Studies Toward Bio-active Macrocyclic Peptides: Teixobactin, Pseudoxylallemycin B, Arthroamide and Fusaristatin C

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In

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By

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Dedicated

To My

Beloved Parents

And

Abhijit

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Thesis Certificate

This is to certify that the work incorporated in this Ph.D. thesis entitled **"Studies toward bio-active macrocyclic peptides: teixobactin, pseudoxylallemycin B, arthroamide and fusaristatin C"** submitted by **Ms. Vidya B. Gunjal** to Academy of Scientific and Innovative Research (AcSIR) in fulfilment of the requirements for the award of the Degree of **Doctor of Philosophy**, embodies original research work under my supervision. I further certify that this work has not been submitted to any other University or Institution in part or full for the award of any degree or diploma. Research material obtained from other sources has been duly acknowledged in the thesis. Any text, illustration, table etc., used in the thesis from other sources, have been duly cited and acknowledged.

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Vidya B. Gunjal	**Dr. D. Srinivasa Reddy**
(Research Student)	**(Research Supervisor)**

Date: 02 July 2019

Place: Pune

Declaration by the Candidate

I hereby declare that the original research work embodied in this thesis entitled, **"Studies toward bio-active macrocyclic peptides: teixobactin, pseudoxylallemycin B, arthroamide and fusaristatin C"** submitted to Academy of Scientific and Innovative Research for the award of degree of **Doctor of Philosophy (Ph.D.)** is the outcome of experimental investigations carried out by me under the supervision of **Dr. D. Srinivasa Reddy**, Senior Principal Scientist, Organic Chemistry Division, CSIR-National Chemical Laboratory, Pune. I affirm that the work incorporated is original and has not been submitted to any other academy, university or institute for the award of any degree or diploma.

July 2019
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Pune-411 008

Vidya B. Gunjal
(Research Student)

During the long period of my research work, I have been acquainted, accompanied and supported by many people. It is a pleasant aspect that I have now the opportunity to express my gratitude to all of them.

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I also place on record, my sense of gratitude to one and all, who directly or indirectly, have lent their hand in this venture.

Above all, I thank the Almighty for His enormous blessings.

Vidya Gunjal

Abbreviations

AcOH	acetic acid
Ac_2O	acetic anhydride
Å	angstrom
Ar	aryl
AMP	antimicrobial peptide
MeCN	acetonitrile
Bn	benzyl
Boc	tertiary-butyloxycarbonyl
brs	broad singlet
Bu	butyl
^{t}Bu	tertiary-butyl
calcd.	Calculated
cm^{-1}	1/centimeter
C–C	carbon-carbon
C–H	carbon-hydrogen
C–N	carbon-nitrogen
C–O	carbon-oxygen
CH_2Cl_2	dichloromethane
$CHCl_3$	chloroform
DEA	diethyl amine
DMTMM	4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride

Abbreviations

DIC	N,N′-Diisopropylcarbodiimide
DIPEA	diisoproyl ethyl amine
DMAP	4-dimethyl aminopyridine
DMF	N,N-dimethylformamide
DMSO	dimethylsulphoxide
DMSO-d_6	deuterated dimethylsulphoxide
dd	doublet of doublet
d	doublet (in NMR) or day(s) (in Scheme)
ee	enantiomeric excess
Et	ethyl
EtOAc	ethyl acetate
EtOH	ethanol
EDT	1,2-ethanedithiol
equiv	equivalent
EDC.HCl	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
FDPP	Pentafluorophenyl diphenylphosphinate
Fmoc	9-Fluorenylmethoxycarbonyl
g	gram(s)
h	hour(s)
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate

Abbreviations

HOBt	Benzotriazol-1-ol
HOAt	1-Hydroxy-7-azabenzotriazole
HPLC	High Performance Liquid Chromatography
HRMS	High resolution mass spectrometry
Hz	hertz
IR	infrared
ichip	isolation chip
J	coupling constant (in NMR)
mass (ESI)	electron spray ionization mass spectroscopy
min	minute(s)
m	multiplet
mL	milliliter(s)
mmol	millimole(s)
mp	melting point
m/z	mass to charge ratio
Me	methyl
MHz	megahertz
MeOH-d_4	deuterated methanol
MRSA	Methicillin-resistant *Staphylococcus aureus*
MNBA	2-Methyl-6-nitrobenzoic anhydride
MALDI	Matrix assisted laser desorption ionization

MIC	minimum inhibitory concentration
N	normality
nM	nanomolar(s)
NMR	nuclear magnetic resonance
NMM	N-methyl morpholine
Oxyma	Ethyl (*2Z*)-2-cyano-2-hydroxyiminoacetate
Ph	phenyl
ppm	parts per million
Pr	propyl
PyBOP	(Benzotriazol-1-yloxy)tripyrrolidinophosphon hexafluorophosphate
q	quartet
Py	pyridine
R_f	retention factor
rt	room temperature
s	singlet
SN	nucleophilic substitution
sec	secondary
t	triplet
tert	tertiary
TBS	tert-Butyldimethyl silyl chloride
TEA	triethyl amine

Abbreviations

THF	tetrahydrofuran
TFA	trifluroacetic acid
TFAA	trifluroacetic anhydride
TLC	thin layer chromatography
TEA	triethyl amine
T3P	propylphosphonic anhydride
Ts	para-toluenesulphonyl
TBAF	tetra butyl ammonium fluoride
TFMSA	Trifluoromethanesulfonic acid
UV	ultraviolet
v/v	volume by volume
wt/v	weight by volume
Z (Cbz)	Benzyl chloroformate
°C	degree celsius
µM	micromolar
mg	Milligram
µmol	Micromolar
in vitro	Outside a living organism
in vivo	Inside a living organism
Ala	alanine
Ile	isoleucine

Gln	glutamine
Val	valine
Phe	phenylalanine
Ser	Serine
Lys	lysine
Leu	leucine
Tyr	tyrosine
End	L-*allo*-enduracididine
Arg	arginine
Thr	threonine
Met	methionine
Cys	cysteine
Orn	ornithine
Pro	proline
QS	Quorum sensing
2-CTC	2-chlorotrityl chloride
Trt	Trityl
TIPS	Triisopropyl silane

- All reagents, starting materials, and solvents were obtained from commercial suppliers and used as such without further purification however solvents were dried using standard protocols or dried using MBRAUN (MB SPS-800) instrument.
- Reactions were carried out in oven-dried glassware under a positive pressure of argon unless otherwise mentioned with magnetic stirring.
- Air sensitive reagents and solutions were transferred via syringe or cannula and were introduced to the apparatus *via* rubber septa.
- The progress of reactions was monitored by thin layer chromatography (TLC) with 0.25 mm pre-coated silica gel plates (60 F254) and visualization was accomplished with either UV light, Iodine adsorbed on silica gel or by immersion in ethanolic solution of phosphomolybdic acid (PMA), *p*-anisaldehyde or $KMnO_4$ followed by heating with a heat gun for ~15 sec.
- Column chromatography was performed on silica gel (100-200 or 230-400 mesh size).
- All the melting points are uncorrected and recorded using a scientific melting point apparatus (Buchi B-540).
- Deuterated solvents for NMR spectroscopic analyses were used as received. All ^{1}H NMR and ^{13}C NMR analysis were obtained using a 200 MHz, 400 MHz, 500 MHz spectrometer. Coupling constants were measured in Hertz. All chemical shifts are quoted in ppm, relative to TMS, using the residual solvent peak as a reference standard. The following abbreviations are used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad.
- HRMS (ESI) were recorded on ORBITRAP mass analyser (Q Exactive) and MALDI were recorded on MALDI-TOF-TOF mass spectrometer
- Infrared (IR) spectra were recorded on a FT-IR spectrometer as thin films in chloroform using NaCl plates.
- Optical rotations were recorded on a P-2000 polarimeter at 589 nm (sodium D-line).
- Chemical nomenclature (IUPAC) and structures were generated using Chem Bio Draw Ultra.
- The purity of products was determined by reverse phase HPLC analysis using Agilent technologies 1200 series; column: ZORBAX Eclipse XBD-C_{18} or sunfire® C_{18} (4.6 X

250 mm, 5 µm). Flow rate 1.00 mL/min, UV 220 nm and 254 nm; using mobile phases, 95/5, ACN/H_2O (0.1% TFA) as linear gradient.

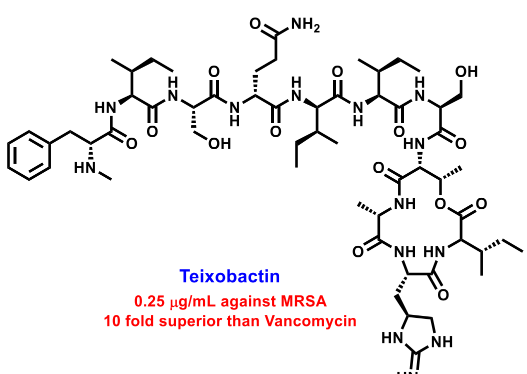
Synopsis

	Synopsis of the Thesis to be submitted to the Academy of Scientific and Innovative Research for Award of the Degree of Doctor of Philosophy in Chemistry
Name of the Candidate	Ms. Vidya B. Gunjal
Degree Enrolment No. & Date	Ph. D in Chemical Sciences (10CC14J26016); January 2014
Title of the Thesis	Studies toward bio-active macrocyclic peptides: teixobactin, pseudoxylallemycin B, arthroamide and fusaristatin C
Research Supervisor	Dr. D. Srinivasa Reddy

The thesis is divided into three chapters. **Chapter 1** is subdivided into two parts; with the introduction to macrocyclic peptide teixobactin and defining objectives of the project followed by efforts toward total synthesis and synthesis of teixobactin analogues, including total synthesis of Met_{10}-teixobactin and their biological evaluation against 'ESKAPE' pathogens. **Chapter 2** describes the role of macrocyclic tetrapeptides in drugs discovery followed by total synthesis of macrocyclic tetrapeptide 3-*epi*-pseudoxylallemycin B. **Chapter 3** deals with the efforts toward synthesis of arthroamide and lipodepsipeptide fusaristatin C.

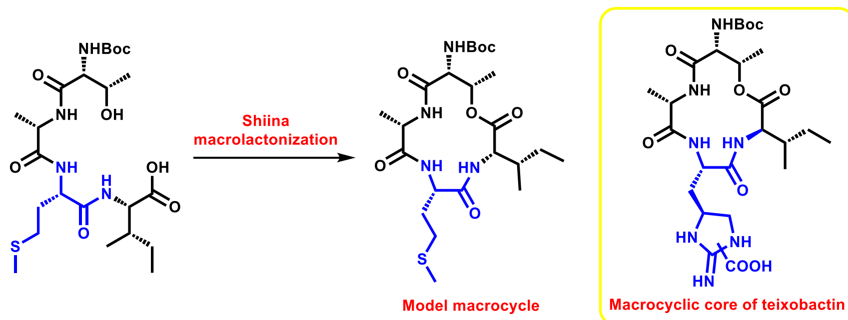
Chapter 1: Design, Synthesis and Biological Evaluation of Potent Antibiotic Peptide Natural Product Teixobactin Analogues

Easy access and overuse of antibiotic drugs leads to the development of resistance against concerned bacteria, which has eventually leading to a global threat and thus, there is a need for new antibiotics with novel modes of action.



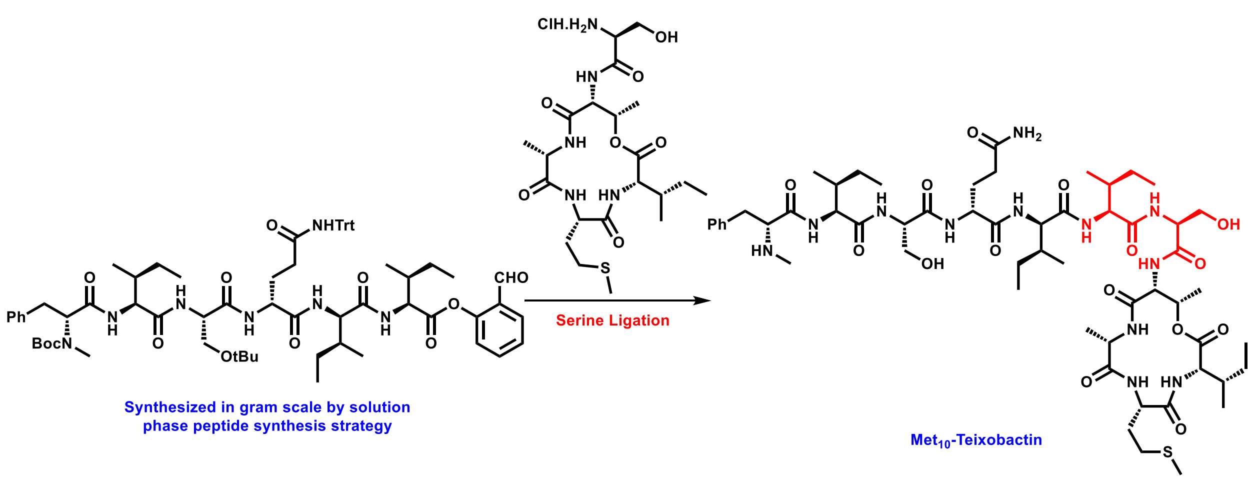
Teixobactin
0.25 μg/mL against MRSA
10 fold superior than Vancomycin

In 2016, Ling and co-workers isolated ‘game changing’ antibiotic teixobactin[1] which exhibited ten-fold better activity than well-known antibiotic vancomycin (*in vivo*) and especially without any detectable resistance when tested in rodent model over 27 days which fascinates synthetic and medicinal chemistry community. Teixobactin is 11-amino acid depsipeptide with 4 unusual amino acids and a rare L-*allo*-enduracididine (End) amino acid which was found to be a key structural feature for its impressive potency. Till date, three total syntheses and several analogues syntheses have been published in the literature which reflects the importance of this molecule.



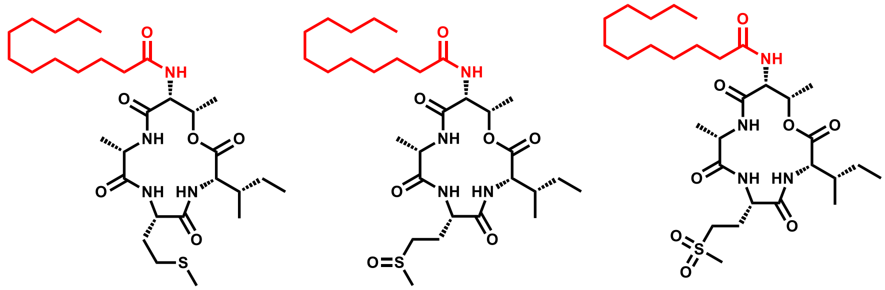
Immediately after isolation in 2016, we prepared the macrocyclic core of this molecule along with model macrocycle in which enduracididine was replaced with methionine using solution phase approach and employing Shiina macrolactonization as a key step.[2]

Total synthesis of Met$_{10}$-teixobactin



In the total synthesis of Met$_{10}$-teixobactin, we carried out solution phase synthesis of linear hexapeptide fragment (gram scale) with salicylate ester. This SAL-hexapeptide on convergent way of serine ligation with methionine macrocycle afforded the target compound Met_{10}-teixobactin which was characterized by ^{1}H, ^{13}C NMR and HRMS data.

Lipopeptides with linear or cyclic peptide sequence are a special class of highly active antibiotics against multi-resistant bacteria. In this aspect, Nowick and Jamison group prepared lipidated teixobactin analogues which were found to retain comparable activity to that of native teixobactin. By developing such lipopeptidomimetics with lipids of different lengths and substituting the synthetically complex End with amino acids of different side-chain functionality, deciphered the role of teixobactin analogues as a potential membrane anchor. With the aim to further explore its SAR, we have synthesized a small library of teixobactin analogues substituting Met in the place of L-*allo*-enduracididine and the linear peptide part with fatty acids of varying chain lengths. In few analogues we also modified the pre-synthesized methionine macrocyclic core with the corresponding sulphones as well as sulphoxides.



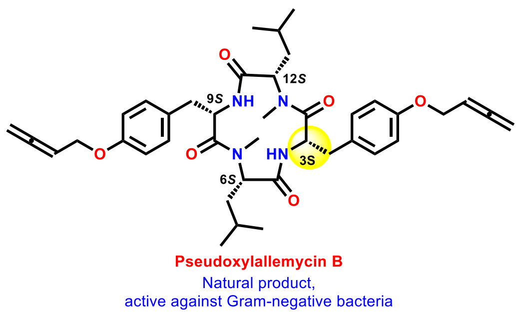
NDS=101103 MIC= 8 μg/mL against *S. aureus*

NDS=101104 MIC= 32 μg/mL against *S. aureus*

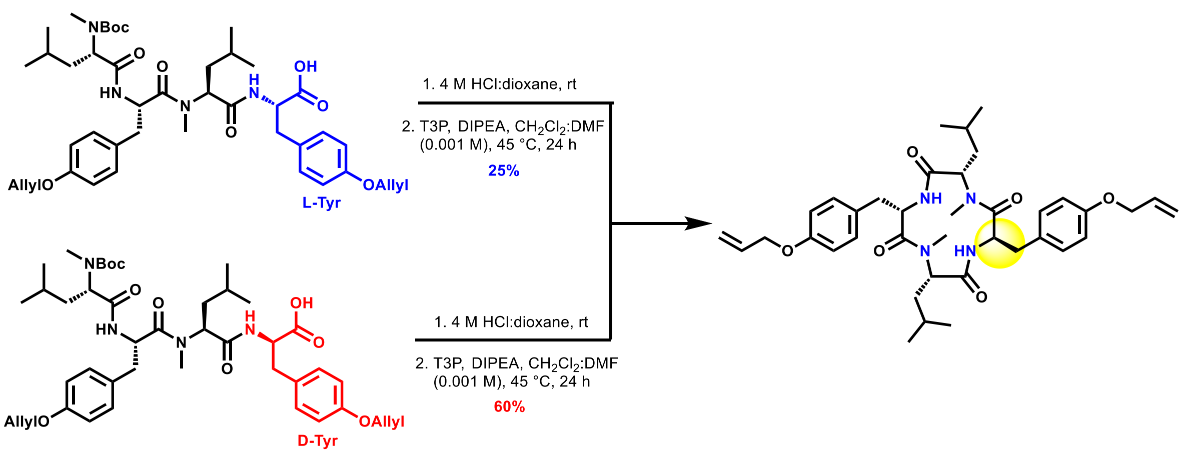
NDS=101105 MIC= 32 μg/mL against *S. aureus*

Another set of analogues were prepared by making macrocyclic part alterations, where we synthesized analogues with Arg and Leu bound with appropriate linkers like Pro, Gly and caproic acid. In most of these linear alterations, we tried to keep Arg at the terminal part as it is morphologically identical to End and can mimic the same. We also chose proline as a linker in some selected analogues which operates as a turn inducer and consequently resulting in a structure morphologically similar to the macrocycle (macrocyclic mimic). In the course of synthesizing these classes of linear analogues, we utilized serine ligation as the key step. We prepared new analogues of teixobactin and screened them with the help of Dr. Sidharth Chopra's group at CSIR-CDRI, Lucknow for their antibacterial potential against 'ESKAPE' pathogens. More details and conclusions are provided at the end of the Chapter.

Chapter 2: Total Synthesis of 3-*epi*-pseudoxylallemycin B



Cyclic peptides are unique as they possess an extensive array of biological properties. In particular, cyclotetrapeptides are attractive pharmacological leads as compared to their larger ring size congeners, due to their close compliance to Lipinski's rules. Pseudoxylallemycin A-F, a group of macrocyclic peptide natural products were isolated from termite associated fungus *Pseudoxylaria sp*. X802 by Beemelmanns' group in 2016. [3]

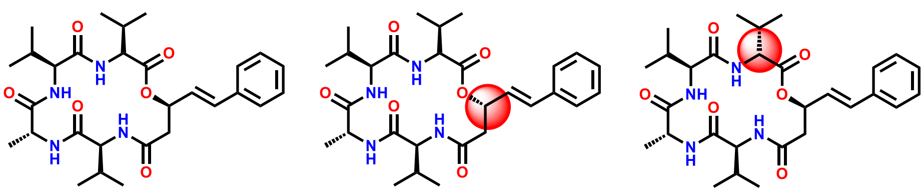


In an attempt towards the total synthesis of Pseudoxylallemycin B, a homo-dimeric, *N*-methylated macrocyclic tetrapeptidic natural product, we came across an unusual observation of complete epimerization of pseudoxylallemycin B which led to the formation of 3-*epi*-pseudoxylallemycin B (D-Tyr instead of L-Tyr).[4] To rule out the possibility of epimerization during the tetrapeptide synthesis, we have synthesized tetrapeptide with D-Tyr at C-terminal and from spectral data comparison we ruled out this possibility. To decipher the cause of such discrepancy, tetrapeptide with D-Tyr at C-terminal undergoes similar macrolactamization conditions and led to the formation of 3-*epi*-pseudoxylallemycin with no epimerization. The absence of any favorable geometrical constrains or structural pre-organization in the linear tetrapeptide as well as the possible development of a 12 membered ring strain might have contributed to this unusual complete epimerization.

Chapter 3: Efforts toward Total Synthesis of Arthroamide and Fusaristatin C

Section I: Efforts toward synthesis of arthroamide

Arthroamide was isolated by Yasuhiro *et al.* in 2015, along with the known compound turnagainolde A. [5] These compounds inhibited the quorum sensing signaling of *Staphylococcus aureus* with an IC_{50} value of 0.3 μM. Arthroamide is a 15 membered cyclic depsipeptide having four common amino acids (three L-valine and one D-Alanine) and a rare structural unit, 3-(*R*)-hydroxy-5-phenyl-4-pentanoic acid (Hppa).



Arthroamide Proposed structure

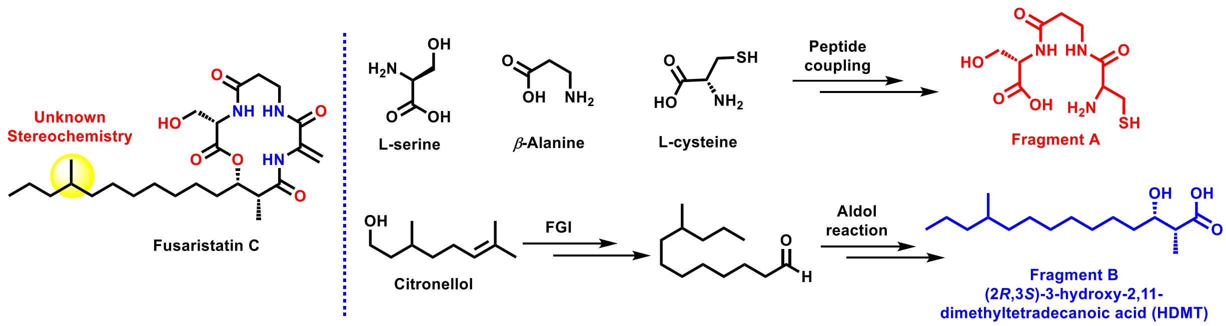
16-*epi*-Arthroamide

3-*epi*-Arthroamide

The enantiopure Hppa was synthesized by the amano PS lipase mediated enzymatic kinetic resolution. The synthesis of arthroamide features an enzymatic kinetic resolution, HATU mediated peptide coupling and lanthanide triflate mediated Shiina macrocyclization as key steps. After synthesis of proposed compound, there were discrepancies in the NMR data when compared to the reported data. With the likelihood of different isomers, we synthesized 16-*epi*-arthroamide and 3-*epi*-arthroamide. However none of the macrocycles spectral data was in agreement with that of published data which warrants further structural characterization of target macrocycles from both the sides (isolation & synthesis).

Section II: Studies toward synthesis of fusaristatin C

Cyclic lipodepsipeptide, fusaristatin C was isolated from the fungus *Pithomyces sp.* RKDO 1698 by Kerr's group in 2018, [6] which was isolated from the Caribbean octocoral *Eunicea fusca*. This macrocyclic tetrapeptide contains serine, *β*-alanine, dehydroalanine and non-peptide (2*R*,3*S*)-3-hydroxyl-2,11-dimethyltetradecanoic acid (HDMT) fragment and the stereochemistry at C-11 position in HDMT fragment was unknown. By accepting it as challenge, we synthesized fragment A in gram scale. After having this fragment, we started synthesis of HDMT fragment



from citronellol. Accordingly, we have synthesized the key component (non-peptidic portion) (2*R*,3*S*)-3-Hydroxy-2,11-dimethyltetradecanoic acid (HDMT), but the spectral data was not in agreement with the proposed structure of HDMT. We have also prepared four other possible structures. However, the NMR data is not exactly matching with reported NMR data. Our efforts suggest that structural revision of the natural product fusaristatin C is inevitable.

Noteworthy Findings

a) Accomplished the synthesis of macrocyclic core of teixobactin.
b) Achieved total synthesis of Met_{10}-teixobactin through native chemical ligation as key step.
c) Synthesized several analogues of teixobactin, out of which **NDS-101103, NDS-101104** and **NDS-101105** came to be active against *S. aureus* in an antibacterial assay against 'ESKAPE' pathogens.
d) Synthesized 3-*epi*-pseoxylallemycin B and observed unusual complete epimerization of one of the amino acid.
e) Accomplished synthesis of proposed structure of arthroamide, along with two possible isomers.
f) During synthetic studies of fusaristatin C, achieved synthesis of peptide fragment in gram scale and HDMT fragment along with five possible isomers.

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Contents

Chapter 1: Design, Synthesis and Biological Evaluation of Potent Antibiotic Peptide Natural Product Teixobactin Analogues

Contents

Chapter 1:

Design, Synthesis and Biological Evaluation of Potent Antibiotic Peptide Natural Product Teixobactin Analogues

1.1. Introduction

Antibiotics are the face of modern medicine to the world in many aspects and their discovery was a turning point in human history. Unfortunately, the easy access and excess use of these wonder drugs led to the widespread problems with antibiotic resistance.[1] Antibiotic resistance is increasing at a dangerous rate because of which growing infections like pneumonia and tuberculosis are becoming challenging to treat.[2] Discovery of penicillin by Alexander Fleming in 1928 gave birth to the modern "Antibiotic era" which led to extended interest in the search of novel antibiotics with similar effectiveness and safety.[3] History of antibiotic drug discovery starting from 1930 to recent times along with time period for the development of resistance with respect to that particular drugs is captured in Figure 1.1.[4,5]

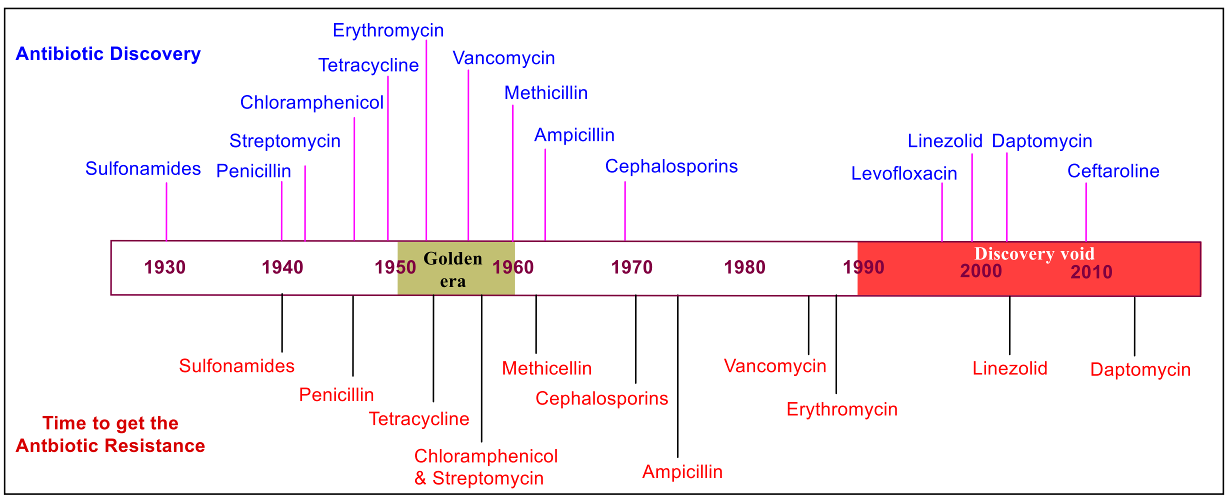


Figure 1.1. Antibiotic discovery and time to get the antibiotic resistance (image source: *Nat. Chem. Biol.* **2007**, *3*, 541–548)[5]

The time between the 1950s and 1960s was considered to be the golden era for drug discovery in which most of the novel antibiotic classes came to the market and from 1990s discovery void as no new class was discoverd.[5] In general, there are four mechanisms which causes resistance to antibiotics:[6]

1) The modification or inactivation of the drugs
2) Diminishing the binding capacity of drug by alteration in binding site
3) Alteration of metabolic pathways

4) Bacteria, either decreases permeability or increases efflux of the drug which results in lowering of intracellular concentration of drug.

The continued evolution of multi-drug-resistant bacteria becomes major concerns which necessitated the discovery of new antibiotics, with novel modes of action. According to the Infectious Diseases Society of America (IDSA), more than 70% of the bacteria causes antibiotic-resistant infections, will resist at least one commonly used drug.[7] According to the report, major pharmaceutical companies have left antibiotic research and development, owing to the weak economic returns on investments for manufacturers.[8] Besides this, an emerging scenario of resistance makes antibiotics less effective and hence less profitable for the pharmaceutical companies.[9] All these cases clearly exemplifies the fact towards the necessity of developing the new and novel class of antibiotic scaffolds. People like us, who work in academic institutions and national laboratories, should have more responsibility to work on this important and challenging area of antibiotic drug discovery.

1.1.1. Macrocyclic peptides in drug discovery

Drug development stream is mostly dominated by small molecules and the road to make them from bench side to bed side has many hurdles related to efficacy and tolerability, which has to be taken into account by the new drug candidates. Conventional small drug molecules, due to their small size, may experience reduced target selectivity that often reflects in side-effects[10]. To overcome some of these concerns, macrocyclics and peptides are now gaining momentum in the pharmaceutical industry which can be explored for new treatments.

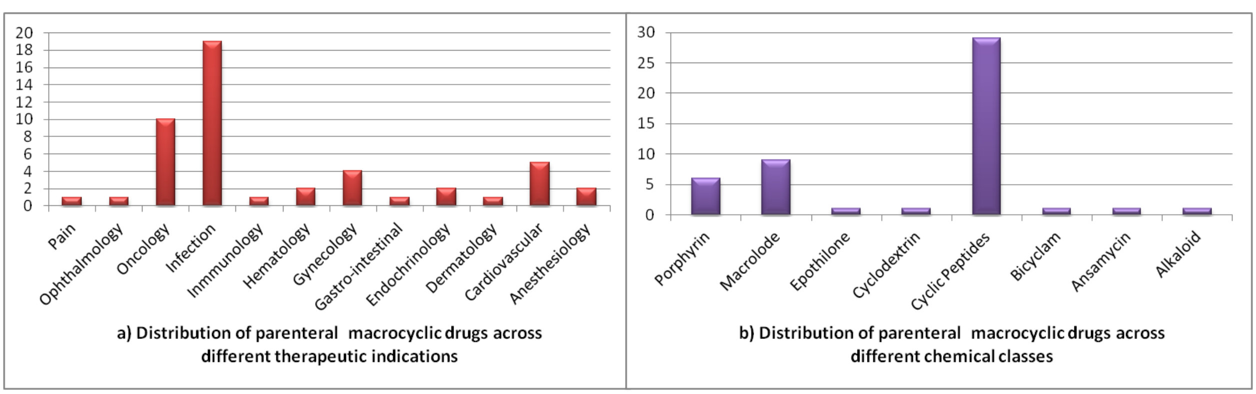


Figure 1.2. a) Distribution of parenteral macrocyclic drugs across different therapeutic indications; b) Distribution of parenteral macrocyclic drugs across different chemical classes. (Data source: *J. Med. Chem.* **2014**, *57*, 278−295)[12]

Because of the size and structure they gained position in between of small molecules and large molecules like biologics.[11] The cyclic nature of these macrocyclics amplifies their conformational rigidity and solubility which increases cell permeability and target specificity, which ultimately resulted in reaching some of them into human clinical trials.[11] Analysis of the 68 macrocyclic drugs which are currently available in the market, revealed that half of them are being used for treating infections (Figure 1.2.a). Figure 1.2.b represents the distribution of drugs with macrocyclic core structure across different chemical classes and broadly most of the macrocyclic drugs distributed equally between cyclic peptides and macrolides.[12]

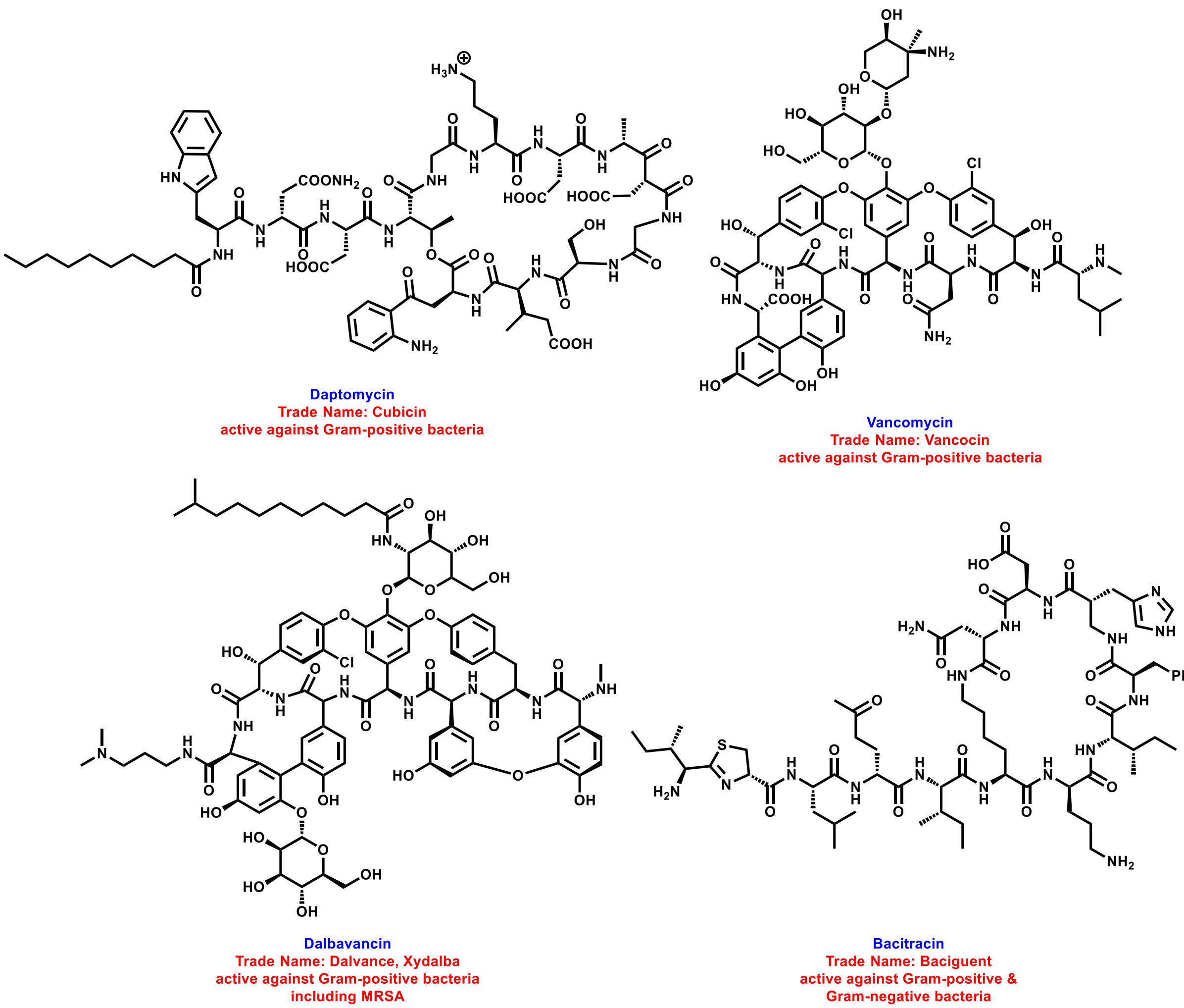


Figure 1.3. Marketed macrocyclic peptide antibiotics

Peptides are identified for being highly selective, efficacious, relatively safe and well tolerated. These features attracted the attention of pharmaceutical research and development (R&D). Till date, more than 7000 naturally occurring peptides have been identified exhibiting wide spectrum of biological activities and nearly 100 peptidic drugs are currently in the market and around 400 novel peptides in clinical development.[13] Owing to the structural features, peptides have some advantages over small drug molecules like degree of selectivity, high potency, lower toxicity, low accumulation in the body, high chemical and biological variety.[14] Peptides are still smaller as compared to large molecules such as proteins and antibodies; which leads to their easy synthesis, optimization, evaluation and biologically they do not cause serious immune responses.[14] Peptide antibiotics are classified into two classes:

1) Ribosomally synthesized peptides
2) Non-ribosomally synthesized peptides

The ribosomally synthesized peptides are often produced by all species of life (including bacteria). Depending upon their origin, these peptides are divided into subtypes as mammalian peptides, amphibian peptides, insect peptides, plant peptides, bacterial peptides, viral peptides and synthetic peptides. Non-ribosomally synthesized peptides are further classified into three types; lipopeptides (eg. daptomycin, polymyxin), glycopeptides (vancomycin, teicoplanin, dalbavancin) and cyclic non-ribosomally synthesized peptides (bacitracin) (Figure 1.3.).[15]

1.2. Teixobactin: introduction and background

Antibiotic resistance is growing at a much faster rate than the rate of development of new drugs for treating bacterial infections. Most of the antibiotics produced by platform developed by Waksman could not be replaced by synthetic approaches to produce antibiotics.

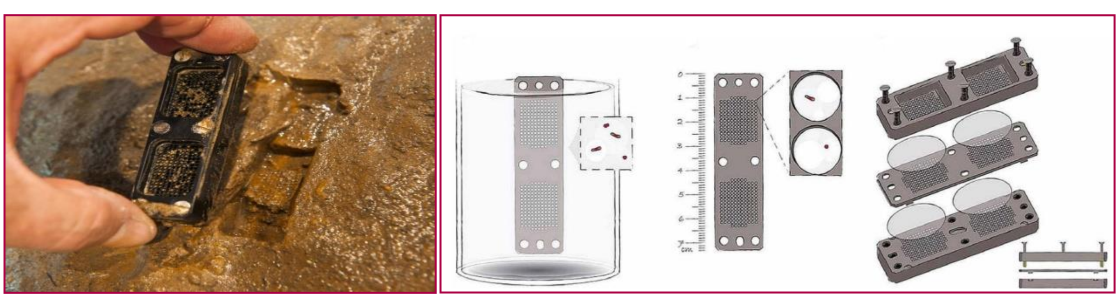


Figure 1.4. Isolation chip (ichip, image source: *Nature* **2015**, *517*, 455−459 and https://www.nature.com/news/promising-antibiotic-discovered-in-microbial-dark-matter-1.16675)[18,19]

Uncultured bacteria are major source of new antibiotics, but 99% of bacterial species are unable to grow under laboratory conditions.[16] Ling and co-workers discovered a multichannel device, the isolation chip (ichip), a novel high-throughput platform for simultaneous isolation and culturing of uncultured bacteria (Figure 1.4.).[17] The sophisticated ichip ultimately cultures bacterial species within its natural environment. This revolutionary tool consists of multiple holes in which diluted soil sample was poured. Diluted soil sample was delivered to each hole (approximately one bacterial cell in each hole), and device wrapped by two semi-permeable membranes and placed back in the soil from where sample was collected, which allows growth of bacteria in their natural environment by dispersing the nutrients through semi-permeable membrane.

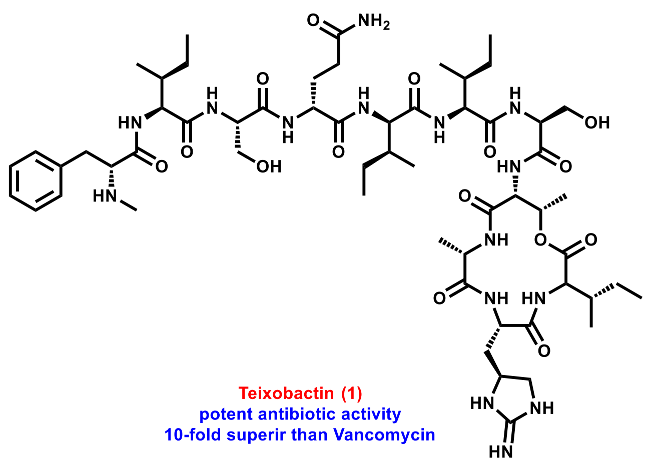


Figure 1.5. Structure of teixobactin

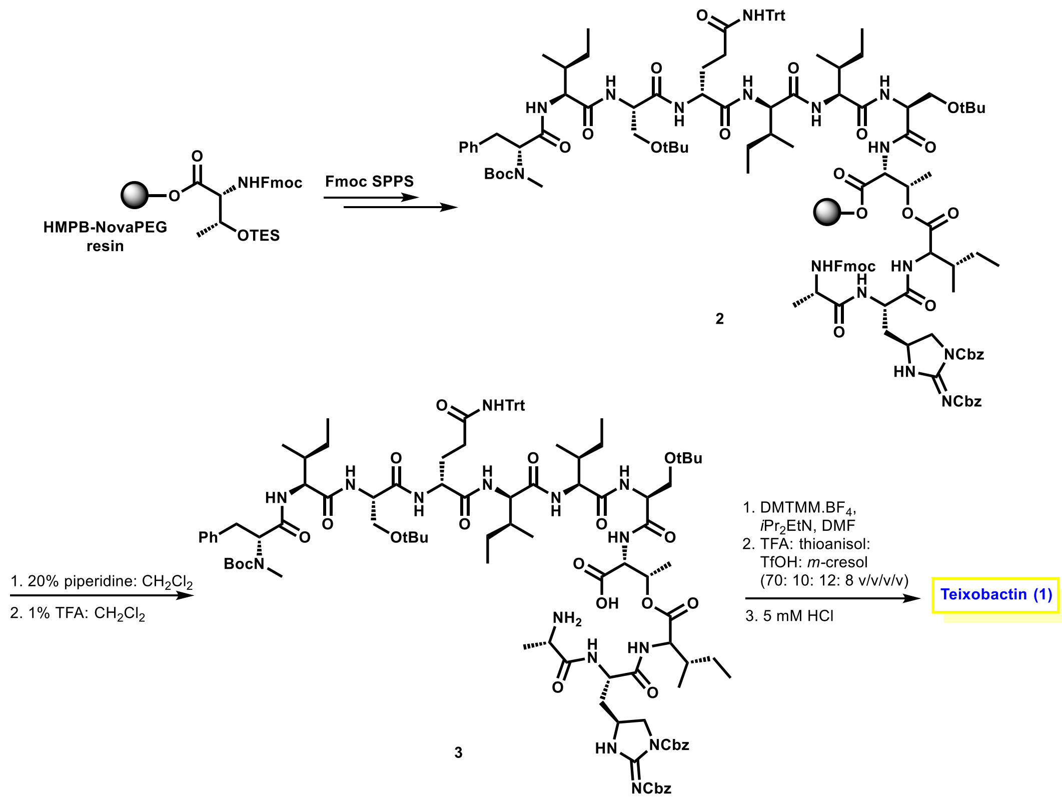
In 2015, Ling and co-workers discovered, "head to side chain" macrocyclic depsipeptide, teixobactin; comprising of 11-amino-acids, using this technology (Figure 1.5.).[18,20a] Teixobactin showed very potent antibacterial activity which is better than well-known drug vancomycin. The discovery and pharmacological characterization of teixobactin was received great attention of scientific and social media across the world with the buzzword "game changing-antibiotic".[20]

1.2.1. Mode of action & antibacterial activity

Teixobactin shows antibacterial activity against various Gram-positive bacteria including *Staphylococcus aureus*, MRSA, *Mycobacterium tuberculosis* and *Streptococcus pneumoniae*. It also showed good activity against *Clostridium difficile* as well as *Bacillus anthracis*. This novel antibiotic lead was found to poses a different mode of action from present line antibiotics currently used to treat bacterial infections. Cell wall biosynthesis is one of the vital processes in maintaining bacterial life. Peptidoglycan and wall teichoic acid are the two essential components for the cell wall construction and rigidity of the same. Most probably, teixobactin targets cell wall biosynthesis process in bacteria. It inhibits the bacterial cell wall synthesis by binding to lipid II which is a building block for peptidoglycan synthesis and lipid III which is a building block of the wall teichoic acid.[18,20a,21] In most of the lipid II binding depsipeptides, the positively charged guanidine side chain might be playing crucial role during an interaction with the phosphate group of lipid II.[20a] Teixobactin shows a faster and efficient killing of *S. aureus* in *in-vitro* models when direct compared to the well-known blockbuster antibiotic vancomycin; a lipid II binder, without detectable cross-resistance.[18] Furthermore, in *in-vitro* toxicological studies, adverse effects like cytotoxicity, hemolysis, hERG inhibition, genotoxicity have not been detected in teixobactin which is an added advantage towards its novelty. Initial pharmacokinetic *in-vitro* studies also displayed a good half-life in plasma (rodents, dogs, humans).[20a] These impressive properties of teixobactin were well translated into *in-vivo* efficacy in a mouse-MRSA-sepsis model and in three rodent infection models.[20a] In both the cases, vancomycin was found to exhibit comparable potency which proves teixobactin to be 10-fold more active than its well-known antibiotic congener. Besides, simpler structure of teixobactin over vancomycin and an easy structural and functional tuning makes it a scaffold persuadable to peripheral modifications with the aim of overcoming the threat of resistance.[18]

1.2.2. Previous synthetic work towards teixobactin

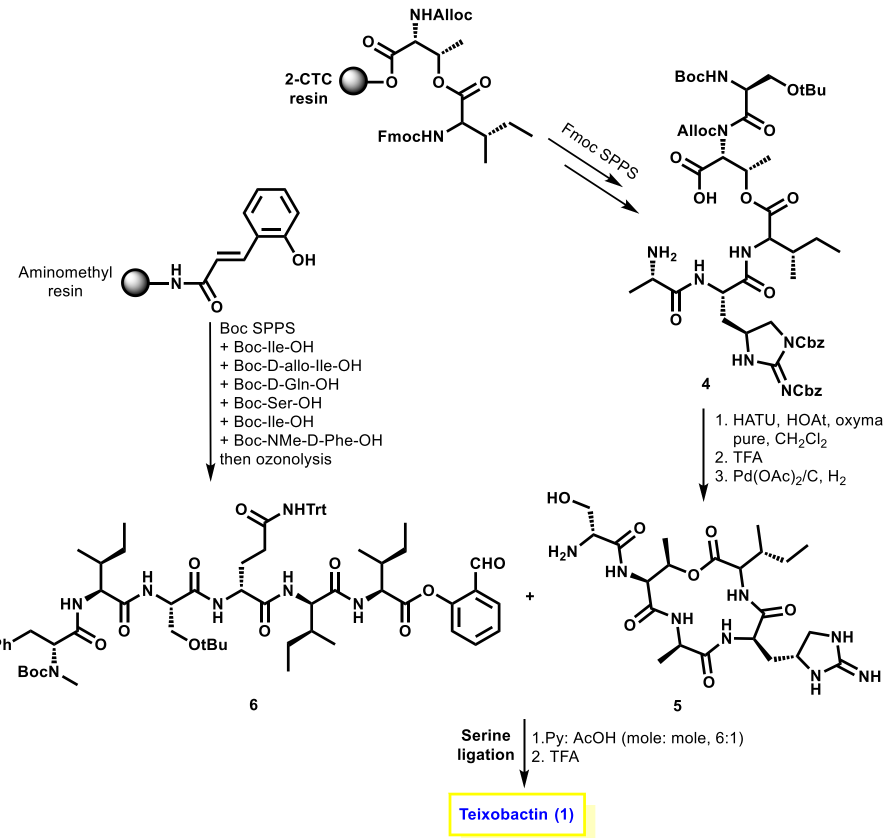
Owing to these promising and impressive features, teixobactin surely holds the scope and potential to be a promising antibiotic lead through proper and systematic structure activity relation (SAR) studies. Hence chemical synthesis of the natural product and related analogues became inevitable. Payne and colleagues published (May 2016) the first total synthesis of teixobactin in 24 steps with an overall yield of 3.3%.[22] A solid-phase strategy was employed, with a triethyl silyl (TES) protected D-threonine on the resin. After building of the peptide chain **2** by Fmoc-SPPS, and cleavage from the resin using 1% TFA/CH_2Cl_2 (v/v) furnished precursor **3**. The ring closure was performed between the D-threonine and alanine residues in the presence of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinum tetrafluoroborate (DMTMM·BF_4)



Scheme 1.1. Total synthesis of teixobactin by Payne's group[21]

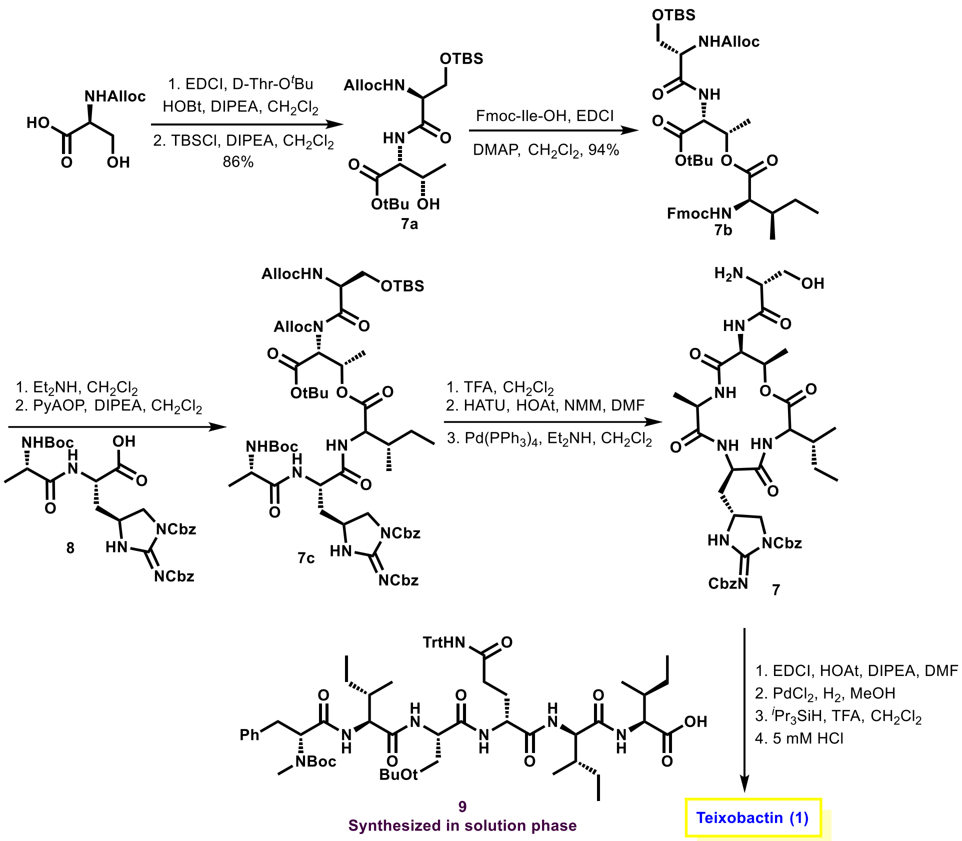
and DIPEA in DMF under dilution (Scheme 1.1.). Final deprotection afforded the HCl salt of teixobactin where all the spectral data was in complete agreement with the reported data, and the synthesized teixobactin was also shown to poses antibacterial properties similar to that of natural one.

Later in 2016, Li's group also reported the total synthesis of teixobactin.[23] This time, a convergent strategy involving Serine/Threonine ligation of the linear peptide fragment containing six amino acid and the cyclic core ring structure with serine at *N*-terminus was employed. For the synthesis, the coupling between Alloc-D-Thr-OH and Fmoc-Ile-OH was performed prior to loading the residue onto the 2-chlorotrityl chloride (2-CTC) resin for solid phase peptide synthesis of **4**. After successful synthesis of cyclization precursor **4**, macrolactamization was proceeded smoothly in presence of mixture of HATU, HOAt and OxymaPure in CH_2Cl_2 with slow addition and high dilution conditions. Removal of protecting groups followed by HPLC purification, furnished key fragment **5** in 17% yield. The second part of the molecule, **6**, was also synthesized using Boc SPPS with a salicylaldehyde ester at the C-terminal position. Ligation of the two pieces (**5** and **6**) was performed in pyridine/AcOH to give 37% yield of teixobactin (**1**) after HPLC purification (Scheme 1.2.).



Scheme 1.2. Total synthesis of teixobactin by Li's group[23]

Very recently, in 2019, Gao and co-workers described the synthesis of teixobactin (**1**) in solution phase for the first time, by utilizing convergent strategy of coupling of linear hexapeptide and macrocycle containing serine at *N*-terminus with aid of coupling reagents (Scheme 1.3.).[24] Alloc-L-Ser-OH was coupled with D-ThrO^{t}Bu in presence of EDC, HOBt and DIPEA in CH_2Cl_2 followed by protection of the primary alcohol in serine afforded dipeptide **7a** in good yield. Secondary alcohol of D-Thr was esterified with Fmoc-L-Ile-OH using EDCI and DMAP in CH_2Cl_2 afforded tripeptide, which under deblocking of Fmoc group followed by coupling with acid **8** delivered pentapeptide **7c** in a 90% yield. **7c** undergoes acidolytic cleavage of Boc and ^{t}Bu ester group. Macrolactamization of corresponding amino acid followed by deprotection of Alloc and TBS group afforded hydroxyl amine **7**.



Scheme 1.3. Total synthesis of teixobactin by Gao's group[24]

Hexapeptide **9** was synthesized in solution phase and linked with **7** followed by removal of all remaining protecting groups, using TFA and hydrogenation in the presence of $PdCl_2$ furnished teixobactin (**1**). The total synthesis of teixobactin was achieved through the longest linear sequence of 20 steps with 5.6% overall yield.

1.2.3. Synthesis of teixobactin analogues and their SAR study

Since the original publication of teixobactin (**1**) in the "Nature" in January 2015, numerous research groups have focused their efforts on synthesis of various teixobactin analogues.[25, 26] The presence of rare and commercially unavailable L-*allo*-enduracididine (End) residue; which containing a five membered cyclic guanidine moiety, was became the first choice of replacement by many research groups. The inherent mutation for L-*allo*-enduracididine is the commercially available residue L-arginine (Arg).[27] Many groups focused on investigating the hypothesis that the macrocycle of teixobactin binds to the pyrophosphate group of lipid II;[37] and the hydrophobic linear portion anchors into the cell membrane.

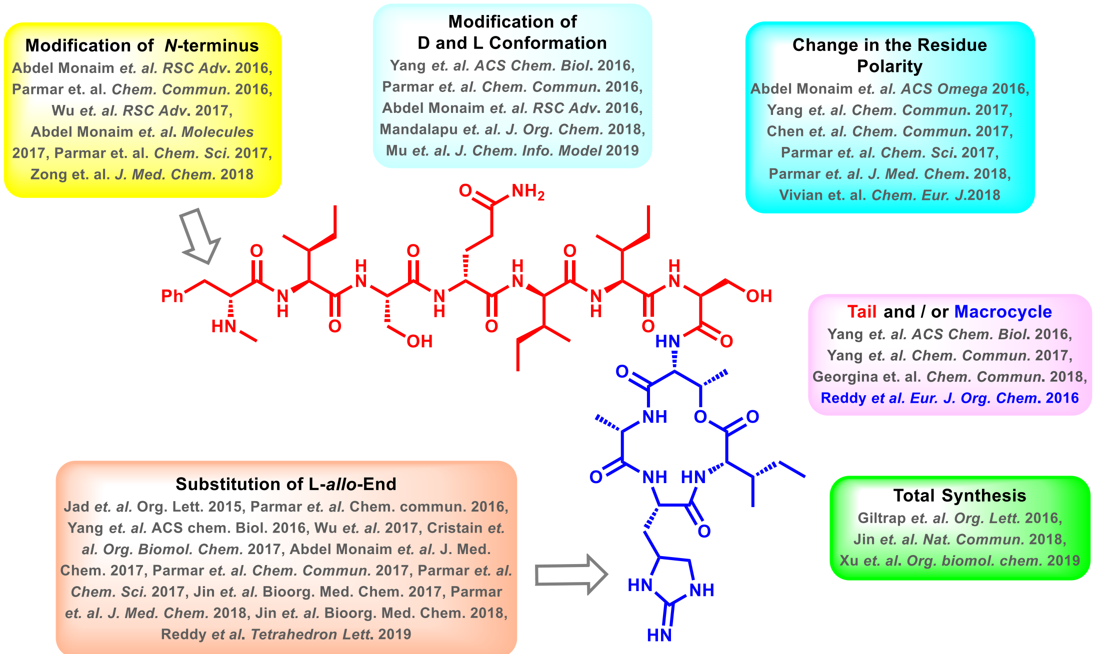


Figure 1.6. Modification in teixobactin analogues for SAR

Journey of teixobactin analogues synthesis started with the synthesis of Arg_{10}-teixobactin analogue (Figure 1.7.), which resulted in 10-fold loss of activity than natural teixobactin.[28,29,30] As Arg is easily available in nature as compared to L-*allo*-enduracididine and to maintain the guanidine group like environment at position 10, it was used as surrogate towards SAR study of teixobactin.

Modification at L-*allo*-enduracididine: The substitution of unnatural L-*allo*-enduracididine by an Arg residue was the first choice of analogues to be chemically synthesized by Fernando's group[28] and Singh's group[30] independently. From SAR studies, it was observed that Arg_{10}-teixobactin is 10-fold less active as compared to the natural teixobactin. These results manifest the super-activity of teixobactin is mainly dependent on the key amino acid L-*allo*-enduracididine but it is not solely important since there is no entire loss in activity. It is well known that basic amino acids (like Arg) remains in protonated form at physiological pH; to find out the contribution of this positive charge at position 10, it was replaced by other basic amino acids like Lys,[29] ornithine,[31,32] Homo-argenine,[33] nor-argenine,[33] diaminopropanoic acid,[33] L-2,4-diaminobutyric acid;[33] which shows comparable activity to that of Arg_{10}-teixobactin analogue. But when the positive charge was increased more by substituting position 10 by amino acids like histidine,[34,35] 2-amino- 4-(tetramethylguanidino)butanoic acid[31] and 2-amino-4-(dimethylmorphilinoguanidino)butanoic acid[31] which were sterically hindered amino acids resulted in complete inactive analogues. While doing alanine scan to find out the contribution of each amino acid towards antibacterial activity, Nowick's group observed that residue Ala_{10}-teixobactin results in retention of activity of nearly 1 µg/mL. These observations runs contrary to popular belief and established that the antibacterial activity is not dependent on the cationic hydrophilic amino acids at position 10 and opens the door to developing many new teixobactin analogues.[36] By looking into these interesting results, various research groups around the world turned their attention on replacing this unnatural L-*allo*-enduracididine by non-polar amino acids. Analogues were prepared by substitution of aromatic amino acids, aliphatic acyclic amino acids, aliphatic cyclic amino acids and SAR was carried out against *S. aureus*.[37,38,39] From these SAR data it was observed that, the substitution by Ile, Leu, Met came out to be equally or more potent than teixobactin (Figure 1.7.). Regardless of the simpler design of these teixobactin analogues, they showed potent antibacterial activity against various bacterial strains. Most importantly, these results ruled out the popular belief that the basic amino acids such as L-*allo*-enduracididine, Arg or Lys present at position 10 are necessitate for antibacterial activity. As teixobactin consist of hydrophobic linear peptide fragment and hydrophilic macrocyclic part, it's important to maintain the equilibrium of polarity in the molecule; with this idea, Singh's group substituted some of the amino acids like Ser_3, D-Gln_4, and Ala_9 with cationic Arg in Leu_{10}-teixobactin and Ile_{10}-teixobactin and revealed highly potent teixobactin derivatives against *S. aureus*, MRSA, and VRE.[39]

Modification at *N*-terminus: To find out the lipophilic contribution of *N*-termiuns towards antibacterial activity, Fernando's group replaced *N*-methyl group on *N*-terminal D-Phe by *N*-acetyl, *N*-benzyl, *N*-decyl, *N*,*N*-didecyl, *N*-guanidino groups and synthesized derivatives in Arg_{10}-teixobactin, which proceeded with inactive teixobactin analogues.[34,40] Further, Su's group and Fernando's group independently demonstrated that the D-Phe analogue was active, while *N*,*N*-dimethyl or D-Tyr derivatives resulted in total loss of activity.[34,40] Later, Rao's group synthesized equipotent analogues of teixobactin by adding phenyl group at *para*-position on D-Phe.[41]

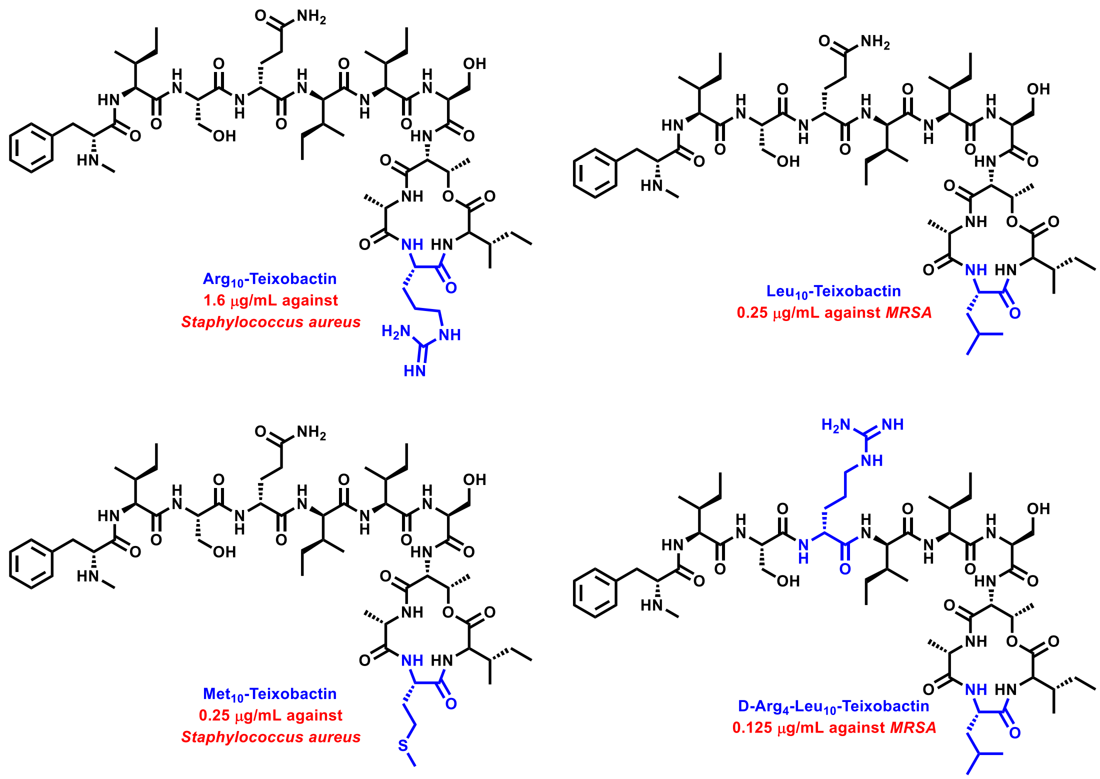


Figure 1.7. Selected potent analogues of teixobactin

Change in residue polarity: To understand role of different residues and their contribution of their polarity towards potency of teixobactin, Albericio group[42] did lysine scan of Arg_{10}-teixobactin and Nowick group[37] did Ala scan of Lys_{10}-teixobactin independently, results from this SAR study showed that the position 3, 4 and 9 in teixobactin are susceptible for modification. Substitution of other amino acids than these positions results in inactive analogues, thereby suggesting that, these amino acids majorly contributing in the activity of teixobactin. In addition to this, the resolution studies of crystal structure underlined the presence of hydrogen bonding between the hydroxyl group of serine present at 7th position and carbonyl group of Ala of 9th position. This result was the output of the understanding of crystal resolution done for truncated version of Arg_{10}- teixobactin.[43] Studies done by Li and co-workers on Arg_{10}- and Orn_{10}- teixobactin successfully demonstrated that the substitution of Ile/Phe residues by non-polar residues afforded inactive analogues, while replacement of D-Gln by D-Asn or by D-Arg conserved the activity of the molecule.[32] These results proved to be the significant output, which highlighted that position 4 can afford different substitutions to retain activity.

D/L modification: Modifications of teixobactin analogues were greatly influenced by easy access of Arg as compared to an unnatural L-*allo*-enduracididine. Therefore, for doing structure activity relationship (SAR) study Arg_{10}-teixobactin was considered as base. However, studies on Orn_{10}- teixobactin analogues were also carried out by Li group.[32] As described earlier, *N*-Me-D-Phe, D-Gln, D-*allo*-Ile, and D-Thr are the four D-amino acids present in teixobactin. Any modification of these four non-proteinogenic D-amino acids with single substitution at any of the centre or the replacement of all the centres by respective L-amino acids afforded non-active compounds;[44] also results turned out to be the same when analogues like *N*-Me-L-Phe-Orn_{10} and L-Gln_4-Orn_{10} were synthesized and tested for activity.[32] However, results with enantiomeric derivative of Arg_{10}-teixobactin, gave similar trend in potency.[29] These findings underscore the presence of achiral targets in bacteria for interaction of teixobactin.

Substitution of linear peptide: Nowick and co-workers came up with an idea to replace 1-5 amino acid residues with long chain fatty acids to figure out the role of linear peptide part of teixobactin in anchoring the plasma membrane.[29] To investigate the workability of the idea, they have synthesised homologue of teixobactin, named as lipobactin, by replacing 1-5 amino acid residues with dodecanoyl group. This synthesized lipobactin was only 2-4 times less active when compared with Arg_{10}-teixobactin.[29] Further, the same group, synthesised a new teixobactin derivatives devoid of 1-5 amino acid, termed as short analogue, which did not show any activity.[29] These findings highlighted the significant role of hydrophobicity of *N*-terminal tail and served as a hint for further development of simpler homologues of teixobactin molecule having increased pharmacological efficacy. Later, Jamieson’s group synthesized prenylated analogues like Lys_{10}-farnesylbactin and Orn_{10}-farnesylbactin which showed less potent activity than lipobactin.[45]

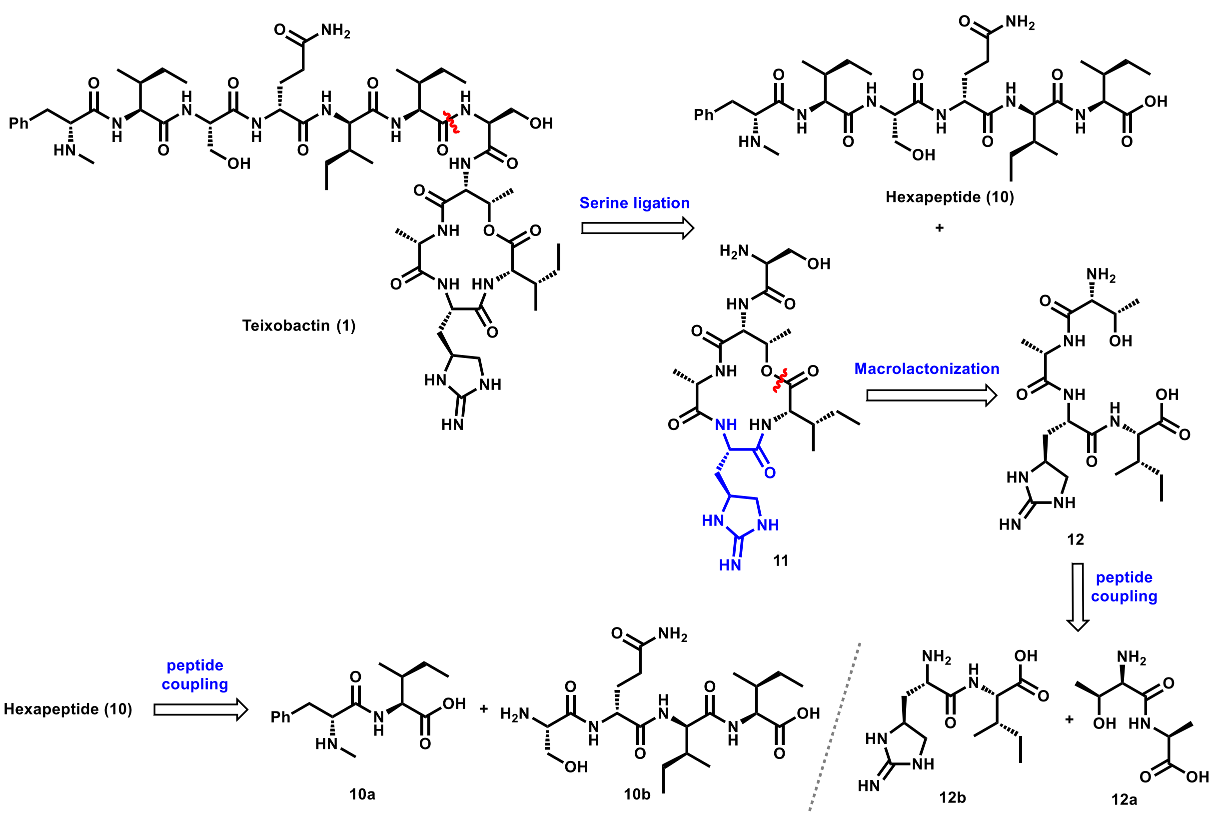
Tail and/or macrocycle: There was one more crucial finding by Nowick’s group; macrocycle of teixobactin is also crucial for biological activity which was underlined by the negative activity results on synthesised acyclic Arg_{10}-teixobactin analogue.[29] An aza macrocyclic derivative (ester bond replaced by an amide bond) prepared by the same group conserved the activity.[29] Furthermore, our group prepared only macrocyclic core of teixobactin along with model macrocycle, where we replaced L-*allo* enduracididine by L-methionine, also resulted inactive analogues.

1.3. Present work

1.3.1. Efforts toward total synthesis of teixobactin

Total synthesis of teixobactin became an interesting project among the synthetic and medicinal chemistry community. Efforts by various groups on total synthesis and analogue synthesis of teixobactin were already discussed in above section. More than 30 publications appeared in a short span of time, which clearly indicates the significance of this molecule among various research groups around the world. However, most of the approaches are based on solid-phase synthesis. In addition, all of them use lactamization as the key step during macrocycle formation, probably, due to failed attempts[28] or raised concerns in lactonization[30] strategy. We were one of the first few among various research groups across the world to initiate total synthesis program on teixobactin and made significant contribution towards the target molecule.

1.3.1.1. Retrosynthesis

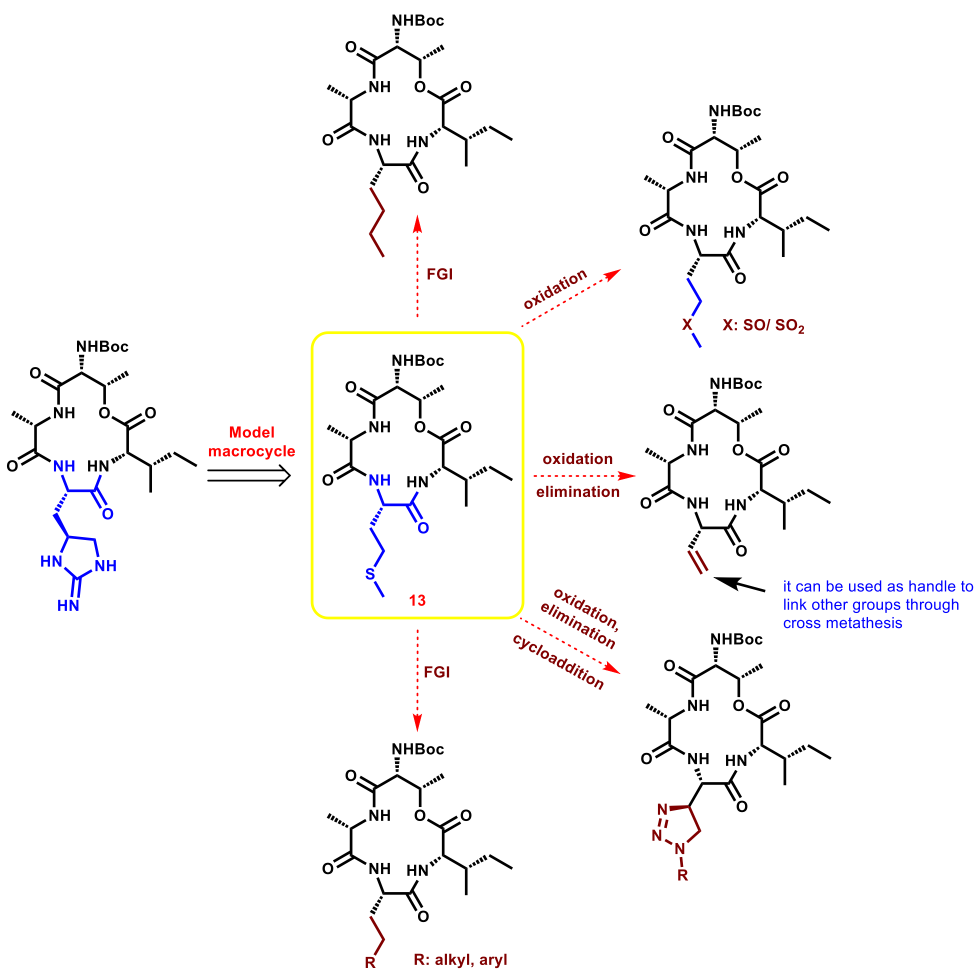


Scheme 1.4. Retrosynthetic analysis of teixobactin

Retrosynthetically, target compound **1** was envisioned by convergent strategy of serine ligation (SL) of two partners, the linear peptide fragment containing six amino acid (**10**) residues and cyclic peptide **11** containing five amino acid residues, in which one of them is rare (Scheme 1.4.). Crucial and challenging parts in this program are:

1) Access to sufficient quantities of the unnatural amino acid L-*allo*-enduracididine
2) Construction of macrocyclic fragment **11** from *seco*-acid **12**

The other component, linear hexapeptide **10** could be synthesized from coupling of dipeptide **10a** and tetrapeptide **10b**, which could be further synthesized from corresponding amino acids by peptide coupling. *Seco* acid **12** could be envisioned from coupling of dipeptide **12a** and **12b** which could be synthesized from corresponding amino acids (Scheme 1.4.). Before commencing the total synthesis, we had to address the challenge of macrocyclization for the construction of

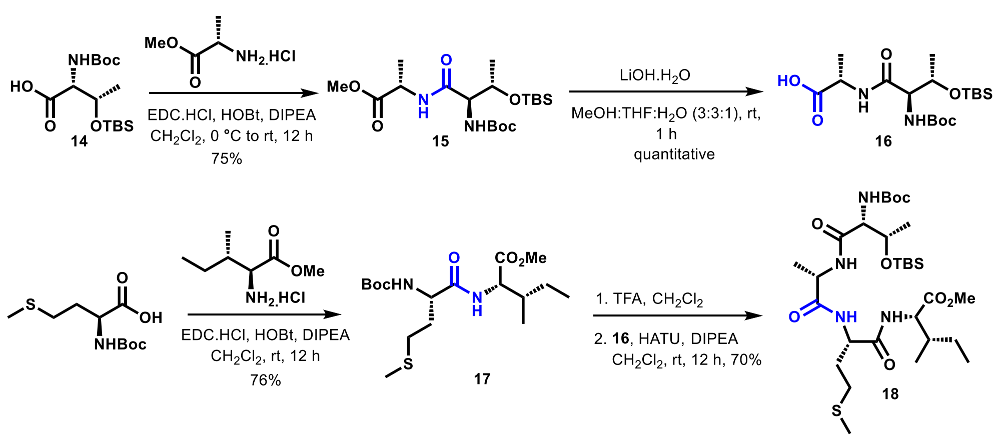


Scheme 1.5. Designing of model macrocycle and its derivatization

the cyclic peptide. For this purpose, we designed synthesis of model macrocycle **13**, in which L-*allo*-enduracididine was substituted with a simple amino acid, L-methionine. While designing this model macrocycle, we also hoped that the L-methionine side chain could be used for further derivatization[47] like oxidation, oxidation followed by elimination and cycloaddition reaction of resulting alkene which in turn could be utilized as handle through cross metathesis with appropriate groups towards lead optimization (Scheme 1.5.).

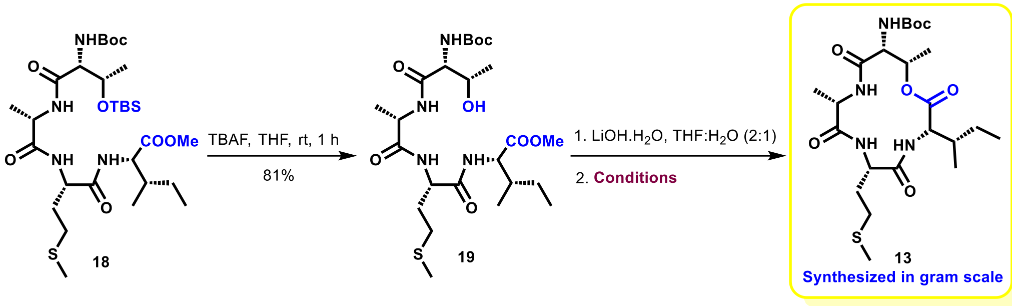
1.3.1.2. Synthesis of model macrocycle

Our synthesis of model macrocycle **13** commenced with the synthesis of dipeptide **15** from appropriately protected serine moiety **14**[48] was coupled with L-alanine methyl ester by using coupling reagent EDC and of hydroxybenzotriazole (HOBt), which upon ester hydrolysis gave desired dipeptide acid **16** in very good yield (Scheme 1.6.).



Scheme 1.6. Synthesis of tetrapeptide

L-isoleucine methyl ester and *N*-Boc-L-methionine were coupled together afforded dipeptide **17**[49], which upon Boc deprotection using 20% solution of TFA in CH_2Cl_2 followed by coupling using HATU and DIPEA as base in CH_2Cl_2 with acid **16** furnished tetrapeptide **18**. Compound **18** was characterized by ^{1}H, ^{13}C NMR and HRMS analysis. *tert*-Butyldimethylsilyl (TBS) group in compound **18** was deprotected by tetrabutylammonium fluoride (TBAF) in THF solution afforded *seco*-ester **19** in 81% and characterized by spectral data where key hydroxyl attached methine proton was appeared at δ 4.46 - 4.61 (m, 1H) ppm in ^{1}H NMR and δ 67.4 ppm in ^{13}C NMR. Compound **19** was subjected to ester hydrolysis to furnish *seco*-acid. The next challenging task was the construction of the macrocycle.[50]

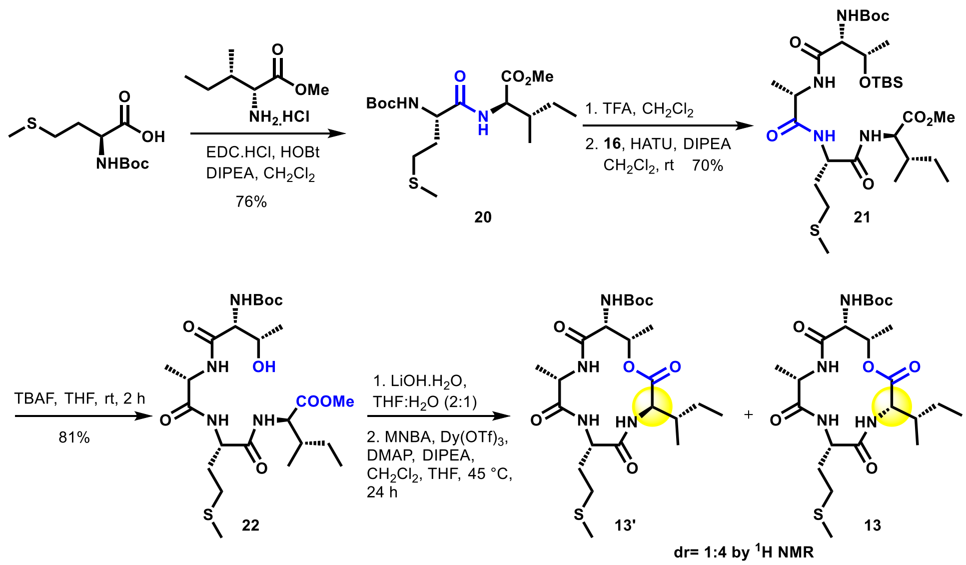


Sr. No.	Conditions	Observations
1	EDC.HCl, DMAP, DIPEA, CH_2Cl_2, 24 h	No desired product
2	2,4,6-trichlorobenzoyl chloride	No desired product
3	MNBA, DMAP, $La(OTf)_3$ (50 mol%), DIPEA, CH_2Cl_2, DMF, THF (2.0 mM)	20-25% product
4	MNBA, DMAP, $Dy(OTf)_3$ (50 mol%), DIPEA, CH_2Cl_2, DMF, THF (2.0 mM)	50-55% product

Scheme 1.7. Synthesis of model macrocycle

For this purpose, we attempted a few conditions like macrolactonization by EDC/ DMAP and Yamaguchi esterification, but in both the cases we did not observe the desired product formation (Scheme 1.7.). However, we found that 2-methyl-6-nitrobenzoic anhydride (MNBA), 4-(dimethylamino)pyridine (DMAP), dysprosium(III) trifluoromethanesulfonate [$Dy(OTf)_3$] (100 mol%), DIPEA, and CH_2Cl_2/THF (2.0 mM conc.) gave the best results for macrocyclization.[51] Synthesized macrocycle **13** was well characterized by using IR, ^1H and ^{13}C NMR spectroscopy where characteristic *O*- attached methine proton was observed at δ 5.44 (dd, *J* = 2.7, 6.4 Hz, 1H) ppm and carbon δ 74.1 ppm and HRMS (ESI) showed peak at 539.2510 for $C_{23}H_{40}O_7N_4NaS$ $[M + Na]^+$ with calculated value 539.2510 which also supports the formation of compound **13** with drawn structure.

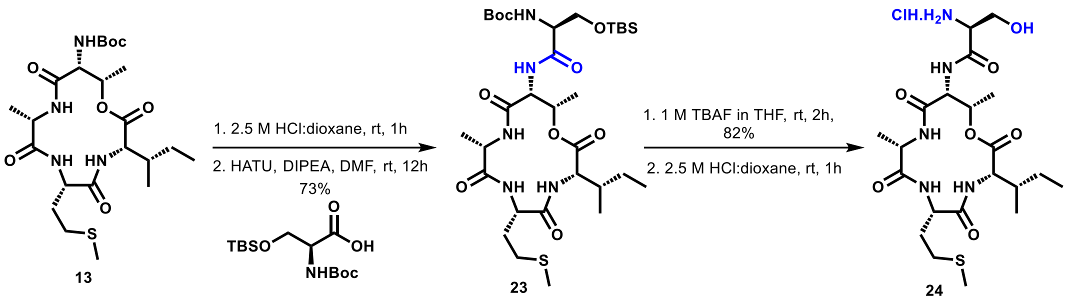
Although we were successful in synthesis of model macrocycle, the major concern in peptide macrocyclization was an epimerization at C-terminal.[50b,52] To make sure that there was no epimerization during the macrocyclization, we planned to synthesize compound **13′**, in which we replaced C-terminal amino acid L-isoleucine with D-*allo*-isoleucine. Boc-methionine on coupling with D-*allo*-Ile methyl ester in presence of EDC, HOBt and DIPEA as base in dichloromethane furnished dipeptide **20** and subjected for acidolytic cleavage of Boc group followed by coupling with acid **16** afforded tetrapeptide **21** in good yield. TBS group in **21** was deprotected using TBAF in THF, and subjected to saponification under presence of LiOH furnished *seco*-acid in good yield. This *seco*-acid was subjected to macrolactonization under similar conditions as shown in Scheme 1.8.



Scheme 1.8. Synthesis of compound **13'**

From the ^{1}H NMR spectroscopic data, we found the presence of compounds **13** and **13′** in an epimeric ratio of 4:1, where in **13'** characteristic *O*-attached methine proton was appeared at δ 6.04 - 6.02 (m, 1H) ppm but in the case of compound **13** it was appeared at δ 5.44 (dd, *J* = 2.7, 6.4 Hz, 1H) ppm. These observations suggested that racemization took place in the case of D-*allo*-isoleucine, but such racemization was not observed in the case of L-isoleucine.[53] Additional details along with HPLC data are provided in the experimental section. Compound **13** was the exact replica of the macrocycle present in teixobactin, in which L-*allo*-enduracididine was replaced with methionine, and this effort gave us the confidence to go forward towards the goal of the total synthesis of teixobactin.

One of the frequent observations in macrocyclic peptides is the intramolecular *O*- to -*N* acyl group migration leading to the emergence of the corresponding macrolactam in similar macrocycles; also a documented phenomenon.[50b] To address this issue, the Boc group in macrocycle **13** was deprotected by using trifluoroacetic acid (TFA), which was followed by

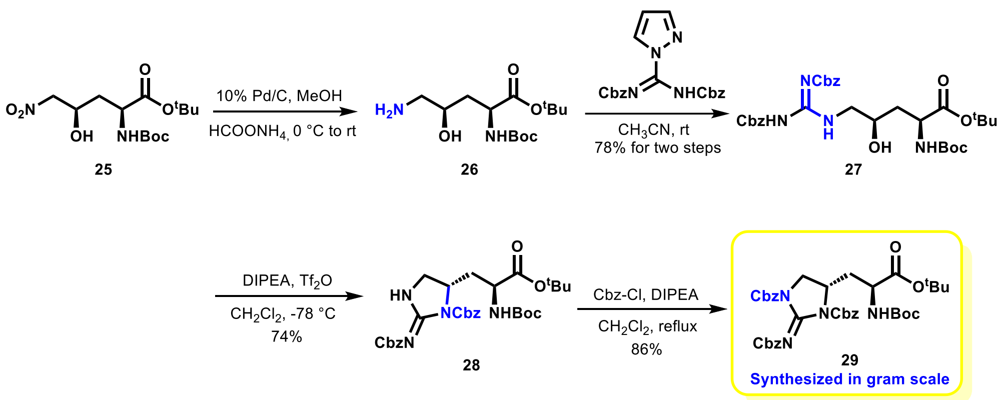


Scheme 1.9. Synthesis of methionine macrocycle with serine

coupling with *N*-Boc-Ser(TBS)-OH under HATU and HOAt in DMF conditions afforded **23** (Scheme 1.9.). The characteristic *O*-attached methine proton from ester was appeared at δ 5.47 (qd, *J* =3.3, 6.4 Hz) ppm and carbon at δ 73.4 ppm. It was clear from NMR spectroscopic analysis that there was no such *O*- to -*N* acyl group migration taking place. Further, formation of desired structure was confirmed by IR and HRMS analysis. Compound **23** was then treated with TBAF in THF for deprotection of TBS group followed by acidolytic cleavage of Boc group in presence of HCl in dioxane furnished compound **24** which we utilized further without purification.

1.3.1.3. Synthesis of L-*allo*-enduracididine and macrocycle

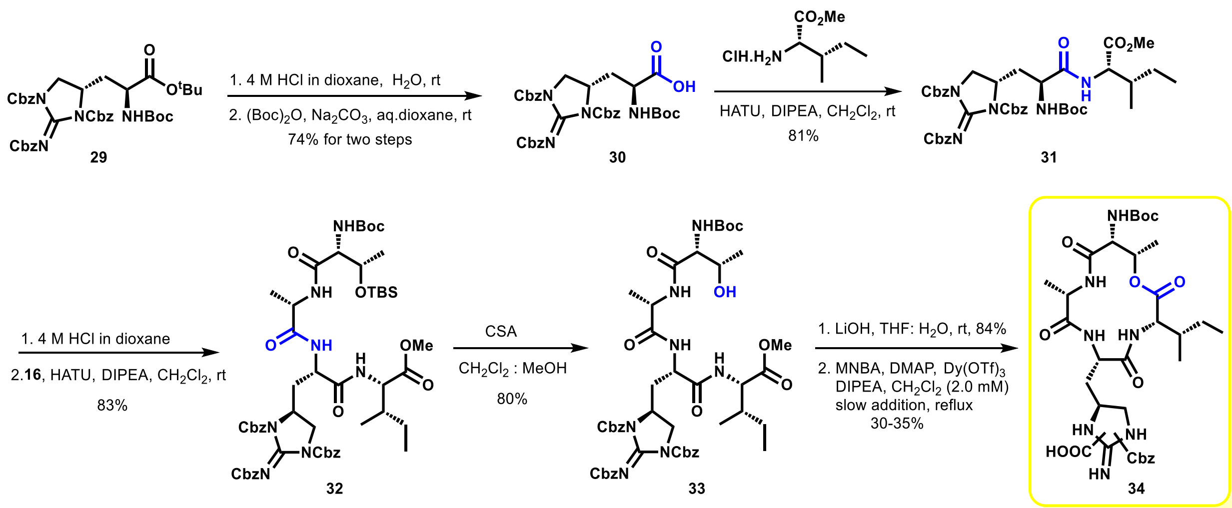
Having addressed the challenges using model macrocycles, efforts were diverted to the synthesis of actual macrocycle. For the synthesis of L-*allo*-enduracididine **29**, a couple of procedures were available in the literature,[54] but they could not be used to access the appropriately protected enduracididine derivative required for the synthesis of the teixobactin macrocycle. We developed an improved and scalable route to an L-*allo*-enduracididine derivative having convenient protecting groups, by modifying of some of the chemistry developed by Rudolph *et al*.[55] and Peoples *et al*.[56] as described in Scheme 1.10. The synthesis of L-*allo*-enduracididine commenced with the reduction of the nitro group in known intermediate **25**, which in turn was prepared from an L-aspartic acid derivative. Amine **26** obtained from this reduction of **25**[55] was treated with benzyloxycarbonyl (Cbz)-protected 1H-pyrazole-1-carboxamidine[57] rendered formation of compound **27** in 78% yield over two steps. The product formation was primarily indicated by presence of broad peak at 3022 cm^{-1} which corresponding to hydroxyl group and peak at 1634 cm^{-1} for imine group in IR spectroscopy. Compound **27** was subjected to cyclization by using triflic anhydride (Tf_2O) in the presence of DIPEA, furnished cyclic guanidine **28** in 74% yield where in ^{1}H NMR, the benzylic protons were appeared at δ 5.27 (s, 2H) and 5.16 - 5.07 (m, 2H) ppm, α-methine protons present at δ 4.37 (m, 1H) ppm. The carbonyl peaks at δ 170.6 ppm from ester carbon, α-methine carbon at δ 53.4 ppm in ^{13}C NMR supports structure. The HRMS analysis showed a mass peak at 597.2911 corresponding to molecular formula $C_{31}H_{41}O_8N_4$ $[M + H]^+$ with calculated mass 597.2919 and confirmed its structure.



Scheme 1.10. Synthesis of L-*allo*-enduracididne

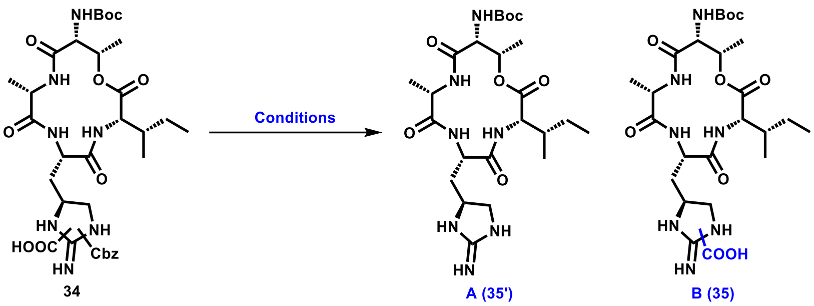
Compound **28** on protection of remaining free NH group by using CbzCl gave fully protected L-*allo*-enduracididine **29** in 86% yield (Scheme 1.10). The formation of product was indicated by TLC, where **29** was appeared non-polar spot than **28**. This white solid gave optical rotation $[\alpha]_D^{28}$ = –7.4 (*c* 0.20, $CHCl_3$). In ^{1}H NMR, the benzylic protons appeared at δ 5.28 - 5.16 (m, 4H), 5.13 - 5.07 (m, 2H) ppm. The presence of calculated number of peaks in ^{13}C NMR supports the drawn structure. The HRMS analysis showed a mass peak at 731.3282 corresponding to $C_{39}H_{47}O_{10}N_4$ $[M + H]^+$ with calculated mass 731.3287, which further confirmed the structure. Compound **29** was prepared in a gram scale which is required for completing total synthesis and also to prepare analogues of teixobactin.

Protected enduracididine **29** was converted into Boc-protected amino acid **30** by treating with 4 M HCl in 1,4-dioxane followed by reprotection of the free amine by using $(Boc)_2O$ and Na_2CO_3. Carboxylic acid **30** was coupled with L-isoleucine methyl ester using HATU and DIPEA in CH_2Cl_2 gave dipeptide **31** in 81% yield. The amine·HCl salt generated from dipeptide **31** by exposing it to 4 M HCl in dioxane was coupled with dipeptide acid **16** (from Scheme 1.6.) by using HATU as the peptide-coupling agent, which resulted in tetrapeptide **32**. The TBS protecting group in **32** was first tried with TBAF in THF, where we ended up with complex reaction mixture. Chemo-selective deprotection of TBS group in **32** was achieved by treatment of **32** with an equimolar amount of 10-camphorsulfonic acid (CSA) in CH_2Cl_2/MeOH (1:1) gave alcohol **33** in 80% yield. The ester group in compound **33** was hydrolyzed by using LiOH to give the *seco*-acid intermediate (not shown in Scheme 1.11.). Interestingly, only two of the three Cbz groups present on enduracididine were removed during the hydrolysis, and we could not determine the position of the Cbz group and the carboxylic acid moiety that remained intact which was confirmed by MALDI.



Scheme 1.11. Synthesis of macrocyclic fragment

At this stage, the *seco*-acid was subjected to macrocyclization under the optimized reaction conditions used for synthesis of model macrocycle **13** to furnish desired macrocycle **34** in 30–35% yield. All the spectral data generated for macrocycle **34** were in full agreement with the structure shown in Scheme 1.11. In ^{1}H NMR, the *O*-attached methine appeared at δ 5.43 - 5.31 (m, 1H) ppm. The presence of eight carbonyl peaks in ^{13}C NMR supports the presence of carbamic acid group. The HRMS analysis showed a mass peak at 740.3234 corresponding to $C_{33}H_{47}O_{11}N_7Na$ [M + Na]$^+$ with calculated mass 740.3231 which further confirms the structure. Although we could not determine the exact locations of the Cbz and COOH groups, we decided to remove the Cbz group under hydrogenation conditions with a hope that the additional COOH group would be removed under the same reaction conditions.



Entry	Conditions	Observations
1	H_2, Pd/C, MeOH, rt, 3 h	Only B (**35**) formed
2	H_2, $Pd(OH)_2$, THF: MeOH: H_2O: HCOOH, rt, 6 h	Only B (**35**) formed
3	H_2, Pd/C, EtOAc, rt, 12 h	Only B (**35**) formed
4	$Pd(OAc)_2$, Et_3N, Et_3SiH, CH_2Cl_2, rt, 3 h	Only B (**35**) formed

Scheme 1.12. Attempts to remove the protecting groups on the enduracididine moiety

Accordingly, compound **34** was subjected to a different reaction conditions to remove the Cbz group and the carbamic acid (Scheme 1.12.), but we were not successful and always ended up with compound **35** (instead of desired compound **35'**). The formation of **35** was indicated by the absence of aromatic signals from Cbz group. Further, the presence of characteristics signals of *O*-attached methine at δ 5.48 - 5.43 (m, 1H) ppm in ^{1}H NMR, imine carbon of enduracididine and carbamic acid were appeared at δ 156.6 and 158.2 ppm respectively in ^{13}C NMR, confirmed the formation of compound **35**. Similarly, the assigned structure was further validated by HRMS (ESI) which showed peak at 606.2845 corresponding to formula $C_{25}H_{41}O_9N_7$ $[M + Na]^+$ with calculated value of 606.2858. Probable intramolecular hydrogen bonding between the nitrogen atom and the carboxylic acid group on the enduracididine ring might be preventing the decarboxylation process. To our knowledge, only on one occasion, this kind of observation was documented.[58] Although we were not successful in the complete deprotection of enduracididine under the limited conditions we tried, we are confident that it would be possible under global deprotection conditions, and the same was observed in recent work from the Nowick group.[29a]

The other option would be to come up with a different protecting group on the enduracididine moiety. During this time (August 2016), two total syntheses of teixobactin appeared in the literature and we decided to drop the idea of total synthesis and focus on the generation of library of compounds with simplified motif around the scaffold to understand SAR as part of medicinal chemistry program towards lead optimization.

1.3.2. Total synthesis of Met_{10}-teixobactin

The L-*allo*-enduracididine unit is not commercially available, which is a limiting factor for the scalable synthesis of teixobactin. Establishment of the C4 chiral centre in a highly stereoselective way was became one of the main challenges in L-*allo*-enduracididine synthesis. Because of which several reports appeared in the literature in a short time span, where they replaced the L-*allo*-enduracididine by a commercially available amino acids in a search for appropriate alternative.[23-46]

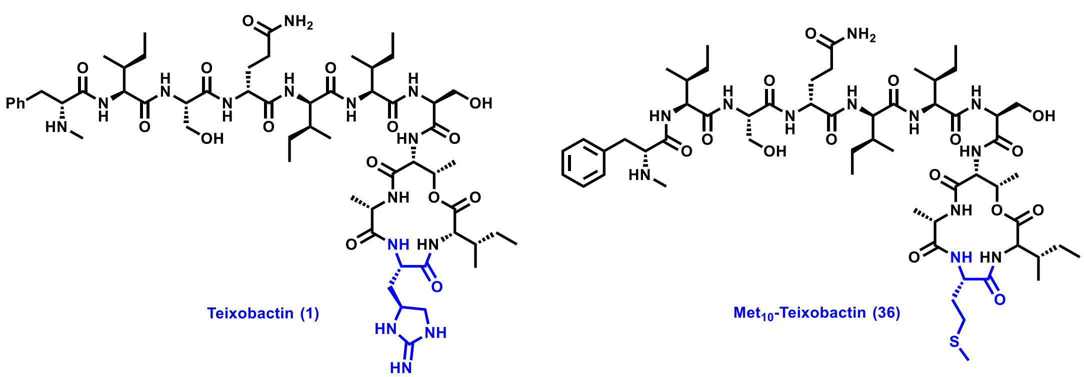


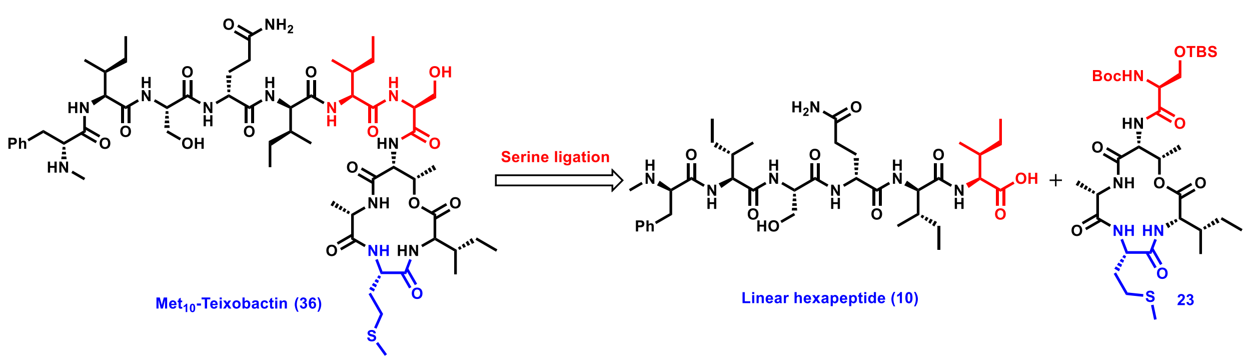
Figure 1.8. Structure of Met_{10}-teixobactin

For example, due to commercial availability and structural similarity between L-Arg and L-*allo*-enduracididine, it was commonly used as a substitute for L-*allo*-enduracididine. While we were working towards the synthesis of teixobactin, Li's group reported the synthesis of End substituted Met_{10}-teixobactin (**36**) which exhibited very promising antibiotic activities (0.25 μg/mL) against *Staphylococcus aureus* strains (Figure 1.8.).[38] Their structure-activity relationship (SAR) studies suggested that the synthetically challenging L-*allo*-enduracididine residue would be replaced with uncharged hydrophobic amino acids. As we were the first in the synthesis of macrocyclic core containing methionine at the place of L-*allo*-enduracididine;[46] these published results by Li's group encouraged us to carry out our efforts towards synthesis of

Met_{10}-teixobactin (**36**). We have accomplished the total synthesis of Met_{10}-teixobactin using solution-phase method and serine ligation strategy. The details are discussed in following sections.

1.3.2.1. Retrosynthesis

Our strategy to access the target compound Met_{10}-teixobactin (**36**) is outlined in Scheme 1.13. We planned the synthesis of target compound *via* convergent strategy of **S**erine **L**igation (SL) of linear hexapeptide **10** and cyclic depsipeptide **23** containing serine at *N*-terminus. Native chemical ligation (NCL) was first discovered by Theodor Wieland's group in 1953, where they did ligation of C-terminus valine-thioester and *N*-terminal cysteine amino acid to yield the Val-Cys dipeptide.[59] Further, in 1990's Stephen Kent and co-workers utilized it for the ligation of large unprotected peptide segments.[60] Ligation enables regio- and chemo-selective merger of two peptides segments which provide access to more complicated and long peptides sequences and would be effective to defeat the challenges of racemization generally occurred in peptide coupling by conventional methods.[61] Serine ligation involves the ligation of a peptide salicylaldehyde ester of one peptide segment and a serine at *N*-terminal as other segment, to afford the formation of an *N*,*O*-benzylidene acetal at the ligation site, followed by simple acidolytic cleavage to release amide bond formation.[62]

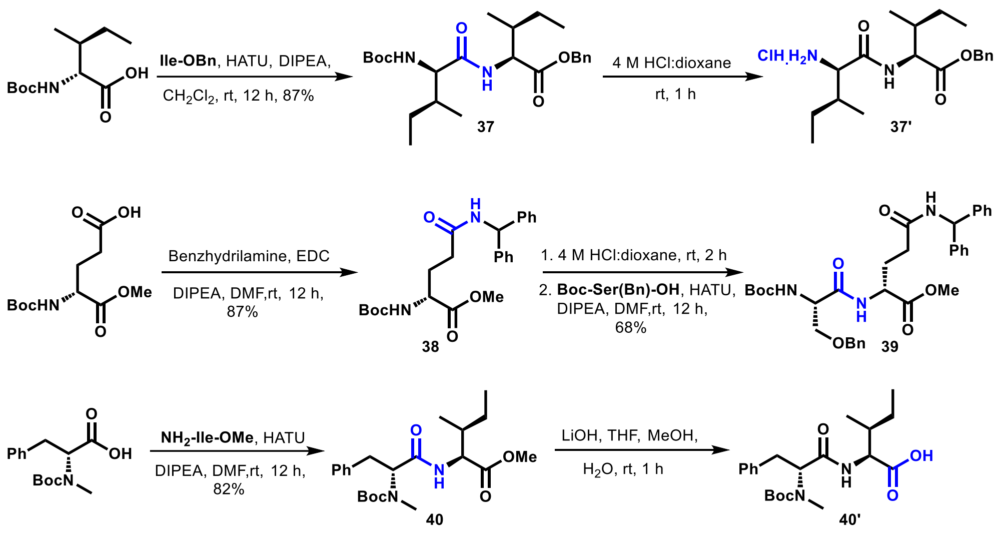


Scheme 1.13. Approach toward synthesis of Met_{10}-teixobactin

This method is more efficient than conventional processes of amide bond formation which able to accommodate side-chain-fully unprotected peptides and is less prone to epimerization.[61] Further, linear hexapeptide **10** could be synthesized from corresponding amino acids using Boc protecting group or Fmoc protecting group strategy in solution phase. Synthesis of compound **23** is discussed previously (see Scheme 1.9.).

1.3.2.2. Attempt toward synthesis of Met$_{10}$-teixobactin

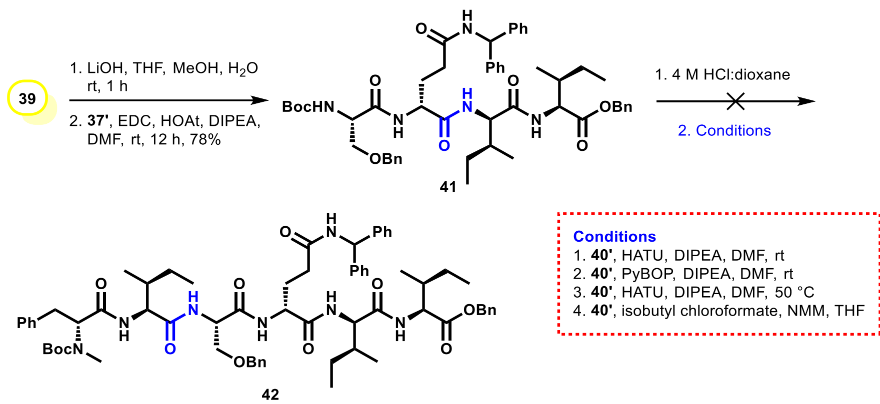
The best way in synthesis of linear hexapeptide was coupling of each dipeptide fragment which will be the convergent route of synthesis. As an onset of our synthesis, we prepared dipeptide **37** *via* coupling of Boc-D-*allo*-Ile[63] and NH_2-Ile-OBn mediated by HATU with 87% yield which undergoes deprotection of Boc group in presence of 4 M HCl in dioxane solution gave dipeptide amine salt **37'** as white solid.



Scheme 1.14. Synthesis of building blocks

On the other hand, Boc-D-Glu-OMe[64] underwent coupling smoothly with benzhydrilamine to afford compound **38** in 87% yield, which under Boc deprotection followed by subsequent coupling with Boc-Ser(Bn)-OH[65] in presence of HATU in DMF furnished dipeptide **39** in 68% yield. Dipeptide **40** was synthesized from coupling of Boc-*N*-Me-D-Phe[66] and isoleucine methyl ester in presence of HATU and DIPEA in 82% yield (Scheme 1.14.). Compound **40** further under methyl ester hydrolysis in presence of LiOH furnished the formation of acid **40'** which was characterized by ^{1}H, ^{13}C NMR and HRMS. Proton NMR clearly indicates the disappearing of methyl ester signal.

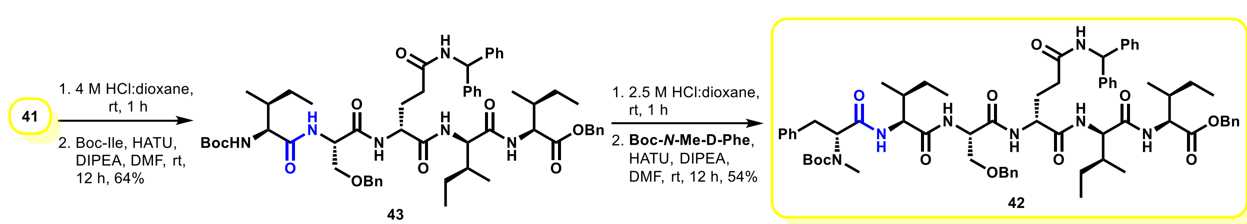
After successful synthesis of all dipeptide fragments, next task was to couple them to have desired hexapeptide. Dipeptide **39** was treated with LiOH to get corresponding acid, followed by linking with **37’** furnished tetrapeptide **41** in good yield.



Scheme 1.15. Attempts to synthesis of hexapeptide

The tetrapeptide **41** was clearly indicated by ^{1}H NMR peaks at δ 6.27 (d, *J*=7.6 Hz, 1H) ppm which corresponds to methine proton of benzhydrilamine. ^{13}C NMR spectra showed the same carbon at δ 53.2 ppm. HRMS (ESI) showed peak at 928.4824 corresponding the molecular formula $C_{52}H_{67}N_5O_9Na$ $[M + Na]^+$ with calculated value 928.4831, which further supports the formation of compound **41**. The tetrapeptide **41** on acidolytic Boc cleavage followed by treatment with **40’** under various coupling conditions (as shown in Scheme 1.15.), but unfortunately, we observed no product formation in all cases. It was difficult for us to rationalize why this reaction did not proceed.

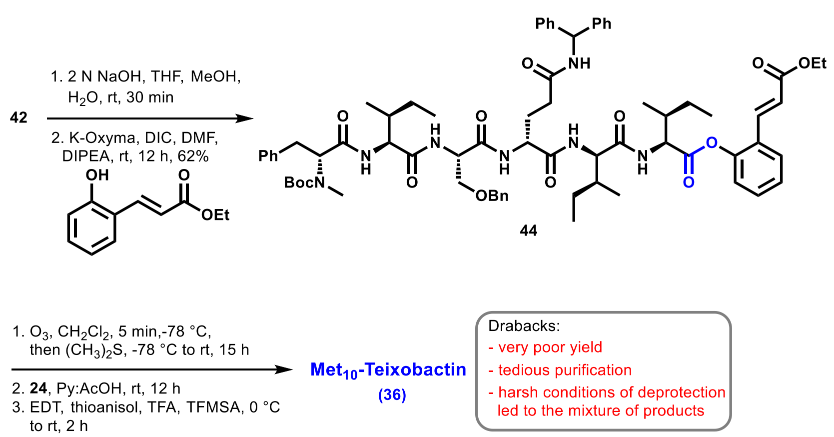
To solve the problem, we planned to go with single amino acid couplings at a time. The tetrapeptide **41** amine salt was reacted with Boc-Ile in presence of HATU and DIPEA in DMF furnished the pentapeptide **43** in 64% yield.



Scheme 1.16. Synthesis of hexapeptide

Compound **43** on treatment with 2.5 M HCl in dioxane provided amine hydrochloride salt which upon HATU mediated coupling with Boc-*N*-Me-Phe in DMF led to the formation of hexapeptide **42**. Compound **42** was synthesized in gram scale and characterized well by using ^{1}H, ^{13}C NMR, IR and HRMS analysis (Scheme 1.16.). The formation of hexapeptide **42** was indicated by the presence of characteristics signals of aromatic protons δ 7.25 - 7.34 (m, 25H), methine proton of benzhydrilamine δ 6.12 (d, *J*=8.3 Hz, 1H), *N*-Me at δ 2.66 (br. s., 3H) ppm in ^{1}H NMR. Similarly, the assigned structure was further confirmed by HRMS (ESI) which showed peak at 1202.6508 corresponding to formula $C_{68}H_{89}N_{7}O_{11}Na$ $[M + Na]^{+}$ with calculated value of 1202.6512.

Towards application of serine ligation method, the benzyl ester of compound **42** was saponified in presence of 2 N NaOH solutions in THF, MeOH and water, furnished the corresponding carboxylic acid which we had coupled with salicylaldehyde in presence of EDC and DMAP in CH_2Cl_2, but we did not observed any product formation. Carboxylic acid was successfully coupled with ethyl (*E*)-3-(2-hydroxyphenyl) acrylate[67] in presence of K-oxyma[68] and DIC[68] in DMF produced the salicylate ester **44** in 62% yield (Scheme 1.17.). Structure of compound **44** was confirmed by ^{1}H, ^{13}C NMR, IR and HRMS analysis.

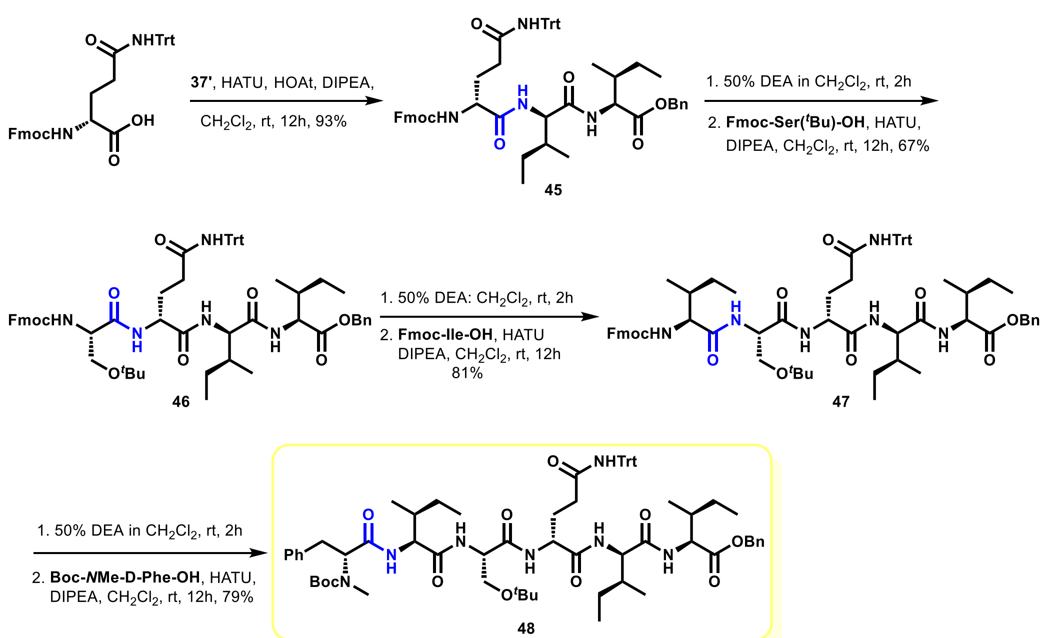


Scheme 1.17. Attempt to synthesis of Met_{10}-teixobactin

In ^{1}H NMR characteristic olefin peaks were appeared at δ 7.64 (d, *J* = 15.87 Hz, 1H) and 6.66 (d, *J* = 16.48 Hz, 1H) ppm, while δ 138.1 and 115.6 ppm in ^{13}C NMR analysis. HRMS (ESI) showed peak at 1286.6282 corresponding to formula $C_{72}H_{93}N_{7}O_{13}Na$ $[M + Na]^{+}$ with calculated value of 1286.6274. Reductive ozonolysis[23] of ester **44** yields corresponding aldehyde which undergoes serine ligation[23] with compound **24** in presence of pyridine acetate buffer followed acidolytic cleavage and global deprotection afforded Met_{10}-teixobactin **36** (as shown in Scheme 1.17.) which was purified by HPLC. MALDI indicated the formation of **36** which was further confirmed by HRMS (ESI) analysis showed peak at 1219.6744 corresponding to $[M + H]^+$ with molecular formula $C_{57}H_{95}N_{12}O_{15}S$ with calculated value 1219.6755, further confirmed the formation of compound **36**. Although we have synthesized the target compound, due to harsh reaction conditions of global deprotection and aerial oxidation of methionine, we ended up with insufficient amount of material for the complete characterization except HRMS.

1.3.2.3. Change of protecting groups and completion of Met_{10}-teixobactin synthesis

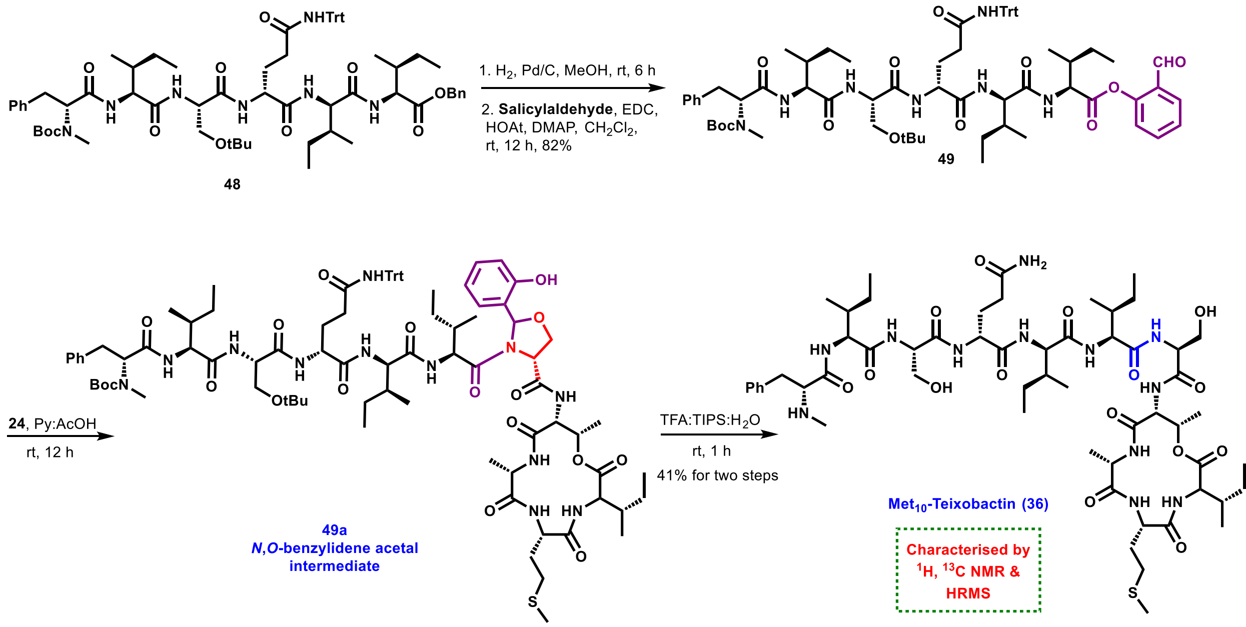
The appropriate protecting group was needed to circumvent the problem of deprotection of benzhydrilamine protecting group on D-Gln. To get the sufficient material, we have modified the scheme with replacement of benzhydrilamine protecting groups of D-Gln by trityl protection which could be deprotected under mild condition. With change in complementary protecting groups, we started the synthesis of target compound. Synthesis of hexapeptide **48** commenced with Fmoc strategy of peptide synthesis as shown in Scheme 1.18.



Scheme 1.18. Synthesis of hexapeptide with change in protecting groups

Fmoc-D-Gln(Trt)-OH on coupling with dipeptide **37'** in presence of HATU and DIPEA as base in dichloromethane furnished tripeptide **45** in 93% yield. This tripeptide **45** was then treated with 50% solution of diethyl amine (DEA) in dichloromethane to afford the amine which was coupled with Fmoc-Ser(^{t}Bu)-OH and HATU as coupling reagent in dichloromethane afford the tetrapeptide **46** in 67% yield. Formation of compound **46** was confirmed by IR, ^{1}H, ^{13}C NMR and HRMS analysis. Compound **46** undergoes Fmoc deprotection in presence of 50% solution of DEA in dichloromethane followed by coupling with Fmoc-Ile-OH afforded pentapeptide **47** in 81% yield. All the physical characterization for compound **47** was in agreement with the drawn structure. Pentapeptide **47** was treated with Et_2NH in CH_2Cl_2 for the deprotection of Fmoc group which further, coupled with the Boc-*N*-Me-D-Phe acid in presence of HATU and DIPEA in CH_2Cl_2, furnished linear hexapeptide **48** in 79% yield. It was characterized by ^{1}H, ^{13}C NMR, IR and HRMS data. The formation of hexapeptide **48** was indicated by the presence of characteristic signals of aromatic and *N*-Me groups in ^{1}H NMR at δ 7.31 - 7.35 (m, 6H), 7.25 (t, *J*=7.3 Hz, 11H), 7.14 - 7.20 (m, 11H) and 2.69 (br. s., 3H) ppm respectively. The assigned structure was further confirmed by HRMS (ESI) which showed peak at 1244.6971 corresponding to formula $C_{71}H_{95}N_7O_{11}Na$ $[M + Na]^+$ with calculated value of 1244.6982.

Compound **48** on catalytic hydrogenation in methanol solvent under the of H_2 atmosphere (balloon pressure) furnished hexapeptide acid which was confirmed by MALDI. The same acid was subjected to esterification using salicylaldehyde in presence of EDC, HOAt and DMAP in CH_2Cl_2 yielded Hex-SAL **49** in 82% yield. The compound **49** was purified by flash column chromatography. In ^{1}H NMR, the characteristic proton of aromatic aldehyde appeared at δ 10.12 (d, *J*=9.5 Hz, 1H) and *N*-Me at δ 2.69 (s, 3H) ppm. The aldehyde carbon appeared at δ 189.2 ppm in ^{13}C NMR. HRMS (ESI) showed peak at 1258.6774 for $C_{71}H_{93}N_7O_{12}Na$ $[M + Na]^+$ 1258.6770. All the NMR values and HRMS analysis were in complete agreement with the structure of **49**. Ligation proceeded efficiently with coupling of compound **49** and **24** in Py: AcOH[23] and generates an *N*,*O*-benzylidene acetal intermediate, **49a** (formation was confirmed by MALDI). Acidolysis of intermediate **49a** in presence of TFA: TIPS: H_2O restored the peptide linkage at ligation site and afforded Met_{10}-teixobactin **36** with 41% yield over two steps (Scheme 1.19.). Purification of crude product by reverse phase HPLC afforded Met_{10}-teixobactin **36** with improved yield which was characterized by ^{1}H and ^{13}C NMR spectral data and HRMS analysis.

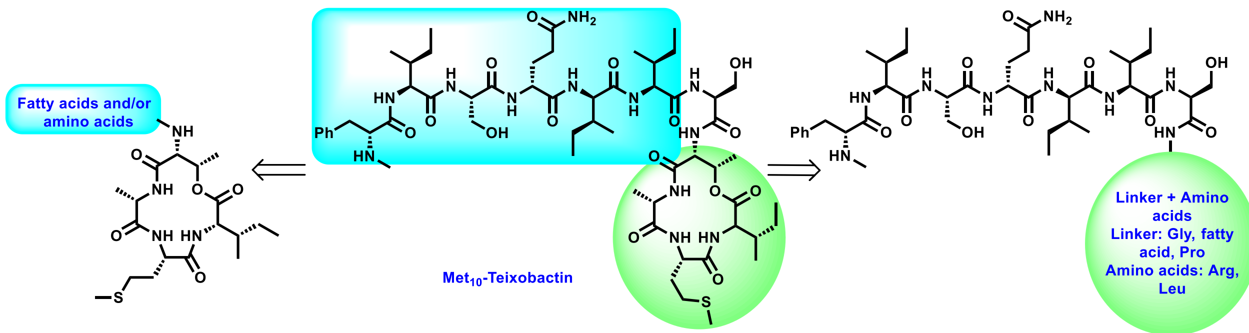


Scheme 1.19. Synthesis of Met_{10}-teixobactin

HRMS (ESI) indicated peak at 1219.6747 for $C_{57}H_{95}N_{15}O_{12}$ $[M + H]^+$ 1219.6755. All the ^1H and ^{13}C NMR values and HRMS data were in complete agreement with the structure **36**. We have successfully synthesized Met_{10}-teixobactin by combinatory approach of ligation-mediated convergent strategy.[69] This strategy can provide beneficial platform for the synthesis of analogues and ultimately helpful for structure activity relationship studies in finding more potent analogues with improved pharmacological properties. The current method of synthesis of Met_{10}-teixobactin in solution phase is convenient for the synthesis of teixobactin and its analogues in good quantities for further biological profiling.

1.3.3. Synthesis of teixobactin analogues

Teixobactin consist of hydrophobic linear heptapeptide and hydrophilic macrocyclic core. To investigate the role of this linear peptide fragment in biological activity of teixobactin, we decided to replace it with fatty acids of different lengths in Met_{10}-teixobactin as base. Lipopeptides with linear or cyclic peptide sequence are a special class of highly active antibiotics against multi-resistant bacteria[70] (eg. Daptomycin is already approved by US-FDA for the infections caused by Gram-positive bacteria in 2013).[71] Peptides with lipophilic domains were found to be responsible for peptide aggregation, membrane association leading to lipid phase transitions and membrane depolarization, which represents the uniqueness of their mode of action.[72] Although teixobactin is a small peptide, synthesis of linear peptide by maintaining orthogonal protecting groups and macrocyclization are the major hurdles on the path of its synthesis. For the synthesis of analogues for SAR, the most convenient approach is to initially synthesize either the linear peptidic part or the macrocyclic unit followed by necessary modifications in the counterpart and subsequent coupling of both. We have successfully synthesized Met_{10}-teixobactin which is an equipotent teixobactin analogue.

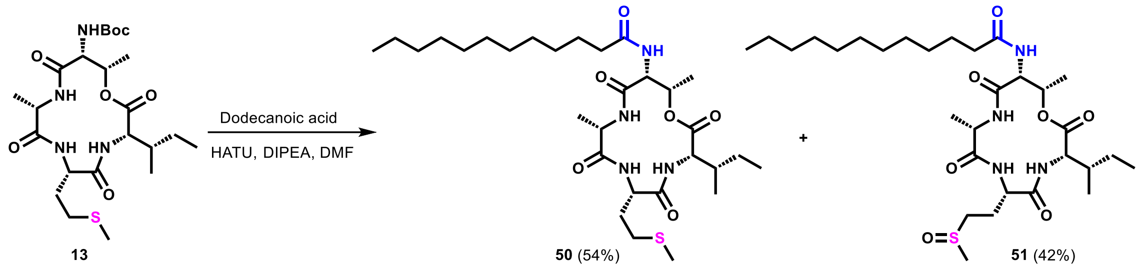


Scheme 1.20. Synthesis of analogues

To find out the role of non-polar linear peptide part and macrocyclic core towards antibacterial activity, we planned to prepare some analogues around this scaffold. In the first part, linear hexapeptide was replaced by fatty acids of different chain length to conclude its contribution in the potency of Met_{10}-teixobactin. On the other side, the macrocyclic core was replaced by acyclic structures with insertion of linkers like glycine, fatty acids or proline which can acts as turn inducer and mimic the macrocycle (Scheme 1.20).

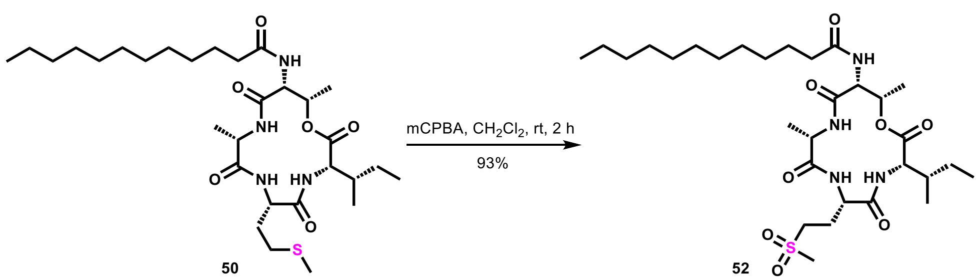
1.3.3.1. Synthesis of teixobactin analogues with replacement of linear hexapeptide by fatty acids

As per the plan, with an aim to investigate contribution of linear hexapeptide to the activity of Met_{10}-teixobactin, we have synthesized a small library of teixobactin analogues; substituting Met in the place of L-*allo*-enduracididine and the linear peptide part with fatty acids of varying chain lengths. As we have synthesized considerable amount of the methionine-containing macrocycle beforehand, we then hooked to the same with various long chain hydrocarbons as well as alkyl moieties.



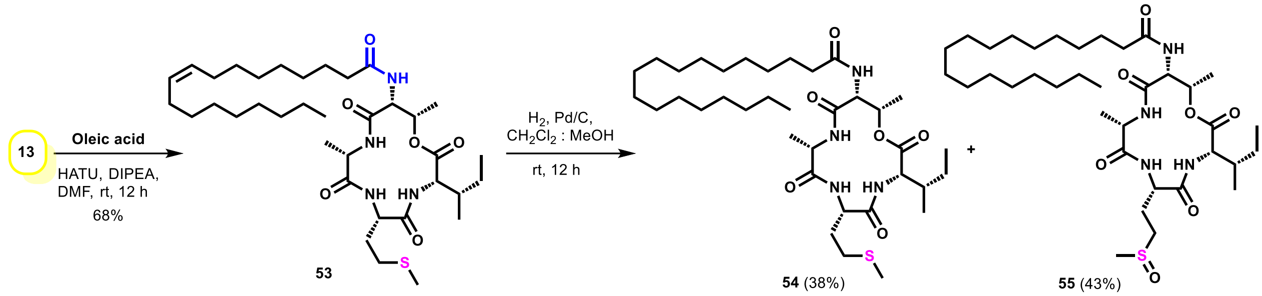
Scheme 1.21. Synthesis of analogues with dodecanoic acid

Synthesis of analogues started with compound **13** which upon acidolytic cleavage of Boc group followed by coupling with dodecanoic acid using HATU and DIPEA in DMF furnished the formation of two spots on TLC (Scheme 1.21.). After column purification, the non-polar spot was desired analogue **50** and polar spot was oxidized compound **51** (might be observed due to aerial oxidation of sulphur) in moderate yield. Both the compound were differentiated by ^{1}H NMR where key *S*-methyl group appeared at δ 2.08 (br. s., 3H) ppm for **50** and at δ 3.15 (br. s., 3H) ppm for **51**. Structures of both the compounds were further confirmed by ^{13}C NMR and HRMS analysis.



Scheme 1.22. Synthesis of sulfone analogues with dodecanoic acid

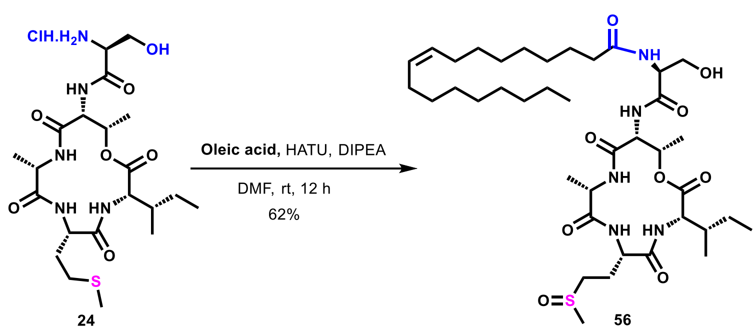
To prepare the sulfone analogue, compound **50** was oxidized in presence of *m*-CPBA in CH_2Cl_2 at room temperature furnished **52**. The formation of sulfone was primarily confirmed by TLC which appeared as non-polar spot than **50**. Sulfone **52** was characterized by ^{1}H, ^{13}C NMR and HRMS analysis. In ^{1}H NMR *S*-methyl was appeared at δ 2.99 (s, 3H) ppm. Here we prepared sulfur, sulfoxide and sulfone analogues (Scheme 1.22.) to find out the role of methionine in antibiotic activity which probably defines the mode of binding to lipid II and lipid III.



