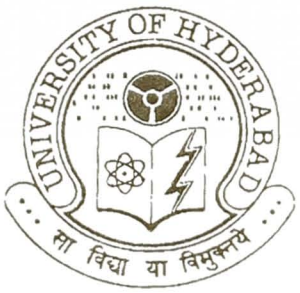
Ring Closing Metathesis Approach To Cyclic β-turn Mimics And Glycon Component Of Novobiocin

A Thesis
Submitted for the Degree of
Doctor of Philosophy

By
Rajesh B. M.



School of Chemistry
University of Hyderabad
Hyderabad 500 046
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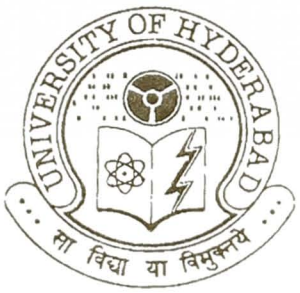

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STATEMENT

I hereby declare that the matter embodied in this thesis entitled "**RING CLOSING METATHESIS APPROACH TO CYCLIC β-TURN MIMICS & GLYCON COMPONENT OF NOVOBIOCIN**" is the result of investigations carried out by me in Dr. Reddy's Laboratories Ltd, Discovery Research, Bollaram Road, Miyapur, Hyderabad and the School of Chemistry, University of Hyderabad, Hyderabad, INDIA, under supervision of Prof. Javed Iqbal and Prof. Ashwini Nangia.

In keeping with the general practice of reporting scientific observations, due acknowledgements have been made wherever the work described is based on findings of other investigators.

Hyderabad
Sept 2008

Rajesh B. M.

CERTIFICATE

This is to certify that the work carried out in the thesis entitled "**RING CLOSING METATHESIS APPROACH TO CYCLIC β-TURN MIMICS & GLYCON COMPONENT OF NOVOBIOCIN"** has been carried out by Mr. Rajesh B.M. under our supervision and the same has not been submitted elsewhere for a degree.

Dean
School of Chemistry

Prof. Javed Iqbal
Thesis Supervisor

Prof. Ashwini Nangia
Thesis Supervisor

ACKNOWLEDGEMENT

Words will be inadequate to express my indebtedness and deep sense of gratitude to my thesis supervisor **Prof. Javed Iqbal**. It is his indispensable guidance, excellent teaching, everlasting encouragement and affection that make the present dissertation possible. The amount of knowledge that I have received from him through every day's discussions is beyond my quantification. I have learnt a lot from him, not only chemistry but about almost every aspect of life. *I do feel proud to be his student*.

I would like to record my sincere thanks to **Prof. Ashwini Nangia**, my thesis supervisor for helping me a great deal in understanding the crystal structures presented in this thesis and in shaping my thesis. He has a pride of place in my scheme of things. *I owe my heart felt gratitude to you Dear Sir!*

I thank Prof. Basavaiah, **Dean**, School of Chemistry, former Deans Prof. M. Periasamy, Prof. E. D. Jemmis, and faculty for their cooperation in providing facilities in the School.

I will always be indebted to Dr. Reddy's Research Foundation for funding my research and Dr. Reddy's Laboratories Ltd for enabling me to use the infrastructure facilities.

I wish to thank **Dr. A. Venkateswarulu** and **Dr K. Vyas** for always encouraging me to pursue my Doctoral studies. I also wish to sincerely thank **Dr. Debnath Bhuniya** for permitting me to do my PhD work in his Laboratory. My thanks are also due to **Dr. D. Srinivasa Reddy** for valuable and critical inputs in chapter VI of this thesis. I am also thankful to Dr. Moses Babu and Vasudev (Analytical department, Dr. Reddy's Laboratories Ltd. Discovery Research) for analytical data. *I will always consider my association with all as a rewarding experience in my career*.

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Being a stickler to niceties, I know that it will be very difficult to make amends later, should I fail to acknowledge anybody's contribution in this Mammoth exercise extending over many years. But, human memory is short and mine is shorter still. Hence, for those people, whose names I have inadvertently left out, I offer my sincerest apologies.

Rajesh B. M.

Synopsis

This thesis entitled "**RING CLOSING METATHESIS APPROACH TO CYCLIC β-TURN MIMICS & GLYCON COMPONENT OF NOVOBIOCIN**" consists of six chapters.

Chapter I

Overview of Peptides and Peptidomimetics

The successful design of peptidomimetics to counteract the detrimental properties inherent with the usage of peptides as drugs followed only after the pioneering work of Ramachandran and co-workers who demonstrated that only limited conformation space was available to most α-amino acids (except glycine) and that the accessible low-energy conformations were the α-helix, β-sheets, extended structures, and β-turns. Since then various efforts to design conformational constraints that bias peptides to one of these low-energy conformations have been successful in eliciting biological activity. The synthesis of conformationally locked Pro containing cyclic peptides mimicking turn motifs using ring closing metathesis is explored in this thesis.

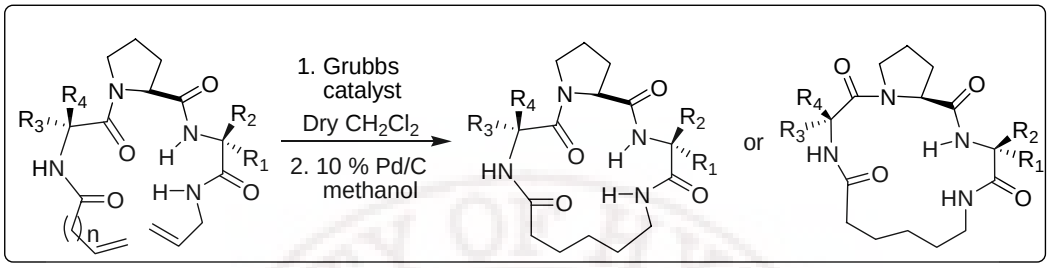
Chapter II

Synthesis of Type VI β-turn Containing Cyclic Tripeptides Using RCM:

The type VI β-turn plays important roles in protein folding and has been implicated in other important recognition events of bioactive peptides. With proline at *i*+2 position, we have synthesized set of tripeptides having the general sequence Xaa-Pro-Yaa residues (where Xaa & Yaa = Gly, D-amino acid or L-amino acid) with olefinic segments in the form of pentenoyl and allyl at N- and C-terminals respectively so as to enable RCM reaction as shown in scheme-**1**. A systematic study about the side chain requirements needed for RCM cyclization reaction led us to generalize that homochiral tripeptides of general formula *N*-pentenoyl-Xaa-Pro-Yaa allylamide nucleates a type VI β-turn, while heterocyclic

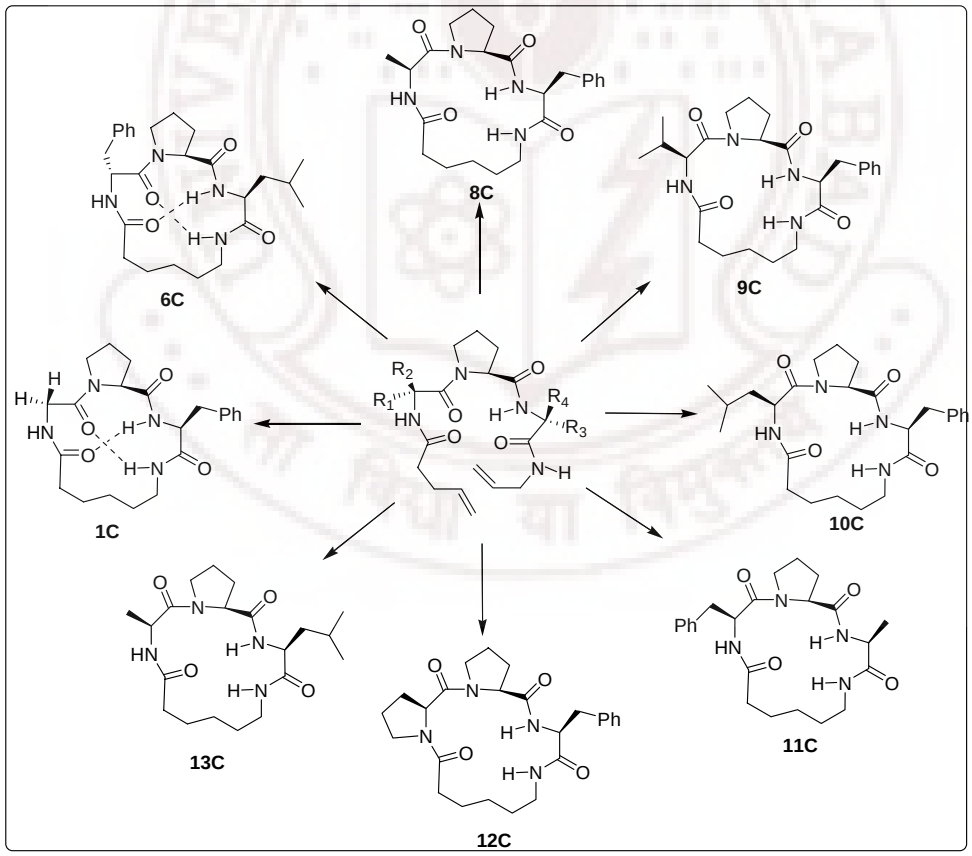
tripeptides with D-amino acid or Gly at *N*-terminal to Pro nucleates a 3_{10} helical geometry.

Scheme-**1** *RCM approach to cyclic peptides*



The following are some of the peptides that were synthesized and its conformation have been studied in this chapter (scheme-**2**).

Scheme-**2** *Some cyclic peptides synthesized and discussed in chapter II*



Chapter III Synthesis and Conformation of β-amino acid Derived Small Cyclic Pseudo 3_{10} Helical/β-Turn Structures *via* RCM

The imide bond between Tyr/Phe-Pro residues is an unique site for hydrolysis by retroviral proteases and are unusual for mammalian proteases. Hence, the design of a molecule, mimicking the stereoelectronic environment of this scissile bond may lead to potent inhibitors based on structural mimicry as shown in figure-**1**. The following cyclic peptides were synthesized and its conformation has been studied in chapter-III (scheme-**2**)

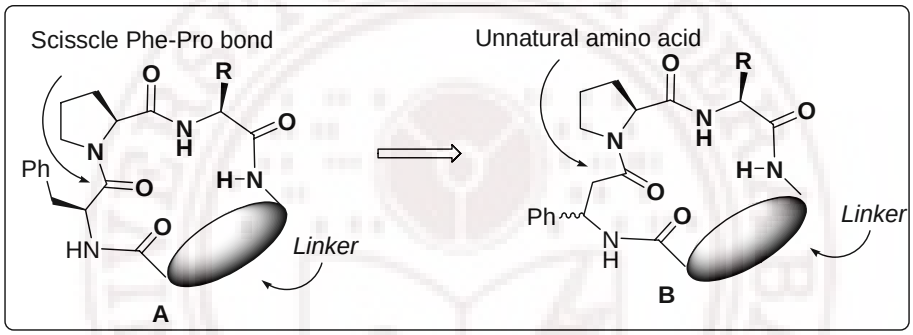
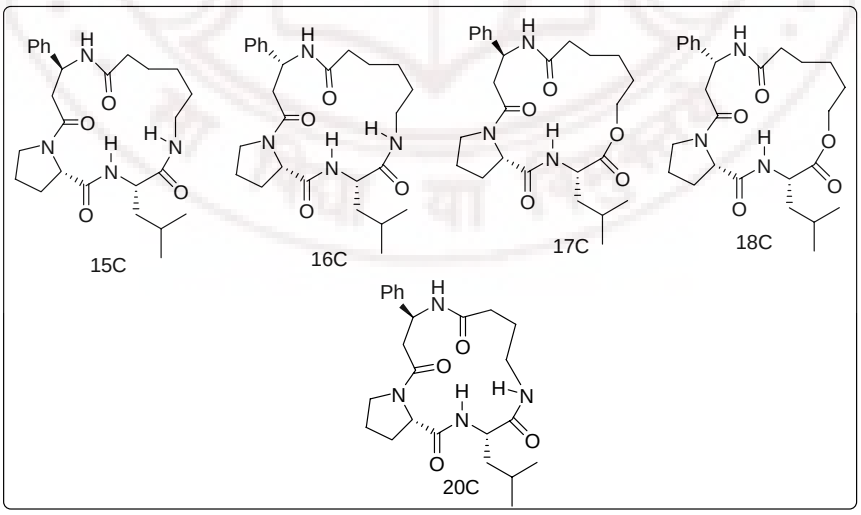


Figure-**1**: *Modification of Phe-Pro bond with β-amino acid-Pro bond*

Scheme-**3**: *β-amino acid containing cyclic peptides discussed in chapter-III*



Chapter 1V

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

Based on the specificity of HIV protease towards cleaving the Phe-Pro bond, we reasoned that incorporation of dehydrophenylalanine, a constrained phenylalanine mimic in the place of Phe in **A** may possess high stereo electronic complementarity resulting in better recognition by the protease (figure-**2**). Both the geometrical isomers of Δ-Phe were synthesized and following cyclic peptides were synthesized and studied in chapter IV (scheme-**4**)

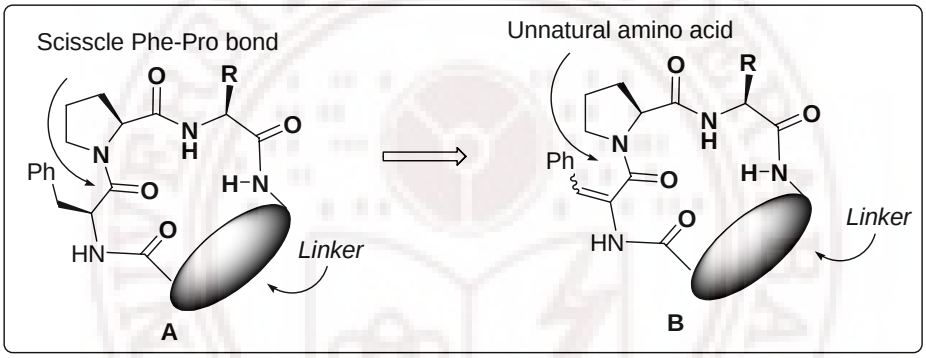
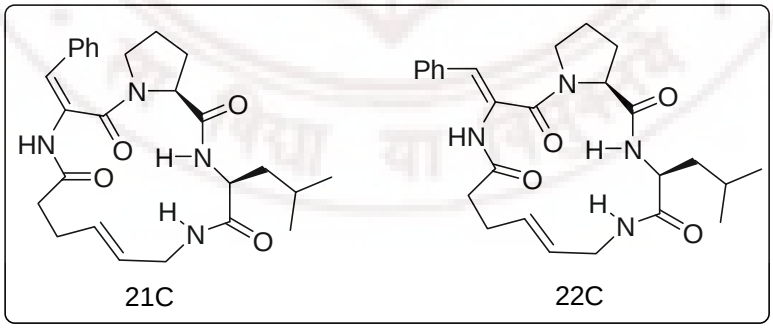


Figure-**2**: *Modification of Phe-Pro bond with ΔPhe-Pro bond*

Scheme-**4** *Δ-Phe containing cyclic peptides discussed in chapterIV*

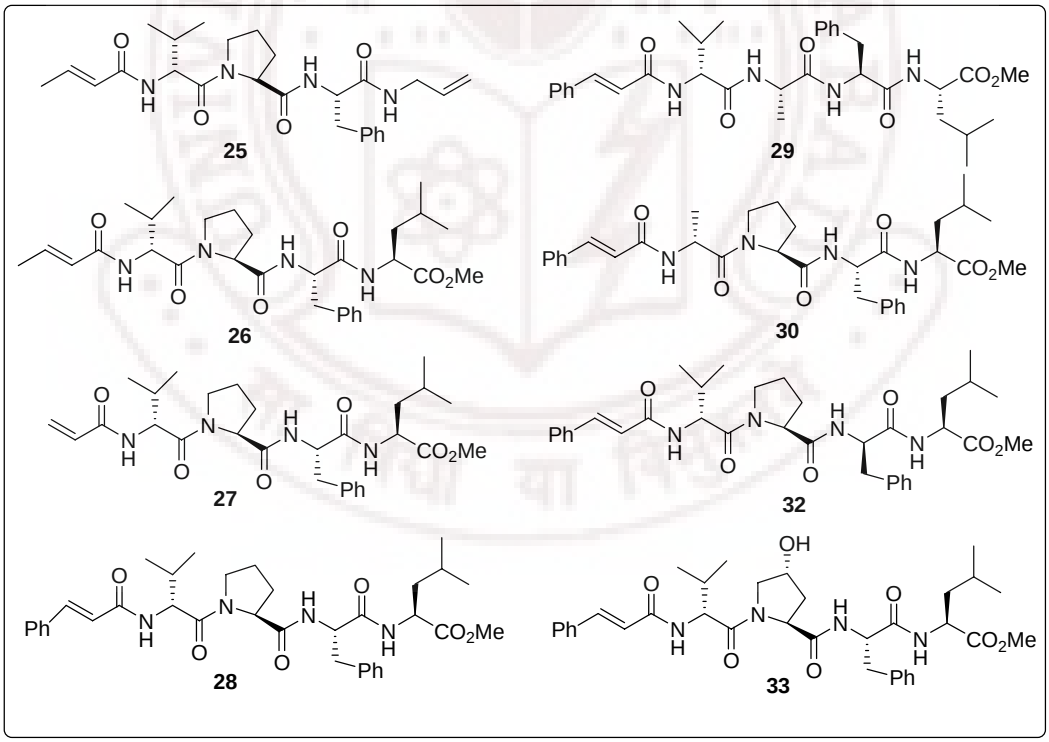


Chapter V

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

The CH/π interactions are considered to be one of the weakest but significant interactions in proteins. A data base study has shown that strong CH/π interactions are observed when an aromatic amino acid residue precedes or follows Pro, which enhance the stability of the system. To understand the role of such interactions in proteins, we have designed Pro-Phe containing tri and tetrapeptides which nucleate 3_{10} helical geometry and are stabilized by CH/π interactions. The following peptides (scheme-**5**) were synthesized and its conformation has been discussed in this chapter.

Scheme-**5** *Tri and Tetrapeptides discussed in chapter V*



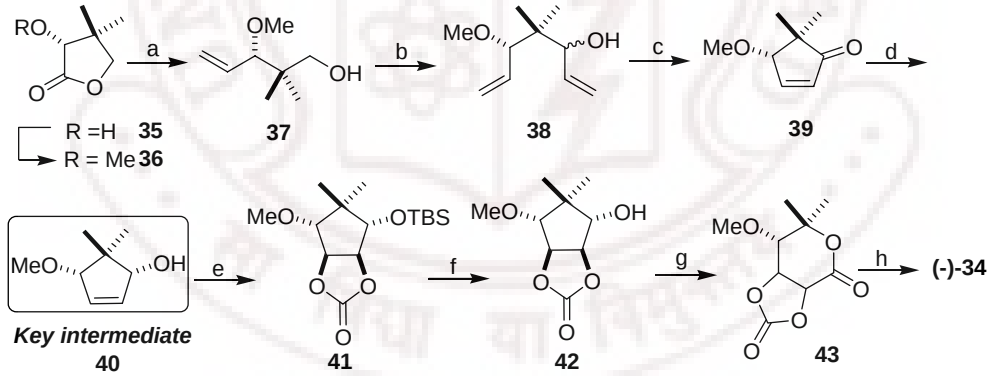
Two CH/π interactions (one between Pro CγH and Phe and the other between π-bond of α,β-unsaturated sy and Phe CβH) were found to be responsible for the folding of the peptides based on NMR studies.

Chapter VI

Enantiodivergent Strategy to Access Both (+) and (–) Novioses from a Single Enantiomer of Pantolactone

Novobiocin is an important antimicrobial and antitumor agent that has been shown to inhibit DNA gyrase and HSP90. It contains a rare sugar moiety called noviose. Most of the approaches towards the synthesis of noviose/noviose derivatives reported till date emanate from sugars or sugar derived scaffolds capable of producing single enantiomer. During an enantiospecific approach to D-(–)-noviose starting from commercially available (–)-pantolactone (a non-sugar scaffold) we identified a key intermediateas shown in scheme-**6**.

Scheme-6 *Synthesis of (–)-Noviose from (–)-Pantolactone*

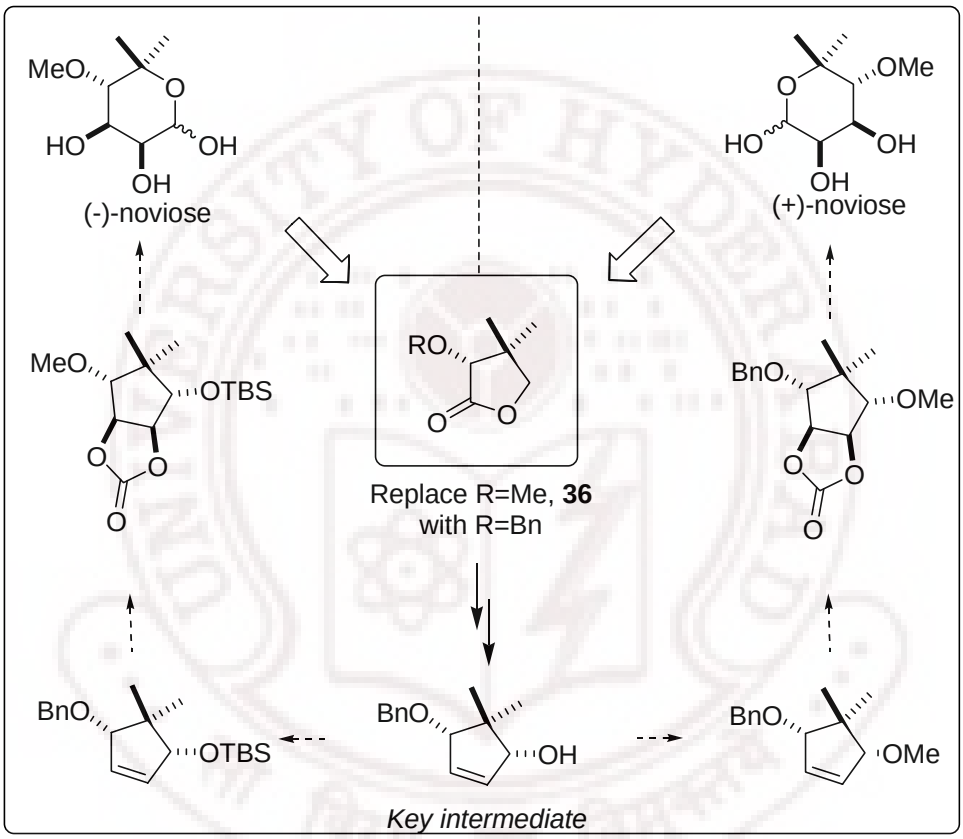


Reagents and conditions: (a)i. Ag_2O, MeI, ACN 58 ^oC ii. DIBAL-H, -78 ^oC, DCM iii. n-Buli, methyl triphenyl phophonium bromide, Dry THF, -78 ^oC (b) i. $(COCl)_2$, DMSO, DCM, -78 ^oC, ii. vinyl magnesium bromide, Dry THF, 0 ^oC (c) i. Grubb's catalyst 5 mol %, DCM, rt. ii. Jones reagent, Et_2O, rt (d) $NaBH_4$, $CeCl_3.7H_2O$, 0 ^oC, (e) i. TBSCl, imidazole, 0 ^oC, ii. OsO_4, NMO, acetone, H_2O, t-BuOH, rt, iii. triphosgene, 0 ^oC - rt; (f)TBAF, THF, (g) i. PDC, DCM; ii. m-CPBA, DCM, 3 days, RT, for two steps; (h) DIBAL-H (known)

Commercailly available (–)-pantolactone had served as a starting material for our synthetic approach to (–)-**34**. Unfortunately, the (+)-enantiomer of pantolactone is very expensive, We incorporated a labile group in place of methyl in intermediate

36 and transformed it to the key intermediate (scheme-**7**), whose masked symmetry was exploited for the synthesis of either (+) or (–)-noviose through selective modifications on either side of the molecule as shown in scheme-**7**. The enantiodivergent strategy is discussed in this chapter.

Scheme-7 *Enantiodivergent strategy to access (+) or (–)-noviose*



CONTENTS

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CHAPTER FOUR

Synthesis and Conformation of Δ-Phe derived Small Cyclic Peptides *via* RCM 247-280

Chapter-I

Introduction to Peptides and Peptidomimetics

1.1 Overview of Peptides

Proteins are at the center of action in biological processes. They function as enzymes, which catalyze the complex set of chemical reactions that are collectively referred to as *life*. Proteins serve as regulators of these reactions, both directly as components of enzymes and indirectly in the form of chemical messengers, known as hormones, as well as receptors for those hormones and hence there is a considerable validity that proteins are the “building blocks” of life.

The construction of complex protein folds relies on the precise conversion of a linear polypeptide chain into a compact 3-dimensional structure. They are synthesized by the sequential addition of activated amino acids to the growing peptide chain and gradually fold up into the structure of the native protein. The structural description of proteins has been traditionally described in terms of four levels of organization. The primary structure represents the linear arrangement of the individual amino acids in the protein structure, while the secondary structure represents the local architecture of linear segments of the polypeptide chain, without regarding the conformations of the side chains. The tertiary structure portrays the overall topology of the folded polypeptide chain and the quaternary structure describes the arrangements of the separate subunits or the monomers into the functional proteins (Figure-**1**).

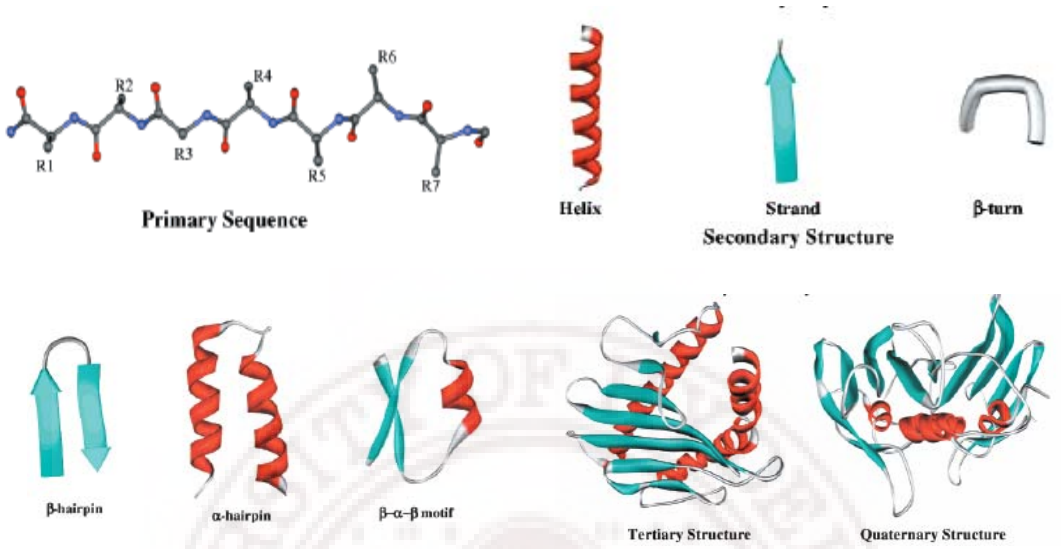


Figure-**1**: *Various levels of structural organization observed in protein structures*

The diversity of the polypeptide chain folding arises because of the multiple conformations that are energetically accessible at each amino acid residue which is defined by three torsional angles namely ϕ (C′-N-Cα-C′), ψ (N-Cα-C′-N) and ω (Cα-C′-N-Cα) (Figure-**2**). Amongst the three torsion angles ω is fixed either in *cis* conformation or in the *trans* conformation, because of the partial double bond character of the amide bond. However, invariably, the thermodynamically feasible *trans* rotamer predominates in solution. Using simple computational methods, Ramachandran and co-workers demonstrated that only limited conformation space was available to most α-amino acids except glycine (Ramachandran plot [1]) and that the accessible low-energy conformations were the α-helix, β-sheets, extended structures and β-turns.

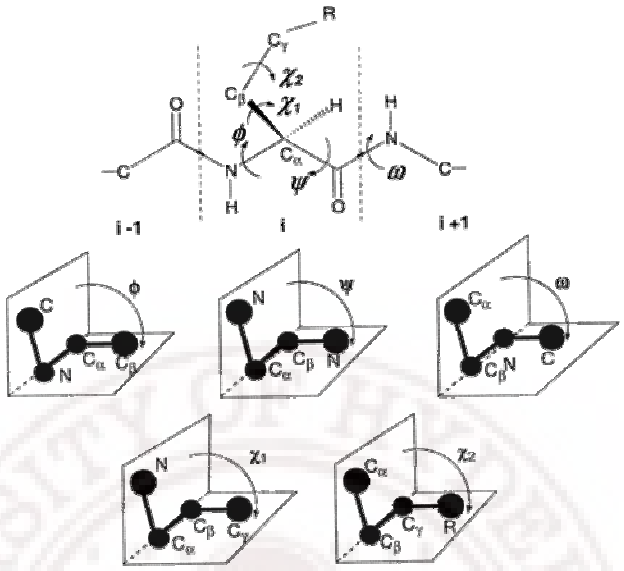


Figure **2**: *Schematic representation of backbone torsion angles ϕ, ψ & ω.*

Helices: Helix formation is the early step in the protein folding, which guides subsequently to the other folding processes. Pauling and Corey proposed in 1951 that helices have the highest propensity amongst the secondary structural elements available in proteins. Comprehensive classification of the helices depending on the number of residues involved per turn or atoms participating in H-bonding, result in 3_{10} helices, α-helices and π-helices. Helix types are usually designated as "x_y" where "x" stands for number of amino acids per helix turn and "y" stands for the number of atoms involve in H-bonding.[2]

α-Helices or 3.6_{13} Helices: In proteins, about 31% of the amino acids are found in α-helices. α-Helices are defined as a repetitive 13-membered intramolecular H-bonding between i^{th} carbonyl oxygen of the i^{th} residue with the amide proton of

the i+4th residue with the standard backbone angles ϕ = -57° and ψ = -47°. The helix rises 5.4 Å per turn or 1.5 Å per residue and contains 3.6 residue per turn, which involves repetitive 13-membered H-bonds (Figure **3**).

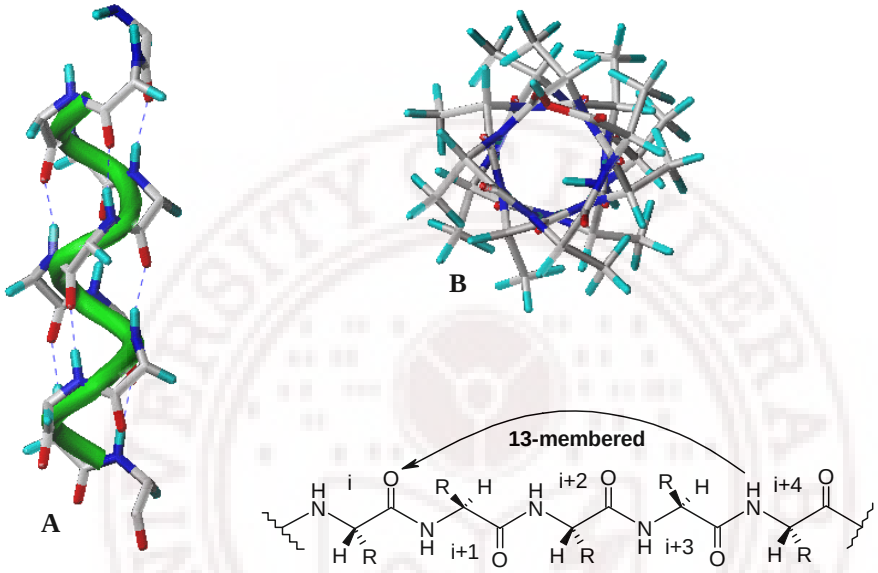
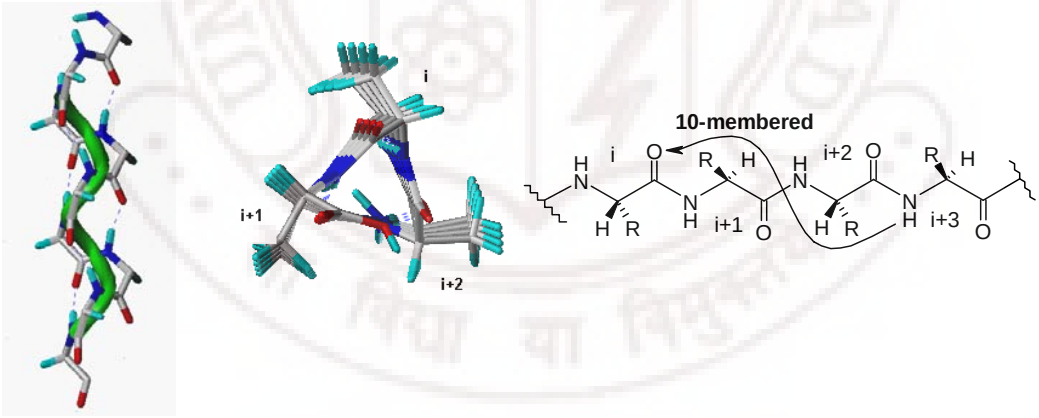


Figure **3**: *Schematic representation of α-Helix: (A) side view (with C-terminal at the top and N-terminal at the bottom), (B) 13 membered H-bonding i (CO) ← i+4 (NH).*

In nature, α-helices are found in right handed form, where as the left handed helices are not allowed energetically because of steric clash between i^{th} residue carbonyl oxygen and the i+4th residue β-carbon. α-Helices play critical determinant role in biological functions.

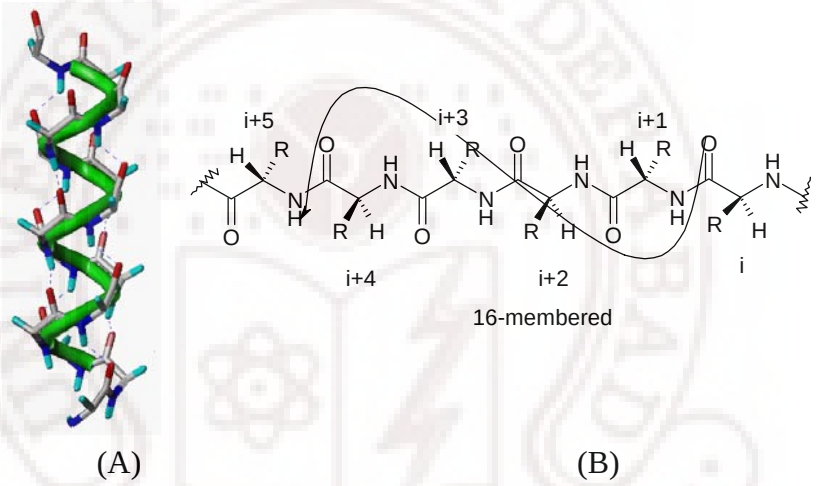
3_{10} Helices: These are rare in proteins and about 4% of amino acids are found with 3_{10} helical conformations with the standard backbone angles ϕ = -60° and ψ = -30° along with 3 residues per turn and repetitive 10 membered intra molecular H-bonding between carbonyl oxygen of the i^{th} residue and amide proton of the i+3rd residue (Figure **4**). Thus, a helix is generated in which the i^{th} and i+3rd α-carbon atoms and side chains align exactly on top of each other. Steric clash between the side chains is the primary cause for the rare occurrence of 3_{10} helical structures in proteins. In 3_{10} helical structures also all amide protons point toward the N-terminus (down) and all carbonyl oxygens point toward the C-terminus (up).



A **B** **C**

Figure **4**: *Schematic representation of 3_{10}-Helix: (A) side view, (B) top view and (C) 10 membered H-bonding i (CO) ← i+3 (NH).*

π-Helices or 4.4_{16} helices: They appear to be extremely rare and unstable, because of their unfavorable ϕ and ψ dihedral angles of -76° and -41° respectively, lying at the edge of the allowed minimum energy region of the Ramachandran plot. The π-helices have repetitive intra molecular H-bonds between carbonyl oxygen of the i^{th} residue to amide proton of the i+5^{th} residue with 16 membered intra molecular H-bondings (Figure **5**).



(A) (B)

Figure **5**: *Schematic representation of π-Helix: (A) side view (B) 16 membered H-bonding i (CO) ← i+5 (NH).*

The helix rise 1.2 A° per residue and has 4.4 residues per turn with an average helix twist of 83° per residue. The data base study has shown that each and every tenth proteins contain a π-helix. It has been found that the residue having large side chains such as Phe, Tyr, Ile, Leu and Met have high propensity

of being at the both terminals of the helix. Polar residues such as Asn, Glu, Thr and Ser are preferred to be at the middle of the helix.[3]

β-sheet structures: These are the second most abundant (~28%) secondary structural elements in proteins, which was first proposed by Linus Pauling and Robert Corey in 1951 along with the helix. Based on the direction of the peptide chain they are classified as parallel and anti parallel β-sheets. In globular proteins, β sheets are found with inter strand intra molecular H-bonding, which result in a compact structure of proteins. β sheets are made up of fully extended conformation with 3.2-3.4 Å/residue and the Cα-Cα distances between the two adjacent strands of about 3.5 Å, along with the backbone angles lying in the β region of Ramachandran plot. For an anti parallel sheet structures the average backbone angles are ϕ = -139° and ψ = +135° where as in the parallel sheet structures ϕ = -119° and ψ = +113° (Figure-**6**)[4] β sheet structures play significant role in biological processes.

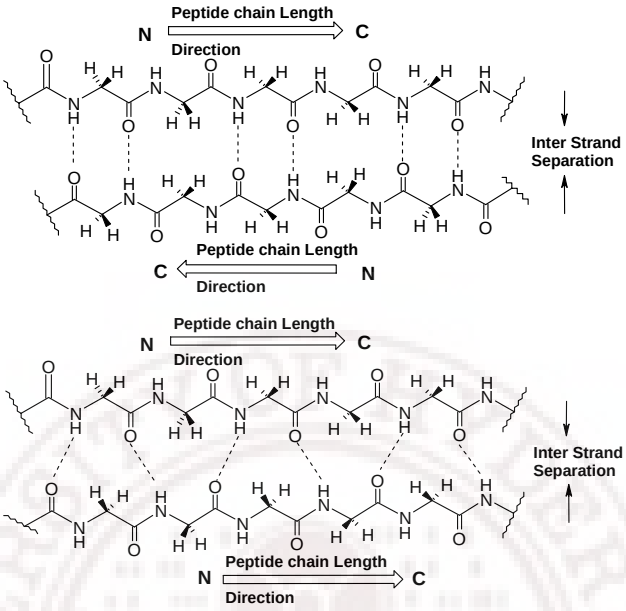


Figure **6**: *Schematic representation of anti parallel and parallel β-sheets.*

Turns are the third most frequently observed secondary structural elements in proteins and peptides after helices and β-sheets. Turns are located primarily on the protein surface and accordingly contain polar and charged residues. Antibody recognition, phosphorylation, glycosylation, hydroxylation, and intron/exon splicing are found frequently at or adjacent to turns. However it is not clear if this is due to specific recognition or the surface location of turn conformations. Turns or loops are responsible for the reversal of polypeptide chains by about 180° to get a compact structure and reduce the size of the proteins. The comprehensive studies of the turns based on the number of residues involved has revealed three classes, such as β-turns, γ-turns and ϕ-turns.[5]

β-Turns are the ubiquitous secondary structural elements of the polypeptides and invariably, locate themselves at the surface of the proteins. According to Venkatachalam who first identified β- turns in 1968,[5c] a β-turn can be defined for four consecutive residues denoted as *i*, *i*+1, *i*+2, *i*+3 and the distance between the Cα of i^{th} residue and the Cα of $i+3^{rd}$ residue must be less than 7 A° and backbone dihedral angles of the central two residues must not be in the helical region of Ramachandran plot. A 10 membered H-bond between the carbonyl of i^{th} residue and amide proton of the $i+3^{rd}$ residue is a secondary requirement. Thus, the β-turns can exist without a 10 membered H-bond. Careful analysis of 205 high-resolution crystal structures of proteins revealed 3,899 β-turns. Based on the central two residues dihedral angles, β-turns has been classified as follows (Table **1**)

Table-I β-*turn classification based on* ϕ, ψ *angles.*

Turn	ϕi+1	ψi+1	ϕi+2	ψi+2
I	-60	-30	-90	0
I’	60	30	90	0
II	-60	120	80	0
II’	60	-120	-80	0
III	-60	-30	-60	-30
VIa1	-60	120	-90	0
VIa2	-120	120	-60	0
VIb1	-135	135	-75	150
VIb2	-135	90	-75	150
VIb3	-90	180	-75	150

γ-Turns: In proteins, the location of the γ-turns is not so easy, because of the lack of well-defined conformational signatures. A γ-turn can be defined for three consecutive residues denoted as *i*, *i*+1, *i*+2. γ-Turns can exists in two forms in proteins, such as classical γ-turn and reverse γ-turn. By definition γ-turns are small loops, which involves only three residues by forming 7-membered intra molecular hydrogen bonding between the carbonyl of i^{th} residue and the amide proton of $i+2^{nd}$ residue with the typical back bone dihedral angles of the central residue of ϕ_{i+1}= 75° and ψ_{i+1}= -64° for classical γ-turn and ϕ_{i+1}= -79° ψ_{i+1}= 69° for inverse γ-turn.

π-turns: π-turns contain 16 membered intra molecular H-bonding between the amide proton of $i+5^{th}$ residue with the carbonyl of i^{th} residue. They are very rare and uncommon because of more conformational flexibility in the amide chain. They are usually located at the C-terminal end of the alpha helices. Further π-turns has been classified in three classes such as $\pi_{\alpha L}$-turn, $\pi_{\alpha R}$-turn and π_{β}-turns.

1.2 Recent advances in Peptidomimetics

The critical roles that peptides & proteins play at virtually all levels of biological regulation ranging from bio-catalytic processes to the transformation of information between cells have opened limitless options for therapeutic intervention. A wide variety of naturally occurring bioactive peptides have been discovered which play a pivotal role in many physiological pathways. Understanding the bioactive conformation of ligands as they are bound to their targets has been the impetus behind much of rational drug design. However, with some notable exceptions, the therapeutic applications of proteinaceous species have been severely limited because of poor bioavailability, protease degradation and clearance. Attempts towards counteracting such detrimental properties by various research groups has helped us to realize that, amino acid sequence is not the only determinant for biological activity, but the secondary structure is also important. The retention of these peptide secondary structures by mimetism has become an important tool and is applied with the goal of fixing these bioactive conformations in a small peptide molecule. Molecules designed and synthesized in such a way are often referred to as ***peptidomimetics***. Some of the major approaches adopted for the mimetism of secondary structural motifs are given below.

1.2.1 Use of non amino acids

This is one of the widely used ways to generate the secondary structural motif in small peptides. Several examples have figured out where a suitable non amino acid segment is used to generate β-strand or β-sheet like arrangements in small peptides. One of the early works was done by Kemp *et al*,[6] who used 2, 8-diaminoepidolidione to nucleate β-sheet formation. This planar aromatic compound served as a template to induce an adjacent strand to form β-sheet like conformation Smith, Hirschman and co-workers studied 3,5-linked pyroline-4-ones as β-strand mimics.[7] These groups of peptidomimetics were studied as HIV protease inhibitors, which has a rigid conformation and duplicates the main chain conformation and side chain placement of β-sheets. Several designs of β-hairpin structure have also been reported. Kelly and co-workers used 4-(2'-aminoethyl)-6-dibenzofuranpropionic acid as a turn nucleator thereby inducing β-sheet hydrogen bonding pattern between adjacent strands. The dibenzofuran moiety not only restricts the movements of amino acid strands but also creates a hydrophobic cluster, which led to the formation of β-hairpin.[8]

1.2.2 Use of unnatural amino acids

In an attempt to expand well-defined two residue loop by insertion of an additional residue without disruption of strand registry, Balaram *et al*[9] reported the successful construction of a peptide hairpin containing a central three residue loop having the sequence ^{D}pro-^{L}pro-^{D}Ala as shown in Figure-7. Their design was based on expanding the two-residue loop established in the peptide β-hairpin Boc-Leu-Phe-Val-^{D}Pro-^{L}Pro-Leu-Phe-Val-OMe thus establishing that expansion of loop size by using two unnatural D-amino acids to nucleate a turn which bias the local conformational choice in a centrally positioned segment.

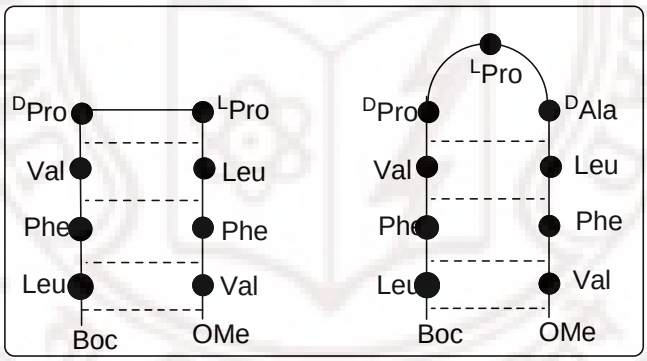


Figure-**7**: *Engineering of larger loops by Balaram et al*

Nowick *et al*[10] in continuation with their studies to coax peptides into well-defined β-sheet like structures introduced a unique amino acid Orn(^{i}PrCO-Hao) consisting of an ornithine residue with β-strand mimicking amino acid Hao

attached to its side chain (figure-**8**), which could be readily incorporated into peptides to make them fold into β-sheet like structures.

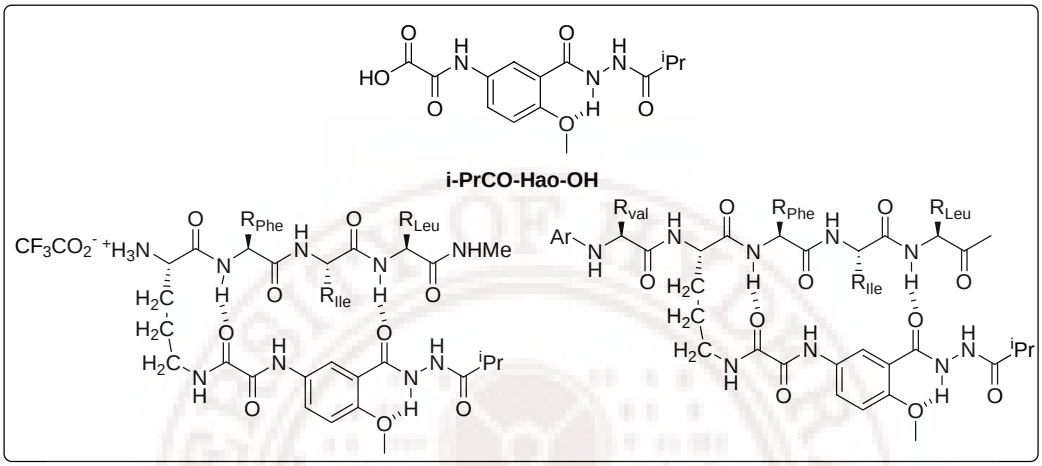


Figure-**8**: *Designed β-sheet structures by Nowick et al*

The development of new unnatural oligomers that mimic natural biopolymers (proteins, nucleic acids and polysaccharides) provide a powerful tool to obtain compounds with potential applications in life sciences, since, biopolymers encompass a broad range of structures and are involved in key aspects of our understanding of life. The pioneering work of Dervan and co-workers[11] on DNA-binding aromatic polyamides based on pyroles and imidazoles provide a glimpse of what can be achieved through sustained multidisciplinary approach to the problem of oligomer design. Since then, foldamers other than aromatic polyamides[12] have also been explored, including nucleic acids with alternative sugar[13] and peptidic backbones.[14] Extensive studies have focused in the recent past on structures that more closely resemble conventional peptides.

Peptides containing ω-amino acids which differ from their α-amino acid counterparts in the number of the C-atoms which separate the two successive peptide linkages have been shown to adopt well defined structural motifs.[15] β-peptides have particular appeal for the synthesis of oligomers because β-amino acids represent the smallest step away from α-amino acids in "backbone space". The 10/12 mixed helix, unique to β-peptides was first reported by Seebech *et al* [16] in β^2/β^3-dipeptide repeats. Subsequently, Gellman and co-workers [17] have reported the formation of 14-helix in β-amino acid oligopeptides (β-peptides) containing conformationally constrained cyclic ACHC residues, while Seebech *et al* showed the formation of such helices in series of β-peptides prepared from acyclic residues with a diverse collection of side chains. Further, Hanessian *et al* [18] has reported the synthesis and study of γ-amino acid oligopeptide and beautifully demonstrated that the folding pattern of the oligopeptide could be controlled by changing the stereochemistry of an α-methyl substituent to give helical or non-helical structures. Adding a new dimension to the design of novel foldamers, Gellman *et al*[19] showed the co-existence of an 11-helix along with a 14/15 helix in the α/β-dipeptide repeats where as Reiser *et al*[20] observed a 13-helix in the α/β-peptides containing *cis*-β-aminocyclopropane carboxylic acid and L-Ala repeats. Off late, the use of cyclic sugar β-amino acids in forming well defined mixed helices has been reported by Kessler *et al*[21] and Sharma *et al*[22].

Expanding the possibility of unnatural amino acids oligomers, recently, Miriam Royo *et al*[23] have developed a synthetic strategy to obtain two new families of γ-peptides formed by the cyclic monomer *cis* γ-amino proline.

1.2.3 Modification of the side chain of a natural amino acid residue

The use of dehydro amino acids in synthesizing peptide analogues with preferred secondary structure is one of the explored methods. Model acyclic peptides containing dehydrophenylalanine (Δ^z Phe) and dehydroleucine (Δ^z Phe) have shown strong tendency to adopt β-turn structures. On the other hand, peptides having 5-8 residues have shown the propensity of Δ^z Phe to form either 3_{10} helical or α-helical conformation depending upon the length of the peptide. For example, the peptide Boc-Val-Δ^zPhe-Phe-Ala-Phe-Δ^zPhe-Val-Δ^zPhe-Gly-OCH_3 was found to nucleate seven consecutive overlapping type III β-turns.[24] The α-aminoisobutyric aid (AIB),[25] also showed similar propensities as dehydro amino acids depending upon the length of the peptide chain.

1.2.4 Constrained bicyclic β-turn dipeptide (BTD) motifs

The replacement of dipeptide component by incorporation of a carboxyl and an amino group in geometrically suitable positions for peptide coupling (figure-**9**) for inducing turns has received much attention in the recent past due to the

success achieved in designing molecules with less peptidic character for therapeutic interventions.

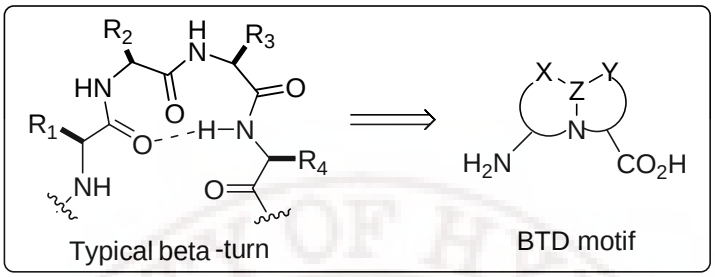


Figure-**9**: *Design of bicyclic β–turn motifs*

The synthesis of a variety of such scaffolds has been reviewed.[26] Recently, Hruby *et al*, identified the core bioactive sequence of novel melanocortin peptide as His-(D/L)-Phe-Arg-Trp having a β-turn structural feature around Phe and Arg residues. This was successfully replaced by dipeptide mimetics, such as azabicyclo[4.3.0]nonane amino acid.[27] In an another interesting study, a small library of external bicyclic β-turn mimetics were designed and synthesized based on Leu-enkephalin, an endogenous opioid peptide whose β-turn conformation around Gly^2-Gly^3 was known to be important for its activity (figure-**10**).[28]

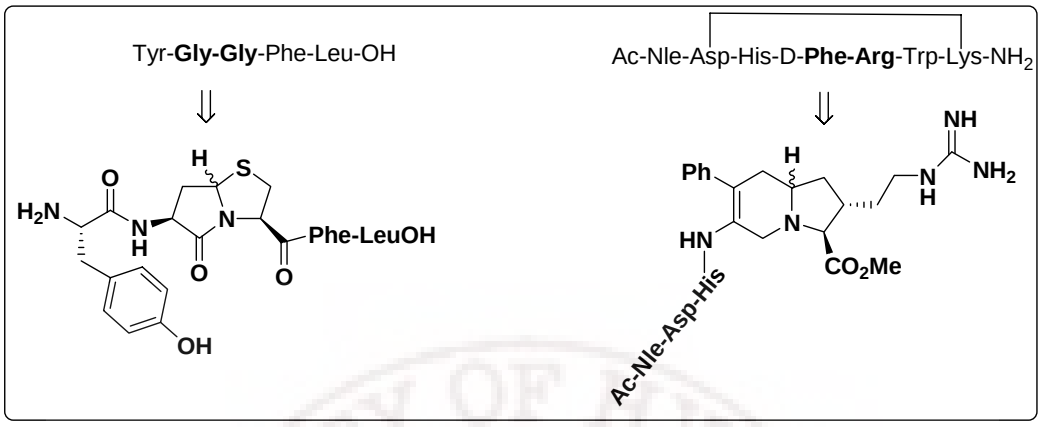


Figure-**10**: *Incorporation of dipeptide mimetics in Melanocortin and Leu-Enkephalin peptides.*

Lubell *et al* [29] in the recent past have used azacycloalkane turn mimics to explore the relationship between conformation and biological activity of peptide ligands to the opioid receptor-like (ORL 1) receptor. In this connection a small library of peptides incorporating dipeptide mimetics (figure-**11**) were synthesized and two antagonists were identified with increased selectivity for the ORL-1 receptor.

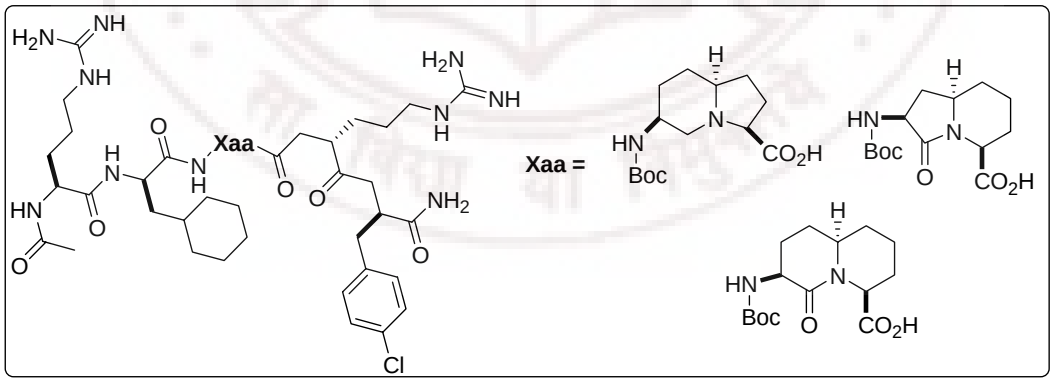


Figure-**11**: *Dipeptide mimics used for the study of ORL-1 receptor*

1.2.5 Cyclization strategy

Off late, Ring Closing Metathesis (RCM) using Grubb's catalyst has been widely used for the preparation of potent bioactive peptides. For example, Grubb's *et al* [30] reported the synthesis of 23-membered macrocyclic helical peptide which was synthesized using RCM involving side chain residues as shown in figure-**12**.

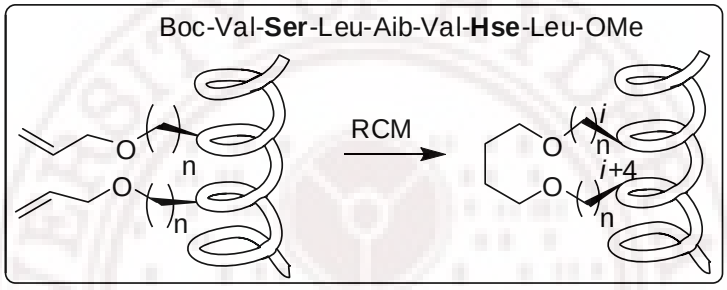


Figure-**12**: *Macrocyclic helical peptide using RCM*

Towards the development of potent and selective HIV protease inhibitors, Fairlie *et al* [31] have reported the synthesis of a series of macrocyclic β-strand mimetics of tripeptides (figure-**13**) having two *trans* amide bonds and a planar aromatic ring with a short non peptidic linker between them.

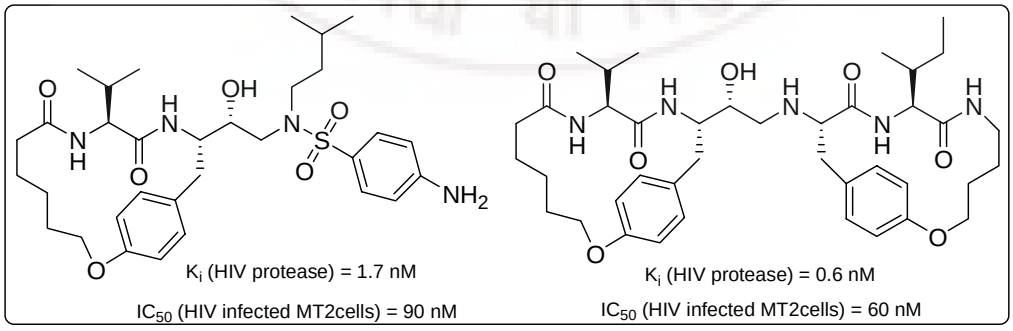


Figure-**13**: β-*strand mimetics as HIV protease inhibitors*

Kevin Burgess *et al*[32] reported the synthesis of several cyclic β-turn peptidomimetics that mimic or disrupt the binding of neurotrophins (eg: nerve growth factors (NGF) and the neurotrophin factor-3 (NT-3) to its transmembrane Trk receptors. The dipeptide units incorporated were chosen to correspond to the turn regions ("hot spots") of the neurotrophins which were locked with a pseudo amino acid like linker synthesized *via* S_NAr macrocyclization reactions as shown in figure-**14**. Some of these peptides were shown to have NT-3 like neurotrophic activity in cell survival assays.

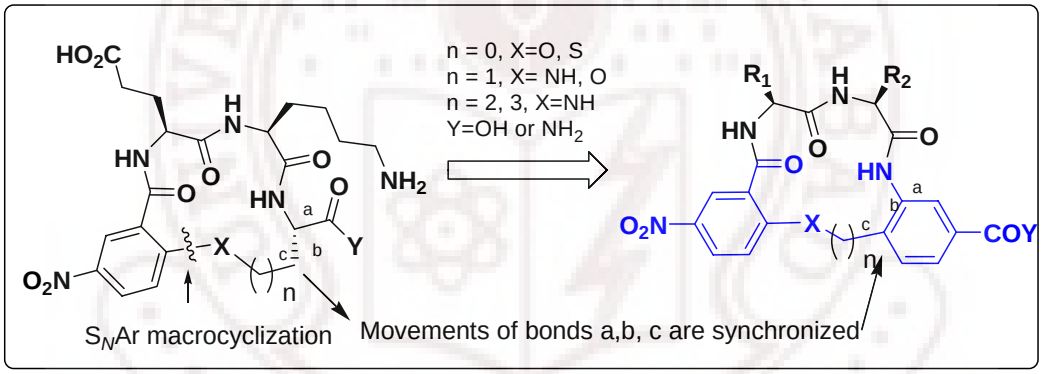


Figure-**14**: *β-turn peptidomimetics possessing neurotrophic activity*

Iqbal and co-workers[33] have employed ring closing metathesis strategy for the synthesis of cyclic β-turn mimics as potent HIV protease inhibitors based on the structural mimicry of Phe-Pro bond which is cleaved by HIV protease. In addition, various palladium-catalyzed C-C bond forming cyclization strategies, such as Heck, Sonogashira, Trost-enyne cycloisomerizations, Buchwald-Hartwig

coupling have been used to constrain acyclic peptides as potential drug leads and/or as models for conformational analysis.

Most of the approaches are aimed at designing conformational constraints that would bias them to one of the low energy conformations to explore the significance of this conformation to the peptide's biological activity. The importance and structural uniqueness of proline[34] coupled with its ability to nucleate turn motifs (*a common feature in biologically active peptides and globular proteins where it is widely thought to act as a molecular recognition site for many biological processes*) prompted us to explore the synthesis of proline containing small cyclic peptides mimicking turn motifs *via* versatile ring closing metathesis (figure-**15**). This study would enable us to determine factors responsible for locking a specific conformation (*cis* or *trans*) in such peptides which may be used as probes to understand the importance of a particular conformation in eliciting biological activity at molecular levels.

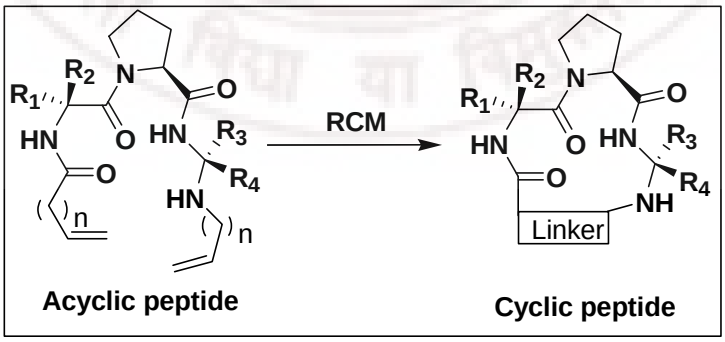


Figure-**15**: *Synthesis of cyclic peptides using RCM*

1.3 References

1. G. N. Ramachandran, G. N.; Sasisekaran, V. *Adv. Protein Chem*, **1968,** *23*, 283.
2. Andrews, M. J. I.; Tabor, A. B. *Tetrahedron*, **1999,** *55*, 11711.
3. Low, B.W.; Baybutt, R.B.; *J. Am. Chem. Soc.* **1952**, *74*, 5806.
4. (a) Pauling, L.; Corey, R. B.; *Proc. Natl. Acad. Sci. USA*, **1951,** *37*, 729 (b) Pauling, L.; Corey, R. B.; Branson, H. R.; *Proc. Natl. Acad. Sci. USA*, **1951,** *37*, 205.
5. (a) Kabsch, W.; Sander, C. *Biopolymers*, **1983,** *22*, 2577 (b) Rose, G. D.; Giersch, L. M.; Smith, J. A.; *Adv. Protein Chem.* **1985,** *37*, 1 (c) Venkatachalam, C. M.; *Biopolymers*, **1968**, *6*, 1425.
6. Kemp, D. S.; Bowen, B. R.; Muendel, C. C. *J.Org. Chem*., **1990**, *55*, 4650 and references listed therein.
7. Smith A. B.; Hirschmann, R.; Pasternak, A.; Guzman,M. C.; Yokoyama, A.; Sprengeler,P.A.; Darke, P.L; Emini, E. A.; Schleif, W. A.; *J. Am. Chem. Soc*., **1995**, *117*, 11113 and references listed therein.
8. Tsang, K. Y.; Diaz, H.; Graciani, N.; Kelly, J. W.; *J. Am. Chem. Soc*., **1994**, *116*, 3988.
9. Mahalakshmi, R.; Raghotama, S.; Balaram, P. *J. Am. Chem. Soc*., **2006**, *128*, 1125.

10. Nowick, J. S.; Lam, K. S.; Khasanova, T. V.; Kemnitzer, W. E; Maitra, S.; Mee, H. T.; Liu, R. *J. Am. Chem. Soc*., **2002**, *124*, 4972.

11. (a) Dervan, P.B. *Science* **1986**, *232*, 464. (b) Dervan, P.B.; Burli, R. W. *Curr. Opin. Chem. Biol.* **1999**, *3*, 688.

12. (a) Hamuro, Y.; Geib, S. J.; Hamilton, A. D. *Angew. Chem., Int. Ed*. *Engl*. **1994**, *33*, 446. (b) Hamuro, Y.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc*. **1996**, *118*, 7529. (c) Hamuro, Y.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1997***, 119,* 10587.

13. (a) Eschenmoser, A.; Loewenthal, E. *Chem. Soc, Rev*. **1992**, *21*, 1. (b) Eschenmoser, A. *Origins Life* **1994**, *24*, 389. (c) Eschenmoser, A. *Science*, **1999**, *284*, 2118.

14. (a) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchhardt, O. *Science* **1991**, *254*, 1497. (b) Nielsen, P. E. *Acc. Chem. Res.* **1999**, *32*, 624. (c) Uhlmann, E.; Peyman, A.; Breipohl, G.; Will, D. W. *Angew. Chem., Int. Ed*. *Engl*. **1998**, *37*, 2796. (d) Diederischsen, U.; Schmitt, H. W. *Angew. Chem., Int. Ed*. *Engl*. **1998**, *37*, 302.

15. For recent reviews see (a) Gellman, S. H. *Acc. Chem .Res* **1998***, 31,* 173. (b) Banerjee, A.; Balaram, P. *Curr. Sci.* **1997,** *73,* 1067. (c) Deming, T. J. *Adv. Mater.* **1997,** *9,* 299. (d) Seebach, D.; Mathews, J. L. *J. Chem. Soc. Chem. Commun*. **1997**, *43*, 219.

16. For representative examples see: Seebech, D.; Abele, S.; Gademann, D.; Guichard, G., Hintermann, T.; Jaun, B.; Mathews, J. L.; Schriber, V. J.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1998**, *81*, 187 and references there in.

17. (a) Chung, Y. J.; Christianson, L. A.; Stanger, H. E.; Powell, D. R.; Gellman , S. H. *J. Am. Chem. Soc*. **1998**, *120*, 10555. (b) Krausthauser, S.; Christianson, L. A.; Powell, Christianson, L. A.; Powell, D. R.; Gellmann, S. H. *J. Am. Chem. Soc.* **1997**, *119*, 11789. (c) Appela, D. H. Christianson, L. A.; Klein, D.A. ; Powel, D. R. Huang, X.; Barchi jr, J. J.; Gellman, S. H. Nature **1997,** *387* 381. (d) Appela, D. H.; Christianson, L. A.; Karle, I. L.; Powel, D. R. ; Gellman, S. H. *J. Am. Chem. Soc* **1997**. *119*, 13071.

18. Hanessian, S.; Yang, H. *Tetrahedron Lett*. **1997**, *38*, 3155. (b) Hanessian, S.; Yang, H.; Schaum, R. *J. Am. Chem. Soc*. **1996**, *118*, 2507.

19. Hayen, A.; Schmitt, M. A.; Ngassa, F. N.; Thomasson, K. A.; Gellman, S. H. *Angew. Chem., Int. Ed*. **2004**, *43*, 505.

20. Pol, V. D.; Zorn, C.; Klein, C. D.; Zerbe, O.; Reiser, O. *Angew. Chem., Int. Ed*. **2004**, *43*, 511.

21. Gruner, S. A. W.; Truffault, V.; Voll, G.; Locardi, E.; Stockle, M.; Kessler, H.; *Chem. Eur. J.* **2002**, *8*, 4366.

22. (a) Sharma, G. V. M.; Reddy, K. R.; P. Krishna, P. R.; Ravi Sankar, A.; Narsimulu, K.; Kiran Kumar, S.; Jayaprakash P.; Jagannadh, B.; Kunwar, A. C. *J. Am. Chem. Soc*. **2003**, *125*, 13670. (b) Sharma, G. V. M.; Reddy, K. R.; P. Krishna, P. R.; Ravi Sankar, A.; Jayaprakash, P.; Jagannadh, B.; Kunwar, A. C. *Angew. Chem.* **2004**, 116, 4051. (c) Sharma, G. V. M.; Nagendar, P.; Jayaprakash, P.; Krishna, P. R.; Ramakrishna, K. V. S.; Kunwar, A. C. *Angew. Chem. Int. Ed.* **2005,** *44*, 5878.

23. Sinfreu, J. F.; Zaccaro L.; Vidal, D.; Salvatella, X.; Giralt, E.; Pons, M.; Albericio, F.; Royo, M. *J. Am. Chem. Soc*. **2004**, *126*, 6048.

24. (a) Rajashankar, K. R.; Ramakumar, S.; Chauhan, V.S. *J. Am. Chem. Soc*. **1992**, *114*, 9225. (b) Rajashankar, K. R.; Ramakumar, S.; Jain R. M.; Chauhan, V.S. *J. Am. Chem. Soc*. **1995**, *117*, 10129.

25. (a) Karle, I. L.; Balaram, P. *Biochemistry*, **1990**, *29*, 6747. (b) Regan, L.; De Grado, W. F. Science **1998,** *241*, 976. (c) Kaumaya, P. T. P.; Berndt, K. D.; Heidron, D. B.; Trewbella, J.; Kezdy, F. J.; Goldberg, E. *Biochemisty*, **1990,** *29*, 13.

26. Hanessian, S.; McNaughton Smith, G.; Lombart, H. G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789.

27. Zhang, J.; Xiong, C.; Ying, J.; Wang, W.; Hruby V. J. *Org. Lett*. **2003**, *5*, 3115.

28. Xuyuan Gu, X.; Jinfa Ying, J.; Agnes, R. S.; Navratilova, E.; Davis, P.; Stahl, G.; Porreca, F.; Yamamura, H. L.; Hruby, V. J. *Org. Lett*, **2004**, *6*, 3285.

29. Halab, L.; Becker, J. A. J.; Darula, Z.; Tourwe, D.; Kieffer, B. L.; Simonin, F. ; Lubell, W. D. *J. Med. Chem.* **2002**; 45, 5353.

30. (a) Blackwell, H. E.; Sadowsky, J. D.; Howard, R. J.; Sampson, J. N.; Chao, J. A.; Steinmetz, W. E.; O'Leary, D. J.; Grubbs, R. H. *J. Org. Chem.* **2001**, *66*, 5291. (b) Boal, A. K.; Guryanov, I.; Moretto, A.; Crisma, M.; Lanni, E. L.; Toniolo, C.; Grubbs, R. H.; O'Leary, D. J. *J. Am. Chem. Soc.* **2007**; *129,* 6986.

31. Glenn, M. P.; Pattenden, L. K.; Reid, R. C.; Tyssen, D. P.; Tyndall, J. D. A.; Birch, C. J.; Fairlie, D. P. *J. Med. Chem.* **2002,** *45*, 371.

32. Pattarawarapan, M.; Zaccaro, M. C.; Saragovi, U. H.; Burgess, K. *J. Med. Chem.* **2002,** *45*, 4387.

33. (a) Reddy, P. R.; Balraju, V.; Madhavan, G. R.; Banerji, B.; Iqbal, J. *Tet. Lett.* **2003**, *44*, 353. (b) Balraju, V.; Reddy, D. S.; Periasamy, M.; Iqbal, J. *J. Org. Chem.* **2005**, *70*, 9626. (c) Balraju, V.; Reddy, D. S.; Periasamy, M.; Iqbal, J. *Tet.Lett*. **2006**, *47*, 3569. (d) Saha, B.; Nandy, J. P.; Shukla, S.;Siddiqui,I.;Iqbal,J. *J. Org. Chem.* **2002**, *67*, 7858 and references cited therein.

34. Williamson, M. P. *Biochem. J.* **1994**, *297*, 249.

Chapter-II

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

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 system 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 ω [Cα(1)-C(1)-N(1′)-Cα(1′)]: *cis* and *trans* (ω= 0 and 180°, respectively)[3] , which are referred to as *s-cis* and *s-trans* respectively (figure-**1)**.

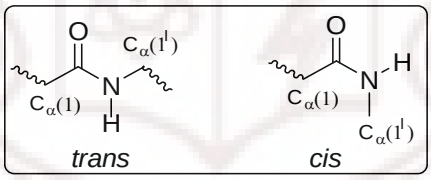


Figure-**1:** *s-trans and s-cis peptide bond.*

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 ϕ dihedral angle; while on the other hand, the imidic bond formed with the preceding residue (Xaa-Pro) is readily subject to

cis-trans isomerization (Figure-**2**). Additionally, the low activation barrier [4] (*ca.* 13 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]

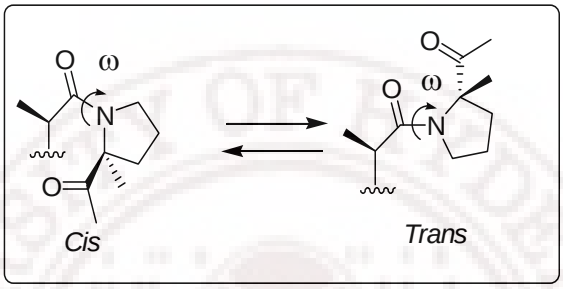


Figure-**2**: *cis/trans isomerization of peptidyl-prolyl bond.*

In 1998, M. S. Weiss *et al* [6] revisited the peptide bonds available from 571 protein crystal structures with resolution ≤ 3.5 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° < ω < 45°). 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* (ω_i=180 ^{o}) or at the *i*+2 position of turn type VI (ω_{i+1}= 0 ^{o}) 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.[7] Two classes of type VI β-turns have been identified [7,8] based on the dihedral angles of central *i*+1, *i*+2 residues (figure-**4**).

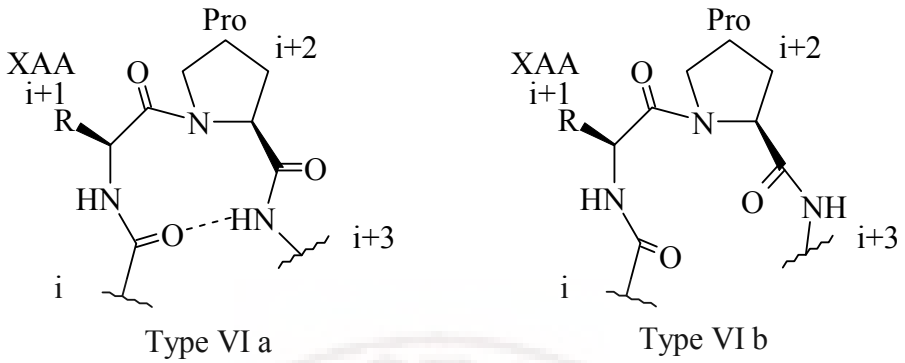


Figure-**4:** *Schematic representation of type-VIa and VIb β-turn structures*

In the type VIa β-turn, the proline ψ-dihedral angle is near 0^o 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^o 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**: *ϕ, ψ and ω backbone torsional angles for type VI β-turn*

β-turns	ϕ	ψ	ω	ϕ	ψ
VIa	-60^o	120^o	0^o	-90^o	0^o
VIb	-120^o	120^o	0^o	-60^o	0^o

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

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,[10] a cyclic depsipeptide is also shown to adopt a *cis*-pro bond in solution. Recently, a novel cyclic hexapeptide, called **segetalin A**,[11] 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 Cycloleonurinin[12] 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 cycloleonurinin consists of two β-turns, a β-turn type VI at Pro[6]-Pro[7], and a β I turn at Pro[11]-Ala[12].

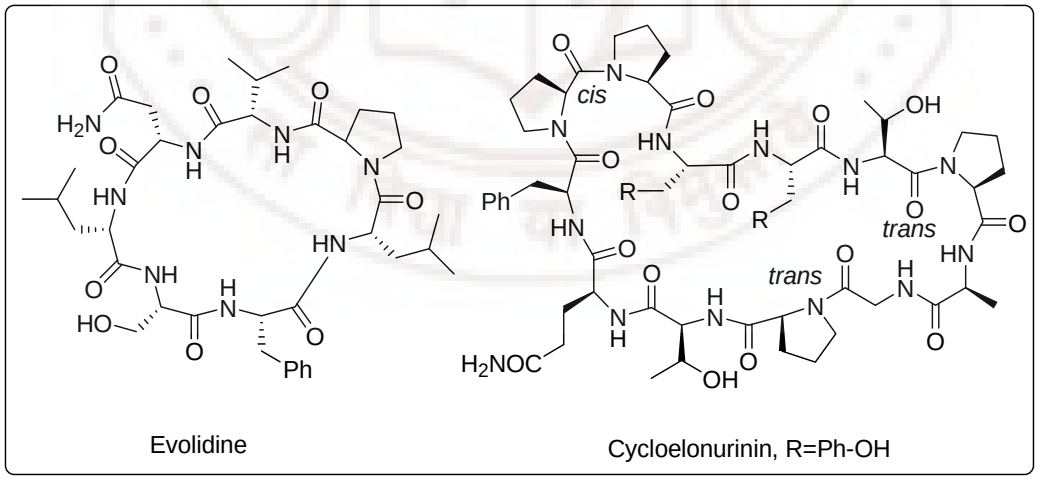


Figure-**5**: *Naturally occurring cis-proline containing peptides*.

Apart from proline itself, a variety of its derivatives are found in nature. Dehydrogenated, mono- and polysubstituted Pro derivatives have been found (Figure-**6**).

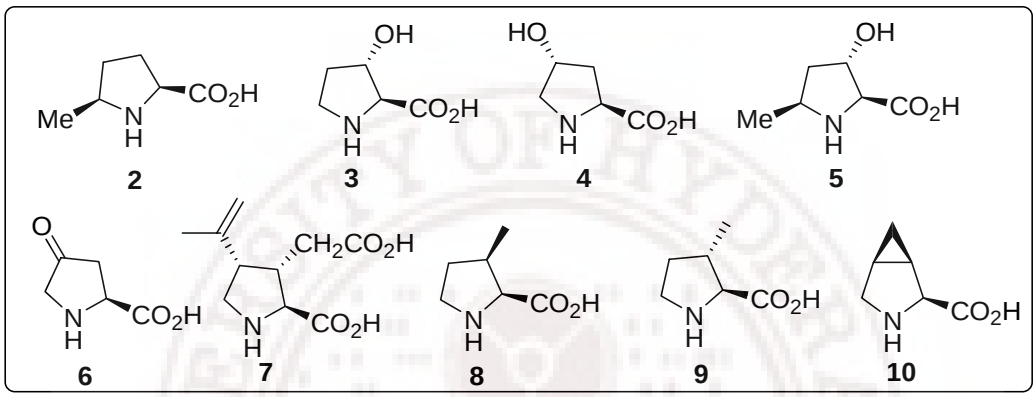


Figure-**6**: *Naturally occurring proline derivatives*

In many cases, the proline derivatives themselves, or peptides containing them, display antibiotic, neurotoxic or anti-tumor activity. The most common proline derivative is (4*R*)-hydroxyproline **3**, which is a major component of collagen and was first discovered in gelatin hydrolysates in 1902.[13] 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.[14] (3*S*)-hydroxyproline **4** was first isolated from hydrolysates of Mediterranean sponge[15] and was later found in human urine resulting from collagen metabolism.[16] Moreover, many members of the class of the actinomycin antibiotics[17] also contain (4*R*)-hydroxyproline[18] as well as 4-

ketoproline **6**,[19] 3-hydroxy-5-methylproline **5**[20] or 5-methylproline **2**.[21] Both diastereomers of 3-methyl proline **8**[22] and **9**[23] are known both as part of cyclic peptides and even *cis* 3,4-methano-L-proline **10**[24] 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 prothrombin**[27] 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-NH_2), 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 GIIIB[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*.[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,[33] particularly Cdc25 phosphatase,[34] a key regulator of the Cdc2/cyclinB complex in mitosis.[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[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 β-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*[38] during their study in testing and refining the receptor bound conformation of the small-ring somatostatin analog c(Pro^{6}-Phe^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}-Phe^{11}) investigated structures constrained within bicyclic systems. Incorporation of an 8-membered -Cys-Cys- unit in place of the Phe^{11}-Pro^{6} segment showed retention of high potency, in confirmation of cyclocystine as a good mimic for *cis* amide (Figure-**7**).

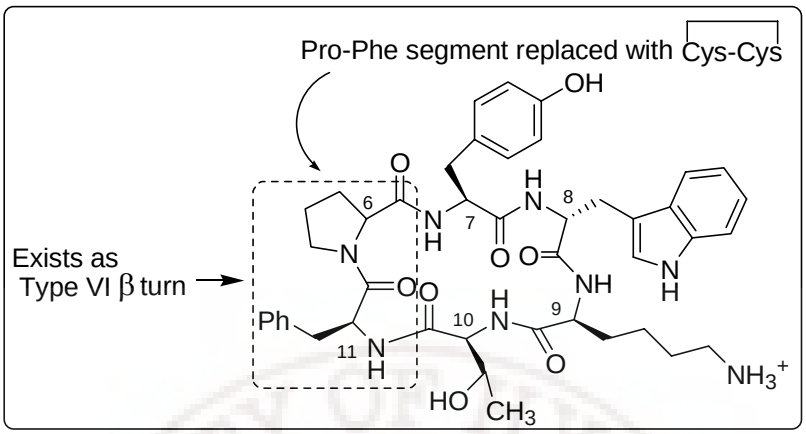


Figure-**7** *Cyclocystine as a mimic for cis-amide bond*

By incorporation of heterocycles and related isosteres:

Rigid sp^2 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*[39] 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*[40] 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*[41] reported the synthesis of 1,2,4-triazole **12** as a *cis*-amide bond surrogate via an easily accessible thiono peptide (Figure-**8**).

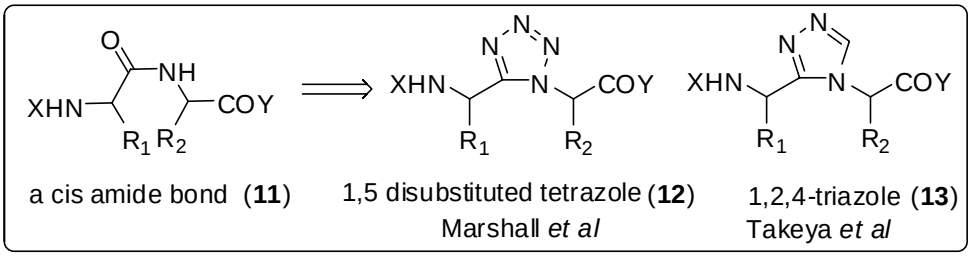
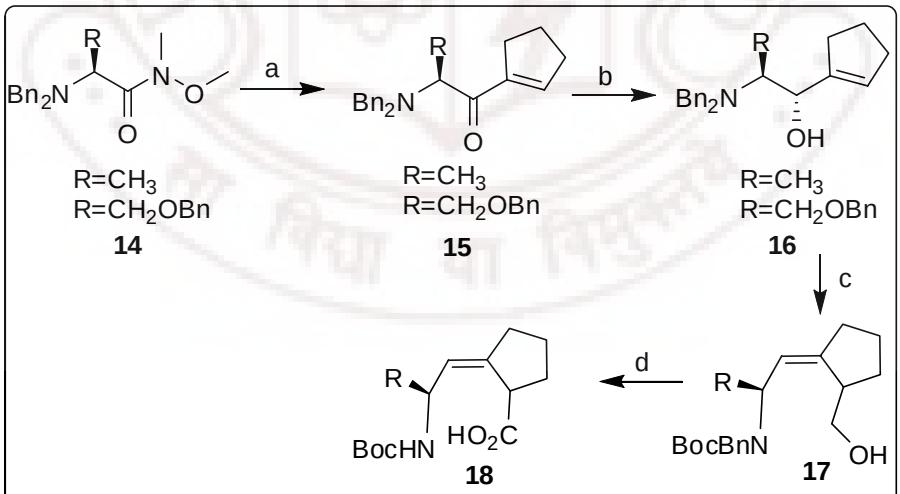


Figure-**8:** *cis-amide bond surrogates*

Etzkorn *et al*[42] 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 isotere*



Reagents and conditions: (a) i. cyclopentenyl lithium, sec-BuLi, THF,-40 °C (b). LAH, THF, (c). (i) Bu_3SnCH_2I, KH 18-crown-6, THF (ii) n-BuLi, THF, -78 °C (d) i. Formic acid, 20 % $Pd(OH)_2/C$, MeOH, ii. Boc_2O, CH_2Cl_2, iii. Jones reagent iv. Na/NH_3, -33 °C.

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**).

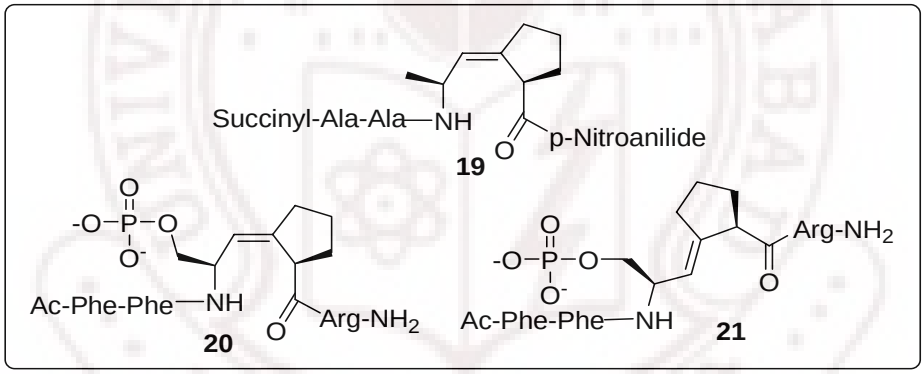


Figure-**9:** *Inhibitors of Cyclophilin and Pin*1

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[43] 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**.

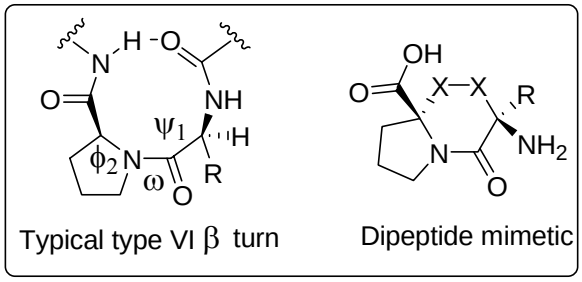
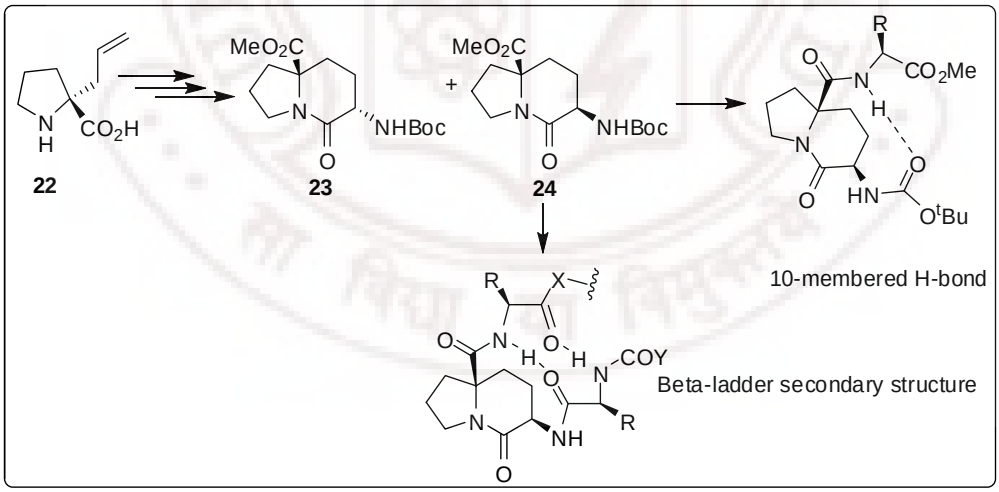


Figure-**10** *Design of dipeptide mimetic*

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 a β-turn and an antiparallel β-ladder formation (Scheme-**2)**.

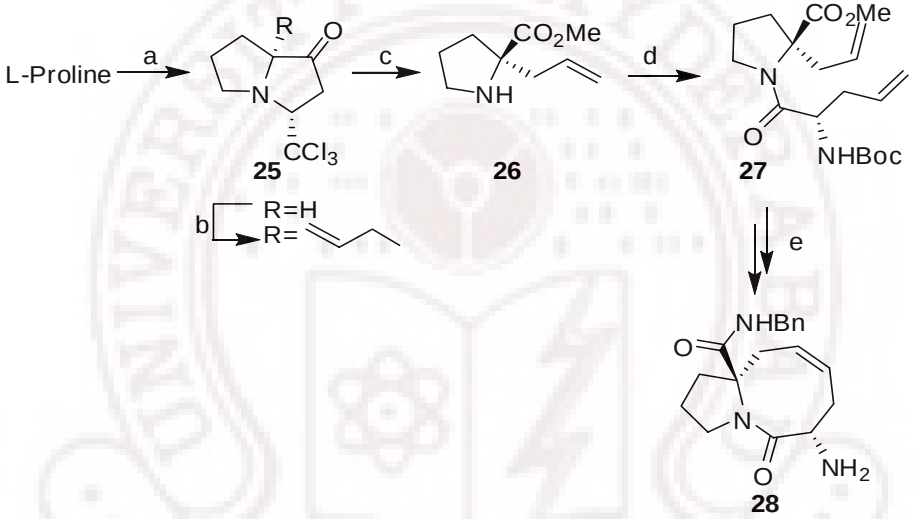
Scheme-**2** *synthetic approach to bicyclic lactam mimicking Type VI* β- *turn*



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.[44] 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*

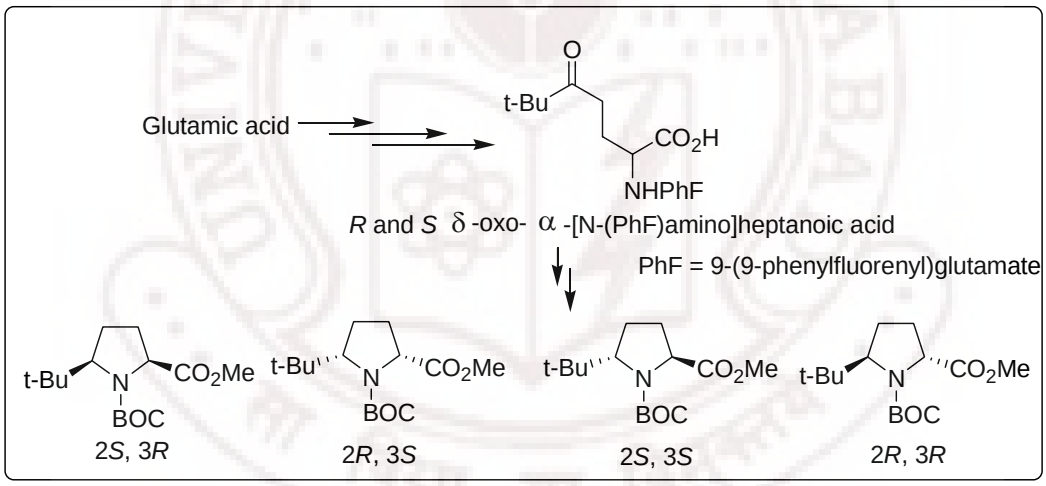


Reagents and conditions: (a) $CCl_3CH(OH)_2$, C_6H_6, 80 ^oC (b) LDA, allylbromide, THF, -78 ^oC (c) HCl, MeOH, $NaHCO_3$ (d) (S)-N-Boc-2-amino-4-pentenoic acid, NMM, $ClCO_2iBu$, THF(e) 7 mol % Grubbs catalyst ii. LiOH, dioxane/H_2O iii. $BnNH_2$, DCC, DCM, HOBt iv. HCl /ether.

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*-(acetyl) proline *N*′-methylamide. This effect was contingent on the relative stereochemistry of the 5-methylproline. These results prompted Lubell *et al* [45] to use bulkier substituents at 5-position in order to prepare X-Pro analogues with greater *cis*-isomer population. Enantiopure 5-^{t}butyl prolines have been synthesized from glutamic acid via an acylation/diastereoselective reductive amination sequence (Scheme-**4**).

Scheme-**4** *Synthetic approach to enantiopure 5 t-butyl prolines*



To support the importance of a particular conformation for bioactivity, Lubell and co-workers[46] designed and synthesized three oxytocin analogues by substituting (2*S*, 5*R*)-5-^{t}butyl proline for proline in the native peptide, the potent agonist [Mpa^{1}]oxytocin and the potent antagonist[dPen1]-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.

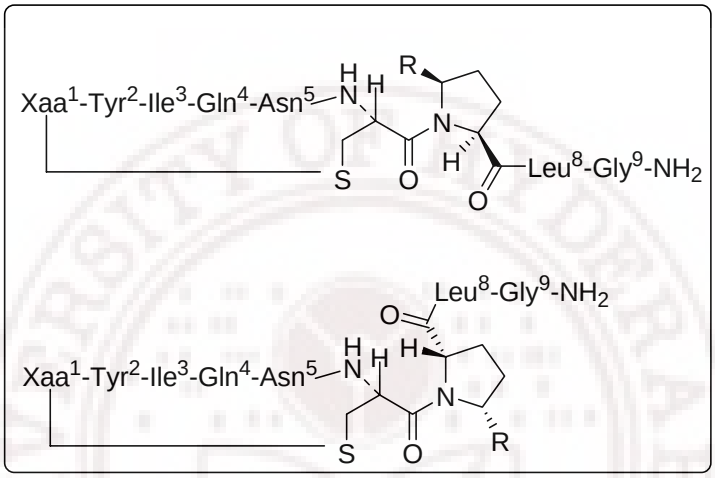


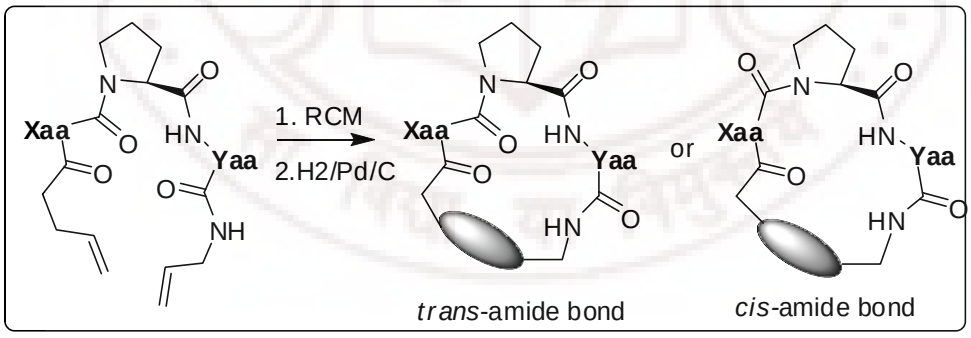
Figure-**11:** *Prolyl amide isomers of Oxytocin and its analogues*

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 a 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.

2.2 Present study

The choice of synthesizing conformationally locked *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,[49] we have explored the syntheses of cyclic tripeptides containing Xaa-Pro-Yaa segment with proline at *i*+2 position (scheme-**5**), which is a prerequisite for the nucleation of type VI β-turn. In the subsequent paragraphs our efforts towards the generation of a library of *cis*-proline containing compounds is discussed.

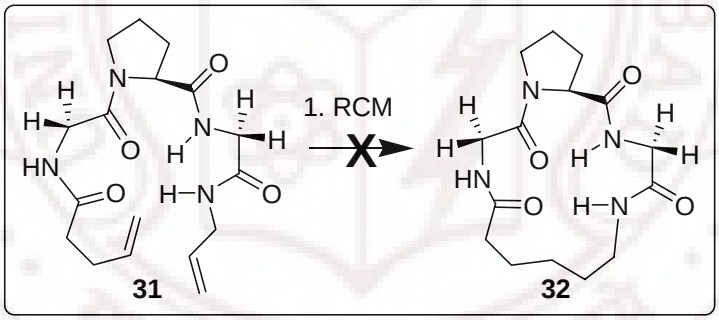
Scheme-**5**: *RCM approach to cyclic peptides*



2.3 Results and Discussions

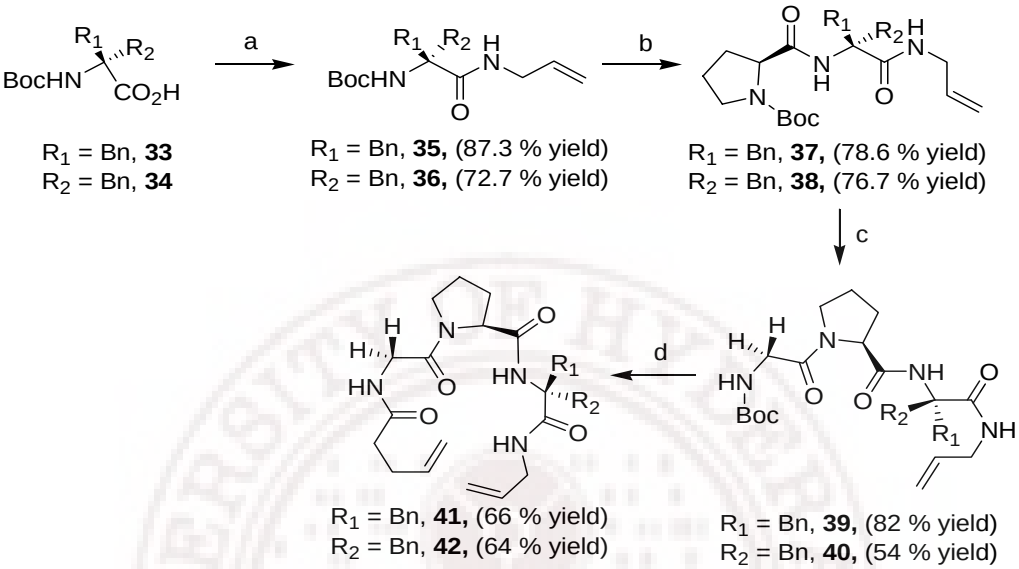
Previous studies in our group have shown that acyclic peptide *N*-pentenoyl-Gly-Pro-Gly allylamide **31** failed to undergo RCM reaction which was attributed to the flexibility in terms of ϕ and ψ angles of Gly residues due to the achiral nature by virtue of which the double bonds move far apart eluding 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**.

Scheme-**7**: *Preparation of acyclic peptides* **41** *and* **42** *from N-Boc* L *and* D*-Phe*

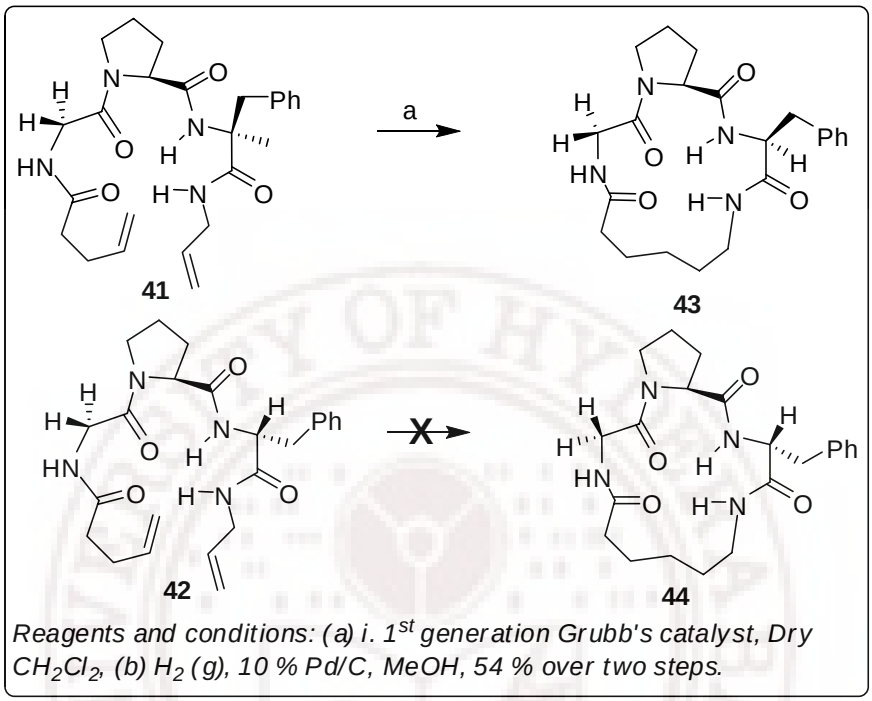


Reagents and conditions: (a) i. Allylamine, $ClCO_2{}^iBu$, NEt_3, CH_2Cl_2, 0 °C-rt (d) i. TFA, CH_2Cl_2, ii. N-Boc Proline, $ClCO_2{}^iBu$, NEt_3, CH_2Cl_2, 0 °C-rt (c) i. TFA, CH_2Cl_2, ii. EDC, HOBt, *CH_2Cl_2*, (d) *i. TFA, CH_2Cl_2, 4-pentenoic acid, $ClCO_2{}^iBu$, NEt_3, CH_2Cl_2, 0 °C-rt*

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 $ClCO_2{}^iBu$, NEt_3 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 NEt_3 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 CH_2Cl_2 yielded the tripeptides *N*-Boc-Gly-Pro-L-Phe allylamide **39** and *N*-Boc-Gly-Pro-D-Phe

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 ($ClCO_2{}^{i}Bu/NEt_3$) 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.[50] 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 intra molecular 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 CH_2Cl_2 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**



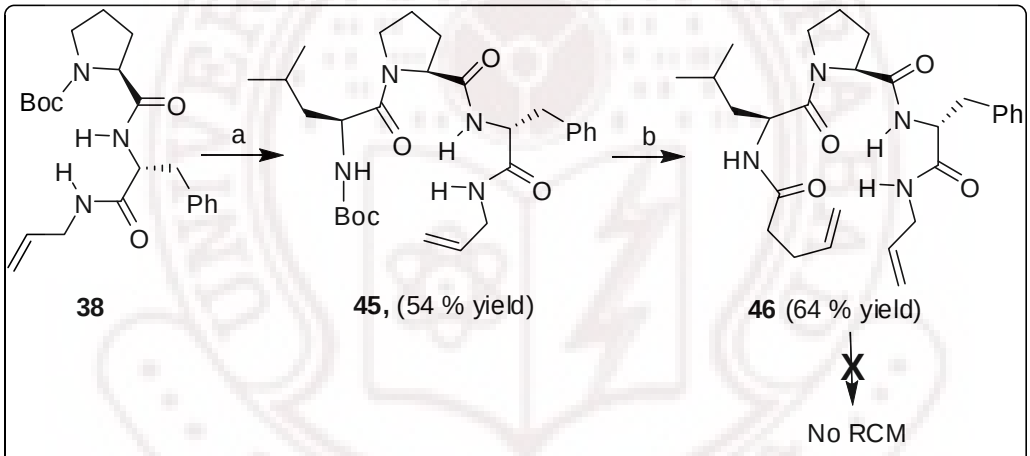
Reagents and conditions: (a) i. 1st generation Grubb's catalyst, Dry CH_2Cl_2, (b) H_2 (g), 10 % Pd/C, MeOH, 54 % over two steps.

The conformation of cyclic peptide **43** in $CDCl_3$ (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 ^{1}H 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 out come 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**



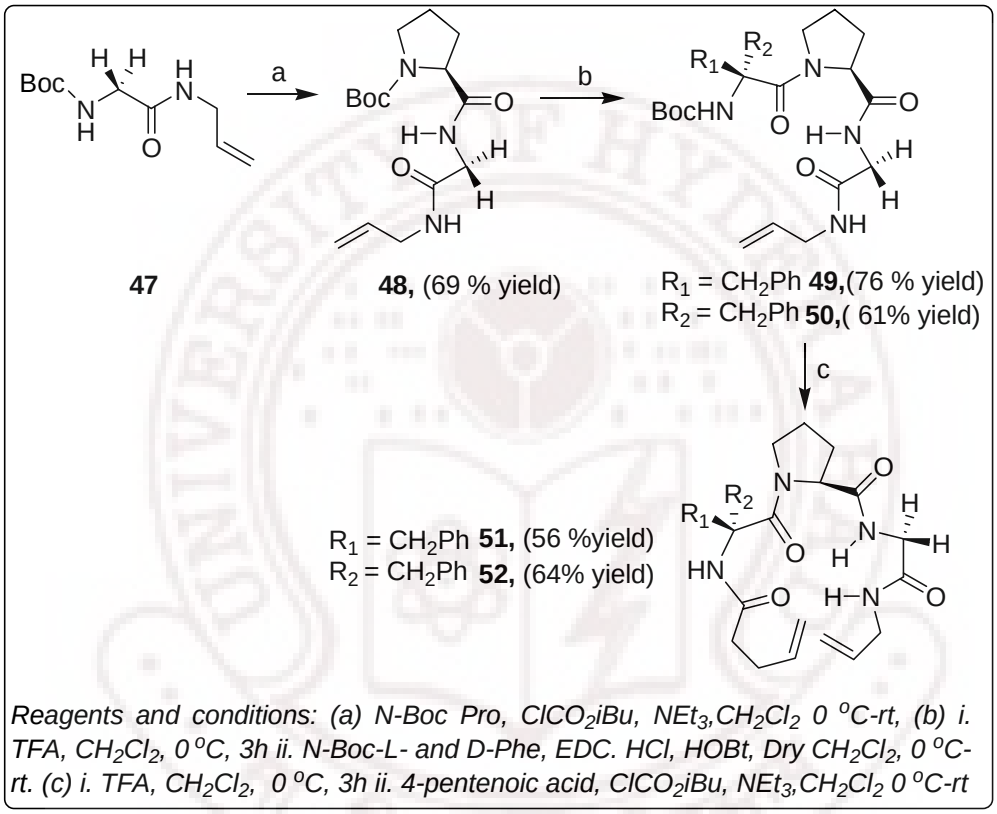
38 **45,** (54 % yield) **46** (64 % yield)

Reagents and conditions: (a) i. TFA (10 eq), CH_2Cl_2, 0C, 3h ii. N-Boc-Leu, $ClCO_2{}^iBu$, NEt_3, CH_2Cl_2, rt, 12h, (b) TFA (10 eq), CH_2Cl_2, 0C, 3h ii. pentenoic acid, $ClCO_2{}^iBu$, NEt_3, CH_2Cl_2, rt, 12h.

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 ($ClCO_2{}^iBu/NEt_3$) afforded the acyclic peptides **51** & **52**.

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

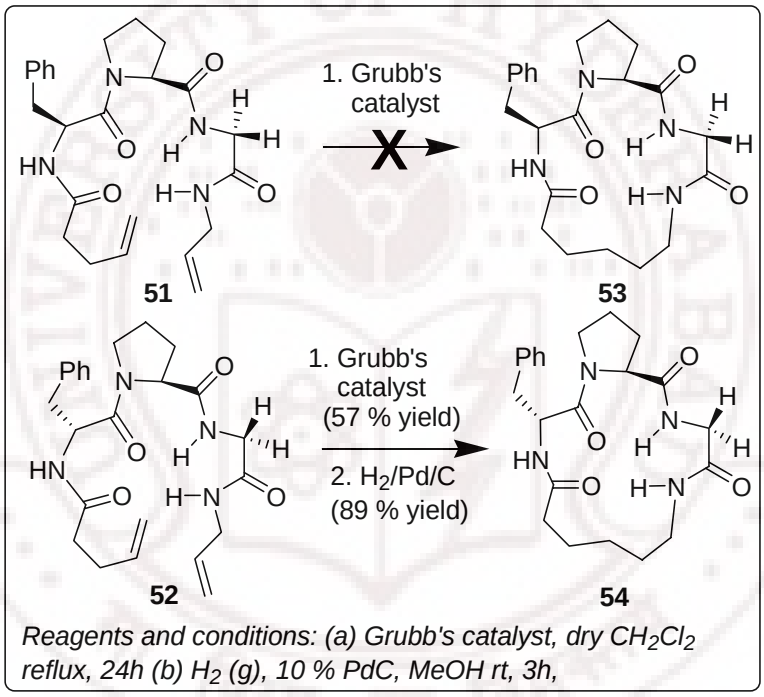


Reagents and conditions: (a) N-Boc Pro, $ClCO_2iBu$, NEt_3,CH_2Cl_2 0 ^oC-rt, (b) i. TFA, CH_2Cl_2, 0 ^oC, 3h ii. N-Boc-L- and D-Phe, EDC. HCl, HOBt, Dry CH_2Cl_2, 0 ^oC-rt. (c) i. TFA, CH_2Cl_2, 0 ^oC, 3h ii. 4-pentenoic acid, $ClCO_2iBu$, NEt_3,CH_2Cl_2 0 ^oC-rt

The solution conformation of these peptides was studied in $CDCl_3$. The down field 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 upfiled shift of Allylic NH

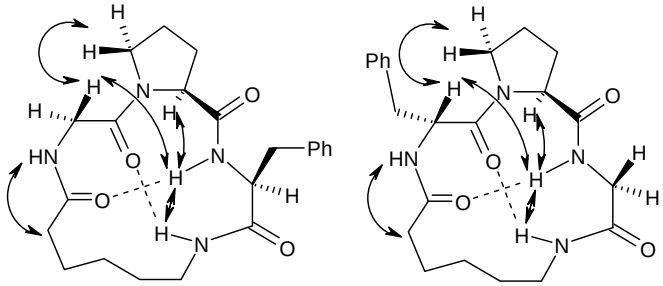
(6.52ppm) in **51** indicated an extended conformation. It is noteworthy, though not surprising, that the peptide **52** preorganized by a β-turn underwent facile RCM in good yields and was converted to cyclic peptide **54** in fairly good yields while **51** did not (Scheme-**11**).

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



Reagents and conditions: (a) Grubb's catalyst, dry CH_2Cl_2 reflux, 24h (b) H_2 (g), 10 % PdC, MeOH rt, 3h,

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.

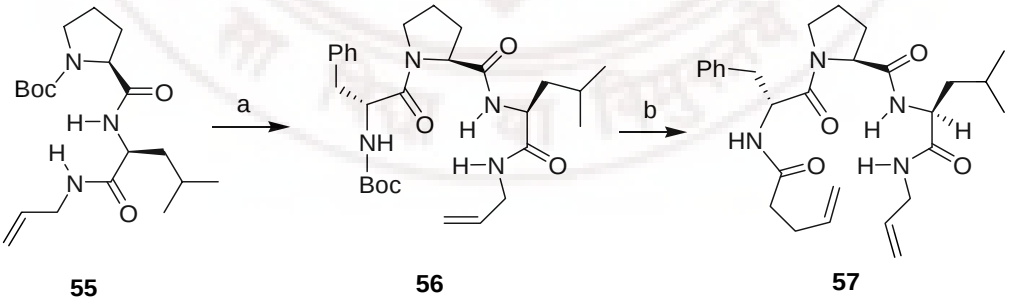


43 **52**

Figure-**12**: *Diagnostic NOEs in cyclic peptides* **43** and **52**

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 ($ClCO_2{}^{i}Bu/NEt_3$).

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

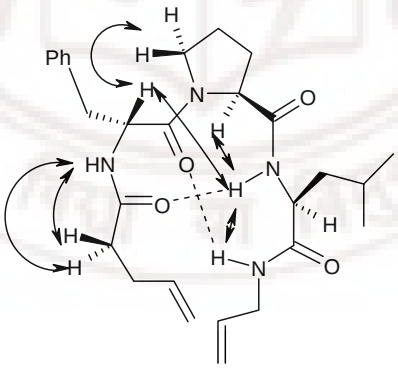


55 **56** **57**

Reagents and conditions: (a) i. TFA, CH_2Cl_2, 0 ^{o}C, 3h ii. N-Boc D-Phe, EDC. HCl, HOBt, Dry CH_2Cl_2, 0 ^{o}C-rt, 81 % (b) i. TFA, CH_2Cl_2, 0 ^{o}C, 3h ii. 4-pentenoic acid, $ClCO_2iBu$, NEt_3, CH_2Cl_2 0 ^{o}C-rt, 70 %

Conformation of 57:

Acyclic peptide **57** yielded well-resolved ^{1}H NMR spectra in both $CDCl_3$ and $DMSOd_6$. 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 CαH/Leu NH suggested a β-turn about D-Phe-Pro residues. On the other hand, the D-Phe CαH/Pro CδH NOE together with the involvement of allylic NH in H-bonding supports the presence of *trans*-amide bond preceding proline with another β-turn around Pro-Leu residues as shown in Figure-**13**.



57

Figure-**13**: *Diagnostic NOEs in Pentenoyl-D-Phe-Pro-Leu allylamide* **(57)**

Crystals required for the X-ray studies of **57** were grown from MeOH-EtoAc-hexane system as colorless blocks in orthorhombic $P2_12_12_1$ space group (Figure-**14**).

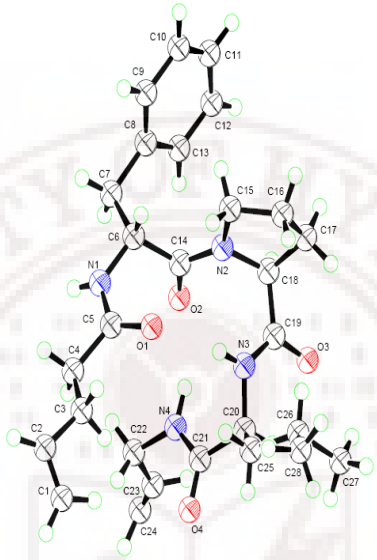


Figure-**14**: *ORTEP plot of Pentenoyl-D-Phe-Pro-Leu allylamide* **(57)**

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 ϕ, ψ angles around Pro-Leu residues (Standard values in brackets)

S.No	Dihedral angles between atoms	ϕ(i+1) ψ(i+1)	Dihedral angles between atoms	ϕ(i+2) ψ(i+2)
1	C14-**N2-C18**-C19	-66.1(-60)	C19-**N3-C20**-C21	-110.0(-90)
2	N2-**C18-C19**-N3	-11(-30)	N3-**C20-C21**-N4	20.0 (0)

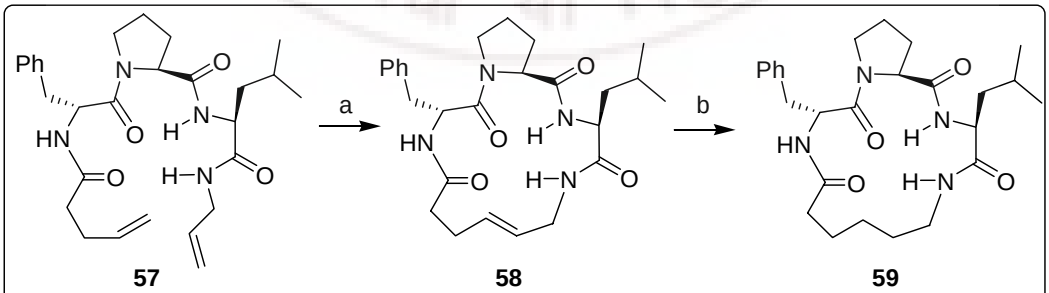
Another intramolecular H-bond (10-membered) between the carbonyl oxygen of pentenoyl(O1) and Leu amide proton(N3-H) was apparent from the interatomic distance of 2.39 Å. The dihedral angles around D-Phe-Pro residues resembled those of the *i*+1 and *i*+2 residues of a type II′ β-turn thereby establishing 3_{10} helical conformation for **57**.

