Chapter V
Synthesis & Conformational Study of $3_{10}$ Helical Model Peptides Induced by CH/π H-Bonding
5.1 Introduction

Although the three-dimensional structure of a protein is determined by its covalent structure, i.e. its amino acid sequence, the forces responsible for the folding and stabilization of the structure are mainly non-covalent in nature. These non-covalent interactions include hydrogen bonds (H-bonds) and other electrostatic interactions and (H-bonds), which involve electronegative atoms such as N and O, are well established in biological systems. A set of somewhat weaker interactions has also been recognized to play an important role in protein structure and stability. This set includes N-H and O-H···π-interactions, interactions between aromatic side-chains. The even weaker interactions, which contribute about 0.5-1 kcal/mol per bond was initially noticed as a weak CH···π interaction and Reeves et al in 1957 have experimentally proved their existence by NMR spectroscopy, drawing parallel with H-bonding. More recently, Brandl et al have carried out a systematic data base study on non-redundant set of 1154 protein crystal structures to speculate the role of CH···π interactions. They chose a systematic procedure to look for such interactions in proteins and gave a definition as “the weak interaction between CH and π system, whose distance between C atom and the centre of phenyl ring is (d_{C-A}) < 3.5 Å and the H-bond angle between C-H---A (∠_{C-H-A}) is <120° (Figure I), naming them as CH···π hydrogen bonds. The total number of C-H-donors was
divided into three groups and the total number of π-acceptors divided into four groups. The donor groups constitute all the Cα-donors, all aliphatic C-H-donors, and all aromatic C-H-donors, and the acceptor groups all aromatic π-systems, the side-chain amide groups, the side-chain carboxylate groups, and the guanidinium groups. They found a total of 31,087 CH···π interactions, which satisfied their criteria for H-bonding. About 40% amongst them were between the side chains separated by 6-9 residues.  

Figure-1: Schematic representation of CH···π H-bonding

A plot of the occurrence of C-H···π-interactions as a function of ΔD,A (distance between the donor and the acceptor amino acid residue along the primary structure) revealed that many (41 %) CH···π interactions involving protein side-chain π-systems are local. This indicates that either direct neighbors along the sequence or close neighbors in α-helices or turns preferably display this kind of interaction. The occurrence of CH···π interactions in proteins and peptides has been extensively reviewed in the past. One of the recent example include
designing structural motifs using ΔPhe by Chauhan et al who reported a two
residue spacer in an α,β-didehydrophenylalanine containing hexapeptide, which
adopted a right handed 3_10 helical structure based on NMR and crystallographic
studies. The aromatic ring of ΔPhe formed the hub of multicentred interactions,
namely as a donor in aromatic CH···π and aromatic CH···O=C interactions and as
an acceptor in a CH_3···π interaction. In some cases, such interactions have been
observed among opposite strands in a β-hairpin peptides for example: Waters et al
investigated a set of β-hairpin peptides by employing double mutant cycles to
determine the interaction energies of residues in diagonal positions and provided
insight into the way weak interactions allow a protein to obtain the specificity
necessary to form a single low energy folded state. The cases in which they have
been described in proteins include the formation of complexes of proteins with
special ligands or cofactors such as the heme group, pyridoxal-50-phosphate,
nucleotides, carbohydrates, bound peptides and in the design of serine
protease inhibitors. An excellent example of a structure displaying a number of
(15) CH···π interactions is γB-eye-lens-crystallin. A recent study by Imamoto et
al has demonstrated that a loss of single CH···π hydrogen bond between the
phenyl ring of Phe6 adjacent to the alkyl chain of Lys123 caused substantial
alteration of the stability and photocycle of the photoactive yellow protein (PYP),
based on characterization of the mutants for these amino acid residues.
5.2 Present Study

Proline-rich regions of proteins, occur widely in both prokaryotes and eukaryotes and have been implicated in binding in a functionally important way.\textsuperscript{17} Chakrabarti \textit{et al} have looked at Pro residues, which are implicated in having a role in molecular association employ CH⋯π interactions found 11 unique protein structures in complexation with one or more protein/peptide(s) in 18 PDB files.\textsuperscript{18} In continuation with our study on Pro containing peptides, we became interested to design peptides with secondary structure elements stabilized by such weak interactions\textsuperscript{19} as we believed that the ultimate goal of the peptide chemists and protein engineers is to design an unnatural protein, which has a well defined secondary / tertiary structure and has the desired biological function. To achieve this goal, it is essential to study several model systems with designed conformation.
5.3 Results and discussions

To understand the role of CH⋯π interactions in stabilizing secondary structures at C-terminal to Pro\textsuperscript{20} in model peptides, we instigated our study with the synthesis of acyclic tripeptide Pent-\textsuperscript{3}Val-Pro-Phe-allylamide according to scheme-1.

Scheme-1: Preparation of acyclic tripeptide 3 from N-Boc-Pro-Phe allylamide

Coupling of N-Boc-Phe allylamide with N-Boc-Pro was mediated by EDC/HOBt as coupling agents to afford dipeptide 1, which was reacted with TFA to deprotect the ‘Boc’ group and coupled with N-Boc-D-Val to yield the tripeptide 2 in good yields. The final amide bond formation between pentenoic acid and 2 was achieved using mixed anhydride protocol (\text{CICO}_2\text{Bu}/\text{NEt}_3) to yield acyclic tripeptide 3. The conformation of the peptide showed the presence of a 3\textsubscript{10} helical geometry based on the down field appearance of Phe NH (7.2 ppm) and Allylic NH (6.9 ppm) in the \textsuperscript{1}H NMR spectrum and diagnostic NOEs (Figure-2) (this
result is in consonance with our previous results\textsuperscript{20} that heterocyclic peptide with D-amino acid at N-terminal to Pro nucleates a $3_{10}$ helical geometry).

![Diagram](image.png)

Figure-2: Long-range nOe correlations and 10 membered H-bonding pattern that support for $3_{10}$ helical structure of peptide 3.

We did not observe CH···π interaction between the aromatic ring of Phe and Pro residues in acyclic peptide 3, which was attributed to the lack of appropriate orientation (of aromatic ring) and distance between Phe and Pro residues. To make the structure more compact, we invoked a modified design based on which, reducing the olefinic segment by two carbon atoms in 3 at N-terminal would place the π-cloud almost parallel to the Phe residue (3A) (at C-terminal) thereby enabling it to be available for a possible π-π interaction\textsuperscript{21} which may in turn force the aromatic ring of Phe residue to participate in a CH···π interaction (figure-3). Further using RCM reaction such peptides could be transformed to cyclic peptides.
Figure-3: Schematic representation of modified acyclic peptide 3A

Based on the above reasoning we designed and synthesized two acyclic peptides namely crotonyl-D-Val-Pro-Phe-allylamide and acrolyl-D-Val-Pro-Phe-allylamide respectively as shown in scheme-2 by deprotecting ‘Boc’ in tripeptide 1 and subsequently coupling it with crotonyl chloride and acrolyl chloride respectively to yield 4 & 5 in good yields.

Scheme-2: Preparation of acyclic peptide 4 and 5 from dipeptide 1

These peptides yielded well-resolved $^1$H NMR spectra in 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 in
the peptides was probed by addition of varying concentrations of the strongly hydrogen-bonding solvent DMSO to peptides in the poorly interacting solvent, CDCl₃. The solvent titration curves and temperature coefficients of NH chemical shifts in DMSO-d₆ are shown in Figure-4.

![Figure-4](image-url)

**Figure-4:** (a) Solvent titration plot of peptide 4 in CDCl₃ (b) and plot of variable temperature experiment study in DMSO-d₆.

