Total Synthesis of Revised Structure of Potent Anti-inflammatory Natural Product Solomonamides and Related Studies
&
Design, Synthesis, and Biological Evaluations of Silicon Incorporated Morpholine Antifungals

Thesis Submitted to AcSIR

For the Award of the Degree of

DOCTOR OF PHILOSOPHY

In

CHEMICAL SCIENCES

By

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Under the guidance of
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January 2019

Dedicated

To My

Parents

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This is to certify that the work incorporated in this Ph.D. thesis entitled "**Total Synthesis of Revised Structure of Potent Anti-inflammatory Natural Product Solomonamides and Related Studies and Design, Synthesis, and Biological Evaluations of Silicon Incorporated Morpholine Antifungals**" submitted by **Mr. Gorakhnath Rajaram Jachak** 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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Declaration by the Candidate

I hereby declare that the original research work embodied in this thesis entitled, **"Total Synthesis of Revised Structure of Potent Anti-inflammatory Natural Product Solomonamides and Related Studies and Design, Synthesis, and Biological Evaluations of Silicon Incorporated Morpholine Antifungals"** 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 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.

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Finally, I bow my head in front of God, the almighty for giving me the strength to overcome all the difficulties in life. I am thankful to him for everything in my life, the good and the bad. Some were blessings and some were lessons.

Gorakh

Abbreviations

AcOH	acetic acid
AcCl	acetyl chloride
Ac_2O	acetic anhydride
Å	angstrom
Ar	aryl
MeCN	acetonitrile
Bn	benzyl
Boc	*tertiary*-butyloxycarbonyl
D_2O	deuterium oxide
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
DMAP	4-dimethyl aminopyridine
DMF	*N*,*N*-dimethyl formamide
DMSO	dimethyl sulfoxide
DMSO-d_6	deuterated dimethyl sulfoxide
dd	doublet of doublet
d	doublet (in NMR)
Et	ethyl
EtOAc	ethyl acetate
EtOH	ethanol
equiv	equivalent
CD_3OD	deuterated methanol

Abbreviations

g	gram(s)
h	hour(s)
Hz	hertz
IR	infrared
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
N	normality
nM	nanomolar(s)
NMR	nuclear magnetic resonance
Ph	phenyl
ppm	parts per million
Pr	propyl
q	quartet
R_f	retention factor
rt	room temperature
s	singlet

Abbreviations

TMS	trimethyl silane
$CDCl_3$	deuterated chloroform
t	triplet
tert	tertiary
TBHP	*tert*-Butyl hydroperoxide
TEA	triethyl amine
THF	tetrahydrofuran
TFA	trifluroacetic acid
TFAA	trifluroacetic anhydride
TLC	thin layer chromatography
UV	ultraviolet
i.p.	intra peritonial
wt/v	weight by volume
^oC	degree celsius
µM	micromolar
mg	milligram
µmol	micromolar
in vitro	outside a living organism
in vivo	inside a living organism
RCM	ring closing metathesis
IL	Interleukin
TNF	Tumor Necrosis Factor

General Remarks

- All reagents and starting materials were obtained from commercial suppliers and used as such without further purification however, solvents are purchased and further dried using MBRAUN (MB-SPS 800).
- 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). 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) or neutral alumina.
- All the melting points are uncorrected and were 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 spectra were obtained using a 200 MHz, 400 MHz or 500 MHz spectrometer. Coupling constants were measured in Hertz. 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 analyzer (Q Exactive).
- Infrared (IR) spectra were recorded on a FT-IR spectrometer as thin films 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.

Synopsis

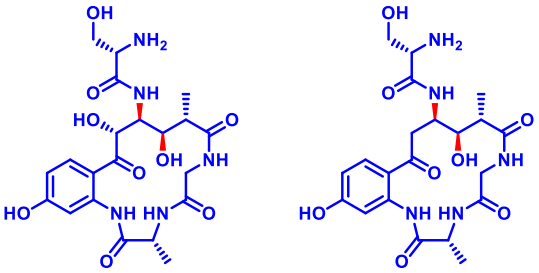
Synopsis of the Thesis to be submitted to the Academy of Scientific and Innovative Research for Award of the Degree of Doctor of Philosophy in Chemistry	
Name of the Candidate	Mr. Gorakhnath R. Jachak
Degree Enrolment No. & Date	Ph. D in Chemical Sciences (10CC13A26013); August 2013
Title of the Thesis	Total Synthesis of Revised Structure of Potent Anti-inflammatory Natural Product Solomonamides and Related Studies & Design, Synthesis, and Biological Evaluations of Silicon Incorporated Morpholine Antifungals.
Research Supervisor	Dr. D. Srinivasa Reddy

The thesis is divided into two major chapters. Initial part of **Chapter 1** introduces to role of macrocyclic peptides in drug discovery and sets the stage for solomonamide natural products. Then describes the total synthesis of revised structure of solomonamide A and solomonamide B. The later part deals with synthesis of solomonamide analogues, their biological evaluation to measure their anti-inflammatory potential. **Chapter 2** deals with the role of silicon incorporation in drugs and agrochemicals which are described in the first part. Second part includes design, synthesis and biological screening of silicon incorporated morpholines towards antifungals.

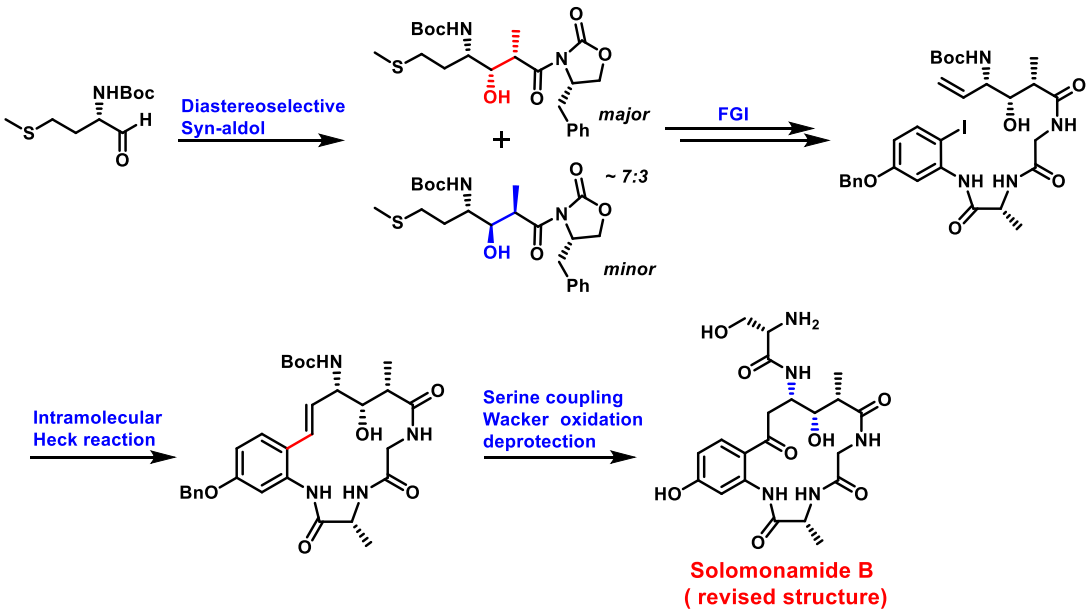
Chapter I: Total Synthesis of Revised Structure of Potent Anti-inflammatory Natural Product Solomonamides and its Related Studies

In recent times, macrocyclic peptides are considered to be an interesting class of compounds in the field of drug discovery. Solomonamides A and B belong to same class with interesting structures were isolated from marine sponge *Theonella swinhoei*, by Zampella's group.[1]On being tested in anti-inflammatory assay, solomonamide A showed ~60% paw edema reduction in mouse model at very low dose(100 µg/kg IP route) whereas, solomonamide B could not be tested due to paucity of the material. Owing to the interesting structural features, promising biological activity and, scarcity of natural products for further evaluation, we have initiated a program towards total synthesis of the natural products followed by medicinal chemistry. Henceforth throughout the timeline our group has achieved the first total synthesis of proposed structure of solomonamide B previous work from our group).[2] However, spectral data was not matching with that of natural product, which necessitated structural revision. As an onset to this challenge of eight

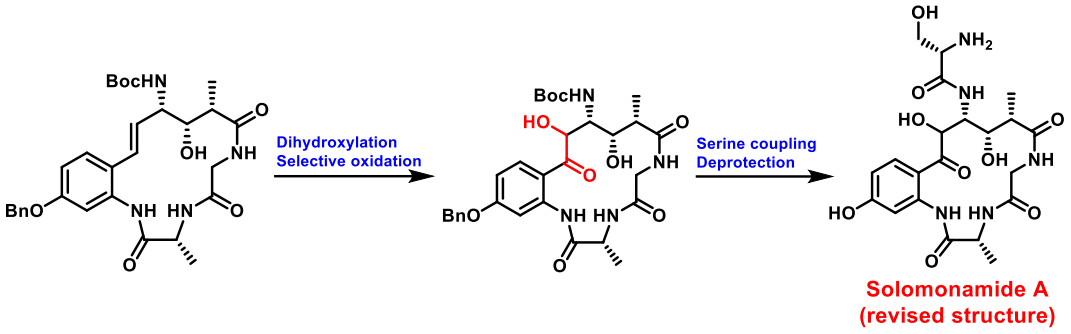
possible stereoisomers, we prioritized a few of them and ultimately, a stereoisomer having 2*S*, 3*S*, 4*S* configuration on non-peptide part has been prioritized and synthesized, which turned out be the configuration present in the solomonamide B natural product. The synthetic route followed to access the natural product with revised structure is shown below. The diastereoselective syn-aldol, intramolecular Heck macrocyclization, regioseletive Wacker oxidationas the key steps used to afford the target compound with the required stereochemistry (2*S*, 3*S*, 4*S*). All the spectral data of this synthesized stereoisomer of solomonamide B found to be in complete agreement with the reported spectral data of natural solomonamide B. Thus, we have accomplished solomonamide B for the first time.



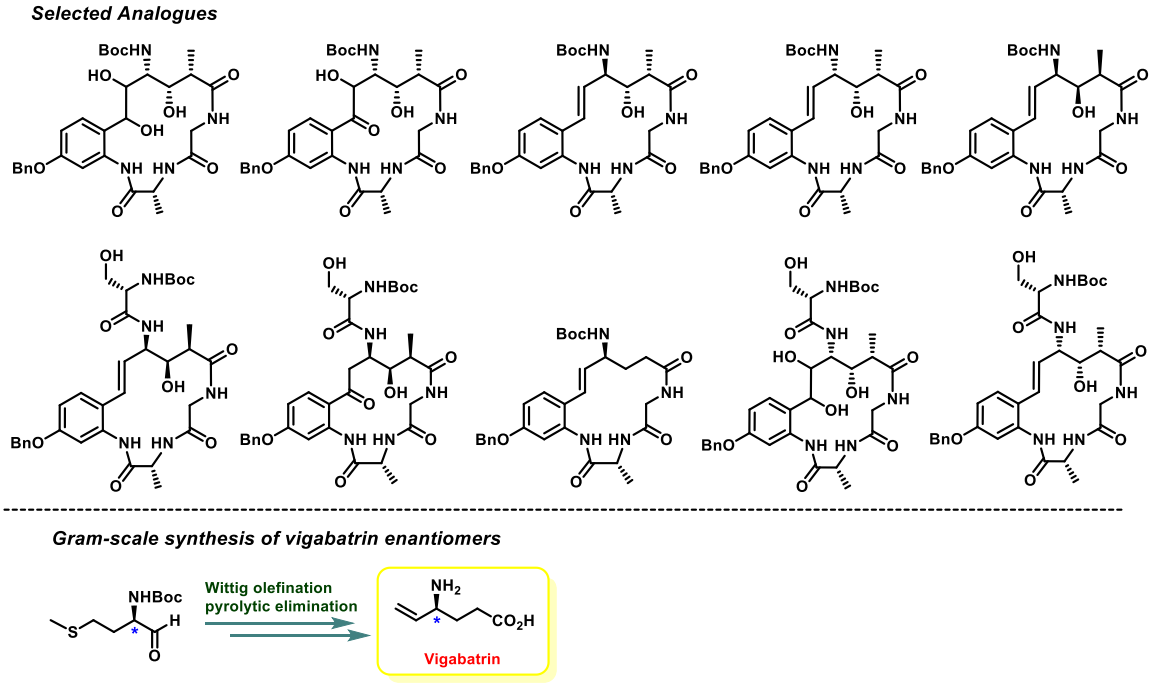
Total synthesis of solomonamide B (revised structure)



Total synthesis of solomonamideA (revised structure)



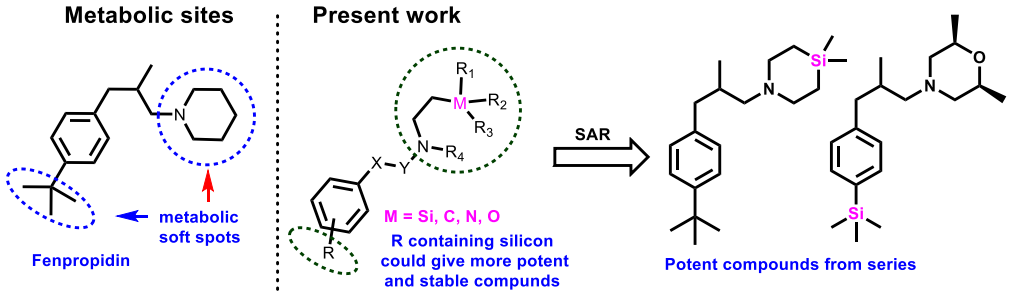
It was proposed in the literature that solomonamide A was biogenetically derived from solomonamide B, which makes the structural revision of solomonamide A inevitable. Accordingly, the three chiral centres (2*S*, 3*S*, 4*S*) present in Solomonamide B expected to be present in Solomonamide A, thus leaving one chiral centre (C5-hydroxy group) in AHMOA fragment, whose absolute stereochemistry needs to be fixed. Accordingly, we have synthesized the solomonamide A with the revised structure by using one of the macrocyclic intermediate employed in synthesis of solomonamide B. All the spectral data were in agreement along with biological evaluation of revised solomonamide A in an anti-inflammatory mouse model further substantiates of its structural revision.



Following successful total synthesis of both the natural products, we planned to synthesize a library of analogs around the scaffold. Accordingly, ~30 analogues were synthesized using the synthetic protocols developed during the total synthesis. Among them, a few selected compounds were profiled using animal models (in collaboration with Dr. Manoj Kumar Barthwal, CSIR-CDRI). Results indicate that synthesized solomonamides also show anti-inflammatory potential in similar lines that of natural products. In addition, we found a more potent macrocyclic analogue with the simplified structure. During the course of analogue synthesis, we have also developed a method to access the anti-epileptic drug vigabatrin with in a gram scale.[3]

Chapter II: Design, Synthesis, and Biological Evaluation of Silicon Incorporated Morpholine Antifungals

The introduction of bioisostere is a key strategy used by medicinal chemists during lead optimization process in order to improve the desired biological and physico-chemical properties of a potential compound without altering its structure significantly. Along these lines, our research group made significant contribution by using the concept of "silicon incorporation" into drug scaffolds. With this background, we have designed and synthesized modified analogues of marketed antiungals amorolfine, fenpropidine and fenpropimorph by incorporating silicon to increase desired properties, in particular their metabolic stability. Synthesized compounds, showed potent antifungal activity against different fungal pathogens, similar to morpholines, by targeting sterol reductase and sterol isomerase of ergosterol biosynthetic pathway.[4] The microsomal stability of selected compound was evaluated in mouse as well as human liver microsomes, which reflected



the incorporation of silicon in the piperidine ring to be beneficial toward improving the metabolic stability. To our delight, one of the synthesized compounds showed better antifungal activity than fenpropidin and amorolfine against different human pathogens and was identified as a potent lead for further profiling.