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

S.No	Dihedral angles between atoms	ϕ(i+1) ψ(i+1)	Dihedral angles between atoms	ϕ(i+2) ψ(i+2)
1	C5-**N1**-**C6**-C14	59(60)	C14-**N2**-**C18**-C19	-66.1(-80)
2	N1-**C6**-**C14**-N2	-127.22(-120)	N2-**C18**-**C19**-N7	20 (0)

As expected, based on previous results, the resulting acyclic peptide underwent smooth cyclization with 10 mol% Grubb's catalyst in dry CH_2Cl_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**



57 **58** **59**

Reagents and conditions: (a) Grubb's catalyst, Dry CH_2Cl_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↔Aha NH, Leu NH↔Pro CδH, Leu NH↔^{D}Phe CαH, Aha NH↔Pro CαH (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 β-turns (Figure**15**).

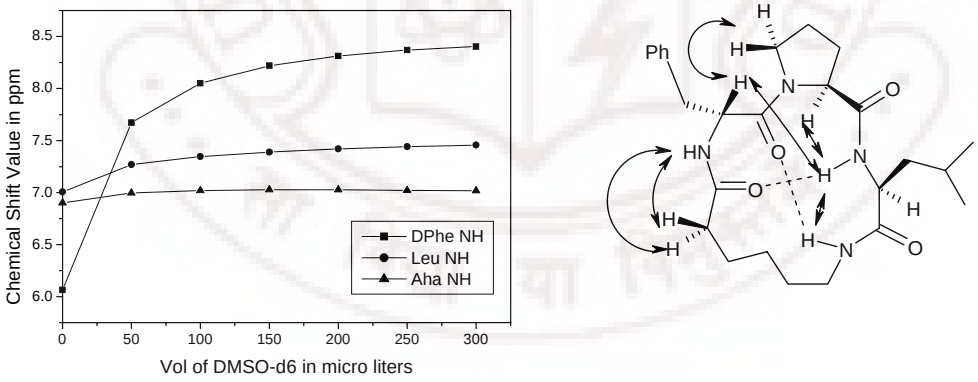


Figure-**15** *NMR titration plot and NOE correlations observed in $CDCl_3$ & $DMSOd_6$ of peptide **59***

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 $P2_12_12_1$ space group (Figure-**16**).

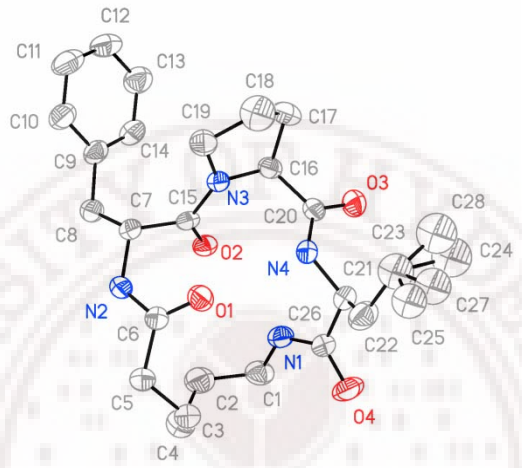


Figure- **16** *ORTEP of cyclo(D-Phe-Pro-Leu-Aha)* ***59*** *(H atoms are not shown for clarity).*

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

S.No	Dihedral angles between atoms	ϕ(i+1) ψ(i+1)	Dihedral angles between atoms	ϕ(i+2) ψ(i+2)
1 2	C6-**N2-C7**-C15 N2-**C17-C15**-N3	48.87(60) -131.47(-120)	C15-**N3-C16**-C20 N3-C16-C20-N4	-69.5(-80) -10.9 (0)

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 β-turn.

Table IV ϕ, ψ angles around Pro-Leu residues (Standard values in brackets)

S.No	Dihedral angles between atoms	ϕ(i+1) ψ(i+1)	Dihedral angles between atoms	ϕ(i+2) ψ(i+2)
1 2	C15-**N3-C16**-C20 N3-**C16-C20**-N4	-69.5(-60) -10.9(-30)	C20-**N4-C21**-C26 N4-**C21-C26**-N1	-101.1(-90) -10.2 (0)

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**).

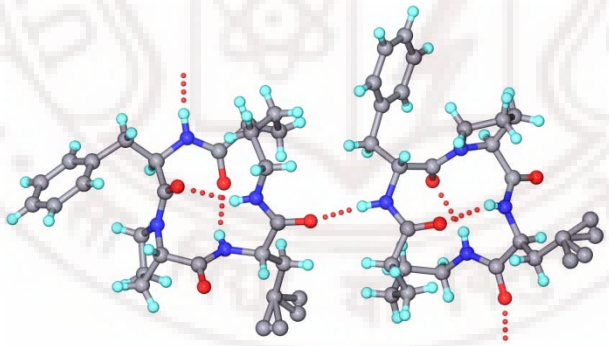
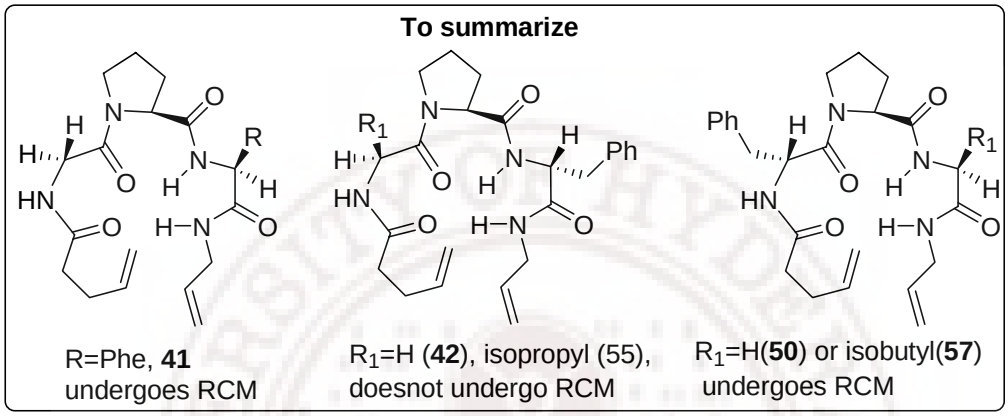


Figure-**17** *Two intramolecular N–H···O H bonds in cyclic peptide* ***59*** *and one intermolecular H bond between such rings.*

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*



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**).

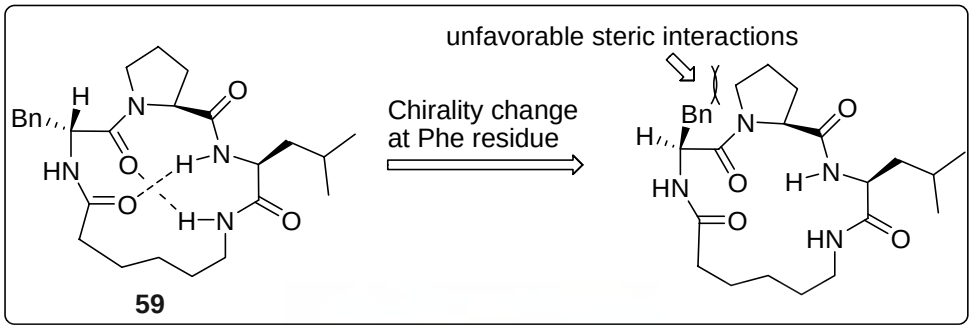
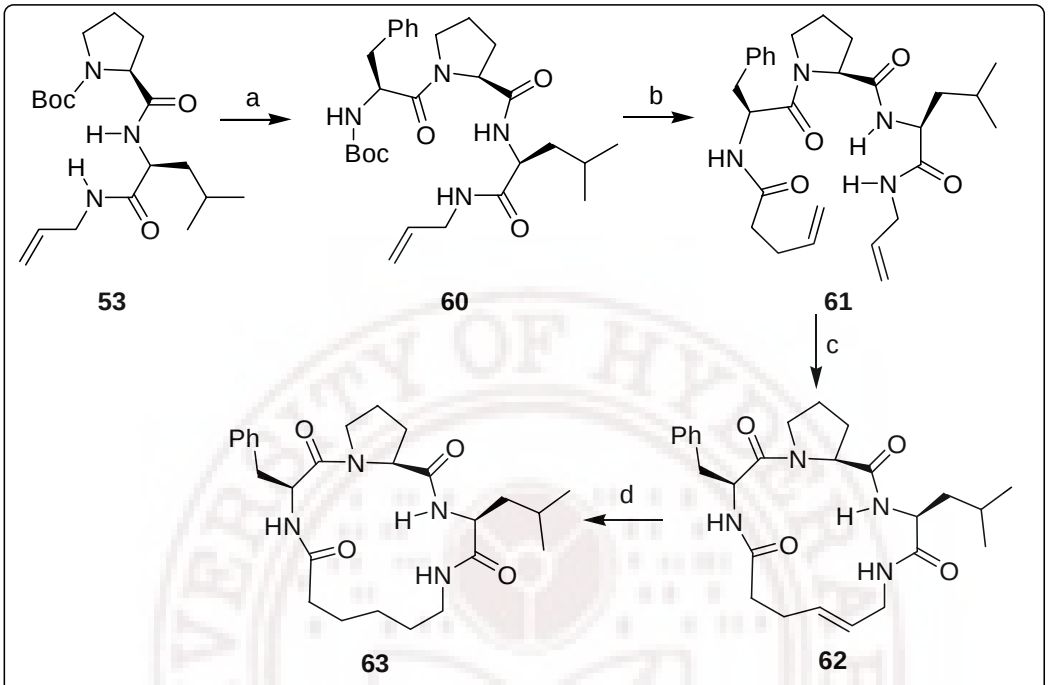


Figure-**18**: *Representation of unfavorable steric interactions due to change of chirality of* D*-Phe residue in* **59.**

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 $ClO_2{}^iBu/NEt_3$ 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, CH_2Cl_2, ii. N-Boc Phe, EDC.HCl, HOBt, CH_2Cl_2, 0 ^oC-rt, 59 % (b) i. TFA, CH_2Cl_2,ii. 4-pentenoic acid, $ClCO_2{}^iBu$, NEt_3, CH_2Cl_2, 0 ^oC-rt, 53 % (c) 10 mol % Grubbs catalyst, Dry CH_2Cl_2, reflux, 24 h, 80 % (d) 10 % Pd/C, H_2 (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 ^{1}H NMR of acyclic peptide **61** showed the presence of two rotamers in the ratio of 7:3 in $CDCl_3$. 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

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 CαH↔Pro CαH 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 Cβ and Cγ ($\Delta\delta_{\beta\gamma}$ = 9.24 ppm using HSQC spectrum **12** on page # 140) was used as a diagnostic tool for the confirmation of *cis*-amide bond (The Cγ-endo (DOWN) pucker is preferable for the *cis*-amide bond and the Cγ-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 CαH↔Pro CαH, Leu NH↔Phe CαH, Leu NH↔Pro CαH 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.

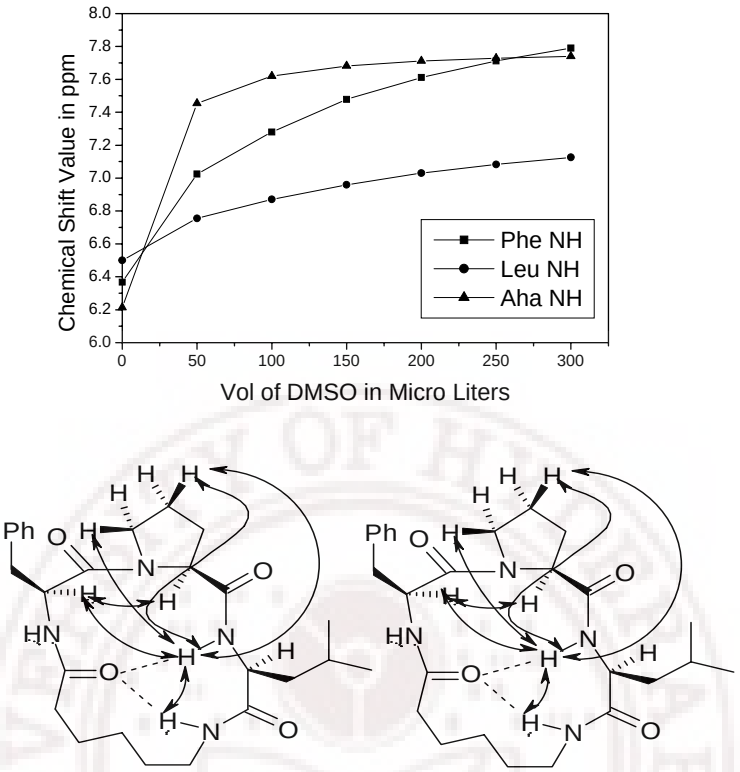


Figure **19**: *Titration plot and diagnostic NOE correlations observed in $CDCl_3$ and DMSO-d_6 for* ***63***

The unprecedented up field shift of the Pro CαH (3.45 ppm), Pro Cβ′H (1.21 ppm) signals were attributed to the ring current effect of phenyl ring. The NOE cross peaks Phe ortho-H↔Pro CαH, Phe ortho-H↔Pro Cδ′H provide strong evidence for the aromatic ring and proline ring CH···π 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/°K) and Aha NH (-3.4 ppb/°K) indicated that these amides participated in intramolecular hydrogen bonding. The observation of NOE correlations Phe CαH↔Pro CαH,

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···O_w, O_w–H···O_w, O_w–H···O=C hydrogen bonds.

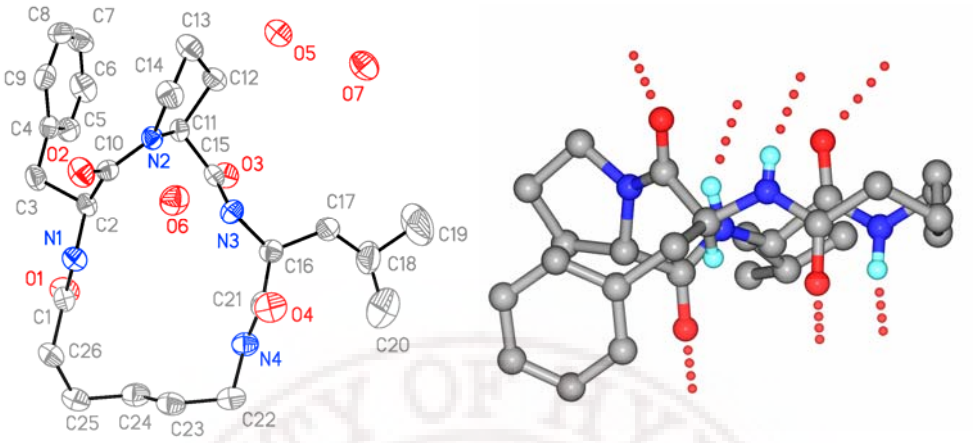


Figure-**20:** *ORTEP plot of cyclic peptide* ***63***

The molecular conformation of cyclo(Phe-Pro-Leu-Aha) has a type VIa2 β-turn around Phe-Pro residues with C–H···π interaction[51] 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 = 4.17°, 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**

S.No	Dihedral angles between atoms	ϕ(*i*+1) ψ(*i*+1)	Dihedral angles between atoms	ϕ(*i*+2) ψ(*i*+2)
1	C1–N1–C2–C10	-169.3(-120)	C10–N2–C11–C15	-75.2(-60)
2	N1–C2–C10–N2	135.62(120)	N2–C11–C15–N3	-19.3 (0)

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**).

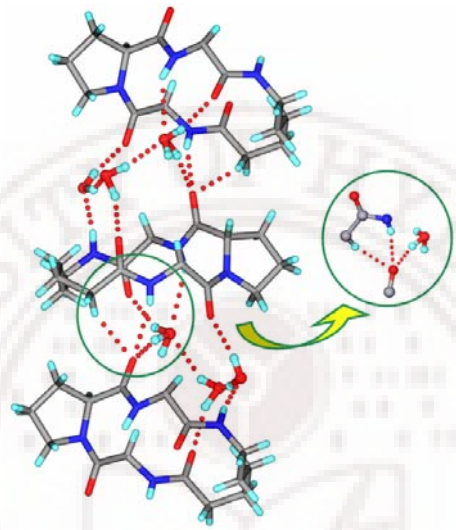


Figure-**21:** *Water mediated crystal structure of the cyclic peptide. Trifurcated acceptor of Pro C=O from Phe NH, O6 water and Aha CH donors. Side chains are omitted for clarity*

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 assemly 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.

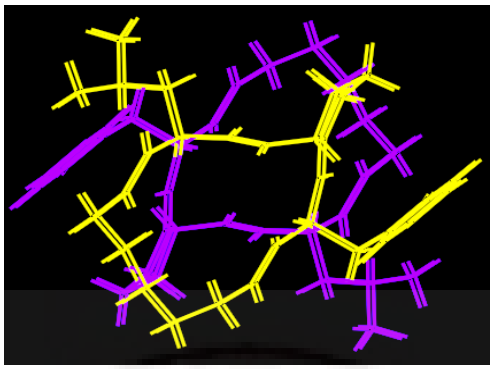
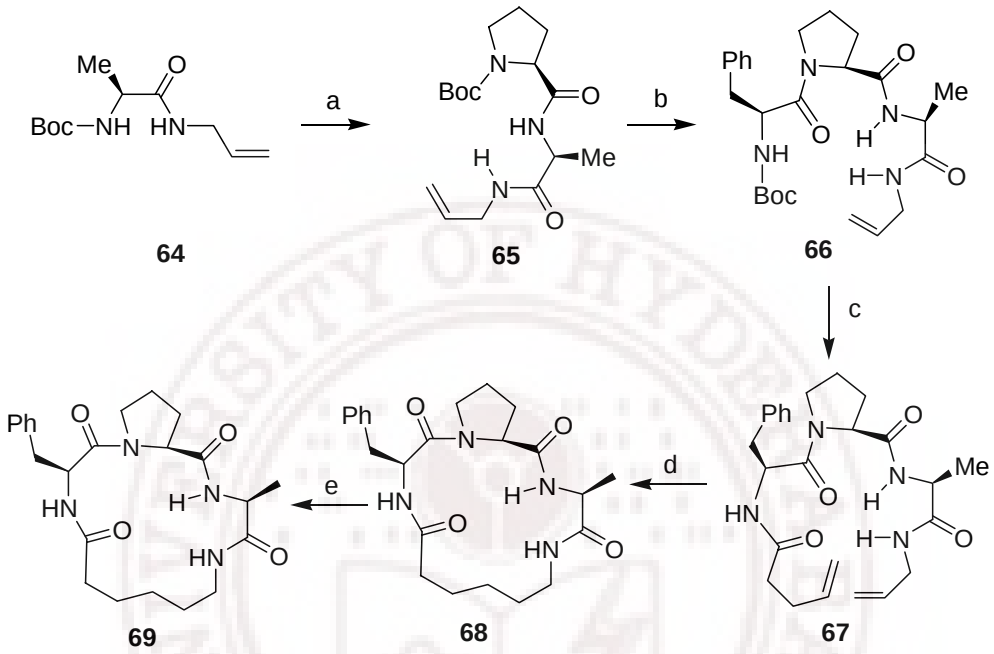


Figure-**22**: *Stacking of adjacent cyclic peptides* ***65*** *with incomplete offset generates a curving tubular architecture. Water molecules are not shown for clarity.*

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.[52] 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).[53] Cyclic peptide **63** 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**.

Scheme-**16**: *Preparation of cyclic peptide* **69** *from N-Boc-Ala allylamide*



Reagents and conditions: (a) i. TFA, CH_2Cl_2, 0 ^oC, 3h ii. N-Boc-Pro, $ClCO_2{}^iBu$, NEt_3, CH_2Cl_2, 0 ^oC-rt, 86 % (b) i. TFA, DCM, ii. N-Boc Phe, EDC.HCl, HOBt, DCM, 0 ^oC-rt, 60 % (c) i. TFA, CH_2Cl_2, 4-pentenoic acid, $ClCO_2{}^iBu$, NEt_3,CH_2Cl_2, 0 ^oC-rt, 57 % (d) 10 mol % Grubbs catalyst, Dry CH_2Cl_2, reflux, 24 h (e) 10 % Pd/C, MeOH, H_2 (g) 54 % over two steps.

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_2Cl_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 CαH↔Pro CαH (spectrum 16, page# 142) and the large difference between the ^{13}C chemical shift of Cβ and Cγ 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↔Phe CαH, Ala NH↔Pro Cδ*(Pro-S)*H and Ala NH↔Pro Cγ*(Pro-S)*H in the NOESY spectrum (page # 142) coupled with H-bonding of Ala NH, supported for the existence of a type-VIa β-turn around Phe-Pro residues (Figure-**23**). The up field shift of proline CαH (3.42 ppm), Cβ*(Pro-S)*H (1.22 ppm), Pro Cδ*(Pro-R)*H (3.38 ppm) and NOE peaks between aromatic ortho H with proline CαH, Cβ*(Pro-S)*H and Cδ*(Pro-R)*H was due to the influence Phe aromatic ring indicating a strong CH$\cdots\pi$ interaction

between the two rings. In DMSO-d_6 the NOE Aha NH↔Phe CαH suggested Aha NH H-bond between Aha NH↔Aha CO resulting in a 9-membered ring.

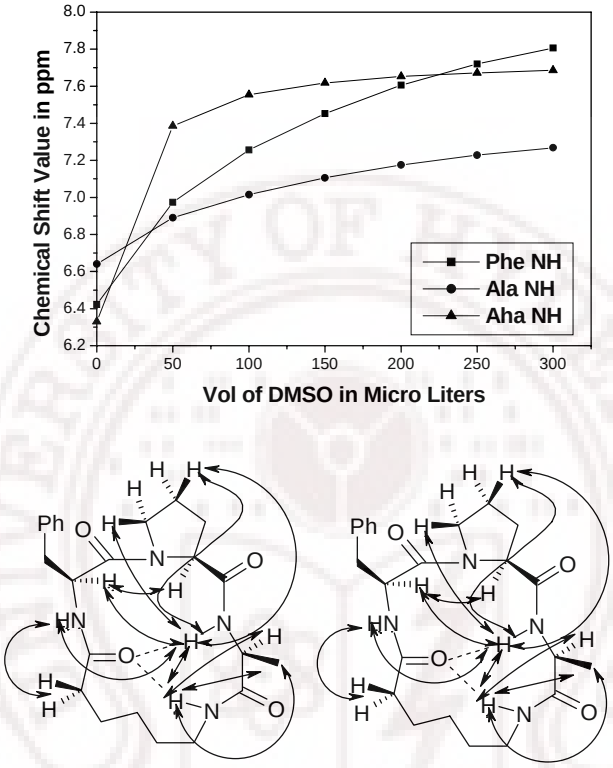
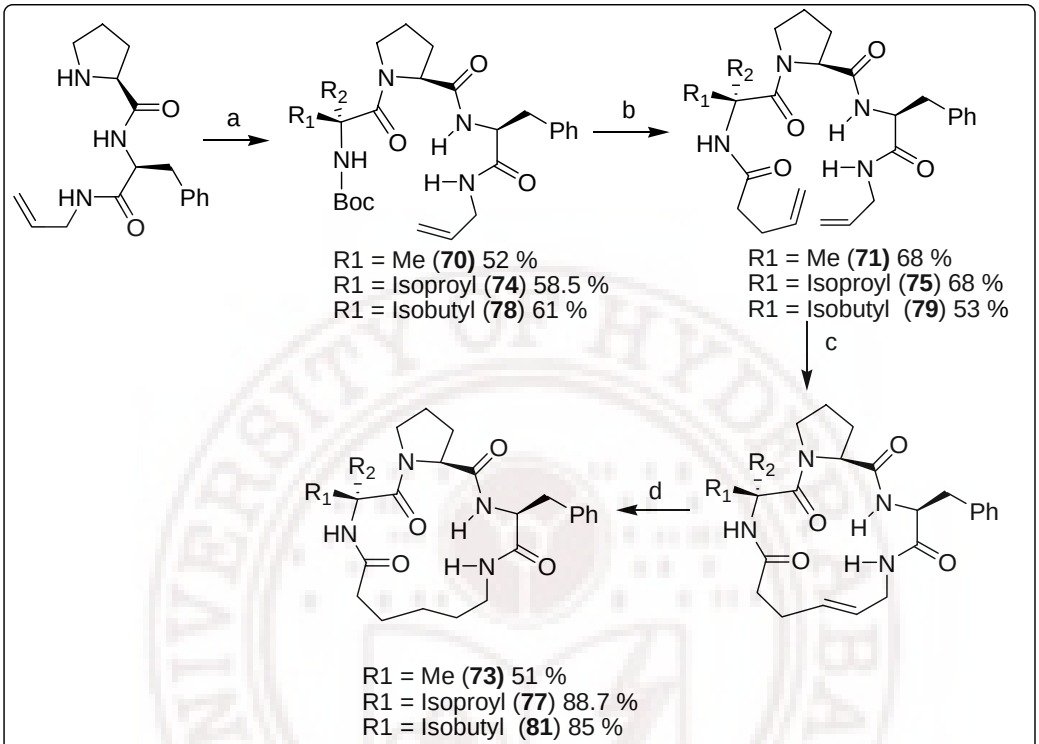


Figure-**23**: *Solvent titration plot and diagnostic NOEs of cyclo(Phe-Pro-Ala-Aha)* ***69*** *in $CDCl_3$ and $DMSOd_6$*

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**



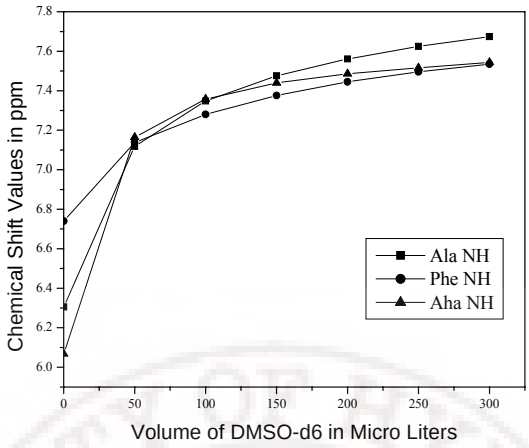
Reagents and conditions: (a) i. TFA, CH_2Cl_2, ii. N-Boc aa (amino acid), EDC.HCl, HOBt, CH_2Cl_2, 0 ^oC-rt, (b) i. TFA, CH_2Cl_2, 4-pentenoic acid, $ClCO_2{}^iBu$, NEt_3,CH_2Cl_2, 0 ^oC-rt, (c) 15 mol % Grubbs catalyst, Dry CH_2Cl_2, reflux, 24 h, (d) 10 % Pd/C, MeOH, H_2 (g).

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 ($ClCO_2{}^iBu/NEt_3$) afforded the acyclic tripeptides **71, 75** and **79**. The ^1H 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 ^{13}C chemical shifts of Pro Cβ and Pro Cγ ($\Delta\delta$= 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 $CDCl_3$ (figure **28**) coupled with moderate magnitude (–4.1 ppb/°K) of $\Delta\delta/\Delta T$ in DMSO-d_6 suggested its involvment 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-VIa β-turn around Ala-Pro residues. The moderate magnitude of –3.1 ppb/°K of Aha NH in **73** in DMSO-d_6 as well as cross correlation between Aha NH↔Ala CαH supporting the existence of 9-membered H-bond Aha C=O–Aha NH.



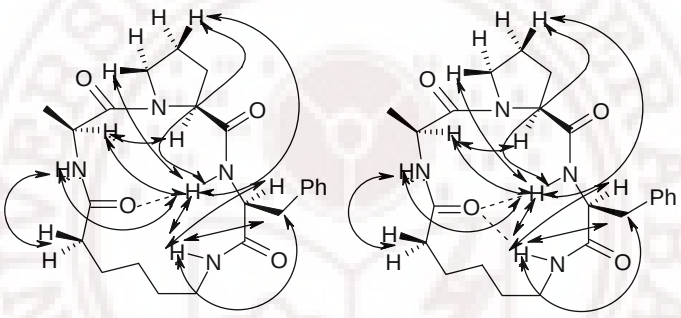


Figure-**24**: *Titration plot and diagnostic nOe correlations observed in $CDCl_3$ and DMSO-d_6 of 73.*

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 ^{13}C chemical shifts of Pro Cβ and Pro Cγ ($\Delta\delta$ = 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 $CDCl_3$ 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**).

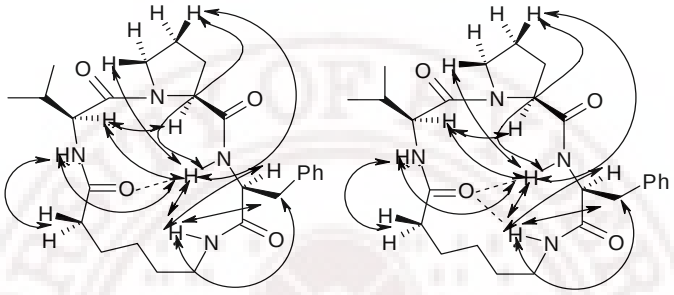


Figure-**25**: *Diagnostic NOE correlations observed in $CDCl_3$ and DMSO-d_6 of peptide **73**.*

Conformation of 81:

Cyclic peptide cyclo(Leu-Pro-Phe-Aha), is a positional isomer of peptide **63**. The conformational analysis in $CDCl_3$ as well as in DMSO-d_6 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 ^{13}C chemical shift values of proline Cβ and Cγ ($\Delta\delta_{\beta\gamma}$ = 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-d_6 and cross peaks Aha NH↔Leu CαH, Aha NH↔Phe NH further support a 9-membered H-bonding between Aha C=O–Aha NH.

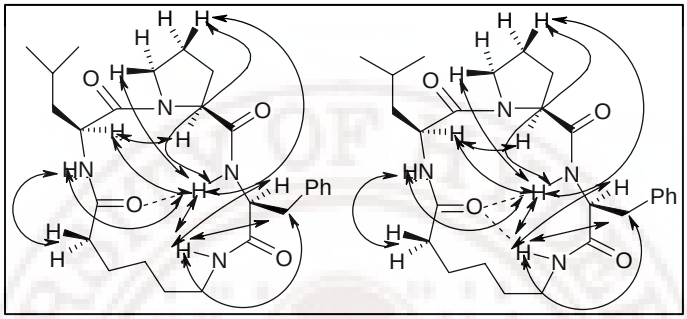
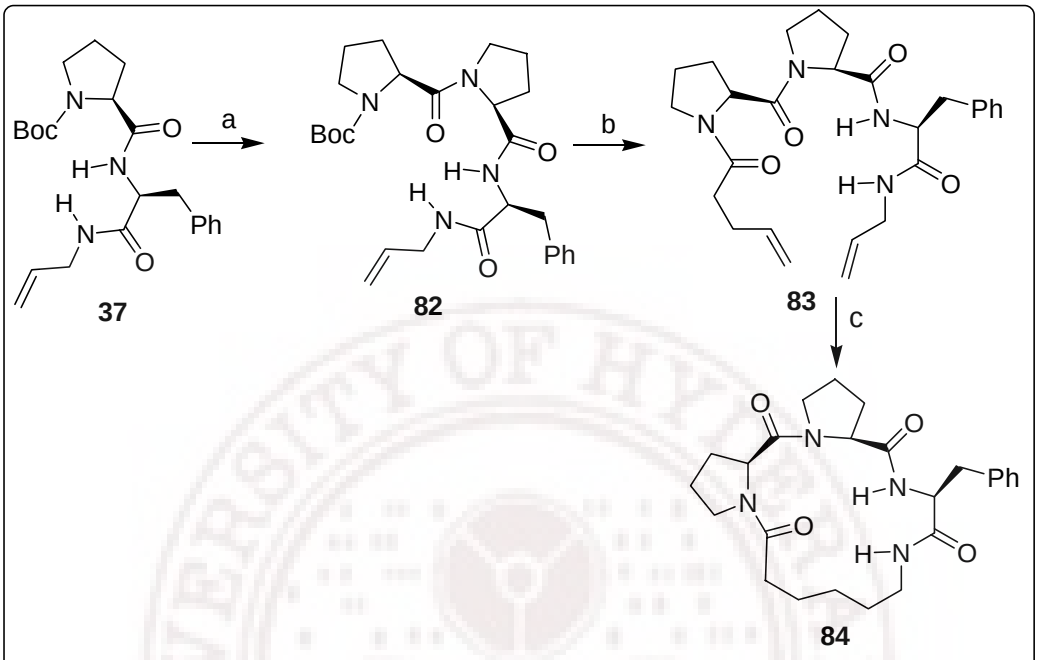


Figure-**26** *Diagnostic NOE correlations of cyclo(Leu-Pro-Phe-Aha)* ***81*** *in* $CDCl_3$ *and* $DMSOd_6$

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, CH_2Cl_2, ii. N-Boc Pro, EDC.HCl, HOBt, CH_2Cl_2 ,0 ^oC-rt, 75%. (b) i. TFA, CH_2Cl_2, ii. 4-pentenoic acid, $ClCO_2{}^iBu$, NEt_3, CH_2Cl_2, 0 ^oC-rt, 57 % (c) i. Grubb's catalyst, Dry CH_2Cl_2, reflux ii. H_2, Pd/C, MeOH, 48 % over two steps.

The ^1H NMR spectrum of **83** in $CDCl_3$ 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 $CDCl_3$ 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↔^{L}Pro1 CδHs and ^{L}Pro1 CαH↔^{L}Pro2 CαH are in agreement with a *trans* amide bond about Aha-^{L}Pro1 and *cis* amide bond about ^{L}Pro1-^{L}Pro2 (spectrum 31B, page # 155). This was further confirmed by the difference between ^{13}C 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 ($\Delta\delta_{C\beta\gamma}$ = 3.00 ppm), where as the second proline ring which has a *cis* amide bond preceding it, showed large chemical shift difference ($\Delta\delta_{C\beta\gamma}$ = 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, ^{L}Pro1 CαH ↔^{L}Pro2 CαH, Phe NH↔^{L}Pro1 CαH, Aha NH↔^{L}Pro1 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**).

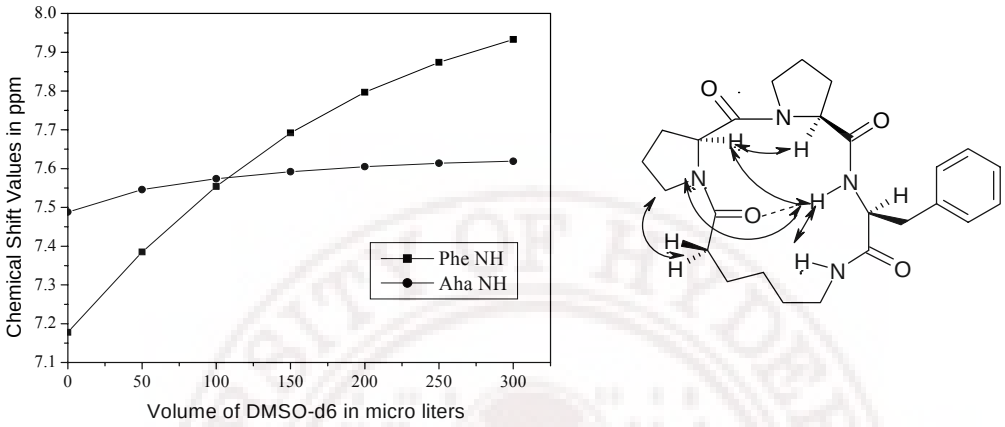


Figure-**27**: *Titration plot and diagnostic NOE correlations observed for peptide **84** in $CDCl_3$.*

In polar solvent, DMSO-d_6 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 (t*rans* – c*is* 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 Δδ/ΔT values ruled out the possibility of H-bonding. The NOE cross correlations Aha CαHs↔^{L}Pro1 CαH and ^{L}Pro1 CαH↔ ^{L}Pro2 CαH confirm the presence of *cis* amide about Aha-Pro1 and *cis* amide about ^{L}Pro1-^{L}Pro2 residues (c*is* – c*is* isomer) (Figure-**28**). The unprecedented up field appearance of Pro2 C*γ(Pro-S)*H at 0.70 in $CDCl_3$ and 0.81 in DMSO-d_6 as well as the NOEs between Pro2 C*γ(Pro-S)*H↔Phenyl aromatic protons unequivocally support the existence of CH···π H-bonding between Pro2 C*γ(Pro-S)*H and Phe aromatic ring.

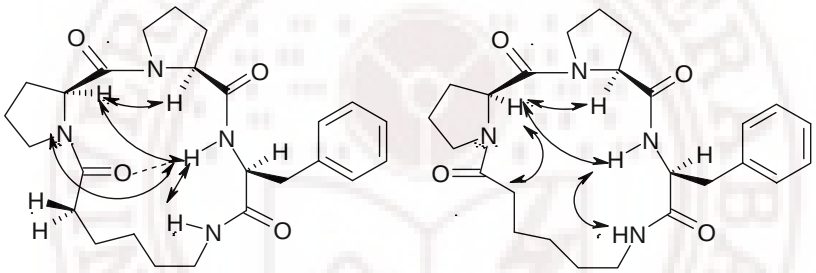
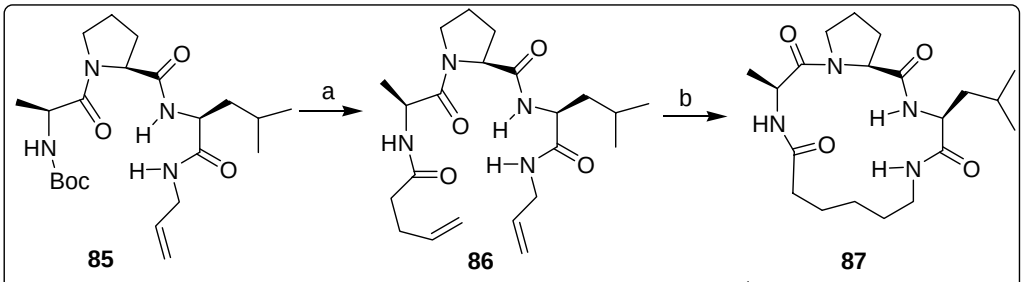


Figure-**28**: *Diagnostic nOe correlations observed for two isomers in DMSO-d_6 of peptide **81** trans-cis and cis-cis isomers.*

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***



Reagents and conditions: (a) i. TFA, CH_2Cl_2, 4-pentenoic acid, $ClCO_2{}^iBu$, NEt_3, CH_2Cl_2, 0 °C-rt, 56.3 % (c) i. Grubb's catalyst, Dry CH_2Cl_2, reflux ii. H_2, Pd/C, MeOH 56 % over two steps.

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 β-turn about Ala-Pro residues as shown in the Figure-**29**.

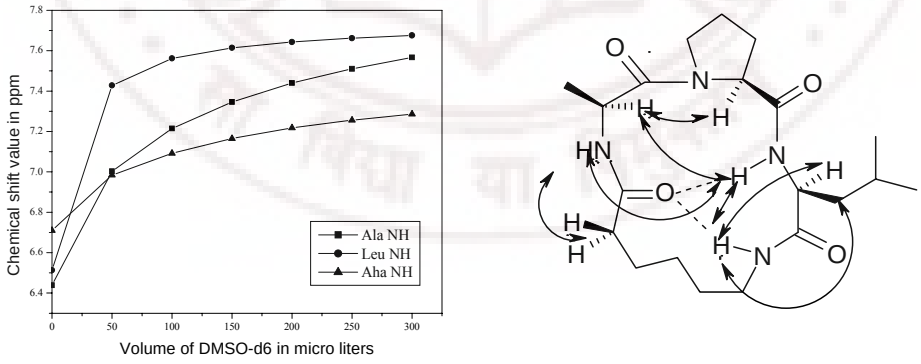
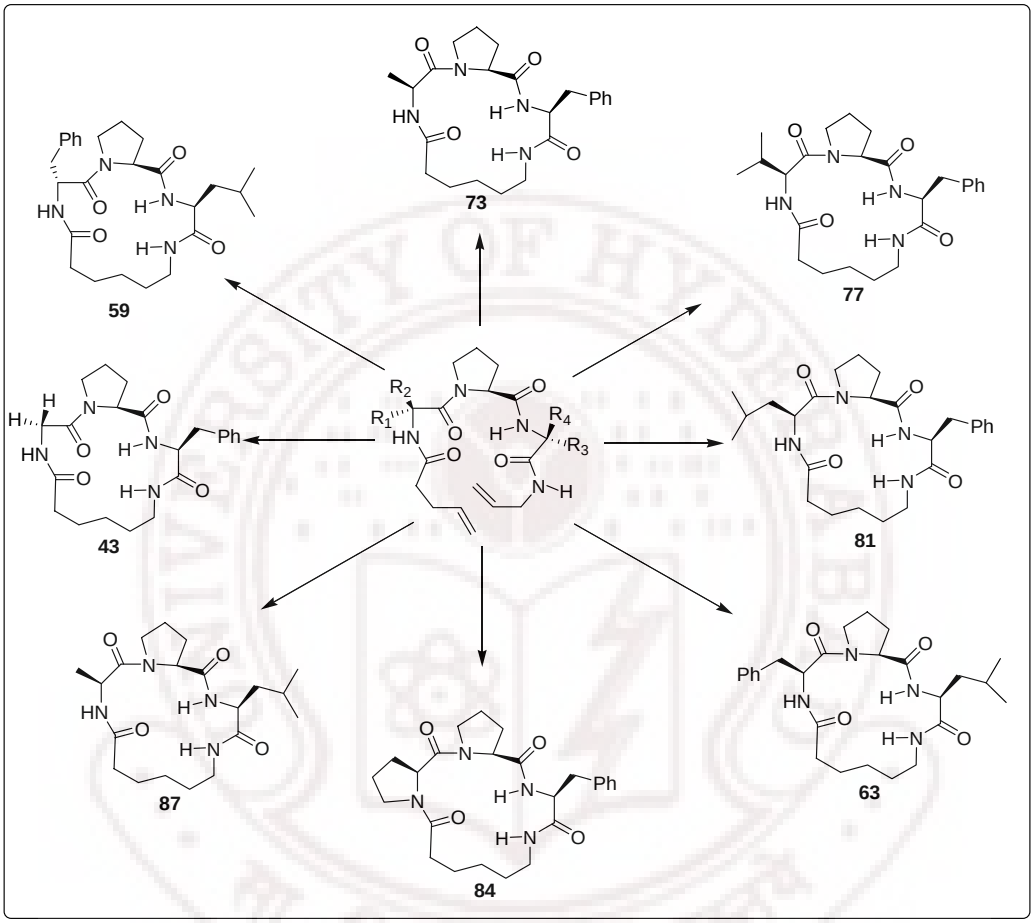


Figure **29**: *Solvent Titration plot and diagnostic NOE correlations observed for cyclo(Ala-Pro-Leu-Aha)* ***87*** *in DMSOd_6*

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.

Results at a Glance



2.5 References

1. Vendruscolo, M., Zurdo, J., MacPhee, C. E. & Dobson, C. M. Protein folding and misfolding: a paradigm of self-assembly and regulation in complex biological systems. *Phil. Trans. R. Soc. Lond.* **2003**, *361***,** 1205.
2. Radford, S. E. & Dobson, C. M. From computer simulations to human disease: emerging themes in protein folding. *Cell* **1999**, *97***,** 291.
3. Pauling, L. The Nature of the Chemical Bond, 3rd edit., **1960,** pp.281, Cornell University Press, Ithaca, NY. (b) Ramachandran, G. N. & Sasisekharan, V. *Advan. Protein Chem*. **1968**, *23*, 283.
4. Schulz, G. D.; Schirmer, R. H. Principles of Protein Structure, **1984**, pp.*25*, Springer-Verlag, Newyork.
5. (a) Brandts, J. F.; Lin, L. N.; *Biochemistry*, **1975**, *14*, 4953. (b) Creighton, T. E.; *J. Mol. Biol*., **1978**, *125*, 401. (c) Schmid, F. X.; Baldwin, R. L. *Proc. Natl Acad. Sci, USA,* **1978,** *75*, 4764. (d) Cook, K. H.; Schmid, F.X.; Baldwin, R. L. *Proc. Natl. Acad. Sci, USA,* **1979,** *76*, 6157. (e) Lin., L. N.; Brandts, J. F. *Biochemistry*, **1984**, *23*, 5713. (f) Brandts, J. F.; Lin., L. N.; *Methods Enzymol.,* **1986**, *131*, 107. (g) Kim, P. S.; Baldwin, R. L. *Annu. Rev. Biochem*., **1990**, *59*, 631.
6. For previous studies before Weiss *et al* see (a) MacArthur, M. W.; Thorton, J. M. *J. Mol. Biol*. **1991**, *218*, 397. (b) Weiss, M. S.; Jabss, A.;

Hilgenfeld, R. *Nature Struct. Biol*., **1998**, 203, 221. (c) Jabs, A.; Weiss, M. S.; Hilgenfeld, R. *J. Mol. Biol*. **1999**, *286*, 291.

7. Muller, G.; Gurrath, M.; Kurz, M.; Kessler, H. *Proteins: Struct. Funct. Genet.* **1993**, *15*, 235.
8. Reviews and leading articles on β-turns include the following: (a) Wilmot, C. M.; Thornton, J. M. *J. Mol. Biol.* **1988**, *203*, 221. (b) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1. (c) Richardson, J. S. *Adv. Protein Chem.* **1981**, *34*, 167. (d) Lewis, P. N.; Momany, F. A.; Scheraga, H. A. *Biochim. Biophys. Acta* **1973**, *303*, 211. (e) Venkatachalam, C. M. *Biopolymers* **1968**, *6*, 1425.
9. Eggleston, D. S.; Baures, P. W.; Peishoff, C. E.; Kopple, K. D. *J. Am. Chem. Soc.* **1991**, *113*, 4410.
10. E, Fujikawa, A.; In, Y.; Inoue, M.; Ishida, T.; Nemoto, N.; Kobayashi, Y.; Kataoka, R.; Ikai, K.; Takesako, K.; Kato, I. *J. Org. Chem.* **1994**, *59*, 570.
11. A, Morita, H.; Yun, Y. S.; Takeya, K.; Itokawa, H.; Shiro, M. *Tetrahedron* **1995**, *51*, 5987.
12. Morita, H.; Gonda, A.; Takeya, K.; Itokawa, H.; Hirano, T.; Oka, K.; Shirota, O. *Tetrahedron* **1997**, *53*, 7469.
13. Fischer, E. *Ber. Dtsch. Chem. Ges.* **1902**, *35*, 2660.
14. Stetten. M. R. *J. Biol. Chem*. **1949**. *181*, 31.

15. Irreverre, F.; Morita, K.; Robertson, A. V.; Witkop, B. *J. Am. Chem. Soc*. **1963**, *85*, 2824.

16. Weiss, M.-T.; Weninger, M.; Hausler, J.; Lubec, G. *Padiatrie Padagogie* **1988**, *23*, 9.

17. Mauger, A. B. *Top. Antibiot. Chem*. **1980**, *5*, 224.

18. Brockmann, H.; Pampus, G.; Manegold, J. H. *Chem. Ber*. **1959,** *92*, 1294.

19. (a) Brockmann, H.; Manegold, J. H. *Chem. Ber*. **1960,** *93*, 2971. (b) Brockmann, H.; Manegold, J. H. *Chem. Ber*. **1962,** *95*, 1081.

20. (a) Katz, E.; Mason, K. T.; Mauger, A. B. *Biochem. Biophys. Res. Commun*. **1975**. *63*, 502. (b) Mauger, A. B.; Stuart, O. A.; Katz, E.; Mason, K. T. *J. Org. Chem*. **1977**, *42*, 1000.

21. (a) Brockmann, H,; Stahler, E. A. *Tetrahedron Lett*. **1973**, 2567. (b) Katz, E.; Mason, K. T.; Mauger, A. B. *Biochem. Biophys. Res. Commun*. **1973**, *52*, 819.

22. Takahashi, Y; Naganawa, H.; Takita, T.; Umezawa, H.; Nakamura, S. *J. Antibiot*. **1976**, *29*, 1120.

23. Schipper, D. *J. Antibiot.* **1983**, *36*, 1076.

24. Fowden, L.; Smith, A.; Millington, D. S.; Sheppard, R. C. *Phytochemistry* **1969**, *8*, 437.

25. (a) Murakami, S.; Takemoto, T.; Shimizu, Z.; Daigo, K. *Jpn. J. Pharm. Chem*. **1953**, *25*, 571. (b) Murakami, S.; Takemoto, T.; Shimizu, Z.;

Daigo, K. *J. Pharm. Soc. Jpn.* **1953**, *73*, 1055-1057. (c) Nadler, J. V. *Life Sci.* **1979**, *24*, 289.

26. Dirr, H.: Reinemer, P.; Huber, R. *Eur. J. Biochem.* **1994**, *220*, 645.
27. Evans, T. C. Jr.; Nelsestuen, G. L.; *Biochemistry*, **1996**, *35*, 8210.
28. M. Oka, G. T. Montelione, and H. A. Scheraga, *J. Am. Chem. Soc*. **1984**, *106*, 7959.
29. Brauer, A. B. E.; Domingo, G. J.; Cooke, R. M.; Matthews, S. J.; Leatherbarrow, R. J. *Biochemistry* **2002**, *41*, 10608.
30. Keller, M.; Boissard, C.; Patiny, L.; Chung, N. N.; Lemieux, C.; Mutter, M. Schiller, P.W. *J. Med. Chem.* **2001**, *44*, 3896.
31. Nielsen, K. J.; Watson, M.; Adams, D. J.; Hammarstrom, A. K.; Gage, P. W.; Hill, J. M.; Craik, D. J.; Thomas, L.; Adams, D.; Alawood, P. F.; Lewis, R. J. *J. Biol. Chem*. **2002**. *277*, 27247.
32. Fischer, G. *Angew.Chem. Int. Ed. Engl*. **1994**, *33*, 1415.
33. Yaffe, M. B.; Schutkowski M.; Shen, M.; Zhou, X. Z.; Stukenberg, P. T.; Rahfeld, J. U.; Xu, J.; Kuang, J.; Kirschner, M. W.; Fischer, G.; Cantley, L. C.; Lu. K. P. *Science* **1997**, *278*, 1957.
34. (a) Crenshaw, D. G.; Yang, J.; Means, A. R.; Kornbluth, S. *EMBO J.* **1998**, *17*, 1315. (b) Stukenberg, P. T.; Kirschner, M. W. *Mol. Cell* **2001**, *7*, 1071.
35. King, R. W.; Jackson, P. K.; Kirschner, M. W. *Cell* **1994**, 79, 563

36. (a) Kallen, J.; Spitzfaden C.; Zurini, M. G. M.; Wider, H.; Wuthrich, K.; Walkinshaw, M. D. *Nature* **1992** *356*, 24. (b) Kallen, J.; Walkinshaw**,** M. D. *FEBS Len*. **1992**, 300, 286-290. (c) Zhao, Y.; Ke, H. *Biochemistry* **1996**, *35*, 7356.

37. Ranganathan, R.; Lu, K. P.; Hunter T.; Noel, J. P. *Cell* **1997**, *89*, 875.

38. Brady, S. F.; Paleveda, W. J.; Arison, B. H.; Saperstein, R.; Brady, E. J.; Raynor, K.; Reisine, T.; Veber, D. F.; Freidinger, R. M. Tet. Lett. **1993**, *49,* 3449.

39. (a) Zabrocki, J.; Smith, G. D.; Dunbar, J. B.; Jr.; Iijima, H.; Marshall, G. R. *J. Am. Chem. Soc*. **1988**, *110*, 5875. (b) Zabrocki, J.; Dunbar, J. B., Jr.; Marshall, K. W.; Toth, M. V.; Marshall, G. R. *J. Org. Chem*. **1992**, *57*, 202.

40. Boteju, L W.; Zalewska, T.; Yamamura, H. I.; Hruby, V. J. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2011.

41. Hitotsuyanagi, Y.; Motegi, S.; Fukaya H.; Takeya K. *J. Org. Chem*. **2002**, *67*, 3266.

42. Hart, S. A.; Sabat, M.; Etzkorn, F. A. *J. Org. Chem*. **1998**, *63*, 7580.

43. (a) Kim, K.; Dumas, J. P.; Germanas, J. P. *J. Org. Chem*. **1996,** *61* 3138. (b) Kim, K.; Germanas, J. P. *J. Org. Chem*. **1997** *62*, 2853.

44. Hoffmann, T.; Lanig, H.; Waibel, R.; Gmeiner, P. *Angew. Chem. Int. Ed* **. 2001,** *40,* 3361.

45. (a) Beausoleil, E.; L'Archeveque, B.; Belec, L.; Atfani, M.; Lubell, W. D. *J. Org. Chem*. **1996**, *61*, 9447. (b) Halab, L; Lubell, W. D. *J. Org. Chem*. **1999,** *64,* 3312.

46. Belec, L.; Slaninova, J.; Lubell, W. D. *J. Med. Chem*. **2000,** *43*, 1448.

47. (a) Haack, T.; Mutter, M. *Tet. Lett.* **1992,** 33, 1589.

48. Wittelsberger, A; Keller, M.; Scarpelino, L.; Patiny, L.; Acha-Orea, H.; Mutter, M. *Angew. Chem. Int. Ed.* **2000,** *39,* 1111.

49. Boruah, A.; Rao, I. N.; Nandy, J. P.; Kumar, S. K.; Kunwar, A. C.; Iqbal, J. *J. Org. Chem.* **2003**; *68,* 5006.

50. Raghothama, S.; Chaddha, M.; Balaram, P. *J. Phys. Chem.* **1996**, *100*, 19666.

51. M. Nishio, M. Hirota and Y. Umezawa, The CH/p Interaction. Evidence, Nature and Consequences, Wiley-VCH, New York, 1998.

52. (a) Infantes, L; Fa´bia´n, L; Motherwell, W. D. S. *Cryst. Eng. Comm*, **2007**, *9*, 65 (b) Gillon, A. L.; Feeder, N.; Davey R. J.; Storey, R.; *Cryst. Growth. Des*. **2003**, *3*, 663.

53. See Table VI on next page

Table VI (Ref codes)

S.No	Water···Peptide H bonds	Water···peptide and peptide···peptide H bonds	Peptide···peptide H bonds
1	ACEBUG	AWONOP	AAGGAG10
2	ADUGAH	BINJIR	CLPGDH
3	BAMLIK	BIHTUH	LACSUD
4	CAHWEN	BIHXUL10	OHUXEU
5	CAZSEB	BIXPAZ10	VOPYUU
6	CEWCIQ10	CGDLLL10[a]	VOYZOY
7	CGLPGL	CHPSAR	YIJDIE01
8	CPSAYL10	CINYED	YILMUB
9	DEWFEQ	CYLATW10	ZARZOH
10	DUPKEE	DUTLAF10	
11	DUYTIA[a]	FAQYIG	
12	GABXEN	FAQYEC	
13	GAGDUQ	FUDWIK	
14	HESFUG	GABWOW	
15	IWUGUC	GABWUC	
16	LTAVAV	GAGFAY	
17	PAANTD	GLSARM	
18	TOSWED	LALWIF01	
19	VOKZUQ	PAWJUT	
20	VORZEH	PEZNAJ	
21	XAZFUA[a]	PHLEGL10	
22		PROGLY20	
23		QOJQAH	
24		SEFTIG	
25		VEGROO	
26		VOKKAH	
27		ZAMNIK	
28		ZOZTUD	

[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 (1equivalent) in dry CH_2Cl_2 was added NEt_3 (2 equivalent) followed by the addition of $ClCO_2{}^{i}Bu$(1.5 equivalent). After 5 min at 0 ^{o}C a solution of allylamine (1.2 equivalent) in dry CH_2Cl_2 was added and stirred at room temperature for a period of 12 h. The reaction mixture was diluted with $CH2Cl_2$, washed with water, brine, dried over Na_2SO_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-L-Pro-Yaa allylamide (B)

A. To an ice cold stirred solution of N-Boc-AA allylamide (1equivalent) in dry dichloromethane was added trifluoroacetic acid (10 equivalent) at 0 ^{o}C under argon atmosphere and stirred at the same temperature for 3h. Solvent was evaporated to afford the TFA salt as a pale yellow gum, which was neutralized with NEt_3 at 0 ^{o}C to obtain the free amine.

B. To an ice cold stirred solution of N-Boc-L-Proline (1equivalent) in dry dichloromethane at 0 ^{o}C was added NEt_3 (2 equivalents) followed by the addition of isobutyl chloroformate (1.5 equivalent). After 5 min at 0 ^{o}C a solution of amine (obtained in part A) (1equivalent) in dry CH_2Cl_2 was added and stirred at room

temperature for a period of 12 h. The reaction mixture was diluted with CH_2Cl_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_2Cl_2 was added trifluoroacetic acid (10 equivalents) at 0 ^oC 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_2Cl_2 was added a solution of L-Pro-Yaa allylamide (obtained in part A) (1equivalent) in CH_2Cl_2. EDC.HCl (1.5 equivalent) was added portion wise to the reaction mixture at 0 ^oC and then stirred at room temperature for a period of 12 h. The reaction mixture was diluted with CH_2Cl_2 washed with water, brine, dried over Na_2SO_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 ^oC 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 4-Pentenoic acid (1equivalent) in dry CH_2Cl_2at 0 ^oC was added NEt_3 (2 equivalents) followed by the addition of $ClCO_2{}^iBu$ (1.5 equivalents). After 5 min a solution of amine (obtained in part A) (1.53 g, 3.7 mmol) in dry CH_2Cl_2 was added at 0 ^oC and then stirred at room temperature for a period of 12 h. The reaction mixture was diluted with CH_2Cl_2 washed with water, brine, dried over Na_2SO_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.

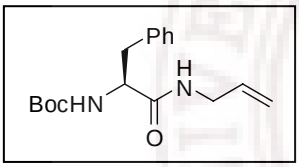
***Ring Closing Metathesis* of Pentenoyl-Xaa-Pro-Yaa allylamide (E)**

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 (1equivalent) in dry CH_2Cl_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 H_2 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.

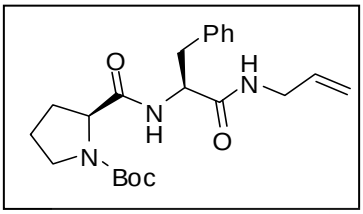
Preparation of N-Boc-L-Phe allylamide (35):



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 $ClCO_2{}^iBu$ and (3.1 ml, 22.62 mmol) of NEt_3 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, $CDCl_3$): 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).

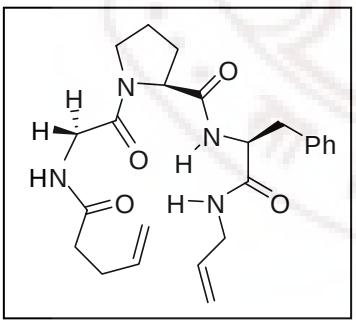
Preparation of N-Boc-Pro-Phe allylamide (37):



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 $ClCO_2{}^iBu$ and (3.9 ml, 27.9 mmol) in 86 % yield as a pale yellow gum.

[α] = -51.00 (c, 0.1, MeOH), IR (Neat): 3297, 2977, 1663, 1548, 1396 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): 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).

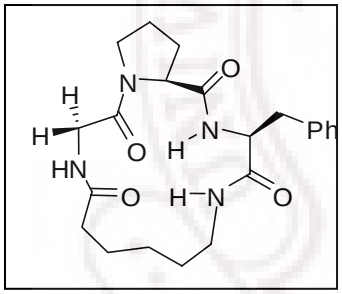
Preparation of *N*-Pentenoyl-Gly-Pro-Phe allylamide (41):



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}, ^1H NMR (400 MHz, $CDCl_3$): δ 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):

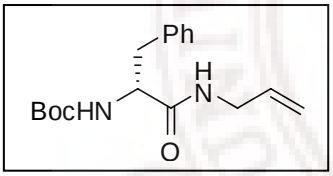


The unsaturated cyclic peptide was obtained by following the general procedure (E) using **41** (250 mg, 0.56 mmol) and 10 mol % Grubbs catalyst in 250 ml of dry CH_2Cl_2 as a mixture of *E*:*Z* (60:40 by TLC) isomers which was transformed to title compound following the general procedure (F) using 30 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}, ^1H NMR (500 MHz, $CDCl_3$): δ 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 CαH), 4.37 (dd, *J* =

8.7 Hz and *J* = 3.8 Hz, 1H, Pro CαH), 4.32 (dd, *J* = 15.9 Hz and *J* = 6.1 Hz, 1H, Gly CαH), 3.75 (m, 1H, Aha CξH), 3.66 (ddd, *J* = 9.7 Hz, 7.8 Hz, 4.1 Hz, 1H, Pro CδH), 3.47 (dd, *J* = 14.2 Hz and *J* = 4.7 Hz, 1H, Phe CβH), 3.43 (m, 1H, Pro Cδ′H), 3.40 (dd, *J* = 15.9 Hz and *J* = 4.8 Hz, 1H, Gly Cα′H), 3.07 (dd, *J* = 14.2 Hz and *J* = 10.5 Hz, 1H, Phe Cβ′H), 2.82 (m, 1H, Aha CξH), 2.35 (m, 1H, Aha CαH), 2.20 (m, 1H, Aha Cα′H), 2.04 (m, 1H, Pro CβH), 1.77 (m, 1H, Pro CγH), 1.72 (m, 1H, Pro Cβ′H), 1.62-1.36 (m, 7H), Mass (CI method (m/z): 415 ($(M+H)^+$, 100).

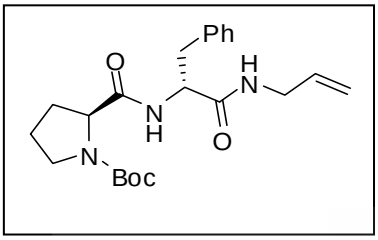
Preparation of *N*-Boc-D-Phe allylamide (36):



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 allylamine (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, [α] = -10.9 (c, 0.5, MeOH), IR (KBr): 3340, 2968, 1686, 1658, 1523, 1169 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): δ 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).

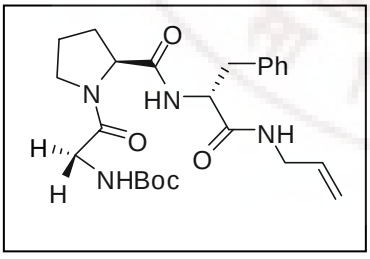
Preparation of *N*-Boc-Pro-D-Phe allylamide (38):



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 $ClCO_2{}^iBu$ and (2.05 ml, 14.65 mmol) of NEt_3 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, $CDCl_3$): 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).

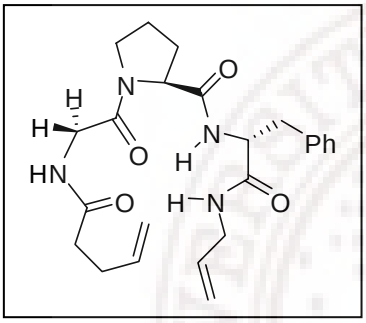
Preparation of *N*-Boc-Gly-Pro-D-Phe allylamide (40):



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}, ^1H NMR (200 MHz, $CDCl_3$): δ 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):

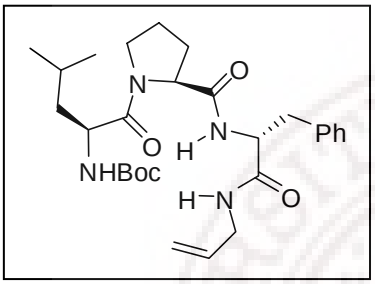


The title product was obtained by following the general procedure (D) using (0.19 ml, 1.90 mmol) of 4-pentenoic acid, (0.68 g, 1.90 mmol) of Gly-Pro-D-Phe allylamide, (0.37 ml, 2.84 mmol) of $ClCO_2{}^iBu$ and (0.8 ml, 5.7 mmol) of NEt_3 to yield (487 mg, 64 %) as a gummy mass.

[α] = -35.0 (c, 0.1, MeOH), IR (Neat): 3305, 3068, 2930, 1640, 1545, 1448 cm^{-1}, ^1H NMR (500 MHz, $CDCl_3$): δ 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 CαH), 4.23 (dd, *J* = 7.8 Hz and *J* = 4.6 Hz, 1H, Pro CαH), 4.09 (dd, *J* = 17.8 Hz and *J* = 4.8 Hz, 1H, Gly CαH), 3.91 (dd, *J* = 17.8 Hz and *J* = 3.7 Hz, 1H, Gly CαH), 3.85-3.81 (m, 2H, Allylic-CH_2), 3.54 (ddd, *J* = 9.8, 7.6, 5.2 Hz, 1H Pro CδH), 3.45 (m, 1H), 3.22 (dd, *J* = 14.1 Hz and *J* = 7.0 Hz, 1H, Phe CβH), 3.14 (dd, *J* = 4.1 and *J* = 6.2

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-D-Phe allylamide (45):

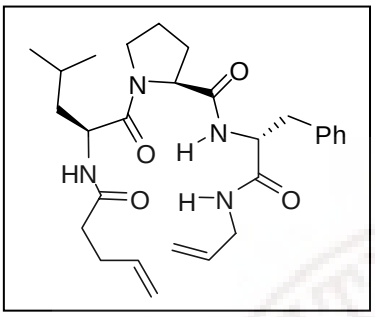


The title compound was obtained following the general procedure (A) using (0.82 g, 3.54 mmol) of N-Boc-Leucine, (1.07 g, 3.54 mmol) of Pro-D-Phe allylamide, of (576 mg, 4.26 mmol) 1-hydroxy benzotriazole and (1.02 g, 5.33 mmol) of EDC.HCl to afford (0.98 g, 54 %) of title product as a white solid.

IR (KBr): 3425, 2925, 2855, 1639, 1533, 1450, 1168 cm^{-1}, ^1H 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* = 13.9Hz and *J* = 6.6 Hz, 1H, Phe CβH), 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).

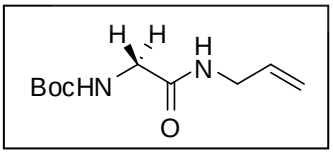
Preparation of *N*-Pentenoyl-Leu-Pro-D-Phe allylamide (46):

The title product was obtained by following the general procedure (D) using (0.19ml, 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 NEt_3 in (0.54 g, 59 %) yield as a colorless gum.



IR (KBr): 3298, 2957, 1635, 1536, 1446 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): 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):

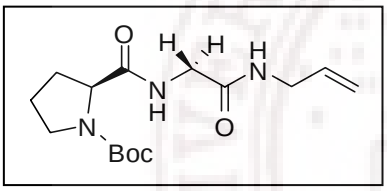


The title compound was obtained following the general procedure (A) using (1.2 g, 6.85 mmol) of *N*-Boc-Glycine, (0.6 ml, 8.22 mmol) of allylamine, (1.1

ml, 8.22 mmol) of $ClCO_2{}^iBu$ 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}, ^1H NMR (200 MHz, $CDCl_3$): δ 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):



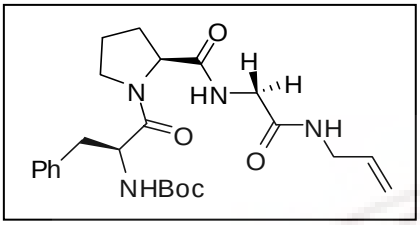
The title compound was obtained following the general procedure (B) using (0.77 g, 3.59 mmol) of *N*-Boc-Proline, (0.41 g, 3.59 mmol) of Glycine allylamide, (0.7 ml, 5.37 mmol) of $ClCO_2{}^iBu$ and (1.50 ml, 10.74 mmol) of NEt_3 to afford (0.78 g, 69 %) of title product as a colorless gum.

IR (Neat): 3321, 3085, 2981, 2933, 1673, 1546, 1410 cm^{-1}, ^1H NMR (200 MHz, $CDCl_3$): δ 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), 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

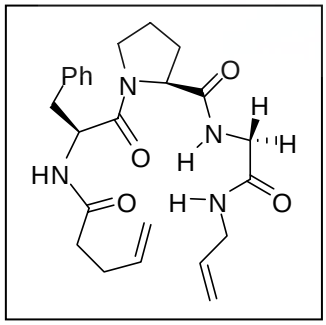
ml, 4.24 mmol) of $ClCO_2{}^iBu$ and (1.20 ml, 8.49 mmol) of NEt_3 to afford (1.0 g, 76 %) of title product as a colorless gum.



[α] = -13.90 (c, 0.18, MeOH), IR (Neat): 3319, 3066, 2979, 1662, 1532, 1448, 1167 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$): δ 7.31-7.20 (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 Hz and *J* = 6.93 Hz, 1H, CαH), 4.33-4.29 (m, 1H, CαH), 3.96-3.69 (m, 4H), 3.54-3.51 (m, 1H), 3.25-3.19 (m, 1H), 2.99 (d, *J* = 6.8 Hz, 2H), 2.16-2.04 (m, 3H), 1.97-1.86 (m, 1H), 1.42 (s, 9H), Mass (CI method) (m/z): 459 ($(M+H)^+$, 48).

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

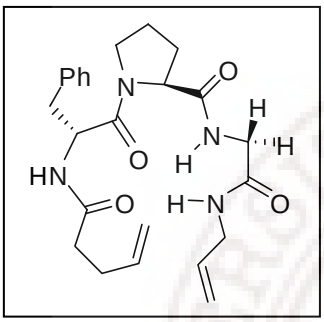
The title product was obtained by following the general procedure (D) using (0.26ml, 2.64 mmol) of 4-pentenoic acid, (860 mg, 2.40 mmol) of Phe-Pro-Gly allylamide, (0.47 ml, 3.60 mmol) of isobutyl chloroformate and (1.0 ml, 7.20 mmol)in (590 mg, 56 %) yield as a gummy mass.



[α] = -7.00 (c, 0.1, MeOH), IR (Neat): 3308, 3077, 2928, 1637, 1538, 1447 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$): δ 7.31-7.17 (m, 5H), 7.06 (bs, 1H), 6.52 (bs, 1H), 6.28 (d, *J* = 7.8 Hz, 1H), 5.88-5.69 (m, 2H),

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):

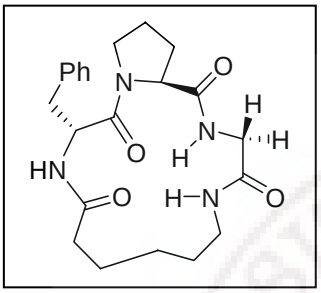


The title product was obtained by following the general procedure (D) using (0.13 ml, 1.25 mmol) of 4-pentenoic acid, (550 mg, 1.25 mmol) of Phe-Pro-Gly allylamide, (0.25 ml, 1.87 mmol) of $ClCO_2{}^iBu$ and (0.53 ml, 3.75 mmol) of NEt_3 to yield (338 mg, 64 %) yield as a gummy mass.

IR (Neat): 3305, 1640, 1545, 1448 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): δ 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-PheCαH), 4.39-4.36 (m, 1H, Pro CαH), 4.19-4.15 (dd, *J* = 16.9 Hz and *J* = 7.7 Hz, Allylic CH), 3.89-3.80 (m, 2H, Gly Cββ′H), 3.74-3.69 (m, 1H, Pro CδH), 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 CβH), 2.97 (dd, *J* = 12.9 Hz and *J* = 6.4 Hz, Phe Cβ′H), 2.69-2.65 (m, 1H, Pro Cδ′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).

Preparation of c(D-Phe-Pro-Gly)(54):

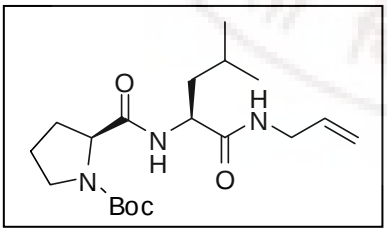
The unsaturated cyclic peptide was obtained by following the general procedure (E) using (280 mg, 0.63 mmol) of **50** and 10 mol % of Grubbs catalyst to yield a



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, $CDCl_3$): 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).

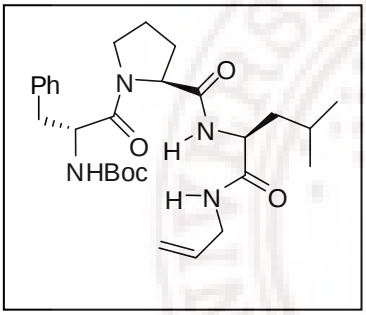
Preparation of *N*-Boc-Pro-Leu allyl amide (55):



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 $ClCO_2{}^iBu$ and (9.6 ml, 68.79 mmol) of NEt_3 to afford (8 g, 95 %) of title product as a white solid.

mp. 92-96 °C, [α] = -89.00 (c, 0.1, MeOH), IR ($CHCl_3$): 3292, 2958, 1652, 1550 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): 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-D-Phe-Pro-Leu allylamide (56):

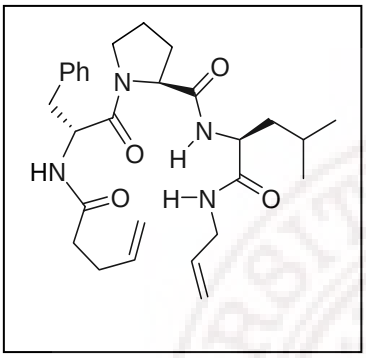


The title product was obtained by following the general procedure (C) using (3.57 g, 13.48 mmol) of N-Boc-D-Phenylalanine and (3.6 g, 13.48 mmol) of Pro-Leu-allylamide in (8.7 g, 81 %) yield as a white solid.

[α] = -89.0 (c, 0.1, MeOH), IR (Neat): 3321, 2926, 1659, 1530, 1450, 11667 cm^{-1},^1H NMR (400 MHz, $CDCl_3$): 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 CH_2), 5.04 (d, *J* = 4.3 Hz, 1H, D-Phe NH), 4.50-4.44 (m, 1H, Leu CαH), 4.40-4.38 (m, 1H, Pro CαH), 4.36-4.33 (m, 1H, D-Phe CαH), 3.94-3.90 (m, 1H, Allylic CH), 3.89-3.87 (m, 1H, Allylic CH′), 3.78-3.63 (m, 1H, Pro CδH), 3.01 (dd, *J* = 12.6 Hz and *J* = 9.4 Hz, 1H, Phe CβH), 2.92 (dd, *J* = 12.8 Hz and *J* = 6.4 Hz, 1H, Phe Cβ′H), 2.61 (dd, *J* = 16.10 Hz and *J* = 8.85 Hz, 1H, Pro Cδ′H), 2.11-2.05 (m, 1H, Pro CβH), 1.93-1.85 (m, 1H, Leu CβH), 1.78-1.71 (m,

2H), 1.62-1.55 (m, 3H), 1.38 (s, 9H, t-Bu), 0.91 (d, *J* = 6.5 Hz, 3H, Leu Cδ*H*), 0.87 (d, *J* = 6.4 Hz, 3H, Leu Cδ′H), Mass (CI method) (m/z): 515 ($(M+H)^+$, 100).

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

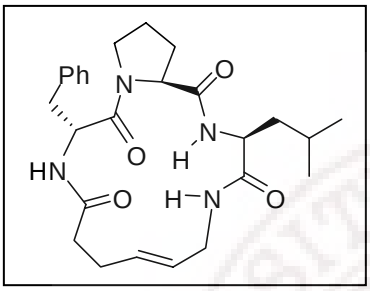


The title product was obtained by following the general procedure (D) using (0.8 ml, 7.78 mmol) of 4-pentenoic acid and (3.22 g, 7.78 mmol) of D-Phe-L-Pro-Leu allyl amide to afford the title compound as a white solid (2.70g, 70 %).

[α] = -118 (c, 0.1, MeOH), IR (KBr): 3293, 1676, 1636, 1544 cm^{-1}, ^1H NMR (500 MHz, $CDCl_3$): 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),

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 (CI method) (m/z): 497 $(M+H)^+$100).

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

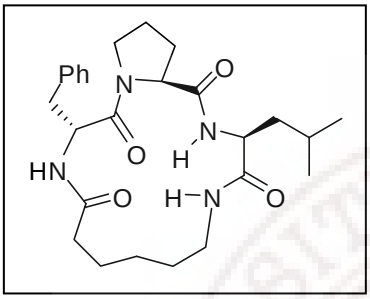


The title product was obtained by following the general procedure (E) using (250 mg, 0.50 mmol) of *N*-Pentenoyl-D-Phe-L-Pro-Leu allylamide and 10 mol% of Grubb's first generation catalyst in 250 ml of dry CH_2Cl_2 to afford cyclic unsaturated peptide as a mixture of *E:Z* (4:1) isomers as an off white 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, $CDCl_3$): 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 CδH), 3.32 (ddd, *J* = 14.9, 6.2, 2.0 Hz, 1H, Aha CαH), 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

(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) .

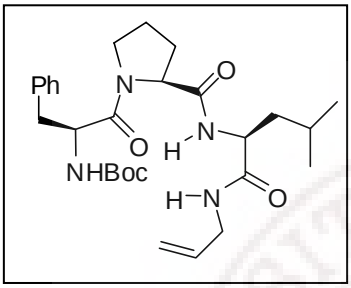
Preparation of c(D-Phe-Pro-Leu) (59):



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, 1451cm^{-1}, ^{1}H NMR (500 MHz, $CDCl_3$): 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 CξH), 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, Cξ′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).

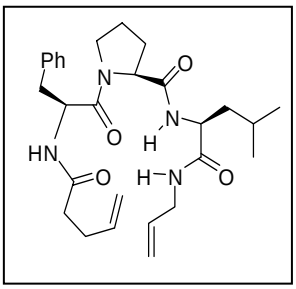
Preparation of *N*-Boc-L-Phe-Pro-Leu allylamide (60):



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.

[α] = -52.0 (c, 0.1, MeOH), IR (Neat): 3300, 2959, 1642 cm^{-1}, ^1H NMR (400 MHz, $DMSOd_6$): δ 7.88 (t, *J* = 5.65 Hz, 1H, allylic NH), 7.83 (d, *J* = 8.3 Hz, 1H, NH), 7.30-7.17 (m, 5H, aromatic), 7.03 (d, *J* = 8.3 Hz, 1H, NH), 5.82-5.73 (m, 1H), 5.14-5.01 (m, 2H), 4.39-4.34 (m, 2H), 4.30-4.24 (m, 1H), 3.69-3.67 (t, *J* = 5.4 Hz, 2H), 3.61-3.55 (m, 2H), 2.95-2.71 (m, 2H), 2.07-2.00 (m, 1H), 1.94-1.80 (m, 3H), 1.65-1.53 (m, 1H), 1.51-1.46 (m, 2H), 1.28 (s, 9H, t-Bu), 0.9 (d, *J* = 6.4 Hz, 3H), 0.84 (d, *J* = 6.5 Hz, 3H), Mass (CI method): 515 ($(M+H)^+$, 10), 514 (65), 415 (100).

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



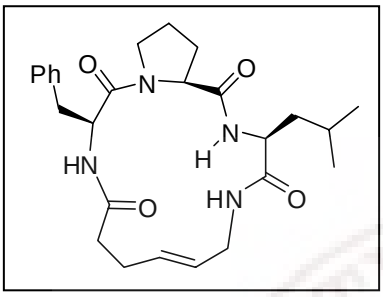
The title compound was obtained following the general procedure (D) using (0.16 ml, 1.55 mmol) of 4-pentenoic

acid, (0.64 g, 1.55 mmol) of Phe-Pro-Leu allylamide (0.3 ml, 2.33 mmol) of $ClCO_2{}^iBu$ and (0.65 ml, 4.66 mmol) of NEt_3 to afford (260 mg, 53 %) of title product as a colorless gum.

[α] = -78.0 (c, 0.1, MeOH), IR (Neat): 3291, 2957, 1636, 1547, 1445 cm^{-1}, ^1H NMR (500 MHz, $CDCl_3$): δ 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 CαH), 4.99 (m, 1H), 4.44 (dd, *J* = 8.2, 4.2 Hz, 1H, Pro CαH), 4.41 (m, 1H. Leu CαH), 3.89-3.91 (m, 2H, allylic CH_2), 3.67 (m, 1H, Pro_CδH), 3.09 (ddd, *J* = 10.0, 6.8, 5.4 Hz, 1H, Pro Cδ′H), 3.01 (dd, *J* = 13.5, 7.8 Hz, 1H, Phe CβH), 2.99 (dd, *J* = 13.5, 6.4 Hz, 1H, Phe Cβ′H), 2.33-2.21 (m, 4H, pentenoyl), 2.12-1.90 (m, 4H, Pro CβγH), 1.85 (m, 1H, Leu CγH), 1.57 (m,1H, Leu CβH), 1.49 (m,1H, Leu Cβ′H), 0.95 (d, *J* = 6.5 Hz, 3H, Leu CδH), 0.91 (d, *J* = 6.5 Hz, 3H, Leu Cδ′H), Mass (CI method): 497 ($(M+H)^+$, 100), 440 (9), 327 (16), 268 (47).

Preparation of unsaturated cyclo(L-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

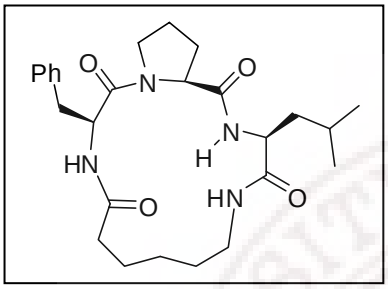


Grubb's first generation catalyst in 240 ml of dry CH_2Cl_2 to afford (180 mg, 80 %) of cyclic unsaturated peptide as a mixture of *E:Z* isomers.

[α] = -31.0 (c, 0.1, MeOH), IR (Neat): 3299, 2925, 2854, 1626 cm^{-1}, ^1H NMR (500 MHz, $CDCl_3$): δ 7.19-7.31 (m, 5H, aromatic), 6.42 (d, *J* = 6.7 Hz, 1H, Phe NH), 6.38 (d, *J* = 8.5 Hz, 1H, Leu NH), 6.00 (dd, *J* = 7.7, 4.6 Hz, 1H, Allylic NH), 5.51 (ddd, *J* = 15.1, 7.5, 5.9 Hz, 1H, Olefinic-CH(γ)-allyl), 5.41 (ddd, *J* = 15.1, 6.8, 4.8 Hz, 1H, Olefinic-CH(β)-allyl), 4.56 (ddd, *J* = 10.3, 4.5 Hz, 1H, Phe CαH), 4.35 (m, 1H, Leu CαH), 4.05 (ddd, *J* = 15.0, 7.7, 6.8 Hz, 1H, Allylic CαH), 3.60 (ddd, *J* = 12.2, 8.5, 2.5 Hz, 1H, Pro CαH), 3.37 (m, 1H, Pro CδH), 3.36 (m, 1H, Pro Cδ′H), 3.33 (dt, *J* = 15.0, 4.7 Hz, 1H, Allylic Cα′H), 3.32 (dd, *J* = 12.5, 4.5 Hz, 1H, Phe CβH), 2.77 (dd, *J* = 12.5, 10.3 Hz, 1H, Phe Cβ′H), 2.55-2.18 (m, 4H, Pentenoyl), 1.83 (m,1H, Pro CβH), 1.71 (m, 3H, Pro Cβ′H + C γγ′H), 1.55-1.42 (m, 2H, Leu Cββ'H), 1.24 (m, 1H, Leu CγH), 0.90 (d, *J* = 5.1 Hz, 3H), 0.88 (d, *J* = 5.1 Hz, 3H), Mass (CI method):469 $(M+H)^+$, 100).

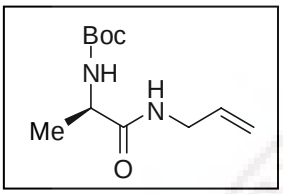
Preparation of cyclo(L-Phe-Pro-Leu) (63):

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, $CDCl_3$): δ 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 CξH), 3.59 (ddd, *J* = 12.3, 8.4, 2.8 Hz, 1H, Pro Cδ′H), 3.45 (dd, *J* = 8.6, 1.9 Hz, 1H, Pro CαH), 3.39 (ddd, *J* = 12.3, 9.9, 7.5 Hz, 1H, Pro CδH), 3.21 (dd, *J* = 12.6, 4.9 Hz, 1H, Phe CβH), 2.90 (m, 1H, Aha Cξ′H), 2.84 (dd, *J* = 12.6, 10.5 Hz, 1H, Phe Cβ′H), 2.32 (ddd, *J* = 14.1, 6.2, 3.8 Hz, 1H, Aha CαH), 2.15 (ddd, *J* = 14.1, 10.6, 3.8 Hz, 1H, Aha Cα′H), 1.84 (m, 1H, Pro CβH), 1.70 (m, 1H, Pro CγH), 1.68 (m,1H, Aha CβH), 1.63 (m, 2H, Aha CH), 1.59 (m, 1H, Pro Cγ′H), 1.57-1.39 (m, 3H), 1.34 (m, 2H), 1.21 (m, 1H, Pro Cβ′H), 1.20 (m, 1H, Aha Cγ′H), 0.90 (d, *J* = 6.2 Hz, 3H, Leu CδH), 0.88 (d, *J* = 6.2 Hz, 3H, Leu Cδ′H), ^{13}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).

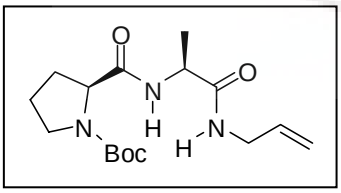
Preparation of *N*-Boc-Ala allylamide (64):



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, (3.10ml, 23.80 mmol) of $ClCO_2{}^iBu$ 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}, ^1H 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).

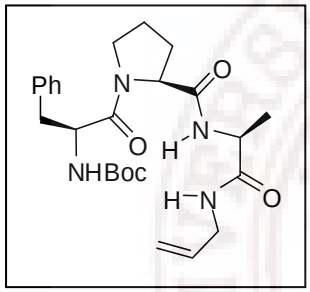
Preparation of *N*-Boc-Pro-Ala allylamide (65):



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 $ClCO_2{}^iBu$ 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, [α] = -84.60 (c, 0.5, MeOH), IR (KBr): 3294, 2926, 1702, 1642, 1401 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): δ 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):

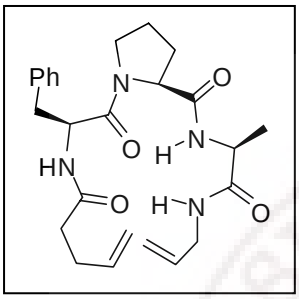


The title product was obtained by following the general procedure (C) using (2.2 g, 9.77 mmol) of *N*-Boc-Phe, (2.62 g, 9.77 mmol) of Pro-Ala allylamide, (1.60 g, 11.70 mmol) of HOBt and (2.81 g, 14.66 mmol) of EDC. HCl to afford the title compound (3.30 g, 60 %) as pale yellow oil.

[α] = -66.5 (c, 0.4, MeOH), IR (Neat): 3307, 2978, 1642, 1523, 1167 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): δ 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):

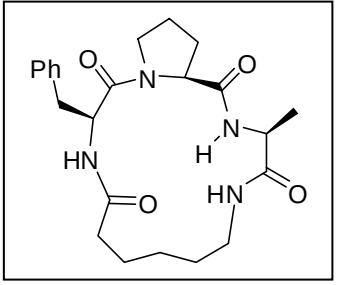
The title product was obtained by following the general procedure (D) using 4-pentenoic acid (0.6 ml, 5.85 mmol) and (2.17g, 5.85 mmol) amine Leu-Pro-Ala allylamide in 57 % yield as a white fluffy hygroscopic solid.



[α] = -68.20 (c, 0.5, MeOH), IR (Neat): 3296, 3077, 1634 cm^{-1}, ^1H NMR (500 MHz, $CDCl_3$): δ 7.32-7.20 (m, 5H, Aromatic), 6.83 (d, *J* = 7.3 Hz, 1H, Ala NH), 6.48 (bs, 1H, Allylic NH), 6.24 (d, *J* = 7.6 Hz, 1H, Phe NH), 5.88-5.81 (m, 1H, Allylic CH), 5.79-5.74 (m, 1H, Pentenoyl CH), 5.22-5.18 (m, 2H, Allylic CH_2), 5.15-5.00 (m, 2H, Pentenoyl CH_2), 4.99-4.97 (m, 1H, Phe CαH), 4.46-4.42 (m, 2H, Pro CαH and Ala CαH), 3.90-3.87 (m, 2H, Allylic CH_2), 3.65-3.62 (m, 1H, Pro CδH), 3.09-2.97 (m, 3H, Phe CβH, Cβ′H & Pro Cδ′H), 2.18-2.14 (m, 1H, Pro CβH), 2.35-2.32 (m, 2H, Pentenoyl), 2.30-2.25 (m, 2H, Pentenoyl), 2.18-2.14 (m, 1H, Pro Cβ′H), 1.99-1.96 (m, 2H, Pro Cγγ′H), 1.38 (d, *J* = 6.9 Hz, 3H), Mass (CI method) (m/z): 455 ($(M+H)^+$, 100).

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

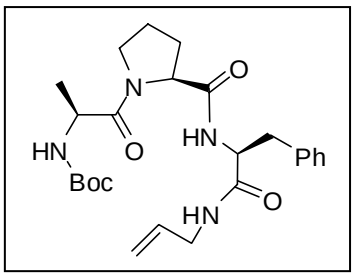
The unsaturated cyclic peptide **68** was obtained by following the general procedure (E) using (180 mg, 0.39 mmol) of **67** and 10 mol % of Grubbs catalyst as a mixture of *E*:*Z* (60:40 by TLC) isomers which was transformed to title product (91 mg, 54 %, white fluffy solid) following the general procedure (F) using 30 mg of 10 % Pd/C in MeOH.



IR (Neat): 3421, 2930, 1636, 1520, 1448 cm^{-1}, ^1H NMR (500 MHz, $CDCl_3$): δ 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 CαH), 4,36 (m, 1H, Ala CαH), 3.74 (m, 1H, Aha CξH), 3.61 (ddd, *J* = 12.3, 8.4, 2.7 Hz, 1H, Pro Cδ′H), 3,42 (dd, *J* = 8.6 Hz and *J* = 1.9 Hz, 1H, Pro CαH), 3.38 (ddd, *J* = 12.3, 10.0, 7.3 Hz, 1H, Pro CδH), 3.19 (dd, *J* =12.5 and *J* = 4.9 Hz, 1H, Phe CβH), 2.91 (m, 1H, Aha Cξ′H), 2,84 (dd, *J* = 12.5 Hz and *J* = 10.7 Hz, 1H, Phe Cβ′H), 2.31 (m, 1H, Aha CαH), 2,16 (m, 1H Aha Cα′H), 1.86 (m, 1H, Pro CβH), 1.69 (m, 1H, Pro CγH), 1.66 (m, 1H, Aha CδH), 1.64 (m, 1H, Aha CβH), 1.60 (m, 1H, Aha Cδ′H), 1.58 (m, 1H), 1.35 (m, 1H, Aha Cβ′H), 1.33 (m, 1H, Aha CγH), 1.27 (d, J = 6.8 Hz, 3H), 1.22 (m, 1H, Pro Cβ′H), 1.18 (m, 1H, Aha Cγ′H), Mass (CI method) (m/z): 429 ($(M+H)^+$, 100).

Preparation of N-Boc-L-Ala-Pro-Phe allylamide(70):

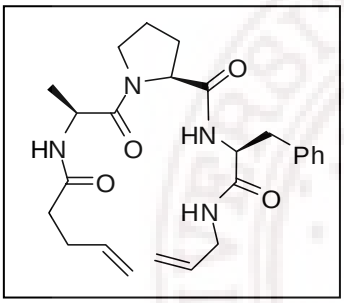
The title product was obtained by following the general procedure (C) using *N*-Boc-L-Ala (630 mg, 3.32 mmol) and (1g, 3.32 mmol) of amine L-Pro-Phe-allyl amide to yield (810 mg, 52 %) as a white solid.



mp. 152-156°C, [α] = -103 (c, 0.5, MeOH), IR (KBr): 3310, 2979, 1643 cm^{-1}, ^1H NMR (400 MHz, $DMSOd_6$): δ 7.81-7.76 (m, 2H), 7.27-7.16 (m, 5H),

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):

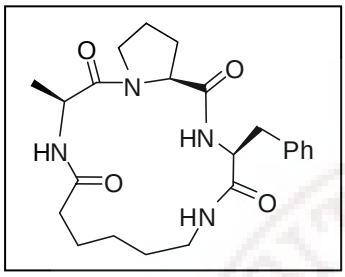


The title product was obtained by following the general procedure (D) using 4-pentenoic acid (0.26 ml, 2.54 mmol) and (0.94 g, 2.54 mmol) amine (Ala-Pro-Phe allylamide) to afford the title compound as a fluffy solid (1.10g, 68 %).

mp. 126-128^{o}C, [α] = -115 (c, 0.1, MeOH), IR (Neat): 3299, 2980, 1637, 1540 cm^{-1}, ^{1}H NMR (400 MHz, DMSOd_6): 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(L-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 CH_2Cl_2 yielded unsaturated cyclic peptide as a mixture of *E:Z* isomers which

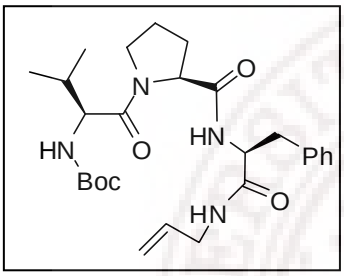


was transformed to title compound following the general procedure (F) using 30 mg of 10 % Pd/C to afford (120 mg, 51 %, over two steps) of title product s white fluffy hygroscopic solid.

[α] = -54 (c, 0.1, MeOH), IR (KBr): 3429, 2925, 1636 cm^{-1}, ^1H NMR (500 MHz, $CDCl_3$): 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 CξH), 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 Cξ′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), ^{13}C 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).

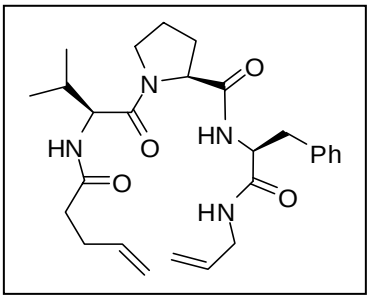
Preparation of *N*-Boc-L-Val-Pro-Phe allyl amide (74):



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

[α] = -125.8 (c, 0.5, MeOH), IR (Neat): 3304, 2972, 1632, 1514, 1444, cm^{-1}, ^1H NMR (400 MHz, $DMSOd_6$): δ 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):

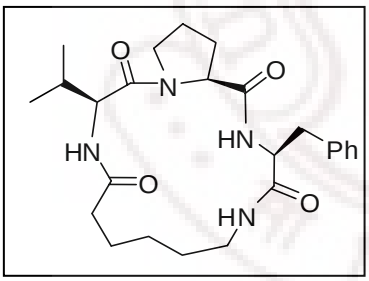


The title product was obtained by following the general procedure (D) using (0.23 ml, 2.20 mmol)

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, $CDCl_3$): 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* = 13.70 Hz and *J* = 6.2 Hz, 1H), 3.04 (dd, *J* = 13.70 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):

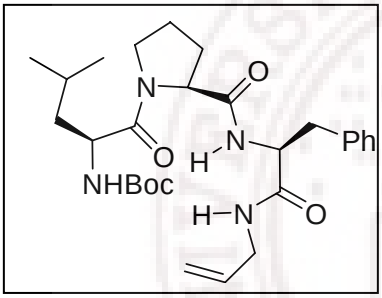


Following the general procedure (E), (250 mg, 0.51 mmol) of *N*-Pentenoyl-Val-Pro-Phe allylamide and 10 mol% of Grubb's first generation catalyst in 250 ml of dry CH_2Cl_2 yielded unsaturated cyclic peptide as a mixture of *E:Z* isomers which was transformed to title compound following the general procedure (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, $CDCl_3$): 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):

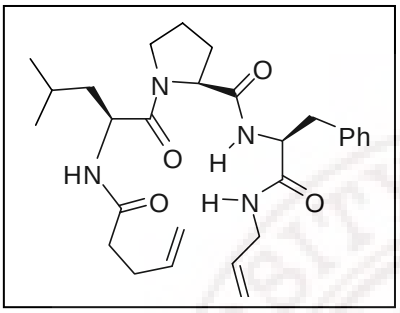


The title product was obtained by following the general procedure (C) using (0.58 g, 2.49 mmol) of *N*-Boc-L-Leu and (0.75 g, 2.49 mmol) of Pro-Phe allylamide (780 mg, 61 %) as white fluffy hygroscopic solid.

[α] = -101 (c, 0.5, MeOH), IR (Neat): 3302, 2958, 1644 cm^{-1}, ^1H NMR (400 MHz, $DMSOd_6$) : δ 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):

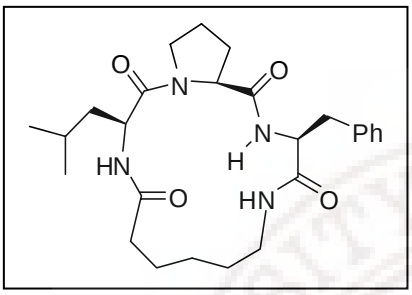
The title product was obtained following the general procedure (D) using (0.1 ml, 0.99 mmol) of 4-pentenoic acid and (0.41g, 0.99 mmol) of Leu-Pro-Phe allylamide in (260 mg, 53 %) yield as a colorless gum.



[α] = -128 (c, 0.1, MeOH), IR (Neat): 3293, 2957, 1637, 1545 cm^{-1}, ^1H NMR (500 MHz, $CDCl_3$): δ 7.36-7.16 (m, 5H, Aromatic), 6.59 (d, *J* = 8.2 Hz, 1H, Phe NH), 6.19 (t, *J* = 5.2 Hz, 1H, Allylic NH), 5.98 (d, *J* = 8.7 Hz, 1H, Leu NH), 5.81 (m, 1H, pentenoyl), 5.72 (m, 1H, Olefinic-CH, Allylic), 5.06 (m, 1H, Olefinic-CH_2 (cis), Allylic), 5.05 (m, 1H, Olefinic-CH (trans), pentenoyl), 5.04 (m, 1H, Olefinic-CH (trans), Allylic), 5.00 (m, 1H, Olefini-CH (cis), pentenoyl), 4.76 (ddd, *J* = 10.3, 3.6 Hz, 1H, Leu CαH), 4.62 (m, 1H, Phe CαH), 4.42 (dd, *J* = 4.1, 8.2 Hz, 1H, Pro CαH), 3.84-3.78 (m, 2H), 3.76 (m, 1H, Pro CδH), 3.44 (m, 1H, Pro Cδ′H), 3.19 (dd, *J* = 13.8, 6.5 Hz, 1H, Phe CβH), 3.08 (dd, *J* = 6.7, 13.8 Hz, 1H, Phe Cβ′H), 2.36-2.29 (m, 4H, pentenoyl), 2.10-1.89 (m, 4H, Pro CβH), 1.62 (m, 1H, Leu CγH), 1.39 (ddd, *J* = 14.4, 10.3, 4.1 Hz, 1H, Leu CβH), 1.17 (ddd, *J* = 14.4, 9.2, 3.6 Hz, 1H, Leu CβH), 0.96 (d, *J* = 6.6 Hz, 3H Leu CδH), 0.96 (d, *J* = 6.6 Hz, 3H, Leu Cδ′H), Mass (CI method) (m/z): 497 ($(M+H)^+$100), 302 (43).

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

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

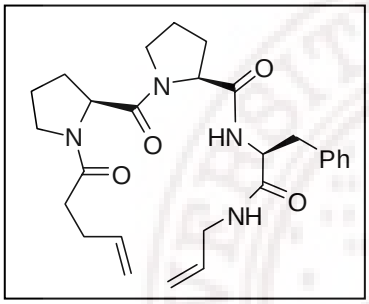


mixture of *E:Z* isomers (60:40 by TLC) which was transformed to title product following the general procedure (F) using 30 mg of 10% Pd/C to afford (120 mg, 88.7 %) of title product s white fluffy hygroscopic solid.

[α] = -71.2 (c, 0.25, MeOH), IR (Neat): 3310, 2929, 1636 cm^{-1}, ^1H NMR (500 MHz, $CDCl_3$): δ 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 CαH), 4.54 (m, 1H, Leu CαH), 4.32 (dd, *J* = 8.3 Hz, and *J* = 2.5 Hz, 1H, Pro CαH), 3.66 (m, 1H, Aha CξH), 3.49 (ddd, *J* = 12.2, 9.5, 7.2 Hz, 1H, Pro CδH), 3.44 (ddd, *J* = 12.2, 8.5, 3.2 Hz, 1H, Pro Cδ′H), 3.19 (dd, *J* = 13.8 Hz and *J* = 6.9 Hz, 1H, Phe CβH), 3.01 (dd, *J* = 13.8 Hz and *J* = 7.7 Hz, 1H), 2.89 (m, 1H, Aha Cξ′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 CβH), 2.05 (m, 1H, Pro Cβ′H), 1.73 (m, 1H, Pro Cγ′H), 1.65-1.57 (m, 3H, Leu Cβ & CγH), 1.62 (m, 1H, Aha Cδ′H), 1.58 (m, 1H, Aha CβH), 1.37 (m, 1H, Aha Cγ′H), 1.34 (m, 1H, Aha CδH), 1.29 (m,1H, Aha Cβ′H), 1.27 (m, 1H, Pro CγH), 1.14 (m, 1H, Aha CγH), 0.96 (d, *J* = 6.5 Hz,

3H, Leu CδH), 0.92 (d, J = 6.5 Hz, 3H, Leu Cδ′H), ^{13}C NMR derived from HSQC and HMBC in $CDCl_3$: δ173.4, 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).

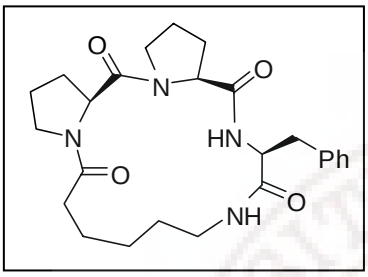
Preparation of *N*-Pentenoyl-Pro-Pro-Phe allylamide (83):



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.

[α] = -95.2 (C, 0.5, MeOH), IR (Neat): 3302, 1640, 1530, 1440 cm^{-1}, ^{1}H NMR (400 MHz, $DMSOd_6$): δ 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).

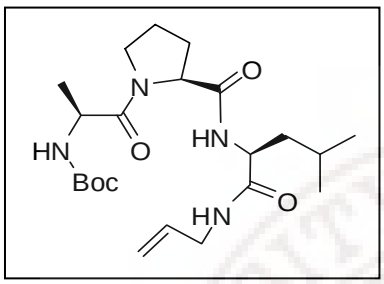
Preparation of cyclo(Pro-Pro-Phe-Aha) (84):



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): δ 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* = 5.9Hz, 1H, Pro CαH), 3.70 (m, 1H, Pro CH), 3.61 (m, 1H, Pro Cδ′H), 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).

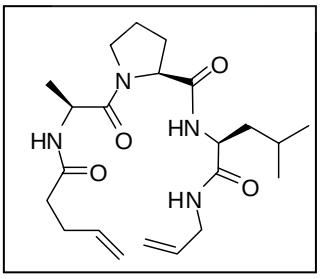
Preparation of *N*-Boc-L-Ala-Pro-Leu allylamide (85):



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 allylamide to yield (800 mg, 56 %) as a white solid.

mp. 132-136^{o}C, IR (KBr): 3406, 3331, 3282, 2960, 1706, 1689, 1658 cm^{-1}, ^{1}H NMR (400 MHz, DMSOd_6): 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):

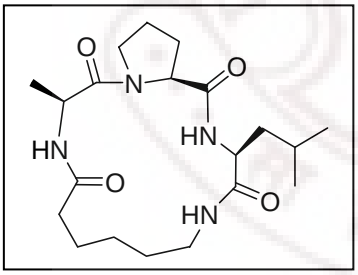


The title product was obtained by following the general procedure (D) using (0.175 ml, 1.71 mmol) of 4-pentenoic acid and (580 mg, 1.71 mmol) of Ala-Pro-Leu allyl amide to afford (405 mg, 56.3 %) yield as a

white solid.

mp. 120-124^{o}C, [α] = -109.4 (c, 0.5, MeOH), IR (KBr): 3290, 2955, 1643, 1548 cm^{-1}, ^{1}H NMR (500 MHz,$CDCl_3$): 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 CH_2), 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 (CI method) (m/z):421 $(M+H)^{+}$, 100).

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

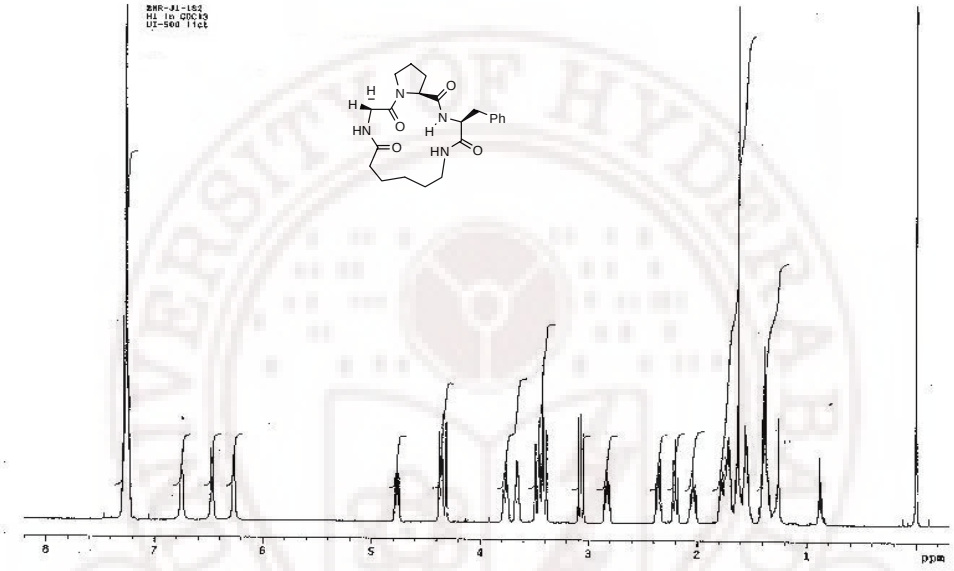


The unsaturated cyclic product was obtained by following the general procedure (E) using (175 mg, 0.41 mmol) of *N*-Pentenoyl-Ala-Pro-Phe allylamide and 10 mol % of Grubb's first generation catalyst to afford of cyclic unsaturated peptide as a mixture of *E:Z* isomers (70:30 by TLC) which was transformed 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.

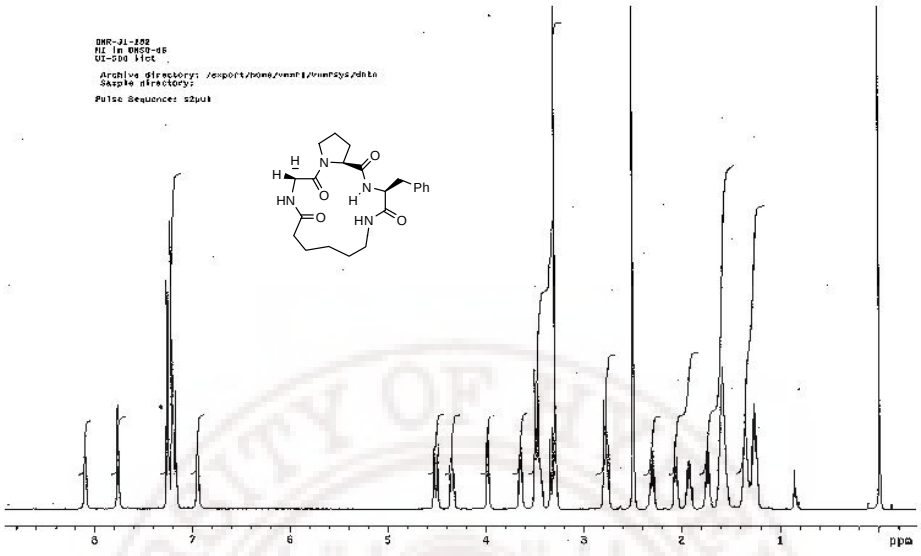
IR (KBr): 3410, 3330, 3284, 2960, 1704 $cm^{-1,}$ ^1H NMR (500 MHz, $CDCl_3$): δ 6.70 (d, *J* = 8.2 Hz, 1H, Leu NH), 6.44 (dd, *J* = 8.2 Hz and *J* = 3.8 Hz, 1H, Aha NH), 6.40 (d, *J* = 6.1 Hz, 1H, Ala NH), 4.45 (m, 1H, Leu CαH), 4.44 (dd, *J* = 8.4 Hz, 2.8 Hz, 1H, Pro CαH), 4.37 (m, 1H, Ala CαH), 3.74 (dd, *J* = 12.2, 8.5, 2.9 Hz, 1H, Pro CδH), 3.67 (m, 1H, Aha CξH), 3.59 (ddd, *J* = 12.2, 9.9, 7.4 Hz, 1H, Pro Cδ′H), 2.91 (m, 1H, Aha Cξ′H), 2.32 (m, 1H, Pro CβH), 2.27 (m, 1H, Aha CαH), 2.25 (m, 1H, Pro Cβ′H), 2.16 (m, 1H, Aha Cα′H), 1.98 (m, 1H, Pro CγH), 1.82 (m, 1H, Pro Cγ′H), 1.63-1.53 (m, 5H, Leu Cββ′γH and Aha Cββ′H), 1.33 (d, *J* = 6.7 Hz, 3H),1.32 (m, 1H, Aha CγH), 1.30 (m, 1H, Aha Cγ′H), 1.22 (m, 2H, Aha Cδδ′H), 0.94 (d, *J* = 6.4 Hz, 3H, Leu CδH), 0.92 (d, *J* = 6.4 Hz, 3H, Leu Cδ′H), Mass (CI method) (m/z): 395 ($(M+H)^+$, 100).