**Conformation of peptide 4:**

In CDCl₃ solution, the solvent titration studies of 4 showed two of three amide protons (Phe NH (7.15 ppm) and Allyl NH (6.81 ppm) to be involved in intramolecular H-bonding based on small change in their chemical shift values (Δδ for Allyl NH = 0.25 ppm and Phe NH = 0.20 ppm when 33% v/v DMSO-d₆ was added in CDCl₃ solution) during solvent titration study (Figure 4). In addition, the observation of cross correlations between Phe NH ↔ Allyl NH, Phe NH ↔ DVal CαH, Phe NH ↔ Pro Cδ (Pro-S)H, Allyl NH ↔ Pro CαH (Figure 5) suggested the
existence of two successive $\beta$-turns about $^D$Val-Pro and Pro-Phe residues, which resembles incipient $3_{10}$ helical conformation for the peptide 4. Furthermore, the unexpected up field appearance of $C_\gamma$(Pro-S)H at 1.27 ppm and the NOEs Pro $C_\gamma$(Pro-S)H / Phe Ar OH (Phe aromatic ortho H), Pro $C_\gamma$(Pro-S)H / Phe Ar MH (Phe aromatic meta H) suggested the eminent presence of CH···π H-bond involving $C_\gamma$(Pro-S)H ← Phenyl ring. In addition the unusual difference of in the chemical shift values of Phe $C_\beta$Hs (3.47 and 2.93 ppm) and the strong NOE cross peak Phe $C_\beta$(Pro-S)H ↔ Crot $C_\beta$H implies the existence of another CH···π H-bond involving Phe $C_\beta$(Pro-S)H → Crot Olefin that further stabilizes the $3_{10}$ helical conformation.

In DMSO-$d_6$ solution $^1$H NMR spectrum showed two sets of resonances in 55:45 ratios. The major resonances were found to have a trans geometry preceding proline, which was confirmed by the appearance of cross correlations between $^D$Val $C_\alpha$H ↔ Pro $C_\delta$s in the NOESY spectrum (spectrum 2, page#326). The NOEs Phe NH ↔ Allyl NH, Phe NH ↔ $^D$Val $C_\alpha$H, Phe NH ↔ Pro $C_\delta$ (Pro-S)H, Allyl NH ↔ Pro $C_\alpha$H and involvement of Phe NH and Allyl NH H-bonds, support two successive $\beta$-turns about Val-Pro and Pro-Phe residues, providing unequivocal evidence for a stable $3_{10}$ helical conformation. For the minor resonances the cross correlations between $^D$Val $C_\alpha$H ↔ Pro $C_\alpha$H supported the cis rotamer preceding proline.
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Figure-5: Long-range nOe correlations and 10 membered H-bonding pattern that support for 3_{10} helical structure of peptide 4.

Conformation of peptide 5:
Inspection of the distribution of NH chemical shifts (Phe NH (7.1 ppm) and Allylic NH (6.8 ppm) as compared to D-Val NH (6.07 ppm) for peptide 5 in CDCl₃ suggested similar conformational signatures as compared to 4. The critical NOEs Phe NH ↔ Allyl NH, Phe NH ↔ D-Val CαH, Phe NH ↔ Pro Cδ (Pro-S)H, Allyl NH ↔ Pro CαH (Figure-6) confirmed the existence of a 3_{10} helical conformation for peptide 5. Most importantly, the unexpected up field appearance of Cγ(Pro-S)H at 1.27 ppm and the NOE cross correlations between Pro Cγ(Pro-S)H ↔ Phe Ar OH (Phe aromatic ortho H), Pro Cγ(Pro-S)H ↔ Phe Ar MH (Phe aromatic meta H), suggested the presence of CH⋯π H-bond involving Cγ(Pro-S)H← Phenyl ring of the Phe residue. In addition, the difference in the chemical shift values of Phe CβHs (3.57 and 2.89 ppm) and the NOEs Phe Cβ(Pro-
S)H→Crot CβH implied the existence of another CH···π H-bond involving Phe Cβ(Pro-S)H → Crot Olefin.

Figure-6: Long-range nOe correlations and 10 membered H-bonding pattern that support for 310 helical structure of peptide 5.

The results on tripeptides 4 & 5 provided a platform for further validating our design in tetrapeptides. Accordingly, the C-terminal in tripeptides (4 & 5) was modified (by incorporating a Leu residue instead of olefinic segment) to acrolyl-D-Val-Pro-Phe-LeuOMe (9) according to scheme-3 using iterative protection-deprotection strategy. According to this, Leucine methyl ester was coupled with N-Boc-Phe using mixed anhydride protocol (EDC/HOBt) to yield the dipeptide (7) in excellent yields. Unmasking ‘Boc’ using TFA followed by coupling with N-Boc-Pro (EDC/HOBt) yielded the tripeptide N-Boc-Pro-Phe-Leu methyl ester (8). The final fragment condensation between Pro-Phe-LeuOMe (obtained by deprotection of ‘Boc’ in 8) and N-acroyl-d-Val24 using EDC/HOBt yielded tetrapeptide 9 in good yield.
Scheme-3 Preparation of acyclic tetrapeptides 9, 10 and 11 from Leucine methylester.HCl

Reagents and conditions: (a) N-Boc-Phe, EDC, HO\text{Bt}, CH\textsubscript{2}Cl\textsubscript{2}, 0 °C-rt, 12h, 71 %
(b) i. TFA, CH\textsubscript{2}Cl\textsubscript{2}, ii. N-Boc-Pro, EDC, HO\text{Bt}, CH\textsubscript{2}Cl\textsubscript{2}, 0°C-rt, 12h, 80 %
(c) i. TFA, CH\textsubscript{2}Cl\textsubscript{2}, N-acroyl or crotonyl or cinnamoyl-D-Val, ClCO\textsubscript{2}Bu, NE\textsubscript{t}\textsubscript{3}, CH\textsubscript{2}Cl\textsubscript{2}, 0 °C-rt.

Conformation of peptide 9:

It was gratifying to observe well-resolved \textsuperscript{1}H NMR spectra for peptides 9 in both CDCl\textsubscript{3} and DMSO\textsubscript{d6}. The down field appearance of Phe NH (7.21 ppm) and Leu NH (6.90 ppm) amide protons in CDCl\textsubscript{3} solution (spectrum 5, Page\# 328), small change in their chemical shift values during the solvent titration study and variable temperature experiments (Δδ/ΔT of −2.4 ppb/°K and −1.3 ppb/°K respectively) confirmed their participation in intra molecular H-bonding (Figure 7).
Figure-7: (a) Solvent titration plots of peptide 9 in CDCl₃ (b) and plot of variable temperature experiment study in DMSO-d₆.

The NOEs Phe NH↔Leu NH, Phe NH↔Val CαH, Leu NH↔Pro CαH, Phe NH↔Pro Cδ(Pro-S)H (spectrum 6, page# 328) was found to be in agreement with the existing H-bonds between Phe NH←acryloyl CO and Leu NH← Val CO (Figure 8) confirming existence of two successive β-turns, which corresponds 3₁₀ helical conformation. The evidence for CH···π H-bonding between Pro Cγ(Pro-S)H and aromatic ring was established based on the NOEs between Pro Cγ(Pro-S)H↔Phe Ar OH (Phenylalanine aromatic ortho H), Pro Cδ(Pro-S)H↔Phe Ar OH. The NOE cross peak between Phe Cβ(Pro-S)H↔Acryloyl CβH supported Phe Cβ(Pro-S)H↔Acryloyl olefin CH···π H-bonding.
Crystallization from EtOAc-MeOH-n-hexane afforded diffraction quality single crystals for X-ray diffraction. Its crystal structure in the $P2_12_12_1$ space group showed two independent molecules in the asymmetric unit (Figure 9).

Figure- 9 ORTEP of acrolyl-$^{3}$Val-Pro-Phe-LeuOMe 9 (H atoms are not shown for clarity).
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An intramolecular H-bond (10-membered) between the amide carbonyl oxygen of Aha residue (Amino hexanoic acid) (O1 in A and O7 in B) and Leu amide proton (N3-H in A and N7-H in B) was apparent from the interatomic distance of 2.87 Å in A and 2.91 Å in B respectively. The dihedral angle around D-Val-Pro residue was in agreement with the standard values of a type II’ β-turn (Table I φ, ψ angles are given for molecule A).