Scheme 1.23. Synthesis of analogues with oleic acid

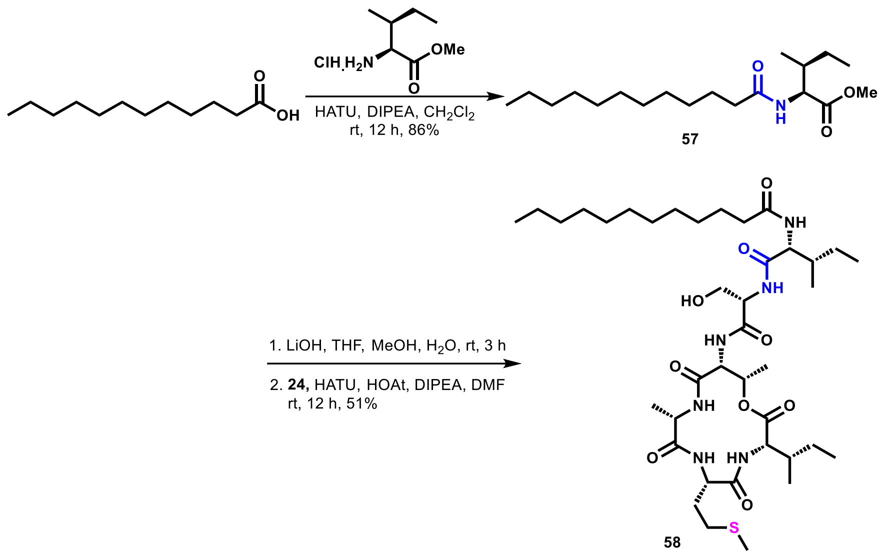
To find out the role of extended hydrophobicity, we increased the length of fatty acid by inserting oleic acid. Compound **13** on Boc deprotection followed by coupling with oleic acid using HATU and DIPEA furnished compound **53**, where characteristic olefin signals were appeared at δ 5.35 - 5.40 (m, 1H), 5.36 (t, J=4.6 Hz, 1H) ppm in ^{1}H NMR and δ 130.0, 129.9 ppm in ^{13}C NMR confirmed the structure. Further **53** upon hydrogenation of olefin gave compound **54** along with oxidized product **55** in moderate yield (Scheme 1.23.). Disappearance of olefin peaks in ^{1}H and ^{13}C indicates formation of products, which further confirmed by HRMS analysis.

Compound **24** underwent coupling with oleic acid in HATU and DIPEA in DMF to give compound **56**. In this case we were unable to isolate the unoxidized product as most of it was converted to **56** by aerial oxidation (Scheme 1.24.).



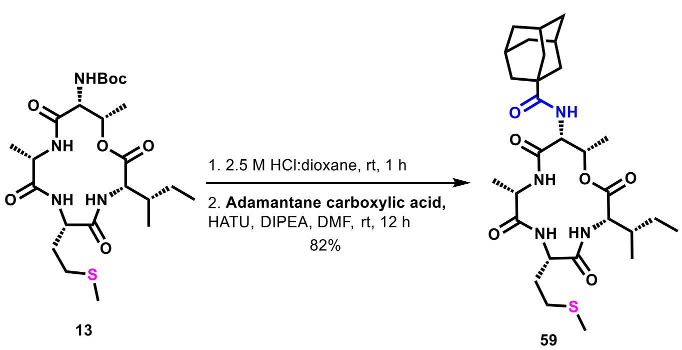
Scheme 1.24. Synthesis of analogue with oleic acid

Lipobactin, a lipidated analogue of teixobactin having comparable potency to that of teixobactin was known in the literature.[29a] We planned the synthesis of similar analogue, where we replaced Arg_{10} by Met_{10}. Dodecanoic acid was reacted with isoleucine methyl ester using coupling



Scheme 1.25. Synthesis of lipobactin homologue

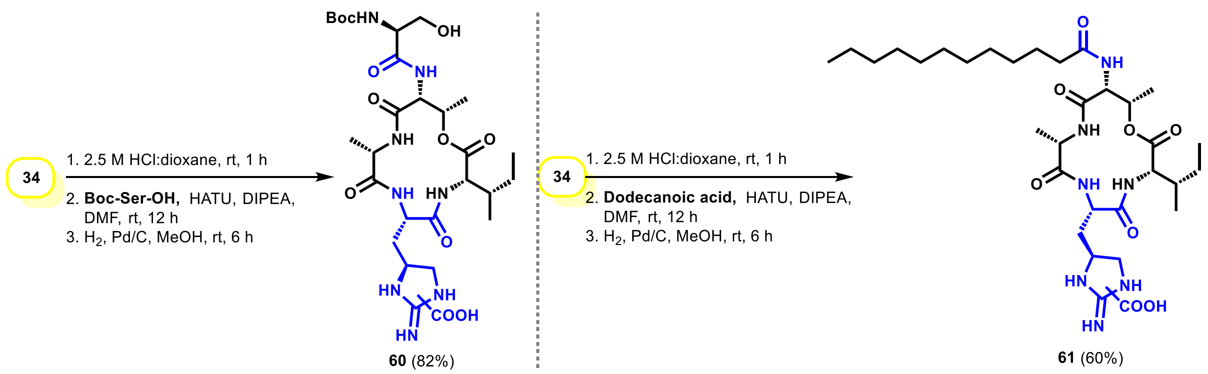
reagents HATU and DIPEA in dichloromethane gave dipeptide 57.[73] Dipeptide **57** on saponification by LiOH followed by coupling of corresponding acid with **24** furnished lipobactin homologue **58** in 52% yield (Scheme 1.25.). In ^{1}H NMR, the characteristic *O*-attached methine signals were appeared at δ 5.47 - 5.32 (m, 1H) and *S*-methyl at δ 2.08 (d, *J* = 3.7 Hz, 3H) ppm while in ^{13}C NMR this *O*-attached methine was appeared at δ 71.7 ppm. HRMS (ESI) indicated peak at 821.4833 for $C_{39}H_{70}N_6O_9SNa$ $[M + Na]^+$ 821.4823.



Scheme 1.26. Synthesis of adamantane based teixobactin analogues

Compound **13** on coupling with adamantine carboxylic acid furnished new compound **59** in 82% yield which was confirmed by ^{1}H, ^{13}C and HRMS (Scheme 1.26.). In ^{1}H NMR *O*-attached methine was appeared at δ 5.55 (d, *J*=5.5 Hz, 1H), four α-protons were coming at δ 4.72 (d, *J*=8.5 Hz, 1H), 4.50 (q, *J*=7.9 Hz, 1H), 4.32 (br. s., 1H), 4.01 - 4.08 (m, 1H) ppm, five carbonyl carbons were present at δ 179.9, 173.8, 171.3, 170.9, and 169.6 ppm while *O*-attached methine was appeared at 70.3 ppm.

L-*allo*-enduracididine macrocycle **34** was treated with 4 M HCl in 1,4-dioxane to afford amine hydrochloric acid salt which on coupling with Boc-Ser-OH afforded product in Cbz-protected form.



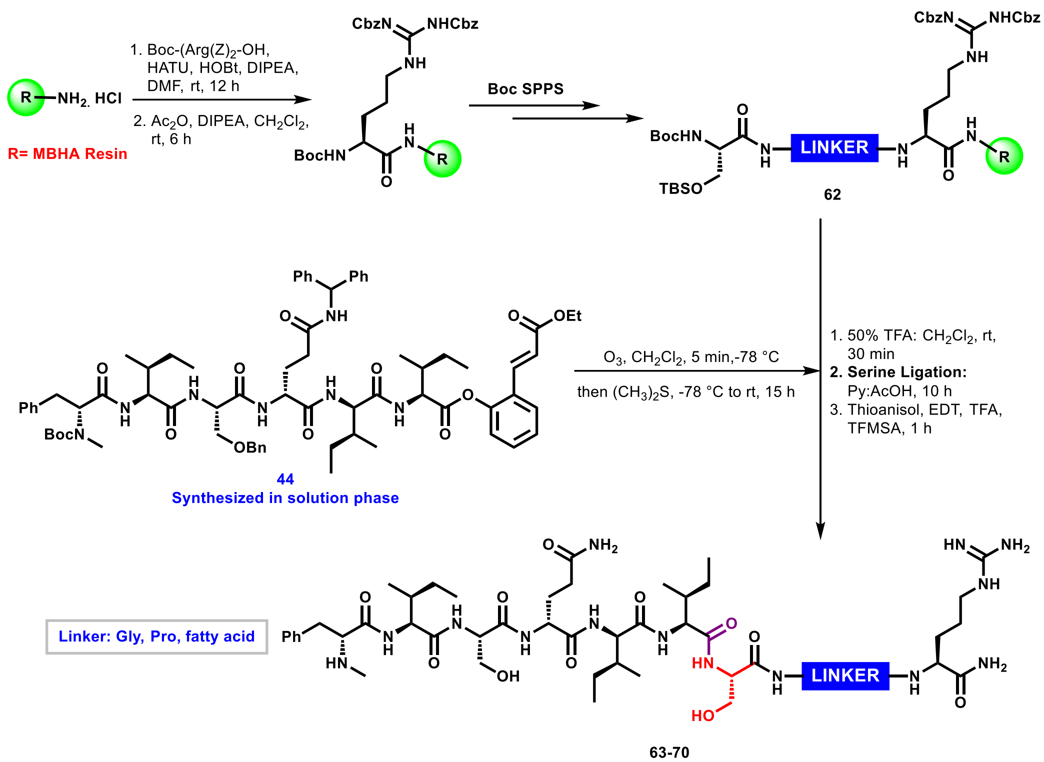
Scheme 1.27. Synthesis of analogues with enduracididine macrocycle

Further, Cbz group was removed under hydrogenolysis condition gave compound **60** in 82% yield. Formation of product was confirmed by ^{1}H, ^{13}C NMR and HRMS analysis. Similarly, compound **34** was coupled with dodecanoic acid in presence of HATU, afforded product with Cbz protection which on hyrogenolysis in presence of H_2 balloon pressure and Pd/C (10%) furnished compound **61**. ^{1}H NMR shows *O*-attached methine proton at δ 5.27 - 5. 27 (m, 1H) ppm, 1.24 - 1.33 (m, 20H) protons from aliphatic long chain and HRMS gave peak at 665.8334 with molecular formula $C_{32}H_{55}N_7O_8$ $[M + H]^+$ calculated for mass 665.8330 was in agreement with the structure (Scheme 1.27.).

1.3.3.2. Synthesis of teixobactin analogues with alteration of macrocyclic core

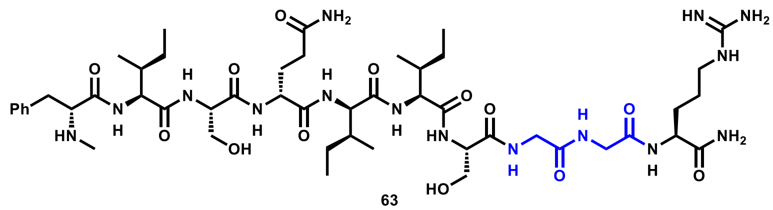
As a part of macrocyclic alterations, we planned to couple mostly Arg and Leu, bound with appropriate linkers like Pro, Gly and caproic acid. In most of these linear alterations, we tried to keep Arg at the C-terminal part as it is morphologically similar to End and can mimic the same. We also chose proline as a linker in some selected analogues which operates as a turn inducer[74] consequently resulting in a structure with morphologically similar to the macrocycle (macrocyclic mimic). Also, it was well known that proline-containing peptides with *cis*-*trans* isomerisation capability affect various biological processes.[74] In the course of synthesizing these classes of linear analogues; we utilized serine ligation as our key step (Scheme 1.28.).[60]

Our synthesis commenced with Boc-Arg(Z)$_2$-OH which was coupled on MBHA resin[75] through solid phase peptide synthesis strategy. Tetrapeptide **62** containing serine at *N*-terminus was synthesized by using Boc SPPS strategy.

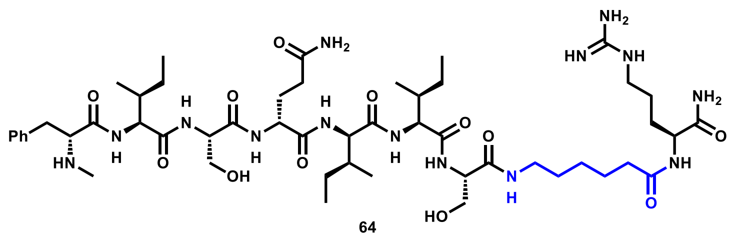


Scheme 1.28. Synthesis of analogues

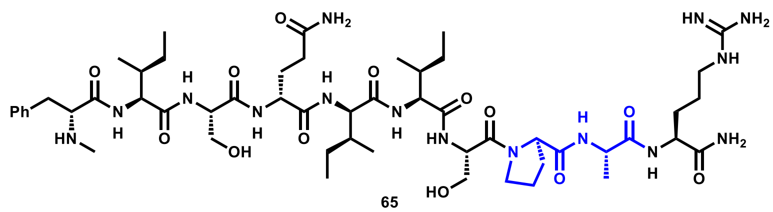
Deprotection of Boc and TBS groups in compound **62** was achieved by using trifluoroacetic acid in CH_2Cl_2 followed by serine ligation with a corresponding aldehyde which was in turn prepared in situ through ozonolysis of salicylate ester **44**. Simultaneous cleavage from resin and global deprotection using TFA and TFMSA in presence of scavengers, furnished the required teixobactin analogues as shown in Scheme 1.28. Here we have used linkers like Gly, 6-amino caproic acid, Pro and Lys. By using this approach, we prepared seven analogues with Arg at C-terminal and one with Leu at C-terminal.



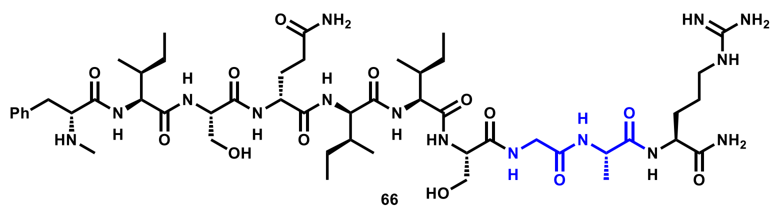
63



64



65



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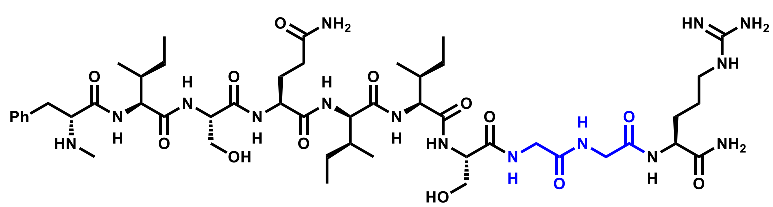

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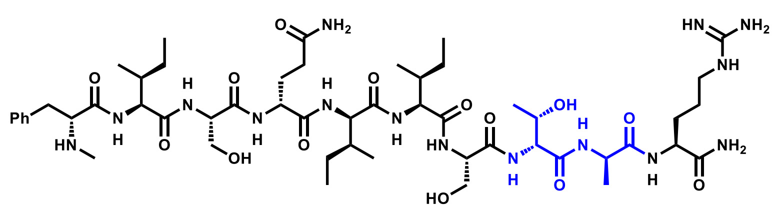

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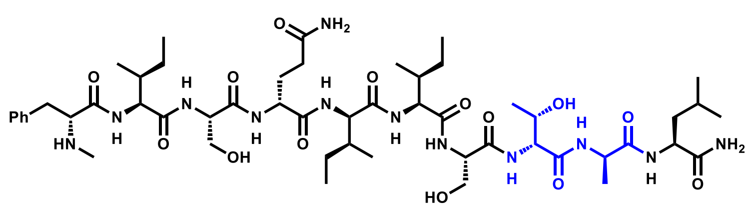
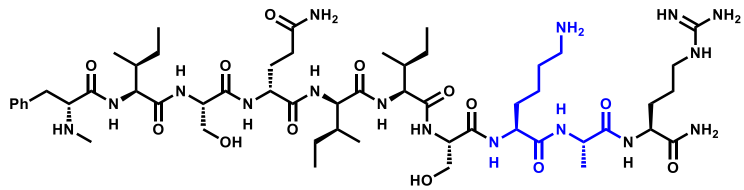


69



70

Figure 1.9. Synthesized teixobactin analogues

All the synthesized analogues from **63** to **70** were purified by preparative HPLC using linear gradient of ACN: H_2O and the products were confirmed by HRMS analysis (Figure 1.9.).

1.3.4. Biological evaluation of teixobactin analogues

Having successfully synthesized the targeted teixobactin analogues, we went forward for the bio-evaluation of the same in antibacterial activities against ESKAPE pathogens. All the biological evaluations are done at CSIR-CDRI with the help of Dr. Sidharth Chopra of Microbiology department.

Compounds and reference bacterial strains: Stock solutions of test compounds were prepared in DMSO. Compounds were screened against a bacterial panel consisting of ESKAPE pathogens, namely Gram-positive pathogen *Staphylococcus aureus* strain ATCC29213 and four Gram-negative pathogens which includes *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli*. All the bacterial strains were subcultured onto Mueller Hinton agar and incubated at 37 °C for 24 hours prior to use in the experiments.

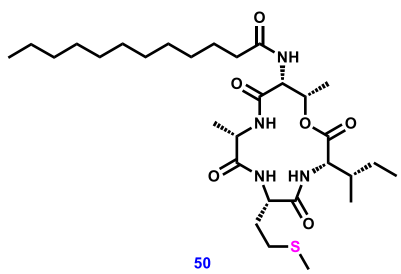
Minimum Inhibitory Concentration (MIC) determination: The MIC was determined using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI) guidelines.[76] Bacterial cultures were inoculated in MHBII, and optical density (OD) was measured at 600 nm, followed by dilution to achieve ~10^6 colony-forming units (CFU)/mL. The compounds were tested from 64 to 0.5 µg/mL in two-fold serial diluted fashion, with 2.5 µL of each concentration added to the wells of a 96-well round bottomed microtitre plate. The plates were incubated at 37 °C for 18–24 h, and then the minimum inhibitory concentration (MIC) was determined. The MIC is defined as the lowest concentration of the compound at which there is absence of visible growth. For each test compound, MIC determinations were carried out independently three times using duplicate samples.

Sr. No.	Compound No.	*S. aureus* ATCC 29213 (µg/mL)	*K. pneumoniae* BAA-1705 (µg/mL)	*A. baumannii* BAA-1605 (µg/mL)	*P. aeruginosa* ATCC 27853 (µg/mL)	*Escherichia coli* ATCC 25922 (µg/mL)
1.	13	>64	>64	>64	>64	>64
2.	Hexapeptide	>64	>64	>64	>64	>64
3.	23	>64	>64	>64	>64	>64
4.	35	>64	>64	>64	>64	>64
5.	**50**	**8**	>64	>64	>64	>64
6.	**51**	**32**	>64	>64	>64	>64
7.	**52**	**32**	>64	>64	>64	>64
8.	53	>64	>64	>64	>64	>64
9.	54	>64	>64	>64	>64	>64
10.	55	>64	>64	>64	>64	>64
11.	56	>64	>64	>64	>64	>64
12.	58	>64	>64	>64	>64	>64
13.	59	>64	>64	>64	>64	>64
14.	60	>64	>64	>64	>64	>64
15.	61	>64	>64	>64	>64	>64
16.	63	>64	>64	>64	>64	>64
17.	64	>64	>64	>64	>64	>64
18.	65	>64	>64	>64	>64	>64
19.	66	>64	>64	>64	>64	>64
20.	67	>64	>64	>64	>64	>64
21.	68	>64	>64	>64	>64	>64
22.	69	>64	>64	>64	>64	>64
23.	70	>64	>64	>64	>64	>64
24.	36	>64	>64	>64	>64	>64

25.	Met_{10}-Teixobactin[38]	0.25				
26.	Teixobactin[18]	0.25				

Table 1.1. MIC values of teixobactin analogues against ESKAPE pathogens.

All the results obtained from antibacterial screening against ESKAP pathogens are captured in Table 1.1. Broadly, all the compounds showed no encouraging results with different bacteria, except *S. aureus* Gram-positive bacteria. However, it is worth to mention that the maximum concentration tested was at 64 microgram per mL. To explore the contribution of *N*-terminus, we checked the activity of compound **13**, **23** and **35**, which proved to be inactive. The replacement of hydrophobic residue at the place of linear hexapeptide, resulting lipopeptide **50** proved only two times less active than lipobactin[29a] against only Gram-positive pathogens and thus followed alike tendency of antibiotic potency to that of teixobactin. Along with this, compound **51** and **52** were 4 fold less active than the compound **50**.



50
Most simplified & potent analogue of teixobactin

The substitution of linear hexapeptide with increased length of fatty acid furnished the completely inactive compounds suggesting that the importance of balanced hydrophobicity of the *N*-terminal tail. Compound **58** was homologue of lipobactin where Arg_{10} was replaced by Met_{10}, surprisingly came out to be inactive. Compound **60** contains fatty acid in replacement of linear heptapetide and enduracididine with carbamic acid was also inactive. The other set of analogues which were prepared from the replacement of macrocyclic part by linear peptide or fatty acid part turned to be inactive at tested concentrations. This SAR information indicates that cyclic depsipeptide structure with balanced lipophilicity is necessary for antibacterial activity, at least in the case of *S. aureus*. The most potent teixobactin analogue (compound **50**) synthesized in present work is shown above.

1.4. Conclusions

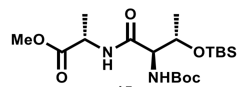
We have accomplished the synthesis of the macrocyclic part (key component) of the antibiotic teixobactin along with model macrocycle by using a solution-phase synthetic approach. The synthesis of the macrocycle in a good scale by using solution-phase techniques, which helped us to access diverse analogues and also to acquire sufficient amounts of material for further biological evaluation. Synthesis features a lanthanide triflate mediated Shiina macrolactonization and gram scale synthesis of the rare amino acid L-*allo*-enduracididine. Unlike previous reports, we utilized macrolactonization for the construction of the macrocycle.

We have successfully synthesized Met_{10}-teixobactin by using the key strategy of "serine ligation". The solution-phase combinatory synthetic strategy described herein is suitable for the synthesis of teixobactin and its analogues in good quantities for further biological profiling.

The prudent design and synthesis of teixobactin lipopeptide analogues has been achieved by using solution phase peptide synthesis. Substituting the linear peptide fragment with fatty acid of different chain length provided an active analogue of teixobactin. A structure activity relationship (SAR) study was done to probe the function of macrocyclic core on biological activity which disclosed the significance of it along with the presence of enduracididine isostere residue. Finally, compound **50** has been identified as most simplified novel lipidated analogue of teixobactin with promising antibacterial activity, which imparts role of lipophilicity at *N*-terminal and represents promising lead compound for further profiling. Present efforts of convergent synthetic strategy are helpful for synthesis of complex peptides and can be used to decipher their SAR. While working on this project, we made a striking observation of a symbiotic relationship in activity between the linear peptidic part and cyclic depsipeptide; which is found to be separately inactive against various bacterial strains.

1.5. Experimental section

Methyl *N*-(*tert*-butoxycarbonyl)-*O*-(*tert*-butyldimethylsilyl)-D-threonyl-L-alaninate (15)



15

To a stirred solution of D-threonine acid **14** (3.0 g, 9.0 mmol) and L-alanine methyl ester hydrochloride (1.4 g, 9.9 mmol) in anhydrous dichloromethane (50 mL) at 0 °C were added EDC.HCl (2.25 g, 11.7 mmol), HOBt (1.37 g, 9.0 mmol) and DIPEA (4.7 mL, 27.0 mmol). After being stirred at room temperature for 12 h, the reaction mixture was quenched with water. The organic layer was washed with 1 N HCl (10 mL) and a saturated $NaHCO_3$ solution (20 mL) dried over anhydrous Na_2SO_4, concentrated under reduced pressure to get the crude product which was purified by silica gel column chromatography using ethyl acetate and hexane (1:4) as mobile phase to afford dipeptide **15** as viscous liquid.

Yield: 75% (2.82 g)

Specific rotation: $[\alpha]_D^{26}$ = −6.6 (*c* 1.11, $CHCl_3$)

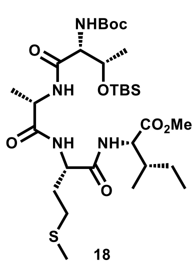
IR ν_{max} (film): 3415, 3016, 2942, 2863, 1741, 1714, 1674, cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20–6.97 (m, 1H), 5.43 (d, *J* = 5.6 Hz, 1H), 4.59 (t, *J* = 7.2 Hz, 1H), 4.44–4.25 (m, 1H), 4.20–3.99 (m, 1H), 3.73 (s, 3H), 1.45 (s, 9H), 1.39 (d, *J* = 7.1 Hz, 3H), 1.08 (d, *J* = 6.1 Hz, 3H), 0.87 (m, 9H), 0.09 (brs., 6H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 172.9, 169.2, 155.7, 79.9, 68.1, 59.1, 52.3, 48.0, 28.3, 25.7, 18.6, 17.8, −4.8, −5.1

HRMS (ESI): calculated for $C_{19}H_{38}O_6N_2NaSi$ $[M + Na]^+$: 441.2391, found: 441.2392.

Methyl *N*-(*tert*-butoxycarbonyl)-*O*-(*tert*-butyldimethylsilyl)-D-threonyl-L-alanyl-Lmethionyl-L-isoleucinate (18)



18

To a solution of *N*-Boc dipeptide **17** (1.0 g, 2.66 mmol) was added TFA (2.0 mL) in anhydrous CH_2Cl_2 (8 mL) at room temperature under argon. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. Coupling was done from the residue of **17** and acid **16** by following similar procedure as utilized in synthesis of **15**. The crude tetrapeptide was purified by silica gel column chromatography using ethyl acetate and hexane (2:3) as mobile phase to afford tetrapeptide **14** as white foam.

Yield: 70%

Specific rotation: $[\alpha]_D^{26}$ = −18.1 (*c* 0.50, $CHCl_3$)

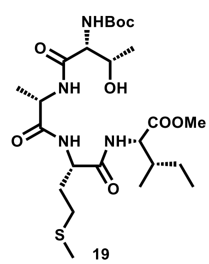
IR ν_{max} (film): 3413, 3328, 3021, 2969, 2403, 1669 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.18 (brs., 1H), 7.14 - 6.97 (m, 1H), 6.86 (brs., 1H), 5.50 (brs., 1H), 4.62 (q, *J* = 6.7 Hz, 1H), 4.53 (dd, *J* = 5.0, 8.4 Hz, 2H), 4.35 - 4.25 (m, 1H), 4.17 - 4.06 (m, 1H), 3.73 (s, 3H), 2.60 (t, *J* = 7.0 Hz, 2H), 2.11 (s, 3H), 2.05 (dt, *J* = 7.3, 13.6 Hz, 3H), 1.91 (tdd, *J* = 4.7, 6.8, 9.0 Hz, 1H), 1.45 (s, 9H), 1.43 - 1.34 (m, 4H), 1.24 - 1.14 (m, 1H), 1.09 (d, *J* = 5.9 Hz, 3H), 0.96 - 0.83 (m, 15H), 0.11 (s, 3H), 0.10 (s, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.9, 171.7, 170.5, 169.9, 155.6, 79.9, 68.3, 59.3, 56.6, 52.1, 52.0, 49.0, 37.5, 30.6, 30.0, 28.3, 25.7, 25.0, 18.6, 18.5, 17.8, 15.5, 14.9, 11.5, −4.8, −5.0

HRMS (ESI): calculated for $C_{30}H_{58}O_8N_4NaSSi$ $[M + Na]^+$: 685.3637, found: 685.3624.

Methyl (*tert*-butoxycarbonyl)-D-threonyl-L-alanyl-L-methionyl-L-isoleucinate (19)



19

To a stirred solution of silyl ether **18** (500 mg, 0.75 mmol) in anhydrous THF (10 mL) was added TBAF (1.50 mL, 1 M solution in THF, 1.50 mmol) at room temperature. The reaction mixture was stirred for 1 h at same temperature. After completion of the reaction (monitored by TLC), it was quenched with aqueous ammonium chloride solution (5 mL). The reaction mixture was extracted with ethyl acetate (3 X 10 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography utilizing MeOH and CH_2Cl_2 (1:49) as mobile phase to afford alcohol **19** as a colorless foam.

Yield: 81%

Specific rotation: $[\alpha]_D^{26}$ = +6.3 (*c* 0.55, $CHCl_3$)

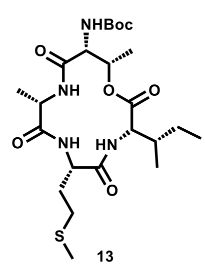
IR ν_{max} (film): 3576, 3369, 3303, 3017, 2974, 1668, 1528 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (d, *J* = 8.1 Hz, 1H), 7.22 (d, *J* = 8.3 Hz, 1H), 7.10 (d, *J* = 6.4 Hz, 1H), 5.63 (d, *J* = 7.3 Hz, 1H), 4.73 (q, *J* = 7.2 Hz, 1H), 4.61 - 4.46 (m, 2H), 4.36 (brs., 1H), 4.22 - 4.11 (m, 1H), 3.73 (s, 3H), 2.54 (t, *J* = 7.1 Hz, 2H), 2.08 (s, 3H), 2.01 (td, *J* = 6.9, 13.9 Hz, 1H), 1.97–1.84 (m, 2H), 1.45 (s, 9H), 1.40 (d, *J* = 6.8 Hz, 3H), 1.40–1.35 (m, 1H), 1.28 - 1.10 (m, 1H), 1.20 (d, *J* = 6.4 Hz, 3H), 0.96 - 0.81 (m, 6H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 172.3, 171.9, 171.6, 171.2, 156.2, 80.4, 67.4, 59.6, 56.7, 52.1, 51.9, 49.3, 37.5, 31.6, 29.8, 28.2, 25.0, 19.2, 18.0, 15.4, 15.0, 11.5

HRMS (ESI): calculated for $C_{24}H_{44}O_8N_4NaS$ $[M + Na]^+$: 571.2772, found: 571.2776.

***tert*-Butyl ((3*S*,6*S*,9*S*,12*R*,13*S*)-3-((*S*)-*sec*-butyl)-9,13-dimethyl-6-(2-(methylthio)ethyl)-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-12-yl) carbamate (13)**



13

Lithium hydroxide monohydrate (39 mg, 0.91 mmol) was added to a vigorously stirring solution of tetrapeptide methyl ester **19** (250 mg, 0.456 mmol) in THF (10 mL) and H_2O (5 mL) at 0 °C. Following complete consumption of the starting material by TLC, the reaction mixture was acidified with 1 N HCl until the resulting solution was acidified to pH 2-3. The reaction mixture was diluted with water and extracted with ethyl acetate (3 X 15 mL). The combined organic extracts were dried over Na_2SO_4, concentrated to dryness under reduced pressure to yield the crude acid which was used for next step without further purification.

MNBA (386 mg, 1.12 mmol) and DMAP (274 mg, 2.12 mmol) were loaded into a round bottom flask equipped with a side arm, dissolved in anhydrous CH_2Cl_2 (130 mL), and Hünig's base

(0.195 mL, 1.12 mmol) and $Dy(OTf)_3$ (227 mg, 0.37 mmol) were successively added. The flask was fitted with a water cooled condenser and heated to reflux under argon atmosphere. To the refluxing reaction mixture was slowly added (through the side arm) the *seco* acid (200 mg, 0.374 mmol) in CH_2Cl_2 (50 mL) and THF (10 mL) *via* syringe pump (2.0 mL/h) over ~30 h. After the addition was complete the reaction was continued for another 6 h under reflux. The reaction mixture was cooled to room temperature and concentrated, giving a crude residue. The crude residue was then purified by column chromatography (gradient elution, 99.5:0.5 to 95:5, CH_2Cl_2: MeOH) to yield the corresponding macrocycle **13** as white solid.

Yield: 55%

Specific rotation: $[\alpha]_D^{26}$ = −24.7 (*c* 0.85, $CHCl_3$)

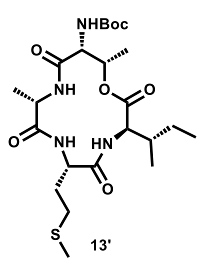
IR ν_{max} (film): 3684, 3411, 3022, 2403, 1781, 1674, 1522 cm^{-1}

^{1}H NMR (400 MHz, MeOH-d_4): δ 5.44 (dd, *J* = 2.7, 6.4 Hz, 1H), 4.64 - 4.55 (m, 1H), 4.52 (d, *J* = 5.6 Hz, 1H), 4.45 (d, *J* = 2.4 Hz, 1H), 4.26 (q, *J* = 7.5 Hz, 1H), 2.50 (dt, *J* = 3.9, 7.1 Hz, 2H), 2.32–2.17 (m, 1H), 2.11 (s, 3H), 2.00 (td, *J* = 6.6, 13.2 Hz, 1H), 1.83 (td, *J* = 7.1, 14.2 Hz, 1H), 1.53 (s, 9H), 1.44 (d, *J* = 7.1 Hz, 3H), 1.43 - 1.36 (m, 1H), 1.27 (d, *J* = 6.6 Hz, 3H), 1.23 - 1.16 (m, 1H), 0.96 (t, *J* = 7.5 Hz, 3H), 0.91 (d, *J* = 6.8 Hz, 3H)

^{13}C NMR (100 MHz, MeOH-d_4): δ 176.0, 172.7, 172.5, 171.6, 158.3, 81.5, 74.1, 58.9, 56.7, 53.3, 52.9, 37.3, 31.4, 31.2, 28.8, 27.8, 16.8, 16.4, 15.4, 15.1, 12.2

HRMS (ESI): calculated for $C_{23}H_{40}O_7N_4NaS$ $[M + Na]^+$: 539.2510, found: 539.2510.

***tert*-Butyl ((3*R*,6*S*,9*S*,12*R*,13*S*)-3-((*S*)-*sec*-butyl)-9,13-dimethyl-6-(2-(methylthio)ethyl)-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-12-yl)carbamate (13')**



13'

Compound **13'** was prepared using the similar experimental procedure as described above for preparation of compound **13**. The ^{1}H NMR spectral data shows *dr* ~1:4 for **13’** and **13** respectively.

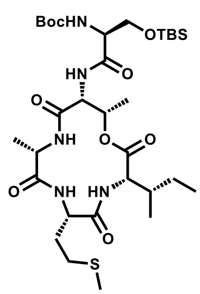
Specific rotation: $[\alpha]_D^{26}$ = −7.8 (*c* 0.24, $CHCl_3$)

IR ν_{max} (film): 3684, 3411, 3022, 2403, 1781, 1674 cm^{-1}

^{1}H NMR (400 MHz, MeOH-d_4): δ 6.04 - 6.02 (m, 1H), 4.59 - 4.54 (m, 1H), 4.32 - 4.26 (m, 1H), 4.14 - 4.12 (m, 1H), 3.94 - 3.92 (m, 1H), 2.83 - 2.78 (m, 1H), 2.66–2.62 (m, 1H), 2.52 - 2.47 (m, 1H), 2.09 (s, 3H), 1.85 - 1.76 (m, 1H), 1.58 - 1.53 (m, 1H), 1.46 (s, 9H), 1.31 (d, *J* = 7.3 Hz, 3H), 1.30 - 1.26 (m, 1H), 1.23 (d, *J* = 6.8 Hz, 3H), 1.20 - 1.18 (m, 1H), 0.99 - 0.88 (m, 6H)

HRMS (ESI): calculated for $C_{18}H_{33}O_5N_4S$ $[M - Boc + H]^+$: 417.2166, found: 417.2155.

***tert*-Butyl ((*S*)-1-(((3*S*,6*S*,9*S*,12*R*,13*S*)-3-((*S*)-*sec*-butyl)-9,13-dimethyl-6-(2-(methylthio)ethyl)-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-12-yl)amino)-3-((*tert*-butyldimethylsilyl)oxy)-1-oxopropan-2-yl)carbamate (23):**



23

Compound **23** was synthesized from compound **13** according to the protocol described above for the synthesis of **15**. The crude product was purified by silica gel column chromatography using MeOH and CH_2Cl_2 (1:49) as mobile phase to afford compound **23** as white foam.

Yield: 72%

Specific rotation: $[\alpha]_D^{26}$ = −18.1 (*c* 0.50, $CHCl_3$)

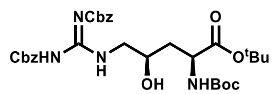
IR ν_{max} (film): 3416, 3333, 3022, 2969, 2401, 1672 cm^{-1}

^{1}H NMR (400 MHz, MeOH-d_4): δ 5.47 (qd, *J* = 3.3, 6.4 Hz, 1H), 4.59 (t, *J* = 7.2 Hz, 1H), 4.51 (d, *J* = 5.6 Hz, 1H), 4.30 - 4.04 (m, 3H), 3.92 - 3.84 (m, 2H), 2.49 (qd, *J* = 6.7, 13.7 Hz, 2H), 2.23 (dd, *J* = 7.1, 14.2 Hz, 1H), 2.09 (s, 3H), 2.01 - 1.95 (m, 1H), 1.81 (dd, *J* = 6.5, 14.1 Hz, 1H), 1.50 (d, *J* = 7.6 Hz, 3H), 1.47 - 1.42 (m, 9H), 1.42 - 1.36 (m, 1H), 1.27 (d, *J* = 7.6 Hz, 3H), 1.21 - 1.14 (m, 1H), 0.98 - 0.87 (m, 6H), 0.92 (s, 9H), 0.13 - 0.07 (m, 6H)

^{13}C NMR (100 MHz, MeOH-d_4): δ 175.9, 174.7, 172.6, 172.2, 171.6, 158.2, 81.2, 73.4, 64.1, 59.2, 56.9, 56.8, 53.7, 53.1, 37.2, 31.5, 31.3, 28.8, 27.8, 26.6, 19.5, 17.4, 17.0, 15.4, 15.2, 12.1, -5.1

HRMS (ESI): calculated for $C_{32}H_{60}O_9N_5SSi$ $[M + H]^+$: 718.3876, found: 718.3863.

***tert*-Butyl(2*S*,4*R*)-5-((*Z*)-2,3-*bis*((benzyloxy)carbonyl)guanidino)-2-((*tert*-butoxycarbonyl)amino)-4-hydroxypentanoate (27)**



27

The amino alcohol **26** was dissolved in 50 mL CH_3CN and added benzyl (*E*)-((((benzyloxy)carbonyl)amino)(1H-pyrazol-1-yl)methylene)carbamate (8.1 g, 21.5 mmol) in 50 mL CH_3CN at room temperature. After stirring for 12 h, the solvent was removed *in vacuo* and crude residue was purified by column chromatography (3:7, ethyl acetate: petroleum ether) afforded **27** as white foam.

Yield: 78% (8.6 g)

Specific rotation: $[\alpha]_D^{28}$ = +13.6 (*c* 0.20, $CHCl_3$)

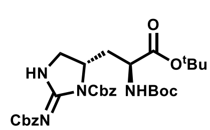
IR ν_{max} (film): 3022, 1634, 1216, 757 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 11.71 (s, 1H), 8.74 (s, 1H), 7.37 - 7.27 (m, 10H), 5.43 (d, *J* = 7.5 Hz, 1H), 5.19 (s, 2H), 5.12 (s, 2H), 4.77 - 4.76 (m, 1H), 4.43 - 4.33 (m, 1H), 3.83 - 3.70 (m, 2H), 3.34 - 3.21 (m, 1H), 1.96 - 1.82 (m, 1H), 1.59 - 1.52 (m, 1H), 1.46 (s, 9H), 1.44 (s, 9H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7, 163.7, 157.0, 156.4, 153.7, 136.8, 134.8, 128.9, 128.8, 128.7, 128.5, 128.2, 128.0, 82.7, 80.7, 68.3, 67.2, 66.3, 51.1, 46.4. 39.4, 28.4, 28.1

HRMS (ESI): calculated for $C_{31}H_{43}O_9N_4$ $[M + H]^+$: 615.3025, found: 615.3023.

Benzyl (*S*,Z)-2-(((benzyloxy)carbonyl)imino)-5-((*S*)-3-(*tert*-butoxy)-2-((*tert*-butoxycarbonyl)amino)-3-oxopropyl)imidazolidine-1-carboxylate (8)



28

To a solution of **27** (8 g, 13.0 mmol) in anhydrous CH_2Cl_2 (400 mL) was added DIPEA (10.9 mL, 62.5 mmol) followed by Tf_2O (2.40 mL, 14.3 mmol) at –78 °C dropwise manner. After stirring at the same temperature for 4 h, the reaction was quenched with saturated aqueous $NaHCO_3$ (100 mL), and extracted with CH_2Cl_2 (2 X 200 mL). The combined organic layer was washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (1: 1, ethyl acetate: petroleum ether as eluent) to afford **28** as light yellow solid.

Yield: 74% (5.75 g)

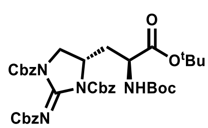
Specific rotation: $[\alpha]_D^{28}$ = +7.6 (*c* 0.20, $CHCl_3$)

IR ν_{max} (film): 3022, 1772, 1217, 765 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.65 (s, 1H), 7.45 - 7.29 (m, 10H), 5.27 (s, 2H), 5.16 - 5.07 (m, 3H), 4.37 (m, 1H), 4.17 - 4.11 (m, 1H), 3.79 (m, 1H), 3.55 - 3.54 (m, 1H), 1.98 (m, 2H), 1.44 (s, 9H), 1.42 (s, 9H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.6, 164.5, 159.6, 155.7, 150.4, 136.7, 135.1, 128.6, 128.3, 128.0, 127.8, 82.7, 80.0, 68.3, 67.3, 60.3, 53.4, 50.6, 44.9, 36.6, 28.2, 27.9, 21.0, 14.1

HRMS (ESI) calculated for $C_{31}H_{41}O_8N_4$ $[M + H]^+$: 597.2919, found: 597.2911.

Dibenzyl (*S*,*Z*)-2-(((benzyloxy)carbonyl)imino)-4-((*S*)-3-(*tert*-butoxy)-2-((*tert*-butoxycarbonyl)amino)-3-oxopropyl)imidazolidine-1,3-dicarboxylate (3)



29

To a stirred solution of **28** (5.7 g, 9.56 mmol) in anhydrous CH_2Cl_2 (200 mL) was added DIPEA (16.6 mL, 95.6 mmol) followed by Cbz-Cl (6.8 mL, 47.8 mmol) at room temperature. The resulting mixture was refluxed for 12 h. After completion, reaction mixture was concentrated *in vacuo*. Purification by column chromatography (4:6; ethyl acetate: petroleum ether as eluent) afforded **29** as white solid. All the spectral data was matching with then reported data for compound **29**.

Yield: 86% (6.0 g)

Specific rotation: $[\alpha]_D^{28}$ = –7.4 (*c* 0.20, $CHCl_3$)

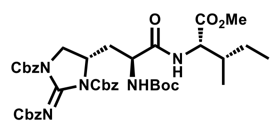
IR ν_{max} (film): 3022, 1772, 1216 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37 - 7.28 (m, 15H), 5.28 - 5.16 (m, 4H), 5.13 - 5.07 (m, 3H), 4.30 (m, 1H), 4.12 - 4.08 (m, 1H), 3.95 - 3.87 (m, 2H), 2.05 - 1.90 (m, 2H), 1.43 (s, 9H), 1.40 (s, 9H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 158.4, 155.9, 151.1, 150.7, 142.0, 136.4, 134.9, 134.8, 128.7, 128.7, 128.7, 128.4, 128.4, 128.2, 127.9, 83.1, 80.3, 68.8, 67.9, 52.1, 50.9, 47.5, 37.1, 28.3, 28.0

HRMS (ESI): calculated for $C_{39}H_{47}O_{10}N_4$ $[M + H]^+$: 731.3287, found: 731.3282.

Dibenzyl (*S*,*Z*)-2-(((benzyloxy)carbonyl)imino)-4-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(((2*S*,3*S*)-1-methoxy-3-methyl-1-oxopentan-2-yl)amino)-3-oxopropyl)imidazolidine-1,3-dicarboxylate (31)



31

Compound **31** was prepared using the similar experimental procedure as described above for preparation of **17**.

Yield: 81%

Specific rotation: $[\alpha]_D^{28}$ = +13.5 (*c* 0.22, $CHCl_3$)

IR ν_{max} (film): 3023, 1793, 1716, 1514, 1218 cm^{-1}

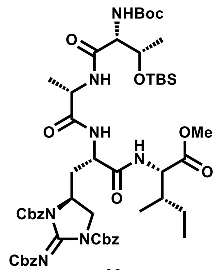
^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers): δ 11.2 -11.1 (m, 1H), 7.37 - 7.29 (m, 15H), 5.71 - 5.66 (m, 1H), 5.33- 5.03 (m, 6H), 4.52 - 4.49 (m, 1H), 4.34 - 4.09 (m, 2H), 4.05 - 3.98 (m, 1H), 3.78 - 3.74 (m, 1H), 3.68 - 3.62 (m, 3H), 1.92 - 1.83 (m, 3H), 1.44 - 1.41 (m, 10H), 1.28 - 1.22 (m, 1H), 0.92 - 0.90 (m, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) (mixture of rotamers): δ 172.4, 172.0, 171.3, 157.6, 155.5, 155.0, 150.5, 150.4, 136.4, 135.1, 134.5, 128.9, 128.8, 128.6, 128.4, 128.0, 127.7, 79.8, 69.6, 67.8, 67.0, 57.1, 53.5, 51.9, 51.1, 48.3, 46.8, 37.6, 37.1, 28.4, 25.1, 15.7, 15.5, 11.7

HRMS (ESI): calculated for $C_{42}H_{51}O_{11}N_5$ $[M + Na]^+$: 824.3474, found: 824.3477.

Dibenzyl (*S*,*E*)-2-(((benzyloxy) carbonyl)imino)-4-((5*S*,6*R*,9*S*,12*S*)-6-((*tert*-butoxy carbonyl)amino)-12-(((2*S*,3*S*)-1-methoxy-3-methyl-1-oxopentan-2-yl)carbamoyl)-

2,2,3,3,5,9-hexamethyl-7,10-dioxo-4-oxa-8,11-diaza-3-silatridecan-13-yl)imidazolidine 1,3-dicarboxylate (32)



32

Compound **32** was prepared using the similar experimental procedure as described above for preparation of **18**.

Yield: 83% (4.2 g)

Specific rotation: $[\alpha]_D^{28}$ = –7.4 (*c* 0.39, $CHCl_3$)

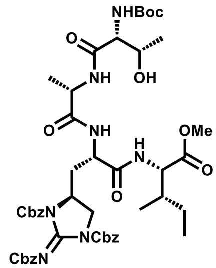
IR ν_{max} (film): 3022, 1792, 1713, 1515 cm^{-1}

^{1}H NMR (400 MHz, MeOH-d_4) (mixture of rotamers): δ 7.45 - 7.22 (m, 15H), 5.29 - 5.18 (m, 4H), 5.07 - 4.93 (m, 2H), 4.61 - 4.48 (m, 1H), 4.44 - 4.39 (m, 1H), 4.36 - 4.23 (m, 3H), 4.10 - 3.92 (m, 2H), 3.86 - 3.73 (m, 1H), 3.68 - 3.62 (m, 3H), 2.21 - 2.03 (m, 1H), 1.91 - 1.87 (m, 1H), 1.86 - 1.71 (m, 1H), 1.43 (s, 9H), 1.35 - 1.32 (m, 3H), 1.31 - 1.29 (m, 2H), 1.15 - 1.14 (m, 3H), 0.91 - 0.88 (m, 15H), 0.09 - 0.06 (m, 6H)

^{13}C NMR (100 MHz, MeOH-d_4) (mixture of rotamers): δ 173.4, 173.2, 173.0, 172.2, 158.9, 158.7, 157.6, 157.0, 152.2, 151.9, 138.2, 136.8, 136.2, 129.7, 129.5, 128.9, 80.9, 70.7, 70.3, 68.7, 67.7, 67.5, 61.4, 58.6, 58.4, 52.5, 38.9, 38.2, 35.9, 30.7, 28.7, 26.3, 20.5, 18.8, 18.7, 18.4, 16.1, 11.9, 11.8, –4.40, –4.73

HRMS (ESI): calculated for $C_{55}H_{78}O_{14}N_7Si$ $[M + H]^+$: 1088.5378, found: 1088.5371.

((*S*)-3-((*S*,*E*)-1,3-bis((benzyloxy)carbonyl)-2-(((benzyloxy)carbonyl)imino)imidazolidin-4-yl)-2-((*S*)-2-((2*R*,3*S*)-2-((*tert*-butoxycarbonyl)amino)-3-hydroxybutanamido)propanamido)propanoyl)-L-isoleucine (33)



33

Compound **33** as white foam was prepared using the similar experimental procedure as described above for preparation of **19**.

Yield: 80% (2.5 g).

Specific rotation: $[\alpha]_D^{28}$ = –10.3 (*c* 0.55, $CHCl_3$)

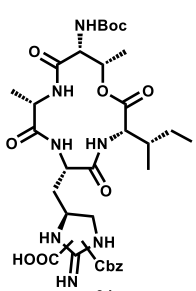
IR ν_{max} (film): 3022, 1791, 1714, 1216 cm^{-1}

^{1}H NMR (400 MHz, MeOH-d_4) (mixture of rotamers): δ 7.40 - 7.23 (m, 15H), 5.23 - 5.18 (m, 4H), 5.08 - 4.92 (m, 2H), 4.50 - 4.47 (m, 1H), 4.42 - 4.31 (m, 2H), 4.16 - 4.05 (m, 2H), 4.02 - 3.95 (m, 1H), 3.93 - 3.82 (m, 1H), 3.80 - 3.73 (m, 1H), 3.69 - 3.62 (m, 3H), 2.06 - 2.01 (m, 1H), 1.89 - 1.71 (m, 2H), 1.45 - 1.43 (m, 9H), 1.38 (d, *J* = 7.8 Hz, 3H), 1.35 - 1.24 (m, 2H), 1.18 (d, *J* = 6.1 Hz, 3H), 0.92 - 0.86 (m, 6H)

^{13}C NMR (100 MHz, MeOH-d_4) (mixture of rotamers): δ 174.6, 173.8, 173.5, 173.2, 158.0, 157.0, 152.2, 151.9, 138.2, 136.8, 136.2, 129.7, 129.6, 129.5, 129.0, 128.9, 80.9, 70.7, 68.7, 67.7, 62.0, 58.5, 52.5, 51.7, 50.5, 38.2, 35.6, 28.7, 26.3, 20.0, 17.5, 16.0, 11.9

HRMS (ESI) calculated for $C_{49}H_{65}O_{15}N_7$ $[M + H_2O]^+$: 991.4539, found: 991.4575.

Benzyl (*S*)-5-(((3*S*,6*S*,9*S*,12*R*,13*S*)-12-((*tert*-butoxycarbonyl)amino)-3-((*S*)-*sec*-butyl)-9,13-dimethyl-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-6-yl)methyl)-2-iminoimidazolidine-1-carboxylate (34)



34

Compound **34** as white foam was prepared using the similar experimental procedure as described above for preparation of **13**. The crude residue was then purified by column chromatography (gradient elution, 99.5:0.5 to 95:5, CH_2Cl_2: MeOH) to yield the corresponding macrocycle **34** as yellow solid.

Yield: 35% (41 mg)

Specific rotation: $[\alpha]_D^{28}$ = –9.7 (*c* 0.55, $CHCl_3$)

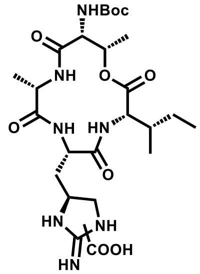
IR ν_{max} (film): 3022, 1728, 1668, 1216 cm^{-1}

^{1}H NMR (400 MHz, MeOH-d_4) (mixture of rotamers) : δ 7.40 - 7.32 (m, 5H), 5.43 - 5.31 (m, 1H), 5.24 - 5.16 (m, 2H), 4.60 - 4.46 (m, 3H), 4.30 - 4.24 (m, 1H), 4.05 - 3.98 (m, 1H), 3.87 - 3.67 (m, 2H), 2.17 - 2.10 (m, 1H), 1.99 - 1.94 (m, 1H), 1.86 - 1.79 (m, 1H), 1.47 (s, 9H), 1.42 (d, *J* = 7.3 Hz, 3H), 1.32 - 1.30 (m, 1H), 1.24 (d, *J* = 6.4 Hz, 3H), 1.20 - 1.12 (m, 1H), 0.94 - 0.87 (m, 6H)

^{13}C NMR (100 MHz, MeOH-d_4): δ 175.7, 172.5, 171.8, 171.4, 158.4, 158.0, 152.0, 150.7, 136.9, 129.6, 129.5, 129.4, 81.3, 74.0, 68.6, 58.7, 56.7, 52.9, 50.2, 47.2, 37.5, 37.2, 28.6, 27.6, 16.7, 16.2, 15.0, 12.1

HRMS (ESI): calculated for $C_{33}H_{47}O_{11}N_7Na$ $[M + Na]^+$: 740.3231, found: 740.3234.

***tert*-Butyl((3*S*,6*S*,9*S*,12*R*,13*S*)-3-((*S*)-*sec*-butyl)-6-(((*S*)-2-iminoimidazolidin-4-yl)methyl)-9,13-dimethyl-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-12-yl) carbamateacetate (35)**



B (35)

To a solution of **34** (15 mg, 0.021 mmol) in 3 mL of MeOH was added Pd/C (10% on carbon, 5 mg) at room temperature. After being stirred at the same temperature for 2 h under hydrogen atmosphere, the reaction mixture was filtered through pad of celite and concentrated *in vacuo*.

Purification by column chromatography (1:9, MeOH: CH_2Cl_2 as eluent) afforded **35** as white solid.

Yield: 85% (10 mg)

Specific rotation: $[\alpha]_D^{26}$ = –7.2 (*c* 0.10, $CHCl_3$)

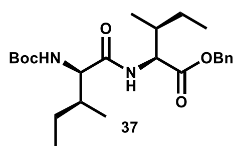
IR ν_{max} (film): 3022, 1730, 1666, 1212, 1110 cm^{-1}

^{1}H NMR (500 MHz, MeOH-d_4) (mixture of rotamers): δ 5.48 - 5.43 (m, 1H), 4.56 - 4.51 (m, 2H), 4.47 - 4.46 (m, 1H), 4.29 - 4.24 (m, 1H), 4.06 - 4.02 (m, 1H), 3.76 - 3.73 (m, 1H), 3.63 - 3.60 (m, 1H), 2.17 - 2.12 (m, 1H), 2.01 - 1.96 (m, 1H), 1.88 - 1.82 (m, 1H), 1.53 (s, 9H), 1.45 (d, *J* = 7.3 Hz, 3H), 1.38 - 1.36 (m, 1H), 1.26 (d, *J* = 6.4 Hz, 3H), 1.23 - 1.17 (m, 1H), 0.97 - 0.91 (m, 6H)

^{13}C NMR (125 MHz, MeOH-d_4): δ 175.8, 172.6, 172.0, 171.4, 159.3, 158.2, 156.6, 81.3, 73.9, 68.6, 58.7, 56.6, 53.0, 50.4, 47.2, 37.8, 37.1, 28.7 (3C), 27.6, 16.7, 16.2, 14.9, 12.1

HRMS (ESI): calculated for $C_{25}H_{41}O_9N_7$ $[M + Na]^+$: 606.2858, found: 606.2845.

Benzyl (*tert*-butoxycarbonyl)-D-alloisoleucyl-L-isoleucinate (37)



37

Dipeptide **37** was synthesized from Boc-D-*allo*-Ile and L-Ile-OBn.HCl by following similar procedure as used for synthesis of compound **15**. The residue was purified by silica gel column chromatography using ethyl acetate and hexane (1:9) as mobile phase to afford dipeptide **37** as a colourless solid.

Yield: 87% (3.65 g)

Specific rotation: $[\alpha]_D^{30}$ = +15.8 (*c* 1.0, $CHCl_3$)

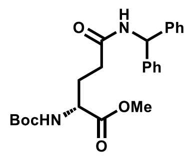
IR ν_{max} (film): 3321, 2964, 1735, 1687, 1650 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers): δ 7.35 (s, 5H), 6.57 - 6.58 (m, 1H), 5.21 (d, *J*=12.2 Hz, 1H), 5.12 (d, *J*=12.2 Hz, 1H), 4.94 (br. s., 1H), 4.65 (dd, *J*=8.6, 4.6 Hz, 1H), 4.15 (br. s., 1H), 2.02 (br. s., 1H), 1.92 - 1.93 (m, 1H), 1.44 (s, 9H), 1.31 - 1.41 (m, 2H), 1.25 (br. s., 1H), 1.08 - 1.15 (m, 1H), 0.83 - 0.95 (m, 12H)

^{13}C NMR (100 MHz, $CDCl_3$) (mixture of rotamers): δ 171.7, 171.6, 155.7, 135.3, 128.5, 128.4, 128.3, 80.0, 67.0, 58.1, 56.3, 37.8, 36.8, 28.2, 26.4, 24.9, 15.5, 14.1, 11.7, 11.4

HRMS (ESI): calculated for $C_{24}H_{36}N_2O_6Na$ $[M + Na]^+$: 471.2460; found 471.2466.

Methyl *N*^{5}-benzhydryl-*N*^{2}-(*tert*-butoxycarbonyl)-D-glutaminate (38)



38

Dipeptide **37** was synthesized from (*R*)-4-((*tert*-butoxycarbonyl)amino)-5-methoxy-5-oxopentanoic acid and benzhydrylamine by following similar procedure as used for synthesis of compound **15**. The residue was purified with column chromatography (eluted in 35% EtOAc in Pet ether) to afford corresponding amide **6** as a white solid.

Yield: 87%

Specific rotation: $[\alpha]_D^{30}$ = -9.9 (*c* 0.835, $CHCl_3$)

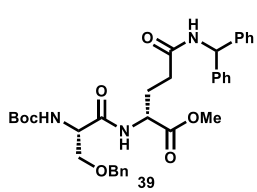
IR ν_{max} (film): 3306, 2978, 1709, 1649, 1495 cm^{-1}

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33 - 7.28 (m, 5 H), 7.26 (br. s., 5 H), 7.09 – 7.06 (m, 1 H), 6.24 (d, *J* = 7.9 Hz, 1 H), 5.41 (br. s., 1 H), 4.29 (br. s., 1 H), 3.70 (br. s., 3 H), 2.35 (br. s., 2 H), 2.19 - 2.18 (m, 1 H), 1.92 (dd, *J* = 6.7, 13.1 Hz, 1 H), 1.46 - 1.42 (m, 9 H)

^{13}C NMR (125 MHz, $CDCl_3$): δ 172.7, 171.0, 155.8, 141.6, 141.5, 128.5, 128.5, 127.3, 127.3, 127.2, 80.1, 57.0, 52.9, 52.4, 32.5, 28.9, 28.2

HRMS (ESI): calculated for $C_{24}H_{30}N_2O_5Na$ $[M + Na]^+$: 449.2047; found 449.2042.

Methyl *N*^{5}-benzhydryl-*N*^{2}-(*O*-benzyl-*N*-(*tert*-butoxycarbonyl)-L-seryl)-D-glutaminate (39)



39

To the compound **38** (1.6 g, 3.89 mmol) was added 4 N HCl in dioxane at 0 °C and reaction mixture was stirred at room temperature for 1 h. After completion of reaction, reaction mixture was concentrated, dried under high vacuum and forwarded for next step without purification.

The coupling reaction was performed from above prepared acid and Boc-Ser(Bn)-OH by following similar procedure to that of compound **15**. Crude compound was purified by column chromatography to afford the dipeptide **39** as white solid.

Yield: 68%

Specific rotation: $[\alpha]_D^{30}$ = +5.7 (*c* 1.65, $CHCl_3$)

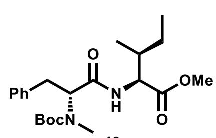
IR ν_{max} (film): 3313, 2927, 1711, 1655, 1526, 1167 cm^{-1}

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.34 - 7.25 (m, 15H), 7.13 (d, *J*=6.7 Hz, 1H), 6.93 (br. s., 1H), 6.25 (d, *J*=8.2 Hz, 1H), 5.44 (br. s., 1H), 4.61 (td, *J*=8.3, 3.8 Hz, 1H), 4.56 (d, *J*=11.6 Hz, 1H), 4.49 (d, *J*=11.6 Hz, 1H), 4.26 - 4.25 (m, 1H), 3.87 - 3.86 (m, 1H), 3.71 (s, 3H), 3.63 (dd, *J*=9.5, 5.5 Hz, 1H), 2.32 - 2.25 (m, 3H), 1.97 - 1.92 (m, 1H), 1.43 (s, 9H)

^{13}C NMR (125 MHz, $CDCl_3$): δ 172.0, 170.8, 170.3, 155.6, 141.5, 137.3, 128.5, 128.4, 128.3, 127.9, 127.7, 127.6, 127.5, 127.3, 127.3, 127.2, 80.5, 73.3, 73.2, 69.4, 57.0, 54.6, 52.5, 51.5, 31.9, 28.4, 28.3, 28.2

HRMS (ESI): calculated for $C_{34}H_{41}N_3O_7Na$ $[M + Na]^+$: 626.2837; found 626.2830.

Methyl *N*-(*tert*-butoxycarbonyl)-*N*-methyl-D-phenylalanyl-L-isoleucinate (40)



40

Dipeptide **40** was synthesized from coupling reaction of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-D-phenylalanine and NH_2-Ile-OH by following similar procedure to that used in synthesis of compound **15**. Crude compound was purified by column chromatography to afford the dipeptide **40** as white solid.

Yield: 82%

Specific rotation: $[\alpha]_D^{25}$ = +62.8 (*c* 1.29, $CHCl_3$)

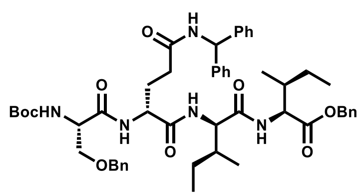
IR ν_{max}(film): 3015, 2917, 1736, 1647, 1526 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers): δ 7.12 - 7.35 (m, 5H), 6.77 (d, *J* = 8.07 Hz, 1H), 4.97 (t, *J* = 7.82 Hz, 1H), 4.49 (dd, *J* = 4.77, 8.19 Hz, 1H), 3.62 - 3.80 (m, 3H), 3.34 (dd, *J* = 6.36, 14.67 Hz, 1H), 2.85 - 3.02 (m, 1H), 2.83 (s, 1H), 2.77 (s, 2H), 1.87 (br. s., 1H), 1.35 - 1.44 (m, 7H), 1.32 (br. s., 4H), 1.04 - 1.20 (m, 1H), 0.80 - 0.96 (m, 7H)

^{13}C NMR (100 MHz, $CDCl_3$) (mixture of rotamers): δ 172.0, 172.0, 170.7, 170.2, 156.7, 155.1, 137.8, 137.5, 129.0, 128.8, 128.5, 128.3, 126.5, 126.4, 80.7, 80.5, 77.3, 76.7, 61.4, 59.3, 56.4, 52.1, 51.9, 37.6, 37.3, 33.8, 33.7, 30.7, 30.5, 30.3, 29.6, 29.6, 28.2, 28.0, 25.0, 24.9, 15.5, 11.5

HRMS (ESI): calculated for $C_{22}H_{34}O_5N_2Na$ $[M + Na]^+$: 429.2360, found: 429.2357.

Benzyl *N*^{5}-benzhydryl-*N*^{2}-(*O*-benzyl-*N*-(*tert*-butoxycarbonyl)-L-seryl)-D-glutaminyl-D-*allo*isoleucyl-L-isoleucinate (41)



41

Compound **41** was synthesized from compound **38** and **39** according to the protocol described above for the synthesis of **15**. After purification by column chromatography, **41** was obtained as a colourless sticky liquid.

Yield: 78 %

Specific rotation: $[\alpha]_D^{25}$ = +2.6 (*c* 1.05, $CHCl_3$: MeOH, 1:1, v/v)

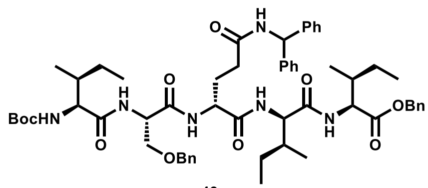
IR ν_{max} (film): 3282, 2964, 1638, 1430, 1529 cm^{-1}

^{1}H NMR (500 MHz, $CDCl_3$) (mixture of rotamers): δ 7.24 - 7.36 (m, 20H), 7.16 - 7.17 (m, 1H), 6.97 – 6.98 (m, 1H), 6.90 (d, *J*=6.9 Hz, 1H), 6.27 (d, *J*=7.6 Hz, 1H), 5.02 - 5.14 (m, 2H), 4.55 - 4.58 (m, 1H), 4.51 - 4.54 (m, 1H), 4.44 - 4.49 (m, 2H), 4.35 - 4.38 (m, 1H), 4.27 - 4.30 (m, 1H), 3.81 - 3.88 (m, 1H), 3.61 (br. s., 1H), 2.35 - 2.45 (m, 3H), 2.12 - 2.14 (m, 1H), 1.87 - 1.98 (m, 2H), 1.38 - 1.43 (m, 9H), 1.24 - 1.34 (m, 2H), 1.10 - 1.20 (m, 2H), 0.83 - 0.91 (m, 12H)

^{13}C NMR (125 MHz, $CDCl_3$) (mixture of rotamers): δ 171.8, 171.7, 171.4, 171.3, 171.2, 171.2, 170.8, 155.6, 155.6, 141.5, 141.4, 141.3, 141.2, 137.4, 137.3, 135.3, 135.2, 128.6, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 127.8, 127.7, 127.5, 127.4, 80.3, 77.3, 76.7, 73.2, 69.4, 67.1, 67.1, 66.8, 57.2, 57.0, 57.0, 56.9, 56.8, 56.7, 56.7, 56.6, 56.5, 53.2, 37.8, 37.6, 37.4, 37.2, 36.2, 32.3, 28.2, 28.0, 26.3, 26.1, 25.1, 25.0, 23.2, 15.5, 14.5, 14.1, 11.6, 11.6, 11.5, 11.4

HRMS (ESI): calculated for $C_{52}H_{67}N_5O_9Na$ $[M + Na]^+$: 928.4831; found 928.4824.

Benzyl *N*^{5}-benzhydryl-*N*^{2}-(O-benzyl-*N*-((*tert*-butoxycarbonyl)-L-isoleucyl)-L-seryl)-D-glutaminyl-D-*allo*isoleucyl-L-isoleucinate (43)



43

Compound **43** was synthesized from compound **41** and Boc-Ile-OH according to the protocol described above for the synthesis of **15**. After purification by column chromatography, afforded pentapeptide **43** as white solid.

Yield: 64%

Specific rotation: $[\alpha]_D^{25}$ = +0.8 (*c* 0.67, $CHCl_3$: MeOH, 1:1, v/v)

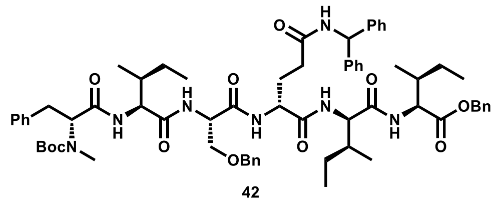
IR ν_{max} (film): 3282, 2964, 1740, 1634, 1531, 1176 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) (mixture of rotamers): δ 8.71 (d, *J*=8.3 Hz, 1H), 8.16 - 8.40 (m, 2H), 7.91 - 7.95 (m, 1H), 7.69 - 7.81 (m, 1H), 7.22 - 7.34 (m, 20H), 6.87 – 6.97 (m, 1H), 6.12 (d, *J*=8.8 Hz, 1H), 5.06 - 5.14 (m, 2H), 4.59 - 4.66 (m, 1H), 4.40 - 4.46 (m, 4H), 4.26 (t, *J*=7.3 Hz, 1H), 3.87 - 4.05 (m, 1H), 3.57 (br. s., 2H), 2.23 - 2.25 (m, 2H), 1.91 (br. s., 1H), 1.77 - 1.85 (m, 4H), 1.29 - 1.36 (m, 9H), 1.02 - 1.27 (m, 6H), 0.77 (dd, *J*=13.7, 6.8 Hz, 18H)

^{13}C NMR (100 MHz, DMSO-d_6) (mixture of rotamers): δ 171.4, 171.2, 170.9, 169.3, 155.4, 142.6, 142.6, 138.0, 135.8, 128.4, 128.3, 128.1, 128.1, 127.5, 127.3, 127.3, 127.2, 126.9, 126.8, 78.1, 72.0, 70.1, 65.9, 58.9, 56.3, 55.8, 55.5, 52.3, 37.3, 36.6, 36.2, 31.9, 28.2, 25.7, 24.6, 24.2, 15.5, 15.4, 14.2, 11.5, 11.1, 10.8

HRMS (ESI): calculated for $C_{58}H_{78}N_6O_{10}Na$ $[M + Na]^+$: 1041.5672; found 1041.5670.

Benzyl *N*^{5}-benzhydryl-*N*^{2}-(*O*-benzyl-*N*-*N*-(*tert*-butoxycarbonyl)-*N*-methyl-D-phenylalanyl-L-isoleucyl-L-seryl)-D-glutaminyl-D-*allo*isoleucyl-L-isoleucinate (42)



42

Compound **42** was synthesized from compound **43** and Boc-*N*-Me-Phe-OH according to the protocol described above for the synthesis of **15**. After purification by column chromatography, afforded hexapeptide **42** as white solid.

Yield: 54%

Specific rotation: $[\alpha]_D^{30}$ = +31.8 (*c* 1.08, $CHCl_3$: MeOH, 1:1, v/v)

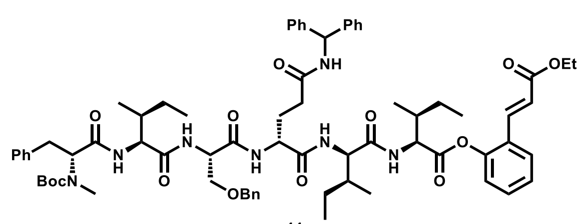
IR ν_{max} (film): 3282, 2964, 2929, 1738, 1631, 1530 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) (mixture of rotamers): δ 8.71 (d, *J*=8.3 Hz, 1H), 8.09 - 8.25 (m, 3H), 7.81 - 7.91 (m, 2H), 7.25 - 7.34 (m, 25H), 6.12 (d, *J*=8.3 Hz, 1H), 5.05 - 5.13 (m, 2H), 4.63 (br. s., 1H), 4.41 - 4.47 (m, 5H), 4.25 - 4.28 (m, 2H), 3.58 (br. s., 2H), 3.16 (d, *J*=13.2 Hz, 1H), 2.84 (br. s., 1H), 2.66 (br. s., 3H), 2.24 (br. s., 2H), 1.91 (br. s., 1H), 1.78 - 1.79 (m, 4H), 1.30 - 1.33 (m, 2H), 1.21 (s, 5H), 1.25 (s, 4H), 0.96 - 1.15 (m, 4H), 0.74 - 0.82 (m, 18H)

^{13}C NMR (100 MHz, DMSO-d_6) (mixture of rotamers): δ 171.7, 171.4, 170.9, 170.9, 170.3, 169.9, 169.2, 155.4, 154.7, 142.6, 142.6, 138.2, 138.0, 135.8, 128.9, 128.4, 128.3, 128.2, 128.1, 127.9, 127.5, 127.4, 127.3, 127.2, 126.9, 126.9, 126.2, 78.9, 72.0, 69.9, 65.9, 56.7, 56.3, 55.8, 55.5, 52.6, 52.2, 37.3, 36.6, 36.2, 34.8, 31.9, 30.4, 29.1, 27.9, 27.8, 25.8, 25.4, 24.6, 24.3, 22.1, 18.5, 15.5, 15.3, 14.9, 14.2, 14.0, 13.7, 11.6, 11.5, 11.4, 10.8

HRMS (ESI): calculated for $C_{68}H_{89}N_7O_{11}Na$ $[M + Na]^+$: 1202.6512; found 1202.6508.

2-((*E*)-3-ethoxy-3-oxoprop-1-en-1-yl)phenyl *N*^{5}-benzhydryl-*N*^{2}-(*O*-benzyl-*N*-*N*-(*tert*-butoxycarbonyl)-*N*-methyl-D-phenylalanyl-L-isoleucyl-L-seryl)-D-glutaminyl-D-*allo*isoleucyl-L-isoleucinate (44)



44

To the solution of compound **42** in THF and MeOH, 2 N aqueous NaOH was added dropwise at 0 °C and reaction mixture was stirred at same temperature for 30 min. After completion of reaction, organic solvent was evaporated under *vacuo* and reaction mixture was diluted with ethyl acetate (10 mL), acidified with 1 N HCl and extracted with ethyl acetate. Organic layer was dried over Na_2SO_4, evaporated under *vacuo* to get the corresponding acid which was utilized for coupling without purification.

To the solution of acid (300 mg, 0.25 mmol) in dry DMF (1.5 mL) was added DIC (0.2 mL, 1.28 mmol) and DIPEA (0.24 mL, 1.28 mmol) at 0 °C and stirred for 15 min. After that K-oxyma (231 mg, 1.28 mmol) and ethyl (*E*)-3-(2-hydroxyphenyl) acrylate (54.4 mg, 0.28 mmol) was added dropwise and reaction mixture was stirred at room temperature for 12 h. After completion of reaction (TLC checked), diluted with ethyl acetate 10 mL and washed with aqueous $NaHCO_3$ and 1 N HCl. Ethyl acetate layer was dried over Na_2SO_4, evaporated under *vacuo* and crude obtained was purified by column chromatography to get compound **11** as white solid.

Yield: 62% (201 mg)

Specific rotation: $[\alpha]_D^{30}$ = +18.4 (*c* 0.74, $CHCl_3$: MeOH, 1:1, v/v)

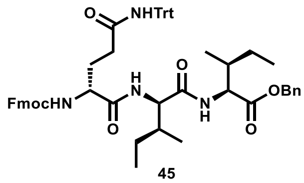
IR ν_{max} (film): 3337, 2967, 1632, 1573, 1169 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) (mixture of rotamers): δ 8.76 (br. s., 1H), 8.24 - 8.28 (m, 1H), 7.89 - 7.93 (m, 1H), 7.78 - 7.84 (m, 1H), 7.64 (d, *J* = 15.87 Hz, 1H), 7.40 - 7.48 (m, 1H), 7.27 (br. s., 23H), 7.04 - 7.09 (m, 1H), 6.66 (d, *J* = 16.48 Hz, 1H), 6.12 (d, *J* = 7.93 Hz, 1H), 5.49 (d, *J* = 7.32 Hz, 1H), 5.03 - 5.10 (m, 1H), 4.79 - 4.80 (m, 1H), 4.68 (br. s., 1H), 4.59 - 4.62 (m, 1H), 4.46 (br. s., 4H), 4.36 - 4.40 (m, 1H), 4.29 (br. s., 1H), 4.15 - 4.20 (m, 2H), 3.58 - 3.59 (m, 2H), 3.14 - 3.16 (m, 1H), 2.81 - 2.83 (m, 1H), 2.65 - 2.67 (m, 4H), 2.26 (br. s., 2H), 1.92 - 1.95 (m, 1H), 1.80 (br. s., 3H), 1.24 (s, 9H), 1.20 (s, 6H), 1.00 (d, *J* = 6.71 Hz, 9H), 0.87 - 0.89 (m, 3H), 0.76 - 0.78 (m, 9H)

^{13}C NMR (100 MHz, DMSO-d_6) (mixture of rotamers): δ 171.9, 171.0, 170.9, 170.9, 170.4, 170.3, 165.7, 156.8, 148.9, 142.6, 138.1, 136.8, 128.9, 128.3, 128.1, 127.5, 127.4, 127.2, 126.9, 126.6, 126.1, 123.0, 120.5, 120.4, 115.6, 78.8, 72.1, 72.1, 72.0, 60.2, 55.8, 52.4, 52.4, 40.7, 40.1, 39.9, 36.6, 34.9, 34.8, 30.7, 27.9, 27.8, 25.6, 24.9, 24.4, 23.3, 15.5, 15.2, 14.9, 14.1, 11.6, 11.5, 11.5, 11.4, 10.8

HRMS (ESI): calculated for $C_{72}H_{93}N_7O_{13}Na$ $[M + Na]^+$: 1286.6724; found 1286.6282.

Benzyl *N*²-(((9H-fluoren-9-yl)methoxy)carbonyl)-*N*⁵-trityl-D-glutaminyl-D-*allo*isoleucyl-L-isoleucinate (45)



45

Compound **45** was synthesized from compound Fomc-D-Gln(Trt)-OH and **37'** according to the protocol described above for the synthesis of **15**. After purification by column chromatography, afforded tripeptide **45** as white solid.

Yield: 93%

Specific rotation: $[\alpha]_D^{30}$ = +5.0 (*c* 0.41, $CHCl_3$: MeOH, 1:1, v/v)

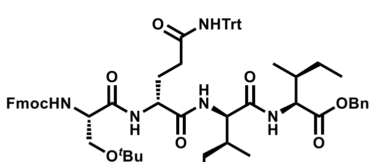
IR ν_{max} (film): 3322, 3061, 2927, 1725, 1662 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers): δ 7.79 (d, *J*=7.3 Hz, 2H), 7.60 (d, *J*=7.2 Hz, 2H), 7.20 - 7.46 (m, 26H), 6.85 (d, *J*=6.3 Hz, 1H), 6.22 (d, *J*=5.3 Hz, 1H), 4.96 - 5.10 (m, 2H), 4.54 - 4.60 (m, 1H), 4.34 - 4.48 (m, 3H), 4.08 - 4.27 (m, 2H), 2.51 - 2.55 (m, 2H), 1.88 - 2.09 (m, 4H), 1.29 (br. s., 2H), 1.10 - 1.18 (m, 2H), 0.76 - 0.93 (m, 12H)

^{13}C NMR (100 MHz, $CDCl_3$) (mixture of rotamers): δ 172.7, 172.1, 171.9, 171.9, 156.7, 144.2, 143.5, 141.1, 135.0, 128.5, 128.4, 128.2, 128.1, 127.7, 127.6, 127.0, 126.8, 124.9, 119.8, 70.4, 66.9, 56.8, 56.7, 54.4, 46.9, 38.4, 36.8, 36.3, 33.1, 32.6, 29.5, 27.8, 26.0, 24.9, 15.3, 14.0, 11.2, 11.1

HRMS (ESI): calculated for $C_{56}H_{62}N_4O_7$ $[M + Na]^+$: 949.4451; found 949.4498.

Benzyl *N*²-(*N*-(((9H-fluoren-9-yl)methoxy)carbonyl)-*O*-(*tert*-butyl)-L-seryl)-*N*⁵-trityl-D-glutaminyl-D-*allo*isoleucyl-L-isoleucinate (46)



46

Compound **45** (1.5 g, 1.62 mmol, 1 equiv.) was dissolved in dichloromethane (20 mL) and diethyl amine (10 mL) was added. The reaction was stirred at room temperature for 2 h. After concentration, the crude amine was dried under high vacuum and used in the next step without further purification. The crude amine and Fmoc-Ser(^{t}Bu)-OH (0.620 g, 1.62 mmol, 1.0 equiv.) were dissolved in dichloromethane (20 mL). DIPEA (0.8 mL, 4.85 mmol, 3.0 equiv.) and HATU (1.231 g, 3.24 mmol, 2.0 equiv.) were added at 0 °C. The reaction was stirred at room temperature for 12 h and quenched with saturated aqueous $NaHCO_3$ solution (40 mL). Organic layer was washed with 1 N HCl solution. The combined organic phase was washed with saturated aqueous NaCl solution (20 mL) and dried with anhydrous Na_2SO_4. After concentration under low pressure, the crude product was purified using flash chromatography afforded tetrapeptide **46** as white solid.

Yield: 67%

Specific rotation: $[\alpha]_D^{30}$ = +20.6 (*c* 0.29, $CHCl_3$)

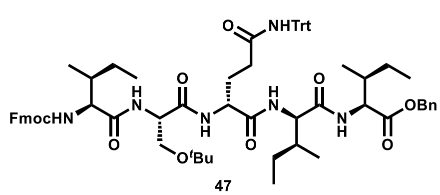
IR ν_{max} (film): 3305, 2966, 2875, 1721, 1659, 1512 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers): δ 7.80 (d, *J*=7.3 Hz, 3H), 7.62 - 7.65 (m, 2H), 7.41 - 7.45 (m, 3H), 7.30 (d, *J*=6.4 Hz, 9H), 7.34 (d, *J*=6.0 Hz, 6H), 7.26 (d, *J*=3.7 Hz, 6H), 7.14 (s, 1H), 7.02 (d, *J*=8.2 Hz, 1H), 6.72 (d, *J*=7.8 Hz, 1H), 5.77 (d, *J*=6.0 Hz, 1H), 5.04 - 5.14 (m, 2H), 4.61 (dd, *J*=8.0, 4.8 Hz, 1H), 4.39 - 4.46 (m, 4H), 4.23 - 4.26 (m, 2H), 3.81 (dd, *J*=8.5, 3.9 Hz, 1H), 3.42 (t, *J*=7.8 Hz, 1H), 2.56 (dd, *J*=14.4, 6.6 Hz, 1H), 2.40 - 2.47 (m, 1H), 1.88 - 2.07 (m, 4H), 1.33 - 1.51 (m, 4H), 1.21 (s, 9H), 0.83 - 0.93 (m, 12H)

^{13}C NMR (100 MHz, $CDCl_3$) (mixture of rotamers): δ 171.6, 171.6, 171.5, 170.9, 170.6, 165.7, 144.4, 143.9, 143.7, 141.2, 135.3, 128.6, 128.5, 128.3, 128.2, 127.9, 127.6, 127.0, 125.1, 119.9, 74.4, 70.6, 67.1, 66.8, 61.5, 56.9, 56.5, 54.9, 53.3, 47.1, 38.5, 37.5, 36.4, 36.1, 33.3, 31.4, 28.4, 27.3, 26.4, 25.0, 15.5, 14.3, 11.6, 11.4

HRMS (ESI): calculated for $C_{65}H_{75}N_5O_9Na$ $[M + Na]^+$: 1092.5457; found 1092.5454.

Benzyl *N*^{2}-(*N*-((((9H-fluoren-9-yl)methoxy)carbonyl)-L-isoleucyl)-*O*-(*tert*-butyl)-L-seryl)-*N*^{5}-trityl-D-glutaminyl-D-*allo*isoleucyl-L-isoleucinate (47):



47

Compound **47** was synthesized from compound Boc-Ile-OH and **46** according to the protocol described above for the synthesis of **46**. After purification by column chromatography, afforded pentapeptide **47** as white solid.

Yield: 81%

Specific rotation: $[\alpha]_D^{30}$ = +5.5 (*c* 0.51, $CHCl_3$: MeOH, 1:1, v/v)

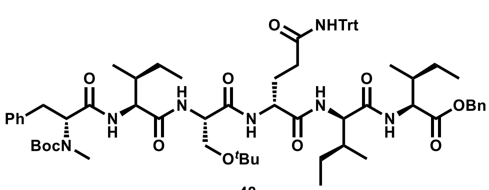
IR ν_{max} (film): 3297, 2964, 1656, 1513 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) (mixture of rotamers): δ 8.53 (s, 1H), 8.29 (d, *J*=8.0 Hz, 1H), 7.93 (d, *J*=7.6 Hz, 1H), 7.88 (d, *J*=7.2 Hz, 2H), 7.78 (d, *J*=9.5 Hz, 1H), 7.72 (t, *J*=8.6 Hz, 2H), 7.49 (d, *J*=8.8 Hz, 1H), 7.41 (td, *J*=7.4, 3.4 Hz, 2H), 7.30 - 7.34 (m, 7H), 7.23 - 7.26 (m, 6H), 7.14 - 7.19 (m, 10H), 5.07 - 5.14 (m, 2H), 4.53 (dd, *J*=9.0, 4.8 Hz, 1H), 4.40 - 4.44 (m, 2H), 4.27 - 4.33 (m, 2H), 4.21 - 4.25 (m, 2H), 3.94 (t, *J*=8.2 Hz, 1H), 3.46 (d, *J*=5.3 Hz, 2H), 2.30 (t, *J*=8.0 Hz, 2H), 1.85 (br. s., 1H), 1.80 (dd, *J*=17.2, 7.6 Hz, 2H), 1.66 - 1.74 (m, 2H), 1.41 - 1.46 (m, 1H), 1.22 - 1.36 (m, 3H), 1.12 - 1.20 (m, 2H), 1.067 - 1.08 (m, 9H), 0.84 (d, *J*=8.0 Hz, 6H), 0.78 - 0.81 (m, 6H), 0.76 (dd, *J*=7.1, 3.2 Hz, 6H)

^{13}C NMR (100 MHz, DMSO-d_6) (mixture of rotamers): δ 171.4, 171.4, 171.2, 171.1, 171.0, 169.4, 156.1, 144.9, 143.9, 143.7, 140.7, 140.7, 139.4, 137.4, 135.8, 128.9, 128.5, 128.4, 128.1, 128.0, 127.6, 127.4, 127.3, 127.1, 126.3, 125.3, 121.4, 120.1, 120.1, 120.0, 72.8, 69.1, 65.9, 65.7, 61.8, 59.3, 56.2, 54.9, 53.2, 52.4, 46.7, 40.1, 39.9, 39.8, 39.6, 39.4, 38.2, 37.5, 36.3, 36.2, 32.6, 27.1, 25.9, 24.6, 24.3, 15.4, 15.4, 14.2, 11.6, 10.9, 10.8

HRMS (ESI): calculated for $C_{71}H_{86}N_6O_{10}Na$ $[M + Na]^+$: 1205.6298; found 1205.6309.

Benzyl *N*^{2}-(*N*-*N*-(*tert*-butoxycarbonyl)-*N*-methyl-D-phenylalanyl-L-isoleucyl-*O*-(*tert*-butyl)-L-seryl)-*N*^{5}-trityl-D-glutaminyl-D-*allo*isoleucyl-L-isoleucinate (48)



48

Compound **48** was synthesized from compound Boc-*N*-Me-D-Phe-OH and **47** according to the protocol described above for the synthesis of **46**. After purification by column chromatography, afforded hexapeptide **48** as white solid.

Yield: 79%

Specific rotation: $[\alpha]_D^{30}$ = +106.2 (*c* 0.45, $CHCl_3$)

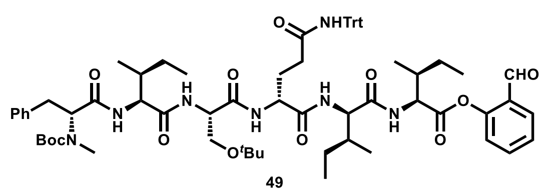
IR ν_{max} (film) 3291, 2966, 2875, 1693, 1628, 1528 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) (mixture of rotamers): δ (ppm) 8.55 (br. s., 1H), 8.29 (d, *J*=6.7 Hz, 1H), 8.10 - 8.11 (m, 1H), 7.98 (dd, *J*=15.6, 7.6 Hz, 1H), 7.88 (br. s., 1H), 7.79 (d, *J*=9.2 Hz, 1H), 7.31 - 7.35 (m, 6H), 7.25 (t, *J*=7.3 Hz, 11H), 7.14 - 7.20 (m, 11H), 5.07 - 5.15 (m, 2H), 4.82 - 5.01 (m, 1H), 4.53 (dd, *J*=8.9, 4.6 Hz, 1H), 4.3540 - 4.41 (m, 2H), 4.28 (t, *J*=7.3 Hz, 2H), 3.46 (d, *J*=4.9 Hz, 2H), 3.15 (br. s., 1H), 2.82 - 2.88 (m, 1H), 2.69 (br. s., 3H), 2.30 (t, *J*=7.9 Hz, 2H), 1.80 (dd, *J*=13.1, 6.4 Hz, 3H), 1.66 - 1.72 (m, 2H), 1.15 - 1.38 (m, 15H), 1.09 (s, 9H), 0.75 - 0.85 (m, 18H)

^{13}C NMR (100 MHz, DMSO-d_6) (mixture of rotamers): δ 171.5, 171.4, 171.3, 171.0, 170.9, 169.3, 155.3, 144.9, 135.8, 128.9, 128.5, 128.4, 128.1, 128.1, 127.4, 126.3, 126.2, 78.8, 72.9, 69.2, 65.9, 61.7, 56.7, 56.2, 55.0, 53.4, 52.4, 40.1, 39.9, 39.7, 38.2, 37.5, 36.2, 34.9, 32.6, 29.0, 27.9, 27.8, 27.1, 25.9, 24.6, 24.3, 15.4, 15.4, 14.2, 11.6, 10.8

HRMS (ESI): calculated for $C_{71}H_{95}N_7O_{11}Na$ $[M + Na]^+$: 1244.6982; found 1244.6971.

2-Formylphenyl *N*^{2}-(*N*-*N*-(*tert*-butoxycarbonyl)-*N*-methyl-D-phenylalanyl-L-isoleucyl-*O*-(*tert*-butyl)-L-seryl)-*N*^{5}-trityl-D-glutaminyl-D-*allo*isoleucyl-L-isoleucinate (49)



49

Compound **48** (60 mg, 0.05 mmol, 1.0 equiv.) was dissolved in MeOH (2 mL) and Pd/C (10%) in catalytic amount was added. The reaction was stirred with a H_2 balloon at room temperature for 12 h. After filtration and concentration, the crude residue of acid (100 mg, 0.08 mmol) was dissolved in dichloromethane (10 mL) and EDC (50 mg, 0.26 mmol) and DMAP (32 mg, 0.26 mmol) followed by salicylaldehyde (11 mg, 5.0 equi) was added at 0 °C. The reaction was stirred at room temperature for 12 h and quenched with saturated aqueous $NaHCO_3$ solution (50 mL). The aqueous phase was extracted using dichloromethane (3 X 10 mL). The combined organic phase was washed with saturated aqueous NaCl solution (10 mL) and dried with anhydrous

Na_2SO_4. After concentration under low pressure, the crude product was purified using flash chromatography to afford compound **49** as white solid.

Yield: 82% (90 mg)

Specific rotation: $[\alpha]_D^{30}$ = +29.6 (*c* 0.35, $CHCl_3$)

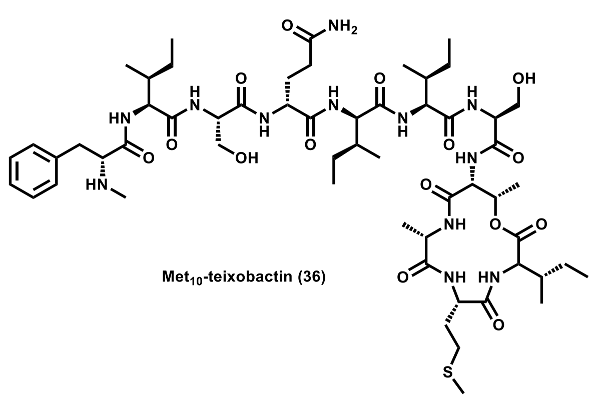
IR ν_{max} (film): 3298, 2968, 1767, 1661, 1518, 1155 cm^{-1}

^{1}H NMR (500 MHz, DMSO-d_6): δ 10.12 (d, *J*=9.5 Hz, 1H), 8.49 - 8.54 (m, 1H), 7.95 (br. s., 1H), 7.86 - 7.89 (m, 2H), 7.75 (dt, *J*=14.5, 7.2 Hz, 1H), 7.45 - 7.50 (m, 1H), 7.15 - 7.25 (m, 24H), 4.712 – 5.01 (m, 1H), 4.27 - 4.60 (m, 5H), 3.44 - 3.45 (m, 2H), 3.11 (dd, *J*=12.2, 6.9 Hz, 1H), 2.83 - 2.88 (m, 1H), 2.69 (s, 3H), 2.29 (br. s., 2H), 2.09 (br. s., 1H), 1.83 (br. s., 2H), 1.73 (br. s., 2H), 1.36 (br. s., 3H), 1.25 - 1.27 (m, 10H), 1.16 - 1.19 (m, 2H), 1.06 - 1.09 (m, 9H), 0.96 - 1.02 (m, 5H), 0. 90 - 0.94 (m, 2H), 0.77 - 0.84 (m, 11H)

^{13}C NMR (125 MHz, DMSO-d_6): δ 189.1, 171.4, 171.4, 170.8, 170.8, 170.3, 169.3, 164.6, 151.6, 149.4, 144.9, 135.8, 128.9, 128.4, 128.0, 127.4, 126.3, 126.1, 123.3, 86.9, 78.8, 72.9, 69.1, 61.7, 59.8, 56.7, 55.1, 53.3, 45.8, 40.1, 40.0, 39.9, 39.8, 39.8, 39.7, 39.6, 39.3, 39.2, 39.0, 38.2, 37.2, 36.4, 35.6, 34.8, 32.6, 30.4, 29.1, 27.8, 27.1, 25.8, 25.6, 24.8, 24.2, 21.2, 15.5, 15.4, 15.0, 14.3, 14.1, 11.5, 11.4, 10.9, 8.6

HRMS (ESI): calculated for $C_{71}H_{93}N_7O_{12}Na$ $[M + Na]^+$ 1258.6770; found 1258.6774.

(*R*)-*N*^{1}-((2*R*,3*S*)-1-(((2*S*,3*S*)-1-(((*S*)-1-(((3*S*,6*S*,9*S*,12*R*,13*S*)-3-((*S*)-*sec*-butyl)-9,13-dimethyl-6-(2-(methylthio)ethyl)-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-12-yl)amino)-3-hydroxy-1-oxopropan-2-yl)amino)-3-methyl-1-oxopentan-2-yl)amino)-3-methyl-1-oxopentan-2-yl)-2-((*S*)-3-hydroxy-2-((2*S*,3*S*)-3-methyl-2-((*R*)-2-(methylamino)-3 phenylpropanamido)pentanamido)propanamido)pentanediamide (36)



To the compound **23** was added 4 N HCl in dioxane and reaction mixture was stirred at room temperature for 1 h. After completion of reaction, solvent was evaporated under vacuo to get the free hydroxyl amine **24** which was forwarded for the next reaction without purification.

Compound **48** (50 mg, 0.04) and above prepared hydroxyl amine **24** were dissolved in pyridine acetate buffer (1:1 mole ratio, 0.8 mL) and stirred for 10 h at room temperature. After that reaction mixture was concentrated in vacuo and forwarded for acidolytic cleavage by using TFA: H_2O: TIPS (94:4:2) and stirred for 1 h at 0 °C. Reaction mixture was then concentrated in *vacuo* and purified by preparative HPLC using an Agilent 1200 system, with a reverse phase column (Column details: Ascentis C_{18}, column size 250 cm X 10 mm, 10 µm). Mobile phase: MeCN (with 0.1% TFA)/ H_2O (with 0.1% TFA) using linear gradients: 15% to 70% MeCN/ H_2O. Flow rate: 4 mL min^{-1}. Retention time: 10.2 min, to get target compound **36**.

Yield: 41% (20 mg)

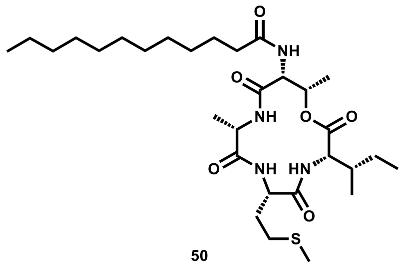
IR ν_{max} (film): 3297, 2963, 2925, 1636, 1456, 1200 cm^{-1}

^{1}H NMR (700 MHz, DMSO-d_6): δ 9.05 (br. s., 1H), 8.90 (br. s., 1H), 8.48 (d, *J*=7.9 Hz, 2H), 8.06 (d, *J*=6.7 Hz, 2H), 8.01 (d, *J*=6.7 Hz, 2H), 7.91 (d, *J*=7.9 Hz, 1H), 7.81 (d, *J*=8.5 Hz, 1H), 7.33 (d, *J*=6.7 Hz, 2H), 7.25 (d, *J*=6.7 Hz, 1H), 7.31 (d, *J*=6.7 Hz, 3H), 6.76 (br. s., 1H), 5.21 - 5.32 (m, 1H), 4.96 (br. s., 1H), 4.42 - 4.52 (m, 3H), 4.30 - 4.36 (m, 5H), 4.10 - 4.19 (m, 5H), 3.94 - 4.03 (m, 1H), 3.61 -3.65 (m, 1H), 3.51 - 3.61 (m, 5H), 3.10 (dd, J = 13.3, 5.3 Hz, 2H), 2.96 (dd, J = 13.0, 9.9 Hz, 2H), 2.46 (s, 3H), 2.07 (d, *J*=7.9 Hz, 3H), 2.04 (br. s., 3H), 1.87 (br. s., 1H), 1.79 - 1.80 (m, 2H), 1.68 (br. s., 1H), 1.53 (br. s., 2H), 1.47 (br. s., 1H), 1.35 - 1.37 (m, 2H), 1.33 (br. s., 1H), 1.27 - 1.30 (m, 3H), 1.15 (d, *J*=4.3 Hz, 2H), 1.08 (br. s., 1H), 1.06 (br. s., 1H), 1.05 (br. s., 1H), 1.03 (br. s., 1H), 0.82 - 0.87 (m, 12H), 0.76 (d, *J*=6.7 Hz, 5H), 0.64 (d, *J*=6.7 Hz, 3H), 0.59 (d, *J*=6.1 Hz, 3H)

^{13}C NMR (175 MHz, DMSO-d_6): δ 174.0, 173.2, 173.1, 171.4, 171.2, 171.2, 171.1, 170.5, 170.5, 169.9, 169.9, 166.6, 134.5, 129.4, 128.6, 127.3, 74.4, 61.7, 61.4, 57.4, 57.3, 56.2, 55.3, 55.1, 55.0, 55.0, 54.9, 54.9, 52.5, 52.4, 39.8, 39.7, 39.6, 39.4, 39.3, 36.3, 36.3, 36.1, 31.5, 31.5, 26.0, 25.8, 25.7, 24.6, 23.9, 15.7, 15.1, 15.1, 15.0, 14.3, 14.2, 11.6, 11.6, 11.1, 10.9

HRMS (ESI): calculated for $C_{57}H_{95}N_{15}O_{12}$ $[M + H]^+$: 1219.6755; found 1219.6747.

***N*-((3*S*,6*S*,9*S*,12*R*,13*S*)-3-((*S*)-*sec*-butyl)-9,13-dimethyl-6-(2-(methylthio)ethyl)-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-12-yl)dodecanamide (50)**



50

To compound of methionine macrocycle **13** was added 4 M HCl in dioxane at 0 °C and stirred at room temperature. After completion of reaction, solvent was evaporated under *vacuo* and forwarded for coupling without purification.

To the solution of dodecanoic acid (24 mg, 0.12 mmol) in dry DMF (1 mL) was added HATU (91 mg, 0.24 mmol) and DIPEA (62 µL, 0.36 mmol) at 0 °C and stirred for 10 min. After that amine salt (50 mg, 0.12 mmol) in DMF (0.2 mL) was added slowly and reaction mixture was stirred at room temperature for 12 h. After completion of reaction, mixture was diluted with ethyl acetate (10 mL) and washed with ice-cold water (3 mL), aqueous $NaHCO_3$ solution (5 mL) and 1 N HCl (5 mL). Organic layer was concentrated under *vacuo* and purified by column chromatography to afford compound **50** as white solid.

Yield: 54% (34 mg)

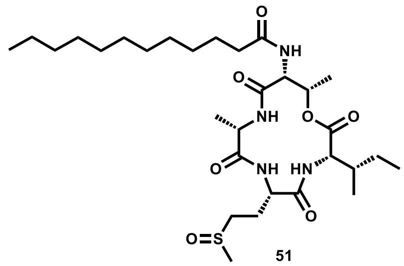
IR ν_{max} (film): 3322, 3061, 2927, 2606, 1662 cm^{-1}

^{1}H NMR (400 MHz, DMF-d_7): δ 8.38 - 8.53 (m, 1H), 8.22 (d, *J*=8.5 Hz, 1H), 7.37 - 7.57 (m, 2H), 5.37 - 5.53 (m, 1H), 4.83 (d, *J*=7.9 Hz, 1H), 4.46 - 4.64 (m, 2H), 4.13 - 4.27 (m, 1H), 2.41 - 2.50 (m, 2H), 2.25 - 2.36 (m, 2H), 2.14 - 2.22 (m, 1H), 2.08 (br. s., 3H), 1.90 - 2.04 (m, 2H), 1.51 - 1.75 (m, 4H), 1.39 (d, *J*=6.7 Hz, 3H), 1.28 (br. s., 16H), 1.19 - 1.23 (m, 3H), 0.84 - 0.94 (m, 9H)

^{13}C NMR (100 MHz, DMF-d_7): δ 173.8, 173.4, 171.1, 170.9, 170.7, 72.9, 55.8, 55.3, 52.2, 51.2, 36.5, 36.0, 32.0, 30.7, 30.5, 29.6, 29.5, 29.4, 29.1, 26.7, 25.7, 22.8, 16.4, 16.0, 14.8, 14.6, 13.9, 11.7

HRMS (ESI): calculated for $C_{26}H_{54}N_4O_6SNa$ $[M + Na]^+$: 621.3656; found 621.3647.

***N*-((3*S*,6*S*,9*S*,12*R*,13*S*)-3-((*S*)-*sec*-butyl)-9,13-dimethyl-6-(2-(methylsulfinyl)ethyl)-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-12-yl)dodecanamide (51)**



51

Compound was prepared by following above procedure. During the synthesis of compound **50**, we observed the oxidation of sulfur group of methionine and from same reaction mixture we isolated both the compounds *i.e.* non-oxidized compound **50** and oxidized compound **51** as white solid.

Yield: 42%

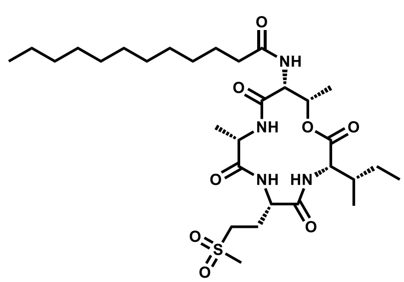
IR ν_{max} (film): 3337, 2967, 1632, 1573 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 5.28 (d, 1H), 4.66 (s, 1H), 4.43 (dd, *J*=7.6, 4.6 Hz, 1H), 4.36 (d, *J*=4.9 Hz, 1H), 4.06 - 4.11 (m, 1H), 3.15 (br. s., 3H), 2.71 - 2.77 (m, 1H), 2.57 (dt, *J*=9.3, 4.8 Hz, 1H), 2.25 - 2.27 (m, 1H), 2.15 (dd, *J*=12.8, 6.1 Hz, 2H), 1.93 - 2.01 (m, 1H), 1.85 (t, *J*=5.2 Hz, 1H), 1.46 - 1.54 (m, 2H), 1.06 - 1.28 (m, 26H), 0.72 - 0.80 (m, 6H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 167.5, 166.4, 163.0, 162.1, 162.1, 64.4, 47.3, 43.7, 42.7, 42.0, 41.8, 28.8, 27.9, 27.8, 23.6, 21.2, 21.2, 21.1, 21.0, 21.0, 18.1, 17.4, 15.9, 14.2, 7.1, 6.9, 5.5, 5.0, 2.6

HRMS (ESI): calculated for $C_{30}H_{54}N_4O_7SNa$ [M + Na]$^+$: 637.3505; found 637.3696.

***N*-((3*S*,6*S*,9*S*,12*R*,13*S*)-3-((*S*)-*sec*-butyl)-9,13-dimethyl-6-(2-(methylsulfonyl)ethyl)-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-12-yl)dodecanamide (52)**



52

To the solution of compound **51** (50 mg, 0.083 mmol) in dichloromethane (5 mL) was added *m*CPBA (43 mg, 0.250 mmol) at 0 °C and stirred at room temperature for 2 h. After completion of reaction, mixture was quenched with saturated solution of $NaHCO_3$ and extracted with dichloromethane, dried over Na_2SO_4, evaporated under *vacuo* and purified by column chromatography to afford compound **52** as a colourless sticky liquid.

Yield: 93%

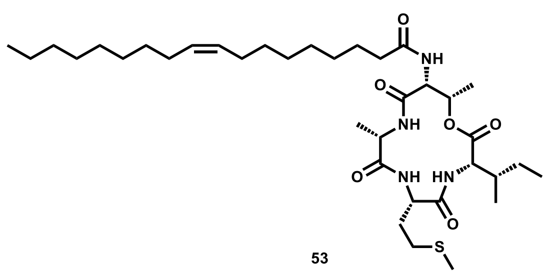
IR ν_{max} (film): 3321, 2964, 1687, 1335, 1169 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.15 (d, *J*=4.9 Hz, 1H), 8.01 (d, *J*=9.8 Hz, 1H), 7.88 - 7.90 (m, 1H), 7.68 (d, *J*=8.5 Hz, 1H), 7.29 (d, *J*=9.2 Hz, 1H), 5.28 (dd, *J*=6.1, 2.4 Hz, 1H), 4.70 - 4.73 (m, 1H), 4.38 - 4.48 (m, 2H), 4.02 - 4.09 (m, 1H), 3.13 (td, *J*=12.5, 4.3 Hz, 1H), 2.99 (s, 3H), 2.85 - 2.93 (m, 1H), 2.34 (t, *J*=6.4 Hz, 2H), 2.15 - 2.29 (m, 2H), 1.88 - 1.98 (m, 3H), 1.57 (br. s., 2H), 1.44 - 1.47 (m, 1H), 1.23 - 1.33 (m, 18H), 1.10 (d, *J*=6.1 Hz, 3H), 0.83 - 0.88 (m, 6H), 0.77 (d, *J*=6.7 Hz, 3H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 173.1, 170.4, 170.0, 169.3, 130.5, 128.8, 127.9, 72.1, 54.8, 54.7, 51.5, 50.8, 50.1, 35.6, 31.3, 29.0, 28.9, 28.8, 28.7, 26.0, 25.0, 23.6, 22.1, 16.5, 15.9, 14.5, 14.0, 11.7

HRMS (ESI): calculated for $C_{30}H_{56}N_4O_8S$ $[M + H]^+$: 631.3735; found 631.3733.

***N*-((3*S*,6*S*,9*S*,12*R*,13*S*)-3-((*S*)-*sec*-butyl)-9,13-dimethyl-6-(2-(methylthio)ethyl)-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-12-yl)oleamide (53)**



53

Compound **53** was synthesized from compound **13** and oleic acid according to the protocol described above for the synthesis of **50**. After purification by column chromatography, afforded compound **53** as colourless sticky liquid.

Yield: 68%

IR ν_{max} (film): 3305, 3085, 2634, 1966, 1659, 1640 cm^{-1}

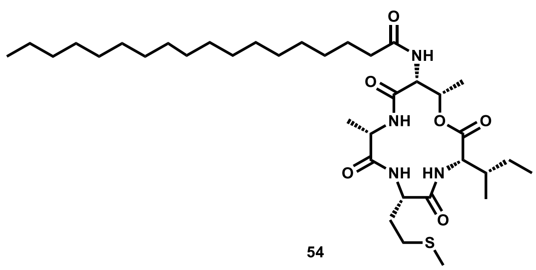
^{1}H NMR (400 MHz, DMF-d_7): δ 8.42 (d, *J*=6.1 Hz, 1H), 8.32 (d, *J*=9.2 Hz, 1H), 7.47 - 7.65 (m, 2H), 5.35 - 5.40 (m, 1H), 5.36 (t, *J*=4.6 Hz, 2H), 4.81 - 4.84 (m, 1H), 4.50 - 4.58 (m, 2H), 4.22

(t, *J*=7.0 Hz, 1H), 2.43 - 2.51 (m, 2H), 2.27 - 2.37 (m, 2H), 2.13 - 2.20 (m, 1H), 2.08 (s, 3H), 1.94 - 2.04 (m, 6H), 1.68 - 1.75 (m, 1H), 1.58 - 1.60 (m, 2H), 1.39 (d, *J*=7.9 Hz, 3H), 1.27 (s, 11H), 1.30 (s, 9H), 1.21 (d, *J*=6.7 Hz, 3H), 1.14 (dd, *J*=14.0, 6.7 Hz, 1H), 0.86 - 0.92 (m, 9H)

^{13}C NMR (100 MHz, DMF-d_7): δ 174.0, 173.5, 170.9, 170.7, 130.0, 129.9, 72.7, 55.7, 55.2, 52.1, 51.3, 36.2, 35.9, 31.9, 30.6, 30.4, 29.5, 29.5, 29.4, 29.3, 29.2, 29.1, 27.1, 27.1, 26.6, 25.6, 22.6, 16.3, 15.8, 14.6, 14.4, 13.8, 11.5

HRMS (ESI): calculated for $C_{36}H_{65}N_4O_6S$ $[M + H]^+$: 681.4619; found 681.4604.

***N*-((3*S*,6*S*,9*S*,12*R*,13*S*)-3-((*S*)-*sec*-butyl)-9,13-dimethyl-6-(2-(methylthio)ethyl)-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-12-yl)stearamide (54):**



54

To a solution of compound **53** (50 mg, 0.0735 mmol) in methanol (10 mL), 10% Pd/C (~ 3 mg) was added and stirred under H_2 atmosphere for 5 h. The reaction mixture was then filtered through pad of celite, concentrated and purified by column chromatography to afford compound **54** as sticky liquid.

Yield: 38%

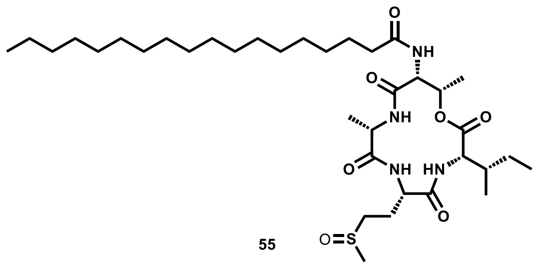
IR ν_{max} (film): 3291, 2964, 2658, 1656, 1513 cm^{-1}

^{1}H NMR (400 MHz, DMF-d_7): δ 8.46 (d, *J*=6.1 Hz, 1H), 8.27 (d, *J*=9.2 Hz, 1H), 7.62 (d, *J*=9.2 Hz, 1H), 7.41 (d, *J*=8.5 Hz, 1H), 5.43 (dd, *J*=6.1, 2.4 Hz, 1H), 4.83 - 4.89 (m, 1H), 4.49 - 4.62 (m, 2H), 4.18 - 4.23 (m, 1H), 2.58 - 2.62 (m, 1H), 2.46 (t, *J*=7.6 Hz, 1H), 2.26 - 2.36 (m, 2H), 2.13 - 2.20 (m, 1H), 2.09 (s, 3H), 1.99 - 2.02 (m, 2H), 1.71 (dt, *J*=14.6, 7.3 Hz, 1H), 1.58 - 1.60 (m, 3H), 1.39 (d, *J*=7.3 Hz, 3H), 1.28 (s, 31H), 1.21 (d, *J*=6.7 Hz, 3H), 0.88 - 0.93 (m, 9H)

^{13}C NMR (125 MHz, DMF-d_7): δ 174.1, 173.5, 170.9, 170.8, 170.7, 72.7, 55.7, 55.3, 55.2, 52.1, 51.3, 36.2, 35.9, 35.5, 35.3, 35.1, 35.0, 31.9, 30.5, 30.3, 29.6, 29.3, 26.6, 26.5, 25.6, 22.6, 16.3, 16.2, 15.8, 14.6, 14.4, 13.8, 11.6, 11.5

HRMS (ESI): calculated for $C_{36}H_{67}N_4O_6S$ $[M + H]^+$: 683.4776; found 683.4764.

***N*-((3*S*,6*S*,9*S*,12*R*,13*S*)-3-((*S*)-*sec*-butyl)-9,13-dimethyl-6-(2-(methylsulfinyl)ethyl)-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-12-yl)stearamide (55)**



55

Compound **55** was prepared by following above procedure for the synthesis of **54**. In the same reaction mixture, we observed oxidation of sulfur group of methionine to corresponding sulfoxide **55** as colorless sticky liquid.

Yield: 43%

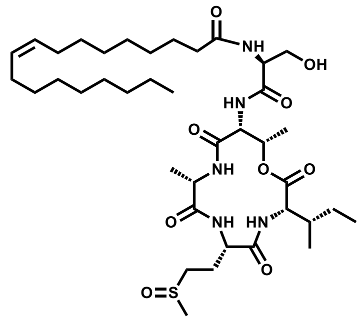
IR ν_{max} (film): 3291, 2966, 2875, 1693, 1328 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.37 (br. s., 2H), 8.05 - 8.12 (m, 1H), 7.99 (d, *J*=9.8 Hz, 1H), 7.72 (d, *J*=9.2 Hz, 1H), 7.22 (dd, *J*=19.2, 8.9 Hz, 1H), 5.27 - 5.36 (m, 1H), 4.71 (d, *J*=9.2 Hz, 1H), 4.368 - 4.49 (m, 2H), 4.04 (d, *J*=4.3 Hz, 1H), 2.64 - 2.797 (m, 1H), 2.53 (br. s., 3H), 2.33 - 2.37 (m, 2H), 2.16 (dt, *J*=13.3, 6.5 Hz, 1H), 1.91 - 1.99 (m, 3H), 1.57 (br. s., 1H), 1.23 - 1.28 (m, 34H), 1.10 (d, *J*=5.5 Hz, 3H), 0.83 - 0.88 (m, 6H), 0.77 (d, *J*=6.7 Hz, 3H)

^{13}C NMR (125 MHz, DMSO-d_6): δ 173.7, 173.3, 173.3, 170.6, 170.3, 170.2, 169.8, 72.3, 55.0, 53.8, 51.7, 51.0, 51.0, 50.6, 50.0, 49.8, 42.1, 40.1, 39.9, 39.8, 39.6, 39.4, 38.1, 37.9, 35.8, 35.7, 32.1, 31.5, 29.2, 29.2, 29.1, 29.0, 29.0, 28.9, 28.7, 26.8, 26.7, 26.2, 25.3, 23.8, 23.4, 22.3, 18.2, 16.9, 16.6, 16.5, 16.1, 14.7, 14.2, 12.7, 11.8

HRMS (ESI): calculated for $C_{36}H_{67}N_4O_7S$ $[M + H]^+$: 699.4725; found 699.4711.

***N*-((2*S*)-1-(((3*S*,6*S*,9*S*,12*R*,13*S*)-3-((*S*)-*sec*-butyl)-9,13-dimethyl-6-(2-(methylsulfinyl)ethyl)-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-12-yl)amino)-3-hydroxy-1-oxopropan-2-yl)oleamide (56)**



56

Compound **56** was prepared by following the procedure as used for the synthesis of **54** from compound **24**. In the same reaction mixture, we observed oxidation of sulfur group of methionine to corresponding sulfoxide **56** as colorless sticky liquid.

Yield: 57%

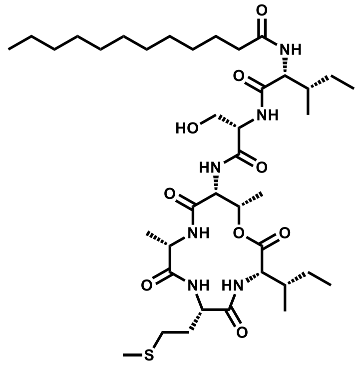
IR ν_{max} (film): 3298, 2968, 1687, 1651 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.65 - 8.57 (m, 1H), 8.41 - 8.38 (m, 1H), 8.29 (br. s., 1H), 7.74 - 7.70 (m, 1H), 6.90 (dd, *J* = 8.9, 19.8 Hz, 1H), 5.27 - 5.25 (m, 1H), 5.20 (br. s., 1H), 4.74 (d, *J* = 9.2 Hz, 1H), 4.51 (dd, *J* = 4.0, 8.9 Hz, 1H), 4.45 (dd, *J* = 4.3, 8.5 Hz, 1H), 4.15 (br. s., 1H), 4.02 (dd, *J* = 4.6, 11.3 Hz, 1H), 3.67 - 3.64 (m, 3H), 3.58 - 3.51 (m, 1H), 2.66 (br. s., 1H), 2.53 (s, 5H), 2.14 (t, *J* = 6.7 Hz, 2H), 1.96 (br. s., 6H), 1.46 (br. s., 4H), 1.37 - 1.35 (m, 3H), 1.23 (br. s., 20H), 1.14 (d, *J* = 6.1 Hz, 3H), 0.86 (d, *J* = 7.9 Hz, 6H), 0.77 (d, *J* = 6.7 Hz, 3H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 173.4, 172.8, 171.7, 170.7, 170.2, 169.4, 129.6, 71.7, 60.5, 56.9, 54.8, 51.8, 51.5, 50.0, 49.7, 37.9, 37.8, 36.0, 34.5, 31.3, 29.1, 28.8, 28.7, 28.6, 28.5, 26.6, 25.9, 24.8, 22.1, 16.2, 15.9, 14.4, 13.9, 11.8

HRMS (ESI): calculated for $C_{39}H_{69}N_5O_9SNa$ [M + Na]$^+$: 806.4708; found 806.4711.

***N*-((2*R*,3*S*)-1-(((*S*)-1-(((3*S*,6*S*,9*S*,12*R*,13*S*)-3-((*S*)-*sec*-butyl)-9,13-dimethyl-6-(2-(methylthio)ethyl)-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-12-yl)amino)-3-hydroxy-1-oxopropan-2-yl)amino)-3-methyl-1-oxopentan-2-yl)dodecanamide (58)**



58

Compound **58** was prepared by following the procedure as used for the synthesis of **54** from compound **13**. In the same reaction mixture, we observed oxidation of sulfur group of methionine to corresponding sulfoxide **58** as colorless sticky liquid.

Yield: 51%

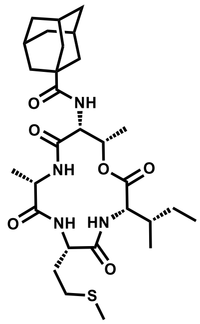
IR ν_{max} (film): 3306, 2978, 1709, 1649, 1395 cm^{-1}

^{1}H NMR (400 MHz, DMF-d_7): δ 8.90 (d, *J* = 16.5 Hz, 1H), 8.61 (d, *J* = 6.7 Hz, 1H), 8.23 (d, *J* = 9.8 Hz, 1H), 7.82 (d, *J* = 9.8 Hz, 1H), 7.63 (d, *J* = 9.2 Hz, 1H), 7.54 (d, *J* = 10.4 Hz, 1H), 5.47 - 5.32 (m, 1H), 5.17 (d, *J* = 6.7 Hz, 1H), 5.07 - 4.99 (m, 1H), 4.86 (d, *J* = 5.5 Hz, 1H), 4.57 - 4.47 (m, 3H), 4.43 - 4.28 (m, 2H), 4.15 (dd, *J* = 7.0, 12.5 Hz, 1H), 2.63 (t, *J* = 7.3 Hz, 1H), 2.52 - 2.45 (m, 2H), 2.28 - 2.24 (m, 1H), 2.11 (s, 2 H), 2.08 (d, *J* = 3.7 Hz, 3H), 2.02 - 1.98 (m, 2H), 1.45 - 1.41 (m, 9H), 1.35 (dd, *J* = 7.0, 10.7 Hz, 7H), 1.27 (br. s., 12H), 0.91 - 0.86 (m, 15H)

^{13}C NMR (100 MHz, DMF-d_7): δ 173.3, 172.7, 172.2, 171.8, 171.7, 171.0, 169.9, 71.7, 69.2, 68.6, 63.7, 62.5, 58.7, 55.4, 55.2, 54.9, 54.5, 51.4, 50.9, 46.4, 42.6, 36.8, 36.2, 35.9, 33.9, 32.5, 32.0, 31.0, 30.6, 28.7, 28.1, 26.8, 26.6, 26.1, 23.7, 22.8, 18.8, 16.9, 16.4, 16.1, 16.1, 15.7, 14.7, 14.5, 13.9, 13.8, 12.2, 11.5, 11.1

HRMS (ESI): calculated for $C_{39}H_{70}N_6O_9SNa$ [M + Na]$^+$: 821.4823; found 821.4828.

(3*R*,5*R*,7*R*)-*N*-((3*S*,6*S*,9*S*,12*R*,13*S*)-3-((*S*)-*sec*-butyl)-9,13-dimethyl-6-(2-(methylthio)ethyl)-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-12-yl)adamantane-1-carboxamide (59)



59

Compound **59** was prepared by following the procedure as used for the synthesis of **54** from compound **13** and adamantine carboxylic acid. After purification by column chromatography, **59** was obtained as colorless sticky liquid.

Yield: 82%.

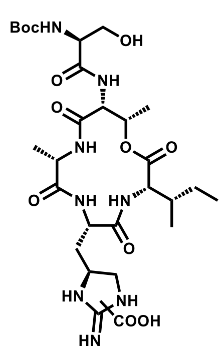
IR ν_{max} (film): 3282, 2964, 1638, 1430, 1392 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 - 7.72 (m, 1H), 7.03 (br. s., 1H), 6.87 (d, *J*=8.5 Hz, 2H), 5.55 (d, *J*=5.5 Hz, 1H), 4.72 (d, *J*=8.5 Hz, 1H), 4.50 (q, *J*=7.9 Hz, 1H), 4.32 (br. s., 1H), 4.01 - 4.08 (m, 1H), 2.63 (br. s., 1H), 2.49 (t, *J*=7.0 Hz, 2H), 2.03 - 2.09 (m, 9H), 1.97 (br. s., 7H), 1.76 (br. s., 6H), 1.39 (d, *J*=6.7 Hz, 3H), 1.24 (d, *J*=6.1 Hz, 3H), 0.89 - 0.92 (m, 6H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 179.9, 173.8, 171.3, 170.9, 169.6, 70.3, 56.5, 55.4, 52.8, 52.5, 41.0, 39.0, 36.3, 34.7, 30.2, 29.6, 28.0, 26.5, 16.5, 16.3, 15.2, 14.6, 11.3

HRMS (ESI): calculated for $C_{29}H_{47}N_4O_6S$ [M + H]$^+$: 579.3211; found 579.3207.

(*S*)-4-(((3*S*,6*S*,9*S*,12*R*,13*S*)-12-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-hydroxypropanamido)-3-((*S*)-*sec*-butyl)-9,13-dimethyl-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-6-yl)methyl)-2-iminoimidazolidine-1-carboxylic acid (60)



60

To compound **34**, 4 M HCl in dioxane was added at 0 °C and reaction mixture was stirred at room temperature for 1 h. After completion of reaction, solvent was evaporated under *vacuo* to afford amine hydrochloric acid salt.

To the solution of Boc-Ser-OH (23 mg, 0.114 mmol) in dry DMF (0.4 mL) was added HATU (57.7 mg, 0.154 mmol), HOAt (10.3 mg, 0.076 mmol) and DIPEA (59 µL, 0.342) were added simultaneously at 0 °C. The amine.HCl salt of **34** (50 mg, 0.076 mmol) was dissolved in DMF (0.2 mL) and added slowly to reaction mixture and mixture stirred at room temperature for 12 h. Then reaction mixture was diluted with ethyl acetate (10 mL), washed with ice-cold water, aqueous $NaHCO_3$ (10 mL) and 1 N HCl (10 mL). Organic layer was concentrated under *vacuo* and crude product was purified by column chromatography to afford product in Cbz-protected form; which on hydrogenolysis in methanol (3 mL), 10% Pd/C (~ 3 mg) was added and stirred under H_2 atmosphere for 12 h. The reaction mixture was then filtered through pad of celite, concentrated and purified by column chromatography (eluted in 4-7 % MeOH in CH_2Cl_2) afforded compound **60** as colourless sticky liquid.

Yield: 88%

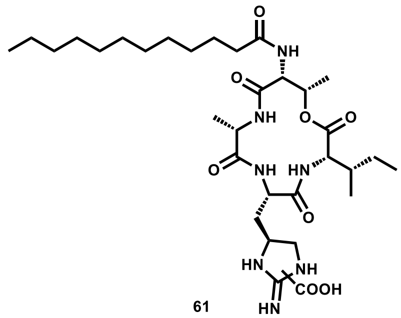
IR ν_{max} (film): 3305, 3022, 2890, 1730, 1666 cm^{-1}

^{1}H NMR (400 MHz, MeOH-d_4): δ 5.48 (dd, *J* = 3.05, 6.10 Hz, 1H), 4.60 (br. s., 1H), 4.51 - 4.54 (m, 2H), 4.19 (q, *J* = 7.12 Hz, 1H), 4.05 - 4.10 (m, 1H), 4.02 (d, *J* = 9.16 Hz, 1H), 3.82 (t, *J* = 6.10 Hz, 2H), 3.74 - 3.76 (m, 1H), 3.61 (dd, *J* = 6.10, 10.38 Hz, 1H), 3.22 - 3.26 (m, 1H), 2.16 (td, *J* = 6.79, 13.89 Hz, 1H), 1.97 - 2.01 (m, 1H), 1.81 - 1.88 (m, 1H), 1.46 (s, 11H), 1.25 - 1.29 (m, 6H), 0.90 - 0.96 (m, 6H)

^{13}C NMR (100 MHz, MeOH-d_4): δ 175.9, 175.0, 172.1, 172.0, 171.6, 159.4, 158.3, 156.8, 81.3, 73.2, 67.0, 62.8, 59.3, 57.2, 56.9, 56.0, 53.4, 53.0, 50.7, 47.4, 38.1, 37.3, 30.9, 28.8, 27.8, 24.9, 17.3, 16.5, 15.1, 14.1, 12.2

HRMS (ESI): calculated for $C_{28}H_{46}N_8O_{11}$ $[M + H]^+$: 670.3211; found 670.3208.

(*S*)-4-(((3*S*,6*S*,9*S*,12*R*,13*S*)-3-((*S*)-*sec*-butyl)-12-dodecanamido-9,13-dimethyl-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-6-yl)methyl)-2-iminoimidazolidine-1-carboxylic acid (61)



61

Compound **61** was prepared by following the procedure as used for the synthesis of **60** from compound **34** and dodecanoic acid. After purification by column chromatography, afforded **61** as colorless sticky liquid.

Yield: 60%

IR ν_{max} (film): 3365, 3029, 2829, 1702, 1661 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 8.08 (br. s., 1H), 7.98 (d, *J* = 9.16 Hz, 1H), 7.66 (s, 1H), 7.61 (d, *J* = 8.54 Hz, 1H), 7.54 (br. s., 1H), 7.13 - 7.15 (m, 1H), 6.94 – 6.99 (m, 1H), 5.27 - 5. 27 (m, 1H), 4.71 (d, *J* = 7.32 Hz, 1H), 4.48 (d, *J* = 5.49 Hz, 1H), 4.39 (d, *J* = 4.88 Hz, 1H), 4.02 - 4.05 (m, 1H), 3.77 - 3.82 (m, 1H), 3.43 (br. s., 1H), 2.35 (t, *J* = 6.41 Hz, 2H), 1.87 - 2.01 (m, 3H), 1.74 - 1.79 (m, 1H), 1.56 (d, *J* = 7.32 Hz, 2H), 1.49 - 1.50 (m, 1H), 1.24 - 1.33 (m, 20H), 1.10 (d, *J* = 6.71 Hz, 3H), 0.84 - 0.85 (m, 6H), 0.78 (d, *J* = 6.10 Hz, 3H)

HRMS (ESI): calculated for $C_{32}H_{55}N_7O_8$ [M + H]$^+$: 665.8330; found 665.8334.

General Boc-SPPS (solid phase peptide synthesis) procedure:

100 mg MBHA.HCl resin (0.7 mmol/g) was swollen in dry CH_2Cl_2 for 30 min and treated with the first 50% DIPEA in CH_2Cl_2 for neutralization, washed with CH_2Cl_2, treated with the first building block (2.0 equiv) and DIPEA (4.0 equiv) in dry CH_2Cl_2. After that, mixture was kept for 1 h, 80 μL Ac_2O, DIPEA and CH_2Cl_2 was added to cap the unreacted resin for another 20 min. The loaded resin was washed by CH_2Cl_2 (3 X 2 mL) and DMF (3 X 2 mL). Boc deprotection was achieved by shaken with 2 mL 20% solution of TFA in CH_2Cl_2 for 15 min X 3. The following Boc-amino acids (4.0 equiv) were coupled using HATU (4.0 equiv) as coupling reagent and DIPEA (8.0 equiv) as base. The mixture was shaken in DMF for 1 h. After each Boc deprotection and coupling reaction, the resin was washed by DMF (3 X 2 mL), CH_2Cl_2 (3 X 2 mL) and DMF (3 X 2 mL).

Side chain deprotection and Ser ligation:

After coupling of the last building block, the resin was washed by CH_2Cl_2 (3 X 2 mL), DMF (3 X 2 mL) and CH_2Cl_2 (5 X 2 mL). Then a cocktail of 50% TFA in CH_2Cl_2 was added to the resin and shaken for 1 h, the resin was washed by CH_2Cl_2 (3 X 2 mL), DMF (3 X 2 mL) and CH_2Cl_2 (5 X 2 mL). Resin and peptide 1–6 SAL aldehyde (prepared by ozonolysis of Hex-SAL ester, **44**) (1.2 equiv) were dissolved in a mixture of pyridine/AcOH (mol: mol = 1:1) at a concentration of 10.0 mmol/L. The reaction mixture was stirred at room temperature for 10 h. After that resin was washed by CH_2Cl_2 (3 X 2 mL), DMF (3 X 2 mL) and CH_2Cl_2 (5 X 2 mL). The residue was treated with TFA/ TFMSA/ 1,2-ethanedithiol (EDT)/ thioanisol (*v/v/v/v* = 20:3:1.3:1.8) for 1 h. Then the crude peptide was blown-dry under a stream of condensed air and purified by preparative HPLC (20–60% CH_3CN [0.1%TFA] in H_2O [0.1%TFA] over 30 min) to afford teixobactin analogues as white solid.

Synthesis of 63-70 teixobactin analogues:

Teixobactin analogues **63**–**70** were synthesized through the same methods as the above mentioned general procedures.

(*R*)-*N*^{1}-((6*S*,15*S*,18*S*,21*R*,22*S*)-1-amino-18-((*S*)-*sec*-butyl)-6-carbamoyl-15-(hydroxymethyl)-1-imino-22-methyl-8,11,14,17,20-pentaoxo-2,7,10,13,16,19-hexaazatetracosan-21-yl)-2-((*S*)-3-hydroxy-2-((2*S*,3*S*)-3-methyl-2-((*R*)-2-(methylamino)-3-phenylpropanamido)pentanamido)propanamido)pentanediamide (63)

Retention time: 11.06 min, white solid, HPLC Gradient: 5-95% CH_3CN/H_2O with 0.1% TFA over 15 min at a flow rate of 1.0 mL/min.

HRMS (ESI): calculated for $C_{32}H_{55}N_7O_8$ $[M - H]^-$ 1088.6211; found 1088.6245.

(*R*)-*N*^{1}-((6*S*,16*S*,19*S*,22*R*,23*S*)-1-amino-19-((*S*)-*sec*-butyl)-6-carbamoyl-16-(hydroxymethyl)-1-imino-23-methyl-8,15,18,21-tetraoxo-2,7,14,17,20-pentaazapentacosan-22-yl)-2-((*S*)-3-hydroxy-2-((2*S*,3*S*)-3-methyl-2-((*R*)-2-(methylamino)-3-phenylpropanamido)pentanamido)propanamido)pentanediamide (64)

Retention time: 10.08 min, white solid, HPLC Gradient: 5-95% CH_3CN/H_2O with 0.1% TFA over 15 min at a flow rate of 1.0 mL/min.