2.7 Spectral data

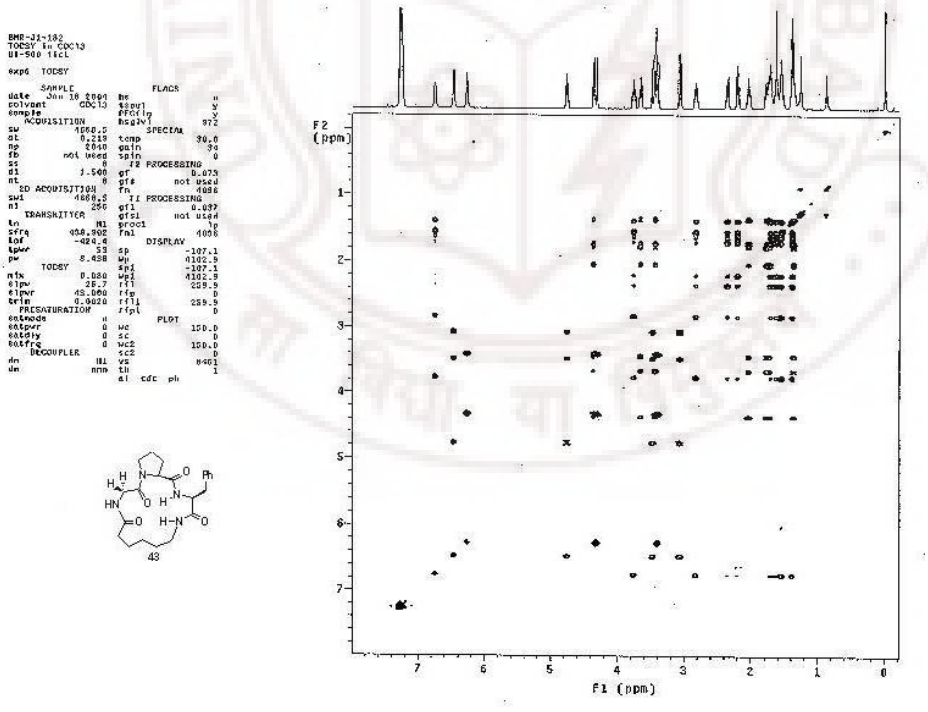
Spectrum-1A: ^{1}H NMR of peptide **43** in $CDCl_3$



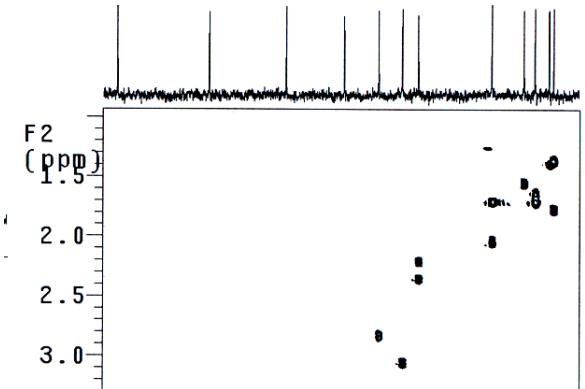
Spectrum-1B: ^{1}H NMR of peptide **43** in DMSO-d_6



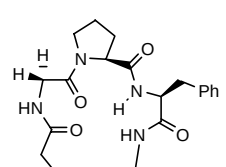
Spectrum-2: TOCSY spectrum of **43** in $CDCl_3$

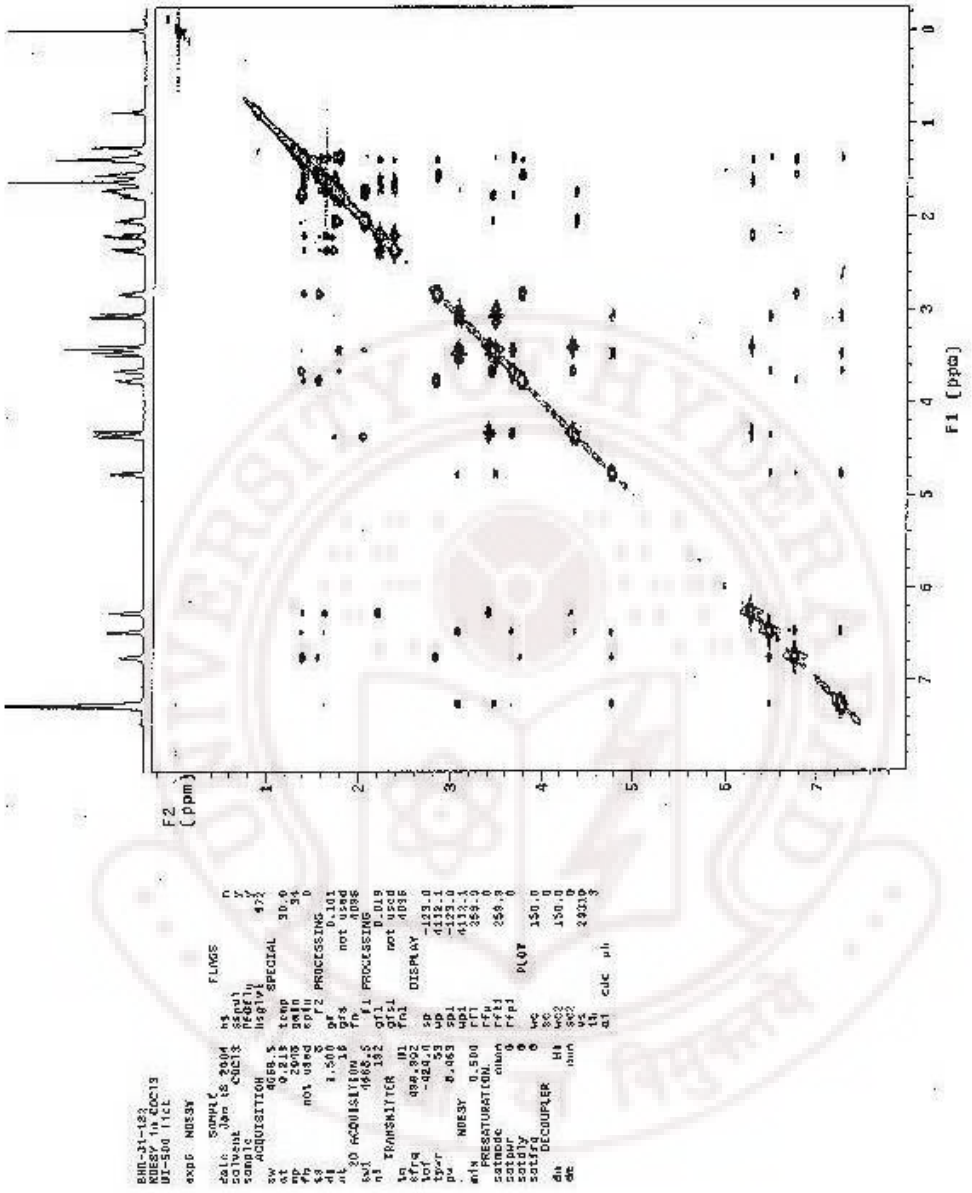


Spectrum-3: Expansion of HSQC spectrum of **43** in $CDCl_3$

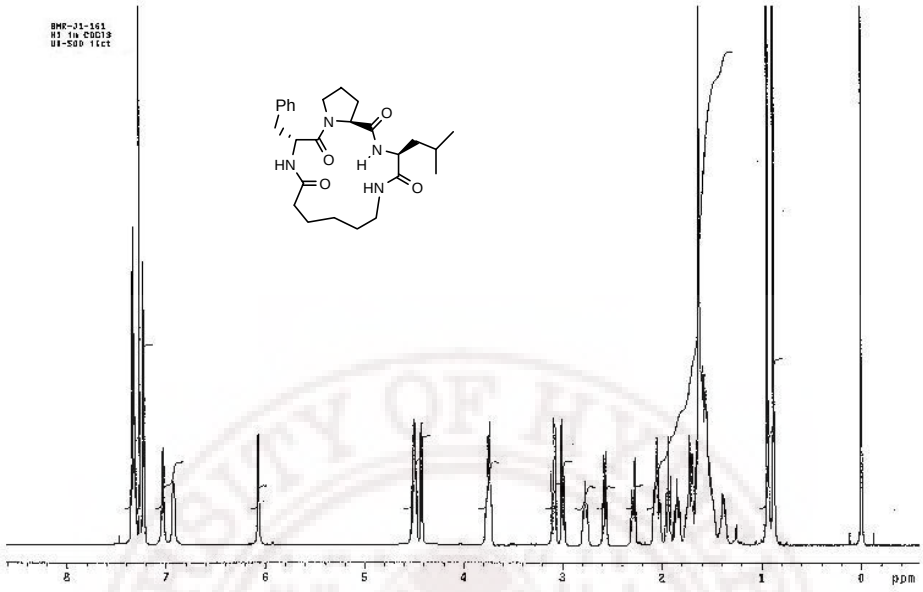


Spectrum-4: NOESY spectrum of peptide **43** in $CDCl_3$

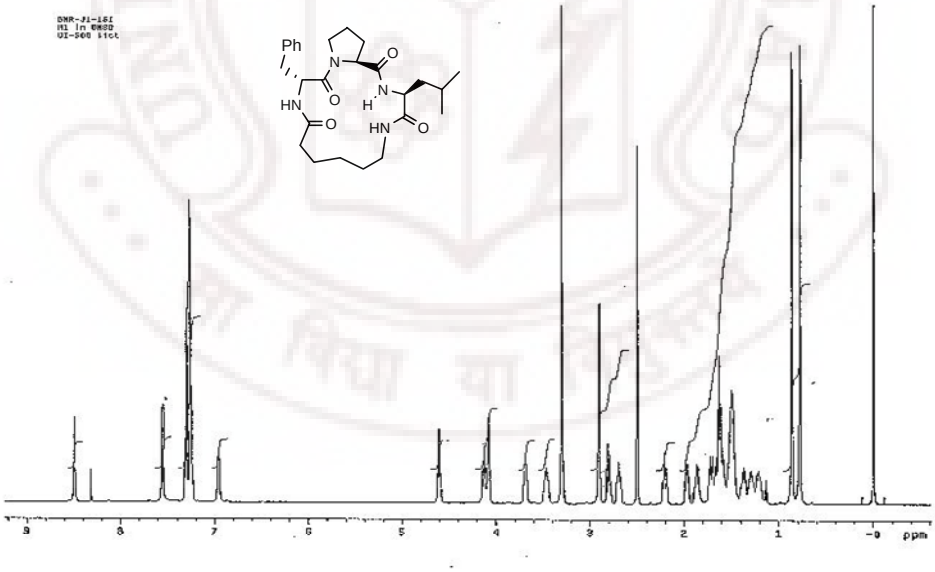




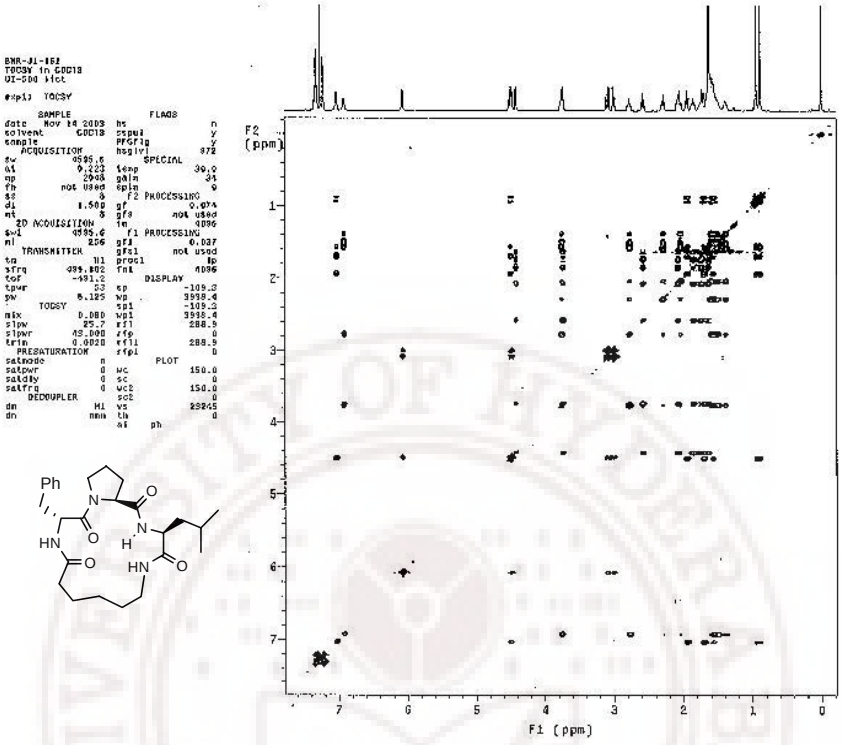
Spectrum-5A: ^{1}H NMR of peptide **59** in $CDCl_3$



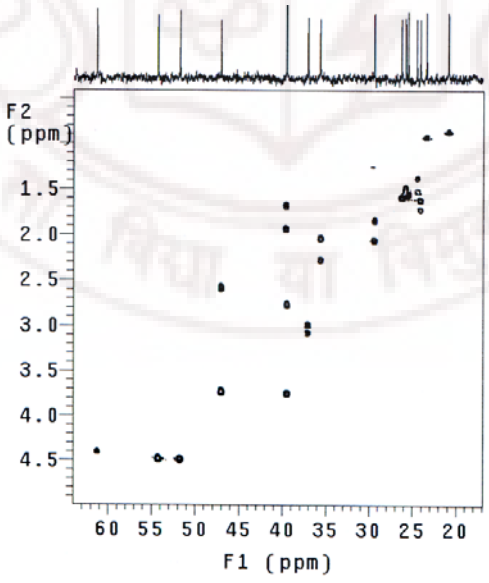
Spectrum-5B: ^{1}H NMR of peptide **59** in DMSO-d_6



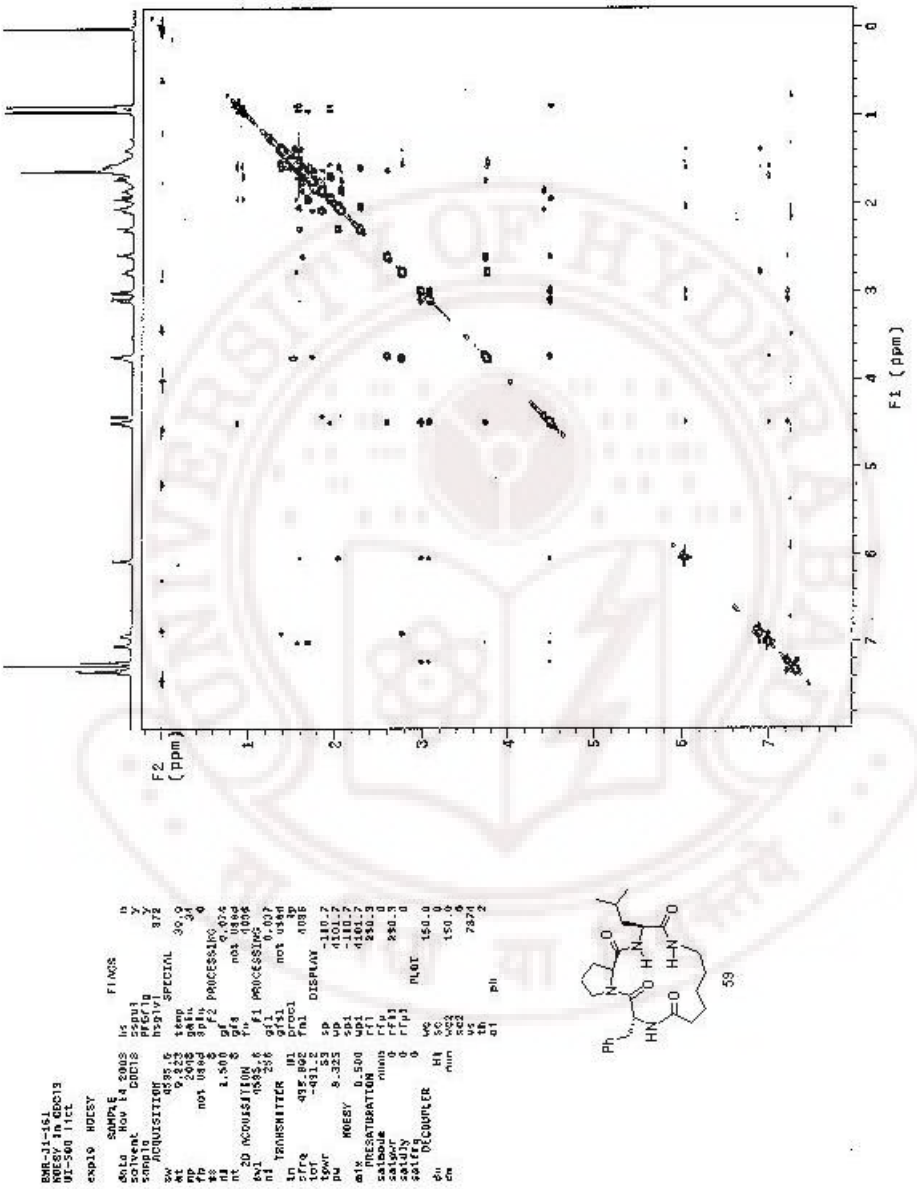
Spectrum-6: TOCSY spectrum of **59** in $CDCl_3$



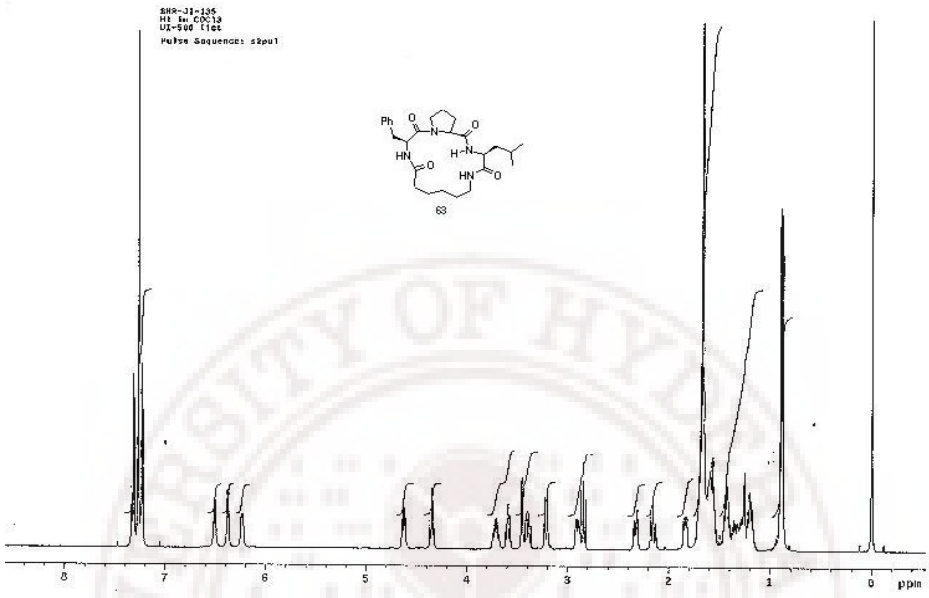
Spectrum-7: Expanded HSQC spectrum of peptide **59** in $CDCl_3$



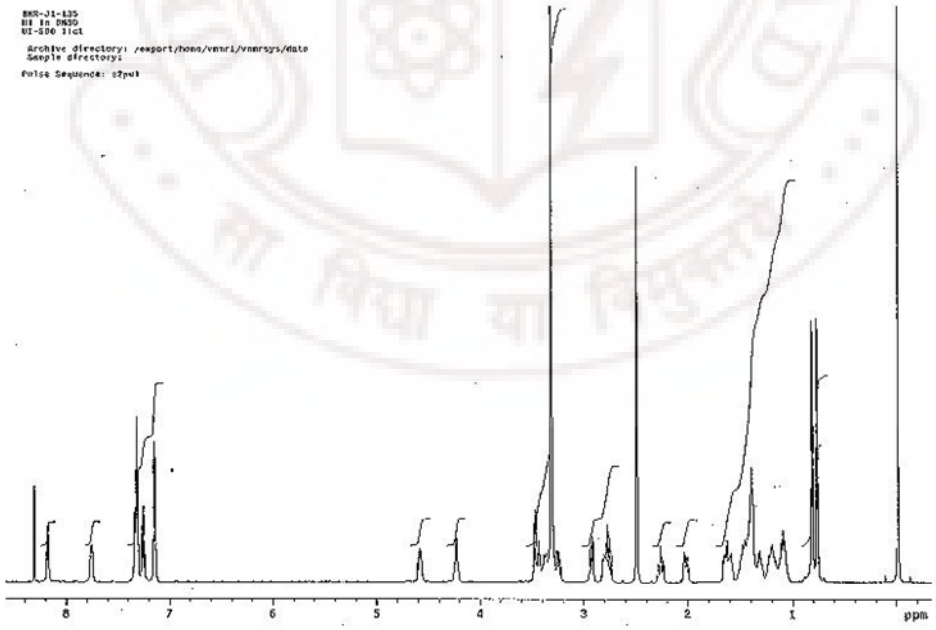
Spectrum-8: NOESY spectrum of peptide **59** in $CDCl_3$



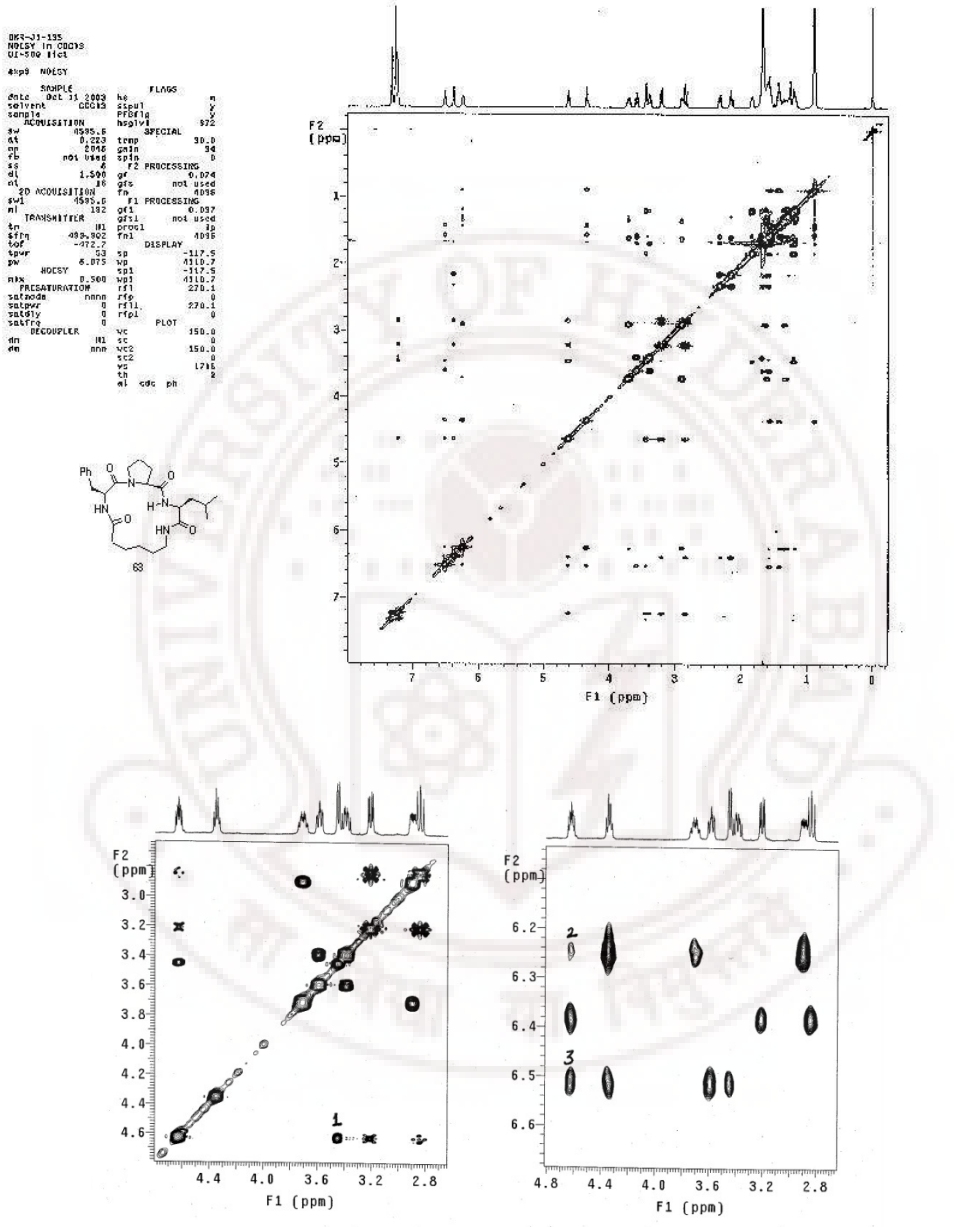
Spectrum-9: ^{1}H NMR Spectrum of **63** in $CDCl_3$



(B) ^{1}H NMR Spectrum of **63** in DMSO-d_6

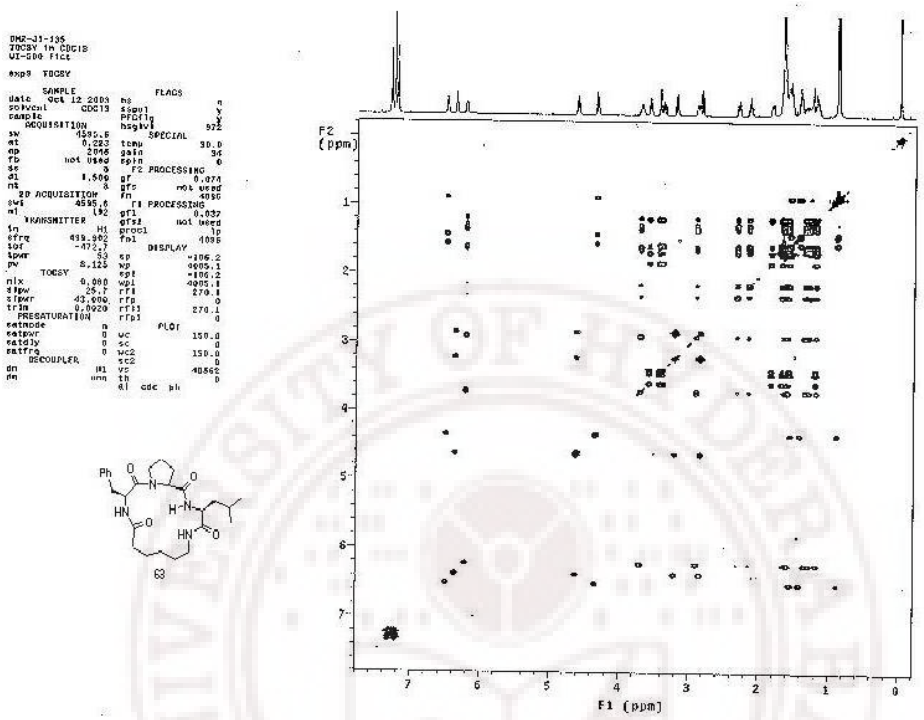


Spectrum-10: NOESY spectrum of **63** in $CDCl_3$

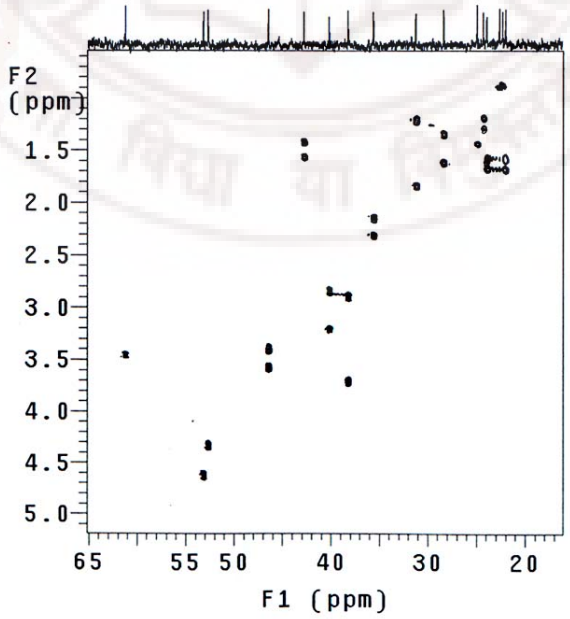


Phe CαH↔Pro CαH, Aha NH↔Phe CαH, LeuNH↔Phe CαH

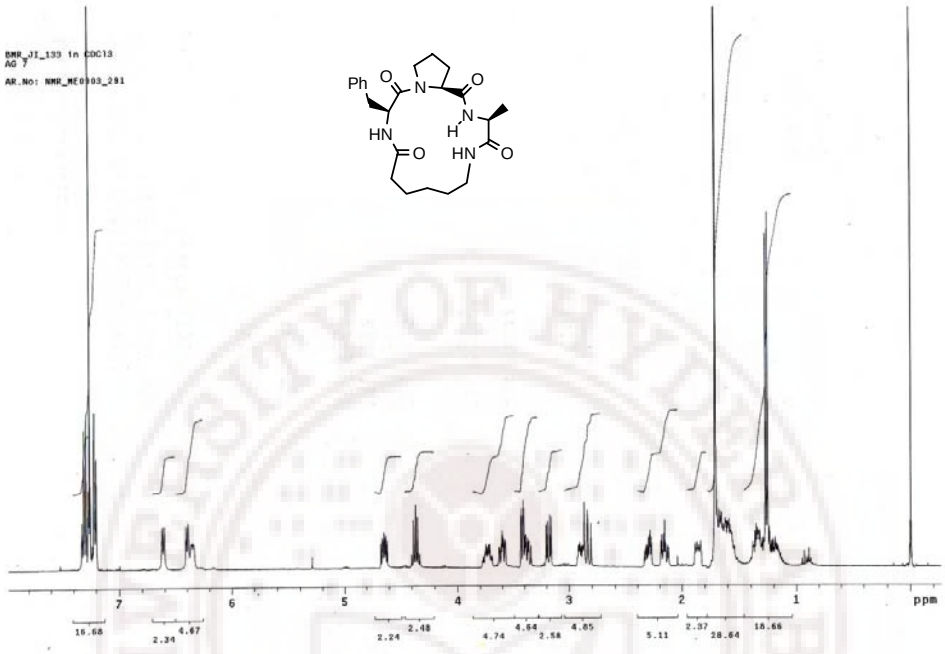
Spectrum-11: TOCSY spectrum of **63** in $CDCl_3$



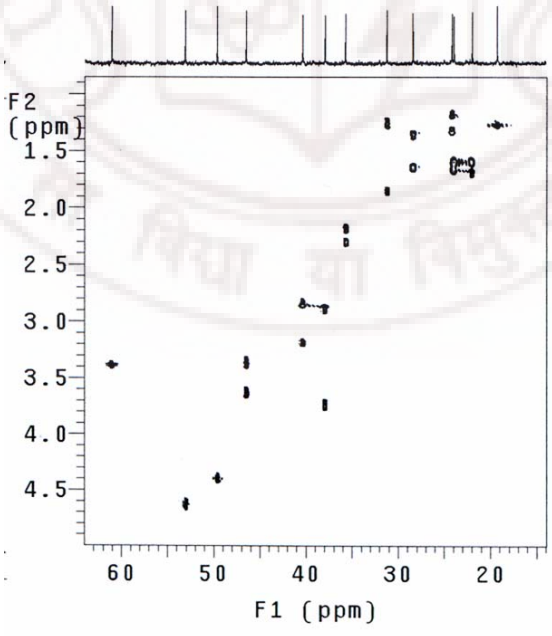
Spectrum-12: HSQC spectrum of 6**3** in $CDCl_3$



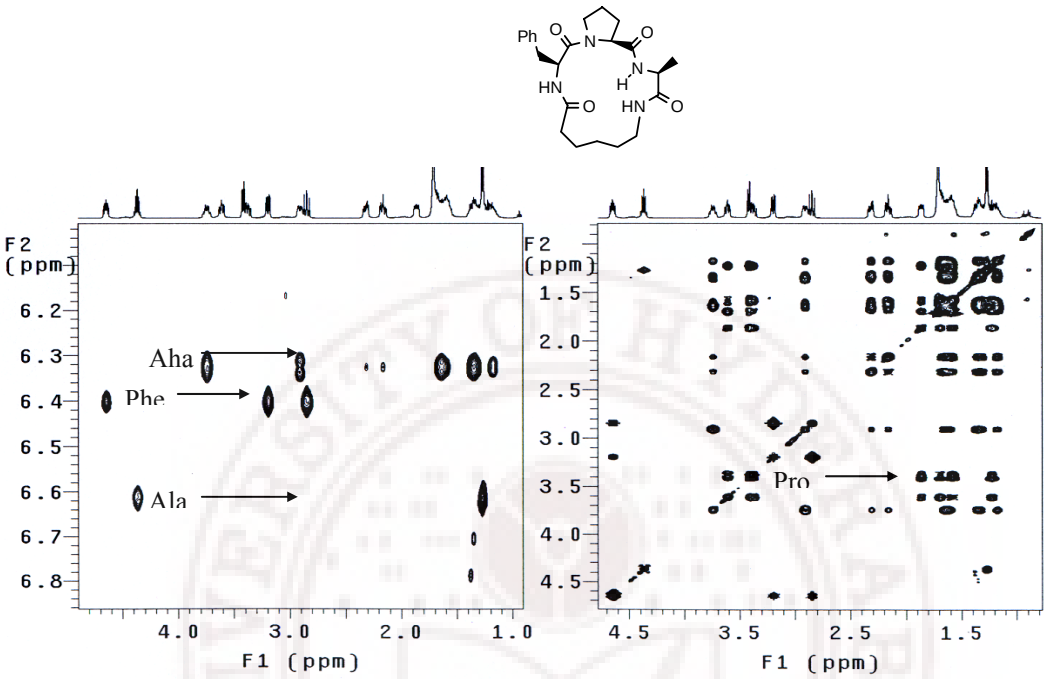
Spectrum-13: ^{1}H NMR of peptide **69** in $CDCl_3$



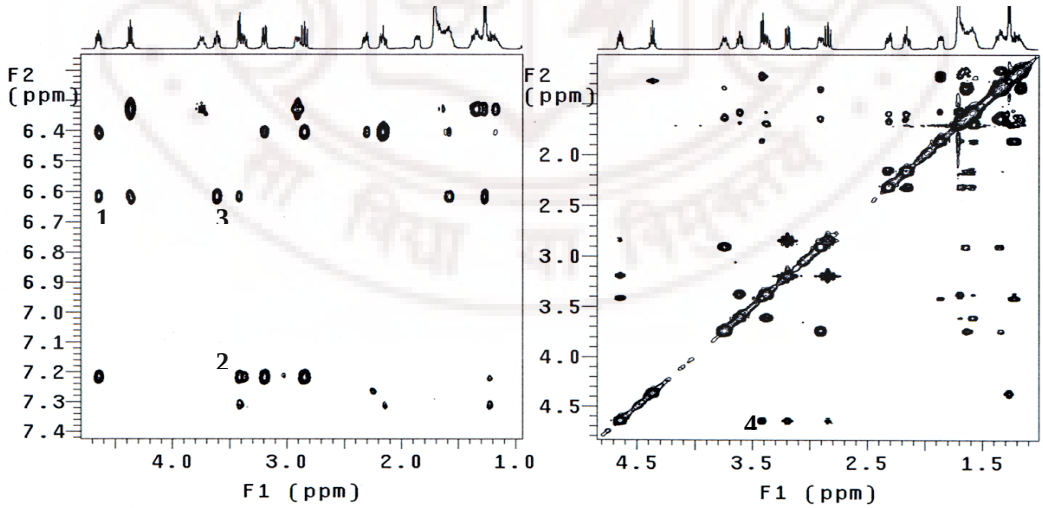
Spectrum-14: Expanded HSQC spectrum of peptide **69** in $CDCl_3$



Spectrum 15: TOCSY spectrum of peptide **69**

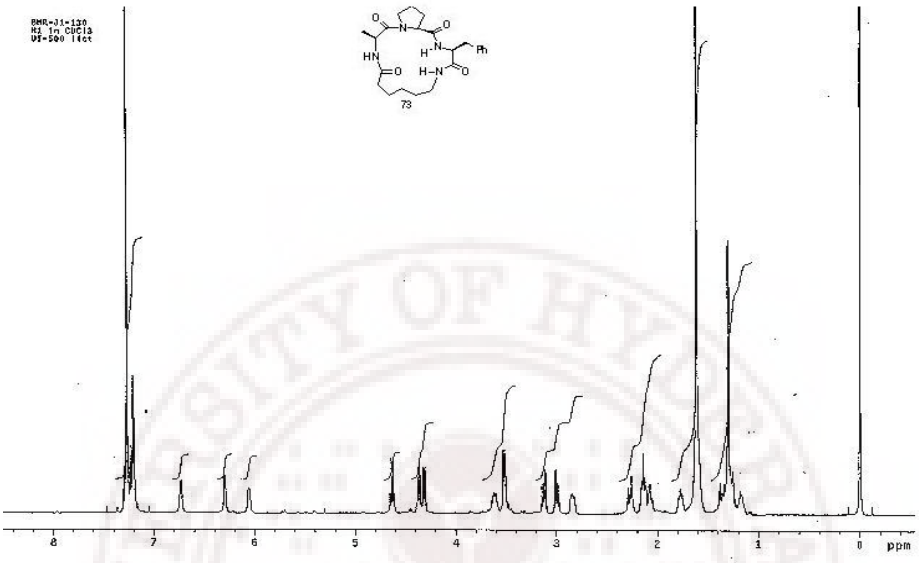


Spectrum 16: Expansions of NOESY spectrum of peptide **69**

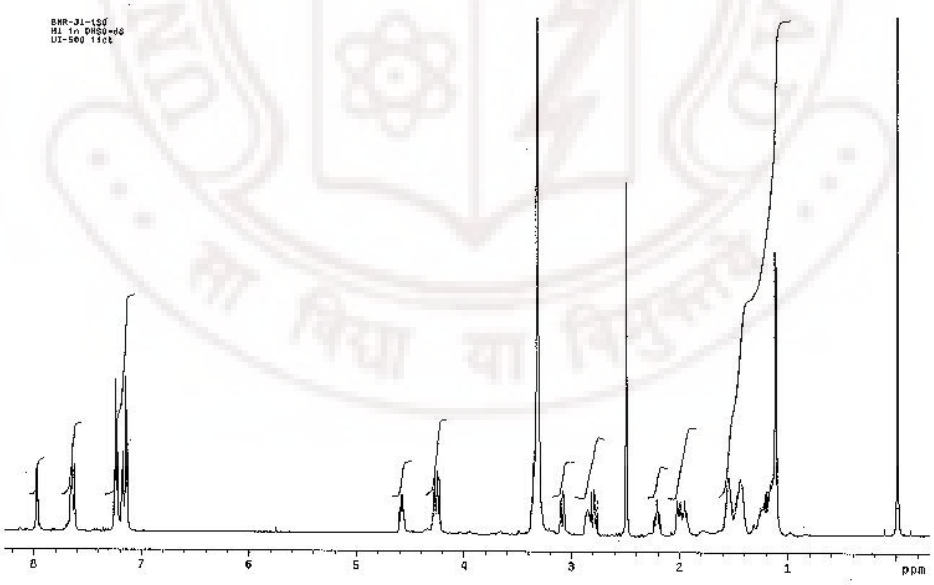


Ala NH↔Phe CαH, Phenyl ArH↔Pro CαH, Ala NH↔Pro CδH, Phe CαH↔Pro CαH).

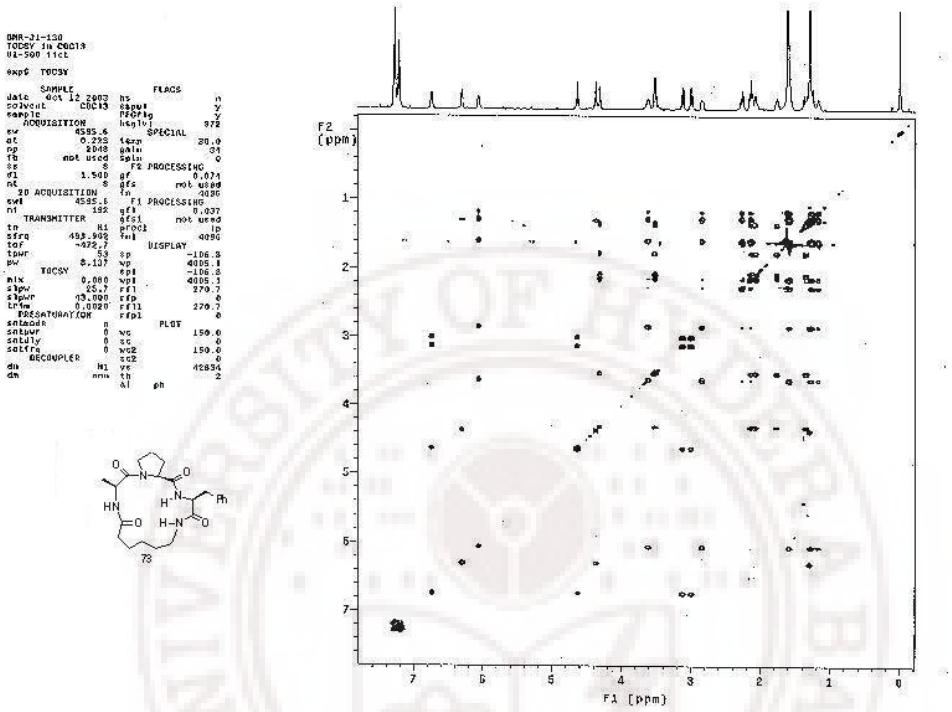
Spectrum-17A: ^{1}H NMR spectrum of peptide **73** in $CDCl_3$



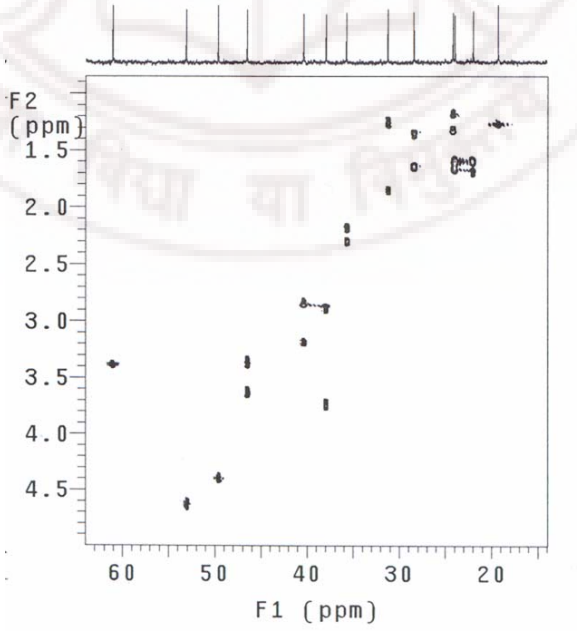
Spectrum-17B: ^{1}H NMR spectrum of peptide **73** in DMSO-d_6



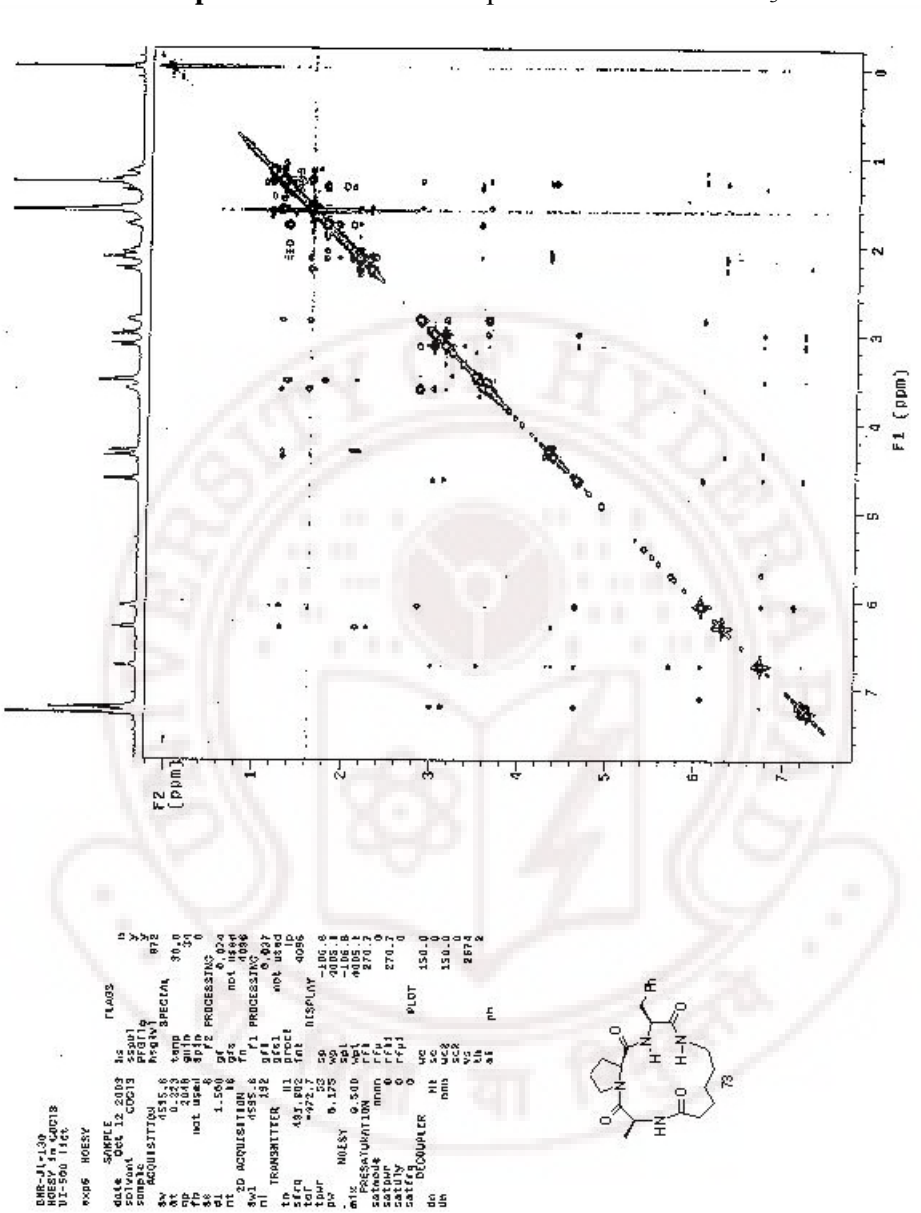
Spectrum-18: TOCSY spectrum of **73** in $CDCl_3$



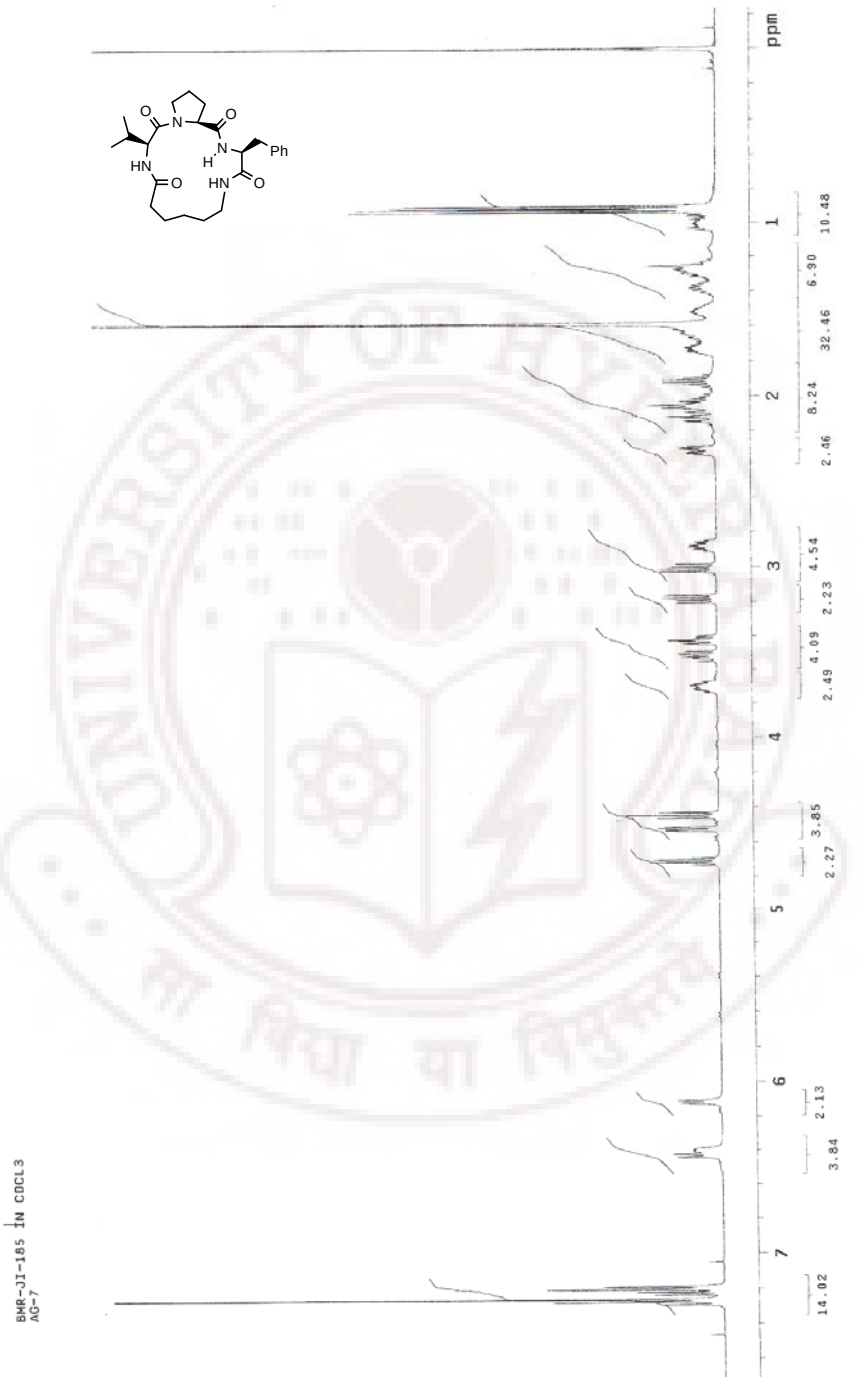
Spectrum-19: Expanded HSQC spectrum of peptide **73** in $CDCl_3$



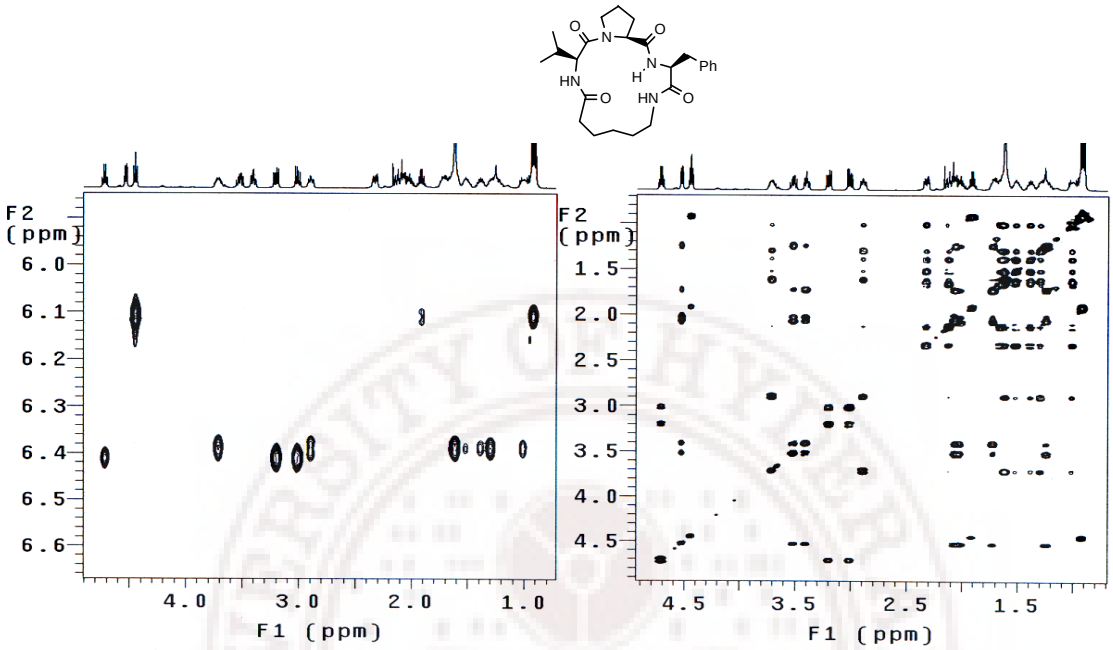
Spectrum-20: NOESY spectrum of **73** in $CDCl_3$



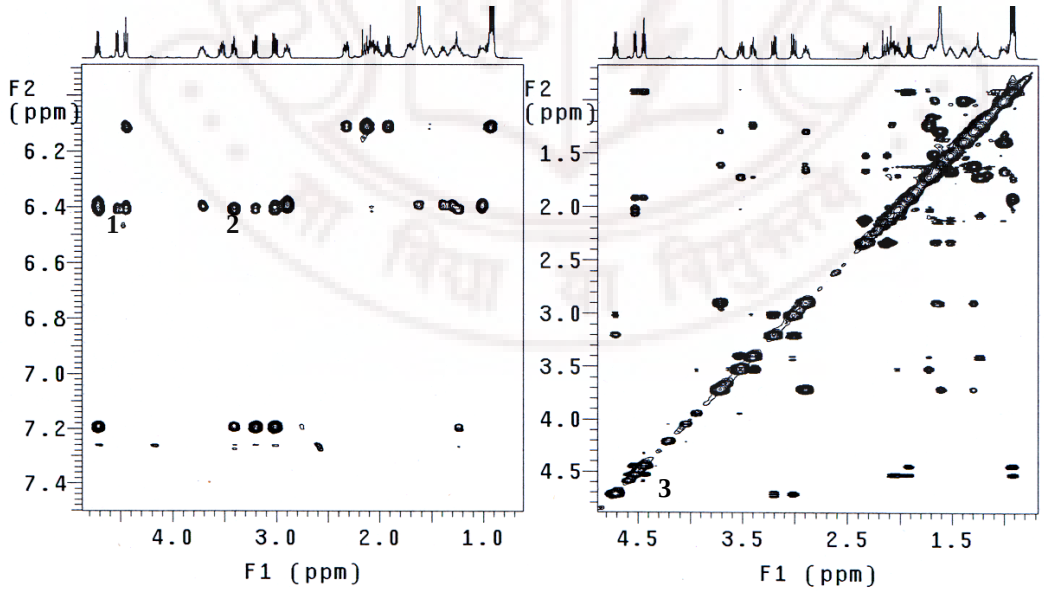
Spectrum 21: ^{1}H NMR of peptide **77** in $CDCl_3$



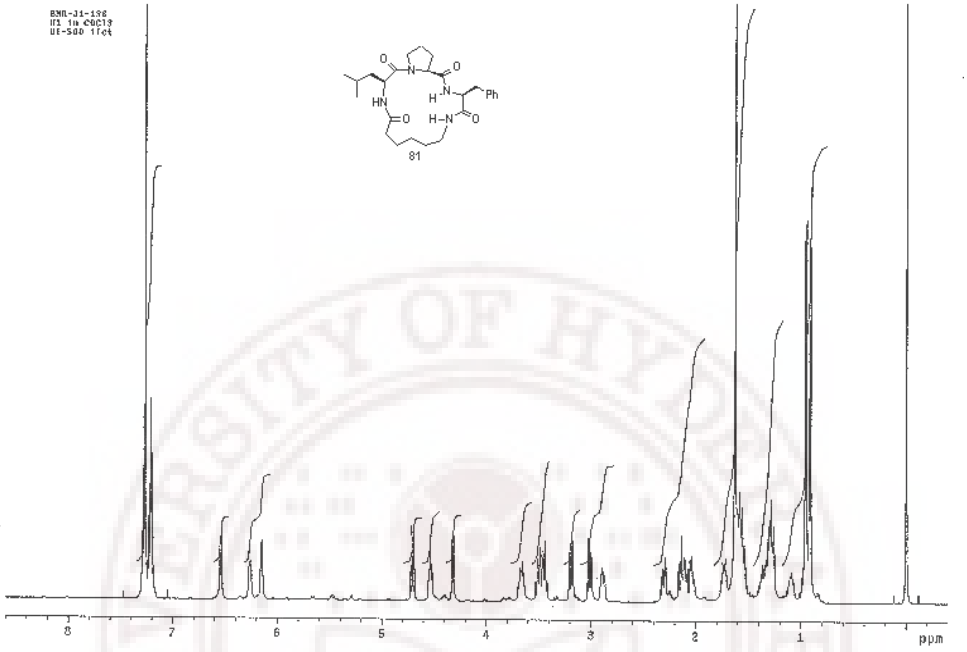
Spectrum 22: Expansions of TOCSY spectrum of peptide **77**.



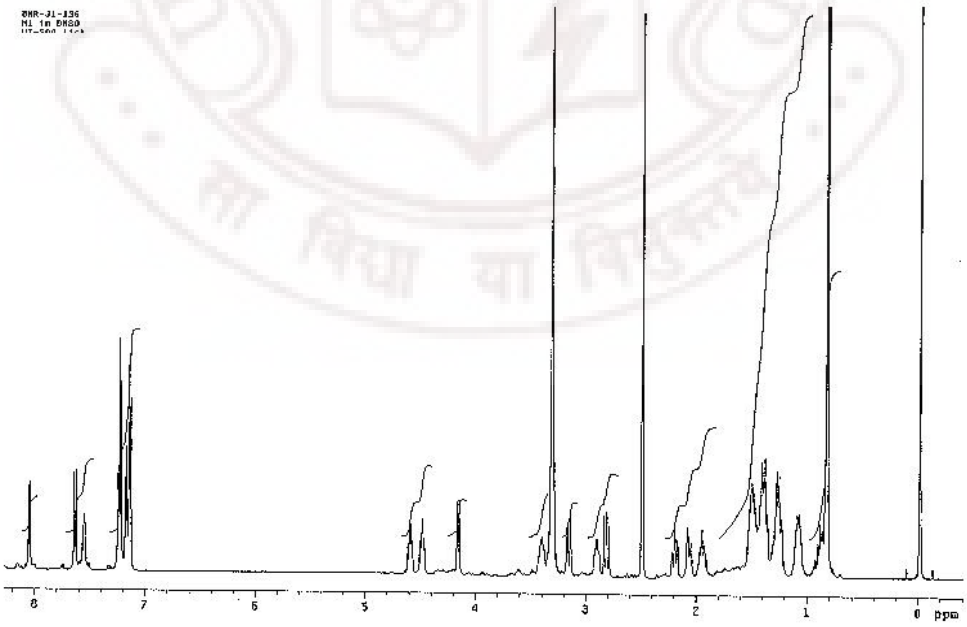
Spectrum 23: Expansions of NOESY spectrum of peptide **77.**



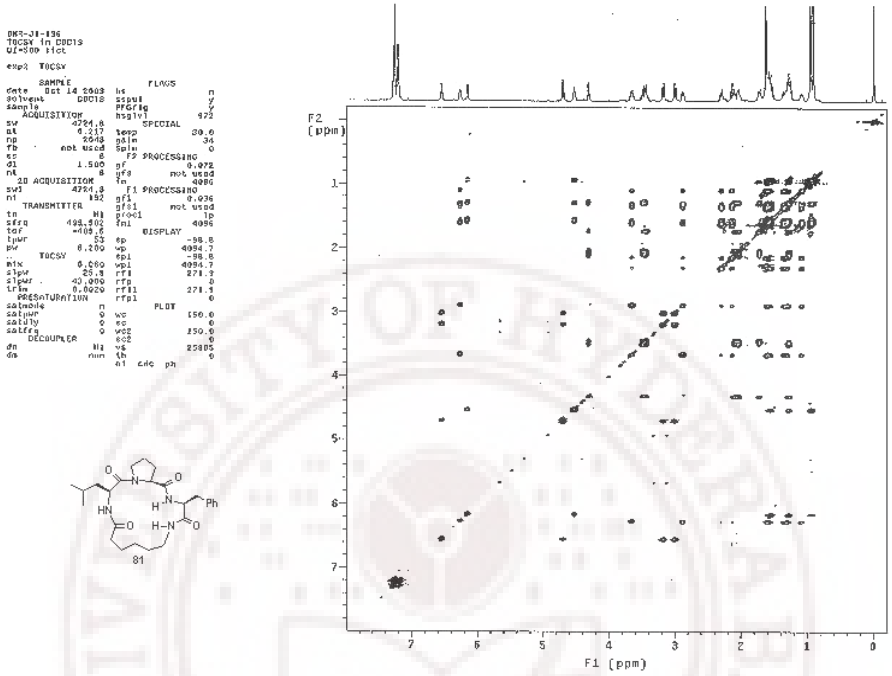
Spectrum-23A: ^{1}H NMR of peptide **81** in $CDCl_3$



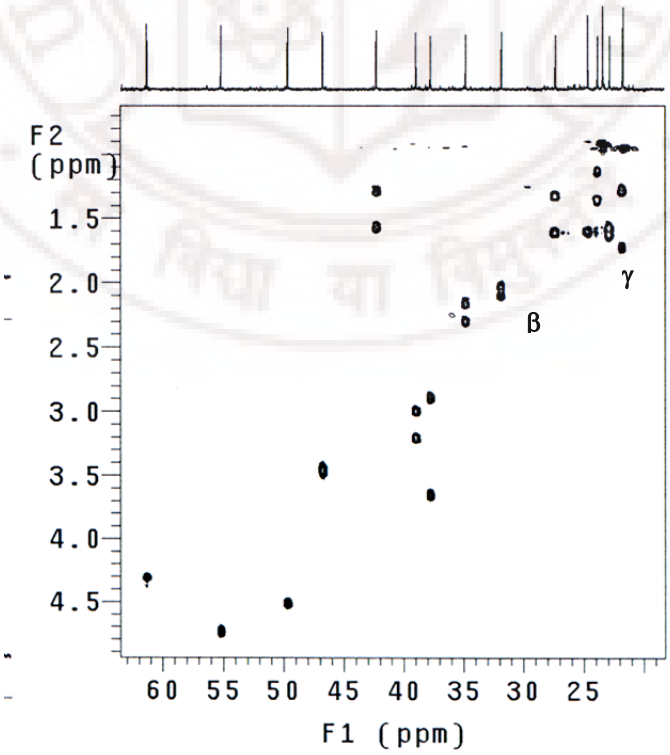
Spectrum-23B: ^{1}H NMR of peptide **81** in DMSO-d_6



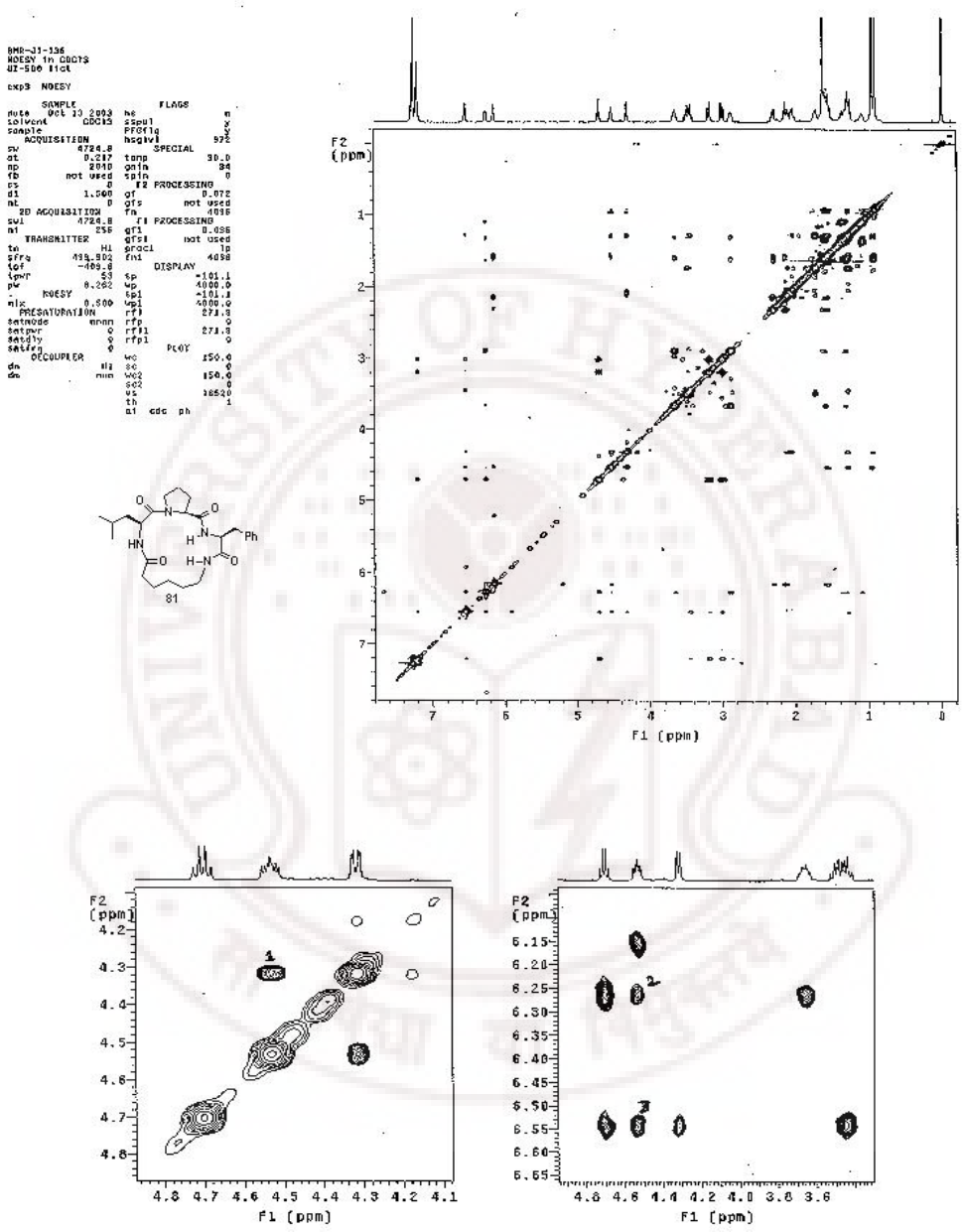
Spectrum-24: TOCSY spectrum of **81** in $CDCl_3$



Spectrum-25: HSQC spectrum of **81** in $CDCl_3$

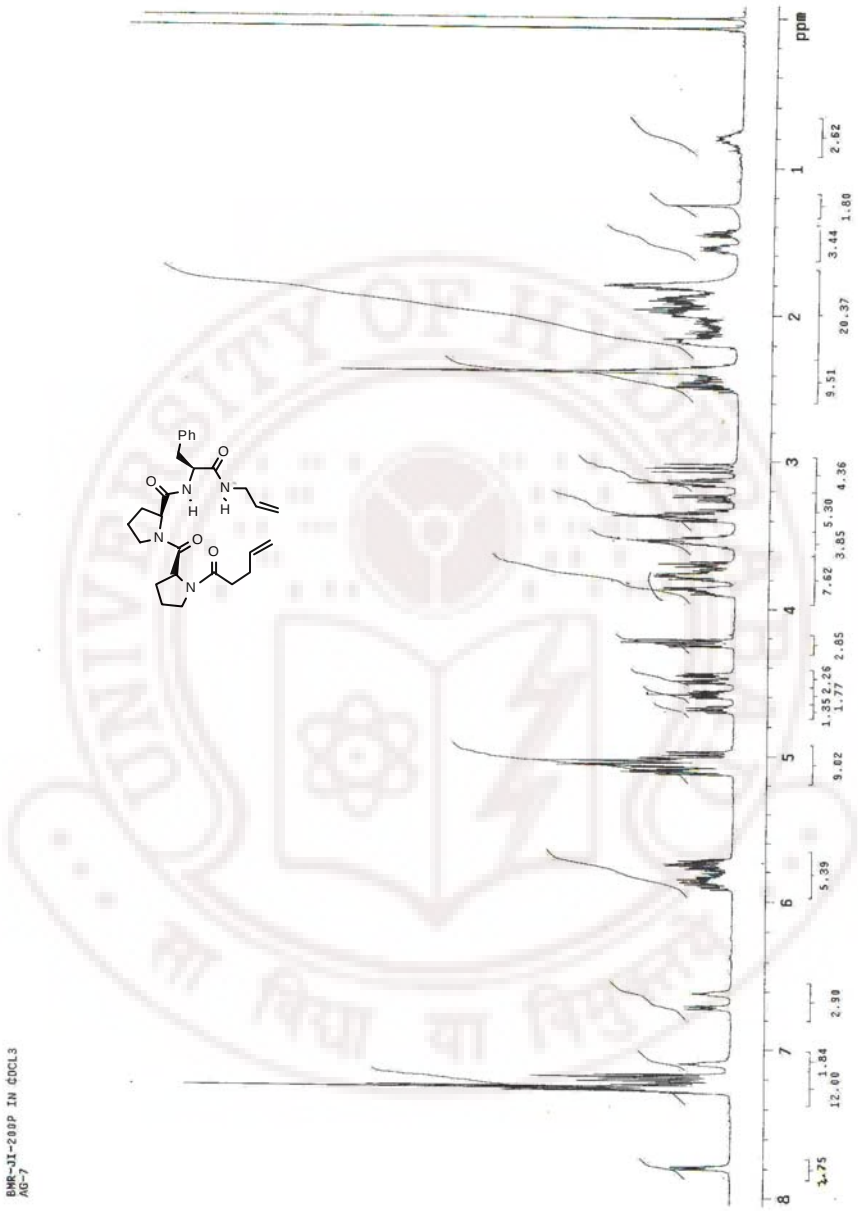


Spectrum-26: NOESY spectrum of **81** in $CDCl_3$

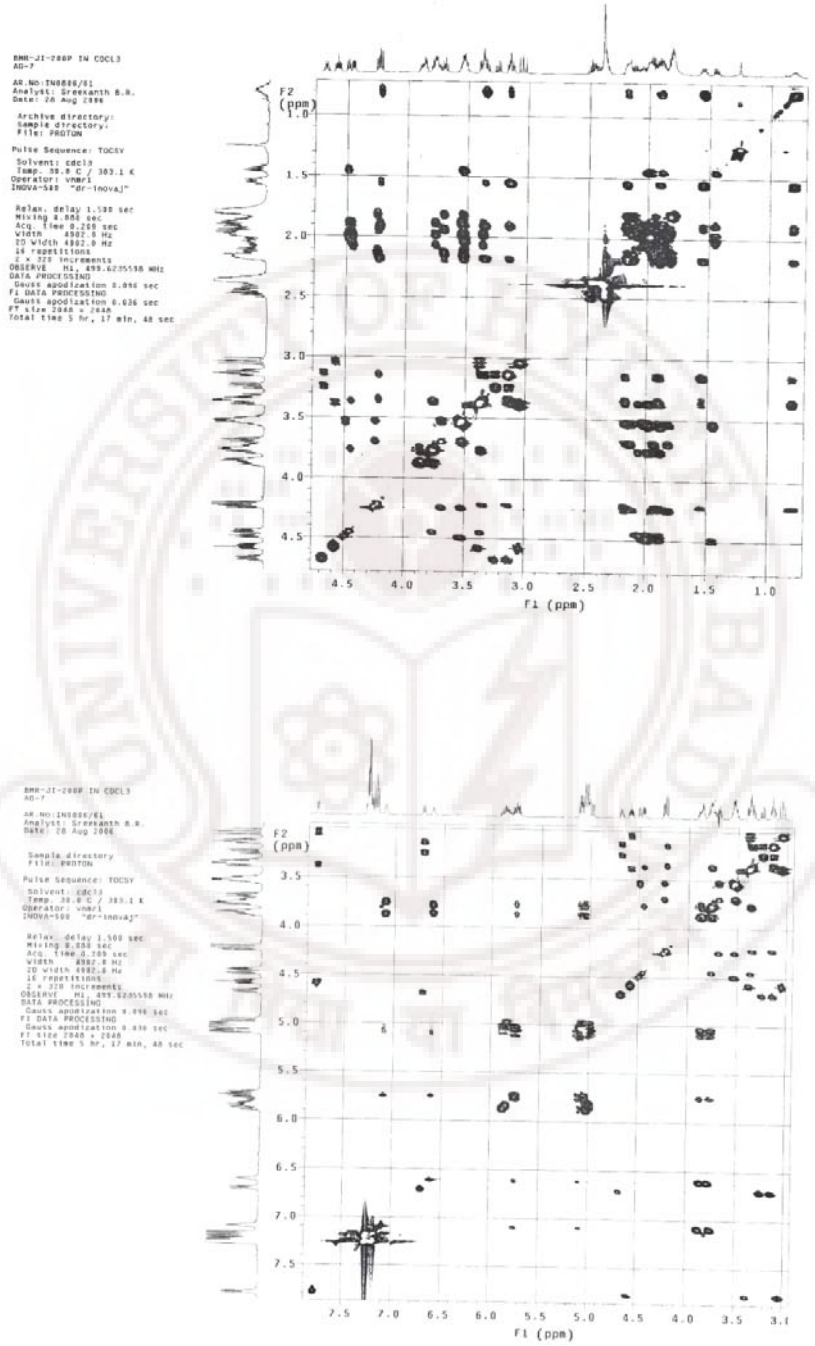


LeuCαH↔ Pro, Aha NH↔Leu CαH, Phe NH↔Leu CαH

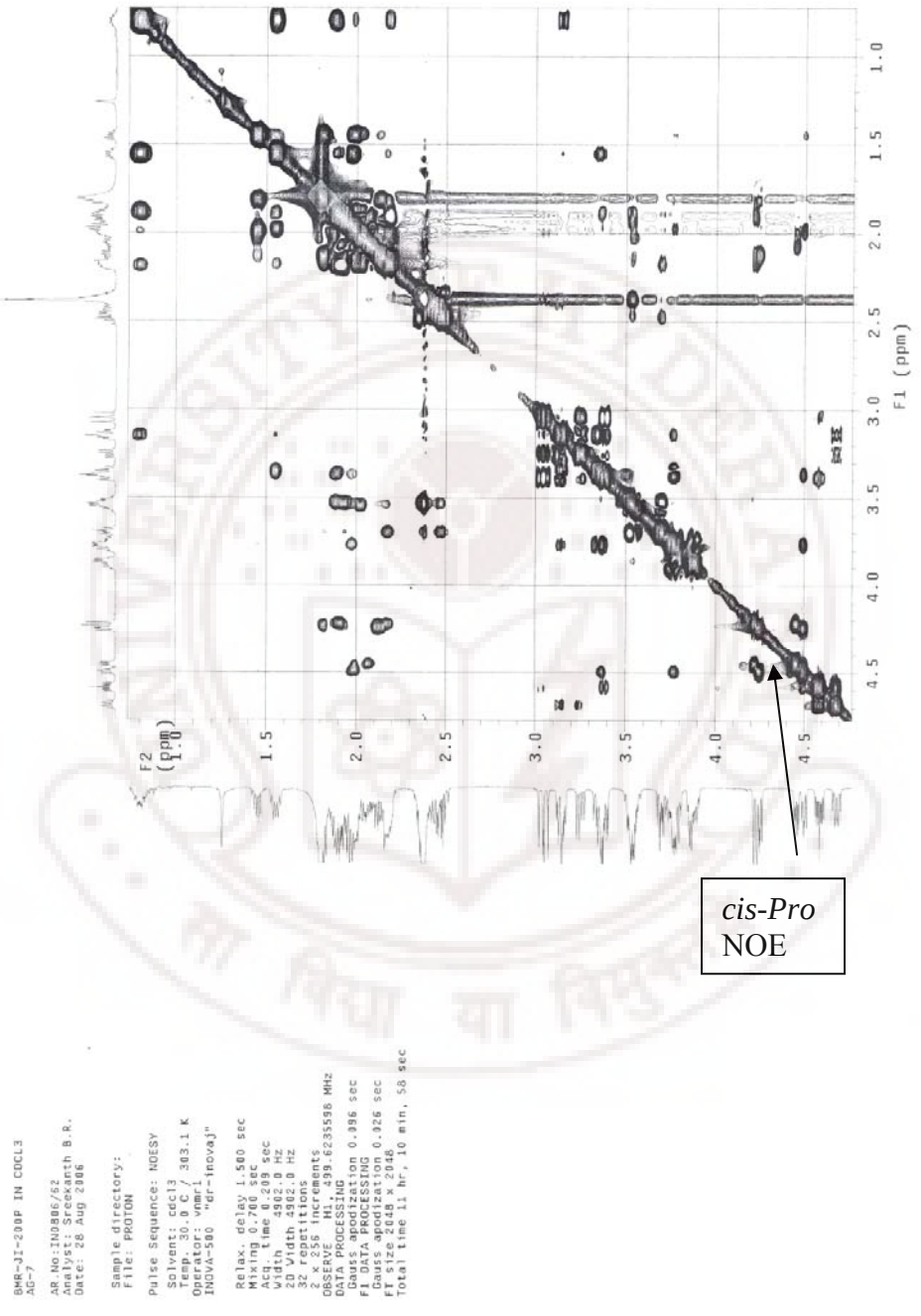
Spectrum-27: ^{1}H NMR spectrum of peptide **83** in $CDCl_3$



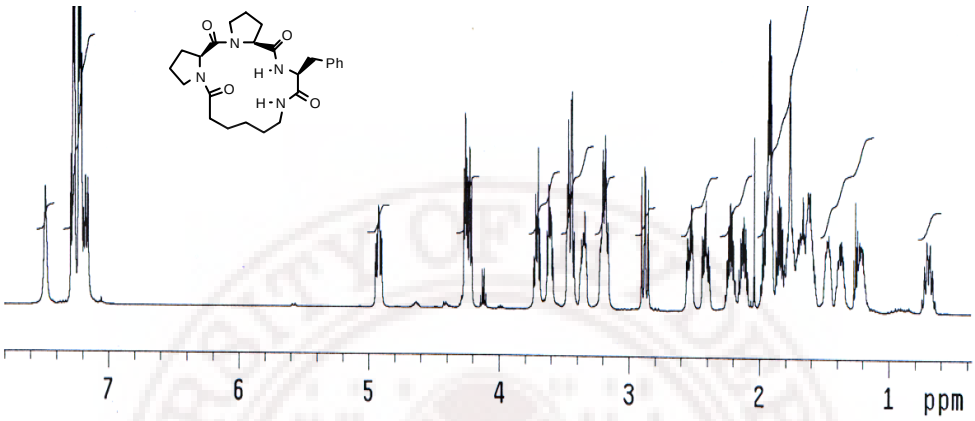
Spectrum-28: Expanded TOCSY spectrum of peptide **83** in $CDCl_3$



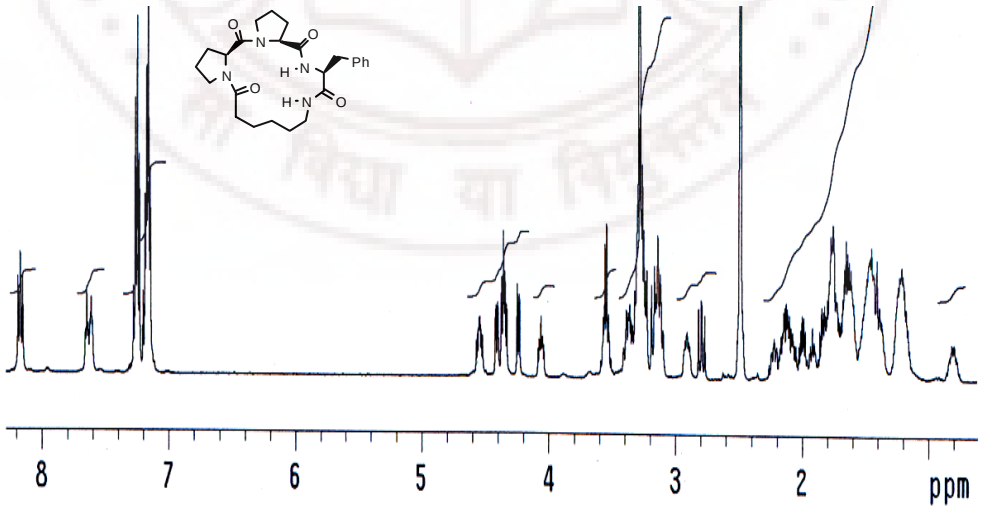
Spectrum-29: Expanded NOESY spectrum of peptide **83** in $CDCl_3$



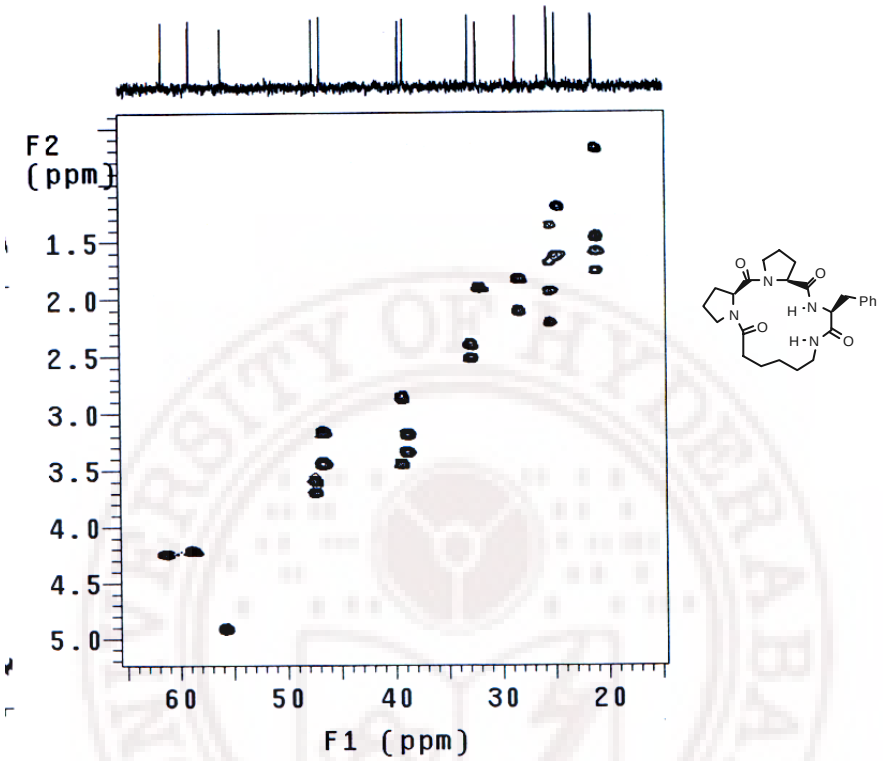
Spectrum-30A: ^{1}H NMR spectrum of peptide **84** in $CDCl_3$



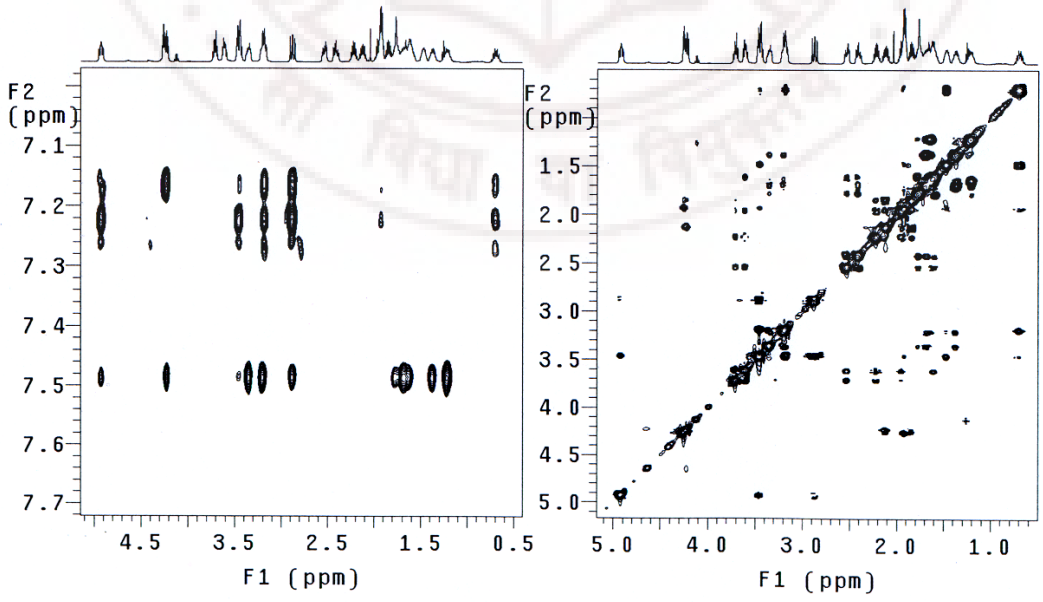
Spectrum-30B: ^{1}H NMR spectrum of peptide **84** in DMSO-d_6



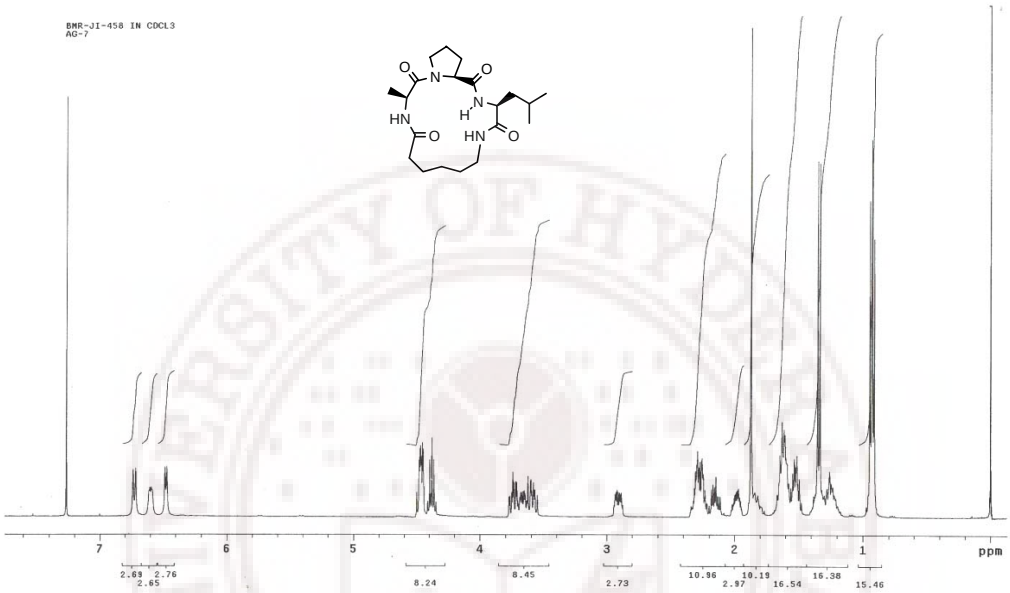
Spectrum-31A: Expanded HSQC spectrum of peptide **84** in $CDCl_3$



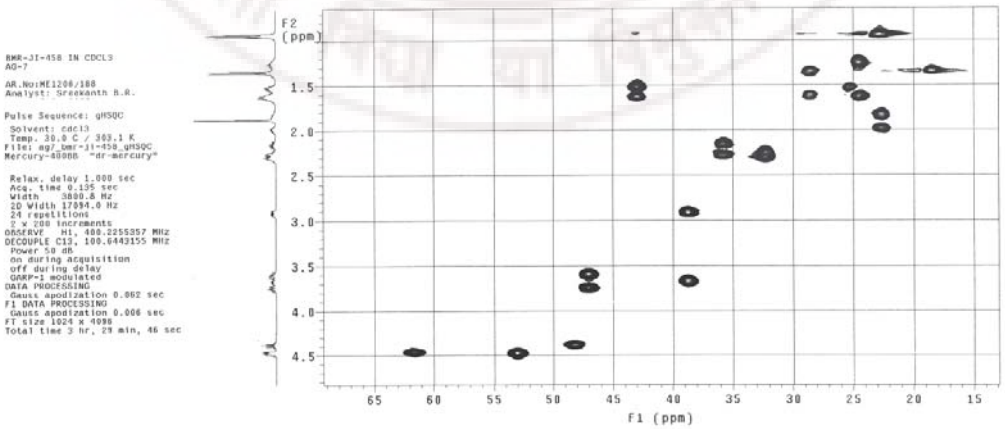
Spectrum-36: Expanded NOESY spectrum of peptide **87**



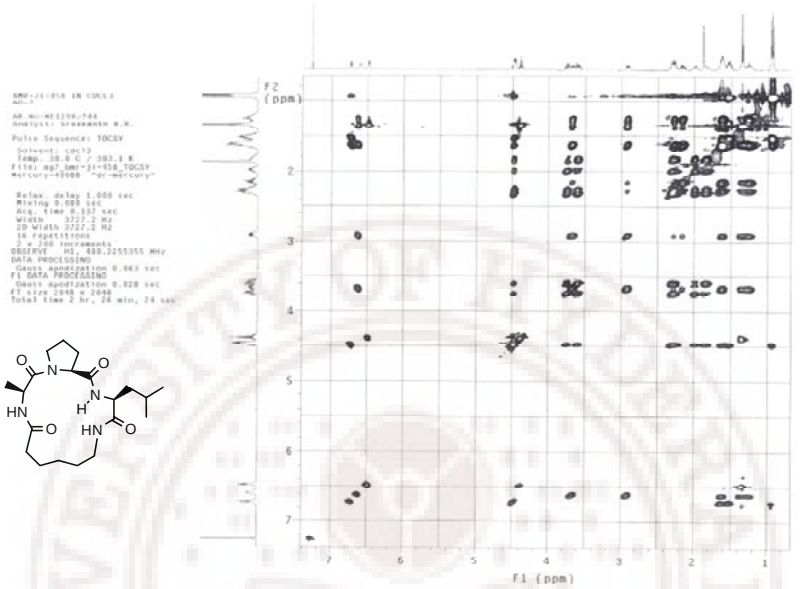
Spectrum-33: ^{1}H NMR of peptide **87** in $CDCl_3$



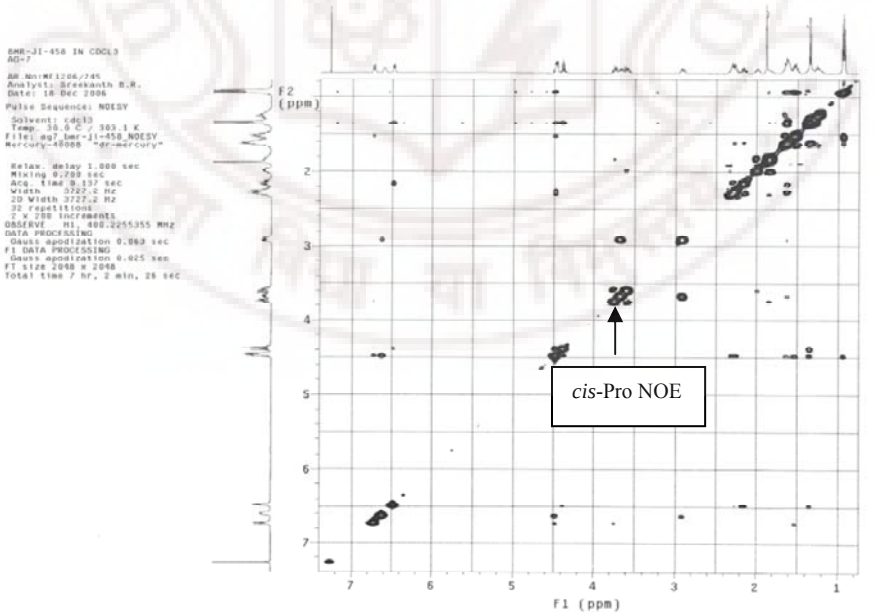
Spectrum-34: Expanded HSQC spectrum of peptide **87** in $CDCl_3$



Spectrum-35: TOCSY spectrum of peptide **87** in $CDCl_3$



Spectrum-36: NOESY spectrum of peptide **87** in $CDCl_3$



Chapter III

Synthesis and Conformation of β-amino acid Derived Small Cyclic Pseudo 3_{10} Helical/β-Turn Structures *via* RCM

3.1 Introduction

Acquired Immunodeficiency Syndrome was first reported by the US Center for Disease Control (CDC) about two decades ago and it still continues to be one of the most threatening epidemics to mankind. The causative agent of this epidemic was identified as a retrovirus (genetic information in the form of RNA) called human immunodeficiency virus (HIV).[1]

3.1.1. HIV life cycle

HIV infects T-cells that carry the CD4 antigen on their surface. The infection of the virus requires fusion of the viral and cellular membranes which is mediated by viral envelope glycoprotein (gp120, gp41) and receptors (CD4 and co-receptors, such as CCR 5 or CXCR 4) on the target cell. As the virus enters the cell, its RNA is reverse-transcribed to DNA by a virally encoded enzyme, the reverse transcriptase (RT). The viral DNA enters the cell nucleus, where it is integrated into the genetic material of the cell by the second virally encoded enzyme, the integrase. Activation of the host cell results in the transcription of the viral DNA into m-RNA, which is then translated into viral proteins. HIV protease, the third virally encoded enzyme, is required in this step to cleave a viral polyprotein precursor into individual mature proteins. The viral RNA and viral proteins assemble at the cell surface into new virions, which then bud from the cell and are released to infect another cell. Intricate analysis of the HIV life

cycle, it is apparent that there are various events in the HIV replicative cycle that are vital to the survival of HIV, but not for the host cell [2] that have been aimed as potential targets for chemotherapeutics, namely

1. Viral adsorption, through binding to the viral envelope glycoprotein gp120
2. Viral entry, through blockade of the viral co-receptors CXCR4 and CCR5
3. Virus-cell fusion
4. Viral assembly and disassembly
5. Reverse Transcriptase Inhibitors
6. Proviral DNA integration
7. Viral mRNA transcription
8. Protease inhibitors

To date, more than 20 anti-HIV agents have been approved by the United States Food and Drug Administration for the management of HIV-1 infected individuals. These antiviral agents can be broadly divided into three different therapeutic classes: three are non-nucleoside reverse transcriptase inhibitors (NNRTIa), eight are nucleoside or nucleotide reverse transcriptase inhibitors and nine are inhibitors of HIV-1 protease. Off late, one fusion inhibitor has also been approved by US FDA.

3.1.2 Reverse Transcriptase Inhibitors

HIV-1 reverse transcriptase (RT) is a multifunctional enzyme responsible for the conversion of the viral single-stranded RNA genome into double-stranded DNA. To facilitate this process, RT exhibits two enzymatically distinct activities: a DNA polymerase activity that synthesizes DNA using either RNA or DNA templates (termed RNA-dependent (RDDP) or DNA dependent DNA polymerase (DDDP) activity, respectively) and a ribonuclease H (RNase H) activity that degrades the RNA strand of RNA/DNA hybrids.

There are two major types of HIV-1 RT inhibitors, nucleoside analogues (NRTIs) and non-nucleoside analogues (NNRTIs). The nucleoside analogues for example, Zidovudine (AZT, Retrovir),[3] Didanosine (ddI, Videx),[4] Zalcitabine[5] (ddC, Hivid) , Stavudine (d4T, Zerit)[6] bind to the active site of the enzyme and can be incorporated into the growing DNA chain. However, further elongation is not possible, as they lack the 3′-OH group normally present in the substrate. This causes premature termination of the growing viral DNA strand. In contrast, NNRTIs are allosteric inhibitors that indirectly interfere with the catalytic mechanism of the enzyme (Nevirapine[7,8], Delavirdine[9], Efavirenz[8,10], and PETT[10,11] compounds). Further, several studies have demonstrated that HIV-1 RT is an obligate dimer and their monomeric forms of both subunits are devoid of DNA polymerase activity. Therefore, dimerization of HIV-1 RT also represents a novel target for the identification of a new therapeutic class of antiviral agents,

and several classes of small molecules and peptides have been identified as RT dimerization inhibitors.[12]

3.1.3 HIV Protease

Structure

HIV PR is a 99 amino acid aspartyl protease which functions as a homodimer with only one active site which is C2-symmetric in the free form (figure-**1**). Each monomer contains an extended β-sheet region (a glycine-rich loop) known as the flap, that constitutes in part the substrate-binding site and plays an important role in substrate binding, and one of the two aspartyl residues, Asp-25 and Asp-25′ which lie on the bottom of the activity. The substrate binds in its extended conformation, in which its interaction with the different amino acid side chains determines the specificity of the enzyme.

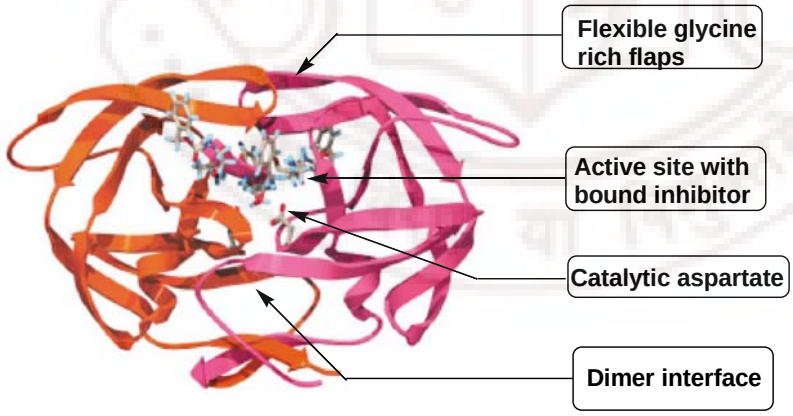


Figure-**1**: *Co-crystal structure of HIV PR complexed with TL-3*

3.1.4 HIV Protease Function

HIV carries most of its genetic information in three genes: *gag, pol* and *env*. *Gag* and *Pol* genes encode the structural and functional proteins, where as *env* encodes the envelop proteins. Host cellular enzymes cleave the *env* polyprotein, while an HIV encoded protease proteolytically cleaves the polyproteins encoded by the *gag* and *pol* genes. As the replicative enzymes (including protease) are formed from this fusion protein, the protease enzyme first leaves itself from the fusion protein and later processes the precursor proteins to structural proteins and replicative enzymes. The HIV protease cleavage sites in the gag and gag-pol precursor proteins ($Pr55^{gag}$ and Pr 160 $^{gag\text{-}pol}$, respectively)[13] was identified and analysis of the proteolytic processing of the HIV-1 PR1-5 revealed that three of the cleavage sites contain Phe-Pro or Tyr-Pro at P1-P1′ residues (notation of Schecter and Berger) and are unusual for mammalian endopeptidases. Thus, *Phe-Pro* unit has been used as the basis of selectivity for most of the HIV protease inhibitors.

The necessity of HIV-1 PR for virus replication was demonstrated through several experiments. Deletion mutagenesis of the gene encoding HIV-1 PR resulted in the production of virus particles that has an immature morphology and were noninfectious.[14] This was confirmed by mutation of the active site aspartic acids[15] and, later, by chemical inhibition.[16] These seminal experiments provided conclusive proof that the viral protease is essential for the life-cycle of HIV, and

highlighted this enzyme as an important target for the design of specific antiviral agents. These findings stimulated further interest in HIV-1 PR, leading to solution of high-resolution 3D structures of HIV-1 PR using X-ray crystallography.[17] Currently FDA approved HIV-1 protease inhibitors,[18] saquinavir, indinavir, ritonavir, nelfinavir, amprenavir, lopinavir, atazanavir, tipranavir, and darunavir (TMC-114),[19] (figure-2) are all competitive inhibitors that bind in the active site of the enzyme. Except the newly approved drug tipranavir, all approved inhibitors have been developed on the basis of the transition state mimetic concept and contain various non-cleavable dipeptide isosteres as core scaffolds to mimic the transition state of HIV-1 protease substrates.

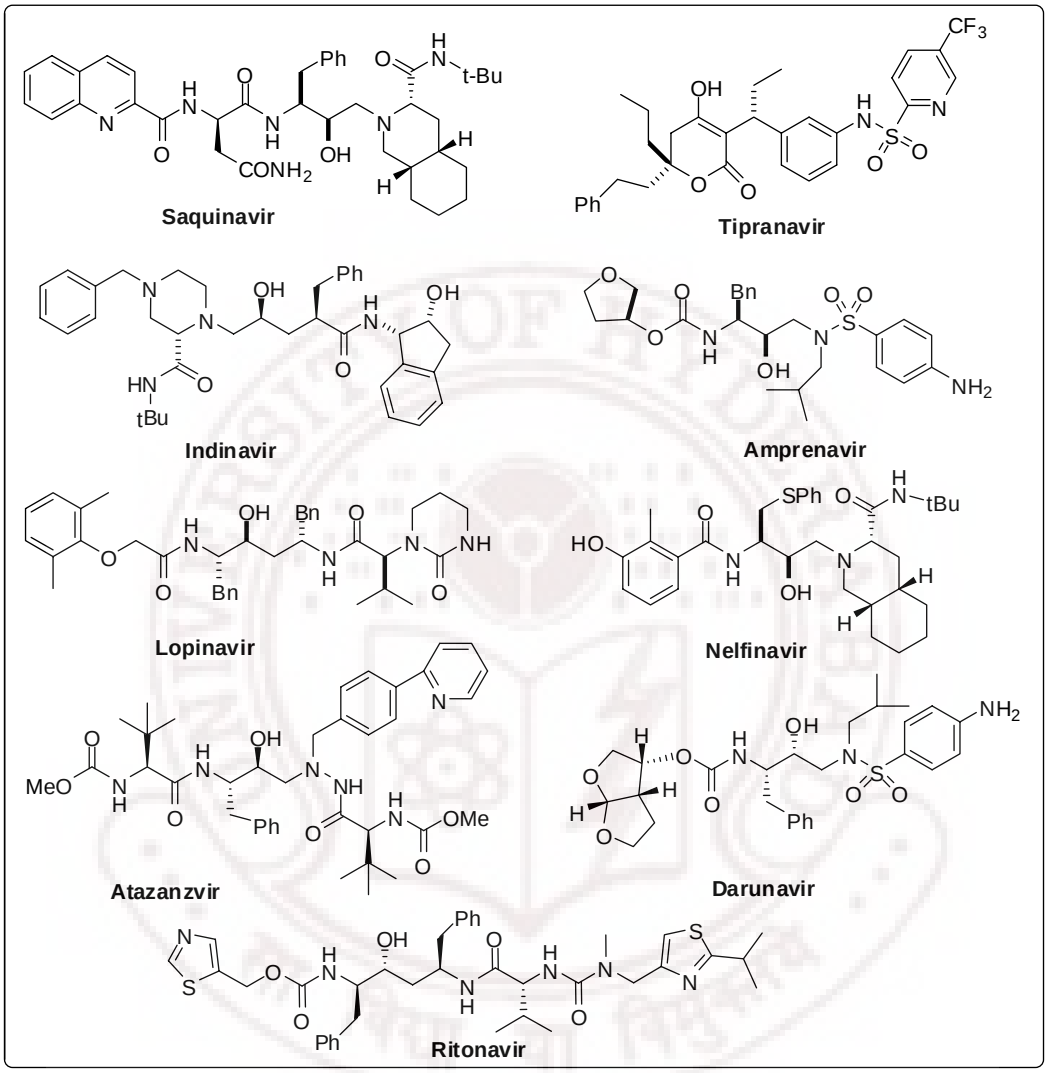


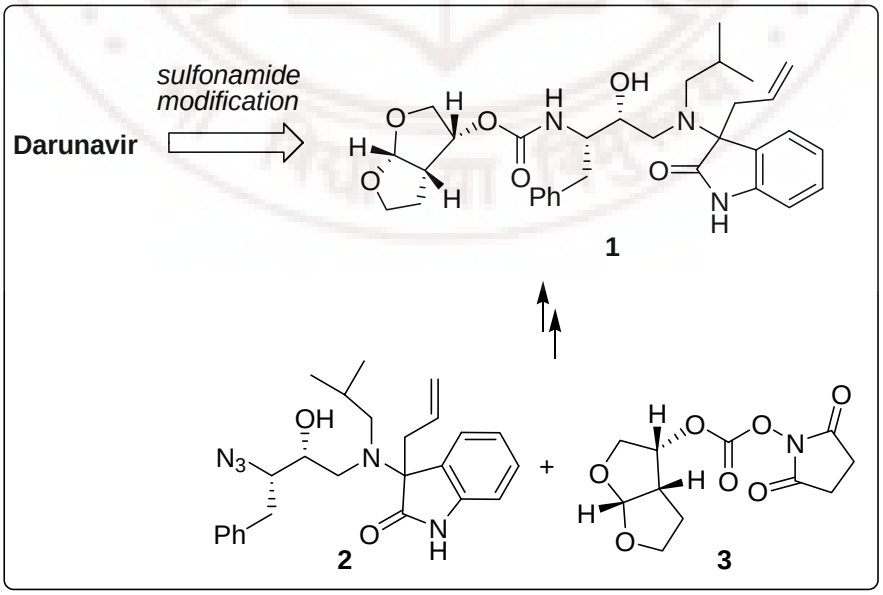
Figure-**2**: *Clinically approved protease inhibitors*

Since their introduction in late 1995, HIV-1 protease inhibitors (PIs) have proven to be an advantageous extension of the existing antiretroviral armamentarium. This introduction has been marked by a profound decrease in mortality rate associated with HIV-1 infection. Today, PIs are considered as

essential components to establish highly active antiretroviral therapy (HAART). Despite this remarkable success, the emergence of mutants that confer multidrug resistance (MDR's) is disturbing and the increasing cross-resistance has necessitated further research with an emphasis on broad spectrum activity against PI-resistant mutants. Some of the recent approaches towards achieving this goal are outlined below.

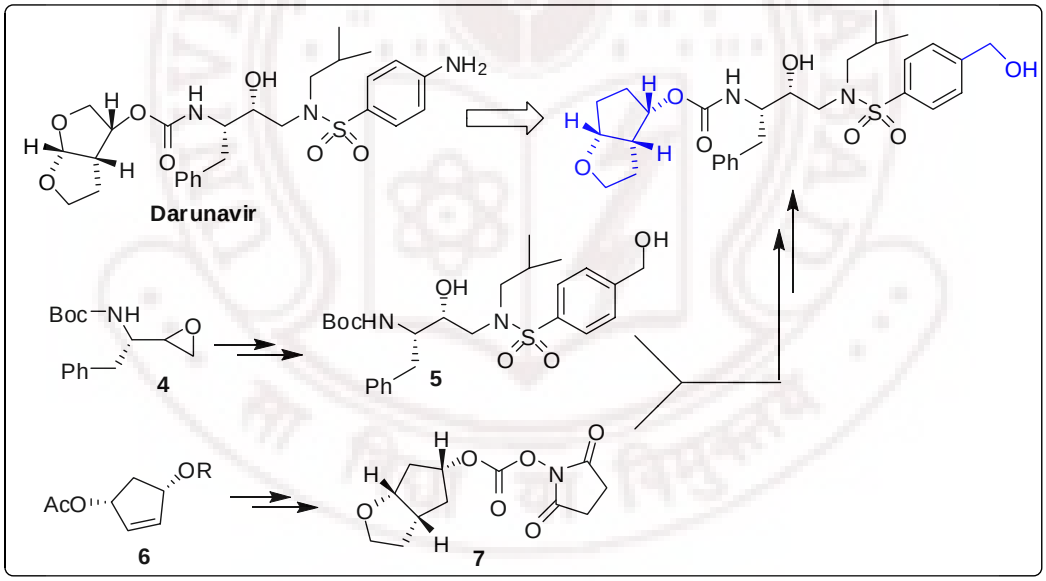
Following the discovery of Darunavir, Ghosh *et al*, made subtle but critical structural modifications and identified novel series of inhibitors. For example, a series of novel oxyindole-derived HIV-1 protease inhibitors.[20] Their synthesis instigated with the coupling of **2** (obtained in multiple steps from Isatin) and **3** (scheme-1). Among a series of inhibitors compound (**1**) exhibited nanomolar inhibitory potencies against HIV protease.

Scheme-**1**: *Synthetic approach to oxyindole derived protease inhibitors*



In an other study by the same group,[21] the inhibitors incorporate a stereochemically defined 5-hexahydrocyclopenta[*b*]-furanyl urethane as the P2-ligand into the (*R*)-(hydroxyethylamino)sulfonamide isostere where the cyclic ether oxygen is positioned to hydrogen bond with the backbone Asp-29 NH. Optically active urethane was prepared by an enzymatic asymmetrization of meso-diacetate with acetyl cholinesterase, radical cyclization, and Lewis acid-catalyzed anomeric reduction as the key steps (scheme-**2**).

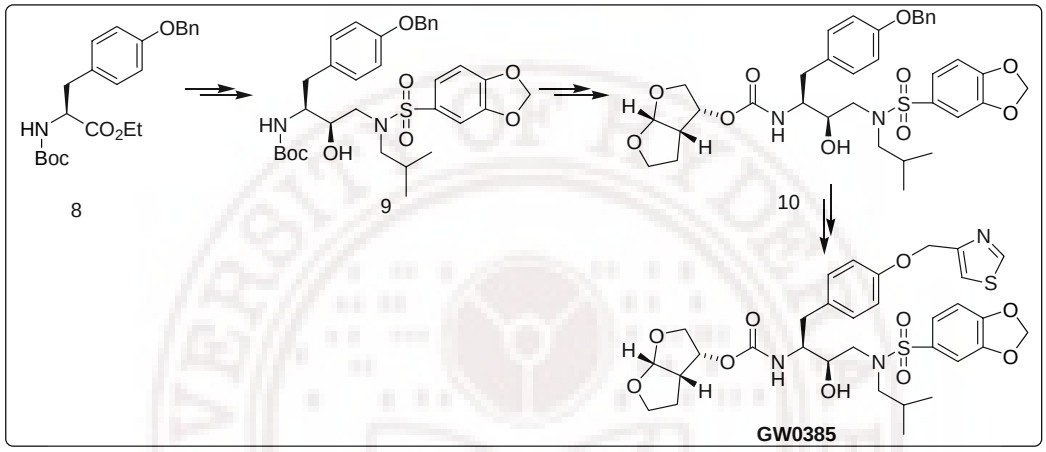
Scheme-**2:** *Synthetic approach to modified Darunavir*



Miller *et al*[22] reported the synthesis of novel series of P1 modified HIV protease inhibitors through iterative modification of the amprenavir scaffold and evaluated for *invitro* antiviral activity against wild-type virus and protease inhibitor-resistant viruses. Optimization of the P1 moiety resulted in compounds with

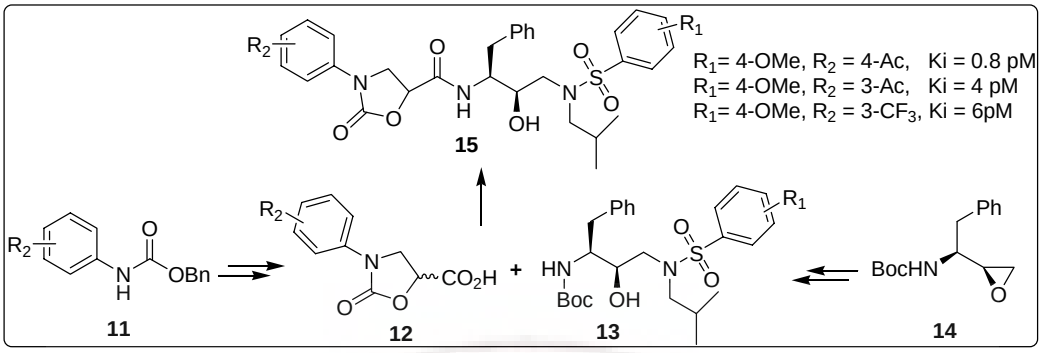
femtomolar enzyme activities and cellular antiviral activities in the low nanomolar range culminating in the identification of clinical candidate GW0385. A brief outline of their synthesis is shown in scheme-**3**.

Scheme-**3:** *Brief synthetic approach to GW0385*



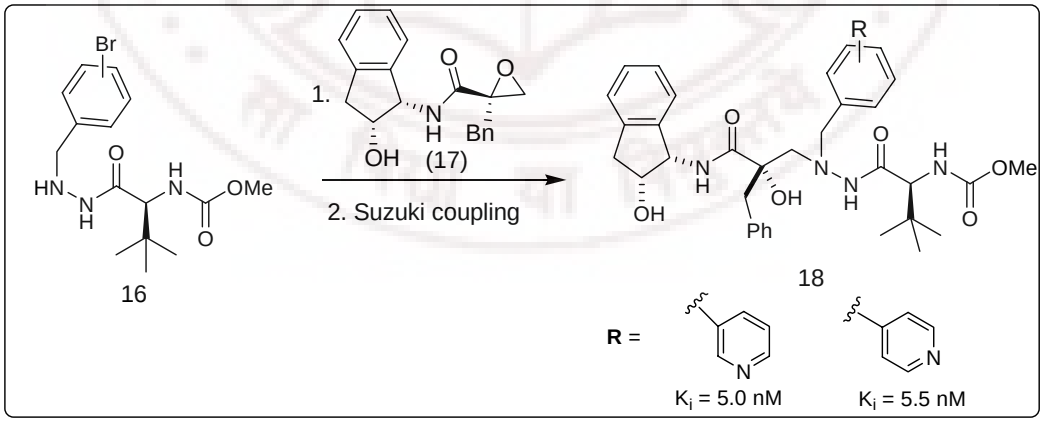
T. M. Rana *et al*[23] designed inhibitors by incorporating *N*-phenyloxazolidinone-5-carboxamides into the (hydroxyethylamino) sulfonamide scaffold. Their synthetic approach is outlined in scheme-**4**. Compounds with (S0-entiomer of substituted phenyloxazolidinones showed inhibitory activities in the picomolar(pM) range.

Scheme-**4:** *Synthetic approach to novel phenyl oxazolidinone derivatives*



Hallberg *et al*[24] recently reported a new class of HIV-1 protease inhibitors which are structurally related to atazanavir and indinavir but comprising a tertiary alcohol as part of the transition-state mimicking unit having a *K*i value of 5.0 and 5.5 nM respectively (Scheme-**5**). Hydrazide coupling, regioselective ring opening of the chiral epoxide and Suzuki coupling were the key transformations used during the synthesis.

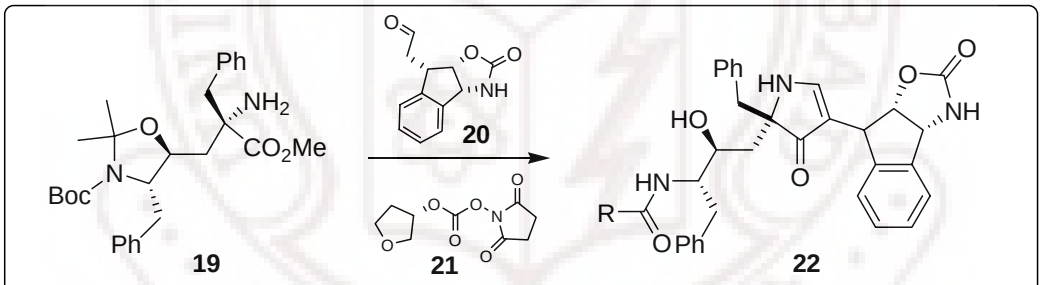
Scheme-**5:** *Synthesis of HIV protease inhibitors encompassing 3° alcohol as transition state mimicking unit*



Based on the fact that proteolytic enzymes (aspartic, serine, metallo, and cysteine proteases) bind to their inhibitors/substrates using the extended β-strand peptide

backbone conformation or equivalent non-peptide structures, a principal goal of the Smith/Hirschmann collaboration at Penn has been the development of the pyrrolinone scaffold as a privileged peptidomimetic. In continuation of their efforts to prepare highly potent pyrrolinone-based HIV-1 protease inhibitors, they have recently reported[25] the design, synthesis, and biological evaluation of a third-generation series of monopyrrolinone-based inhibitors. The most potent inhibitor displayed subnanomolar potency *in vitro* for the wild-type HIV-1 protease (scheme-**6**). Notably, the monopyrrolinone inhibitors retained potency in cellular assays against clinically significant mutant forms of the virus.

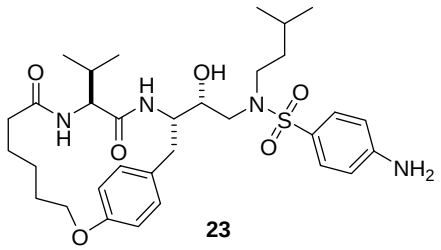
Scheme-**6:** *Brief* **s***ynthetic approach to pyrrolinone based HIV protease inhibitors*



Reagents and conditions: *(a) (i)20, KHMDS, C6H6, THF, (ii) 1N HCl.MeOH, (iii)* ***21****, DIEA*

Fairlie *et al* reported the synthesis of several macrocyclic peptidomimetics[26] mimicking β-strand conformation using RCM that are potent and selective inhibitors of HIV protease (figure-).

