from dipeptide 37

The synthesis of $N$-pentenoyl-Ala-Pro-Phe allylamide (71), $N$-pentenoyl-Val-Pro-Phe allylamide (75) and $N$-pentenoyl-Leu-Pro-Phe allylamide (79) emanated with the deprotection of dipeptide 37 followed by coupling (EDC/HOBt) with $N$-Boc-Ala, $N$-Boc-Val and $N$-Boc-Leu respectively to afford the tripeptide 70, 74 and 78 in good yields. Unmasking of the ‘Boc’ group followed by subsequent coupling with pentenoic acid ($\text{ClC}O_2\text{Bu/NEt}_3$) afforded the acyclic tripeptides 71, 75 and 79. The $^1$H NMR spectrum of these peptides showed the presence of two
rotamers (in ratio of 4:1 for 71, 9:1 for 75 and 4:1 for 79). Based on the assignments of the major isomer using TOCSY spectrum, the upfield resonances of NHs and lack of well defined NOEs implied the absence of H-bonding and hence extended conformation for all three peptides. However, RCM reaction of 71, 75 and 79 followed by the reduction of the resulting double bond (10 % Pd/C in methanol) proceeded smoothly furnishing the cyclic peptides 73, 77 and 81 in good yields (Scheme-17).

**Conformation of 73:**
Well resolved spectra were obtained in both the solvents. The NOE cross correlation Ala CαH↔Pro CαH and the large chemical shift difference between the 13C chemical shifts of Pro Cβ and Pro Cγ (∆δ= 9.95 ppm) confirmed the Ala-Pro amide bond as cis configuration (spectrum 19, page# 144). Further, Phe NH showing moderate shift (0.79 ppm) in solvent titration study in CDCl3 (figure 28) coupled with moderate magnitude (–4.1 ppb/°K) of ∆δ/∆T in DMSO-d6 suggested its involvement in H-bonding. Further, the NOE cross correlations (spectrum 20, page# 145) between Phe NH↔Aha NH, Ala CαH↔Pro CαH, Phe NH↔Ala CαH, Phe NH↔Pro Cδ(Pro-S) H (Figure 24) were in agreement with a type-VIα β-turn around Ala-Pro residues. The moderate magnitude of –3.1 ppb/°K of Aha NH in 73 in DMSO-d6 as well as cross correlation between Aha NH↔Ala CαH supporting the existence of 9-membered H-bond Aha C=O–Aha NH.
Figure-24: Titration plot and diagnostic nOe correlations observed in CDCl₃ and DMSO-d₆ of 73.

Conformation of 77:

In both solvents the amide bond preceding proline in cyclo(Val-Pro-Phe-Aha) takes a cis geometry, which was supported by the NOE correlation between Val CαH ↔ Pro CαH (spectrum 23, page# 147) and confirmed by large chemical shift difference between the ¹³C chemical shifts of Pro Cβ and Pro Cγ (Δδ = 10.03 ppm). An additional support for cis amide bond was the NOE correlation between Phe NH ↔ Pro CγH (Figure-25). Hydrogen bonding studies in CDCl₃ solution
suggested none of the amide protons to be involved in H-bonding. However, the NOEs between Val CαH↔Pro CαH, Phe NH↔Val CαH, Phe NH↔Aha NH, Aha NH↔Val CαH and Aha NH (Δδ/ΔT=-1.6 ppb/°K) suggested its participation in a 9-membered H-bonding (Figure-27).

![Diagram](image.jpg)

Figure-25: *Diagnostic NOE correlations observed in CDCl₃ and DMSO-d₆ of peptide 73.*

**Conformation of 81:**

Cyclic peptide cyclo(Leu-Pro-Phe-Aha), is a positional isomer of peptide 63. The conformational analysis in CDCl₃ as well as in DMSO-d₆ solutions showed that a single rotamer with a *cis* amide geometry preceding proline. Based on the amide proton signals and NMR titration study, Phe NH was found to be involved in H-bonding with Aha C=O group forming a β-turn. In both solvent media, the observation of characteristic NOE cross peak between Leu CαH↔Pro CαH and the large difference between the ¹³C chemical shift values of proline Cβ and Cγ (Δδ_{βγ} = 10.06 ppm) (spectrum 25 and 26, page# 149 and 150), corroborates the existence of *cis* amide bond. In addition the small magnitude of Aha NH
temperature coefficient (-1.5 ppb/°K) in DMSO-\textsubscript{d6} and cross peaks Aha NH\textrightarrow Leu CoH, Aha NH\textrightarrow Phe NH further support a 9-membered H-bonding between Aha C=O\textrightarrow Aha NH.

![Figure 26](image)

**Figure-26 Diagnostic NOE correlations of cyclo(Leu-Pro-Phe-Aha) 81 in CDCl\textsubscript{3} and DMSO\textsubscript{d6}**

These results established that side chain residue that precedes proline, play an important role in the nucleation of cis amide bond. Database study containing Pro-Pro dipeptide sequences has shown that maximum amount of cis population exists between Pro-Pro linkage. We became interested to investigate the role of Proline preceding Pro residue situated at \(i+2\) position since Pro-Pro dipeptide template has the possibility of forming cis/trans isomerisation about two imide bonds that results in the likely presence of four isomers corresponding to trans-trans, trans-cis, cis-trans and cis-cis imide bonds. Consequently, we started the synthesis of the cyclic peptide cyclo(Pro-Pro-Phe-Aha) following the protection-deprotection strategy as shown in the scheme-18.
Scheme 18 Preparation of cyclic peptide 84 from dipeptide 37

Reagents and conditions: (a) i. TFA, CH₂Cl₂, ii. N-Boc Pro, EDC.HCl, HOBT, CH₂Cl₂, 0 °C-rt, 75%. (b) i. TFA, CH₂Cl₂, ii. 4-pentenoic acid, ClCO₂iBu, NEt₃, CH₂Cl₂, 0 °C-rt, 57 % (c) i. Grubb's catalyst, Dry CH₂Cl₂, reflux ii. H₂, Pd/C, MeOH, 48 % over two steps.

The ¹H NMR spectrum of 83 in CDCl₃ showed the presence of two rotamers in the ratio of ~55:45 (spectrum 27, page #151). The relatively down field shifting of both the NH’s (Phe NH and Aha NH) implied the presence of preorganised structure which prompted us to investigate this peptide in detail. The resonances were assigned using a TOCSY spectrum (spectrum 28, page# 152) based on which the NOE cross correlation between Pro CαH of the two Pro residues (spectrum 29, page# 153) confirmed the presence of the cis-amide bond in the major isomer while the minor isomer was devoid of a preorganised structure.
Anticipating an interesting result, we subjected it to RCM reaction, which yielded the cyclic peptide in good yields. Reduction of the double bond using 10% Pd/C in methanol afforded the corresponding saturated cyclic peptide 84 in excellent yield. In CDCl₃ solution the appearance of single set of resonances in ¹H NMR spectrum (spectrum 30A, page# 154) implies the presence of a single rotamer in solution. The NOE cross correlations Aha CαHs↔¹Pro1 CδHs and ¹Pro1 CαH↔¹Pro2 CαH are in agreement with a trans amide bond about Aha-¹Pro1 and cis amide bond about ¹Pro1-¹Pro2 (spectrum 31B, page # 155). This was further confirmed by the difference between ¹³C chemical shift values of Cβ and Cγ of proline rings (spectrum 31A, page# 155). First proline ring, which has a trans amide bond showed small difference (Δδ_Cβγ = 3.00 ppm), where as the second proline ring which has a cis amide bond preceding it, showed large chemical shift difference (Δδ_Cβγ = 10.82 ppm). The down field appearance of both Phe NH (7.17 ppm) and Aha NH (7.49 ppm) protons in ¹H NMR spectrum indicates the possibility of their involvement in H-bonding (Figure 29). This was further confirmed by the small change in their chemical shift values during solvent titration study. The nOe cross correlations Phe NH↔Aha NH, ¹Pro1 CαH ↔¹Pro2 CαH, Phe NH↔¹Pro1 CαH, Aha NH↔¹Pro1 CαH suggest the possibility of the observed H-bonds between Aha CO←Phe NH with a type VIa
β-turn about L-Pro1-L-Pro2 and Aha CO←Aha NH with unusual 9-membered three centre H-bonding (Figure-27).

**Figure-27:** *Titration plot and diagnostic NOE correlations observed for peptide 84 in CDCl₃.*

In polar solvent, DMSO-d₆ two distinctive sets of resonances in the ratio of 55:45 indicate the existence of two conformations in solution. In the major isomer the NOEs, Aha CαHs↔L-Pro1 CδHs and L-Pro1 CαH↔L-Pro2 CαH support a *trans* amide bond about Aha-Pro and *cis* amide about L-Pro1-L-Pro2 residues (*trans* – *cis* isomer). The Δδ/ΔT of −1.9 and −3.5 ppb /° K for Aha NH and Phe NH, respectively, are in agreement with their participation in intramolecular H-bonding. In addition, the NOE correlations Phe NH↔L-Pro1 CαH, L-Pro1 CαH↔L-Pro2 CαH, Phe NH↔Aha NH and Aha NH↔L-Pro1 CαH and involvement of Phe NH in intra molecular H-bonding is in agreement with a type-VIa β-turn about L-Pro1-L-Pro2 residues. On the other hand in the minor isomer the
large $\Delta \delta/\Delta T$ values ruled out the possibility of H-bonding. The NOE cross correlations Aha $\alpha$Hs $\leftrightarrow$ $^L$Pro1 $\alpha$H and $^L$Pro1 $\alpha$H $\leftrightarrow$ $^L$Pro2 $\alpha$H confirm the presence of cis amide about Aha-Pro1 and cis amide about $^L$Pro1-$^L$Pro2 residues (cis – cis isomer) (Figure-28). The unprecedented up field appearance of Pro2 $\gamma$(Pro-S)H at 0.70 in CDCl$_3$ and 0.81 in DMSO-$d_6$ as well as the NOEs between Pro2 $\gamma$(Pro-S)H $\leftrightarrow$ Phenyl aromatic protons unequivocally support the existence of CH$^\cdots$$\pi$ H-bonding between Pro2 $\gamma$(Pro-S)H and Phe aromatic ring.

![Chemical Structure]

Figure-28: Diagnostic nOe correlations observed for two isomers in DMSO-$d_6$ of peptide 81 trans-cis and cis-cis isomers.

Our study of synthesizing a cyclic peptide devoid of aromatic ring substituent was imperative to prove that cyclization is the primary governing factor for inducing cis-amide bonds preceding Pro residue and the aromatic interaction is just a stabilizing factor in this series of peptides. Hence, we substituted Leucine in place of Phenylalanine in 73 and accordingly synthesized cyclic peptide cyclo(Ala-Pro-Leu-Aha) as shown below.

Scheme-19: Preparation of cyclic peptide 87 from dipeptide 53
It is noteworthy that in spite of the absence of aromatic side chain, acyclic peptide 86 underwent smooth RCM reaction and was transformed to 87 using 10% Pd/C in methanol. The cis amide geometry preceding proline which was confirmed by the NOE correlation between Ala CαH→Pro CαH and $^{13}$C chemical shifts ($\Delta \delta_{C\beta\gamma} = 9.50$ ppm). In both CDCl$_3$ and DMSO-$d_6$ solvents, the participation of Leu NH in H-bonding as well as NOEs (spectrum 35 and 36, page# 157), were in agreement with a type-VIa $\beta$-turn about Ala-Pro residues as shown in the Figure-29.

Figure 29: Solvent Titration plot and diagnostic NOE correlations observed for cyclo(Ala-Pro-Leu-Aha) 87 in DMSO$_d_6$
2.4 Conclusion

In conclusion, we have demonstrated a chirality based nucleation of cis-Pro amide bonds in a series of tripeptides tethered by amino hexanoic acid as linker which was generated using an RCM reaction. Our initial studies of probing side chain requirements laid the foundation for our study following which, based on logical reasoning, we have successfully demonstrated the nucleation of type VIa2 β-turn. We believe that such peptides may either help in understanding critical protein-protein interactions and/or such tailor made molecules may also be used to mimic bioactive conformations of peptides where such conformation are necessary in eliciting a biological response.
Chapter II

Results at a Glance

![Chemical Structures Diagram]

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2.5 References


Chapter II


Chapter II


Chapter II


45. (a) Beausoleil, E.; L’Archeveque, B.; Belec, L.; Atfani, M.; Lubell, W. D.  


49. Boruah, A.; Rao, I. N.; Nandy, J. P.; Kumar, S. K.; Kunwar, A. C.; Iqbal,  


53. See Table VI on next page
### Table VI (Ref codes)

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\(^a\) Water and MeOH contacts
2.6 Experimental

General procedure for the preparation of N-Boc-L-Yaa allylamide (A)

To an ice cold stirred solution of N-Boc protected amino acid (1 equivalent) in dry CH₂Cl₂ was added NEt₃ (2 equivalent) followed by the addition of ClCO₂Bu (1.5 equivalent). After 5 min at 0 °C a solution of allylamine (1.2 equivalent) in dry CH₂Cl₂ was added and stirred at room temperature for a period of 12 h. The reaction mixture was diluted with CH₂Cl₂, washed with water, brine, dried over Na₂SO₄ and evaporated the solvent to afford crude product which was purified using MeOH-CHCl₃ as the eluent using 100-200 mesh silica gel to afford the title compound.

