Design, Synthesis & Structural Investigations of Template-Assisted Peptides and Synthesis of Novel Peptide Derivatives as Potential Central Nervous System Active Agents

Thesis Submitted to AcSIR For the Award of
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In Chemical Sciences

By
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Abbreviations

A
Å Ångström
α Alpha
ACN Acetonitrile
AcOH Acetic acid
AcOEt Ethyl acetate
Aib α-amino isobutyric acid
Ala Alanine
Ant Anthranilic acid
B
β beta
Boc tert-Butyloxycarbonyl
C
Calcd Calculated
$CDCl_3$ Chloroform-*d*
COSY Correlated spectroscopy
Cbz Benzyl carbamate
D
d doublet (NMR)
δ Chemical shift (NMR)
DBU 1,8-Diazabicycloundec-7-ene
DCM Dichloromethane
DMF Dimethylformamide
DIPEA N,N-Diisopropylethylamine
DMSO Dimethyl sulfoxide
DMAP 4-Dimethylaminopyridine
E
ESI Electron spray ionization
EDC 1-Ethyl-3(3-dimethylamino Propyl) carbodiimide
G
γ Gamma
Gly Glycine
H
H-bond Hydrogen bond
HBTU O-benzotriazol-1-yl-N,N, N',N'-tetramethyluronium hexafluorophosphate
HOBt 1-Hydroxybenzotriazole
Hz Hertz
HRMS High Resolution Mass Spectrometry

K
K_{dim} Dimerization constant
K_{taut} Tautomeric constant
L
Leu Leucine
LCMS Liquid chromatography–mass spectrometry
M
m Multiplet (NMR)
Me Methyl
MHz Megahertz
MS Mass spectrometry
ms millisecond

N
NOESY Nuclear Overhauser Effect Spectroscopy
O
Orn Ornithine

P
Piv Pivaloyl
Pd/C palladium 10 % on activated carbon
Pro Proline
Pet ether Petroleum ether
R
r.f. Radio frequency
S
s Singlet (NMR)
S second
T
t Triplet (NMR)
TFA Trifluroacetic acid
TEA Triethyl amine
THF Tetrahydrofuran
TOCSY Total Correlation Spectroscopy
τ Tau
V
Val Valine
ω Omega

ABSTRACT

Name of the Candidate	**Krishna Prasad M**
Research Supervisors	**Dr. G. J. Sanjayan**
Title of the Ph. D. thesis	**Design, Synthesis & Structural Investigations of Template-Assisted Peptides and Synthesis of Novel Peptide Derivatives as Potential Central Nervous System Active Agents**

Preamble: Template assisted peptide folding is an important phenomenon that allows nature to carry many important biological functions. It is a process in which molecules adopt a well-defined low energy minimum conformation through non-covalent interactions like van der Wall forces, π- π interactions, hydrogen bonding and so on. Of the several non-covalent interactions present, hydrogen bonding finds several applications due to its strength, directionality and specificity. Synthetic peptide analogues are also widely recognized as important lead compounds for the generation of novel CNS active agents. NMDA receptor has drawn particular interest as it covers a wide range of CNS disorders. Non-proteinogenic amino acids designed and synthesized by chemists are largely used to mimic the secondary structure of peptides.

Contents: This thesis is divided into three chapters. The first chapter deals with the design of dimedone-based rigid synthetic scaffold for organizing symmetrical peptide chains attached on a single carbon. It describes the synthesis and conformational investigations of single stranded and double stranded peptide chains attached to dimedone as an organic scaffold. The second chapter deals with the design, synthesis and characterization of novel unnatural amino acid incorporated close structural mimics of potent antidepressant drug rapastinel. The last chapter chronicles the design, synthesis and characterization of novel azepine and norquetiapine derived hybrid drug analogues as potential central nervous system active agents.

Chapter 1: Design, synthesis and conformational investigations of novel single stranded and double stranded peptide chains attached to dimedone as an organic scaffold.

This chapter describes the synthesis and its structural investigations of symmetrical peptide chains attached to scaffold containing dimedone. There are a large number of template molecules that are found in the literature which stimulates and stabilizes secondary and

tertiary structures of the peptides covalently attached to them. The main hurdle in handling secondary structures of small peptides is their inherently flexible nature, thus posing a serious problem in controlling the molecular orientation, a prerequisite for creating ordered supramolecular assemblies. Dimedone-sulfur intermediate **A** as a template consisting of two carbonyls as hydrogen bond acceptor is a simple synthetic molecule that plays a major role in the synthesis of various supramolecular architectures (Fig. 1.1).

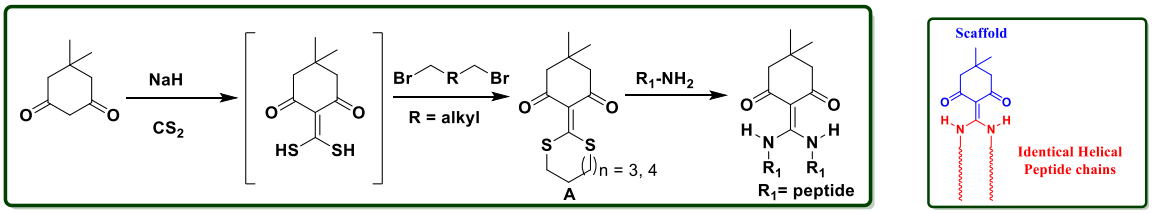


Figure 1.1: Synthetic route for template-assisted synthesis of double stranded peptides from dimedone-sulfur intermediate **A**.

Tripeptide Boc-Lue-Aib-Val-OMe **3a**, tetrapeptide Boc-Ala-Lue-Aib-Val-OMe **4a**, heptapeptide Boc-Gly-Lue-Aib-Val-Ala-Lue-Aib-Val-OMe **5a**, octapeptide Boc-Gly-Lue-Aib-Val-Ala-Lue-Aib-Val-OMe **6**, pentadecapeptide Boc-Gly-Lue-Aib-Val-Ala-Lue-Aib-Val-Lue-Aib-Val-Ala-Lue-Aib-Val-OMe **7** and hexadecapeptide Boc-Gly-Lue-Aib-Val-Ala-Lue-Aib-Val-Gly-Lue-Aib-Val-Ala-Lue-Aib-Val-OMe **8** were synthesized using EDC.HCl as coupling agent in solution phase which were then Boc-deprotected using TFA and treated with dimedone-sulfur intermediate **10a** and **10b** to furnish corresponding symmetrical double stranded peptide derivatives in good yields as shown in Fig. 1.2a.

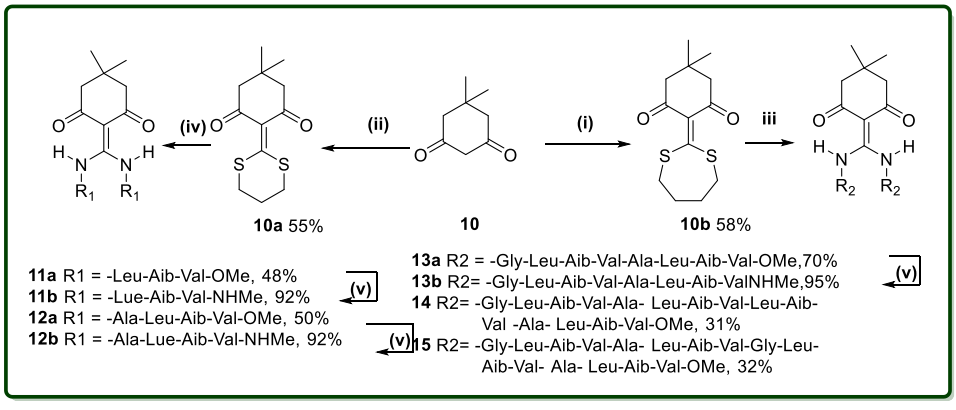


Figure 1.2a: Synthesis of dimedone-assisted symmetrical double stranded peptides.

Dimedone based template assisted single stranded peptides were also prepared by treating dimedone-sulfur intermediate **9** with various peptides as shown in Fig. 1.2b.

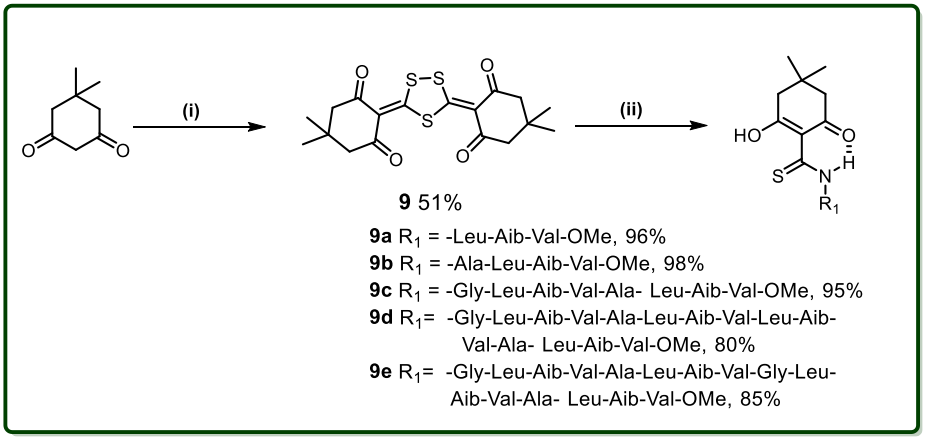


Figure 1.2b: Synthesis of dimedone-assisted single stranded peptides.

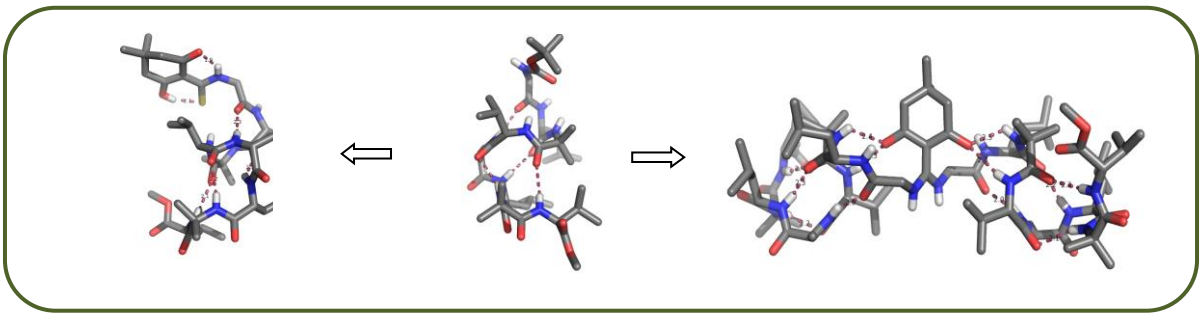


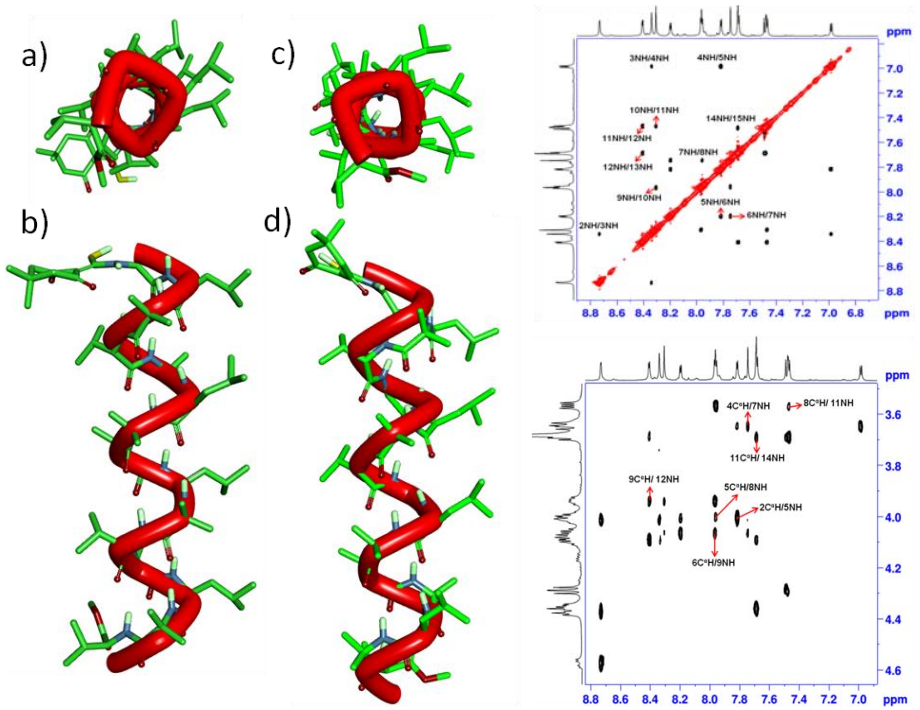
Figure 1.3a: PyMOL rendered crystal structures of dimedone-assisted single stranded octapeptide **9c** (left), Boc-octapeptide **6** (middle), dimedone-attached symmetrical double stranded peptide **13a** (right).

When two symmetrical peptide chains are attached to the scaffold containing dimedone, peptide chains gets oriented in such a way that it forms a strong C-12 and C-15 intramolecular bifurcated hydrogen bonding with the dimedone's carbonyl all together adopting a different conformation in solid state as shown for **13a** (Fig. 1.3a). This is also evident in short peptides in solid state as shown for **11b** in Fig. 1.3b.

11b

Figure 1.3b: PyMOL rendered crystal structure of dimedone-assisted double stranded peptide **11b**.

We have also investigated the conformational preferences of higher oligopeptides by solution state NMR spectroscopy and MD simulation studies. Extensive NMR investigations revealed the existence of 13-helix conformation for the higher oligopeptides **7**, **8**, **9d** and **9e**.



(A) **(B)**

Figure 1.4 : (A) Minimum energy structures obtained from the ROEs derived distances for compounds **9d** and **9e** . Top view of **9d** (a) side view of **9d** (b) topview of **9e** (c) and side view of **9e** (d); (B) characteristic nOe's of Compound **9d**.

Chapter 2: Design and synthesis of novel unnatural amino acid incorporated structural mimics of potent antidepressant drug Rapastinel.

Part A: Novel silaproline (Sip)-incorporated close structural mimics of potent antidepressant peptide drug rapastinel.

Silaproline (Sip) is a proline (Pro) analogue in which the γ-methylene carbon is substituted by dimethyl silyl group. Sip exhibits and induces similar conformational properties as the natural amino acid Pro. This part describes the synthesis of a new class of rapastinel drug analogs carrying silaproline (Sip) - a well known surrogate for proline, using standard peptide coupling strategy in the solution-phase.

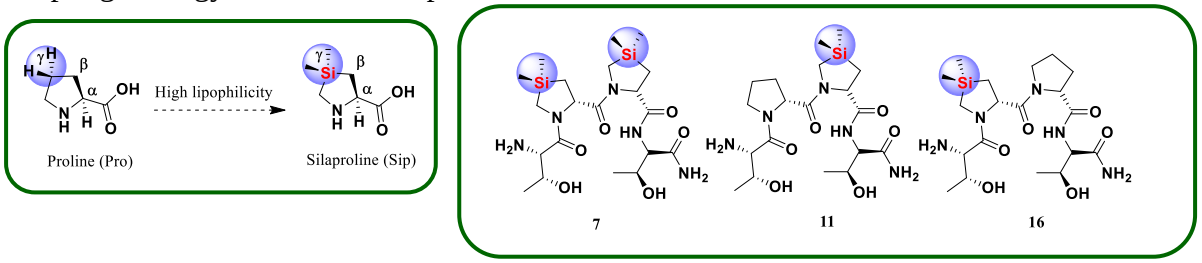


Figure 2.1: Structure of proline and silaproline (left). Molecular structures of peptide analogues 7, 11, 16 containing silaproline as proline surrogate(right).

Silaproline was incorporated into rapastinel peptide sequence, considering its utility to enhance the pharmacokinetic profiles of proline-containing peptides such as lipophilicity, hydrophobicity, cell permeability and conformational stability. All novel peptides were well characterized by spectroscopic tools.

Part B: Novel Aib, Fenclonine and L-threo-3-phenylserine incorporated close structural mimics of potent antidepressant peptide drug rapastinel.

Rapastinel analogues containing Aib, p-chlorophenylalanine and L-threo-3-phenylserine were synthesized using standard peptide coupling strategy in the solution-phase as shown in Fig. 2.2. All novel peptides were well characterized by spectroscopic tools.

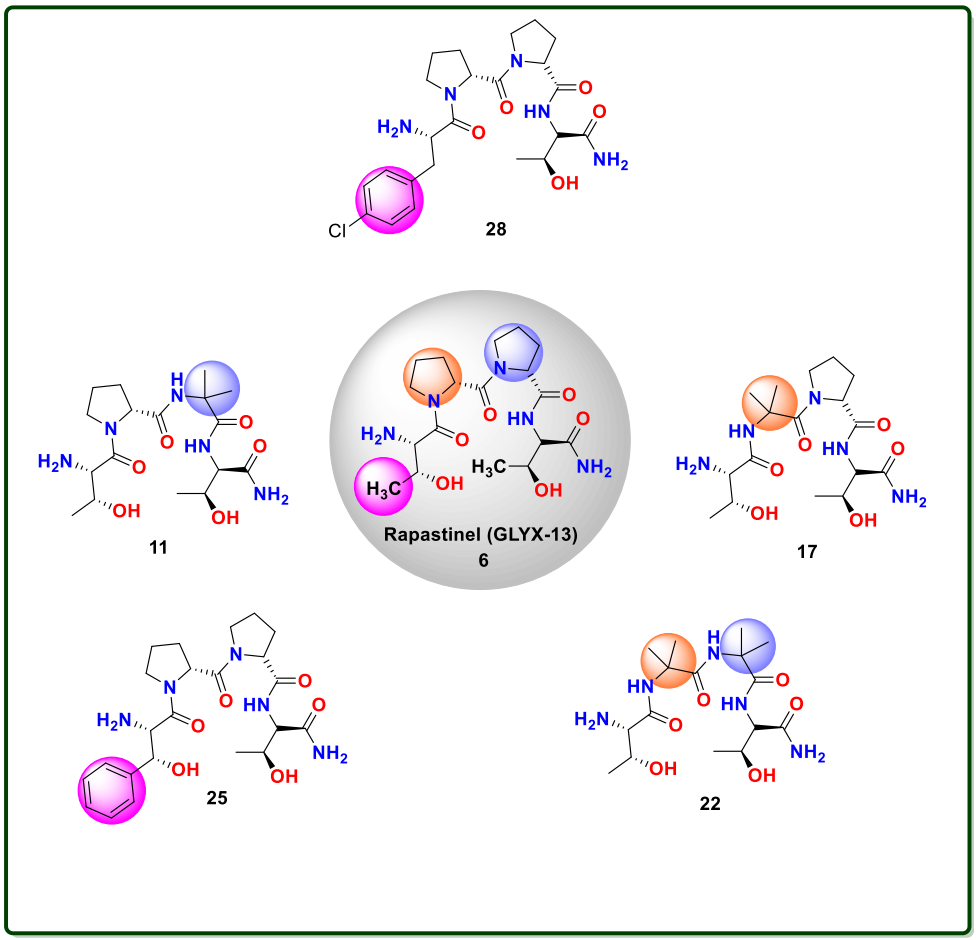


Figure 2.2: Molecular structures of potent antidepressant drug rapastinel analogues synthesized.

Chapter 3: Design and synthesis of novel 10,11-dihydro-5H-dibenz[b,f]azepine and Norquetiapine derived hybrid drug molecules as potential CNS active agents.

Tricyclic antidepressants (TCAs) and Tetracyclic antidepressants (TeCAs) are a class of medications that are used primarily as antidepressants. Novel 10,11-dihydro-5H-dibenz[b,f]azepine and Norquetiapine derived hybrid drug analogues containing molecules such as Gabapentin, Fenclonine, L-threo phenyl serine and Boc-Glu(OtBu)OH were

synthesized using standard peptide coupling strategy in the solution-phase as shown in Fig. 3.1. All novel hybrid molecules were well characterized by spectroscopic tools.

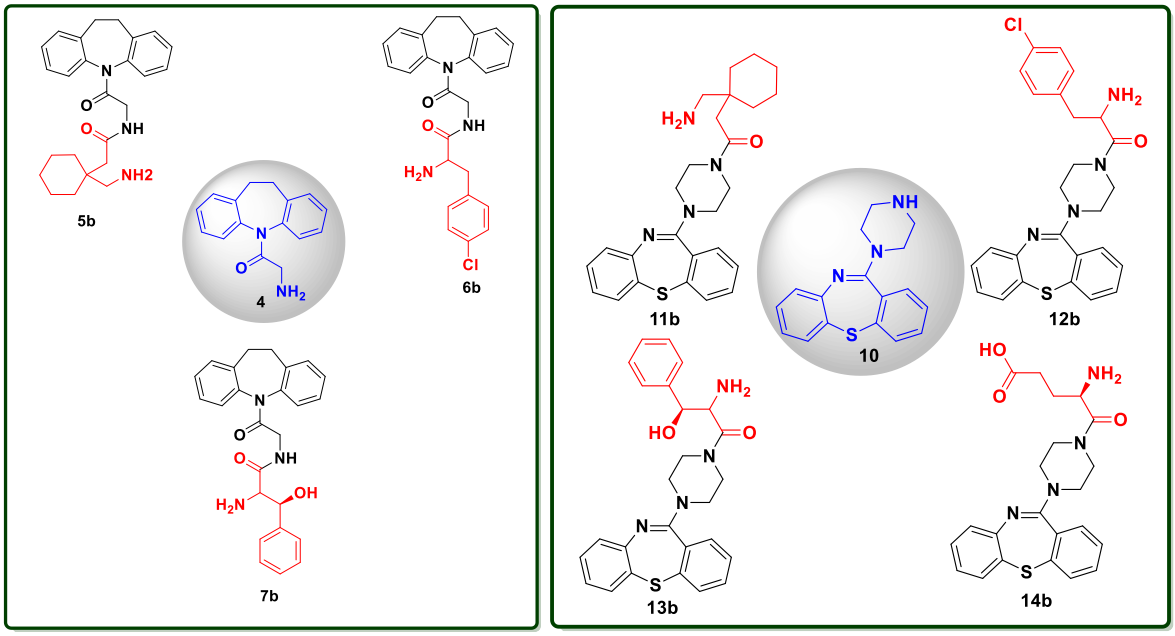


Figure 3.1: Molecular structures of potent azepine and norquetiapine containing analogues synthesized.

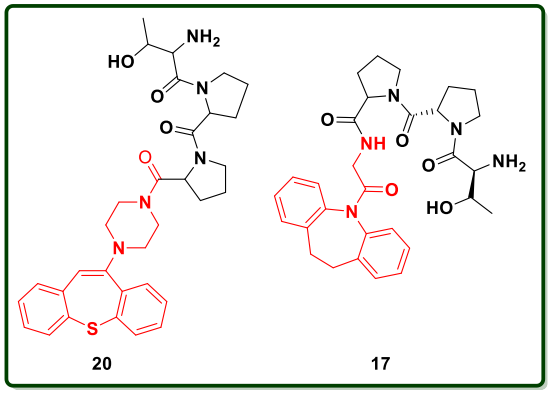


Figure 3.2: Molecular structures of potent antidepressant drug azepine and norquetiapine containing rapastinel analogues synthesized.

Azepine and Norquetapine incorporated rapastinel analogues were also synthesized using standard peptide coupling strategy in the solution-phase as shown in Fig. 3.2. All novel molecules were well characterized by spectroscopic tools. These molecules were synthesized considering its utility to enhance the pharmacokinetic profiles such as lipophilicity, hydrophobicity, cell permeability and conformational stability.

General Remarks

- Unless otherwise stated, all the chemicals and reagents were obtained commercially.
- Required dry solvents and reagents were prepared using the standard procedures.
- All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel plates (Kieselgel 60F254, Merck) with UV, I_2 or ninhydrin solution as the developing reagents in the concerned cases.
- Column chromatographic purifications were done with 100-200 Mesh silica gel or with flash silica gel (230-400 mesh) in special cases.
- NMR spectra were recorded on AV 400 MHz, AV 500 MHz and AV 700 MHz Bruker NMR spectrometers. All chemical shifts are reported in δ ppm downfield to TMS and peak multiplicities as singlet (s), doublet (d), quartet (q), broad (br), broad singlet (bs) and multiplet (m).
- High-resolution mass spectrometric analyses (HRMS) was carried out using a Thermo Scientific Q-Exactive, Accela 1250 pump mass spectrometer.
- nOe-restrained molecular modeling studies were carried out using Insight II (97.0)/Discover program on a Silicon Graphics Octane workstation and MacroModel, Maestro version from Schrodinger software in CSIR-IICT, Hyderabad.
- Single crystal X-ray data were collected on a Bruker SMART APEX CCD Area diffractometer and Bruker D8 VENTURE Kappa Duo PHOTON II CPAD diffractometer.
- Circular dichroism (CD) was performed using JASCO 2000 spectrometer and Spectra were smoothened and plotted using Origin Pro 6.0 software.

List of Publications

1. Krishna Chaitanya Nadimpally, **Krishnaprasad Madica,** Aswathi Chakrapani, Priyanka Prabhu, Dr. Gangadhar J. Sanjayan. Rigid Peptide Scaffold-Incorporated Structural Analogs of the Potent Antidepressant Peptide Drug Rapastinel (GLYX-13). *ChemistrySelect.* **2017**, *2*, 3594-3596.
2. **Krishnaprasad Madica**, Krishna Chaitanya Nadimpally and Gangadhar J. Sanjayan. "Novel silaproline (Sip)-incorporated close structural mimics of potent antidepressant peptide drug rapastinel (GLYX-13)." *Tetrahedron Lett.* **2017**, *58*, 1568-1571.
3. **Krishnaprasad Madica**, Krishna Chaitanya, Rajesh Gonnade and Gangadhar J Sanjayan "Helically Structured Peptide Self-Assembly Engineered Using Dimedone as a Rigid Organic Scaffold" *ChemistrySelect.* **2018**, *3*, 2776–2780.
4. **Krishnaprasad Madica,** Jerripothula Lakshmi, Krishna Chaitanya Nadimpally, Ekta Sangtani, Rajesh Gonnade, Bharatham Jagadeesh and Gangadhar J Sanjayan “Dimedone-Based Rigid Organic Scaffold for Organizing Symmetrical Helical Peptide Chains". **(*Manuscript under preparation*)**.

Chapter 1

Design, synthesis and conformational investigations of novel single stranded and double stranded peptide chains attached to dimedone as an organic scaffold.

1.1 Introduction

Biopolymers adopt a well-defined low energy minimum conformation through non-covalent interactions like van der Wall forces, π- π interactions, hydrogen bonding and electrostatic interactions.[1] Among them, peptides and proteins play a prominent role in the growth of a cell or an organism by these interactions.[2] A protein is composed of one or more linear chains of amino acids, each of which is called a polypeptide. These polypeptides consist mainly of twenty natural amino acids and some rare amino acids. There exists a different combination of amino acids in the nature which makes the polypeptide chain to orient in different shapes. This leads to enormous structural diversity of proteins and each structure gives different information which is useful in many important physiological functions.

According to Linderstrom Lang, peptide folding in nature is divided mainly in to four types namely primary protein structure, secondary protein structure, tertiary protein structure and quaternary protein structure.[3]Primary protein structure consists of a sequence of a chain of amino acids. Secondary protein structure occurs when the sequence of amino acids is linked by hydrogen bonds to generate β-sheet, turns and helices.[4]Tertiary protein structure occurs when certain attractions are present between pleated sheets and helices i.e. between the secondary structures. Finally quaternary protein structure can be described as a protein containing more than one or two amino acid chain as shown in Fig. 1.11

Synthetic peptides containing non-natural peptide chain architecture which tend to fold into regular secondary structures are valuable tools in understanding protein-protein interactions, protein folding etc.[5]The designed peptides that fold into α-helical conformation assumes importance as it is the most abundant secondary structural motif present in nature constituting of 30% of protein secondary structure.[6] Non proteinogenic amino acids help in controlling the helical structure by imparting their conformational preferences either by hydrogen bonding or by a combination of hydrogen bonding and hydrophobic interactions.

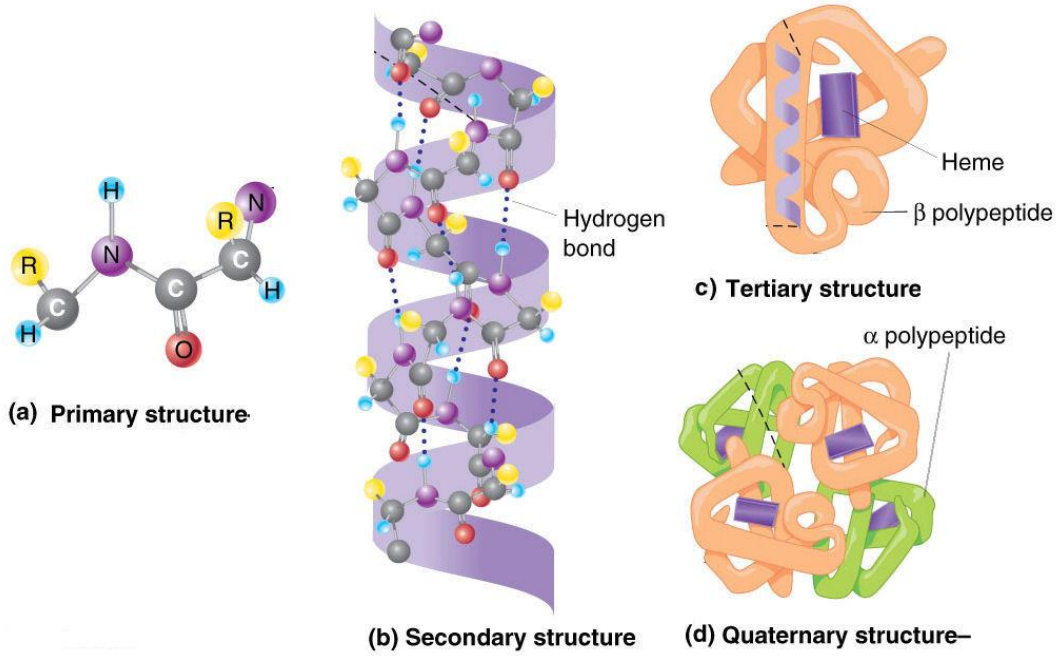
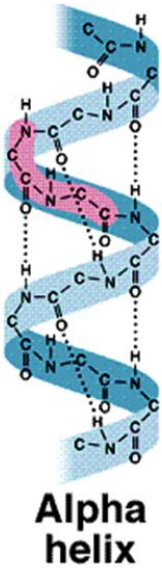


Figure 1.11 Four levels of protein structure (Image source: internet) [33]

1.1.1 α-helix (Secondary protein structure)

The alpha Helix (α-helix) is a secondary structural motif present in proteins and it exists in a right handed-spiral conformation (i.e. helix). It has regular periodic structure and there exists hydrogen bonding of backbone N-H group with the backbone C=O group of the amino acid located four residues earlier along the peptide sequence. It is also most abundant structural motif among the existing secondary structures in nature. It consists of 13 member hydrogen bond rings and it is generally denoted as 13-helix as 13 atoms are involved in the ring formation. The general average number of residues per helical turn is around 3.6.



Generally amino acids that predominantly prefer α-helical conformation in proteins include methionine, alanine, leucine, glutamate and lysine. Proline and glycine tend to disrupt α-helices and is generally considered as α-helix breaker. Proline lacks N-H bond to donate to the carbonyl group thereby destabilizing the helical architecture and glycine tends to disrupt α-helices due to their built-in conformational flexibility.[7]

1.1.2 Peptides containing non-natural oligomers

Peptides are composed of amino acids with short and length specific oligomers. These bio-molecules are found everywhere in living cells and participate in interactions including cell receptor ligand etc. There exists a three-dimensional structure for each bioactive peptide resulting in various supramolecular structures. In this way, there has been significant interest in making full use of these bioactive peptides through nature's evolution in designing pharmaceutical lead compounds. However, unfortunately, these bioactive peptides themselves are inferior drug candidates because of their low cell permeability, susceptibility, low bioavailability, conformational flexibility and inferior metabolic stability.[8]

In order to overcome adverse peptide characteristics and generate library of pharmaceutical agents, there has been a considerable focus on the creation of non-natural peptide mimics. Any oligomer that mimics primary structure of peptides by means of amide bond isosteres is called peptidomimetics. These peptidomimetics are generated by modifying native peptide backbone either by heteroatom incorporation or chain extension or by using template. Oligomers which are peptidomimetics are often protease-resistant and could have improved bioavailability and cell permeability compared to their native peptide analogues. In addition to mimicking peptide primary structure, chemists have designed and synthesized sequence-specific non-natural peptide oligomers called foldamers[6] which exhibits well-defined secondary structural motifs such as helices, sheet-like structures and turns. Examples of simple peptidomimetic oligomers include oligoureas, azapeptides and oligocarbamates and some common foldamers include vinylogous sulfonopeptides, γ-peptides, β-peptides, phenylene ethynylene, and peptoids as shown in Fig 1.12.

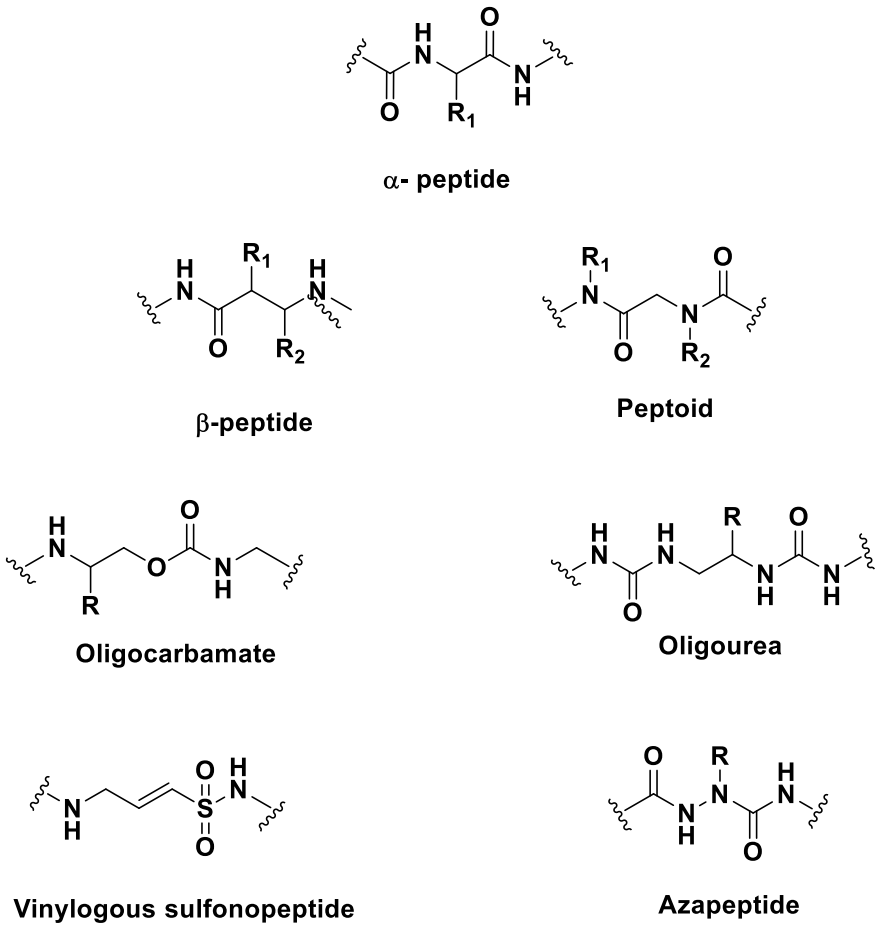


Figure 1.12 Representative examples for sequence-specific peptidomimetic oligomers.

Sequence-specific non-natural oligomers are beginning to find important biomimetic applications and also these non natural peptides also find use as drug therapeutics.

1.1.3 Non-natural helical oligomers:

The identification of new polymer backbones with compact and conventional folding propensities provides a source for design of biomimetics with potential applications. Polyamide family favouring sequence specific helical secondary structures serves as a building block for stable tertiary structures.[9]

Lehn *et al* reported helical molecular chains constituted of extended hyz-pym sequences with 3.3 units per turn whereas I Huc *et al* reported oligoamides of 8-amino-2-carboxy-quinoline and it adopted a very stable helical conformation with only 2.5 units per turn as shown in Fig. 1.13.[10]

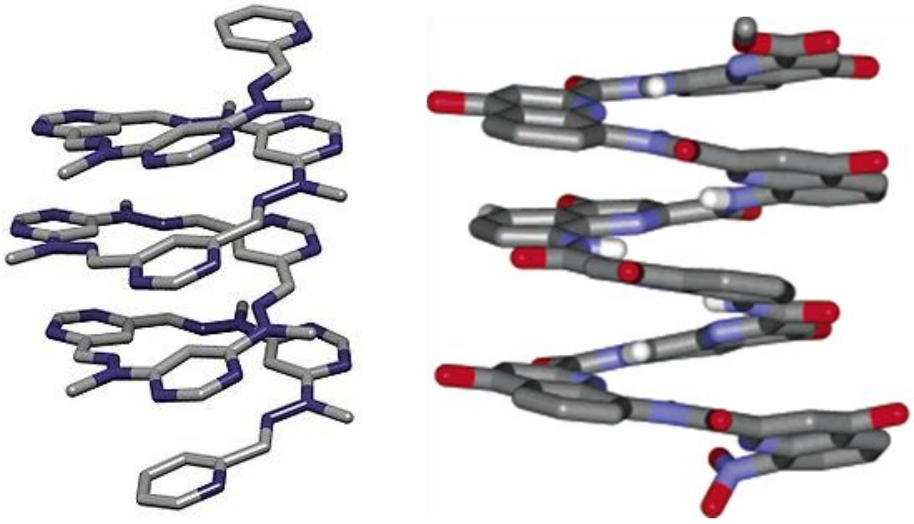


Fig 1.13: Representative examples for helical aromatic foldamers: hyz-pym sequences with 3.3 units per turn[10a] (left), oligoamides of 8-amino-2-carboxy-quinoline with 2.5 units per turn[10b] (right).

1.1.4 Aib Residues in Peptaibiotics:

The achiral residue α-aminoisobutyric acid (Aib) has been found to be major constituent in synthesized fungal peptides.[11]The word called peptaibiotics was established to depict certain class of natural products, which contains the unnatural amino acid Aib and possess antibiotic activities.[12]

The theoretical analysis of the unusual conformational properties of Aib residues were first predicted in 1970s.[13]The existence of geminal di-methyl groups at the tetrahedral C(α)-atom enforces major steric limitations on the energetically available conformational space, thereby restraining the Aib residue to the helical region of the Ramachandran map ($\phi = \pm 60° \pm 20°$; $\psi = \pm 30° \pm 20°$).[14]

The structural determination of the Aib-containing small peptides was well studied and experimental evidence showed that these peptides had the ability to stabilize helical conformations.[15-16] There has also been considerable discussion in the literature on the distinction between 3_{10} and α-helical conformation of Aib-containing peptides.[17]

1.1.4.1 Helix forming propensity of α-amino isobutyric acid (Aib)

It is known that Aib-containing peptides generally fold their backbone into α-helix, 3_{10} or mixed 3_{10}/ α-helix. The folding generally depends on the total number of residues in the peptide sequence and also the number of Aib residues present in it. Balaram *et al* reported the crystal structure of an ideal α-helix formed by pentadecapeptide Boc–Val–Ala–Leu–Aib–Val–Ala–Leu–Val–Ala–Leu–Aib-Val-Ala-Lue-Aib–OMe.[18]

Due to the strong helix-forming propensity of Aib residues in peptide sequences, disruptive residues barely have any effect on their helicities. Proline is generally considered α-helix-breaker as it provides rigidity to its conformation by its inability of the nitrogen atom to participate in a hydrogen bond. In spite of poor helix forming propensities of the Pro residue, Balaram *et al* have shown that heptapeptide Boc–Val–Val–Aib–Pro–Val–Val–Val–OMe still folded into distorted 3_{10}-helix[19] Some of the other examples that show these type of helices are from Aib residues combined with several hydroxyproline residues.[20] These types of helices are somewhat distorted and have a considerable curvature to their helix axes due to the presence of the hydroxyproline (Hyp) and proline (pro) residues.

In order to further prove the strong helix generating propensities of Aib residue, Balaram *et al* synthesized Aib containing peptides containing a Gly-Gly residue in the middle of the sequence. As we know that glycine has got higher tendency to occur in β-turns[21] because of its conformational flexibility, it is least expected to fold in to helices in peptide sequences. But incorporation of glycine in Aib-containing peptides like peptaibol tricogin, resulted in helical conformation.[22] One such example is for octa-peptide sequence Boc–Leu–Aib–Val–Gly–Gly–Leu–Aib–Val–OMe.[22a]

1.1.4.2 Helix reversal in Aib-containing peptides

Helical conformations have specific beginning and an end. Helices are terminated by helix reversal at the C-terminus due to which torsional angles φ and ψ change from negative values to positive values. This type of helix reversal is often found in protein structures and occurs when there are Gly and Asn segment present in the penultimate residues.[23]

When Aib is the penultimate residue, designed peptides tend to undergo helix reversal at the C-terminus. Balaram *et al* have shown this kind of helix reversal in several Aib-containing peptides and one such model peptide is heptapeptide Boc-Lue-Aib-Val-Ala-Lue-Aib-Val-OMe where Aib is the penultimate residue.[24]

In general, the presence of one or more dialkylated glycine residues mainly Aib and also Dpg (dipropylglycyl) in an oligopeptide of reasonable length equal to 18 or more residues will make sure the formation of an α-helix or 3_{10} helix. The Aib residue not only is involved in instigating helix formation but also carry out another task by terminating a helix reversal provided that the Aib residue is placed in the penultimate position in the peptide sequence.

1.1.5 Template-assisted peptide folding

α-Amino acid containing linear peptides does not always have ordered structure. In such cases, there has been considerable interest to use templates that can nucleate and propagate a desired ordered structure and stabilize them to form regular secondary structures. This strategy has been established to be a feasible method for accomplishing folding in the absence of a thorough understanding of how to design a peptide sequence that will instinctively fold. The entropy factor can be readily lowered by this kind of folding through the introduction of conformationally rigid scaffolds thereby converting the unfolded state of a peptide to a folded state. These templates impart their conformational inclination through non-covalent interactions like hydrogen bonding, electrostatic interactions and hydrophobic interactions to nucleate a specific structure. This concept which was first coined by Hirshmann and their team introduced a rigid molecule to stabilize a structure and reduce their conformational entropy.[25]They introduced a conformationally rigid disulfide bridge in the synthesis of analogues of somastatin and increased their biological activity.

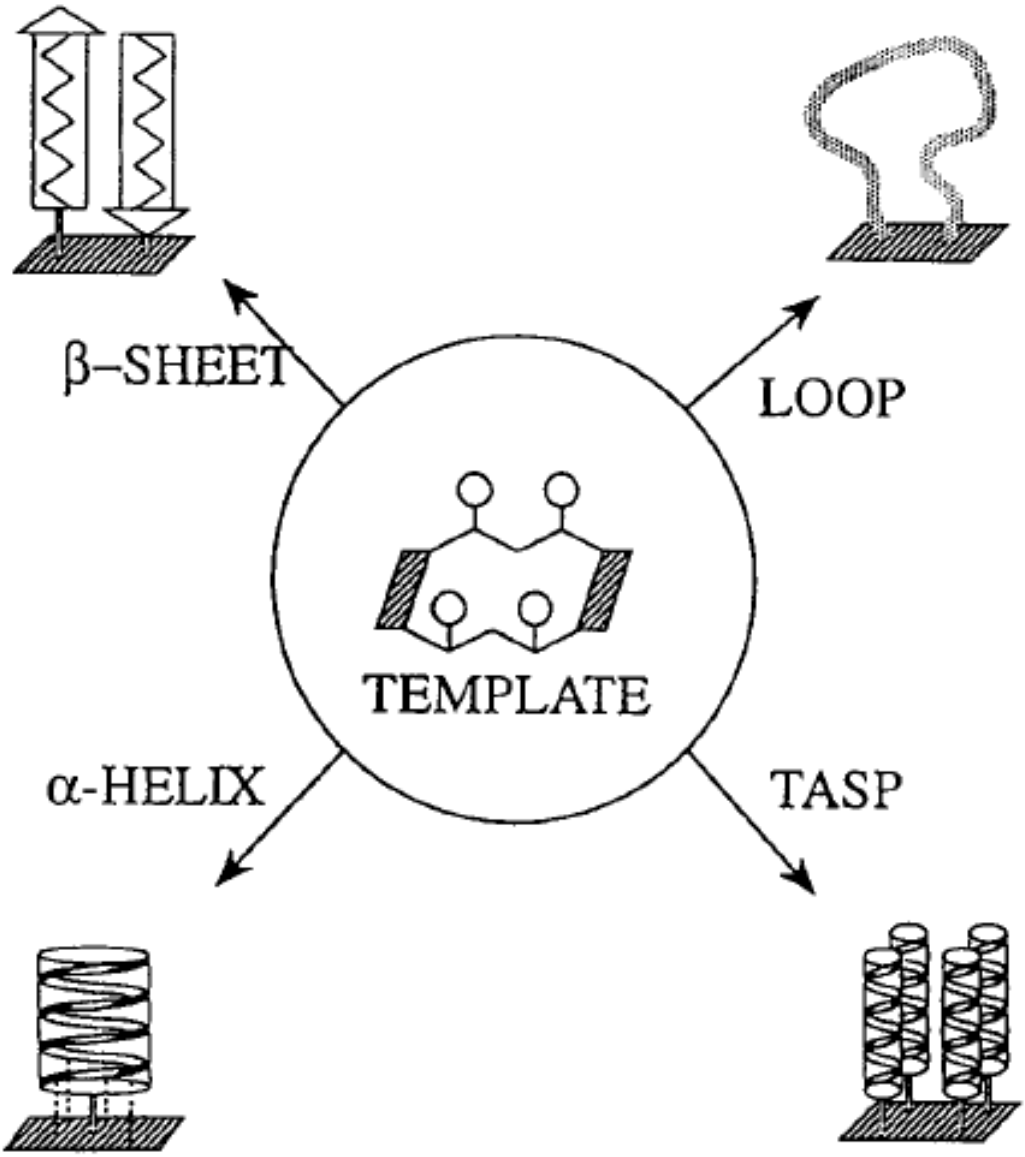


Fig 1.14: Template as device for induction and stabilization of protein folds

1.1.5.1Templates that stimulate α-helical folding in peptides

1.1.5.1.1 Porphyrin Templates

Tetra substituted coproporphyrin acts as a template to organize the peptide chains attached to them forming a helichrome i.e. a four helix bundle.[26] Poryphirin containing four acid linkers were used to couple with amine counterpart of amphilic peptides by segment condensation strategy to afford this helichrome as shown in Fig 1.5.

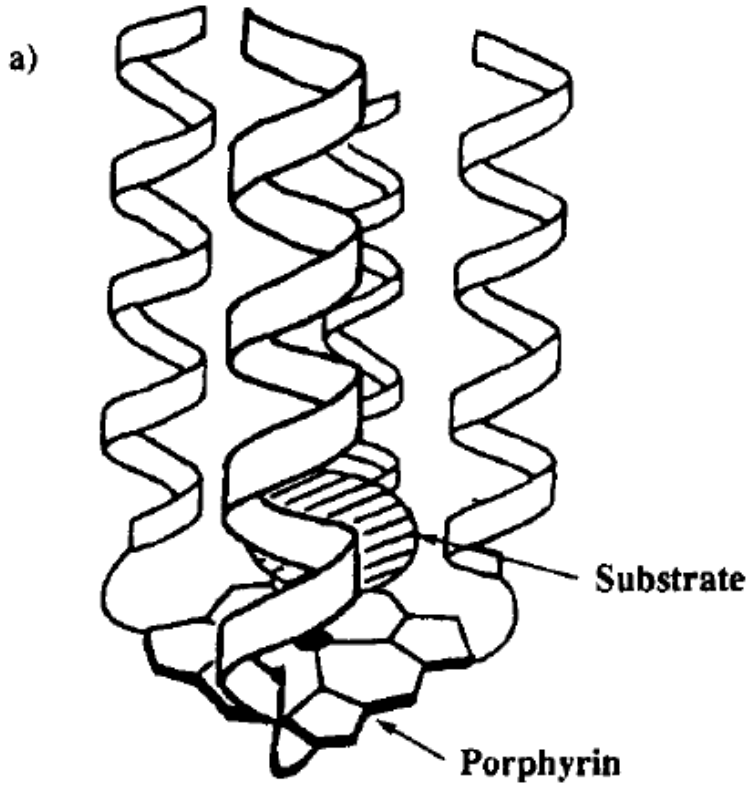


Fig 1.15: Porphyrin template containing four helix bundle.[26]

This helichrome was synthesized to impersonate the hydroxlase activity of cytochrome P-450.[26]The peptide sequences were chosen such that bundle formed had hydrophobic pocket at the base of the porphyrin. Circular dichroism (CD) studies were carried out to further confirm the presence of helical conformation and at pH 7.5 in aqueous buffer, it showed a pronounced double minima at 209 nm and 222 nm. Porphyrin as a template had a huge impact on the stabilization of folds because isolated peptide segments adopted random conformations under similar conditions.

1.1.5.1.2 Pyridine and bipyridine-based molecular scaffolds that stimulates helical folding.

Ghadiri *et al* have shown that incorporation of pyridine-based ruthenium binding amino acid residues in to ampiphilic peptide sequences resulted in the formation of a four helix bundle by transmitting its folding properties.[27]When the pyridyl moiety was attached to the N-terminus of the amphiphilic peptides and was complexed with Ru(II), folding occurred to form a four helix bundle metalloprotein as shown in Fig. 1.16. Hydrophobic interactions between the helical chains and strong binding energy

upon ruthenium complexation played a major role in stabilizing the formation of four helix bundle. Circular dichroism spectroscopy also revealed that there existed a helical conformation of this metalloprotein.

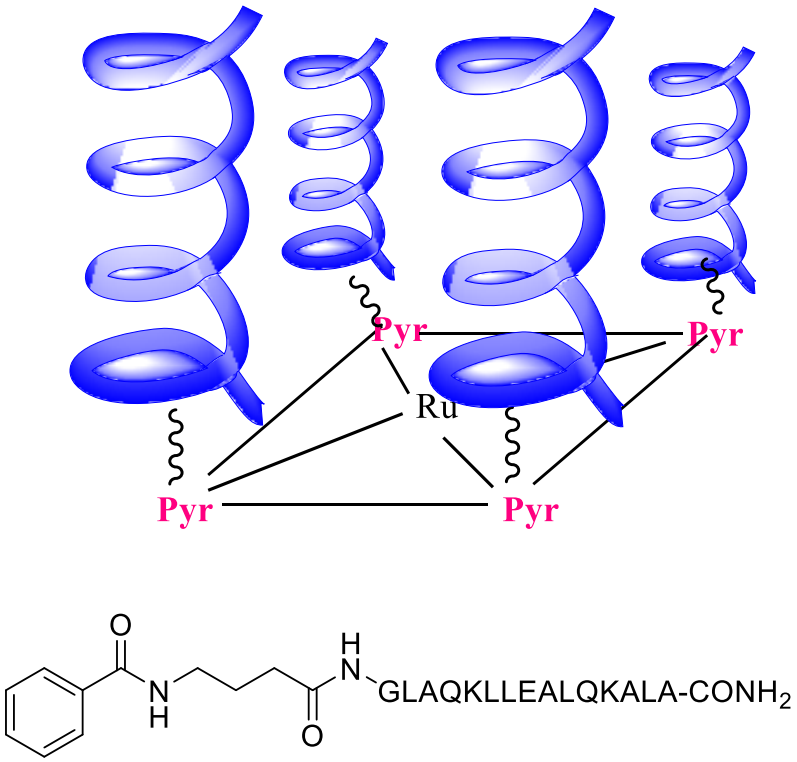
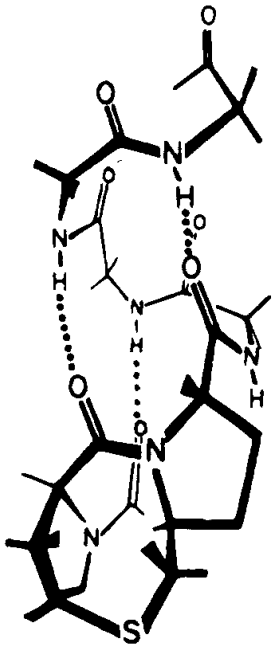
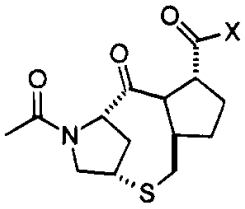


Figure 1.16: Four helix bundle formation by Ru-pyridyl based complexation with amphilic peptides.

1.1.5.1.3 Helix nucleated by Ac-Pro-Pro Sequence

A conformationally restricted Ac-Pro-Pro (acetyl-prolylproline) sequence was incorporated in the peptide sequences by Kemp *et al.*[28] In order to obtain helical architecture of these peptide chains, sulfur was incorporated in Ac-Pro-Pro sequence so as to prevent the fraying at the helical ends. The three carbonyl group of this sequence gets oriented in such a way as to hydrogen bond with NH's of the peptide chains to adopt helical conformation as shown in Fig. 1.17. With the use of this template, authors have synthesized stable helical peptides up to 11 residues in length.





Template **5**

X = OH

X = Peptide

Figure 1.17 Helix nucleated by Ac-Pro-Pro Sequence.[28]

1.1.5.1.4 Helix nucleated by template-assembly of synthetic protein

Small acyclic and cyclic peptides as templates have been used for generating tertiary structures by converting them to ordered state and thereby reducing the conformational entropy of the assembled protein. Mutter *et al*[29] have shown that attachment of peptide sequences to the templates containing acyclic and cyclic peptides produced super secondary structures. This approach has been termed as "template assembled synthetic protein"(TASP). It is very helpful in generating several helical structures. One such example is the formation of a four helix bundle as depicted in fig 1.18. Peptides at their carboxy-terminus acting as template are covalently attached to the €-amino groups of lysine residues as shown in figure 1.18.

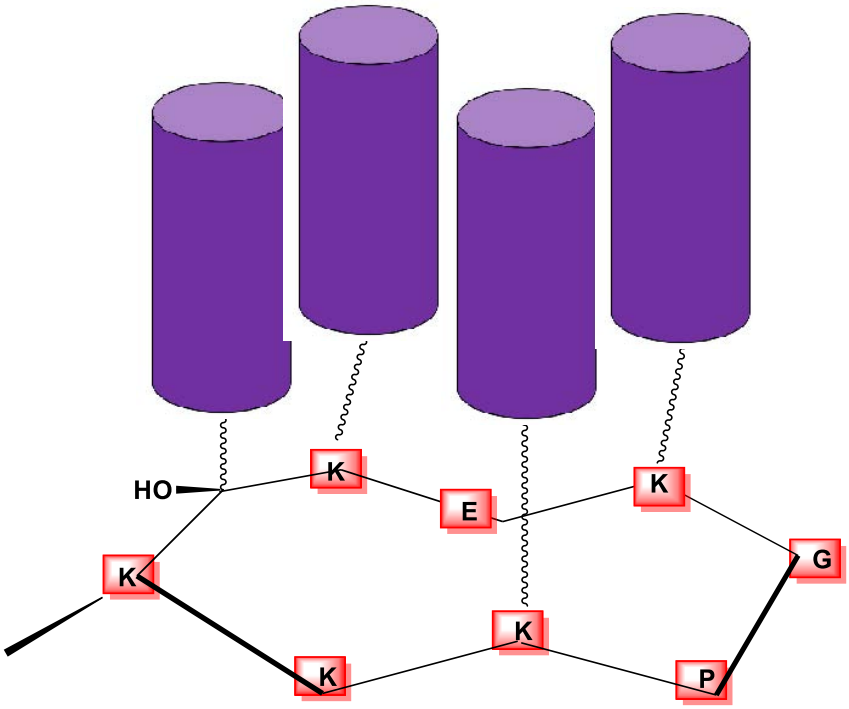


Figure 1.18 Schematic representation of TASP: Attachment of four peptide chains (amphiphilic) to the acyclic template leading to four helix bundle.

1.2 Objective of the present work

The main aim of the work described in this chapter is to introduce a conformationally rigid synthetic scaffold in to helical peptides. We also aim to investigate the conformation of oligopeptides as well as their scaffold tethered oligopeptides.

1.3 Design strategy

There are a large number of template molecules that are found in the literature which stimulates and stabilizes secondary and tertiary structures of the peptides covalently attached to them. Dimedone-sulfur intermediate as a template consisting of two carbonyls as hydrogen bond acceptor is a simple synthetic molecule that would play a major role in the synthesis of various supramolecular architectures. We have designed the concept of synthesizing non-natural oligopeptides and their dimedone-tethered single stranded and symmetrical double stranded helical peptide derivatives to investigate their conformational changes as shown in fig 1.19.

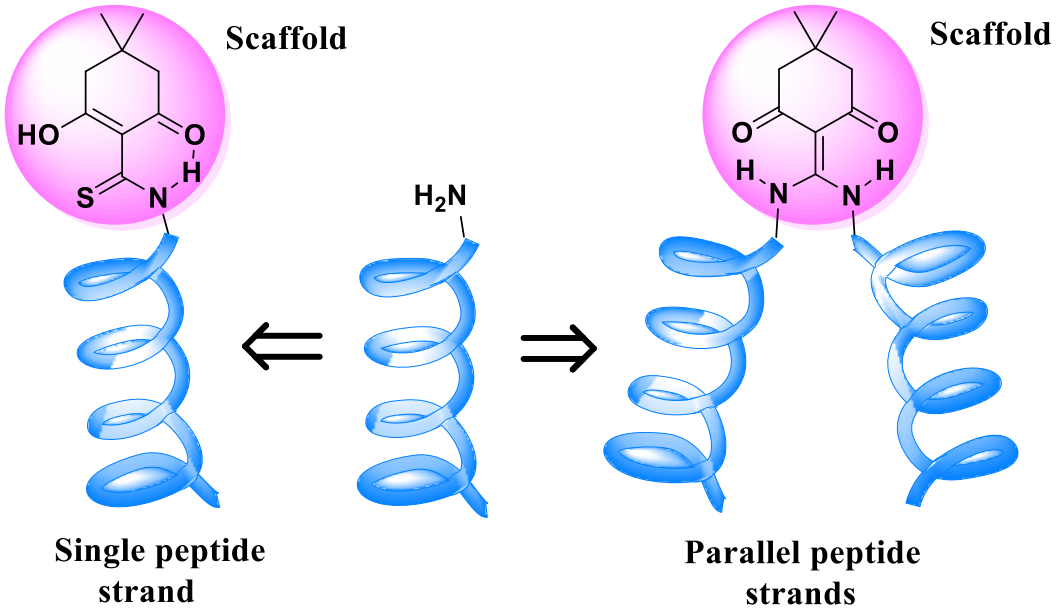


Figure 1.19: Concept of attachment of peptide chains to dimedone scaffold in a parallel fashion.

Dimedone-based single stranded peptides can be prepared from dimedone-sulfur intermediate A which can be obtained by treating dimedone with iodopentaflurobenzene as shown in Fig 1.20.

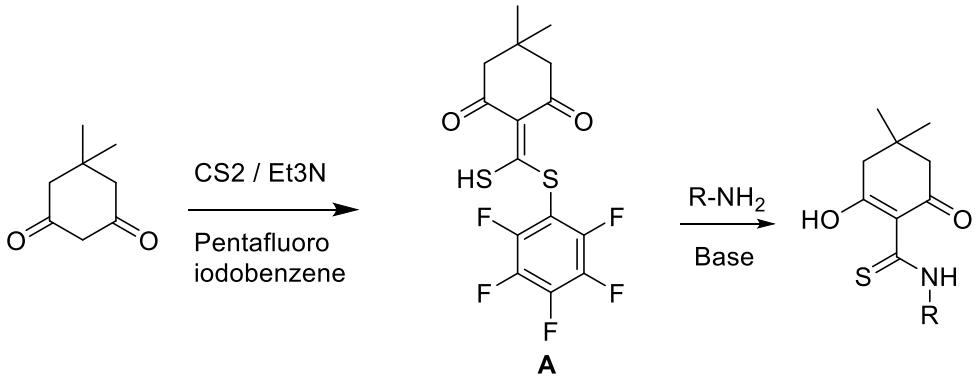


Figure 1.20: Schematic diagram for the synthesis of dimedone-tethered single stranded peptides.

Dimedone-based double stranded peptides can be prepared from dimedone-sulfur cyclic intermediate **B** which can be synthesized by treating dimedone with alkyl dihalides as shown in figure 1.21.

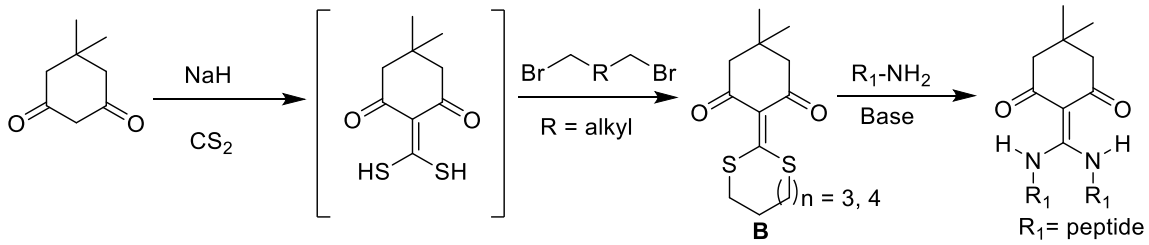


Figure 1.21: Schematic diagram for the synthesis of dimedone-tethered double stranded symmetrical peptides.

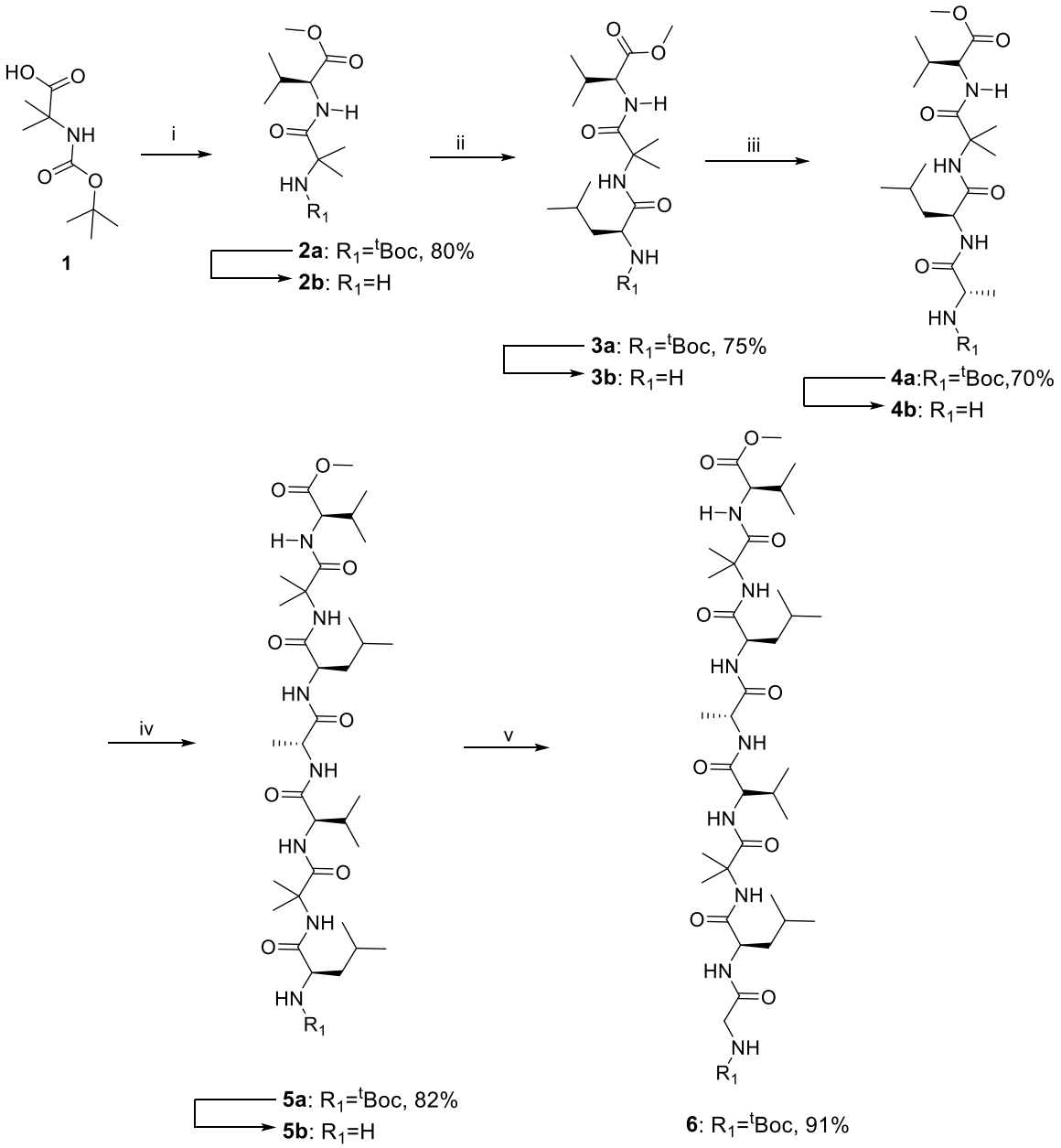
1.4 Synthesis

Herein, we describe the synthesis of oligopeptides and their dimedone-tethered single stranded and symmetrical double stranded helical peptide derivatives in solution phase.

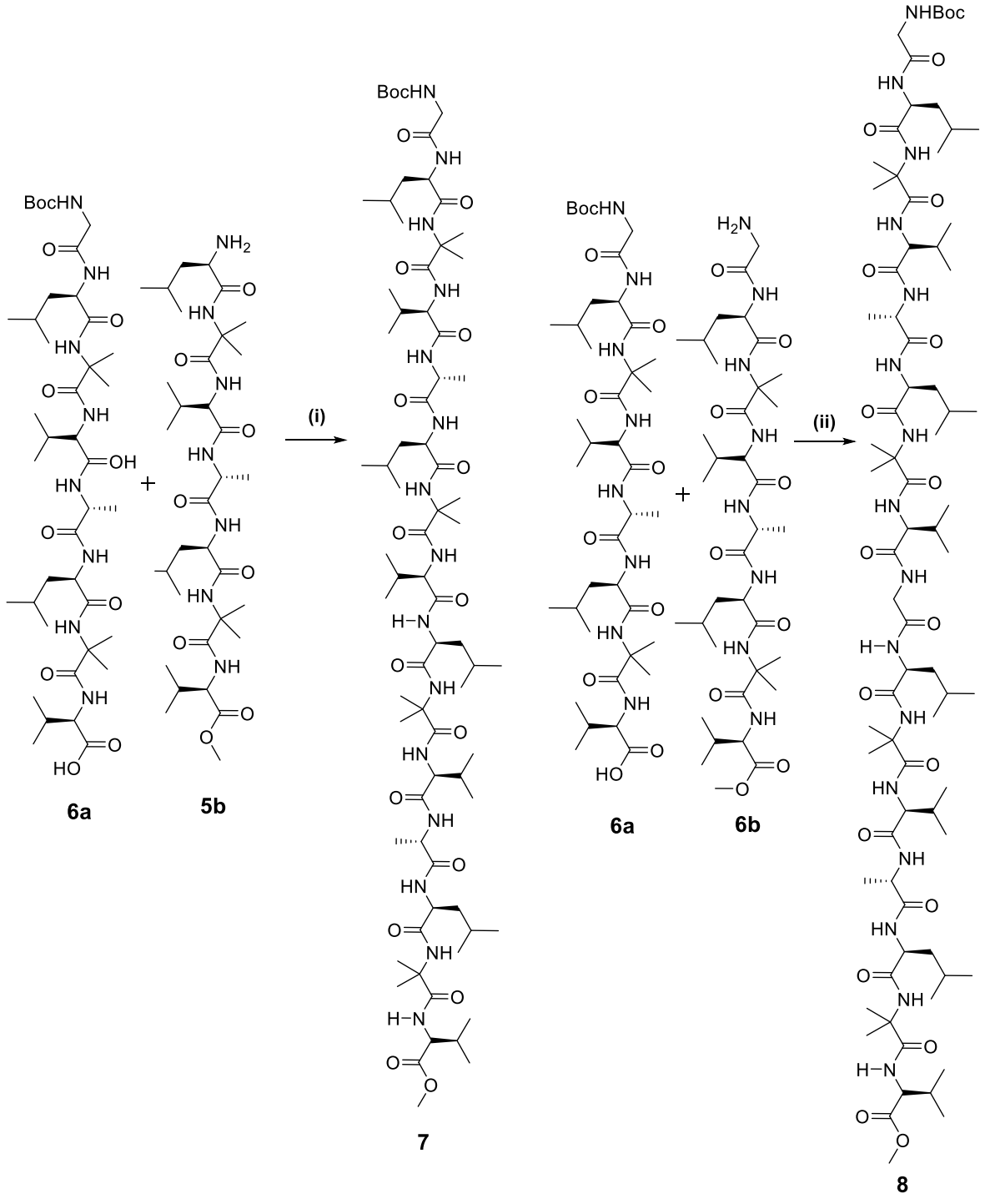
1.4.1 Synthesis of oligopeptides:

Firstly, known peptides Boc-L-Leu-Aib-L-Val-OMe[22] **3a** and Boc-L-Ala-L-Leu-Aib-L-Val-OMe[24] **4a** (Boc = tert butoxy carbonyl; OMe = methyl ester) were synthesized using conventional solution-phase methods mediated by EDC/HOBt. EDC.HCl as coupling agent aided in increasing the yields of the resultant compounds and also synthesis was carried out in a hassle free manner. Later, octa-peptide, Boc-Gly-L-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-OMe **6** was synthesized via a fragment condensation strategy involving a racemisation-free coupling of tri-peptide acid and tetra-peptide amine **4b** mediated by EDC/HOBt to yield hepta-peptide **5a** first,[24] after which the resultant compound **5a** was Boc deprotected and coupled with Boc-glycine as shown in scheme 1.1.

Considering the hassle free synthesis of these peptides, synthesis of pentadecapeptide Boc-Gly-Lue-Aib-Val-Ala-Lue-Aib-Val-Lue-Aib-Val-Ala-Lue-Aib-Val-OMe **7** was carried out by coupling of octa-peptide acid **6a** and hepta-peptide amine **5b** via a fragment condensation strategy mediated by EDC/HOBt using solution-phase methods in good yield. Similarly, octa-peptide acid **6a** was coupled with octa-peptide amine **6b** by fragment condensation strategy to obtain hexadecapeptide Boc-Gly-Lue-Aib-Val-Ala-Lue-Aib-Val-Gly-Lue-Aib-Val-Ala-Lue-Aib-Val-OMe **8** in good yield as shown in scheme 1.2.



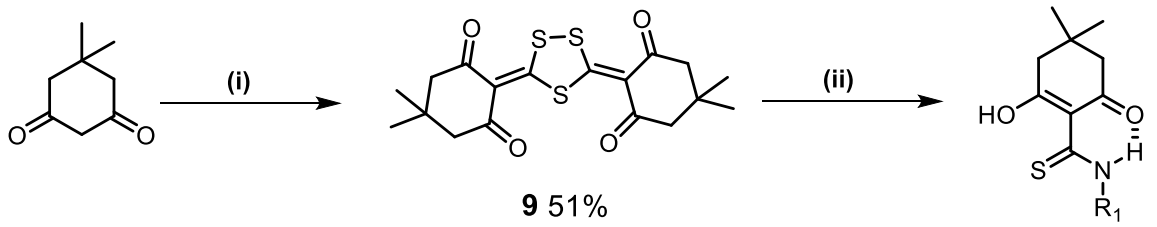
Scheme 1.1 #Synthesis of Oligomers. Reagents and conditions: (i) amine H-Val-OMe, EDC, HOBt, DMF, rt, 12 h; (ii) **2b**, Boc-Lue-OH, EDC, HOBt, rt, 12h; (iii)**3b**, Boc-Ala-OH, EDC, HOBt, DMF, rt, 24 h; (iv) **4b**, amine Boc-Lue-Aib-Val-OH, EDC, HOBt, DMF, rt, 24 h; (v) **5b**, Boc-Gly-OH, EDC, HOBt, DMF, rt, 24 h;



Scheme 1.2 [#]Synthesis of higher oligopeptides: Reagents and conditions: (i) DIPEA, EDC, HOBt, DMF, rt, 12 h, 72 %, (ii) DIPEA, EDC, HOBt, DMF, rt, 12 h, 70 %

1.4.2 Syntheis of dimedone-sulphur intermediate for the preparation of single stranded peptides.

The synthesis of **9** was carried out by reacting dimedone with excess of carbon disulfide and triethyl amine at room temperature in THF solvent, followed by treatment of the reaction mixture with iodopentafluorobenzene in THF under nitrogen atmosphere.

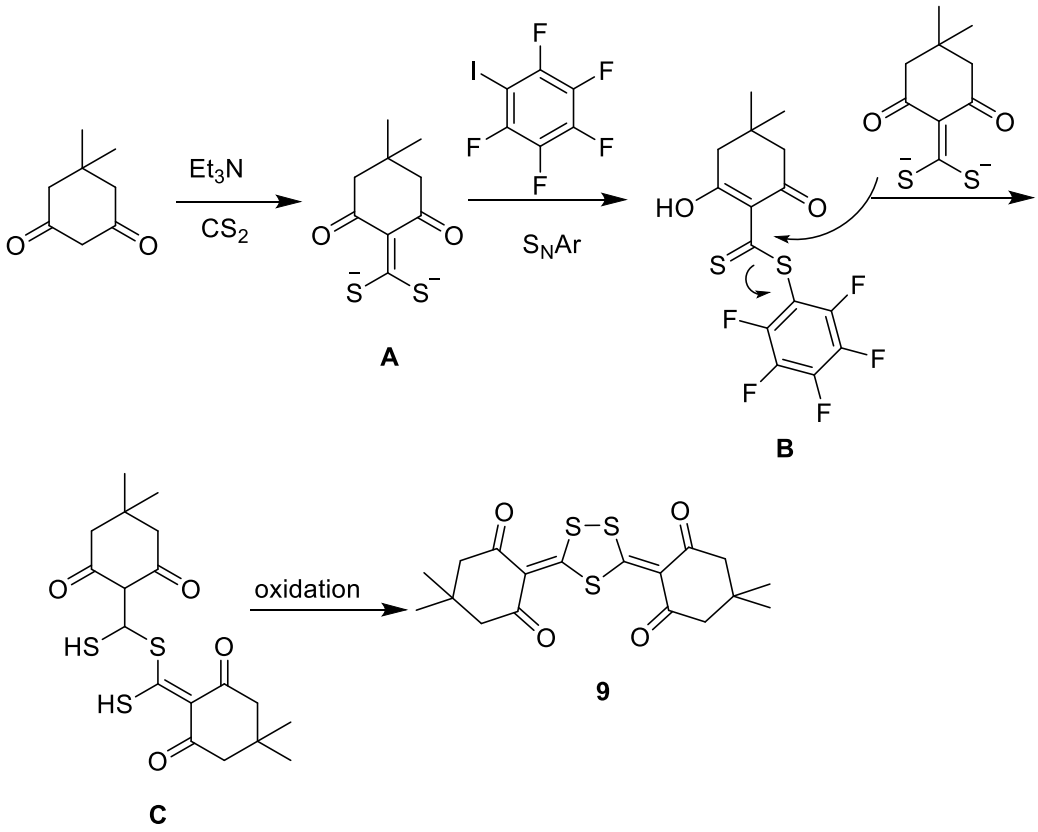


9 51%

9a R_1 = -Leu-Aib-Val-OMe, 96%
9b R_1 = -Ala-Leu-Aib-Val-OMe, 98%
9c R_1 = -Gly-Leu-Aib-Val-Ala- Leu-Aib-Val-OMe, 95%
9d R_1= -Gly-Leu-Aib-Val-Ala-Leu-Aib-Val-Leu-Aib-Val-Ala- Leu-Aib-Val-OMe, 80%
9e R_1= -Gly-Leu-Aib-Val-Ala-Leu-Aib-Val-Gly-Leu-Aib-Val-Ala- Leu-Aib-Val-OMe, 85%

Scheme 1.3: #Synthesis of dimedone-tethered peptides: Reagents and conditions; (i)(a) Et_3N, CS_2, rt, 1 h (b)Iodopentafluorobenzene, rt, 12 h, THF, 50 % (ii) R_1-NH_2, DIPEA, rt, 6 h, DMF.

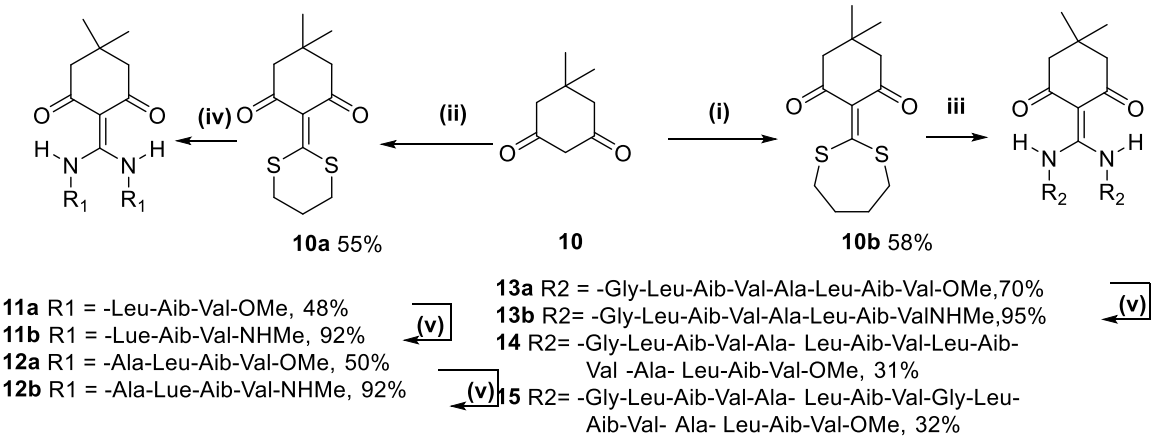
In a mechanistic point of view, the reaction is expected to follow a classical route as shown in Fig 1.20. Firstly, dimedone on reaction with carbon disulfide and triethylamine generates bisthiolate **A** which on subsequent aromatic nucleophilic substitution reaction with iodopentafluorobenzene produces mono arylated intermediate **B**. Mono S-arylated intermediate could further react with another enolate of dimedone in THF to produce intermediate **C** which finally undergoes aerial oxidation to liberate the product **9** (Scheme 1.4). Compound **9** was further reacted with peptide amines to yield dimedone-tethered single stranded peptides as shown in scheme 1.3.



Scheme 1.4: Plausible mechanism of trithiolane formation

1.4.3 Synthesis of dimedone-sulphur intermediate for the preparation of double stranded peptides.

The next objective was to attach two symmetrical peptide strands on a single carbon containing dimedone. In order to achieve this, we synthesized 2-(1,3-dithian-2-ylidene)-5,5-dimethylcyclohexane-1,3dione **10a** and 2-(1,3-dithiepan-2-ylidene)-5,5-dimethylcyclohexane-1,3-dione **10b** from dimedone **10**, according to the general method of Gompper *et al*[29] from dimedone but with slightly modified condition using CS_2, Et_3N, and 1,3-dibromopropane/1,4-dibromo butane in good yields. These intermediates **10a** and **10b** reacted efficiently with peptide amino esters furnishing novel dimedone-tethered symmetrical double stranded peptide derivatives. Compounds **11a**, **12a** and **13a** were then treated with methanolic methylamine to afford amidated double stranded dimedone-tethered peptides **11b**, **12b** and **13b** respectively, in excellent yields as shown in scheme 1.5.



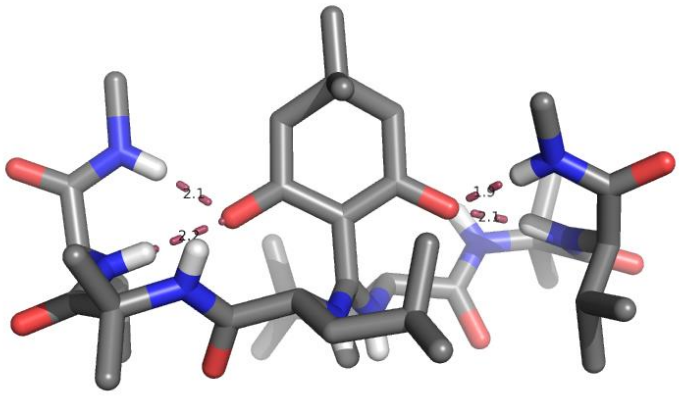
Scheme 1.5 Synthesis of dimedone-tethered peptides: Reagents and conditions : i) (a) Et_3N, CS_2, rt, 1 h (b) 1,4-dibromobutane, rt, 12 h, THF, 75 %; ii) (a) Et_3N, CS_2, rt, 1 h (b) 1,3-dibromopropane, rt, 12 h, THF, 72 %; (iii)R_2-NH_2, DIPEA, rt, 12 h, DMSO; (iv)R_1-NH_2, DIPEA, rt, 12 h, DMF; (v) methanolic methylamine, rt, 2 h.

1.5 Conformational analyses

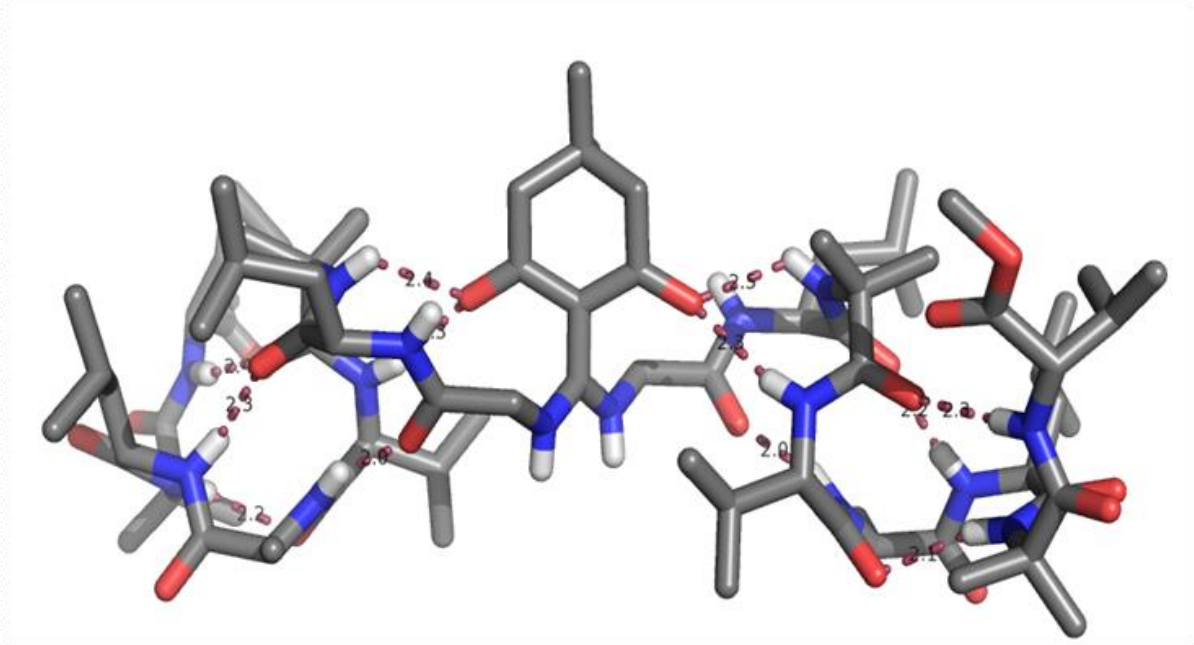
The conformational features of the synthetic peptides were investigated by X-ray crystallography, solution state NMR and CD studies.

1.5.1 Single crystal X-ray diffraction studies

After many trials, single crystals obtained from double stranded peptides **11b** (by slow evaporation of methanol) and **13a** (by slow evaporation of isopropanol) as solvents revealed the solid state structure, showing the presence of unusual C-12 and C-15 membered bifurcated intramolecular hydrogen bonding of peptide chains with the scaffold dimedone as shown in figure 1.22. It is noteworthy that compound **11b** and **13a** showed similar hydrogen bonding pattern in spite of different N-terminal amino acid residue attached to dimedone. Compound **13a** consisted of three 4→1 type and one 6→1 type intramolecular hydrogen bonds suggesting that helical conformation of the peptide chains are maintained on both sides even after the scaffold is attached.



11b



13a

Figure 1.22: Single-crystal X-ray structure of compound **11b** and **13a.** Hydrogen bonding is highlighted in dashes (salmon coloured), above which hydrogen bond distances (N-H···O are displayed in Aº. All hydrogens, other than those at the hydrogen-bonding sites, have been removed for clarity.

We also obtained single crystals from dimedone-Gly-L-Leu-Aib-L-Val-L-Ala-L-Lue-Aib-L-Val-OMe **9c** and Boc-Gly-L-Leu-Aib-L-Val-L-Ala-L-Lue-Aib-L-Val-OMe **6** by slow evaporation of methanol. Compound **6** adopted a right-handed 3_{10}-helical structure, terminated by a left-handed helical conformation (αL) at Aib(6) resulting in a 6→1 hydrogen bond at the C-terminus whereas compound **9c** adopted left-handed 3_{10}-helical structure, terminated by a right-handed helical conformation (αR) at Aib(6)

resulting in a 6→1 hydrogen bond at the C-terminus. It also showed the presence of strong C-6 hydrogen bonding between peptide chain and dimedone for **9c** (Fig. 1.23).

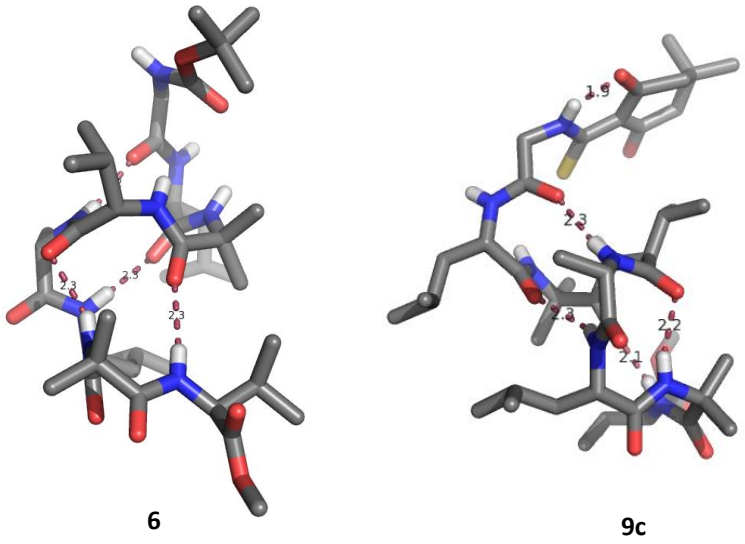


Figure 1.23: Single-crystal X-ray structure of compound **6** and **9c.** Hydrogen bonding is highlighted in dashes (salmon coloured), above which hydrogen bond distances (N-H···O are displayed in Aº. All hydrogens, other than those at the hydrogen-bonding sites, have been removed for clarity.

1.5.2 CD Studies.

CD spectroscopy was used to study the secondary structure content of the higher oligopeptides and their dimedone-tethered peptides. Compounds **11a** and **11b** did not show any characteristic helical signature due to the shorter peptide chain length. However, we observed characteristic maxima near 195 nm and pronounced double minima at 208 nm and 222 nm suggesting helical conformation for higher oligopeptides and their dimedone-tethered oligopeptides (Fig. 1.24). Although glycine is having poor helix-forming propensities,[7]compound **8** and **9d** with central Gly segment in the sequence preferred to adopt α-helical conformation since Aib-containing peptides have a tendency for promoting helix formation.[22a]

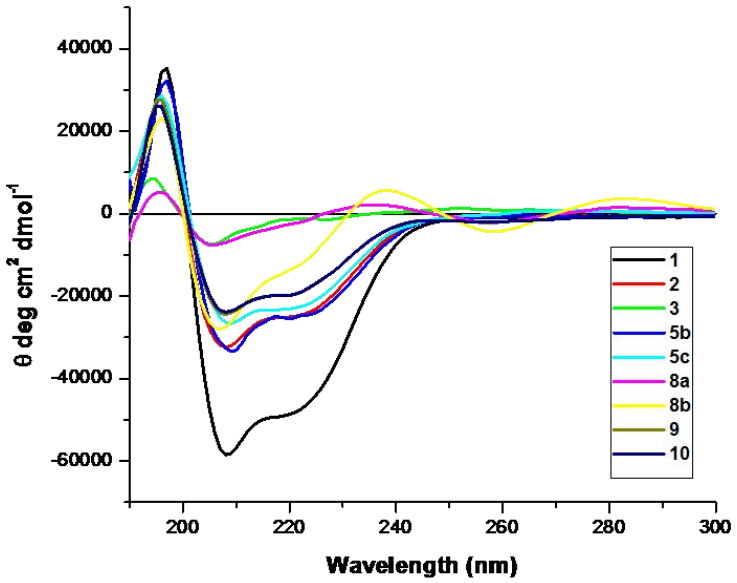


Figure 1.24: CD studies of higher oligopeptides in CH_3OH

1.5.3 NMR Studies.

The structural elucidation of higher oligopeptides and its dimedone-tethered peptides were carried out *via* solution state NMR studies, since they were highly resistant to yield to crystal formation, despite several efforts. In order to investigate the solution state conformation of these peptides, we performed 2D ROESY NMR studies in CD_3OH. The signal assignments were carried out with 2D TOCSY.

1.5.3.1 Variable Temperature Studies

Variable temperature NMR experiments were used to probe the strength of intra-molecular hydrogen bonding in these higher oligopeptides. Temperature coefficients of NHs calculated from different temperature studies of the compounds have given initial information about the specific NHs that is involved in hydrogen bonding. Temperature coefficients measured over a range of 43K (from 283K to 323K) are listed in Table 1. Values of −2 ppb/K to −4 ppb/K of NHs suggested their involvement in strong hydrogen bonding. A marginally higher value −4.1 ppb/K for NHs is indicative of its involvement in H-bonding with intermediate strength, while the remaining NHs are not participating in any H-bonding.

1.5.3.1.1 Variable Temperature studies of Compound 7

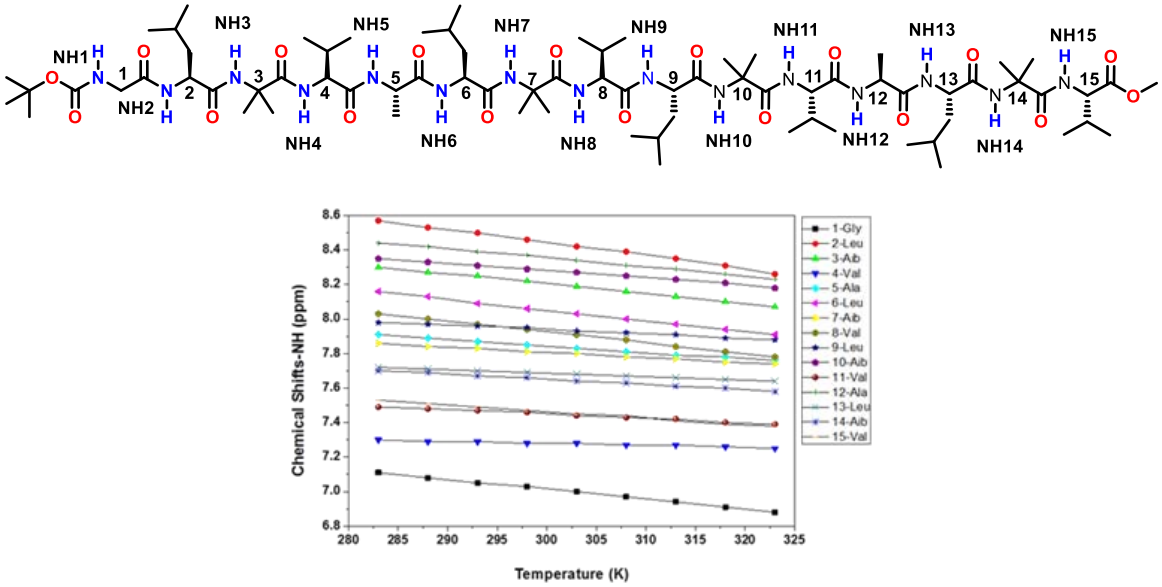


Figure 1.25: Variation of NH chemical shifts of compound **7** with respect to the temperature change from 283 K to 323 K in CD_3OH (700 MHz).

Temperature (K)	Table 2: NH chemical shifts (ppm)														
	Gly 1-NH	Leu 2-NH	Aib 3-NH	Val 4-NH	Ala 5-NH	Leu 6-NH	Aib 7-NH	Val 8-NH	Leu 9-NH	Aib 10-NH	Val 11-NH	Ala 12-NH	Leu 13-NH	Aib 14-NH	Val 15-NH
283	7.11	8.57	8.30	7.30	7.91	8.16	7.86	8.03	7.98	8.35	7.49	8.44	7.72	7.70	7.53
288	7.08	8.53	8.27	7.29	7.89	8.13	7.84	8.00	7.97	8.33	7.48	8.42	7.71	7.69	7.51
293	7.05	8.50	8.25	7.29	7.87	8.09	7.83	7.97	7.96	8.31	7.47	8.39	7.70	7.67	7.49
298	7.03	8.46	8.22	7.28	7.85	8.06	7.81	7.94	7.95	8.29	7.46	8.37	7.69	7.66	7.47
303	7.00	8.42	8.19	7.28	7.83	8.03	7.80	7.91	7.93	8.27	7.44	8.34	7.68	7.64	7.45
308	6.97	8.39	8.16	7.27	7.81	8.00	7.78	7.88	7.92	8.25	7.43	8.31	7.67	7.63	7.44
313	6.94	8.35	8.13	7.27	7.79	7.97	7.77	7.84	7.91	8.23	7.42	8.29	7.66	7.61	7.41
318	6.91	8.31	8.10	7.26	7.78	7.94	7.75	7.81	7.89	8.21	7.40	8.26	7.65	7.60	7.39
323	6.88	8.26	8.07	7.25	7.76	7.91	7.74	7.78	7.88	8.18	7.39	8.23	7.64	7.58	7.38
Temperature Coefficient (Δδ/ΔT) (in ppb/K)	-5.7	-7.7	-5.7	-1.2	-3.7	-6.2	-3.0	-6.2	-2.5	-4.2	-2.5	-5.2	-2.0	-3.0	-3.7

Table 1.11 Variable temperature study of **7** (CD_3OH, 700MHz)

1.5.3.1.2 Variable Temperature studies of Compound 8

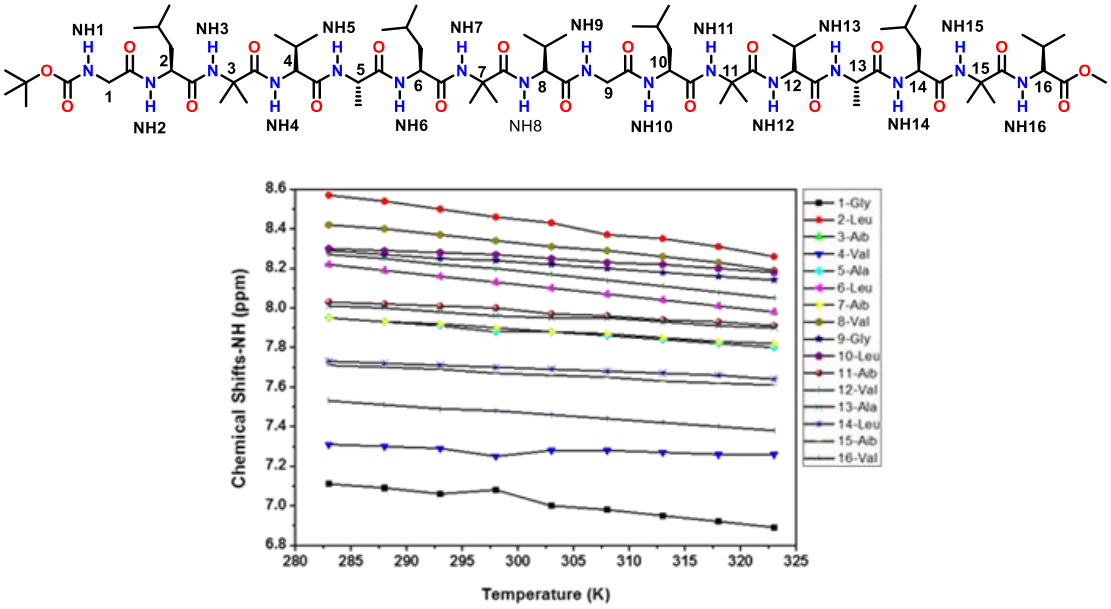


Figure 1.26: Variation of NH chemical shifts of compound **8** with respect to the temperature change from 283 K to 323 K in CD_3OH (700 MHz)

Temperature (K)	Table 08: NH chemical shifts (ppm)															
	Gly 1-NH	Leu 2-NH	Aib 3-NH	Val 4-NH	Ala 5-NH	Leu 6-NH	Aib 7-NH	Val 8-NH	Gly 9-NH	Leu 10-NH	Aib 11-NH	Val 12-NH	Ala 13-NH	Leu 14-NH	Aib 15-NH	Val 16-NH
283	7.11	8.57	8.22	7.31	7.95	8.22	7.95	8.42	8.29	8.30	8.03	8.27	8.01	7.73	7.71	7.53
288	7.09	8.54	8.19	7.30	7.93	8.19	7.93	8.40	8.27	8.29	8.02	8.25	8.00	7.72	7.70	7.51
293	7.06	8.50	8.16	7.29	7.91	8.16	7.92	8.37	8.25	8.28	8.01	8.22	7.98	7.71	7.69	7.49
298	7.08	8.46	8.13	7.29	7.89	8.13	7.90	8.34	8.24	8.27	8.00	8.2	7.96	7.70	7.67	7.47
303	7.00	8.43	8.10	7.28	7.88	8.10	7.88	8.31	8.22	8.25	7.97	8.17	7.95	7.69	7.66	7.46
308	6.98	8.37	8.07	7.28	7.86	8.07	7.87	8.29	8.2	8.23	7.96	8.14	7.95	7.68	7.65	7.44
313	6.95	8.35	8.04	7.27	7.84	8.04	7.85	8.26	8.18	8.22	7.94	8.11	7.93	7.67	7.63	7.42
318	6.92	8.31	8.01	7.26	7.82	8.01	7.83	8.23	8.16	8.20	7.93	8.08	7.91	7.66	7.62	7.40
323	6.89	8.26	7.98	7.26	7.80	7.98	7.82	8.19	8.14	8.18	7.91	8.05	7.90	7.64	7.61	7.38
Temperature Coefficient (Δδ/ΔT) (in ppb/K)	-5.5	-7.7	-6.0	-1.2	-3.7	-6.0	-3.2	-5.7	-3.7	-4.0	-3.0	-5.5	-3.2	-2.2	-2.5	-3.7

Table 1.12 Variable temperature studies of **8** (CD_3OH, 700MHz)

1.5.3.1.3 Variable Temperature studies of Compound 9d

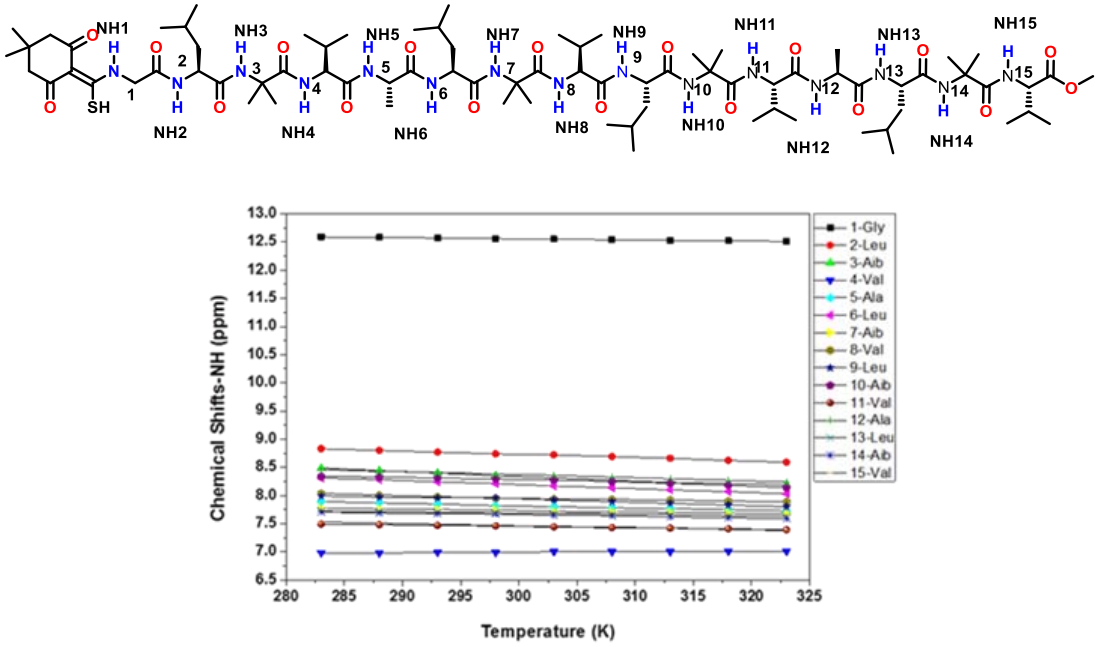


Figure 1.27: Variation of NH chemical shifts of compound **9d** with respect to the temperature change from 283 K to 323 K in CD_3OH (700 MHz)

Temperature (K)	Table 05: NH chemical shifts (ppm)														
	Gly 1-NH	**Leu 2-NH**	**Aib 3-NH**	**Val 4-NH**	**Ala 5-NH**	**Leu 6-NH**	**Aib 7-NH**	**Val 8-NH**	**Leu 9-NH**	**Aib 10-NH**	**Val 11-NH**	**Ala 12-NH**	**Leu 13-NH**	**Aib 14-NH**	**Val 15-NH**
283	12.59	8.83	8.49	6.98	7.89	8.31	7.77	8.04	7.99	8.34	7.49	8.46	7.72	7.71	7.53
288	12.58	8.80	8.44	6.98	7.87	8.28	7.76	8.01	7.97	8.33	7.48	8.43	7.71	7.69	7.51
293	12.57	8.77	8.40	6.99	7.85	8.24	7.75	7.98	7.96	8.31	7.47	8.41	7.70	7.68	7.49
298	12.56	8.74	8.35	6.99	7.82	8.21	7.74	7.95	7.96	8.29	7.46	8.38	7.69	7.67	7.47
303	12.55	8.72	8.31	7.00	7.80	8.17	7.72	7.94	7.92	8.27	7.44	8.36	7.68	7.65	7.45
308	12.54	8.69	8.27	7.00	7.78	8.14	7.71	7.93	7.89	8.25	7.43	8.33	7.67	7.64	7.44
313	12.53	8.66	8.23	7.00	7.77	8.10	7.70	7.92	7.86	8.22	7.42	8.30	7.66	7.62	7.42
318	12.52	8.62	8.21	7.01	7.75	8.07	7.69	7.90	7.83	8.18	7.41	8.27	7.64	7.60	7.40
323	12.51	8.59	8.19	7.01	7.73	8.03	7.68	7.89	7.80	8.14	7.39	8.24	7.63	7.59	7.38
Temperature Coefficient (Δδ/ΔT) (in ppb/K)	-2.0	-6.0	-7.5	0.7	-4.0	-7.0	-2.2	-3.7	-4.7	-5.0	-2.5	-5.5	-2.2	-3.0	-3.7

Table 1.13 Variable temperature studies of **9d** (CD_3OH, 700MHz)

1.5.3.1.4 Variable Temperature studies of Compound 9e

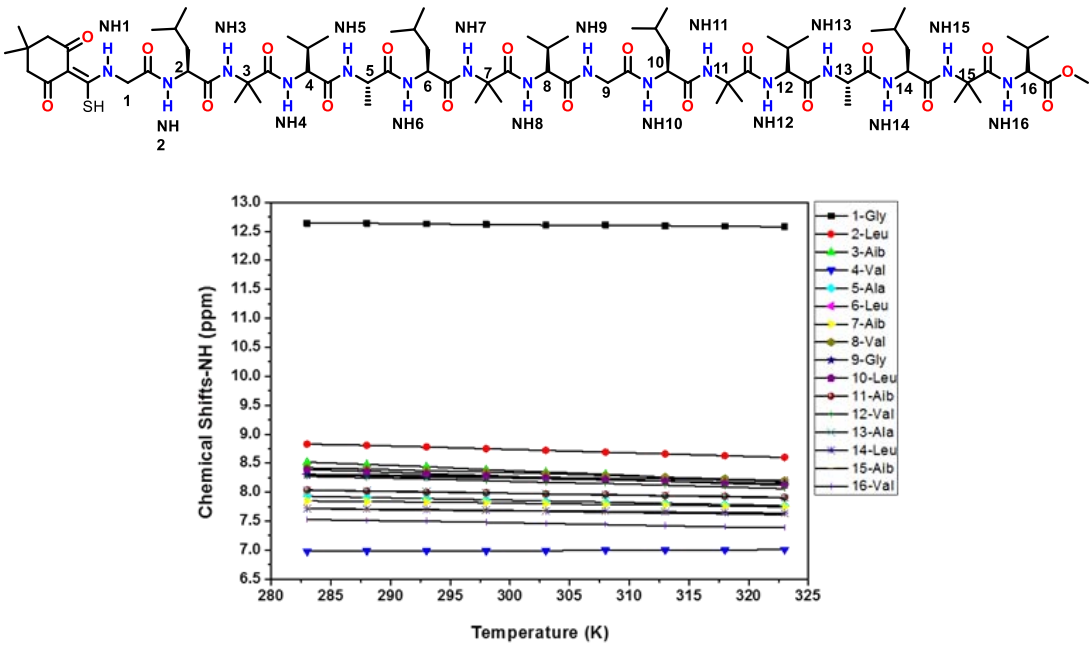


Figure 1.28: Variation of NH chemical shifts of compound **9e** with respect to the temperature change from 283 K to 323 K in CD_3OH (700 MHz)

Temperature (K)	Table-11: NH chemical shifts (ppm)															
	Gly 1-NH	**Leu 2-NH**	**Aib 3-NH**	**Val 4-NH**	**Ala 5-NH**	**Leu 6-NH**	**Aib 7-NH**	**Val 8-NH**	**Gly 9-NH**	**Leu 10-NH**	**Aib 11-NH**	**Val 12-NH**	**Ala 13-NH**	**Leu 14-NH**	**Aib 15-NH**	**Val 16-NH**
283	12.64	8.83	8.52	6.98	7.93	8.31	7.85	8.42	8.30	8.39	8.04	8.27	8.03	7.72	7.72	7.53
288	12.64	8.81	8.48	6.99	7.91	8.30	7.84	8.40	8.28	8.36	8.02	8.25	8.02	7.71	7.70	7.51
293	12.63	8.78	8.44	6.99	7.89	8.29	7.83	8.37	8.26	8.32	8.00	8.23	8.01	7.70	7.69	7.50
298	12.63	8.75	8.39	6.99	7.87	8.27	7.82	8.35	8.25	8.29	7.99	8.20	7.99	7.69	7.68	7.48
303	12.61	8.72	8.35	6.99	7.85	8.26	7.80	8.32	8.23	8.25	7.97	8.17	7.98	7.68	7.67	7.46
308	12.61	8.69	8.31	7.00	7.83	8.24	7.79	8.29	8.21	8.22	7.96	8.15	7.95	7.67	7.65	7.44
313	12.60	8.66	8.26	7.00	7.81	8.23	7.78	8.26	8.19	8.19	7.94	8.12	7.94	7.66	7.64	7.42
318	12.59	8.63	8.22	7.00	7.79	8.21	7.76	8.23	8.17	8.15	7.93	8.09	7.92	7.65	7.63	7.40
323	12.58	8.60	8.18	7.01	7.77	8.19	7.75	8.20	8.15	8.12	7.91	8.06	7.90	7.64	7.61	7.39
Temperature Coefficient (Δδ/ΔT) (in ppb/K)	-1.5	-5.7	-8.5	0.7	-4.0	-3.0	-2.5	-5.5	-3.7	-6.7	-3.2	-5.2	-3.2	-2.0	-2.7	-3.5

Table 1.14 Variable temperature studies of **9e** (CD_3OH, 700MHz)

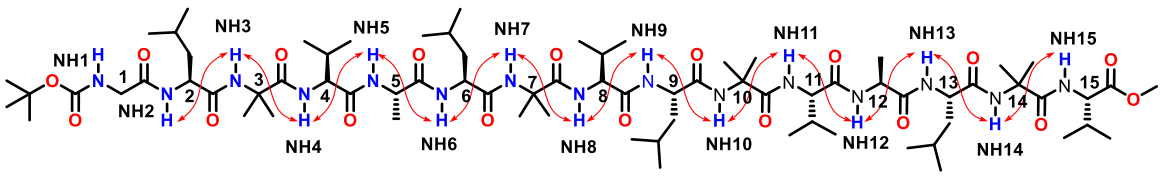
1.5.3.2 ROESY Analysis and MD simulation studies

Conformational investigations of the higher oligopeptides and their dimedone-tethered peptides were undertaken using 2D-NOESY studies (CD_3OH, 700 MHz). It revealed a 16-membered α-helix for all the compounds.

For MD simulation studies, the structure of compound **7**, compound **9d**, compound **8** and compound **9e** were initially optimized using different minimisers to achieve a convergence in Insight-II 2005 software. In order to search the conformations of the peptides, they were equilibrated in room temperature for 5ps. A simulated annealing protocol was adopted in order to delineate low energy structures. The structure was rapidly heated from 300K to 900K allowing the structure to remain at 300K, 600K and 900K for 5ps. The velocity scaling temperature controlling method was adopted for heating and the temperature was allowed to vary by an order of 10K. The velocity verlet integration method was used for integration. Further the structures were slowly cooled from 900K to 300K by allowing the structures to remain for 5ps at 800K, 700K, 600K, 500K, 400K and 300K. The minimum energy structures obtained during the trajectory satisfied all the ROEs obtained from ROESY spectra within 0.2 Å error limit.

1.5.3.2.1 ROESY Analysis and MD simulation studies of Compound 7

2D ROESY NMR reveals characteristic NOEs of NH-NH protons and also reveals the characteristic NOEs of NH-alpha and alpha-beta as shown in fig. Based on these analyses, it reveals that 16-membered α-helix is maintained throughout the peptide chain. Inter residual NOE interactions between $2C^{\alpha}H/5NH$, $4C^{\alpha}H/7NH$, $5C^{\alpha}H/8NH$, $6C^{\alpha}H/9NH$, $8C^{\alpha}H/11NH$, $9C^{\alpha}H/12NH$ and $11C^{\alpha}H/14NH$ as well as NOE interactions between $2C^{\alpha}H/5C^{\beta}H$, $4C^{\alpha}H/7C^{\beta}H$, $5C^{\alpha}H/8C^{\beta}H$, $6C^{\alpha}H/9C^{\beta}H$, $8C^{\alpha}H/11C^{\beta}H$, $9C^{\alpha}H/12C^{\beta}H$ and $11C^{\alpha}H/14C^{\beta}H$ suggested that compound **7** adopted well-defined folded conformation (C16 turn).



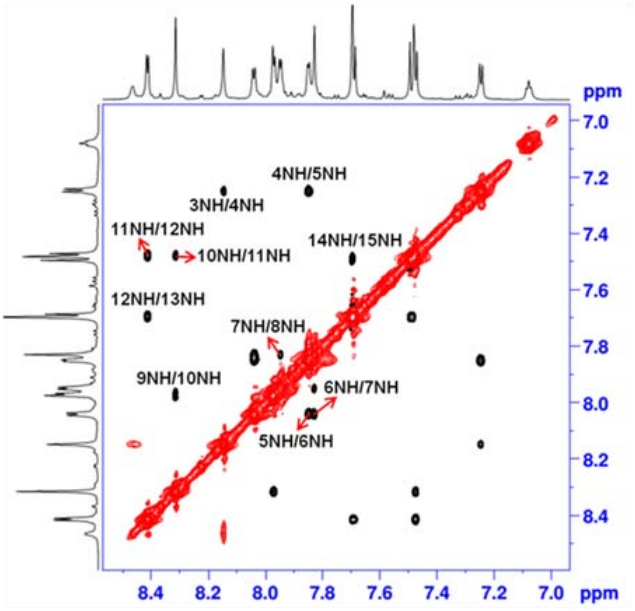
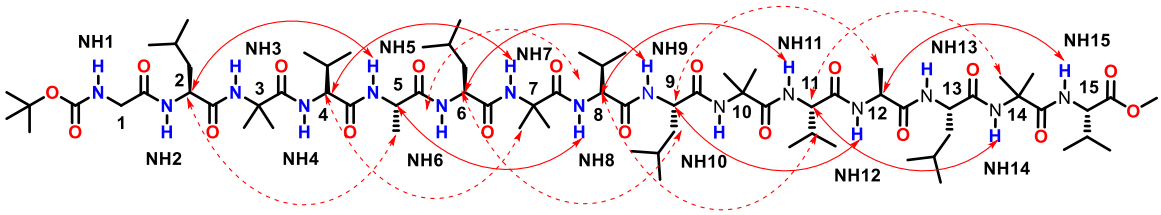


Figure 1.29 Expanded ROESY NMR spectra of Compound **7** [700 MHz, 273 K, CD_3OH] depicting the characteristic NOEs of NH-NH protons.



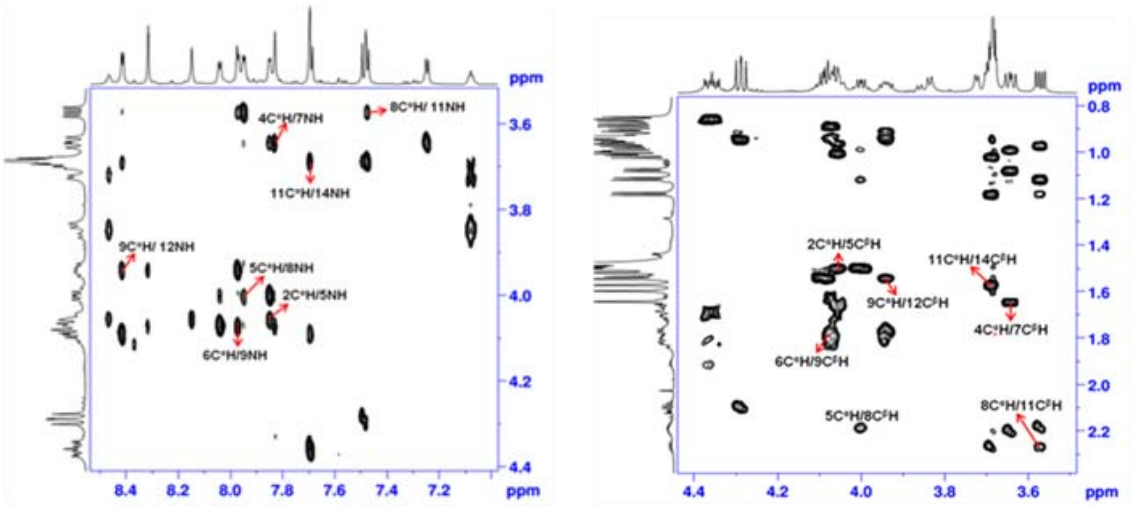


Figure 1.30 Expanded ROESY NMR spectra of Compound **7** [700 MHz, 273 K, CD_3OH] depicting the characteristic NOEs of NH-alpha and alpha-beta.

For estimating the ^1H-^1H distance restraints from the ROESY cross-peak intensities, the corresponding integrals were calibrated with respect to the ROESY cross-peak

integral for the *beta geminal* protons of Leu-13 residue, for which the inter-nuclear distance was taken as 1.78 Å as shown in table 1.15.

S. No	Atom	Residue	Atom	Residue	Distance range (Å)	Type
1	NH	2	NH	3	2.8 – 3.4	Sequential
2	NH	3	NH	4	2.8 – 3.4	Sequential
3	NH	4	NH	5	2.4 – 30	Sequential
4	NH	5	NH	6	2.5 – 3.1	Sequential
5	NH	6	NH	7	2.5 – 3.1	Sequential
6	NH	7	NH	8	2.6 – 3.2	Sequential
7	NH	9	NH	10	2.5 – 3.1	Sequential
8	NH	10	NH	11	2.6 – 3.2	Sequential
9	NH	11	NH	12	2.5 – 3.1	Sequential
10	NH	12	NH	13	2.5 – 3.1	Sequential
11	NH	14	NH	15	2.4 – 3.0	Sequential
12	$C^{\alpha}H$	2	NH	5	2.4 – 3.0	Sequential
13	$C^{\alpha}H$	4	NH	7	3.1 – 3.7	Sequential
14	$C^{\alpha}H$	5	NH	8	3.6 – 4.4	Sequential
15	$C^{\alpha}H$	6	NH	9	3.1 – 3.9	Sequential
16	$C^{\alpha}H$	8	NH	11	4.2 – 5.0	Sequential
17	$C^{\alpha}H$	9	NH	12	3.3 – 4.0	Sequential
18	$C^{\alpha}H$	11	NH	14	2.8 – 3.4	Sequential
19	$C^{\alpha}H$	2	$C^{\beta}H$	5	1.8 – 2.4	Sequential
20	$C^{\alpha}H$	4	$C^{\beta}H$	7	2.1 – 2.7	Sequential
21	$C^{\alpha}H$	5	$C^{\beta}H$	8	2.7 – 3.3	Sequential
22	$C^{\alpha}H$	6	$C^{\beta}H$	9	2.0 – 2.6	Sequential
23	$C^{\alpha}H$	8	$C^{\beta}H$	11	2.6 – 3.2	Sequential
24	$C^{\alpha}H$	9	$C^{\beta}H$	12	2.3 – 2.9	Sequential

Table 1.15: ROESY distance bounds for ^{1}H-^{1}H ROESY spectrum of compound **7** in CD_3OH solvent (700 MHz, 298 K)

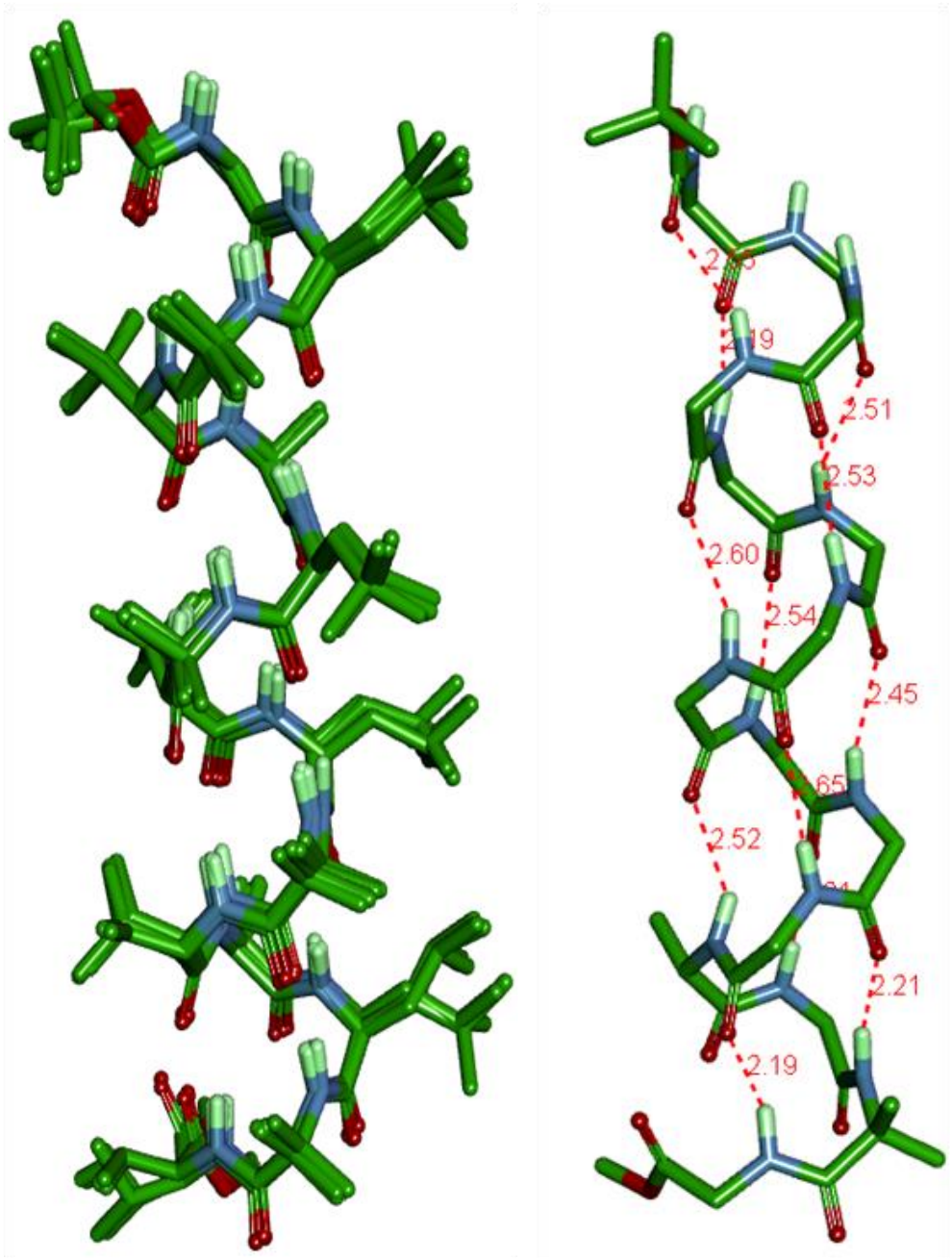


Figure 1.31 Superimposition of the minimum energy structures obtained from ROE Molecular Dynamics simulations showing side view of compound **7** in polar solvent medium (hydrogen bonds are shown in red dotted lines).

1.5.3.2.2 ROESY analysis and MD simulation studies of Compound 9d.

2D ROESY NMR reveals characteristic NOEs of NH-NH protons and also reveals the characteristic NOEs of NH-alpha and alpha-beta as shown in fig. Based on these

analyses, it reveals that 16-membered α-helix is maintained throughout the peptide chain. Inter residual NOE interactions between 2C$^{\alpha}$H/5NH, 4C$^{\alpha}$H/7NH, 5C$^{\alpha}$H/8NH, 6C$^{\alpha}$H/9NH, 8C$^{\alpha}$H/11NH, 9C$^{\alpha}$H/12NH and 11C$^{\alpha}$H/14NH as well as NOE interactions between 2C$^{\alpha}$H/5C$^{\beta}$H, 4C$^{\alpha}$H/7C$^{\beta}$H, 5C$^{\alpha}$H/8C$^{\beta}$H, 6C$^{\alpha}$H/9C$^{\beta}$H, 8C$^{\alpha}$H/11C$^{\beta}$H, 9C$^{\alpha}$H/12C$^{\beta}$H and 11C$^{\alpha}$H/14C$^{\beta}$H suggested that compound **9d** adopted well-defined folded conformation (C16 turn).

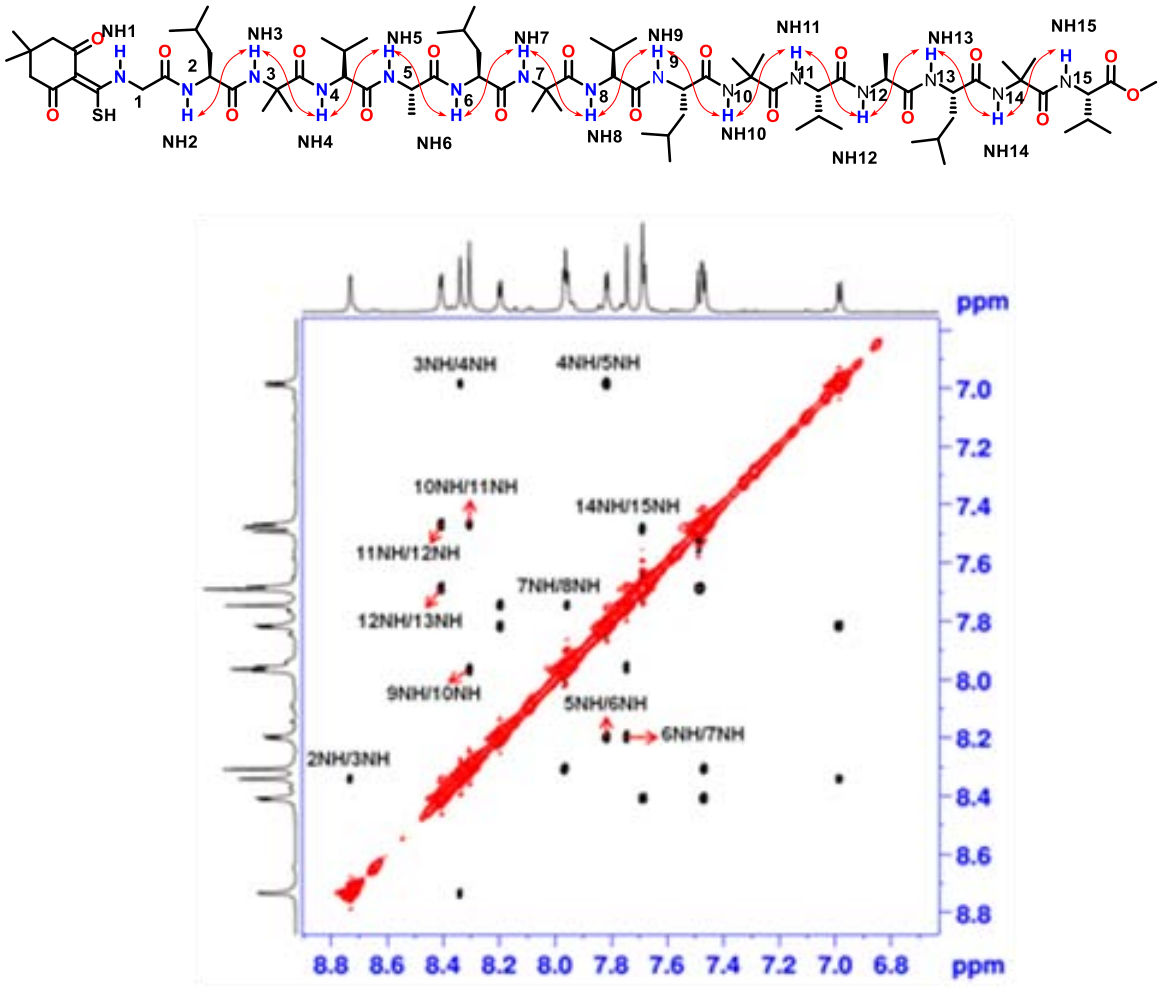


Figure 1.32 Expanded ROESY NMR spectra of Compound **9d** [700 MHz, 273 K, CD_3OH] depicting the characteristic NOEs of NH-NH protons.

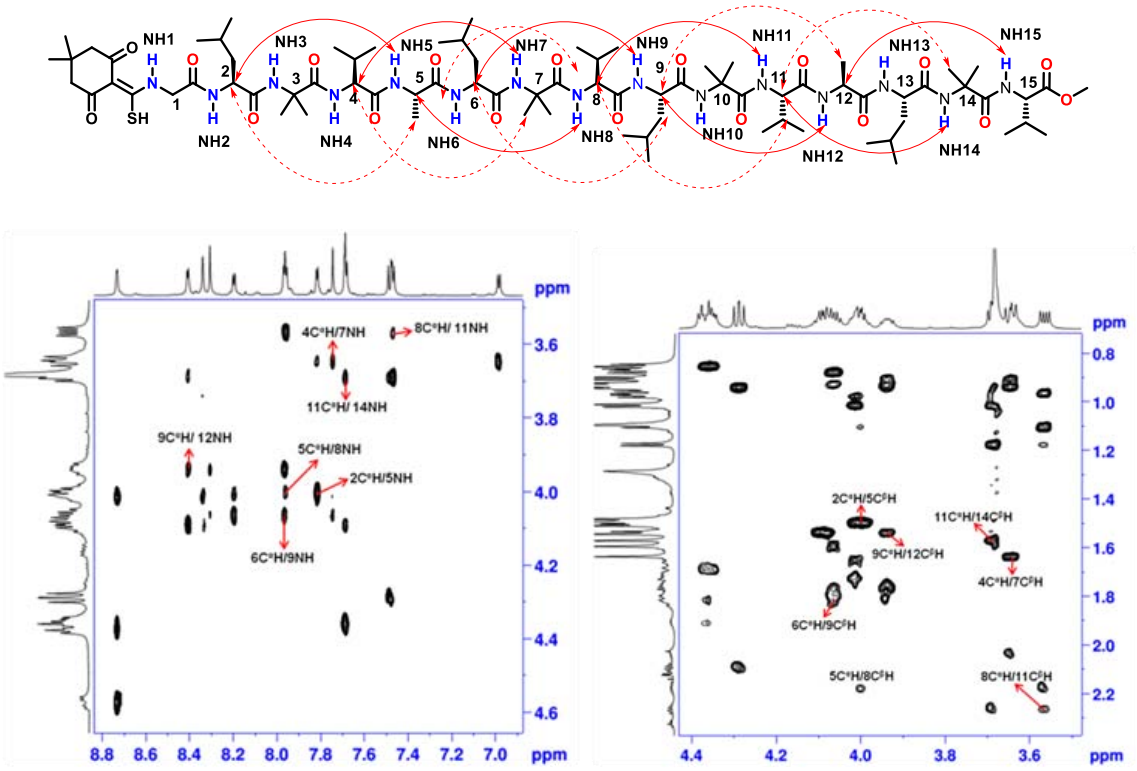


Figure 1.33 Expanded ROESY NMR spectra of Compound **9d** [700 MHz, 273 K, CD_3OH] depicting the characteristic NOEs of NH-alpha and alpha-beta.

For estimating the ^{1}H-^{1}H distance restraints from the ROESY cross-peak intensities, the corresponding integrals were calibrated with respect to the ROESY cross-peak integral for the *beta geminal* protons of Leu-13 residue, for which the inter-nuclear distance was taken as 1.78

S. No	Atom	Residue	Atom	Residue	Distance range (Å)	Type
1	NH	2	NH	3	2.8 – 3.4	Sequential
2	NH	3	NH	4	2.8 – 3.4	Sequential
3	NH	4	NH	5	2.4 – 3.0	Sequential
4	NH	5	NH	6	2.5 – 3.1	Sequential
5	NH	6	NH	7	2.5 – 3.1	Sequential
6	NH	7	NH	8	2.6 – 3.2	Sequential
7	NH	9	NH	10	2.5 – 3.1	Sequential
8	NH	10	NH	11	2.6 – 3.2	Sequential
9	NH	11	NH	12	2.5 – 3.1	Sequential
10	NH	12	NH	13	2.5 – 3.1	Sequential
11	NH	14	NH	15	2.4 – 3.0	Sequential
12	$C^{\alpha}H$	2	NH	5	2.4 – 3.0	Sequential
13	$C^{\alpha}H$	4	NH	7	3.1 – 3.7	Sequential
14	$C^{\alpha}H$	5	NH	8	3.6 – 4.4	Sequential
15	$C^{\alpha}H$	6	NH	9	3.1 – 3.9	Sequential
16	$C^{\alpha}H$	8	NH	11	4.2 – 5.0	Sequential
17	$C^{\alpha}H$	9	NH	12	3.3 – 4.0	Sequential
18	$C^{\alpha}H$	11	NH	14	2.8 – 3.4	Sequential
19	$C^{\alpha}H$	2	$C^{\beta}H$	5	1.8 – 2.4	Sequential
20	$C^{\alpha}H$	4	$C^{\beta}H$	7	2.1 – 2.7	Sequential
21	$C^{\alpha}H$	5	$C^{\beta}H$	8	2.7 – 3.3	Sequential
22	$C^{\alpha}H$	6	$C^{\beta}H$	9	2.0 – 2.6	Sequential
23	$C^{\alpha}H$	8	$C^{\beta}H$	11	2.6 – 3.2	Sequential
24	$C^{\alpha}H$	9	$C^{\beta}H$	12	2.3 – 2.9	Sequential

Table 1.16: ROESY distance bounds for ^{1}H-^{1}H ROESY spectrum of compound **9d** in CD_3OH solvent (700 MHz, 298 K)

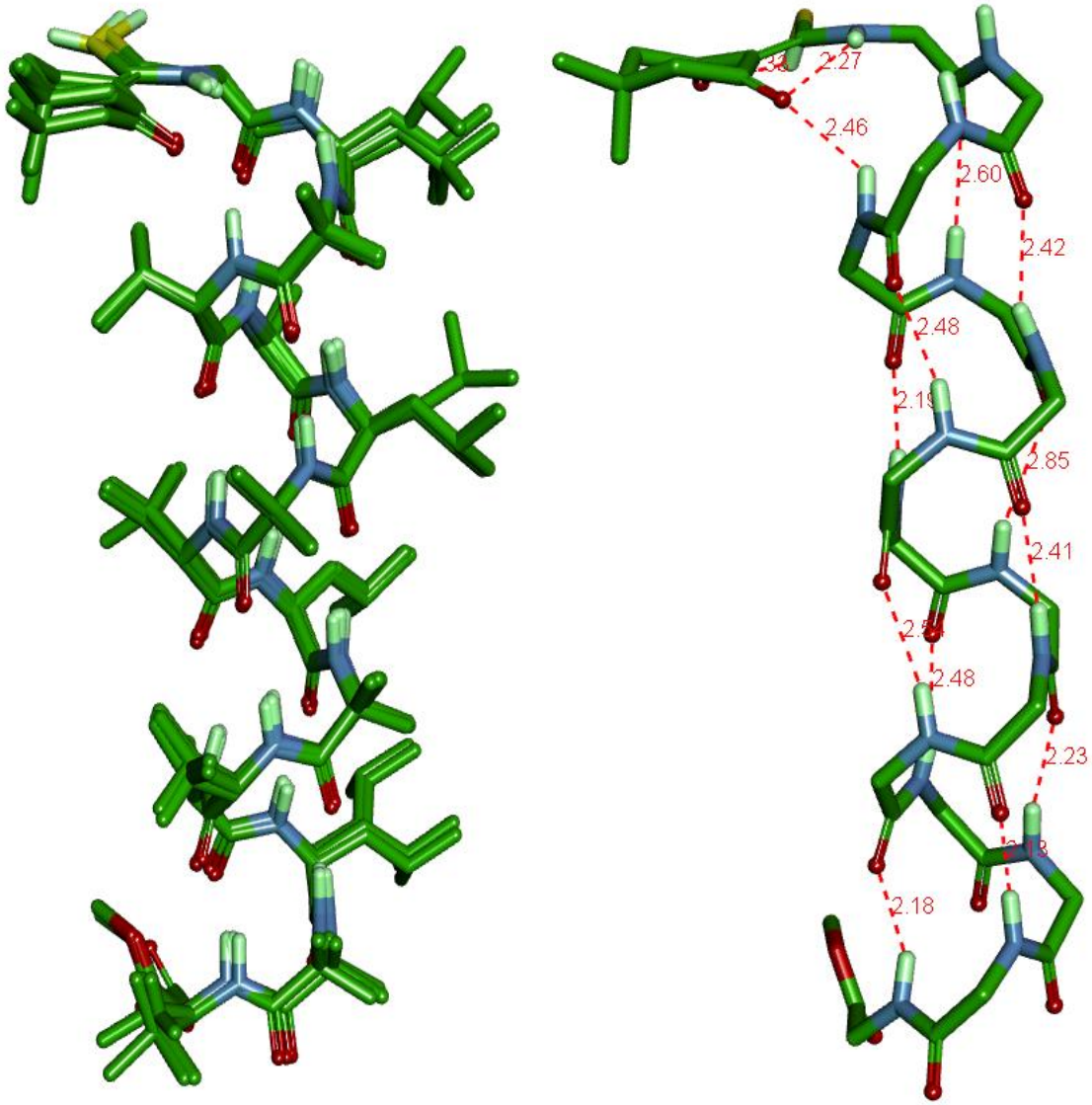


Figure 1.34 Superimposition of the minimum energy structures obtained from ROE Molecular Dynamics simulations showing side view of compound **9d** in polar solvent medium (hydrogen bonds are shown in red dotted lines).