**Table I φ, ψ angles around D-Val-Pro residues** (Standard values in parenthesis)

<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>C3-N1-C4-C8</td>
<td>56.13(60)</td>
<td>-127.54(-120)</td>
<td>C8-N2-C12-C13</td>
<td>-68.6(-80)</td>
<td>-11.87 (0)</td>
</tr>
<tr>
<td>2</td>
<td>N1-C4-C8-N2</td>
<td></td>
<td></td>
<td>N2-C12-C13-N3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Another intramolecular H-bond (10-membered) between the amide carbonyl oxygen of D-Val(O2 in A and O8 in B) and Aha amide proton(N4-H in A and N8-H in B) was obvious from the interatomic distance of 3.03 Å in A and 2.95 Å in B respectively. The dihedral angle around Pro-Leu residue were in consonance with the i+1 and i+2 residues of a type I β-turn (φ, ψ angles are given for molecule A).

**Table II φ, ψ angles around Pro-Leu residues** (Standard values in parenthesis)

<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>C8-N2-C12-C13</td>
<td>-68.66(-60)</td>
<td>-11.87(-30)</td>
<td>C13-N3-C14-C22</td>
<td>-94.07(-90)</td>
<td>2.37 (0)</td>
</tr>
<tr>
<td>2</td>
<td>N2-C12-C13-N3</td>
<td></td>
<td></td>
<td>N3-C14-C22-N4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Although the molecular conformation (in terms of H-bonds) of acrolyl-D-Val-Pro-Phe-LeuOMe is consistent with a $3_{10}$ helical conformation found in solution, we observed only one intramolecular C-H···π interaction between Pro C$_\gamma$H and aromatic ring of Phe residue of 3.04 Å, 164.8° (C10–H101···π, π = C17–C18 centroid) in the crystal structure. The distance between the Phe C$_\beta$H and C1-C2 centroid was too long (3.71 Å).

With these encouraging results we became interested to investigate the role of peptide sequence/substituent’s in stabilizing or destabilizing the secondary structure and C-H···π interaction. Accordingly, we continued our study by synthesizing mutant peptides with modified N-terminal namely crotonyl-D-Val-Pro-Phe-LeuOMe (10) and N-cinnamoyl-D-Val-Pro-Phe-LeuOMe (11) following scheme-3. Deprotection of ‘Boc’ in dipeptide 8 followed by coupling with N-crotonyl-d-Val$^{24}$ and N-cinnamoyl-d-Val$^{25}$ using EDC/HOBt yielded the tetrapeptides 10 and 11 respectively in good yields.

**Conformation of peptide 10:**

The solvent titration study for peptide 10 in CDCl$_3$ and variable temperature experiments in DMSO-d$_6$(Δδ/ΔT of $-2.4$ ppb/°K and $-1.3$ ppb/°K respectively) confirmed their involvement in intra molecular H-bonding (Figure 10). The NOEs between Phe NH↔Leu NH, Phe NH↔Pro Cδ(Pro-S)H, Phe NH↔DVal CαH, Leu NH↔Pro CαH (spectrum 10, Page# 330) were in agreement with two
successive \( \beta \)-turns about \( ^{\text{D}} \text{Val-Pro} \) and Pro-Phe residues (Figure-11). As observed in peptide 9, the up field appearance of Pro \( C_{\gamma}(\text{Pro-S})H \) at 1.28 ppm as well as cross peaks between Pro \( C_{\gamma}(\text{Pro-S})H \leftrightarrow \text{Phe Ar OH} \) (Phenylalanine aromatic ortho H), Pro \( C_{\delta}(\text{Pro-S})H \leftrightarrow \text{Phe Ar OH} \), imply a CH–\( \pi \) H-bond between Pro \( C_{\gamma}(\text{Pro-S})H \) and aromatic ring, while, the strong NOE cross peak between Phe \( C_{\beta}(\text{Pro-S})H \leftrightarrow \text{Acrolyl C\( \beta \)H} \) supports Phe \( C_{\beta}(\text{Pro-S})H \leftrightarrow \text{Acrolyl olefin CH–\( \pi \) H-bonding.}

**Figure-10:** (a) Solvent titration plots of peptide 10 in CDCl\(_3\) (b) and plot of variable temperature experiment study in DMSO-\( d_6\).

**Figure-11:** Long-range NOE correlations and 10 membered H-bonding patterns that support \( 3_{10} \) helical structure of peptide 10.
Conformation of peptide 11:

$^1$H NMR spectrum showed sharp and well dispersed resonances, indicating the presence of well defined secondary structure in solution. In CDCl$_3$ solution (spectrum 11, page# 331), exclusive appearance of only one set of resonances imply the existence of single rotamer in CDCl$_3$ solution, whereas in DMSO-$d_6$ solution two sets of resonances with 7:1 ratio were observed. The NOEs Val C$\alpha$H$\leftrightarrow$Pro C$\delta$(Pro-S) H and Val C$\alpha$H$\leftrightarrow$Pro C$\delta$(Pro-R) H in the major isomer were in agreement with a trans amide bond preceding proline. The H-bonding studies in both solvents indicated Phe NH(7.31 ppm) and Leu NH (6.89 ppm) to be involved in intra molecular H-bonding (Figure 12). The NOEs Phe NH$\leftrightarrow$Leu NH, Phe NH$\leftrightarrow$$^3$Val C$\alpha$H, Leu NH$\leftrightarrow$Pro C$\alpha$H, Phe NH$\leftrightarrow$Pro C$\delta$(Pro-S) H (spectrum 14, page# 332) corroborated the existence of H-bonds Phe NH $\leftrightarrow$Cinn C=O and Leu NH $\leftrightarrow$Val C=O (Figure 13). In addition the long range correlations Cinn C$\beta$H$\leftrightarrow$Phe C$\beta$(Pro-S) H, Cinn C$\alpha$H$\leftrightarrow$Leu C$\beta$H, Cinn Ar H$\leftrightarrow$Leu C$\delta$Me’s, Cinn C$\alpha$H$\leftrightarrow$Leu C$\delta$Me’s, Val NH$\leftrightarrow$Leu C$\beta$H strongly supported 3$_{10}$ helical structure in peptide 11. It was interesting to note the significant upfield shift of proline C$_\gamma$(Pro-S)H proton at 1.35 ppm due to the ring current of phenyl ring. The spatial correlation between Pro C$_\gamma$(Pro-S)H/ Phe Ar OH (phenyl alanine aromatic
ortho H), Pro C<sub>δ</sub>(Pro-S)H / Phe Ar OH, provides the strong evidence for the existence of CH···π interaction between Pro-Phe residues.

Figure-12: (a) Solvent titration plot of peptide 11 in CDCl₃ (b) and plot of variable temperature experiment study in DMSO-d₆.

Figure-13: Long-range NOE correlations and 10 membered H-bonding pattern that support for 3₁₀ helical structure of peptide 11.

It is known that Ala has high helix forming propensity, while, Val has one of the highest propensities among branched amino acids to nucleate β-sheet conformation in peptides and proteins. We reasoned that replacing Ala, a helix
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nucleating residue, with Val in peptide 12 should favor \(3_{10}\) helical structure and synthesized Cinn-\(^D\)Ala-Pro-Phe-Leu-OMe (12) following scheme-4.

**Scheme-4 Preparation of acyclic tetrapeptide 12 from dipeptide 8**

According to this, dipeptide 8 after unmasking ‘Boc’ was coupled with \(N\)-cinnamoyl-d-Ala to yield tetrapeptide 12 in good yields. It was gratifying to observe the involvement of Phe NH (7.33 ppm) and Leu NH (6.87 ppm) in intramolecular H-bonding in both solvents (Figure 14) as ascertained by H-bonding studies. Further, the spatial correlations between Phe NH\(\leftrightarrow\)Leu NH, Phe NH\(\leftrightarrow\)^DAla C\(\alpha\)H, Leu NH\(\leftrightarrow\)Pro C\(\alpha\)H, Phe NH\(\leftrightarrow\)Pro C\(\delta\)(Pro-S) H (spectrum 15 and 18, Page #334 & 335) strongly supported the H-bonds Phe NH\(\leftrightarrow\)Cinn C=O and Leu NH\(\leftrightarrow\)Ala C=O, corresponding to \(3_{10}\) helical conformation (Figure 15). Moreover, Unusually large up field shift of Pro C\(\gamma\) (Pro-S)H proton at 1.41 ppm and the spatial correlation between Pro C\(\gamma\) (Pro-S)H\(\leftrightarrow\)Phe Ar OH (Phe aromatic
ortho H), Pro C8(Pro-S)H ↔ Phe Ar OH, supports the CH···π intra molecular H-bond Pro Cγ(Pro-S)H ↔ Phenyl ring in peptide 12.