Figure-**3** *Representative HIV protease inhibitors mimicking* β*-strand*



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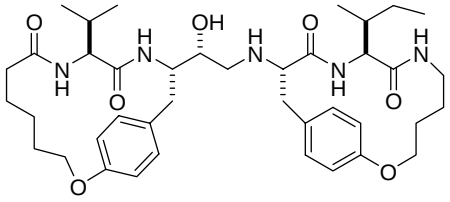
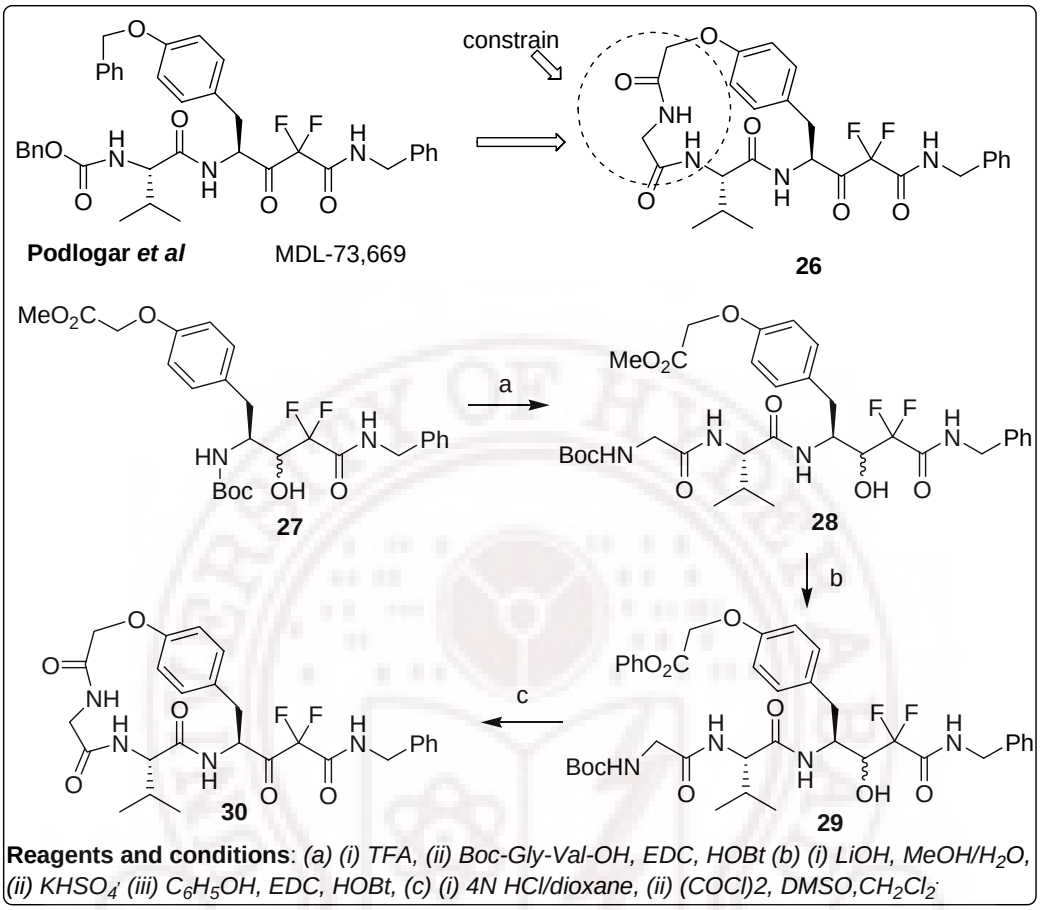


24

K_i = 1.7 nM, IC_{50} (HIV infected MT2cells) = 90 nM. *K_i = 0.6 nM, IC_{50} (HIV infected MT2cells) = 60 nM.*

Podlogar and co-workers[27] designed a macrocyclic inhibitor based on the molecular modeling inputs from the binding mode of MDL 73,669 (K_i = 5 nM) (scheme-**7**) with HIV protease. The design of introducing suitable constraint into the flexible molecule to mimic the bioactive conformation of its linear counterpart resulted in favorable binding.

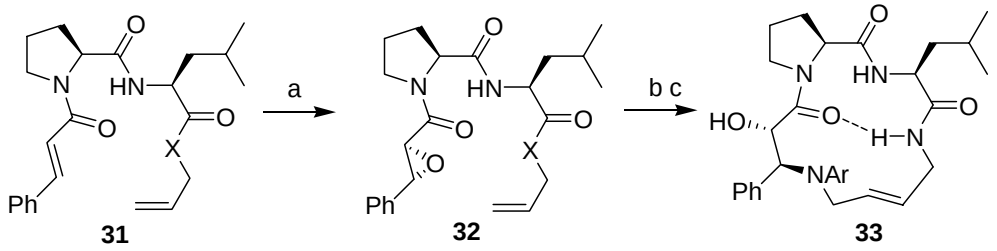
Scheme-**7:** *Synthetic approach to macrocycli inhibitor with difluoroketone isostere*



Reagents and conditions: *(a) (i) TFA, (ii) Boc-Gly-Val-OH, EDC, HOBt (b) (i) LiOH, MeOH/H_2O, (ii) $KHSO_4$, (iii) C_6H_5OH, EDC, HOBt, (c) (i) 4N HCl/dioxane, (ii) (COCl)2, DMSO,CH_2Cl_2.*

Iqbal and co-workers [28] developed norstatine containing macrocycles obtained by ruthenium alkylidene catalyzed ring closing metathesis (scheme-**8**). Synthesis of these macrocycles involved a diastereofacial selective epoxidation of the cinnamoyl proline derived peptides followed by ring opening with allylamine to afford acyclic precursors leading to facile RCM reaction to afford designed macrocyclic peptides.

Scheme-8: *Synthetic approach to cyclic peptides using RCM*



Reagents and conditions: (a) Polyaniline supported Cobalt salen, O_2/isobutyraldehyde, (b) Allylamine, $CoCl_2$ (cat), (c) Grubb's catalyst 10 mol %, dry DCM.

Despite the success achieved in the design of various HIV protease inhibitors, a significant problem with current antiviral treatments based on inhibition of HIV-1 protease is the rapid onset of viral resistance[29] and the limited number of options available after initial failure with mono or combination drug therapy. A peptidomimetic approach with significant potential that has emerged in recent years to overcome the inherent fundamental limitations associated with the use of peptides as therapeutics *viz* poor permeability across membranes, proteolytic degradation, rapid clearance and in some cases poor solubility and a tendency to aggregate, oral bioavailability is by the incorporation of unnatural amino acids namely β-amino acids. There are number of examples of naturally occurring biologically active peptides containing substituted β-amino acids isolated from marine organisms and various prokaryotes (Figure-**4**).

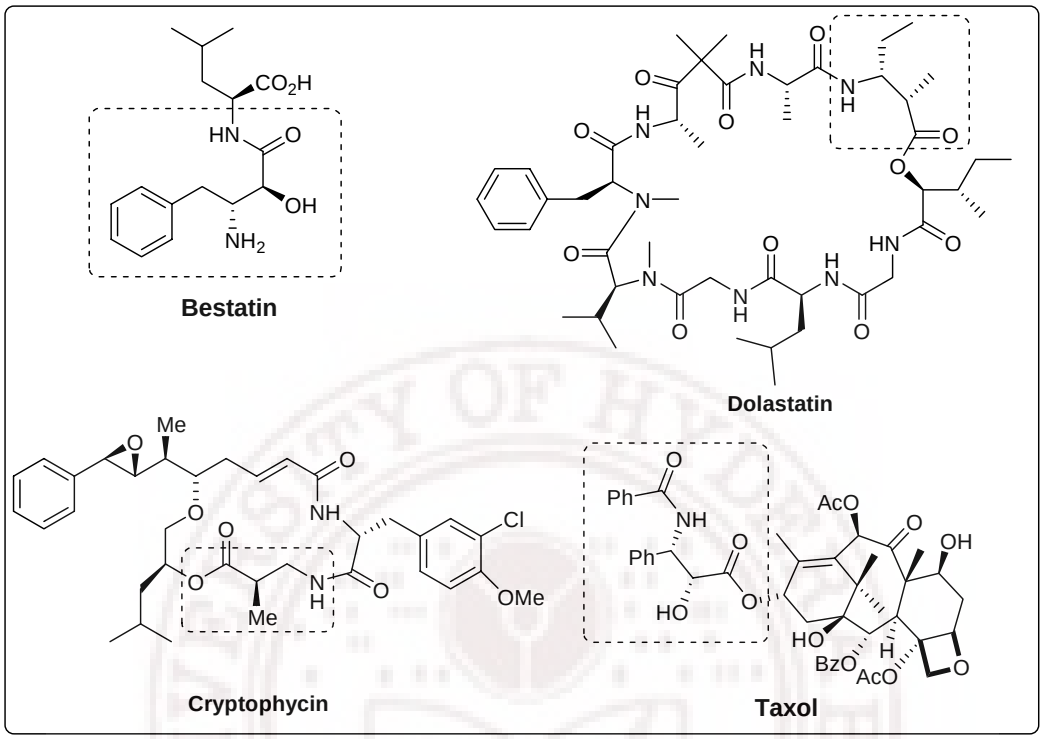


Figure-**4**: *Some naturally occurring β-amino acids*

Given the significance of β-amino acids, it is not surprising that their enantioselective synthesis has become an important and challenging endeavor for organic chemists. The conscientious effort of various research groups towards the synthesis of diverse β-amino acid derivatives is a reflection of their importance in the development of peptidomimetics. A plethora of methods developed for the synthesis of various β-amino acid derivatives have been reviewed in the past.[30] The study of β-peptides has accelerated over the past decade propelled by demonstrations that they can be programmed to adopt protein-like secondary structures which have given rise to a variety of biological activities. Since then,

several papers have been published exploiting the possibility of synthesizing potent bio-active peptides. For example, β-peptides composed entirely of β-amino acids were investigated as inhibitors of cholesterol and lipid absorption through the brush border membrane in small intestines.[31] Recently, the structural properties of β-peptides have been exploited in the *denovo* design of antimicrobial agents. Notably, Gellman *et al* [32] have recently shown that 12-helical β-peptides are capable of selectively killing a variety of bacterial species including 2 clinical isolates that are resistant to antibiotics. Helical amphilicity along with 40 % cationic face was found to be best for activity.

In the recent past, oligomers of β-peptides have been used as probes in understanding critical protein-protein interactions. For example, a set of β^3-peptides were shown to recognize a cleft on the surface of the human oncogene product double minute 2 (hDM2) by Schepartz *et al*.[33] Gellman *et a*l [34] have designed and synthesized a set of β-peptides containing repeating triads of (X-β^3hArg- β^3hArg), where the choice of X was intended to influence 14-helical stability. Molecular designs with the unique control of helix stability offered by β-peptides allow designing of cationic oligomers with improved cargo-delivery ability. Of late, the same authors reported the synthesis of (14/15 helical secondary structures responsible for the tight binding to Bcl-x_L) chimeric (α/β+α)-peptide ligands for the BH3-recognition cleft of Bcl-x_L.[35]

The protease resistance of β-peptides has been exploited in the synthesis of protease inhibitors. For example, the α-hydroxy β-amino acids are important class of β-amino acid peptidomimetics due to their action as transition state mimics and are found in inhibitors of hydrolyzing enzymes for example: Apstatin-an inhibitor of aminopeptidase.[36] Other work using α-hydroxy β-amino acids include the use of α-hydroxy β-phenylalanine to stabilize the scissile Leu-Val bond in an analogue of the peptide Pro-Phe-His-Leu-Val.[37] This compound was a potent inhibitor of renin, an aspartate protease that generates angiotensin I from angiotensinogen. Another interesting biological application for β-amino acids in protease inhibitor design is the phosphoramidate-β-amino acid-containing inhibitor of the serine protease β-lactamase.[38] An alternate approach to the design of enzyme inhibitors has been the use of a substrate peptide sequence as the basis for inhibitor design and then stabilisation of the scissile bond *via* a number of possible chemical modifications. In a recent study,[39] β-amino acids were used to stabilise an inhibitor of the enzyme endopeptidase EC 3.4.24.15 (EP24.15) against proteolysis by the related enzyme neprilysin (EP24.11). In another approach to the design of inhibitors of EP24.15 and EP24.16, β-amino acids were incorporated into the scissile bond of the substrate bradykinin.[40] β-Glycine substitution at or near the scissile bond (Phe^{s}-Ser^{s}) completely prevented cleavage by either enzyme. These studies clearly demonstrate that the incorporation of β-amino acids into peptide sequences represents a powerful

approach to stabilizing peptides against proteolytic attack, and demonstrates the potential of β-amino acids in the design of novel peptidomimetic peptidase inhibitors.

3.2 Present study

The unpredictability of the induced fit, with the shape of both ligand and enzyme changing cooperatively and unpredictably in response to subtle changes within a ligand has been a major problem in the *de novo* design of enzyme inhibitors. While others have postulated receptor-based conformational selection of ligands and successfully designed inhibitors to emulate the β-strand binding motif of native ligands, [41] *novel protein conformations* (not observed in either native or enzyme-inhibitor complexes) can also be exploited to create enzyme inhibitors. [42] Driven by the fact that incorporation of β-amino acids has been successful in creating peptidomimetics that not only have potent biological activity but are also stable to proteolysis, we reasoned that, the turn inducing propensity of β-amino acids can be exploited in creating potent cyclic peptides based on the recent developments in the area of drug discovery, which have shown small conformationally constrained peptides with certain structural features to be more potent as leads.[43] Based on this hypothesis, we believe that constrained cyclic peptides can be exploited in designing selective ligands as potential drug candidates.[44]

Hence, in an ongoing project in our laboratory on the discovery of new chemical entities (NCE) inhibiting HIV-I protease, we have undertaken the synthesis of small cyclic peptides based upon the structural mimicry of the 'Phe-Pro' bond

present in the 'gag-pol' polyprotein. It is well known that HIV protease specifically cleaves the imide bond between phenylalanine and proline. So the design of a molecule, mimicking the stereo electronic environment of this scissile bond coupled with protein secondary structural motifs may lead to potent inhibitors. In an attempt to access a conformational mimic of 'Phe-Pro' bond, where aziridine peptide **I** was conceptualized to function as the surrogate of the 'bioactive' conformation during the cleavage by protease (Figure-**5**)

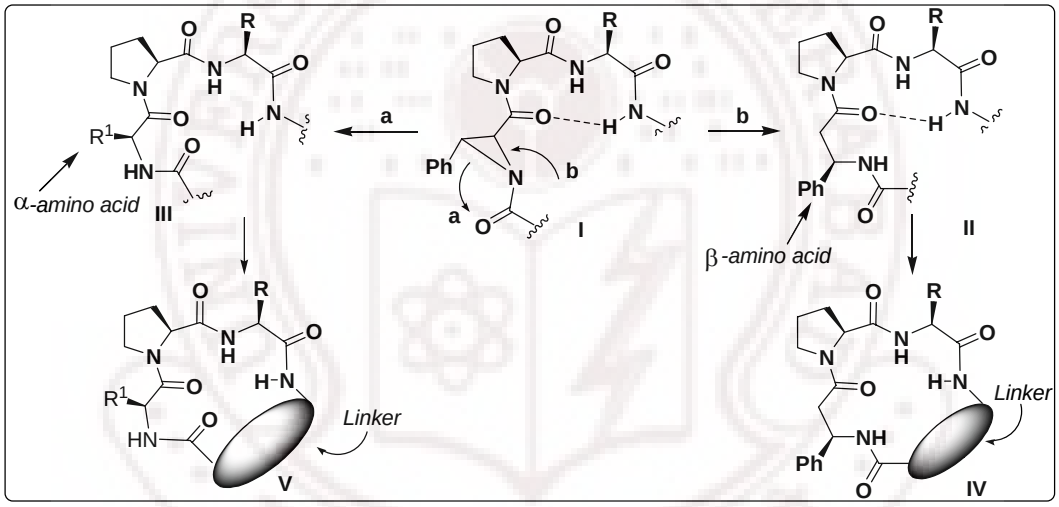


Figure-**5**: *Mimicry of different protein secondary structure in small peptides*

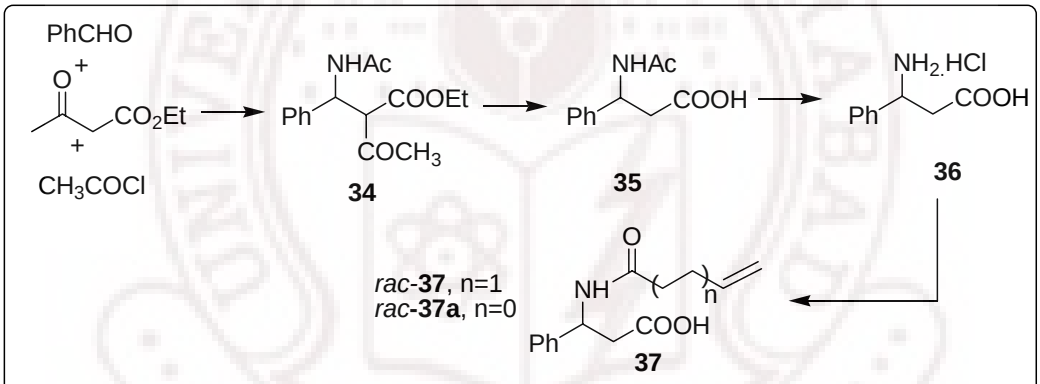
Conceivably, the breaking of bond **'a**' may lead to α-amino acid-L-proline derived acyclic peptides **III** which were shown by us earlier to exist in an organized conformation (β-turn) involving intramolecular hydrogen bond. On the other hand, the breaking of aziridine bond '**b**' in **I** may lead to homophenylglycine (β Phg)-L-proline derived acyclic peptides **II** which can be

converted in to cyclic form possessing a conformational mimic of a 'Phe-Pro' bond in an entropically advantageous fashion. In order to achieve this objective, we have developed a strategy to synthesize β-amino acid (β Phg)-L-proline derived cyclic peptides IV via ring closing metathesis reaction (RCM).

3.3 Results and Discussion

We initially focused our attention towards the synthesis of β-amino acid namely homophenylglycine residue (*rac*-**37 and** rac-**37a)** using a multi-component coupling procedure developed earlier[45] in our laboratory from benzaldehyde, ethyl acetoacetate and acetyl chloride using catalytic anhydrous cobaltous chloride in acetonitrile (scheme-**9**).

Scheme-**9:** *Preparation of* (±)-β-*amino acid derivatives*

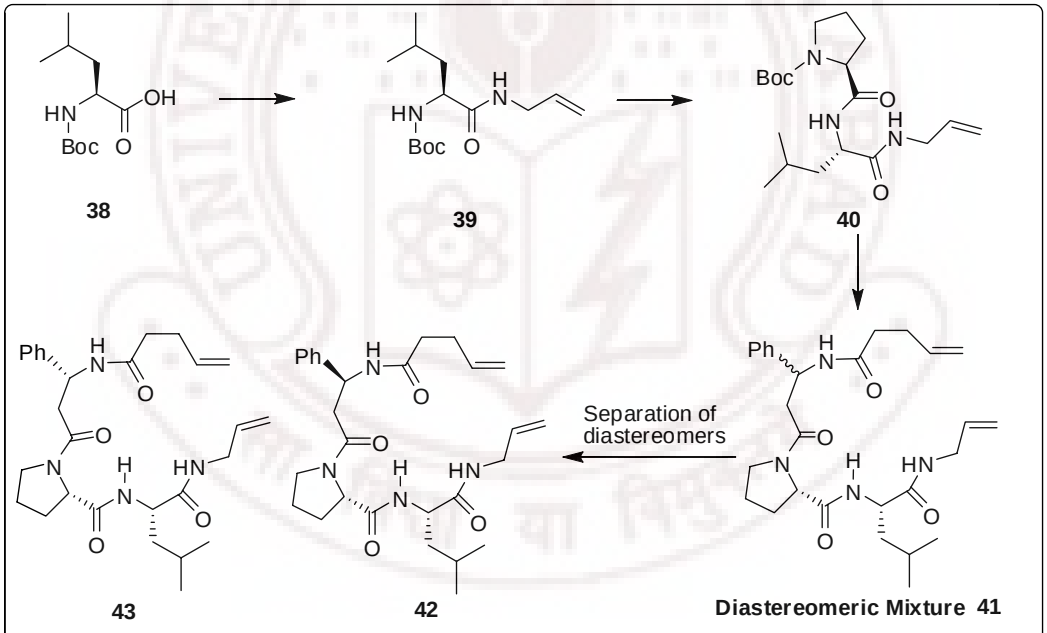


Reagents and Disscussion: (a) $CoCl_2$, CH_3CN, rt, 6 days, 45 % (b) $LiOH.H_2O$, $MeOH:H_2O$, reflux, 11h, 21 % (c) 6N Hcl, refl;ux, 13 h, 67 % (d) pentenoyl chloride,K_2CO_3, acetone, 0^o-RT, 5 h, 59 % (e) acrolyl chloride, K_2CO_3, acetone, 0^o-RT, 15 h, 47 %

Our next aim was to synthesize acyclic peptides with olefinic segments in the form of pentenoyl and allyl at *N*- and *C*- terminal respectively and cyclize it using RCM to generate surrogate amino acid as linker. The dipeptide required for the coupling was synthesized as shown in scheme-**10**, where *N*-Boc-Leu was coupled with allylamine using mixed anhydride protocol to give *N*-Boc-Leu allylamide **39** in 65% yield. The 'Boc' group in **39** was deprotected using TFA and then

coupled with *N*-Boc-L-Pro to afford dipeptide (*N*-Boc-Pro-Leu allylamide) **40** in good yields. This was subsequently coupled with the **rac 37** using the mixed anhydride protocol to yield the tripeptide as a mixture of diastereomers. The diastereomers were separated using column chromatography to afford the acyclic peptides **42** and **43** respectively. These peptides contain a homophenylglycine (β Phg) with L-Pro-L-Leu dipeptide template with olefinic segments that enable cyclization *via* ring closing metathesis reaction.

Scheme-**10:** *Preparation of acyclic peptides* **42** *and* **43** *from N-Boc-Leucine*



Reagents and conditions: (a) Allylamine, $ClCO_2{}^iBu$, NEt_3, Dry CH_2Cl_2, 0 ^oC-RT, 12h, 87.3 % (b) N-Boc Pro $ClCO_2{}^iBu$, NEt_3, Dry CH_2Cl_2, 0 ^oC-RT, 12h, 95 % (c) ***rac-37,*** *$ClCO_2{}^iBu$, NEt_3, Dry CH_2Cl_2, 0 ^oC-RT, 12h.*

Earlier studies (chapter II) have shown that the chirality at *N*-terminal to Pro residue has a profound influence on the conformation of the acyclic and cyclic

tripeptides consisting of α-amino acids. Hence, we became interested in investigating the role of chirality of β-amino acids on the conformation of acyclic peptides by NMR. Peptides **42** and **43** yielded well-resolved ^{1}H NMR spectra in both $CDCl_3$ and $DMSOd_6$. Sequential resonance assignments were achieved using a combination of TOCSY (spectrum **2**, page# 223 and spectrum **5**, page# 226) respectively) and NOESY spectra (spectrm **3**, page# 224 and spectrum **6**, page#227 respectively). 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, $CDCl_3$. The solvent titration curves and temperature coefficients of NH chemical shifts in DMSO-d_6 are shown in Figure-**6**.

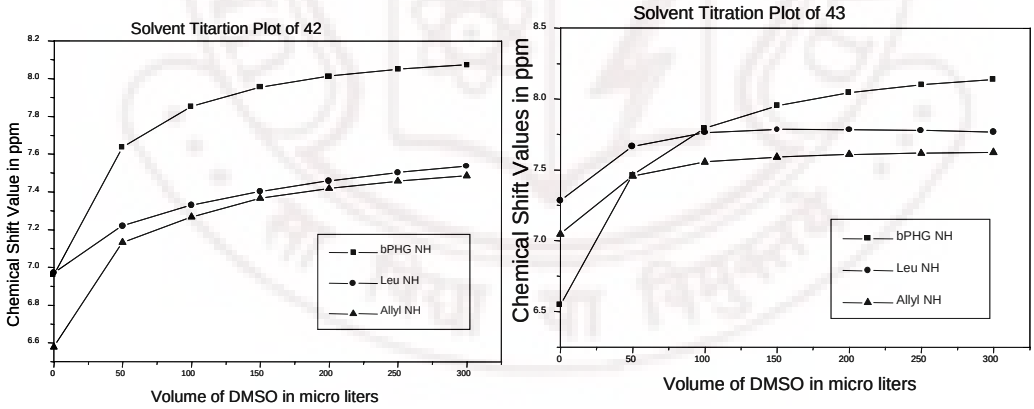


Figure**-6:** *Solvent titration plot of acyclic tripeptides **42** and **43**.*

Conformation of 42 and 43:

The ^{1}H NMR spectrum of **42** (spectrum **1**, page # 222) and **43** (spectrum **4**, page # 225) in $CDCl_3$ showed downfield shifted resonances of the Leu NH and Allyl

NH in the NMR spectrum followed by solvent titration studies, showed small shifts of 0.56 ppm and 0.90 ppm respectively (Figure **6**), when 33% v/v of DMSO-d_6 was added to 600 µl $CDCl_3$ solution, thus confirming the presence of H-bonds. The inter residue NOE cross peak involving between both β-Phg CαH↔Pro CδH confirmed a *trans* imide bond while, the cross correlations between Leu NH↔Allyl NH, Allyl NH↔ Pro CαH, Leu NH↔Pro CδH, β Phg CαH↔Pro CδH, Leu NH↔β Phg CβH, βPhg CβH↔Pro CδH strongly supported for pseudo 3_{10} helical conformation(11/10) in solution (Figure-**7**).

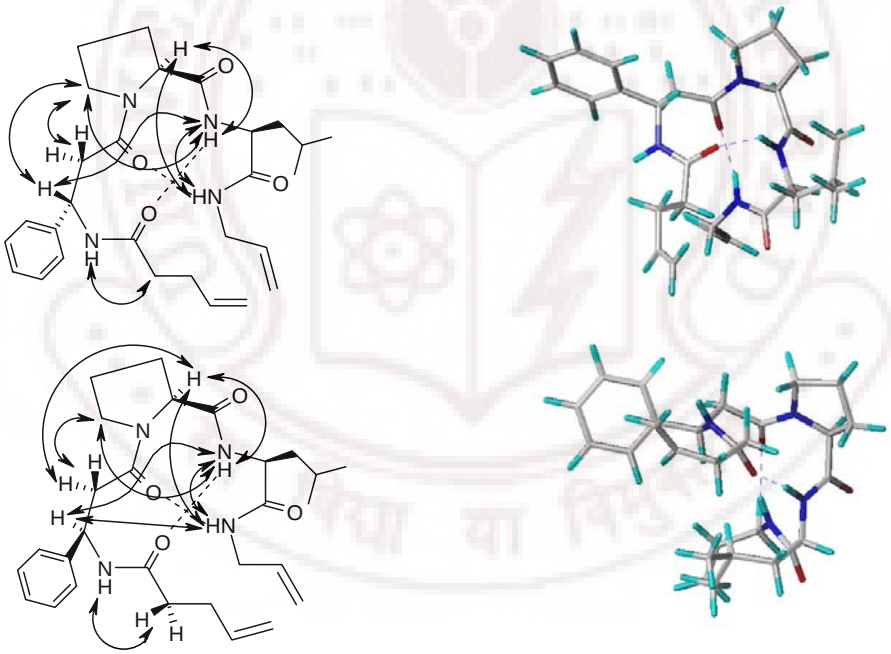
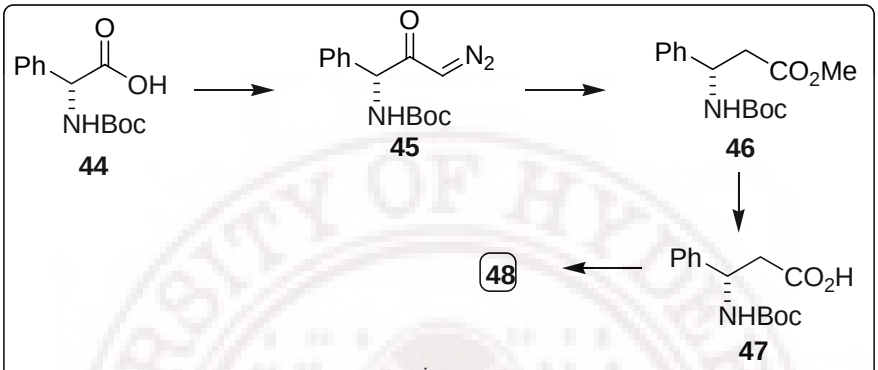


Figure-**7**: *Diagnostic NOEs and one of the lowest energy conformations collected in the MD simulation study of* **42** and **43**.

The absolute stereochemistry of the stereocenter in the β-amino acid residue in **42 and 43** was proved by chemical correlation studies as shown in scheme-**11**.

Scheme-**11**: *Arndt-Eistert homologation*

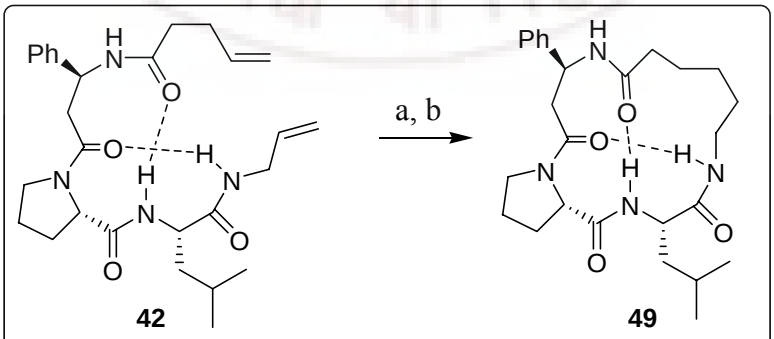


*Reagents and condtions:(a) $ClCO_2{}^iBu$, NEt_3, excess CH_2N_2, Dry CH_2Cl_2, 0 ^oC,1h, 78 % (b) Ag(OAC), NEt, MeOH, rt, 0.5 h, 75 % (c) LiOH, MeOH-H_2O, RT, 2h, 85 % (d) Dipeptide **40**, LiOH,MeOH-H_2O, rt, 2h, 72 % (e) i. TFA, Dry CH_2Cl_2, 0 ^oC, 3h, ii. 4-Pentenoic acid, $ClCO_2{}^iBu$, NEt_3, Dry CH_2Cl_2, 0 ^oC-rt, 6 h, 70 % over two steps*

N-Boc-D-*phenylglycine* was converted to the homologated ester **46** *via* diazoketone **45** following Arndt-Eistert homologation and hydrolysis to afford the corresponding β-amino acid **47**. The homologated acid was coupled with dipeptide (**40**) using EDC/HOBt afforded tripeptide in good yields which on deprotection followed by coupling with 4-pentenoic acid yielded diastereomer **48**. HPLC studies and optical rotation indicated that the absolute configuration of the β-amino acid residue obtained by Arndt-Eistert homologation $[\alpha]_D$ = -61.00 (c, 0.5, MeOH) was identical to the diastereomer **42** $[\alpha]_D$ = -58.00 (c, 0.5, MeOH) obtained in scheme-**10** and was assigned the '*S*' absolute stereochemistry and '*R*' was assigned by analogy to the other diastereomer **43** for the asymmetric center

in the β-amino acid residue. We have demonstrated earlier that the acyclic tripeptides derived from α-amino acids with double bond at both terminal generally preorganise themselves leading to a β-turn or a 3_{10} helix and have a strong propensity to undergo cyclization when subjected to a RCM reaction. Similar study on acyclic peptides **42** and **43** with homo & heterochiral β-amino acids at *N*-terminal to L-Pro would enable us to understand the influence of β-amino acids in invoking turns in small cyclic peptides. Accodingly, we subjected **42** to RCM reaction in the presence of first generation Grubb's ruthenium catalyst (10 mol %) to afford the unsaturated cyclic peptide as a mixture of *E*:Z isomers (70:30 by TLC), which on reduction using 10 % Pd/C in MeOH afforded cyclic peptide **49** in good yield (55 % over 2 steps) (Scheme-**12**) It is interesting to note that **49** is a 17-membered macrocyclic peptide with one β-amino acid and the cyclization results in the formation of an amino acid spacer, 6-amino hexanoic acid (*Aha*).

Scheme-**12:** *Preparation of cyclic peptide* **49** *from acyclic peptide* **42**



Reagents and conditions: (a) 10 mol% Grubbs catalyst, CH_2Cl_2, reflux, 12h (b) H_2/Pd/C, MeOH, 55 % over two steps.

Conformation of 49:

Low field appearance of Leu NH (7.01 ppm) and Aha NH (6.93 ppm) and solvent titration studies confirmed their participation in intramolecular H-bonding. Small magnitude of temperature coefficient for Aha NH (-1.8 ppb/°K) and medium value for Leu NH (-4.2 ppb/°K) confirmed that most of the molecular population has Aha NH participating in H-bonding, while much smaller population has Leu NH H-bonds respectively (Figure-**7**). ^{1}H NMR spectrum of **49** in $CDCl_3$ (spectrum **7**, page# 228) showed only one set of resonances implying the presence of a single isomer. The diagnostic NOEs between β Phg CαHs↔Pro Cδs confirmed a *trans* imide bond-preceding Pro, Further, the cross peaks, Leu NH↔Aha NH, Leu NH↔β Phg CβH, Leu NH↔Pro Cδ(Pro-S) H, and Aha NH↔Pro CαH in the NOESY spectrum (spectrum **9**, page# 230), indicates pseudo 3_{10} helical conformation with two intra molecular H-bonds between Leu NH ← Aha CO and Aha NH ← β Phg C=O (Figure-**8**).

In polar solvent DMSO-d_6 the appearance of two sets of resonances with 56:44 ratio, and the presence of exchange peaks between the two sets of resonances implied that rotamerisation takes place about β Phg-Pro amide linkage. In the major isomer the NOE cross peaks between β Phg CαH(H & H′)↔Pro CδH(H & H′) confirm the presence of *trans* amide bond preceding Pro. In addition, the cross peaks Leu NH↔Aha NH, Leu NH↔Pro Cδ(Pro-S) H, Leu

NH↔β Phg CβH and Aha NH↔Pro CαH supported the existence of pseudo 3_{10} helical conformation with Leu NH←Aha CO and Aha NH←β Phg CO H-bonds. In the minor isomer, the NOEs β Phg CαHs↔Pro CαHs supported the presence of *cis* amide-preceding Pro. While the Δδ/ΔT for all the amide protons ruled out the existence of intramolecular H-bonding, the NOE cross peaks between Leu NH↔β Phg CαH, Leu NH↔β Phg Cα′H, β Phg CαH↔Pro CαH, Leu NH↔Pro CδH, indicated a turn structure in the molecule resembling a type-VI β turn, without any intramolecular H-bond.

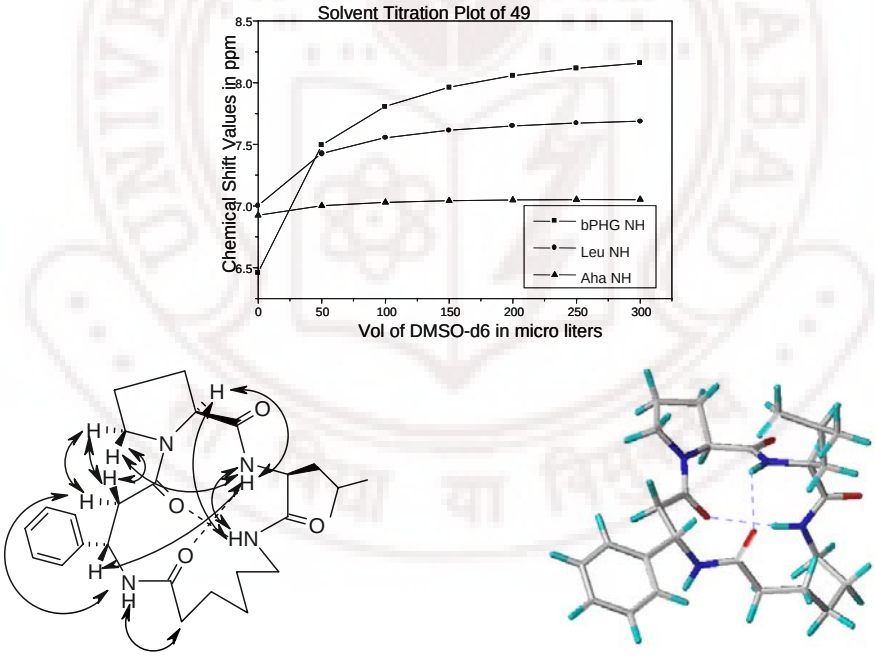
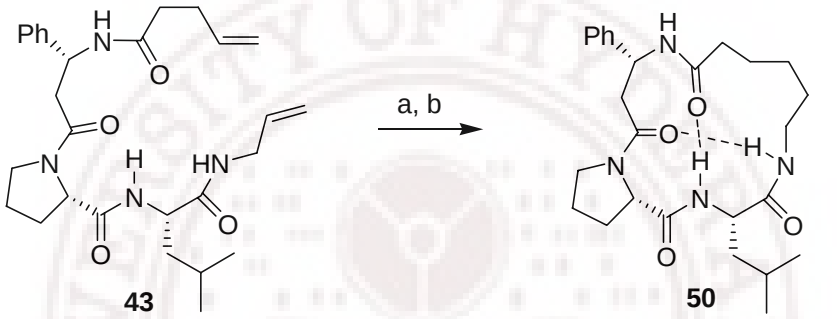


Figure **8:** *Solvent titration plot, diagnostic NOEs and one of the low energy conformation by MD simulation study of* ***49****.*

The RCM of **43** was found to be comparatively slower and sluggish than **42**, which may be attributed to the influence of (*R*)-β-amino acid in **43**. Hydrogenation of unsaturated cyclic peptide yielded the saturated **50** (27 % over 2 steps).

Scheme-13: *Preparation of cyclic peptide* **50** *from acyclic peptide* **43**



Reagents and conditions: (a) 10 mol% Grubbs catalyst, CH_2Cl_2, reflux (b) H_2/Pd/C, MeOH, 27 % yield over two steps.

Conformation of 50:

Single set of proton resonances in both solvents ($CDCl_3$ & $DMSOd_6$) in peptide **50** indicated that only a single rotamer in *trans* geometry exists about β Phg-Pro amide linkage, which was confirmed by the diagnostic NOE cross peak between β Phg CαH-Pro CδH (spectrum **13**, page# 234). In $CDCl_3$ solution, the solvent titration studies showed that Leu NH shifted 0.96 ppm, Aha NH shifted 0.51 ppm and βPhg shifted 0.05 ppm (Figure-**9**), which suggested that βPhg NH and Aha NH protons are H-bonded and Leu NH does not find a CO to form H-bond or may be exposed to solvent. However, based on the chirality of βPhg an unprecedented upfield appearance of Leu NH at 5.57 ppm was expected due to its

orientation in the upfield zone of phenyl ring current and similarly the down field appearance of βPhg NH at 7.92 ppm was attributed because it lies in the deshielding zone of phenyl ring current. The cross peaks between Leu NH↔Ar H (ortho), Leu NH↔ Ar H (meta) suggested for non-covalent aromatic π...NH interaction between Leu NH and βPhg phenyl ring, which leads to βPhg NH↔Ar H(ortho) NOE that is responsible for the downfield appearance of βPhg NH. The NOE cross peaks between Leu NH↔Aha NH, β Phg NH↔Aha NH, Aha NH↔Pro CαH, Leu NH ↔Pro CδH and intramolecular hydrogen bonding of Leu NH and Aha NH supports for the existence of (11/10) pseudo 3_{10} helical conformation in solution.

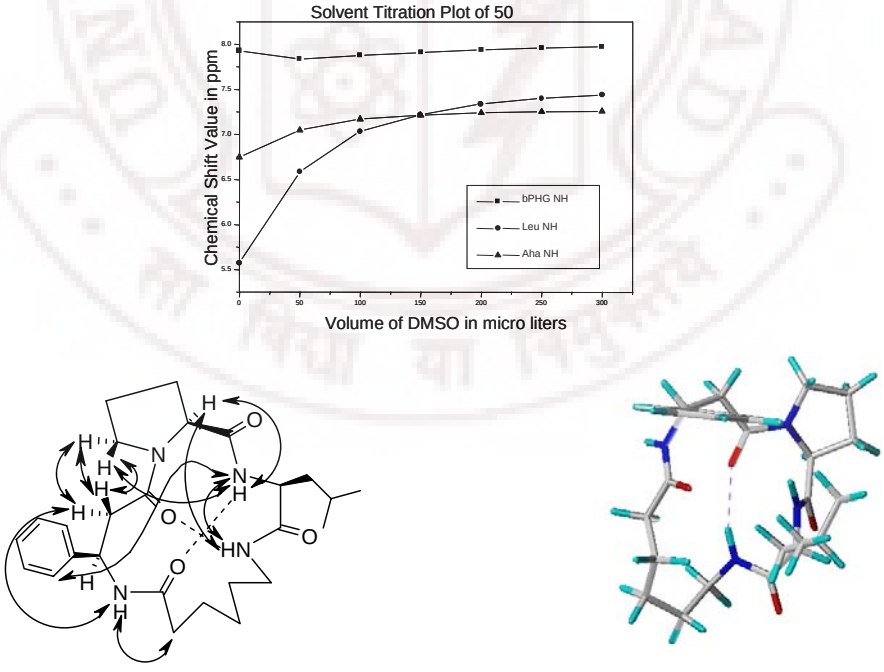
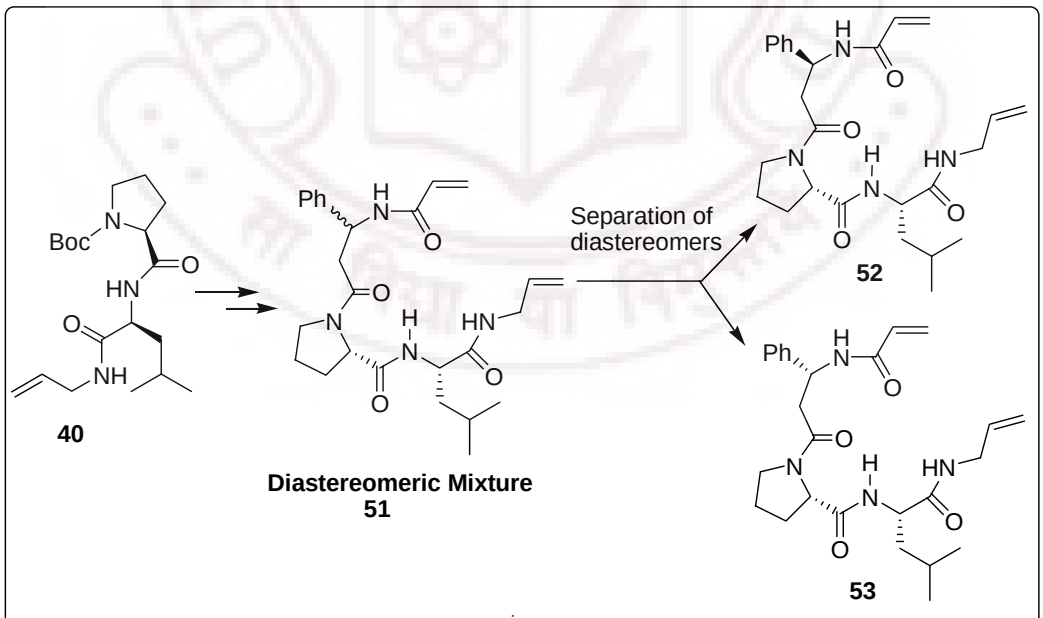


Figure-**9:** *Solvent titration plot, diagnostic NOEs and one of the lowest energy conformation collected in the MD simulation study of* ***50****.*

In polar solvent DMSO-d_6, the hydrogen bonding partners ($\Delta\delta/\Delta T$ of -3.1 ppb /°K and -1.8 ppb/°K for Leu NH and Aha NH) and the NOE peaks Leu NH↔Aha NH, Aha NH↔Pro CαH, Leu NH↔Pro CδH, Aha NH↔β Phg CβH confirmed H-bonds between Leu NH←Aha C=O and Aha NH← β Phg C=O, a geometry consistent with pseudo 3_{10} helical conformation (Figure-**9**).

We then focused our attention towards forming cyclic peptides having shortened linkers as this would enable us to not only construct smaller macrocycles but also explore the role of linker towards the conformation of the cyclic peptide. Accordingly we decided to have linkers in the form of acrolyl and allyl at *N*- and C-terminal respectively as shown in scheme-**14**.

Scheme-**14:** *Preparation of acyclic tripeptides* **52** *and* **53** *from dipeptide* **40**



Reagents and conditions: (a) ***rac-37a,*** *$ClCO_2{}^iBu$, NEt_3, Dry CH_2Cl_2, 0 ^oC-RT, 12h, 46 %*

The acyclic peptides were synthesized by coupling the dipeptide *N*-Boc-Pro-Leu allylamide with **rac 37a** to yield a mixture of diasteromers which were separated to yield tripeptides **52** and **53**. Based on the chemical correlation study, the absolute stereochemistry of β-amino acid in **52** and **53** was assigned as '*S*' and '*R*' respectively.

Conformation of 52 and **53**:

The downfield shifted resonances of the Leu NH and Allyl NH were indicative of their involvement in hydrogen bonding (spectrum 14, page# 235 and spectrum 17, page# 238). Solvent titration studies showed small shifts of 0.54 ppm for Leu NH, 0.87 ppm for Allyl NH and 0.53 ppm for Leu NH, 0.63 for Allyl NH in **52** and **53** respectively (Figure **10**), when 33% v/v of DMSO-d6 was added to 600 µl $CDCl_3$ solution, thus confirming the presence of H-bonds.

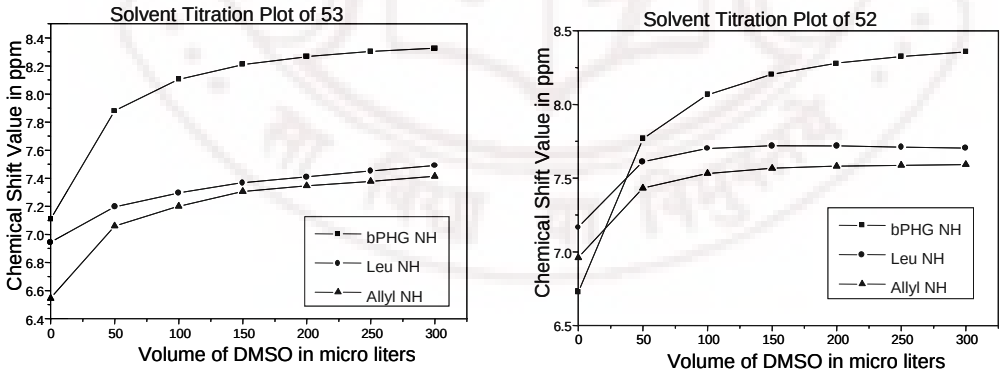


Figure-**10** *Solvent titration plots of* ***52 and 53.***

The diagnostic NOE cross peak between β-Phg CαH↔Pro CδH confirmed a *trans* imide bond (spectrum **16**, Page# 237 and spectrum **19,** page# 240). In

addition, Leu NH↔Allyl NH, Allyl NH↔Pro CαH, Leu NH↔Pro CδH, β Phg CαH↔Pro CδH, Leu NH↔β Phg CβH, β Phg CβH↔Pro CδH NOE correlations strongly supported a pseudo 3_{10} helical conformation in solution (Figure **10**).

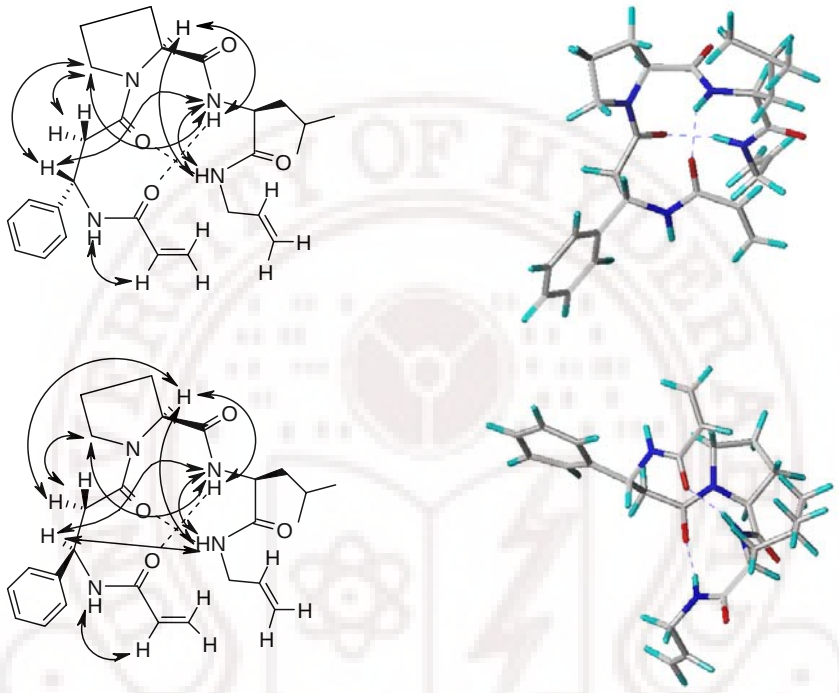
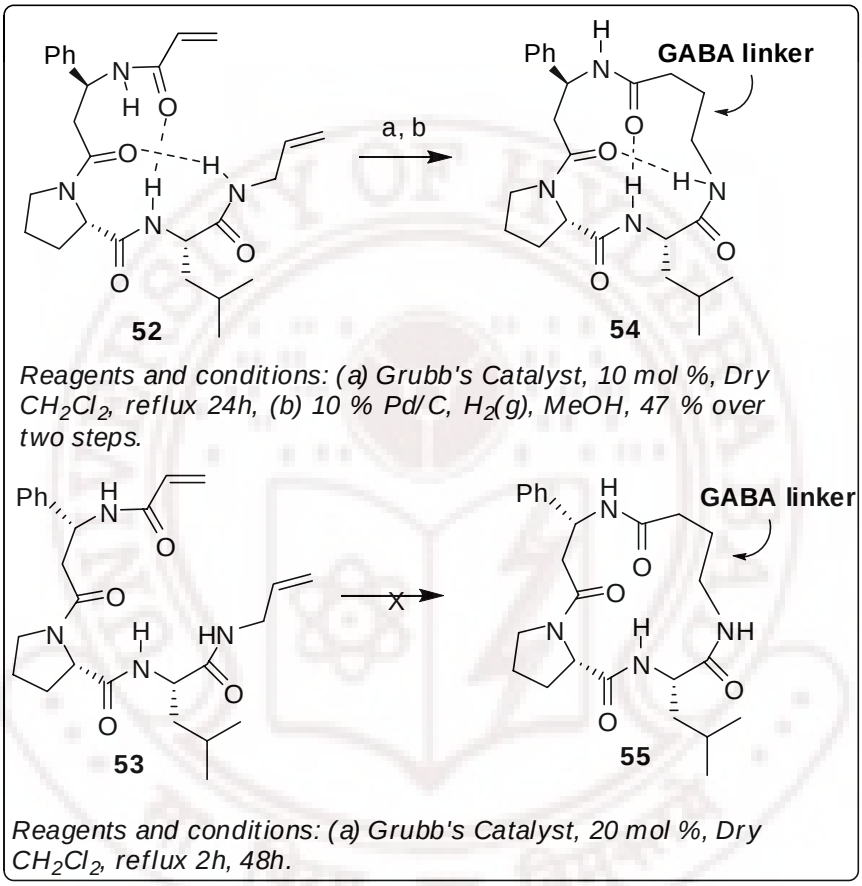


Figure-**11:** *Diagnostic NOEs and one of the lowest energy conformations collected in the MD simulation study of* ***52*** *and* ***53****.*

It is noteworthy that acyclic peptide **52** (similar to the cyclization of **42** having 'S'-chirality at βPhg) underwent smooth RCM reaction using first generation Grubb's ruthenium catalyst (10 mol%) to afford the cyclic peptide which on hydrogenation gave **55** in good yields (45 % over 2 steps) while **53** failed to cyclize using first and second generation Grubb's ruthenium catalyst with up to 30 mol % of catalyst loading in dry DCM and dry DME clearly

indicating the influence of chiral center during cyclization (*This result is in consonance with the RCM reactions of acyclic peptide* ***42*** *and* ***43***).

Scheme-**15**: *Preparation of cyclic peptides* **54** *and* **55**



Conformation of 54:

Inspection of the distribution of NH chemical shifts in 15-membered macrocycle in $CDCl_3$ solution (Page# 241) showed small variation in Leu NH (0.23 ppm) and 2Aba NH (amino butyric acid) (0.20 ppm) chemical shift values during solvent titration study. In addition, small magnitude of temperature coefficients of 3Aba

NH (-1.6 ppb/°K, Leu NH (–4.6 ppb/°K) confirmed their involvement in intramolecular H-bonds. Further, the cross correlations Leu NH↔3Aba NH, Leu NH↔β Phg CβH, Leu NH↔Pro Cδ(Pro-S) H, β Phg CβH↔Pro Cδ(Pro-S) H, 3Aba NH↔Pro CαH in $CDCl_3$ and DMSO-d_6 (Page# 242&243) indicated propensity of pseudo 3_{10} helical conformation in the molecule (Figure-**12**).

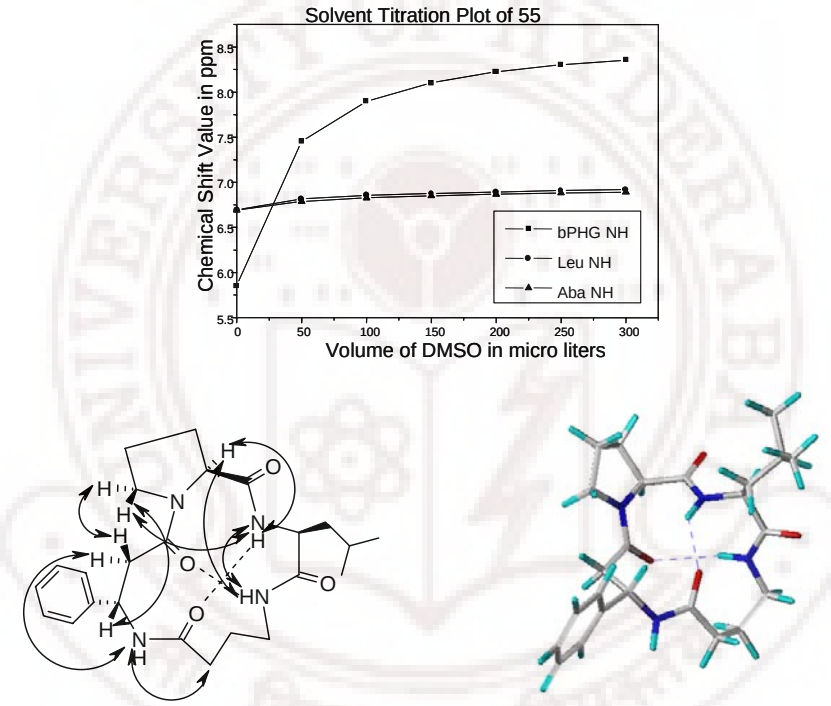
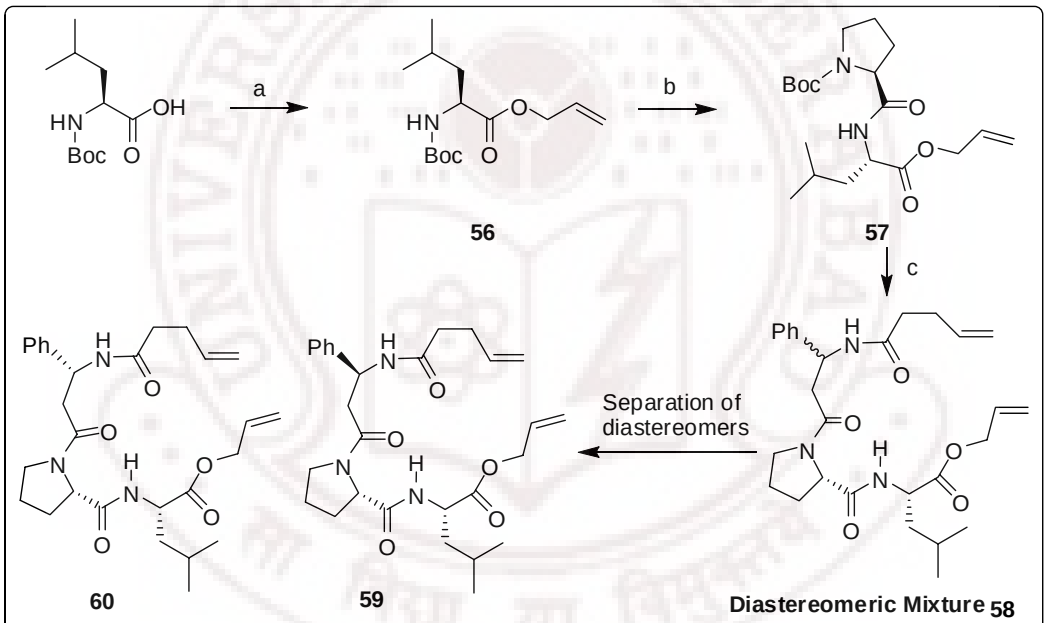


Figure-**12** *Solvent titration plot Diagnostic NOEs and one of the lowest energy conformation collected in the MD simulation study of* ***55***.

Encouraged with the results, we intended to substitute one of the donor partner (allylic NH) in acyclic peptides **42** and **43** with oxygen and expected it to result in peptides having an 11-membered pseudo β-turn. Accordingly, we synthesized two acyclic peptides by replacing the donor atom (NH) at *C*-terminal with

oxygen as shown in scheme-**17**. Unmasking the protecting group in dipeptide **57** with TFA yielded amine, which was coupled with **rac37** to afford a mixture of diastereomers which were separated using column chromatography to yield tripeptides **59** and **60** respectively. The absolute configuration of the β-amino acid in **59** was assigned as 'S' and '*R*' was assigned for **60** based on chemical correlation study.

Scheme-**16**: *Preparation of acyclic peptides* **59** *and* **60**



Reagents and conditions: (a) Allylbromide, K_2CO_3, Dry acetone, reflux, 12h, 61% (b) N-Boc Pro $ClCO_2{}^iBu$, NEt_3, Dry CH_2Cl_2, 0 ^oC-rt, 12h, 64 % (c) ***rac-37,*** *$ClCO_2{}^iBu$, NEt_3, Dry CH_2Cl_2, 0 ^oC-rt, 12h.*

Conformation of 59 and 60:

In both the peptides, the observation of NOE cross peaks involving both β Phg CαH as well as Pro CδH showed predominant population of a *trans* imide bond. The diagnostic NOEs Leu NH↔Pro CδH, Leu NH↔Pro CαH, Leu NH↔β Phg

CβH, Leu NH↔β Phg CαH coupled with involvement of Leu NH hydrogen bond suggests that the peptide has folded into a pseudo β-turn conformation (Figure-**12**) with a 11 membered H-bond between Leu NH-pentenoyl C=O.

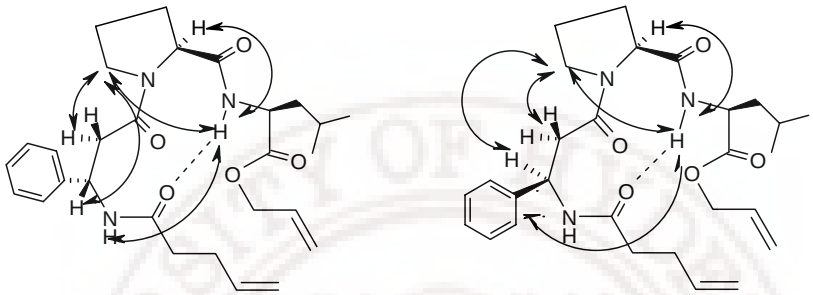
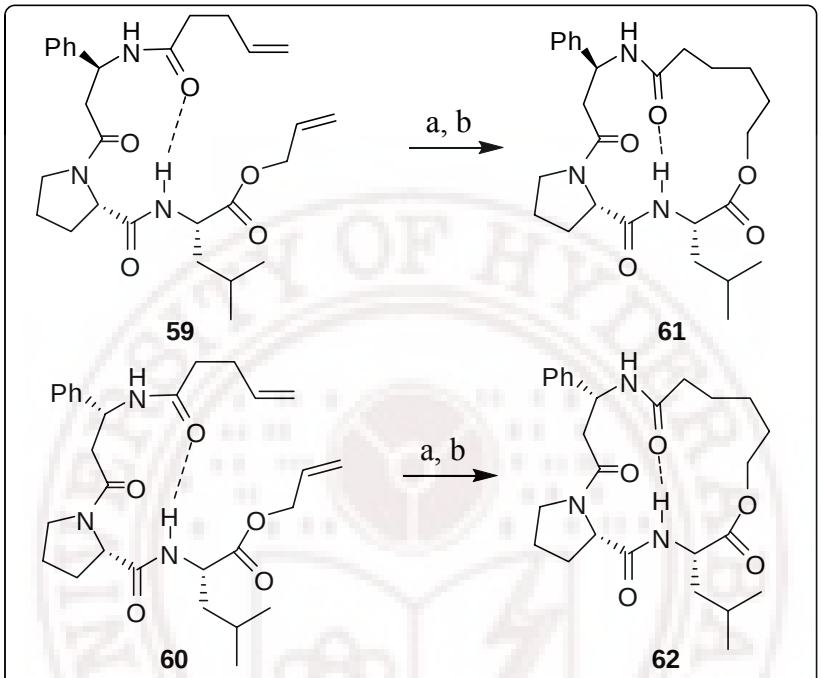


Figure-**13**: *Diagnostic NOEs observed in* ***59*** *and* ***60.***

The ring closing metathesis of **59** and **60** (0.01M solution in CH_2Cl_2) in the presence of Grubb's ruthenium catalyst (10 mol %) afforded unsaturated cyclic peptides as a mixture of *E*:Z isomers (70:30 by TLC), which was reacted with 10 % Pd/C in MeOH and transformed to cyclic peptides **61** (55 % over two steps) and **62** (38 % over two steps). The cyclization results in the formation of 17-membered macrocyclic peptides with spacer, 6-hydroxy hexanoic acid (*Hha*) (Scheme-**17**).

Scheme-**17:** *Preparation of cyclic peptides* **61** *and* **62** *from acyclic tripeptides* **59** *and* **60**



Reagents and conditions: (a) 10 mol% Grubbs catalyst, CH_2Cl_2, reflux, 12h (b) H_2/Pd/C, MeOH, 50.7 % for ***61*** *and 38 % for* ***62*** *over two steps.*

Conformation of 61 and 62:

In $CDCl_3$ solution, only one set of resonances were observed, implying presence of single isomer (spectrum **24**, Page# 245 and spectrum **26**, page#246). NOEs between β Phg CαHs↔Pro Cδs confirmed a *trans* imide bond preceding Pro. The cross peaks, Leu NH↔Aha NH, Leu NH↔β Phg CβH and Leu NH↔Pro CδH, in the NOESY spectrum, coupled with Leu NH H-bond indicated the retention of a pseudo β-turn conformation between Leu NH ← Hha C=O (Figure-**14**).

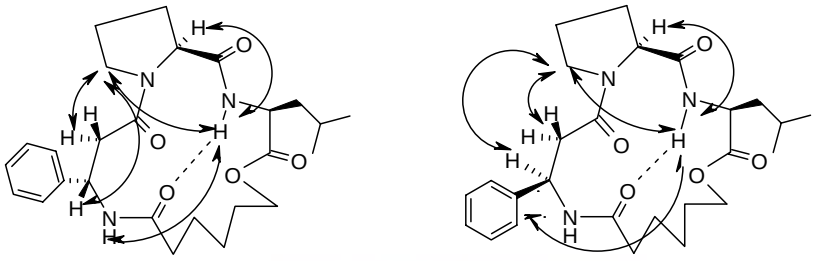


Figure-**13**: *Diagnostic NOEs observed for cyclic peptides* ***61*** *and* ***62***.

3.4 Conclusion

In conclusion, the acyclic peptides derived from L-proline and the β-amino acid show interesting conformational properties as they can be easily transformed into the corresponding cyclic pseudo 3_{10} helical and pseudo β-turn structures via ring closing metathesis reaction. The RCM cyclization leads to the concomitant synthesis of unnatural linker amino acids, *AHA* and *GABA* and the resulting 3_{10} helix contains a type-I about the Pro-Leu residues where the chirality of the homophenylglycine (β Phg) residue in the β-amino acid controls the formation of the pseudo 3_{10} helical structure in the cyclic peptide. These small cyclic peptides mimic the protein secondary structures and may act as useful pharmaceutical probes in understanding the protein-protein or protein-DNA interactions.

3.5 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 CH_2Cl_2 was added NEt_3 (2 equivalent) followed by the addition of $ClCO_2{}^iBu$ (1.5 equivalent). After 5 min, a solution of Allylamine (1.2 equivalent) in dry CH_2Cl_2 was added and stirred at room temperature for a period of 12 h. The reaction mixture was diluted with CH_2Cl_2 and washed with water, brine, dried over Na_2SO_4 and evaporated to afford crude product which was purified using MeOH-$CHCl_3$ as 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 (1equivalent) in dry CH_2Cl_2 was added TFA (10 equivalent) under argon atmosphere and stirred at 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-L-Proline (1equivalent) in dry CH_2Cl_2 at 0^oC was added NEt_3 (2 equivalents) followed by the addition of $ClCO_2{}^iBu$ (1.5 equivalent). After 5 min at 0^oC a solution of amine (obtained in part A) (1equivalent) in dry $ClCO_2{}^iBu$ was added and stirred at room temperature for a

period of 12 h. The reaction mixture was diluted with $ClCO_2{}^iBu$ and washed with water, brine, dried over Na_2SO_4 and evaporated to yield crude compound which was purified using 100-200 mesh silica and MeOH-$CHCl_3$ as eluent afford the required dipeptide.

Preparation of Pentenoyl-Xaa-Pro-Yaa allylamide (C)

A. To a stirred solution of *N*-Boc-Pro-Yaa allylamide (1equivalent) in dry CH_2Cl_2 was added TFA (10 equivalents) under argon atmosphere and stirred 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 rac 37 or rac 37a (1equivalent) in dry CH_2Cl_2 at 0^oC was added NEt_3 (2 equivalents) followed by the addition of $ClCO_2{}^iBu$ (1.5 equivalents). After 5 min a solution of amine (obtained in part A) (1.53 g, 3.7 mmol) in dry CH_2Cl_2 was added at 0^oC and then stirred at room temperature for a period of 12 h. The reaction mixture was diluted with CH_2Cl_2 and washed with water, brine, dried over Na_2SO_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 (D)

To a stirred solution of Grubb's ruthenium catalyst (10 mol%) in dry CH_2Cl_2 (300 ml) under nitrogen was added a solution of Pentenoyl-Xaa-Pro-Yaa allylamide (1equivalent) in dry CH_2Cl_2 slowly over a period of 15 min and the

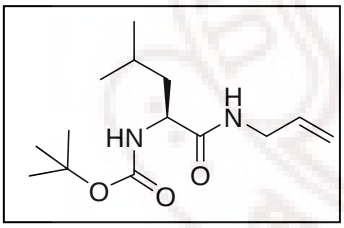
mixture was refluxed for 12 h. The solvent was evaporated and residue was purified to afford the cyclic compound as a mixture of *E* and *Z* isomers.

Reduction of the double bond in the cyclic peptide (E)

To a stirred solution of the unsaturated cyclic peptide (*E* and *Z mixture)* (100 mg) in MeOH (5 ml) was added 20 mg of 10 % Pd/C. The mixture was hydrogenated using a H_2 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 purified to afford the saturated analogue of the cyclic peptide.

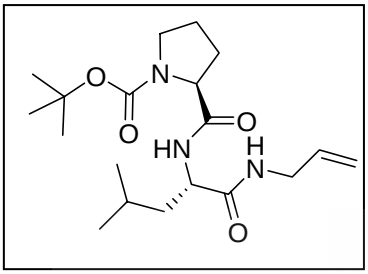
Preparation of N-Boc-L-Leu allylamide:

The title compound was obtained following the general procedure (A) using (6.1 g, 26.4 mmol) of N-Boc-L-Leucine, (2.20 ml, 29.40 mmol) of allylamine, (5.20 ml, 39.6 mmol) of $ClCO_2{}^iBu$ and (11ml, 79.22 mmol) of NEt_3 to afford (2g, 87.3 %) of product as a white solid.



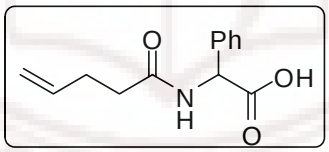
mp. 66-70^{o}C, [α] = -27 (c, 0.5, MeOH), IR (KBr): 3352, 2966, 1666, 1535, 1244 cm^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.27 (bs, 1H, NH), 5.87-5.77 (m, 1H), 5.21-5.10 (m, 2H), 4.88 (bs, 1H), 4.10-4.09 (m, 1H), 3.87 (t, *J* = 5.5 Hz, 2H), 1.70-1.67 (m, 2H), 1.50-1.45 (m, 1H), 1.44 (s, 9H), 0.94 (d, *J* = 4.0 Hz, 3H,), 0.92 (d, *J* = 3.7 Hz, 3H), Mass (CI method): 271 ($(M+H)^+$, 100).

Preparation of N-Boc-L-Pro-Leu allylamide:

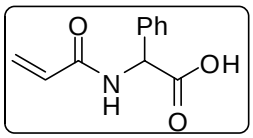


The title compound was obtained following the general procedure (B) using (4.93 g, 22.96 mmol) of N-Boc-L-Pro, (3.90 g, 22.96 mmol) of Leucine allylamide, (4.50 ml, 34.41 mmol) of $ClCO_2{}^iBu$ and (9.6 ml, 68.79 mmol) of NEt_3 to afford (8 g, 95 %) of title product as a white solid.

mp. 92-96°C, [α] = -89.00 (c, 0.1, MeOH), IR ($CHCl_3$): 3292, 2958, 1652, 1550 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): 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).



Compound 37: IR (Neat): 3298, 3069, 1718, 1641, 1545 cm^{-1}, ^1H NMR (200 MHz, $CDCl_3$) : δ 7.28 (m, 5H), 6.68 (bs, 1H), 5.85-5.63 (m, 1H), 5.44-5.40 (m, 1H), 5.03-4.97 (m, 2H), 2.9-2.86 (m, 2H), 2.30 (bs, 4H)., Mass (CI method)(m/z): 248 (M^+ +1, 100), 204, 164.

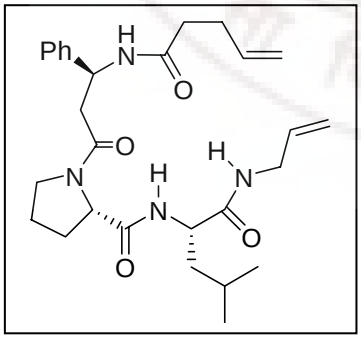


Compound 37a: IR (KBr): 3349, 2923, 2599, 2522, 1718 cm^{-1}, ^1H NMR (200 MHz, $CDCl_3$) : δ 7.32-7.26 (m, 5H), 6.57 (bs, 1H), 6.35-6.05 (m, 2H), 5.70-5.65 (m, 1H), 5.54-5.50 (m2.97-2.56 (m, 2H), Mass (CI method) (m/z): 220 ($(M+H)^+$, 100), 202, 164.

Preparation of Pentenoyl-(S)-βPhg-Pro-Leu allylamide (42) and Pentenoyl-(S)-βPhg-Pro-Leu allylamide (43):

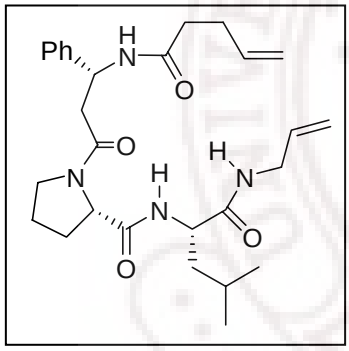
The title compound was obtained following the general procedure (C) using (515 mg, 1.92 mmol) of Pro-Leu allylamide, (0.48 g, 1.92 mmol) of **rac37**, (0.37 ml, 2.89 mmol) of $ClCO_2{}^iBu$ and (0.822 ml, 5.76 mmol) of NEt_3 to afford (475 mg, 49 %) of **42** and (267 mg, 27%) of **43** as a white solid.

[α]:-84.80 (c, 0.1, MeOH), IR (Neat): 3300, 2958, 1647, 1543 cm^{-1}, ^{1}HNMR(500



MHz, $CDCl_3$) δ 7.36-7.27 (m, 5H, Aromatic), 6.99 (d, *J* =8.6 Hz, 1H, Leu N*H*), 6.97 (d, *J* = 8.3 Hz, 1H, βPhg N*H*), 6.61 (t, *J* = 5.6 Hz, 1H, Allyl N*H*), 5.83-5.79 (m, 2H), 5.45 (ddd, *J* = 4.5, 7.0, 8.3 Hz, 1H, βPhg Cβ*H*), 5.19 (m, 1H, Allyl Cγ*H*(t)), 5.12 (dq, *J* = 10.4 and *J* = 1.9 Hz, 1H, Allyl Cγ′*H*(c)), 5.04 (dq, *J* = 1.8, 17.1 Hz, 1H, Pentenoyl Cε*H*), 4.99(dq, *J* = 1.8, 10.3 Hz, 1H, Pentenoyl Cε′*H*), 4.44 (ddd, *J* =

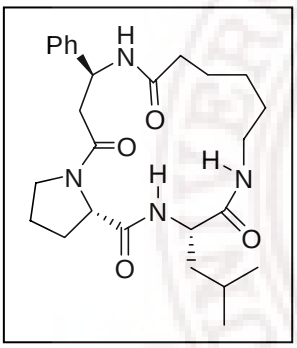
5.0, 8.6, 9.7 Hz, 1H, Leu Cα*H*), 4.40 (dd, *J* = 3.5, 7.8 Hz, 1H, Pro Cα*H*), 3.90 (ttd, *J* = 1.7, 5.6, 15.7 Hz, 1H, Allyl Cα*H*), 3.80 (m, 1H, Allyl Cα′*H*), 3.61 (m, 1H, Pro Cδ*H*), 3.13 (m, 1H, Pro Cδ′*H*), 2.90 (dd, *J* = 7.0, 14.3 Hz, 1H, βPhg Cα*H*), 2.83 (dd, *J* = 4.5, 14.3 Hz, 1H, βPhg Cα′*H*), 2.37(m, 1H, Pentenoyl Cα*H*), 2.28 (m, 1H, Pentenoyl Cα′*H*), 2.16-2.14 (m, 2H), 1.96 (m, 1H), 1.83 (m, 1H), 1.82 (m, 1H, Leu Cβ*H*), 1.63 (m, 1H, Pentenoyl Cβ*H*), 1.61 (m, 2H, Leu Cγ*H*, & Leu Cβ′*H*), 1.31 (m, 1H, Pentenoyl Cβ′*H*), 0.94 (d, *J* = 6.3 Hz, 3H, Leu Cδ*CH₃*), 0.90 (d, *J* = 6.3 Hz, 3H, Leu Cδ′*CH₃*), Mass (m/z): 497($(M+H)^+$, 100).



mp. 206-208°C, [α]: -61.00 (c, 0.5, MeOH), IR (Neat): 3299, 2957, 1643, 1548 cm^{-1}, ^{1}HNMR($CDCl_3$, 500 MHz) δ 7.38-7.28 (m, 5H, Aromatic), 7.28 (d, *J* =8.8 Hz, 1H, Leu N*H*), 7.04 (t, *J* = 5.6 Hz, 1H, Allyl N*H*), 6.53 (d, *J* = 5.6 Hz, 1H, βPhg N*H*), 5.88 (m, 1H, Allyl Cβ*H*), 5.80 (m, 1H, Pentenoyl Cγ*H*), 5.27 (ddd, *J* = 3.3, 5.6, 6.6 Hz, 1H, βPhg Cβ*H*), 5.21 (dq, *J* = 1.6, 17.1 Hz, 1H, Allyl Cγ*H*(t)), 5.11 (dq, *J* = 1.6, 10.4 Hz, 1H, Allyl Cγ'*H*(c), 5.06 (dq, *J* = 1.6, 17.1 Hz, 1H, Pentenoyl Cε*H*), 5.02(dq, *J* = 1.6, 10.1 Hz, 1H, Pentenoyl Cε'*H*), 4.48 (ddd, *J* = 4.6, 8.8, 10.7 Hz, 1H, Leu Cα*H*), 4.44(dd, *J* = 4.1, 8.5 Hz, 1H, Pro Cα*H*), 3.90 (m, 1H, Allyl Cα*H*), 3.85 (m, 1H), 3.51 (m, 2H, Pro Cδ*H* & Cδ′*H*), 3.09 (dd, *J* = 6.6, 15.3 Hz, 1H, βPhg Cα*H*), 2.70 (dd, *J* = 3.3, 15.3 Hz, 1H, βPhg Cα′*H*), 2.38(m, 2H, Pentenoyl Cα*H*

& Cα′*H*), 2.29(m, 2H, Pentenoyl Cβ*H* & Cβ′*H*), 2.16 (m, 1H, Pro Cβ*H*), 2.16(m, 1H, Pro Cγ*H*), 1.96 (m, 2H, Pro Cβ′*H* & Cγ′*H*), 1.85 (ddd, *J* = 4.6, 9.1, 13.7 Hz, 1H, Leu Cβ*H*), 1.60(m, 1H, Leu Cγ*H*), 1.52 (ddd, *J* = 5.2, 10.7, 13.7 Hz, 1H, Leu Cβ′*H*),0.93 (d, *J* = 6.6 Hz, 3H, Leu Cδ*CH₃*), 0.90 (d, *J* = 6.6 Hz, 3H, Leu Cδ′*CH₃*), Mass (CI method) (m/z): 497 ($(M+H)^+$, 100), 440 (32).

Preparation of c((S)-βPhg-Pro-Leu-aha) (49):

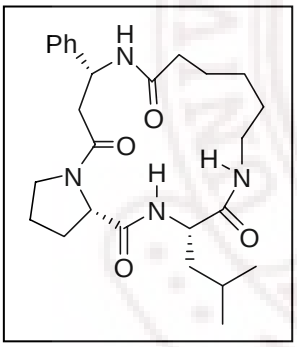


The title compound was obtained following the general procedure (D) and (E) using (300 mg, 0.60 mmol) of *N*-pentenoyl-(S)-βPhg-Pro-Leu allylamide, (50 mg, 10 mol%) of Grubbs catalyst (first generation) and 300 ml of dry CH_2Cl_2 to afford (313 mg, 55 %) of product as a white solid.

mp.110-114°C, [α] = -51.1 (c, 0.26, MeOH), IR (KBr): 3346, 2955, 1637.2 cm^{-1}, ^{1}HNMR($CDCl_3$, 500 MHz) δ 7.42-7.31(m, 5H, Aromatic), 7.01 (d, *J* = 9.2 Hz, 1H, Leu N*H*), 6.93 (dd, *J* =4.0, 7.5 Hz, 1H, Aha N*H*), 6.46 (d, *J* = 8.3 Hz, 1H, βPhg N*H*), 5.49 (ddd, *J* = 2.9, 8.3, 9.5 Hz, 1H, βPhg Cβ*H*), 4.58 (ddd, *J* = 3.8, 9.2, 11.5 Hz, 1H, Leu Cα*H*), 4.46 (dd, *J* = 5.0, 8.0 Hz, 1H, Pro Cα*H*), 3.80 (dt, *J* = 5.9, 9.8 Hz, 1H, Pro Cδ*H*), 3.69 (m, 1H, Aha Cε*H*), 3.45 (dt, *J* = 7.5, 9.8 Hz, 1H, Pro Cδ′*H*), 2.94 (dd, *J* = 2.9, 13.8 Hz, 1H, βPhg Cα*H*), 2.86 (m, 1H, Aha

Cε′*H*), 2.81 (dd, *J* = 9.5, 13.8 Hz, 1H, βPhg Cα′*H*), 2.32 (m, 1H, Aha Cα*H*), 2.18 (m, 1H, Pro Cβ*H*), 2.18 (m, 1H, Pro Cγ*H*), 2.15 (m, 1H, Aha Cα′*H*), 1.97 (m, 1H, Pro Cβ′*H*), 1.97 (m, 1H, Pro Cγ′*H*), 1.97 (ddd, *J* = 3.8, 9.8, 14.0 Hz, 1H, Leu Cβ*H*), 1.82 (ddd, *J* = 4.7, 11.5, 14.0 Hz, 1H, Leu Cβ′*H*), 1.66-1.41 (m, 6H, Aha Cβ*H* & Cβ′*H* & Cγ*H* & Cγ′*H* & Cδ*H* & Cδ′*H*), 1.62 (m, 1H, Leu Cγ*H*), 0.97 (d, *J* = 6.6 Hz, 3H, Leu Cδ*CH*$_3$), 0.92 (d, *J* = 6.6 Hz, 3H, Leu Cδ*CH*$_3$), Mass (EI method) (m/z): 470 ((M+H)$^+$, 41), 414 (100).

Preparation of c((*R*)-βPhg-Pro-Leu-aha) (50):

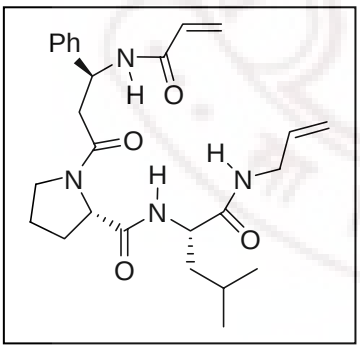


The title compound was obtained following the general procedures (D) and (E) using (250 mg, 0.50 mmol) of *N*-pentenoyl-(S)-βPhg-Pro-Leu allylamide, (42 mg, 10 mol%) of Grubbs catalyst (first generation) and 250 ml of dry CH_2Cl_2 to afford (64 mg, 27 %) of product as a white solid.

mp.122-126^{o}C, [α]: 4.23 (c, 0.26, MeOH), IR (KBr) : 3413, 2955, 1643 cm^{-1}, ^{1}HNMR($CDCl_3$, 500 MHz) δ 7.92 (d, *J* = 5.8 Hz, 1H, βPhg N*H*), 7.41-7.28 (m, 5H, Aromatic), 6.76 (dd, *J* =2.4, 7.6 Hz, 1H, Aha N*H*), 5.58 (d, *J* = 8.9 Hz, 1H, Leu N*H*), 5.32 (ddd, *J* = 4.4, 5.2, 5.8 Hz, 1H, βPhg Cβ*H*), 4.49 (ddd, *J* = 4.0, 8.9, 11.6 Hz, 1H, Leu Cα*H*), 4.38 (dd, *J* = 3.9, 9.0 Hz, 1H, Pro Cα*H*), 3.69 (m, 1H,

Aha Cε*H*), 3.41 (dt, *J* = 6.7, 9.6 Hz, 1H, Pro Cδ*H*), 3.21 (dd, *J* = 5.2, 14.6 Hz, 1H, βPhg Cα*H*), 3.04 (m, 1H, Aha Cε′*H*), 3.03 (m, 1H, Pro Cδ′*H*), 2.73 (dd, *J* = 4.4, 14.6 Hz, 1H, βPhg Cα′*H*), 2.46 (m, 1H, Aha Cα*H*), 2.31 (m, 1H, Aha Cα′*H*), 2.16 (m, 1H, Pro Cβ*H*), 2.04 (m, 1H, Pro Cβ′*H*), 1.98 (ddd, *J* = 4.0, 9.8, 14.4 Hz, 1H, Leu Cβ*H*), 1.89 (m, 1H, Pro Cγ*H*), 1.77-1.47 (m, 6H, Aha Cβ*H* & Cβ′*H* & Cγ*H* & Cγ′*H* & Cδ*H* & Cδ′*H*), 1.62 (m, 1H, Pro Cγ′*H*), 1.46 (ddd, *J* = 5.2, 11.6, 14.4 Hz, 1H, Leu Cβ′*H*), 1.35 (m, 1H, Leu Cγ*H*), 0.91 (d, *J* = 6.6 Hz, 3H, Leu Cδ*CH₃*), 0.84 (d, *J* = 6.6 Hz, 3H, Leu Cδ*CH₃*), Mass (m/z): 471 ($(M+H)^+$, 100)

Preparation of *N*-acrolyl-(S)-βPhg-Pro-Leu allylamide (52) and *N*-acrolyl-(*R*)-βPhg-Pro-Leu allylamide (53):

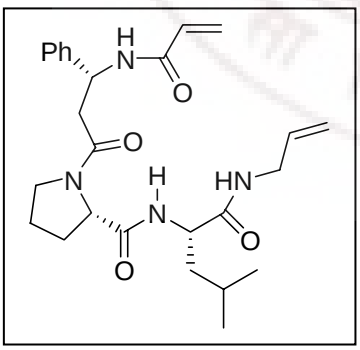


The title compound was obtained following the general procedure (D) using (1.05 g, 3.93 mmol) of Pro-Leu allylamide, (0.87 g, 3.93 mmol) of **rac37**, (0.77 ml, 5.89 mmol) of $ClCO_2{}^iBu$ and (1.65 ml, 11.79 mmol) of NEt_3 to afford (865 mg, 47 %) of **52** and (736 mg, 40%) of **53** as a white solid.

mp.70-72°C**,** [α]: -114° (c, 0.1, MeOH), IR (Neat): 3296, 2957, 1655 cm^{-1}, ^{1}HNMR($CDCl_3$, 500 MHz) δ 7.37-7.28 (m, 5H, Aromatic), 7.12 (d, *J* = 8.2 Hz, 1H, βPhg N*H*), 6.97 (d, *J* = 8.6 Hz, 1H, Leu N*H*), 6.60 (t, *J* = 5.9 Hz, 1H, Allyl

NH), 6.23 (dd, *J* = 1.5, 17.1 Hz, 1H, crotonyl Cβ*H*), 6.12 (dd, *J* = 10.3, 17.1 Hz, 1H, crotonyl Cα*H*), 5.82 (m, 1H, Allyl Cβ*H*), 5.67 (dd, *J* = 1.5, 10.1 Hz, 1H, crotonyl Cβ'*H*), 5.52 (ddd, *J* = 4.5, 7.1, 8.2 Hz, 1H, β Phg Cβ*H*), 5.18 (dq, *J* = 1.8, 17.3 Hz, 1H, Allyl Cβ*H*), 5.11 (dq, *J* = 1.8, 10.3 Hz, 1H, Allyl Cβ′*H*), 4.44 (ddd, *J* = 4.8, 8.6, 9.6 Hz, 1H, Leu Cα*H*), 4.41 (dd, *J* = 3.6, 7.8 Hz, 1H, Pro Cα*H*), 3.88 (m, 1H, Allyl Cα*H*), 3.71 (m,1H, Allyl Cα′*H*), 3.63 (m, 1H, Pro Cδ*H*), 3.17 (m, 1H, Pro Cδ′*H*), 2.93 (dd, *J* = 7.1, 14.4 Hz, 1H, βPhg Cα*H*), 2.89 (dd, *J* = 4.5, 14.4 Hz, 1H, βPhg Cα′*H*), 2.15 (m, 1H, Pro Cβ*H*), 1.96 (m, 1H, Pro Cβ′*H*), 1.96 (m, 1H, Pro Cγ*H*), 1.83 (m, 1H, Pro Cγ′*H*), 1.83 (m, 1H, Leu Cβ*H*), 1.63 (m, 1H, Leu Cβ′*H*), 1.63 (m, 1H, Leu Cγ*H*), 0.94 (d, *J* = 6.4 Hz, 3H, Leu Cδ*CH₃*), 0.90 (d, *J* = 6.5 Hz, 3H, Leu Cδ′*CH₃*), Mass (CI method): 469 ($(M+H)^+$, 100).

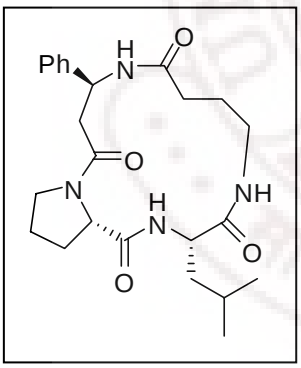
Compound **53**:



[α]: -57.0 (c, 0.1, MeOH), IR (KBr): 3296, 2954, 1647, 1534cm^{-1}, ^{1}HNMR($CDCl_3$, 500 MHz) δ 7-39-7.29 (m, 5H, Aromatic), 7.21 (d, *J* =8.7 Hz, 1H, Leu N*H*), 7.01 (t, *J* = 5.7 Hz, 1H, Allyl N*H*), 6.72 (d, *J* = 6.0 Hz, 1H, βPhg N*H*), 6.27 (dd, *J* = 1.5, 17.0 Hz, 1H, crotonyl Cβ*H*), 6.12 (dd, *J* = 10.2, 17.0 Hz, 1H, crotonyl Cα*H*), 5.87 (tdd, *J* = 5.6, 10.4, 17.1 Hz, 1H, Allyl Cβ*H*), 5.69 (dd, *J* = 1.5, 10.2 Hz, 1H, crotonyl

Cβ′*H*), 5.36 (ddd, *J* = 3.4, 6.0, 6.7 Hz, 1H, βPhg Cβ*H*), 5.21 (dq, *J* = 1.8, 17.1 Hz, 1H, Allyl Cγ*H*(t)), 5.10 (dq, *J* = 1.8, 10.4 Hz, 1H, Allyl Cγ′*H*(c)), 4.48 (ddd, *J* = 4.7, 8.7, 10.6 Hz, 1H, Leu Cα*H*), 4.45 (dd, *J* = 4.4, 8.2 Hz, 1H, Pro Cα*H*), 3.90-3.84 (m, 2H), 3.51 (m, 2H, Pro Cδ*H* & Cδ′*H*), 3.17 (dd, *J* = 6.7, 15.4 Hz, 1H, βPhg Cα*H*), 2.75 (dd, *J* = 3.4, 15.4 Hz, 1H, βPhg Cα′*H*), 2.15 (m, 1H, Pro Cβ*H*), 2.15(m, 1H, Pro Cγ*H*), 1.97 (m, 2H, Pro Cβ′*H* & Cγ′*H*), 1.85 (ddd, *J* = 4.7, 9.1, 13.8 Hz, 1H, Leu Cβ*H*), 1.61(m, 1H, Leu Cγ*H*), 1.51 (ddd, *J* = 5.3, 10.6, 13.8 Hz, 1H, Leu Cβ′*H*), 0.93 (d, *J* = 6.6 Hz, 3H, Leu Cδ*CH*$_3$), 0.90(d, *J* = 6.6 Hz, 3H, Leu Cδ′*CH*$_3$), Mass (m/z): 469 ((M +H)$^+$, 100), 398 (62).

Preparation of c((S)-βPhg-Pro-Leu-Aha) (54):

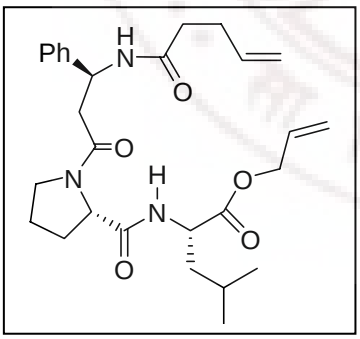


The title compound was obtained following the general procedures (D) and (E) using (250 mg, 0.53 mmol) of *N*-acrolyl-(S)-βPhg-Pro-Leu allylamide, (45 mg, 10 mol%) of Grubbs catalyst (first generation) and 250 ml of dry CH_2Cl_2 to afford (110 mg, 47 %) of product as a white solid.

mp. >200 ^oC, [α]: -55.20 (c, 0.25, MeOH), IR (KBr): 3312, 2928, 1642, 1388 cm^{-1}, ^{1}HNMR($CDCl_3$, 500 MHz) δ 7.42-7.31 (m, 5H, Aromatic), 6.70 (d, *J* = 9.7 Hz, 1H, Leu NH), 6.69(m, 1H, Gaba N*H*), 5.86 (d, *J* = 9.3 Hz, 1H, βPhg N*H*), 5.56 (ddd, *J* = 2.4, 9.3, 12.6 Hz, 1H, βPhg Cβ*H*), 4.73 (ddd, *J* = 3.4, 9.7, 11.4 Hz, 1H,

Leu Cα*H*), 4.48 (dd, *J* = 3.9, 8.8 Hz, 1H, Pro Cα*H*), 4.01 (m, 1H, Gaba Cγ*H*), 3.89 (ddd, *J* = 3.3, 7.2, 9.8 Hz, 1H, Pro Cδ*H*), 3.59 (ddd, *J* = 6.6, 9.4, 9.8 Hz, 1H, Pro Cδ′*H*), 2.99 (dd, *J* = 2.4, 12.6 Hz, 1H, βPhg Cα*H*), 2.88 (m, 1H, Gaba Cγ′*H*), 2.66 (t, *J* = 12.6 Hz, 1H, βPhg Cα′*H*), 2.29 (m, 1H, Gaba Cα*H*), 2.24 (m, 1H, Pro Cβ*H*), 2.24 (m, 1H, Pro Cβ′*H*), 2.17 (m, 1H, Gaba Cα′*H*), 2.11 (ddd, *J* = 3.4, 10.4, 14.3 Hz, 1H, Leu Cβ*H*), 2.04 (m, 1H, Pro Cγ*H*), 2.04 (m, 1H, Pro Cγ′*H*), 2.02 (m, 1H, Gaba Cβ*H*), 1.85 (m, 1H, Gaba Cβ′*H*), 1.73 (ddd, *J* = 4.4, 11.4, 14.3 Hz, 1H, Leu Cβ′*H*), 1.60 (m, 1H, Leu Cγ*H*), 0.99 (d, *J* = 6.7 Hz, 3H, Leu Cδ*CH₃*), 0.96 (d, *J* = 6.6 Hz, 3H, Leu Cδ′*CH₃*), Mass (EI method) (m/z): 442 ($(M+H)^+$24), 386 (100).

Preparation of *N*-Pentenoyl-(S)-βPhg-Pro-Leu allylester (59) and *N*-Pentenoyl-(*R*)-βPhg-Pro-Leu allylester (60):



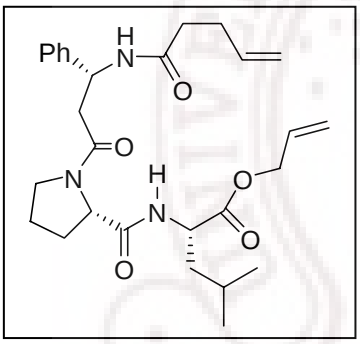
The title compound was obtained following the general procedure (D) using (780 mg, 2.91 mmol) of Pro-Leu allylamide, (0.72 g, 2.91 mmol) of **rac37**, (0.57 ml, 4.38 mmol) of $ClCO_2{}^iBu$ and (1.22 ml, 8.76 mmol) of NEt_3 to afford (447 mg, 31 %) of **59** and (390 mg, 27 %) of **60** as a colorless gum.

IR (Neat): 3288, 2958, 1743, 1651 1543 cm-^{1}, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59 (d, *J* = 7.8 Hz, 1H), 7.32-7.21 (m, 5H), 6.99 (d, *J* = 7.8 Hz, 1H), 5.95-5.78

(m, 2H), 5.44-5.39 (m, 1H), 5.37-5.30 (m, 2H), 5.27-4.93 (m, 2H), 4.66-4.60 (m, 3H), 4.54-4.9 (m, 1H), 4.45-4.43 (m, 1H), 3.43-3.38 (m, 1H), 2.97-2.87 (m, 2H), 2.79-2.74 (m, 1H), 2.43-2.34 (m, 4H), 2.31-2.21(m, 2H), 2.04-1.95(m, 1H), 1.77-1.54 (m, 3H), 0.98-0.96 (m, 6H), Mass (CI method): 498 ($(M+H)^+$, 100).

Compound **60**:

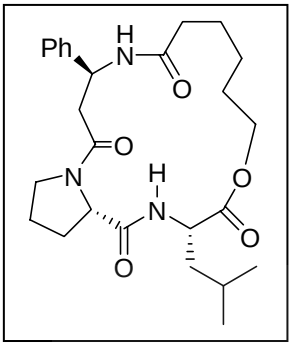
IR (Neat): 3272, 3082, 2957, 1743, 1688, 1640, 1558 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): δ 7.32-7.22 (m, 6H), 7.05 (d, *J* = 7.5 Hz, 1H), 5.91-5.80 (m, 2H), 5.43-5.41 (m, 1H), 5.35-5.31 (m, 2H), 5.26-5.23 (m, 2H), 5.08-4.97 (m, 2H), 4.63-4.60



(m, 2H), 4.52-4.50 (m, 1H), 4.49-4.50 (m, 1H), 4.49-4.45 (m, 1H), 3.38-3.19 (m, 1H), 2.95-2.83 (m, 1H), 2.81-72 (m, 1H), 2.42-2.32 (m, 4H), 2.16-1.85 (m, 2H), 1.83-1.72 (m, 1H), 1.59-1.55 (m, 1H), 1.42-1.39 (m, 1H), 0.95-0.89 (m, 6H), Mass (CI method): 498 ($(M+H)^+$, 100).

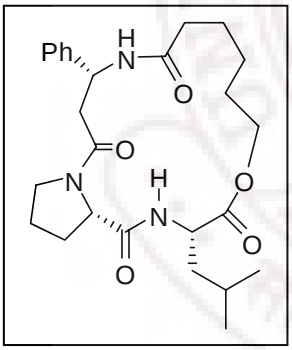
Preparation of c((*S*)-βPhg-Pro-Leu-Hha) (61):

Following the general procedure (D) (200 mg, 0.40 mmol) of *N*-pentenoyl-(S)-βPhg-Pro-Leu allylester, (10 mol%) of Grubbs catalyst (first generation) in 250 ml of dry CH_2Cl_2 afforded pale pink solid which was transformed to the title compound (96 mg, 50.7 %) using general procedure (E) (30 mg of 10 % Pd/C) in MeOH as a white solid.



IR (Neat): 3321, 2958, 1744, 1686, 1623 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): δ 7.38-7.27 (m, 7H), 5.52-5.50 (m, 1H), 4.68-4.61 (m, 2H), 4.39-4.34 (m, 1H), 4.06-4.01 (m, 1H), 3.63-3.58 (m, 1H), 3.42-3.35 (m, 1H), 2.93 (dd, *J* = 15.3 Hz and *J* = 4.3 Hz, 1H), 2.83 (dd, *J* = 15.1 Hz and *J* = 6.5 Hz, 1H), 2.43-2.39 (m, 2H), 2.38-2.33 (m, 1H), 1.99-1.91 (m, 2H), 1.89-1.77 (m, 4H), 1.70 (t, *J* = 7.0 Hz, 2H), 1.64-1.60 (m, 4H), 0.96-0.91 (m, 6H). Mass (CI method): 472 ($(M+H)^+$, 100).

Preparation of c((*R*)-βPhg-Pro-Leu-Hha) (62):



Following the general procedure (D) (150 mg, 0.30 mmol) of *N*-pentenoyl-(*R*)-βPhg-Pro-Leu allylester, (10 mol%) of Grubbs catalyst (first generation) in 250 ml of dry CH_2Cl_2 afforded pale pink solid which was transformed to the title compound (80 mg, 56.3 %) using general procedure (E) (30 mg of 10 % Pd/C) in MeOH as a white solid.

IR (Neat): 3322, 2959, 1743, 1670, 1658 cm-^{1}, ^1H NMR (400 MHz, $CDCl_3$): δ 8.24 (d, *J* = 5.3 Hz, 1H), 7.51-7.47 (m, 1H), 7.31-7.28 (m, 5H), 5.27-5.26 (m, 1H), 4.61-4.55 (m, 2H), 4.30-4.29 (m, 1H), 4.24-4.22 (m, 2H), 3.63-3.57 (m, 1H), 3.29-3.22 (m, 1H), 3.04-3.00 (m 1H), 2.78-2.73 (m, 1H), 2.71-2.63 (m, 1H), 2.50-2.40 (m, 1H), 2.34-2.27 (m, 2H), 2.16-2.11 (m, 2H), 1.92-1.81 (m, 2H),

1.79-1.60 (m, 4H), 1.58-1.56 (m, 2H), 0.98-.93 (m, 6H). Mass (CI method): 472 ($(M+H)^+$, 100).

3.6 References

1. (a) Barresinoussi, F. *Science* **1983,** *220*, 868. (b) Gallo, R.C. *Science* **1983**, *220*, 865
2. Garg, R; Gupta, S.P.; Gao, H.; Tessmer, U.; Hohenberg, H.; Krausslich, H. G. *J. Virol.,* **1998**, *72*, 2846.
3. Fischl, M.A.; Richman D.D.; Grieco M. H.; Gottlieb M. S.; Volberding, P. A.; Laskin, O. L.; Leedom, J. M.; Groopman, J. E.; Mildvan, D.; Schooley, R.T.; *N Engl J Med* **1987**, *317,* 185.
4. Yarchoan, R.; Mitsuya, H.; Thomas, R.V.; Pluda, J. M.; Hartman, N. R.; Perno, C. F.; Marczyk, K. S.; Allian, J.P.; Johns, D. G.; Broader, S. Science, **1989**, *245*, 412.
5. Merigan, T. C.; Skoworn, G.; Bozzette, S. A.; Richman, D.; Uttamchandani, R.; Fischl, M.; Schooley, R.; Hirsch, M.; Soo, W.; Pettinelli, C. *Ann. Intern. Med.* **1989**, *245*, 412.
6. Riddler, S. A.; Anderson, R. E.; Mellors, J.W. *Antiviral Res.,* **1995**, *27*, 189.
7. Kohlstaedt, L. A.; Wang, J.; Friedman, J. M.; Rice, P. A.; Steitz, T. A. *Science* **1992**, *256*, 1783.
8. Ren, J.; Milton, J.; Weaver, K. L.; Short, S. A.; Stuart, D. I.; Stammers, D. K. *Struct. Fold. Des.* **2000**, *8*, 1089.

9. Esnouf, R. M.; Ren, J.; Hopkins, A. L.; Ross, C. K.; Jones, E. Y.; Stammers, D. K.; Stuart, D. I. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 3984.

10. Lindberg, J.; Sigurdsson, S.; Lowgren, S.; Andersson, H. O.; Sahlberg, C.; Nore´en, R.; Fridborg, K.; Zhang, H.; Unge, T. *Eur. J. Biochem.* **2002**, *269*, 1670.

11. Ren, J.; Diprose, J.; Warren, J.; Esnouf, R. M.; Bird, L. E.; Ikemizu, S.; Slater, M.; Milton, J.; Balzarini, J.; Stuart, D. I.; Stammers, D. K. *J. Biol. Chem*. **2000**, *275*, 5633.

12. Cremer, N.; Hamamouch, N.; Félix, A. S.; Velazquez, S.; Jan Balzarini, J.; and María-José Camarasa, M. J.; *J. Med. Chem.,* **2006**, *49*, 4834 and references cited therein.

13. (a) De Clercq, E. *J. Med. Chem*., 19995, 38, 2491. (b) Huff, J. R.; *J. Med. Chem*., **1991**, *34*, 2305.

14. Kohl, N.E.; *Proc. Natl. Acad. Sci. U. S. A*., **1988**, *85*, 4686.

15. Seelmeier, S.; Proc. Natl. Acad. Sci. U. S. A., **1988**, *85*, 6612.

16. Mcquade, T.J. *Science*., **1990**, *247*, 454.

17. Vondrasek, J.; Wlodawer, A. *Proteins Struct. Funct. Genet.*, **2002**, *49*, 429.

18. De Clercq, E. *Expert Opin. Emerging Drugs* **2005**, *10*, 1 and references cited therein.

19. De Meyer, S.; Azijn, H.; Surleraux, D.; Jochmans, D.; Tahri, A.; Pauwels, R.; Wigerinck, P.; de Bethune, M. P. *Antimicrob. Agents Chemother.* **2005**, *49*, 2314 and references cited therein.

20. Arun K. Ghosh, A. K.; Schiltz, G.; Perali, R. S.; Leshchenko, S.; Kay, S.; Walters, D. E.; Koh, Y.; Maedad, K.; Mitsuyad, H. *Bioorg. Med. Chem. Lett.* **2006***, 16,* 1869.

21. Ghosh, A. K.; Perali, R. S.; Leshchenko, S.; Hussain, H. K.; Li, J.; Kovalevsky, A. Y.; Walters, D. E.; Wedekind, J. E.; Tokars, V. G.; Das, D.; Koh, Y.; Maeda, K.; Gatanaga, H.; Weber, I. T.; Mitsuya. H. *J. Med. Chem*., **2006**, *49*, 5252.

22. Miller, J. F.; Andrews, C. W.; Brieger, M.; Furfine, E. S.; Hale, M. R.; Hanlon, M. H.; Hazen, R. J.; Kaldor, I.; McLean, E. W.; Reynolds, D. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1788 and references cited therein.

23. Ali, A.; Reddy, G. S. K. K; Cao, H.; Anjum, S. G.; Nalam, M. N. L.; Schiffer, C. A.; Rana, T. M. *J. Med. Chem.*, **2006**, *49*, 7342.

24. Ekegren, J. K.; Ginman, N.; Johansson, A.; Wallberg, H.; Larhed, M.; Samuelsson, B.; Unge, T.; Hallberg, A. *J. Med. Chem.* **2006,** *49,* 1828.

25. Smith, A. B.; Charnley, A. K.; Harada, H.; Beiger, J. J.; Cantin, L, D.; Kenesky, C. S.; Hirschmann, R.; Munshi, S.; Olsen, D. B.; Stahlhut, M. W.; Schleif W. A.; Lawrence C. Kuo, L. C. *Bioorg. Med. Chem. Lett*., **2006,** *16*, 859.

26. (a) Tyndall, J. D. A.; Reid, R. C.; Tyssen, D. P.; Jardine, D. K.; Todd, B.; Passmore, M.; March, D. R.; Pattenden, L. K.; Bergman, D. A.; Alewood, D.; Hu, S.-H.; Alewood, P. F.; Birch, C. J.; Martin, J. L.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 3495. (b) Tyndall, J. D. A.; Fairlie, D. P. *Curr. Med. Chem.* **2001**, *8*, 893. (c) Reid, R. C.; March, D. R.; Dooley, M. J.; Bergman, D. A.; Abbenante, G.; Fairlie, D. P. *J. Am. Chem. Soc.* **1996**, *118*, 8511.

27. Podoglar, B.L.; Farr, R. A.; Friedrich, D.; Tarnus, C.; Huber, E. W.; Cregge, R. J.; Schrilin, D. *J. Med. Chem*., **1994**, *37*, 3684.

28. Prabhakaran, E. N.; Nageswararao, I.; Boruah, A.; Iqbal, J. *J. Org. Chem,* **2002**, *67*, 8247.

29. (a) Graham, D. J.; Quintero, J. C.; Rhodes, A.; Robbins, H. L.; Roth, E.; Shivaprakash, M. *Nature.,* **1995**, *374*, 569. (b) Clavel, F.; Hance, A. J. *N. Engl. J. Med.* **2004**, *350*, 1023.

30. (a) Abdel-Magid, A. F., Cohen, J. H., Maryanoff, C. A. *Curr. Med. Chem.* **1999**, *6*, 955. (b) Juaristi, E., Lopez-Ruiz, H. *Curr. Med. Chem*. **1999**, *6*, 955 (c) Sewald, N. *Amino Acids* **1996**, *11*, 397. (d) Cole, D. C. *Tetrahedron* **1994**, *50*, 9517 (e) Abele, S., Seebech, D. *Eur. J. Org. Chem.* 2000, 1.

31. Werder, M., Hauser, H., Abele, S., Seebach, D. *Helv. Chim. Acta* **1999**, *82*, 1774.

32. Porter, E. A., Weisblum, B., Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 11516.

33. Kritzer, J. A., Lear, J. D., Hodsdon, M. E., Schepartz, A. *J. Am. Chem. Soc.* **2004**, *126*, 9468.

34. Potocky, T. B., Menon, A. K., Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 3686.

35. Sadowsky, J. D., Schmitt, M. A., Lee, H. S., Umezawa, N., Wang, S., Tomita, Y., Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 11966.

36. Cardillo, G., Tomasini, C. *Chem. Soc. Rev.* **1996**, *25*, 117.

37. Iizuka, K., Kamijo, T., Harada, H., Akahane, K., Kubota, T., Umeyama, H., Kiso, Y *J. Chem. Soc. Chem. Commun.* **1989**, 1678.

38. Bateson, J. H., Gassson, B. C., Khushi, T., Neale, J. E., Payne, D. J., Tolson, D. A., Walker, G. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1667

39. Steer, D. L.; Lew, R. A., Permutter, P., Smith, A. I., Aguilar, M. I. *J. Pept. Sci.* **2000**, *6*, 470.

40. Lew, R. A.; Boulos, E.; Stewart, K. M.; Permutter, P.; Harte, M. F.; Bond, S.; Aguilar, M. I.; Smith, A. I; *J. Pept. Sci.* **2000**, *6*, 440.

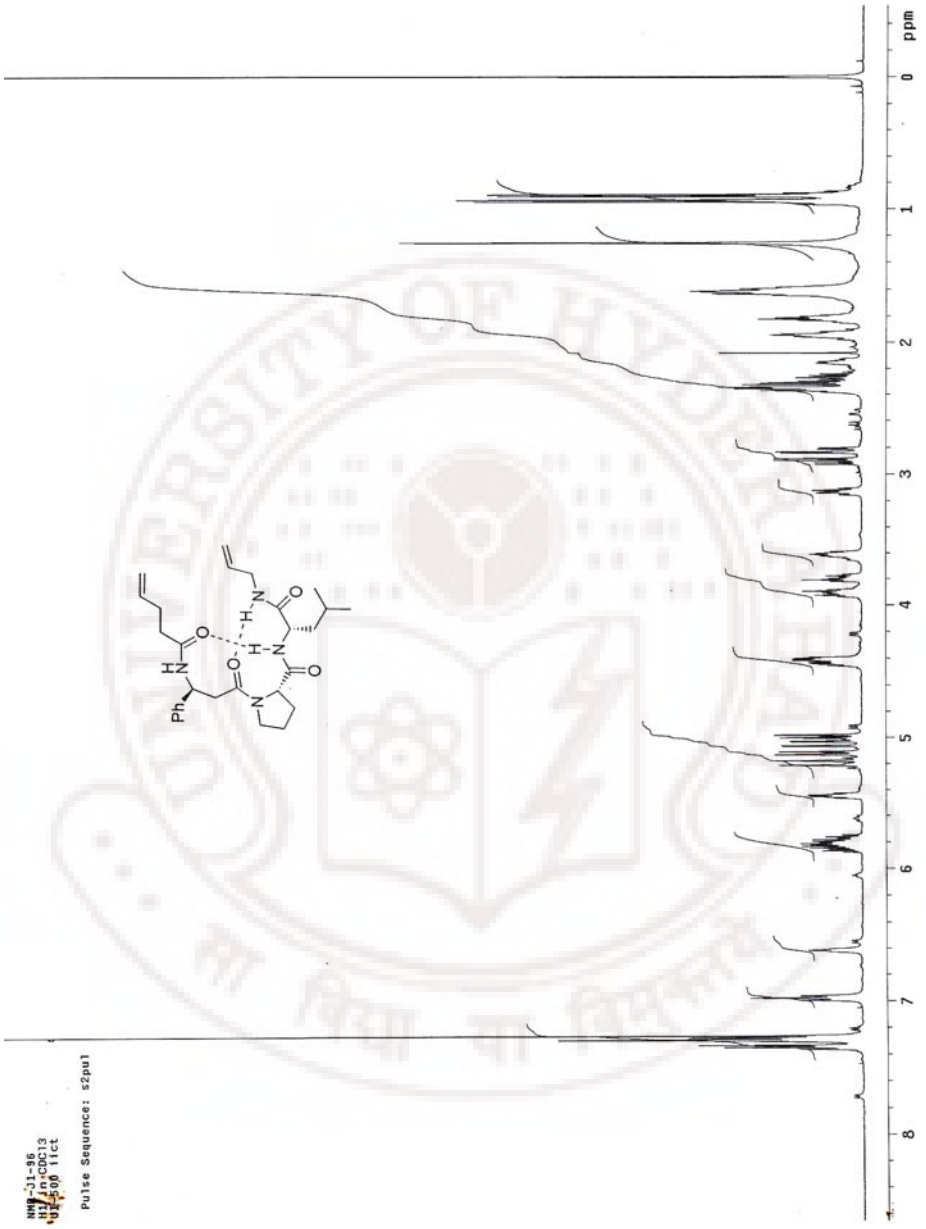
41. (a) Ref. 26(a) (b) Smith, A. B.; Keenan, T. P.; Holcomb, R. C.; Sprengeler, P. A.; Guzman, M. C.; Wood, J. L.; Carroll, P. J.; Hirschmann, R. *J. Am. Chem. Soc.* **1992**, *114*, 10672. (c) Smith, A. B.; Hirschmann, R.; Pasternak, A.; Cuzman, M. C.; Yokoyama, A.;

Sprengeler, R. A.; Darke, P. L.; Emini, E. A.; Schleif, W. A. *J. Am. Chem. Soc.* **1995**, *117*, 11113.