General procedure for the preparation of N-Boc-L-Pro-Yaa allylamide (B)

A. To an ice cold stirred solution of N-Boc-AA allylamide (1 equivalent) in dry dichloromethane was added trifluoroacetic acid (10 equivalent) at 0 °C under argon atmosphere and stirred at the same temperature for 3 h. 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-L-Proline (1 equivalent) in dry dichloromethane at 0 °C was added NEt₃ (2 equivalents) followed by the addition of isobutyl chloroformate (1.5 equivalent). After 5 min at 0 °C a solution of amine (obtained in part A) (1 equivalent) in dry CH₂Cl₂ was added and stirred at room
temperature for a period of 12 h. The reaction mixture was diluted with CH$_2$Cl$_2$ and washed with water and brine. Solvent evaporation under reduced pressure followed by purification using 100-200 mesh silica and MeOH-CHCl$_3$ as eluent afforded the required dipeptide

**Preparation of N-Boc-Xaa-pro-Yaa allylamide (C)**

**A.** To a ice cold stirred solution of N-Boc- L-Pro-Yaa allylamide (1equivalent) in dry CH$_2$Cl$_2$ was added trifluoroacetic acid (10 equivalents) at 0 °C under argon atmosphere and stirred at 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 ºC to obtain the free amine.

**B.** To an ice cold stirred solution of N-Boc-Xaa-OH (1equivalent) and HOBt (1.2 equivalent) of dry CH$_2$Cl$_2$ was added a solution of L-Pro-Yaa allylamide (obtained in part A) (1equivalent) in CH$_2$Cl$_2$. EDC.HCl (1.5 equivalent) 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$_2$ washed with water, brine, dried over Na$_2$SO$_4$ 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.

**Preparation of Pentenoyl-Xaa-Pro-Yaa allylamide (D)**

**A.** To a stirred solution of N-Boc-Xaa-Pro-Yaa allylamide (1equivalent) in dry v was added trifluoroacetic acid (10 equivalents) at 0 °C under argon atmosphere
and stirred at 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 4-Pentenoic acid (1 equivalent) in dry CH$_2$Cl$_2$ at 0 °C was added NEt$_3$ (2 equivalents) followed by the addition of ClCO$_2$Bu (1.5 equivalents). After 5 min a solution of amine (obtained in part A) (1.53 g, 3.7 mmol) in dry CH$_2$Cl$_2$ was added at 0 °C and then stirred at room temperature for a period of 12 h. The reaction mixture was diluted with CH$_2$Cl$_2$ washed with water, brine, dried over Na$_2$SO$_4$ 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.

**Ring Closing Metathesis of Pentenoyl-Xaa-Pro-Yaa allylamide (E)**

To a stirred solution of Grubb’s ruthenium catalyst (10 mol%) in dry dichloromethane (300 ml) under nitrogen was added a solution of Pentenoyl-Xaa-Pro-Yaa allylamide (1 equivalent) in dry CH$_2$Cl$_2$ slowly over a period of 15 min and the mixture was refluxed for 12 h after. After 16-28 h, the reaction was exposed to air and directly subjected to column purification to afford the corresponding cyclic compound as a mixture of $E$ and $Z$ isomers in 50-60 % yield as an off white solid.
Reduction of the double bond in the cyclic peptide (F)

To a stirred solution of the unsaturated cyclic peptide (100 mg) in 5 ml of methanol was added 20 mg of 10 % Pd/C. The mixture was hydrogenated using a H₂ gas balloon at 20 psi for 3h. Pd/C was filtered off using celite bed and the celite pad was washed thoroughly with methanol. Combined organic layers were evaporated and the crude compound was chromatographed to afford the saturated analogue of the cyclic peptide.

Preparation of N-Boc-L-Phe allylamide (35):

This experiment was carried out following the general procedure (A) using (2g, 7.5 mmol) N-Boc-L-Phenylalanine, (0.6 ml, 8.30 mmol) of allylamine, (1.5 ml, 11.32 mmol) of ClCO₂Bu and (3.1 ml, 22.62 mmol) of NEt₃ to afford (2g, 87.3 %) of title product as a white solid.

mp. 96-100 °C, [α] = -9.00 (c, 0.1, MeOH), IR (KBr): 3336, 3323, 2981, 2968, 1685, 1658, 1525, 1170, 771 cm⁻¹, ¹H NMR (500 MHz, CDCl₃): δ 7.32-7.19 (m, 6H), 5.79 (bs, 1H), 5.74-5.64 (m, 1H), 5.07-5.01 (m, 2H), 4.30 (dd, J = 14.0 and J = 7.1 Hz, 1H), 3.80 (t, J = 5.6 Hz, 2H), 3.10-3.01 (m, 2H), 1.41 (s, 9H), Mass (ES mass): 305 ((M+H)⁺, 100).
Preparation of N-Boc-Pro-Phe allylamide (37):

The title product was obtained by following the general procedure (B) using (2g, 9.30 mmol) of N-Boc-Proline, (1.89g, 9.30 mmol) of L-phenylalanine allylamide, (1.81 g, 13.9 mmol) of ClCO₂Bu and (3.9 ml, 27.9 mmol) in 86 % yield as a pale yellow gum.

\[ \alpha = -51.00 \] (c, 0.1, MeOH), IR (Neat): 3297, 2977, 1663, 1548, 1396 cm⁻¹, \(^1\)H NMR (400 MHz, CDC\(_3\)): δ 7.31-7.16 (m, 5H), 6.77 (bs, 1H), 6.38 (bs, 1H), 5.74-5.70 (m, 1H), 5.10-5.02 (m, 2H), 4.73 (m, 1H), 4.18-4.16 (m, 1H), 3.82-3.72 (m, 2H), 3.42-3.20 (m, 3H), 3.07-3.0 (m, 1H), 2.07-2.03 (m, 3H), 1.83-1.71 (m, 1H), 1.42 (s, 9H), Mass CI method): 402 (M+H\(^+\), 64), 346 (100), 302 (95).

Preparation of N-Pentenoyl-Gly-Pro-Phe allylamide (41):

Following the general procedure (C) (460 mg, 2.61 mmol) of N-Boc-Gly, (788 mg, 2.61 mmol) of Pro-Phe allylamide was transformed to N-Boc-Gly-Pro-Phe allylamide (39) (980 mg, 82 %) as a white hygroscopic solid. This was transformed to title product following general procedure (D) using (0.22 ml, 2.15 mmol) of 4-pentenoic acid and (770 mg, 2.15 mmol) of Gly-Pro-Phe allylamide in (620 mg, 66 %) yield as a white solid.
mp. 151-152 °C, [α] = -63.0 (c, 0.1, MeOH), IR (KBr): 3308, 3074, 2929, 1635, 1536, 1456, 1431 cm\(^{-1}\), \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.28-7.15 (m, 5H), 6.96 (d, \(J = 8.3\) Hz, 1H), 6.40 (t, \(J = 5.1\) Hz, 1H), 6.35 (bs, 1H), 5.87-5.71 (m, 2H), 5.25-5.01 (m, 4H), 4.67-4.62 (m, 1H), 4.48-4.44 (m, 1H), 3.98-3.82 (m, 4H), 3.51-3.40 (m, 1H), 3.48 -3.45 (m, 1H), 3.27 (dd, \(J = 13.96\) Hz and \(J = 5.65\) Hz, 1H), 3.05 (dd, \(J = 13.96\) Hz, and \(J = 9.4\) Hz, 1H), 2.44-2.33 (m, 4H), 2.12-2.05 (m, 1H), 1.99-1.82 (m, 3H), Mass (CI method) (m/z): 441((M+H)\(^+\), 37), 384 (48), 356 (65), 237 (100).

**Preparation of c(Gly-Pro-Phe) (43):**

The unsaturated cyclic peptide was obtained by following the general procedure (E) using 41 (250 mg, 0.56 mmol) and 10 mol % Grubbs catalyst in 250 ml of dry CH\(_2\)Cl\(_2\) as a mixture of E:Z (60:40 by TLC) isomers which was transformed to title compound following the general procedure (F) using 30 mg of 10 % Pd/C to afford (127 mg, 54 %, over two steps) of title product as a white fluffy solid.

mp. 241-243 °C, [α] = -29.4 (c, 0.5, MeOH), IR (KBr): 3306, 2930, 1641, 1555, 1449 cm\(^{-1}\), \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.36-7.21 (m, 5H, Aromatic), 6.75 (dd, \(J = 7.9\) Hz and \(J = 4.4\) Hz, 1H, Aha NH), 6.47 (d, \(J = 9.3\) Hz, 1H, Phe NH), 6.27 (dd, \(J = 6.1\) and \(J = 4.8\) Hz, 1H, Gly NH), 4.76 (m, 1H, Phe C\(\alpha\)H), 4.37 (dd, \(J =\)
8.7 Hz and \( J = 3.8 \) Hz, 1H, Pro C\( \alpha \)H), 4.32 (dd, \( J = 15.9 \) Hz and \( J = 6.1 \) Hz, 1H, Gly C\( \alpha \)H), 3.75 (m, 1H, Aha C\( \varepsilon \)H), 3.66 (ddd, \( J = 9.7 \) Hz, 7.8 Hz, 4.1 Hz, 1H, Pro C\( \delta \)H), 3.47 (dd, \( J = 14.2 \) Hz and \( J = 4.7 \) Hz, 1H, Phe C\( \beta \)H), 3.43 (m, 1H, Pro C\( \delta ' \)H), 3.40 (dd, \( J = 15.9 \) Hz and \( J = 4.8 \) Hz, 1H, Gly C\( \alpha ' \)H), 3.07 (dd, \( J = 14.2 \) Hz and \( J = 10.5 \) Hz, 1H, Phe C\( \beta ' \)H), 2.82 (m, 1H, Aha C\( \varepsilon \)H), 2.35 (m, 1H, Aha C\( \alpha \)H), 2.20 (m, 1H, Aha C\( \alpha ' \)H), 2.04 (m, 1H, Pro C\( \beta \)H), 1.77 (m, 1H, Pro C\( \gamma \)H), 1.72 (m, 1H, Pro C\( \beta ' \)H), 1.62-1.36 (m, 7H), Mass (CI method (m/z): 415 ((M+H)+, 100).

**Preparation of N-Boc-d-Phe allyl amide (36):**

\[
\begin{array}{c}
\text{BocHN} \quad \overset{\text{Ph}}{\text{N}} \quad \overset{\text{O}}{\text{N}} \\
\end{array}
\]

The title compound was obtained following the general procedure (A) using (2.1 g, 7.92 mmol) of N-Boc-d-Phenylalanine (0.7 ml, 8.71 mmol) of allyl amine (1.34 ml, 10.30 mmol) of ClCO\(_2\)iBu and (3.3 ml, 23.7 mmol) of NEt\(_3\) to afford (1.6 g, 72.7 %) of title product as a cream colored solid.

mp. 96-98 °C, [\( \alpha \)] = -10.9 (c, 0.5, MeOH), IR (KBr): 3340, 2968, 1686, 1658, 1523, 1169 cm\(^{-1}\), \(^{1}\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 7.31-7.19 (m, 6H), 5.82-5.64 (m, 2H), 5.09-5.01 (m, 2H), 4.30 (dd, \( J = 14.50 \) Hz and \( J = 7.0 \) Hz, 1H), 3.79 (t, \( J = 5.75 \) Hz, 2H), 3.11-3.01 (m, 2H), 1.40 (s, 9H), Mass (CI method (m/z): 305 ((M+H)+, 31).
Preparation of N-Boc-Pro-d-Phe allylamide (38):

The title compound was obtained following the general procedure (B) using (1.05 g, 4.90 mmol) of N-Boc-L-Proline, (1 g, 4.90 mmol) of d-Phe allylamide, (0.95 ml, 7.35 mmol) of ClCO₂Bu and (2.05 ml, 14.65 mmol) of NE₃ to afford (1.5 g, 76.9 %) of title product as a cream colored solid.

IR (KBr): 3267, 2926, 1700, 1643, 1546, 1396, 1162 cm⁻¹, ¹H NMR (300 MHz, CDCl₃): δ 7.27-7.16 (m, 5H), 7.05 (bs, 1H), 6.77 (bs, 1H), 5.78-5.67 (m, 1H), 5.05-4.98 (m, 2H), 4.72-4.64 (m, 1H), 3.99-3.77 (m, 1H), 3.76-3.72 (m, 2H), 3.42-3.21 (m, 2H), 3.18-2.95 (m, 2H), 1.95-1.62 (m, 4H), 1.41 (s, 9H), Mass (CI method (m/z): 402 ((M+H)+, 100).