Figure-14: (a) Solvent titration plots of peptide 12 in CDCl3 and plot of variable temperature experiment study in DMSO-d6.

Figure 15: Long-range NOE correlations and 10 membered H-bonding pattern that support 310 helical structure of peptide 12.

Based on the crystal structure of 9, we conceived the pyrrolidine ring puckering to play a pivotal role in CH···π interaction between Pro-Phe residues. To probe this, we designed Cinn-DVal-Hyp-Phe-Leu-OMe (13) (Hyp = trans-4-hydroxy Pro)
anticipating that the hydroxyl group in ‘Hyp’ may distort the ring pucker and may in turn lead to the destabilization of the secondary structure.

Scheme-5 *Preparation of acyclic tetrapeptide 16 from dipeptide 8*

Reagents and conditions: (a) i. TFA, CH₂Cl₂, ii. 13, EDC, HOBt, CH₂Cl₂, 0 °C-RT, 12h, 56 % (b) i. 10 % PdC, MeOH, 40 psi pressure ii. N-cinnamoyl-D-Val, EDC, HOBt, CH₂Cl₂, 0 °C-RT, 12h, 39 % (c) TBAF, dry THF, 0 °C to rt, 68 %

The synthesis instigated with the transformation of *trans*-4-hydroxy proline to compound 13 following literature procedures²² (scheme-5). It was then coupled with dipeptide Pro-Phe-LeuOMe (obtained by deprotection of 8) (EDC/HOBt) to yield tripeptide 14 in decent yields. Deprotection of ‘Cbz’ (H₂(g) /10 % Pd/C in methanol at 40 psi) followed by final fragment coupling with *N*-cinnamoyl-D-Val.
yielded tetrapeptide 15 which was desilylated using TBAF to afford the designed peptide 16 in near quantitative yields. At the outset, the absence of diagnostic chemical shift difference in the Phe CβHs and pro CγHs in the 1H NMR spectrum (spectrum 19, page# 335) of 16 suggested an extended conformation for the peptide. The lack of intra molecular H-bonding and distinctive cross correlations, supporting 3_{10} helical conformations were absent in both the solvents thereby proving the importance of Pro puckering in inducing folded conformation and C-H···π interaction.

To ascertain the role of CH···π interaction between Pro-Phe residues in nucleation of 3_{10} helical conformations, we continued sequence mutation studies by replacing one of the residues participating in CH···π interaction and expected such peptides to exist in an extended conformation. To validate this, we synthesized Cinn-^{D}Val-Ala-Phe-Leu-OMe (18) (Ala replacing Pro) and Cinn-^{D}Val-Ala-^{D}Phe-Leu-OMe (21) (D-Phe replacing L-Phe). The synthesis of 18 initiated with the condensation of dipeptide Phe-LeuOMe and N-Boc-Ala using EDC/HOBt as coupling agents. The tripeptide 17 was in turn prepared by unmasking of the ‘Boc’ group in 17 followed by its coupling with N-cinnamoyl-D-val in good yields (Scheme-6). Tetrapeptide 21 was synthesized by fragment coupling of N-cinnamoyl-D-Val with tripeptide Pro-D-Phe-LeuOMe using EDC/HOBt in good yields (Scheme-7).
Scheme-6 Preparation of acyclic tetrapeptide 18 from dipeptide 8

\[
\text{Reagents and conditions: (a) } i. \text{ TFA, CH}_2\text{Cl}_2, \text{ ii. N-Boc-Ala, EDC, HOBr, CH}_2\text{Cl}_2, 0^\circ\text{C-RT, 12h, 56}\% \text{ (b) N-cinnamoyl-D-Val, EDC, HOBr, CH}_2\text{Cl}_2, 0^\circ\text{C-RT, 12h, 39}\%.
\]

Scheme-7 Preparation of acyclic tetrapeptide 21 from dipeptide 8

\[
\text{Reagents and conditions: (a) N-Boc-Pro, EDC, HOBr, CH}_2\text{Cl}_2, 0^\circ\text{C-rt, 12h, 61}\% \text{ (ii) N-cinnamoyl-D-Val, EDC, HOBr, CH}_2\text{Cl}_2, 0^\circ\text{C-rt, 12h, 39}\%}.
\]

Conformation of peptide 18 and 21:

The poor solubility of tetrapeptide 18 restricted us to study its conformation only in DMSO-$d_6$ solution (spectrum 21, page# 336). The large magnitudes of temperature coefficients for all amide protons (-7.1 ppb/°K for Leu NH, -5.8 ppb/°K for Ala NH, -5.4 ppb/°K for Val NH and -4.8 ppb/°K for Phe NH) ruled out their involvement in intra molecular H-bonding. The lack of characteristic
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spatial correlations supporting $3_{10}$ helical conformation in NOESY spectrum indicates the absence of well defined structure in solution.

As expected, the absence of distinctive cross correlations, supporting $3_{10}$ helical folded conformations, intramolecular H-bonding and unusual chemical shift difference of Pro CγHs and Phe CβHs in the $^1$H NMR spectrum (spectrum 22, page# 336) in tetrapeptide 21 suggested an extended conformation in both the solvents.
5.4 Conclusion

In conclusion, we have designed and synthesized acyclic tri and tetrapeptides that fold into well organized $3_{10}$ helical structures stabilized by non-covalent weak CH···π H-bonding. Based on our studies on a series of peptides with single point and double point sequence mutations we established that, folding of the peptides depends on two types of CH···π interactions (Pro-Phe aliphatic and aromatic interactions as well as olefin aliphatic CH···π interactions) in solution. Such small peptides with well defined secondary structures should be useful in understanding critical protein-protein interactions.
5.5 References


19. β-turns and antiparallel β-sheets are known to be stabilized by Pro-aromatic interactions.


5.6 Experimental

**General procedure for the preparation of N-Boc-Yaa-Leu methyl ester (A)**

To an ice cold stirred solution of N-Boc protected amino acid (1 equivalent) in dry CH$_2$Cl$_2$ was added HOBt (1.2 equivalent) and a solution of Leucine methyl ester (1 equivalent) in dry CH$_2$Cl$_2$. After 5 min, EDC.HCl (1.5 equivalent) was added portion wise over a period of 10 min at 0 °C and then stirred the reaction mixture at room temperature for 12h (The reaction was monitored by quenching small aliquots in water and then extracted with small amount of EtOAc. The organic layer was spotted on an analytical silicagel TLC plate (10% MeOH in CHCl$_3$, using I$_2$ and KMnO$_4$ stain to visualize the spots). The reaction mixture was diluted with CH$_2$Cl$_2$ and washed with water, brine, dried over Na$_2$SO$_4$ and evaporated the solvent to afford crude product, which was purified using MeOH – CHCl$_3$ as the eluent using 100-200 mesh silica gel to afford the title compound.

**General procedure for the preparation of N-Boc-Pro-Yaa-Leu methyl ester (B)**

A. To an ice cold stirred solution of N-Boc-Yaa-Leu methyl ester (1 equivalent) in dry CH$_2$Cl$_2$ was added trifluoroacetic acid (TFA) (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.
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B. To an ice cold stirred solution of N-Boc-Proline (1 equivalent) and HOBt (1.2 equivalent) of dry CH₂Cl₂ was added a solution of Yaa-Leu methyl ester (obtained in part A) (1 equivalent) in dry CH₂Cl₂ ad stirred for 5 min. 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 CH₂Cl₂ and washed with water, brine, dried over Na₂SO₄ and evaporated to afford crude product which was purified using 100-200 mesh silica and MeOH-CHCl₃ as the eluent to afford the title compound.