1.5.3.2.3 ROESY analysis and MD simulation studies of compound 8

2D ROESY NMR reveals characteristic NOEs of NH-NH protons and also reveals the characteristic NOEs of NH-alpha and alpha-beta as shown in fig. Based on these analyses, it reveals that 16-membered α-helix is maintained throughout the peptide chain. Inter residual NOE interactions between 2C$^{\alpha}$H/5NH, 4C$^{\alpha}$H/7NH, 5C$^{\alpha}$H/8NH, 6C$^{\alpha}$H/9NH, 8C$^{\alpha}$H/11NH, 10C$^{\alpha}$H/113NH and 12C$^{\alpha}$H/15NH as well as NOE interactions between 2C$^{\alpha}$H/5C$^{\beta}$H, 4C$^{\alpha}$H/7C$^{\beta}$H, 5C$^{\alpha}$H/8C$^{\beta}$H, 8C$^{\alpha}$H/11C$^{\beta}$H, 9C$^{\alpha}$H/12C$^{\beta}$H, 10C$^{\alpha}$H/13C$^{\beta}$H and 12C$^{\alpha}$H/15C$^{\beta}$H suggested that compound **8** adopted well-defined folded conformation (C16 turn).

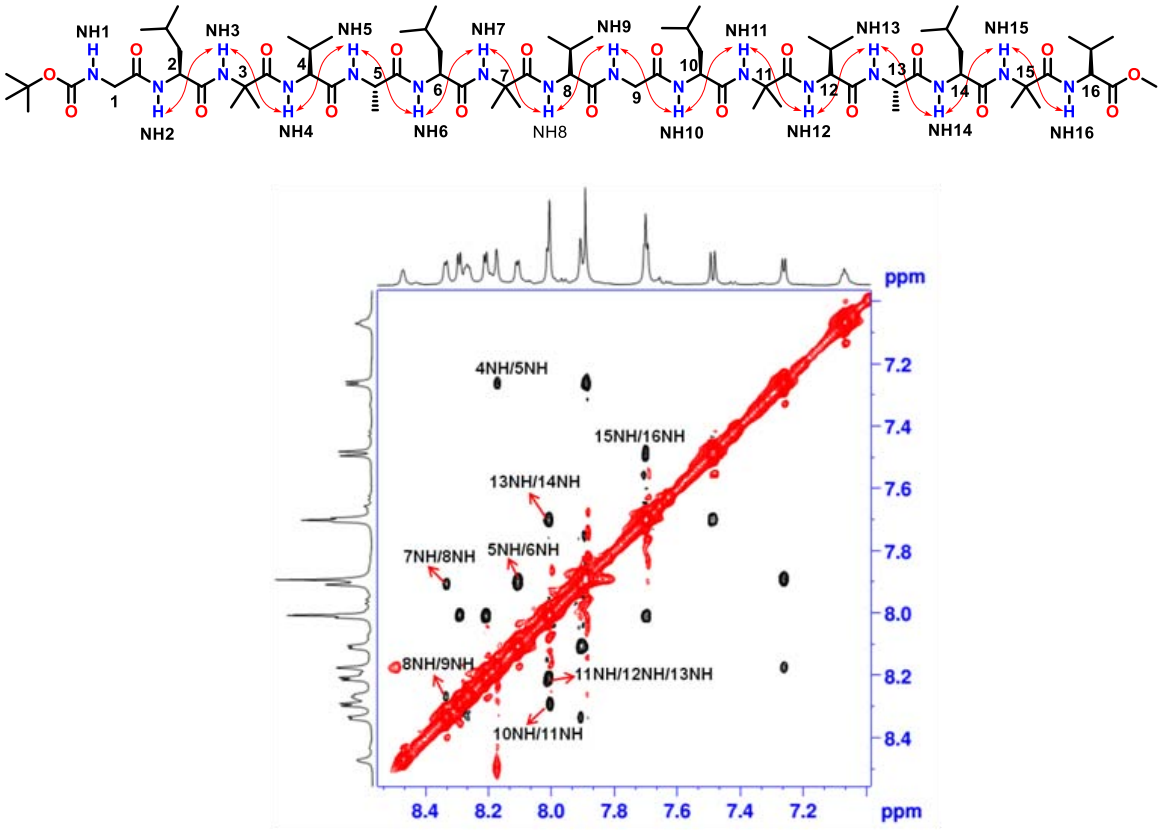


Figure 1.35 Expanded ROESY NMR spectra of Compound **8** [700 MHz, 273 K, CD_3OH] depicting the characteristic NOEs of NH-NH protons.

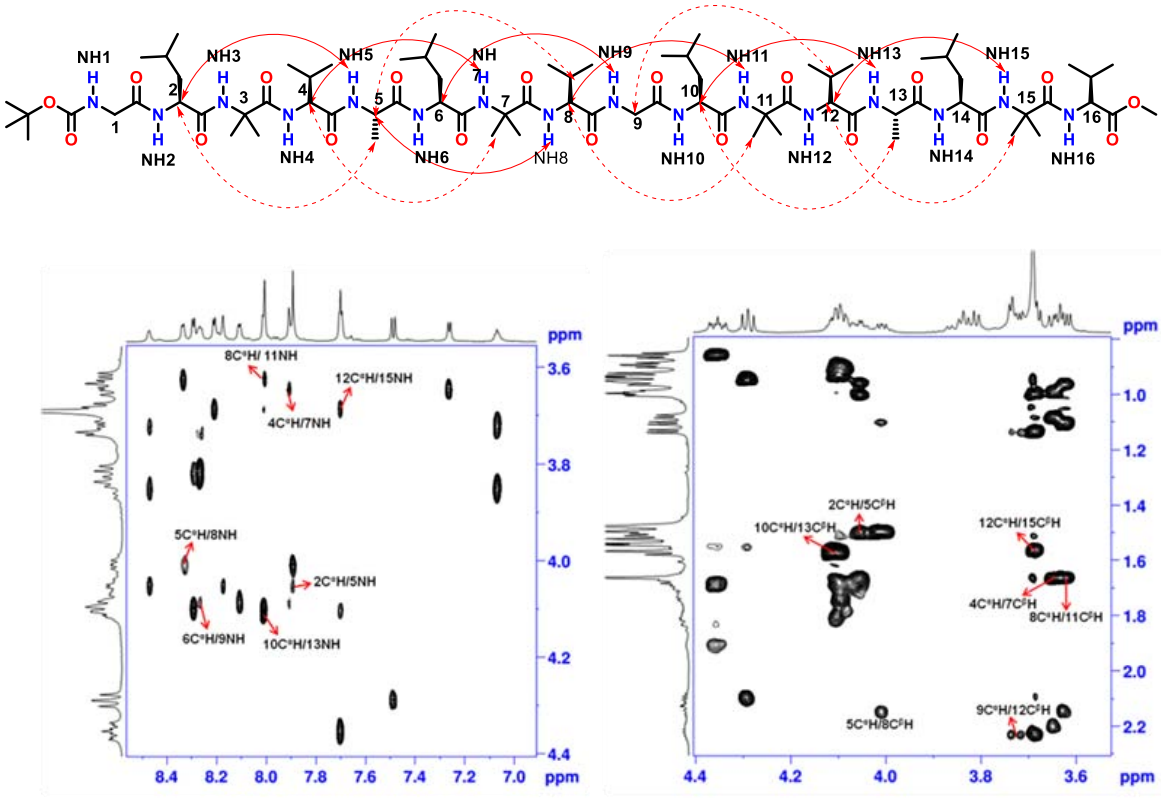


Figure 1.36 Expanded ROESY NMR spectra of Compound **8** [700 MHz, 273 K, CD_3OH] depicting the characteristic NOEs of NH-alpha and alpha-beta.

For estimating the ^{1}H-^{1}H distance restraints from the ROESY cross-peak intensities, the corresponding integrals were calibrated with respect to the ROESY cross-peak integral for the *beta geminal* protons of Leu-14 residue, for which the inter-nuclear distance was taken as 1.78 Å.

S. No	Atom	Residue	Atom	Residue	Distance range (Å)	Type
1	NH	2	NH	3	2.8 – 3.4	Sequential
2	NH	3	NH	4	2.8 – 3.4	Sequential
3	NH	4	NH	5	2.4 – 3.0	Sequential
4	NH	5	NH	6	2.5 – 3.1	Sequential
5	NH	6	NH	7	2.5 – 3.1	Sequential
6	NH	7	NH	8	2.6 – 3.2	Sequential
7	NH	9	NH	10	2.5 – 3.1	Sequential
8	NH	10	NH	11	2.6 – 3.2	Sequential
9	NH	11	NH	12	2.5 – 3.1	Sequential
10	NH	12	NH	13	2.5 – 3.1	Sequential
11	NH	14	NH	15	2.4 – 3.0	Sequential
12	$C^{\alpha}H$	2	NH	5	2.4 – 3.0	Sequential
13	$C^{\alpha}H$	4	NH	7	3.1 – 3.7	Sequential
14	$C^{\alpha}H$	5	NH	8	3.6 – 4.4	Sequential
15	$C^{\alpha}H$	6	NH	9	3.1 – 3.9	Sequential
16	$C^{\alpha}H$	8	NH	11	4.2 – 5.0	Sequential
17	$C^{\alpha}H$	9	NH	12	3.3 – 4.0	Sequential
18	$C^{\alpha}H$	11	NH	14	2.8 – 3.4	Sequential
19	$C^{\alpha}H$	2	$C^{\beta}H$	5	1.8 – 2.4	Sequential
20	$C^{\alpha}H$	4	$C^{\beta}H$	7	2.1 – 2.7	Sequential
21	$C^{\alpha}H$	5	$C^{\beta}H$	8	2.7 – 3.3	Sequential
22	$C^{\alpha}H$	6	$C^{\beta}H$	9	2.0 – 2.6	Sequential
23	$C^{\alpha}H$	8	$C^{\beta}H$	11	2.6 – 3.2	Sequential
24	$C^{\alpha}H$	9	$C^{\beta}H$	12	2.3 – 2.9	Sequential

Table 1.17: ROESY distance bounds for ^{1}H-^{1}H ROESY spectrum of compound **8** in CD_3OH solvent (700 MHz, 298 K)

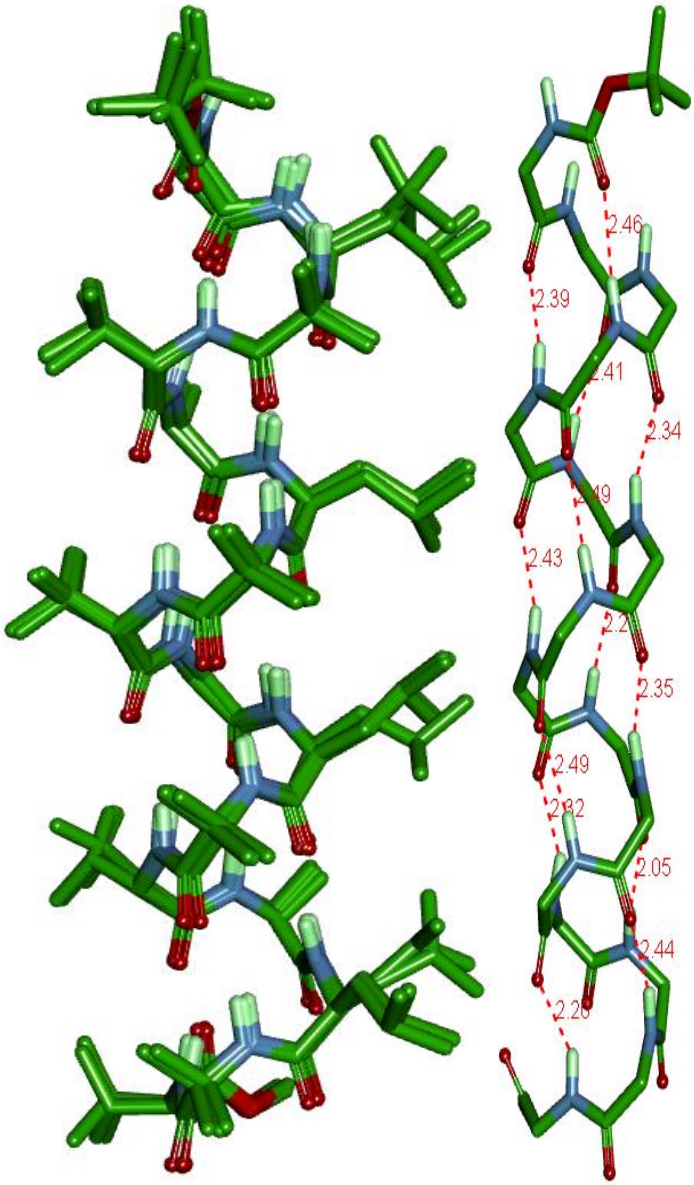


Figure 1.37 Superimposition of the minimum energy structures obtained from ROE Molecular Dynamics simulations showing side view of compound **8** in polar solvent medium (hydrogen bonds are shown in red dotted lines).

1.5.3.2.4 ROESY analysis and MD studies of compound 9e

2D ROESY NMR reveals characteristic NOEs of NH-NH protons and also reveals the characteristic NOEs of NH-alpha and alpha-beta as shown in fig. Based on these analyses, it reveals that 16-membered α-helix is maintained throughout the peptide chain. Inter residual NOE interactions between 2C$^{\alpha}$H/5NH, 4C$^{\alpha}$H/7NH, 5C$^{\alpha}$H/8NH, 6C$^{\alpha}$H/9NH, 8C$^{\alpha}$H/11NH, 10C$^{\alpha}$H/113NH and 12C$^{\alpha}$H/15NH as well as NOE interactions between 2C$^{\alpha}$H/5C$^{\beta}$H, 4C$^{\alpha}$H/7C$^{\beta}$H, 5C$^{\alpha}$H/8C$^{\beta}$H, 8C$^{\alpha}$H/11C$^{\beta}$H, 9C$^{\alpha}$H/12C$^{\beta}$H, 10C$^{\alpha}$H/13C$^{\beta}$H and 12C$^{\alpha}$H/15C$^{\beta}$H suggested that compound **8** adopted well-defined folded conformation (C16 turn).

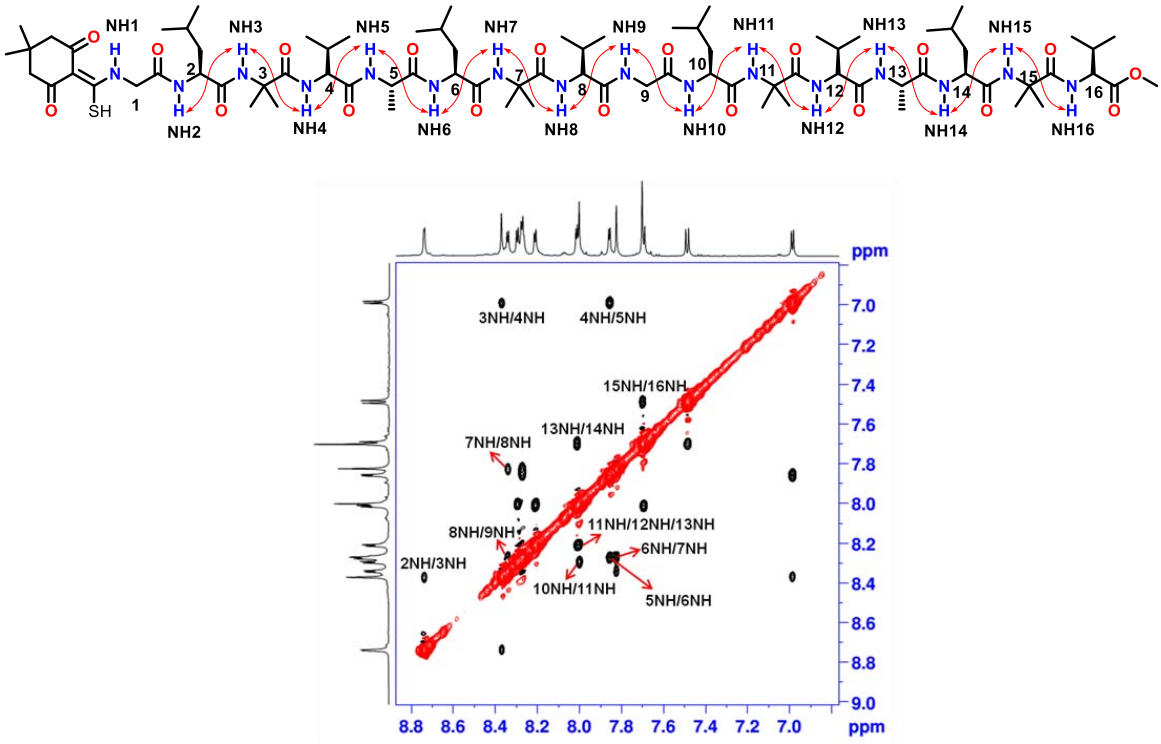
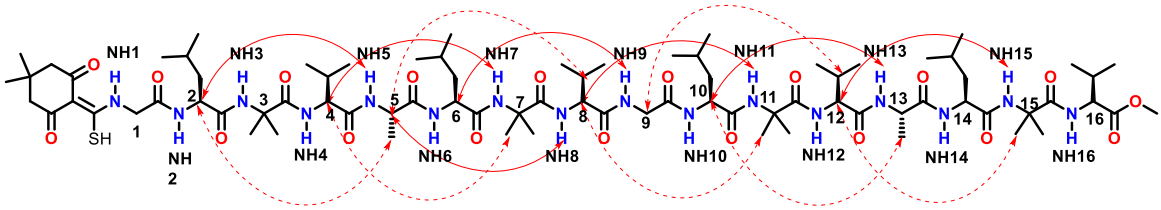


Figure 1.38 Expanded ROESY NMR spectra of Compound **9e** [700 MHz, 273 K, CD_3OH] depicting the characteristic NOEs of NH-NH protons.



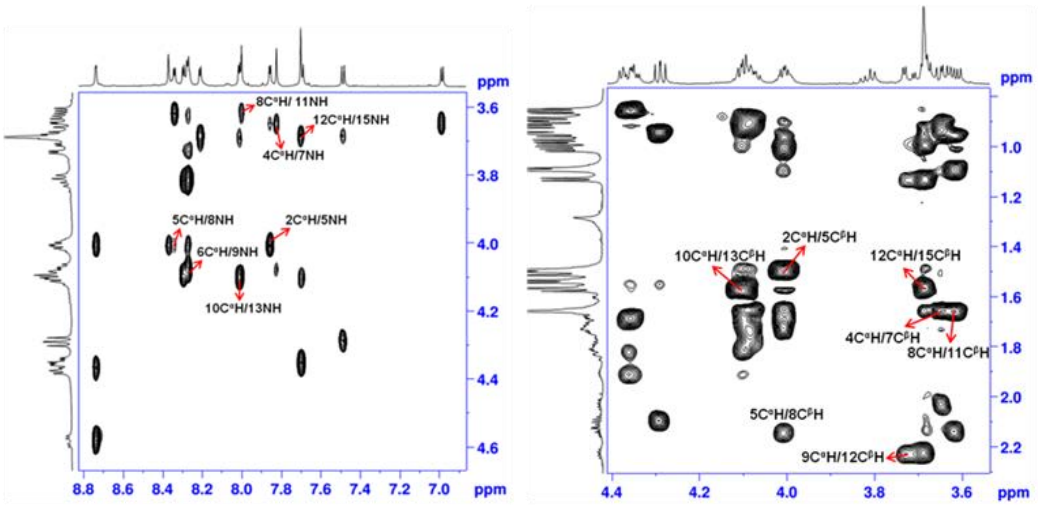


Figure 1.39 Expanded ROESY NMR spectra of compound **9e** [700 MHz, 273 K, CD_3OH] depicting the characteristic NOEs of NH-alpha and alpha-beta.

For estimating the ^1H-^1H distance restraints from the ROESY cross-peak intensities, the corresponding integrals were calibrated with respect to the ROESY cross-peak integral for the *beta geminal* protons of Leu-14 residue, for which the inter-nuclear distance was taken as 1.78 Å.

S. No	Atom	Residue	Atom	Residue	Distance range (Å)	Type
1	NH	2	NH	3	2.8 – 3.4	Sequential
2	NH	3	NH	4	2.8 – 3.4	Sequential
3	NH	4	NH	5	2.4 – 3.0	Sequential
4	NH	5	NH	6	2.5 – 3.1	Sequential
5	NH	6	NH	7	2.5 – 3.1	Sequential
6	NH	7	NH	8	2.6 – 3.2	Sequential
7	NH	9	NH	10	2.5 – 3.1	Sequential
8	NH	10	NH	11	2.6 – 3.2	Sequential
9	NH	11	NH	12	2.5 – 3.1	Sequential
10	NH	12	NH	13	2.5 – 3.1	Sequential
11	NH	14	NH	15	2.4 – 3.0	Sequential
12	$C^{\alpha}H$	2	NH	5	2.4 – 3.0	Sequential
13	$C^{\alpha}H$	4	NH	7	3.1 – 3.7	Sequential
14	$C^{\alpha}H$	5	NH	8	3.6 – 4.4	Sequential
15	$C^{\alpha}H$	6	NH	9	3.1 – 3.9	Sequential
16	$C^{\alpha}H$	8	NH	11	4.2 – 5.0	Sequential
17	$C^{\alpha}H$	9	NH	12	3.3 – 4.0	Sequential
18	$C^{\alpha}H$	11	NH	14	2.8 – 3.4	Sequential
19	$C^{\alpha}H$	2	$C^{\beta}H$	5	1.8 – 2.4	Sequential
20	$C^{\alpha}H$	4	$C^{\beta}H$	7	2.1 – 2.7	Sequential
21	$C^{\alpha}H$	5	$C^{\beta}H$	8	2.7 – 3.3	Sequential
22	$C^{\alpha}H$	6	$C^{\beta}H$	9	2.0 – 2.6	Sequential
23	$C^{\alpha}H$	8	$C^{\beta}H$	11	2.6 – 3.2	Sequential
24	$C^{\alpha}H$	9	$C^{\beta}H$	12	2.3 – 2.9	Sequential

Table-1.18: ROESY distance bounds for ^{1}H-^{1}H ROESY spectrum of compound **9e** in CD_3OH solvent (700 MHz, 298 K)

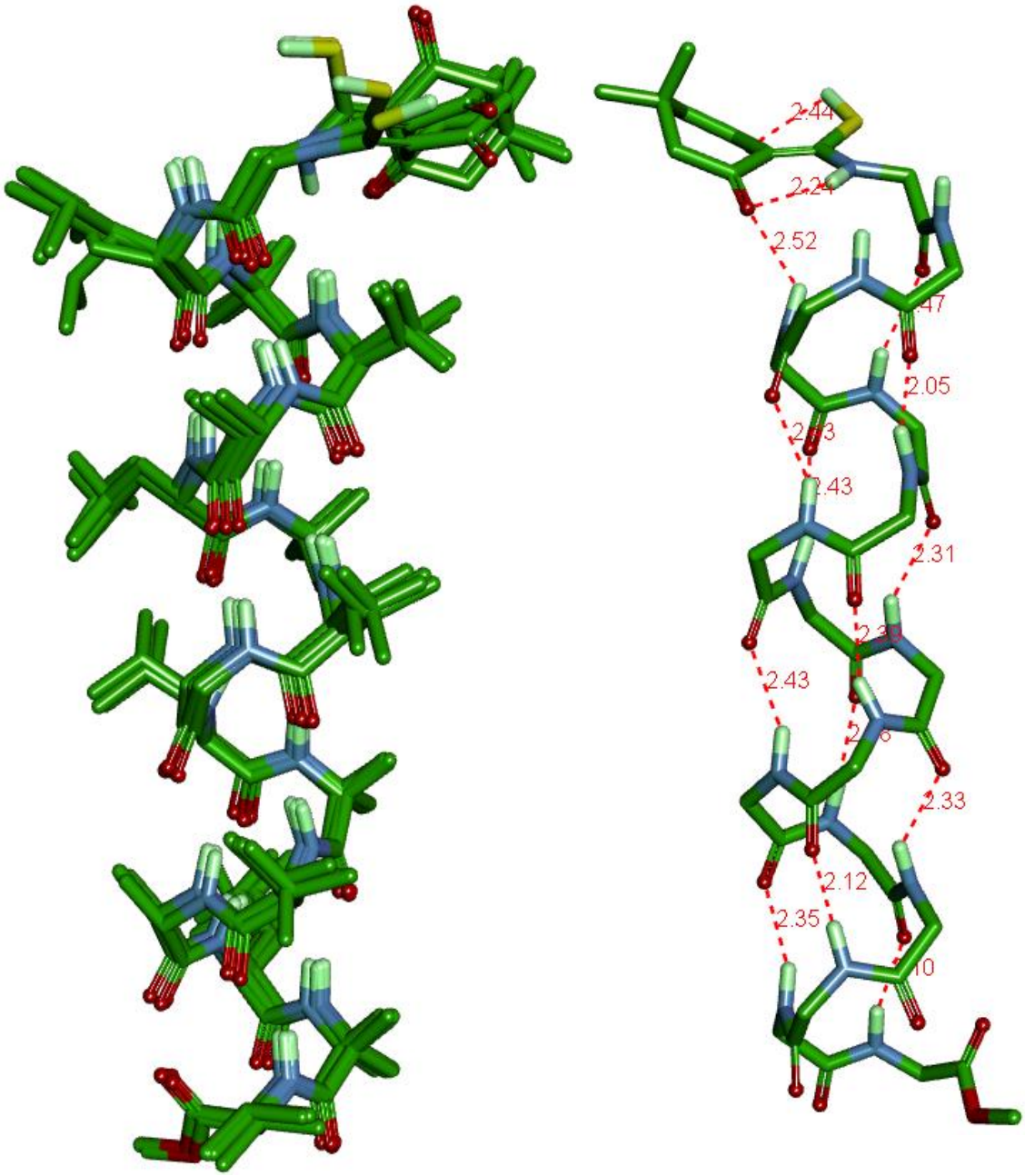


Figure 1.40 Superimposition of the minimum energy structures obtained from ROE Molecular Dynamics simulations showing side view of compound **9e** in polar solvent medium (hydrogen bonds are shown in red dotted lines).

1.6 Conclusions

In conclusion, the molecular scaffold provides a pre-organized folding nucleus which can position hydrogen-bonding groups in a manner that permits unusual hydrogen bonding maintaining the overall regular secondary structure. When two identical peptide chains are attached to the scaffold containing dimedone, the dimedone carbonyls get oriented in such a way that it forms a C-12 and C-15 intramolecular bifurcated hydrogen bonding with the peptide chains attached all together maintaining the helical conformation. This is evident in single crystal X-ray crystallographic studies for compounds **11b** and **13a**. Extensive solution state NMR studies suggests α-helical conformation for higher oligopeptides **7**, **8**, **9d** and **9e**.Variable temperature studies were carried out to determine the strength of the hydrogen bonding. CD spectroscopy also suggested α-helical conformation for higher oligopeptides. Dimedone as a template comprising of folding properties creates an opportunity to synthesize various supramolecular architectures featuring oligopeptides.

The chemical shift assignments for compounds **7**, **8**, **9d** and **9e** were made by using standard 1D and 2D NMR (TOCSY and ROESY/NOESY) techniques. The dispersion of the downfield NH chemical shifts around 7.0 ppm to 9.0 ppm is consistent with the possibility of these molecules adopting secondary structure and the NH's being involved in hydrogen bonding. Furthermore, the oligomers have exhibited ROEs patterns: CαH(i)--N-H(i+3), CαH(i)--CβH(i+3) and CβH(i)-CαH(i+3), which are characteristic of 13-helix favouring CO(i-3)--N-H(i) H-bonding. The observed ROE patterns are consistent for compounds **7**, **8**, **9d** and **9e**, suggesting the propagation of the 13-helix conformation with the increase in the length of the backbone. Furthermore, these oligomers have exhibited several sequential, CαH(i)--N-H(i+1), CβH(i)--N-H(i+1) and CγH(i)--N-H(i+1) NOEs that strongly support the 13-helical folding. Subsequently, for compounds **7**, **8**, **9d** and **9e**, about 24 ROE-derived distances, respectively, have been used as distance constraints in restrained MD-simulations (Insight-II). The minimum energy structures thus obtained have shown good convergence to periodic strong CO(i-3)--N-H(i) H-bonding (2.2Å-2.5Å). The

observed H-bonding and its orientation along the backbone are consistent with the natural α-peptides.

1.7 Experimental Section

Single Crystal X-ray Crystallographic studies

X-ray intensity data measurement of compound **1a** was carried out on a Bruker D8 VENTURE Kappa Duo PHOTON II CPAD diffractometer equipped with Incoatech multilayer mirrors optics. The intensity measurements were carried out with Mo micro-focus sealed tube diffraction source (MoK_α = 0.71073Å) at 100(2) K temperature. The X-ray generator was operated at 50 kV and 1.4 mA. A preliminary set of cell constants and an orientation matrix were calculated from three sets of 36 frames. Data were collected with ω scan width of 0.5° at different settings of φ and 2θ with a frame time of 40 secs keeping the sample-to-detector distance fixed at 4 cm. The X-ray data collection was monitored by APEX3 program (Bruker, 2016).[31] All the data were corrected for Lorentzian, polarization and absorption effects using SAINT and SADABS programs (Bruker, 2016). ShelX-97 was used for structure solution and full matrix least-squares refinement on F^2.[32] All the hydrogen atoms were placed in geometrically idealized position and constrained to ride on their parent atoms.

Crystal data for **6** ($C_{41}H_{74}N_8O_{11}$): M = 855.08, crystal dimensions 0.280 x 0.140 x 0.040 mm^3, Orthorhombic, space group $P2_12_12_1$, a = 9.8016(3) Å, b = 20.9059(7) Å, c = 23.1280(8) Å, α = 90°., β = 90°, γ = 90°, V = 4739.2(3) $Å^3$, Z = 4, D_c = 1.198 Mg/m^3, μ = 0.087 mm^{-1}, F(000) = 1856, 51340 reflections collected, Independent reflections 14131 [R(int) = 0.0357], index ranges- -13<=h<=11, -29<=k<=27, -30<=l<=33, Final R indices [I>2sigma(I)], R1 = 0.0407, wR2 = 0.0925, R indices (all data) R1 = 0.0452, wR2 = 0.0959, Maximum and mininum transmission = -0.997 and 0.976.

Crystal data for **9c** ($C_{46}H_{81}N_8O_{13}S$): M = 986.24, crystal dimensions 0.420 x 0.370 x 0.310 mm^3, Orthorhombic, space group $P2_12_12_1$, a = 9.6556(19) Å, b = 23.130(5) Å, c = 24.789(5) Å, α = 90°., β = 90°, γ = 90°, V = 5536(2) $Å^3$, Z = 4, D_c = 1.183 Mg/m^3, μ

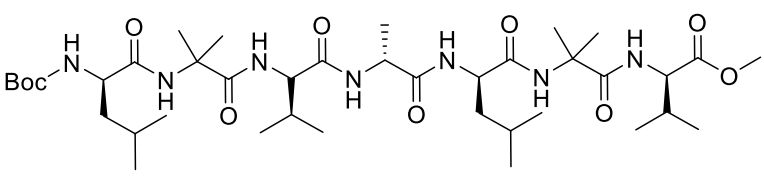
= 0.122 mm^{-1}, F(000) = 2132, 39187 reflections collected, Independent reflections 14565 [R(int) = 0.0599], Index ranges -13<=h<=11, -31<=k<=31, -33<=l<=22, Final R indices [I>2sigma(I)] R1 = 0.0737, wR2 = 0.1579, R indices (all data) R1 = 0.1152, wR2 = 0.1783, Maximum and mininum transmission = 0.963 and 0.950.

Crystal data for **11b** ($C_{47}H_{82}N_{10}O_{10}$): M = 947.24, crystal dimensions 0.410 x 0.350 x 0.290 mm3, Orthorhombic, space group $P2_12_12_1$, a = 9.257(16) Å, b = 24.152(7) Å, c = 25.769(3) Å, α = 90º., β = 90º, γ = 90º, V = 5840(2) Å3, Z = 4, Dc = 1.687 Mg/m3, μ = 0.135 mm-1, F(000) = 2345, 43187 reflections collected, Independent reflections 15676 [R(int) = 0.0622], Index ranges -13<=h<=11, -32<=k<=29, -31<=l<=25, Final R indices [I>2sigma(I)] R1 = 0.0825, wR2 = 0.1789, R indices (all data) R1 = 0.1552, wR2 = 0.1854, Maximum and mininum transmission = 0.980 and 0.910.

Crystal data for **13a** ($C_{81}H_{140}N_{16}O_{20}$): *M* = 1657.24, crystal dimensions 0.10 x 0.15 x 0.23 mm^3, Orthorhombic P, space group : $P2_12_12_1$, partial merohedral twin (0 1 0 1 0 0 0 0 -1), a = 19.36 Å, b = 19.36 Å, c = 43.64 Å, α = 90º., β = 90º, γ = 90º, V = 16352 $Å^3$, Z = 4, Largest residual density peaks: 0.765 and -0.419 e $Å^{-3}$, 270829 reflections were collected up to 0.90 Å; 44911 unique reflections; averaged multiplicity 6.03; completeness 99 %, 84 % reflections had I > 2σ(I), μ = 0.122 mm^{-1}, R_{int} = 5.14 %; R_{sig} = 3.91 %, Preliminary quality criteria (undetermined residual electron density): R1 = 10.8 % (I > 2σ(I)); wR2 = 27.0 % (all data). The twin ratio was refined to 82:18. The D8 VENTURE with Cu IμS 3.0 in combination with the most sensitive PHOTON II CPAD detector made the structure determination possible.

Synthetic procedures and data

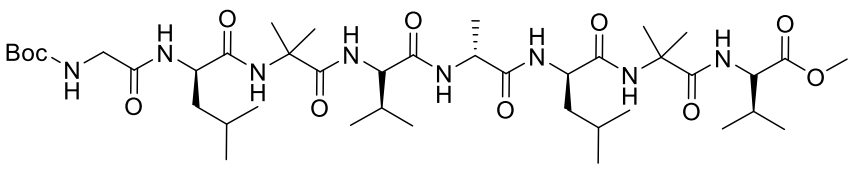
Compound 5[2] (Boc-L-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-OMe): To a solution of Boc protected tripeptide ester **3a** (5.0 g, 11.65 mmol, 1 equiv) in methanol



(20 mL), $LiOH.H_2O$ (1.96 g, 46.56 mmol, 4 equiv) dissolved in water (10 mL) was added and the reaction

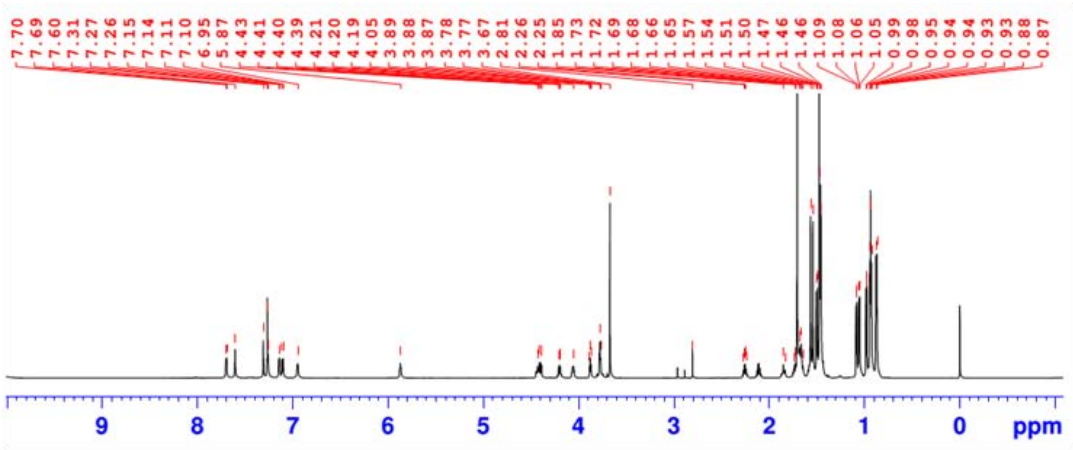
mixture was stirred for 6 h at room temperature. After completion of the reaction, solvent was evaporated under reduced pressure and the residue obtained was treated with saturated $KHSO_4$ solution, followed by extraction with ethyl acetate (50 mL x 2). The tripeptide acid derivative (Boc-L-Leu-Aib-L-Val-OH) obtained after evaporation of the solvent under reduced pressure, was carried forward for the next reaction, without further purification. Trifluoroacetic acid (2 mL) was added to a solution of Boc protected tetra peptide ester **4a** (6.0 g, 11.00 mmol) in CH_2Cl_2 (2 mL) at 0 °C, and the resulting mixture was stirred at room temperature for 1 h. Later, solvent was evaporated under reduced pressure to obtain H-L-Ala-Leu-Aib-L-Val-OMe, **4b** and was used directly for the next coupling reaction. To a solution of **4b** (3.45 g, 8.67 mmol, 1.2 equiv) in DMF (10 mL), DIPEA (5 mL, 21.6 mmol, 3 equiv) was added. Subsequently, (Boc-L-Leu-Aib-L-Val-OH, 3.0 g, 7.22 mmol, 1 equiv), HOBt (0.48 g, 3.6 mmol, 0.5 equiv) and EDC.HCl (2.07 g, 10.83 mmol, 1.5 equiv) were added. After completion of the reaction (12 h), the reaction mixture was taken in EtOAc and the organic layer was washed sequentially with aq. solutions of sat. $KHSO_4$, brine and $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was purified by silica gel column chromatography (petroleum ether/ethyl acetate 1:9) to afford (Boc-L-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-OMe) **5a** as an off-white solid (3.6 g, 67%).[2]

Compound 6: (Boc-Gly-L-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-OMe): Trifluoroacetic acid (2 mL) was added to a solution of heptapeptide **5a** (3.0 g, 3.76

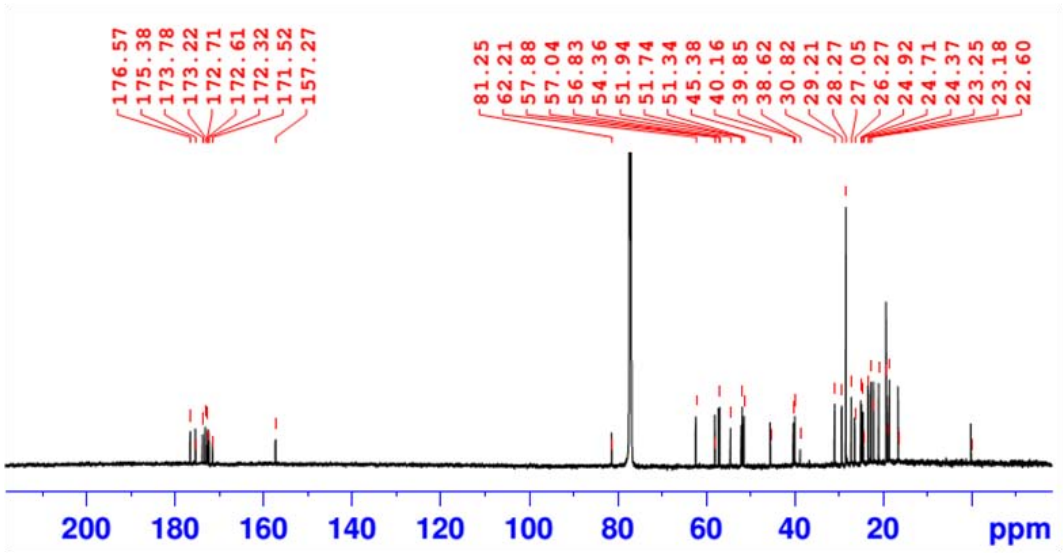


mmol) in CH_2Cl_2 (4 mL) at 0 °C and resulting mixture was stirred at room temperature for 1 h. Removal of the solvent afforded the free amine **5b** (H-L-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-OMe) and was used directly for the next coupling reaction. To a solution of **5b** (2.3 g, 3.4 mmol, 1.2 equiv) in DMF (3 mL), DIPEA (1 mL, 8.5 mmol, 3 equiv) was added. Subsequently, Boc-Gly-OH (0.5 g, 2.8 mmol, 1 equiv), HOBt (0.192 g, 1.42 mmol, 0.5 equiv) and EDC.HCl (0.818 g, 4.2 mmol, 1.5 equiv) were added. After completion of the reaction (12 h), the reaction

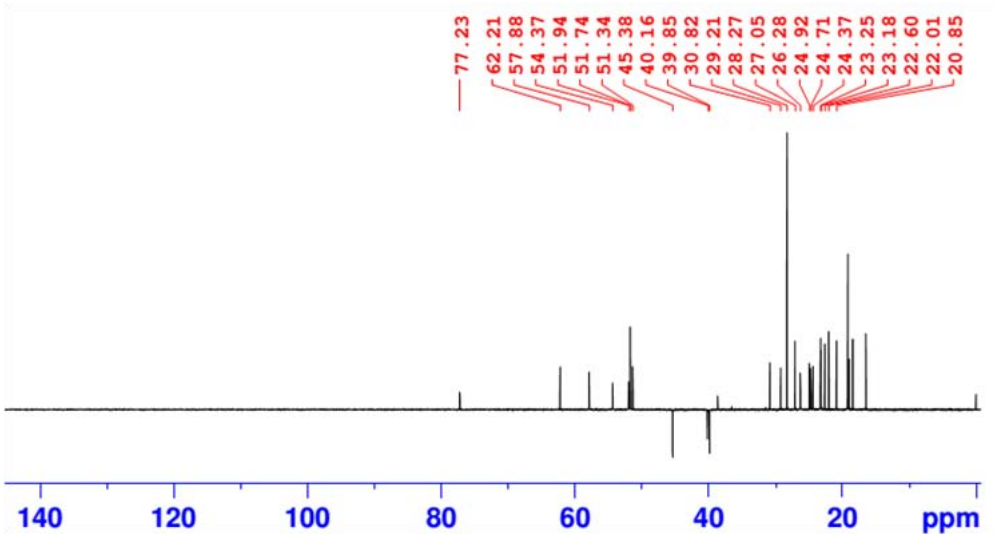
mixture was taken in EtOAc and the organic layer was washed sequentially with aq. solutions of sat. $KHSO_4$, brine and $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was purified by silica gel column chromatography (ethyl acetate) to afford (Boc-Gly-L-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-OMe) **6** as an off-white solid (2.91 g, 91 %). $[\alpha]^{22}_D$: -13.654° (c= 0.23, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.75-7.72 (m, 2H), 7.41 (bs, 1H), 7.30-7.23 (m, 3H), 7.14-7.12 (d, *J* = 8.5, 1H), 6.39 (bs, 1H), 4.44-4.33 (m, 2H), 4.21-4.14 (m, 1H), 4.03-4.02 (m, 1H), 3.84-3.70 (m. 3H), 3.68 (s, 3H), 2.27-2.22 (m, 1H), 2.16-2.09 (m, 1H), 1.82-1.69 (4H), 1.64 (s, 6H), 1.46 (s, 14H), 1.10-1.08 (d, *J* = 6.71 Hz, 3H), 1.06-1.04 (d, *J* = 6.71 Hz, 3H), 0.98-0.92 (m, 12H), 0.87-0.86 (d, *J* = 4.2 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 176.7, 175.4, 174.0, 173.4, 173.0, 172.4, 172.2, 157.3, 80.6, 62.4, 58.1, 56.8, 56.7, 54.6, 52.0, 51.7, 51.4, 45.3, 40.0, 39.8, 30.6, 29.2, 28.2, 27.0, 26.1, 24.7, 24.6, 24.3, 23.1, 22.4, 22.1, 20.7, 19.1, 18.5, 16.3. HRMS: calculated for $C_{41}H_{75}N_8O_{11}$ $[M+H]^+$: 855.5561, observed : 855.5551 $[M+H]^+$.



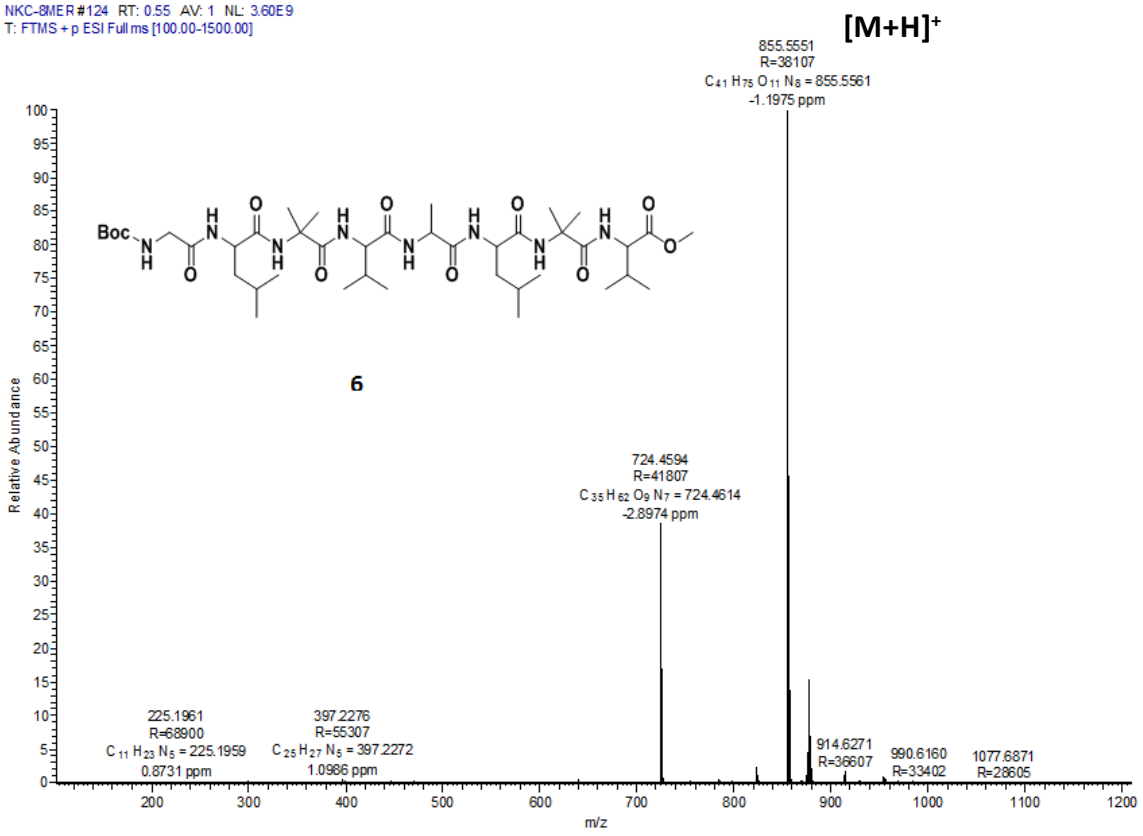
^{1}H NMR spectrum of compound **6** ($CDCl_3$, 400 MHz, 298 K)



^{13}C NMR spectrum of compound **6** ($CDCl_3$, 400 MHz, 298 K)

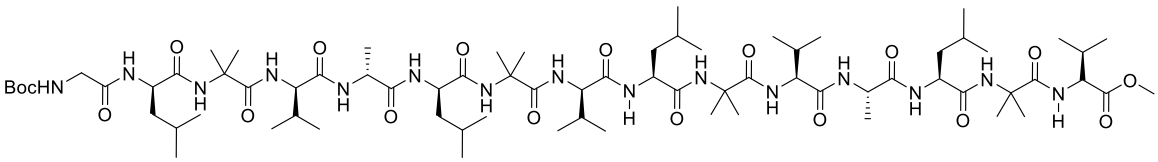


DEPT-135 spectrum of compound **6** ($CDCl_3$, 175 MHz, 273 K)



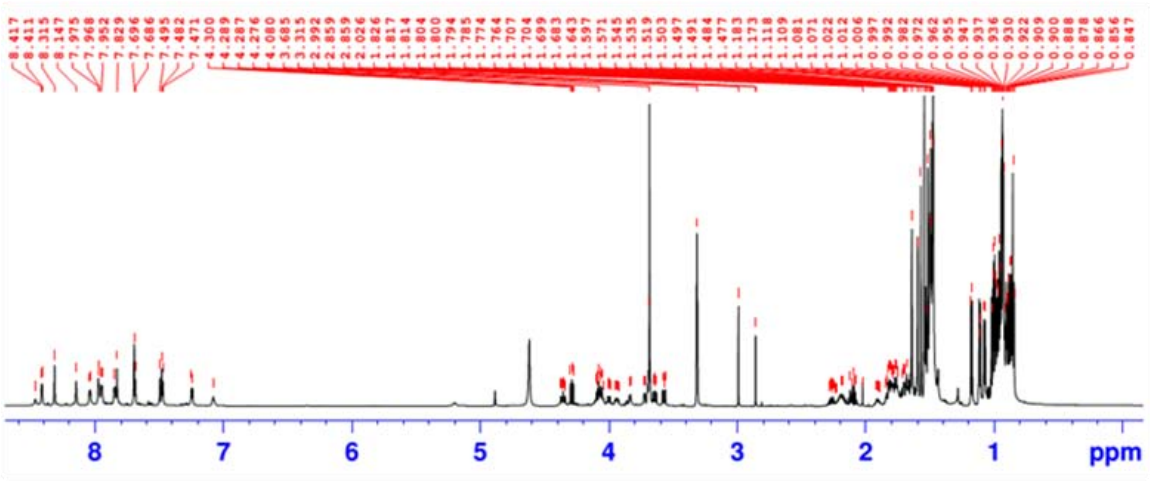
HRMS of Compound **6**

Compound 7 (Boc-Gly-L-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val- Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-OMe):

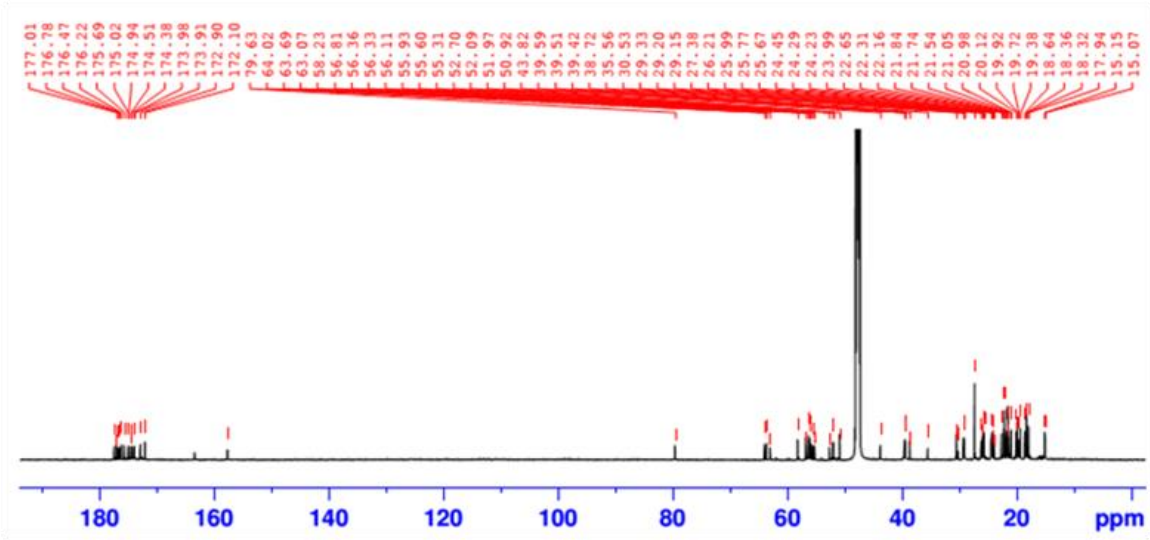


To a solution of Boc protected octapeptide ester **6** (2.0 g, 4.66 mmol, 1 equiv) in methanol (20 mL), $LiOH.H_2O$ (0.78 g, 18.64 mmol, 4 equiv) dissolved in water (10 mL) was added and the reaction mixture was stirred for 6 h at room temperature. After completion of the reaction, solvent was evaporated under reduced pressure and the residue obtained was treated with saturated $KHSO_4$ solution, followed by extraction with ethyl acetate (50 mL x 2). The octapeptide acid derivative **6a** obtained after evaporation of the solvent under reduced pressure, was carried forward for the next reaction, without further purification. Trifluoroacetic acid (2 mL) was added to a solution of Boc protected hepta-peptide ester **5a** (2.0 g, 4 mmol) in CH_2Cl_2 (2 mL) at

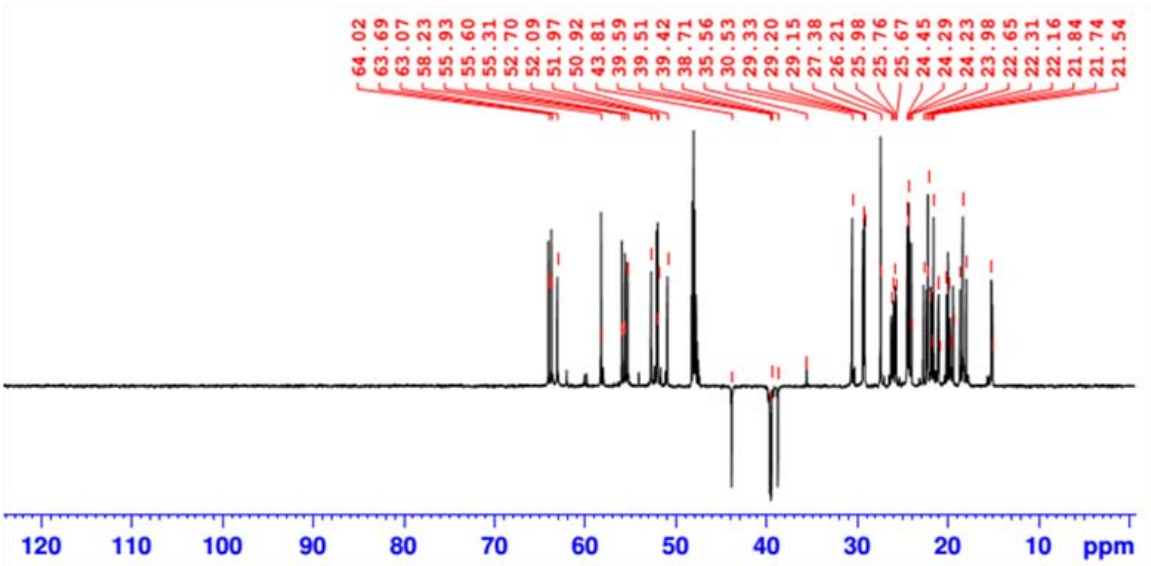
0 °C, and the resulting mixture was stirred at room temperature for 1 h. Later, solvent was evaporated under reduced pressure to obtain **5b** and was used directly for the next coupling reaction. To a solution of **5b** (1.15 g, 2.89 mmol, 1.2 equiv) in DMF (5 mL), DIPEA (1.25 mL, 7.2 mmol, 3 equiv) was added. Subsequently, **6a** (1.0 g, 2.4 mmol, 1 equiv), HOBt (0.162 g, 1.2 mmol, 0.5 equiv) and EDC.HCl (0.687 g, 3.6 mmol, 1.5 equiv) were added. After completion of the reaction (12 h), the reaction mixture was taken in EtOAc and the organic layer was washed sequentially with aq. solutions of sat. $KHSO_4$, brine and $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was purified by silica gel column chromatography (eluent:2% MeOH/DCM) to afford (Boc-L-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-L-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-OMe) **7** as an off-white solid (1.3 g, 72%). $[\alpha]^{26}_D$: -23.0° (c = 0.1, MeOH); ^{1}H NMR (700MHz ,CD_3OH) chemical shift values are given in table 00. ^{13}C NMR (175MHz ,CD_3OH) δ = 178.9, 178.4, 178.2, 177.9, 177.6, 177.1, 175.9, 175.8, 175.4, 175.3, 174.4, 174.3, 173.5, 164.9, 159.1, 81.1, 65.4, 65.1, 64.5, 59.7, 58.2, 57.8, 57.6, 57.5, 57.3, 57.0, 56.7, 54.1, 53.5, 53.4, 52.3, 45.2, 40.9, 40.1, 37.0, 32.0, 31.7, 30.8, 30.6, 28.8, 27.6, 27.4, 27.2, 27.1, 25.9, 25.7, 25.7, 25.4, 24.1, 23.8, 23.6, 23.3, 23.2, 23.0, 22.5, 22.4, 21.5, 21.3, 21.1, 20.8, 20.1, 19.8, 19.4, 16.5; HRMS: $C_{73}H_{123}N_{15}O_{18}Na$, calculated: 1520.9063 $[M+Na]^+$, observed: 1521.0017 $[M+Na]^+$.



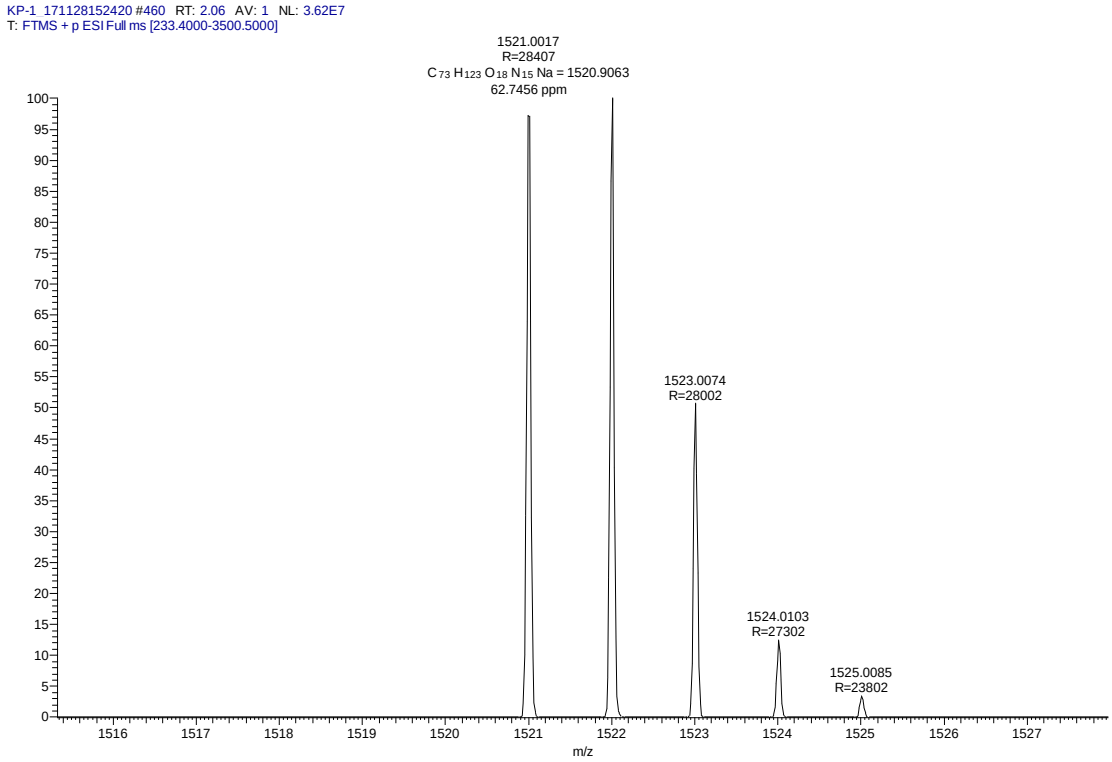
^{1}H NMR spectrum of compound **7** (CD_3OH, 700 MHz, 273 K)



^{13}C NMR spectrum of compound **7** (CD_3OH, 175 MHz, 273 K)

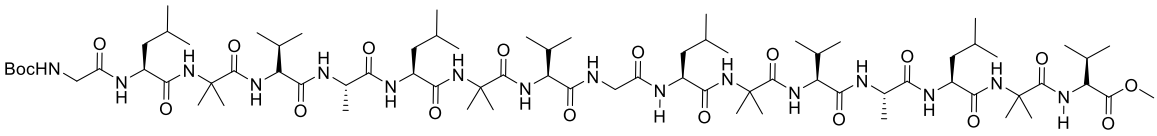


DEPT-135 NMR spectrum of compound **7** (CD_3OH, 175 MHz, 273 K)



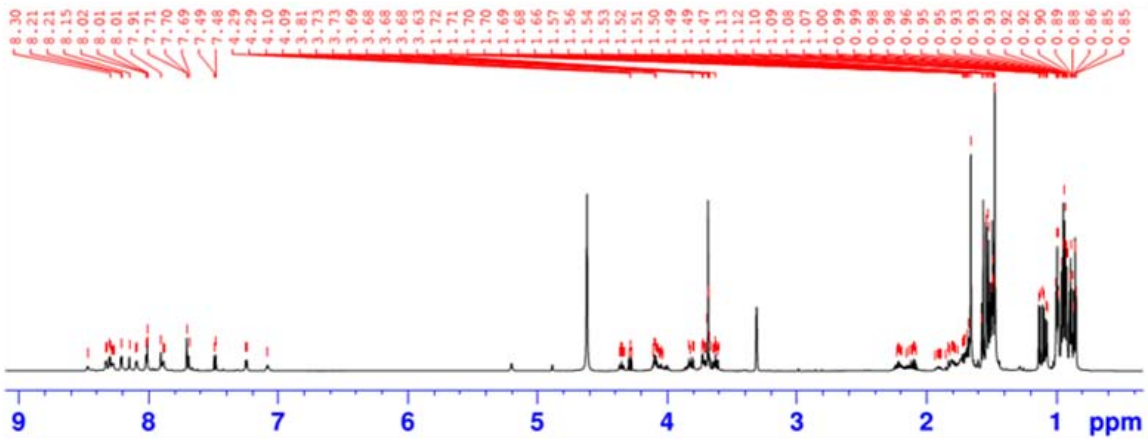
HRMS of Compound **7**

Compound 8 (Boc-Gly-L-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-Gly-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-OMe):

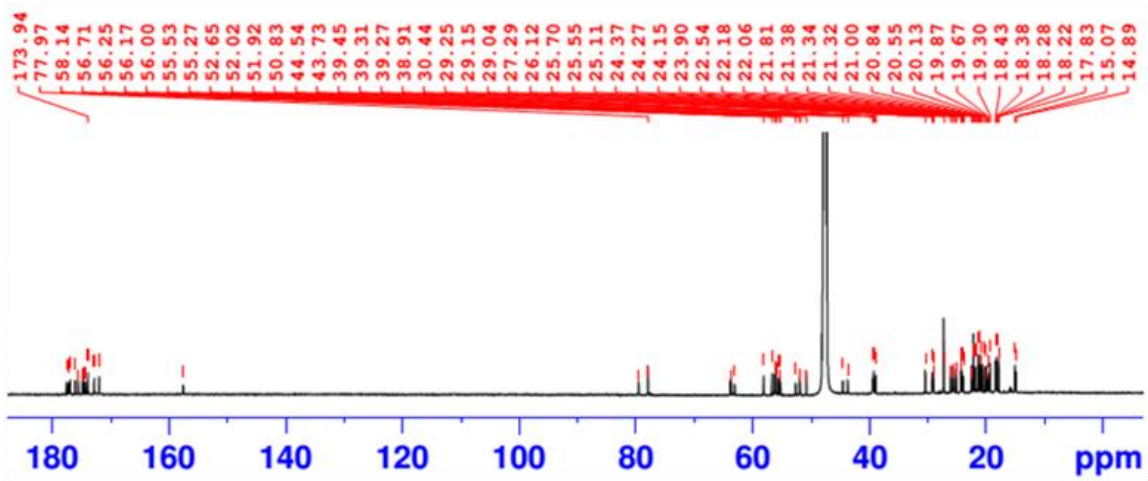


To a solution of Boc protected octapeptide ester **6** (2.0 g, 4.66 mmol, 1 equiv) in methanol (20 mL), $LiOH.H_2O$ (0.78 g, 18.64 mmol, 4 equiv) dissolved in water (10 mL) was added and the reaction mixture was stirred for 6 h at room temperature. After completion of the reaction, solvent was evaporated under reduced pressure and the residue obtained was treated with saturated $KHSO_4$ solution, followed by extraction with ethyl acetate (50 mL x 2). The octapeptide acid derivative **6a** obtained after evaporation of the solvent under reduced pressure, was carried forward for the next reaction, without further purification. Trifluoroacetic acid (2 mL) was added to a solution of Boc protected octa-peptide ester **6a** (2.0 g, 4 mmol) in CH_2Cl_2 (2 mL) at 0 °C, and the resulting mixture was stirred at room temperature for 1 h. Later, solvent

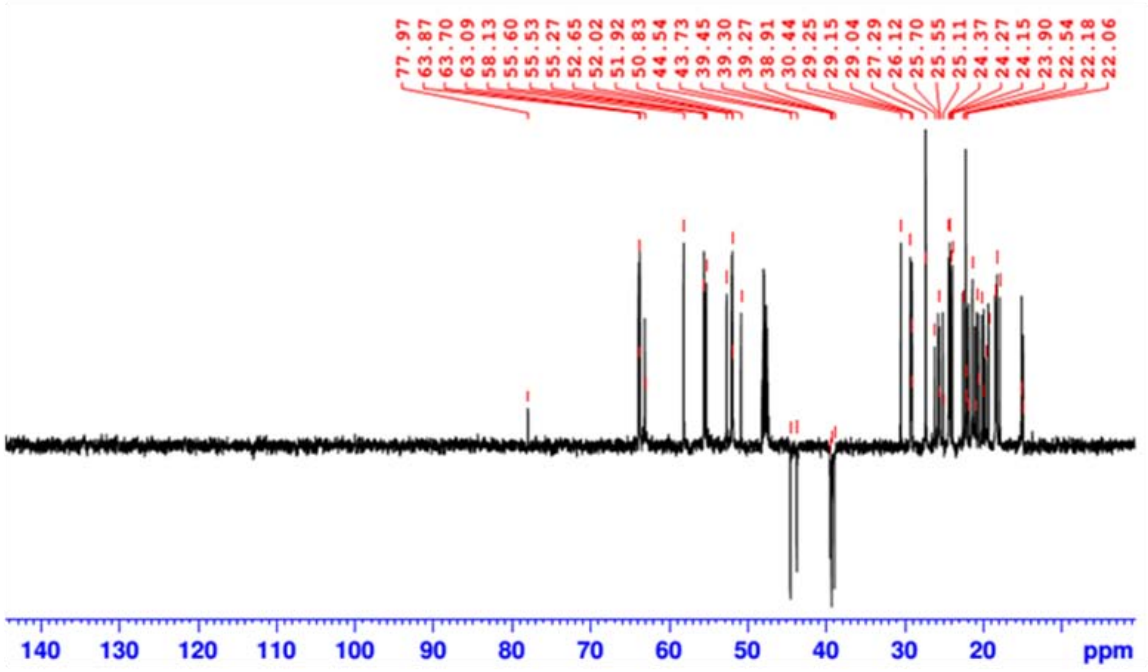
was evaporated under reduced pressure to obtain **6b** and was used directly for the next coupling reaction. To a solution of **6b** (1.15 g, 2.89 mmol, 1.2 equiv) in DMF (5 mL), DIPEA (1.25 mL, 7.2 mmol, 3 equiv) was added. Subsequently, **6a** (1.0 g, 2.4 mmol, 1 equiv), HOBt (0.162 g, 1.2 mmol, 0.5 equiv) and EDC.HCl (0.687 g, 3.6 mmol, 1.5 equiv) were added. After completion of the reaction (12 h), the reaction mixture was taken in EtOAc and the organic layer was washed sequentially with aq. solutions of sat. $KHSO_4$, brine and $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was purified by silica gel column chromatography (eluent:2% MeOH/ AcOEt, R_f: 0.5) to afford (Boc-L-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-L-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-OMe) **8** as an off-white solid (1.28 g, 70%).$[\alpha]^{26}_D$: -32.0°(c = 0.1, MeOH); ^{1}H NMR (700MHz ,CD_3OH) chemical shift values are given in table 00. ^{13}C NMR (175MHz, CD_3OH) δ =^{1}H NMR (700MHz ,CD_3OH) chemical shift values are given in table 00. ^{13}C NMR (175MHz, CD_3OH) δ = 179.1, 178.8, 178.6, 178.5, 177.6, 177.1, 176.4, 176.1, 175.9, 175.5, 175.4, 174.4, 174.4, 173.5, 159.1, 81.1, 81.1, 79.5, 79.2, 65.4, 65.4, 65.2, 65.2, 64.6, 64.5, 59.7, 58.2, 58.2, 57.8, 57.7, 57.6, 57.5, 57.1, 57.0, 56.8, 54.2, 53.5, 53.4, 52.3, 46.1, 45.3, 41.0, 40.8, 40.4, 32.0, 30.7, 28.8, 27.6, 27.2, 27.1, 26.6, 25.8, 25.4, 24.1, 23.7, 23.6, 23.3, 22.9, 22.8, 22.5, 22.4, 22.1, 21.7, 21.4, 19.9, 19.8, 19.4, 16.6, 16.4; HRMS: $C_{76}H_{137}N_{16}O_{19}$, calculated: 1578.0240 $[M+H]^+$, observed: 1578.0228 $[M+H]^+$.



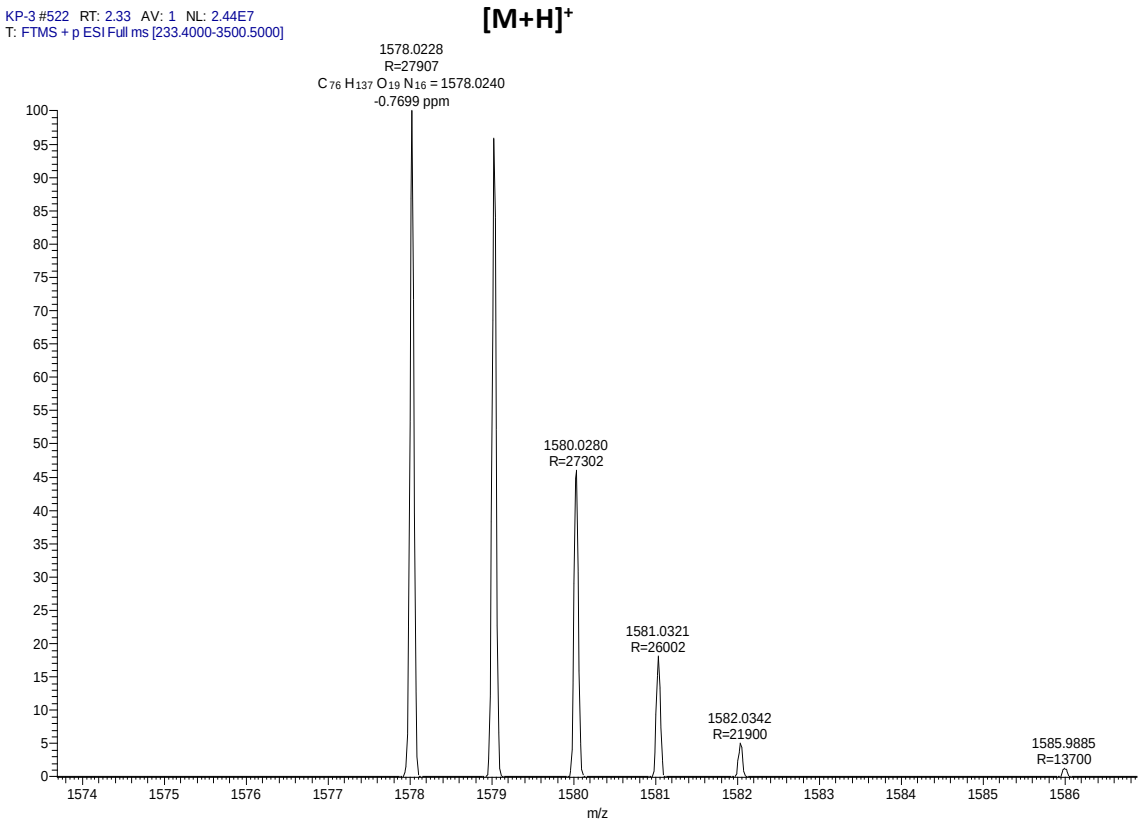
^{1}H NMR spectrum of compound **8** (CD_3OH, 700 MHz, 273 K)



^{13}C NMR spectrum of compound **8** (CD_3OH, 175 MHz, 273 K)



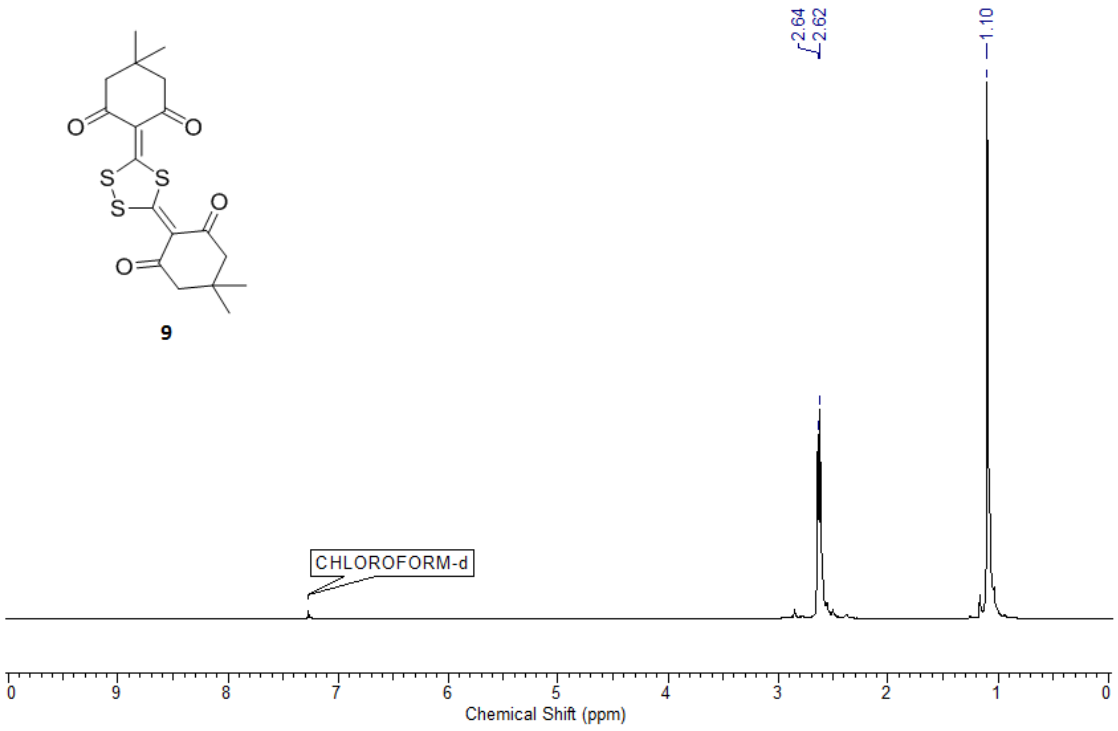
DEPT-135 NMR spectrum of compound **8** (CD_3OH, 175 MHz, 273 K)



HRMS of Compound **8**

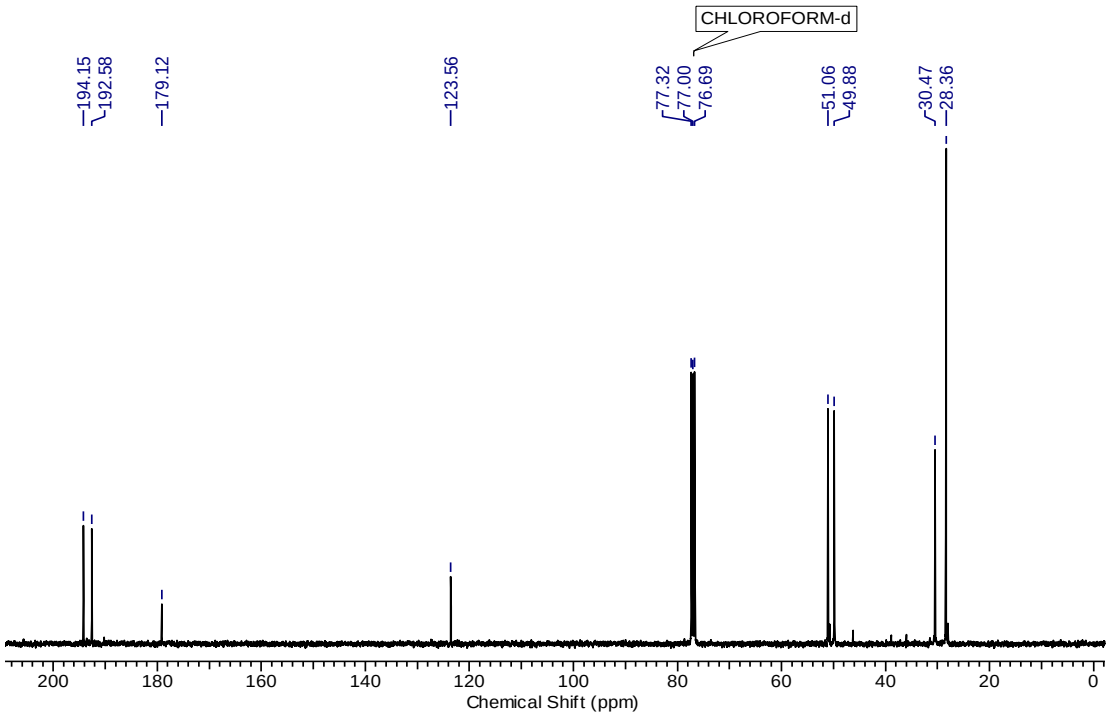
Compound 9 2,2'-(1,2,4-trithiolane-3,5-diylidene)bis(5,5-dimethylcyclohexane-1,3-dione): To a nitrogen flushed 250 mL two-neck round bottom flask, equipped with magnetic stir bar, dimedone (1.0 g, 7.1 mmol, 1 equiv) was added. Then, anhydrous THF (40 mL) was added *via* syringe followed by Et_3N (1.8 g, 17.8 mmol, 2.5 equiv) and CS_2 (0.81 g, 10.7 mmol, 1.5 equiv). After stirring for 1 h, iodopentafluorobenzene (3.1 g, 10.7 mmol, 1.5 equiv) dissolved in THF (10 ml) was added *via* syringe and the reaction mixture was stirred for 12 h at room temperature. After removal of solvent under reduced pressure, the residue was taken into dichloromethane and the organic layer was washed with aq. solution of sat. $KHSO_4$ and brine. Organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was purified by silica gel column chromatography (dichloromethane) to afford **9** as pale-green solid. (1.3 g, 51%). ^1H NMR (400 MHz, $CDCl_3$) δ 2.64-2.62 (d, J = 7.3 Hz, 4H), 1.10 (s, 6H), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 194.1, 192.5, 123.5,

51.0, 49.8, 30.4, 28.3. HRMS: calculated for $C_{18}H_{20}O_4S_3$ $[M+H]^+$ 397.0596, observed: 397.0593. $[M+H]^+$

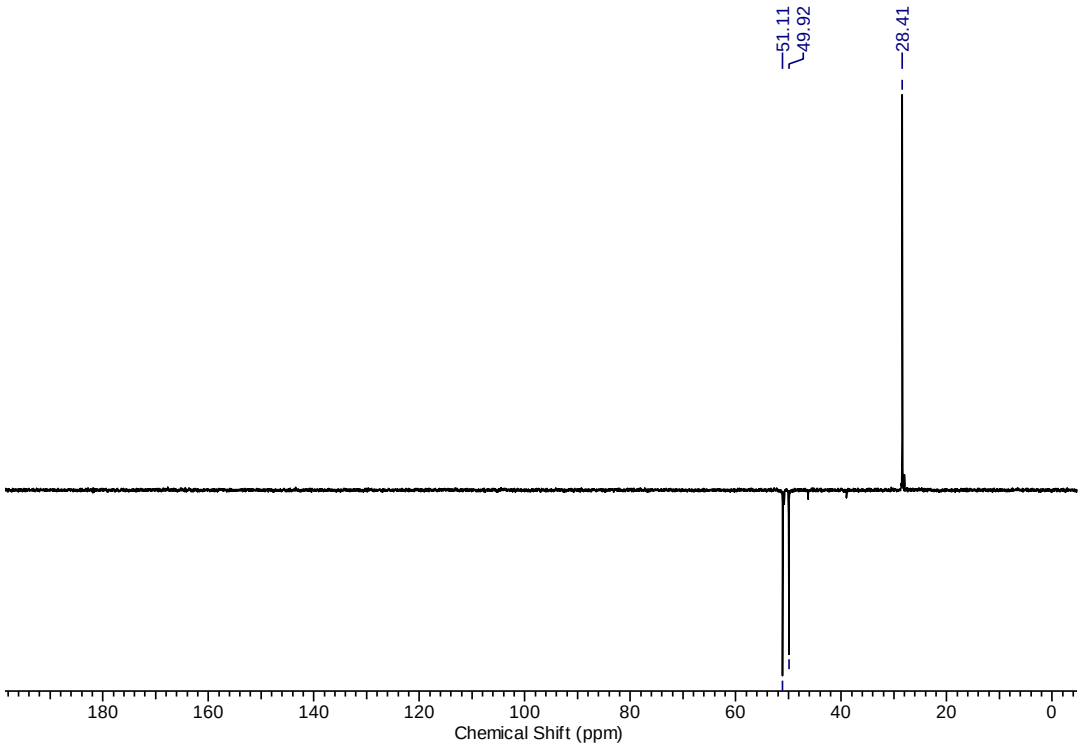


9

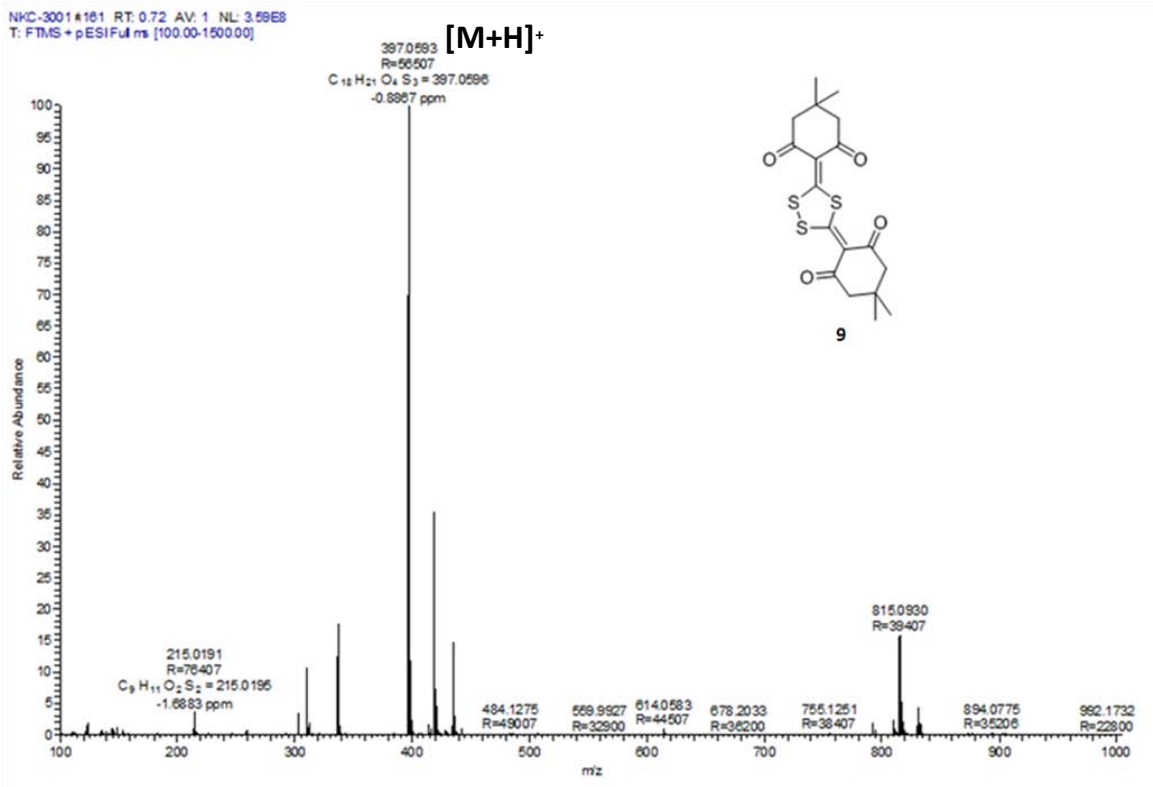
^{1}H NMR spectrum of compound **9** ($CDCl_3$, 400 MHz, 298 K)



^{13}C NMR spectrum of compound **9** ($CDCl_3$, 400 MHz, 298 K)

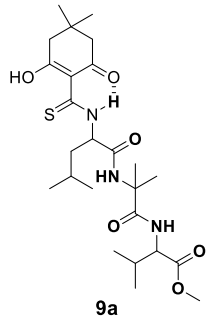


DEPT-135 spectrum of compound **9** ($CDCl_3$, 100 MHz, 298K)



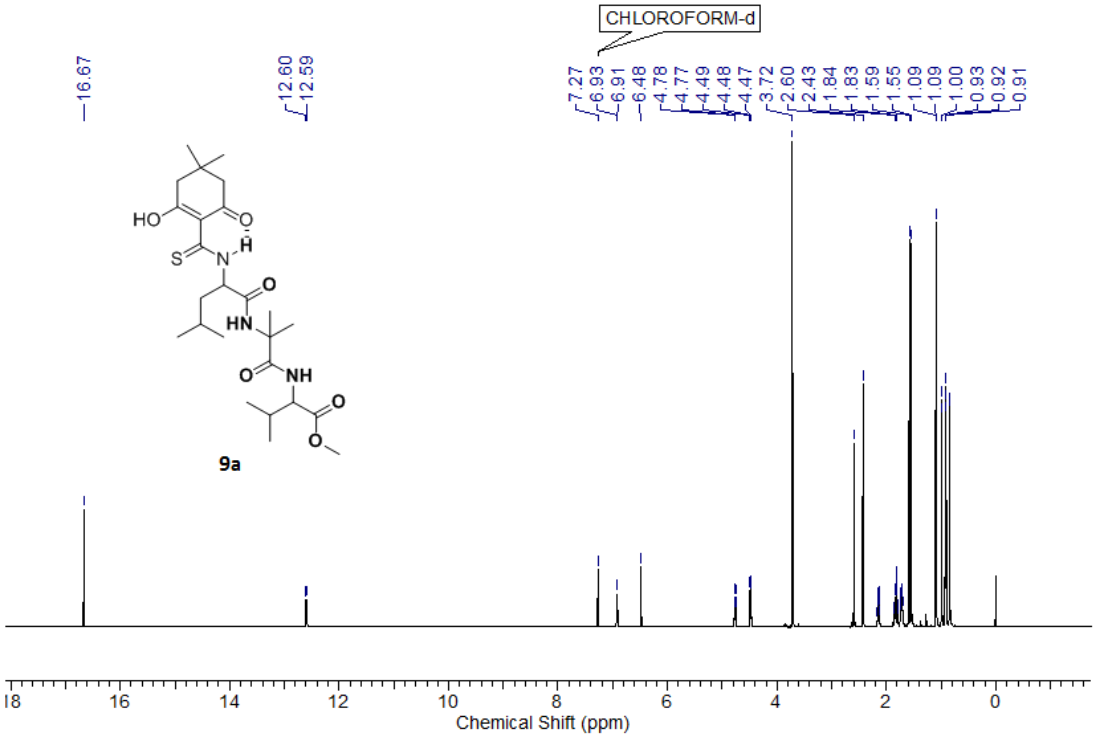
HRMS of Compound **9**

Compound 9a (Methyl (2-(2-(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-ene-1-carbothioamido)-4-methylpentanamido)-2-methylpropanoyl)valinate):

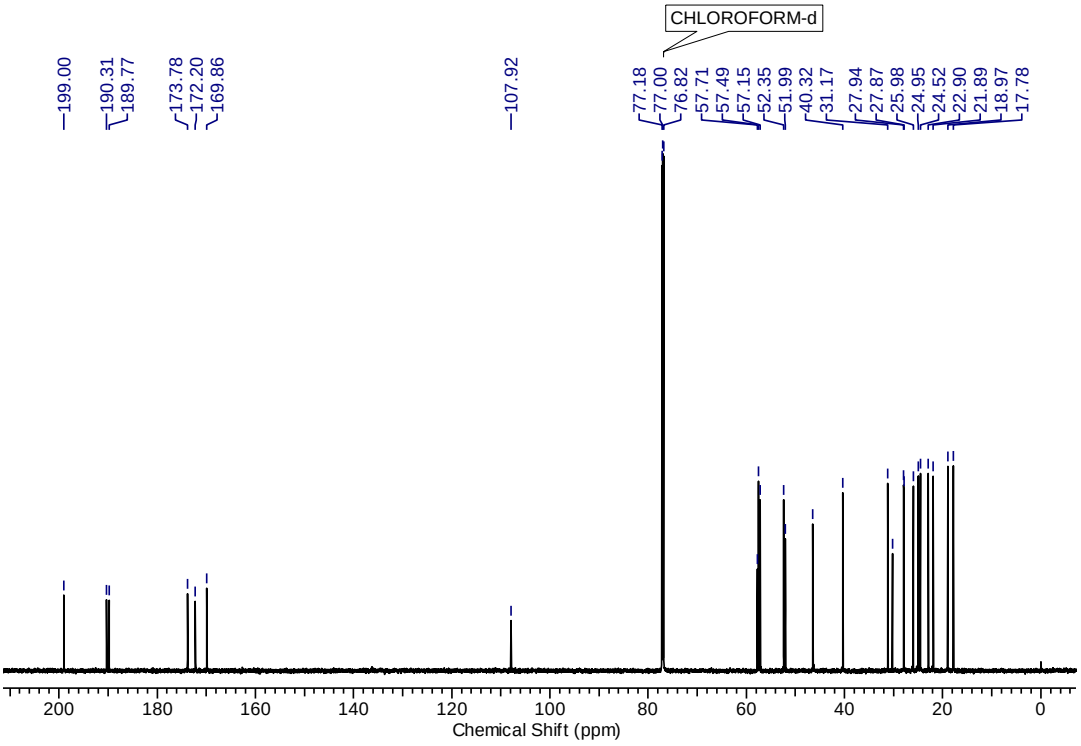


9a

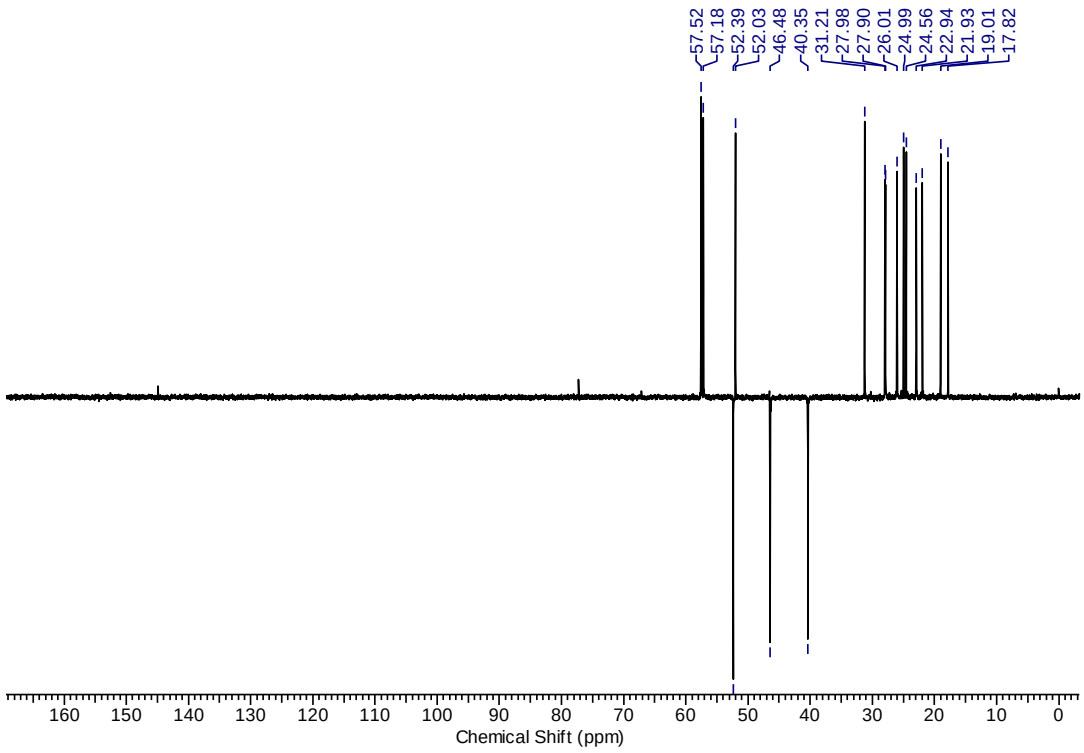
Boc-protected tripeptide methyl ester (Boc-L-Leu-Aib-L-Val-OMe, 0.25 g, 0.585 mmol, 1.5 equiv) was subjected to Boc-deprotection using 2 mL of TFA: CH_2Cl_2 (1:1) at 0 °C. After complete conversion of starting material (1 h), TFA was evaporated under reduced pressure to obtain TFA salt of H-^{L}Leu-Aib-^{L}Val-OMe and was used directly without further purification. To a solution of H-^{L}Leu-Aib-^{L}Val-OMe in DMF (5 ml), DIPEA (0.1g, 0.78 mmol, 2 equiv) was added and was stirred for 0.5 h at room temperature followed by addition of compound **9** (0.15 g, 1 equiv). After completion of the reaction (6 h), the reaction mixture was taken in AcOEt (30 mL) and the organic layer was washed sequentially with aq. solutions of sat. $KHSO_4$ and brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was purified by silica gel column chromatography (ethyl acetate/petroleum ether: 2:5) to afford **9a** as off-white solid (0.19 g, 96%). $[\alpha]^{26}_{D}$: -19.7° (c = 0.1, MeOH); ^{1}H NMR (700MHz, $CDCl_3$) δ: 16.67 (s, 1H), 12.60-12.59 (d, *J* = 6.4 Hz, 1H), 6.93-6.91 (d, *J* = 8.5 Hz, 1H), 6.48 (s, 1H), 4.78-4.75 (m, 1H), 4.49-4.47 (q, *J* = 4.8 Hz, 1H), 3.72 (s. 3H), 2.60 (s, 2H), 2.43 (s, 2H), 2.17-2.12 (m, 1H), 1.87-1.79 (m, 2H), 1.76-1.71 (m, 2H), 1.59 (s, 3H), 1.55 (s, 3H), 1.09-1.09 (d, *J* = 3.73 Hz, 6H), 1.00-0.99 (d, *J* = 6.4 Hz, 3H), 0.93-0.91 (m, 6H), 0.85-0.84 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (175MHz, $CDCl_3$) δ: 199.0, 190.3, 189.7, 173.7, 172.2, 169.8, 170.9, 57.7, 57.4, 57.1, 52.3, 51.9, 46.4, 40.3, 31.1, 30.3, 27.9, 27.8, 25.9, 24.9, 24.5, 22.9, 21.8, 18.9, 17.7. HRMS: calculated for $C_{25}H_{42}N_3O_6S$ $[M+H]^+$: 512.2787, observed : 512.2789 $[M+H]^+$.



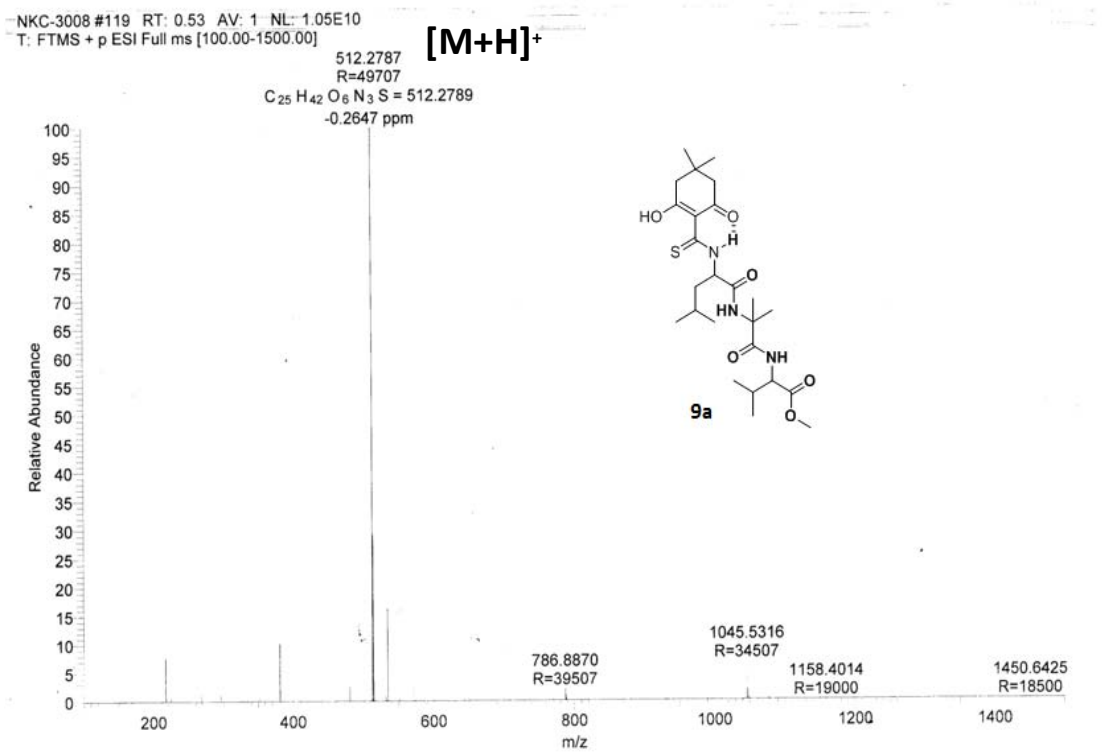
^{1}H NMR spectrum of compound **9**a ($CDCl_3$, 400 MHz, 298 K)



^{13}C NMR spectrum of compound **9a** ($CDCl_3$, 400 MHz, 298 K)

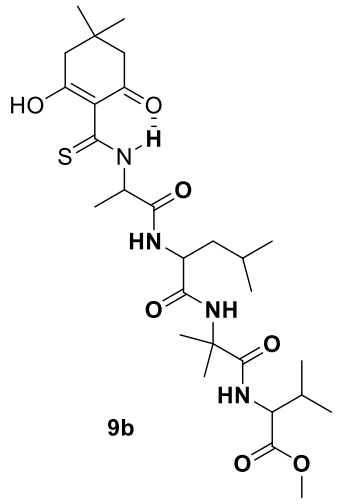


DEPT-135 spectrum of compound **9a** ($CDCl_3$, 100 MHz, 298K)

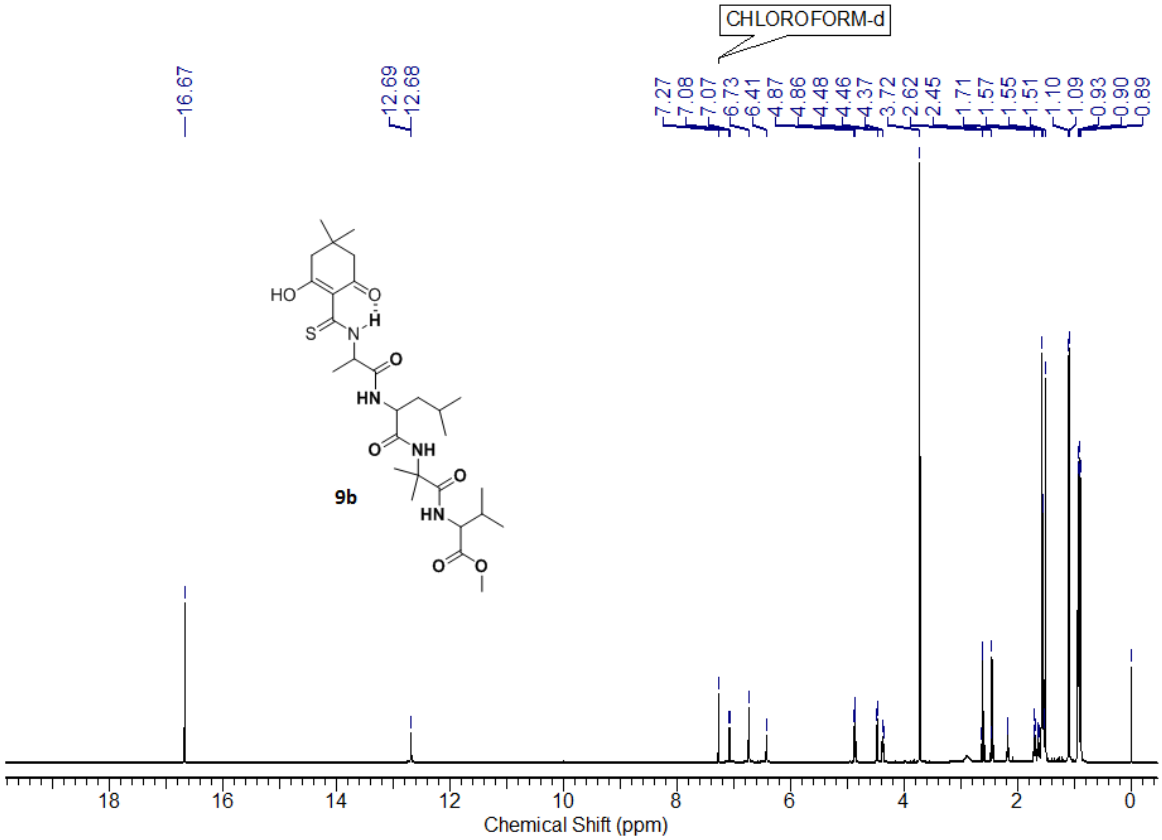


HRMS of Compound **9a**

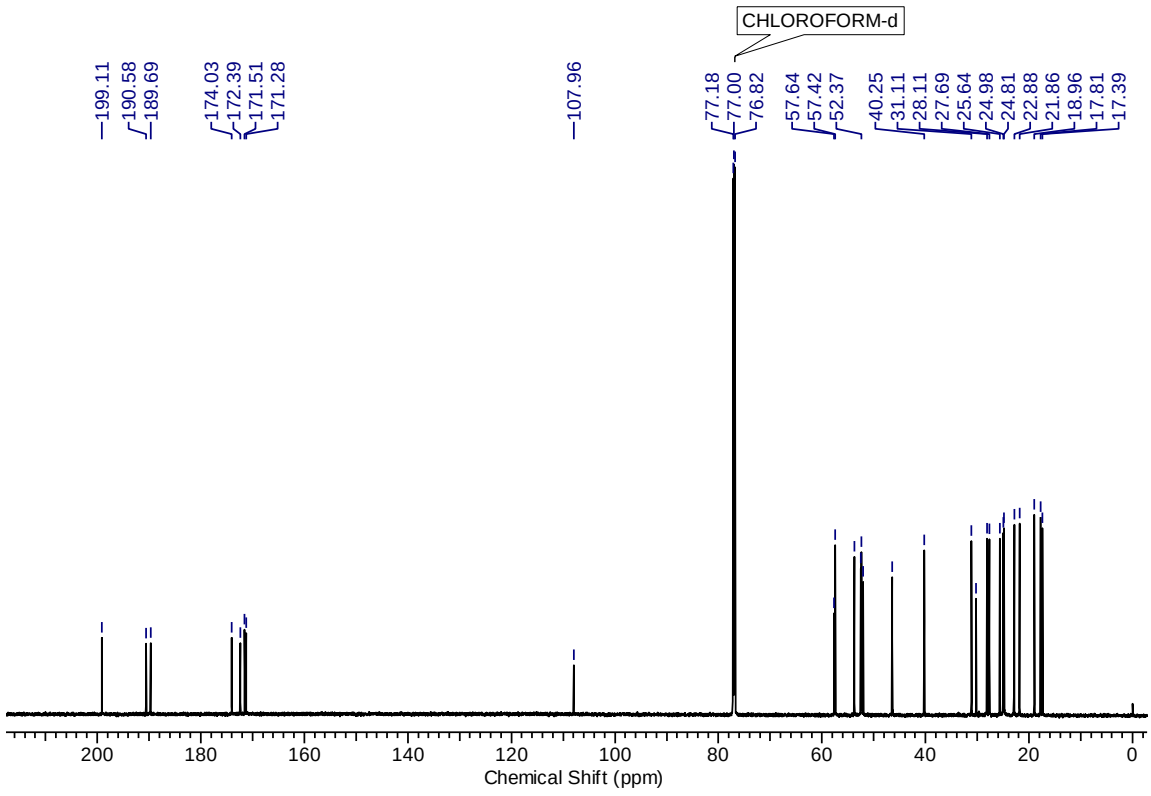
Compound 9b (Methyl (2-(2-(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-ene-1-carbothioamido)propanamido)-4-methylpentanamido)-2-methylpropanoyl)valinate): Compound **9b** was obtained using Boc-L-Ala-L-Leu-Aib-L-Val-OMe (0.292 g, 0.585 mmol, 1.5 equiv) following the procedure for synthesizing **9a**, as described earlier. **9b** was obtained as an off-white solid (0.22 g, 98%) after purification by silica gel column chromatography (ethyl acetate/petroleum ether 1:1); $[\alpha]^{26}_{D}$: -31.7° (c = 0.15, MeOH); ^{1}H NMR (700MHz, $CDCl_3$) δ: 16.67 (s, 1H), 12.69-12.68 (d, *J* = 6.4 Hz, 1H), 7.08-7.07 (d, *J* = 8.5 Hz, 1H), 6.73 (s, 1H), 6.43-6.41 (d, *J* = 7.4 Hz, 1H), 4.88-4.84 (q, *J* = 6.9 Hz, 1H), 4.48-4.46 (m, 1H), 4.38-4.35 (m, 1H), 3.72 (s, 3H), 2.62-2.61 (d, *J* = 6.9 Hz, 2H), 2.45-2.44 (d, *J* = 6.4 Hz, 2H), 2.20-2.15 (m, 1H), 1.73-1.69 (m, 1H), 1.66-1.60 (m, 1H), 1.57 (s, 3H), 1.56-1.55 (d, *J* = 6.94 Hz, 4H), 1.51 (s, 3H), 1.10-1.09 (d, *J* = 6.4 Hz, 6H), 0.94-0.92 (dd, *J* = 6.9 Hz, *J* = 2.6 Hz, 6H), 0.9-0.89 (dd, *J* = 6.9 Hz, *J* = 2.6 Hz, 6H). ^{13}C NMR (175MHz, $CDCl_3$) δ: 199.1, 190.5, 189.6, 174.0, 172.3, 171.5, 171.2, 170.9, 57.6, 57.4, 53.7, 52.0, 40.2, 31.1, 28.1, 27.6, 25.6, 24.9, 24.8, 22.8, 21.8,18.9, 17.8, 17.3. HRMS: calculated for $C_{28}H_{47}N_4O_7S$ $[M+H]^+$: 583.3162, observed : 583.3160 $[M+H]^+$.



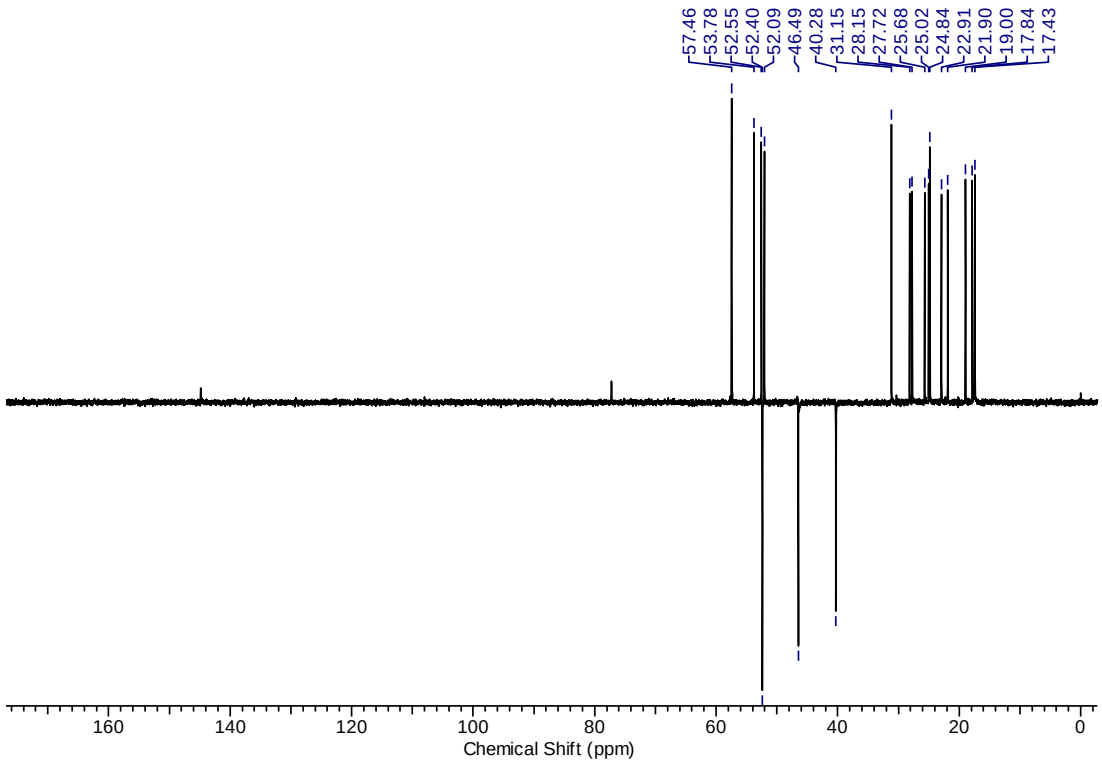
9b



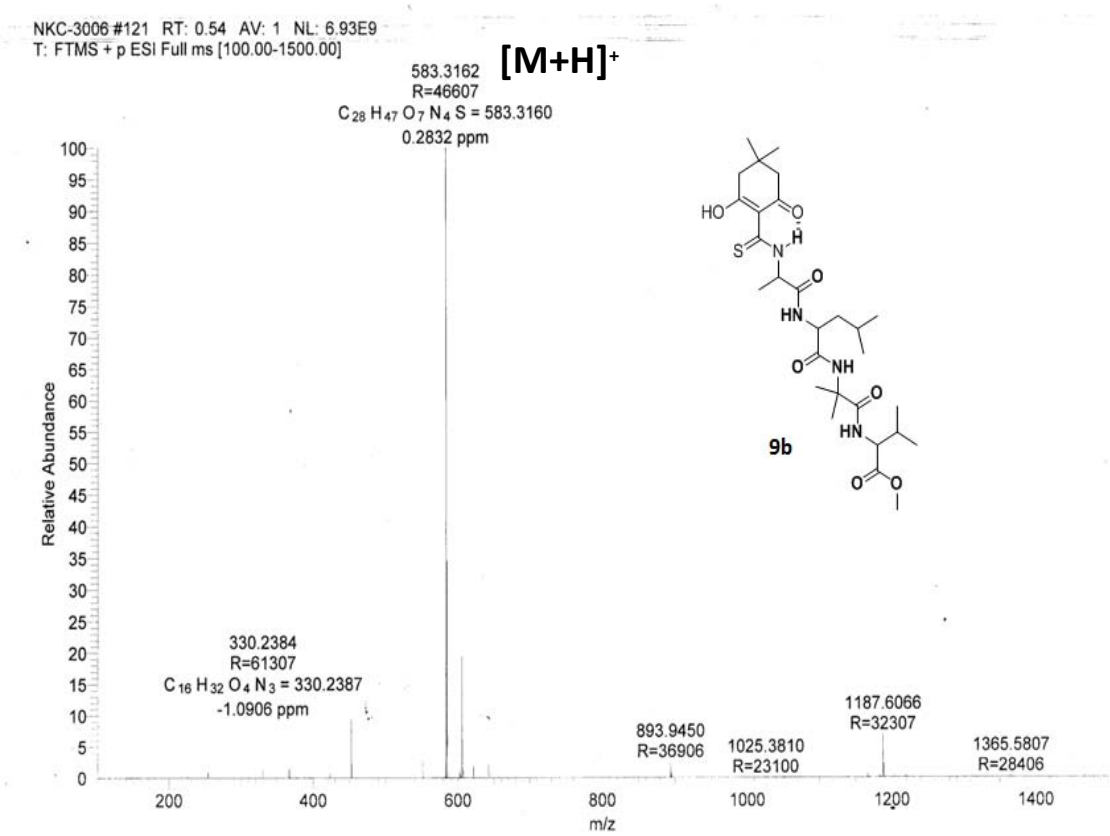
^{1}HNMR spectrum of compound **9b** ($CDCl_3$, 100 MHz, 298K)



^{13}CNMR spectrum of compound **9b** ($CDCl_3$, 100 MHz, 298K)

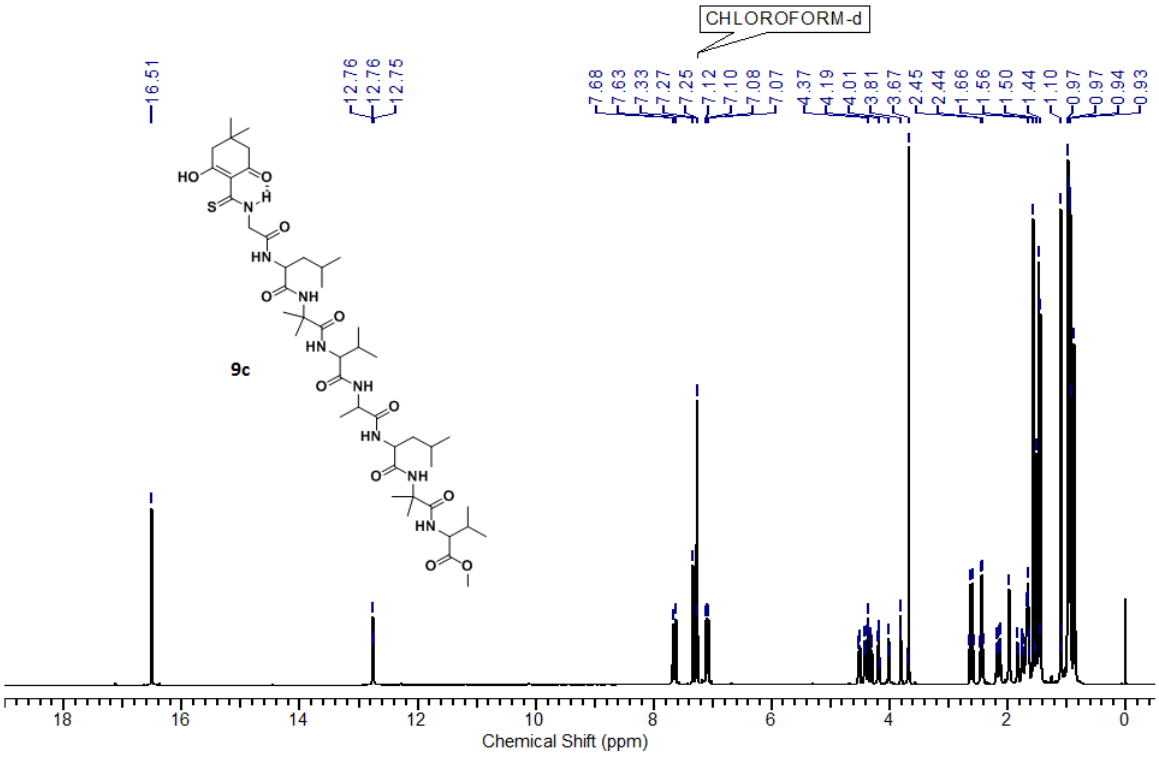


DEPT-135 spectrum of compound **9b** ($CDCl_3$, 100 MHz, 298K)

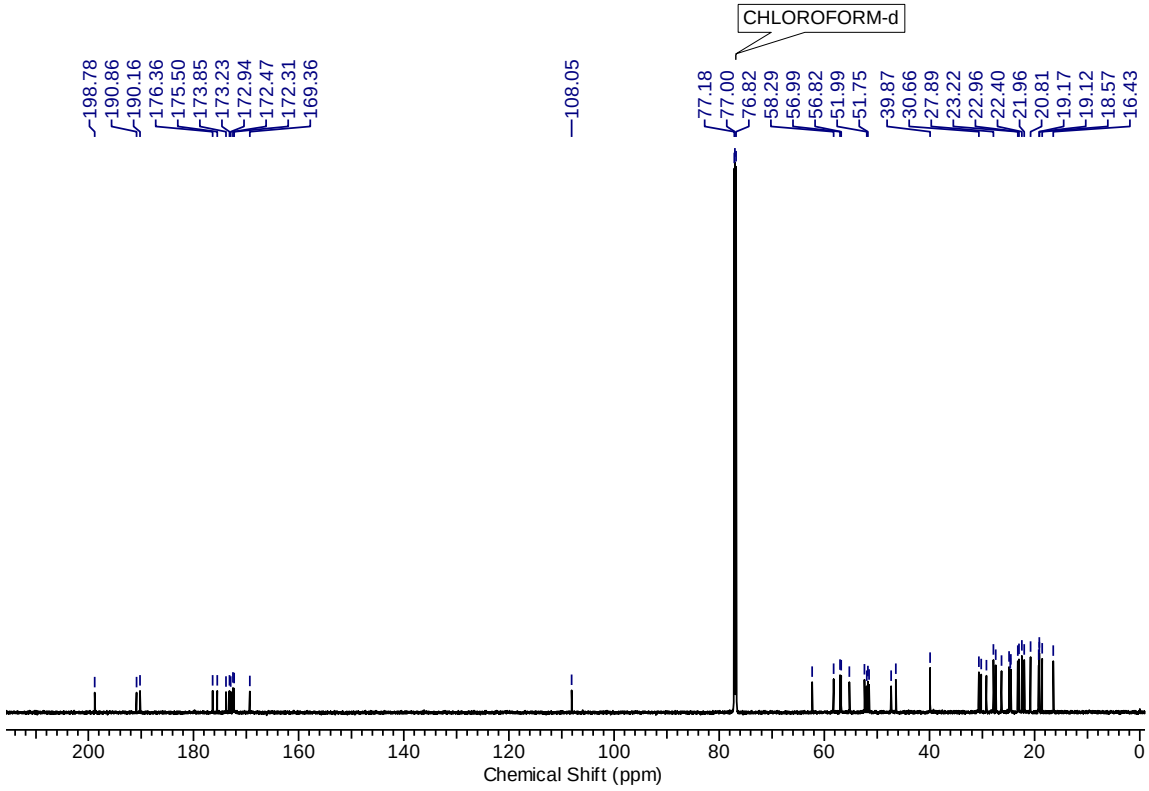


HRMS of compound **9b**

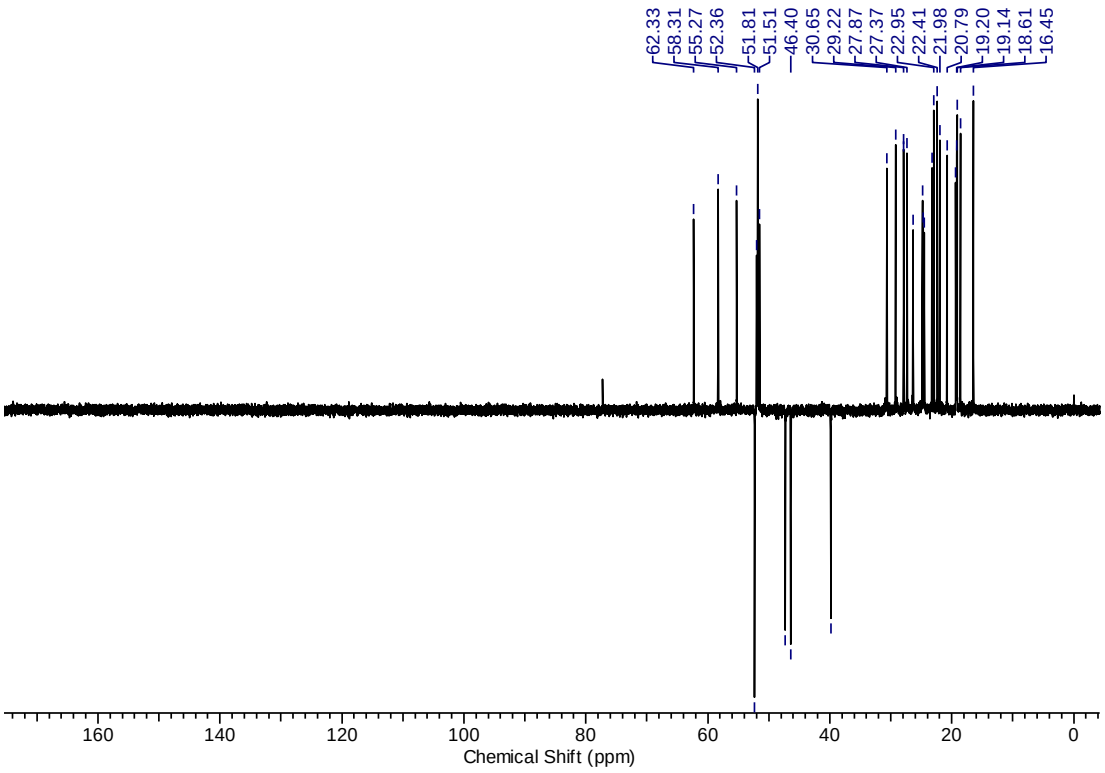
Compound 9c (Methyl (2-(2-(2-(2-(2-(2-(2-(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-ene-1-carbothioamido)acetamido)-4-methylpentanamido)2-methylpropanamido)-3-methylbutanamido)propanamido)-4-methylpentanamido)-2 methyl propanoyl) valinate) Compound **9c** was obtained using Boc-Gly-L-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-OMe (1.0 g, 1.17 mmol, 1.5 equiv) following the procedure for synthesizing **9a**, as described earlier. **9c** was obtained as an off-white solid (0.595 g, 95%) after purification by silica gel column chromatography (ethyl acetate/petroleum ether 4:5) : $[\alpha]^{26}_{D}$: -21.4° (c = 0.13, MeOH); ^{1}H NMR (700MHz, $CDCl_3$) δ: 16.51 (s, 1H), 12.76-12.75 (t, *J* = 5.3 Hz, 1H), 7.68 (s, 1H), 7.63-7.63 (d, *J* = 4.8 Hz, 1H), 7.33 (s, 1H), 7.27-7.25 (m, 2H), 7.12-7.10 (d, *J* = 8.5 Hz, 1H), 7.08-7.07 (d, *J* = 5.3 Hz, 1H), 4.53-4.50 (dd, *J* = 17 Hz, *J* = 5.3 Hz, 1H), 4.44-4.41 (m, 1H), 4.38-4.36 (t, *J* = 7.4 Hz, 1H), 4.32-4.29 (dd, *J* = 16.5 Hz, *J* = 5.3 Hz, 1H), 4.28-4.18 (m, 1H), 4.02-4.0 (m, 1H), 3.82-3.81 (t, *J* = 5.8 Hz, 1H), 3.67 (s, 3H), 2.64-2.57 (q, *J* = 14 Hz, 2H), 2.47-2.41 (q, *J* = 10 Hz, 2H), 2.20-2.10 (m, 2H), 1.97 (bs, 2H), 1.84-1.81 (m, 1H), 1.77-1.71 (m, 1H), 1.68-1.62 (m, 4H), 1.56-1.56 (d, *J* = 3Hz, 6H), 1.51-1.50 (d, *J* = 7.4 Hz, 3H), 1.47 (s, 3H), 1.44 (s, 3H), 1.10-1.10 (d, *J* = 4.8 Hz, 6H), 0.97-0.92 (m, 18H), 0.87-0.86 (m, 6H). ^{13}C NMR (175MHz, $CDCl_3$) δ: 198.7, 190.8, 190.1, 176.3, 175.5, 173.8, 173.2, 172.9, 172.4, 172.3, 169.3, 108.0, 62.3, 58.2, 56.9, 56.8, 55.2, 52.3, 47.3, 46.4, 39.8, 27.8, 27.3, 23.2, 22.9, 22.4, 21.9, 20.8, 19.1, 19.1, 18.5, 16.4. HRMS: calculated for $C_{45}H_{77}N_8O_{11}S$ $[M+H]^+$: 937.5438, observed : 937.5430 $[M+H]^+$.



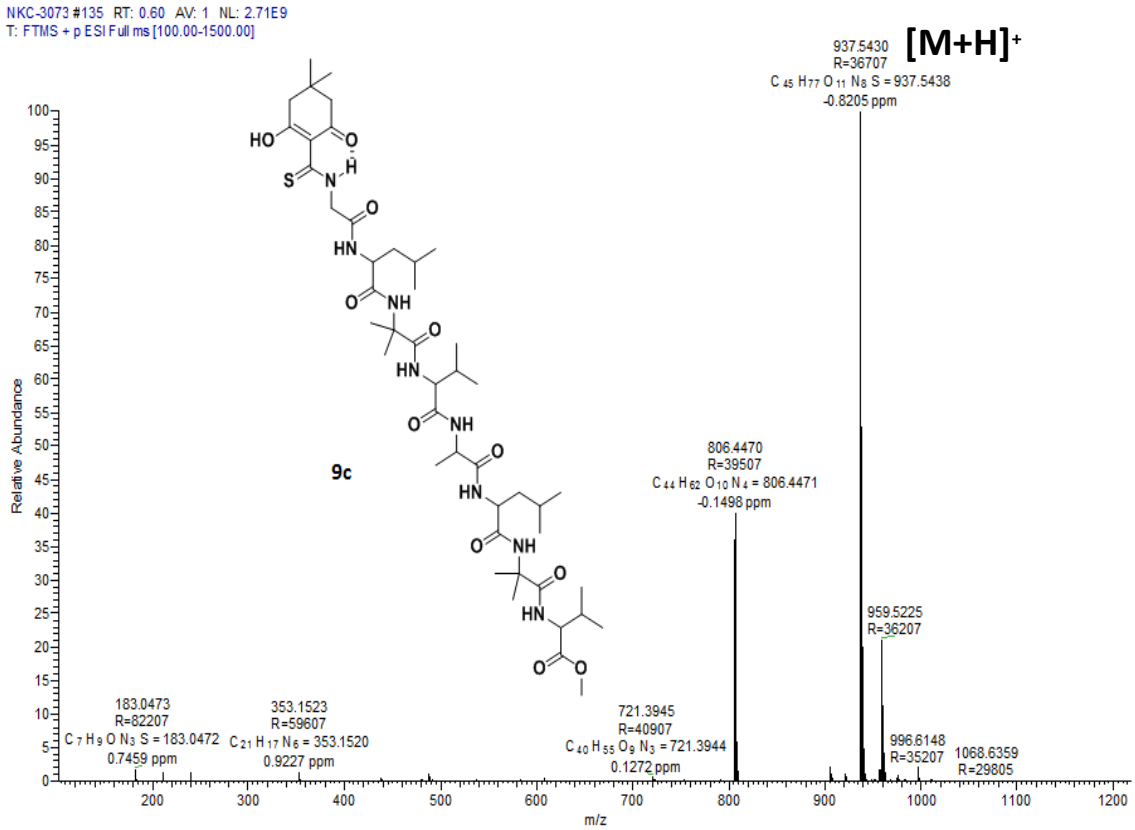
^{1}HNMR spectrum of compound **9c** ($CDCl_3$, 100 MHz, 298K)



^{13}CNMR spectrum of compound **9c** ($CDCl_3$, 100 MHz, 298K)

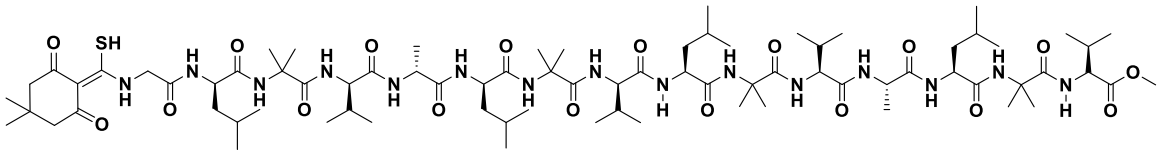


DEPT-135 spectrum of compound **9c** ($CDCl_3$, 100 MHz, 298K)

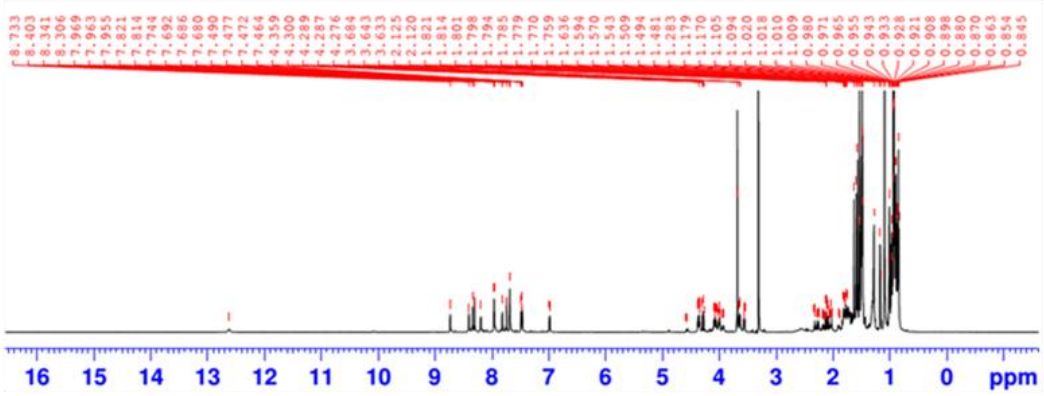


HRMS of compound **9c**

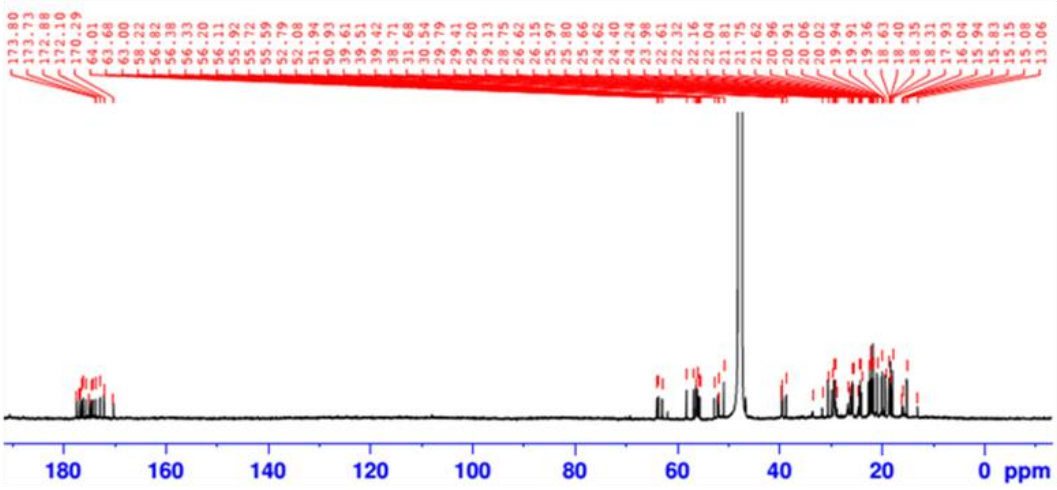
Compound 9d (Dimedone-Gly-L-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-OMe):



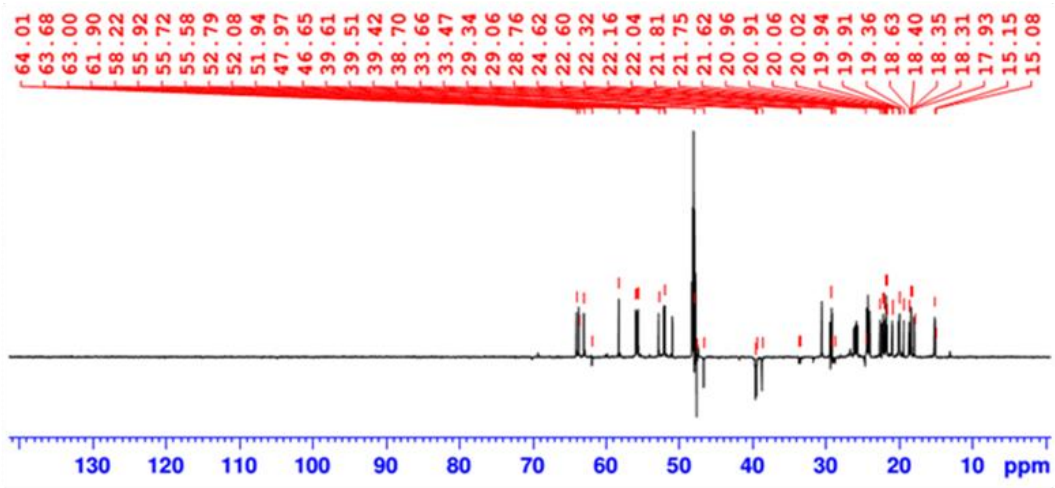
Boc-protected pentadecapeptide methyl ester **7** (0.5 g, 0.32 mmol) was subjected to Boc-deprotection using 2 mL of TFA: CH_2Cl_2 (1:1) at 0 °C. After complete conversion of starting material (1 h), TFA was evaporated under reduced pressure to obtain TFA salt of **7** and was used directly without further purification. To a solution of TFA salt of **7** (0.46 g, 0.32 mmol, 1 equiv) in DMF (3 mL), DIPEA (0.17 mL, 0.97 mmol, 3 equiv) was added and was stirred for 0.5 h at room temperature followed by addition of compound **9** (0.13 g, 0.32 mmol, 1 equiv). After completion of the reaction (6 h), the reaction mixture was taken in AcOEt (30 mL) and the organic layer was washed sequentially with aq. solutions of sat. $KHSO_4$ and brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was purified by silica gel column chromatography (3% Methanol/DCM) to afford **9d** as a white solid (0.41 g, 80%). $[\alpha]^{26}_D$: -19.7° (c = 0.1, MeOH); ^{1}H NMR (700MHz, CD_3OH, 273K) chemical shift values are given in table 00. ^{13}C NMR (175MHz, CD3OH) δ = ^{13}C NMR (176MHz ,METHANOL-d_4) δ = 178.9, 178.3, 178.3, 177.9, 177.6, 177.1, 176.5, 176.4, 176.0, 175.8, 175.2, 175.1, 174.3, 173.5, 171.7, 109.1, 65.4, 65.1, 64.4, 59.6, 58.2, 57.8, 57.8, 57.6, 57.5, 57.3, 57.1, 57.0, 54.2, 53.5, 53.4, 52.3, 48.1, 40.9, 40.1, 32.0, 31.2, 30.8, 30.6, 30.5, 28.1, 27.2, 25.9, 25.6, 25.3, 24.0, 23.7, 23.2, 22.4, 21.5, 21.4, 20.8, 20.1, 19.8, 19.4, 16.5; HRMS: calculated for $C_{78}H_{135}N_{15}O_{18}SNa$ $[M+Na]^+$: 1624.9722, observed : 1624.9696 $[M+Na]^+$.