Preparation of N-Boc-Gly-Pro-d-Phe allylamide (40):

The title compound was obtained following the general procedure (A) using (0.61 g, 3.49 mmol) of N-Boc-Glycine, (1.05 g, 3.49 mmol) of Pro-d-Phe allylamide, (0.56 g, 4.18 mmol) of HOBt and (1 g, 5.23 mmol) of EDC.HCl to afford (0.86 g, 54 %) of title product as a pale yellow gum.
IR (KBr): 3314, 3066, 2978, 1648, 1530, 1453, 1167 cm$^{-1}$, $^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 7.30-7.18 (m, 5H), 7.00 (bs, 1H), 6.41 (bs, 1H), 5.77-5.71 (m, 1H), 5.24 (bs, 1H), 5.16-5.08 (m, 2H), 4.65 (m, 1H), 4.18-4.15 (m, 1H), 3.93-3.76 (m, 4H), 3.50-3.43 (m, 2H), 3.22-3.21 (m, 2H), 2.15-1.92 (m, 4H), 1.44 (s, 9H).

**Preparation of N-pentenoyl Gly-Pro-d-Phe-allylamide (42):**

The title product was obtained by following the general procedure (D) using (0.19 ml, 1.90 mmol) of 4-pentenoic acid, (0.68 g, 1.90 mmol) of Gly-Pro-d-Phe allylamide, (0.37 ml, 2.84 mmol) of ClCO$_2$Bu and (0.8 ml, 5.7 mmol) of NEt$_3$ to yield (487 mg, 64 %) as a gummy mass.

[$\alpha$] = -35.0 (c, 0.1, MeOH), IR (Neat): 3305, 3068, 2930, 1640, 1545, 1448 cm$^{-1}$, $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.31-7.19 (m, 5H, Aromatic), 6.69 (t, $J = 6.0$ Hz, 1H, Allylic NH), 6.51 (d, $J = 8.3$ Hz, 1H, d-Phe NH), 6.29 (t, $J = 4.8$ Hz, 1H, Gly NH), 5.88-5.67 (m, 2H), 5.17-5.03 (m, 4H), 4.66 (m, 1H, d-Phe C$\alpha$H), 4.23 (dd, $J = 7.8$ Hz and $J = 4.6$ Hz, 1H, Pro C$\alpha$H), 4.09 (dd, $J = 17.8$ Hz and $J = 4.8$ Hz, 1H, Gly C$\alpha$H), 3.91 (dd, $J = 17.8$ Hz and $J = 3.7$ Hz, 1H, Gly C$\alpha$H), 3.85-3.81 (m, 2H, Allylic-CH$_2$), 3.54 (ddd, $J = 9.8$, 7.6, 5.2 Hz, 1H Pro C$\delta$H), 3.45 (m, 1H), 3.22 (dd, $J = 14.1$ Hz and $J = 7.0$ Hz, 1H, Phe C$\beta$H), 3.14 (dd, $J = 4.1$ and $J = 6.2$ Hz, 1H).
Hz, 1H, Phe β'H), 2.39-2.32 (m, 4H, pentenoyl), 3.13-1.95 (m, 4H), Mass (CI method) (m/z): 441 (M+H)^+, 100).

**Preparation of N-Boc-Leu-Pro-δ-Phe allylamide (45):**

The title compound was obtained following the general procedure (A) using (0.82 g, 3.54 mmol) of N-Boc-Leucine, (1.07 g, 3.54 mmol) of Pro-δ-Phe allylamide, of (576 mg, 4.26 mmol) 1-hydroxy benzotriazole and (1.02 g, 5.33 mmol) of EDC.HCl to afford (0.98 g, 54 %) of title product as a white solid.

IR (KBr): 3425, 2925, 2855, 1639, 1533, 1450, 1168 cm\(^{-1}\), \(^1\)H NMR (400 MHz, CDCl\(_3\)): δ 7.31-7.18 (m, 5H), 6.98 (t, \(J = 4.5\) Hz, 1H), 6.28 (t, \(J = 8.8\) Hz, 1H), 5.80-5.72 (m, 1H), 5.12-5.06 (m, 2H), 4.96 (d, \(J = 8.8\) Hz, 1H), 4.73-4.68 (m, 1H), 4.44-4.42 (m, 1H), 4.16-4.12 (m, 1H), 3.84-3.82 (m, 2H), 3.79-3.73 (m, 1H), 3.57-3.51 (m, 1H), 3.25 (dd, \(J = 13.9\)Hz and \(J = 6.6\) Hz, 1H, Phe Cβ'H), 3.11 (dd, \(J = 13.9\) Hz and \(J = 5.1\) Hz, 1H, Phe Cβ'H), 2.17-2.11 (m, 1H), 2.08-1.99 (m, 2H), 1.98-1.90 (m, 1H), 1.73-1.65 (m, 1H), 1.41 (s, 9H), 1.38-1.34 (m, 2H), 0.95 (d, \(J = 6.6\) Hz, 3H), 0.92 (d, \(J = 6.3\) Hz, 3H), Mass (CI method) (m/z): 515 ((M+H)^+, 100).

**Preparation of N-Pentenoyl-Leu-Pro-δ-Phe allylamide (46):**

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The title product was obtained by following the general procedure (D) using (0.19 ml, 1.84 mmol) of 4-pentenoic acid, (0.76 g, 1.84 mmol) of Leu-Pro-d-Phe allylamide, (0.35 ml, 2.75 mmol) of isobutyl chloroformate and (0.77 ml, 5.50 mmol) of NEt₃ in (0.54 g, 59 %) yield as a colorless gum.

IR (KBr): 3298, 2957, 1635, 1536, 1446 cm⁻¹, ¹H NMR (400 MHz, CDCl₃): δ 7.32-7.18 (m, 5H, Aromatic), 6.85 (t, J = 5.15 Hz, 1H, NH), 6.33 (d, J = 8.3 Hz, 1H, NH), 5.89 (d, J = 8.8 Hz, 1H, NH), 5.83-5.71 (m, 2H), 5.11-4.98 (m, 4H), 4.80-4.72 (m, 1H, CαH), 4.70-4.67 (m, 1H), 4.16-4.13 (m, 1H), 3.85-3.70 (m, 3H), 3.58-3.52 (m, 1H), 3.23 (dd, J = 14.16 Hz and J = 6.85 Hz, 1H, Phe CβH), 3.10 (dd, J = 13.9 Hz and J = 6.1 Hz, 1H, Phe Cβ'H), 2.38-2.33 (m, 2H), 2.29-2.25 (m, 2H), 2.17-2.09 (m, 1H), 2.06-1.95 (m, 3H), 1.63-1.59 (m, 1H), 1.41-1.39 (m, 2H), 0.95 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H), Mass (CI method (m/z):498 ((M+H)⁺, 49), 302 (100).

**Preparation of N-Boc-Gly-allylamide (47):**

The title compound was obtained following the general procedure (A) using (1.2 g, 6.85 mmol) of N-Boc-Glycine, (0.6 ml, 8.22 mmol) of allylamine, (1.1
ml, 8.22 mmol) of ClCO$_2$Bu and (2.8 ml, 20.57 mmol) of NEt$_3$ to afford (1.04 g, 71.2 %) of title product as a colorless gum.

IR (Neat): 3322, 3084, 2980, 2932, 1667, 1531, 1368, 1250, 1170 cm$^{-1}$, $^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 6.33 (bs, 1H), 5.93-5.74 (m, 1H), 5.24-5.12 (m, 2H), 4.10 (t, $J = 7.0$ Hz, 2H), 3.90-3.80 (m, 2H), 1.45 (s, 9H), Mass (CI method) (m/z): 215 ((M+H)$^+$, 23), 159 (100), 115 (15).

**Preparation of N-Boc-Pro-Gly-allylamide (48):**

The title compound was obtained following the general procedure (B) using (0.77 g, 3.59 mmol) of N-Boc-Proline, (0.41 g, 3.59 mmol) of Glycine allylamide, (0.7 ml, 5.37 mmol) of ClCO$_2$Bu and (1.50 ml, 10.74 mmol) of NEt$_3$ to afford (0.78 g, 69 %) of title product as a colorless gum.

IR (Neat): 3321, 3085, 2981, 2933, 1673, 1546, 1410 cm$^{-1}$, $^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 7.33 (bs, 1H), 6.99 (bs, 1H), 5.88-5.74 (m, 1H), 5.22-5.07 (m, 2H), 4.17 (t, $J = 6.1$ Hz, 1H), 3.98-3.86 (m, 2H), 3.50-3.41 (m, 2H), 2.13-1.83 (m, 4H), 1.44 (s, 9H), Mass (CI method) (m/z): 312 ((M+H)$^+$, 10), 256 (16), 212 (100).

**Preparation of N-Boc-Phe-Pro-Gly-allylamide (49):**

The title compound was obtained following the general procedure (B) using (0.75 g, 2.83 mmol) of N-Boc-l-Phe, (0.6 g, 2.83 mmol) of Glycine allylamide, (0.55
ml, 4.24 mmol) of ClCO₂Bu and (1.20 ml, 8.49 mmol) of NEt₃ to afford (1.0 g, 76 %) of title product as a colorless gum.

\[
[\alpha] = -13.90 \text{ (c, 0.18, MeOH)}, \text{ IR (Neat): } 3319, 3066, 2979, 1662, 1532, 1448, 1167 \text{ cm}^{-1}, \text{ } ^1\text{H NMR (300 MHz, CDCl}_3\text{): } \delta 7.31-7.20 \text{ (m, 5H), 7.06 (bs, 1H, NH), 6.99 (bs, 1H, NH), 6.50 (bs, 1H, NH), 5.88-5.70 (m, 1H), 5.44-5.08 (m, 2H), 4.69 (dd, } J = 14.67 \text{ Hz and } J = 6.93 \text{ Hz, 1H, C} \alpha \text{H), 4.33-4.29 (m, 1H, C} \alpha \text{H), 3.96-3.69 (m, 4H), 3.54-3.51 (m, 1H), 3.25-3.19 (m, 1H), 2.99 (d, } J = 6.8 \text{ Hz, 2H), 2.16-2.04 (m, 3H), 1.97-1.86 \text{ (m, 1H), 1.42 (s, 9H), Mass (CI method) (m/z): 459 ((M+H)^+, 48).}
\]

**Preparation of N-Pentenoyl-Phe-Pro-Gly-allylamide (51):**

The title product was obtained by following the general procedure (D) using (0.26 ml, 2.64 mmol) of 4-pentenoic acid, (860 mg, 2.40 mmol) of Phe-Pro-Gly allylamide, (0.47 ml, 3.60 mmol) of isobutyl chloroformate and (1.0 ml, 7.20 mmol) in (590 mg, 56 %) yield as a gummy mass.

\[
[\alpha] = -7.00 \text{ (c, 0.1, MeOH)}, \text{ IR (Neat): } 3308, 3077, 2928, 1637, 1538, 1447 \text{ cm}^{-1}, \text{ } ^1\text{H NMR (300 MHz, CDCl}_3\text{): } \delta 7.31-7.17 \text{ (m, 5H), 7.06 (bs, 1H), 6.52 (bs, 1H), 6.28 (d, } J = 7.8 \text{ Hz, 1H), 5.88-5.69 (m, 2H),}
\]

105
5.19-4.97 (m, 4H), 4.32 (t, \(J = 6.5\) Hz, 1H), 3.91-3.80 (m, 5H), 3.30-3.18 (m, 1H), 3.03-2.99 (m, 3H), 2.35-2.26 (m, 4H), 2.09-1.90 (m, 4H), Mass (CI method) (m/z): 441 ((M+H)+, 100).

**Preparation of N-Pentenoyl-d-Phe-Pro-Gly-allylamide (52):**

The title product was obtained by following the general procedure (D) using (0.13 ml, 1.25 mmol) of 4-pentenoic acid, (550 mg, 1.25 mmol) of Phe-Pro-Gly allylamide, (0.25 ml, 1.87 mmol) of CICO\(_2\)iBu and (0.53 ml, 3.75 mmol) of NEt\(_3\) to yield (338 mg, 64 %) yield as a gummy mass.

IR (Neat): 3305, 1640, 1545, 1448 cm\(^{-1}\), \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.34-7.27 (m, 6H, Aromatic + Allylic NH), 6.92 (bs, 1H Gly NH), 6.07 (bs, 1H, d-Phe NH), 5.90-5.82 (m, 1H, pentenoyl), 5.79-5.72 (m, 1H, allylic), 5.24-5.23 (m, 2H, pentenoyl), 5.19-4.98 (m, 2H, allylic), 4.56-4.51 (m, 1H, d-PheC\(\alpha\)H), 4.39-4.36 (m, 1H, Pro C\(\alpha\)H), 4.19-4.15 (dd, \(J = 16.9\) Hz and \(J = 7.7\) Hz, Allylic CH), 3.89-3.80 (m, 2H, Gly C\(\beta\)\(\beta'\)H), 3.74-3.69 (m, 1H, Pro C\(\delta\)H), 3.63 (dd, \(J = 17.2\) Hz and \(J = 5.1\) Hz, Allylic CH), 3.05 (dd, \(J = 12.9\) Hz and \(J = 6.4\) Hz, Phe C\(\beta\)H), 2.97 (dd, \(J = 12.9\) Hz and \(J = 6.4\) Hz, Phe C\(\beta'\)H), 2.69-2.65 (m, 1H, Pro C\(\delta'\)H), 2.28-2.24 (m, 4H, pentenoyl), 2.09-2.05 (m,1H), 1.90-1.61 (m, 3H), Mass (CI method) (m/z): 441 (M+H)+, 100).
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Preparation of c(D-Phe-Pro-Gly)(54):

The unsaturated cyclic peptide was obtained by following the general procedure (E) using (280 mg, 0.63 mmol) of 50 and 10 mol % of Grubbs catalyst to yield a mixture of E:Z (60:40 by TLC) isomers which was transformed to title compound 54 following the general procedure (F) using 30 mg of 10% Pd/C to afford (150 mg, 57 %) of title product as a white fluffy hygroscopic solid.