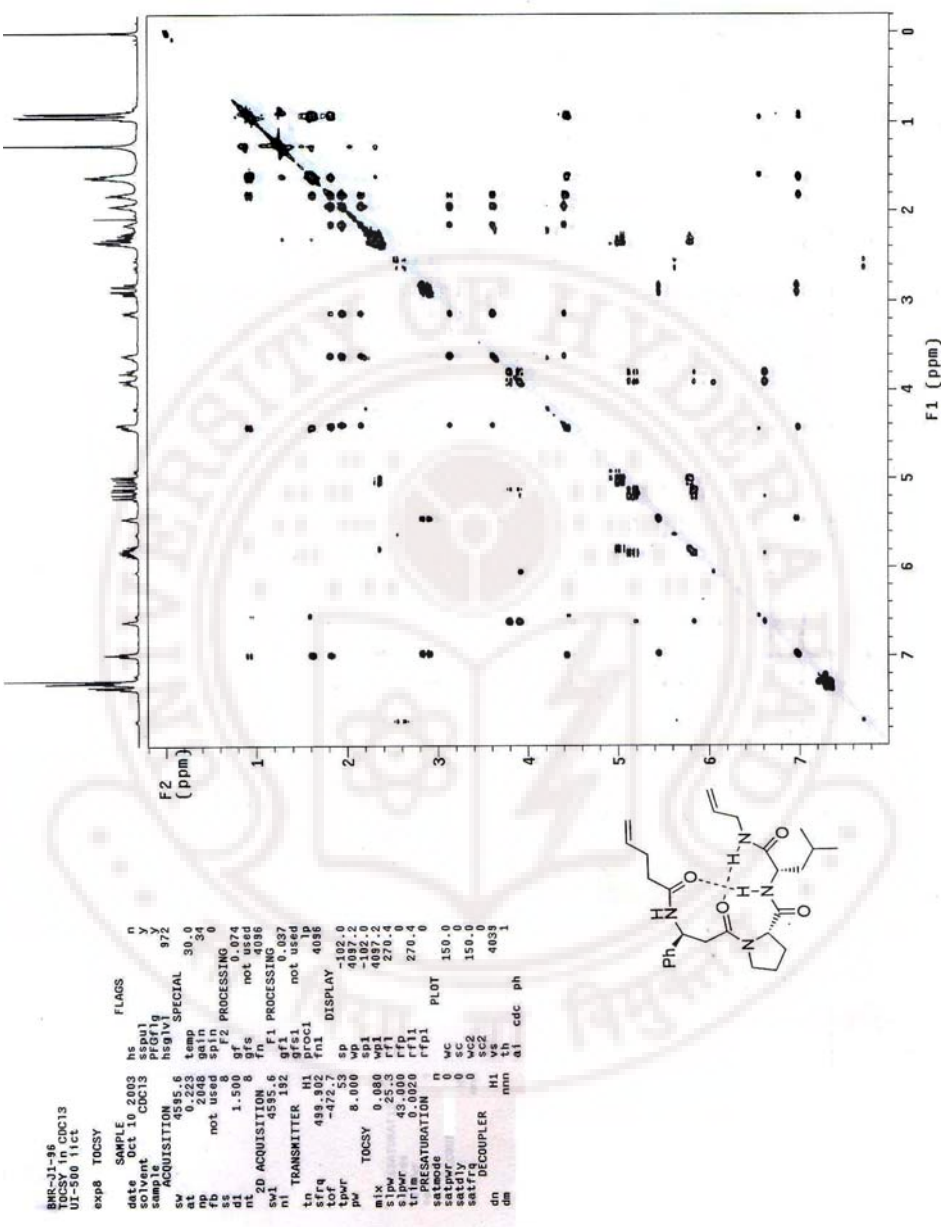
42. Bursavich, M. G., Rich, D. H. *J. Med. Chem.* **2002**, *457*, 541.
43. (a) Smith, A. B.; Hirschmann, R.; Pasternak, A.; Guzman, M. C.; Yokohama, A.; Sprengeler, P. A.; Darke, P. L.; Emini, E. A.; Schleif, W. A. *J. Am. Chem. Soc*. **1995***, 117*, 11113. (b) Martin, S. F.; Austin, R. E.; Oalmann, C. J.; Baker, W. R.; Condon, S. L.; DeLara, E.; Rosenberg, S. N.; Spina, K. P.; Stein, H. H.; Cohen, J.; Kleinert, H. D. *J. Med. Chem*. **1992**, *35*, 1710. (c) Kim, E. E.; Baker, C. T.; Dwyer, M. D.; Murcko, M. A.; Rao, B. G.; Tung, R. D.; Navia, M. A. *J. Am. Chem. Soc*. **1995**, *117*, 1181. (d) Martin, S. F.; Dorsey, D. O.; Gane, T.; Hillier, M.; Kessler, H.; Baur, M.; Matha, B.; Erickson, J.; Bhat, T. N.; Munshi, S.; Gulnik, S. V.; Topol, I. A. *J. Med. Chem*. **1998**, *41*, 1581. (e) Emmons, K. T.; Murali, R.; Greene, M. I. *Curr. Opin. Biotechnol*. **1997**, *8*, 433. (f) Reineke, U.; Mergener, J. S.; *Angew. Chem. Int. Ed*. **1998**, *37*, 769.
44. Furnster, A. *Angew. Chem. Int. Ed*, **2000**, *39*, 3012 and references cited therein.
45. Prabhakaran, E. N., Iqbal, J. *J. Org. Chem.* **1999**, *64*, 3339

3.7 Spectral data

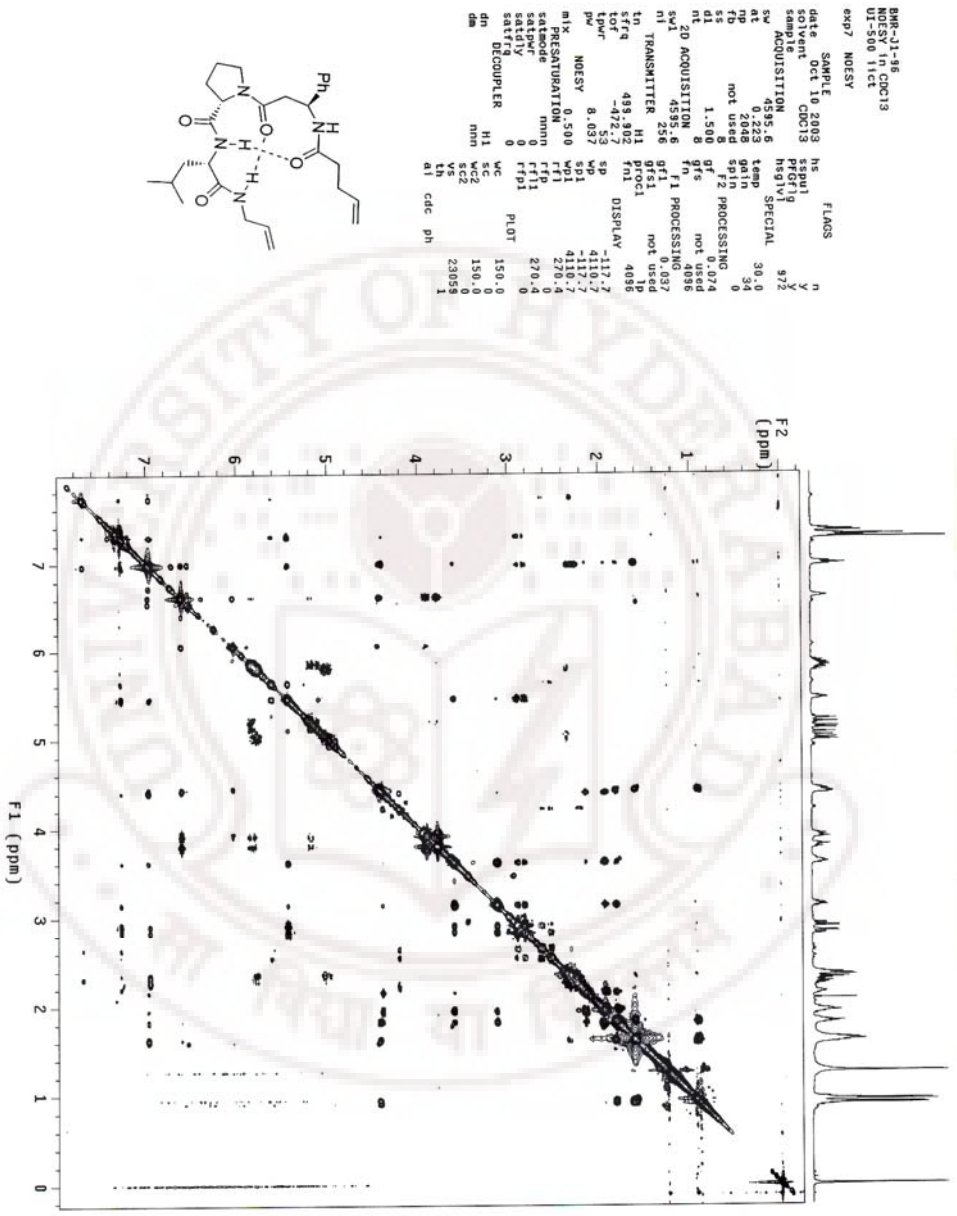
Spectrum **1**: ^{1}H NMR of **42** in $CDCl_3$



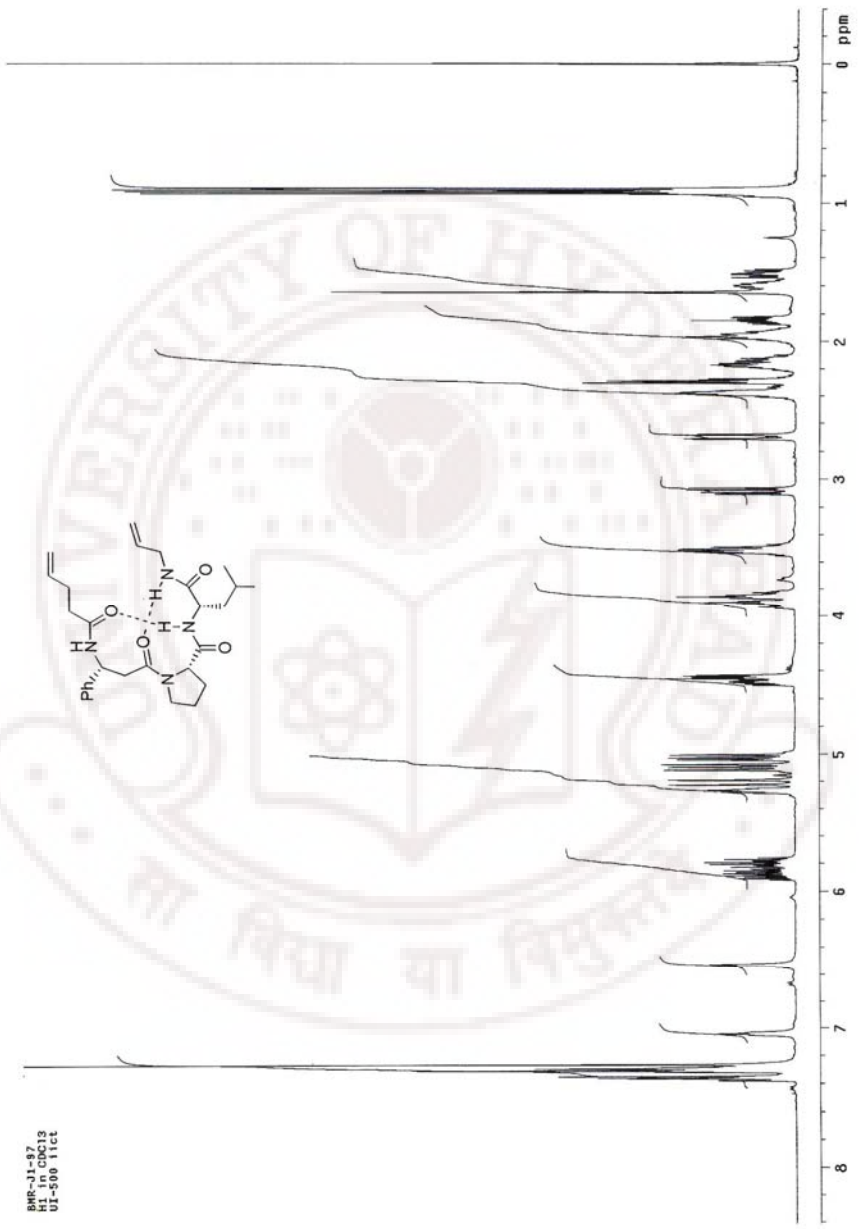
Spectrum **2** TOCSY spectrum of **42** in $CDCl_3$



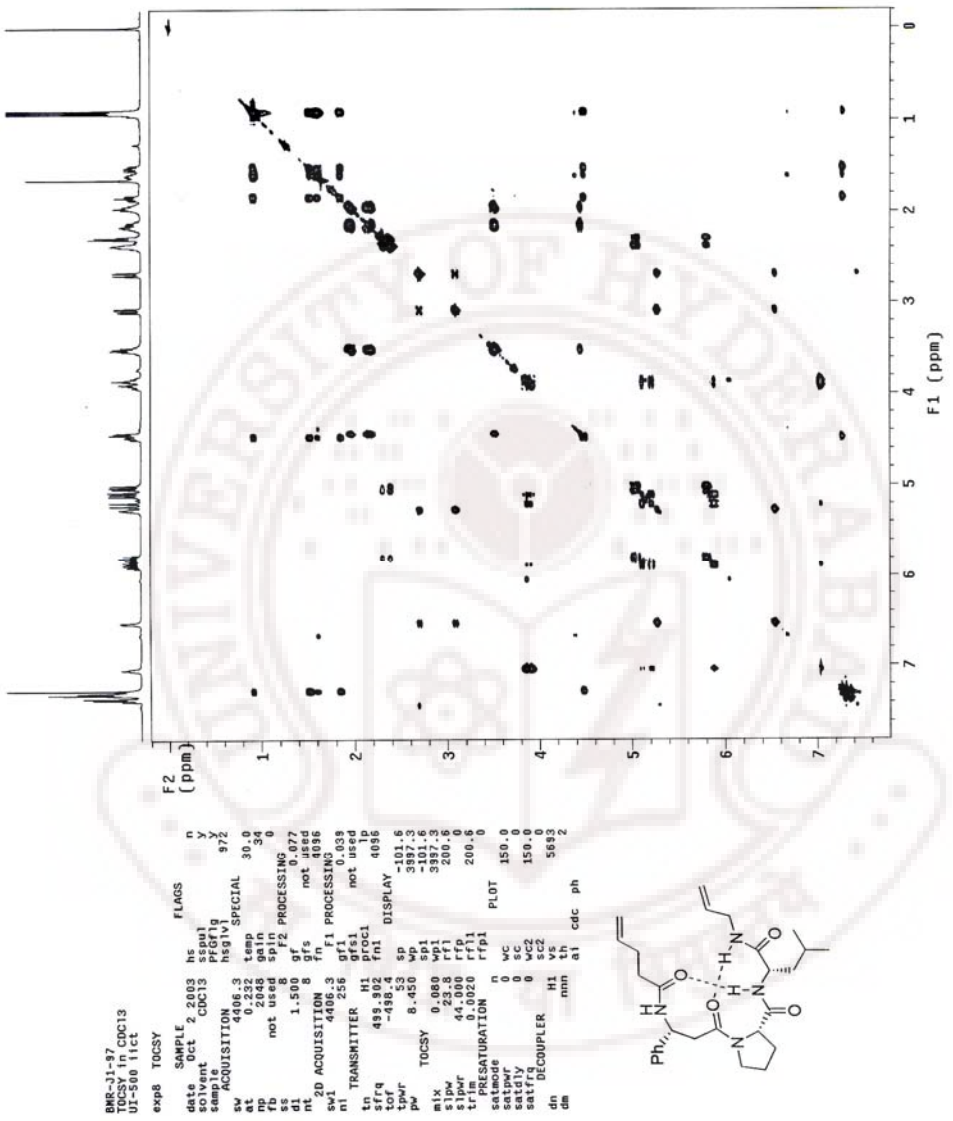
Spectrum **3** NOESY of **42** in $CDCl_3$



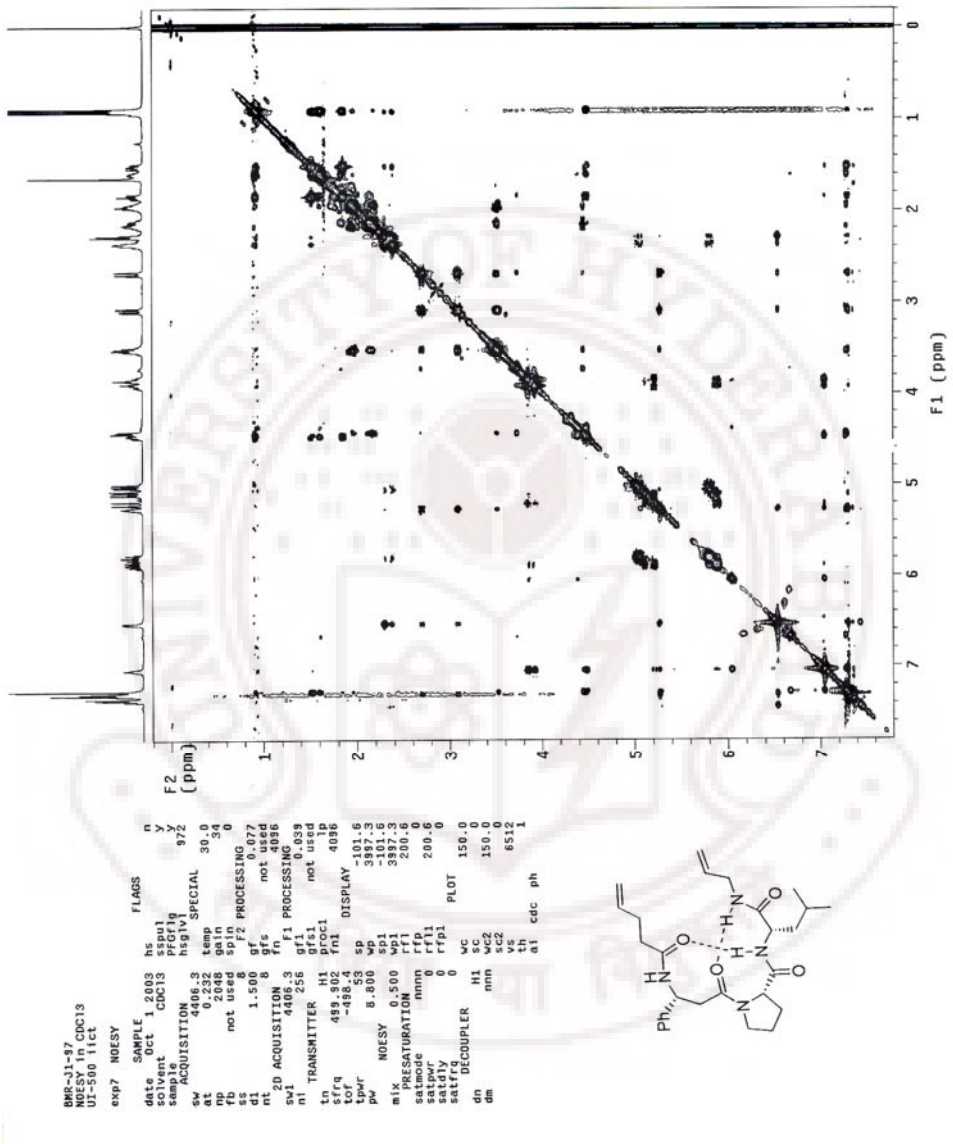
Spectrum **4** ^{1}H NMR of **43** in $CDCl_3$



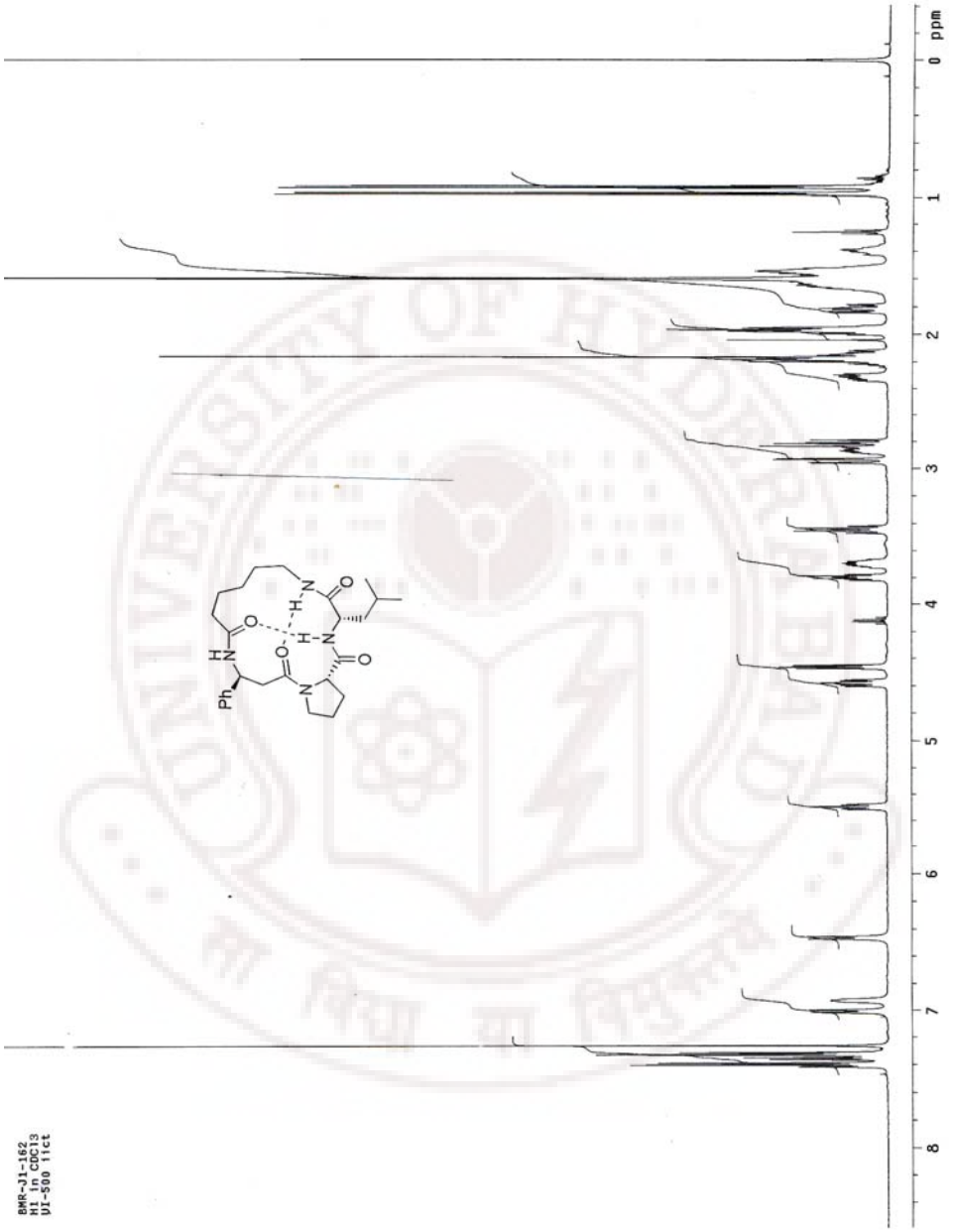
Spectrum **5** TOCSY spectrum of **43** in $CDCl_3$



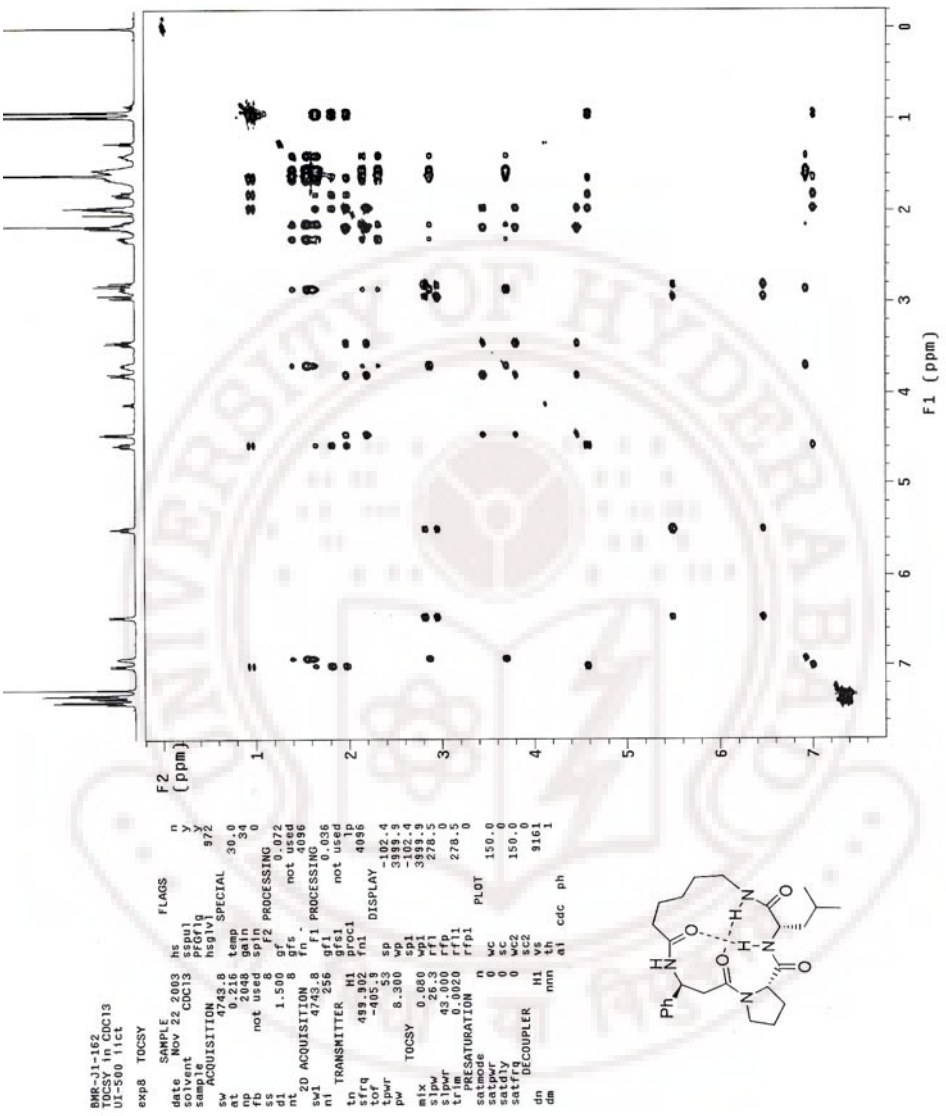
Spectrum **1** NOESY spectrum of **43** in $CDCl_3$



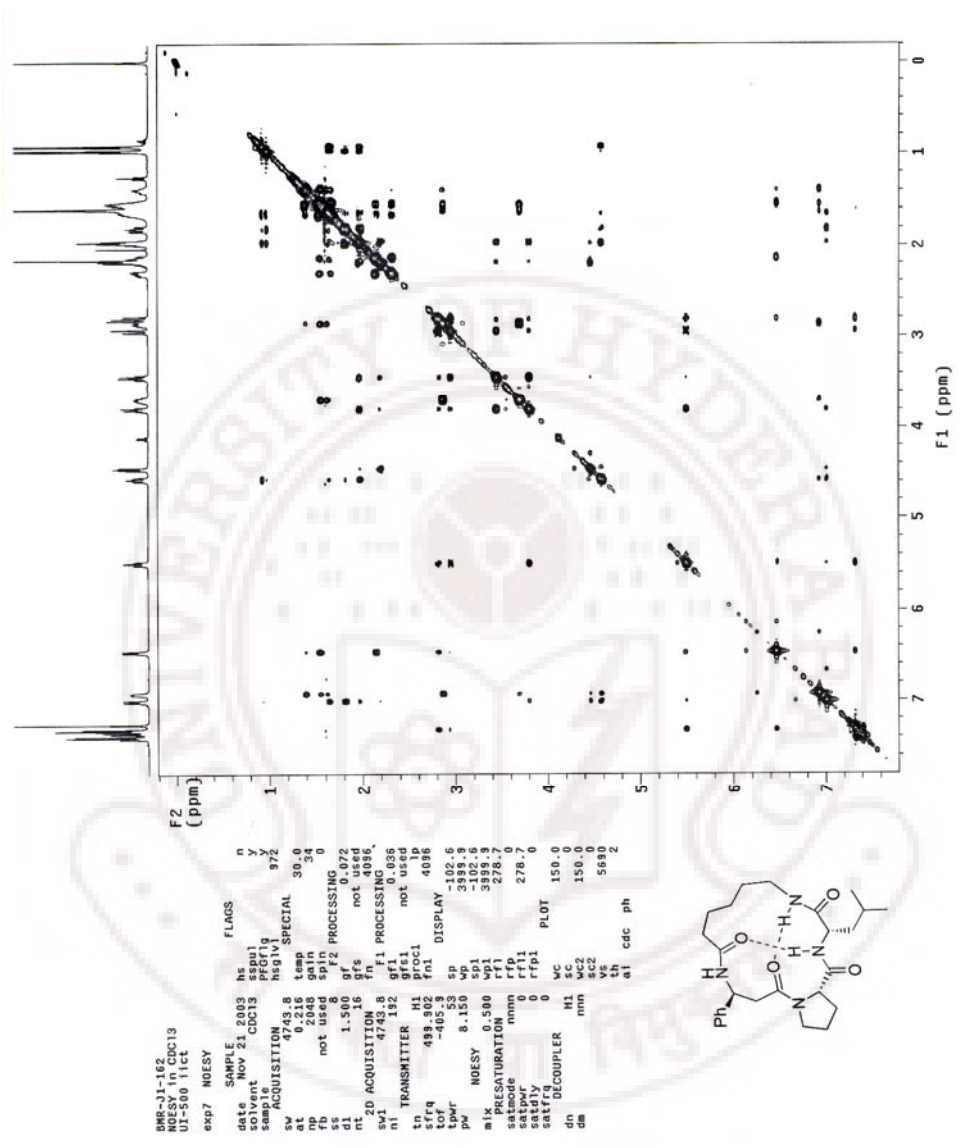
Spectrum **7** 500 MHz ^{1}H NMR of **49** in $CDCl_3$



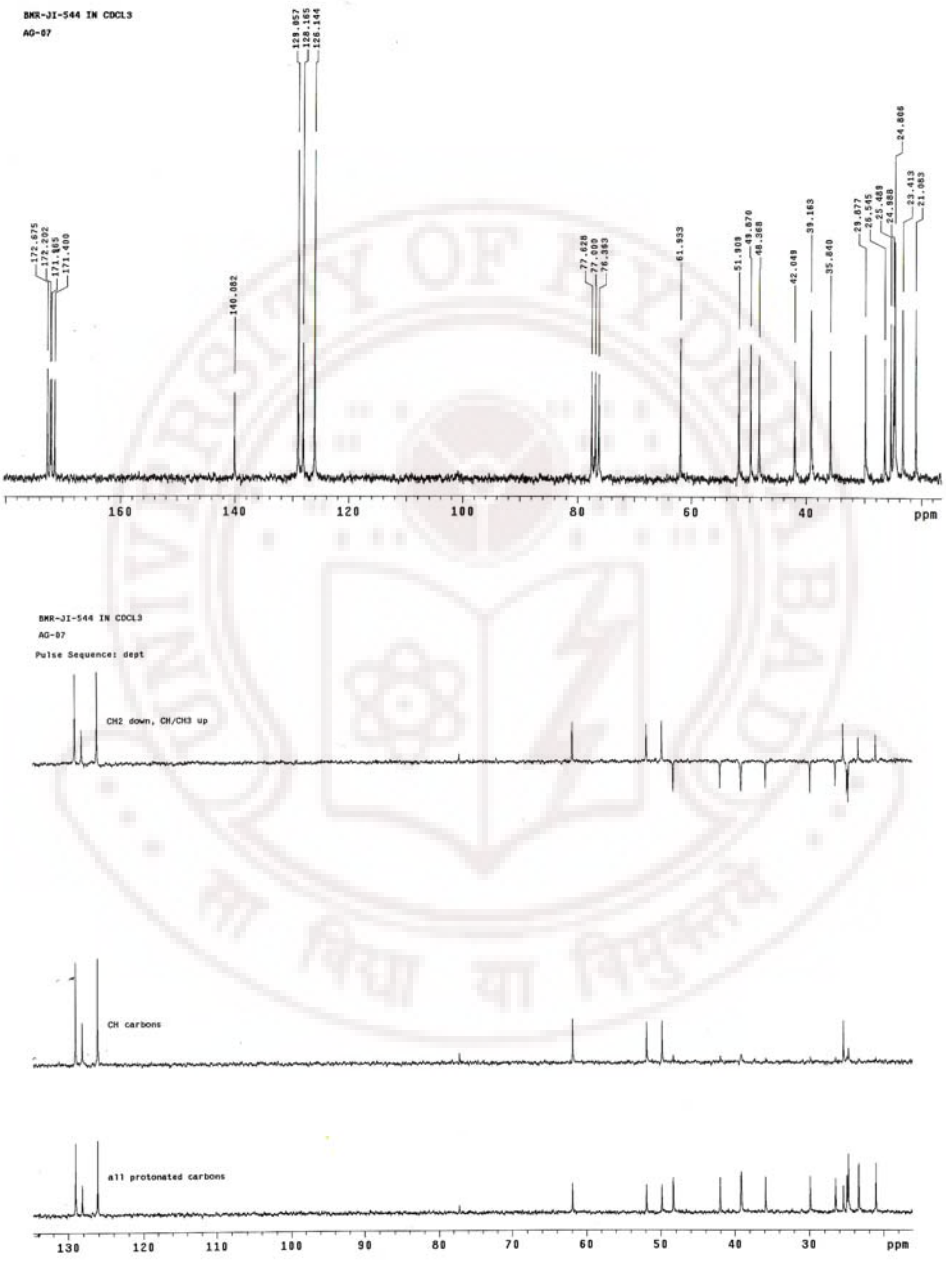
Spectrum **8** TOCSY of **49** in $CDCl_3$



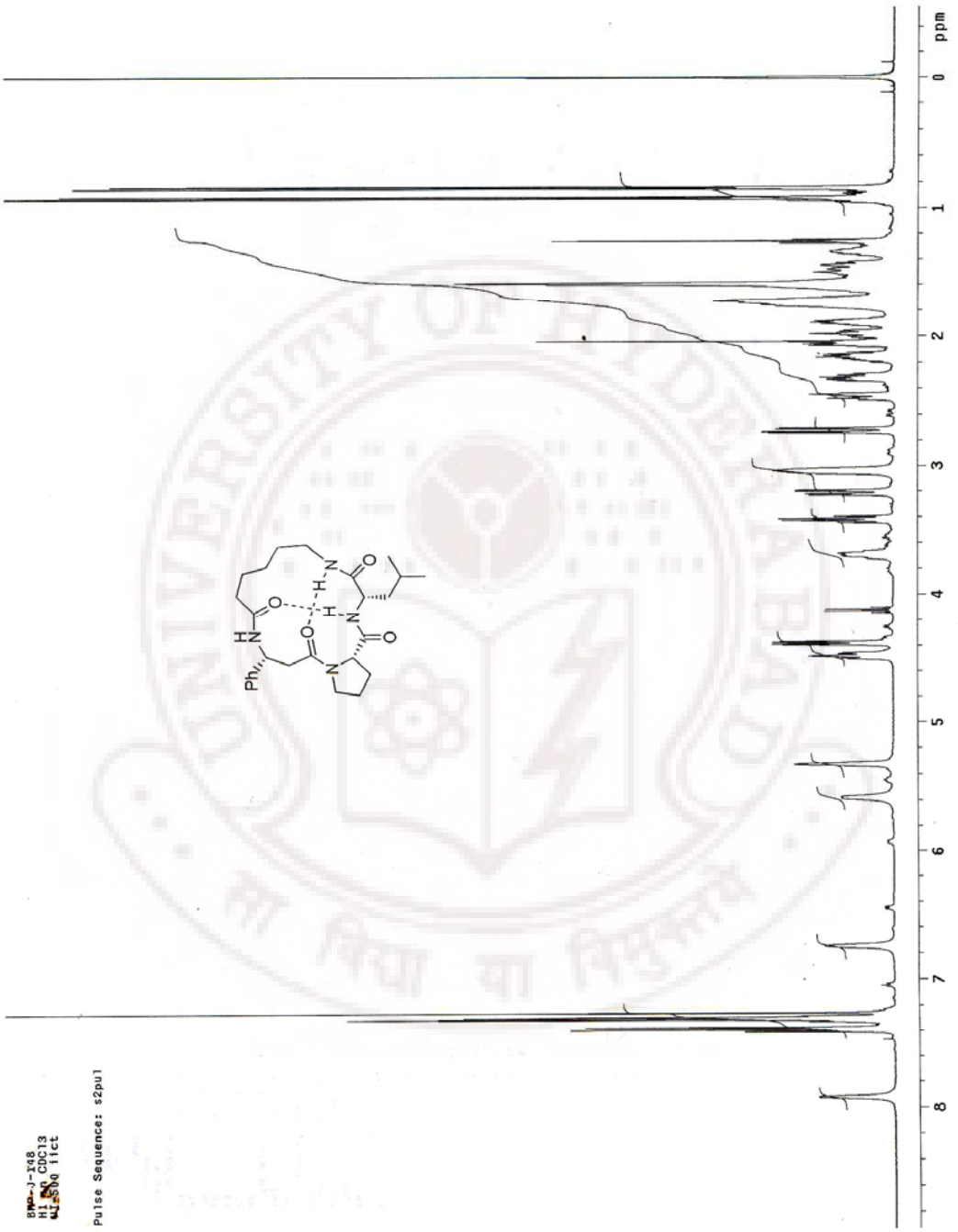
Spectrum **9** NOESY of **49** in $CDCl_3$



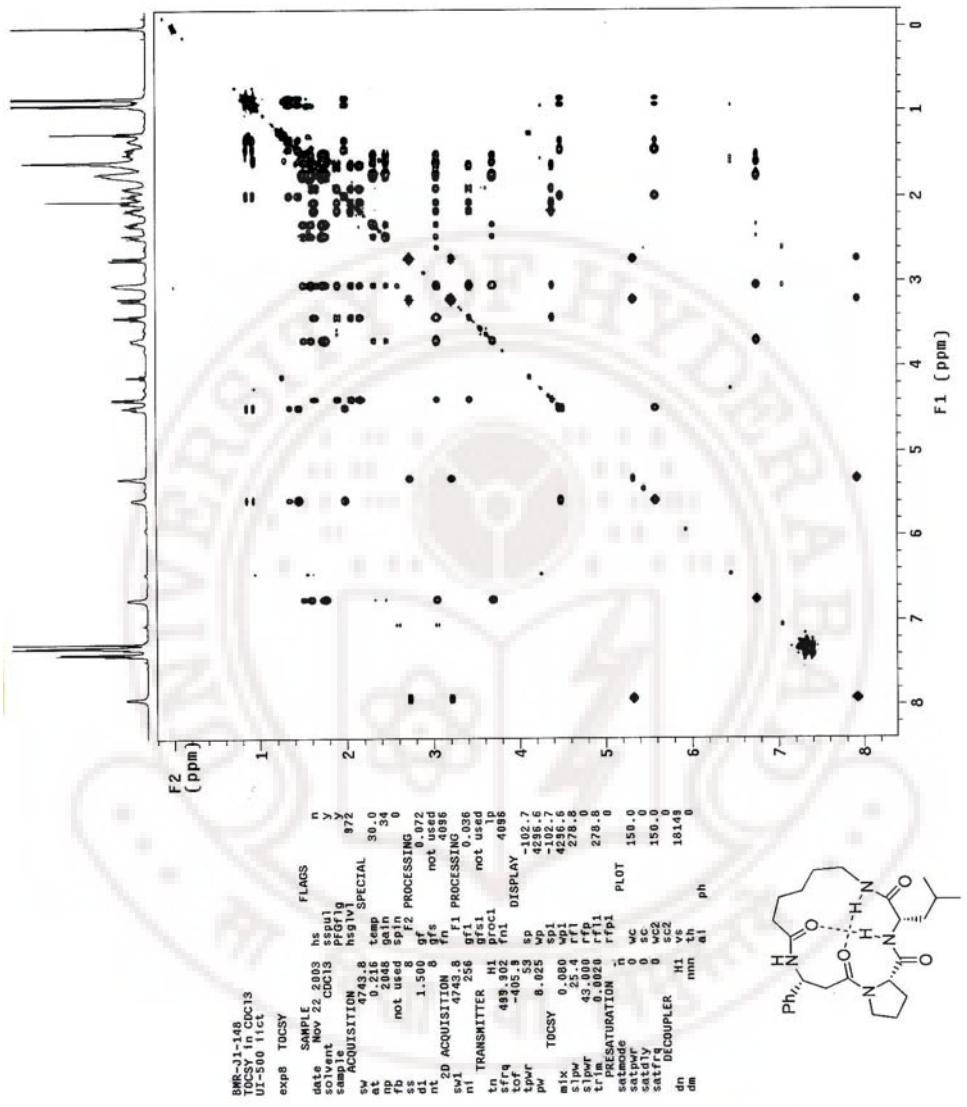
Spectrum **10** ^{13}C NMR and DEPT spectra of peptide **49** in $CDCl_3$



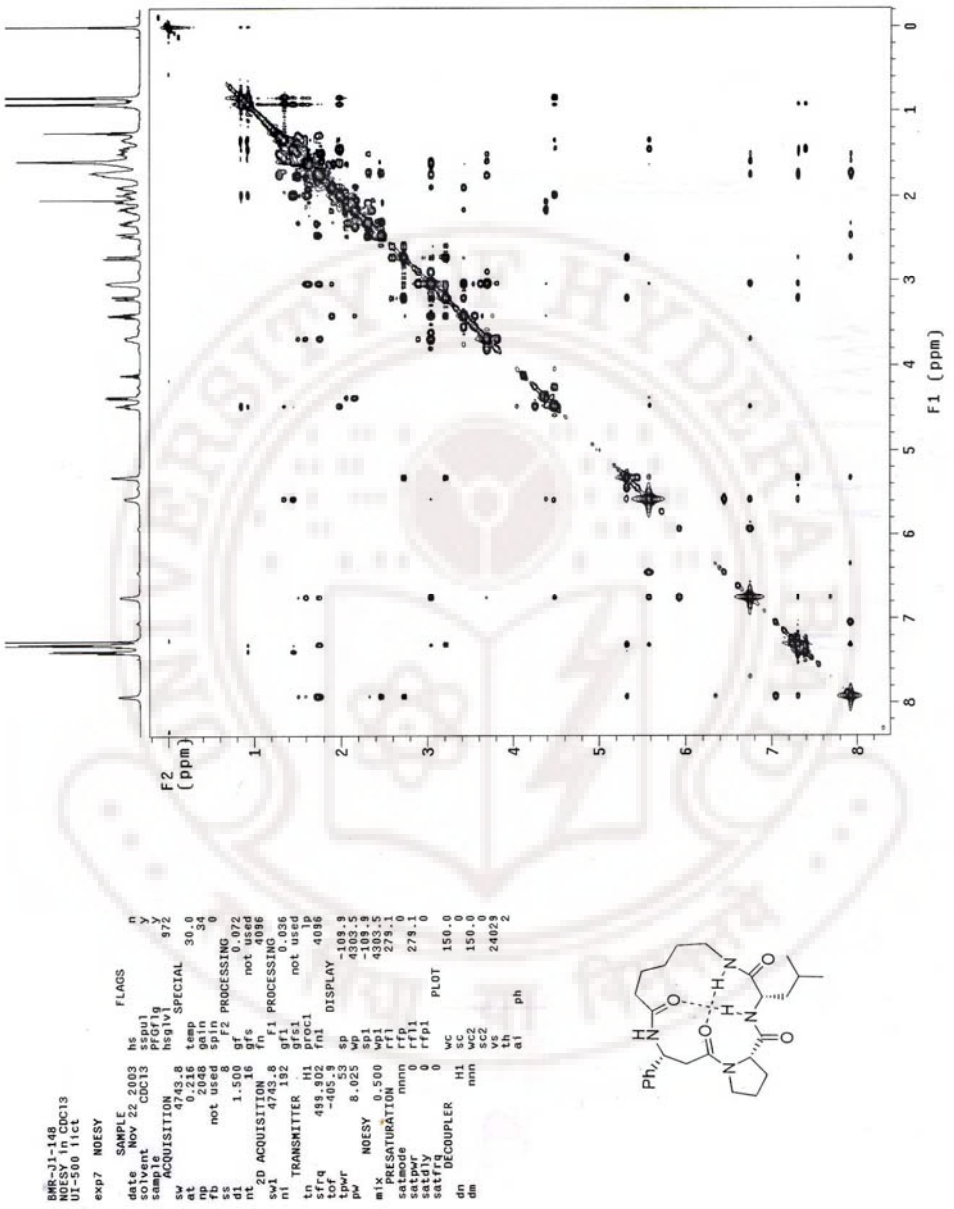
Spectrum **11** ^{1}H NMR spectrum of **50** in $CDCl_3$



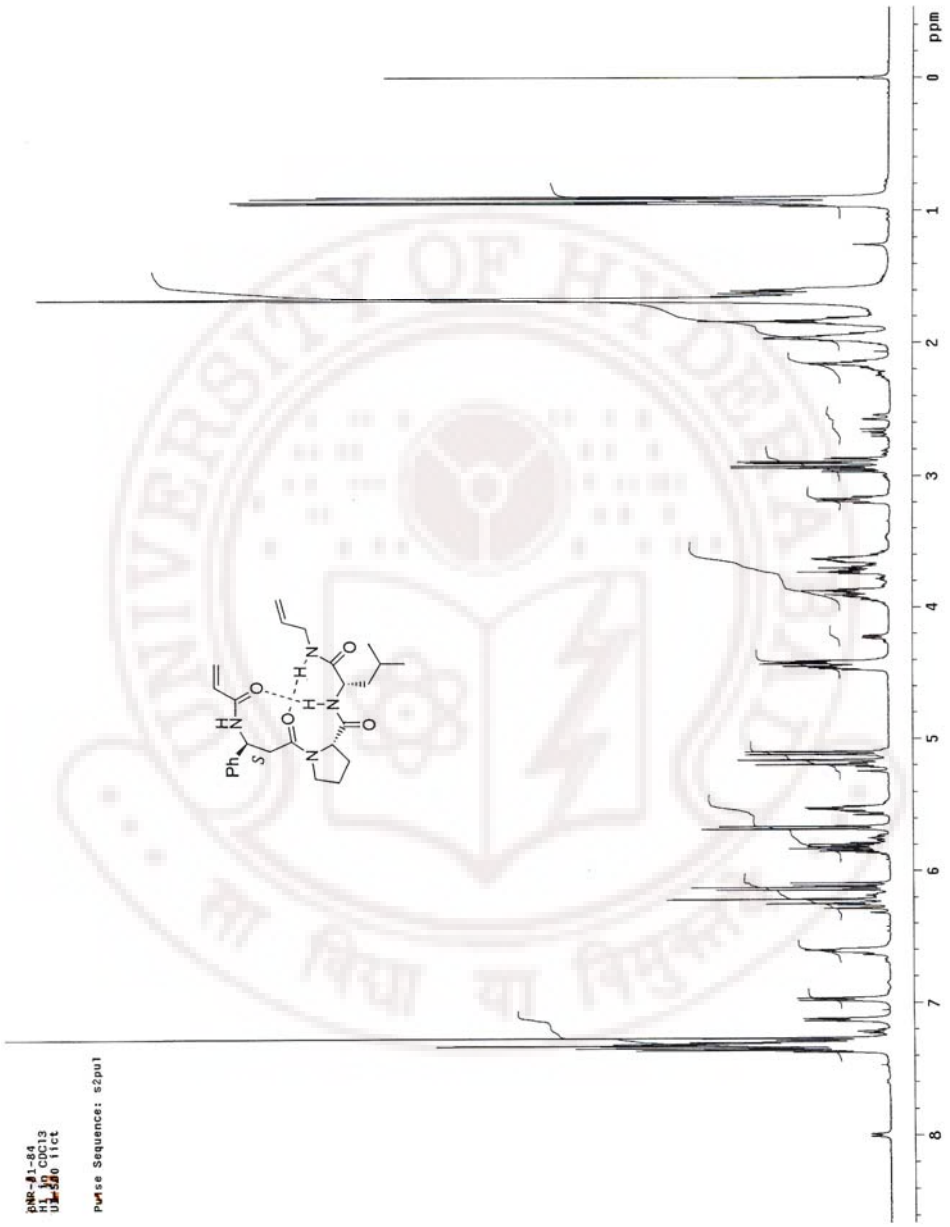
Spectrum **12** TOCSY spectrum of **50** in $CDCl_3$



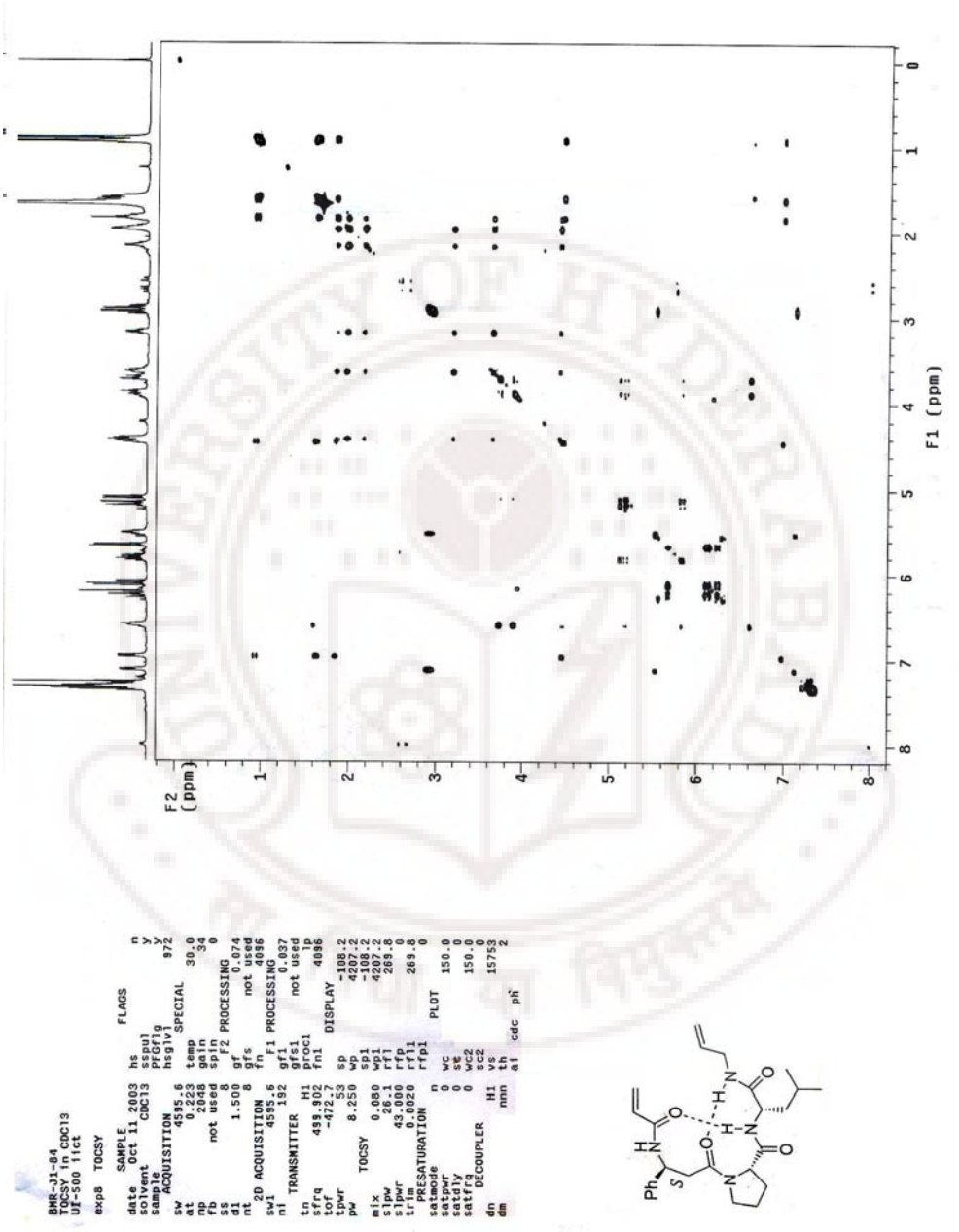
Spectrum **13** NOESY spectrum of **50** in $CDCl_3$



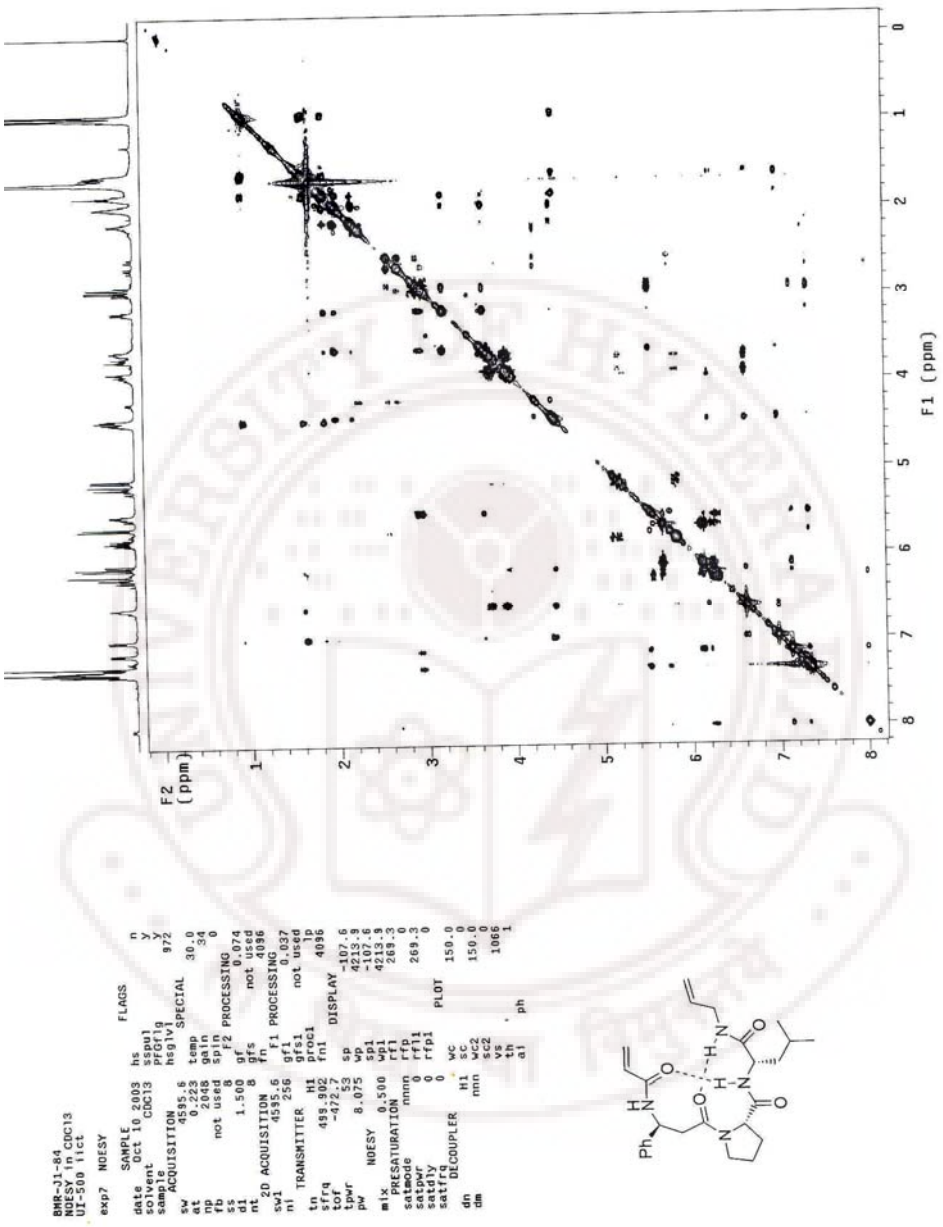
Spectrum **14** 500 MHz ^{1}H NMR spectrum of **52** in $CDCl_3$



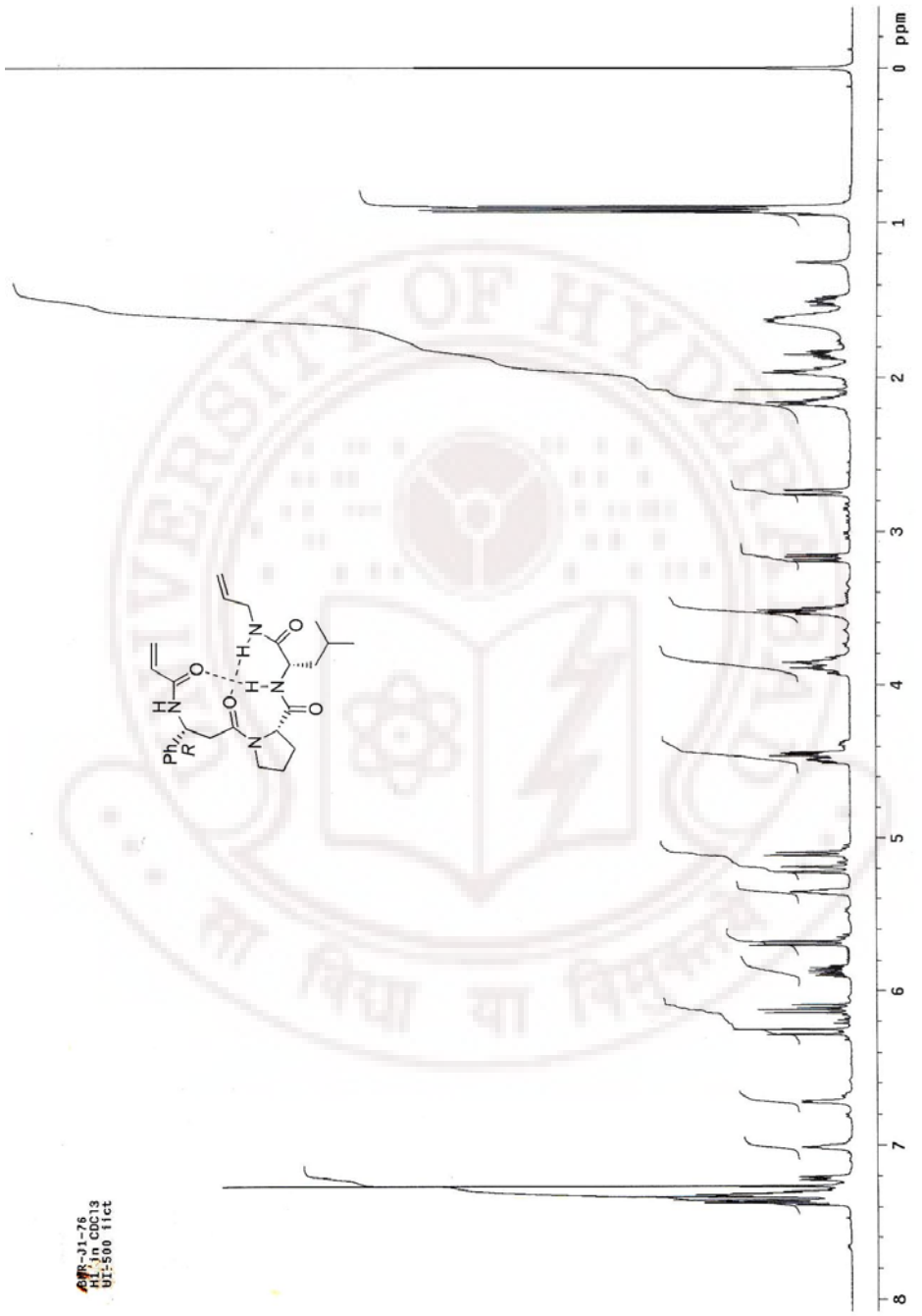
Spectrum **15** TOCSY spectrum of **52** in $CDCl_3$



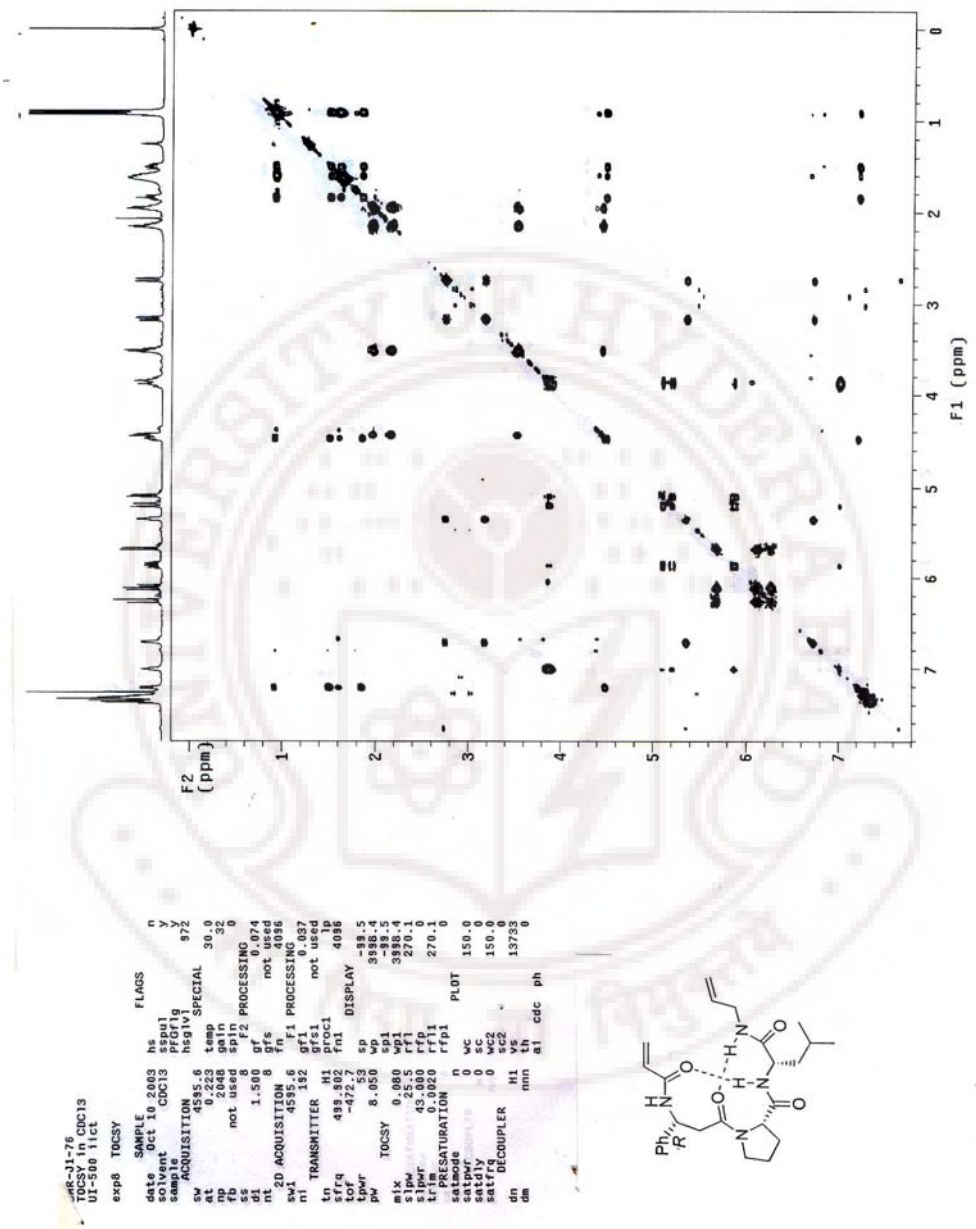
Spectrum **16** NOESY spectrum of **52** in $CDCl_3$



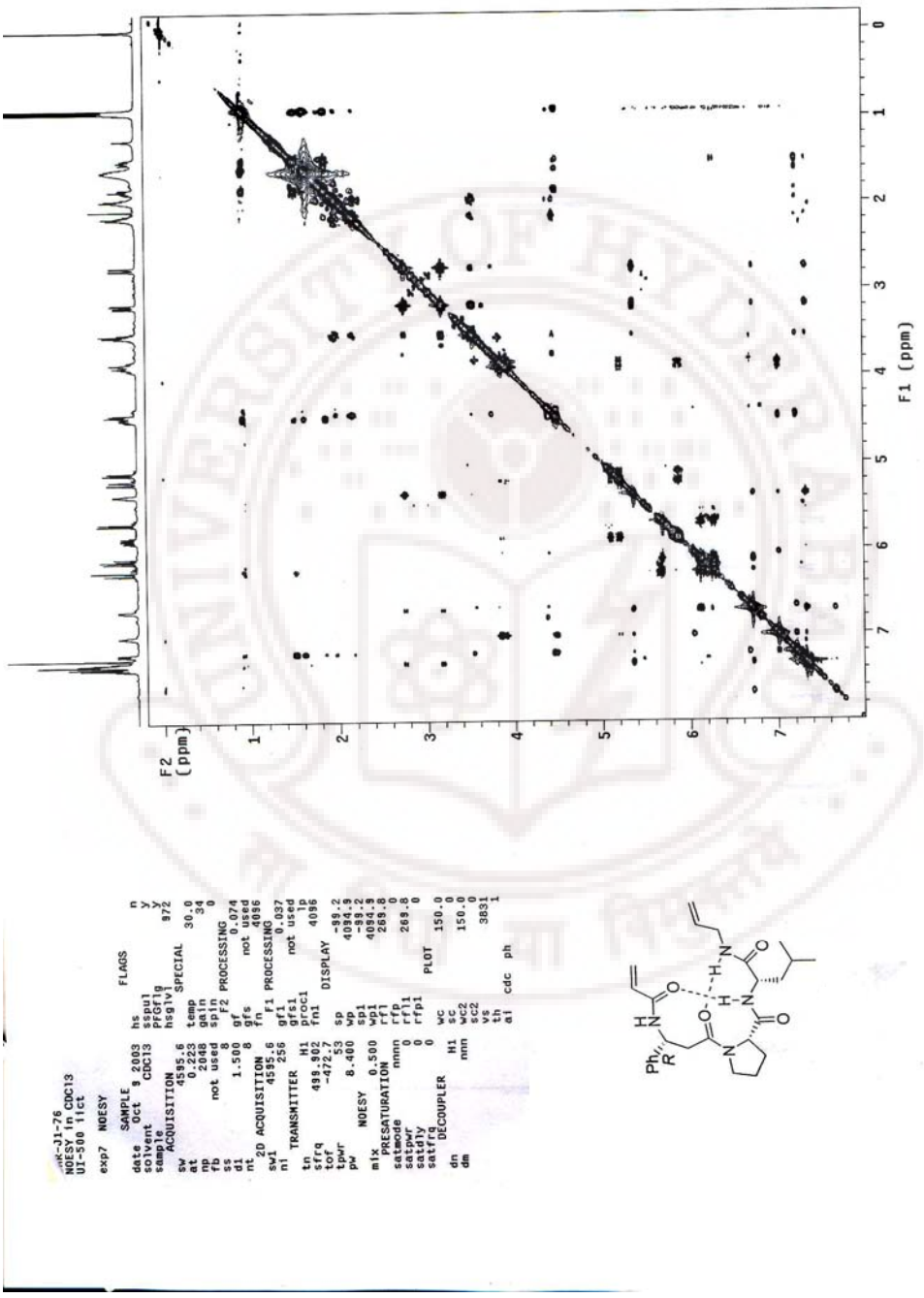
Spectrum **17** ^{1}H NMR spectrum of **53** in $CDCl_3$



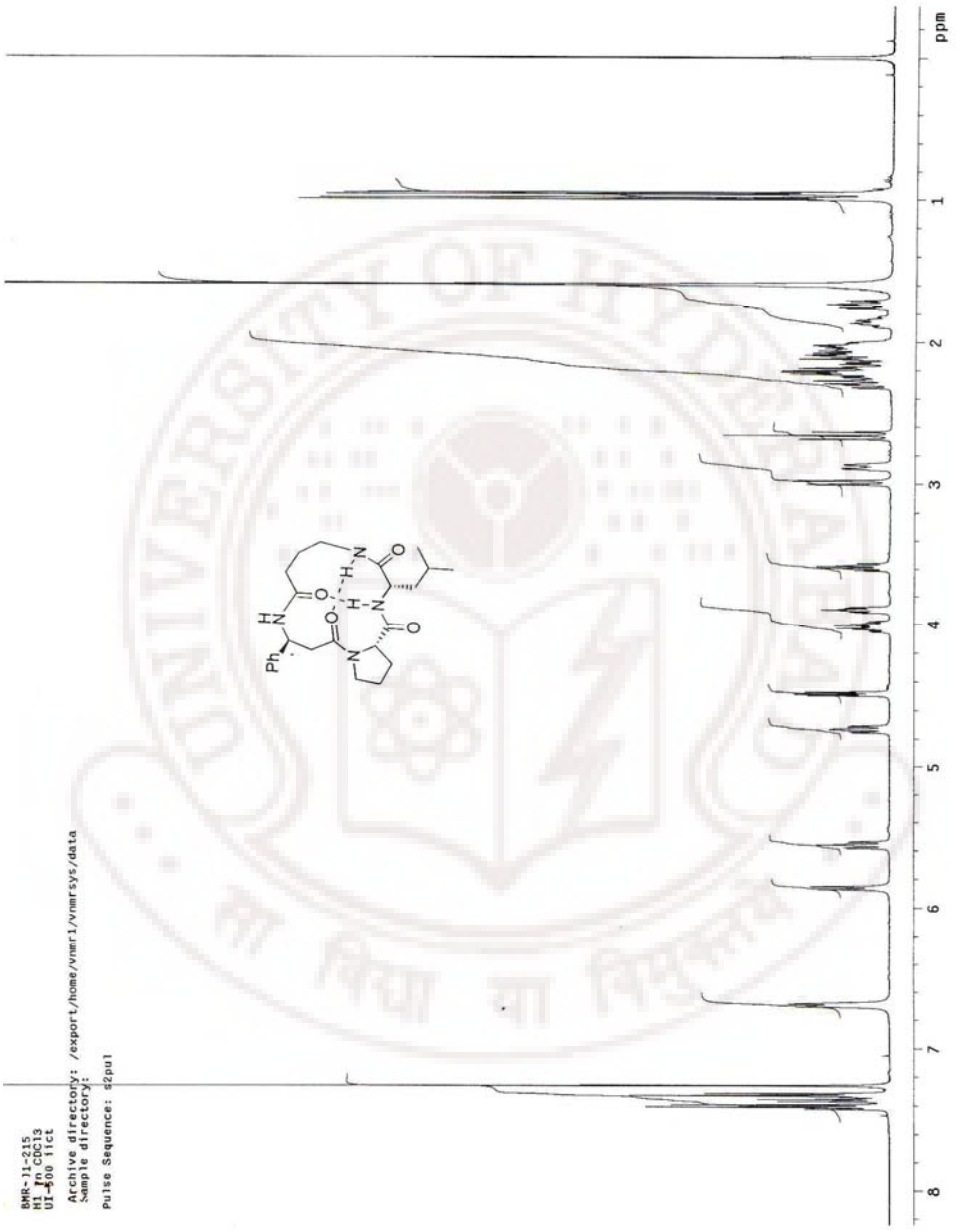
Spectrum **18** TOCSY spectrum of **53** in $CDCl_3$



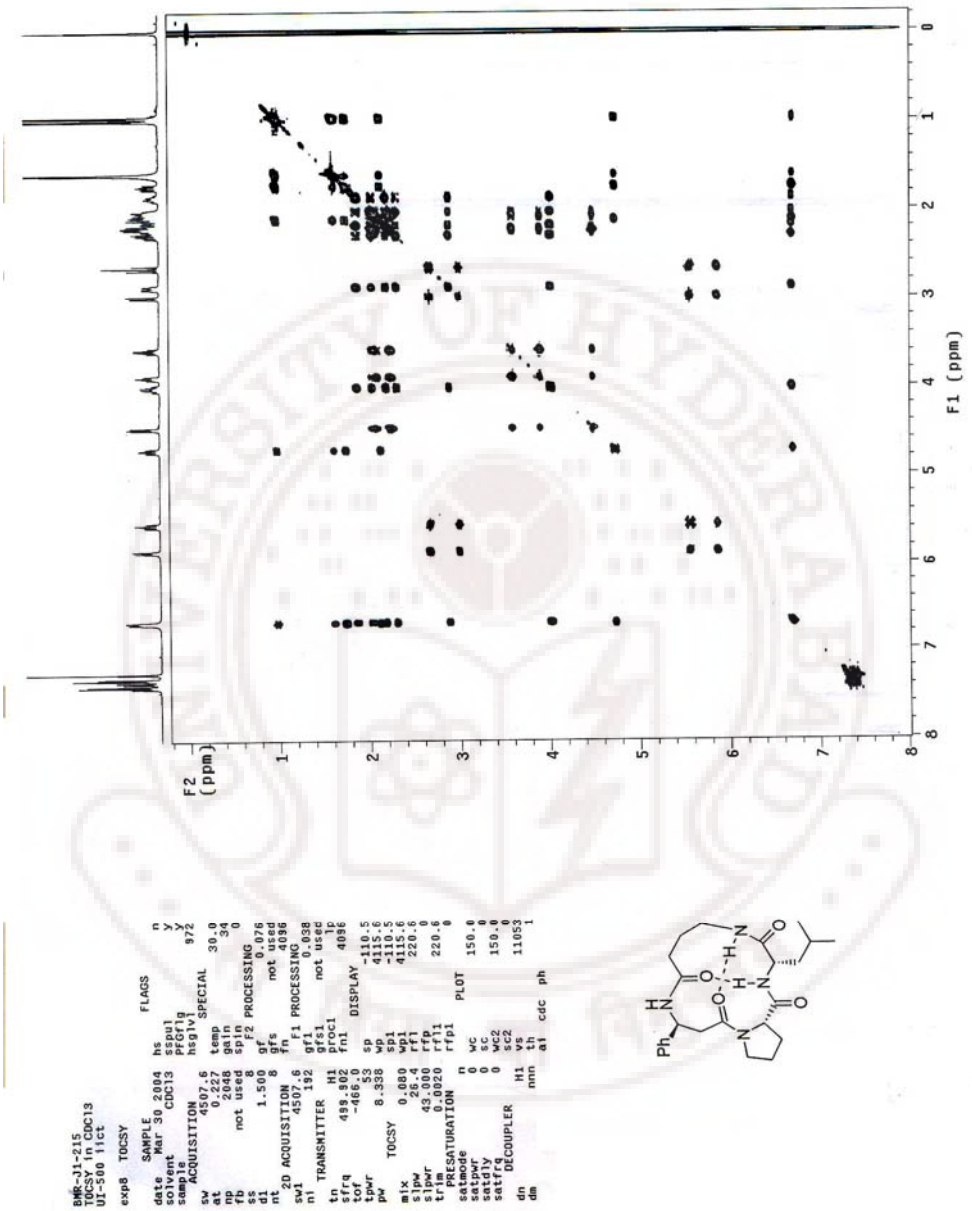
Spectrum **19** NOESY spectrum of **53** in $CDCl_3$



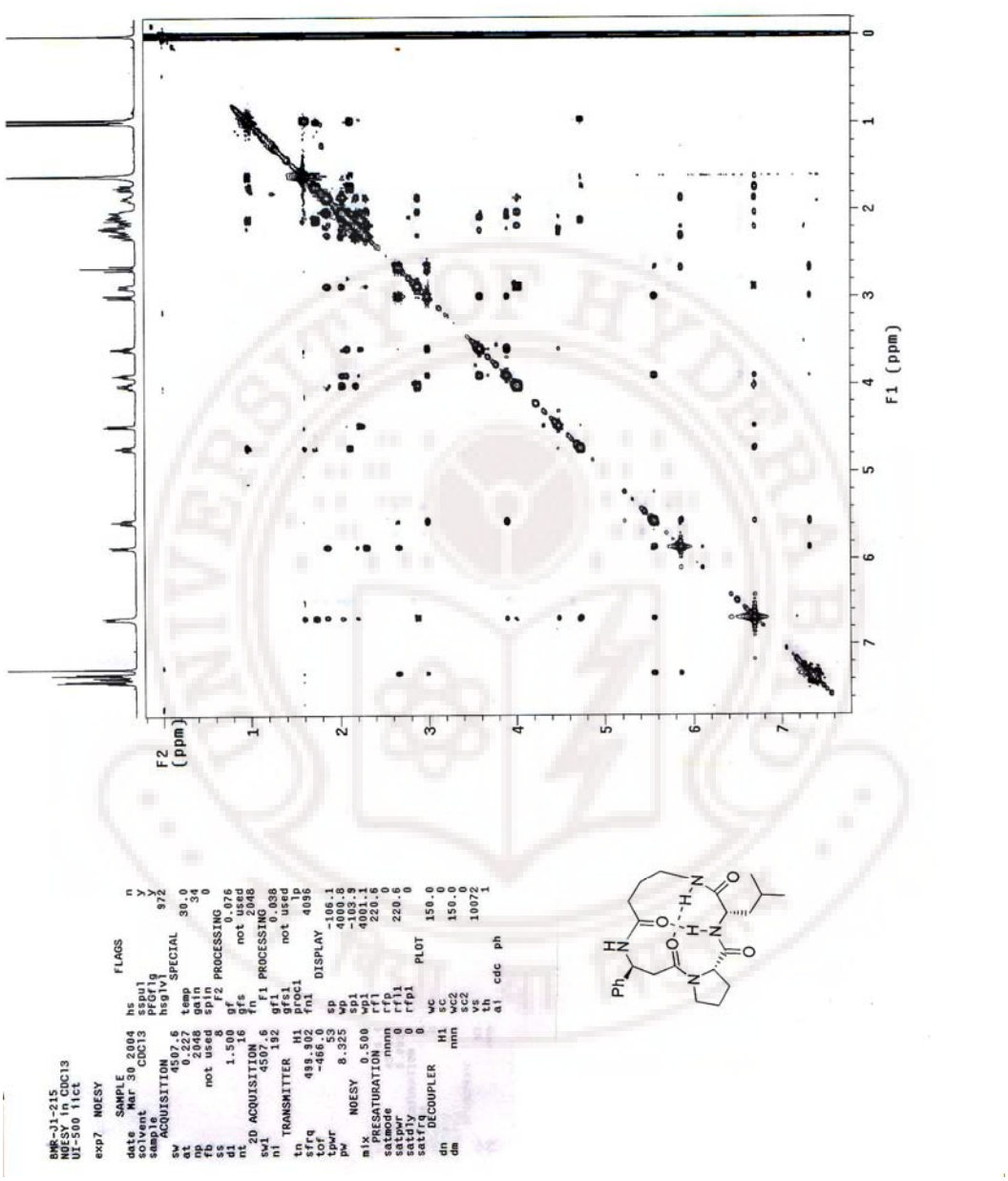
Spectrum **20** ^{1}H NMR spectrum of **54** in $CDCl_3$



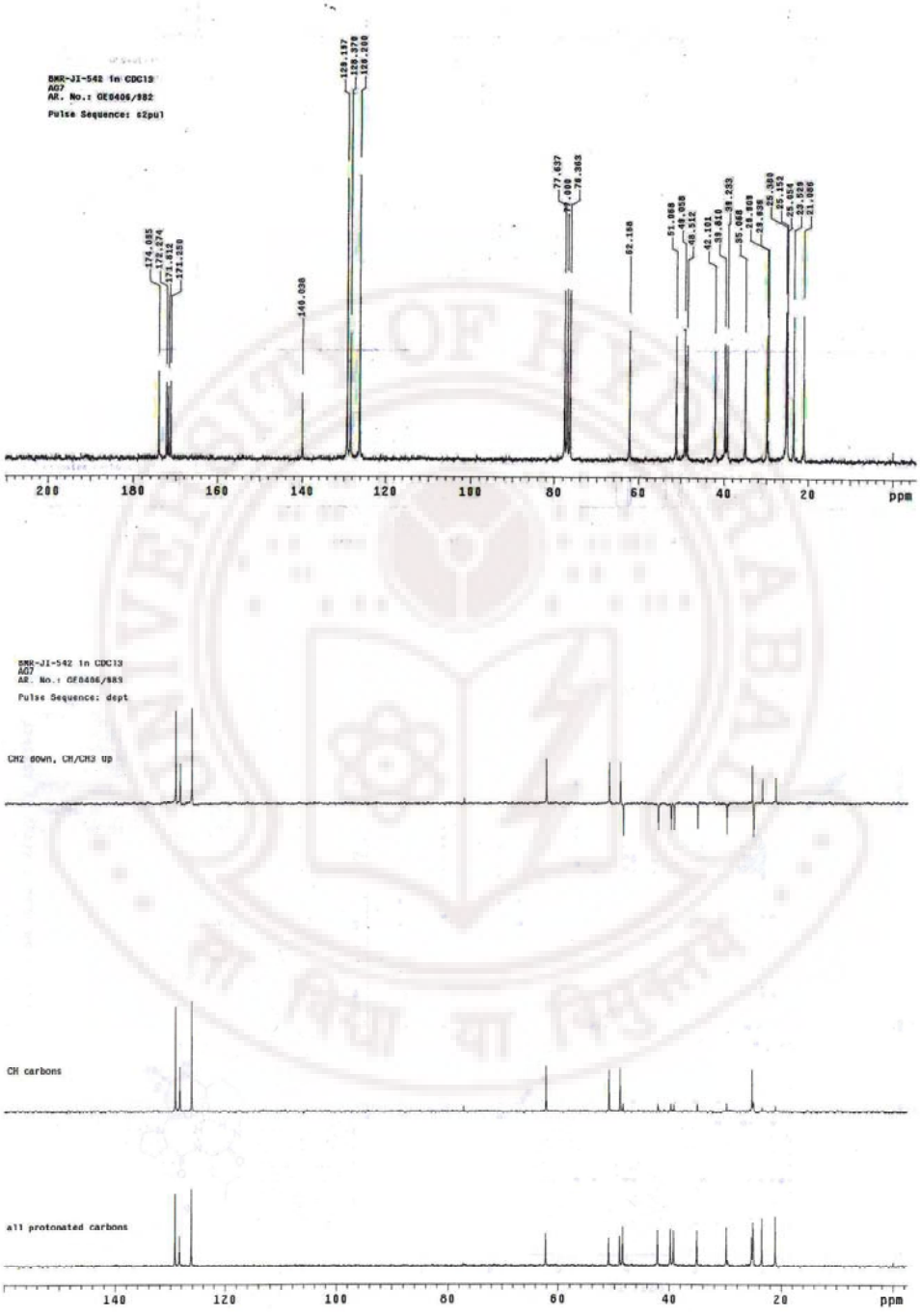
Spectrum **21** TOCSY spectrum of **54** in $CDCl_3$



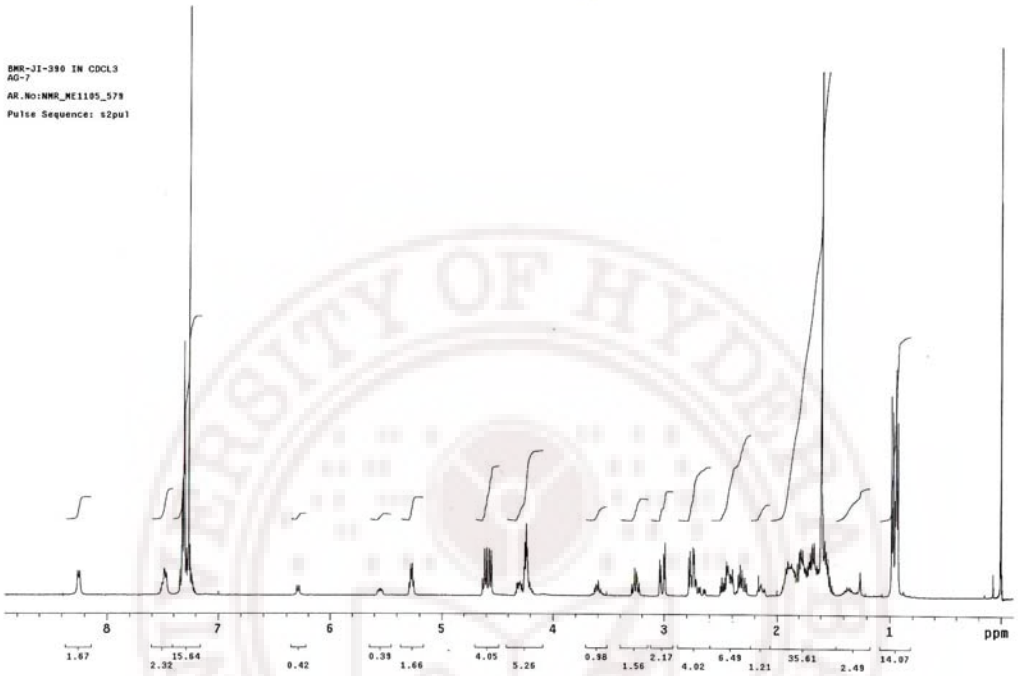
Spectrum **22** NOESY spectrum of **54** in $CDCl_3$



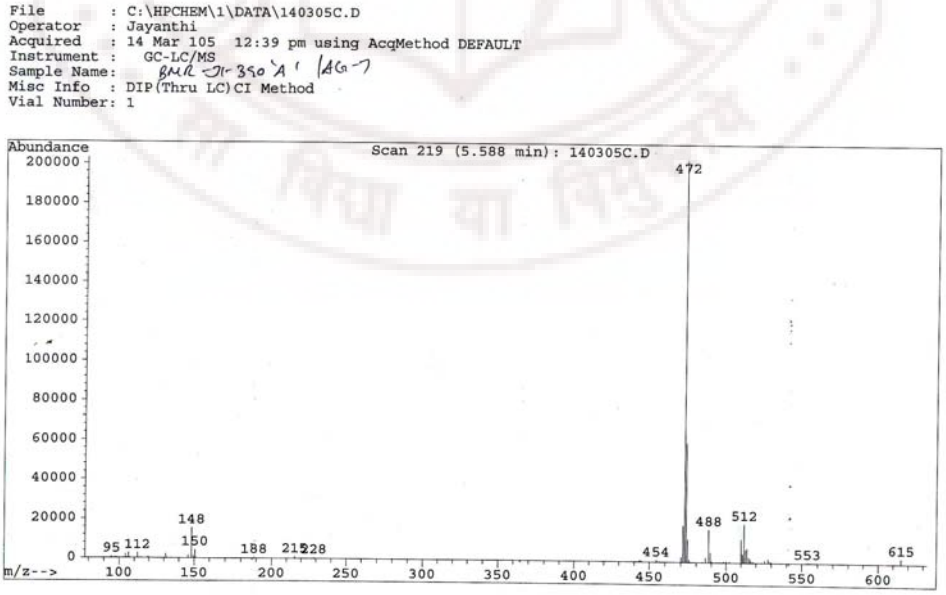
Spectrum **23** ^{13}C spectra and DEPT of peptide 54 in $CDCl_3$



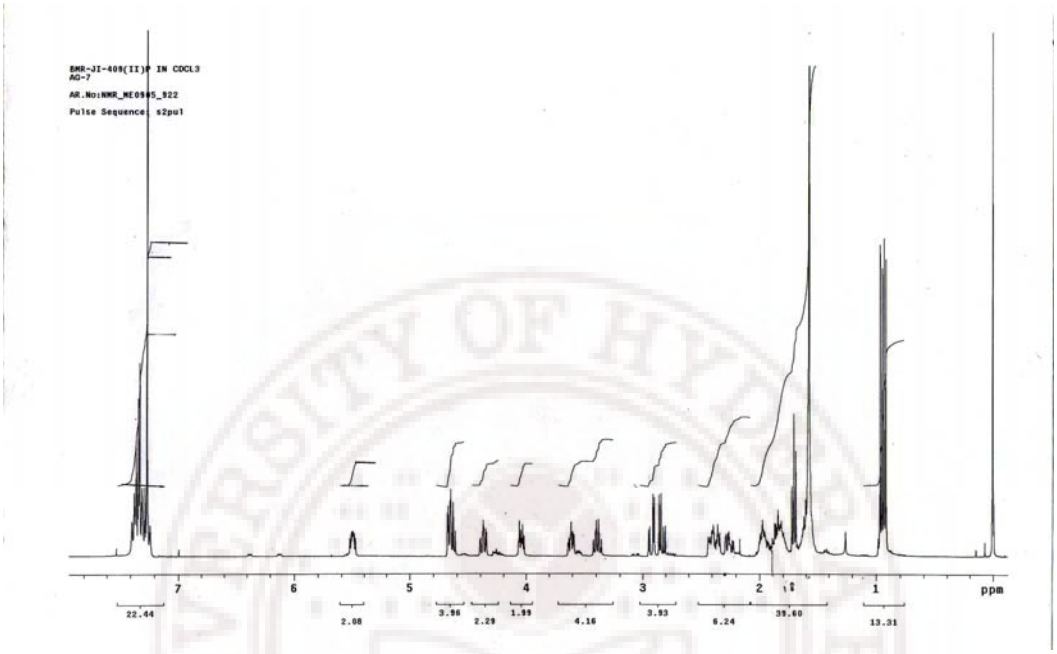
Spectrum **24** ^{1}H NMR of peptide 61 in $CDCl_3$



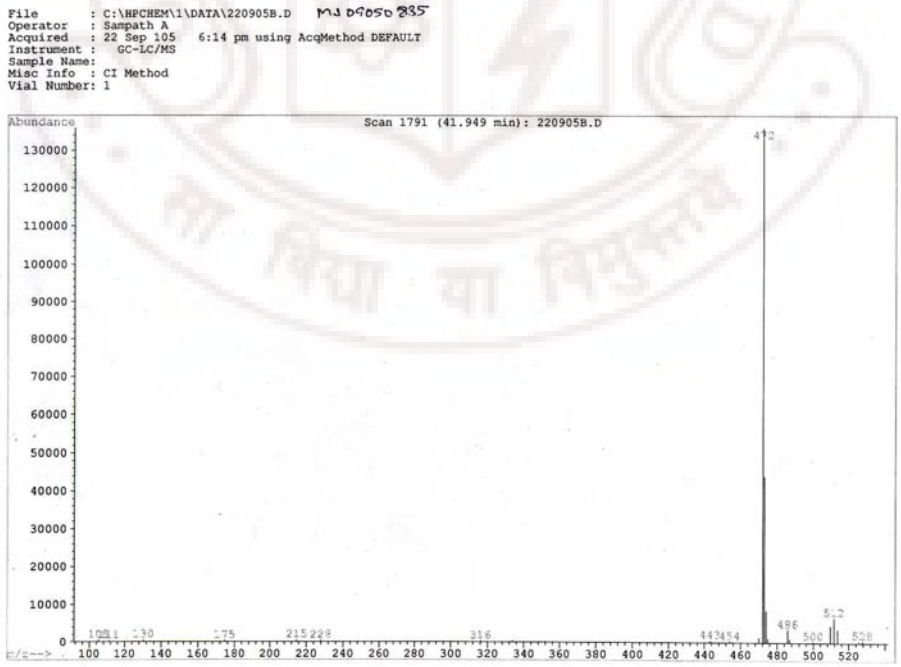
Spectrum **25** Mass spectrum of peptide 61



Spectrum **26** ^{1}H NMR spectrum of peptide 62 in $CDCl_3$



Spectrum **27** Mass spectrum of peptide **62**



Chapter IV

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

4.1 Introduction

Dehydro (or α,β-unsaturated) amino acids have been frequently found in naturally occurring peptides of microbial origin[1] and in some proteins, e.g., histidine ammonia lyase from bacterial and mammalian sources and phenylalanine ammonia lyase from plants.[2] They are also constituents of a separate class of polycyclic peptide antibiotics, lantibiotics,[3] such as nisin, epidermin, and subtilin.[4] Nisin and subtilin both contain dehydroalanine (ΔAla) and dehydrobutyrine (ΔAbu). A recent study suggests that nisin, well known as a food preservative, latches to a molecule known as lipid-II on bacterial cell membrane and kills the hosts by punching a hole in the membrane.[5] Dehydroleucine (ΔLeu) and dehydrophenylalanine (ΔPhe) are present in albonoursin,[6] while dehydrovaline (ΔVal) is found in penicillin[7] and cephalosporin (antibacterials11). Dehydrotryptophan (ΔTrp) is contained in neochinulins[8] (growth inhibitor) and dehydrophenylalanine is found in tentoxin[9] (phytotoxic). In some cases, dehydroalanine residues form a part of the active site in the protein and lack of these residues result in certain diseases.[10] The presence of dehydroresidues in peptides confer altered bioactivity as well as increased resistance to enzymatic degradation.[11] Dehydroresidues have been introduced in several bioactive sequences in order to obtain highly active agonist and antagonist analogs, and this modification has become one of the most promising methods to study structure–

function relationships in biologically active peptides.[12] Based on this precedence, we recognized dehydroamino acid (Dhaa) residues as one of the important conformational constraint in the design of cyclic peptides because the presence of a sp^2 hybridized carbon atom in the backbone, the altered electronic distribution (conjugation) caused by the α, β π-system, and the change in the side-chain rotamer populations all contribute significantly to the conformation of the peptide backbone and may in turn increase peptide-receptor affinity by reducing the entropic costs of binding.

4.2 Present Study

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

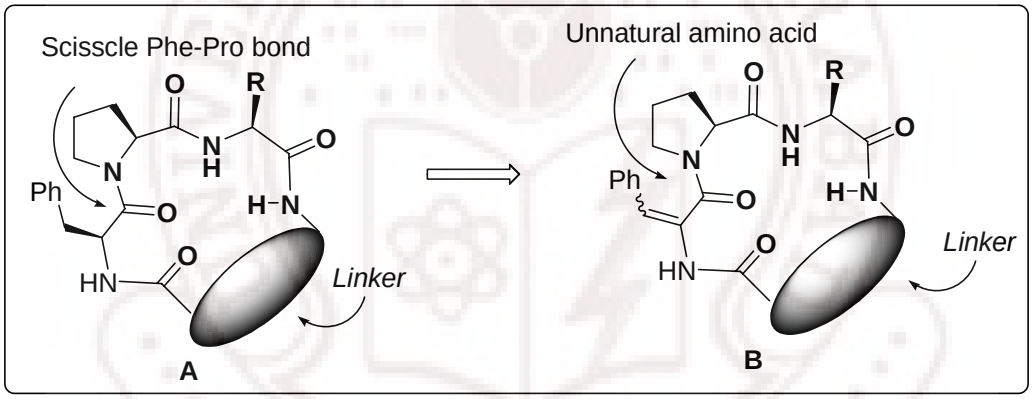


Figure-**1**: *Schematic representation of ΔPhe containing cyclic peptide*

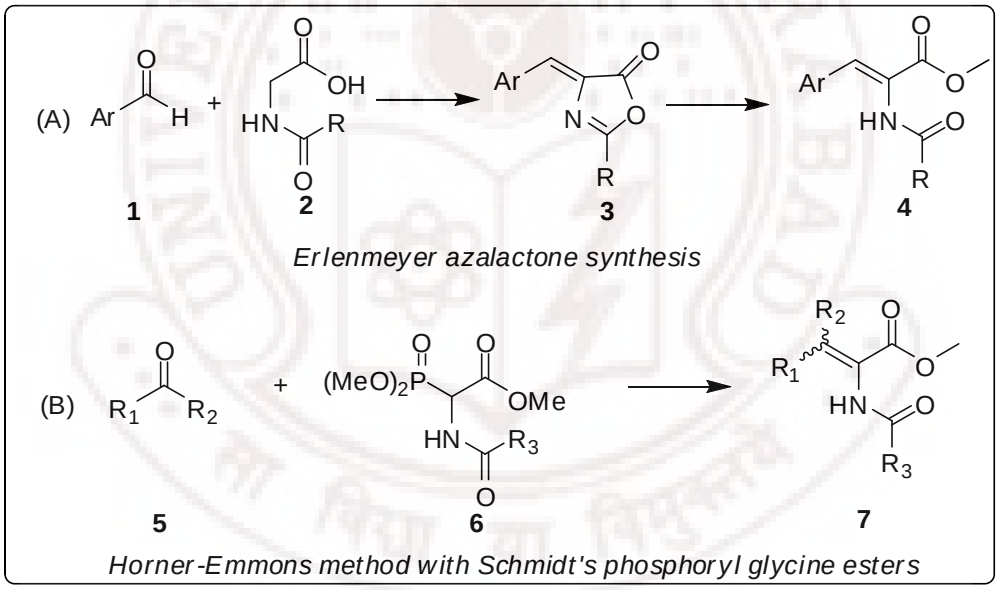
It is also noteworthy that the presence of two constrained amino acids alpha to each other (namely L-proline and constrained phenylalanine) may lead to either a β-strand conformation or a turn conformation thereby rendering an additional element of recognition for the protease active site. We believed that the configuration of the double bond in the dehydro residue (ΔPhe in this case) may control the conformations of proximal amino acid residues and will afford

information on conformations acceptable to the bioreceptor and hence, it is important that we be able to incorporate both double bond isomers into cyclic peptides. Therefore, we became interested to synthesize ΔPhe-Pro containing cyclic peptides.[15]

4.2.1 Synthetic approaches to dehydroamino acids:

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

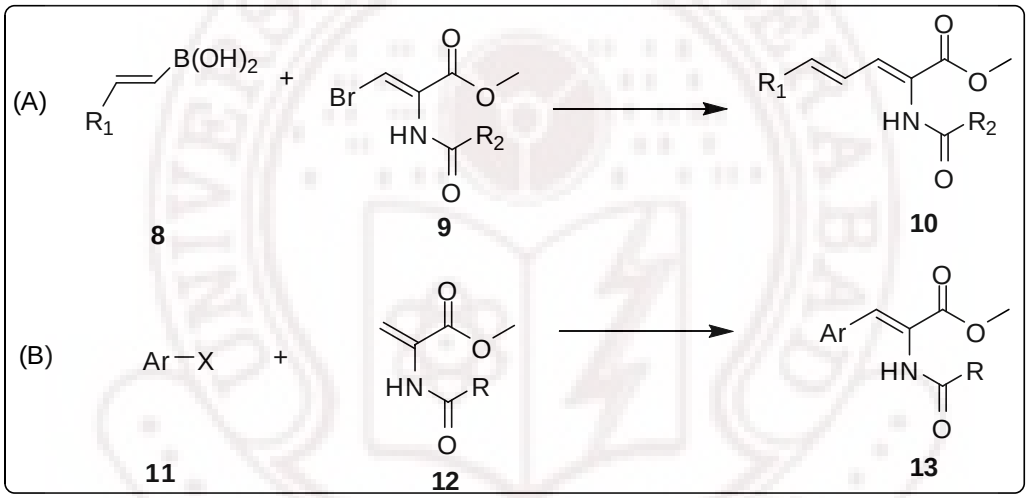
Scheme-**1**: *Synthetic approach to dehydro amino acid derivatives*



The Erlenmeyer synthesis *via* azlactone [16] (method A), condensation of aldehydes with phosphorylglycine esters **6** (method B) are the most frequently used.[17] The main limitation of approach (A) lies in the harsh reaction conditions, which limits its use to aromatic aldehydes devoid of acid sensitive groups. In

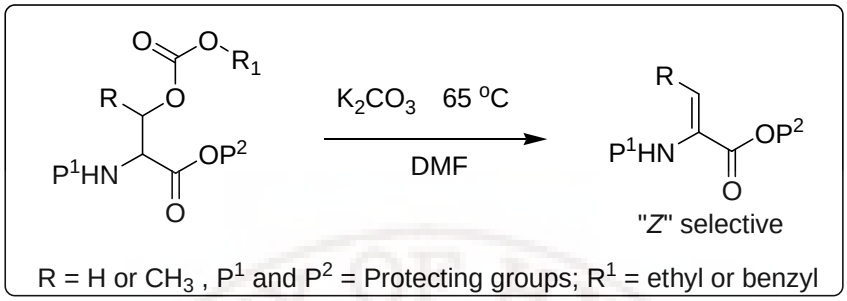
addition, substrates with carbamate protecting groups cannot be prepared (method A). The use of phosphorylglycine esters **6** is instead more versatile and the synthesis is milder. Both β-alkenyl (**10**) and β-aryl (**13**) substituted α-dehydroamino acids derivatives can be conveniently obtained through Suzuki coupling (Scheme-**2**, method A).[18] Nevertheless, the preparation of the starting methyl β-bromo-(acetamido)acrylate **9** is quite laborious and low yielding.

Scheme-**2**: *Synthetic approach to dehydro amino acid derivatives*



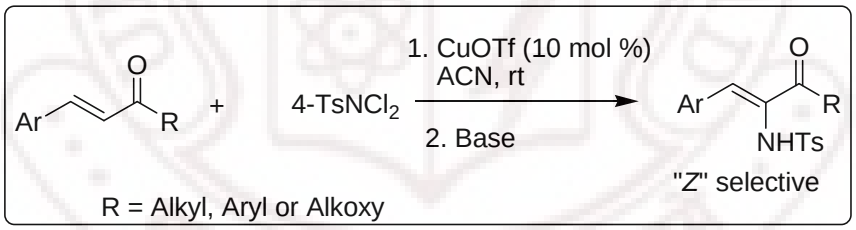
The other common procedures for the preparation of dehydroamino acids are by the β-elimination of α-amino acid derived alcohols or halides,[19] Recently, a similar but modified protocol for the synthesis of ΔAla and ΔAbu (*Z*-selective) in excellent yields was reported by Chandrasekaran *et al*.[20] Their method is based on the anti-selective β-elimination of O-Cbz and O-Eoc derivatives of serine and threonine, using K_2CO_3 in DMF (Scheme-**3**).

Scheme-**3**: *Synthetic approach to 'Z' selective* ΔAla *and* ΔAbu *dehydro amino acid derivatives*



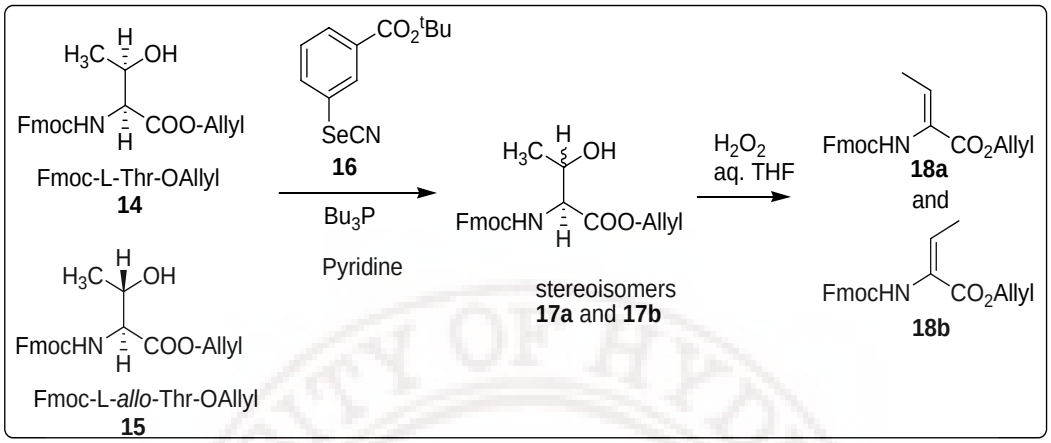
On the other hand, Li *et al* [21] have reported a new facile and highly stereoselective protocol toward α,β-dehydroamino acid derivatives by using the aminohalogenation reaction of α,β-unsaturated esters and ketones followed by treatment with specific bases (Scheme-**4**)

Scheme-**4**: *Synthetic approach to 'Z' selective dehydroamino acid derivatives*



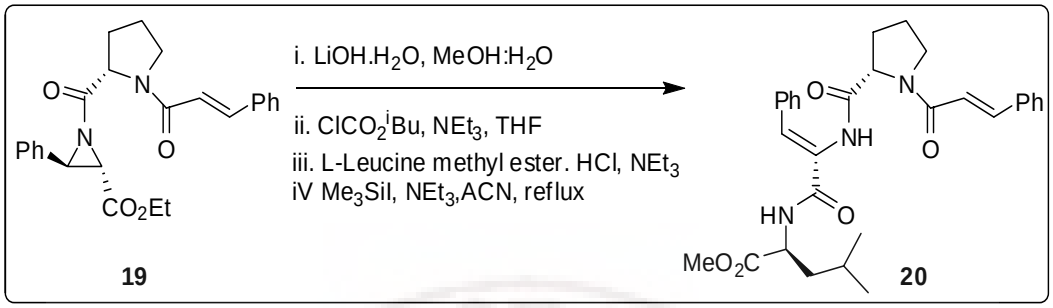
Nakamura *et al* [22] has reported the selective synthesis of *Z*- and *E*-ΔAbu (**18a** and **18b**) from L-Threonine (**14**) and L-allo-threonine (**15**) as starting materials through selenation and oxidative elimination processes with a selenyl linker (scheme-**5**). The usefulness of this linker for solid-phase synthesis of dehydropeptides has been demonstrated.[23]

Scheme-**5**: *Synthetic approach to 'E' and 'Z' ΔAbu derivatives*



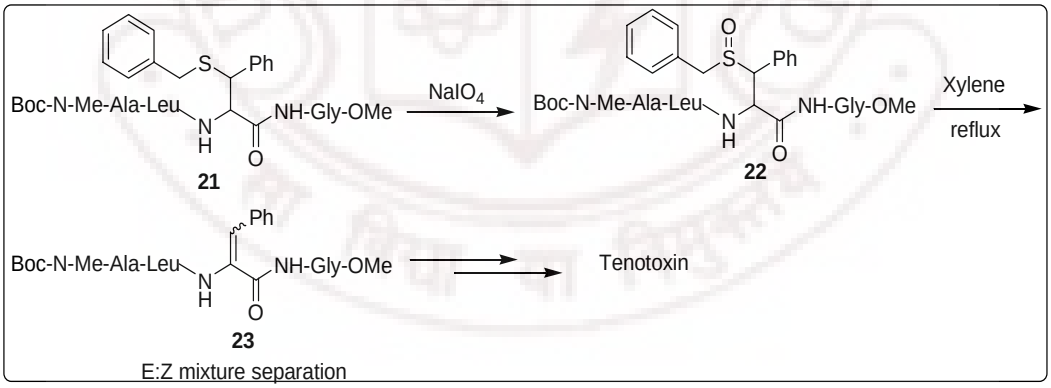
An expedient stereo selective rearrangement of the acyl aziridines to the corresponding Δ^z Phe was developed in our group[24] (scheme-**6**). *N*-cinnamoyl proline was treated with a diastereomeric mixture of 3-phenyl-aziridine-2-carboxylic acid ethyl ester under kinetic resolution conditions to obtain peptide **19** in diastereomerically pure form which was converted to compound-**20** *via* novel rearrangement of acyl aziridine mediated by iodotrimethylsilane in the presence of NEt_3. The geometry of the double bond was assigned as 'Z' based on nOe studies. This methodology was applied for the synthesis of Pro-Δ^z Phe containing cyclic peptides using RCM.

Scheme-**6**: *Synthetic approach to 'Z'-ΔPhe containing peptides*



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

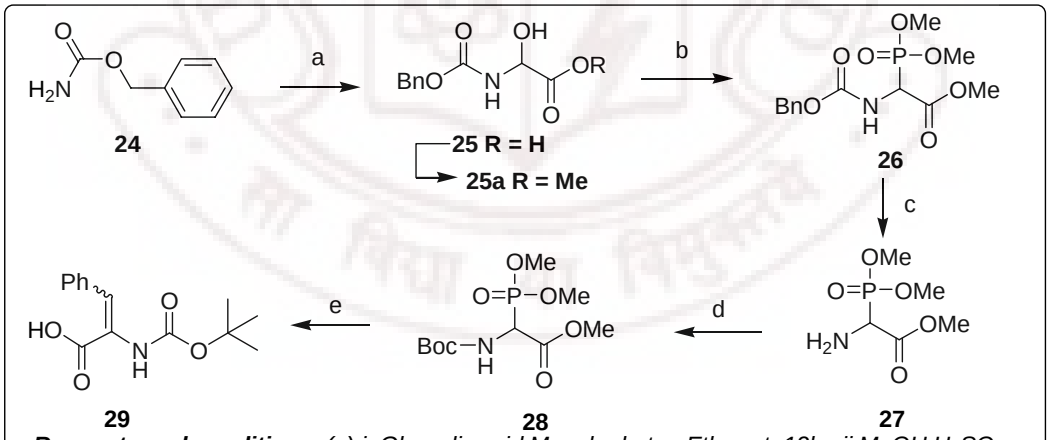
Scheme-**7:** *Brief synthetic approach to Tenotoxin*



4.3 Results and discussion

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

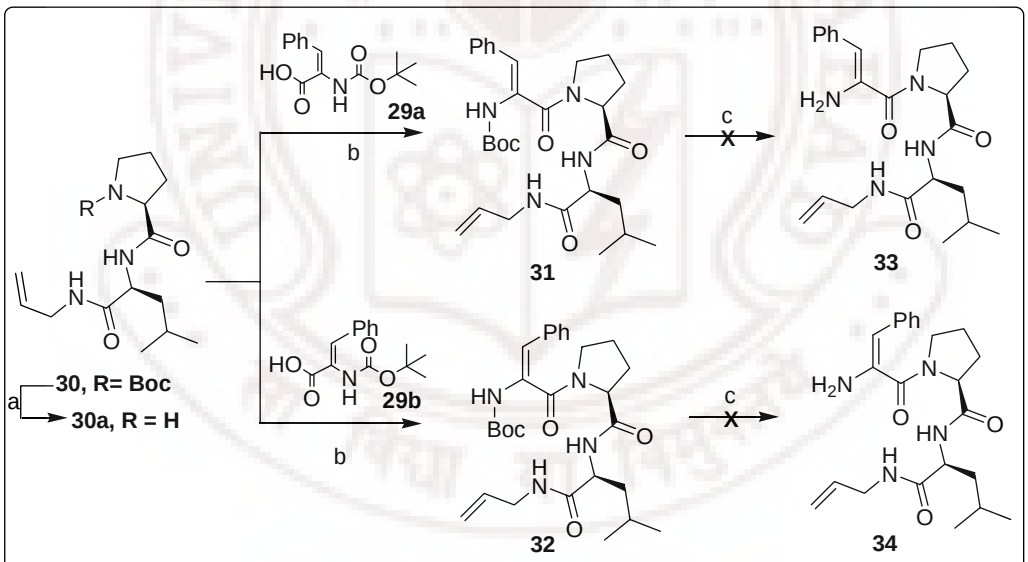
Scheme-**8:** *Preparation of N-Boc-ΔPhe from benzyl carbamate*



***Reagents and conditions**: (a) i. Glyoxylic acid.Monohydrate, Ether, rt, 12h, ii.MeOH,H_2SO_4 61.4 % (b) i. $POCl_3$, Toluene, 70 ^oC, 18 h ii. $P(OEt)_3$, 70 ^oC, 2h , 61 % (c) 10 % Pd/C, MeOH, 40 psi, 4h, rt (d) $(Boc)_2O$, CH_2Cl_2, rt, 12h, 49 % (e) KOtBu, Dry THF, -60 ^oC-rt, 2.5 h, 67 %*

This was achieved by reacting **26** initially with 10 % Pd/C in MeOH and protecting the resultant amine with 'Boc' to yield compound-**28** which was transformed to a mixture of *E:Z* dehydro amino acids (**29)** using Horner-Emmons reaction. The isomers **29a** and **29b** were confirmed based on the ^{1}H NMR values reported in the literature.[26] These residues where then coupled with dipeptide **30a** (obtained by deprotecting 'Boc' group in **30** with TFA/NEt_3) as shown in scheme-**9** using standard mixed anhydride protocol ($ClCO_2{}^{i}Bu/NEt_3$) to yield corresponding tripeptides **31** and **32** respectively.

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

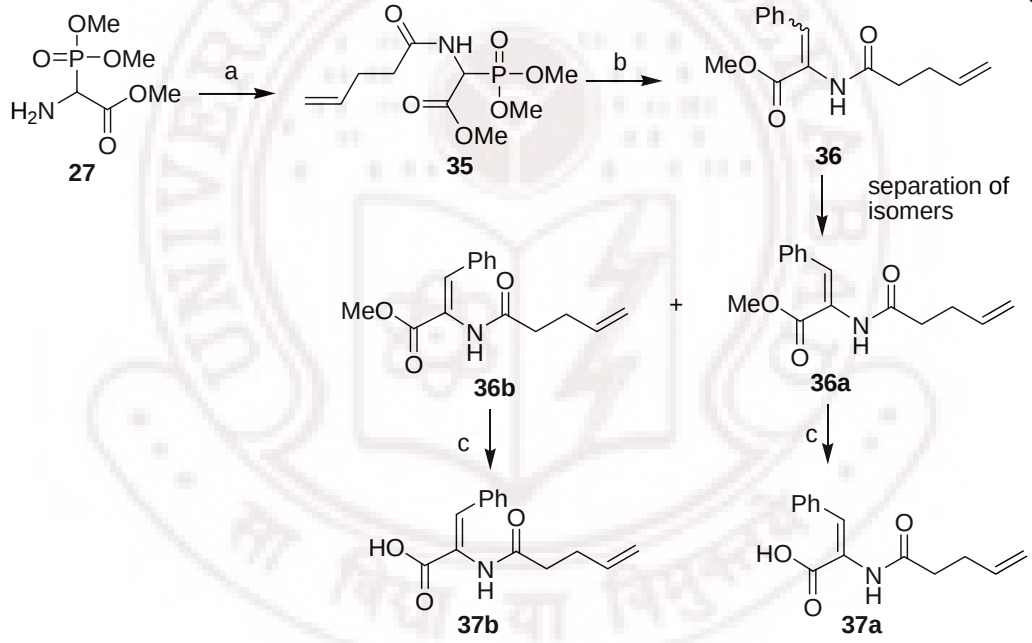


Reagents and conditions: (a) i. TFA, dry CH_2Cl_2, 0 ^{o}C, 3h (b) $ClCO_2{}^{i}Bu$, NEt_3, CH_2Cl_2, 0^{o}C-rt, 12h, 79.1 % for 31 and 66 % for 32 (c) TFA dry CH_2Cl_2, 0 ^{o}C.

At this stage, we attempted to deprotect the 'Boc' in **31** and **32** to install the pentenoyl group required for RCM reaction. However, acid mediated deprotection proved futile, leading to decomposition of the starting material under the reaction

conditions. Alternatively, after unmasking the 'Cbz' the resulting amine **27** was coupled with 4-pentenoic acid using ($ClCO_2{}^iBu/NEt_3$) to yield compound **35** which upon Horner-Emmons reaction with benzaldehyde yielded a mixture of geometrical isomers in good yields. The isomers were separated using column chromatography and hydrolysis using LiOH yielded '*E*' and '*Z*' N-pentenoyl dehydrophenylalanine **37a** and **37b** respectively as shown in scheme-**10**.