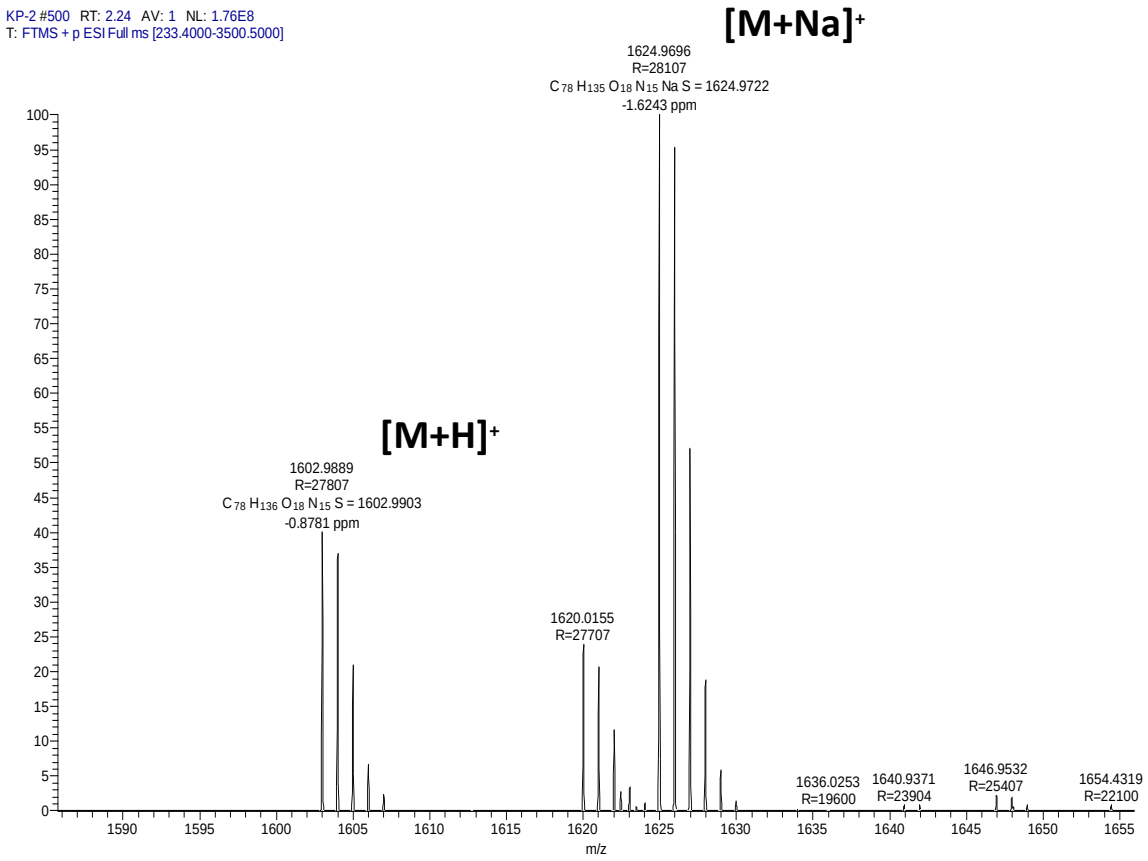
^{1}H NMR spectrum of compound **9d (**CD_3OH, 700 MHz, 273 K)



^{13}C NMR spectrum of compound **9d** (CD_3OH, 700 MHz, 273 K)

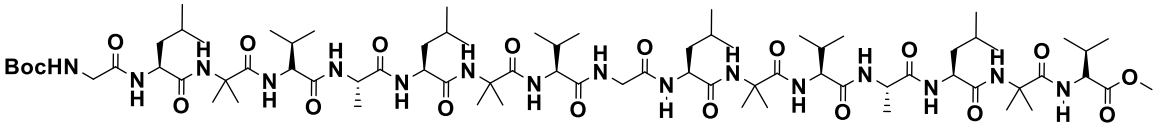


DEPT-135 NMR spectrum of compound **9d** (CD_3OH, 175 MHz, 273 K)



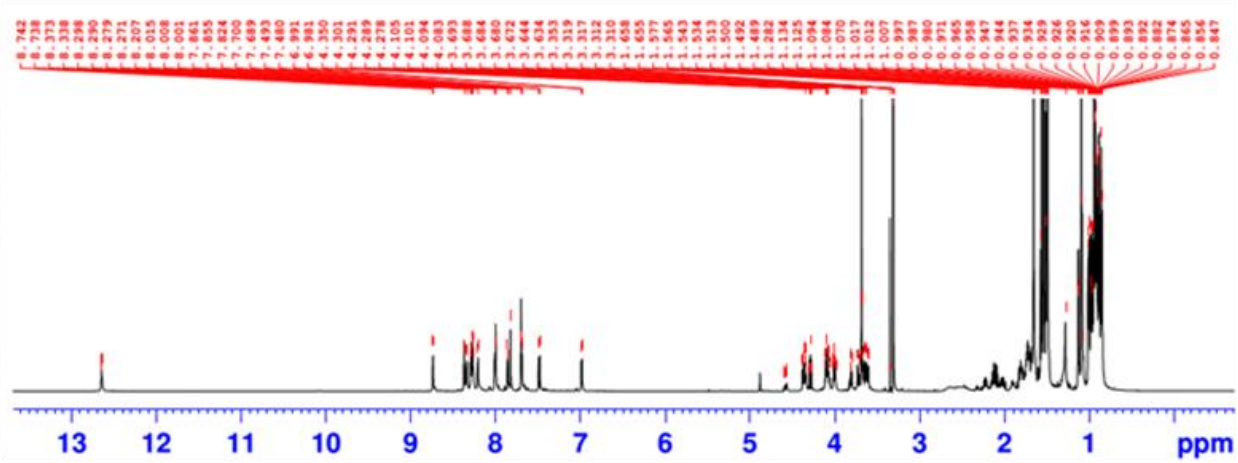
HRMS of compound **9d**

Compound 9e (Dimedone-Gly-L-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-Gly-Leu-Aib-L-Val-L-Ala-L-Leu-Aib-L-Val-OMe):

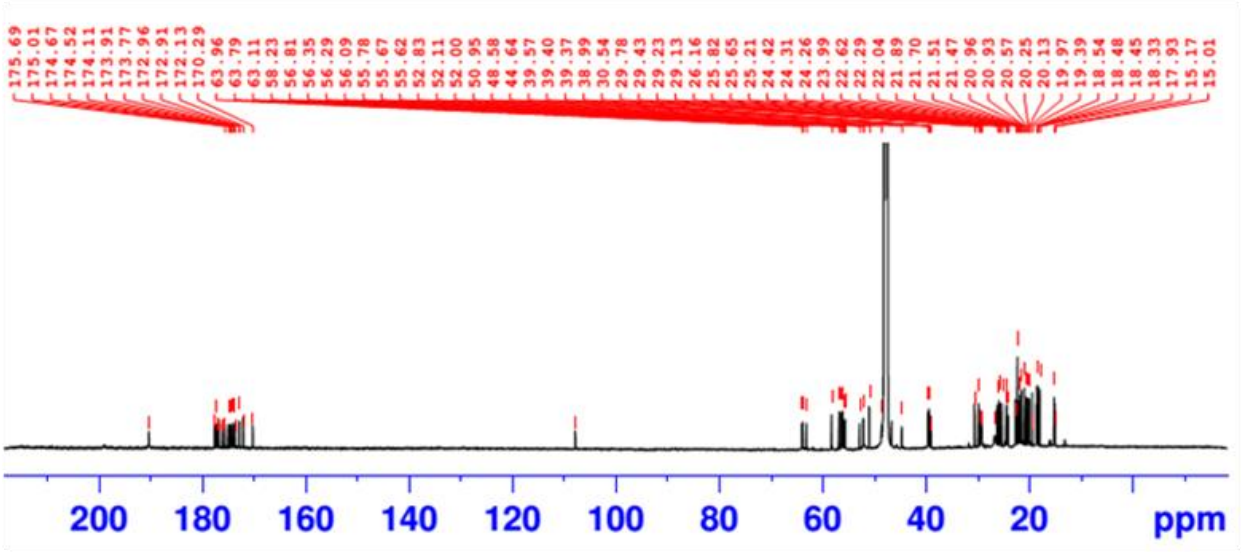


Boc-protected hexadecapeptide methyl ester **8** (0.5 g, 0.318 mmol) was subjected to Boc-deprotection using 2 mL of TFA: CH_2Cl_2 (1:1) at 0 °C. After complete conversion of starting material (1 h), TFA was evaporated under reduced pressure to obtain TFA salt of **8** and was used directly without further purification. To a solution of TFA salt of **2** (0.45 g, 0.30 mmol, 1 equiv) in DMF (3 mL), DIPEA (0.16 mL, 0.91 mmol, 3 equiv) was added and was stirred for 0.5 h at room temperature followed by addition of compound **9** (0.14 g, 0.30 mmol, 1 equiv). After completion of the reaction (6 h), the reaction mixture was taken in AcOEt (30 mL) and the organic layer was

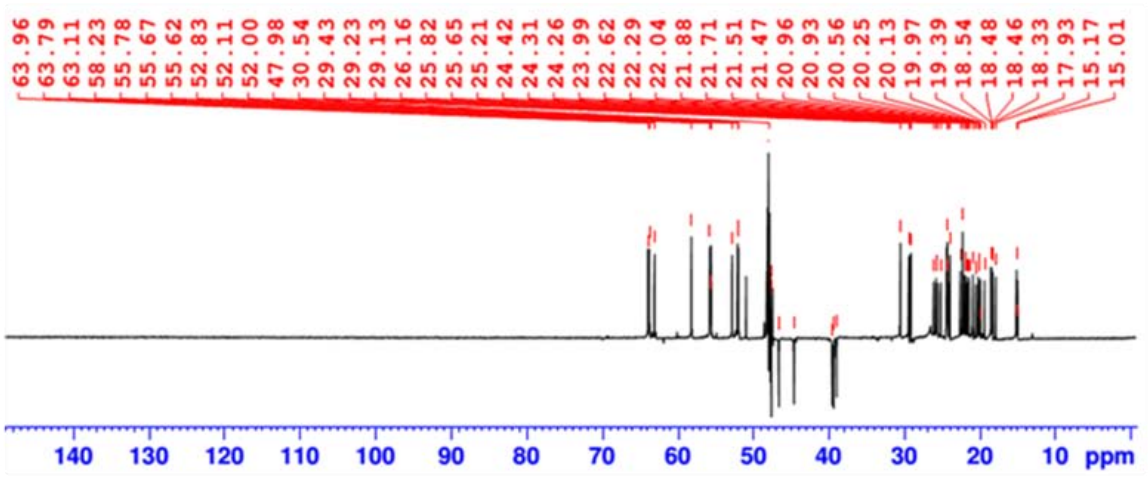
washed sequentially with aq. solutions of sat. $KHSO_4$ and brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was purified by silica gel column chromatography (4% Methanol/DCM) to afford **9e** as off-white solid (0.42 g, 85%). $[\alpha]^{26}_D$: -20.7° (c = 0.1, MeOH) ^{1}H NMR (700MHz, CD_3OH, 273K) chemical shift values are given table 00. ^{13}C NMR (175MHz, CD3OH) δ = 179.1, 178.9, 178.6, 178.3, 177.6, 177.1, 176.4, 176.1, 175.9, 175.5, 175.3, 175.2, 174.4, 174.3, 173.5, 171.7, 109.3, 65.4, 65.2, 64.5, 59.6, 58.2, 57.8, 57.7, 57.6, 57.5, 57.2, 57.1, 57.0, 54.2, 53.5, 52.4, 48.1, 46.1, 41.0, 40.8, 40.4, 32.0, 31.2, 30.9, 30.6, 30.6, 27.6, 27.2, 27.1, 26.6, 25.8, 25.4, 24.0, 23.7, 23.5, 23.3, 23.1, 22.9, 22.4, 22.0, 21.7, 21.5, 21.4, 20.8, 20.0, 19.9, 19.7, 19.4, 16.6, 16.4; HRMS: calculated for $C_{80}H_{139}N_{16}O_{19}S$ $[M+H]^+$: 1660.0118, observed : 1660.0106 $[M+H]^+$.



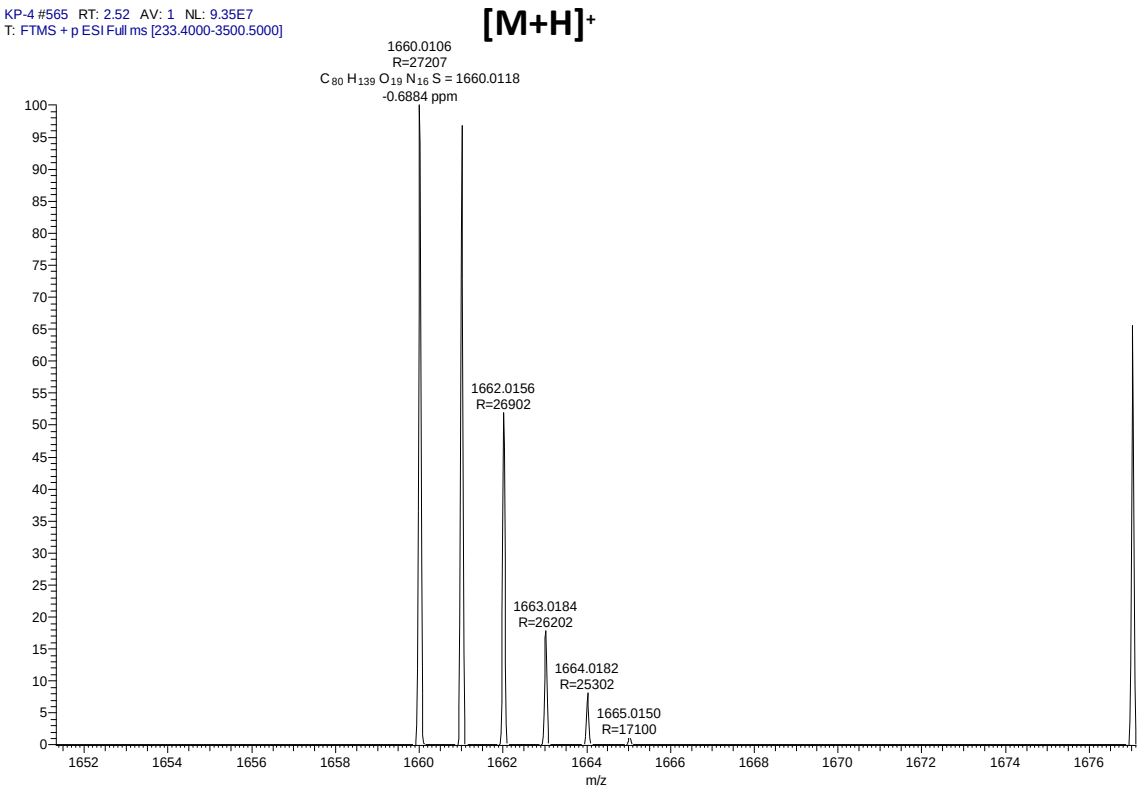
^{1}H NMR spectrum of compound **9e** (CD_3OH, 700 MHz, 273 K)



^{13}C NMR spectrum of compound **9e** (CD_3OH, 700 MHz, 273 K)

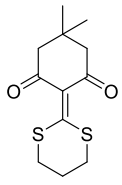


DEPT-135 NMR spectrum of compound **9e** (CD_3OH, 175 MHz, 273 K)



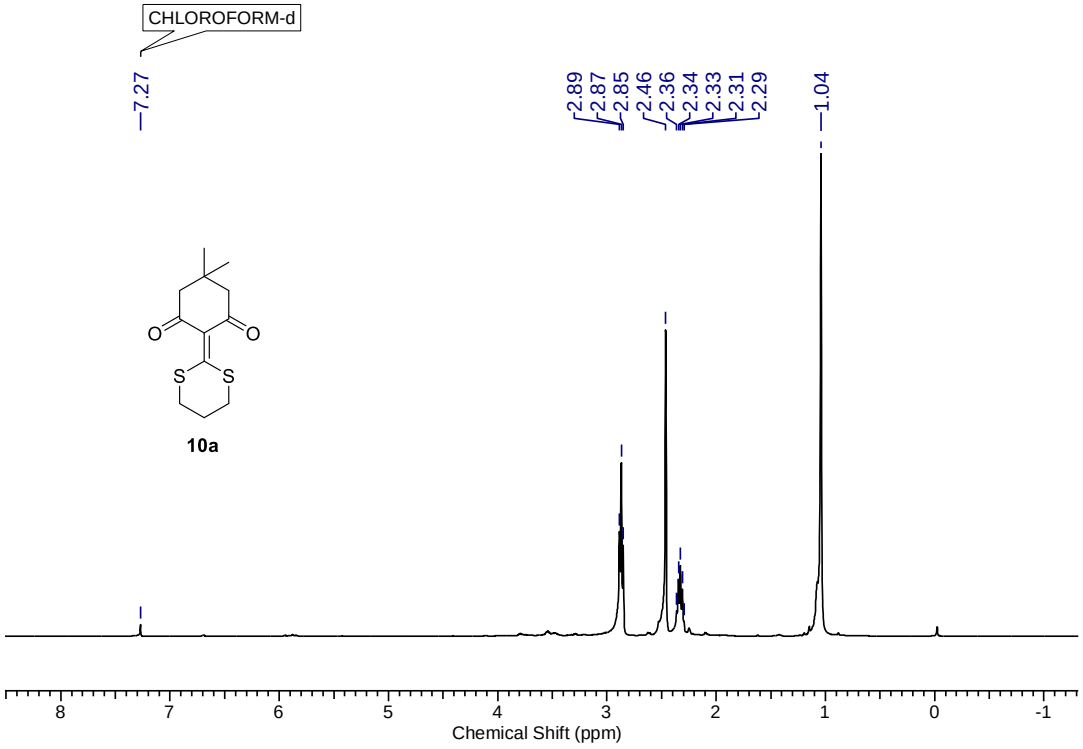
HRMS of Compound **9e**

Compound 10a (2-(1,3-dithian-2-ylidene)-5,5-dimethylcyclohexane-1,3-dione):

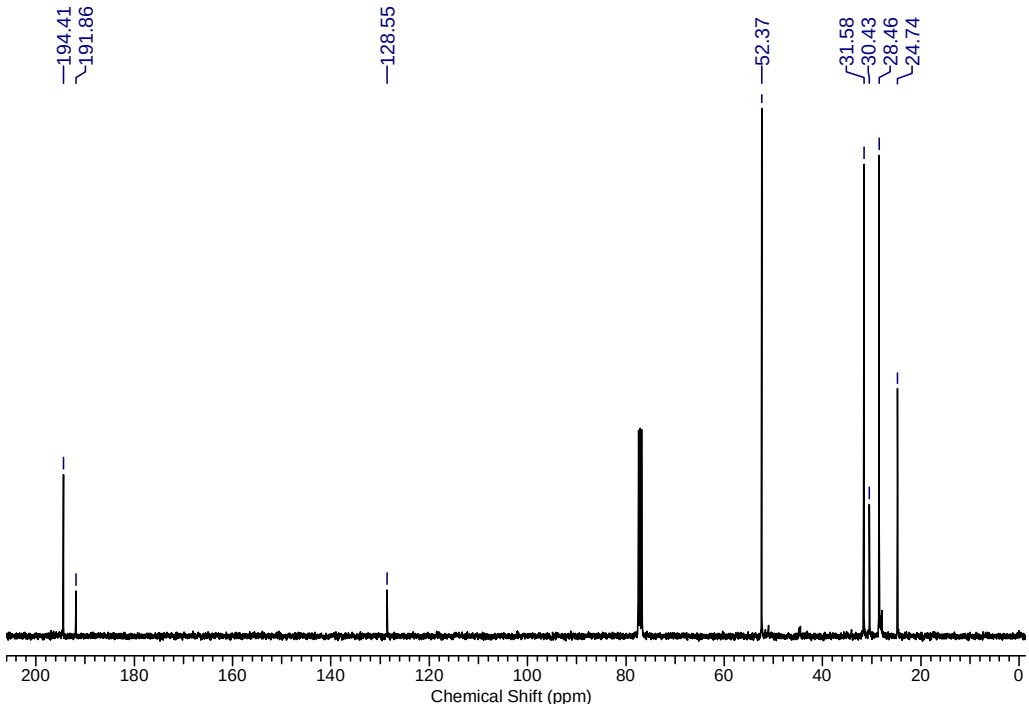


10a

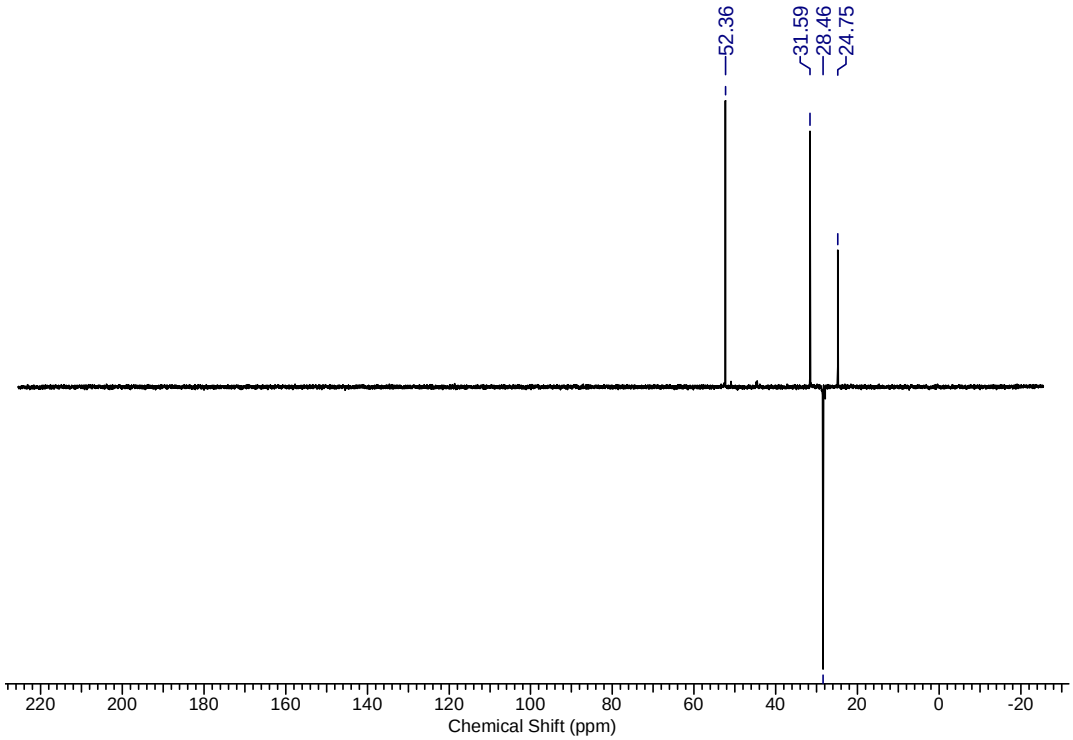
This was prepared according to the general method of Gompper *et al*[1] but with a slightly modified procedure where Et_3N was used as a base in place of NaH. To a solution of compound **10** (2 g, 14.2 mmol, 1 equiv) in DMSO (5 mL), Et_3N (4.3 mL, 42.6 mmol, 3 equiv) was added followed by carbon disulfide (1.3 mL, 21.4 mmol, 1.5 equiv) and kept at room temperature for 1 h. Finally, 1,3-dibromopropane (2.17 mL, 21.4 mmol, 1.5 equiv) was added and kept at room temperature for 12 h. The resulting mixture was extracted with ethyl acetate and organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude products which was purified by column chromatography (eluent: 2% AcOEt/ Pet ether, R_f : 0.5) to furnish compound **10a** (2.1 g, 58 %) as a yellow solid. ^{1}H NMR (400MHz, $CDCl_3$ δ = 2.87 (t, *J* = 7.0 Hz, 4 H), 2.55 - 2.41 (m, 4 H), 2.40 - 2.24 (m, 2 H), 1.13 - 0.98 (m, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 194.4, 191.9, 128.6, 52.4, 31.6, 30.4, 28.5, 24.7; HRMS: calculated for $C_{12}H_{17}O_2S_2$ $[M+H]^+$: 257.0664, observed : $[M+H]^+$: 257.1285.



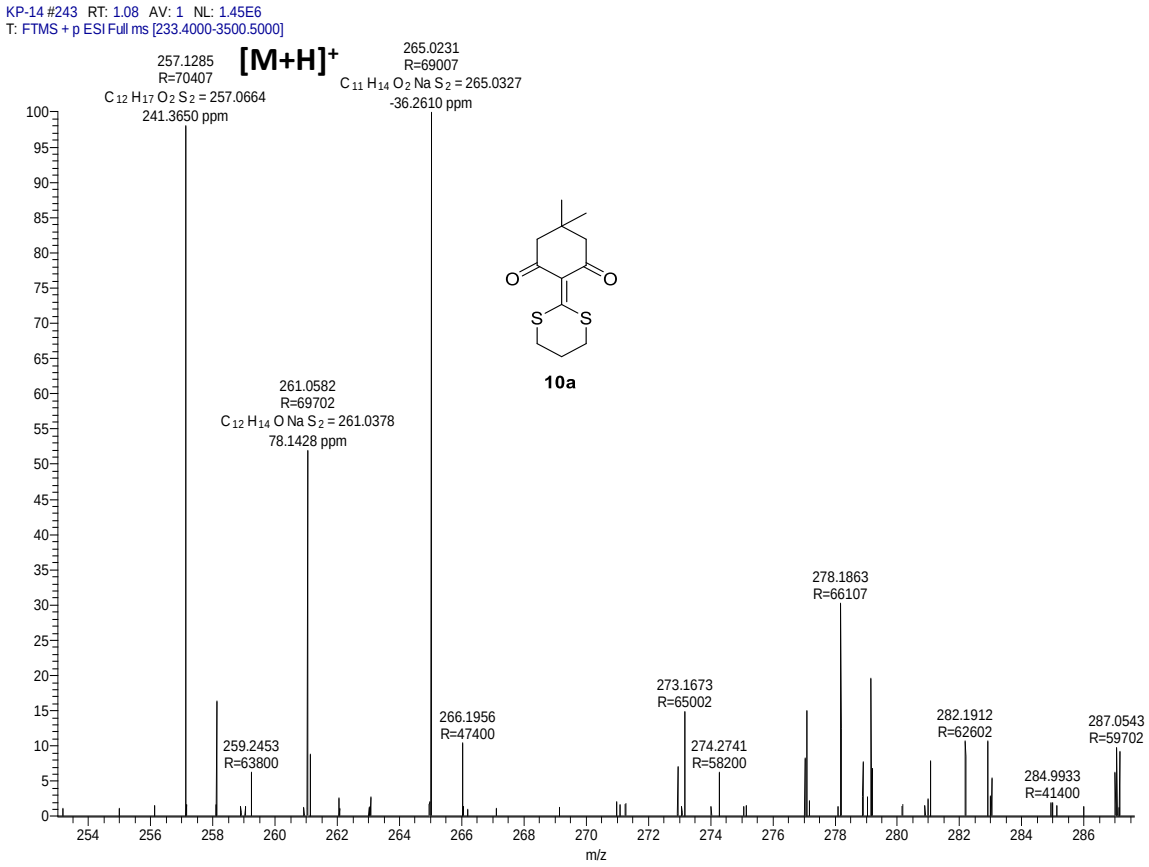
^{1}H NMR spectrum of compound **10a** ($CDCl_3$, 400 MHz, 298 K)



^{13}C NMR spectrum of compound **10a** ($CDCl_3$, 100 MHz, 298 K)

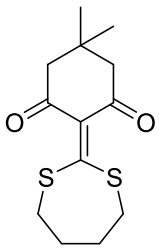


DEPT-135 spectrum of compound **10a** ($CDCl_3$, 100 MHz, 298 K)



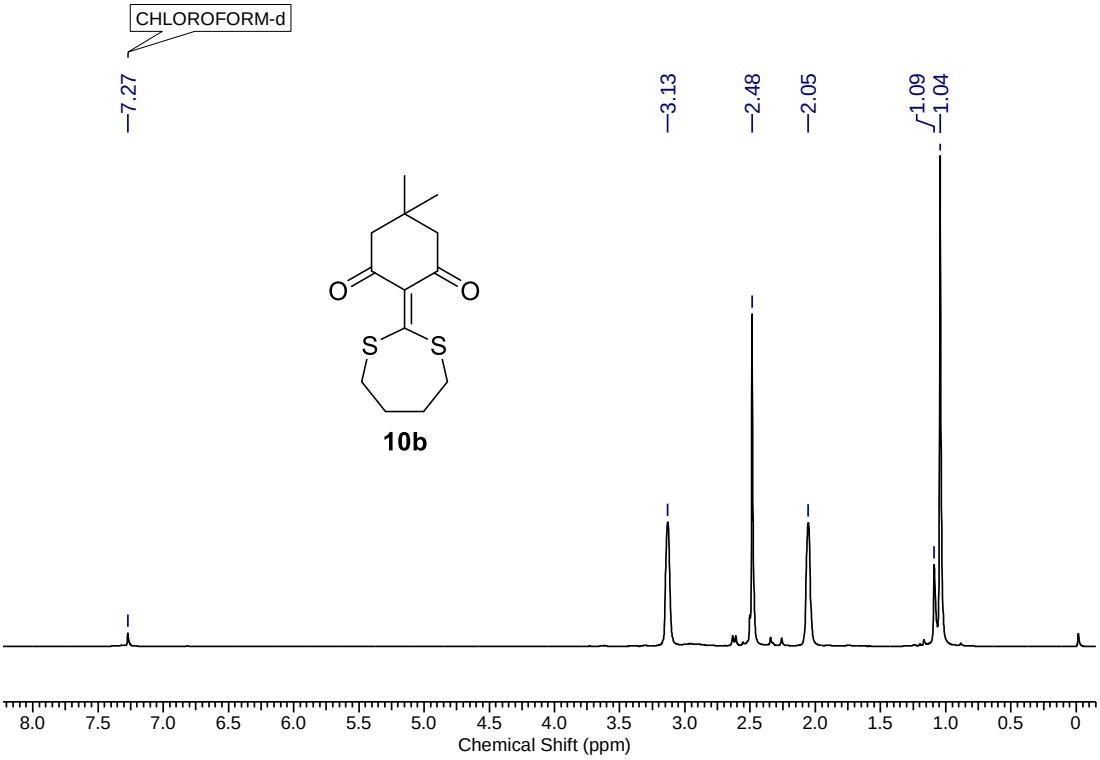
HRMS of Compound **10a**

Compound 10b (2-(1,3-dithiepan-2-ylidene)-5,5-dimethylcyclohexane-1,3-dione):

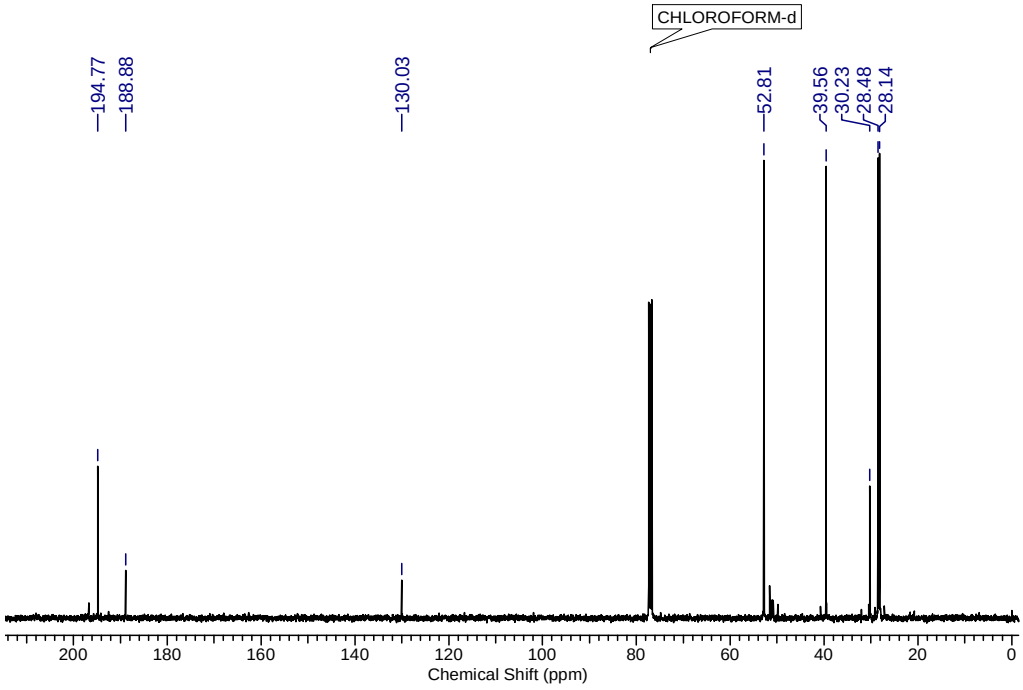


10b

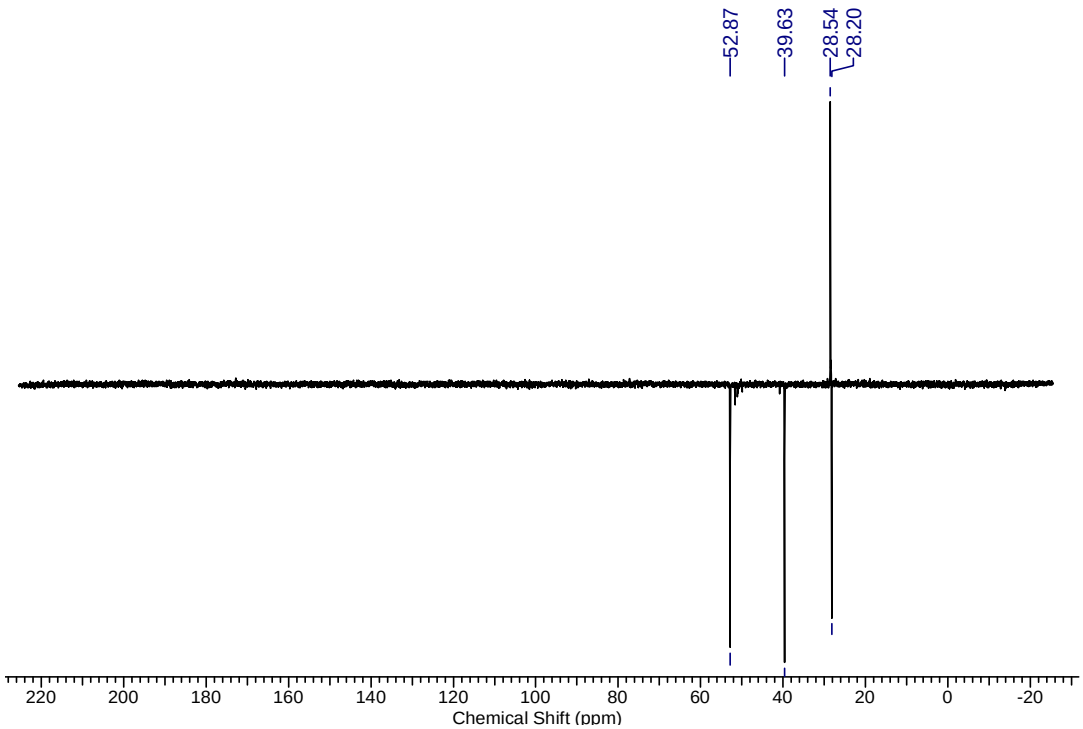
This was prepared according to the general method of Gompper *et al*[1] but with a slightly modified procedure where Et_3N was used as a base in place of NaH. To a solution of compound **10** (2 g, 14.2 mmol, 1 equiv) in DMSO (5 mL), Et_3N (4.3 mL, 42.6 mmol, 3 equiv) was added followed by carbon disulfide (1.3 mL, 21.4 mmol, 1.5 equiv) and kept at room temperature for 1 h. Finally, 1,4-dibromobutane (2.55 mL, 21.4 mmol, 1.5 equiv) was added and kept at room temperature for 12 h. After the usual work up, organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was purified by column chromatography (eluent:5% AcOEt\Petether, Rf: 0.5) to furnish compound **10b** (2.1 g, 55 %) as a yellow solid. ^{1}H NMR (400MHz, CDCl3) δ = 3.13 (br. s., 4 H), 2.53 - 2.43 (m, 4 H), 2.05 (br. s., 4 H), 1.04 (s, 6 H); ^{13}C NMR (100 MHz, CDCl3) δ = 194.8, 188.9, 130.1, 52.9, 39.6, 30.3, 28.5, 28.2; HRMS: calculated for $C_{13}H_{19}O_2S_2$ $[M+H]^+$: 271.0821, observed : $[M+H]^+$: 271.0822.



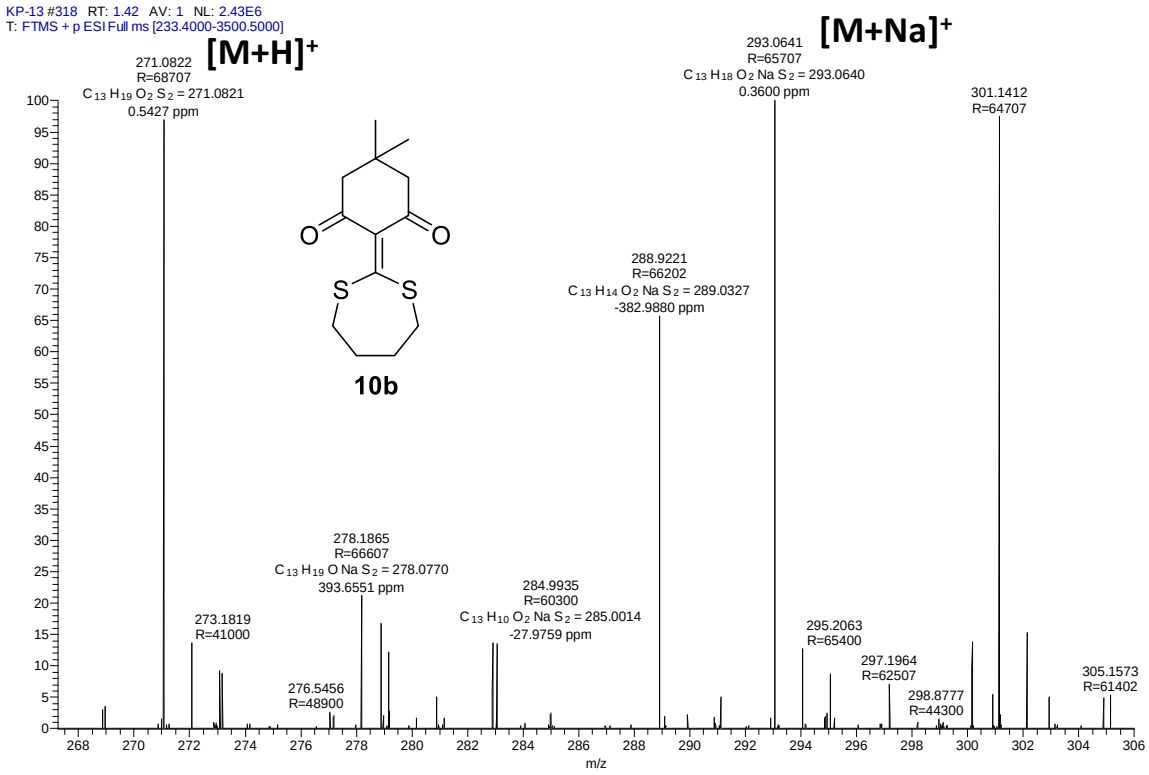
^{1}H NMR spectrum of compound **10b** ($CDCl_3$, 400 MHz, 298 K)



^{13}C NMR spectrum of compound **10b** ($CDCl_3$, 100 MHz, 298 K)



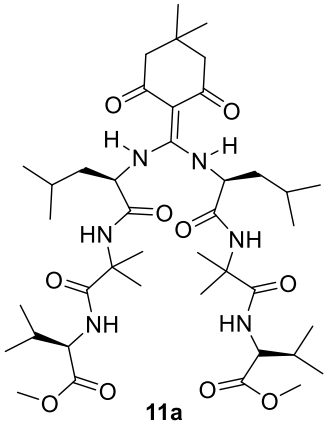
DEPT-135 spectrum of compound **10b** ($CDCl_3$, 100 MHz, 298 K)



HRMS of Compound **10b**

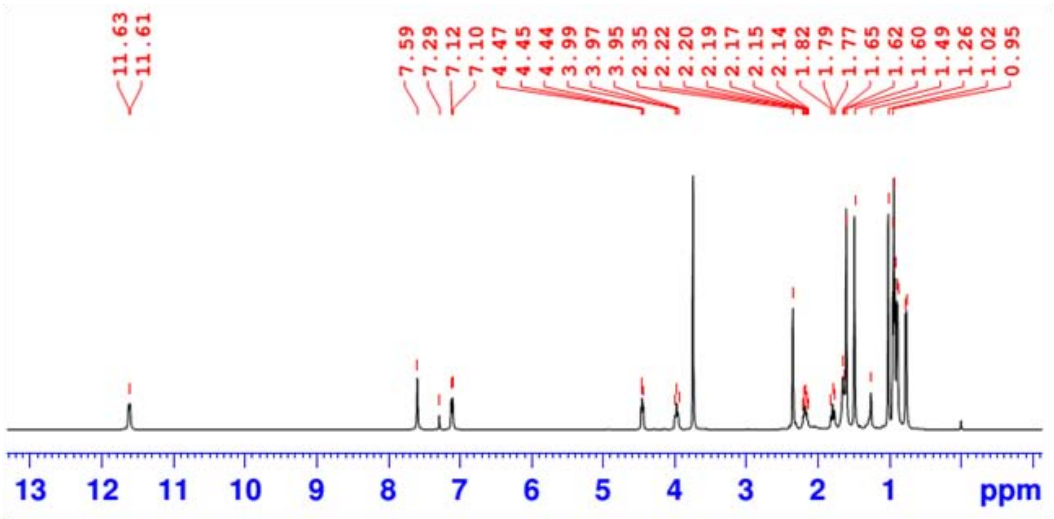
Experimental data and synthetic procedures for symmetrical dimedone-tethered peptides.

Compound 11a (OMe-Val-Aib-Lue-Dimedone-Lue-Aib-Val-OMe):

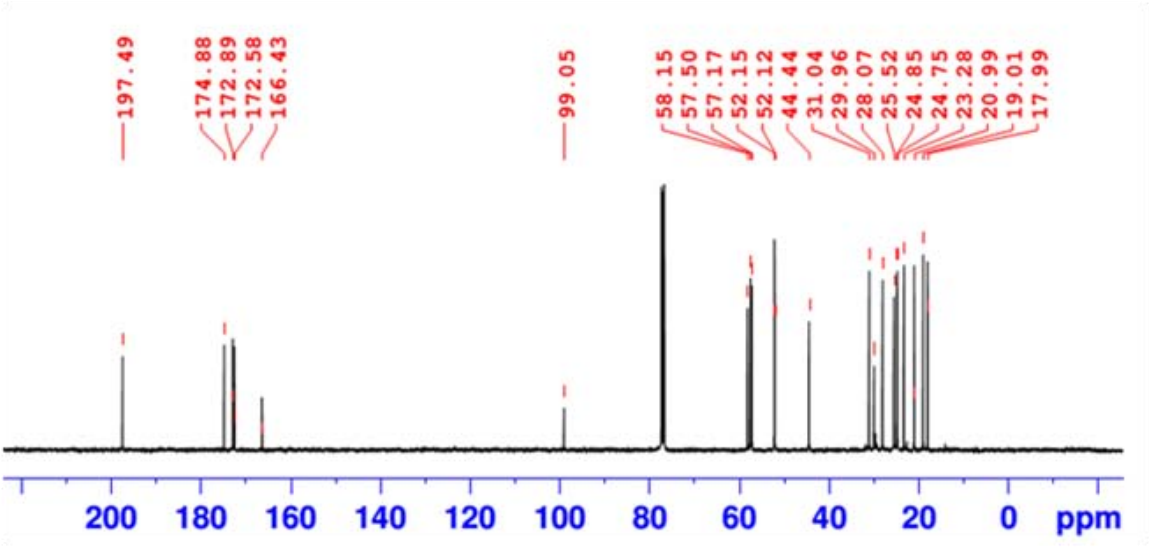


Boc-protected tripeptide ester (1.0 g, 2.33 mmol) was dissolved in 1 mL of DCM followed by addition of 1 mL of TFA (1M) at 0 °C and stirred for 30 min at room temperature. After completion of the reaction, the solvent was stripped off and co-evaporated using toluene to obtain the TFA salt of tripeptide ester which was used for the next step without further purification. Tripeptide TFA salt (0.76g, 2.33mmol, 1 equiv) was dissolved in DMSO in a round bottomed flask followed by the addition of DIPEA (2 mL) and finally compound **10a** (0.18g, 0.6mmol, 0.3 equiv) was added and heated at 50°C for 2 h. After the completion of the reaction, ice cold water was added to the reaction mixture and it was then taken in DCM and organic layer was washed sequentially with saturated

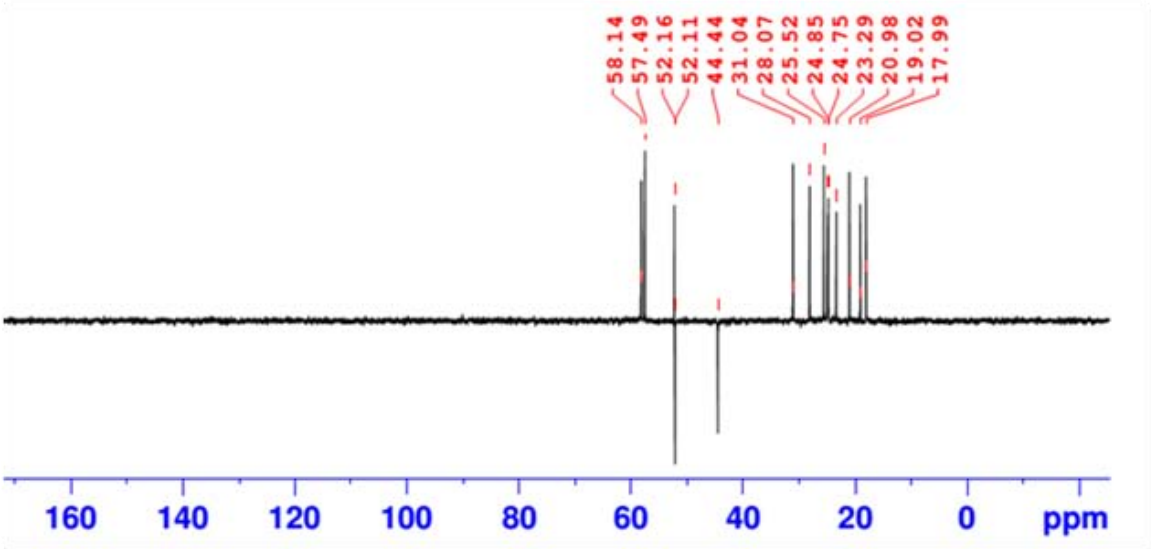
$KHSO_4$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 70% AcOEt/Petether, R_f : 0.5) to furnish compound **11a** (0.84 g, 48 %) as a white solid; $[\alpha]^{26}_D$: -79.0° (c = 0.1, MeOH), ^{1}H NMR (400 MHz, $CDCl_3$) δ = 11.61 (d, J = 7.3 Hz, 2 H), 7.59 (s, 2 H), 7.10 (d, J = 8.5 Hz, 2 H), 4.44 (dd, J = 5.2, 7.6 Hz, 2 H), 3.96 (t, J = 8.5 Hz, 2 H), 3.73 (s, 6 H), 2.40 - 2.28 (m, 4 H), 2.17 (qd, J = 6.4, 12.6 Hz, 2 H), 1.85 - 1.72 (m, 2 H), 1.62 (d, J = 11.0 Hz, 3 H), 1.59 (s, 5 H), 1.52 - 1.43 (m, 6 H), 1.01 (s, 6 H), 0.93 (m, 12 H), 0.88 (d, J = 5.5 Hz, 6 H), 0.76 (d, J = 4.9 Hz, 6 H)^{13}C NMR (100 MHz, $CDCl_3$) δ = 197.5, 174.9, 172.9, 172.6, 166.4, 99.0, 58.1, 57.5, 57.1, 52.1, 44.4, 31.0, 29.9, 28.0, 25.5, 24.8, 24.7, 23.3, 21.0, 19.0, 18.0, HRMS: $C_{41}H_{71}N_6O_{10}$, calculated: 807.5150 $[M+H]^+$, observed: 807.5227 $[M+H]^+$.



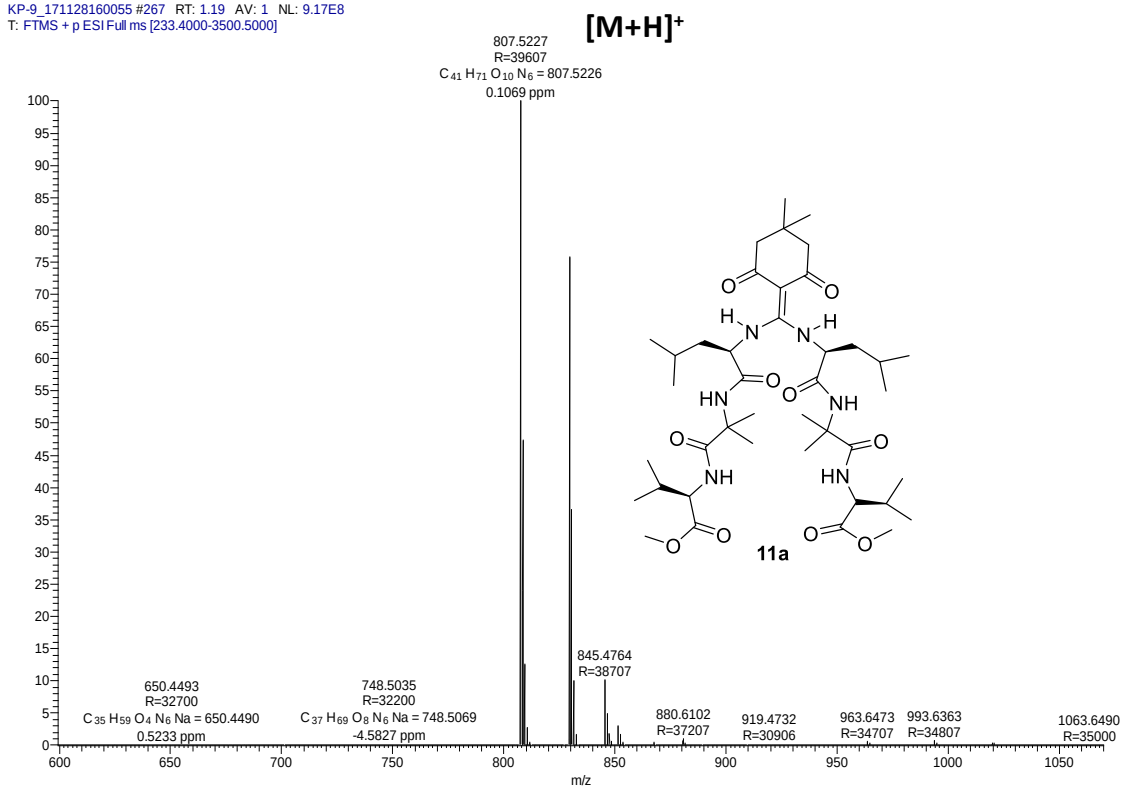
^{1}H NMR Spectrum of compound **11a** [700 MHz, 273 K, $CDCl_3$]



^{13}C NMR Spectrum of compound **11a** [175 MHz, 273 K, $CDCl_3$]

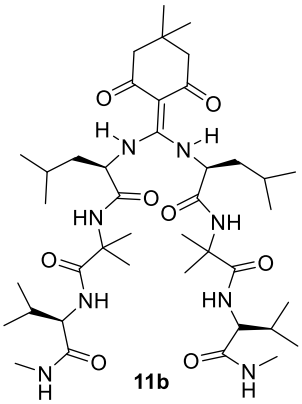


DEPT-135 NMR Spectrum of compound **11a** [175 MHz, 273 K, $CDCl_3$]

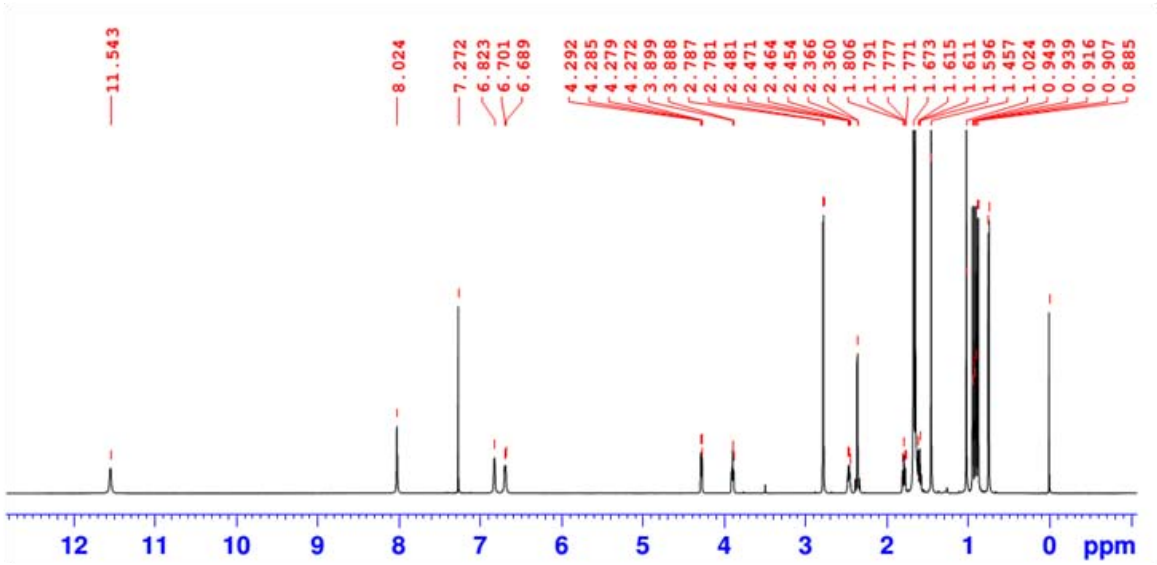


HRMS of Compound **11a**

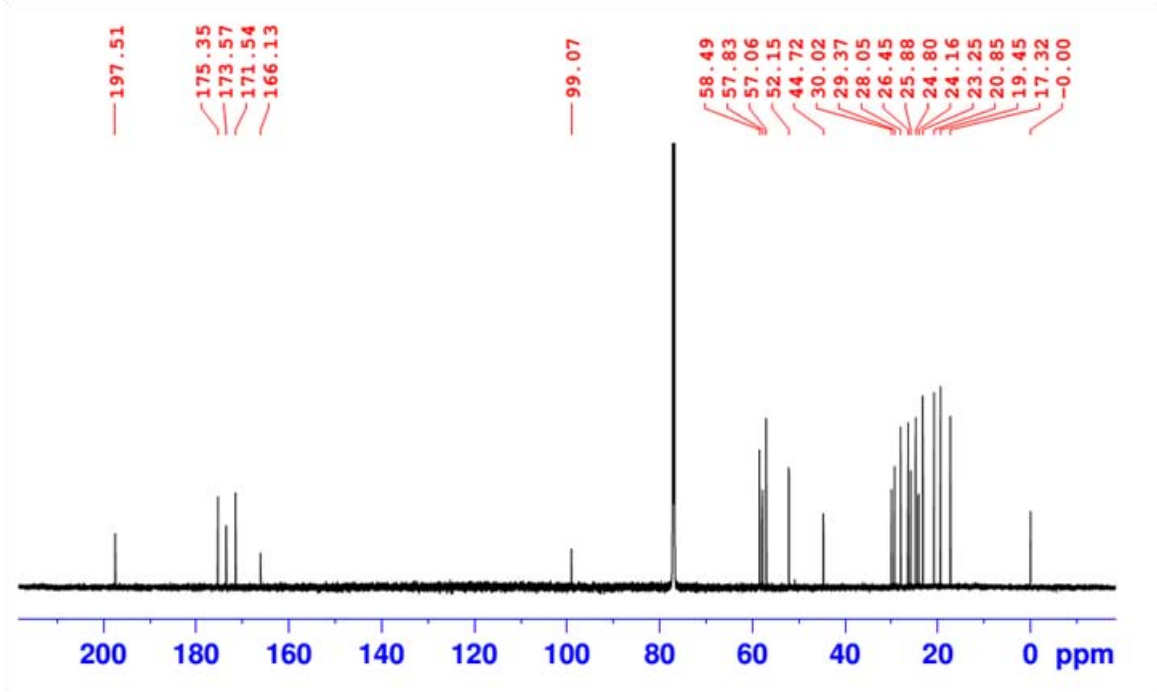
Compound 11b (NHMe-Val-Aib-Lue-Dimedone-Lue-Aib-Val-NHMe): To a solution of **11a** (0.5g, 0.6 mmol) in methanol, saturated methanolic methylamine solution (3 ml) was added and reaction mixture was stirred for 24 h at room temperature. Later, the volatiles were stripped off under reduced pressure and the product was further purified by column chromatography and preparative thin layer chromatography (eluent: 2% MeOH/DCM, R_f : 0.5) to furnish compound **11b** (0.46 g, 92%) as a white solid; $[\alpha]^{26}_D$: - 65.5°(c = 0.1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 11.53 (d, *J* = 6.1 Hz, 2 H), 8.01 (s, 2 H), 6.89 (d, *J* = 4.3 Hz, 2 H), 6.75 (d, *J* = 9.2 Hz, 2 H), 4.28 (dd, *J* = 4.9, 8.5 Hz, 2 H), 3.90 (t, *J* = 8.5 Hz, 2 H), 2.77 (d, *J* = 4.3 Hz, 6 H), 2.53 - 2.37 (m, 2 H), 2.37 - 2.26 (m, 4 H), 1.78 (t, *J* = 10.1 Hz, 2 H), 1.67 - 1.54 (m, 10 H), 1.50 - 1.37 (m, 6 H), 1.01 (s, 6 H), 0.98 - 0.80 (m, 18 H), 0.74 (d, *J* = 5.5 Hz, 6 H); ^{13}C NMR (100MHz, $CDCl_3$) δ = 197.4, 175.3, 173.5, 171.6, 166.2, 99.0, 58.2, 57.1, 52.1, 44.7, 30.0, 29.4, 28.0, 26.4, 25.8, 24.8, 24.2, 23.2, 20.8,



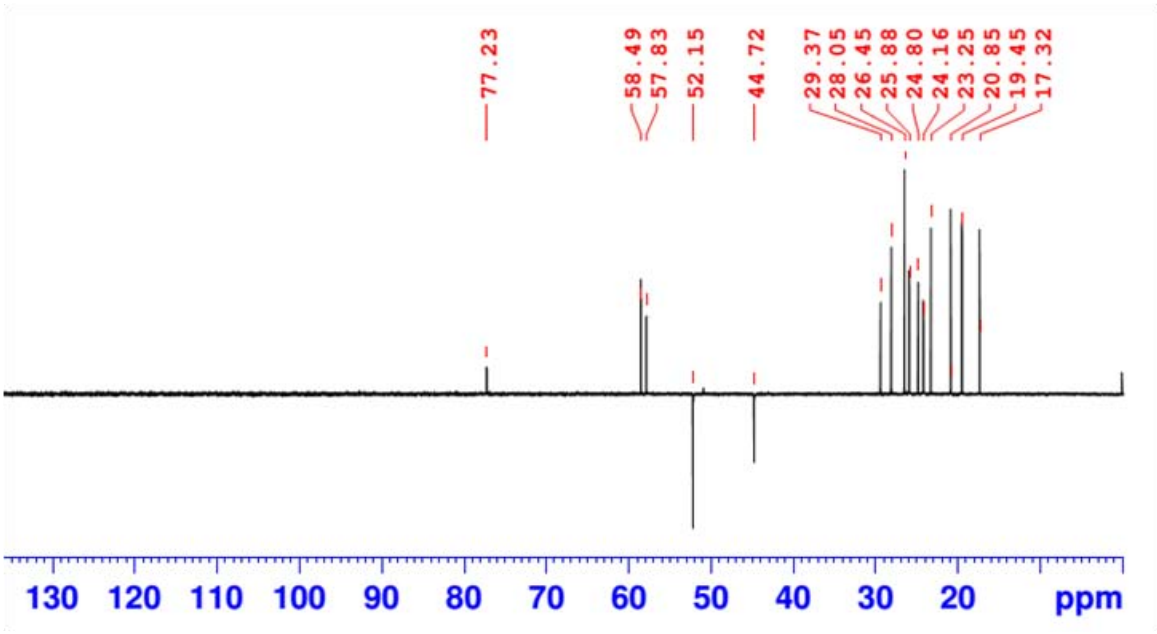
19.4, 17.4; HRMS: $C_{41}H_{73}N_8O_8$, calculated: 827.0750 $[M+Na]^+$, observed: 827.5365$[M+Na]^+$.



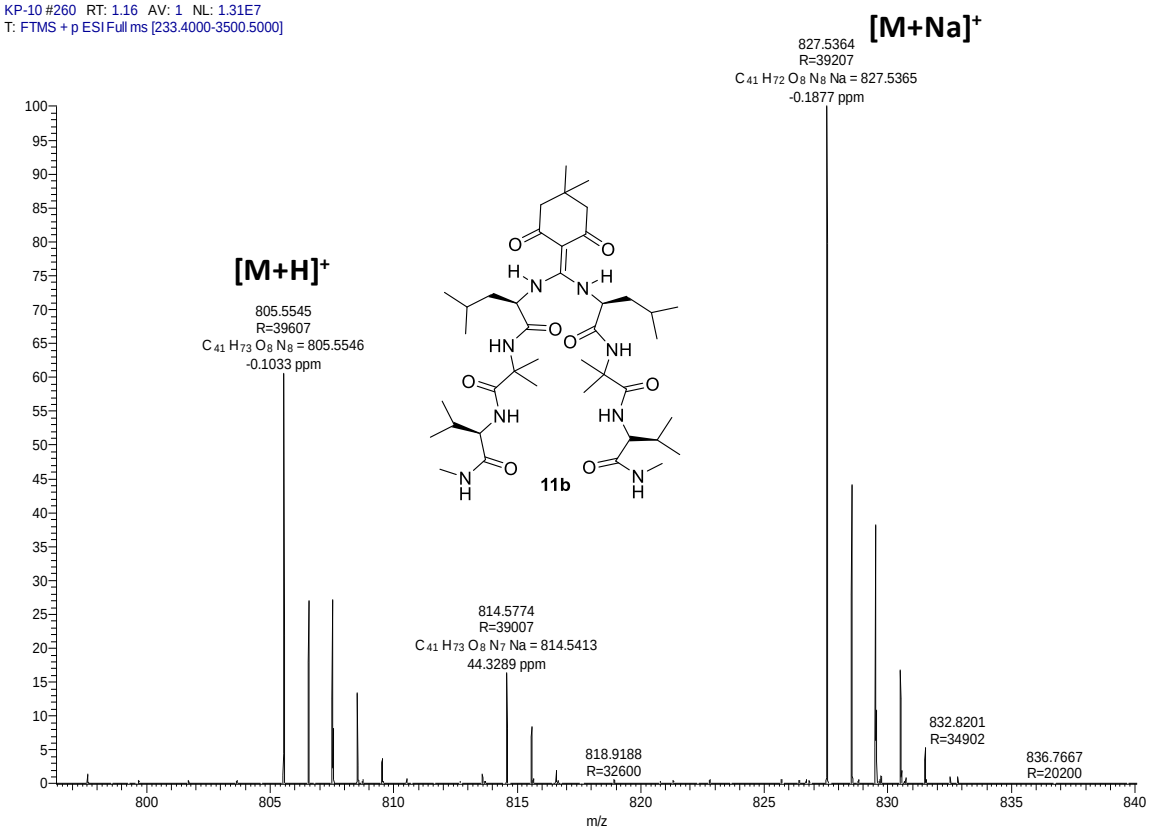
^{1}H NMR Spectrum of compound **11b** [700 MHz, 273 K, $CDCl_3$]



^{13}C NMR Spectrum of compound **11b** [175 MHz, 273 K, $CDCl_3$]

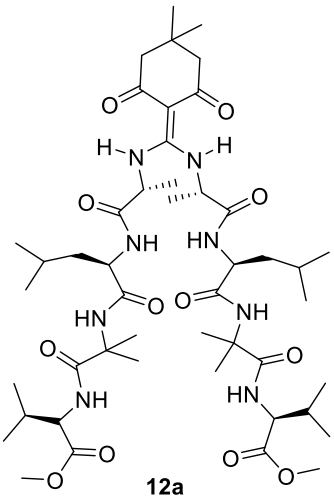


DEPT-135 spectrum of compound **11b** ($CDCl_3$, 175 MHz, 298 K)

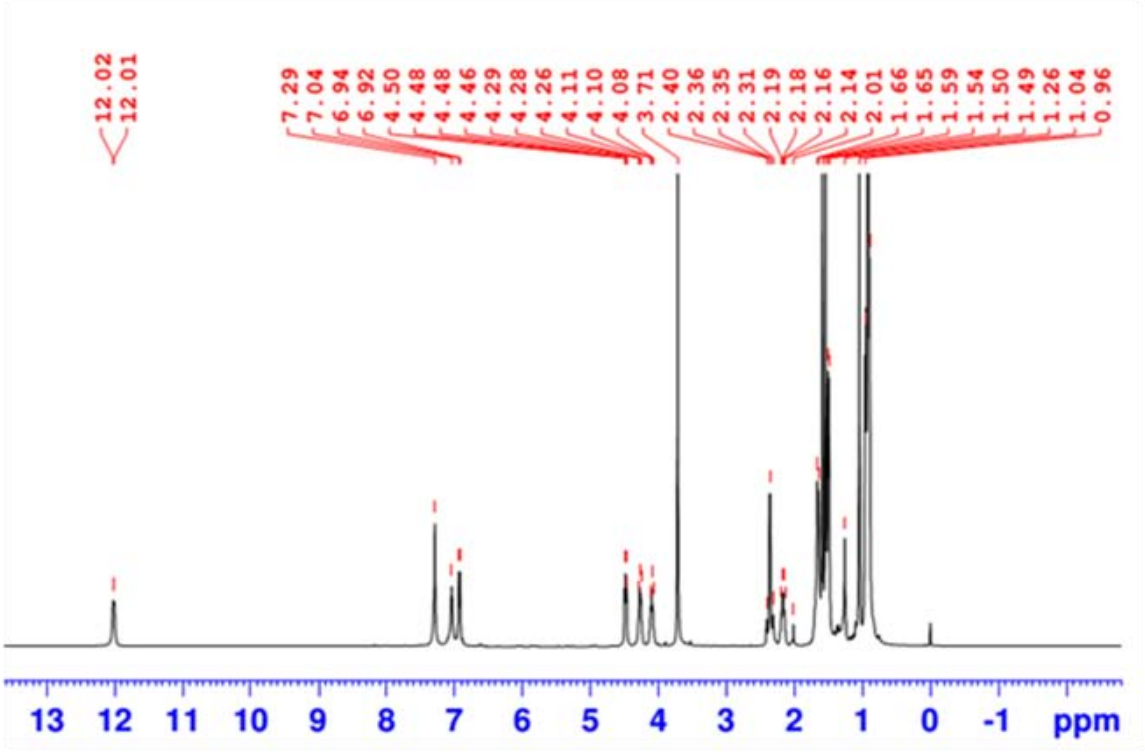


HRMS of Compound **11b**

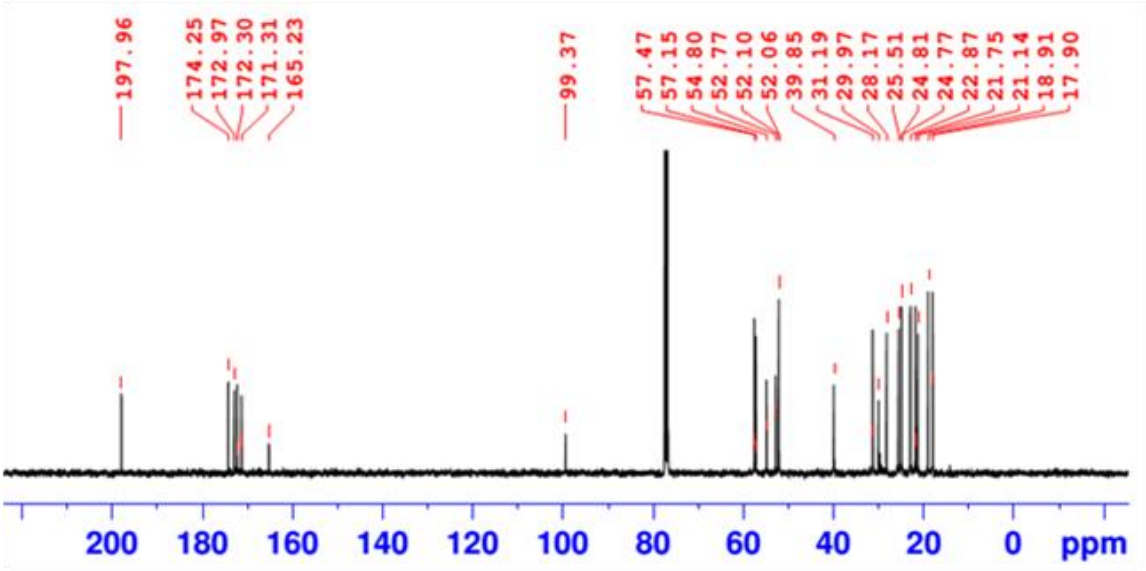
Compound **12a (OMe-Val-Aib-Lue-Ala-Dimedone-Ala-Lue-Aib-Val-OMe):**



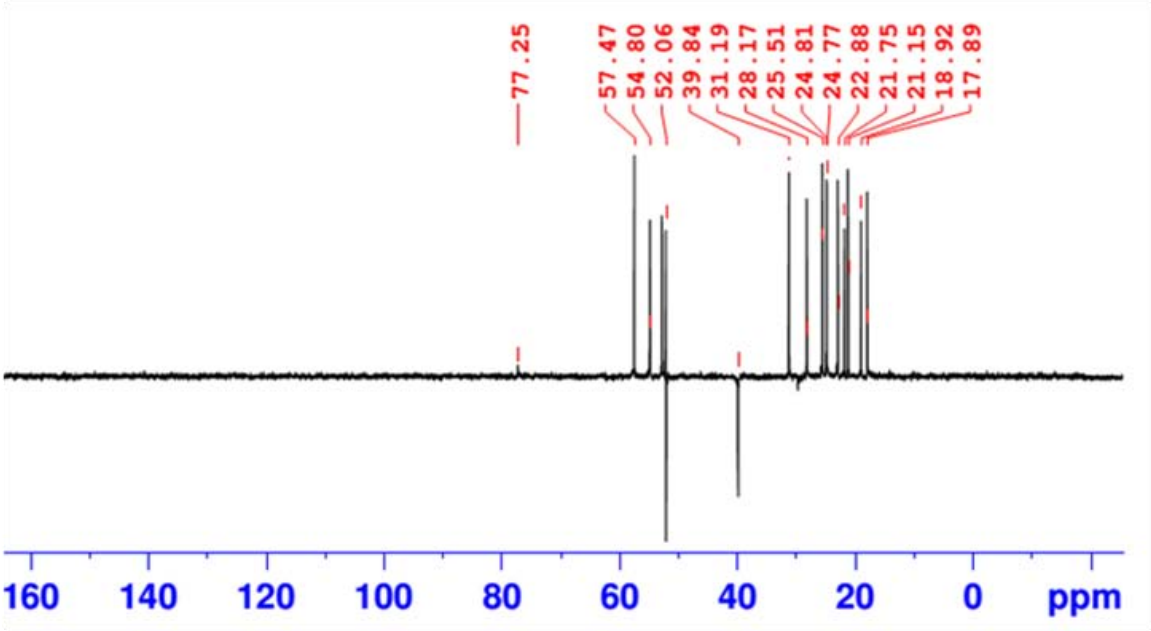
Boc-protected tetrapeptide ester (1.0 g, 2.0 mmol) was dissolved in 1 mL of DCM followed by addition of 1 mL of TFA (1M) at 0 °C and stirred for 30 min at room temperature. After completion of the reaction, the solvent was stripped off and co-evaporated using toluene to obtain the TFA salt of tetrapeptide ester which was used for the next step without further purification. Tetrapeptide TFA salt (0.8g, 2.33mmol, 1 equiv) was dissolved in DMSO in a round bottomed flask followed by the addition of DIPEA (2 mL) and finally compound **10a** (0.15 g, 0.6 mmol, 0.3 equiv) was added and heated at 50°C for 2 h. After the completion of the reaction, ice cold water was added to the reaction mixture and it was then taken in DCM and organic layer was washed sequentially with saturated $KHSO_4$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: AcOEt, R_f : 0.4) to furnish compound **12a** (0.94 g, 50 %) as a white solid; $[\alpha]^{26}_D$: -85.63° (c = 0.1, MeOH), ^{1}H NMR (400 MHz, $CDCl_3$) δ = 11.99 (d, J = 5.5 Hz, 2 H), 7.02 (br. s., 2 H), 6.91 (d, J = 7.9 Hz, 2 H), 4.46 (dd, J = 5.5, 7.9 Hz, 2 H), 4.32 - 4.20 (m, 2 H), 4.08 (t, J = 6.7 Hz, 2 H), 3.70 (s, 6 H), 2.42 - 2.25 (m, 4 H), 2.15 (dd, J = 6.7, 12.8 Hz, 2 H), 1.71 - 1.60 (m, 6 H), 1.57 (s, 6 H), 1.55 - 1.44 (m, 12 H), 1.02 (s, 6 H), 0.98 - 0.80 (m, 26 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 197.9, 174.2, 172.9, 172.3, 171.3, 165.2, 99.3, 57.4, 57.1, 54.8, 52.7, 52.0, 39.8, 31.2, 29.8, 28.1, 25.4, 24.7, 22.8, 21.7, 21.0, 18.9, 17.9; HRMS: $C_{47}H_{80}N_8O_{12}$, calculated: 971.5788$[M+Na]^+$, observed: 971.5793 $[M+H]^+$.



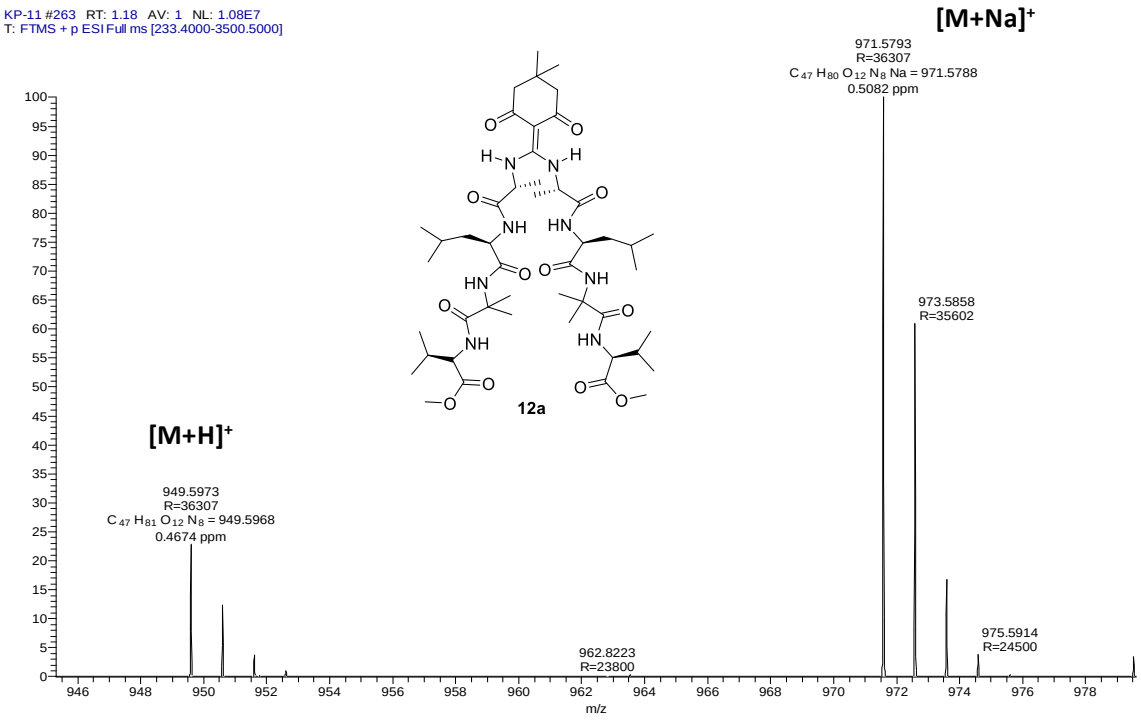
^{1}H NMR Spectrum of **12a** [400 MHz, 273 K, $CDCl_3$]



^{13}C NMR Spectrum of **12a** [400 MHz, 273 K, $CDCl_3$]

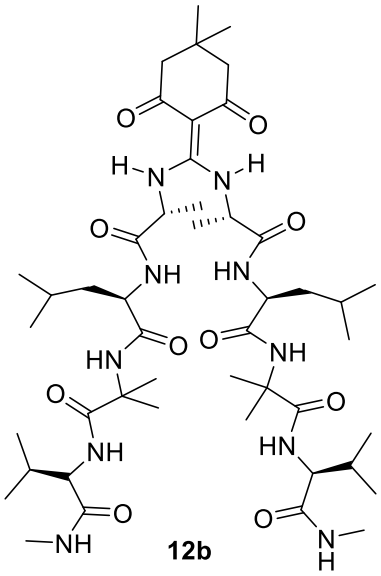


DEPT-135 NMR Spectrum of **12a** [100 MHz, 273 K, $CDCl_3$]

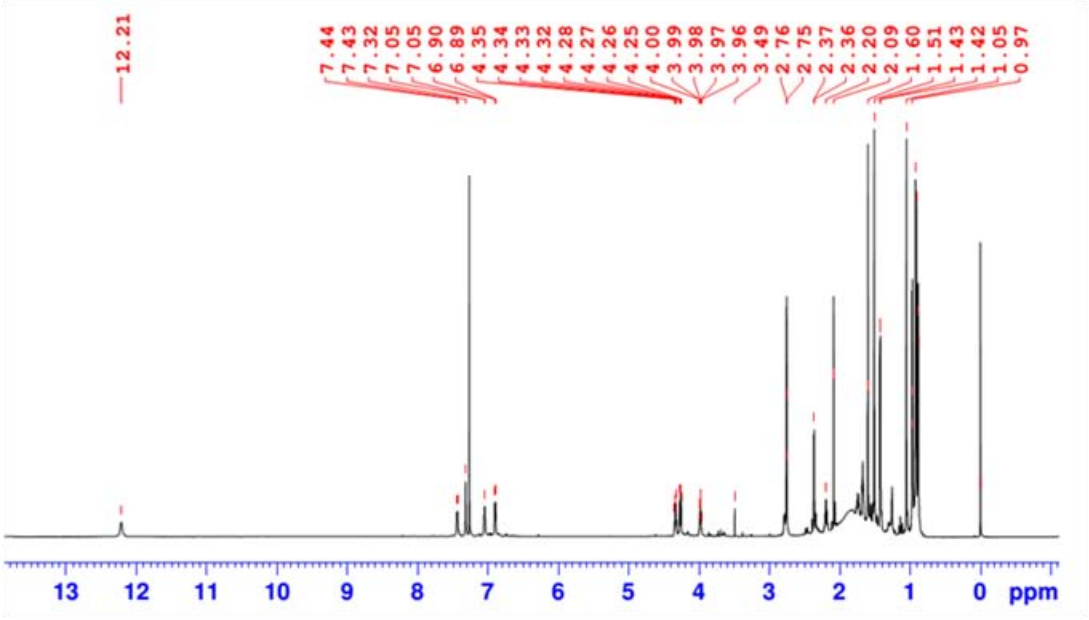


HRMS of Compound **12a**

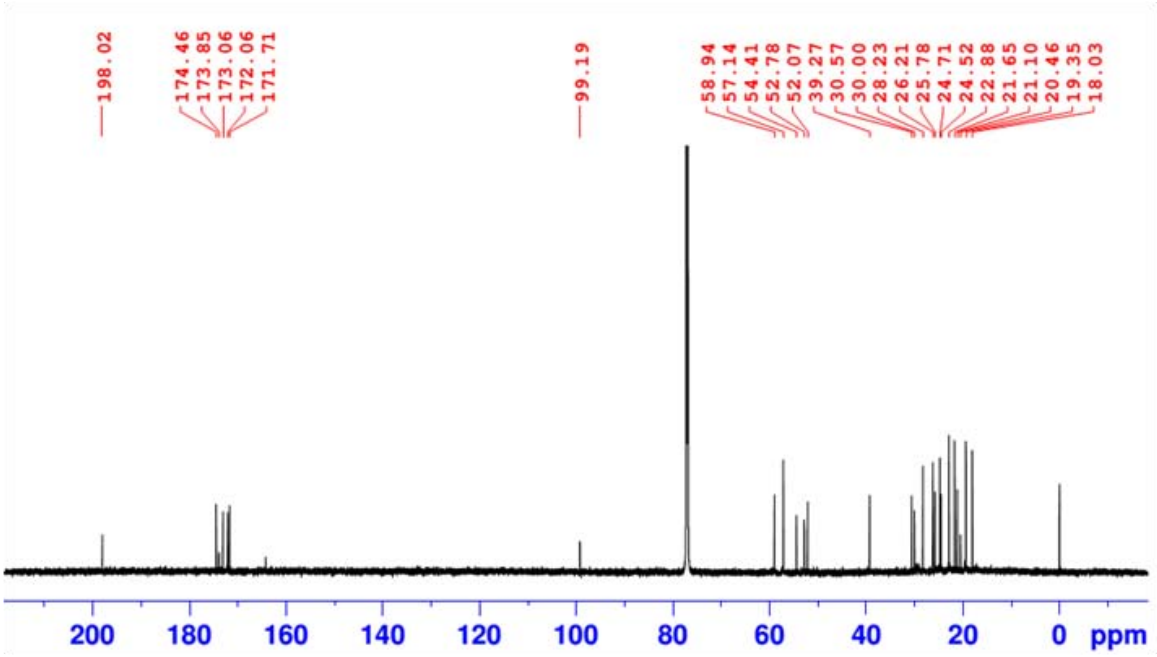
Compound 12b (NHMe-Val-Aib-Lue-Ala-Dimedone-Ala-Lue-Aib-Val-NHMe):



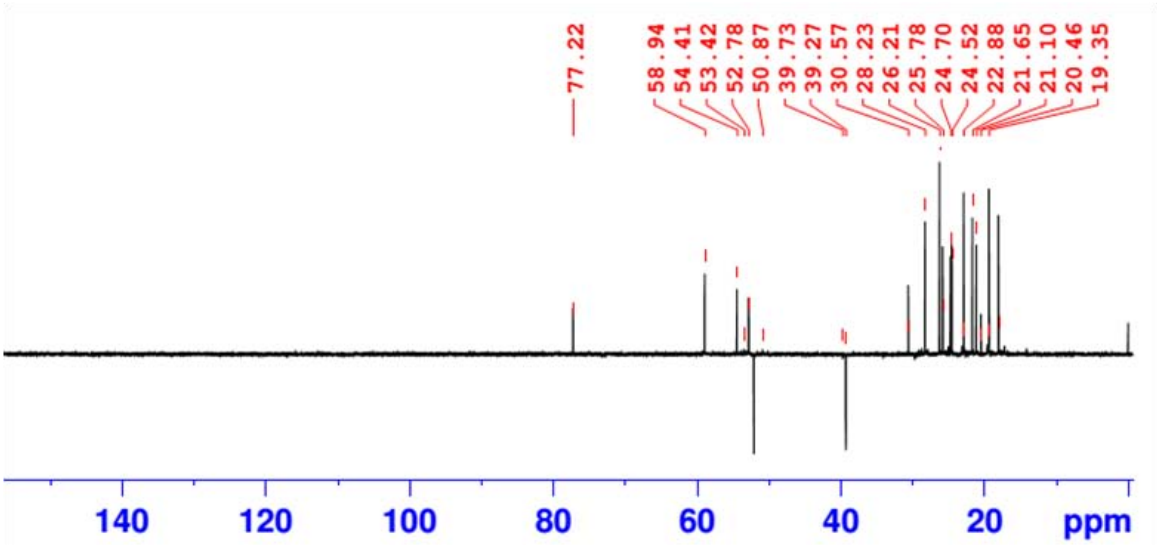
To a solution of **12a** (0.5 g, 0.6 mmol) in methanol, saturated methanolic methylamine solution (3 ml) was added and reaction mixture was stirred for 24 h at room temperature. Later, the volatiles were stripped off under reduced pressure and the product was further purified by column chromatography and preparative thin layer chromatography (eluent: 2% MeOH/DCM, R_f : 0.5) to furnish compound **12b** (0.46 g, 92%) as a white solid; $[\alpha]^{26}_D$: -85.5° (c = 0.1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 12.21 (br. s., 2 H), 7.44 (s., 2 H), 7.43-7.32 (br. s., 2 H), 7.05-6.95 (d, J = 8.4 Hz, 2 H), 6.90-6.85 (m, 2H), 4.33 (q, J = 7.2 Hz, 2 H), 4.27 (t, J = 7.6 Hz, 2 H), 4.05 - 3.96 (m, 2 H), 3.65-3.50 (m, 2H) 2.75 (d, J = 4.2 Hz, 6 H), 2.41 - 2.29 (m, 6 H), 2.23 (dt, J = 6.7, 13.3 Hz, 4 H), 1.78 - 1.70 (m, 2 H), 1.70 - 1.64 (m, 6 H), 1.55 - 1.48 (m, 8 H), 1.47 - 1.39 (m, 6 H), 0.94 - 0.84 (m, 26 H); ^{13}C NMR (100MHz, $CDCl_3$) δ = 197.9, 174.4, 173.0, 172.1, 171.6, 99.1, 59.0, 57.1, 54.4, 52.9, 52.0, 39.2, 30.4, 30.0, 29.7, 28.2, 26.2, 25.5, 24.8, 24.7, 22.9, 21.7, 21.1, 19.4, 17.9HRMS: $C_{47}H_{82}N_{10}O_{10}Na$, calculated: 969.6108 $[M+Na]^+$, observed: 969.6103$[M+Na]^+$.



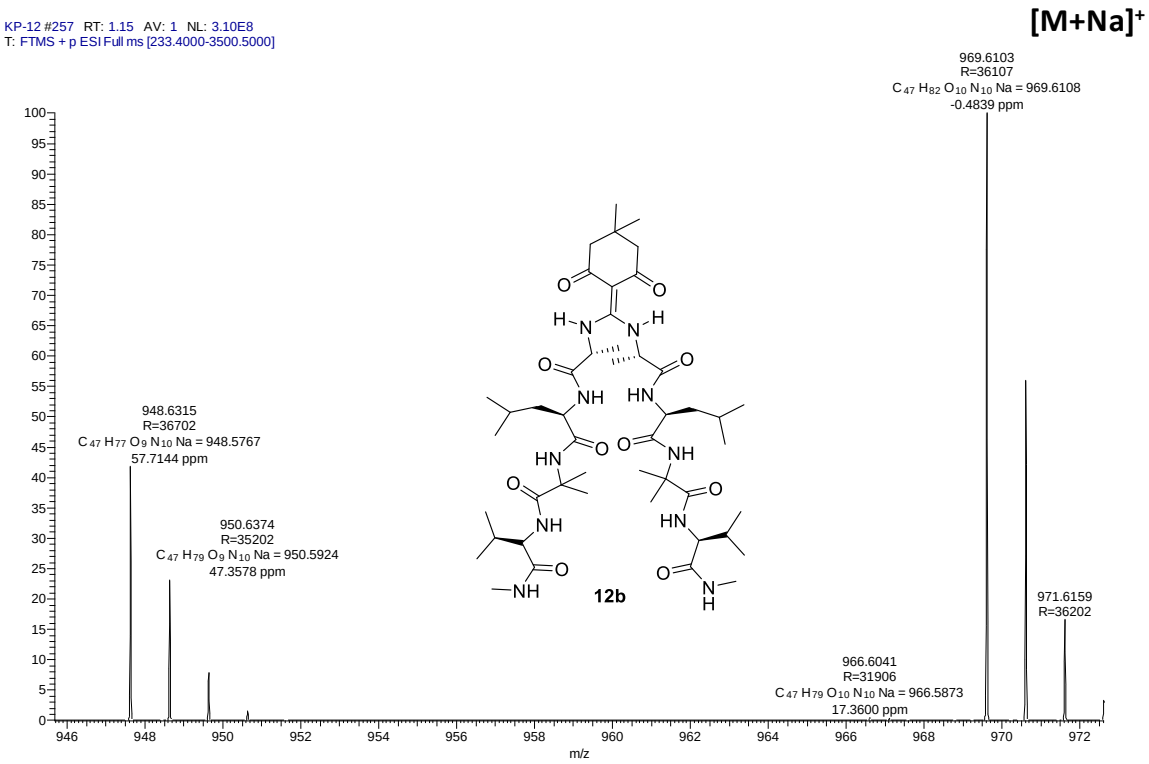
^{1}H NMR Spectrum of **12b** [400 MHz, 273 K, $CDCl_3$]



^{13}C NMR Spectrum of **12b** [400 MHz, 273 K, $CDCl_3$]

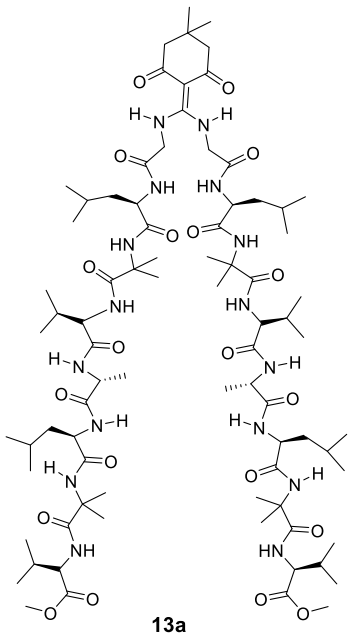


DEPT-135 NMR Spectrum of **12b** [100 MHz, 273 K, $CDCl_3$]

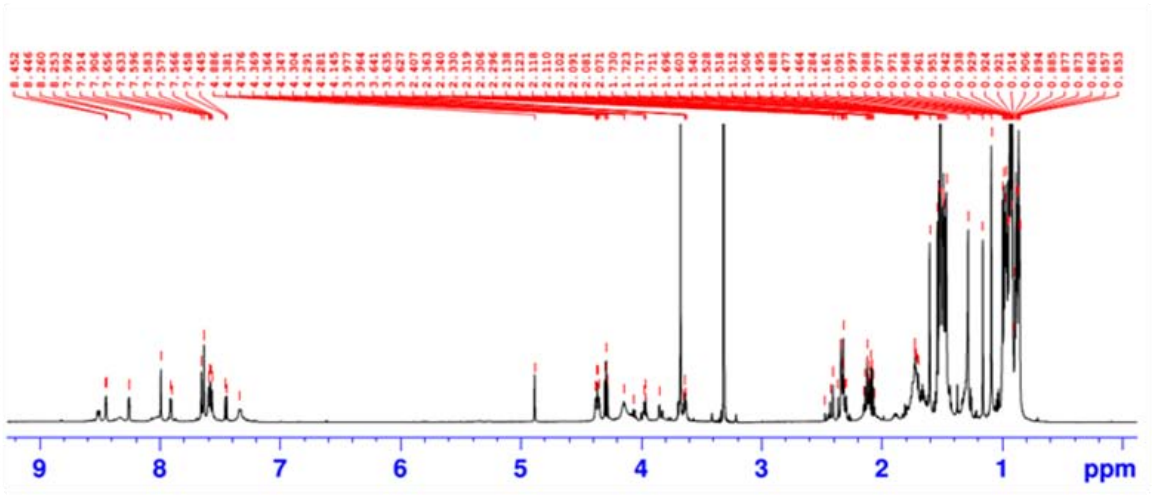


HRMS of Compound **12b**

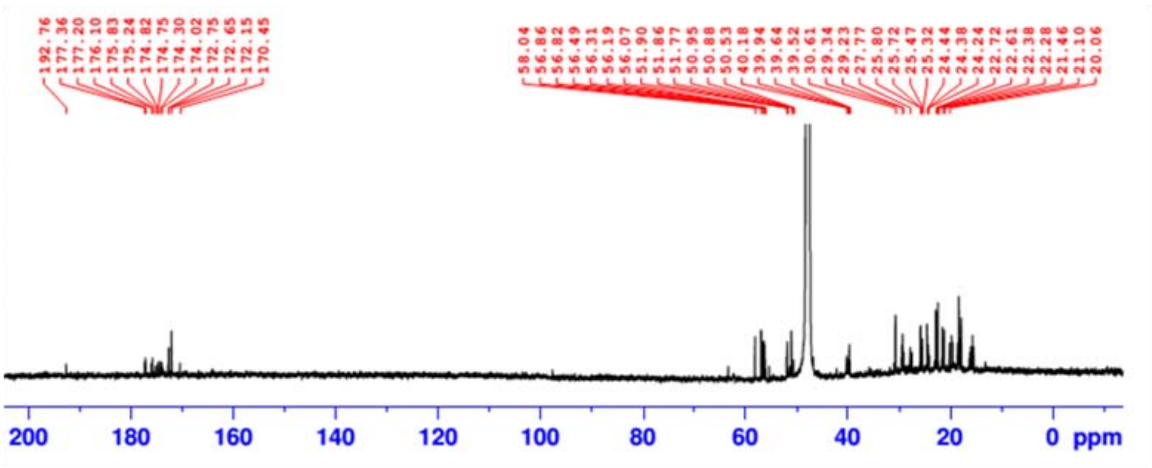
Compound 13a (OMe-Val-Aib-Lue-Ala-Val-Aib-Lue-Dimedone-Lue-Aib-Val-Ala-Lue-Aib-Val-OMe): Boc-protected octa-peptide ester **6** (0.5 g, 0.50 mmol) was dissolved in 1 mL of DCM followed by addition of 1 mL of TFA (1M) at 0 °C and stirred for 30 min at room temperature. After completion of the reaction, the solvent was stripped off and co-evaporated using toluene to obtain the TFA salt of octa-peptide ester **6** which was used for the next step without further purification. Octa-peptide TFA salt of **6** (0.44 g, 0.5mmol, 1 equiv) was dissolved in DMF in a round bottomed flask followed by the addition of DIPEA (3 mL) and finally compound **10b** (0.04 g, 0.17mmol, 0.3 equiv) was added and heated at 50°C for 2 h. After the completion of the reaction, ice cold water was added to the reaction mixture and it was then taken in DCM and organic layer was washed sequentially with saturated $KHSO_4$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude


13a

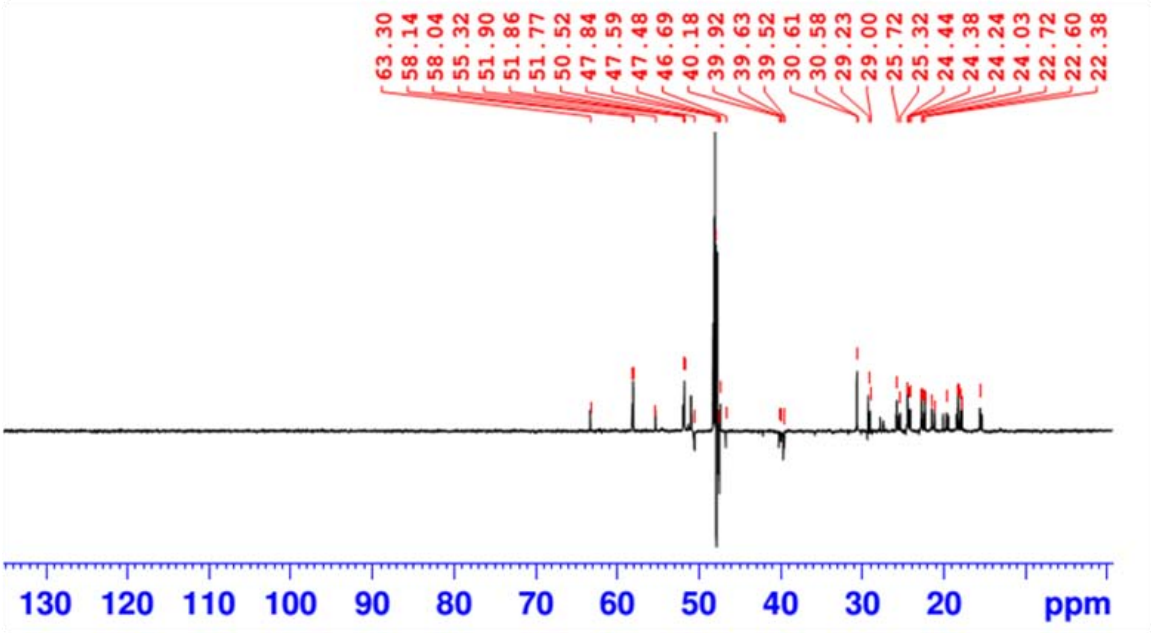
product which was further purified by column chromatography (eluent: 4% MeOH/DCM , R_f : 0.5) to furnish compound **13a** (0.75 g, 70%) as a white solid; $[\alpha]^{26}_D$: -35.5° (c = 0.1, MeOH), ^{13}C NMR (100 MHz, CD_3OH) δ = 194.2, 178.8, 178.6, 177.5, 177.2, 176.7, 176.2, 176.2, 175.7, 175.4, 174.2, 174.1, 173.6, 64.7, 59.5, 58.3, 57.9, 57.7, 57.6, 57.5, 57.4, 56.7, 53.3, 53.2, 52.7, 52.4, 52.3, 52.0, 41.6, 41.4, 41.1, 41.0, 37.2, 32.2, 30.7, 29.2, 28.8, 27.2, 26.8, 25.8, 24.2, 23.7, 22.9, 22.5, 21.5, 21.0, 19.7, 19.2, 17.0; HRMS: $C_{81}H_{141}N_{16}O_{20}Na$, calculated: 1681.0333 $[M+Na]^+$, observed: 1681.0400 $[M+Na]^+$.



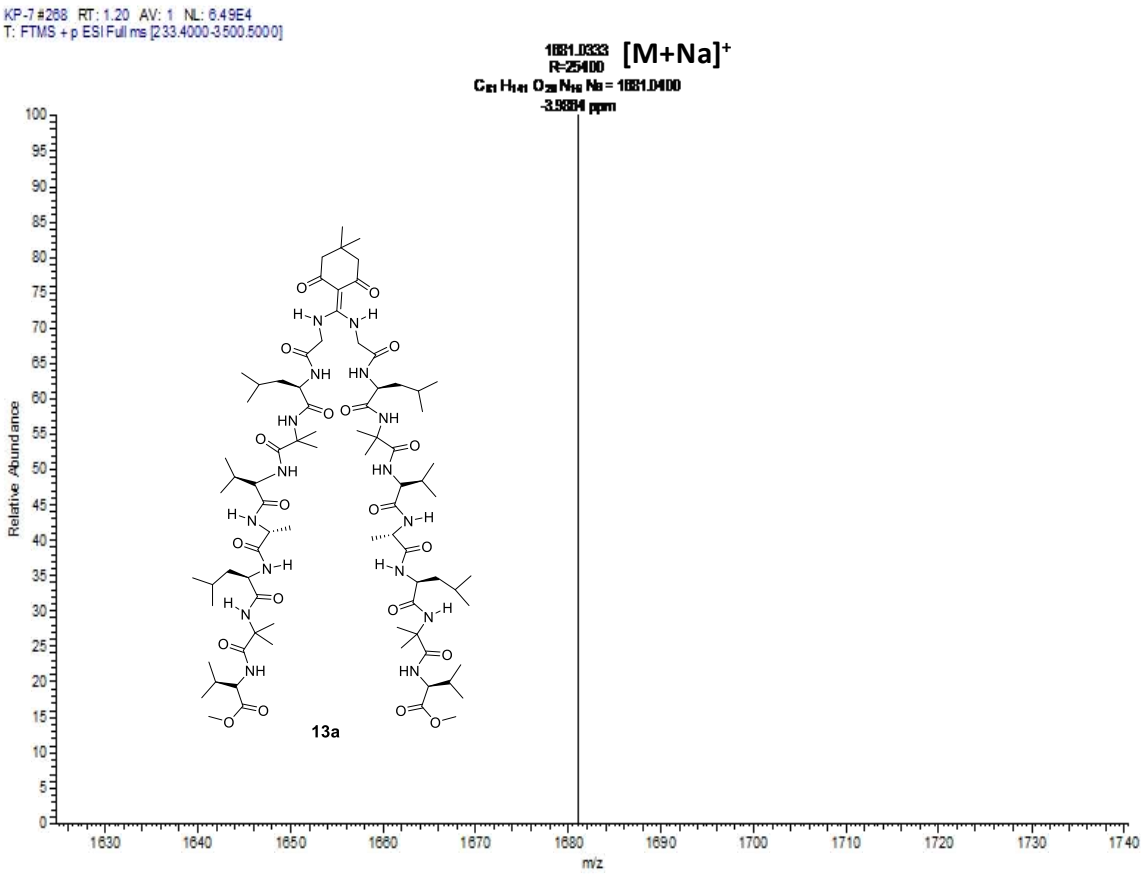
^{1}H NMR Spectrum of compound **13a** [700 MHz, 273 K, CD_3OH]



^{13}C- NMR Spectrum of Compound **13a** [175 MHz, 273 K, CD_3OH]

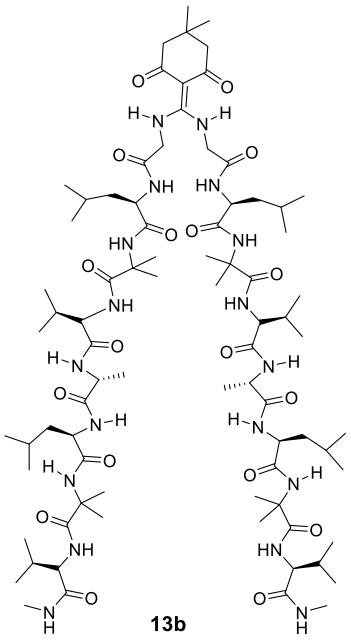


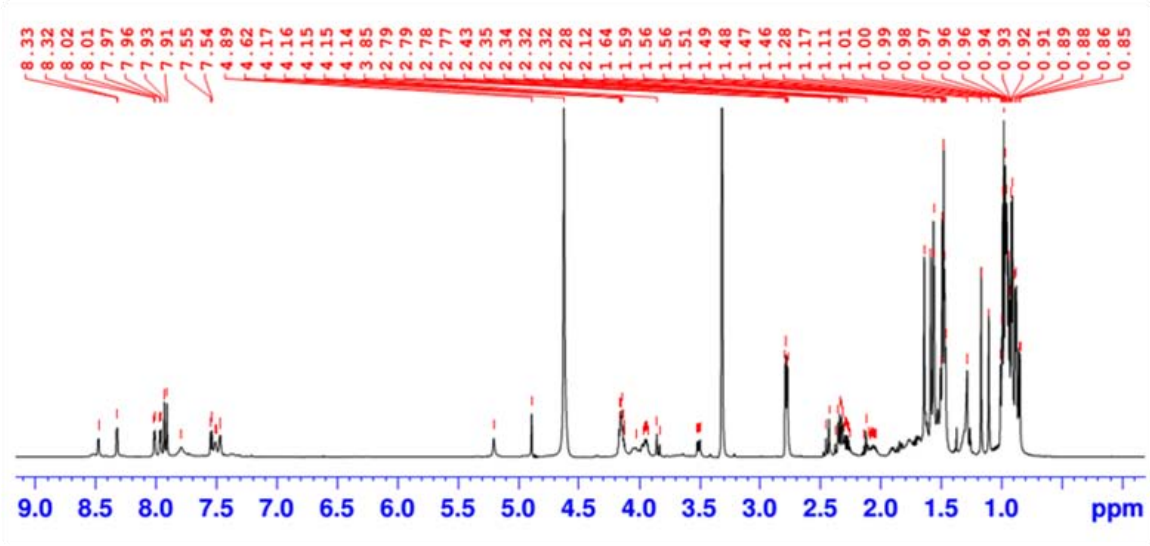
DEPT-135 NMR Spectrum of Compound **13a** [175 MHz, 273 K, CD_3OH]



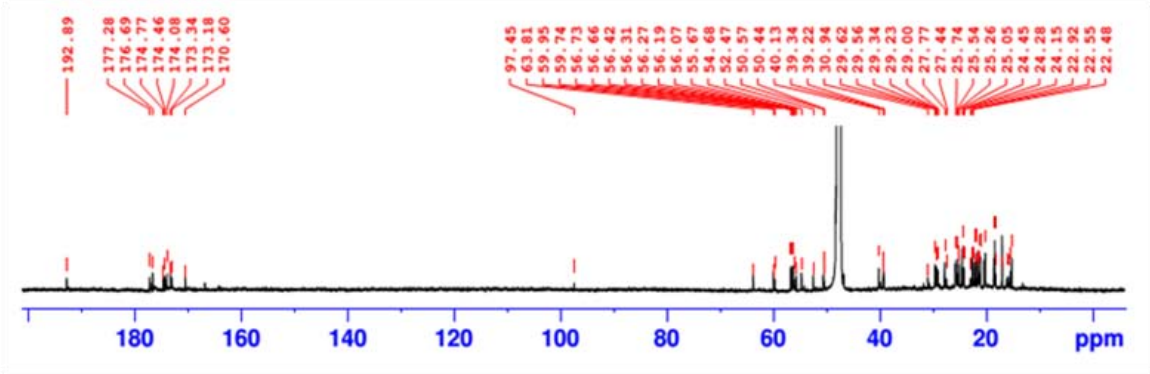
HRMS of Compound **13a**

Compound **13b** (NHMe-Val-Aib-Lue-Ala-Val-Aib-Lue-Dimedone-Lue-Aib-Val-Ala-Lue-Aib-Val-NHMe): To a solution of **13a** (0.5 g, 0.3 mmol) in methanol, saturated methanolic methylamine solution (3 ml) was added and reaction mixture was stirred for 24 h at room temperature. Later, the volatiles were stripped off under reduced pressure and the product was further purified by column chromatography and preparative thin layer chromatography (eluent: 5% MeOH/DCM, R_f : 0.5) to furnish compound **13b** (0.47 g, 95%) as a white solid; $[\alpha]^{26}_D$: -78.6° (c = 0.1, MeOH); ^{13}C NMR (175MHz, CD_3OH) δ = 191.8, 179.1, 178.9, 178.6, 178.3, 177.6, 177.1, 176.4, 176.1, 175.9, 175.5, 175.3, 175.2, 174.4, 174.3, 173.5, 171.7, 109.3, 65.4, 65.2, 64.5, 59.6, 58.2, 57.8, 57.7, 57.6, 57.5, 57.2, 57.1, 57.0, 54.2, 53.5, 52.4, 48.1, 46.1, 41.0, 40.8, 40.4, 32.0, 31.2, 30.9, 30.6, 30.6, 27.6, 27.2, 27.1, 26.6, 25.8, 25.4, 24.0, 23.7, 23.5, 23.3, 23.1, 22.9, 22.4, 22.0, 21.7, 21.5, 21.4, 20.8, 20.0, 19.9, 19.7, 19.4, 16.6; HRMS: $C_{81}H_{139}N_{16}O_{20}Na$, calculated: 1679.0244 $[M+Na]^+$, observed: 1679. 0638 $[M+Na]^+$.

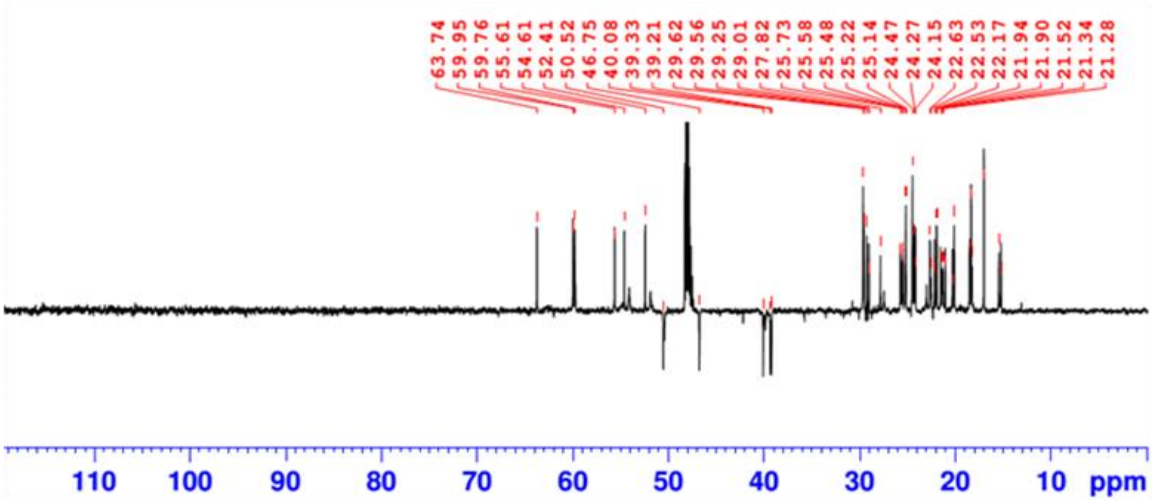




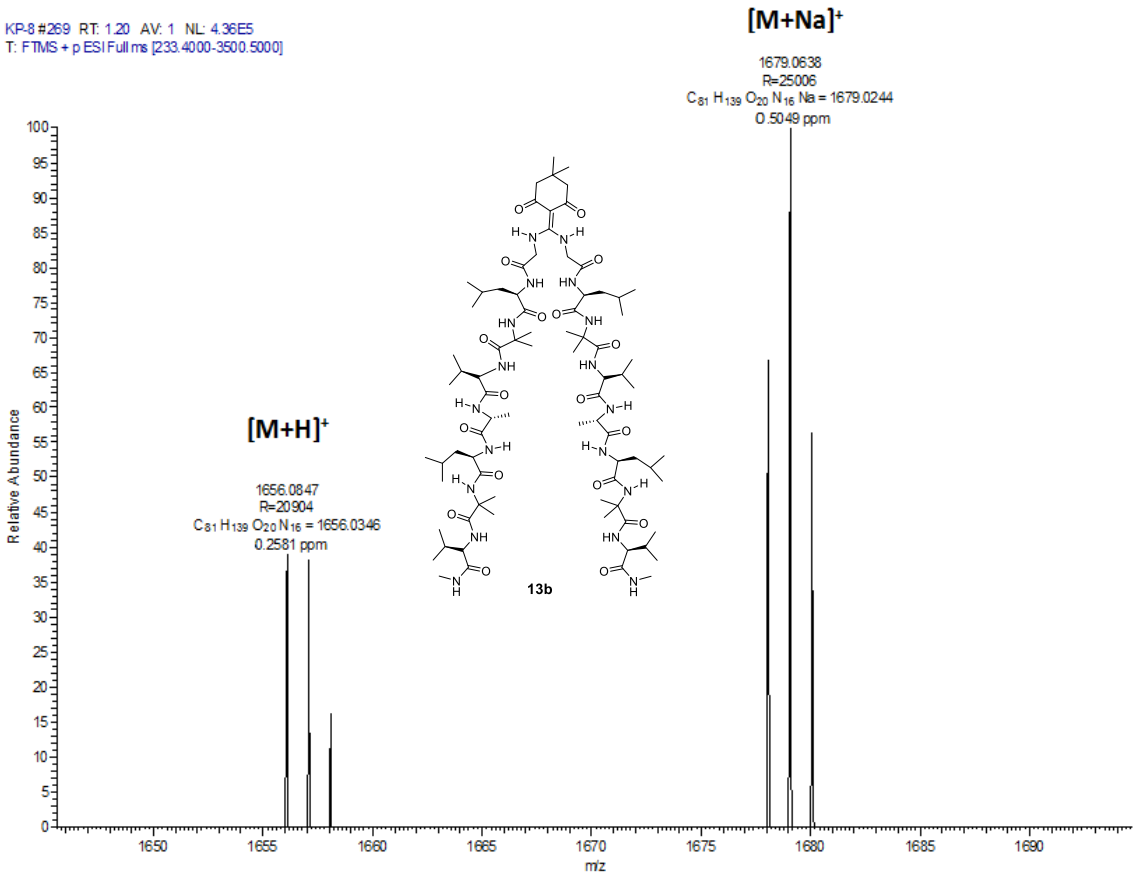
^1H NMR Spectrum of Compound **13b** [700 MHz, 273 K, CD_3OH]



^{13}C- NMR Spectrum of Compound **13b** [175 MHz, 273 K, CD_3OH]



DEPT-135- NMR Spectrum of Compound **13b** [175 MHz, 273 K, CD_3OH]

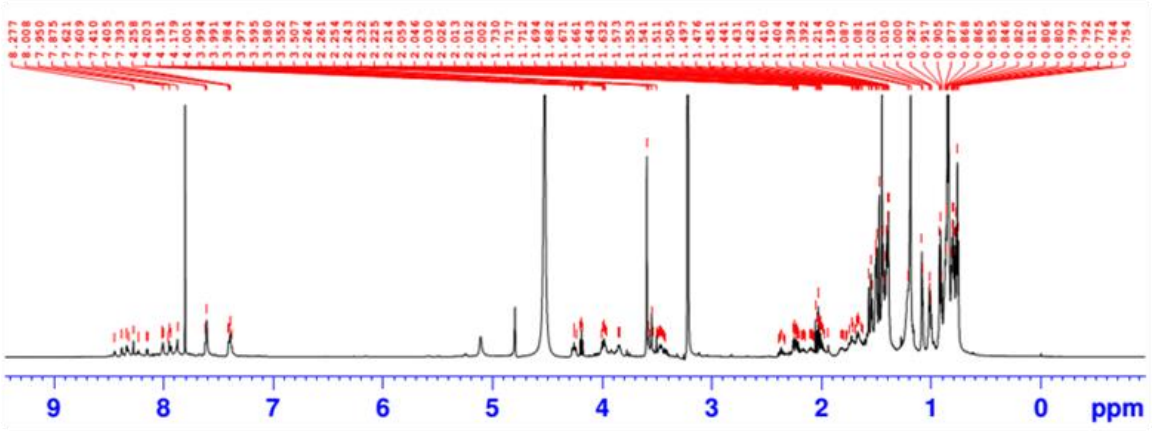


HRMS of Compound **13b**

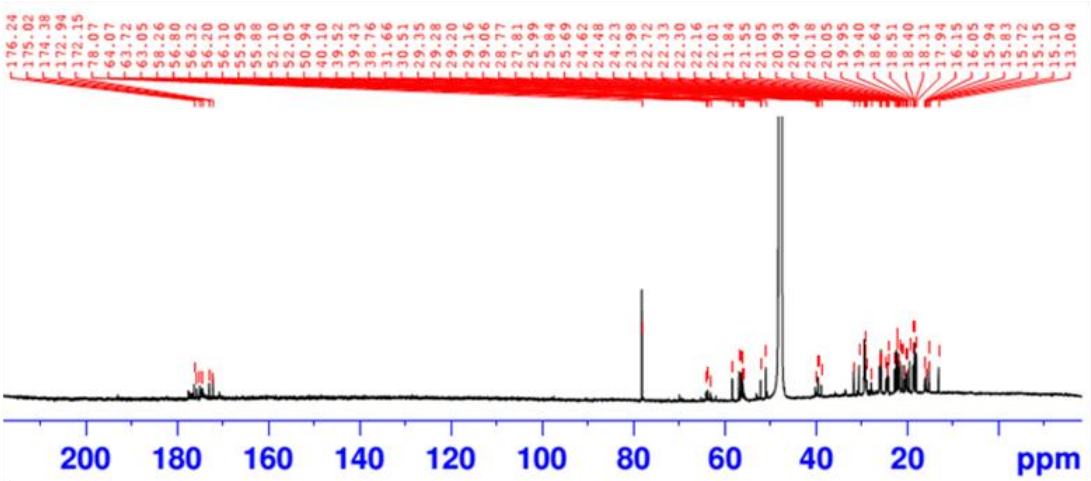
Compound 14 (OMe-Val-Aib-Lue-Ala-Val-Aib-Lue-Val-Aib-Lue-Ala-Val-Aib-Lue-Dimedone-Lue-Aib-Val-Ala-Lue-Aib-Val-Lue-Aib-Val-Ala-Lue-Aib-Val-OMe):

Boc-protected pentadecapeptide ester **7** (0.5 g, 0.32 mmol) was dissolved in 1 mL of DCM followed by addition of 1 mL of TFA (1M) at 0 °C and stirred for 30 min at room temperature. After completion of the reaction, the solvent was stripped off and co-evaporated using toluene to obtain the TFA salt of pentadecapeptide ester which was used for the next step without further purification. Pentadecapeptide TFA salt of **7** (0.46g, 0.32mmol, 1 equiv was dissolved in DMF in a round bottomed flask followed by the addition of DIPEA (2 mL) and finally compound **10b** (0.03 g, 0.09 mmol, 0.3 equiv) was added and heated at 50°C for 2 h. After the completion of the reaction, ice cold water was added to the reaction mixture and it was then taken in DCM and organic layer was washed sequentially with saturated $KHSO_4$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced

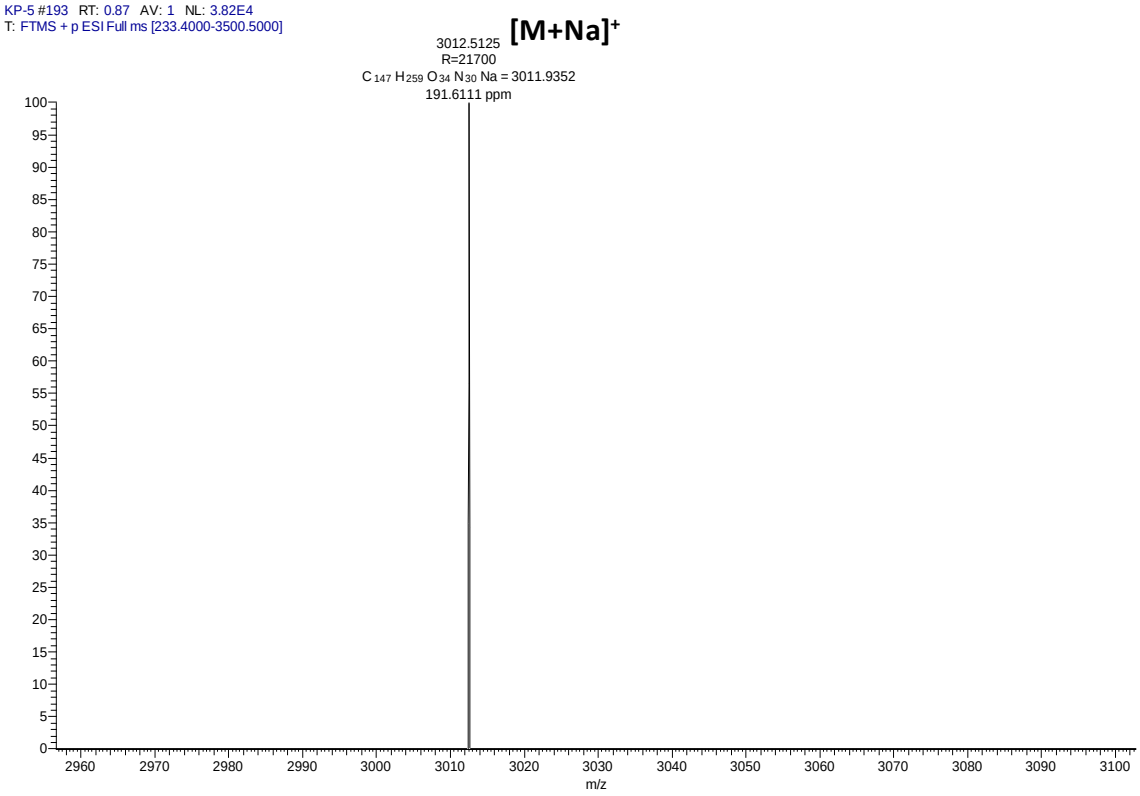
pressure to get the crude product which was further purified by column chromatography (eluent: 4% MeOH/DCM , R_f : 0.5) to furnish compound **14** (0.3 g, 31%) as a white solid; $[\alpha]^{26}_D$: -119° (c = 0.1, MeOH), ^{13}C NMR (175MHz, CD_3OH) = 194.4, 178.9, 178.7, 178.4, 178.1, 177.9, 177.7, 177.1, 176.4, 176.1, 176.0, 175.8, 175.7, 175.3, 174.9, 174.4, 173.6, 172.1, 171.8, 171.4, 164.8, 161.8, 161.6, 159.8, 152.4, 149.8, 140.5, 140.0, 134.9, 129.9, 128.9, 79.5, 71.3, 65.5, 65.3, 65.1, 64.6, 64.5, 63.3, 59.7, 58.2, 57.9, 57.7, 57.6, 57.5, 57.4, 57.3, 57.2, 57.0, 54.4, 53.5, 52.4, 52.0, 48.3, 41.4, 40.9, 40.2, 37.2, 34.9, 33.1, 32.4, 31.9, 30.6, 29.8, 28.9, 27.7, 25.8, 24.1, 23.6, 23.0, 22.4, 21.9, 21.5, 20.8, 19.9, 19.4, 17.4, 16.6; HRMS: $C_{147}H_{259}N_{30}O_{34}Na$, calculated: 3012.9352 $[M+Na]^+$, observed: 3012.5125 $[M+Na]^+$.



^{1}H NMR Spectrum of Compound **14** [700 MHz, 273 K, CD_3OH]



^{13}C NMR Spectrum of Compound **14** [700 MHz, 273 K, CD_3OH]

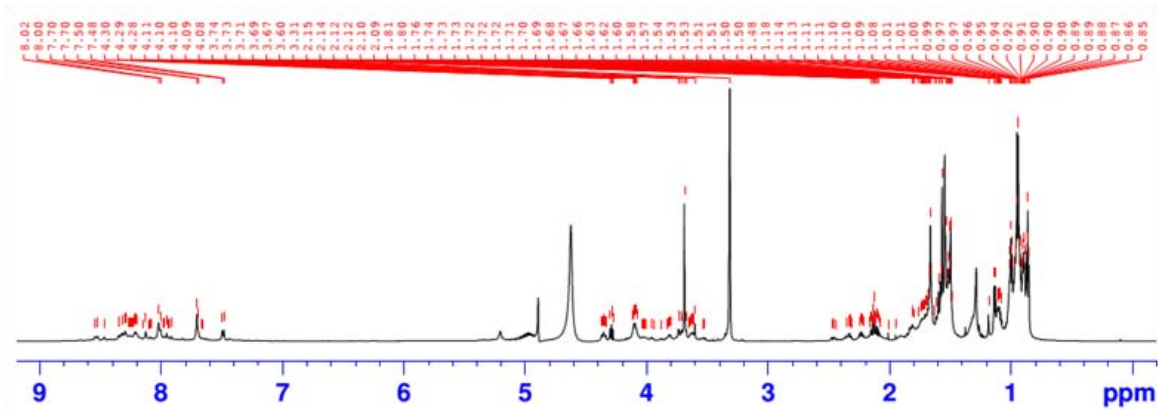


HRMS of Compound **14**

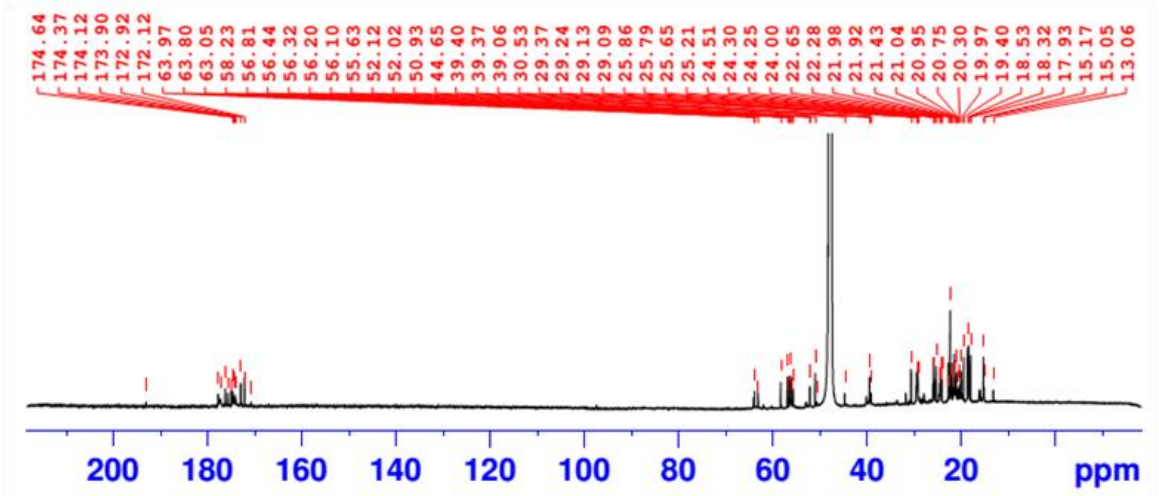
Compound 15 (OMe-Val-Aib-Lue-Ala-Val-Aib-Lue-Gly-Val-Aib-Lue-Ala-Val-Aib-Lue-Dimedone-Lue-Aib-Val-Ala-Lue-Aib-Val- Gly-Lue-Aib-Val-Ala-Lue-Aib-Val-OMe):

Boc-protected hexadecapeptide ester **8** (0.5 g, 0.31 mmol) was dissolved in 1 mL of DCM followed by addition of 1 mL of TFA (1M) at 0 °C and stirred for 30 min at room temperature. After completion of the reaction, the solvent was stripped off and co-evaporated using toluene to obtain the TFA salt of hexadecapeptide ester **8** which was used for the next step without further purification. Hexadecapeptide TFA salt of **8** (0.46g, 0.31 mmol, 1 equiv) was dissolved in DMF in a round bottomed flask followed by the addition of DIPEA 3 mL) and finally compound **10b (**0.025 g, 0.094 mmol, 0.3 equiv) was added and heated at 50°C for 2 h. After the completion of the reaction, ice cold water was added to the reaction mixture and it was then taken in DCM and organic layer was washed sequentially with saturated $KHSO_4$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column

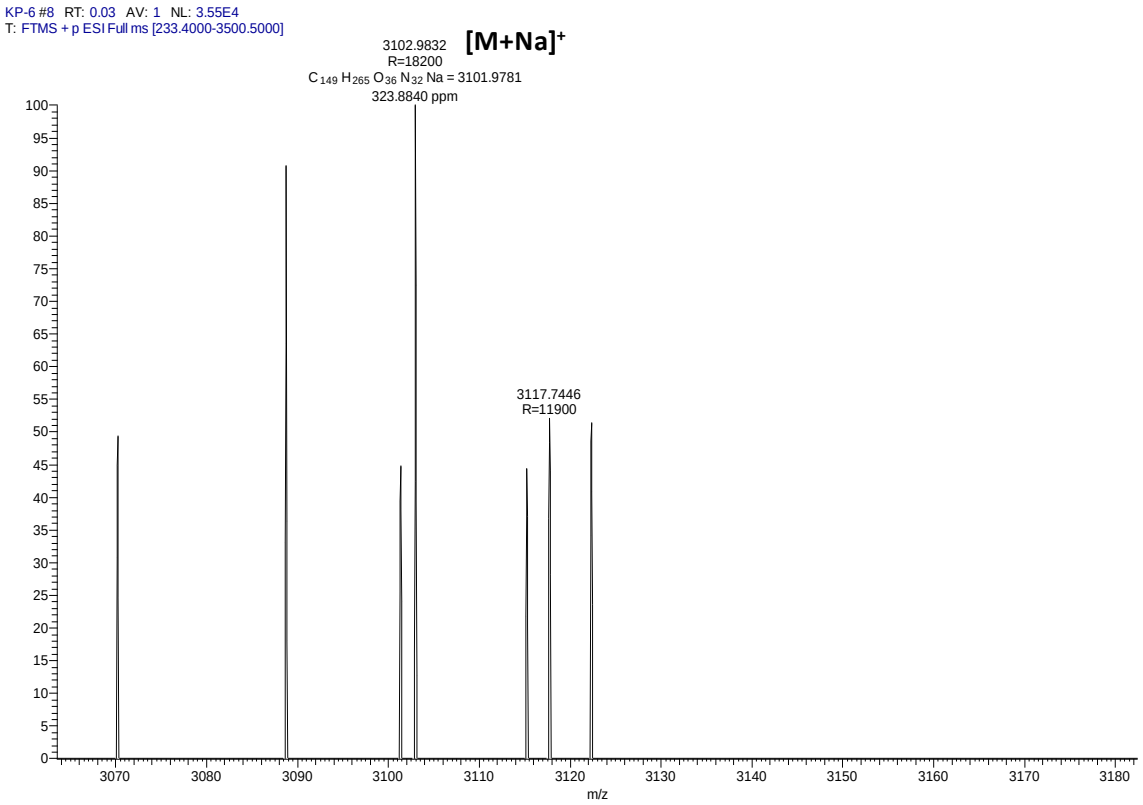
chromatography (eluent: 4% MeOH/DCM , R_f : 0.5) to furnish compound **15** (0.32 g, 32 %) as a white solid; $[\alpha]^{26}_D$: -123° (c = 0.1, MeOH), ^{13}C NMR (175MHz, CD_3OH) = 194.4, 179.1, 178.7, 177.6, 177.1, 176.4, 176.1, 175.5, 174.3, 173.5, 172.2, 65.3, 64.5, 59.7, 58.2, 57.8, 57.4, 57.1, 53.5, 52.2, 46.1, 41.5, 40.7, 37.2, 34.9, 33.1, 32.1, 30.6, 29.5, 27.0, 27.5, 25.8, 25.6, 23.6, 22.5, 21.8, 21.0, 19.7, 17.4, 16.5; HRMS: $C_{149}H_{265}N_{32}O_{36}Na$, calculated: 3102.9781 $[M+Na]^+$, observed: 3102.9832$[M+Na]^+$.



^1H NMR Spectrum of Compound **15** [700 MHz, 273 K, CD_3OH]

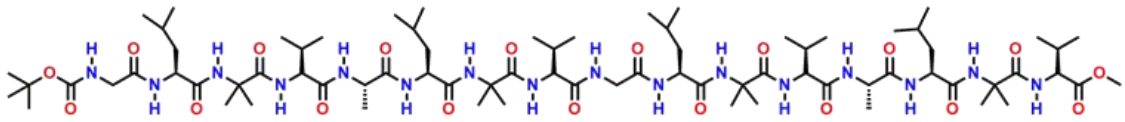


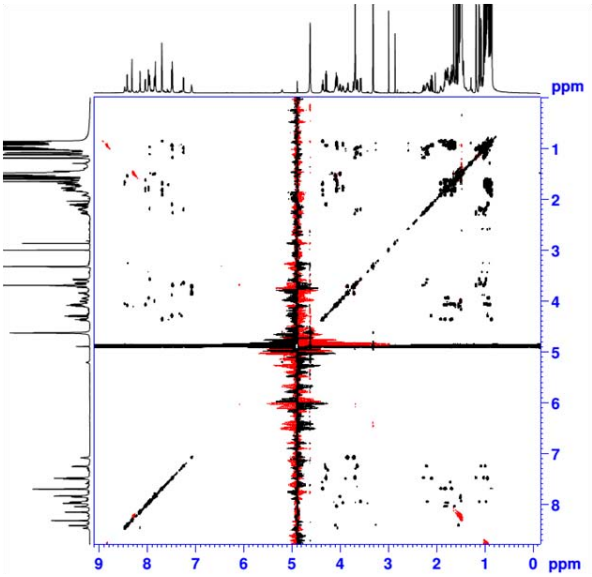
^{13}C- NMR Spectrum of Compound **15**[175 MHz, 273 K, CD_3OH]



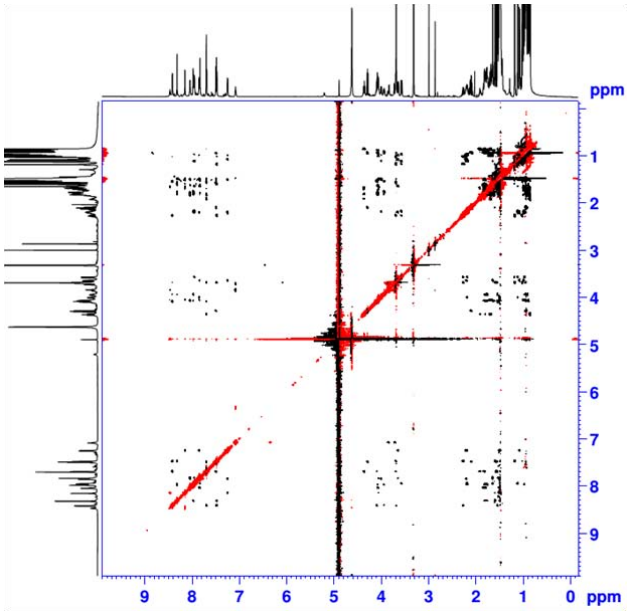
HRMS of Compound **15**

2D NMR spectra of Compound 7





Full TOCSY NMR spectrum of Compound **7** [700 MHz, 273 K, CD_3OH].



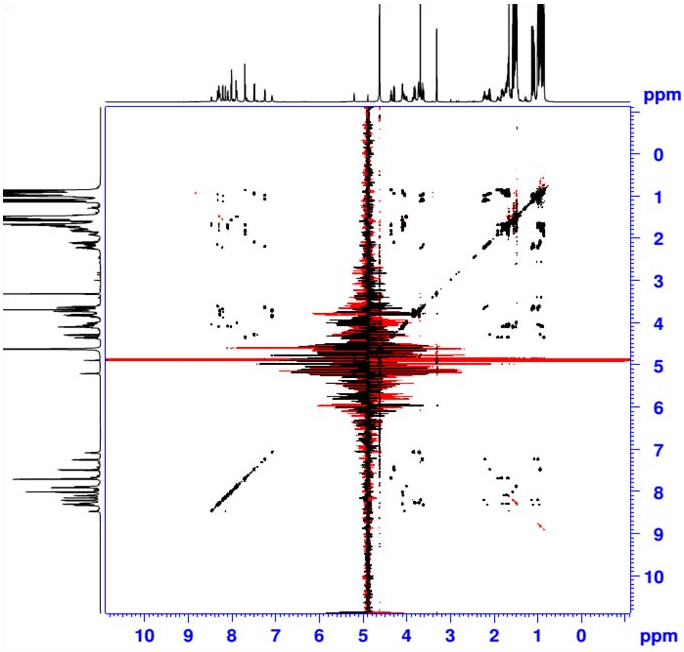
Full ROESY NMR spectrum of Compound **7** [700 MHz, 273 K, CD3OH].

Table-01: Chemical shift assignment of Compound **7** in CD_3OH at 298K.