IR (Neat): 3321, 1640, 1545, 1448 cm⁻¹, ¹H NMR (200 MHz, CDCl₃): δ 7.23-7.18 (m, 6H), 6.74 (bs, 1H), 6.61 (bs, 1H), 4.89-4.82 (m, 1H) 4.13-4.09 (m, 3H), 3.77-3.67 (m, 1H), 3.68-3.54 (m, 3H), 3.13-3.12 (m, 1H), 3.06-2.82 (m,1H), 2.36 (bs, 4H), 2.04-1.85 (m, 6H), 1.50-1.38 (m, 2H) Mass (CI method) (m/z): 441 (M⁺+1, 100).

Preparation of N-Boc-Pro-Leu allyl amide (55):

The title compound was obtained following the general procedure (B) using (4.93 g, 22.96 mmol) of N-Boc-L-Proline, (3.90 g, 22.96 mmol) of Leucine allylamide, (4.50 ml, 34.41 mmol) of ClCO₂Bu and (9.6 ml, 68.79 mmol) of NEt₃ to afford (8 g, 95 %) of title product as a white solid.
mp. 92-96 °C, [α] = -89.00 (c, 0.1, MeOH), IR (CHCl₃): 3292, 2958, 1652, 1550 cm⁻¹, ¹H NMR (400 MHz, CDCl₃): δ 6.78 (bs, 2H), 5.85-5.76 (m, 1H), 5.19-5.09 (m, 2H), 4.46-4.40 (m, 1H), 4.26 (bs, 1H), 3.85-3.80 (m, 2H), 3.43 (bs, 2H), 2.19-2.08 (m, 2H), 1.90-1.74 (m, 2H), 1.63-1.62 (bs, 1H), 1.62-1.49 (m, 2H), 1.46 (s, 9H), 0.97-0.88 (m, 6H), Mass (CI method): 368 ((M +H)⁺, 56), 312 (100).

Preparation of N-Boc-δ-Phe-Pro-Leu allylamide (56):

The title product was obtained by following the general procedure (C) using (3.57 g, 13.48 mmol) of N-Boc-δ-Phenylalanine and (3.6 g, 13.48 mmol) of Pro-Leu-allylamide in (8.7 g, 81 %) yield as a white solid.

[α] = -89.0 (c, 0.1, MeOH), IR (Neat): 3321, 2926, 1659, 1530, 1450, 11667 cm⁻¹ ¹H NMR (400 MHz, CDCl₃): δ 7.33-7.19 (m, 5H), 6.97 (bs, 1H, Allylic NH), 6.85 (d, J = 8.9 Hz, 1H, Leu NH), 5.91-5.81 (m, 1H, Allylic CH), 5.25-5.09 (m, 2H, Allylic CH₂), 5.04 (d, J = 4.3 Hz, 1H, D-Phe NH), 4.40-4.38 (m, 1H, Pro CH₂), 4.36-4.33 (m, 1H, D-Phe CH₂), 3.94-3.90 (m, 1H, Allylic CH), 3.89-3.87 (m, 1H, Allylic CH''), 3.78-3.63 (m, 1H, Pro CH₁), 3.01 (dd, J = 12.6 Hz and J = 9.4 Hz, 1H, Phe CH₂), 2.92 (dd, J = 12.8 Hz and J = 6.4 Hz, 1H, Phe CH₂), 2.61 (dd, J = 16.10 Hz and J = 8.85 Hz, 1H, Pro CH₁), 2.11-2.05 (m, 1H, Pro CH₂), 1.93-1.85 (m, 1H, Leu CH₂), 1.78-1.71 (m,
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2H), 1.62-1.55 (m, 3H), 1.38 (s, 9H, t-Bu), 0.91 (d, J = 6.5 Hz, 3H, Leu CδH), 0.87 (d, J = 6.4 Hz, 3H, Leu Cδ'H), Mass (Cl method) (m/z): 515 ((M+H)+, 100).

**Preparation of Pentenoyl-D-Phe-Pro-Leu allylamide (57):**

The title product was obtained by following the general procedure (D) using (0.8 ml, 7.78 mmol) of 4-pentenoic acid and (3.22 g, 7.78 mmol) of D-Phe-L-Pro-Leu allyl amide to afford the title compound as a white solid (2.70g, 70%).

[α] = -118 (c, 0.1, MeOH), IR (KBr): 3293, 1676, 1636, 1544 cm⁻¹, ¹H NMR (500 MHz, CDCl₃): δ 7.19-7.31 (m, 5H, Aromatic), 7.03 (d, J = 8.6 Hz, 1H, Leu NH), 6.92 (t, J = 5.5 Hz, 1H, Allylic NH), 6.01 (d, J = 4.3 Hz, 1H, D-Phe NH), 5.86 (ddt, J = 17.2, 10.3, 5.5 Hz, 1H, Olefinic-CH, allyl), 5.79 (m, 1H, Olefinic-CH-pentenoyl), 5.20 (dq, J =17.2, 1.6 Hz, 1H, Olefinic-CH (trans), allyl), 5.09 (dq, J = 10.3, 1.6 Hz, 1H, Olefinic-CH (cis), allyl), 5.03 (dq, J = 17.3, 1.6 Hz, 1H, Olefinic-CH (trans), pentenoyl), 5.00 (dq, J = 10.3, 1.6 Hz, 1H, Olefinic-CH (cis), pentenoyl), 4.46 (dt, J = 9.6, 6.5 Hz, 1H, D-Phe CαH), 4.41 (m, 1H, Leu CαH), 4.39 (m, 1H, Pro CαH), 3.85 (dt, J = 15, 5.5 Hz, 2H, Allylic CαH), 3.80 (m, 1H, Aha Cα'H) 3.77 (m, 1H, Pro CδH), 3.07 (dd, J = 12.8, 9.6 Hz, 1H, Phe CβH), 2.96 (dd, J = 12.8, 6.5 Hz, 1H, Phe Cβ'H), 2.65 (dt, J = 9.3, 7.0 Hz, 1H, Pro Cδ'H), 2.33-2.17 (m, 4H, pentenoyl), 2.07 (m, 1H, Pro CβH), 1.87-1.65 (m, 3H),
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1.83 (m, 1H, Leu CβH), 1.72-1.60 (m, 1H), 0.95 (d, J = 6.5 Hz, 3H, Leu CδH), 0.88 (d, J = 6.5 Hz, 3H Leu Cδ'H), Mass (Cl method) (m/z): 497 (M+H)+100).

Preparation of c(D-Phe-Pro-Leu) (58):

The title product was obtained by following the general procedure (E) using (250 mg, 0.50 mmol) of N-Pentenoyl-d-Phe-L-Pro-Leu allylamide and 10 mol% of Grubb’s first generation catalyst in 250 ml of dry CH₂Cl₂ to afford cyclic unsaturated peptide as a mixture of E:Z (4:1) isomers as an off white solid (150 mg, 64 %).

mp. 234-236 °C, [α] = -35.6 (c, 0.5, MeOH), IR (Neat): 3312, 2928, 1644 cm⁻¹. 

¹H NMR (500 MHz, CDCl₃): δ 7.31-7.19 (m, 5H, Aromatic), 6.80 (dd, J = 9.0, 2.0 Hz, 1H, Aha NH), 6.27 (d, J = 6.5 Hz, 1H, Phe NH), 6.10 (d, J = 9.4 Hz, 1H, Leu NH), 5.65 (dt, J = 15.7, 5.9 Hz, 1H, Olefinic-CH(β), allyl), 5.54 (dt, J = 15.7, 5.6 Hz, 1H, Olefinic-CH(γ), allyl), 4.86 (dt, J = 8.9, 6.5 Hz, 1H, Phe CαH), 4.56 (ddd, J = 11.1, 3.9 Hz, 1H, Leu CαH), 4.23 (dd, J = 7.0 and 5.9 Hz, 1H, Pro CαH), 4.30 (ddd, J = 14.9, 9.0, 5.4 Hz, 1H, Aha CαH), 3.67 (ddd, J = 9.9, 7.5, 4.3 Hz, 1H, Pro CβH), 3.32 (ddd, J = 14.9, 6.2, 2.0 Hz, 1H, Aha CβH), 3.04 (dd, J = 13.1, 6.0 Hz, 1H, Phe CβH), 2.98 (dd, J = 13.1, 8.9 Hz, 1H, Phe Cβ'H), 2.71 (ddd, J = 9.9, 8.4, 6.7 Hz, 1H, Pro CδH), 2.37-2.29 (m, 4H, pentenoyl), 2.01 (m, 1H, Leu CβH), 1.95 (m, 1H), 1.80-1.58 (m, 3H), 1.53-1.50 (m, 2H, Leu Cβ'H), 0.93
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(d, J = 6.5 Hz, 3H, Leu CδH), 0.89 (d, J = 6.5 Hz, 3H, Leu Cδ'H), Mass (CI Method) (m/z): 427 ([M+H]+, 100).

Preparation of c(d-Phe-Pro-Leu) (59):

The title product was obtained by following the general procedure (F) using 110 mg of unsaturated cyclic peptide (RCM product) and 25 mg of 10 % Pd/C to afford (110 mg, 88.7 %) of title product white solid.

[α] = -89.8 (c, 0.5, MeOH), IR (KBr): 3422, 1635, 1451 cm⁻¹, ¹H NMR (500 MHz, CDCl₃): δ 7.36-7.21 (m, 5H, Aromatic), 7.03 (d, J = 9.4 Hz, 1H, Leu NH), 6.92 (dd, J = 7.9 Hz, 4.4 Hz, 1H, Aha NH), 6.07 (d, J = 4.2 Hz, 1H, d-Phe NH), 4.50 (m, 1H, Leu CαH), 4.48 (m, 1H, d-Phe CαH), 4.42 (dd, J = 8.7 Hz and J = 2.7 Hz, 1H, Pro CαH), 3.75 (m, 1H, Aha CζH), 3.73 (m, 1H, Pro CδH), 3.08 (dd, J = 12.9 Hz and J = 9.7 Hz, 1H, Phe CβH), 2.99 (dd, J = 12.9 Hz and J = 6.4 Hz, 1H, Phe Cβ'H), 2.77 (m, 1H, Cζ'H), 2.57 (dt, J = 9.5 Hz and J = 6.9 Hz, 1H), 2.28 (m, 1H, Aha CαH), 2.16 (m, 1H, Aha Cα'H), 2.07 (m, 1H, Pro CβH), 1.94 (m, 1H, Leu CβH), 1.85 (m, 1H, Pro Cβ'H), 1.74 (m, 1H, Pro CγH), 1.70 (m, 1H, Leu CβH), 1.63 (m, 1H, Pro Cγ'H), 1.62-1.36 (m, 4H), 1.56 (m, 1H, Leu CγH), 0.94 (d, J = 6.6 Hz, 3H, Leu CδH), 0.88 (d, J = 6.6 Hz, 3H, Leu Cδ'H), Mass (CI method) 471 ([M+H]+, 100).
Preparation of N-Boc-L-Phe-Pro-Leu allylamide (60):

The title compound was obtained following the general procedure (C) using (1.60 g, 5.99 mmol) of N-Boc-d-Phenylalanine, (1.60 g, 5.99 mmol) of Pro-Leu allylamide, (970 mg, 7.19 mmol) of HOBt, and (1.72 g, 8.98 mmol) of EDC.HCl to afford (1.84 g, 59 %) of title product as a pale yellow gum.

\[ \alpha = -52.0 \, (c, \; 0.1, \; \text{MeOH}) \], IR (Neat): 3300, 2959, 1642 cm\(^{-1}\), \(^1\)H NMR (400 MHz, DMSO\(_d6\)): \( \delta 7.88 \, (t, \; J = 5.65 \, \text{Hz}, \; 1\, \text{H}, \; \text{allylic NH}), \; 7.83 \, (d, \; J = 8.3 \, \text{Hz}, \; 1\, \text{H}, \; \text{NH}), \; 7.30-7.17 \, (m, \; 5\, \text{H}, \; \text{aromatic}), \; 7.03 \, (d, \; J = 8.3 \, \text{Hz}, \; 1\, \text{H}, \; \text{NH}), \; 5.82-5.73 \, (m, \; 1\, \text{H}), \; 5.14-5.01 \, (m, \; 2\, \text{H}), \; 4.39-4.34 \, (m, \; 2\, \text{H}), \; 4.30-4.24 \, (m, \; 1\, \text{H}), \; 3.69-3.67 \, (t, \; J = 5.4 \, \text{Hz}, \; 2\, \text{H}), \; 3.61-3.55 \, (m, \; 2\, \text{H}), \; 2.95-2.71 \, (m, \; 2\, \text{H}), \; 2.07-2.00 \, (m, \; 1\, \text{H}), \; 1.94-1.80 \, (m, \; 3\, \text{H}), \; 1.65-1.53 \, (m, \; 1\, \text{H}), \; 1.51-1.46 \, (m, \; 2\, \text{H}), \; 1.28 \, (s, \; 9\, \text{H}, \; \text{t-Bu}), \; 0.9 \, (d, \; J = 6.4 \, \text{Hz}, \; 3\, \text{H}), \; 0.84 \, (d, \; J = 6.5 \, \text{Hz}, \; 3\, \text{H}), \] Mass (CI method): 515 ((M+H)^+\, 10), 514 (65), 415 (100).