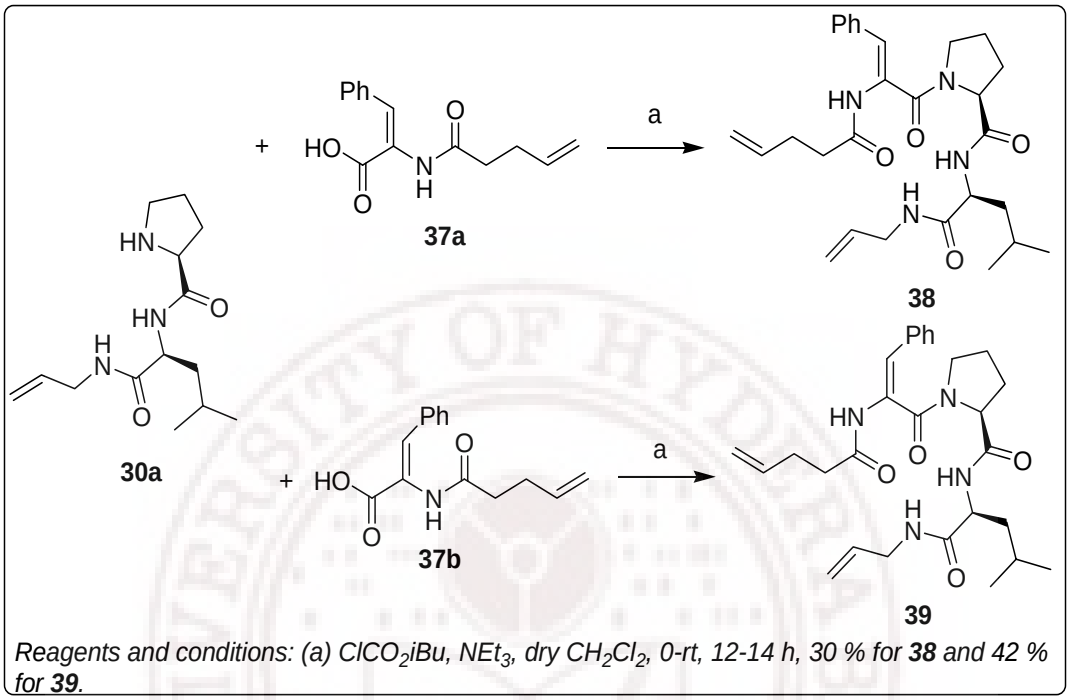
Scheme-**10**: *Preparation of* ***"E"*** and ***'Z'*** *N-pentenoyl dehydrophenylalanine*



Reagents and conditions*: (a) 4-pentenoic acid, $ClCO_2iBu$, NEt_3, CH_2Cl_2, rt, 12h, 40 % (b) KO^tBu, Dry CH_2Cl_2 , -60^oC-rt, 2.5 h, 86 % (c) LiOH, MeOH:H_2O,rt, 4h, 71.4 % for 37a and 66 % for 37b.*

With both geometrical isomers of ΔPhe in hand, these residues were coupled ($ClCO_2{}^iBu/NEt_3$) independently with dipeptide Pro-Leu allylamide (**30a)** to yield corresponding tripeptides in good yields (scheme-**11**).

Scheme-**11**: *Preparation of acyclic tripeptides **38** and **39** from dipeptide **30a***



*Reagents and conditions: (a) $ClCO_2iBu$, NEt_3, dry CH_2Cl_2, 0-rt, 12-14 h, 30 % for **38** and 42 % for **39**.*

Well resolved diagnostic signals in the ^{1}H NMR and molecular ion in the mass spectrum (spectrum **1** and **2**, page# 275, spectrum **3** and **4**, page# 276) confirmed the formation of product **38** and **39** in good yields. Low field appearance of Leu NH and allylic NH in $CDCl_3$ solution suggested their participation in intramolecular H-bonding which was confirmed solvent titration studies. The evaluation of the NOESY spectrum of these peptides reveals a number of critical NOEs. In particular, the NOESY cross peaks Leu NH↔Aha NH, Leu NH↔Pro CδH, and Aha NH↔Pro CαH confirmed the presence of a *trans* imide bond preceding proline residue with a 10-membered hydrogen bonding network

involving Aha NH ← Δ-Phe CO. Another 10-membered H-bonding network was evident between Leu NH ← Pentenoyl C=O indicating a 3_{10} helical conformation with two intra molecular H-bonds (Figure-**2**).

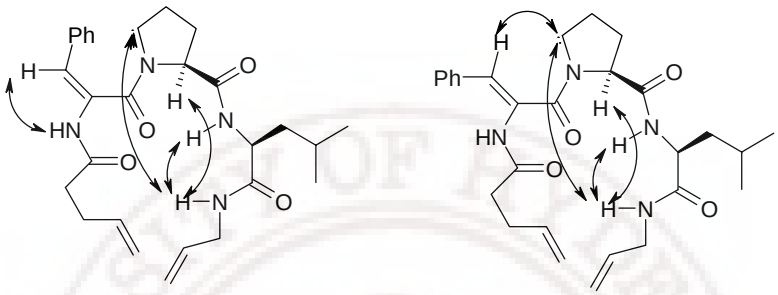
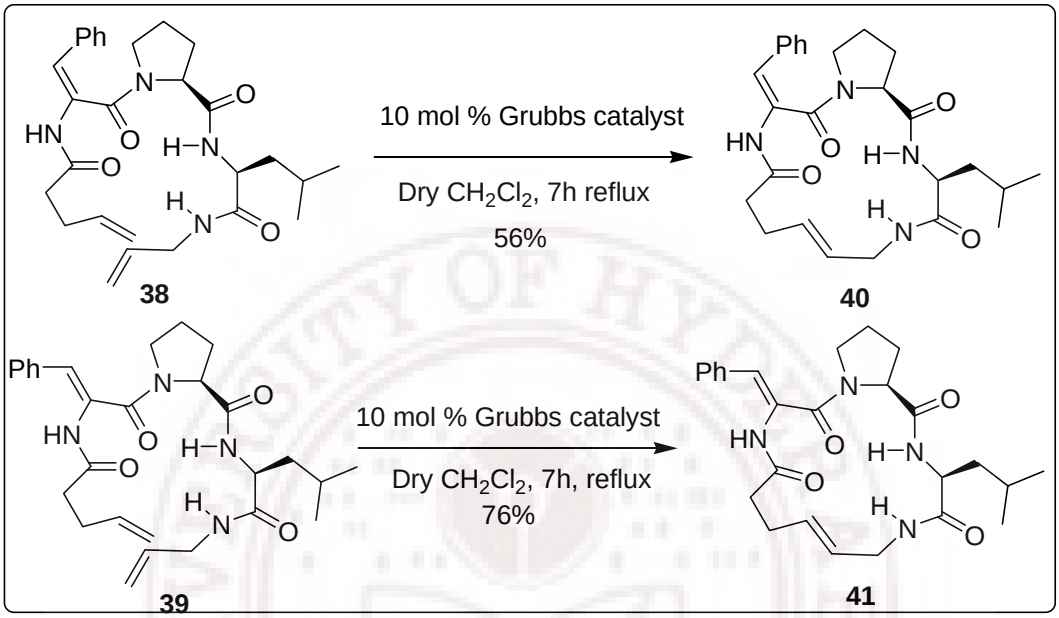


Figure-**2**: *Diagnostic NOEs observed in* **38** *and* **39**

Recent studies from our laboratory have shown that preorganized peptides folded into a 3_{10} helical/ β-turn structures undergo facile RCM reaction. It was gratifying but not surprising to observe peptides **38** and **39** undergo smooth cyclization in a stereospecific manner to afford *E*-isomer exclusively in excellent yields when subjected to ring closing metathesis reaction using 10 mol % of Grubb's first generation catalyst in good yields (scheme-**11**). It is noteworthy that in the process of cyclization a new unnatural ω-amino acid was created and the resulting cyclic peptides now contain 2-natural and 2-unnatural amino acids. Being a cyclic peptide with Δ-Phe-Pro linkage, **40** and **41** can potentially belong to a new class of structural analogues of HIV protease inhibitors.

Scheme-**12**: *Synthesis of cyclic peptides* ***40*** *and* ***41*** *from acyclic tripeptides* ***38*** *and* ***39***



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

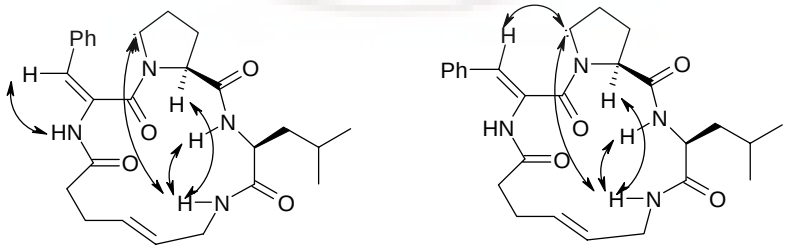


Figure-**3**: *Diagnostic NOEs observed in* **40** *and* **41**

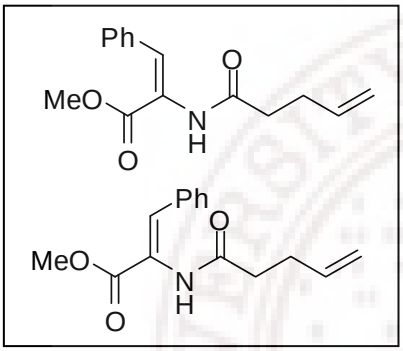
4.4 Conclusion

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

4.5 Experimental

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

To an cold (-15°C) stirred solution **35** (3 g, 9.77 mmol) in dry THF was added NaH (0.58 g, 14. 65 mmol) portion wise over a period of 10 min. After 30 min at

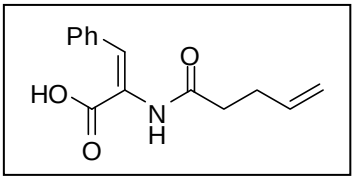


the same temperature was added freshly distilled benzaldehyde in 10 ml of dry THF and stirred at RT for 12h. THF was evaporated completely and the residue was purified using EtOAc-hexane system to afford 1.1 g of 'Z' and 1.1 g of '*E*' isomer in 86 % yield.

IR (Neat): 3287, 2925, 2854, 1731, 1664, 1631 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) of **36a**: 7.96 (s, 1H), 7.53-7.49 (m, 1H), 7.37-7.22 (m, 5H), 5.92-5.82 (m, 1H), 5.15-5.04 (m, 2H), 3.63 (s, 3H), 2.49-2.42 (m, 4H), Mass (CI method): 260 (M+H, 100). IR (Neat): 3267, 2927, 1716 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) of **36b**: 7.44 (bs, 1H), 7.38-7.32 (m, 5H), 6.96 (bs, 1H), 5.85 (bs, 1H), 5.12-5.03 (m, 2H), 3.85 (s, 3H), 2.44 (bs, 4H), Mass (CI method): 260 (M+H, 100).

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

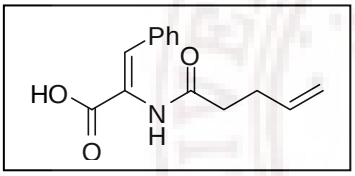
To a stirred solution of compound-**36a** (1g, 3.85 mmol) in ml of MeOH (16 ml) was added an aqueous solution of LiOH (80 mg, 4.22 mmol) in 4 ml of water and stirred for a period of 6h. Methanol was evaporated completely and the residue



was diluted with water. The aqueous layer was cooled and acidified using 2N HCl to yield a white solid which was filtered off and dried (695 mg, 71.4 %).

IR (KBr): 3301, 1670, 1650 cm^{-1}, ^1H NMR(400 MHz, $CDCl_3$) of 25 a: δ 7.53 (s, 1H), 7.51 (bs, 2H), 7.46-7.36 (m, 3H), 7.07 (bs, 1H), 5.85 (m, 1H), 5.13-5.03 (m, 2H), 2.46-2.26 (m, 4H), Mass (CI method): 246 (M+H, 100).

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

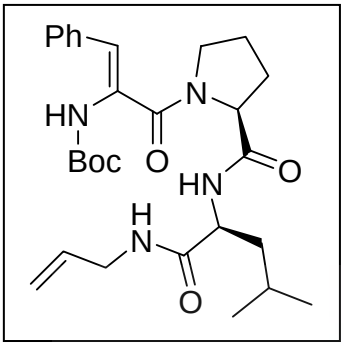


To a stirred solution of compound-**36b** (1.1 g, 4.24 mmol) in 16 ml of MeOH was added a solution of LiOH (196 mg, 4.66 mmol) in 4 ml of water and stirred for a period of 6h. Methanol was evaporated completely and the residue was diluted with water, acidified using 2N HCl to yield a white solid which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

IR (KBr): 3310, 1679, 1640 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) of 25 b: δ 8.15 (s, 1H), 7.57 (bs, 1H), 7.33-7.26 (m, 5H), 5.89-5.82 (m, 1H), 5.13-5.04 (m, 2H), 2.46-2.42 (m, 4H), Mass (CI method): 246 (100).

Preparation of *N*-Boc-Δ^{Z}-Phe-Pro-Leu allylamide (31):

A. To an ice cold stirred solution of compound-**30** (1.0 g, 2.72 mmol) in dry CH_2Cl_2 was added trifluoroacetic acid (TFA) (10 eq) at 0°C under argon



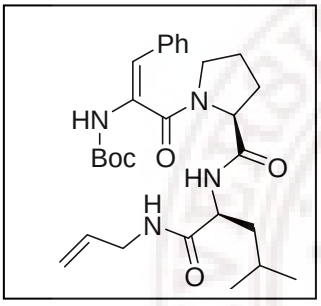
atmosphere and stirred at the same temperature for 3h. Solvent was evaporated to afford the TFA salt as a pale yellow gum, which was neutralized with NEt_3 at 0°C to obtain the free amine.

B. To an ice cold stirred solution of *N*-Boc-Z-Δ-Phe-OH **37b** (716 mg, 2.72 mmol) and HOBt (440 mg, 3.26 mmol) in dry CH_2Cl_2 was added a solution of Pro-Leu allylamide (obtained in part A) (1equivalent) in dry CH_2Cl_2. EDC.HCl (780 mg, 4.08 mmol) was added portion wise to the reaction mixture at 0°C and then stirred at room temperature for a period of 12 h. The reaction mixture was diluted with CH_2Cl_2 and washed with water, brine, dried over Na_2SO_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 (1.10 g, 79.13 %).

IR (KBr): 3320, 2954, 1698, 1665, 1659, 1612 cm^{-1}, (400 MHz, $CDCl_3$): δ7.57 (d, *J* = 9.1 Hz, 1H), 7.45-7.41 (m, 2H), 7.35-7.26 (m, 4H), 6.66 (s, 1H), 6.10 (s, 1H), 5.87-5.78 (m, 1H), 5.21-5.02 (m, 2H), 4.62-4.56 (m, 2H), 3.94-3.87 (m, 1H), 3.78-3.66 (m, 3H), 2.38-2.31 (m, 1H), 2.22-2.15 (m, 1H), 2.03-1.94 (m, 2H), 1.73-1.66 (m, 3H), 1.45 (s, 9H), 0.95 (d, *J* = 6.4 Hz, 3H), 0.92 (d, *J* = 6.3 Hz, 3H), Mass (CI method): 513 ($(M+H)^+$, 100).

Preparation of *N*-Boc-Δ^{E}Phe-Pro-Leu allylamide (32):

A. To an ice cold stirred solution of compound-**30** (300 mg, 0.81 mmol) in dry $CH_2Cl_2$was added trifluoroacetic acid (10 eq) at 0 °C under argon atmosphere and stirred at the same temperature for 3h. Solvent was evaporated to afford the TFA salt as a pale yellow gum, which was neutralized with NEt_3 at 0 °C to obtain the free amine.



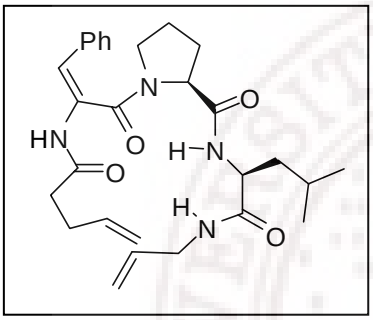
B. To an ice cold stirred solution of *N*-Boc-*E*-Δ-Phe-OH **37a** and HOBt (125 mg, 0.81 mmol) in dry CH_2Cl_2 was added a solution of Pro-Leu allylamide (obtained in part A) (1equivalent) in dry CH_2Cl_2. EDC.HCl (200 mg, 1.017 mmol) was added portion wise to the reaction mixture at 0°C and then stirred at room temperature for a period of 12 h. The reaction mixture was diluted with CH_2Cl_2 and washed with water, brine. The organic layer was dried over sodium sulfate and evaporated to afford crude product which was purified using 100-200 mesh silica and MeOH-$CHCl_3$ as the eluent to afford the title compound (139 mg, 42 %).

IR (KBr): 3330, 2967, 1667, 1520 cm^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 (d, *J* = 8.1 Hz, 1H), 7.43-7.21 (m, 6H), 6.65 (s, 1H), 6.17 (s, 1H), 5.91-5.81 (m, 1H), 5.24-5.07 (m, 2H), 4.59-4.53 (m, 1H), 4.38-4.35 (m, 1H), 3.94-3.85 (m, 1H), 3.80-3.73 (m, 1H), 3.60-3.56 (m, 1H), 2.89-2.82 (m, 1H), 2.08-2.0 (m, 1H), 1.99-

1.93 (m, 1H), 1.79-1.70 (m, 5H), 1.40 (s, 9H), 0.88 (m, 6H), Mass (CI method): 513 ($(M+H)^+$, 100).

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

A. To an ice cold stirred solution of compound-**30** (300 mg, 0.817 mmol) in dry $CH_2Cl_2$was added trifluoroacetic acid (10 eq) at 0 °C under argon atmosphere and



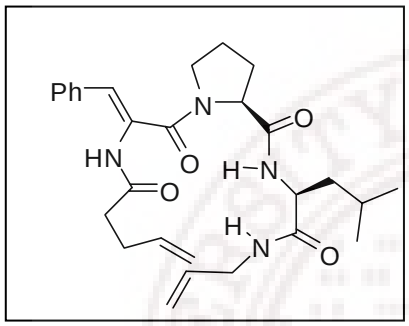
stirred at the same temperature for 3h. Solvent was evaporated to afford the TFA salt as a pale yellow gum, which was neutralized with NEt_3 at 0°C to obtain the free amine (181 mg) in 83 % yield.

B. To an ice cold stirred solution of *N*-Pentenoyl-Δ^E-Phe-OH (**37a**) (181 mg, 0.677 mmol) and HOBt (125 mg, 0.813 mmol) in dry CH_2Cl_2 was added a solution of Pro-Leu allylamide (obtained in part A) (1equivalent) in dry CH_2Cl_2. EDC.HCl (200 mg, 1.017 mmol) was added portion wise to the reaction mixture at 0 °C and then stirred at room temperature for a period of 12h. The reaction mixture was diluted with CH_2Cl_2 and washed with water, brine, dried over Na_2SO_4 and evaporated to afford crude product which was purified using 100-200 mesh silica gel and MeOH-$CHCl_3$ as the eluent to afford the title compound (140 mg, 42 %) as a hygroscopic solid.

IR (KBr):3315, 2956, 1651, 1520 cm^{-1}, (400 MHz, CDCl3): δ 7.66 (d, *J* = 8.6 Hz, 1H), 7.44-7.33 (m, 6H), 7.15 (bt, 1H), 6.21 (s, 1H), 5.84-5.77 (m, 2H), 5.18-5.01

(m, 4H), 4.58-4.49 (m, 2H), 3.86-3.84 (m, 3H), 3.82-3.81 (m, 1H), 2.41-2.36 (m, 6H), 1.89-1.86 (m, 5H), 0.97 (d, J = 6.8 Hz, 3H), 0.92 (d, J = 6.7 Hz, 3H), Mass (CI method): 495 ($(M+H)^+$, 100).

Preparation of *N*-Pentenoyl-Δ^z-Phe-Pro-Leu-allylamine (39): 413

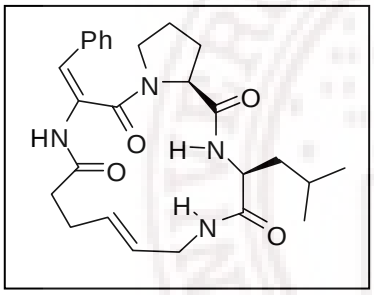


A. To an ice cold stirred solution of compound-**30** (250 mg, 0.68mmol) in dry CH_2Cl_2 was added trifluoroacetic acid (10 eq) at 0°C under argon atmosphere and stirred at the same temperature for 3h. Solvent was evaporated to afford the TFA salt as a pale yellow gum, which was neutralized with NEt_3 at 0°C to obtain the free amine in quantitative yield.

B. To an ice cold stirred solution of *N*-Pentenoyl-Δ^Z Phe-OH (166 mg, 0.677 mmol) (**37b)** and HOBt (125 mg, 0.81 mmol) in dry CH_2Cl_2 was added a solution of Pro-Leu allylamide (obtained in part A) (1eq) in dry CH_2Cl_2. EDC.HCl (200 mg, 1.01 mmol) was added portion wise to the reaction mixture at 0 °C and then stirred at room temperature for a period of 12h. The reaction mixture was diluted with CH_2Cl_2 and washed with water, brine. The organic layer was dried over sodium sulfate and evaporated to afford crude product which was purified using 100-200 mesh silica and MeOH-$CHCl_3$ as the eluent to afford the title compound (100 mg, 30 %) yield.

IR (KBr): 3319, 2960, 1659, 1520 cm^{-1}, ^1H NMR(400 MHz, $CDCl_3$): δ7.68 (d, *J* = 8.8 Hz, 1H), 7.52 (s, 1H), 7.45-7.27 (m, 5H), 7.20 (t, *J* = 5.3 Hz, 1H), 6.22 (s, 1H), 5.86-5.77 (m, 2H), 5.18-5.00 (m, 4H), 4.57-4.54 (m, 1H), 4.51-4.45 (m, 1H), 3.88-3.71 (m, 3H), 3.69-3.62 (m, 1H), 2.44-2.24 (m, 6H), 1.98-1.76 (m, 5H), 0.98 (d, *J* = 6.3 Hz, 3H), 0.86 (d, *J* = 6.3 Hz, 3H), Mass (CI method): 495 ($(M+H)^+$, 100)

Preparation of cyclo(Δ^{E}-Phe-Pro-Leu-aha) (40):

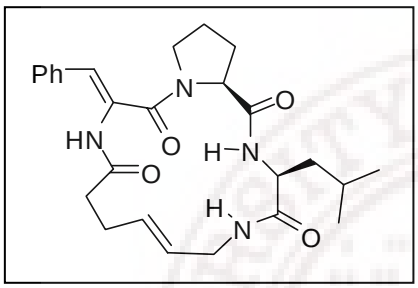


To a stirred solution of Grubb's ruthenium catalyst (10 mol%) in dry CH_2Cl_2 (in high dilution) under nitrogen was added a solution of compound-**38** (132 mg, 0.26 mmol) slowly over a period of 10 min and the mixture refluxed for 7 h. The reaction was exposed to air and purified by column using 100-200 mesh silica gel and EtOAc:hexane as eluent to afford the title product (70 mg, 56 %) yield as a brown solid.

IR (Neat): 3329, 2956, 1651, 1518 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): δ 7.44-7.41 (m, 4H), 7.36-7.32 (m, 2H), 7.15 (d, *J* = 9.7 Hz, 1H), 6.98 (d, *J* = 9.4 Hz, 1H), 6.15 (s, 1H), 5.85-5.78 (m, 1H), 5.67-5.60 (m, 1H), 4.66-4.60 (m, 1H), 4.55-4.53 (m, 1H), 4.33-4.26 (m, 1H), 4.01-3.96 (m, 1H), 3.71-3.64 (m, 1H), 3.10-3.06 (m, 1H), 2.55-2.52 (m, 1H), 2.43-2.39 (m, 1H), 2.30-2.23 (m, 4H), 2.05-1.91 (m, 3H), 1.68-1.56 (m, 2H), 0.98-0.95 (m, 6H), ^{13}CNMR (100 MHz, $CDCl_3$): δ 172.1,

171.2, 166.9, 133.1, 131.4, 130.2, 129.5, 129.1, 128.6, 128.4, 119.12, 61.4, 51.22, 50.37, 39.46, 38.65, 37.08, 30.2, 28.7, 25.3, 24.3, 23.5, 20.8, Mass (CI method): 467 ($(M+H)^+$ 100)

Preparation of cyclo(Δ^z-Phe-Pro-Leu-Aha)(41): 426



To a stirred solution of Grubb's ruthenium catalyst (10 mol%) in dry CH_2Cl_2 (in high dilution) under nitrogen was added a solution of compound **39** (200 mg, 0.40 mmol) slowly over a period of 10 min and the mixture refluxed for 7 h while monitoring the reaction by TLC. The reaction was exposed to air and directly subjected to column chromatography (silica gel, EtOAc:hexane) to afford the product (*E-isomer)* (143 mg, 76 %) yield.

IR (Neat): 3330, 2956, 1651, 1626, 1518 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): δ 7.45-7.41 (m, 2H), 7.39-7.30 (m, 3H), 7.27 (s, 1H), 7.14 (d, *J* = 9.7 Hz, 1H), 6.98 (d, *J* = 9.4 Hz, 1H), 6.16 (s, 1H), 5.86-5.80 (m, 1H), 5.68-5.60 (m, 1H), 4.68-4.62 (m, 1H), 4.56-4.54 (m, 1H), 4.35-4.27 (m, 1H), 4.01-3.99 (m, 1H), 3.72-3.65 (m, 1H), 3.12-3.07 (m, 1H), 2.54-2.29 (m, 2H), 2.22-2.04 (m, 4H), 2.03-1.91 (m, 2H), 1.68-1.57 (m, 3H), 0.97 (d, *J* = 6.6 Hz, 3H), 0.92 (d, *J* = 6.5 Hz, 3H), ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.8 171.2, 171.1, 166.7, 133.1, 131.1, 130.5, 129.6,

129.2, 128.6, 128.4, 118.8, 61.5, 51.2, 50.3, 39.5, 38.6, 37.1, 30.3, 28.8, 25.4, 24.3, 23.5, 20.9, Mass (CI method): 467 ($(M+H)^+$, 100).

4.6 References

1. Gross, E.; Morell, J. L. *J. Am. Chem. Soc.* **1967**, *89*, 2791. (b) Gross, E.; Morell, J. L. *FEBS Lett* **1968**, *2*, 61–64. (c) Gross, E.; Morell, J. L.; Craig, G. T. *Proc. Natl. Acad. Sci.USA.* **1969**, *62*, 952. (d) Aydin, M.; Lucht, N.; Koenig, W. A.; Lupp, R.; Jung, G.; Winkelmann, G. *Liebigs Ann Chem* **1985**, 2285.(e) Allagier, H.; Jung, G.; Werner, R. G.; Schneider, U.; Zahner, H. Eur J Biochem **1986**, *160*, 9.
2. Noda, K.; Shimohigashi, Y.; Izumiya, N. *The Peptides*, 5th ed.; Academic Press: New York, **1983**; pp 285.
3. Jung, G. *Angew Chem Int Ed Engl* **1991**, *30*, 1051.
4. Breukink, E.; Viedemann, I.; Karaaij, C. V.; Kuipers, O. P.; Shal, H. G.; de Kruijff, B. *Science* **1999**, *286*, 2361.
5. Komatsubara, S.; Kisumi, M.; Chibata, I.; Gregario, M. M. V.; Miller, V. S.; Crout, D. H. G. *J Chem Soc Chem Commun* **1977**, 839.
6. Stammer, C. H. *Chemistry and Biochemistry of Amino Acid, Peptides and Proteins, Vol. VI*; Weinstein, B., Ed.; Marcel Dekker: New York, **1982**; 33.
7. Tsutsui, M.; Ichikawa, M.; Vohwinkel, F.; Susuki, K. *J. Am. Chem. Soc.* **1966**, *88*, 852.
8. Dossena, A.; Marcelli, R.; Pochini, J. *J Chem Soc Chem Commun* **1974**, 771.
9. Meyer, W. L.; Templeton, G. E.; Grable, C. I.; Siegel, C. W.; Jones, R.; Woodhead, S. H.; Sauer, C. *Tet. Lett.* **1971**, *12*, 2357.

10. Langer, M.; Pauling, A.; Retey, J. *Angew Chem Int Ed Engl* **1995**, *34*, 1464.

11. English, M. L.; Stammer, C. H. *Biochem Biophys Res Comm* **1978**, *83*, 1464.

12. Joshi, R. M.; Chauhan, C. H. *Synthesis of Peptides and Peptidomimetics*, E22, Hoben-Weyl, **2003**.

13. Banerji, B.; Mallesham, B.; Kiran Kumar, S.; Kunwar, A. C. ; Javed Iqbal *Tet. Lett.* **2002,** *43,* 6479.

14. Flexner, C. *The New England Journal of Medicine*, **1998**, *338*, 1281.

15. Sastry, T. V. R. S.; Biswadip Banerji, B.; Kiran Kumar, S.; Kunwar, A. C.; Das, J.; Nandy, J. P.; Javed Iqbal *Tet. Lett.* **2002**, *43,* 7621.

16. (a) Herbst, R. M.; Shemin, D. Organic Syntheses **1943**, Coll. Vol. II, 1. (b) Vogel, A. I.Practical Organic Chemistry; Longmans, 3rd Ed., 1956, 907.

17. Schmidt, U.; Lieberknecht, A.; Schanbacher, U.; Beuttler, T.; Wild, J. *Angew. Chem., Int. Ed.* **1982**, *10*, 776.

18. (a) Miossec, B.; Danion-Bougot, R.; Danion, D. *Synthesis-Stuttgart* **1994**, *11*, 1171. (b) Burk, M. J.; Allen, J. G.; Kiesman, W. F.; Stoffan, K. M. *Tetrahedron Lett.* **1997**, *38*, 1309.

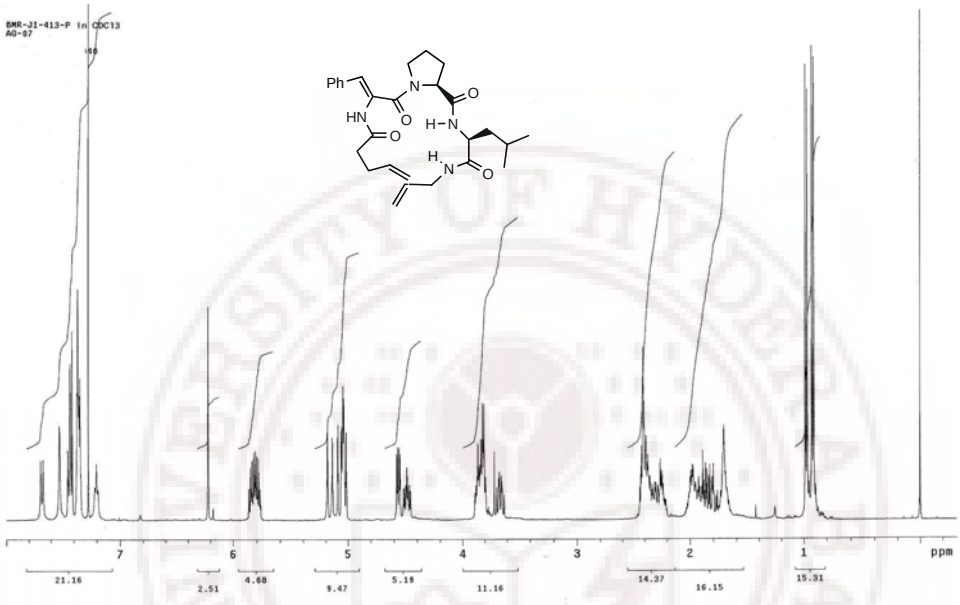
19. (a) Noda K, Shimohigashi Y, Izumiya N, *The Peptides, Analysis, Synthesis, Biology, Vol. 5, New York: Academic Press,* **1983**, 286. (b) Schmidt U, Lieberknecht A, Wild J, *Synthesis* **1988**, 159. (c) Berti F, Ebert C, Gardossi L, *Tetrahedron Lett*. **1992**, *33*, 8145. (d) Balsamini A, Duranti E, Mariani L, Salvatori A, Spadoni G, *Synthesis* **1990**, 779. (e) Ranganathan D, Shah K. Vaish

N, *J. Chem. Soc., Chem. Commun*. **1992**, 1145. (f) Goodall K, Parsons AF, *Tetrahedron Lett*. **1995**, *36,* 3259.

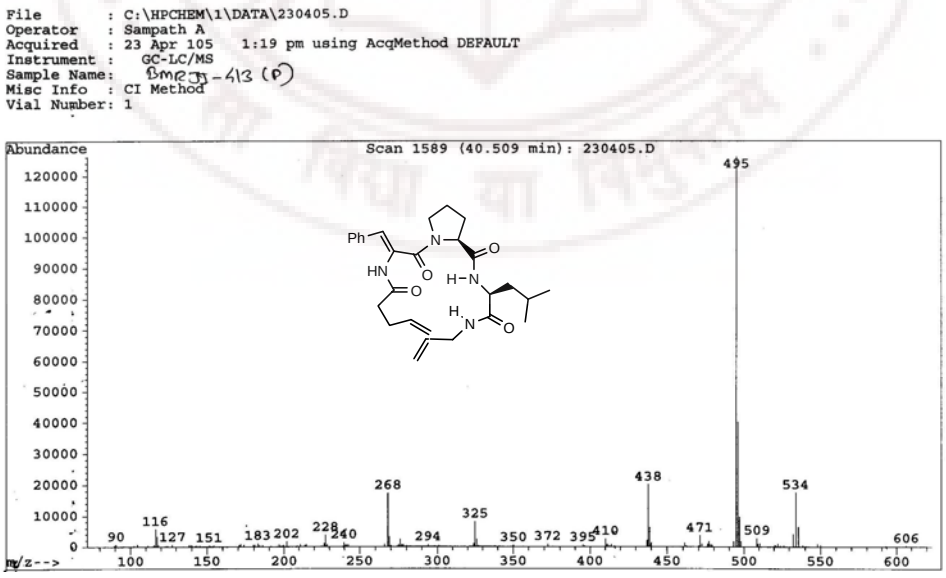
20. Ramesh, R.; Kavita, De.; Chandrasekaran, S. *Tetrahedron Lett*. **2007**, *63,* 10534.
21. Chen, D.; Guo, L.; Liu, J.; Kirtane, S.; Cannon, J. F.; Li, G. *Org. Lett*, **2005**, *7*, 921.
22. Nakamura, K.; Isaka, K.; Toshima, K.; Kodaka, M. *Tetrahedron Lett*. **2004**, *45,* 7221.
23. Nakamura, K.; Ohnishi, Y.; Horikawa, E.; Konakahara, T.; Kodaka, M.; Okuno, H. Tetrahedron Lett. **2003**, *44*, 5445.
24. Prabhakaran, E. N.; Nandy, J. P.; Shukla, S.; Tewari, A.; Das S. K.; Iqbal, J. *Tet. Lett.* **2002**, *43,* 6461.
25. Rich, D. H.; Mathiaparanam, P. *Tett. Lett.* **2002**, 43, 6473.
26. Schmidt, U.; Griesser, H.; Leitenberger, V.; Lieberknecht, A.; Mangold, R.; Meyer, R.; Riedl, B. *Synthesis*, **1992**, 487.
27. Arbuzov, B. A. *Pure Appl. Chem.* **1964**, *9*, 307-353.

4.7 Spectral Data

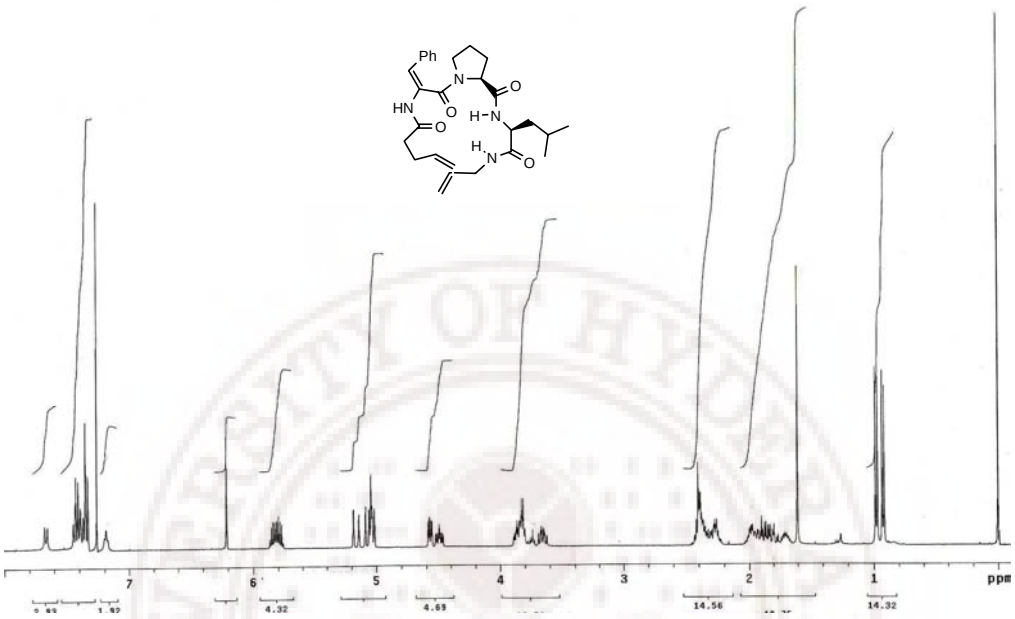
Spectrum 1: ^{1}H NMR spectrum of peptide 38 in $CDCl_3$



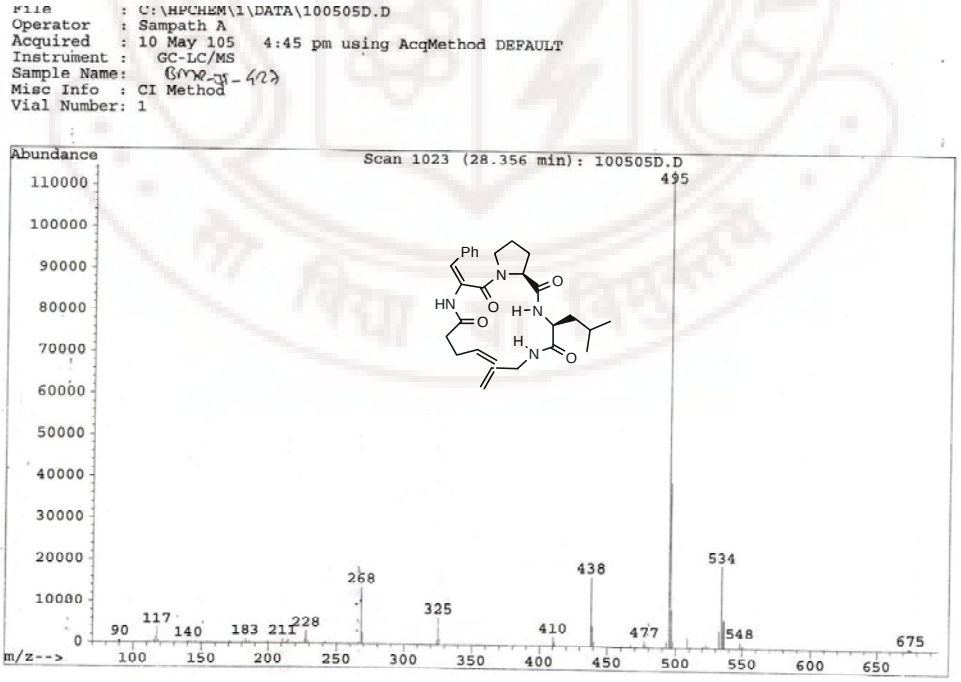
Spectrum 2: Mass spectrum of peptide 38



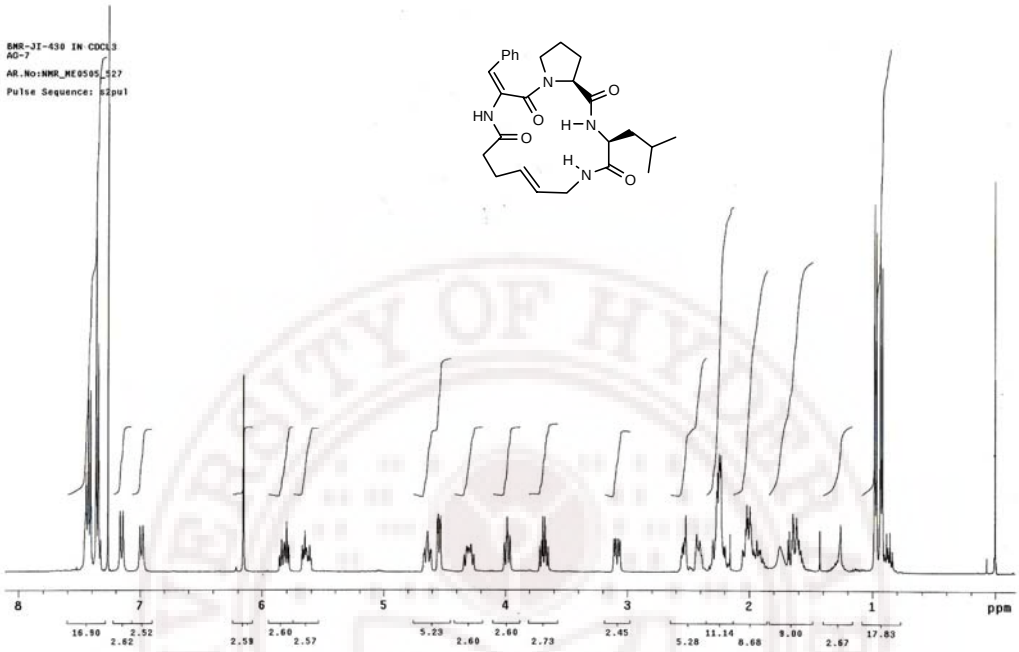
Spectrum 3: ^{1}H NMR of peptide in 39 $CDCl_3$



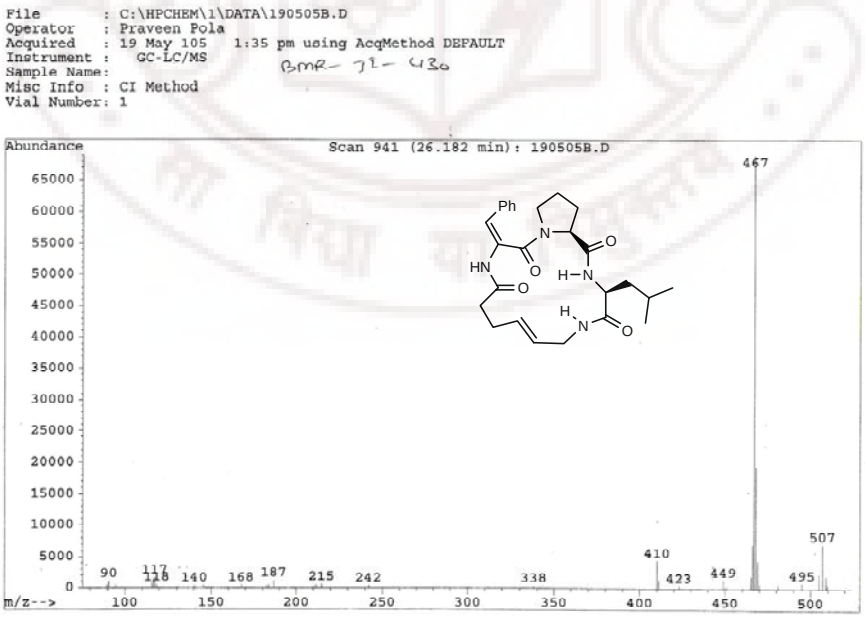
Spectrum 4: Mass spectrum of peptide 39



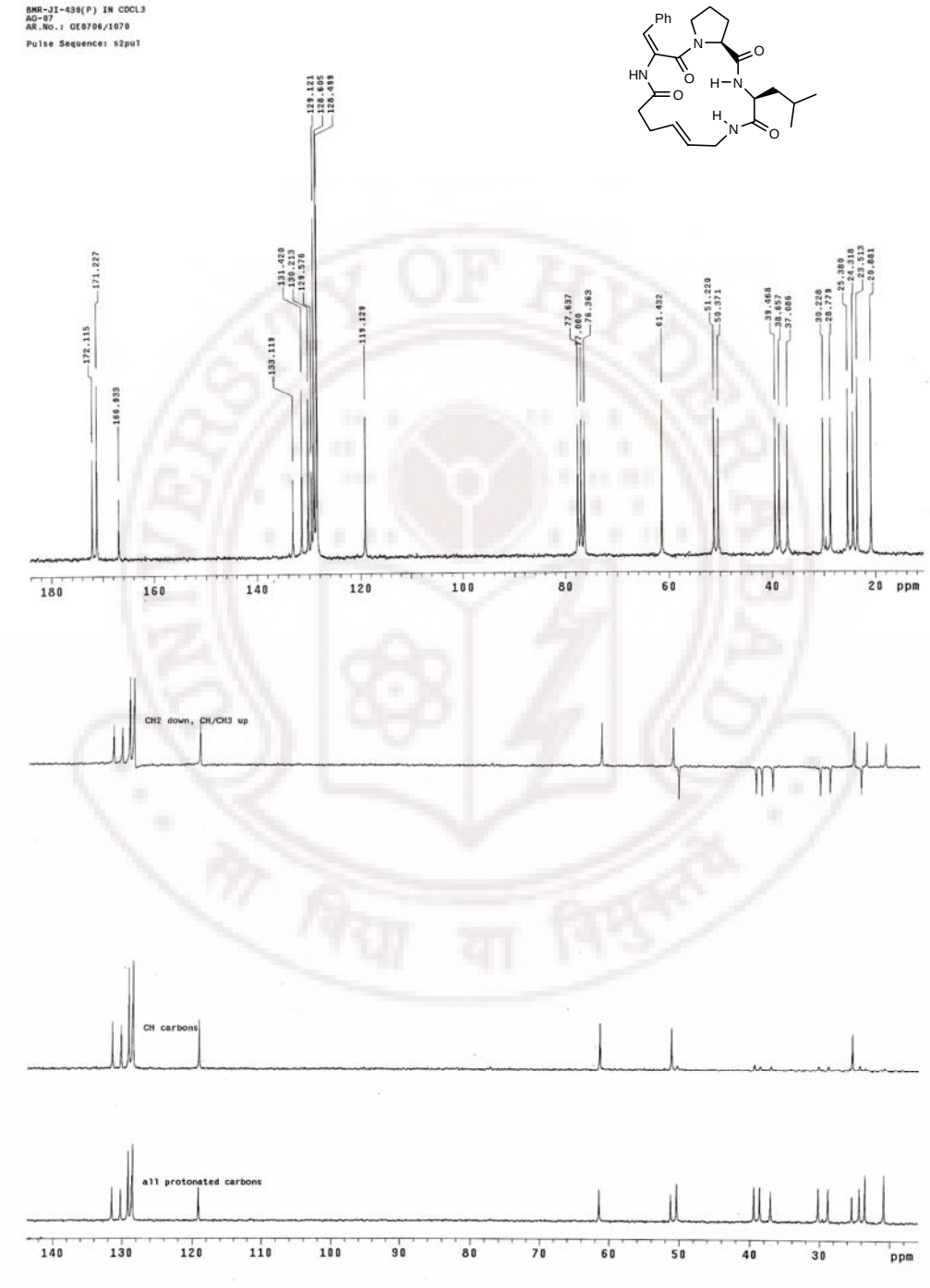
Spectrum 5: ^{1}H NMR spectrum of cyclic peptide 40 in $CDCl_3$



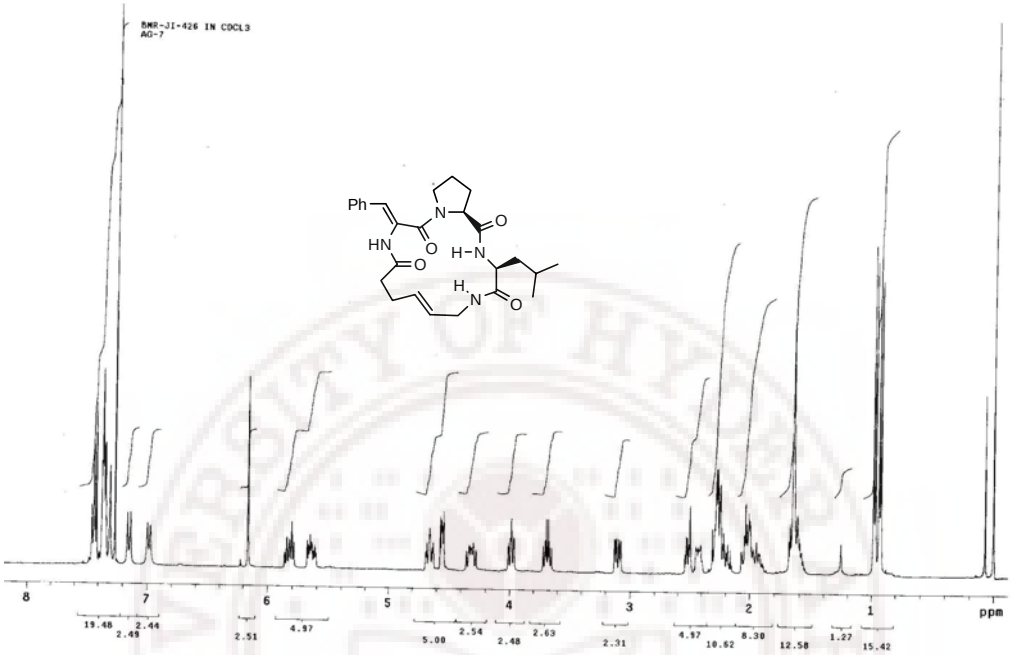
Spectrum 6: Mass spectrum of cyclic peptide 40



Spectrum 7: ^{13}C NMR spectrum of cyclic peptide 40 in $CDCl_3$

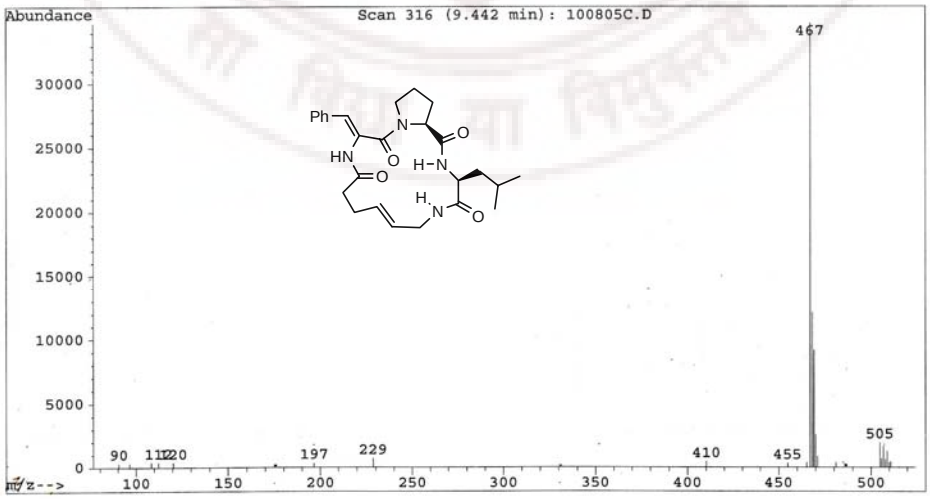


Spectrum 8: ^{1}H NMR spectrum of cyclic peptide 41 in $CDCl_3$

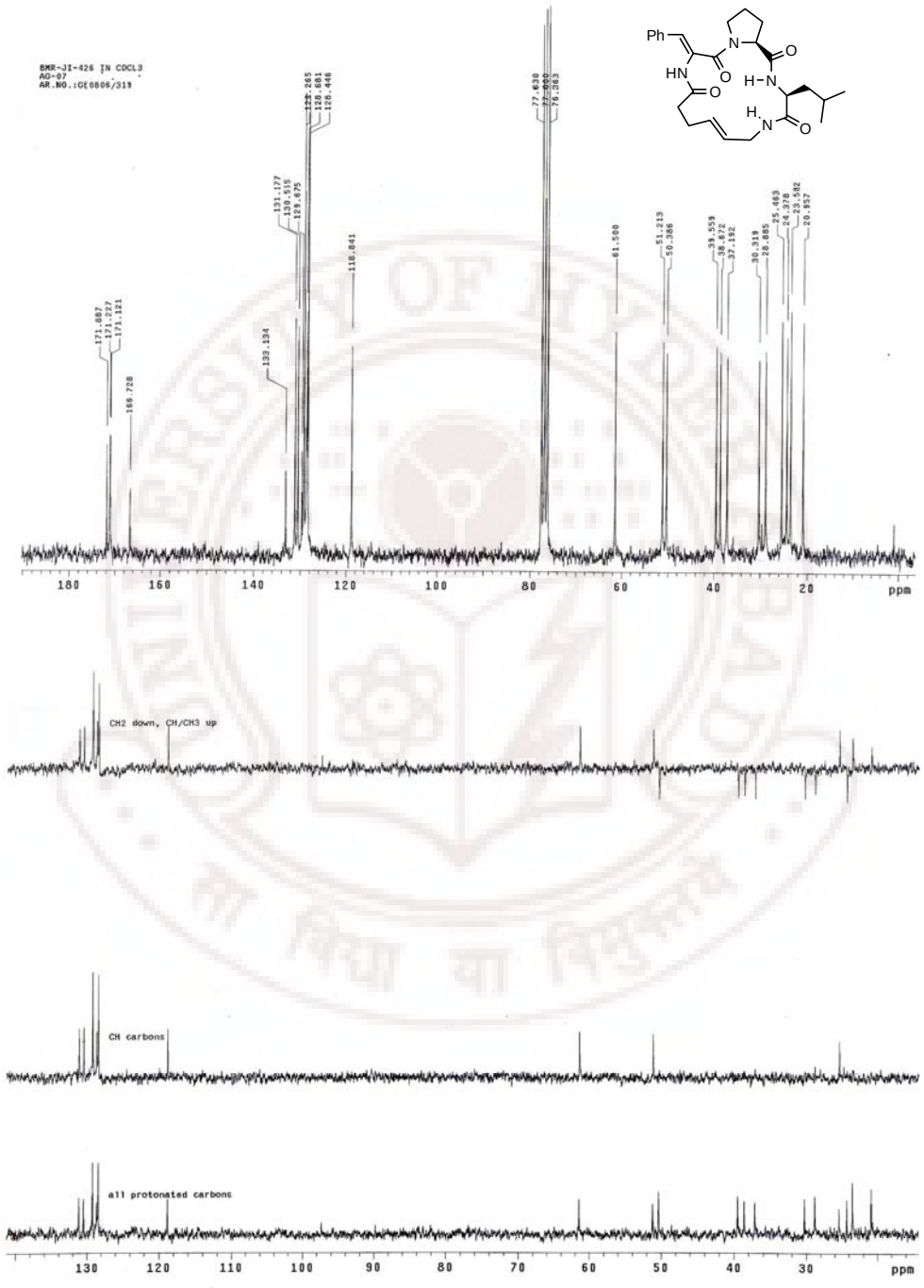


Spectrum 9: Mass spectrum of cyclic peptide 41

File : C:\HPCHEM\1\DATA\100805C.D MSa805035
Operator : Sampath A
Acquired : 10 Aug 105 4:36 pm using AcqMethod DEFAULT
Instrument : GC-LC/MS
Sample Name: BME-JI-126,
Misc Info : CI Method
Vial Number: 1



Spectrum 10: ^{13}C NMR spectrum of cyclic peptide 41 in $CDCl_3$



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.[1] 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, [2] interactions between aromatic side-chains.[3]

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,[4] 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 ($d_{C\text{-}A}$) < 3.5 A° and the H-bond angle between C-H---A ($\angle_{C\text{-}H\text{-}A}$) is <120° (Figure **1**), naming them as CH···π hydrogen bonds.[5] 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.[5]

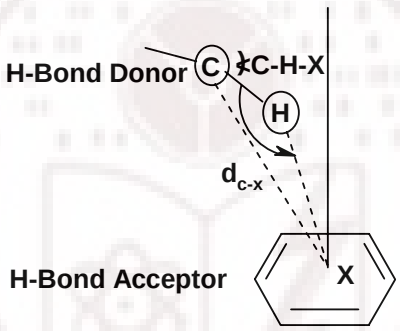


Figure-**1**: *Schematic representation of CH···π H-bonding*

A plot of the occurrence of C-H···π-interactions as a function of Δ_{D-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.[6] 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.[7] 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.[8] 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,[9] pyridoxal-50-phosphate,[10] nucleotides,[11] carbohydrates,[12] bound peptides[13] and in the design of serine protease inhibitors[14] An excellent example of a structure displaying a number of (15) CH···π interactions is γB-eye-lens-crystallin.[15] 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.[16]

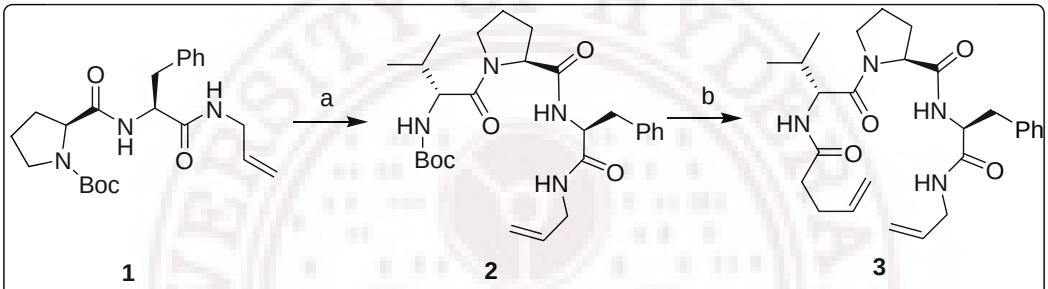
5.2 Present Study

Proline-rich regions of proteins, occur widely in both prokaryotes and eukaryotes and have been implicated in binding in a functionally important way.[17] Chakrabarthi *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.[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[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[20] in model peptides, we instigated our study with the synthesis of acyclic tripeptide Pent-^{D}Val-Pro-Phe-allylamide according to scheme-**1**.

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



Reagents and conditions: (a) i. TFA, CH_2Cl_2, ii. N-Boc-D-Val, EDC, HOBt, CH_2Cl_2, *0 ^{o}C-rt, 12h, 59 %* (b) *i. TFA, CH_2Cl_2, 4-pentenoic acid, $ClCO_2{}^{i}Bu$, NEt_3, CH_2Cl_2, 0 ^{o}C-RT, 47 %*

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 ($ClCO_2{}^{i}Bu/NEt_3$) to yield acyclic tripeptide **3.** The conformation of the peptide showed the presence of a 3_{10} helical geometry based on the down field appearance of Phe NH (7.2 ppm) and Allylic NH (6.9 ppm) in the ^{1}H NMR spectrum and diagnostic NOEs (Figure-**2**) (this

result is in consonance with our previous results[20] that heterocyclic peptide with D-amino acid at *N*-terminal to Pro nucleates a 3_{10} helical geometry).

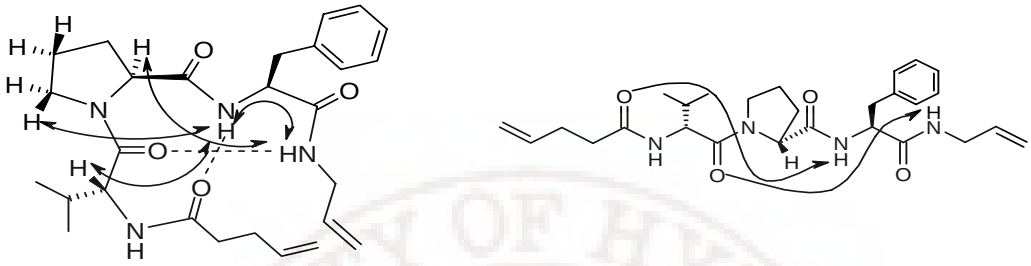


Figure-**2**: *Long-range nOe correlations and 10 membered H-bonding pattern that support for 3_{10} helical structure of peptide **3**.*

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[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.

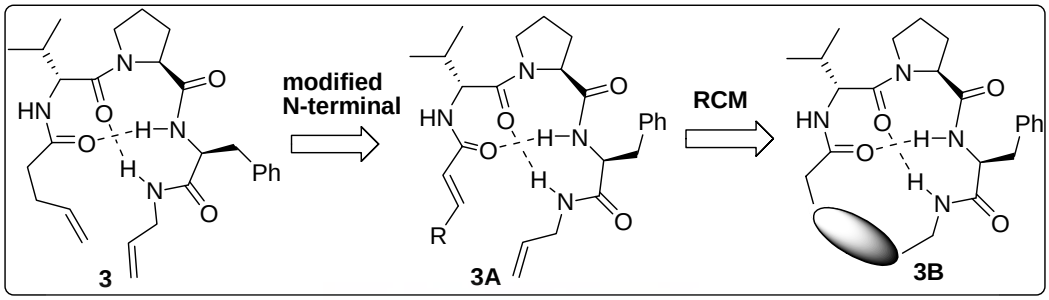
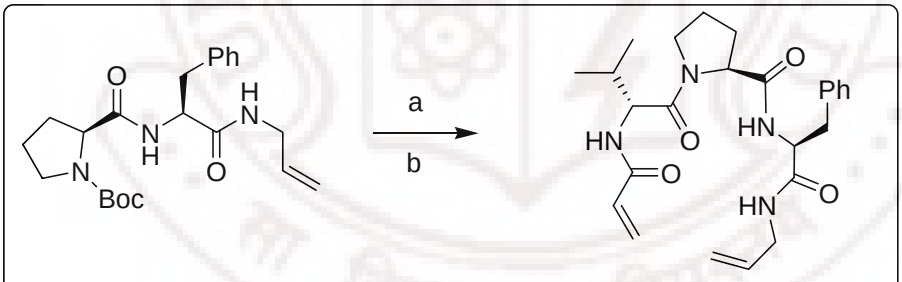


Figure-**3**: *Schematic representation of modified acyclic peptide* **3A**

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**



Reagents and conditions: (a) i. TFA, CH_2Cl_2*, ii. N-crotonyl -D-Val,* NEt_3*,* CH_2Cl_2*, 0* ^{o}*C-rt, 47 % (c) i. TFA,* CH_2Cl_2*, ii. N-acrolyl-D-Val,* NEt_3*,* CH_2Cl_2*, 0 C-rt, 41 %*

These peptides yielded well-resolved ^{1}H NMR spectra in $CDCl_3$ and $DMSOd_6$. 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, $CDCl_3$. The solvent titration curves and temperature coefficients of NH chemical shifts in DMSO-d_6 are shown in Figure-**4**.

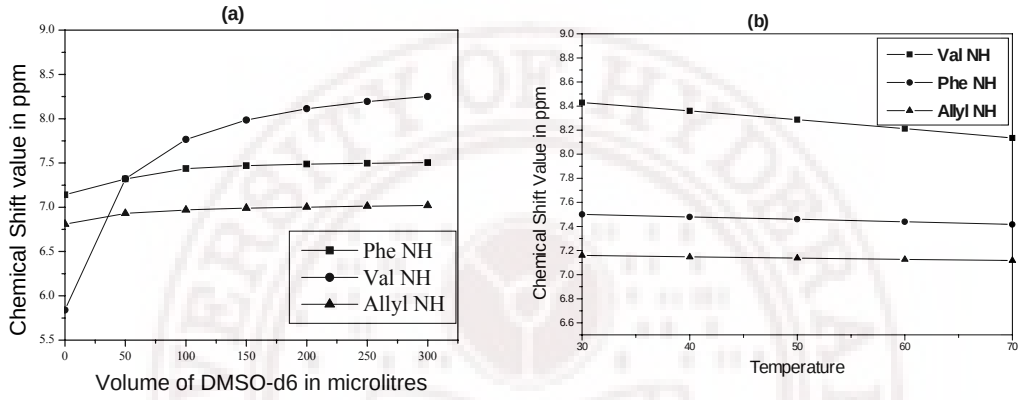


Figure-**4**: *(a) Solvent titration plot of peptide **4** in $CDCl_3$ (b) and plot of variable temperature experiment study in DMSO-d_6.*

Conformation of peptide 4:

In $CDCl_3$ 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 intra molecular 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-d_6 was added in $CDCl_3$ solution) during solvent titration study (Figure **4**). In addition, the observation of cross correlations between Phe NH↔Allyl NH, Phe NH↔^{D}Val CαH, Phe NH↔Pro Cδ (Pro-S)H, Allyl NH↔Pro CαH (Figure **5**) suggested the

existence of two successive β-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 Cγ(Pro-S)H at 1.27 ppm and the NOEs 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 eminent presence of CH···π H-bond involving Cγ(Pro-S)H←Phenyl ring. In addition the unusual difference of in the chemical shift values of Phe CβHs (3.47 and 2.93 ppm) and the strong NOE cross peak Phe Cβ(Pro-S)H↔Crot CβH implies the existence of another CH···π H-bond involving Phe Cβ(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 CαH↔Pro Cδs in the NOESY spectrum (spectrum **2**, page#326). The NOEs Phe NH↔Allyl NH, Phe NH↔^{D}Val CαH, Phe NH↔Pro Cδ (Pro-S)H, Allyl NH↔ Pro CαH and involvement of Phe NH and Allyl NH H-bonds, support two successive β-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 CαH↔Pro CαH supported the *cis* rotamer preceding proline.

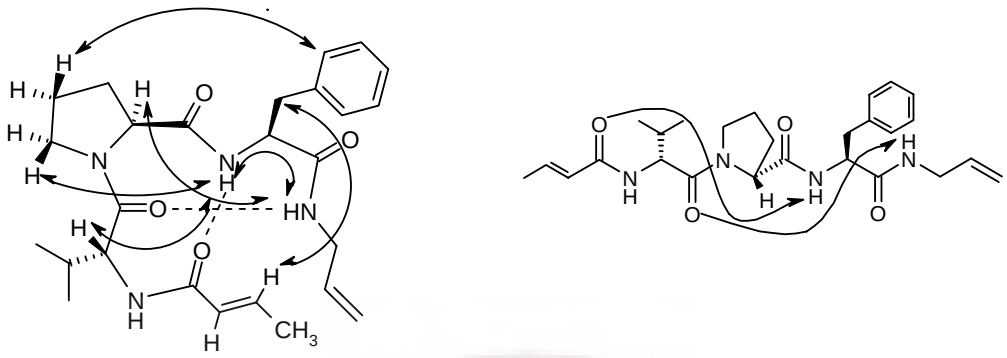


Figure-**5**: *Long-range nOe correlations and 10 membered H-bonding pattern that support for 3_{10} helical structure of peptide **4**.*

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 $CDCl_3$ 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.

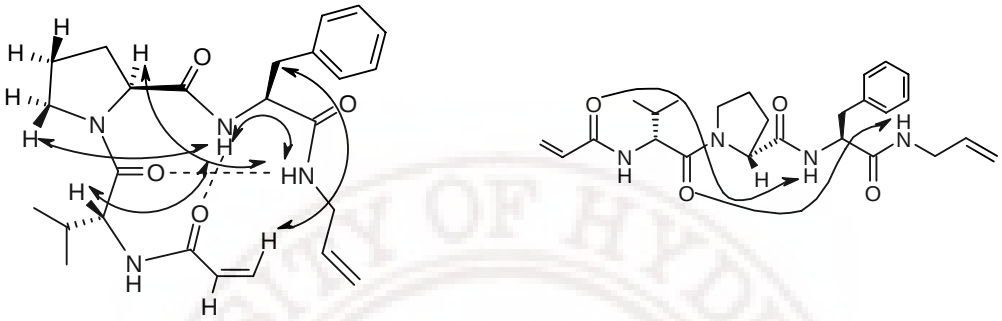
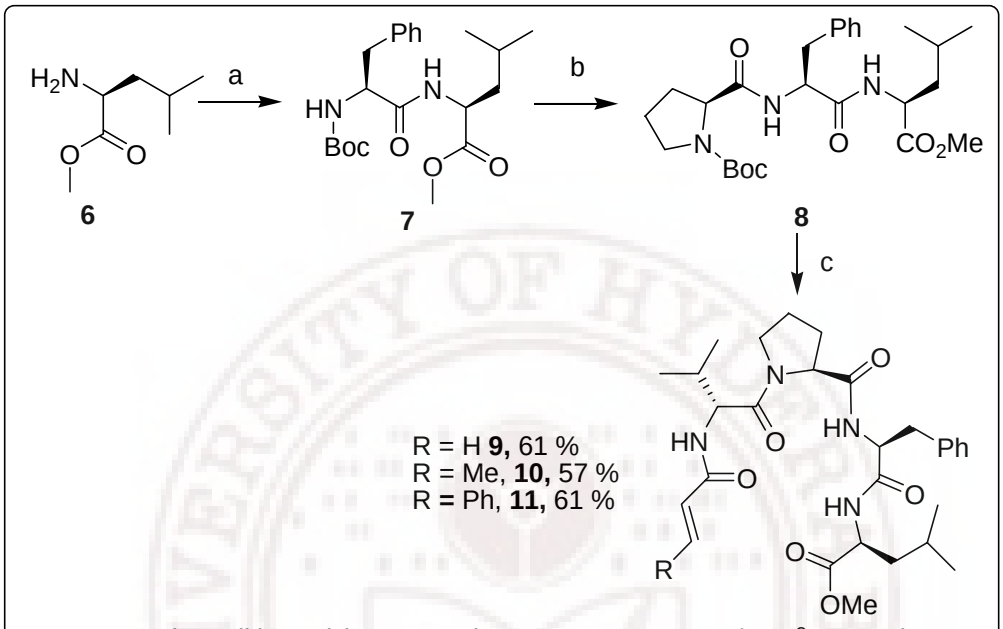


Figure-**6**: *Long-range nOe correlations and 10 membered H-bonding pattern that support for 3_{10} helical structure of peptide* ***5.***

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 deproection of 'Boc' in **8**) and *N*-acroyl-D-Val[24] 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, HOBt, CH_2Cl_2, *0 °C-rt, 12h, 71 %* (b) *i. TFA, CH_2Cl_2, ii. N-Boc-Pro,* EDC, HOBt, CH_2Cl_2, *0°C-rt, 12h, 80 %* (c) *i. TFA,* CH_2Cl_2, *N-acrolyl or crotonyl or cinnamoyl-D-Val, $ClCO_2{}^iBu$, NEt_3,* CH_2Cl_2, *0 °C-rt.*

Conformation of peptide 9:

It was gratifying to observe well-resolved ^{1}H NMR spectra for peptides **9** in both $CDCl_3$ and $DMSOd_6$. The down field appearance of Phe NH (7.21 ppm) and Leu NH (6.90 ppm) amide protons in $CDCl_3$ solution (spectrum 5, Page# 328), small change in their chemical shift values during the solvent titration study and variable temperature experiments ($\Delta\delta/\Delta T$ of –2.4 ppb/°K and –1.3 ppb/°K respectively) confirmed their participation in intra molecular H-bonding (Figure **7**).

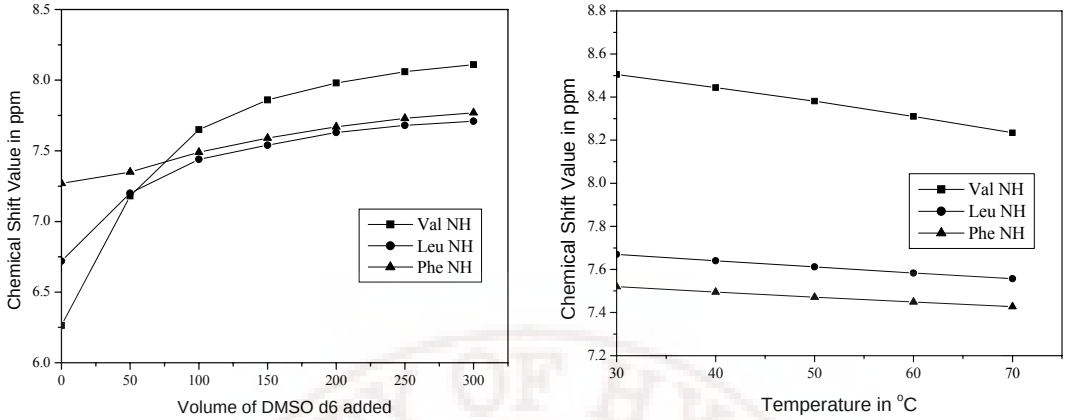


Figure-**7**: *(a) Solvent titration plots of peptide **9** in $CDCl_3$ (b) and plot of variable temperature experiment study in DMSO-d_6.*

The NOEs Phe NH↔Leu NH, Phe NH↔^{D}Val CαH, Leu NH↔Pro CαH, Phe NH↔Pro C*δ(Pro-S)*H (spectrum **6**, page# 328) was found to be in agreement with the existing H-bonds between Phe NH←acrolyl CO and Leu NH← Val CO (Figure **8**) confirming existence of two successive β-turns, which corresponds 3_{10} helical conformation. The evidence for CH···π H-bonding between Pro C*γ(Pro-S)*H and aromatic ring was established based on the NOEs between Pro C*γ(Pro-S)*H↔Phe Ar OH (Phenylalanine aromatic ortho H), Pro C*δ(Pro-S)*H↔Phe Ar OH. The NOE cross peak between Phe Cβ(*Pro-S*)H↔Acrolyl CβH supported Phe Cβ(*Pro-S*)H↔Acrolyl olefin CH···π H-bonding.

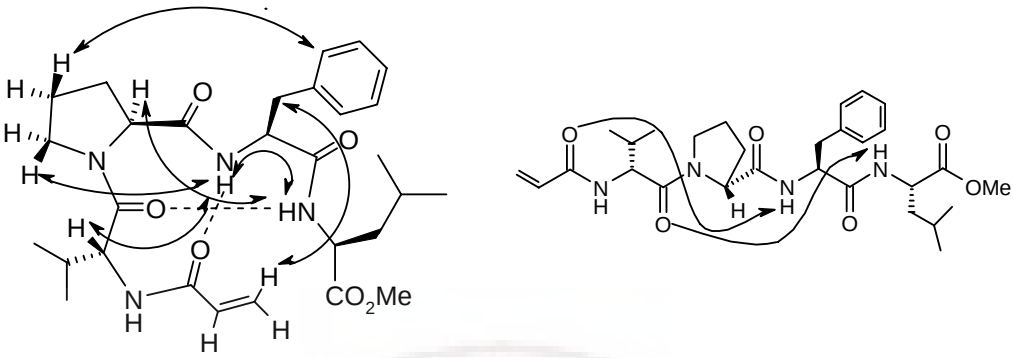


Figure-**8**: *Long-range nOe correlations and 10 membered H-bonding pattern that support for 3_{10} helical structure of peptide* **9**.

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**).

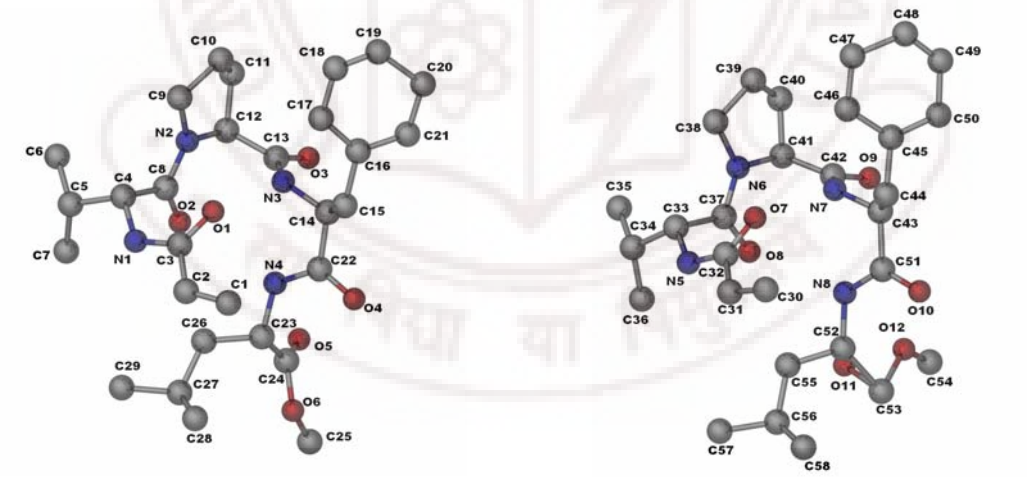


Figure- **9** *ORTEP of acrolyl-^{D}Val-Pro-Phe-LeuOMe* **9** *(H atoms are not shown for clarity).*

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)

S.No	Dihedral angles between atoms	φ(i+1) ψ(i+1)	Dihedral angles between atoms	φ(i+2) ψ(i+2)
1 2	C3-**N1-C4**-C8 N1-**C4-C8**-N2	56.13(60) -127.54(-120)	C8-**N2-C12**-C13 N2-C12-C13-N3	-68.6(-80) -11.87 (0)

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)

S.No	Dihedral angles between atoms	φ(i+1) ψ(i+1)	Dihedral angles between atoms	φ(i+2) ψ(i+2)
1 2	C8-**N2-C12**-C13 N2-**C12-C13**-N3	-68.66(-60) -11.87(-30)	C13-**N3-C14**-C22 N3-C14-C22-N4	-94.07(-90) 2.37 (0)

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 CγH and aromatic ring of Phe residue of 3.04 Å, 164.8º (C10–H101···π, π = C17–C18 centroid) in the crystal structure. The distance between the Phe CβH 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($\Delta\delta/\Delta T$ 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↔^{D}Val CαH, Leu NH↔Pro CαH (spectrum **10**, Page# 330) were in agreement with two

successive β-turns about ^{D}Val-Pro and Pro-Phe residues (Figure-**11**). As observed in peptide **9,** the up field appearance of Pro C*γ(Pro-S)*H at 1.28 ppm as well as cross peaks between Pro C*γ(Pro-S)*H↔Phe Ar OH (Phenylalanine aromatic ortho H), Pro C*δ(Pro-S)*H↔Phe Ar OH, imply a CH···π H-bond between Pro C*γ(Pro-S)*H and aromatic ring, while, the strong NOE cross peak between Phe Cβ(*Pro-S*)H↔Acrolyl CβH supports Phe Cβ(*Pro-S*)H↔Acrolyl olefin CH···π H-bonding.

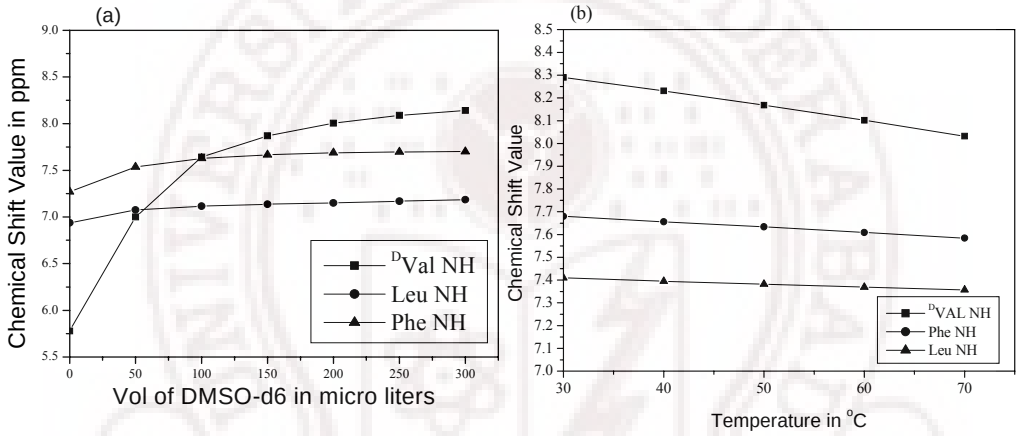


Figure-**10**: *(a) Solvent titration plots of peptide **10** in $CDCl_3$ (b) and plot of variable temperature experiment study in DMSO-d_6.*

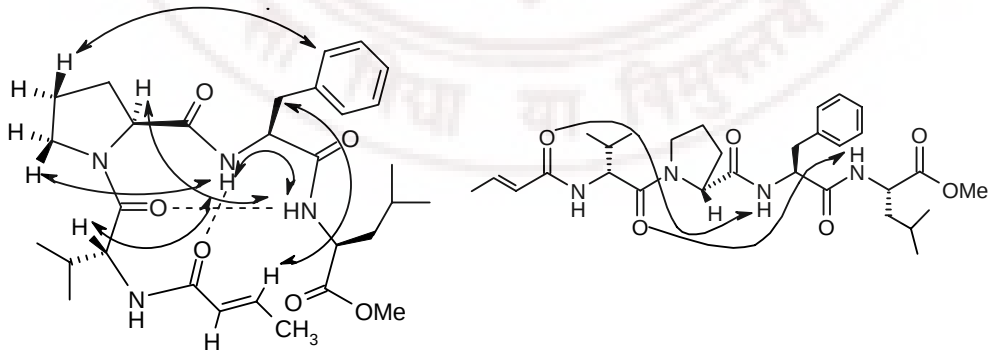


Figure-**11**: *Long-range NOE correlations and 10 membered H-bonding patterns that support 3_{10} helical structure of peptide **10**.*

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, where as in DMSO-d_6 solution two sets of resonances with 7:1 ratio were observed. The NOEs Val CαH↔Pro Cδ*(Pro-S)* H and Val CαH↔Pro Cδ*(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↔Leu NH, Phe NH↔^{D}Val CαH, Leu NH↔Pro CαH, Phe NH↔Pro Cδ*(Pro-S)* H (spectrum 14, page# 332) corroborated the existence of H-bonds Phe NH ←Cinn C=O and Leu NH←Val C=O (Figure **13**). In addition the long range correlations Cinn CβH↔Phe Cβ*(Pro-S)* H, Cinn CαH↔Leu CβH, Cinn Ar H↔Leu CδMe's, Cinn CαH↔ Leu CδMe's, Val NH↔Leu CβH strongly supported 3_{10} helical structure in peptide **11**. It was interesting to note the significant upfield shift of proline Cγ*(Pro-S)*H proton at 1.35 ppm due to the ring current of phenyl ring. The spatial correlation between Pro Cγ*(Pro-S)*H/ Phe Ar OH (phenyl alanine aromatic

ortho H), Pro Cδ*(Pro-S)*H / Phe Ar OH, provides the strong evidence for the existence of CH···π interaction between Pro-Phe residues.

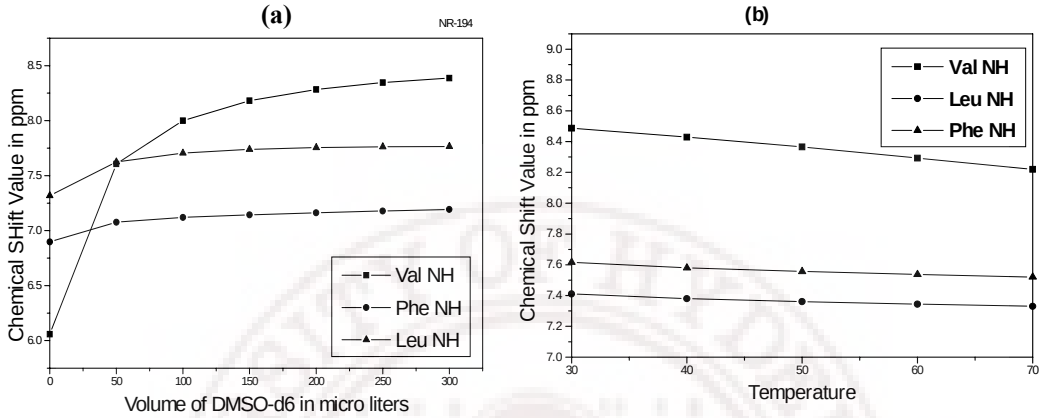


Figure-**12**: *(a) Solvent titration plot of peptide **11** in $CDCl_3$ (b) and plot of variable temperature experiment study in DMSO-d_6.*

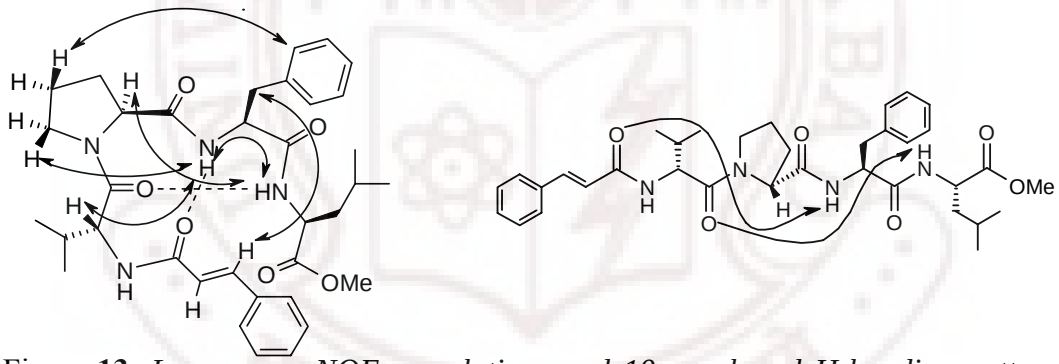
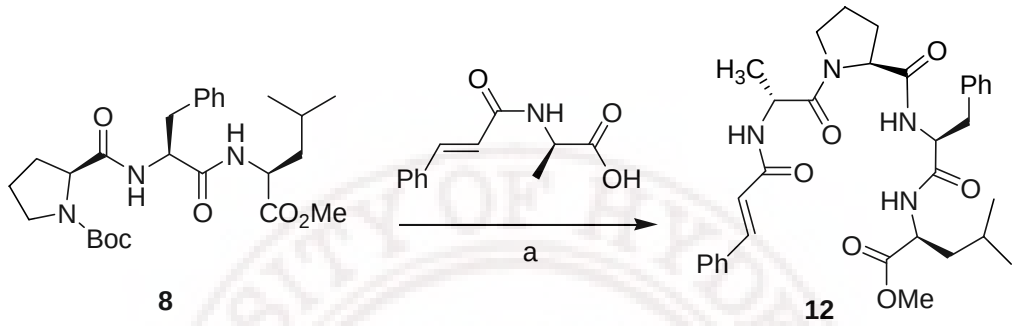


Figure-**13**: *Long-range NOE correlations and 10 membered H-bonding pattern that support for 3_{10} helical structure of peptide **11**.*

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*

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**



Reagents and conditions: (a) i. TFA, CH_2Cl_2*, N-cinnamoyl-D-Ala, EDC.HCl, HOBt,* CH_2Cl_2*, 0* ^{o}*C-rt, 47 %.*

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↔Leu NH, Phe NH↔^{D}Ala CαH, Leu NH↔Pro CαH, Phe NH↔Pro Cδ*(Pro-S)* H (spectrum **15** and **18**, Page #334 & 335) strongly supported the H-bonds Phe NH←Cinn C=O and Leu NH←Ala C=O, corresponding to 3_{10} helical conformation (Figure **15**). Moreover, Unusually large up field shift of Pro Cγ*(Pro-S)*H proton at 1.41 ppm and the spatial correlation between Pro Cγ*(Pro-S)*H↔Phe Ar OH (Phe aromatic

ortho H), Pro Cδ*(Pro-S)*H ↔Phe Ar OH, supports the CH···π intra molecular H-bond Pro C*γ(Pro-S)*H←Phenyl ring in peptide **12**.

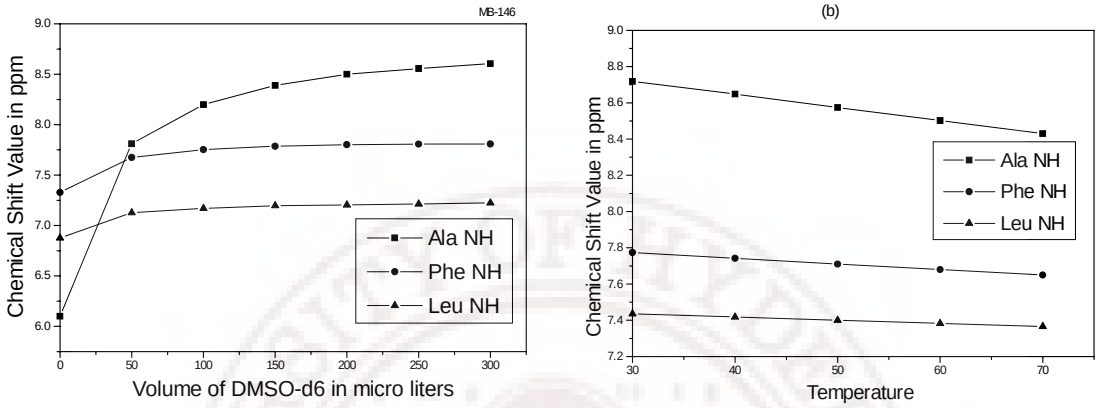


Figure-**14**: *(a) Solvent titration plots of peptide **12** in $CDCl_3$ and plot of variable temperature experiment study in DMSO-d_6.*

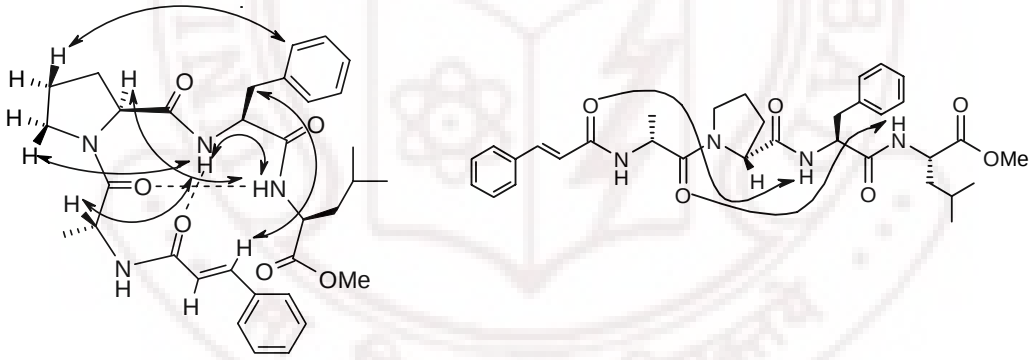
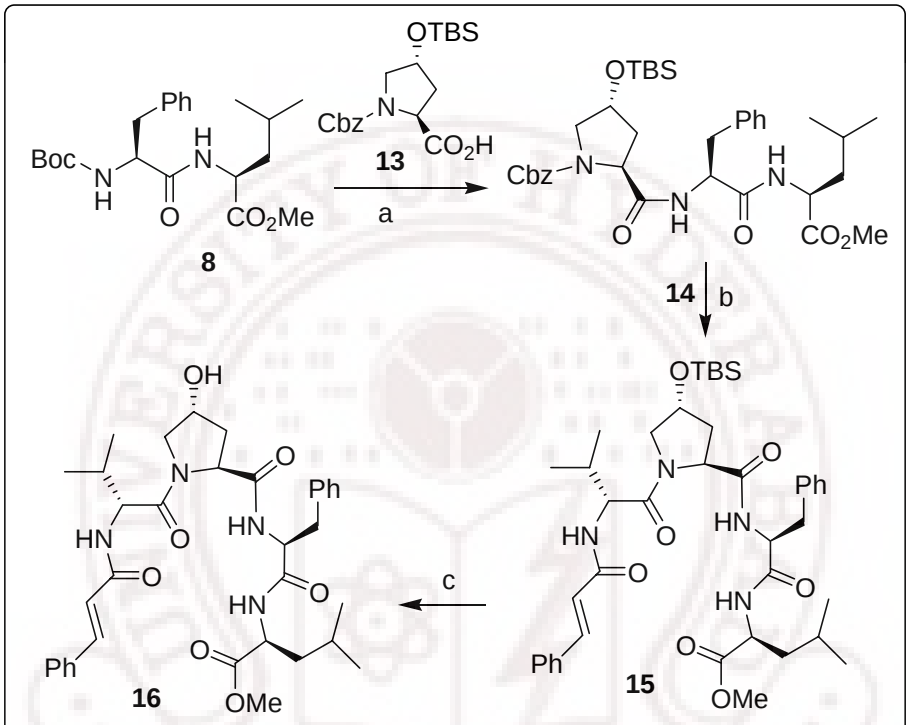


Figure **15**: *Long-range NOE correlations and 10 membered H-bonding pattern that support 3_{10} helical structure of peptide **12**.*

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-^{D}Val-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**



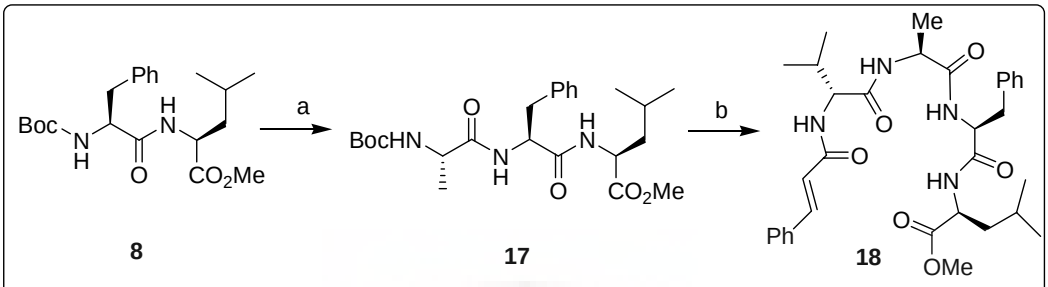
Reagents and conditions: (a) i. TFA, CH_2Cl_2*, ii.* ***13****, EDC, HOBt,* CH_2Cl_2*, 0* ^oC*-RT,12h, 56 % (b) i. 10 % PdC, MeOH, 40 psi pressure ii. N-cinnamoyl-D-Val, EDC, HOBt,* CH_2Cl_2*, 0* ^oC*-RT,12h, 39 % (c) TBAF, dry THF, 0* ^oC *to rt, 68 %*

The synthesis instigated with the transformation of *trans*-4-hydroxy proline to compound **13** following literature procedures[22] (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' (H_2 (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 out set, the absence of diagnostic chemical shift difference in the Phe CβHs and pro CγHs in the ^{1}H 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**).

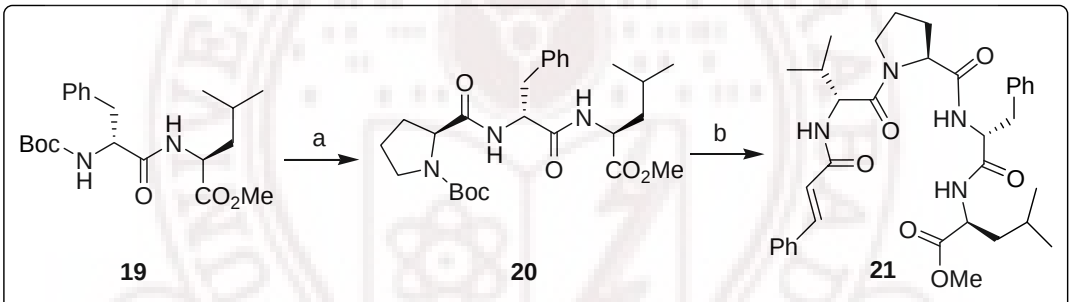
Scheme**-6** *Preparation of acyclic tetrapeptide* **18** *from dipeptide* **8**



8 **17** **18**

Reagents and conditions: (a)) i. TFA, CH_2Cl_2, ii. *N*-Boc-Ala*, EDC, HOBt,* CH_2Cl_2*, 0* ^oC*-RT,12h, 56 % (b) N-cinnamoyl-D-Val, EDC, HOBt,* CH_2Cl_2*, 0* ^oC*-RT,12h, 39 %*

Scheme**-7** *Preparation of acyclic tetrapeptide* **21** *from dipeptide* **8**



19 **20** **21**

Reagents and conditions: (a) N-Boc-Pro, EDC, HOBt, CH_2Cl_2*, 0* ^oC*-rt, 12h, 61% (ii)N-cinnamoyl-D-Val, EDC, HOBt,* CH_2Cl_2*, 0* ^oC*-rt, 12h,39 %.*

Conformation of peptide18 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

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, intrammolecular 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

1. (a) Baker, E. N.; Hubbard, R. E. *Prog. Biophys. Mol. Biol.* **1984**, *44*, 97-179.
2. (a) Burley, S. K.; Petsko, G. A. Science, 1985, *229*, 23. (b) Desiraju, G.; Steiner, T. *The Weak Hydrogen Bond in Structural Chemistry and Biology, Oxford University Press*, **1999**, Oxford.
3. Burley, S. K.; Petsko, G. A. *FEBS Letters*, **1986**, *203*, 139.
4. Reeves, L. W.; Schneider, W. G. *Can. J. Chem*. **1957**, *35*, 251.
5. Brandl, M., Weiss, M. S., Jabs, A., Suhnel, J., Hilgenfeld, R. *J. Mol Biol*. **2001**, *307*, 357.
6. (a) Ref **5** (b) Umezawa, Y.; Tsuboyama, S.; Takahashi, H.; Uzawa, J.; Nishio, M. *Bioorg. Med. Chem.* **1999**, *7,* 2021.
7. Mathur, P.; Ramakumar, S.; Chauhan V. S. *Biopolymers*, **2004**, *76*, 150.
8. Tatko, C. D.; Waters, M. L. *J. Am. Chem. Soc.* **2004**, *126*, 2028.
9. Nishio, M., Hirota, M. & Umezawa, Y. *The CH/pi Interaction. Evidence, Nature and Consequences*, **1998**, Wiley-VCH, New York.
10. Matsui, I., Matsui, E., Sakai, Y., Kikuchi, H., Kawarabayasi, Y., Ura, H., Kawaguchi, S., Kuramitsu, S.; Harata, K. *J. Biol.Chem.* **2000**, *275*, 4871.
11. (a) Chakrabarti, P. & Samanta, U. J. Mol. Biol. **1995**, *251*, 9. (b) Umezawa, Y.; Nishio, M. *Bioorg. Med. Chem.* **1998**, *6*, 493.

12. Muraki, M.; Harata, K.; Sugita, N. Sato, K. I., *Biochemistry*, **2000**, *39*, 292.

13. Umezawa, Y.; Nishio, M. *Bioorg. Med. Chem.* **1998**, *6*, 2507.

14. Shimohigashi, Y.; Nose, T.; Yamauchi, Y; Maeda, I. *Biopolymers*, **1999**, *51*, 9.

15. Kumaraswamy, V. S.; Lindley, P. F.; Slingsby, C.; Glover, I. D. *Acta Crystallog. Sect. D,* **1996**, 52, 611-622.

16. Harigai, M.; Kataoka, M.; Imamoto, Y *J. Am. Chem. Soc.* **2006**, *128*, 10646.

17. Williamson, M. P. *Biochem. J.* **1994**, *297*, 249.

18. Rajasri Bhattacharyya, R.; Chakrabarti, P. *J. Mol Biol*. **2003**, *331*, 925.

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

20. Rajesh B. M.; Javed, I; Jagdeesh, B.; Nangia A. *Cryst. Eng. Comm*. **2007**, *10*, 860.

21. Hunter, C. A.; Lawson, K. R.; Perkins, J.; Urch, C. J. *J. Chem. Soc. Perkin Trans* **2001**, *2*, 651.

22. Mayer, S. C.; Ramanjulu, J.; Vera, M. D.; Pfizenmayer, A. J.; Joullie, M. M. *J.Org. Chem.* **1994**, *59*, 5182.

23. Tsutomu, N; Shigemitsu. A; Toshio. F. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 2778.

24. Dibyendu. K; Rabindranath, M.; Joykrishna, D. *Chem, Comm*. **2006**, *47*, 4903.

25. Jean Michel, B.; Chanaz, S.; Yves, L. *Tetrahedron Lett.* **2005**, *46*, 217.

26. Elliott, R. L.; Marks, N.; Berg, M. J.; Portoghese C.; *J. Med. Chem* **1985**, *28*, 1208.

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 (1equivalent) in dry CH_2Cl_2 was added HOBt (1.2 equivalent) and a solution of Leucine methyl ester (1 equivalent) in dry CH_2Cl_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_2Cl_2 and washed with water, brine, dried over Na_2SO_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 (1equivalent) in dry CH_2Cl_2 was added trifluoroacetic acid (TFA) (10 equivalents) at 0^oC 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-Proline (1equivalent) and HOBt (1.2 equivalent) of dry CH_2Cl_2 was added a solution of Yaa-Leu methyl ester (obtained in part A) (1equivalent) in dry CH_2Cl_2 ad stirred for 5 min. EDC.HCl (1.5 equivalent) was added portion wise to the reaction mixture at 0^oC and then stirred at room temperature for a period of 12 h. The reaction mixture was diluted with CH_2Cl_2 and washed with water, brine, dried over Na_2SO_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.

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 CH_2Cl_2 was added NEt_3 (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 12h. The reaction mixture was diluted with CH_2Cl_2, washed with water, dried over Na_2SO_4 and evaporated to yield crude compound which was purified using MeOH-$CHCl_3$ 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-H_2O (4:1) (8 ml) was added an aqueous solution of LiOH (1.2 equivalent) and stirred for 4h 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 (P^H~2.0) and extracted with CH_2Cl_2. The organic layer was washed with brine (20 ml), dried over Na_2SO_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-L-Pro-Yaa allylamide (1equivalent) in dry CH_2Cl_2 was added trifluoroacetic acid (TFA) (10 equivalents) at 0^oC 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.

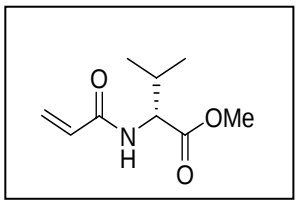
B. To an ice cold stirred solution of appropriately *N*-substituted (*acrolyl or crotonyl or cinnamoyl*)-Xaa (aa= amino acid) (1equivalent) and HOBt (1.2 equivalent) of dry CH_2Cl_2 was added a solution of Pro-Yaa allylamide (obtained in part A) (1equivalent) in dry CH_2Cl_2. EDC.HCl (1.5 equivalent) was added portion wise to the reaction mixture at 0 ^oC and then stirred at room temperature for a period of 12 h. The reaction mixture was diluted with CH_2Cl_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 (1equivalent) in dry CH_2Cl_2 was added trifluoroacetic acid (TFA) (10 equivalents) at 0^oC 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 appropriately *N*-substituted (*acrolyl or crotonyl or cinnamoyl*)-Xaa (aa= amino acid) (1equivalent) and HOBt (1.2 equivalent) of dry CH_2Cl_2 was added a solution of Pro-Yaa-Leu methyl ester (obtained in part A) (1equivalent) in dry CH_2Cl_2. EDC.HCl (1.5 equivalent) was added portion wise to the reaction mixture at 0 ^oC and then stirred at room temperature for a period of 12 h. The reaction mixture was diluted with CH_2Cl_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]:

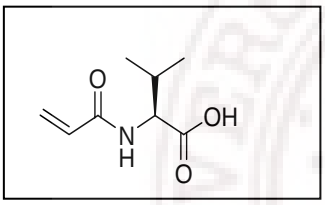


The title compound was obtained following the general procedure (C) using (2.4 g, 14.3 mmol) of D-Valine methylester.HCl, (1.55 g, 17.2 mmol) of acrolyl chloride

and (6 ml, 43 mmol) of NEt_3 to afford (1.32 g, 50 %) of title product as pale yellow oil.

[α] = 44 (c, 0.1, MeOH); IR (KBr): 3299, 2966, 1715, 1661, 1628, 1537 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.31 (dd, *J* = 16.9 Hz and *J* = 1.3 Hz, 1H), 6.19-6.12 (m, 1H), 6.03 (m, 1H), 4.68-4.64 (m, 1H), 5.69 (dd, *J* = 10.2 Hz and *J* = 1.3 Hz, 1H), 3.77 (s, 3H), 2.22-2.15 (m, 1H), 0.96-0.91 (m, 6H).

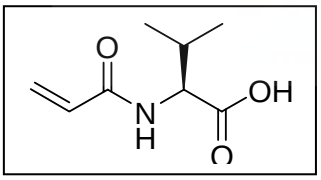
Preparation of *N*-acrolyl-L-valine OH [24]:



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-H_2O to afford (0.45 g, 71 %) of title product as colorless gum.

[α] = -13.0 (c, 0.5, MeOH); ^1H NMR (400 MHz, $CDCl_3$): δ 6.326.19 (m, 2H), 570-5.67 (dd, J = 102 Hz and J = 1.6 Hz, 1H), 2.27-2.17 (m, 2H), 0.99-0.98 (m, 6H). Mass (CI method): 172 $((M+H)^+$, 16), 126 (100).

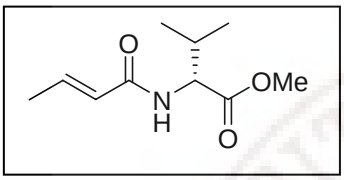
Preparation of *N*-acrolyl-D-valine OH[24]:



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-H_2O 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).

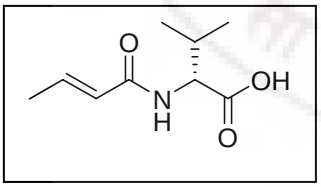
Preparation of *N*-crotonyl-D-valine methyl ester[24]:



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).

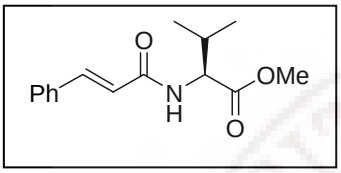
Preparation of *N*-crotonyl-D-valine[24]:



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_2O (4:1) (10 ml) to afford (0.61 g, 66 %) of title product as colorless gum.

^{1}H NMR (200 MHz, $CDCl_3$): δ7.76 (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).

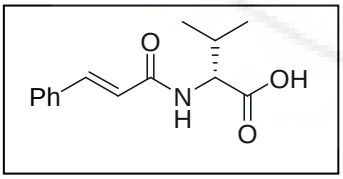
Preparation of *N*-cinnamoyl-D-valine methyl ester[25]:



The title compound was obtained following the general procedure (C) using (4.47g, 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, $CDCl_3$): δ 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).

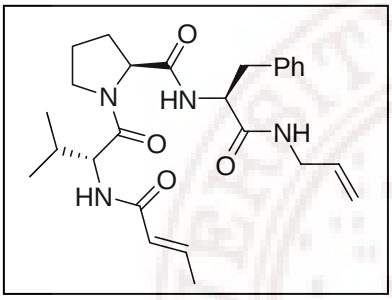
Preparation of *N*-cinnamoyl-D-valine[25]:



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_2O (4:1) (10 ml) to afford (0.45 g, 71 %) of title product as white solid.

mp: 206 °C; [α] = -22.4 (c, 0.5, MeOH); IR (Neat): 3319, 2967, 1721, 1653, 1212 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 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).

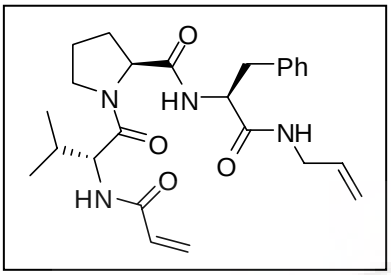
Preparation of *N*-crotonyl-D-Val-Pro-Phe-allyl amide (4):



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, $DMSOd_6$): 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, ^{D}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 (CI method): 469 ($(M+H)^+$,100).

Preparation of *N*-acrolyl-D-Val-Pro-Phe-allyl amide (5):

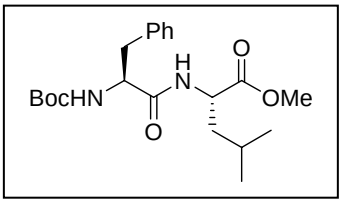


The title compound was obtained following the general procedure (E) using (465 mg, 2.71 mmol) of *N*-acrolyl-D-Val, (818 mg, 2.71 mmol) of Pro-Phe-allylamide, (440 mg, 3.26 mmol) of HOBt and (781 mg, 4.07 mmol) of EDC.HCl to afford (500 mg, 41 %) of title product as fluffy solid.

IR (Neat); 3300, 2965, 1650, 1543, 1192 cm^{-1}; ^1H NMR (400 MHz, $DMSOd_6$): δ 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 $(M+H)^+$, 100).

Preparation of *N*-Boc-L-Phe-Leu methyl ester (7)[26]:

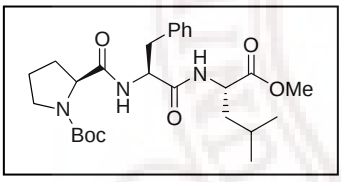
The title compound was obtained following the general procedure (A) using (7.42 g, 28.0 mmol) of *N*-Boc-Phe, (4.06 g, 28.0 mmol) of Leu methyl ester, (4.54g,



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}; ^1H 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).

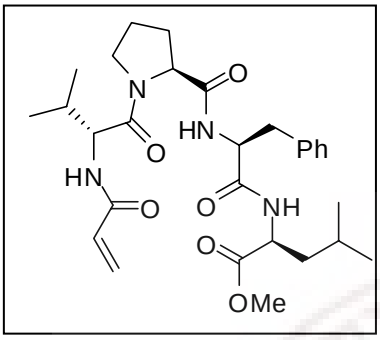
Preparation of *N*-Boc-Pro-Phe-Leu methyl ester (8):



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}; ^1H NMR (400 MHz, $DMSOd_6$): δ 8.38 (d, *J* = 7.5 Hz, IH), 7.76 (d, *J* = 8.5 Hz, IH), 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*-acrolyl-D-Val-Pro-Phe-Leu methyl ester (9):

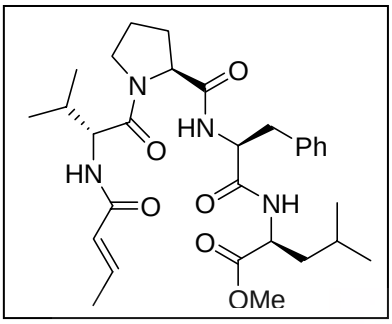


The title compound was obtained following the general procedure (F) using (610 mg, 3.57 mmol) of *N*-acrolyl-D-Val (1.39 g, 3.57 mmol) of Pro-Phe-Leu methyl ester (580 mg, 4.28 mmol) of HOBt and (1.02 g, 5.35 mmol) of EDC.HCl to afford (1.17 g, 61 %) of title product as fluffy solid.

IR (Neat); 3300, 2965, 1650, 1543, and 1192 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 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, AcrylCβ´H), 6.15 (dd, *J* = 16.9 Hz & *J* =10.2 Hz, AcrylCαH), 6.07 (d, J = 5.3 Hz, ^{D}ValNH), 5.75 (dd, *J* = 10.2 Hz and J = 1.4 Hz, AcrylCβH), 4.64 (m, 1H, Phe CαH), 4.48 (m, 1H, Pro CαH), 4.39 (m, 1H, Leu CαH), 4.16 (m, 1H, ^{D}ValCαH), 3.96 (m, 1H, Pro CδH), 3.69 (s, LeuOMe), 3.48 (m, 1H, Pro Cδ´H), 3.44 (dd, *J* = 14.1 Hz and *J* = 4.2 Hz, Phe CβH), 2.94 (dd, *J* = 14.1 Hz and *J* = 11.8 Hz, 1H, Phe Cβ´H), 2.08-2.03 (m, 1H, Val CβH), 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-



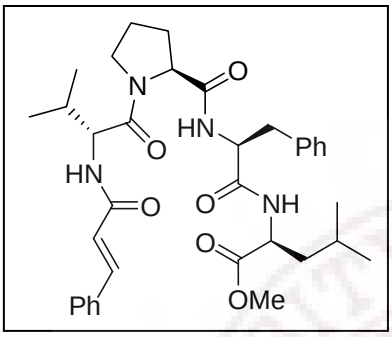
Phe-Leu methyl ester, (490 mg, 3.56 mmol) of HOBt and (860 mg, 4.45 mmol) of EDC.HCl as a fluffy solid.

[α] = -44 (c, 0.1, MeOH), IR (Neat); 3340, 2966, 1745, 1691, 1632, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 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 CβH), 5.87 (m, 1H, Crot CαH), 5.77 (d, J = 4.9 Hz, 1H, Val NH), 4.65 (m, 1H, Phe CαH), 4.48 (dd, J = 7.3 Hz and *J* = 3.4 Hz, Pro CαH), 4.38 (m, 1H, Leu CαH), 4.09 (m, 1H, Val CαH), 3.99 (m, 1H, Pro CδH), 3.69 (s, 3H, LeuOMe), 3.48 (m, 1H, Pro Cδ′H), 3.47 (dd, *J* = 13.7 Hz and *J* = 4.0 Hz, 1H, Phe CβH), 2.93 (dd, *J* = 12.2 Hz and *J* = 12.2 Hz, Phe Cβ′H), 2.06-2.00 (m, 1H), 1.94-1.84 (m, 4H), 1.74-1.71 (m, 1H, Pro CγH), 1.42-1.31 (m, 4H), 1.29-1.25 (m, 1H, Pro Cγ′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), Mass (CI 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-

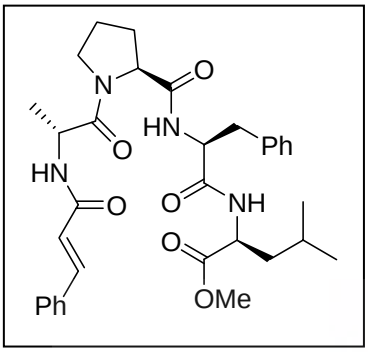
Phe-Leu methyl ester, (247 mg, 1.81 mmol) of HOBt and (430 mg, 2.27 mmol) of EDC.HCl as a fluffy solid.



IR (Neat); 3324, 2955, 1650, 1515, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.63 (d, *J* = 15.8 Hz, 1H, Cinn CβH), 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 CαH), 6.07 (d, *J* = 5.4 Hz,1H, Val NH), 4.66 (m, 1H, Phe CαH), 4.50 (dd, *J* = 7.0 Hz & *J* = 3.5 Hz, 1H, Pro CαH), 4.33 (m,1H, Leu CαH), 4.22 (m, 1H, Val CαH), 4.01-3.96 (m, 1H, Pro CδH), 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 Cβ´H), 2.09-2.06 (m, 1H, Val CβH), 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).

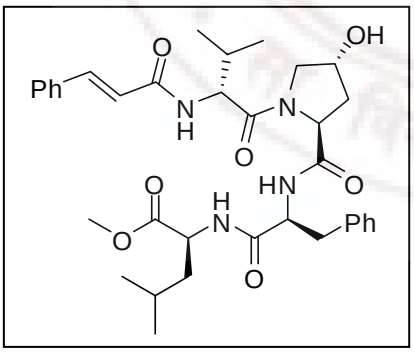
Preparation of N-cinnamoyl-D-Ala-Pro-Phe-Leu methyl ester (12):

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}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.63 (d, *J* = 15.6 Hz, 1H, cinnamoyl CβH), 7.51-7.18 (m, 11H), 6.87 (d, *J* = 8.0 Hz, 1H, LeuNH), 6.43 (d, *J* = 15.6 Hz, 1H, cinnamoyl CαH), 6.10 (d, *J* = 5.0 Hz, 1H, ^{D}Ala NH), 4.67-4.60 (m, 1H, Phe CαH), 4.60-4.57 (m, 1H, ^{D}Ala CαH), 4.49 (dd, *J* = 14.0 Hz and *J* = 4.3 Hz, 1H, Phe CβH), 4.41-4.38 (m, 1H, Leu CαH), 3.87-3.80 (m, 1H, Pro CδH), 3.66 (s, 3H, LeuOMe), 3.47-3.40 (m, 2H) 3.11 (dd, *J*= 14.0 Hz and *J* = 11.3 Hz, Phe Cβ′H), 1.99-1.97 (m, 2H), 1.80 (m, 1H, Pro CγH), 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 CδH), 0.71 (d, *J* = 6.3 Hz, 3H, Leu Cδ′H), mass (CI method): 591 ($(M+H)^+$, 100).