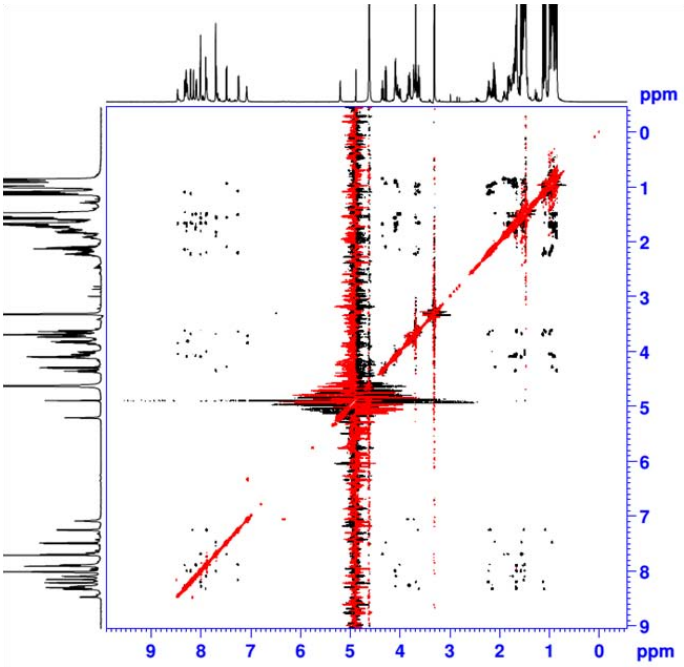
Residue	NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	$C^{\delta}H$
1-Gly	7.07 (t) $^{3}J_{NH\text{-}C^{\alpha}H}$=4.3	3.84 (not resolved) 3.70 (dd) $^{2}J_{C^{\alpha}H\text{-}C^{\alpha}H}$=12.0 $^{3}J_{C^{\alpha}H\text{-}NH}$=6.5	-	-	-
2-Leu	8.46 (d) $^{3}J_{NH\text{-}C^{\alpha}H}$=3.3	4.05 (m)	1.66 (m) 1.65 (m)	1.71 (m)	1.00 (d) $^{3}J_{C^{\delta}H\text{-}C^{\gamma}H}$=6.7 0.95 (d) $^{3}J_{C^{\delta}H\text{-}C^{\gamma}H}$=6.7
3-Aib	8.14 (s)	-	1.51 (s) 1.50 (s)	-	-
4-Val	7.24 (d) $^{3}J_{NH\text{-}C^{\alpha}H}$=6.5	3.64 (dd) $^{3}J_{C^{\alpha}H\text{-}C^{\beta}H}$=9.9 $^{3}J_{C^{\alpha}H\text{-}NH}$=6.5	2.19 (m)	1.07 (d) $^{3}J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.8 0.98 (d) $^{3}J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.8	-
5-Ala	7.85 (d) $^{3}J_{NH\text{-}C^{\alpha}H}$=4.2	3.99 (m)	1.49 (d) $^{3}J_{C^{\beta}H\text{-}C^{\alpha}H}$ =7.6	-	-
6- Leu	8.04 (d) $^{3}J_{NH\text{-}C^{\alpha}H}$=5.7	4.07 (m) $J_{H1,\ H2}$=3.9	1.79 (m) 1.62 (m)	1.83 (m)	0.93 (d) $^{3}J_{C^{\delta}H\text{-}C^{\gamma}H}$=6.7 0.88 (d) $^{3}J_{C^{\delta}H\text{-}$

					$^{C^{\gamma}H}$=6.7
7-Aib	7.82 (s)	-	1.64 (s) 1.49 (s)	-	-
8-Val	7.94 (d) $^{3}J_{NH-C^{\alpha}H}$=4.7	3.56 (dd) $^{3}J_{C^{\alpha}H-C^{\beta}H}$=9.8 $^{3}J_{C^{\alpha}H-NH}$=4.7	2.18 (m)	1.11 (d) $^{3}J_{C^{\gamma}H-C^{\beta}H}$ =6.7 0.96 (d) $^{3}J_{C^{\gamma}H-C^{\beta}H}$ =6.7	-
9-Leu	7.97 (d) $^{3}J_{NH-C^{\alpha}H}$=4.4	3.93 (m)	1.80 (m) 1.76 (m)	1.75 (m)	0.92 (d) $^{3}J_{C^{\delta}H-C^{\gamma}H}$ =6.7 0.91 (d) $^{3}J_{C^{\delta}H-C^{\gamma}H}$ =6.4
10-Aib	8.31 (s)	-	1.59 (s) 1.48 (s)	-	-
11-Val	7.47 (d) $^{3}J_{NH-C^{\alpha}H}$=6.0	3.68 (not resolved)	2.26 (m)	1.17 (d) $^{3}J_{C^{\gamma}H-C^{\beta}H}$ =6.7 1.01 (d) $^{3}J_{C^{\gamma}H-C^{\beta}H}$ =6.7	-
12-Ala	8.41 (d) $^{3}J_{NH-C^{\alpha}H}$=4.5	4.08 (m)	1.53 (d) $^{3}J_{C^{\beta}H-C^{\alpha}H}$ =7.1	-	-
13-Leu	7.69 (d) $^{3}J_{NH-C^{\alpha}H}$=8.3	4.35(ddd) $^{3}J_{C^{\alpha}H-C^{\beta}H}$=12.0 $^{3}J_{C^{\alpha}H-NH}$=8.3 $^{3}J_{C^{\alpha}H-C^{\beta}H}$=3.5	1.82 (m) 1.68 (m)	1.90 (m)	0.86 (d) $^{3}J_{C^{\delta}H-C^{\gamma}H}$ =6.7 0.85 (d) $^{3}J_{C^{\delta}H-C^{\gamma}H}$ =6.7
14-Aib	7.69 (s)	-	1.57 (s) 1.54 (s)	-	-
15-Val	7.48 (d) $^{3}J_{NH-C^{\alpha}H}$=9.0	4.28 (dd) $^{3}J_{C^{\alpha}H-NH}$=9.0 $^{3}J_{C^{\alpha}H-C^{\beta}H}$=7.4	2.09 (m)	0.93 (d) $^{3}J_{C^{\gamma}H-C^{\beta}H}$ =6.7 0.92 (d) $^{3}J_{C^{\gamma}H-C^{\beta}H}$ =6.7	

2D NMR spectra of Compound 8



Full TOCSY NMR spectrum of Compound **8** [700 MHz, 273 K, CD_3OH].



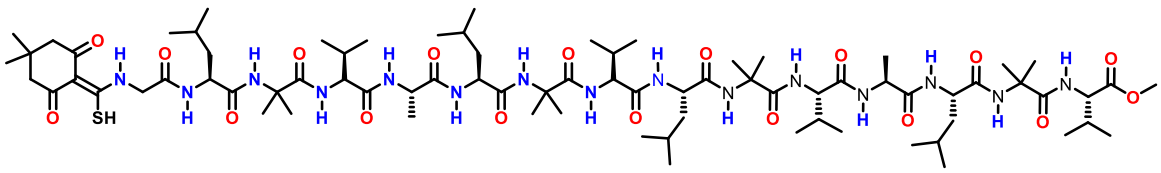
Full ROESY NMR spectrum of Compound **8** [700 MHz, 273 K, CD_3OH].

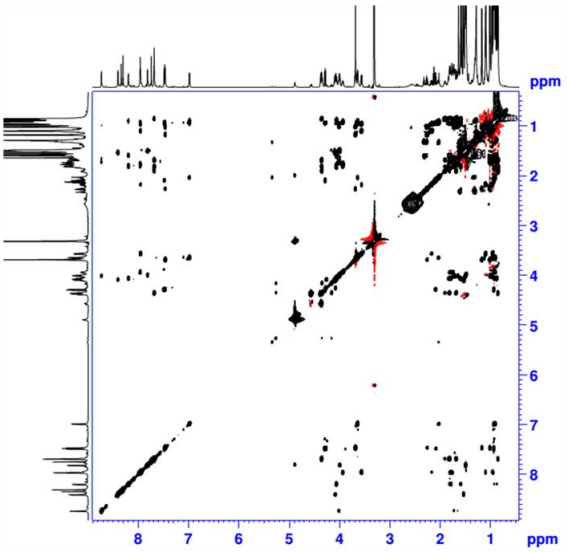
Table-03: Chemical shift assignment of Compound **8** in CD_3OH at 298K.

Residue	NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	$C^{\delta}H$
1-Gly	7.08 (t) $^3J_{NH\text{-}C^{\alpha}H}$=4.3	3.84 3.70 (not resolved)	-	-	-
2-Leu	8.46 (d) $^3J_{NH\text{-}C^{\alpha}H}$=4.3	4.04 (m)	1.66 (m) 1.65 (m)	1.71 (m)	0.99 (d) $^3J_{C^{\delta}H\text{-}C^{\gamma}H}$=6.7 0.95 (d) $^3J_{C^{\delta}H\text{-}C^{\gamma}H}$=6.7
3-Aib	8.15 (s)	-	1.51 (s) 1.50 (s)	-	-
4-Val	7.25 (d) $^3J_{NH\text{-}C^{\alpha}H}$=6.5	3.63 (dd) $^3J_{C^{\alpha}H\text{-}C^{\beta}H=}$10.0 $^3J_{C^{\alpha}H\text{-}NH=}$6.5	2.20 (m)	1.07 (d) $^3J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.8 0.98 (d) $^3J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.8	-
5-Ala	7.88 (d) $^3J_{NH\text{-}C^{\alpha}H}$=4.7	4.00 (m)	1.49 (d) $^3J_{C^{\beta}H\text{-}C^{\alpha}H}$ =7.3	-	-
6- Leu	8.09 (d) $^3J_{NH\text{-}C^{\alpha}H}$=5.8	4.08 (m) $J_{H1,\ H2}$=3.9	1.73 (m) 1.68 (m)	1.78 (m)	0.92 (d) $^3J_{C^{\delta}H\text{-}C^{\gamma}H}$=6.7 0.88(d) $^3J_{C^{\delta}H\text{-}C^{\gamma}H}$=6.7
7-Aib	7.90 (s)	-	1.65 (s) 1.53 (s)	-	-
8-Val	8.32 (d) $^3J_{NH\text{-}C^{\alpha}H}$=5.2	3.61(dd) $^3J_{C^{\alpha}H\text{-}C^{\beta}H=}$10.3 $^3J_{C^{\alpha}H\text{-}NH=}$5.2	2.14 (m)	1.10 (d) $^3J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.6 0.96 (d) $^3J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.6	-
9-Gly	8.27 (dd) $^3J_{NH\text{-}C^{\alpha}H}$=4.3, 6.6	3.81 3.72 (not resolved)	-	-	-

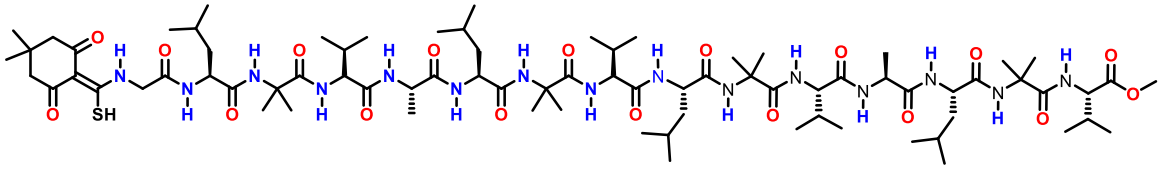
10-Leu	8.29 (d) $^3J_{NH\text{-}C^{\alpha}H}$=5.5	4.09 (m)	1.80 (m) 1.67 (m)	1.73 (m)	0.92(d) $^3J_{C^{\delta}H\text{-}C^{\gamma}H}$ =6.4 0.88(d) $^3J_{C^{\delta}H\text{-}C^{\gamma}H}$ =6.4
11-Aib	8.00 (s)	-	1.65 (s) 1.49 (s)	-	-
12-Val	8.20 (d) $^3J_{NH\text{-}C^{\alpha}H}$=5.2	3.68 (not resolved)	2.23 (m)	1.13 (d) $^3J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.7 0.99 (d) $^3J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.7	-
13-Ala	8.01 (d) $^3J_{NH\text{-}C^{\alpha}H}$=4.7	4.09 (m)	1.57 (d) $^3J_{C^{\beta}H\text{-}C^{\alpha}H}$ =7.8	-	-
14-Leu	7.69 (d) $^3J_{NH\text{-}C^{\alpha}H}$=8.3	4.35(ddd) $^3J_{C^{\alpha}H\text{-}C^{\beta}H}$=12.0 $^3J_{C^{\alpha}H\text{-}NH}$=8.3 $^3J_{C^{\alpha}H\text{-}C^{\beta}H}$=3.5	1.82 (m) 1.68 (m)	1.90 (m)	0.85(d) $^3J_{C^{\delta}H\text{-}C^{\gamma}H}$ =6.4 0.84(d) $^3J_{C^{\delta}H\text{-}C^{\gamma}H}$ =6.4
15-Aib	7.70 (s)	-	1.56 (s) 1.54 (s)	-	-
16-Val	7.48 (d) $^3J_{NH\text{-}C^{\alpha}H}$=9.0	4.28 (dd) $^3J_{C^{\alpha}H\text{-}NH}$=9.0 $^3J_{C^{\alpha}H\text{-}C^{\beta}H}$=7.4	2.09 (m)	0.93 (d) $^3J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.6 0.92 (d) $^3J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.6	

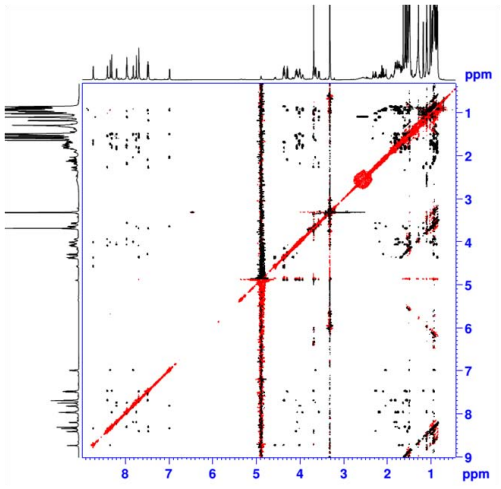
2D NMR studies of 9d





Full TOCSY NMR spectrum of Compound **9d** [700 MHz, 273 K, CD_3OH].





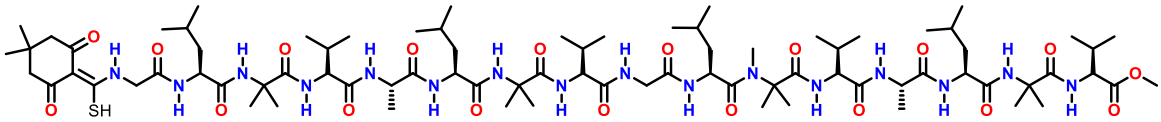
ROESY NMR spectrum of Compound **9d** [700 MHz, 273 K, CD_3OH].

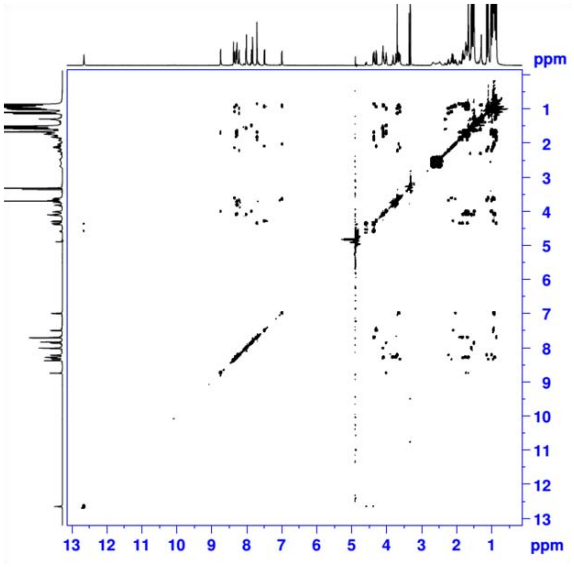
Table-02: Chemical shift assignment of Compound **9d**-CD_3OH at 298K.

Residue	NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	$C^{\delta}H$
1-Gly	12.62 (t) $^{3}J_{NH\text{-}C^{\alpha}H}$=5.7	4.57 $^{2}J_{C^{\alpha}H\text{-}C^{\alpha}H}$=16.4 $^{3}J_{C^{\alpha}H\text{-}NH}$=7.0 4.37 (not resolved)	-	-	-
2-Leu	8.73 (d) $^{3}J_{NH\text{-}C^{\alpha}H}$=2.6	4.01 (m)	1.72 (m) 1.65 (m)	1.72 (m)	1.00 (d) $^{3}J_{C^{\delta}H\text{-}C^{\gamma}H}$=6.4 0.97 (d) $^{3}J_{C^{\delta}H\text{-}C^{\gamma}H}$=6.4
3-Aib	8.33 (s)	-	1.50 (s) 1.49 (s)	-	-
4-Val	6.98 (d) $^{3}J_{NH\text{-}C^{\alpha}H}$=7.1	3.64 (dd) $^{3}J_{C^{\alpha}H\text{-}C^{\beta}H}$=9.9 $^{3}J_{C^{\alpha}H\text{-}NH}$=7.1	2.04 (m)	0.93 (d) $^{3}J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.7 0.90 (d) $^{3}J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.7	-
5-Ala	7.81 (d) $^{3}J_{NH\text{-}C^{\alpha}H}$=4.6	3.99 (m)	1.49 (d) $^{3}J_{C^{\beta}H\text{-}C^{\alpha}H}$ =7.3	-	-
6- Leu	8.19(d) $^{3}J_{NH\text{-}C^{\alpha}H}$=5.7	4.05 (m) $J_{H1,\ H2}$=3.9	1.78 (m) 1.59 (m)	1.83 (m)	0.92 (d) $^{3}J_{C^{\delta}H\text{-}C^{\gamma}H}$=6.7 0.87 (d) $^{3}J_{C^{\delta}H\text{-}C^{\gamma}H}$=6.7
7-Aib	7.74 (s)	-	1.63 (s) 1.49 (s)	-	-
8-Val	7.95 (d) $^{3}J_{NH\text{-}C^{\alpha}H}$=4.9	3.56(dd) $^{3}J_{C^{\alpha}H\text{-}C^{\beta}H}$=9.8 $^{3}J_{C^{\alpha}H\text{-}NH}$=4.9	2.18 (m)	1.09 (d) $^{3}J_{C^{\gamma}H\text{-}C^{\beta}H}$ =7.0 0.95 (d) $^{3}J_{C^{\gamma}H\text{-}C^{\beta}H}$ =7.0	-

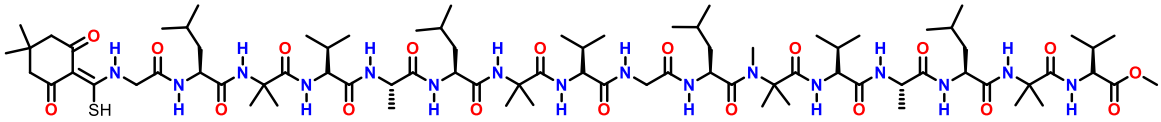
9-Leu	7.56 (d) $^3J_{NH\text{-}C^{\alpha}H}$=4.5	3.93 (m)	1.80 (m) 1.75 (m)	1.75 (m)	0.92 (d) $^3J_{C^{\delta}H\text{-}C^{\gamma}H}$ =6.8 0.90 (d) $^3J_{C^{\delta}H\text{-}C^{\gamma}H}$ =6.8
10-Aib	8.30 (s)	-	1.59 (s) 1.48 (s)	-	-
11-Val	7.46 (d) $^3J_{NH\text{-}C^{\alpha}H}$=6.0	3.68 (dd) (not resolved)	2.22 (m)	1.17 (d) $^3J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.7 1.01 (d) $^3J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.7	-
12-Ala	8.40 (d) $^3J_{NH\text{-}C^{\alpha}H}$=4.4	4.08 (m)	1.53 (d) $^3J_{C^{\beta}H\text{-}C^{\alpha}H}$ =7.1	-	-
13-Leu	7.68 (d) $^3J_{NH\text{-}C^{\alpha}H}$=8.2	4.35(m)	1.81 (m) 1.68 (m)	1.90 (m)	0.86 (d) $^3J_{C^{\delta}H\text{-}C^{\gamma}H}$ =6.6 0.85 (d) $^3J_{C^{\delta}H\text{-}C^{\gamma}H}$ =6.6
14-Aib	7.68 (s)	-	1.57 (s) 1.53 (s)	-	-
15-Val	7.48 (d) $^3J_{NH\text{-}C^{\alpha}H}$=9.0	4.28 (dd) $^3J_{C^{\alpha}H\text{-}NH=}$9.0 $^3J_{C^{\alpha}H\text{-}C^{\beta}H=}$7.5	2.09 (m)	0.94 (d) $^3J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.7 0.93 (d) $^3J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.7	

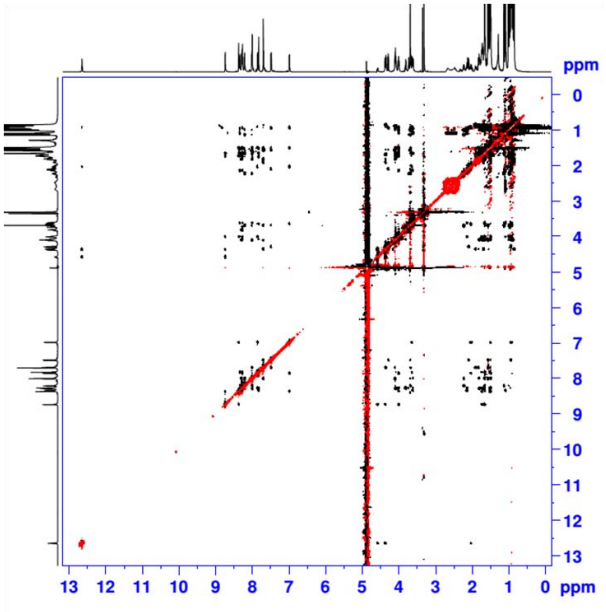
2D NMR Spectra of 9e





Full TOCSY NMR spectrum of Compound **9e** [700 MHz, 273 K, CD_3OH].





Full ROESY NMR spectrum of Compound **9e** [700 MHz, 273 K, CD_3OH].

Table-04: Chemical shift assignment of Compound **9e**-CD_3OH at 298K.

Residue	NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	$C^{\delta}H$
1-Gly	12.64 (t) $^{3}J_{NH\text{-}C^{\alpha}H}$=4.3	4.58 4.37 (not resolved)	-	-	-
2-Leu	8.73 (d) $^{3}J_{NH\text{-}C^{\alpha}H}$=4.3	4.04 (m)	1.66 (m) 1.65 (m)	1.72 (m)	0.91 (d) $^{3}J_{C^{\delta}H\text{-}C^{\gamma}H}$=6.7 0.90 (d) $^{3}J_{C^{\delta}H\text{-}C^{\gamma}H}$=6.7
3-Aib	8.37 (s)	-	1.50 (s) 1.49 (s)	-	-
4-Val	6.98 (d) $^{3}J_{NH\text{-}C^{\alpha}H}$=7.0	3.63 (dd) $^{3}J_{C^{\alpha}H\text{-}C^{\beta}H=}$10.0 $^{3}J_{C^{\alpha}H\text{-}NH=}$7.0	2.03 (m)	0.92 (d) $^{3}J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.6 0.91 (d) $^{3}J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.6	-
5-Ala	7.85 (d) $^{3}J_{NH\text{-}C^{\alpha}H}$=4.5	4.00 (m)	1.49 (d) $^{3}J_{C^{\beta}H\text{-}C^{\alpha}H}$ =7.3	-	-
6- Leu	8.27(d) $^{3}J_{NH\text{-}C^{\alpha}H}$=5.4	4.07 (m) $J_{H1,\ H2}$=3.9	1.73 (m) 1.64 (m)	1.78 (m)	0.90 (d) $^{3}J_{C^{\delta}H\text{-}C^{\gamma}H}$=6.7 0.89(d) $^{3}J_{C^{\delta}H\text{-}C^{\gamma}H}$=6.7
7-Aib	7.82 (s)	-	1.65 (s) 1.52 (s)	-	-
8-Val	8.34 (d) $^{3}J_{NH\text{-}C^{\alpha}H}$=5.3	3.61(dd) $^{3}J_{C^{\alpha}H\text{-}C^{\beta}H=}$10.3 $^{3}J_{C^{\alpha}H\text{-}NH=}$5.3	2.14 (m)	1.09 (d) $^{3}J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.7 0.95 (d) $^{3}J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.7	-
9-Gly	8.27 (d) $^{3}J_{NH\text{-}C^{\alpha}H}$=5.2	3.80 3.72 (not resolved)	-	-	-
10-Leu	8.29 (d) $^{3}J_{NH\text{-}C^{\alpha}H}$=5.4	4.09 (m)	1.82 (m) 1.67 (m)	1.73 (m)	0.92(d) $^{3}J_{C^{\delta}H\text{-}C^{\gamma}H}$ =6.4 0.88(d)$^{3}J_{C^{\delta}H\text{-}C^{\gamma}H}$ =6.4

11-Aib	8.00 (s)	-	1.65 (s) 1.50 (s)	-	-
12-Val	8.21 (d) $^3J_{NH\text{-}C^{\alpha}H}$=5.2	3.68 (dd) $^3J_{C^{\alpha}H\text{-}C^{\beta}H=}$10.0 $^3J_{C^{\alpha}H\text{-}NH=}$5.1	2.22 (m)	1.13 (d) $^3J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.7 0.99 (d) $^3J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.7	-
13-Ala	8.01 (d) $^3J_{NH\text{-}C^{\alpha}H}$=4.6	4.09 (m)	1.57 (d) $^3J_{C^{\beta}H\text{-}C^{\alpha}H}$ =7.8	-	-
14-Leu	7.69 (d) $^3J_{NH\text{-}C^{\alpha}H}$=8.3	4.35(m)	1.83 (m) 1.68 (m)	1.90 (m)	0.85(d) $^3J_{C^{\delta}H\text{-}C^{\gamma}H}$ =6.4 0.84(d) $^3J_{C^{\delta}H\text{-}C^{\gamma}H}$ =6.4
15-Aib	7.70 (s)	-	1.56 (s) 1.55 (s)	-	-
16-Val	7.48 (d) $^3J_{NH\text{-}C^{\alpha}H}$=8.9	4.28 (dd) $^3J_{C^{\alpha}H\text{-}NH=}$8.9 $^3J_{C^{\alpha}H\text{-}C^{\beta}H=}$7.5	2.09 (m)	0.94 (d) $^3J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.8 0.93 (d) $^3J_{C^{\gamma}H\text{-}C^{\beta}H}$ =6.8	

1.8 References and Notes:

1. (a) Salonen, L. M.; Ellermann; Miederich, F. *Angew. Chem. Int. Ed*. **2011**, *50*, 4808; (b) Scott L. C.; Christopher A. H.; *Chem. Soc. Rev.* **2007**, *36*, 172-188; (c) Karshikoff, A. *Non-covalent interactions in proteins*, Imperial College press, United Kingdom, **2006**.

2. (a) Berg, J. M.; Tymoczko, J. L.; Stryer, L. *Biochemistry. 5th. New York: WH Freeman*. **2002**, *38*, p.76. (b) Beck, Martin, *et al.* "The quantitative proteome of a human cell line." *Molecular systems biology* **2011**, *7*, 549.

3. Linderstrom-Lang, K. U. *'Proteins and Enzymes', Lane Medical Lectures*, Stannford University Publications, University Series, Medical Sciences, Stanford University Press, **1952**, 6.

4. (a) Braker, D. *et al Proc. Natl. Acad. Sci.* **2003**, *100*, 12105; (b) Dobson, C. M. *et al*. *Nature* **2001**, *410*, 165; (c) Branden, C.; *Introduction to protein structure*, 2nd Ed. Newyork, NY: Garland, **1988**. (d) Blundell, T. *et al*. *Annu. Rev. Biophys. Bioeng*. **1984**, *13*, 453; (e) Richardson, D. C. *et al*. *Proc. Natl. Acad. Sci.* **2002**, *99*, 2754.

5. a) Wood, E. J.; *Proteins: Structures and molecular properties:* By Creighton, T. E. pp 515. W H Freeman, New York. 1983. (b) Degrado, W. F.; Regan, L. *Science*, **1988**, *241*, 976-978; (c) Richardson, D. C.; Richardson, J. S. *Biochem. Sci.* **1989**, *14*, 304-309.

6. (a) Gorp, J. J. V; Vekemans, J. A. M.; Meijer, E. W. *Chem. Commun.* **2004**, *120*, 60–61; (b) Claridge, T. D. W.; Long, D. D.; Baker, C. M.; Odell, B.; Grant, G. H.; Edwards, A. A.; Tranter, G. E., Fleet, G. W.; Smith, M. D. *J. Org. Chem.* **2005**, *70*, 2082–2090; (c) Violette, A; Averlant-Petit, M. C.; Semetey, V. C; Hemmerlin, Casimir, R.; Graff, R.; Marraud, M. J.; Briand, -P; Rognan, D; Guichard, G. *J. Am. Chem. Soc.* **2005**, *127*, 2156–2164.

7. Pace, C. N; Scholtz, J. M. *Biophys J.* **1998**, *75*, 422–427.

8. Latham, P.W. *Nat Biotechnol.* **1999**, *17*, 755-7.

9. (a) Seebach, D.; Overhand, M.; Kuehnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. *Hel*V. *Chim. Acta* **1996**, *79*, 913–941; (b) Nelson, J. C.; Saven, J. G.; Moore, J. S.; Wolynes, P. G. *Science.* **1997**, *277*, 1793–1796. (c) Yuan, L.; Zeng, H.; Yamato,K.; Sanford, A. R.; Feng, W.; Atreya, H. S.; Sukumaran, D. K.; Szyperski, T.; Gong, B. *J. Am. Chem. Soc.* **2004**, *126*, 16528–16537

10. (a) Lehn, *et al*. *Helv. Chim. Acta.* **2003**, *86*, 1598-1624; (b) Jiang, H.; Le´ger, J.-M.; Huc, I. *J. Am. Chem. Soc.* **2003**, *125*, 3448–3449.

11. (a) Kenner, G. W; Sheppard, R. C. *Nature.* **1958**, *181*,48; (b) Bruckner, H.; Graf, H. *Experientia*. **1983**, *39*, 528; (c) Thirumalachur, M. J. *Hindustan Antibiot. Bull.* **1968**, *10*, 287; (d) Vaidya, M. G; Deshmukh, P. V; Chari, S.N.

Hindustan Antibiot. Bull. **1968**, *11*, 81; (e) Pandey, R. C.; Meng, H; Cook Jr., J. C.; Rinehart Jr., K. L. *J. Antibiot.* **1978**, *31*, 241; (f) Fox, R. J.; Richards, F. M. *Nature* **1982**, *300*, 325; (g) Whitmore, L.; Chugh, J. K.; Snook, C. F.; Wallace, B. A. *J. Pept. Sci.* **2003**, *9*, 663; (h) Whitmore, L.; Wallace, B. A. *Nucleic Acids Res.* **2004**, *32*, 593. (i) Daniel, J. F.; Filho, E. R. *Nat. Prod. Rep.* **2007**, *24*, 1128.

12. (a) Degenkolb, T.; Berg, A.; Gams, W.; Schlegel, B.; Grafe, U. *J. Pept. Sci.* **2003**, *9*, 666; (b) Szekeres, A.; Leitgeb, B.; Kredics, L.; Antal, Z.; Hatvani, L.; Manczinger, L.; Vagvclgyi, C. *Acta Microbiol. Immunol.Hung.* **2005**, *52*, 137; (c) Duclohier, H.; *Chem. Biodivers.* **2007**, *4*, 1023; (d) Degenkolb, T.; Kirschbaum, J.; Bruckner, H. *Chem. Biodivers.* **2007**, *4*, 1052.

13. (a) Marshall, R.; Bosshard, H. E. *Circ. Res.* **1972**, 30–31; (b) Ramachandran, G. N.; Chandrasekaran, R.; in 'Progress in Peptide Research Volume II: Proceedings of the Second American Peptide Symposium, Cleveland, **1970**', Ed. S. Lande, Gordon and Breach, New York, **1972**, p. 195.

14. Ramachandran, G. N.; Sasisekharan, V. *Adv. Protein Chem.* **1968**, *23*, 283.

15. (a) Shamala, N.; Nagaraj, R.; Balaram, P. *Biochem. Biophys. Res. Commun.* **1977**, *79*, 292; (b) Shamala, N.; Nagaraj, R.; Balaram, P. *J. Chem. Soc., Chem. Commun.* **1978**, 996; (c) Benedetti, E.; Bavoso, A.; Blasio, B. Di.; Pavone, V.; Pedone, C.; Crisma, M.; Bonora, M.; Toniolo, C. *J. Am. Chem. Soc.* **1982**, *104*, 2437; (d) Toniolo, C.; Bonora, G. M.; Bavoso, A.; Benedetti, E.; Blasio, B. Di.; Pavone, V.; Pedone, C. *Biopolymers*. **1983**, *22*, 205. (e) Prasad, B. V. V.; Balaram, P. *CRC Crit. Rev. Biochem*. **1984**, *16*, 307. (f) Karle, I. L.; Balaram, P.; *Biochemistry*. **1990**, *29*, 6747.

16. (a) Toniolo, C.; Benedetti, E. ISI Atlas of Science: *Biochemistry.* **1988**, *1*, 225; (b) Toniolo, C.; Benedetti, E. *Trends Biochem. Sci.* **1991**, *16*, 350; (c) Toniolo, C.; Benedetti, E. *Macromolecules*. **1991**, *24*, 4004; (d) Karle, I. L. *Acta Crystallogr, Sect. B.* **1992**, *48*, 341; (e) Benedetti, E. *Biopolymers.* **1996**, 40, 3;

(f) Karle I. L. *et al*. *Biopolymers*. **1996**, *40*, 157; (g) Kaul, R.; Balaram, P. *Bioorg. Med. Chem*. **1999**, 7, 105; (h) Venkatraman, J.; Shankaramma, S. C.; Balaram, P. *Chem. Rev.* **2001**, *101*, 3131; (i) Toniolo, C.; Crisma, M.; Formaggio, F.; Peggion, C. *Biopolymers.* **2001**, *60*, 396; (j) Aravinda, S.; Shamala, N.; Roy, R. S.; Balaram, P. *Proc. Indian Acad. Sci. Chem. Sci.* **2003**, *115*, 373.

17. (a) Karle, I. L.; Flippen-Anderson, J. L.; Gurunath, R.; Balaram, P. *Protein Sci.* **1994**, *3*, 1547; (b) Millhauser, G. L. *Biochemistry*. **1995**, *34*, 3873; (c) Yoder, G.; Polese, A.; Silva, R. A. G. D.; Formaggio, F.; Crisma, M.; Broxterman, Q. B.; Kamphuis, J.; Toniolo, C.; Keiderling, T. A. *J. Am. Chem. Soc*. **1997**, *119*, 10278; (d) Mammi, S.; Rainaldi, M.; Bellanda, M.; Schievano, E.; Peggion, E.; Broxterman, Q. B.; Formaggio, F.; Crisma, M.; Toniolo, C. *J. Am. Chem. Soc.* **2000**, *122*, 11735; Silva, R. A. G.; Yasui, S. C.; Kubelka, J.; Formaggio, F.; Toniolo, C.; Keiderling, T. *Biopolymers.* **2002**, *65*, 229.

18. Karle, I. L.; Flippen-Anderson, J. L.; Uma, K.; Sukumar,M.; Balaram, P. *J Amer Chem Soc.* **1990**, *112*, 9350–9356.

19. Karle, I. L.; Flippen-Anderson, J. L.; Uma, K.; Sukumar, M.; Balaram, P. *Biopolymers.* **1990**, *29*, 1433–1442.

20. (a) Fox, R. O.; Richards, F. M. *Nature* (London). **1982**, *300*, 325–330; (b) Karle, I. L.; Flippen-Anderson, J. L.; Agarwalla, S.; Balaram, P. *Biopolymers.* **1994**, *34*, 721–735.

21. (a)Richardson, J. S. *Adv Protein Chem*. **1981**, *34*, 167–339; (b) Wilmot, C. M.; Thornton, J. M. *J Mol Biol*. **1988**, *20*, 221–232. (c) Ramakrishnan, C.; Srinivasan, N. *Curr Sci*. **1990**, *59*, 851–861.

22. (a) Karle, I. L.; Banerjee, A.; Bhattacharjya, S.; Balaram, P. *Biopolymers.* **1996**, 38, 515–526. (b) Crisma, M.; Monaco, V.; Formaggio, F.; Toniolo, C.; George, C.; Flippen-Anderson, J. L. *Lett Pept Sci*. **1997**, *4*, 213–218.

23. (a) Schellman, C. In Protein Folding; Jaenicke, R., Ed.; Elsevier/North Holland Medical Press: Amsterdam, **1980**, 53–61; (b) Richardson, J. S.; Richardson, D. C. *Science.* **1988**, *240*, 1648–1652; (c) Milner-White, E. J. *J Mol Biol*. **1988**, *199*, 503–511; (d) Nagarajaram, H. S.; Sowdhamini, R.;

Ramakrishnan,C.; Balaram, P. *FEBS Lett*. **1993**, *321*, 79–83; (e) Aurora, R.; Srinivasan, R.; Rose, G. D. *Science*. **1994**, *264*, 1126–1130.

24. Banerjee, A.; Datta, S.; Pramanik, A; Shamala N.; Balaram P. *J. Am. Chem. Soc*. **1996,** *118*, 9477-9483.

25. Vgber, D. F.; Strachan, R. G.; Bergstrand, S. J.; Holly, F. W.; Homnick, C. F.; Hirschmann, R. *J. Am. Chem. Soc.* **1976**, *98*, 2367. Freidinger, R. M.; Veber, D. F.; Perlow, D. S.; Brooks, J. R.; Saperstein, R. *Science*. **1980**, *210*, 656.

26. Sasaki, T.; Kaiser, E. T. *J. Am. Chem. Soc*. **1989**, *111*, 380.

27. Ghadiri, M. R.; Soares, C.; Choi, C. *J. Am. Chem. Soc.* **1992,** *114*, 4000.

28. Kemp, D. S.; Curran, T. P. *Tetrahedron Lett.* **1988**, *29*, 4935.

29. Carey, R.I., Altmann, K.H. and Mutter, M. Protein design: template-assembled synthetic proteins. *Host-Guest Molecular Interactions: From Chemistry to Biology*. **1991**, *82*, 187.

30. Gompper, A; Topfl, W. *Chem. Ber.*, **1962**, *95*, 2861.

31. Bruker (**2016**). *APEX2*, *SAINT* and *SADABS*. Bruker AXS Inc., Madison, Wisconsin, USA.

32. Sheldrick, G. M. *Acta Crystallogr*., **2008**, A64, 112.

33. https://www.mun.ca/biology/scarr/iGen3_06-04.html.

Chapter 2

Design and synthesis of novel unnatural amino acid incorporated structural mimics of potent antidepressant drug Rapastinel

2.1 Introduction

2.1.1 NMDA receptor:

There are three types of ionotropic glutamate receptor namely NMDAR, kainate and AMPA receptors. NMDAR gets activated when glycine or D-Serine bind to it permitting the positive charged ions to glide through the cell membrane.[1] NMDA receptor has drawn considerable interest as it covers a wide range of CNS disorders.[2] NMDAR finds importance in controlling function of memory as well as synaptic plasticity.[3] The agonist molecule N-methyl-D-aspartate (NMDA) binds selectively and specifically to one receptor and not to other glutamate receptors which is why it has been named as NMDA receptor.

2.1.2 Activation of NMDA receptor:

NMDARs activation involves binding of two coagonists, glycine or D-Serine and L-glutamate.[4] NMDAR activation leads to opening of ion channel but it is non-selective to cations with reverse potential near 0 mV. Ligand binding is primarily responsible for opening and closing of the ion channel and current flow through the ion channel is voltage dependent. Mg^{2+} and Zn^{2+} can bind to the specific sites on the receptor thereby disrupting the flow of other cations through the open ion channel. Depolarization of the cell leads to discharge of Mg^{2+} and Ca^{2+} ions which leads to voltage dependent flow of Na^{+} and small amounts of Ca^{2+} ions through the open ion channel which is responsible for synaptic plasticity.[5]

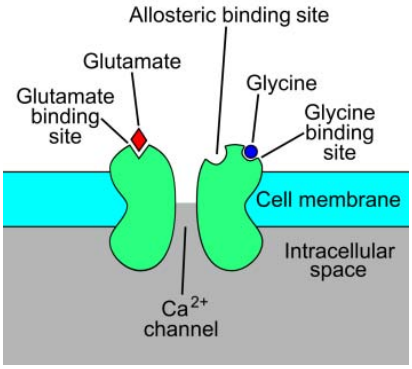


Figure 2.1.1 Depiction of an activated NMDAR (Image Source: internet)[28]

2.1.3 Glutamate and Glycine : Coagonists

Binding sites of glutamate and glycine are structurally similar and seem to have similar role in receptor activation. However, glycine and glutamate have distinct functions physiologically. Thus glycine is considered to be NMDA modulator setting apart from the agonist glutamate. Glycine and glutamate binding sites on the NMDAR illustrate two different therapeutic targets since glycine acts more as a modulatory role. Efforts are also been carried out in the development of partial agonists for the glycine site thereby acting as negative modulators of the NMDAR function.[6]

NMDARs configure to tetrameric complexes by interacting with multiple sub units namely NR1, NR2, NR3 etc.[7] Each subunit has its own signaling, biophysical and pharmacological properties, which determines specific functions to be carried in the CNS in different conditions. All these sub types constitute a common extracellular N terminus, a membrane region and an intracellular C terminus as depicted in the figure 2.1.2

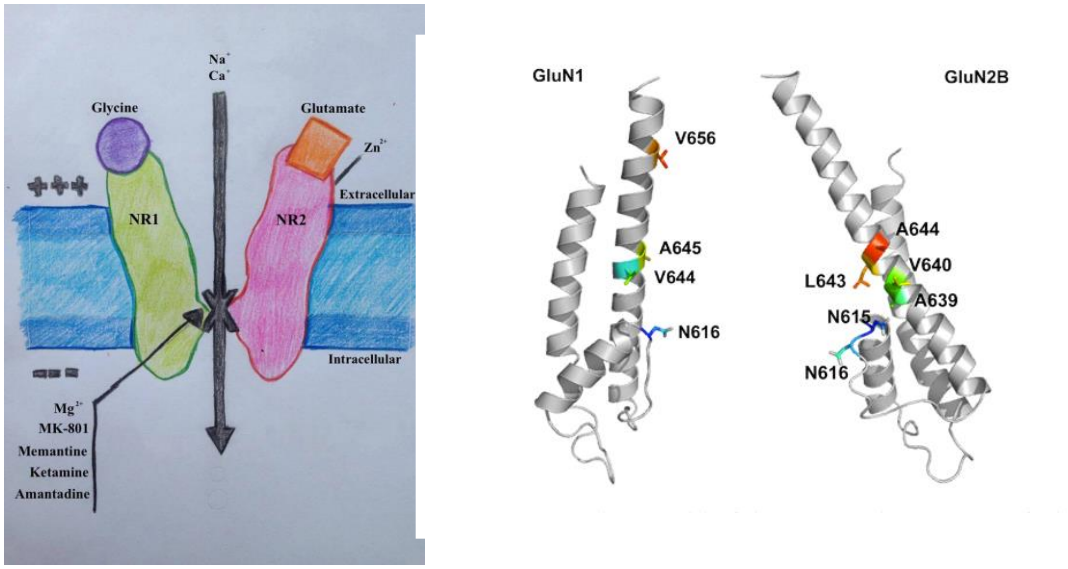
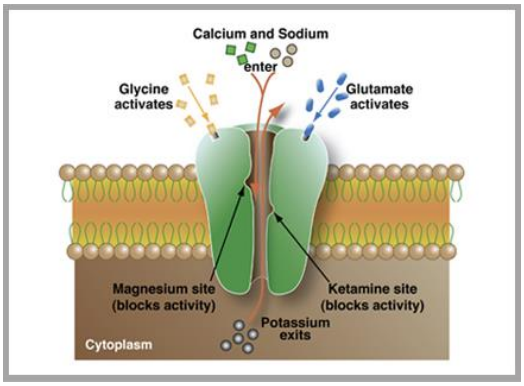


Figure 2.1.2 NR1/NR2B subunit (left). Transmembrane region of NR1 [left][29a] and NR2B [right] subunits of NMDA receptor (right)[29b] (Image Source: internet)

Excessive amounts of excitatory amino acid glutamate may be released from neurons deprived of oxygen which leads to brain ischemia.[8]This excess glutamate binds to the receptor leading to over activation of NMDA receptor which allows excessive flow of Ca^{2+} ion through open ion channel. This phenomenon is called excitotoxicity. These have general implications in causing neurological disorders such as Huntington's disease, Alzheimer's disease and Parkinson's disease. However it is necessary to preserve the NMDA receptor activity from excitotoxicity by trying to block the excess flow of Ca^{2+} ions. This can only be achieved by developing some antagonists which can block the receptor ion channel if it is excessively open.[9]

The balance in opening and closing of the open ion channel stimulates the activation of NMDA receptor. When the binding of the ligands to the allosteric sites of protein occupying on the extracellular surface takes place, it effects on the overall conformation of the protein structure and hence contemplating in the channel closing or opening or partially closing or partially opening. This regulatory mode of action controls the flow of ions through open ion channel thereby enhancing the NMDA receptor activity. Hence, NMDA receptor compounds are called partial agonists (agonists/antagonists) as they exert dual effect on it through allosteric sites. A partial agonist displaces some of the ligands in the presence of principle site ligand thereby decreasing the flow of Ca^{2+} ion through the open ion channel. On the other side, the flow of Ca^{2+} ion increases in the absence of or lowered level of principal site ligand.[9]



In order to provide pharmaceutical benefits, there exists a need to discover potent antidepressant drugs that are capable of binding glycine binding site of the NMDA receptors.

Figure 2.1.3: The NMDA (N-Methyl D-Aspartate) receptor (Image Source: internet)[30]

2.1.4 Rapastinel (GLYX-13)

Major depressive disorder (MDD) has affected 250 million people worldwide. There are numerous medications ranging from amitriptyline to venlafaxine to treat MDD. However current prescribed drugs are not effective to almost 70% of people suffering from MDD. Hence MDD has become the leading issue in the medical world.

Allergen Company is currently testing a compound called rapastinel previously known as GLYX-13 for MDD.[10] The initial screening showed promising results from the traditional drug compounds and it has been found to be almost two fold increase in the activity for MDD without untoward side effects.[10]

Rapastinel is a novel potent antidepressant drug that is under clinical development for depression. Rapastinel is known to bind the glycine site of the NMDA receptor. It is a NMDA receptor modulator that has partial agonist properties and hence enhances the antidepressant effects in both humans and in animal models. Structurally, GLYX-13 is simple amidated tetra peptide having the amino acid sequence threonine-proline-proline-threonine. Rapastinel has been granted breakthrough therapy designation by the food and drug administration (FDA) and it is currently in phase III clinical development for treatment-resistant depression.[11] Rapastinel has found to be well tolerated and there exists no hallucinogenic side effects as compared to ketamine which makes rapastinel to be a potent drug candidate.

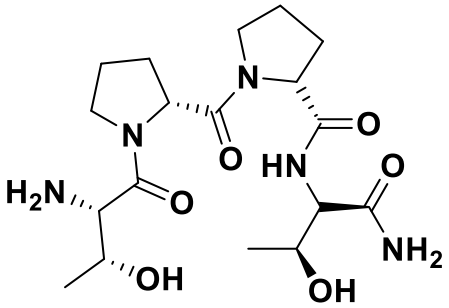


Figure 2.1.4: Molecular structure of potent antidepressant drug rapastinel (GLYX-13).

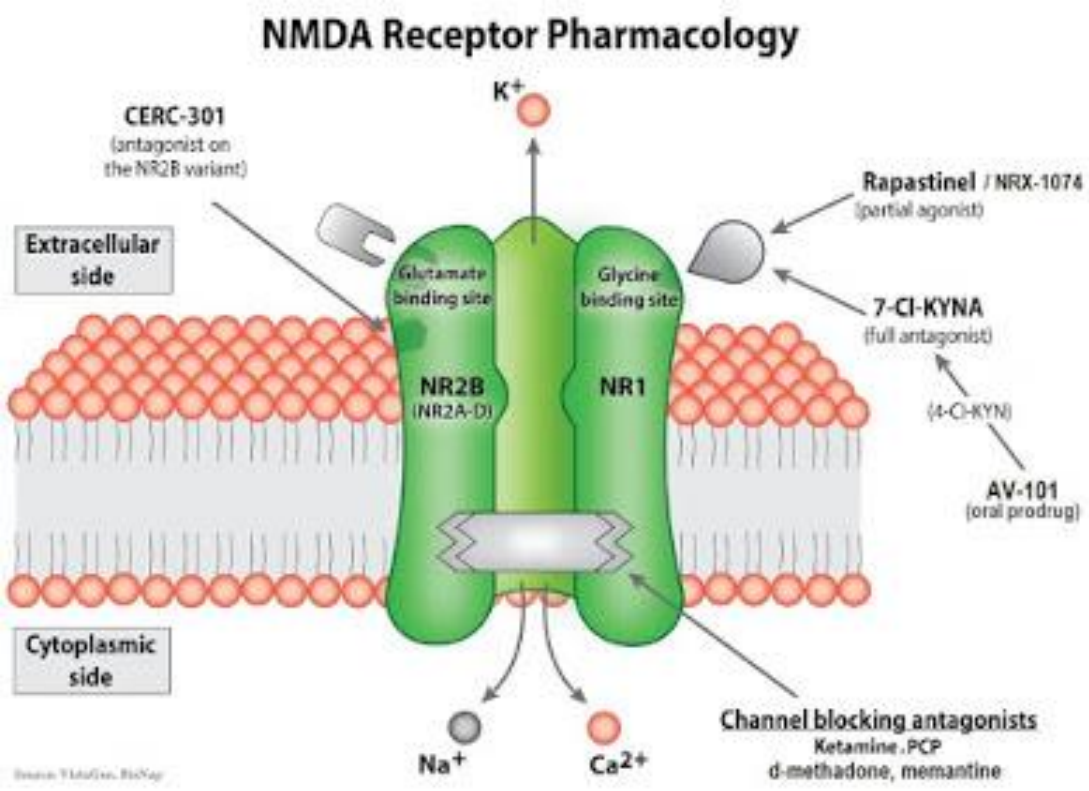


Figure 2.1.5: Rapastinel binding the glycine site of the NMDA (N-Methyl D-Aspartate) receptor. (Image Source: internet).[31]

2.1.5 Apimostinel (NRX-1074)

Apimostinel is similar to rapastinel structurally and mechanistically where it acts as a partial agonist of an allosteric site of the glycine site of the NMDA receptor. It is under study by Naurex for treatment of major depressive disorder.[12]Apimostinel is orally active potent drug and also found to be administered through intravenous injection. It is also found to be hundred fold more potent by weight compared to rapastinel. Structurally, apimostinel is also an amidated tetrapeptide but slightly modified with an additional benzyl group centered at the proline alpha carbon thereby increasing the hydrophobicity of the molecule. Apimostinel is also well tolerated and is devoid of common side effects that are naturally found in the drugs like ketamine (schizophrenia-like psychotomimetic effects).

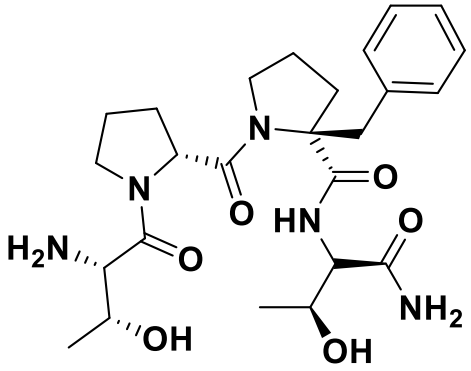


Figure 2.1.6: Molecular Structure of apimostinel (rapastinel mimic)

Some other NMDA receptor modulators are

1. Esketamine : It is a NMDA receptor antagonist (Glycine site)[13]
2. 4-Chlorokynurenine : It is also a NMDA receptor antagonist (Glycine site)[14]
3. Ketamine : It is also a NMDA receptor antagonist and also an indirect AMPA receptor activator.[15]

Chapter 2

Part A

Novel Silaproline (Sip)-incorporated Close Structural Mimics of Potent Antidepressant Peptide Drug Rapastinel (GLYX-13).

2.2 Introduction

2.2.1 Silicon in Medicinal Chemistry

Silicon (Atomic number 14) is positioned below carbon and belongs to third row of the periodic table. Similar properties between carbon and silicon makes silicon as natural choice as carbon bioisostere in drug design and development.

2.2.2 Difference between Silicon and Carbon

1. C-Si bond length is 1.87 A° compared to C-C bond length of 1.54 A° which makes C-Si bond longer than C-C bond which in turn would change the conformation of the molecule.
2. Silicon compounds are more lipophilic compared to carbon compounds. Replacing carbon by silicon would lead to enhanced cell penetration thereby generating more potent molecules.[16]
3. There is also a difference in polarity between carbon and silicon as silicon is more electropositive than carbon. This difference can cause varied activity in their compounds.

2.2.3 Silicon containing amino acids

Silicon containing amino acids are unnatural amino acids that find wide applications in drug discovery.[17]There are only a few silicon containing unnatural amino acids known in the literature.[18] Silicon incorporation increases lipophilicity which in turn stabilizes the peptides towards biodegradation.

There are few silicon containing amino acids found in the literature. Some of them are shown in Fig. 2.1.7.

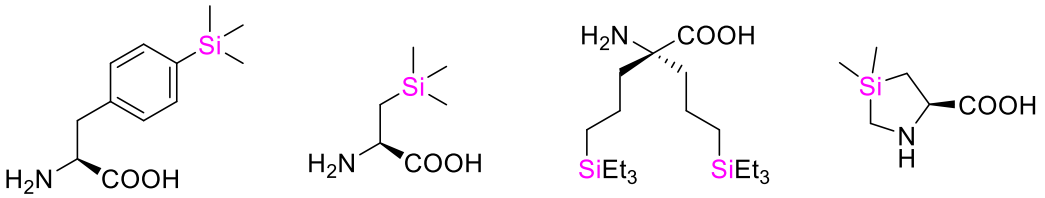


Figure 2.1.7 Sila amino acids known in the literature.

2.2.4 Silaproline as a proline surrogate:

Silaproline is an analogue of proline and it differs only by substituting γ-carbon with dimethylsilyl group. The carbon-silicon bond in the silaproline is longer than carbon-carbon bond of proline (about 0.35 A°) as shown by X-ray crystallography of silaproline containing model peptides. The angle between C-Si-C of silaproline (93°) is also small compared to C-C-C of proline containing peptides (105°). Slight alteration in the bond angles and bond lengths provides silaproline similar conformational properties as proline in model peptides.[19]Moreover, presence of dimethylsilyl group induces enhanced lipophilicity as compared to proline which in turn encourages enhanced cell membrane permeability. The cLogP value of Fmoc-silaproline was calculated and it was found experimentally that it is 14 times greater than Fmoc-proline confirming the increased lipophilicity.[19]

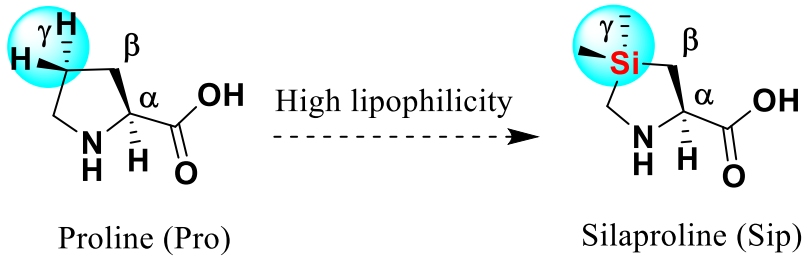


Figure 2.1.8 Structure of proline and silaproline.

2.3 Objective and design strategy:

Here in, we have designed rapastinel analogues containing silaproline as a proline surrogate as it is known to have similar conformational properties like that of proline. Rapastinel is a polar molecule with hydrophilic character. Sip incorporation is believed to improve its pharmacokinetic properties like hydrophobicity, cell permeability, lipophilicity and conformational stability.

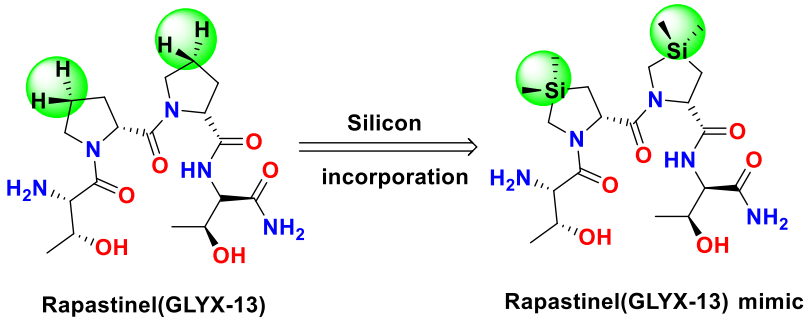


Figure 2.1.9 Incorporation of silaproline as a proline surrogate.

2.4 Synthesis:

Boc-Sip-OH was commercially obtained from BOC Sciences and was used as starting material for all the peptide sequences synthesized. In order to obtain free amine Thr residues at N-terminus, coupling conditions were standardized accordingly. It is noteworthy that HATU proved to be best coupling agent for all the reactions involving Thr residues. The molecular structures of the compounds **7**, **11** and **16** synthesized by solution phase peptide synthesis are shown in Fig. 2.2.10.

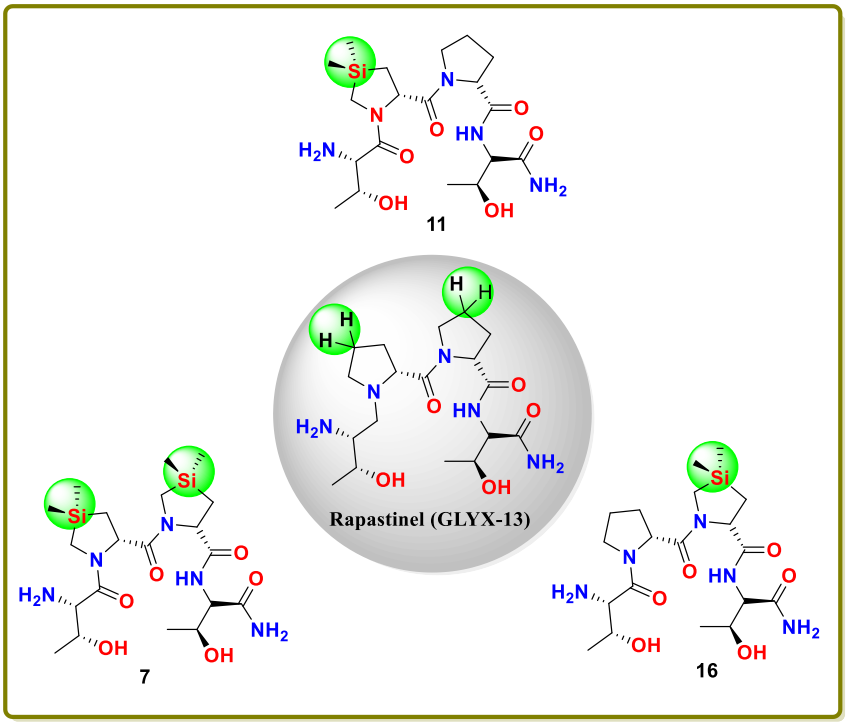
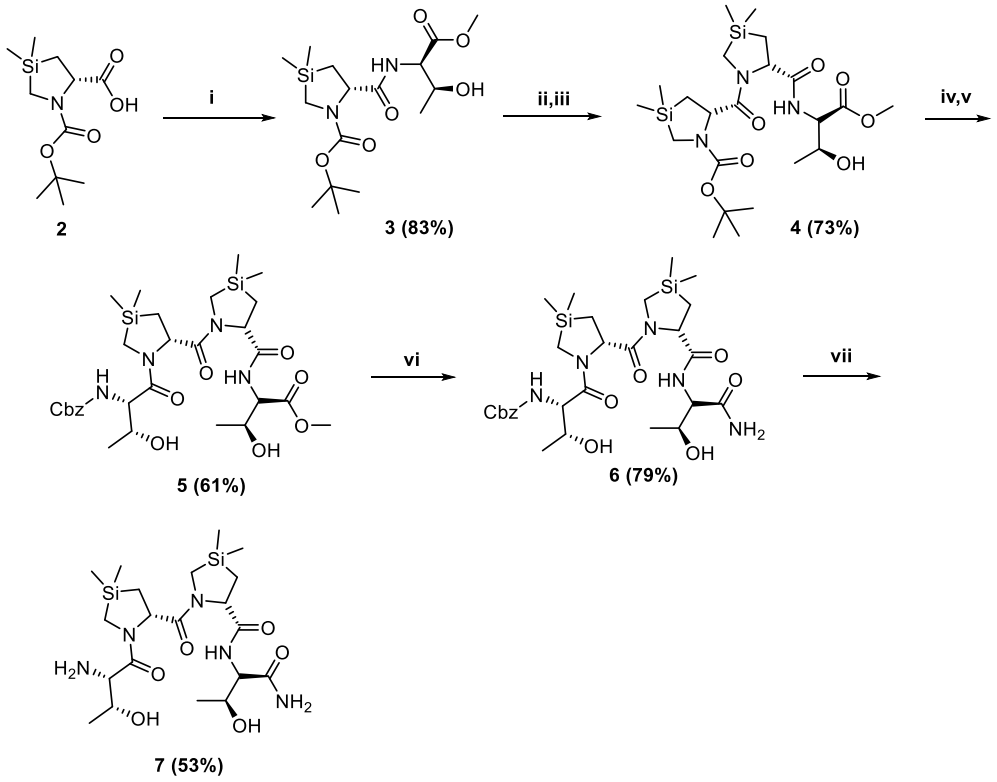


Figure 2.2.10 Molecular structures of peptide analogues synthesized containing silaproline as a proline surrogate**.**

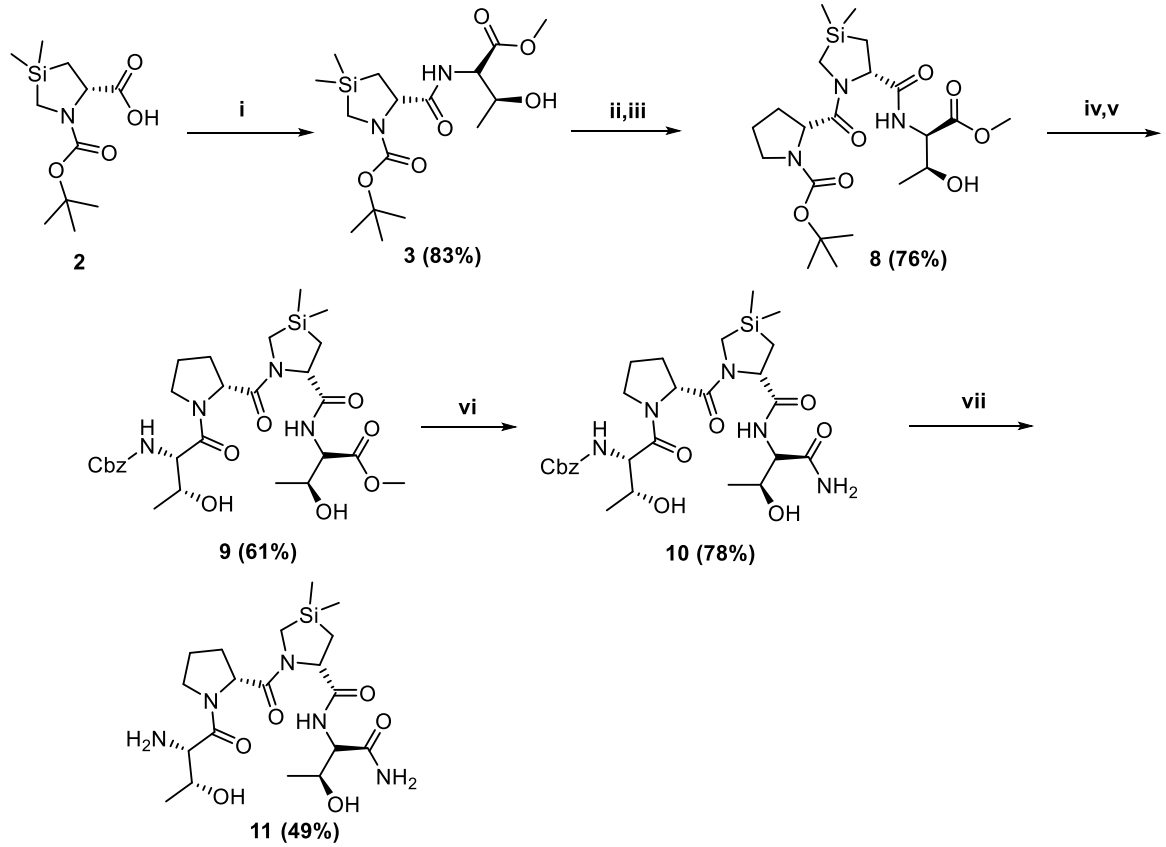
Tetrapeptide sequence **7** was synthesized following the procedure starting from Boc-Sip-OH as shown in scheme 2.1.

Boc-Sip-OH **2** was coupled with threonine methyl ester hydrochloride using HATU as a coupling agent to obtain the dipeptide ester **3** which was then boc-deprotected using TFA to obtain the TFA salt. The TFA salt of compound **3** was further coupled with Boc-Sip-OH **2** using HATU as coupling agent to obtain the tripeptide ester **4**. The tripeptide ester **4** was then treated with TFA to obtain Boc deprotected tripeptide ester which was then coupled with Cbz-Thr-OH using HATU to obtain tetrapeptide ester **5** in reasonable yield. Compound **5** was then amidated using methanolic ammonia to obtain amidated tetrapeptide which was then Cbz-deprotected to obtain the Sip-incorporated peptide **7**.



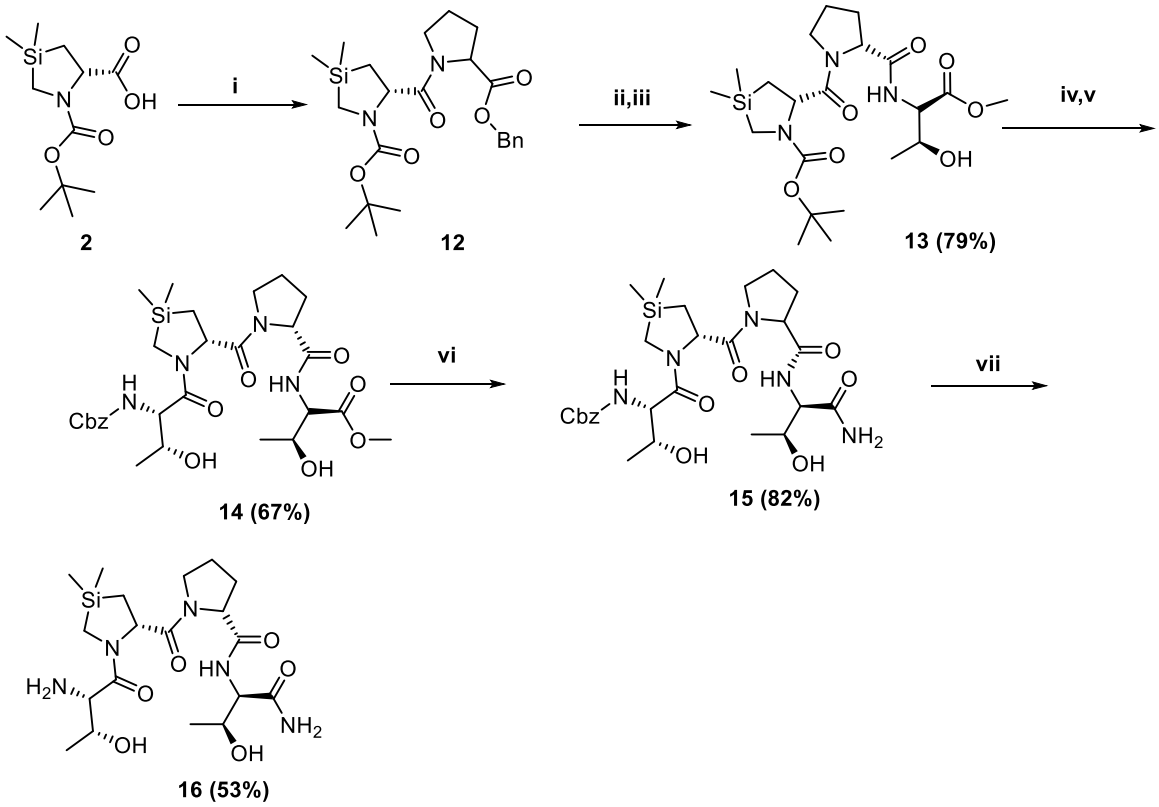
Scheme 2.1. Reagents and conditions: (i) H-Thr-OMe.HCl, HATU, HOBt, DIPEA, DCM, rt, 12 h; (ii) TFA, DCM, 0° C -rt, 30 min.; (iii) Boc-Sip-OH, HATU, DIPEA, DMF, rt, 24 h; (iv) TFA, DCM, 0 °C - rt, 30 min.; (v) Cbz-Thr-OH, HATU, DIPEA, DMF, 24 h, rt; (vi) NH_3/methanol, rt, 12 h,; (vii) H_2-Pd/C, rt, 2 h.

Sip-incorporated peptide **11** was also synthesized in the same manner as shown for peptide **7** where in Sip was incorporated at the third position of rapastinel (Thr-Pro-Pro-Thr-NH_2).All the coupling conditions were similar as shown for peptide **7**.



Scheme 2.2 Reagents and conditions: (i) H-Thr-OMe.HCl, HATU, HOBt, DIPEA, DCM, rt, 12 h; (ii) TFA, DCM, 0 °C - rt, 30 min; (iii) Boc-Pro-OH, HATU, DIPEA, DMF, rt, 12 h; (iv) TFA, DCM, 0 °C - rt, 30 min; (v) Cbz-Thr-OH, HATU, DIPEA, DMF, rt, 24 h; (vi) NH_3/methanol, rt, 2 h; (vii) H_2-Pd/C, rt, 2 h.

Peptide **16** was synthesized with a slightly modified conditions where in Sip has been introduced at the second position of rapastinel (Thr-Pro-Pro-Thr-NH_2) as shown in scheme **3**. The dipeptide **12** Boc-Sip-Pro-OBn was subjected to Cbz-deprotection using H_2/Pd-C followed by coupling with Thr-methyl ester hydrochloride using HATU as a coupling agent to obtain **13**, which was then converted to **16**, with the chain of reactions similar to the preparation of peptide **7** and peptide **11.**



Scheme 2.3. Reagents and conditions: (i) H-Pro-OBn, HBTU, Et_3N, DMF, rt, 12 h; (ii) H_2, Pd/C, rt, 2 h; (iii) H-Thr-OMe.HCl, HATU, DIPEA, DMF, rt, 24 h; (iv) TFA, DCM, 0 °C - rt, 30 min.; (v) Cbz-Thr-OH, HATU, DIPEA, DMF, rt, 24 h; (vi) NH_3/methanol, rt, 12 h; (vii) H_2-Pd/C, rt, 2 h.

2.5 Results and discussion:

Herein, we describe the solution phase synthesis of a new class of novel Sip-incorporated rapastinel analogues (Figure 2.2.1) using standard peptide coupling strategy. Three structurally close analogues of rapastinel were synthesized by replacing proline residues. The resulting peptides are believed to be conformationally similar to that of original potent drug rapastinel as it is evidenced that replacing Sip by Pro does not alter the overall conformation of the resultant peptide.[19] Hence, the silicon containing compounds synthesized would have increasing cell permeability compared to the original potent drug rapastinel as the incorporation of silicon is known to enhance the lipophilicity of the peptides.

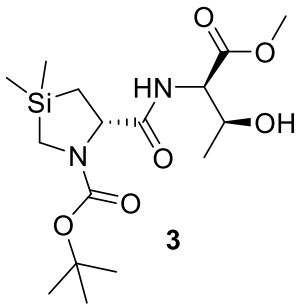
2.6 Conclusion

In summary, a new class of potent rapastinel analogues was synthesized using normal peptide coupling strategy with HATU as coupling agent in the solution-phase. Peptides could be readily synthesized in solution phase by using column chromatography and preparative thin layer chromatography in spite of increase in the polarity of the peptides synthesized. There has been increase in the use of silicon bearing heterocyclic amino acids in recent times. Dimethylsilyl group was incorporated into potent drug rapastinel, inorder to enchance the pharmacokinetic profiles of proline-containing peptides such as hydrophobicity, lipophilicity, conformational stability and cell permeability.

2.7 Experimental section

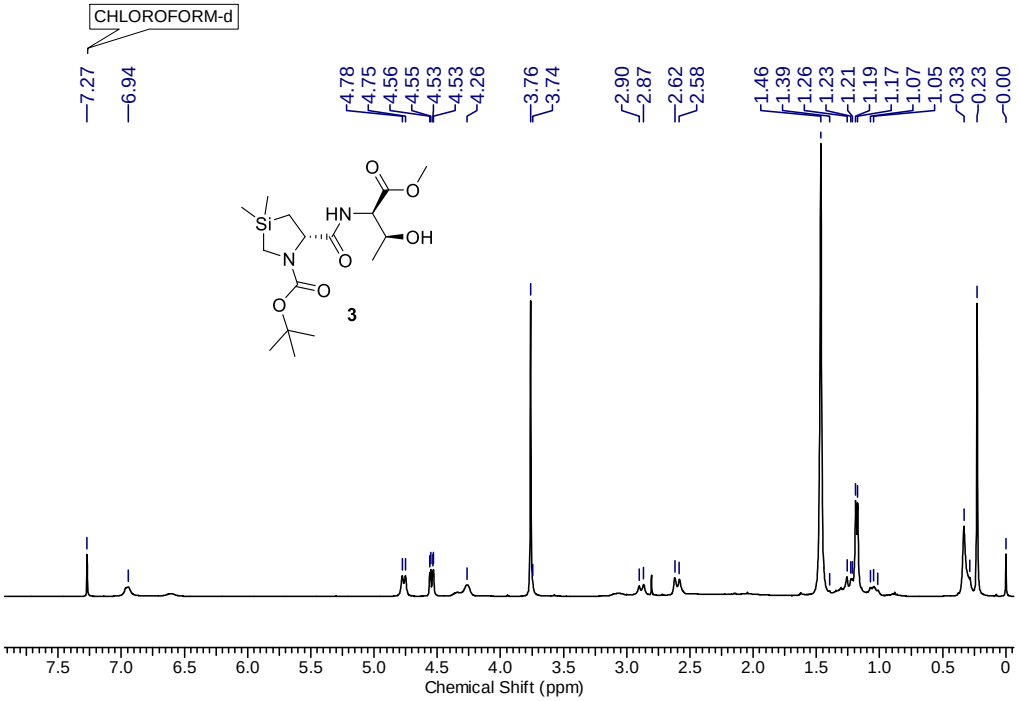
Synthetic procedures and data:

Compound 3 (Boc-Sip-Thr-OMe): Boc-Sip-OH **2** (0.50 g, 1.92 mmol) was dissolved in DMF (1 mL) in a two neck round bottom flask and cooled to 0˚C. Next, threonine methyl ester hydrochloride (0.80 g, 4.8 mmol, 2.5 equiv) was dissolved in DMF (1 mL) and added to the above round bottom flask containing **2**, followed by the addition of DIPEA (1.4 mL, 7.7 mmol, 4 equiv), HATU (1.08 g, 2.88 mmol, 1.5 equiv), and HOBt (0.14 g, 0.96 mmol, 0.5 equiv) and kept for 12 h at room temperature. Later, the reaction mixture was taken in AcOEt and the organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under *vacuo* to obtain the crude product which was further purified by column chromatography (eluent: 30% AcOEt/pet. ether, R_f : 0.4) to furnish compound **3** (0.60 g, 83%) as a white solid; $[\alpha]^{25}_D$ = -49.0^0 (c = 0.1, MeOH); ^{1}H NMR (400 MHz ,$CDCl_3$) δ: 6.94 (bs, 1 H), 4.76 (d, *J* = 9.8 Hz, 1 H), 4.54 (dd, *J* = 9.2, 2.4 Hz, 1 H), 4.26 (bs, 1 H), 3.76 (s, 3 H), 2.96-2.80 (m, 1 H), 2.60 (d, *J* = 14.0 Hz, 1 H), 1.46 (s, 9 H), 1.24 (m, 1 H), 1.18 (d, *J* = 6.1 Hz, 3 H), 1.05 (bs, 1 H), 0.41-0.27 (m, 3 H), 0.23 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ = 173.4,

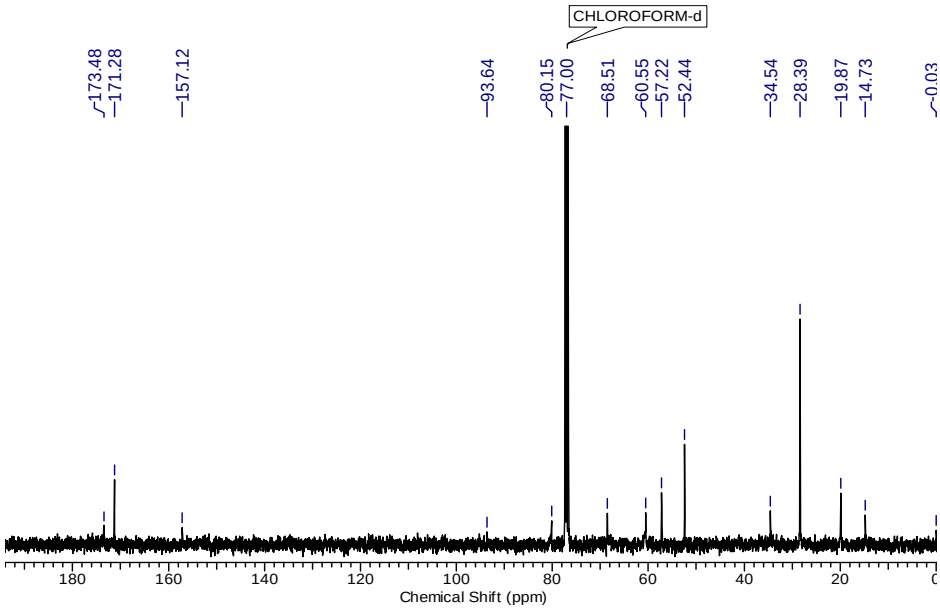


3

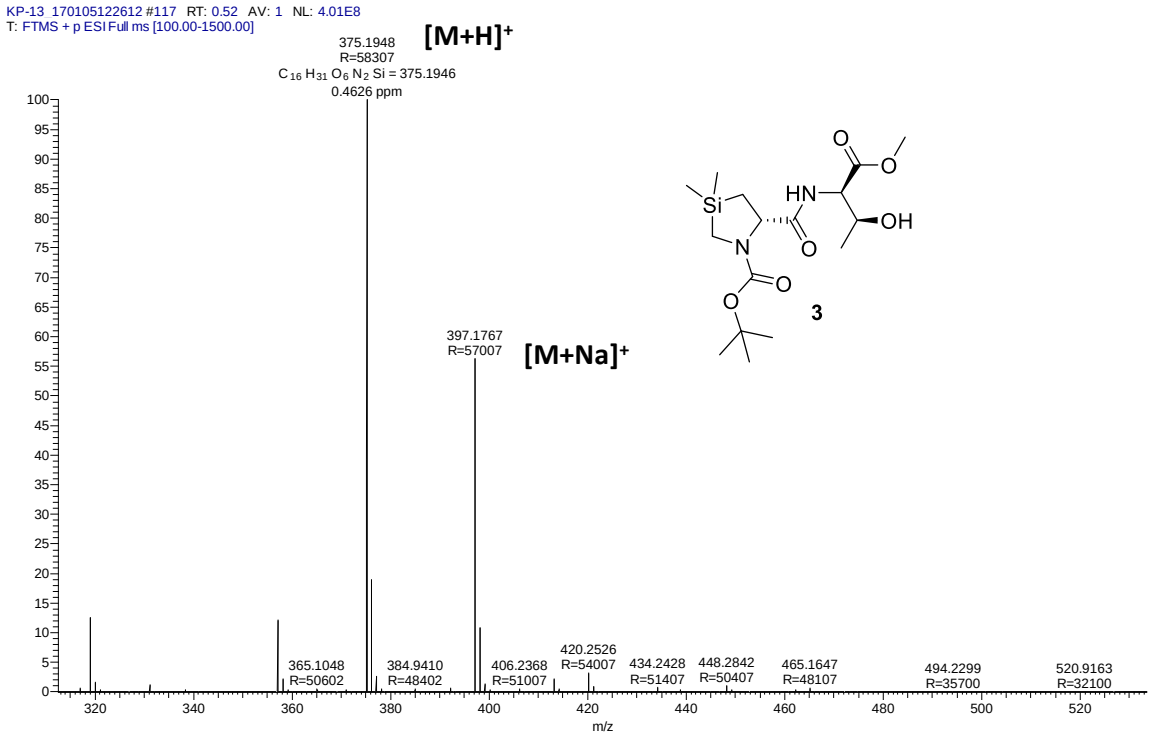
171.2, 157.1, 93.6, 80.1, 68.5, 60.5, 57.2, 52.4, 34.5, 28.3, 19.8, 14.7. HRMS: $C_6H_{31}N_2O_6Si$, Calculated: 375.1946 $[M+H]^+$, observed : 375.1948 $[M+H]^+$.



^{1}H NMR spectrum of compound **3** ($CDCl_3$, 400 MHz, 298 K)

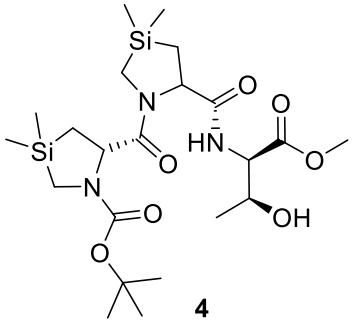


^{13}C NMR spectrum of compound **3** ($CDCl_3$, 100 MHz, 298 K)

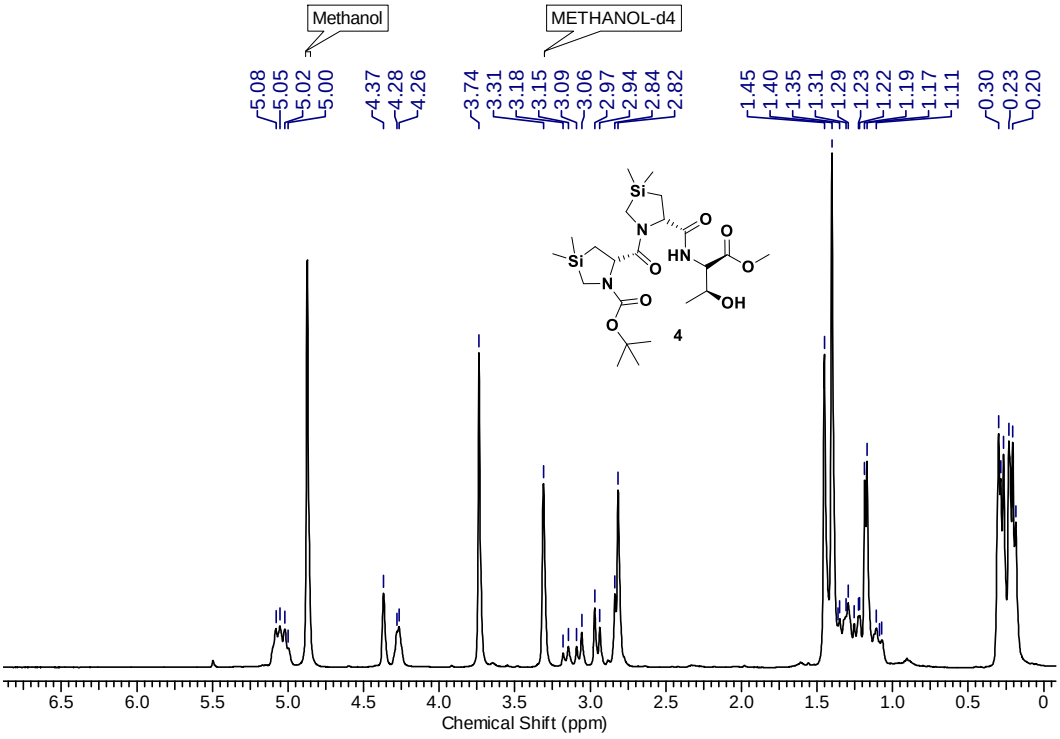


HRMS of compound **3**

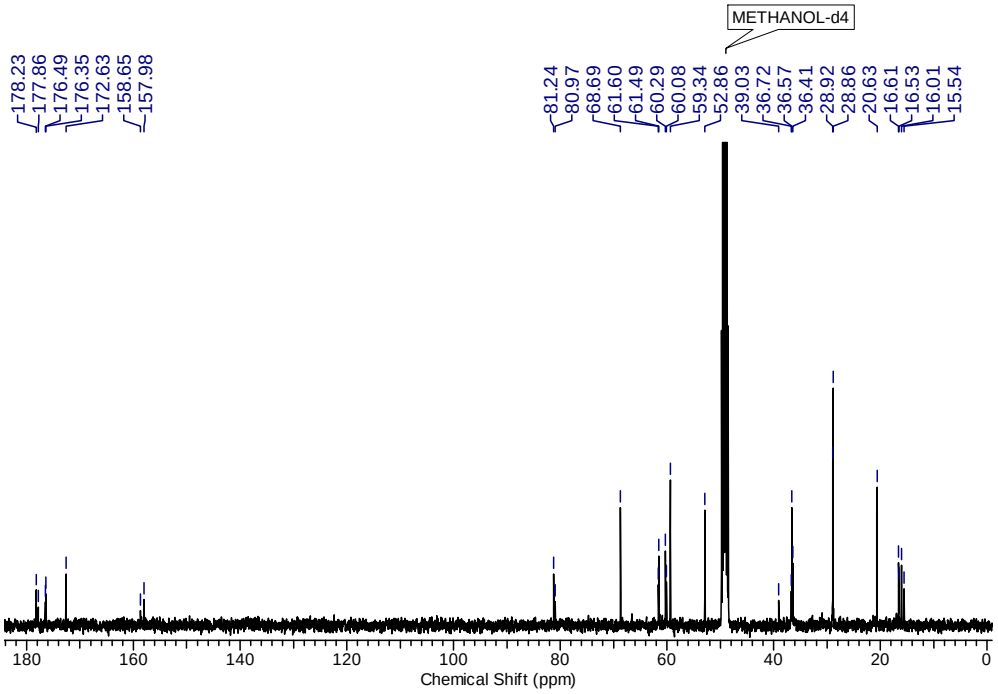
Compound 4 (Boc-Sip-Sip-Thr-OMe): Boc-protected dipeptide ester **3** (0.28 g, 0.75 mmol) was dissolved in 1 mL of dichloromethane followed by addition of 1 mL of TFA (1 M) at 0 °C. The reaction mixture was stirred for 30 min at room temperature. After completion of the reaction, the solvent was stripped off and co-evaporated using toluene to obtain the TFA salt which was used for the next step without further purification. Boc-Sip-OH (0.22 g, 0.84 mmol, 1 equiv) was coupled with dipeptide ester TFA salt (0.23 g, 0.84 mmol, 1 equiv) using HATU (0.47 g, 1.2 mmol, 1.5 equiv) and DIPEA (0.6 mL, 3.3 mmol, 4 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in AcOEt and the organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 60% AcOEt/ pet. ether, R_f: 0.5) to furnish compound **4**



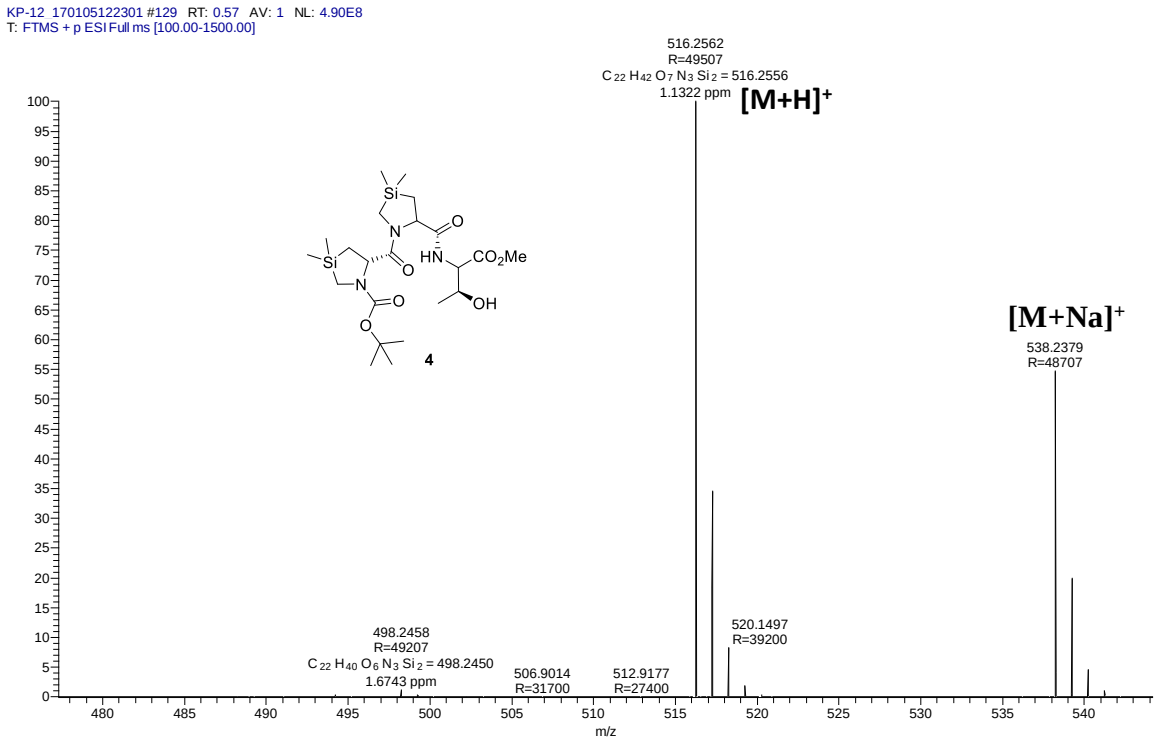
(0.32 g, 73%) as a hygroscopic white solid; $[\alpha]^{26}_{D}$: -72.6° (c = 0.1, MeOH); ^{1}H NMR (400MHz ,CD_3OD) δ = 5.14-4.92 (m, 2 H), 4.37 (bs, 1 H), 4.27 (d, *J* = 5.5 Hz, 1 H), 3.74 (s, 3 H), 3.16 (d, *J* = 13.4 Hz, 1 H), 3.11-3.03 (m, 1 H), 2.95 (d, *J* = 13.4 Hz, 1 H), 2.88-2.75 (m, 2 H), 1.45 $_{rotamer}$ (4 H), 1.40 $_{rotamer}$ (5 H), 1.36-1.21 (m, 2 H), 1.18 (d, *J* = 6.1 Hz, 3 H), 1.13-1.04 (m, 2 H), 0.34-0.25 (m, 6 H), 0.25-0.15 (m, 6 H), ^{13}C NMR (100 MHz ,CD_3OD) δ = 178.2, 177.9, 176.5, 176.4, 172.6, 158.6, 157.9, 81.2, 81.0, 68.7, 61.6, 61.5, 60.3, 60.1, 59.3, 52.9, 39.0, 36.7, 36.6, 36.4, 28.9, 28.9, 20.6, 16.6, 16.5, 16.0, 15.5; HRMS: $C_{22}H_{42}N_2O_7Si_2$, Calculated: 516.2556 $[M+H]^+$, observed: 516.2562 $[M+H]^+$



^{1}H NMR spectrum of compound **4** (CD_3OD, 400 MHz, 298 K)

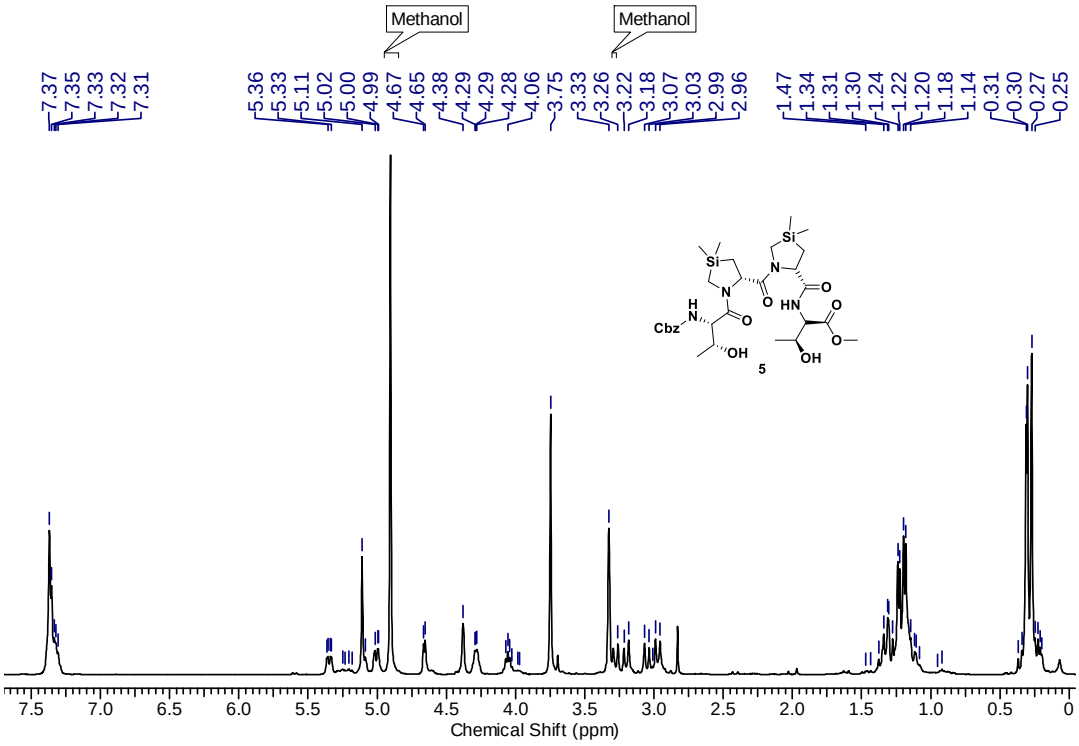


^{13}C NMR spectrum of compound **4** (CD_3OD, 100 MHz, 298 K)

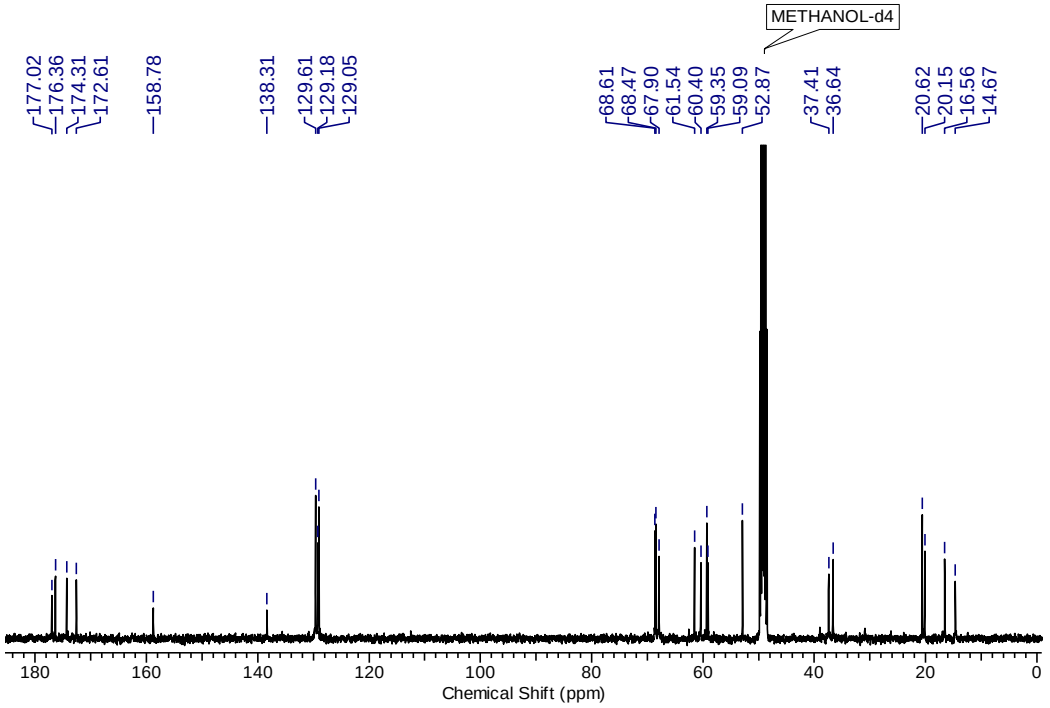


HRMS of compound **4**

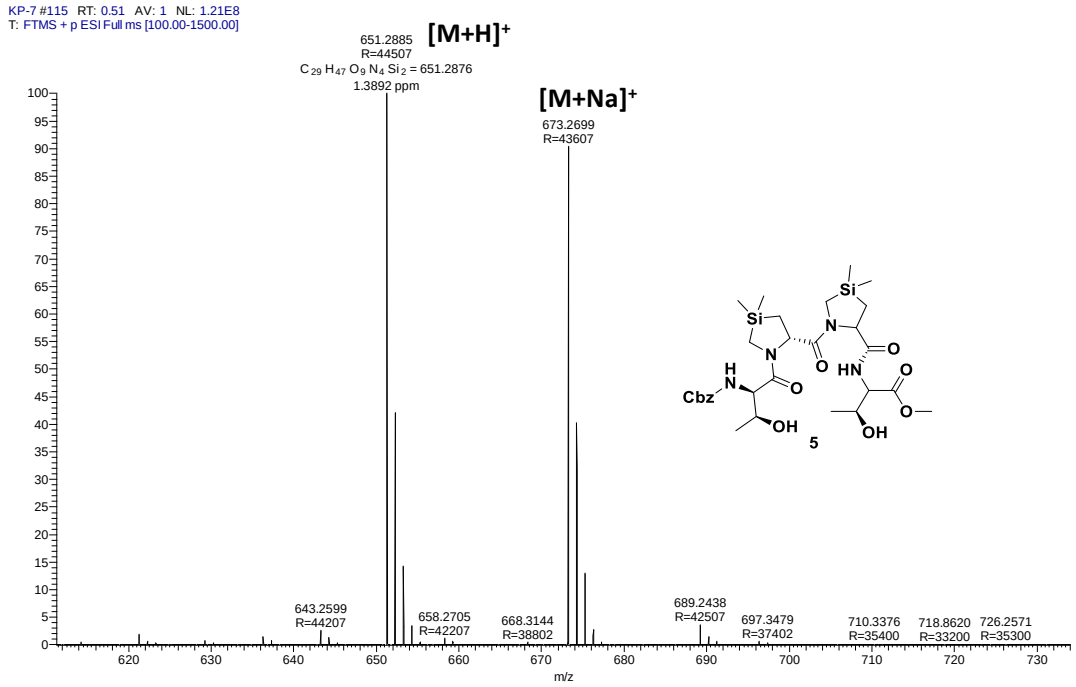
Compound 5 (Cbz-Thr-Sip-Sip-Thr-OMe): Boc-protected tripeptide ester **4** (0.25 g, 0.48 mmol) was dissolved in 1 mL of dichloromethane followed by addition of 1 mL of TFA (1M) at 0°C. The reaction was stirred for 30 min at room temperature. After completion of reaction, the solvent was stripped off and co-evaporated using toluene to obtain the TFA salt which was used for the next step without further purification. Cbz-Thr-OH (0.13 g, 0.52 mmol, equiv) was coupled with tripeptide ester TFA salt (0.22 g, 0.52 mmol, 1 equiv) using HATU (0.29 g, 0.78 mmol, 1.5 equiv) and DIPEA (0.4 mL, 2 mmol, 4 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in 2 % MeOH/DCM and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 2% MeOH/DCM, R_f : 0.5) to furnish compound **5** (0.21 g, 61%) as a hygroscopic white solid; $[\alpha]^{26}_D$: -51.7° (c = 0.1, MeOH); ^{1}H NMR (400 MHz ,CD_3OD) δ = 7.43-7.24 (m, 5 H), 5.33 (dd, *J* = 10.1, 2.7 Hz, 1 H), 5.29-5.16 (m, 1 H), 5.14-5.04 (m, 2 H), 5.05-4.95 (m, 1 H), 4.64 (d, *J* = 5.5 Hz, 1 H), 4.36 (bs, 1 H), 4.31-4.22 (m, 1 H), 4.11-4.00 (m, 1 H), 3.99-3.89 (m, 1H), 3.75 (s, 3 H), 3.29-3.13 (m, 2 H), 3.08-2.91 (m, 2 H), 1.39-1.26 (m, 2 H), 1.26-1.21 (m, 3 H), 1.21-1.17 (3 H), 1.14-1.05 (m, 2 H), 0.41-0.27 (m, 6 H), 0.27-0.14 (m, 6H); ^{13}C NMR (100 MHz ,CD_3OD), δ = 177.0, 176.4, 174.3, 172.6, 158.9, 138.6, 129.6, 129.2, 129.0, 68.6, 68.5, 67.9, 61.5, 60.4, 59.4, 59.1, 52.9, 37.4, 36.6, 20.6, 20.2, 16.6, 14.7; HRMS: $C_{29}H_{47}N_4O_9Si_2$, Calculated: 651.2876 $[M+H]^+$, observed: 651.2885 $[M+H]^+$.



^{1}H NMR spectrum of compound **5** (CD_3OD, 400 MHz, 298 K)



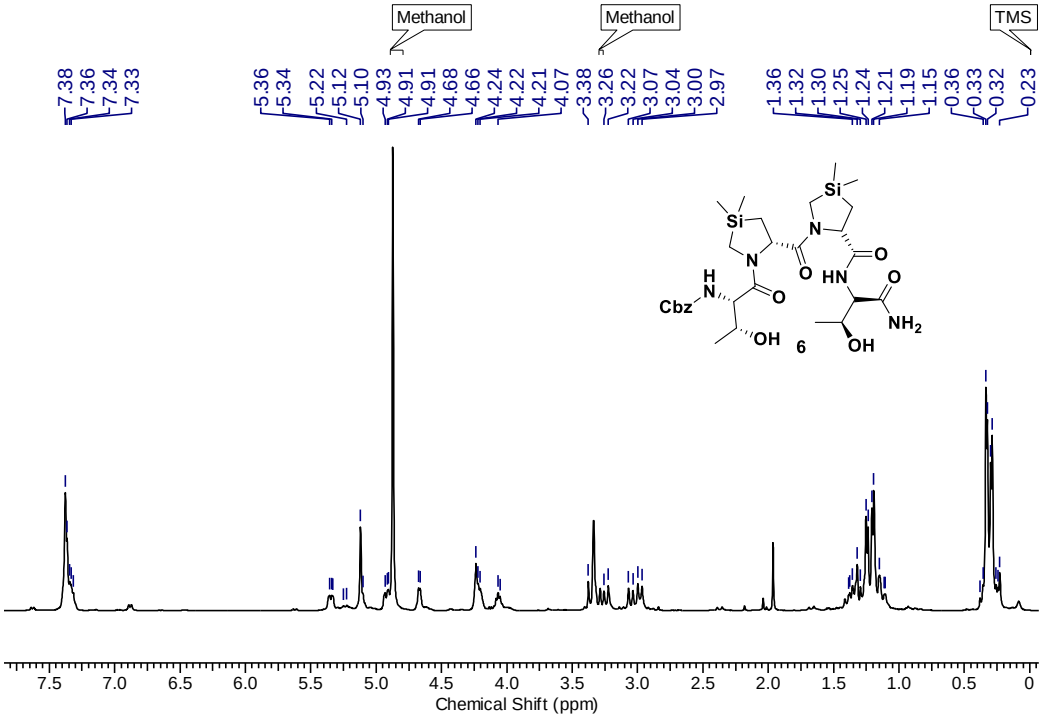
^{13}C NMR spectrum of compound **5** (CD_3OD, 100 MHz, 298 K)



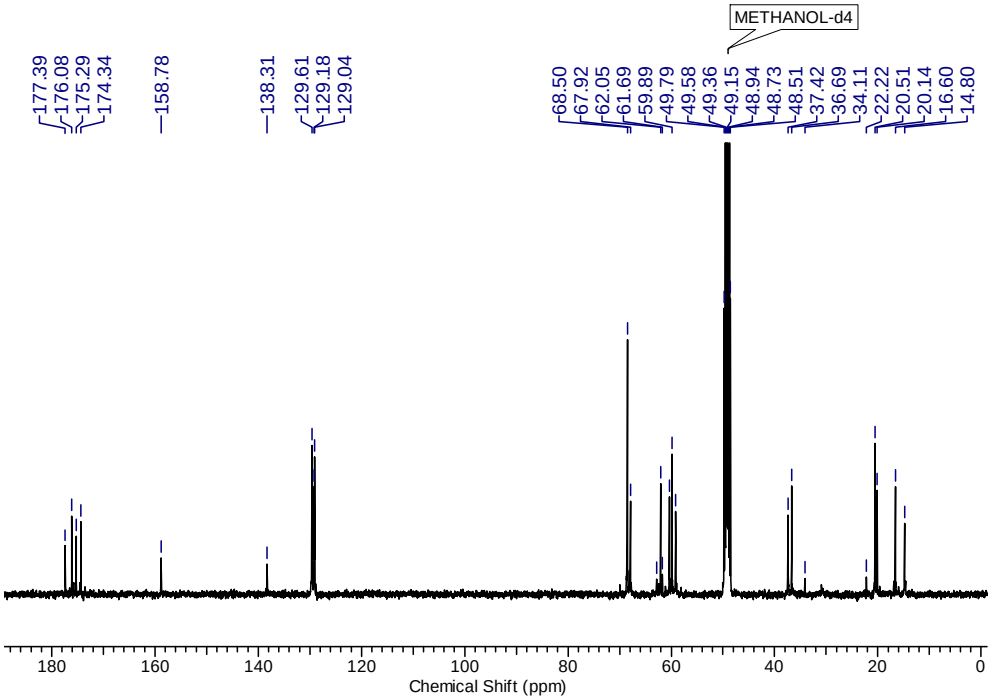
HRMS of compound **5**

Compound 6: (Cbz-Thr-Sip-Sip-Thr-NH$_2$): To a solution of tetrapeptide ester **5** (0.20 g, 0.30 mmol) in methanol, saturated methanolic ammonia solution (3 ml) was added and the reaction mixture was stirred for 24 h at room temperature. Later, the volatiles were stripped off under reduced pressure and the product was further purified by column chromatography and preparative thin layer chromatography (eluent: 3% MeOH/ DCM, R_f : 0.5) to furnish compound **6** (0.15 g, 79%) as a white solid; $[\alpha]^{26}_D$: -41.6° (c = 0.1, MeOH); ^{1}H NMR (400 MHz , CD_3OD) δ = 7.43-7.24 (m, 5 H), 5.33 (dd, *J* = 10.1, 2.1 Hz, 1 H), 5.22-5.25 (m, 1 H) 5.14-5.10 (m, 2 H), 4.97-4.93 (m, 1 H), 4.64 (d, *J* = 5.5 Hz, 1 H), 4.24-4.22 (m, 1 H), 4.23-4.21 (m, 1H), 4.07-4.05 (m, 1 H), 3.38-3.28 (m, 1H), 3.26-3.22 (m, 2 H), 3.08-2.91 (m, 2 H), 1.40-1.26 (m, 2 H), 1.26-1.21 (m, 3 H), 1.21-1.17 (3 H), 1.14-1.05 (m, 2 H), 0.41-0.27 (m, 6 H), 0.27-0.14 (m, 6H); ^{13}C NMR (100 MHz ,CD_3OD) δ = 177.4, 176.1, 175.3, 174.3, 158.8, 138.3, 129.6, 129.2, 129.0, 68.5, 67.9, 62.0, 60.4, 59.9, 59.1,

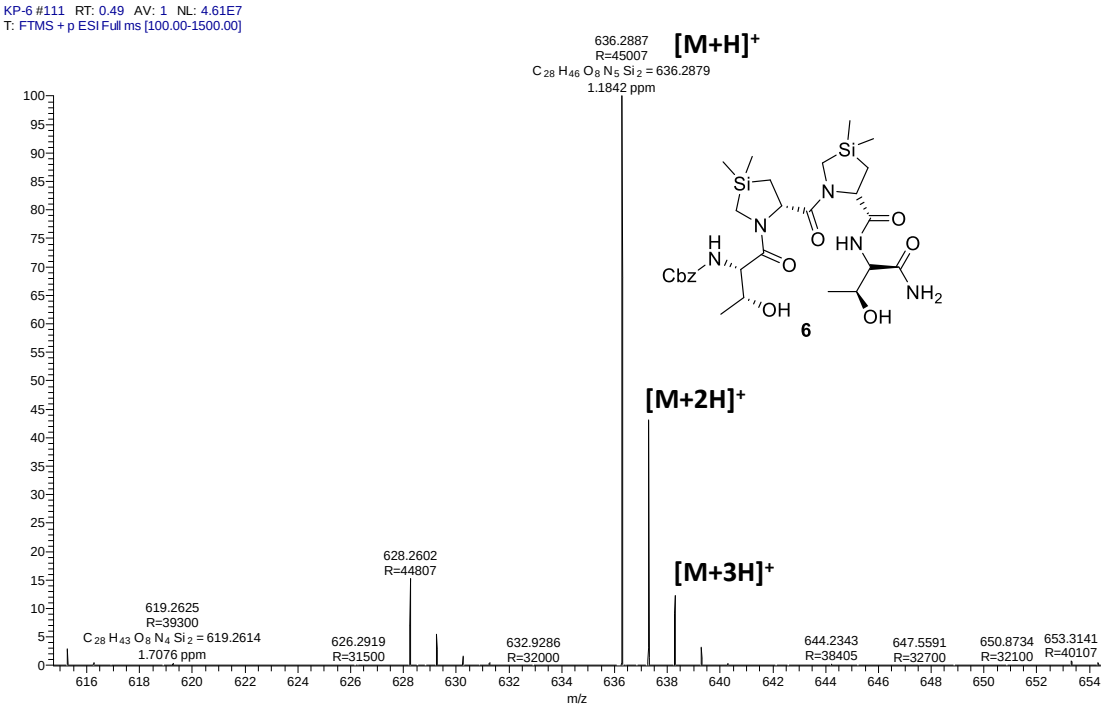
37.4, 36.7, 20.5, 20.1, 16.6, 14.8; HRMS: $C_{28}H_{46}N_5O_8Si_2$, calculated: 636.2879 $[M+H]^+$, observed: 636.2887 $[M+H]^+$.



^{1}H NMR spectrum of compound **6** (CD_3OD, 400 MHz, 298 K)

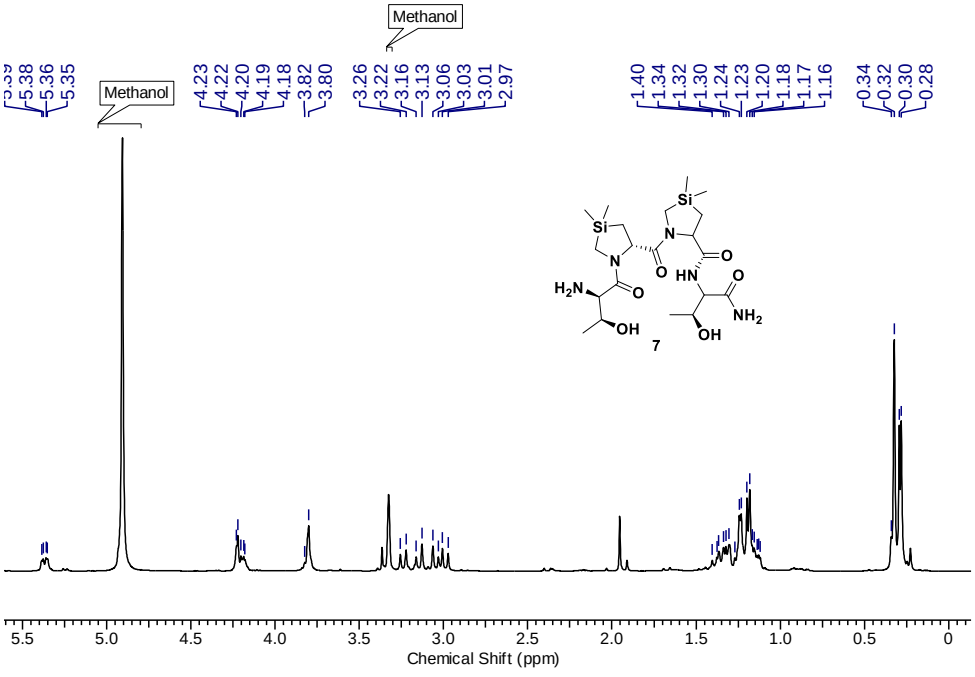


^{13}C NMR spectrum of compound **6** (CD_3OD, 100 MHz, 298 K)

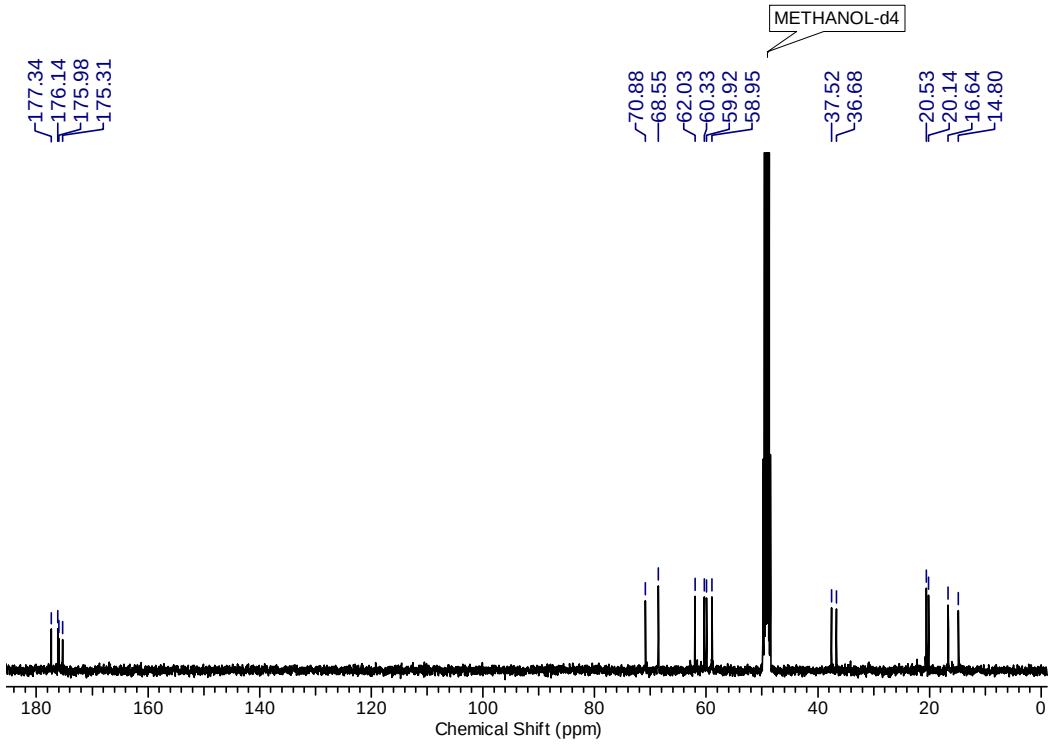


HRMS of compound **6**

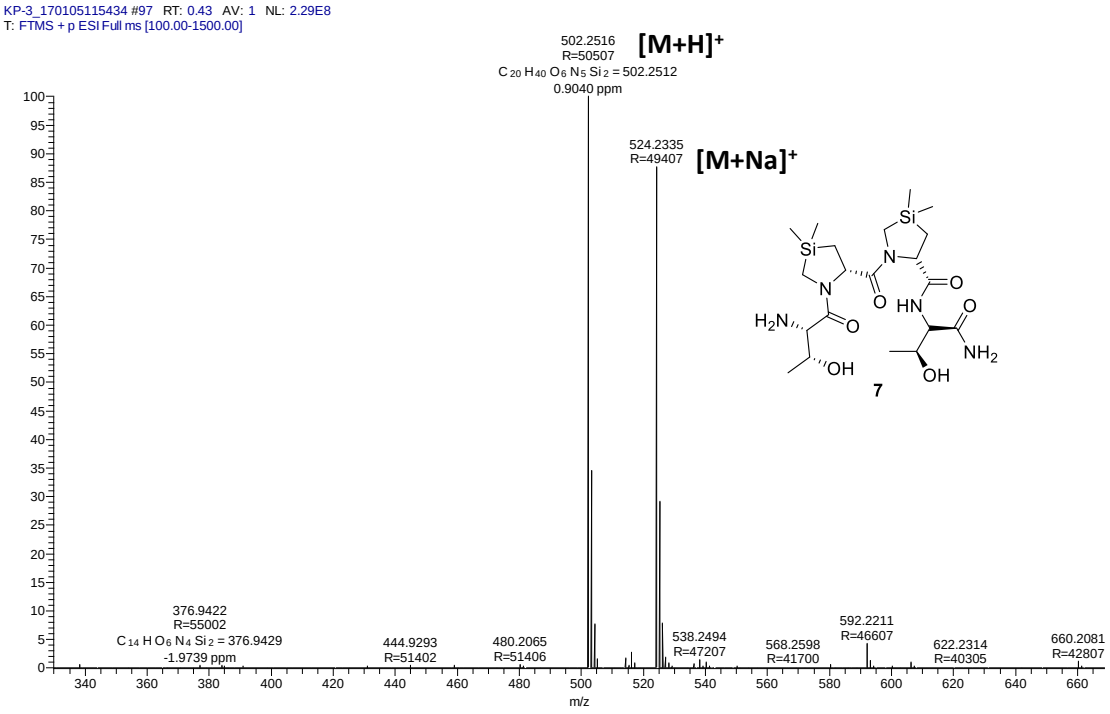
Compound 7 (H-Thr-Sip-Sip-Thr-NH_2): To a solution of **6** (0.13 g, 0.2 mmol) in methanol (0.1M) was added 10% Pd/C (0.1 equiv). The resulting mixture was stirred under an atmosphere of H_2 for 2 h. The mixture was then filtered through a celite pad, washed with methanol and the filtrate was concentrated *in vacuo* and purified by preparative thin layer chromatography (eluent: 8% MeOH/ 2% NH_3 solution/DCM, R_f : 0.4) to furnish the compound **7** (0.05 g, 53%) as a white solid; $[\alpha]^{26}_D$: -72.0^0 (c = 0.1, MeOH), ^{1}H NMR (400 MHz, CD_3OD) δ = 5.37 (dd, *J* = 10.7, 3.4 Hz, 1 H), 4.22 (d, *J* = 3.1 Hz, 1 H), 4.21 - 4.16 (m, 1 H), 3.85 - 3.75 (m, 2 H), 3.24 (d, *J* = 13.4 Hz, 1 H), 3.18 - 3.10 (m, 1 H), 3.08 - 3.02 (m, 1 H), 2.99 (d, *J* = 13.4 Hz, 1 H), 1.44-1.29 (m, 2 H), 1.35-1.28 (m, 2 H), 1.28-1.21 (m, 3 H), 1.21-1.17 (m, 3 H), 1.16-1.10 (m, 2 H), 0.36-0.31 (m, 6 H), 0.30-0.25 (m, 6 H). ^{13}C NMR (100 MHz, CD_3OD) δ = 177.3, 176.1, 176.0, 175.3, 70.9, 68.6, 62.0, 60.3, 59.9, 59.0, 37.5, 36.7, 20.5, 20.1, 16.6, 14.8; HRMS: $C_{20}H_{40}N_5O_6Si_2$, calculated: 502.2512 $[M+H]^+$, observed: 502.2516 $[M+H]^+$.



^{1}H NMR spectrum of compound **7** (CD_3OD, 400 MHz, 298 K)

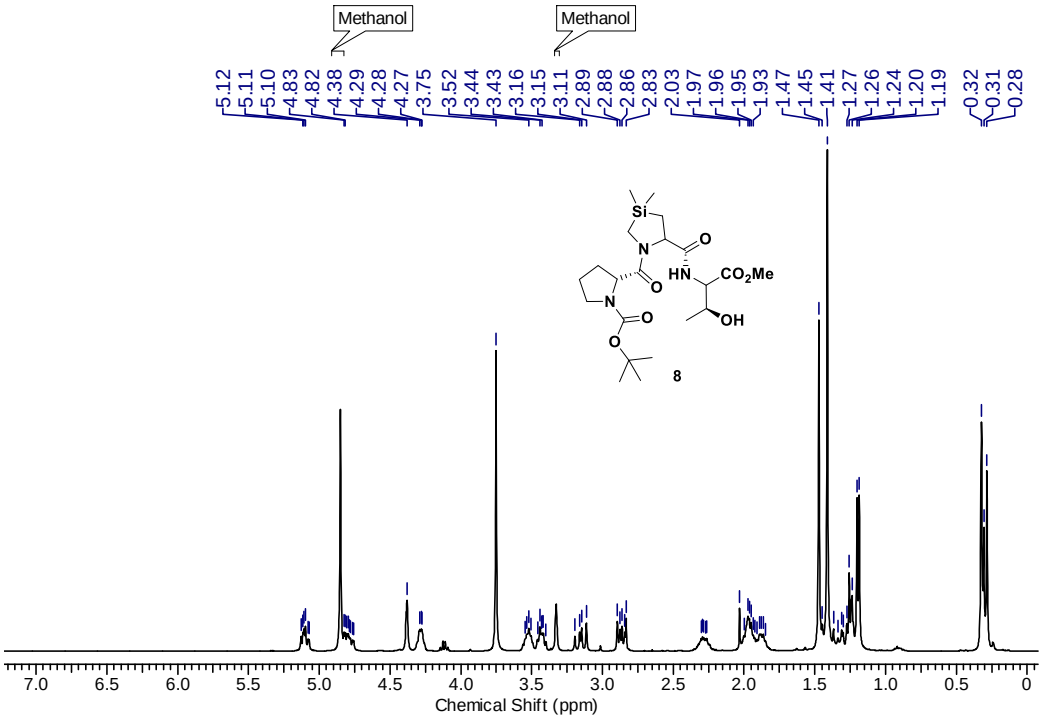


^{13}C NMR spectrum of compound **7** (CD_3OD, 100 MHz, 298 K)

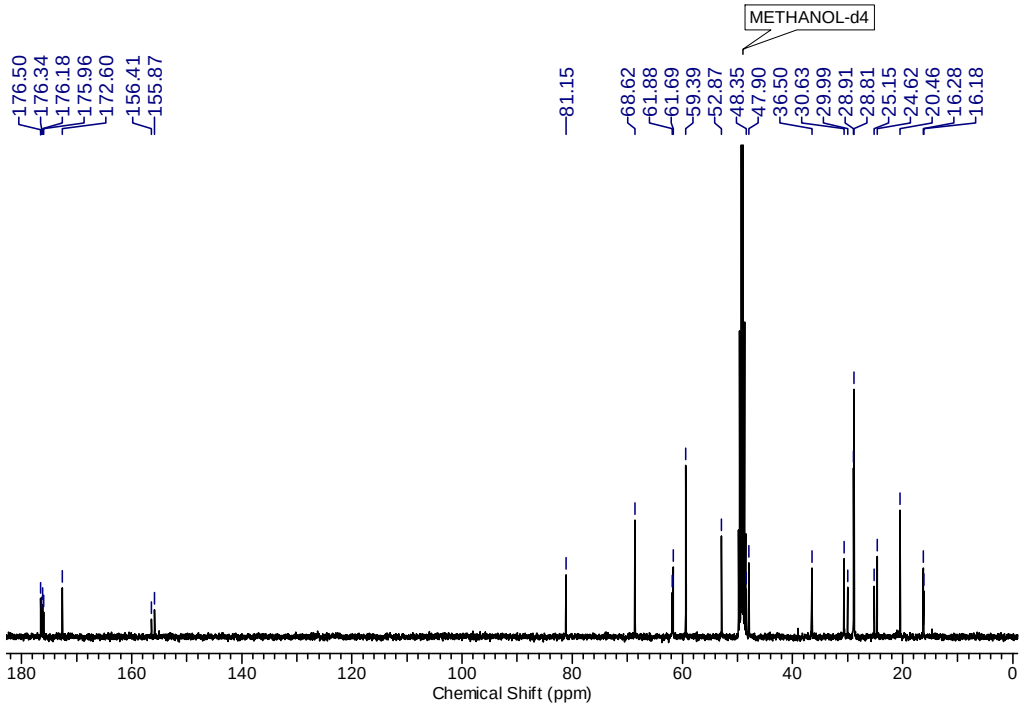


HRMS of compound **7**

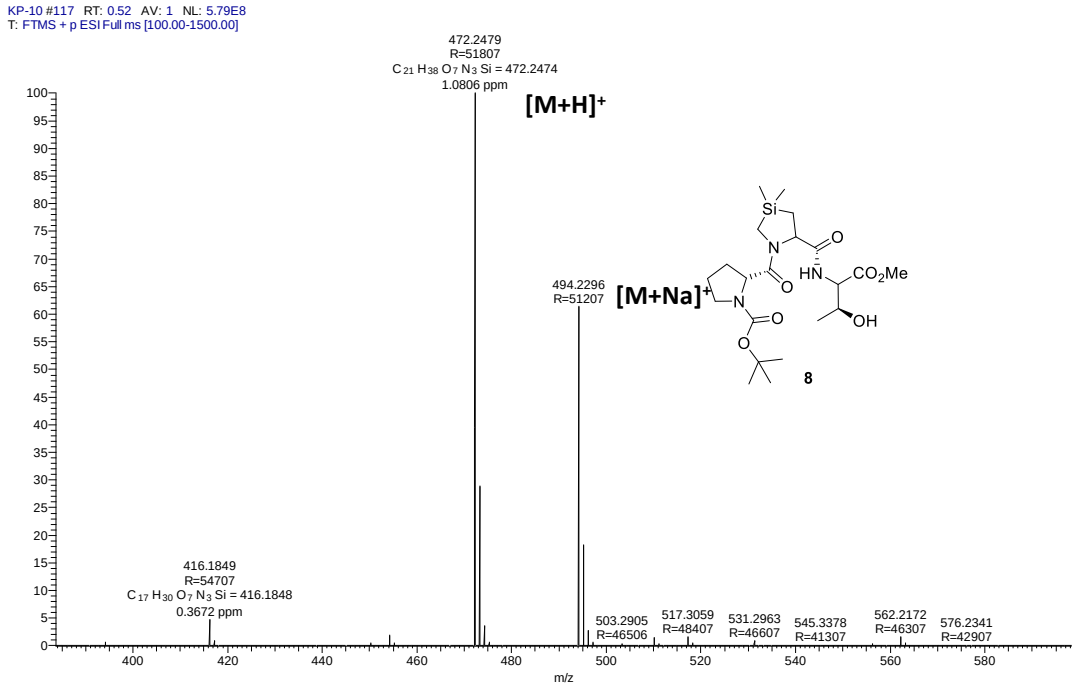
Compound 8 (Boc-Pro-Sip-OMe): Boc-protected dipeptide ester **3** (0.28 g, 0.75 mmol) was dissolved in 1 mL of DCM followed by addition of 1 mL of TFA (1M) at 0 °C and stirred for 30 min at room temperature. After completion of reaction, the solvent was stripped off and co-evaporated using toluene to obtain the TFA salt which was used for the next step without further purification. Boc-Pro-OH (0.18 g, 0.84 mmol, 1 equiv) was coupled with dipeptide ester TFA salt (0.23 g, 0.84 mmol, 1 equiv) using HATU (0.47 g, 1.2 mmol, 1.5 equiv) and DIPEA (0.6 mL, 3.3 mmol, 4 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in AcOEt and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 70% AcOEt/pet. ether, R_f: 0.5) to furnish compound **8** (0.3 g, 76%) as a hygroscopic white solid. $[\alpha]^{26}_D$: -91.8° (c = 0.1, MeOH); ^{1}H NMR (400 MHz ,CD_3OD) δ = 5.20-5.03 (m, 1 H), 4.85-4.77 (m, 1 H), 4.40 (bs, 1 H), 4.36-4.23 (m, 1 H), 3.77 (s, 3 H), 3.62-3.50 (m, 1 H), 3.50-3.39 (m, 1 H), 3.25-3.11 (m, 1 H), 2.94-2.82 (m, 1 H), 2.43-2.22 (m, 1 H), 2.02-1.93 (m, 2 H), 1.92-1.83 (m, 1 H), 1.53-1.46 (m, 4 H), 1.43 (s, 5 H), 1.39-1.24 (m, 3 H), 1.21 (d, *J* = 6.1 Hz, 3 H), 0.38-0.28 (m, 6 H). ^{13}C NMR (100 MHz ,CD3OD) δ = 176.5, 176.3, 176.2, 176.0, 172.6, 156.4, 155.9, 81.2, 68.6, 61.9, 61.7, 59.4, 59.3, 52.9, 48.3, 47.9, 36.5, 30.6, 30.0, 28.9, 28.8, 25.2, 24.6, 20.5, 16.3, 16.2. HRMS: $C_{21}H_{38}N_3O_7Si$, calculated: 472.2474 $[M+H]^+$, observed: 472.2479 $[M+H]^+$.



^{1}H NMR spectrum of compound **8** (CD_3OD, 400 MHz, 298 K)

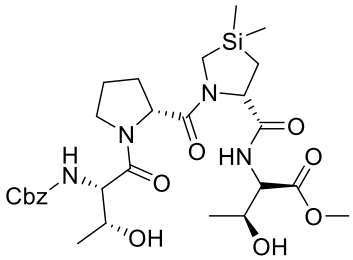


^{13}C NMR spectrum of compound **8**(CD_3OD, 100 MHz, 298 K)

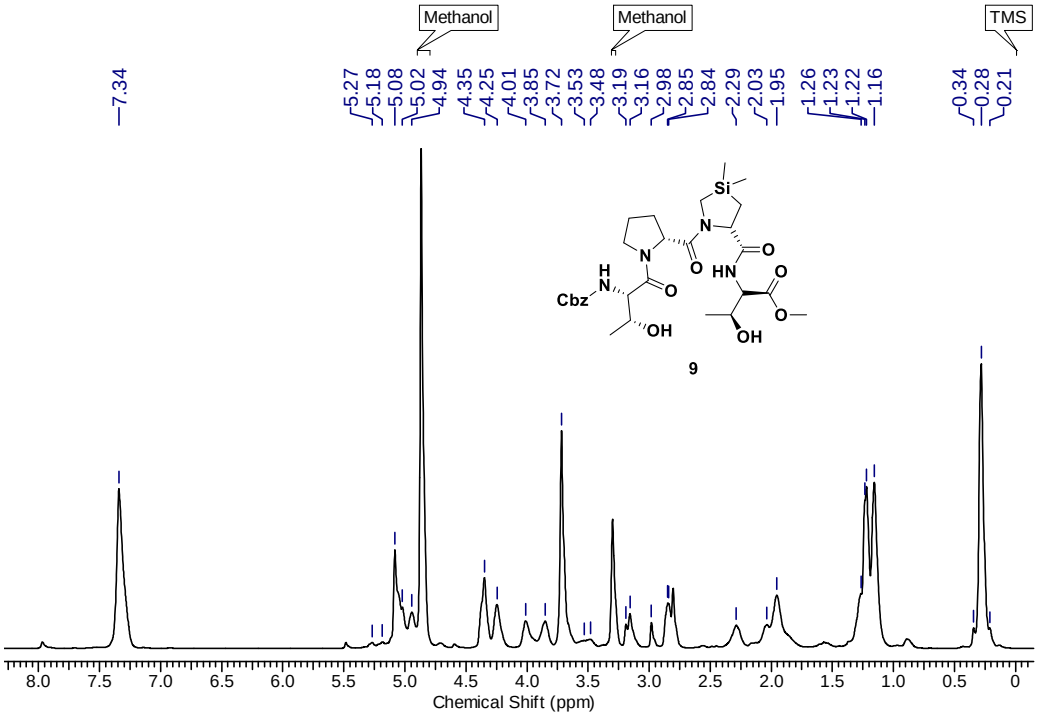


HRMS of compound **8**

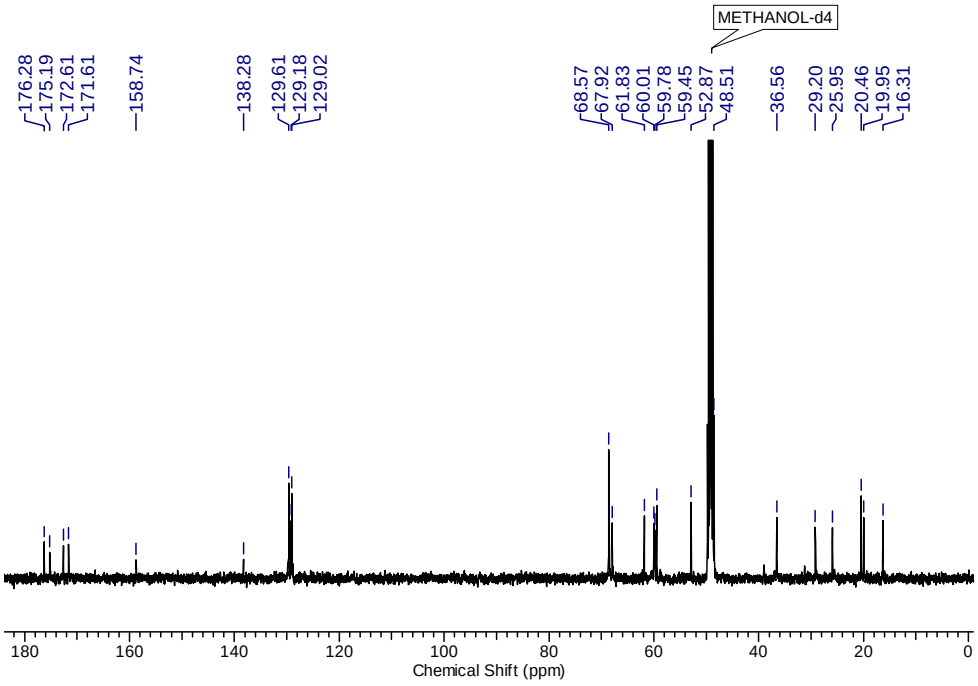
Compound 9 (Cbz-Thr-Pro-Sip-Thr-OMe): Boc-protected tripeptide ester **8** (0.25 g, 0.53 mmol) was dissolved in 1 mL of DCM followed by addition of 1 mL of TFA (1M) at 0 °C and stirred for 30 min at room temperature. After completion of reaction, the solvent was stripped off and co-evaporated using toluene to obtain the TFA salt which was used for the next step without further purification. Cbz-Thr-OH (0.14 g, 0.58 mmol, 1 equiv) was coupled with tripeptide TFA salt (0.21 g, 0.56 mmol, 1 equiv) using HATU (0.32 g, 0.84 mmol, 1.5 equiv) and DIPEA (0.4 mL, 2 mmol, 4 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in 2 % MeOH/DCM and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 2% MeOH/DCM, R_f : 0.5) to furnish compound **9** (0.21 g, 61%) as a white solid; $[\alpha]^{26}_D$: -40.5°(c = 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ = 7.35-7.29 (m, 5 H), 5.33-5.18



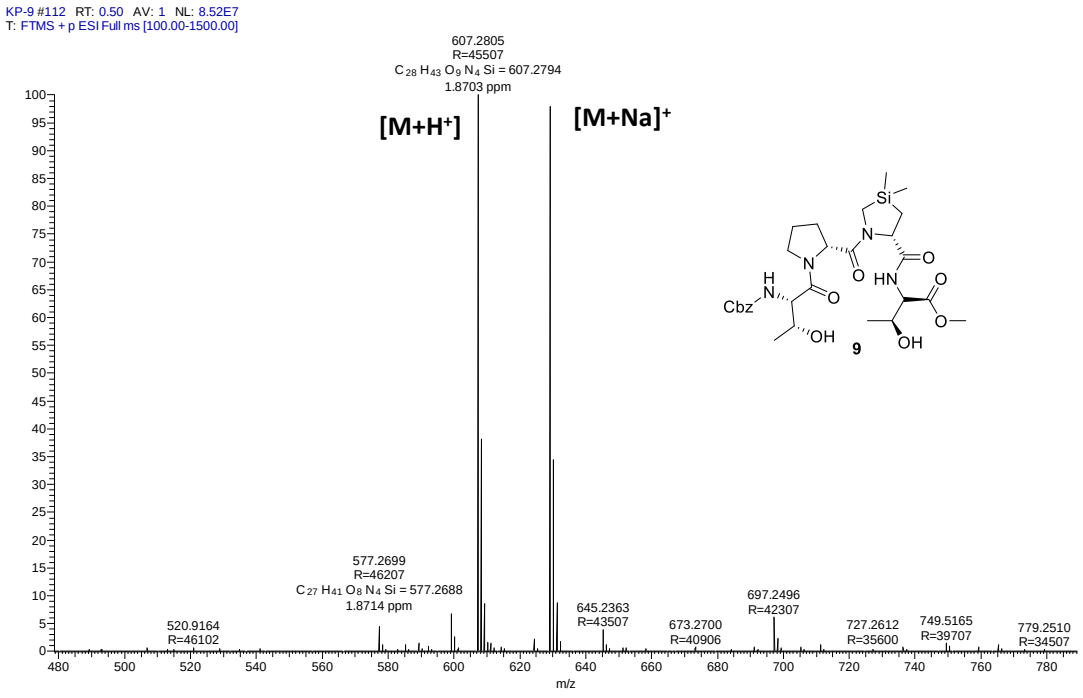
(m,1 H) ,5.07-5.03 (m, 2 H), 5.02-4.95 (m, 1 H), 4.35 (bs, 2 H), 4.25 (bs, 1 H), 4.02 (bs., 1 H), 3.85 (bs., 1 H), 3.72 (s., 3 H), 3.53-3.48 (m, 1H), 3.18 (d, *J* = 12.8 Hz, 1 H), 3.03-2.95 (m, 1 H) 2.94-2.74 (m, 2 H), 2.29 (bs, 1 H), 2.13-1.80 (m, 3 H), 1.38-1.27 (m, 1 H), 1.23 (d, *J* = 5.5 Hz, 3 H), 1.16 (bs, 3 H), 0.99-1.10 (m, 1 H), 0.42-0.17 (m, 6 H); ^{13}C NMR (100 MHz, CD_3OD) δ = 176.3, 175.2, 172.6, 171.6, 158.7, 138.3, 129.4, 129.0, 68.6, 67.9, 61.8, 60.0, 59.8, 59.5, 52.9, 36.6, 29.2, 25.9, 20.5, 20.0, 16.3. HRMS: $C_{28}H_{43}N_4O_9Si$, calculated: 607.2805 $[M+H]^+$, observed: 607.2794 $[M+H]^+$.