Preparation of Pentenoyl-Phe-Pro-Leu allylamide (61):

The title compound was obtained following the general procedure (D) using (0.16 ml, 1.55 mmol) of 4-pentenoic
acid, (0.64 g, 1.55 mmol) of Phe-Pro-Leu allylamide (0.3 ml, 2.33 mmol) of
ClCO₂Bu and (0.65 ml, 4.66 mmol) of NEt₃ to afford (260 mg, 53 %) of title
product as a colorless gum.

\[ \alpha \] = -78.0 (c, 0.1, MeOH), IR (Neat): 3291, 2957, 1636, 1547, 1445 cm⁻¹, ¹H
NMR (500 MHz, CDCl₃): \( \delta \) 7.31-7.19 (m, 5H, aromatic), 6.47 (t, \( J = 8.8 \) Hz, 1H,
Leu NH), 6.45 (dd, \( J = 5.8 \) Hz, 1H, Allylic NH), 6.15 (d, \( J = 7.8 \) Hz, 1H, Phe
NH), 5.86 (ddt, \( J = 17.3, 10.3, 5.6 \) Hz, 1H, olefinic-CH, allyl), 5.76 (ddt, \( J = 17.3,
10.3, 1.7 \) Hz, 1H, olefinic-CH, pentenoyl), 5.22 (dq, \( J = 17.3, 1.6 \) Hz, 1H,
olefinic-CH (trans), allyl), 5.15 (dq, \( J = 10.3, 1.6 \) Hz, 1H, olefinic-CH (cis), allyl),
5.03 (dq, \( J = 17.3, 1.7 \) Hz, 1H, olefinic-CH (trans), pentenoyl), 5.01 (dt, \( J = 7.8,
6.4 \) Hz, 1H, Phe \( \alpha \)H), 4.99 (m, 1H), 4.44 (dd, \( J = 8.2, 4.2 \) Hz, 1H, Pro \( \alpha \)H ),
4.41 (m, 1H, Leu \( \alpha \)H), 3.89-3.91 (m, 2H, allylic CH₂), 3.67 (m, 1H, Pro\( \delta \)H),
3.09 (ddd, \( J = 10.0, 6.8, 5.4 \) Hz, 1H, Pro\( \delta \)'H), 3.01 (dd, \( J = 13.5, 7.8 \) Hz, 1H,
Phe \( \beta \)H), 2.99 (dd, \( J = 13.5, 6.4 \) Hz, 1H, Phe \( \beta \)'H), 2.33-2.21 (m, 4H,
pentenoyl), 2.12-1.90 (m, 4H, Pro\( \betaγ \)H), 1.85 (m, 1H, Leu\( γ \)H), 1.57 (m,1H,
Leu\( β \)H), 1.49 (m,1H, Leu\( β' \)H), 0.95 (d, \( J = 6.5 \) Hz, 3H, Leu\( δ \)H), 0.91 (d, \( J
= 6.5 \) Hz, 3H, Leu\( δ' \)H), Mass (CI method): 497 ((M+H)+, 100), 440 (9), 327
(16), 268 (47).

**Preparation of unsaturated cyclo(t.-Phe-Pro-Leu) (62):**
The title product was obtained by following the general procedure (E) using (240 mg, 0.48 mmol) of N-Pentenoyl-Ala-Pro-Phe allylamide and (10 mol %) of Grubb’s first generation catalyst in 240 ml of dry CH\textsubscript{2}Cl\textsubscript{2} to afford (180 mg, 80 %) of cyclic unsaturated peptide as a mixture of \( E:Z \) isomers.

\[ \alpha ] = -31.0 \ (c, 0.1, \text{MeOH}), \ \text{IR (Neat):} \ 3299, \ 2925, \ 2854, \ 1626 \ \text{cm}^{-1}, \ \text{\textsuperscript{1}H NMR (500 MHz, CDCl} \textsubscript{3}): \ \delta \ 7.19-7.31 \ (m, \ 5H, \ \text{aromatic}), \ 6.42 \ (d, \ J = 6.7 \ Hz, \ 1H, \ \text{Phe NH}), \ 6.38 \ (d, \ J = 8.5 \ Hz, \ 1H, \ \text{Leu NH}), \ 6.00 \ (dd, \ J = 7.7, \ 4.6 \ Hz, \ 1H, \ \text{Allylic NH}), \ 5.51 \ (ddd, \ J = 15.1, \ 7.5, \ 5.9 \ Hz, \ 1H, \ \text{Olefinic-CH(\textgamma)-allyl}), \ 5.41 \ (ddd, \ J = 15.1, \ 6.8, \ 4.8 \ Hz, \ 1H, \ \text{Olefinic-CH(\textbeta)-allyl}), \ 4.56 \ (ddd, \ J = 10.3, \ 4.5 \ Hz, \ 1H, \ \text{Phe C\alpha H}), \ 4.35 \ (m, \ 1H, \ \text{Leu C\alpha H}), \ 4.05 \ (ddd, \ J = 15.0, \ 7.7, \ 6.8 \ Hz, \ 1H, \ \text{Allylic C\alpha H}), \ 3.60 \ (ddd, \ J = 12.2, \ 8.5, \ 2.5 \ Hz, \ 1H, \ \text{Pro C\alpha H}), \ 3.37 \ (m, \ 1H, \ \text{Pro C\delta H}), \ 3.36 \ (m, \ 1H, \ \text{Pro C\delta'H}), \ 3.33 \ (dt, \ J = 15.0, \ 4.7 \ Hz, \ 1H, \ \text{Allylic C\alpha'H }), \ 3.32 \ (dd, \ J = 12.5, \ 4.5 \ Hz, \ 1H, \ \text{Phe C\beta H}), \ 2.77 \ (dd, \ J = 12.5, \ 10.3 \ Hz, \ 1H, \ \text{Phe C\beta'H}), \ 2.55-2.18 \ (m, \ 4H, \ \text{Pentenoyl}), \ 1.83 \ (m,1H, \ \text{Pro C\beta H}), \ 1.71 \ (m, \ 3H, \ \text{Pro C\beta'H + C\gamma'H}), \ 1.55-1.42 \ (m, \ 2H, \ \text{Leu C\beta'\beta'H}), \ 1.24 \ (m, \ 1H, \ \text{Leu C\gamma H}), \ 0.90 \ (d, \ J = 5.1 \ Hz, \ 3H), \ 0.88 \ (d, \ J = 5.1 \ Hz, \ 3H), \ \text{Mass (CI method):} 469 (M+H\textsuperscript{+}, 100).

**Preparation of cyclo(\textgamma-Pro-Leu) (63):**
The title product was obtained by following the general procedure (F) using (240 mg, 0.51 mmol) of unsaturated cyclic peptide and 10 % Pd/C (48 mg) in 5 ml of MeOH to afford (200 mg, 80 %) of saturated cyclic peptide.

mp. 68-72 °C, [α] = -45 (c, 0.1, MeOH), IR (Neat): 3312, 2959, 1633, 754 cm\(^{-1}\), \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.33-7.22 (m, 5H, Aromatic), 6.51 (d, \(J = 8.5\) Hz, 1H, Leu NH), 6.38 (d, \(J = 7.0\) Hz, 1H, Phe NH), 6.23 (dd, \(J = 8.8, 4.0\) Hz, 1H, Aha NH), 4.63 (m, 1H), 4.35 (m, 1H), 3.71 (m, 1H, Aha C\(\varepsilon\)H), 3.59 (ddd, \(J = 12.3, 8.4, 2.8\) Hz, 1H, Pro C\(\delta\)'H), 3.45 (dd, \(J = 8.6, 1.9\) Hz, 1H, Pro C\(\alpha\)H), 3.39 (ddd, \(J = 12.3, 9.9, 7.5\) Hz, 1H, Pro C\(\delta\)H), 3.21 (dd, \(J = 12.6, 4.9\) Hz, 1H, Phe C\(\beta\)H), 2.90 (m, 1H, Aha C\(\varepsilon\)'H), 2.84 (dd, \(J = 12.6, 10.5\) Hz, 1H, Phe C\(\beta\)'H), 2.32 (ddd, \(J = 14.1, 6.2, 3.8\) Hz, 1H, Aha C\(\alpha\)H), 2.15 (ddd, \(J = 14.1, 10.6, 3.8\) Hz, 1H, Aha C\(\alpha\)'H), 1.84 (m, 1H, Pro C\(\beta\)H), 1.70 (m, 1H, Pro C\(\gamma\)H), 1.68 (m, 1H, Aha C\(\beta\)H), 1.63 (m, 2H, Aha CH), 1.59 (m, 1H, Pro C\(\gamma\)'H), 1.57-1.39 (m, 3H), 1.34 (m, 2H), 1.21 (m, 1H, Pro C\(\beta\)'H), 1.20 (m, 1H, Aha C\(\gamma\)'H), 0.90 (d, \(J = 6.2\) Hz, 3H, Leu C\(\delta\)H), 0.88 (d, \(J = 6.2\) Hz, 3H, Leu C\(\delta\)'H), \(^1\)C NMR derived from HSQC and HMBC spectrum: 172.8, 171.9, 171.5, 170.8, 136.0, 129.5, 129.1, 127.65, 61.5, 53.4, 52.8, 46.7, 43, 40.6, 38.5, 35.8, 31.4, 28.6, 25.1, 24.5, 24.3, 22.7, 22.2. Mass (CI method) (m/z): 472 ((M+H)\(^+\), 100).

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Preparation of N-Boc-Ala allylamide (64):

The title compound was obtained following the general procedure (A) using (3g, 15.87 mmol) of N-Boc-Alanine, (1.80 ml, 23.80 mmol) of allylamine, (3.10 ml, 23.80 mmol) of ClCO$_2$Bu and (6.70 ml, 47.61 mmol) of NEt$_3$ to afford (2.66 g, 72.7 %) of title product as a white solid.

mp. 88-92 °C, [α] = -26.20 (c, 0.5, MeOH), IR (KBr): 3362, 3238, 2983, 1702, 1654, 1542, 1516 cm$^{-1}$, $^1$H NMR (400 MHz, CDCl$_3$): δ 6.25 (bs, 1H), 5.87-5.78 (m, 1H), 5.21-5.11 (m, 2H), 4.96 (bs, 1H), 4.17-4.14 (m, 1H), 3.88 (m, 2H), 1.44 (s, 9H), 1.36 (d, J = 6.98 Hz, 3H), Mass (CI method) (m/z): 229 ((M+H)$^+$20), 173 (100), 128 (35).

Preparation of N-Boc-Pro-Ala allylamide (65):

The title compound was obtained following the general procedure (A) using (2.45 g, 11.40 mmol) of N-Boc-Pro, (1.45 g, 11.40 mmol) of Ala allylamide, (2.30 ml, 17.10 mmol) of ClCO$_2$Bu and (4.76 ml, 33.98 mmol) of NEt$_3$ to afford (3.20 g, 86 %) of title product as a white solid.
mp. 128-130 °C, \([\alpha] = -84.60\) (c, 0.5, MeOH), IR (KBr): 3294, 2926, 1702, 1642, 1401 cm\(^{-1}\), \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta 6.81\) (bs, 1H), 5.86-5.76 (m, 1H), 5.29-5.09 (m, 2H), 4.50-4.43 (m, 1H), 4.25 (bs, 1H), 3.86-3.81 (m, 2H), 3.46-3.43 (m, 2H), 2.16-2.11 (m, 3H), 1.90-1.87 (m, 2H), 1.46 (s, 9H), 1.38 (d, \(J = 6.9\) Hz, 3H), Mass (CI method) (m/z): 325 (4), 270 (30), 226 (100).