Noteworthy Findings

a) Accomplished the first total synthesis of revised structure of solomonamide A and solomonamide B.
b) Synthesized several macrocyclic analogues of solomonamides, one being structurally simple and more potent than the natural product in anti-inflammatory assay.
c) Developed a synthetic route to access an epileptic drug vigabatrin in a gram scale in both enantiomeric forms.
d) Designed and synthesized several analogues of marketed antifungals through silicon incorporation; one of which showed superior antifungal activity than the marketed congeners.

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Chapter 1

Total Synthesis of Revised Structure of Potent Anti-inflammatory Natural Product Solomonamides and Related Studies

Section I Structural revision of solomonamide A and B

1.1. Introduction

Natural products have enormous structural, chemical diversity and three-dimensional arrangements, which are inimitable to any synthetic small molecules and thus continue to encourage novel discoveries in chemistry, biology, and medicines.[1] Natural products are the origin of significant portion of the active ingredients of currently available medicines for treatment of various diseases and illnesses of humans and animals. Because of diversified structures with specific conformational patterns of natural products they interact with particular targets in a specific manner within the biological systems.

1.1.1. Macrocyclic peptides in drug discovery

Among the natural products, the macrocycles occupy a special place as they have diverse features which make them fascinating, for example, to tackle "difficult" targets with extended binding sites.[2,3] Literature report suggests that cyclization has a distinctive influence on other necessary parameters required for druggable candidates, such as permeability, metabolic stability, and overall pharmacokinetics.[4] The macrocycles are not rigid, instead they have a balance between preorganization and desired flexibility that could induce interactions with protein targets which are in dynamic in nature. Thus, macrocycles are presently one of the important class of compounds in the domain of medicinal chemistry as well as drug discovery. In this context, peptides happens to be a prominent class of naturally occurring mediators for biological processes that have gained greater attention as therapeutics during the past few years.[3b,5] From this class of compound around 60 peptide based drugs reached the market and several hundreds of novel peptides are in clinical trials.[3b,5] The key behind the success of the peptide drug is that they are very potent and target specific with the minimal side effects and having novel mode of action. For the present thesis perspective, the discussion is limited to macrocyclic peptides. There are a few marketed drugs derived from macrocyclic class of peptides which includes cyclosporin A (**1**), pasireotide (**2**), daptomycin (**3**) and clinical candidates like plitidepsin (**4**), SCY-635, voclosporin, linopristin (Figure 1.1.).[6-8] Our group has continued interest in macrocyclic natural products where our focus is on synthesis of natural products as well as

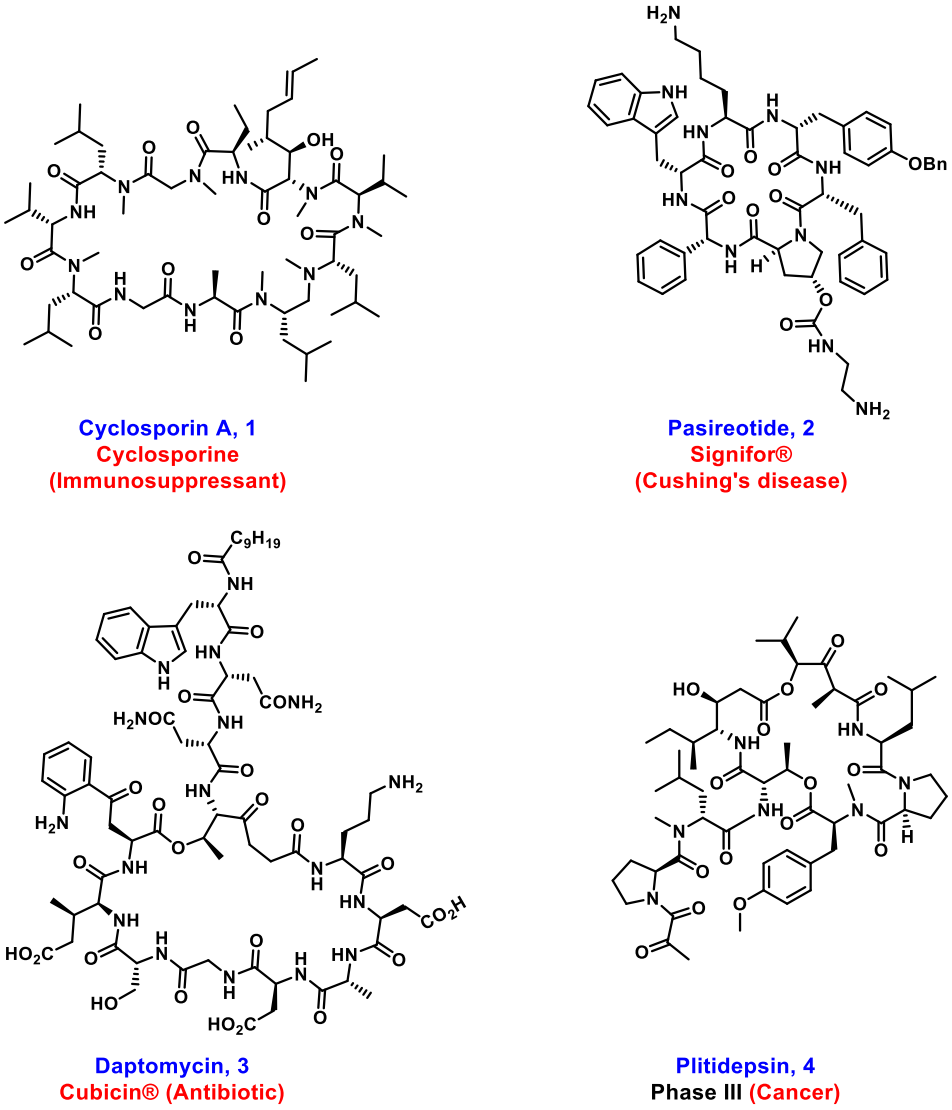
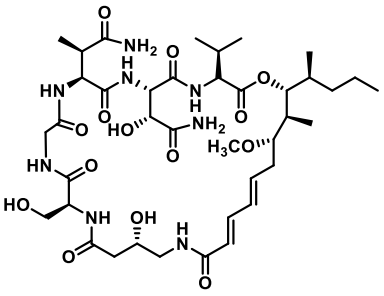


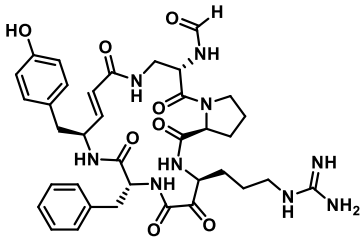
Figure 1.1. Structures of selected marketed drugs/clinical candidates based on macrocyclic peptides

their analogues towards identifying lead molecules for various diseases. Thus, we have been working on macrocyclic targets such as gliomasolides (anti-cancer),[9] palmyrolide (sodium channel blocker),[10] teixobactin (antibiotic),[11] and cyanolide A (antiparasitic).[12] Along these lines, we planned and performed our studies on natural products solomonamides for the last seven years.

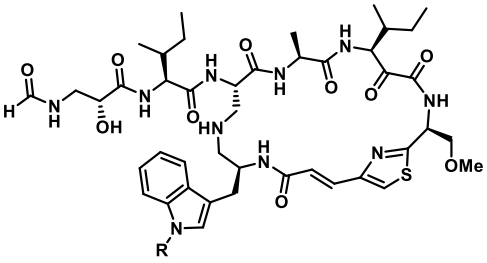
1.1.1.2. Natural products isolated from *Theonella* species



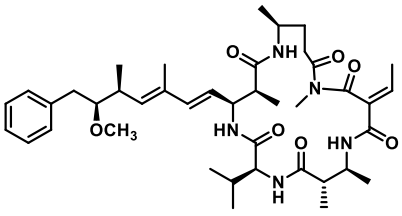
Nagahamide A, 5



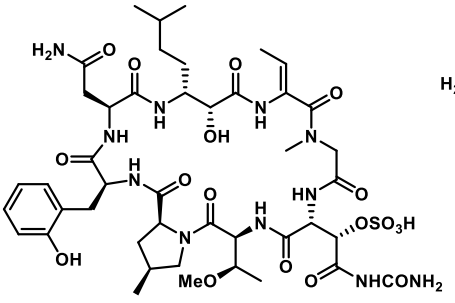
Cyclotheonamide A, 6



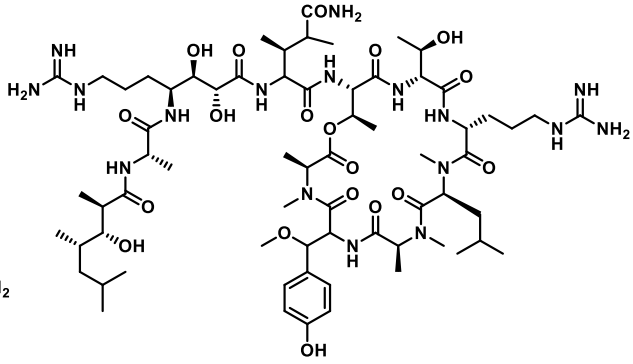
R = Me (Keramamide K), 7
R = H (Keramamide J), 8



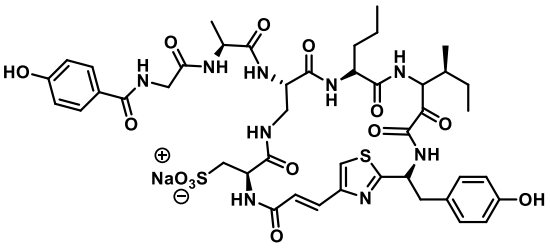
Isomotuporin, 9



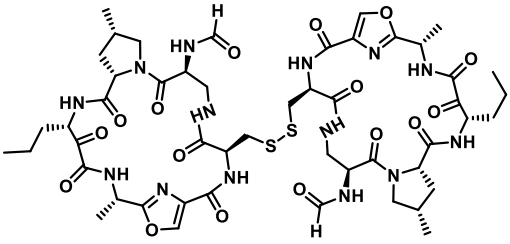
Perthamide C, 10



Callipeltin A, 11



Cyclotheonellazole A, 12



Nazumazole A, 13

Figure 1.2. Selected macrocyclic peptides isolated from *Theonella sp.*

Among the widespread sources of natural products, sponges are one of the richest and ancient source of natural products and have provided potential lead molecules for the development of new drugs. *Theonellidae* family of sponges have been recognized as a rich source of structurally novel secondary metabolites. More than 100 metabolites have been isolated till date from the family which includes polypeptides, macrolides, polyene derivatives and uncommon steroids with potent and varied biological activities. *Theonella swinhoei* in particular has yielded a number of unusual peptides with unique structures (Figure 1.2.) including cyclolithistide A,[13] theopalauamide,[14] papuamides,[15] polytheonamides, cyclotheonamide A (**6**), keramamide K (**7**) and keramamide J (**8**),[17] nazumazole A (**13**),[16] callipeltin A (**11**), theonegramide, cyclotheonellazole A (**12**), isomotuporin.

1.1.2. Isolation details of solomonamides

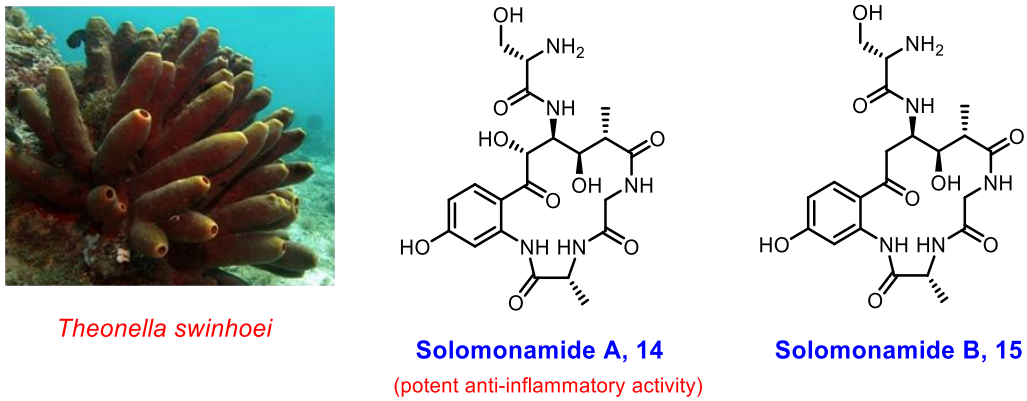


Figure 1.3. Natural products solomonamide A and B

Solomonamide A and B happens to be two of the most potent anti-inflammatory natural products isolated from *Theonella swinhoei*, which were collected from Solomon Islands by Zampella and co-workers from Italy in 2011 (Figure 1.3.).[18] As depicted in Figure 1.3. both the natural products bear amino acids serine, alanine, and glycine whose absolute stereochemistry were determined using Marfey's method as L-Serine and D-Alanine. Both

the compounds were isolated from 207 g of lypolized sponge material which was extracted with methanol and purified with the help of HPLC on a C-12 Jupiter proteo column

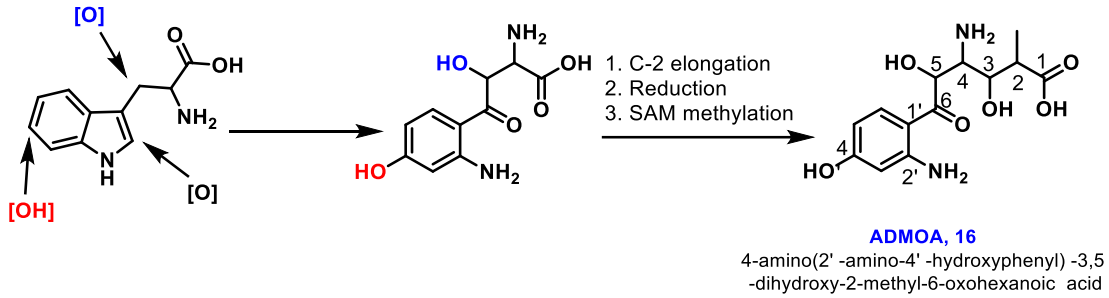
ADMOA, 16 (Solomonamide A)
4-amino(2' -amino-4' -hydroxyphenyl) -3,5 -dihydroxy-2-methyl-6-oxohexanoic acid

AHMOA, 17 (Solomonamide B)
4-amino-6-(2' -amino-4' - hydroxyphenyl) -3-hydroxy-2-methyl-6-oxohexanoic acid

Figure 1.4. Non-amino acid portions in solomonamide A and solomonamide B.

using 20% MeOH/H_2O containing 0.1% of TFA as eluent. From this experiment, solomonamide A and solomonamide B were obtained in 6.2 mg and 3.6 mg of qunatities as white amorphous solids with low specific rotation of + 2.3 (*c* 0.17, CH_3OH) and + 4.8 (*c* 0.28, CH_3OH) respectively. The non-amino acid portion of solomonamide A possess four contiguous chiral centers, which were established by extensive 2D NMR studies and defined as 4-amino(2'-amino-4'-hydroxyphenyl)-3,5-dihydroxy-2-methyl-6-oxohexanoic acid (**16)** (ADMOA). The most challenging task of stereochemical assignment of the ADMOA unit of solomonamide A was carried out with the help of QM *J* based analysis and DFT $J/^{13}C$ calculations. Comparison of 2D NMR data of solomonamide A and solomonamide B revealed that, there is absence of hydroxy group at C5 position of non-amino acid partner of solomonamide B. Hence, the residue was named as 4-amino-6-(2'-amino-4'-hydroxyphenyl) -3-hydroxy-2-methyl-6-oxohexanoic acid (AHMOA) (**17)** (Figure 1.4.). Biogenesis of ADMOA in solomonamide A was, proposed by Zampella and co-workers from basic unit tryptophan mentioned in scheme 1.1. Tryptophan on β-hydroxylation followed by oxidative cleavge of indole ring afforded dihydroxykynurenine unit followed by subsequent transformation of the ketone to alcohol and alkylation of the acetate carbon (C-2) using SAM (S-adenosyl methionine) would finally produce the ADMOA residue (**16**). Solomonamide A was evaluated for its anti-inflammmatory potential using male Swiss albino mice and it was identified with a potent anti-inflammatory activity with 60% paw edema reduction in mice which was observed at

concentration of 100 μg/kg (i.p.). However, Zampella's group mentioned that biological evaluation of solomonamide B was not possible due to the paucity of isolated material.