^{1}H NMR spectrum of compound **9** (CD_3OD, 400 MHz, 298 K)

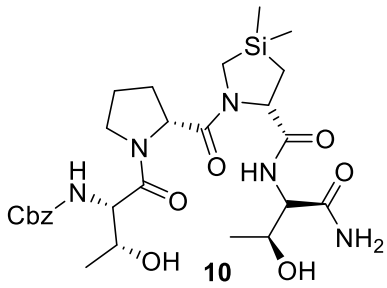


^{13}C NMR spectrum of compound **9** (CD_3OD, 100 MHz, 298 K)

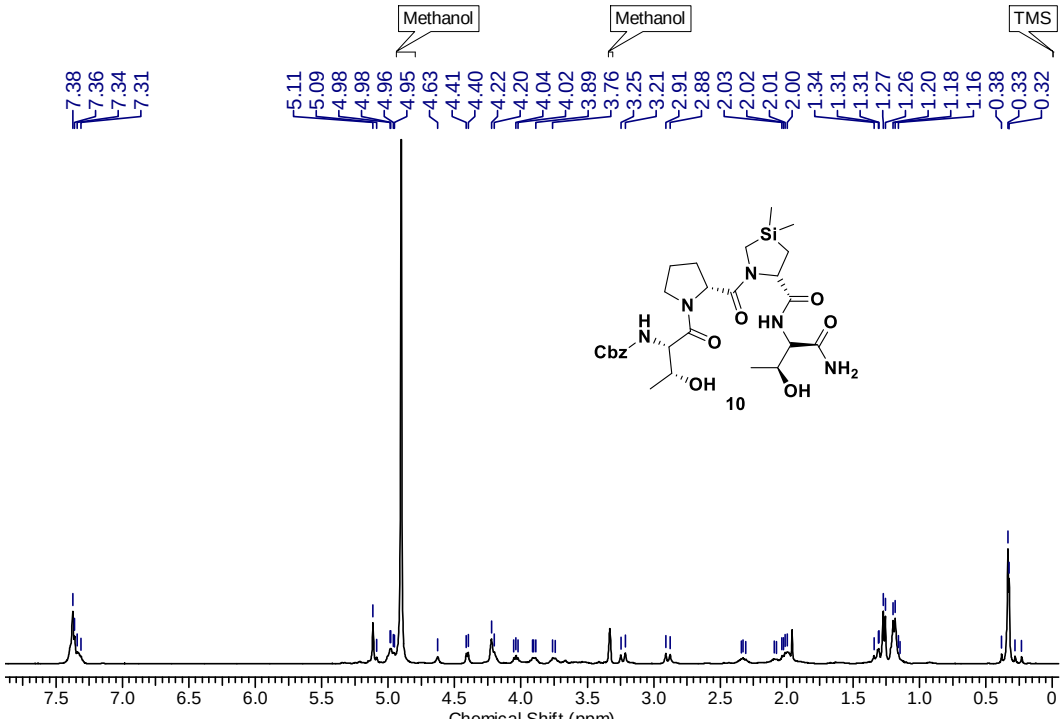


HRMS of compound **9**

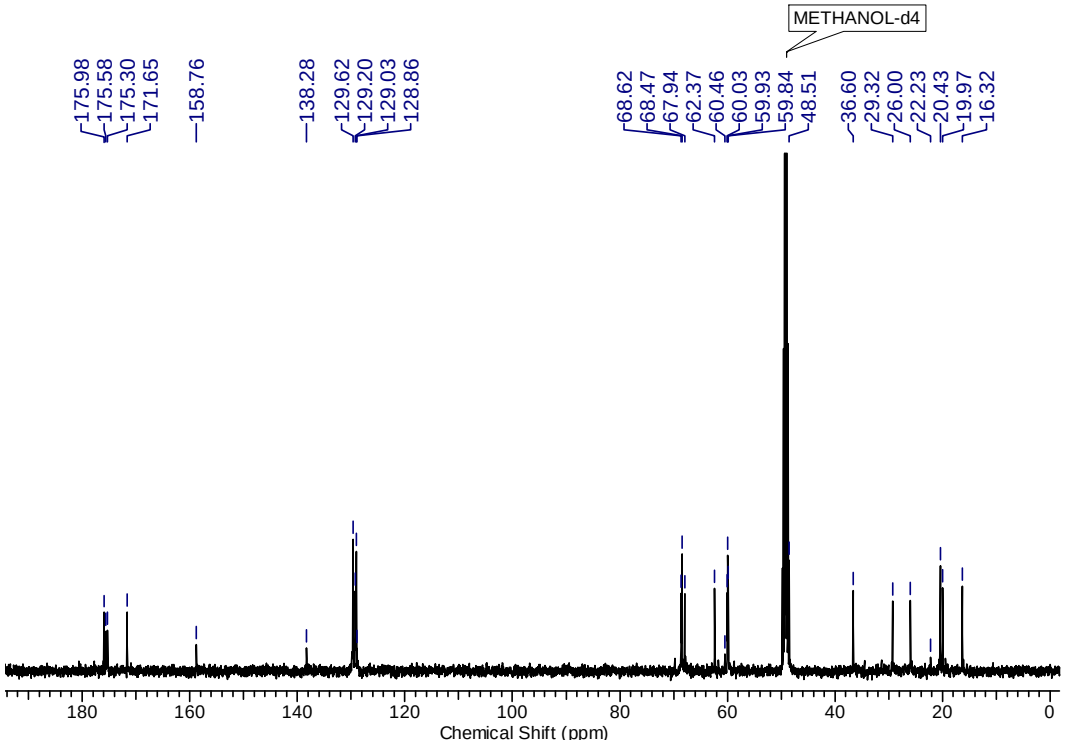
Compound 10 (Cbz-Thr-Pro-Sip-Thr-OMe): To a solution of tetrapeptide ester **9** (0.20 g, 0.32 mmol) in methanol, saturated methanolic ammonia solution (3 ml) was added and reaction mixture was stirred for 24 h at room temperature. Later, the volatiles were stripped off under reduced pressure and the product was further purified by column chromatography and preparative thin layer chromatography (eluent: 5% MeOH/DCM, R_f : 0.5) to furnish compound **6** (0.15 g, 78%) as a white solid; $[\alpha]^{26}_D$: -91.3° (c = 0.1, MeOH); ^{1}H NMR (400 MHz ,CD_3OD) δ = 7.47-7.25 (m, 5 H), 5.16-5.05 (m, 2 H), 4.97 (m, 1 H), 4.63 (m, 1 H), 4.40 (d, *J* = 5.5 Hz, 1 H), 4.30-4.14 (m, 2 H), 4.10-3.98 (m, 1 H), 3.97-3.85 (m, 1 H), 3.81-3.62 (m, 1 H), 3.23 (d, *J* = 13.4 Hz, 1 H), 2.89 (d, *J* = 13.4 Hz, 1 H), 2.45-1.96 (m, 4 H), 1.36-1.30 (m, 2 H), 1.26 (d, *J* = 6.1 Hz, 3 H), 1.19 (d, *J* = 6.1 Hz, 3 H), 1.16-1.10 (m, 2 H), 0.45-0.24 (m, 6 H); ^{13}C NMR (100 MHz ,CD_3OD) δ = 176.0, 175.6, 175.3, 171.6, 158.8, 138.3, 129.6, 129.2, 129.0, 128.9, 68.6, 68.5, 67.9, 62.4, 60.5, 60.0, 59.9, 59.8, 36.6, 29.3, 26.0, 22.2, 20.4, 20.0, 16.3. HRMS: $C_{27}H_{42}N_5O_8Si$, calculated: 592.2797 $[M+H]^+$, observed: 592.2797 $[M+H]^+$.



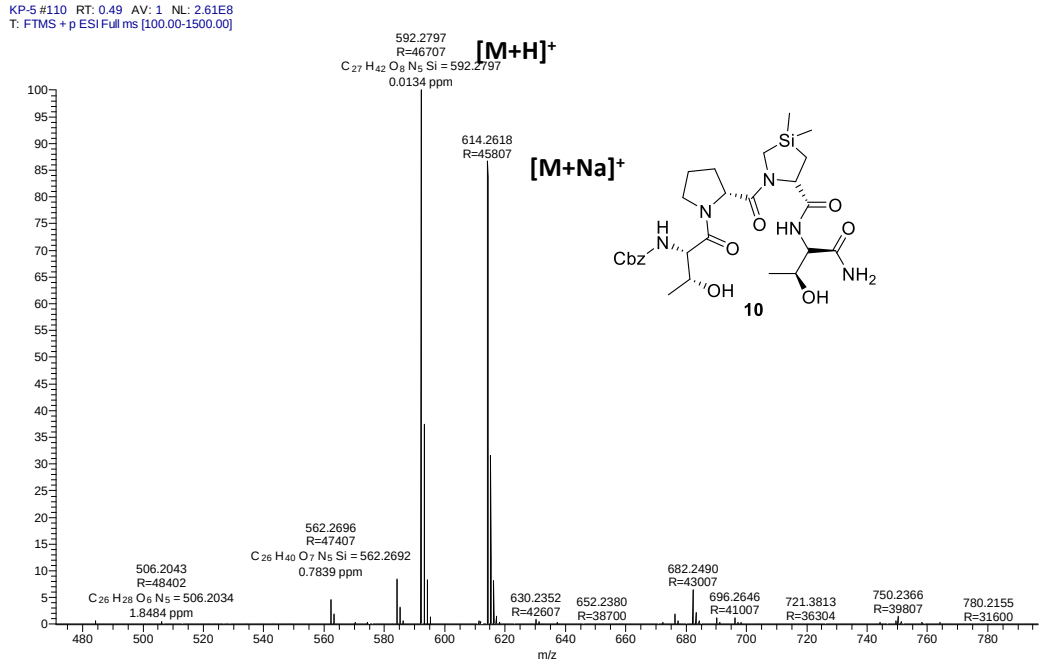
10



^{1}H NMR spectrum of compound **10** (CD_3OD, 400 MHz, 298 K)

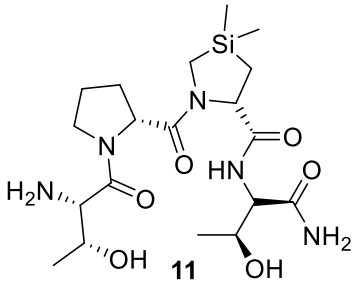


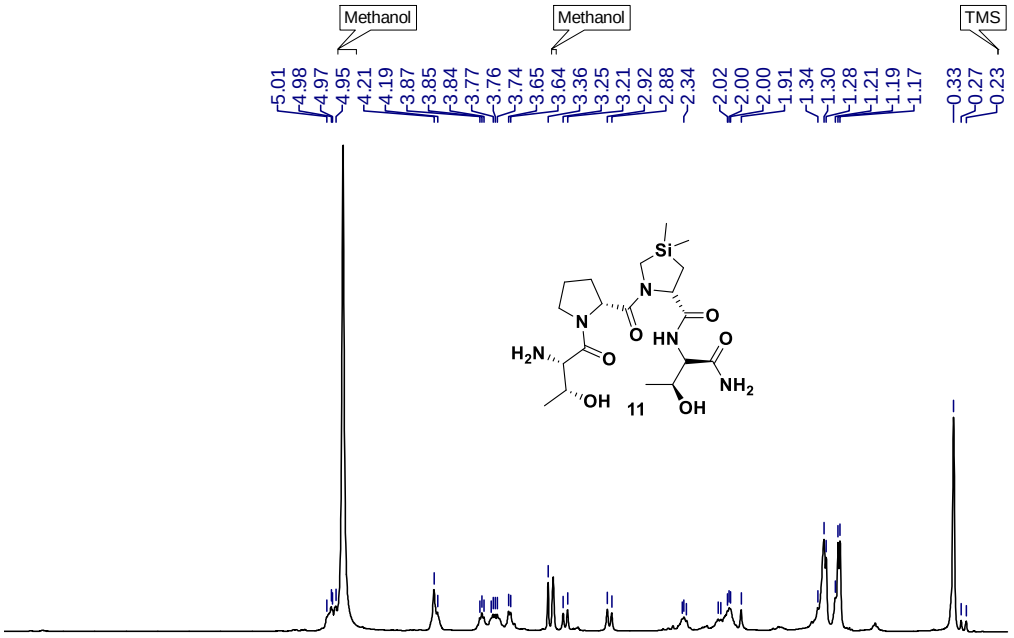
^{13}C NMR spectrum of compound **10** (CD_3OD, 100 MHz, 298 K)



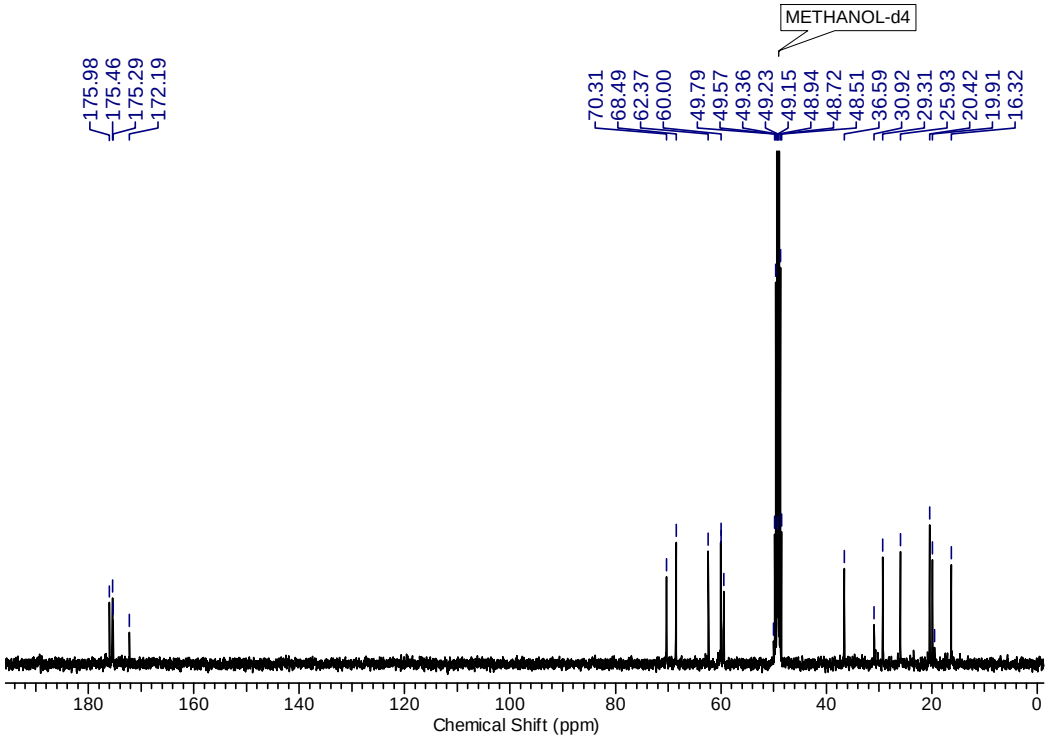
HRMS of compound **10**

Compound 11: **(Cbz-Thr-Pro-Sip-Thr-NH_2):**To a solution of **10** (0.13 g, 0.21 mmol) in methanol (0.1M) was added 10% Pd/C (0.1 equiv). The resulting mixture was stirred under an atmosphere of H_2 for 2 h and the mixture was filtered through a celite pad. The celite pad was then washed with methanol and the filtrate was concentrated in *vacuo* and purified by preparative thin layer chromatography (eluent: 10% MeOH/ 2% NH_3 solution/DCM, R_f : 0.4) to obtain the product **11** (0.049 g, 49%) as a white solid. $[\alpha]^{26}{}_D$: -38.8^0 (c = 0.1, MeOH), ^{1}H NMR (400 MHz ,CD_3OD) Shift = 5.06 - 4.95 (m, 2 H), 4.28 - 4.13 (m, 2 H), 3.92-3.80 (m, 1 H), 3.79 - 3.76 (m, 1 H), 3.68-3.58 (m, 1 H), 3.23 (d, *J* = 13.4 Hz, 1 H), 2.90 (d, *J* = 13.4 Hz, 1 H), 2.49-2.29 (m, 1 H), 2.13-1.88 (m, 3 H), 1.40-1.35 (m, 2H), 1.30-1.25 (m, 3 H), 1.23-1.20 (m, 3 H), 1.19-1,14 (m, 2H), 0.4-0.21 (m, 6 H); ^{13}C NMR (100 MHz ,CD_3OD) δ = 176.0, 175.5, 175.3, 172.2, 70.3, 68.5, 62.4, 60.0, 59.4, 49.0, 36.6, 30.9, 29.3, 25.9, 20.4, 19.9, 19.4, 16.3. HRMS: $C_{19}H_{36}N_5O_6Si$, calculated: 458.2429 $[M+H]^+$, observed: 458.2436 $[M+H]^+$.

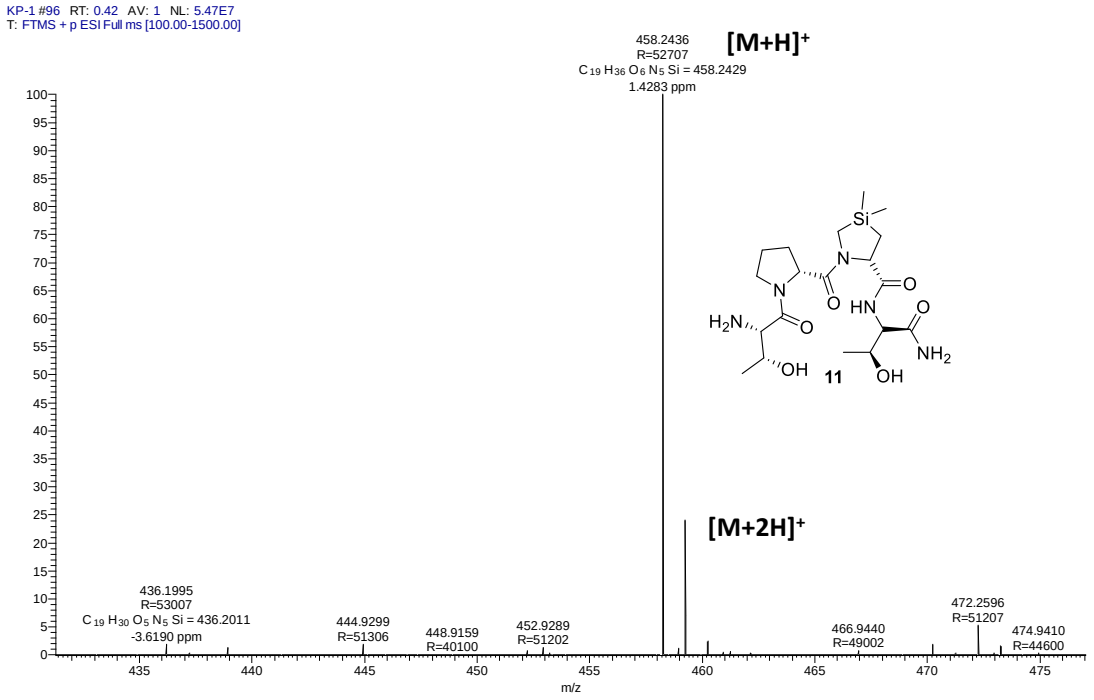




^{1}H NMR spectrum of compound **11** (CD_3OD, 400 MHz, 298 K)



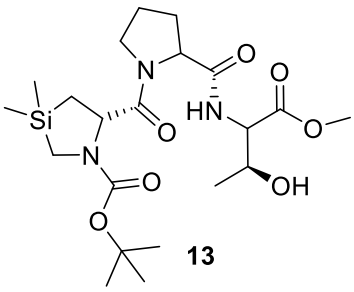
^{13}C NMR spectrum of compound **11** (CD_3OD, 100 MHz, 298 K)



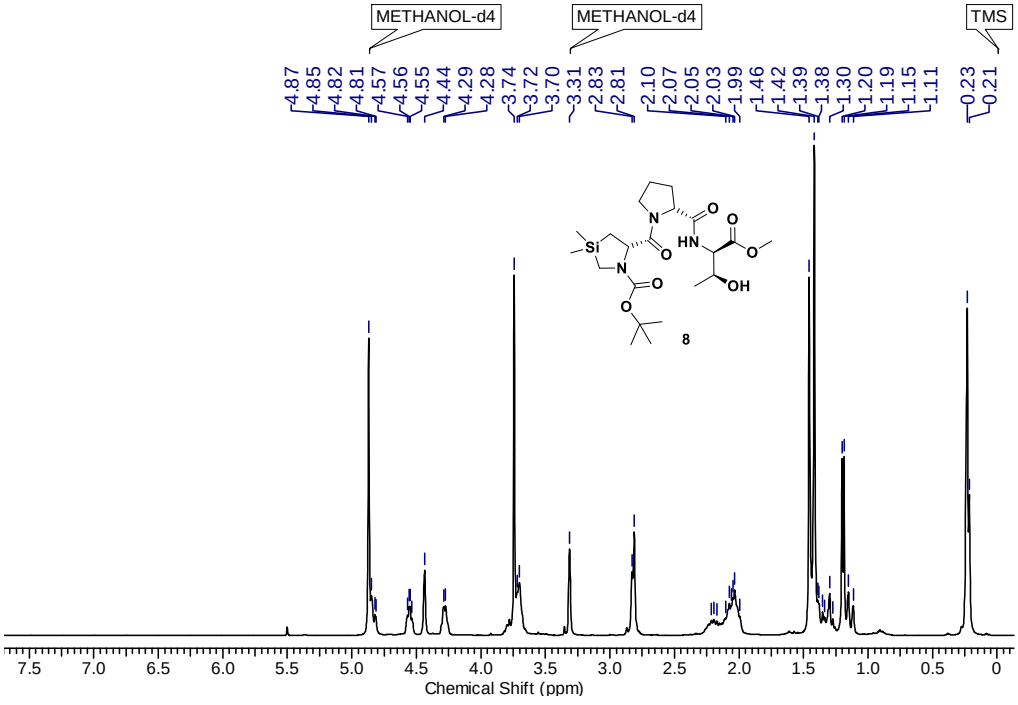
HRMS of Compound **11**

Compound **12** was prepared according to the reported procedure.[1]

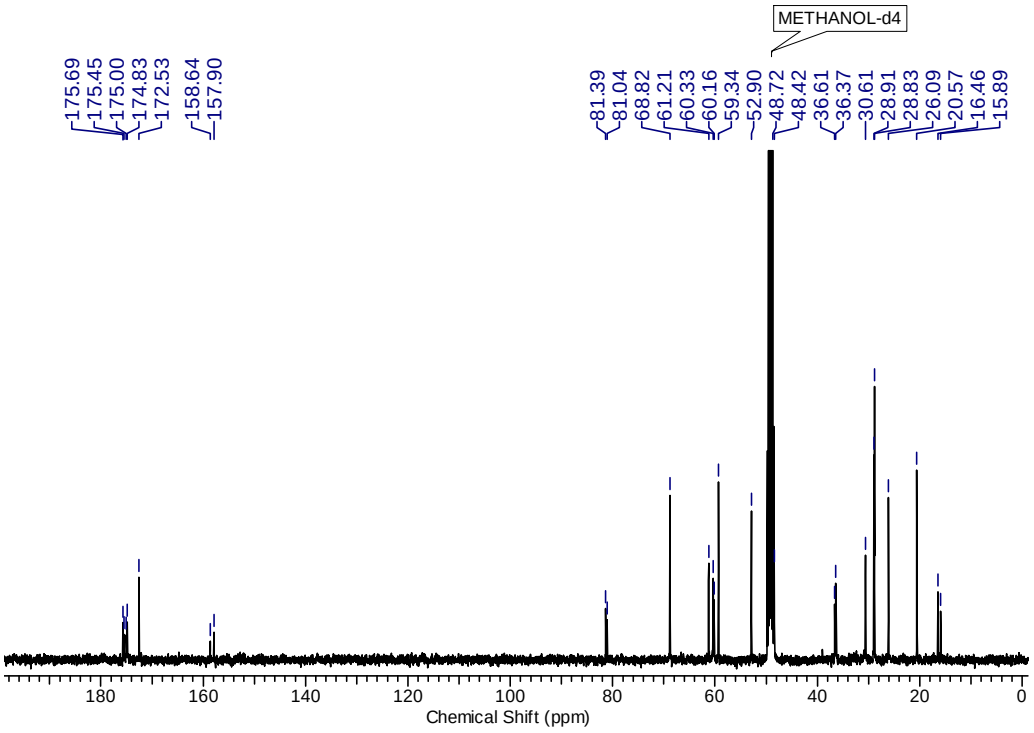
Compound 13 (Boc-Sip-Pro-OMe): To a solution of **12** (0.35 g, 0.7 mmol) in methanol (0.1M) was added 10% Pd/C (0.1 equiv). The resulting mixture was stirred under an atmosphere of H_2 for 2 h, and was filtered through a celite pad. The celite pad was then washed with methanol and the filtrate was concentrated in *vacuo* to obtain the free acid which was used for the next step without further purification. Dipeptide acid (0.25 g, 0.7 mmol, 1 equiv) was then coupled with threonine methyl ester hydrochloride (0.29 g, 1.7 mmol, 2.5 equiv) using HATU (0.40 g, 1 mmol, 1.5 equiv) and DIPEA (0.5 mL, 2.8 mmol, 4 equiv) in DMF for 12 h at room temperature. Reaction mixture was then taken in EtOAc and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. Organic layer was dried over anhydrous Na_2SO_4 and evaporated under



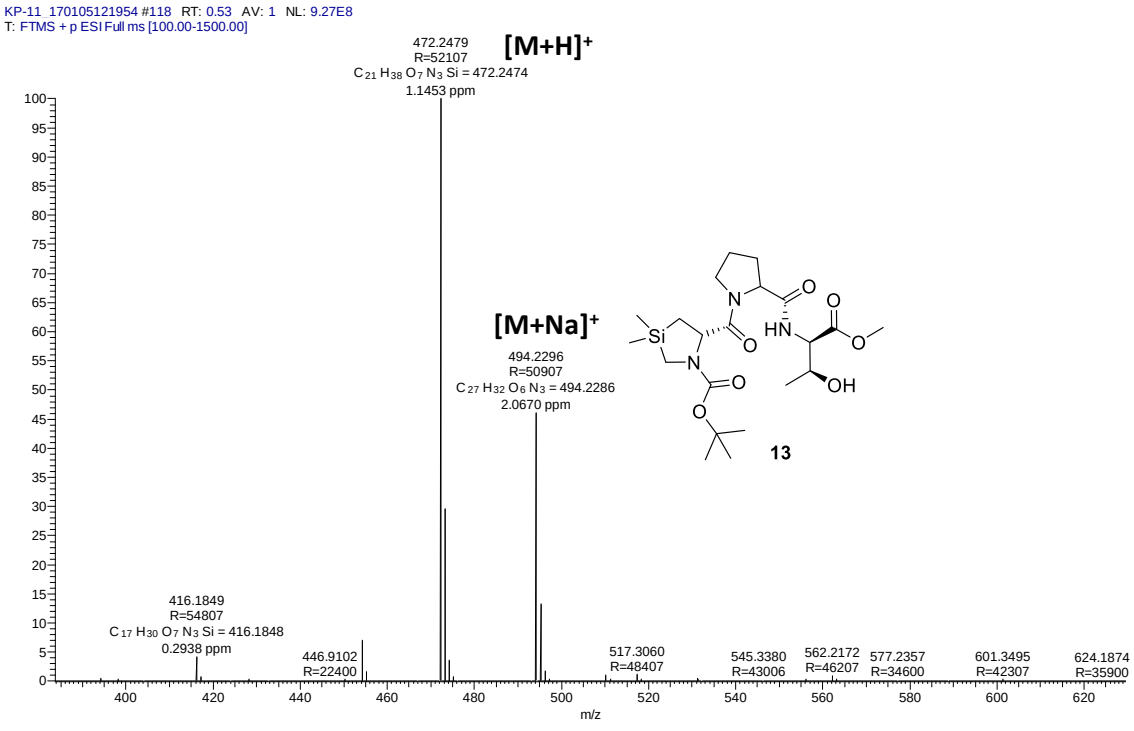
reduced pressure to get the crude product which was purified by column chromatography (eluent: 70% AcOEt/pet. ether, Rf : 0.5) to furnish compound **13** (0.26 g, 79%) as a hygroscopic white solid; $[\alpha]^{26}_{D}$: -75.5° (c = 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ = 4.85-4.80 (m, 1 H), 4.61-4.50 (m, 1 H), 4.43 (bs, 1 H), 4.28 (d, *J* = 4.9 Hz, 1 H), 3.74 (s, 3 H), 3.71 (d, *J* = 6.7 Hz, 2 H), 2.85-2.78 (m, 2 H), 2.29-2.10 (m, 2 H), 2.09-1.97 (m, 2 H), 1.45 (s, 4 H), 1.43-1.39 (m, 5 H), 1.34-1.25 (m, 1 H), 1.19 (d, J = 6.1 Hz, 3 H), 1.13 (d, *J* = 15.3 Hz, 1 H), 0.31-0.17 (m, 6 H). ^{13}C NMR (100 MHz ,CD_3OD) δ = 175.7, 175.4, 175.0, 174.8, 172.5, 158.6, 157.9, 81.4, 81.0, 68.8, 61.2, 60.3, 60.2, 59.3, 52.9, 48.4, 36.6, 36.4, 30.6, 28.9, 28.8, 26.1, 20.6, 16.5, 15.9. HRMS: $C_{21}H_{38}N_3O_7Si$, calculated: 472.2474 $[M+H]^+$, observed: 472.2479 $[M+H]^+$.



^{1}H NMR spectrum of compound **13** (CD_3OD, 400 MHz, 298 K)

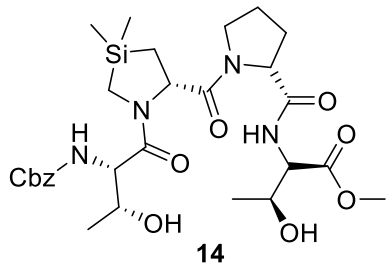


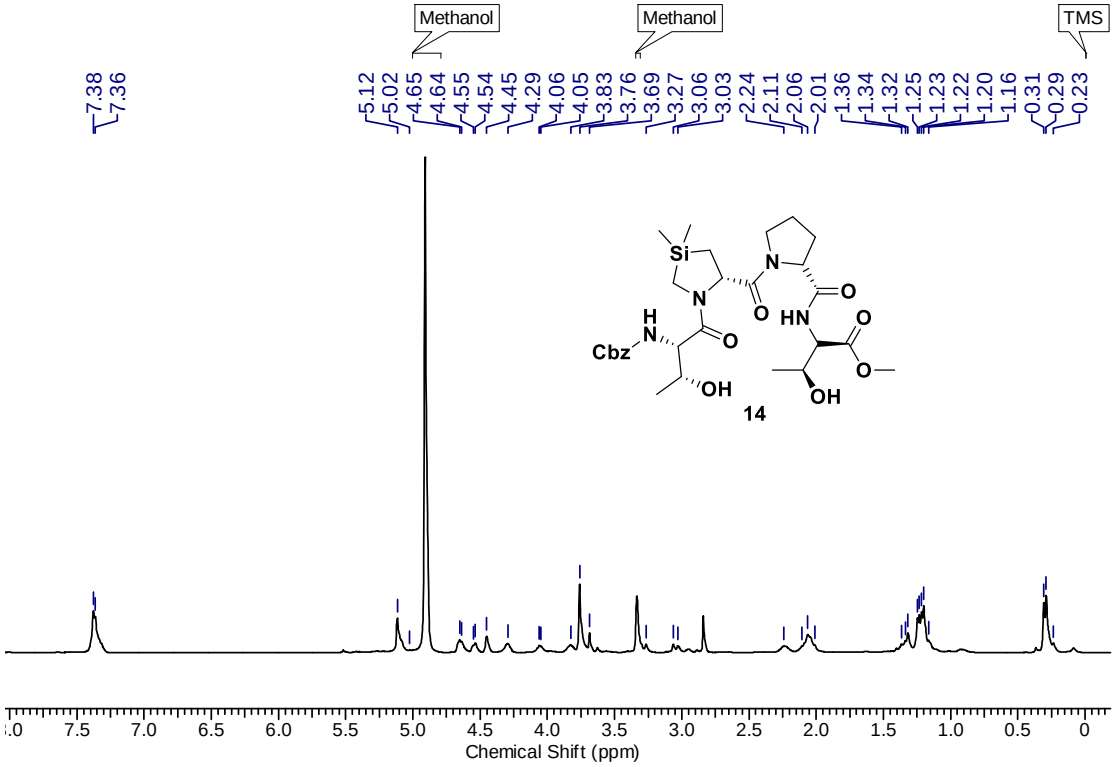
^{13}C NMR spectrum of compound **13** (CD_3OD, 100 MHz, 298 K)



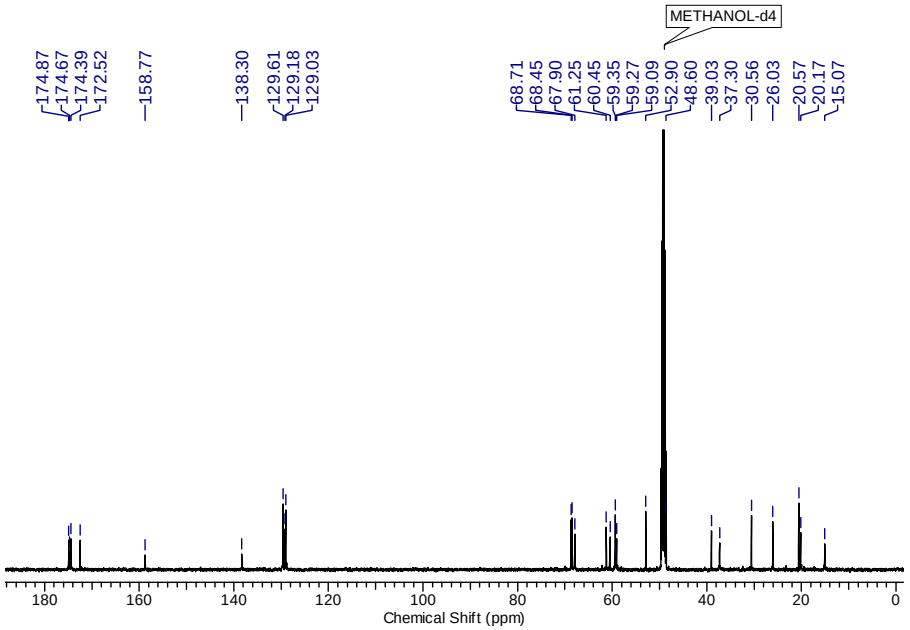
HRMS of compound **13**

Compound 14 (Cbz-Thr-Sip-Pro-Thr-OMe): Boc-protected tripeptide ester **13** (0.23 g, 0.4 mmol) was dissolved in 1 mL of dichloromethane followed by addition of 1 mL of TFA (1M) at 0° C. Reaction mixture was stirred for 30 min at room temperature. After completion of reaction the solvent was stripped off and co-evaporated using toluene to obtain the TFA salt which was used for the next step without further purification. Cbz-Thr-OH (0.13 g, 0.51 mmol, equiv) was coupled with tripeptide ester TFA salt (0.19 g, 0.51 mmol, 1 equiv) using HATU (0.29 g, 0.76 mmol, 1.5 equiv) and DIPEA (0.37 mL, 2 mmol, 4 equiv) in DMF for 12 h at room temperature. Reaction mixture was then taken in EtOAc and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. Organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude products which was purified by column chromatography (eluent: 3% MeOH/DCM, R_f: 0.5) to furnish compound **14** (0.21g, 67%) as a hygroscopic white solid; $[\alpha]^{26}_D$: -60.1° (c = 0.1, MeOH), ^{1}H NMR (400 MHz ,CD_3OD) δ = 7.35-7.29 (m, 5 H), 5.09 (m, 2H), 5.11-5.07 (m, 1H), 4.75-4.64 (m, 1 H), 4.58-4.51(m. 1 H), 4.49-4.42 (m, 1 H), 4.35-4.25 (m, 1 H), 4.15-4.03 (m, 1 H), 3.89-3.80 (m, 1H), 3.77 (bs, 3 H), 3.75-3.69 (m, 1H), 3.32-3.24 (m, 1 H), 3.13-2.97 (m, 1H), 2.28-2.00 (m, 4H), 1.31-1.28 (m, 2 H), 1.25 (d, *J* = 5.5 Hz, 3 H), 1.18 (d, *J* = 6.7 Hz, 3 H), 1.14 (m, 2 H), 0.39-0.15 (m, 6 H). ^{13}C NMR (100 MHz ,CD_3OD) δ = 174.8, 174.4, 158.8, 138.3, 129.6, 129.2, 129.0, 128.8, 68.7, 68.4, 67.9, 61.3, 60.4, 59.3, 59.1, 52.9, 48.6, 39.0, 37.3, 30.6, 26.0, 20.6, 20.2, 15.1. HRMS: $C_{28}H_{43}N_4O_9Si$, calculated: 607.2794 $[M+H]^+$, observed: 607.2797 $[M+H]^+$.

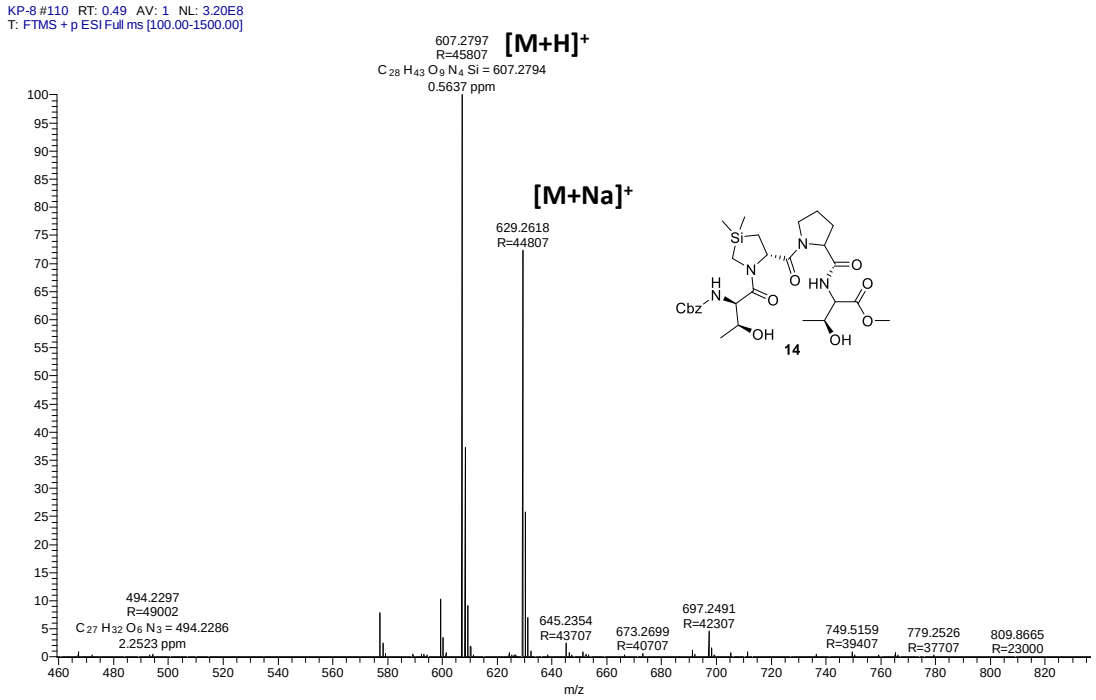




^{1}H NMR spectrum of compound **14** (CD_3OD, 400 MHz, 298 K)

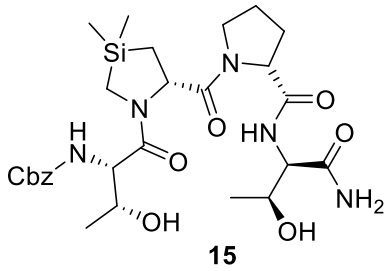


^{13}C NMR spectrum of compound **14** (CD_3OD, 100 MHz, 298 K)



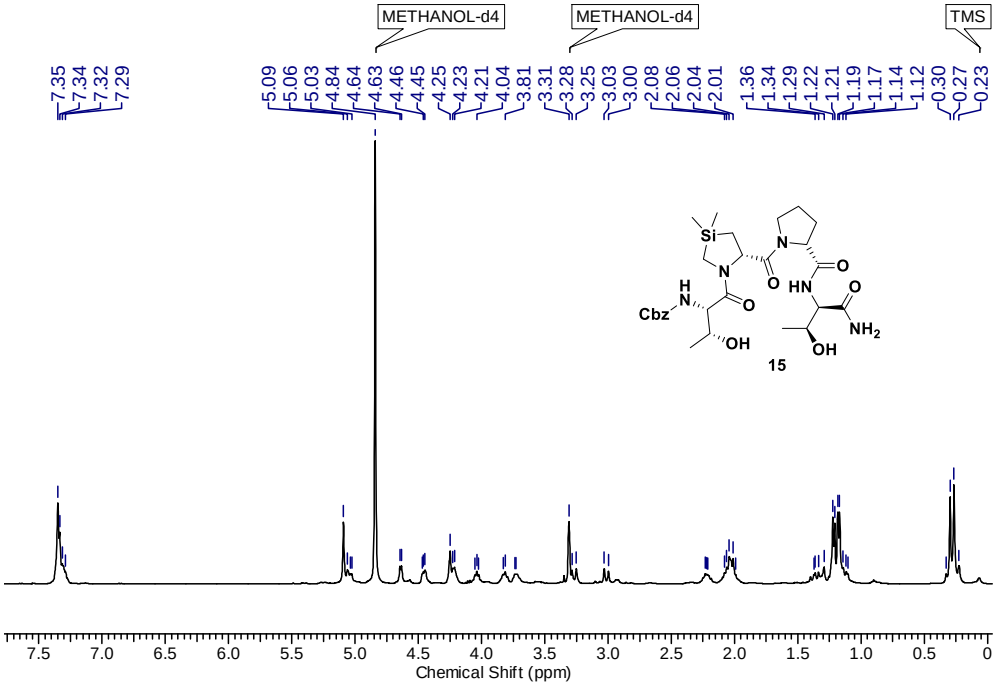
HRMS of compound **14**

Compound 15 (Cbz-Thr-Sip-Thr-NH_2): To a solution of **14** (0.20 g, 0.3 mmol) in methanol (0.1M) was added 10% Pd/C (0.1 equiv). The resulting mixture was stirred under an atmosphere of H_2 for 2 h, after which time the mixture was filtered through a celite pad. The celite pad was washed with methanol and the filtrate was concentrated *in vacuo*. It was then further purified by column chromatography and preparative thin layer chromatography (eluent : 3% MeOH/DCM, Rf: 0.4) to furnish compound **15**. (0.16 g, 82%) as a hygroscopic white solid; $[\alpha]^{26}_D$: -92.7° (c = 0.1, MeOH), ^{1}H NMR (400 MHz, CD_3OD) δ = 7.47-7.16 (m, 5 H), 5.09 (s, 2 H), 5.07-5.00 (m, 1 H), 4.64 (d, *J* = 4.9 Hz, 1 H), 4.52-4.40 (m, 1 H), 4.31-4.25 (m, 1 H), 4.24-4.17 (m, 1 H), 4.14-3.99 (m, 1 H), 3.87-3.65 (m, 2H), 3.27 (d, *J* = 13.4 Hz, 1 H), 3.01 (d, *J* = 13.4 Hz, 1 H), 2.34-1.88 (m, 4 H), 1.44-1.26 (m, 2 H), 1.22 (d, *J* = 6.1 Hz, 3 H), 1.18 (d, *J* = 6.1 Hz, 3 H), 1.16 - 1.06 (m, 2 H), 0.36-0.18 (m, 6 H). ^{13}C NMR (100 MHz ,CD_3OD) δ = 175.3, 175.2, 174.6, 174.5, 158.8, 138.1, 129.6, 129.2,

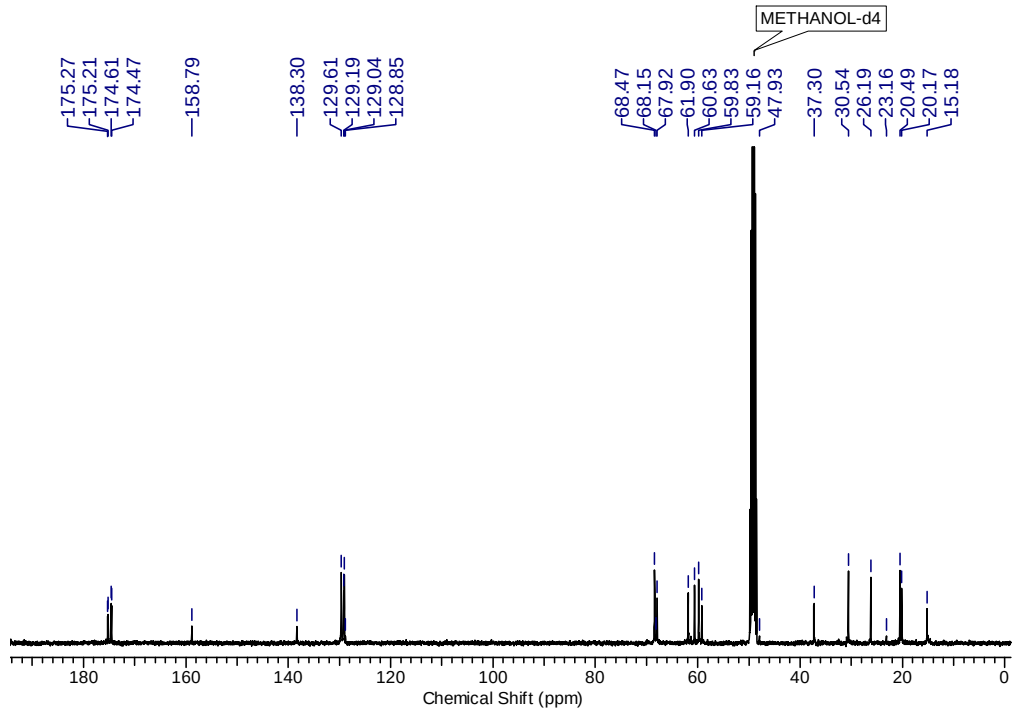


15

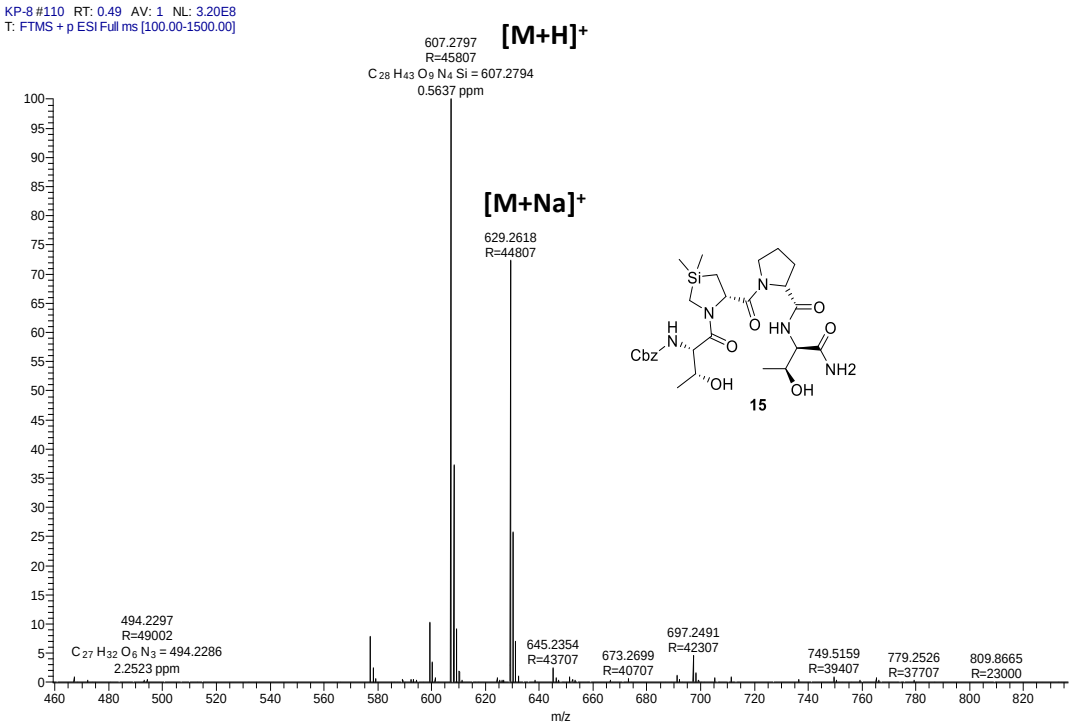
129.0, 128.8, 68.5, 68.4, 67.9, 61.9, 60.6, 59.8, 59.2, 47.9, 37.3, 30.5, 26.2, 20.5, 20.2, 15.2 HRMS: $C_{27}H_{42}N_5O_8Si$, calculated: 592.2797 $[M+H]^+$, observed: 592.2805 $[M+H]^+$.



^{1}H NMR spectrum of compound **15** (CD_3OD, 400 MHz, 298 K)

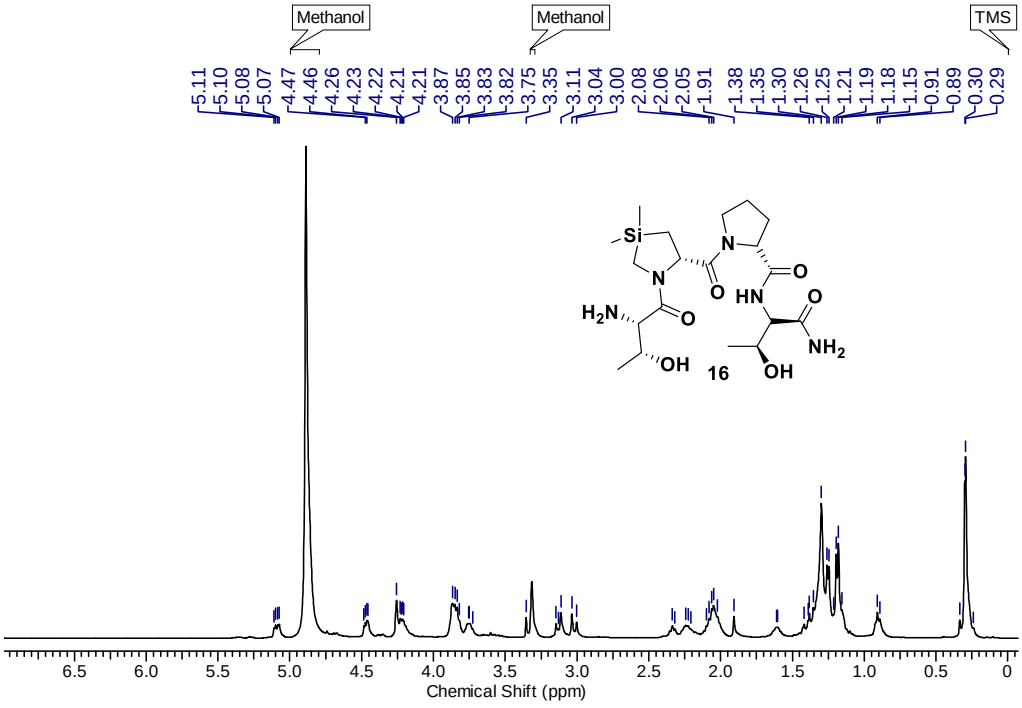


^{13}C NMR spectrum of compound **15** (CD_3OD, 100 MHz, 298 K)

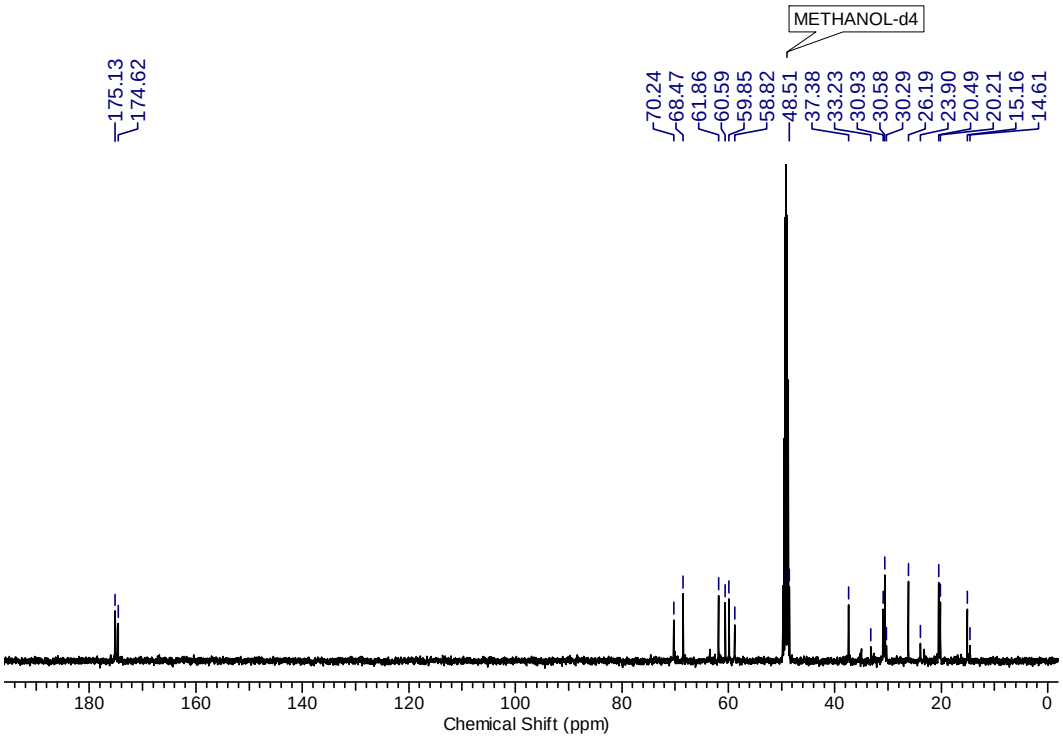


HRMS of compound **15**

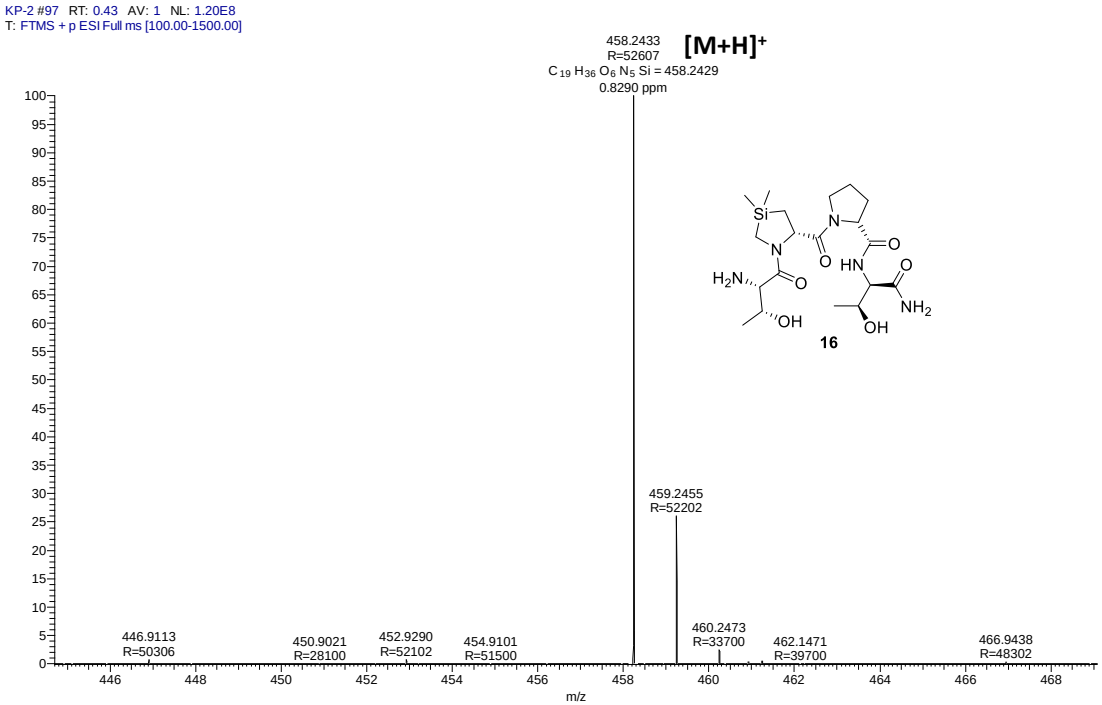
Compound 16 (H-Thr-Sip-Pro-Thr-NH_2): To a solution of **15** (0.14 g, 0.23 mmol) in methanol (0.1M) was added 10% Pd/C (0.1 equiv). The resulting mixture was stirred under an atmosphere of H_2 for 2 h, and was filtered through a celite pad. The celite pad was then washed with methanol and the filtrate was concentrated in *vacuo* and purified by preparative thin layer chromatography (eluent: 10% MeOH/ 2% NH_3 solution/DCM, R_f : 0.4) to obtain the product **16** (0.057 g, 53 %) as a white solid; $[\alpha]^{26}_D$: -76.4^0 (c = 0.1, MeOH), ^{1}H NMR (400 MHz ,CD_3OD) δ = 5.09 (dd, *J* = 10.1, 4 Hz, 1 H), 4.47 (dd, *J* = 8.2, 3.4 Hz, 1 H), 4.32 - 4.14 (m, 2 H), 3.94-3.79 (m, 2 H), 3.77-3.69 m (1 H), 3.19-3.07 (m, 1 H), 3.05-2.95 (m, 1 H), , 2.49 -1.88 (m, 4 H), 1.44-1.35 (m, 2H), 1.30-1.25 (m, 3 H), 1.23-1.20 (m, 3 H), 1.12- 0.89 (m, 2H), 0.42-0.21 (m, 6 H); ^{13}C NMR (100 MHz ,CD_3OD) δ = 175.2, 175.1, 174.6, 70.2, 68.5, 61.9, 60.6, 59.9, 58.8, 37.4, 30.9, 26.2, 20.5, 20.2, 15.2, 14.6. $C_{19}H_{36}N_5O_6Si$, calculated: 458.2429 $[M+H]^+$, observed: 458.2433 $[M+H]^+$.



^{1}H NMR spectrum of compound **16** (CD_3OD, 400 MHz, 298 K)



^{13}C NMR spectrum of compound **16** (CD_3OD, 100 MHz, 298 K)



HRMS of compound **16**

Chapter 2

Part B

Novel 2-Aminoisobutyric acid (Aib), Fenclonine and L-threo-3-phenylserine incorporated close Structural Mimics of Potent Antidepressant Peptide Drug Rapastinel (GLYX-13).

2.3 Introduction

2.3.1 Aib-containing peptide analogs:

α-Aminoisobutyric acid (Aib) is an unnatural hydrophobic amino acid, in which its α-carbon is substituted by methyl group. Its incorporation in to peptide sequence imparts helicity.[20] It is also known to be resistant to protease and strongly binds to cell membrane.[21] S. Wada *et al.* have shown that cell uptake was more for Aib-containing amphipathic helix peptide than for the non-Aib amphipathic helix peptide.[22] Aib-containing peptides shows 3_{10}, α and mixed 3_{10}/α-helical structures predominantly. The semi-extended polyproline II (PII) conformation is also often observed in protected Aib containing peptides. So it is generally considered as helicogenic residue because of its ability to impart helical folding in many natural as well as unnatural peptide sequences.

2.3.2 Fenclonine and L-threo-3-phenylserine as useful intermediates in drug discovery

Para-chlorophenylalanine which is also known as fenclonine is a inhibitor of trytophan hydroxylase which is used as rate limiting enzyme in the biosynthesis of seriotonin. It is also helpful in treating carcinoid syndrome.[23]

In enzymology, a phenylserine aldolase is an enzyme that catalyzes the chemical reaction

L-threo-3-phenylserine → glycine + benzaldehyde

This enzyme has one substrate, L-threo-3-phenylserine and it has two products namely, benzaldehyde and glycine. Both these molecules are structurally similar to that of threonine found in the N-terminus of rapastinel (H-Thr-Pro-Pro-Thr-NH_2).

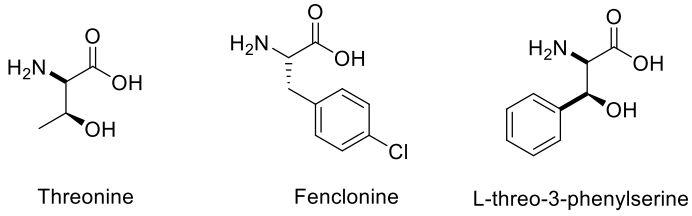


Figure 2.3.1 Molecular structures of threonine, fenclonine and L-threo-3-phenylserine

2.3.2 Objective and design strategy

α-Aminoisobutyric acid (Aib), fenclonine (p-chlorophenylalanine) and L-threo-3-phenylserine incorporated close structural analogues of rapastinel were designed to enchance the hydrophobicity, cell permeability and conformational stability. The molecular structures of rapastinel analogues designed are as shown below.

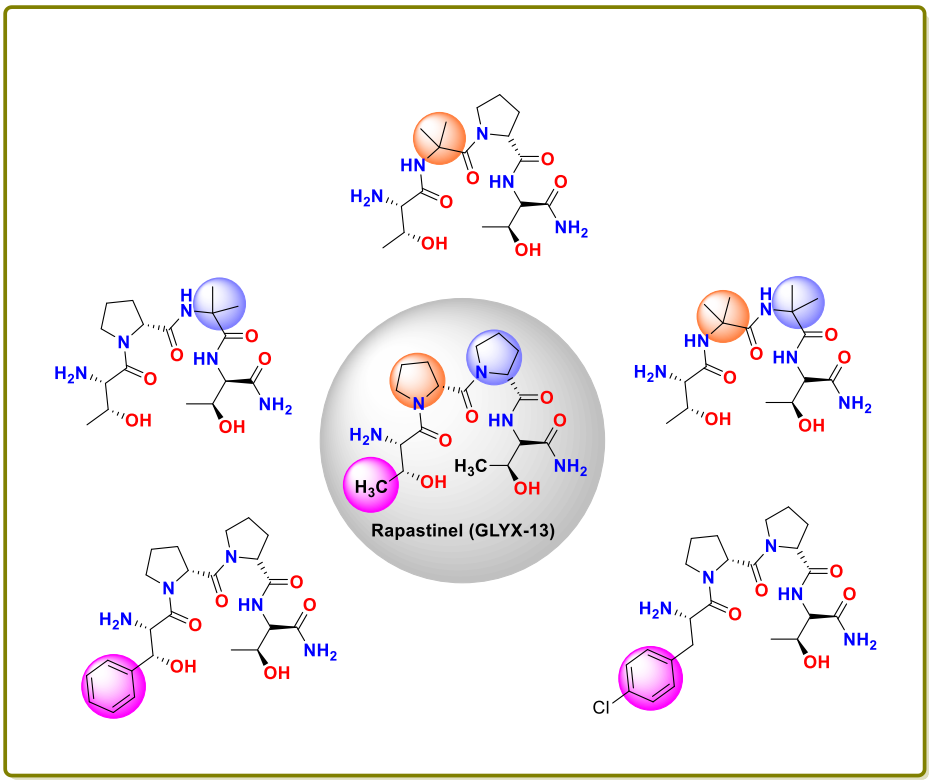


Figure 2.3.2: Molecular structures of the peptides featuring rapastinel analogues synthesized.

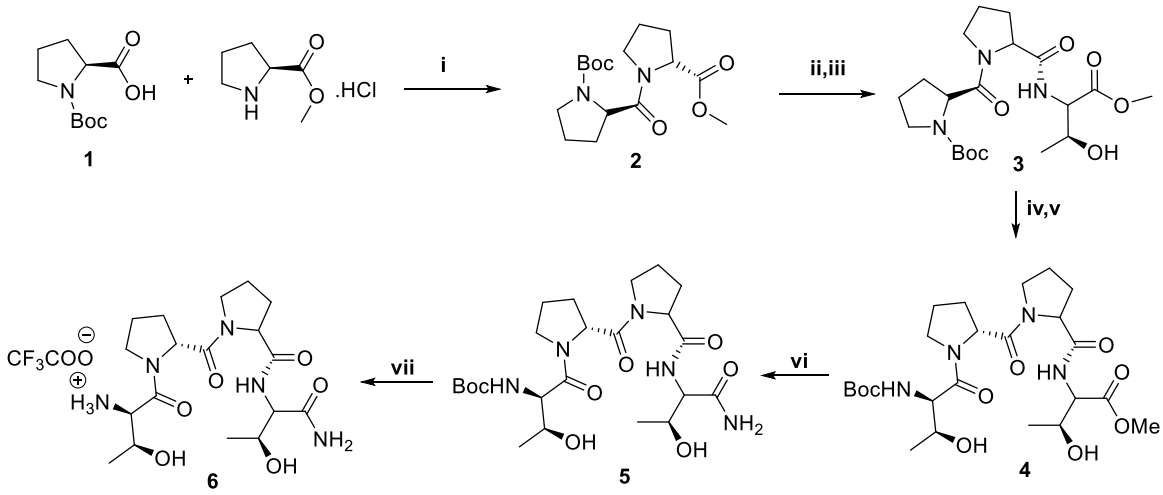
2.3.3 Synthesis

Aib containing peptides were synthesized by standard peptide coupling strategy in solution phase. Rapastinel peptide was synthesized initially using peptide coupling strategy and the same was followed for Aib-containing peptides.

2.3.3.1 Synthesis of rapastinel peptide sequence (TFA.H-Thr-Pro-Pro-Thr-NH_2)

Compound **2** was synthesized according to the reported procedure. Compound **2** was hydrolyzed using LiOH and was then coupled with H-Thr-OMe.HCl using EDC.HCl and

HOBt as coupling agent to obtain tripeptide ester **3** in good yield. Tripeptide ester **3** was then Boc-deprotected using TFA in DCM and then coupled with Boc-Thr-OH to furnish tetrapeptide ester **4** in good yield. Compound **4** was then subjected to amidation to obtain tetrapeptide amide **5** which was then Boc-deprotected to furnish compound **6** in reasonable yield.



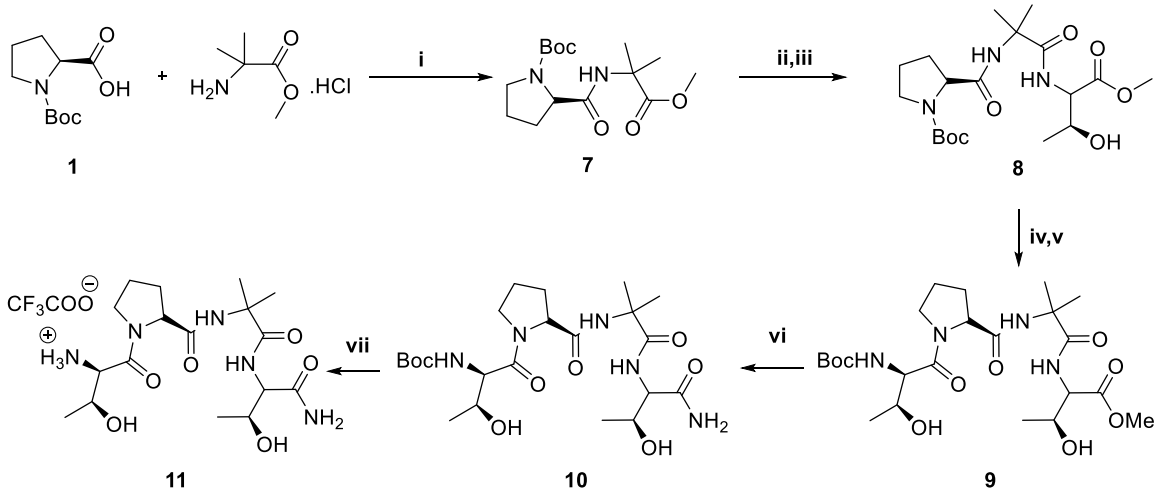
Scheme 2.4: Reagents and conditions : (i) DCC, HOBt, DIPEA, DCM, 24 h, rt, 50%; (ii) LiOH, MeOH/H_2O, rt, 100%; (iii) H-Thr-OMe.HCl, EDC.HCl, HOBt, DIPEA, DMF, 24h, rt, 73%; (iv) TFA, DCM, 0^0 C-rt; (v) Boc-Thr-OH, HATU, DIPEA, DMF, 16h, rt, 62%; (vi) NH_3/methanol, rt, 87%. (vii) TFA, DCM, 0^0C-rt, 93 %.

Following the synthetic scheme for the preparation of rapastinel, Aib-containing rapastinel analogues were synthesized.

2.3.3.2 Synthesis of TFA.H-Thr-Pro-Aib-Thr-NH_2

Boc-Pro-OH **1** was coupled with H-Aib-OMe.HCl using EDC.HCl and HOBt as coupling agent to obtain dipeptide ester **7** which was then hydrolyzed by LiOH to obtain dipeptide acid. Without further purification, dipeptide acid was coupled with H-Thr-OMe.HCl using HATU as coupling agent to furnish tripeptide ester 8 in good yield. Tripeptide ester **8** was then Boc-deprotected using TFA in DCM and then coupled with Boc-Thr-OH to furnish tetrapeptide ester **9** in good yield. Compound **9** was then subjected to amidation

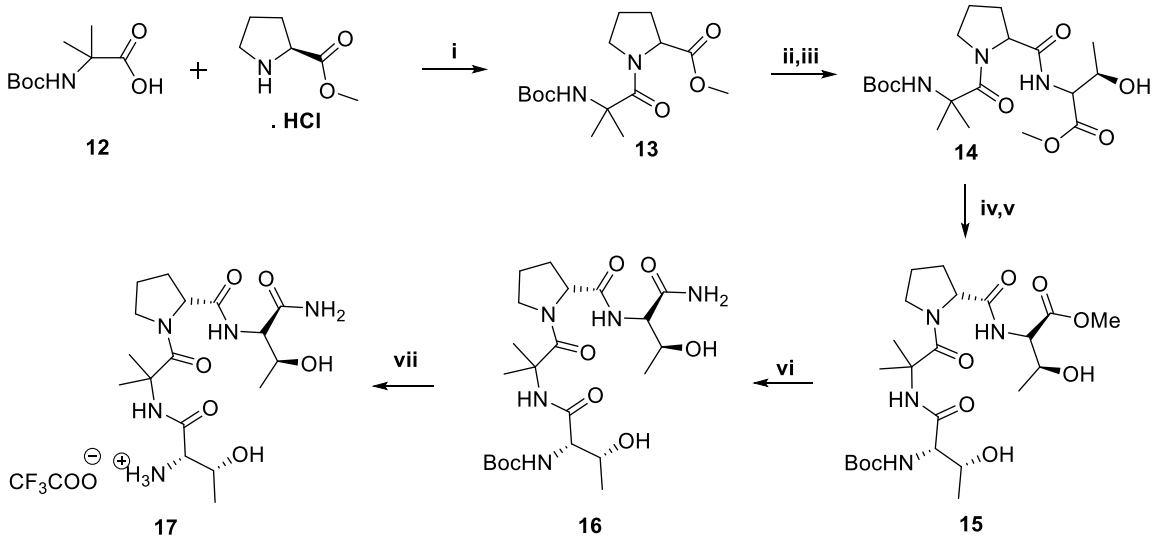
to obtain tetrapeptide amide **10** which was then Boc-deprotected to furnish compound **11** in reasonable yield.



Scheme 2.5: Reagents and conditions **:** (i) EDC.HCl, HOBt, Et_3N, DCM, 40h, rt, 70%; (ii) LiOH, MeOH/H_2O, rt, 95%; (iii) H-Thr-OMe.HCl, EDC.HCl, HOBt, DIPEA, DMF, 24h, rt, 73%; (iv) TFA, DCM, 0^0C-rt; (v) Boc-Thr-OH, HATU, DIPEA, DMF, 16 h, rt, 76%; (vi) NH_3/methanol, rt. 86%; (vii) TFA, DCM, 0^0C-rt, 94 %.

2.3.3.3 Synthesis of TFA.H-Thr-Aib-Pro-Thr-NH_2

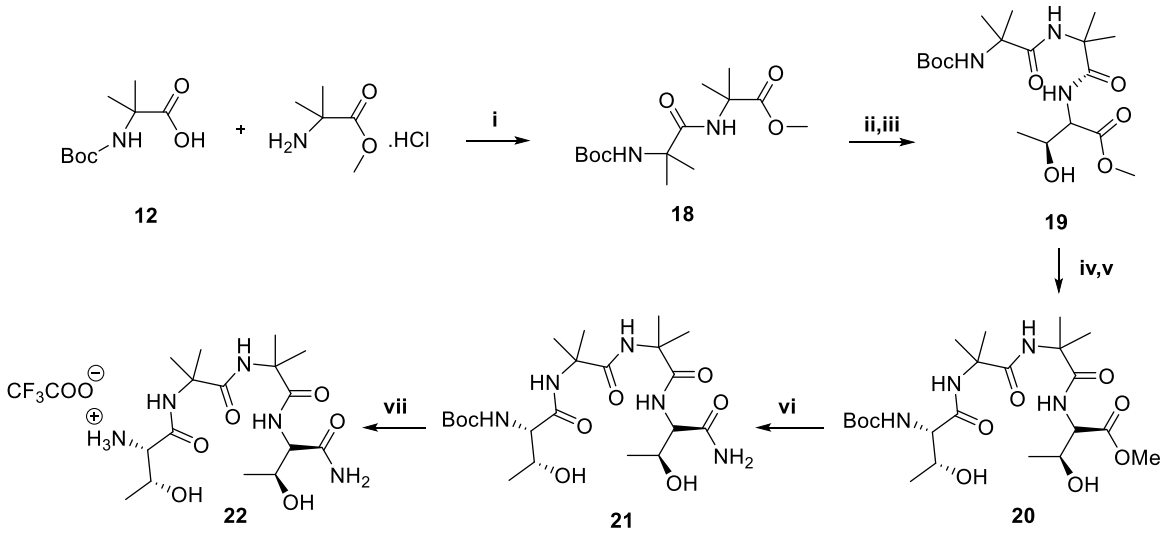
Boc-Aib-OH **2** was coupled with H-Pro-OMe.HCl using EDC.HCl and HOBt as coupling agent to obtain dipeptide ester **13** which was then hydrolyzed by LiOH to obtain dipeptide acid. Without further purification, dipeptide acid was coupled with H-Thr-OMe.HCl using HATU as coupling agent to furnish tripeptide ester **14** in good yield. Tripeptide ester **14** was then Boc-deprotected using TFA in DCM and then coupled with Boc-Thr-OH to furnish tetrapeptide ester **15** in good yield. Compound **15** was then subjected to amidation to obtain tetrapeptide amide **16** which was then Boc-deprotected to furnish compound **17** in reasonable yield.



Scheme 2.6: Reagents and conditions : (i) EDC.HCl, HOBt, Et_3N, DCM, 40h, rt, 45%; (ii) LiOH, MeOH/H_2O, rt, 98 %; (iii) H-Thr-OMe.HCl, EDC.HCl, HOBt, DIPEA, DMF, 24 h, rt, 80%; (iv) TFA, DCM, 0 °C-rt; (v) Boc-Thr-OH, HATU, DIPEA, DMF, 16 h, rt, 80%; (vi) NH_3/methanol, rt, 75%; (vii) TFA, DCM, 0 °C -rt, 93 %.

2.3.3.4 Synthesis of TFA.H-Thr-Aib-Aib-Thr-NH_2

Boc-Aib-OH **12** was coupled with H-Pro-OMe.HCl using EDC.HCl and HOBt as coupling agent to obtain dipeptide ester **18** which was then hydrolyzed by LiOH to obtain dipeptide acid. Without further purification, dipeptide acid was coupled with H-Thr-OMe.HCl using HATU as coupling agent to furnish tripeptide ester **19** in good yield. Tripeptide ester **19** was then Boc-deprotected using TFA in DCM and then coupled with Boc-Thr-OH to furnish tetrapeptide ester **20** in good yield. Compound **20** was then subjected to amidation to obtain tetrapeptide amide **21**which was then Boc-deprotected to furnish compound **22** in reasonable yield.

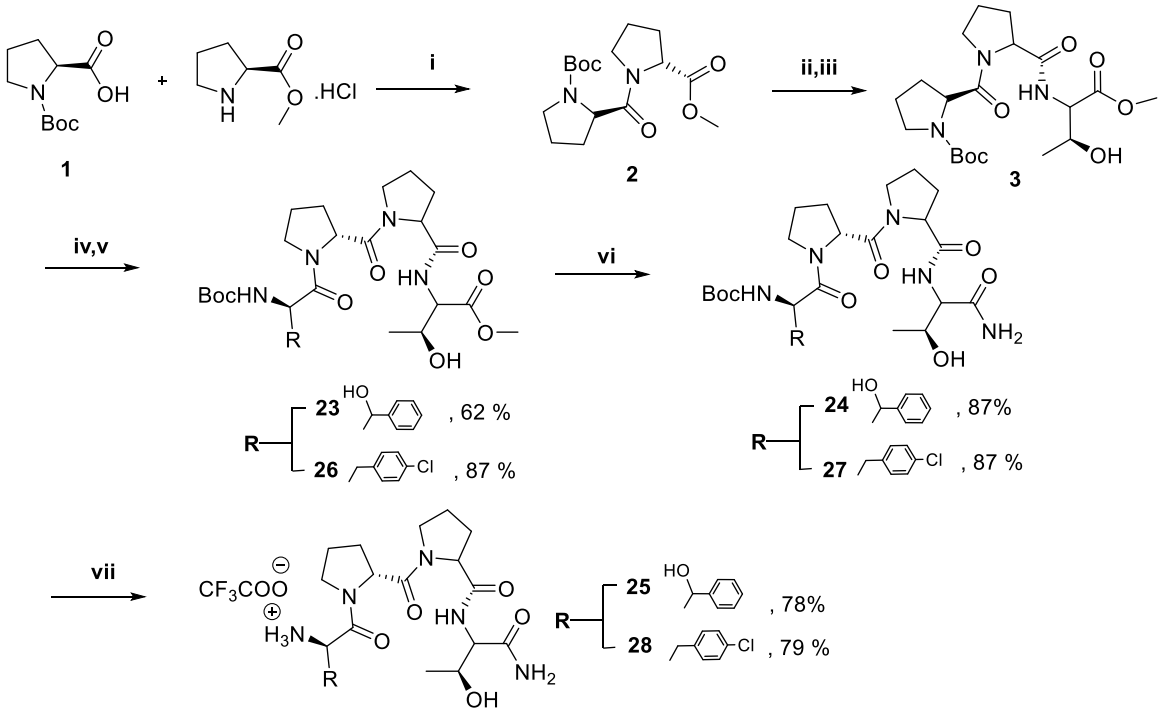


Scheme 2.7: Reagents and conditions : (i) EDC.HCl, HOBt, DIPEA,DMF, 24 h, rt, 75%; (ii) LiOH, MeOH/H_2O, rt, 90%; (iii)H-Thr-OMe.HCl, EDC.HCl, HOBt, DIPEA, DMF, 24 h, rt, 74%; (iv) TFA, DCM, 0 °C-rt; (v) Boc-Thr-OH, HATU, DIPEA, DMF, 16 h, rt, 76%; (vi) NH_3/methanol, rt, 93 %; (vii) TFA, DCM, 30 min, 0 °C-rt, 93 %.

Synthesis of rapastinel peptide analogs modified at N-terminus (H-Thr-Pro-Pro-Thr-NH_2)

2.3.3.5 Synthesis of peptide sequences H-PCPA-Pro-Pro-Thr-NH_2 and H-PheSer-Pro-Pro-Thr-NH_2

Compound **3** was synthesized according to the procedure as shown in scheme 2.4. It was then Boc-deprotected using TFA in DCM and was used for the next step without further purification. Boc-fenclonine and Boc-L-threophenylserine was coupled with tripeptide acid using HATU as coupling agent to furnish compound **23** and **24** respectively. Compounds **23** and **24** were then subjected to amidation using methanolic ammonia to furnish compounds **25** and **26** respectively. Finally compounds **25** and **26** were Boc-deprotected using TFA (1M) in DCM to furnish compounds **27** and **28** as their TFA salts respectively in reasonable yields.



Scheme 3.1: Reagents and conditions : (i) DCC, HOBt, DIPEA, DCM, 24 h, rt, 50 %; (ii) LiOH, MeOH/H_2O, rt, 100 %; (iii) H-Thr-OMe.HCl, EDC.HCl, HOBt, DIPEA, DMF, 24 h, rt, 70%; (iv) TFA, DCM, 0 °C-rt; (v) amino acid, HATU, DIPEA, DMF, 16 h, rt; (vi) NH_3/methanol, 2 h, rt; (vii)TFA, DCM, 30 min, 0 °C-rt.

2.3.4 Result and discussion

Modification of rapastinel peptide was done at the first position of rapastinel as well as at the middle positions of rapastinel. Fenclonine and L-threophenylserine was incorporated at the first position of rapastinel i.e at **H-Thr**-Pro-Pro-Thr-NH_2 respectively. Incorporation lead to close structural analogues of rapastinel.

Incorporation of Aib residues were carried out at the middle positions of rapastinel replacing Pro residues systematically. Three analogues of rapastinel containing Aib residues are synthesized namely H-Thr-Pro-**Aib**-Thr-NH_2, H-Thr-**Aib**-Pro-Thr-NH_2 and H-Thr-**Aib-Aib**-Thr-NH_2. All the synthesized molecules are carried out in solution phase peptide synthesis, purified by column chromatography and well characterized by spectroscopic tools like ^{1}H-NMR, ^{13}C-NMR and HRMS.

2.3.5 Conclusion

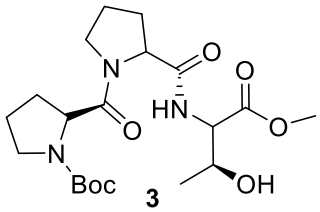
Structurally close analogues of rapastinel were prepared by replacing N-terminus of Thr residue by L-PCPA and L-threo-3-phenylserine using standard peptide coupling strategy in the solution-phase. L-PCPA & L-threo-3-phenylserine were incorporated in to rapastinel sequence to enhance the pharmacokinetic profiles of rapastinel drug such as cell permeability, hydrophobicity and bioactivity.

2.3.6 Experimental Section

Synthetic procedures and data:

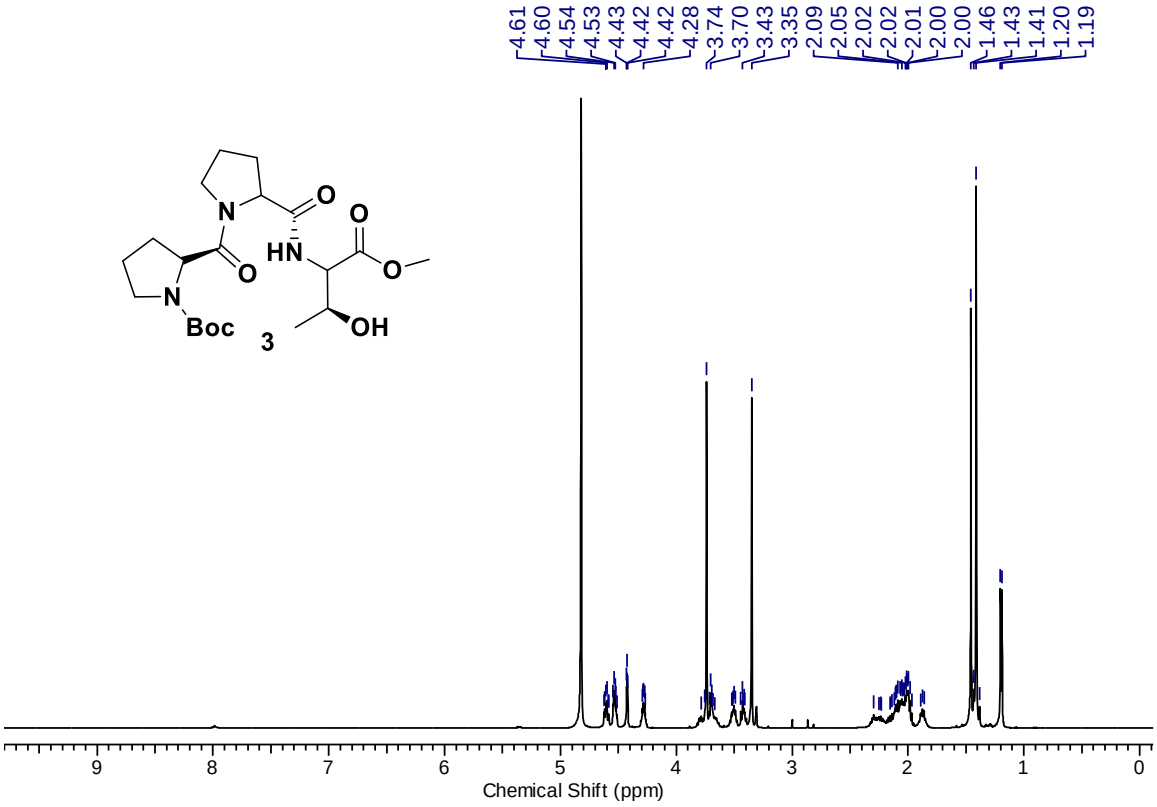
Compound 2 was synthesized according to the literature procedure.[24]

Compound 3 (Boc-Pro-Pro-Thr-OMe): Compound **2** (0.5 g, 1.53 mmol) was taken in a RBF and was dissolved in methanol (1 mL). LiOH (0.18 g, 4.6 mmol) was dissolved in 4 mL of water and added drop wise to the solution containing compound **2**. After checking the TLC, volatiles were stripped off and neutralized with $KHSO_4$ solution. Later, the reaction mixture was extracted with AcOEt and the organic layer was dried over anhydrous Na_2SO_4 and evaporated under vacuo to obtain the crude product which was used for the next reaction without further purification. Boc-Pro-Pro-OH (0.4 g, 1.28 mmol, 1 equiv) was coupled with H-Thr-OMe.HCl (0.21 g, 1.28 mmol, 1 equiv) using EDC.HCl (0.36 g, 1.92 mmol, 1.5 equiv), HOBt (0.09 g, 0.64 mmol, 0.5 equiv) and DIPEA (0.6 mL, 3.8 mmol, 3 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in AcOEt and the organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 70% AcOEt/ pet. ether, R_f: 0.5) to furnish compound **3** (0.41g, 73 %) as a hygroscopic white solid; $[\alpha]^{26}_D$: -55.0° (c = 0.1, MeOH); ^{1}H NMR (400MHz ,CD_3OD) δ = 4.60 (m, 1 H), 4.57 - 4.50 (m, 1 H), 4.42 (t, J = 2.3 Hz, 1 H), 4.28 (m, 1 H), 3.81 - 3.73 (m, 3 H), 3.72 -

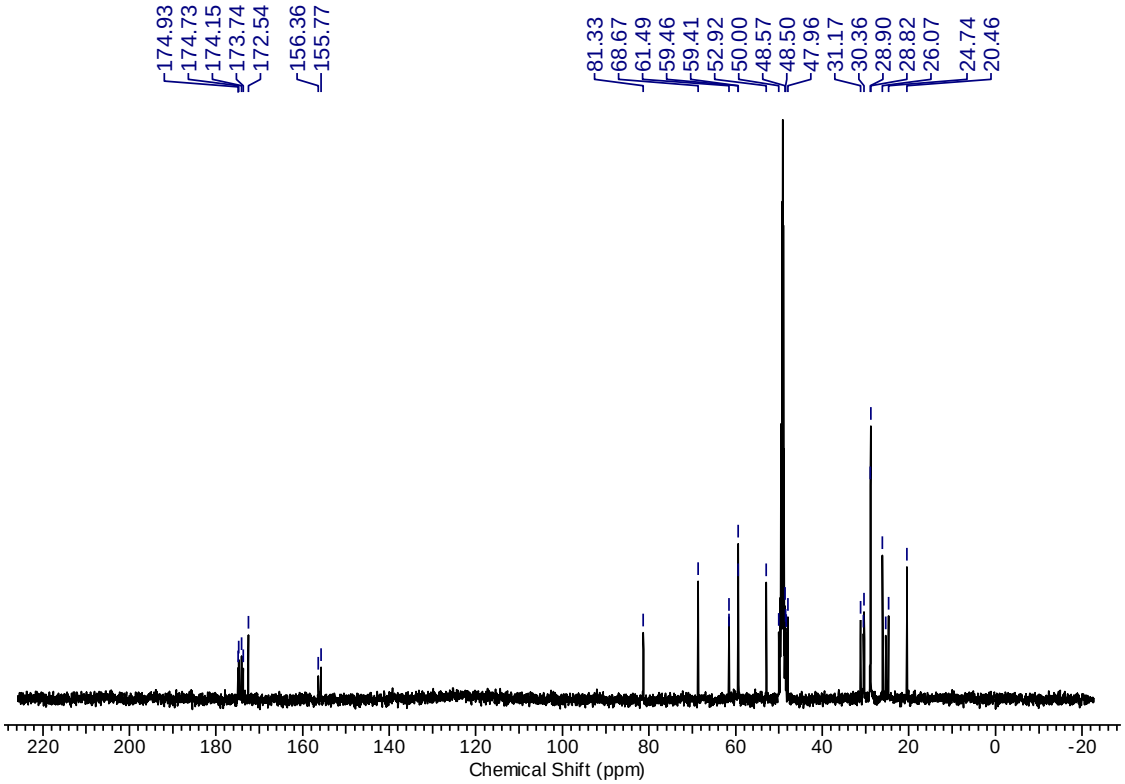


3

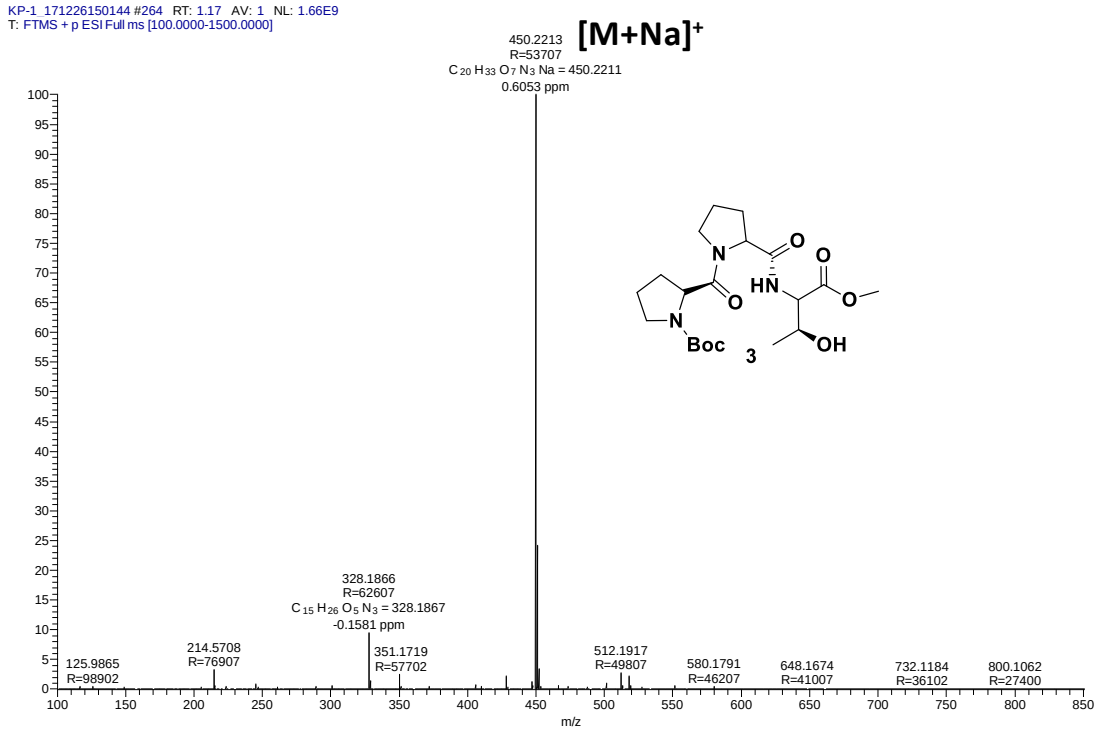
3.67 (m, 1 H), 2.34 - 2.24 (m, 2 H), 2.23- 2.14 (m, 2 H), 2.10 1.99 (m, 2 H), 1.98-1.80 (m, 2 H), 1.50 - 1.36 (m, 9 H), 1.20 (d, J = 6.5 Hz, 3 H); ^{13}C NMR (100 MHz ,CD_3OD) δ = 174.9, 174.7, 174.1, 173.7, 172.5, 156.4, 155.8, 81.3, 68.7, 61.5, 59.4, 52.9, 50.0, 48.7, 48.6, 48.5, 48.3, 48.0, 31.2, 30.4, 30.3, 30.2, 28.9, 28.8 26.1, 25.3, 24.7, 20.5; HRMS: $C_{20}H_{33}N_3O_7$, Calculated: 450.2211 $[M+Na]^+$, observed: 450.2213 $[M+Na]^+$.



^{1}H NMR spectrum of compound **3** (CD_3OD, 400 MHz, 298 K)

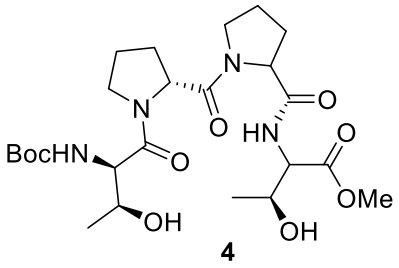


^{13}C NMR spectrum of compound **3** (CD_3OD, 100 MHz, 298 K)

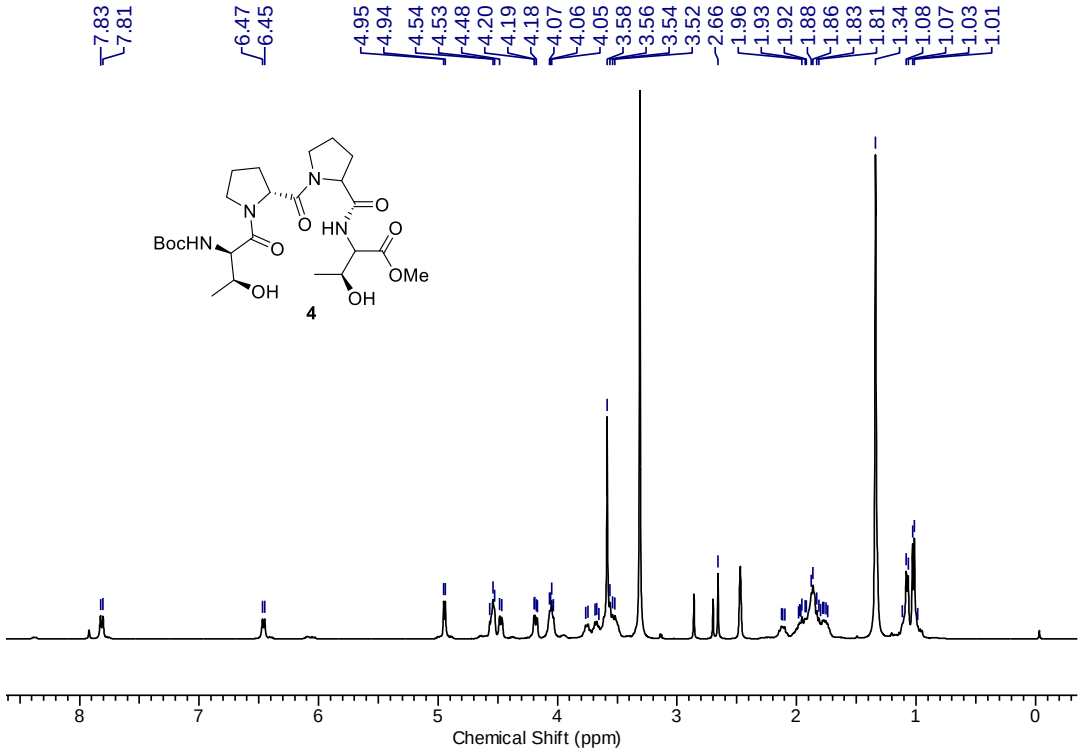


HRMS of compound **3**

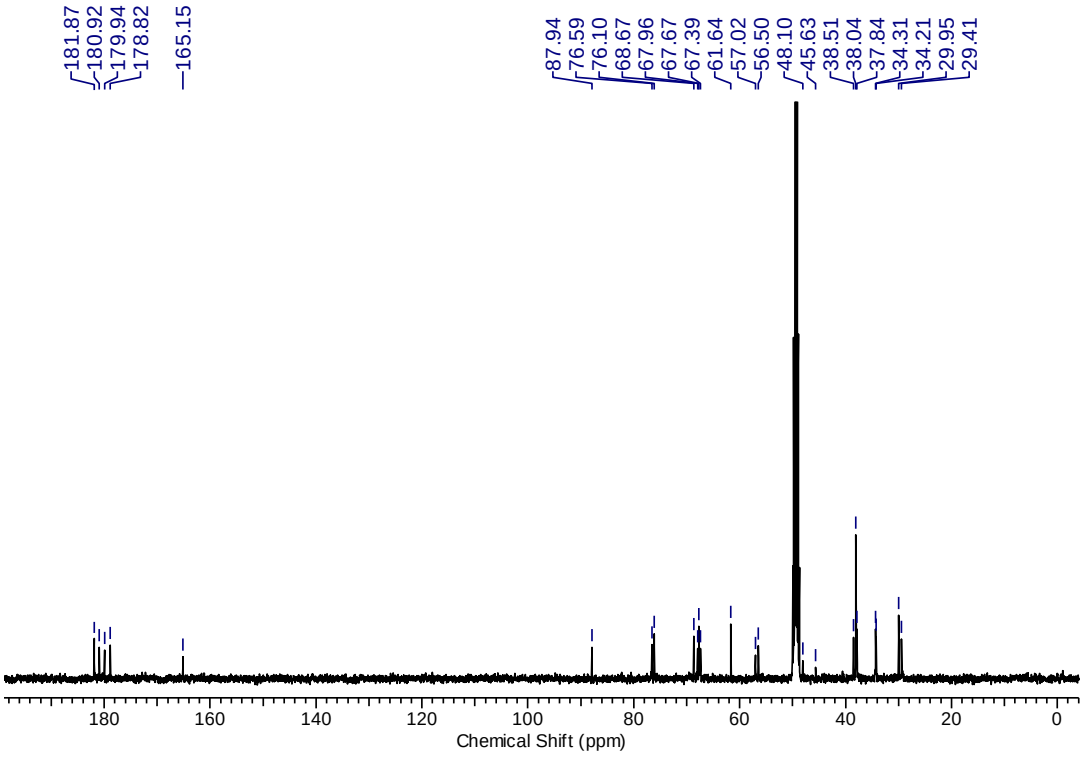
Compound 4 (Boc-Thr-Pro-Pro-Thr-OMe): Boc-protected tripeptide ester **3** (0.3 g, 0.70 mmol) was dissolved in 1 mL of dichloromethane followed by addition of 1 mL of TFA (1M) at 0°C. The reaction was stirred for 30 min at room temperature. After completion of reaction, the solvent was stripped off and co-evaporated using toluene to obtain the TFA salt which was used for the next step without further purification. Boc-Thr-OH (0.16 g, 0.76 mmol, equiv) was coupled with tripeptide ester TFA salt (0.25 g, 0.76 mmol, 1 equiv) using HATU (0.4 g, 1.14 mmol, 1.5 equiv) and DIPEA (0.4 mL, 2.2 mmol, 4 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in 2 % MeOH/DCM and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 2% MeOH/DCM, R_f : 0.5) to furnish compound **5** (0.25 g, 62 %) as a hygroscopic white solid; $[\alpha]^{26}_D$: -74.7° (c = 0.1, MeOH); ^{1}H NMR (400 MHz ,CD_3OD) δ = 7.82 (d, J = 7.9 Hz, 1 H), 6.46 (d, J = 7.3 Hz, 1 H), 4.95 (d, J = 5.5 Hz, 1 H), 4.59 - 4.43 (m, 2 H), 4.18 (dd, J = 2.7, 8.2 Hz, 1 H), 4.12 - 4.00 (m, 2 H), 3.76 (d, J = 8.5 Hz, 1 H), 3.69 - 3.63 (m, 1 H), 3.63 - 3.55 (m, 4 H), 2.19 - 1.68 (m, 8 H), 1.34 (s, 9 H), 1.14 - 0.93 (m, 6 H); ^{13}C NMR (100 MHz ,CD_3OD) = 181.9, 180.9, 179.9, 178.8, 165.2, 87.9, 76.6, 76.1, 68.7, 68.0, 67.7, 67.4, 61.6, 57.0, 56.5, 48.1, 45.6, 38.5, 38.0, 37.8, 34.3, 34.2, 29.9, 29.4; HRMS: $C_{24}H_{40}N_4O_9$, Calculated: 551.2688 $[M+Na]^+$, observed: 551.2687 $[M+Na]^+$.



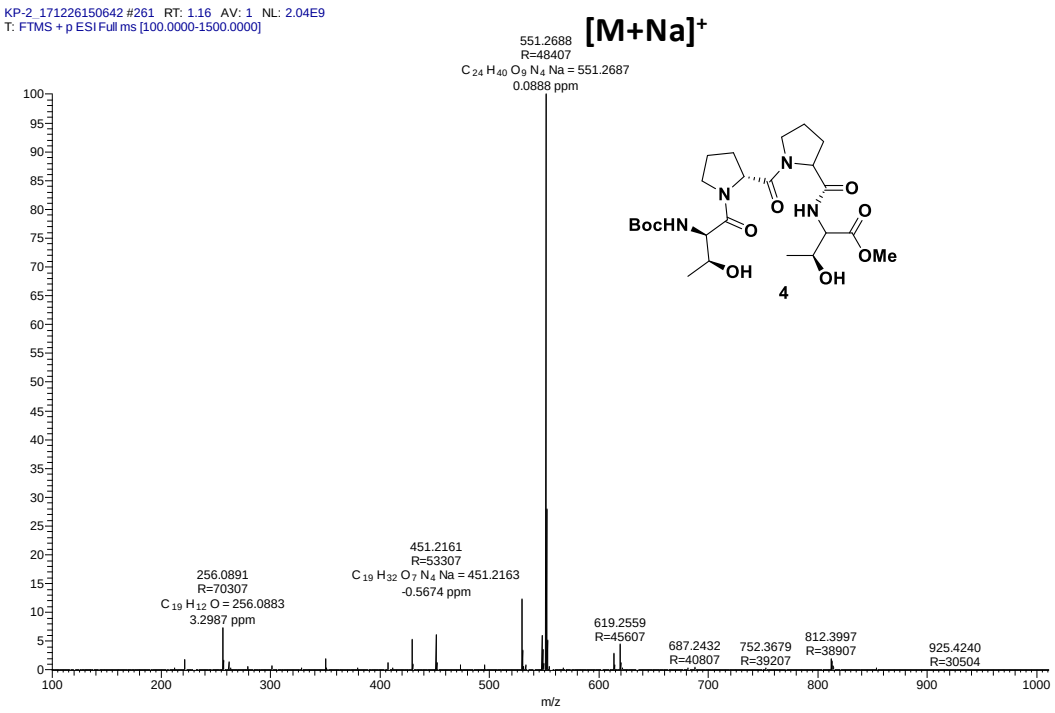
4



^{1}H NMR spectrum of compound **4** (CD_3OD, 400 MHz, 298 K)

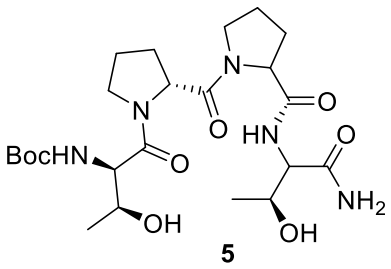


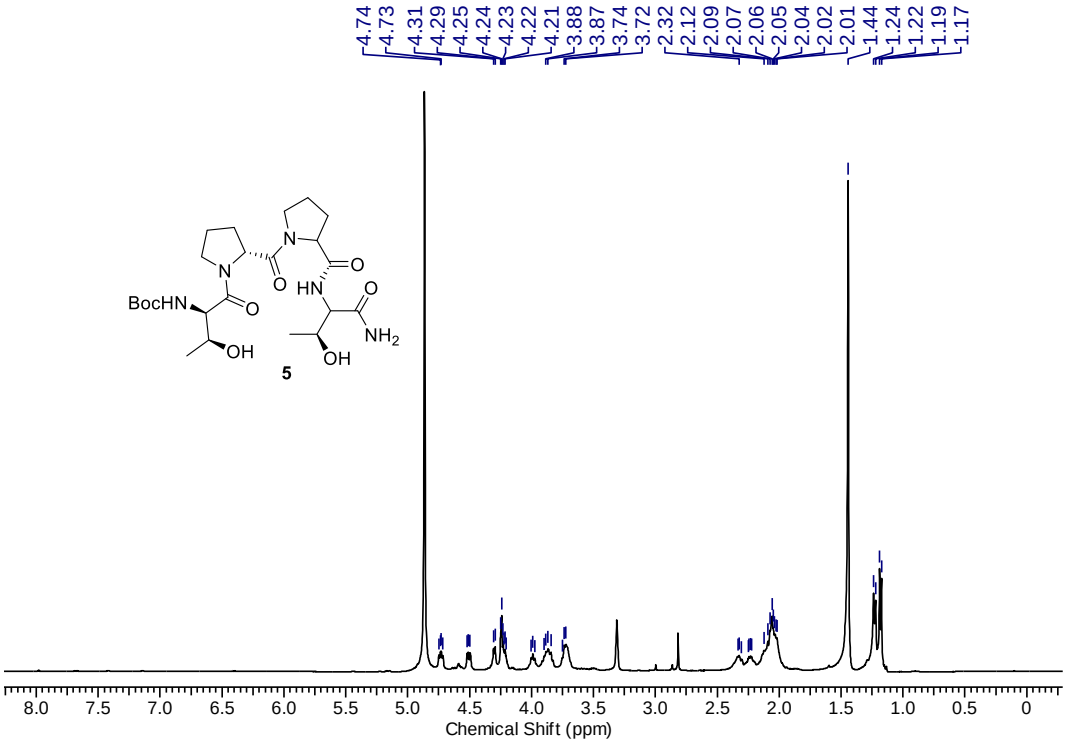
^{13}C NMR spectrum of compound **4** (CD_3OD, 100 MHz, 298 K)



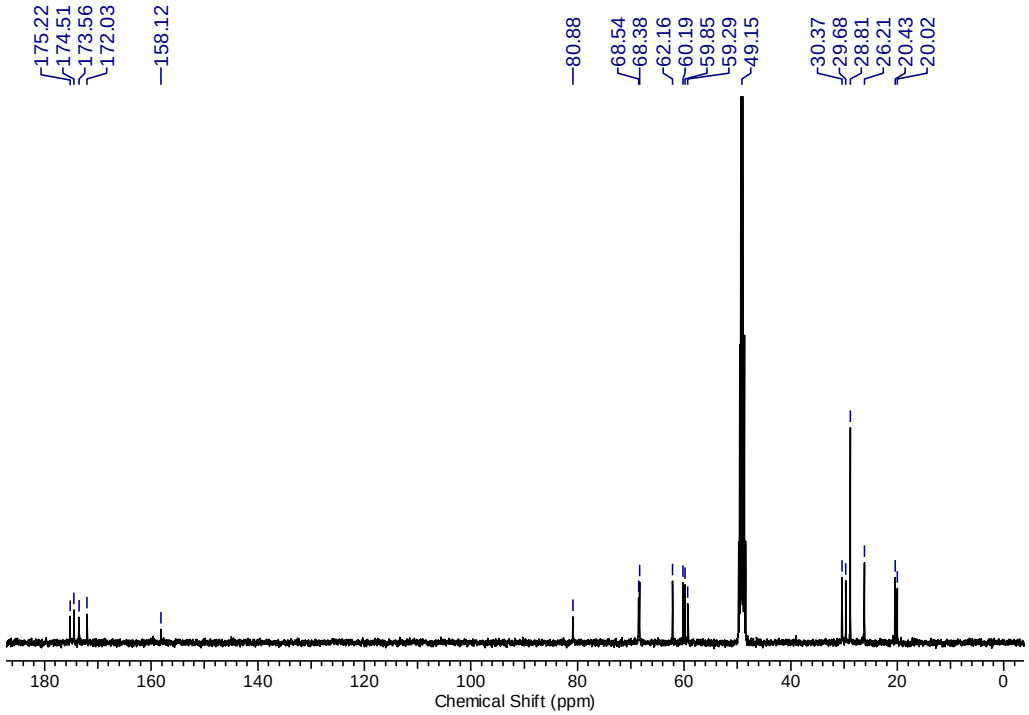
HRMS of Compound **4**

Compound 5 (Boc-Thr-Pro-Pro-Thr-NH$_2$): To a solution of tetrapeptide ester **4** (0.20 g, 0.37 mmol) in methanol, saturated methanolic ammonia solution (3 ml) was added and the reaction mixture was stirred for 24 h at room temperature. Later, the volatiles were stripped off under reduced pressure and the product was further purified by column chromatography and preparative thin layer chromatography (eluent: 4% MeOH/ DCM, R_f : 0.5) to furnish compound **5** (0.17 g, 87%) as a white solid; $[\alpha]^{26}_D$: -85.5° (c = 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 4.73 (dd, J = 4.9, 7.9 Hz, 1 H), 4.54 - 4.47 (m, 1 H), 4.30 (d, J = 5.5 Hz, 1 H), 4.27 - 4.18 (m, 2 H), 4.04 - 3.94 (m, 1 H), 3.93 - 3.80 (m, 2 H), 3.77 - 3.66 (m, 2 H), 2.39 - 2.20 (m, 2 H), 2.18 - 1.96 (m, 6 H), 1.44 (s, 9 H), 1.23 (d, J = 6.1 Hz, 3 H), 1.18 (d, J = 6.1 Hz, 3 H), exchangeable hydrogen's = 6; ^{13}C NMR (100 MHz ,CD_3OD) δ = 175.2, 174.5, 173.6, 172.0, 157.9, 80.9, 68.5, 68.4, 62.2, 60.2, 59.9, 59.3, 30.4, 29.7, 28.8, 26.2, 26.1, 20.4, 20.0; HRMS: $C_{23}H_{39}N_5O_8$, calculated: 536.2691$[M+Na]^+$, observed: 536.2690 $[M+Na]^+$.

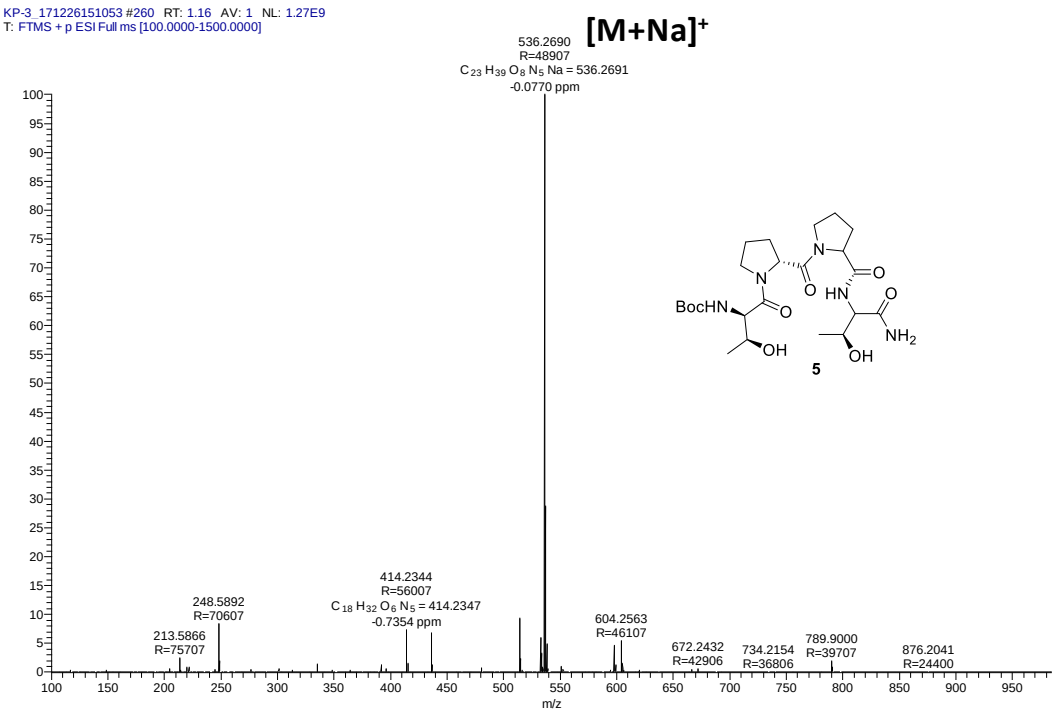




^{1}H NMR spectrum of compound **5** (CD_3OD, 400 MHz, 298 K)

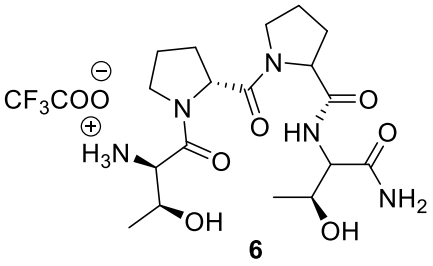


^{13}C NMR spectrum of compound **5** (CD_3OD, 400 MHz, 298 K)



HRMS of Compound **5**

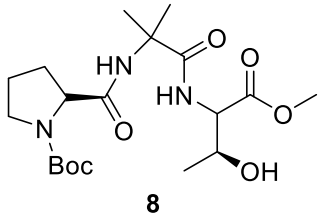
Compound 6 (TFA.H-Thr-Pro-Pro-Thr-NH_2):[8]



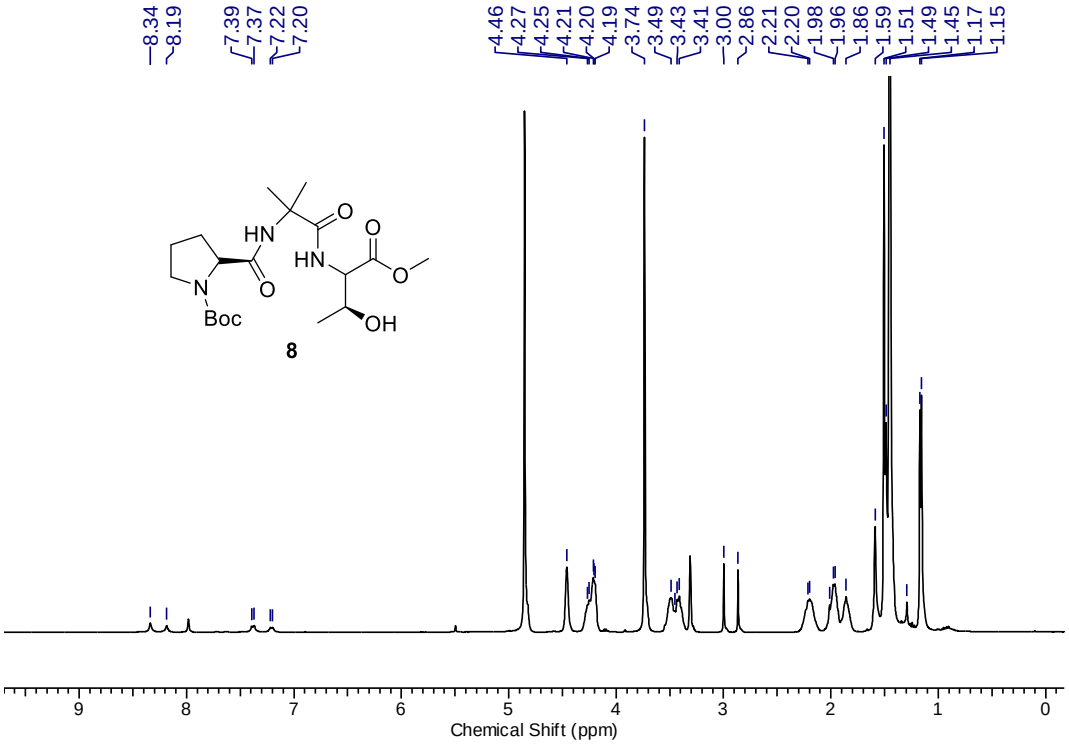
To the solution of compound **5** (0.1 g, 0.19 mmol) in DCM, TFA (1M) was added and stirred for 30 min. After the completion of the reaction, the volatiles were stripped off and co evaporated with toluene to obtain compound **6** as a white solid (0.075 g, 93 %).[8]

Compound **7** was synthesized according the reported literature procedure.[25]

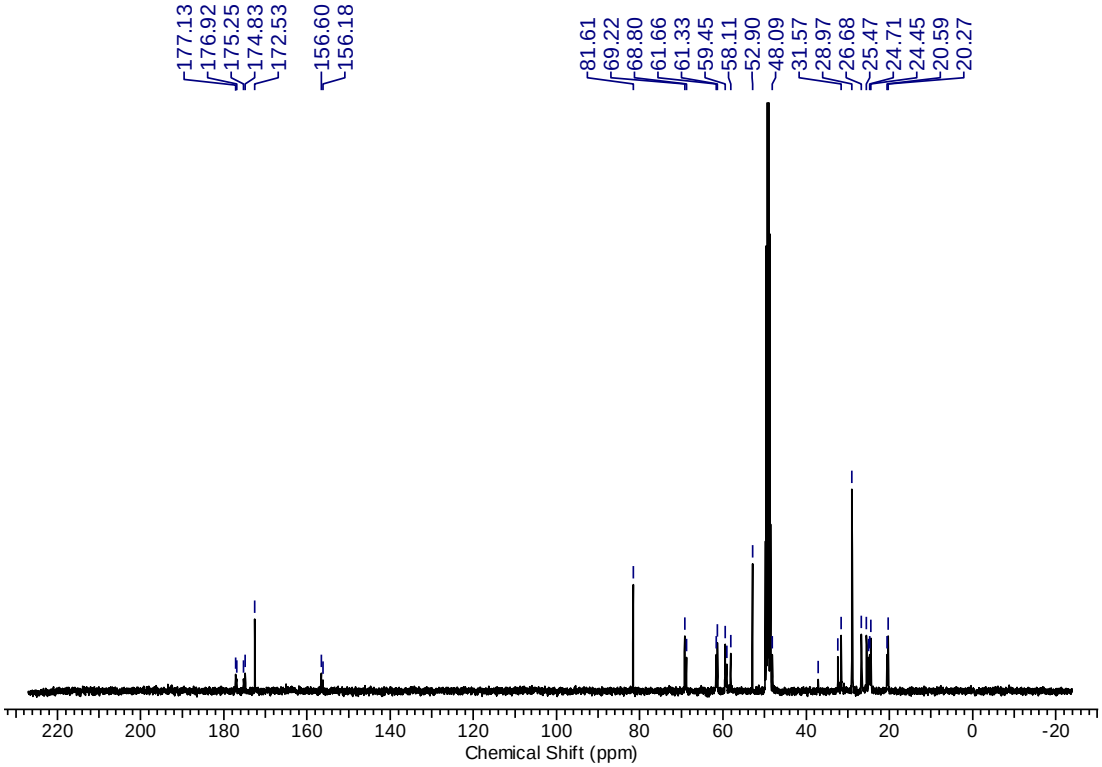
Compound 8 (Boc-Pro-Aib-OMe): Compound **7** (0.5 g, 1.59 mmol) was taken in a RBF and was dissolved in methanol (1 mL). LiOH (0.19 g, 4.7 mmol) was dissolved in 4 mL of water and added drop wise to the solution containing compound **7**. After checking the TLC, volatiles were stripped off and neutralized with $KHSO_4$ solution. Later, the reaction mixture was extracted with AcOEt and the organic layer was dried over anhydrous Na_2SO_4 and evaporated under vacuum to



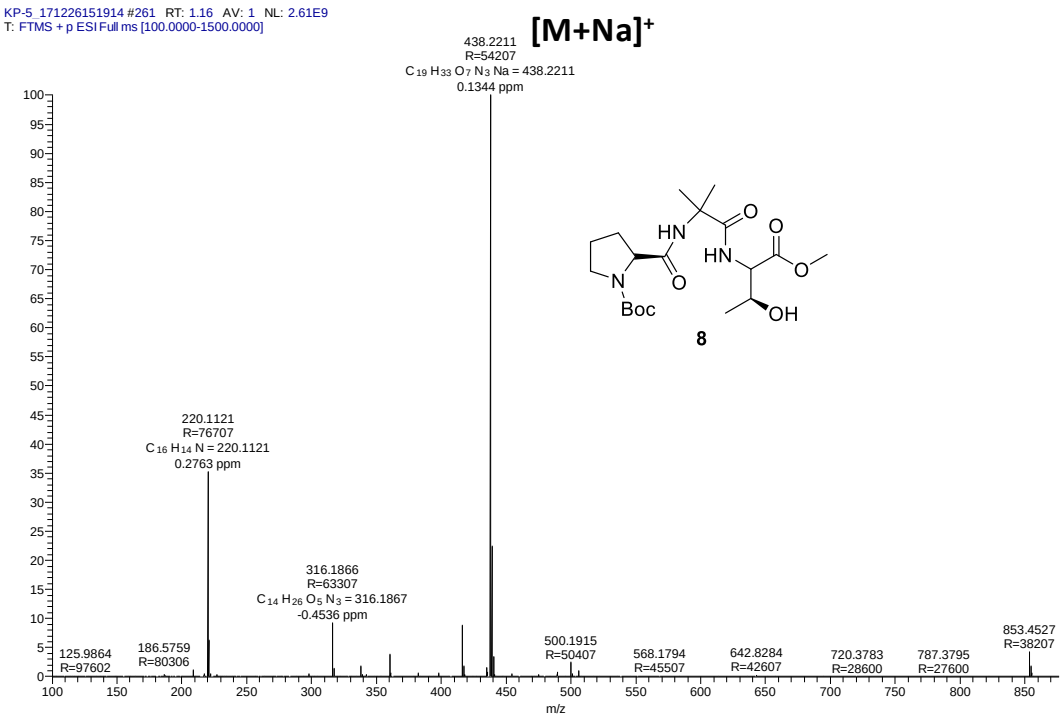
obtain the crude product which was used for the next reaction without further purification. Boc-Pro-Aib-OH (0.4 g, 1.27 mmol, 1 equiv) was coupled with H-Thr-OMe.HCl (0.215 g, 1.27 mmol, 1 equiv) using EDC.HCl (0.38 g, 1.90 mmol, 1.5 equiv), HOBt (0.091 g, 0.63 mmol, 0.5 equiv) and DIPEA (0.6 mL, 3.8 mmol, 3 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in AcOEt and the organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 60% AcOEt/ pet. ether, R_f: 0.5) to furnish compound **8** (0.41g, 73 %) as a hygroscopic white solid; $[\alpha]^{26}_D$: -88.9° (c = 0.1, MeOH); ^{1}H NMR (400MHz ,CD_3OD) = 8.41 - 8.12 (m, 1 H), 7.46 - 7.13 (m, 1 H), 4.46 (br. s., 1 H), 4.33 - 4.13 (m, 2 H), 3.74 (s, 3 H), 3.56 - 3.34 (m, 2 H), 2.21 (d, J = 5.5 Hz, 2 H), 2.06 - 1.78 (m, 2 H), 1.59 (br. s., 1 H), 1.54 - 1.36 (m, 14 H), 1.16 (d, J = 6.7 Hz, 3 H)δ ^{13}C NMR (100 MHz, CD_3OD) δ = 177.2, 176.9, 175.3, 174.8, 172.5, 156.6, 156.1, 81.6, 69.2, 68.8, 61.7, 61.3, 59.5, 59.1, 58.1, 52.9, 48.1, 32.4, 31.6, 28.9, 26.7, 25.5, 25.0, 24.7, 24.4, 20.6, 20.3 HRMS: $C_{19}H_{33}N_3O_7$, Calculated: 438.2211 $[M+Na]^+$, observed: 438.2211 $[M+Na]^+$.



^{1}H NMR spectrum of compound **8** (CD_3OD, 400 MHz, 298 K)



^{13}C NMR spectrum of compound **8** (CD_3OD, 100 MHz, 298 K)

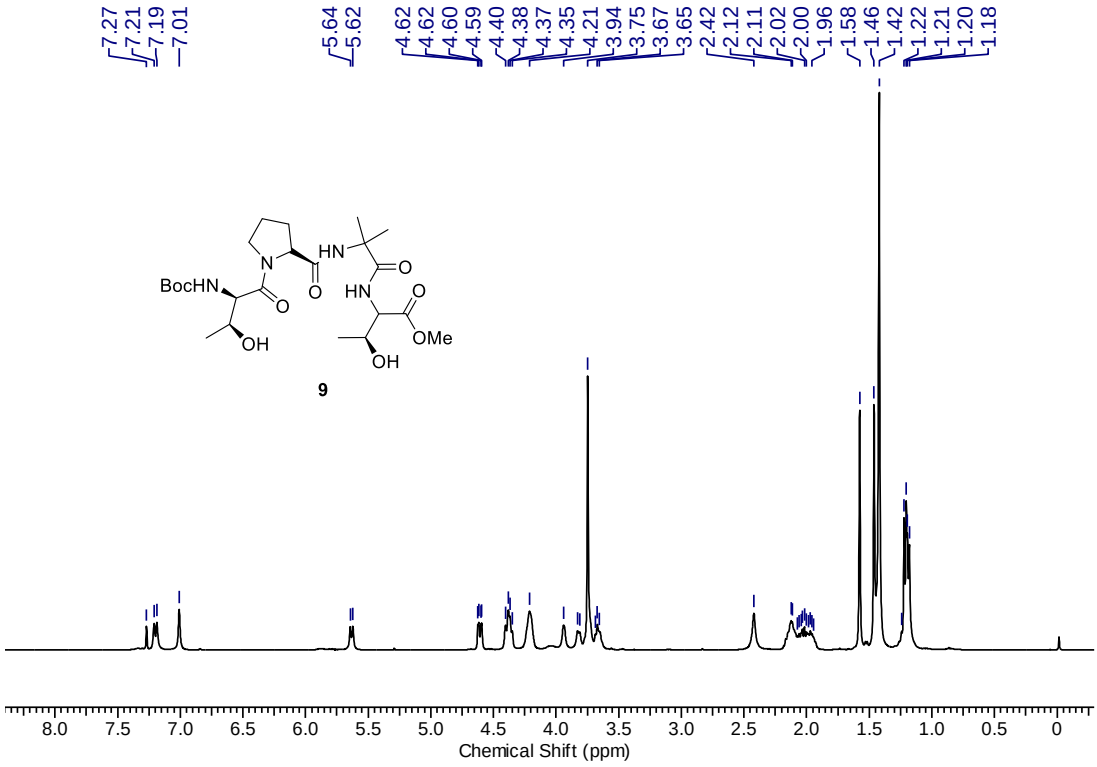


HRMS of Compound **8**

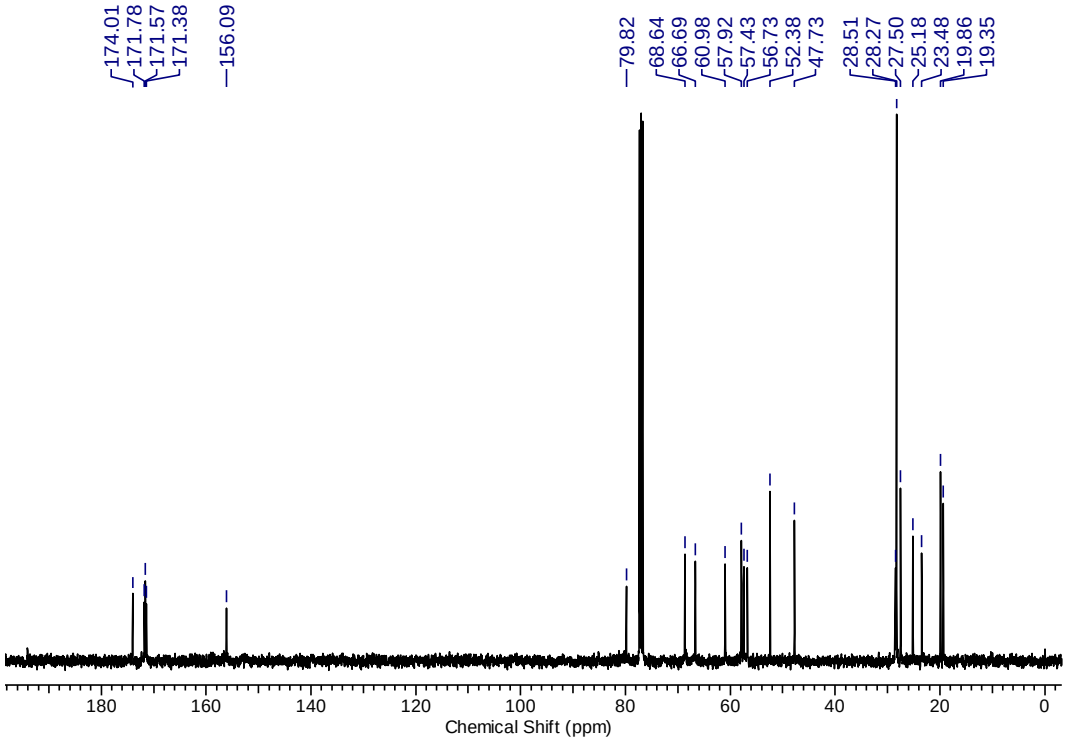
Compound 9 (Boc-Thr-Pro-Aib-Thr-OMe): Boc-protected tripeptide ester **8** (0.35 g, 0.84 mmol) was dissolved in 1 mL of dichloromethane followed by addition of 1 mL of TFA (1M) at 0°C. The reaction was stirred for 30 min at room temperature. After completion of reaction, the solvent was stripped off and co-evaporated using toluene to obtain the TFA salt which was used for the next step without further purification. Boc-Thr-OH (0.13 g, 0.63 mmol, equiv) was coupled with tripeptide ester TFA salt (0.27 g, 0.63 mmol, 1 equiv) using HATU (0.36 g, 0.95 mmol, 1.5 equiv) and DIPEA (0.4 mL, 1.8 mmol, 4 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in 2 % MeOH/DCM and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 2% MeOH/DCM, R_f : 0.5) to furnish compound **9** (0.25 g, 76

9

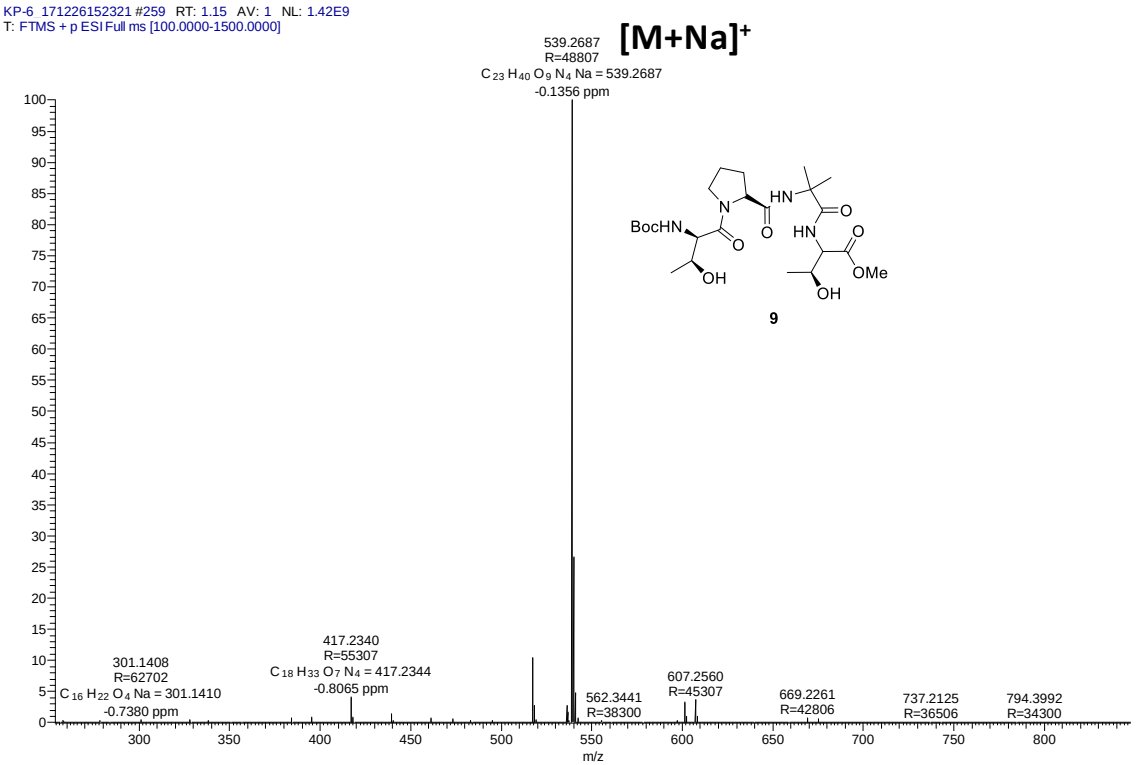
%) as a hygroscopic white solid; $[\alpha]^{26}_{D}$: -95.6° (c = 0.1, MeOH); ^{1}H NMR (400 MHz ,CD_3OD) δ = 7.21 (d, J = 9.2 Hz, 1 H), 7.02 (br. s., 1 H), 5.64 (d, J = 8.5 Hz, 1 H), 4.62 (dd, J = 3.1, 9.2 Hz, 1 H), 4.46 - 4.32 (m, 2 H), 4.22 (br. s., 2 H), 3.95 (br. s., 1 H), 3.83 (d, J = 6.1 Hz, 1 H), 3.76 (s, 3 H), 3.68 (t, J = 6.1 Hz, 1 H), 2.43 (br. s., 1 H), 2.20 - 1.89 (m, 4 H), 1.59 (s, 3 H), 1.51 - 1.38 (m, 12 H), 1.29 - 1.13 (m, 6 H); ^{13}C NMR (100 MHz, CD_3OD) = 174.0, 171.8, 171.6, 171.4, 156.1, 79.8, 68.6, 66.7, 61.0, 57.9, 57.4, 56.7, 52.4, 47.7, 28.5, 28.3, 27.5, 25.2, 23.5, 19.9, 19.3; HRMS: $C_{23}H_{40}N_4O_9$, Calculated: 539.2687 $[M+Na]^+$, observed: 539.2687 $[M+Na]^+$.