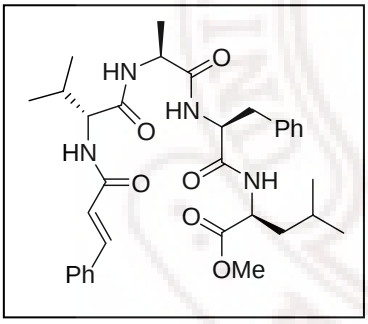
Preparation of Cinnamoyl-Val-*trans*-4-OH-Pro-Phe-Leu methyl ester (16)



Using general procedure (F) (210 mg, 0.40 mmol) of *trans*-4-[{t-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_2Cl_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}; ^1H NMR (400 MHz, $CDCl_3$): δ 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):

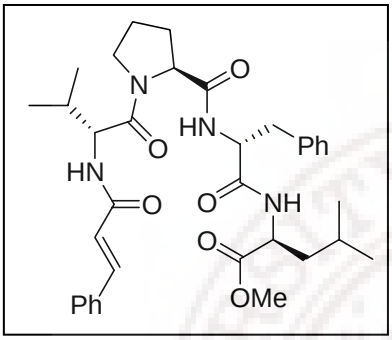


The title compound (935 mg, 39%) was obtained following the general procedure (F) using (1 g, 4.12 mmol) of *N*-cinnamoyl-D-Val, (1.46 g, 4.12 mmol) of Pro-Phe-LeuOMe, (668 mg, 4.94 mmol) of HOBt and (1.18 g, 6.19 mmol) of EDC.HCl.

^1H NMR (400 MHz, $CDCl_3$): δ 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

(m, 1H, Val CβH), 1.62-1.44 (m, 4H), 1.12 (d, *J* = 6.3 Hz, 3H, Ala CβH)), 096-0.90 (m, 12H).

Preparation of *N*-cinnamoyl-D-Val-Pro-D-Phe-Leu methyl ester (21)

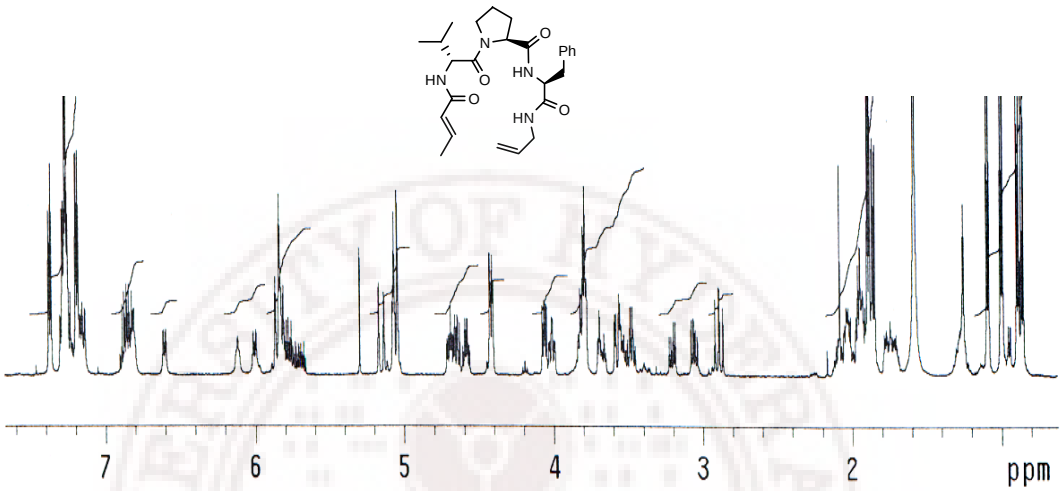


The title compound (470 mg, 39 %) was obtained following the general procedure (F) using (480 mg, 1.94 mmol) of *N*-cinnamoyl-D-Val, (745 mg, 1.94 mmol) of Pro-D-Phe-LeuOMe, (410 mg, 3.04 mmol) of HOBt and (730 mg, 3.80 mmol) of EDC.HCl as a gum.

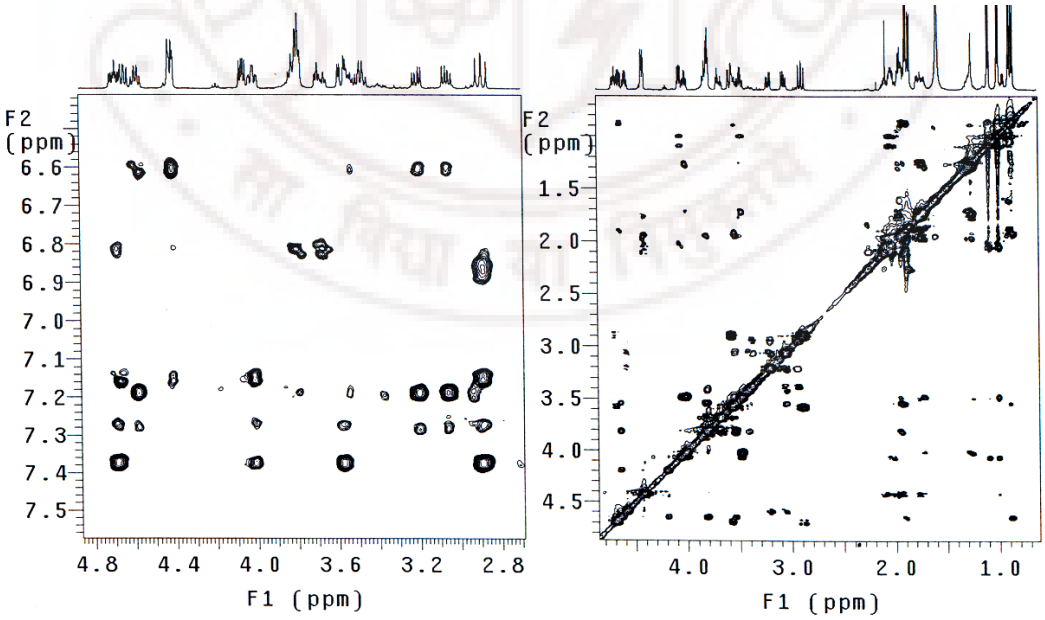
IR (Neat); 3297, 2959, 1655, 1207, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.6 (d, *J* = 15.6 Hz, 1H), 7.52-7.49 (m, 2H), 7.36-7.34 (m, 3H,), 7.27-7.10 (m, 7H), 6.56 (d, J = 7.8 Hz, 1H), 6.51 (d, J = 15.6 Hz, 1H,), 4.66-4.64 (m, 1H), 4.59-4.55 (m, 2H), 4.39-4.37 (m, 1H), 3.72-3.68 (m, 1H), 3.68 (s, 3H), 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

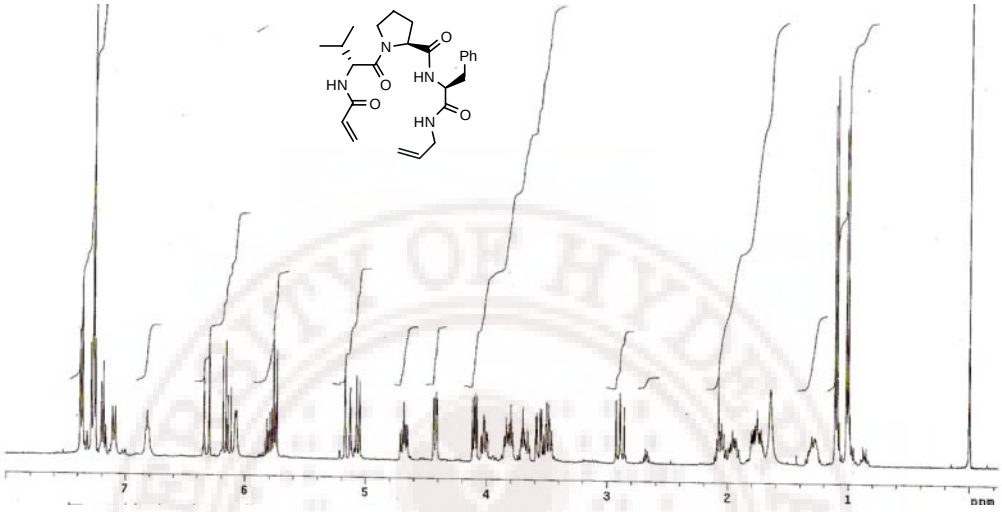
Spectrum 1: **^{1}H NMR spectrum of peptide 4 in $CDCl_3$**



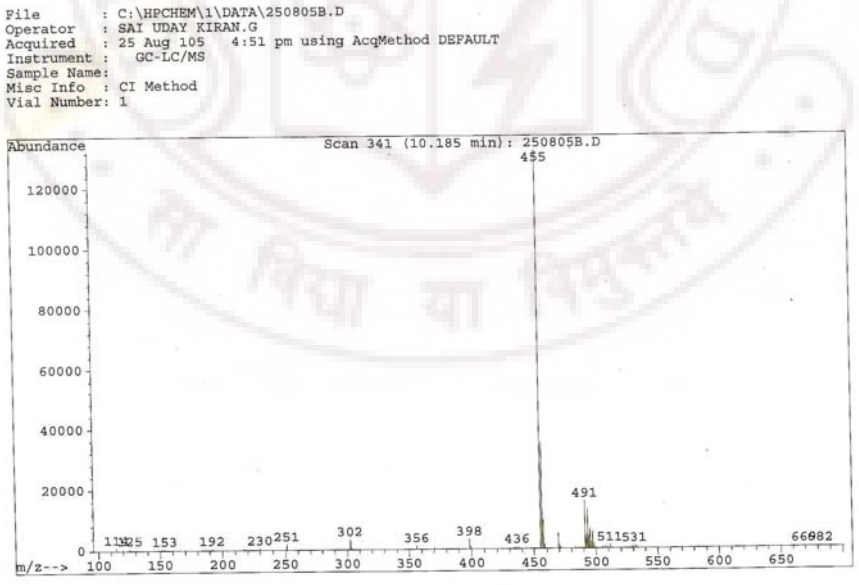
Spectrum 2: **Expansion of NOESY spectrum of peptide 4.**



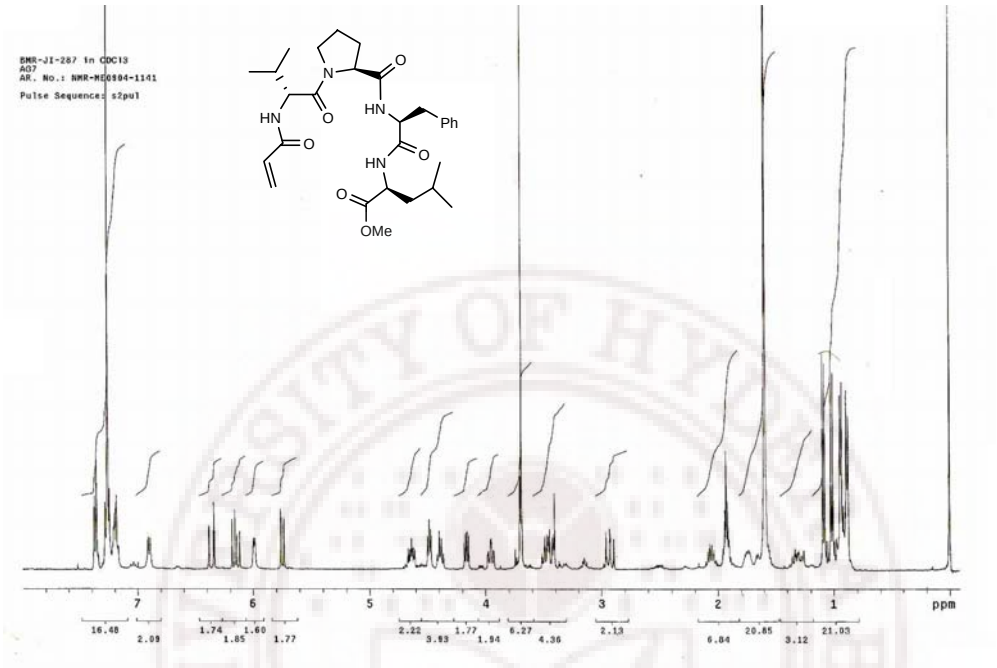
Spectrum 3: **^{1}H NMR spectra of peptide 5 in $CDCl_3$**



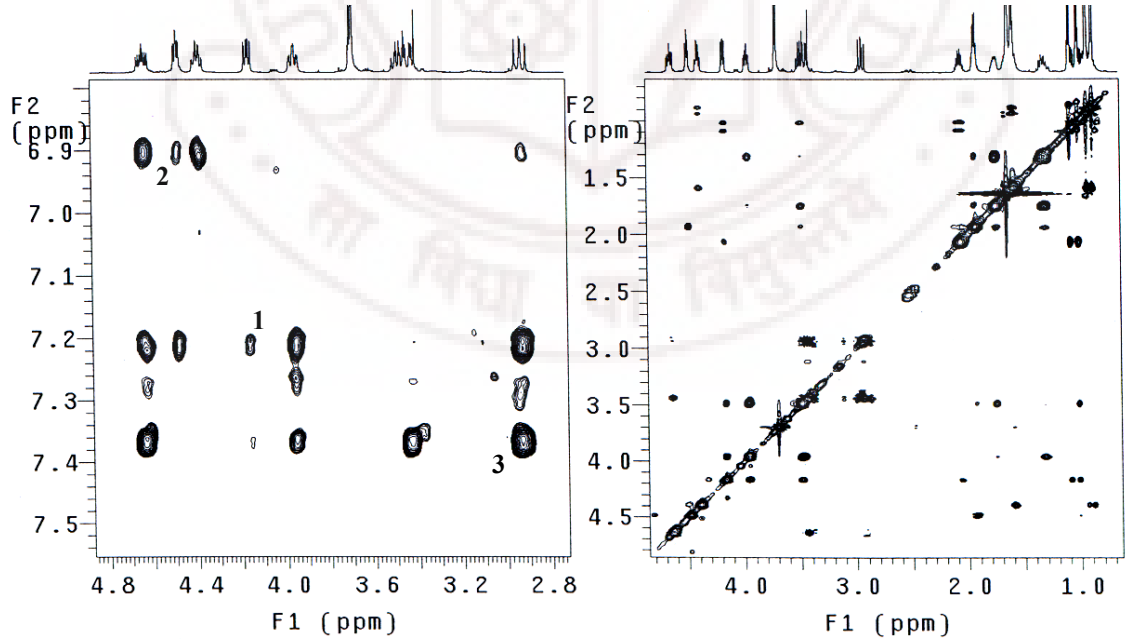
Spectrum 4: **Mass spectrum of peptide 5**



Spectrum 5: ^{1}H NMR spectrum of peptide 9 in $CDCl_3$

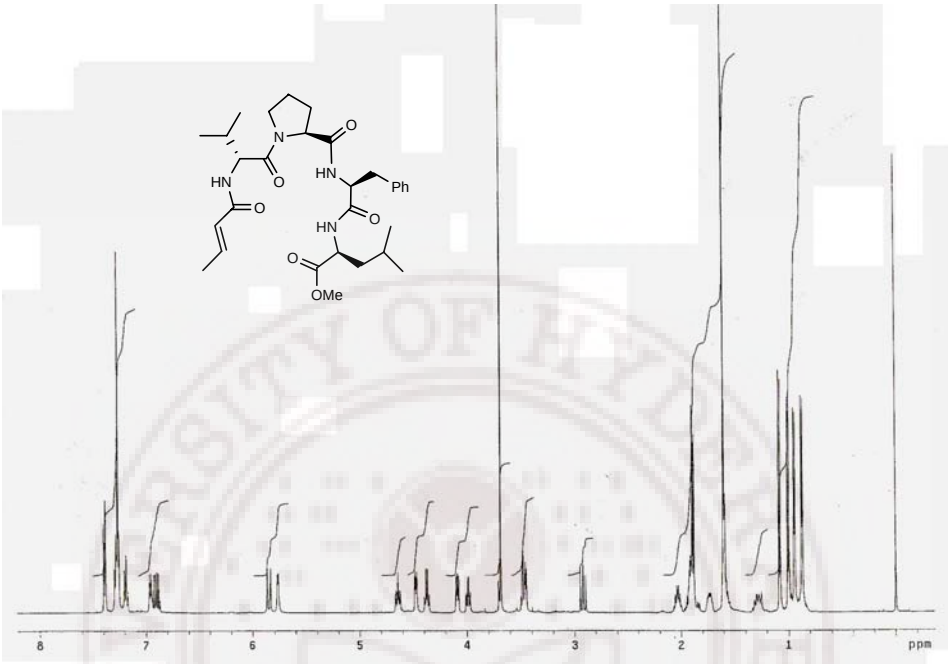


Spectrum 6: Expanded NOESY spectrum of 9

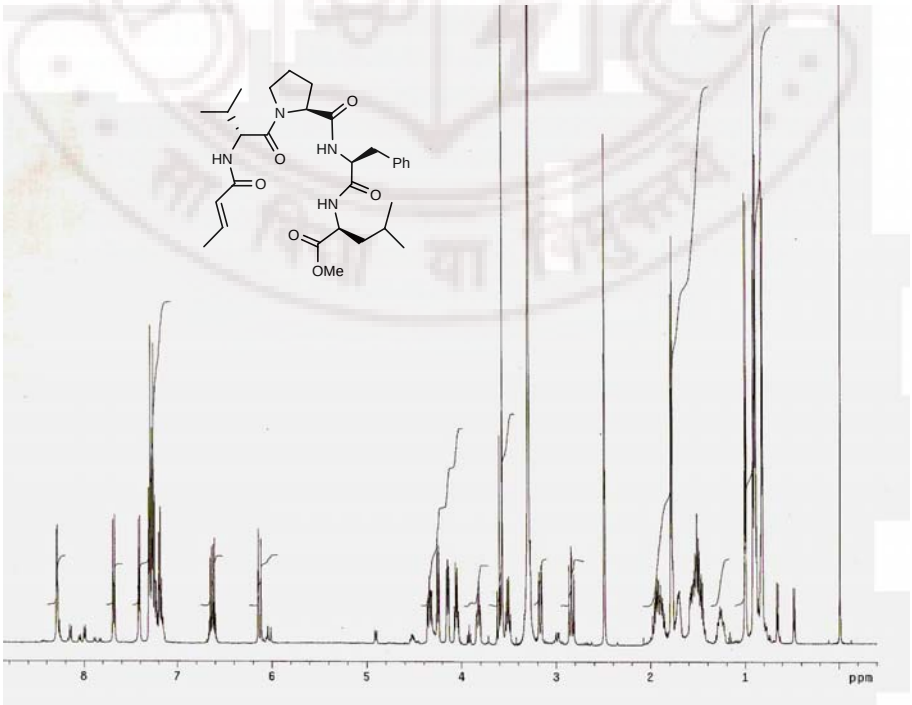


1-Phe NH↔^{D}Val CαH, 2-Leu NH↔Pro CαH, 3-Acryl CβH↔Phe Cβ*(Pro-S)*H).

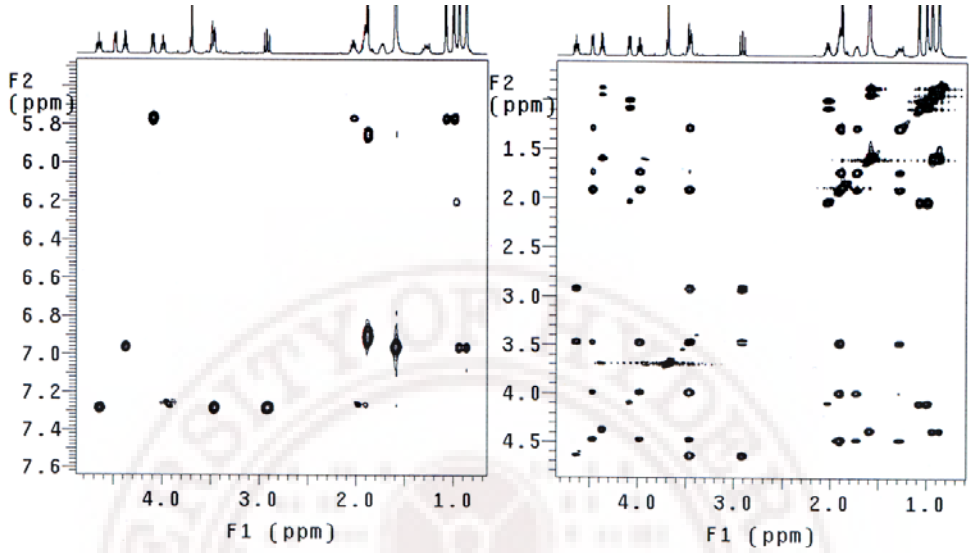
Spectrum 7: ^{1}H NMR of peptide 10 in $CDCl_3$



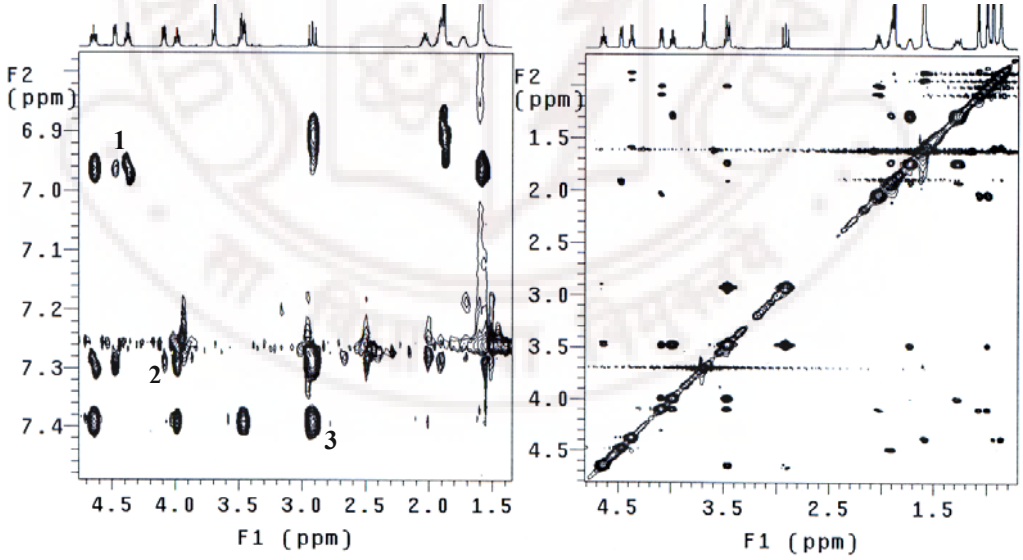
Spectrum 8: ^{1}H NMR of peptide 10 in DMSO-d_6



Spectrum 9: Expanded TOCSY spectrum of peptide 10

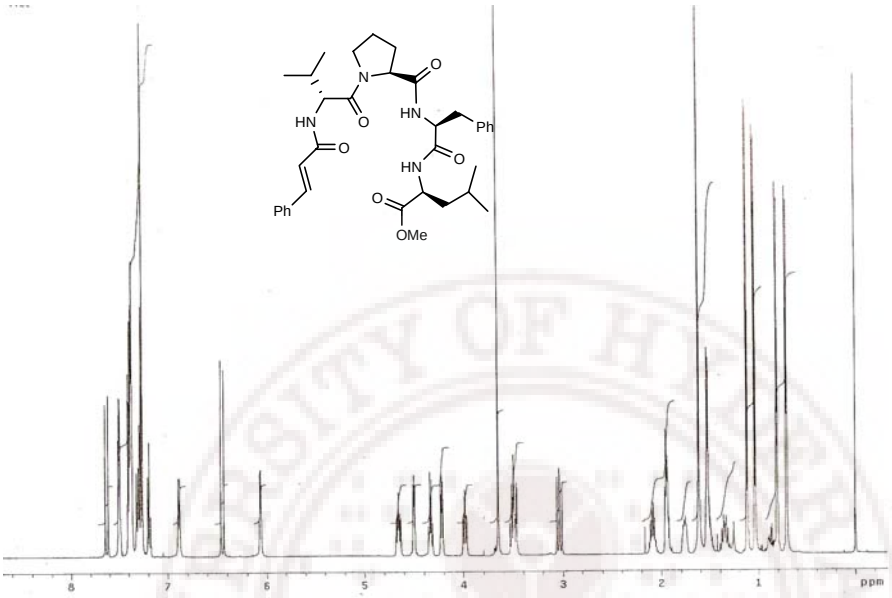


Spectrum 10: Expanded NOESY spectrum of peptide 10

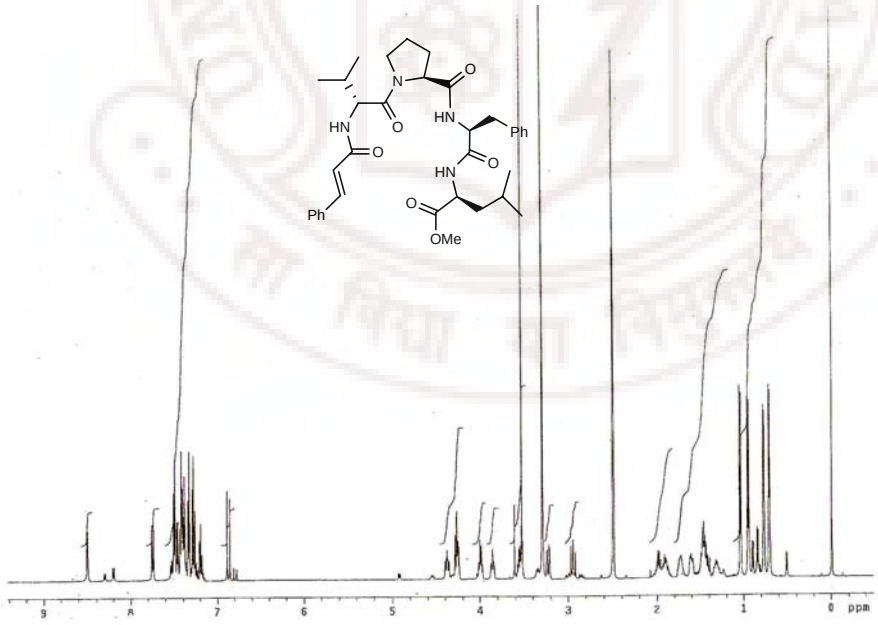


1-Leu NH / Pro CαH, 2-Phe NH / Val CαH, 3-Crot CβH / Phe Cβ*(Pro-S)*H).

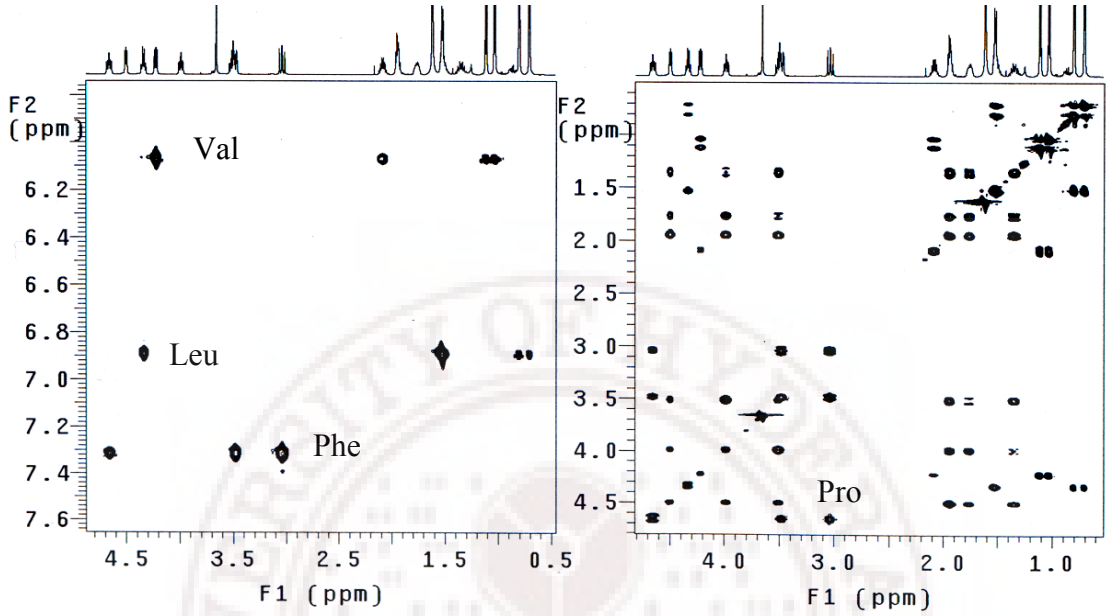
Spectrum 11: ^{1}H NMR spectrum of peptide 11 in $CDCl_3$



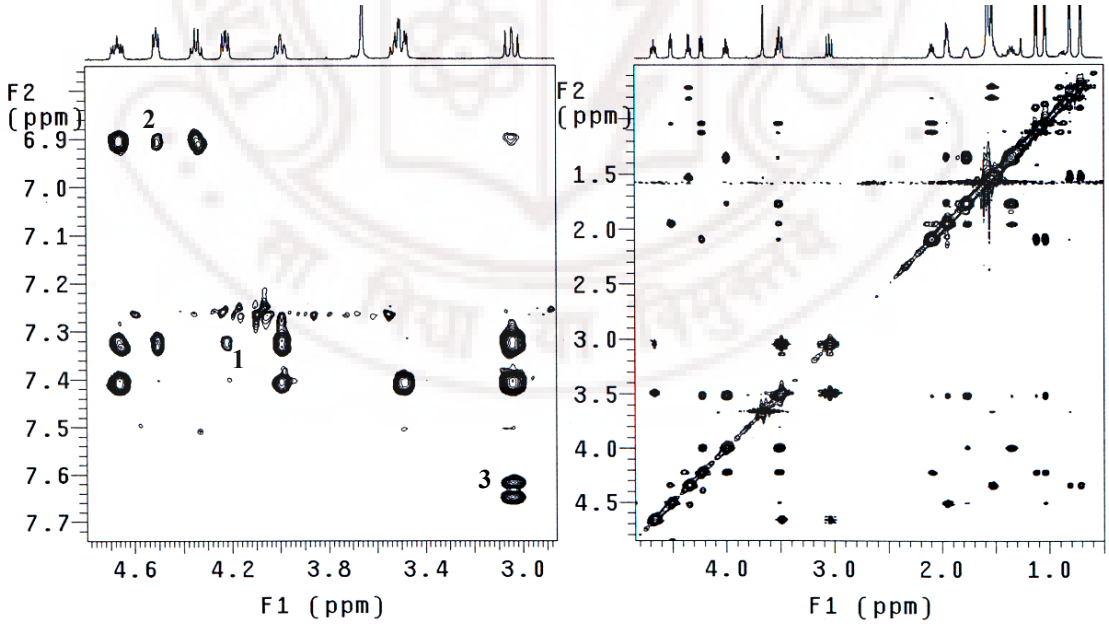
Spectrum 12: ^{1}H NMR spectrum of peptide 11 in DMSO-d_6



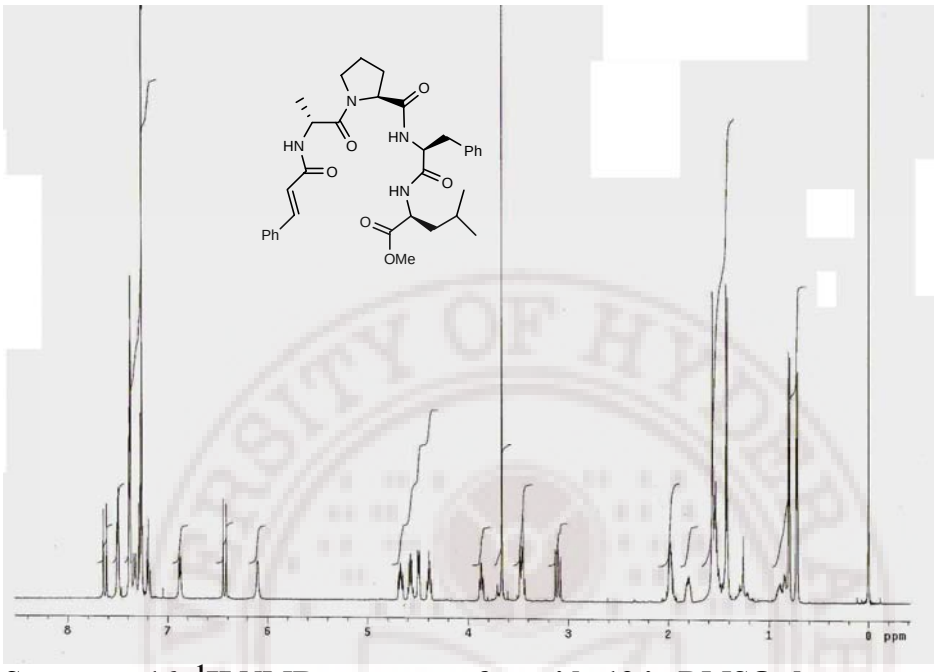
Spectrum 13: Expanded TOCSY spectrum of peptide 11



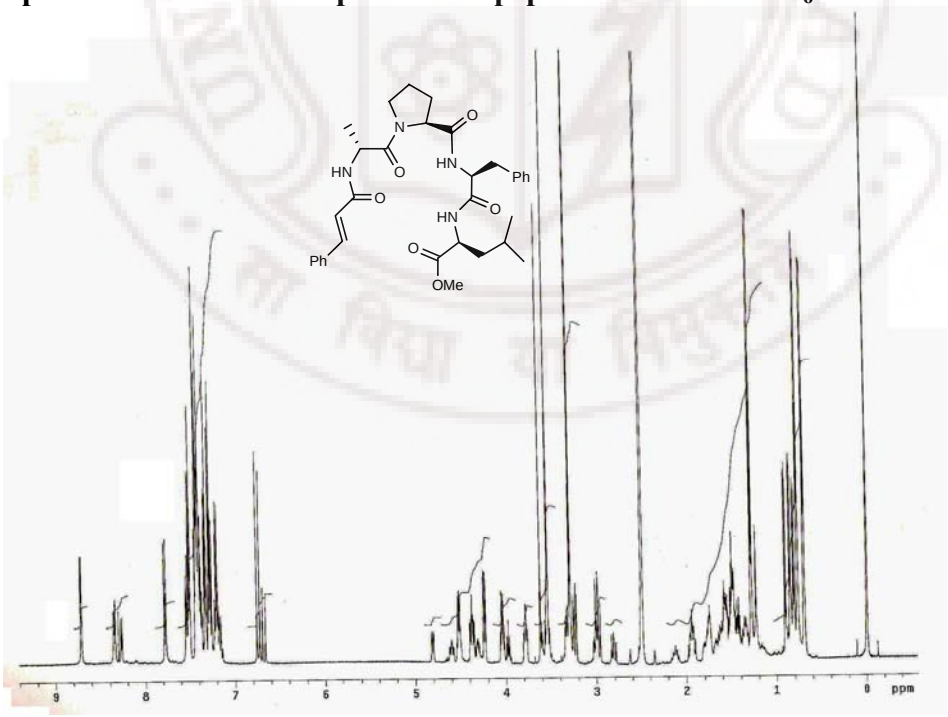
Spectrum 14: Expanded NOESY spectrum of peptide 11



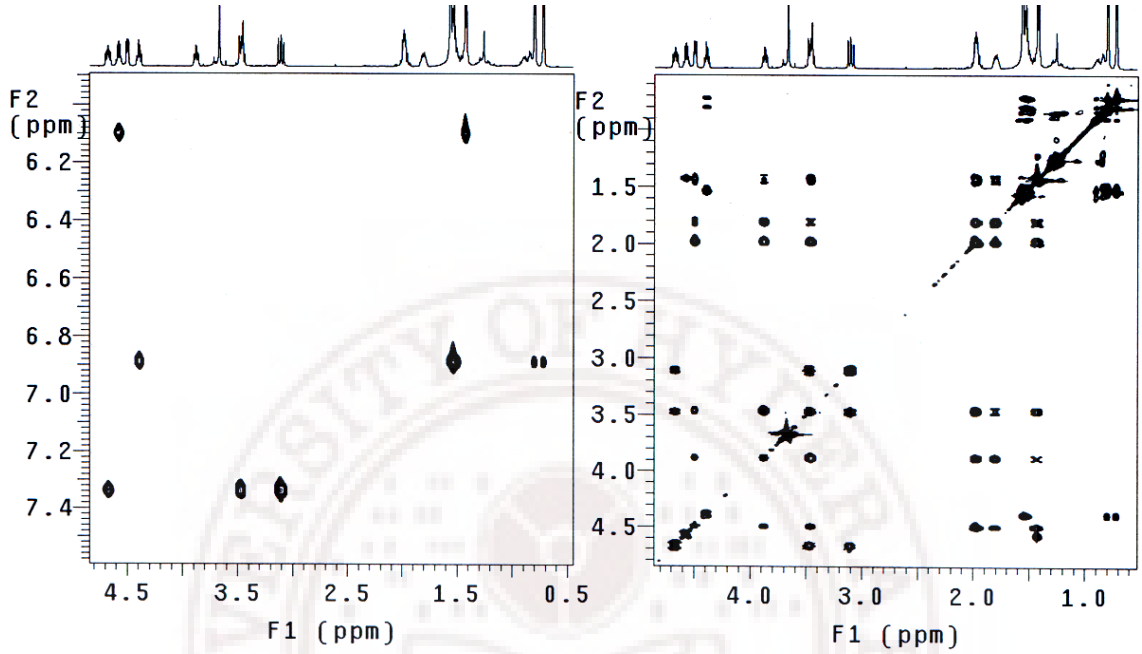
Spectrum 15: ^{1}H NMR spectrum of peptide 12 in $CDCl_3$



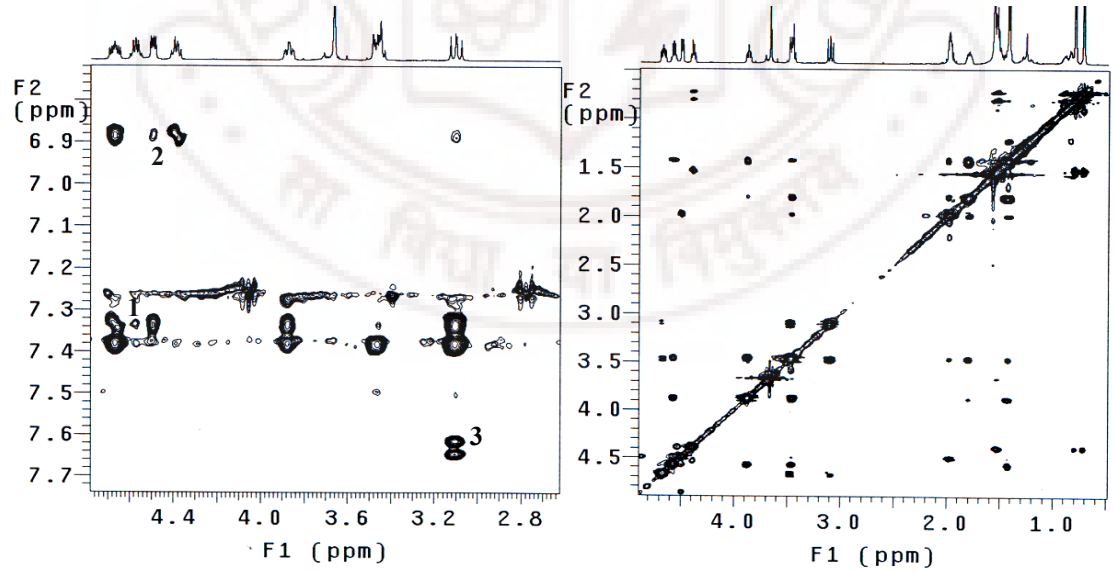
Spectrum 16: ^{1}H NMR spectrum of peptide 12 in DMSO-d_6



Spectrum 17: Expanded TOCSY spectrum of peptide 12

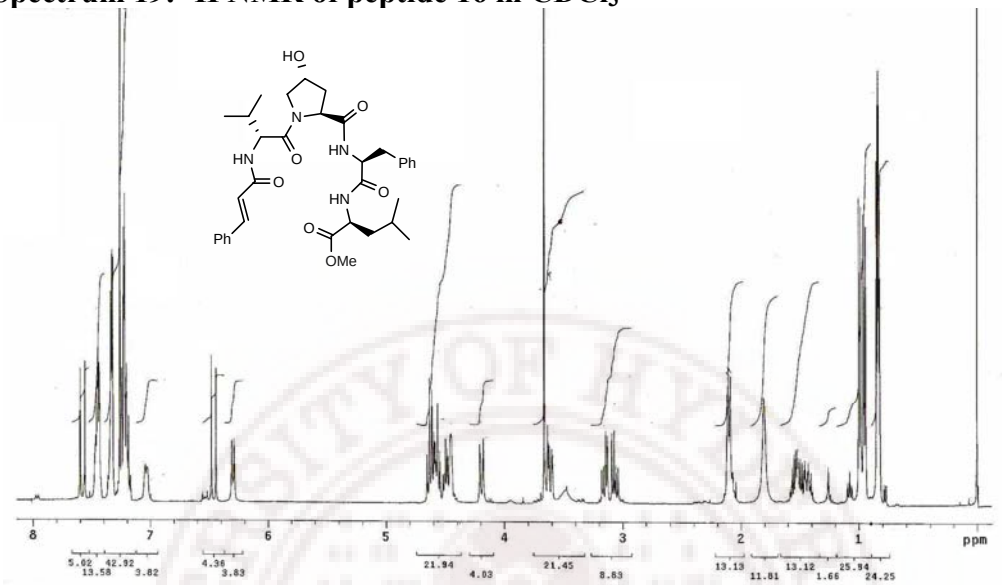


Spectrum 18: Expanded NOESY spectrum of peptide 12

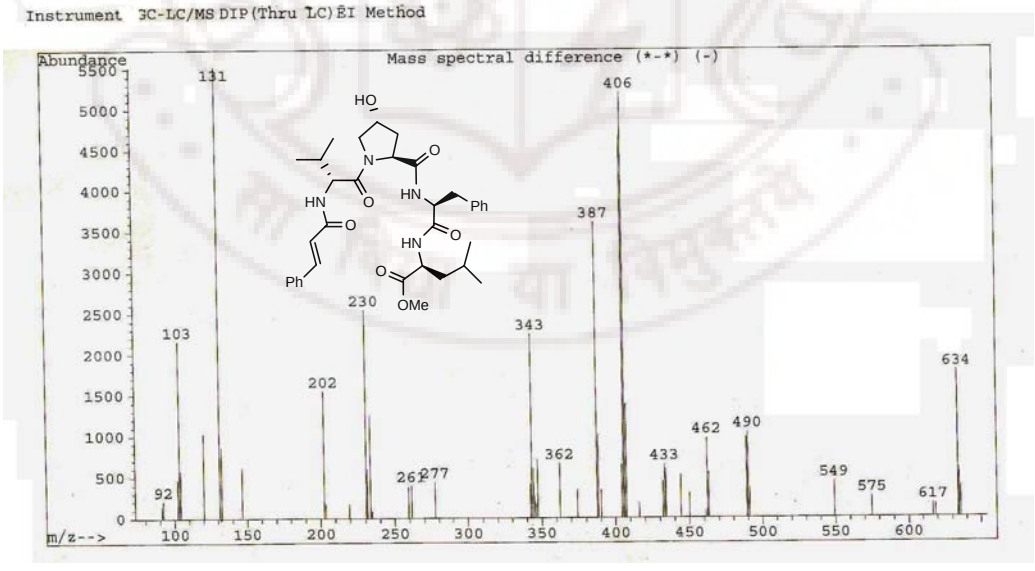


1-Phe NH / ^{D}Ala CαH, 2-Leu NH / Pro CαH, 3-Cinn CβH / Phe Cβ*(Pro-S)*H).

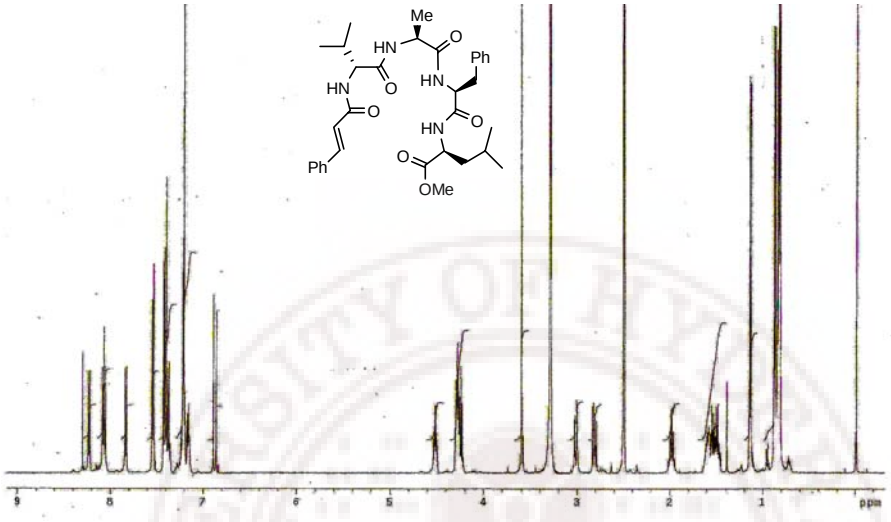
Spectrum 19: ^{1}H NMR of peptide 16 in $CDCl_3$



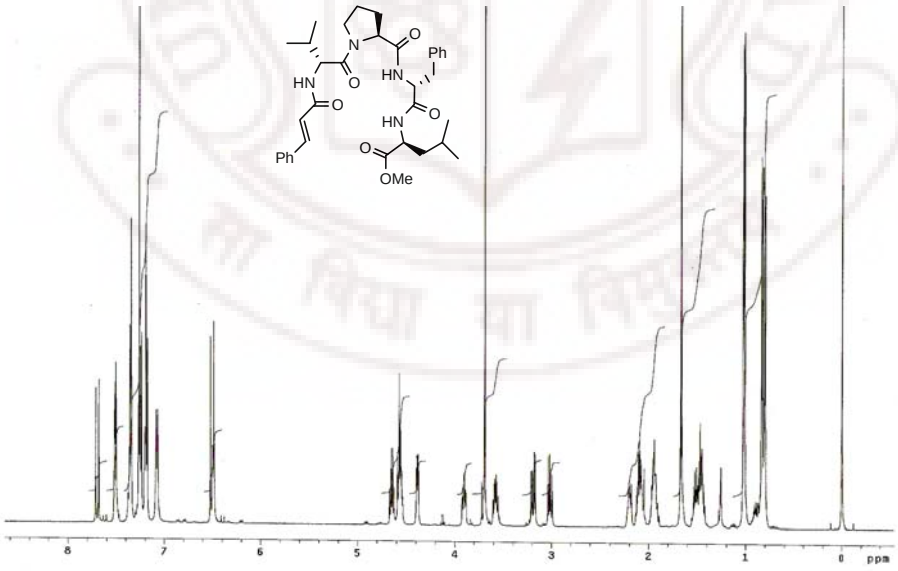
Spectrum 20: Mass spectrum of peptide 16



Spectrum 21: ^{1}H NMR of peptide 18 in $CDCl_3$



Spectrum 22: ^{1}H NMR spectrum of peptide 21 in $CDCl_3$



Chapter VI

Enantiodivergent Strategy to Access Both (+) and (-) Novioses from a Single Enantiomer of Pantolactone

6.1 Introduction

Natural products have inspired chemists and physicians for millennia. Their rich structural diversity and complexity has prompted synthetic chemists to produce them in the laboratory, often with therapeutic applications in mind. The natural product landscape offers entry into the drug discovery process in a number of ways. In the most direct case, a natural product may itself possess all of the potency, selectivity, and pharmacokinetic traits required to render it a clinically useful agent. More often are the instances where the natural products themselves serve as lead agents, providing the chemist with a structural platform which can be elaborated upon, or simplified, to yield a therapeutically valuable pharmaceutical. Analogues that can be accessed through modification of the natural product itself are considered to be "natural product derived." Alternatively, a biologically active natural product may serve as an inspiration for the medicinal discovery chemist, by providing insight into types of structural features that may prove valuable.

The impact of natural products on drug development can be felt across virtually every major therapeutic area. For instance, between 1981 and 2002, of the 90 antibacterial new chemical entities (NCE) approved by the FDA, 10 % were natural products while another 68 % were natural products derived.[1] Indeed, many of the most prevalent antibiotic agents used today are members of well

known natural product classes, including β-lactams (penicillins and cephalosporin C), macrolides (such as erythromycin), aminoglycosides (such as streptomycin), and glycopeptides (including vancomycin) (Figure-1).[2] The glycosylated units present in these natural products are required for the associated antibiotic and/or antitumor activities.[3]

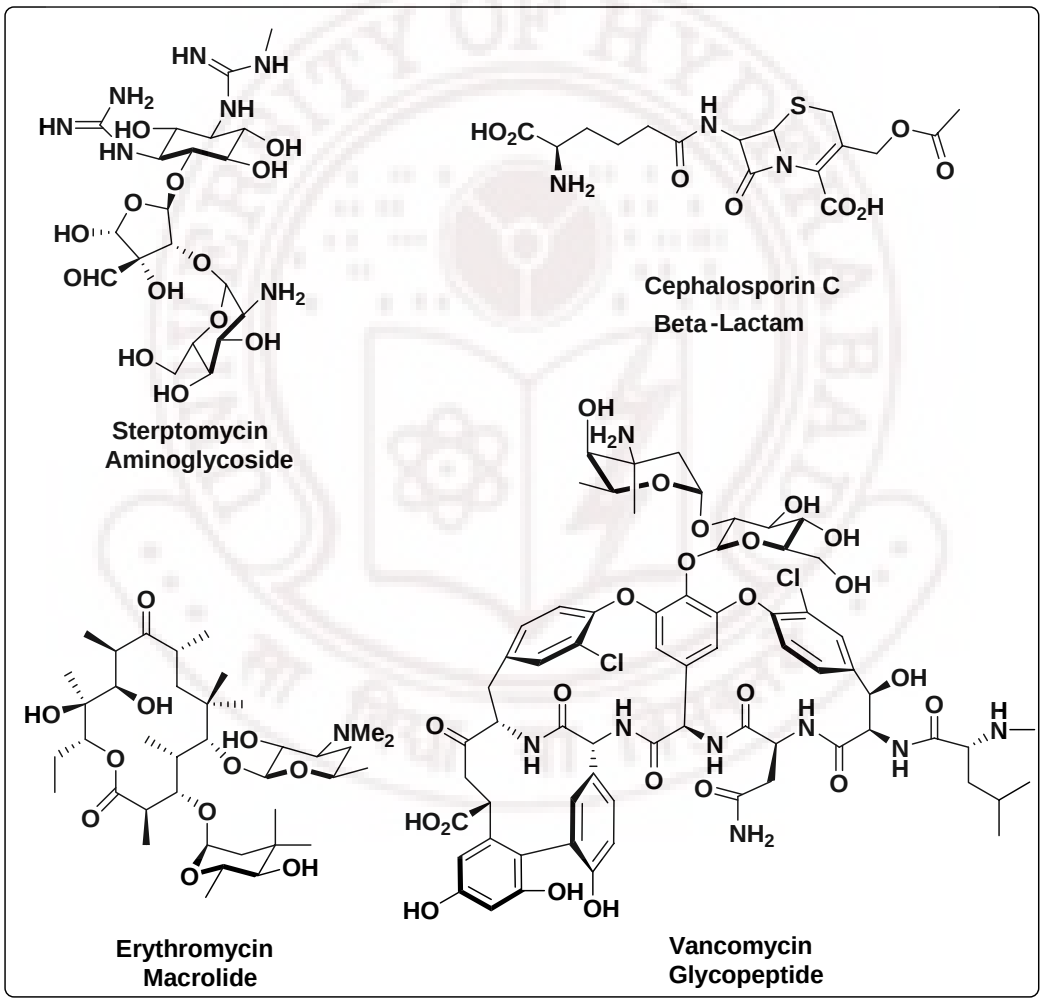


Figure-**1**: *Some naturally occurring antibiotics*

Owing to the bacterial resistance, many other antibiotics (both natural and synthetic) have also been discovered which act through different mechanisms (Figure-2).

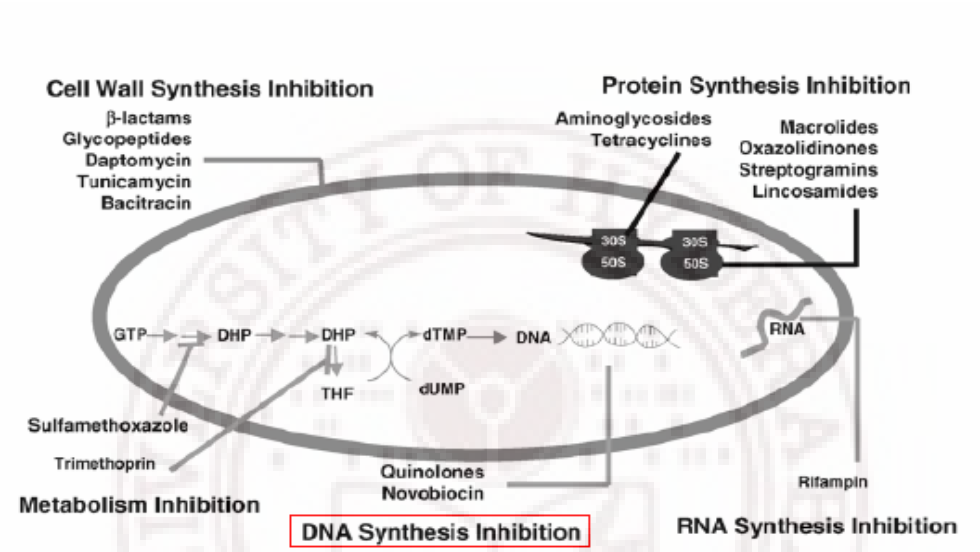


Figure-**2**: *Mode of action of different antibiotics*

The coumarin class of antibiotics (Novobiocin, Clorobiocin and Coumermycin A_1) (Figure-3) are members produced from various streptomyces species,[4] and each bears a noviosyl sugar component that imparts the functionality essential for biological activity. This family of antibiotics exerts its antibacterial activity by inhibiting ATPase activity of subunit B of DNA gyrase, a tetrameric A_2B_2 enzyme that belongs to a family of enzymes known as Topoisomerase. These ubiquitous enzymes play an important role in resolving topological problems that arise during the various processes of DNA metabolism, including transcription,

recombination, replication and chromosome portioning during cell division. Specifically, the aminocoumarins inhibit ATP hydrolysis in the GyrB subunit,[5] the same mode of action exhibited by the quinolone antibacterials such as ciprofloxacin. In general, these antibiotics have had limited use in the clinic because of problems associated with poor solubility and pharmacological properties.[6] Novobiocin has, however, received renewed attention as a result of its potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA) bacterial strains.[7]

The coumarin antibiotics contain three structural moieties: a noviose sugar, a 3-substituted coumarin ring, and a prenylated 4-hydroxybenzoic acid moiety. Clorobiocin differs structurally from novobiocin at two positions: C-8 of the coumarin ring and the 3-position of the noviose moiety. Novobiocin bears a carbamoyl group at the 3-position of the noviose moiety, while clorobiocin bears a 5-methyl-2-pyrrolecarboxy group, a structural modification that results in enhanced inhibitory activity of clorobiocin relative to novobiocin.[8] Coumermycin A1 is a dimer of the noviosyl coumarin components linked by a 3-methyl-2,4-dicarboxylpyrrole moiety and bears the 5-ethyl-2-pyrrolecarboxy group at the 3-position of the noviose ring. Apart from coumarin antibiotics, noviose can be found (in modified form) in Lipiaramycin and Endiyne toxin C-1027 Chr (Figure-**3**).

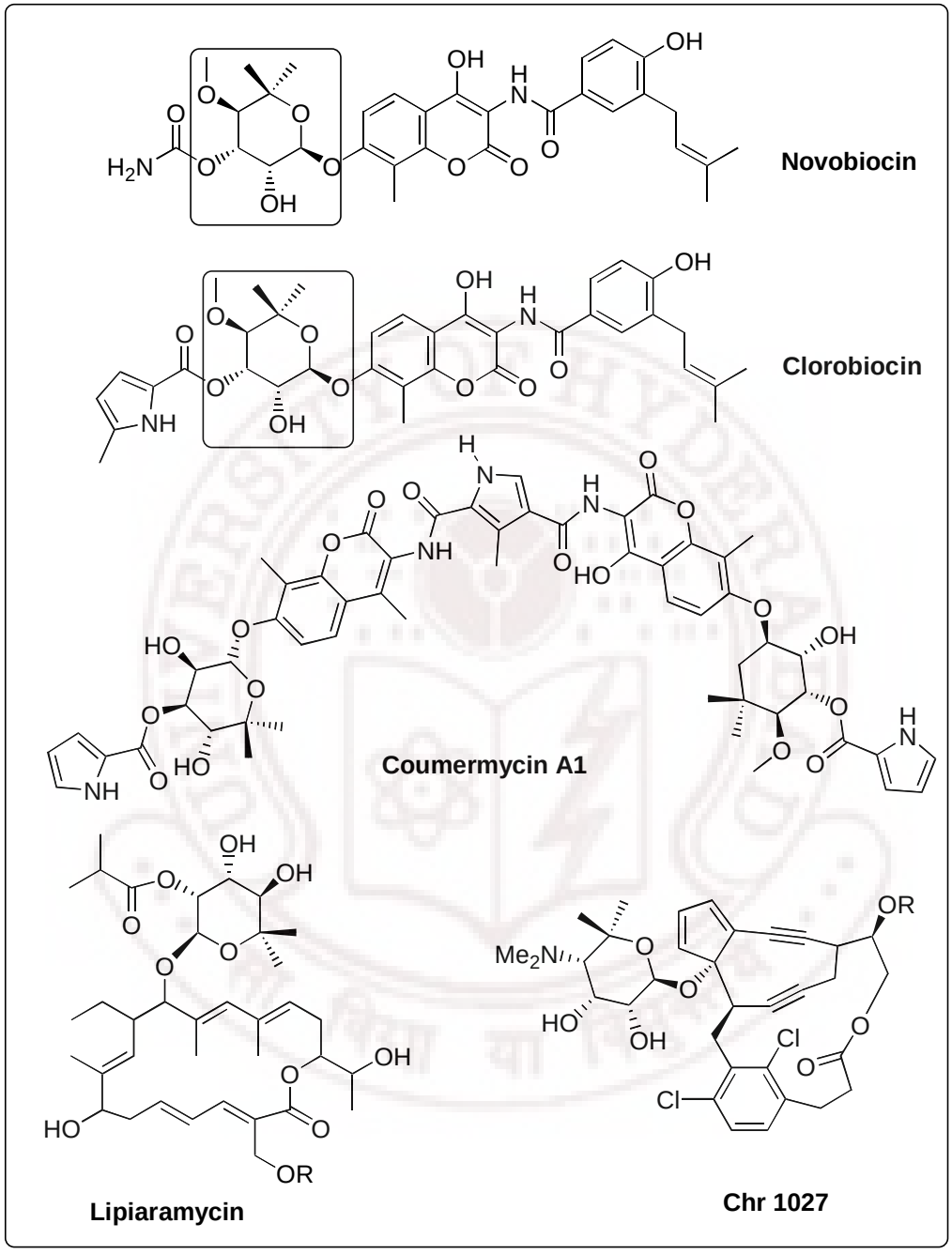


Figure-**3**: *Coumarin class of antibiotics*

Although originally identified as an inhibitor of type II topoisomerase, novobiocin has been used as an anticancer agent for over a decade.[9] Recent investigations have shown novobiocin to be an inhibitor of the 90 kDa heat shock proteins (Hsp90).[10] Heat shock proteins contain two nucleotide-binding site; the *N*-terminal ATP binding site is the region to which geldanamycin (GDA) and radicicol bind[11] and the *C*-terminus, which was recently shown to bind novobiocin. Hsp90 is a molecular chaperone responsible for the refolding of denatured proteins following cellular stress as well as for the conformational maturation of nascent polypeptides into biologically active three dimensional structures.[12] Multiple signaling pathways that are constitutively activated or up regulated in malignant cells leading to (a) self-sufficiency in growth signals, (b) insensitivity to antigrowth signals, (c) evasion of apoptosis, (d) limitless replicative potential, (e) sustained angiogenesis, and (6) tissue invasion/metastasis [12] are controlled by multiple oncogenic proteins such as Raf-1, HER2, Src-family kinases, steroid hormone receptors, polo-1-kinase, death domain kinase, protein kinase B, focal adhesion kinase, telomerase, hypoxia inducible factor, and MET kinase which are Hsp90 dependent.[12] Consequently, Hsp90 has emerged as a promising biological target for the development of cancer therapeutics because signaling nodes regulating all six hallmarks of cancers can be simultaneously disrupted by the inhibition of Hsp90 folding machinery.[13]

In the course of degradation studies of novobiocin,[14] a sugar component was isolated as an anomeric mixture of methyl or ethyl glycosides.[15] The structure of this monosaccharide, which was named noviose (Figure-**4**), was established as 4-*O*-methyl-5,5-dimethyl-L-lyxose.[16, 17]

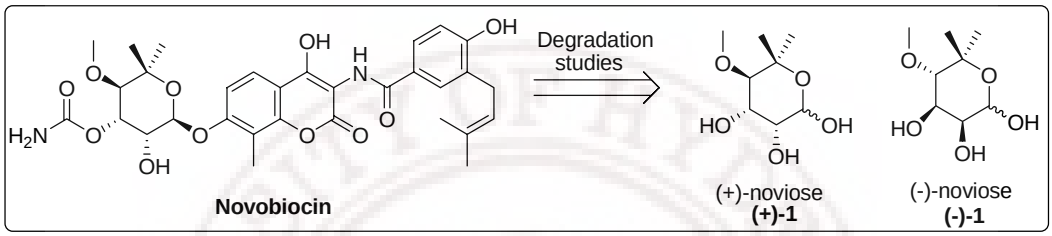


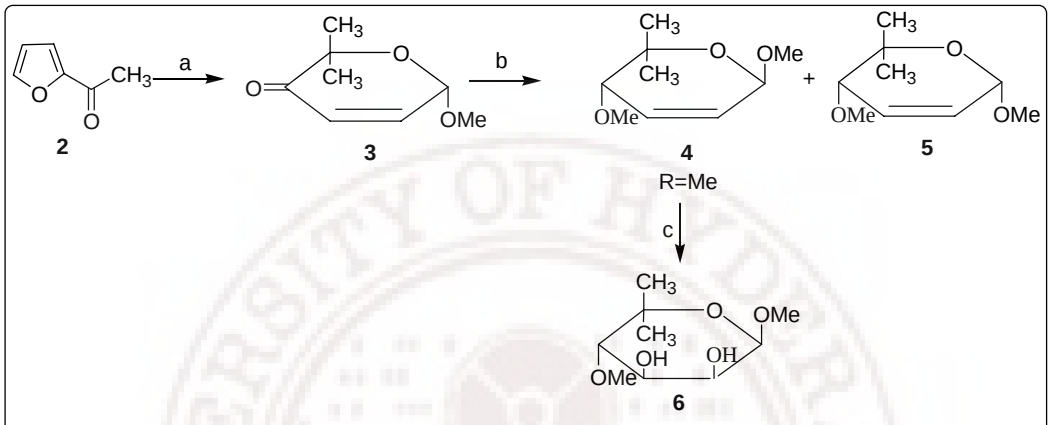
Figure-**4**: *Structure of Novobiocin and Noviose*

6.1.1 Previous Approaches

Several efforts for the synthesis of unique sugar moiety, noviose, has been made in the past based on the findings that the sugar component in novobiocin plays a critical role in eliciting biological activity. A brief prelude of previous approaches towards the synthesis of such moieties is mandatory before discussing our approach. Following the identification of (+)-L-noviose as the sugar moiety of novobiocin by Hinman *et al* [18] it was first synthesized by Kiss *et al* [19] starting from 1,2:5,6-di-*O*-isopropylidiene-α-D-glucofuranose, which was transformed into 1,1-dimethyl-D-glucitolderivative, then oxidized to epi-noviose and isomerized to (+)-1 in 15 steps. In 1976, Achmatowicz Jr. *et al* [20] reported the total synthesis in the racemic series starting from aetyl furan **2**, which after Grignard reaction, acetylation, and hydrolysis yielded the ketosugar derivative **3**.

Reduction followed by methylation and *cis*-hydroxylation afforded methyl β-DL-novioside **6** (Scheme-**1**).

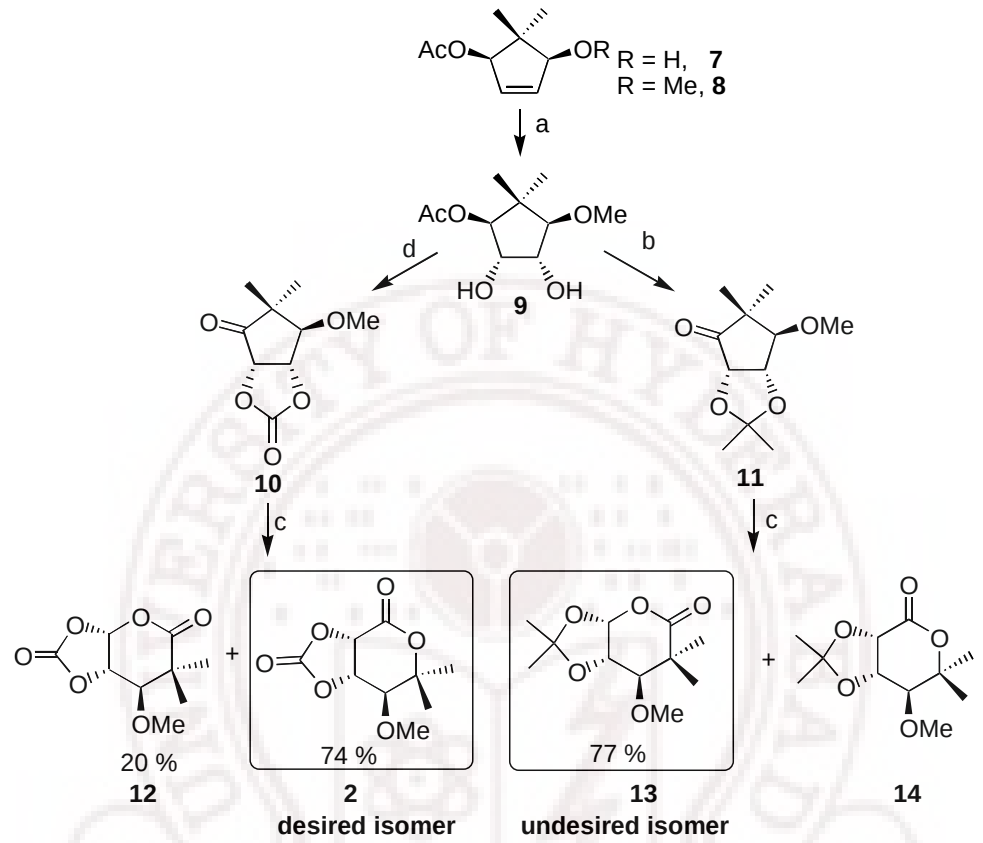
Scheme-**1**: *Synthetic approach of Achmatowicz et al*



Reagents and conditions: (a) i. MeMgBr, ii. CH_3OH/Br_2, iii. $H_3\overset{\oplus}{O}$ iv. $(OMe)_3CH$, $BF_3(OEt)_2$ (b) i LAH/ether, ii. $(OMe)_2SO_2$, (c) H_2O_2/OSO_4.

Subsequently Klemer *et al* [21] reported an efficient seven step synthesis starting from L-rhamnose in 33 % overall yield. However, it suffered from high costs of the starting material and the fact that only one antipode was accessible. The *de novo* synthesis of (–)-noviose, the unnatural antipode, was reported by Kreiser *et al* [22] for the first time in seven steps.The formation of methyl ether **8** under Lewis-acidic conditions by carbene insertion[23] (utilizing diazomethane in Et_2O catalyzed by $BF_3.Et_2O$) in the presence of the acetate group in **7** and influence of carbonate **10** and acetonide **11** towards migration during the Baeyer-Villiger oxidation reaction to afford the desired isomer were key transformations during their synthesis. (scheme-**2**).

Scheme-**2**: *Synthetic approach of Klemer et al*

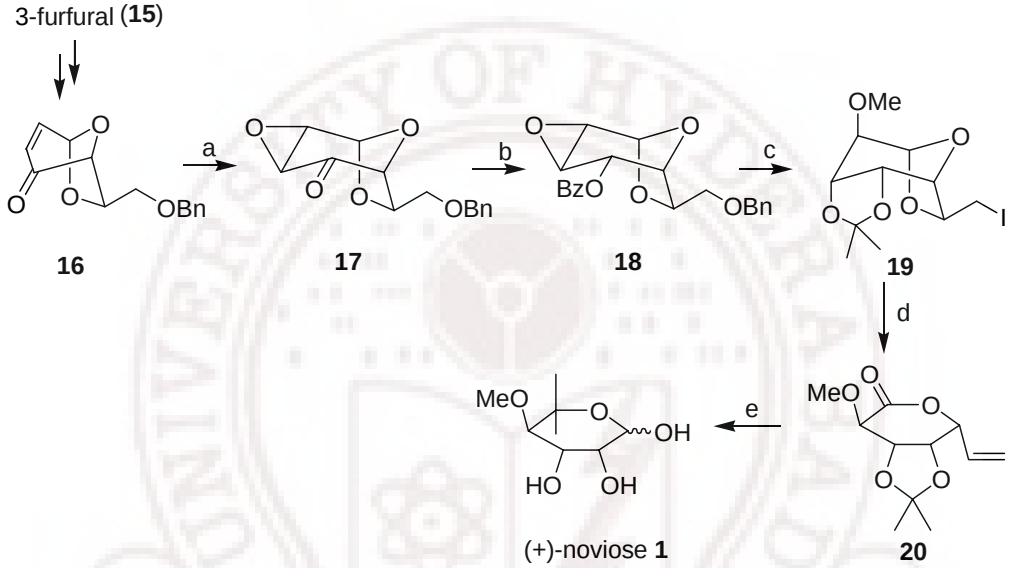


Reagents and conditions: (a) OsO_4, NMO, acetone/H_2O, 0 ^oC (b) i. $Me_2C(OMe)_2$, TsOH, acetone, rt ii. KOH, MeOH/H_2O, rt iii. $COCl_2$, DMSO, NEt_3, CH_2Cl_2, -78 ^oC (c) mCPBA, CH_2Cl_2 rt, 3 days (d) i. KOH, MeOH/H_2O, rt ii. triphosgene, pyridine, CH_2Cl_2, -70 ^oC, iii. PDC, CH_2Cl_2, rt, 3 days.

Ogasawara and co-workers[24] reported a stereocontrolled synthesis of (+)-L-noviose using a sugar building block,[25] which was prepared from furfural in enantiomerically pure form by chemical or enzymatic procedures.[26] The dioxabicyclo[3.2.1]octane (**16**) convex face selectivity and high functionality was exploited by diastereoselective epoxidation followed by diastereoselective reduction resulting in the formation of three consecutive oxygen bearing chiral

centers **18**. Regioselective epoxide opening, Internal acetal cleavage, oxidation and simple functional group transformations accomplished the synthesis of (+)-noviose as shown in scheme-**3**.

Scheme-**3**: *Synthetic approach of Ogasawara et al*

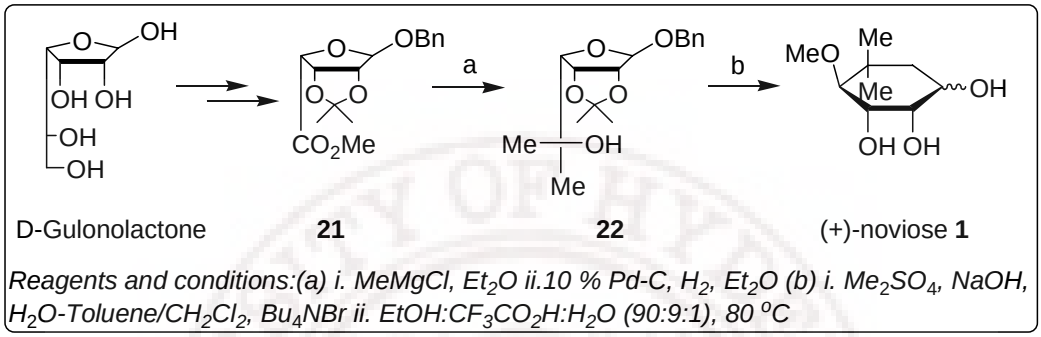


Reagents & conditions: (a) 30 % H_2O_2, 0.5 N NaOH, THF, 0 ^oC (b) i. $NaBH_4$-$CeCl_37H_2O$, MeOH, 0 ^oC, ii, BzCl, pyridine, CH_2Cl_2, 0 ^oC, (c) i. $BF_3(OEt)_2$, toluene, ii. NaOMe, MeOH iii. $Me_2C(OMe)_2$, PPTS, toluene, reflux, iv. MeI, NaH, THF v. H_2 Pd-C, MeOH vi. MsCl, NEt_3, CH_2Cl_2 vii. LiI, THF reflux (d) i. Zn, AcOH:MeOH (1:10) ii. TPAP, NMO, mol. sieves, CH_2Cl_2 (e) i. MeLi, THF, 0 ^oC, ii. OsO_4 (cat), $NaIO_4$, 50 % aq. THF, iii. Dowex 50-W, H_2O, 70 ^oC.

Utilizing commercially available L-gulonolactone, Kocevar *et al*[27] reported the synthesis of L-noviose by converting it into the key ester derivative 1-*O*-benzyl methyl 2,3-*O*-(1-methylethylidene)-α-L-lyxofuranosiduronate **21**. An appropriate selection of protecting groups enabled transformation of **21** to 4-*O*-demethyl-L-

noviofuranose **22** which was further converted to L-lyxopyranoses using phase transfer conditions (Scheme-4).

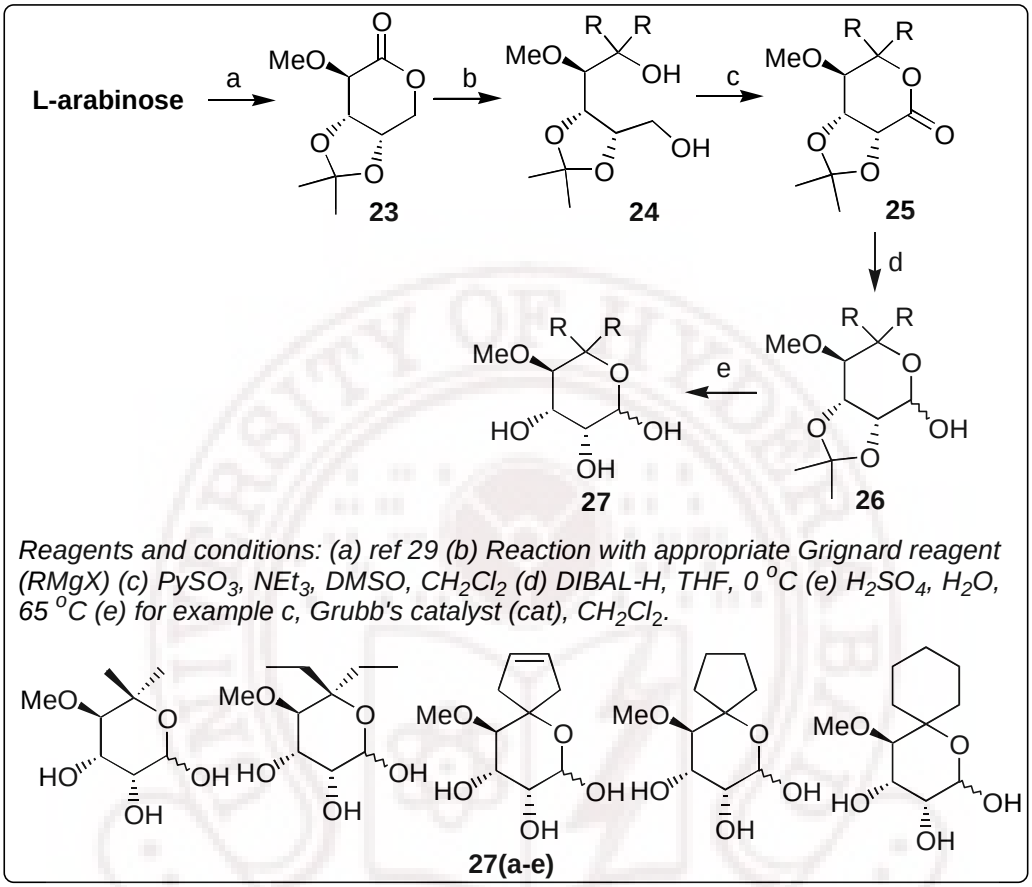
Scheme-**4**: *Synthetic approach of Kocevar et al*



Reagents and conditions:(a) i. MeMgCl, Et_2O ii.10 % Pd-C, H_2, Et_2O (b) i. Me_2SO_4, NaOH, H_2O-Toluene/CH_2Cl_2, Bu_4NBr ii. EtOH:CF_3CO_2H:H_2O (90:9:1), 80 ^oC

During studies aimed at identifying potent gyrase inhibitors, Musicki *et al* [28] reported the marked influence of the 5,5′-dimethyl group of noviose in determining the antibacterial spectrum of antibiotics, which prompted them to the stereoselective synthesis of 5-monosubstituted and 5,5-disubstituted noviose derivatives starting from L-arabinose.[29] The preparation of 5,5-disubstituted noviose derivatives instigated from the lactone **23** (readily available from L-arabinose in 5 steps) which was reacted with different Grignard reagents to provide corresponding diols **24**. Oxidative cyclization, DIBAL-H reduction and acetonide cleavage afforded noviose derivatives. Particularly noteworthy is the accessibility of spirocyclopentene noviose derivative (**27** c-e) *via* ring closing metathesis reaction as shown in scheme-**5**.

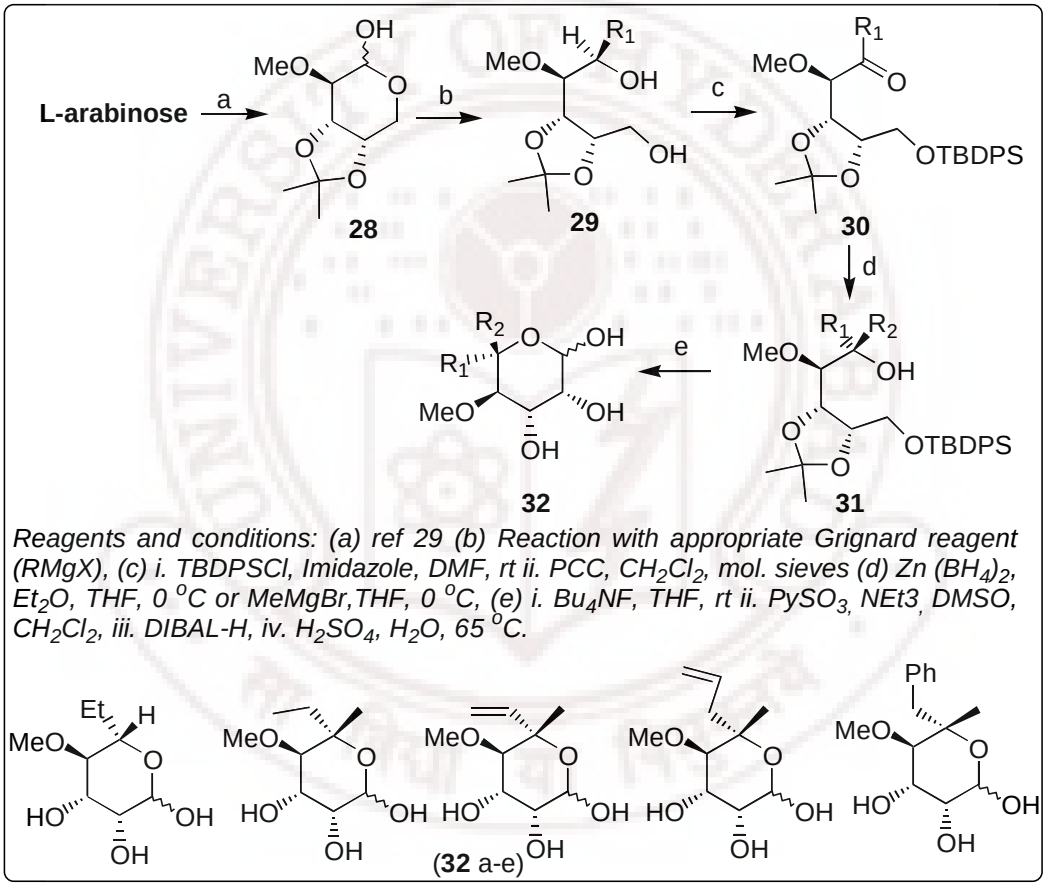
Scheme-**5**: *Synthetic approach of Noviose derivatives by Musicki et al*



A modified approach for the stereoselective introduction of the 5-equatorial alkyl substituent's while keeping the methyl group or the hydrogen atom in the alkyl position was adopted involving regioselective protection of the primary alcohol **29** with TBDPS group followed by oxidation of secondary hydroxyl group to ketone **30**. Diastereoselective reduction of keto group using $Zn(BH_4)_2$ or Grignard addition led to the equatorial attack of alkyl group from the equatorial side based on Cram's rule to give **31**. Simple functional group manipulations led

to noviose analogues **(32 a-e)** having different alkyl groups (Scheme-6). These noviose analogues were used for SAR studies by coupling with the coumarin building block which led to the identification of a most potent analogue RU79115 possessing antibacterial activity.[30]

Scheme-**6**: *Synthetic approach of Noviose derivatives by Musicki et al*

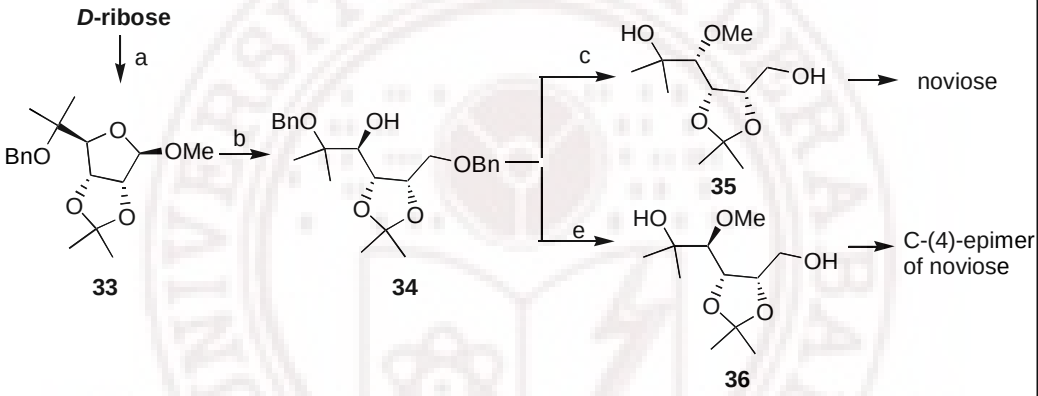


Reagents and conditions: (a) ref 29 (b) Reaction with appropriate Grignard reagent (RMgX), (c) i. TBDPSCl, Imidazole, DMF, rt ii. PCC, CH_2Cl_2, mol. sieves (d) Zn $(BH_4)_2$, Et_2O, THF, 0 ^oC or MeMgBr,THF, 0 ^oC, (e) i. Bu_4NF, THF, rt ii. $PySO_3$, NEt3, DMSO, CH_2Cl_2, iii. DIBAL-H, iv. H_2SO_4, H_2O, 65 ^oC.

The C(4)-epimer of noviose was synthesized by Gammon *et al* .[31] Their synthesis emanated with the conversion of D-ribose to the tertiary alcohol which was protected as its benzyl ether **33**. The ribose ring cleavage and subsequent protection of the primary alcohol resulted in the formation of key intermediate

34. On one hand, the inversion in stereochemistry of secondary alcohol by oxidation-reduction sequence followed by pyranose ring formation led to the synthesis of noviose. On the other hand, methylation of secondary alcohol followed by pyranose ring formation led to the synthesis of C-(4)-epimer of noviose (Scheme-**7**).

Scheme-7: *Synthetic approach of Gammon et al*

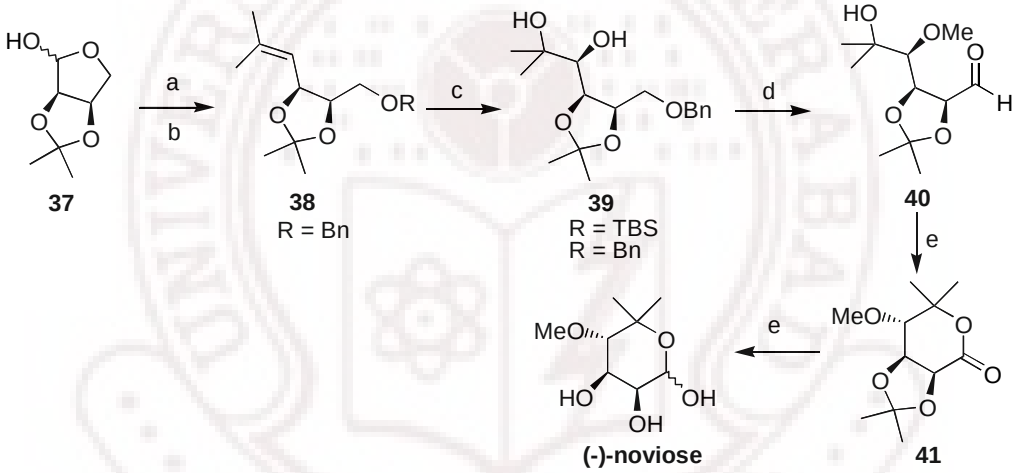


Reagents and conditions: (a) i. MeOH, H_2SO_4, 0 ^oC, ii. acetone, H_2SO_4, 0 ^oC-rt iii. $RuO_2.H_2O$, aq. $NaIO_4$ (10 % m/v) ACN, CCl_4 (1:1), iv.K_2CO_3, MeI, DMF, rt v. MeMgI Et_2O, reflux, vi. NaH, BnBr, nBu_4NI, THF, reflux, (b) i. HCl (1M) dioxane, 60 ^oC, ii. acetone, H_2SO_4, 0 ^oC, iii. LAH, THF, 0 ^oC, iv. NaH, BnBr, THF, rt (c) i. NaH, THF, reflux, then MeI, ii Pd-C, H_2, EtOH,(f) i. Swern oxidation ii. DIBAL-H, THF, -78 ^oC, iii. EtOH: CF_3CO_2H : H_2O (90:9:1), 80 ^oC (d) i. Swern oxidation, ii. K-selectride, toluene, 0 ^oC-rt iii. NaH, MeI, THF, iv. Pd-C, H_2, EtOH, (d) i. TPAP (cat), NMO, mol.sieves, ii. DIBAL-H, THF, -78 ^oC, iv. EtOH: CF_3CO_2H : H_2O (90:9:1), 80 ^oC

Of late, Blagg *et al* [32] reported that both enantiomers of noviose could be readily prepared from commercially available 2,3-*O*-isopropylidene-D-erythronolactol or readily preparable 2,3-*O*-isopropylidene-L-erythronolactol respectively.[33] Their synthesis for (–)-noviose started with a Wittig reaction between the hemiacetal and the ylide of isopropyl triphenylphosphonium bromide providing the

trisubstituted olefinic product **38** (scheme-**8**). After initial attempts to oxidize this double bond in the presence of tert-butyldimethylsilyl ether resulted in poor diastereoselectivity, the benzyl ether containing olefin **38** was hydroxylated using OsO_4/NMO conditions to afford the desired alcohol **39** in 2:1 ratio as the key intermediate. Subsequently, simple functional group transformations yielded (–)-noviose **2**.

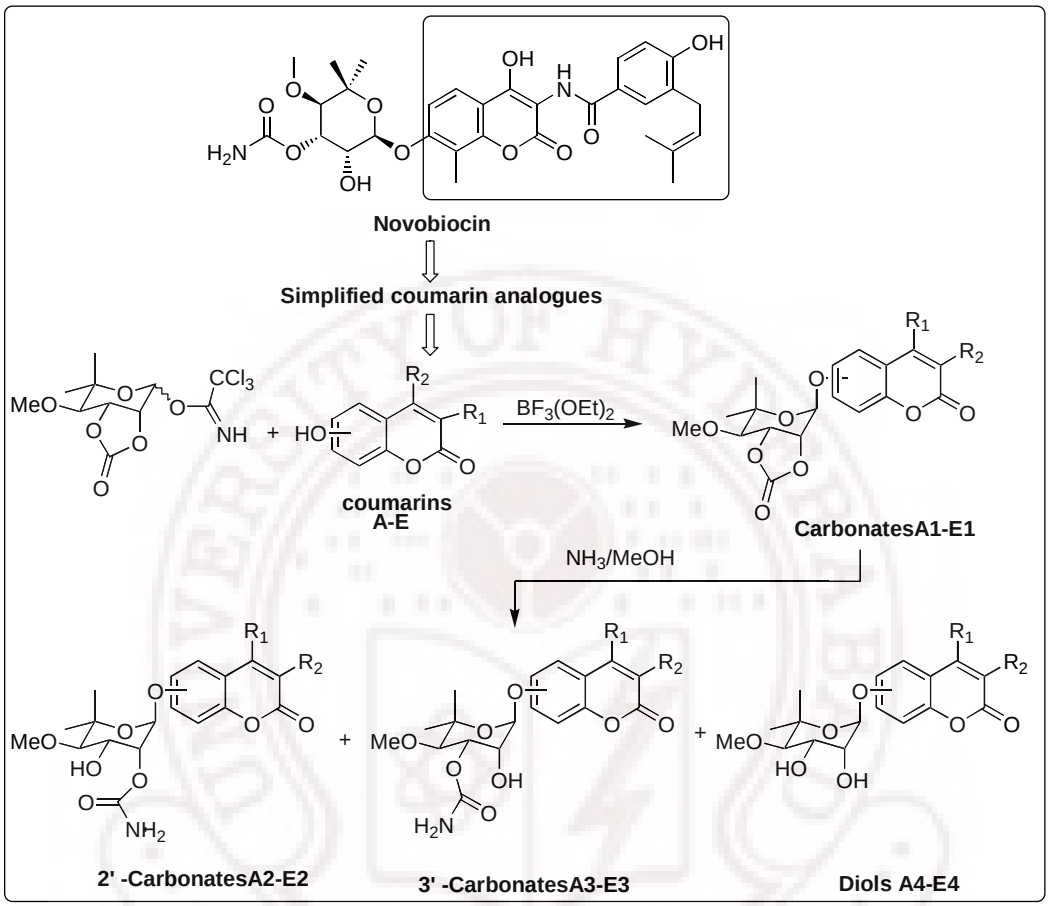
Scheme-8: *Synthetic approach of Blagg et al*



Reagents and conditions: (a) Isopropyl triphenylphosphonium bromide, n BuLi, dry THF, (b) NaH, BnBr, THF, rt (c) OsO_4/NMO, acetone:H_2O, (d) i. KO^tBu, MeI, ii. PCC, CH_2Cl_2, rt (e) i Pd/ C EtOAc (f)i. DIBAL-H, ii. H_2SO_4

It is noteworthy that their synthetic protocol was followed by a small library of novobiocin analogues by coupling noviose with simplified coumarin derivatives.[34] These analogues were found to be more potent than novobiocin in inhibiting Hsp90 protein folding machinery (Scheme-**9**).

Scheme-9: *Schematic representation of novel Novobiocin analogues*



Our interest towards finding a novel route to synthesize noviose stemmed from Blagg's approach of identifying novel Hsp90 inhibitors. The fact that novobiocin binds to and inhibits a previously unrecognized C-terminal ATP binding pocket of Hsp90 (*novel mechanism of action*), low yields and poor selectivity encountered during the key dihydroxylation reaction in the synthesis of noviose by Blagg's approach and the fact that most of the reported syntheses of noviose

emanate from sugar or sugar derived scaffolds (Figure-**5**) prompted us to look into an alternative route.

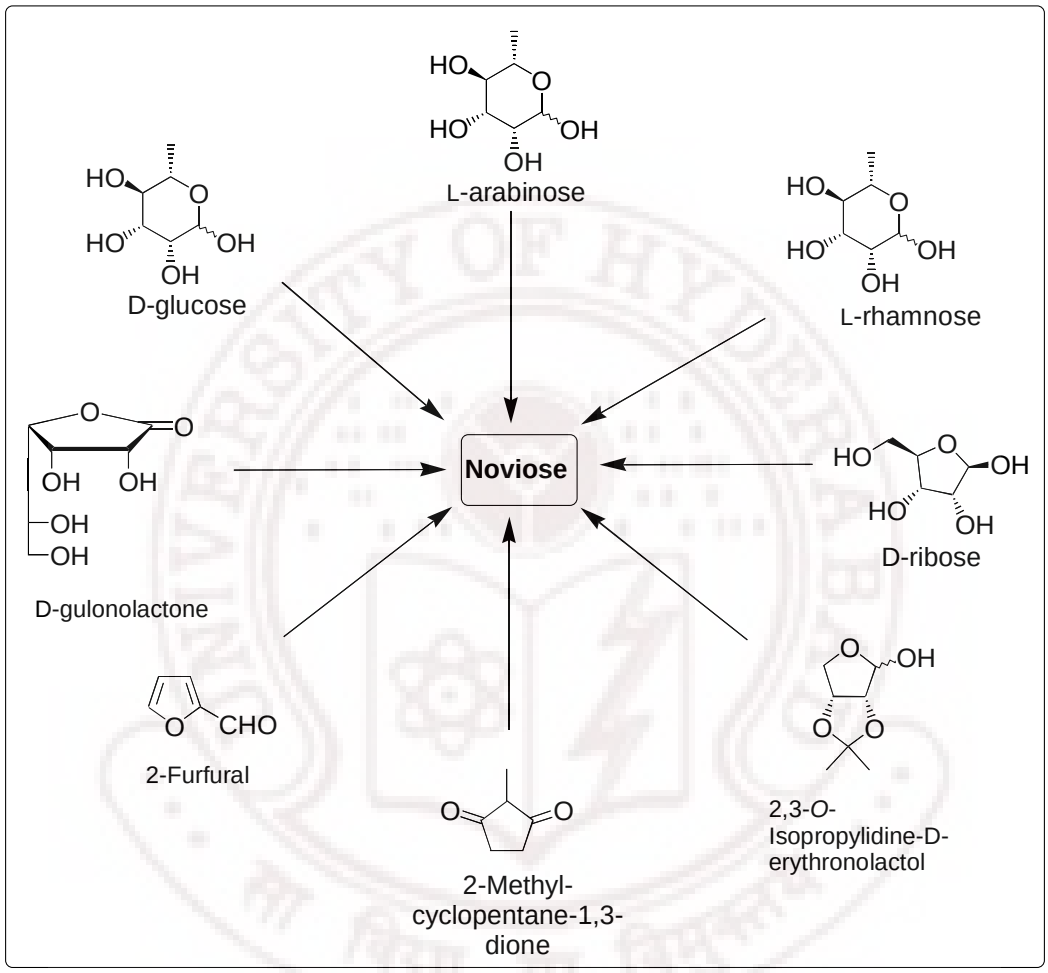


Figure-**5**: *Different approaches for the synthesis of noviose*

We believed that any approach capable of yielding both the enantiomers from a relatively cheap starting material (preferably a non sugar scaffold) in minimal number of steps is highly desirable as this would enable us to carry out further SAR studies in this area to gain insights into the structural requirements needed

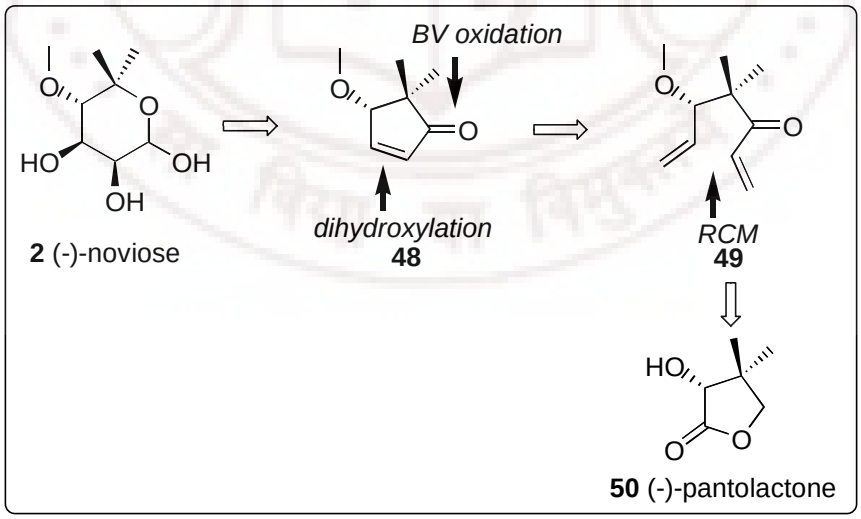
for the development of more potent and therapeutically useful small molecule for disrupting Hsp90.

6. 2 Results and Discussion

6 .2.1 Retrosynthetic Analysis

We looked into literature for commercially available gem-dimethyl group containing compounds and found both (*R*) and (*S*)-pantolactone (non-sugar scaffolds) to be an ideal choice to access both the antipodes of noviose. We first aimed at synthesizing (–)-noviose **2** which was thought to retrosynthetically, originate from cyclopentenone **48 (**Scheme-10**)** *via* dihydroxylation of the double bond and Baeyer-Villiger (BV) oxidation of the corresponding ketone. It was proposed that ring-closing metathesis (RCM) could generate this α, β-unsaturated ketone from its acyclic precursor **49**, which in turn could be prepared from commercially available (–)-pantolactone **50**.

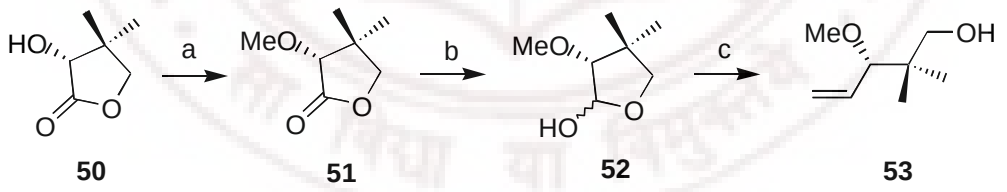
Scheme-10: *Retrosynthetic analysis*



6.2.2. Synthesis

As a first step of our synthesis, we needed to alkylate the secondary hydroxy group of (–)-pantolactone. Previous attempts to alkylate using a strong base such as NaH or milder base such as Cs_2CO_3 have led to partial or complete racemization of the chiral center owing to the presence of a carbonyl group adjacent to the chiral center. Following the procedure developed by Trost *et al*, [35] the alkylation of (–)-pantolactone was accomplished using Ag_2O and MeI under high pressure conditions in almost quantitative yields with high enantiomeric excess. The resulting lactone **51** was then reduced to lactol **52** using DIBAL-H which underwent a smooth Wittig reaction using methyl triphenylphosphonium bromide and n-butyl lithium at -78 °C to furnish the primary alcohol **53** in good yields (scheme 11).[36]

Scheme-11: *Preparation of compound* **53** *from (D)-Pantolactone*

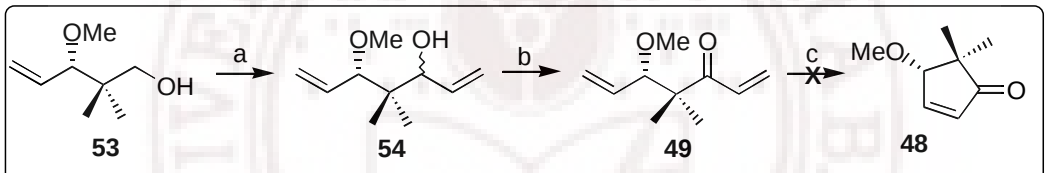


Reagents & conditions: (a) Ag_2O, MeI, MeCN, 58 ^oC, 6h, 86 % (b) DIBAL-H, -78 ^oC, CH_2Cl_2, 4h (c)n-BuLi, methyl triphenyl phophonium bromide, dry THF, -78 ^oC 1h, rt 18h, 81 % over two steps

The primary alcohol was oxidized to aldehyde using Swern conditions and subsequently reacted with vinylmagnesium bromide to furnish the corresponding diene **54** as a 1:1 diastreomeric mixture in excellent yield. As per our

retrosynthetic strategy, we oxidized the secondary hydroxyl group using Jones reagent to afford acyclic enone with an aim of performing ring closing metathesis reaction to obtain **48**. However, the dienone failed to cyclize with first and second generation Grubb's catalyst even under forcing conditions in DCM (scheme-**12**). The failure of the enone to undergo RCM reaction was attributed to the difference in the reactivity of the acrolyl double bond (pseudo double bond because of conjugation) and the double bond present adjacent to the methoxy group in **49.**

Scheme-12: *Preparation of enone* **48** *from compound* **53**

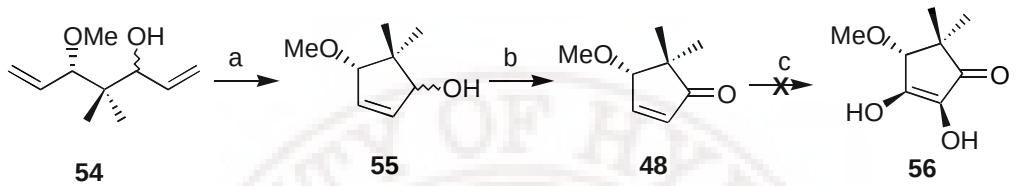


Reagents and conditions: (a) i. $(COCl)_2$, DMSO, dry CH_2Cl_2, -78 ^oC,4h ii. vinyl magnesium bromide, dry THF, 0 ^oC, 30 min, 82 % over two steps (b) Jones reagent, Et_2O, rt, 30 min, 82 % (c) Grubb's catalyst 5 mol %, CH_2Cl_2, rt, 10-12h.

We then resorted to cyclize the diastereomeric diene **54**, which underwent smooth cyclization in near quantitative yield when 5 mol % of Grubb's first generation catalyst was used at room temperature to furnish **55**, which upon oxidation with Jones reagent[37] gave cyclopentenone **48**. The strong carbonyl absorption (1711 cm^{-1}) in the IR spectrum, characteristic olefinic signals at 7.15 ppm and 6.18 ppm in the ^1H NMR spectrum and the 8 line ^{13}C spectrum were in agreement with the structure of enone. At this stage we attempted to dihydroxylate the double bond using OsO_4 conditions. However, we ended up

with an undesired product with a loss of methyl singlet corresponding to the methoxy group in the ^{1}H NMR spectrum. A valiant attempt to characterize this product failed (Scheme-**13**).

Scheme-13: *Preparation of compound* **56** *from compound* **54**

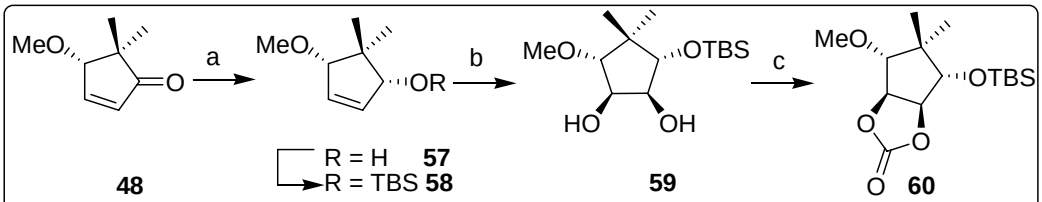


54 **55** **48** **56**

Reagents and conditions: (a)Grubb's catalyst, CH_2Cl_2, rt, 10-12h, 91 % (b) Jones reagent, Et_2O, rt, 30 min, 86 % (c) i. OsO_4, NMO, acetone, H_2O, t-BuOH (1:1:1), rt, 2h.

To circumvent this problem we adapted an alternative approach as described in Scheme-**14**. Luche reduction[38] of cyclopentenone **48** using $NaBH_4$-$CeCl_3.7H_2O$ in methanol gave the allylic alcohol **57** with excellent diastereoselectivity, which was protected as the silyl ether to give compound **58.** The *syn*-stereochemistry of the alcohol with respect to the methoxy group is predictable based on hydride approach from the opposite side to the methoxy group. Interestingly, dihydroxylation of the resulting alkene using a combination of osmium tetroxide and *N*-methylmorpholine-*N*-oxide monohydrate[39] afforded the diol **59** in good yield and selectivity. The presence of pertinent signals (δ 3.84-3.97 ppm, m, 2H) in the ^{1}H NMR spectrum coupled with 14 line ^{13}C NMR spectrum confirmed the assigned structure. The diol was subsequently protected as its carbonate derivative **60** using triphosgene and NEt_3 in dry DCM in excellent yields.

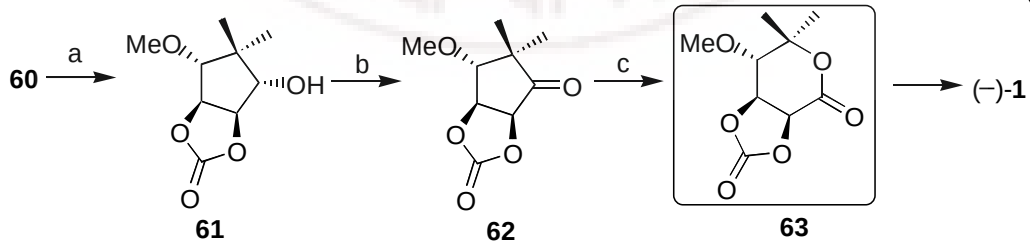
Scheme-**14** *Preparation of compound* **60** *from enone* **48**



Reagents and conditions: (a) i. $NaBH_4$, $CeCl_3.7H_2O$, MeOH, 0 ^oC, 45 min, 96%; (b) TBSCl, imidazole, dry CH_2Cl_2, 0 ^oC, 2h, 83%; (c) i. OsO_4, NMO, acetone, H_2O, t-BuOH, rt, 2h, 89%; (d) triphosgene, NEt_3, CH_2Cl_2, 0 ^oC - rt, 2h, 92%.

With the required stereocenters installed, the silyl group was deprotected using 1M TBAF solution in excellent yields to furnish the alcohol **61**. Following Swern oxidation conditions[40] or by using pyridinium dichromate, the alcohol was converted to 5-membered ketone **62** and then subjected to Baeyer-Villiger oxidation[41] with *m*-CPBA to yield the lactone **63** without any trace of regioisomeric lactone (scheme-**15**). The spectral data of synthetic **63** was compared with reported data and found to be identical $[\alpha]_D$ = +11.1 (*c* = 0.45, $CHCl_3$), lit.: $[\alpha]_D$ = +12.3 (*c*, 1.44, $CHCl_3$).

Scheme-**15** *Preparation of compound* **(**–**)-1** *from compound* **60**



Reagents and conditions: (a) TBAF, THF, 0 ^oC, 1h, 94%; (b) i. $(COCl)_2$, DMSO, NEt_3, CH_2Cl_2, rt, 4h ii. m-CPBA, CH_2Cl_2, 3 days, rt, 90% for two steps (c) DIBAL-H, ref.22

Having accomplished the synthesis of (–)-**1** starting from (–)-pantolactone**,** one could synthesize (+)-**1** from the corresponding (+)-pantolactone following a similar synthetic strategy.

6.2.3. Enantiodivergent Strategy

Commercially available (–)-pantolactone had served as a starting material for our synthetic approach to (–)-1. Unfortunately, the (+)-enantiomer of pantolactone is very expensive[42] and we were confronted with the possibility that our synthesis would not be amenable to the preparation of multigram quantities of (+)-noviose and its mimetics. We therefore conceptualized that incorporation of a labile group in place of methyl in intermediate **51** would enable us to transform it to the key intermediate (Figure-**6**), a molecule that would serve as a two-edged sword because it possesses a masked symmetry which can be exploited for the synthesis of either (+) or (–)-noviose through selective modifications on either side of the molecule. This novel approach is in contrast to the previous approaches which use two different starting materials to access both enantiomers of noviose.

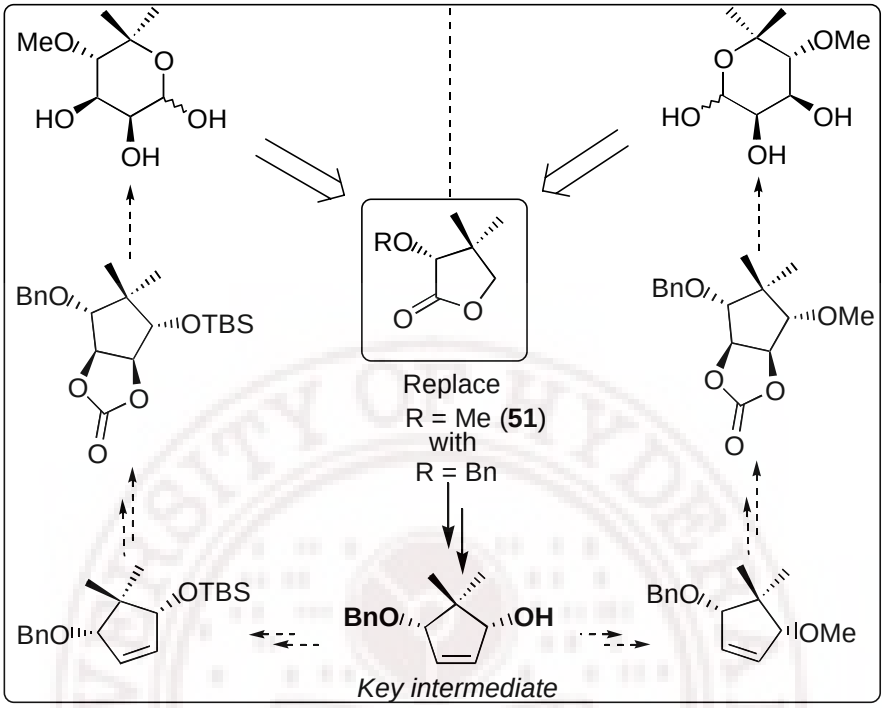
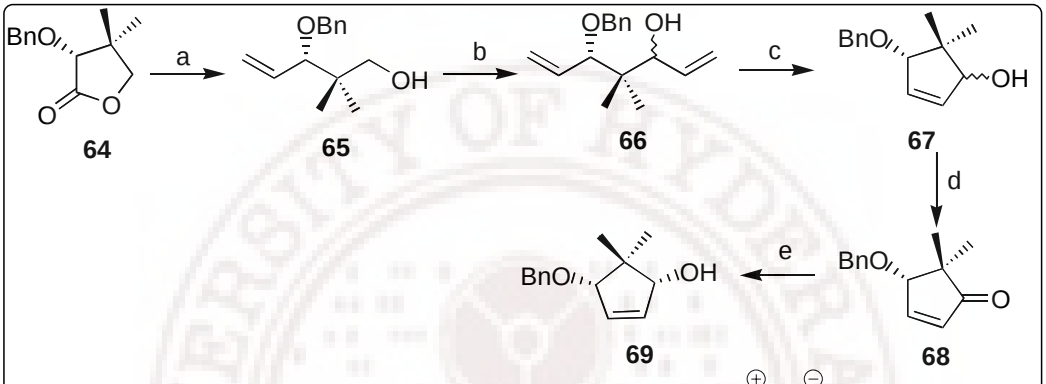


Figure-**6** *Enantiodivergent strategy for accessing both enantiomers of noviose*

The alkylation of (*R*)-pantolactone using benzyl bromide and Ag_2O in dry DMF gave the *O*-alkylated product **64** in good yields. The protected pantolactone was reduced to the corresponding lactol on treatment with DIBAL-H followed by a Wittig reaction with methyltriphenylphosphonium bromide to furnish the acyclic primary alcohol **65.**[43] Swern oxidation of **65** was followed by addition of vinylmagnesium bromide in dry THF at 0 °C furnished diene **66** as a mixture of diastereomers (1:1) in excellent yields. Following this, the diastereomeric diene was then subjected to RCM cyclization using 5 mol % of Grubb's first generation catalyst to furnish **69** in near quantitative yields, which upon oxidation with Jones

reagent gave cyclopentenone **68.** Luche reduction of cyclopentenone gave the allylic alcohol **69** with excellent diastereoselectivity (scheme-**16**). With this key intermediate, a stage for accessing both the enantiomers of noviose was set.