Scheme 1.1. Proposed biogenesis for ADMOA residue

1.1.3. Inflammation

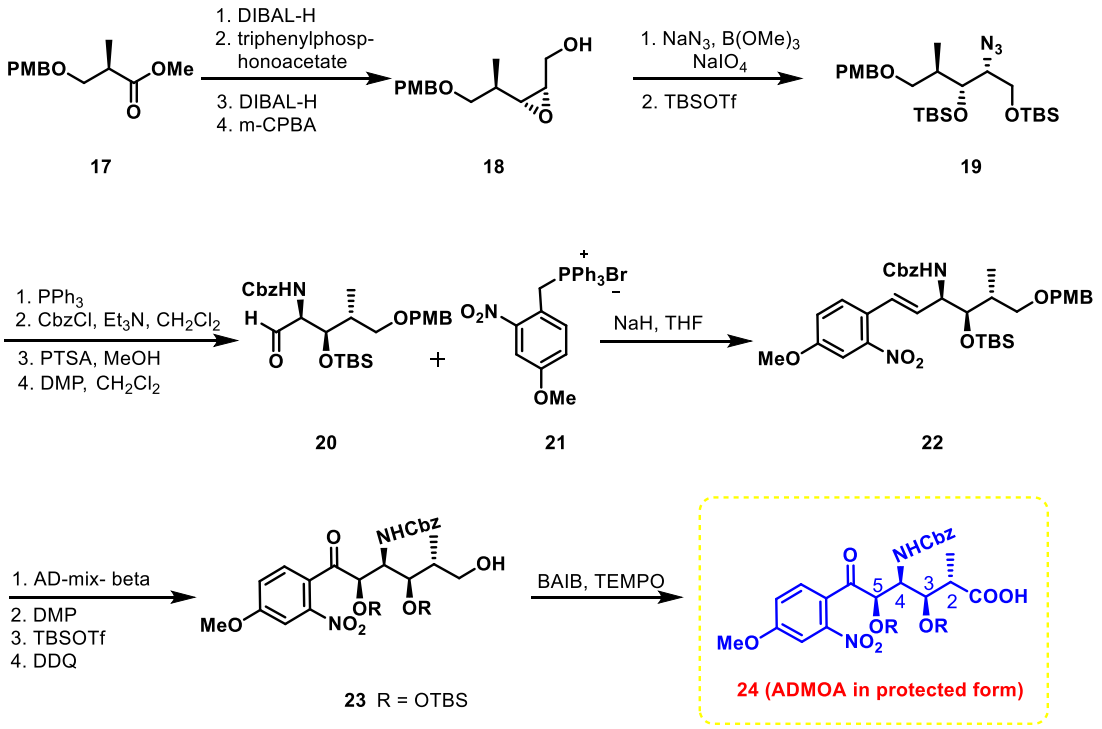
Inflammation is defensive response provided by the immune system that ensures the pathogenic invasion or tissue injury and is intrinsically a beneficial process which restores the physiological balance by eliminating the factors harmful to body. However, when inflammation persists longer than necessary, can be alarming factor which results in causing more harms to body than help, which can lead to diseases such as asthma, atherosclerosis, cancers, allergies, autoimmune disorders and rheumatoid arthritis. The entire process of the inflammatory response is mediated by several key regulators like pro-inflammatory cytokines (IL-1β (IL-1β), IL-6, IL-12, and IL-8), interferons (IFN-γ), eicosanoids (prostaglandins and leukotrienes), TNF-α & TNF-β) and histamine.[19] All the listed factors play important role in the defensive inflammatory mechanism and are useful biomarkers for inflammation. Depending on response and duration inflammation is classified as acute or chronic, and thus research efforts have been directed to develop safer and potent anti-inflammatory drugs in recent years. As a part of our continuous interest in search of biologically active natural molecules, in particular anti-inflammatory agents like nitrosporeucines A and B,[20] periconianone B, [21] peribysin E,[22] botryosphaeridione and pleodendione,[20] we became interested in novel compounds solomonamide A and solomonamide B with potent biological activity as well as interesting structural features.

1.1.4. Reported approaches towards the synthesis of solomonamides

The solomonamides has attracted immense attention of many synthetic chemists because of their potential bilogical activity and challenging structural features. Immiditely after isolation, Natural Product Reviews, a reputed publication from Royal Society of chemistry highlited solomonamides as "Hot off the press" subject matter in 2011.[23] Ever since disclosure of its structure, a total of eight synthetic attempts towards the natural product have been published in peer reviewed journals by various research groups, including Chandrasekhar group,[24] Sarabia group[25] and our own group.[26] Before proceding further for the discussion of present work on this project, here we have summarized previous approaches of synthesis in following section.

1.1.4.1. Approach by Chandrasekhar research group

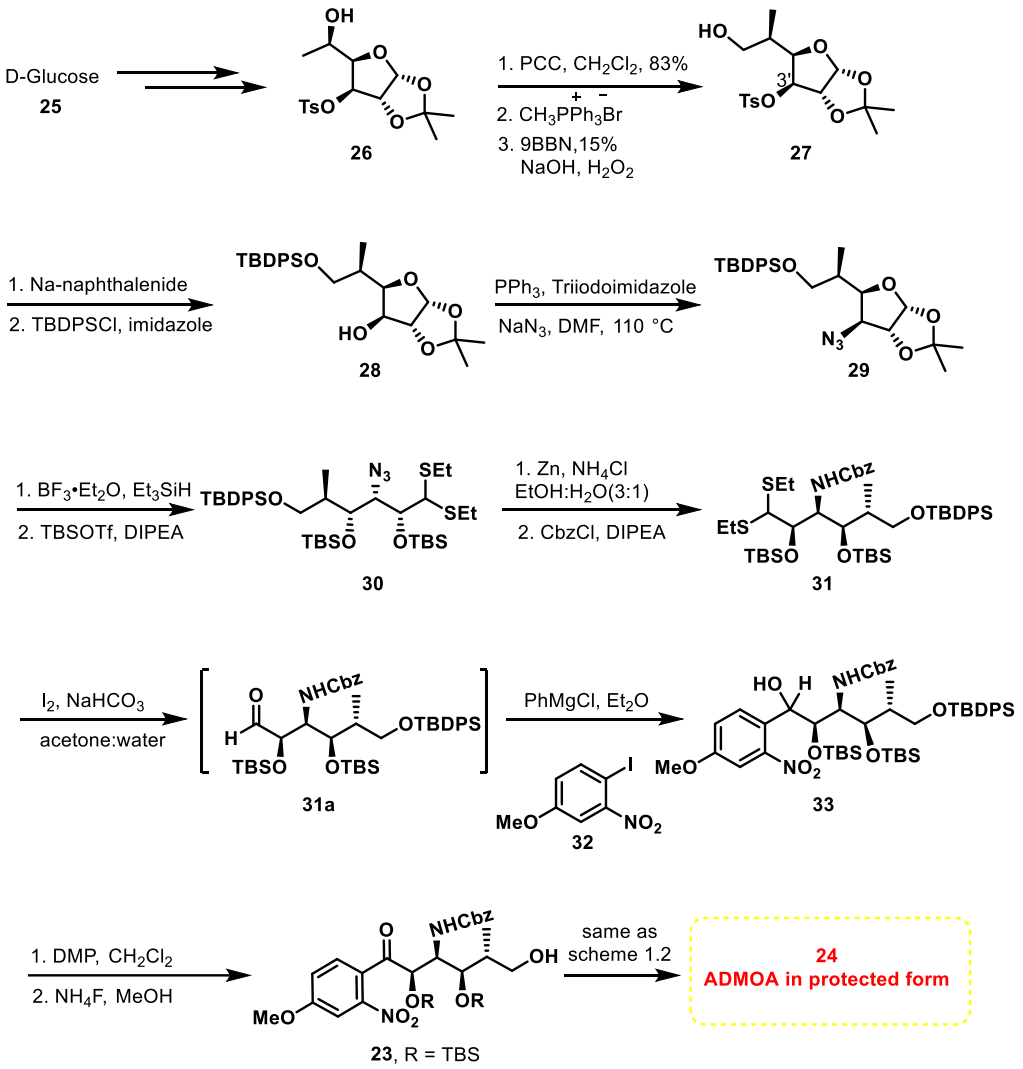
From the CSIR-Indian Institute of Chemical Technology, India Chandrasekhar and co-workers were able to forge the functionalized synthesis of an unusual γ-amino acid part (in protected form) of solomonamide A in 2013.[24a] Their synthesis commenced from *R*-Roche ester having PMB protection **17**, which on four step sequence namely reduction of ester to aldehyde, Wittig homologation reaction, reduction and epoxidation gave epoxy alcohol **18**. Regioselective opening of epoxy alcohol **18** using sodium azide furnished azido alcohol followed by TBS protection of diol afforded compound **19**, with three contiguous chiral centre with required stereochemistry. Reduction of azide to amine followed by functional group interconversion and oxidation of one of the primary alcohol to aldehyde using Dess-Martin periodinane afforded compound **20**. The aldehyde **20** on treatment with Wittig salt **21** using sodium hydride in THF afforded styrene derivative **22** as 7:3 *E*/*Z* diastereomeric mixture in good yields. Further, Sharpless asymmetric dihydroxylation was carried out on major *E*-diastereomer with the help of AD-mix-β to furnished diol which upon regioselective oxidation followed by protection-deprotection furnished the benzylic ketone **23** with all the four requisite stereocenters. Compound **23** was converted to acid **24** under



Scheme 1.2. Synthesis of ADMOA in fully protected form

neutral conditions which resulted in desired unusual amino acid **24** (ADMOA in protected form) with good yields (Scheme 1.2.). In summary, Chandrasekhar group accomplished the synthesis of ADMOA, in protected form, a key component of solomonamide A. It was accomplished in 16 steps from known compound **17**.

In 2015, Chandrasekhar group reported another approach[24b] for the synthesis of the ADMOA fragment starting from D-glucose. In this route, the synthesis commenced from a known furanose derivative **26** obtained from D-Glucose **25**. The secondary alcohol in compound **26** was oxidized to methyl ketone which on one carbon Wittig homologation reaction followed by diastereoselective hydroboration of the olefin furnished furano alcohol **27**, with 20:1 *d.e.* in favor of the required isomer. Further, using sodium napthalenide tosyl group in **27** was deprotected to get the diol followed by protection of alcohol as silyl ether afforded **28** with 98% yields for two steps. The secondary alcohol in compound **28** was converted into azide **29** using a double inversion protocol. Treatment of



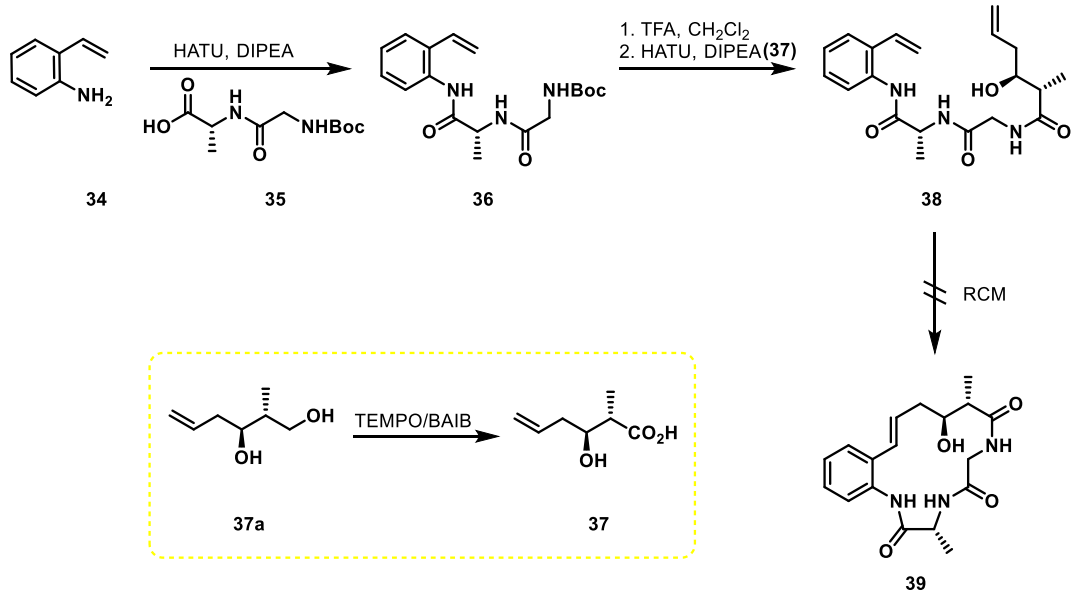
Scheme 1.3. Synthesis of ADMOA fragment from glucose

29 with triethyl silyl hydride and $BF_3 \cdot OEt_2$ followed by protection of alcohol groups as TBS resulted in the formation of compound **30** with 92% yield over 2 steps. The azide was reduced (Zn/NH_4Cl) to amine and protected as Cbz gave compound **31** with 79% yield over two steps. The dithiane derivative **31** on treatment with $I_2/NaHCO_3$ to release free aldehyde **31a** which on Grignard reaction with iodo derivative **32** in the presence of phenyl magnesium chloride (halogen-metal exchange) to furnish the secondary alcohol **33** as a diastereomeric mixture in 58% yield over two steps. Oxidation of secondary alcohol **33** using Dess-Martin periodinane to benzylic ketone and chemoselective removal of the

TBDPS group using NH_4F afforded the primary alcohol **23** with 82% yield for 2 steps. The compound **23** was converted into acid **24** in a similar manner to that of their previous approach (Scheme 1.3.). Thus, synthesis of ADMOA fragment was reported from D-glucose in total 15 steps from known compound and it is better than previous approach.