^{1}H NMR spectrum of compound **9** (CD_3OD, 400 MHz, 298 K)

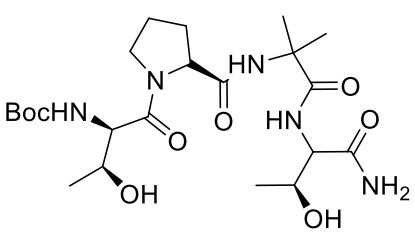


^{13}C NMR spectrum of compound **9** (CD_3OD, 100 MHz, 298 K)

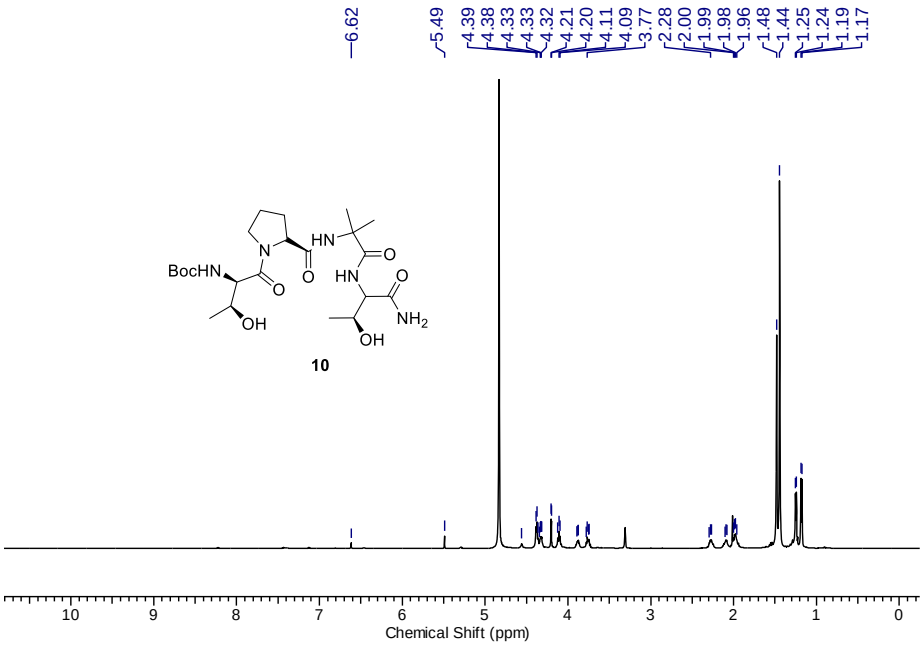


HRMS of Compound **9**

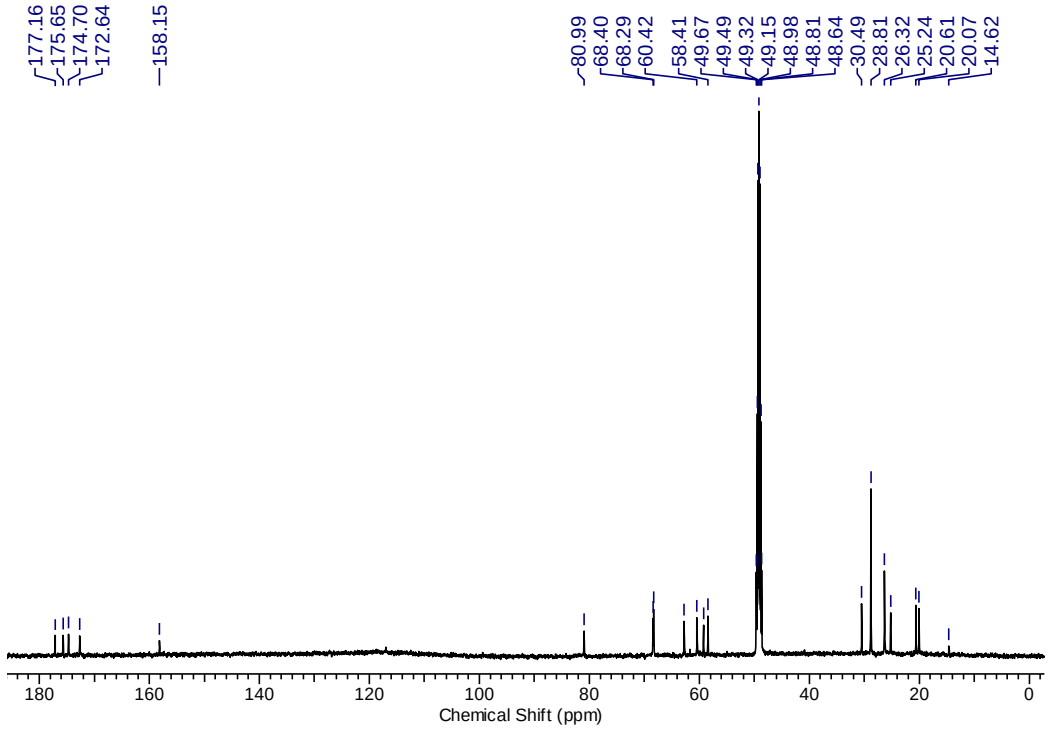
Compound 10 (Boc-Thr-Pro-Aib-Thr-NH_2): To a solution of tetrapeptide ester **9** (0.20 g, 0.38 mmol) in methanol, saturated methanolic ammonia solution (3 ml) was added and the reaction mixture was stirred for 24 h at room temperature. Later, the volatiles were stripped off under reduced pressure and the product was further purified by column chromatography and preparative thin layer chromatography (eluent: 4% MeOH/ DCM, R_f : 0.5) to furnish compound **10** (0.17 g, 86 %) as a white solid; $[\alpha]^{26}_D$: -66.6° (c = 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ = 6.67 - 6.55 (m, 1 H), 5.49 (s, 1 H), 4.61 - 4.51 (m, 1 H), 4.41 - 4.35 (m, 2 H), 4.32 (dd, J = 2.9, 6.3 Hz, 1 H), 4.20 (d, J = 3.1 Hz, 1 H), 4.11 (t, J = 7.1 Hz, 1 H), 3.88 (d, J = 2.7 Hz, 1 H), 3.80 - 3.70 (m, 1 H), 2.27 (d, J = 6.9 Hz, 1 H), 2.09 (d, J = 5.0 Hz, 1 H), 2.04 - 1.92 (m, 2 H), 1.48 (s, 6 H), 1.44 (s, 9 H), 1.28 - 1.23 (m, 3 H), 1.18 (d, J = 6.5 Hz, 3 H)^{13}C NMR (100 MHz ,CD_3OD) δ = 177.2, 175.6, 174.7, 172.6, 158.2, 81.0, 68.4, 68.3, 62.8, 60.4, 59.2, 58.4, 30.5, 28.8, 26.3, 25.2, 20.6, 20.1 HRMS: $C_{22}H_{39}N_5O_8$, calculated: 524.5891 $[M+Na]^+$, observed: 524.2689 $[M+Na]^+$.



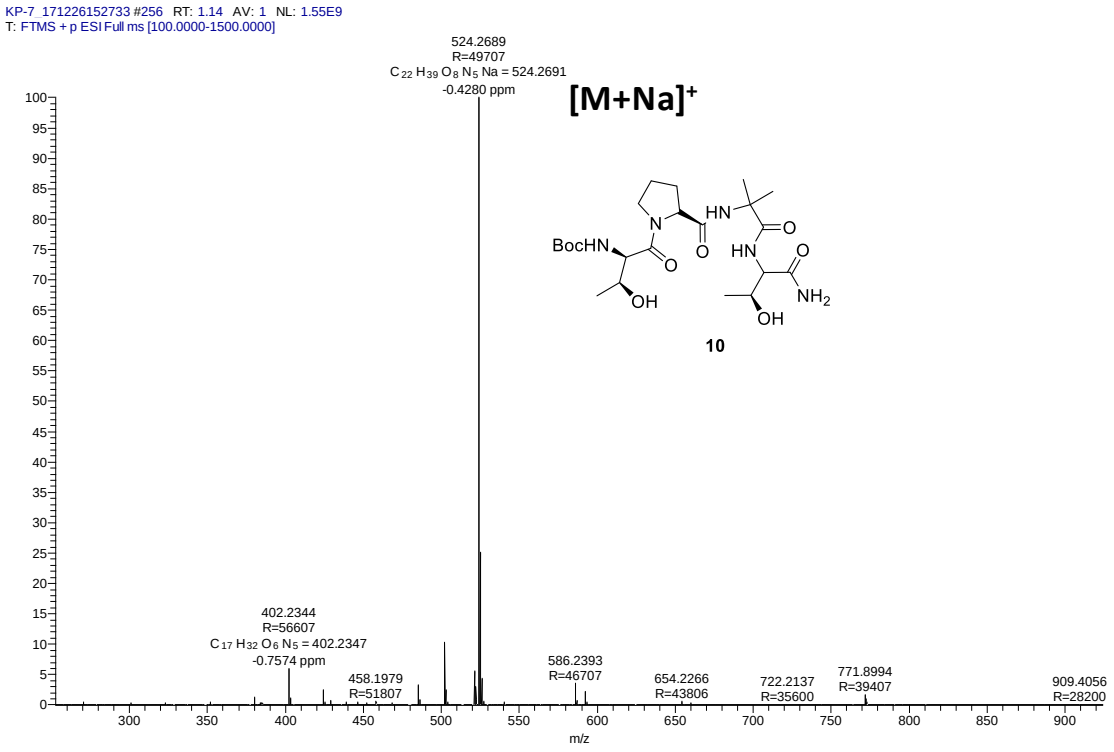
10



^{1}H NMR spectrum of compound **10** (CD_3OD, 400 MHz, 298 K)

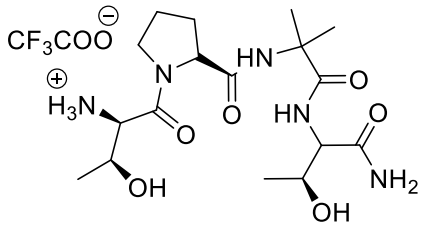


^{13}C NMR spectrum of compound **10** (CD_3OD, 400 MHz, 298 K)

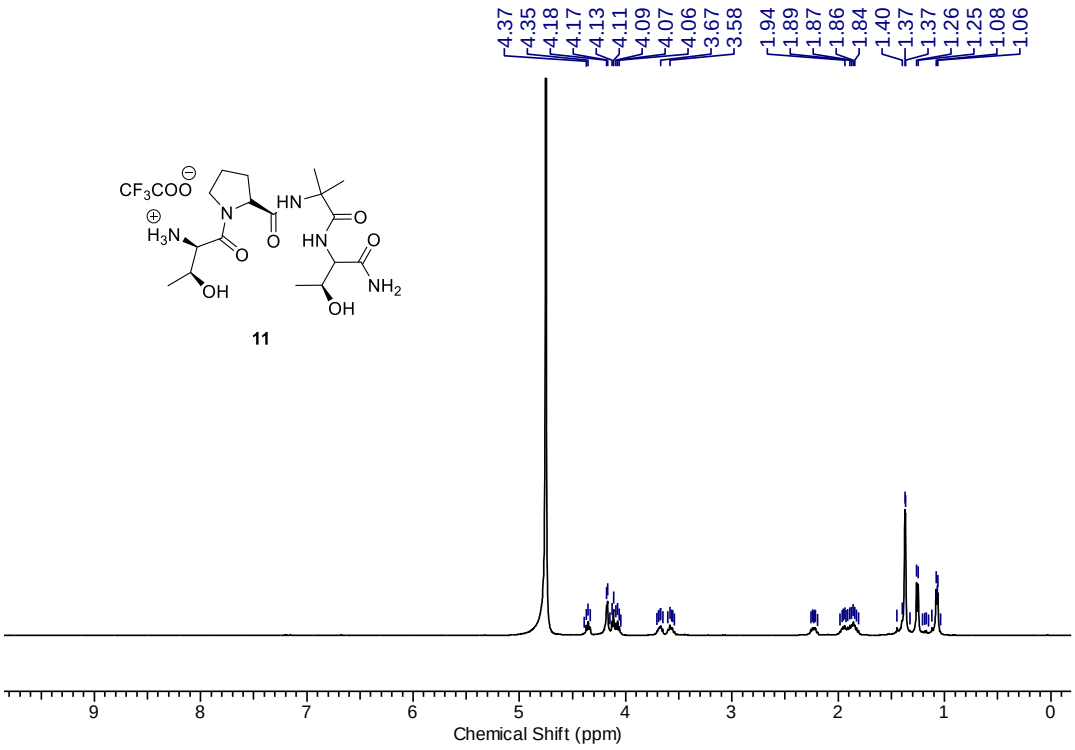


HRMS of Compound **10**

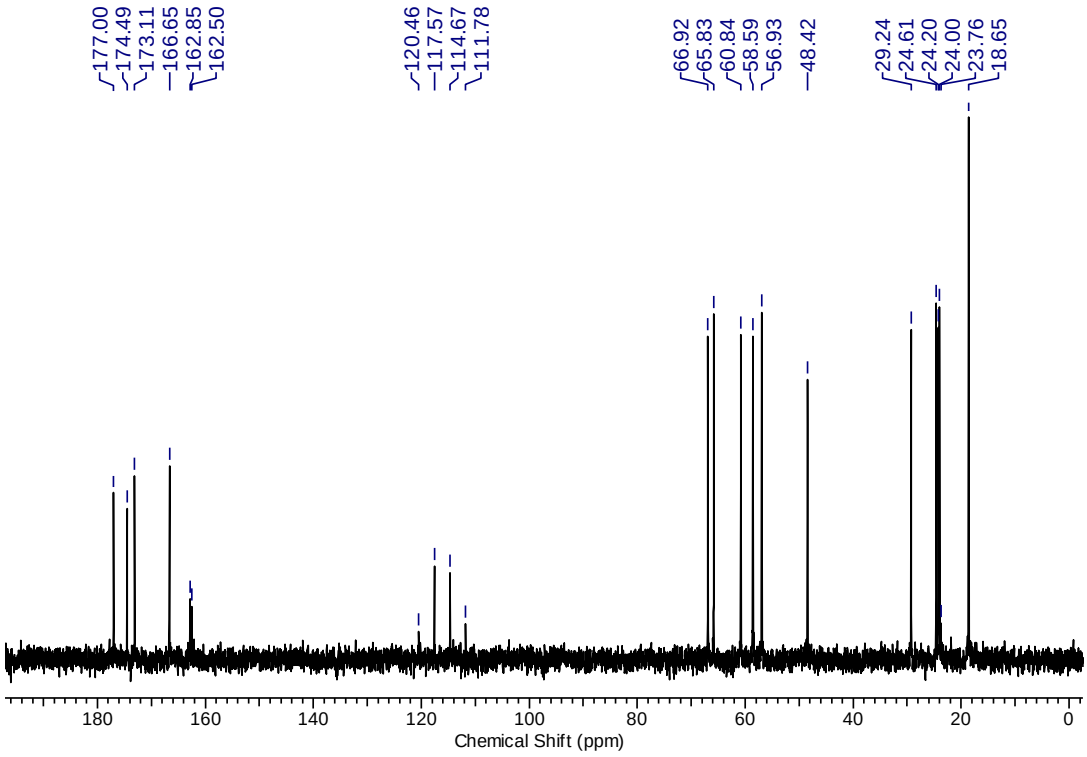
Compound 11 (TFA.H-Thr-Pro-Aib-Thr-NH_2): To the solution of compound **5** (0.1 g, 0.20 mmol) in DCM, TFA (1M) was added and stirred for 30 min. After the completion of the reaction, the volatiles were stripped off and co evaporated with toluene to obtain compound **11** as a hygroscopic white solid (0.075 g, 94 %). $[\alpha]^{26}_{D}$: -101.6° (c = 0.1, MeOH); ^{1}H NMR (400 MHz, D_2O) δ = 4.43 - 4.30 (m, 1 H), 4.17 (d, J = 3.1 Hz, 2 H), 4.14 - 4.05 (m, 2 H), 3.73 - 3.63 (m, 1 H), 3.61 - 3.52 (m, 1 H), 2.30 - 2.16 (m, 1 H), 2.02 - 1.79 (m, 3 H), 1.37 (d, J = 1.8 Hz, 6 H), 1.25 (d, J = 6.1 Hz, 3 H), 1.10 - 1.04 (m, 3 H); ^{13}C NMR (100 MHz ,D_2O) δ = 177.0, 174.5, 173.1, 166.6, 162.9, 162.5, 120.5, 117.6, 114.7, 111.8, 66.9, 65.8, 60.8, 58.6, 56.9, 48.4, 29.2, 24.6, 24.2, 24.0, 23.8, 18.7 HRMS: $C_{17}H_{31}N_5O_6$, calculated: 424.2167 $[M+Na]^+$, observed: 424.2162 $[M+Na]^+$.



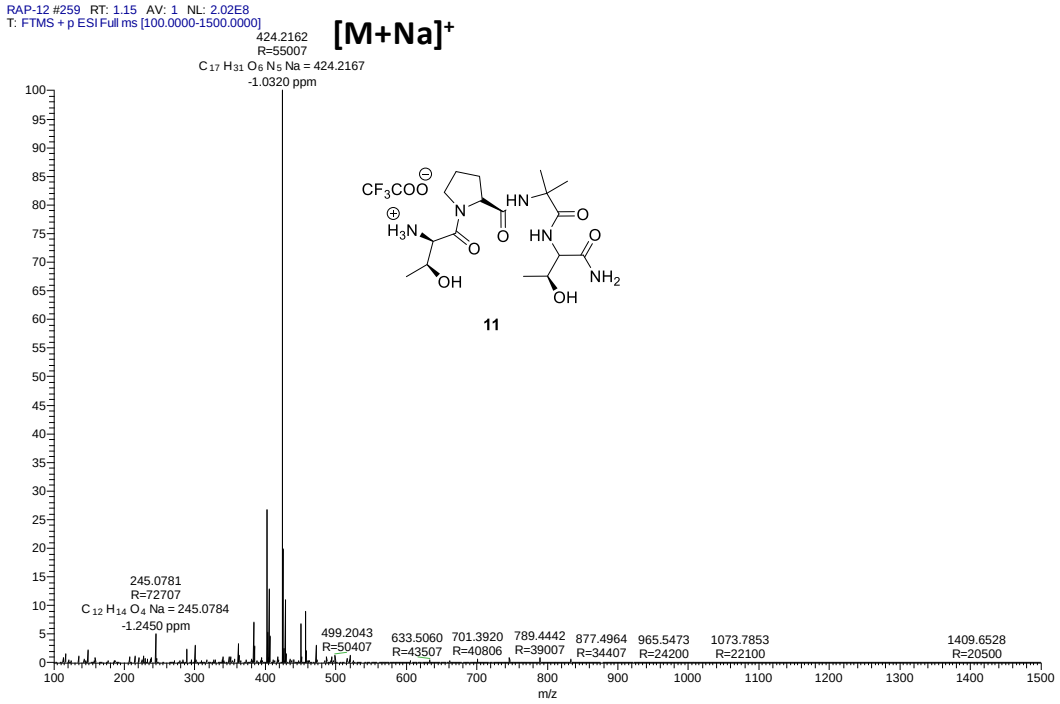
11



^{1}H NMR spectrum of compound **11**(D_2O, 400 MHz, 298 K)



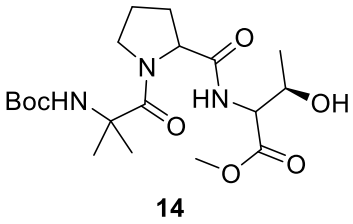
^{13}C NMR spectrum of compound **11**(D_2O, 400 MHz, 298 K)



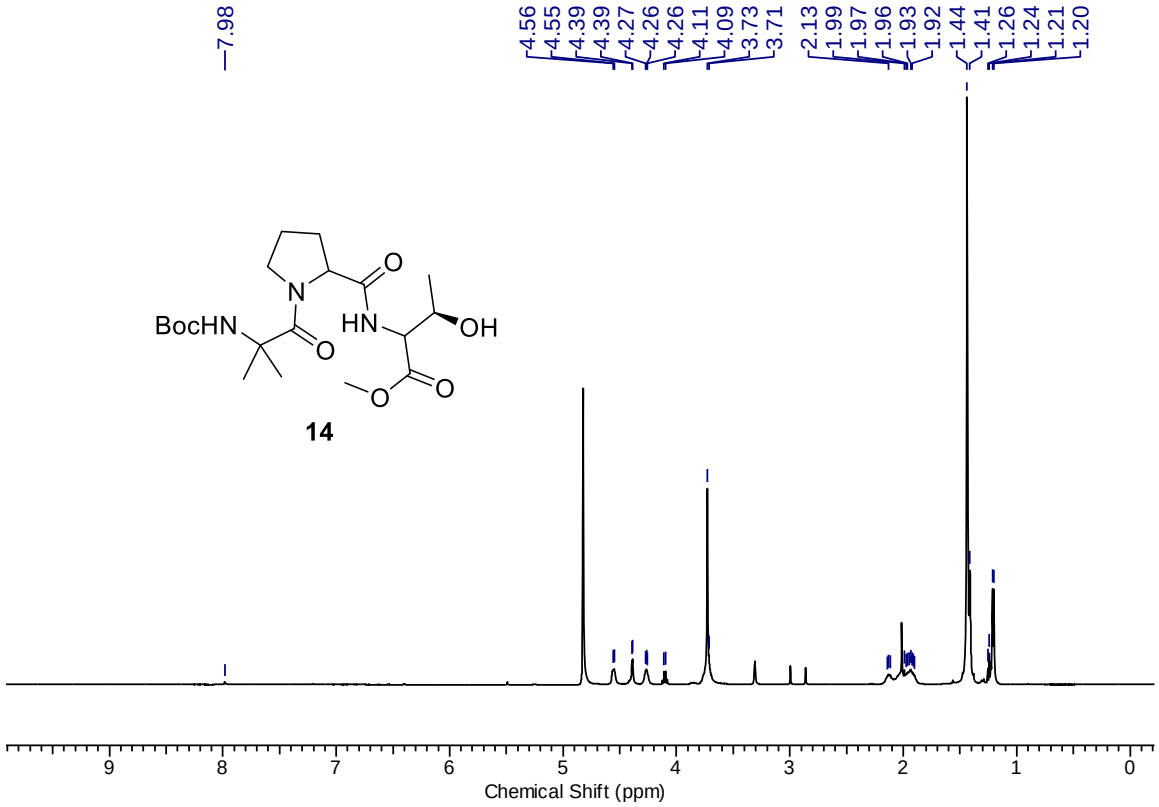
HRMS of Compound **11**

Compound **13** was synthesized according to the literature procedure.[26]

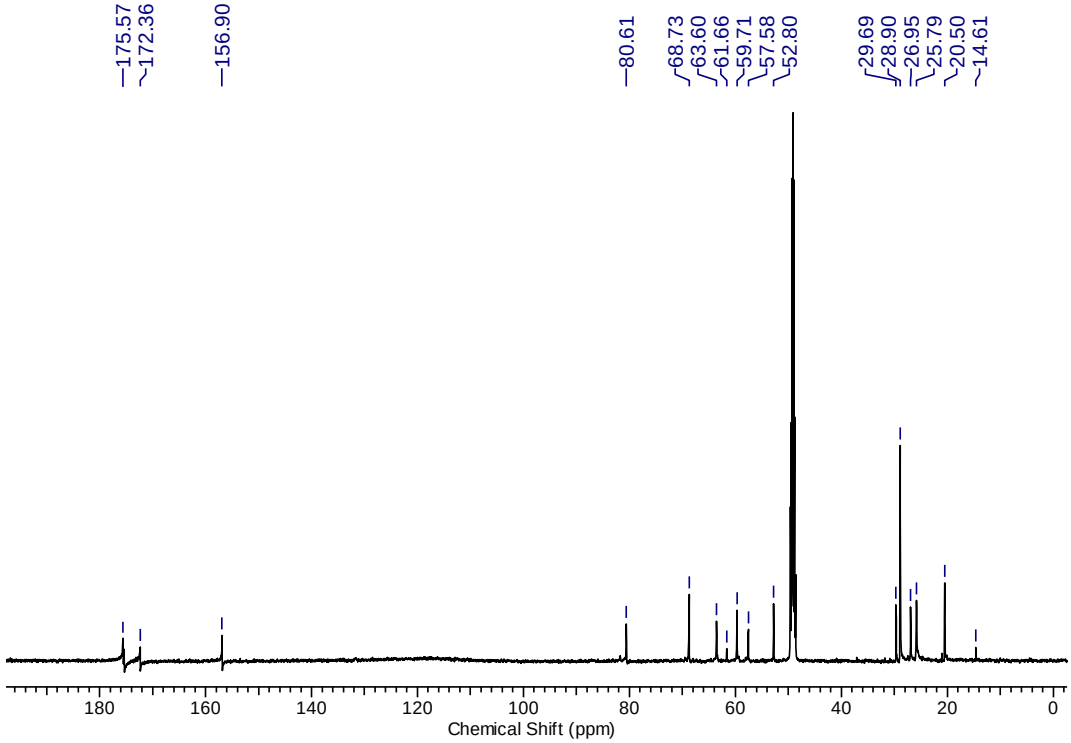
Compound 14 (Boc-Aib-Pro-Thr-OMe): Compound **13** (0.5 g, 1.59 mmol) was taken in a RBF and was dissolved in methanol (1 mL). LiOH (0.19 g, 4.7 mmol) was dissolved in 4 mL of water and added drop wise to the solution containing compound **7**. After checking the TLC, volatiles were stripped off and neutralized with $KHSO_4$ solution. Later, the reaction mixture was extracted with AcOEt and the organic layer was dried over anhydrous Na_2SO_4 and evaporated under vacuum to obtain the crude product which was used for the next reaction without further purification. Boc-Aib-Pro-OH (0.4 g, 1.27 mmol, 1 equiv) was coupled with H-Thr-OMe.HCl (0.215 g, 1.27 mmol, 1 equiv) using EDC.HCl (0.38 g, 1.90 mmol, 1.5 equiv), HOBt (0.091 g, 0.63 mmol, 0.5 equiv) and DIPEA (0.6 mL, 3.8 mmol, 3 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in AcOEt and the organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 60% AcOEt/ pet. ether, R_f: 0.5) to furnish compound **14** (0.45 g, 80 %) as a hygroscopic white solid; $[\alpha]^{26}_D$: -77.2° (c = 0.1, MeOH); ^{1}H NMR (400MHz ,CD_3OD) = 7.98 (m, 1 H) 4.55 (d, J = 3.4 Hz, 1 H), 4.39 (d, J = 3.1 Hz, 1 H), 4.26 (dd, J = 3.6, 5.5 Hz, 1 H), 4.12 - 4.09 (m, 1 H), 3.81 - 3.63 (m, 5 H), 2.18 - 1.85 (m, 4 H), 1.51 - 1.42 (s, 9 H), 1.41 - 1.35 (m, 6 H), 1.26 - 1.18 (m, 3 H)δ ^{13}C NMR (100 MHz, CD_3OD) δ = 175.6, 175.3, 172.4, 156.9, 80.6, 68.7, 63.6, 61.7, 59.7, 57.6, 52.8, 29.7, 28.9, 27.0, 25.8, 20.5, 14.6; HRMS: $C_{19}H_{33}N_3O_7$, Calculated: 438.2211 $[M+Na]^+$, observed: 438.2210 $[M+Na]^+$.



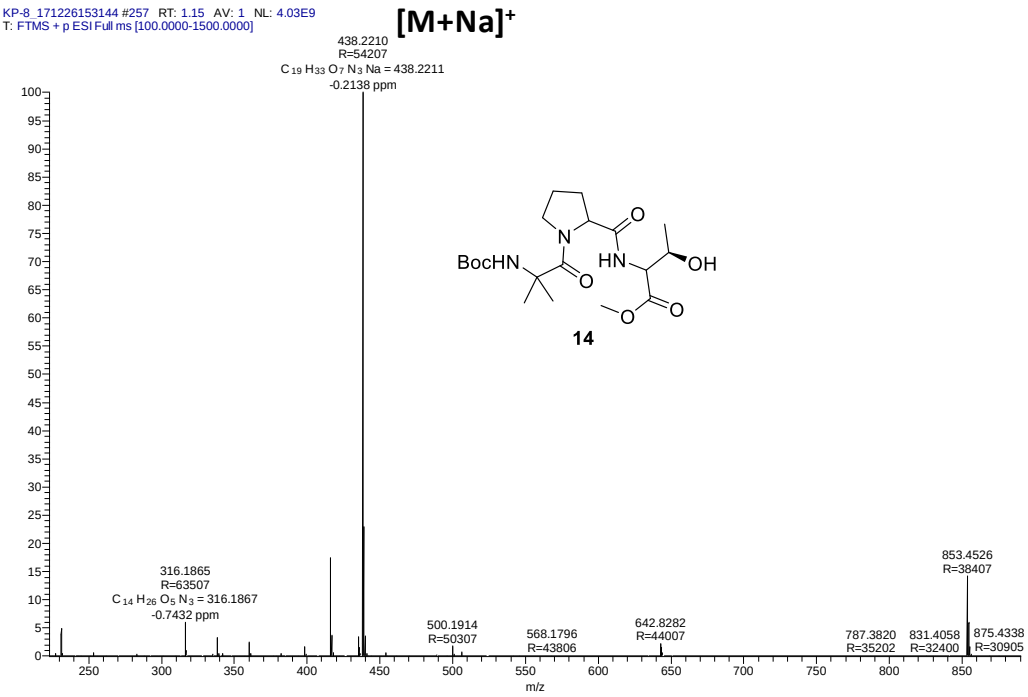
14



^{1}H NMR spectrum of compound **14** (CD_3OD, 400 MHz, 298 K)

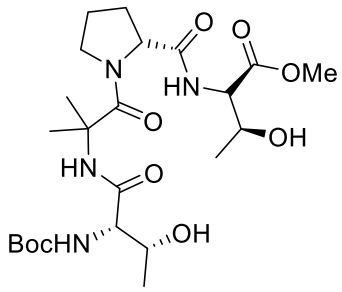


^{13}C NMR spectrum of compound **14** (CD_3OD, 400 MHz, 298 K)



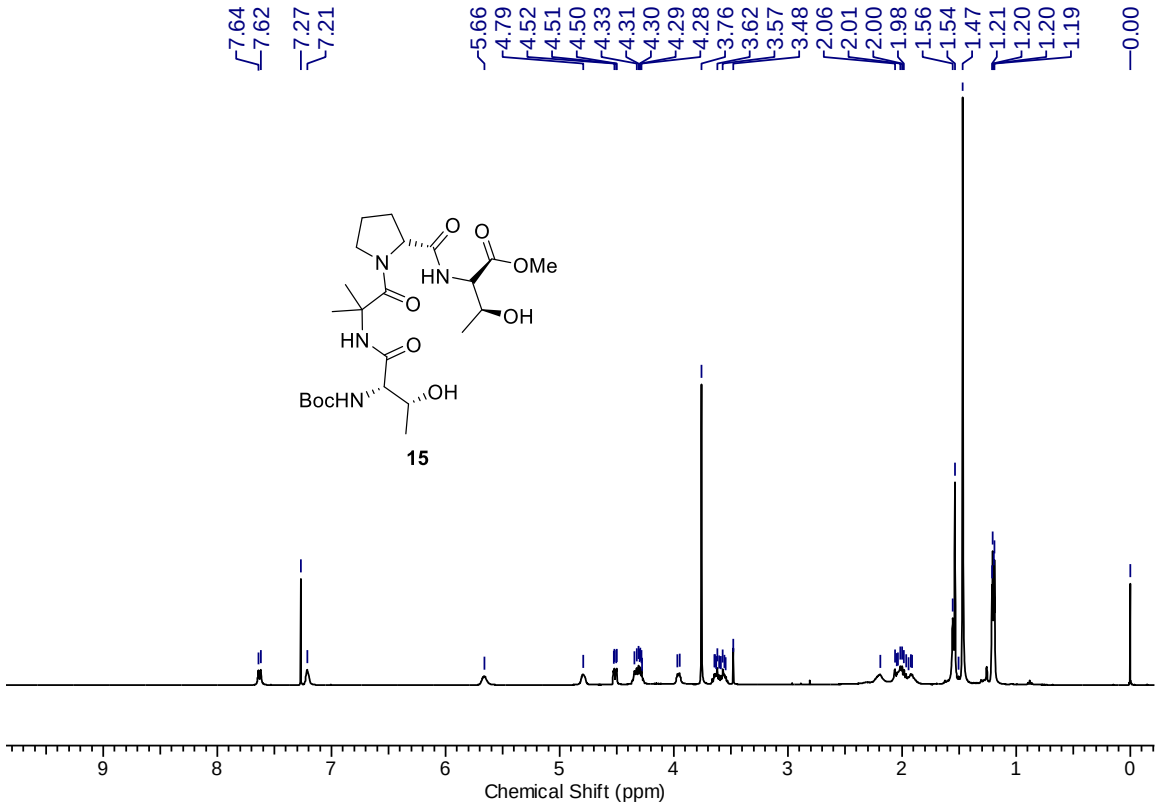
HRMS of Compound **14**

Compound 15 (Boc-Thr-Aib-Pro-Thr-OMe): Boc-protected tripeptide ester **14** (0.35 g, 0.84 mmol) was dissolved in 1 mL of dichloromethane followed by addition of 1 mL of TFA (1M) at 0°C. The reaction was stirred for 30 min at room temperature. After completion of reaction, the solvent was stripped off and co-evaporated using toluene to obtain the TFA salt which was used for the next step without further purification. Boc-Thr-OH (0.13 g, 0.63 mmol, 1 equiv) was coupled with tripeptide ester TFA salt (0.27 g, 0.63 mmol, 1 equiv) using HATU (0.36 g, 0.95 mmol, 1.5 equiv) and DIPEA (0.4 mL, 1.8 mmol, 4 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in 2 % MeOH/DCM and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 2% MeOH/DCM, R_f : 0.5) to furnish compound **15** (0.27 g, 80 %) as a hygroscopic white solid; $[\alpha]^{26}_D$: -75.5° (c = 0.1,

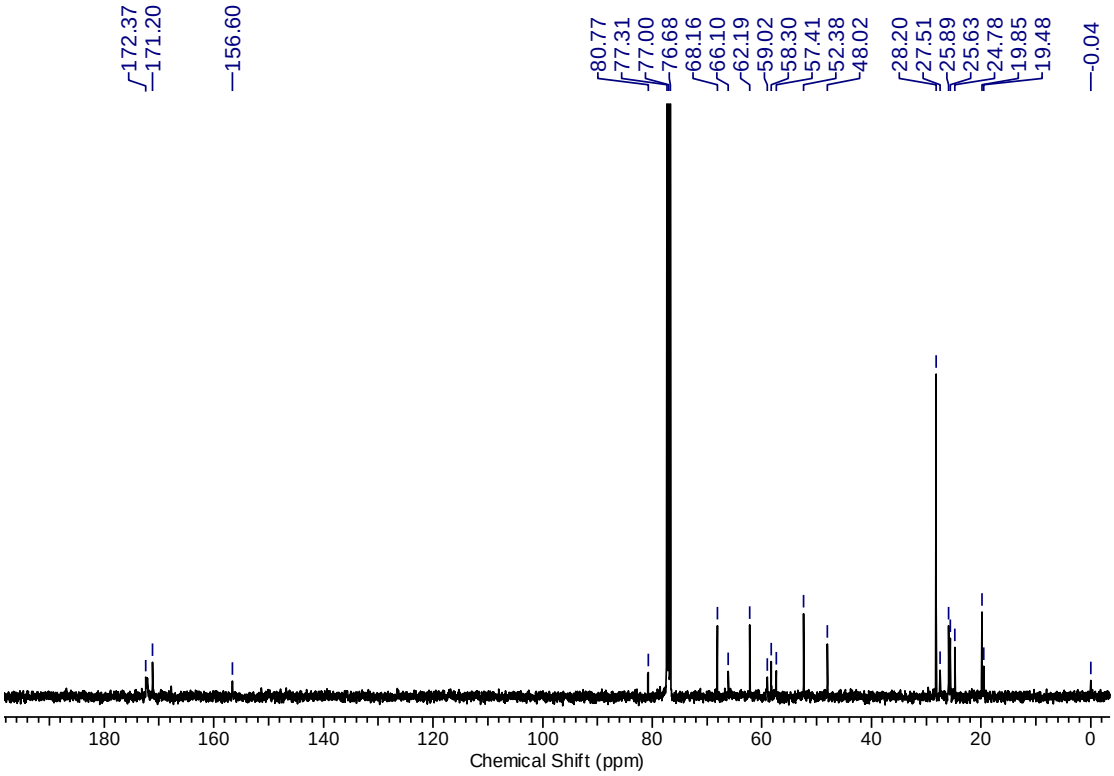


15

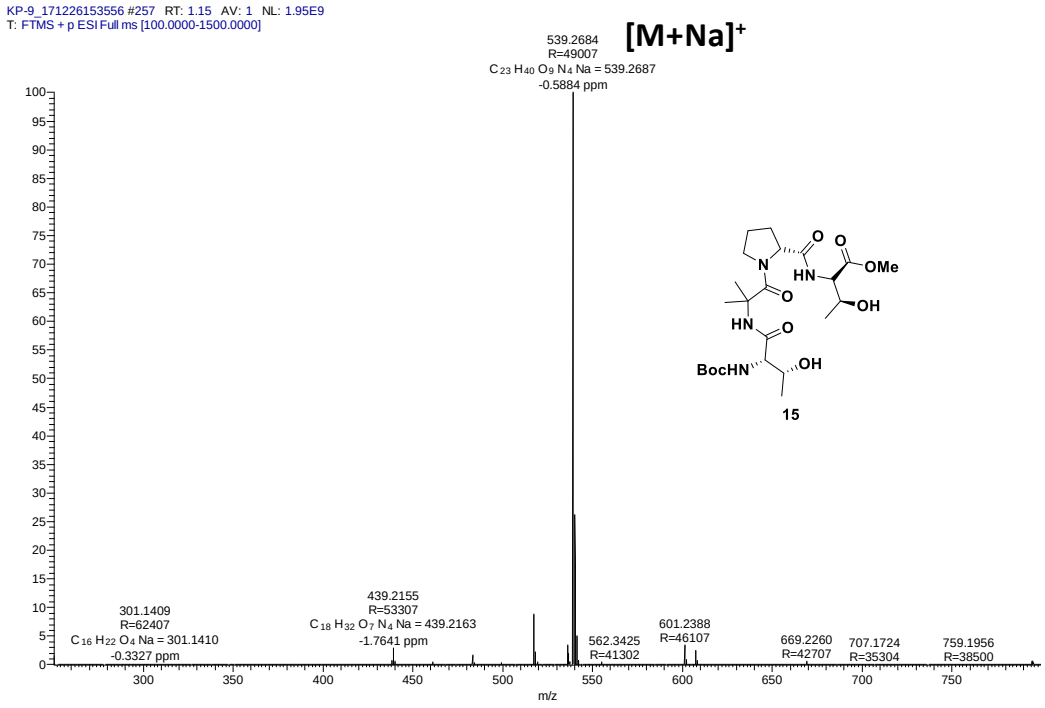
MeOH); ^{1}H NMR (400 MHz ,CD_3OD) = 7.63 (d, J = 8.7 Hz, 1 H), 7.21 (br. s., 1 H), 5.73 - 5.55 (m, 1 H), 4.84 - 4.74 (m, 1 H), 4.51 (dd, J = 2.3, 8.7 Hz, 1 H), 4.37 - 4.26 (m, 2 H), 3.95 (br. s., 1 H), 3.76 (s, 3 H), 3.59 (d, J = 19.7 Hz, 2 H), 2.33 - 2.12 (m, 2 H), 2.09 - 1.90 (m, 4 H), 1.59 - 1.51 (m, 6 H), 1.47 (s, 9 H), 1.20 (dd, J = 3.2, 6.4 Hz, 6 H) ^{13}C NMR (100 MHz, CD_3OD) = 172.2, 171.2, 167.6, 156.6, 80.8, 68.2, 66.1, 62.2, 59.0, 58.3, 57.4, 52.4, 48.0, 28.2, 27.5, 25.9, 25.6, 24.8, 19.8, 19.5 ; HRMS: $C_{23}H_{40}N_4O_9$, Calculated: 539.2687 $[M+Na]^+$, observed: 539.2684 $[M+Na]^+$.



^{1}H NMR spectrum of compound **15** (CD_3OD, 400 MHz, 298 K

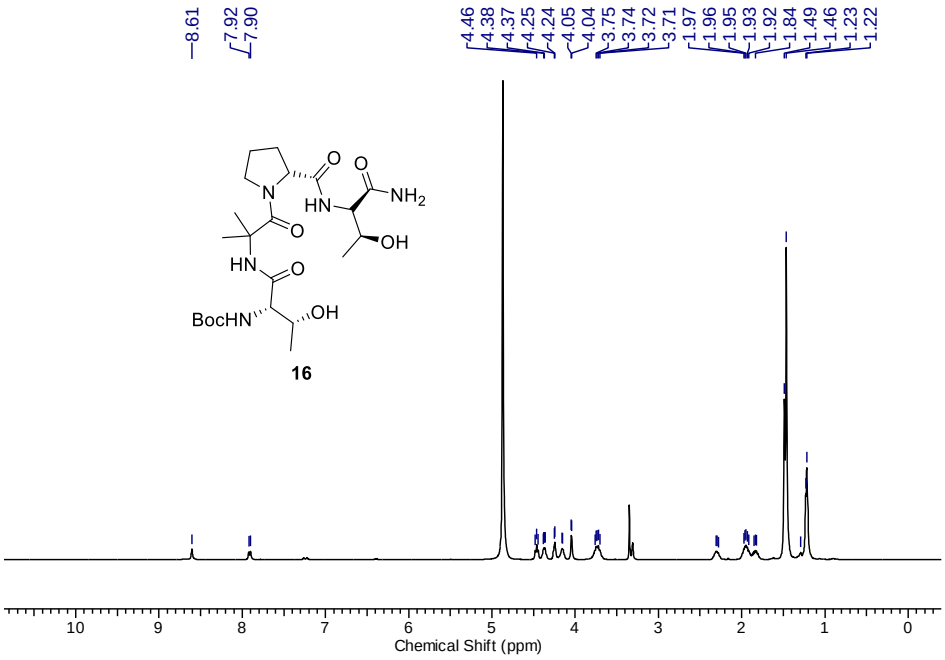


^{13}C NMR spectrum of compound **15** (CD_3OD, 400 MHz, 298 K)

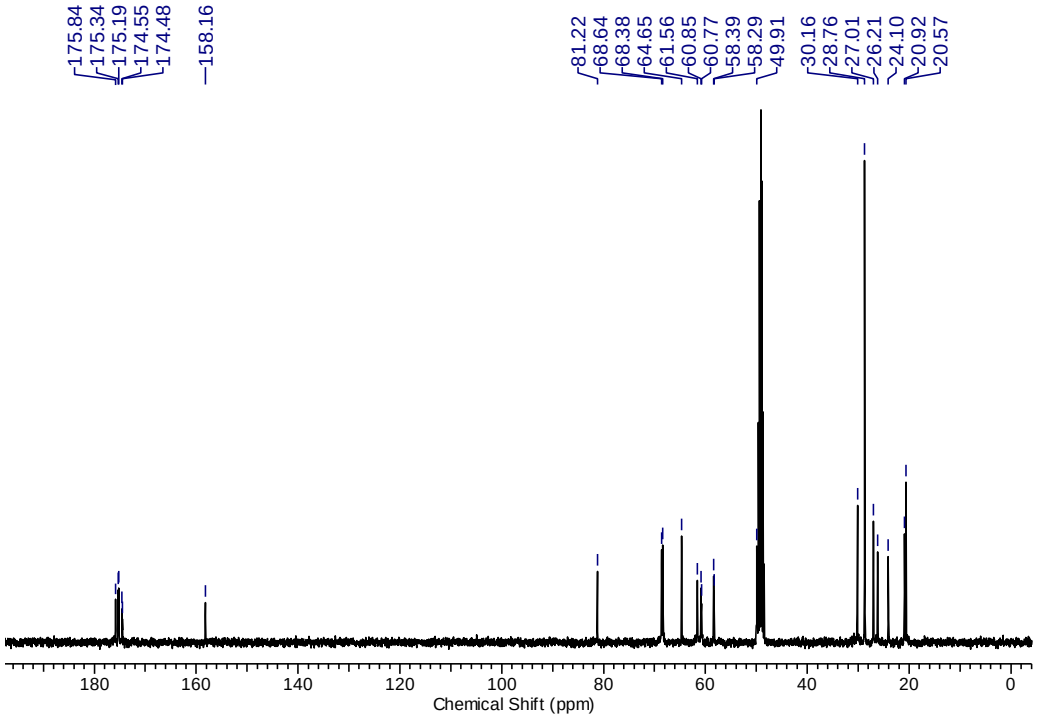


HRMS of Compound **15**

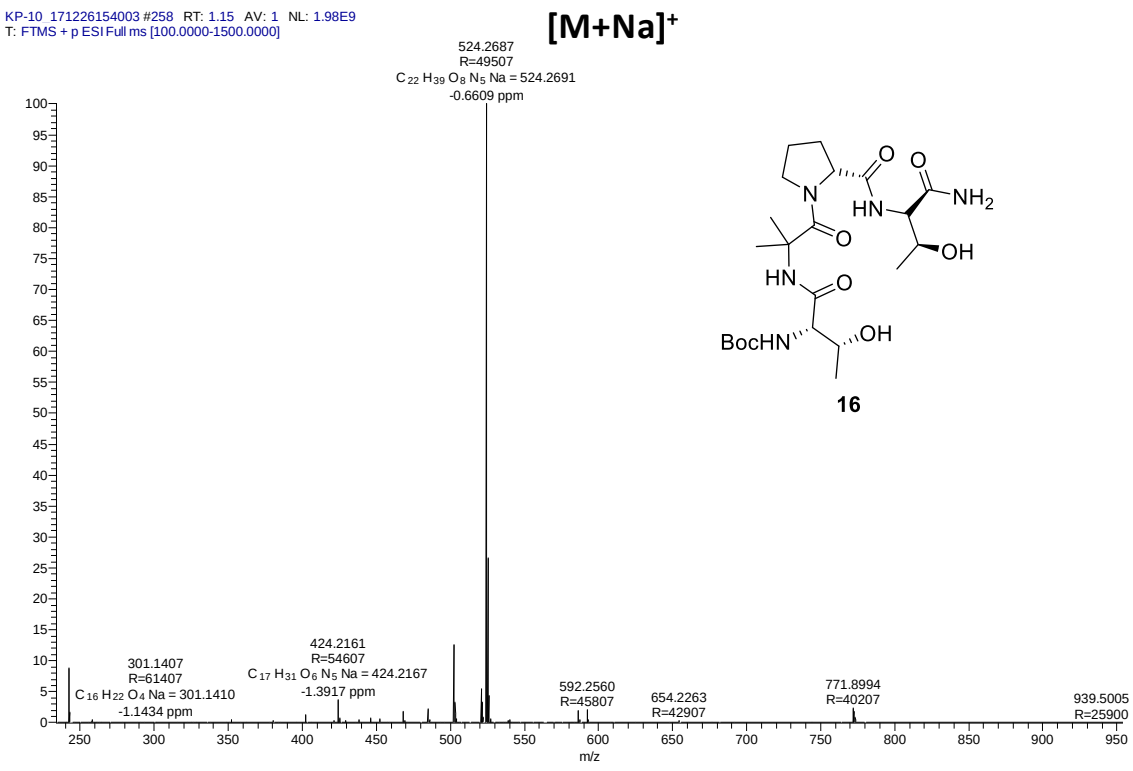
Compound 16 (Boc-Thr-Aib-Pro-Thr-NH_2): To a solution of tetrapeptide ester **15** (0.22 g, 0.42 mmol) in methanol, saturated methanolic ammonia solution (3 ml) was added and the reaction mixture was stirred for 24 h at room temperature. Later, the volatiles were stripped off under reduced pressure and the product was further purified by column chromatography and preparative thin layer chromatography (eluent: 4% MeOH/ DCM, R_f : 0.5) to furnish compound **16** (0.16 g, 75 %) as a white solid; $[\alpha]^{26}_D$: -56.4° (c = 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) = 8.61 (br. s., 1 H), 7.97 - 7.82 (m, 1 H), 4.46 (br. s., 1 H), 4.37 (dd, J = 3.1, 6.1 Hz, 1 H), 4.25 (d, J = 3.1 Hz, 1 H), 4.15 (d, J = 3.1 Hz, 1 H), 4.04 (d, J = 2.4 Hz, 1 H), 3.84 - 3.63 (m, 2 H), 2.38 - 2.22 (m, 1 H), 1.95 (dd, J = 5.8, 10.7 Hz, 3 H), 1.54 - 1.40 (m, 15 H), 1.22 (d, J = 5.5 Hz, 6 H) ^{13}C NMR (100 MHz ,CD_3OD) δ = 175.8, 175.3, 175.3, 175.2, 174.5, 158.2, 81.2, 68.6, 68.4, 64.6, 61.6, 60.9, 60.8, 58.4, 58.3, 49.9, 30.2, 28.8, 27.0, 26.2, 24.1, 20.9, 20.6; HRMS: $C_{22}H_{39}N_5O_8$, calculated: 524.2691 $[M+Na]^+$, observed: 524.2687 $[M+Na]^+$.



^{1}H NMR spectrum of compound **16** (CD_3OD, 400 MHz, 298 K)

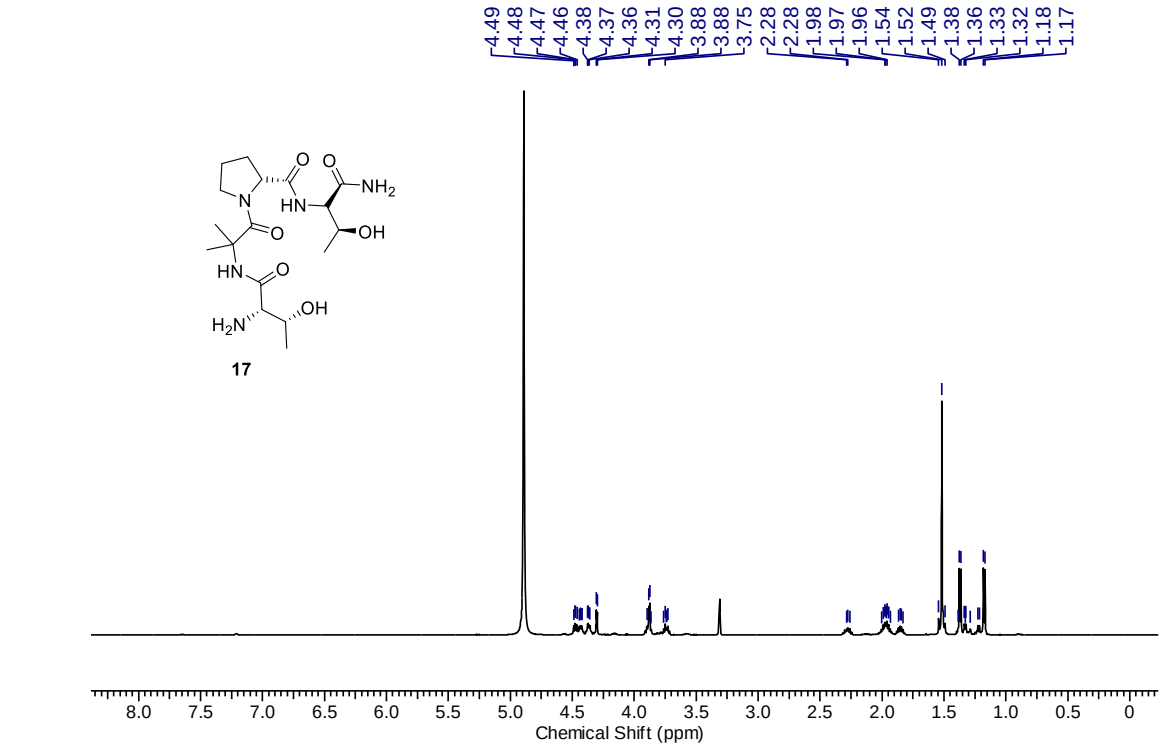


^{13}C NMR spectrum of compound **16** (CD_3OD, 400 MHz, 298 K)

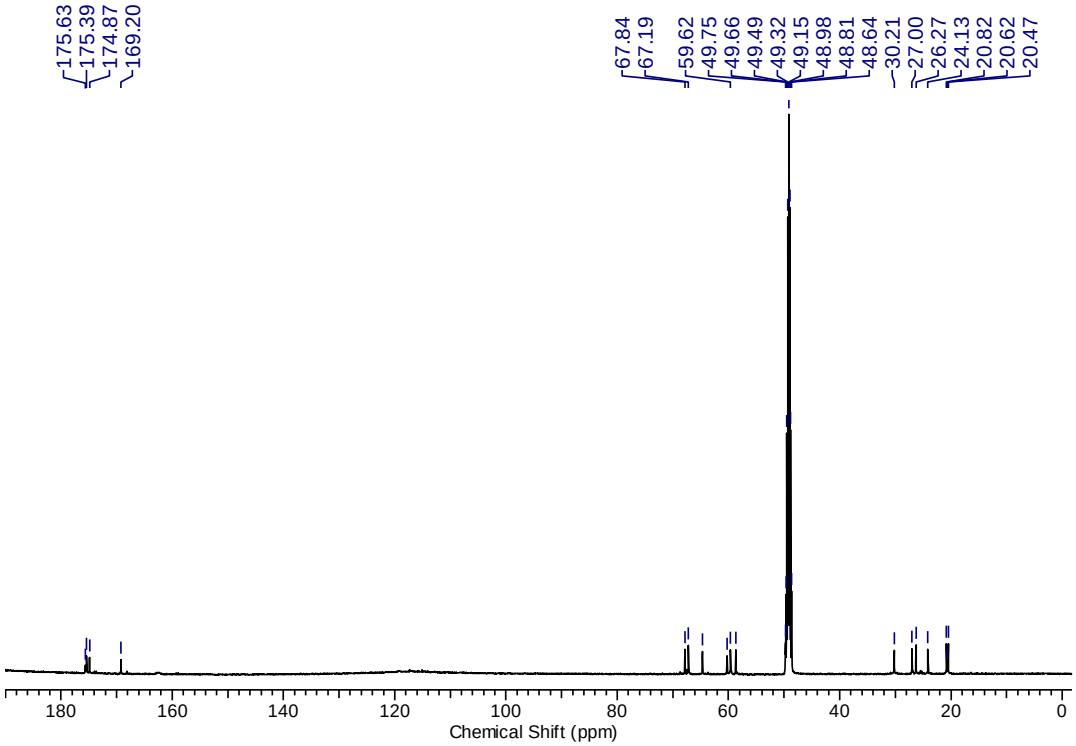


HRMS of Compound **16**

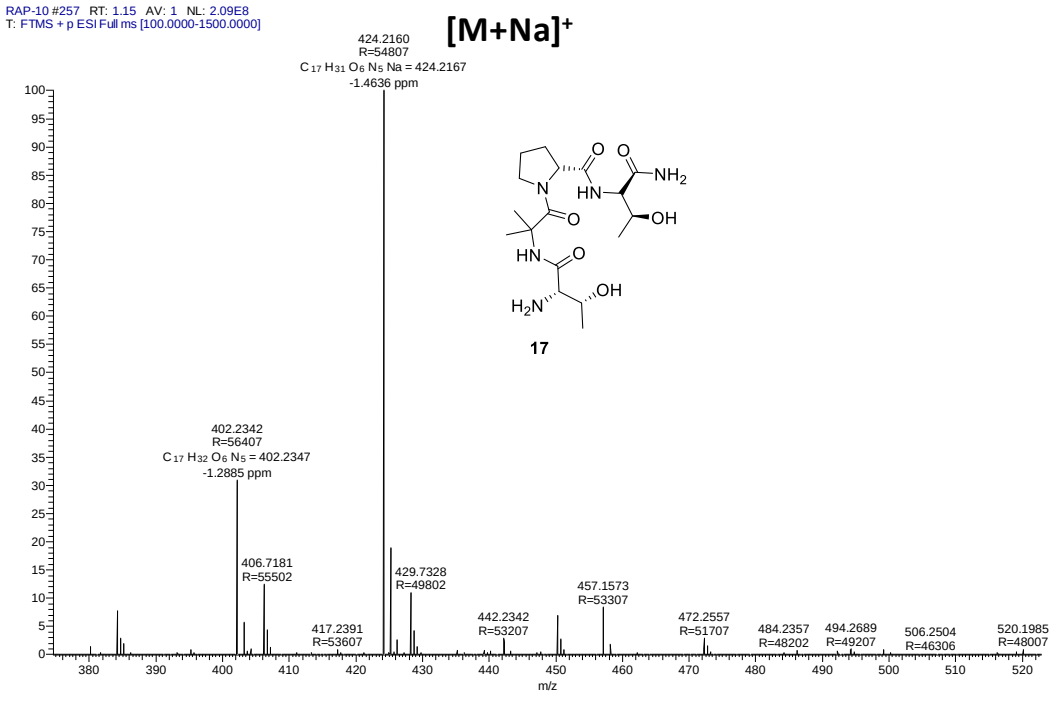
Compound 17 (H-Thr-Aib-Pro-Thr-NH$_2$): To the solution of compound **16** (0.1 g, 0.19 mmol) in DCM, TFA (1M) was added and stirred for 30 min. After the completion of the reaction, the volatiles were stripped off and co evaporated with toluene to obtain compound **17** as a hygroscopic white solid (0.075 g, 93 %). $[\alpha]^{26}_{D}$: -95.5° (c = 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ = 4.47 (d, J = 2.7 Hz, 1 H), 4.45 - 4.39 (m, 1 H), 4.37 (d, J = 3.1 Hz, 1 H), 4.30 (d, J = 3.4 Hz, 1 H), 3.87 (d, J = 2.7 Hz, 2 H), 3.75 (s, 1 H), 2.34 - 2.21 (m, 1 H), 2.03 - 1.90 (m, 2 H), 1.89 - 1.80 (m, 1 H), 1.56 - 1.47 (m, 6 H), 1.39 - 1.31 (m, 3 H), 1.17 (d, J = 6.5 Hz, 3 H); ^{13}C NMR (100 MHz, CD_3OD) δ = 175.6, 175.4, 174.9, 169.2, 67.8, 67.2, 64.7, 60.2, 59.6, 58.7, 30.2, 27.0, 26.3, 24.1, 20.8, 20.5; HRMS: $C_{17}H_{31}N_5O_6$, calculated: 424.2167 $[M+Na]^+$, observed: 424.2160 $[M+Na]^+$.



^{1}H NMR spectrum of compound **17** (CD_3OD, 400 MHz, 298 K)



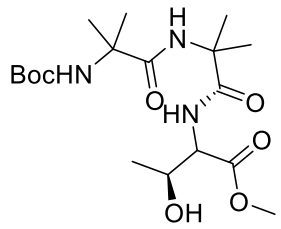
^{13}C NMR spectrum of compound **17** (CD_3OD, 400 MHz, 298 K)



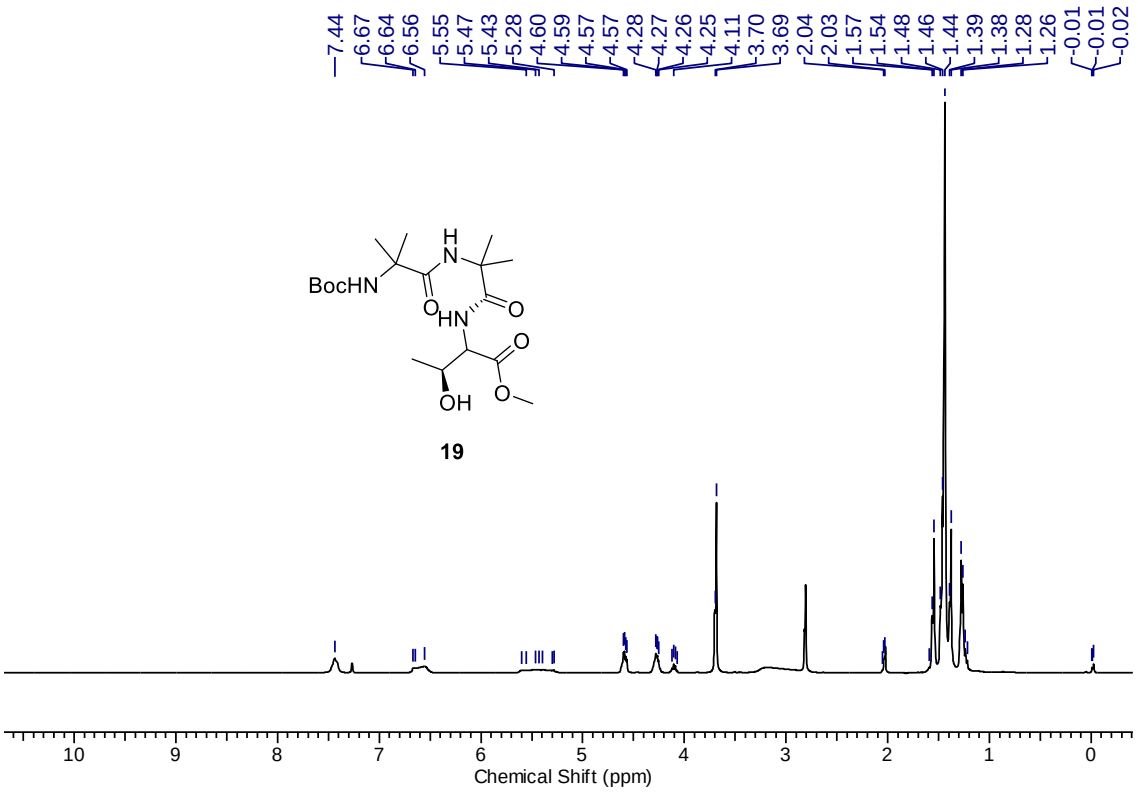
HRMS of Compound **17**

Compound **18** was synthesized according to the literature procedure.[27]

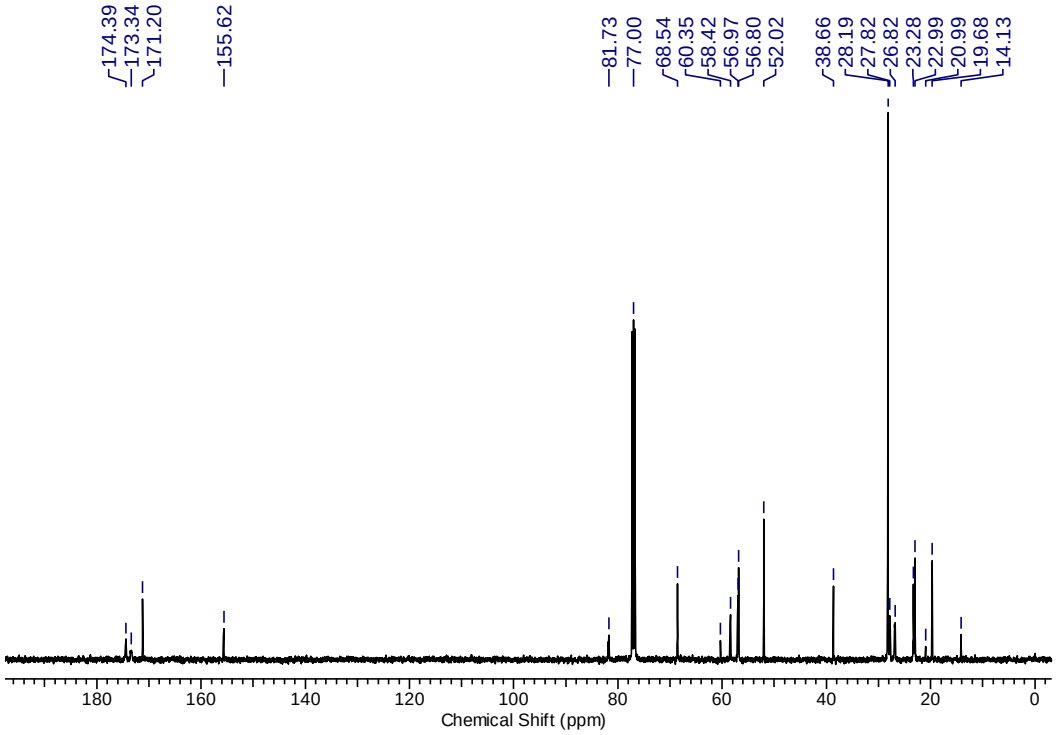
Compound 19 (Boc-Aib-Aib-Thr-OMe): Compound **18** (0.5 g, 1.65 mmol) was taken in a RBF and was dissolved in methanol (1 mL). LiOH (0.2 g, 4.9 mmol) was dissolved in 4 mL of water and added drop wise to the solution containing compound **7**. After checking the TLC, volatiles were stripped off and neutralized with $KHSO_4$ solution. Later, the reaction mixture was extracted with AcOEt and the organic layer was dried over anhydrous Na_2SO_4 and evaporated under vacuum to obtain the crude product which was used for the next reaction without further purification. Boc-Aib-Aib-OH (0.4 g, 1.38 mmol, 1 equiv) was coupled with H-Thr-OMe.HCl (0.23 g, 1.27 mmol, 1 equiv) using EDC.HCl (0.39 g, 2.08 mmol, 1.5 equiv), HOBt (0.10 g, 0.69 mmol, 0.5 equiv) and DIPEA (0.6 mL, 3.8 mmol, 3 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in AcOEt and the organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 60% AcOEt/pet. ether, R_f: 0.5) to furnish compound **19** (0.41 g, 74%) as a hygroscopic white solid; $[\alpha]^{26}_D$: -60.0° (c = 0.1, MeOH); ^{1}H NMR (400MHz ,$CDCl_3$) δ = 7.44 (br. s., 1 H), 6.75 - 6.42 (m, 1 H), 5.66 - 5.22 (m, 1 H), 4.69 - 4.53 (m, 1 H), 4.27 (dd, J = 4.0, 6.4 Hz, 1 H), 3.75 - 3.65 (m, 3 H), 1.59 - 1.53 (m, 3 H), 1.50 - 1.42 (m, 15 H), 1.42 - 1.37 (m, 3 H), 1.27 (d, J = 6.7 Hz, 3 H) ^{13}C NMR (100 MHz, $CDCl_3$) δ = 174.4, 173.3, 171.2, 155.6, 81.7, 68.5, 60.3, 58.4, 57.0, 56.8, 52.0, 38.7, 28.2, 27.8, 26.8, 23.3, 23.0, 21.0, 19.7, 14.1; HRMS: $C_{18}H_{33}N_3O_7$, Calculated: 426.2211 $[M+Na]^+$, observed: 426.2207 $[M+Na]^+$.



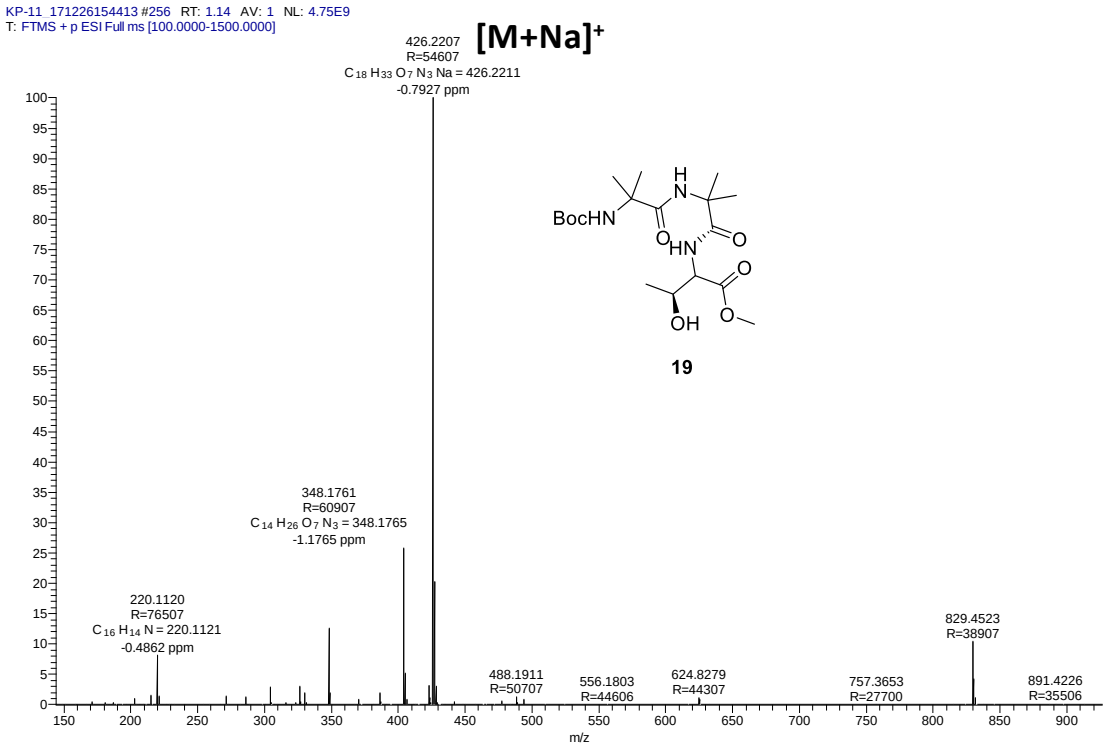
19



^{1}H NMR spectrum of compound **19** ($CDCl_3$, 400 MHz, 298 K)

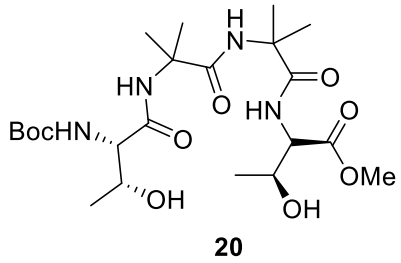


^{13}C NMR spectrum of compound **19** ($CDCl_3$, 400 MHz, 298 K)



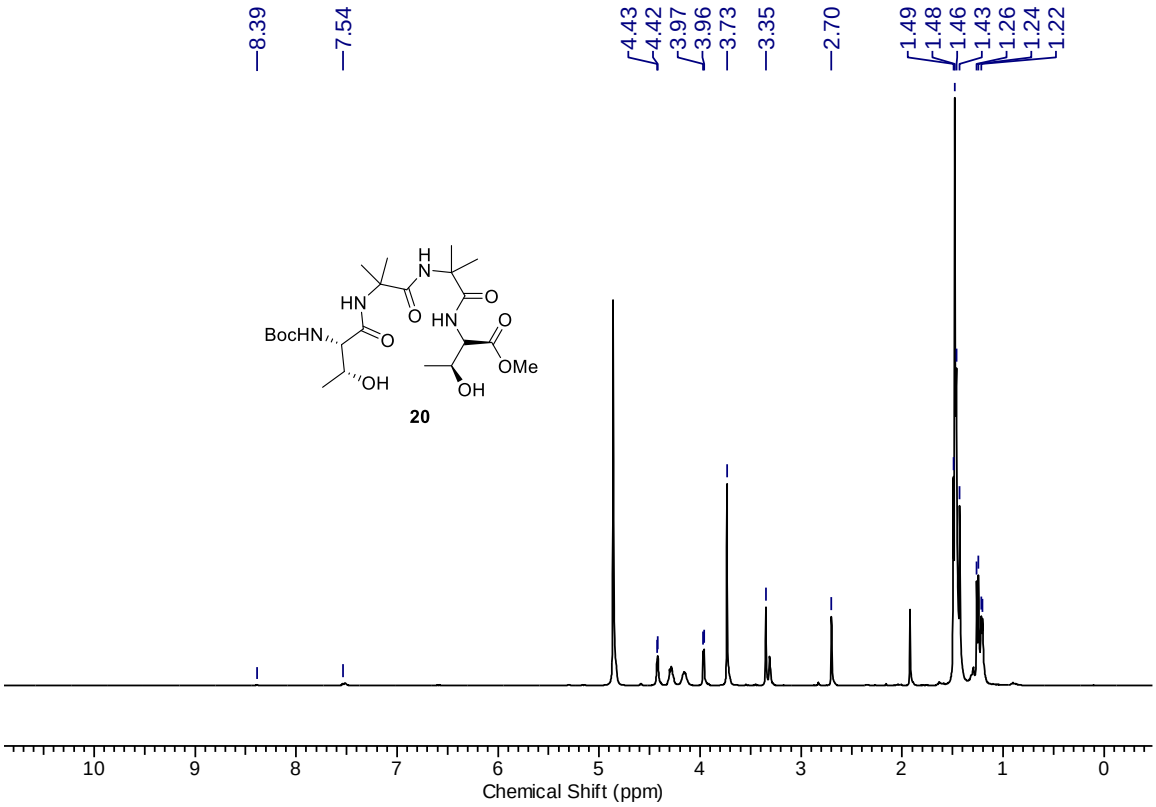
HRMS of compound **19**

Compound 20 (Boc-Thr-Aib-Aib-Thr-OMe): Boc-protected tripeptide ester **8** (0.35 g, 0.84 mmol) was dissolved in 1 mL of dichloromethane followed by addition of 1 mL of TFA (1M) at 0°C. The reaction was stirred for 30 min at room temperature. After completion of reaction, the solvent was stripped off and co-evaporated using toluene to obtain the TFA salt which was used for the next step without further purification. Boc-Thr-OH (0.13 g, 0.63 mmol, equiv) was coupled with tripeptide ester TFA salt (0.27 g, 0.63 mmol, 1 equiv) using HATU (0.36 g, 0.95 mmol, 1.5 equiv) and DIPEA (0.4 mL, 1.8 mmol, 4 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in 2 % MeOH/DCM and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 2% MeOH/DCM, R_f : 0.5) to furnish compound **9** (0.25 g, 76

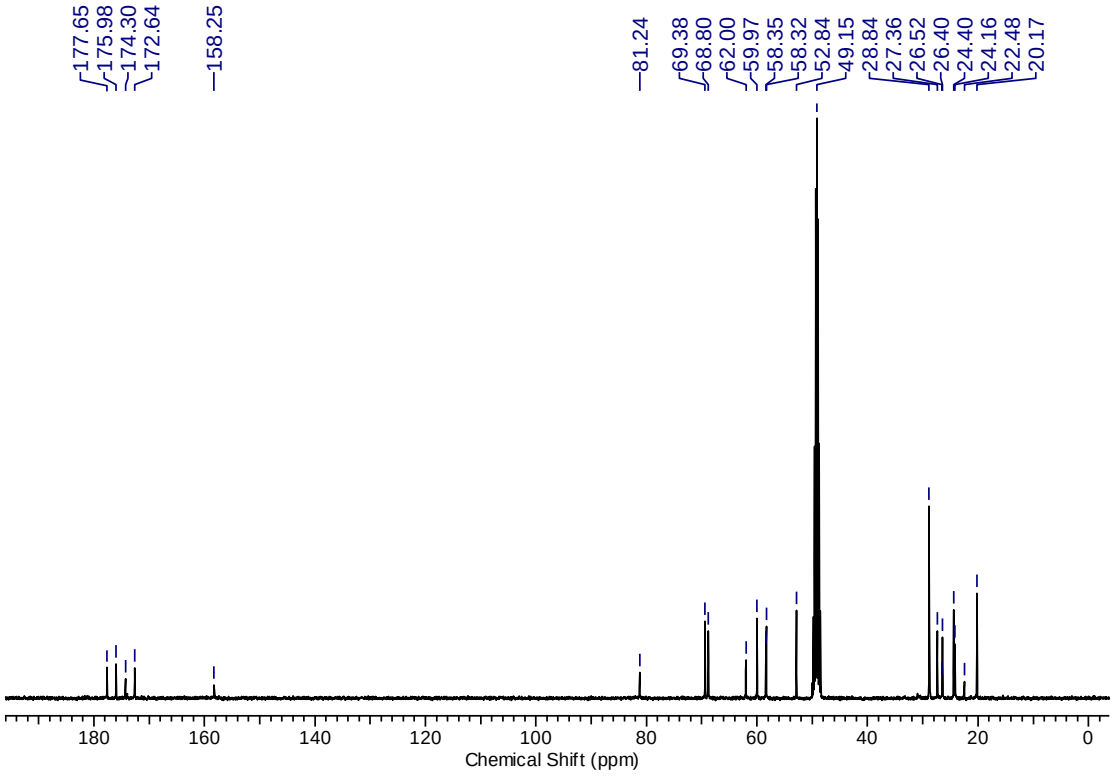


20

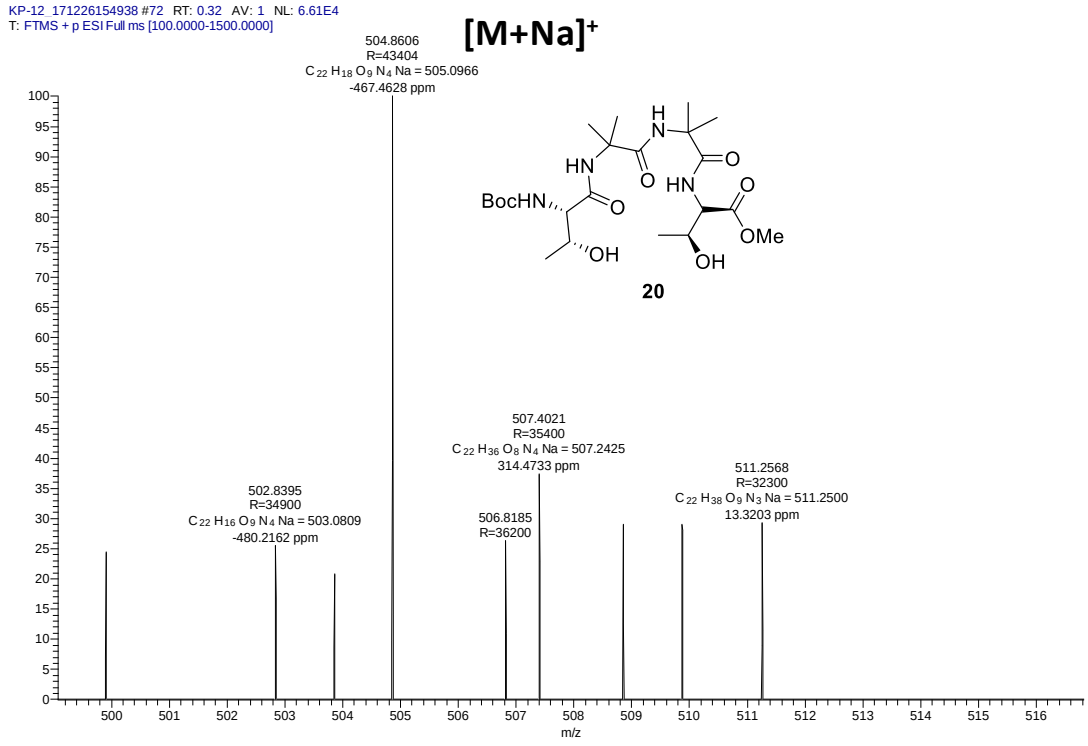
%) as a hygroscopic white solid; $[\alpha]^{26}_{D}$: -30.6° (c = 0.1, MeOH) ^{1}H NMR (400 MHz ,CD_3OD) δ = 8.42 - 8.34 (m, 1 H), 7.59 - 7.44 (m, 1 H), 4.42 (d, J = 3.1 Hz, 1 H), 4.29 (dd, J = 3.4, 6.4 Hz, 1 H), 4.15 (d, J = 4.9 Hz, 1 H), 3.97 (d, J = 3.7 Hz, 1 H), 3.35 (s, 1 H), 2.70 (s, 1 H), 1.52 - 1.40 (m, 21 H), 1.25 (d, J = 6.7 Hz, 3 H), 1.21 (d, J = 6.1 Hz, 3 H) ^{13}C NMR (100 MHz, CD_3OD) = 177.6, 176.0, 174.3, 172.6, 158.3, 81.2, 69.4, 68.8, 62.0, 60.0, 58.3, 58.3, 52.8, 28.8, 27.4, 26.5, 26.4, 24.4, 24.2, 22.5, 20.2, 20.2; HRMS: $C_{22}H_{18}N_4O_9$, Calculated: 504.8806 $[M+Na]^+$, observed: 504.8606 $[M+Na]^+$.



^{1}H NMR spectrum of compound **20** (CD_3OD, 400 MHz, 298 K)

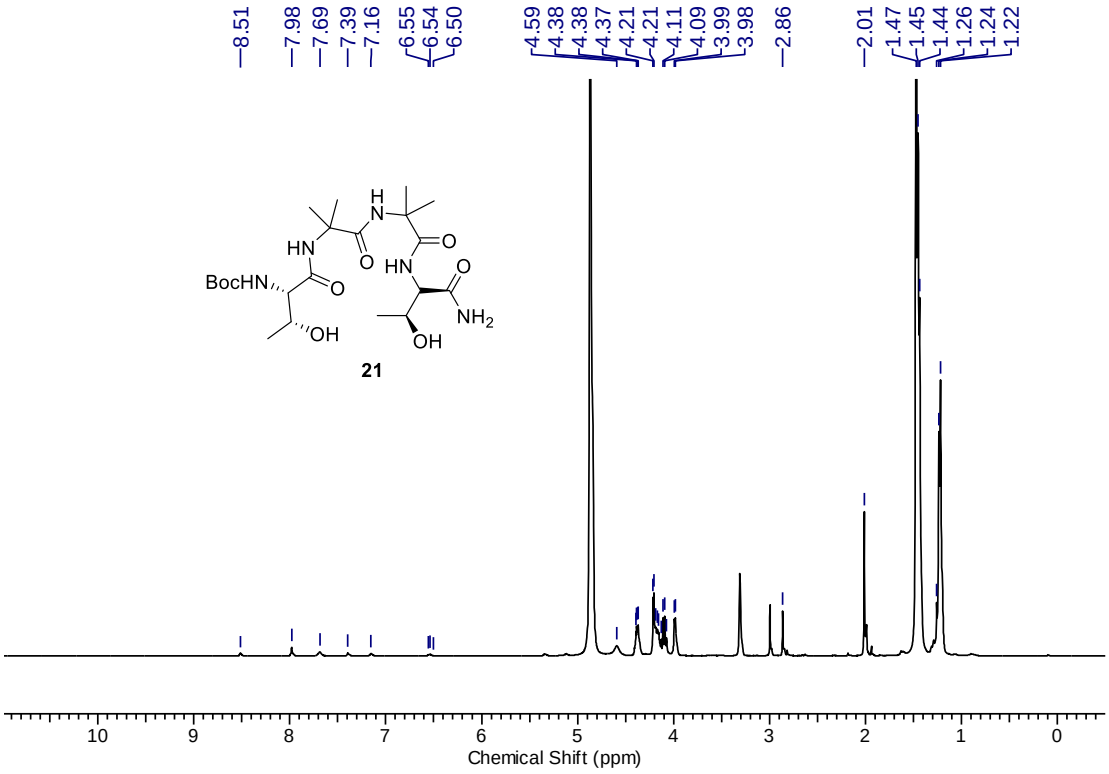


^{13}C NMR spectrum of compound **20** (CD_3OD, 400 MHz, 298 K)

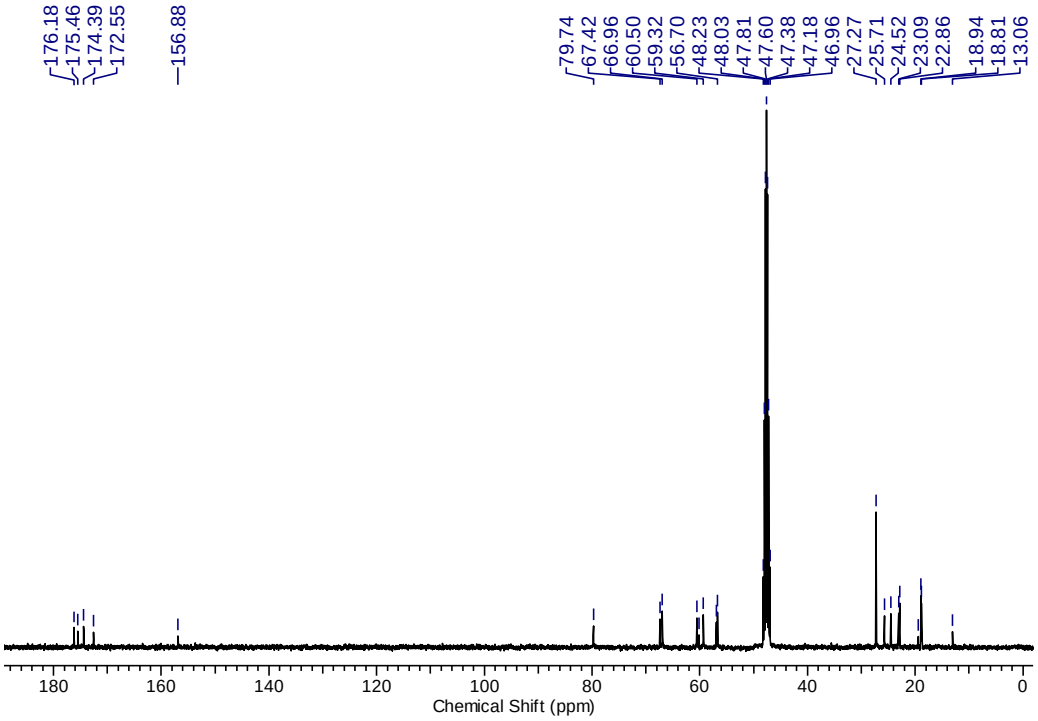


HRMS of Compound **20**

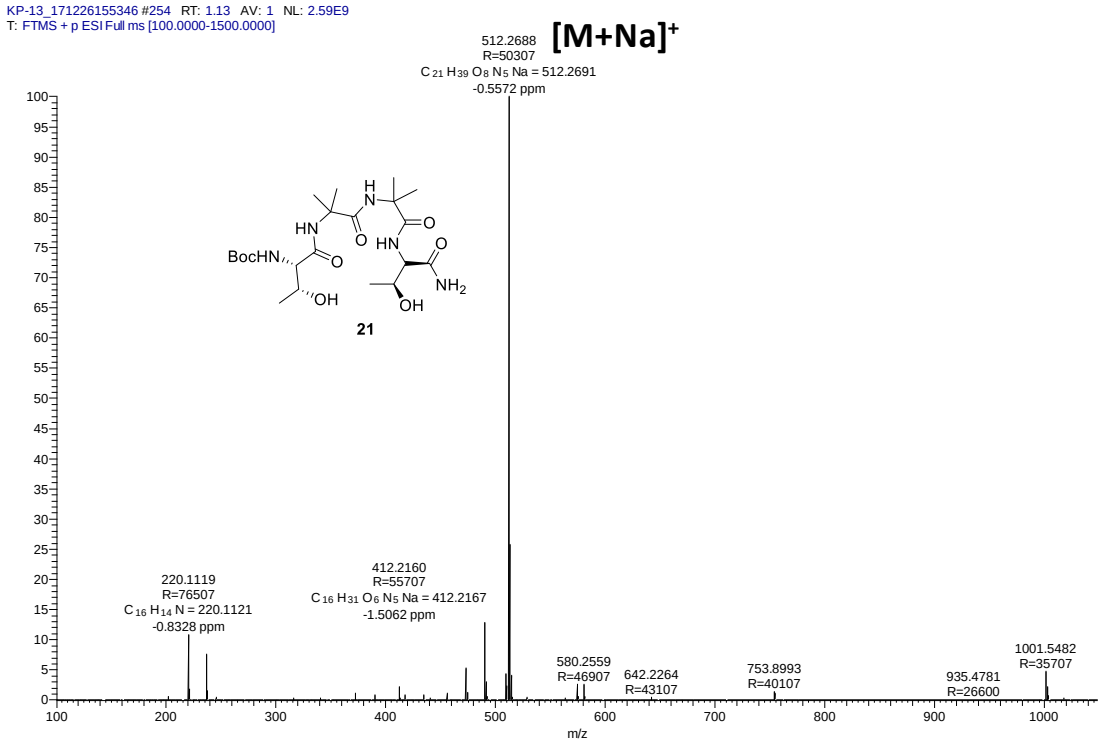
Compound 21 (Boc-Thr-Aib-Aib-Thr-NH_2): To a solution of tetrapeptide ester **20** (0.20 g, 0.39 mmol) in methanol, saturated methanolic ammonia solution (3 ml) was added and the reaction mixture was stirred for 24 h at room temperature. Later, the volatiles were stripped off under reduced pressure and the product was further purified by column chromatography and preparative thin layer chromatography (eluent: 4% MeOH/ DCM, R_f : 0.5) to furnish compound **10** (0.18 g, 93 %) as a white solid; $[\alpha]^{26}_D$: -55.8° (c = 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ = 8.56 - 8.44 (m, 1 H), 8.03 - 7.92 (m, 1 H), 7.76 - 7.63 (m, 1 H), 7.43 - 7.34 (m, 1 H), 7.20 - 7.10 (m, 1 H), 4.38 (dd, *J* = 3.1, 6.1 Hz, 1 H), 4.24 - 4.15 (m, 1 H), 4.14 - 4.05 (m, 1 H), 3.99 (d, *J* = 3.7 Hz, 1 H), 1.68 - 1.34 (m, 17 H), 1.31 - 1.14 (m, 6 H)**,** ^{13}C NMR (100 MHz ,CD_3OD) δ = 176.2, 175.5, 174.4, 172.6, 156.9, 79.7, 67.4, 67.0, 60.5, 60.1, 59.3, 56.9, 56.7, 27.3, 25.7, 24.5, 23.1, 22.9, 19.5, 18.9, 18.8, 13.1; HRMS: $C_{21}H_{39}N_5O_8$, calculated: 512.2691 $[M+Na]^+$, observed: 512.2688 $[M+Na]^+$.



^{1}H NMR spectrum of compound **21** (CD_3OD, 400 MHz, 298 K)

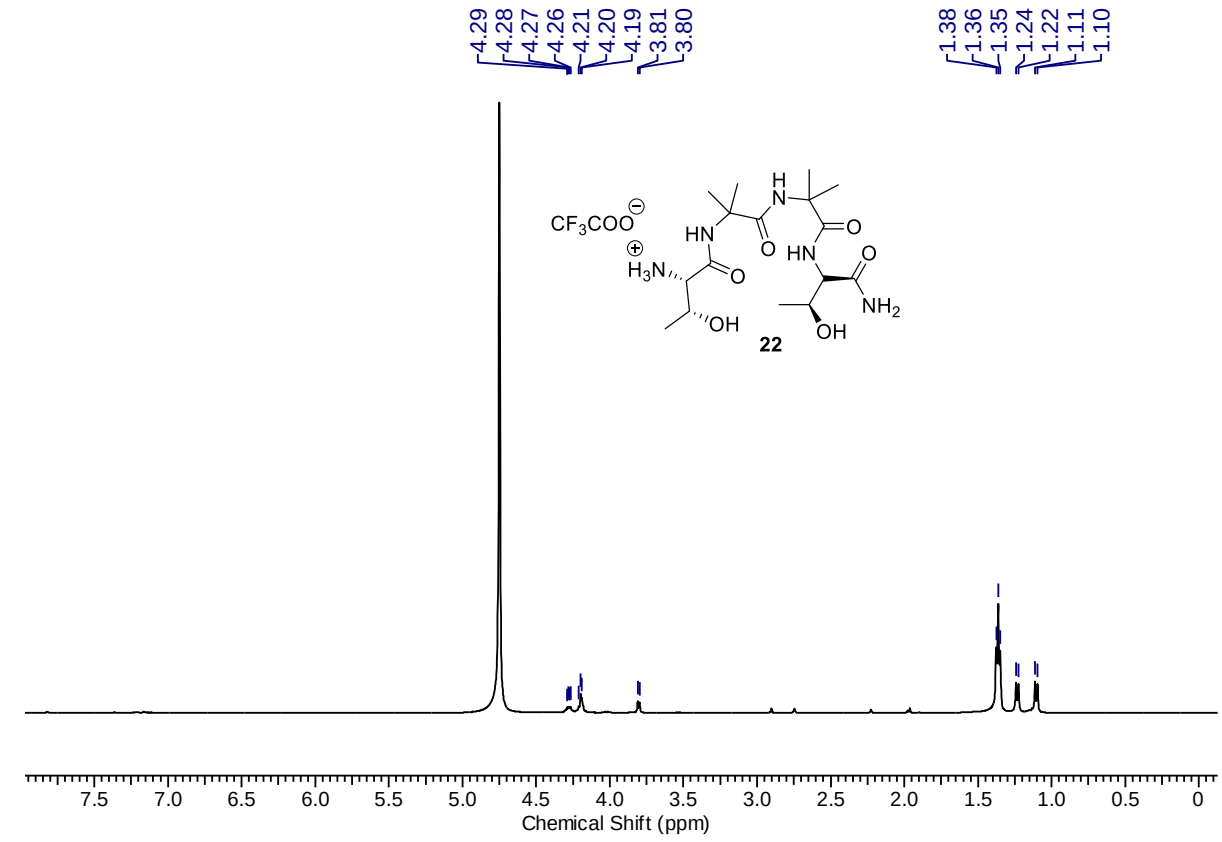


^{13}C NMR spectrum of compound **21** (CD_3OD, 400 MHz, 298 K)

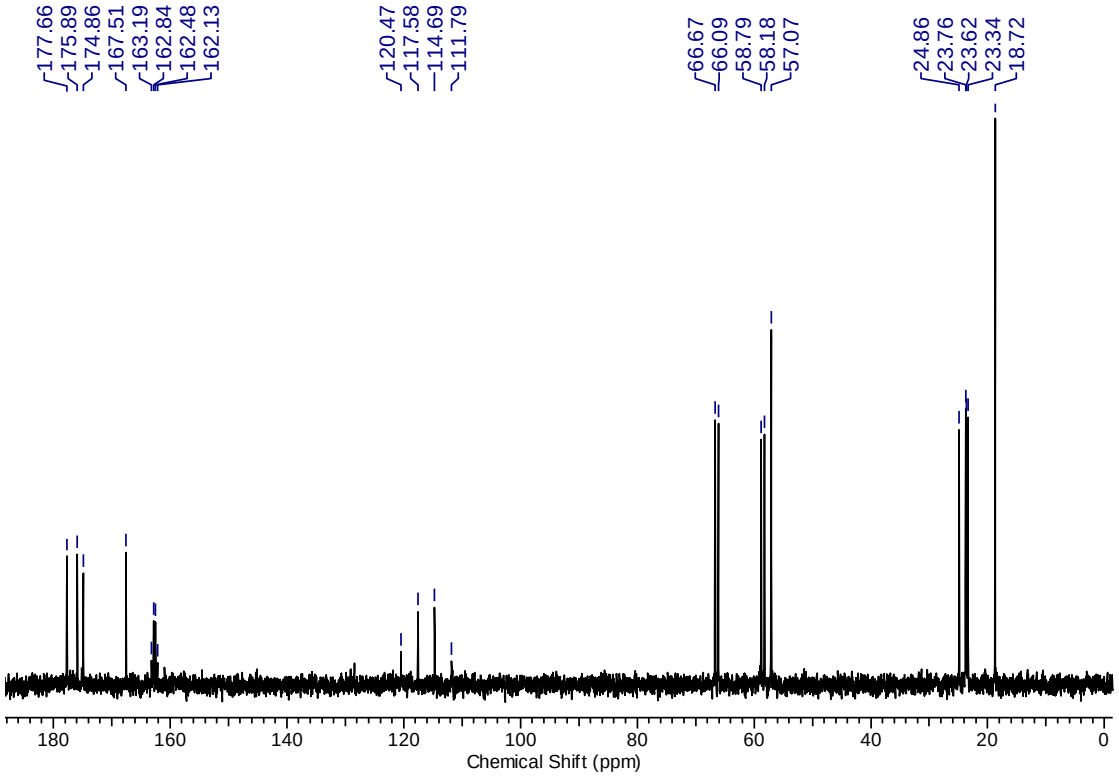


HRMS of Compound **21**

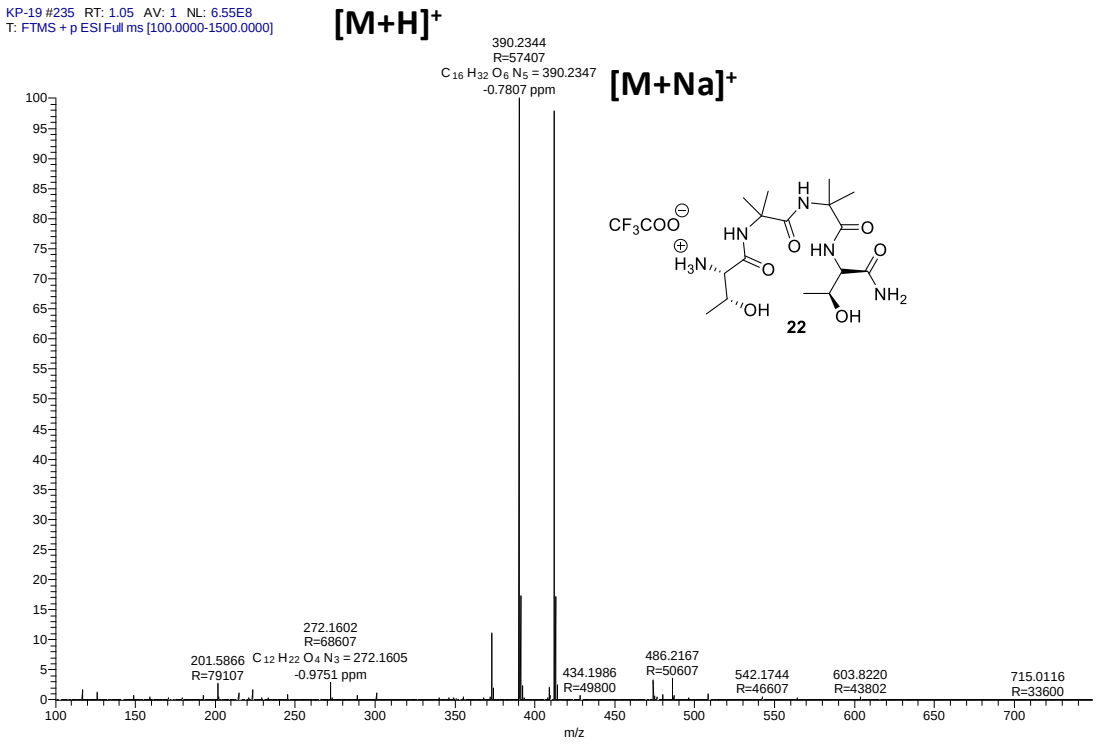
Compound 22 (TFA.H-Thr-Aib-Aib-Thr-NH_2): To the solution of compound **16** (0.1 g, 0.19 mmol) in DCM, TFA (1M) was added and stirred for 30 min. After the completion of the reaction, the volatiles were stripped off and co evaporated with toluene to obtain compound **17** as a hygroscopic white solid (0.075 g, 93 %). $[\alpha]^{26}_{D}$: -95.0° (c = 0.1, MeOH); H NMR (400 MHz, D_2O) δ = 4.32 - 4.25 (m, 1 H), 4.19 (d, J = 3.1 Hz, 2 H), 3.84 - 3.77 (m, 1 H), 1.36 (t, J = 5.2 Hz, 12 H), 1.23 (d, J = 6.7 Hz, 3 H), 1.10 (d, J = 6.1 Hz, 3 H) ^{13}C NMR (100 MHz, D_2O) δ = 177.7, 175.9, 174.9, 163.2, 162.8, 162.5, 162.1, 120.5, 117.6, 114.7, 111.8, 66.7, 66.1, 58.8, 58.2, 57.1, 24.9, 23.8, 23.6, 23.3, 18.7 HRMS: $C_{16}H_{32}N_5O_6$, calculated: 390.2347 $[M+H]^+$, observed: 390.2344 $[M+H]^+$.



^{1}H NMR spectrum of compound **22** (D_2O, 400 MHz, 298 K

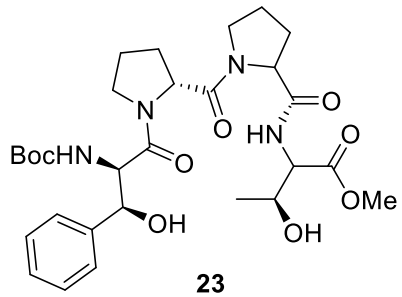


^{13}C NMR spectrum of compound **22** (D_2O, 400 MHz, 298 K)

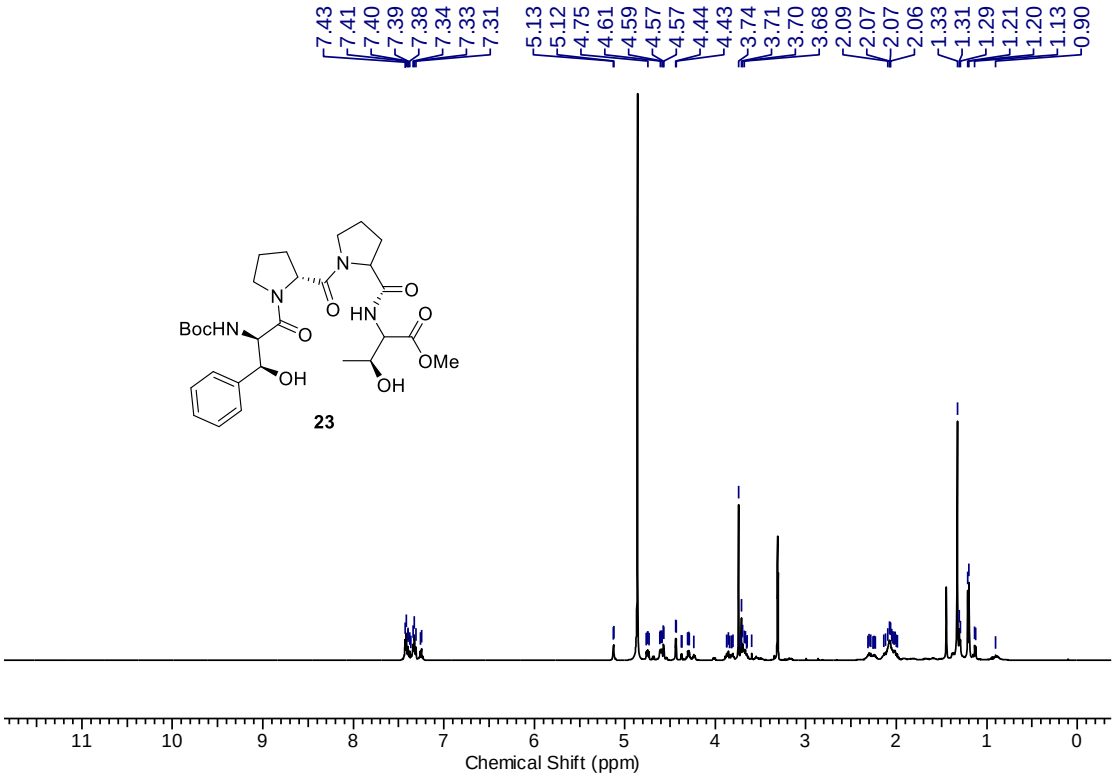


HRMS of Compound **22**

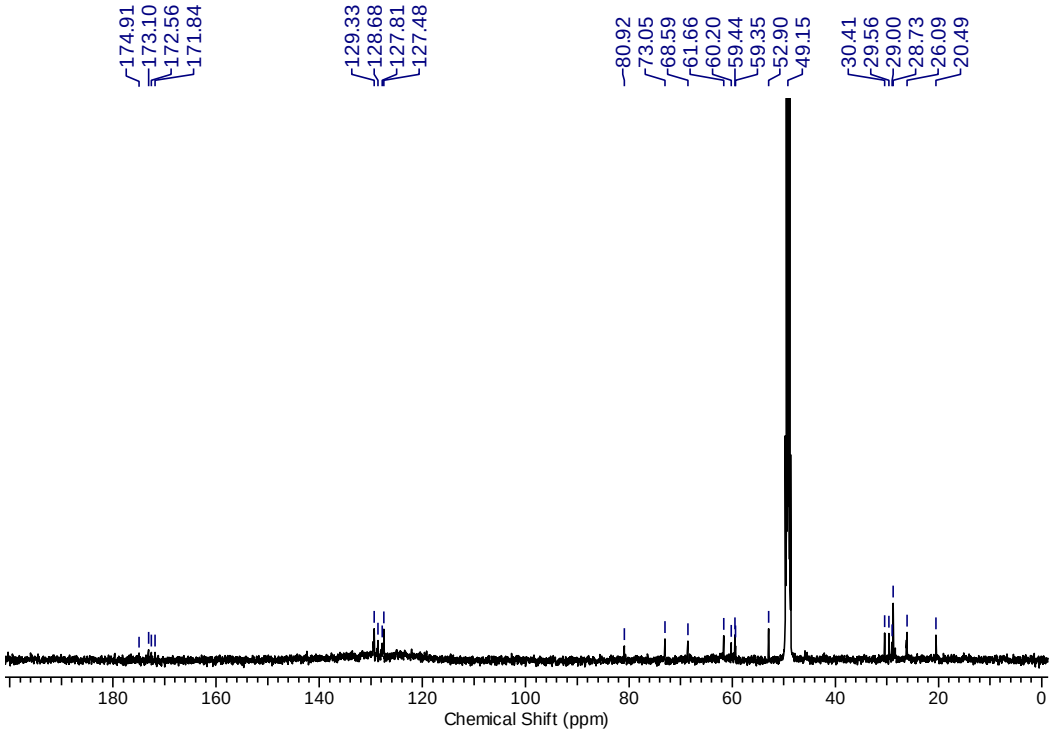
Compound 23 (Boc-PheSer-Pro-Pro-Thr-OMe): Boc-protected tripeptide ester **3** (0.3 g, 0.70 mmol) was dissolved in 1 mL of dichloromethane followed by addition of 1 mL of TFA (1M) at 0°C. The reaction was stirred for 30 min at room temperature. After completion of reaction, the solvent was stripped off and co-evaporated using toluene to obtain the TFA salt which was used for the next step without further purification. Boc-L-threophenylserine (0.16 g, 0.76 mmol, equiv) was coupled with tripeptide ester TFA salt (0.25 g, 0.76 mmol, 1 equiv) using HATU (0.4 g, 1.14 mmol, 1.5 equiv) and DIPEA (0.4 mL, 2.2 mmol, 4 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in 2 % MeOH/DCM and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 2% MeOH/DCM, R_f : 0.5) to furnish compound **23** (0.25 g, 62 %) as a hygroscopic white solid; $[\alpha]^{26}_D$: -77.9° (c = 0.1, MeOH); ^{1}H NMR (400 MHz ,CD_3OD) δ = 7.47 - 7.17 (m, 5 H), 5.13 (d, J = 2.7 Hz, 1 H), 4.77 - 4.67 (m, 1 H), 4.57 (d, J = 2.7 Hz, 2 H), 4.44 (d, J = 2.7 Hz, 1 H), 4.33 - 4.21 (m, 1 H), 3.91 - 3.76 (m, 2 H), 3.74 (s, 3 H), 3.72 - 3.65 (m, 2 H), 3.65-3.61 (m, 1 H), 3.60 - 3.44 (m, 1 H), 2.35 - 2.18 (m, 2 H), 2.15 - 1.94 (m, 6 H), 1.48 - 1.30 (m, 9 H), 1.24 - 1.10 (m, 3 H) ^{13}C NMR (100 MHz ,CD_3OD) =174.9, 173.1, 172.5, 171.8, 129.3, 128.6, 127.8, 127.4, 80.9, 73.0, 68.6 , 61.6, 59.4, 52.9, 30.4, 29.5, 29.0, 28.7, 26.0, 20.4; HRMS: $C_{29}H_{42}N_4O_9$, Calculated: 613.2844 $[M+Na]^+$, observed: 613.2843 $[M+Na]^+$.



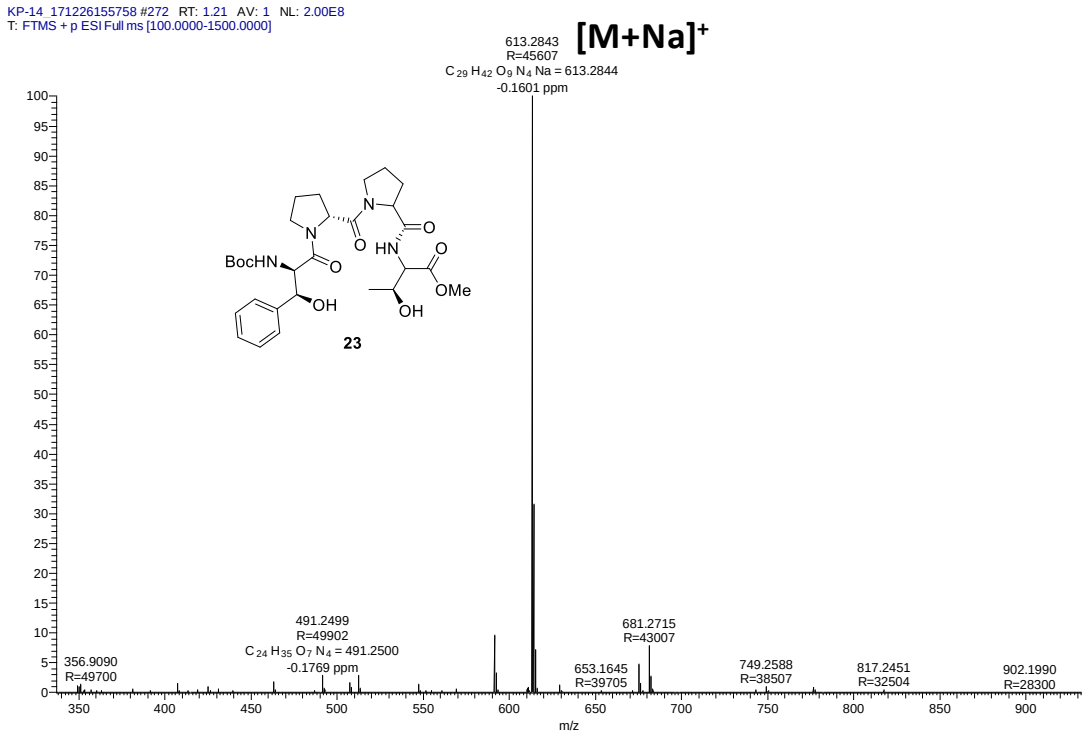
23



^{1}H NMR spectrum of compound **23** (CD_3OD, 400 MHz, 298 K)

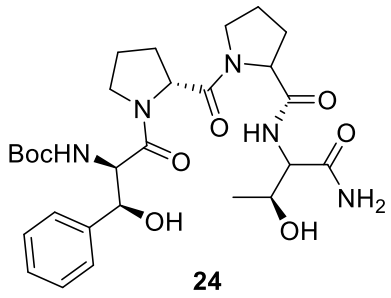


^{13}C NMR spectrum of compound **23** (CD_3OD, 400 MHz, 298 K)

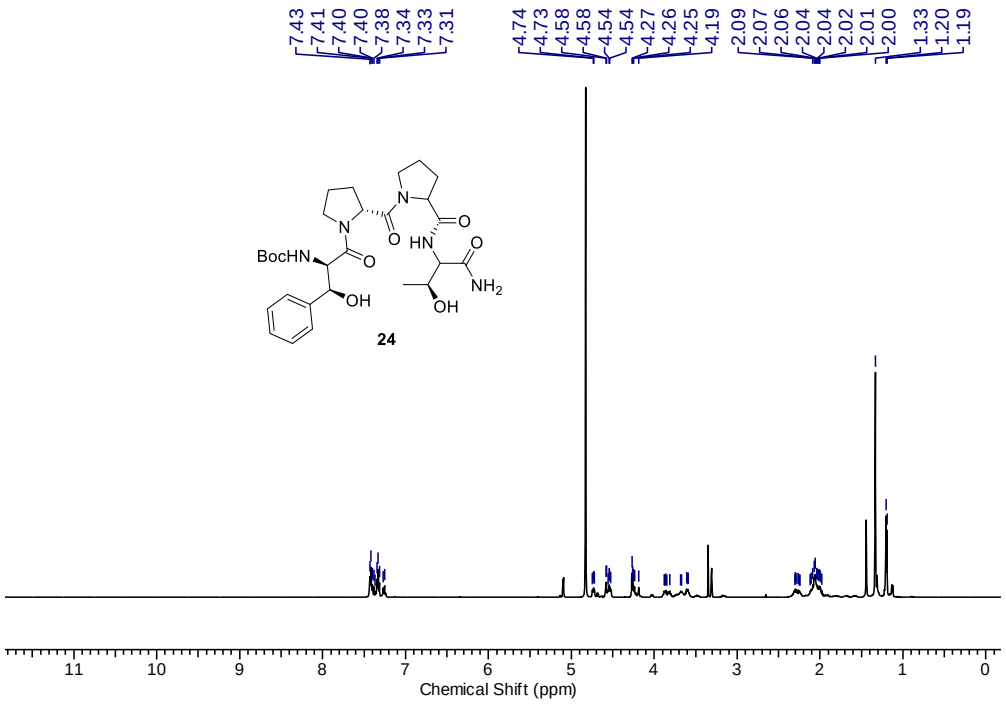


HRMS of Compound **23**

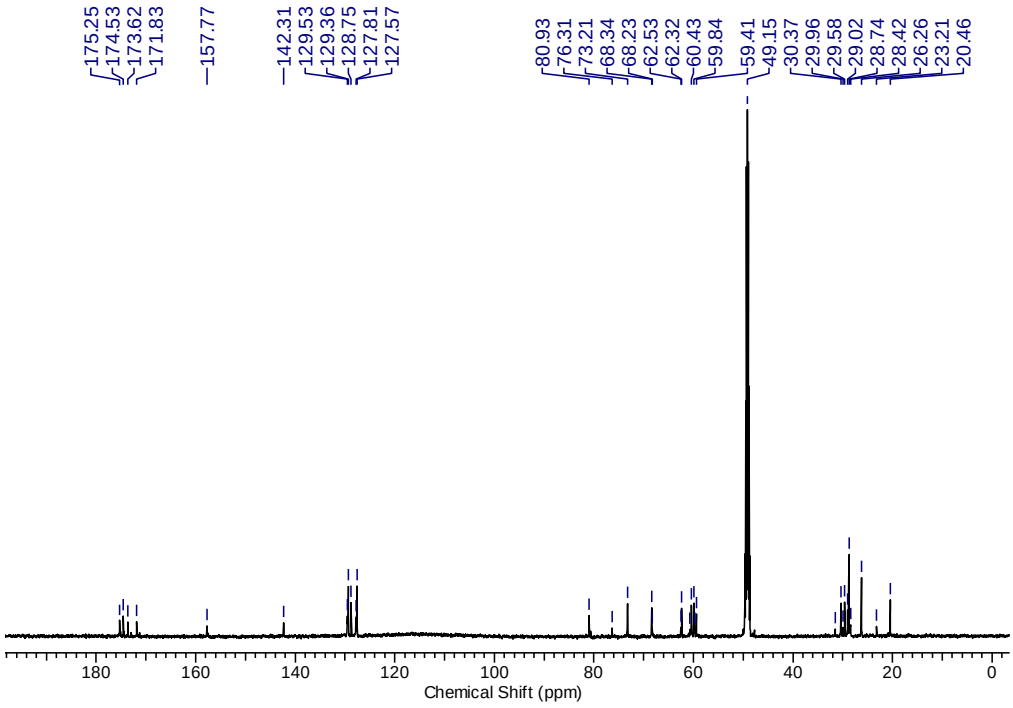
Compound 24 (Boc-PheSer-Pro-Pro-Thr-NH$_2$): To a solution of tetrapeptide ester **4** (0.20 g, 0.37 mmol) in methanol, saturated methanolic ammonia solution (3 ml) was added and the reaction mixture was stirred for 24 h at room temperature. Later, the volatiles were stripped off under reduced pressure and the product was further purified by column chromatography and preparative thin layer chromatography (eluent: 4% MeOH/ DCM, R_f : 0.5) to furnish compound **5** (0.17 g, 87%) as a white solid; $[\alpha]^{26}_D$: -22.2° (c = 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ = 7.47 - 7.39 (m, 3 H), 7.39 - 7.35 (m, 1 H), 7.33 (t, J = 7.6 Hz, 2 H), 7.25 (s, 1 H), 4.76 - 4.65 (m, 2 H), 4.56 (dd, *J* = 3.8, 19.8 Hz, 2 H), 4.31 - 4.15 (m, 2 H), 3.92 - 3.76 (m, 2 H), 3.72 - 3.54 (m, 2 H), 2.36 - 2.21 (m, 2 H), 2.13 - 1.97 (m, 6 H), 1.36 - 1.26 (m, 9 H), 1.20 (d, *J* = 6.1 Hz, 3 H), ^{13}C NMR (100 MHz ,CD_3OD) δ = 175.3, 174.5, 173.6, 171.8, 157.8, 142.3, 129.5, 129.4, 128.8, 127.8, 127.6, 80.9, 76.4, 73.2, 68.3, 62.5, 62.3, 60.8, 60.4, 59.8, 59.4, 30.4, 30.0,



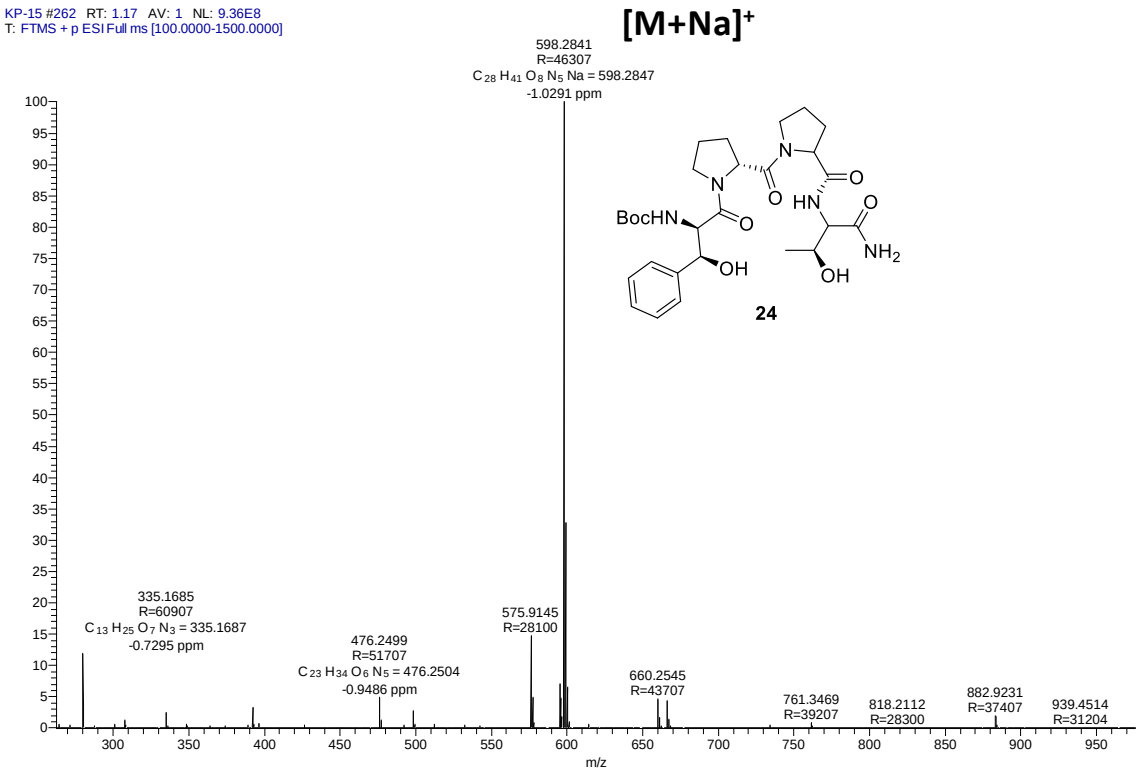
29.6, 28.9, 28.4, 26.3, 22.9, 20.5; HRMS: $C_{28}H_{41}N_5O_8$, calculated: 598.2847 $[M+Na]^+$, observed: 598.2841 $[M+Na]^+$.



^{1}H NMR spectrum of compound **24** (CD_3OD, 400 MHz, 298 K)



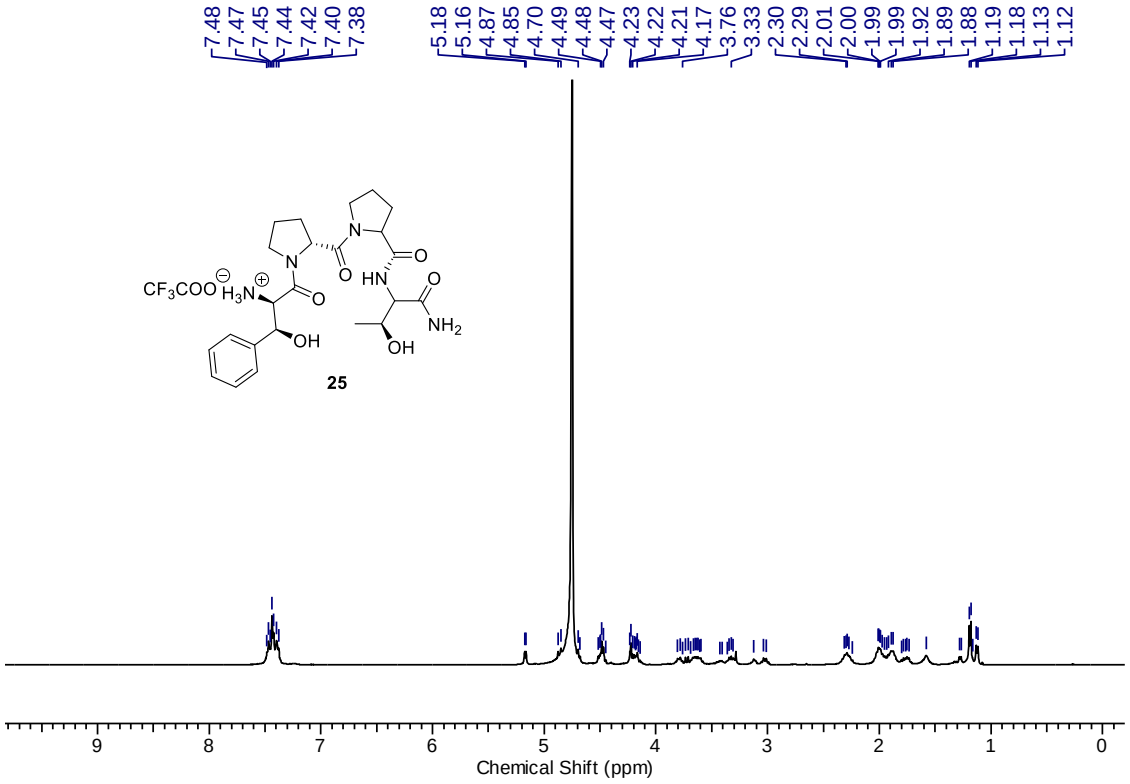
^{13}C NMR spectrum of compound **24** (CD_3OD, 400 MHz, 298 K)



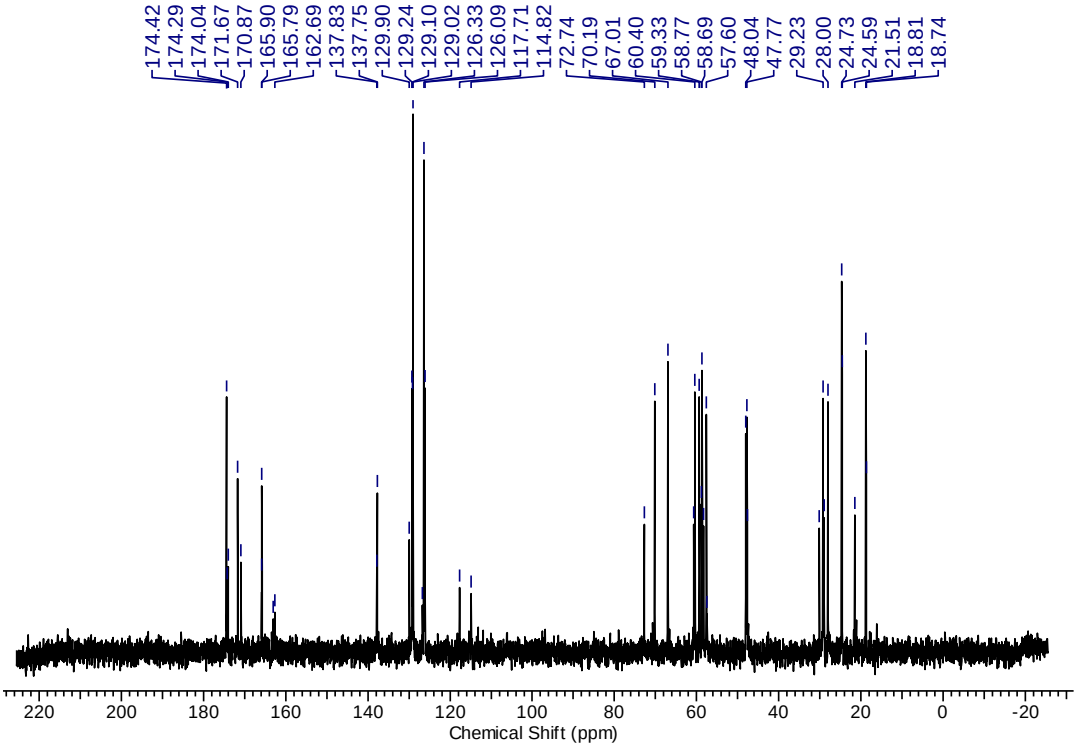
HRMS of Compound **24**

Compound 25 (TFA.H-PheSer-Pro-Pro-Thr-NH_2): To the solution of compound **24** (0.1 g, 0.17 mmol) in DCM, TFA (1M) was added and stirred for 30 min. After the completion of the reaction, the volatiles were stripped off and co evaporated with toluene to obtain compound **25** as a hygroscopic white solid (0.08 g, 78 %). $[\alpha]^{26}_{D}$: -45.7° (c = 0.1, MeOH); ^{1}H NMR (400 MHz, D_2O) δ = 7.52 - 7.33 (m, 5 H), 4.56 - 4.41 (m, 2 H), 4.27 - 4.09 (m, 2 H), 3.87 - 3.57 (m, 2 H), 3.44 - 3.25 (m, 2 H), 3.18 - 2.97 (m, 1 H), 2.38 - 2.16 (m, 2 H), 2.06 - 1.95 (m, 2 H), 1.91 - 1.81 (m, 2 H), 1.78 - 1.72 (m, 1 H), 1.62 - 1.54 (m, 1 H), 1.19 (d, J = 6.1 Hz, 3 H); ^{13}C NMR (100 MHz ,D_2O) δ = 174.4, 174.3, 174.0, 171.7, 170.9, 165.9, 165.8, 163.0, 162.7, 137.8, 129.9, 129.2, 129.1, 129.0, 126.3, 126.1, 117.7, 114.8, 72.7, 70.2, 67.0, 60.7, 60.4, 59.3, 58.8, 58.7, 58.6, 58.3, 57.6, 48.0, 47.8, 47.6, 30.1, 29.2,

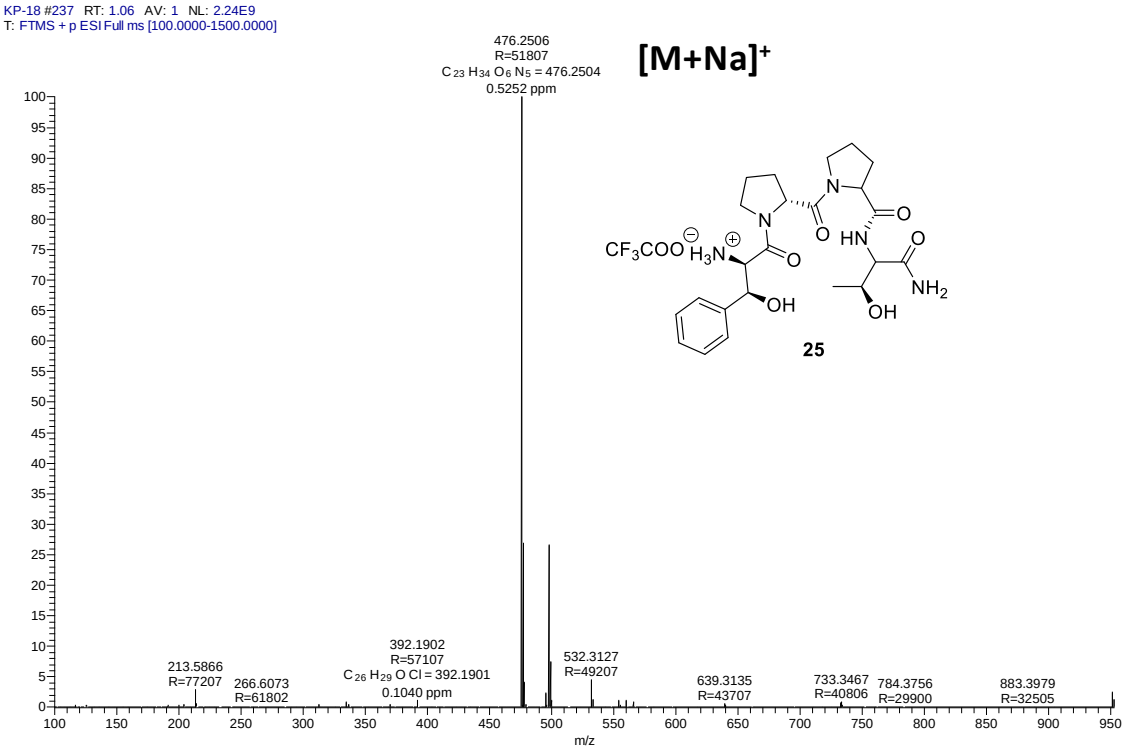
29.0, 28.0, 24.7, 21.5, 18.8; HRMS: $C_{23}H_{36}N_5O_6$, calculated: 476.2504 $[M+H]^+$, observed: 476.2506 $[M+H]^+$.



^{1}H NMR spectrum of compound **25** (D_2O, 400 MHz, 298 K)

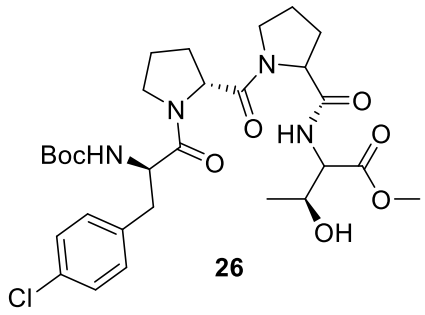


^{13}C NMR spectrum of compound **25** (D_2O, 400 MHz, 298 K)

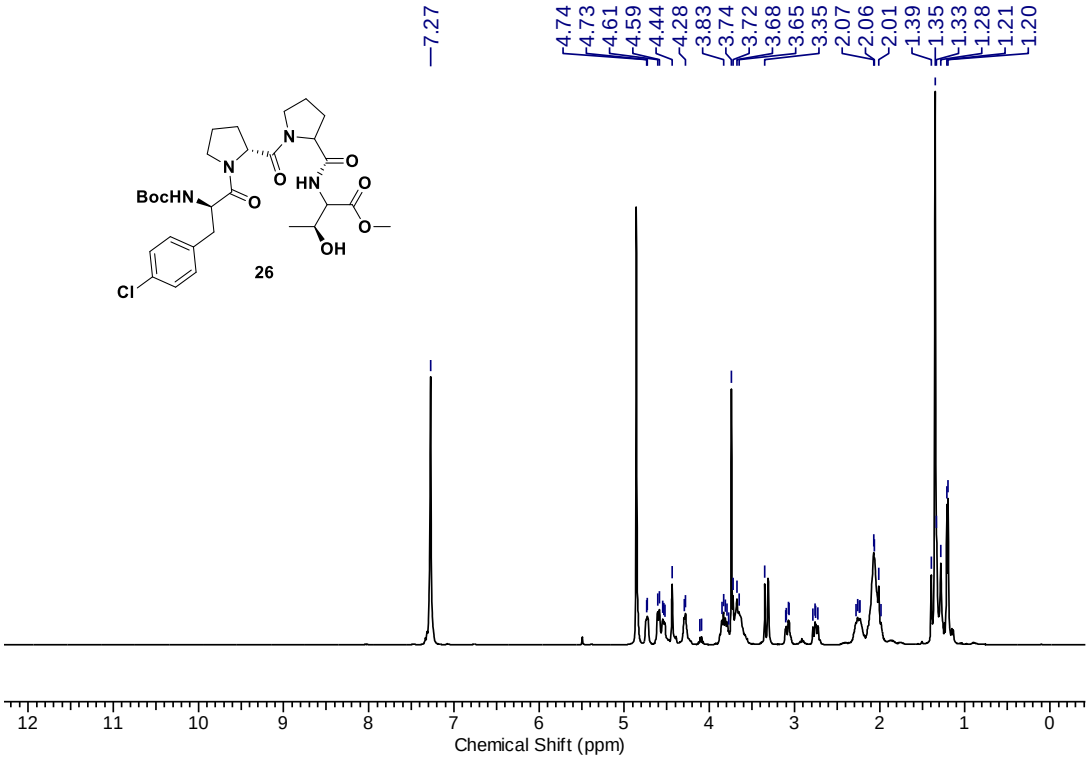


HRMS of Compound **25**

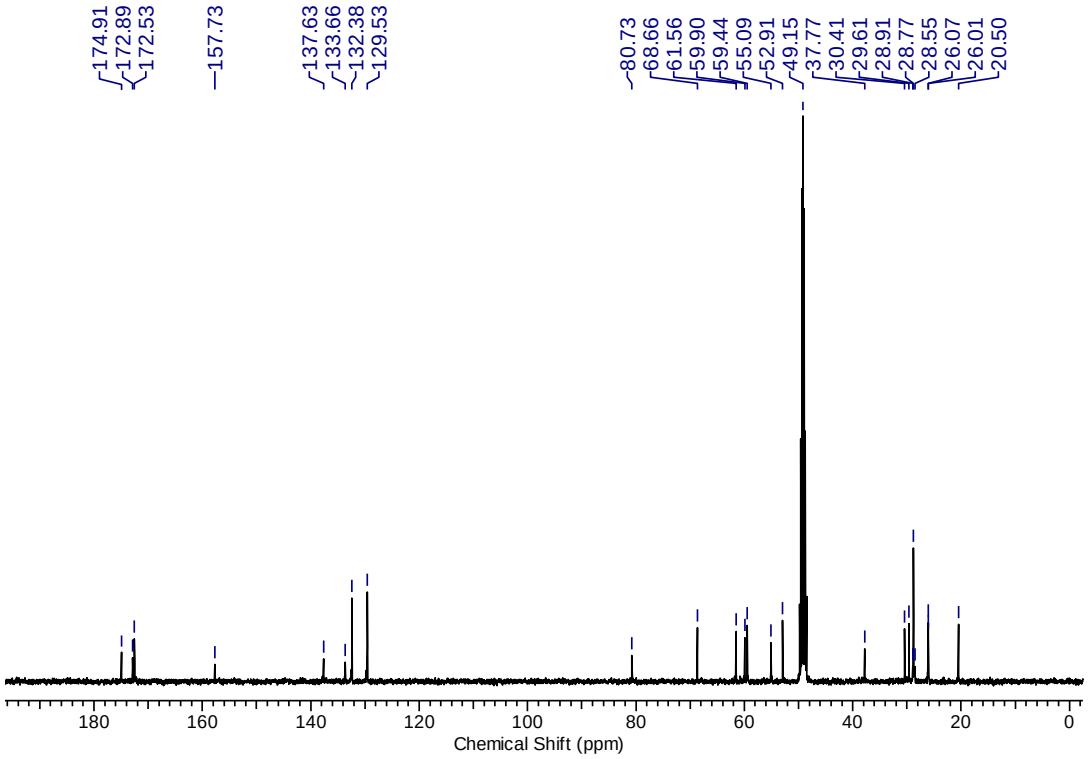
Compound 26 (Boc-PCPA-Pro-Pro-Thr-OMe): Boc-protected tripeptide ester **3** (0.3 g, 0.70 mmol) was dissolved in 1 mL of dichloromethane followed by addition of 1 mL of TFA (1M) at 0°C. The reaction was stirred for 30 min at room temperature. After completion of reaction, the solvent was stripped off and co-evaporated using toluene to obtain the TFA salt which was used for the next step without further purification. Boc-fenclonine (0.15 g, 0.76 mmol, equiv) was coupled with tripeptide ester TFA salt (0.25 g, 0.76 mmol, 1 equiv) using HATU (0.4 g, 1.14 mmol, 1.5 equiv) and DIPEA (0.4 mL, 2.2 mmol, 4 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in 2 % MeOH/DCM and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 2% MeOH/DCM, R_f : 0.5) to furnish compound **26** (0.40 g, 87 %) as a hygroscopic white solid; $[\alpha]^{26}_D$: -89.8° (c = 0.1, MeOH); ^{1}H NMR (400 MHz ,CD_3OD) δ = 7.58 - 6.95 (m, 4 H), 4.73 (d, *J* = 4.9 Hz, 1 H), 4.60 (d, *J* = 8.5 Hz, 1 H), 4.56 - 4.49 (m, 1 H), 4.44 (br. s., 1 H), 4.29 (d, *J* = 6.1 Hz, 1 H), 4.14 - 4.02 (m, 1 H) 3.89 - 3.78 (m, 2 H), 3.77 - 3.70 (m, 3 H), 3.67 (d, *J* = 9.8 Hz, 2 H), 3.35 (s, 1 H), 3.09 (dd, *J* = 3.4, 13.7 Hz, 1 H), 2.75 (dd, *J* = 9.8, 13.4 Hz, 1 H), 2.35 - 2.17 (m, 2 H), 2.16 - 1.95 (m, 6 H), 1.42 - 1.27 (m, 9 H), 1.20 (d, *J* = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz ,CD_3OD) = 173.4, 171.3, 171.0, 156.2, 136.1, 132.1, 130.8, 128.0, 79.2, 67.1, 60.0, 58.4, 57.9, 53.5, 51.4, 36.2, 28.9, 28.1, 27.3, 24.5, 19.0; HRMS: $C_{29}H_{41}ClN_4O_8$, Calculated: 631.2496 $[M+Na]^+$, observed: 631.2505 $[M+Na]^+$.