**Preparation of N-Boc-Phe-Pro-Ala allylamide (66):**

The title product was obtained by following the general procedure (C) using (2.2 g, 9.77 mmol) of N-Boc-Phe, (2.62 g, 9.77 mmol) of Pro-Ala allylamide, (1.60 g, 11.70 mmol) of HOBT and (2.81 g, 14.66 mmol) of EDC. HCl to afford the title compound (3.30 g, 60 %) as pale yellow oil.

\([\alpha] = -66.5\) (c, 0.4, MeOH), IR (Neat): 3307, 2978, 1642, 1523, 1167 cm\(^{-1}\), \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta 7.32-7.17\) (m, 6H), 6.84 (d, \(J = 7.5\) Hz, 1H), 6.59 (bs, 1H), 5.90-5.81 (m, 1H), 5.23-5.13 (m, 2H), 4.66-4.64 (m, 1H), 4.49-4.40 (m, 2H), 3.90-3.87 (m, 2H), 3.60-3.46 (m, 2H), 3.09-2.91 (m, 2H), 2.16-2.10 (m, 1H), 2.03-1.96 (m, 1H), 1.90-1.85 (m, 2H), 1.41-1.38 (m, 12H), Mass (CI method) (m/z): 473 ((M+H\(^+\)), 75), 399 (57), 373 (100).

**Preparation of N-Pentenoyl-Phe-Pro-Ala allylamide (67):**

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The title product was obtained by following the general procedure (D) using 4-pentenoic acid (0.6 ml, 5.85 mmol) and (2.17 g, 5.85 mmol) amine Leu-Pro-Ala allylamide in 57 % yield as a white fluffy hygroscopic solid.

\[ \alpha = -68.20 \text{ (c, 0.5, MeOH), IR (Neat): 3296, 3077, 1634 cm}^{-1} \text{, } ^1\text{H NMR (500 MHz, CDCl}_3\text{): } \delta 7.32-7.20 \text{ (m, 5H, Aromatic), } 6.83 \text{ (d, } J = 7.3 \text{ Hz, 1H, Ala NH), } 6.48 \text{ (bs, 1H, Allylic NH), } 6.24 \text{ (d, } J = 7.6 \text{ Hz, 1H, Phe NH), } 5.88-5.81 \text{ (m, 1H, Allylic CH), } 5.79-5.74 \text{ (m, 1H, Pentenoyl CH), } 5.22-5.18 \text{ (m, 2H, Allylic CH}_2\text{), } 5.15-5.00 \text{ (m, 2H, Pentenoyl CH}_2\text{), } 4.99-4.97 \text{ (m, 1H, Phe C}_\alpha\text{H), } 4.46-4.42 \text{ (m, 2H, Pro C}_\alpha\text{H and Ala C}_\alpha\text{H), } 3.90-3.87 \text{ (m, 2H, Allylic CH}_2\text{), } 3.65-3.62 \text{ (m, 1H, Pro C}_8\text{H), } 3.09-2.97 \text{ (m, 3H, Phe C}_\beta\text{H, C}_\beta\text{'H & Pro C}_8\text{'H), } 2.18-2.14 \text{ (m, 1H, Pro C}_\beta\text{H), } 2.35-2.32 \text{ (m, 2H, Pentenoyl), } 2.30-2.25 \text{ (m, 2H, Pentenoyl), } 2.18-2.14 \text{ (m, 1H, Pro C}_\beta\text{'H), } 1.99-1.96 \text{ (m, 2H, Pro C}_\gamma\text{'H), } 1.38 \text{ (d, } J = 6.9 \text{ Hz, 3H), Mass (CI method) (m/z): 455 ((M+H})^+, 100). \]

**Preparation of cyclo(Phe-Pro-Ala-Aha) (69):**

The unsaturated cyclic peptide 68 was obtained by following the general procedure (E) using (180 mg, 0.39 mmol) of 67 and 10 mol % of Grubbs catalyst as a mixture of E:Z (60:40 by TLC) isomers which was transformed to title product (91 mg, 54 %, white fluffy solid) following the general procedure (F) using 30 mg of 10 % Pd/C in MeOH.
IR (Neat): 3421, 2930, 1636, 1520, 1448 cm\(^{-1}\), \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.33-7.21 (m, 5H, Aromatic), 6.62 (d, \(J = 7.8\) Hz, 1H, Ala NH), 6.40 (d, \(J = 7.2\) Hz, 1H, Phe NH), 6.33 (dd, \(J = 9.1, 3.7\) Hz, 1H, Aha NH), 4.64 (m, 1H, Phe CoH), 4.36 (m, 1H, Ala CoH), 3.74 (m, 1H, Aha C\(\xi\)H), 3.61 (ddd, \(J = 12.3, 8.4, 2.7\) Hz, 1H, Pro C\(\delta\)'H), 3.42 (dd, \(J = 8.6\) Hz and \(J = 1.9\) Hz, 1H, Pro CoH), 3.38 (ddd, \(J = 12.3, 10.0, 7.3\) Hz, 1H, Pro C\(\delta\)H), 3.19 (dd, \(J = 12.5\) and \(J = 4.9\) Hz, 1H, Phe C\(\beta\)H), 2.91 (m, 1H, Aha C\(\xi\)'H), 2.84 (dd, \(J = 12.5\) Hz and \(J = 10.7\) Hz, 1H, Phe C\(\beta\)'H), 2.31 (m, 1H, Aha CoH), 2.16 (m, 1H Aha Co'H), 1.86 (m, 1H, Pro C\(\beta\)H), 1.69 (m, 1H, Pro C\(\gamma\)H), 1.66 (m, 1H, Aha C\(\delta\)H), 1.64 (m, 1H, Aha C\(\beta\)H), 1.60 (m, 1H, Aha C\(\delta\)'H), 1.58 (m, 1H), 1.35 (m, 1H, Aha C\(\beta\)'H), 1.33 (m, 1H, Aha C\(\gamma\)H), 1.27 (d, \(J = 6.8\) Hz, 3H), 1.22 (m, 1H, Pro C\(\beta\)'H), 1.18 (m, 1H, Aha C\(\gamma\)'H), Mass (CI method) (m/z): 429 ((M+H)+, 100).

**Preparation of N-Boc-L-Ala-Pro-Phe allyl amide(70):**

The title product was obtained by following the general procedure (C) using N-Boc-L-Ala (630 mg, 3.32 mmol) and (1g, 3.32 mmol) of amine L-Pro-Phe-allyl amide to yield (810 mg, 52 %) as a white solid.

mp. 152-156°C, \([\alpha] = -103\) (c, 0.5, MeOH), IR (KBr): 3310, 2979, 1643 cm\(^{-1}\), \(^1\)H NMR (400 MHz, DMSOd\(_6\)): \(\delta\) 7.81-7.76 (m, 2H), 7.27-7.16 (m, 5H), 7.33-7.21 (m, 5H, Aromatic), 6.62 (d, \(J = 7.8\) Hz, 1H, Ala NH), 6.40 (d, \(J = 7.2\) Hz, 1H, Phe NH), 6.33 (dd, \(J = 9.1, 3.7\) Hz, 1H, Aha NH), 4.64 (m, 1H, Phe CoH), 4.36 (m, 1H, Ala CoH), 3.74 (m, 1H, Aha C\(\xi\)H), 3.61 (ddd, \(J = 12.3, 8.4, 2.7\) Hz, 1H, Pro C\(\delta\)'H), 3.42 (dd, \(J = 8.6\) Hz and \(J = 1.9\) Hz, 1H, Pro CoH), 3.38 (ddd, \(J = 12.3, 10.0, 7.3\) Hz, 1H, Pro C\(\delta\)H), 3.19 (dd, \(J = 12.5\) and \(J = 4.9\) Hz, 1H, Phe C\(\beta\)H), 2.91 (m, 1H, Aha C\(\xi\)'H), 2.84 (dd, \(J = 12.5\) Hz and \(J = 10.7\) Hz, 1H, Phe C\(\beta\)'H), 2.31 (m, 1H, Aha CoH), 2.16 (m, 1H Aha Co'H), 1.86 (m, 1H, Pro C\(\beta\)H), 1.69 (m, 1H, Pro C\(\gamma\)H), 1.66 (m, 1H, Aha C\(\delta\)H), 1.64 (m, 1H, Aha C\(\beta\)H), 1.60 (m, 1H, Aha C\(\delta\)'H), 1.58 (m, 1H), 1.35 (m, 1H, Aha C\(\beta\)'H), 1.33 (m, 1H, Aha C\(\gamma\)H), 1.27 (d, \(J = 6.8\) Hz, 3H), 1.22 (m, 1H, Pro C\(\beta\)'H), 1.18 (m, 1H, Aha C\(\gamma\)'H), Mass (CI method) (m/z): 429 ((M+H)+, 100).
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6.97 (d, J = 7.5 Hz, 1H), 5.77-5.68 (m, 2H), 5.08-4.98 (m, 2H), 4.45-4.41 (m, 1H), 4.28-4.21 (m, 2H), 3.68-3.65 (m, 2H), 3.55-3.50 (m, 1H), 3.06 (dd, J = 13.70, 5.3 Hz, 1H), 2.83 (dd, J = 13.83, 9.05 Hz, 1H), 1.97-1.92 (m, 1H), 1.83-1.80 (m, 2H), 1.77-1.61 (m, 1H), 1.36 (s, 9H), 1.13 (d, J = 6.7 Hz, 3H), Mass (CI method) (m/z): 473 (M^+1, 100), 373 (90).

Preparation of N-Pentenoyl-Ala-Pro-Phe allylamide (71):

The title product was obtained by following the general procedure (D) using 4-pentenoic acid (0.26 ml, 2.54 mmol) and (0.94 g, 2.54 mmol) amine (Ala-Pro-Phe allylamide) to afford the title compound as a fluffy solid (1.10g, 68%).

mp. 126-128°C, [α] = -115 (c, 0.1, MeOH), IR (Neat): 3299, 2980, 1637, 1540 cm⁻¹, ¹H NMR (400 MHz, DMSOd₆): δ 7.30-7.16 (m, 5H), 6.45 (d, J = 9.3 Hz, 1H, Phe NH), 6.24 (d, J = 7.4 Hz, 1H, Ala NH), 6.19 (t, J = 5.15 Hz, 1H, Allylic NH), 5.84-5.68 (m, 2H), 5.12-4.98 (m, 4H), 4.68-4.62(m, 2H), 4.45-4.22 (m, 1H), 3.90-3.67 (m, 2H), 3.66-3.63 (m, 1H), 3.45-3.37 (m, 1H), 3.25 (dd, J = 13.9 and J = 6 Hz, 1H), 3.03 (dd, J = 13.9 Hz, and J = 6 Hz, 1H), 2.99-2.31 (m, 2H), 2.28-2.25 (m, 2H), 2.14-2.03 (m, 2H), 1.99-1.97 (m, 2H), 1.05 (d, J = 6.9 Hz, 3H), Mass (CI method) (m/z): 455 (M+H)⁺, 100).

Preparation of saturated cyclo(t-Ala-Pro-Phe-Aha) (73):
Following the general procedure (E), (250 mg, 0.55 mmol) of N-Pentenoyl-Ala-Pro-Phe allylamide and 10 mol% of Grubb’s first generation catalyst in 250 ml of dry CH₂Cl₂ yielded unsaturated cyclic peptide as a mixture of E:Z isomers which was transformed to title compound following the general procedure (F) using 30 mg of 10 % Pd/C to afford (120 mg, 51 %, over two steps) of title product as white fluffy hygroscopic solid.

[α] = -54 (c, 0.1, MeOH), IR (KBr): 3429, 2925, 1636 cm⁻¹, ¹H NMR (500 MHz, CDCl₃): δ 7.30-7.19 (m, 5H), 6.73 (d, J = 8.2 Hz, 1H, Phe NH), 6.30 (d, J = 6.1 Hz, 1H, Ala NH), 6.06 (dd, J = 7.9 Hz and J = 3.6 Hz, 1H, Ala NH), 4.63 (m, 1H, Phe CαH), 4.37 (m, 1H, Ala CαH), 4.31 (dd, J = 8.5 and J = 2.4 Hz, 1H, Pro CαH), 3.62 (m, 1H, Aha CζH), 3.53 (m, 1H, Pro CδH), 3.51 (m, 1H, Pro Cδ′H), 3.12 (dd, J = 13.6 Hz and J = 7.3 Hz, 1H, Phe CβH), 2.99 (dd, J = 13.6 Hz, and J = 7.5 Hz, 1H), 2.84 (m, 1H, Aha Cζ′H), 2.27 (m, 1H, Aha CαH), 2.14 (m, 1H, Pro CβH), 2.13 (m, 1H, Aha Aha Cα′H), 2.08 (m, 1H, Pro Cβ′H), 1.77 (m, 1H, Pro CγH), 1.59 (m, 1H, Aha CβH), 1.35 (m, 1H, Pro Cγ′H), 1.30 (d, J = 6.7 Hz, 1H, Ala CβH), 1.29 (m, 1H, Aha Cβ′H), 1.23 (m, 1H, Aha Cγ′H), 1.18 (m, 1H, Aha CγH), 1.15 (m, 1H, Aha Cδ′H), 1.13 (m, 3H), ¹³C NMR data: δ 173.2, 172.3, 171.1, 170.8, 137, 129.5, 128.7, 127.2, 61.5, 55.7, 48.0, 47.0, 39.5, 35.5, 35.1,
32.1, 28.0, 24.4, 24.0, 22.2, and 18.3, Mass (CI method) (m/z): 429 (M+H)$^+$, 100).