General procedure for the preparation of N-acrolyl/N-cinnamoyl/N-crotonyl Xaa methyl ester (C):
To an ice cold stirred solution of Valine methyl ester hydrochloride (1.0 equivalent) in dry CH₂Cl₂ was added NEt₃ (3.0 equivalent) drop wise over a period of 10 min followed by the addition of appropriate acid chloride (acrolyl, cinnamoyl or crotonyl chloride) (1.2 equivalent) at same temperature and then stirred at room temperature for 12 h. The reaction mixture was diluted with CH₂Cl₂, washed with water, dried over Na₂SO₄ and evaporated to yield crude compound which was purified using MeOH-CHCl₃ as eluent to yield product.

Preparation of N-acrolyl/ N-cinnamoyl/N-crotonyl Xaa (D):
To a stirred solution of N-acrolyl, N-cinnamoyl or N-crotonyl-L-Xaa methyl ester (1.0 equivalent) in MeOH-H₂O (4:1) (8 ml) was added an aqueous solution of LiOH (1.2 equivalent) and stirred for 4 h at room temperature. Methanol was
evaporated completely under reduced pressure and the reaction mixture was
diluted with water. The aqueous layer was cooled to 0°C and acidified using cold
1N HCl (pH~2.0) and extracted with CH$_2$Cl$_2$. The organic layer was washed with
brine (20 ml), dried over Na$_2$SO$_4$ and evaporated to yield the product

**Preparation of N-acrolyl or N-cinnamoyl or N-crotonyl Xaa-Pro-Yaa-
allylamide (E)**

**A.** To an ice cold stirred solution of N-Boc-$\alpha$-Pro-Yaa allylamide (1 equivalent) in
dry CH$_2$Cl$_2$ was added trifluoroacetic acid (TFA) (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 appropriately N-substituted (acrolyl or
crotonyl or cinnamoyl)-Xaa (aa= amino acid) (1 equivalent) and HOBt (1.2
equivalent) of dry CH$_2$Cl$_2$ was added a solution of Pro-Yaa allylamide (obtained
in part A) (1 equivalent) in dry 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$_2$ and washed
with water, brine. The organic layer was dried over sodium sulfate and evaporated
to afford crude product which was purified using 100-200 mesh silica and MeOH-
CHCl$_3$ as the eluent to afford the title compound.
General procedure for the preparation of \(N\)-acrolyl or \(N\)-cinnamoyl or \(N\)-crotonyl \(Xaa\)-Pro-\(Yaa\)-Leu methyl ester (F):

A. To an ice cold stirred solution of \(N\)-Boc-Pro-\(Yaa\)-Leu methyl ester (1 equivalent) in dry CH\(_2\)Cl\(_2\) was added trifluoroacetic acid (TFA) (10 equivalents) at 0\(^\circ\)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\(^\circ\)C to obtain the free amine.

B. To an ice cold stirred solution of appropriately \(N\)-substituted (acrolyl or crotonyl or cinnamoyl)-\(Xaa\) (aa= amino acid) (1 equivalent) and HOBt (1.2 equivalent) of dry CH\(_2\)Cl\(_2\) was added a solution of Pro-\(Yaa\)-Leu methyl ester (obtained in part A) (1 equivalent) in dry CH\(_2\)Cl\(_2\). EDC.HCl (1.5 equivalent) was added portion wise to the reaction mixture at 0 \(^\circ\)C and then stirred at room temperature for a period of 12 h. The reaction mixture was diluted with CH\(_2\)Cl\(_2\) and washed with water, brine. The organic layer was dried over sodium sulfate and evaporated to afford crude product which was purified using 100-200 mesh silica and MeOH-CHCl\(_3\) as the eluent to afford the title compound.

Preparation of \(N\)-acrolyl-\(D\)-Valine methyl ester\(^{23}\):
The title compound was obtained following the general procedure (C) using (2.4 g, 14.3 mmol) of \(d\)-Valine methylester.HCl, (1.55 g, 17.2 mmol) of acrolyl chloride
and (6 ml, 43 mmol) of NEt₃ to afford (1.32 g, 50 %) of title product as pale yellow oil.

\[ \alpha = 44 \text{ (c, 0.1, MeOH)}; \text{IR (KBr): 3299, 2966, 1715, 1661, 1628, 1537 cm}^{-1}; \text{ }^1H \text{ NMR (400 MHz, CDCl}_3\text{): } \delta 6.31 \text{ (dd, } J = 16.9 \text{ Hz and } J = 1.3 \text{ Hz, 1H), 6.19-6.12 (m, 1H), 6.03 \text{ (m, 1H), 4.68-4.64 (m, 1H), 5.69 (dd, } J = 10.2 \text{ Hz and } J = 1.3 \text{ Hz, 1H), 3.77 (s, 3H), 2.22-2.15 (m, 1H), 0.96-0.91 (m, 6H).} \]

Preparation of N-acrolyl-L-valine OH\textsuperscript{24}:

The title compound was obtained following the general procedure (D) using (680 mg, 3.67 mmol) of N-acrolyl-D-valine methyl ester and (185 mg, 4.40 mmol) of LiOH in MeOH-H₂O to afford (0.45 g, 71 %) of title product as colorless gum.

\[ \alpha = -13.0 \text{ (c, 0.5, MeOH)}; \text{ }^1H \text{ NMR (400 MHz, CDCl}_3\text{): } \delta 6.32 \text{ (m, 2H), 5.70-5.67 (dd, } J = 102 \text{ Hz and } J = 1.6 \text{ Hz, 1H), 2.27-2.17 (m, 2H), 0.99-0.98 (m, 6H). Mass (CI method): 172 ((M+H)+, 16), 126 (100).} \]

Preparation of N-acrolyl-D-valine OH\textsuperscript{24}:

The title compound was obtained following the general procedure (D) using (1g, 5.4 mmol) of N-acrolyl-D-Valine methyl ester and (272 mg, 6.4 mmol) of LiOH in MeOH-H₂O to afford (0.68 g, 73.9 %) of title product as colorless solid.
Mp. 118-119 °C; [α] = 12.8 (c, 0.5, MeOH); \(^1\)H NMR (400 MHz, CDCl\(_3\)): δ 6.32-6.19 (m, 2H), 5.70-5.67 (dd, \(J = 10.2\) Hz and \(J = 1.6\) Hz, 1H), 2.27-2.17 (m, 2H), 0.99-0.98 (m, 6H). Mass (CI method): 172 ((M+H)+, 7), 126 (100).

**Preparation of N-crotonyl-d-valine methyl ester\(^{24}\):**

The title compound was obtained following the general procedure (C) using (3 g, 25.6 mmol) of d-Valine methylester.HCl, (3.19 g, 30.7 mmol) of crotonyl chloride and (7.76 g ml, 76.9 mmol) of NEt\(_3\) to afford (2.94 g, 62 %) of title product as a white solid.

\(^1\)H NMR (200 MHz, CDCl\(_3\)): δ 6.91-6.88 (m, 2H), 5.90-5.82 (m, 1H), 5.81-5.79 (bs, 1H), 4.64-4.60 (m, 1H), 3.73 (s, 3H), 2.18-2.12 (m, 1H), 1.87-1.84 (m, 3H), 0.95-0.88 (m, 6H), Mass (CI method): 200 ((M+H)+, 100).

**Preparation of N-crotonyl-d-valine\(^{24}\):**

The title compound was obtained following the general procedure (D) using (1g, 5.02 mmol) of N-cinnamoyl-d-Valine methyl ester and (253 mg, 6.03 mmol) of LiOH in MeOH-H\(_2\)O (4:1) (10 ml) to afford (0.61 g, 66 %) of title product as colorless gum.

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$^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 7.76 (bs, 1H), 6.95-6.81 (m, 1H), 6.19(d, $J = 8.3$ Hz, 1H), 5.95-.587 (m, 1H), 4.68-4.61(m, 1H), 2,31-2.22 (m, 1H), 1.89-1.86 (m, 3H), 0.98-.95 (m, 6H), Mass (CI method): 186 ((M+H)$^+$, 100).