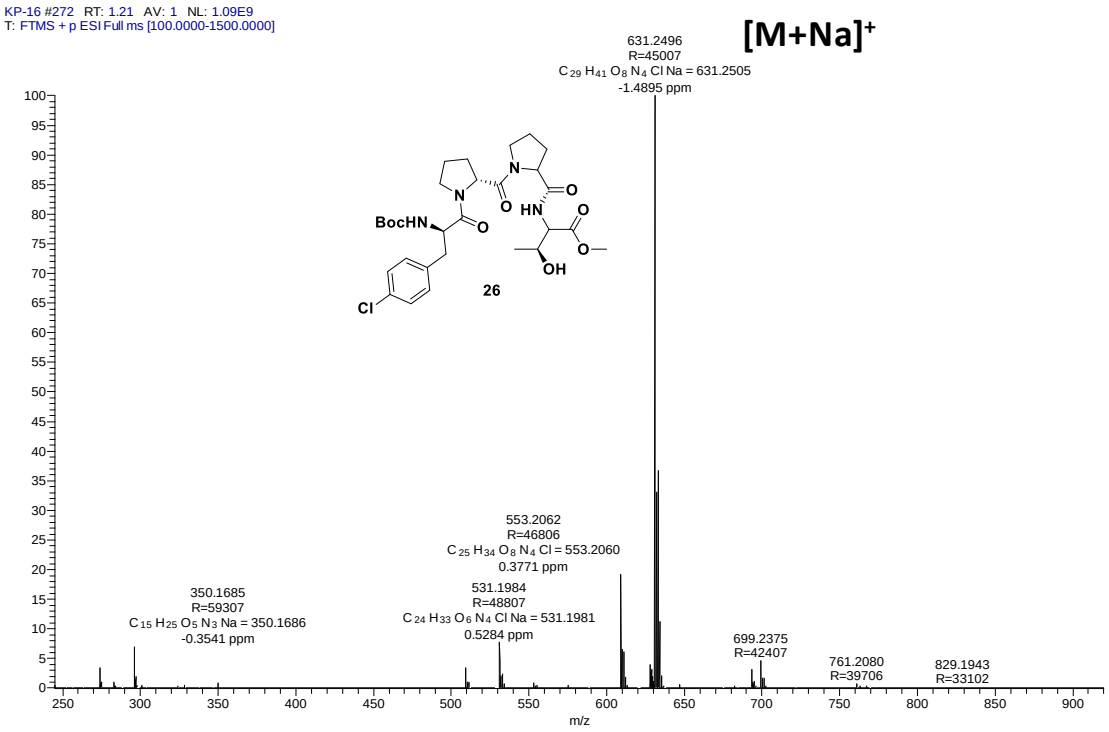
26



^{1}H NMR spectrum of compound **26** (CD_3OD, 400 MHz, 298 K)

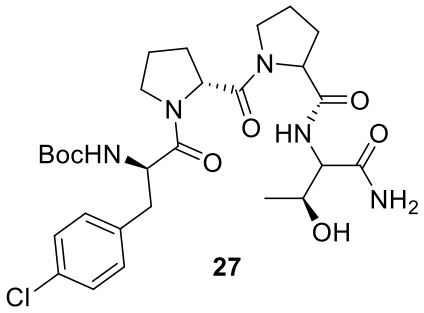


^{13}C NMR spectrum of compound **26** (CD_3OD, 400 MHz, 298 K)



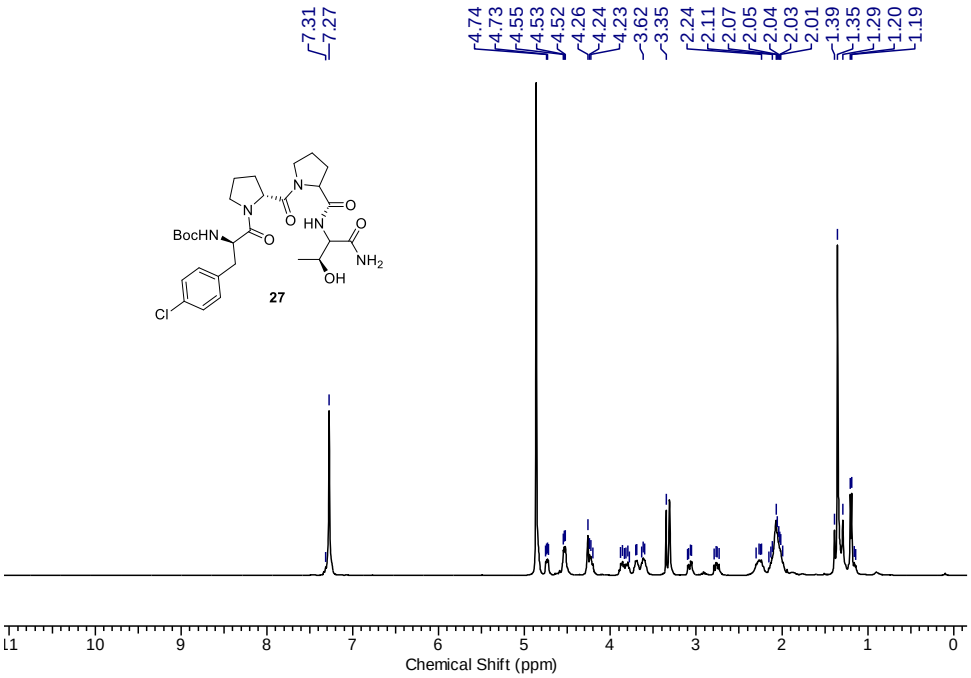
HRMS of Compound **26**

Compound 27 (Boc-PCPA-Pro-Pro-Thr-NH_2): To a solution of tetrapeptide ester **4**

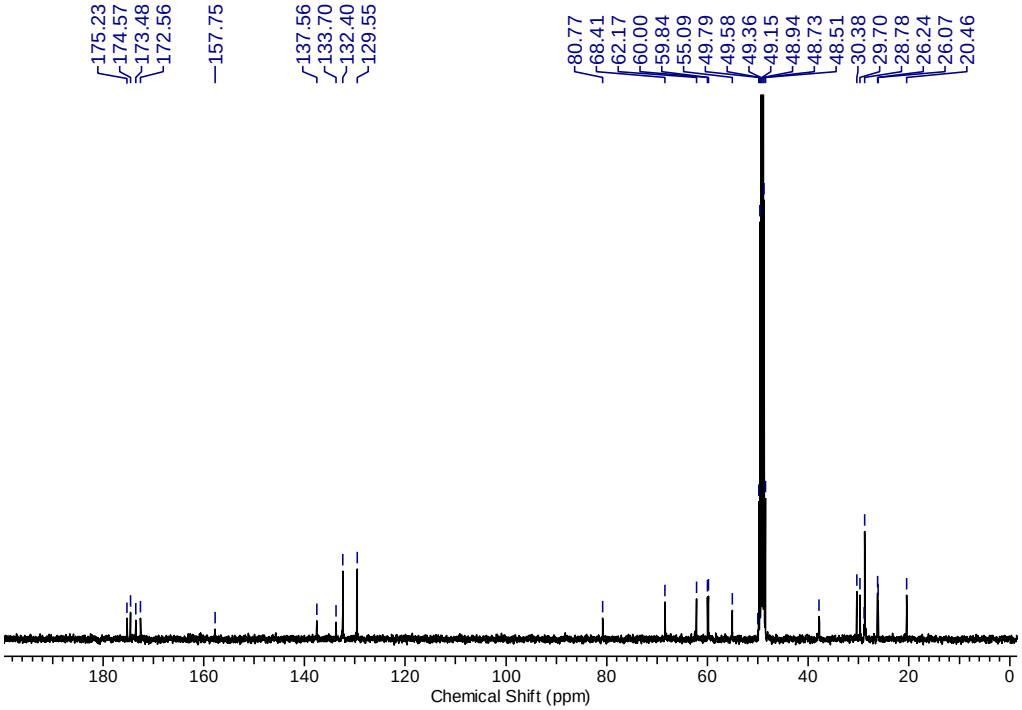


(0.20 g, 0.37 mmol) in methanol, saturated methanolic ammonia solution (3 ml) was added and the reaction mixture was stirred for 24 h at room temperature. Later, the volatiles were stripped off under reduced pressure and the product was further purified by column chromatography and preparative thin layer chromatography (eluent: 4% MeOH/ DCM, R_f : 0.5) to furnish compound **5** (0.17 g, 87%) as a white solid; $[\alpha]^{26}_D$: (c = 0.1, MeOH); IR (Nujol) ν (cm^{-1}): ^{1}H NMR (400 MHz, CD_3OD) δ = 7.36 - 7.19 (m, 4 H), 4.83 (m, 1 H), 4.74 (dd, J = 4.0, 8.2 Hz, 1 H), 4.63 - 4.43 (m, 2 H), 4.33 - 4.14 (m, 2 H), 3.92 - 3.75 (m, 2 H), 3.74 - 3.56 (m, 2 H), 3.35 (s, 1 H), 3.08 (dd, J = 4.3, 14.0 Hz, 1 H), 2.76 (dd, J = 9.2, 14.0 Hz, 1 H), 2.36 - 2.18 (m, 2 H), 2.16 - 1.90 (m, 6 H), 1.41 - 1.27 (m, 9 H), 1.24 - 1.13 (m, 3 H) ^{13}C NMR (100 MHz ,CD_3OD) δ = 175.2, 174.6, 173.5, 172.6, 157.7, 137.6, 133.8, 132.4, 129.6, 80.8, 68.4,

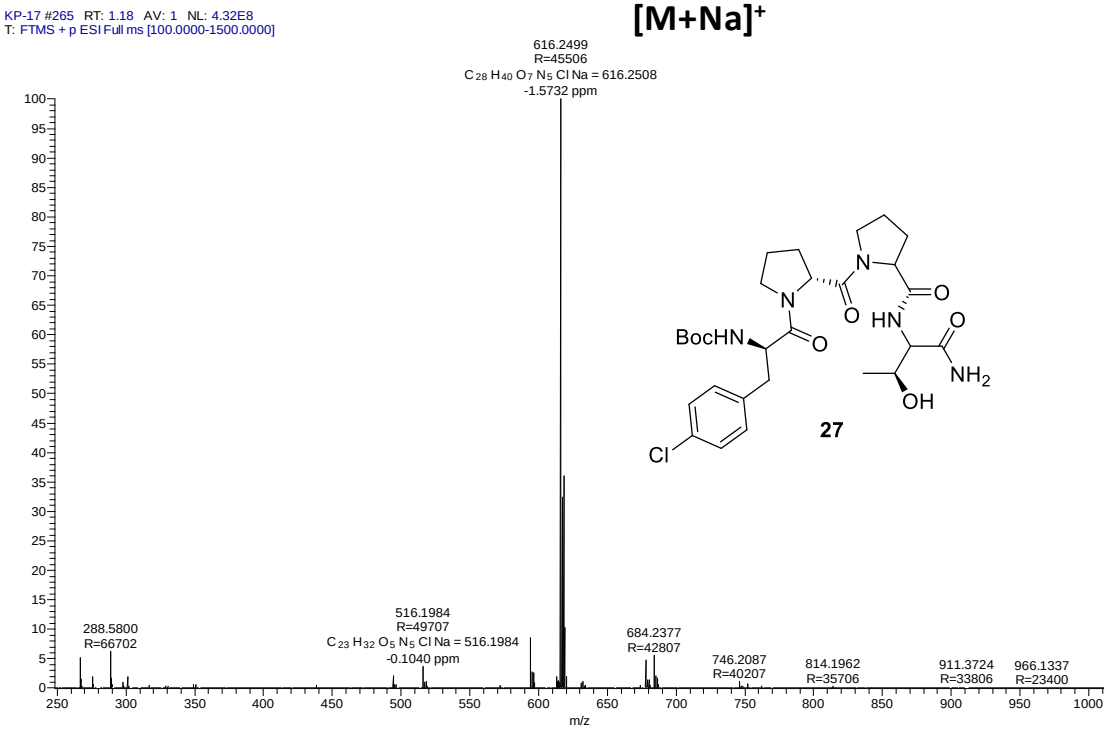
62.2, 60.0, 59.8, 55.1, 37.8, 30.4, 29.7, 28.9, 28.8, 26.2, 26.1, 20.5; HRMS: $C_{28}H_{41}N_5O_8$, calculated: 616.2508 $[M+Na]^+$, observed: 616.2499 $[M+Na]^+$.



^{1}H NMR spectrum of compound **27** (CD_3OD, 400 MHz, 298 K)

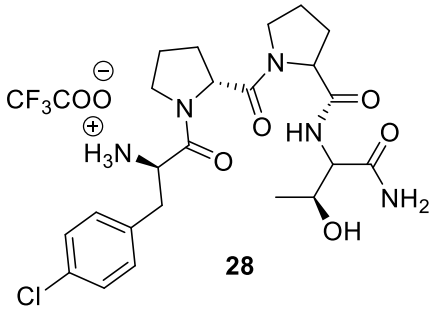


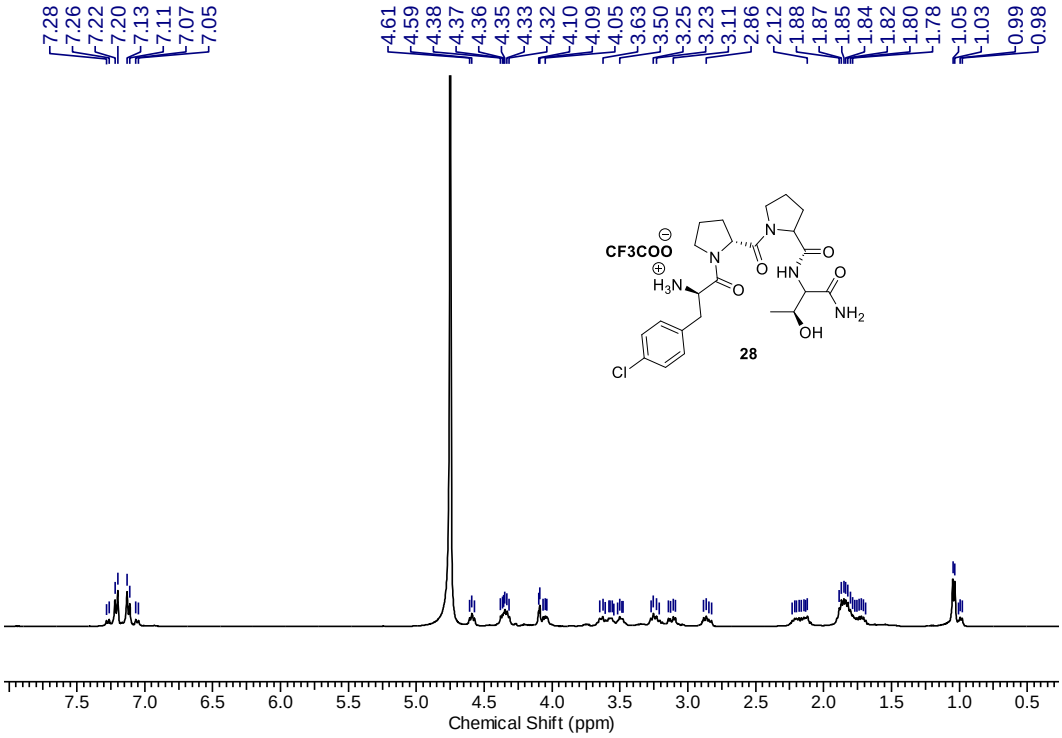
^{13}C NMR spectrum of compound **27** (CD_3OD, 400 MHz, 298 K)



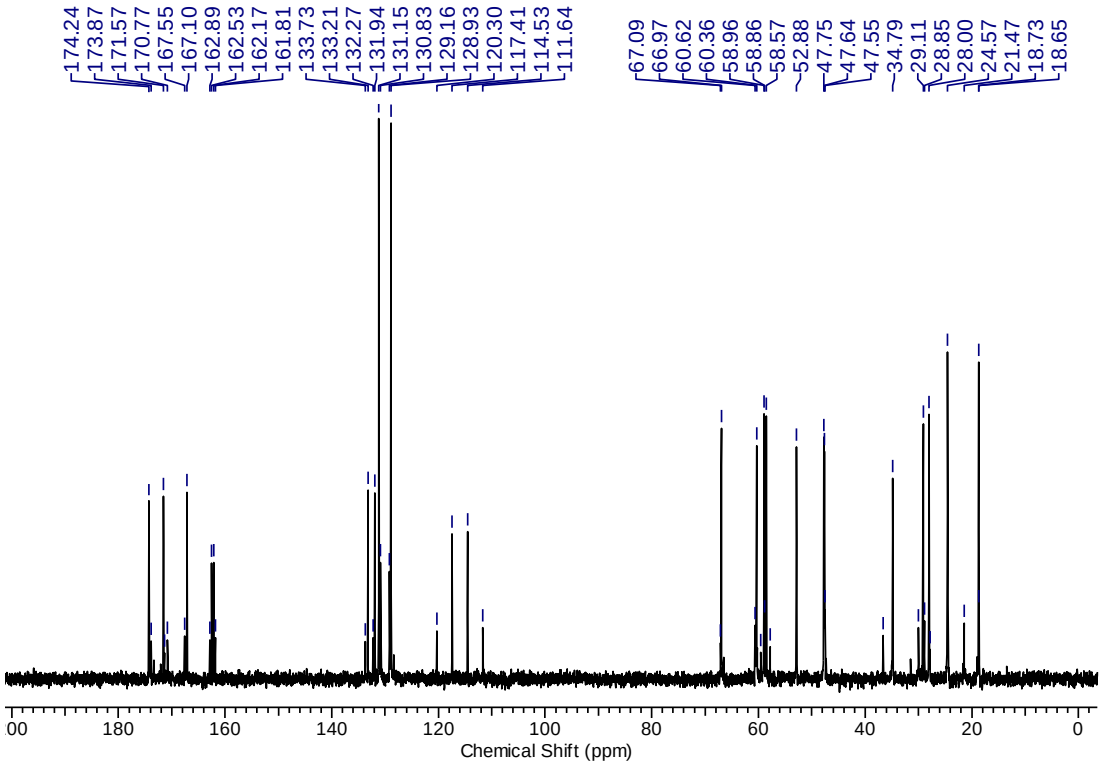
HRMS of Compound **27**

Compound 28 (TFA.H.PCPA-Pro-Pro-Thr-NH_2): To the solution of compound **24** (0.1 g, 0.17 mmol) in DCM, TFA (1M) was added and stirred for 30 min. After the completion of the reaction, the volatiles were stripped off and co evaporated with toluene to obtain compound **25** as a hygroscopic white solid (0.08 g, 78 %). $[\alpha]^{26}_D$: -75.7°(c = 0.1, MeOH); ^{1}H NMR (400 MHz, D_2O) δ =7.29 - 7.18 (m, 2 H), 7.16 - 7.02 (m, 2 H), 4.59 (t, J = 7.0 Hz, 1 H), 4.43 - 4.29 (m, 2 H), 4.16 - 3.99 (m, 2 H), 3.68 - 3.44 (m, 3 H), 3.32 - 3.21 (m, 1 H), 3.12 (dd, J = 5.5, 14.6 Hz, 1 H), 2.91 - 2.80 (m, 1 H), 2.26 - 2.07 (m, 2 H), 1.93 - 1.63 (m, 6 H), 1.08 - 0.96 (m, 3 H) ^{13}C NMR (100 MHz, D_2O) δ = 174.3, 174.2, 173.9, 171.6, 170.8, 167.6, 167.1, 162.9, 162.5, 162.2, 161.8, 133.7, 133.2, 132.3, 131.9, 131.1, 130.8, 129.2, 128.9, 120.3, 117.4, 114.5, 111.6, 67.1, 60.6, 60.4, 58.9, 58.6, 52.9, 47.6, 36.6, 34.8, 30.0, 29.1, 28.9, 28.0, 24.6, 24.5, 21.5, 18.7; HRMS: $C_{23}H_{32}N_5O_5ClNa$, calculated: 516.1984 $[M+Na]^+$, observed: 516.1982 $[M+Na]^+$.

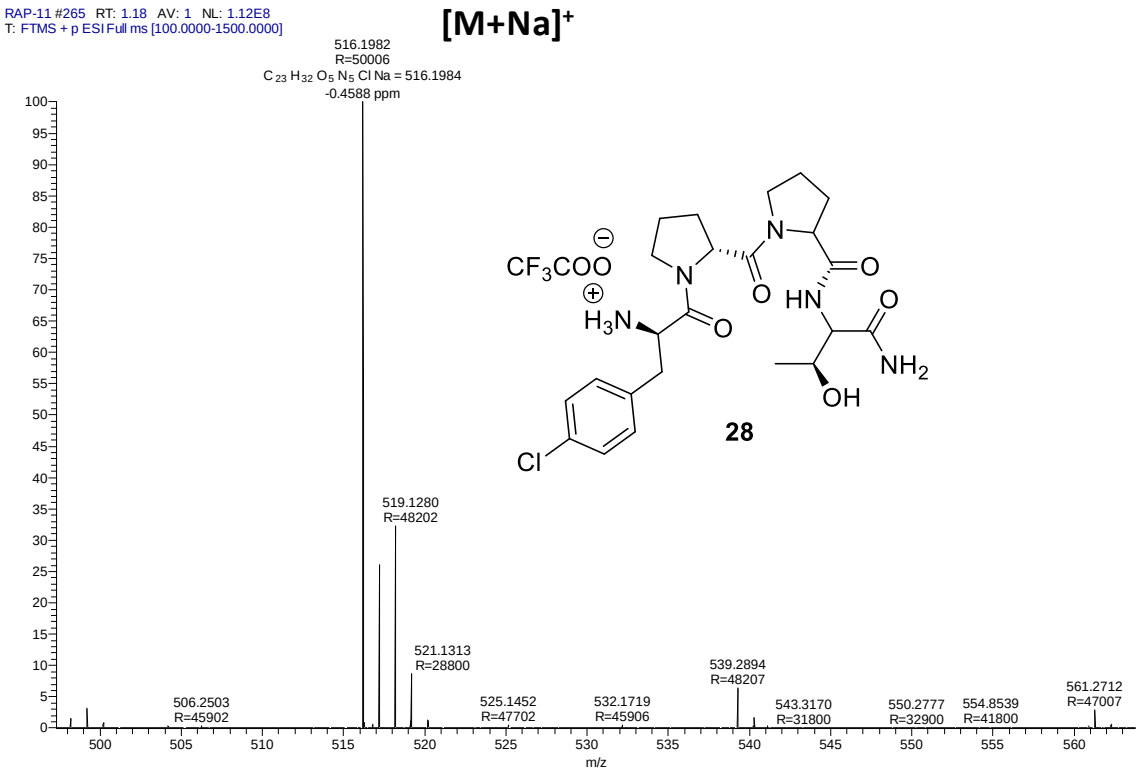




^{1}H NMR spectrum of compound **28** (D_2O, 400 MHz, 298 K)



^{13}C NMR spectrum of compound **28** (D_2O, 400 MHz, 298 K)



HRMS of Compound **28**

2.7 References and notes

1. Furukawa, H.; Singh, S. K.; Mancusso, R.; & Gouaux, E. *Nature* **2005**, *438*, 185.
2. (a) Paoletti, P.; Bellone, C.; Zhou, Q. *Nat. Rev. Neurosci.* **2013,** *14*, 383; (b) Nicholson, K. L.; Balster, R. L. *Psychopharmacology* **2009,** *203*, 441; (c) Malenka, R. C.; Bear, M. F. *Neuron* **2004,** *44*, 5; (d) Berman, R. M.; Cappiello, A.; Anand, A.; Oren, D. A.; Heninger, G. R.; Charney, D. S.; Krystal, J. H. *Biol. Psychiatry* **2000,** *47*, 351; (e) Li, N.; Lee, B.; Liu, R. J.; Banasr, M.; Dwyer, J. M.; Iwata, M.; Li, X.Y.; Aghajanian, G.; Duman, R. S. *Science* **2010**, *329*, 959. (f) Madica, K.; Nadimpally, K. C; and Sanjayan, G. J; *Tetrahedron Letters* **2017,** 58, 1568.
3. Li, Fei; and Joe, Z. Tsien. *The New England journal of medicine*, **2009**, 361, 302.

4. (a) Johnson, J. W., and P. Ascher. *Nature* **1987**, *325*, 529; (b) Kleckner, N. W., Dingledine, R. *Science* **1988**, *241*, 835; (c) Dingledine, Raymond, Nancy; Kleckner, W.; McBain, J, C. *Springer US*, **1990**, 17-26.

5. (a) Dingledine and Raymond, *et al*. *Pharmacological reviews* **1999**, *51*, 7-62; (b) Liu, Yun, Juntian, Z. *Chinese medical journal* **2000**, *113*, 948-956. (c) Cull, C., Stuart, S. B., and Mark, F. *Current opinion in neurobiology* **2001**, *11*, 327-335.

6. (a) Ascher, P. *Springer US*, **1990**, 13-16; (b) Hood, W. F., Robert P. C., and Joseph B. M. *Neurosci Lett.***1989**, *98*, 91-95; (c) Henderson, G., J. W. J., and P. Ascher. *J Physiol.***1990**, *430*, 189-212; (d) Baran, Halina, Wolfgang, L., and Meike, M. *Brain Res.* **1994**, *652*, 195-200; (e) Rundfeldt, C.; Wlaz, P. ; Lo, W. *Brain Res.* **1994**, *653*, 125-130; (f) Seguin, Laure, and Mark, J. M. *Eur. J. Pharmacol*. **1994**, *253*, R1-R3. (g) Tricklebank, M. D.; Bristow, L. J.; Hutson, P. H.; Leeson, P. D, Rowley, M., Saywell, K; Singh, L.; Tattersall, F. D; Thorn, L.; Williams, B. J. *Brit J Pharmacol*. **1994**, *113*, 729-36. (h) Pussinen, R.; Nieminen, S.; Koivisto, E.; Haapalinna, A.; Riekkinen, P.; Sirviö, J. *Neurobiol Learn Mem.* **1997**, *67*, 69-74. (i) Priestley, T.; Marshall, G. R; Hill, R. G.; Kemp, J. A. *Brit J Pharmacol*.**1998**, *124*, 1767-73.

7. (a) Loftis J. M., Janowsky, A. *et al*; *Pharmacol Ther*. **2003**, *97*, 55–85; (b) Kristiansen, L. V.; Huerta, I.; Beneyto, M.; Meador, W.; James, H. *Current Opinion in Pharmacology*. **2007**, *7*, 48–55;

8. Khan, A.M.; Wood, P.; Moskal, J. R. U.S. Patent 0 306 586. 2011.

9. (a) Chen, H. V.; Lipton, S. A. *J. Neurochem*, **2006**, *97*, 1611–1626; (b) Kemp J. A., McKernan R. M. *Nat. Neurosci*. **2002**, *5*, 1039–1042; (c) Lipton S.A. *Nat. Rev. Drug Discov*. **2006**, *5*, 160–170; (d) Koch, H.; Szecsey, A.; Haen, E. *Curr. Pharm. Des*. **2004,** *10*, 253–259.

10. http://naurex.com/wpcontent/uploads/2014/12/Naurex_P2b_Data_Press_Release_FINAL_Approved.pdf

11. (a) Hashimoto, K.; Malchow, B.; Falkai, P.; Schmitt , A. *Eur Arch Psychiatry Clin Neurosci.* **2013**, *263*, 367–77; (b) Moskal, J. R; Burgdorf J. S., Stanton, P. K; Kroes, R.A; Disterhoft, J. F.; Burch, R. M.; Khan, M. A. *Curr Neuropharmacol.* **2017**, *15*, 47-56.

12. Hayley, S; Litteljohn, D; *Front Cell Neurosci.* **2013**, *7*, 218; (b) PR Newswire (2010). "Naurex's Novel Antidepressant GLYX-13 Recognized as One of Windhover's Top 10 Neuroscience Projects to Watch".; (c) Dang, Y. H, Ma, X. C, Zhang, J. C. *et al. Curr. Pharm. Des*. **2014,** *20*, 5151–9.

13. Rakesh, G; Pae, C. U; Masand P, S. *Expert Rev Neurother.* **2017**, *17*, 777-790.

14. Vécsei, L; Szalárdy, L; Fülöp, F; Toldi, J. *Nat Rev Drug Discov.* **2013**, *12*, 64-82.

15. Tyler, M. W; Yourish, H. B; Ionescu, D. F; Haggarty, S. J. *ACS Chem Neurosci.* **2017**, *8*, 1122-1134.

16. (a) Millership, J. S.; Shanks, M. L. *Int. J. Pharm.* **1986**, *28*, 1−9; (b) Fujii, S. *MedChemComm* **2016**, *7*, 1082−1092.

17. (a) Stevenazzi, A.; Marchini, M.; Sandrone, G.; Vergani, B.; Lattanzio, M. *Bioorg.Med. Chem. Lett.* **2014**, *24*, 5349−5356; (b) Blaskovich, M. A.T. *J. Med. Chem*. **2016**, *59*, 10807−10836.

18. Remond, E.; Martin, C.; Martinez, J.; Cavelier, F. *Chem. Rev.* **2016**, *116*, 11654−11684.

19. Cavelier, F; Vivet, B.; Martinez, J. *et al. J Am Chem Soc*, **2002**, *124*, 2917–2923.

20. (a) Nagaraj, R.; Balaram, P. *Acc. Chem. Res.* **1981**, *14*, 356; (b) Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747; (c) Toniolo, C.; Benedetti, E. *Macromolecules* **1991**, *24*, 4004.

21. (a) Yamaguchi, H.; Kodama, H.; Osada, S.; Kato, F.; Jelokhani N. M.; Kondo, M. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 2269; (b) Zikou, S., Koukkou, A. I.; Mastora, P.; Sakarellos, M.; Sakarellos, C.; Drainas, C.; Panou, E. *J. Pept. Sci.* **2007**, *13*, 481; (c) Taylor, J. W.; Kaiser, T. *Methods Enzym.* **1987**, *154*, 473; (d) Voges, K. P.; Jung, G.; Sawyer, W. H. *Biochim. Biophys. Acta* **1987**, *826*, 64.

22. S. Wada *et al*. *Bioorg. Med. Chem. Lett.* **2011,** *21*, 5688–5691.

23. (a) Jouvet M *et al. Neuropsychopharmacology.* **1999,** *2*, 24S-27S; (b) Kvols, L. K. *et al. J Med.* **1986**, *81*, 49-55.

24. Gauchot, V.; Andreea R. S.; *J. Org. Chem.* **2014**, *79*, 2694-2701.

25. Moir, E. M., Yoshiizumi, K., Cairns, J., Cowley, P., Ferguson, M., Jeremiah, F., York, M. *Bioorganic Med. Chem. Lett*. **2010**, *20*, 7327-7330.

26. Kawai, M., Butsugan, Y., Fukuyama, K., & Taga, T. *Biopolymers*, **1987**, *26*, 83-94.

27. Jacobsen, O., Maekawa, H., Ge, N. H., Görbitz, C. H., Rongved, P., Ottersen, O. P., Klaveness, J *J. Org. Chem.* **2011**, *76*, 1228-1238.

28. https://en.wikipedia.org/wiki/Ibotenic_acid#/media/File:Activated_NMDAR.svg

29. (a)https://en.wikipedia.org /wiki/NMDA_receptor# /media/File: NR1 NR2B_subunit.jpg(b)https://en.wikipedia.org/wiki/NMDA_receptor#/media/File: NR1-NR2B_subunit.png.

30. http://profrontal.com/nmda/ The NMDA (N-Methyl D-Aspartate) Receptor

31. https://bionap.whotrades.com/blog/43474819255?iid=454951

Chapter 3

Design and Synthesis of Novel 10,11-Dihydro-5H-dibenz[b,f]azepine and Norquetiapine Derived Hybrid Drug Analogues as Potential CNS Active Agents.

3.1 Introduction

3.1.1 Tricyclic antidepressants

The tricyclic antidepressants (TCAs) were developed in the course of the growth of the field of psychopharmacology in 1950. Paul Charpentier synthesized chlorpromazine from synthetic antihistamines which was developed by Rhône-Poulenc in the early 1940's. Its psychiatric effects were first observed in 1952. In 1955, chlorpromazine was already producing noteworthy revenue as an antipsychotic.[2]Later, scientists began to explore different analogues of chlorpromazine to improve its efficiency.

Imipramine, the close analogue of chlorpromazine is also a dibenzazepine analogue but it is used for the treatment of depression and it was code named as G22355. This drug was inclined to induce some side effects as it was not originally treated for depression. This led to devastating effects in some patients. Thus, inconsistent observation of a sedative inducing mania directed towards the testing of this drug with depressed patients. In 1955, imipramine's first trial had taken place and its antidepressant effects were first reported by Roland Kuhn, a well known Swiss psychiatrist.[1]

Chemical structure of tricyclic compounds contains three rings of atoms whereas tetracyclic antidepressants (TeCAs) are a closely related group containing four rings of atoms. Most of the TCAs are sometimes prescribed for depressive disorders but they have been largely replaced in clinical use by newer antidepressants such as SSRIs (selective serotonin reuptake inhibitors), SNRIs (serotonin–norepinephrine reuptake inhibitors) and NRIs (norepinephrine reuptake inhibitors.[3]

The TCAs are used mainly in the treatment of mood disorders such as dysthymia and major depressive disorder (MDD).[4] They are also used in the treatment of a various anxiety disorders such as generalized anxiety disorder (GAD), social anxiety disorder (SAD), obsessive-compulsive disorder (OCD), body dysmorphic disorder (BDD), post-traumatic stress disorder (PTSD) and some eating disorders like bulimia nervosa and anorexia nervosa.

However, tricyclic antidepressants are perhaps more efficient in treating melancholic depression than other antidepressant drug classes.[5]There is considerably less side effects in newer antidepressants and they are reflected to be less liable to result in major injury or even death if used in a suicide attempt, as the doses required for clinical treatment are far wider in comparison.

Nevertheless, the TCAs are still seldom used for treatment-resistant depression that are unsuccessful to react to therapy with newer antidepressants.[6] The side effects of the TCAs relatively come to significance before the therapeutic benefits against depression. Hence they may be dangerous for the patient who have great desire to commit suicide.[7]

There are mainly two groups of tricyclic antidepressants classified based on the chemical structure.[8] They are as follows.

3.1.1.1 Dibenzazepines

(a) Imipramine, (b) Desipramine, (c) Clomipramine, (d) Trimipramine, (e) Lofepramine

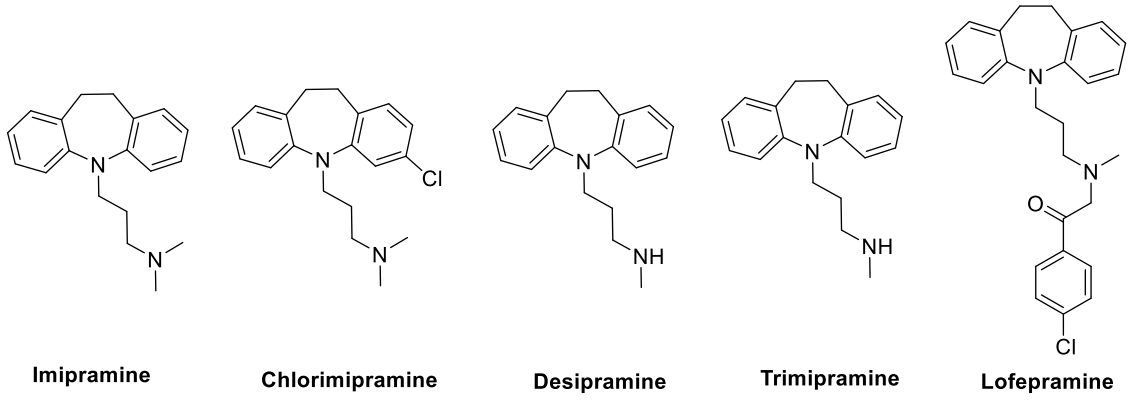


Figure 3.1: Molecular structures of dibenzazepine class of drugs.

3.1.1.2 Dibenzocycloheptadienes

(a) Amitriptyline, (b) Nortriptyline, (c) Protriptyline, (d) Butriptyline.[7]

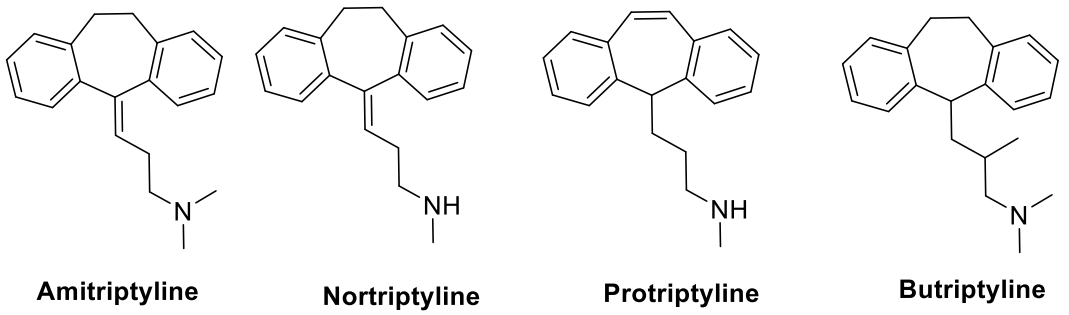


Figure 3.2: Molecular structures of dibenzocycloheptadienes class of drugs.

3.1.2 Tetracyclic antidepressants

In 1970, tetracyclic antidepressants (TeCAs) were first introduced as class of antidepressants. They are named as TeCAs due to their tetra cyclic structure, containing four rings of atoms. They are also very much related to the tricyclic antidepressants which consists of three rings of atoms.

TeCAs have miscellaneous pharmacology and differ from TCAs in many instances. Excluding amoxapine, TeCAs does not restrain from reuptaking of serotonin. However, apart from mirtazapine, they do hinder the reuptake of norepinephrine. TeCAs also block the serotonin 5-HT2 receptors in the same way as TCAs. Excluding mirtazapine, TeCAs also block the α1-adrenergic receptor. TeCAs block the histamine H1 receptor in the same way as the TCAs, but lean to be even more stronger antihistamines than TCAs. Mianserin and mirtazapine show a reduced amount of toxicity than TCAs in overdose.[9]

Figure 3.3 Molecular structures of tetra cyclic antidepressants marketed as drugs.

3.1.3.1 Quetiapine

Quetiapine is a tetracyclic compound and is closely related structurally to clozapine, olanzapine, loxapine, and other tetracyclic antipsychotics.

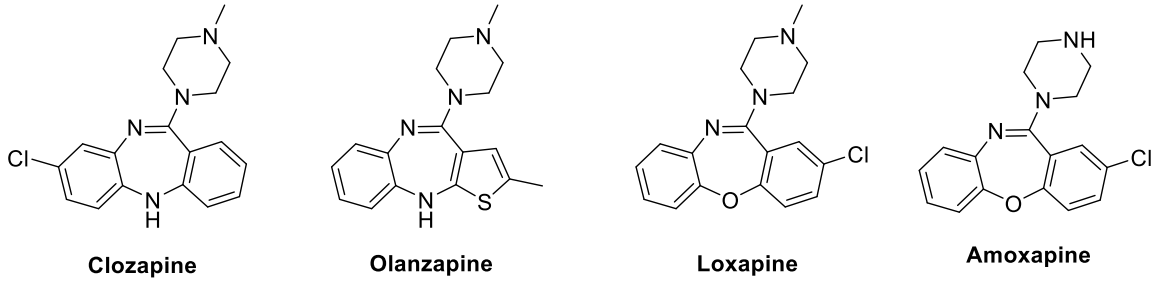


Figure 3.4 Molecular structures of tetracyclic antipsychotics.

Quetiapine which is marketed as Seroquel is a tetracyclic atypical antipsychotic utilized for the treatment of MDD, schizophrenia and bipolar disorder.[10] It contains sedating effect which is why it is sometimes used in aiding sleep.[11]It is orally taken by mouth.[10]

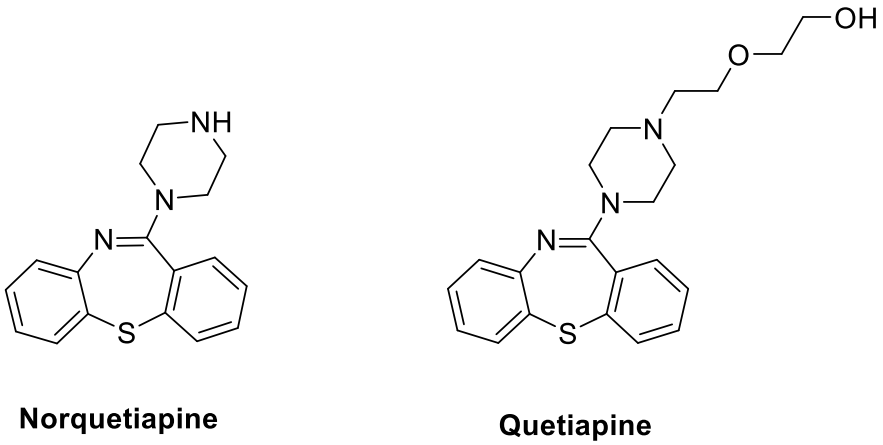


Figure 3.5 Molecular structures of norquetiapine and Quetiapine

Quetiapine drug is associated with common side effects which include weight gain, sleepiness, dry mouth, constipation.[10] Some other side effects like prolonged erection, low blood pressure with standing, high blood sugar etc. Quetiapine is supposed to work by blocking a number of receptors which include dopamine and serotonin receptors.[10]The major active metabolite of quetiapine is norquetiapine (N-desalkylquetiapine) as shown in figure 3.5.

3.2 Objective of present work

The aim of the present work is to design and synthesize molecules containing dibenzazepine and norquetiapine conjugated with drug analogues such as Gabapentin, Fenclonine and L-threo-3-phenylserine to create potential hybrid drug analogues and also to synthesize dibenzazepine and norquetiapine derived rapastinel analogues for their potenial CNS activity.

3.3 Design strategy

Dibenzazepine and norquetiapine derived compounds have been found to be promising drugs as they are related to tricyclic and tetracyclic antidepressants respectively. There has been extensive research carried out in synthesizing various analogues containing dibenzazepine and norquetiapine moiety. However, creating drug conjugates of these molecules with unnatural amino acids is not well known. We have designed these molecules to prepare hybrid drug conjugates containing dibenazepine and unnatural amino acids or norquetiapine and unnatural amino acids(Figure 3.6 and Figure 3.7) for potential CNS activity.

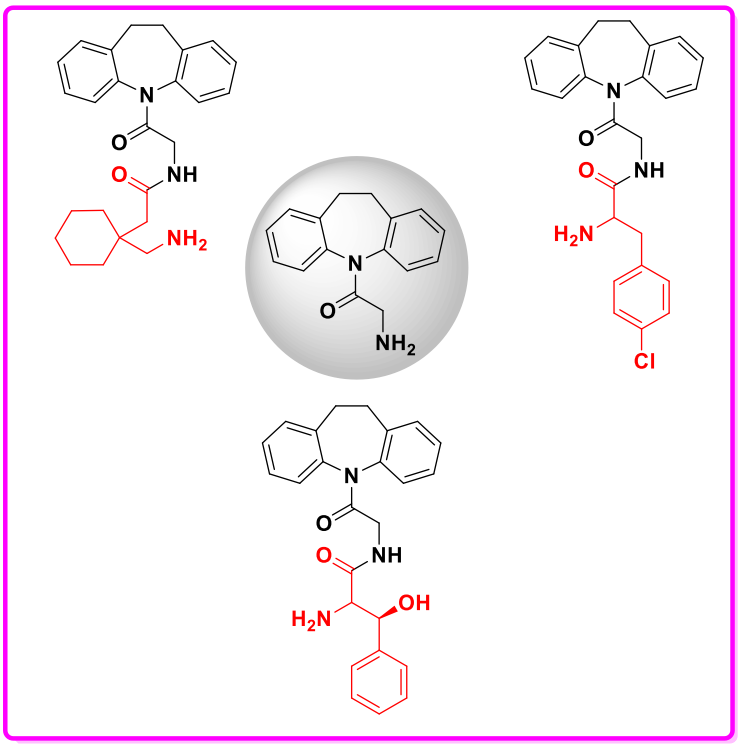


Figure 3.6 Molecular structures of the designed hybrid drug analogues containing dibenzazepine.

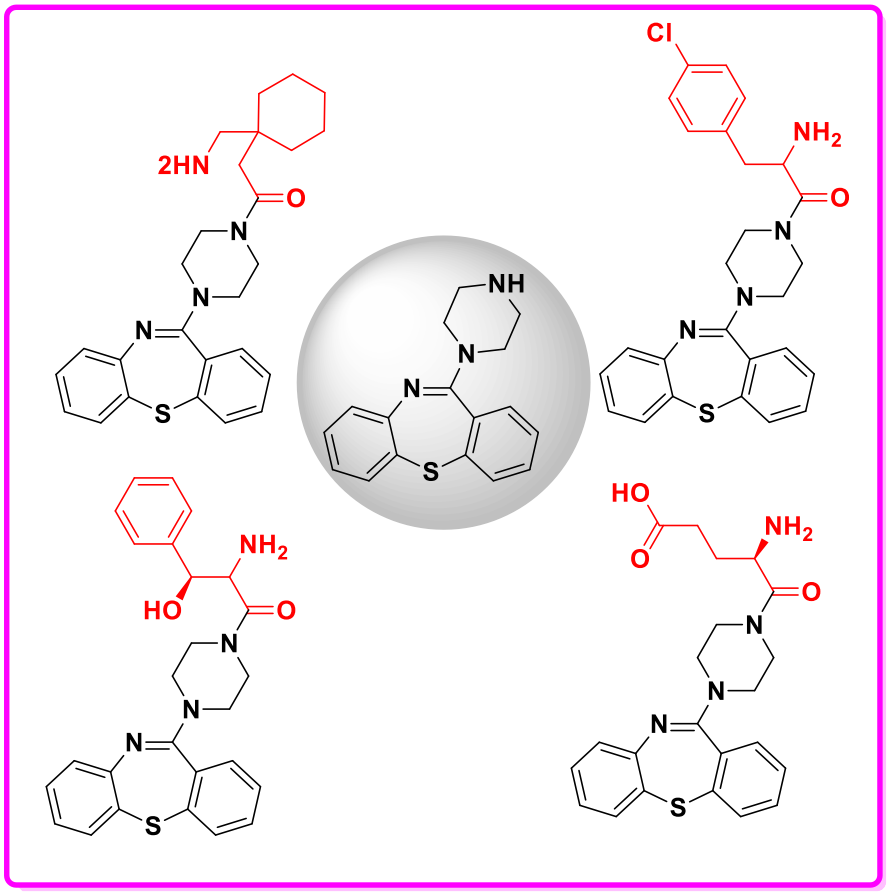


Figure 3.7 Molecular structures of the designed hybrid drug analogues containing norquetiapine.

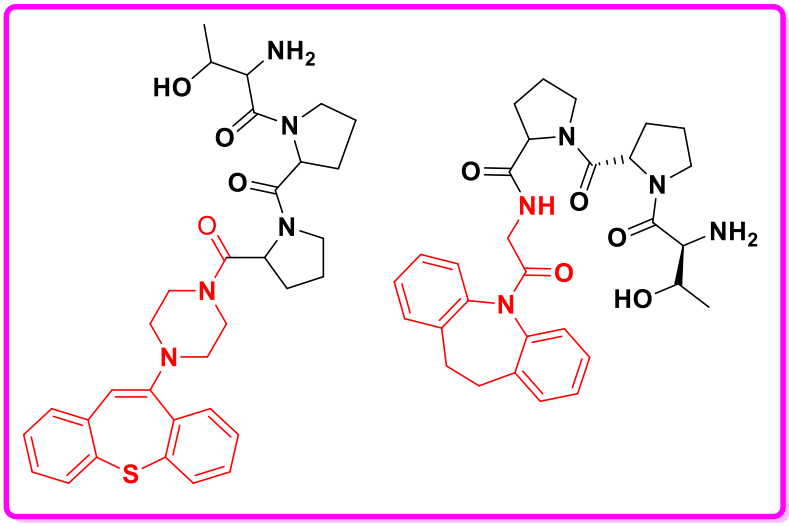
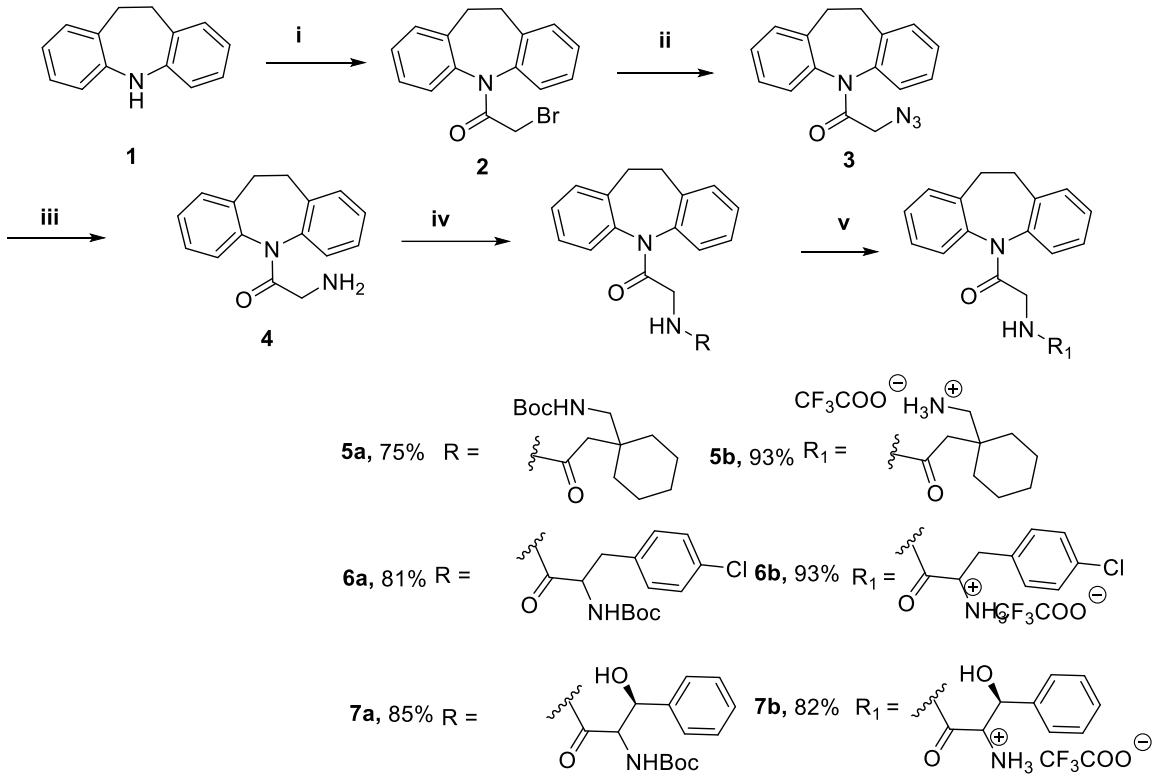


Figure 3.8 Molecular structures of De novo Designed Azepine-based and norquetiapine-based rapastinel anologues as potential CNS Active agents.

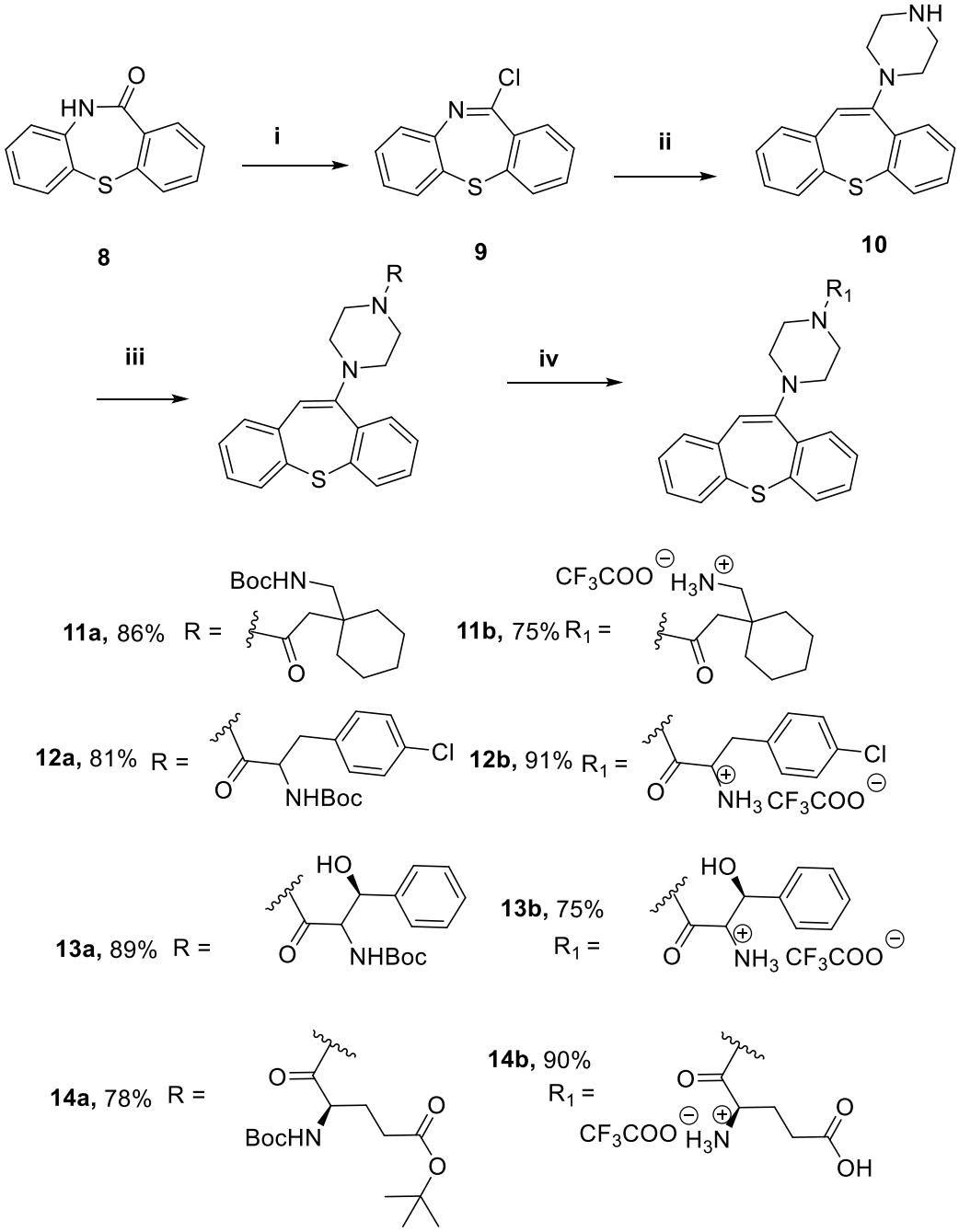
3.4 Synthesis

Synthesis of designed dibenzazepine-based hybrid conjugates was started by treating dibenzazepine with bromoacetyl bromide to obtain compound **2** which was then azidated with sodium azide to furnish azidated compound **3**. Reduction of compound **3** was carried out using hydrogen in palladium over carbon to furnish compound **4** which was used as synthetic intermediate for synthesizing hybrid conjugates as shown in scheme 3.1.

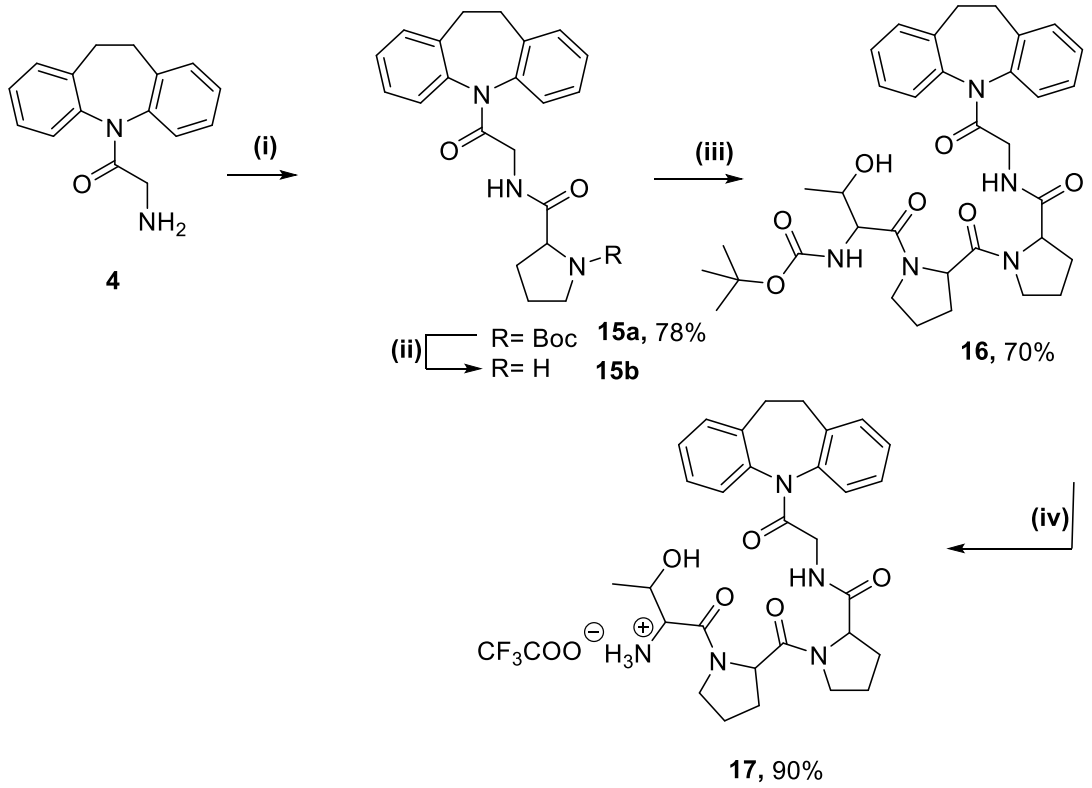


Scheme 3.1: Reagents and conditions : (i) Bromoacetylbromide, Toluene, reflux, 3 h, 80%; (ii) NaN_3, rt, 12 h, 95%; (iiiH_2-Pd/C, 2 h, 70% (iv) Boc-amino acids, HATU, DIPEA, DMF, overnight (v) TFA, DCM, 0°C-rt.

Synthesis of norquetiapine based analogues was carried out by starting with the synthesis of norquetiapine **10**. Dibenzo[b,f][1,4]thiazepin-11(10H)-one **8** was chlorinated using $POCl_3$ to obtain 11-chloro-dibenzo[b,f][1,4]thiazepine **9** which was then treated with piperidine to obtain norquetiapine **10**. This synthetic intermediate was treated with unnatural amino acids to furnish hybrid analogues as shown in scheme 3.2.



Scheme 3.2: Reagents and conditions : (i) $POCl_3$, N,N-dimethyl aniline, toluene, reflux, 3 h, 70 %; (ii) Piperidine, toluene, reflux , 5 h, 50 %; (iii H_2-Pd/C, 2 h, 70% (iv) Boc aminoacids, HATU, DIPEA, DMF, overnight (v) TFA, DCM, 0°C-rt.

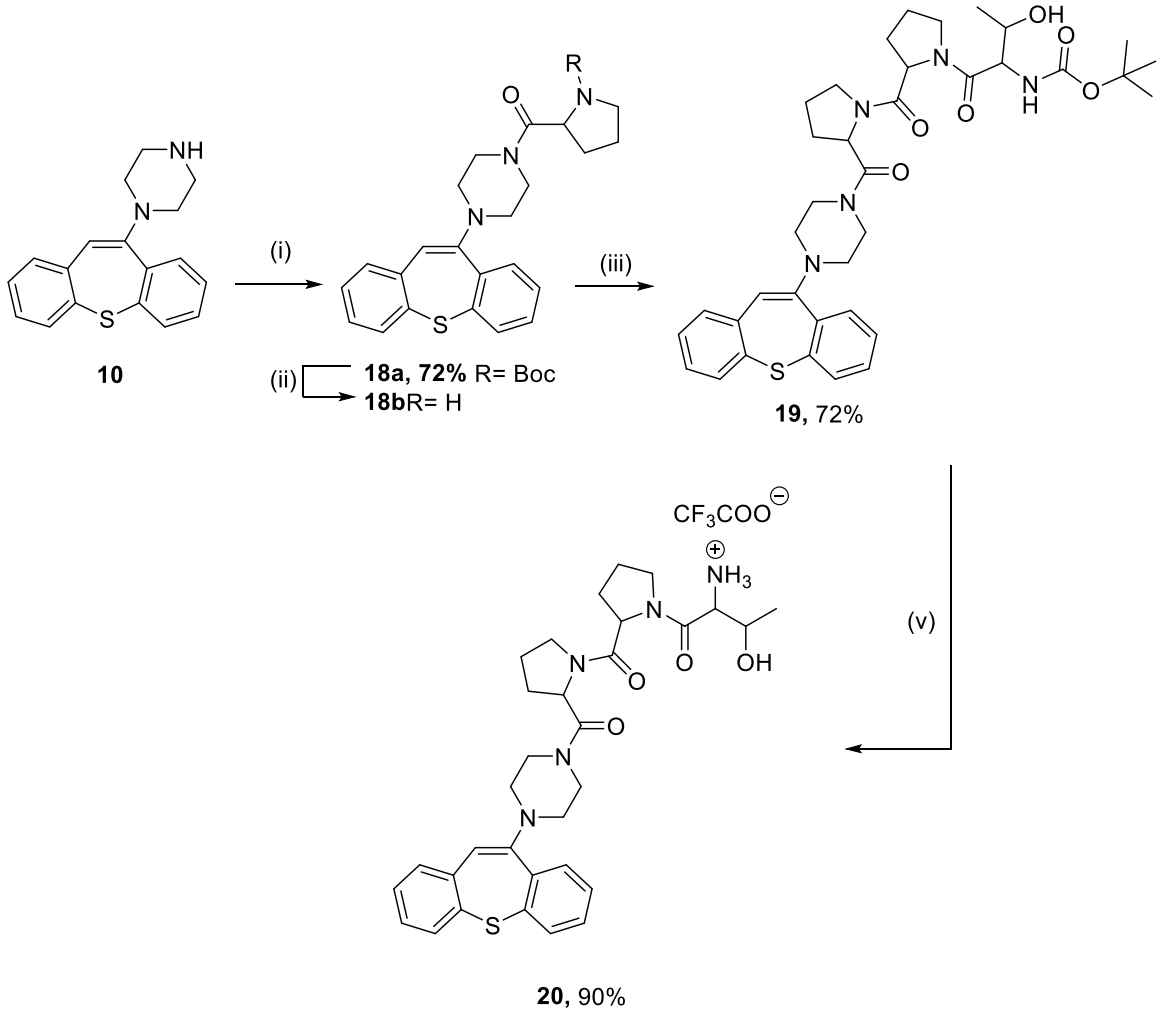


Scheme 3.3 Reagents and conditions: (i) Boc-Pro-OH, HATU, DIPEA, DMF, 12h, rt; (ii) TFA, DCM, 0°C-rt, 30 min; (iii) Boc-Thr-Pro-OH, HATU, DIPEA, DMF, 12h, rt; (iv) TFA, DCM, 0°C-rt, 30 min;

Further, dibenzazepine based rapastinel analogues were synthesized starting from synthetic intermediate **4**. Boc-Pro-OH was coupled with compound **4** using HATU and HOBt as coupling agent to obtain compound 15a which was then Boc-deprotected using TFA in DCM and then coupled with Boc-Thr-OH and Boc-Pro-OH to furnish compound **16a** and **17a** respectively in good yield. Compound **17a** was then Boc-deprotected using TFA in DCM and then coupled with Boc-Thr-OH to furnish compound **18** in good yield. Compound **18** was then Boc-deprotected using TFA in DCM to furnish compound **19** in reasonable yield as shown in scheme 3.3.

Also, norquetiapine based rapastinel analogues were synthesized starting from synthetic intermediate **10**. Boc-Pro-OH was coupled with compound **10** using HATU and HOBt as coupling agent to obtain compound 20a which was then Boc-deprotected using TFA in DCM and then coupled with Boc-Thr-OH and Boc-Pro-OH to furnish compound **21a** and **22a** respectively in good yield. Compound **22a** was then Boc-

deprotected using TFA in DCM and then coupled with Boc-Thr-OH to furnish compound **23** in good yield. Compound **23** was then Boc-deprotected using TFA in DCM to furnish compound **24** in reasonable yield as shown in scheme 3.3.



Scheme 3.4 Reagents and conditions: (i)Boc-Pro-OH, HATU, DIPEA, DMF, 12h, rt; (ii) TFA, DCM, 0 °C-rt, 30 min; (iii) Boc-Thr-Pro-OH, HATU, DIPEA, DMF, 12h, rt; (iv) TFA, DCM, 0 °C-rt, 30 min;

3.5 Result and Discussion

Herein, we describe the solution phase synthesis of a new class of hybrid drug conjugates containing dibenzazepine and norquetapine moiety as potential CNS active agents using standard peptide coupling strategy. Recently, there has been increase in the use of hybrid drug conjugates as drugs in the market. Incorporation of unnatural amino acids in the dibenzazepine and norquetapine are also not well known.

Gabapentin (neurontin) has been widely used as medication for the treatment of hot flashes, epilepsy, restless legs syndrome and also neuropathic pain. Para-chlorophenylalanine (PCPA), also widely known as fenclonine operates as a selective and irreversible inhibitor of tryptophan hydroxylase, which is a rate-limiting enzyme in the biosynthesis of serotonin. Neboglamine is functional modulator of the glycine site of the NMDA receptor to treat mainly schizophrenia and also dependence with cocaine. Rottapharm is currently undertaking investigation for the treatment of schizophrenia and cocaine dependence. L-threo-phenylserine is a constituent of peptide antibiotics and a precious chiral synthon. It has also been used for synthesizing β-mercaptophenylalanine which is used for native chemical ligation.

The above mentioned unnatural amino acid drugs i.e Gabapentin, Fenclonine and L-threo-Phenylserine were conjugated to dibenzapine and norquetapine for potential central nervous system active agents. All the synthesized analogues were well characterized by solution state nuclear magnetic resonance spectroscopy which includes ^{1}H NMR and ^{13}C NMR and also by high resolution mass spectrometry (HRMS).

We have also synthesized dibenzazepine and norquetapine derived rapastinel analogues by substituting threonine with dibenzazepine and norquetapine molecules. The synthesized molecules has potential to increase the hydrophobicity of the rapastinel for better CNS activity. These molecules were well characterized by spectroscopic tools like solution state NMR and high resolution mass spectrometry.

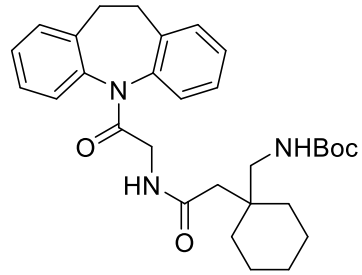
3.6 Conclusions

The novel dibenzazepine and norquetapine conjugated with various unnatural amino acids were successfully synthesized. All synthesized hybrid drug analogues were well characterized by spectroscopic tools. Solutoin phase synthesis of rapasitnel analgues containing dibenzazepine and norquetapine moiety also have synthesized and well characterized in order to increase hydrophobicity of the molecules.

3.7 Experimental section

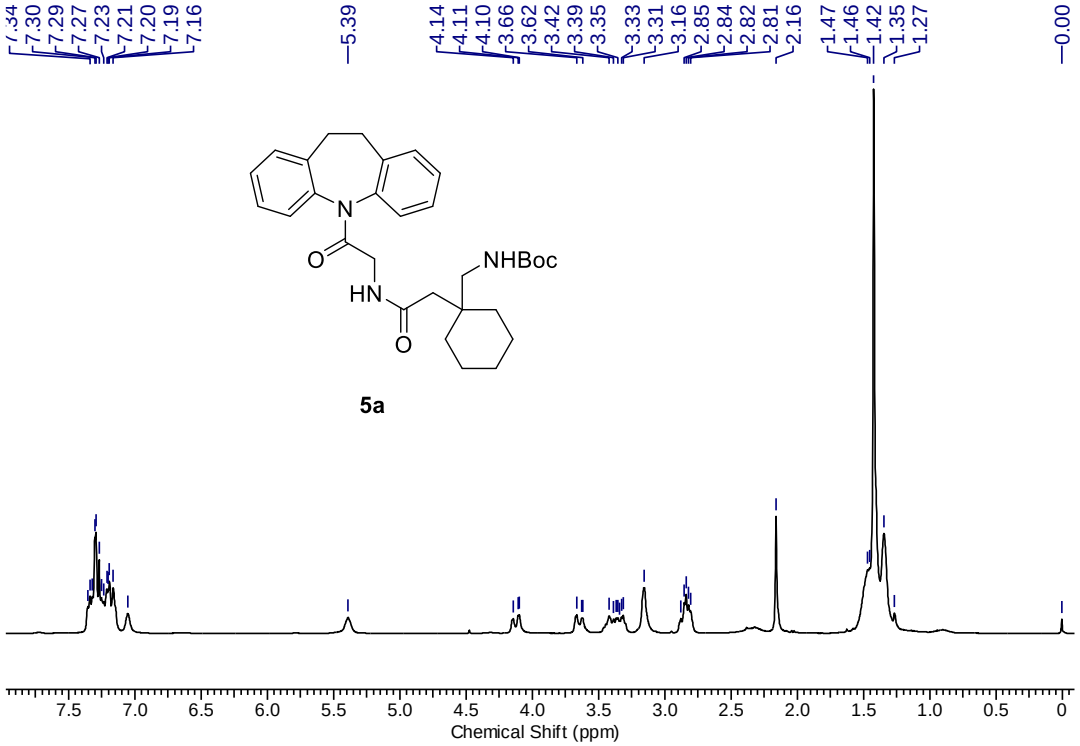
Compound **4** was synthesized as per the reported procedure.

Compound 5a (tert-butyl ((1-(2-((2-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-2-oxoethyl)amino)-2-oxoethyl)cyclohexyl)methyl)carbamate): Boc-gabapentin

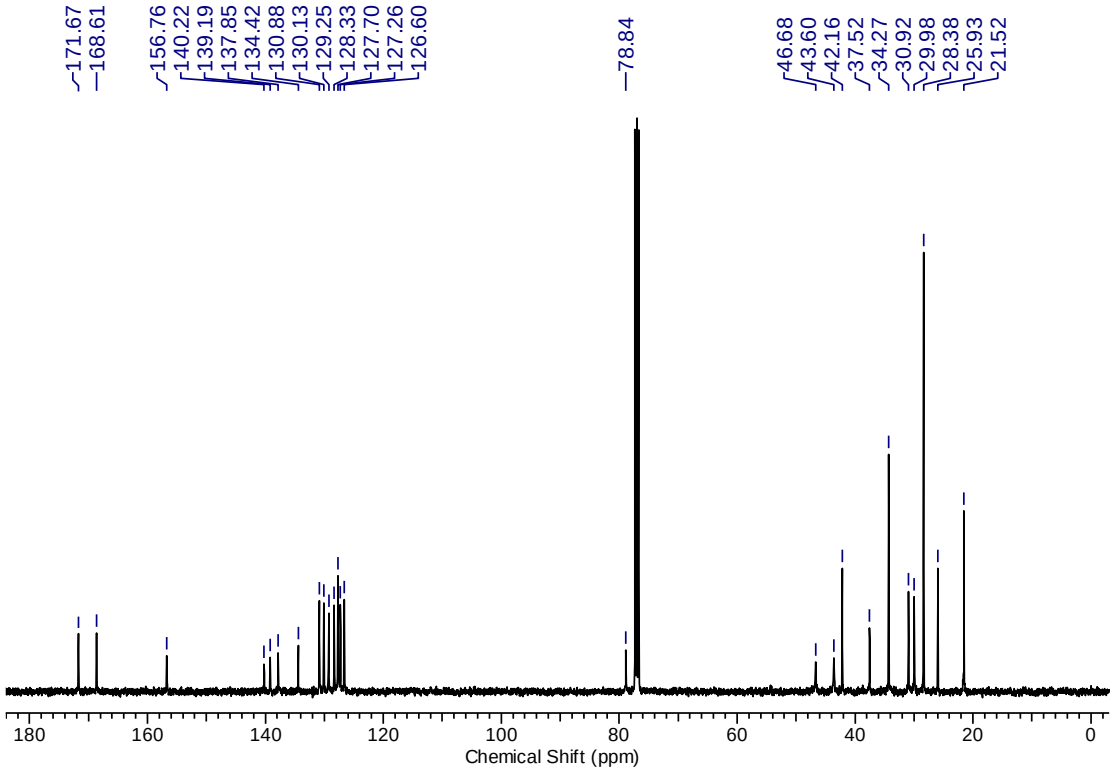


5a

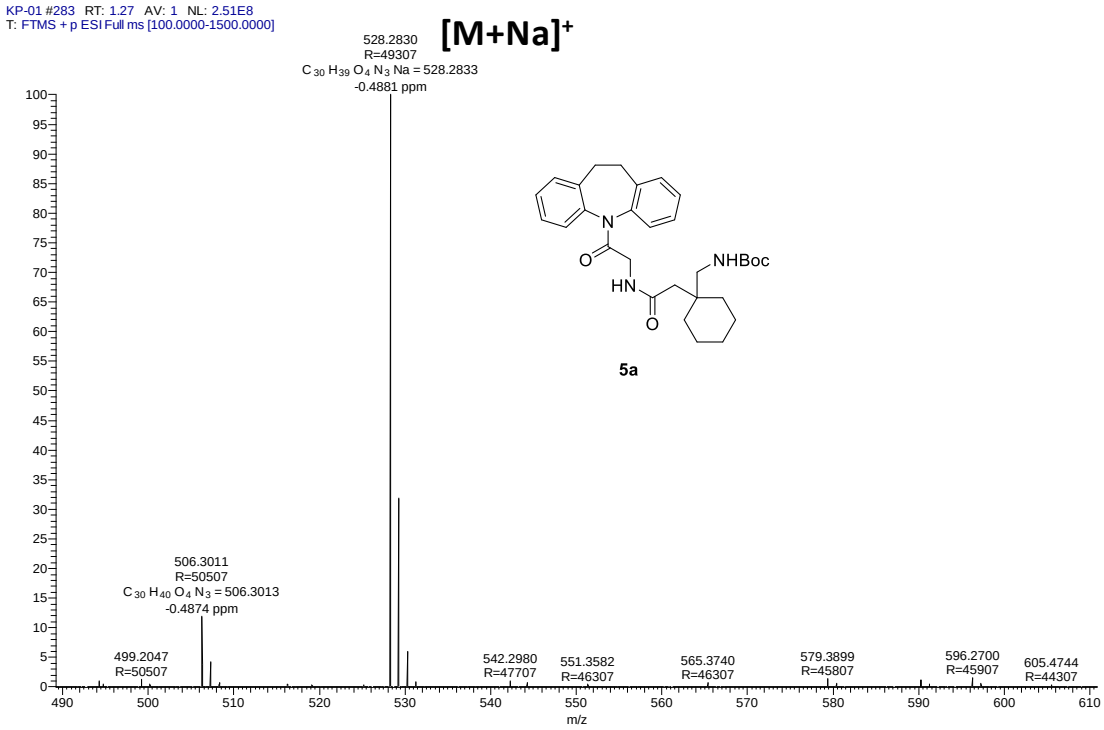
(0.26 g, 0.95 mmol, 1.2 equiv) was coupled with dibenzazepine intermediate **4** (0.2 g, 0.79 mmol, 1 equiv) using HATU (0.45 g, 1.19 mmol, 1.5 equiv) and DIPEA (0.5 mL, 3.95 mmol, 5 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in ethyl acetate and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 20% AcOEt/Petether, Rf : 0.4) to furnish compound **5a** (0.3 g, 75 %) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.38 - 7.31 (m, 2 H), .30 - 7.20 (m, 5 H), 7.05 (br. s., 1 H), 5.39 (br. s., 1 H), 4.22 - 4.02 (m, 1 H), 3.72 - 3.57 (m, 1 H), 3.49 - 3.25 (m, 2 H), 3.16 (br. s., 2 H), 2.94 - 2.76 (m, 2 H), 2.16 (s, 2 H), 1.61 - 1.22 (m, 19 H); ^{13}C NMR (100 MHz ,$CDCl_3$) δ =171.7, 168.6, 156.8, 140.2, 139.2, 137.8, 134.4, 130.9, 130.1, 129.3, 128.3, 127.7, 127.3, 126.6, 78.8, 46.7, 43.6, 42.2, 37.5, 34.3, 30.9, 30.0, 28.4, 25.9, 21.5; HRMS: $C_{30}H_{39}N_3O_4$, calculated: 528.2833 $[M+Na]^+$, observed: 528.2830 $[M+Na]^+$.



^{1}H NMR spectrum of compound **5a** ($CDCl_3$, 400 MHz, 298 K)

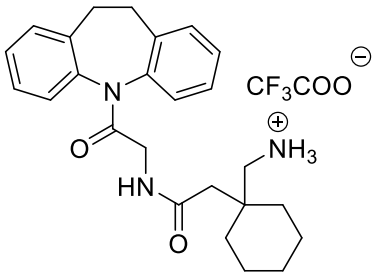


^{13}C NMR spectrum of compound **5a** ($CDCl_3$, 400 MHz, 298 K)

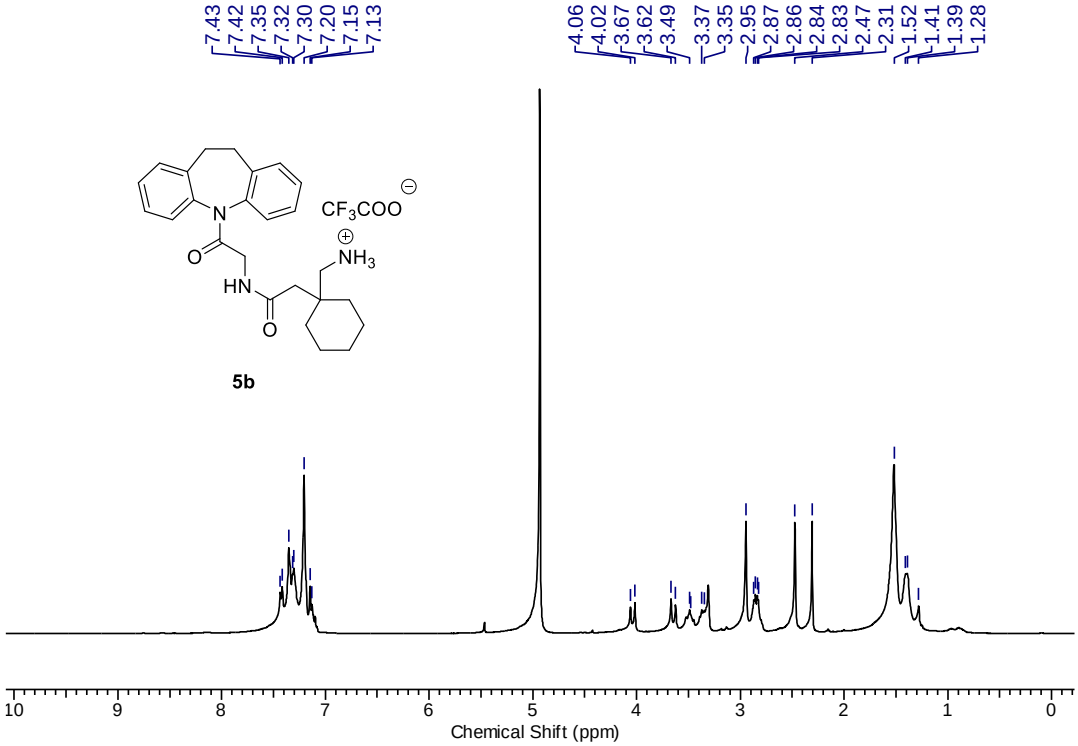


HRMS of Compound **5a**

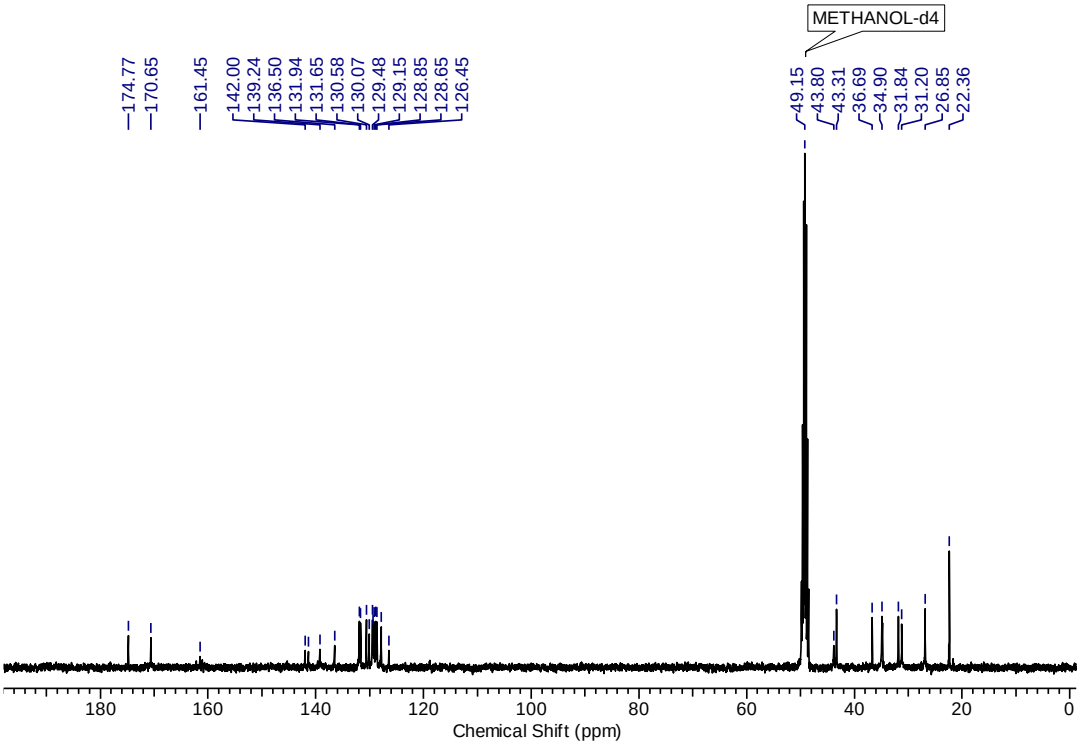
Compound **5b** (1-(2-((2-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-2-oxoethyl)amino)-2-oxoethyl)cyclohexyl)methanaminium: To the solution of compound **5a** (0.2 g, mmol) in DCM, TFA (1M) was added and stirred for 30 min. After the completion of the reaction, the volatiles were stripped off and co-evaporated with toluene to obtain compound **5b** as a hygroscopic white solid (0.19 g, 93 %). ^{1}H NMR (400 MHz, CD_3OD) δ = 7.43 (d, J = 7.3 Hz, 1 H), 7.38 - 7.26 (m, 3 H), 7.20 (m, 3 H), 7.16 - 7.06 (m, 1 H), 4.04 (d, J = 17.1 Hz, 1 H), 3.65 (d, J = 17.1 Hz, 1 H), 3.57 - 3.44 (m, 1 H), 3.42 - 3.32 (m, 1 H), 2.95 (m., 2 H), 2.89 - 2.76 (m, 2 H), 2.47 (s, 1 H), 2.31 (s, 1 H), 1.52 (br. s., 6 H), 1.40 (m, 3 H), 1.28 (br. s., 1 H); ^{13}C NMR (100 MHz, CD_3OD) = 174.8, 170.6, 161.4, 142.0, 141.4, 139.2, 136.5, 131.9, 131.7, 130.6, 129.5, 129.4, 128.8, 128.6, 126.4, 43.8, 43.3, 36.7, 34.9, 31.8, 31.2, 26.9, 22.4; HRMS: $C_{25}H_{31}N_3O_2$, calculated: 406.2489 $[M+H]^+$, observed: 406.2484 $[M+H]^+$.



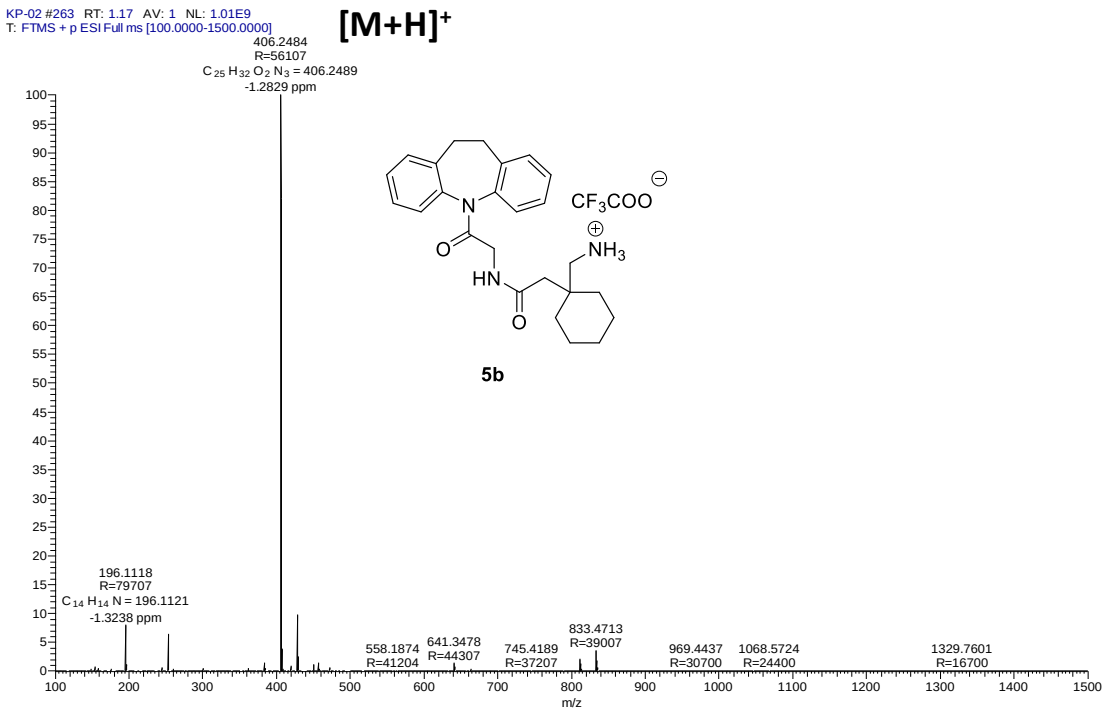
5b



^{1}H NMR spectrum of compound **5b** (CD_3OD, 400 MHz, 298 K)

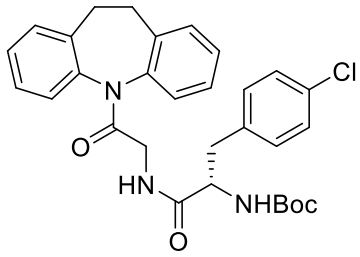


^{13}C NMR spectrum of compound **5b** (CD_3OD, 400 MHz, 298 K)



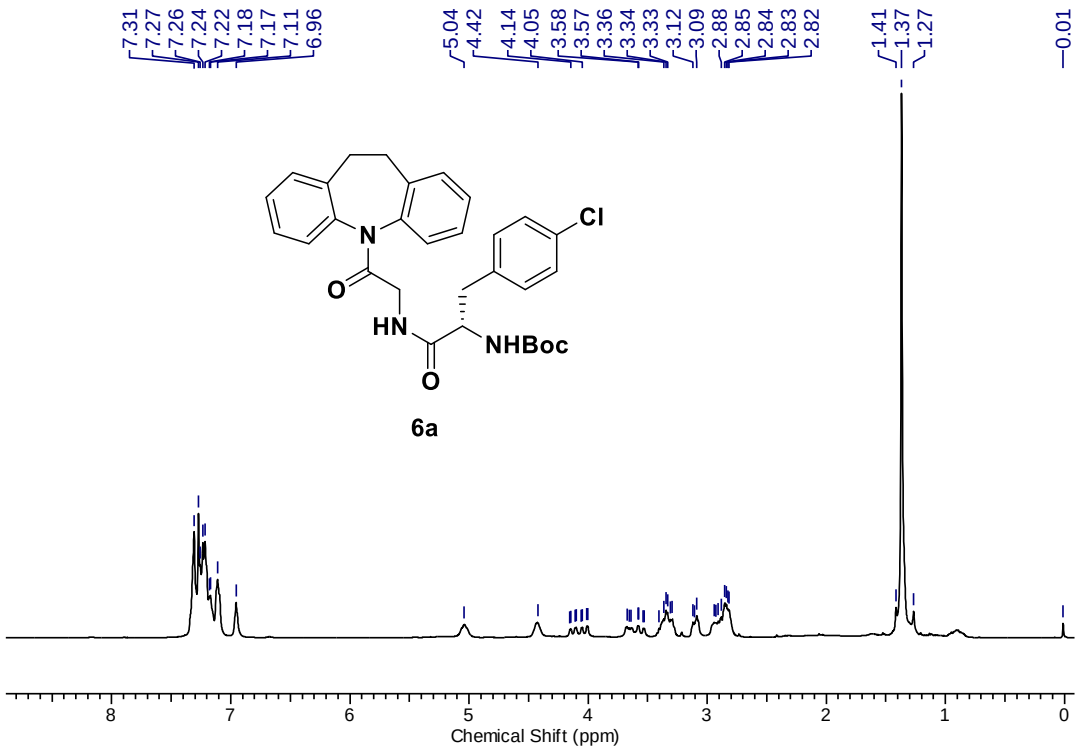
HRMS of Compound **5b**

Compound **6a** (tert-butyl (S)-(3-(4-chlorophenyl)-1-((2-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-2-oxoethyl)amino)-1-oxopropan-2-yl)carbamate): Boc-fenclonine (0.28 g, 0.94 mmol, 1.2 equiv) was coupled with dibenzazepine intermediate **4** (0.2 g, 0.79 mmol, 1 equiv) using HATU (0.45 g, 1.18 mmol, 1.5 equiv) and DIPEA (0.5 mL, 3.95 mmol, 5 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in ethyl acetate and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 15% AcOEt/Petether, Rf : 0.4) to furnish compound **6a** (0.35 g, 81 %) as a white solid; ; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.31 (br. s., 3 H), 7.26 - 7.15 (m, 6H), 7.11 (br. s., 2 H), 6.96 (br. s., 1 H), 5.04 (br. s., 1 H), 4.42 (br. s., 1 H), 4.01 (d, *J* = 3.1 Hz, 1 H), 3.76 - 3.49 (m, 1 H), 3.46 - 3.22 (m, 2 H), 3.16 - 3.04 (m, 1 H), 3.00 - 2.73 (m, 3 H), 1.45 - 1.24 (m, 9 H); ^{13}C NMR (100 MHz ,$CDCl_3$) δ = 171.0, 168.1, 155.2, 140.0, 139.0,

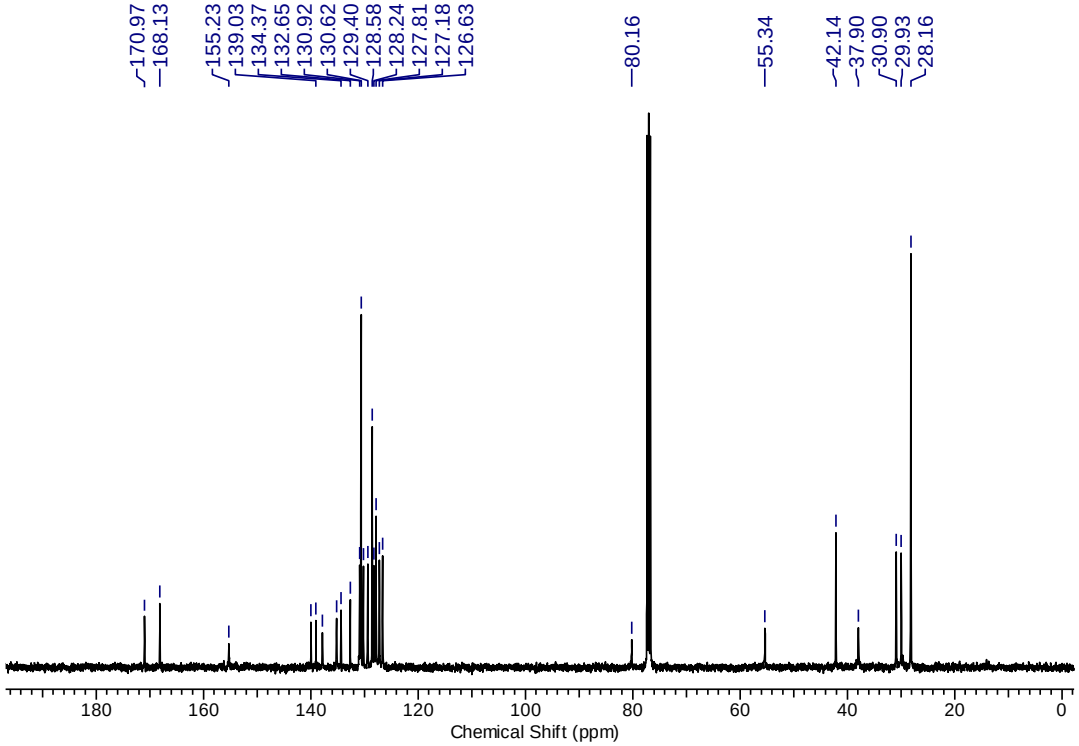


6a

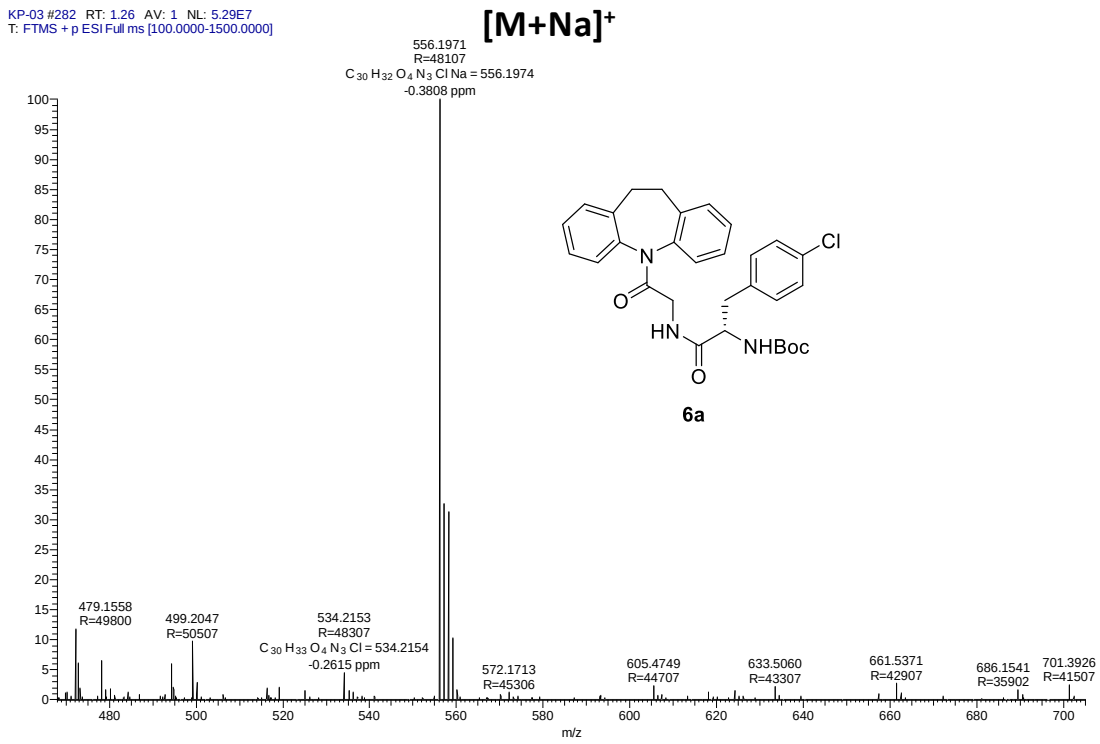
137.8, 135.2, 134.4, 132.6, 130.9, 130.6, 130.2, 129.4, 128.6, 128.2, 127.8, 127.2, 126.6, 80.2, 55.3, 42.1, 37.9, 30.9, 29.8, 27.3; HRMS: $C_{30}H_{32}ClN_3O_4$, calculated: 556.1971 [M+Na]+, observed: 556.1974 $[M+Na]^+$.



^{1}H NMR spectrum of compound **6a** ($CDCl_3$, 400 MHz, 298 K)

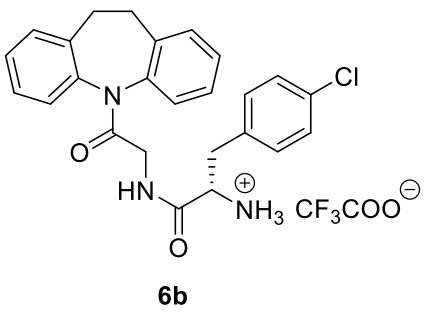


^{13}C NMR spectrum of compound **6a** ($CDCl_3$, 400 MHz, 298 K)

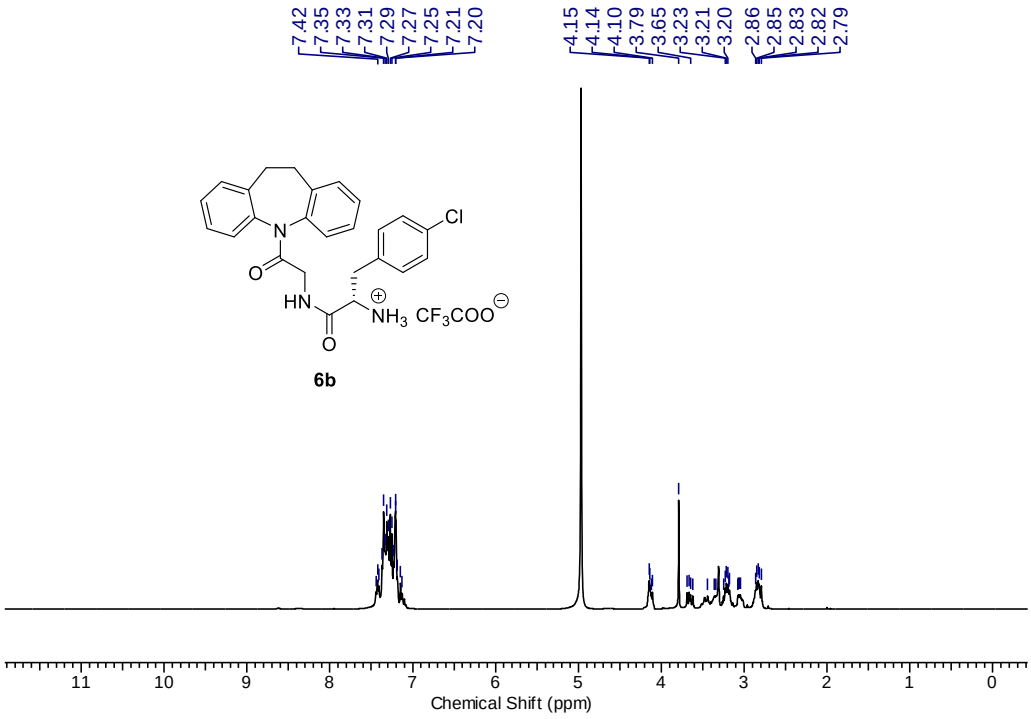


HRMS of Compound **6a**

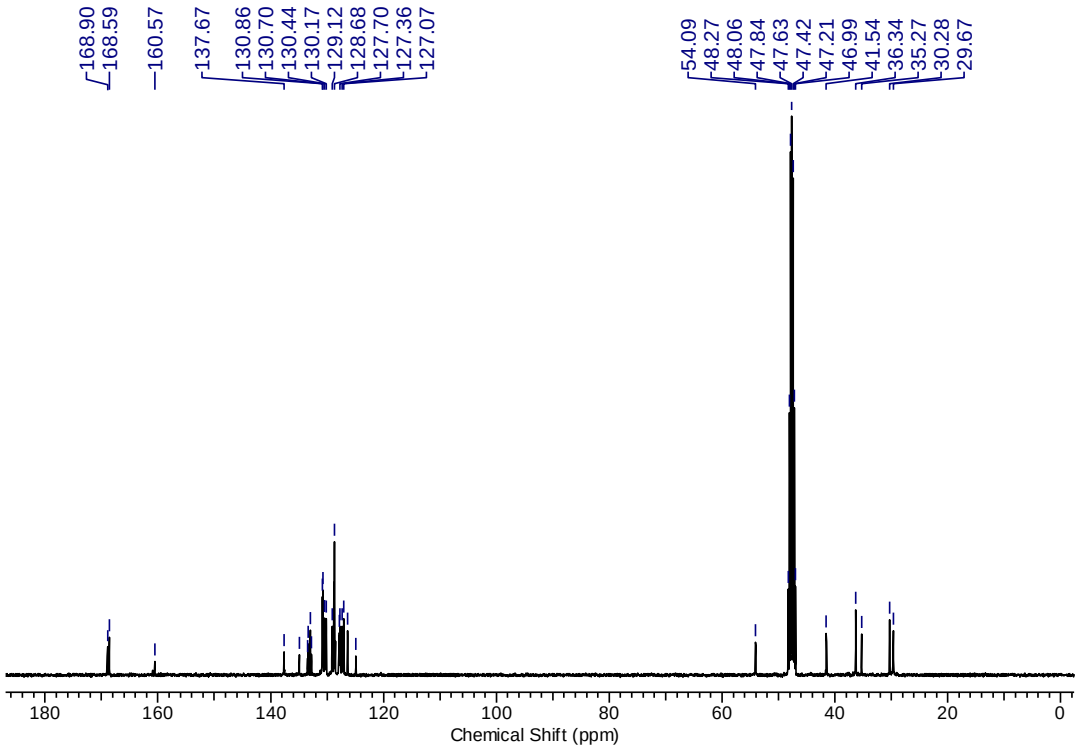
Compound 6b ((S)-3-(4-chlorophenyl)-1-((2-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-2-oxoethyl)amino)-1-oxopropan-2-aminium): To the solution of compound **6a** (0.3 g, mmol) in DCM, TFA (1M) was added and stirred for 30 min. After the completion of the reaction, the volatiles were stripped off and co evaporated with toluene to obtain compound **6b** as a hygroscopic white solid (0.28 g, 93 %); ^{1}H NMR (400 MHz, D_2O) δ = 7.45 - 7.39 (m, 1 H), 7.38 - 7.32 (m, 3 H), 7.30 (d, J = 7.3 Hz, 3 H), 7.26 (d, J = 7.3 Hz, 2 H), 7.24 - 7.18 (m, 2 H), 7.16 - 7.07 (m, 1 H), 4.21 - 4.06 (m, 2 H), 3.66 (dd, J = 10.4, 17.1 Hz, 1 H), 3.54 - 3.41 (m, 1 H), 3.28 - 3.13 (m, 2 H), 3.06 (dd, J = 3.4, 7.6 Hz, 1 H), 2.92 - 2.74 (m, 2 H); ^{13}C NMR (100 MHz, D_2O) δ = 168.9, 168.6, 137.7, 134.9, 133.5, 133.3, 133.0, 132.8, 130.8, 130.7, 130.4, 130.2, 129.1, 128.7, 127.8, 127.7, 127.4, 127.1, 126.3, 124.9, 54.1, 41.5, 36.3, 35.3, 30.3, 29.7; HRMS: $C_{25}H_{25}N_3O_2Cl$, calculated: 434.1630 $[M+H]^+$, observed: 434.1628 $[M+H]^+$.



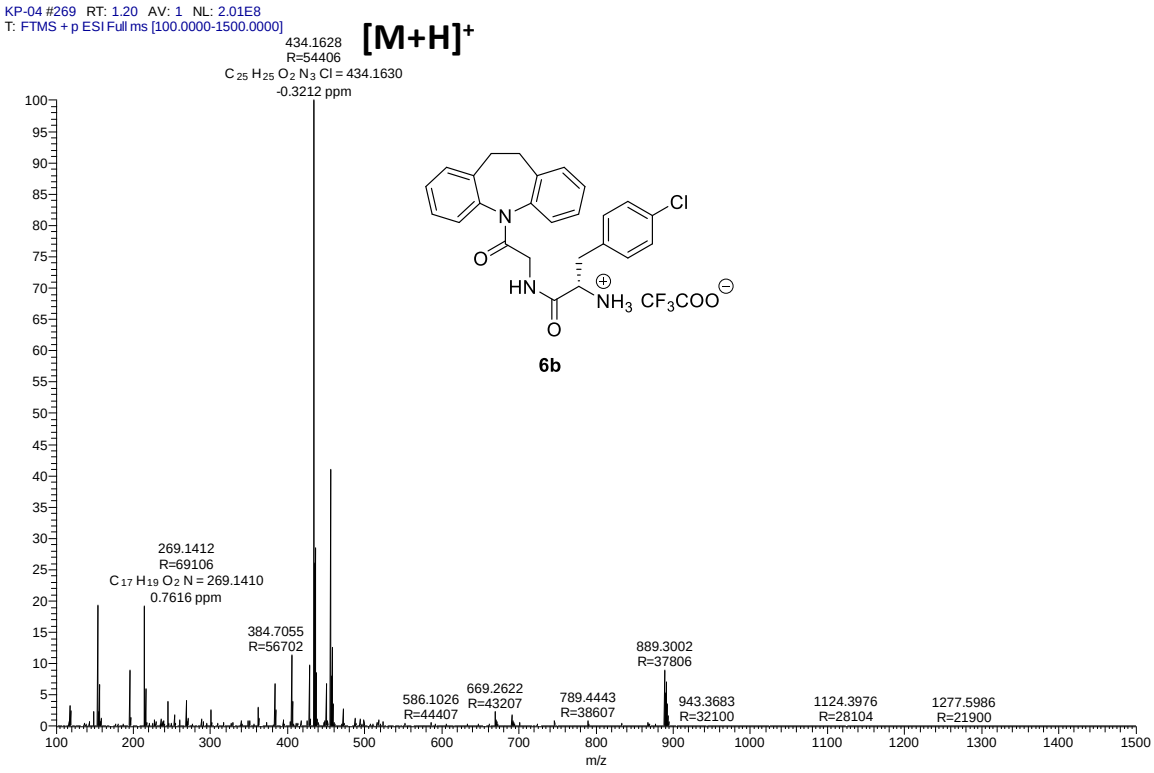
6b



^{1}H NMR spectrum of compound **6b** (D_2O, 400 MHz, 298 K)

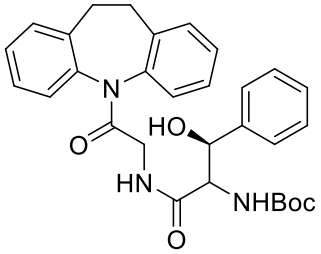


^{13}C NMR spectrum of compound **6b** (D_2O, 400 MHz, 298 K)

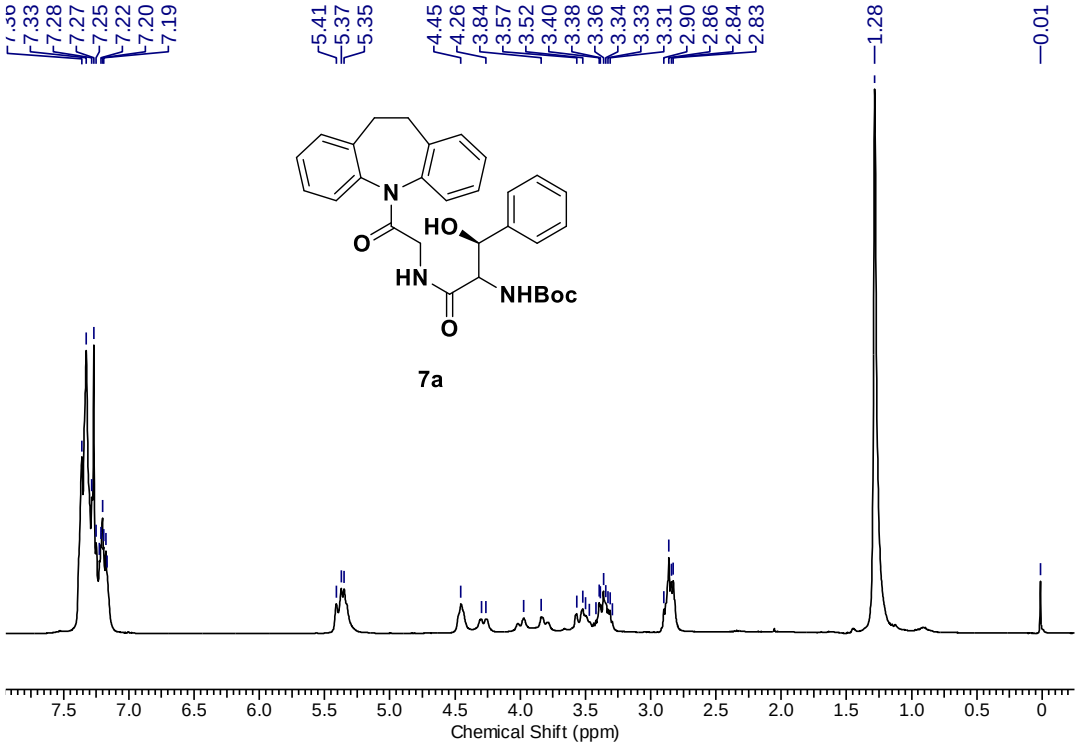


HRMS of Compound **6b**

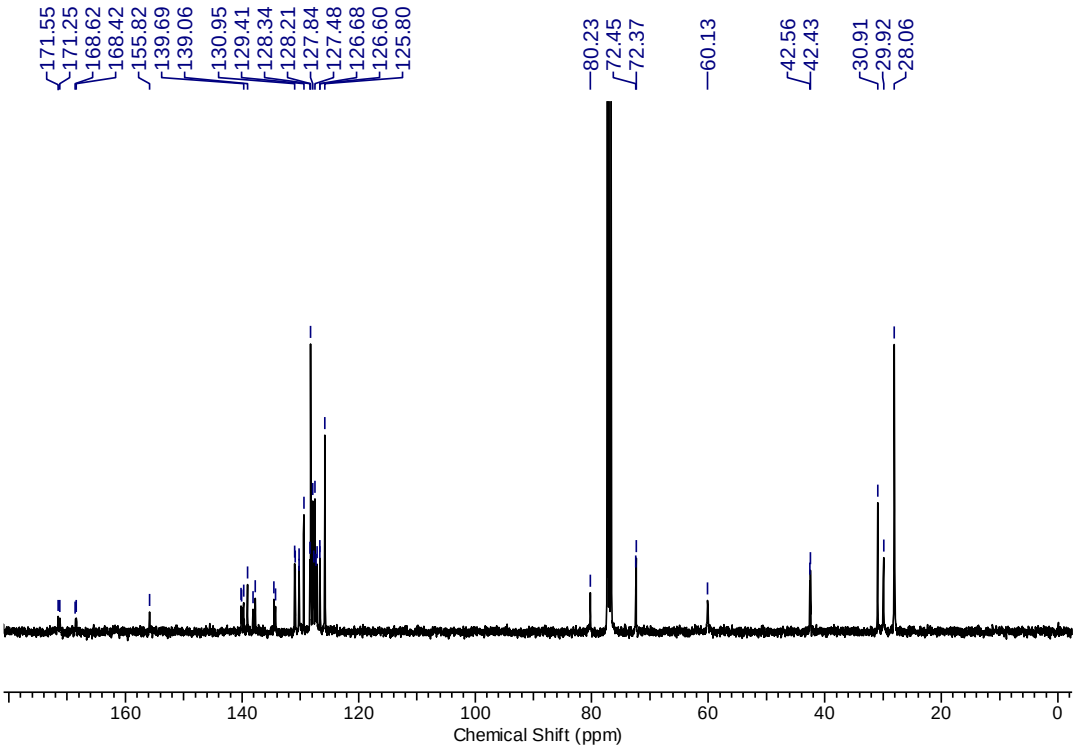
Compound 7a (tert-butyl ((3S)-1-((2-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-2-oxoethyl)amino)-3-hydroxy-1-oxo-3-phenylpropan-2-yl)carbamate): Boc-L-threo-3-phenylserine (0.26 g, 0.95 mmol, 1.2 equiv) was coupled with dibenzazepine intermediate **4** (0.2 g, 0.79 mmol, 1 equiv) using HATU (0.45 g, 1.18 mmol, 1.5 equiv) and DIPEA (0.5 mL, 3.95 mmol, 5 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in ethyl acetate and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 20% AcOEt/Petether, Rf : 0.4) to furnish compound **7a** (0.34 g, 85 %) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.42 - 7.29 (m, 8 H), 7.26 - 7.09 (m, 5 H), 5.51 - 5.25 (m, 2 H), 4.45 (br. s., 1 H), 4.28 (d, J = 14.0 Hz, 1 H), 4.05 - 3.76 (m, 1 H), 3.60 - 3.45 (m, 1 H), 3.42 - 3.32 (m, 2 H), 2.97 - 2.76 (m, 2 H), 1.28 (br. s., 9 H); ^{13}C NMR (100 MHz ,$CDCl_3$) δ = 171.7, 171.2, 168.6, 168.4, 155.8, 140.1, 140.0, 139.7, 139.1, 138.1, 137.8, 134.5, 134.3, 131.0, 130.9, 130.2, 130.2, 129.4, 128.3, 128.2, 127.8, 127.8, 127.7, 127.5, 127.3, 127.1, 126.7, 126.6, 125.8, 80.2, 72.4, 60.1, 42.6, 42.4, 30.9, 30.0, 29.9, 28.1; HRMS: $C_{30}H_{33}N_3O_5$, calculated: 538.2312 M+Na]+, observed: 538.2310 $[M+Na]^+$.



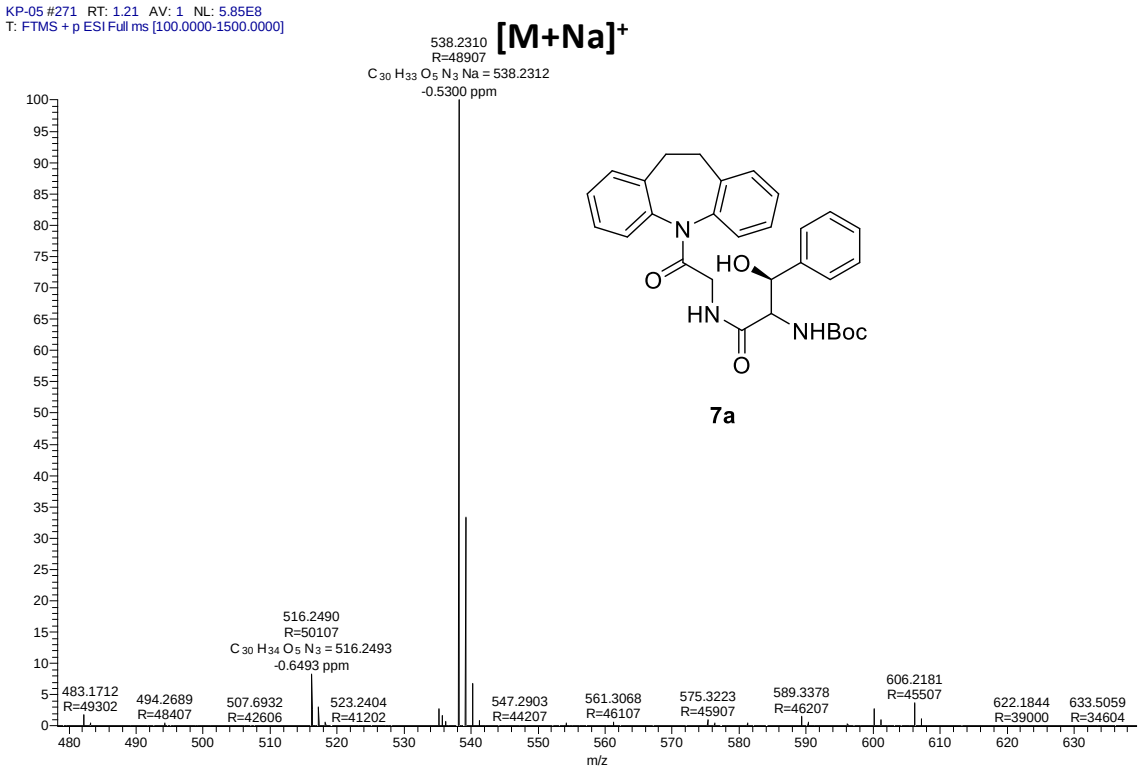
7a



^{1}H NMR spectrum of compound **7a** ($CDCl_3$, 400 MHz, 298 K)

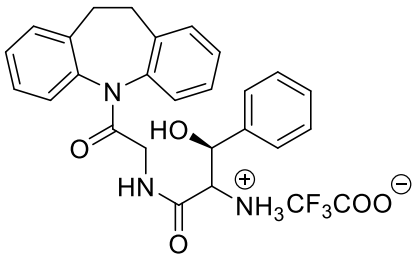


^{13}C NMR spectrum of compound **7a**($CDCl_3$, 400 MHz, 298 K)

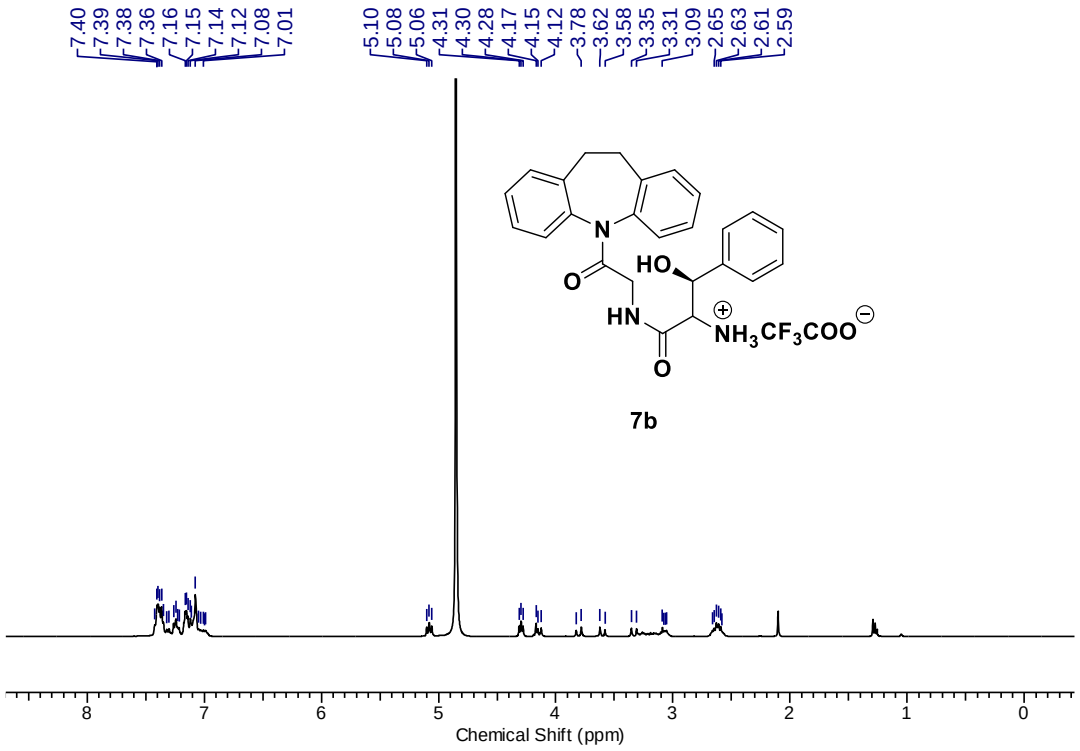


HRMS of Compound **7**

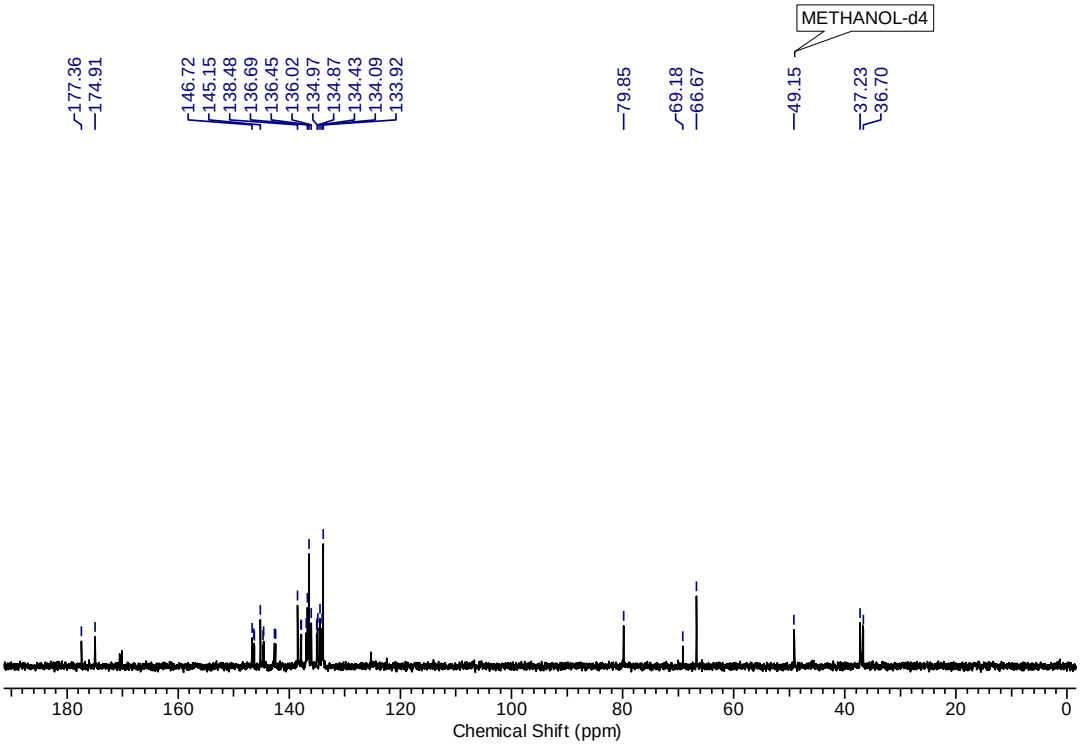
Compound 7b ((S)-3-(4-chlorophenyl)-1-((2-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-2-oxoethyl)amino)-1-oxopropan-2-aminium): To the solution of compound **7a** (0.25 g, mmol) in DCM, TFA (1M) was added and stirred for 30 min. After the completion of the reaction, the volatiles were stripped off and co evaporated with toluene to obtain compound **7b** as a hygroscopic white solid (0.21g, 82 %).[1]H NMR (400 MHz, CD_3OD) δ = 7.52 - 6.93 (m, 13 H), 5.13 - 5.04 (m, 1 H), 4.34 - 4.25 (m, 1 H), 4.20 - 4.09 (m, 1 H), 3.87 - 3.53 (m, 1 H), 3.31 (s, 2 H), 2.69 - 2.53 (m, 2 H), [13]C NMR (100 MHz, CD_3OD) δ = 177.3, 174.1, 170.4, 146.7, 145.9, 144.7, 142.6, 138.4, 136.8, 136.4, 136.0, 134.9, 134.4, 134.1, 133.9, 79.3, 69.8, 66.6, 37.2, 36.7; HRMS: $C_{25}H_{26}N_3O_3$, calculated: 416.1969 $[M+H]^+$, observed: 416.1966 $[M+H]^+$.



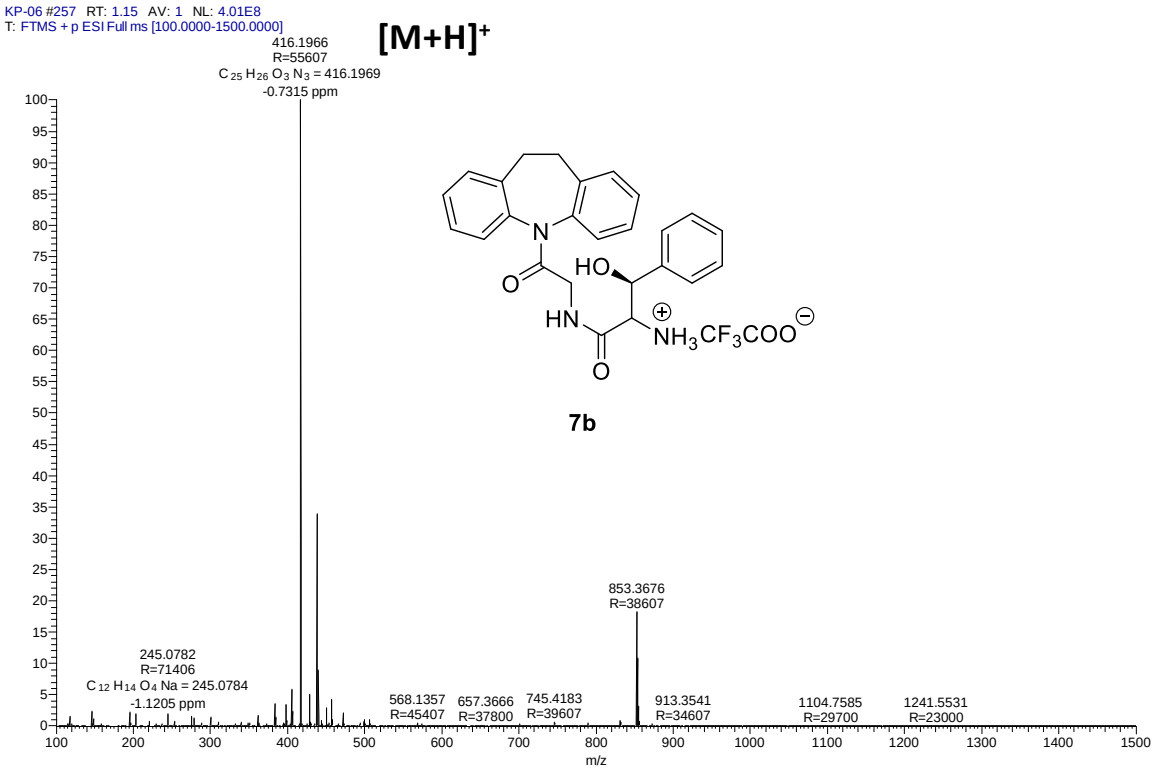
7b



^{1}H NMR spectrum of compound **7b** (CD_3OD, 400 MHz, 298 K)

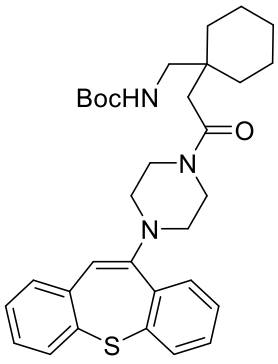


^{13}C NMR spectrum of compound **7b** (CD_3OD, 400 MHz, 298 K)



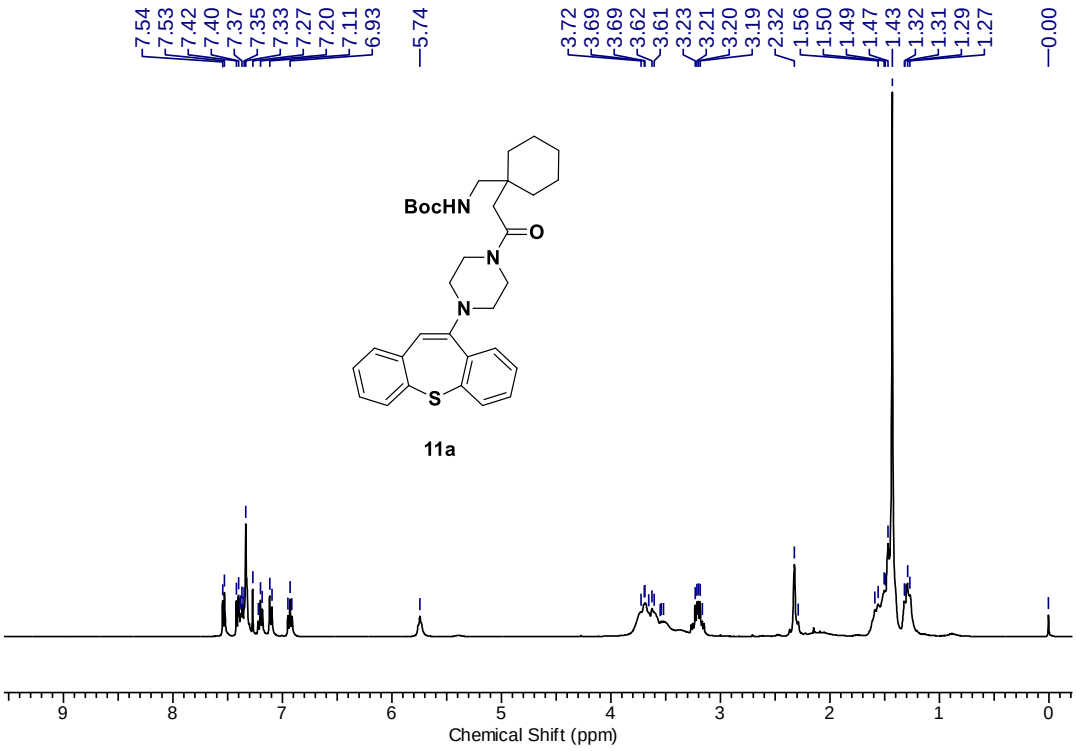
HRMS of Compound **7b**

Compound 11a (tert-butyl ((1-(2-(4-(dibenzo[b,f]thiepin-10-yl)piperazin-1-yl)-2-oxoethyl)cyclohexyl)methyl)carbamate): Boc-gabapentin (0.2 g, 0.73 mmol, 1 equiv) was coupled with TFA salt of norquetiapine **10** (0.90 g, 2.21 mmol, 3 equiv) using HATU (0.42 g, 1.10 mmol, 1.5 equiv) and DIPEA (0.64 mL, 3.6 mmol, 5 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in ethyl acetate and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 15% AcOEt/Petether, Rf : 0.4) to furnish compound **11a** (0.35 g, 86 %) as a white solid; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.54 (d, J = 7.3 Hz, 1 H), 7.44 - 7.39 (m, 1 H), 7.38 - 7.31 (m, 2 H), 7.23 - 7.15 (m, 1 H), 7.10 (d, J = 7.9 Hz, 1 H), 6.93 (t, J = 7.0 Hz, 1 H), 5.74 (d, J = 6.1 Hz, 1 H), 3.82 -

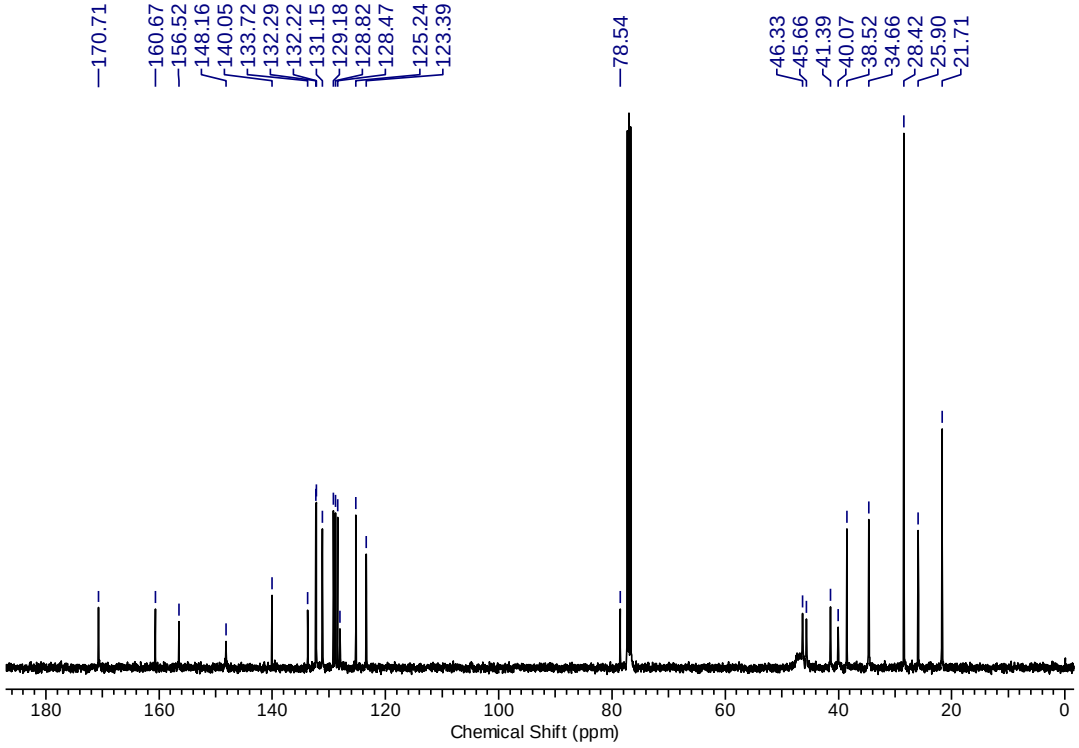


11a

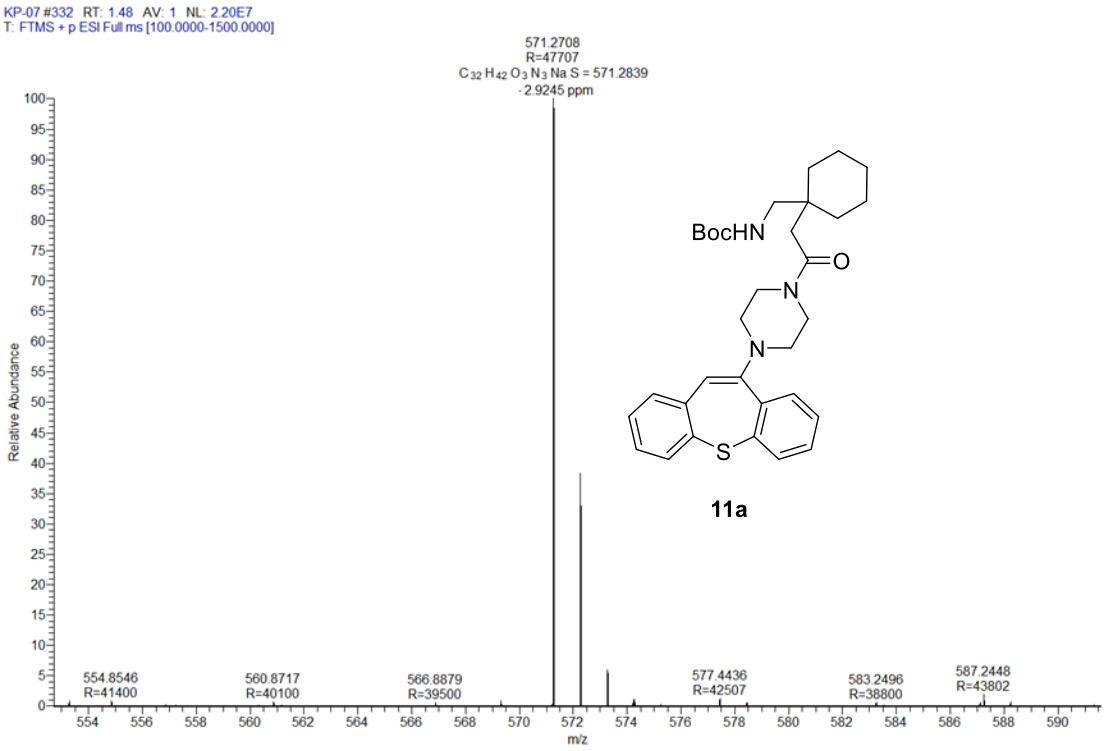
3.36 (m, 6 H), 3.29 - 3.11 (m, 2 H), 2.38 - 2.24 (m, 2 H), 1.65 - 1.37 (m, 16 H), 1.36 - 1.21 (m, 3 H); ^{13}C NMR (100 MHz ,$CDCl_3$) δ = 170.7, 160.7, 156.5, 148.2, 140.0, 133.7, 132.3, 132.2, 131.1, 129.2, 128.8, 128.5, 128.0, 125.2, 123.4, 78.5, 46.3, 45.7, 41.4, 40.1, 38.5, 34.7, 28.4, 25.9, 21.7 HRMS: $C_{32}H_{42}N_3O_3S$, calculated: 571.2839 $[M+Na]^+$, observed: 571.2704 $[M+Na]^+$.