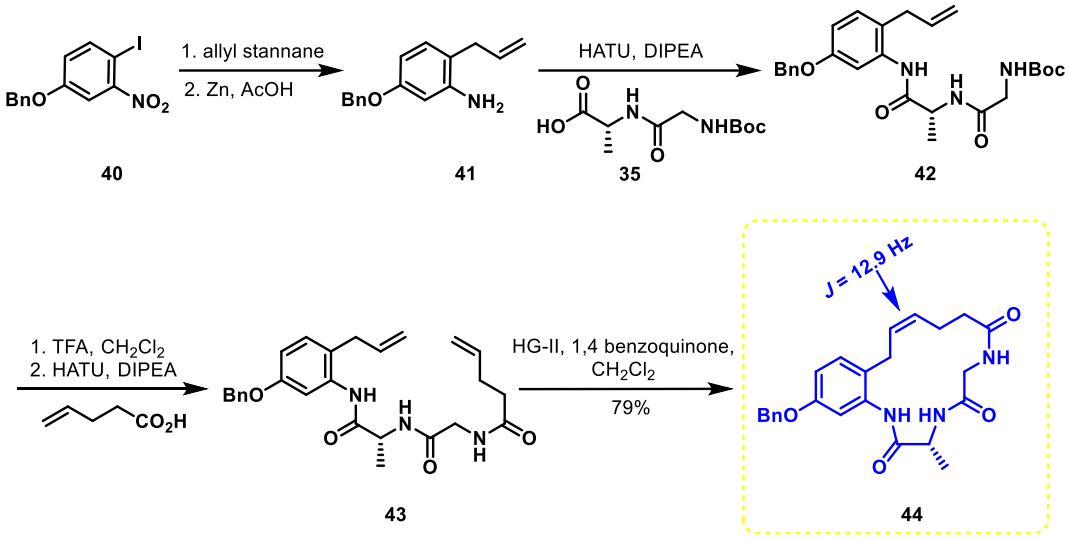
1.1.4.2. Approach by Sarabia research group

In 2016, Sarabia and co-workers reported the synthesis of macrocyclic skeleton of solomonamides using ring closing metathesis (RCM) as key step.[25a] Synthesis of the model macrocycle **39**, commensed with coupling of aniline derivative **34** and Boc-Gly-D-Ala-OH (**35)** resulted in the formation of **36** in 78% yield. Further, Boc deprotection of **36** and coupling with acid **37**, which was synthesized from diol **37a** using TEMPO/BAIB, gave the metathesis precursor **38** with a yield of 92% for two steps. However, in the next step ring closing metathesis did not work to produce macrocycle **39** (Scheme 1.4.). After this unsuccessful attempt they revised the strategy by keeping ring closing metathesis as key step by changing substrate from **34** to **41**. The nitrobenzene derivative **40** on Stille coupling



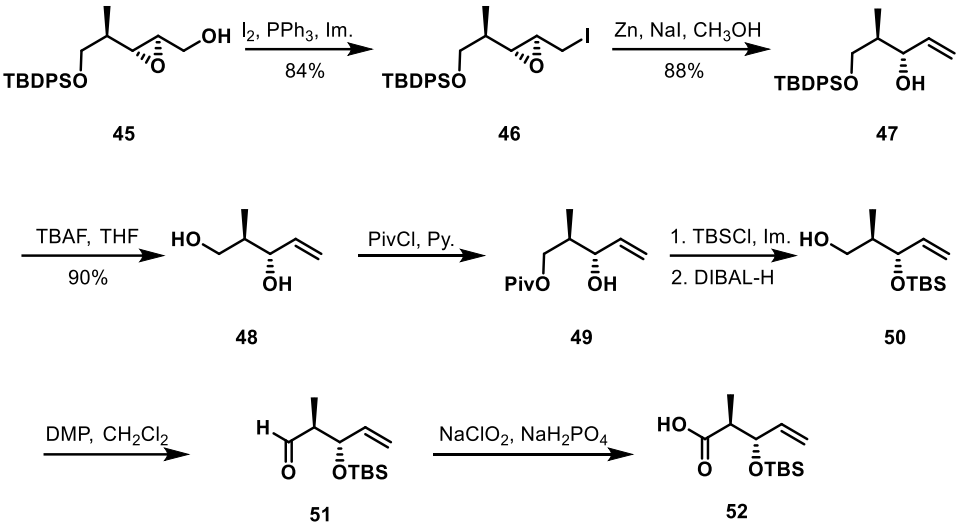
Scheme 1.4. Attempt towards synthesis of model compound

with allyl stannane using palladium catalyst followed by reduction of nitro group gave aniline derivative **41**. Compound **41** was coupled with Boc-Gly-D-Ala-OH (**35**) using standard coupling protocol to furnish **42** in good yields. Compound **42** on Boc removal and subsequent coupling with pentenoic acid using HATU, DIPEA furnished diolefin compound **43** with good yield. Then compound **43** was subjected for ring closing metathesis using 10 mol% of HG-II, *P*-benzoquinone in dichloromethane afforded macrocycle **44** with 79% yield. After synthesis of model macrocycle in an efficient



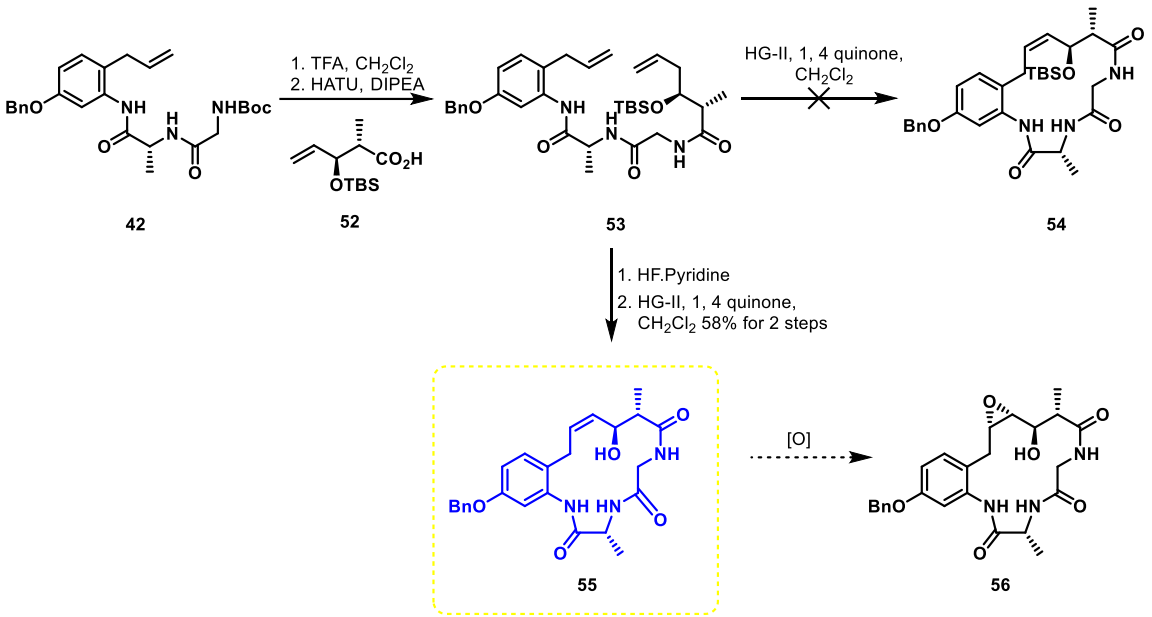
Scheme 1.5. Synthesis of model compound using ring closing metathesis

manner they extended this optimized protocol to the synthesis of desired system. Accordingly, olefinic acid **52** was synthesized from known epoxy alcohol **45**. The primary alcohol **45** was converted to iodo derivative **46** by Appel reaction. Iodo derivative **46** on treatment with zinc, sodium iodide in methanol gave desired alkenol **47** in good yield. Alkenol **47** on TBDPS deprotection resulted in diol **48** which was subjected to selective protection of primary hydroxyl using pivaloyl chloride in presence of pyridine resulted in compound **49**. Protection of **49** using TBS chloride and subsequent treatment with DIBAL-H furnished primary alcohol **50** which on oxidation using DMP afforded aldehyde **51**



Scheme 1.6. Synthesis of olefinic acid

followed by Pinnick oxidation resulted in the formation of acid **52** with 43% yield over 3 steps (Scheme 1.6.). After having acid **52** and dipeptide derivative **42**, which on coupling resulted in the formation of compound **53**. Initially, ring closing metathesis was attempted on compound **53** under the previously optimized conditions.



Scheme 1.7. Synthesis of macrocyclic compound

Unfortunately, they did not observe the formation of macrocycle **54**. Alternatively, TBS was deprotected in compound **53** followed by ring closing metathesis in dichloromethane at reflux furnished the macrocycle **55** in 58% yield for 2 steps. With macrocycle **55** in hand, various conditions were attempted to get epoxy alcohol **56** but formation of the desired product was not observed (Scheme 1.7.).

Very recentlty, the same group reported full account of their work on diverse routes for the synthesis of a variety of macrocyclic analogues around solomonamide scaffold.[25b] By using previously established protocol, they have synthesized several analogues around solomonamide macrocyle (**57**, **44**, **58**, **55**, **59**, **60**, **61** and **55**). However, geometry of double bond in case of macrocycle **44** and **55** were determined and it was exclusively in *E* configuration, as revealed by a coupling constant (J = 15.9 Hz), therefore they revised previous assignment of double bond, which was misassigned as *Z* configuration. After synthesis of analogues their biological potential was evaluated in cancer cell lines. The tested compounds showed moderate cytotoxicities against various tumor cell lines, out of them compound **55** showed moderate activity IC_{50} = 17.2 μM (Figure 1.5.). In summary, Sarabia and co-workers developed a route to access various macrocycles of solomonamide scaffold using RCM as the key step followed with evaluation of their anticancer potential.

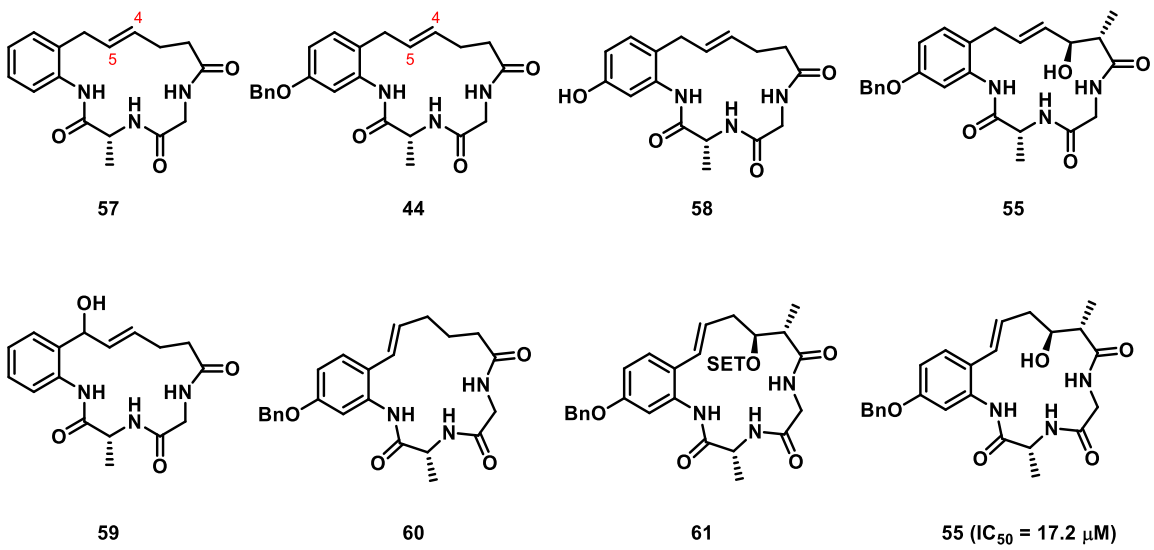
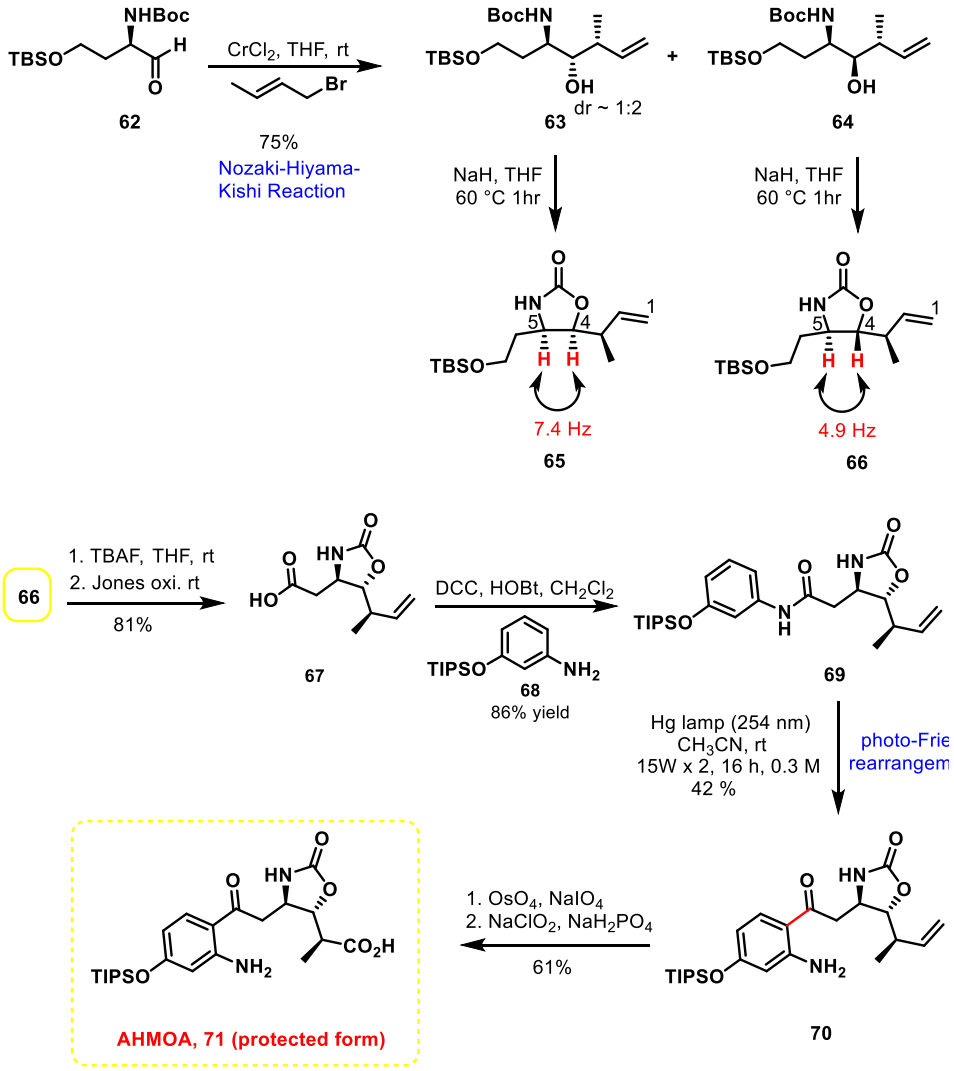


Figure 1.5. Synthesized structures of solomonamide analogues.