HRMS (ESI): calculated for $C_{32}H_{55}N_7O_8$ $[M - H]^-$ 1087.6622; found 1087.6635.

(*R*)-*N*[1]-((2*R*,3*S*)-1-(((2*S*,3*S*)-1-(((*S*)-1-((*R*)-2-(((*S*)-1-(((*S*)-1-amino-5-guanidino-1-oxopentan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)pyrrolidin-1-yl)-3-hydroxy-1-oxopropan-2-yl)amino)-3-methyl-1-oxopentan-2-yl)amino)-3-methyl-1-oxopentan-2-yl)-2-((*S*)-3-hydroxy-2-((2*S*,3*S*)-3-methyl-2-((*R*)-2-(methylamino)-3-phenylpropanamido)pentanamido)propanamido)pentanediamide (65)

Retention time: 9.07 min, white solid, HPLC Gradient: 5-95% CH_3CN/H_2O with 0.1% TFA over 15 min at a flow rate of 1.0 mL/min.

HRMS (ESI): calculated for $C_{32}H_{55}N_7O_8$ $[M - H]^-$ 1142.6681; found 1142.6703.

(*R*)-*N*[1]-((6*S*,9*S*,15*S*,18*S*,21*R*,22*S*)-1-amino-18-((*S*)-*sec*-butyl)-6-carbamoyl-15-(hydroxymethyl)-1-imino-9,22-dimethyl-8,11,14,17,20-pentaoxo-2,7,10,13,16,19-hexaazatetracosan-21-yl)-2-((*S*)-3-hydroxy-2-((2*S*,3*S*)-3-methyl-2-((*R*)-2-(methylamino)-3-phenylpropanamido)pentanamido)propanamido)pentanediamide (66)

Retention time: 9.61 min, white solid, HPLC Gradient: 5-95% CH_3CN/H_2O with 0.1% TFA over 15 min at a flow rate of 1.0 mL/min.

HRMS (ESI): calculated for $C_{32}H_{55}N_7O_8$ $[M - H]^-$ 1102.6451; found 1102.6485.

(*S*)-*N*[1]-((6*S*,15*S*,18*S*,21*R*,22*S*)-1-amino-18-((*S*)-*sec*-butyl)-6-carbamoyl-15-(hydroxymethyl)-1-imino-22-methyl-8,11,14,17,20-pentaoxo-2,7,10,13,16,19-hexaazatetracosan-21-yl)-2-((*S*)-3-hydroxy-2-((2*S*,3*S*)-3-methyl-2-((*R*)-2-(methylamino)-3-phenylpropanamido)pentanamido)propanamido)pentanediamide (67)

Retention time: 11.02 min, white solid, HPLC Gradient: 5-95% CH_3CN/H_2O with 0.1% TFA over 15 min at a flow rate of 1.0 mL/min.

HRMS (ESI): calculated for $C_{32}H_{55}N_7O_8$ $[M - H]^-$ 1088.6211; found 1088.6295.

(*R*)-*N*[1]-((6*S*,9*R*,12*R*,15*S*,18*S*,21*R*,22*S*)-1-amino-18-((*S*)-*sec*-butyl)-6-carbamoyl-12-((*S*)-1-hydroxyethyl)-15-(hydroxymethyl)-1-imino-9,22-dimethyl-8,11,14,17,20-pentaoxo-2,7,10,13,16,19-hexaazatetracosan-21-yl)-2-((*S*)-3-hydroxy-2-((2*S*,3*S*)-3-methyl-2-((*R*)-2-(methylamino)-3-phenylpropanamido)pentanamido)propanamido)pentanediamide (68)

Retention time: 8.38 min, white solid, HPLC Gradient: 5-95% CH_3CN/H_2O with 0.1% TFA over 15 min at a flow rate of 1.0 mL/min.

HRMS (ESI): calculated for $C_{32}H_{55}N_7O_8$ $[M - H]^-$ 1143.6630; found 1143.6647.

(*R*)-*N*[1]-((4*S*,7*R*,10*R*,13*S*,16*S*,19*R*,20*S*)-16-((*S*)-*sec*-butyl)-4-carbamoyl-10-((*S*)-1-hydroxyethyl)-13-(hydroxymethyl)-2,7,20-trimethyl-6,9,12,15,18-pentaoxo-5,8,11,14,17-pentaazadocosan-19-yl)-2-((*S*)-3-hydroxy-2-((2*S*,3*S*)-3-methyl-2-((*R*)-2-(methylamino)-3-phenylpropanamido)pentanamido)propanamido)pentanediamide (69)

Retention time: 10.48 min, white solid, HPLC Gradient: 5-95% CH_3CN/H_2O with 0.1% TFA over 15 min at a flow rate of 1.0 mL/min.

HRMS (ESI): calculated for $C_{32}H_{55}N_7O_8$ $[M - H]^-$ 1103.6459; found 1103.6675.

(*R*)-*N*[1]-((6*S*,9*S*,12*S*,15*S*,18*S*,21*R*,22*S*)-1-amino-12-(4-aminobutyl)-18-((*S*)-*sec*-butyl)-6-carbamoyl-15-(hydroxymethyl)-1-imino-9,22-dimethyl-8,11,14,17,20-pentaoxo-2,7,10,13,16,19-hexaazatetracosan-21-yl)-2-((*S*)-3-hydroxy-2-((2*S*,3*S*)-3-methyl-2-((*R*)-2-(methylamino)-3-phenylpropanamido)pentanamido)propanamido)pentanediamide (70)

Retention time: 7.82 min, white solid, HPLC Gradient: 5-95% CH_3CN/H_2O with 0.1% TFA over 15 min at a flow rate of 1.0 mL/min.

HRMS (ESI): calculated for $C_{32}H_{55}N_7O_8$ $[M - H]^-$ 1173.7186; found 1173.7167.

1.6. References

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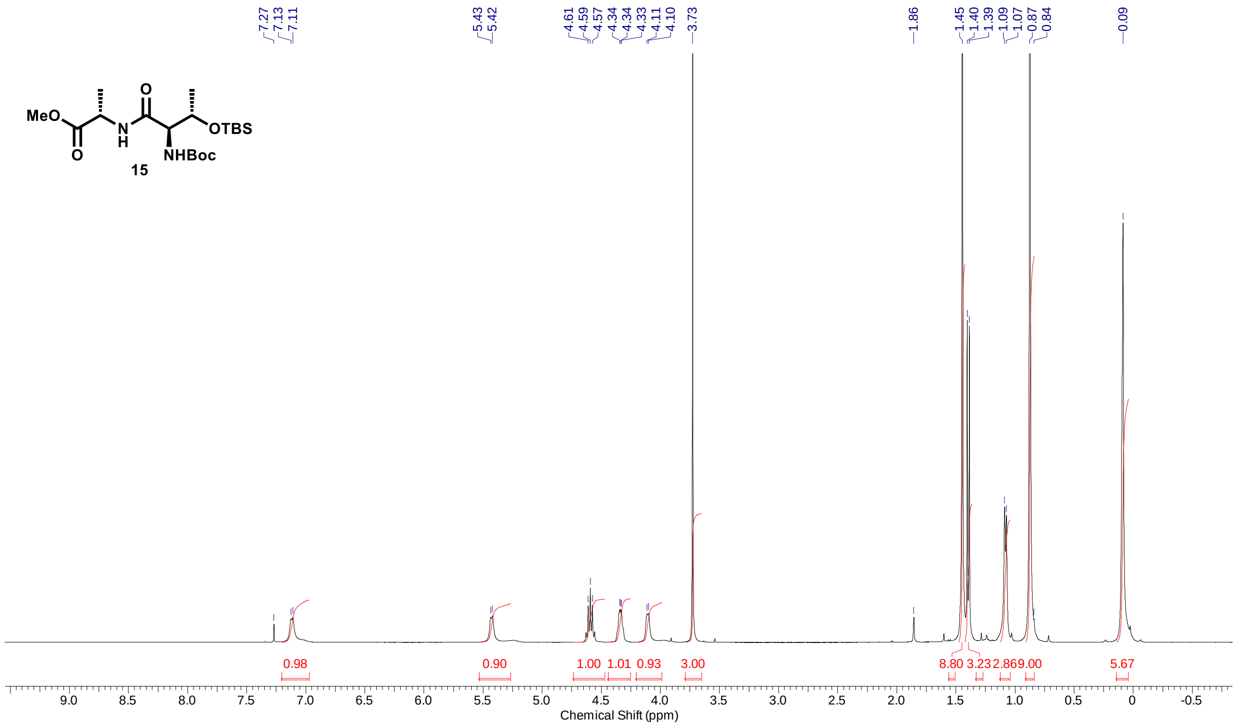
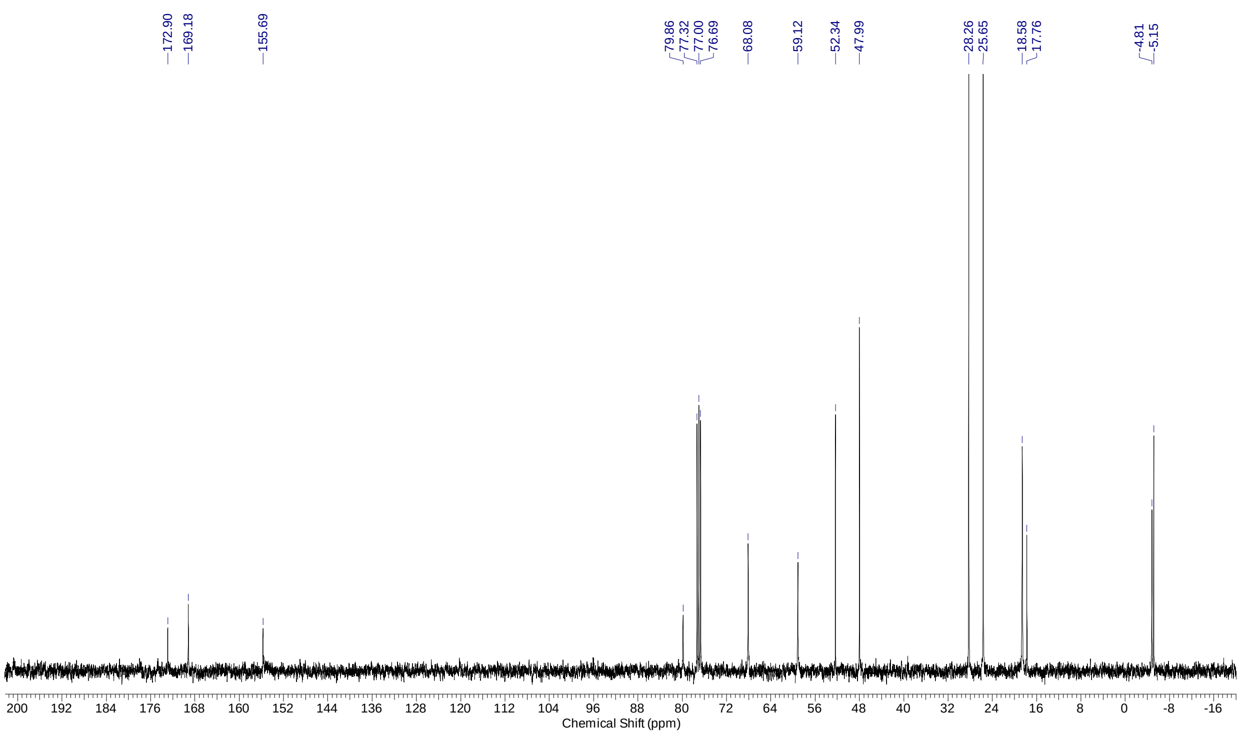
1.7. Copies of NMR spectra

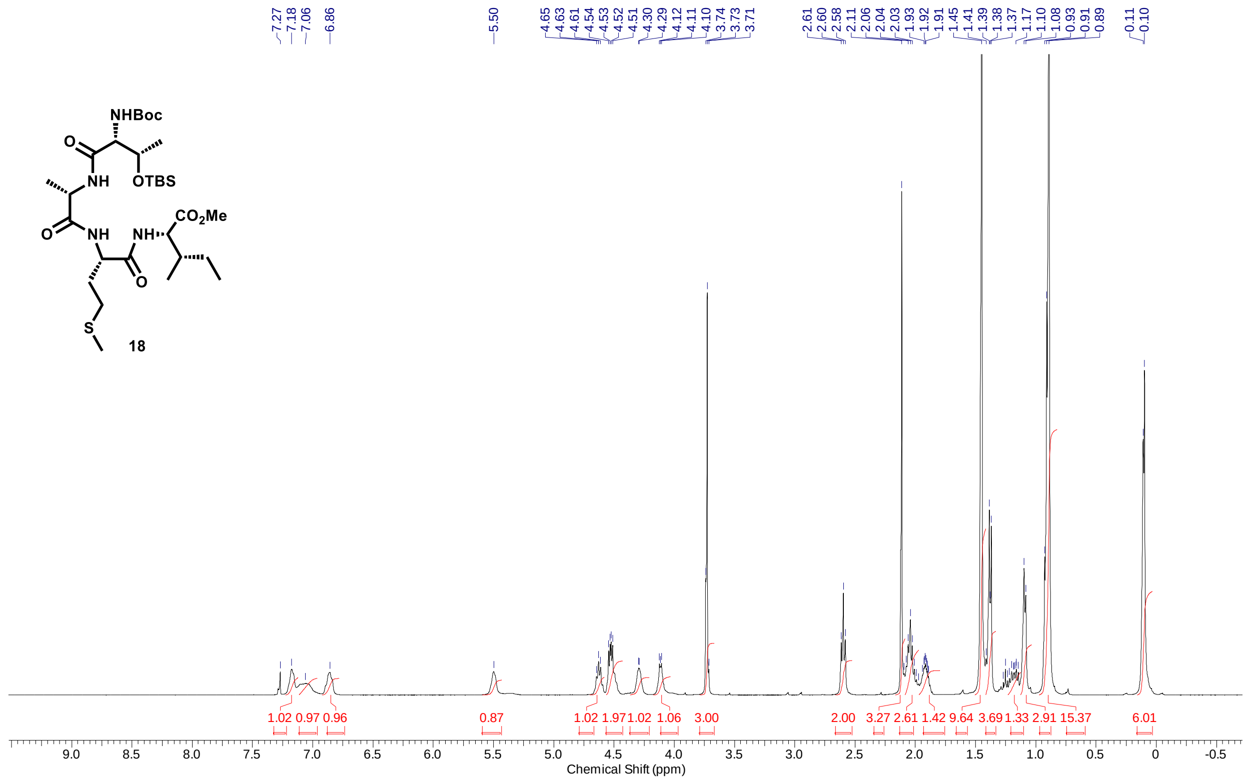


^{1}H NMR of **15** (400 MHz, $CDCl_3$)

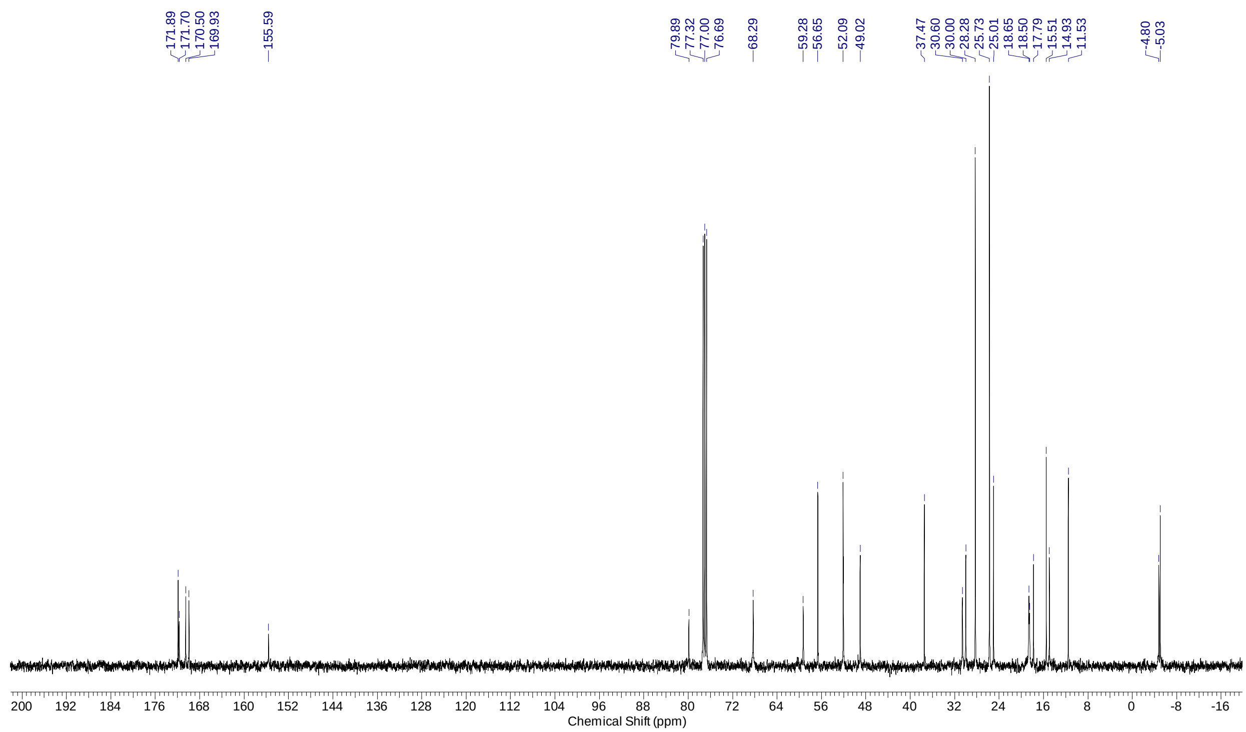


^{13}C NMR of **15** (100 MHz, $CDCl_3$)

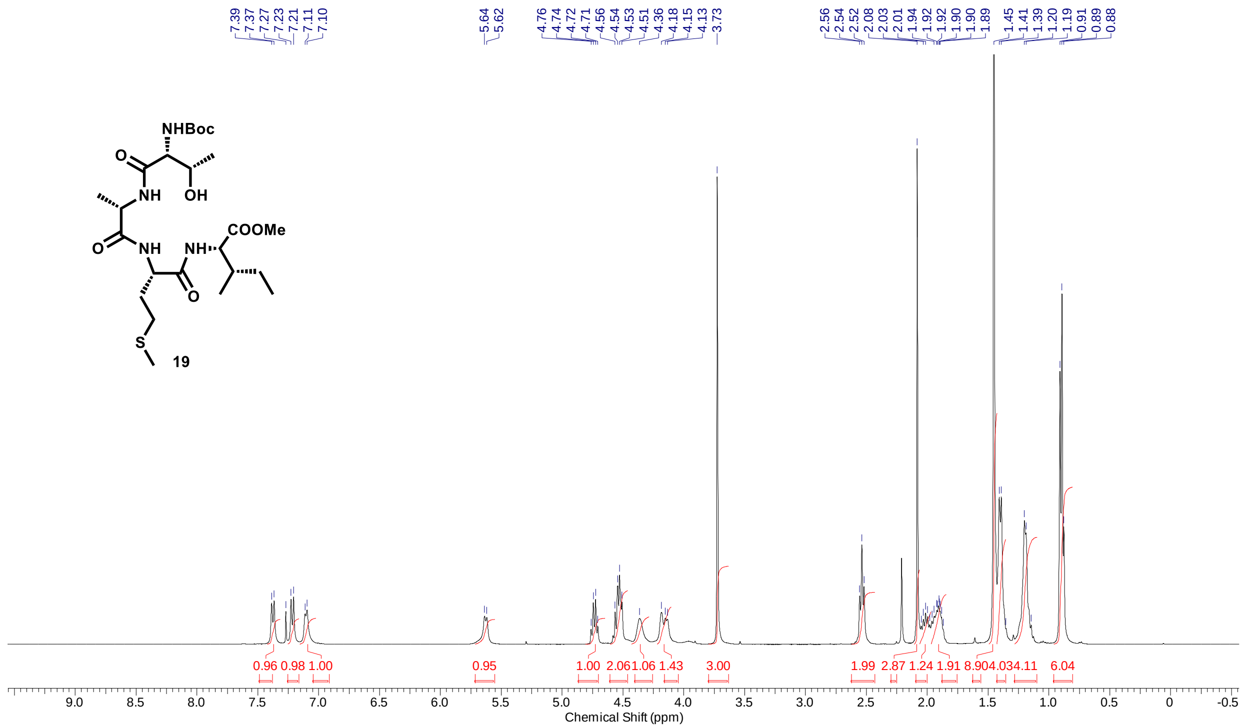
18



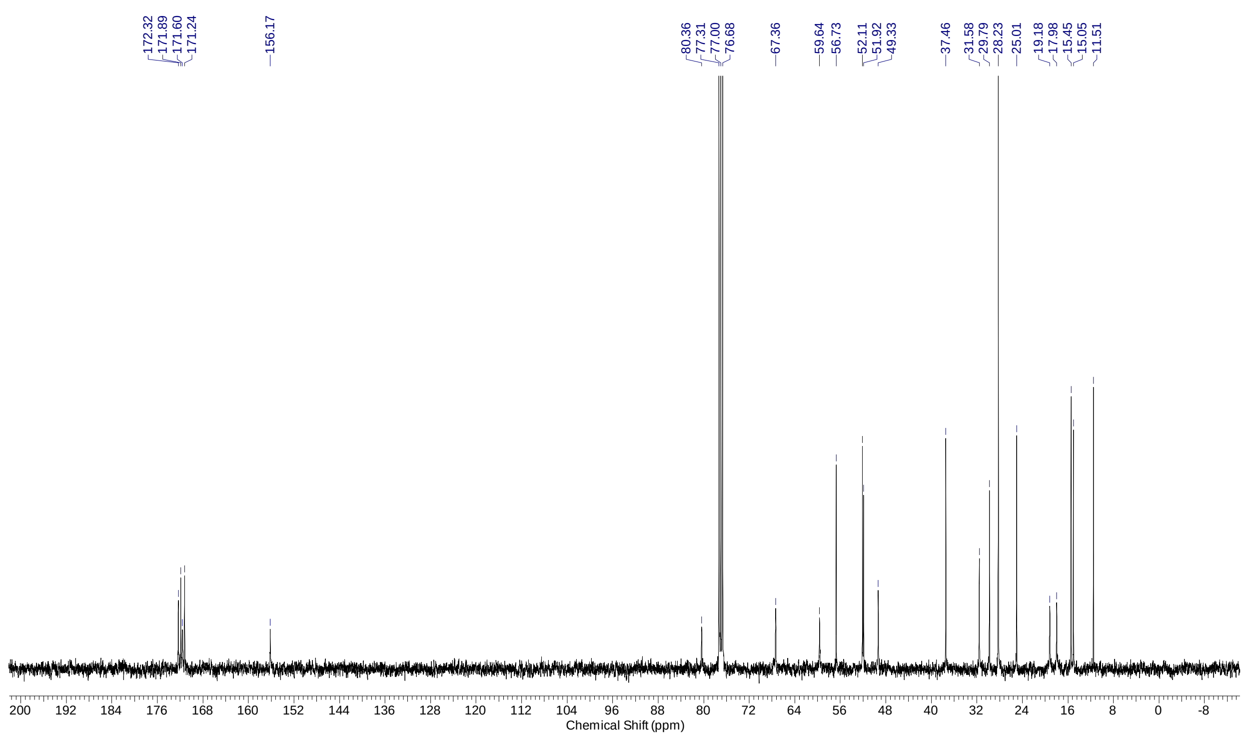
^{1}H NMR of **18** (400 MHz, $CDCl_3$)



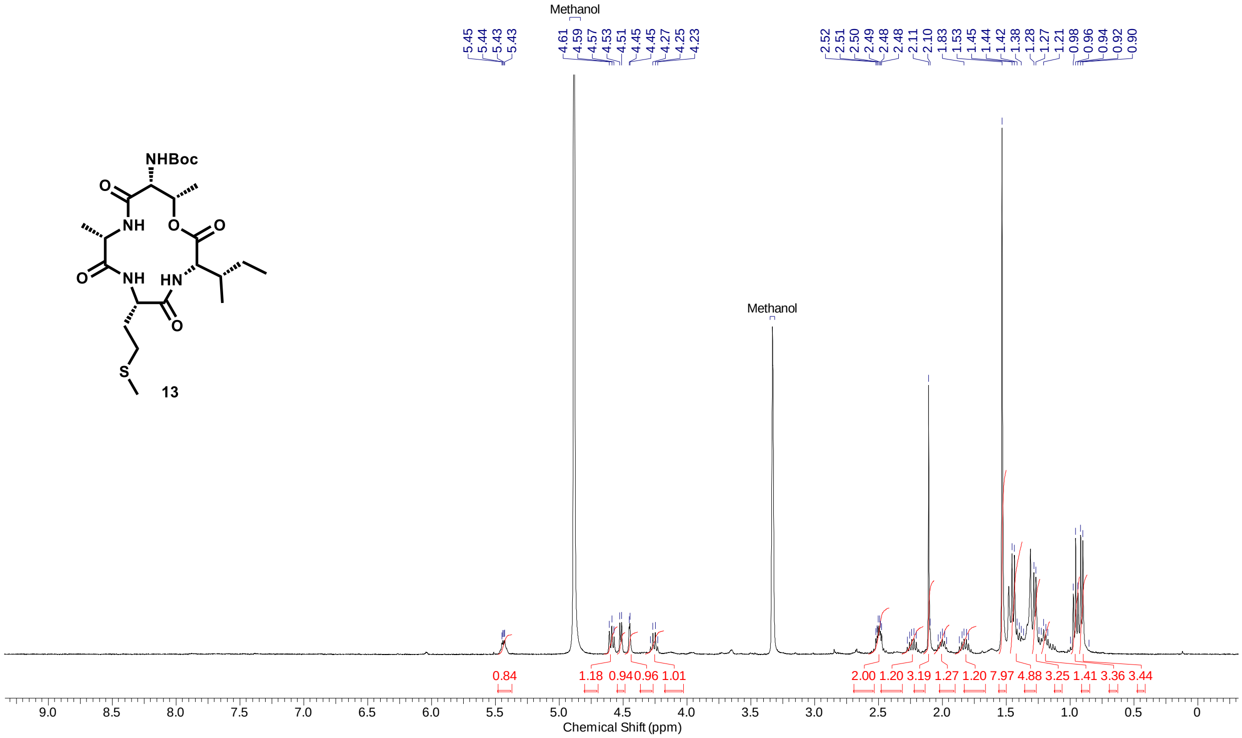
^{13}C NMR of **18** (100 MHz, $CDCl_3$)



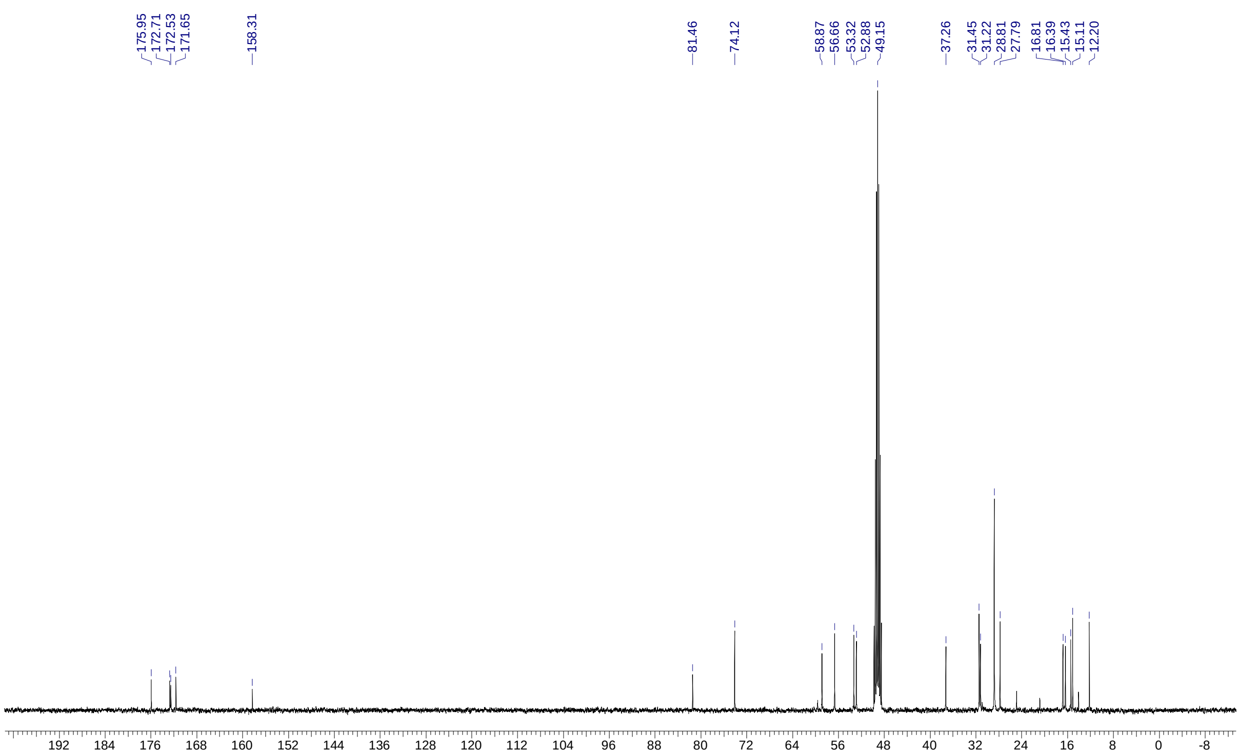
^{1}H NMR of **19** (400 MHz, $CDCl_3$)



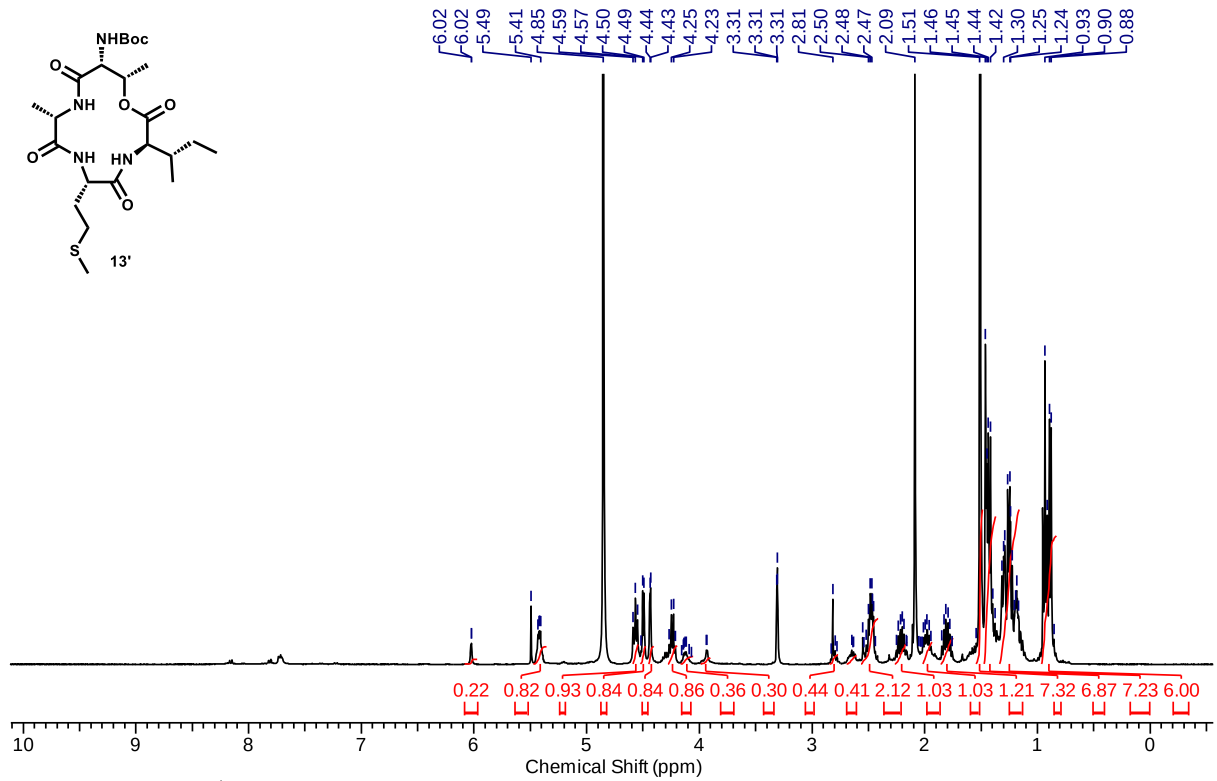
^{13}C NMR of **19** (100 MHz, $CDCl_3$)



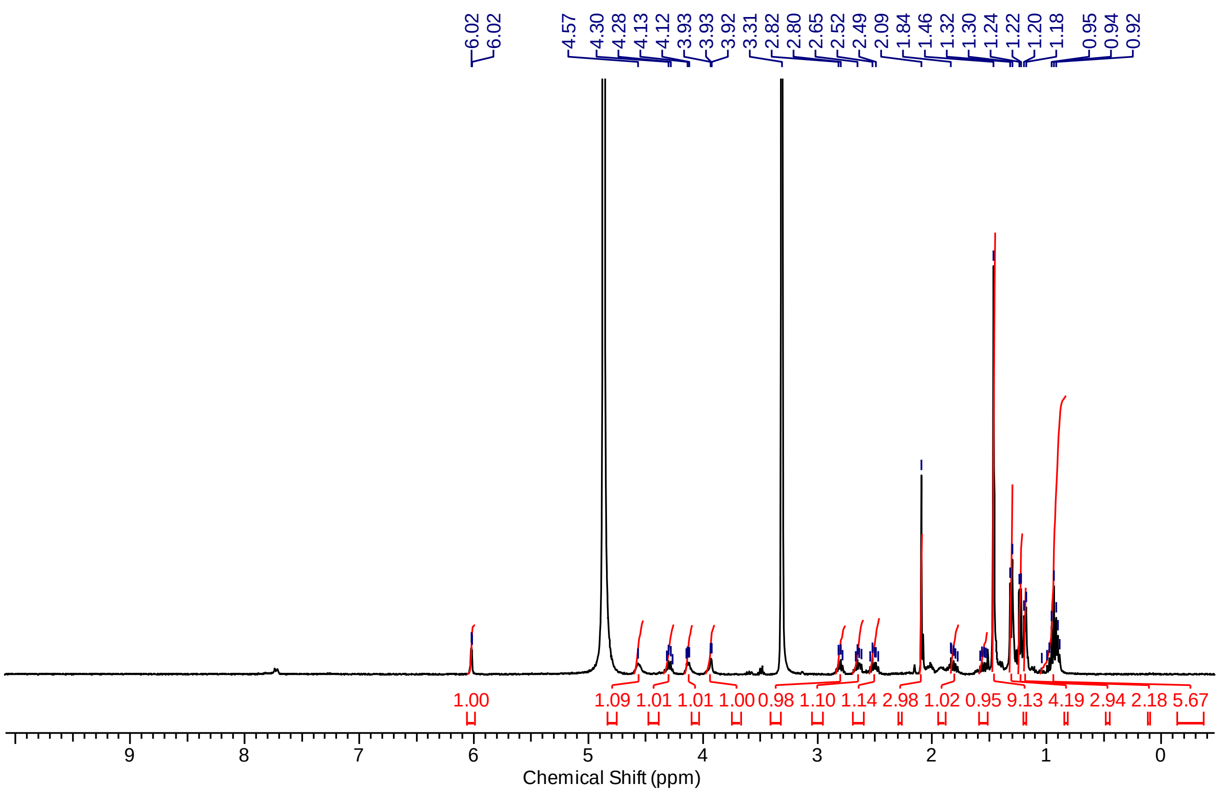
^{1}H NMR of **13** (400 MHz, MeOH-d_4)



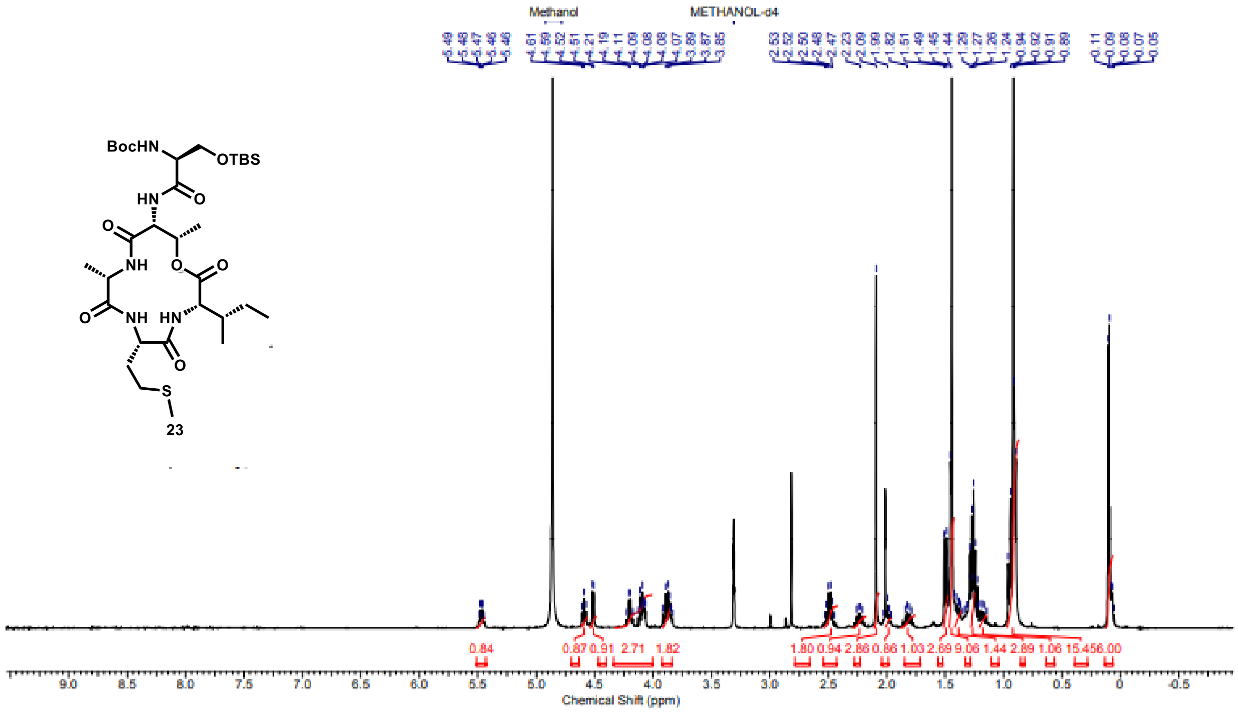
^{13}C NMR of **13** (100 MHz, MeOH-d_4)



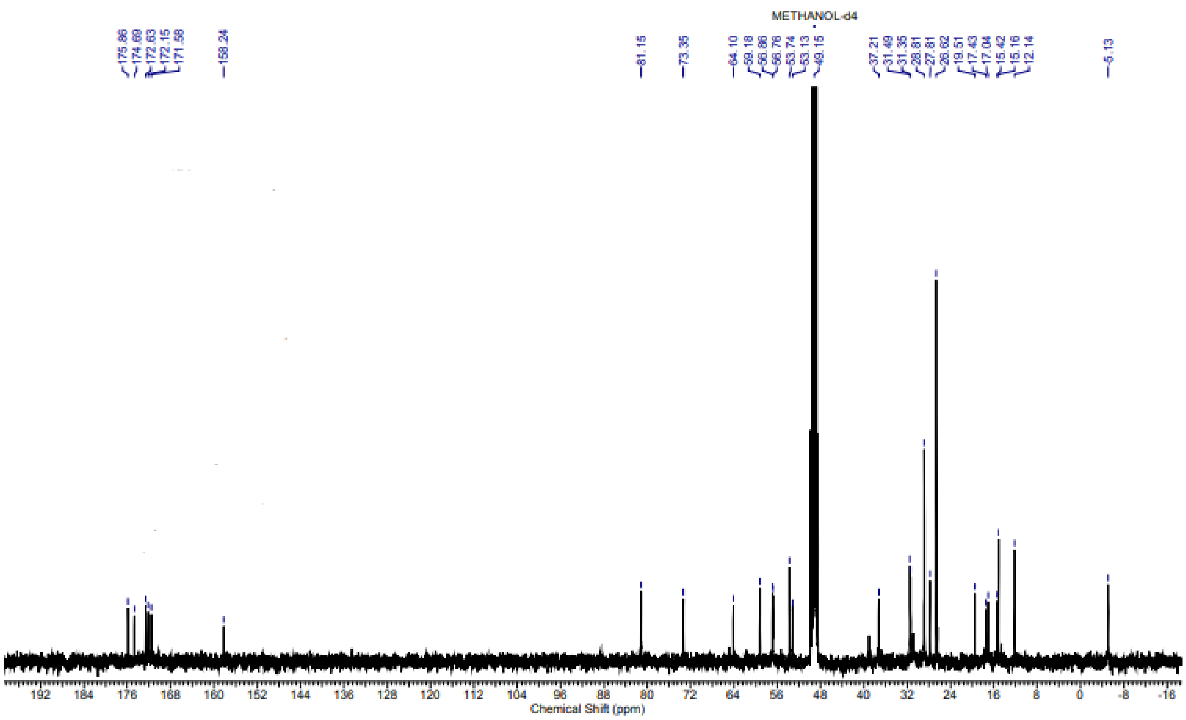
[1]H NMR of reaction mixture-**13** and **13’** (400 MHz, MeOH-d_4)



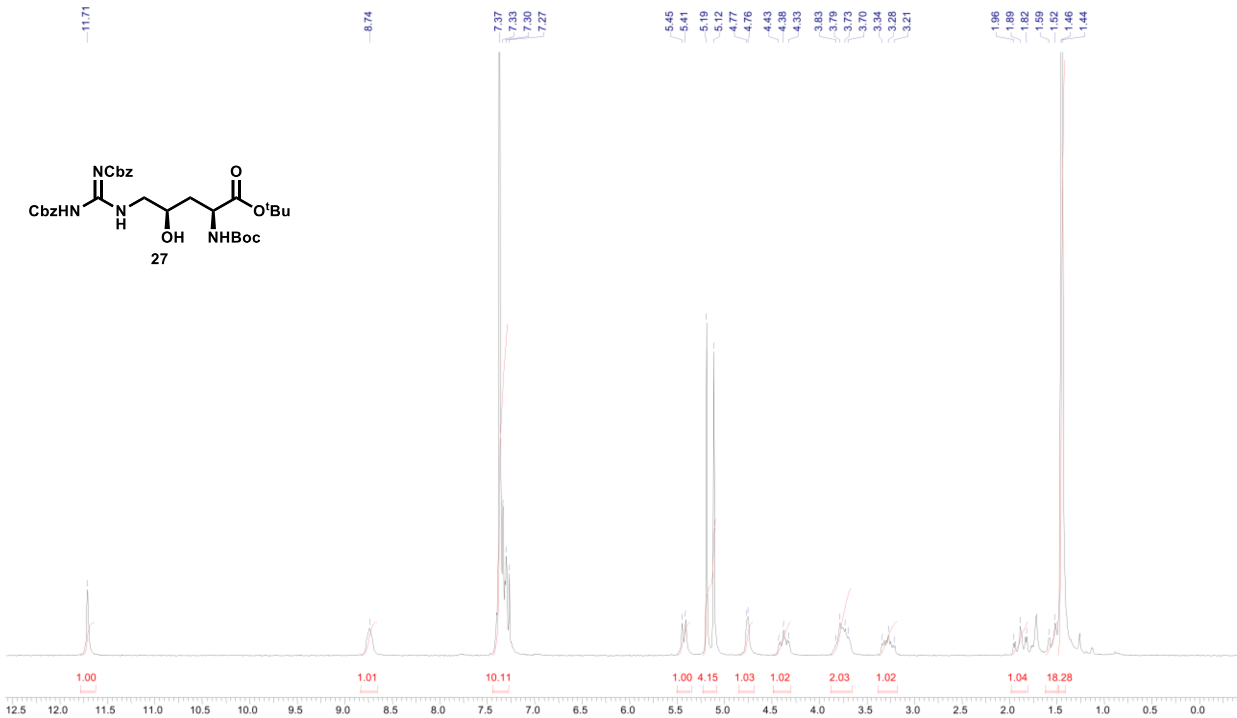
[1]H NMR of **13’** (400 MHz, MeOH-d_4)



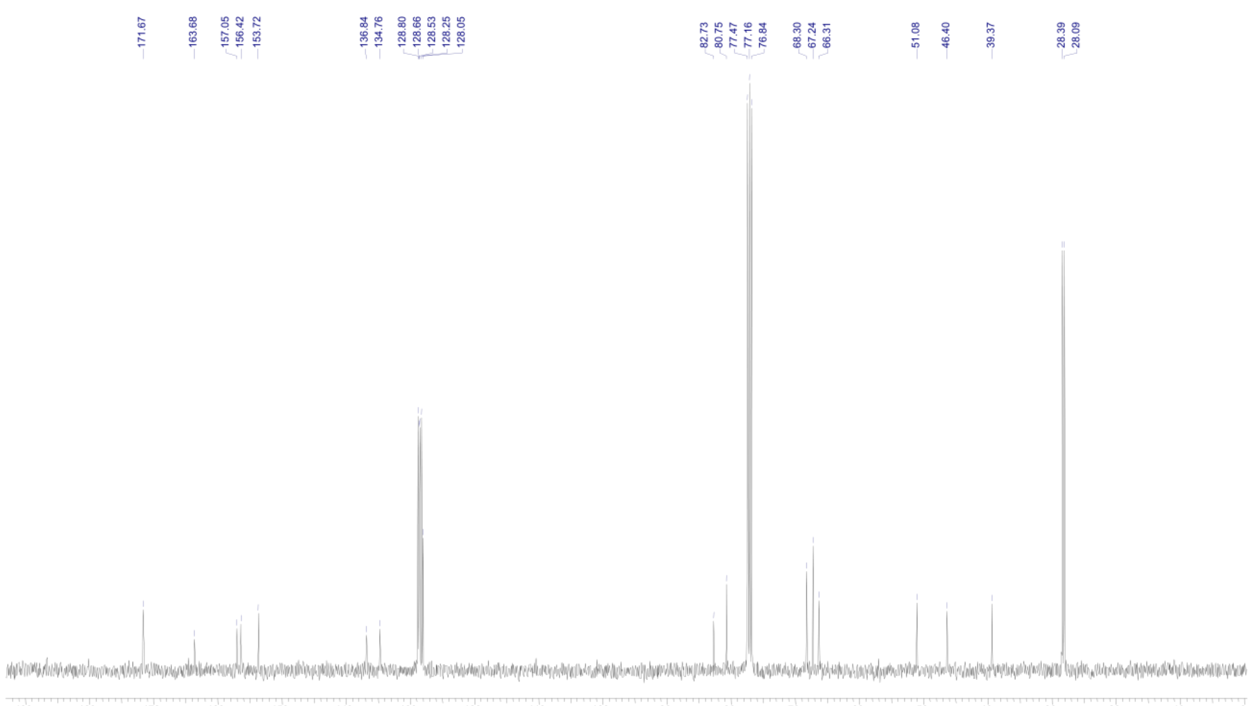
^{1}H NMR of **23** (400 MHz, MeOH-d_4)



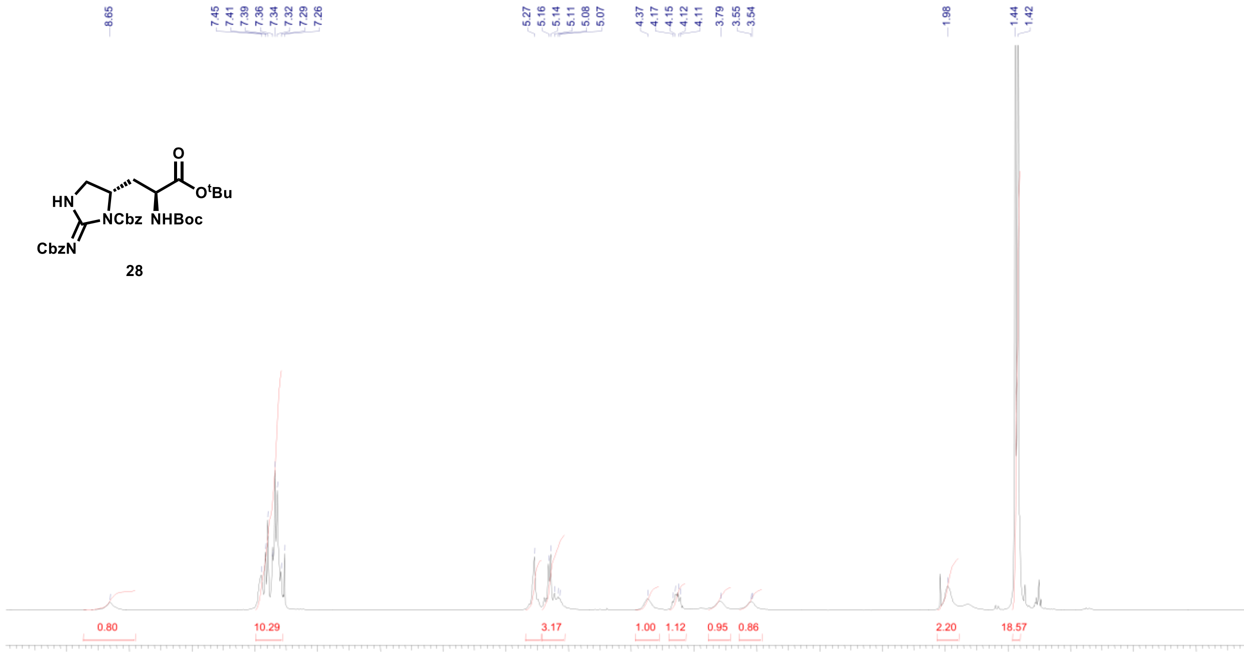
^{13}C NMR of **23** (100 MHz, MeOH-d_4)



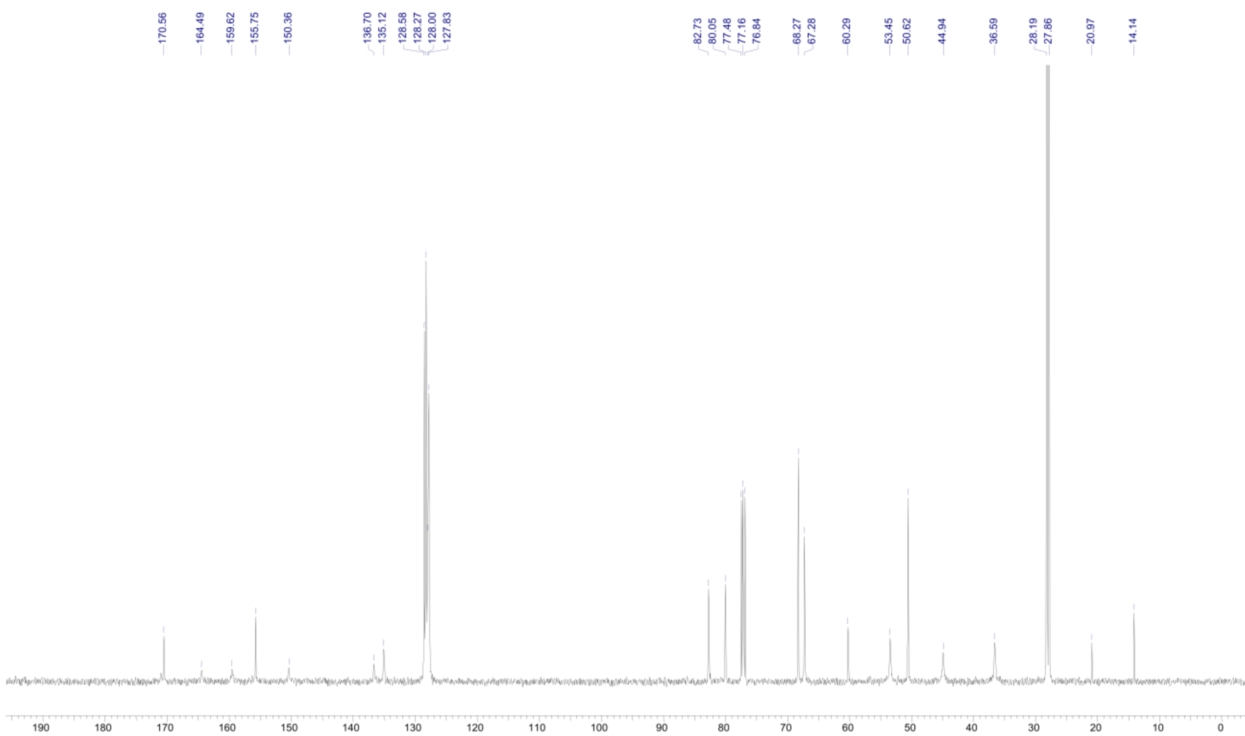
^{1}H NMR of **27** (400 MHz, $CDCl_3$)



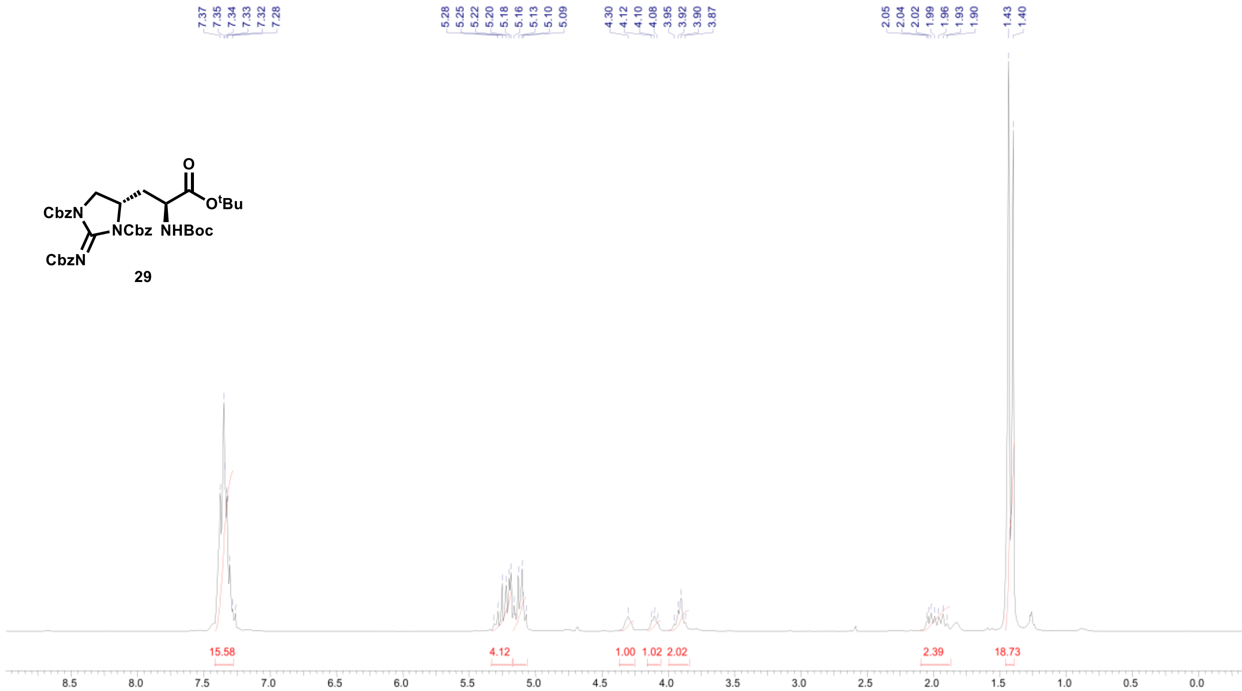
^{13}C NMR of **27** (100 MHz, $CDCl_3$)



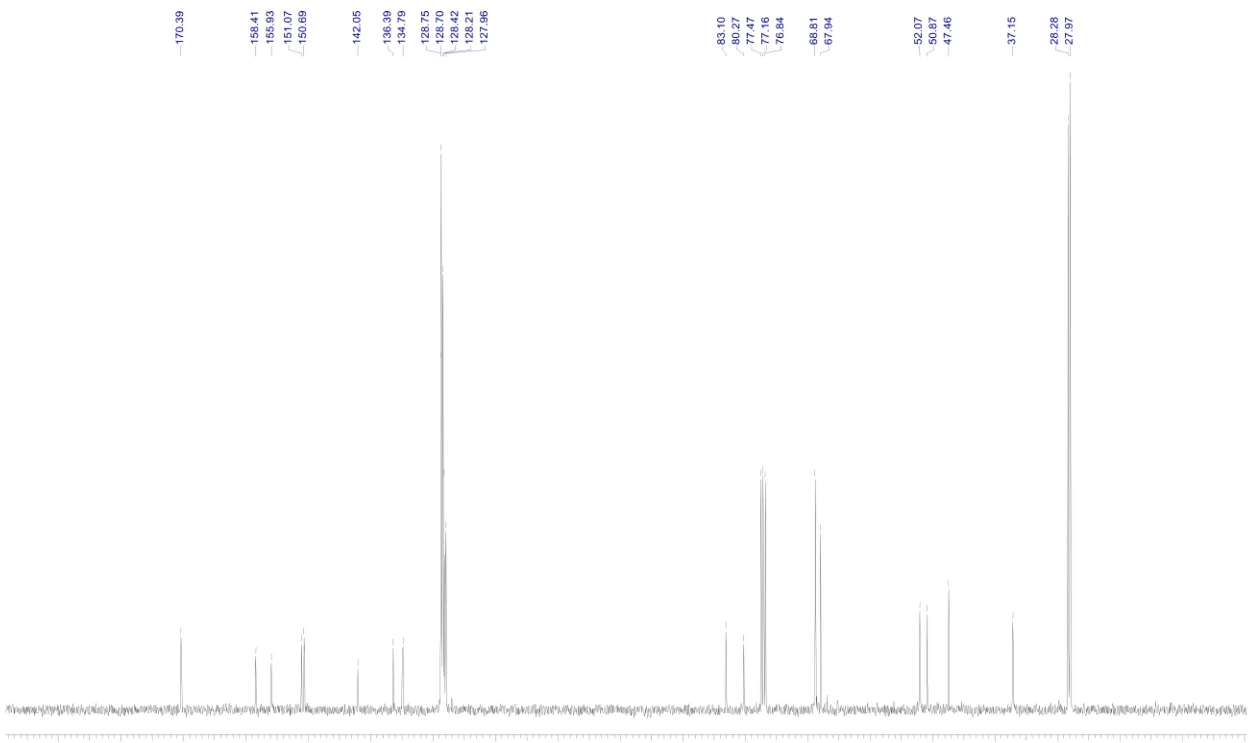
^{1}H NMR of **28** (400 MHz, $CDCl_3$)



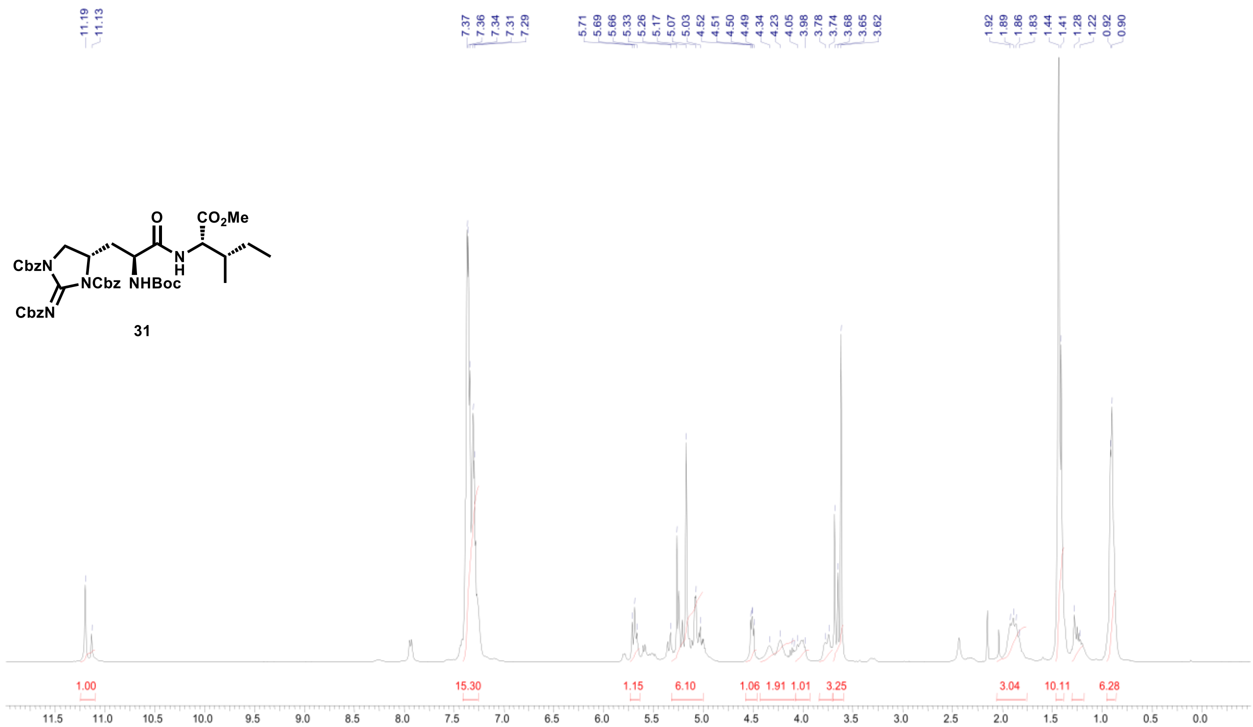
^{13}C NMR of **28** (100 MHz, $CDCl_3$)



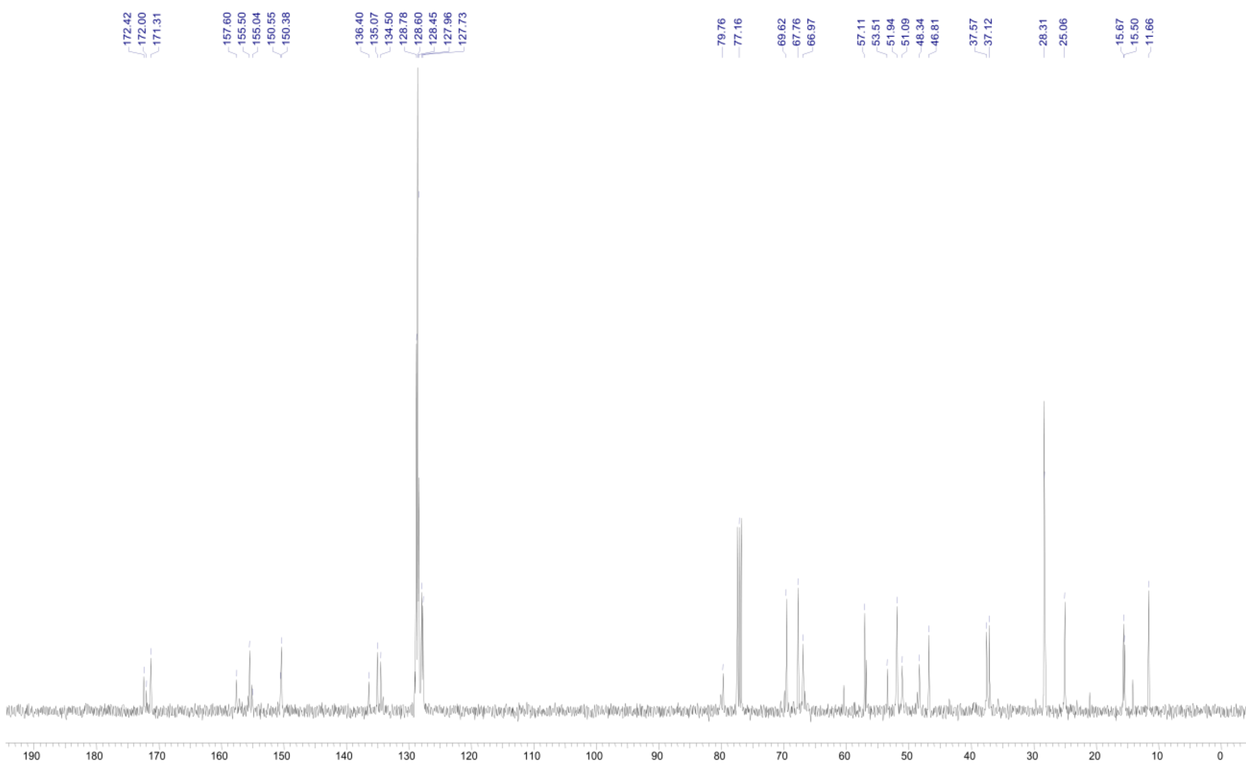
^{1}H NMR of **29** (400 MHz, $CDCl_3$)



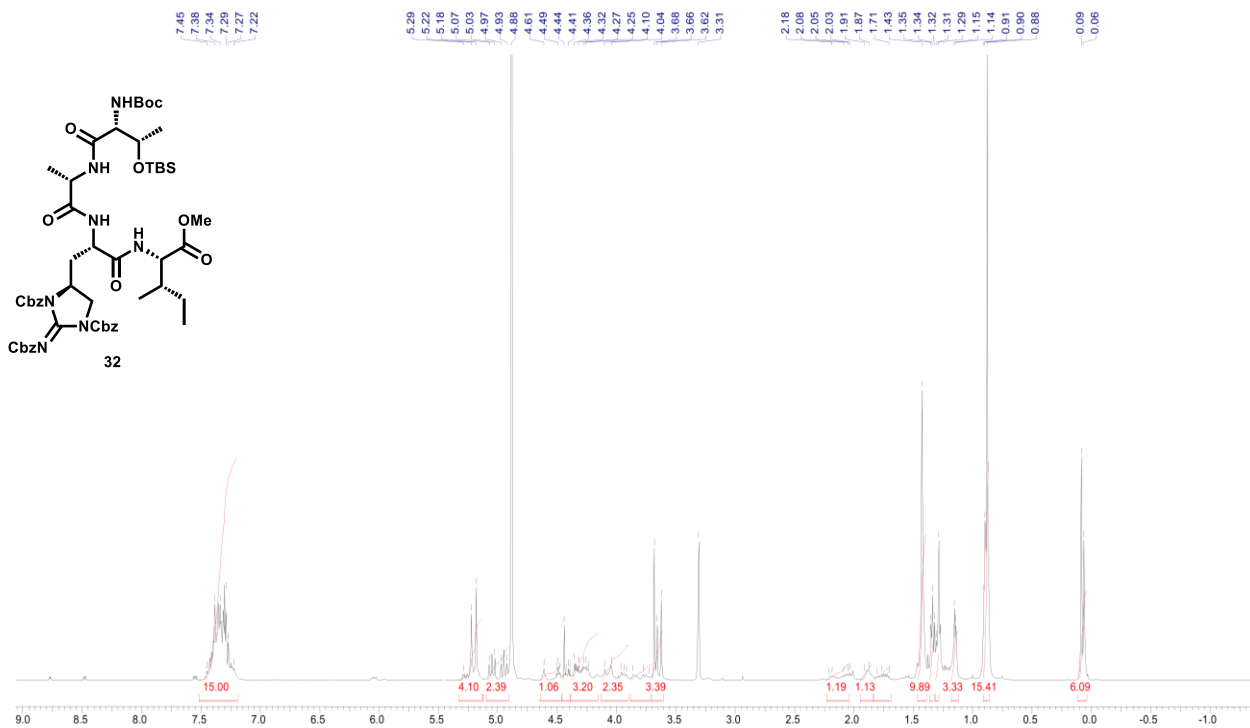
^{13}C NMR of **29** (100 MHz, $CDCl_3$)



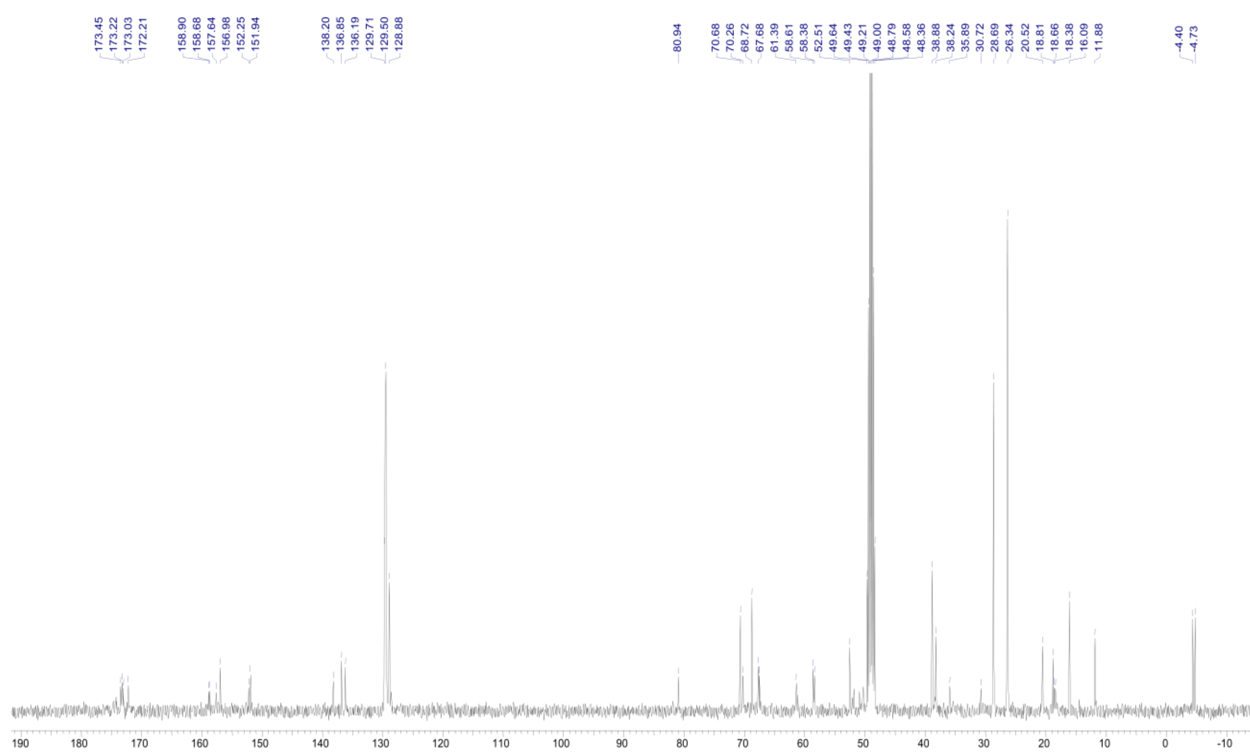
^{1}H NMR of **31** (400 MHz, $CDCl_3$)



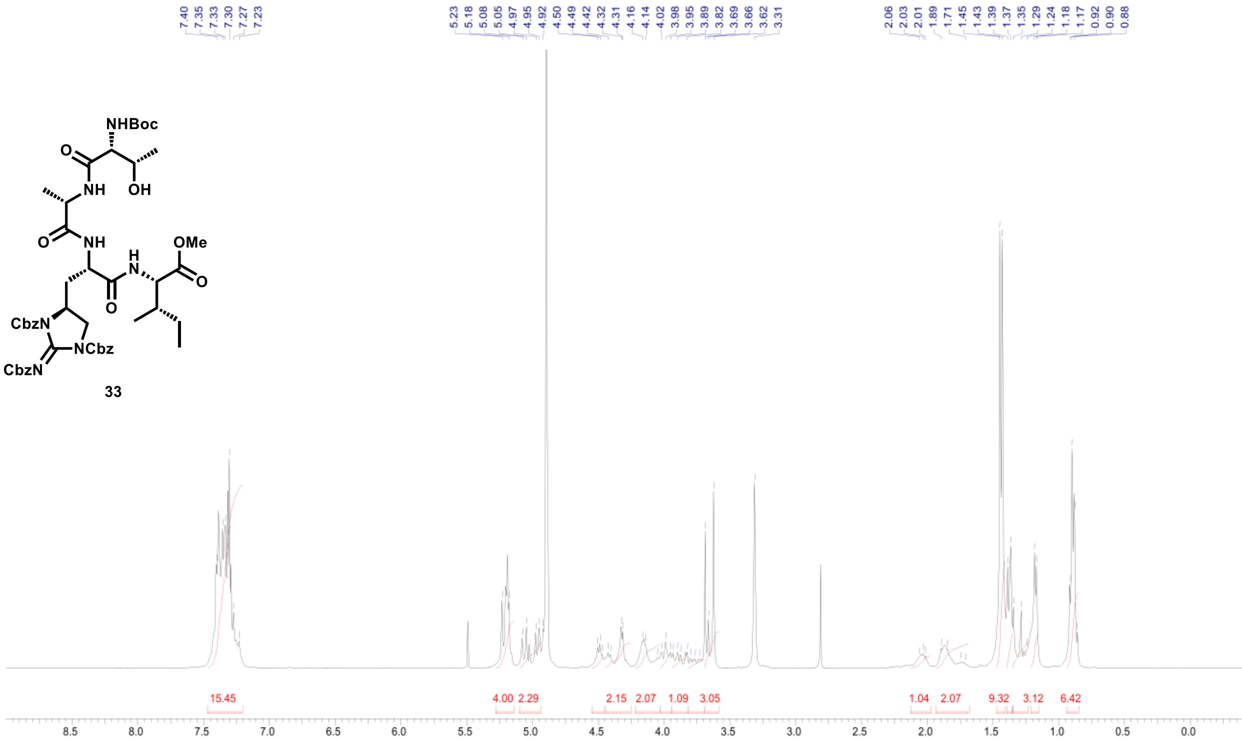
^{13}C NMR of **31** (100 MHz, $CDCl_3$)



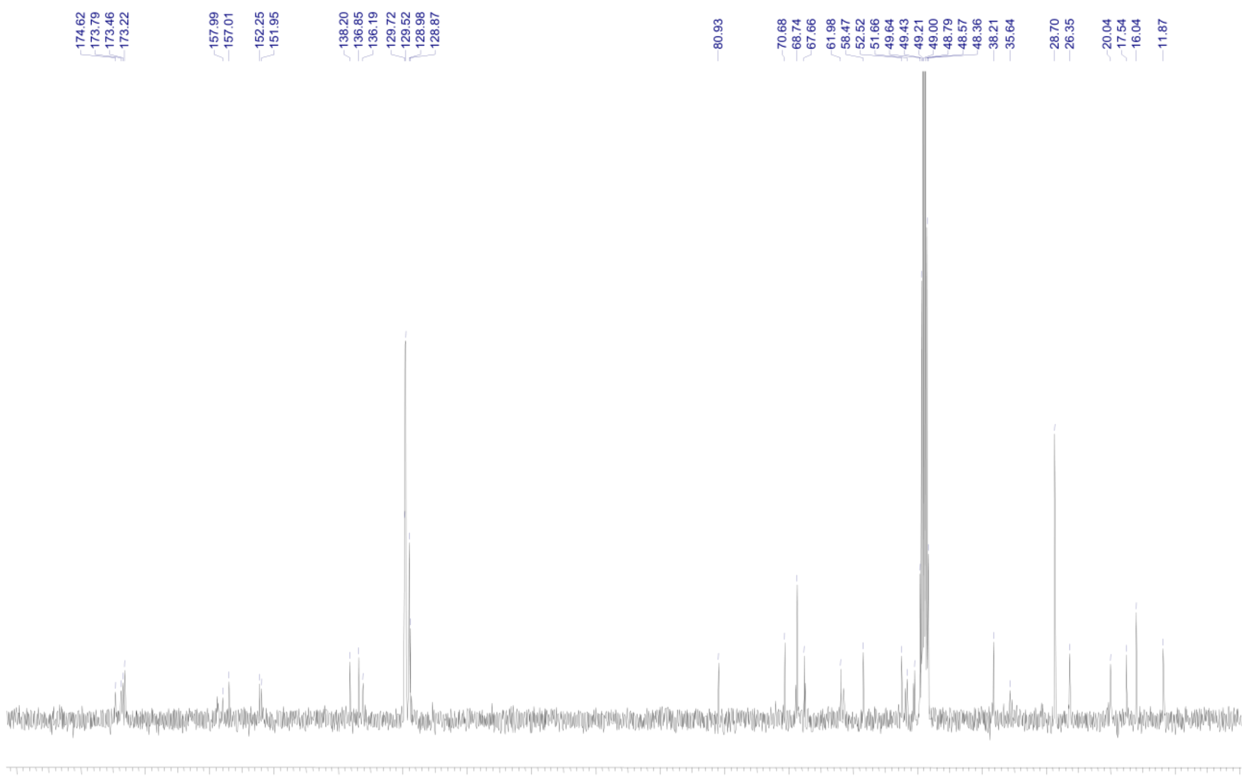
^{1}H NMR of **32** (400 MHz, MeOH-d_4)



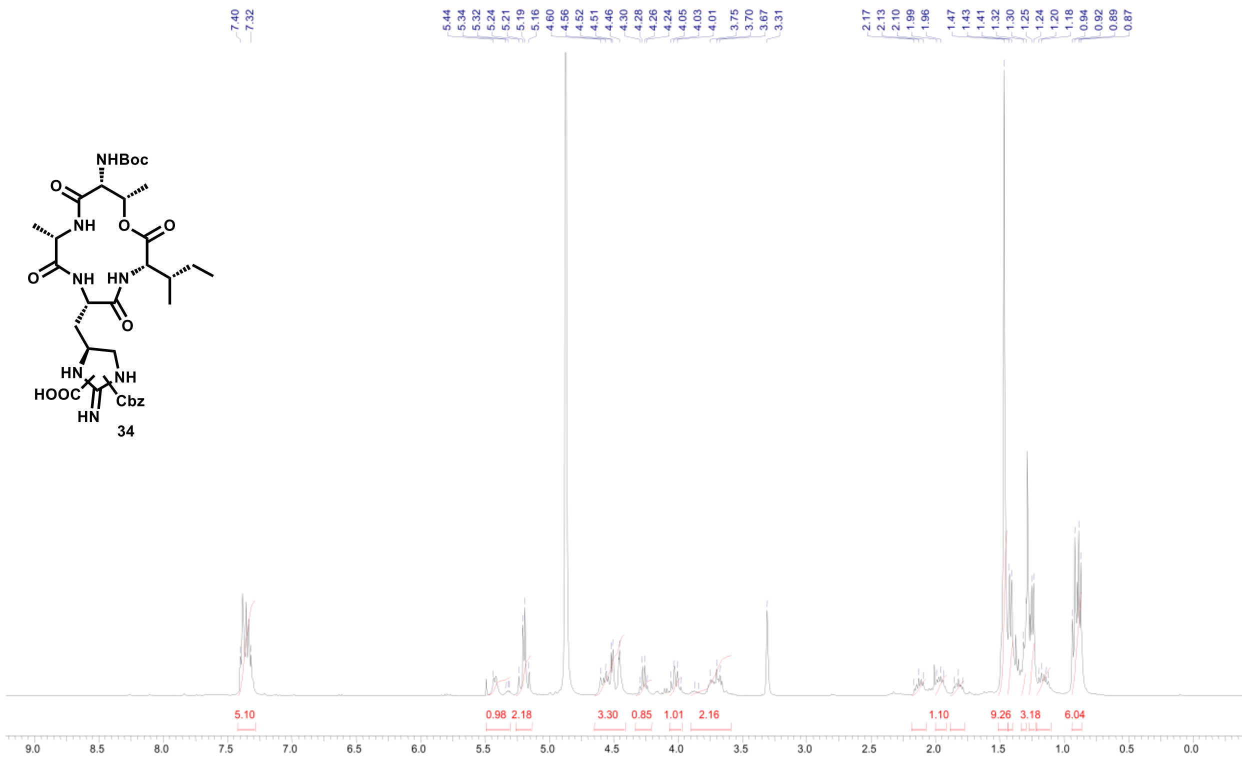
^{13}C NMR of **32** (100 MHz, MeOH-d_4)



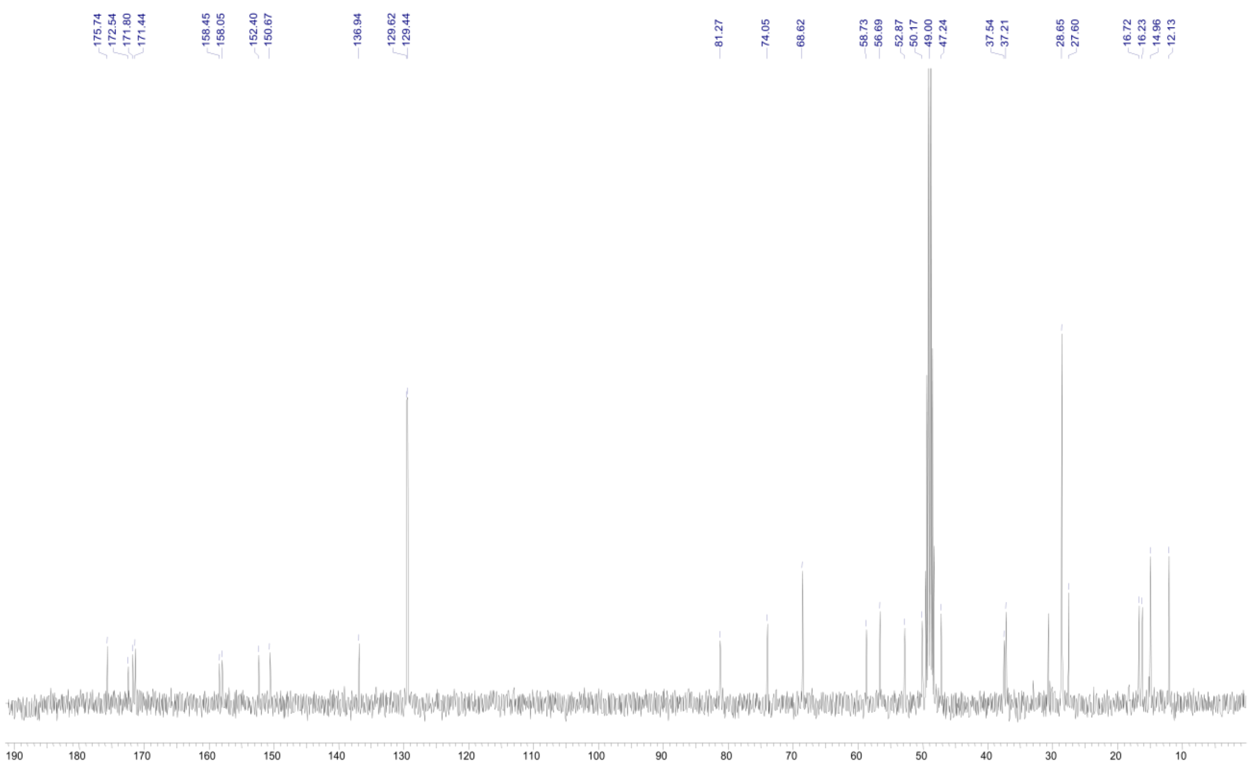
[1]H NMR of **33** (400 MHz, MeOH-d_4)



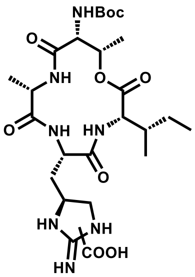
[13]C NMR of **33** (100 MHz, MeOH-d_4)



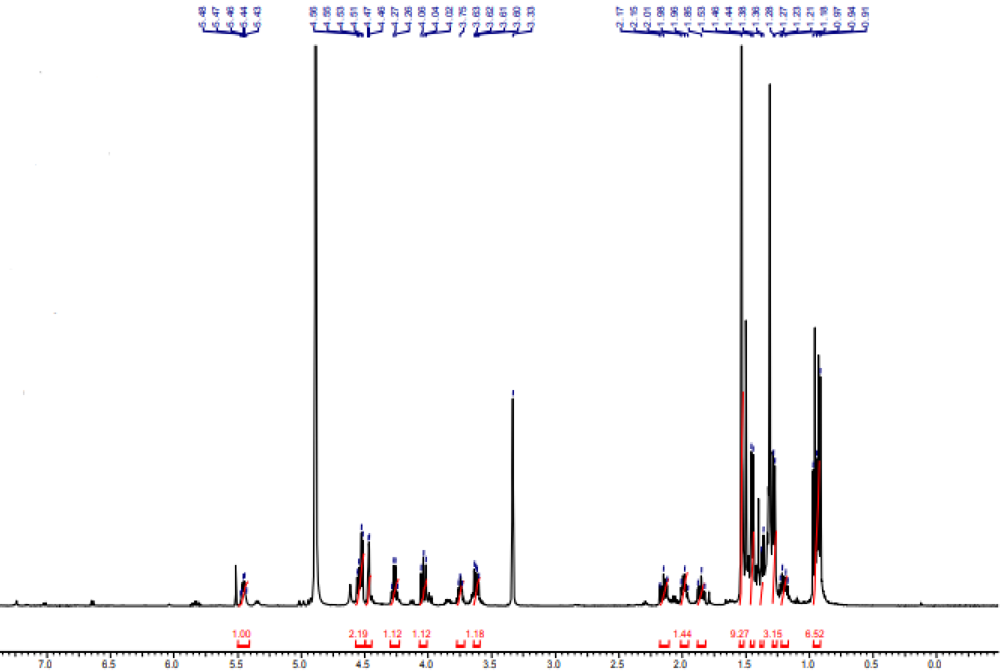
^{1}H NMR of **34** (400 MHz, MeOH-d_4)



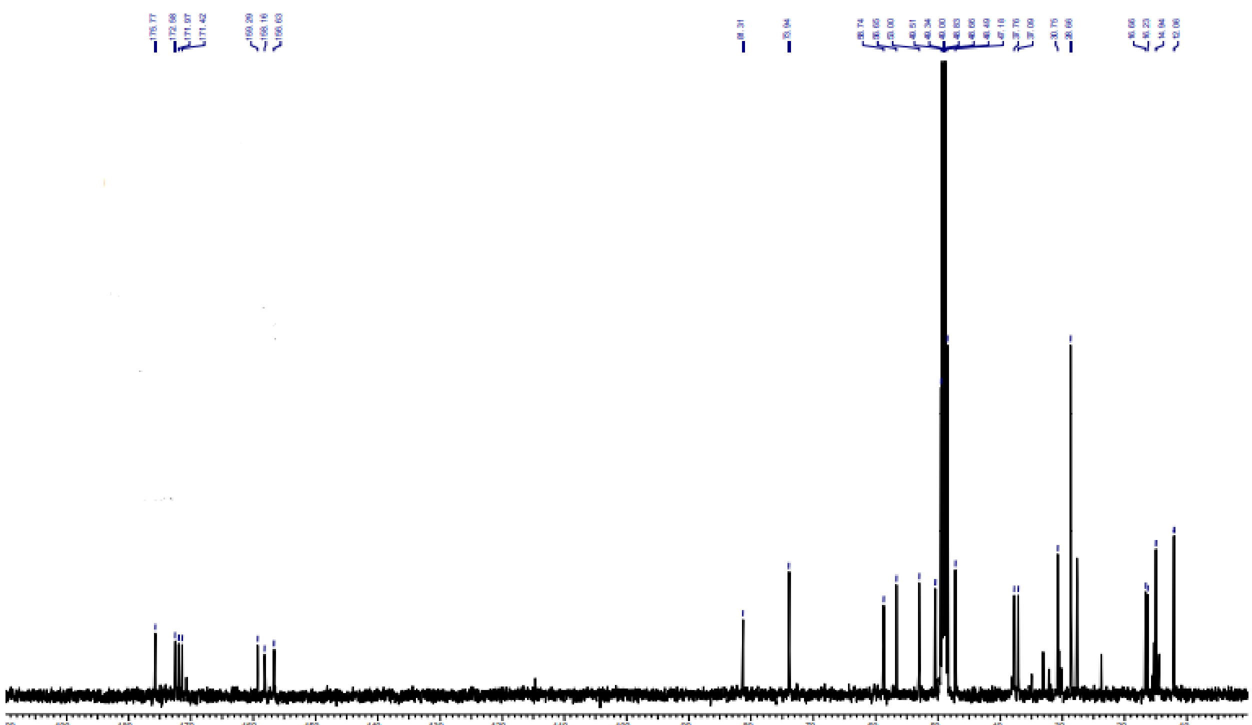
^{13}C NMR of **34** (100 MHz, MeOH-d_4)



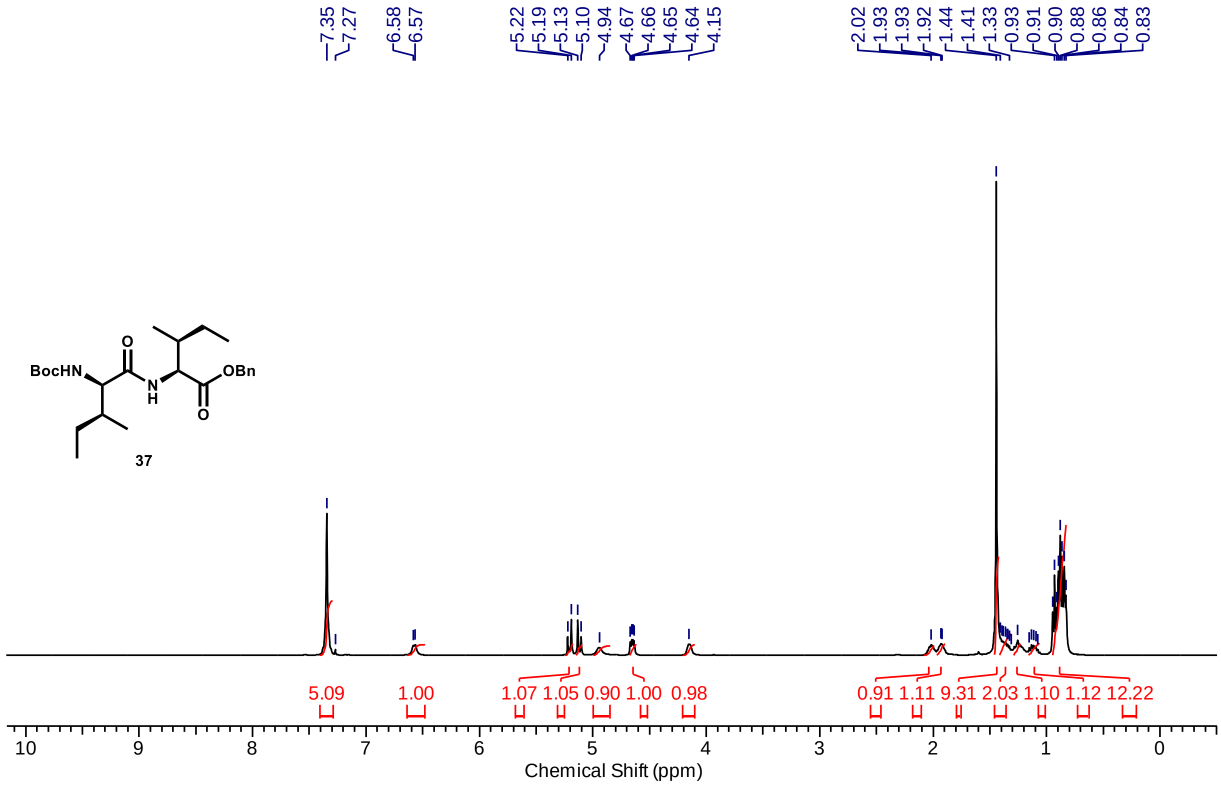
B (35)



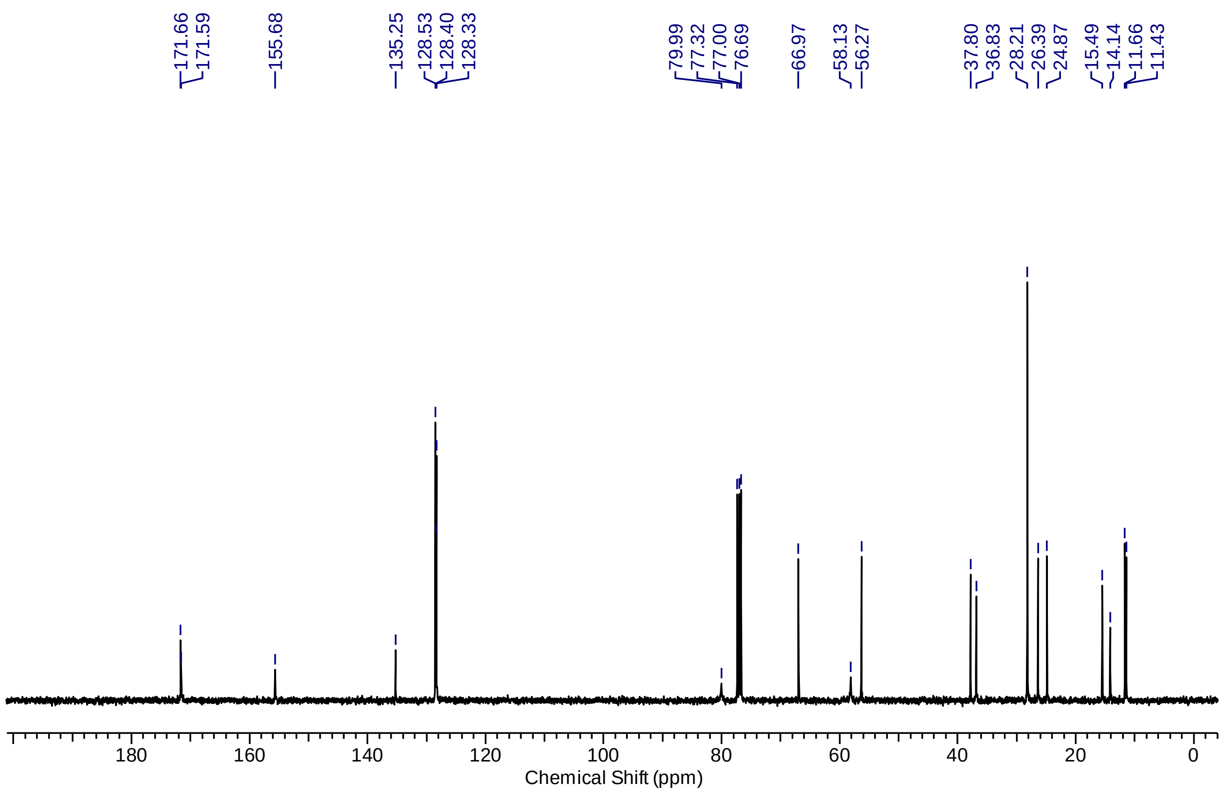
^{1}H NMR of **35** (500 MHz, MeOH-d_4)



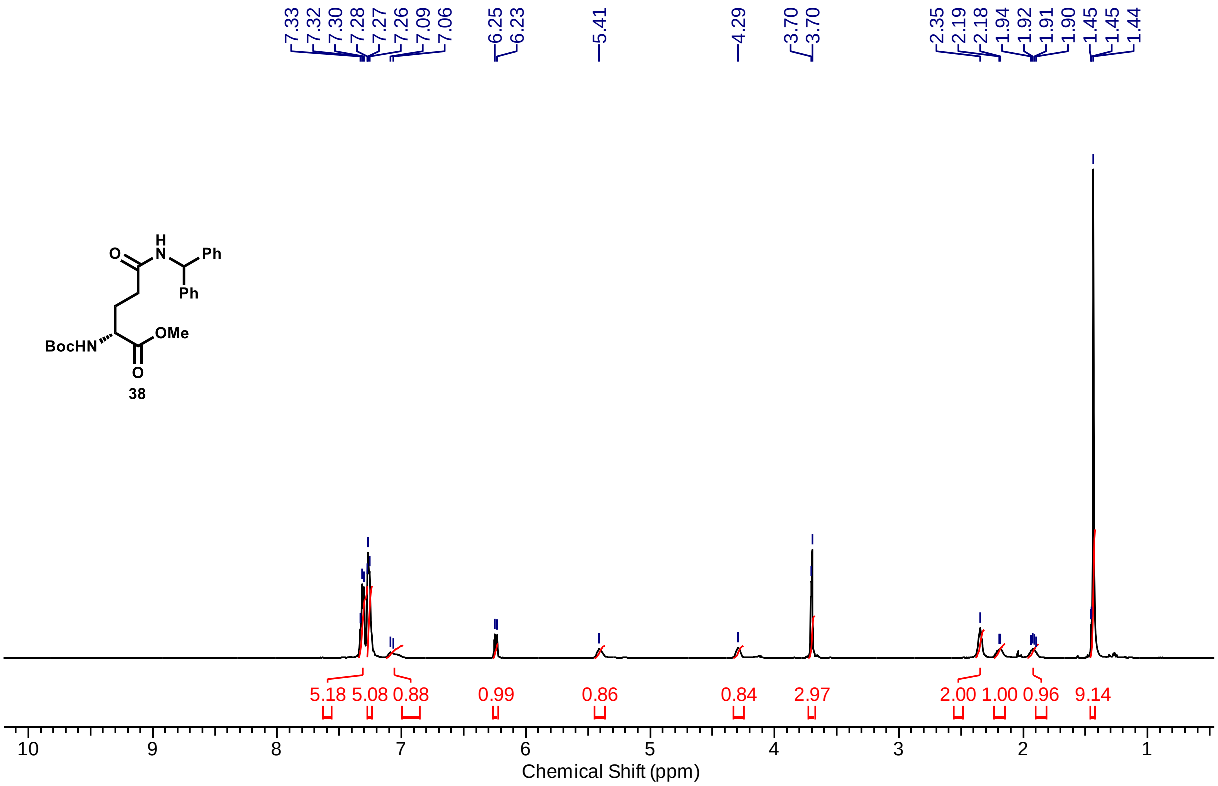
^{13}C NMR of **35** (125 MHz, MeOH-d_4)



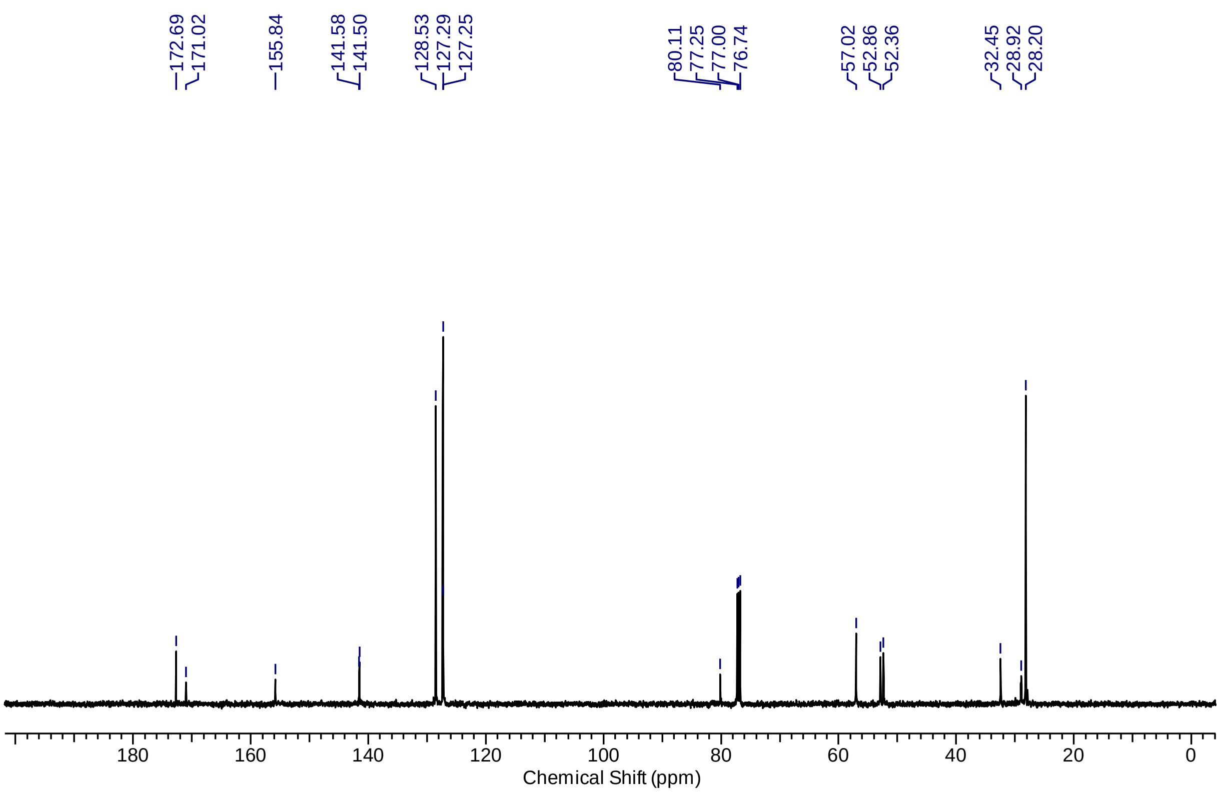
^{1}H NMR of **37** (400 MHz, $CDCl_3$)



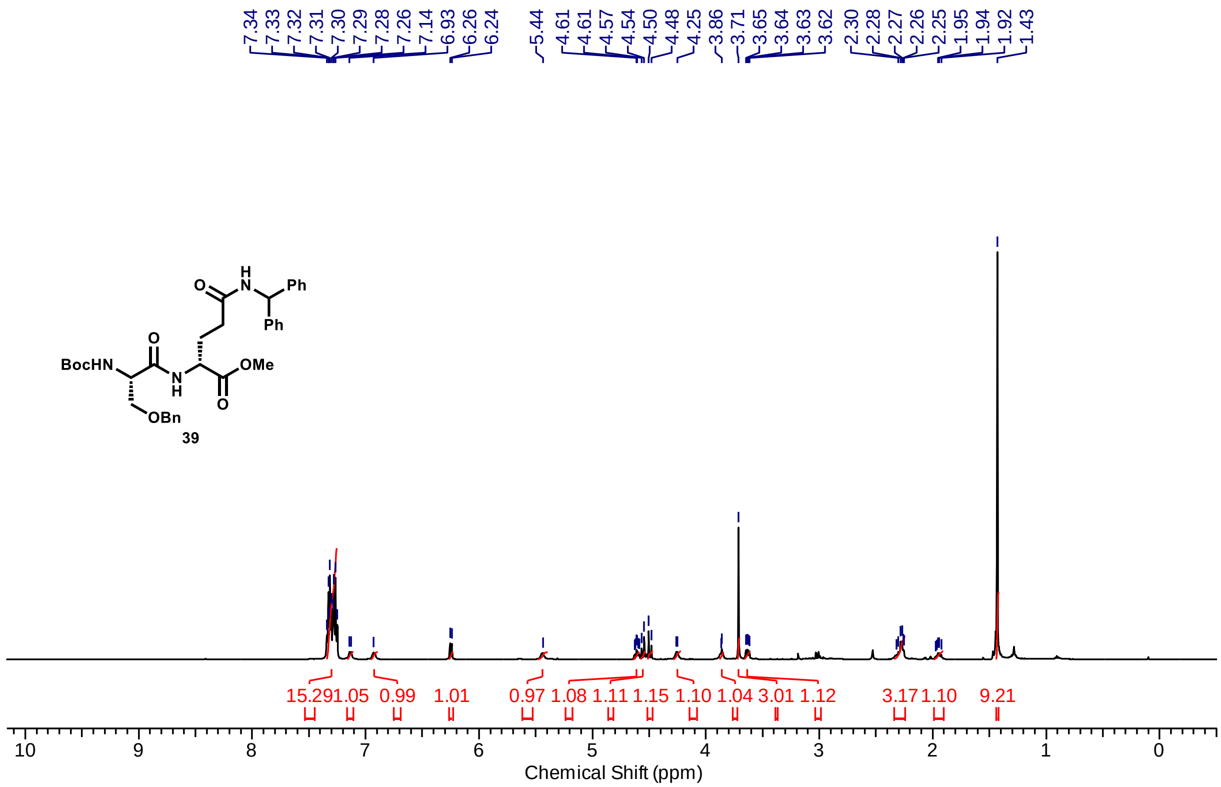
^{13}C NMR of **37** (100 MHz, $CDCl_3$)



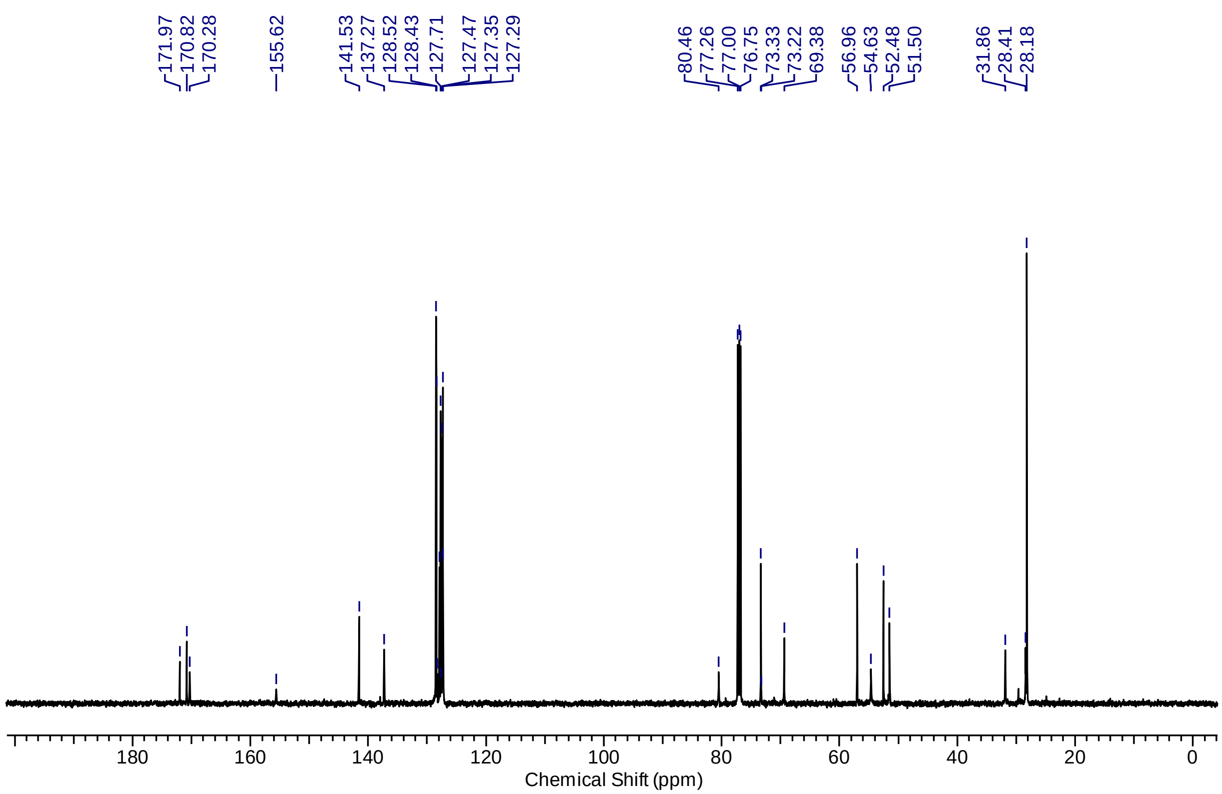
^{1}H NMR of **38** (500 MHz, $CDCl_3$)



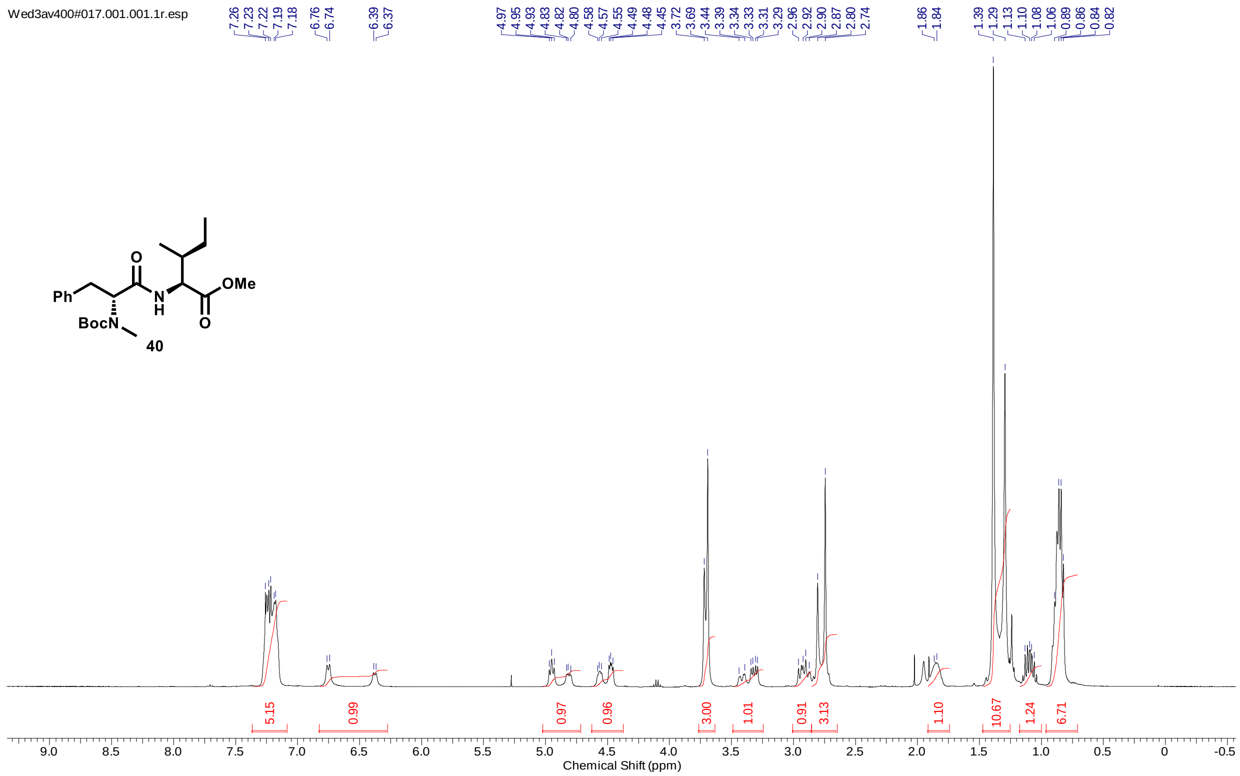
^{13}C NMR of **38** (125 MHz, $CDCl_3$)



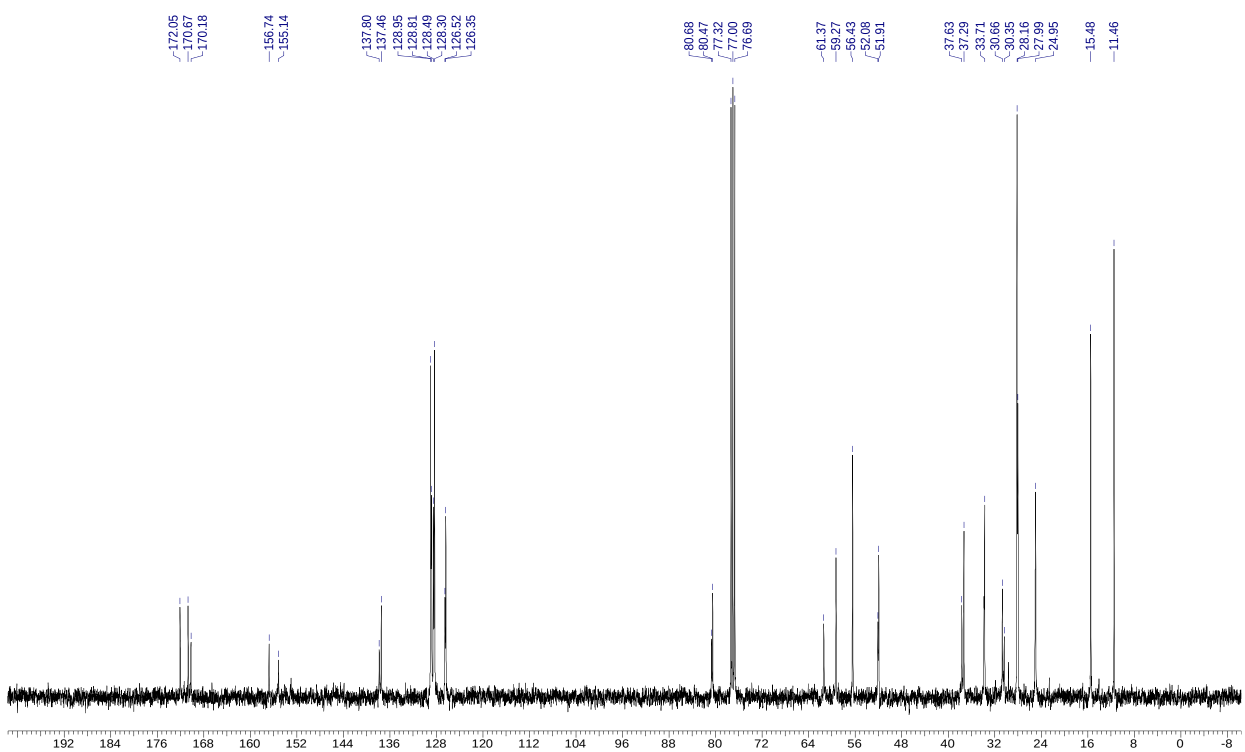
^{13}C NMR of **39** (500 MHz, $CDCl_3$)



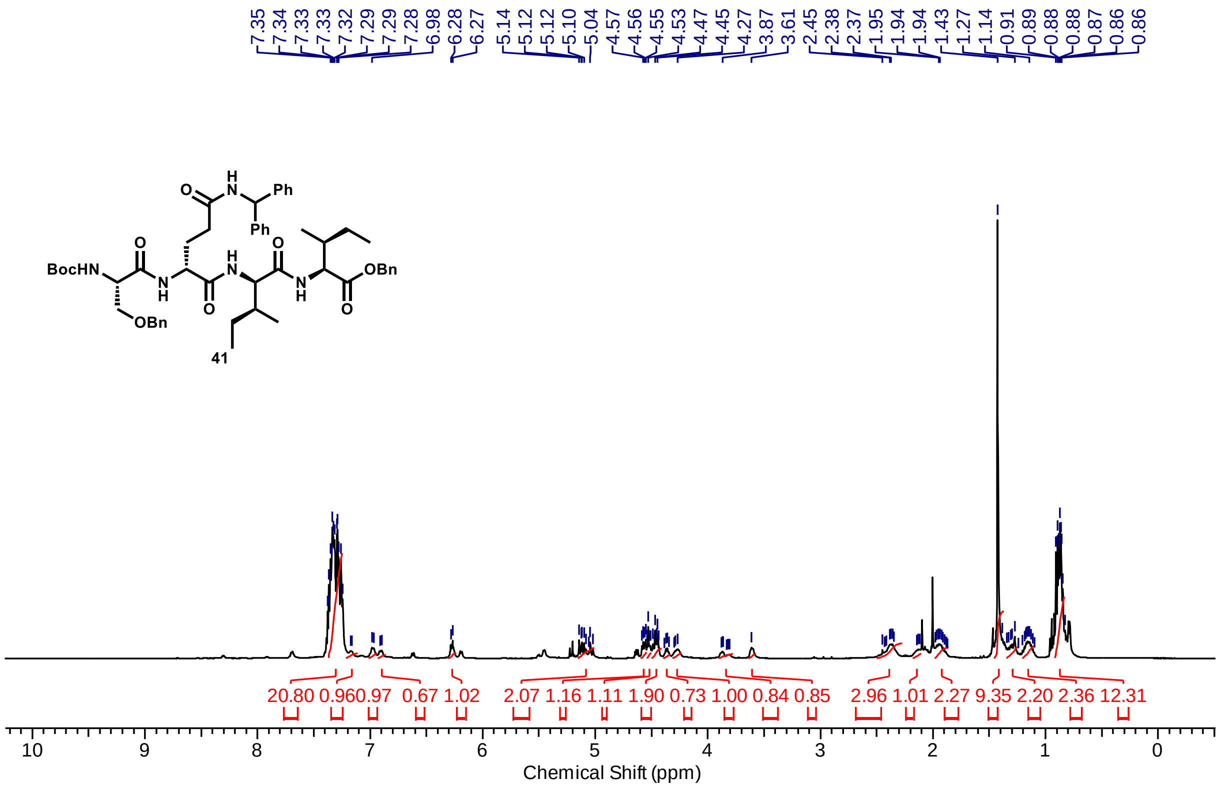
^{13}C NMR of **39** (125 MHz, $CDCl_3$)



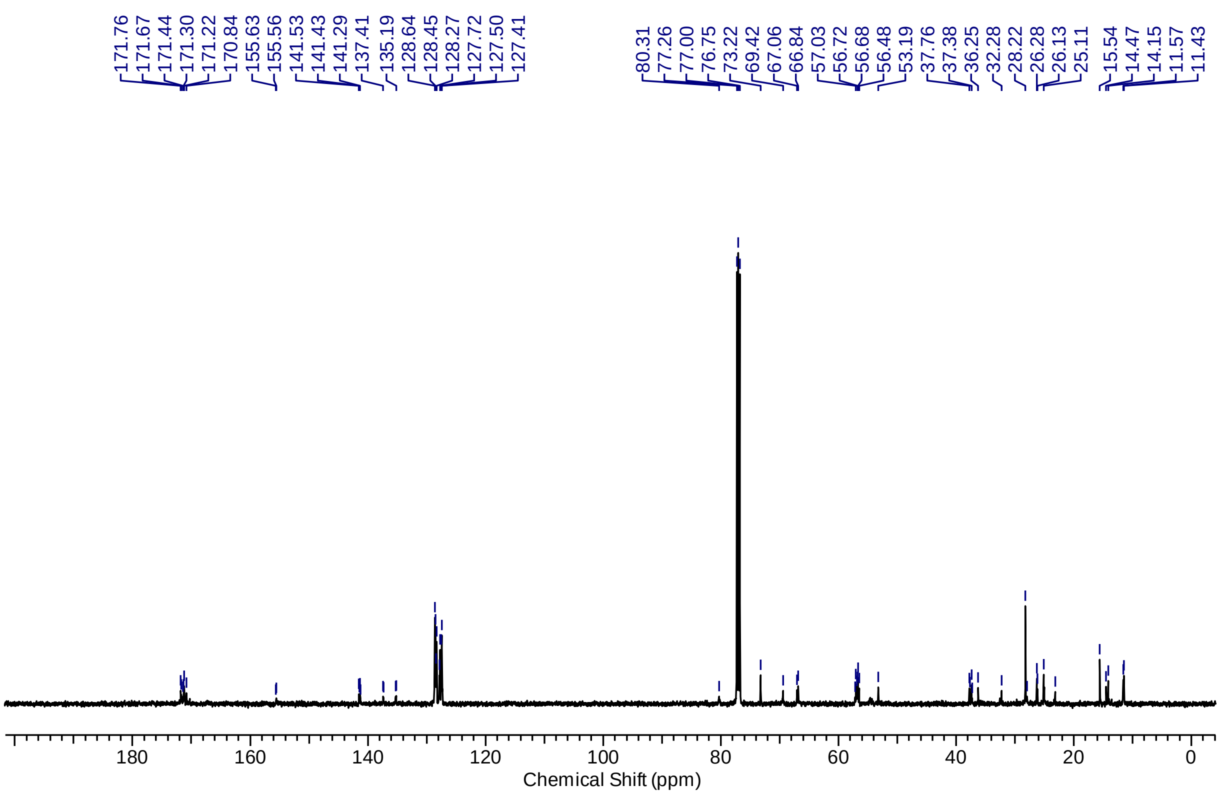
^{1}H NMR of **40** (400 MHz, CDCl$_3$)



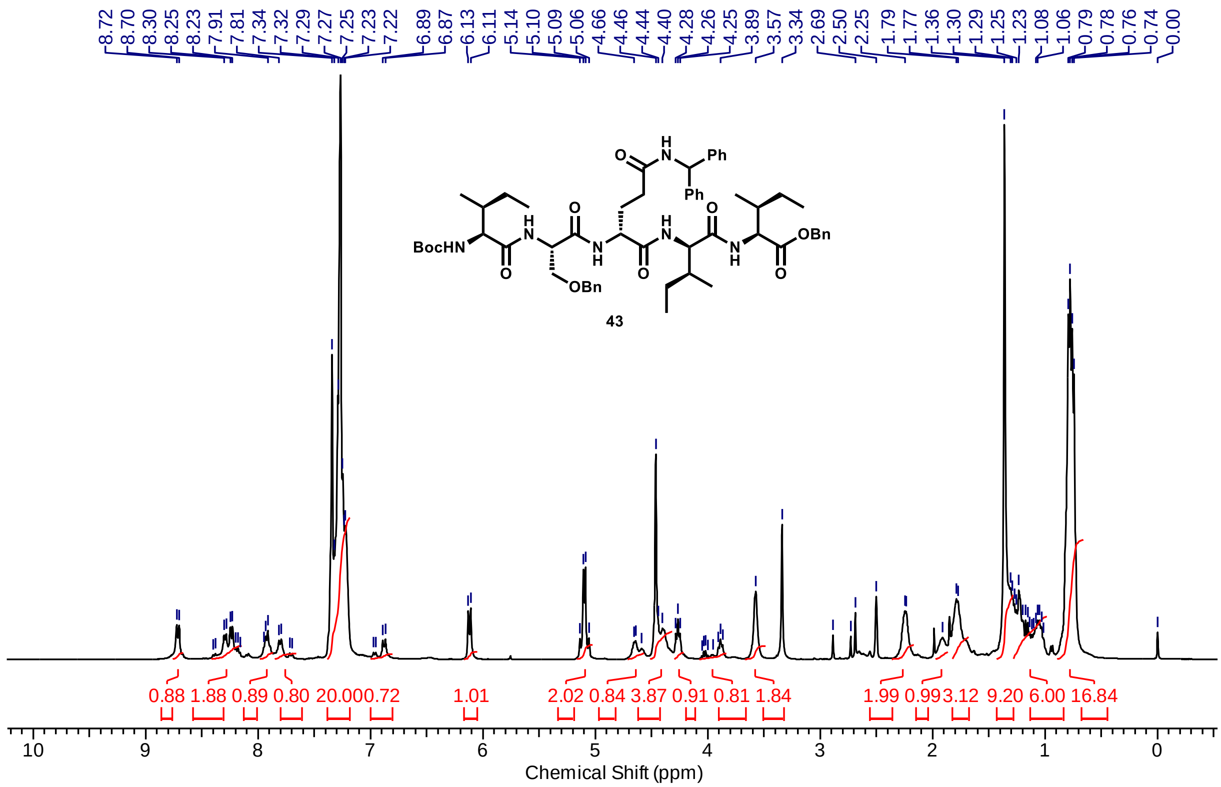
^{13}C NMR of **40** (100 MHz, CDCl$_3$)



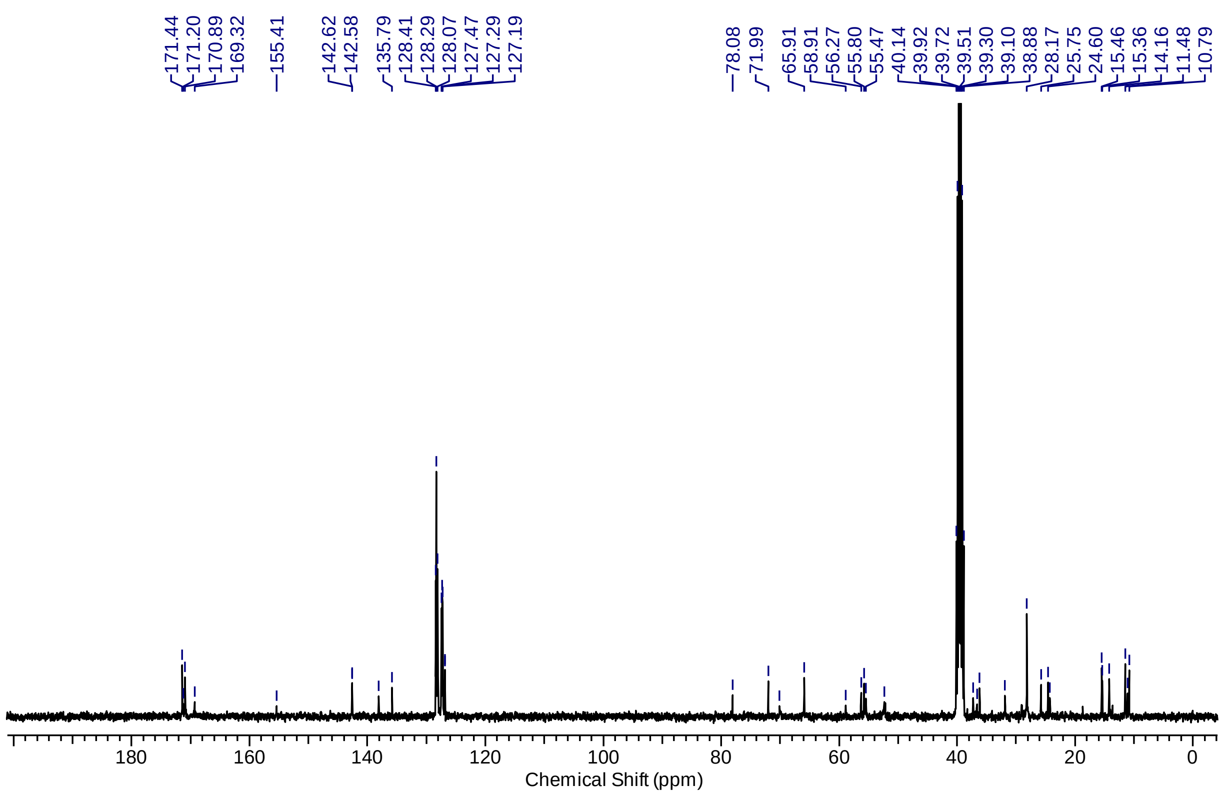
[1]H NMR of **41** (500 MHz, $CDCl_3$)



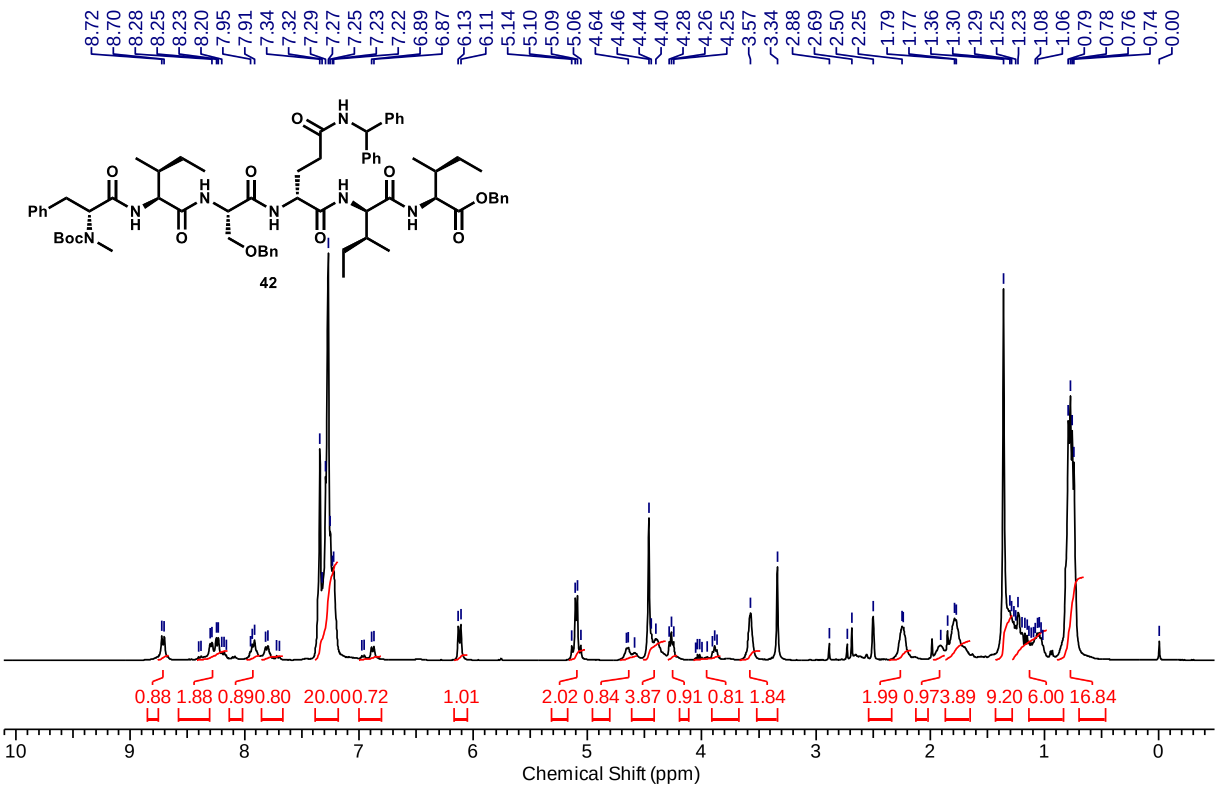
[13]C NMR of **41** (125 MHz, $CDCl_3$)



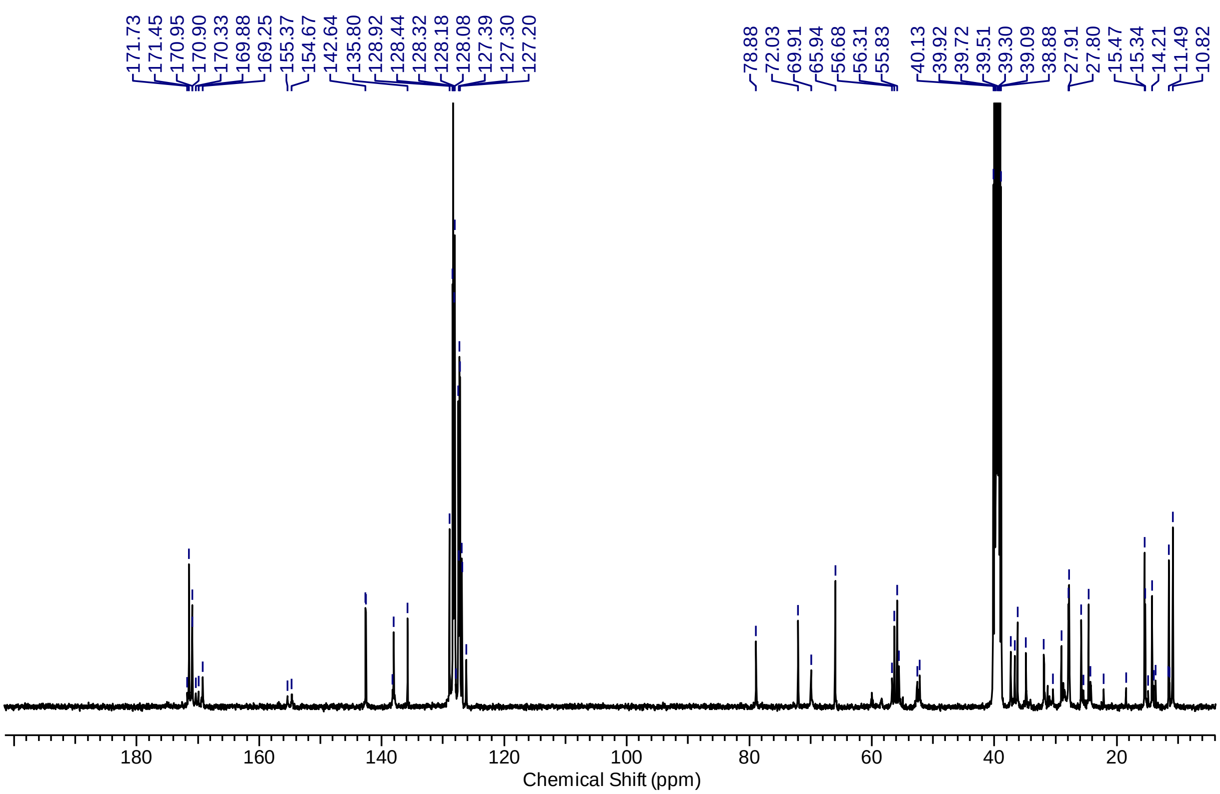
^{1}H NMR of **43** (400 MHz, DMSO-d_6)