**Preparation of N-Boc-L-Val-Pro-Phe allyl amide (74):**

The title product was obtained by following the general procedure (C) using (890 mg, 4.08 mmol) N-Boc-L-Val and (1.23 g, 4.08 mmol) of Pro-Phe-allylamide in (1.20 g, 58.5 %) yield as a white flufhygroscopic solid

[\alpha] = -125.8 (c, 0.5, MeOH), IR (Neat): 3304, 2972, 1632, 1514, 1444, cm$^{-1}$, $^1$H NMR (400 MHz, DMSO$\text{d}_6$): $\delta$ 7.93-7.91 (m, 2H), 7.27-7.16 (m, 5H), 6.76 (d, $J$ = 7.30 Hz, 1H), 5.74-5.55 (m, 1H), 5.05-4.97 (m, 2H), 4.42-4.37 (m, 1H), 4.33-4.31 (m, 1H), 3.68-3.61 (m, 3H), 3.59-3.50 (m, 1H), 2.99 (dd, $J$ = 13.8, 6.0 Hz, 1H), 2.90 (dd, $J$ = 13.70 Hz, 8.5 Hz, 1H), 1.98-1.91 (m, 1H), 1.90-1.82 (m, 3H), 1.80-1.71 (m, 1H), 1.36 (s, 9H), 1.24-1.21 (m, 1H), 0.88-0.83 (m, 6H), Mass (CI method) (m/z): 501 ((M+H)$^+$, 52), 401 (100).

**Preparation of N-Pentenoyl-Val-Pro-Phe allyl amide (75):**

The title product was obtained by following the general procedure (D) using (0.23 ml, 2.20 mmol)
of 4-pentenoic acid and (0.88 g, 2.20 mmol) of Val-Pro-Phe allyl amide to afford
the title compound as a fluffy solid (720 mg, 68 %)

IR (Neat): 3294, 3077, 2962, 2926, 1629, 1540, 1445, 1255, 1217 cm⁻¹, ¹H NMR
(400 MHz, CDCl₃): δ 7.30-7.16 (m, 5H, Aromatic), 6.66 (d, J = 8.1 Hz, 1H), 6.10
(d, J = 8.6 Hz, 1H), 5.83-5.77 (m, 1H), 5.71-5.65 (m, 1H), 5.08-4.98 (m, 4H),
4.60-4.55 (m, 2H), 4.43-4.40 (m, 1H), 3.80-3.75 (m, 3H), 3.53-3.51 (m, 1H), 3.52
(dd, J = 6.3 Hz and 3.9 Hz, 1H), 3.18 (dd, J = 13.7 Hz and J = 6.2 Hz, 1H), 3.04
(dd, J = 13.7 Hz and J = 7.5 Hz, 1H), 2.39-2.27 (m, 4H), 2.10-2.0 (m, 2H), 1.97-
1.85 (m, 3H), 0.96 (d, J = 6.5 Hz, 3H, Leu CδH), 0.92 (d, J = 6.5 Hz, 3H, Leu
Cδ′H).

Preparation of saturated c(Val-Pro-Phe-Aha) (77):

Following the general procedure (E), (250 mg, 0.51
mmol) of N-Pentenoyl-Val-Pro-Phe allylamide and
10 mol% of Grubb’s first generation catalyst in 250
ml of dry CH₂Cl₂ yielded unsaturated cyclic peptide
as a mixture of E:Z isomers which was transformed to title compound following
the general procedure (F) using 50 mg of 10 % Pd/C to afford (210 mg, 88.7 %,
over two steps) of title product s white fluffy hygroscopic solid.

IR (KBr): 3291, 1636, 1216 cm⁻¹, ¹H NMR (500 MHz, CDCl₃): δ 7.28-7.18 (m, 5H),
6.43 (d, J = 7.8 Hz, 1H), 6.41-6.39 (m, 1H), 6.11 (d, J = 7.8 Hz, 1H), 4.73-
4.68 (m, 1H), 4.53-4.51 (m, 1H), 4.46-4.43 (m, 1H), 3.72-3.64 (m, 1H), 3.55-3.50 (m, 1H), 3.49-3.40 (m, 1H), 3.18 (dd, $J = 13.6$ Hz and $J = 6.9$ Hz, 1H), 3.00 (dd, $J = 14.14$ Hz and $J = 7.8$ Hz, 1H), 2.90-2.85 (m, 1H), 2.35-2.30 (m, 1H), 2.14-2.01 (m, 4H), 1.93-.189 (m, 1H), 1.75-1.62 (m, 3H), 1.53-1.50 (m, 1H), 1.39-1.36 (m, 1H), 1.31-1.28 (m, 2H), 0.86-0.92 (m, 6H).

Preparation of $N$-Boc-l-Leu-Pro-Phe allylamide (78):

The title product was obtained by following the general procedure (C) using (0.58 g, 2.49 mmol) of $N$-Boc-l-Leu and (0.75 g, 2.49 mmol) of Pro-Phe allylamide (780 mg, 61 %) as white fluffy hygroscopic solid.

$[\alpha] = -101$ (c, 0.5, MeOH), IR (Neat): 3302, 2958, 1644 cm$^{-1}$, $^1$H NMR (400 MHz, DMSOd$_6$) : $\delta$ 7.85 (d, $J = 7.8$ Hz, 1H), 7.27-7.16 (m, 6H), 6.91 (d, $J = 8.3$ Hz, 1H), 5.76-5.66 (m, 1H), 5.07-4.97 (m, 2H), 4.42-4.36 (m, 1H), 4.29-4.26 (m, 1H), 4.24-4.18 (m, 1H), 3.67-3.58 (m, 3H), 3.58-3.43 (m, 1H), 3.02 (dd, $J = 13.7$, 5.5 Hz, 1H), 2.85 (dd, $J = 13.7$, 8.5 Hz, 1H), 1.98-1.90 (m, 1H), 1.87-1.80 (m, 3H), 1.73-1.60 (m, 2H), 1.48-1.41 (m, 1H), 1.36 (s, 9H), 0.89-0.87 (m, 6H), Mass (EI method) (m/z): 514 (58), 415 (100).

Preparation of $N$-pentenoyl-l-Leu-Pro-Phe allylamide (79):
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The title product was obtained following the general procedure (D) using (0.1 ml, 0.99 mmol) of 4-pentenoic acid and (0.41g, 0.99 mmol) of Leu-Pro-Phe allylamide in (260 mg, 53 %) yield as a colorless gum.

\[ \alpha = -128 \text{ (c, 0.1, MeOH), IR (Neat): 3293, 2957, 1637, 1545 cm}^{-1}, ^1\text{H NMR (500 MHz, CDCl}_3\text{): } \delta \text{ 7.36-7.16 (m, 5H, Aromatic), 6.59 (d, } J = 8.2 \text{ Hz, 1H, Phe NH), 6.19 (t, } J = 5.2 \text{ Hz, 1H, Allylic NH), 5.98 (d, } J = 8.7 \text{ Hz, 1H, Leu NH), 5.81 (m, 1H, pentenoyl), 5.72 (m, 1H, Olefinic-CH, Allylic), 5.06 (m, 1H, Olefinic-CH}_2\text{ (cis), Allylic), 5.05 (m, 1H, Olefinic-CH (trans), pentenoyl), 5.04 (m, 1H, Olefinic-CH (trans), Allylic), 5.00 (m, 1H, Olefinic-CH (cis), pentenoyl), 4.76 (ddd, } J = 10.3, 3.6 \text{ Hz, 1H, Leu } \alpha\text{H), 4.62 (m, 1H, Phe } \alpha\text{H), 4.42 (dd, } J = 4.1, 8.2 \text{ Hz, 1H, Pro } \alpha\text{H), 3.84-3.78 (m, 2H), 3.76 (m, 1H, Pro C} \delta\text{H), 3.44 (m, 1H, Pro C} \delta'\text{H), 3.19 (dd, } J = 13.8, 6.5 \text{ Hz, 1H, Phe C} \beta\text{H), 3.08 (dd, } J = 6.7, 13.8 \text{ Hz, 1H, Phe C} \beta'\text{H), 2.36-2.29 (m, 4H, pentenoyl), 2.10-1.89 (m, 4H, Pro C} \beta\text{H), 1.62 (m, 1H, Leu } \gamma\text{H), 1.39 (ddd, } J = 14.4, 10.3, 4.1 \text{ Hz, 1H, Leu C} \beta\text{H), 1.17 (ddd, } J = 14.4, 9.2, 3.6 \text{ Hz, 1H, Leu C} \beta\text{H), 0.96 (d, } J = 6.6 \text{ Hz, 3H Leu C} \delta\text{H), 0.96 (d, } J = 6.6 \text{ Hz, 3H, Leu C} \delta'\text{H), Mass (Cl method) (m/z): 497 ((M+H})^+\text{100), 302 (43).}

Preparation of saturated cyclo(Leu-Pro-Phe) (81):

\[125\]
The unsaturated cyclic product was obtained by following the general procedure (E) using (250 mg, mmol) of N-Pentenoyl-Leu-Pro-Phe allylamide and 10 mol % of Grubb’s first generation catalyst to afford of cyclic unsaturated peptide as a mixture of $E:Z$ isomers (60:40 by TLC) which was transformed to title product following the general procedure (F) using 30 mg of 10% Pd/C to afford (120 mg, 88.7 %) of title product s white fluffy hygroscopic solid.

$[\alpha] = -71.2$ (c, 0.25, MeOH), IR (Neat): 3310, 2929, 1636 cm$^{-1}$, $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.29-7.20 (m, 5H, Aromatic), 6.54 (d, $J = 8.1$ Hz, 1H, Phe NH), 6.26 (dd, $J = 7.8$ Hz and $J = 3.8$ Hz, 1H, Aha-NH), 6.15 (d, $J = 7.4$ Hz, 1H, Leu NH), 4.70 (m, 1H, Phe C$\alpha$H), 4.54 (m, 1H, Leu C$\alpha$H), 4.32 (dd, $J = 8.3$ Hz, and $J = 2.5$ Hz, 1H, Pro C$\alpha$H), 3.66 (m, 1H, Aha C$\xi$H), 3.49 (ddd, $J = 12.2$, 9.5, 7.2 Hz, 1H, Pro C$\delta$H), 3.44 (ddd, $J = 12.2$, 8.5, 3.2 Hz, 1H, Pro C$\delta'$H), 3.19 (dd, $J = 13.8$ Hz and $J = 6.9$ Hz, 1H, Phe C$\beta$H), 3.01 (dd, $J = 13.8$ Hz and $J = 7.7$ Hz, 1H), 2.89 (m, 1H, Aha C$\xi'$H ), 2.30 (ddd, $J = 14.5$, 6.1, 4.0 Hz, 1H), 2.14 (ddd, $J = 14.5$, 10.7, 4.0 Hz, 1H), 2.10 (m, 1H, Pro C$\beta$H), 2.05 (m, 1H, Pro C$\beta'$H), 1.73 (m, 1H, Pro C$\gamma$H), 1.65-1.57 (m, 3H, Leu C$\beta$ & C$\gamma$H), 1.62 (m, 1H, Aha C$\delta$H), 1.58 (m, 1H, Aha C$\beta$H), 1.37 (m, 1H, Aha C$\gamma$H), 1.34 (m, 1H, Aha C$\delta$H), 1.29 (m,1H, Aha C$\beta'$H), 1.27 (m, 1H, Pro C$\gamma$H), 1.14 (m, 1H, Aha C$\gamma$H), 0.96 (d, $J = 6.5$ Hz, 3H, Methine).
3H, Leu CδH), 0.92 (d, J = 6.5 Hz, 3H, Leu Cδ′H), \(^{13}\)C NMR derived from HSQC and HMBC in CDCl\(_3\): \(\delta\) 173.4, 172.5, 171.2, 170.1, 138, 129.5, 128.8, 127.3, 61.6, 55.6, 49.9, 47.0, 42.7, 39.3, 38.0, 35.2, 32.1, 29.9, 27.6, 24.9, 24.1, 23.7, 23.0, 22.1, 22.0, Mass (CI method) (m/z): 471 ((M+H)^+, 100).

**Preparation of N-Pentenoyl-Pro-Pro-Phe allylamide (83):**

Following the general procedure (C) (785 mg, 3.65 mmol) of N-Boc-Pro, (1.1 g, 3.65 mmol) of Pro-Phe allylamide was transformed to N-Boc-Pro-Pro-Phe allylamide (1.36 g, 75 %) using EDC.HCl (1.05 g, 5.48 mmol) and HOBt (600 mg, 4.38 mmol) as a white solid. This was transformed to title product following general procedure (D) using (0.26 ml, 2.61 mmol) of 4-pentenoic acid and (1.03 g, 2.61 mmol) of Pro-Pro-Leu allylamide (700 mg, 57 %) as a hygroscopic solid.