Scheme-16 *Preparation of compound* **69** *from compound* **64**

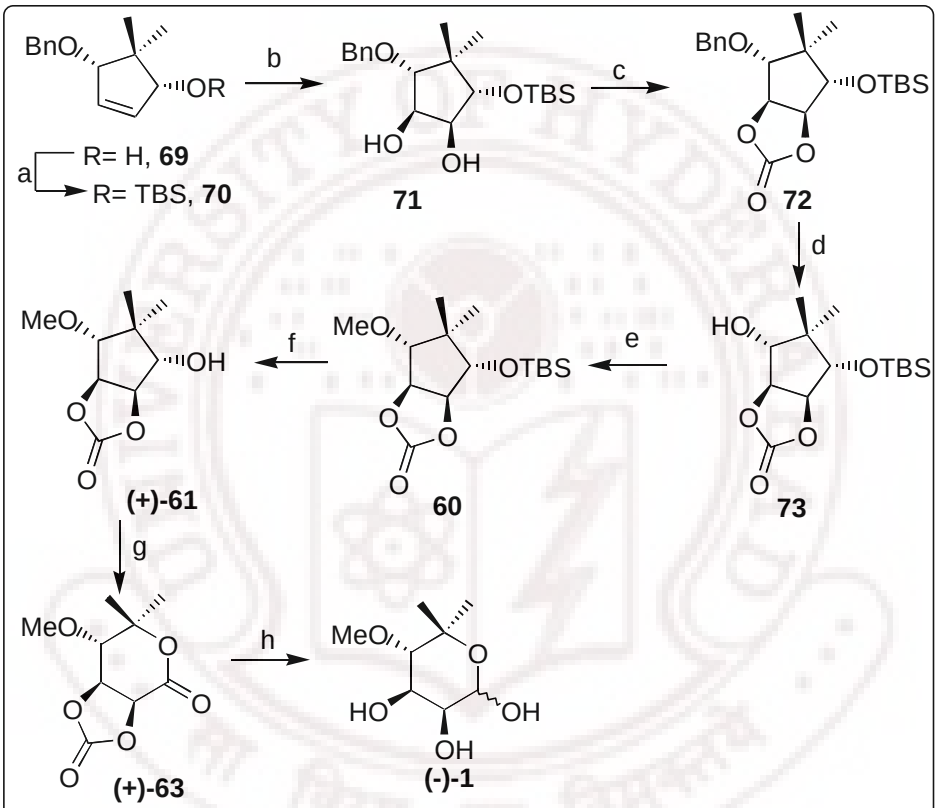


Reagents & conditions: (a) i. DIBAL-H, -78 ^{o}C,CH_2Cl_2 ii. n-BuLi, $MePPh_3Br$, Dry THF, -78 ^{o}C (c) i. $(COCl)_2$, DMSO, CH_2Cl_2, -78 ^{o}C, ii. vinyl magnesium bromide, Dry THF, 0 ^{o}C (d) Grubb's catalyst 5 mol %, CH_2Cl_2 (e) Jones reagent, Et_2O, rt, 30 min,(e) $NaBH_4$-$CeCl_3.7H_2O$, MeOH, 0 ^{o}C, 45 min

To access (–)-noviose, the allylic alcohol **69** was protected as its silyl derivative using TBS chloride and imidazole to afford **70**. Dihydroxylation of alkene using a combination of osmium tetroxide and *N*-methylmorpholine-*N*-oxide monohydrate furnished the diol with excellent diastereoselectivity which was protected as its carbonate derivative **71** using triphosgene/NEt_3. At this point selective deprotection of the benzyl group was achieved using 10% Pd/C resulting in the formation of a secondary alcohol which on alkylation with methyl iodide furnished **60** in excellent yields. Deprotection of silyl group with TBAF afforded alcohol **(+)-61** which was transformed to ketone in good yields. Subsequently,

Baeyer-Villiger oxidation of the resulting ketone with *m*-CPBA gave lactone **(+)-63**, which has earlier been converted to (–)-**1,** using DIBAL-H reduction (Scheme-**17**).

Scheme-**17** Preparation of **(**–)-**1** from compound **69**

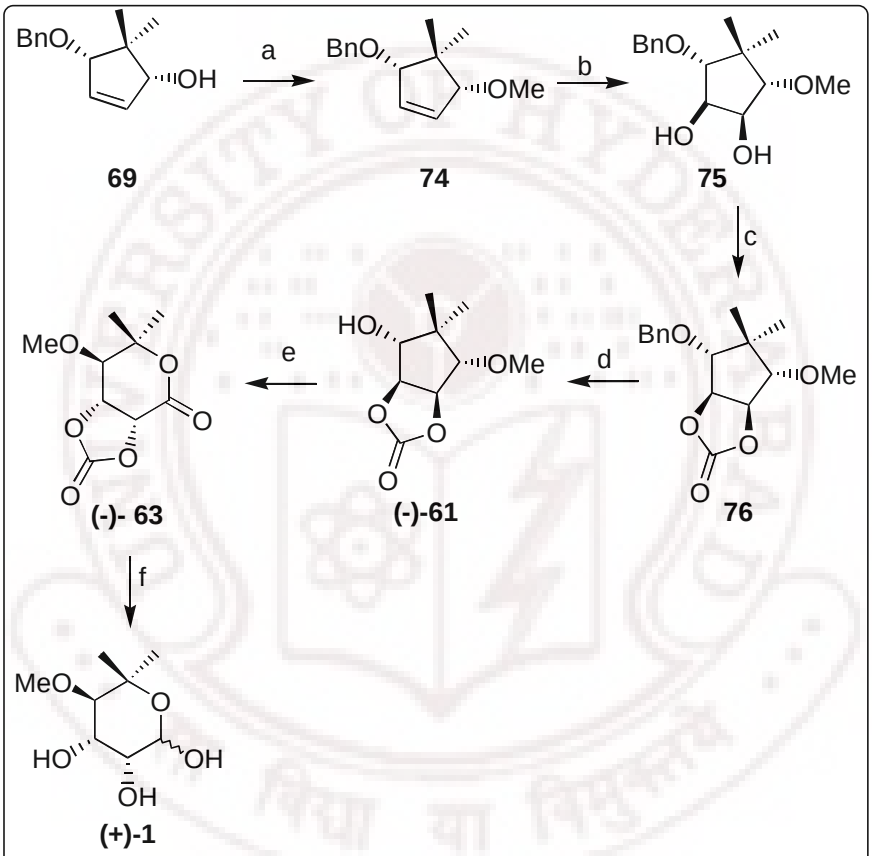


Reagents & conditions: (a) TBSCl, imidazole, CH_2Cl_2, 0 ^oC, 92%; (b) i. OsO_4, NMO, acetone, H_2O, t-BuOH, rt, 71 %; (c) triphosgene,NEt_3, CH_2Cl_2, 0 ^oC - rt, 79%; (d) 10% Pd/C, EtOAc, rt, 2h, 97 %; (e) NaH, MeI, Dry THF, 0 ^oC-rt, 12h, 76% (f) TBAF, THF, 94%; (g) i. $(COCl)_2$,DMSO, NEt_3, rt, 4h (ii) mCPBA, DCM, rt, 3days 90% for two steps (h) DIBAL-H ref.22.

The spectral data of synthetic **(+)-63** was compared with reported data and found to be identical $[\alpha]_D$ = +11.1 (*c* = 0.45, $CHCl_3$), lit. $[\alpha]_D$ = + 12.4 (c 1.44, $CHCl_3$). On the other hand, the secondary alcohol of the key intermediate **69** was

methylated to furnish the corresponding alkylated derivative **74** in good yields thereby installing the methyl group required for the synthesis of (+)-noviose **1** (scheme-**18**).

Scheme-**18** Preparation of **(**+)-**1** from compound **69**



Conditions: (a) i. NaH, MeI, dry THF, 86% (b) OsO_4, NMO, acetone, H_2O, t-BuOH, rt, 74%; (c) triphosgene, NEt_3, CH_2Cl_2 0 ^oC - rt, 80%; (d)10 % Pd/C, EtoAc, rt, 2h, 91 % (e) i. PDC, CH_2Cl_2, rt, 12h (ii). m-CPBA, DCM, 3 days, rt, 90% for two steps; (f) DIBAL-H, -78 ^oC, 60% ref.22.

Dihydroxylation of **74** using a combination of osmium tetroxide and *N*-methylmorpholine-*N*-oxide monohydrate followed by protection of the diol,

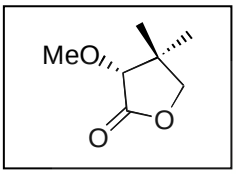
furnished **76** which on debenzylation of using 10% Pd/C gave the corresponding secondary alcohol (–)-**61** in almost quantitative yields. Oxidation of the resulting secondary alcohol to ketone using pyridinium dichromate was followed by Baeyer-Villiger oxidation using *m*-CPBA to give lactone (–)-**63** in excellent yields (scheme-**16**). The spectral data of synthetic (–)-**63** was compared with reported data and was found to be identical.

6.3 Conclusion

In conclusion, we initially developed an enantiospecific synthetic route for the synthesis of (–)-noviose starting from (–)-pantolactone employing ring closing metathesis, Luche reduction, osmylation and Baeyer-Villiger oxidation as key reactions during our synthesis. The failure of the enone **48** to undergo osmylation compelled us to look for an alternative procedure, which led us to accomplish the synthesis of (–)-**1** via Luche reduction. This reaction served as a blessing in disguise, since, we employed this as a key intermediate helped us to develop a novel enantiodivergent strategy for the synthesis of both (+) and (–)-novioses. Our approach of accessing both the enantiomers from a cheap and commercially available common starting material coupled with the synthesis of noviose analogues offers a distinct advantage of accessing library of molecules aimed at inhibiting Hsp90 protein folding machinery.

6.4 Experimental

Preparation of (3*R*)-3-methoxy-4,4-dimethyl-dihydro-furan-2-one (51):

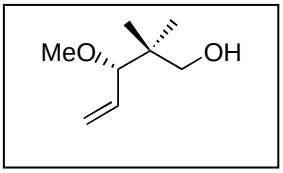


Silver (I) oxide (19.6 g, 84.5 mmol) was added to a solution of (*R*)-pantolactone (5.5 g, 42.3 mmol) and iodomethane (60 g, 422.8 mmol) in acetonitrile (27 ml) in a 100 ml pressure tube. The tube was sealed and heated to 58 °C. After stirring for 5h, the contents was cooled to room temperature, filtered and concentrated. The residue was diluted with diethyl ether, filtered through celite and concentrated under reduced pressure. The crude compound was purified using 100-200 mesh silica gel and 20 % EtoAc-petether as eluent to afford title product as colorless oil (5.23 g, 86 % yield).

[α] = +64.0 (c 3.16, $CHCl_3$), IR (Neat): 2963, 2927, 2897, 1786, 1466, 1370, 1297, 1118, 1009, 990 cm^{-1}, ^1H NMR ($CDCl_3$, 400 MHz): δ 3.98 (d, *J* = 8.6 Hz, 1H), 3.89 (d, J = 8.8 Hz, 1H), 3.64 (s, 3H), 3.57 (s, 1H), 1.20 (s, 3H), 1.08 (s, 3H), ^{13}C NMR ($CDCl_3$, 50MHz): δ174.9, 83.4, 76.3, 76.0, 59.1, 40.1, 23.2, 18.8. Mass (CI method): 145 $(M+H)^+$, 100).

Preparation of (*3S*)-3-methoxy-2,2-dimethyl-pent-4-en-1-ol (53):

DIBAL-H (18 ml, 20 % solution in toluene, 24.98 mmol) was added drop wise to a stirred solution of **51** (3g, 21.0 mmol) in methylene chloride (50 ml) at -78 °C.



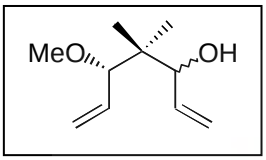
After stirring for 3h, the reaction was quenched with aqueous sodium phosphate monobasic (4M solution). The dry ice-acetone bath was removed. The content was warmed to room temperature, stirred for 3h and then filtered. The filtrate was dried over Na_2SO_4, filtered and concentrated to give 3g of crude lactol which was used as such for the next step.

n-Butyl lithium (22 ml, 15 % solution in hexane, 51.36 mmol) was added drop wise to a suspension of methyl triphenylphosphonium bromide (18.35 g, 51.36 mmol) in 50 ml of dry THF at -78°C followed by the addition of a solution of lactol (2.5 g, 17.12 mmol) in 15 ml of dry THF at the same temperature. After addition, the reaction was stirred at -78°C for 1h and then stirred at room temperature for 18h. The reaction mixture was quenched by slow addition of aqueous NH_4Cl solution and then extracted with CH_2Cl_2. The organic layer was dried over sodium sulfate and evaporated under reduced pressure to afford the crude compound which was purified using 20 % EtOAc-pet-ether as eluent to afford the title compound as colorless oil (2.42 g, 81 % over two steps).

$[\alpha]$ = 16.24 (c 1.25, $CHCl_3$), IR (Neat): 3422, 2966, 2824, 1469, 1421, 1091, 1048 cm^{-1}, ^1H NMR ($CDCl_3$, 400 MHz): δ 5.76-5.67 (m, 1H), 5.34-5.31 (m, 1H), 5.24-5.19 (m, 1H), 3.50 (d, *J* = 10.7 Hz, 1H), 3.40 (d, *J* = 9.1 Hz, 2H), 3.26 (s,

3H), 0.89 (s, 3H), 0.88 (s. 3H), ^{13}C NMR ($CDCl_3$, 50MHz): δ 134.7, 119.3, 90.9, 71.3, 56.55, 38.4, 22.3, 19.55, ES-MS (+ve): 167 $(M+Na)^+$.

Preparation of (5*S*)-5-Methoxy-4,4-dimethyl-octa-1,7-dien-3-ol (54):

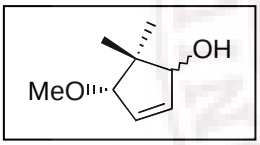


A stirred solution of dimethylsulfoxide (2.35ml, 33.31mmol) in CH_2Cl_2 (20 ml) was cooled to -78 °C using dry ice-acetone bath. oxalyl chloride (1.45 ml, 16.85 mmol) in dry CH_2Cl_2 (20 ml) was then added dropwise. After 30 min, a solution of **53** (2.0 g, 13.88 mmol) in dry CH_2Cl_2 (20 ml) was added slowly and stirred for 1h at -78 °C followed by the addition of NEt_3 (9.6 ml, 69.4 mmol) at the same temperature and stirred for an additional 1.5h. Reaction mixture was diluted with CH_2Cl_2, washed with water, dried over Na_2SO_4 and evaporated. Crude compound was taken for next reaction without purification.

To an ice cold stirred solution of above aldehyde (1.7 g, 11.8 mmol) in dry THF (50ml) under nitrogen, was added vinyl magnesium bromide (1M solution in THF) (23.9 ml, 23.9 mmol) and stirred for 15 min. The reaction was quenched with saturated aqueous NH_4Cl solution and extracted with ether (25 mlx3). Combined organic layers was dried over Na_2SO_4 and evaporated to afford the crude compound which was purified by column chromatography using 20% EtoAc-hexane to afford the title compound as a mixture of diastereomers (1.93 g, 82 %).

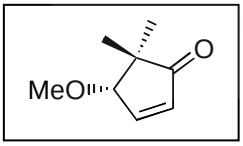
[α] = +1.90 (c 1, $CHCl_3$), IR (Neat): 3445, 3078, 1638, 1421, 1195, 1087, 1053 cm^{-1}, ^1H NMR ($CDCl_3$, 400MHz): δ5.84-5.97 (m, 2H), 5.66-5.77 (m, 2H), 5.16-5.35 (m, 8H), 4.04 (d, *J* = 6.7 Hz, 1H), 3.94 (d, *J* = 5.9 Hz, 1H), 3.48 (d, *J* = 8.3 Hz, 1H), 3.45 (d, *J* = 8.33 Hz, 1H), 3.27 (s, 3H), 3.25 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H), 0.86 (s, 3H), 0.73 (s, 3H), ^{13}C NMR ($CDCl_3$, 50MHz): δ 137.64, 137.33, 134.40, 134.14, 119.62, 119.53, 116.54, 115.98, 91.42, 89.77, 80.29, 79.34, 56.26, 56.19, 40.63, 40.46, 21.47, 21.15, 20.60, 15.35, Mass (CI method): 171 $(M+H)^+$, 100).

Preparation of (4S)-4-methoxy-5,5-dimethyl-cyclopent-2-enol (55):



(A) To a stirred solution of **54** (1.0 g, 5.88 mmol) in dry $CH2Cl_2$ (300 ml) under nitrogen, was added Grubb's catalyst 1st generation (250 mg) and stirred at room temperature for 10-12 h. Reaction mixture was evaporated to afford crude compound (0.76 g, 91 %) which was taken for the next step without purification.

Preparation of (4S)-4-methoxy-5,5-dimethyl-cyclopent-2-enone (48):

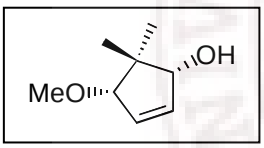


To a stirred solution of **55** (1.0 g, 7.04 mmol) in Et_2O (15 ml) was added Jones reagent (10 ml) and stirred at room temperature (28 °C) for 30 min. Reaction mixture was

extracted with Et_2O, dried over Na_2SO_4 and evaporated. Crude compound was purified by column using 15% EtOAc-Hexane to afford title compound as volatile liquid (0.85g, 86 %.).

$[\alpha]$ = +39.70 (c 1.0, $CHCl_3$), IR (Neat) 1711, 1462, 1377, 1195, 1054, 759 cm^{-1}, ^1H NMR ($CDCl_3$): δ 1.06 (s, 3H), 1.21 (s, 3H), 3.52 (s, 3H), 4.09-4.10 (m, 1H), 6.18 (dd, J = 6.2 Hz, J = 1.6 Hz, 1H), 7.50 (dd, J = 5.9 Hz, J = 1.9 Hz, 1H), ^{13}C NMR ($CDCl_{3)}$: δ 211.2, 158.4, 132.4, 88.5, 586, 48.2, 23.3, 20.2, Mass(CI method): 140 $(M+H)^+$, 100).

Preparation of (*1R, 4S*)-4-methoxy-5,5-dimethyl-cyclopent-2-enol (57):

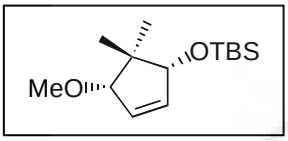


To an ice cold stirred solution of **48** (1.0g, 7.14 mmol) and $CeCl_3.7H_2O$ (3.98 g, 10.71 mmol) in dry MeOH (40ml) under nitrogen, was added $NaBH_4$ (0.29g, 7.85 mmol) portion wise and stirred for 30 min. The reaction was diluted with EtOAc, washed with water, organic layer dried over Na_2SO_4 and evaporated, crude compound was purified by column chromatography using 20% EtOAc-hexane to afford the title compound as volatile liquid (0.97 g, 96%).

$[\alpha]$ = +29.20 (c 1.0, $CHCl_3$), IR (Neat) 3423, 1466, 1359, 1197, 1091, 1063 cm^{-1}, ^1H NMR ($CDCl_3$): δ 1.00 (s, 3H), 1.09 (s, 3H), 3.40 (s, 3H), 3.67-3.68 (m, 1H), 4.02-4.04 (m, 1H), 5.99-6.02 (m, 1H), 6.05-6.07 (m, 1H), ^{13}C NMR ($CDCl_{3)}$: δ

136.30, 133.03, 91.46, 82.88, 57.92, 46.68, 27.10, 16.34. ES-MS (+ve): 165 $(M+Na)^+$.

Preparation of (1*R*,4*S*)4-methoxy-5,5-dimethyl-cyclopentenyloxy)-t-butyl-dimethylsilane (58):

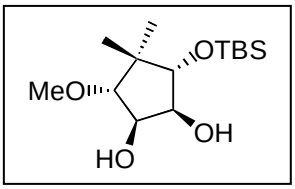


To an ice cold stirred solution of **57** (1.0 g, 7.04 mmol) and imidazole (0.96 g, 14.08 mmol) in dry CH_2Cl_2 (35 ml) under nitrogen, was added TBSCl (1.60 g, 10.56 mmol) and stirred for 2h. The reaction mix was diluted with CH_2Cl_2, washed with water, organic layer dried over Na_2SO_4 and evaporated, crude compound was purified by column using 10 % EtoAC-hexane to afford the title compound as thick liquid (1.5 g, 83 %).

[α] = -17.80 (c 1.0, $CHCl_3$), IR (Neat) 1464, 1362, 1253, 1198, 1099 cm^{-1}, ^1H NMR ($CDCl_3$): δ 0.07 (s, 6H), 0.84 (s, 6H), 0.90 (s, 6H) 1.16 (s, 3H), 3.40 (s, 3H), 3.75-3.76 (m, 1H), 4.16-4.17 (m, 1H), 5.72-5.74 (m, 1H), 5.83-5.86 (m, 1H), ^{13}C NMR ($CDCl_3$): δ 135.46, 130.85, 91.53, 82.74, 57.94, 49.4, 26.92, 26.43, 25.85, 22.68, 22.56, 21.23, 18.18, 16.07, ES-MS (+ve): 279 $(M+Na)^+$.

Preparation of (1*R*,2*S*,3*S*,5*R*)-3-(t-butyl-dimethyl-silanoxy)-5-methoxy-4,4-dimethyl-cyclopentane-1,2-diol (59):

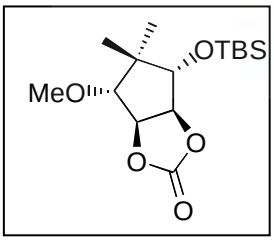
To a stirred solution of **58** (1.0 g, 3.90 mmol) and NMO (0.8 g, 5.85 mmol) in acetone-water-t-butanol (4ml: 4ml: 4ml) was added osmium tetraoxide 7ml (1%



in t-butanol) (4 ml, 1mol %) and stirred at room temperature for 2h. Reaction mixture was quenched with aqueous sodium meta bisulphate solution and extracted with chloroform (25 ml x 3). Combined organic layers was dried over Na_2SO_4 and evaporated to afford the crude compound which was purified by column using 20% EtOAc-hexane to afford the title compound as volatile liquid (1.0 g, 89 %).

[α] = +1.90 (c 1.0, $CHCl_3$), IR (Neat): 3405, 1465, 1365, 1254, 1146, cm^{-1}, ^1H NMR ($CDCl_3$): δ 0.08 (s, 3H), 0.12 (s, 3H), 0.77 (s, 3H) 0.92 (s, 9H), 1.05 (s, 3H), 3.11 (d, *J* = 5.9 Hz, 1H), 3.45-3.49 (m, 1H), 3.51 (s, 3H), 3.84-3.97 (m, 2H), ^{13}C NMR ($CDCl_3$): δ 92.88, 83.58, 74.51, 73.11, 58.88, 41.32, 29.66, 29.33, 26.12, 25.79, 21.01, 18.06, 16.05, 14.16, ES-MS (+ve): m/z 291 $(M+Na)^+$.

Preparation of (1*S*,2*S*,3*S*,6*R*)-4-(t-butyl-dimethyl silanyloxy)-6-methoxy-5,5-dimethyl-tetrahydro-cyclopenta[1,3]dioxol-2-one (60):

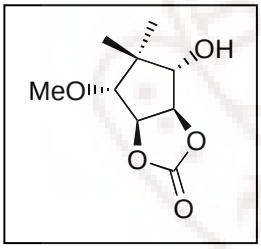


To an ice cold stirred solution of **59** (1.0 g, 3.44 mmol) and NEt_3 (2.8 ml, 20.6 m mol) in dry CH_2Cl_2 (34 ml) under nitrogen, was added triphosgene (2.0 g, 6.88 mmol) and stirred for 2h. The reaction was quenched with saturated aqueous NH_4Cl solution and extracted with CH_2Cl_2 (25 mlx3).

Combined organic layers was dried over Na_2SO_4 and evaporated to afford the crude compound which was purified by column chromatography using 10% EtOAc-hexane to afford the title compound as thick liquid (1.0 g, 92 %).

[α] = +8.20 (c 1.0, $CHCl_3$), IR (Neat): 1812, 1462, 1363, 1255, 1179, 1112, 1066 cm^{-1}, ^1H NMR ($CDCl_3$): δ 0.098 (s, 3H), 0.14 (s, 3H), 0.79 (s, 3H) 0.92 (s, 9H), 1.07 (s, 3H), 3.32 (d, *J* = 5.4 Hz, 1H), 3.48 (s, 3H), 3.71 (d, *J* = 5.6 Hz, 1H), 4.66 (dd, *J* = 9.4 Hz, *J* = 5.4 Hz, 1H), 4.75 (dd, *J* = 9.6 Hz, *J* = 5.3 Hz, 1H), ^{13}C NMR ($CDCl_3$): δ 154.61, 90.14, 84.08, 82.35, 82.21, 58.48, 43.77, 25.60, 24.38, 17.88, 15.38, ES-MS: 334 $(M+NH_4)^+$, 339 $(M+Na)^+$

Preparation of (1*S*,2*R*,3*S*,6*R*)-4-hydroxy-6-methoxy-5,5-dimethyl-tetrahydro-cyclopenta[1,3]dioxol-2-one (+)-61:

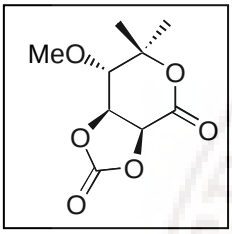


To an ice cold stirred solution of **60** (0.5 g, 1.58 mmol) dry THF (16 ml) under nitrogen, was added TBAF (1M in THF) (1.75 ml, 1.74 mmol) and stirred for 1h. The reaction mixture was diluted with $CHCl_3$, washed with water, dried over anhydrous Na_2SO_4 and evaporated the solvent under reduced pressure to yield crude compound. Column purification using 25 % EtOAc-hexane afforded title compound as thick liquid (0.3 g, 94 %).

[α] = +22.0 (c 2.6, $CHCl_3$), IR (Neat): 3331, 1803, 1599, 1464, 1376, 1160, 1104 cm^{-1}, ^1H NMR ($CDCl_3$): δ 0.86 (s, 3H), 1.14 (s, 3H), 3.36 (d, *J* = 4.3 Hz, 1H),

3.48 (s, 3H), 3.82 (d, J = 4.3 Hz, 1H), 4.76-4.83 (m, 2H), ^{13}C NMR ($CDCl_3$): δ 154.97, 90.40, 83.81, 82.50, 81.43, 58.52, 43.38, 24.18, 15.16, ES-MS: 220 $(M+NH_4)^+$.

Preparation of (1*S*,3*S*,7*S*)-7-methoxy-6,6-dimethyl-tetrahydro-[1,3]dioxolo[4,5-c]pyran-2,4-dione (+)-63:

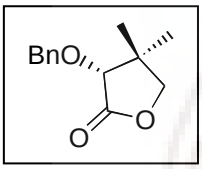


A stirred solution of dimethylsulfoxide (100 μl, 1.36 mmol) in dry CH_2Cl_2 (20 ml) was cooled to -78 °C followed by the addition of oxalylchloride (85 μl, 0.92 mmol). After 30 min, a solution of **61** (125 mg, 0.61 mmol) in dry CH_2Cl_2 (20 ml) was added slowly and stirred for 1.5 h at -78 °C and NEt_3 (0.43 ml, 3.09 mmol) was added drop wise at same temperature and stirred for an additional 1.5 h. Reaction mixture was diluted with CH_2Cl_2, washed with water, dried over Na_2SO_4 and evaporated to yield ketone which was taken for next reaction without purification.

A mixture of ketone and mCPBA in 10 ml of dry CH_2Cl_2 was stirred at room temperature for a period of 3 days. The reaction mixture was diluted with 25 ml of CH_2Cl_2 and washed with aq. $Na_2S_2O_3$ solution followed by aq. $NaHCO_3$ solution. Dried over Na_2SO_4 and evaporated to afford the crude compound, which was purified using 20 % EtOAc: hexane system to afford the title compound as a crystalline solid (120 mg, 90 % over two steps).

[α] = + 11.1 (c 0.45 $CHCl_3$), IR (KBr): 2965, 1835, 1818, 1806, 1793 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): δ 1.38 (s, 3H), 1.52 (s, 3H), 3.44 (d, *J* = 6.9 Hz, 1H), 3.60 (s, 3H), 4.98 (dd, *J* = 10.0 Hz *J* = 7.0 Hz, 1H), 5.21 (d, *J* = 9.7 Hz, 1H), ^{13}C NMR (50 MHz, $CDCl_3$): 22.9, 26.5, 59.6, 70.2, 77.9, 82.2, 82.8, 152.2, 163.2, Mass (CI method): 217 $(M+H)^+$, 100).

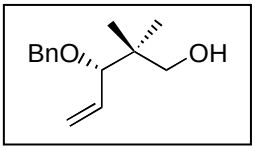
Preparation of (3*R*)-3-Benzyloxy-4, 4-dimethyl-dihydro-furan-2-one (64):



Silver oxide (3.6 g, 15.3 mmol) and benzyl bromide (1.4 g, 8.4 mmol) were added to a solution of D-pantolactone (1.0 g, 7.7 mmol) in dry DMF (25 ml) at 0 °C under nitrogen. The mixture was stirred at 0 °C for 2h, then warmed to room temperature and stirred for an additional 20 h. The solution was diluted with CH_2Cl_2 (100 ml) and filtered. The filtrate was concentrated in vacuo, diluted with EtOAc, and washed with 0.5 N HCl, water and brine. The solvent was removed under reduced pressure and purified using 100-200 mesh silicagel and 20 % EtOAc-pet-ether as eluent to afford title compound as crystalline compound (1.3 g, 77 %)

[α] = 100 (c 1, $CHCl_3$), IR(Neat): 2965, 1788, 1120 cm^{-1}, ^1H NMR ($CDCl_3$): δ 7.38-7.25 (m, 5H), 5.03 (d, *J* = 12.2 Hz, 1H), 4.75 (d, *J* = 12.2 Hz, 1H), 4.00 (d, *J* = 8.9 Hz, 1H), 3.85 (d, *J* = 8.9 Hz, 1H), 3.73 (s, 1H), 1.13 (s, 3H), 1.13 (s, 3H), ES-MS (+ve): 221 $(M+H)^+$.

Preparation of (3*S*)-3-Benzyloxy-2, 2-dimethyl-pent-4-en-1-ol (65):

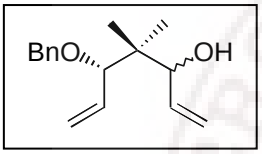


To a stirred solution of lactone **64** (3.00 g, 13.6 mmol) in CH_2Cl_2 (50 ml) at -78°C was added DIBAL-H (1 M in hexanes, 16.3 ml, 16.3 mmol) over 30 min. After 2 h, the reaction was quenched slowly at first within 60 ml of a 1:1 diethyl ether/1 M H_2SO_4 (10 ml) of saturated $NaHCO_3$ solution, 10 ml of water, and twice with 20 ml of brine. The organic phase was then dried with Na_2SO_4 and concentrated *in vacuo.* The crude oil was purified by flash chromatography to yield lactol (2.85 g, 94%) as a clear oil that solidified to white clumps after it was removed from the freezer. The product turned out to be an inseparable mixture of anomers in an approximate 2:3 ratio.

n-Butyl lithium (15 % solution in n-hexane, 5.8 ml, 13.5 mmol) was added drop wise to a suspension of methyl triphenylphosphonium bromide (4.82 g, 13.5 mmol) in 10 ml of dry THF at -78 °C for a period of 30 minutes, followed by the addition of a solution of lactol (1g, 4.5 mmol) in 5 ml of dry THF at the same temperature. After addition, the reaction was stirred at -78 °C for 1h and then stirred at room temperature for 18h. The reaction mixture was quenched by slow addition of aqueous NH_4Cl solution and then extracted with CH_2Cl_2. The organic layer was dried over sodium sulfate and evaporated under reduced pressure to afford the crude compound which was purified using 20 % EtOAc-pet-ether as the eluent to afford the title compound as colorless oil (0.8 g, 80 %).

IR (Neat): 3446, 2963, 2872 cm^{-1}, ^1H NMR ($CDCl_3$): δ 7.36-7.25 (m, 5H), 5.85-5.76 (m, 1H), 5.38-5.35 (m, 1H), 5.26-5.21 (m, 1H), 4.60 (d, *J* = 11.8 Hz, 1H), 4.28 (d, *J* = 11.8 Hz, 1H), 3.62 (d, *J* = 8.3 Hz,1H), 3.53 (d, *J* = 11.0 Hz, 1H), 3.35 (d, *J* = 11.0 Hz, 1H), 2.27 (bs, 1H), 0.90 (s, 3H), 0.89 (s, 3H), ES-MS (+ve): 221 $(M+H)^+$, 243 $(M+Na)^+$.

Preparation of (5*S*)-5-Benzyloxy-4, 4-dimethyl-hepta-1, 6-dien-3-ol (66):



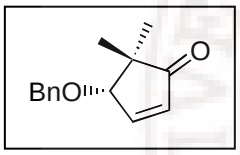
A stirred solution of dimethylsulfoxide (1.42 g, 19.9 mmol) in dry CH_2Cl_2 (20 ml) was cooled to -78 °C followed by the addition of oxalyl chloride (1.37 g, 10.8 mmol). After 0.5 h, a solution of **65** (2.0 g, 9.0 mmol) in dry CH_2Cl_2 (20 ml) was added slowly and stirred for 1h at -78 °C and NEt_3 (5.0 ml, 36.0 mmol) was added at the same temperature and stirred for an additional 1.5 h. Reaction mixture was diluted with CH_2Cl_2, washed with water, dried over Na_2SO_4 and evaporated. The crude compound was taken for next reaction without purification.

To an ice cold stirred solution of aldehyde (obtained above) (1.8 g, 7.75 mmol) in dry THF (50ml) under nitrogen, was added vinylmagnesiumbromide (1M solution in THF) (16.5 ml, 16.5 mmol) and stirred for 15 min. The reaction was quenched with saturated aqueous NH_4Cl solution and extracted with Et_2O (25 ml x3). Combined organic layers was dried over Na_2SO_4 and evaporated to afford the crude compound which was column purified using 20% EtOAc-hexane to

yield the title compound as a mixture of diastereomers (1.7 g, 76.2 %) over two steps.

[α] = 26.30 (c 1.0, $CHCl_3$), IR (Neat): 3474, 2971, 2876 cm^{-1}, ^1H NMR ($CDCl_3$): δ 7.35-7.24 (m, 10H), 5.85-5.75 (m, 4H), 5.40-5.29 (m, 2H), 5.28-5.21 (m, 4H), 5.18-5.14 (m, 2H), 4.62-4.57 (m, 2H), 4.33-4.25 (m, 2H), 4.05-4.00 (m, 2H), 3.76-3.55 (m, 4H), 0.96 (s, 3H), 0.93 (s, 3H), 0.87 (s, 3H), 0.75 (s, 3H). ES-MS: 247 $(M+H)^+$.

(4*S*)-4-Benzyloxy-5, 5-dimethyl-cyclopent-2-enone (68):



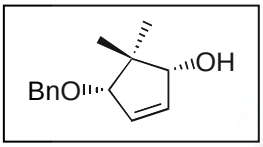
To a stirred solution of **66** (2.0 g, 8.13 mmol) in dry CH_2Cl_2 (600 ml) under nitrogen atmosphere at room temperature was added (5 mol %, 337 mg) of Grubb's catalyst 1st generation and stirred at RT (~28 °C) for 2h. Solvent was evaporated completely and crude compound was taken for the next step without purification.

To a stirred solution of alcohol (obtained above) in Et_2O (15 ml) was added Jones reagent (10 ml) and stirred at room temperature for 20 min. Reaction mixture was extracted with Et_2O (2x50 ml), dried over Na_2SO_4 and evaporated. The crude compound was column purified by using 15% EtOAc-Hexane to afford title compound as pale yellow oil (1.5 g, 85.7 %).

[α] = 62.33 (c 0.6, MeOH), IR (Neat): 2973, 2869, 1715, 1103 cm^{-1}, ^1H NMR ($CDCl_3$): δ 7.46 (dd, *J* = 5.9 Hz, *J* = 1.9 Hz, 1H), 7.39-7.34 (m, 5H), 6.18 (dd, *J* =

5.9 Hz, *J* = 1.3 Hz, 1H), 4.76 (d, *J* = 11.9 Hz, 1H), 4.66 (d, *J* = 11.9 Hz, 1H), 4.32 (t, *J* = 2.0 Hz, 1H), 1.18 (s, 3H), 1.15 (s, 3H), ^{13}C NMR ($CDCl_3$): δ 211.2, 159.0, 137.8, 132.4, 128.4, 127.8, 86.1, 72.7, 53.36, 23.2, 20.6, ES-MS: 217$(M+H)^+$.

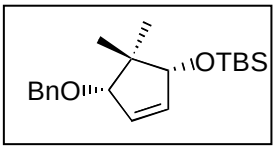
Preparation of (1*R*, 4*S*)-4-benzyloxy-5, 5-dimethyl-cyclopent-2-enol (69):



To an ice cold stirred solution of **68** (1.0g, 4.62 mmol) and $CeCl_3.7H_2O$ (2.58 g, 6.94 mmol) in dry MeOH (40ml) under nitrogen, was added $NaBH_4$ (0.19g, 5.09 mmol) portion wise and stirred for 0.5 h. The reaction was diluted with EtOAc, washed with water, dried over Na_2SO_4 and evaporated. The crude compound was purified by column using 20% EtOAc-hexane to afford the title compound as volatile liquid (0.97 g, 97 %).

[α] = 45.2 (c 1.0, MeOH), IR (Neat): 3406, 2956, 2867, 1062 cm^{-1}, ^{1}H NMR ($CDCl_3$): δ 7.37-7.26 (m, 5H), 6.04-6.00 (m, 2H), 4.6 (s, 2H), 4.04-4.02 (m, 1H), 3.89-3.88 (m, 1H), 1.47-1.45 (m, 1H), 1.09 (s, 3H), 1.08 (s, 3H), ^{13}C NMR ($CDCl_3$): δ 138.5, 136.1, 133.23, 128.2, 127.4, 127.3, 89.2, 82.7, 71.9, 46.8, 27.0, 16.6, ES-MS : 236 $(M+NH_4)^+$.

(1*R*,2*S*)-4-Benzyloxy-5,5-dimethyl-cyclopent-2-enyloxy)-tert-butyl-dimethyl-silane(70):

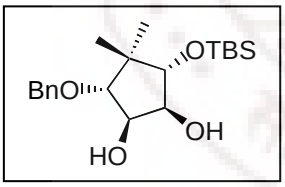


To a solution of **69** (1g, 4.58 mmol), imidazole (620 mg, 9.17 mmol) in dry CH_2Cl_2 was added TBSCl (1.1 g, 6.88

mmol) and stirred for a period of 6h at RT (~28 °C). The reaction mixture was diluted with CH_2Cl_2, washed with water, dried over Na_2SO_4. Evaporation of the solvent followed by column purification afforded the title compound (1.1 g, 92 %).

[α] = 7.1 (c 1.0, MeOH), IR (Neat): 2955, 2857, 1463, 1361, 1252, 1078 cm^{-1}, ^1H NMR ($CDCl_3$): δ 7.37-7.25 (m, 5H), 5.83-5.81 (m, 1H), 5.74-5.71 (m, 1H), 4.61 (s, 2H), 4.17-4.15 (m, 1H), 3.98-3.97 (m, 1H), 1.14 (s, 3H), 0.92 (s, 3H), 0.9 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H), ^{13}C NMR ($CDCl_3$): δ 139.0, 135.4, 131.3, 128.2, 127.4, 127.3, 89.2, 82.6, 71.7, 49.5, 26.3, 25.8, 18.1, 16.5, -4.5, -4.8, ES-MS: 350 $(M+NH_4)^+$.

Preparation of (1*S*, 2*R*, 3*R*, 5*S*)-3-Benzyloxy-5-(tert-butyl-dimethyl-silanloxy)-4,4-dimethyl-cyclopentane-1,2-diol (71):

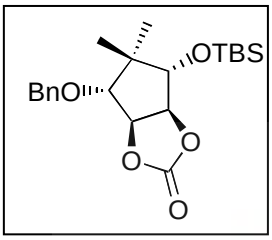


To a stirred solution of **70** (2.5 g, 7.5 mmol) and NMO (2.0 g, 15.06 mmol) in acetone- water- t-butanol (11 ml:11 ml:11 ml) at room temperature was added osmium tetraoxide (14 ml) (1% in t-butanol) and stirred at room temperature for 6h. Reaction mixture was quenched with aqueous $NaHSO_3$ solution and extracted with $CHCl_3$ (50 ml x 3). The combined organic layers was dried over Na_2SO_4 and evaporated to afford the crude compound which was purified by column

chromatography using 20% EtOAc-hexane to afford the title compound as pale yellow liquid (1.95 g, 71 %).

[α] = 11.7 (c 1.0, $CHCl_3$), IR (Neat): 3396, 3032, 2990, 2858,1464, 1255, 1098 cm^{-1}, ^1H NMR ($CDCl_3$): δ 7.39-7.26 (m, 5H), 4.77 (d, *J* = 12.4 Hz, 1H), 4.67 (d, *J* =12.0 Hz, 1H), 4.05-4.00 (m, 1H), 3.90-3.85 (m, 1H), 3.45 (d, *J* = 6.7 Hz, 1H), 3.31 (d, *J* =6.9 Hz, 1H), 2.41 (d, *J* = 4.6 Hz, 1H), 2.23 (d, *J* = 5.3 Hz, 1H), 1.02 (s, 3H), 0.91 (s, 9H), 0.84 (s, 3H), 0.11 (s, 3H), 0.07 (s, 3H), ^{13}C NMR ($CDCl_3$): δ 138.7, 128.2, 127.4, 90.2, 83.6, 74.5, 73.3, 72.4, 41.4, 25.8, 18.04, 16.3, -4.1, -5.0, ES-MS (+ve): 367 $(M+H)^+$.

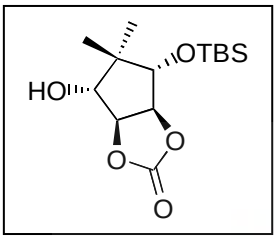
Preparation of (2S,3S,4R,6S)-4-Benzyloxy-6-(tert-butyl-dimethyl-silanyloxy)-5,5-dimethyl-tetrahydro-cyclopenta[1,3]dioxol-2-one (72):



To an ice cold stirred solution of **71** (710 mg, 1.93 mmol) and triethylamine (1.65 ml, 11.6 mmol) in dry CH_2Cl_2 (40 ml) under nitrogen, was added triphosgene (1.15 g, 3.87 mmol) portion wise and stirred for 2h. The reaction was quenched with aq. NH_4Cl solution and extracted with CH_2Cl_2 (40 ml x 3). Combined organic layers was dried over Na_2SO_4 and evaporated to afford the crude compound which was purified by column using 10% EtOAc-hexane to afford the title compound as a white solid (597 mg, 78.6 %).

$[\alpha]$ = 27.6 (c 1.0, $CHCl_3$), ^{1}H NMR ($CDCl_3$): δ 7.37-7.29 (m, 5H), 4.82 (dd, *J* = 9.4 Hz, and *J* = 5.4 Hz, 1H), 4.78 (d, *J* = 12.1 Hz, 1H), 4.67 (dd, *J* = 9.4 Hz, and *J* = 5.7 Hz, 1H), 4.55 (d, *J* = 12.1 Hz, 1H), 3.69 (d, *J* = 5.7 Hz, 1H), 3.51 (d, *J* = 5.6 Hz, 1H), 1.02 (s, 3H), 0.91 (s, 9H), 0.85 (s, 3H), 0 .12 (s, 3H), 0.08 (s, 3H), ^{13}C NMR ($CDCl_3$): δ154.5, 137.3, 128.4, 127.8, 127.6, 87.2, 84.1, 82.5, 82.1, 72.0, 43.8, 25.6, 24.2, 17.8, 15.5, -4.6, -5.2, ES-MS (+ve): 393 $(M+H)^+$, 410 $(M+NH_4)^+$.

Preparation of (2S,3S,4S,6R)-4-(tert-Butyl-dimethyl-silanyloxy)-6-hydroxy-5,5-dimethyl-tetrahydro-cyclopenta[1,3]dioxol-2-one (73):

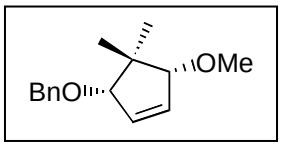


A mixture of **72** (500mg, 1.27 mmol) and 10% Pd/C (100 mg) in ethyl acetate(10 ml) was hydrogenated using a H_2 gas balloon at 20 psi pressure for a period of 2h. Pd/C was filtered off and the filtrate was evaporated and the residue was purified by column using 20 % EtOAc-pet-ether as eluent to afford the title compound as a white solid (375 mg, 97 %).

IR (KBr): 3455, 2959, 2859, 1804, 1080 cm^{-1}, ^{1}H NMR ($CDCl_3$): δ 4.78 (dd, *J* =9.4 Hz and *J* = 5.1 Hz, 1H), 4.68 (dd, *J* = 9.4 Hz and 5.1 Hz, 1H), 3.81 (t, *J* = 5.5 Hz, 1H), 3.74 (d, *J* = 5.1 Hz, 1H), 2.19 (d, *J* = 5.7 Hz, 1H), 1.06 (s, 3H), 0.92 (s, 9H), 0.85 (s, 3H), 0.14 (s, 3H), 0.1 (s, 3H), ^{13}C NMR ($CDCl_3$): δ 154.9, 84.3, 83.8, 82.3, 81.3, 44.1, 25.6, 23.9, 17.9, 15.2, -4.6, -5.2. ES-MS (+ve): 303 $(M+H)^+$, 320 $(M+NH_4)^+$.

Prepartion of (1*R*,4*S*))-4-methoxy-5,5-dimethyl-cyclopent-2-enyloxymethyl)-benzene (74):

A solution of **69** (2g, 9.17 mmol) in dry THF was added drop wise to an ice cold

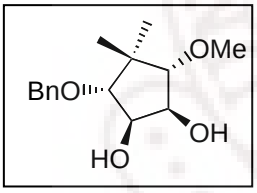


stirred suspension of NaH (450 mg, 11.0 mmol) in dry THF and stirred for 20 minutes. Methyl iodide (6.15 g, 45.85 mmol) was added and then stirred at room temperature for 2h. The reaction

mixture was diluted with EtoAc and washed with water and brine. The organic layer was dried over sodium sulfate and evaporated under reduced pressure to afford the crude compound which was purified by column using 20 % EtOAc-pet-ether to furnish the title compound as pale yellow oil (1.83 g 86.3 %).

[α] = 21.1 (c 1.0, MeOH), IR (Neat): 2932, 2821, 1096 cm^{-1}, ^{1}H NMR ($CDCl_3$): δ 7.36-7.27 (m, 5H), 5.95-5.90 (m, 2H), 4.61-4.56 (m, 2H), 3.94-3.93 (m, 1H), 3.73-3.72 (m, 1H), 3.41 (s, 3H), 1.20 (s, 3H), 1.00 (s, 3H), ^{13}C NMR ($CDCl_3$): δ 138.8, 132.7, 132.2, 128.1, 127.8, 127.6, 127.4, 127.3, 91.6, 89.4, 71.9, 57.9, 48.3, 27.4, 16.3, ES-MS (+ve): 250 $(M+NH_4)^+$.

Preparation of (1*S*,2*R*,3*R*,5*S*)-3-Benzyloxy-5-methoxy-4,4-dimethyl-cyclopentane-1,2-diol (75):

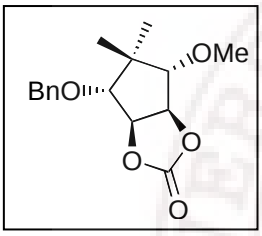


To a stirred solution of **74** (2.32 g, 10 mmol) and NMO (2.0 g, 15 mmol) in acetone-water-t-butanol (1:1:1, 30 ml) at room temperature (~28 °C) was added osmium tetraoxide (10 ml) (1% in t-BuOH) (1 mol %) and stirred at RT (28 °C) for 4h. Reaction mixture was quenched with aqueous $NaHSO_3$ solution and extracted with $CHCl_3$ (50 ml x 3). The combined organic layers was dried over Na_2SO_4 and evaporated to afford the crude compound which was purified by column using 50% EtOAc-hexane to afford the title compound as pale yellow oil (1.7 g, 65.3 %).

[α] = 17 (c 0.9, MeOH), IR (Neat): 3406, 2933, 1100 cm^{-1}, ^{1}H NMR ($CDCl_3$): δ 7.38-7.26 (m, 5H), 4.77 (d, *J* = 12.1 Hz, 1H), 4.69 (d, *J* = 12.1 Hz, 1H), 4.03 (dd,

J = 8.6 Hz and *J* = 6.5 Hz, 1H), 3.97 (dd, *J* = 8.6 Hz and *J* = 6.2 Hz, 1H), 3.51 (s, 3H), 3.33 (d, J = 6.2 Hz, 1H), 3.11 (d, *J* = 6.2 Hz, 1H), 1.1 (s, 3H), 0.8 (s, 3H), ^{13}C NMR ($CDCl_3$): δ 138.5, 128.14, 127.32, 92.4, 89.9, 73.0, 72.9, 72.5, 59, 40.8, 26.5, 16.3, ES-MS: 284 $(M+NH_4)^+$.

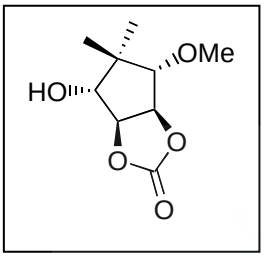
Preparation of (1*R*,3*S*,4*R*,6*S*)-4-benzyloxy-6-methoxy-5,5-dimethyl-tetrahydro-cylopenta[1,3]dioxol-2-one (76):



To an ice cold stirred solution of **75** (1.5 g, 5.63 mmol) and NEt_3 (4.75 ml, 33.8 mmol) in dry CH_2Cl_2 (30 ml) under nitrogen, was added triphosgene (3.34g, 11.27 mmol) portion wise and stirred for 2h. The reaction was quenched with aq. NH_4Cl solution and extracted with CH_2Cl_2 (50 ml x 3). Combined organic layers was dried over Na_2SO_4 and evaporated to afford the crude compound which was purified by column using 10% EtOAc-hexane to afford the title compound as white solid (1.32 g, 80.5 %).

[α] = 13.9 (c 1.0, $CHCl_3$), IR (Neat): 2960, 1808, 1140, 1061 cm^{-1}, ^{1}H NMR ($CDCl_3$): δ 7.37-7.26 (m, 5H), 4.85 (dd, *J* = 9.7 Hz, and *J* = 5.7 Hz, 1H), 4.8-4.76 (m, 2H), 4.56 (d, *J* = 12.0 Hz, 1H), 3.53 (d, *J* = 5.4 Hz, 1H), 3.47 (s, 3H), 3.32 (d, *J* = 5.3 Hz 1H), 1.10 (s, 3H), 0.86 (s, 3H), ^{13}C NMR ($CDCl_3$): δ 154.5, 137.2, 128.4, 127.8, 127.6, 90.2, 87.5, 82.6, 82.5, 72.1, 58.5, 43.2, 24.5, 15.7. Mass (CI method):293 $(M+H)^+$.

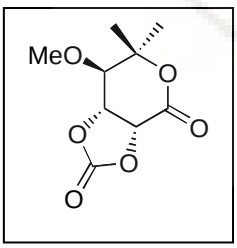
Preparation of (1*R*,3*S*,4*R*,6*S*)-4-Hydroxy-6-methoxy-5,5-dimethyl-tetrahydro-cyclopenta[1,3]dioxol-2-one ((–)-61):



A mixture of **76** (1.2 g, 4.10 mmol) and 10% Pd/C (300 mg) in ethyl acetate was hydrogenated using a H_2 gas balloon at 20 psi pressure for a period of 2h. Pd/C was filtered off and the filtrate was concentrated and purified to afford the title compound as a colorless liquid (755 mg, 91 %).

[α] = -19.4 (c 1.03, $CHCl_3$), IR (Neat): 3444, 2969, 1803, 1099, 1062 cm^{-1}, ^1H NMR ($CDCl_3$): δ 4.80-4.78 (m, 2H), 3.83-3.81 (m, 1H), 3.48 (s, 3H), 3.36-3.35 (m, 1H), 2.20-2.04 (m, 1H), 1.13 (s, 3H), 0.86 (s, 3H), ^{13}C NMR ($CDCl_3$): δ 154.8, 90.4, 83.7, 82.4, 81.5, 58.5, 43.4, 24.2, 15.2, Mass (ES Mass): 220 $(M+NH_4)^+$.

Preparation of (1*R*,3*R*,7*R*)-7-Methoxy-6,6-dimethyl-tetrahydro-[1,3]dioxolo[4,5-*c*]pyran-2,4-dione ((–)-63):



Pyridinium dichromate (PDC) (2.70 g, 7.2 mmol) was added to a solution of **77** (480 mg, 2.37 mmol) in CH_2Cl_2 (40 ml) and the resulting suspension was stirred for 3 days at room temperature (~28 °C). The RM was filtered and the filtrate was evaporated to furnish the corresponding ketone which was used for further step without any purification.

A solution of ketone obtained above and 3-chloroperbenzoic acid (1.7 g, 6.9 mmol) in 15 ml of dry CH_2Cl_2 was stirred for 3 days at room temperature. The reaction mixture was diluted with 100 ml of CH_2Cl_2 and washed with 100 ml of aqueous $Na_2S_2O_3$ and $NaHCO_3$ solution, dried over Na_2SO_4 and evaporated to afford crude compound which was purified to obtain the title compound as a white solid.

[α] = -11.9 (c 1.03, MeOH), IR (Neat): 3421, 2988, 1818, 1749, 1168, 1104 cm^{-1}, ^{1}H NMR ($CDCl_3$): δ 5.22 (d, *J* =10 Hz, 1H), 4.99 (dd, *J* = 10.0Hz and *J* = 7.2 Hz, 1H), 3.60 (s, 3H), 3.44 (d, *J* = 7.0 Hz, 1H), 1.52 (s, 3H), 1.38 (s, 3H), ^{13}C NMR ($CDCl_3$): δ163.5, 152.3, 82.7, 77.9, 70.2, 59.4, 26.8, 22.1, Mass (ES Mass): 234 $(M+NH_4)^+$.

6.5 References

1. For lead references on the role of natural products in drug development, see (a) Newmann, D.J.; Cragg, G.M.; Snader, K.M. *J. Nat. Prod* **2003**, *66*, 1022. (b) Butler, M.S. *J. Nat. Prod* **2004**, 67, 2141. (c) Shu, Y. Z. *J. Nat. Prod* **1998**, *61*, 1053. (d) Newmann, D.J.; Cragg, G.M.; Snader, K. M. *Nat. Prod. Rep.* **2000**, *17*, 215.
2. Wainwright, M. Miracle Cure: *The strory of Penicillin and the Golden Age of Antibiotics*; Blackwell: Oxford, UK, 1990. (b) Sneader, W. *Drug Prototypes and their Expliotation*; Wiley: Chichester, UK, 1996. (c) Mann. J. *The Elusive Magic Bullet: The search of the Perfect Drug*: Oxford University Press: Oxford, UK, **1999**; pp 39-78. (e) Crowley, B. M.; Boger, D. L.; *J. Am. Chem. Soc*. **2006**, *128*, 2885.
3. Thorson, J. S.; Hoster, T. J.; Jiang, J.; Biggins, J. B.; and Ahlert, J. *Curr. Org. Chem.* **2001**, *5*, 139.
4. (a) Maxwell, A. *Biochem. Soc. Trans.* **1999**, *27*, 48. (b) Hooper, D. C.; Wolfson, J. S.; McHugh, G. L.; Winters, M. B.; Swartz, M. N. *Antimicrob. Agents Chemother.* **1982**, *22*, 662. (c) Maxwell, A. *Trends Microbiol.* **1997**, *5*, 102.

5. (a)Lewis, R. J.; Singh, O. M.; Smith, C. V.; Skarzynski, T.; Maxwell, A.; Wonacott, A. J.; Wigley, D. B.; *EMBO J.* **1996** *15*, 1412. (b)Tsai, F. T.; Singh, O. M.; Skarzynski, T.; Wonacott, A. J.; Weston, S.; Tucker, A.; Pauptit, R. A.; Breeze; A. L.; Poyser, J. P.; O'Brien, R.; Ladbury, J. E.; and Wigley, D. B. *Proteins* **1997**, *28*, 41. (c) Kampranis, S. C.; Gormley, N. A.; Tranter, R.; Orphanides, G.; and Maxwell, A. *Biochemistry* **1999**, *38*, 1967-1976. (d) Gormley, N. A.; Orphanides, G.; Meyer, A.; Cullis, P. M.; Maxwell, A. *Biochemistry* **1996**, *35*, 5083. (e) Chatterji, M.; Unniraman, S.; Maxwell, A.; Nagaraja, V. *J. Biol. Chem.* **2000**, *275*, 22888.
6. (a) Downes, C. S.; Ord, M. J.; Mullinger, A. M.; Collins, A. R.; Johnson, R. T. *Carcinogenesis* **1985**, *6*, 1343. (b) Castora, F. J.; Vissering, F. F.; Simpson, M. V. *Biochim. Biophys. Acta* **1983**, *740*, 417. (c) Sung, S. C. *Biochim. Biophys. Acta* **1974**, *361*, 115.
7. Martin, M. A. *Curr. Clin. Top. Infect. Dis.* **1994**, *14*, 170.
8. Hooper, D. C.; Wolfson, J. S.; McHugh, G. L.; Winters, M. B.; Swartz, M. N. *Antimicrob. Agents Chemother.* **1982**, *22*, 662.
9. Blagosklonny, M. V. *Leukemia* **2002**, *16*, 455.
10. (a) Maloney, A.; Workman, P. *Expert Opin. Biol. Ther.* **2002**, *2*, 3. (b) Walter, S.; Buchner, J. *Angew. Chem., Int. Ed.* **2002**, *41*, 1098. (c) Frydman, *J. Annu. Rev. Biochem.* **2001**, *70*, 603. (d) Marcu, M. G.;

Chadli, A.; Bouhouche, I.; Catelli, M.; Neckers, L. M. *J. Biol. Chem.* **2000**, *276*, 37181. (b) Yu, X. M.; Shen, G.; Neckers, L.; Blake, H.; Holzbeierlein, J.; Cronk, B.; Blagg, B. S. J. *J. Am. Chem. Soc.* **2005**, *127*, 12778.

11. Roe, S. M.; Prodromou, C.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *J. Med. Chem.* **1999**, *42*, 260.
12. Zhang, H.; Burrows, F. *J. Mol. Med.* **2004**, *82*, 488.
13. Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57.
14. (a) Shunk, C. H.; Stammer, C. H.; Kaczka, E. A.; Walton, E.; Spencer, C. F.; Wilson, A. N.; Richter, J. W.; Holly, F. W.; Folkers, K. *J. Am. Chem. Soc.* **1956**, *78*, 1770. (b) Kaczka, E. A.; Shunk, C. H.; Richter, I. W.; Wolf, F. I.; Gasser, M. M.; Folkers, K. *ibid*, **1956**, *78*, 4125.
15. Hinman, J. W.; Caron, E. L.; Hoeksema, H. *ibid*, **1957**, *79*, 3789.
16. Hoeksema, H.; Caron, E. L.; Hinman, J. W. *ibid*, **1956**, *78*, 2019.
17. Walton, E.; Rodin, J. O.; Stammer, C. H.; Holly, F. H.; Folkers, K. *ibid*, **1956**, *78*, 5454. (b) Walton, E.; Rodin, J. O.; Stammer, C. H.; Holly, F. H.; Folkers, K. *ibid*, **1958**, *80*, 5168.
18. Hinman, J. W.; Caron, E. L.; Hoeksema, H. *J. Am. Chem. Soc.* **1957**, *79*, 5321.
19. Kiss, J.; Speigelberg, H.; *Helv. Chim. Acta* **1964**, 47, 398.

20. Achmatowicz, O., Jr.; Grynkiewicz, G.; Szechner, B. *Tetrahedron* **1976**, *32*, 1051.

21. Klemer, A.; Waldmann, M. *Liebigs Ann. Chem*. **1986**, *2*, 221.

22. Pankau, W. M.; Kreiser, W. *Helv. Chim. Acta* **1998**, *81*, 1997.

23. Chavis, C.; Dumont F.; Wightman, R. H.; Ziegler, J. C.; Imbach, J. L. *J. Org. Chem.* **1982,** *47*, 202.

24. Takeuchi, M.; Taniguchi, T.; Ogasawara, K. *Tetrahedron Lett.* **2000**, *41*, 2609.

25. (a) Takeuchi, M.; Taniguchi, T.; Ogasawara, K. *Synthesis* **1999**, 341. (b) Taniguchi, T.; Takeuchi, M.; Kadota, K.; ElAzab, A. S.; Ogasawara, K. *Synthesis* **1999**, 1325.

26. (a) Takeuchi, M.; Taniguchi, T.; Ogasawara, K. *Synthesis* **1999**, 341 (b) Taniguchi, T.; Ohnishi, H.; Ogasawara, K. *Chem. Commun*. **1996**, 1477.

27. Jeselnik, M.; Leban, I.; Polanc, S.; Kocevar, M. *Org. Lett.* **2003**, *5*, 2651.

28. (a) Laurin, P.; Ferroud, D.; Klich, M.; Dupuis-Hamelin, C.; Mauvais, P.; Lassaigne, P.; Bonnefoy, A.; Musicki, B. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 2079. (b) Periers, A.-M.; Laurin, P.; Benedetti, Y., Lachaud, S.;

Ferroud, D.; Iltis, A.; Haesslein, J.-L.; Klich, M.; L'Hermite, G.; Musicki, B. *Tetrahedron Lett.* **2000**, *41*, 867.

29. Laurin, P.; Ferroud, D.; Klich, M.; Dupuis-Haelin, C.; Mauvais, P.; Lassaigne, P.; Bonnefoy, A.; Musicki, B. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 2079.

30. Musicki, B.; Periers, A.-M.; Laurin, P.; Ferroud, D. ; Benedetti, Y.; Lachaud, S.; Chatreaux, F.; Haesslein, J-L.; Iltis, A.; Pierre, C. *Bioorganic & Medicinal Chemistry Letters*, **2000**, 10**,** 1695.

31. Gammon, D. W.; Hunter, R.; Wilson, S. *Tetrahedron Lett.* **2002**, *43*, 3141.

32. Yu, X. M.; Shen, G.; Blagg, B. S. J. *J. Org. Chem.* **2004**, *69*, 7375.

33. (a) Hudlicky, T.; Luna, H.; Price, J. D.; Rulin, F. *J. Org. Chem*.**1990**, *55*, 4683 (b) Kotsuki, H.; Miyazaki, A.; Ochi, M. *Tetrahedron Lett.* **1991**, *32*, 4503 (c) Kotsuki, H.; Araki, T.; Miyazaki, A.; Iwasaki, M.; Datta, P. K. *Org. Lett.* **1999**, *1*, 499.

34. Yu, X. M.; Shen, G.; Neckers, L.; Blake, H.; Holzbeierlein, J.; Cronk, B.; Blagg, B S. J. *J. Am. Chem. Soc.* **2005**, *127*, 12778.

35. Trost, B. M.; Yang, H.; Probst, G. D. *J. Am. Chem. Soc.* **2004**, *126*, 48.

36. Pirrung, F.O.H.; Hiemstra, Speckemp, W. N.; Kaptien, B.; Schoemaker, H. E. *Synthesis*, **1995**, 458.

37. Jones, E. R. H. *J. Chem. Soc.* **1953**, 457.

38. Luche, J. L.; Gemal, A. L. *J. Am. Chem. Soc.* **1979**, *101*, 5848.

39. Deardorff, D. R.; Shambayati, S.; Myles, D. C.; Heerding, D. *J. Org. Chem.* **1988**, *53*, 3614.

40. Omura, K.; Swern**,** D. *Tetrahedron,* **1978**, *34, 1651.*

41. For a recent review on Baeyer-Villiger oxidation see ten Brink, G. J.; Arends, I. W. C. E.; Sheldon, R. A. *Chem. Rev*. **2004**, *104*, 4105 and references cited therein.

42. The cost of (–)-pantolactone (Fluka) 10g = $41 whereas, the cost of 1g of (+)-pantolactone is $85.

43. (a) Mandel, A. L.; La Clair, J. J.; and Michael D. Burkart *Org. Lett.* **2004**, *6,* 4801 (b) Matthew O'Brien, Nicholas H. Taylor, and Eric J. Thomas *Tetrahedron Lett.* **2002**, *43*, 5491.

44. ref. 26 (b)

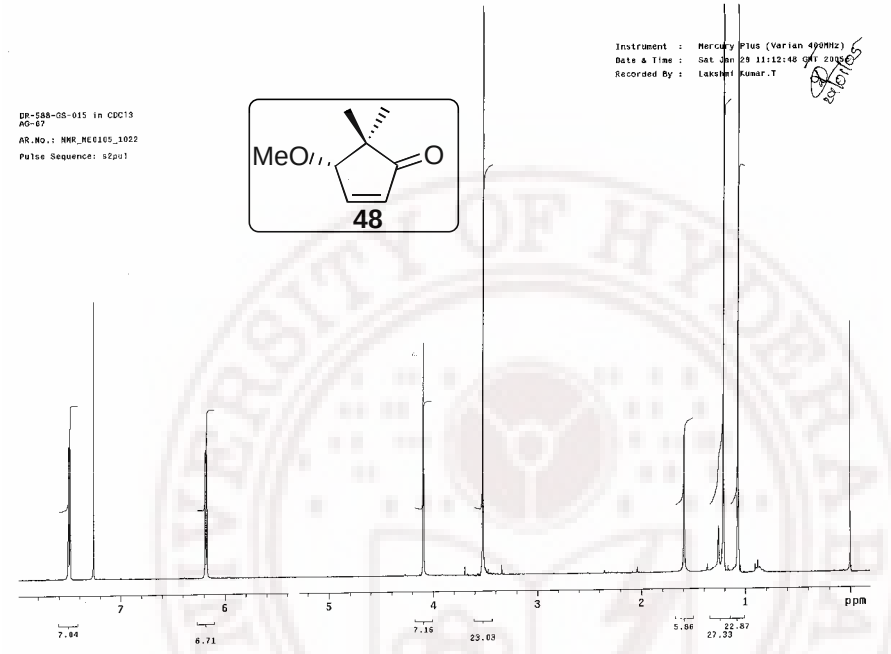
45. For reviews, see: (a) Gawly, R. E. *Org. React.* **1988**, *35*, 1 and references therein (b) Smith, M. B.; March J. In *Advanced Organic Chemistry*, 5th ed.; John Wiley & Sons: New York, 2001; p 1415 and references therein. For selected examples of catalytic Beckmann rearrangement, see: (a) Narasaka, K.; Kusama, H.; Yamashita, Y.; Sato, H. *Chem. Lett*. **1993**, 489. (b) Ichihashi, H.; Kitamura, M. *Catal. Today* **2002**, *73*, 23. (c) Yadav, J. S.; Reddy, B. V. S.; Madhavi, A V.; Ganesh, Y. S. S. *J. Chem. Res. (S)* **2002**, 236. (d) Srinvas, K. V. N. S.; Reddy, E. B.; Das, B. *Synlett* **2002**, 625. (e) Ikushima, Y.; Sato, O.; Sato, M.; Hatakeda, K.; Arai, M. *Chem. Eng. Sci.* **2003**, *58*, 935. (f) Ferna´ndez, A. B.; Boronat, M.; Blasco, T.; Corna, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 2370.

46. Furuya, Y.; Ishihara, K.; Yamamoto, H. *J. Am. Chem. Soc.* **2005**, *127*, 11240.

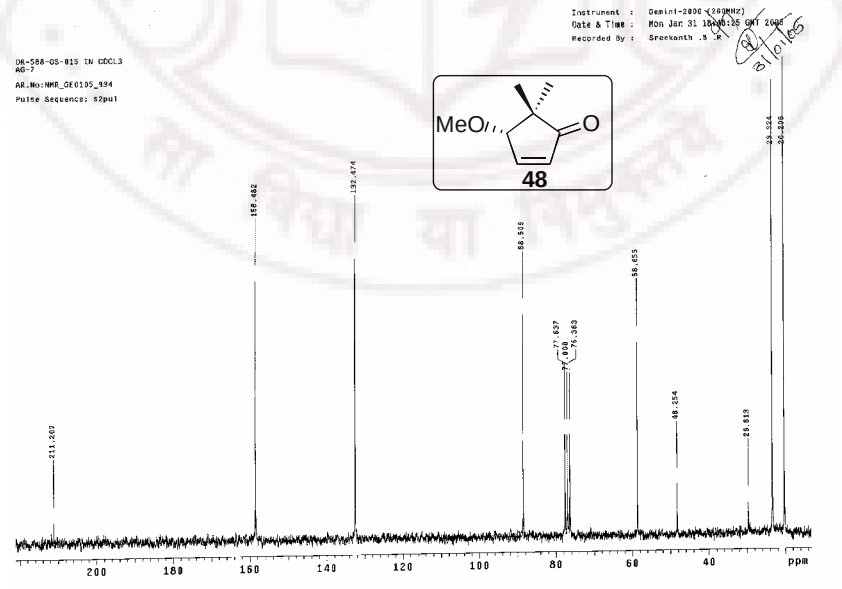
47. Desai, P.; Schildknegt, K.; Agrios, K. A.; Mossman, C.; Milligan, G. L.; Aube, J. *J. Am. Chem. Soc.* **2000**; *122*, 7226.

6.6 Spectral data ^{1}H and ^{13}C NMR data

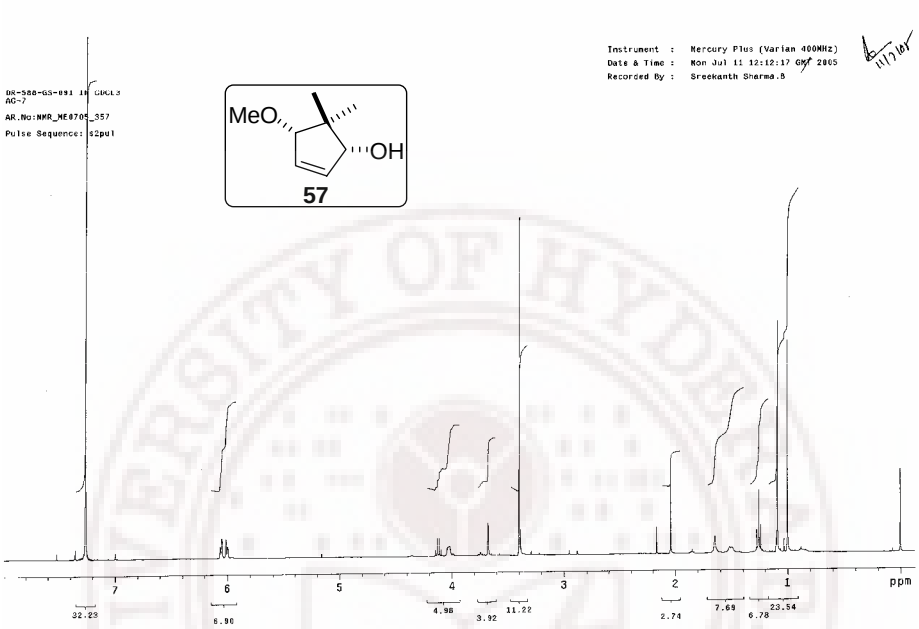
Spectrum 1 ^{1}H NMR Spectrum of 48 in $CDCl_3$



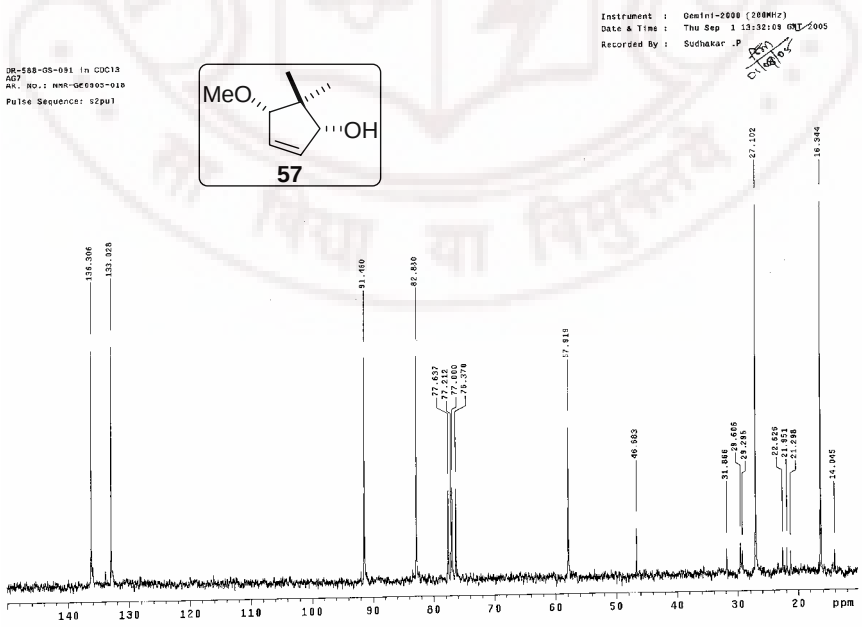
Spectrum 2 ^{13} C NMR Spectrum of 48 in $CDCl_3$



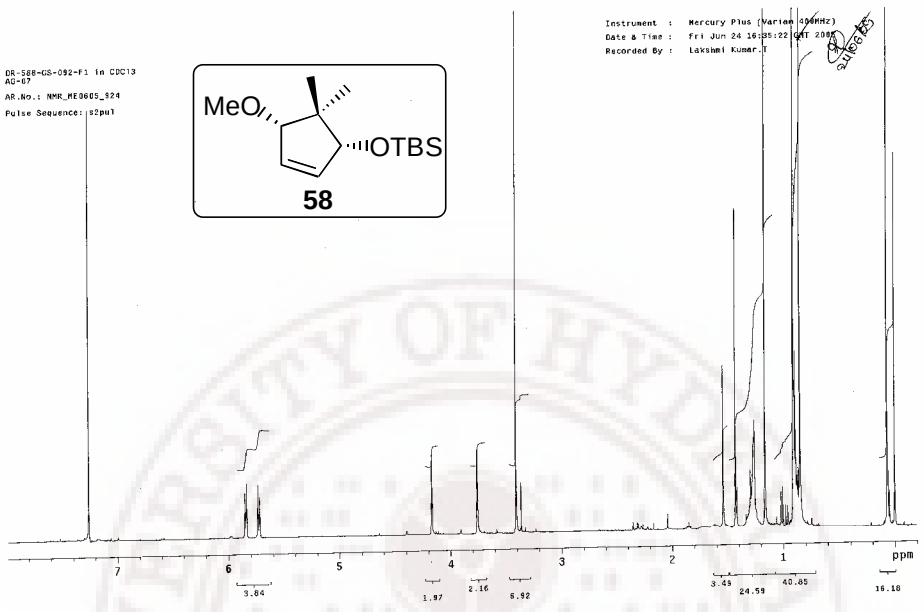
Spectrum 3 ^{1}H NMR Spectrum of 57 in $CDCl_3$



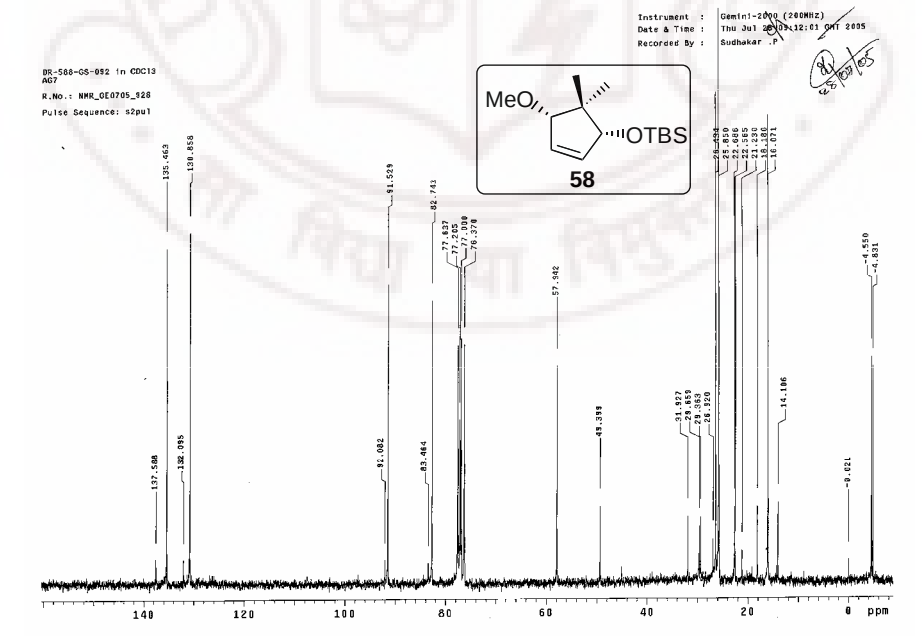
Spectrum 4 ^{13}C NMR Spectrum of 57 $CDCl_3$



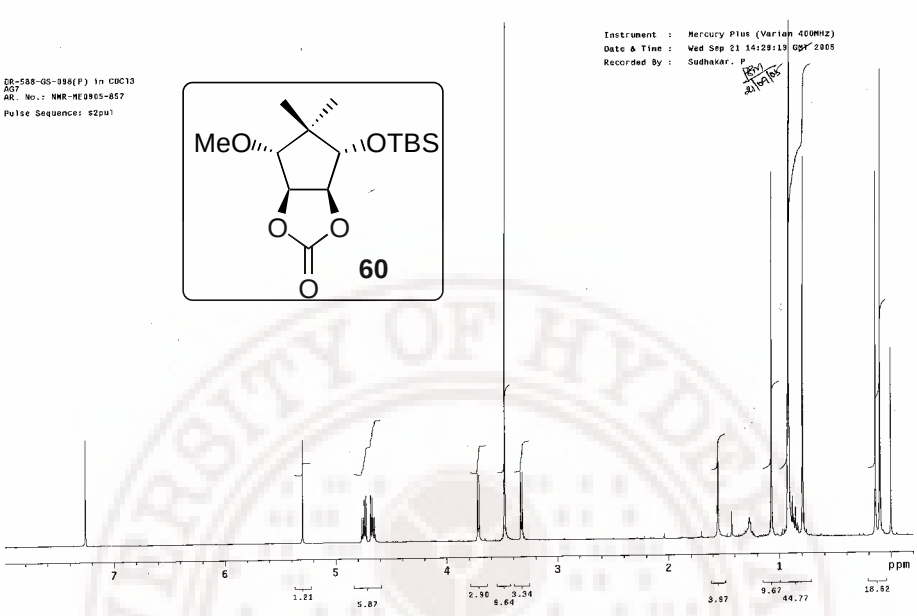
Spectrum 5 ^{1}H NMR Spectrum of 58 in $CDCl_3$



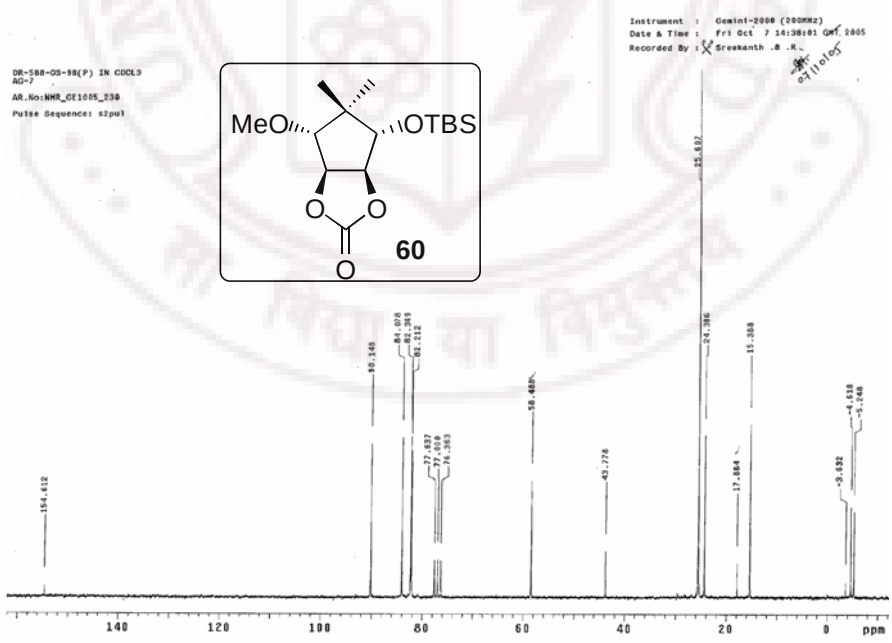
Spectrum 6 ^{13}CNMR Spectrum of 58 in $CDCl_3$



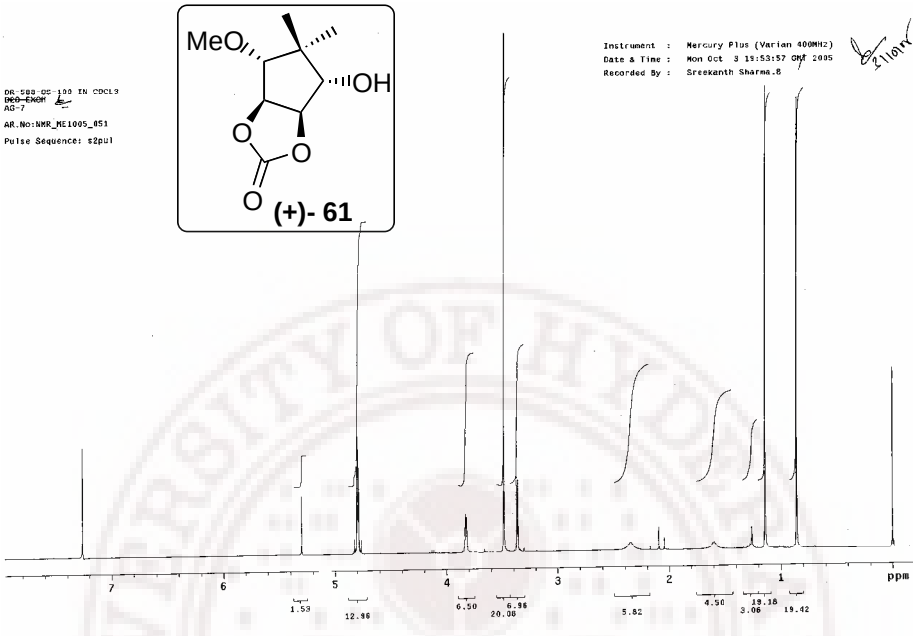
Spectrum 7 ^{1}H NMR Spectrum of 60



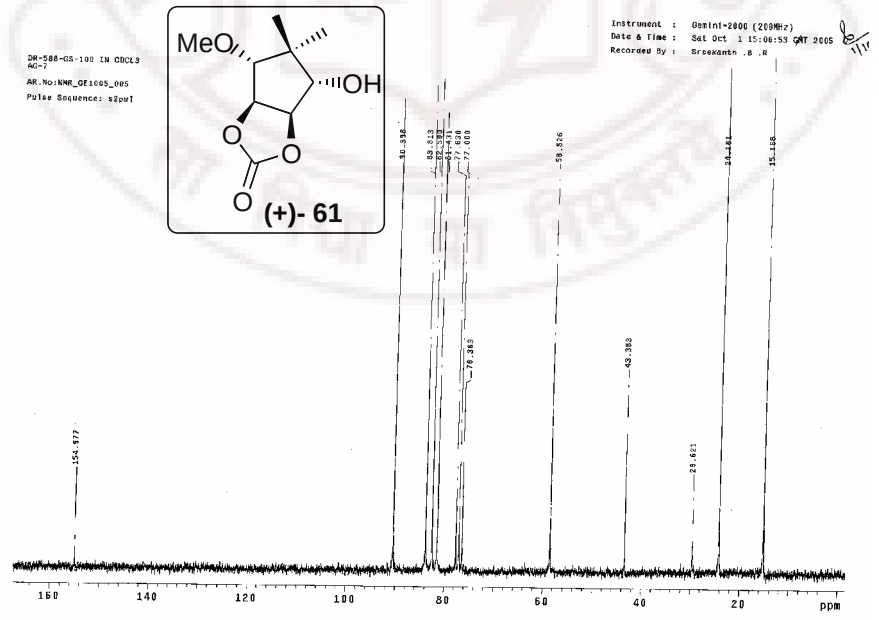
Spectrum 8 ^{13}C NMR Spectrum of 60 in $CDCl_3$



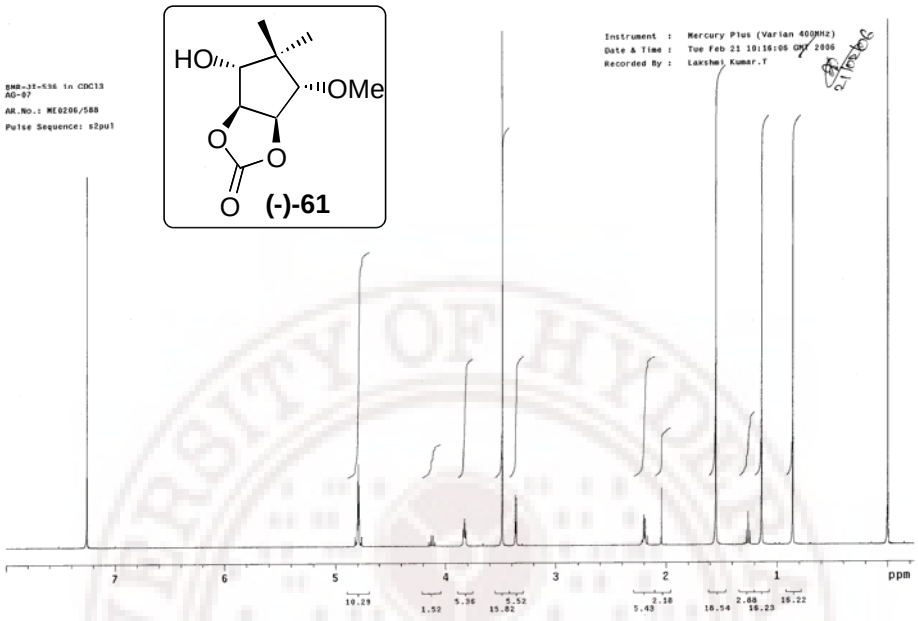
Spectrum 9 ^{1}H NMR Spectrum of (+)-61 in $CDCl_3$



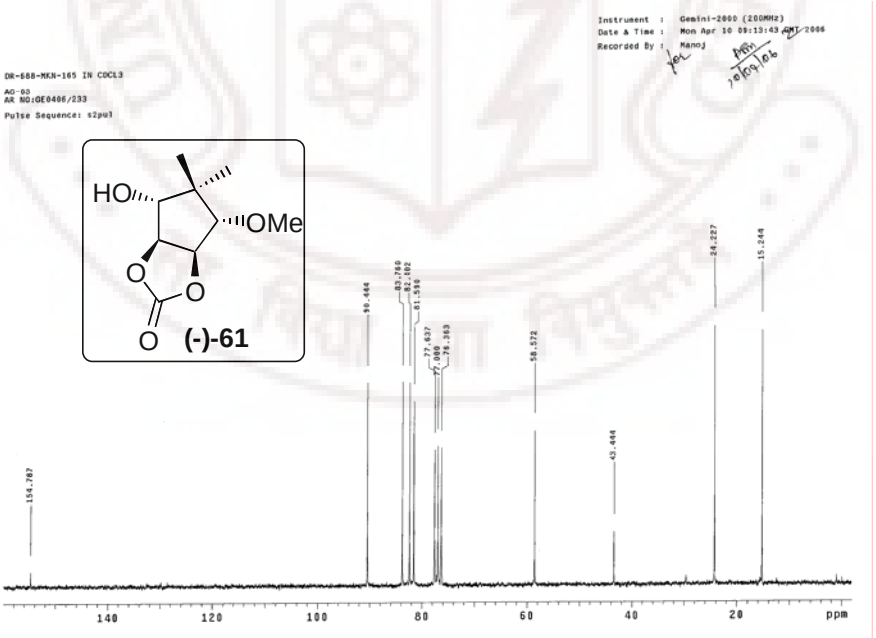
Spectrum 10 ^{13}C NMR Spectrum of (+)-61 in$CDCl_3$



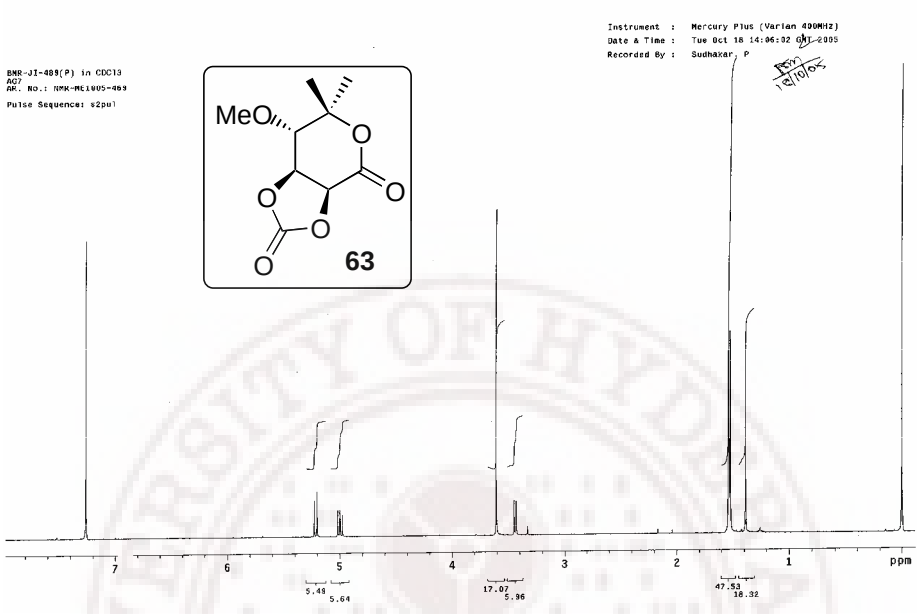
Spectrum 11 ^{1}H NMR Spectrum of (–)-61 in $CDCl_3$



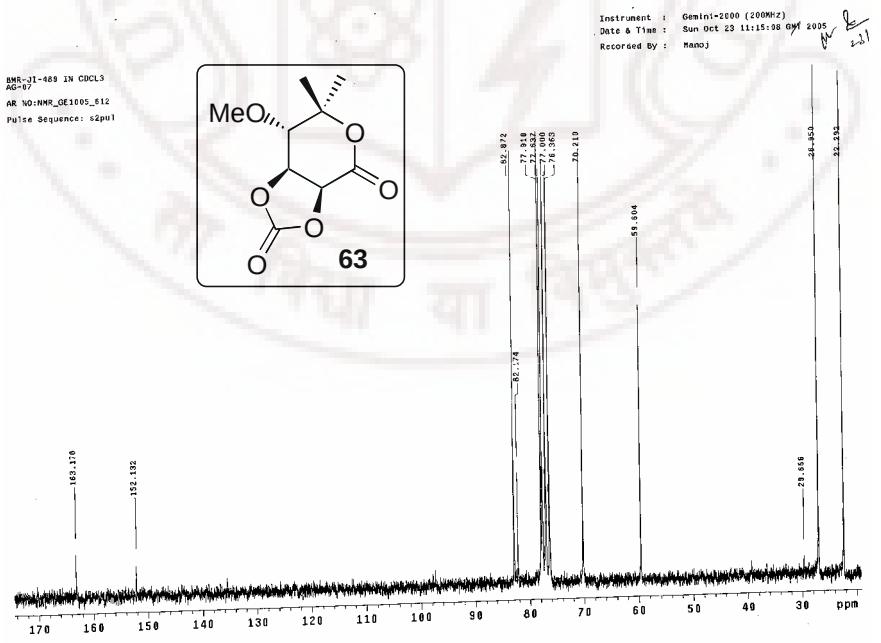
Spectrum 12: ^{13}C NMR spectrum of (–)-61 in $CDCl_3$)



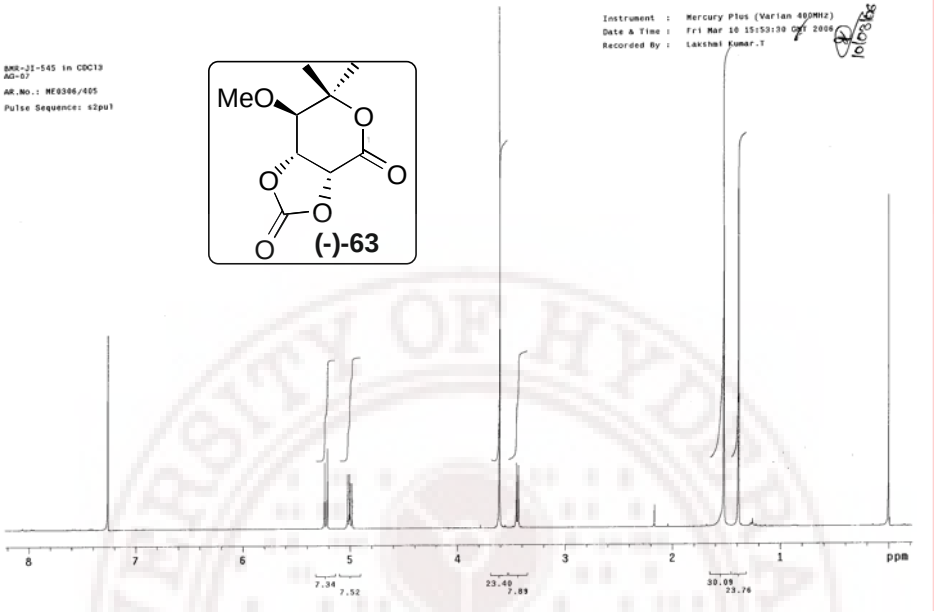
Spectrum 13 ^{1}H NMR Spectrum of (+)-63 in $CDCl_3$



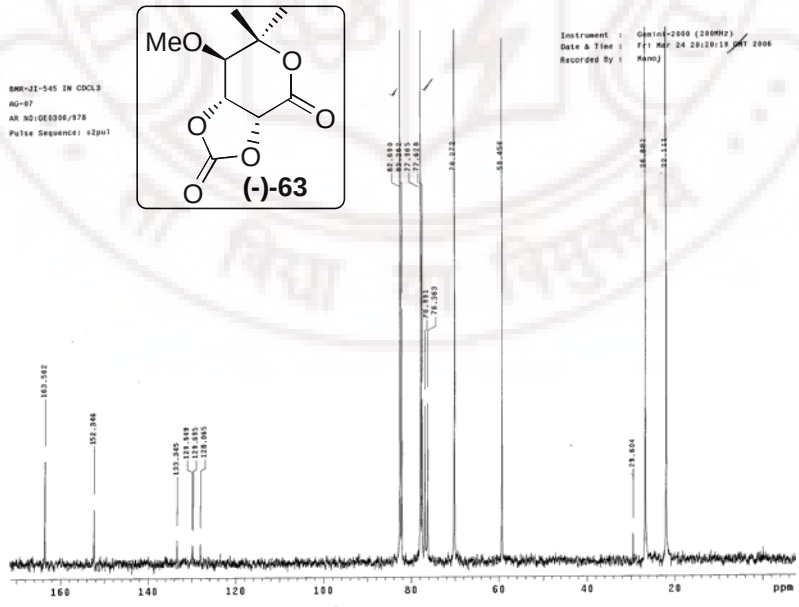
Spectrum 14 ^{13}C NMR Spectrum of (+)-63 in $CDCl_3$



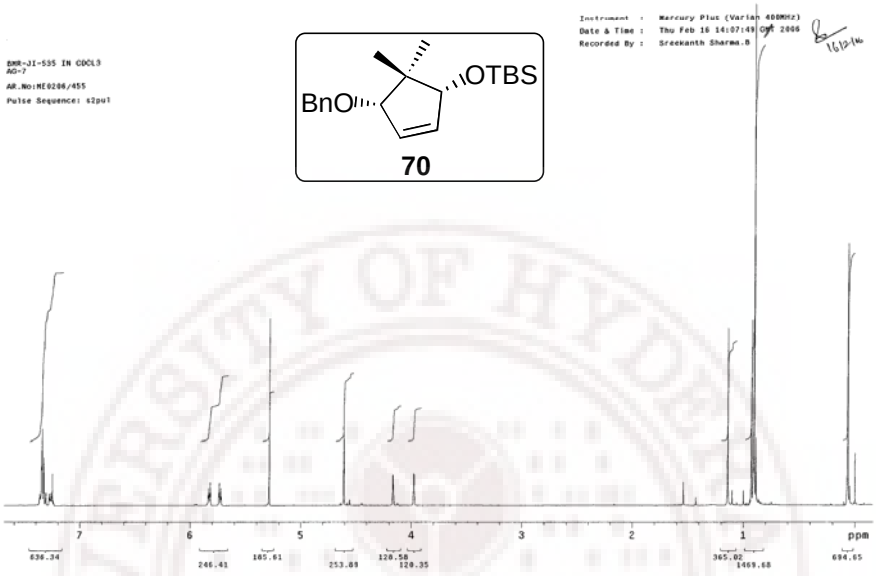
Spectrum 15 ^{1}H NMR Spectrum of (–)-63 in $CDCl_3$



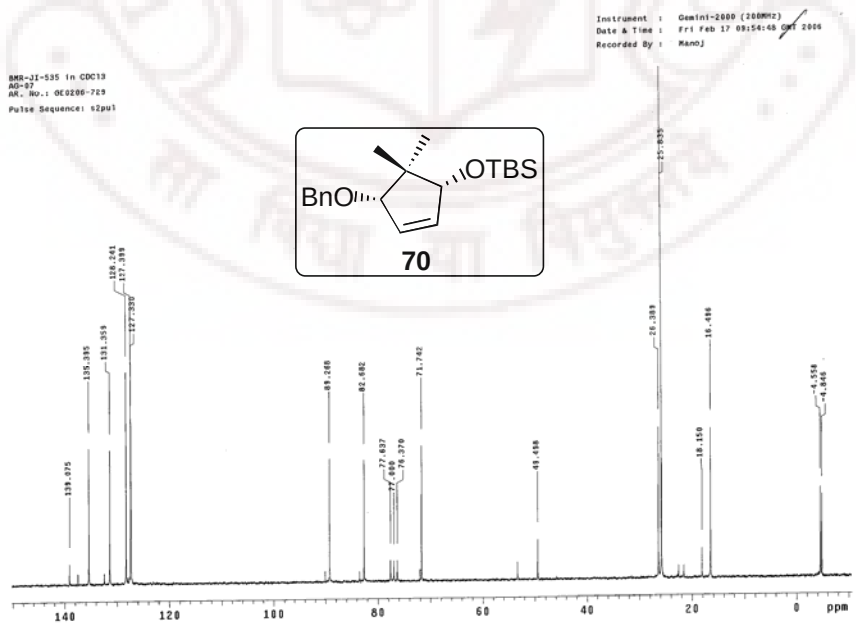
Spectrum 16 ^{13}C NMR Spectrum of (–)-63 $CDCl_3$



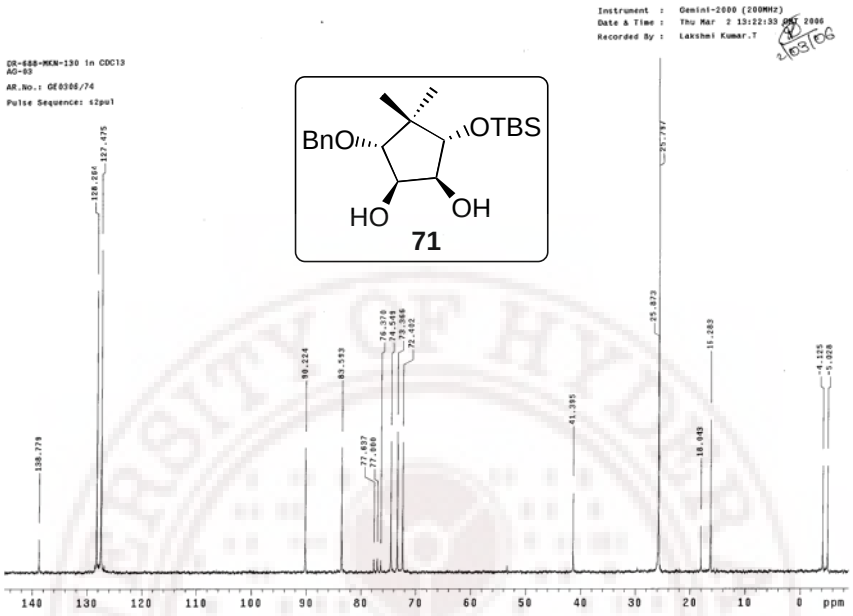
Spectrum 17 ^{1}H NMR Spectrum of (–)-70 in $CDCl_3$



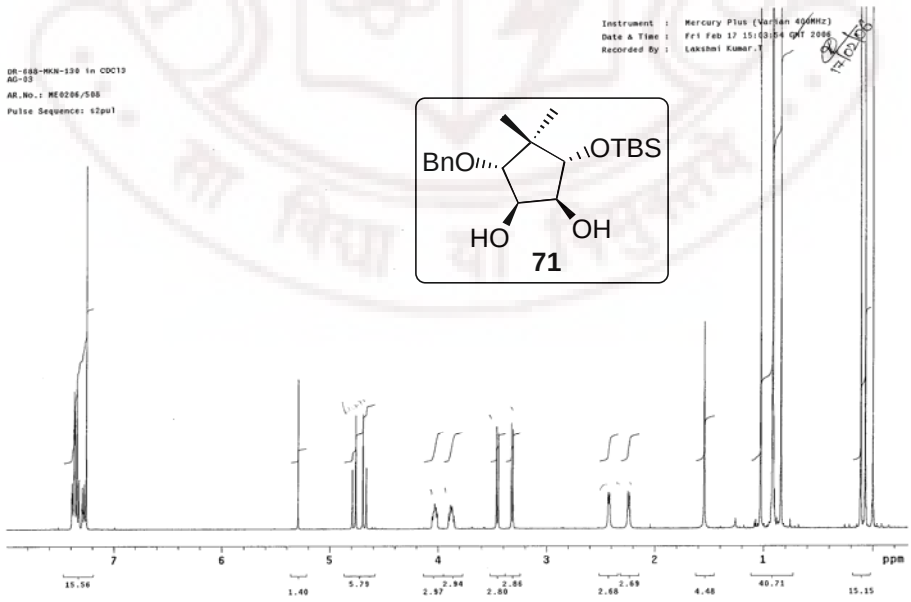
Spectrum 18 ^{13}C NMR Spectrum of (–)-70 in $CDCl_3$



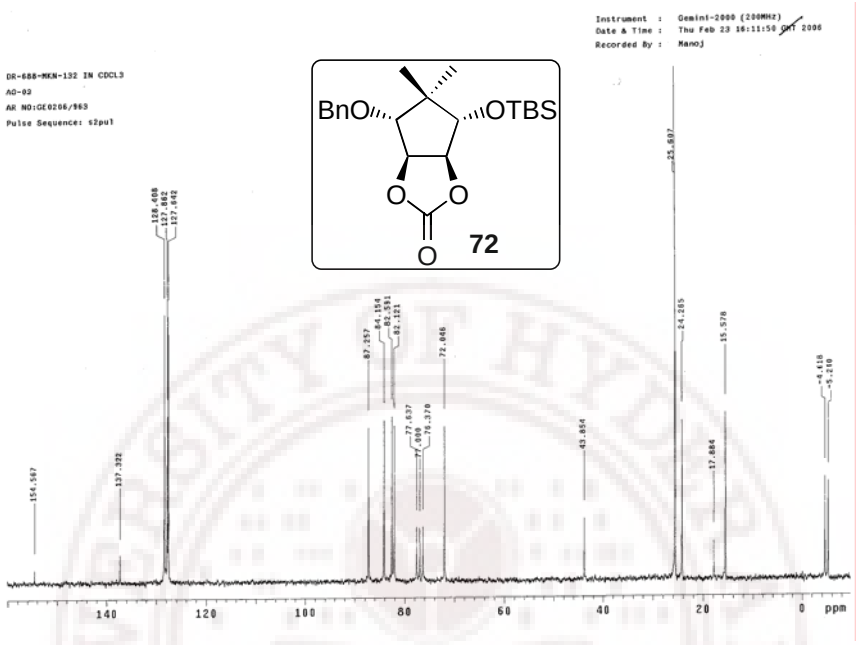
Spectrum 19 ^{1}H NMR Spectrum of 71 in $CDCl_3$



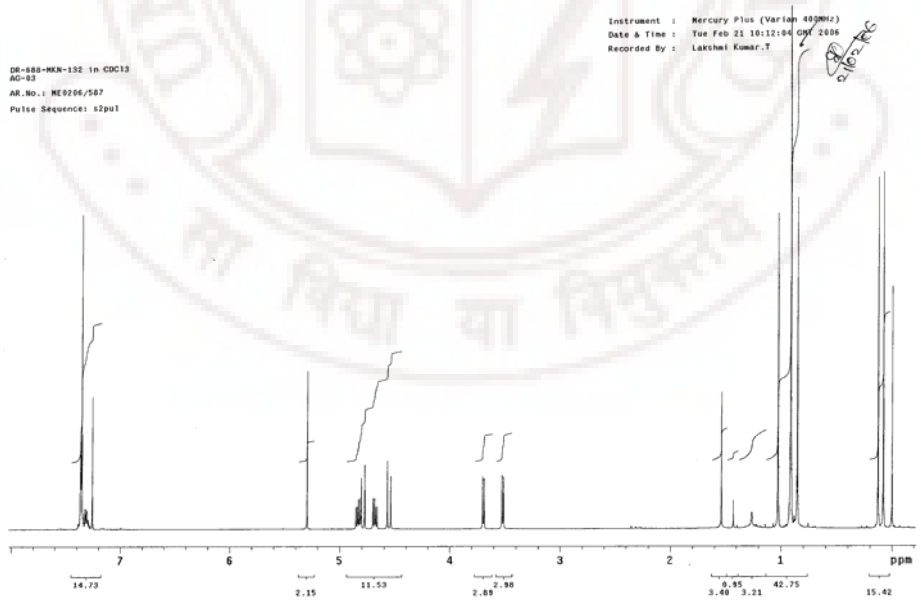
Spectrum 20 ^{1}H NMR Spectrum of 71 in $CDCl_3$



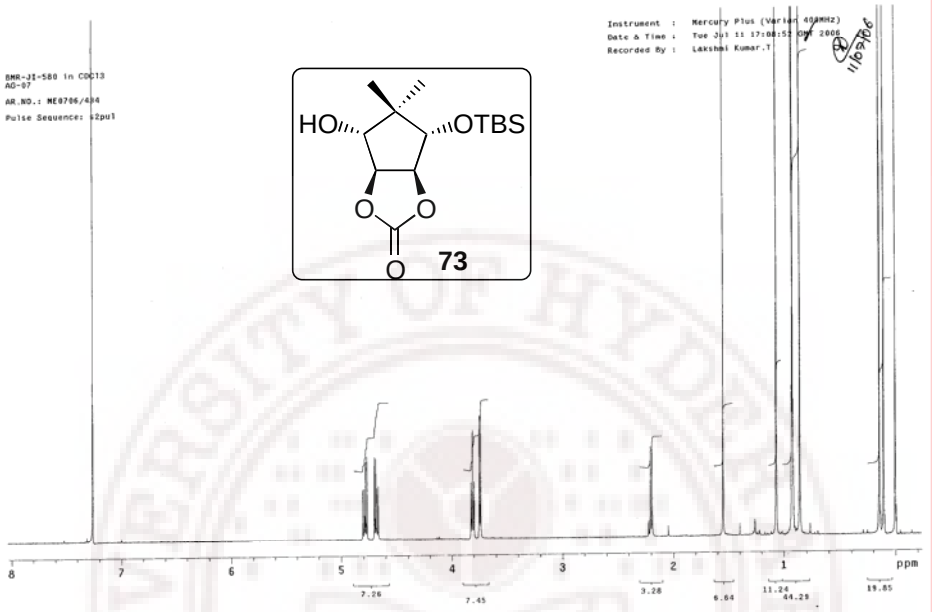
Spectrum 21 ^{13}C NMR Spectrum of 72 in $CDCl_3$



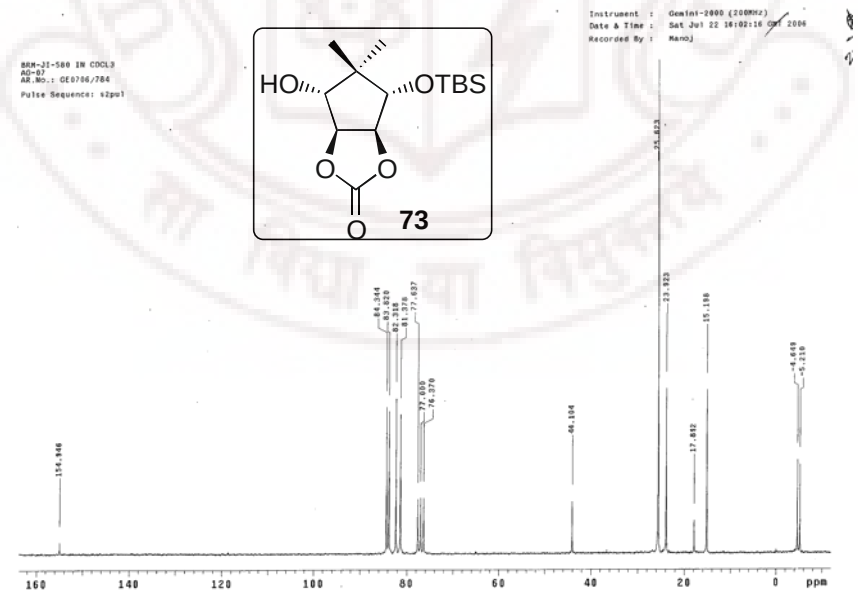
Spectrum 22 ^{1}H NMR Spectrum of 72 in $CDCl_3$



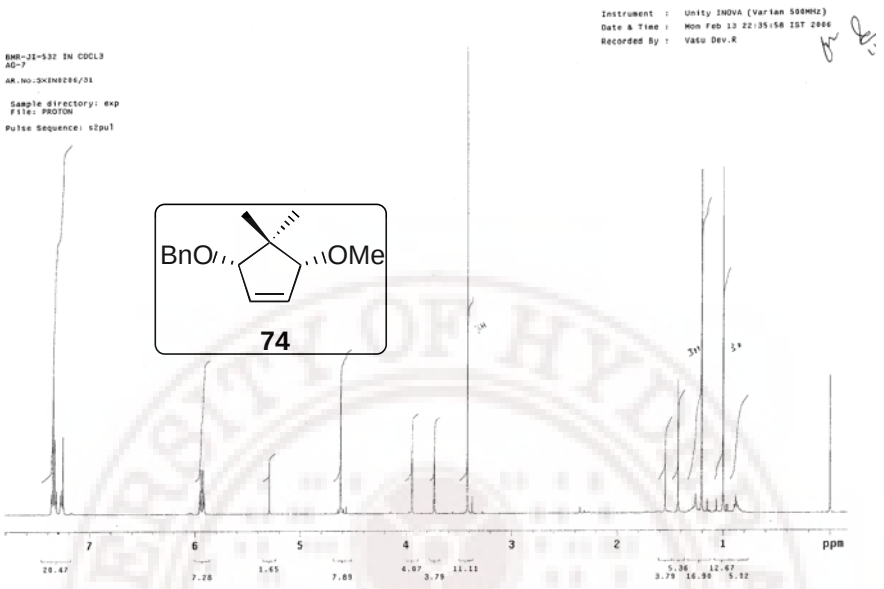
Spectrum 23 ^{1}H NMR Spectrum of 73 in $CDCl_3$



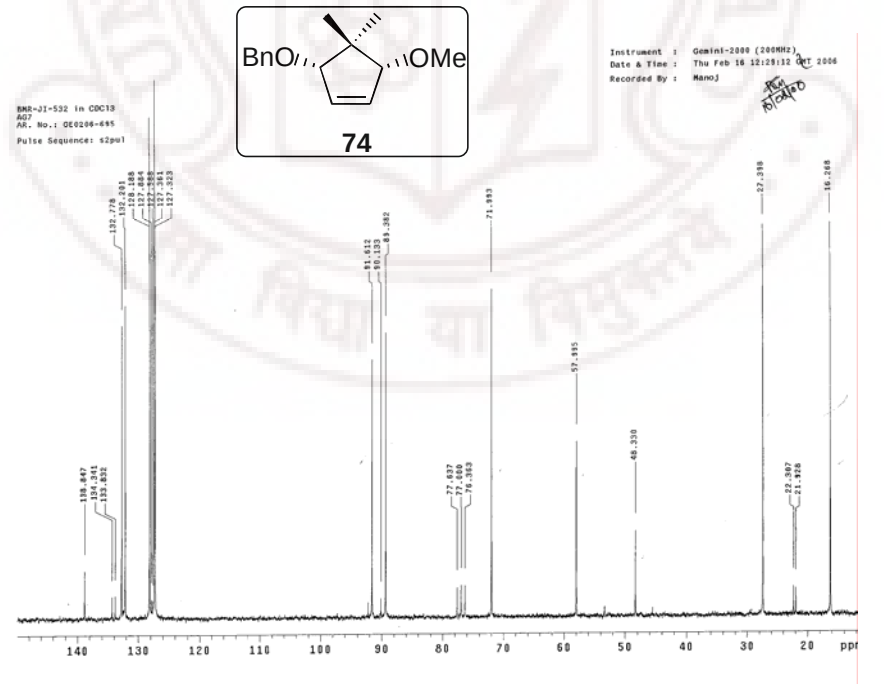
Spectrum 24 ^{13}C NMR Spectrum of 73 in$CDCl_3$



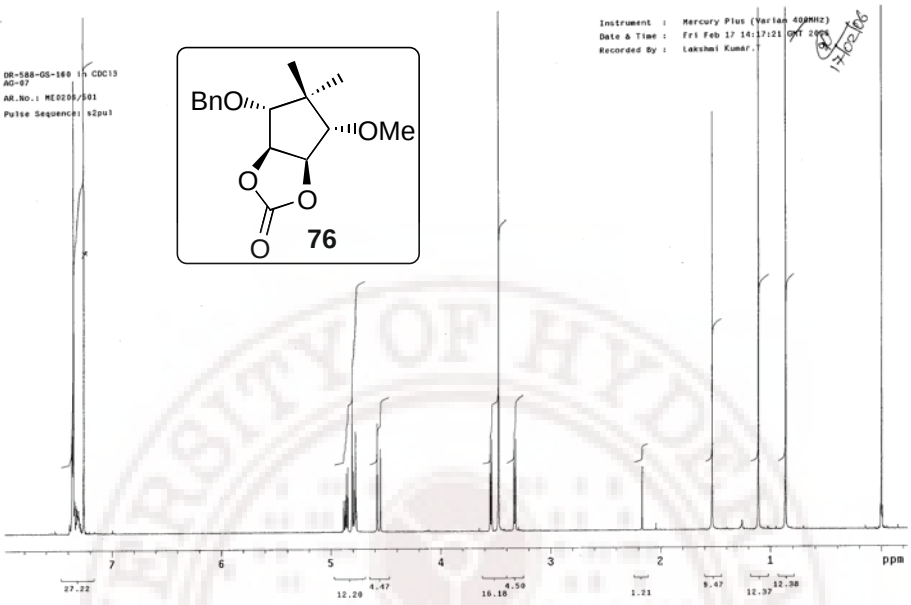
Spectrum 25 ^{1}H NMR Spectrum of 74 in $CDCl_3$



Spectrum 26 , ^{13}C NMR Spectrum of 74 in$CDCl_3$



Spectrum 27 ^{1}H NMR Spectrum of 76 in $CDCl_3$



Spectrum: ^{13}C NMR Spectrum of 76 in $CDCl_3$