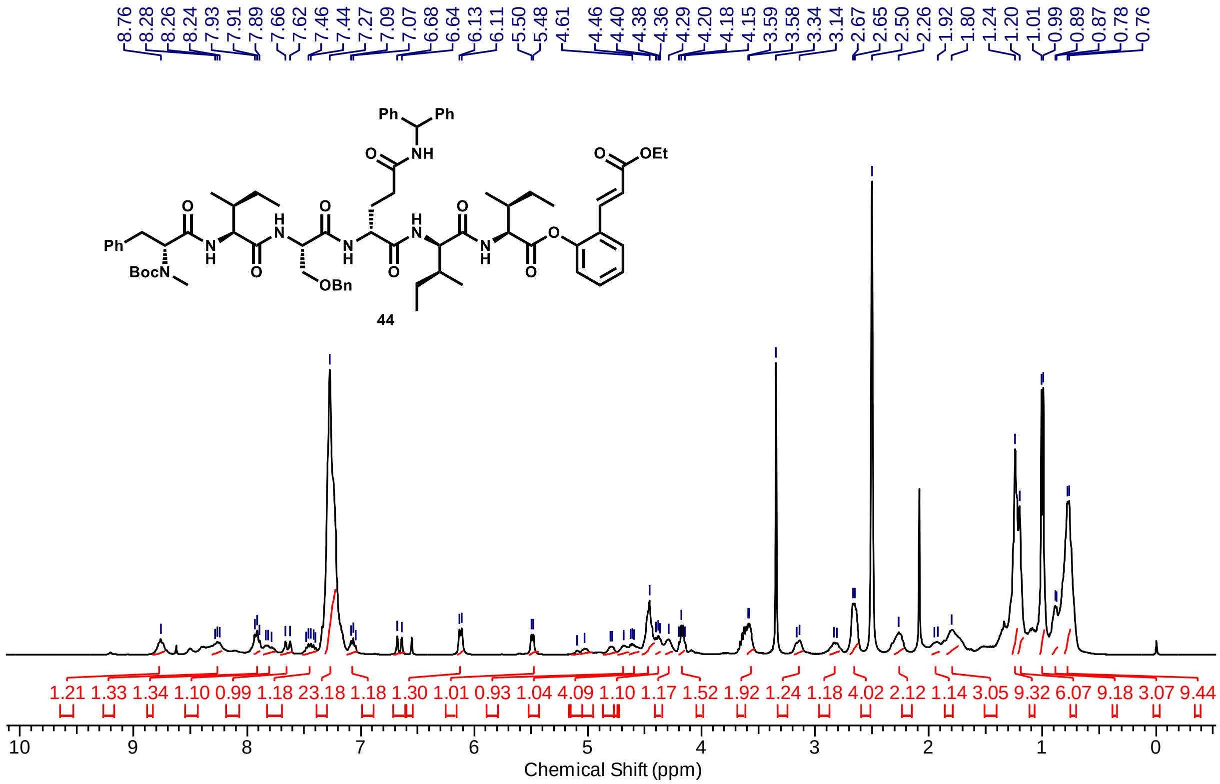
^{13}C NMR of **43** (100 MHz, DMSO-d_6)



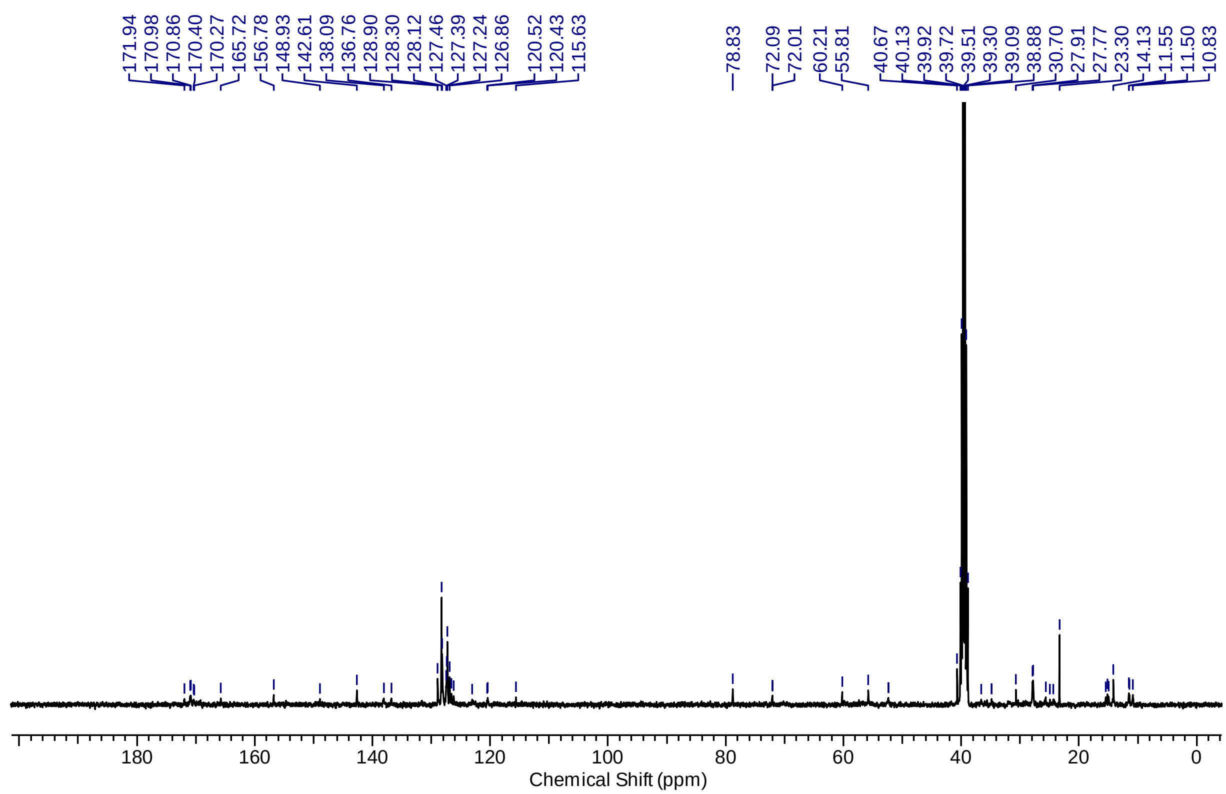
^{1}H NMR of **42** (400 MHz, DMSO-d_6)



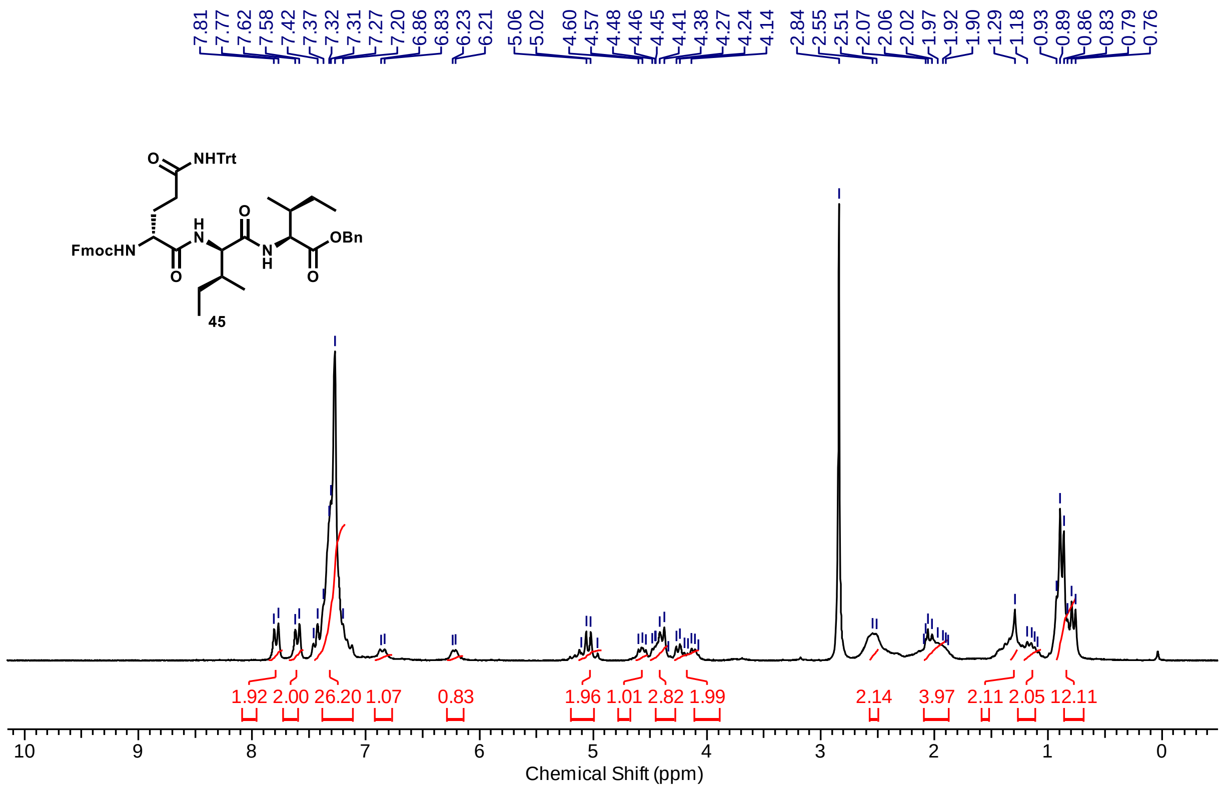
^{13}C NMR of **42** (100 MHz, DMSO-d_6)



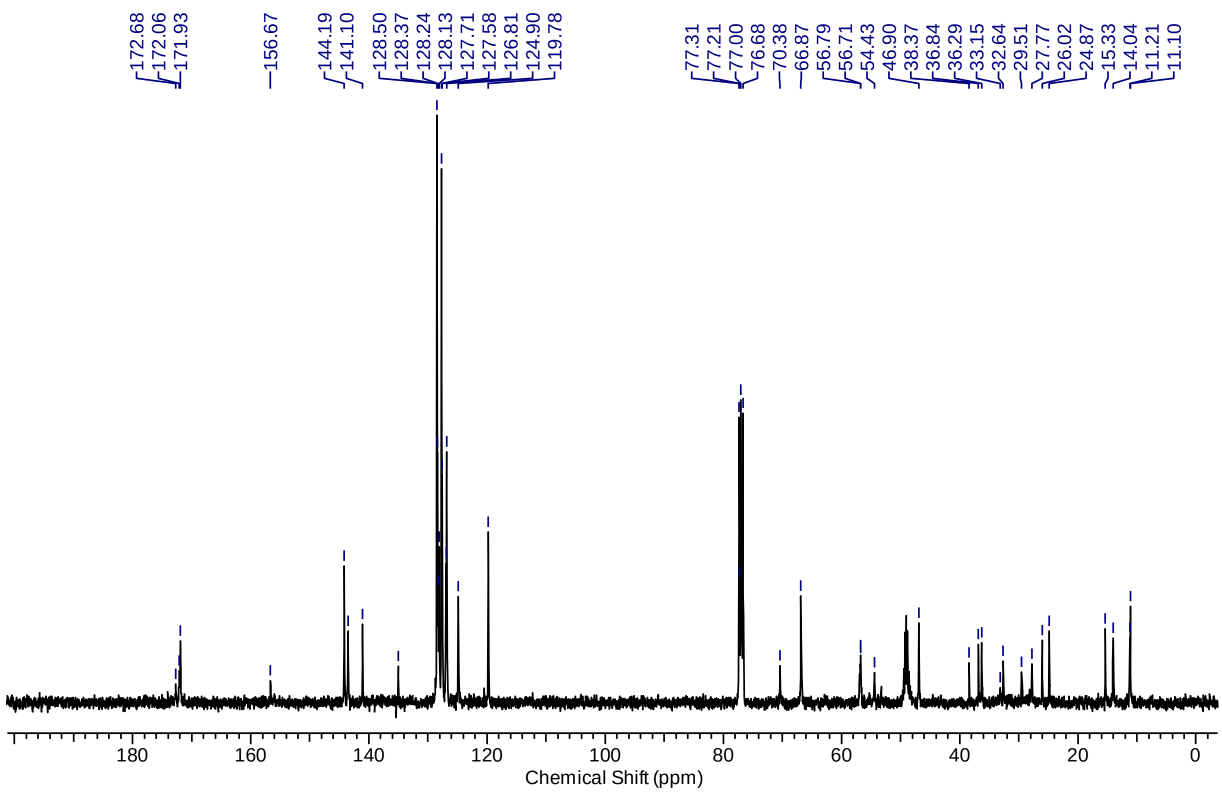
[1]H NMR of **44** (400 MHz, DMSO-d_6)



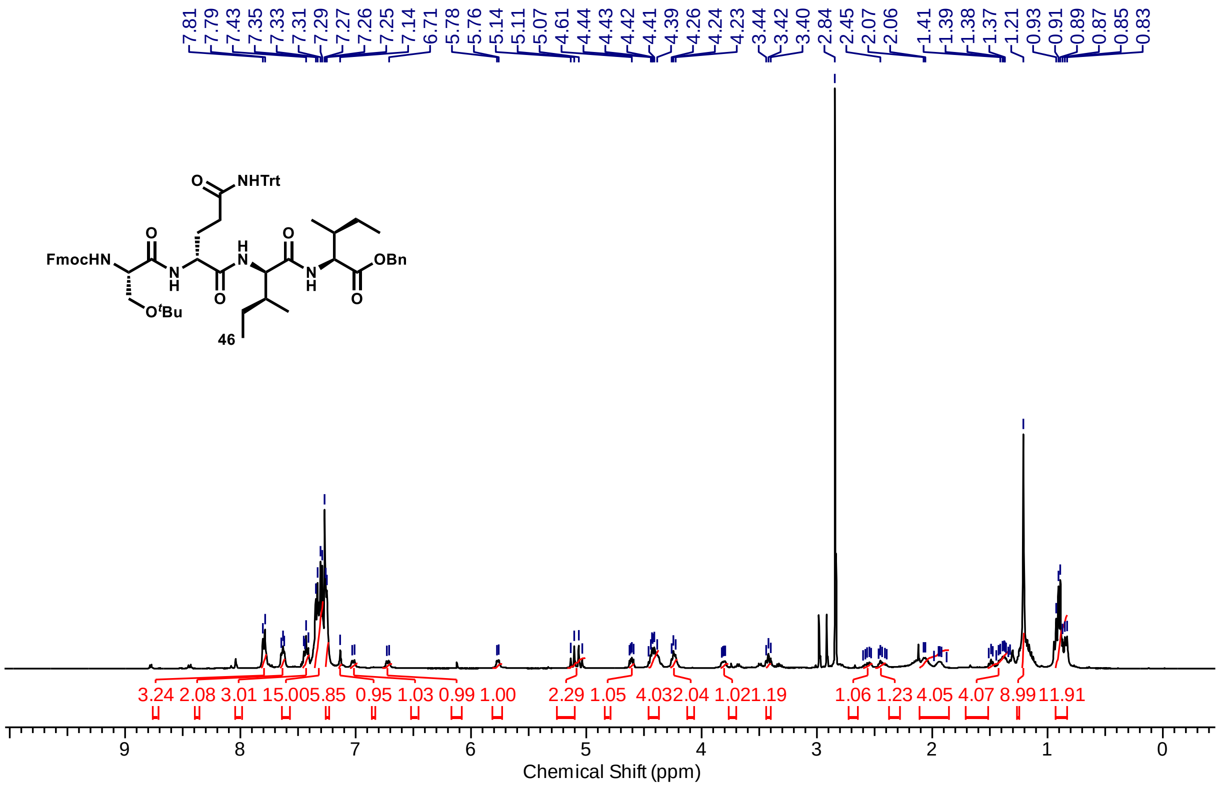
[13]C NMR of **44** (100 MHz, DMSO-d_6)



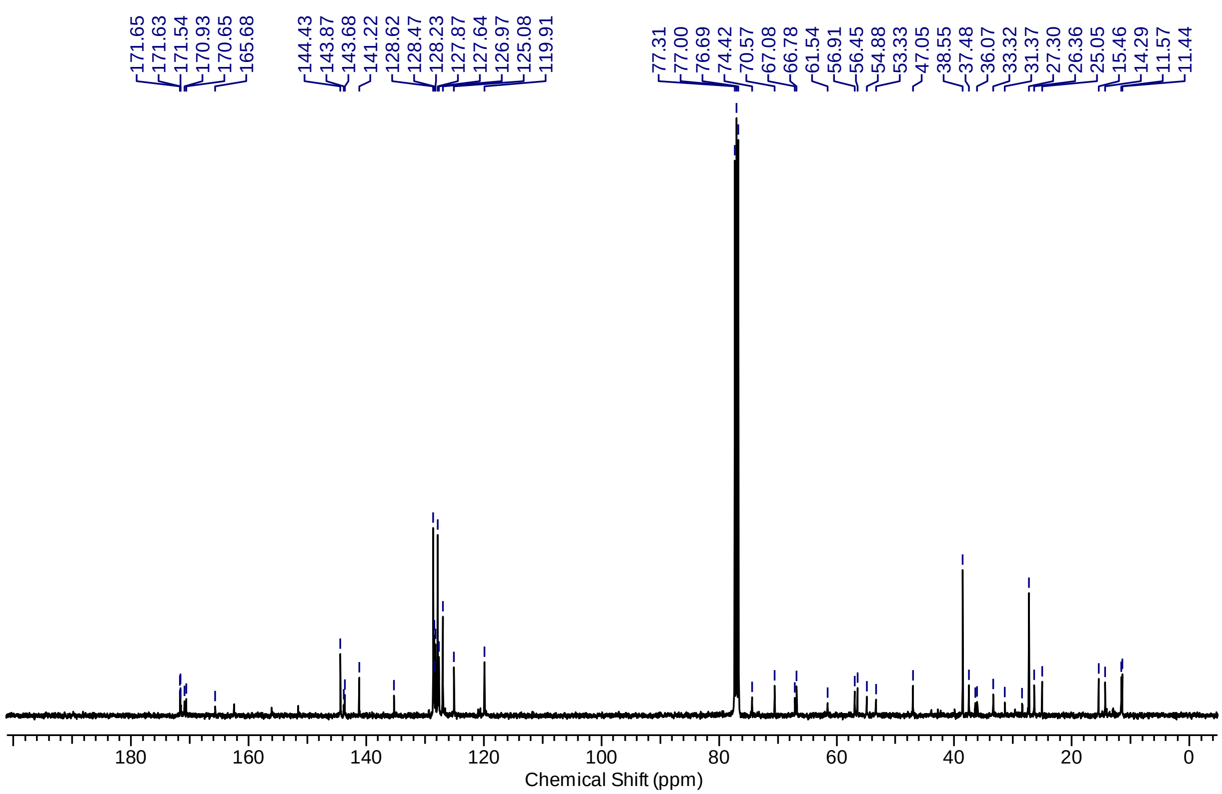
^{1}H NMR of **45** (400 MHz, $CDCl_3$)



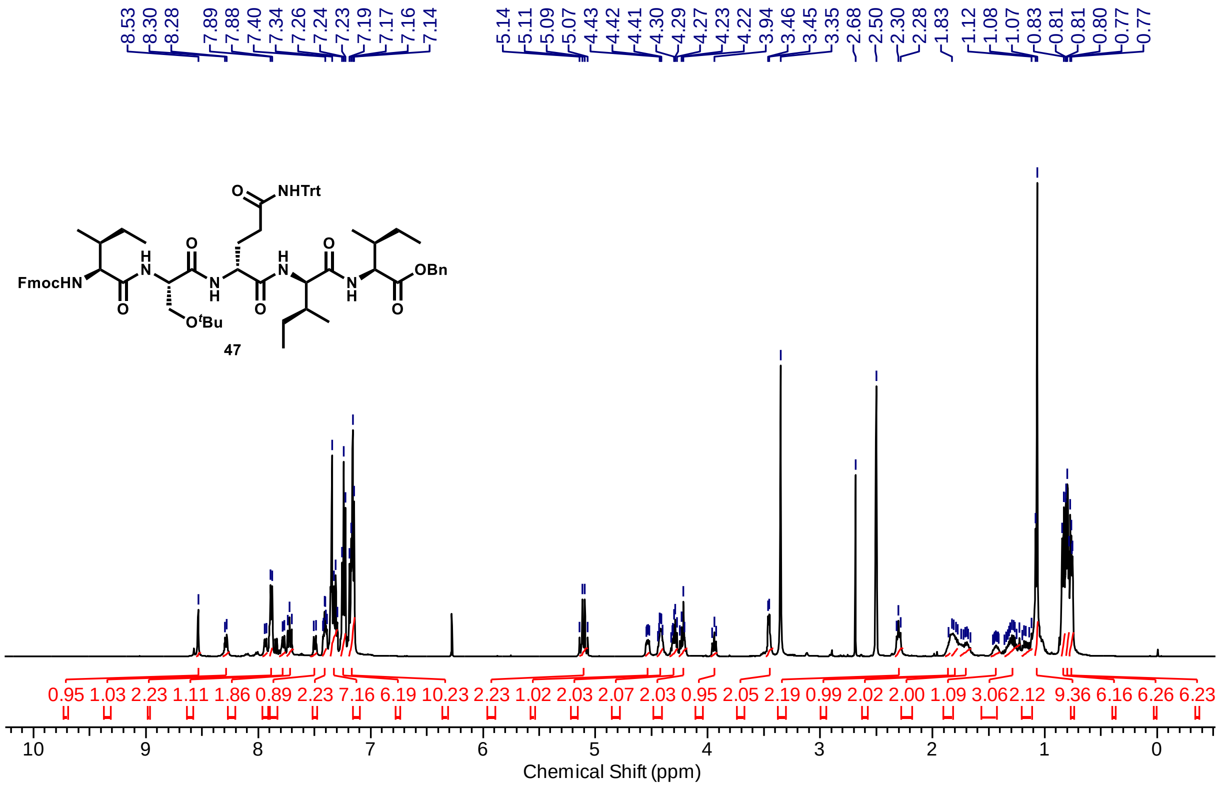
^{13}C NMR of **45** (100 MHz, $CDCl_3$)



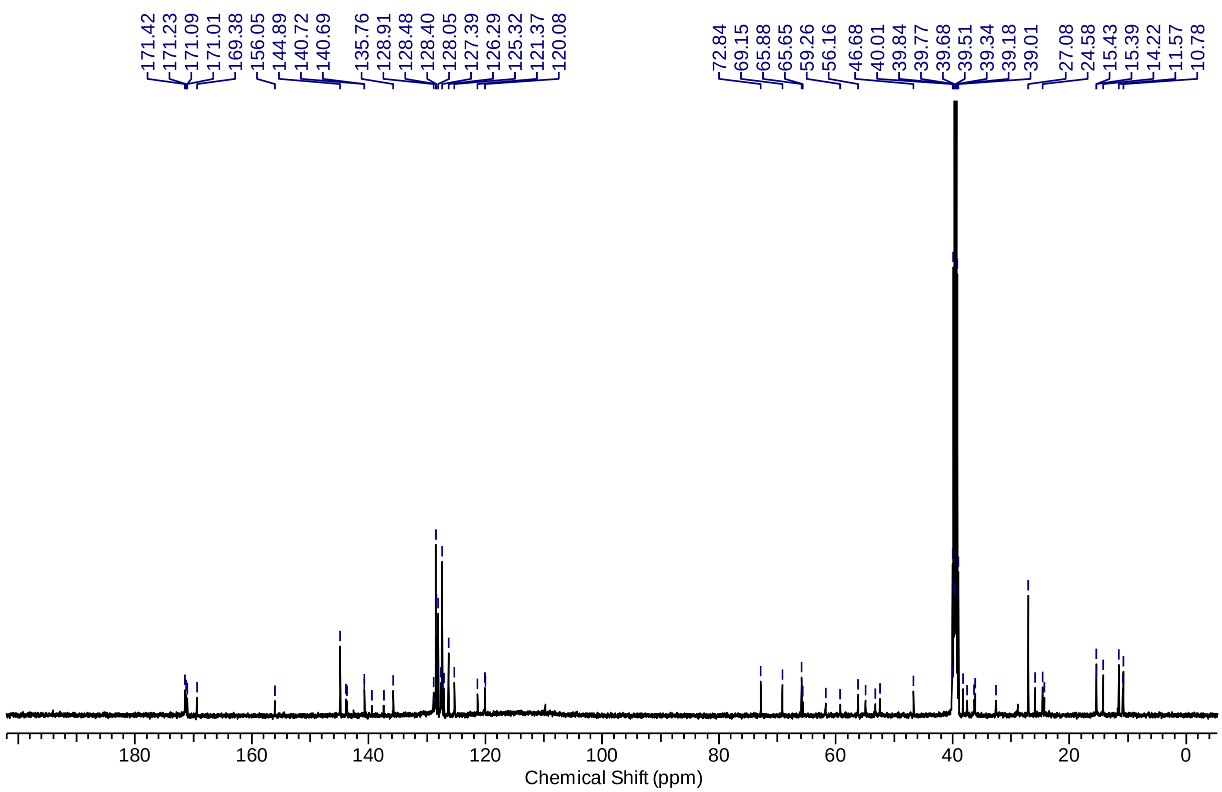
^{1}H NMR of **46** (400 MHz, CDCl$_3$)



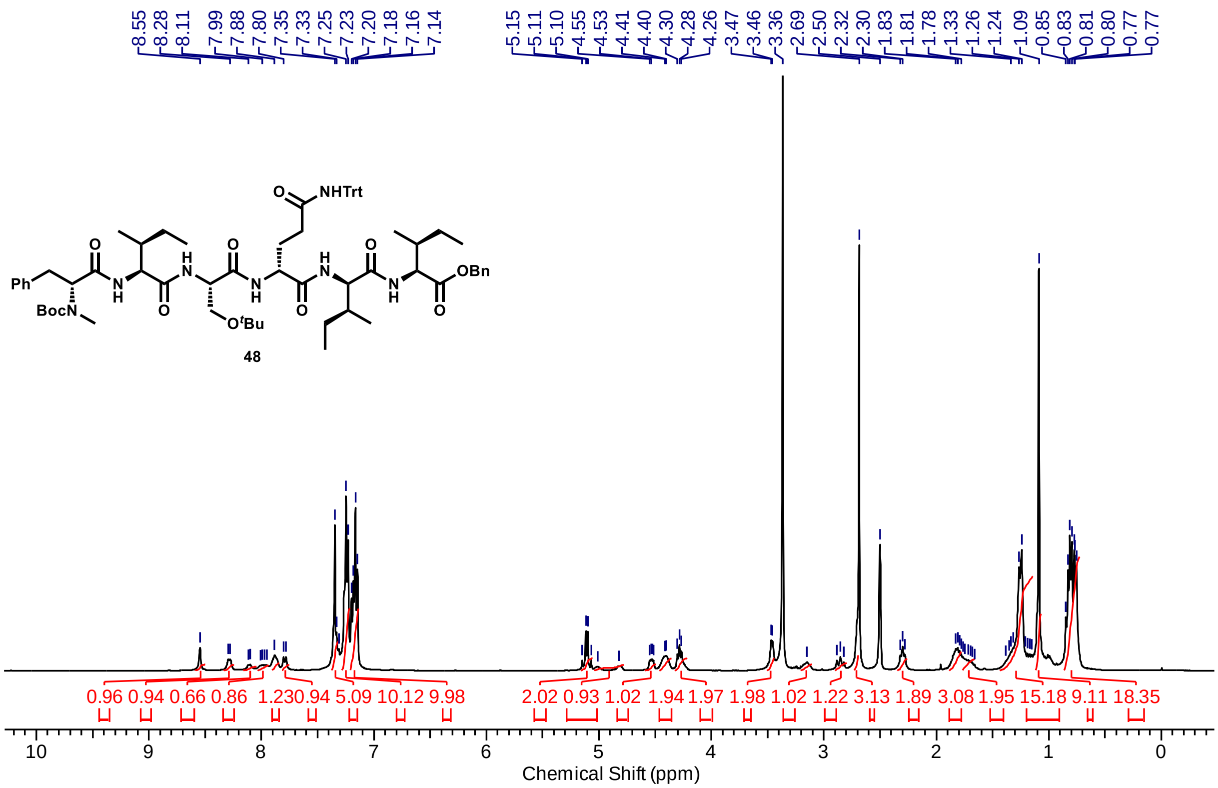
^{13}C NMR of **46** (100 MHz, CDCl$_3$)



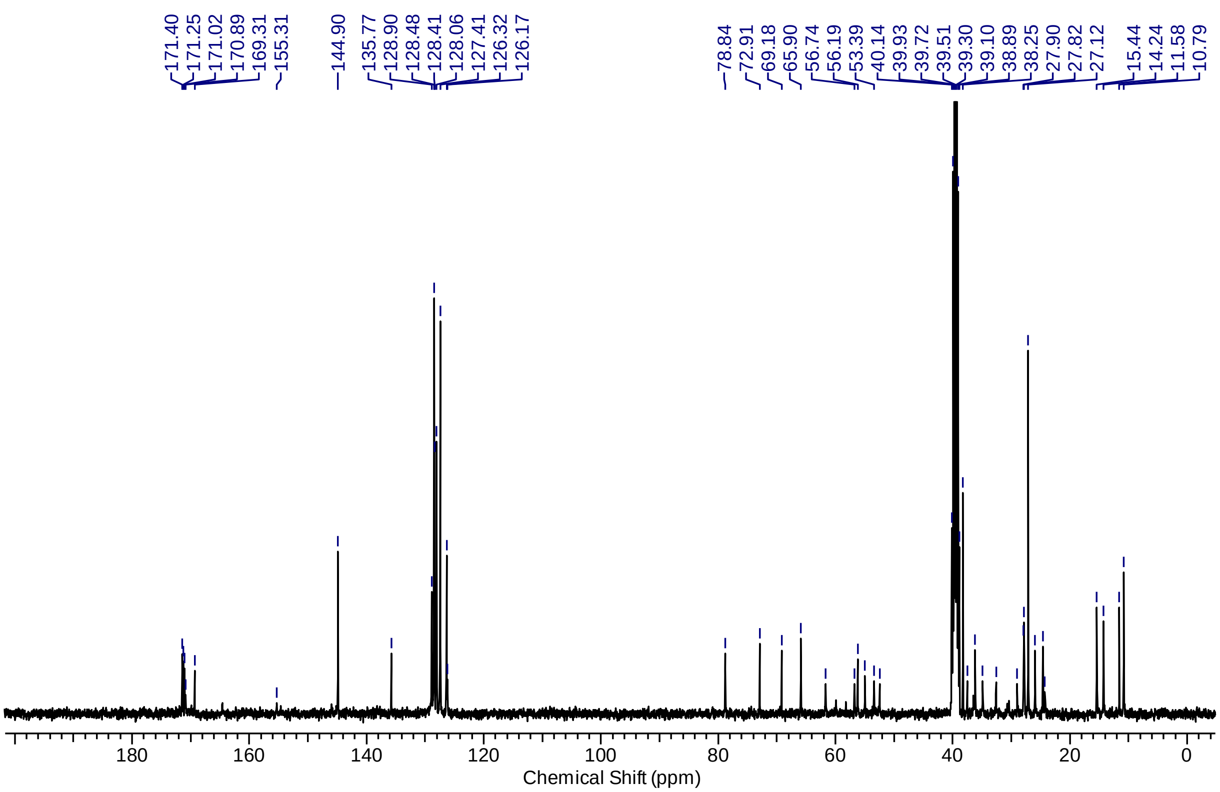
^{1}H NMR of **47** (400 MHz, DMSO-d_6)



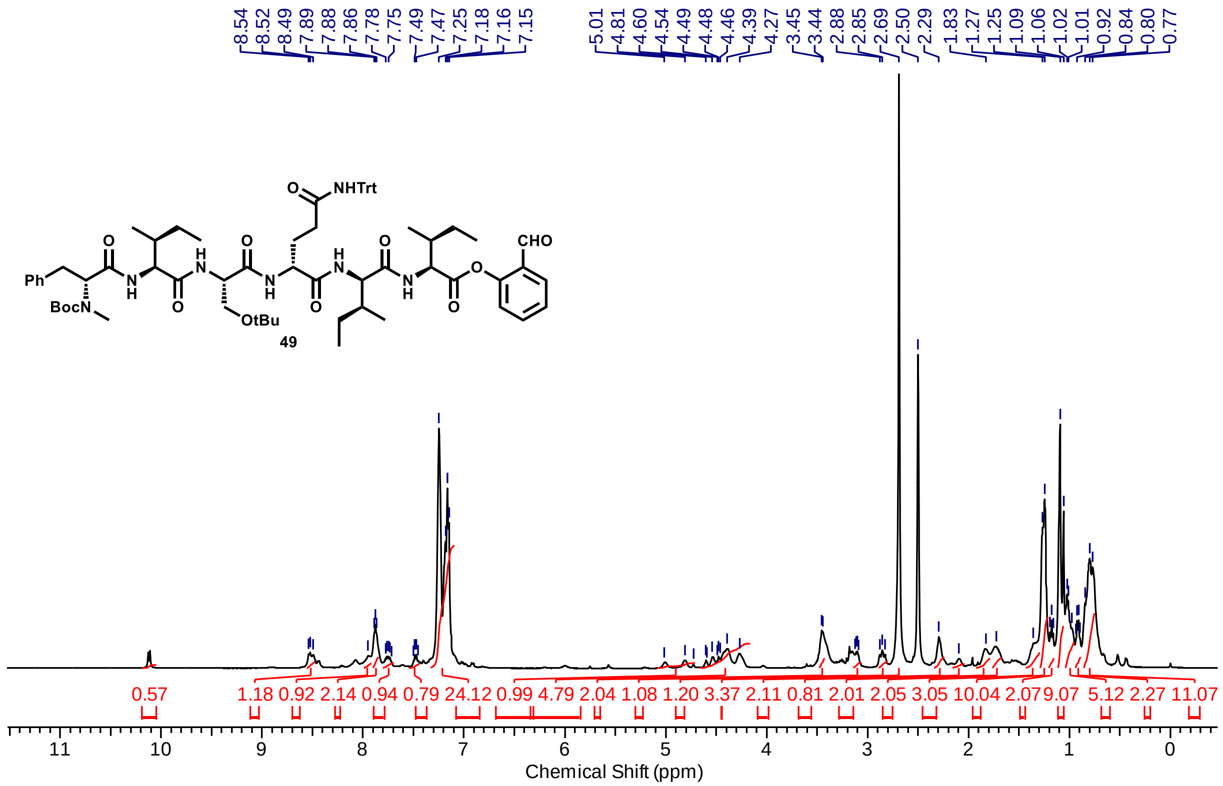
^{13}C NMR of **47** (100 MHz, DMSO-d_6)



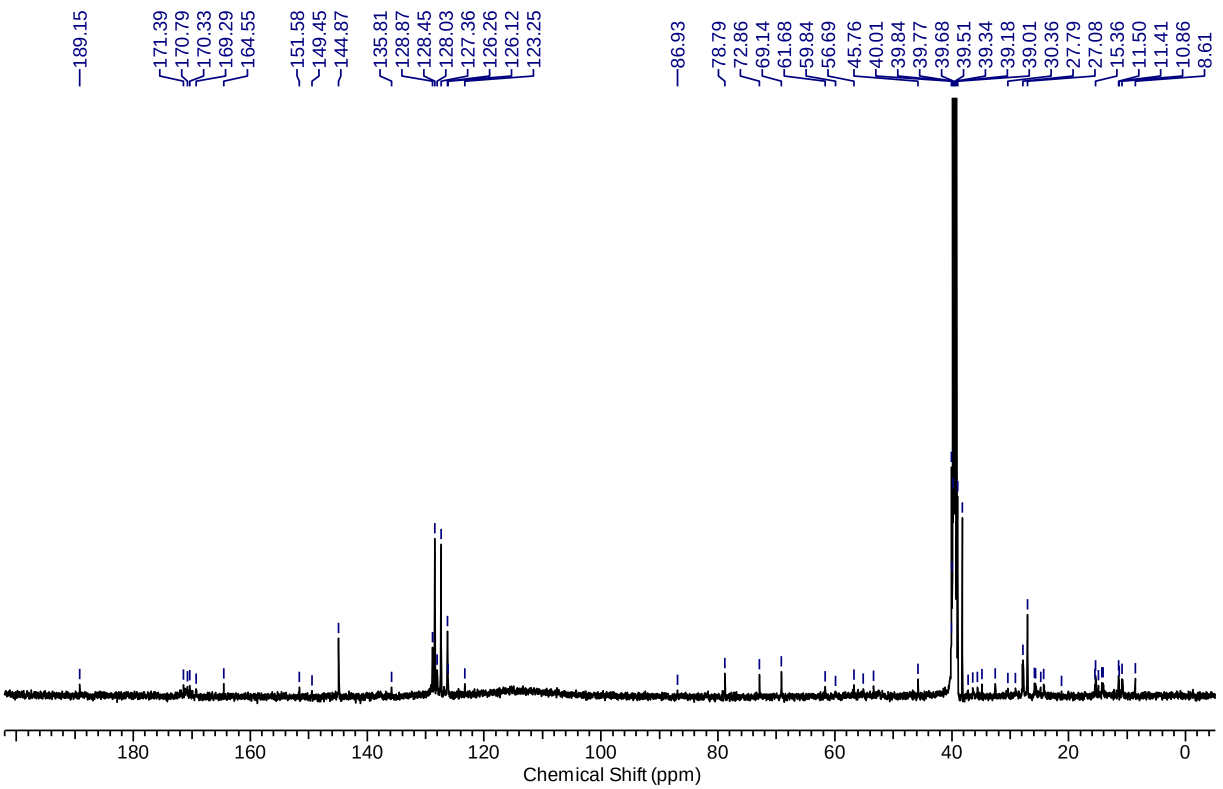
^{1}H NMR of **48** (400 MHz, DMSO-d_6)



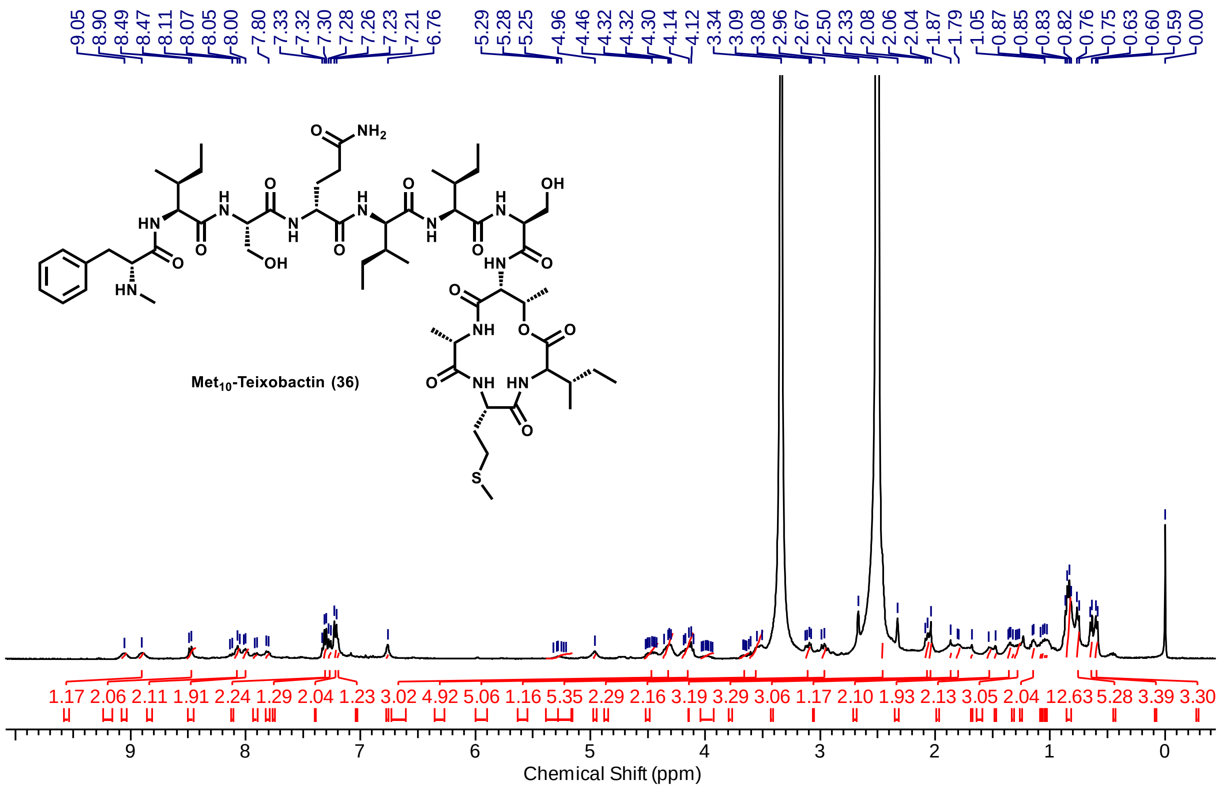
^{13}C NMR of **48** (100 MHz, DMSO-d_6)



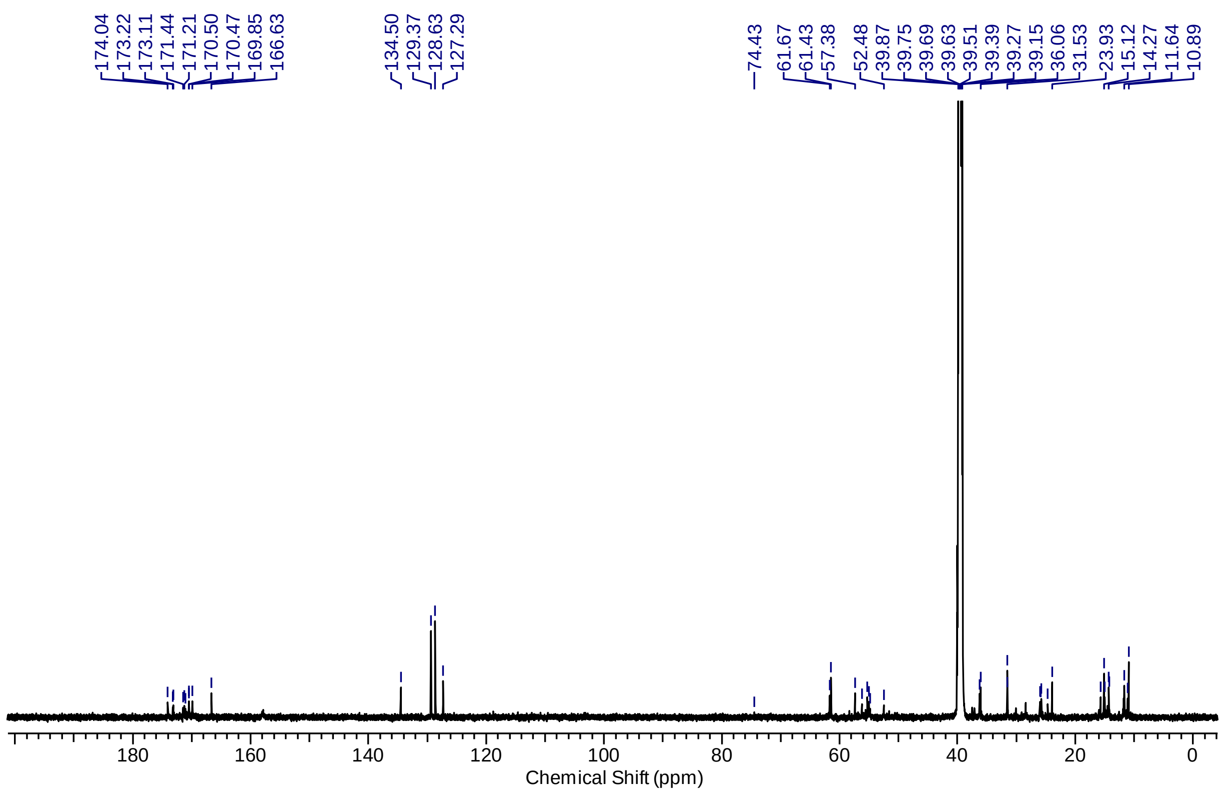
^{1}H NMR of **49** (500 MHz, DMSO-d_6)



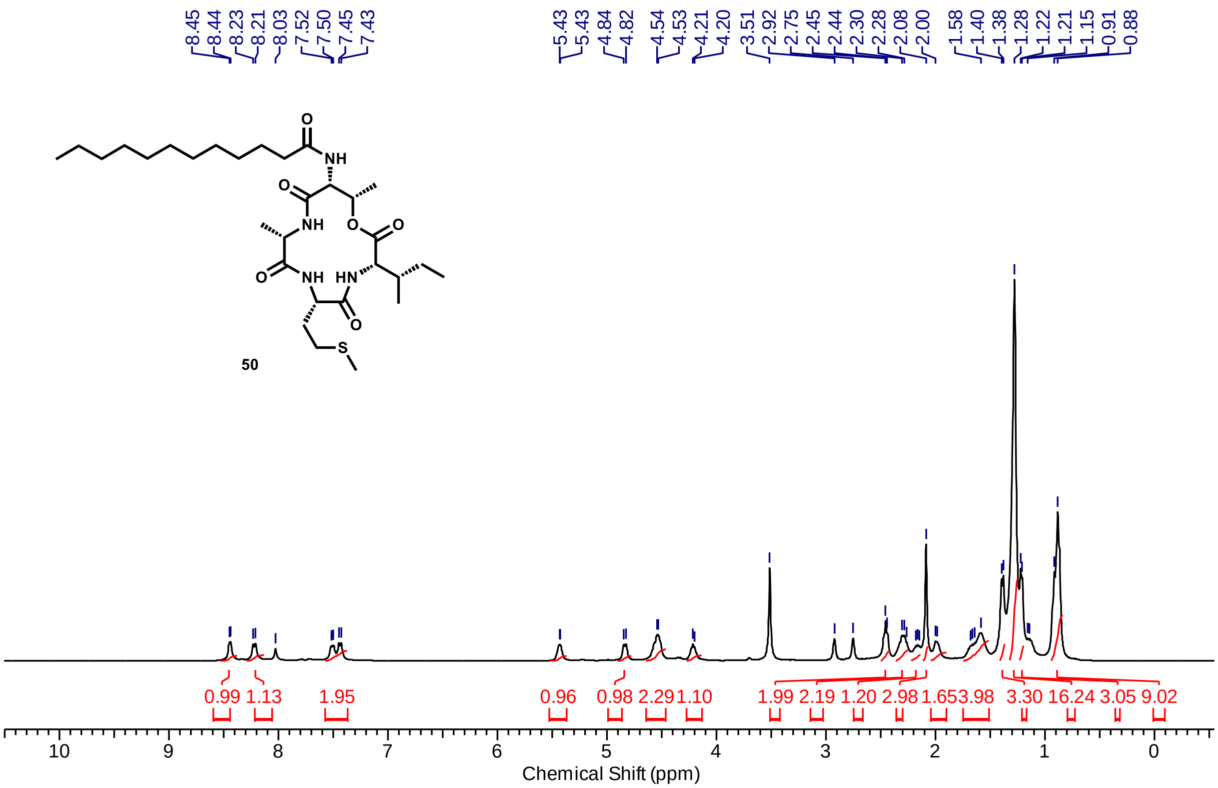
^{13}C NMR of **49** (125 MHz, DMSO-d_6)



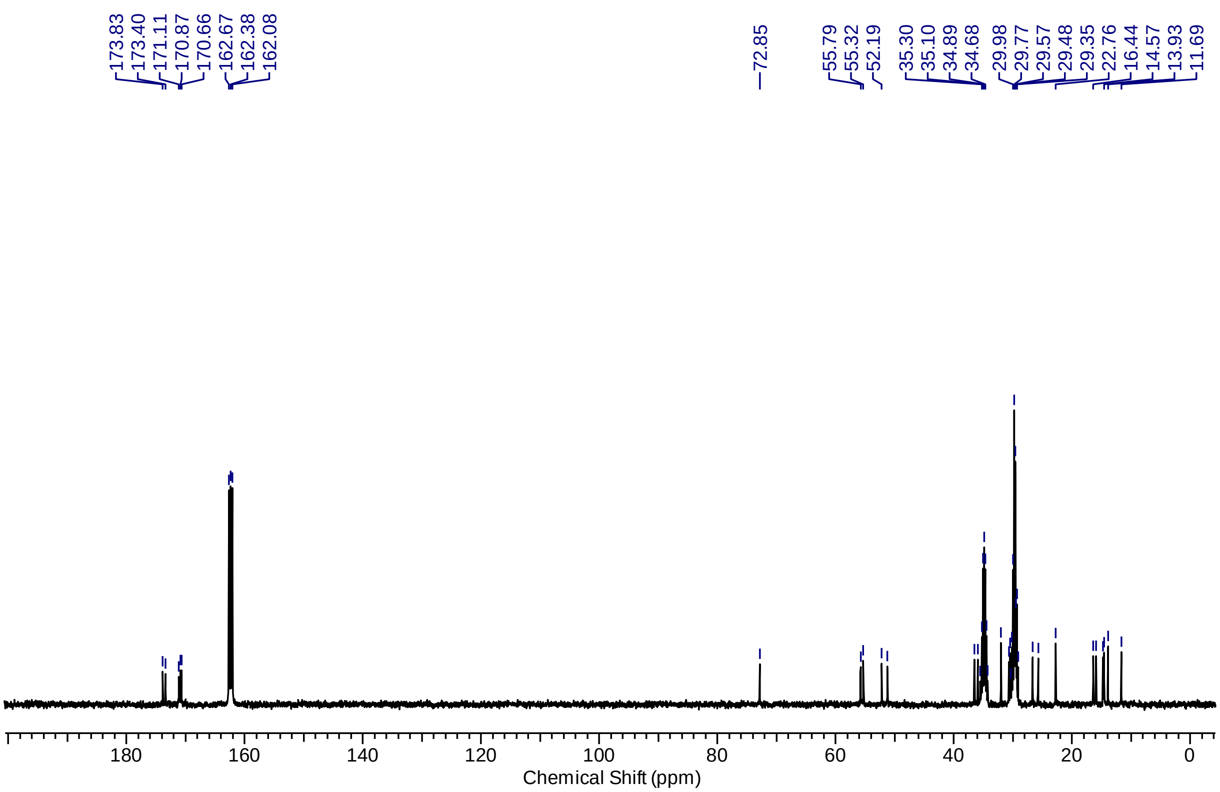
^{1}H NMR of **36** (700 MHz, DMSO-d_6)



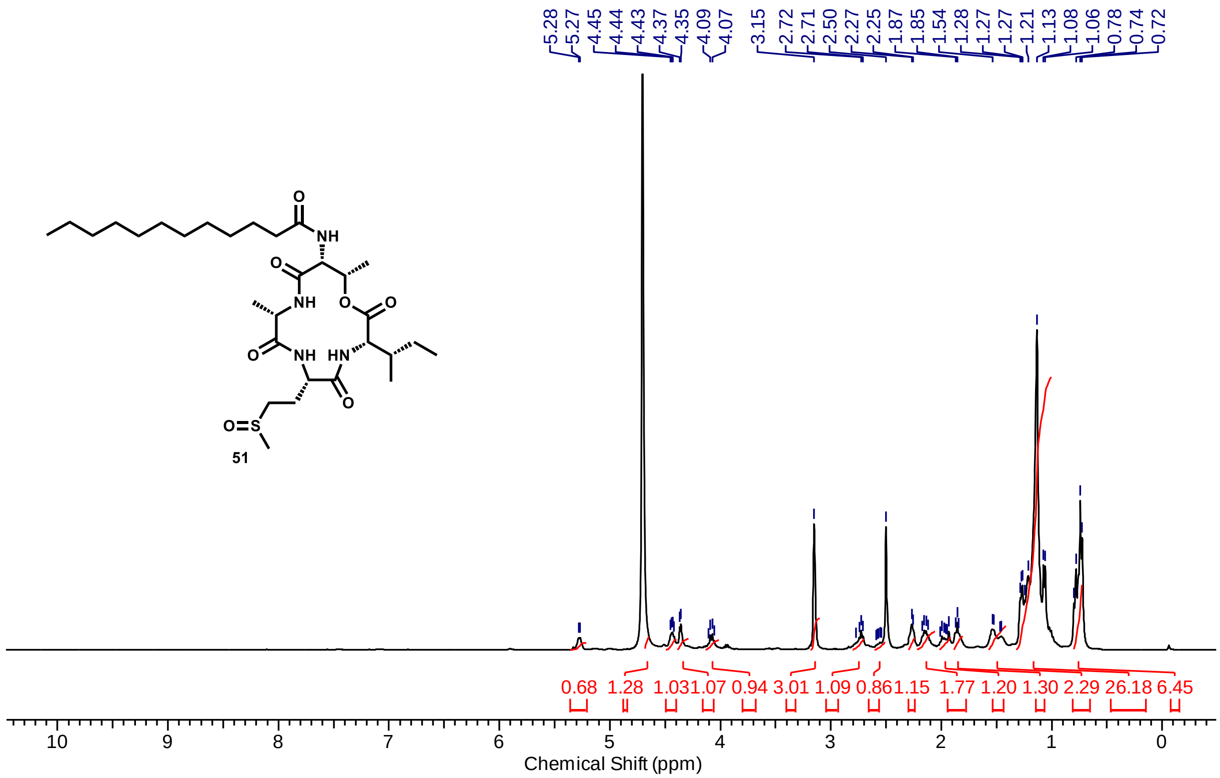
^{13}C NMR of **36** (175 MHz, DMSO-d_6)



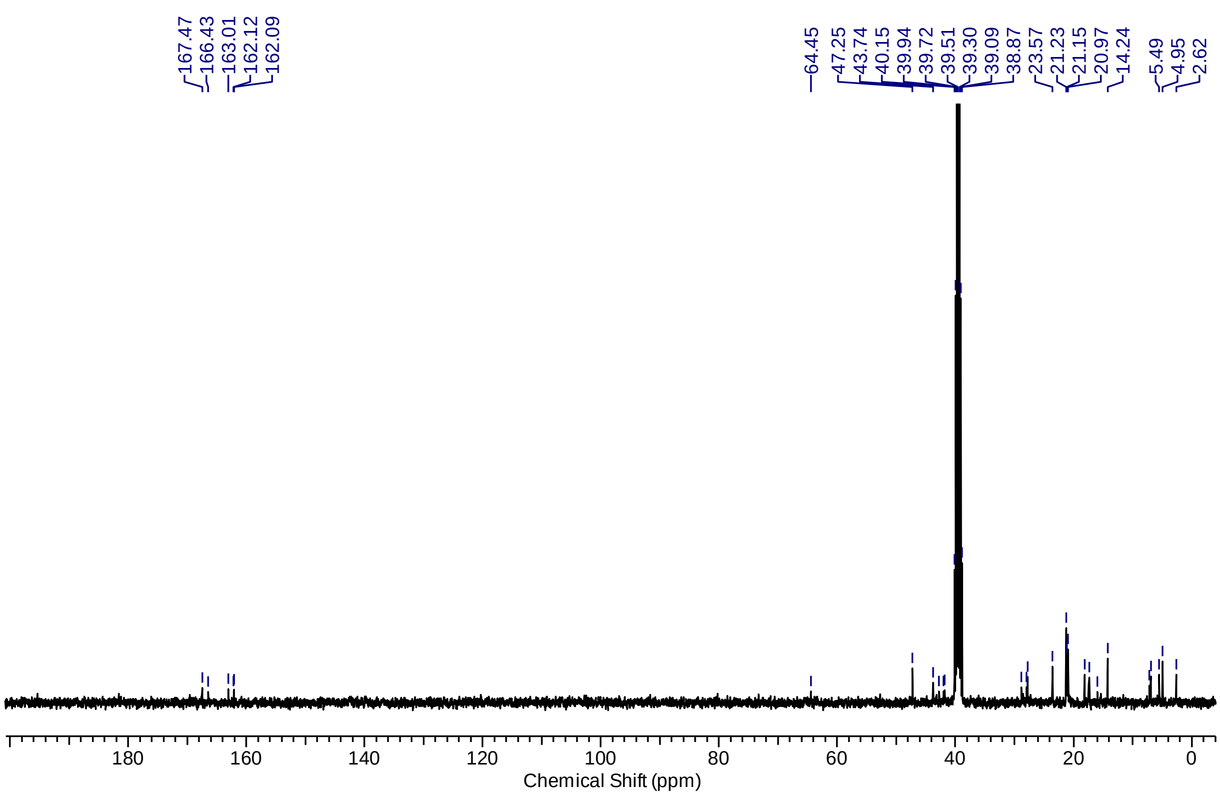
^{1}H NMR of **50** (400 MHz, DMF-d_7)



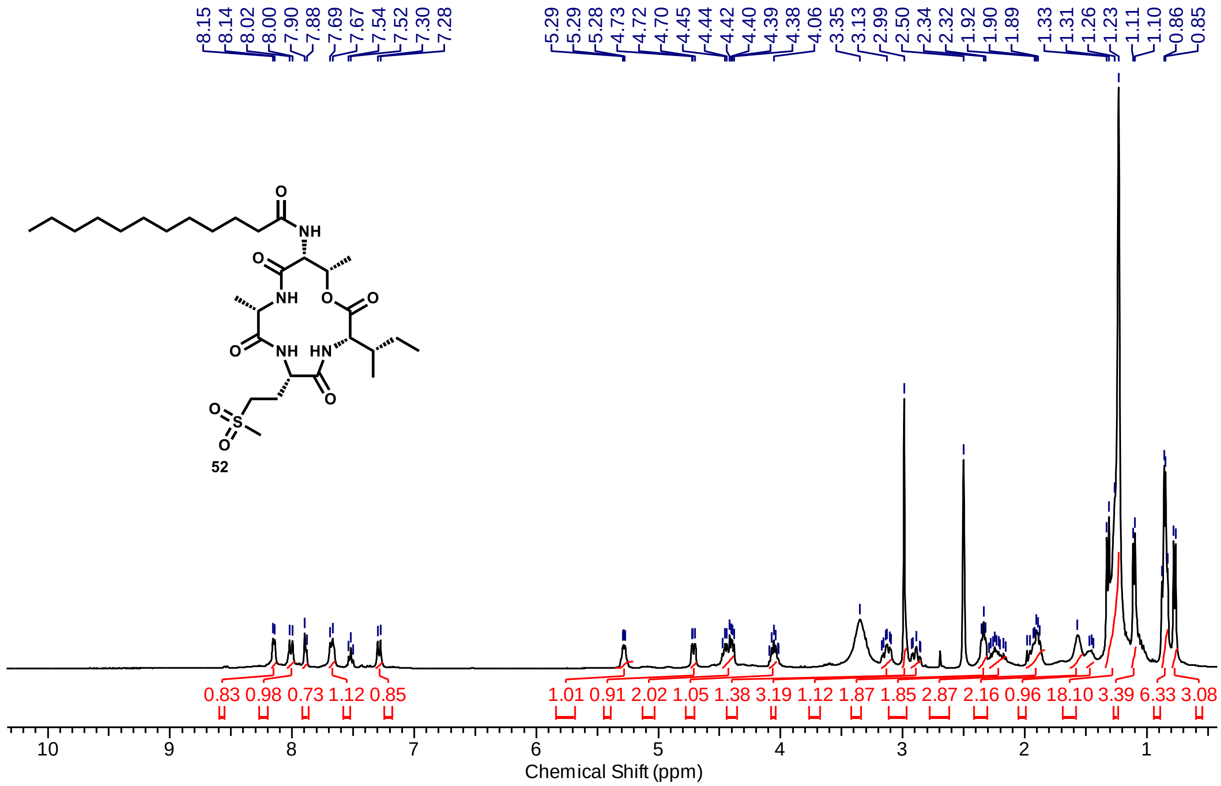
^{13}C NMR of **50** (100 MHz, DMF-d_7)



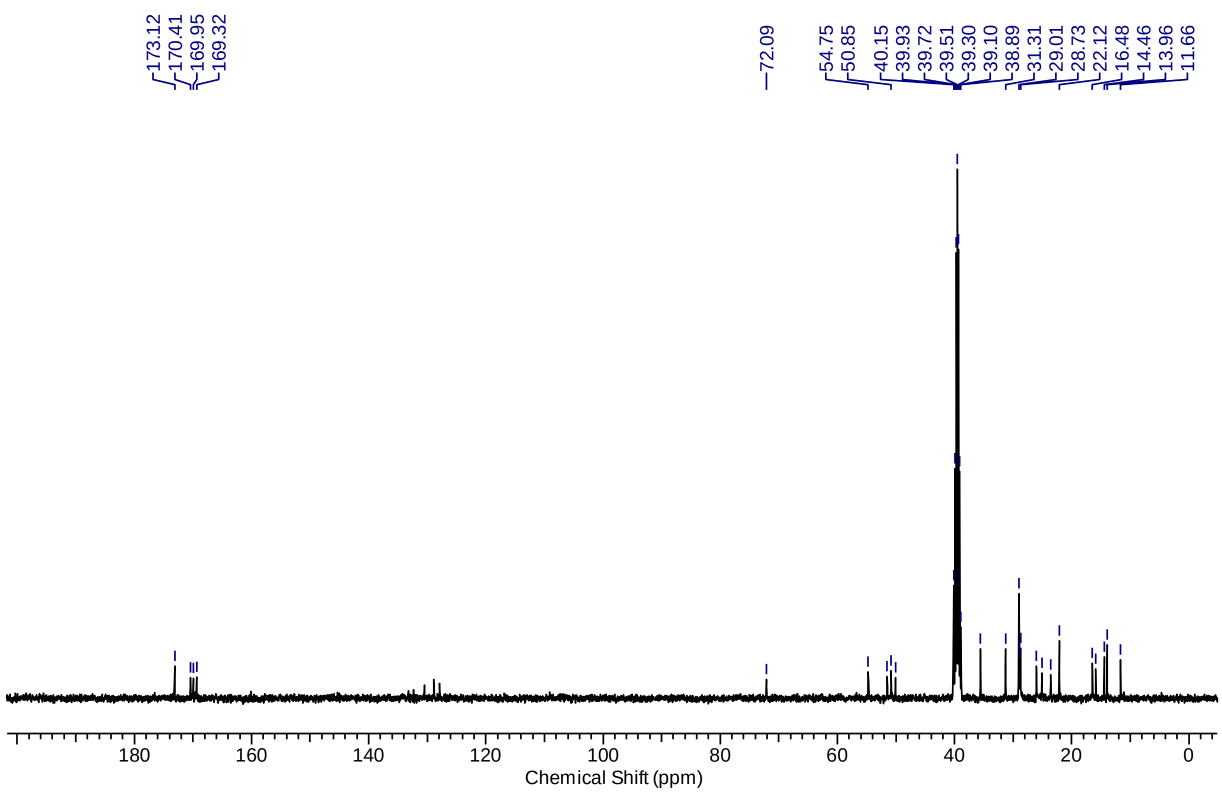
[1]H NMR of **51** (400 MHz, DMSO-d_6)



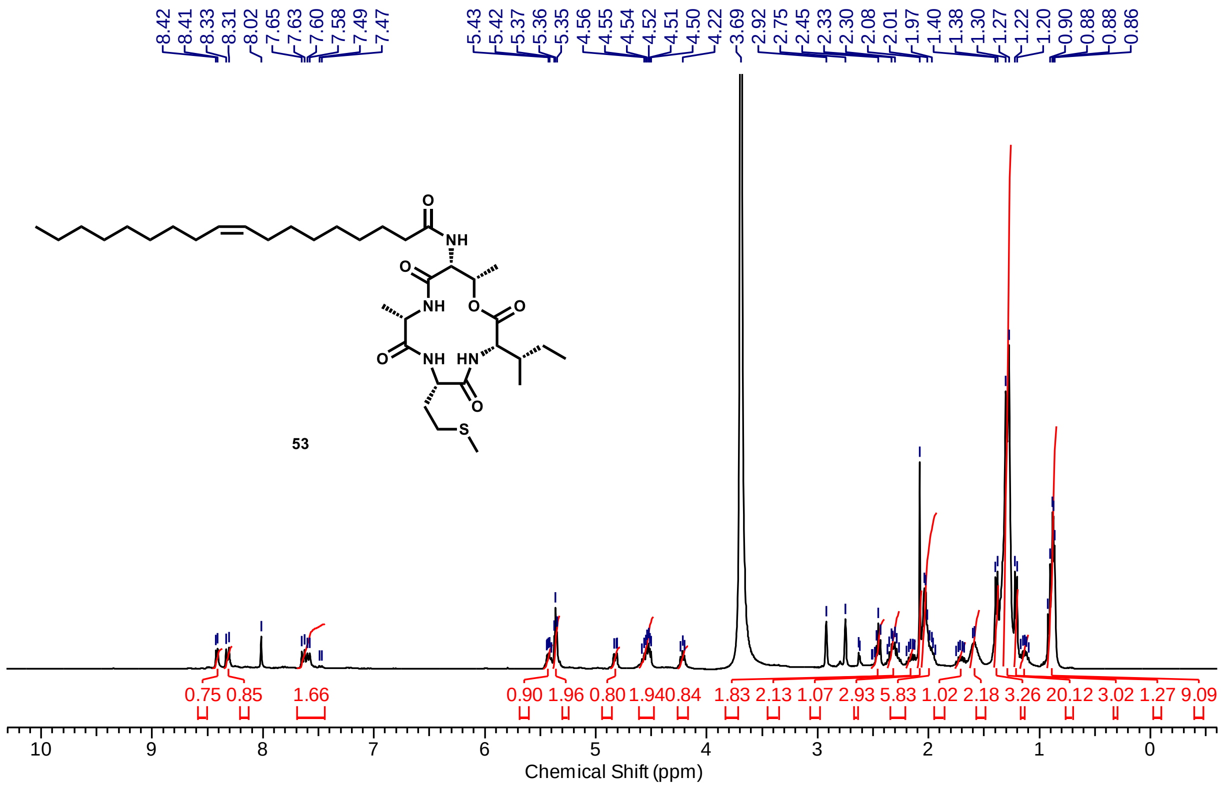
[13]C NMR of **51** (100 MHz, DMSO-d_6)



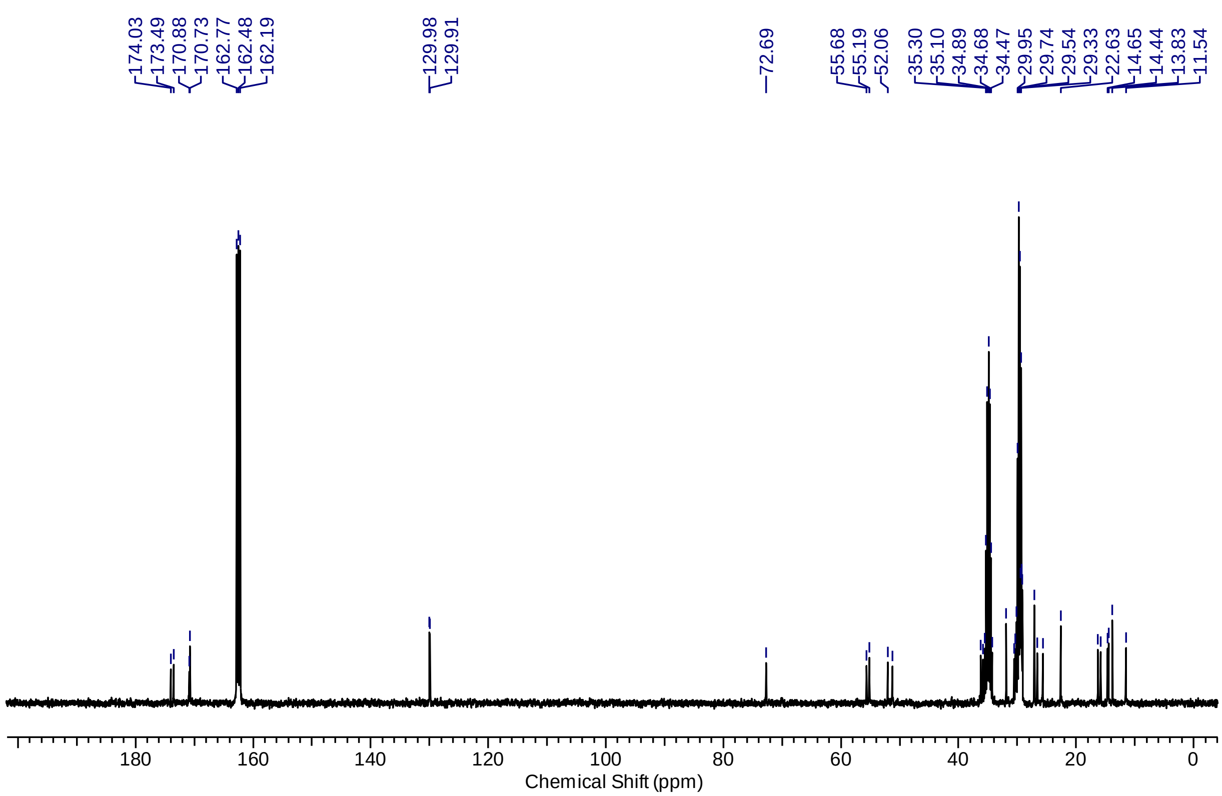
^{1}H NMR of **52** (400 MHz, DMSO-d_6)



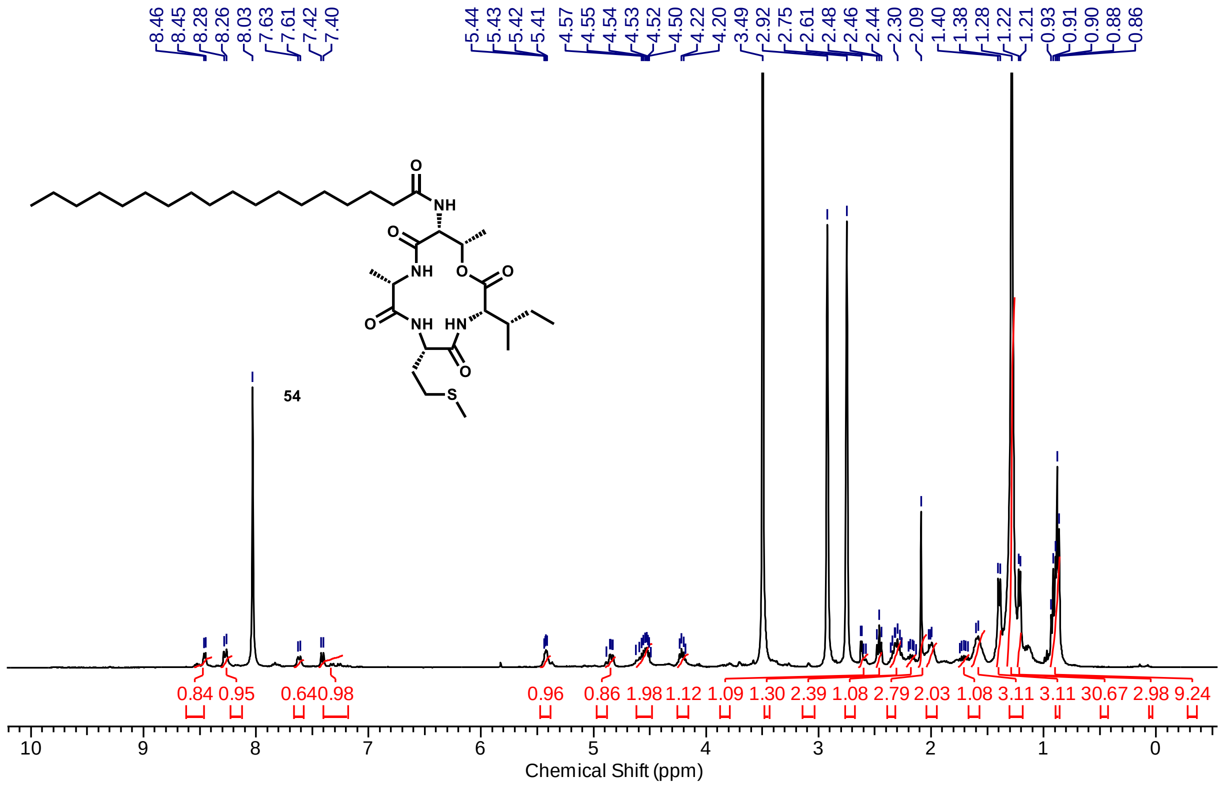
^{13}C NMR of **52** (100 MHz, DMSO-d_6)



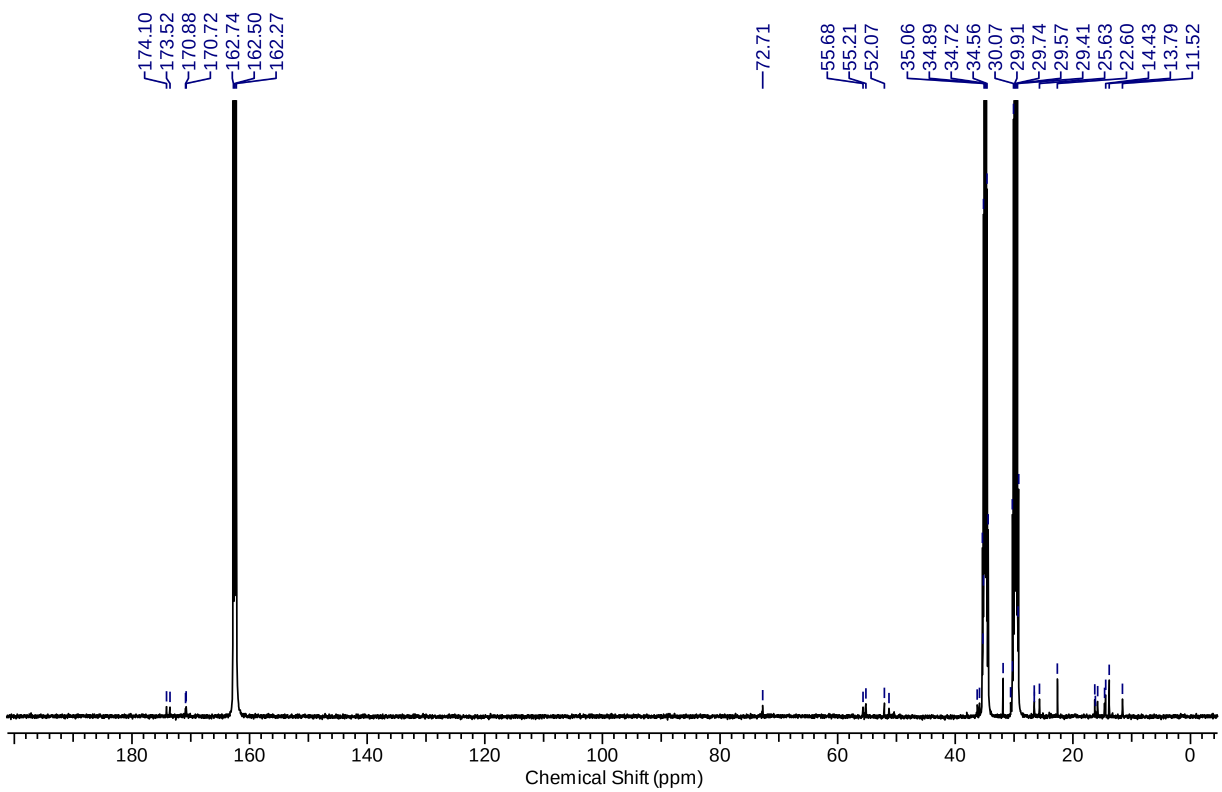
[1]H NMR of **53** (400 MHz, DMF-d_7)



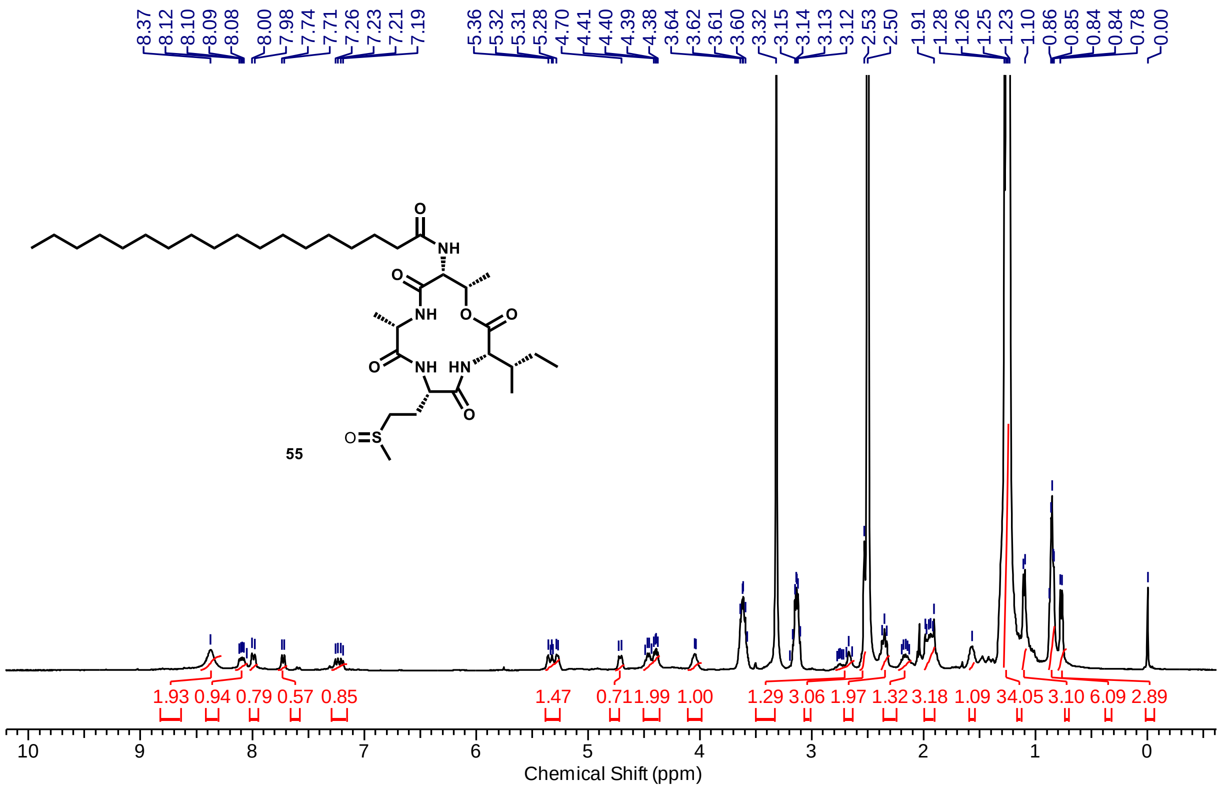
[13]C NMR of **53** (100 MHz, DMF-d_7)



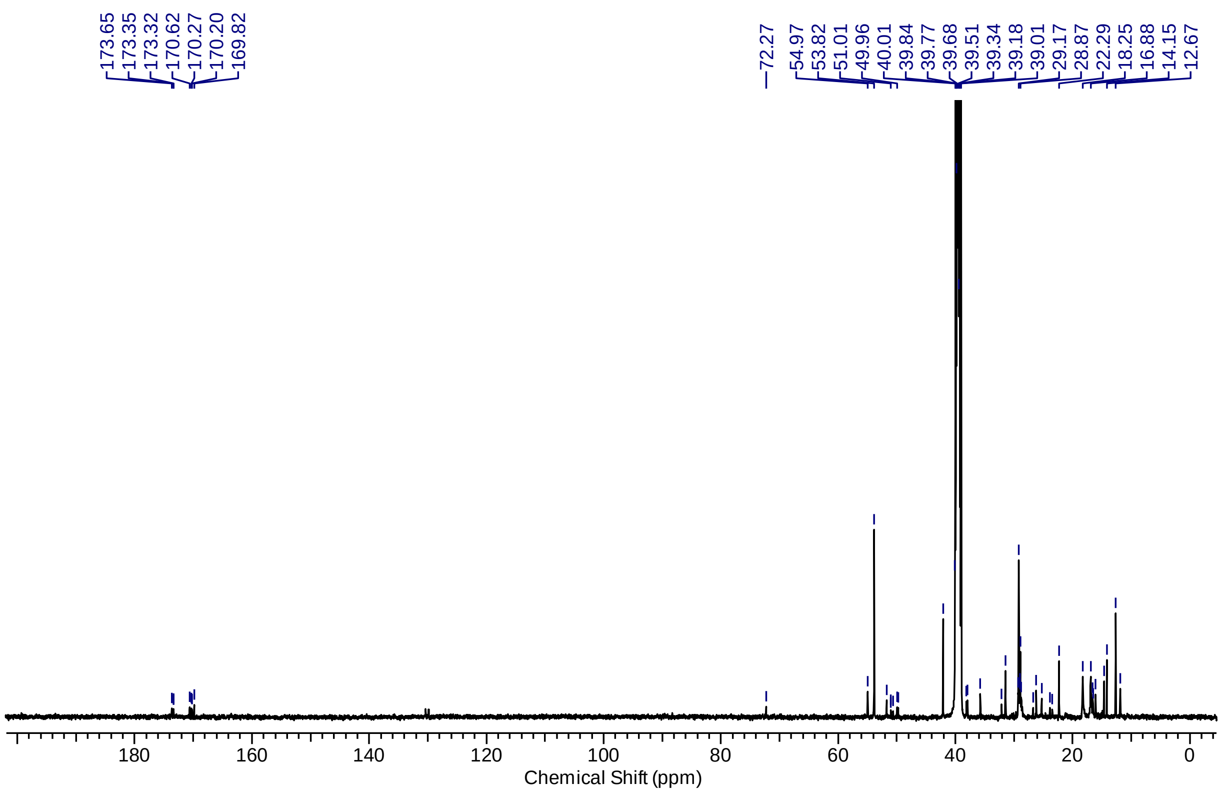
^{1}H NMR of **54** (400 MHz, DMF-*d*$_7$)



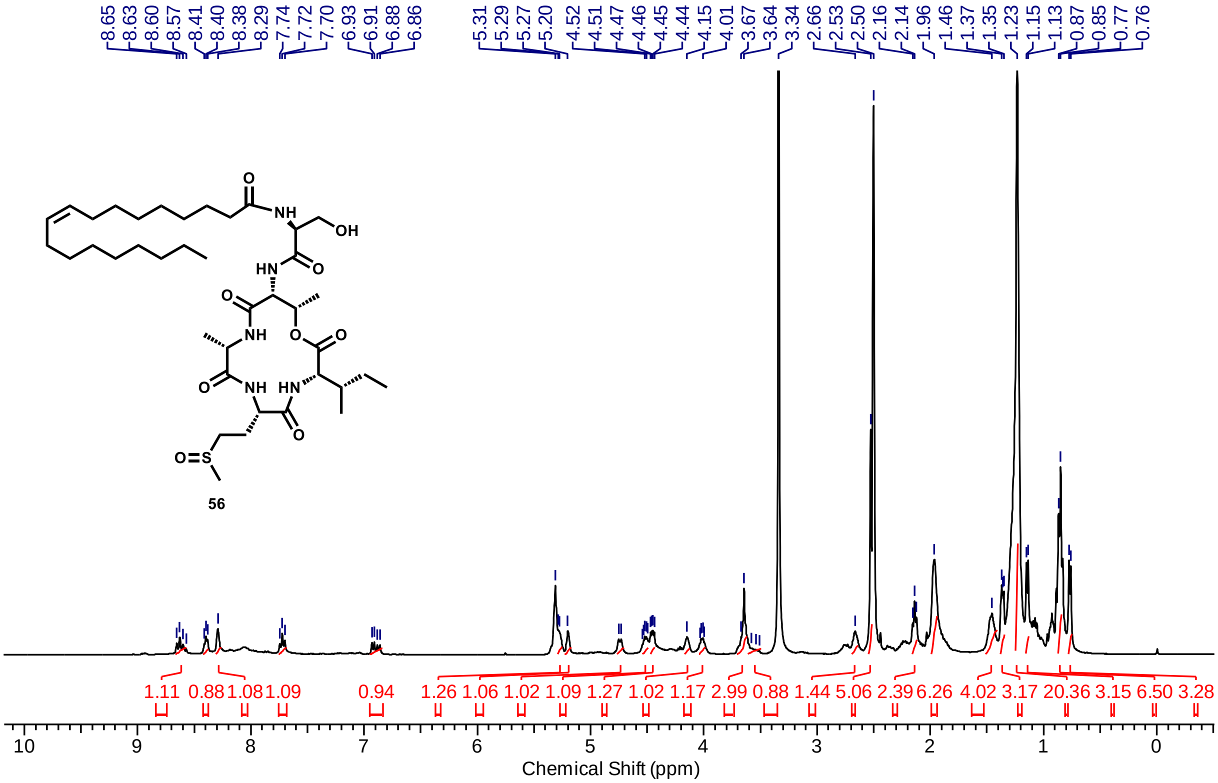
^{13}C NMR of **54** (100 MHz, DMF-*d*$_7$)



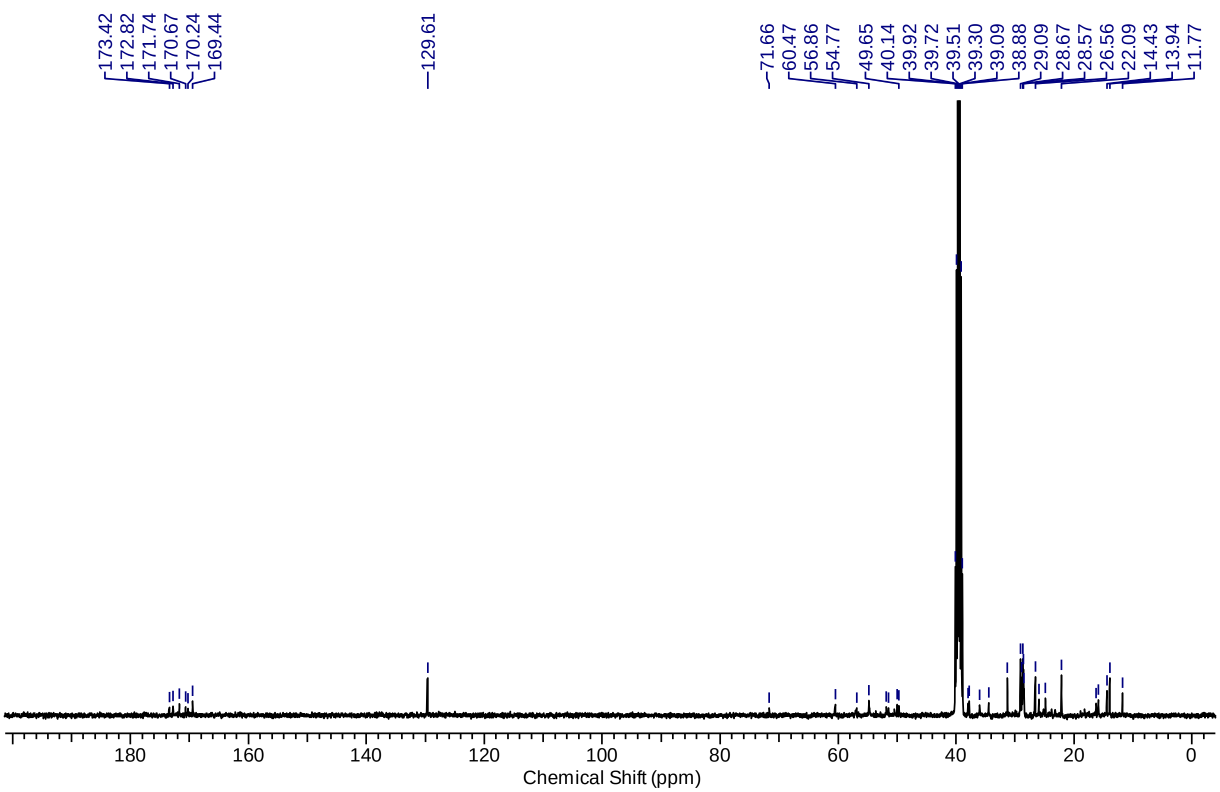
^{1}H NMR of **55** (500 MHz, DMSO-d_6)



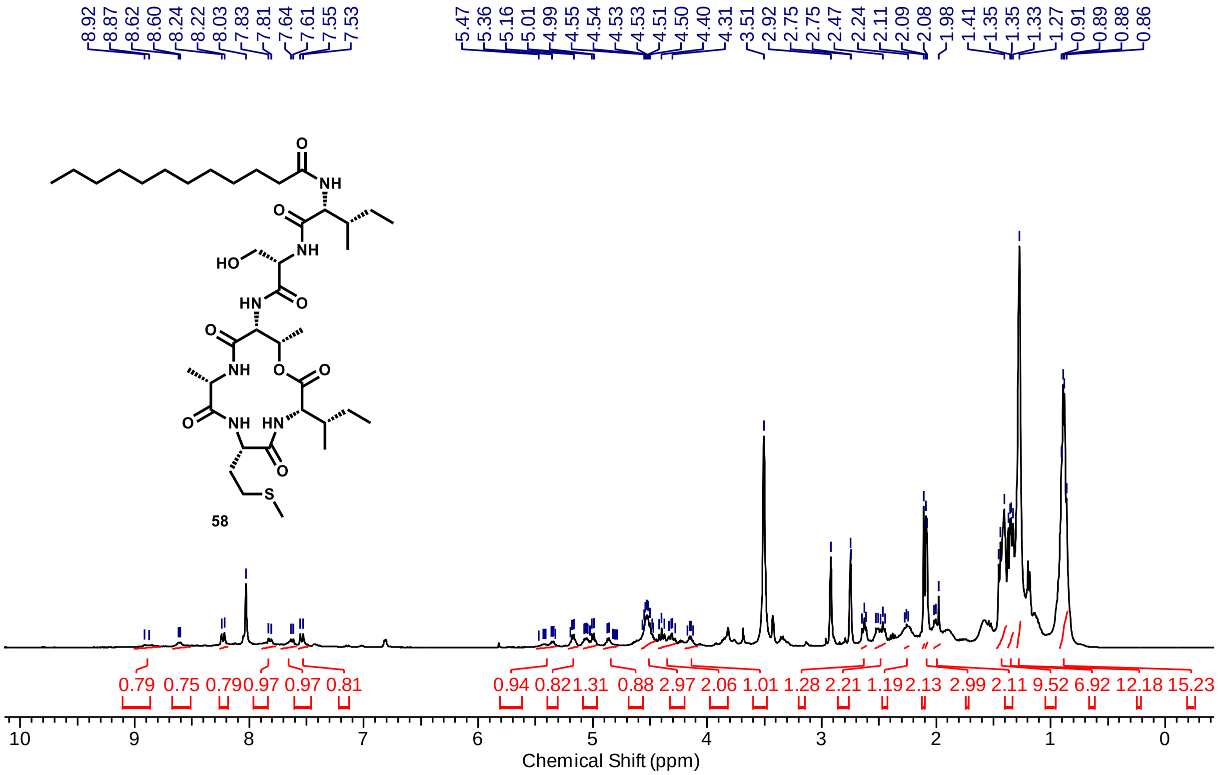
^{13}C NMR of **55** (125 MHz, DMSO-d_6)



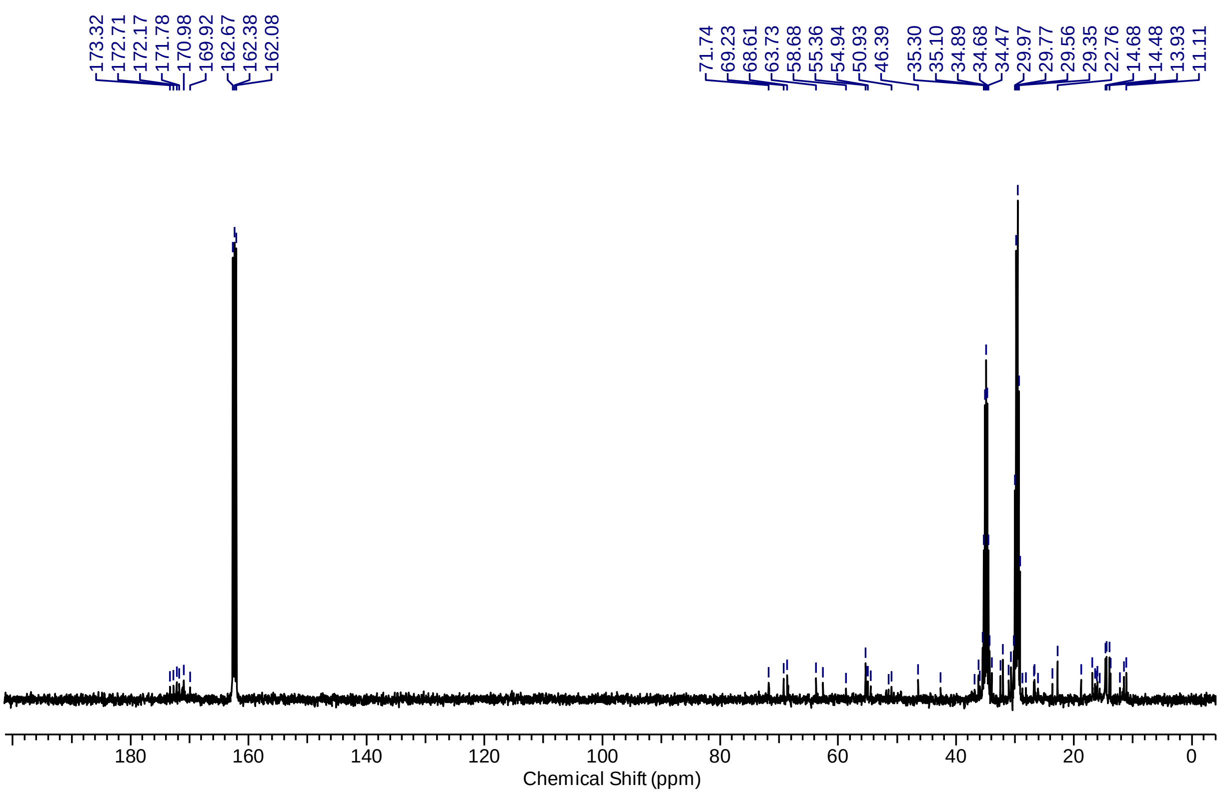
[1]H NMR of **56** (400 MHz, DMSO-d_6)



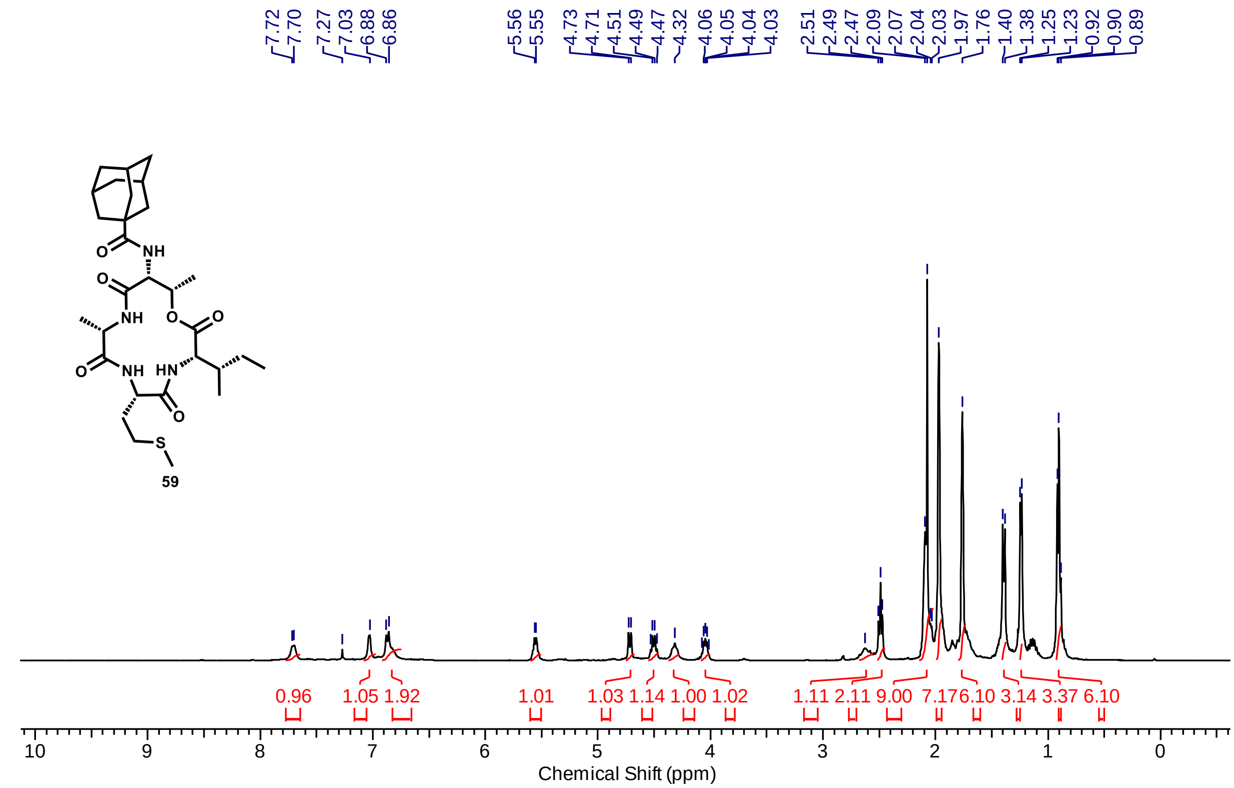
[13]C NMR of **56** (100 MHz, DMSO-d_6)



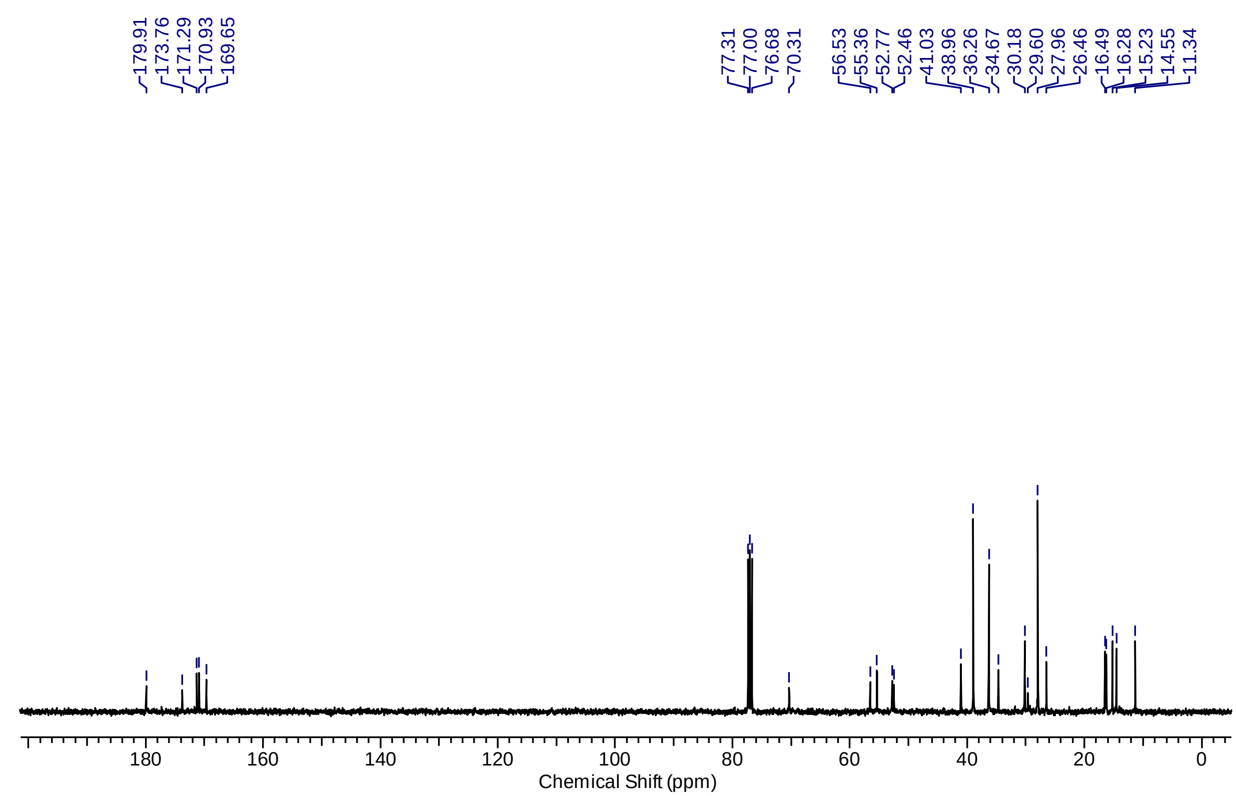
^{1}H NMR of **58** (400 MHz, DMF-d_7)



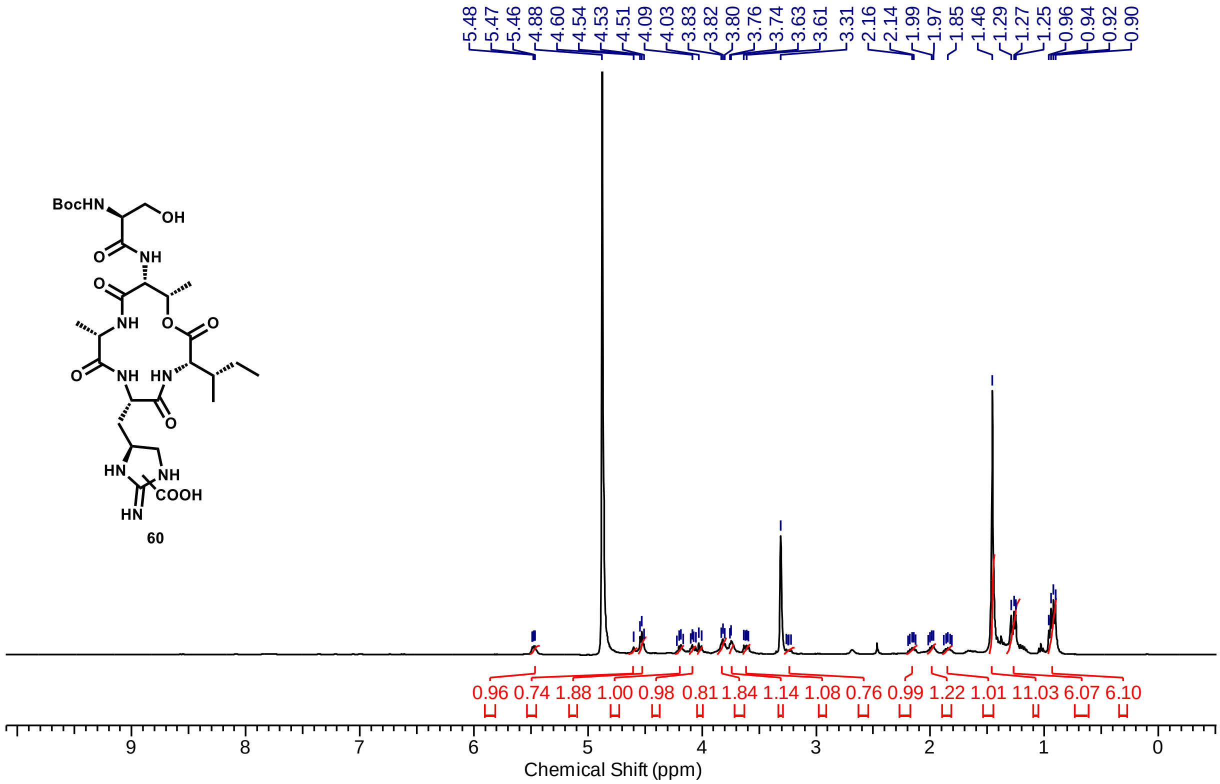
^{13}C NMR of **58** (100 MHz, DMF-d_7)



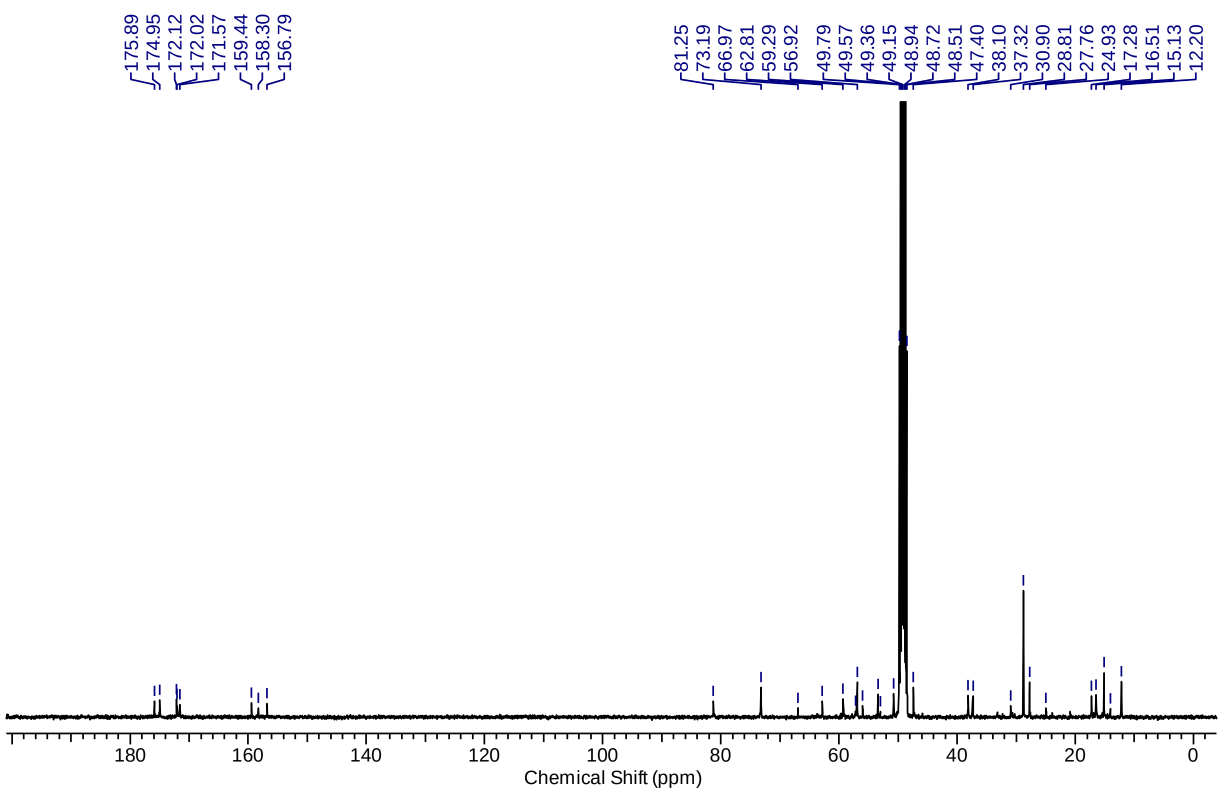
^{1}H NMR of **59** (400 MHz, $CDCl_3$)



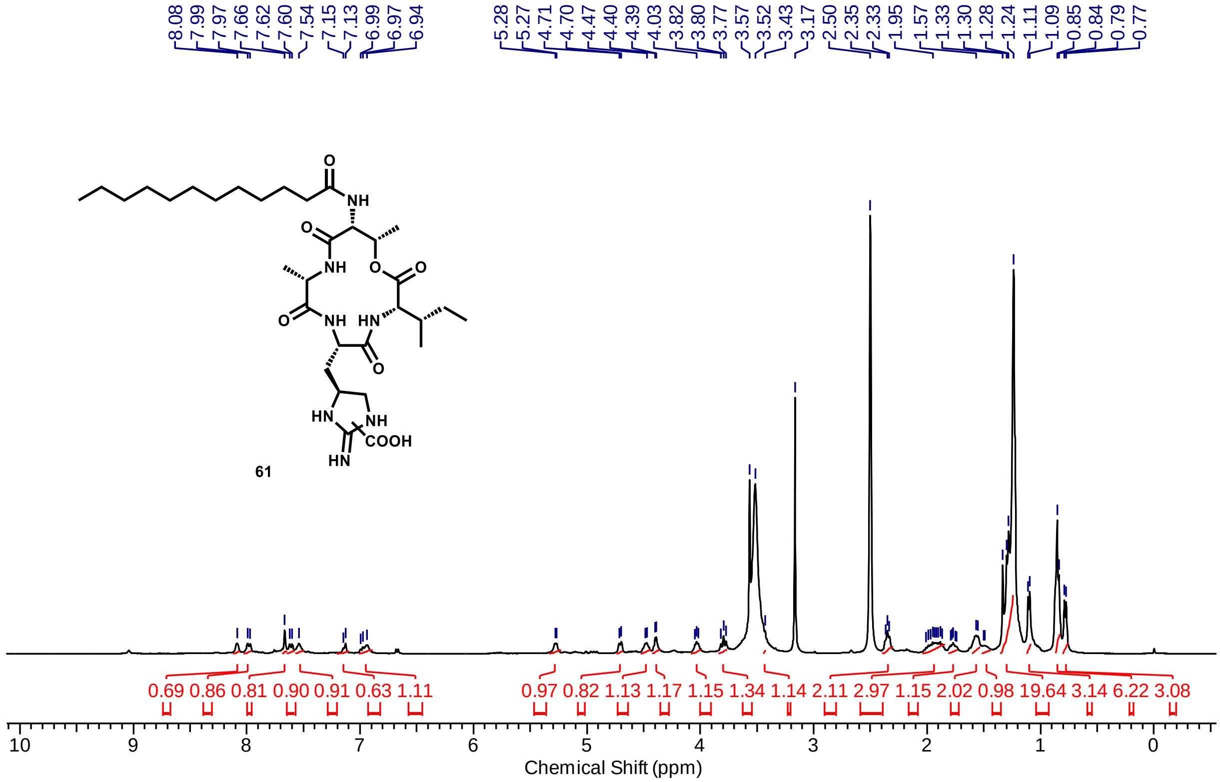
^{13}C NMR of **59** (100 MHz, $CDCl_3$)



^{1}H NMR of **60** (400 MHz, MeOH-d_4)



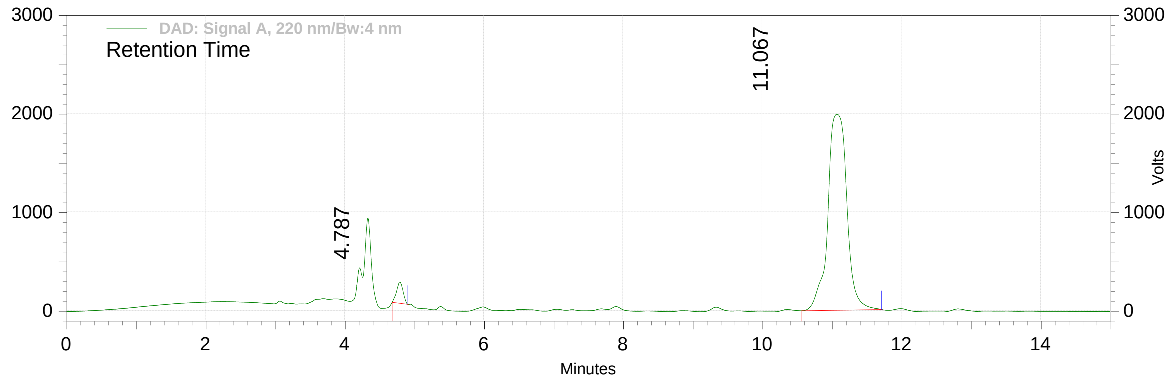
^{13}C NMR of **60** (100 MHz, MeOH-d_4)



^{1}H NMR of **61** (400 MHz, DMSO-d_6)

1.8. HPLC spectra

HPLC of compound **63**



DAD: Signal A,

220 nm/ Results

Retention Time	Area	Area %	Height	Height %
4.787	2934492	3.55	454012	9.81
11.067	79650287	96.45	4171750	90.19
Totals	82584779	100.00	4625762	100.00

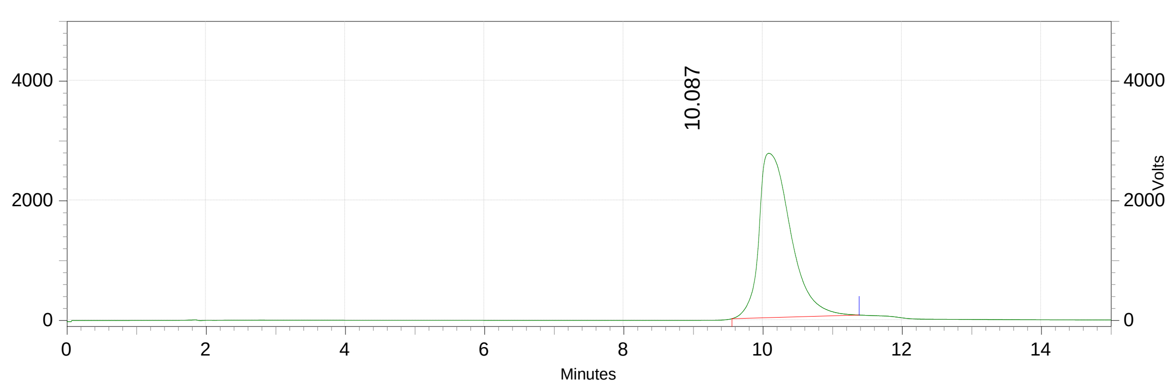
Column: Sunfire® C_{18}, (4.6 X 250 mm, 5 µm)

Mobile phase: ACN: H_2O (0.1% TFA)

Flow rate: 1.00 mL/min

Wavelength: 220 nm.

HPLC of compound **64**



DAD: Signal A, 254 nm/ Results

Retention Time	Area	Area %	Height	Height %
10.087	182380176	100.00	5758305	100.00
Totals	182380176	100.00	5758305	100.00

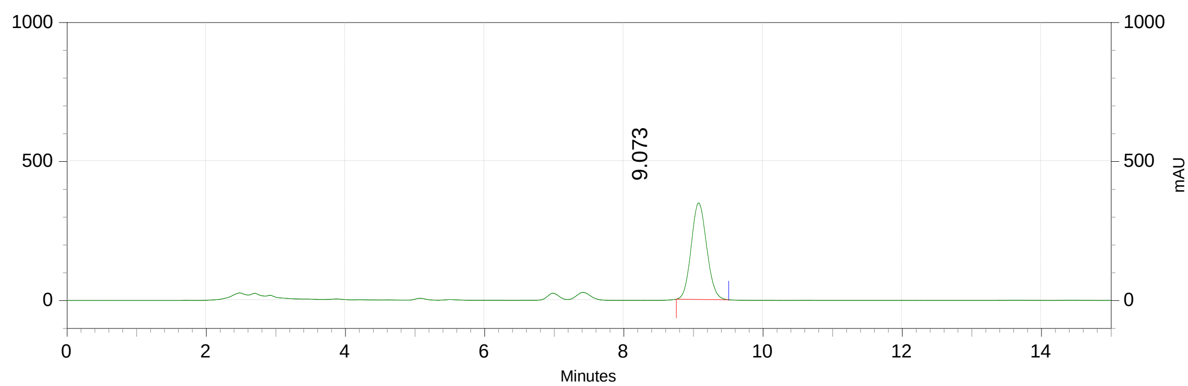
Column: Sunfire® C_{18}, (4.6 X 250 mm, 5 µm)

Mobile phase: ACN: H_2O (0.1% TFA)

Flow rate: 1.00 mL/min

Wavelength: 220 nm.

HPLC of compound **65**



DAD: Signal A, 254 nm/ Results

Retention Time	Area	Area %	Height	Height %
9.073	11251195	80.14	727723	100.00
Totals	14038610	100.00	818871	100.00

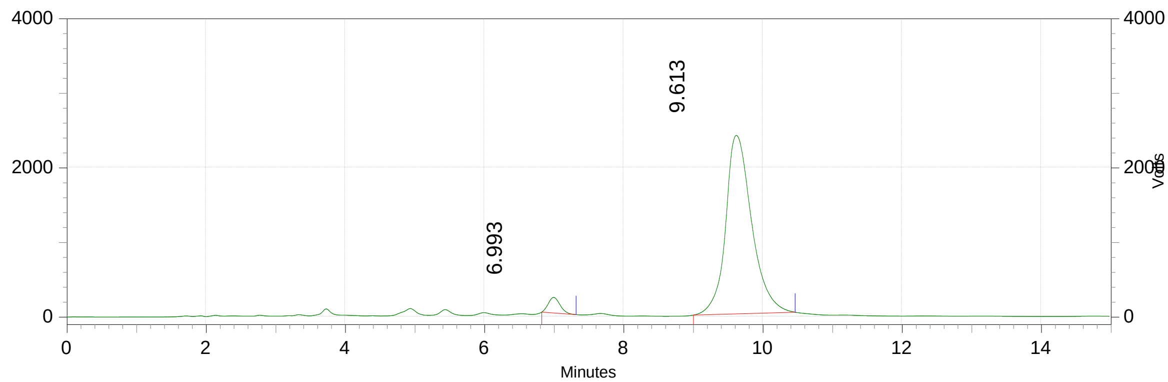
Column: Sunfire® C_{18}, (4.6 X 250 mm, 5 µm)

Mobile phase: ACN: H_2O (0.1% TFA)

Flow rate: 1.00 mL/min

Wavelength: 220 nm.

HPLC of compound **66**



DAD: Signal A, 254 nm/ Results

Retention Time	Area	Area %	Height	Height %
6.993	5133028	3.78	438139	8.03
9.613	130705802	96.22	5017336	91.97
Totals	135838830	100.00	5455475	100.00

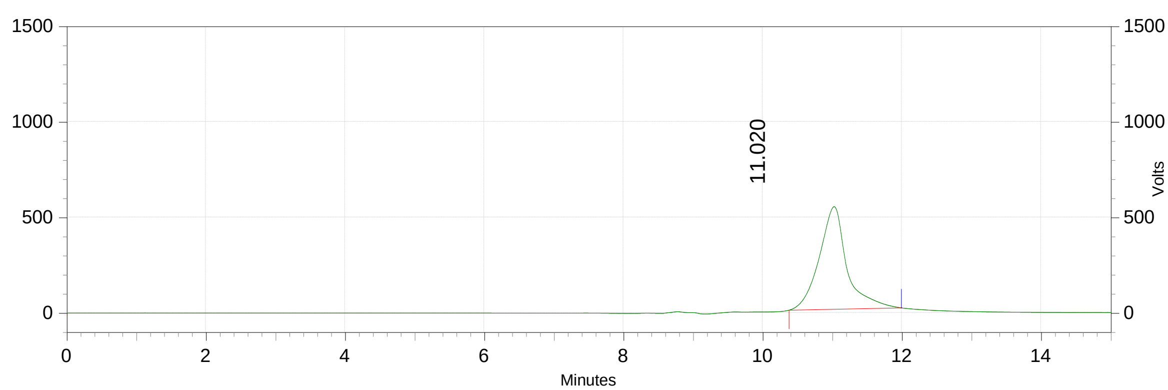
Column: Sunfire® C_{18}, (4.6 X 250 mm, 5 µm)

Mobile phase: ACN: H_2O (0.1% TFA)

Flow rate: 1.00 mL/min

Wavelength: 220 nm.

HPLC of compound **67**



DAD: Signal A, 240 nm/ Results

Retention Time	Area	Area %	Height	Height %
11.020	30906301	100.00	1125577	100.00
Totals	30906301	100.00	1125577	100.00

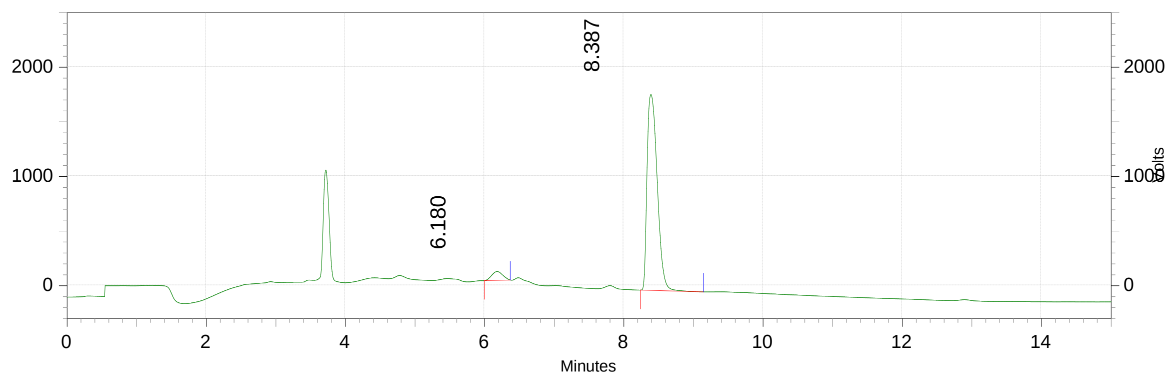
Column: Sunfire® C_{18}, (4.6 X 250 mm, 5 µm)

Mobile phase: ACN: H_2O (0.1% TFA)

Flow rate: 1.00 mL/min

Wavelength: 220 nm.

HPLC of compound **68**



DAD: Signal A, 215 nm/ Results

Retention Time	Area	Area %	Height	Height %
6.180	1853856	4.63	168758	4.29
8.387	38228434	95.37	3760464	95.71
Totals	40082290	100.00	3929222	100.00

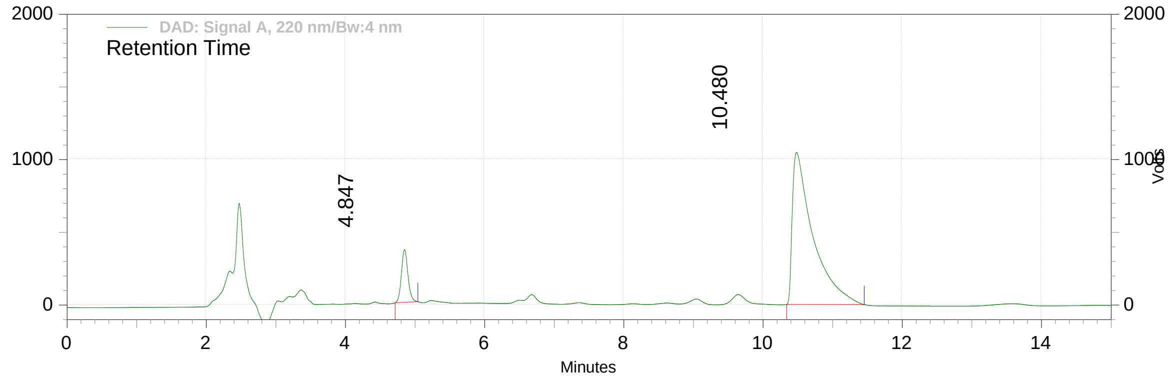
Column: Sunfire® C_{18}, (4.6 X 250 mm, 5 µm)

Mobile phase: ACN: H_2O (0.1% TFA)

Flow rate: 1.00 mL/min

Wavelength: 220 nm.

HPLC of compound **69**



DAD: Signal A,

220 nm/ Results

Retention Time	Area	Area %	Height	Height %
4.847	4956131	9.89	760454	6.95
10.480	45141966	90.11	2192583	93.05
Totals	50098097	100.00	2953037	100.00

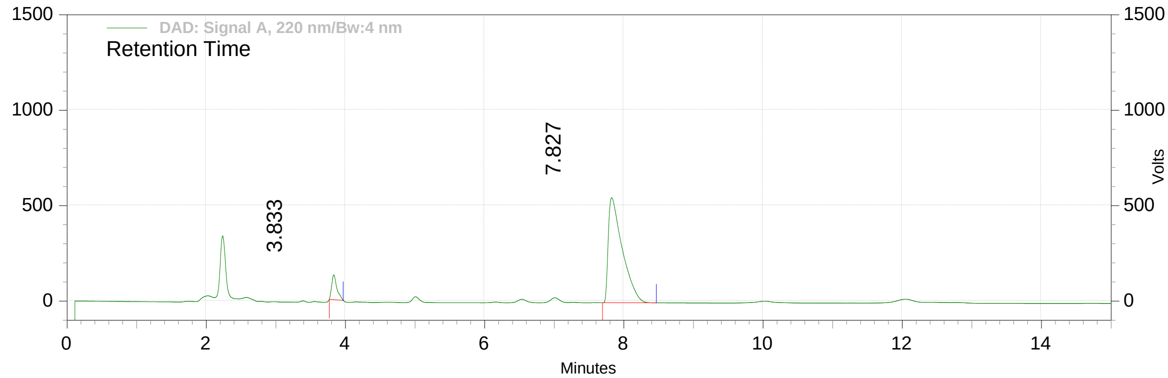
Column: Sunfire® C_{18}, (4.6 X 250 mm, 5 µm)

Mobile phase: ACN: H_2O (0.1% TFA)

Flow rate: 1.00 mL/min

Wavelength: 220 nm.

HPLC of compound **70**



DAD: Signal

A, 220 nm/

Results

Retention Time	Area	Area %	Height	Height %
3.833	1406942	8.00	272338	09.08
7.827	16175322	92.00	1155066	90.92
Totals	17582264	100.00	1427404	100.00

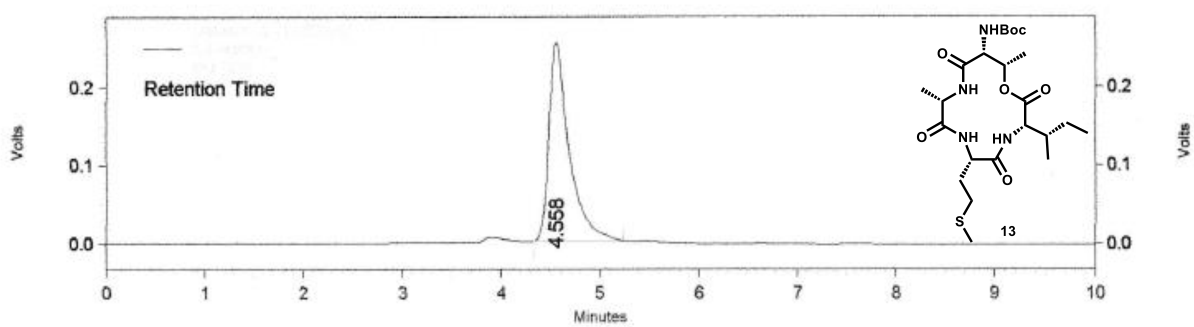
Column: Sunfire® C_{18}, (4.6 X 250 mm, 5 µm)

Mobile phase: ACN: H_2O (0.1% TFA)

Flow rate: 1.00 mL/min

Wavelength: 220 nm.

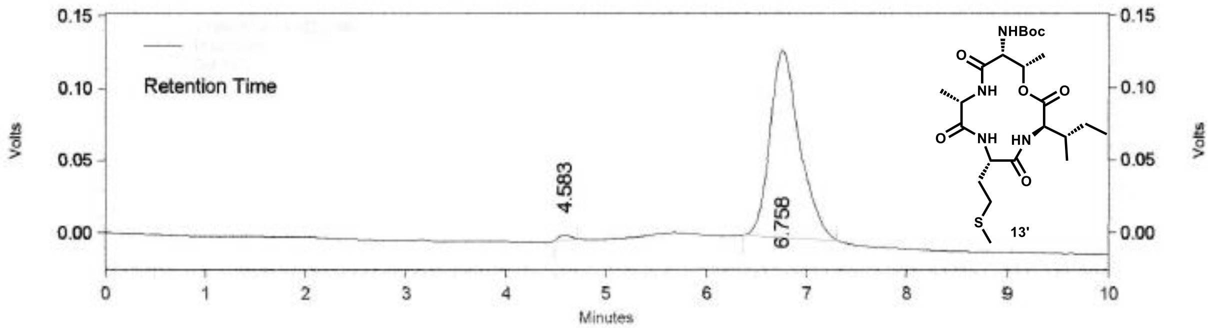
HPLC of compound **13**



Detector A - 1 (220nm) Retention Time	C Area	Area %
4.558	1802615	100.000
Totals	1802615	100.000

Project Leader : Dr.D. S. REDDY
Column : Chiralcel OD-H (0.46cm X 25cm)
Mobile Phase : n-Hexane:EtOH (90:10)
Flow Rate : 1ml/min 550psi
Wavelength : 220nm
Con. : xmg /100ml
Inject vol. : 20ul

HPLC of compound **13'**

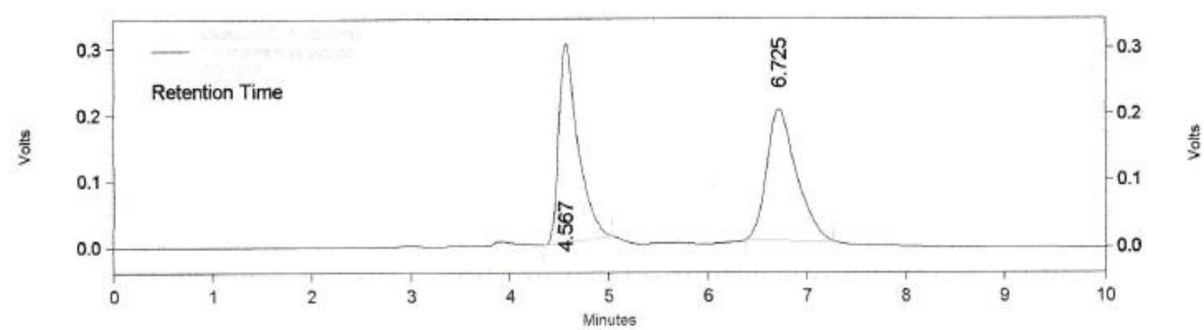


Detector A - 1 (220nm)

Retention Time	C Area	Area %
4.583	11391	0.852
6.758	1325358	99.148
Totals	1336749	100.000

Project Leader : Dr.D. S. REDDY
Column : Chiralcel OD-H (0.46cm X 25cm)
Mobile Phase : n-Hexane:EtOH (90:10)
Flow Rate : 1ml/min 550psi
Wavelength : 220nm
Con. : xmg /100ml
Inject vol. : 20ul

HPLC co-injection of compound **13** and **13’**



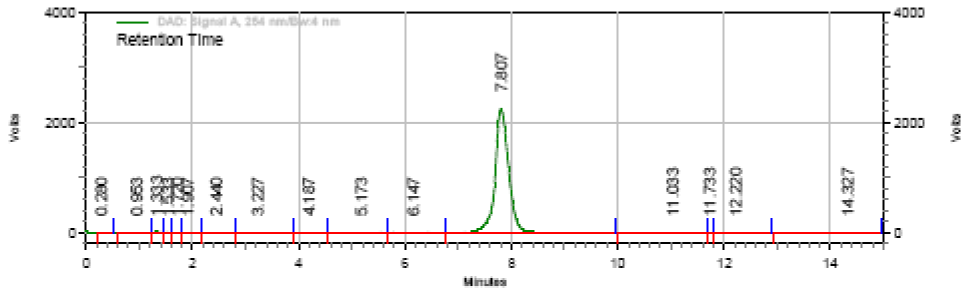
Detector A - 1 (220nm)

Retention Time	C Area	Area %
4.567	2005848	49.640
6.725	2034917	50.360
Totals	4040765	100.000

Project Leader : Dr.D. S. REDDY
Column : Chiralcel OD-H (0.46cm X 25cm)
Mobile Phase : n-Hexane:EtOH (90:10)
Flow Rate : 1ml/min 550psi
Wavelength : 220nm
Con. : xmg /100ml
Inject vol. : 20ul

HPLC purity of Met_{10}-teixobactin, **36**

Data File: E:\VIDYA\TXB PURITY.dat
Method: E:\VIDYA\Report template 3 13 April 19.met
Printed: 4/13/2019 12:25:42 PM



DAD: Signal A,
254 nm/Bw:4 nm
Results

Retention Time	Area	Area %	Height	Height %
0.280	34007	0.04	2739	0.06
0.953	271071	0.31	10664	0.22
1.333	246388	0.28	34805	0.71
1.533	36859	0.04	6566	0.13
1.720	115638	0.13	15680	0.32
1.907	285137	0.32	14082	0.29
2.440	466805	0.53	16632	0.34
3.227	713264	0.81	19181	0.39
4.187	344275	0.39	10472	0.21
5.173	901713	1.02	25493	0.52
6.147	600872	0.68	16999	0.35
7.807	83864349	95.07	4705614	96.32
11.033	165003	0.19	3015	0.06
11.733	2096	0.00	298	0.01
12.220	18804	0.02	442	0.01
14.327	150849	0.17	2508	0.05
Totals	88217130	100.00	4885190	100.00

Column: Eclipse XDB C18, 5µ (250*4.6 mm)
Flow Rate: 1 mL/min
Solvent system: A: 95% H2O (0.1% TFA:
B: 95% ACN (0.1% TFA)
Gradient: 0 to 1 min 95% A
1 to 10 min 95% B
10 to 15 min 5% B

Injection volume: 20µL
Wavelength: 254 nm

Chapter 2:

Total Synthesis of 3-*epi*-pseudoxylallemycin B

2.1. Introduction

Infections caused by Gram-negative bacteria have been emerged as a serious problem due to their continuous development of resistance to the available drugs. Examples of these multi-drug resistant bacteria are *Acinetobacter*, *Pseudomonas*, *Escherichia coli*, *Klebsiella*, *Salmonella* which causes infection like pneumonia, bloodstream infections, wound or surgical site infections, and bacterial meningitis.[1] Among them, infections caused by *Pseudomonas aeruginosa*, particularly in patients with low immunity are becoming problematic leading to the high death rate.[1] According to the reports of Centers for Disease Control and Prevention (CDC) published in 2013, nearly 8% healthcare related infections were caused by *Pseudomonas aeruginosa* and about 13% of them are multidrug resistant.[2] Therefore, the main attention have been focused on to development of new antibiotics against Gram-negative bacteria with novel mode of action.