1.1.4.3. Previous approach by Reddy research group (our group)

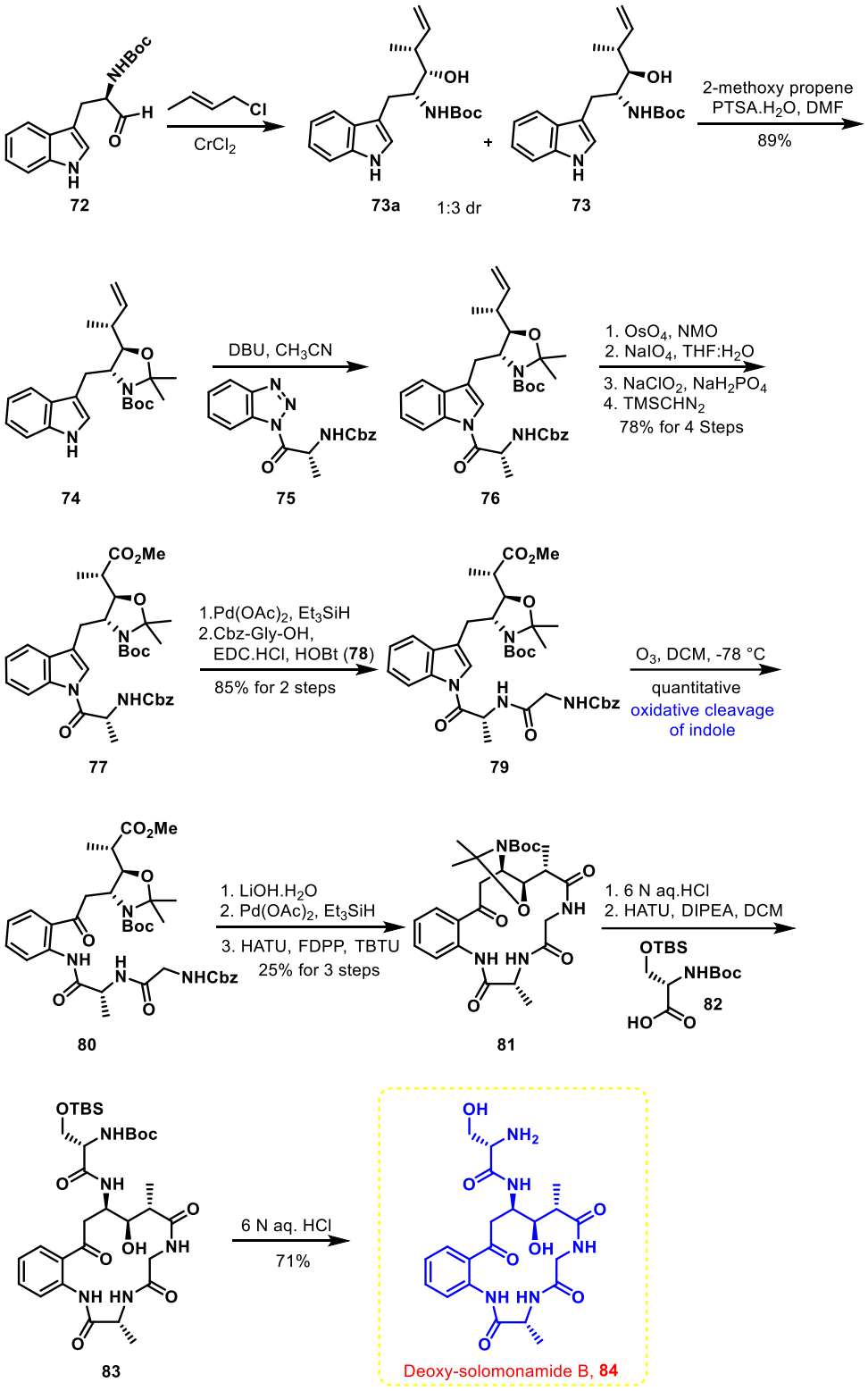
Reddy and co-workers documented the synthesis of key fragment of solomonamide B (AHMOA) in 2012 using photo Fries rearrangement as a key step.[26a] Synthesis of AHMOA fragment commenced with homoserine aldehyde **62** which was then subjected to a crotylation reaction using crotyl bromide and chromium chloride afforded diastereomers **63** and **64** with 1:2 ratio. The stereochemistry of **63** and **64** were determined by making



Scheme 1.8. Synthesis of AHMOA in protected form

their corresponding cyclic carbamates **65** and **66** and measured coupling constants. In addition, stereochemistry of carbamate **66** was confirmed by using single crystal X-ray anlaysis (Scheme 1.8.). Cyclic carbamate **66** on deprotection of TBS followed by oxidation of primary alcohol to acid **67** was achieved using Jones oxidation. TIPS protected phenol derivative **68** was coupled with carboxylic acid **67** using DCC, HOBt in DCM afforded compound **69**. Attempts to form carbon-carbon bond formation through ortho CH activation of the amide **69** using a Mercury lamp (254 nm) at 0.3 M concentration in acetonitrile afforded the photo Fries rearranged product **70** in 42% yield. Compound **70** on oxidative cleavage using Upjohn dihydroxylation resulted in the formation of aldehyde which on further Pinnick oxidation furnished AHMOA (**71)** fragment with in 61% yield over two steps (Scheme 1.8.). In summary, Reddy's group reported synthesis of the key fragment AHMOA in a protected form using photo Fries rearrengment as the key step.

In 2014, same group reported the synthesis of deoxy-solomonamide B based on biogenesis of solomonamides (proposed by Zampella's group) using oxidative cleavage of indole as a key step (Scheme 1.9.).[26c] Synthesis of deoxysolomonamide B commenced with crotylation on known tryptophan aldehyde **72**, resulted in the formation of diastereomer **73** and **73a** with a 3:1 ratio.[27] Required diastereomer **73** on treatment with 2-methoxy propene in presence of catalytic amount of PTSA.H_2O resulted in formation of acetonide protected compound **74** with 89% yield. Compound **74** on coupling with Cbz-D-Ala-OH (**75**) using DBU resulted in formation of **76** in excellent yield. Compound **76** was converted to the ester **77** via Upjohn dihydroxylation, diol cleavage, oxidation of alcohol to acid followed by esterification with 78% yield over 4 steps. Further, Cbz was deprotected in **77** and which was coupled with Cbz-Gly-OH (**78**) using standard coupling protocol afforded the desired dipeptide **79** in 85% yield over two steps (Scheme 1.9.). Oxidative cleavage of indole moiety in compound **79** using ozonolysis resulted in the formation of dipeptide coupled ortho-keto aniline derivative **80** in quantitative yield. Deprotection of Cbz and ester using palladium acetate and LiOH.H_2O respectively, followed by macrocyclization using HATU, TBTU and FDPP resulted in macrocyclic core of natural product **81**. Macrocyclic compound **81** was subjected for acetonide deprotection

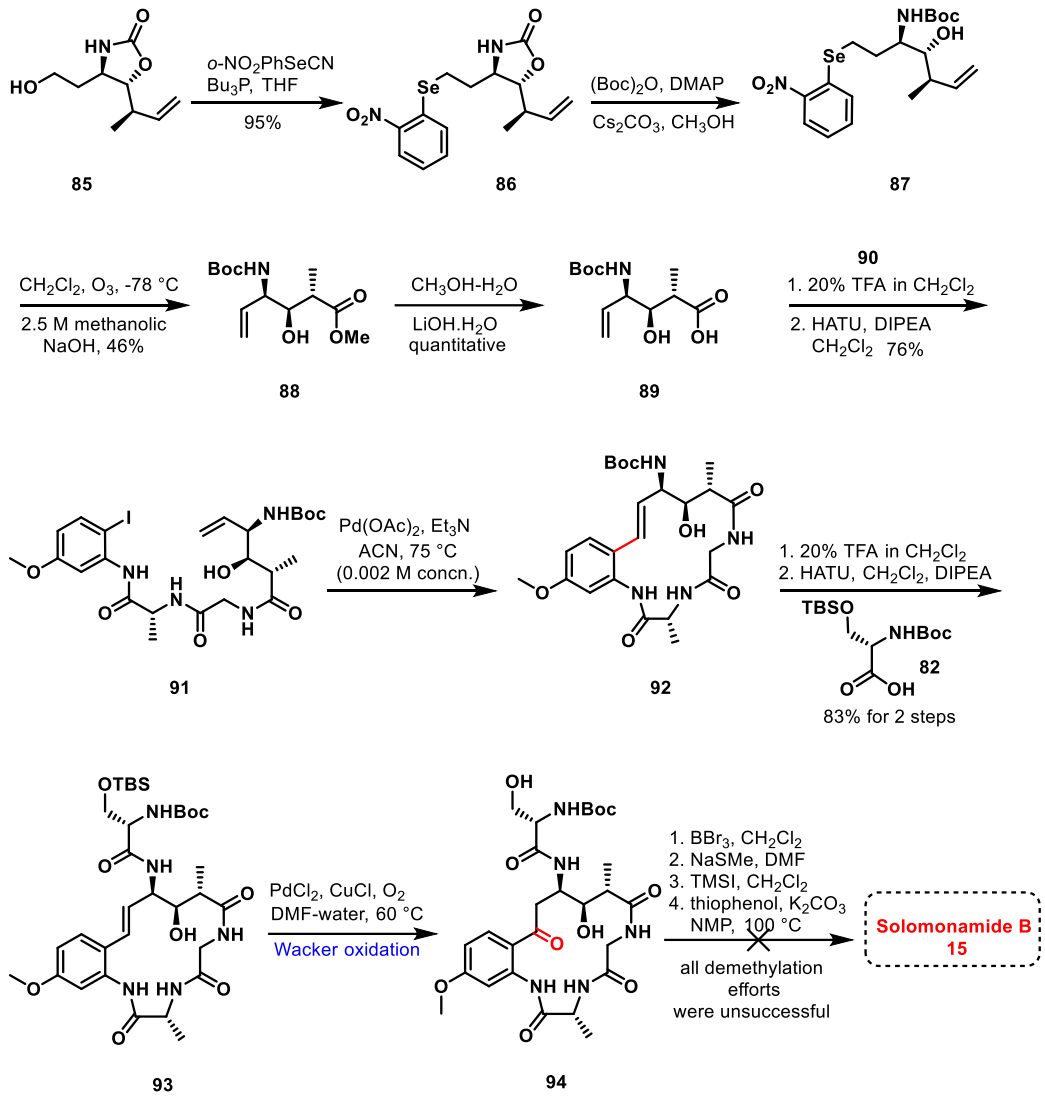


Scheme 1.9. Synthesis of deoxy-solomonamide B

followed by coupling with BocNH-OTBS-L-ser-OH (**82**) lead to synthesis of **83** in 30% yield for two steps. In end game both the protecting groups (TBS and Boc) were removed using 6 N aq.HCl afforded deoxy-solomonamide B (**84)** with 71% yield (Scheme 1.9.). In short, it was reported that synthesis of deoxysolomonamide B using biomimetic approach using oxidative cleavage of indole and crotylation reaction in 16 steps from tryptophan aldehyde.

In 2015, Reddy and co-workers reported a simple one-pot, two-way approach to get terminal olefin and esters via breaking and making of olefins simultaneously using ozonolysis of alkenyl aryl selenides.[26d] In this methodology, alkenol was transformed to the corresponding aryl selenide using *o*-NO_2PhSeCN and Bu_3P followed by ozonolysis using methanolic solution of NaOH to furnish the olefinic ester in single pot. The reaction proceeds through intermediate selenoxide which undergoes elimination to form olefin. Using optimized protocol several compounds with olefin and ester at the terminals of molecule were synthesized. With the optimized condition in hand, they prepared the key intermediate **88** which was then utilized for the core structure of solomonamides. Synthesis of key fragment started from known compound **85** which on treatment with *o*-NO_2PhSeCN and Bu_3P furnished the aryl selenide **86** in 95% yield. Compound **86** on protection of free NH group as *N*-Boc using Boc anhydride, DMAP followed by hydrolysis of carbamate using cesium carbonate in methanol resulted in formation of **87** with 71% yield for two steps. Ozonolysis of **87** using optimized protocol gaveolefinic ester **88** in 46% yield. Ester **88** on basic hydrolysis gave carboxylic acid **89** in quantitative yield (Scheme 1.10.). The iodo aniline containing dipeptide **90** on Boc deprotection followed by coupling with carboxylic acid **89** resulted in the formation of iodo olefin compound **91** in 76% yield for two steps. Macrocyclic precursor **91** on intramolecular ligand free Heck reaction ($Pd(OAc)_2$, Et_3N) at 0.002 M concentration, afforded the macrocyclic compound **92** with 42% yield. After having the macrocyclic compound **92** in hand, next Boc group was deprotected using TFA in CH_2Cl_2 followed by coupling with BocNH-OTBS-L-ser-OH (**82)** resulting in formation of compound **93** with 83% yield for 2 steps. Serine coupled compound **93** was subjected to Wacker oxidation ($PdCl_2$, CuCl) in DMF/H_2O mixture under atmosphere of oxygen at 60 °C resulted in regioselective formation of benzylic

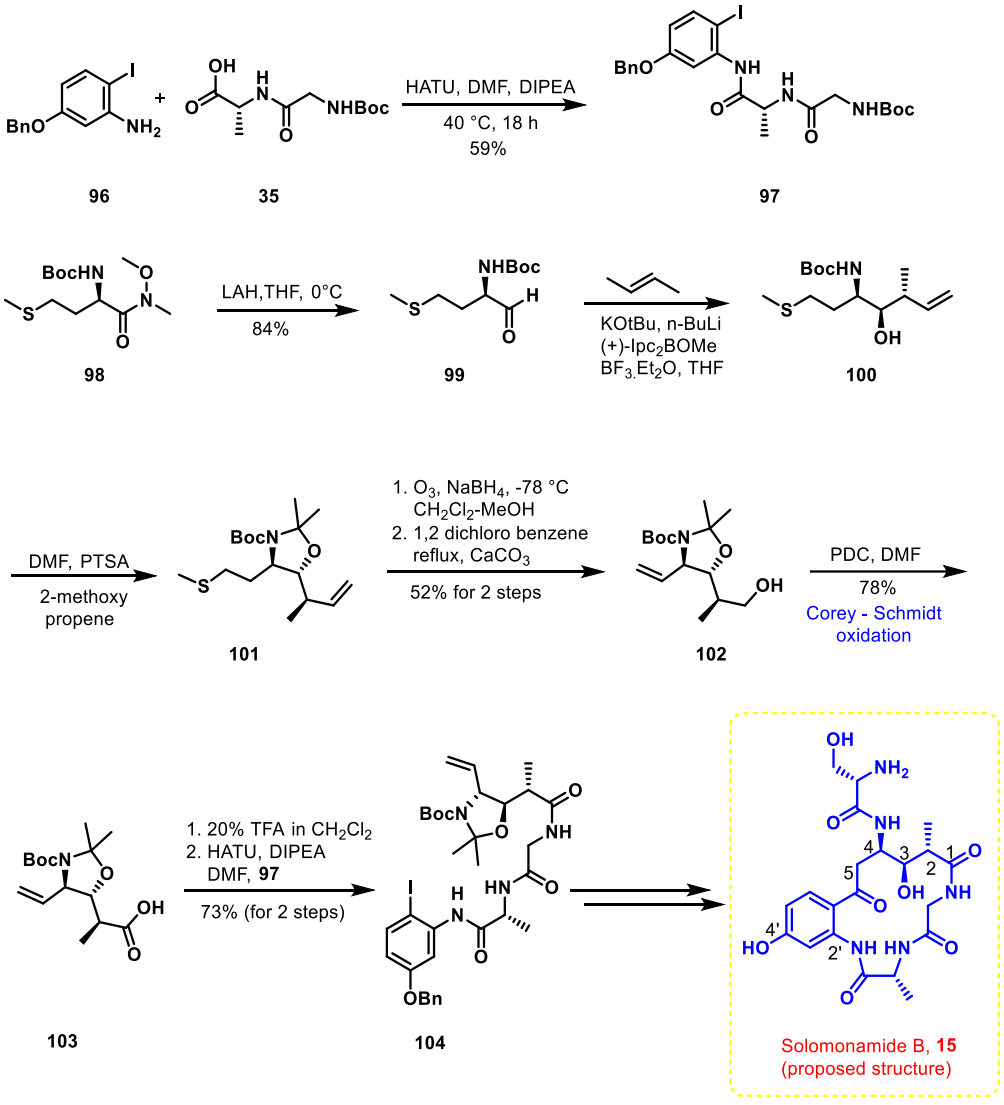
ketone **94**. Attempts for O-methyl deprotection under various conditions (Scheme 1.10.) were unsuccesful, in most of cases decomposition of starting material was observed.