Preparation of N-cinnamoyl-d-valine methyl ester$^{25}$:

The title compound was obtained following the general procedure (C) using (4.47 g, 26.7 mmol) of d-Val methylester.HCl, (5.33 g, 32.1 mmol) of cinnamoyl chloride and (8.1 g, 80.1 mmol) of NEt$_3$ to afford (5.45 g, 61 %) of title product as a white solid.

mp: 130°C; IR (KBr): 3319, 2963, 1743, 1610, 1207 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.64 (d, $J = 15.8$ Hz, 1H), 7.52-7.49 (m, 2H), 7.39-7.34 (m, 3H), 6.47 (d, $J = 15.6$ Hz, 1H), 6.14 (d, $J = 8.3$ Hz, 1H), 4.75-4.71 (m, 1H), 3.76 (s, 3H), 2.25-2.20 (m, 1H), 0.99-0.95 (m, 6H); Mass (CI method): 262 ((M+H)$^+$, 100).

Preparation of N-cinnamoyl-d-valine$^{25}$:

The title compound was obtained following the general procedure (D) using (590 mg, 2.26 mmol) of N-cinnamoyl-d-Valine methyl ester and (115 mg, 2.71 mmol) of LiOH in MeOH-H$_2$O (4:1) (10 ml) to afford (0.45 g, 71 %) of title product as white solid.
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mp: 206 °C; [α] = -22.4 (c, 0.5, MeOH); IR (Neat): 3319, 2967, 1721, 1653, 1212 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.66 (d, J = 15.6 Hz, 1H), 7.52-7.49 (m, 2H), 7.38-7.34 (m, 3H), 6.48 (d, J = 15.6 Hz, 1H), 6.21 (d, J = 8.5 Hz, 1H), 4.74-4.70 (m, 1H), 2.34-2.30 (m, 1H), 1.05-1.00 (m, 6H).

Preparation of N-crotonyl-D-Val-Pro-Phe-allyl amide (4):

The title compound was obtained following the general procedure (E) using (500 mg, 2.70 mmol) of N-crotonyl-D-Val, (812 mg, 2.70 mmol) of Pro-Phe-allylamide, (438 mg, 3.24 mmol) of HOBt and (932 mg, 4.86 mmol) of EDC.HCl to afford (594 mg, 47 %) of title product as a gum.

¹H NMR (400 MHz, DMSO-d₆): δ 7.51-7.17 (m, 5H+ Phe NH), 6.86 (m, 1H, Crot CβH), 6.81 (d, J = 4.7 Hz, 1H, Allyl NH), 5.85-5.84 (m, 1H, Crot CαH), 5.84 (d, J = 5.3 Hz, 1H), 5.78-5.75 (m, 1H), 5.15-5.12 (m, 1H), 5.09-5.06 (m, 1H), 4.69 (m, 1H, Phe NH), 4.42 (dd, J = 7.3 Hz and J = 3.4 Hz, 1H, Pro CαH), 4.06-4.03 (m, 1H), 4.01-3.98 (m, 1H), 3.82-3.78 (m, 2H), 3.69-3.67 (m, 1H), 3.57 (dd, J = 13.9 Hz and J = 4.0Hz, 1H, Phe CβH), 3.48-3.51 (m, 1H), 2.89 (dd, J = 13.9 Hz and J = 12.6 Hz, 1H, Phe Cβ’H), 2.04 (m, 1H, β-Val CβH), 1.95 (m, 1H, Pro CβH), 1.89 (m, 2H), 1.74 (m, 2H), 1.27 (m, 1H), 1.09 (d, J = 6.7 Hz, 3H), 1.00 (d, J = 6.7 Hz, 3H), Mass (Cl method): 469 ((M+H)+,100).
Preparation of \textit{N}-acrolyl-d-Val-Pro-Phe-allyl amide (5):

The title compound was obtained following the general procedure (E) using (465 mg, 2.71 mmol) of \textit{N}-acrolyl-d-Val, (818 mg, 2.71 mmol) of Pro-Phe-allylamide, (440 mg, 3.26 mmol) of HO\textit{B}t and (781 mg, 4.07 mmol) of EDC.HCl to afford (500 mg, 41\%) of title product as fluffy solid.

\textbf{IR (Neat);} 3300, 2965, 1650, 1543, 1192 cm\textsuperscript{-1}; \textbf{\textit{H NMR (400 MHz, DMSO\textsubscript{d}6):} }\delta 7.34 (d, J = 15.0 Hz, 1H), 7.28-7.20 (m, 3H), 7.18-7.16 (m, 2H), 7.09 (d, J = 8.9 Hz, 1H, Phe NH), 6.80 (bt, 1H, allyl NH), 6.32 (d, J = 15.6 Hz, 1H), 6.29-6.11 (m, 2H), 6.07 (d, J = 4.3 Hz, 1H), 5.83-5.73 (m, 1H), 5.17-5.04 (m, 2H), 4.71-4.65 (m, 1H), 4.43-4.40 (m, 1H), 4.11-4.07 (m, 2H), 4.04-3.99 (m, 1H), 3.85-3.78 (m, 1H), 3.71-3.64 (m, 1H), 3.58-3.57 (m, 1H), 3.52-3.46 (m, 1H), 2.90 (dd, J = 13.9 Hz and J = 2.4 Hz, 1H), 2.08-2.01 (m, 1H), 1.99-1.90 (m, 1H), 1.80-1.70 (m, 2H), 1.32-1.26 (m, 1H), 1.07 (d, J = 6.7 Hz, 3H), 0.97 (d, J = 6.7 Hz, 3H); Mass (CI method): 455 (M+H\textsuperscript{+}, 100).

\textbf{Preparation of \textit{N}-Boc-l-Phe-Leu methyl ester (7)\textsuperscript{26}:}

The title compound was obtained following the general procedure (A) using (7.42 g, 28.0 mmol) of \textit{N}-Boc-Phe, (4.06 g, 28.0 mmol) of Leu methyl ester, (4.54g,
33.6 mmol) of HOBT and (8.05 g, 42.0 mmol) of EDC. HCl to afford (7.0 g, 71 \%) of title product as white solid.

mp. 104-105 °C; [α] = -26.2 (c, 0.5, MeOH); IR (Neat): 3296, 2962, 1754, 1687, 1649, 1542, 1172 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): δ 7.31 -7.19 (m, 5H), 6.18 (d, \(J = 8.00 \) Hz, 1H), 4.99 (bs, 1H), 4.55-4.50 (m, 1H), 4.57-4.35 (bt, 1H), 3.69 (s, 3H), 311-3.05 (d, \(J = 8.1 \) Hz, 2H), 1.56-1.48 (m, 1H), 1.44-1.43 (m, 2H), 1.40 (s, 9H), 0.86-0.84 (m, 6H); Mass (CI method): 393 ((M+H)+, 58), 337 (100).

**Preparation of N-Boc-Pro-Phe-Leu methyl ester (8):**

The title compound was obtained following the general procedure (B) using (1.10 g, 5.10 mmol) of N-Boc-Pro (1.48 g, 5.10 mmol) of Phe-Leu methyl ester, (0.93 g, 6.15 mmol) of HOBT and (1.46 g, 7.65 mmol) of EDC. HCl to afford (2.0 g, 80 \%) of title product as fluffy hygroscopic solid.

IR (Neat); 2965, 1755, 1662, 1211 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO\(_d_6\)): δ 8.38 (d, \(J = 7.5 \) Hz, 1H), 7.76 (d, \(J = 8.5 \) Hz, 1H), 7.29- 7.23 (m, 5H), 4.63-4.61 (m, 1H), 4.31 (bs, 1H), 3.99-3.97 (m, 1H), 3.61 (5, 3H), 3.32- 3.30 (m, 1H), 3.29-3.21 (m, 1H), 3.00-2.94 (m, 1H), 2.82-2.77 (m, 1H), 2.01-2.00 (m, 1H), 1.66-1.60 (m, 4H), 1.57-1.46 (m, 2H), 1.16 (m, 9H), 0.90-0.82 (m, 6H); Mass (CI method): 490(44), 434(68), 390 (100).
Preparation of \( N \)-acryloyl-D-Val-Pro-Phe-Leu methyl ester (9):

The title compound was obtained following the general procedure (F) using (610 mg, 3.57 mmol) of \( N \)-acryloyl-D-Val (1.39 g, 3.57 mmol) of Pro-Phe-Leu methyl ester (580 mg, 4.28 mmol) of HOBt and (1.02 g, 5.35 mmol) of EDC.HCl to afford (1.17 g, 61 %) of title product as fluffy solid.