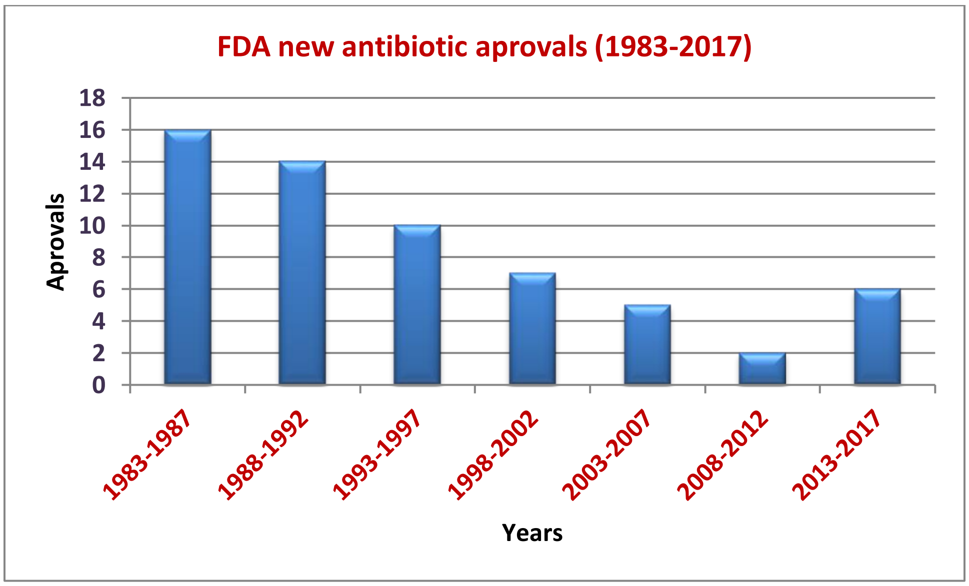


Figure 2.1. New antibiotics approved in USA, 1983-2017 (image source: *Antimicrob. Agents Chemother*. **2013**, *57*, 4605–4607)[3]

Various classes of antimicrobial drugs are used in the treatment of multidrug resistant Gram-negative pathogens, but increased rate of resistance placed limitations in front of pharmaceutical industry.[4] New antibiotics are coming to the market but most of them are for Gram-positive bacteria which show significant scarcity of new antibiotics for Gram-negative pathogens.[5] Figure 2.1. indicates the rate of new antibiotics approved by US-FDA has continuously falling to desperately low levels.[3] There are a few drugs belonging to different chemical scaffolds which are being presently used or under clinical evaluation for treating infections caused by Gram-negative bacteria. Among them, peptides can be a good alternative to small synthetic molecules owing to their lesser toxicity (as metabolites are amino acids), low organ accumulation, rapid degradation catalyzed by enzymes, target selectivity and specificity.[6]

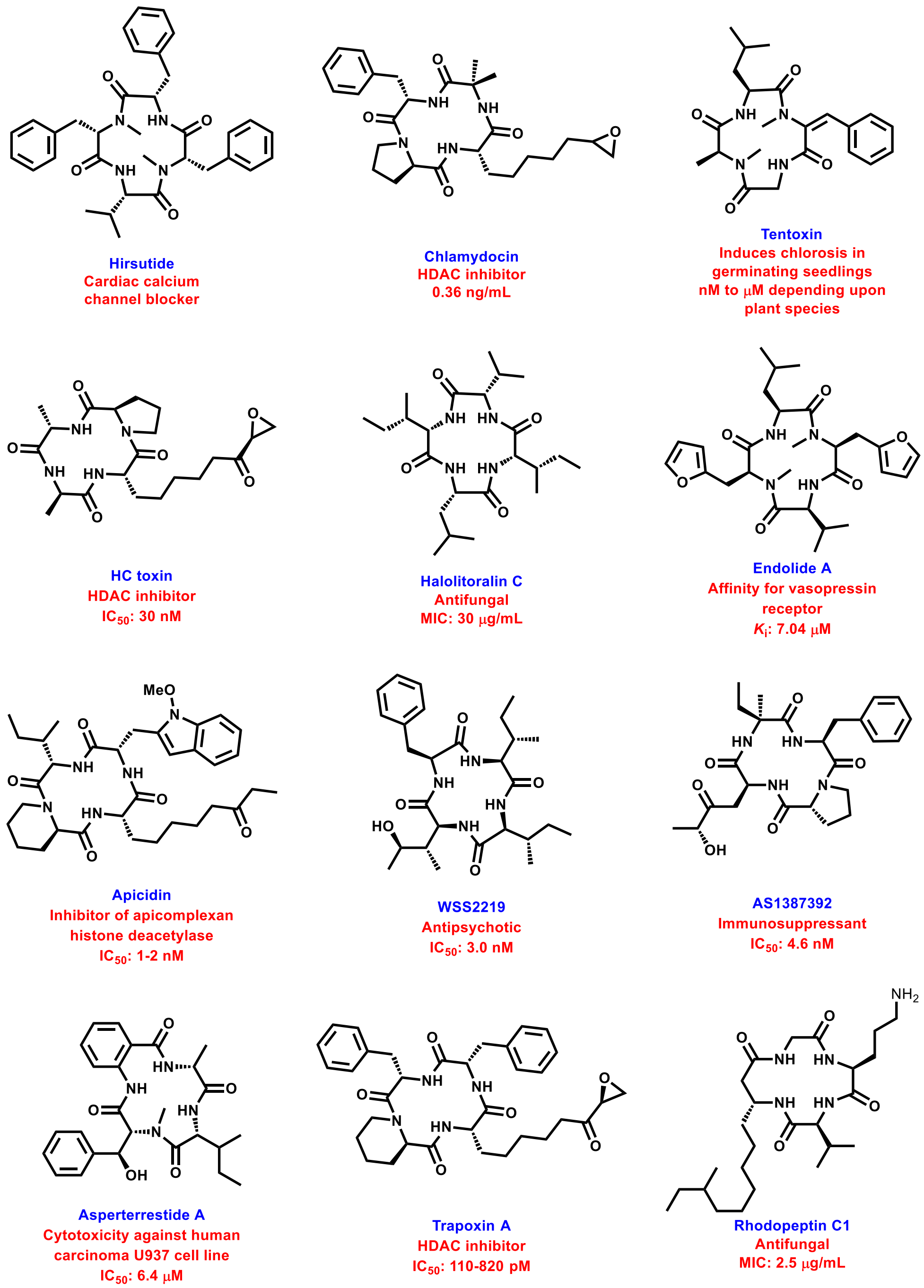


Figure 2.2. Bioactive macrocyclic tetrapeptides

Till date, US Food and Drug Administration (FDA) approved nearly 100 peptide drugs and are available in the market for the treatment of various diseases and above 400 are in various phases of clinical trials.[7] Particularly, macrocyclic peptides are gaining interest due to their potent pharmacological properties.[6] Among them, cyclic tetrapeptides are attractive pharmacological leads as compared to their larger ring size congeners attributed to their constrained structure; which provide them selectivity as well as specificity towards the target and most likely their close compliance to Lipinski's rule (less than 5 hydrogen bond donors, 10 hydrogen bond acceptors and a molecular mass less than 500 Da).[8,9] Along with such fascinating properties, there were certain limitations to them like short half-life and poor oral bioavailability. 12-Membered head-to-tail macrocyclic tetrapeptides are constrained in nature and poses slightly twisted amide bonds because of which their synthesis and lead optimization is the challenging task for the medicinal chemist.[10] Many potent and selective molecules exhibiting a wide spectrum of biological activities are known in the literature;[11] examples include, hirsutide, a cardiac calcium channel blocker,[12] potent HDAC inhibitors like chlamydocin,[13] HC toxin,[14] apicidine,[15] trapoxin A[16] then tentoxin which induces chlorosis in germinating seeds and acts as natural herbicide,[17] endolide A found to exhibit selective affinity for vasopressin receptor,[18] WSS2219 shows potent antipsychotic activity,[19] AS1387392 acts as immunosuppressant,[20] halolitoralin C shows antifungal activity,[21] potent cytotoxic tetrapeptide asperterrestide A,[22] and antifungal rhodopeptin C[23] (Figure 2.2.).

2.2. Isolation and structural confirmation of pseudoxylallemycin B

Pseudoxylallemycin A–F, a group of macrocyclic peptide natural products was isolated from termite associated fungus *Pseudoxylaria sp.* X802 by Beemelmanns' research group in 2016.[24] Cultivation of *Pseudoxylaria sp.* X802 followed by extraction and HPLC purification afforded pseudoxylallemycins A−F (Figure 2.3.). Among the six isolated natural products, pseudoxylallemycin B (**2**) is a structurally symmetric molecule, contains a rare allenyl moiety, whose structure was determined by the combination of HRMS and extensive spectroscopic studies, while amino acid configuration was determined by Marfey's method and CD spectroscopy. Detailed analysis of ^{1}H and ^{13}C NMR spectral data shows the presence of rare allenyl ether on the hydroxyl group of tyrosine.

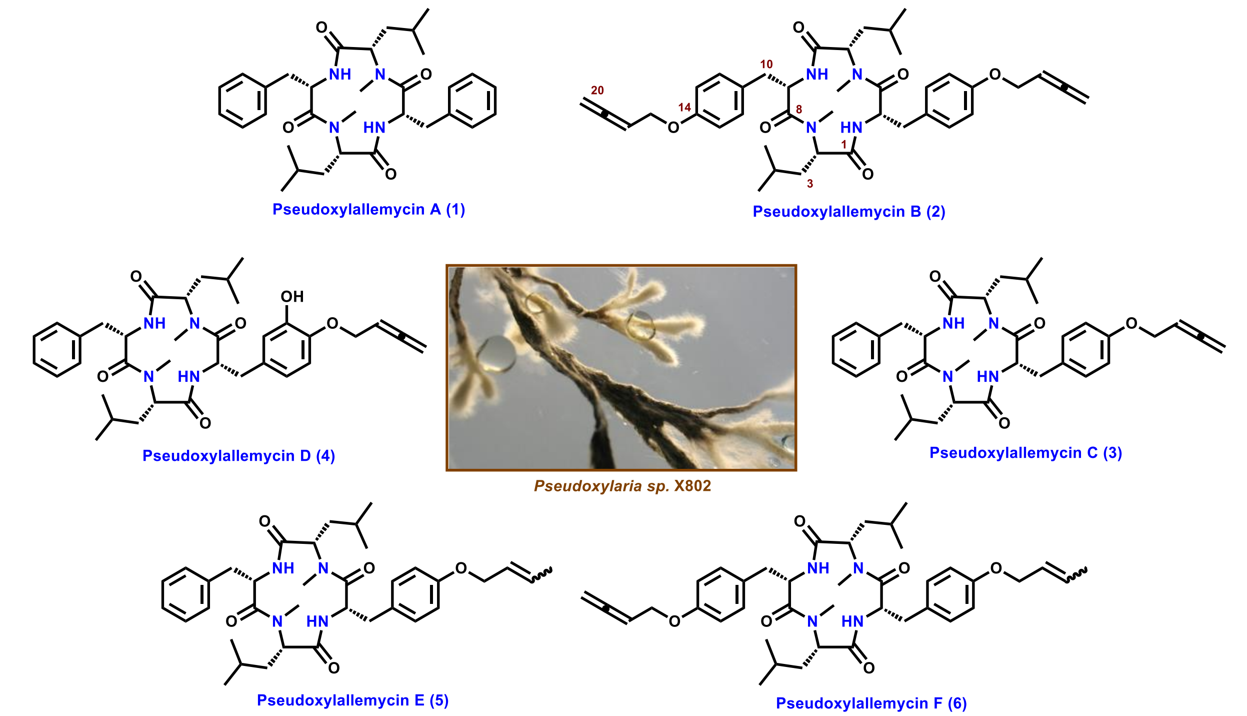


Figure 2.3. Structure of pseudoxylallemycins[24]

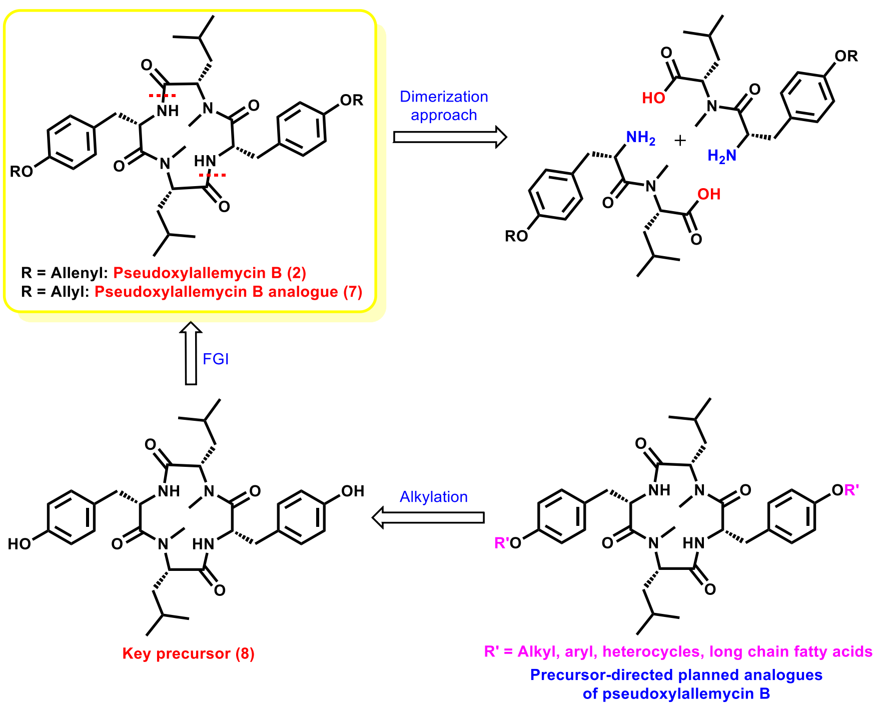
The appearance of characteristic peak at 1955 cm^{-1} further validated the presence of allene functionality by IR spectroscopy. Besides, this macrocyclic natural product contains an alternate pattern of *N*-methyl group present at L-leucine. Pseudoxylallemycin B shows moderately potent antimicrobial activity against Gram-negative bacteria *Pseudomonas aeruginosa* with MIC: 12.5 μg/mL. In addition, this molecule is also found to possess antiproliferative activity (GI_{50}: 9.8 μg/mL and 25.5 μg/mL in HUVEC and K-562 cell lines, respectively).[24] Unique structural complexity with an allenyl moiety as well as potency against Gram-negative pathogen makes pseudoxylallemycin as an interesting as well as attractive synthetic target.

Looking into these interesting features, we became interested in the total synthesis of pseudoxylallemycin B followed by synthesis of its analogues. In an attempt toward the total synthesis of pseudoxylallemycin B (**2**), a homo-dimeric, *N*-methylated macrocyclic tetrapeptidic natural product, synthesis of its epimer at position 3 (D-Tyr instead of L-Tyr) is described here. During the course of synthesis we came across an unusual observation of complete epimerization which led to the formation of 3-*epi*-pseudoxylallemycin B. Our efforts toward the synthesis of pseudoxylallemycin B are described in the following section.

2.3. Total synthesis of 3-*epi*-pseudoxylallemycin B

2.3.1. Retrosynthetic analysis

Our strategy to access the target natural product pseudoxylallemycin B and its analogues is outlined in Scheme 2.1. One of the best strategies to access homo-dimeric cyclic tetrapeptide is the dimerization of corresponding dipeptides where reactive *N*- and C-termini comes in close spatial proximity thereby facilitating ring closure.[25] We planned a dimerization approach using the dipeptide fragment to build the macrocyclic tetrapeptide by utilizing solution phase peptide synthesis. It is important to note that, variations in the required alkylating agents will lead to the synthesis of a library of analogues around the scaffold.

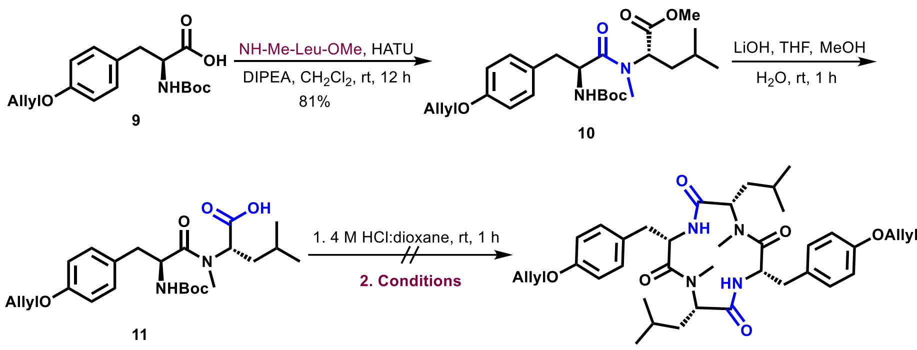


Scheme 2.1. Retrosynthetic analysis

2.3.2. Macrolactamization approach through dimerization

According to the plan, our synthesis began with the preparation of dipeptide **10** from **9**[26] and *N*-Me-L-leucine methyl ester[27] using the solution phase peptide coupling. Formation of dipeptide **10** was confirmed by IR, ^{1}H, ^{13}C NMR, and HRMS spectral analysis. In ^{1}H NMR olefin peaks of allyl group appeared at δ 6.11 - 5.92 (m, 1H), 5.41 - 5.21 (m, 2H) ppm respectively, methyl ester protons were appeared at δ 3.65 (s, 3H) and corresponding *N*-methyl peak observed at δ 2.78 (s, 3H). Two amide carbonyl carbons showed peaks at δ 172.6, 171.8 ppm respectively, whereas

Boc carbonyl group was present at δ 157.4 ppm and methyl ester at δ 51.6 ppm. Methyl ester of **10** was hydrolyzed using LiOH in THF: MeOH: H_2O (3:2:1 v/v/v) to get acid **11**. On preliminary analysis, TLC showed complete consumption of **10** with formation of new polar dragging natured spot. Acid **11** was confirmed by IR, ^1H, ^{13}C NMR and HRMS analysis. ^1H NMR shows disappearance of characteristic methyl ester peak at δ 3.65 (s, 3H). HRMS (ESI) showed peak at 471.2466 with molecular formula $C_{24}H_{36}N_2O_6Na$ $[M + Na]^+$ calculated for mass 471.2460. After successful synthesis of acid **11**, the main task was to dimerization of this amino acid. Towards this, compound **11** was treated with 4 M HCl in dioxane for 1 h in order to deprotect Boc group and forwarded for dimerization without purification.



Sr. No.	Conditions	Observations
1	HATU, DIPEA, CH_2Cl_2: DMF, rt, 24 h	No reaction
2	HATU, HOAt, DIPEA, CH_2Cl_2: DMF, rt, 24 h	No reaction
3	PyBOP, DIPEA, CH_2Cl_2: DMF, rt, 48 h	No reaction
4	FDPP, DIPEA, DMF, rt, 72 h	No reaction
5	T3P, DIPEA, CH_2Cl_2, rt, 24 h	No reaction

Scheme 2.2. Attempts for dimerization macrolactamization

Dimerization of acid **11** was performed using HATU and DIPEA in mixture of CH_2Cl_2 and DMF at room temperature. TLC analysis showed no product formation even after 24 h. Other reagents such as PyBOP, FDPP and T3P were also failed to yield the desired dimerization product. At most care, we also tried several other dimerization macrolactamization conditions on the concerned dipeptide but were unable to achieve the required macrocycle (Scheme 2.2.). We also checked for product formation by means of mass spectral analysis in crude reaction mixtures and found no product formation.

After attempting all above said conditions to dimerization approach that went in vein, we decided to change the strategy to linear approach through a macrocyclization step at the end.

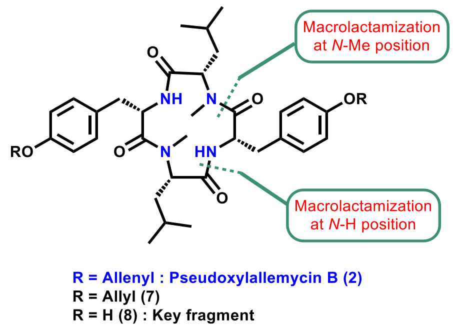
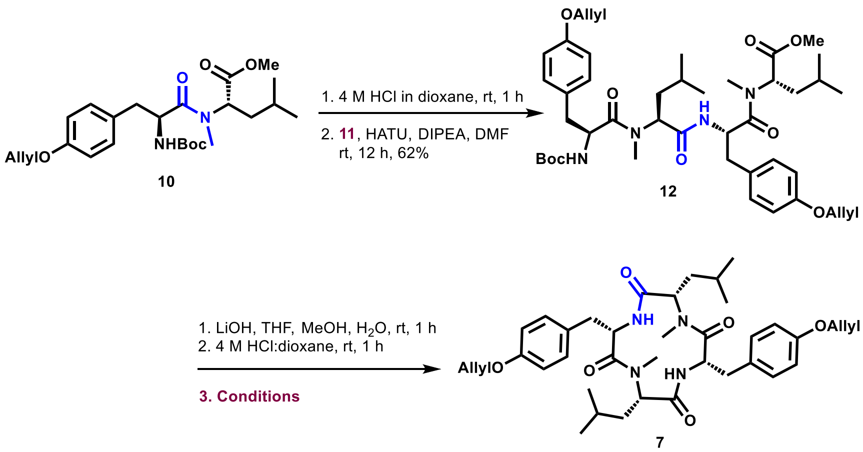


Figure 2.4. Possible ways for macrolactamization

As pseudoxylallemycin B (**2**) is a homo-dimeric cyclic tetrapeptide there are two possible ways of macrolactamization: (1) macrolactamization at *N*-H position, and (2) macrolactamization at *N*-Me position as shown in figure 2.4.

2.3.3. Macrolactamization at *N*-H position

The linear peptide **12** was successfully synthesized using 4 M HCl in dioxane as Boc deblocking agent for dipeptide **10** followed by coupling with acid **11** using HATU as the coupling reagent in 62% yield. The structure of tetrapeptide was confirmed by IR, ^{1}H, ^{13}C NMR, and HRMS analysis. Aromatic protons were appeared at δ 7.04 (d, *J* = 7.9 Hz, 2H), 7.09 (d, *J* = 7.9 Hz, 2H) and 6.90 - 6.74 (m, 4H), respectively. Methyl ester group was present at δ 3.67 (m, 3H) as mixture of rotamers. A careful ^{13}C NMR analysis also confirmed the product formation as mixture of rotamers. HRMS (ESI) showed peak at 715.4476 with molecular formula $C_{39}H_{56}N_4O_7Na$ $[M + Na]^+$ for calculated value 715.4460 further confirmed the structure. Tetrapeptide **12** on saponification using LiOH in THF: MeOH: H_2O (3:2:1) afforded acid which was forwarded for acidolytic cleavage of Boc group to give amine hydrochloride salt (forwarded for cyclization without purification). We anticipated cyclization of synthesized peptide with coupling reagents such as HATU, PyBOP, FDPP or DMTMM.BF_4 will work but all our attempts were proved to be unsuccessful to get the desired compound **7**.



Sr. No.	Conditions	Observations
1	HATU, DIPEA, CH_2Cl_2: DMF, rt, 24 h	No reaction
2	HATU, HOAt, DIPEA, CH_2Cl_2: DMF, rt, 24 h	No reaction
3	PyBOP, DIPEA, CH_2Cl_2: DMF, rt, 48 h	No reaction
4	FDPP, DIPEA, DMF, rt, 72 h	No reaction
5	DMTMM.BF_4, DIPEA, DMF, rt, 24 h	No reaction

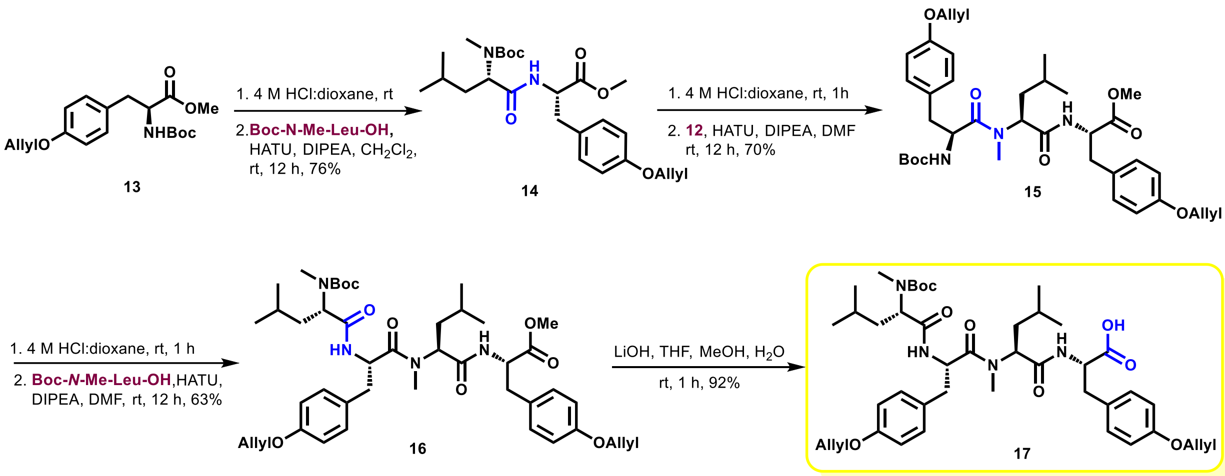
Scheme 2.3. Macrocyclization at *N*-H position

Similar unsuccessful efforts of cyclization at *N*-H position were also reported by Brimble's group during the cyclization of linear *N*-methylated tetrapeptides[28] like pseudoxylallemycin A[29]. We therefore focused our attention on the strategy of cyclization of at *N*-Me position.

2.3.4. Macrolactamization at *N*-Me position

During the course of this project, we happened to come across an elegant study by Brimble's group which reflects the feasibility of macrolactamization at *N*-Me position using propylphosphonic anhydride (T3P) to construct similar tetrapeptidic macrocycles.[28] We thus planned to synthesize our key macrocycle *via* T3P mediated macrolactamization at *N*-Me position.

Boc deprotection of compound **13**[26] using 4 M HCl in dioxane followed by coupling with Boc-*N*Me-leu-OH[30] using HATU and DIPEA in dichloromethane afforded dipeptide **14** in 76% yield. Structure of compound **14** was confirmed by IR, ^{1}H, ^{13}C, and HRMS analysis. In ^{1}H NMR characteristic olefin peaks were appeared at δ 6.05 - 5.96 (m, 1H), 5.36 (d, *J* = 17.1 Hz, 1H), 5.27 - 5.23 (m, 1H), methyl ester was appeared at δ 3.70 (br. s, 3H) and *N*-methyl at δ 2.55 (br. s, 3H) ppm, while δ 133.1 and 117.4 for olefins of allyl group and δ 36.1 for *N*-Me carbon in ^{13}C NMR analysis. HRMS (ESI) showed peak at 485.2630 corresponding to formula $C_{25}H_{38}N_2O_6Na$ [M + Na]$^+$ with calculated value of 485.2644.

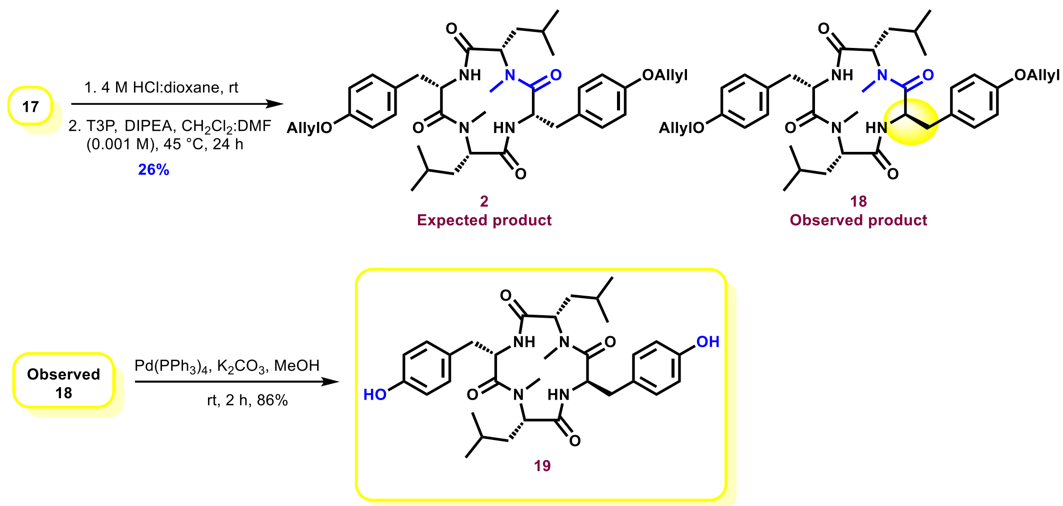


Scheme 2.4. Synthesis of tetrapeptide acid

Deprotection of Boc group in **14** by HCl in dioxane followed by coupling the same with Boc-tyr(allyl)-OH using HATU gave tripeptide **15** in 70% yield which was confirmed by IR, ^{1}H, ^{13}C, and HRMS analysis. The total number of protons in ^{1}H NMR confirmed the formation of desired tripeptide **15**. ^{13}C NMR appeared as mixture of rotamers with three amide carbonyls at δ 173.2, 171.8, 170.1, 168.7 and peak corresponding to carbamate carbonyl at δ 157.8 ppm. It was further confirmed by HRMS, which showed exact mass value at 688.3568 corresponding to molecular formula $C_{37}H_{51}N_3O_8Na$ [M + Na]$^+$ with calculated value 688.3592. Compound **15** was treated with 4 M HCl in dioxane and coupled with Boc-*N*Me-leu-OH to obtain tetrapeptide **16** in 63% yield. IR, ^{1}H, ^{13}C NMR, and HRMS analysis confirmed structure of tetrapeptide **16**. Ester hydrolysis of **16** furnished the required tetrapeptide acid cyclization precursor **17** as colourless sticky liquid in 92% yield. In ^{1}H NMR characteristic aromatic peaks and olefins of allyl group were appeared at δ 6.96 - 7.09 (m, 4H), 6.72 - 6.82 (m, 4H) and 5.99 - 6.02 (m, 2H), 5.23 - 5. 41 (m, 4H) ppm respectively. Also, peaks appearing in the region δ 0.81 - 0.87 (m, 12H) ppm were found to be associated with four methyl groups of leucine. Disappearance of peak at δ 3.64 - 3.69

(m, 3H) ppm indicates formation of acid. HRMS (ESI) showed peak at 777.4433 corresponding to formula $C_{43}H_{61}N_4O_9$ $[M - H]^-$ with calculated value of 777.4517.

After having the requisite cyclization precursor **17** in hand, attempted key macrocyclization step. Compound **17** was treated with 4 M HCl in dioxane to afford the corresponding amino acid which on treatment with T3P and DIPEA in CH_2Cl_2 and DMF (9:1) mixture for 24 h resulted in the formation of the macrocyclic compound **18** as white solid with a melting point 201-203 °C.

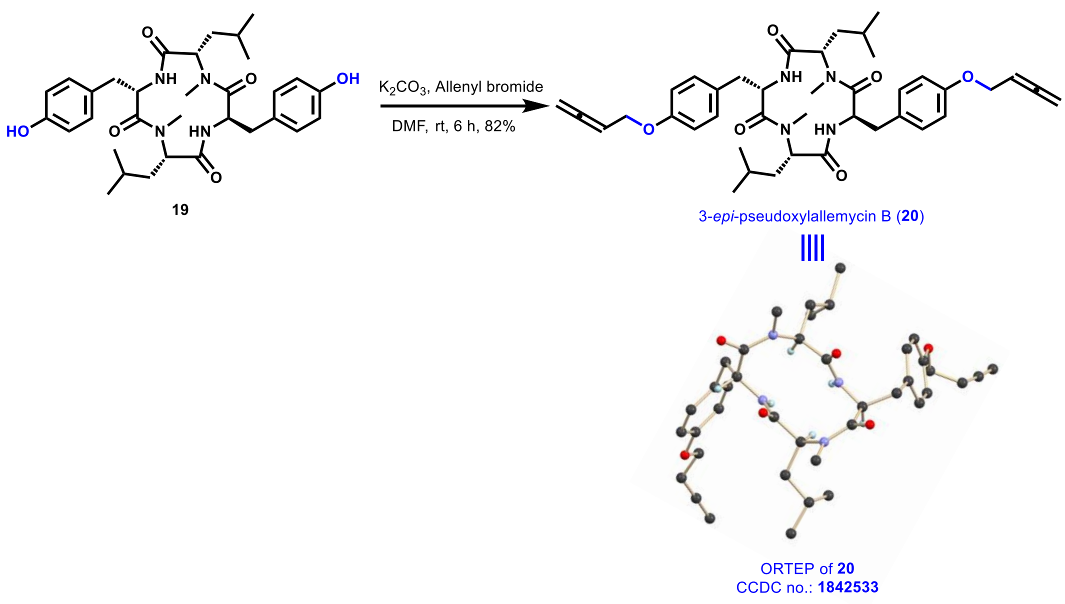


Scheme 2.5. Macrocyclization and synthesis of key fragment

Structure of compound **18** was confirmed by IR, ^{1}H, ^{13}C, and HRMS analysis. HRMS (ESI) showed peak at 661.3960 corresponding to formula $C_{38}H_{52}N_4O_6$ $[M + H]^+$ with calculated value 661.3978. Macrocycle **18** was subjected to allyl deprotection in the presence of K_2CO_3 and $Pd(PPh_3)_4$ in methanol afforded the key macrocycle **19** which in preliminary characterization showed peak at 3444 cm^{-1} for hydroxyl group in IR spectroscopy. The characteristics α-methine peaks were appeared at δ 4.96 (br. s, 1H), 4.32 (br. s, 1H), 4.14 (br. s, 1H), 3.97 (br. s, 1H) ppm in ^{1}H NMR and δ 65.2, 65.1, 56.5 ppm in ^{13}C NMR. In HRMS (ESI) we observed peak at 603.3153 for molecular formula $C_{32}H_{44}N_4O_6Na$ $[M + Na]^+$ with calculated mass 603.3181.

At this stage, key macrocycle **19** underwent alkylation smoothly with allenyl bromide (synthesized from allenyl alcohol and immediately used for coupling without purification due to volatile and lachrymatory nature of the compound)[31] using potassium carbonate in DMF furnished compound **20**. The characteristics α-methine peaks were appeared at δ 4.51 - 4.53 (m, 1H), 4.36 (br. s, 1H), 4.14 (br. s, 1H), 3.96 (br. s, 1H) ppm, two *N*-methyl were appeared at δ

2.87 (br. s, 6H) ppm and allenyl protons at δ 5.40 - 5.48 (m, 2H) and 4.96 (t, *J* = 6.7 Hz, 4H) ppm in ^{1}H NMR. The characteristic allenyl carbons were appeared at δ 208.7, 208.6, 87.1, 87.0 and two carbons at δ 76.9 ppm in ^{13}C NMR. HRMS (ESI) showed peak at 707.3779 which was calculated for $C_{40}H_{52}N_4O_6Na$ $[M + Na]^+$ with mass 707.3799.



Scheme 2.6. Synthesis of 3-*epi*-pseudoxylallemycin B

However, ^{1}H and ^{13}C NMR of **20** was not in good agreement with the reported NMR data of natural pseudoxylallemycin B **(2)**.[24] The original pseudoxylallemycin B (**2**) reported to be a white solid with specific rotation of –232.5 (*c* 0.035, MeOH) and as it is a homo-dimeric cyclic tetrapeptide, shows two sets of α-methine protons in ^{1}H NMR; one is at δ 4.76 (m, 2H) and other at δ 3.83 (m, 2H) ppm.[24] For synthesized compound **20**, optical rotation was $[\alpha]_D^{25}$ = –91.1 (*c* 0.6, MeOH) and all the *α*-protons were appeared separately as δ 4.5 - 4.53 (m, 1H), 4.36 (br. s, 1H), 4.14 (br. s, 1H) and 3.96 (br. s, 1H) ppm. This observation implies that there might be presence of unsymmetry in the molecule due to which all *α*-protons were appeared separately. To our delight, compound **20** could be crystallized from a mixture of *n*-hexane and THF. To decipher the reasons for such NMR discrepancies, we diffracted the single crystal of compound **20** and the X-ray crystal structure disclosed the surprising fact of epimerization of one of the L-Tyrosine stereocenter to D-Tyrosine (Scheme 2.6). We came across a striking yet unusual observation of complete epimerization of pseudoxylallemycin B which led to the formation of 3-*epi*-

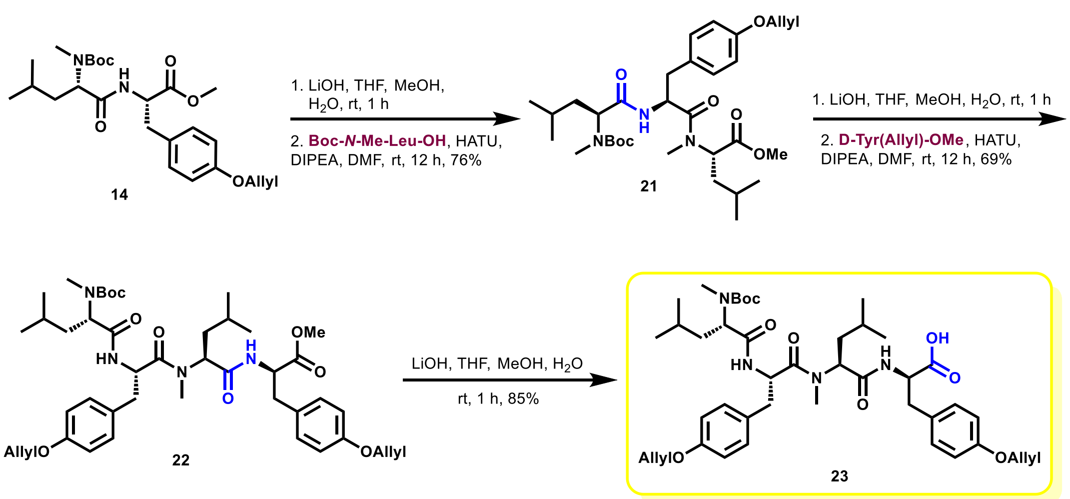
pseudoxylallemycin B (D-Tyr instead of L-Tyr). To suppress or minimize the extent of racemization of that particular amino acid, we also tried macrolactamization at room temperature. However, we did not observe any changes in the outcome of the reaction.

2.3.5. Macrolactamization at *N*-Me position with D-Tyr at C-terminal

Though epimerization during amino acid coupling is well-reported[32], complete epimerization during macrolactamization is unprecedented with only one report in the literature[33]. According to our observations, there could be two possibilities for epimerization:

1) Epimerization during amino acid coupling or
2) Epimerization under macrocyclization condition.

To address this issue, we planned to synthesize tetrapeptide with D-tyrosine at C-terminal.
Also, to rule out the possibility of epimerization during amino acid coupling, we commenced the synthesis of tetrapeptide containing D-Tyr at C-terminal.

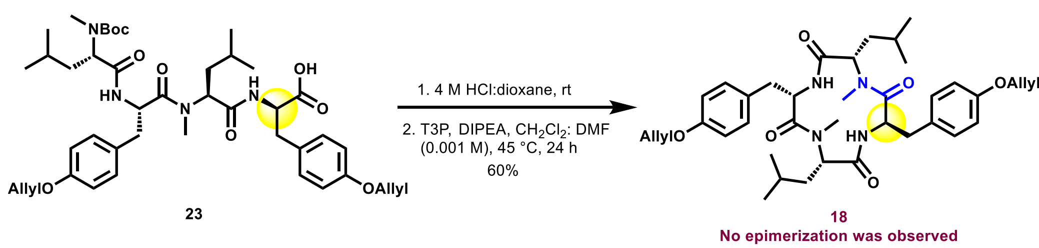


Scheme 2.7. Synthesis of tetrapeptide acid with D-Tyr at C-terminal

Dipeptide **14** on saponification followed by coupling with *N*-Me-leu-OMe using HATU and DIPEA in DMF furnished **21**. In ^{1}H NMR peaks at δ 6.58 - 6.69 (m, 1H), 5.92 - 6.02 (m, 1H), 5.33 (d, 1H) ppm appeared for olefin protons of allyl group, singlet at δ 3.63 ppm corresponds to methyl ester and two broad singlet at δ 2.77 and 2.53 ppm corresponds to two *N*-methyl. The HRMS analysis revealed a peak at 589.3737 corresponding to the molecular ion $C_{32}H_{51}N_3O_7$ [M + H]$^+$ with calculated mass 589.3727 further confirmed the structure. Methyl ester of **21** was hydrolyzed to its corresponding acid and was coupled with NH_2-D-tyr(allyl)-OMe[34] to yield **22**.

^{1}H and ^{13}C NMR analysis manifest the formation of structure which further supported by HRMS (ESI) where a peak appeared at 815.4572 correspond to molecular formula $C_{44}H_{64}N_4O_9Na$ $[M + Na]^+$ with calculated value of 815.4598. Ester hydrolysis of **22** with LiOH successfully afforded the required D-tyr-tetrapeptide acid **23**. In ^{1}H NMR characteristic aromatic peaks and olefins of allyl group were appeared at δ 6.97 (br. s, 2H), 6.82 (br. s, 4H), 6.74 (br. s, 2H) and 5.96 - 6.11 (m, 2H), 5.38 (d, *J* = 15.6 Hz, 2H), 5.27 (br. s, 2H) ppm respectively. Also, peaks at δ 0.82 - 0.91 (m, 12H) ppm was appeared for four methyl groups from leucine. Disappearance of peak at δ 3.62 - 3.72 (m, 3H) ppm indicates formation of acid. ^{1}H NMR comparison of both L-tyr tetrapeptide acid **17** and D-tyr tetrapeptide acid **23** exhibited substantial differences in chemical shifts with respect to compound **23**, which rules out the possibility of epimerization during amino acid coupling.

Next, to find out the epimerization possibility under T3P mediated macrolactamization reaction conditions, compound **23** was treated with HCl in dioxane to produce corresponding cyclization precursor, which was further subjected to cyclization under conditions similar to those we have previously employed successfully afforded the macrocycle **18** in 60% yield.



Scheme 2.8. Synthesis of macrocycle with D-tyr

All the spectral data in complete agreement with the structure and exactly matching with that of previously synthesized compound **18** (in Scheme 2.5.). This observation suggests that no epimerization took place in present case as it is matching exactly with the previously synthesized macrocycle **18**. Improved yield in the case of D-tyr tetrapeptide when compared with L-tyr tetrapeptide also suggests that formation of 3-*epi*-pseudoxylallemycin B (**20**) is more favorable than the natural product **2**. These observations also evidenced concept of less synthetic difficulties in cyclotetrapeptides containing at least one D-amino acids.[35] The absence of any favorable geometrical constrains or structural pre-organization in the linear tetrapeptide in the transition state as well as the possible development of a 12 membered ring strain might have contributed to this unusual complete epimerization.[35] Very recently, Brimble's group also observed the similar phenomenon of epimerization during the synthesis of endolide[36] and pseudoxylallemycin A[29]. During the synthesis of endolide, Brimble's group observed that tetrapeptides with L/D amino acid at C-terminal undergoes epimerization during macrocyclization and this could be controlled by varying reagents from PyAOP to T3P.[36] It was also reported that in case of pseudoxylallemycin A, macrolactamization under T3P or PyAOP conditions afforded epimerized product as major isomer but the extent of epimerization can be minimized by the use of polar solvents.[29]

2.4. Conclusions

We have successfully synthesized 3-*epi*-pseudoxylallemycin B. Being a homo-dimeric cyclic peptide; our initial approach of dimerization strategy did not produce the desired macrocycle. *N*-methylated residues at the *N*-terminus of linear tetrapeptides were found to be the most effective precursors for solution-phase cyclization. During the course of synthesis, we also observed an unusual epimerization at C-3 amino acid center of L-tyrosine bearing tetrapeptide. Similar phenomenon was not detected during lactamization of D-tyrosine bearing tetrapeptide.

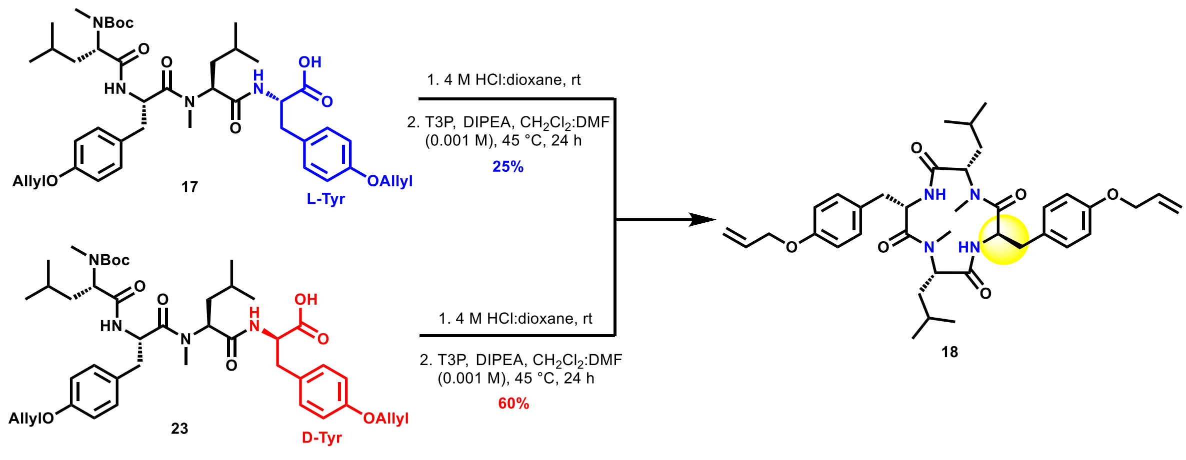
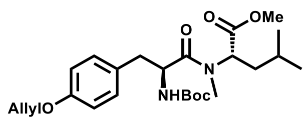


Figure 2.5. Synthesis of macrocycle

The probable cause for this complete epimerization can be cited to the developed strain during the formation of a 12-membered ring. Besides, it can also be addressed to the unfeasibility in cyclization during the formation of cyclic tetrapeptides bearing all L-amino acids. Late stage alkylation of the macrocyclic unit was performed to have analogues around pseudoxylallemycin B scaffold. Although we have not accomplished the total synthesis of target natural product, the present work carried out in this project helped us in understanding the requirements for efficient macro cyclization and synthetic strategy to access library of analogs towards the development of antibacterial agents.

2.5. Experimental section

Methyl *N*-((*S*)-3-(4-(allyloxy)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)-*N*-methyl-L-leucinate (10)



10

HATU (3.5 g, 9.27 mmol) was added to compound **9** (1.6 g, 6.18 mmol) in CH_2Cl_2 (20 mL), at 0 °C followed by the addition of *N*-Me-Leu-OMe.HCl (1.98 g, 6.18 mmol) and DIPEA (3.2 mL, 18.53 mmol). The reaction mixture was stirred at room temperature for 12 h, after which it was diluted with CH_2Cl_2 (50 mL), washed with H_2O (30 mL), saturated $NaHCO_3$ solution (30 mL), 1 N HCl (30 mL) and brine (25 mL). It was then dried over Na_2SO_4 and concentrated under *vacuo*. The crude product was purified *via* column chromatography (10% EtOAc/ PE, R_f = 0.5 in 20% EtOAc/PE) to afford compound **10** as a colorless sticky liquid.

Yield: 81% (2.52 g)

IR ν_{max} (film): 3019, 1705, 1645, 1499, 1218 cm^{-1}

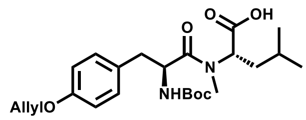
Specific rotation: $[\alpha]_D^{22}$ = –13.1 (*c* 1.92, $CHCl_3$)

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.12 - 7.05 (m, 2H), 6.82 - 6.78 (m, *J* = 8.6 Hz, 2H), 6.11 - 5.92 (m, 1H), 5.41 - 5.21 (m, 4H), 4.82 - 4.71 (m, 1H), 4.49 - 4.46 (m, 2H), 3.65 (s, 3H), 3.06 - 2.95 (m, 1H), 2.85 (d, *J* = 6.1 Hz, 1H), 2.78 (s, 3H), 1.69 - 1.61 (m, 2H), 1.52 - 1.43 (m, 1H), 1.37 - 1.35 (m, 9H), 0.87 (t, *J* = 6.4 Hz, 6H)

^{13}C NMR (50 MHz, $CDCl_3$): δ 172.6, 171.8, 157.4, 155.1, 133.2, 130.4, 128.3, 117.4, 114.5, 79.4, 77.2, 68.6, 54.3, 51.9, 51.6, 37.8, 37.0, 30.9, 29.5, 28.1, 24.5, 23.1, 21.3

HRMS (ESI): calculated for $C_{25}H_{38}N_2O_6Na$ $[M + Na]^+$: 485.2644, found 485.2622.

***N*-((*S*)-3-(4-(allyloxy)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)-*N*-methyl-L-leucine (11)**



11

To compound **10** (0.20 g, 0.43 mmol) in THF and MeOH (3:2, 5 mL) at 0 °C, aqueous solution of lithium hydroxide monohydrate (28 mg, 0.52 mmol) was added. After completion, the reaction mixture was concentrated under *vacuo*, acidified with 1 N HCl and extracted with ethyl acetate (5 mL X 3). The collected organic layers were dried over Na_2SO_4, concentrated under *vacuo* to afford product **11** as colorless sticky liquid.

Yield: 89% (0.173 g)

IR ν_{max} (film): 3130, 2407, 1710, 1637, 1499 cm^{-1}

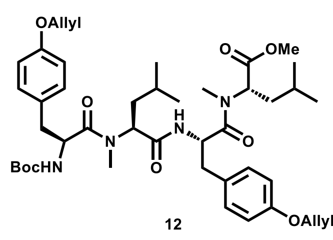
Specific rotation: $[\alpha]_D^{26}$ = –9.33 (*c* 0.786, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.14 (br. s, 1H), 7.12 (d, *J*=7.9 Hz, 2H), 6.81 (d, *J*=7.9 Hz, 2H), 6.02 (ddt, *J*=16.3, 10.8, 5.0 Hz, 1H), 5.66 (d, *J*=8.5 Hz, 1H), 5.24 - 5.40 (m, 3H), 4.79 (d, *J*=7.3 Hz, 1H), 4.47 (br. s, 2H), 3.02 (dd, *J*=13.1, 7.0 Hz, 1H), 2.84 (br. s, 4H), 1.72 - 1.74 (m, 2H), 1.48 (br. s, 1H), 1.36 - 1.39 (m, 9H), 0.83 - 0.93 (m, 6H)

^{13}C NMR (50 MHz, $CDCl_3$): δ 175.7, 173.6, 157.5, 155.5, 133.3, 130.5, 128.3, 117.5, 114.7, 79.7, 68.7, 54.8, 52.0, 37.6, 36.9, 31.2, 29.6, 28.2, 24.6, 23.2, 21.3

HRMS (ESI): calculated for $C_{24}H_{36}N_2O_6Na$ $[M + Na]^+$: 471.2460, found 471.2466.

Methyl *N*-((S)-3-(4-(allyloxy)phenyl)-2-((*S*)-2-((*S*)-3-(4-(allyloxy)phenyl)-2-amino-*N*-methylpropanamido)-4-methylpentanamido)propanoyl)-*N*-methyl-L-leucinate (12)



12

To compound **10** (3.00 g, 6.49 mmol) HCl in dioxane (4 M, 10 mL) was added and stirred at room temperature for 1 h. Upon completion of reaction (monitored by TLC) it was concentrated under *vacuo* and forwarded for coupling without further purification. Coupling was done by following similar procedure as did for synthesis of compound **10** to afford **12** as a colorless sticky liquid (eluted in 25% EtOAc/ PE, R_f = 0.35 in 50% EA/PE).

Yield: 62%

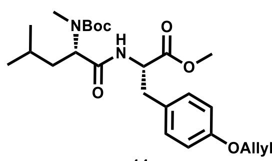
IR ν_{max} (film): 3418, 3020, 1742, 1679 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers): δ 7.09 - 7.02 (m, 4 H), 6.85 - 6.74 (m, 4 H), 6.04 - 5.93 (m, 2 H), 5.38 - 5.31 (m, 2 H), 5.31 - 5.21 (m, 4 H), 5.08 (td, *J* = 7.1, 14.5 Hz, 1H), 4.71 - 4.60 (m, 1 H), 4.48 - 4.38 (m, 4 H), 3.67 - 3.61 (m, 3 H), 3.02 (dd, *J* = 7.3, 13.4 Hz, 1 H), 2.84 - 2.79 (m, 4 H), 2.76 - 2.68 (m, 2 H), 2.62 (s, 3 H), 1.69 - 1.57 (m, 3 H), 1.51 - 1.47 (m, 1 H), 1.43 (s, 2 H), 1.39 - 1.35 (m, 9 H), 0.91 - 0.81 (m, 12 H)

^{13}C NMR (100 MHz, $CDCl_3$) (mixture of rotamers): δ 173.0, 172.0, 171.9, 171.7, 171.0, 169.8, 168.1, 157.7, 157.4, 157.4, 157.2, 155.1, 133.2, 133.1, 133.1, 130.3, 130.2, 130.1, 128.5, 128.4, 128.1, 128.0, 117.5, 117.4, 117.4, 114.9, 114.8, 114.8, 114.7, 114.5, 80.7, 79.5, 68.6, 68.6, 68.6, 60.2, 58.0, 54.6, 54.5, 54.2, 52.3, 52.0, 51.9, 51.8, 51.2, 50.7, 50.1, 37.9, 37.3, 37.1, 37.0, 36.4, 30.9, 30.3, 28.3, 28.2, 28.1, 24.6, 24.6, 24.4, 23.2, 23.1, 23.0, 21.8, 21.6, 21.3, 20.9, 14.1

HRMS (ESI): calculated for $C_{39}H_{56}N_4O_7Na$ $[M + Na]^+$: 715.2644, found 715.2630.

Methyl (*S*)-3-(4-(allyloxy)phenyl)-2-((*S*)-2-((*tert*-butoxycarbonyl)(methyl)amino)-4 methylpentanamido)propanoate (14)



14

Compound **14** was synthesized from compound **13** and Boc-*N*-Me-Leu-OH by following similar procedure as that for the synthesis of compound **10**.

Yield: 76%

IR ν_{max} (film): 3418, 3020, 1742, 1679 cm^{-1}

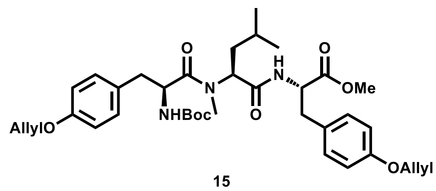
Specific rotation: $[\alpha]_D^{23}$ = –37.6 (*c* 0.91, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.06 - 6.96 (m, 2H), 6.80 (d, *J* = 8.5 Hz, 2H), 6.49- 6.30 (m, 1H), 6.05 - 5.96 (m, 1H), 5.36 (d, *J* = 17.1 Hz, 1H), 5.27 - 5.23 (m, 1H), 4.77 (br. s, 1H), 4.65 - 4.56 (m, 1H), 4.47 (d, *J* = 4.9 Hz, 2H), 3.70 (br. s, 3H), 3.10 - 3.06 (m, 1H), 2.94 (br. s, 1H), 2.55 (br. s, 3H), 1.63 - 1.59 (m, 2H), 1.52 (s, 1H), 1.42 (br. s, 9H), 0.91 - 0.86 (m, 6H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7, 170.9, 157.6, 156.4, 133.1, 130.0, 128.0, 127.6, 117.4, 114.8, 80.7, 80.2, 80.1, 68.6, 57.0, 55.9, 53.0, 52.8, 52.2, 37.2, 37.0, 36.1, 29.6, 29.5, 29.2, 28.2, 27.6, 24.6, 24.4, 23.1, 21.7, 21.2, 21.2

HRMS (ESI): calculated for $C_{25}H_{38}N_2O_6Na$ $[M + Na]^+$: 485.2644, found 485.2630.

Methyl (6*S*,9*S*,12*S*)-6,12-bis(4-(allyloxy)benzyl)-9-isobutyl-2,2,8-trimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oate (15)



15

Compound **15** was synthesized from compound **14** and Boc-Tyr(allyl)-OH by following similar procedure of compound **12**.

Yield: 70%

IR ν_{max} (film): 3330, 1593, 1418, 1217 cm^{-1}

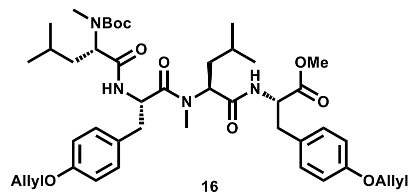
Specific rotation: $[\alpha]_D^{22}$ = –32.6 (*c* 0.63, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers): δ 6.97 - 7.09 (m, 4H), 6.78 - 6.86 (m, 4H), 6.15 (d, *J*=7.9 Hz, 1H), 5.93 - 6.08 (m, 2H), 5.33 - 5.41 (m, 2H), 5.24 - 5.29 (m, 2H), 5.18 (d, *J*=8.5 Hz, 1H), 5.10 (dd, *J*=9.2, 6.1 Hz, 1H), 4.66 - 4.74 (m, 2H), 4.46 - 4.51 (m, 3H), 4.40 (d, *J*=4.3 Hz, 1H), 3.68 - 3.72 (m, 3H), 3.00 - 3.12 (m, 1H), 2.80 - 2.85 (m, 2H), 2.69 (dd, *J*=13.4, 6.7 Hz, 1H), 2.60 - 2.62 (m, 2H), 2.38 (s, 1H), 1.61 - 1.68 (m, 1H), 1.48 - 1.55 (m, 1H), 1.44 (br. s, 1H), 1.37 - 1.41 (m, 9H), 0.79 - 0.89 (m, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) (mixture of rotamers): δ 173.2, 171.8, 170.1, 168.7, 157.8, 157.6, 157.5, 157.4, 156.0, 155.1, 133.3, 133.1, 130.3, 130.2, 130.0, 129.9, 128.8, 128.5, 128.0, 127.9, 117.7, 117.6, 117.5, 117.5, 115.0, 114.8, 114.7, 80.7, 79.7, 68.7, 68.7, 68.6, 58.3, 54.7, 53.9, 53.2, 52.3, 52.1, 51.8, 51.2, 37.9, 36.9, 36.8, 36.1, 30.4, 28.8, 28.3, 28.2, 24.6, 24.4, 23.1, 23.0, 22.1, 21.8

HRMS (ESI): calculated for $C_{37}H_{51}N_3O_8Na$ $[M + Na]^+$: 688.3592, found 688.3568.

Methyl (6*S*,9*S*,12*S*,15*S*)-9,15-bis(4-(allyloxy)benzyl)-6,12-diisobutyl-2,2,5,11-tetramethyl-4,7,10,13-tetraoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-oate (16)



16

Compound **16** was synthesized from compound **15** and Boc-*N*-Me-Leu-OH by following similar procedure to that of compound **12** to afford **16** as a colorless sticky liquid.

Yield: 63%

IR ν_{max} (film): 3411, 3021, 2963, 2402, 1676 cm^{-1}

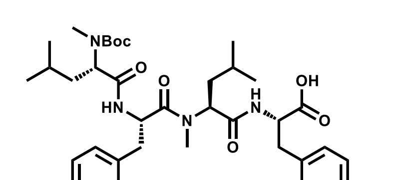
Specific rotation: $[\alpha]_D^{23}$ = –65.6 (*c* 1.12, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers): δ 6.93 - 7.05 (m, 4H), 6.73 - 6.82 (m, 4H), 6.47 - 6.62 (m, 1H), 6.20 (d, *J*=7.9 Hz, 1H), 5.88 - 6.01 (m, 2H), 5.28 - 5.37 (m, 2H), 5.18 - 5.24 (m, 2H), 5.06 (t, *J*=7.6 Hz, 1H), 4.87 - 5.00 (m, 1H), 4.67 - 4.72 (m, 1H), 4.62 (br. s, 1H), 4.42 - 4.45 (m, 3H), 4.34 (d, 1H), 3.64 - 3.69 (m, 3H), 3.05 (d, *J*=13.4 Hz, 1H), 2.81 (dd, *J*=12.2, 6.7 Hz, 2H), 2.63 - 2.70 (m, 3H), 2.50 (s, 3H), 2.41 - 2.43 (m, 1H), 1.60 - 1.67 (m, 1H), 1.50 - 1.54 (m, 2H), 1.44 - 1.45 (m, 9H), 1.29 - 1.40 (m, 3H), 0.80 - 0.91 (m, 12H)

^{13}C NMR (100 MHz, $CDCl_3$) (mixture of rotamers): δ 172.2, 171.6, 170.9, 170.4, 169.9, 168.5, 157.5, 157.4, 157.2, 133.2, 133.0, 132.9, 130.1, 130.0, 129.8, 127.8, 117.4, 117.4, 117.3, 117.3, 115.0, 114.7, 114.6, 80.1, 80.0, 68.6, 68.5, 68.4, 58.3, 54.6, 53.9, 53.0, 52.2, 51.9, 50.1, 49.9, 37.4, 37.0, 36.7, 36.5, 36.3, 36.0, 30.3, 29.5, 28.7, 28.2, 24.5, 23.1, 22.8, 22.2, 21.7

HRMS (ESI): calculated for $C_{44}H_{64}N_4O_9Na$ $[M + Na]^+$: 815.4598, found 815.4566.

(6*S*,9*S*,12*S*,15*S*)-9,15-bis(4-(allyloxy)benzyl)-6,12-diisobutyl-2,2,5,11-tetramethyl-4,7,10,13-tetraoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-oic acid (17)



17

Compound **17** was synthesized from compund **16** by following similar procedure used for the synthesis of compound **11** to afford **11** as a colorless sticky liquid (R_f = 0.56 in 100% EA).

Yield: 92%

IR ν_{max} (film): 3341, 3021, 1723, 1676, 1513 cm^{-1}

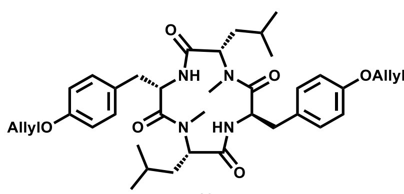
Specific rotation: $[\alpha]_D^{22}$ = –60.2 (*c* 0.31, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers): δ 7.41 (br. s, 1H), 7.12 - 7.29 (m, 1H), 6.96 - 7.09 (m, 4H), 6.72 - 6.82 (m, 4H), 5.99 - 6.02 (m, 2H), 5.23 - 5. 41 (m, 4H), 5.01 (br. s, 1H), 4.90 (br. s, 1H), 4.67 - 4.75 (m, 1H), 4.44 - 4.55 (m, 3H), 3.19 (dd, J = 4.9, 14.0 Hz, 1H), 3.94 - 3.12 (m, 1H), 2.86 - 2.77 (m, 3H), 2.68 - 2.74 (m, 2H), 2.46 - 2.55 (m, 3H), 1.59 - 1.68 (m, 3H), 1.47 (br. s, 9H), 1.25 - 1.33 (m, 3H), 0.81 - 0.87 (m, 12H)

^{13}C NMR (100 MHz, $CDCl_3$) (mixture of rotamers): δ 174.3, 174.2, 172.2, 170.9, 170.5, 170.3, 157.5, 133.3, 133.2, 133.1, 130.3, 130.1, 130.0, 128.7, 128.5, 128.5, 117.6, 117.5, 117.4, 115.1, 114.8, 114.5, 80.9, 77.2, 68.7, 68.7, 68.5, 58.2, 57.5, 55.7, 54.9, 52.9, 50.5, 50.5, 37.3, 37.0, 36.7, 31.9, 30.8, 29.6, 29.4, 29.3, 29.2, 28.3, 24.6, 23.1, 23.0, 22.6, 22.2, 21.9, 21.3, 20.7, 14.1

HRMS (ESI): calculated for $C_{43}H_{61}N_4O_9$ $[M - H]^-$: 777.4517, found 777.4433.

(3*R*,6*S*,9*S*,12*S*)-3,9-Bis(4-(allyloxy)benzyl)-6,12-diisobutyl-1,7-dimethyl-1,4,7,10 tetraazacyclododecane-2,5,8,11-tetraone (18)



18

4 M HCl in dioxane was added to compound **17** (50.0 mg, 64.3 μmol) and stirred at room temperature for 1 h. It was then concentrated under *vacuo*. The amino acid hydrochloride salt thus obtained was dissolved in CH_2Cl_2/DMF (9:1, 65 mL) and treated with propylphosphonic anhydride (T3P, 95.6 μL, 321.3 μmol) followed by the addition of DIPEA (67.0 μL, 385.6 μmol). The reaction mixture was stirred at 45 °C for 24 h. After evaporation of CH_2Cl_2 under reduced pressure, the residue was dissolved in ethyl acetate and washed with chilled saturated $NaHCO_3$ solution and 1 N HCl (5 mL). The collected organic layer was dried over Na_2SO_4, concentrated under *vacuo* and purified by column chromatography (3% MeOH/CH_2Cl_2, R_f = 0.6 in 5% MeOH/CH_2Cl_2) to give **18** as a white powder.

Yield: 26% (from L-Tyr tetrapeptide acid, **17**) and 60% (from D-Tyr tetrapeptide acid, **23**)

Melting point: 201-203˚C

IR ν_{max} (film): 3415, 3022, 1650, 1514, 1216 cm^{-1}

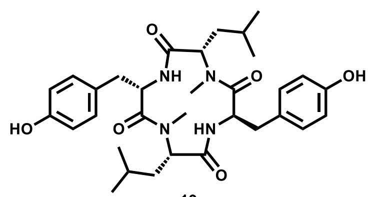
Specific rotation: $[\alpha]_D^{23}$ = –69.5 (*c* 0.28, $CHCl_3$)

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.09 (d, *J*=8.8 Hz, 2H), 7.11 (d, *J*=8.8 Hz, 2H), 6.87 (d, *J*=8.0 Hz, 4H), 5.98 - 6.07 (m, 2H), 5.34 - 5.40 (m, 2H), 5.22 - 5.26 (m, 2H), 4.52 (d, *J*=5.0 Hz, 5H), 4.35 - 4.37 (m, 1H), 4.14 (br. s, 1H), 3.96 (br. s, 1H), 3.00 - 3.03 (m, 1H), 2.91 (br. s, 2H), 2.85 - 2.87 (m, 4H), 2.76 - 2.83 (m, 3H), 1.48 - 1.60 (m, 1H), 1.41 (br. s, 1H), 1.30 - 1.36 (m, 2H), 1.23 - 1.26 (m, 2H), 0.88 - 0.91 (m, 6H), 0.82 (br. s, 6H)

^{13}C NMR (125 MHz, DMSO-d_6): δ 171.0, 170.8, 170.1, 170.1, 156.9, 156.7, 133.9, 133.8, 130.7, 130.1, 129.5, 117.2, 117.1, 114.5, 114.2, 114.2, 79.1, 78.9, 68.1, 68.1, 56.5, 40.1, 39.9, 39.8, 39.6, 35.1, 34.7, 30.5, 30.0, 24.5, 22.9, 22.0

HRMS (ESI): calculated for $C_{38}H_{52}N_4O_6$ $[M + H]^+$: 661.3978, found 661.3960.

(3*R*,6*S*,9*S*,12*S*)-3,9-Bis(4-hydroxybenzyl)-6,12-diisobutyl-1,7-dimethyl-1,4,7,10-tetraazacyclododecane-2,5,8,11-tetraone (19)



19

$Pd(PPh_3)_4$ (0.9 mg, 0.76 μmol) was added to a solution of compound **18** (50.0 mg, 75.76 μmol) in MeOH at 0 °C, followed by the addition of K_2CO_3 (27.182 mg, 196.9 μmol). The reaction mixture was stirred at room temperature untill the starting material was completly consumed. After complete evaporation of solvent, the reaction mixture was diluted with ethyl acetate and washed with H_2O. The collected organic layer was then dried over Na_2SO_4 and concentrated under *vacuo* and purified by column chromatography (5% MeOH/CH_2Cl_2, R_f = 0.4 in 5% MeOH/CH_2Cl_2) to give **19** as a white sticky solid.

Yield: 86%

IR ν_{max} (film): 3444, 2255, 2130, 1646 cm^{-1}

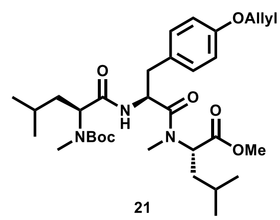
Specific rotation: $[\alpha]_D^{26}$ = –111.3 (*c* 0.136, MeOH)

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.26 (br. s, 2H), 7.08 - 7.11 (m, 1H), 6.96 - 6.98 (m, 4H), 6.87 (br. s, 1H), 6.67 (br. s, 4H), 4.96 (br. s, 1H), 4.32 (br. s, 1H), 4.14 (br. s, 1H), 3.97 (br. s, 1H), 2.87 - 2.95 (m, 10H), 1.40 (br. s, 1H), 1.31 (br. s, 2H), 1.23 (br. s, 2H), 1.17 (br. s, 1H), 0.90 (br. s, 6H), 0.81 (br. s, 6H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 170.1, 170.0, 169.9, 156.1, 155.8, 130.8, 130.6, 130.5, 129.5, 129.4, 115.0, 114.9, 114.7, 114.6, 65.2, 65.1, 56.5, 45.6, 40.1, 39.9, 34.8, 30.5, 30.0, 24.6, 24.4, 23.0, 22.6, 22.1

HRMS (ESI): calculated for $C_{32}H_{44}N_4O_6Na$ $[M + Na]^+$: 603.3181, found 603.3153.

Methyl *N*-((*S*)-3-(4-(allyloxy)phenyl)-2-((*S*)-2-((tert-butoxycarbonyl)(methyl)amino)-4-methylpentanamido)propanoyl)-*N*-methyl-*L*-leucinate (21)



21

Aqueous solution of lithium hydroxide monohydrate (0.21 g, 3.88 mmol) was added dropwise to a solution of compound **14** (1.50 g, 3.24 mmol) in THF: MeOH (3:2, 10 mL) at 0 °C and stirred at room temperature. Upon reaction completion, solvent was removed under reduced pressure and the reaction mass was acidified with 1 N HCl, extracted with ethyl acetate (30 mL x 3) and dried over Na_2SO_4. The organic layer was concentrated under *vacuo* to furnish the corresponding acid. To the solution of this acid in DMF, HATU (3.40 g, 8.96 mmol) was added at 0 °C followed by the addition of *N*-Me-Leu-OMe.HCl (0.63 g, 3.24 mmol) (prepared from the Boc deprotection of corresponding ester using HCl in dioxane at room temperature for 1 h) and DIPEA (1.69 mL, 9.72 mmol). The resultant mixture was stirred at room temperature for 12 h after which it was diluted with CH_2Cl_2 (50 mL), washed with H_2O (20 mL), saturated $NaHCO_3$ solution (20 mL), 1 N HCl (20 mL), and brine (15 mL). The collected organic layer was dried over Na_2SO_4 and concentrated under *vacuo*. The crude product thus furnished was purified by column chromatography (18% EtOAc/PE, R_f = 0.5 in 40% EA/PE) to afford **21** as a colorless sticky liquid.

Yield: 76%

IR ν_{max} (film): 3410, 3018, 1737, 1649, 1505 cm^{-1}

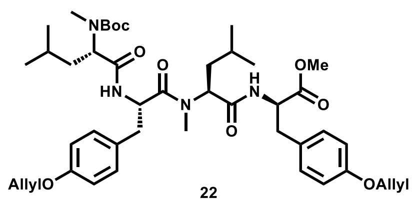
Specific rotation: $[\alpha]_D^{22}$ = –67.4 (*c* 1.47, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers): δ 7.02 (d, *J*=6.7 Hz, 2H), 6.76 (d, *J*=8.5 Hz, 2H), 6.58 - 6.69 (m, 1H), 5.92 - 6.02 (m, 1H), 5.33 (d, 1H), 5.19 - 5.24 (m, 2H), 5.06 (br. s, 1H), 4.62 (br. s, 1H), 4.44 (d, *J*=4.9 Hz, 2H), 3.63 (s, 3H), 3.02 (dd, *J*=14.0, 6.7 Hz, 1H), 2.75 - 2.88 (m, 4H), 2.53 (br. s, 3H), 1.53 - 1.67 (m, 4H), 1.41 - 1.44 (m, 9H), 1.30 - 1.39 (m, 2H), 0.82 - 0.87 (m, 12H)

^{13}C NMR (100 MHz, $CDCl_3$) (mixture of rotamers): δ 171.8, 171.6, 170.8, 170.5, 170.4, 157.3, 156.1, 155.0, 133.1, 130.3, 130.1, 128.1, 127.7, 117.3, 114.4, 80.5, 80.1, 68.5, 60.1, 57.6, 56.8, 55.8, 54.4, 53.3, 52.2, 51.9, 50.0, 49.3, 38.0, 37.9, 37.4, 36.8, 36.3, 31.7, 30.8, 29.5, 29.1, 29.0, 28.1, 24.5, 24.2, 23.0, 22.5, 22.0, 21.6, 21.2, 20.8, 14.0, 13.9

HRMS (ESI): calculated for $C_{32}H_{51}N_3O_7$ $[M + H]^+$ 589.3727, found 589.3737.

Methyl (6*S*,9*S*,12*S*,15*R*)-9,15-bis(4-(allyloxy)benzyl)-6,12-diisobutyl-2,2,5,11-tetramethyl-4,7,10,13-tetraoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-oate (22)



22

Compound **22** was synthesized from **21** by folowing similar procedure used for the synthesis of compound **21**. The crude product thus obtained was purified by column chromatography (50% EA/PE, R_f = 0.4 in 50% EA/PE) to afford **16** as a colorless sticky liquid.

Yield: 69%

IR ν_{max} (film): 3391, 3021, 1658, 1430, 1217 cm^{-1}

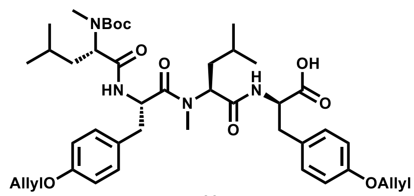
Specific rotation: $[\alpha]_D^{26}$ = –34.2 (*c* 0.925, MeOH)

^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers): δ 7.01 - 7.13 (m, 4H), 6.76 - 6.85 (m, 4H), 6.34 - 6.52 (m, 1H), 5.97 - 6.07 (m, 2H), 5.35 - 5.41 (m, 2H), 5.23 - 5.28 (m, 2H), 4.93 - 5.07 (m, 2H), 4.57 - 4.76 (m, 2H), 4.46 - 4.50 (m, 4H), 3.62 - 3.72 (m, 3H), 3.03 - 3.15 (m, 1H), 2.82 - 2.99 (m, 5H), 2.71 (d, *J*=2.7 Hz, 1H), 2.62 (br. s, 1H), 2.52 (s, 2H), 1.57 - 1.86 (m, 3H), 1.41 - 1.56 (m, 9H), 1.19 - 1.31 (m, 3H), 0.71 - 0.93 (m, 12H)

^{13}C NMR (100 MHz, $CDCl_3$) (mixture of rotamers): δ 172.6, 171.7, 171.8, 171.6, 170.7, 170.0, 169.6, 168.4, 157.7, 157.5, 157.4, 133.3, 133.2, 133.1, 130.1, 130.1, 130.0, 127.9, 127.8, 117.6, 117.5, 117.5, 115.0, 114.8, 114.6, 80.4, 77.2, 68.6, 58.1, 55.9, 54.5, 53.1, 52.3, 52.2, 51.2, 50.2, 37.4, 36.8, 36.7, 36.4, 35.8, 31.9, 30.6, 30.4, 29.6, 29.3, 29.1, 28.3, 24.6, 24.5, 23.1, 23.0, 22.9, 22.6, 22.0, 21.8, 21.7, 21.6, 21.4, 1.0

HRMS (ESI): calculated for $C_{44}H_{64}N_4O_9Na$ $[M + Na]^+$: 815.4598, found 815.4572.

(6*S*,9*S*,12*S*,15*R*)-9,15-bis(4-(allyloxy)benzyl)-6,12-diisobutyl-2,2,5,11-tetramethyl-4,7,10,13-tetraoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-oic acid (23)



23

Compound **23** was synthesized from compund **22** by following similar procedure used for the synthesis of compound **11**. The crude product was then purified by column chromatography (2% $MeOH/CH_2Cl_2$, R_f = 0.6 in 100% EA) to afford **23** as a colorless sticky liquid.

Yield: 85%

IR ν_{max} (film): 3409, 3022, 1674, 1517, 1216 cm^{-1}

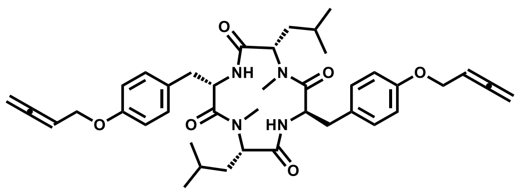
Specific rotation: $[\alpha]_D^{23}$ = –51.2 (*c* 0.19, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$) (mixture of rotamers): δ 7.17 (br. s, 1H), 7.03 - 7.11 (m, 2H), 6.97 (br. s, 2H), 6.82 (br. s, 4H), 6.74 (br. s, 2H), 5.96 - 6.11 (m, 2H), 5.38 (d, *J*=15.6 Hz, 2H), 5.27 (br. s, 2H), 4.98 (br. s, 2H), 4.61 - 4.84 (m, 2H), 4.41 - 4.52 (m, 4H), 3.18 (d, *J*=13.7 Hz, 1H), 2.92 - 3.13 (m, 2H), 2.86 (br. s, 3H), 2.60 - 2.72 (m, 2H), 2.45 - 2.56 (m, 2H), 1.56 (br. s, 4H), 1.48 (br. s, 9H), 1.28 (d, *J*=14.9 Hz, 2H), 0.82 - 0.91 (m, 12H)

^{13}C NMR (100 MHz, $CDCl_3$) (mixture of rotamers): δ 171.0, 170.9, 170.1, 170.0, 157.6, 157.5, 133.3, 133.1, 130.3, 130.2, 130.1, 128.4, 117.7, 117.6, 115.0, 114.7, 114.7, 80.9, 80.7, 77.2, 68.7, 68.7, 55.1, 53.4, 50.6, 38.6, 37.7, 37.2, 36.7, 36.5, 31.9, 31.0, 30.2, 29.7, 29.6, 29.3, 28.4, 28.3, 24.6, 23.3, 23.1, 23.0, 22.8, 22.7, 22.1, 21.9, 21.8, 21.3, 14.1

HRMS (ESI): calculated for $C_{43}H_{61}N_4O_9$ $[M - H]^-$: 777.4517, found 777.4433.

(3*R*,6S,9S,12S)-3,9-bis(4-(buta-2,3-dien-1-yloxy)benzyl)-6,12-diisobutyl-1,7-dimethyl-1,4,7,10-tetraazacyclododecane-2,5,8,11-tetraone (20)



3-*epi*-Pseudoxylallemycin B (**20**)

K_2CO_3 (5.9 mg, 0.04 mmol) was added to a solution of compound **13** (10 mg, 0.02 mmol) in 1 mL of dry DMF at 0 °C followed by the slow addition of allenyl bromide[1] (6.8 mg, 0.05 mmol) dissolved in DMF. The reaction mixture was then stirred at room temperature for 6h. After completion of reaction, small pieces of ice were pored into the reaction mixture and diluted with ethyl acetate (10 mL). The organic layer collected separately was dried over Na_2SO_4 and concentrated under *vacuo*. The crude product thus obtained was purified by column chromatography (4% MeOH/CH_2Cl_2, R_f = 0.6 in 5% MeOH/ CH_2Cl_2) to afford **14** as a white solid.

Yield: 82%

Melting point: 220-222 ˚C

IR ν_{max} (film): 3346, 3023, 1945, 1653, 1522 cm^{-1}

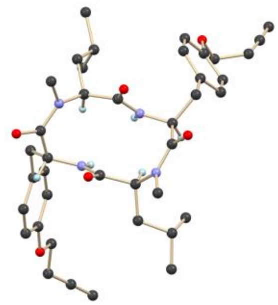
Specific rotation: $[\alpha]_D^{25}$ = –91.1 (*c* 0.6, MeOH)

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.10 (t, *J*=8.2 Hz, 4H), 6.86 (d, *J*=7.3 Hz, 4H), 5.40 - 5.48 (m, 2H), 4.96 (t, *J*=6.7 Hz, 4H), 4.51 - 4.53 (m, 5H), 4.36 (br. s, 1H), 4.14 (br. s, 1H), 3.96 (br. s, 1H), 2.97 - 3.04 (m, 2H), 2.87 (br. s, 6H), 2.76 - 2.80 (m, 2H), 1.37 - 1.40 (m, 2H), 1.31 (dd, *J*=11.6, 6.7 Hz, 2H), 1.23 - 1.26 (m, 2H), 0.89 (d, *J*=5.5 Hz, 6H), 0.83 (br. s, 6H)

^{13}C NMR (125 MHz, DMSO-d_6): δ 208.7, 208.6, 170.3, 170.1, 170.1, 169.8, 156.7, 156.5, 130.8, 129.6, 114.6, 114.4, 114.3, 114.0, 87.1, 87.0, 76.9, 65.2, 65.1, 59.8, 56.5, 34.7, 30.6, 30.1, 29.0, 24.6, 23.0, 22.1, 20.8, 14.1

HRMS (ESI): calculated for $C_{40}H_{52}N_4O_6Na$ $[M + Na]^+$: 707.3799, found 707.3779.

X-ray crystallographic analysis of 3-*epi*-Pseudoxylallemycin B (20)



ORTEP of **20**, CCDC no.: **1842533**

Compound **20** was crystallized from mixture of THF and hexane solvents. An X-ray intensity data measurement of compound 20 was carried out on a Bruker D8 VENTURE Kappa Duo PHOTON II CPAD diffractometer equipped with Incoatech multilayer mirrors optics. The intensity measurements was carried out at 100(2) K temperature with Mo micro-focus sealed tube diffraction source (MoK_α= 0.71073 Å). 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 12 frames (total 36 frames). Data were collected with ω scan width of 0.5° at different settings of φ and 2θ with a frame time of 15 secs keeping the sample-to-detector distance fixed at 5.00 cm. The X-ray data collection was monitored by APEX3 program (Bruker, 2016). All the data were corrected for Lorentzian, polarization and absorption effects using SAINT and SADABS programs (Bruker, 2016). Using APEX3 (Bruker) program suite, the structure was solved with the ShelXS-97 (Sheldrick, 2008) structure solution program, using direct methods. The model was refined with version of ShelXL-2013 (Sheldrick, 2015) using Least Squares minimization. All the hydrogen atoms were placed in a geometrically idealized position and constrained to ride on its parent atoms. An *ORTEP* III (Farrugia, 2012) view of both compound were drawn with 30% probability displacement ellipsoids and H atoms are not included for clarity. The compound $C_{40}H_{52}N_4O_6$, CH_3SOCH_3, H_2O crystallizes in the monoclinic $P2_1$ chiral space group containing a molecule of dimethyl sulfoxide (DMSO) and water in the asymmetric unit. The DMSO molecule showed statistical disorder over two positions with occupancies roughly 60% and 40%. The absolute configuration for compound **20** was established by the structure determination of a compound containing a chiral reference molecule of known absolute configuration and confirmed by anomalous dispersion effects in diffraction measurements on the crystal (Flack parameter, 0.09(4)). The single crystal X-ray diffraction data analysis clearly established that the synthesized compound has *R*, *S*, *S*, and *S*, configurations at C1, C3, C5, and C7 positions respectively. The terminal groups of the macrocycle showed more vibrational motions compared to the core moiety. In the crystal structure, the DMSO molecule is associated with the core moiety of the macrocycle through bifurcated N-H…O hydrogen bond engaging the amine (N-H) moieties of the core and oxygen of DMSO. Whereas the water molecules engage the carbonyl oxygen's of the core moiety through O-H…O hydrogen bonds. The crystal data and structure refinement details are given below.

Crystal data of 20: $C_{40}H_{52}N_4O_6$, CH_3SOCH_3, H_2O, M = 781.00, colourless needle, 0.43 x 0.31 x 0.18 mm^3, monoclinic, $P2_1$ chiral space group, a = 9.5688(4) Å, b = 21.9169(13) Å, c = 10.6833(6) Å, β = 105.573(2)°, V = 2158.2(2) Å^3, Z = 2, T = 100(2) K, $2\theta_{max}$= 59.374°, D_{calc} (g cm^{-3}) = 1.202, $F(000)$ = 840, μ (mm^{-1}) = 0.129, 129324 reflections collected, 12128 unique reflections (R_{int} = 0.1397, R_{sig} = 0.0588), 9707 observed ($I > 2\sigma(I)$) reflections, multi-scan absorption correction, T_{min} = 0.947, T_{max} = 0.977, 561 refined parameters, no. of restraints = 144, Good of Fit = S = 1.110, $R1$ = 0.0934, $wR2$ = 0.1721 (all data R = 0.1170, $wR2$ = 0.1170), maximum and minimum residual electron densities; $\Delta\rho_{max}$ = 0.805, $\Delta\rho_{min}$= -0.365 (e Å^{-3}). **CCDC no.: 1842533**.

2.6. References

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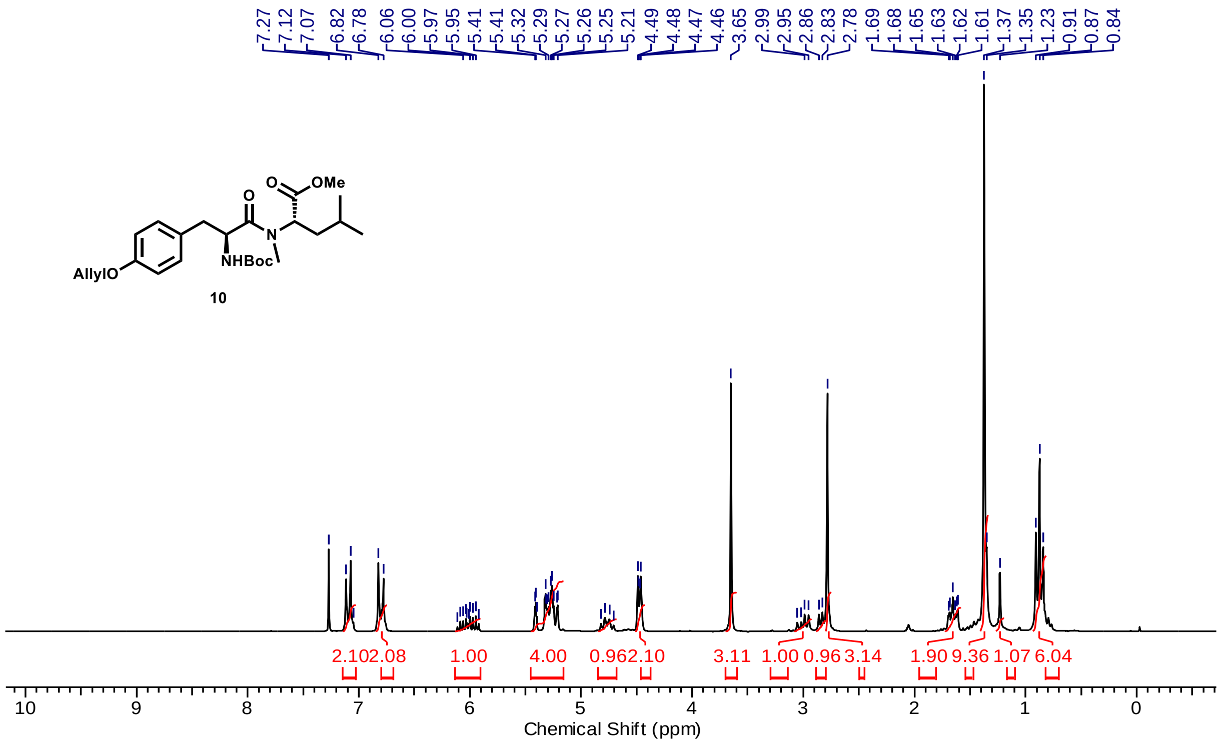
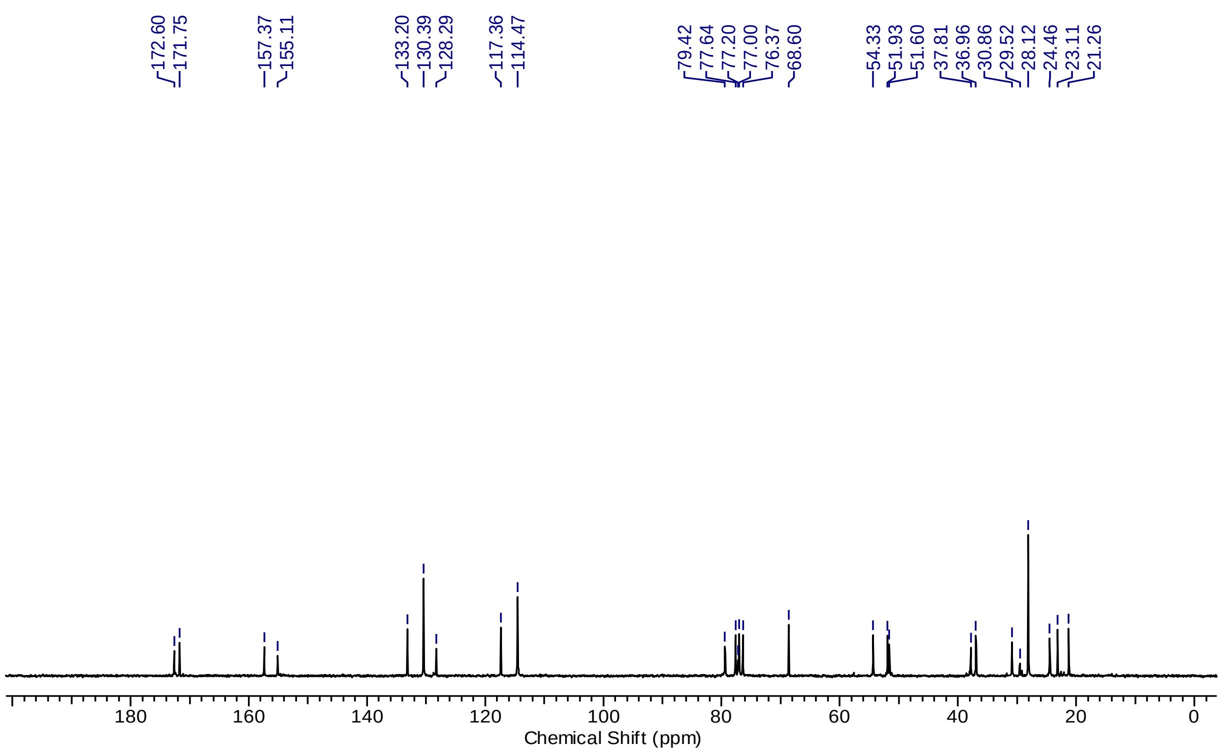
2.7. Copies of NMR spectra



^{1}H NMR of **10** (200 MHz, CDCl$_3$)



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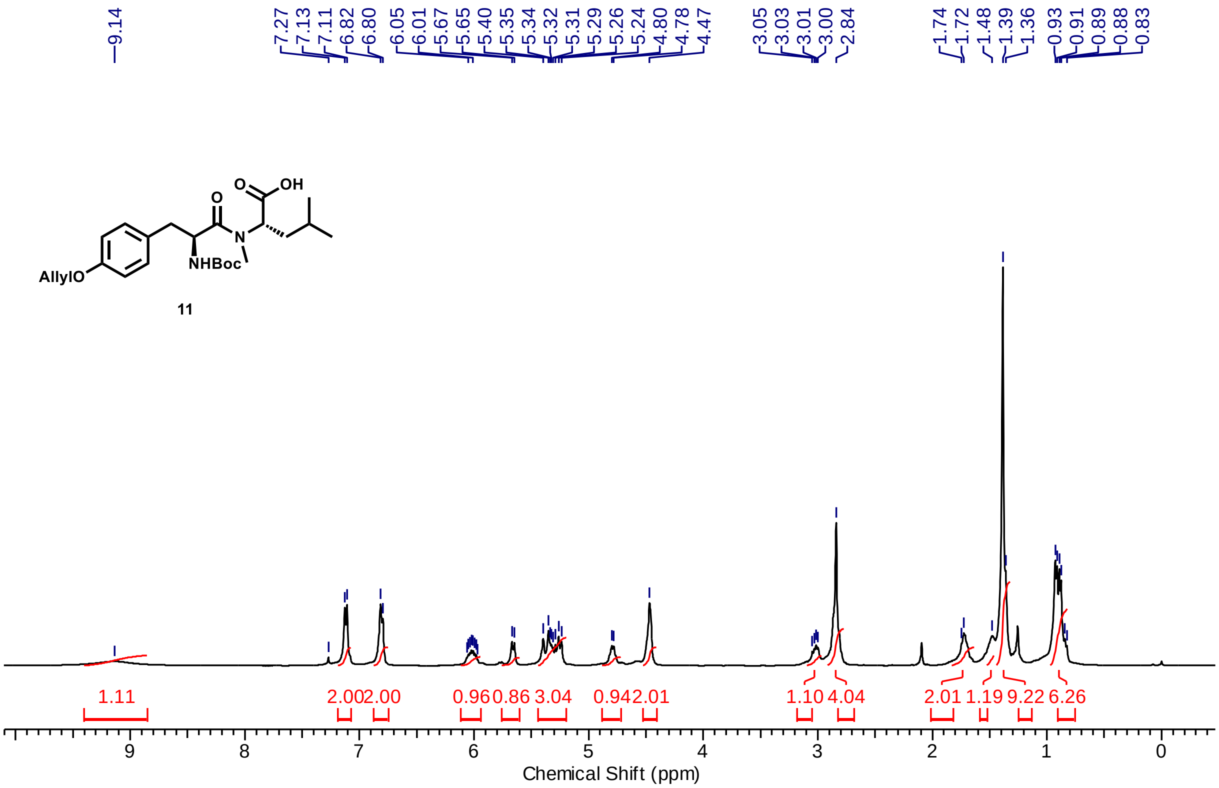
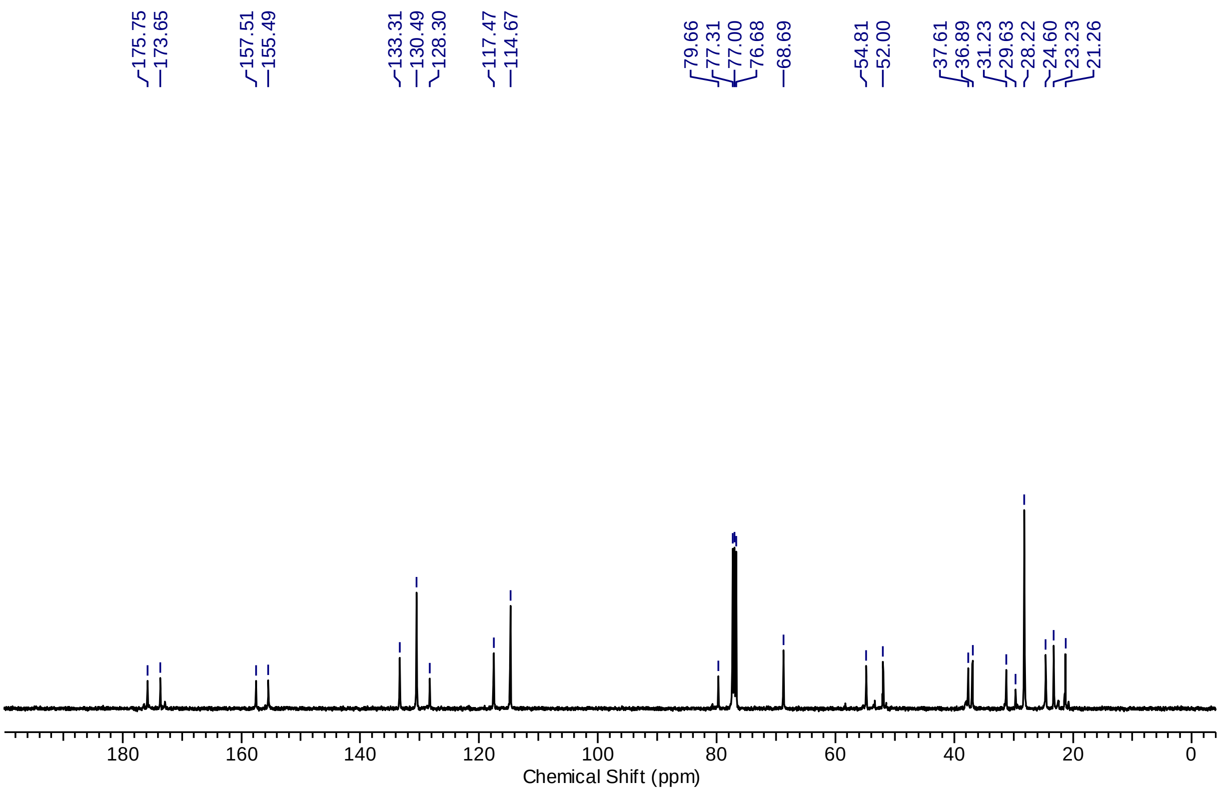


11

^{1}H NMR of **11** (400 MHz, $CDCl_3$)



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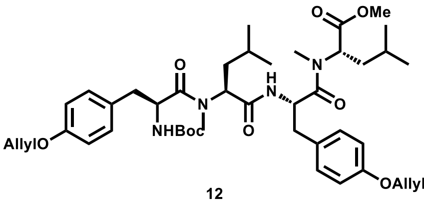

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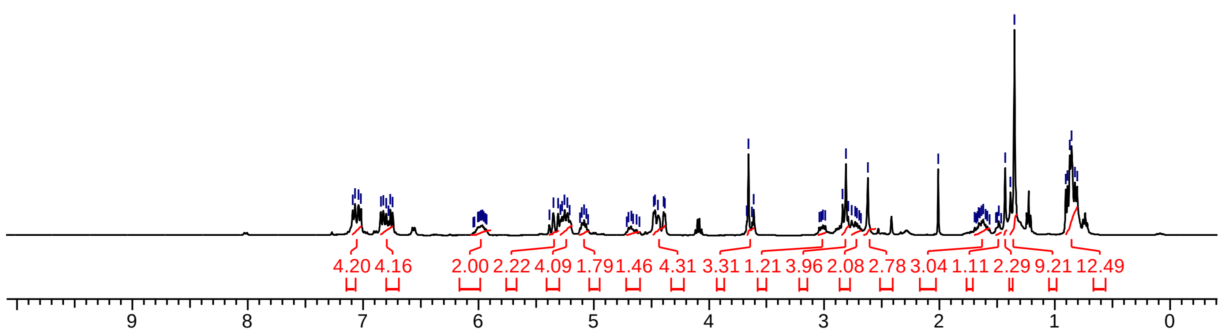

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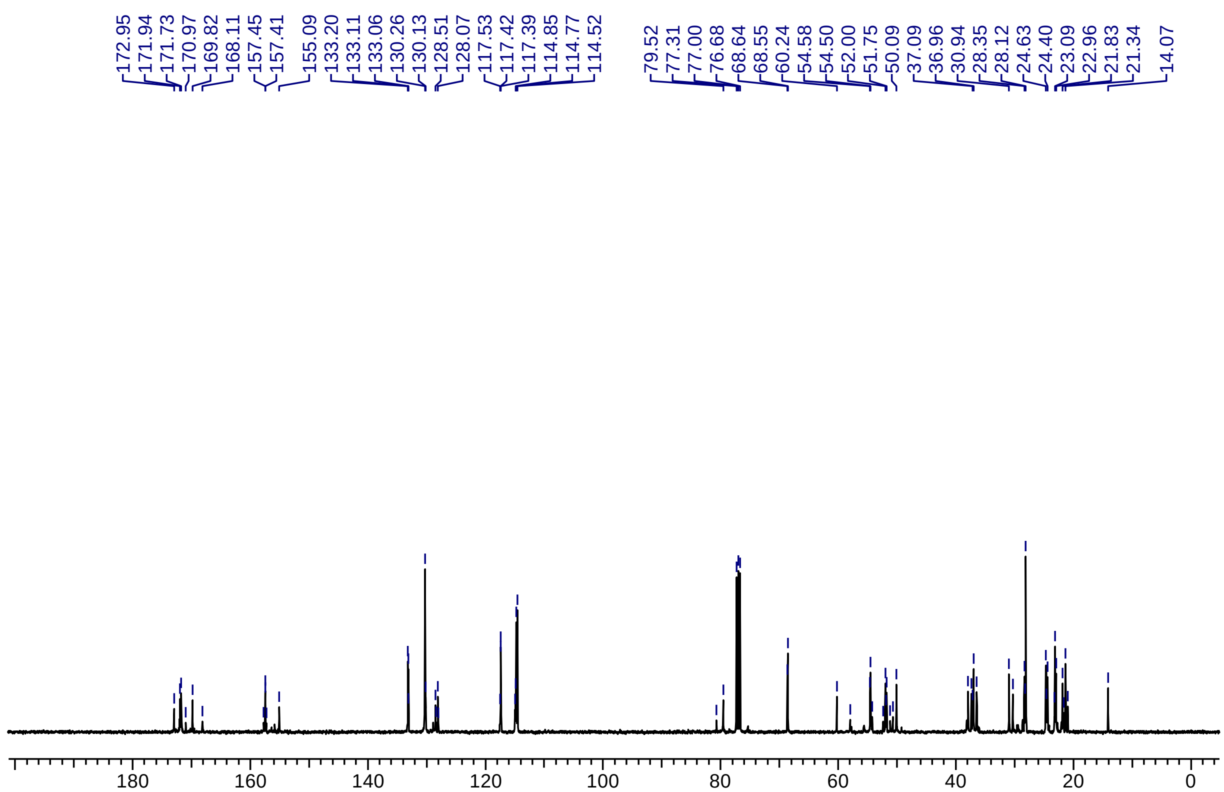

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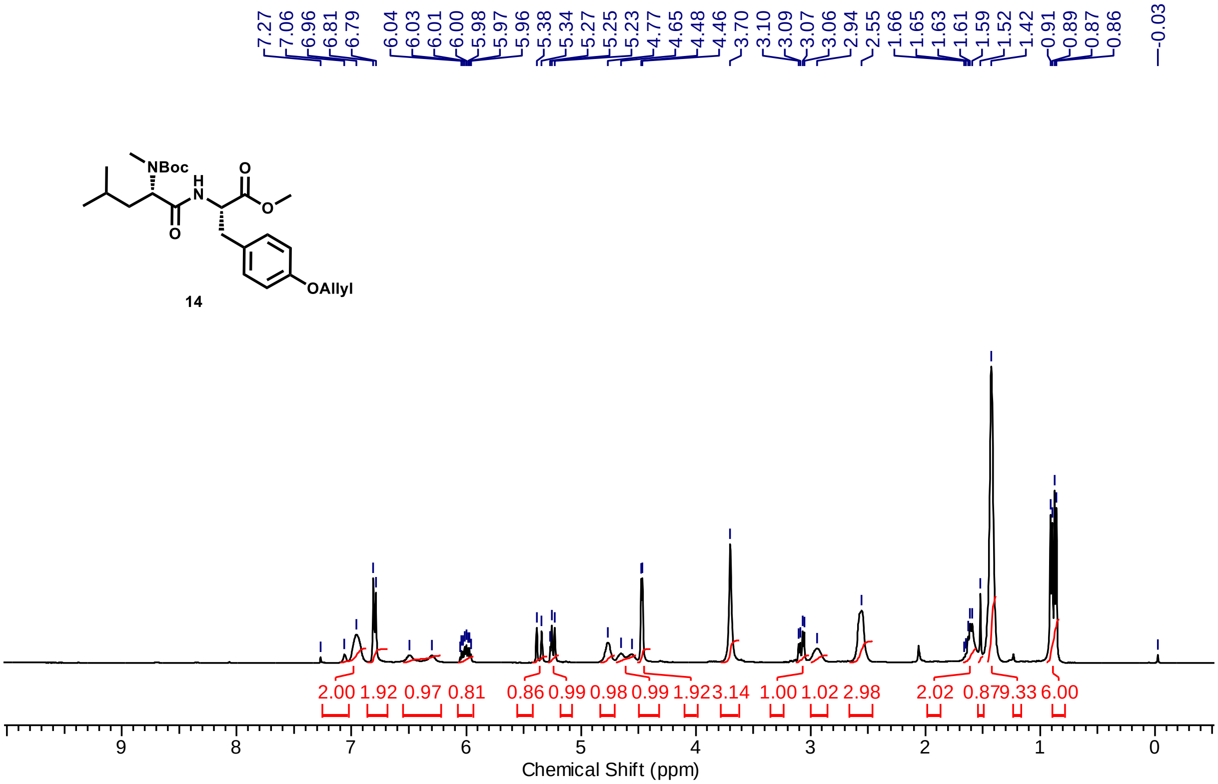

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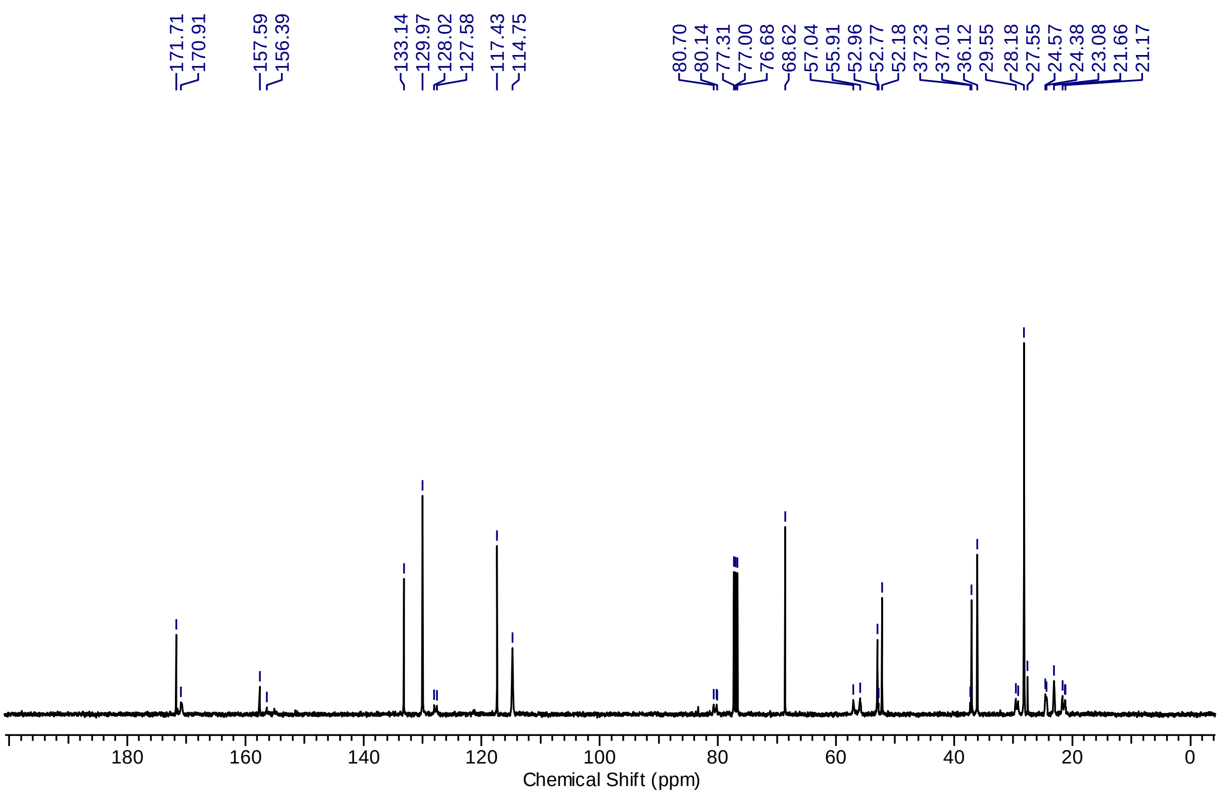

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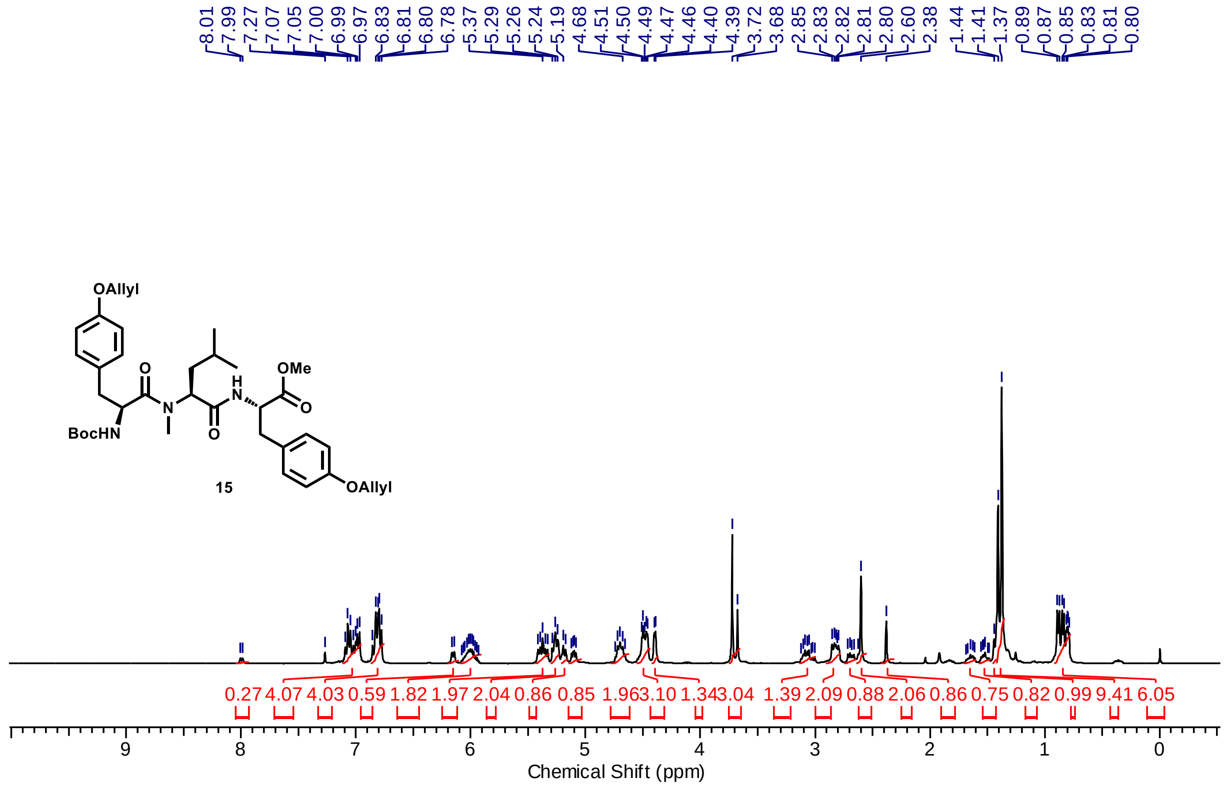

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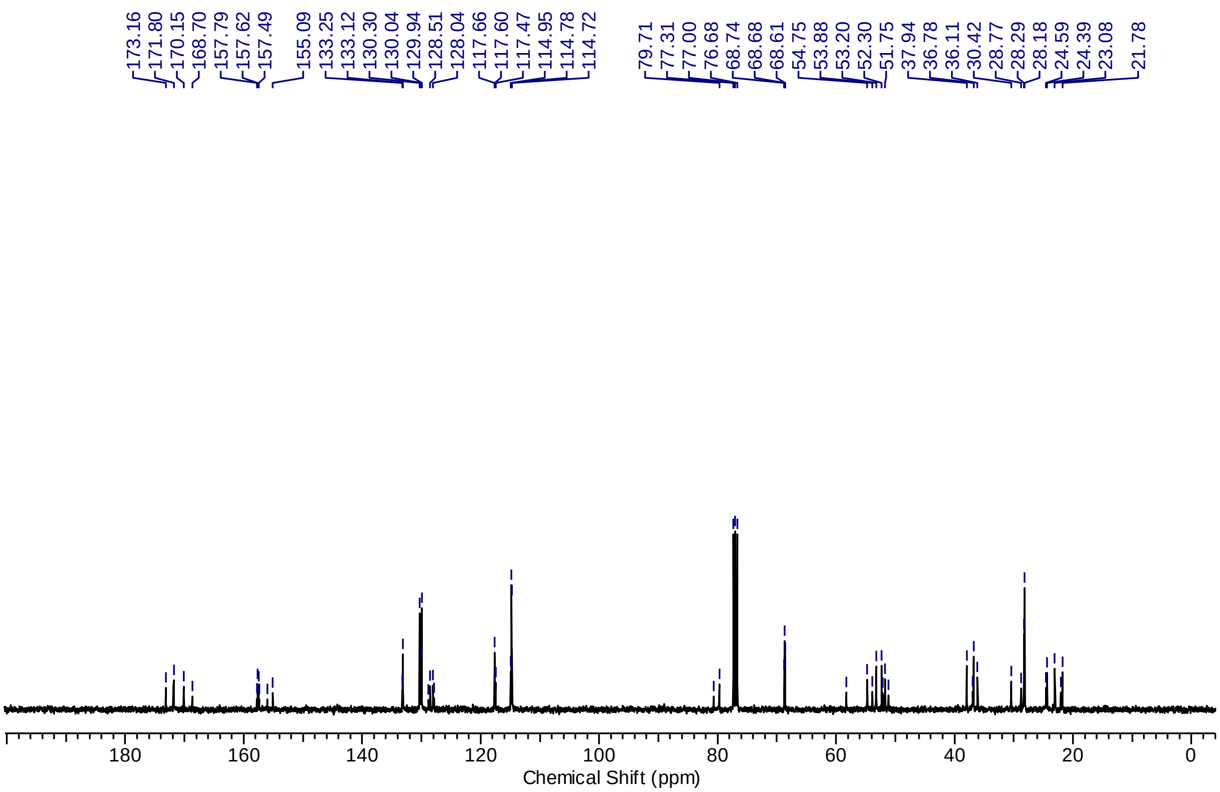

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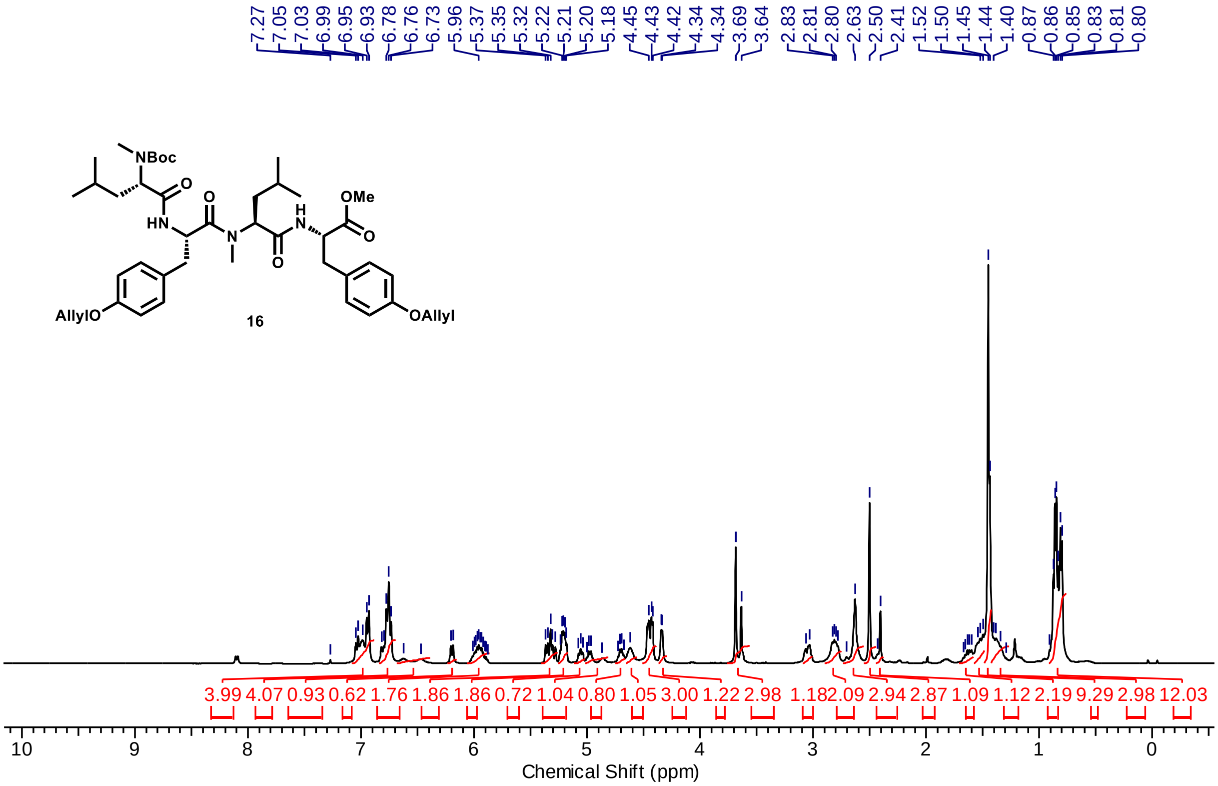

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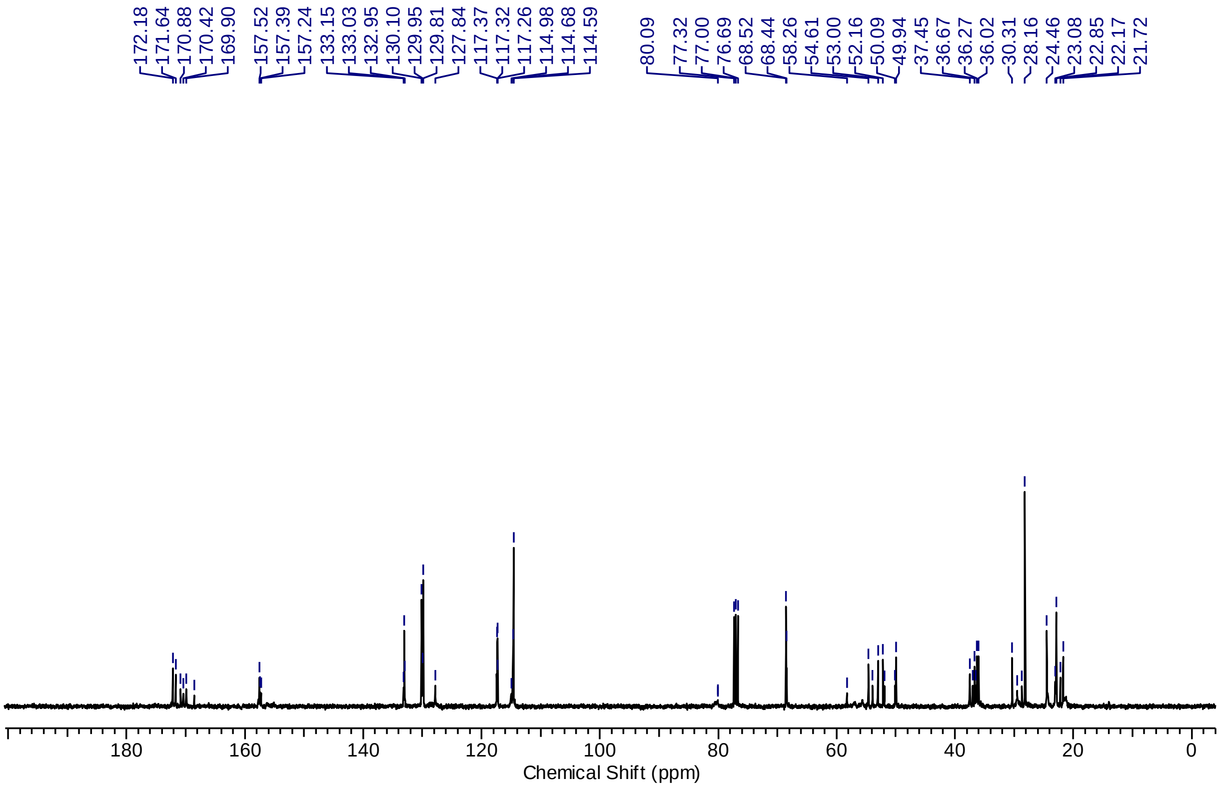

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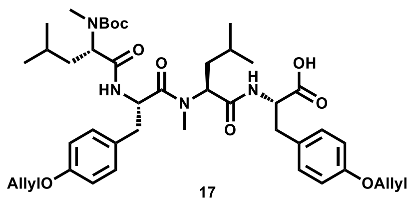
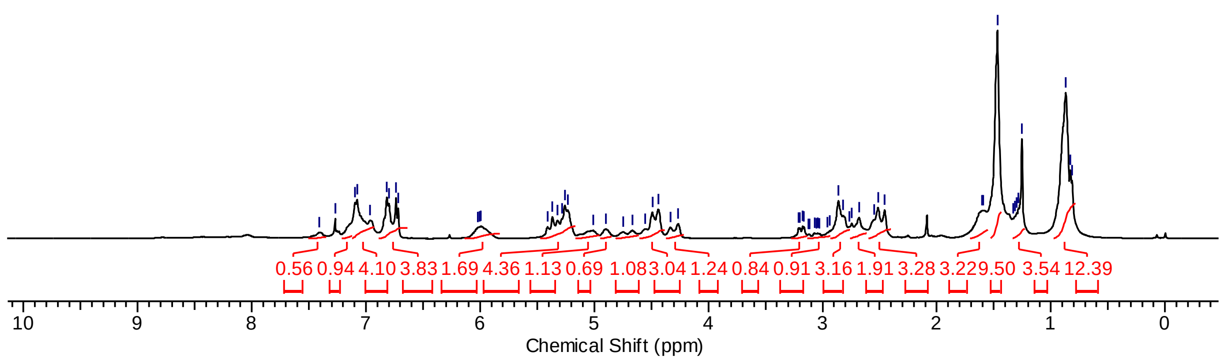


17



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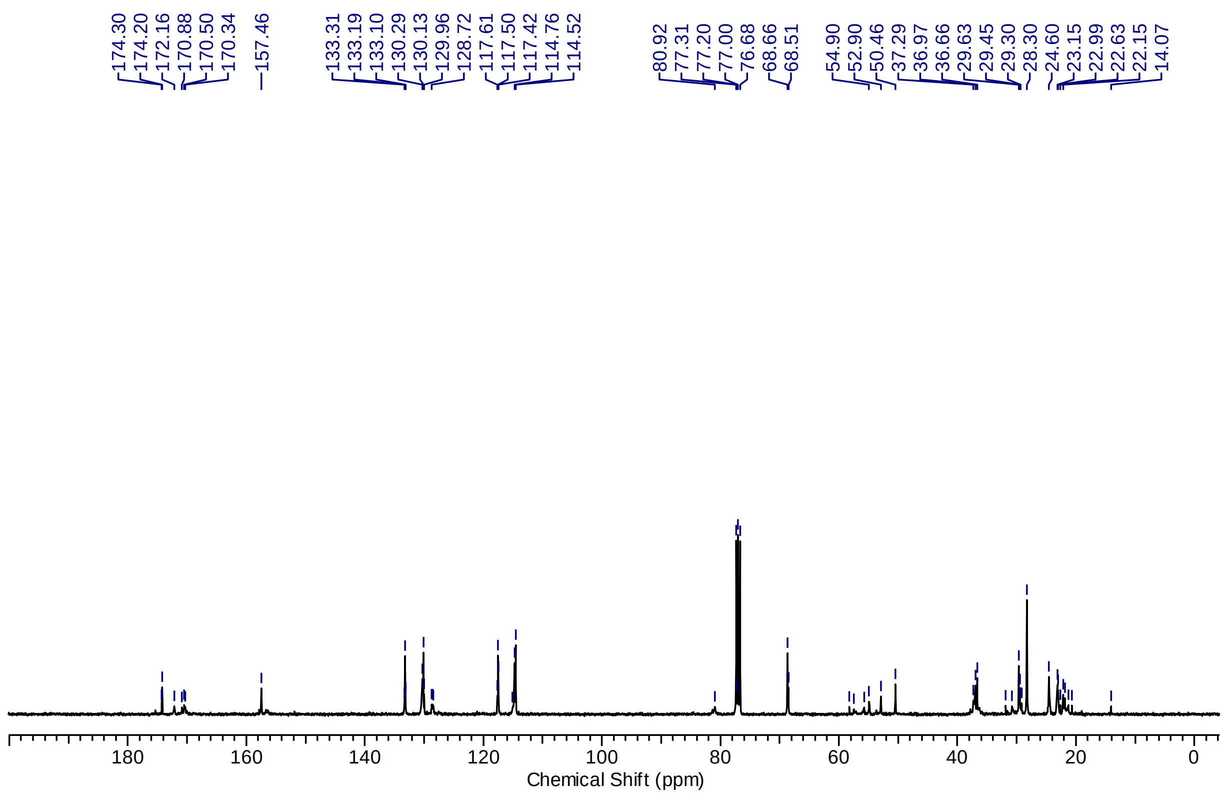

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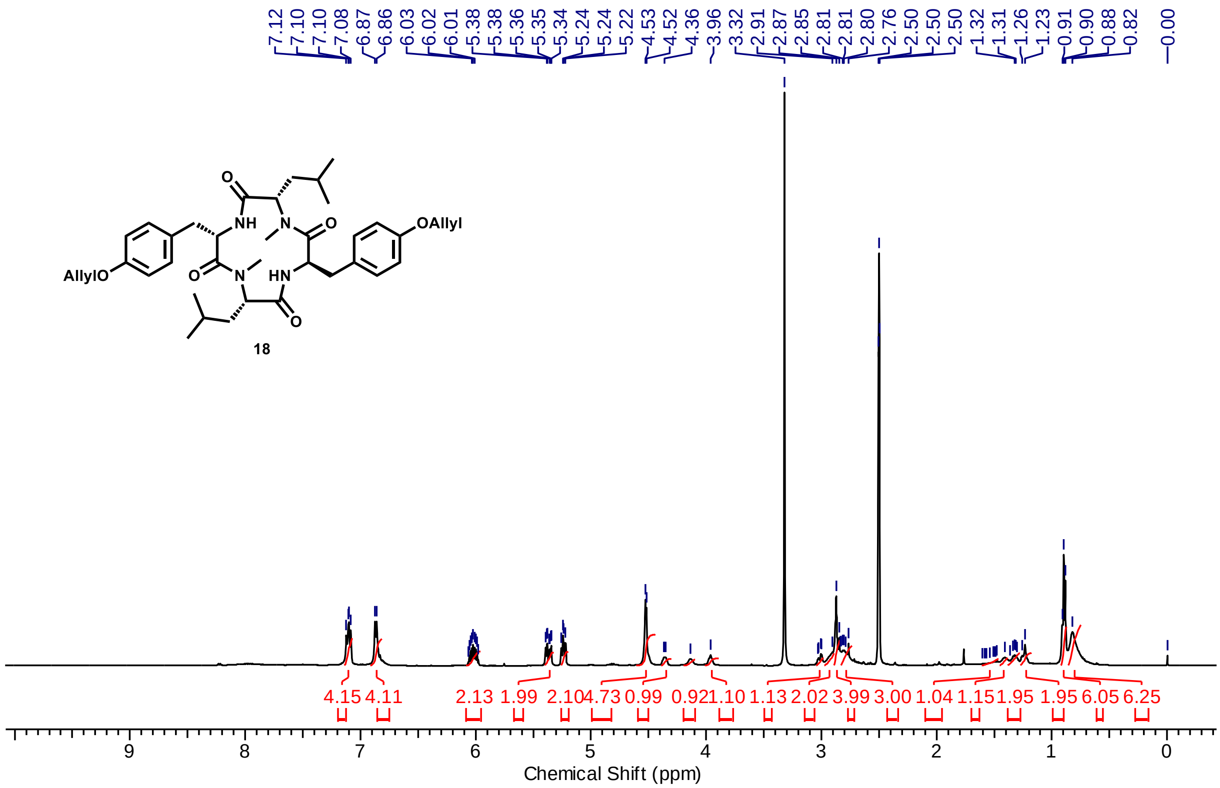