Scheme 1.10. Towards synthesis of solomonamide B

An appropriate protecting group was needed to solve the problem of deprotection of phenolic group. Accordingly, it was decided to use benzyl protection over methoxy because it could be removed under neutral conditions. Subsequently, iodo aniline derivative **97** was prepared by using known protocol with benzyl protecting group. Then it was coupled with the dipeptide, Boc-Gly-D-Ala-OH (**35**) using standard acid amine

coupling protocol to obtain **97** in 59% yield (Scheme 1.11.). This time, Reddy and co-workers used another approach for the synthesis of non-amino acid partner **103** to improve the overall efficiency of total synthesis. Synthesis of non amino acid **103** started from known Weinreb amide **98**, which was reduced to aldehyde **99** by lithium aluminium hydride in THF. The crude aldehyde **99** on Brown crotylation reaction using (+)-(B)-*E*-crotyl diisopinacamphenyl borane[28] afforded compound **100** with desired stereochemistry of three contiguous centres in 61% yield as a single diastereomer. The stereochemistry of



Scheme 1.11. Synthesis of solomonamide B (proposed structure)

compound **100** was assigned based on literature reports. The amino alcohol **100** on acetonide protection gave the compound **101** with yield of 95%. The compound **101** on reductive ozonolysis (O_3, $NaBH_4$), followed by refluxing in 1, 2-dichlorobenzene in presence of $CaCO_3$ afforded alkenol **102** with 52% yield for 2 steps. The alkenol **102** was converted into carboxylic acid **103** (Scheme 1.11.) in one pot using Corey Schmidt protocol (PDC/DMF). The alkenoic acid **103** was then coupled with the free amine which was prepared from **97** by Boc deprotection to furnish the compound **104** with 73% yield over 2 steps. Following same synthetic sequence they have synthesized the target compound solomonamide B (**15**) (Scheme 1.11.). The NMR spectroscopic data for the compound **15** was obtained in DMSO-d_6 at 300 K on a 500 MHz spectrometer. Clearly, the chemical shifts data for the synthesized solomonomide B did not match with the reported values. Hence, it was concluded that it is more likely due to the mis-assignment of the stereochemistry in the natural product by Zampella's group.[18]

With this background, present thesis work was initiated. The first goal was to take up total synthesis of actual natural solomonamides (revised structures), a challenging task as they have multiple chiral centers. The next task was to synthesize a focused library of macrocycles based on solomonamide scaffold and evaluate their biological activity followed by mechanism of action and lead optimization. All the efforts are discussed below in detail.

1.1.5. Present work

1.1.5.1. Towards structural revision of solomonamide B

Before taking up the synthesis of prioritized structures towards structural revision of solomonamides, we have anlyzed the known information about their structural features. There are three amino acids (serine, alanine and glycine) are present in solomonamide B. Using Marfey's method absolute configuration of alanine and serine were determined. We have analysed the published data again and it was found that the reported R_f values[18] in Marfey's method were very clear and distinguishable. Therefore, the assignment of amino

acids, stereochemistry was correct. This leaves three stereocenters present on the nonpeptide portion, for which the absolute and relative stereochemistry was carried out by

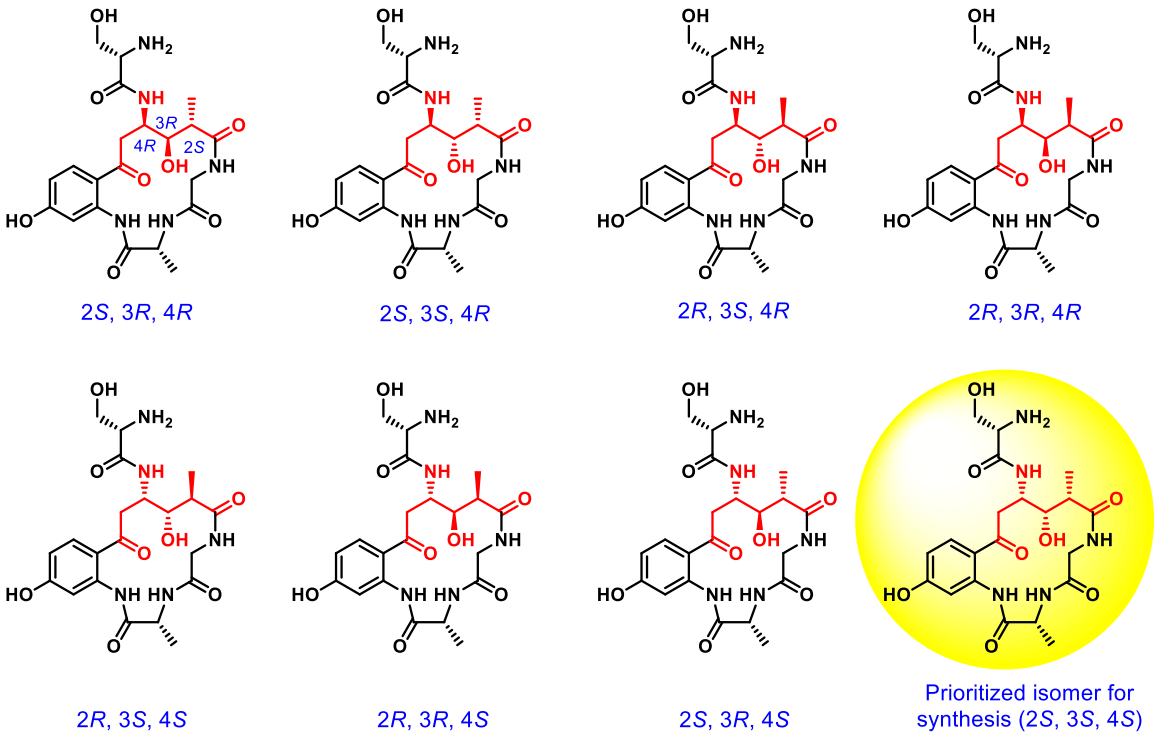
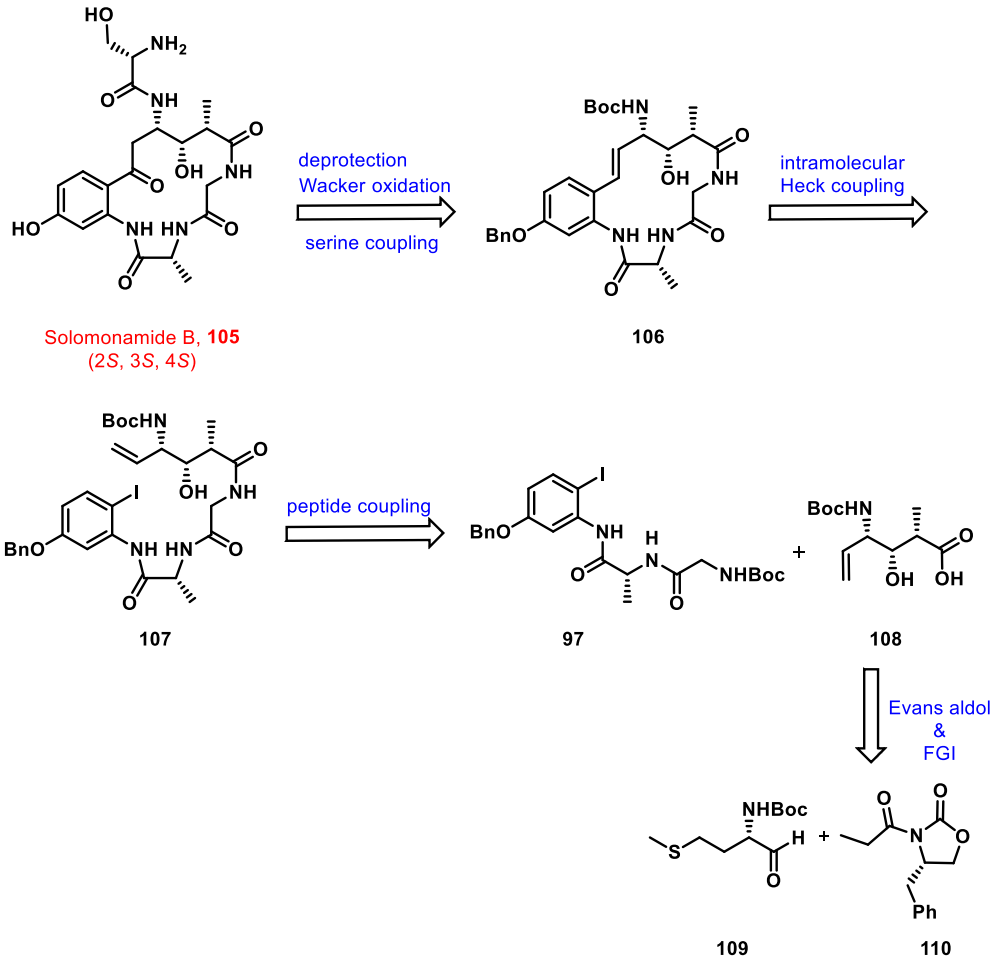


Figure 1.6. Possible eight stereoisomers of solomonamide B

QM and *J* based analysis and DFT *J*/^{13}C calculations.[18] According to 2^n rule for three chiral centre (2^3) in non peptide portion of solomonamide B hence, total eight stereoisomers are possible for solomonamide B (Figure 1.6.). Among them, the first compound with 2*S*, 3*R*, 4*R* configuration was synthesized in our lab previously as the structure was proposed by Zampella's group (see previous section). Endeavor to synthesize the remaining seven possible stereoisomers is a big task that involves changes of the synthetic routes. Therefore, we wanted to prioritize the structures for the synthesis. Based on careful analysis of spectral data of natural solomonamide B and synthesized solomonamide B as well as analysis of Zampella's group publication[18] and Festa's doctoral thesis,[29] we planned synthesis of the macrocycle having 2*S*, 3*S*, 4*S* configuration in non peptide portion as prioritized structure.

1.1.5.1.1. Retrosynthesis of Solomonamide B

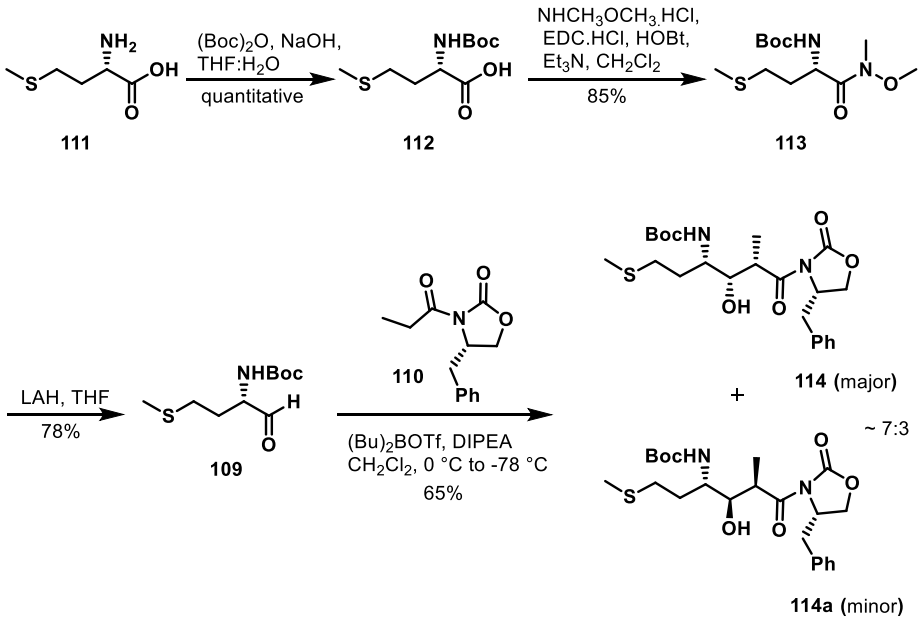


Scheme 1.12. Retrosynthetic analysis for solomonamide B (2*S*, 3*S*,4*S*)

1.1.5.1.2. Synthesis of key fragment

The synthesis of the planned isomer with 2*S*, 3*S*, 4*S* configuration on non-peptide portion began with L-methionine **111** which on *N*-Boc protection using Boc anhydride gave compound **112** in quantitative yield. The Boc protected compound **112** was coupled with methoxymethyl amine hydrochloride using standard acid amine coupling condition to give Weinreb amide **113** with good yield. Weinreb amide **113** was converted to aldehyde

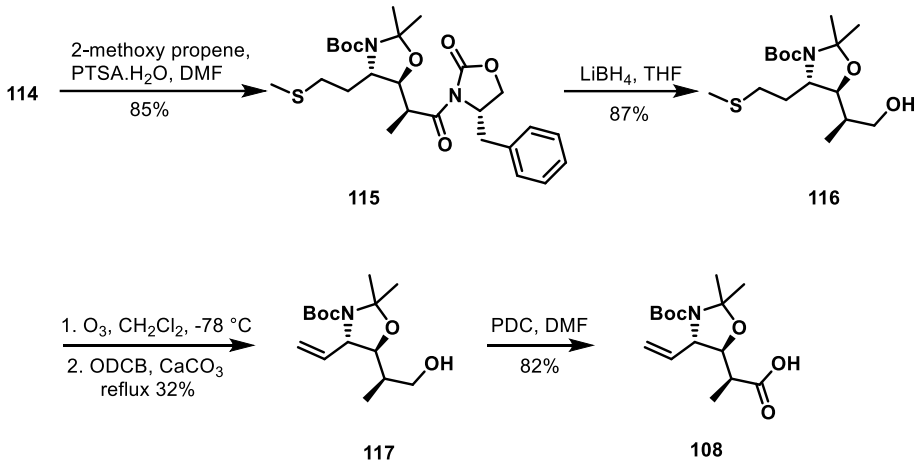
109 using LAH[30] in THF with quantitative yield. The crude aldehyde **109** was subjected to diastereoselective Evans' aldol reaction using dibutylboron triflate to get (*Z*)-enolate derived from (*S*)-4-benzyloxazolidin-2-one derivative **110** to afford compounds **114** and **114a** with moderate selectivity (7:3 diastereomeric ratio) in 65% yield (Scheme 1.13.).[31] However, we could cleanly separate both diastereomers by simple silica gel column chromatography. The formation of aldol products **114** and **114a** was confirmed by the



Scheme 1.13. Synthesis of evans aldol product **114** with 2*S*, 3*S*, 4*S* configuration

presence of aromatic protons as well as number of proton counts in ^{1}H NMR. The carbonyl peak corresponding to amide in compound **114** and **114a** appeared at δ 175.8 and 177.0 while peaks corresponding to carbamate carbonyl appeared at δ 156.3 and 155.7 in ^{13}C NMR confirmed the formation of aldol products **114** and **114a**. In addition to this, the HRMS (ESI) analysis showed a peak at m/z 489.2027 corresponding to $[M+H]^+$ with a molecular formula $C_{23}H_{34}O_6N_2NaS$ which supported the formation of product. The stereochemistry of compounds **114** and **114a** was assigned on the basis of the literature precedence. With optimized condition in hand, we scaled up the reaction to gram scale and used the required major diastereomer **114** for the total synthesis. Acetonide protection of

114 using 2-methoxy propene in presence of catalytic amount of PTSA.H_2O in DMF solvent furnished compound **115** in 85% yield. Structure of compound **115** was confirmed by number of proton count in ^{1}H NMR. In ^{13}C NMR additional peak corresponding to two methyls appeared at δ 27.5, 27.3 depicted the acetonide protection. Compound **115** on treatment with $LiBH_4$ in THF to remove the auxiliary furnished the desired alcohol **116** in 87% yield. The formation of **116** was indicated by formation of polar spot on TLC compared with the starting material. Disappearance of peak from δ 7.35-7.21 (m, 5H) in ^{1}H NMR corresponding to aromatic proton and appearance of an additional peak at δ 3.91 - 3.89 (m, 2H) confirmed the reduction of compound **115**. While in ^{13}C NMR, peak



Scheme 1.14. Synthesis of non-peptide acid **108** with 2*S*, 3*S*, 4*S* configuration