\([\alpha] = -95.2\) (C, 0.5, MeOH), IR (Neat): 3302, 1640, 1530, 1440 cm\(^{-1}\), \(^1\)H NMR (400 MHz, DMSOD\(_6\)): \(\delta\) 7.79 and 6.73 (d, J = 8.2 Hz, 1H), 7.30-7.17 (m, 5H), 7.11 and 6.63 (t, 1H), 5.93-5.70 (m, 2H), 5.13-4.97 (m, 4H), 4.70-4.60 (m, 1H), 4.59-4.56 (m, 1H), 4.51-4.44 (m, 1H), 4.26-4.21 (m, 2H), 3.91-3.67 (m, 2H), 3.57-3.49 (m, 1H), 3.40-3.32 (m, 1H), 3.27-2.99 (m, 2H), 2.39-2.30 (m, 4H), 2.20-2.09 (m, 5H), 1.80-1.45 (m, 2H), 0.82-0.70 (m, 1H), Mass (CI method) (m/z): 481 ((M+H)^+, 100).
Preparation of cyclo(Pro-Pro-Phe-Aha) (84):

The unsaturated cyclic product was obtained by following the general procedure (E) using (200 mg, 0.41 mmol) of N-Pentenoyl-Pro-Pro-Phe allylamide and 10 mol % of Grubb’s first generation catalyst to afford of cyclic unsaturated peptide as a mixture of E:Z isomers (70:30 by TLC) which was transformed to title product following the general procedure (F) using 30 mg of 10% Pd/C to afford (90 mg, 48 %) of title product’s white fluffy hygroscopic solid.

$^1$H NMR (400 MHz, DMSOd$_6$): $\delta$ 7.49 (t, $J = 4.6$ Hz, 1H, AhaNH), 7.25-7.13 (m, 6H, Aromatic 5H + Phe NH), 4.92 (m, 1H, Phe CαH), 4.26 (dd, $J = 5.7$ Hz & $J = 4.6$ Hz, 1H, Pro CαH), 4.22 (dd, $J = 8.3$ Hz & $J = 5.9$Hz, 1H, Pro CαH), 3.70 (m, 1H, Pro CH), 3.61 (m, 1H, Pro CδH), 3.46 (dd, $J = 14.4$ Hz & $J = 6.4$ Hz, Pro CδH), 3.45 (m, 1H), 3.4 (m, 2H), 3.18 (m, 1H), 2.87 (dd, $J = 14.4$ Hz and $J = 11.2$ Hz, 1H, Phe CβH), 2.52 (m, 1H, Aha CαH), 2.41 (m, 1H, Aha, Cα’ H), 2.21 (m, 1H), 2.13 (m, 1H), 1.98-1.78 (m, 5H), 1.76-1.66 (m, 3H), 1.58 – 1.52 (m, 1H), 1.51-1.46 (m, 1H), 1.34- 1.20 (m, 1H), 0.70-0.84 (m, 1H), Mass (CI method) : 455 ((M+H)$^+$, 100).
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Preparation of N-Boc-L-Ala-Pro-Leu allyl amide (85):

The title product was obtained by following the general procedure (C) using (620 mg, 3.27 mmol) of N-Boc-L-Ala and (875 mg, 3.27 mmol) of Pro-Leu allyl amide to yield (800 mg, 56 %) as a white solid.

mp. 132-136°C, IR (KBr): 3406, 3331, 3282, 2960, 1706, 1689, 1658 cm⁻¹, ¹H NMR (400 MHz, DMSOδ): δ 7.81 (t, J = 5.6 Hz, 1H), 7.75 (d, J = 8.3 Hz, 1H), 7.00 (d, J = 7.2 Hz, 1H), 5.81-5.71 (m, 1H), 5.13-5.00 (m, 2H), 4.32-4.30 (m, 1H), 4.26-4.19 (m, 1H), 3.68-3.65 (m, 2H), 3.59-3.53 (m, 2H), 2.06-2.01 (m, 1H), 1.90-1.87 (m, 2H), 1.82-1.76 (m, 1H), 1.61-1.58 (m, 1H), 1.56-1.45 (m, 2H), 1.36 (s, 9H), 1.12 (d, J = 7.0 Hz, 3H), 0.83 (d, J = 6.5 Hz, 3H), 0.82 (d, J = 6.4 Hz, 3H), Mass (CI method) (m/z): 439 (M+H)+, 100), 339 (60).

Preparation of N-pentenoyl-L-Ala-Pro-Leu allyl amide (86):

The title product was obtained by following the general procedure (D) using (0.175 ml, 1.71 mmol) of 4-pentenoic acid and (580 mg, 1.71 mmol) of Ala-Pro-Leu allyl amide to afford (405 mg, 56.3 %) yield as a
white solid.

mp. 120-124°C, [α] = -109.4 (c, 0.5, MeOH), IR (KBr): 3290, 2955, 1643, 1548 cm⁻¹, ¹H NMR (500 MHz,CDCl₃): δ 6.95 (d, J = 7.8 Hz, 1H, Leu NH), 6.3 (d, J = 7.3 Hz, 1H, Ala NH), 6.29 (m, 1H, Aha NH), 5.86-5.77 (m, 2H, Olefinic), 5.19-5.00 (m, 4H, olefinic), 4.77-4.74 (m, 1H, Ala CαH), 4.57-4.55 (m, 1H), 4.37-4.32 (m, 1H, Leu CαH), 3.88-3.85 (m, 2H, Allylic CH₂), 3.71-3.67 (m, 1H, Pro CδH), 3.58-3.54 (m, 1H, Pro Cδ’H), 2.39-2.27 (m, 4H, pentenoyl), 2.12-1.97 (m, 4H, Pro ββ’γγ’H), 1.75-1.70 (m, 1H, Leu CβH), 1.59-1.52 (m, 2H, Leu Cβ,γH), 1.34 (d, J = 7.0 Hz, 3H, Ala CβH), 0.92 (d, J = 6.4 Hz, 3H, Leu CδH), 0.88 (d, J = 6.5 Hz, 3H, Leu Cδ’H), Mass (Cl method) (m/z):421 (M+H)⁺, 100).

Preparation of cyclo(Ala-Pro-Leu-Aha) (87):

The unsaturated cyclic product was obtained by following the general procedure (E) using (175 mg, 0.41 mmol) of N-Pentenoyl-Ala-Pro-Phe allylamide and 10 mol % of Grubb’s first generation catalyst to afford of cyclic unsaturated peptide as a mixture of E:Z isomers (70:30 by TLC) which was transformed to title product following the general procedure (F) using 30 mg of 10% Pd/C to afford (92 mg, 56 %) of title product as white fluffy hygroscopic solid.
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IR (KBr): 3410, 3330, 3284, 2960, 1704 cm\(^{-1}\). \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 6.70 (d, \(J = 8.2\) Hz, 1H, Leu NH), 6.44 (dd, \(J = 8.2\) Hz and \(J = 3.8\) Hz, 1H, Aha NH), 6.40 (d, \(J = 6.1\) Hz, 1H, Ala NH), 4.45 (m, 1H, Leu C\(\alpha\)H), 4.44 (dd, \(J = 8.4\) Hz, 2.8 Hz, 1H, Pro C\(\alpha\)H), 4.37 (m, 1H, Ala C\(\alpha\)H), 3.74 (dd, \(J = 12.2, 8.5, 2.9\) Hz, 1H, Pro C\(\delta\)H), 3.67 (m, 1H, Aha C\(\xi\)H), 3.59 (ddd, \(J = 12.2, 9.9, 7.4\) Hz, 1H, Pro C\(\delta'\)H), 2.91 (m, 1H, Aha C\(\xi'\)H), 2.32 (m, 1H, Pro C\(\beta\)H), 2.27 (m, 1H, Aha C\(\alpha\)H), 2.25 (m, 1H, Pro C\(\beta'\)H), 2.16 (m, 1H, Aha C\(\alpha'\)H), 1.98 (m, 1H, Pro C\(\gamma\)H), 1.82 (m, 1H, Pro C\(\gamma'\)H), 1.63-1.53 (m, 5H, Leu C\(\beta\beta'\gamma\)H and Aha C\(\beta\beta'\)H), 1.33 (d, \(J = 6.7\) Hz, 3H), 1.32 (m, 1H, Aha C\(\gamma\)H), 1.30 (m, 1H, Aha C\(\gamma'\)H), 1.22 (m, 2H, Aha C\(\delta\delta'\)H), 0.94 (d, \(J = 6.4\) Hz, 3H, Leu C\(\delta\)H), 0.92 (d, \(J = 6.4\) Hz, 3H, Leu C\(\delta'\)H). Mass (CI method) (m/z): 395 (M+H\(^+\), 100).
2.7 Spectral data

**Spectrum-1A:** $^1$H NMR of peptide 43 in CDCl$_3$

**Spectrum-1B:** $^1$H NMR of peptide 43 in DMSO-d$_6$
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Spectrum-2: TOCSY spectrum of 43 in CDCl$_3$

Spectrum-3: Expansion of HSQC spectrum of 43 in CDCl$_3$
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Spectrum-4: NOESY spectrum of peptide 43 in CDCl₃
Spectrum-5A: $^1$H NMR of peptide 59 in CDCl$_3$
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Spectrum-5B: $^1$H NMR of peptide 59 in DMSO-d$_6$

Spectrum-6: TOCSY spectrum of 59 in CDCl$_3$
Spectrum-7: Expanded HSQC spectrum of peptide 59 in CDCl₃.
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Spectrum-8: NOESY spectrum of peptide 59 in CDCl₃
Spectrum-9: $^1$H NMR Spectrum of 63 in CDCl$_3$

(B) $^1$H NMR Spectrum of 63 in DMSO-d$_6$
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**Spectrum-10:** NOESY spectrum of 63 in CDCl$_3$

Phe $\overset{\leftrightarrow}{\text{CaH}}$ Pro $\overset{\leftrightarrow}{\text{CaH}}$, Aha $\overset{\leftrightarrow}{\text{NH}}$ Phe $\overset{\leftrightarrow}{\text{CaH}}$, LeuNH $\overset{\leftrightarrow}{\text{Phe CaH}}$

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**Spectrum-11:** TOCSY spectrum of 63 in CDCl₃

**Spectrum-12:** HSQC spectrum of 63 in CDCl₃
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Spectrum-13: $^1$H NMR of peptide 69 in CDCl$_3$

Spectrum-14: Expanded HSQC spectrum of peptide 69 in CDCl$_3$
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**Spectrum 15**: TOCSY spectrum of peptide 69

![Spectrum 15: TOCSY spectrum of peptide 69](image)

**Spectrum 16**: Expansions of NOESY spectrum of peptide 69

![Spectrum 16: Expansions of NOESY spectrum of peptide 69](image)

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Spectrum-17A: $^1$H NMR spectrum of peptide 73 in CDCl$_3$

Spectrum-17B: $^1$H NMR spectrum of peptide 73 in DMSO-d$_6$
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**Spectrum-18:** TOCSY spectrum of 73 in CDCl₃

**Spectrum-19:** Expanded HSQC spectrum of peptide 73 in CDCl₃
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**Spectrum-20:** NOESY spectrum of 73 in CDCl₃
Spectrum 21: $^1$H NMR of peptide 77 in CDCl$_3$
Spectrum 22: Expansions of TOCSY spectrum of peptide 77.

Spectrum 23: Expansions of NOESY spectrum of peptide 77.
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Spectrum-23A: $^1$H NMR of peptide 81 in CDCl$_3$

Spectrum-23B: $^1$H NMR of peptide 81 in DMSO-d$_6$
Chapter II

Spectrum-24: TOCSY spectrum of 81 in CDCl₃

Spectrum-25: HSQC spectrum of 81 in CDCl₃
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Spectrum-26: NOESY spectrum of 81 in CDCl₃

LeuCaH ↔ Pro, Aha NH ↔ Leu CaH, Phe NH ↔ Leu CaH
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Spectrum-27: $^1$H NMR spectrum of peptide 83 in CDCl$_3$.
Spectrum-28: Expanded TOCSY spectrum of peptide 83 in CDCl₃
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**Spectrum-29**: Expanded NOESY spectrum of peptide 83 in CDCl₃
Chapter II

Spectrum-30A: $^1$H NMR spectrum of peptide 84 in CDCl$_3$

Spectrum-30B: $^1$H NMR spectrum of peptide 84 in DMSO-d$_6$
Chapter II

**Spectrum-31A:** Expanded HSQC spectrum of peptide 84 in CDCl$_3$

**Spectrum-36:** Expanded NOESY spectrum of peptide 87
Chapter II

**Spectrum-33:** $^1$H NMR of peptide 87 in CDCl$_3$

**Spectrum-34:** Expanded HSQC spectrum of peptide 87 in CDCl$_3$
Chapter II

**Spectrum-35:** TOCSY spectrum of peptide 87 in CDCl₃

**Spectrum-36:** NOESY spectrum of peptide 87 in CDCl₃