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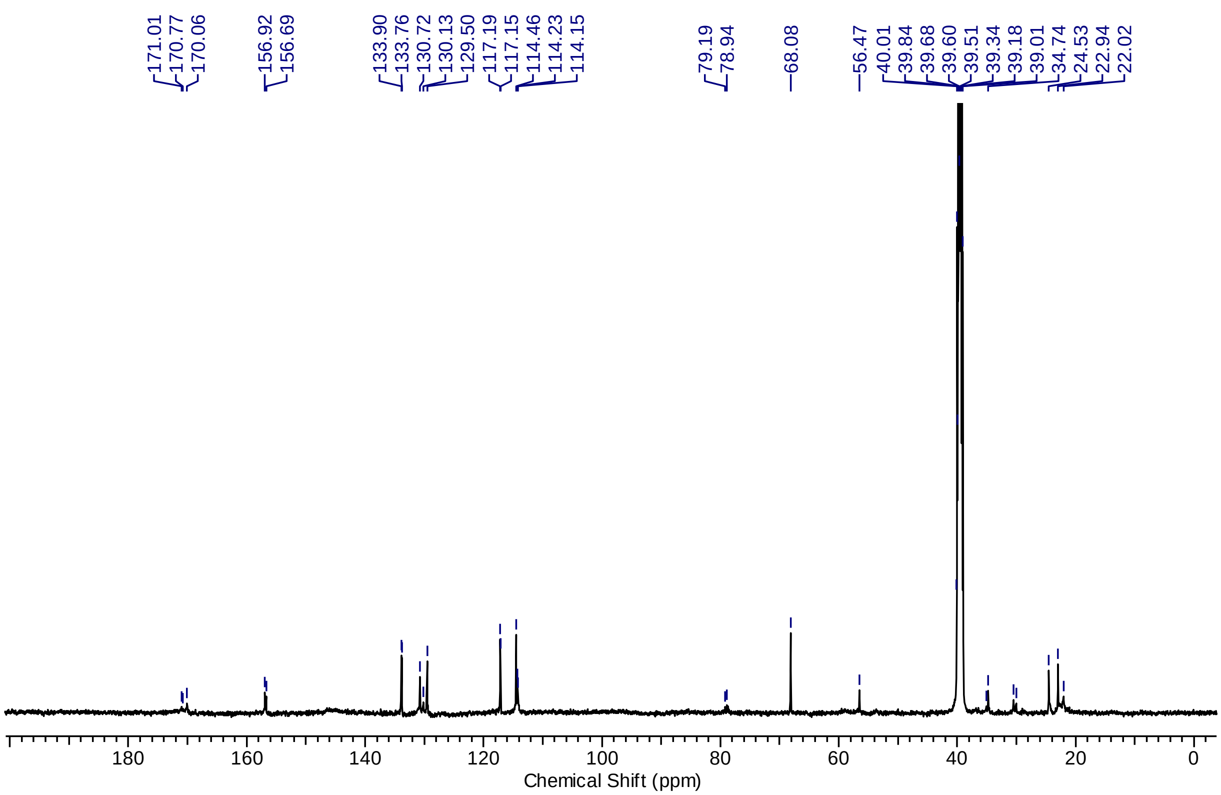

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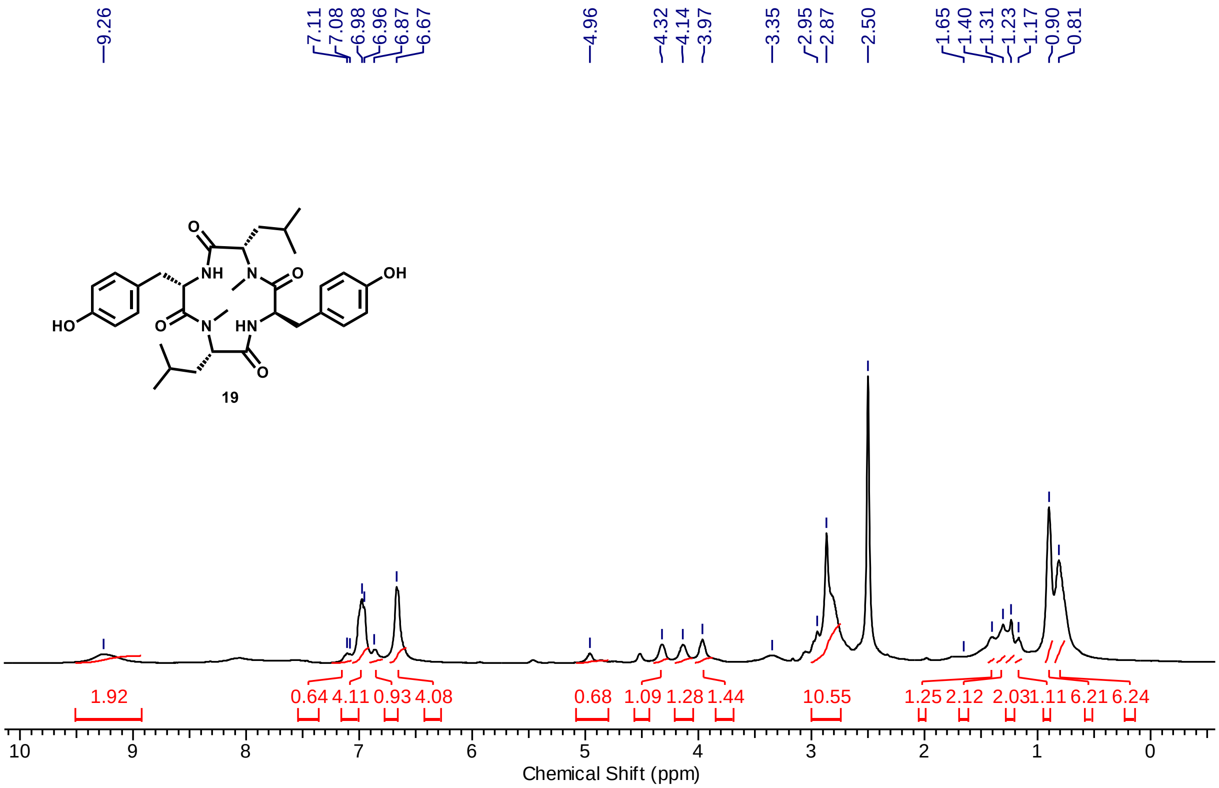

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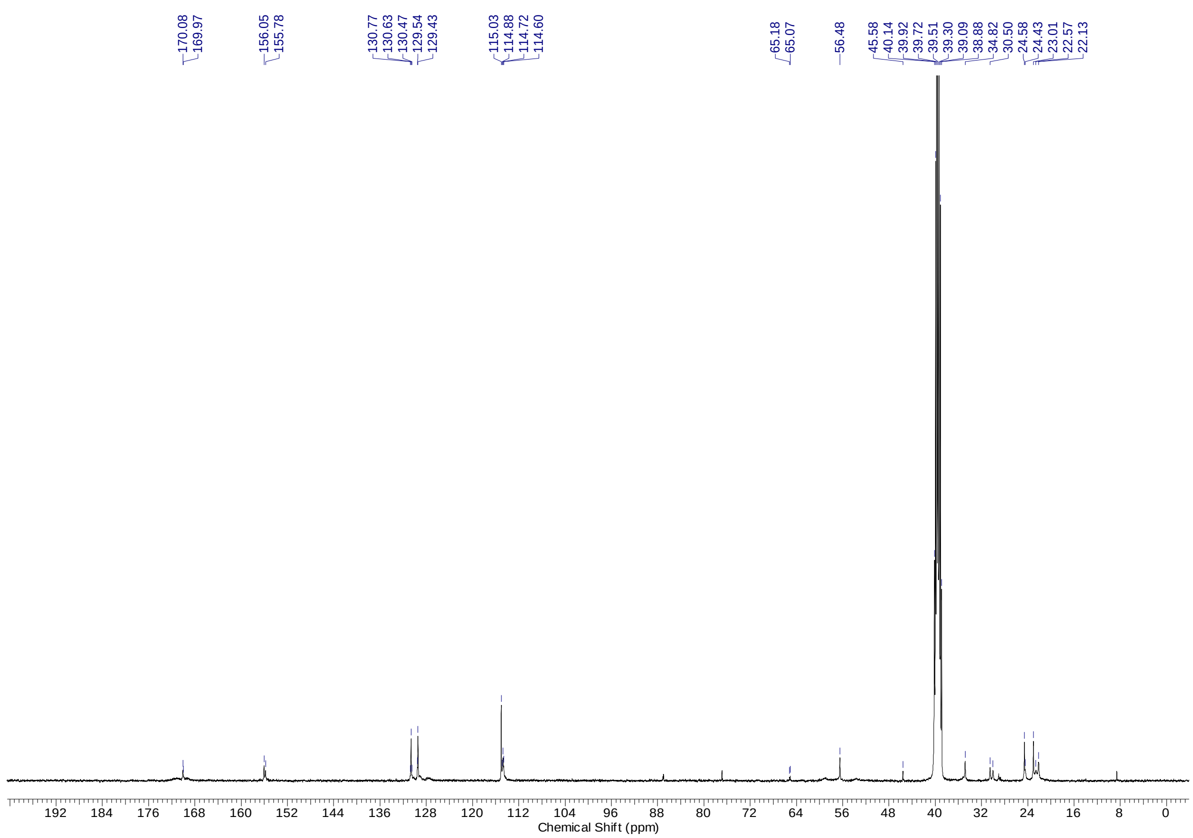

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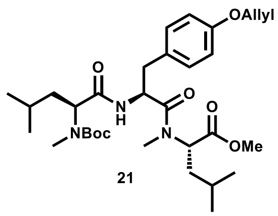

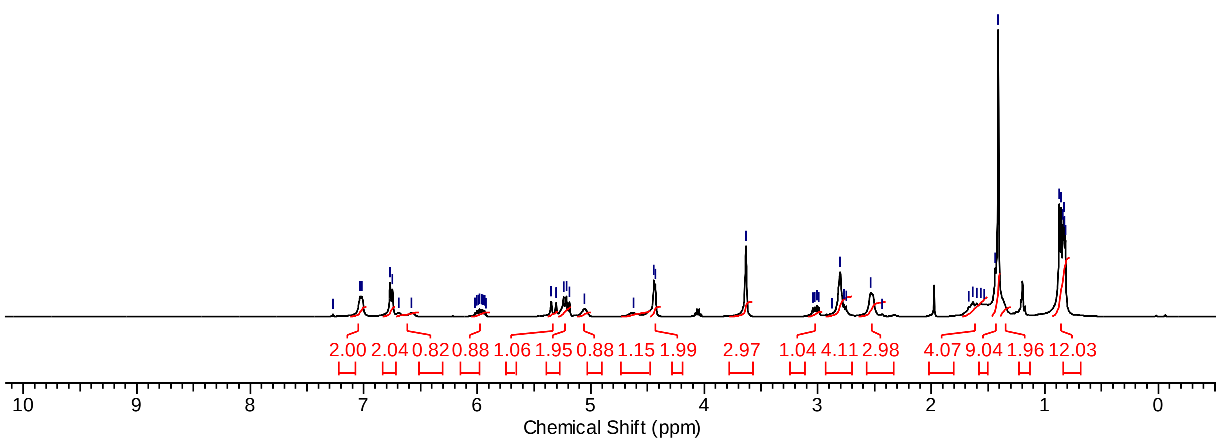

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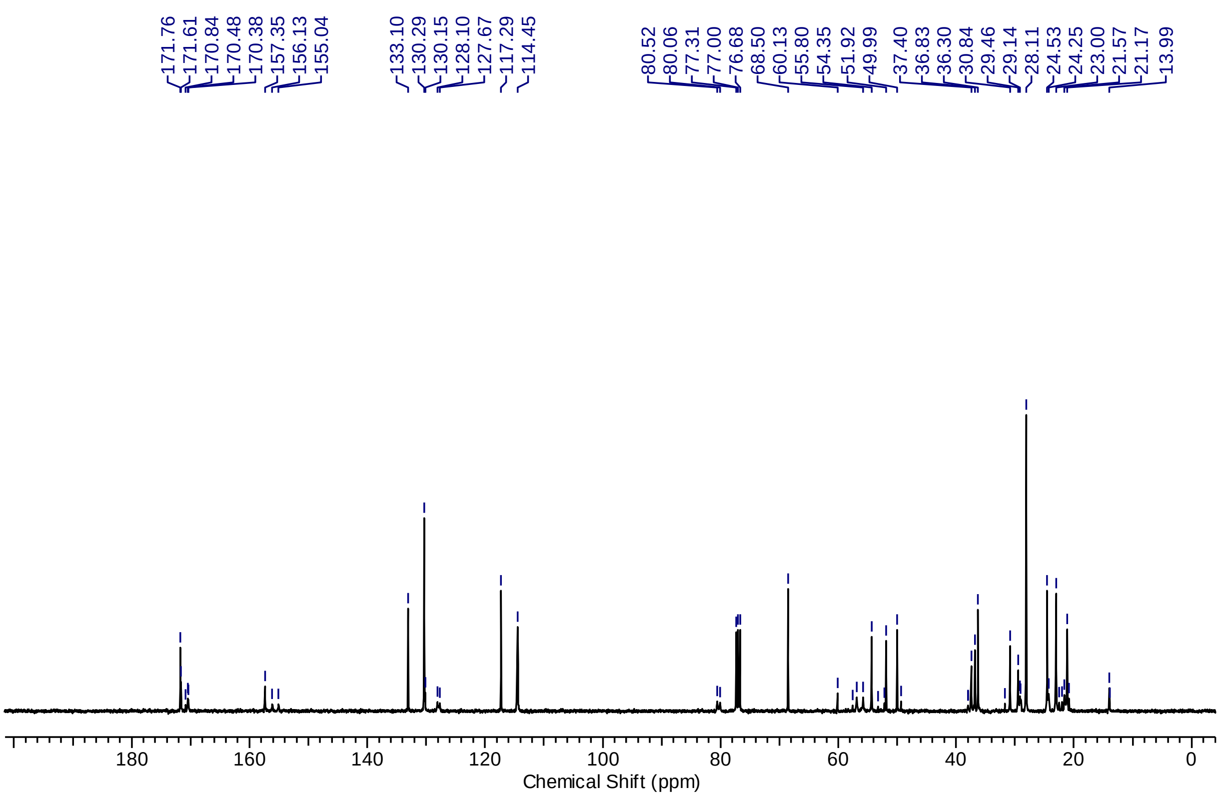

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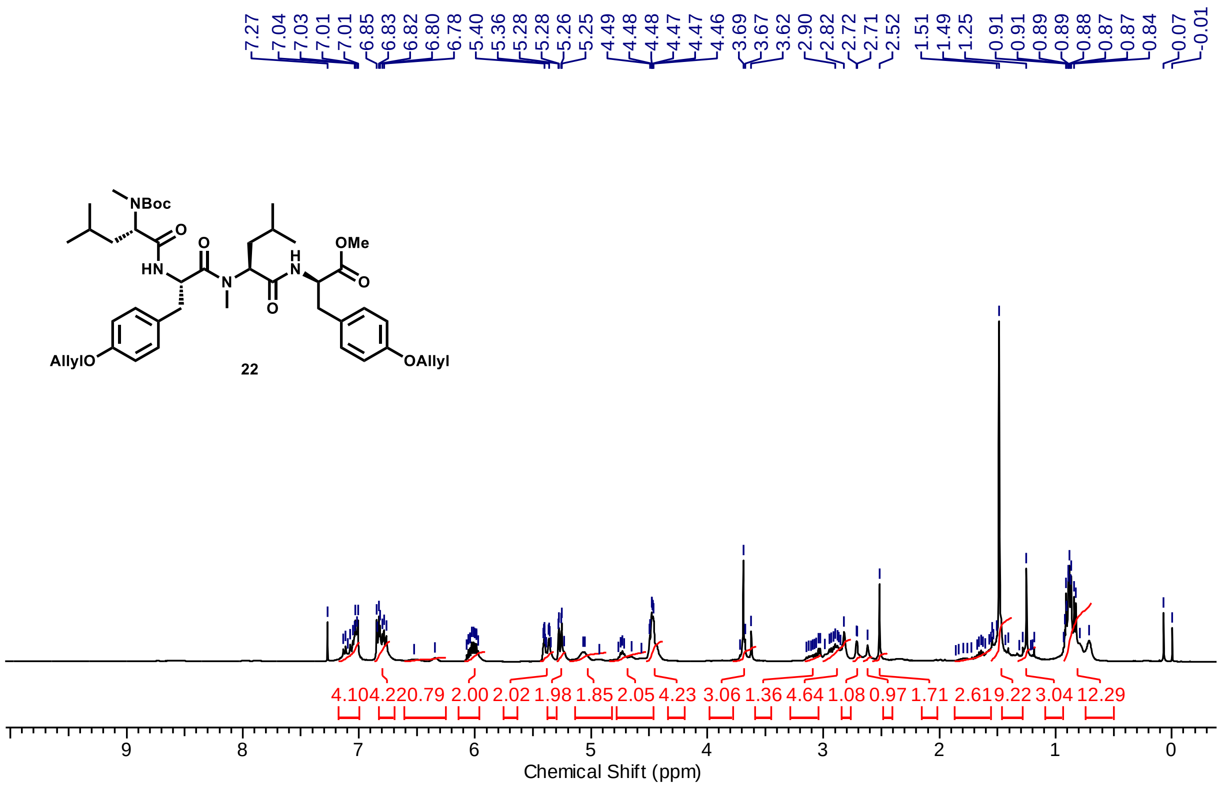

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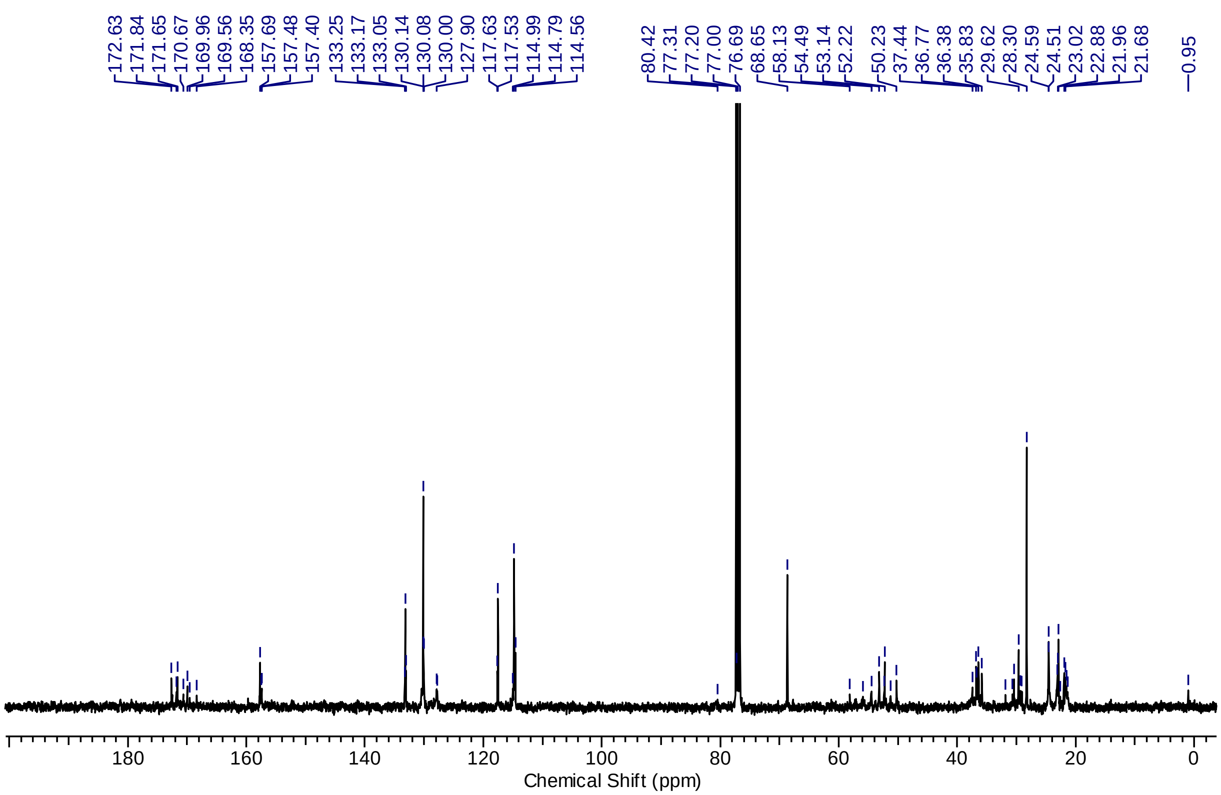
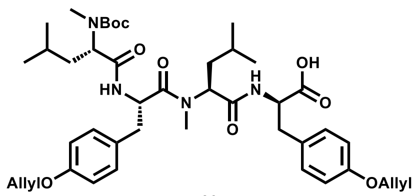
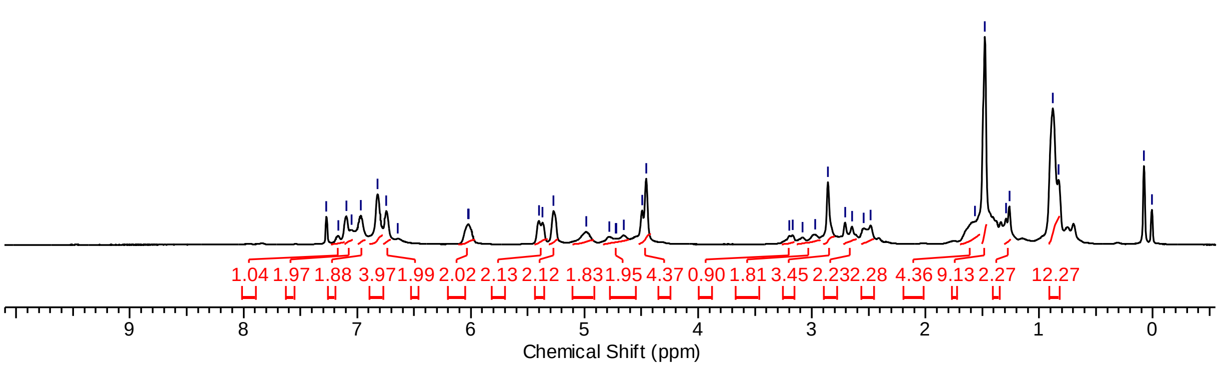


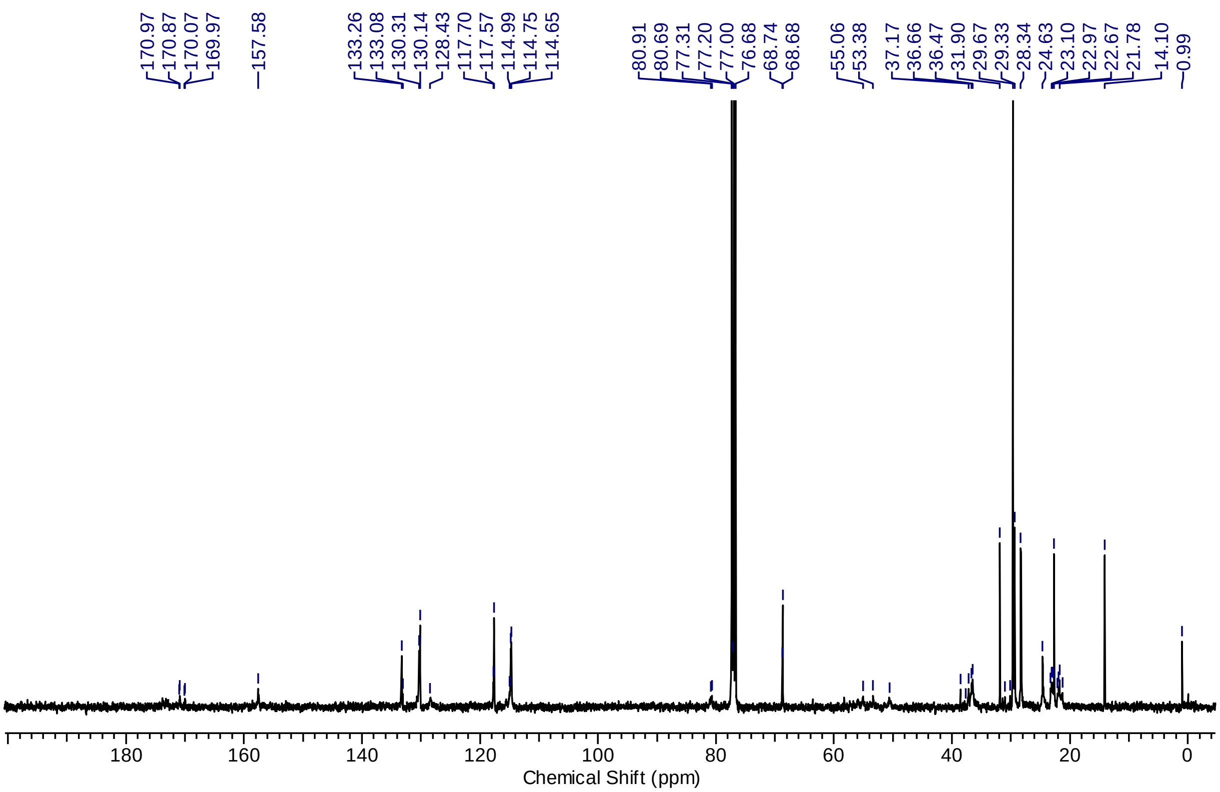
^{13}C NMR of **22** (100 MHz, $CDCl_3$)



23



^{1}H NMR of **23** (500 MHz, $CDCl_3$)



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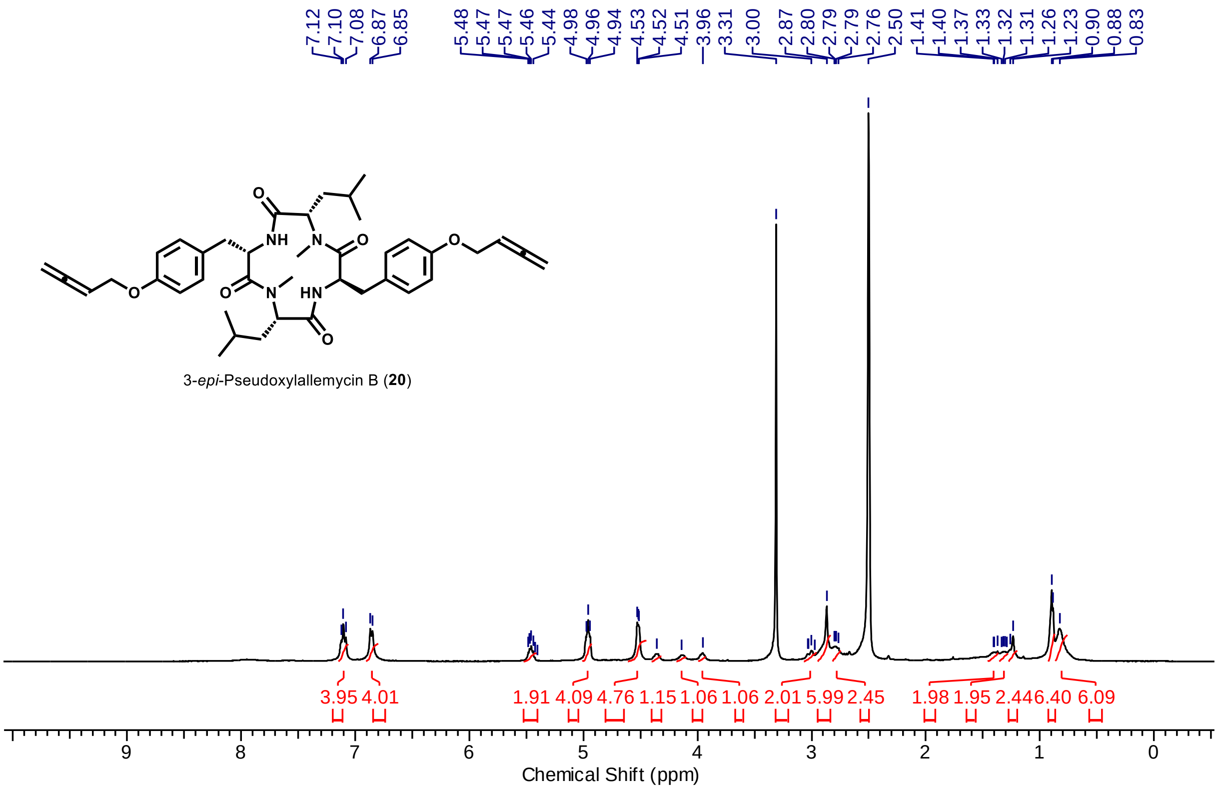

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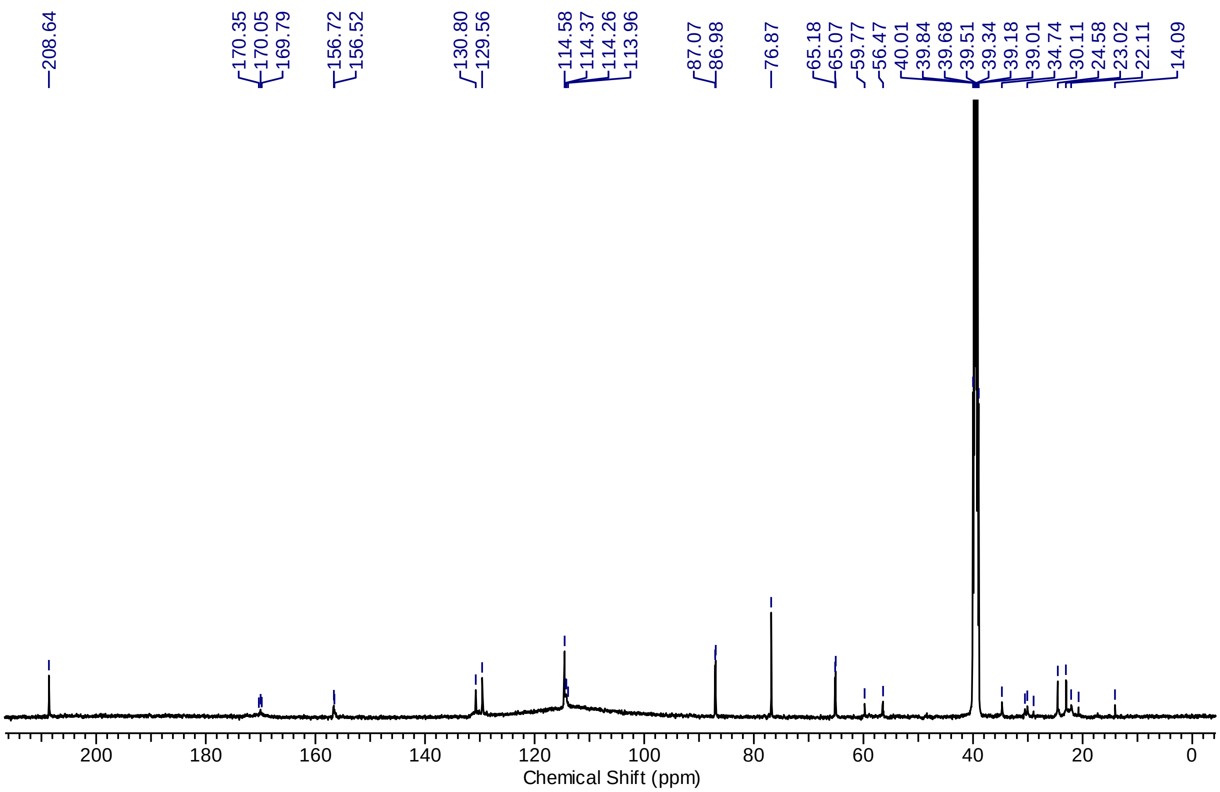


^{13}C NMR of **20** (100 MHz, DMSO-d_6)

Chapter 3:

Efforts toward Total Synthesis of Arthroamide and Fusaristatin C

3.1.1. Introduction

Infections in human, animals and plants with multidrug resistant bacteria became worrisome, since very few or even no treatment options remained for them. In this scenario, as discussed in previous sections, there is an urgent need of identification of novel antimicrobial compounds with distinct modes of action or which can be used along with conventional therapies to lower down the dose of them for the treatment.[1] The increasing complications of antibiotic resistance is calling for the development of novel compounds which must have mechanism of action other than bacteriostatic or bacteriolytic; as an example quorum sensing (QS) pathways is a new way of such treatment.[2] Quorum sensing (QS) is the process of communication between bacteria through diffusible chemical signals produced by autoinducers and useful in various vital functioning in bacteria including biofilm formation.[3] Quorum sensing inhibition (QSI) provides attractive surrogate for the antimicrobials.

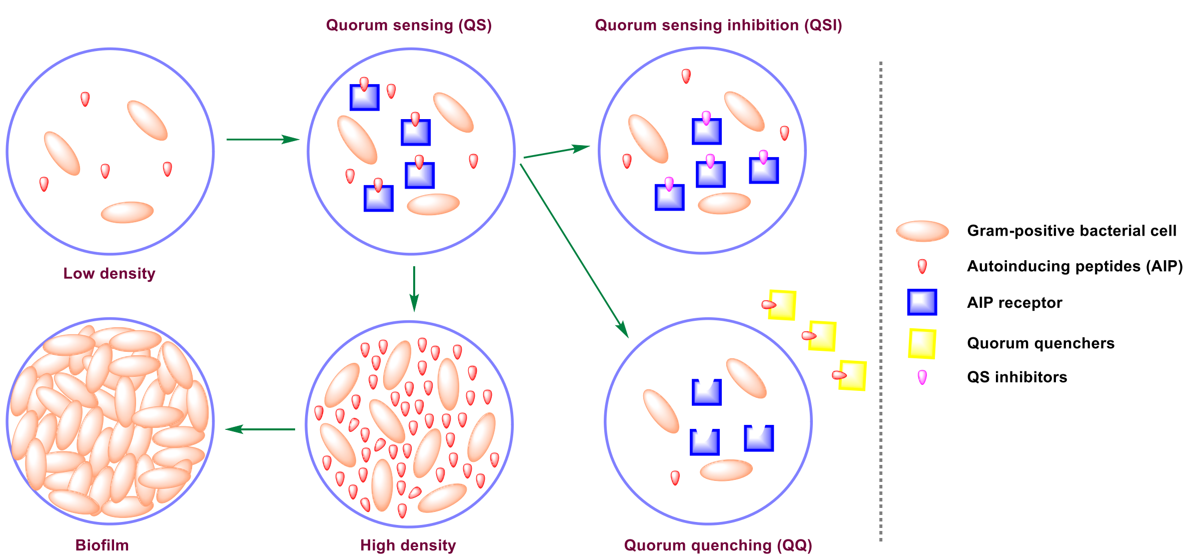


Figure 3.1.1. Quorum sensing in Gram-positive bacteria (Source: *Molecules* **2016**, *21*, 1211-1221)[4]

Biofilms are microbial community held together in a self-generated matrix; highly resistant to most of the available treatments and host's immune system. Quorum sensing triggers the formation of biofilms as a burdensome biomedical problem with yet unfound therapeutic solutions.[5] QSIs do not kill the bacteria but defeat the biofilm formation without affecting bacterial growth and therefore are less prone to develop long term resistance in bacteria.[6] As process of QS is specific to bacteria and not observed in humans, because of which it became highly specific to antibacterials.[3] Till date, many compounds were identified and developed as quorum sensing inhibitors. Some of them are listed below in figure 3.1.2.[7]

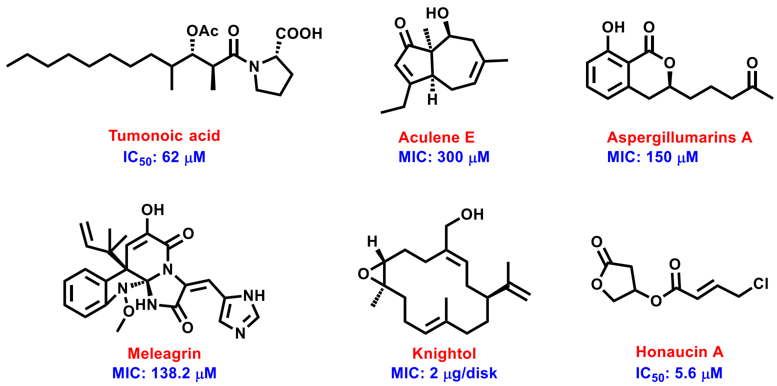


Figure 3.1.2. Structures of selected quorum sensing inhibitors (QSIs)

Recently, macrocyclic peptides were also gaining interest in the field of antibiotics and some of them have potential of inhibiting the process of quorum sensing. Some of the QSIs are solonamide A and B,[8] Avellanin C,[9] AIP-III D4A,[10] tAIP-III D2A[10] and WS9326A[11] (Figure 3.1.3.); these are active against *Staphylococcus aureus*.

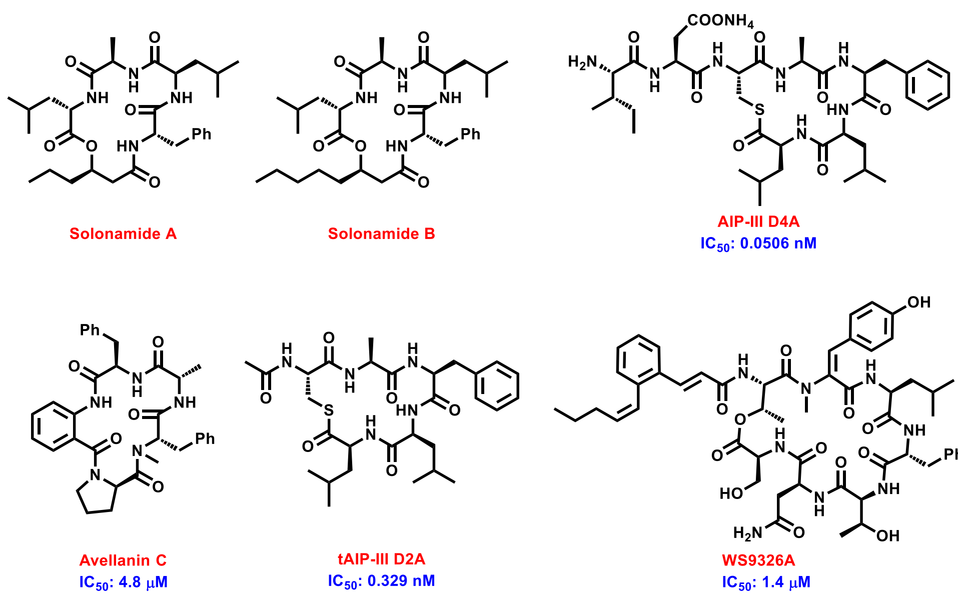


Figure 3.1.3. Structures of macrocyclic quorum sensing inhibitors

In 2015, Yasuhiro group isolated macrocyclic depsipeptide, arthroamide (**1**) form the non-filamentous actinobacteria *Arthrobacter* in addition with known natural product turnagainolide A

(**2**)[12] (Figure 3.1.4.).[13] *Arthrobacter* strains were cultured in liquid medium followed by extraction and purification by HPLC afforded arthroamide as white powder. HRMS (ESI) analysis showed peak at 565.3000 corresponding to the molecular formula $C_{29}H_{42}N_4O_6Na$ $[M + Na]^+$ with calculated mass 565.2997. Structure of this macrocyclic depsipeptide was confirmed by spectroscopic analysis. It contains tetrapeptide containing three L-valine and one D-alanine; whose stereochemistry was confirmed by Marfey's amino acid analysis and chiral anisotropic analysis methods. Non-peptidic fragment comprises of 3-hydroxy-5-phenyl-4-pentenoic acid (Hppa) unit where the absolute configuration at C-3 hydroxyl group was determined by Mosher's method and the geometry of C-C double bond was evaluated on the basis of coupling constant (J= 16.0 Hz) which is characteristic for trans geometry (E-) of double bond.

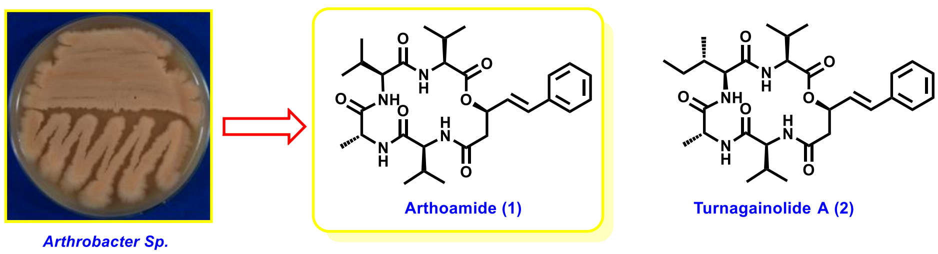


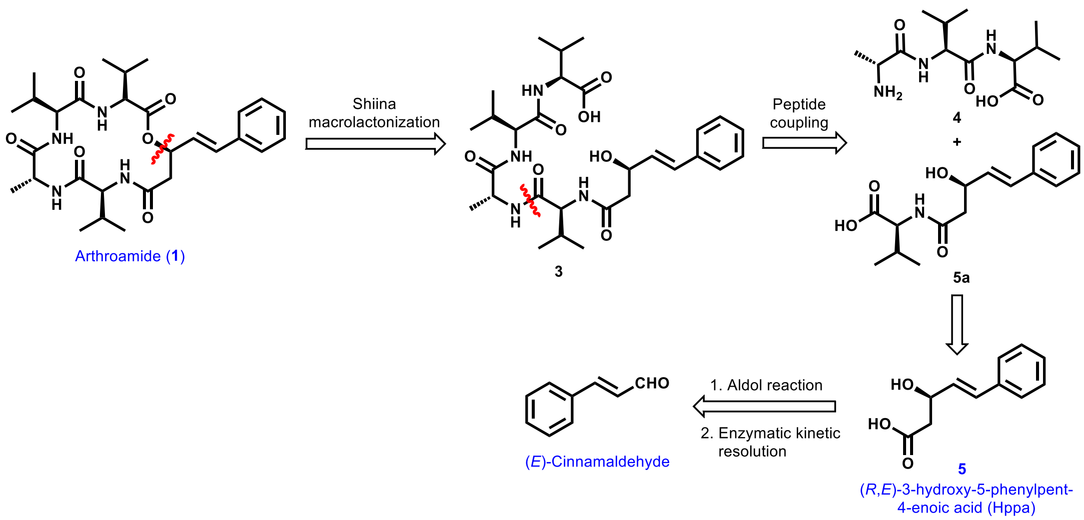
Figure 3.1.4. Structures of arthroamide and turnagainolide A

Interestingly, Arthroamide showed potent quorum sensing inhibitory activity through the agr-signaling pathway in *Staphylococcus aureus* with IC_{50} of 0.3 μM. With our bit experience in peptide synthesis and antibacterial compounds; fascinating structural features and interesting biological activity prompted us to work on the synthesis of arthroamide towards identifying new scaffolds with different mechanistic approaches.

3.1.2. Efforts toward synthesis of arthroamide

3.1.2.1. Retrosynthesis

Retrosynthetic analysis for the synthesis of arthroamide (**1**) is illustrated in Scheme 3.1.1. We envisioned that arthroamide could be synthesized using Shiina macrolactonization[14] of *seco* acid **3**, a key step in the synthesis. Compound **3** could be assembled from the acid **5a** and tripeptide amine **4** *via* peptide coupling.

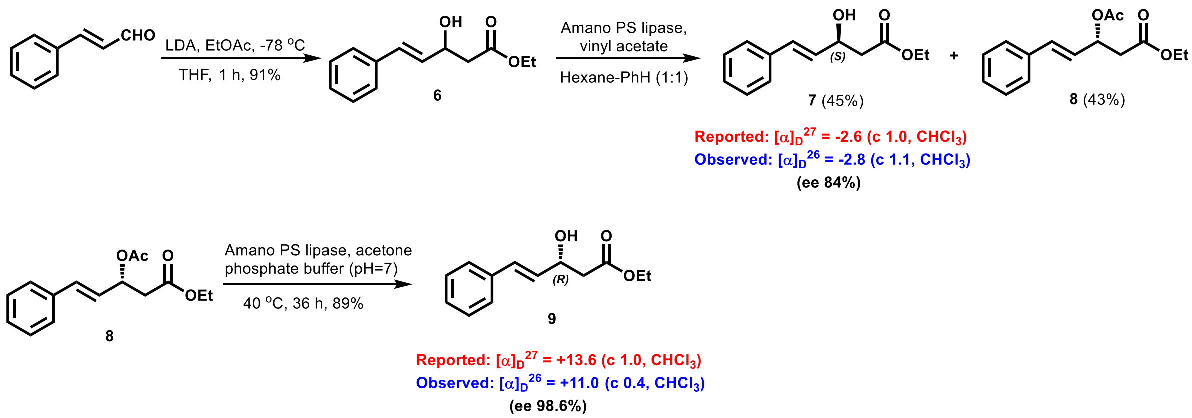


Scheme 3.1.1. Retrosynthetic analysis for arthroamide

Tripeptide **4** could be achieved by peptide coupling of corresponding amino acids. Acid **5a** could be synthesized from L-valine methyl ester and 3-hydroxy-5-phenyl-4-pentanoic acid (Hppa), **5**, which in turn could be prepared from aldol reaction of (*E*)-cinnamaldehyde with EtOAc, followed by enzymatic kinetic resolution.[15] Having this plan in hand, we ventured into the total synthesis of target natural product arthroamide.

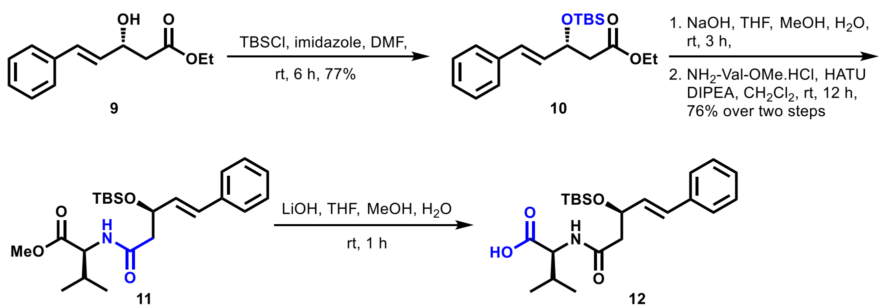
3.1.2.2. Synthesis of proposed structure of arthroamide

The Hppa residue **5** was synthesized from (*E*)-cinnamaldehyde *via* aldol reaction with EtOAc using LDA to give the *β*-hydroxy ester **6** as a racemic mixture in high yield by a known protocol.[12] To have the enantiopure hppa, the Amano PS lipase mediated enzymatic kinetic resolution was carried out using vinyl acetate in benzene-hexane (1:2) solvent mixture produced enantiopure alcohol **7** (84% ee, determined using the chiral HPLC method by using Chiralpack IB column) and corresponding acetate **8** in almost equal quantities.[16] Compound **7** gave specific rotation $[\alpha]_D^{26}$ = −3.1 (*c* 1.1, $CHCl_3$) which was consistent with reported data.[17] The acetate compound was then hydrolyzed using Amano PS lipase and phosphate buffer (pH = 7) in acetone to get compound **9** (>98.6% ee) with specific rotation $[\alpha]_D^{26}$ = +11.0 (*c* 0.4, $CHCl_3$).[18] In compound **9**, characteristic two olefin protons were appeared at δ 6.71 (d, *J* = 14.8 Hz, 1H) and 6.27 (dd, *J* = 14.8, 6.1 Hz, 1H) ppm, hydroxyl attached methine was present at δ 4.77 (m, 1H) and disappearance of acetate peak at δ 2.06 (s, 3H) ppm supports the formation of structure and further, spectral data was compared with the reported data (Scheme 3.1.2.).



Scheme 3.1.2. Synthesis of Hppa fragment

Having optically pure compound **9** in hand, it was converted to its TBDMS ether using TBSCl, imidazole and DMAP in DMF afforded ester **10** in 77% yield which was confirmed by IR, ^{1}H, ^{13}C NMR and HRMS analysis.[12,19] Ethyl ester in compound **10** was hydrolyzed using aqueous sodium hydroxide in THF and MeOH, afforded corresponding acid which was coupled with methyl L-valinate hydrochloride in presence of HATU and DIPEA in dichloromethane afforded dipeptide **11** in 76% yield over two steps. Formation of **11** was confirmed by IR, ^{1}H, ^{13}C NMR and HRMS analysis.

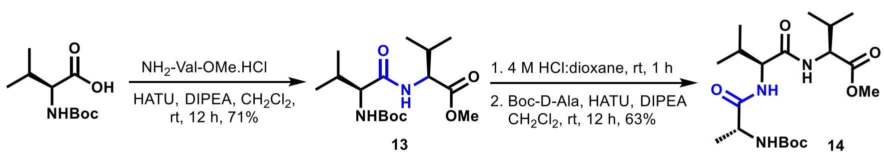


Scheme 3.1.3. Synthesis of peptide-Hppa fragment

In ^{1}H NMR characteristic olefin protons were appeared at δ 6.53 (d, J = 15.8 Hz, 1H) and 6.15 (dd, J = 15.8, 5.5 Hz, 1H) ppm, methyl ester at δ 3.65 (s, 3H) ppm and two methyl from valine at δ 0.80 (d, J = 6.7 Hz, 6H) ppm. ^{13}C NMR also shows presence of two carbonyl carbons at δ 172.3 and 170.0 ppm along with hydroxyl attached carbon at δ 70.4 ppm ensures the formation of structure **11**. Further, it was validated by HRMS analysis, which showed peak at 442.2393 corresponding to the molecular formula $C_{23}H_{37}NO_4SiNa$ $[M + Na]^+$ with calculated mass

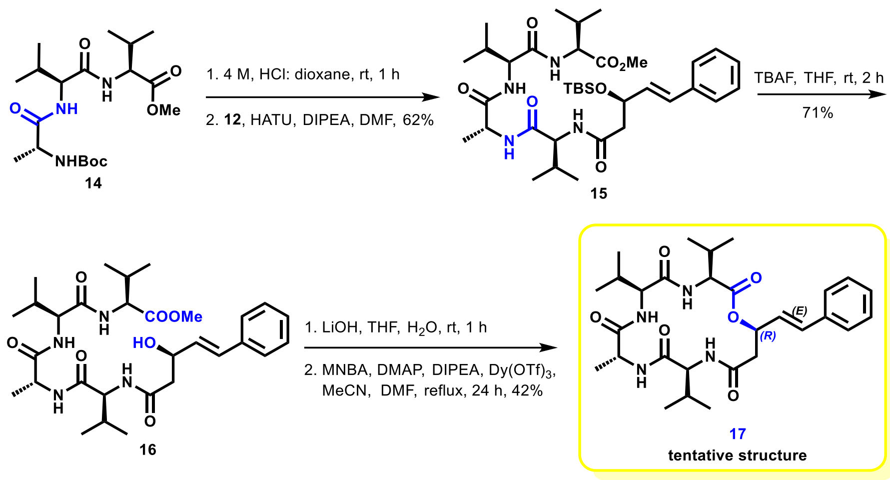
442.2389. Methyl ester in compound **11** was hydrolysed in presence of lithium hydroxide, afforded key fragment **12** as shown in Scheme 3.1.3.

To synthesize other peptidic fragment, we started with Boc-Val-OH, which undergoes coupling with methyl valinate hydrochloride in presence of HATU and DIPEA in dichloromethane, afforded dipeptide **13** in 71% yield, whose structure was confirmed by ^{1}H, ^{13}C NMR and matched with reported spectral data.[20] Dipeptide **13** was subjected under acidic conditions to remove Boc group and corresponding amine hydrochloride salt was linked with Boc-D-Ala-OH in existence of HATU and DIPEA furnished tripeptide **14** in 63% yield over two steps as shown in Scheme 3.1.4.[21] Structure of tripeptide **14** was established by IR, ^{1}H, ^{13}C NMR and HRMS analysis which was in consistent with the reported spectral data.



Scheme 3.1.4. Synthesis of peptidic fragment **14**

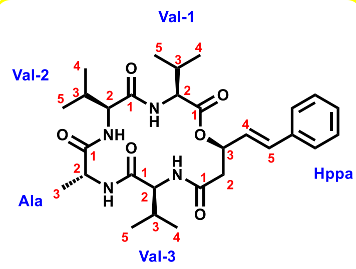
After successful synthesis of both the fragments **12** and **14**, next task was to couple them and complete the synthesis of target compound. With this in mind, tripeptide **14** was treated with 4 M HCl in dioxane to remove Boc group and corresponding amine hydrochloride salt was linked with required acid **12** along with HATU and DIPEA in DMF, yielded pentapeptide **15** in 62%. IR, ^{1}H, ^{13}C NMR data was in complete agreement with structure. Further, HRMS (ESI) showed a peak at 689.4305 equivalent to the molecular formula $C_{36}H_{61}N_4O_7Si$ $[M + H]^+$ with calculated mass 689.4309. Compound **15** was treated with tetrabutylammonium fluoride (TBAF) solution in THF for desilylation, afforded *seco* ester **16**, which was on preliminary analysis on TLC showed formation of polar spot than compound **15**. Disappearance of TBS group signal from the region δ 0.94–0.75 (m, 27H) and 0.02 (s, 6H) ppm as well as total number of protons in ^{1}H NMR confirmed the formation of **16**. Presence of characteristic peaks of five amide carbonyls at δ 172.1, 171.8, 171.4, 170.8, 170.3 ppm and hydroxyl attached methine at δ 68.2 ppm validate the formation of product. HRMS (ESI) showed peak at 597.3268 corresponding to molecular formula $C_{30}H_{46}N_4O_7Na$ $[M + Na]^+$ with calculated value 597.3264. All the spectral data was in complete accordance with the spectral data reported by Yasuhiro group.



Scheme 3.1.5. Synthesis of proposed structure of arthroamide

Then, compound **16** was forwarded for ester hydrolysis in presence of lithium hydroxide to give corresponding *seco* acid which was subjected to Shiina macrolactonization conditions in presence of MNBA, DMAP and Dy(OTf)$_3$ under dilution of ACN and DMF afforded desired compound **17** in 42% yield over two steps (Scheme 3.1.5.). In preliminary analysis by IR spectroscopy, signals at 3313 and 1666 cm^{-1} confirms the presence of amide linkage. In ^{1}H NMR, characteristic olefin protons were appeared at δ 6.66 (d, J = 15.8 Hz, 1H), 6.52 (dd, J = 6.7, 16.8 Hz, 1H), hydroxyl attached methine at δ 5.68 (m, 1H) and four α-methine of amide at δ 4.30 (m, 1H), 4.17 - 3.99 (m, 2H), 3.92 (t, J = 5.8 Hz, 1H) ppm. Also, in ^{13}C NMR, five carbonyl carbons were present at δ 171.6, 170.7, 170.5, 170.4 and 168.9 ppm indicated the formation of structure **17**. HRMS (ESI) showed peak at 565.3007 corresponding to molecular formula $C_{30}H_{46}N_4O_7Na$ [M + Na]$^+$ with calculated value 565.3002 validated the formation of product. But, when we compared the spectral data of the synthesized compound **17** with reported data of arthroamide **1**, there were major differences in the spectral data at olefin region of Hppa along with some minor differences (see table 3.1.1.). Along with this, the specific rotation of natural product **1** ($[\alpha]_D^{26}$ = +33 (*c* 0.050, 1:1, CH_2Cl_2: MeOH))[13] and synthetic compound **17** ($[\alpha]_D^{26}$ = +0.849 (*c* 0.50, CH_2Cl_2: MeOH, 1:1)) were also not matching with each other. Therefore, we assume the structure of macrocycle (**17**) as tentative and needs further confirmation. We also tried various conditions for crystallization of compound **17**, but unfortunately in all the conditions we observed formation of gel kind of mass. Also, there is possibility of migration of

C4-C5 double bond to C2-C3 position, but extensive NMR studies with the help of Dr. Uday Kiran Marelli (CSIR-NCL, Pune), ruled out the possibility of migration of double bond.



Arthroamide
proposed structure (1)

Residue	Position	Comparison of ^{1}H NMR of arthroamide		Comparison of ^{13}C NMR of arthroamide	
		Natural (**1**)	Synthetic (**17**)	Natural (**1**)	Synthetic (**17**)
Val-1	1 C=O			168.67	169.5
	2, CH	4.25, dd (9.7, 9.7)	4.127, t (8.4)	58.3	59.4
	3, CH	2.25, m	2.01, m	28.5	30.6
	4, CH_3	0.90, d (6.5)	0.85, d (6.9)	19.4	18.3
	5, CH_3	0.89, d (6.5)		19.6	
	NH	7.57, d (9.6)	7.61, d (8.4)		
Val-2	1 C=O			170.4	170.4
	2, CH	4.21, dq (9.7, 4.3)	4.05, dd (9.7, 8.2)	57.3	60.4
	3, CH_2	2.37, m	2.03, m	28.4	30.9
	4, CH_3	0.83, d (7.0)	0.86, d	16.6	17.2
	5, CH_3	0.83, d (7.0)		18.2	
	NH	8.10, d (9.7)	7.83, d (9.7)		
Ala	1 C=O			173.1	171.9
	2, CH	4.33, dq (6.9, 5.7)	4.30, dq (6.9, 6.7)	48.8	48.96

	3, CH_3	1.19, d (6.9)	1.22, d (6.9)	16.3	17.12
	NH	8.55, d (5.7)	7.92, d (6.8)		
Val-3	1 C=O			172.3	170.94
	2, CH	4.14, dd (8.3, 6.9)	3.92, t (6.0)	57.5	60.8
	3, CH	1.97, m	2.11, m	29.8	29.8
	4, CH_3	0.88, d (6.5)	0.89, d (6.5)	19.1	18.3
	5, CH_3	0.88, d (7.0)	0.89, d (6.5)	18.8	
	NH	7.73, d (8.6)	8.008, d (6.7)		
Hppa	1 C=O			168.7	170.6
	2, CH_2	2.89, dd (14.3, 11.6)	2.87, dd (15.7, 3.3)	39.5	
		2.41, dd (14.3, 2.6	2.69, dd (15.8, 6.9)		
	3, CH	5.49, m	5.68, m	73.0	73.22
	4, CH	6.28, dd (16.0, 7.0)	6.53, dd (16.0, 7.2)	126.7	127.7
	5, CH	6.68, d (16.0)	6.66, d (16.0)	132.5	132.5
	Cq			135.7	135.9
	Ar, CH	7.44, d (7.4)	7.42, d (7.4)	126.5	128
	CH	7.34, t (7.4)	7.35, t (7.2)	128.7	129
	CH	7.27, t (7.4)	7.29, t (7.2)	128.1	128.5

Table 3.1.1. Comparison of spectral data for arthroamide (**1**)[13] with synthetic compound (**17**)

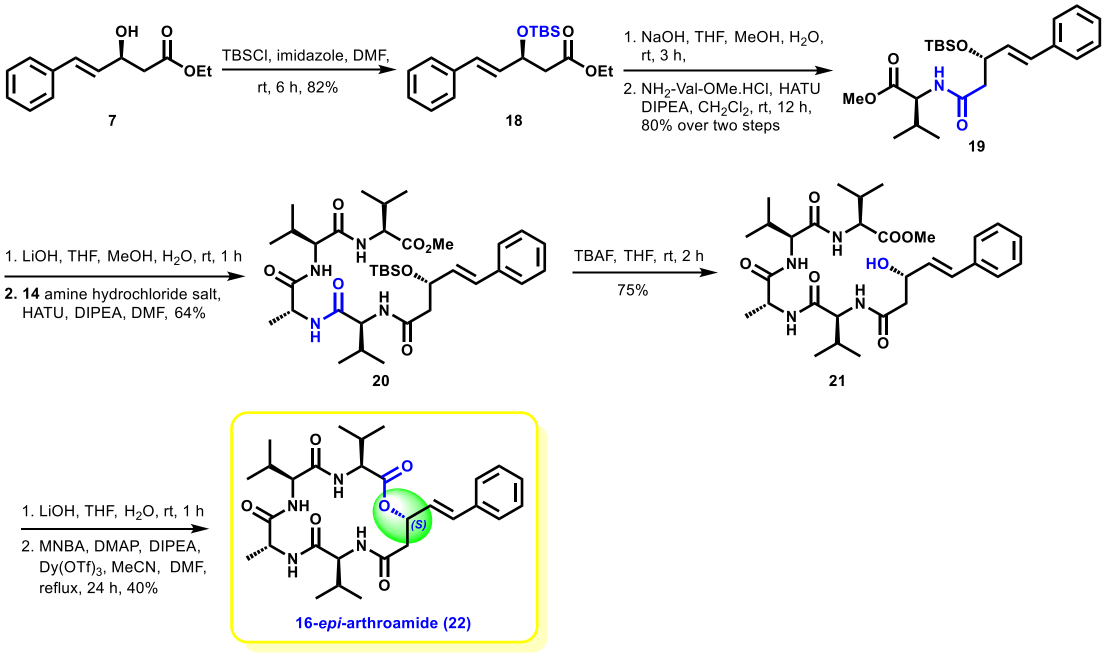
According to our observations, there are two probable causes for the observed discrepancies in the spectral data;

1) Due to the misassignment at C-16 stereocenter or
2) Epimerization at C-terminus under macrolactonization conditions. (Similar observation was also made in other projects (see chapter 2, scheme 2.5)).

To find out exact structure of arthroamide and observed ambiguity in the spectral data, we started with synthesis of 16-*epi*-arthroamide and 3-*epi*-arthroamide.

3.1.2.3. Synthesis of 16-*epi*-arthroamide

Hydroxyl group in compound **7** was protected with TBSCl, gave compound **18** in good yield. Compound **18** was treated with sodium hydroxide in presence of THF, MeOH and water afforded acid which was forwarded for coupling with methyl valinate hydrochloride, afforded dipeptide **19** in 80% yield. The structure of compound **19** was confirmed by IR, ^{1}H, ^{13}C NMR and HRMS analysis. Further, methyl ester was hydrolysed by lithium hydroxide afforded corresponding acid which was coupled with tripeptide **14** amine hydrochloride salt in presence of HATU and DIPEA in DMF afforded pentapeptide **20** in good yield. Structure of **20** was confirmed by IR, ^{1}H, and ^{13}C NMR analysis and further, it was validated by HRMS (ESI) which showed peak at 689.4305 with molecular formula $C_{36}H_{61}N_4O_7Si$ $[M + H]^+$ calculated for 689.4309.

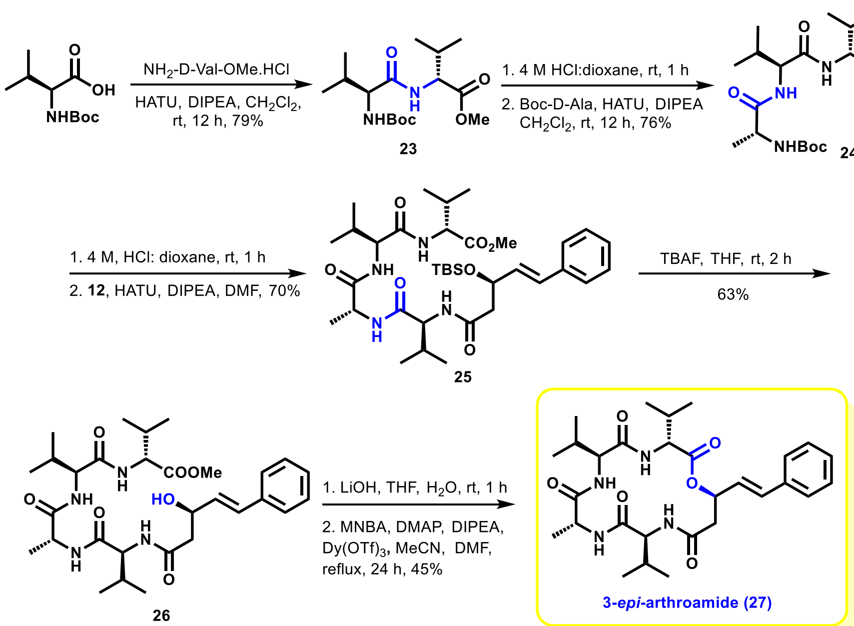


Scheme 3.1.6. Synthesis of 16-*epi*-arthroamide

TBS group in compound **20** was deprotected to furnish *seco* ester **21** in good yield. ^{1}H, ^{13}C, IR and HRMS data was in complete agreement with compound **21**. *Seco* ester **21** was subjected for methyl ester hydrolysis in presence of lithium hydroxide and cyclised under previously standardized Shiina macrolactonization condition; yielded target compound **22** in moderate amount (Scheme 3.1.6.). Macrocycle **22** was characterized by IR, ^{1}H, ^{13}C NMR and HRMS analysis. In ^{1}H NMR characteristic olefins were appeared at δ 6.70 (dd, *J* = 16.1, 6.8 Hz, 1H) and 6.57 (d, *J* = 16.1 Hz, 1H) ppm, *O*-attached methine at δ 5.58 (m, 1H) ppm along with rest of the protons in respected regions assures the formation of **22**. Five carbonyl carbons were present at δ 178.9, 171.7, 170.7, 170.1, 168.1 ppm and *O*-attached methine at δ 72.8 ppm supports the formation of 16-*epi*-arthroamide, **22**. It was further validated by HRMS analysis which showed base peak at 565.3002 corresponding to the molecular formula $C_{30}H_{46}N_4O_7Na$ $[M + Na]^+$ with calculated mass 565.3002.

3.1.2.4. Synthesis of 3-*epi*-arthroamide

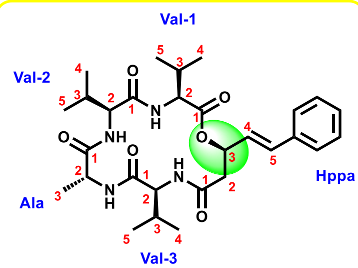
As per the plan, after successful synthesis of 16-*epi*-arhtromaide, we started synthesis of 3-*epi*-arthroamide. Tripeptide **24** was synthesized from dipeptide **23**[22] which was further constructed from methyl-D-valinate hydrochloride and Boc-Val-OH by HATU and DIPEA in dichloromethane.



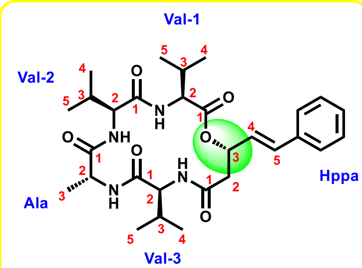
Scheme 3.1.7. Synthesis of 3-*epi*-arthroamide

Boc group in tripeptide **24** was removed under acidic conditions followed by coupling with acid **12**, rendered pentapeptide **25** in good yield which was confirmed by ^{1}H, ^{13}C, IR and HRMS analysis. Compound **25** subjected to TBS group cleavage followed by methyl ester hydrolysis and macrolactonization under previously standardised conditions of Shiina macrolactonization afforded 3-*epi*-arthroamide (**27**) in 45% yield (Scheme 3.1.7.). The structure of **27** was confirmed by IR, ^{1}H NMR and HRMS analysis and further studies on structural confirmation are in progress.

After successful synthesis of 16-*epi*-arthoamide (**22**) and 3-*epi*-arthroamide (**27**), we compared the spectral data of both the isomers with the spectral data of proposed structure of arthroamide (**1**), but they are not in agreement with each other and showed significant differences with respect to natural one (Table 3.1.2.).



Arthroamide
proposed structure (1)



16-*epi*-arthroamide (22)

Residue	Position	Comparison of ^{1}H NMR of arthroamide		Comparison of ^{13}C NMR of arthroamide	
		Natural (**1**)	Synthetic (**22**)	Natural (**1**)	Synthetic (**22**)
Val-1	1 C=O			168.67	169.5
	2, CH	4.25, dd (9.7, 9.7)	3.98, t (7.5)	58.3	59.5
	3, CH	2.25, m	2.07, m	28.5	29.72
	4, CH_3	0.90, d (6.5)	0.87, d (6.68)	19.4	18.92
	5, CH_3	0.89, d (6.5)		19.6	
	NH	7.57, d (9.6)	8.04, d (7.64)		
Val-2	1 C=O			170.4	171.33
	2, CH	4.21, dq (9.7, 4.3)	4.31, t (7.35)	57.3	57.98
	3, CH_2	2.37, m	1.93, m	28.4	30.95

	4, CH_3	0.83, d (7.0)	0.85, d (6.2)	16.6	18.73
	5, CH_3	0.83, d (7.0)		18.2	
	NH	8.10, d (9.7)	7.78, d (7.7)		
Ala	1 C=O			173.1	172.0
	2, CH	4.33, dq (6.9, 5.7)	4.42, dq (7.9,6.9)	48.8	47.75
	3, CH_3	1.19, d (6.9)	1.16, d (6.9)	16.3	16.06
	NH	8.55, d (5.7)	8.43, d (7.9)		
Val-3	1 C=O			172.3	172.2
	2, CH	4.14, dd (8.3, 6.9)	3.90, t (8.7)	57.5	59.02
	3, CH	1.97, m	1.86, m	29.8	29.48
	4, CH_3	0.88, d (6.5)	0.83, d (7.0)	19.1	19.57
	5, CH_3	0.88, d (7.0)		18.8	
	NH	7.73, d (8.6)	8.035, d (8.42)		
Hppa	1 C=O			168.7	168.67
	2, CH_2	2.89, dd (14.3, 11.6)	2.82, dd (15.8,3.8)	39.5	40.01
		2.41, dd (14.3, 2.6	2.44, dd (15.42,5.1)		
	3, CH	5.49, m	5.58, m	73.0	70.0
	4, CH	6.28, dd (16.0, 7.0)	6.70, dd (16.14,7.4)	126.7	128.43
	5, CH	6.68, d (16.0)	6.57, d (16.1)	132.5	131.27
	Cq			135.7	135.9
	Ar, CH	7.44, d (7.4)	7.39, d (7.54)	126.5	126.92
	CH	7.34, t (7.4)	7.33, t (7.40)	128.7	129.03
	CH	7.27, t (7.4)	7.25, t (7.42)	128.1	128.26

Table 3.1.2. Comparison of spectral data for arthroamide (**1**)[13] with 16-*epi*-arthroamide (**22**)

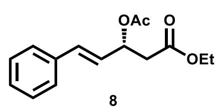
In all the cases there were discrepancies in spectral data at the olefin region and α-methine region of corresponding amino acids. These observations, ruled out the possibility of misassignment at C-16 stereocenter. Further, NMR studies on 3-*epi*-arthroamide are in progress.

3.1.3. Conclusions

We have successfully synthesized the proposed structure of arthroamide. Along this line, we also synthesized 16-*epi*-arthroamide and 3-*epi*-arthroamide. Comparison of spectral data of all synthesized isomers with natural arthroamide, revealed major differences in spectral data at olefin region of Hppa and α-methine protons. Further work and analysis is needed to make conclusions on the structural assignment part, which is underway presently in our group.

3.1.4. Experimental section

Ethyl (*R*,*E*)-3-acetoxy-5-phenylpent-4-enoate (8)



A suspension of (±)-**6** (4.0 g, 19.2 mmol), *Amano PS* (1.5 g) and vinyl acetate (8.8 mL, 96.0 mmol) in benzene–hexane (50 mL, 1:2) was heated at 40 °C for 36 h. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10-15% ethyl acetate in pet ether) to afford alcohol (***S***)-**7** (1.8 g, 45%, ee 94%) and acetate (***R***)-**8** as pale yellow oils.

Yield: 43% (2.1 g)

IR ν_{max} (film): 3045, 1738, 1660, 1468 cm^{-1}

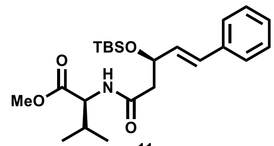
Specific rotation: $[\alpha]_D^{26}$ = +48.3 (*c* 0.31, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29 (s, 2H), 7.22 (t, *J* = 7.3 Hz, 2H), 7.17 - 7.14 (m, 1H), 6.58 (d, *J* = 15.7 Hz, 1H), 6.08 (dd, *J* = 7.3, 15.7 Hz, 1H), 5.72 (q, *J* = 6.8 Hz, 1H), 4.06 (q, *J* = 7.0 Hz, 2H), 2.70 (dd, *J* = 7.8, 15.7 Hz, 1H), 2.59 (dd, *J* = 5.6, 15.4 Hz, 1H), 1.98 (s, 3H), 1.16 (t, *J* = 7.3 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 169.8, 169.7, 135.9, 133.3, 128.5, 128.1, 126.6, 125.9, 70.9, 60.7, 39.8, 21.1, 14.2

HRMS (ESI): calculated for $C_{15}H_{18}O_4Na$ $[M + Na]^+$: 285.1102, found: 285.1106.

Methyl ((*R*,*E*)-3-((*tert*-butyldimethylsilyl)oxy)-5-phenylpent-4-enoyl)-L-valinate (11)



11

To a solution of compound **10** (1.2 g, 3.59 mmol) in THF (5 mL), MeOH (5 mL) and H_2O (5 mL) was added NaOH (0.377 g, 8.98 mmol) and the reaction mixture was stirred for 3 h at room temperature. The reaction mixture was acidified with 1 M aqueous HCl and extracted with EtOAc. The organic layer was dried in *vacuo* to get the acid which was used for the next reaction without further purification.

To a stirred solution of above prepared acid (0.90 g, 2.94 mmol) and L-valine methyl ester hydrochloride (0.621 g, 3.52 mmol) in anhydrous dichloromethane (35 mL) at 0 °C were added EDC.HCl (0.842 g, 4.41 mmol), HOBt (0.584 g, 3.82 mmol) and Hünig's base (2.5 mL, 14.7 mmol). After being stirred at room temperature for 12 h, the reaction mixture was quenched with water and extracted with CH_2Cl_2. The organic layer was washed with 1 N HCl (30 mL) and a saturated $NaHCO_3$ solution (30 mL), dried over anhydrous Na_2SO_4, concentrated under reduced pressure to get the crude product which was purified by silica gel column chromatography using ethyl acetate and hexane (3:17) as mobile phase to obtain compound **11** as sticky liquid.

Yield: 76% (0.936 g)

IR ν_{max} (film): 3317, 3079, 2969, 1745, 1654 cm^{-1}

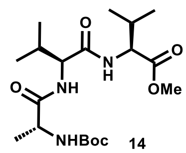
Specific rotation: $[\alpha]_D^{26}$ = +52 (*c* 1.0, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 (q, *J* = 8.1 Hz, 4H), 7.25 - 7.22 (m, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.61 (d, *J* = 15.9 Hz, 1H), 6.23 (dd, *J* = 5.5, 15.9 Hz, 1H), 4.76 (q, *J* = 4.9 Hz, 1H), 4.60 (dd, *J* = 4.9, 9.2 Hz, 1H), 3.73 (s, 3H), 2.67 (dd, *J* = 4.9, 15.3 Hz, 1H), 2.54 (dd, *J* = 5.5, 15.3 Hz, 1H), 2.15 - 2.06 (m, 1H), 0.95 (s, 9H), 0.87 (d, *J* = 6.7 Hz, 6H), 0.13 (d, *J* = 5.5 Hz, 6H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 172.3, 170.0, 136.4, 130.5, 130.3, 128.5, 127.6, 126.4, 70.4, 56.9, 51.9, 44.8, 31.4, 25.8, 18.9, 18.2, 17.8, -4.5, -5.1

HRMS (ESI): calculated for $C_{23}H_{37}NO_4SiNa$ $[M + Na]^+$: 442.2389, found: 442.2393.

Methyl (*tert*-butoxycarbonyl)-D-alanyl-L-valyl-L-valinate (14)



14

To a solution of *N*-Boc dipeptide **13** (1.32 g, 4.0 mmol) was added TFA (2.0 mL) in anhydrous CH_2Cl_2 (8 mL) at room temperature under argon. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To the solution of *N*-Boc-D-alanine (0.638 g, 3.38 mmol) in anhydrous CH_2Cl_2 (40 mL) were added DIPEA (2.35 mL, 13.5 mmol) and HATU (1.96 g, 5.07 mmol) at room temperature under argon. Then above synthesized dipeptide amine.HCl salt (1.2 g, 3.38 mmol) in CH_2Cl_2 (20 mL) was added to the reaction mixture. After being stirred at the same temperature for 12 h, the reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with 1 N HCl (25 mL), saturated solution of $NaHCO_3$ (25 mL), brine (30 mL) and then evaporated to dryness. The crude tripeptide was purified by silica gel column chromatography using ethyl acetate and hexane (2:3) as mobile phase to obtain tripeptide **14**.

Yield: 63% (0.853 g, for two steps)

IR ν_{max} (film): 3413, 3328, 3021, 2969, 2403, 1669 cm^{-1}

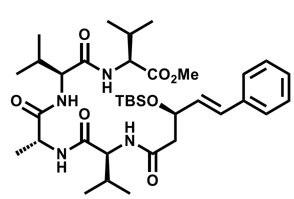
Specific rotation: $[\alpha]_D^{26}$ = +5.8 (*c* 1.0, $CHCl_3$)

^{1}H NMR (400 MHz, MeOH-d_4): δ 4.29 (d, *J* = 6.8 Hz, 2H), 4.08 (q, *J* = 7.5 Hz, 1H), 3.70 (s, 3H), 2.20 - 2.07 (m, 2H), 1.44 (s, 9H), 1.30 (d, *J* = 6.8 Hz, 3H), 0.97 - 0.92 (m, 12H)

^{13}C NMR (100 MHz, MeOH-d_4): δ 176.1, 173.9, 173.4, 80.8, 59.6, 59.5, 52.5, 52.1, 32.3, 31.7, 28.8, 19.8, 19.6, 18.8, 18.5, 18.4

HRMS (ESI): calculated for $C_{19}H_{35}O_6N_3Na$ $[M + Na]^+$: 424.2425, found: 424.2422.

Methyl ((*R*,*E*)-3-((*tert*-butyldimethylsilyl)oxy)-5-phenylpent-4-enoyl)-L-valyl-D-alanyl-L-valyl-L-valinate (15)



15

To a solution of *N*-Boc tripeptide **14** (0.864 g, 2.17 mmol) was added TFA (2.0 mL) in anhydrous CH_2Cl_2 (8 mL) at room temperature under argon. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of methyl ester of compound **11** (0.9 g, 2.14 mmol) in MeOH (6 mL), THF (6 mL) and H_2O (2 mL) was added LiOH.H_2O (0.299 g, 6.42 mmol) at room temperature. After being stirred at the same temperature for 2 h, the reaction mixture was concentrated to remove THF and MeOH. Acidified to pH 3 with 1 N HCl, extracted with ethyl acetate (20 mL X 3).Combined organic layers were dried over Na_2SO_4, concentrated under reduced pressures to afford crude acid **12**, which was used for the next reaction without further purification.

To the solution of the *N*-Boc deprotected residue of **14** in DMF (40 mL) was added acid (Hppa-val-OH) **12**, DIPEA (1.3 mL, 7.88 mmol), HATU (1.14 g, 2.99 mmol) and HOAt (0.267 g) at room temperature under argon. After being stirred at the same temperature for 12 h, the reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with 1 N HCl (25 mL), saturated solution of $NaHCO_3$ (25 mL), brine (30 mL) and then evaporated to dryness. The crude tetra peptide was purified by silica gel column chromatography using ethyl acetate and hexane (2:3) as mobile phase to obtain tetra peptide **15** as white foam.

Yield: 62% (0.928 g, for two steps)

IR ν_{max} (film): 3413, 3328, 3021, 2969, 2403, 1669 cm^{-1}

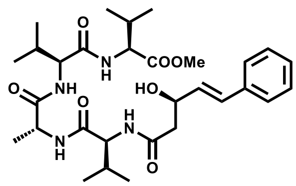
Specific rotation: $[\alpha]_D^{26}$ = +7.4 (*c* 0.3, MeOH: CH_2Cl_2, 1:1)

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.17 (t, *J* = 7.6 Hz, 2H), 7.89 - 7.84 (m, 2H), 7.37 (d, *J* = 7.8 Hz, 2H), 7.32 (t, *J* = 7.3 Hz, 2H), 7.25 - 7.21 (m, 1H), 6.51 (d, *J* = 16.1 Hz, 1H), 6.27 (dd, *J* = 6.4, 16.1 Hz, 1H), 4.68 (q, *J* = 6.4 Hz, 1H), 4.46 - 4.38 (m, 1H), 4.33 - 4.29 (m, 1H), 4.19 (t, *J* = 8.1 Hz, 1H), 4.10 (t, *J* = 6.8 Hz, 1H), 3.60 (s, 3H), 2.46 - 2.39 (m, 2H), 2.00 (td, *J* = 6.7, 13.0 Hz, 2H), 1.86 - 1.79 (m, 1H), 1.19 (d, *J* = 6.8 Hz, 3H), 0.91 - 0.84 (m, 18H), 0.80 - 0.74 (m, 9H), 0.02 (s, 6H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 171.9, 171.8, 171.3, 170.5, 169.1, 136.5, 132.1, 128.8, 128.6, 127.5, 126.2, 70.8, 57.6, 56.9, 51.5, 47.9, 44.5, 30.8, 30.6, 29.5, 29.0, 25.7, 19.1, 19.0, 18.9, 18.4, 18.3, 17.9, 17.9, -4.6, -4.9

HRMS (ESI): calculated for $C_{36}H_{61}N_4O_7Si$ $[M + H]^+$: 689.4309, found: 689.4305.

Methyl ((*R*,*E*)-3-hydroxy-5-phenylpent-4-enoyl)-L-valyl-D-alanyl-L-valyl-L-valinate (16)



16

To a stirred solution of silyl ether **15** (0.9 g, 1.30 mmol) in anhydrous THF (10 mL) was added TBAF (2.6 mL, 1 M solution in THF, 2.6 mmol). The reaction mixture was stirred for 1 h at room temperature. After completion of the reaction (monitored by TLC), it was quenched with aqueous ammonium chloride solution (5 mL). The reaction mixture was extracted with ethyl acetate (3 X 10 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography utilizing MeOH and CH_2Cl_2 (1:49) as mobile phase to afford alcohol **16** as a colourless foam.

Yield: 71% (0.540 g)

IR ν_{max} (film): 3576, 3369, 3303, 3017, 2974, 1667, 1529 cm^{-1}

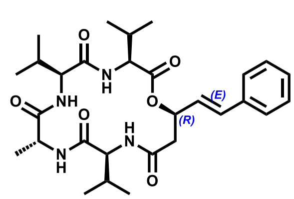
Specific rotation: $[\alpha]_D^{26}$ = -1.8 (*c* 0.5, MeOH: CH_2Cl_2, 1:1)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.21 - 8.17 (m, 1H), 8.11 - 8.05 (m, 1H), 7.92 - 7.86 (m, 1H), 7.84 - 7.76 (m, 1H), 7.39 (d, *J* = 7.3 Hz, 2H), 7.31 (t, *J* = 7.6 Hz, 2H), 7.23 - 7.20 (m, 1H), 6.54 (d, *J* = 16.1 Hz, 1H), 6.33 - 6.25 (m, 1H), 5.20 - 5.15 (m, 1H), 4.52 - 4.50 (m, 1H), 4.39 - 4.30 (m, 2H), 4.22 - 4.19 (m, 1 H), 4.11 (t, *J* = 6.8 Hz, 1H), 3.60 (s, 3H), 2.43 - 2.34 (m, 2H), 1.96 - 1.93 (m, 3H), 1.20 - 1.16 (m, 3H), 0.91 - 0.79 (m, 18H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 172.0, 171.7, 171.3, 170.6, 170.1, 136.8, 133.3, 128.6, 128.0, 127.2, 126.2, 68.2, 57.6, 57.4, 56.8, 51.5, 48.1, 43.5, 30.9, 30.5, 29.5, 19.3, 19.0, 18.9, 18.6, 18.3, 18.1, 17.8

HRMS (ESI): calculated for $C_{30}H_{46}N_4O_7Na$ $[M + Na]^+$: 597.3264, found: 597.3268.

(3*S*,6*S*,9*R*,12*S*,16*R*)-3,6,12-triisopropyl-9-methyl-16-((*E*)-styryl)-1-oxa-4,7,10,13-tetraazacyclohexadecane-2,5,8,11,14-pentaone(17)



17

Lithium hydroxide monohydrate (0.182 g, 4.35 mmol) was added to a vigorously stirring solution of tetra peptide methyl ester, **16** (0.5 g, 0.871 mmol) in THF (10 mL) and H_2O (5 mL). Following complete consumption of the starting material by TLC, the reaction mixture was acidified with 1 N HCl until the resulting solution was acidified to pH 2–3. The reaction mixture was diluted with water and extracted with ethyl acetate (3 X 15 mL). The combined organic extracts were dried over Na_2SO_4, concentrated to dryness under reduced pressure to yield the crude acid quantitatively which was used for next step without further purification.

MNBA (0.185 g, 0.535 mmol) and DMAP (0.131 g, 1.07 mmol) were loaded into a round bottom flask (RBF) equipped with a side arm, dissolved in anhydrous CH_2Cl_2 (60 mL), and Hünig's base (0.093 mL, 0.535 mmol) and $Dy(OTf)_3$ (0.108 g, 0.178 mmol) were successively added. The flask was fitted with a water cooled condenser and heated to reflux under argon atmosphere. To the refluxing reaction mixture was slowly added (through the side arm) the *seco* acid (0.1 g, 0.178 mmol) in CH_2Cl_2 (17.5 mL) and DMF (2.5 mL) *via* syringe pump (1.0 mL/h) over ~20 h. After the addition was complete the reaction was continued for another 6 h under reflux. The reaction mixture was cooled to room temperature and concentrated, giving a crude residue. The crude residue was then purified by column chromatography (gradient elution, 99.5:0.5 to 95:5 CH_2Cl_2: MeOH) to yield the corresponding macrocycle **17** as white solid.

Yield: 42% (0.044 g)

IR ν_{max} (film): 3686, 3411, 3024, 2403, 1783, 1675, 1525 cm^{-1}

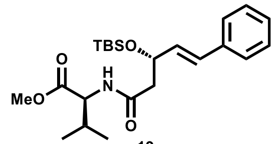
Specific rotation: $[\alpha]_D^{26}$ = +0.85 (*c* 0.50, MeOH: CH_2Cl_2, 1: 1)

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.01 (d, *J* = 6.1 Hz, 1H), 7.92 (d, *J* = 6.7 Hz, 1H), 7.83 (d, *J* = 9.8 Hz, 1H), 7.62 (d, *J* = 8.5 Hz, 1H), 7.43 - 7.41 (m, 2H), 7.35 (t, *J* = 7.3 Hz, 2H), 7.29 (d, *J* = 7.3 Hz, 1H), 6.67 (d, *J* = 15.9 Hz, 1H), 6.53 (dd, *J* = 7.0, 16.2 Hz, 1H), 5.68 (br.s., 1H), 4.31 (quin, *J* = 6.7 Hz, 1H), 4.12 (t, *J* = 8.2 Hz, 1H), 4.05 (t, *J* = 8.9 Hz, 1H), 3.92 (t, *J* = 5.8 Hz, 1H), 2.89 - 2.85 (m, 1H), 2.69 (dd, *J* = 6.4, 15.6 Hz, 1H), 2.13 - 2.09 (m, 1H), 2.01 (td, *J* = 7.2, 13.7 Hz, 2H), 1.22 (d, *J* = 6.7 Hz, 3H), 0.93 - 0.84 (m, 18H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 171.6, 170.7, 170.5, 170.4, 168.9, 135.9, 132.2, 128.8, 128.1, 127.0, 126.5, 72.6, 60.1, 60.1, 59.1, 48.7, 30.3, 30.3, 29.3, 19.3, 19.1, 18.9, 18.6, 18.3, 17.9, 17.1

HRMS (ESI): calculated for $C_{30}H_{46}N_4O_7Na$ $[M + Na]^+$: 565.3002, found: 565.3007.

Methyl ((*S*,*E*)-3-((*tert*-butyldimethylsilyl)oxy)-5-phenylpent-4-enoyl)-L-valinate (19)



19

Compound **19** was synthesized from Hppa **18** and methyl valinate hydrochloride by following similar procedure for the synthesis of compound **11**.

Yield: 80%

IR ν_{max} (film): 3317, 3079, 2969, 1745, 1654 cm^{-1}

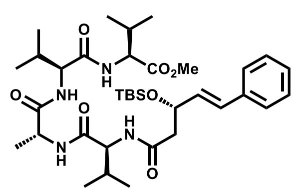
Specific rotation: $[\alpha]_D^{26}$ = -32.8 (*c* 1.25, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (d, *J* = 7.3 Hz, 2H), 7.31 (t, *J* = 7.3 Hz, 2H), 7.25 - 7.22 (m, 1H), 6.93 (d, *J* = 7.9 Hz, 1H), 6.61 (d, *J* = 15.9 Hz, 1H), 6.30 (dd, *J* = 6.1, 15.9 Hz, 1H), 4.76 (q, *J* = 5.3 Hz, 1H), 4.53 (dd, *J* = 5.5, 8.5 Hz, 1H), 3.64 (s, 3H), 2.68 (dd, *J* = 4.6, 15.0 Hz, 1H), 2.53 (dd, *J* = 5.2, 15.0 Hz, 1H), 2.14 (qd, *J* = 6.6, 12.7 Hz, 1H), 0.97 - 0.93 (m, 15H), 0.14 (d, *J* = 4.3 Hz, 6H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 170.3, 136.5, 130.4, 128.4, 127.5, 126.5, 70.6, 57.1, 51.8, 44.8, 31.1, 25.8, 18.9, 18.1, 18.1, -4.4, -5.0

HRMS (ESI): calculated for $C_{23}H_{37}NO_4SiNa$ [M + Na]$^+$: 442.2389, found: 442.2393.

Methyl ((*S*,*E*)-3-((*tert*-butyldimethylsilyl)oxy)-5-phenylpent-4-enoyl)-L-valyl-D-alanyl-L-valyl-L-valinate (20)



20

Compound **20** was synthesized from **19** and tripeptide **14** by following similar procedure for the synthesis of compound **15**.

Yield: 64%

IR ν_{max} (film): 3413, 3328, 3021, 2969, 2403, 1669 cm^{-1}

Specific rotation: $[\alpha]_D^{26}$ = -11.9 (*c* 0.51, MeOH: CH_2Cl_2, 1:1)

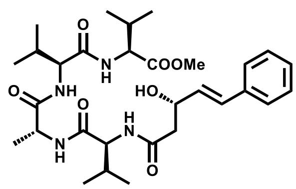
^{1}H NMR (400 MHz, DMSO-d_6): δ 7.40 - 7.38 (m, 2H), 7.31 (t, *J* = 7.3 Hz, 2H), 7.24 - 7.21 (m, 1H), 6.53 (d, *J* = 15.6 Hz, 1H), 6.30 (dd, *J* = 5.4, 15.7 Hz, 1H), 4.74 (br. s., 1H), 4.38 (q, *J* = 6.4

Hz, 1H), 4.28 (d, *J* = 6.8 Hz, 1H), 4.10 (d, *J* = 4.9 Hz, 2H), 3.60 (s, 3H), 2.29 (d, *J* = 14.2 Hz, 1H), 2.10 - 1.87 (m, 4H), 1.18 (d, *J* = 6.8 Hz, 3H), 0.90 - 0.86 (m, 24H), 0.78 (d, *J* = 6.4 Hz, 3H), 0.03 (s, 6H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 171.8, 171.8, 171.2, 170.8, 169.4, 136.5, 132.6, 128.6, 128.4, 127.4, 126.3, 70.3, 58.2, 57.5, 56.9, 51.5, 47.9, 44.2, 30.6, 30.0, 29.5, 29.0, 25.8, 19.1, 19.0, 18.9, 18.8, 18.4, 18.0, 17.9, -4.5, -4.8

HRMS (ESI): calculated for $C_{36}H_{61}N_4O_7Si$ $[M + H]^+$: 689.4309, found: 689.4305.

Methyl ((S,E)-3-hydroxy-5-phenylpent-4-enoyl)-L-valyl-D-alanyl-L-valyl-L-valinate (21)



21

Compound **21** was synthesized from compound **20** by following similar procedure for the synthesis of compound **16**.

Yield: 75%

IR ν_{max} (film): 3576, 3369, 3303, 3017, 2974, 1667, 1529 cm^{-1}

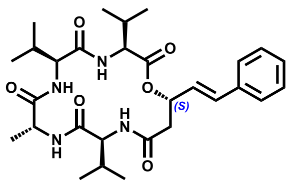
Specific rotation: $[\alpha]_D^{26}$ = -4.1 (*c* 0.9, MeOH: CH_2Cl_2, 1: 1)

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.17 (d, *J* = 7.3 Hz, 2H), 7.89 (d, *J* = 8.3 Hz, 1H), 7.81 (d, *J* = 8.8 Hz, 1H), 7.40 - 7.37 (m, 2H), 7.31 (t, *J* = 7.6 Hz, 2H), 7.23 - 7.20 (m, 1H), 6.54 (d, *J* = 16.1 Hz, 1H), 6.32 (dd, *J* = 5.4, 15.7 Hz, 1H), 5.14 (d, *J* = 4.9 Hz, 1H), 4.52 (br. s., 1H), 4.40 (t, *J* = 7.3 Hz, 1H), 4.30 (dd, *J* = 7.1, 8.6 Hz, 1H), 4.18 (t, *J* = 7.6 Hz, 1H), 4.10 (t, *J* = 6.8 Hz, 1H), 3.60 (s, 3H), 2.42 (d, *J* = 7.8 Hz, 1H), 2.35 - 2.30 (m, 1H), 2.03 - 1.91 (m, 3H), 1.19 (d, *J* = 7.3 Hz, 3H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.87 - 0.84 (m, 12H), 0.79 (d, *J* = 6.8 Hz, 3H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 172.1, 171.8, 171.4, 170.8, 170.3, 136.8, 133.4, 128.6, 128.0, 127.3, 126.3, 68.2, 58.0, 57.7, 57.0, 51.6, 48.1, 43.5, 30.8, 30.5, 29.6, 19.2, 19.1, 18.9, 18.8, 18.4, 18.0

HRMS (ESI): calculated for $C_{30}H_{46}N_4O_7Na$ $[M + Na]^+$: 597.3264, found: 597.3268.

(3*S*,6*S*,9*R*,12*S*,16*S*)-3,6,12-triisopropyl-9-methyl-16-((*E*)-styryl)-1-oxa-4,7,10,13-tetraazacyclohexadecane-2,5,8,11,14-pentaone (22)



22

Compound **22** was synthesized from *seco* ester **21** by following similar procedure for the synthesis of compound **17**.

Yield: 40%

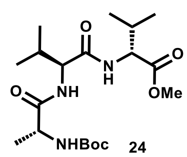
IR ν_{max} (film): 3686, 3411, 3024, 2403, 1783, 1675, 1525 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.45 (d, *J* = 7.82 Hz, 1H), 8.05 (d, *J* = 8.31 Hz, 1H), 8.09 (d, *J* = 7.34 Hz, 1H), 7.78 (d, *J* = 7.34 Hz, 1H), 7.39 - 7.41 (m, 2H), 7.34 (t, *J* = 7.34 Hz, 2H), 7.27 (d, *J* = 6.85 Hz, 1H), 6.70 (dd, *J* = 7.09, 15.89 Hz, 1H), 6.57 (d, *J* = 16.14 Hz, 1H), 5.58 (br. s., 1H), 4.40 - 4.44 (m, 1H), 4.21 (t, *J* = 7.09 Hz, 1H), 3.98 (t, *J* = 7.09 Hz, 1H), 3.90 (t, *J* = 8.56 Hz, 1H), 2.82 (d, *J* = 13.20 Hz, 1H), 2.44 (br. s., 1H), 2.05 - 2.10 (m, 1H), 1.95 (dd, *J* = 6.60, 12.96 Hz, 1H), 1.84 - 1.87 (m, 1H), 1.17 (d, *J* = 6.36 Hz, 3H), 0.83 - 0.87 (m, 18H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 171.9, 171.7, 170.7, 170.2, 168.1, 136.2, 130.9, 128.7, 127.8, 126.4, 72.8, 58.9, 58.5, 57.5, 47.2, 30.7, 29.3, 29.1, 29.0, 19.4, 19.2, 19.1, 18.8, 18.5, 18.4, 15.7

HRMS (ESI): calculated for $C_{30}H_{46}N_4O_7Na$ $[M + Na]^+$: 565.3002, found: 565.3007.

Methyl (*tert*-butoxycarbonyl)-D-alanyl-L-valyl-D-valinate (24)



24

Compound **24** was synthesized from dipeptide **23** by following similar procedure for the synthesis of compound **14**.

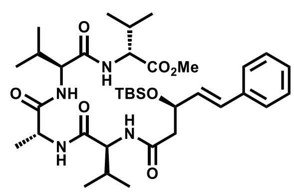
Yield: 76%

IR ν_{max} (film): 3424, 3238, 3011, 2979, 2413, 1670 cm^{-1}

Specific rotation: $[\alpha]_D^{26}$ = +3.2 (*c* 0.8, $CHCl_3$)

^{1}H NMR (400 MHz, MeOH-*d*$_4$): δ 8.12 (d, *J* = 6.7 Hz, 1H), 7.72 (d, *J* = 8.5 Hz, 1H), 4.37 - 4.30 (m, 2H), 4.10 (q, *J* = 6.3 Hz, 1H), 3.70 (s, 3H), 2.19 - 2.14 (m, 2H), 1.44 (s, 9H), 1.31 (d, *J* = 7.3 Hz, 3H), 0.96 - 0.91 (m, 12H)
^{13}C NMR (100 MHz, MeOH-*d*$_4$): δ 176.2, 173.8, 173.4, 157.7, 80.7, 59.6, 59.5, 52.6, 52.1, 39.0, 32.4, 31.7, 28.8, 20.0, 18.9, 18.4, 18.1
HRMS (ESI): calculated for $C_{19}H_{35}O_6N_3Na$ $[M + Na]^+$: 424.2425, found: 424.2421.

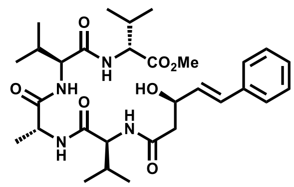
Methyl ((*R*,*E*)-3-((tert-butyldimethylsilyl)oxy)-5-phenylpent-4-enoyl)-L-valyl-D-alanyl-L-valyl-D-valinate (25)



25

Compound **25** was synthesized from tripeptide **24** and acid **12** by following similar procedure for the synthesis of compound **15**.
Yield: 70%
IR ν_{max} (film): 3385, 3268, 3028, 2379, 1669, 1638 cm^{-1}
Specific rotation: $[\alpha]_D^{26}$ = +9.2 (*c* 0.29, MeOH: CH_2Cl_2, 1:1)
^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.23 (br. s., 1H), 8.17 (br. s., 1H), 8.01 - 7.88 (m, 1H), 7.85 - 7.74 (m, 1H), 7.38 - 7.31 (m, 4H), 7.22 (br. s., 1H), 6.53 (t, *J* = 13.4 Hz, 1H), 6.32 - 6.29 (m, 1H), 4.75 (br. s., 1H), 4.39 (br. s., 2H), 4.19 (br. s., 1H), 4.10 (br. s., 1H), 3.62 (br. s., 3H), 2.30 (d, *J* = 11.6 Hz, 2H), 2. 01 - 1.86 (m, 3H), 1.19 (br. s., 3H), 0.86 (br. s., 27H), 0.03 (br. s., 6H)
^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 172.0, 171.1, 170.9, 169.7, 169.2, 136.5, 132.6, 132.1, 128.9, 128.6, 128.4, 127.4, 126.3, 126.2, 70.7, 70.3, 58.5, 57.3, 57.0, 51.6, 48.1, 44.3, 31.1, 31.0, 30.6, 29.9, 25.8, 25.7, 19.2, 19.1, 19.0, 18.8, 18.6, 18.2, 18.2, 17.9, 17.6, 17.5, -4.5, -4.9
HRMS (ESI): calculated for $C_{36}H_{61}N_4O_7Si$ $[M + H]^+$: 689.4309, found: 689.4312.

Ethyl ((*R*,*E*)-3-hydroxy-5-phenylpent-4-enoyl)-L-valyl-D-alanyl-L-valyl-D-valinate (26)



26

Compound **26** was synthesized from compound **25** by following similar procedure for the synthesis of compound **16**.

Yield: 63%

IR ν_{max} (film): 3540, 3219, 3028, 1672, 1640 cm^{-1}

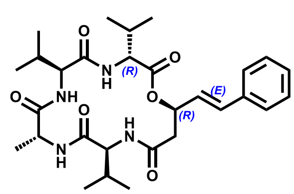
Specific rotation: $[\alpha]_D^{26}$ = -1.8 (*c* 0.15, MeOH: CH_2Cl_2, 1:1)

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 (d, *J* = 7.2 Hz, 1H), 8.07 - 7.01 (m, 1H), 7.94 - 7.85 (m, 1H), 7.78 (d, *J* = 8.8 Hz, 1H), 7.41 - 7.21 (m, 5H), 6.54 (d, *J* = 16.0 Hz, 1H), 6.29 (td, *J* = 5.2, 15.9 Hz, 1H), 5.23 - 5.14 (m, 1H), 4.53 (br. s., 1H), 4.35 (dt, *J* = 7.2, 9.2 Hz, 2H), 4.25 - 4.08 (m, 2H), 3.60 (s, 3H), 2.45 - 2.38 (m, 2H), 2.08 - 1.88 (m, 3H), 1.23 - 1.16 (m, 3H), 0.90 - 0.79 (m, 18H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 172.7, 172.0, 171.2, 171.2, 169.0, 136.8, 133.4, 128.6, 127.9, 127.2, 126.2, 68.2, 57.5, 57.4, 57.1, 51.7, 48.3, 43.5, 30.9, 29.8, 29.8, 19.2, 19.2, 19.0, 18.7, 18.5, 18.3, 17.6, 17.4

HRMS (ESI): calculated for $C_{30}H_{46}N_4O_7Na$ $[M + Na]^+$: 597.3264, found: 597.3252.

(3*R*,6*S*,9*R*,12*S*,16*R*)-3,6,12-triisopropyl-9-methyl-16-((*E*)-styryl)-1-oxa-4,7,10,13-tetraazacyclohexadecane-2,5,8,11,14-pentaone (27)



27

Compound **27** was synthesized from compound **26** by following similar procedure for the synthesis of compound **17**.

Yield: 45%

IR ν_{max} (film): 3676, 3411, 2989, 2428, 1783, 1678, 1564 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.01 (d, *J* = 6.7 Hz, 1H), 7.92 (d, *J* = 6.7 Hz, 1H), 7.83 (d, *J* = 9.2 Hz, 1H), 7.62 (d, *J* = 8.5 Hz, 1H), 7.41 - 7.43 (m, 2H), 7.35 (t, *J* = 7.3 Hz, 2H), 7.29 (d, *J* = 7.3 Hz, 1H), 6.67 (d, *J* = 16.5 Hz, 1H), 6.53 (dd, *J* = 7.0, 16.2 Hz, 1H), 5.70 - 5.66 (m, 1H), 4.34 - 4.29 (m, 1H), 4.15 - 4.03 (m, 2H), 3.92 (t, *J* = 6.1 Hz, 1H), 2.87 (dd, *J* = 3.1, 15.3 Hz, 1H), 2.69 (dd, *J* = 7.0, 15.6 Hz, 1H), 2.19 - 1.98 (m, 3H), 1.22 (d, *J* = 6.7 Hz, 3H), 0.93 - 0.83 (m, 18H)

HRMS (ESI): calculated for $C_{30}H_{46}N_4O_7Na$ $[M + Na]^+$: 565.3002, found: 565.2995.

3.1.5. References

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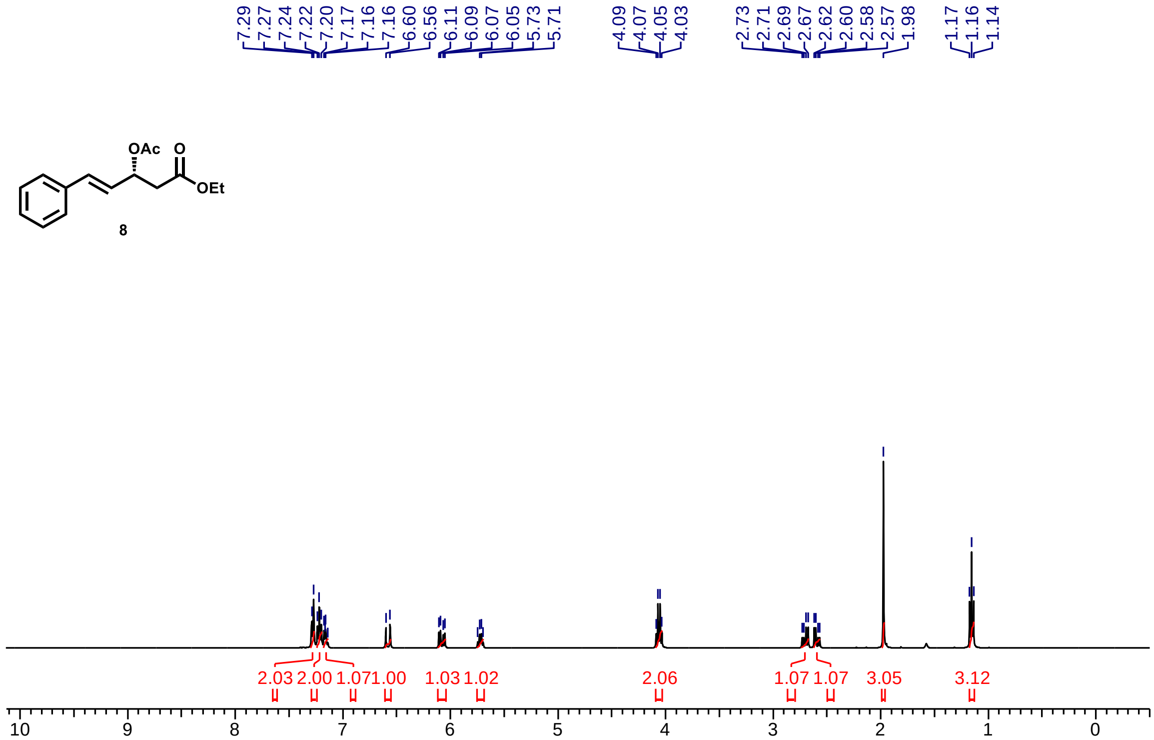
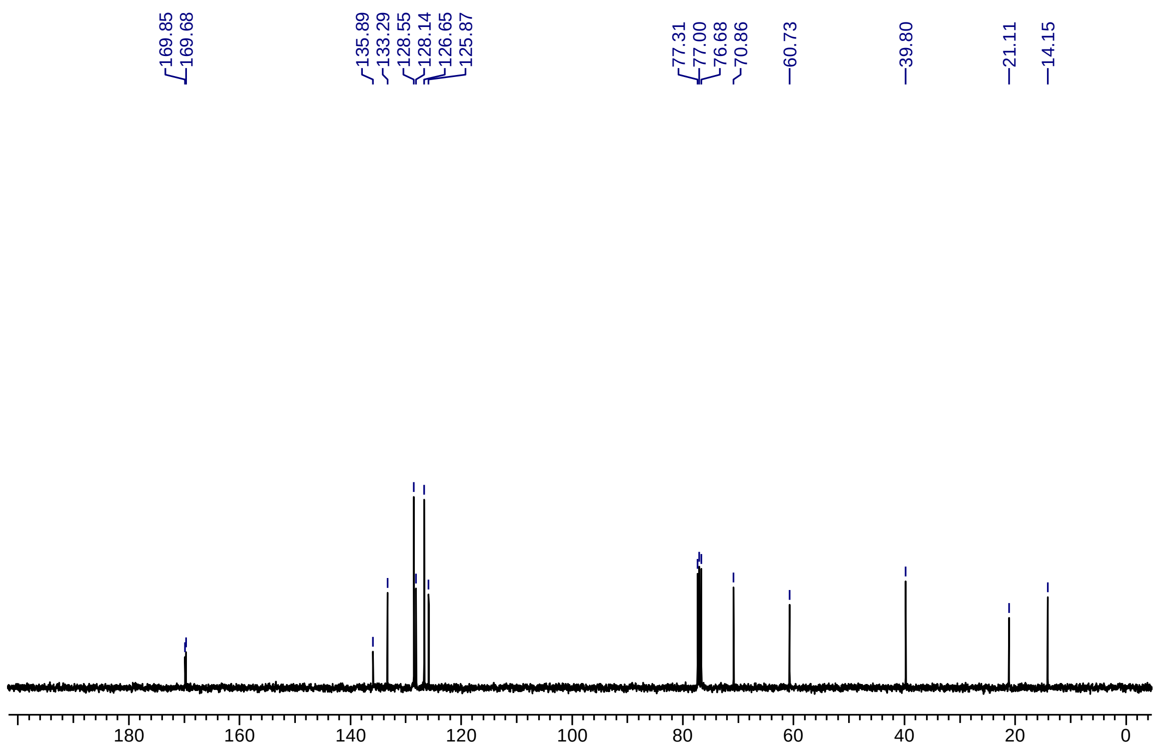
3.1.6. Copies of NMR spectra

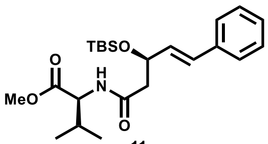


8

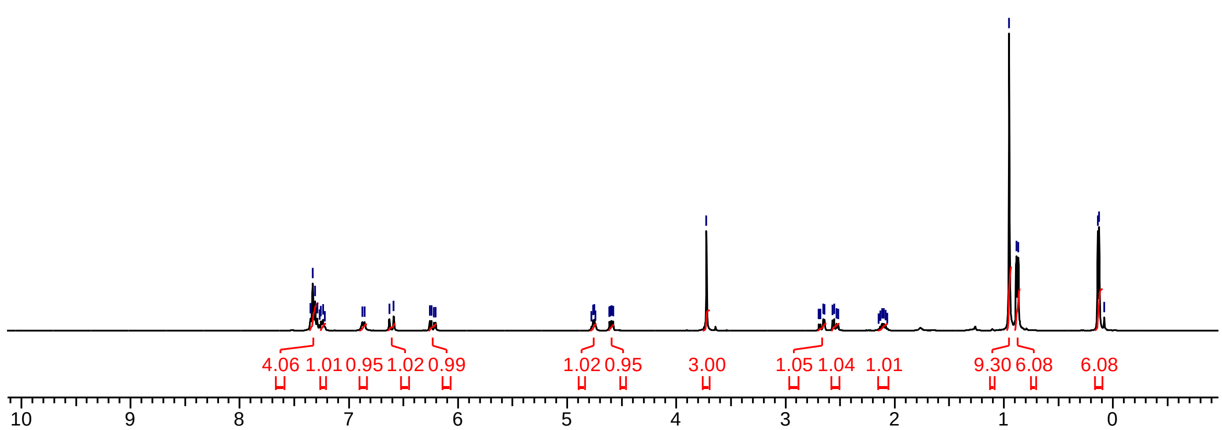
^{1}H NMR of **8** (400 MHz, $CDCl_3$)



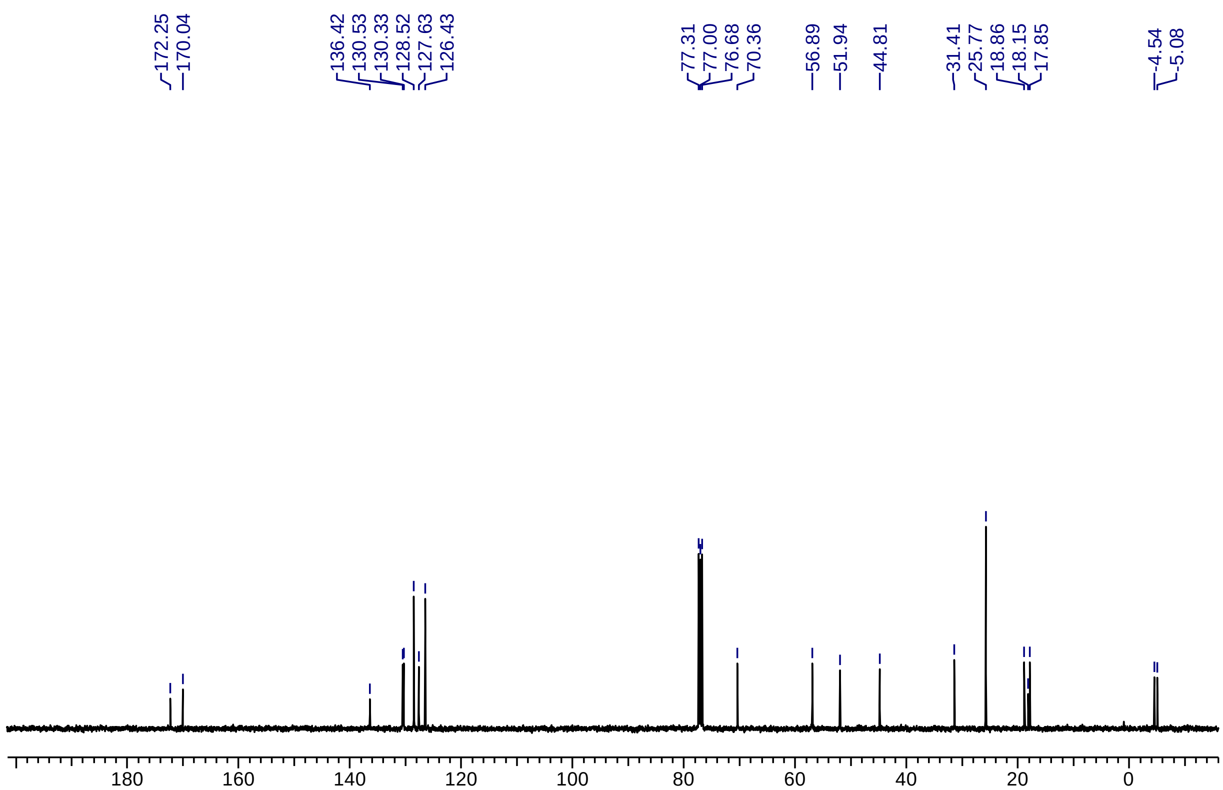
^{13}C NMR of **8** (100 MHz, $CDCl_3$)



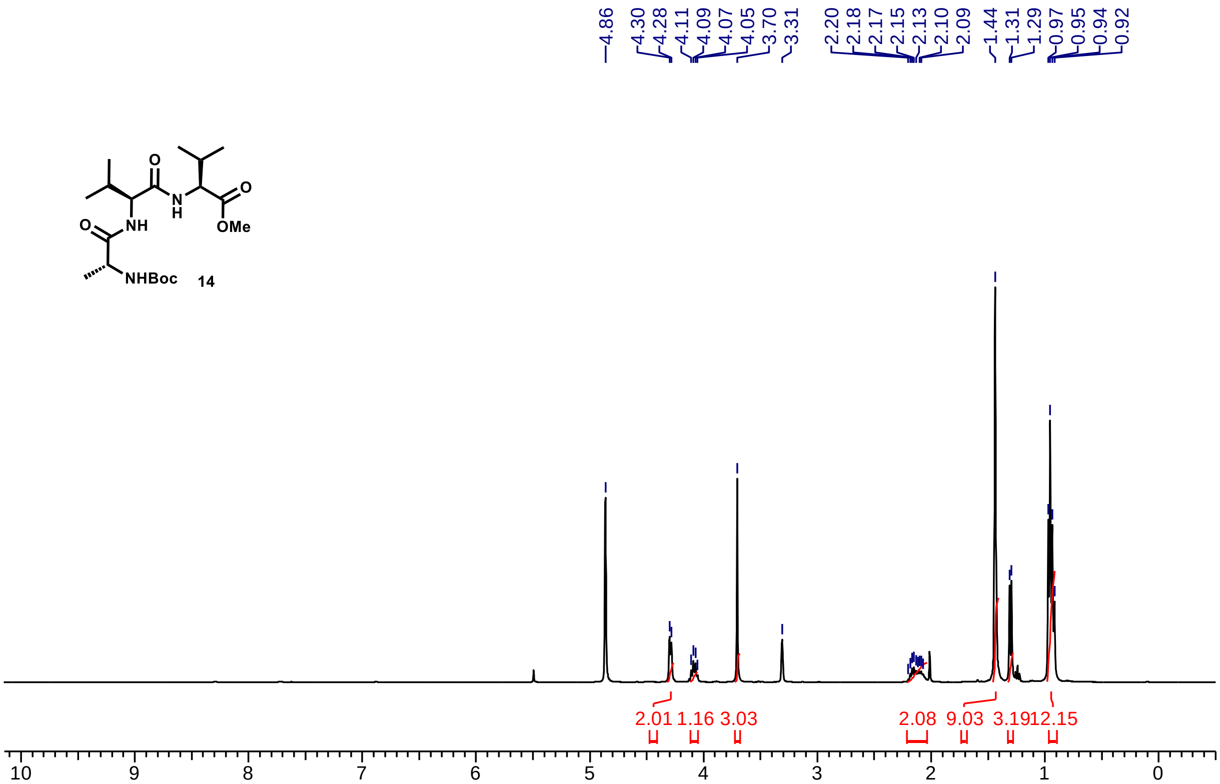
11



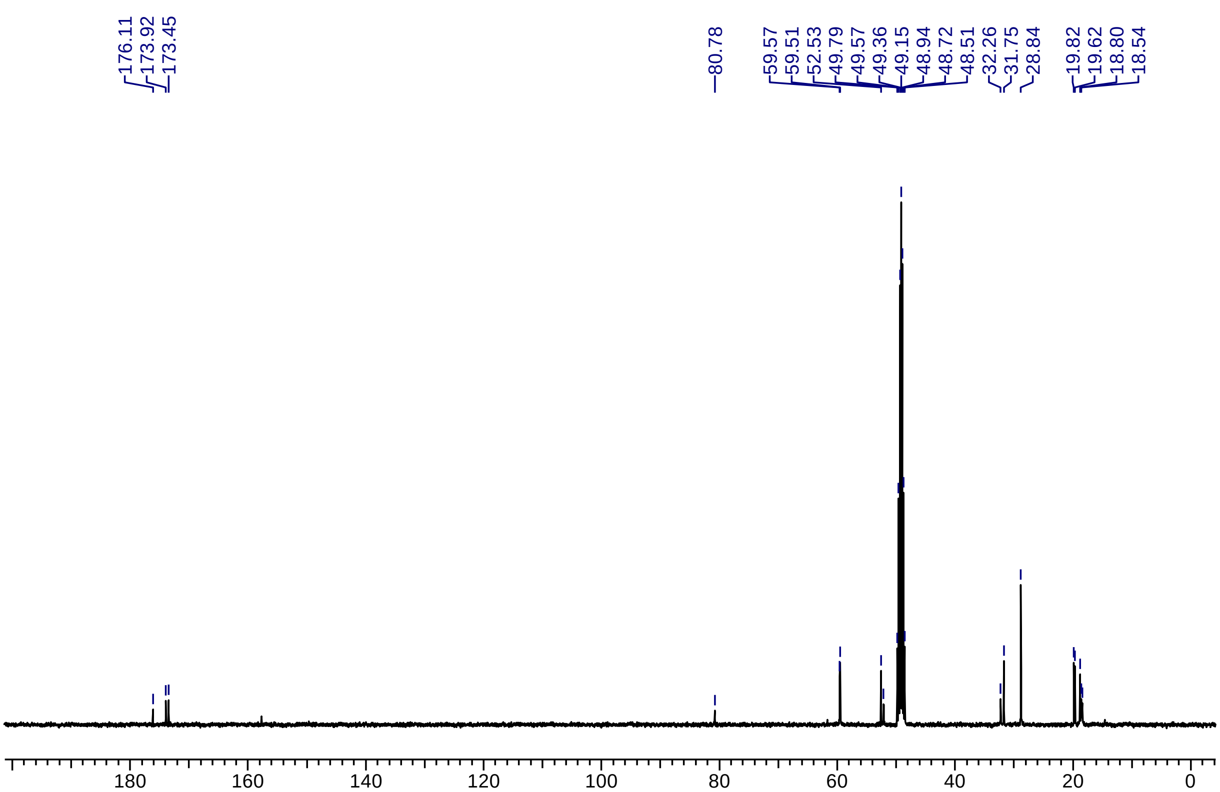
^{1}H NMR of **11** (400 MHz, $CDCl_3$)



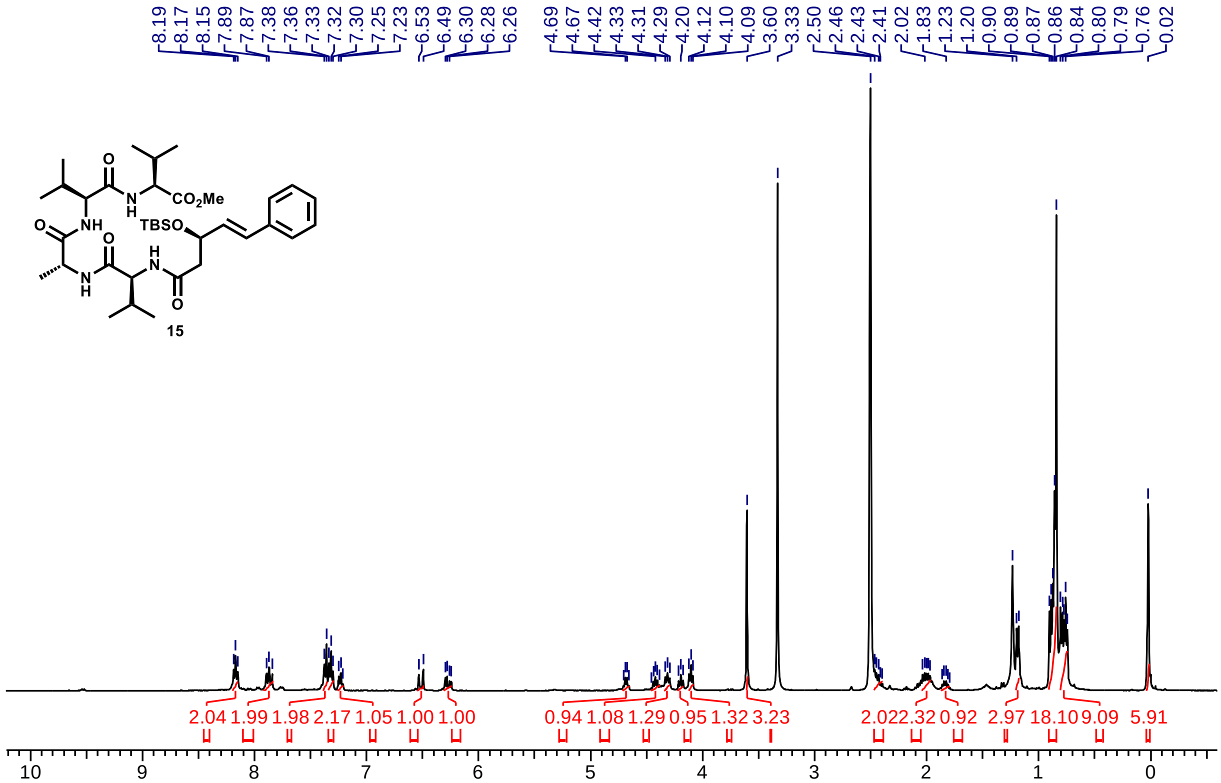
^{13}C NMR of **11** (100 MHz, $CDCl_3$)



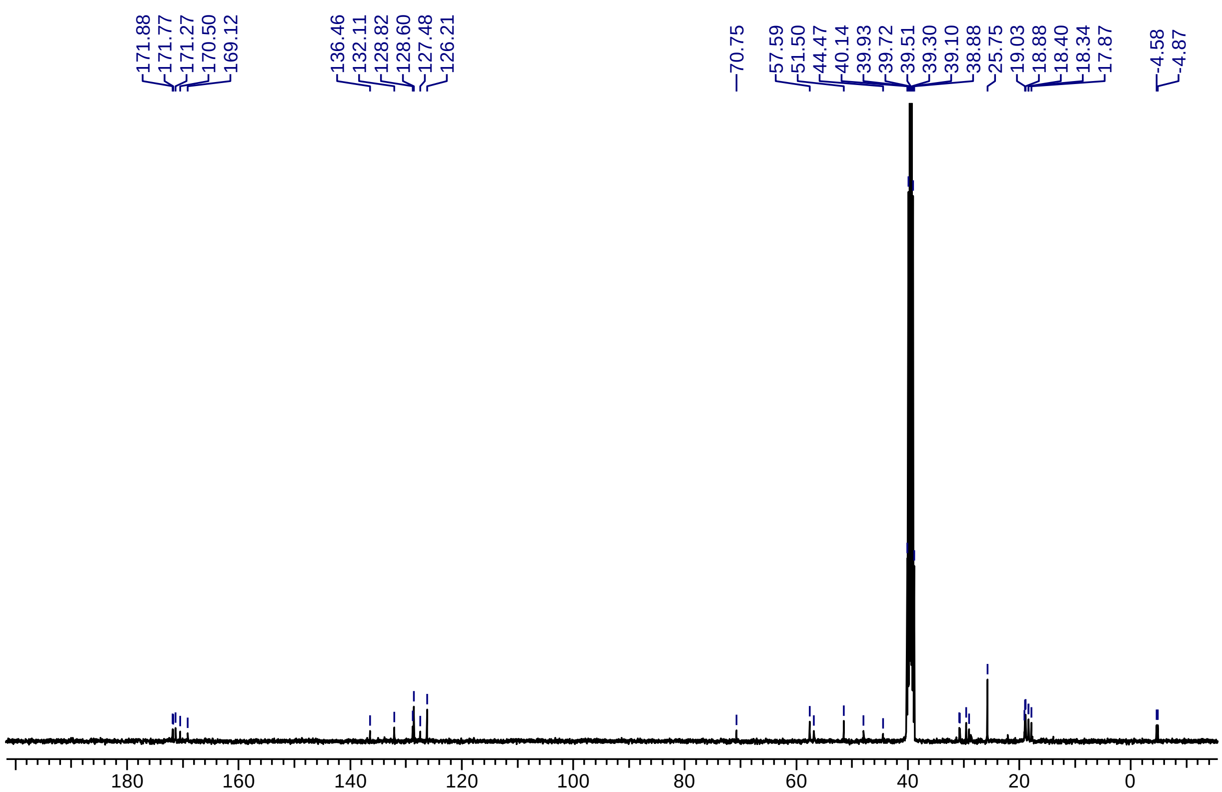
^{1}H NMR of **14** (400 MHz, MeOH-d_4)



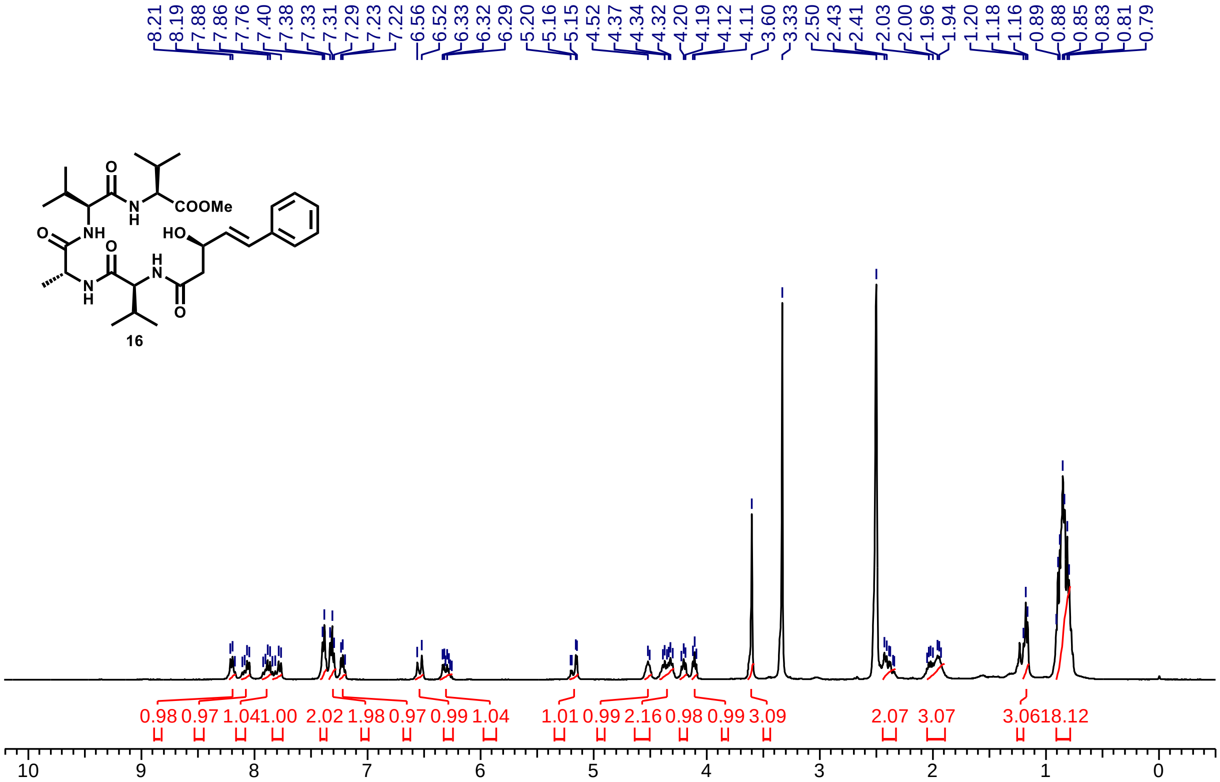
^{13}C NMR of **14** (100 MHz, MeOH-d_4)