IR (Neat); 3300, 2965, 1650, 1543, and 1192 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)):
\[ \delta \] 7.38-7.17 (m, 5H, Aromatic protons), 7.21 (d, \( J = 8.6 \) Hz, PheNH), 6.90 (d, \( J = 7.6 \) Hz, LeuNH), 6.35 (dd, \( J = 16.9 \) Hz and \( J = 1.4 \) Hz, AcrylC\( \beta \)'H), 6.15 (dd, \( J = 16.9 \) Hz & \( J = 10.2 \) Hz, AcrylC\( \alpha \)H), 6.07 (d, \( J = 5.3 \) Hz, \(^{b}\)ValNH), 5.75 (dd, \( J = 10.2 \) Hz and \( J = 1.4 \) Hz, AcrylC\( \beta \)H), 4.64 (m, 1H, Phe C\( \alpha \)H), 4.48 (m, 1H, Pro C\( \alpha \)H), 4.39 (m, 1H, Leu C\( \alpha \)H), 4.16 (m, 1H, \(^{b}\)ValC\( \alpha \)H), 3.96 (m, 1H, Pro C\( \delta \)H), 3.69 (s, LeuOMe), 3.48 (m, 1H, Pro C\( \delta \)'H), 3.44 (dd, \( J = 14.1 \) Hz and \( J = 4.2 \) Hz, Phe C\( \beta \)H), 2.94 (dd, \( J = 14.1 \) Hz and \( J = 11.8 \) Hz, 1H, Phe C\( \beta \)'H), 2.08-2.03 (m, 1H, Val C\( \beta \)H), 1.95-1.90 (m, 2H), 1.76-1.72 (m, 1H), 1.66-1.54 (m, 3H), 1.35-1.25 (m, 1H); Mass (CI method): 543 ((M+H)\(^+\), 100).

Preparation of \( N \)-crotonyl-D-Val-Pro-Phe-Leu methyl ester (10):

The title compound (940 mg, 57 %) was obtained following the general procedure (F) using (550 mg, 2.97 mmol) of \( N \)-crotonyl-D-Val, (1.15 g, 2.97 mmol) of Pro-
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Phe-Leu methyl ester, (490 mg, 3.56 mmol) of HOBt and (860 mg, 4.45 mmol) of EDC.HCl as a fluffy solid.

\[ \alpha = -44 \ (c, 0.1, \text{MeOH}), \ \text{IR (Neat)}; \ 3340, 2966, 1745, 1691, 1632, \text{cm}^{-1}; \ \text{^1H NMR (500 MHz, CDCl}_3\]): \ \delta \ 7.51-7.17 \ (m, 5H), \ 7.26 \ (d, J = 8.6 \ Hz, 1H, Phe NH), \ 6.96 \ (d, J = 7.3 Hz, 1H, Leu NH), \ 6.91 \ (m, 1H, Crot C\beta H), \ 5.87 \ (m, 1H, Crot C\alpha H), \ 5.77 \ (d, J = 4.9 Hz, 1H, Val NH), \ 4.65 \ (m, 1H, Phe C\alpha H), \ 4.48 \ (dd, J = 7.3 Hz and J = 3.4 Hz, Pro C\alpha H), \ 4.38 \ (m, 1H, Leu C\alpha H), \ 4.09 \ (m, 1H, Val C\alpha H), \ 3.99 \ (m, 1H, Pro C\delta H), \ 3.69 \ (s, 3H, LeuOMe), \ 3.48 \ (m, 1H, Pro C\delta' H), \ 3.47 \ (dd, J = 13.7 Hz and J = 4.0 Hz, 1H, Phe C\beta H), \ 2.93 \ (dd, J = 12.2 Hz and J = 12.2 Hz, Phe C\beta' H), \ 2.06-2.00 \ (m, 1H), \ 1.94-1.84 \ (m, 4H), \ 1.74-1.71 \ (m, 1H, Pro C\gamma H), \ 1.42-1.31 \ (m, 4H), \ 1.29-1.25 \ (m, 1H, Pro C\gamma' H), \ 1.08 \ (d, J = 6.8 Hz, 3H), \ 1.00 \ (d, J = 6.5 Hz, 3H), \ 0.94 \ (d, J = 6.3 Hz, 3H), \ 0.87 \ (d, J = 6.0 Hz, 3H), \ \text{Mass (Cl method)}. \ 557 ((M+H)^+, \ 100).

**Preparation of N-cinnamoyl-d-Val-Pro-Phe-Leu methyl ester (11):**

The title compound (572 mg, 61%) was obtained following the general procedure (F) using (375 mg, 1.51 mmol) of N-acrolyl-d-Val, (0.59 g, 1.51 mmol) of Pro-
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Phe-Leu methyl ester, (247 mg, 1.81 mmol) of HOBt and (430 mg, 2.27 mmol) of EDC.HCl as a fluffy solid.

IR (Neat); 3324, 2955, 1650, 1515, cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.63 (d, \(J = 15.8\) Hz, 1H, Cinn C\(\beta\)H), 7.31 (d, \(J = 9.1\) Hz, 1H, PheNH), 7.51-7.17 (m, 10H, Aromatic), 6.89 (d, \(J = 7.8\) Hz, 1H, LeuNH), 6.45 (d, \(J = 15.8\) Hz, 1H, Cinn C\(\alpha\)H), 6.07 (d, \(J = 5.4\) Hz, 1H, Val NH), 4.66 (m, 1H, Phe C\(\alpha\)H), 4.50 (dd, \(J = 7.0\) Hz & \(J = 3.5\) Hz, 1H, Pro C\(\alpha\)H), 4.33 (m, 1H, Leu C\(\alpha\)H), 4.22 (m, 1H, Val C\(\alpha\)H), 4.01-3.96 (m, 1H, Pro C\(\delta\)H), 3.65 (s, 3H, LeuOMe), 3.54-3.46 (m, 4H), 3.07-3.00 (dd, \(J = 13.9\) Hz & \(J = 11.7\) Hz, Phe C\(\beta\)'H), 2.09-2.06 (m, 1H, Val C\(\beta\)H), 1.96-1.91 (m, 1H), 1.78-1.74 (m, 2H), 1.56-1.50 (m, 1H), 1.38-1.34 (m, 1H), 1.12 (d, \(J = 6.6\) Hz, 3H), 1.03 (d, \(J = 6.9\) Hz, 3H), 0.80 (d, \(J = 6.3\) Hz, 3H), 0.70 (d, \(J = 6.0\) Hz, 3H) Mass (CI method): 619 ((M+H)+24), 390 (100).

Preparation of N-cinnamoyl-d-Ala-Pro-Phe-Leu methyl ester (12):

The title compound (867 mg, 47 %) was obtained following the general procedure (F) using (700 mg, 3.19 mmol) of N-cinnamoyl-d-Val, (1.22 g, 3.19 mmol) of Pro-Phe-LeuOMe, (518 mg, 3.83 mmol) of HOBt and (917 mg, 4.78 mmol) of EDC.HCl.
IR (Neat); 3297, 3014, 2963, 1738, 1660, 1629, cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.63 (d, \(J = 15.6\) Hz, 1H, cinnamoyl C\(\beta\)H), 7.51-7.18 (m, 11H), 6.87 (d, \(J = 8.0\) Hz, 1H, LeuNH), 6.43 (d, \(J = 15.6\) Hz, 1H, cinnamoyl C\(\alpha\)H), 6.10 (d, \(J = 5.0\) Hz, 1H, \(^{\text{D}}\)Ala NH), 4.67-4.60 (m, 1H, Phe C\(\alpha\)H), 4.60-4.57 (m, 1H, \(^{\text{D}}\)Ala C\(\alpha\)H), 4.49 (dd, \(J = 14.0\) Hz and \(J = 4.3\) Hz, 1H, Phe C\(\beta\)H), 4.41-4.38 (m, 1H, Leu C\(\alpha\)H), 3.87-3.80 (m, 1H, Pro C\(\delta\)H), 3.66 (s, 3H, LeuOMe), 3.47-3.40 (m, 2H) 3.11 (dd, \(J = 14.0\) Hz and \(J = 11.3\) Hz, Phe C\(\beta'\)H), 1.99-1.97 (m, 2H), 1.80 (m, 1H, Pro C\(\gamma\)H), 1.54-1.50 (m, 2H), 1.44-1.42 (m, 2H), 1.40 (d, \(J = 7.0\) Hz, 3H), 0.80 (d, \(J = 6.3\) Hz, 3H, Leu C\(\delta\)H), 0.71 (d, \(J = 6.3\) Hz, 3H, Leu C\(\delta'\)H), mass (CI method): 591 ((M+H\\(^+\)), 100).