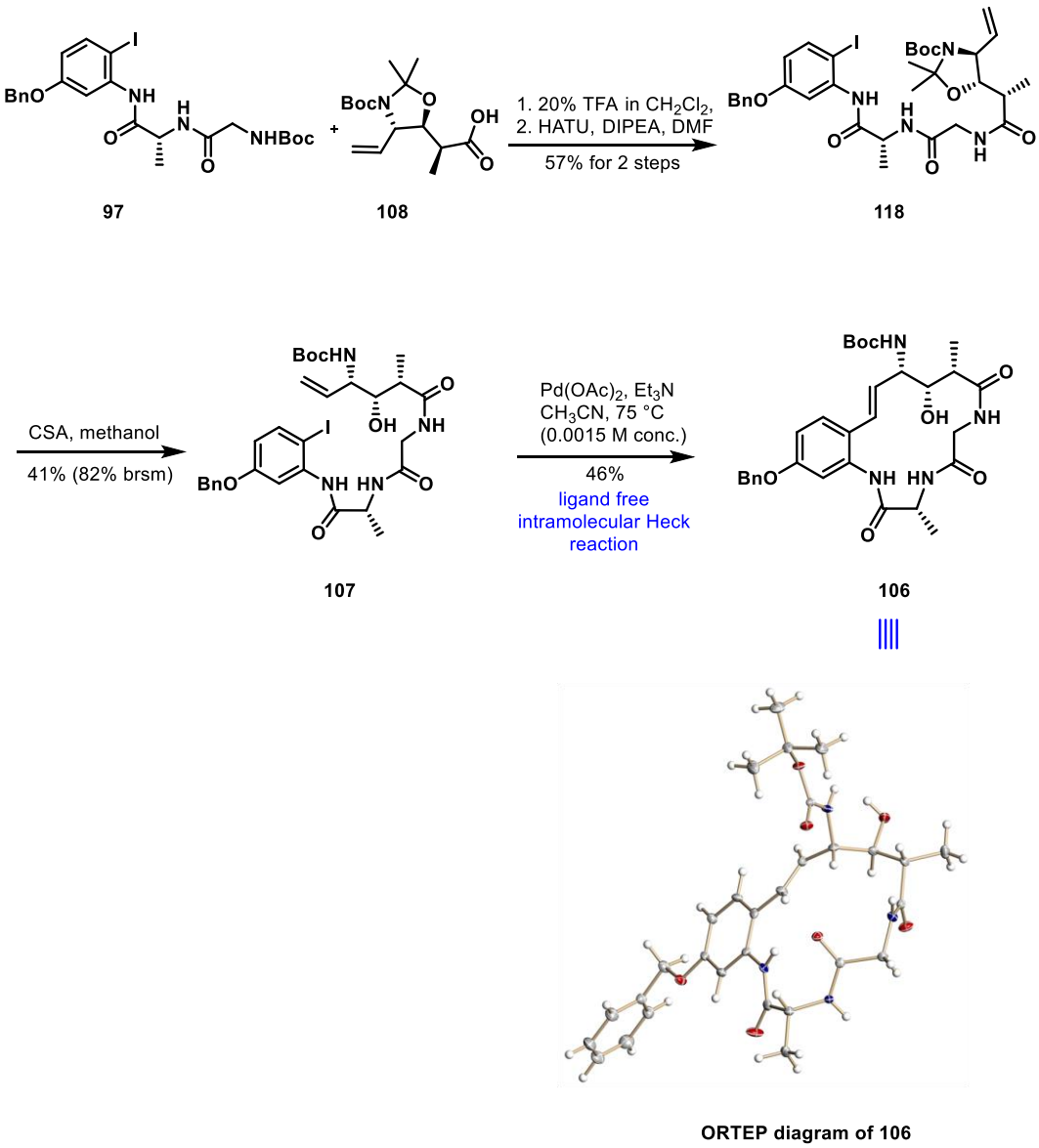
corresponding to carbonyls at δ 175.8 and 153.2 disappeared and appearance of new peak at δ 80.1 confirmed the reduction to alcohol. In addition, the HRMS (ESI) analysis showed mass at m/z 356.1861 corresponding to $[M+Na]^+$ with a molecular formula $C_{16}H_{31}O_4NNaS$ further confirmed the formation of **116**. The oxidation of sulphur to sulphoxide was achieved by using ozonolysis in DCM. The formation of sulphoxide was indicated by checking TLC, formed sulphoxide is more polar than the starting material. After completion of reaction, DCM was evaporated and then crude sulphoxide was forwarded for next step without further purification for pyrolytic elimination using $CaCO_3$ as base in 1,2-dichloro benzene as solvent under reflux condition resulted in formation of alkenol **117**

in 32% yield for 2 steps.[32] The presence of peaks at δ 5.72-5.64 (m, 2H), 5.18 - 5.16 (m, 1H) in ^{1}H NMR confirmed the formation of terminal olefin in **117**. In ^{13}C NMR peaks corresponding to terminal olefins appeared at δ 138.3 and 117.0 supported the formation of alkenol. In addition, the HRMS analysis observed mass at m/z 308.1831 corresponding to $[M+Na]^{+}$with a molecular formula $C_{15}H_{27}O_4NNa$ further asserted the formation of **117**. The alkenol **117** was converted to the desired key unnatural amino acid **108** in one pot in presence of PDC in DMF solvent using Corey-Schmidt protocol (Scheme 1.16).[33] The formation of carboxylic acid **108** was monitored by TLC and it is more polar than alkenol **117** stipulated the formation of same. The disappearance of peak in ^{1}H NMR at δ 3.91-3.89 (m, 2H) confirmed the oxidation of primary alcohol while in ^{13}C NMR peak corresponding to CH_2 at δ 80.1 attached to hydroxy disappeared and appearance of new peak corresponding to acid carbonyl at δ 179.1 confirmed the formation of compound **108**. A mass peak of m/z 322.1625 corresponding to $[M+Na]^{+}$ was observed in HRMS analysis which correspond to a molecular formula $C_{15}H_{25}O_5NNa$ further confirmed the formation of carboxylic acid **108** (Scheme 1.14.).

1.1.5.1.3. Synthesis of macrocycle

After having the required nonpeptide acid **108** in hand, the next task was to complete the total synthesis. For this purpose, dipeptide **97** on Boc deprotection using TFA in DCM followed by coupling with non peptide acid **108** using (HATU, DIPEA, DMF) resulted in formation of compound **118** in 57% yield for 2 steps. The formation of product was evident from TLC, where the product **118** was more polar than acid **108**. In ^{1}H NMR, peaks at δ 1.50 (s, 3H), 1.48 (s, 3H) corresponding to methyls of acetonide and proton as well as carbon count in ^{13}C NMR confirmed the structure of compound **118**. In addition, the HRMS (ESI) analysis showed a peak at m/z 757.2044 corresponding to $[M+Na]^{+}$ with a molecular formula $C_{33}H_{43}O_7N_4NaI$ reaffirmed the formation of **118**. Chemoselective deprotection of acetonide in compound **118** was achieved by using CSA (catalytic) in methanol afforded the macrocyclic precursor **107** with 82% yield based on recovery of starting material. Formation of **107** was asserted by absence of peaks at δ 1.50 (s, 3H), 1.48

(s, 3H) corresponding to acetonide methyls in ^{1}H NMR. It was further confirmed by HRMS, which showed a peak at 717.1755 corresponding to molecular formula $C_{30}H_{39}O_7N_4NaI$ $[M+Na]^+$, with calculated value 717.1756. With macrocyclic precursor **107** in hand, we performed ligand free intramolecular Heck reaction ($Pd(OAc)_2$, Et_3N) in acetonitrile (0.0015 M conc.) under reflux condition resulted in formation of desired macrocycle **106** with 48% yield (Scheme 1.15.).[26d] The formation of macrocyclic compound **106** could be clearly seen in ^{1}H NMR peaks at δ 5.88 (ddd, *J* = 5.3, 10.7, 16.8 Hz, 1H), 5.21-5.13 (m, 2H), corresponding to terminal olefin disappeared and new peak corresponding to internal olefin appeared at δ 6.61 and 5.96. ^{13}C NMR spectra showed the presence of internal olefin carbons at δ 136.7 and 129.7 confirming the formation of macrocycle **106**. It was further supported by HRMS, which showed a peak at 589.2635 corresponding to molecular formula $C_{30}H_{38}O_7N_4Na$ $[M+Na]^+$, with calculated value 589.2633. Although we know configurations of chiral centers present on macrocycle **106**, we wanted to confirm the stereochemistry without any ambiguity. To our delight, the macrocycle **106** crystallized in ethyl acetate- hexane mixture resulting in suitable crystals for the diffraction. The single crystal X-ray structure analysis of **106** clearly indicatedt that three contiguous stereocentres of non-peptide portion in macrocycle **106** are 2*S*, 3*S*, 4*S* thus, we affirmed stereochemistry of synthesized macrocycle without any ambiguity (Scheme 1.15.).

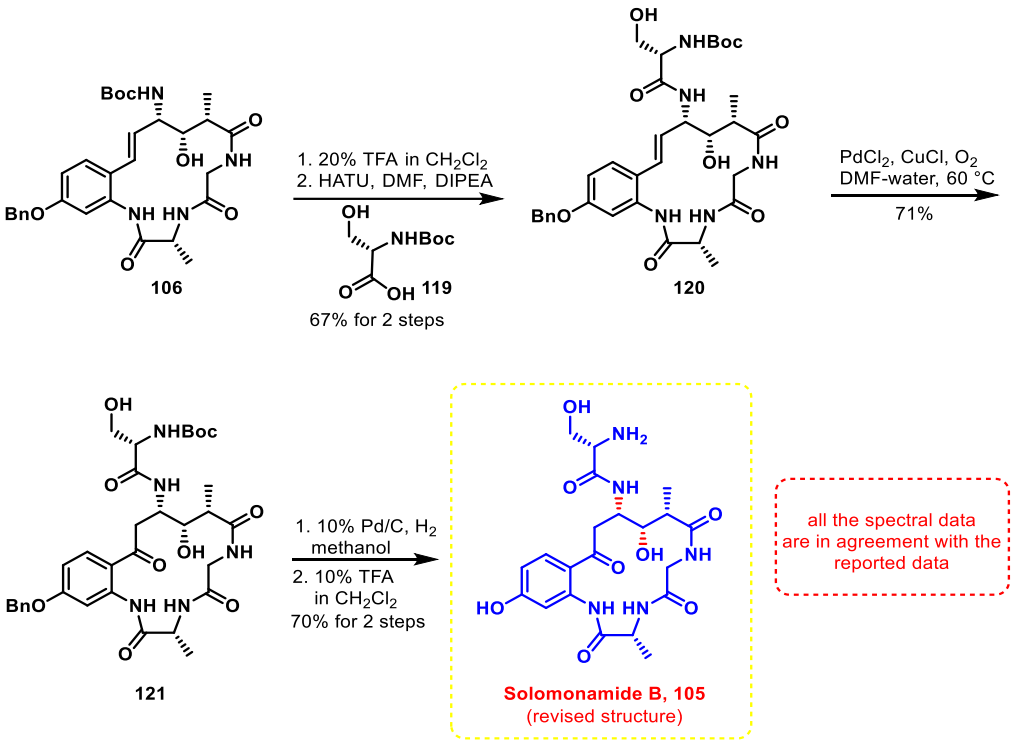


Scheme 1.15. Synthesis of solomonamide B macrocycle

1.1.5.1.4. Synthesis of solomonamide B

After confirmation of stereochemistry, Boc protection was removed using 20% trifluoroacetic acid in DCM followed by coupling with BocNH-L-Ser-OH (**119**) using optimized condition afforded serine coupled compound **120** with 67% yield for two steps.[34] Formation of product was indicated by TLC; serine coupled compound **120** is more polar

than macrocycle **106**. The presence of certain ^{1}H NMR signals in **120** when compared to **106** (serine CH_2 4.16 - 4.14 (m, 1H), 4.02 (d, *J* = 14.7 Hz, 1H)), and total number of protons also confirmed the formation of **120**. Similarly, ^{13}C NMR also supported the formation of **120** with appearance of four amide carbonyl peak at δ 176.5, 172.9, 171.4, 171.3 and carbonyl carbamate peak at δ 158.7 ppm. It was further confirmed by HRMS, which showed a peak at 676.2945 corresponding to molecular formula $C_{33}H_{43}O_9N_5Na[M+Na]^+$ with calculated value 676.2953. Next task was to selectively introduce the oxygen functionality at the benzylic carbon in compound **120**. Accordingly double bond present in compound **120** was converted to benzylic ketone using Wacker type of oxidation with the help of $PdCl_2$, CuCl in DMF/water mixture under atmosphere of oxygen to furnish the macrocyclic ketone **121**[35] in which an exclusive regioselectivity was observed with yield of 71%. Probably, the success of the desired regioselective benzylic ketone formation on macrocycle can be explained by presence of allylic OH group and its co-ordination with a double bond through a Pd species followed by nucleophilic attack of water molecule from more stabilized benzylic site and β-hydride elimination resulted in the formation of bezylic ketone **121.** Formation of benzylic ketone **121** was clearly seen in ^{1}H NMR, peak corresponding to internal olefin disappeared and new peak corresponding to CH_2 appeared at δ 2.89 (d, *J* = 17.1 Hz, 1H), and 3.31 (ovl, 1H). ^{13}C NMR spectra showed presence of ketone carbonyl at δ 201.6 confirming the formation of benzylic ketone **121**. It was further validated by HRMS, which showed a mass peak at 692.2892 corresponding to molecular formula $C_{30}H_{43}O_{10}N_5Na$ $[M+Na]^+$ with calculated value 692.2902. Deprotection of the benzyl group in compound **121** was achieved by using hydrogenation reaction to furnish the phenolic compound, which was further treated with 10% TFA in CH_2Cl_2 to afford the target compound **105** with 2*S*, 3*S*, 4*S* stereochemistry (Scheme 1.16.). To our delight, all the spectral data of the compound **105** was in complete agrrement with the natural product.[18] The proton and carbon NMR of both the compounds are compared in table 1 and 2. In ^{1}H NMR, we observed that CH_3 of AHMOA fragment in proposed solomonamide B (**15**) was shown at δ 0.95 (d, *J* = 6.1 Hz, 3H) while in case of revised solomonamide B (**105**) it appeared at δ 1.08 (d, *J* = 6.8 Hz, 3H) which is in complete matching with the



Scheme 1.16. Total synthesis of revised solomonamide B (with 2*S*, 3*S*, 4*S* stereochemistry)

reported value. In addition, we observed major difference in AHMOA fragment corresponding to C2-H in proposed solomonamide B (**15**) which was shown at δ 2.73 (brs, 1H) while in case of revised solomonamide B (**105**) the same appeared at δ 2.35 (m, 1H) which is also in complete agreement with the reported value. Besides, in ^{13}CNMR of solomonamide B we observed that glycine carbonyl (δ 169.8), alanine carbonyl (δ 172.1), serine carbonyl (δ 165.6), C4 of AHMOA (δ 45.2) and C5 of AHMOA (δ 42.3) are reported whereas in case of revised solomonamide B it appeared as glycine carbonyl (δ 169.1), alanine carbonyl (δ 171.2), serine carbonyl (δ 166.7), C4 of AHMOA (δ 48.6) C5 of AHMOA (δ 41.2) which is in complete agreement with reported values. The measured optical rotation $[\alpha]_D^{26}$ + 9.99 (*c* 0.23, CH_3OH) of the synthetic solomonamide B are in agreement with that of the natural solomonamide B ($[\alpha]_D^{25}$ + 4.8 (*c* 0.28, CH_3OH)).