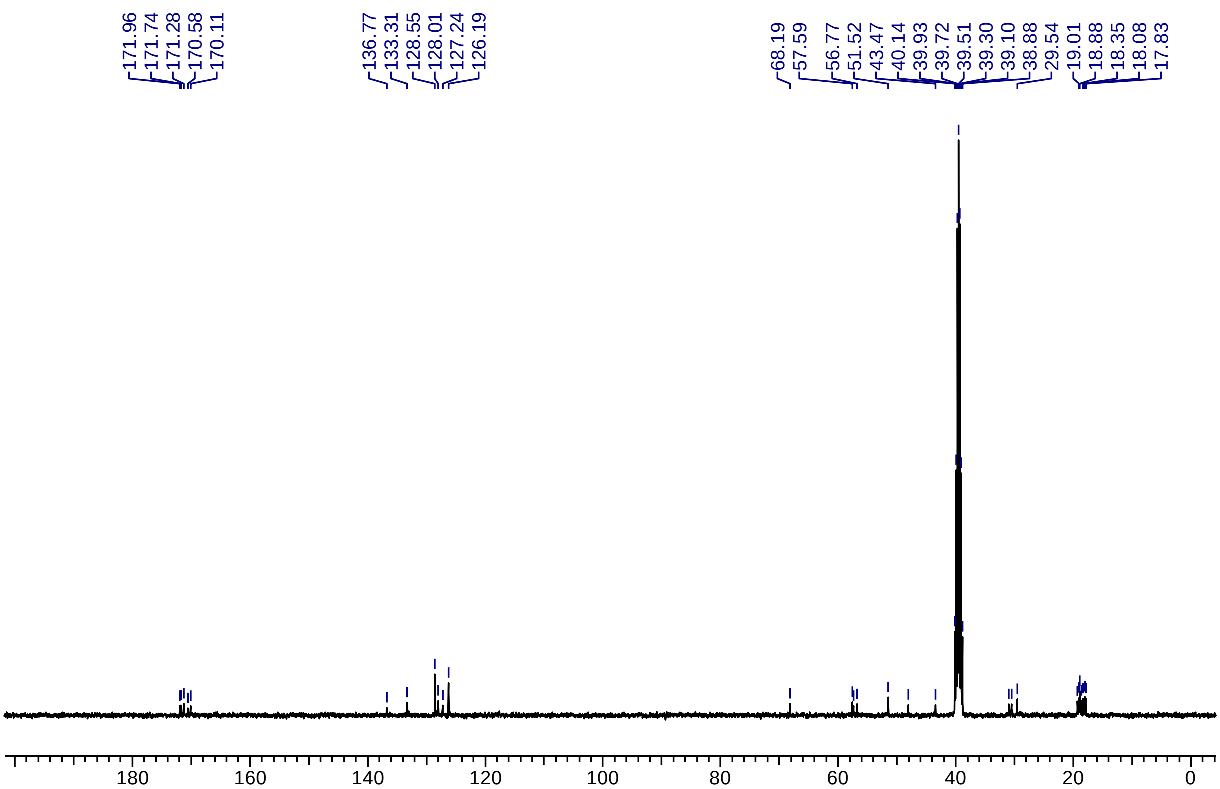
^{1}H NMR of **15** (400 MHz, DMSO-d_6)



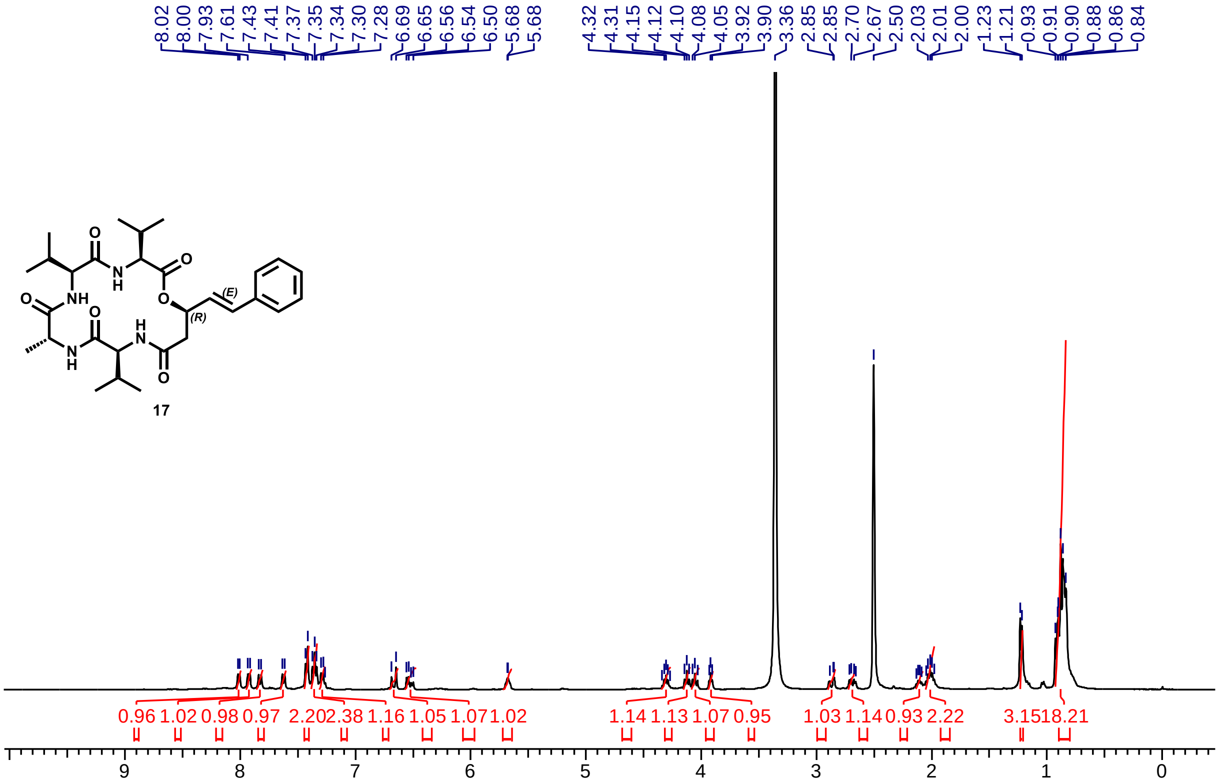
^{13}C NMR of **15** (100 MHz, DMSO-d_6)



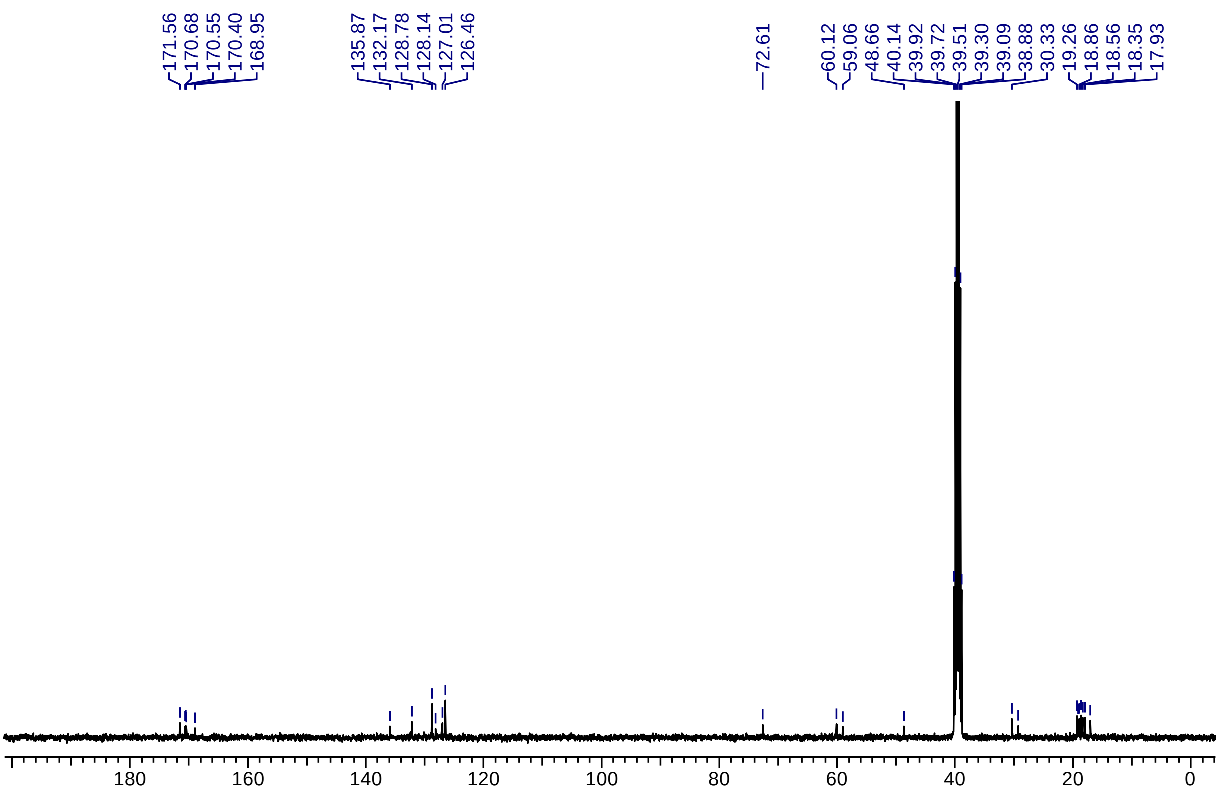
^{1}H NMR of **16** (400 MHz, DMSO-d_6)



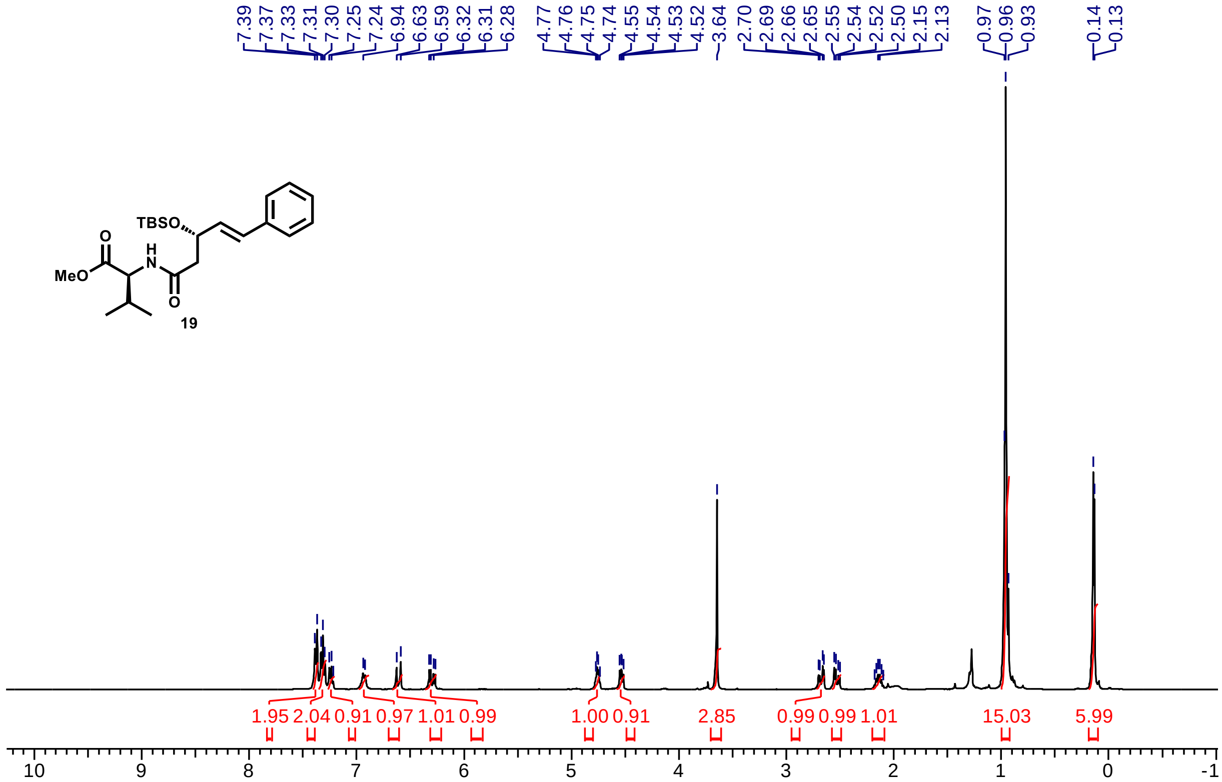
^{13}C NMR of **16** (100 MHz, DMSO-d_6)



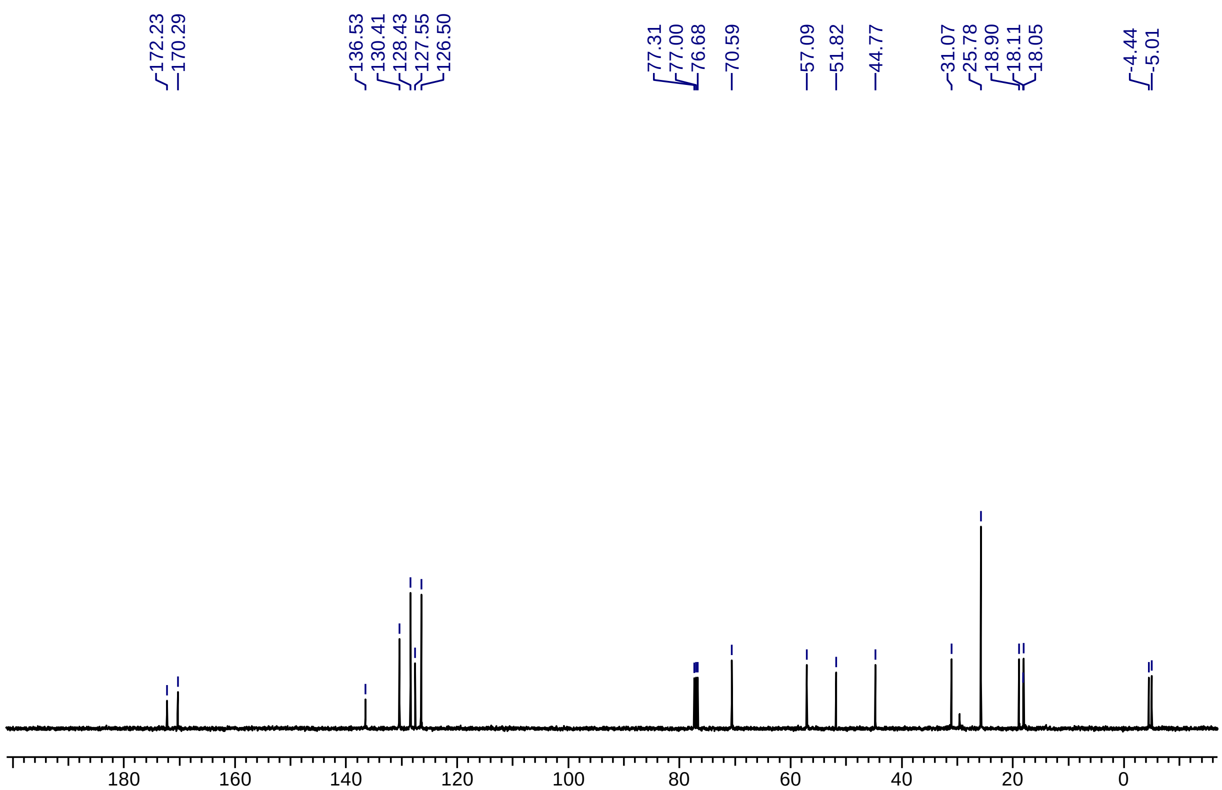
^{1}H NMR of **17** (400 MHz, DMSO-d_6)



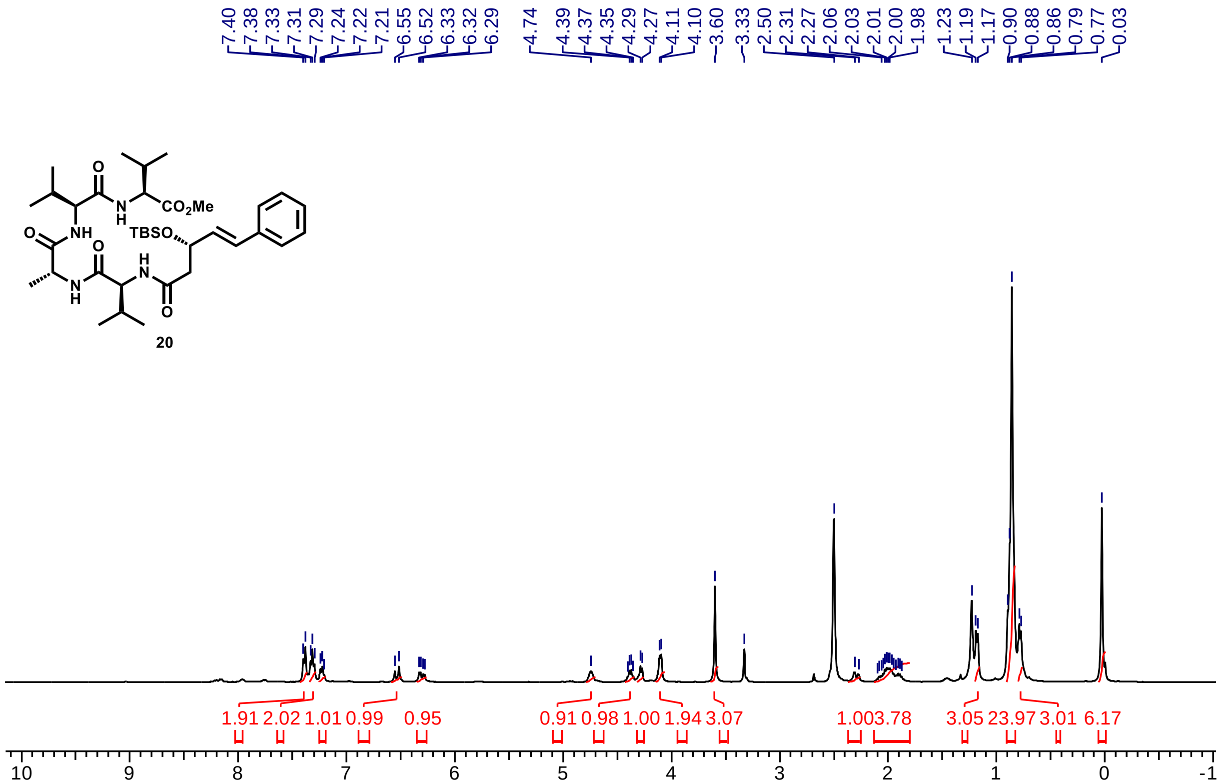
^{13}C NMR of **17** (100 MHz, DMSO-d_6)



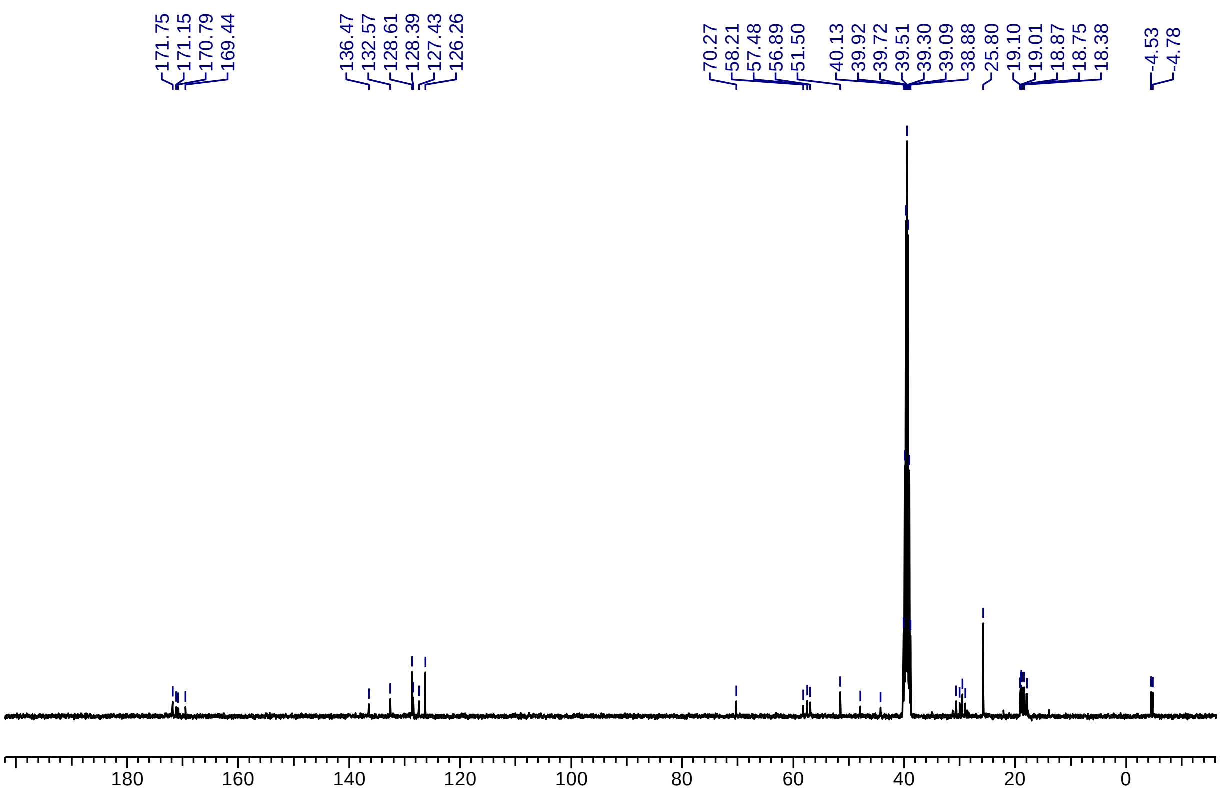
^{1}H NMR of **19** (400 MHz, $CDCl_3$)



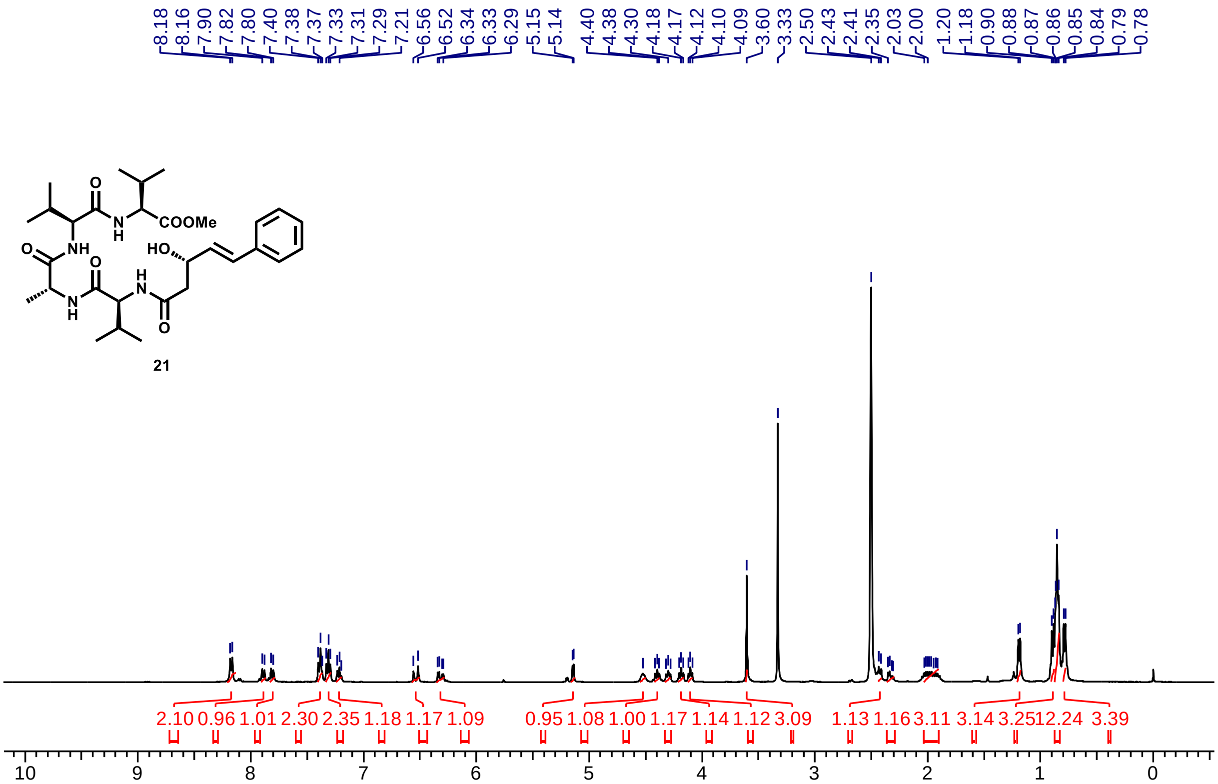
^{13}C NMR of **19** (100 MHz, $CDCl_3$)



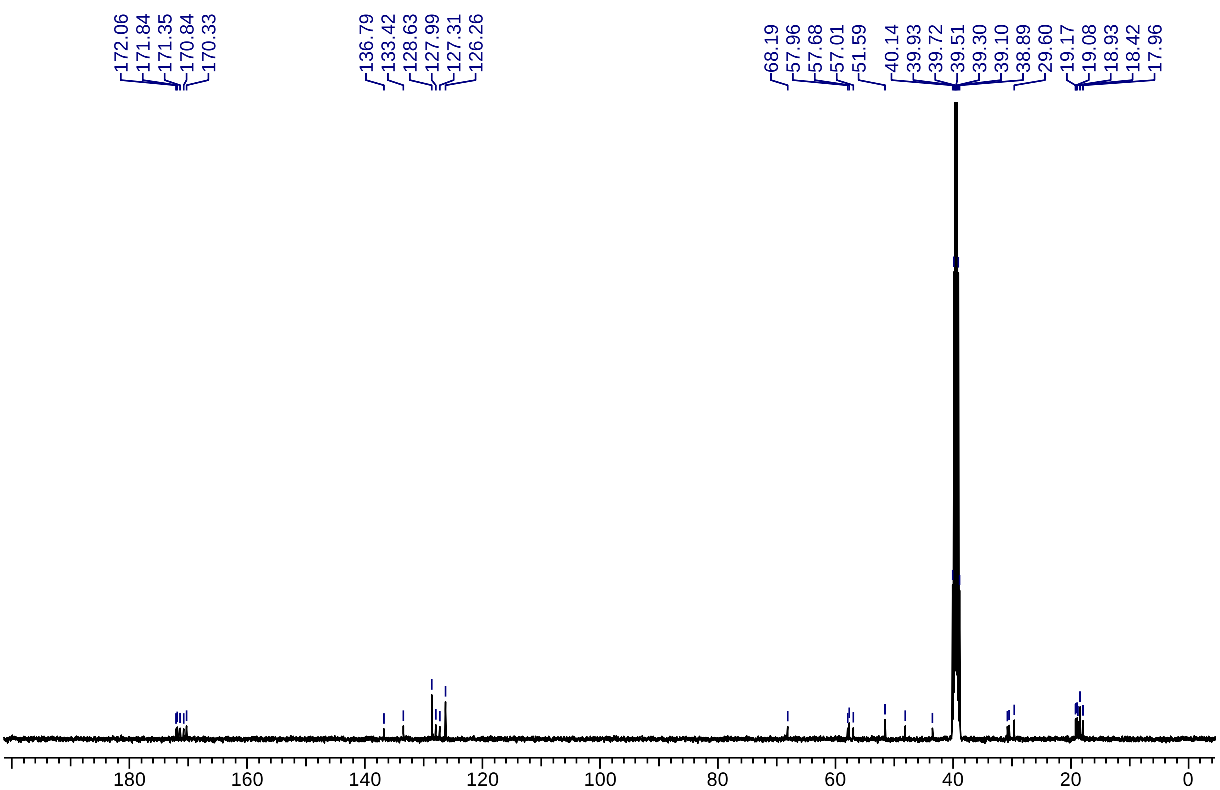
[1]H NMR of **20** (400 MHz, DMSO-d_6)



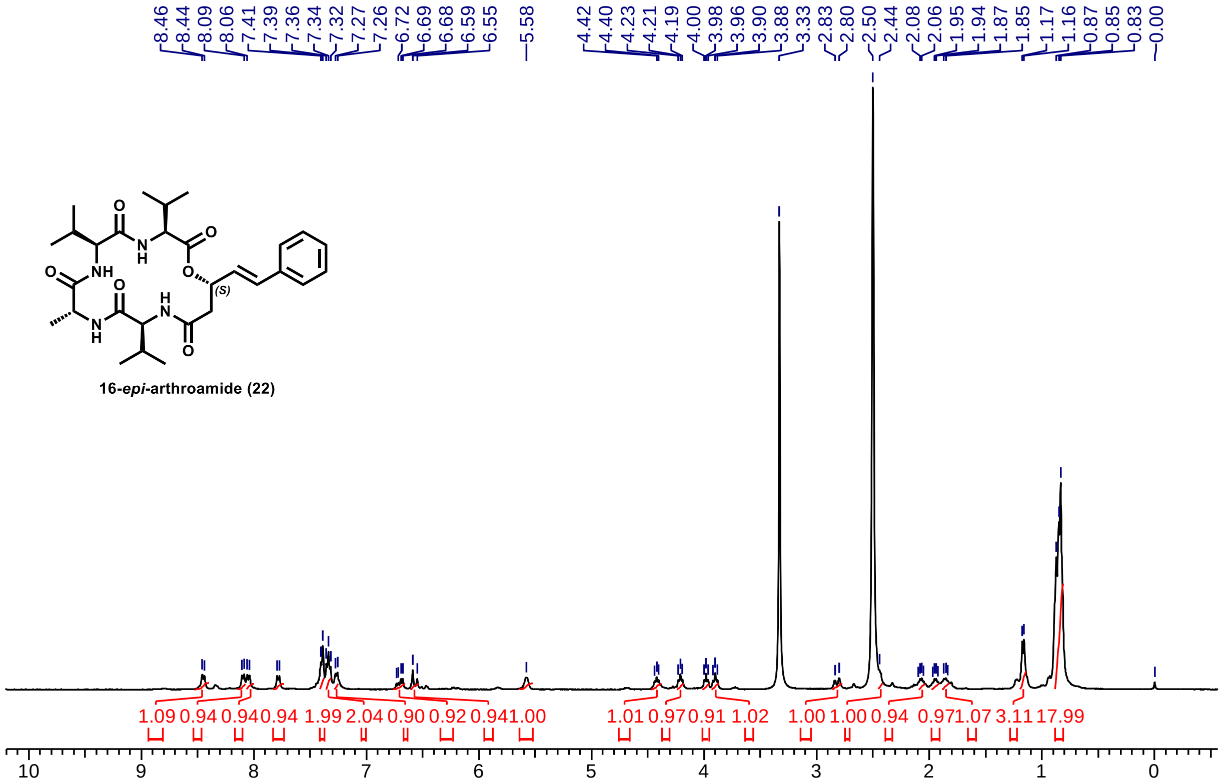
[13]C NMR of **20** (100 MHz, DMSO-d_6)



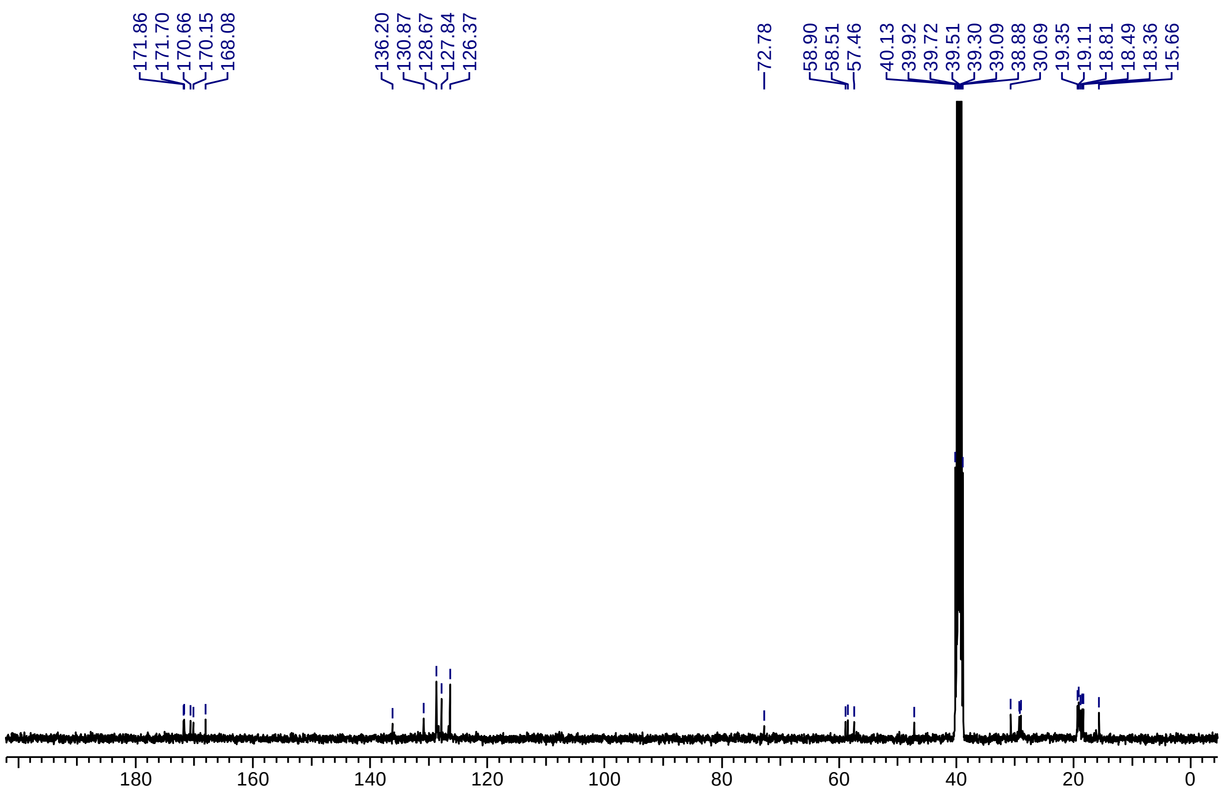
^{1}H NMR of **21** (400 MHz, DMSO-d_6)



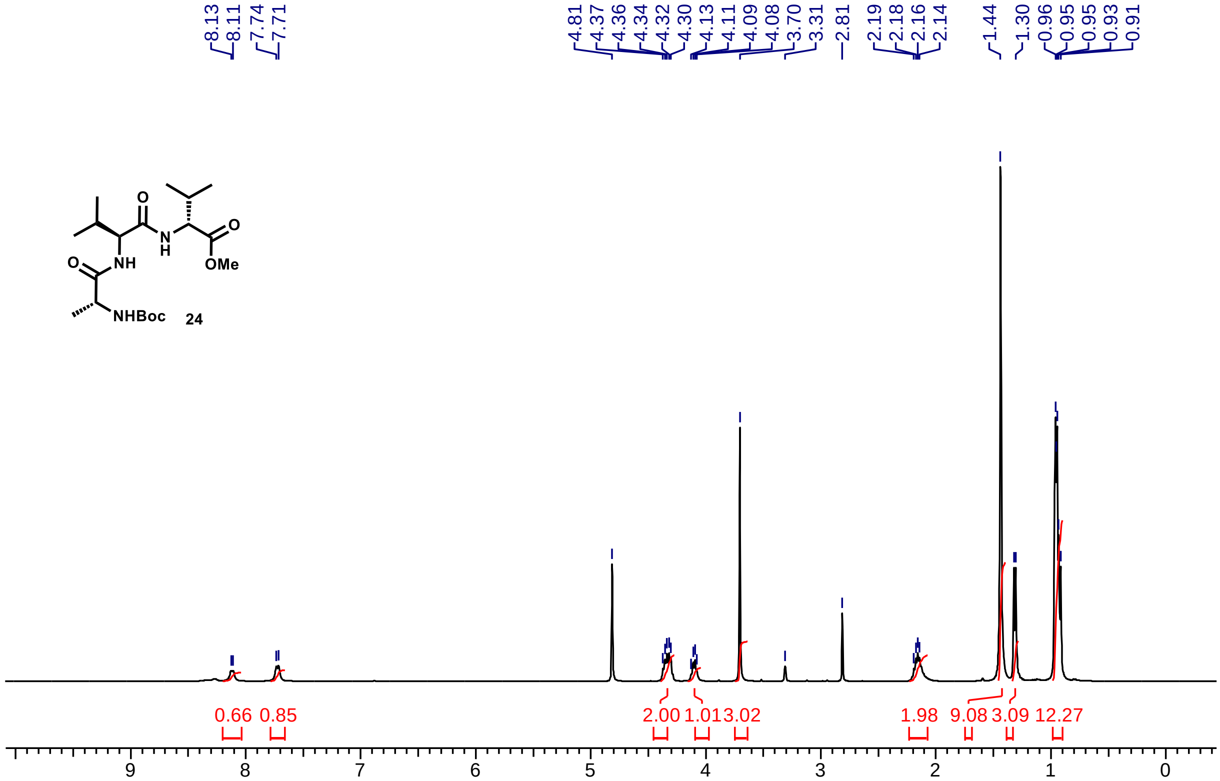
^{13}C NMR of **20** (100 MHz, DMSO-d_6)



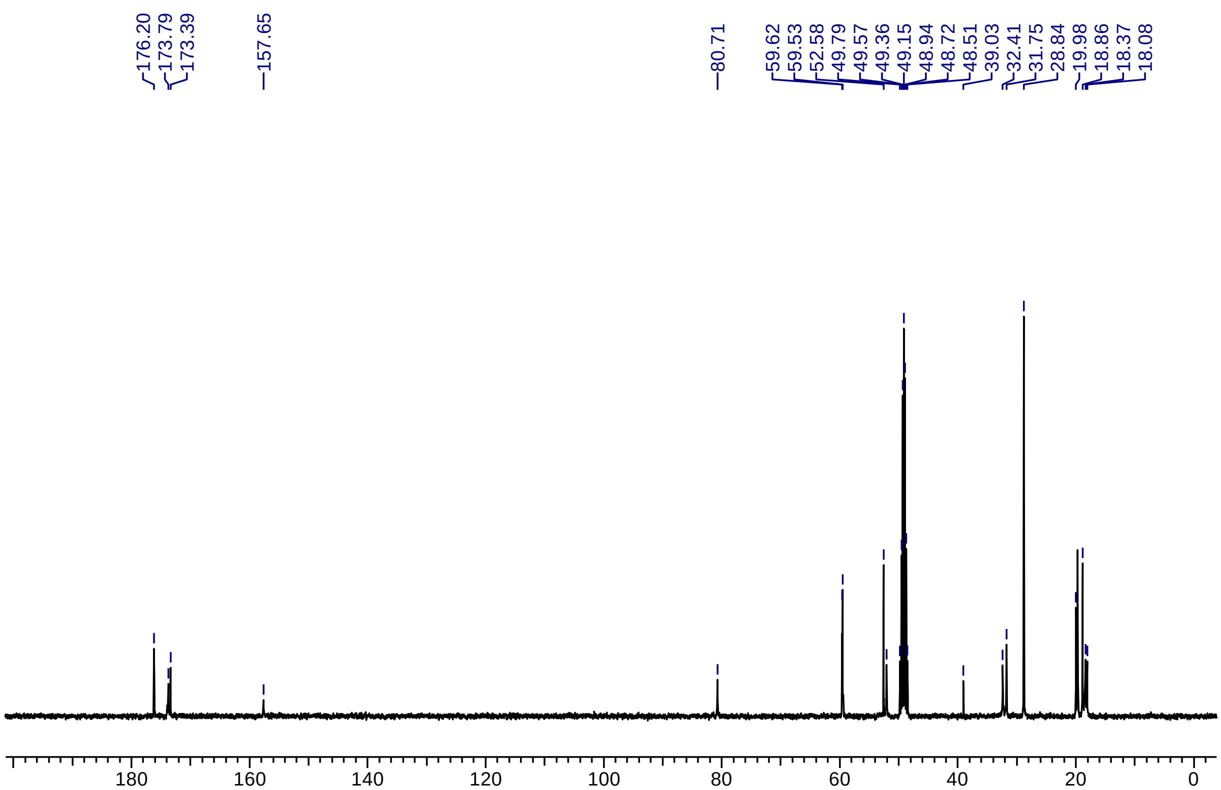
[1]H NMR of **22** (400 MHz, DMSO-d_6)



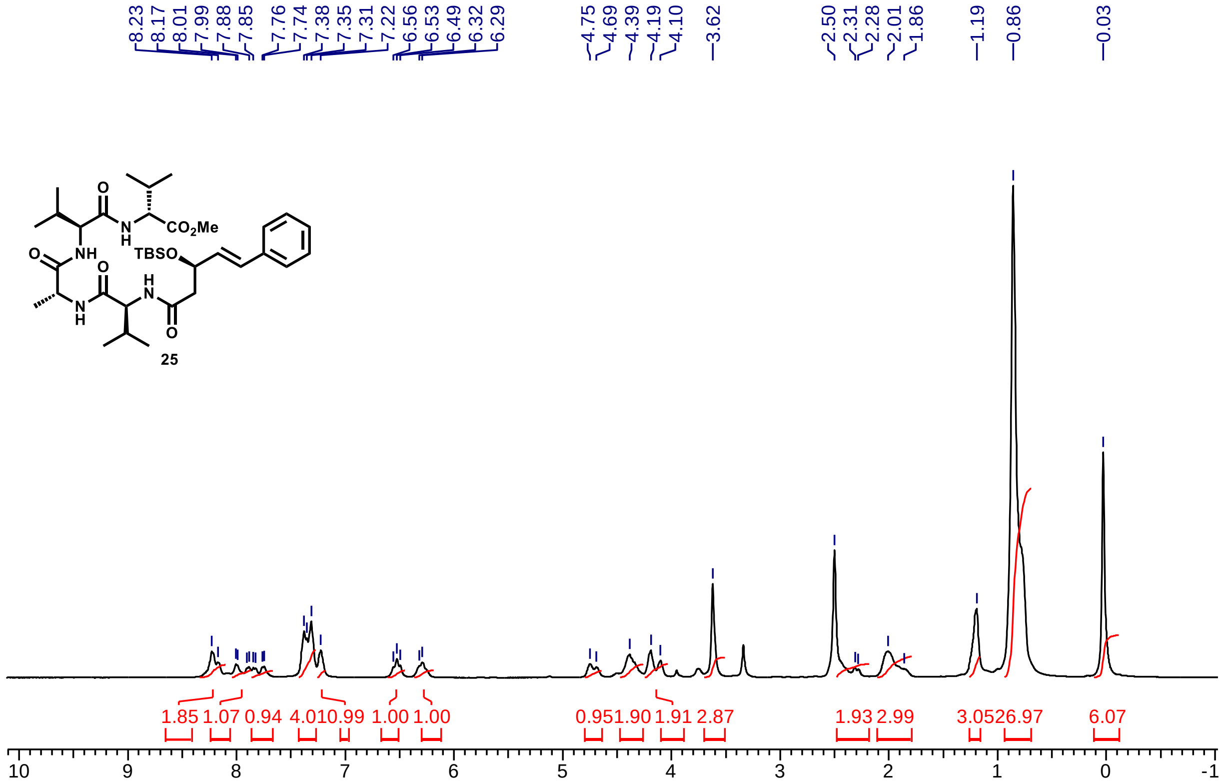
[13]C NMR of **22** (100 MHz, DMSO-d_6)



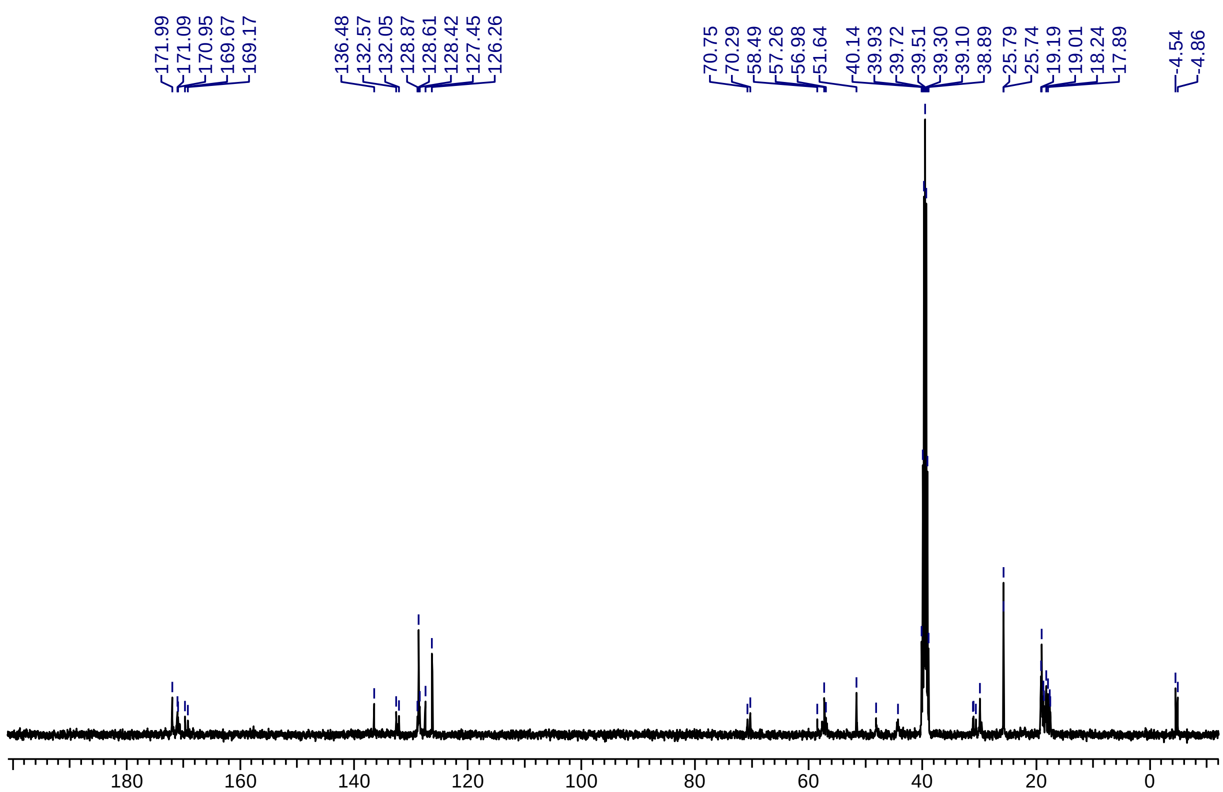
^{1}H NMR of **24** (400 MHz, MeOH-d_4)



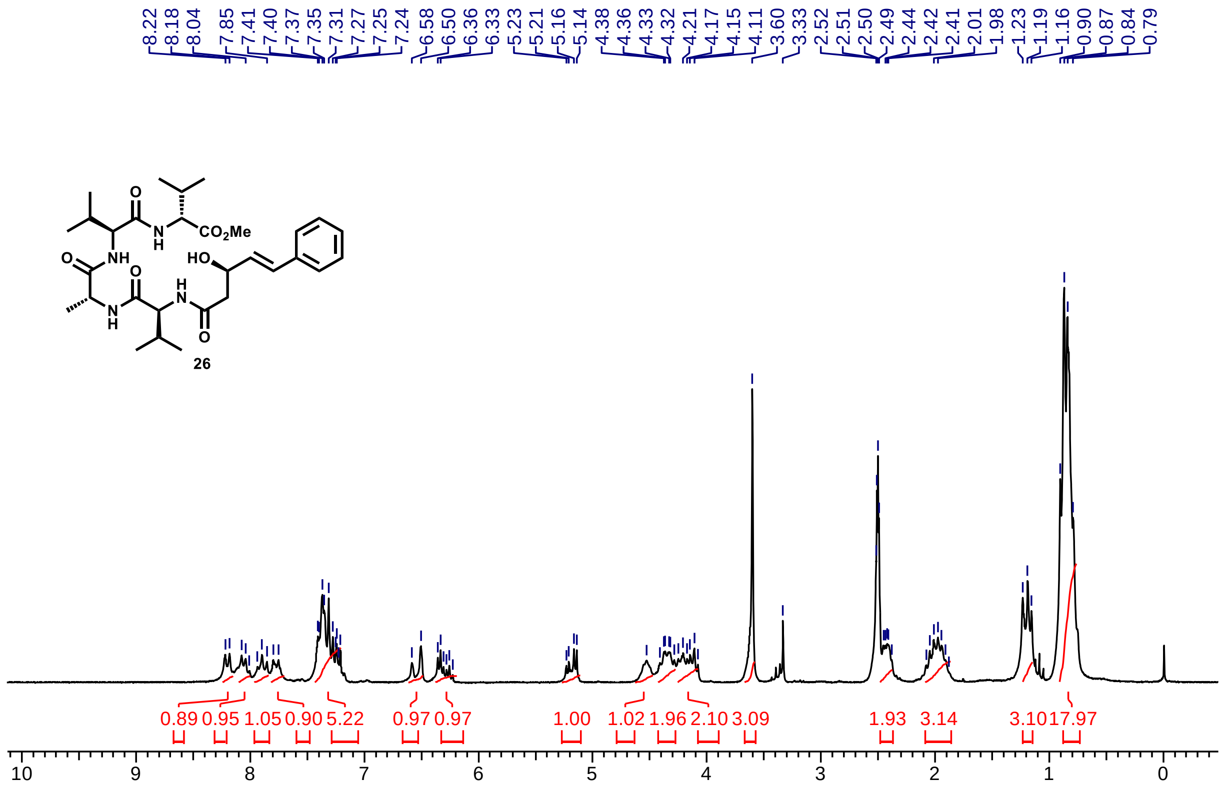
^{13}C NMR of **24** (100 MHz, MeOH-d_4)



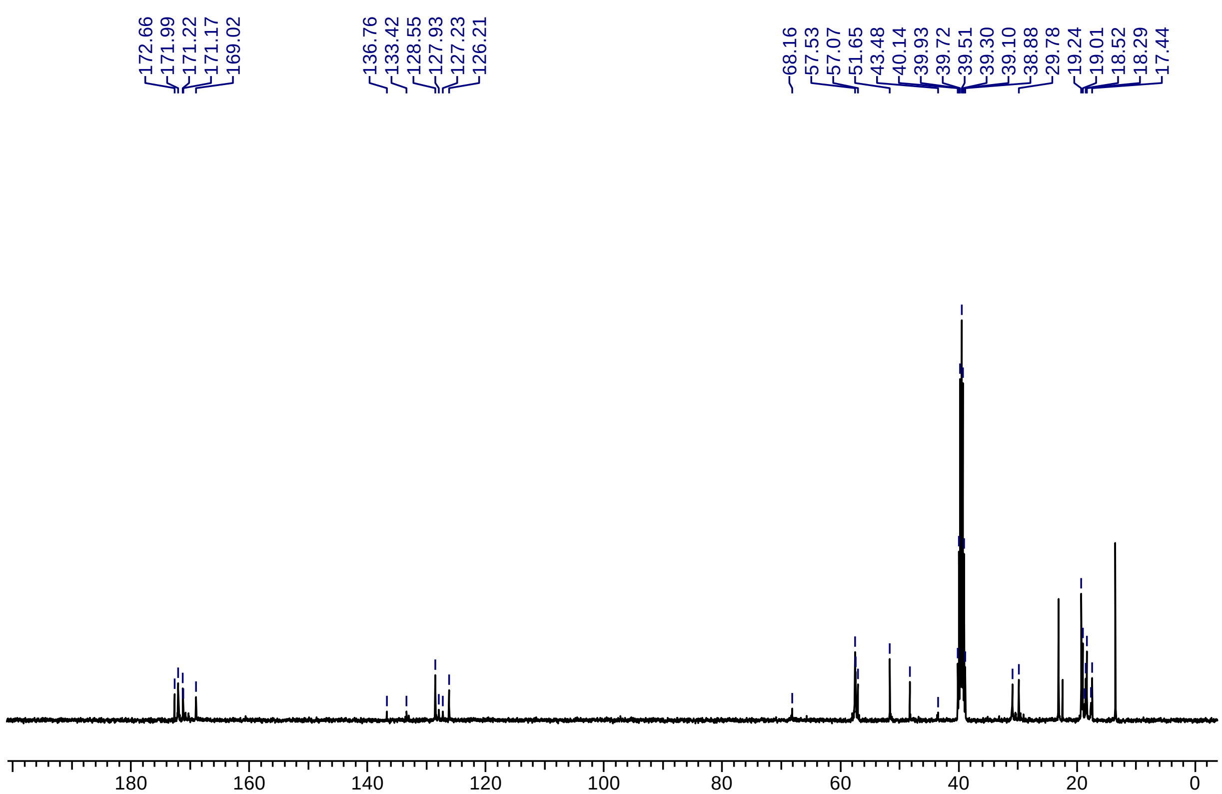
[1]H NMR of **25** (400 MHz, DMSO-d_6)



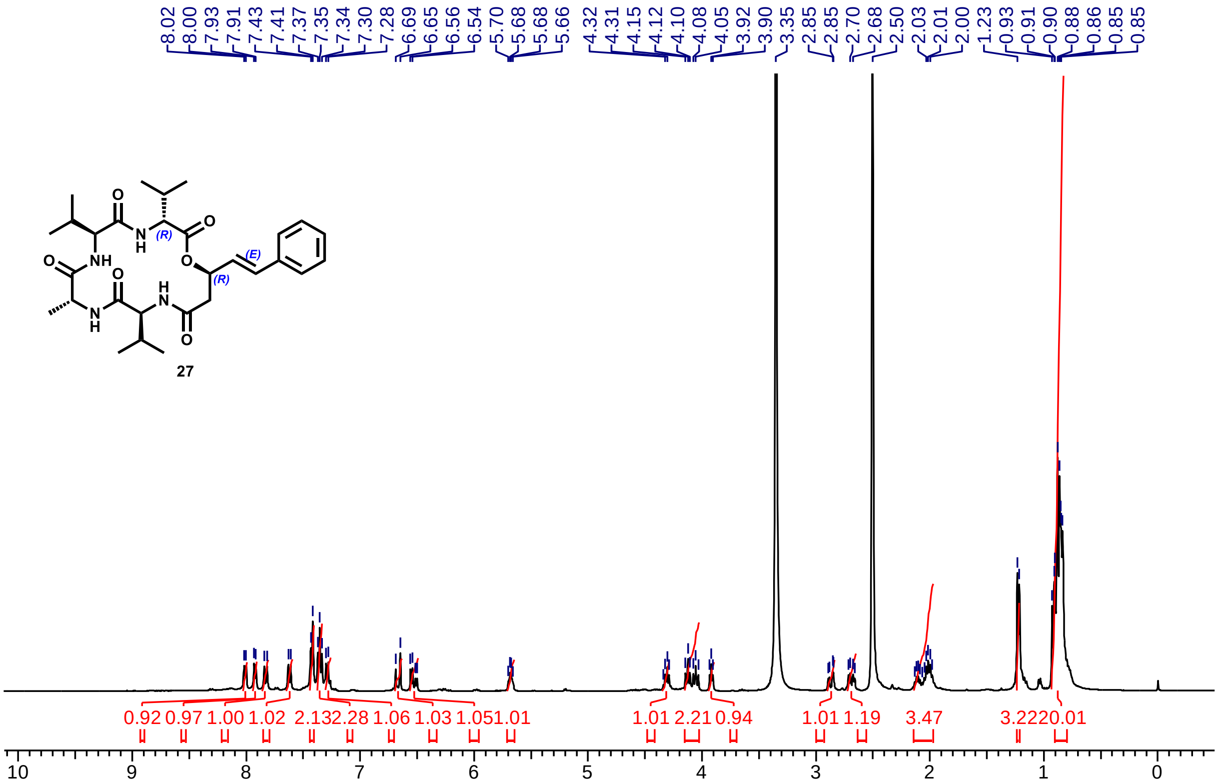
[13]C NMR of **25** (100 MHz, DMSO-d_6)



^{1}H NMR of **26** (400 MHz, DMSO-d_6)



^{13}C NMR of **26** (100 MHz, DMSO-d_6)



^{1}H NMR of **27** (400 MHz, DMSO-d_6)

3.2.1. Introduction

Macrocyclic peptides are becoming an important class of compounds in the field of drug discovery as they occupy a chemical space between small molecules and large molecules like biologics by possessing a number of different pharmacological properties from other entrenched therapeutic molecular classes.[1] The macrocyclization gives large surface area to the cyclic peptide as compared to their liner counterparts, along with limited conformational freedom that yields high selectivity and affinity towards the concerned biological targets.[2]

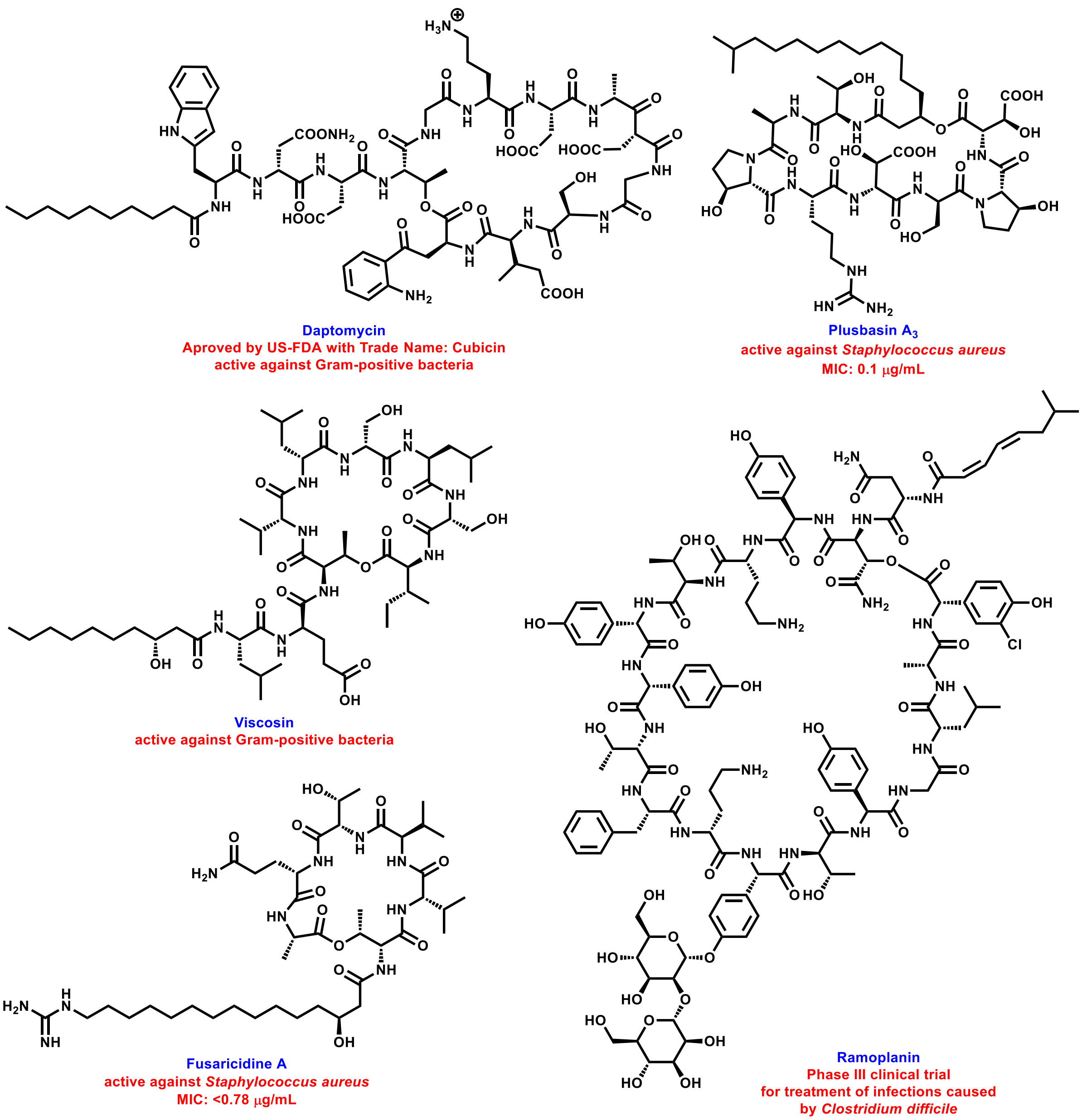


Figure 3.2.1. Structures of selected macrocyclic lipodepsipeptides

Among these, cyclic lipodepsipeptides are interesting ones in the development of new drugs which owns amide bonds along with ester bonds, and fatty acid linkages which are useful in interaction with bacterial cell membrane.[3] They also exhibit a broad spectrum of biological activities including insecticidal, antiviral, antimicrobial, antitumor, anti-inflammatory, and immunosuppressive actions.[3] Some of these natural products are either already marketed or under clinical development. Examples of these cyclic lipodepsipeptide include viscosin,[4] plusbacin,[5] fusaricidin,[6] ramoplanin[7] and daptomycin[8] listed in Figure 3.2.1.

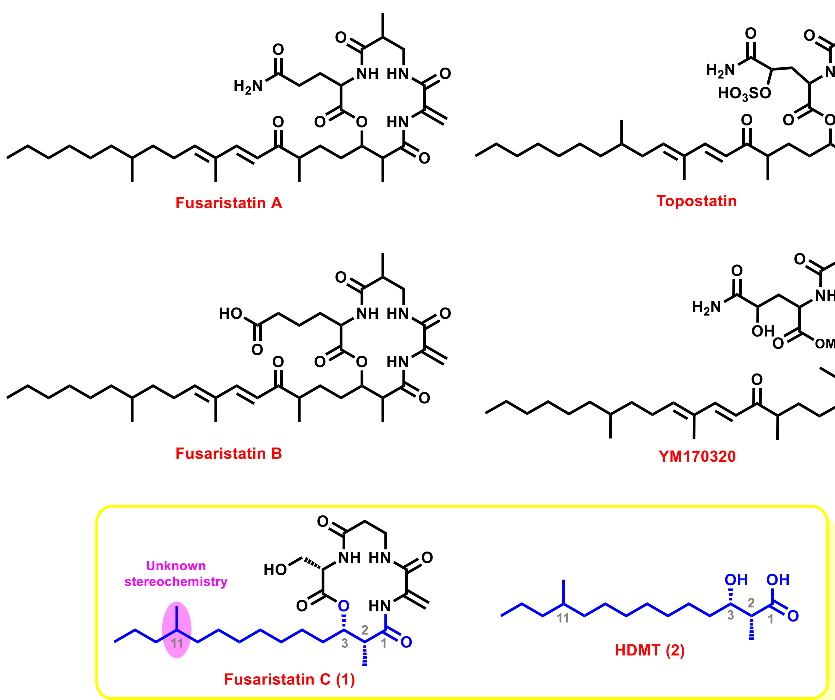
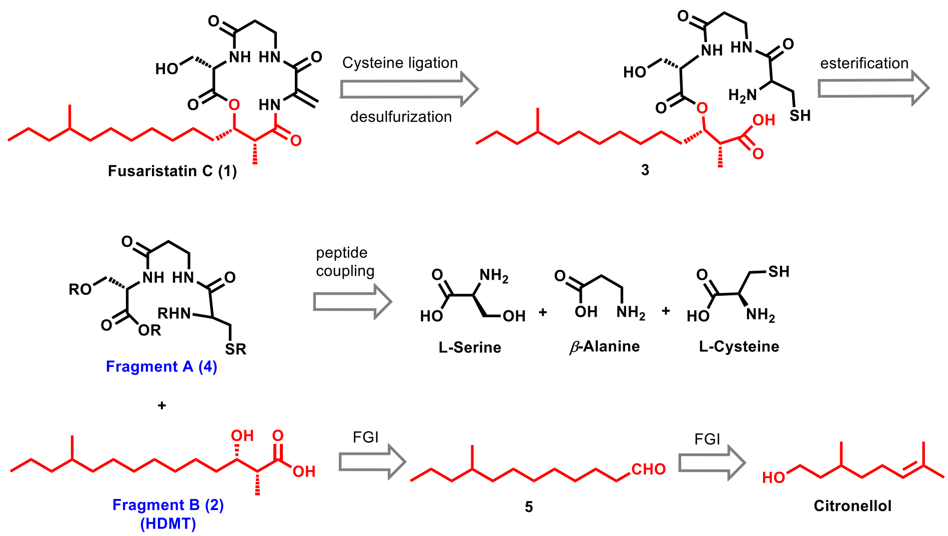


Figure 3.2.2. Structures of fusaristatin family natural products

Very recently, Kerr's group isolated new cyclic lipodepsipeptide fusaristatin C (**1**) from a fungal strain called *Pithomyces sp.* RKDO 1698, which was obtained from the Caribbean octocoral *Eunicea fusca*.[9] The fusaristatin family of natural products consisting of fusaristatin A, fusaristatin B, topostatin and YM-117032 which were isolated earlier from *Fusarium sp.* YG-45 and *Thermomonospora alba* respectively have become enriched by addition of new natural product fusaristatin C.[10] Fusaristatin family natural products are 14-membered macrocyclic depsipeptides containing lipid chain of varied length and YM170320 is considered as biogenetic precursor for synthesis of rest of them. The fungus *Pithomyces sp.* RKDO 1698 was fermented in yeast extract sucrose broth, extracted with ethyl acetate which predominately gives the formation of compound **1**, analyzed by mass spectrometry. Further, purification by combiflash using C_{18} column and linear gradient of 5% MeOH: H_2O over 50 min afforded **1** in 240 mg quantities as white amorphous solid with low specific rotation of $[\alpha]_D^{24}$ = +1.4 (*c* 0.28, CH_3OH). Combination of NMR spectroscopy and chemical derivatization techniques elucidated the structure of fusaristatin C.[9] In chemical aspects, fusaristatin C is a 14-membered cyclic lipodepsipeptide, bears three amino acids as serine, *β*-alanine and dehydroalanine (Dha). The stereochemistry of only chiral amino acid was determined by Marfey's method as L-serine. The non-amino acid fragment is 3-hydroxyl-2,11-dimethyltetradecanoic acid (HDMT) poses two contiguous chiral centers and the stereochemistry at C-11 position was unknown. The challenging task of stereochemical assignment of HDMT fragment was done by various techniques. The HDMT fragment was cleaved from fusaristatin C by acidolytic cleavage and characterized separately. The methyl position at C-11 was determined by ^3J HMBC correlation but they could not determine its stereochemistry as it is challenging task with no chiral centers in the proximity. The absolute configuration at hydroxyl attached C-3 position was fixed by Mosher's ester analysis and relative stereochemistry of hydroxyl group C-3 and methyl group (C-2) was determined by *J*-based configurational analysis. As such fusaristatin C does not exhibit any antimicrobial activity against bacterial strains including MRSA, *Mycobacterium tuberculosis* and VRE, or cytotoxicity. However, previously reported fusaristatins show biological activity (Figure 3.2.2.).[10] As part of our research group interests on synthesis macrocyclic peptides and the stereochemistry of methyl group at C-11 position was found unknown, a challenging task, we undertook this particular project to accomplish total synthesis and establishment of absolute stereochemistry at C-11 position. It is also worth mentioning that there are no synthetic efforts has been carried out or documented for the syntheses of fusaristatin family of natural products. Our efforts toward synthesis of fusaristatin C are described in the following sections.

3.2.2. Retrosynthetic analysis

We envisioned that the target compound **1** could be achieved through macrocyclization followed by desulfurization[11] of acyclic precursor **3**. This method is also called "cysteine ligation" which was used previously by other groups. Since Fusaristatin C is a macrocyclic depsipeptide, we preferred cyclization by macrolactamization rather than macrolactonization which could be efficiently achieved by cysteine ligation[12] of **3**. In turn compound **3** could be synthesized by utilizing intermolecular esterification between peptide fragment A (**4**) and non-peptidic fragment

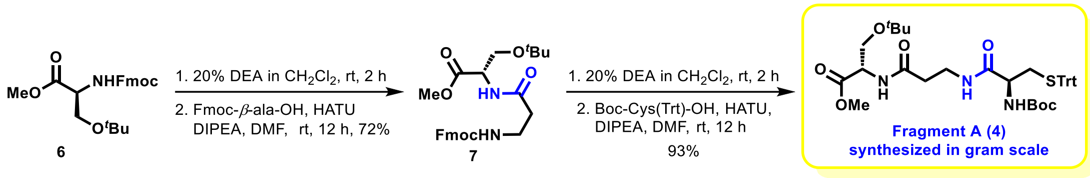


Scheme 3.2.1. Retrosynthetic analysis of fusaristatin C

B (**2**). Further, peptidic fragment **4** could be synthesized from corresponding amino acids by standard peptide coupling in solution phase. Non-peptidic fragment **2** we intended through stereoselective Evans aldol reaction of aldehyde **5**, this could be further synthesized from citronellol over functional group interconversion strategy. We envisioned that the citronellol with proper stereochemistry could be employed to fix the C-11 methyl stereocenter as both enantiomers of citronellal are commercially available (Scheme 3.2.1).

3.2.3. Synthesis of fragment A

As stated, our synthetic plan of dipeptide **7** commenced from compound **6** which is serine derivative with appropriate orthogonal protection and it was prepared as per the procedures known in the literature.[13] The compound **6** was treated with diethyl amine in dichloromethane solution to deprotect Fmoc group followed by coupling of corresponding amine with Fmoc-*β*-Ala-OH[14] in presence of HATU and DIPEA in DMF afforded dipeptide **7** in 72% yield. Dipeptide **7** was characterized by IR, ^{1}H, ^{13}C NMR, and HRMS analysis. Characteristic methyl ester protons were appeared at δ 3.63 (br. s., 3H), α-protons of serine and *β*-alanine appeared at δ 4.22 (d, J = 6.1 Hz, 1H) and δ 2.40 (t, J = 6.4 Hz, 2H) ppm respectively while *N*-attached CH_2 of *β*-alanine at δ 3.22 - 3.21 (m, 2H) ppm in ^{1}H NMR. ^{13}C NMR also supported the formation of desired compound with three carbonyl carbons peaks at δ 170.9, 170.6 and 156.0 ppm, respectively. In addition to this, HRMS (ESI) analysis showed peak at 469.2337 corresponding to molecular formula $C_{26}H_{33}N_2O_6$ $[M + H]^+$ with calculated mass 469.2333 further confirmed the

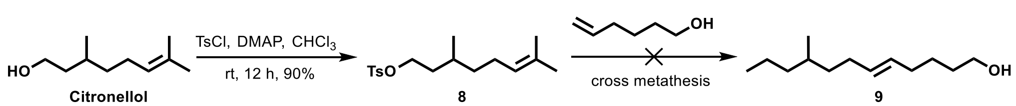


Scheme 3.2.2. Synthesis of fragment A

drawn structure. Fmoc deprotection in dipeptide **7** was carried out in diethyl amine and dichloromethane cocktail and corresponding amine was coupled with Boc-Cys(Trt)-OH[15] in DMF afforded fragment A (**4**) with 93% yield in protected form (Scheme 3.2.2.). Structure of tripeptide **4** was confirmed by IR, ^{1}H, ^{13}C NMR, and HRMS analysis. In ^{1}H NMR α-protons were appeared at δ 4.44 - 4.39 (m, 1H), 3.92 - 3.84 (m, 1H) ppm, β-CH_2 of cysteine and α-CH_2 of ala appeared at δ 2.36 - 2.29 (m, 4H) while ^{t}Bu protons from serine ether and Boc group comes at δ 1.09 (s, 9H) and 1.37 (s, 9H) ppm respectively. ^{13}C NMR supported the formation of **4** with three amide carbonyls at δ 170.9, 170.6 and 169.9 and peak corresponding to carbamate carbonyl at δ 154.9 ppm. It was further validated by HRMS (ESI) which showed peak at 714.3181 with molecular formula $C_{36}H_{49}N_3O_7SNa$ $[M + Na]^+$ with calculated mass 714.3183. The fragment A was successively synthesized in a gram scale.

3.2.4. Synthesis of HDMT fragment (B)

3.2.4.1. Attempt toward synthesis of fragment B

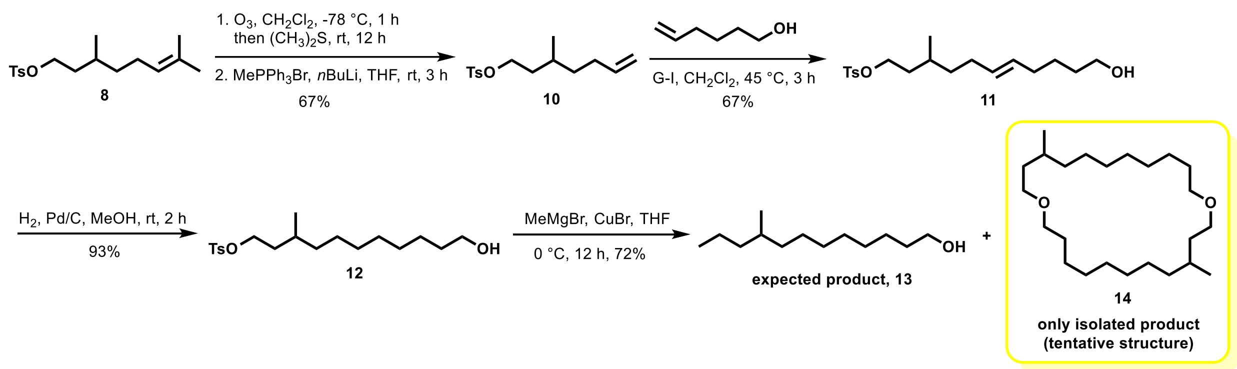


Sr. No	Cross metathesis reaction conditions	Observations
1	G-I (5 mol%), CH_2Cl_2, 45 °C, upto 24 h	Only dimer of hexenol
2	G-I (5 mol%), toluene, 80 °C, upto 24 h	Only dimer of hexenol
3	G-II (5 mol%), CH_2Cl_2, 45 °C, upto 24 h	Only dimer of hexenol
4	G-II (5 mol%), toluene, 80 °C, upto 24 h	Only dimer of hexenol
5	HG-II (5 mol%), CH_2Cl_2, 45 °C, upto 24 h	Only dimer of hexenol

Scheme 3.2.3. Attempt toward synthesis of fragment B

After successful synthesis of fragment A, the next task was to synthesize fragment B for which we started with citronellol (initially, we have chosen racemic mixture) which on tosylation of hydroxyl group using TsCl and DMAP in $CHCl_3$ afforded compound **8** in 90% yield.[16] Tosylate compound **8** was subjected under cross metathesis reaction conditions in presence of Grubbs' Ist generation catalyst (5 mol%), but we did not get any traces of product **9**. With variation of solvent and temperature as well as catalyst, we observed formation of only dimer of hexenol as shown in Scheme 3.2.3. Presence of trisubstituted olefin in compound **8** might have been contributing to its inactivity in cross metathesis.

To move forward, we converted compound **8** into terminal olefin by reductive ozonolysis of **8** followed by Wittig reaction of corresponding aldehyde with $MePPh_3Br$ afforded alkene **10** in 67% yield over two steps.[17] Cross metathesis between alkene **10** and 5-hexen-1-ol in Ist generation Grubbs' catalyst (5 mol%) rendered alkene **11** in 67% yield.

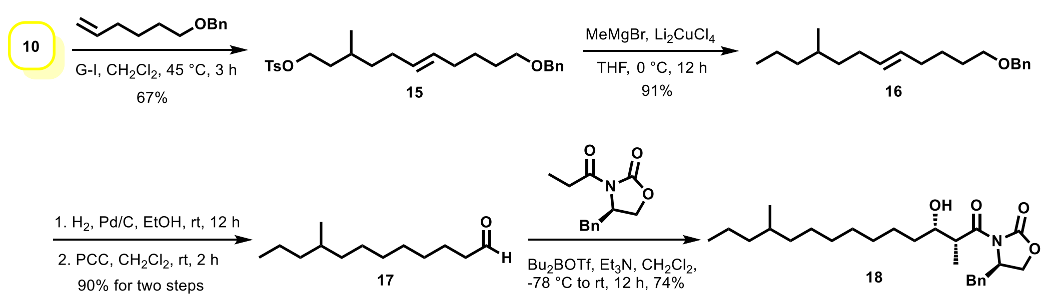


Scheme 3.2.4. Attempt toward synthesis of fragment B

The formation of alkene **11** was primarily confirmed by TLC which appeared as polar spot than both the starting materials. In ^{1}H NMR, the olefin protons were appeared at δ 5.40 - 5.27 (m, 2H) ppm, hydroxyl attached methylene protons present at δ 4.09 - 4.03 (m, 2H) and 3.66 - 3.62 (m, 2H) ppm and methyl present aromatic group comes at δ 2.45 (s, 3H) ppm. Peaks appeared at δ 130.2 and 129.8 ppm were of olefin carbons and δ 69.0, 62.8 ppm for hydroxyl attached carbons in ^{13}C NMR, fully confirmed the structure. The HRMS analysis showed a mass peak at 377.1861 corresponding to molecular formula $C_{19}H_{30}O_4SNa$ $[M + Na]^+$ with calculated mass 377.1865 validated the structure. Olefin in compound **11** was hydrogenated under the blanket of H_2 atmosphere (balloon) using Pd/C (10%) catalyst. The product formation was primarily indicated by TLC, where the product spot appeared just above the starting material. Further, structure of compound **12** was confirmed by ^{1}H, ^{13}C NMR and HRMS analysis. To carry out chain elongation of compound **12**, it was treated with Grignard reagent MeMgBr and copper bromide in THF to afford desired compound **13**[18]. However, we could isolate unexpected cyclic ether **14** in 72% yield (Scheme 3.2.4.). In ^{1}H NMR, presence of two hydroxyl attached methylene protons at δ 3.65 (t, *J* = 6.5 Hz, 2H) and 3.51 - 3.38 (m, 2H) and only one methyl signal at δ 0.89 (d, *J* = 6.4 Hz, 3H) and in ^{13}C NMR, hydroxyl attached carbons were appeared at δ 63.0 ppm supports the formation of compound **14** and explained that there was no Grignard addition product. The formation of macrocyclic ether **14** could be explained by the displacement of terminal tosylate of one molecule with free hydroxyl group of another molecule of **12**. These observations indicated the necessity for the hydroxyl group protection. Further, formation of macrocyclic dimeric ether was assisted by HRMS (ESI) which showed peak at 369.2770 corresponding to the molecular formula $C_{24}H_{49}O_2$ $[M + H]^+$ with calculated mass 369.2769. It is worth noting that formation of dimeric symmetrical ether in high yields is very interesting and warrants further efforts to understand the scope of the reaction.

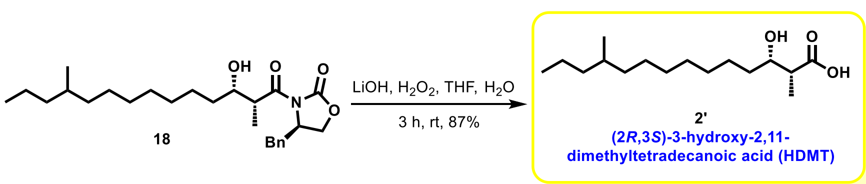
3.2.4.2. Synthesis of (2*R*,3*S*)-HDMT

Accordingly, 5-hexen-1-ol was treated with sodium hydride and benzyl bromide in dry THF afforded ((hex-5-en-1-yloxy)methyl)benzene[19] which on cross metathesis with alkene **10** using G-I catalyst in dichloromethane at 45 °C afforded compound **15** in 67% yield. Formation of **15** was confirmed by ^{1}H, ^{13}C NMR, IR and further validated by HRMS (ESI) which showed peak at 467.2226 for molecular formula $C_{26}H_{36}O_4SNa$ $[M + Na]^+$ with calculated mass 467.2227.



Scheme 3.2.5. Efforts toward synthesis of fragment B

Compound **15** on coupling with Grignard reagent MeMgBr (1.0 M in THF) in presence of catalyst dilithium tetrachlorocuprate (Li_2CuCl_4) (a well known protocol for the coupling of alkyl tosylate and Grignard reagent developed by Schlosser in 1974)[20] in THF under dilution afforded compound **16** in 91% yield which was confirmed by IR, ^{1}H, ^{13}C NMR, and HRMS analysis. In ^{1}H NMR disappearance of peaks in aromatic region corresponding to tosylate group and presence of two methyl groups at δ 0.93 - 0.88 (m, 6H) ppm clearly indicated the formation of product. Hydrogenation of compound **16** under H_2 pressure and Pd/C catalyst in afforded corresponding alcohol which was oxidized using PCC in dichloromethane furnished aldehyde **17** in 90% yield over two steps.[18] In ^{1}H NMR, the characteristic aldehyde protons was appeared at *δ* 9.77 (t, *J* = 1.8 Hz, 1H) ppm while in ^{13}C this carbonyl was present at *δ* 203.1 ppm which clearly supports the formation of structure **17**. The HRMS analysis showed a mass peak at 199.1908 for molecular formula $C_{13}H_{27}O$ $[M + H]^+$ with calculated mass 199.1900 confirmed the structure. After successful synthesis of aldehyde **17**, it was subjected to stereoselective aldol reaction with Evans chiral auxiliary coupled propionic acid derivative in presence of Bu_2BOTf and triethyl amine in dichloromethane at -78 °C gave aldol product **18** in 74% yield (Scheme 3.2.5.) which was characterized by IR, ^{1}H, ^{13}C NMR, and HRMS analysis.[21] In ^{1}H NMR, key hydroxyl attached methine appeared at δ 3.96 - 3.94 (m, 1H), while methyl attached methine at δ 3.77 (dq, *J* = 2.4, 7.1 Hz, 1H) and *N*-attached methine at δ 4.75 - 4.69 (m, 1H) ppm. In ^{13}C NMR two carbonyl groups were at δ 177.6 and 153.0 ppm and hydroxyl attached carbon at δ 71.5 ppm.



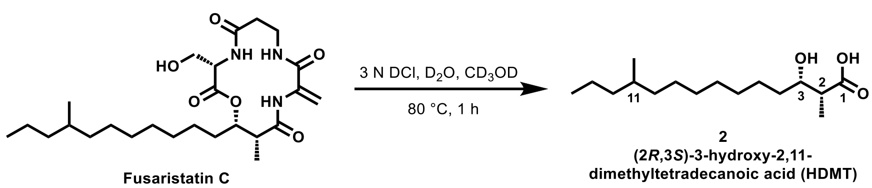
Scheme 3.2.6. Synthesis of fragment B

The stereochemistry of newly created chiral centers was assigned based on aldol reactions of related substrates documented in the literature.[22] Finally, auxiliary in compound **18** was removed using lithium hydroxide and aqueous hydrogen peroxide in THF and water furnished acid **2’** in 87% yield which was characterized by spectroscopic techniques (Scheme 3.2.6.). On preliminary analysis, product formation was indicated by typical TLC pattern of carboxylic acid and disappearance of starting material. In ^{1}H NMR, characteristic hydroxyl attached methine was appeared at δ 3.76 (br. s., 1H), methyl attached methine at δ 2.41 (quint, *J* = 6.4 Hz, 1H) and two terminal methyl protons at δ 0.89 (t, *J* = 6.7 Hz, 3H) and 0.86 (d, *J* = 6.1 Hz, 3H) ppm. ^{13}C NMR showed carbonyl carbon at δ 179.1 ppm, hydroxyl attached carbon at δ 73.4 ppm, and methyl attached carbon at δ 46.9 ppm. HRMS (ESI) in negative mode gave peak at 271.2284 corresponds to molecular formula $C_{16}H_{31}O_3$ $[M - H]^-$ with calculated value 271.2284.

After successful synthesis of both the fragments, the next task was intermolecular esterification towards the total synthesis of target natural product fusaristatin C.

3.2.4.3. Synthesis of fragment B by Kerr's group

As Kerr's group reported the synthesis of HDMT fragment from the natural fusaristatin C by acidic hydrolysis at 80 °C (Scheme 3.2.7.) and it was well characterized using spectral data;[9] we decided to compare the same with that of synthesized HDMT fragment **2'**.

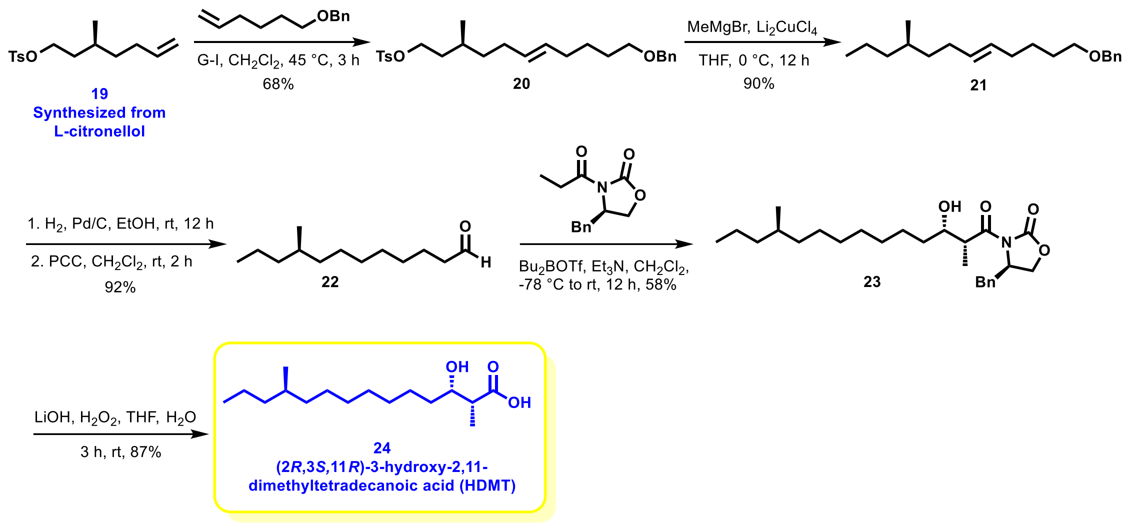


Scheme 3.2.7. Synthesis of HDMT by Kerr's group[9]

To our surprise, comparison of the ^{1}H and ^{13}C NMR spectra of the synthetic (2*R*,3*S*)-HDMT **2'** with literature data of HDMT **2**; we observed significant differences, in particular, at δ 3.76 *vs* 3.66 ppm (3-CH) and 2.41 *vs* 2.52 ppm (2-CH) in ^{1}H NMR and at δ 73.4 *vs* 74.5 ppm (C-3) and δ 46.9 *vs* 47.7 ppm (C-2) and at δ 179.1 *vs* 177.5 ppm (C-1) in ^{13}C NMR. With these observed discrepancies in the spectral data, we were curious to fix the stereochemistry at C-11 position from L-citronellol.

3.2.4.4. Synthesis of (2*R*,3*S*,11*R*)-HDMT

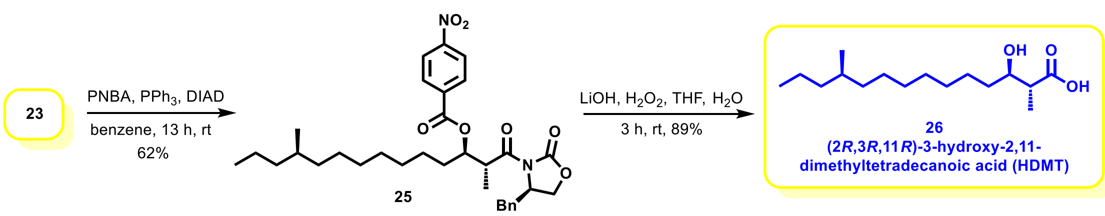
Based on continuation of our studies, synthesis of compound **24** commenced with compound **19**[23] which in turn derived from L-citronellol. We synthesized HDMT acid **24** in 87% yield using a sequence of reaction conditions as described in Scheme 3.2.8. Formation of compound **24** was confirmed by ^{1}H, ^{13}C, IR and HRMS analysis. Comparison of spectral details of synthesized compound **24** was not in agreement with the reported data of **2** and we observed similar difference in spectral data as that for compound **2'**. As expected, we did not observe any difference in the spectral data of compound **24** and **2'** as the C-11 methyl chiral centre is far away from the other two chiral centres.



Scheme 3.2.8. Synthesis of (2*R*,3*S*,11*R*)-HDMT

3.2.4.5. Synthesis of (2*R*,3*R*,11*R*)-HDMT

To inspect the observed discrepancies in the spectral data due to the probable stereochemical misassignment at C-2 methyl and C-3 hydroxyl centre, we decided to change the stereochemistry pattern at these two positions. For that purpose, we synthesised of HDMT stereoisomer with anti aldol configuration. Aldol product **23** was subjected to inversion of stereocenter of hydroxyl group under Mitsunobu reaction conditions. The planned reaction was carried out in presence of 4-nitrobenzoic acid (PNBA), triphenylphosphine and DIAD in dry benzene to give compound **25** with stereochemical inversion at hydroxyl centre.[24]

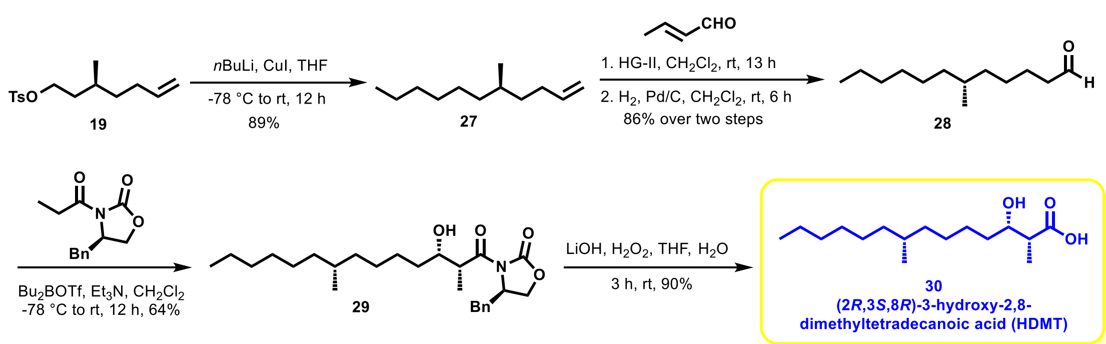


Scheme 3.2.9. Synthesis of (2*R*,3*R*,11*R*)-HDMT

Preliminary analysis by TLC; product **25** was appeared as non-polar spot and with strong UV activity than compound **23**. In ^{1}H NMR spectrum, hydroxyl attached methine was appeared more desheilded at δ 5.54 (dt, *J* = 3.2, 8.2 Hz, 1H), aromatic protons from PNBA at δ 8.30 (d, 2H) and 8.21 (d, 2H), benzylic methylene protons at 3.24 (dd, *J* = 3.2, 13.3 Hz, 1H) and 2.77 (dd, *J* = 9.4, 13.5 Hz, 1H) ppm; while in ^{13}C NMR, two carbonyl carbons present at δ 174.1, 163.7 ppm and oxazolidinone carbonyl appeared at δ 153.0 ppm, two *O*-attached carbons at δ 76.5 and 66.0 ppm in confirms the formation of structure **25**. Further formation of product was validated by HRMS (ESI) which showed peak at 603.3043 for molecular formula $C_{33}H_{44}N_2O_7Na$ $[M + Na]^+$ with calculated mass 603.3041. Auxiliary and benzoyl group in compound **25** were hydrolyzed using LiOH and aqueous hydrogen peroxide solution in THF and water to afford (2*R*,3*R*,11*R*)-HDMT, **26** in 89% yield (Scheme 3.2.9). In ^{1}H NMR, hydroxyl attached methine was appeared at δ 3.71 (br. s., 1H), C-2 methine at δ 2.49 (t, *J* = 6.9 Hz, 1H) ppm while carbonyl carbon at δ 179.1, C-3 carbon at δ 73.9 and C-2 at δ 47.4 ppm confirms the formation of structure which was further verified by HRMS (ESI). However, the chemical shift data for synthesized HDMT **26** also did not match with the reported values of **2**. These observations made us to look at other possible structures; in particular, change is the position of methyl group present on lipid chain. Although, it was fixed at C-11 using HMBC correlation, we decided to examine other possibilities.

3.2.4.6. Synthesis of (2*R*,3*S*,8*R*)-HDMT

According to the available literature of fusaristatins, where in case of fusaristatin A, fusaristatin B and YM170320, methyl group was assigned at C-7 position (counted from left side).[10] With this background information, we decided to change the position of methyl from C-11 to C-8 in the present studies. In this context, we have synthesized the HDMT fragment as shown in scheme 3.2.10.



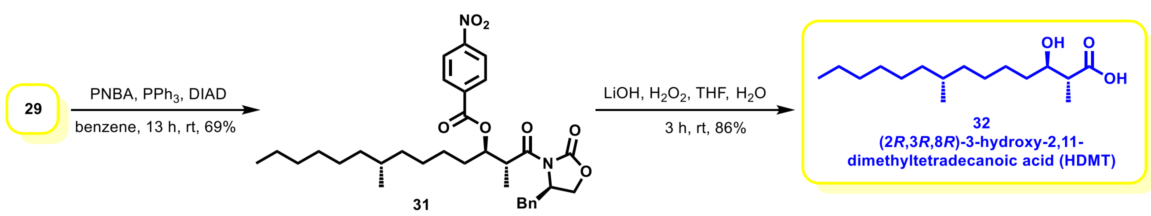
Scheme 3.2.10. Synthesis of (2*R*,3*S*,8*R*)-HDMT

Compound **19** underwent homologation smoothly with *n*BuLi in presence of CuI in THF and afforded alkene **27** which was very non-polar on TLC and compound was purified by column chromatography, eluted in pet ether. The assigned structure for compound **27** was confirmed by

[1]H, [13]C NMR and HRMS analysis. Compound **27** and trans-crotonaldehyde on cross metathesis using Hovyeda-Grubbs' catalyst (2nd generation) furnished alkene which on hydrogenation in the presence of H_2, 10% Pd/C in dichloromethane yielded aldehyde **28** in 86% over two steps. Stereoselective aldol reaction of aldehyde **28** with Evans chiral auxiliary in dibutylboron triflate and triethyl amine in dichloromethane rendered aldol product **29** in 64% yield (Scheme 3.2.10.).[21] Structure of compound **29** was confirmed by IR, [1]H, [13]C NMR, and HRMS analysis. Auxiliary hydrolysis of **29** afforded (2*R*,3*S*,8*R*)-HDMT, **30** in 90% yield. In [1]H NMR, hydroxyl attached methine was appeared at δ 3.75 (br. s., 1H), C-2 methine at 2.39 (quin, *J* = 6.9 Hz, 1H) while carbonyl carbon at δ 179.1, C-3 carbon at δ 73.4 and C-2 at δ 47.0 ppm confirms the formation of structure which was further verified by HRMS (ESI) (Scheme 3.2.10.).

3.2.4.7. Synthesis of (2*R*,3*R*,8*R*)-HDMT

Compound **29**, on stereochemical inversion at hydroxyl group (C-3 stereocenter) under Mitsunobu reaction condition, afforded compound **31**. Formation of benzoyl ester clearly seen in [1]H NMR, peak corresponding to PNBA appeared at δ 8.20 (d, *J* = 9.2 Hz, 2H), 8.12 (d, *J* = 9.2 Hz, 2H) ppm, new peak in deshielded region corresponding to hydroxyl attached methine appeared at δ 5.45 (dt, *J* = 3.4, 8.2 Hz, 1H) ppm. [13]C NMR spectra showed presence of ester carbonyl at δ 174.2, amide carbonyl at δ 163.7 and oxazolidinone carbonyl at δ 153.0 ppm confirming the formation of product. It was further validated by HRMS (ESI), which showed peak at 581.3226 corresponding to molecular formula $C_{33}H_{45}N_2O_7S$ $[M + H]^+$ with calculated value 581.3221.



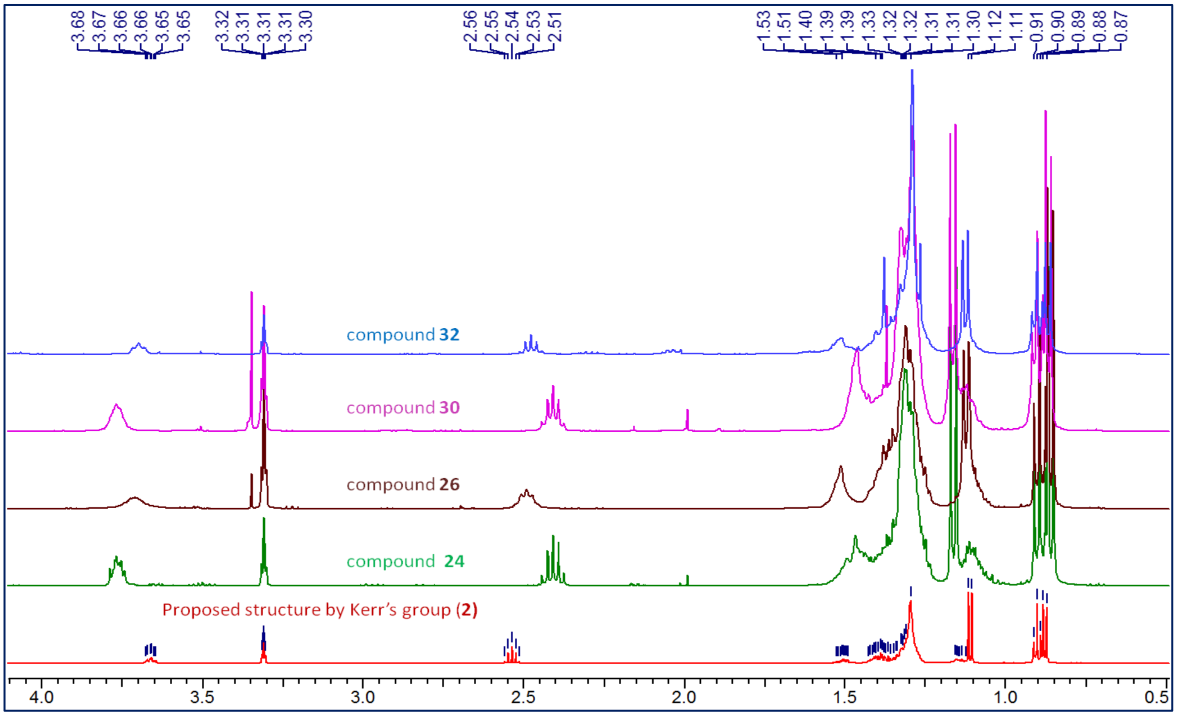
Scheme 3.2.11. Synthesis of (2*R*,3*R*,8*R*)-HDMT

Auxiliary and benzoyl ester hydrolysis of compound **31** using LiOOH yielded (2*R*,3*R*,8*R*)-HDMT, **32** (Scheme 3.2.11.) which was again confirmed by spectral data as well as HRMS analysis. In [1]H NMR, hydroxyl attached methine was appeared at δ 3.68 (t, *J* = 6.5 Hz, 1H), C-2 methine at δ 2.46 (quin, *J* = 6.9 Hz, 1H) while carbonyl carbon at δ 179.4, C-3 carbon at δ 74.0

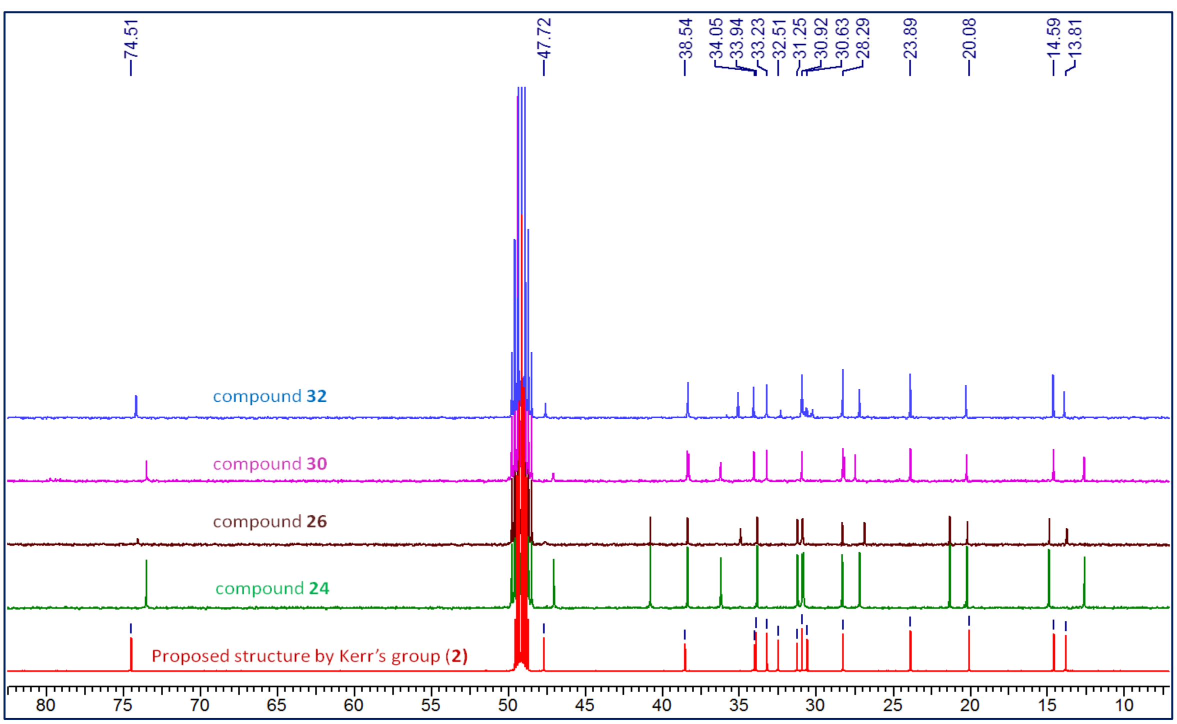
and C-2 at δ 47.5 ppm confirms the formation of structure which was further verified by HRMS (ESI) with peak at 271.2261 corresponding to molecular formula $C_{16}H_{31}O_3$ $[M - H]^-$ with calculated value 271.2268. Again, the spectral data of both isomers **30** and **32** was compared with that of compound **2** (prepared from natural fusaristatin C)[9] and synthetic HDMTs **2'**, **24**, and **26** prepared in our lab (Figure 3.2.3.); it was found that they are not in agreement with assigned structure by Kerr's group, suggesting additional efforts are warranted.

3.2.5. Comparison of the HDMT spectral data

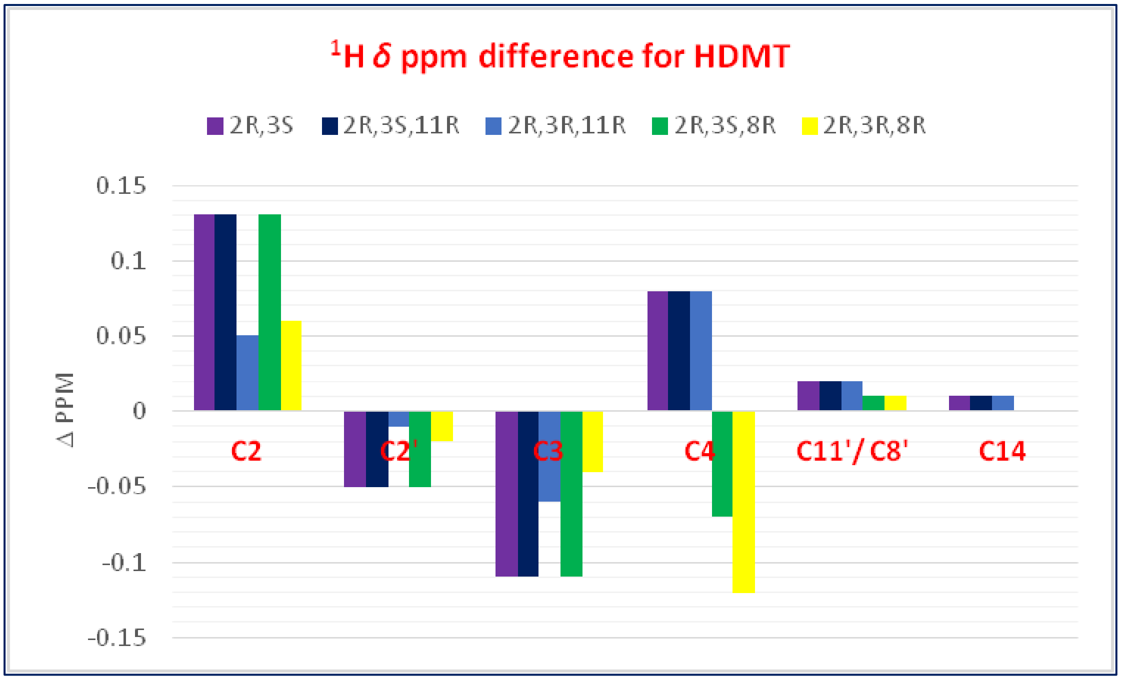
We have compared the spectral data of all synthesized compounds with the proposed structure of HDMT (FID of NMR spectra provided by Kerr's group), there were substantial discrepancies in spectral data. In ^{1}H NMR, of compound **2** (reported by Kerr's group[9]) hydroxyl attached methine present at δ 3.66 ppm and methyl attached methine (C-2) at δ 2.54 ppm showing major discrepancies with all synthesized isomers. Also, C-2 attached methyl was reported at δ 1.11(d, *J* = 7.1 Hz) ppm which was observed in close only with anti isomers **26** and **32** (δ 1.12, d, 6.9) which indicates the relative configuration at C-2 methyl at C-3 hydroxyl must be anti to each other. In case of ^{13}C NMR spectral data, carbonyl carbon of all synthesized isomers appeared more deshielded than reported at δ 177.5 ppm by kerr's group. In case of both the syn HDMT isomers, C-2 carbon appeared at 46.9 (for **24**), 47.0 (for **30**) and C-3 carbon at 74.0 ppm while in case of anti isomers, C-2 at 47.9 (**26**) and 47.5 **(32**) and C-3 at 73.9 (**26**) and 74.1 (**32**) which indicates that spectral data of anti isomers are in close proximity to reported spectral data (C-2, δ 47.7 and C-3, δ 74.5 ppm) than syn isomers. The discrepancies at C-13 methylene (23.9 ppm, **2**), for **24** and **26**, δ 21.2 ppm, than for **30** and **32**, δ 23.8 ppm, indicate that C-8 methyl isomer is in more close vicinity than C-11 methyl isomer but not exactly matching with that of HDMT isomer reported by Kerr's group. These observations clearly indicate that the C-11 methyl position as assigned by Kerr's group as well as the relative stereochemistry at C-2 and C-3 centre needs to be reassigned again; which manifest the structural revision of fusaristatin C.



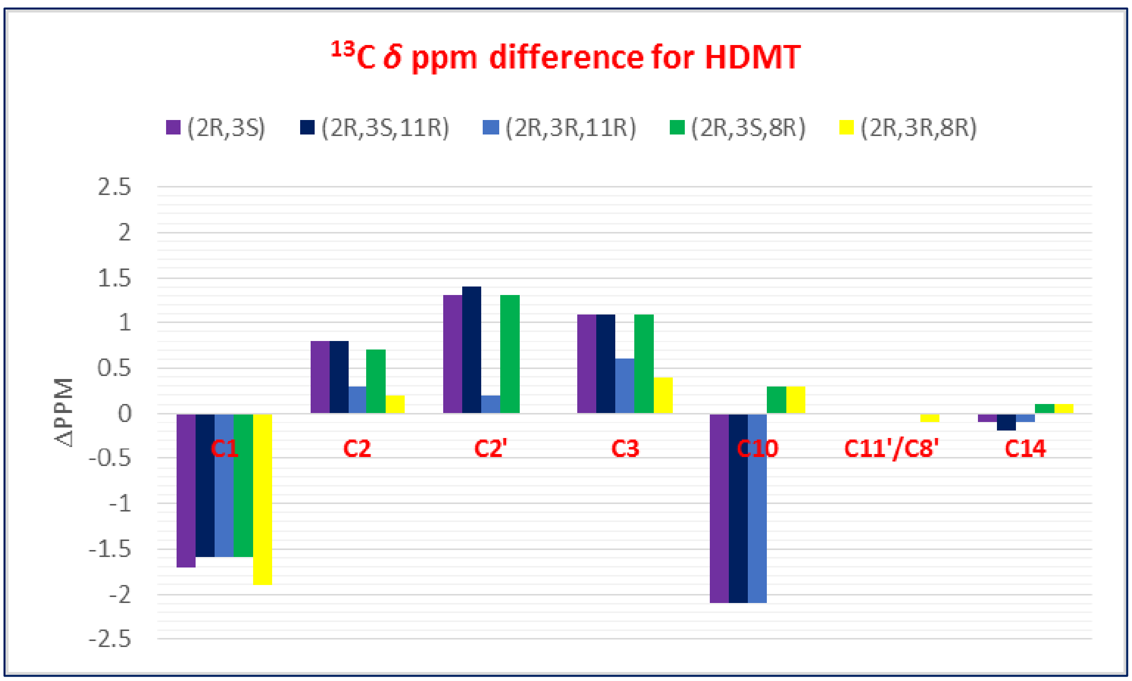
(a) ^{1}H NMR comparison of synthesized HDMT isomers with respect to HDMT fragment of Kerr's group



(b) ^{13}C NMR comparison of synthesized HDMT isomers with respect to HDMT fragment of Kerr's group



(c) ^{1}H NMR chemical shift difference of selected protons of synthesized HDMT isomers with respect to HDMT fragment of Kerr's group



(d) ^{13}C NMR chemical shift difference of selected carbons of synthesized HDMT isomers with respect to HDMT fragment of Kerr's group.

Figure 3.2.3. Comparison of HDMT spectral data

3.2.6. Conclusions

In conclusion, our efforts towards total synthesis of fusaristatin C, a macrocyclic lipodepsipeptide are described in this Chapter. We have successfully synthesized peptidic fragment required for the synthesis of fusaristatin C in a gram scale.

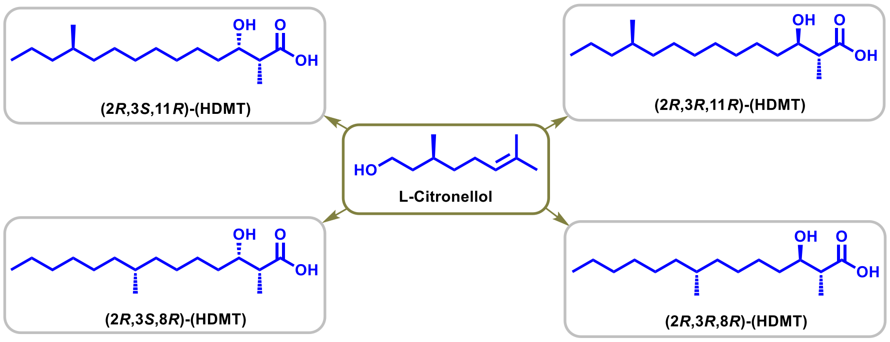
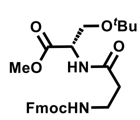


Figure 3.2.4. Synthesized isomers of HDMT

We have also accomplished the synthesis of proposed structure of HDMT fragment where spectral data were not in agreement with the reported data. In an attempt towards finding the correct structure of HDMT, we synthesized four other isomers, including the movement of methyl group on lipid chain, but in all the cases there were clear discrepancies of selected ^{1}H and ^{13}C NMR signals, suggesting that the structural revision of fusaristatin C, in particular HDMT fragment is necessary.

3.2.7. Experimental section

Methyl *N*-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoyl)-O-(*tert*-butyl)-L-serinate (7)



7

To the solution of Fmoc-β-Ala-OH (156 mg, 0.50 mmol) in DMF (1 mL) was added HATU (383 mg, 1.01 mmol) and DIPEA (0.175 mL, 1.01 mmol) at 0 °C and stirred for 10 min. The solution of NH_2-Ser(^{t}Bu)-OMe (200 mg, 0.50 mmol; synthesized from compound **6** by Fmoc deprotection) in DMF (0.5 mL) was added dropwise and reaction mixture was stirred at room temperature for 12 h. After completion of reaction, mixture was diluted with ethyl acetate (10 mL); organic layer was washed with aqueous $NaHCO_3$ solution (5 mL) followed by 1 N HCl (5 mL); dried over Na_2SO_4, concentrated under *vacuo*, purified by column chromatography gave dipeptide **7** as white solid.

Yield: 72% (169 mg)

IR ν_{max} (film): 3322, 2961, 2927, 1716, 1662 cm^{-1}

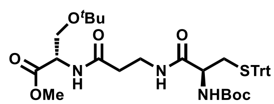
Specific rotation: $[\alpha]_D^{26}$ = –11.3 (*c* 0.136, MeOH)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.24 (d, *J* = 7.3 Hz, 1H), 7.88 (d, *J* = 7.3 Hz, 2H), 7.69 (d, *J* = 7.3 Hz, 2H), 7.42 (t, *J* = 7.0 Hz, 2H), 7.34 - 7.31 (m, 2H), 7.27 (br. s., 1H), 4.47 (br. s., 1H), 4.30 (d, *J* = 6.7 Hz, 2H), 4.22 (d, *J* = 6.1 Hz, 1H), 3.63 (br. s., 4H), 3.50 - 3.48 (m, 1H), 3.22 - 3.21 (m, 2H), 2.40 (t, *J* = 6.4 Hz, 2H), 1.10 (s, 9H)

^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 170.9, 170.6, 156.0, 143.9, 140.7, 127.6, 127.0, 125.1, 120.1, 72.9, 65.4, 61.5, 52.9, 51.7, 46.7, 37.0, 35.2, 27.1

HRMS (ESI): calculated for $C_{26}H_{33}N_2O_6$ [M + H]$^+$: 469.2333; found 469.2337.

Methyl *N*-(3-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(tritylthio)propanamido)propanoyl)-O-(*tert*-butyl)-L-serinate (4)



4

Dipeptide **7** (2.0 g, 4.32 mmol) was dissolved in dichloromethane (10 mL) and diethyl amine (DEA, 10 mL) was added at 0 °C. Reaction mixture was stirred at room temperature until the starting material completely consumed. After completion, solvent was evaporated under *vacuo* and amine was forwarded for coupling without purification.

Coupling was done by following similar procedure as did for synthesis of compound **7** and purified by column chromatography, furnished tripeptide **4** as white solid.

Yield: 93% (2.8 g)

IR ν_{max} (film): 3309, 2971, 2931, 1719, 1660, 1491 cm^{-1}

Specific rotation: $[\alpha]_D^{28}$ = +18.70 (*c* 0.701, $CHCl_3$: MeOH, 1:1)

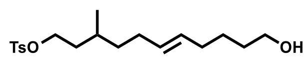
^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.27 (d, *J* = 7.8 Hz, 1H), 7.76 (t, *J* = 5.5 Hz, 1H), 7.34 - 7.22 (m, 15H), 6.92 (d, *J* = 8.2 Hz, 1H), 4.44 - 4.39 (m, 1H), 3.92 - 3.84 (m, 1H), 3.62 (s, 3H), 3.58

(dd, *J* = 5.3, 9.4 Hz, 1H), 3.45 (dd, *J* = 4.8, 9.4 Hz, 1H), 3.20 (dt, *J* = 6.2, 12.7 Hz, 2H), 2.36 - 2.29 (m, 4H), 1.37 (s, 9 H), 1.09 (s, 9H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 170.9, 170.6, 169.9, 154.9, 144.4, 129.1, 128.1, 126.8, 78.4, 73.0, 65.9, 61.5, 53.5, 52.8, 51.8, 35.2, 34.5, 34.0, 28.1, 27.1

HRMS (ESI): calculated for $C_{36}H_{49}N_3O_7SNa$ $[M + Na]^+$: 714.3183; found 714.3181.

(*E*)-11-Hydroxy-3-methylundec-6-en-1-yl 4-methylbenzenesulfonate (11)



11

The solution of 3-methylhept-6-en-1-yl 4-methylbenzenesulfonate (**10**, synthesized by known protocol from citronellol) (530 mg, 1.87 mmol) and 5-hexen-1-ol (1.879 mg, 18.79 mmol) in dichloromethane (50 mL) was purged with Ar gas for 15 min. Grubbs catalyst of Ist generation was added to the solution, and reaction mixture stirred at 45 °C for 3 h. After complete consumption of tosylate compound, solvent was evaporated under *vacuo*. The crude product was purified by column chromatography afforded compound **11** as colourless liquid.

Yield: 67% (445 mg)

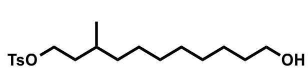
IR ν_{max} (film): 3642, 3065, 2937, 2616, 1652, 1640 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (d, *J* = 8.2 Hz, 2H), 7.35 (d, *J* = 8.2 Hz, 2H), 5.40 - 5.27 (m, 2H), 4.09 - 4.03 (m, 2H), 3.66 - 3.62 (m, 2H), 2.45 (s, 3H), 2.05 - 1.92 (m, 4H), 1.72 - 1.63 (m, 2H), 1.58 - 1.54 (m, 2H), 1.43 - 1.39 (m, 2H), 1.31 - 1.23 (m, 2H), 1.18 - 1.10 (m, 1H), 0.81 (t, *J* = 6.9 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 144.6, 133.1, 130.2, 130.2, 129.8, 129.7, 129.6, 127.8, 69.0, 69.0, 62.8, 36.5, 36.4, 35.5, 32.3, 32.2, 32.2, 29.7, 28.8, 28.5, 26.9, 25.8, 25.6, 24.4, 21.6, 19.0, 18.9

HRMS (ESI): calculated for $C_{19}H_{30}O_4SNa$ $[M + Na]^+$: 377.1865; found 377.1861.

11-Hydroxy-3-methylundecyl 4-methylbenzenesulfonate (12)



12

Compound **11** (300 mg, 0.86 mmol) was dissolved in MeOH (20 mL), catalytic amount of Pd/C (10 mol%) was added and reaction mixture was stirred under H_2 balloon pressure until the starting material consumed completely. After completion, reaction mixture was filtered through celite pad, washed with MeOH, concentrated under *vacuo* and furnished product **12** as colourless liquid.

Yield: 93% (280 mg)

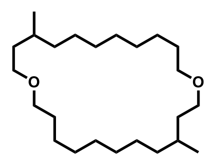
IR ν_{max} (film): 3622, 3061, 2927, 2606, 1662, 1480 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (d, *J* = 8.2 Hz, 2H), 7.34 (d, *J* = 8.2 Hz, 2H), 4.08 - 4.04 (m, 2H), 3.64 (t, *J* = 6.6 Hz, 2H), 2.45 (s, 3H), 1.68 - 1.51 (m, 5H), 1.43 (dd, *J* = 6.2, 13.5 Hz, 1H), 1.28 - 1.22 (m, 10H), 0.80 (d, *J* = 6.9 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 144.6, 133.1, 129.8, 127.8, 69.1, 63.0, 36.5, 35.6, 32.7, 29.7, 29.5, 29.3, 29.1, 26.7, 25.7, 21.6, 19.1

HRMS (ESI): calculated for $C_{19}H_{32}O_4SNa$ $[M + Na]^+$: 379.2356; found 379.2360.

4-Methyloxacyclododecane (14)



14

To the solution of methyl magnesium bromide (1.3 mL, 1.26 mmol) and CuBr (120 mg, 0.84 mmol) in dry THF (30 mL) was added compound **12** (150 mg, 0.42 mmol) in dry THF (2 mL) at 0 °C. Reaction mixture was allowed to warm to room temperature and stirred for 12 h. After completion of reaction, solvent was evaporated under reduced pressure, crude was purified by column chromatography afforded compound **14** as colourless liquid.

Yield: 72% (56 mg)

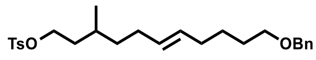
IR ν_{max} (film): 3343, 2924, 2853, 1462, 1378, 1260 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.65 (t, *J* = 6.5 Hz, 2H), 3.51 - 3.38 (m, 2H), 1.94 - 1.82 (m, 1H), 1.72 - 1.54 (m, 5H), 1.30 (br. s., 11H), 0.89 (d, *J* = 6.4 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 63.0, 63.0, 40.0, 36.4, 32.8, 32.2, 31.6, 29.7, 29.4, 26.7, 25.7, 18.9

HRMS (ESI): calculated for $C_{24}H_{49}O_2$ $[M + H]^+$: 369.2769; found 369.2770.

(*E*)-11-(benzyloxy)-3-methylundec-6-en-1-yl 4-methylbenzenesulfonate (15)



15

Compound **15** was synthesized from compound **13** and ((hex-5-en-1-yloxy)methyl)benzene by following similar procedure as that for the synthesis of compound **11**. The crude product was purified by column chromatography gave compound **15** as colourless liquid.

Yield: 65%

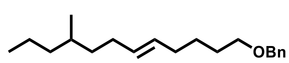
IR ν_{max} (film): 2923, 2853, 1725, 1599, 1455 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (d, *J* = 8.3 Hz, 2H), 7.29 (d, *J* = 2.3 Hz, 2H), 7.25 - 7.17 (m, 3H), 7.15 - 7.03 (m, 2H), 5.27 (qd, *J* = 3.7, 5.0 Hz, 2H), 4.44 (s, 2H), 4.00 (t, *J* = 7.0 Hz, 2H), 3.41 (t, *J* = 6.5 Hz, 2H), 2.38 (s, 3H), 1.99 - 1.79 (m, 4H), 1.65 - 1.53 (m, 4H), 1.45 - 1.29 (m, 5H), 0.75 (d, *J* = 6.4 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 144.6, 138.6, 133.1, 130.2, 130.1, 129.8, 128.3, 127.8, 127.6, 127.4, 72.8, 70.3, 69.0, 36.4, 35.6, 32.3, 29.8, 29.2, 28.7, 26.1, 21.6, 18.9

HRMS (ESI): calculated for $C_{26}H_{36}O_4SNa$ [M + Na]$^+$: 467.2227; found 467.2226.

(*E*)-(((9-methyldodec-5-en-1-yl)oxy)methyl)benzene (16)



16

A solution of **15** (1.5 g, 3.37 mmol) in dry THF (500 mL) was added cooled solution of methyl magnesium bromide in THF (1 M,6.74 mL, 6.74 mmol) at 0 °C under argon. Next a solution of Li_2CuCl_4 in THF (0.1 M, 4 mL) was added dropwise *via* syringe to the stirred solution 0 °C. The mixture was stirred at 0 °C for 1 h, and then left to stand overnight to reach room temperature. It was then quenched with sat. NH_4Cl solution, THF was evaporated under *vacuo*. Crude was diluted with EtOAc and washed successively with water, $NaHCO_3$ solution and brine, dried (Na_2SO_4), and concentrated in *vacuo*. The residue was purified by column chromatography afforded **16** as a colorless oil.

Yield: 91% (0.89 g)

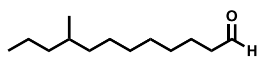
IR ν_{max} (film): 2925, 2852, 1454, 1361, 1104 cm^{-1}

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38 (d, *J* = 4.2 Hz, 4H), 7.33 - 7.29 (m, 1H), 5.42 - 5.38 (m, 2H), 4.54 (s, 2H), 3.50 (t, *J* = 6.1 Hz, 2H), 2.11 - 1.97 (m, 4H), 1.69 - 1.63 (m, 2H), 1.48 - 1.45 (m, 3H), 1.410 - 1.35 (m, 2H), 1.33 - 1.30 (m, 2H), 1.22 - 1.11 (m, 2H), 0.93 - 0.88 (m, 6H)

^{13}C NMR (125 MHz, $CDCl_3$): δ 138.7, 131.0, 130.4, 129.7, 129.2, 128.3, 127.6, 127.4, 72.8, 70.3, 39.3, 37.0, 36.9, 32.4, 32.1, 32.0, 30.1, 29.4, 29.2, 27.0, 26.3, 26.2, 24.8, 20.1, 20.0, 19.5, 14.4

HRMS (ESI): calculated for $C_{20}H_{33}O$ $[M + H]^+$: 289.2526; found 289.2524.

9-Methyldodecanal (17)



17

Compound **16** was hydrogenation under similar procedure as did for compound **12** to afford alcohol which was forwarded for oxidation without purification.

To the solution of alcohol (1.0 g, 5.00 mmol) in dichloromethane (50 mL) was added PCC (1.6 g, 7.51 mmol) at 0 °C and stirred at room temperature for 3 h. After completion of reaction, mixture was diluted with diethyl ether (50 mL) and decanted. The residue was washed with diethyl ether (20 mL X 3). Solvent was evaporated under *vacuo* at low temperature, purified by flash column chromatography afforded aldehyde **17** over two steps.

Yield: 90% (630 mg)

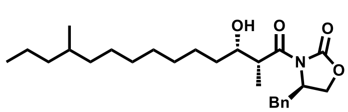
IR ν_{max} (film): 2925, 2854, 1708, 1461, 1259 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.77 (t, *J* = 1.8 Hz, 1H), 2.43 (dt, *J* = 1.8, 7.3 Hz, 2H), 1.67 - 1.62 (m, 2H), 1.36 - 1.22 (m, 13H), 1.12 - 1.04 (m, 2H), 0.88 (t, *J* = 7.1 Hz, 3H), 0.84 (d, *J* = 6.4 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 203.1, 43.9, 39.4, 37.0, 32.4, 29.8, 29.4, 29.2, 27.0, 22.1, 20.1, 19.6, 14.4

HRMS (ESI): calculated for $C_{13}H_{27}O$ $[M + H]^+$: 199.1900; found 199.1908.

(4*R*)-4-benzyl-3-((2*R*,3*S*)-3-hydroxy-2,11-dimethyltetradecanoyl)oxazolidin-2-one (18)



18

To the solution of (*R*)-4-benzyl-3-propionyloxazolidin-2-one (117 mg, 0.50 mmol) in CH_2Cl_2 (10 mL) was added *n*-Bu_2BOTf (0.61 mL, 1 M in CH_2Cl_2) followed by Et_3N (0.11 mL, 0.75 mmol) at -78 °C and stirred at same temperature for 30 min. After that, mixture was stirred at 0 °C for 20 min, again cooled to -78 °C and solution of aldehyde **17** (100 mg, 0.50 mmol) in CH_2Cl_2 (2 mL) was added slowly. Then reaction was stirred at room temperature for overnight. After quenching of reaction with phosphate buffer (pH 7.0, 1 mL), cooled to 0 °C, MeOH (1 mL) and 30% H_2O_2 solution (1 mL) was added. After being stirred for 30 min at same temperature, the mixture was extracted with CH_2Cl_2 (20 mL) and organic extracts were washed with aqueous $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated in *vacuo*. The residue was purified by column chromatography yielded **18** as colourless sticky liquid.

Yield: 74% (160 mg)

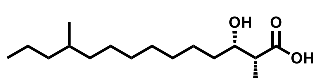
IR ν_{max} (film): 2923, 2853, 1782, 1697, 1456 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36 - 7.33 (m, 2H), 7.30 (d, *J* = 6.7 Hz, 1H), 7.21 (d, *J* = 6.7 Hz, 2H), 4.75 - 4.69 (m, 1H), 4.26 - 4.18 (m, 2H), 3.96 - 3.94 (m, 1H), 3.77 (dq, *J* = 2.4, 7.1 Hz, 1H), 3.26 (dd, *J* = 3.7, 13.4 Hz, 1H), 2.88 (br. s., 1H), 2.80 (dd, *J* = 9.5, 13.1 Hz, 1H), 1.44 - 1.23 (m, 20H), 1.11 - 1.07 (m, 2H), 0.88 (t, *J* = 7.0 Hz, 3H), 0.84 (d, *J* = 6.1 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 177.6, 153.0, 135.0, 129.4, 129.0, 127.4, 71.5, 66.1, 55.1, 42.0, 39.4, 37.8, 37.0, 33.8, 32.5, 29.9, 29.6, 27.0, 26.0, 20.1, 19.6, 14.4, 10.3

HRMS (ESI): calculated for $C_{26}H_{42}NO_4$ $[M + H]^+$: 432.3108; found 432.3105.

(2*R*,3*S*)-3-hydroxy-2,11-dimethyltetradecanoic acid (2')



2'

To the solution of oxazolidinone **18** (100 mg, 0.23 mmol) in THF-water (3:1 *v*/*v*, 4 mL) was added H_2O_2 (30% aqueous solution, 0.186 mL, 2.32 mmol) at 0 °C. This was followed by addition of LiOH (37 mg, 0.69 mmol) dissolved in H_2O (0.5 mL). After stirring for 1 h at 0 °C and 2 h at room temperature, the solvent was evaporated under *vacuo*. Aqueous layer was acidified by 1 N HCl solution and extracted with ethyl acetate (10 mL X 2). The combined ethyl acetate extracts were dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography gave **2'** as colourless liquid.

Yield: 87% (55 mg)

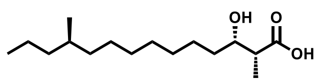
IR ν_{max} (film): 3296, 2924, 2854, 1731, 1457, 1377 cm^{-1}

^{1}H NMR (500 MHz, MeOH-d_4): δ 3.76 (br. s., 1 H), 2.41 (quin, J = 6.4 Hz, 1H), 1.49 - 1.39 (m, 4H), 1.32 (br. s., 13H), 1.16 (d, J = 6.9 Hz, 3H), 1.11 - 1.10 (m, 2H), 0.89 (t, J = 6.7 Hz, 3H), 0.86 (d, J = 6.1 Hz, 3H)

^{13}C NMR (125 MHz, MeOH-d_4): δ 179.1, 73.4, 46.9, 40.6, 38.2, 36.1, 33.7, 31.1, 30.7, 30.7, 28.2, 27.0, 21.2, 20.1, 14.7, 12.5

HRMS (ESI): calculated for $C_{16}H_{31}O_3$ $[M - H]^-$: 271.2268; found 271.2284.

(2*R*,3*S*,11*R*)-3-hydroxy-2,11-dimethyltetradecanoic acid (24)



24

Compound **24** was synthesized from compound **23** by following similar procedure as that for the synthesis of compound **2'**. The residue was purified by column chromatography to give **24** as colourless liquid.

Yield: 87%

IR ν_{max} (film): 3280, 2921, 2852, 1723, 1411 cm^{-1}

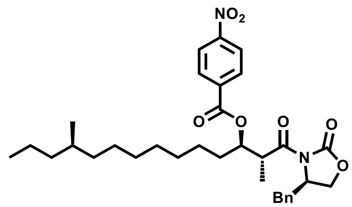
Specific rotation: $[\alpha]_D^{28}$ = −9.46 (c 0.2, $CHCl_3$)

^{1}H NMR (400 MHz, MeOH-d_4): δ 3.79 - 3.74 (m, 1H), 2.41 (quin, J = 6.9 Hz, 1H), 1.50 - 1.38 (m, 4H), 1.36 - 1.26 (m, 13H), 1.16 (d, J = 6.9 Hz, 3H), 1.13 - 1.08 (m, 2H), 0.89 (t, J = 6.9 Hz, 3H), 0.86 (d, J = 6.1 Hz, 3H)

^{13}C NMR (100 MHz, MeOH-d_4): δ 179.1, 73.4, 46.9, 40.6, 38.2, 36.1, 33.7, 31.1, 30.8, 30.7, 28.2, 27.1, 21.2, 20.1, 14.8, 12.4

HRMS (ESI): calculated for $C_{16}H_{31}O_3$ $[M - H]^-$: 271.2268; found 271.2282.

(2*R*,3*R*,11*R*)-1-((*R*)-4-benzyl-2-oxooxazolidin-3-yl)-2,11-dimethyl-1-oxotetradecan-3-yl 4-nitrobenzoate (25)



25

To the solution of compound **23** (300 mg, 0.69 mmol) in dry benzene (30 mL) was added triphenyl phosphine (364 mg, 1.39 mmol) and 4-nitrobenzoic acid (140 mg, 0.83 mmol) at 0 °C. After 10 min, solution of DIAD (0.27 mL) in dry benzene (2 mL) cooled to 0 °C and added via syringe over the period of 5 min. Reaction was stirred at room temperature for 12 h. After completion of reaction, solvent was evaporated under *vacuo*, mixture was diluted with ethyl acetate (30 mL), washed with aqueous $NaHCO_3$ solution. Organic layer was dried over Na_2SO_4, concentrated and purified by column chromatography afforded compound **25** as colourless liquid.