**Preparation of Cinnamoyl-Val-trans-4-OH-Pro-Phe-Leu methyl ester (16)**

Using general procedure (F) (210 mg, 0.40 mmol) of trans-4-[\(\text{t}-\text{butyldimethylsilyl}\)-oxy]-L-Pro-Phe-Leu methyl ester, (100 mg, mmol) of \(N\)-Cinnamoyl-d-valine were coupled using (66 mg, 0.48 mmol) of HOBT and (116 mg, 0.60 mmol) of EDC.HCl in dry CH\(_2\)Cl\(_2\) to afford (120 mg, 39 %) of title product as fluffy hygroscopic solid. This was transformed to the tile compound (69 mg, 68 %) by reacting with TBAF (1M Solution in THF) at 0°C for 1h.
IR (Neat); 3419, 2925, 1742, 1656, 1624 cm⁻¹; \(^1\)H NMR (400 MHz, CDCl₃): \(\delta\) 7.58 (d, \(J = 15.8\) Hz, 1H), 7.46 - 7.42 (m, 2H), 7.36 - 7.31 (m, 3H), 7.24 - 7.17 (m, 5H + 1H), 7.03 (d, \(J = 7.2\) Hz, 1H), 6.46 (d, \(J = 15.6\) Hz, 1H), 6.30 (d, \(J = 8.0\) Hz, 1H), 4.65 - 4.61 (m, 1H), 4.59 - 4.54 (m, 2H), 4.51 - 4.45 (m, 2H), 4.20 - 4.17 (m, 1H), 3.66 (s, 3H), 3.63 - 3.59 (m, 1H), 3.14 (dd, \(J = 13.9\) Hz and \(J = 6.2\) Hz, 1H), 3.06 (dd, \(J = 13.8\) Hz and \(J = 7.8\) Hz, 1H), 2.11 - 2.08 (m, 3H), 1.56 - 1.40 (m, 4H), 0.99 - 0.84 (m, 6H), 0.84 - 0.82 (m, 6H); Mass (EI method): 634 (M, 32), 406 (93), 131 (100).

Preparation of \(N\)-cinnamoyl-d-Val-Ala-Phe-Leu methyl ester (18):

The title compound (935 mg, 39%) was obtained following the general procedure (F) using (1 g, 4.12 mmol) of \(\text{N}\)-cinnamoyl-d-Val, (1.46 g, 4.12 mmol) of Pro-Phe-LeuOMe, (668 mg, 4.94 mmol) of HOBt and (1.18 g, 6.19 mmol) of EDC.HCl.

\(^1\)H NMR (400 MHz, CDCl₃): \(\delta\) 8.21 (d, \(J = 7.6\) Hz, 1H, LeuNH), 8.10 (m, 1H, AlaNH), 7.28 (d, \(J = 8.6\) Hz, Phe NH), 7.56 - 7.52 (m, 1H, cinnamoyl CβH), 7.42 - 7.34 (m, 5H), 7.23 - 7.16 (m, 5H), 6.82 (d, \(J = 15.6\) Hz, cinnamoyl CαH), 4.56 - 4.52 (m, 1H, Phe CαH), 4.33 - 4.20 (m, 3H), 3.60 (s, 3H), 3.00 (dd, \(J = 11.9\) Hz, and \(J = 4.1\) Hz, Phe CβH), 2.80 (dd, \(J = 14.1\) Hz, and \(J = 11.8\) Hz, Phe Cβ’H), 2.00...
Preparation of N-cinnamoyl-d-Val-Pro-d-Phe-Leu methyl ester (21)

The title compound (470 mg, 39 %) was obtained following the general procedure (F) using (480 mg, 1.94 mmol) of N-cinnamoyl-d-Val, (745 mg, 1.94 mmol) of Pro-d-Phe-LeuOMe, (410 mg, 3.04 mmol) of HOBt and (730 mg, 3.80 mmol) of EDC.HCl as a gum.

IR (Neat); 3297, 2959, 1655, 1207, cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.6 (d, J = 15.6 Hz, 1H), 7.52-7.49 (m, 2H), 7.36-7.34 (m, 3H), 7.27-7.10 (m, 7H), 6.56 (d, J = 7.8 Hz, 1H), 6.51 (d, J = 15.6 Hz, 1H), 4.66-4.64 (m, 1H), 4.59-4.55 (m, 2H), 4.39-4.37 (m, 1H), 3.72-3.68 (m, 1H), 3.68 (s, 3H), 3.17-3.15 (m, 1H), 3.03-3.01 (m, 1H), 2.10-2.04 (m, 3H), 1.94-1.86 (m, 1H), 1.48-1.45 (m, 5H), 1.02-1.00 (m, 6H), 0.82 (d, J =6.2 Hz, 3H), 0.80 (d, J = 6.1 Hz, 3H) Mass (CI method): 619 ((M+H)⁺, 100), 390 (90).
5.7 Spectral Data

Spectrum 1: $^1$H NMR spectrum of peptide 4 in CDCl$_3$

Spectrum 3: $^1$H NMR spectra of peptide 5 in CDCl$_3$

Spectrum 4: Mass spectrum of peptide 5
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Spectrum 5: $^1$H NMR spectrum of peptide 9 in CDCl$_3$

Spectrum 6: Expanded NOESY spectrum of 9

1-Phe NH$\rightleftharpoons$ Val C$\alpha$H, 2-Leu NH$\rightleftharpoons$ Pro C$\alpha$H, 3-Acryl C$\beta$H$\rightleftharpoons$ Phe C$\beta$(Pro-$S$)H).
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Spectrum 7: $^1$H NMR of peptide 10 in CDCl$_3$

Spectrum 8: $^1$H NMR of peptide 10 in DMSO-d$_6$
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Spectrum 9: Expanded TOCSY spectrum of peptide 10

Spectrum 10: Expanded NOESY spectrum of peptide 10

1-Leu NH / Pro CαH, 2-Phe NH / Val CαH, 3-Crot CβH / Phe Cβ(Pro-S)H.
Spectrum 11: $^1$H NMR spectrum of peptide 11 in CDCl$_3$

Spectrum 12: $^1$H NMR spectrum of peptide 11 in DMSO-d$_6$
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Spectrum 13: Expanded TOCSY spectrum of peptide 11

Spectrum 14: Expanded NOESY spectrum of peptide 11
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Spectrum 15: $^1$H NMR spectrum of peptide 12 in CDCl$_3$

Spectrum 16: $^1$H NMR spectrum of peptide 12 in DMSO-d$_6$
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Spectrum 17: Expanded TOCSY spectrum of peptide 12

Spectrum 18: Expanded NOESY spectrum of peptide 12

1-Phe NH / $^3$Ala CαH, 2-Leu NH / Pro CαH, 3-Cinn CβH / Phe Cβ(Pro-S)H.

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Spectrum 19: $^1$H NMR of peptide 16 in CDCl$_3$

Spectrum 20: Mass spectrum of peptide 16
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Spectrum 21: $^1$H NMR of peptide 18 in CDCl$_3$

Spectrum 22: $^1$H NMR spectrum of peptide 21 in CDCl$_3$