^{1}H NMR spectrum of compound **11a** ($CDCl_3$, 400 MHz, 298 K)

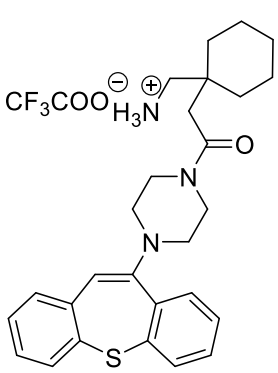


^{13}C NMR spectrum of compound **11a** ($CDCl_3$, 400 MHz, 298 K)

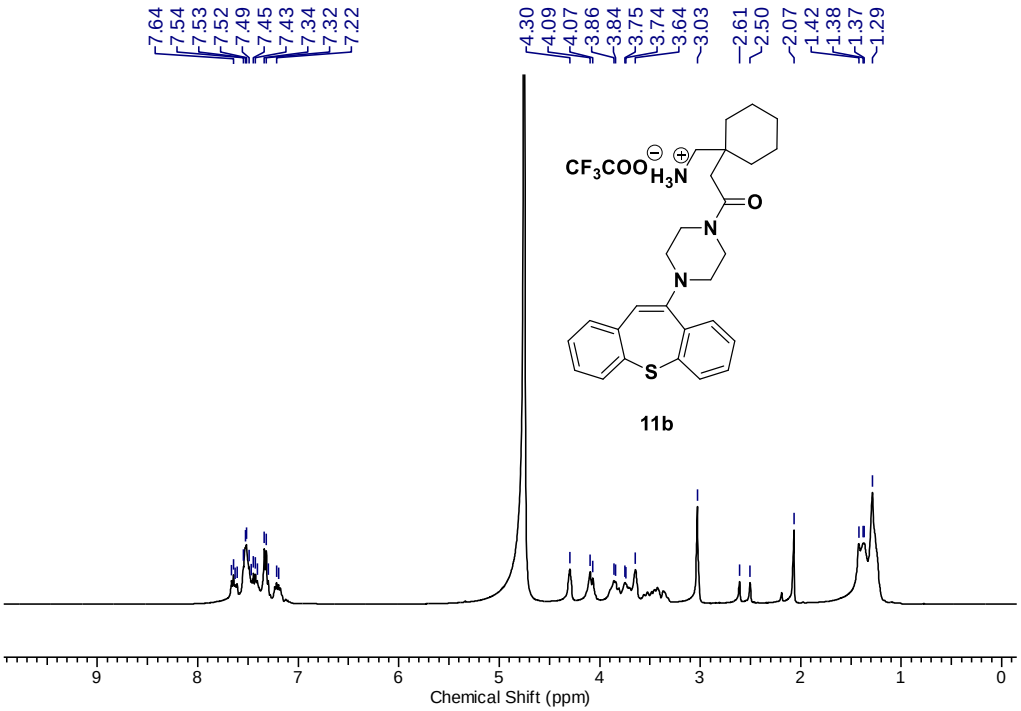


HRMS of Compound **11a**

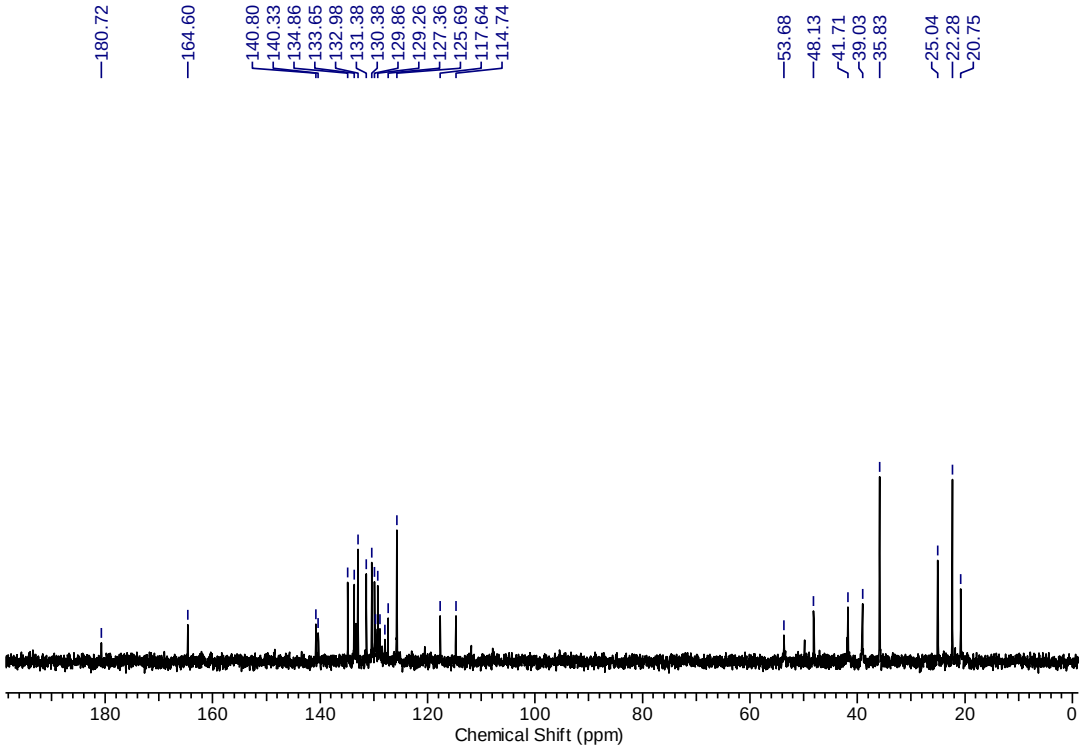
Compound 11b ((1-(2-(4-(dibenzo[b,f]thiepin-10-yl)piperazin-1-yl)-2-oxoethyl)cyclohexyl)methanaminium): To the solution of compound **11a** (0.2 g, mmol) in DCM, TFA (1M) was added and stirred for 30 min. After the completion of the reaction, the volatiles were stripped off and co evaporated with toluene to obtain compound **11b** as a hygroscopic white solid (0.15 g, 75 %).[1]H NMR (400 MHz, CD_3OD) δ = 7.64 (dd, J = 7.3, 14.0 Hz, 1 H), 7.57 - 7.36 (m, 4 H), 7.35 - 7.28 (m, 2 H), 7.25 - 7.17 (m, 1 H), 4.30 (br. s., 1 H), 4.35 - 4.25 (m, 1 H), 4.08 (d, J = 9.8 Hz, 2 H), 3.93 - 3.79 (m, 1 H), 3.77 - 3.70 (m, 1 H), 3.64 (br. s., 1 H), 3.56 - 3.31 (m, 1 H), 3.03 (br. s., 2 H), 2.64 - 2.48 (m, 1 H), 2.21 - 2.05 (m, 1 H), 1.51 - 1.16 (m, 10 H) [13]C NMR (100 MHz, D_2O)= 180.7, 164.6, 140.8, 135.3, 134.9, 133.7, 132.0, 131.4, 130.4, 129.9, 129.3, 128.8, 127.4, 125.7, 117.6, 114.7, 53.7, 48.1, 41.7, 35.8, 25.0, 22.3; HRMS: $C_{27}H_{33}N_3O_3S$, calculated: 471.2315 $[M+Na]^+$, observed: 471.2184 $[M+Na]^+$.



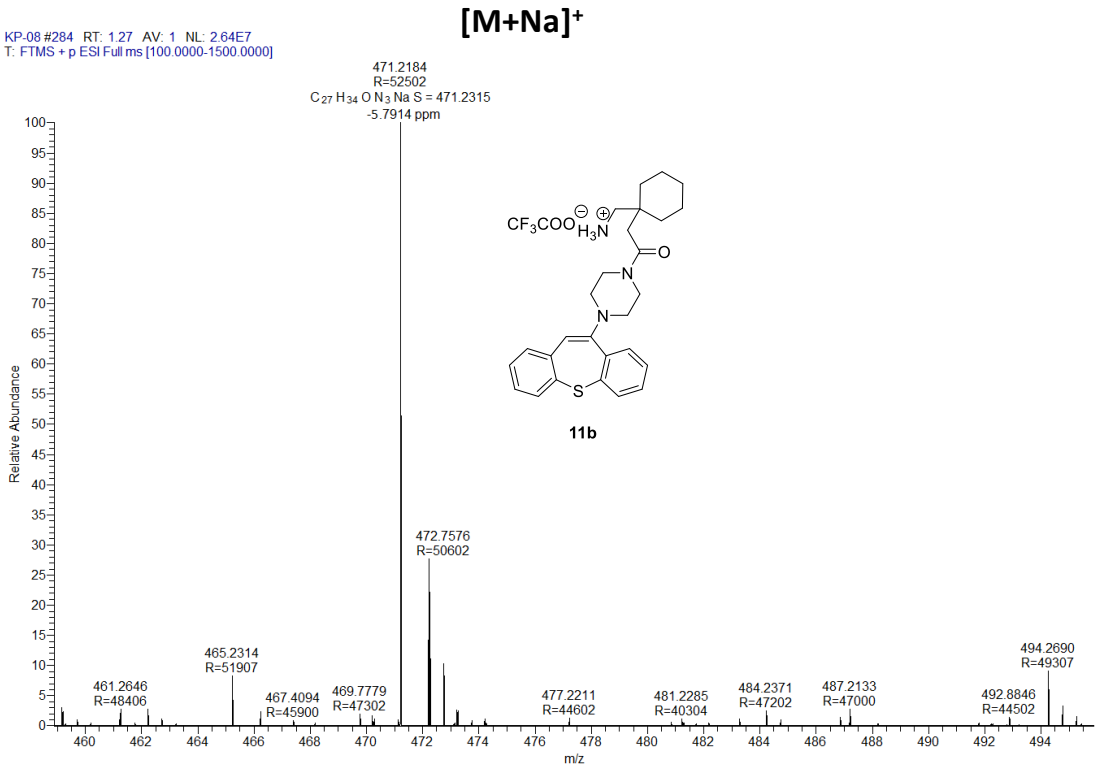
11b



[1]H NMR spectrum of compound **11b** (D_2O, 400 MHz, 298 K)

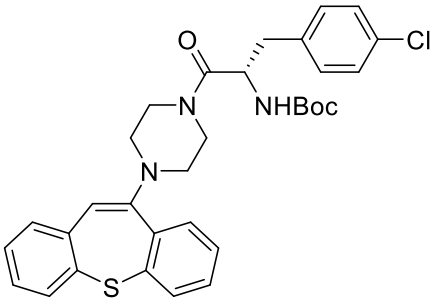


^{13}C NMR spectrum of compound **11b** (D_2O, 400 MHz, 298 K)

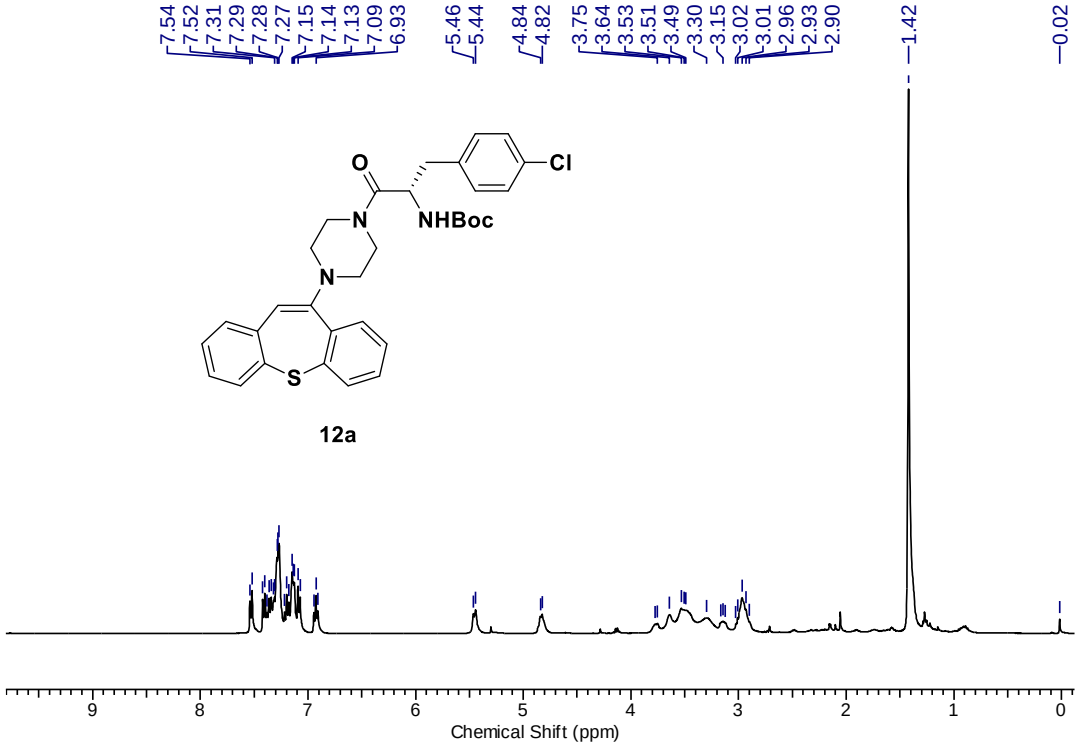


HRMS of Compound **11b**

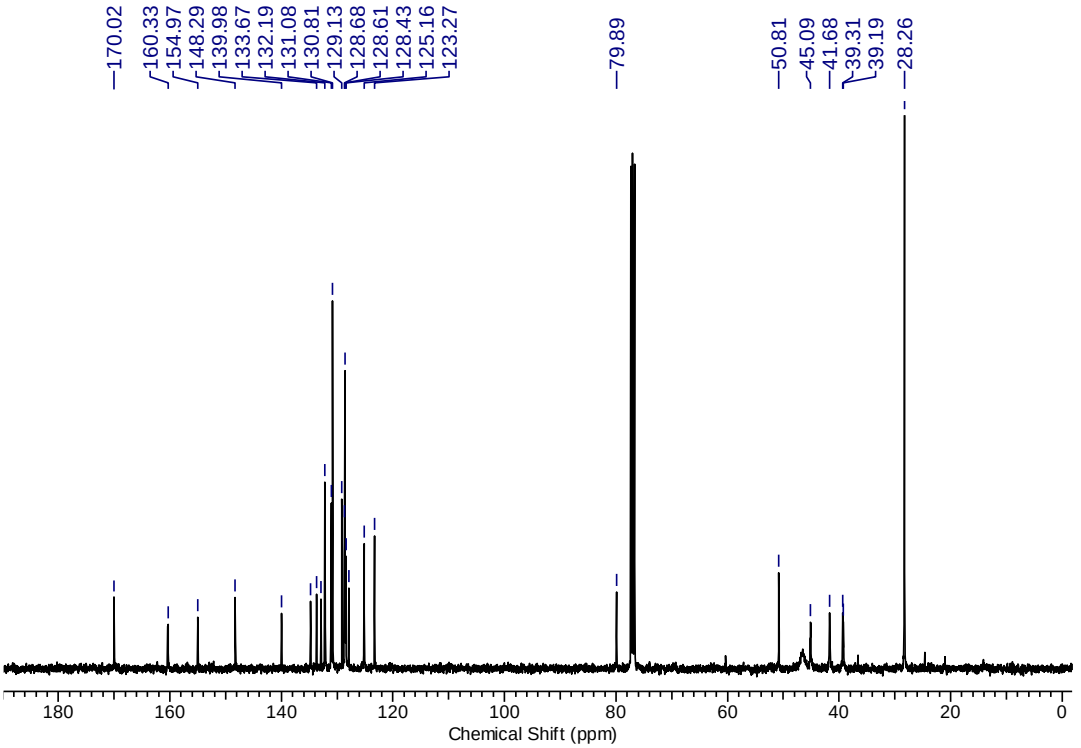
Compound 12a (tert-butyl (S)-(3-(4-chlorophenyl)-1-(4-(dibenzo[b,f]thiepin-10-yl)piperazin-1-yl)-1-oxopropan-2-yl)carbamate): Boc-fenclonine (0.2 g, 0.67 mmol, 1 equiv) was coupled with TFA salt of norquetiapine **10** (0.81 g, 2.0 mmol, 3 equiv) using HATU (0.38 g, 1.0 mmol, 1.5 equiv) and DIPEA (0.61 mL, 3.3 mmol, 5 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in ethyl acetate and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 20% AcOEt/Petether, Rf : 0.4) to furnish compound **12a** (0.31 g, 81 %) as a white solid; ; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.53 (d, J = 7.3 Hz, 1 H), 7.44 - 7.33 (m, 2 H), 7.27 (br. s., 4 H), 7.22 - 7.11 (m, 3H), 7.08 (d, J = 7.9 Hz, 1 H), 6.93 (t, J = 7.3 Hz, 1 H), 5.45 (d, J = 7.9 Hz, 1 H), 4.83 (d, J = 6.7 Hz, 1 H), 3.87 - 2.72 (m, 10 H), 1.42 (s, 9 H); ^{13}C NMR (100 MHz ,$CDCl_3$) δ = 170.0, 160.4, 155.0, 148.3, 140.0, 134.8, 133.7, 132.9, 132.2, 132.2, 131.1, 130.8, 129.1, 128.7, 128.6, 128.4, 127.9, 125.2, 123.3, 79.9, 50.8, 45.1, 41.7, 39.3, 39.2; HRMS: $C_{32}H_{35}ClN_3O_4S$, calculated: 577.1974 $[M+H]^+$, observed:577.2036 $[M+H]^+$.



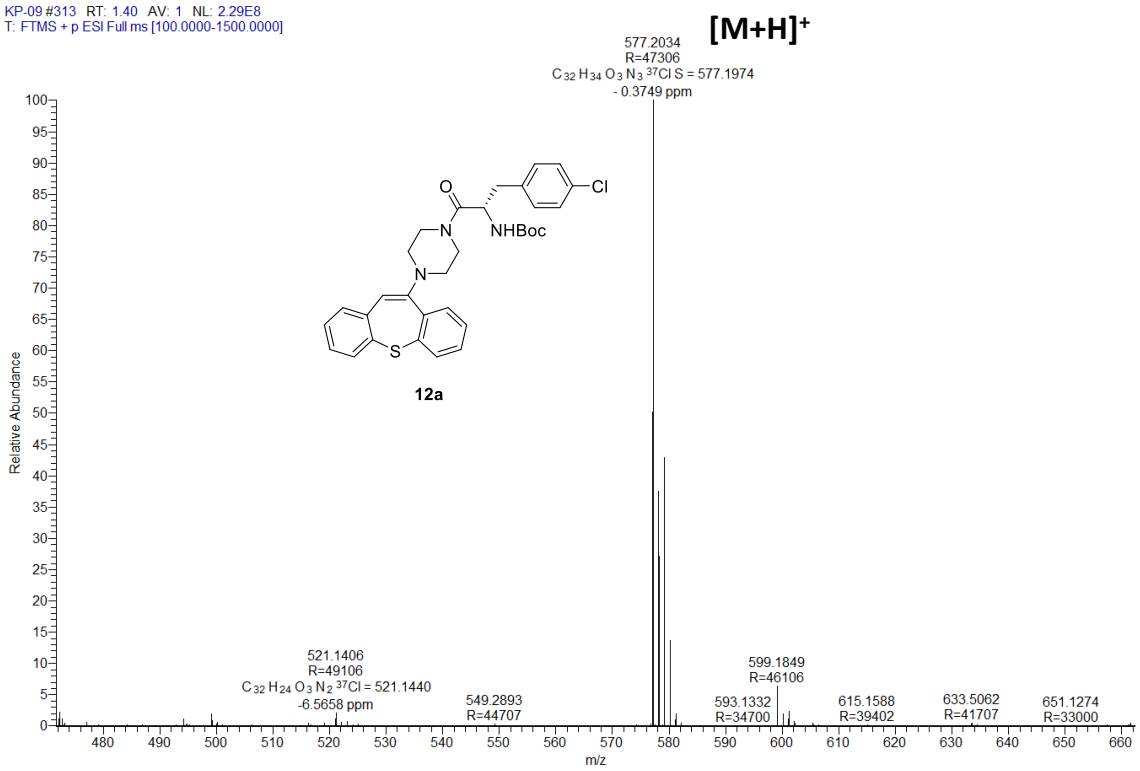
12a



^{1}H NMR spectrum of compound **12a** ($CDCl_3$, 400 MHz, 298 K)

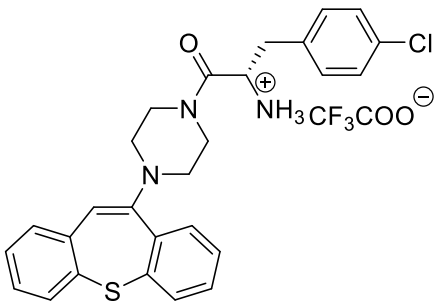


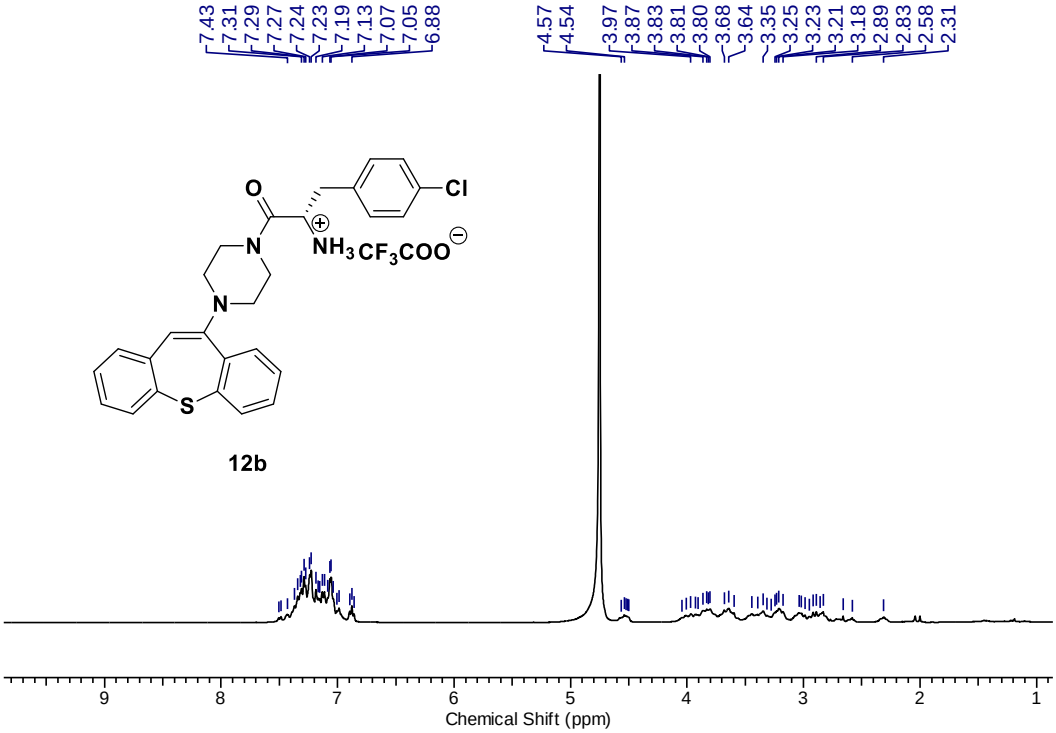
^{13}C NMR spectrum of compound **12a** ($CDCl_3$, 400 MHz, 298 K)



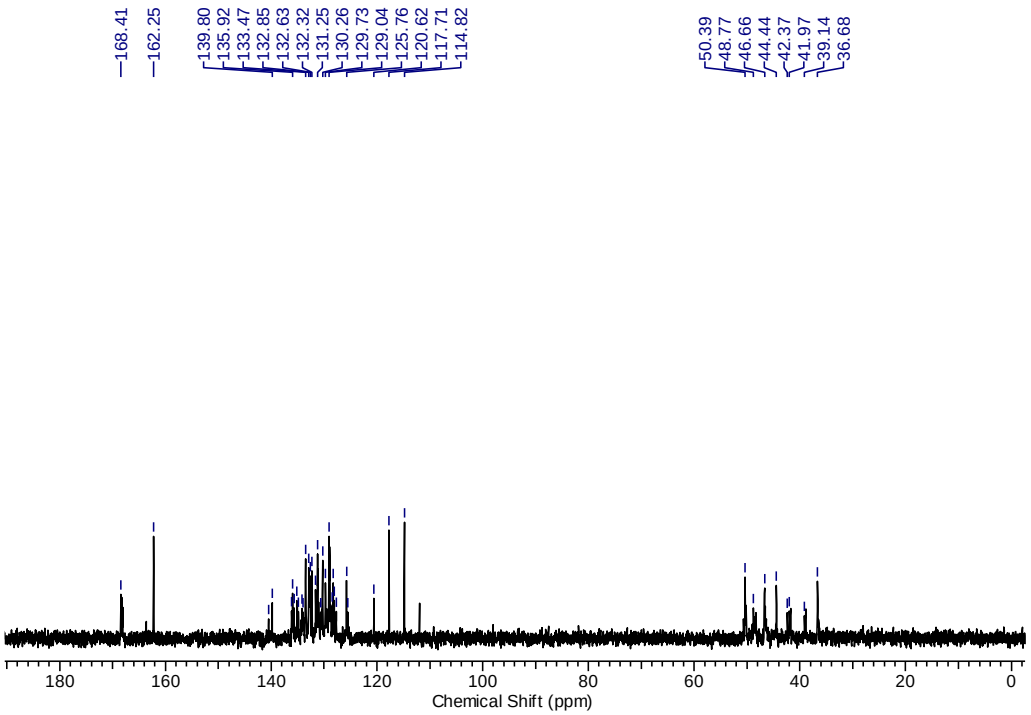
HRMS of Compound **12a**

Compound 12b (tert-butyl (S)-(3-(4-chlorophenyl)-1-(4-(dibenzo[b,f]thiepin-10-yl)piperazin-1-yl)-1-oxopropan-2-yl)carbamate): To the solution of compound **12a** (0.23 g, 0.40 mmol) in DCM, TFA (1M) was added and stirred for 30 min. After the completion of the reaction, the volatiles were stripped off and co-evaporated with toluene to obtain compound **12b** as a hygroscopic white solid (0.21 g, 91 %).[1]H NMR (400 MHz, D_2O) = 7.53 - 6.82 (m, 12 H), 4.66 - 4.42 (m, 1 H), 4.11 - 3.79 (m, 2 H), 3.76 - 3.52 (m, 2 H), 3.50 - 3.29 (m, 2 H), 3.27 - 3.13 (m, 1 H), 3.09 - 2.98 (m, 1 H), 2.93 - 2.76 (m, 1 H), 2.74 - 2.47 (m, 1 H), 2.45 - 2.21 (m, 1 H); ^{13}C NMR (100 MHz, D_2O) δ = 168.4, 162.9, 162.6, 140.52, 139.8, 135.9, 133.5, 132.7, 132.3, 131.6, 131.3, 130.3, 129.7, 129.0, 128.9, 125.8, 120.6, 117.7, 114.8, 50.4, 48.8, 46.7, 44.4, 42.4, 41.9, 39.0, 36.7; HRMS: $C_{27}H_{26}ClN_3O_5S$, calculated: 477.1450$[M+H]^+$, observed: 477.1510 $[M+H]^+$.

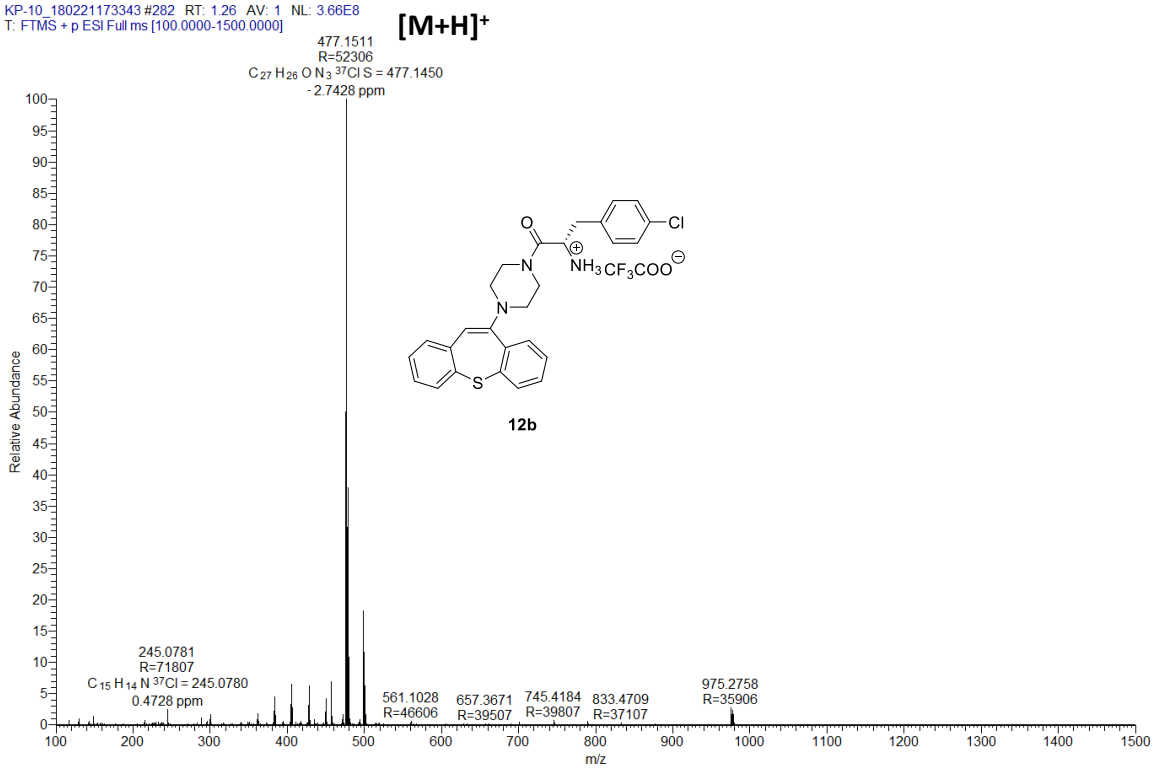




^{1}H NMR spectrum of compound **12b** (D_2O, 400 MHz, 298 K)

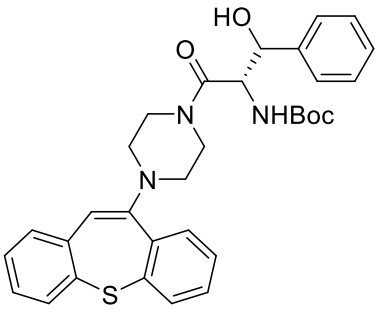


^{13}C NMR spectrum of compound **12b** (D_2O, 400 MHz, 298 K)



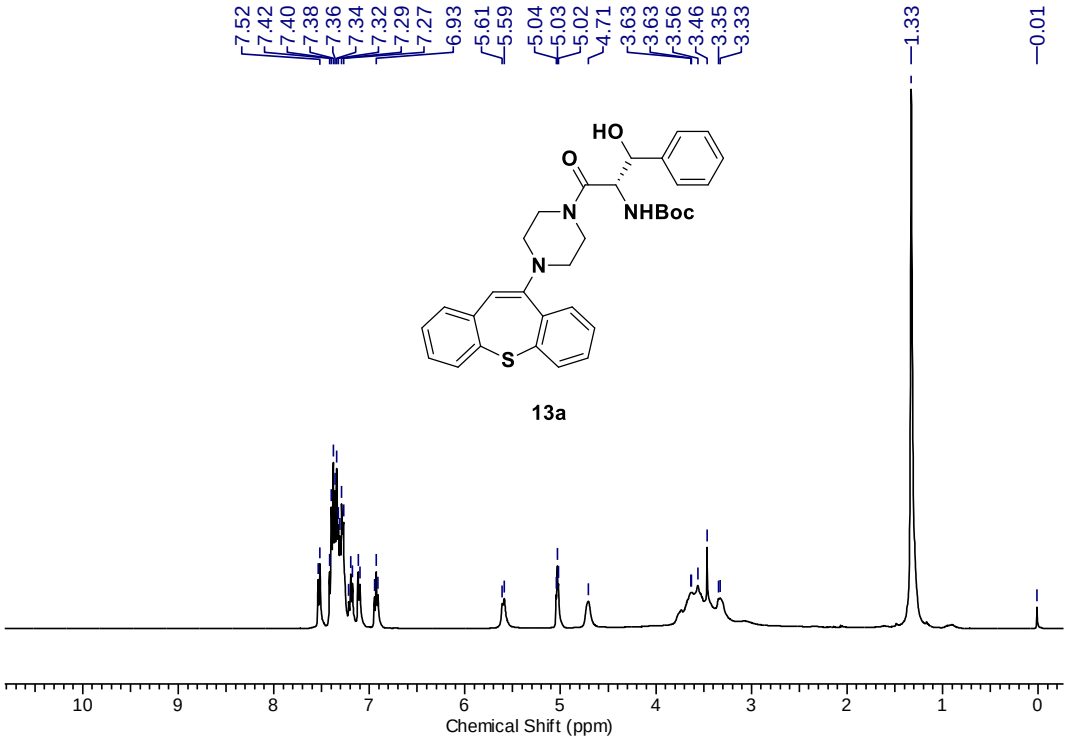
HRMS of Compound **12b**

Compound 13a (tert-butyl ((2S,3S)-1-(4-(dibenzo[b,f]thiepin-10-yl)piperazin-1-yl)-3-hydroxy-1-oxo-3-phenylpropan-2-yl)carbamate): Boc-L-threo-3-phenylserine (0.2 g, 0.71 mmol, equiv) was coupled with TFA salt of norquetiapine (0.87 g, 2.13 mmol, 3 equiv) using HATU (0.40 g, 1.06 mmol, 1.5 equiv) and DIPEA (0.65 mL, 3.5 mmol, 5 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in ethyl acetate and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 20% AcOEt/Petether, Rf : 0.4) to furnish compound **13a** (0.35 g, 89 %) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ = δ = 7.53 (d, *J* = 7.9 Hz, 1 H), 7.27 (br. s., 9 H), 7.23 - 7.16 (m, 1 H), 7.11 (d, *J* = 7.9 Hz, 1 H), 6.93 (t, *J* = 7.3 Hz, 1 H), 5.60 (d, *J* = 8.5 Hz, 1 H), 5.12 - 4.96 (m, 1 H), 4.71 (br. s., 1 H), 3.87 -

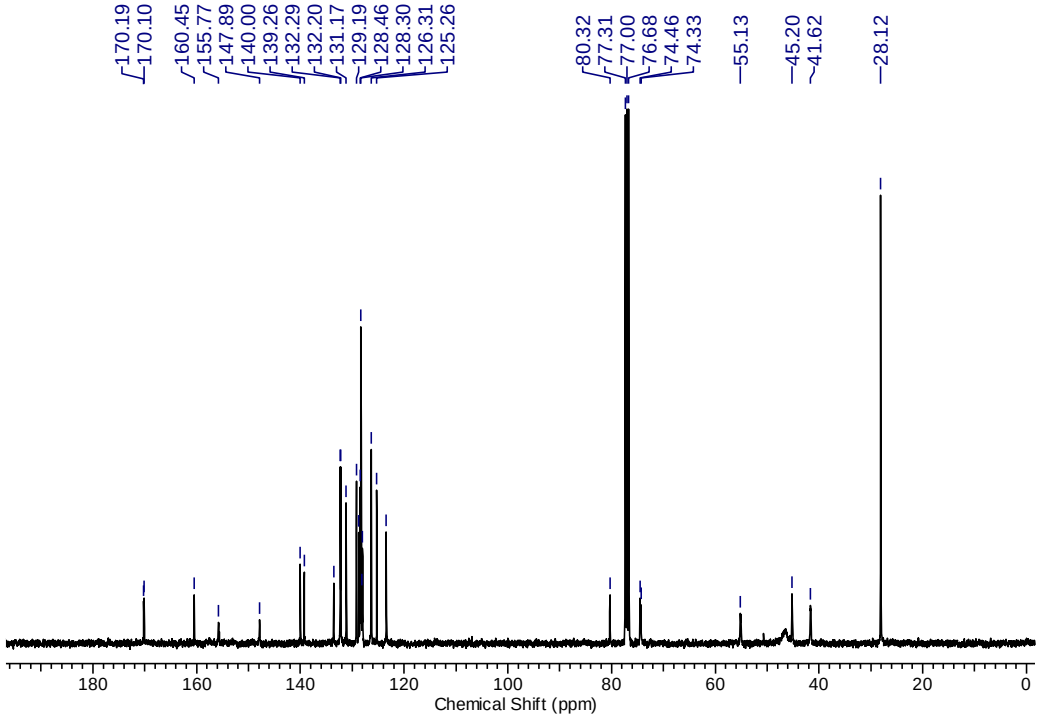


13a

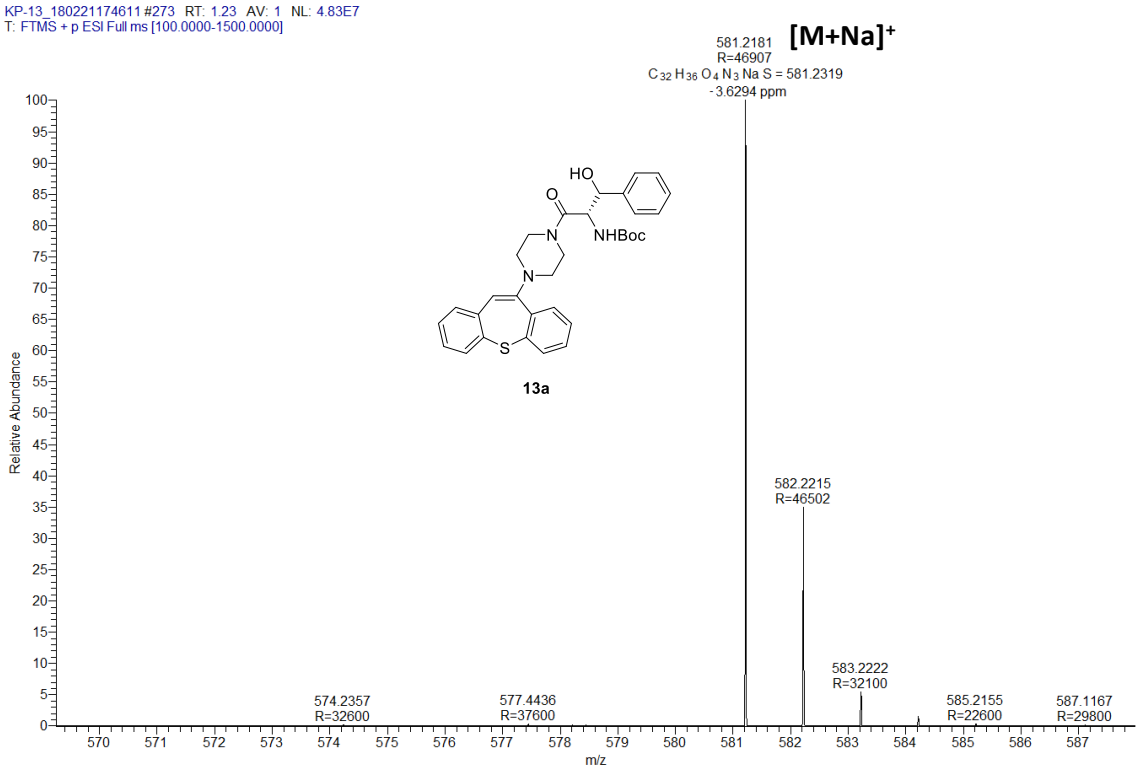
3.15 (m, 8 H), 1.33 (br. s., 9 H) ^{13}C NMR (100 MHz ,$CDCl_3$) δ = 170.1, 160.5, 155.8, 147.9, 140.0, 139.3, 133.5, 132.3, 132.2, 131.2, 129.2, 128.7, 128.5, 128.3, 128.1, 128.0, 127.9, 126.3, 125.3, 123.4, 80.3, 74.5, 74.3, 55.1, 45.2, 41.6, 28.1; HRMS: $C_{32}H_{35}N_3O_4S$, calculated: 581.2319 [M+Na]+, observed: 581.2181 $[M+Na]^+$.



^{1}H NMR spectrum of compound **13a** ($CDCl_3$, 400 MHz, 298 K)

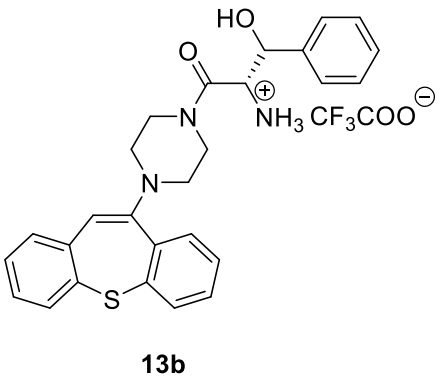


^{13}C NMR spectrum of compound **13a** ($CDCl_3$, 400 MHz, 298 K)

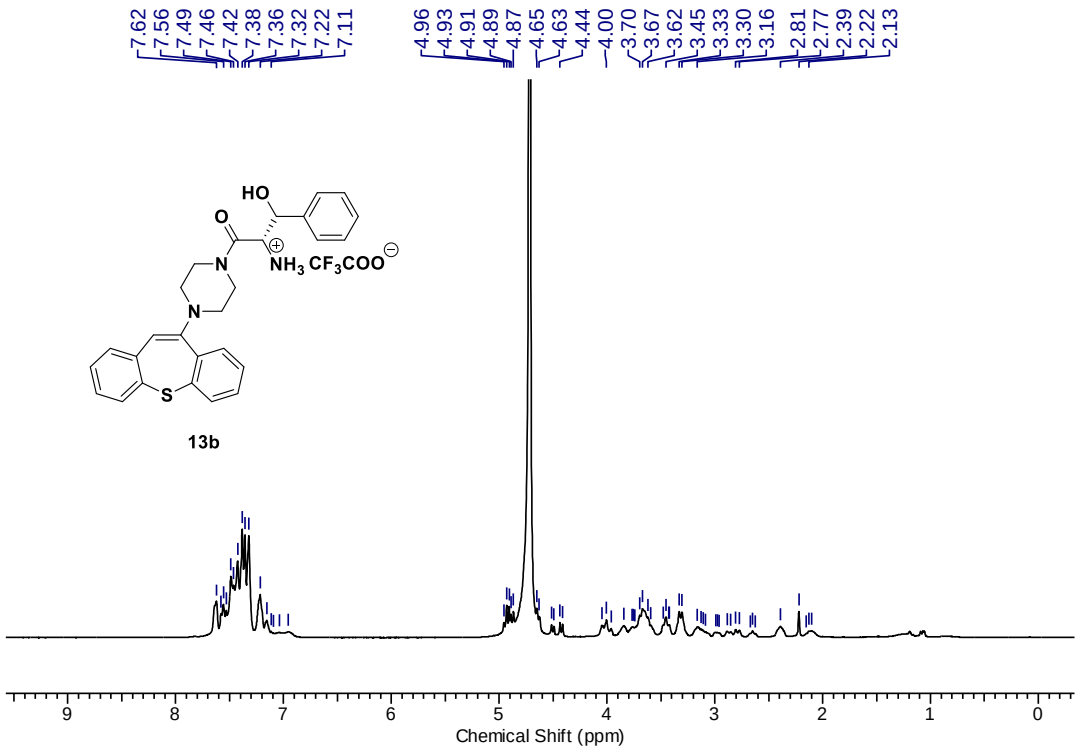


HRMS of Compound **13a**

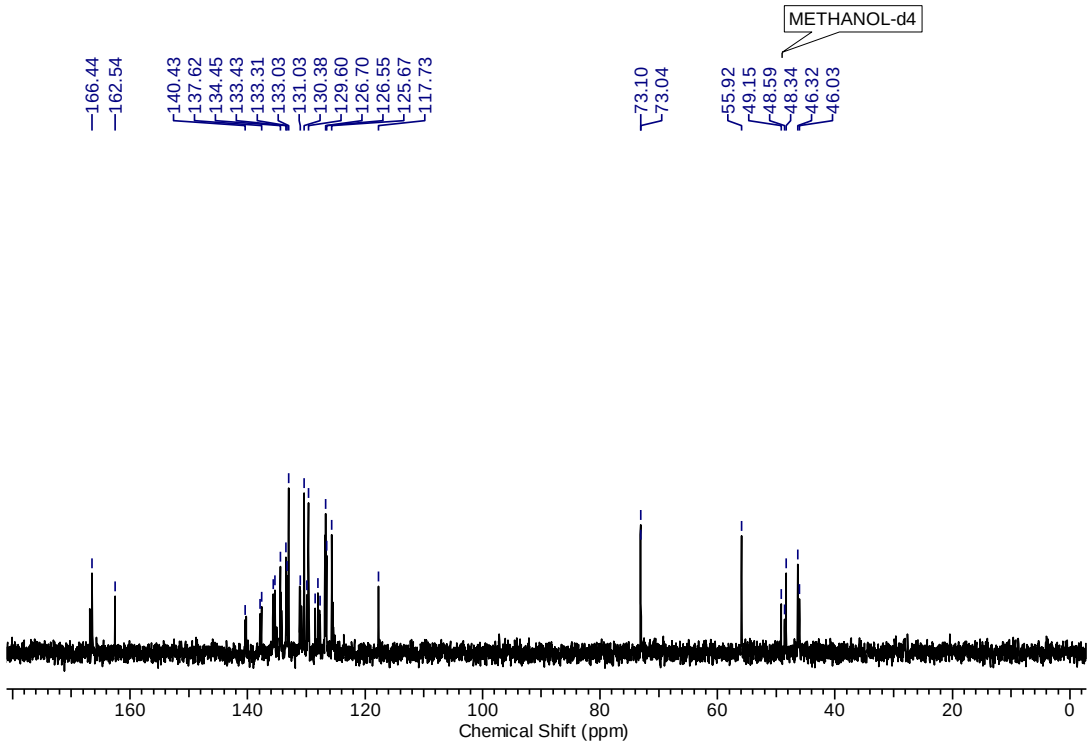
Compound 13b (2S,3S)-1-(4-(dibenzo[b,f]thiepin-10-yl)piperazin-1-yl)-3-hydroxy-1-oxo-3-phenylpropan-2-aminium 2,2,2-trifluoroacetate: To the solution of compound **13a** (0.2 g, 0.36 mmol) in DCM, TFA (1M) was added and stirred for 30 min. After the completion of the reaction, the volatiles were stripped off and co evaporated with toluene to obtain compound **13b** as a hygroscopic white solid (0.15 g, 75 %).[1]H NMR (400 MHz, D_2O) δ = 7.74 - 6.81 (m, 12 H), 4.99 - 4.83 (m, 1 H), 4.65 - 4.37 (m, 1 H), 4.11 - 3.93 (m, 1 H), 3.91 - 3.52 (m, 3 H), 3.51 - 3.38 (m, 1 H), 3.38 - 3.25 (m, 1 H), 3.22 - 2.88 (m, 1 H), 2.86 - 2.56 (m, 1 H), 2.50 - 2.28 (m, 1 H), 2.19 - 1.98 (m, 1 H), [13]C NMR (100 MHz, D_2O) δ = 166.4, 162.5, 140.2, 137.8, 135.6, 134.5, 133.4, 133.3, 131.2, 130.8, 129.6, 126.9, 125.7, 117.7, 73.1, 55.9, 48.5, 46.3; HRMS: $C_{27}H_{26}ClN_3O_5S$, calculated: 481.1556 $[M+Na]^+$, observed: 481.1663 $[M+Na]^+$.



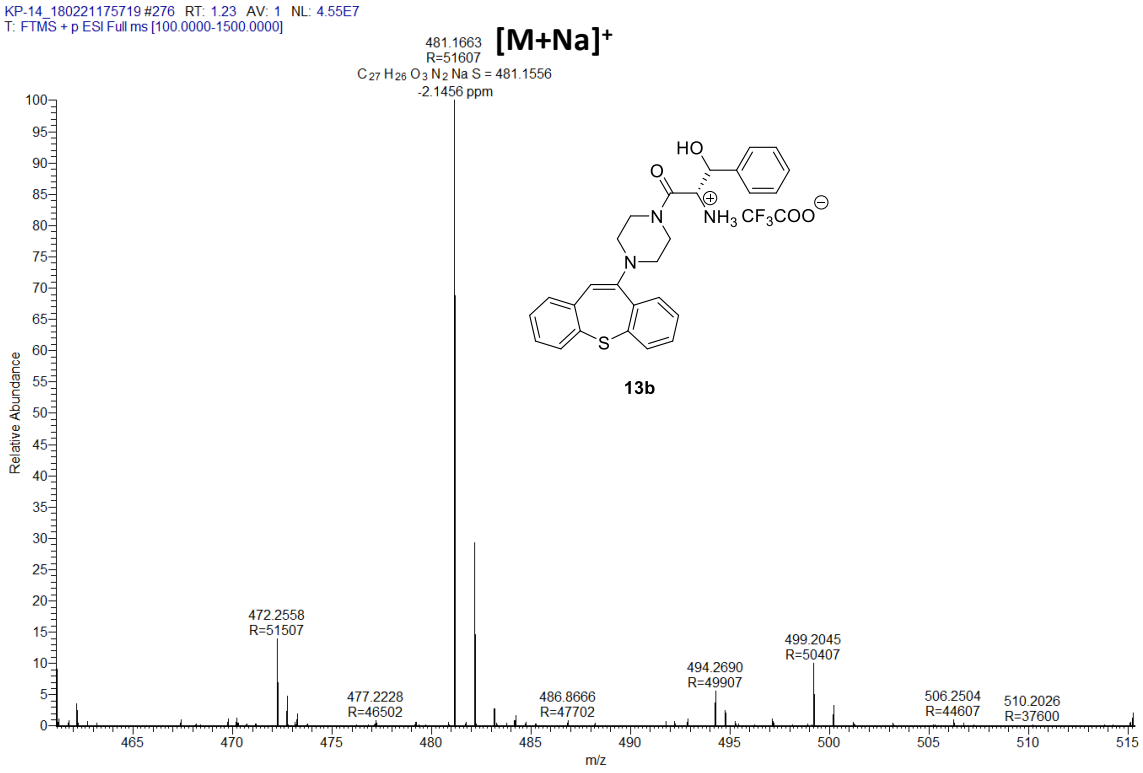
13b



[1]H NMR spectrum of compound **13b** (CD_3OD, 400 MHz, 298 K)

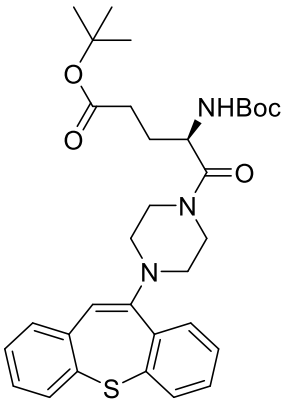


^{13}C NMR spectrum of compound **13b** (CD_3OD, 400 MHz, 298 K)



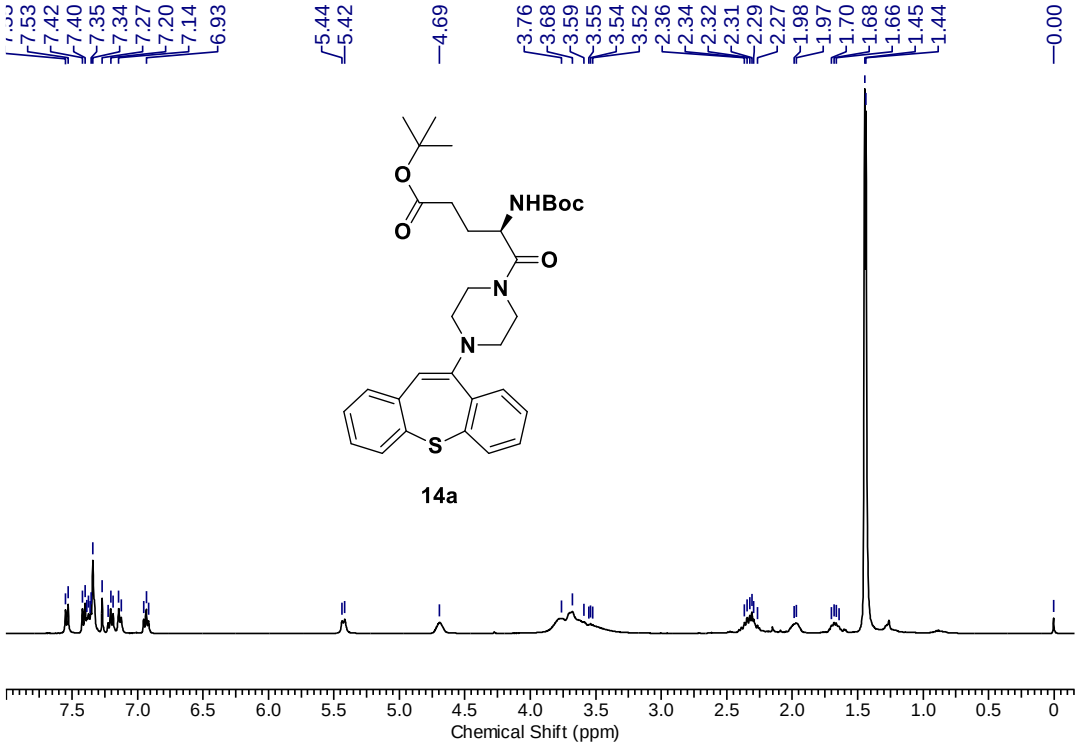
HRMS of Compound **13b**

Compound 14a (tert-butyl (R)-4-((tert-butoxycarbonyl)amino)-5-(4-(dibenzo[b,f]thiepin-10-yl)piperazin-1-yl)-5-oxopentanoate): Boc-Glu(OtBu)OH

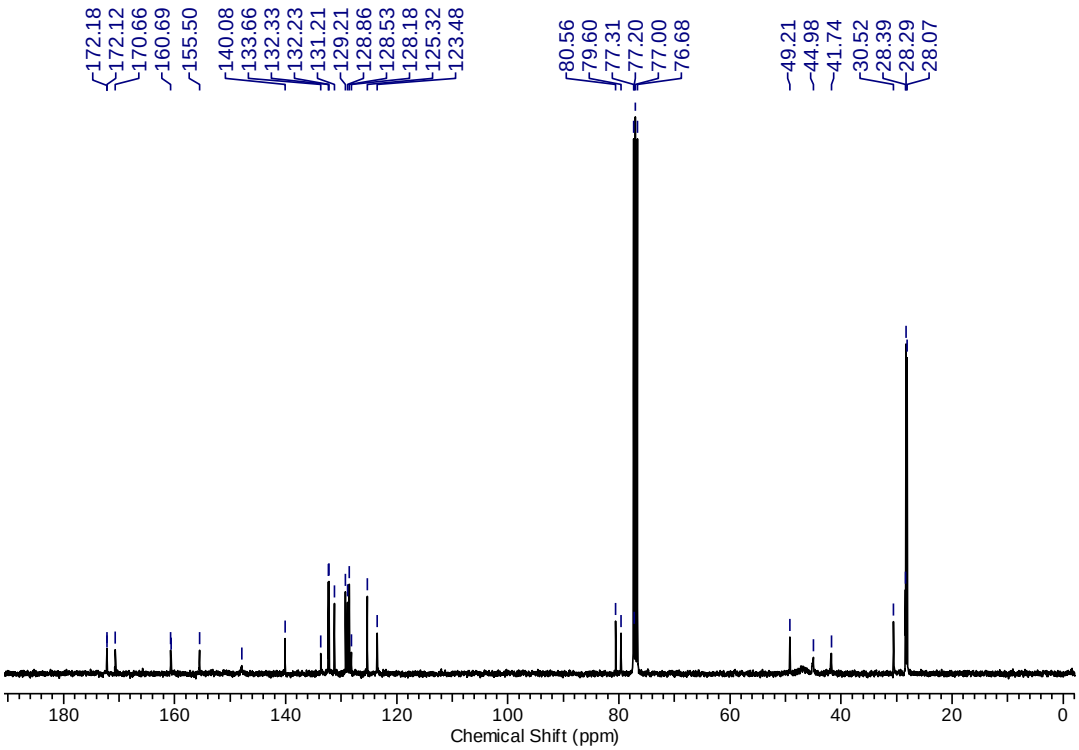


14a

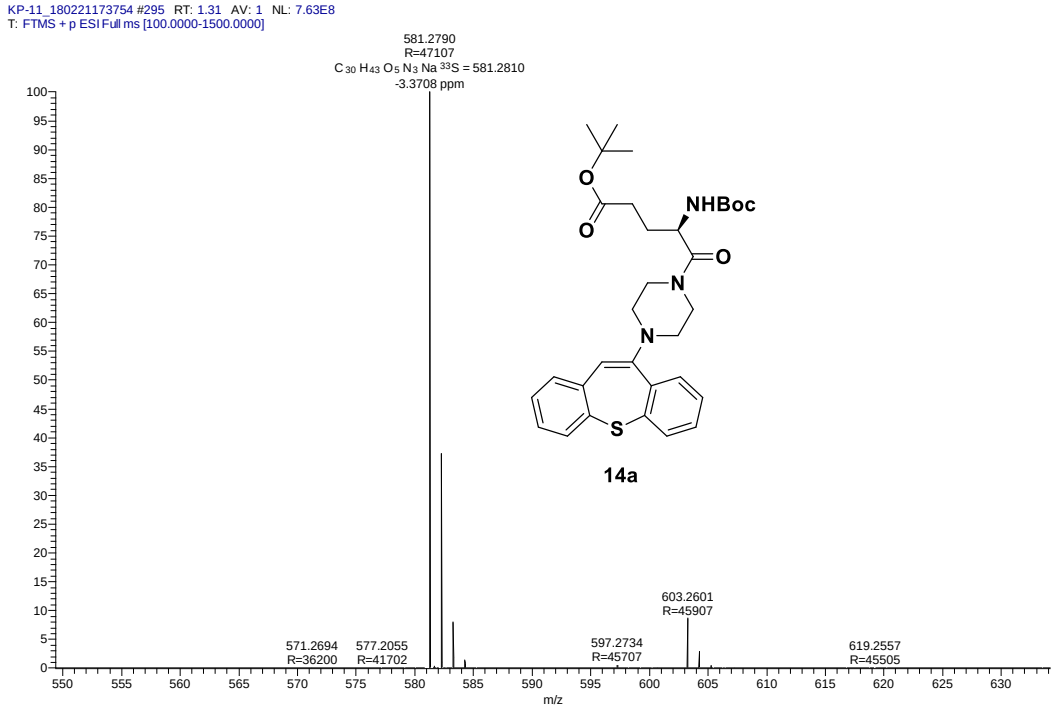
(0.1 g, 0.32 mmol, equiv) was coupled with TFA salt of norquetiapine (0.40 g, 0.98 mmol, 3 equiv) using HATU (0.18 g, 0.49 mmol, 1.5 equiv) and DIPEA (0.3 mL, 1.6 mmol, equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in ethyl acetate and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 20% AcOEt/Petether, Rf : 0.4) to furnish compound **14a** (0.15 g, 78 %) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.54 (d, *J* = 7.3 Hz, 1 H), 7.41 (d, *J* = 7.9 Hz, 1 H), 7.39 - 7.36 (m, 1 H), 7.34 (s, 2 H), 7.23 - 7.17 (m, 1 H), 7.15 - 7.11 (m, 1 H), 6.93 (t, *J* = 7.6 Hz, 1 H), 5.43 (d, *J* = 7.9 Hz, 1 H), 4.69 (br. s., 1 H), 3.89 - 3.44 (m, 8 H), 2.40 - 2.24 (m, 2 H), 1.98 (d, *J* = 6.1 Hz, 1 H), 1.67 (dd, *J* = 8.5, 14.6 Hz, 1 H), 1.44 (d, *J* = 3.7 Hz, 18 H) ^{13}C NMR (100 MHz ,$CDCl_3$) δ = 172.2, 170.7, 160.7, 160.6, 155.5, 147.9, 140.1, 133.7, 132.3, 132.2, 131.2, 129.2, 128.9, 128.5, 128.2, 125.3, 123.5, 80.6, 79.6, 49.2, 45.0, 41.7, 30.5, 28.4, 28.3, 28.1; HRMS: $C_{32}H_{41}N_3O_5S$, calculated: 581.2810 [M+Na]+, observed: 581.2790 $[M+Na]^+$.



^{1}H NMR spectrum of compound **14a** ($CDCl_3$, 400 MHz, 298 K)

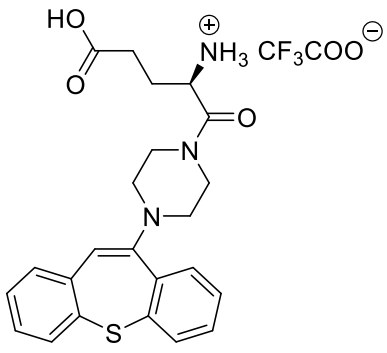


^{13}C NMR spectrum of compound **14a** ($CDCl_3$, 400 MHz, 298 K)

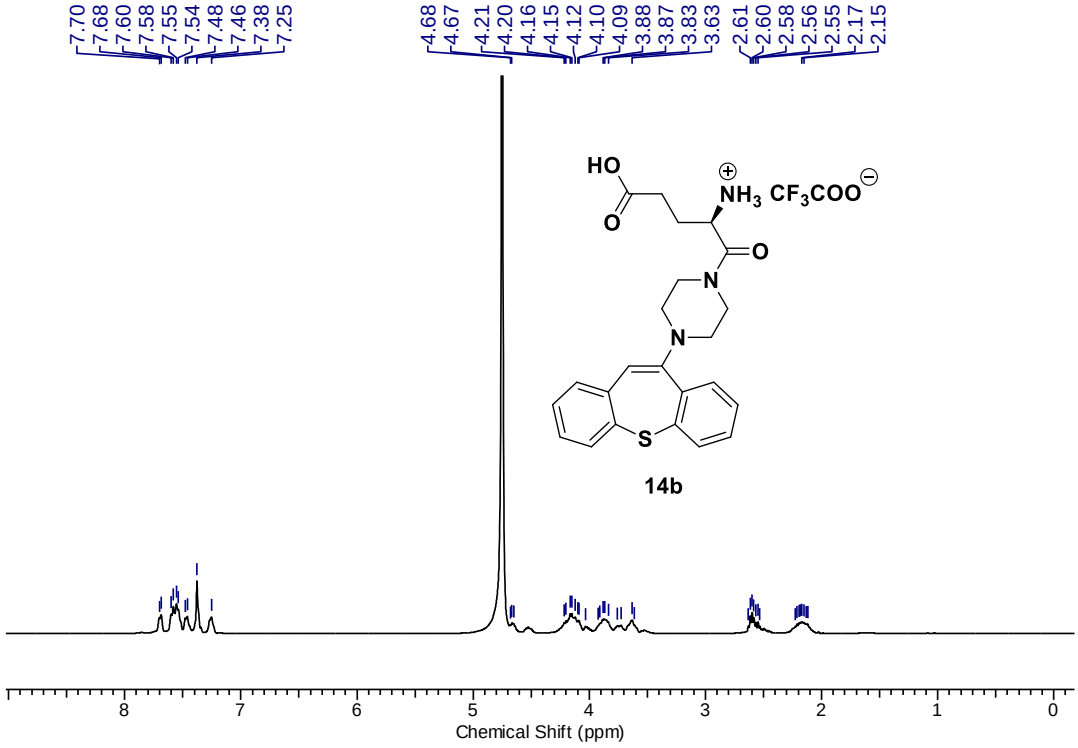


HRMS of Compound **14a**

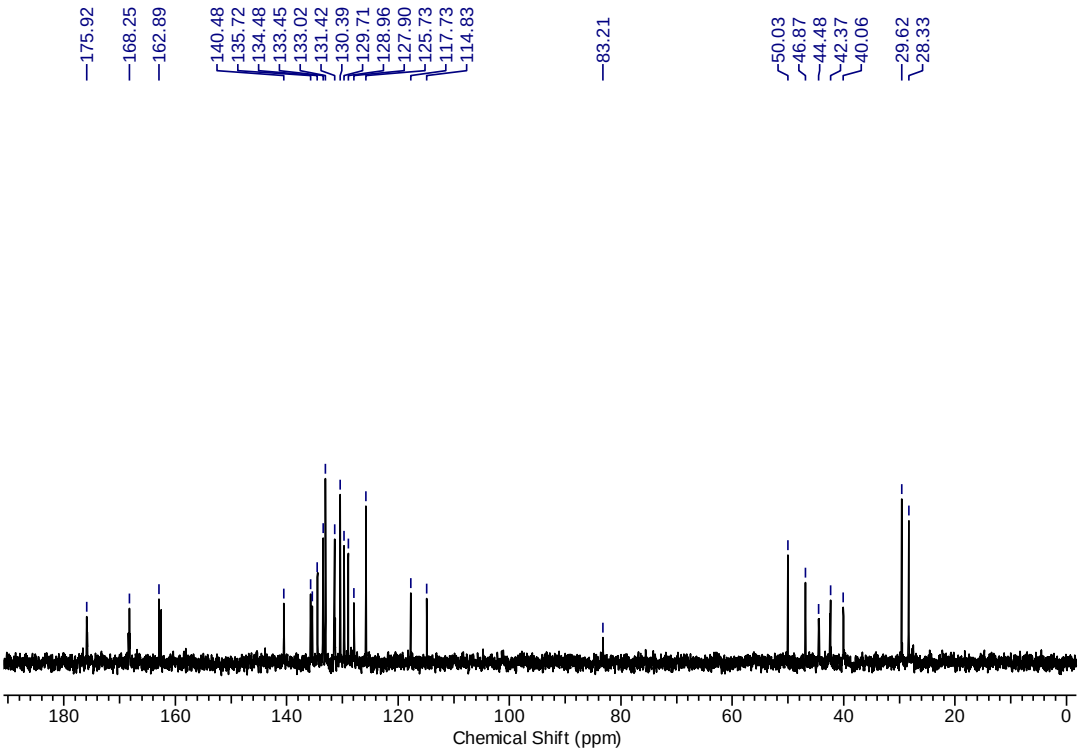
Compound 14b ((R)-4-carboxy-1-(4-(dibenzo[b,f]thiepin-10-yl)piperazin-1-yl)-1-oxobutan-2-aminium 2,2,2-trifluoroacetate): To the solution of compound **14a** (0.12 g, 0.2 mmol) in DCM, TFA (1M) was added and stirred for 30 min. After the completion of the reaction, the volatiles were stripped off and co evaporated with toluene to obtain compound **14b** as a hygroscopic white solid (0.10 g, 90 %).^{1}H NMR (400 MHz, D_2O) δ = 7.73 - 7.66 (m, 1 H), 7.55 (br. s., 3 H), 7.49 - 7.43 (m, 1 H), 7.38 (br. s., 2 H), 7.31 - 7.20 (m, 1 H), 4.70 - 4.41 (m, 2 H), 4.31 - 4.06 (m, 3 H), 4.03 - 3.80 (m, 2 H), 3.78 - 3.53 (m, 2 H), 2.71 - 2.40 (m, 2 H), 2.33 - 2.02 (m, 2 H); ^{13}C NMR (100 MHz, D_2O) δ = 175.9, 168.3, 163.7, 162.9, 162.5, 140.5, 135.7, 135.4, 134.5, 133.4, 133.0, 131.4, 130.4, 129.7, 129.0, 127.9, 125.7, 120.6, 117.7, 114.8, 83.2, 50.0, 49.9, 49.8, 49.4, 49.1, 46.9, 46.6, 46.4, 44.4, 42.5, 42.4, 42.1, 40.1, 29.6, 28.3, 27.3, 25.1, 24.9 ; HRMS: $C_{23}H_{27}N_3O_3S$, calculated: 447.1587$[M+Na]^+$, observed: 447.1455 $[M+Na]^+$.



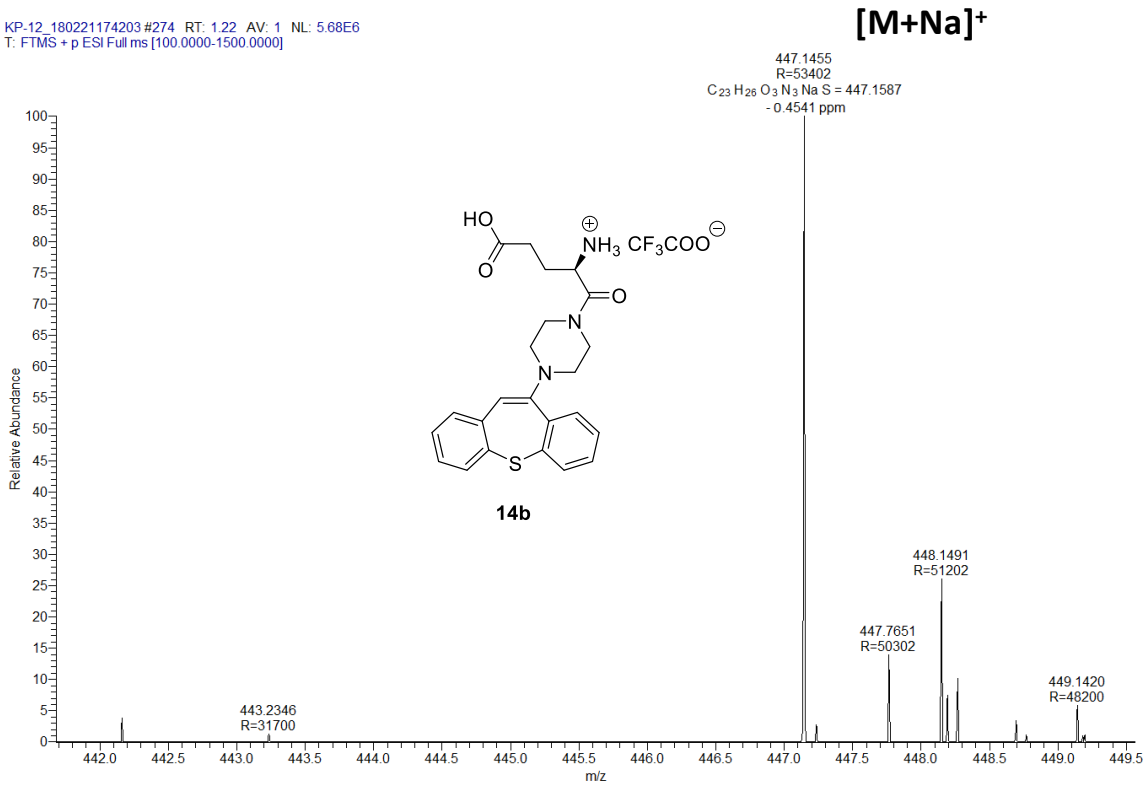
14b



^{1}H NMR spectrum of compound **14b** (D_2O, 400 MHz, 298 K)

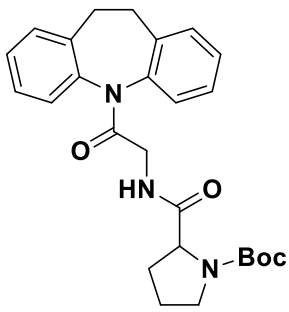


^{13}C NMR spectrum of compound **14b** (D_2O, 400 MHz, 298 K)



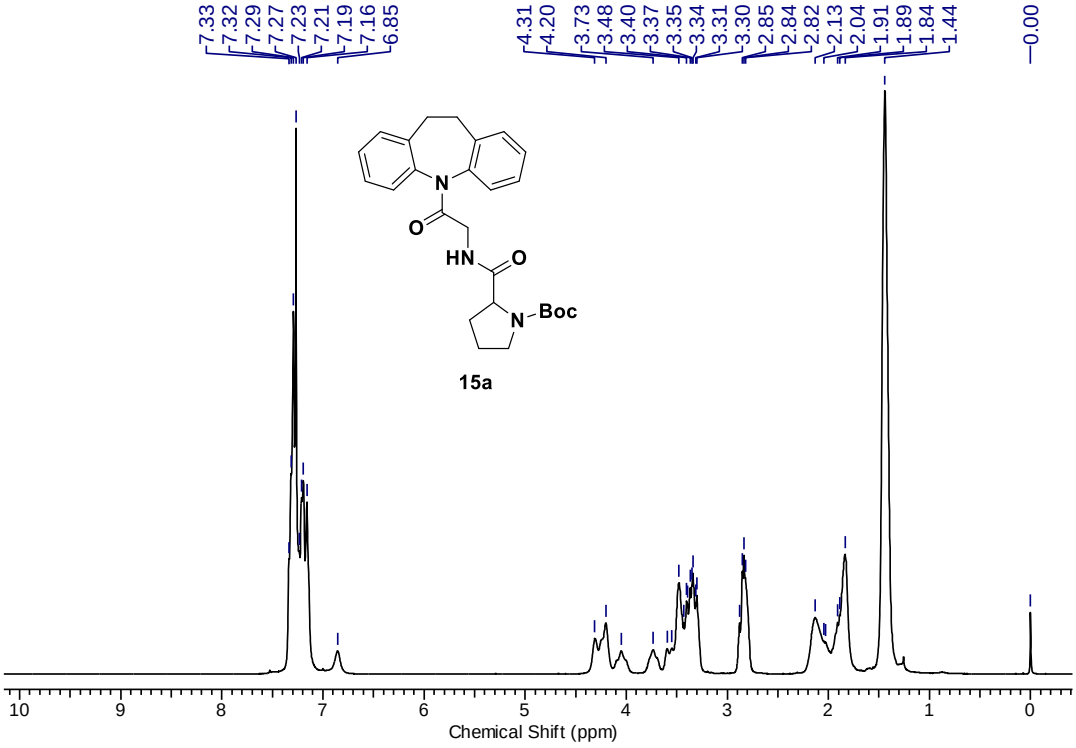
HRMS of Compound **14b**

Compound 15a (tert-butyl 2-((2-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-2-oxoethyl)carbamoyl)pyrrolidine-1-carboxylate): Boc-Pro-OH (0.502 g, mmol, 1.2 equiv) was coupled with dibenzazepine intermediate **4** (0.5 g, 1.98 mmol, 1 equiv) using EDC.HCl (0.49 g, 3.17 mmol, 1.5 equiv), HOBt (0.14 g, 0.91 mmol, 0.5 equiv) and DIPEA (1 mL, 5.9 mmol, 3 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in ethyl acetate and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 20% AcOEt/Petether, Rf : 0.4) to furnish compound **15a** (0.7 g, 78%) as a white solid; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.36 - 7.28 (m, 3 H), 7.24 (br. s., 2 H), 7.23 - 7.12 (m, 3 H), 6.86 (br. s, 1 H), 4.40 - 3.95 (m, 2 H), 3.84 - 3.53 (m, 1 H), 3.53 - 3.25 (m, 4 H),

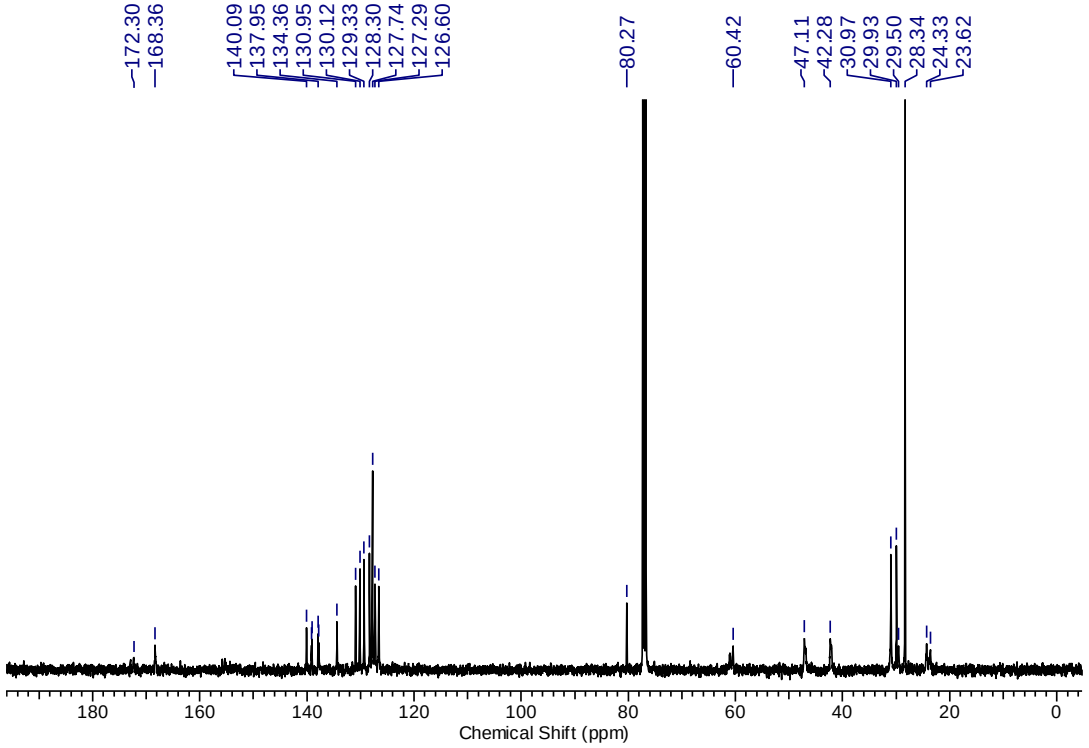


15a

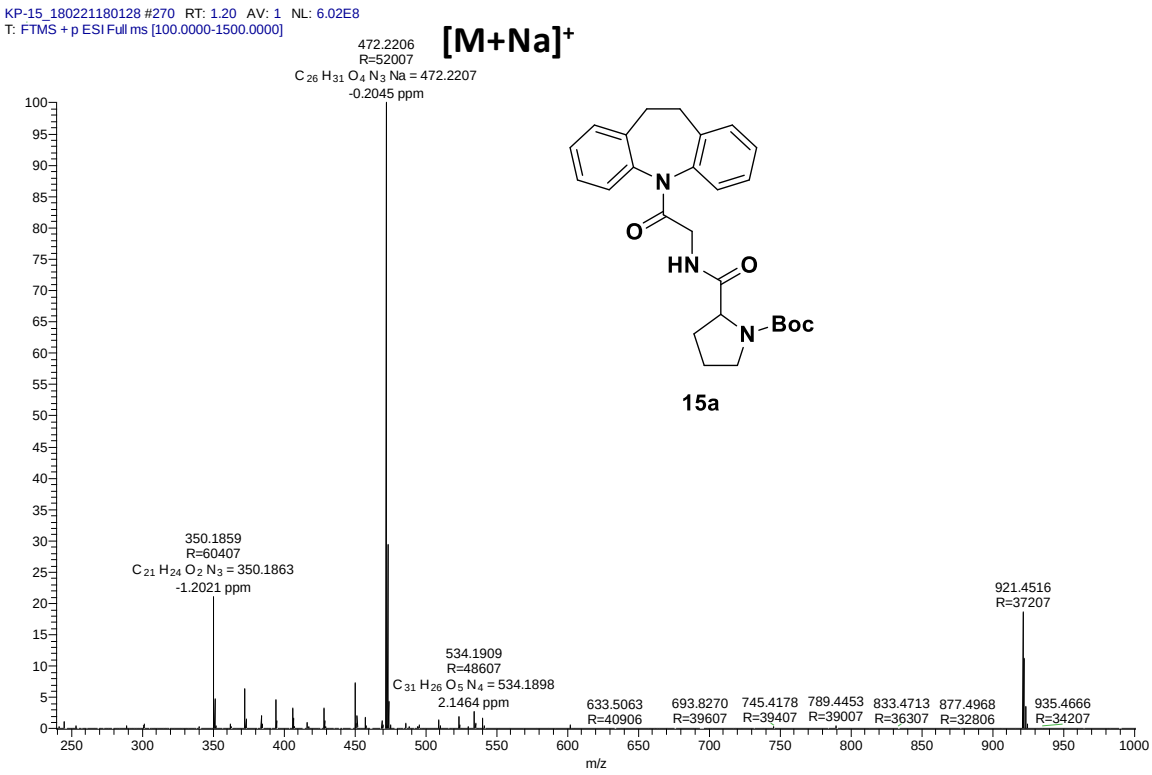
2.93 - 2.74 (m, 2 H), 2.25 - 1.99 (m, 2 H), 1.96 - 1.80 (m, 2 H), 1.45 (br. s., 8 H) ^{13}C NMR (100 MHz, $CDCl_3$)= 172.3, 168.4, 140.1, 139.1, 139.0, 137.9, 137.8, 134.4, 130.9, 130.9, 130.1, 129.3, 128.3, 127.7, 127.3, 126.6, 80.3, 61.0, 60.4, 47.1, 42.3, 31.0, 29.7, 28.3, 24.3, 23.6; HRMS: $C_{26}H_{31}N_3O_4$, calculated: 472.2206 $[M+Na]^+$, observed: 472.2207 $[M+Na]^+$.



^1H NMR spectrum of compound **15a** ($CDCl_3$, 400 MHz, 298 K)

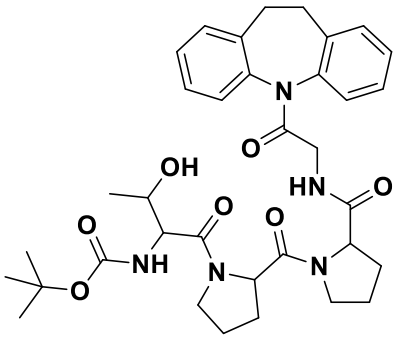


^{13}C NMR spectrum of compound **15a** ($CDCl_3$, 400 MHz, 298 K)

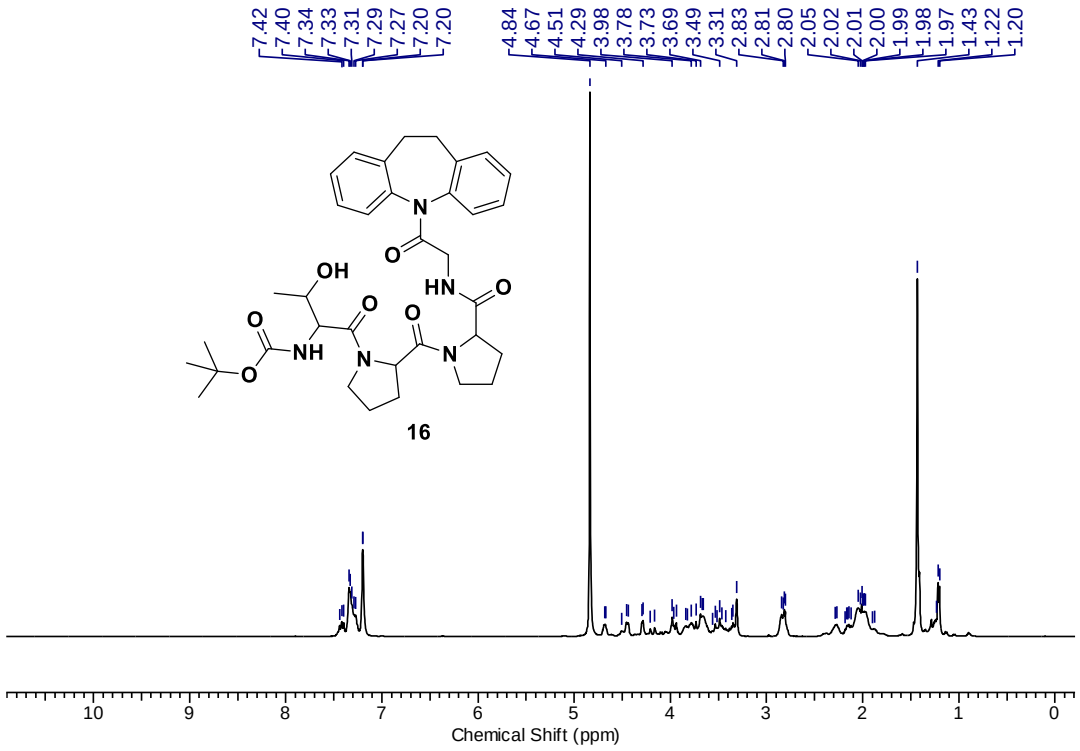


HRMS of Compound **15a**

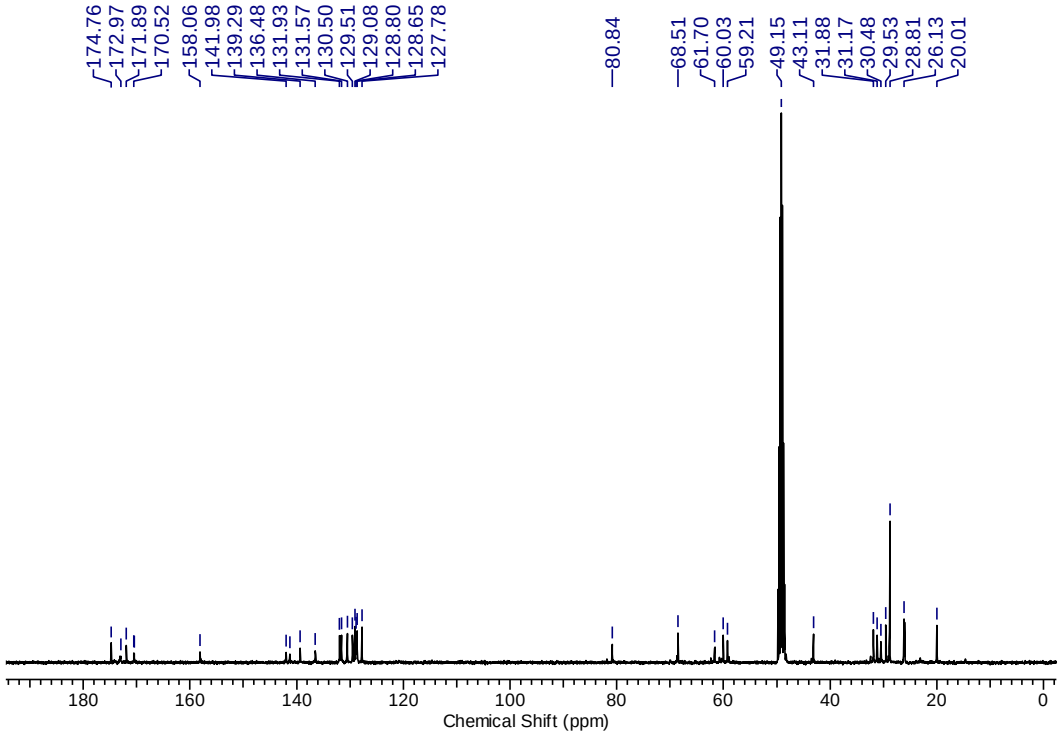
Compound 16 (tert-butyl (1-(2-(2-((2-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-2-oxoethyl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidin-1-yl)-3-hydroxy-1-oxobutan-2-yl)carbamate): To the solution of compound **15a** (0.5 g, 1.11 mmol) in DCM, TFA (1M) was added and stirred for 30 min. After the completion of the reaction, the volatiles were stripped off and co evaporated with toluene to obtain compound **15b** which was used for next step without further purification. Boc-Thr-Pro-OH (0.41 g, 1.30 mmol, 1.2 equiv) was coupled with TFA salt of **15a** (0.35 g, 1.0 mmol, equiv) using HATU (0.57 g, 1.50 mmol, 1.5 equiv) and DIPEA (0.55 mL, 3.0 mmol, equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in ethyl acetate and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 20% AcOEt/Petether, Rf : 0.4) to furnish compound **16** (0.45 g, 70 %) as a white solid; ^{1}H NMR (400 MHz, CD_3OD) = 7.41 (d, J = 8.5 Hz, 1 H), 7.37 - 7.25 (m, 4 H), 7.20 (d, J = 1.8 Hz, 3 H), 4.68 (d, J = 3.1 Hz, 1 H), 4.45 (d, J = 6.1 Hz, 1 H), 4.29 (d, J = 4.3 Hz, 1 H), 4.22 - 4.02 (m, 1 H), 4.01 - 3.91 (m, 1 H), 3.78 (m, 2 H), 3.72 - 3.60 (m, 2 H), 3.56 - 3.41 (m, 2 H), 3.35 (br. s., 1 H), 2.89 - 2.75 (m, 2 H), 2.35 - 2.13 (m, 2 H), 2.10 - 1.86 (m, 6 H), 1.51 - 1.40 (m, 9 H), 1.27 - 1.16 (m, 3 H) ^{13}C NMR (100 MHz, CD_3OD) δ = 174.8, 173.0, 171.9, 170.5, 158.1, 142.0, 141.3, 139.3, 136.5, 131.9, 131.6, 130.5, 129.5, 129.1, 128.8, 128.6, 127.8, 80.8, 68.6, 61.6, 60.8, 60.0, 59.2, 43.1, 32.4, 31.9, 31.2, 30.5, 29.5, 28.9, 26.1, 20.0; HRMS: $C_{35}H_{45}ClN_5O_7$, calculated: 670.3211$[M+Na]^+$, observed: 670.3198 $[M+Na]^+$.



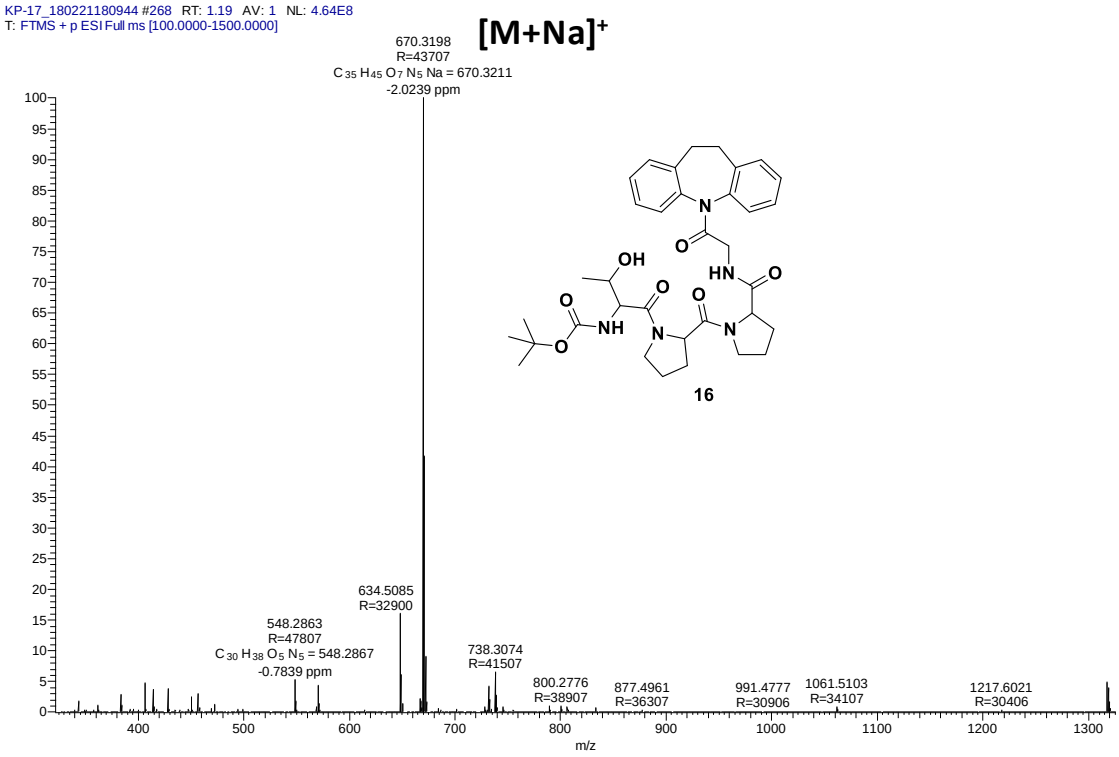
16



^{1}H NMR spectrum of compound **16**(CD_3OD, 400 MHz, 298 K)

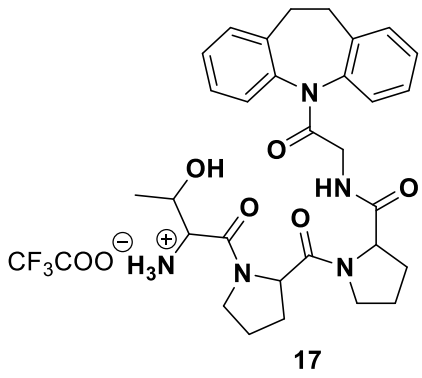


^{13}C NMR spectrum of compound **16**(CD_3OD, 400 MHz, 298 K)

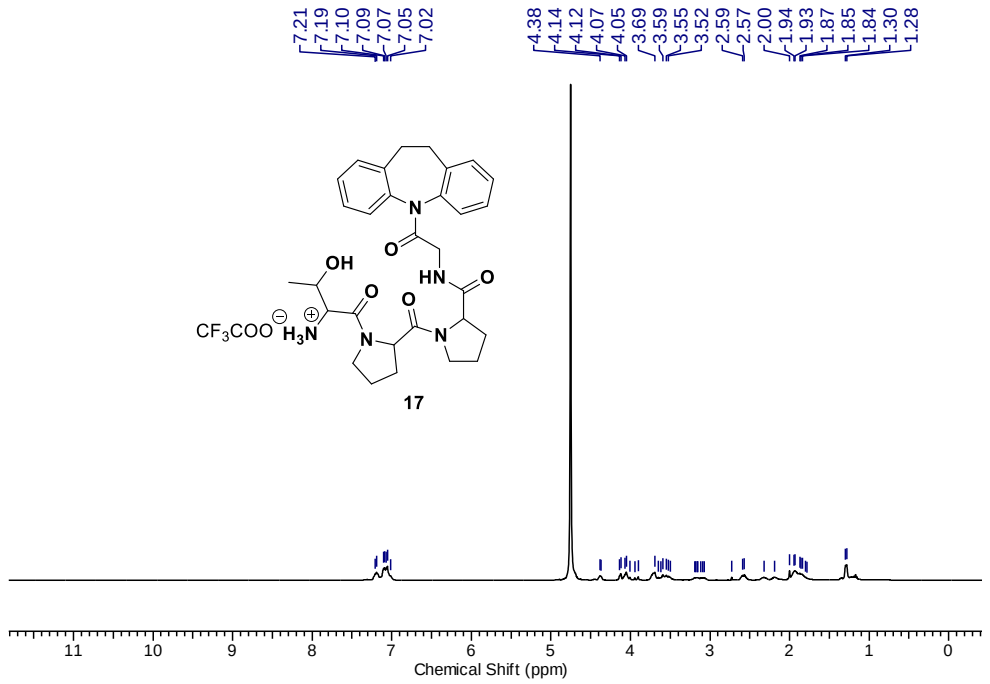


HRMS of Compound **16**

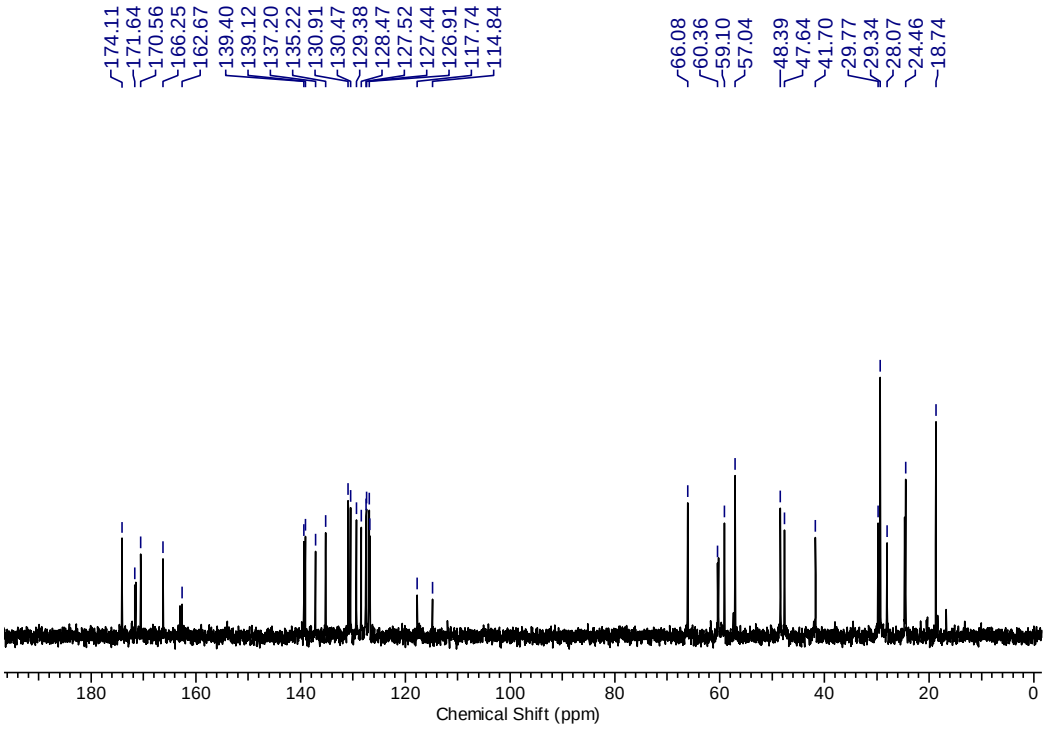
Compound 17 (1-(2-(2-((2-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-2-oxoethyl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidin-1-yl)-3-hydroxy-1-oxobutan-2-aminium 2,2,2-trifluoroacetate): To the solution of compound **16** (0.2 g, 0.3 mmol) in DCM, TFA (1M) was added and stirred for 30 min. After the completion of the reaction, the volatiles were stripped off and co evaporated with toluene to obtain compound **17** as a hygroscopic white solid (0.18 g, 90%).[1]H NMR (400 MHz, D_2O) 7.25 - 7.16 (m, 2 H), 7.15 - 6.97 (m, 6 H), 4.49 - 4.33 (m, 1 H), 4.16 - 4.10 (m, 1 H), 4.09 - 3.97 (m, 2 H), 3.96 - 3.85 (m, 1 H), 3.76 - 3.66 (m, 2 H), 3.66 - 3.44 (m, 3 H), 3.25 - 3.01 (m, 2 H), 2.66 - 2.46 (m, 2 H), 2.37 - 2.13 (m, 2 H), 2.01 - 1.80 (m, 6 H), 1.29 (d, J = 5.5 Hz, 3 H); ^{13}C NMR (100 MHz, D_2O) δ = 174.1, 171.5, 170.6, 166.3, 162.7, 139.4, 139.1, 137.2, 135.2, 131.0, 130.5, 129.4, 128.5, 127.5, 127.4, 126.9, 126.8,



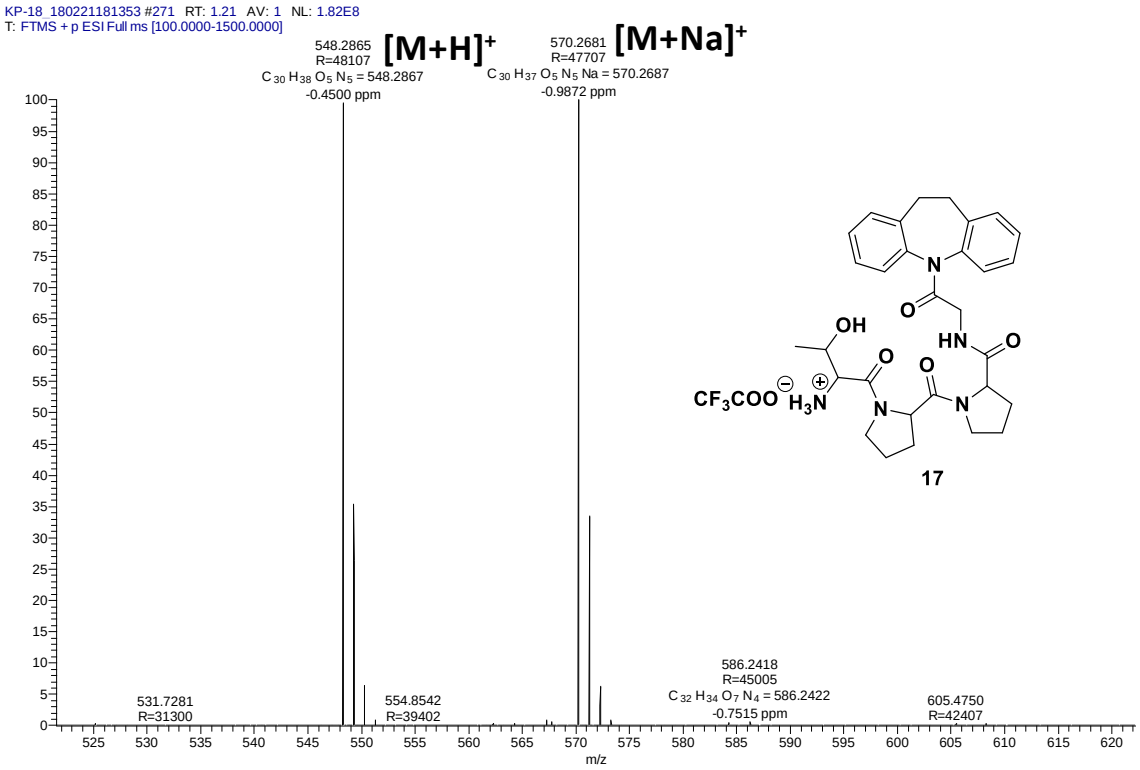
117.7, 114.8, 66.1, 60.2, 59.1, 57.0, 48.4, 47.6, 41.7, 29.8, 29.3, 28.1, 24.6, 24.5, 18.7; HRMS: $C_{23}H_{27}N_3O_3S$, calculated: 548.2865 $[M+H]^+$, observed: 425.2867 $[M+H]^+$.



^{1}H NMR spectrum of compound **17** (D_2O, 400 MHz, 298 K)

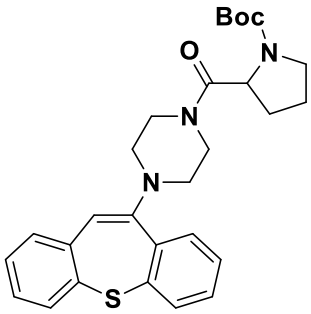


^{13}C NMR spectrum of compound **17** (D_2O, 400 MHz, 298 K)



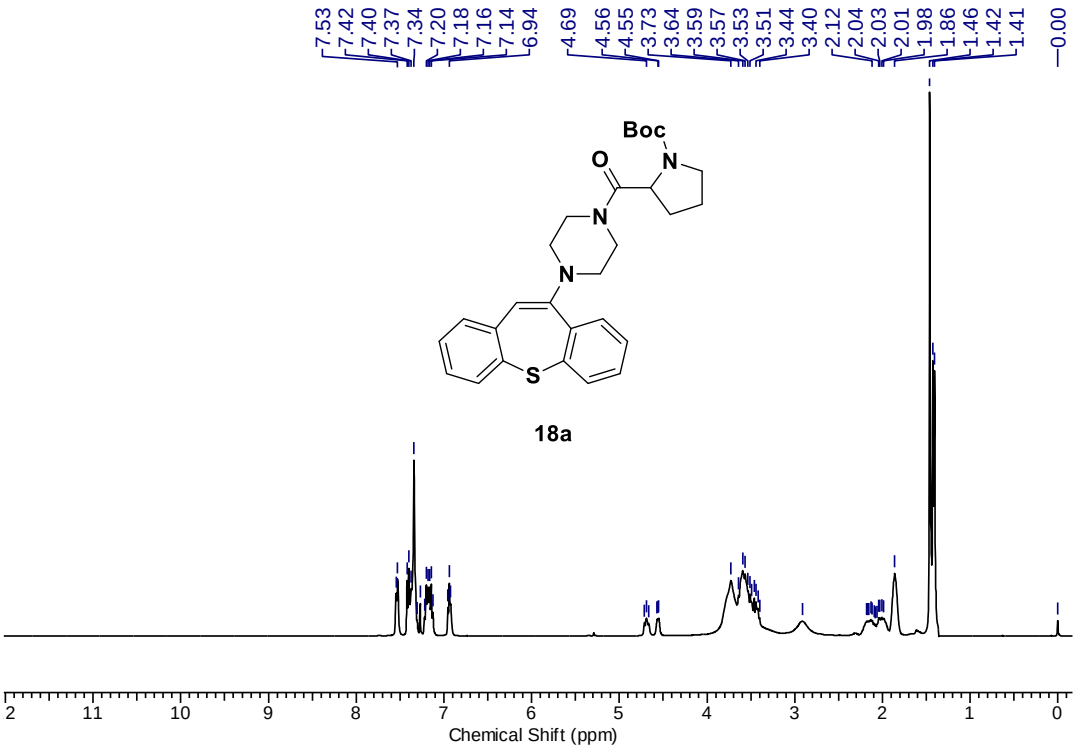
HRMS of Compound **17**

Compound 18a (tert-butyl 2-(4-(dibenzo[b,f]thiepin-10-yl)piperazine-1-carbonyl)pyrrolidine-1-carboxylate): Boc-Pro-OH (0.5 g, 2.3 mmol, 1 equiv) was coupled with norquetiapine **10** (2.09 g, 5.1 mmol, 2.2equiv) using HATU (1.3 g, 3.4 mmol, 1.5 equiv), and DIPEA 1.2 (mL, 6.9 mmol, 3 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in ethyl acetate and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 20% AcOEt/Petether, Rf : 0.4) to furnish compound **18a** (0.8 g, 72 %) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.54 (d, J = 6.7 Hz, 1 H), 7.44 - 7.29 (m, 4 H), 7.24 - 7.10 (m, 2 H), 6.94 (t, J = 6.7 Hz, 1 H), 4.76 - 4.49 (m, 1 H), 3.85 - 3.47 (m, 8 H), 3.47 - 3.39 (m, 1 H), 2.91 (br. s., 2 H), 2.23 - 1.93 (m, 2 H),

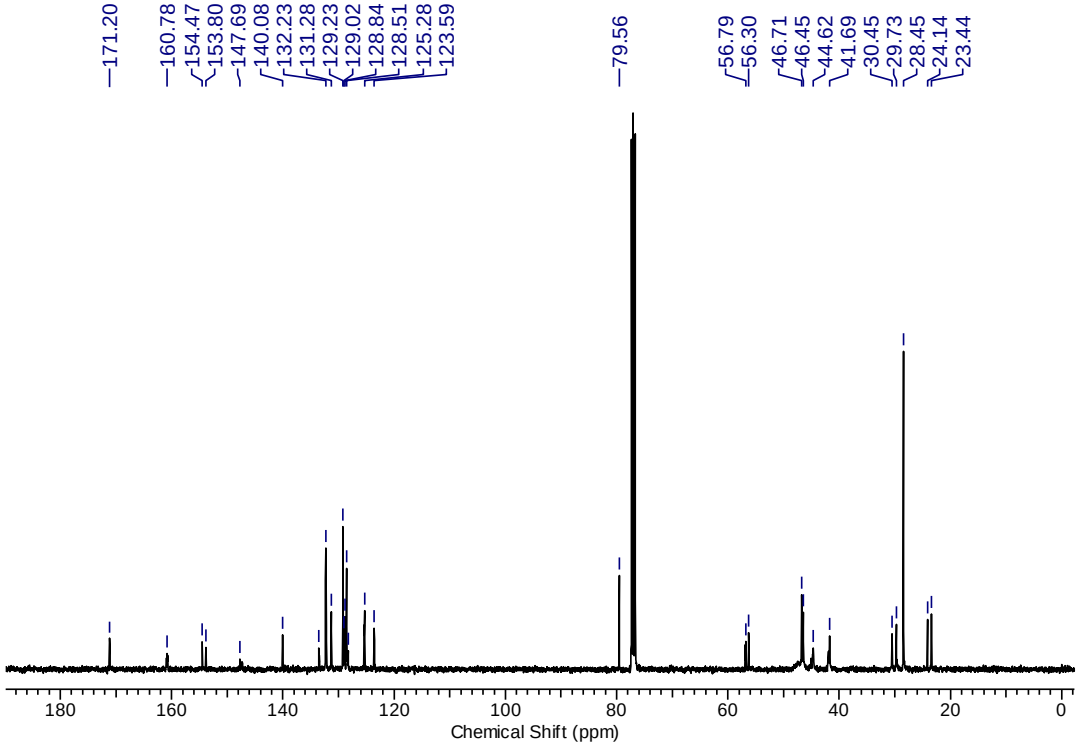


18a

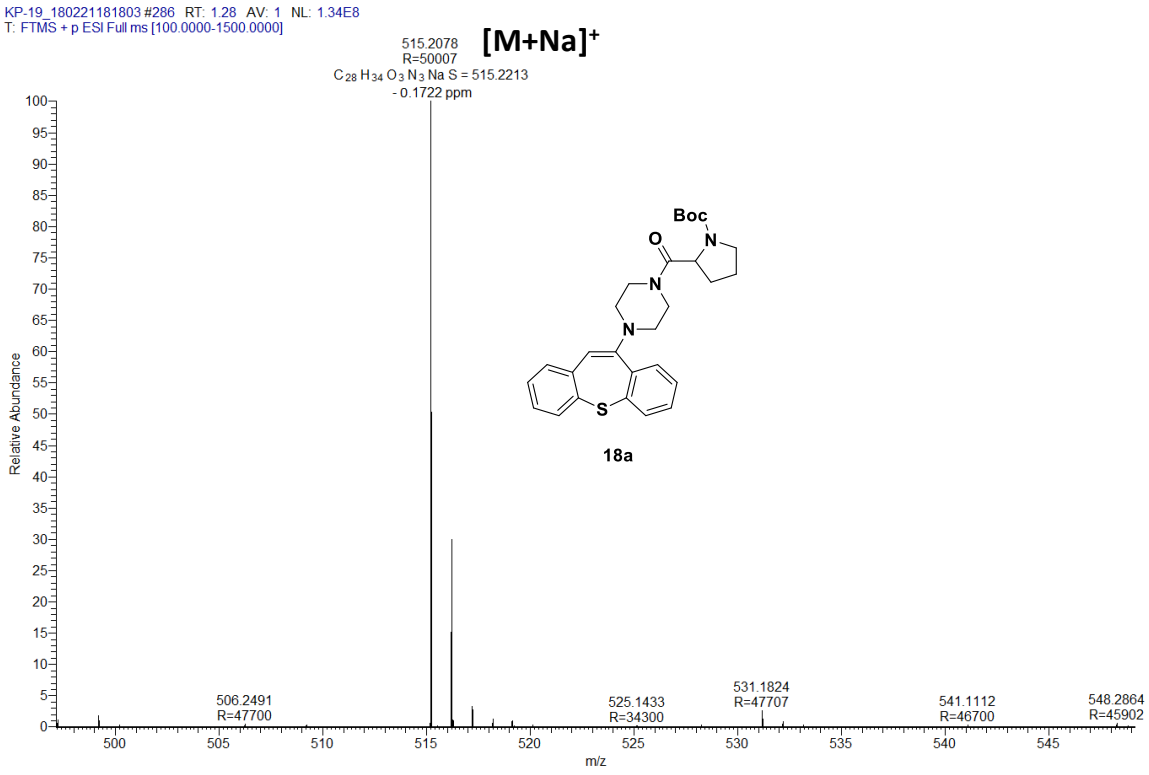
1.86 (br. s., 2 H), 1.52 - 1.33 (m, 9 H) ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.2, 160.8, 154.5, 153.8, 147.7, 140.1, 133.5, 132.3, 131.3, 129.2, 129.0, 128.8, 128.5, 128.2, 125.3, 123.6, 79.6, 56.8, 56.3, 46.7, 46.4, 44.6, 41.7, 30.5, 29.7, 28.4, 24.1, 23.4; HRMS: $C_{28}H_{34}N_3O_3$, calculated: 515.2213 $[M+Na]^+$, observed: 515.2078 $[M+Na]^+$.



^{1}H NMR spectrum of compound **18a** ($CDCl_3$, 400 MHz, 298 K)

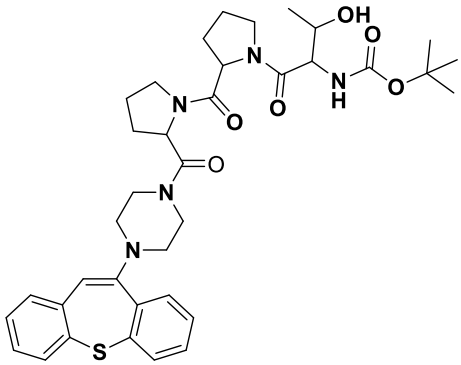


^{13}C NMR spectrum of compound **18a** ($CDCl_3$, 400 MHz, 298 K)

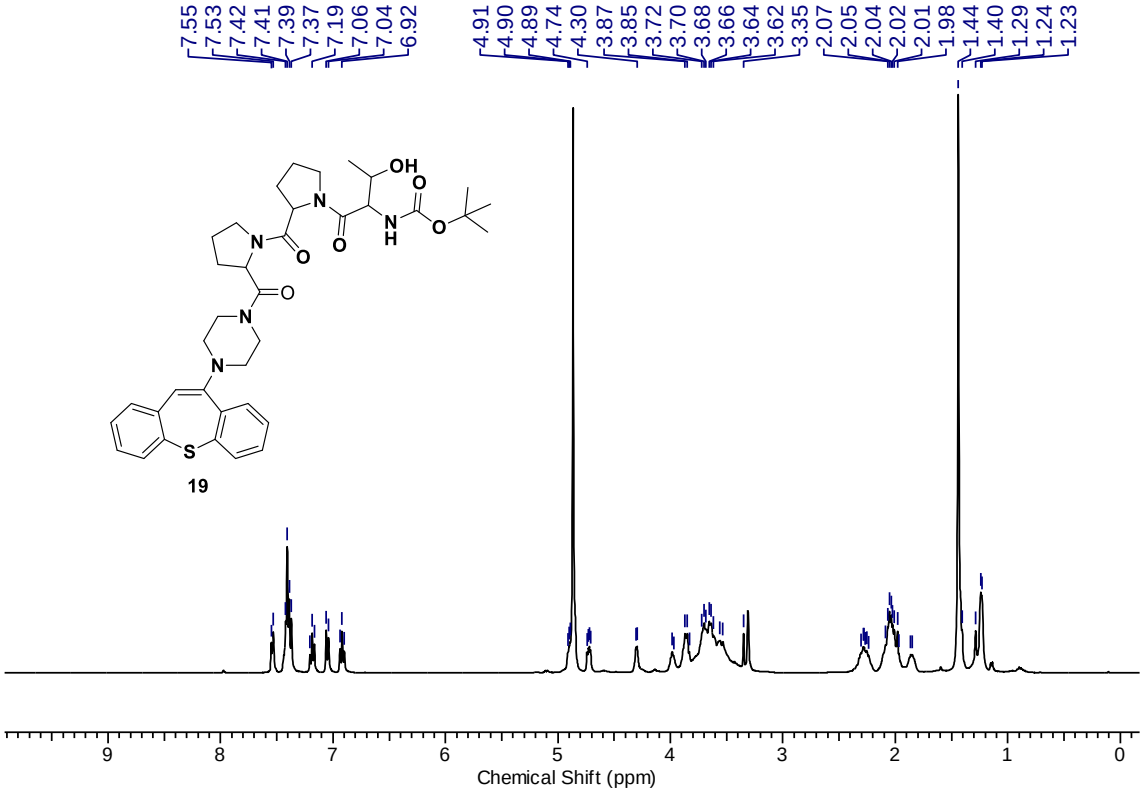


HRMS of Compound **18a**

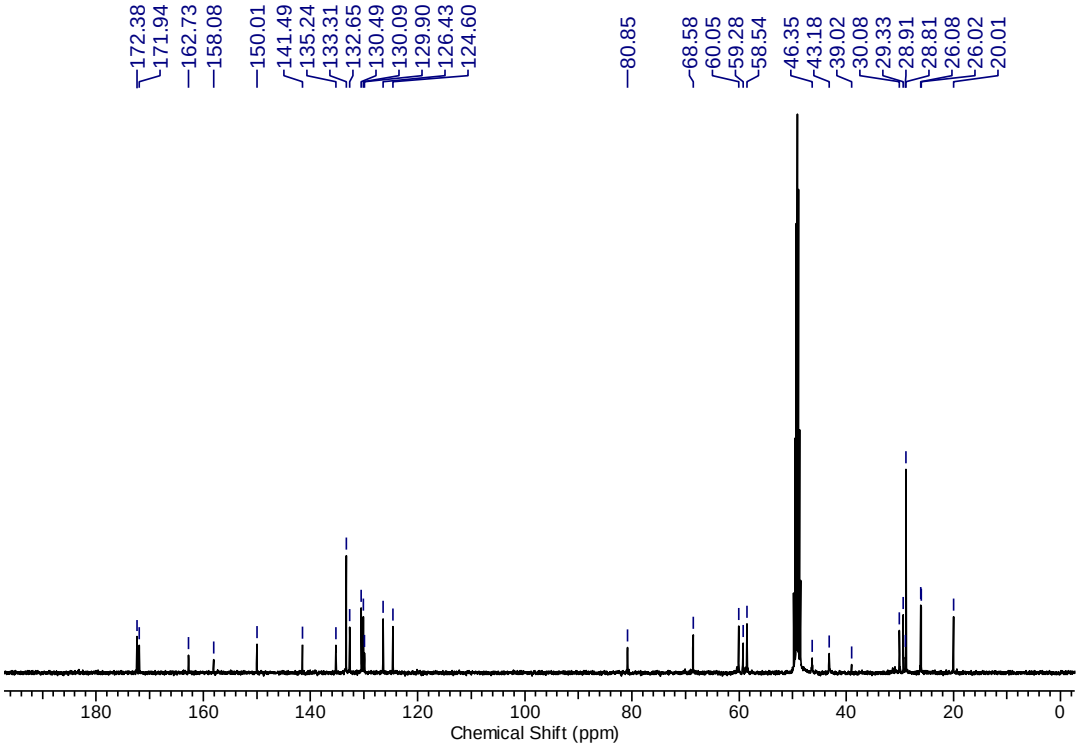
Compound 19 (tert-butyl (1-(2-(2-(4-(dibenzo[b,f]thiepin-10-yl)piperazine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidin-1-yl)-3-hydroxy-1-oxobutan-2-yl)carbamate: To the solution of compound **18a** (0.5 g, 1.0 mmol) in DCM, TFA (1M) was added and stirred for 30 min. After the completion of the reaction, the volatiles were stripped off and co evaporated with toluene to obtain compound **18b** which was used for next step without further purification. Boc-Thr-Pro-OH (0.18 g, 0.5 mmol, 1 equiv) was coupled with TFA salt of **18a** (0.26 g, 0.60 mmol, 1.5 equiv) using HATU (0.28 g, 0.75 mmol, 1.5 equiv) and DIPEA (0.27 mL, 1.5 mmol, 3 equiv) in DMF for 12 h at room temperature. The reaction mixture was then taken in ethyl acetate and organic layer was washed sequentially with saturated $KHSO_4$, $NaHCO_3$ and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which was further purified by column chromatography (eluent: 20% AcOEt/Petether, Rf : 0.4) to furnish compound **19** (0.25 g, 72 %) as a white solid; ^{1}H NMR (400 MHz, CD_3OD) = 7.54 (d, J = 7.9 Hz, 1 H), 7.46 - 7.34 (m, 4 H), 7.23 - 7.15 (m, 1 H), 7.05 (d, J = 7.9 Hz, 1 H), 6.92 (t, J = 7.6 Hz, 1 H), 4.73 (dd, J = 4.6, 7.6 Hz, 1 H), 4.30 (d, J = 4.9 Hz, 1 H), 3.98 (d, J = 4.9 Hz, 1 H), 3.91 - 3.82 (m, 2 H), 3.80 - 3.44 (m, 10 H), 3.35 (s, 1 H), 2.39 - 2.19 (m, 2 H), 2.18 - 1.78 (m, 7 H), 1.51 - 1.38 (m, 9 H), 1.23 (d, J = 2.4 Hz, 3 H) ^{13}C NMR (100 MHz, CD_3OD) δ = 172.4, 171.9, 162.7, 158.1, 150.0, 141.5, 135.2, 133.3, 132.6, 130.5, 130.1, 129.9, 126.4, 124.6, 80.8, 68.6, 60.0, 59.3, 58.5, 46.3, 43.2, 39.0, 30.1, 29.3, 28.8, 26.0, 20.0 HRMS: $C_{47}H_{48}N_5O_6NaS$, calculated: 713.3018 $[M+Na]^+$, observed: 713.3079 $[M+Na]^+$.



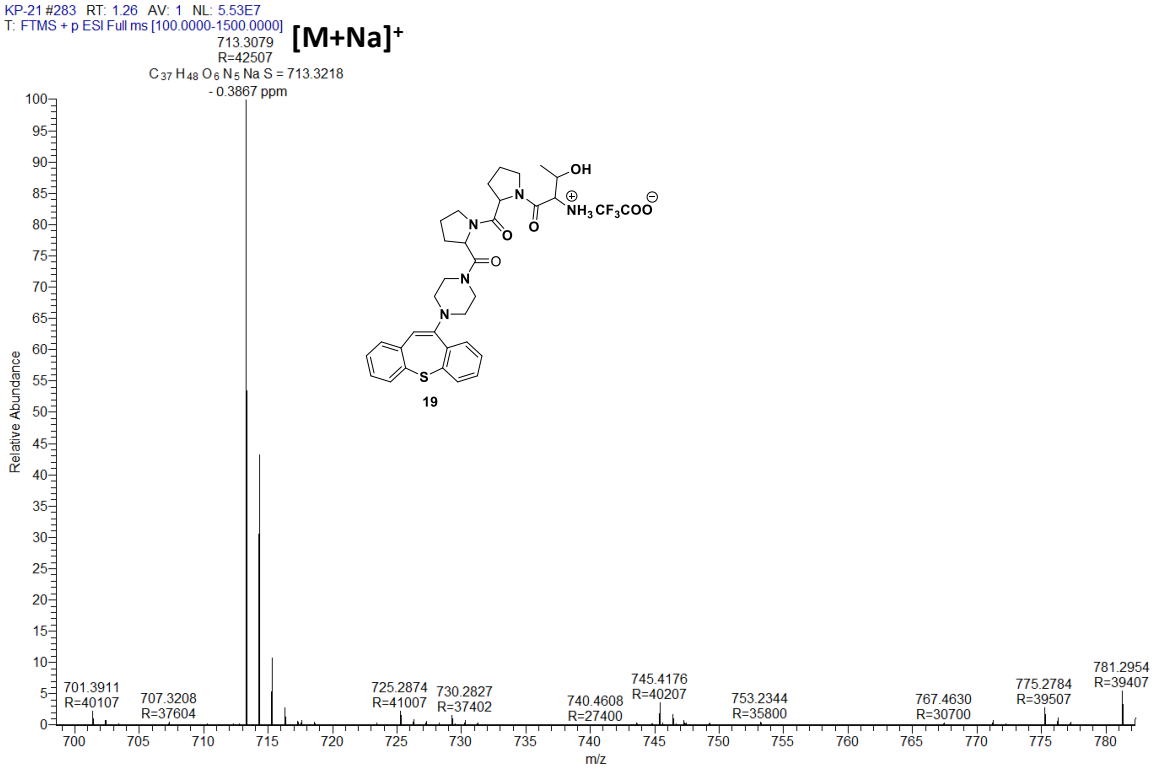
19



^{1}H NMR spectrum of compound **19** (CD_3OD, 400 MHz, 298 K)

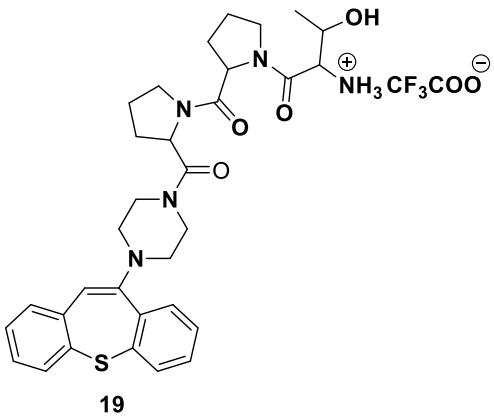


^{13}C NMR spectrum of compound **19** (CD_3OD, 400 MHz, 298 K)

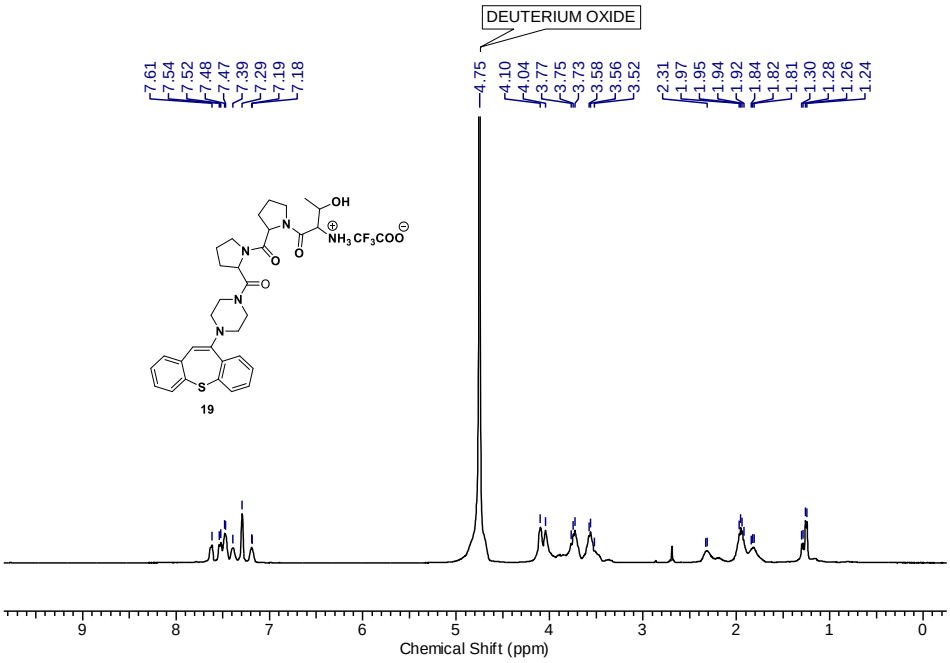


HRMS of Compound **19**

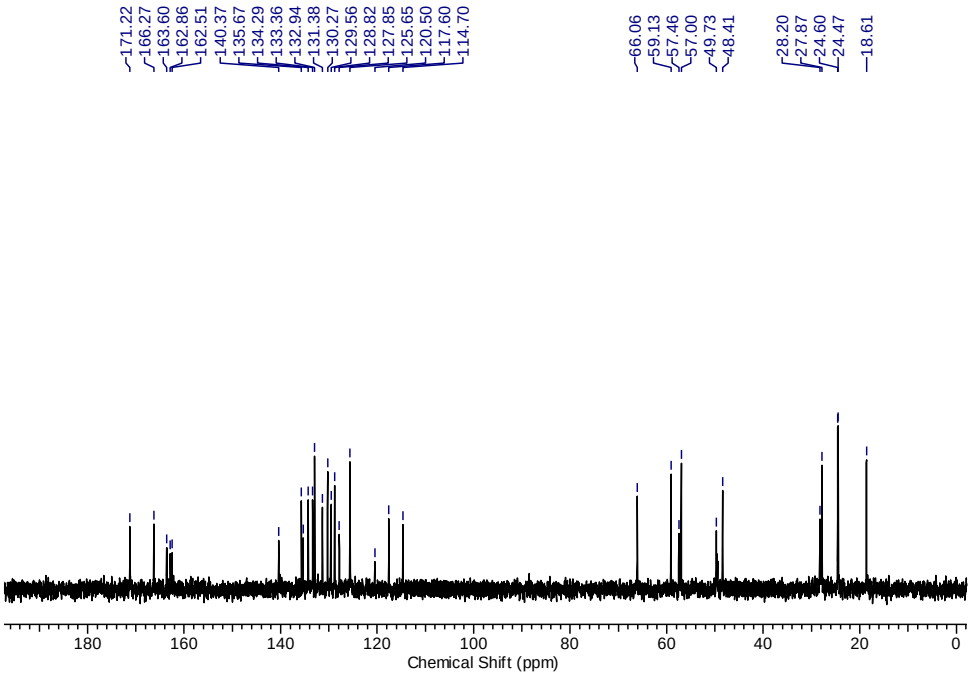
Compound 20 (1-(2-(2-(4-(dibenzo[b,f]thiepin-10-yl)piperazine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidin-1-yl)-3-hydroxy-1-oxobutan-2-aminium 2,2,2-trifluoroacetate): To the solution of compound **19** (0.2 g, 0.2 mmol) in DCM, TFA (1M) was added and stirred for 30 min. After the completion of the reaction, the volatiles were stripped off and co evaporated with toluene to obtain compound **20** as a hygroscopic white solid (0.18 g, 90 %). ^{1}H NMR (400 MHz, D_2O) δ = 7.67 - 7.59 (m, 1 H), 7.57 - 7.50 (m, 1 H), 7.50 - 7.43 (m, 2 H), 7.43 - 7.35 (m, 1 H), 7.29 (br. s., 2 H), 7.23 - 7.14 (m, 1 H), 4.18 - 3.93 (m, 6 H), 3.73 (br. s., 4 H), 3.64 - 3.42 (m, 4 H), 2.39 - 2.22 (m, 2 H), 1.95 (d, *J* = 6.7 Hz, 4 H), 1.88 - 1.70 (m, 2 H), 1.25 (d, *J* = 5.5 Hz, 3 H) ^{13}C NMR (100 MHz, D_2O) δ = 171.2, 166.3, 163.6, 162.9, 140.4, 135.7, 135.4, 134.3,



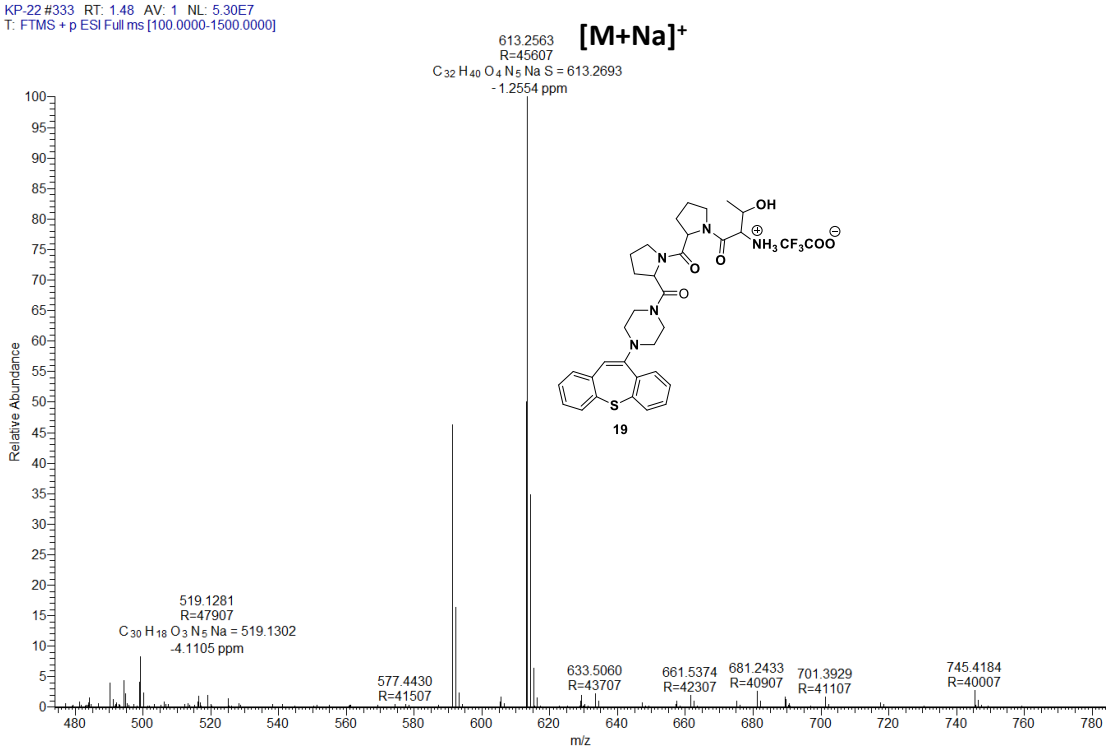
133.4, 132.9, 131.4, 130.3, 129.6, 128.8, 125.6, 117.6, 114.7, 57.5, 56.9, 49.7, 48.4, 27.9, 24.6, 18.6; HRMS: $C_{32}H_{40}N_5O_4NaS$, calculated: 613.2693$[M+Na]^+$, observed: 613.2559 $[M+Na]^+$.



^{1}H NMR spectrum of compound **20** (D_2O, 400 MHz, 298 K)



^{13}C NMR spectrum of compound **20** (D_2O, 400 MHz, 298 K)



HRMS of Compound **20**

3.8 References and Notes:

1. Owens, D.C., **2014**. *A guide to the extrapyramidal side-effects of antipsychotic drugs*. Cambridge University Press. http://assets.cambridge.org/97805216/33536/excerpt/9780521633536_excerpt.pdf

2. Rose, N., **2004.** Becoming neurochemical selves. *Biotechnology, commerce and civil society*, pp.89-128.

3. Trindade, E., Menon, D., Topfer, L.A. and Coloma, C., Adverse effects associated with selective serotonin reuptake inhibitors and tricyclic antidepressants: a meta-analysis. *Can. Med. Assoc. J.* **1998**, *159*, 1245-1252.

4. (a) Arroll, B., Macgillivray, S., Ogston, S., Reid, I., Sullivan, F., Williams, B. & Crombie, I. *Ann Fam Med.* **2005**, *3(5)*, 449-455. (b) Grisel, J. E., Rasmussen, P. R. & Sperry, L. *J Individ Psychol.* **2006**, *62(4)*, 397-413 (c)

Solomon, D. A., Keller, M. B., Leon, A. C, Mueller, T. I., Lavori, R W., Shea, M. T , Coryell, W., Warshaw, M., Turvey, C , Maser, J. D., & Endicott, J. *Am. J. Psychiatry*. **2000**, *157*, 229-233.

5. Mitchell, P.B. and Mitchell, M.S. *Australian family physician*, **1994,** *23*, 1771-3**.**

6. Broquet, K.E. *South. Med. J*. **1999**, *92*, 846-856.

7. Teicher, M.H., Glod, C.A. and Cole, J.O. *Drug Saf.* **1993**, *8*, 186-212.

8. (a) Ghose, K., **2013** Antidepressants for Elderly People. *Springer*. pp.182-; (b) Aronson, J.K., **2009** Meyler's side effects of psychiatric drugs. *Elsevier*. pp. 7-; (c) Anthony, P.K., **2002** Pharmacology secrets. *Elsevier Health Sciences*,pp.39-.

9. Roth, BL; Driscol, J. "PDSP Ki Database". **2017,** Psychoactive Drug Screening Program (PDSP). University of North Carolina at Chapel Hill and the United States National Institute of Mental Health.

10. (a) "Quetiapine-fumarate". The American Society of Health-System Pharmacists. **2011,** https://www.drugs.com/monograph/quetiapine-fumarate.html. (b) Komossa, K., Depping, A.M., Gaudchau, A., Kissling, W. and Leucht, S., **2010,** Second-generation antipsychotics for major depressive disorder and dysthymia. *Cochrane Libr*.

11. Gugger, J. J. and Cassagnol, M. Low-dose quetiapine is not a benign sedative-hypnotic agent. *Am J Addict.* **2008**, *17*, 454-455.