Yield: 62% (243 mg)

IR ν_{max} (film): 2925, 2855, 1779, 1701, 1605, 1528 cm^{-1}

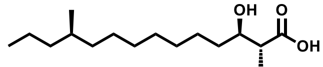
Specific rotation: $[\alpha]_D^{28}$ = −41.04 (*c* 0.737, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (d, 2H), 8.21 (d, 2H), 7.34 - 7.29 (m, 3H), 7.20 - 7.18 (m, 2H), 5.54 (dt, *J* = 3.2, 8.2 Hz, 1H), 4.60 - 4.54 (m, 1H), 4.35 - 4.28 (m, 1H), 4.17 - 4.11 (m, 2H), 4.00 - 3.95 (m, 1H), 3.24 (dd, *J* = 3.2, 13.3 Hz, 1H), 2.77 (dd, *J* = 9.4, 13.5 Hz, 1H), 1.91 - 1.85 (m, 1H), 1.75 (dd, *J* = 7.3, 15.6 Hz, 1H), 1.42 - 1.38 (m, 3H), 1.35 - 1.32 (m, 6H), 1.29 - 1.24 (m, 14H), 1.11 - 1.05 (m, 2H), 0.88 (t, *J* = 7.3 Hz, 3H), 0.83 (d, *J* = 6.9 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.1, 163.7, 153.0, 150.5, 135.5, 134.8, 130.7, 129.4, 128.9, 127.4, 123.5, 76.5, 66.0, 60.3, 55.1, 41.1, 39.3, 37.7, 37.0, 32.4, 31.4, 29.8, 29.5, 29.4, 26.9, 24.6, 20.1, 19.6, 14.3, 14.2

HRMS (ESI): calculated for $C_{33}H_{44}N_2O_7Na$ $[M + Na]^+$: 603.3041; found 603.3043.

(2*R*,3*R*,11*R*)-3-hydroxy-2,11-dimethyltetradecanoic acid (26)



26

Compound **26** was synthesized from compound **25** by following similar procedure as that for the synthesis of compound **2'**. The residue was purified by column chromatography to give **26** as colourless liquid.

Yield: 89%

IR ν_{max} (film): 3473, 2924, 2854, 1738, 1460, 1376 cm^{-1}

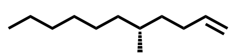
Specific rotation: $[\alpha]_D^{28}$ = +3.04 (*c* 0.437, $CHCl_3$)

^{1}H NMR (400 MHz, MeOH-d_4): δ 3.71 (br. s., 1H), 2.49 (t, *J* = 6.9 Hz, 1H), 1.51 (br. s., 2H), 1.39 - 1.25 (m, 16H), 1.12 (d, *J* = 6.9 Hz, 3H), 1.10 - 1.078 (m, 1H), 0.90 (t, *J* = 7.2 Hz, 3H), 0.86 (d, *J* = 6.9 Hz, 3H)

^{13}C NMR (100 MHz, MeOH-d_4): δ 179.1, 73.9, 47.4, 40.6, 38.2, 34.8, 33.7, 31.1, 30.8, 30.7, 28.2, 26.7, 21.2, 20.1, 14.7, 13.6

HRMS (ESI): calculated for $C_{16}H_{31}O_3$ $[M - H]^-$: 271.2268; found 271.2283.

(*R*)-5-methylundec-1-ene (27)



27

To a suspension of CuI (1.51 g, 7.92 mmol) in dry diethyl ether (500 mL) at -78 °C was added *n*-butyl lithium (9.0 mL of a 1.6 M solution in hexane; 15.91 mmol). Then reaction was stirred at 0 °C for 1 h. Prior to the addition of tosylate, reaction was cooled to -78 °C and (*R*)-3-methylhept-6-en-1-yl 4-methylbenzenesulfonate (1.5 g, 5.32 mmol) in diethyl ether (50 mL) was added dropwise. The resulting solution was stirred for 12 h at room temperature. After this time, the mixture was quenched with aqueous NH_4Cl solution, diluted with Et_2O (60 mL) and with water (3 X 30 mL). The organic phase was separated, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel using hexane as the eluent afforded compound **27** as colourless sticky liquid.

Yield: 89% (800 mg)

IR ν_{max} (film): 2925, 1696, 1638, 975 cm^{-1}

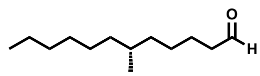
Specific rotation: $[\alpha]_D^{28}$ = +1.54 (*c* 0.45, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.82 (tdd, *J* = 6.8, 10.2, 17.1 Hz, 1H), 5.03 - 4.96 (m, 1H), 4.964 - 4.91 (m, 1H), 2.08 - 2.01 (m, 2H), 1.43 - 1.39 (m, 2H), 1.30 - 1.27 (m, 11H), 0.90 - 0.86 (m, 6H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 139.5, 113.9, 77.3, 76.7, 36.9, 36.2, 32.3, 31.9, 31.4, 29.7, 27.0, 22.7, 19.5, 14.1

HRMS (ESI): calculated for $C_{12}H_{25}$ $[M + H]^+$: 169.1817; found 168.1721.

(*R*)-6-methyldodecanal (28)



28

Compound **28** was synthesized from compound **27** and trans-crotonaldehyde by following similar procedure as that used for the synthesis of compound **11**. The crude product was forwarded for the hydrogenation without purification which was dissolved in dry CH_2Cl_2 and catalytic amount of Pd/C (10 mol %) was added. Mixture was stirred under H_2 balloon pressure for 6 h. After completion of reaction, mixture was filtered through celite pad, purified by column chromatography afforded aldehyde **28** as colourless liquid.

Yield: 86% over two steps

IR ν_{max} (film): 2956, 2855, 1694, 1638, 1462 cm^{-1}

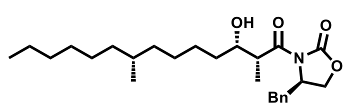
Specific rotation: $[\alpha]_D^{28}$ = +0.57 (*c* 0.938, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.77 (t, *J* = 1.9 Hz, 1H), 2.43 (dt, *J* = 1.8, 7.3 Hz, 2H), 1.65 - 1.54 (m, 2H), 1.35 - 1.26 (m, 13H), 1.14 - 1.05 (m, 2H), 0.91 - 0.82 (m, 6H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 180.3, 37.0, 36.6, 34.1, 32.6, 31.9, 29.7, 27.0, 26.5, 25.0, 22.7, 19.6, 14.1

HRMS (ESI): calculated for $C_{13}H_{27}O$ $[M + H]^+$: 199.1900; found 1901.

(*R*)-4-benzyl-3-((2*R*,3*S*,8*R*)-3-hydroxy-2,8-dimethyltetradecanoyl)oxazolidin-2-one (29):



29

Compound **29** was synthesized from compound **28** according to the protocol described above for the synthesis of **18**. After purification by column chromatography, **29** was afforded as a colourless sticky liquid.

Yield: 64%

IR ν_{max} (film): 2929, 2849, 1770, 1705, 1617, 1423 cm^{-1}

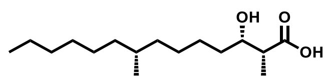
Specific rotation: $[\alpha]_D^{28}$ = −52.34 (*c* 0.644, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36 - 7.33 (m, 2H), 7.30 (d, *J* = 7.2 Hz, 1H), 7.21 (d, *J* = 6.9 Hz, 2H), 4.74 - 4.69 (m, 1H), 4.26 - 4.18 (m, 2H), 3.97 - 3.94 (m, 1H), 3.77 (dq, *J* = 2.7, 7.0 Hz, 1H), 3.26 (dd, *J* = 3.2, 13.5 Hz, 1H), 2.80 (dd, *J* = 9.3, 13.5 Hz, 1H), 1.32 - 1.26 (m, 17H), 1.15 - 1.06 (m, 3H), 0.89 (t, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 6.5 Hz, 3H)

^{13}C NMR (125 MHz, $CDCl_3$): δ 177.6, 153.0, 135.0, 129.4, 129.0, 127.4, 71.5, 66.1, 55.1, 42.1, 37.8, 37.1, 36.9, 33.9, 32.7, 31.9, 29.7, 27.0, 26.3, 22.7, 19.7, 14.1, 10.3

HRMS (ESI): calculated for $C_{26}H_{42}NO_4$ $[M + H]^+$: 454.3108; found 454.3106.

(2*R*,3*S*,8*R*)-3-hydroxy-2,8-dimethyltetradecanoic acid (30)



30

Compound **30** was synthesized from compound **29** according to the protocol described above for the synthesis of **2’**. After purification by column chromatography, **30** was afforded as a colourless sticky liquid.

Yield: 90%

IR ν_{max} (film): 3282, 2957, 2864, 1723 cm^{-1}

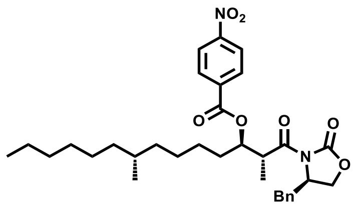
Specific rotation: $[\alpha]_D^{28}$ = −8.81 (*c* 0.456, $CHCl_3$)

^{1}H NMR (400 MHz, MeOH-d_4): δ 3.75 (br. s., 1H), 2.39 (quin, *J* = 6.9 Hz, 1H), 1.44 (br. s., 2H), 1.37 - 1.27 (m, 15H), 1.14 (d, *J* = 6.9 Hz, 3H), 1.11 - 1.06 (m, 2H), 0.88 (t, *J* = 6.1 Hz, 3H), 0.85 (d, *J* = 6.1 Hz, 3H)

^{13}C NMR (100 MHz, MeOH-d_4): δ 179.1, 73.4, 47.0, 38.2, 38.1, 36.1, 33.9, 33.1, 30.8, 28.1, 28.0, 27.3, 23.7, 20.1, 14.5, 12.5

HRMS (ESI): calculated for $C_{16}H_{31}O_3$ $[M - H]^-$: 271.2268; found 271.2274.

(2*R*,3*R*,8*R*)-1-((*R*)-4-benzyl-2-oxooxazolidin-3-yl)-2,8-dimethyl-1-oxotetradecan-3-yl 4-nitrobenzoate (31)



31

Compound **31** was synthesized according to the protocol described above for the synthesis of **25**. After purification by column chromatography, **31** was afforded as a colourless sticky liquid.

Yield: 69%

IR ν_{max} (film): 2929, 2849, 1770, 1705, 1617, 1523 cm^{-1}

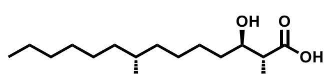
Specific rotation: $[\alpha]_D^{28}$ = −54.53 (*c* 0.19, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (d, *J* = 9.2 Hz, 2H), 8.12 (d, *J* = 9.2 Hz, 2H), 7.25 - 7.20 (m, 3H), 7.09 (d, *J* = 6.9 Hz, 2H), 5.45 (dt, *J* = 3.4, 8.2 Hz, 1H), 4.51 - 4.45 (m, 1H), 4.26 - 4.18 (m, 1H), 4.04 (dd, *J* = 2.3, 9.2 Hz, 1H), 3.88 (t, *J* = 8.4 Hz, 1H), 3.15 (dd, *J* = 3.4, 13.4 Hz, 1H), 2.67 (dd, *J* = 9.5, 13.4 Hz, 1H), 1.79 (dt, *J* = 3.1, 7.6 Hz, 1H), 1.71 - 1.63 (m, 1H), 1.29 (d, *J* = 4.6 Hz, 2H), 1.25 - 1.15 (m, 16H), 1.03 - 0.95 (m, 2H), 0.79 (t, *J* = 6.9 Hz, 4H), 0.74 (d, *J* = 6.9 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.2, 163.7, 153.0, 150.5, 135.5, 134.9, 130.7, 129.4, 129.4, 128.9, 127.4, 123.6, 76.6, 66.0, 55.2, 41.1, 37.7, 36.9, 36.8, 32.7, 31.9, 31.5, 29.6, 27.0, 26.9, 25.0, 22.7, 19.6, 14.2, 14.1

HRMS (ESI): calculated for $C_{33}H_{45}N_2O_7S$ $[M + H]^+$: 581.3221; found 581.3226.

(2*R*,3*R*,8*R*)-3-hydroxy-2,8-dimethyltetradecanoic acid (32)



32

Compound **32** was synthesized from compound **31** according to the protocol described above for the synthesis of **2'**. After purification by column chromatography, **32** was obtained as a colourless sticky liquid.

Yield: 86%

IR ν_{max} (film): 3322, 3061, 2927, 2806, 1732, 1249 cm^{-1}

Specific rotation: $[\alpha]_D^{28}$ = +2.32 (*c* 0.56, $CHCl_3$)

^{1}H NMR (400 MHz, MeOH-d_4): δ 3.68 (t, J = 6.5 Hz, 1H), 2.46 (quin, J = 6.9 Hz, 1H), 1.54 - 1.46 (m, 2H), 1.31 - 1.27 (m, 17H), 1.10 (d, J = 6.9 Hz, 3H), 0.88 (t, J = 6.9 Hz, 3H), 0.85 (d, J = 6.1 Hz, 3H)

^{13}C NMR (100 MHz, MeOH-d_4): δ 179.4, 74.0, 47.5, 38.2, 38.2, 34.9, 33.9, 33.1, 30.8, 30.8, 28.2, 27.1, 23.8, 20.1, 14.5, 13.8

HRMS (ESI): calculated for $C_{16}H_{31}O_3$ $[M - H]^-$: 271.2268; observed 271.2261.

3.2.8. References

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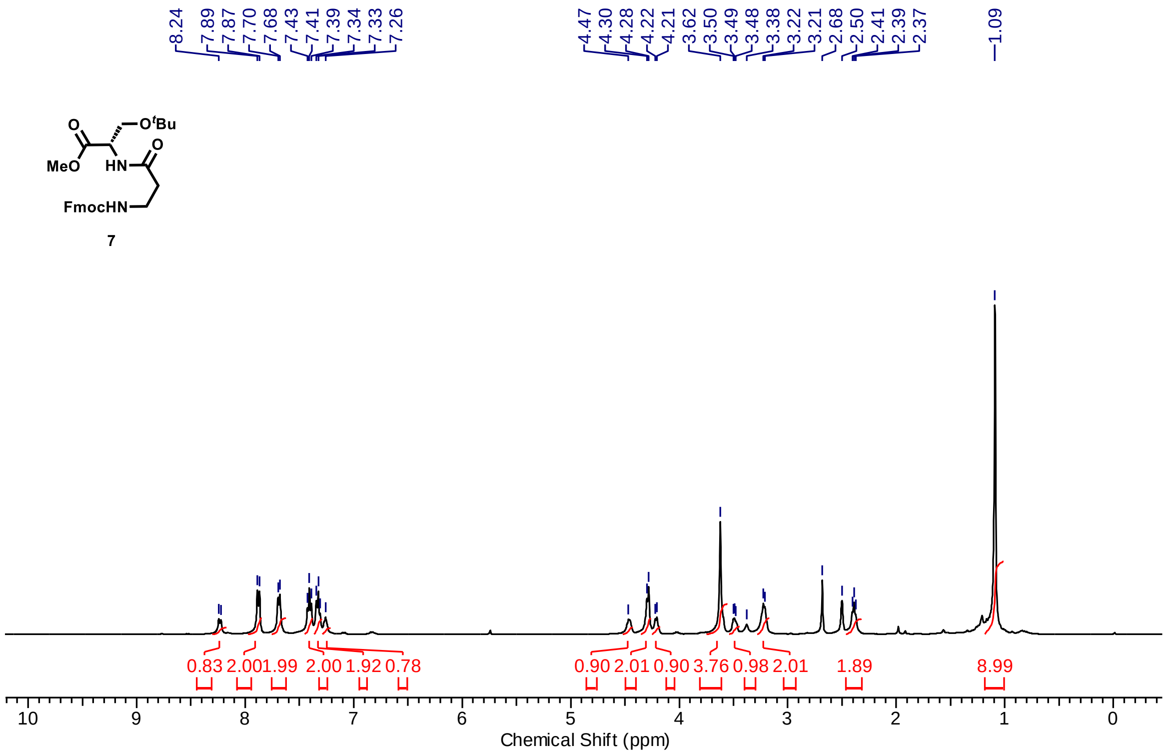
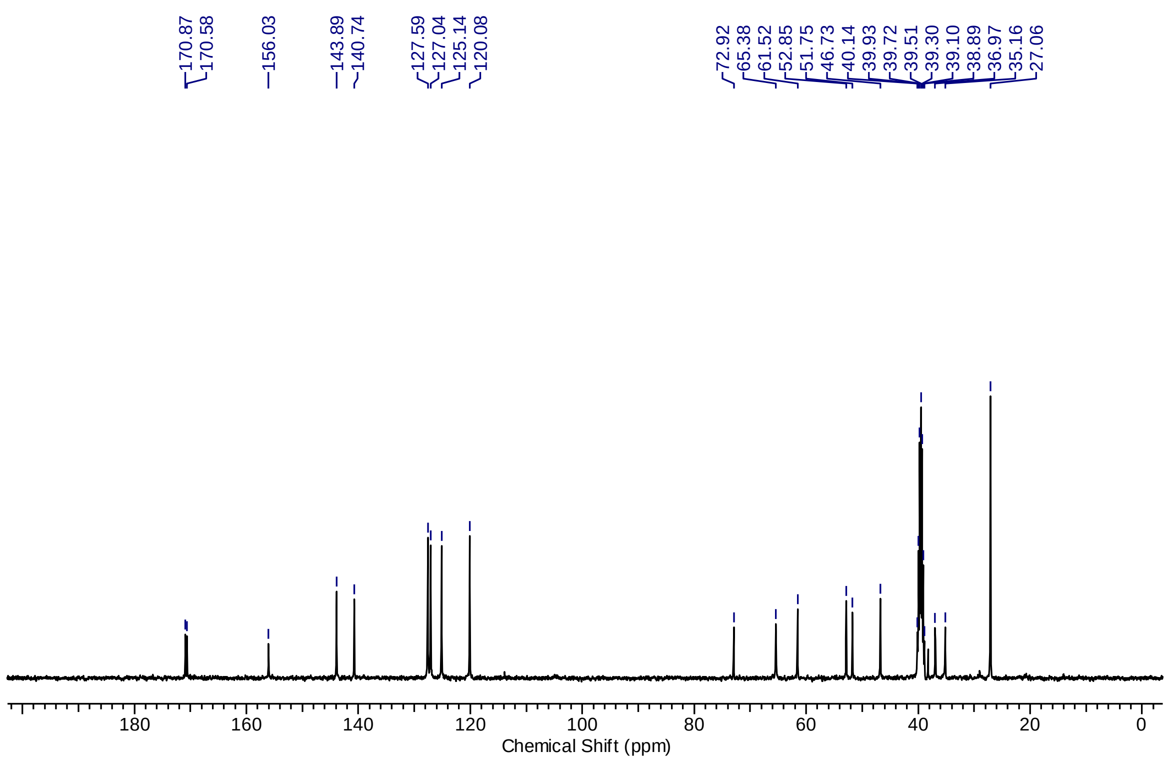
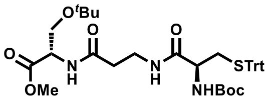
3.2.9. Copies of NMR spectra



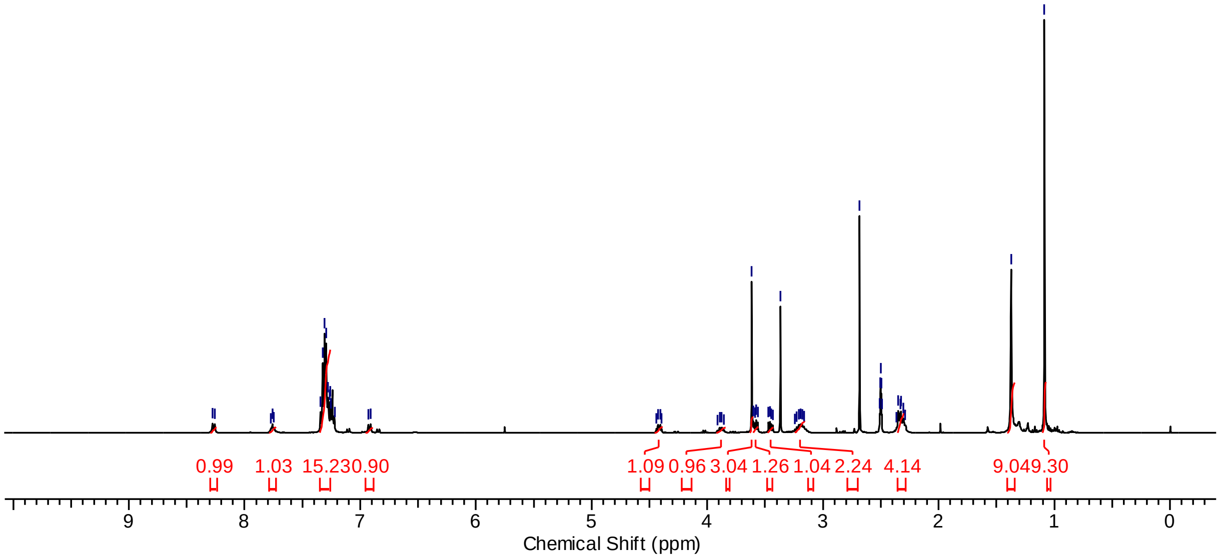
^{1}H NMR of **7** (400 MHz, DMSO-d_6)



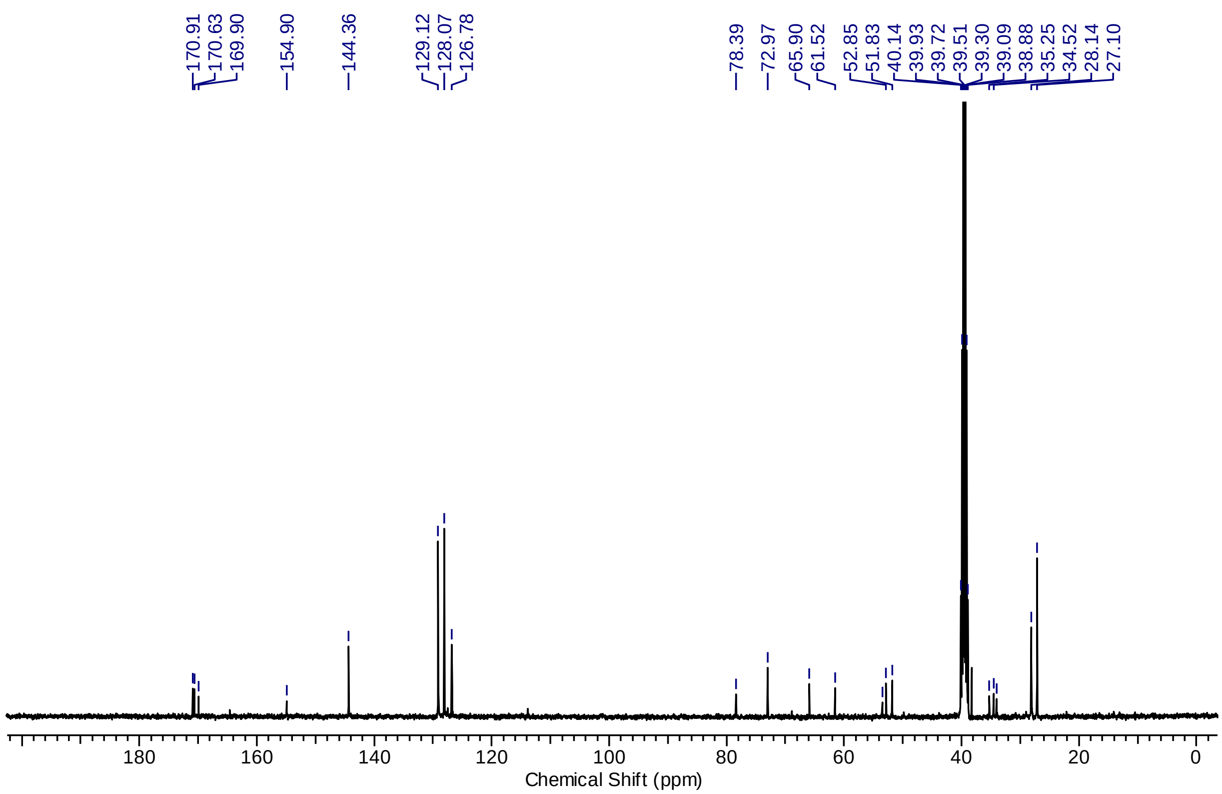
^{13}C NMR of **7** (100 MHz, DMSO-d_6)



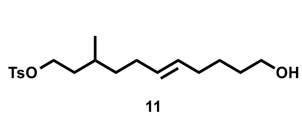
4

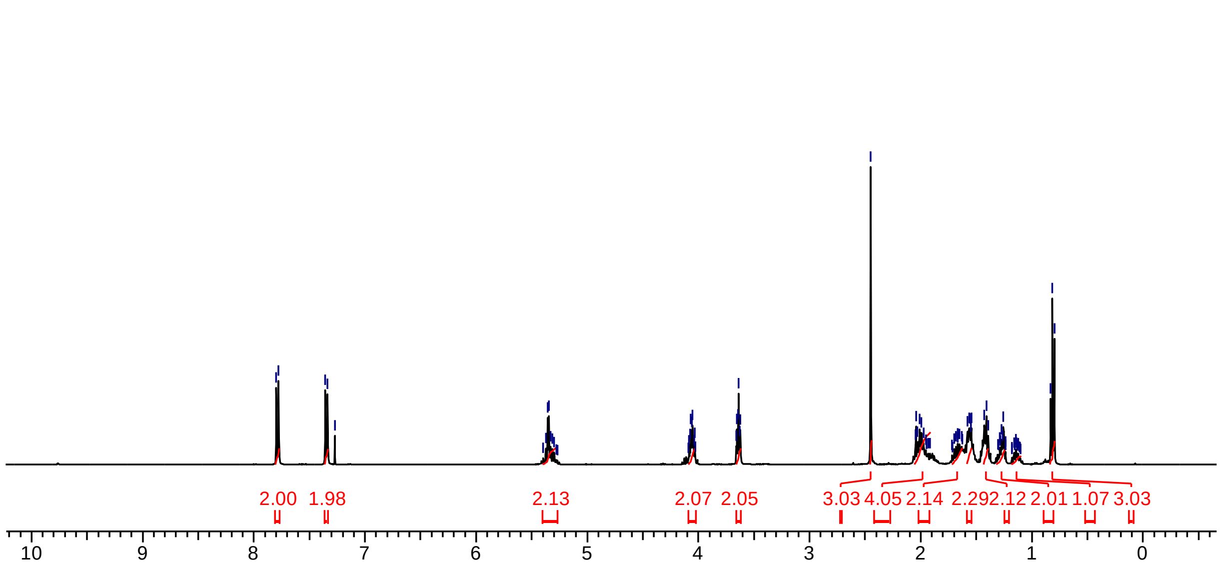


^{1}H NMR of **4** (400 MHz, DMSO-d_6)

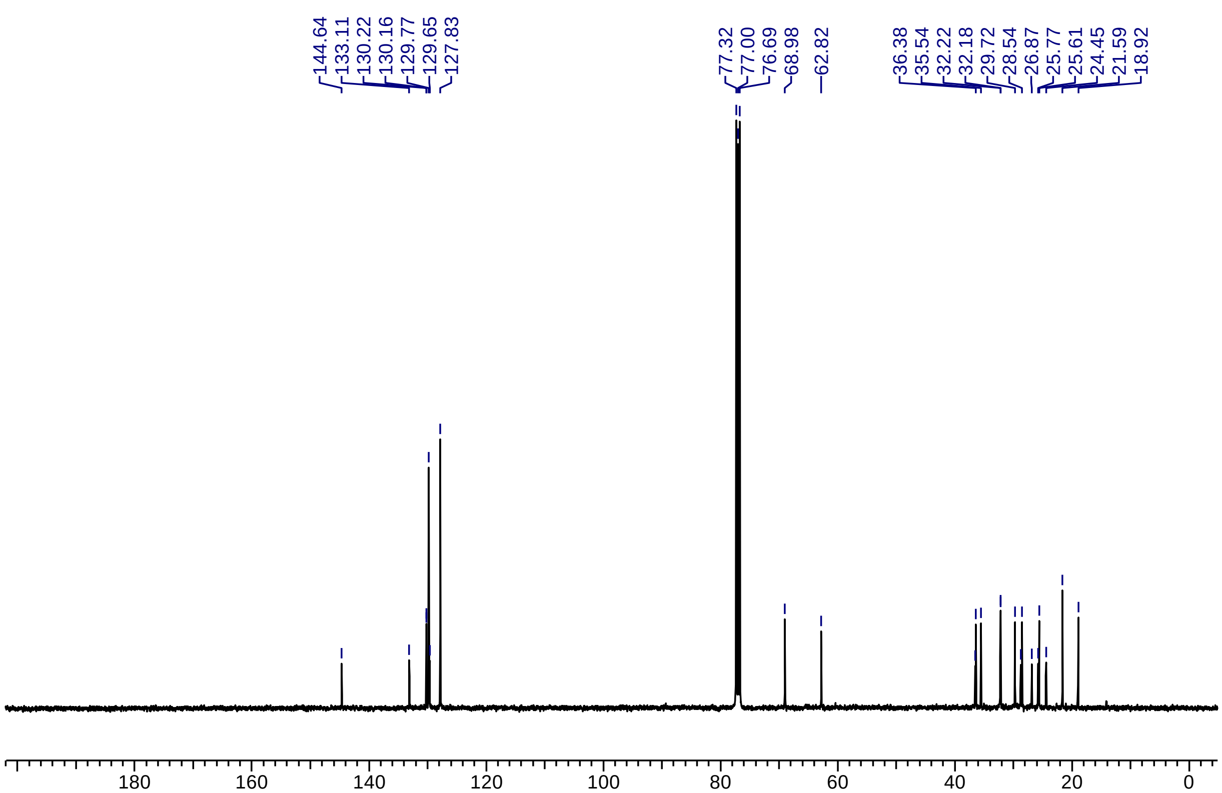


^{13}C NMR of **4** (100 MHz, DMSO-d_6)

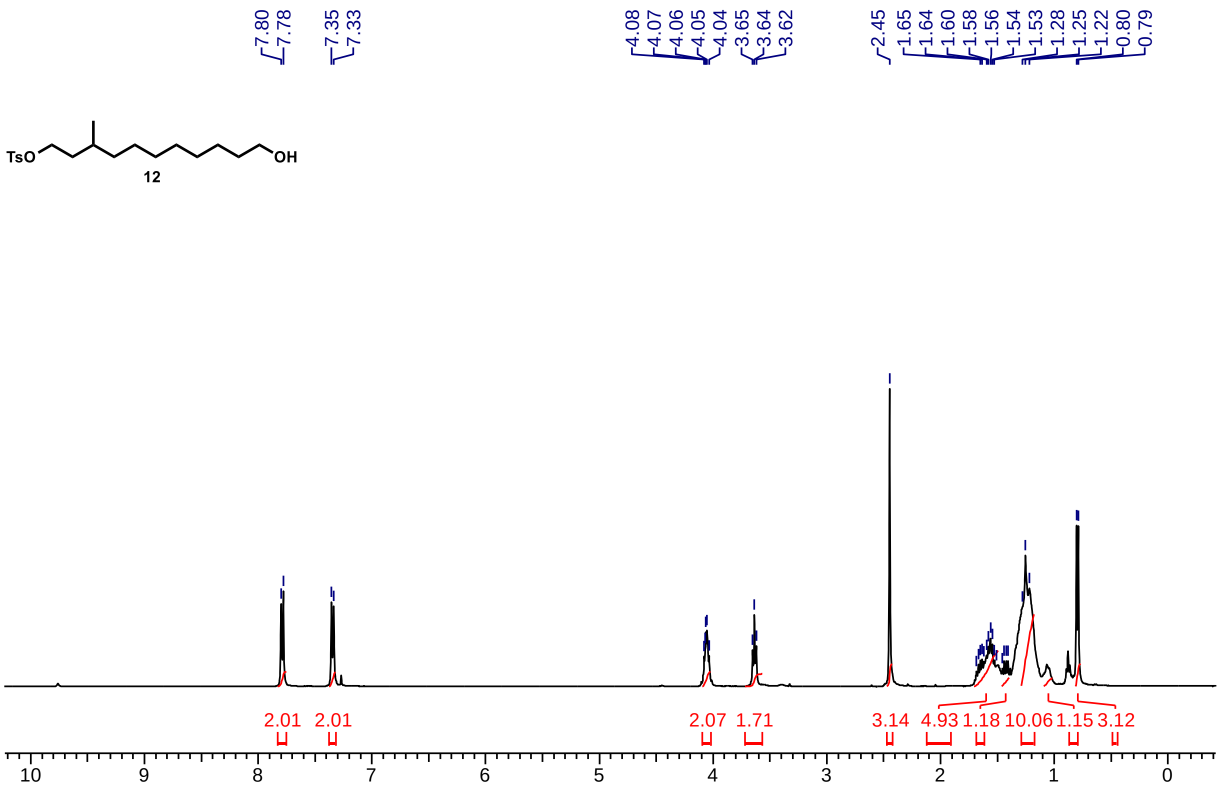




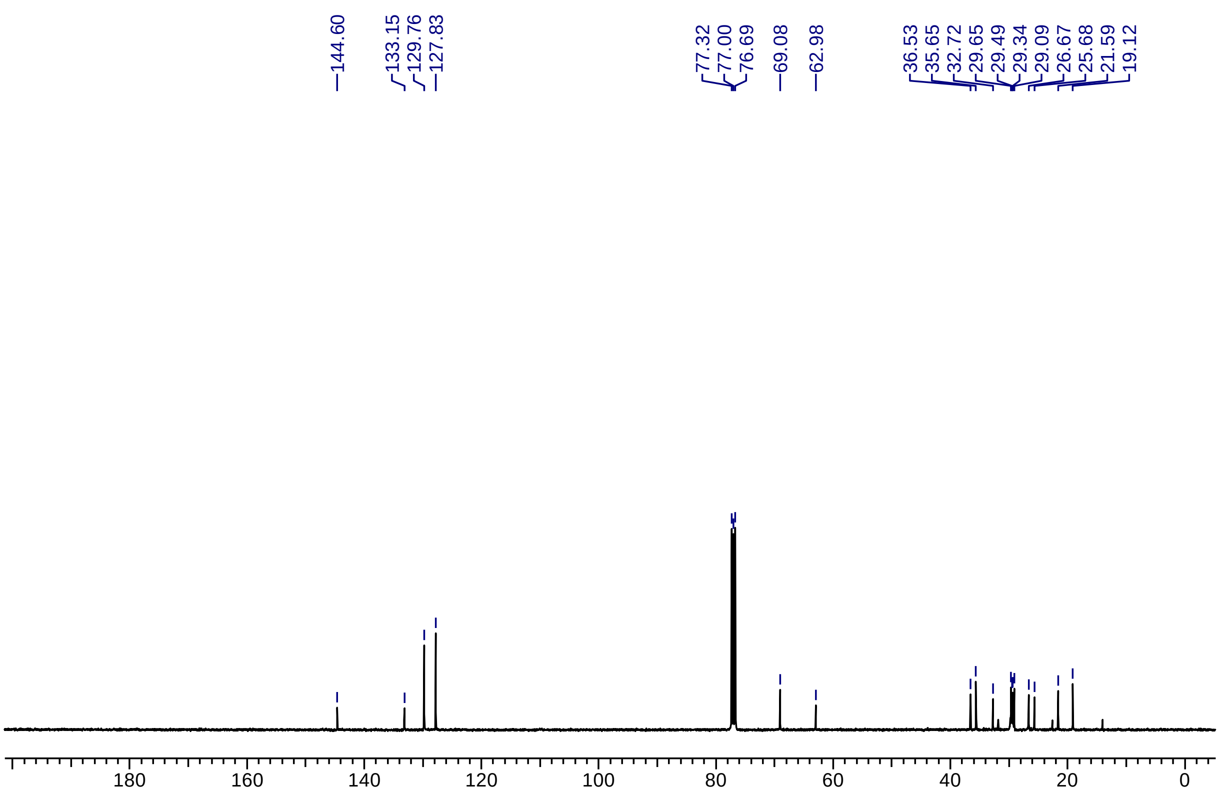
^{1}H NMR of **11** (400 MHz, CDCl$_3$)



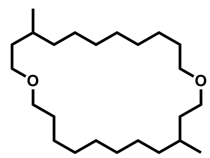
^{13}C NMR of **11** (100 MHz, CDCl$_3$)



[1]H NMR of **12** (400 MHz, $CDCl_3$)



[13]C NMR of **12** (100 MHz, $CDCl_3$)



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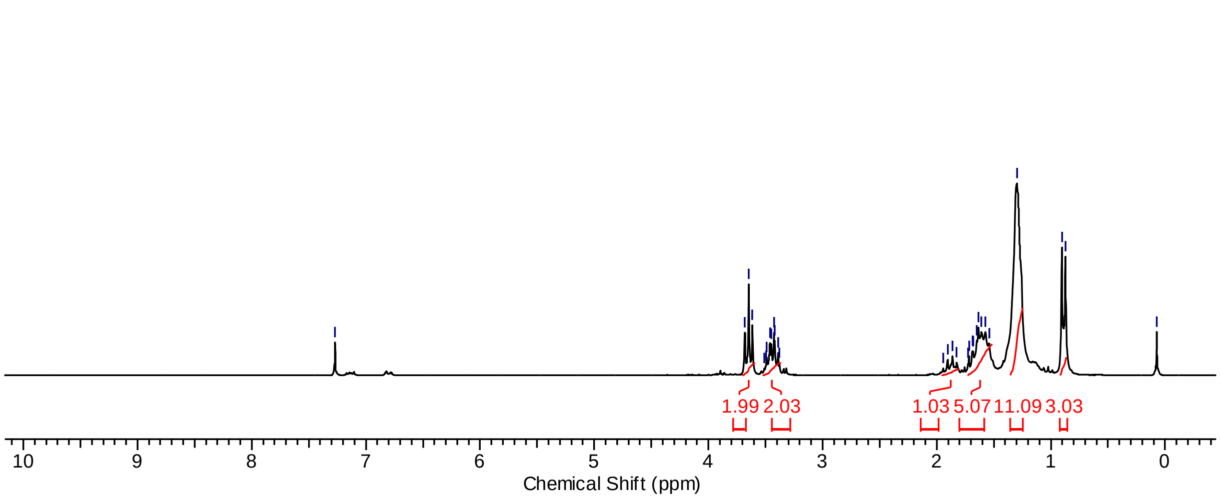
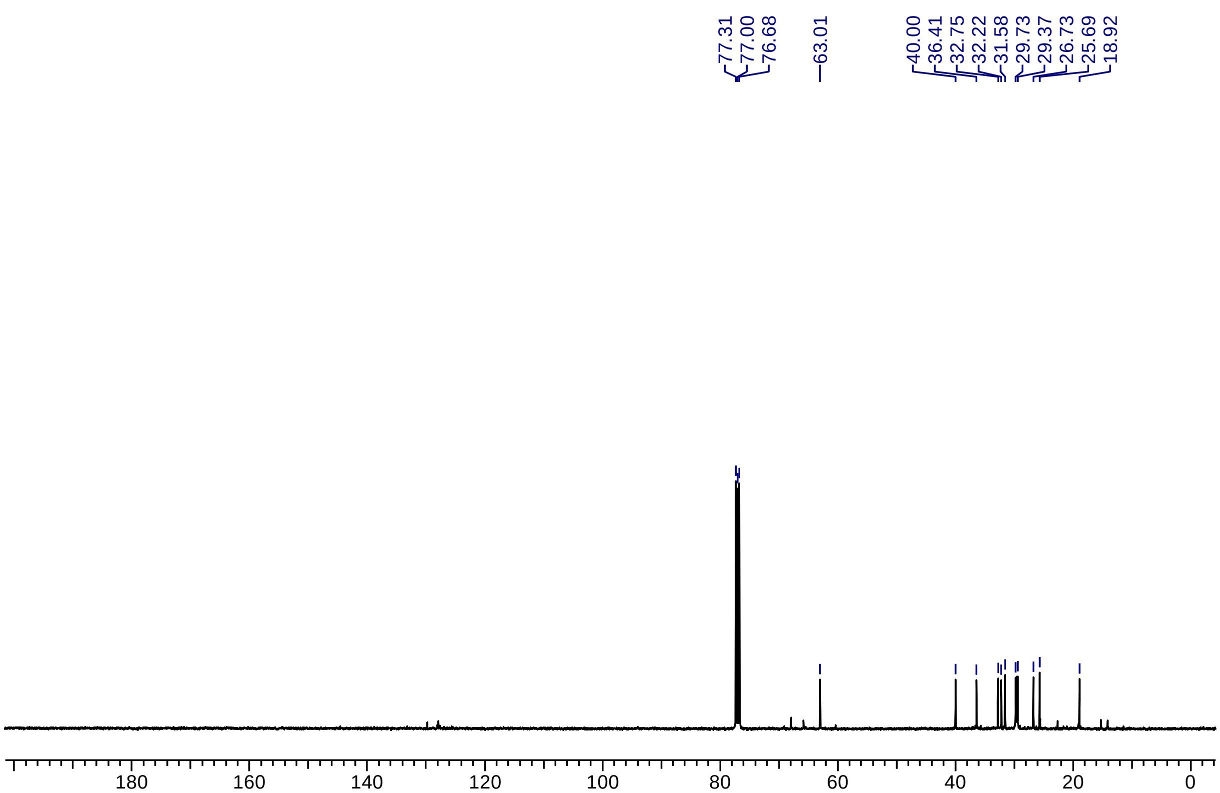
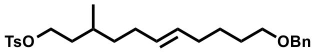


^{1}H NMR of **14** (400 MHz, $CDCl_3$)



^{13}C NMR of **14** (100 MHz, $CDCl_3$)



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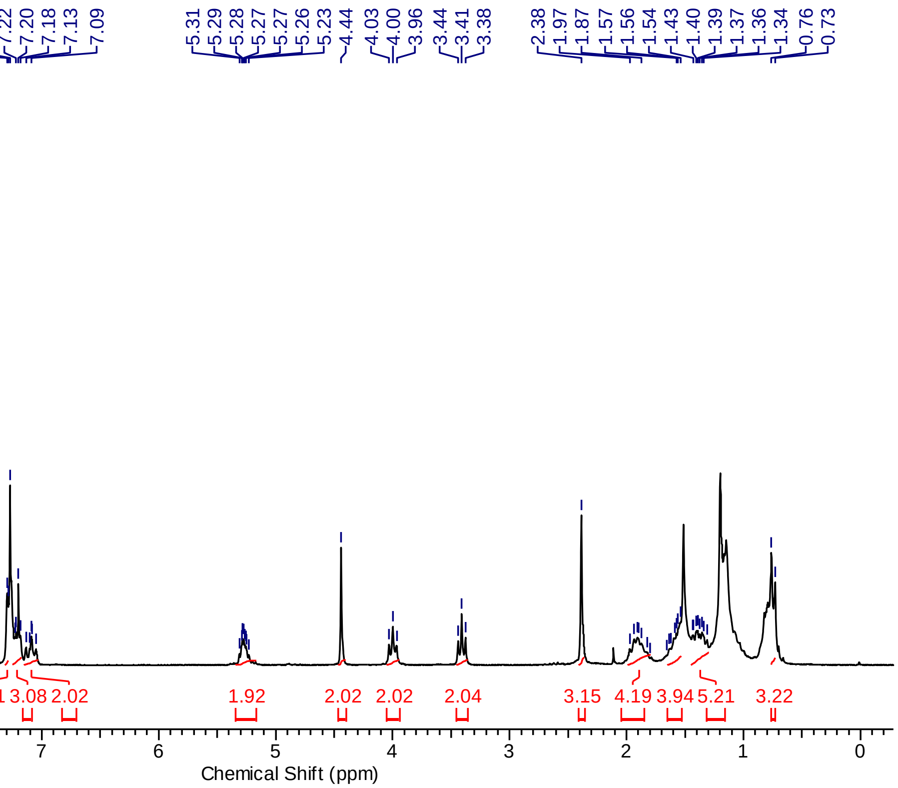
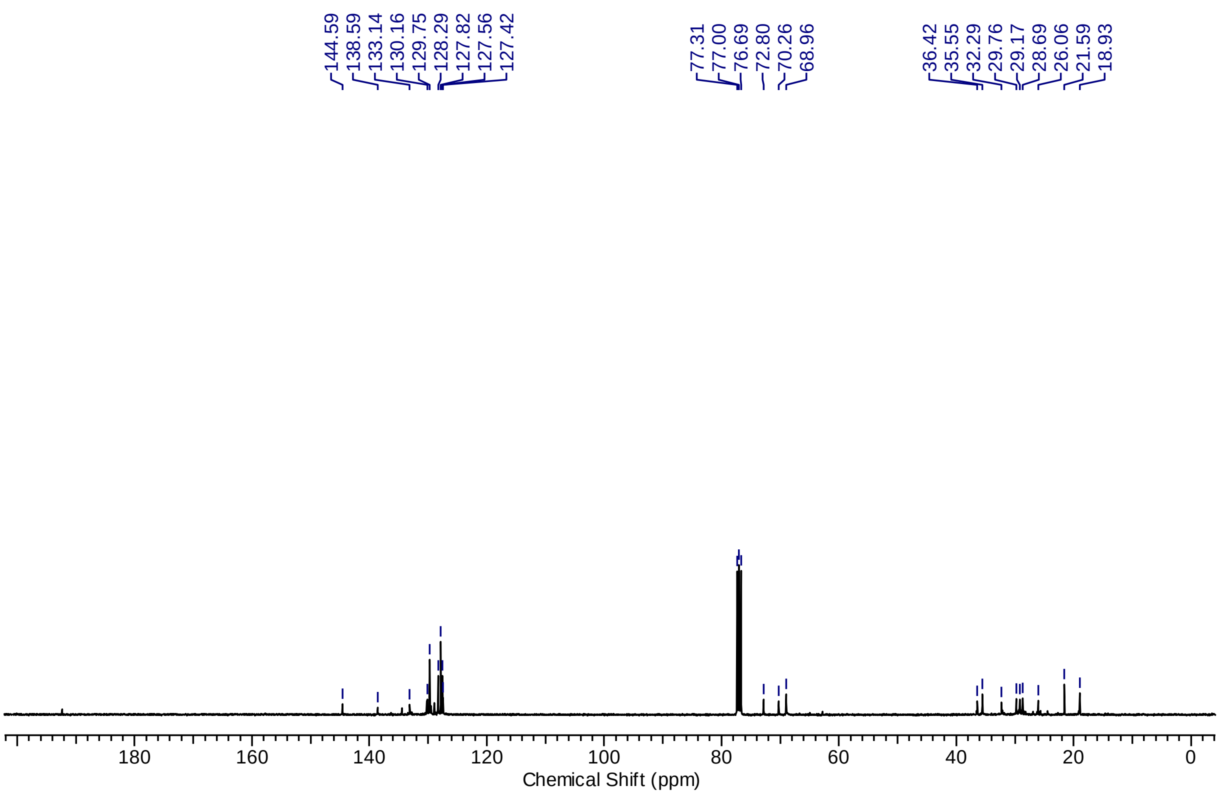
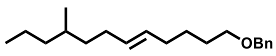


[1]H NMR of **15** (400 MHz, $CDCl_3$)



[13]C NMR of **15** (100 MHz, $CDCl_3$)



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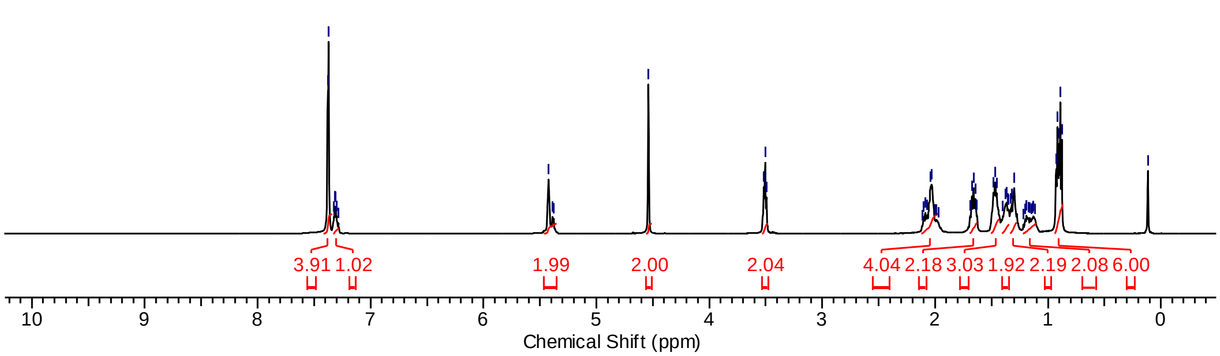
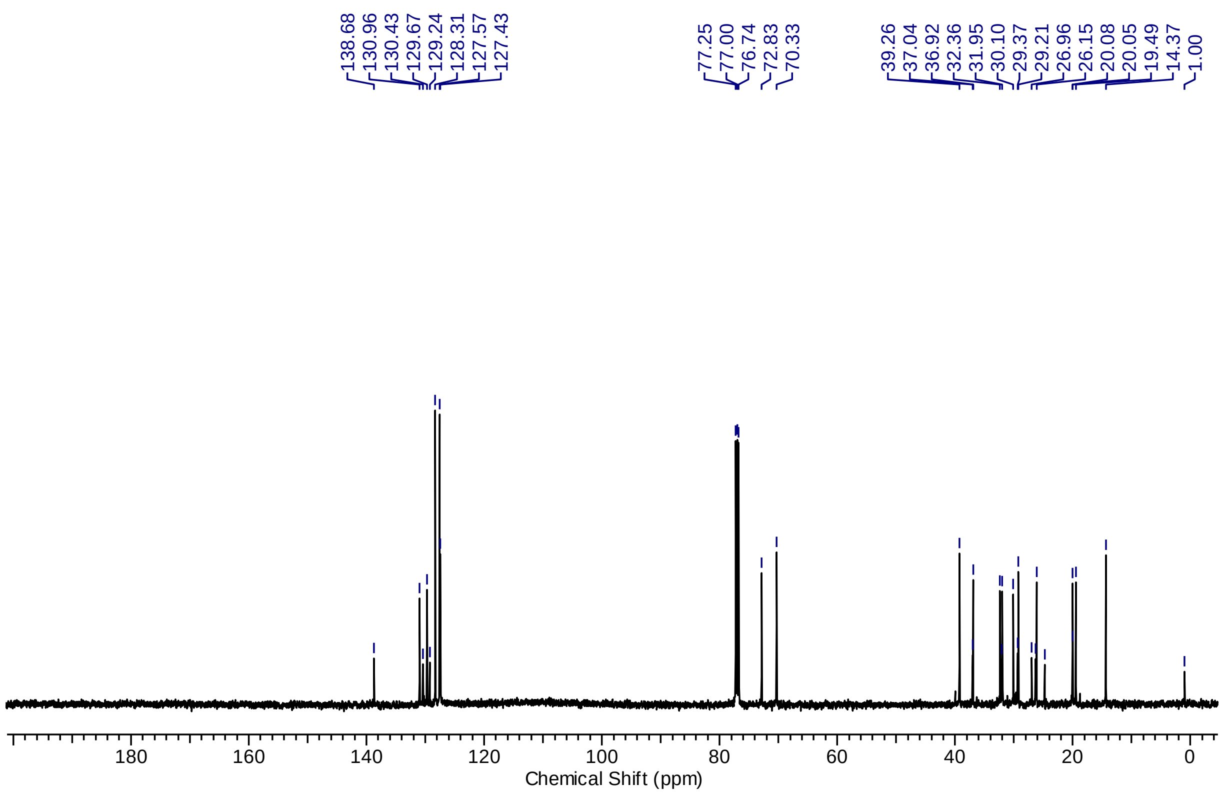
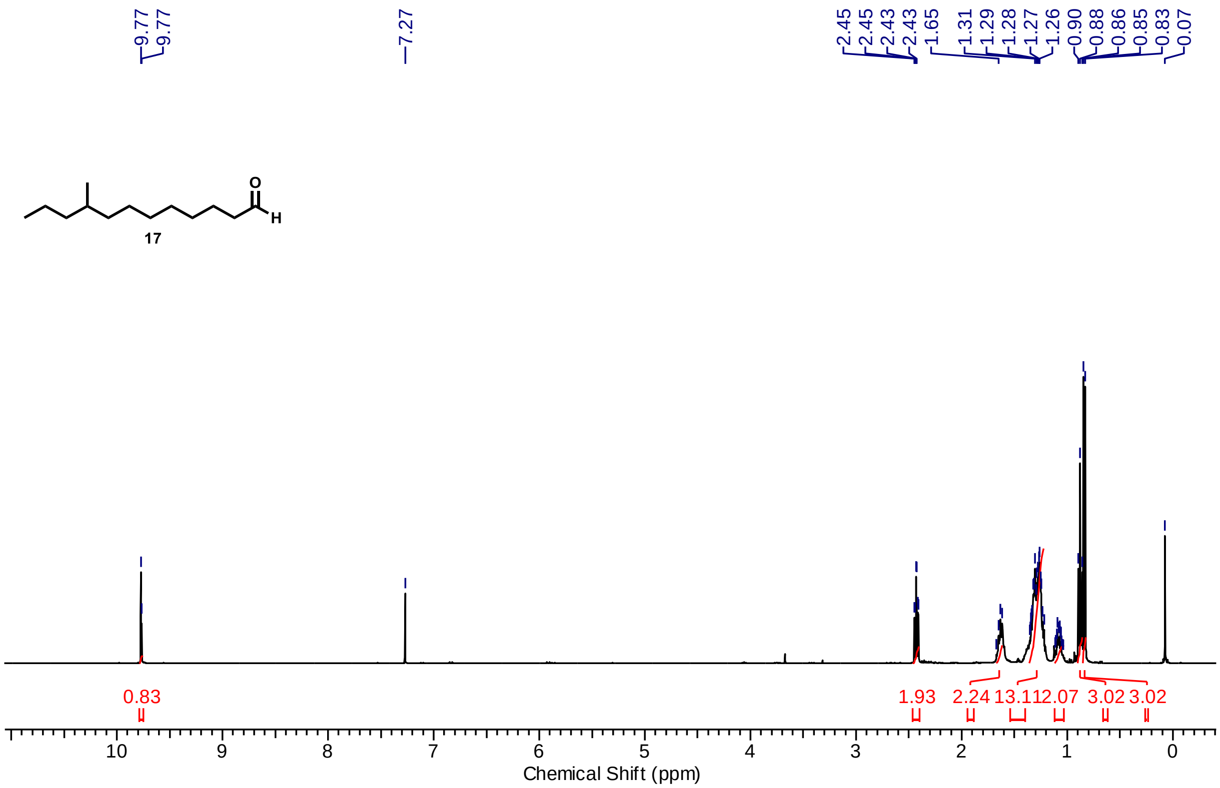


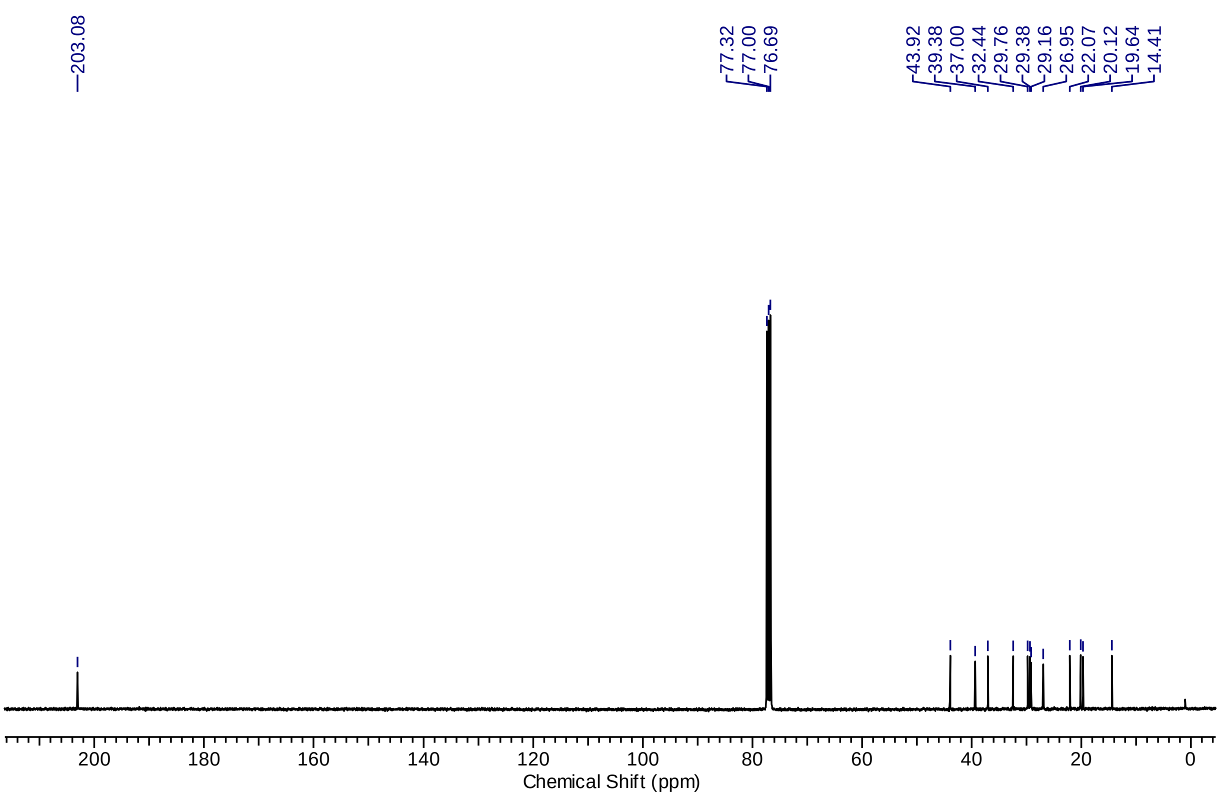
^{1}H NMR of **16** (500 MHz, $CDCl_3$)



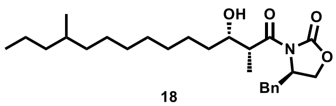
^{13}C NMR of **16** (125 MHz, $CDCl_3$)



^{1}H NMR of **17** (400 MHz, $CDCl_3$)



^{13}C NMR of **17** (100 MHz, $CDCl_3$)



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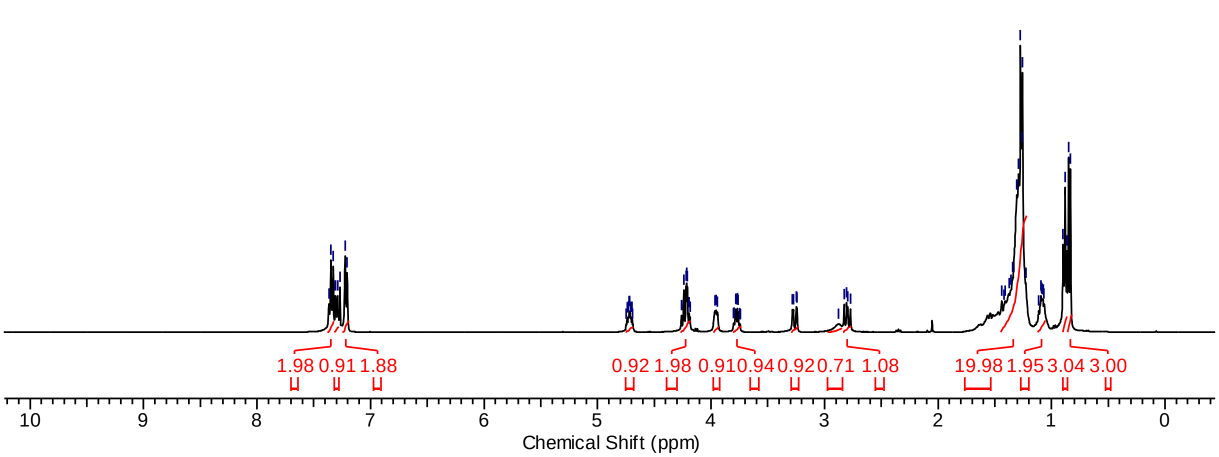
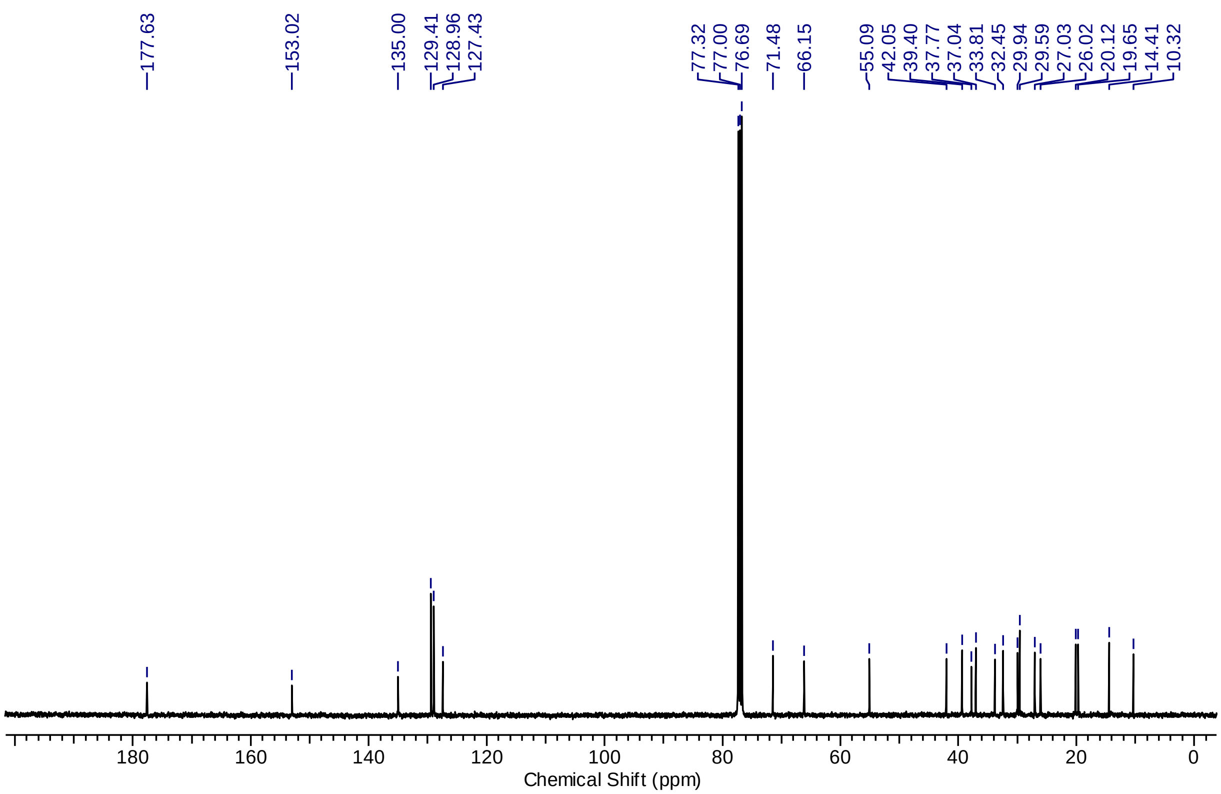
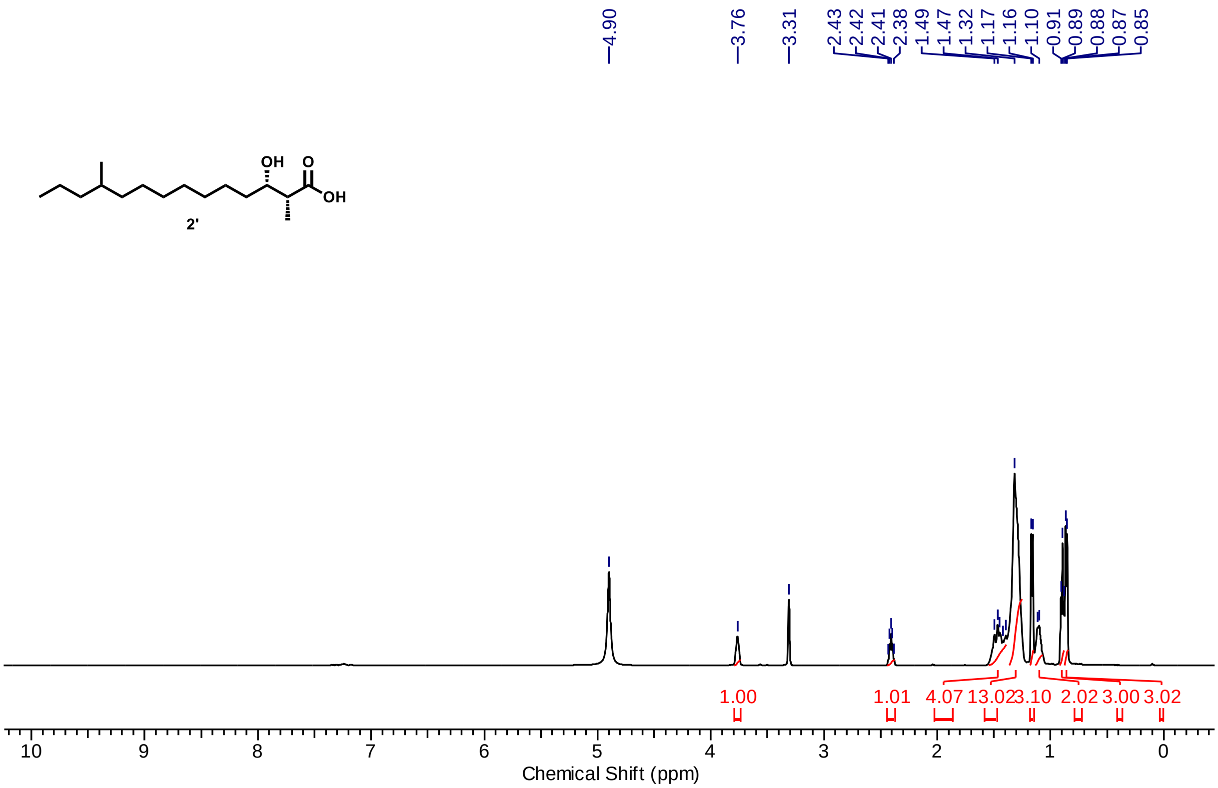


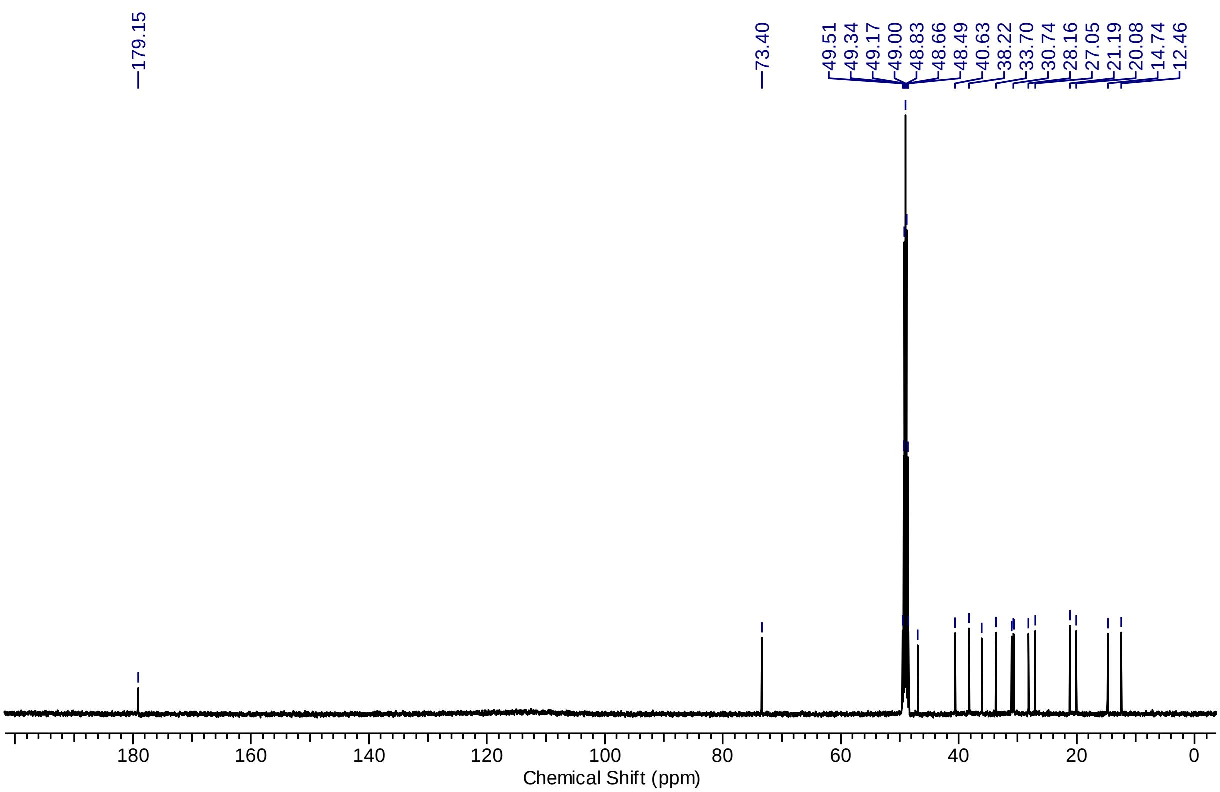
^{1}H NMR of **18** (400 MHz, $CDCl_3$)



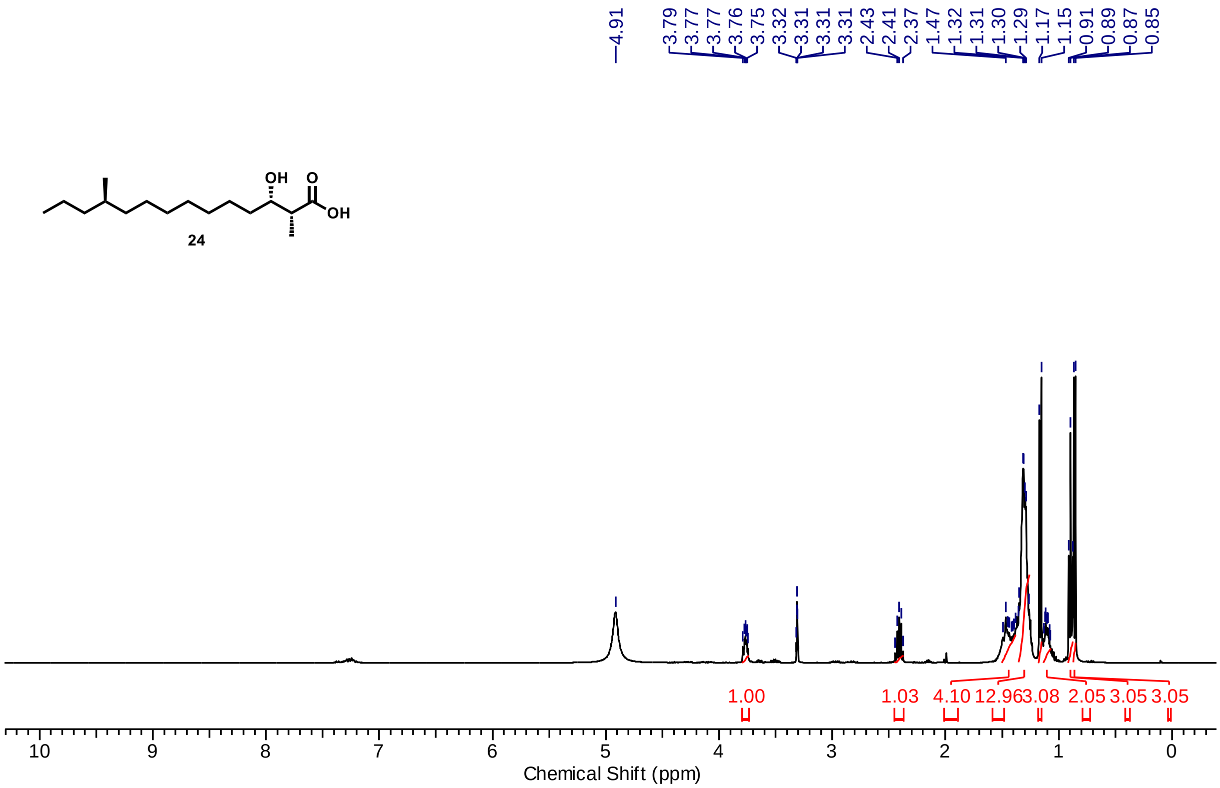
^{13}C NMR of **18** (100 MHz, $CDCl_3$)



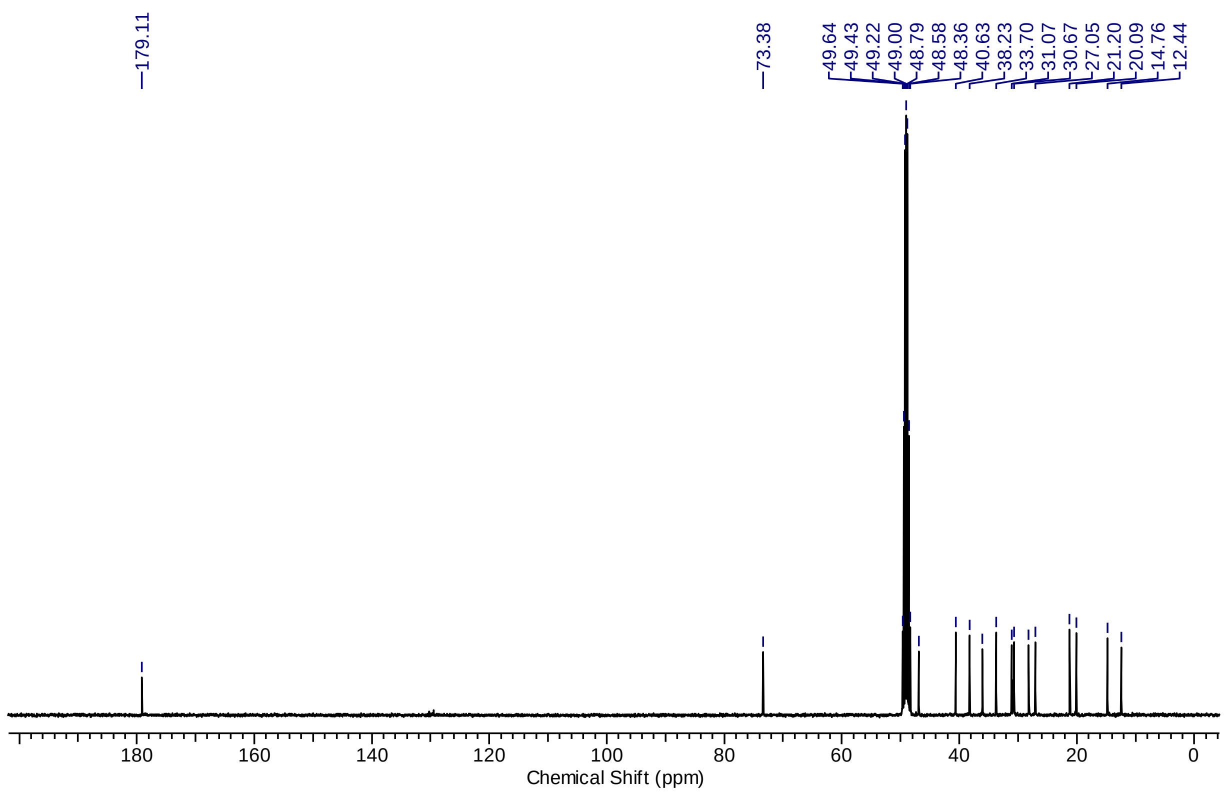
^{1}H NMR of **2'** (500 MHz, MeOH-d_4)



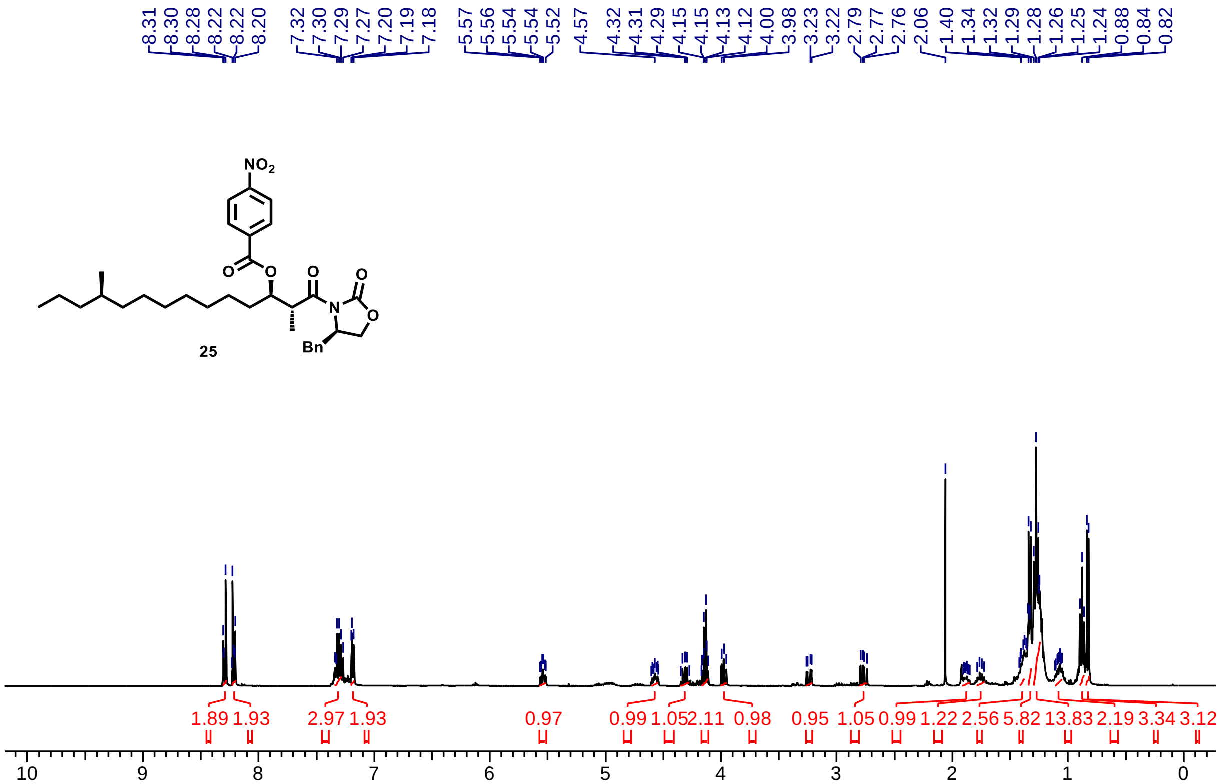
^{13}C NMR of **2'** (125 MHz, MeOH-d_4)



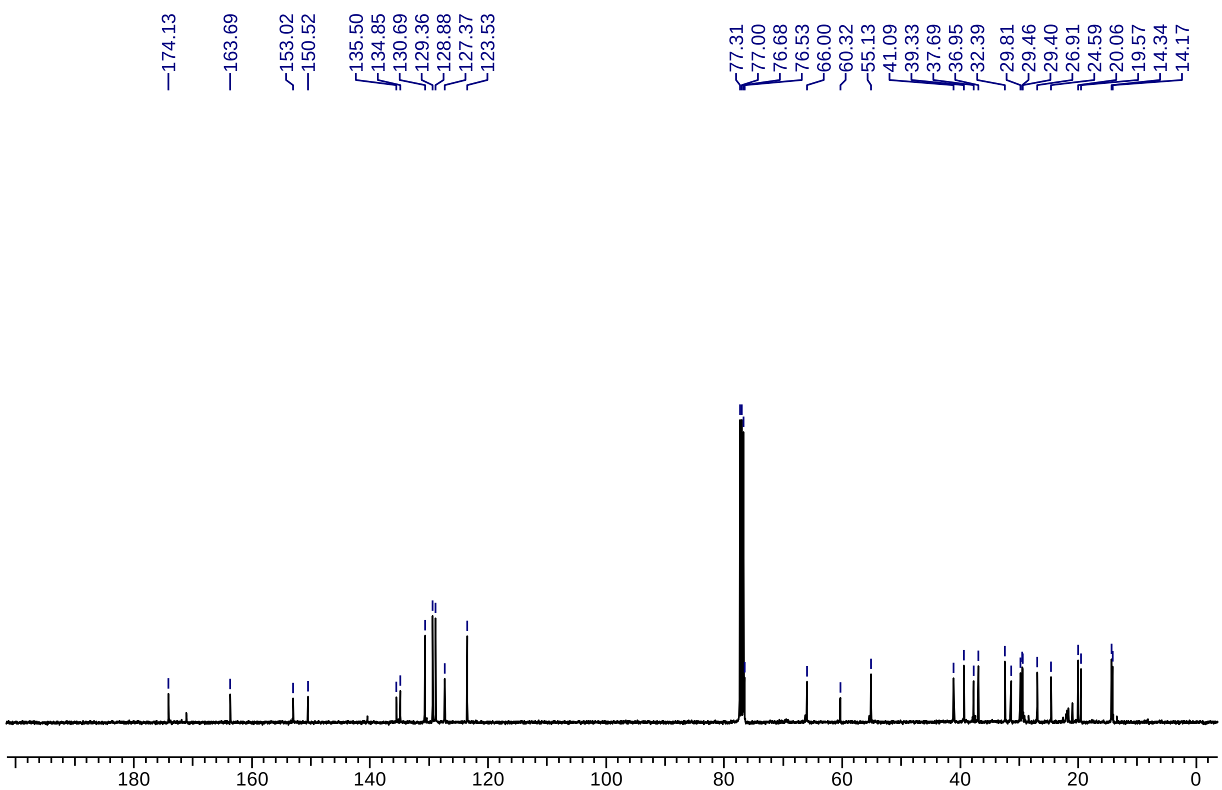
[1]H NMR of **24** (400 MHz, MeOH-d_4)



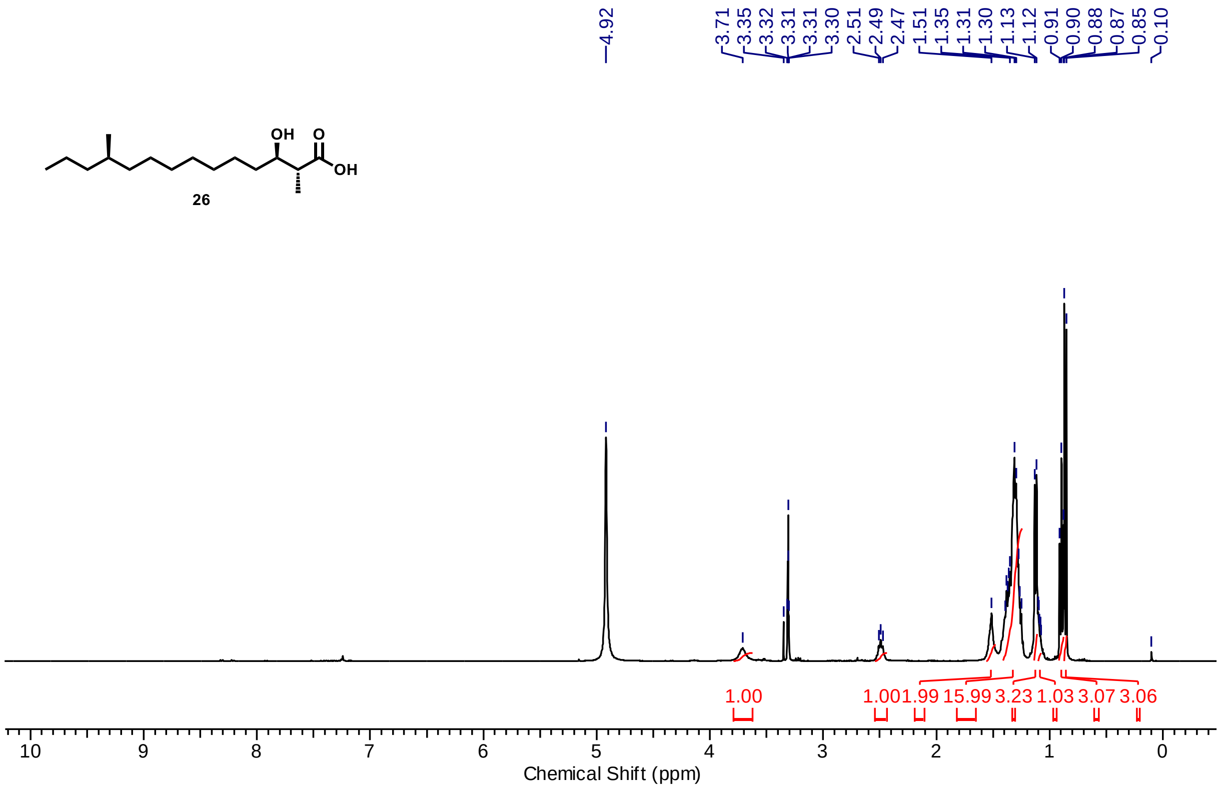
[13]C NMR of **24** (100 MHz, MeOH-d_4)



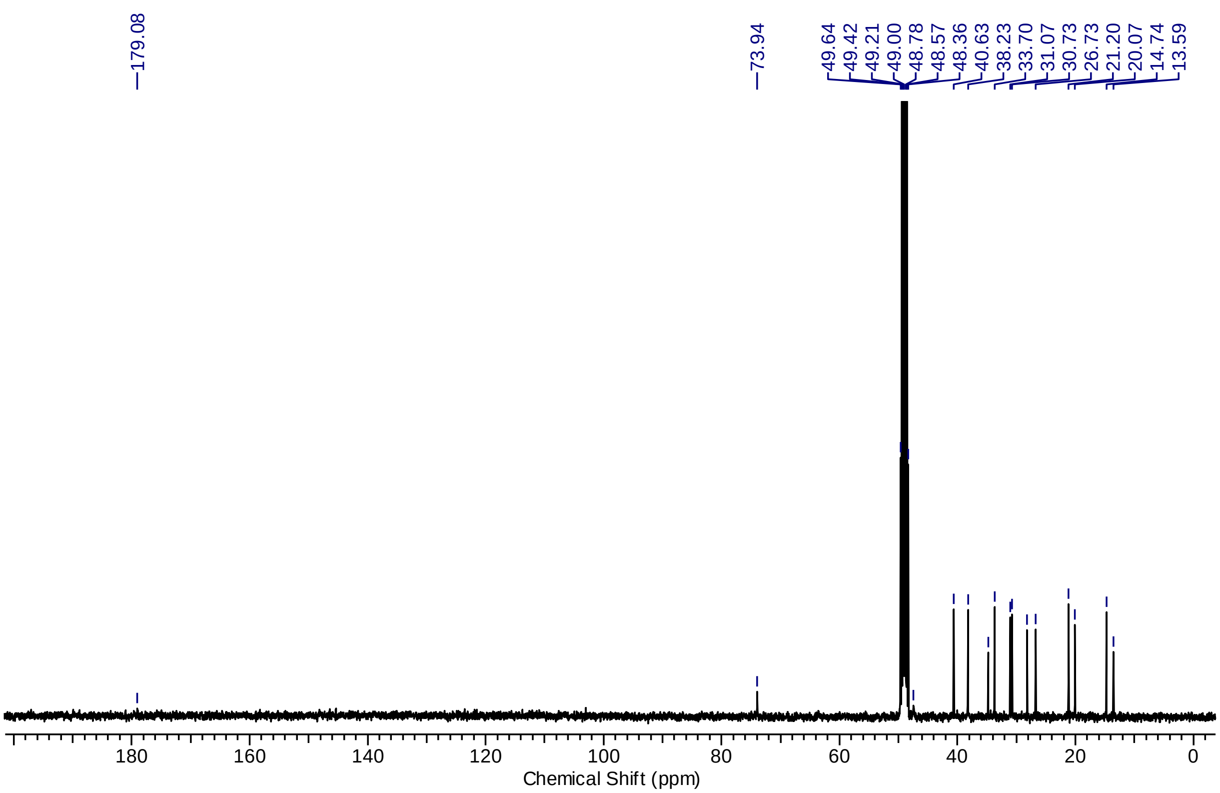
^{1}H NMR of **25** (400 MHz, CDCl$_3$)



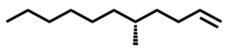
^{13}C NMR of **25** (100 MHz, CDCl$_3$)



^{1}H NMR of **26** (400 MHz, MeOH-d_4)



^{13}C NMR of **26** (100 MHz, MeOH-d_4)



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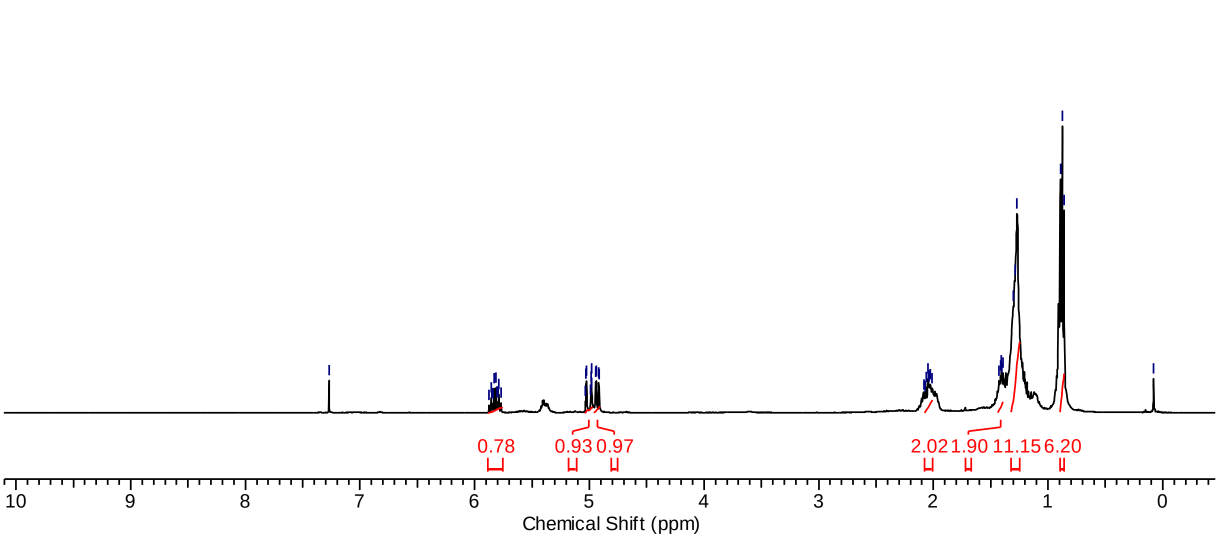
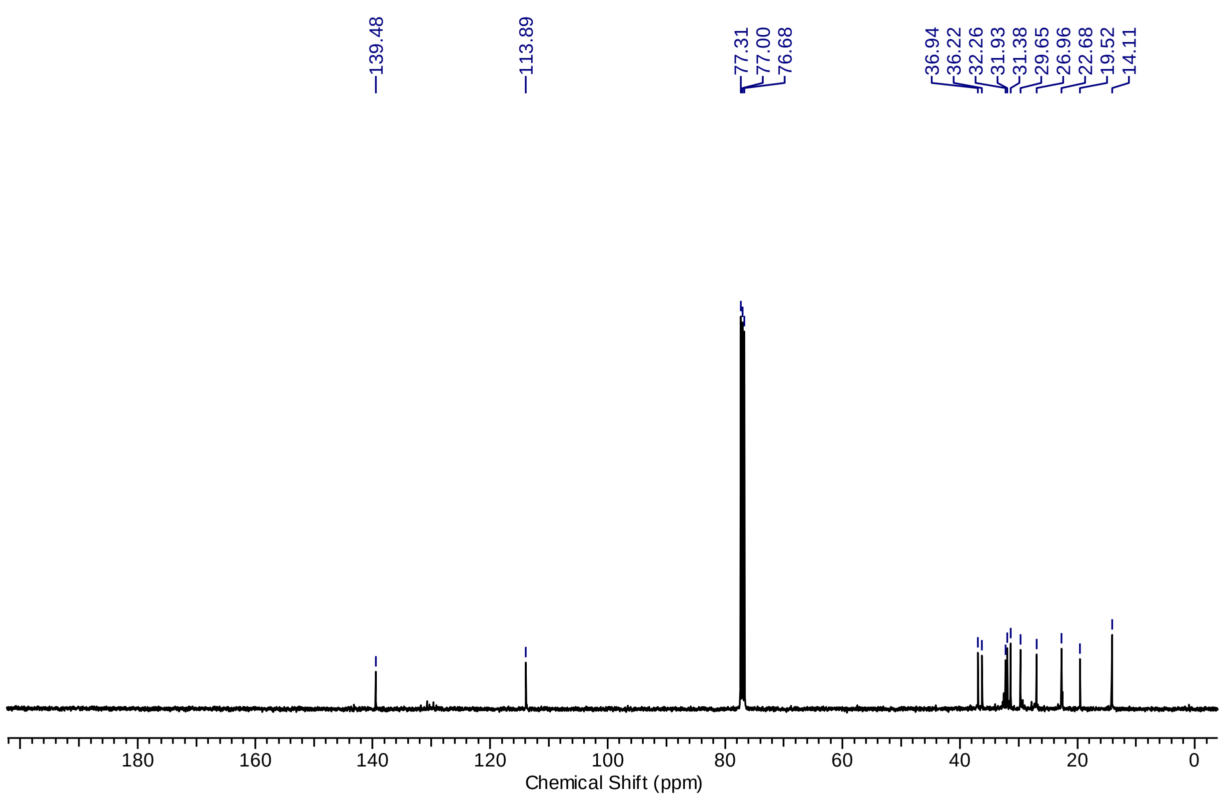
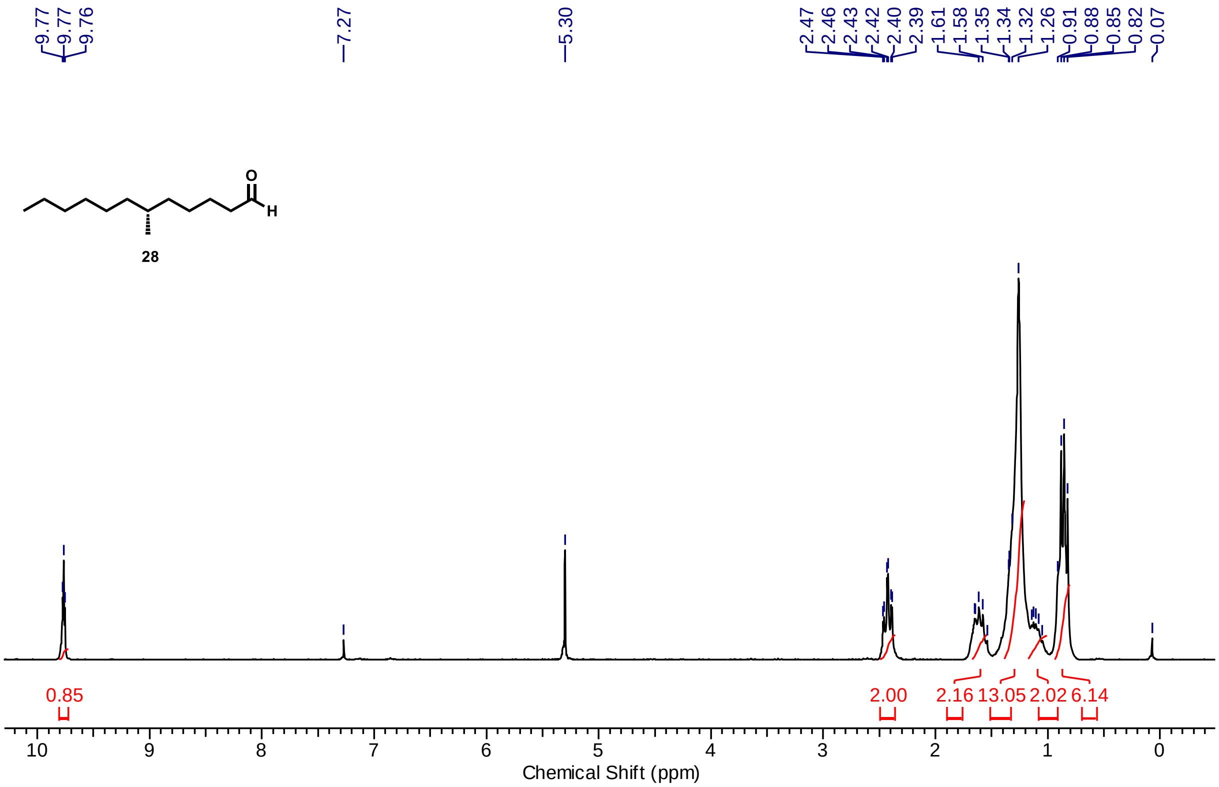


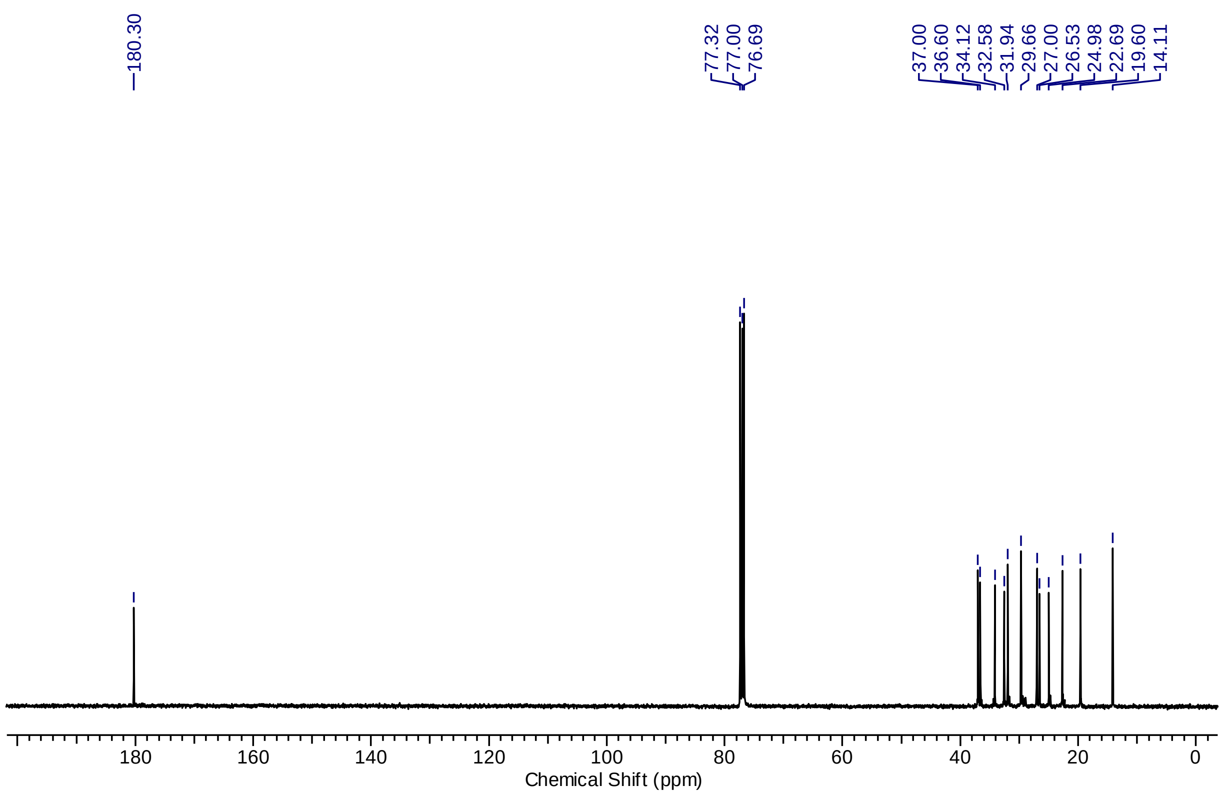
^{1}H NMR of **27** (400 MHz, $CDCl_3$)



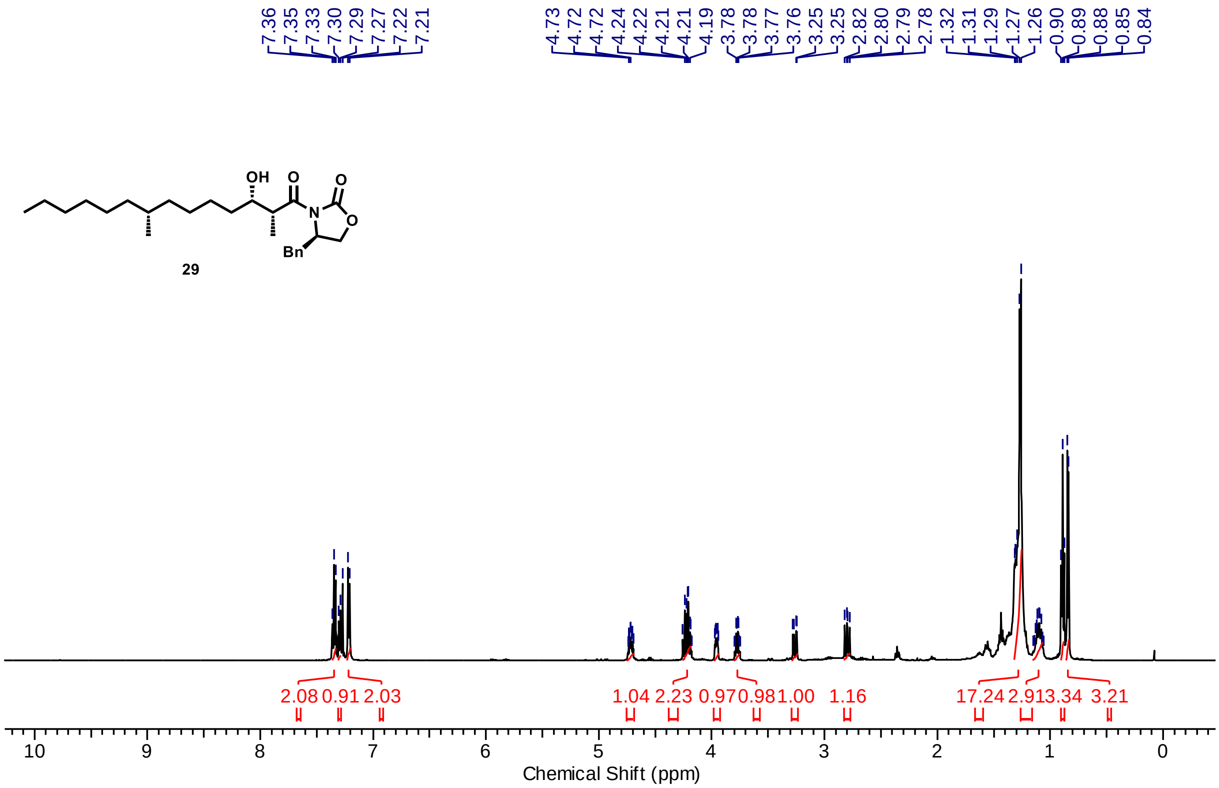
^{13}C NMR of **27** (100 MHz, $CDCl_3$)



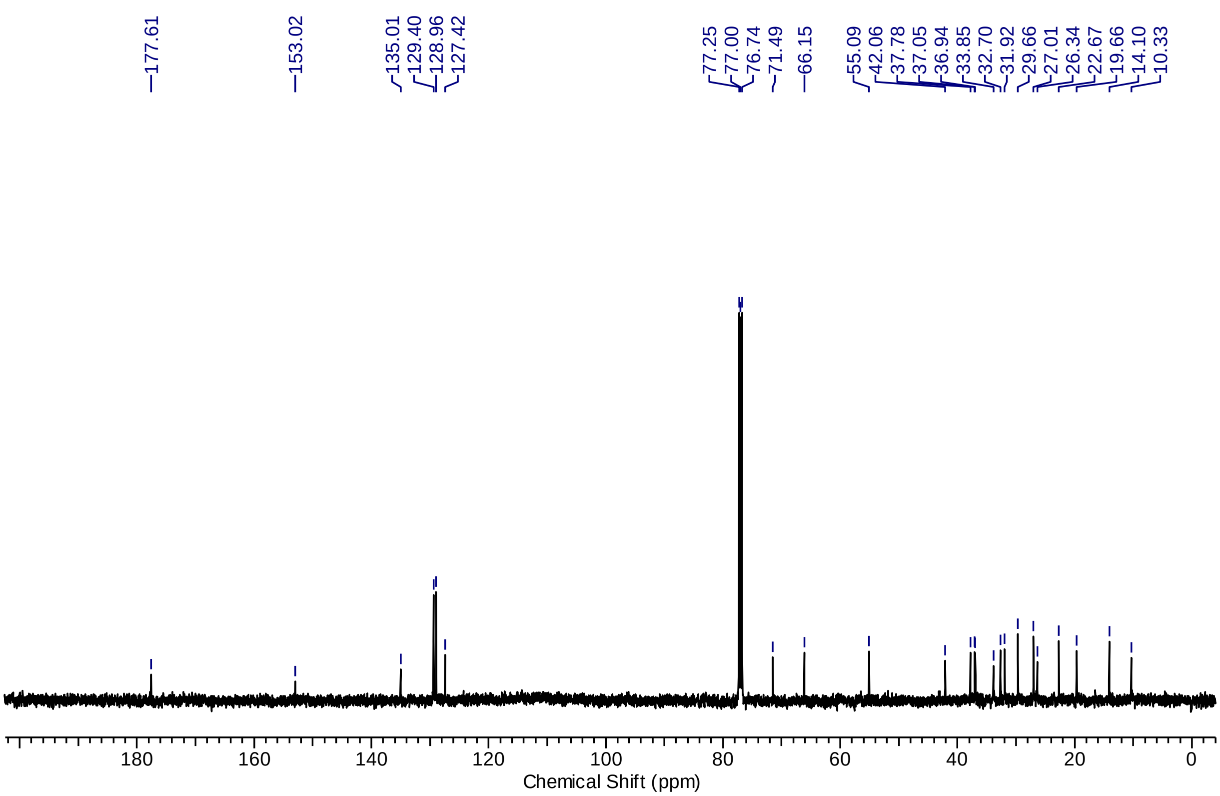
^{1}H NMR of **28** (400 MHz, $CDCl_3$)



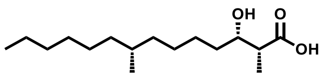
^{13}C NMR of **28** (100 MHz, $CDCl_3$)



^{1}H NMR of **29** (500 MHz, $CDCl_3$)



^{13}C NMR of **29** (125 MHz, $CDCl_3$)



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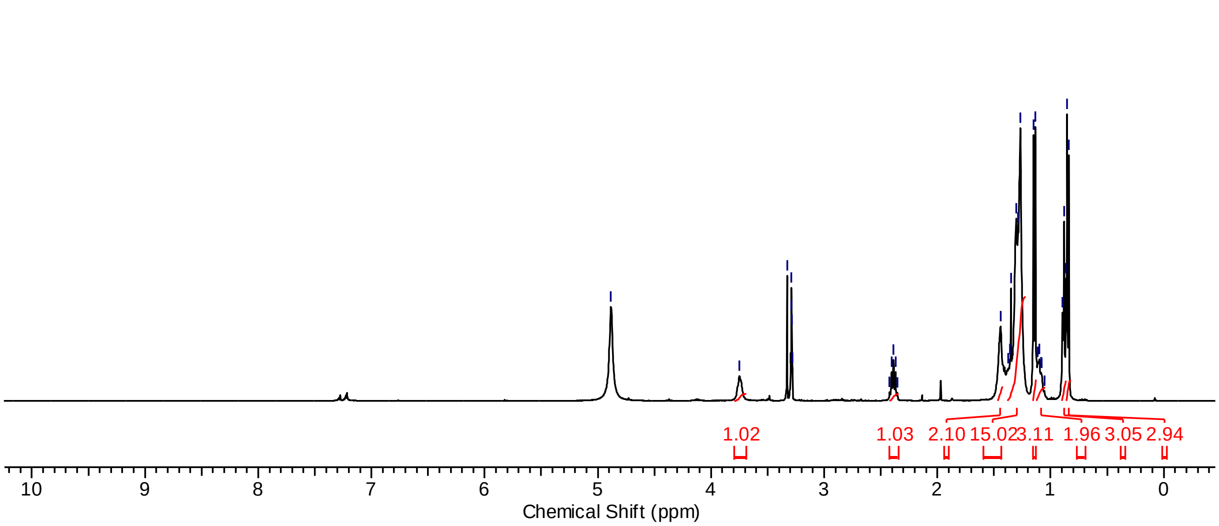
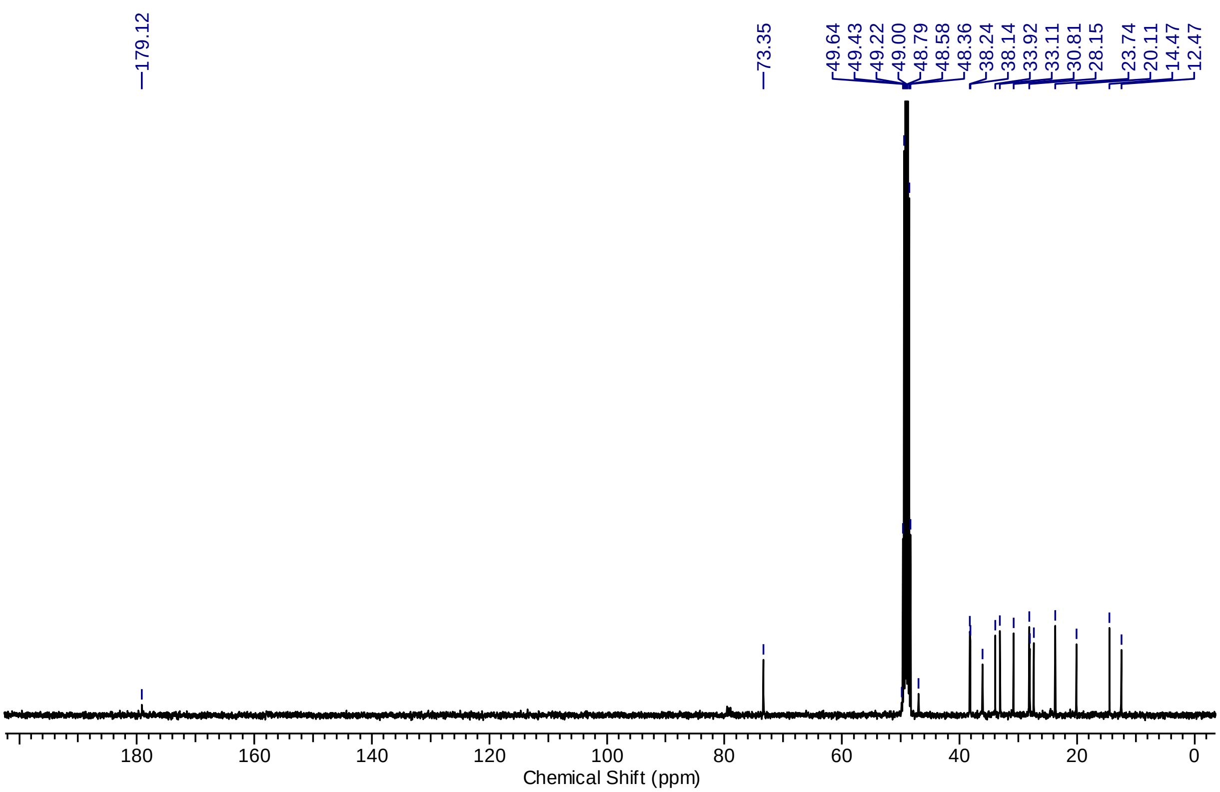
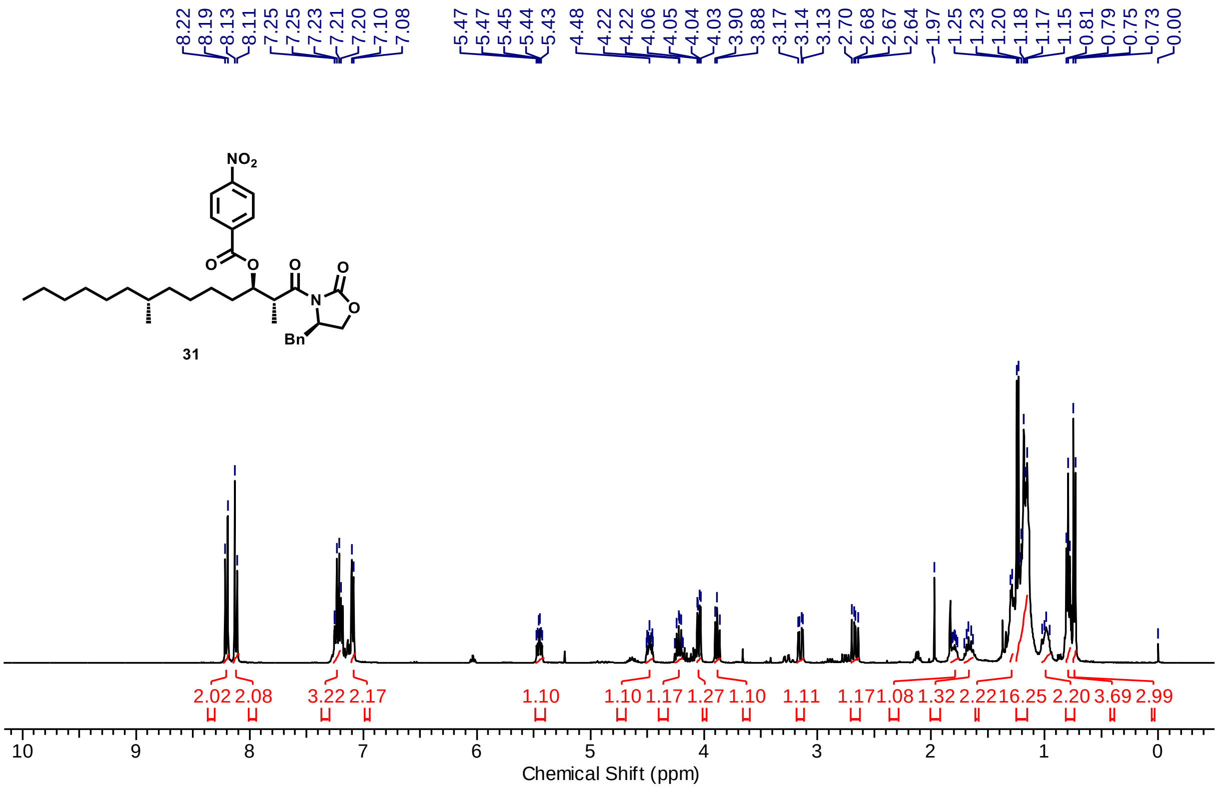


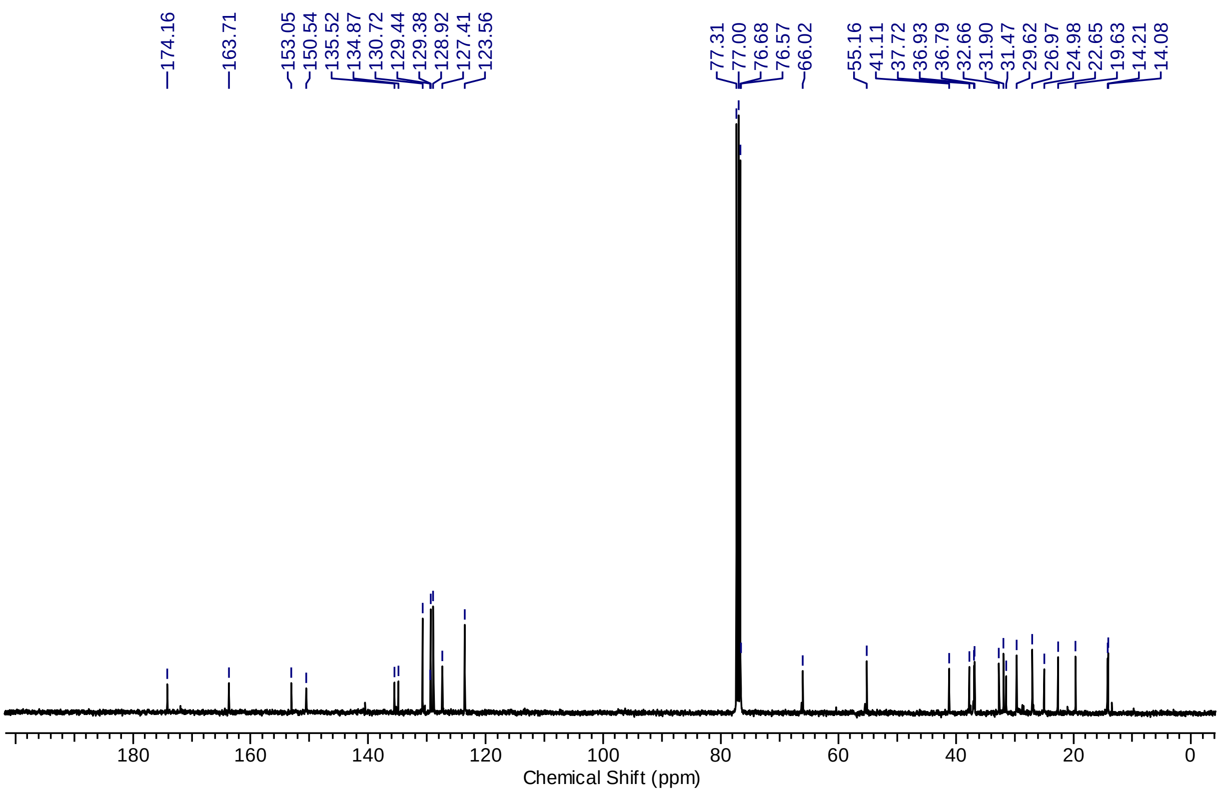
^{1}H NMR of **30** (500 MHz, MeOH-*d*$_4$)



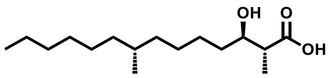
^{13}C NMR of **30** (125 MHz, MeOH-*d*$_4$)



^{1}H NMR of **31** (400 MHz, $CDCl_3$)



^{13}C NMR of **31** (100 MHz, $CDCl_3$)



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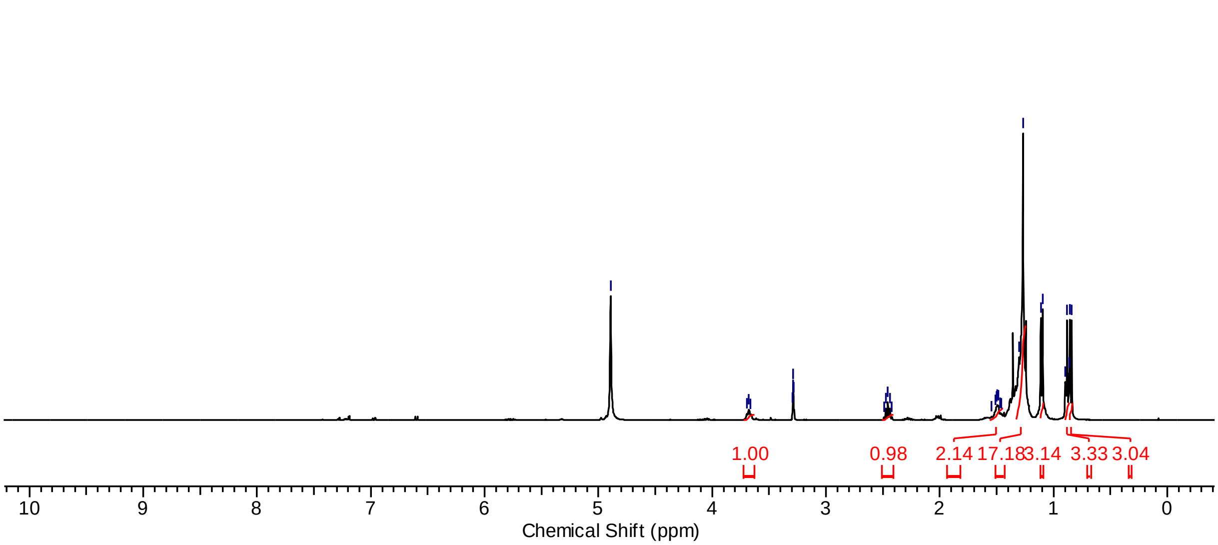
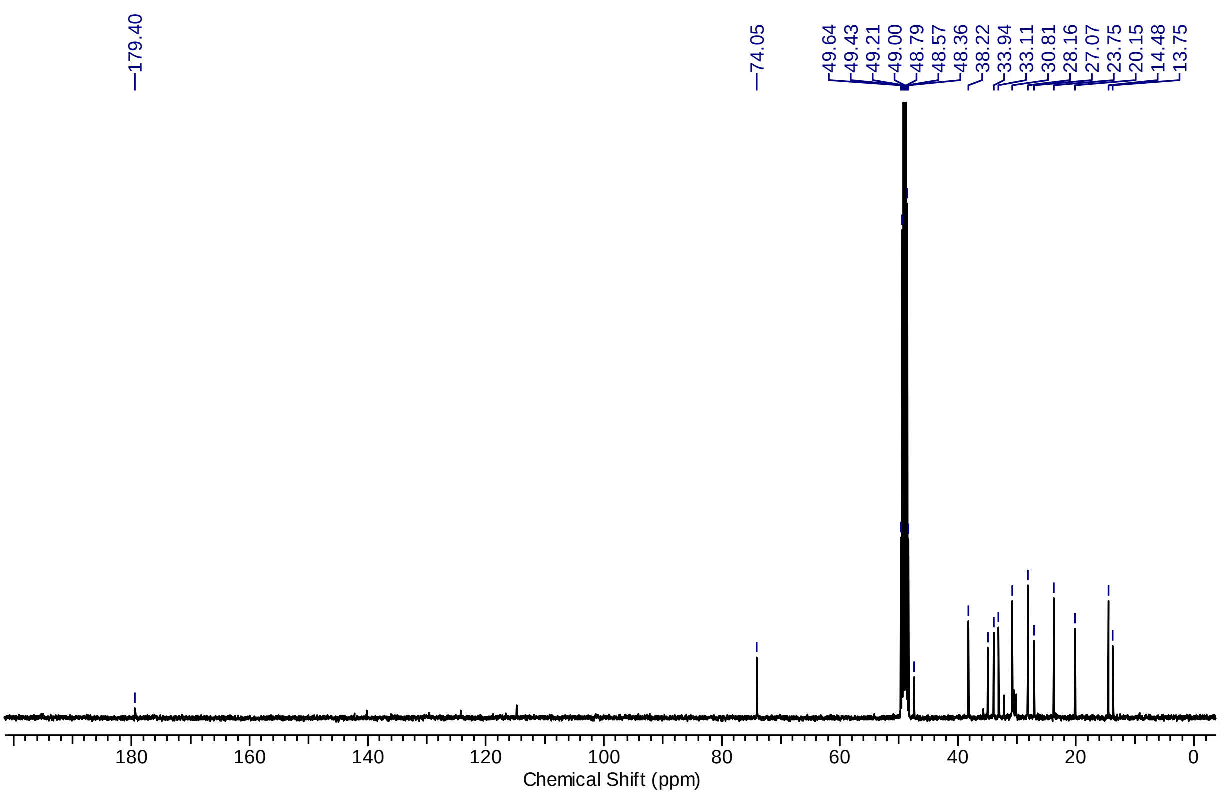


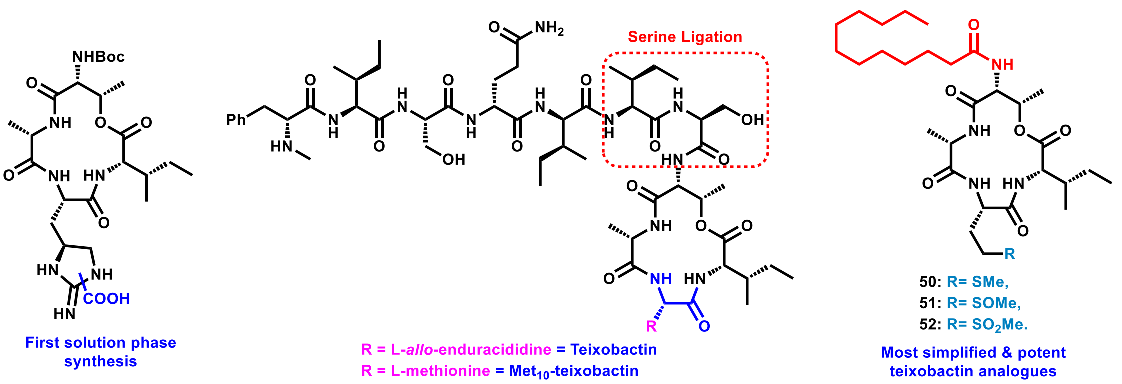
^{1}H NMR of **32** (400 MHz, MeOH-d_4)



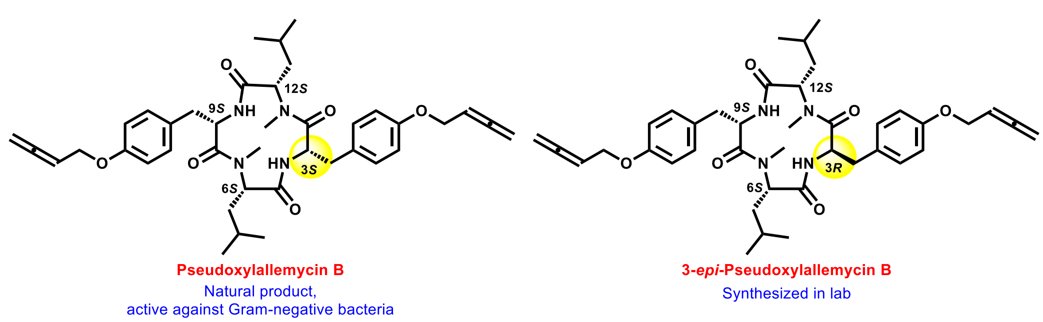
^{13}C NMR of **32** (100 MHz, MeOH-d_4)

In short, present thesis work has been divided in to three major chapters and third one further sub divided as two sections. The highlights of the present work are captured below.

- Total synthesis Met$_{10}$-teixobactin, an equipotent analogue of teixobactin, in which a rare amino acid, L-*allo*-enduracididine was replaced by readily available methionine.
- Synthesis of macrocyclic core of teixobactin for the first time.
- Prepared a library of analogues with methionine macrocycle and screened against ESKAPE pathogens, with the help of Dr. Sidharth Chopra, CSIR-CDRI, Lucknow.
- Identified a new, most simplified and potent analogue (compound **50**) of teixobactin.

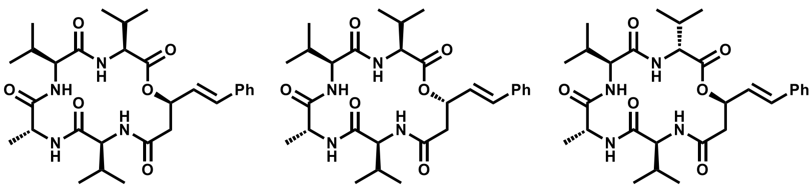


- Total synthesis of 3-*epi*-pseudoxylallemycin B (D-Tyr instead of L-Tyr).
- In an attempt towards the total synthesis of pseudoxylallemycin B, we came across an unusual observation of complete epimerization which led to the formation of 3-*epi*-pseudoxylallemycin B and the structure was disclosed by X-ray crystallography.



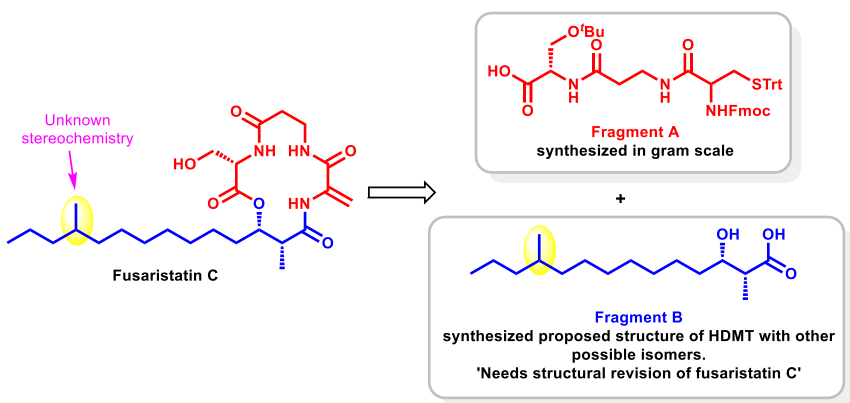
- Total synthesis of arthroamide natural product (proposed structure), 16-*epi*-arthroamide and 3-*epi*-arthroamide. Further work and analysis is needed to make conclusions on the structural assignment part, which is presently under progress in our group.

- Our route features an enzymatic kinetic resolution of Hppa fragment, HATU mediated peptide couplings and lanthanide triflate mediated Shiina macrocyclization as key steps.



Synthesized proposed structure of arthroamide — 16-*epi*-Arthroamide — 3-*epi*-Arthroamide

- In our efforts toward total synthesis of fusaristatin C, we have synthesized peptidic fragment required for the synthesis of fusaristatin C, in gram scale.
- The key non-peptidic portion, HDMT and its isomers were synthesized and characterized but in all the cases, there were clear discrepancies of selected ^{1}H and ^{13}C NMR signals with respect to HDMT fragment derived from fusaristatin C, suggesting that the structural revision of fusaristatin C, in particular, HDMT fragment is necessary.



Publications and Patents

1. **Gunjal, V. B.**; Reddy, D. S. "Total synthesis of Met_{10}-teixobactin". *Tetrahedron Lett*. **2019**, *60*, 1909-1912.
2. **Gunjal, V. B.**; Reddy, D. S. "Synthetic studies towards Pseudoxylallemycin B, an antibiotic active against Gram-negative bacteria: Total synthesis of 3-*epi*-Pseudoxylallemycin B". *Tetrahedron Lett*. **2018**, *59*, 2900-2903.
3. Dhara, S.; **Gunjal, V. B.**; Handore, K. L.; Reddy, D. S. "Solution-phase synthesis of the macrocyclic core of teixobactin". *Eur. J. Org. Chem*. **2016**, *2016*, 4289-4293. (All the authors contributed equally).
4. Philkhana, S. C.; Jachak, G. R.; **Gunjal, V. B.**; Dhage, N. M.; Bansode, A. H.; Reddy, D.S. "First synthesis of nitrosporeusines, alkaloids with multiple biological activities". *Tetrahedron Lett*. **2015**, *56*, 1252-1254.
5. Philkhana, S. C.; Jachak, G. R.; **Gunjal, V. B.**; Reddy, D. S. "Benzenecarbothioccyclopenta [c]pyrrole-1,3-dione compounds and process for synthesis thereof". WO2016051425A1.
6. **Gunjal, V. B.**; Roy, R. C.; Reddy, D. S. "Fusaristatin C needs structural revision: Synthesis of key non-peptidic fragment 3-hydroxyl-2,11-dimethyltetradecanoic acid (HDMT) and its isomers". Manuscript under revision.
7. **Gunjal, V. B.**; Chopra, S.; Reddy, D. S. "Teixobactin: A Paving Stone towards New Class of Antibiotics?" under preparation of *J. Med. Chem*. perspective (invited).