Table 1. ^{1}H chemical shift data of **15** and **105** in comparison to the reported values

^{1}H NMR values of solomonamide B (natural)	^{1}H NMR values of proposed structure of solomonamide B **15** (synthesized)	^{1}H NMR values of revised structure of solomonamide B **105** (synthesized)
1.08 (d, *J* = 7.2, 3H)	0.95 (d, *J* = 6.1 Hz, 3H)	1.08 (d, *J* = 6.8 Hz, 3H)
1.36 (d, *J* = 7.2, 3H)	1.38 (d, *J* = 7.1 Hz, 3H)	1.36 (d, *J* = 7.3, 3H)
2.35 (m, 1H)	2.73 (brs, 1H)	2.35 (m, 1H)
2.87 (brdd, *J* = 17.6, 1.7, 1H)	2.90 (brd,1H)	2.84 (brdd, *J* = 16.4, 1.5 Hz 1H)
3.34 (ovl, 1H)	might be merged with moisture peak	3.34 (ovl, 1H)
3.39 (ovl, 1H)	might be merged with moisture peak	3.39 (ovl, 1H)
3.69 (brs, 2H)	3.70 (brs, 2H)	3.64 (brs, 1H) 3.77 (dd, 1H)
3.78 (dd, *J* = 15.7, 3.4, 1H)	3.58 (brs, 1H)	3.82 (dd, *J* = 15.7, 3.4, 1H)
3.98 (m 1H)	3.93 (brs, 1H)	4.01 (m 1H)
4.19 (dd, *J* = 15.7, 7.1, 1H)	4.20 - 4.06 (m, 1H)	4.14 (dd, *J* = 15.6, 6.9, 1H)
4.29 (quint, *J* = 7.1, 1H)		4.32 (quint, *J* = 7.3, 1H)
4.52 brdt (9.5, 1.7)	4.51 (brs, 1H)	4.49 brdt (1 H)
5.53 (br d, *J* = 5.3 Hz, 1H)	4.79 (dd, J = 9.0, 14.9 Hz, 1H)	5.54 (d, *J* = 5.3 Hz, 1H)
6.57 (dd, *J* = 8.8, 2.1, 1H)	5.58-5.34 (m, 2H)	6.58 (dd, *J* = 8.8, 2.0, 1H)
7.30 (brdd, *J* = 7.0, 3.4, 1H)	6.56 (d, *J* = 8.1 Hz,1H)	7.42 (NH, 1H)
7.77 (d, *J* = 8.8, 1H)	7.32 - 7.13 (m, 1H)	7.75(d, *J* = 8.8, 1H)
7.92 (d, *J* = 2.1, 1H)	7.68 (d, *J* = 8.3 Hz, 1H)	7.94 (d, *J* = 2.0, 1H)
7.98 (d, *J* = 9.5, 1H)	7.84 (d, *J* = 8.3 Hz, 1H)	8.06 (d, *J* = 8.31Hz, 1H)
8.08 (br d, *J* = 4.4, 2H)	8.11 (brs,1H)	8.12 (2H) NH_2
8.79 (d, *J* = 7.2, 1H)	8.05 (brs, 2H)	8.83 (d, *J* = 7.3, 1H) NH
10.7 s	9.06 (brs,1H)	10.74 s
11.5 s	10.72 (brs,1H)	12.0 s
	12.49 (brs,1H)	

Table 2. ^{13}C NMR chemical shift data of **15** and **105** in comparison to the reported values

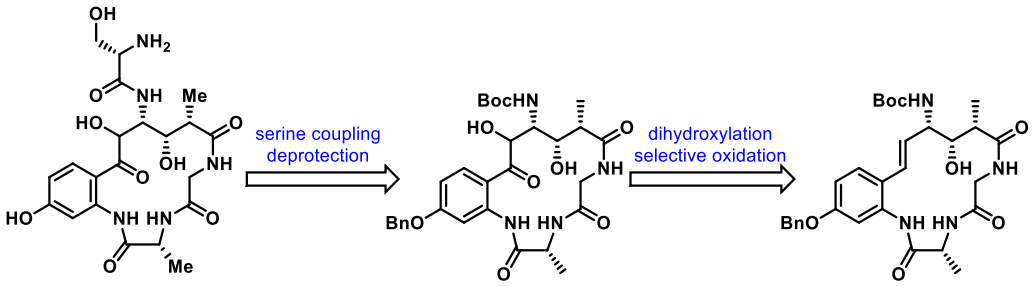
Residue		^{13}C NMR values of solomonamide B (Natural)	^{13}C NMR values of proposed structure of solomonamide B **15** (Synthesized)	^{13}C NMR values of revised structure of solomonamide B **105** (Synthesized)
Glycine	carbonyl	169.0	169.8	169.1
	CH_2	42.4	42.0	42.6
D-alanine	carbonyl	171.2	172.1	171.2
	CH	49.7	50.7	49.9
	methyl	16.0	16.9	16.2
L- serine	carbonyl	166.7	165.6	166.7
	CH	53.6	53.6	54.1
	CH_2	60.3	60.6	60.6
AHMOA	1 carbonyl	173.2	172.6	173.3
	2 CH attached to CH_3	45.2	45.2	45.6
	3 CH attached to OH	72.2	71.4	72.3
	4 CH attached NH	48.0	45.2	48.6
	5 CH_2	41.2	42.3	41.2
	6 keto carbonyl	201.1	200.2	201.0
	7 methyl	13.6	13.6	13.8
Aromatic	1’	115.8	113.9	116.0
	2’	141.3	142.4	141.3
	3’	106.1	105.6	106.3
	4’	162.9	163.3	162.9
	5;	110.0	110.1	110.2
	6’	132.9	133.6	132.9

1.1.5.2. Towards synthesis of revised solomonamide A

After successful stereochemical revision of the solomonamide B,[36] our next aim was to synthesize solomonamide A, in which additional hydroxy functional group is present at the C5-position. During the initial characterization of natural products it was reported that solomonamide A was biogenetically derived from solomonamide B, which makes the structural revision of solomonamide A unavoidable. Accordingly, the three chiral centres of non peptide key fragment (2*S*, 3*S*, 4*S*) present in solomonamide B are expected to retain in solomonamide A, thus leaving one chiral centre (C5-Hydroxy group) in ADMOA fragment, whose absolute stereochemistry needs to be fixed. Accordingly, we designed a structure of solomonamide A having 2*S*, 3*S*, 4*R*, *5R/S* as drawn (Scheme 1.17.).

1.1.5.2.1. Retrosynthesis of revised solomonamide A

The planned strategy to access solomonamide A (**122**) with revised structure is showed in scheme 1.17. Solomonamide A could be furnished from ketone **123** by coupling of serine and subsequent removal of all protecting groups. Ketone **123** could be obtained from macrocycle **106**[36] using dihydroxylation followed by selective oxidation of benzylic alcohol (Scheme 1.17.). The key macrocyclic intermediate **106** was prepared in sufficient quantities starting from L-methionine as described in synthesis of revised structure of solomonamide B.

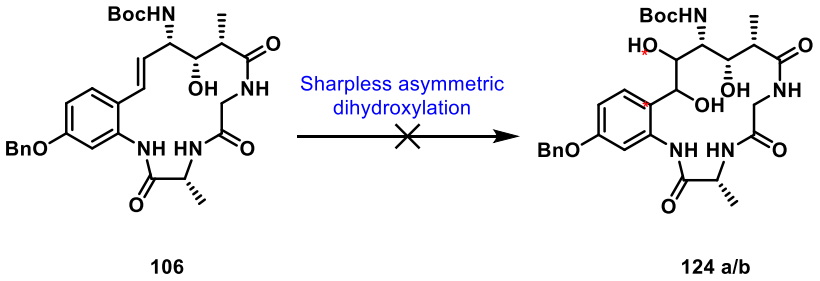


Scheme 1.17. Retrosynthesis of revised solomonamide A (2*S*, 3*S*, 4*R*, *5R/S*)

1.1.5.2.2. Synthesis of revised solomonamide A

We have attempted a Sharpless asymmetric dihydroxylation[37] on macrocycle to access both the diastereomeric triols, (**124a/124b**) using readily available AD-mix-α and AD-mix-β as they will be providing stereoselective dihydroxylations. Stereochemical assignments can be made using well established empirical rules which will play a crucial role in spectral data comparison towards a successful structural revision of solomonamide A. Although we can not apply the rules strictly as the macrocycles posses some bias, we went ahead and tried planned reactions. But to our misfortune, we did not observe triol (**124a/124b**) formation despite a few attempts and we always recovered the starting material (Scheme 1.18.). After failing to obtain the desired product using Sharpless asymmetric dihydroxylations, we turned our attention on Upjohn dihydroxylation.[38] Accordingly, macrocyclic intermediate **106** on treatment with 2.5% OsO_4 and NMO in *t*-BuOH and water mixture furnished the triol **124** in good yield. To our pleasing surprise, we observed the formation of a single diastereomer. The reason behind formation of single diastereomeric triol **124** can possibly be explained by the rigid conformation of macrocycle through hydrogen bonding. The formation of single diastereomer was confirmed by single set of protons and carbons. Dihydroxylated product formation was primarily indicated by presence of broad peak at 3600-3400 cm^{-1} in IR spectroscopy corresponding to a hydroxyl group. Further diol was confirmed by the disappearance of olefin signals and appearance of signals for two CH attached to hydroxyl group in ^{1}H NMR and ^{13}C NMR. It was further confirmed by HRMS, which showed a peak at 623.2687 corresponding to molecular formula $C_{30}H_{40}O_9N_4Na$ $[M+Na]^+$ with calculated value 623.2679. The next task was to perform selective benzylic oxidation of triol **124** and it was achieved using DMP, in presence of $NaHCO_3$ in DCM as solvent to get α-Hydroxyketone **123** with a yield of 64%.[39] The obtained ketone **123** was non polar than triol **124** on TLC in addition it showed prominent charring in 2,4-DNP stains indicates the presence of ketone. The formation of ketone **123** was clearly evident in its ^{1}H NMR, in particular the difference in total number of protons between the starting material and product. The presence of peak at δ 202.1 in ^{13}C NMR asserted the formation of ketone **123**. It was further confirmed by HRMS, which

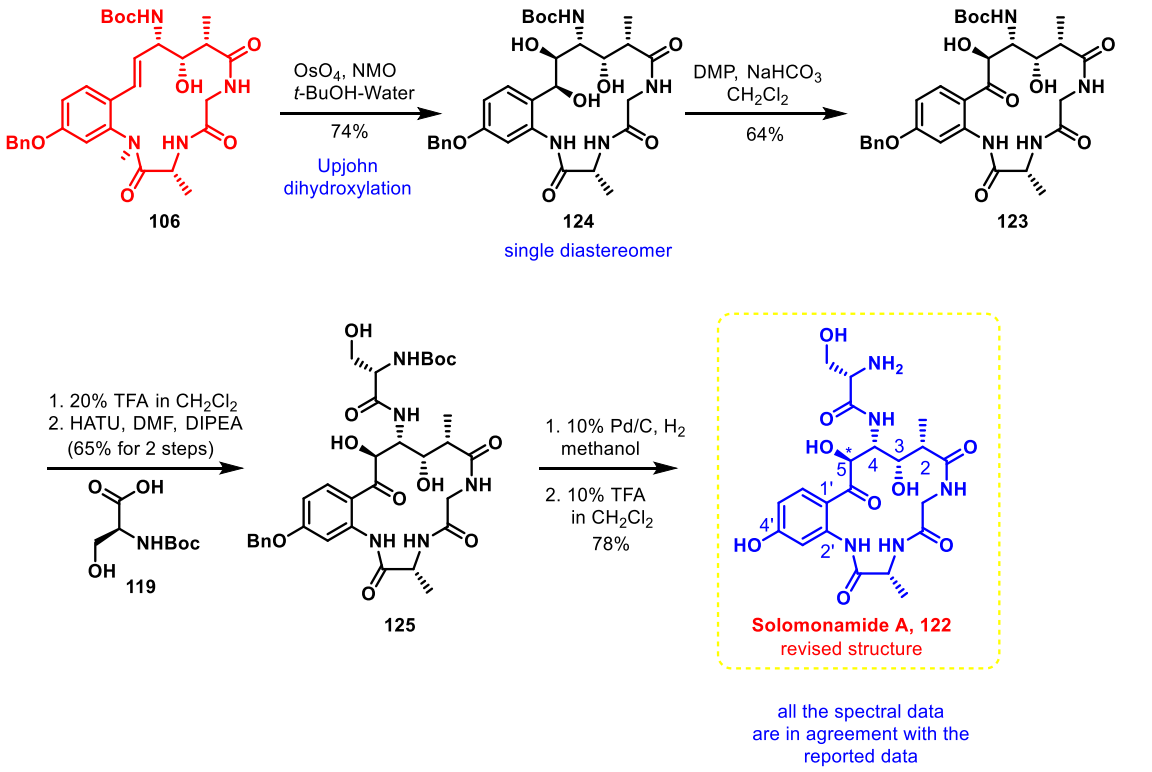
showed a peak at 599.2712 corresponding to molecular formula $C_{30}H_{39}O_9N_4[M+H]^+$ with calculated value 599.2706. After having benzylic ketone **123** in hand, it was subjected for Boc deprotection using trifluoroacetic acid in DCM followed by coupling with BocNH-L-Ser-OH (**119**) using standard coupling protocol (HATU, DIPEA in DMF) afforded serine coupled compound **125** with 65% yield over two steps. The total number of protons in ^1H NMR confirmed the formation of desired serine coupled compound **125**. In addition, ^{13}C NMR also supported the formation of **125** with four amide carbonyls at δ 173.8, 171.1, 169.3, 162.6 and peak corresponding to carbamate carbonyl at δ 156.0 as well as additional number of signals.



Scheme 1.18. Attempts towards asymmetric dihydroxylation

It was further confirmed by HRMS, which showed exact mass value at 708.2851 corresponding to molecular formula $C_{33}H_{43}O_{11}N_5Na$ $[M+Na]^+$ with calculated value 708.2842. Liberation of phenolic group in **125** was carried out using 10% Pd/C under the blanket of H_2 atmosphere in methanol afforded phenolic compound which was indicateded by TLC, followed by treatment with 10% TFA in CH_2Cl_2 to remove the Boc group to furnish the natural product solomonamide A **(122)** with 78% yield for two steps (Scheme 1.19.). We obtained the corrected ^{13}C NMR values from Prof. Zampella in a private communication (through email exchange), where eight carbons namely (glycine CH_2, alanine CH_3, ketone carbonyl, C2 of ADMOA, aromatic 2', 4', 5'and 6') have slightly different values as compared with the original report from her group. To our delight all the analytical data of the compound **122** was in complete agreement with the reported data of the natural product. Comparison of proton and carbon NMR spectra of both the compounds

are provided in table 3. Though the magnitude of optical rotation $[\alpha]_D^{26}$ + 3.4 (*c* 0.52, CH_3OH) of the synthetic solomonamide A (**122)** is higher than that of the natural solomonamide A ($[\alpha]_D^{25}$ + 2.3 (*c* 0.17, CH_3OH)), however, the sign of optical rotation is the same. Thus, we have successfully achieved the synthesis of revised solomonamide A for the first time. However, the assignemnt of stereochemistry at C5-hydroxy position was a challenging task as it was difficult for us to come up with an explanation during the dihydroxylation reaction which gave exclusive selectivity because of conformation of the macrocycle **106**. To find out the stereochemistry at C5-hydroxy position, we tried crystallization efforts on intermediates such as triol **124** as well as α-hydroxy ketone **123** but all our efforts went in vein, otherwise, it could have proved the structure without any ambiguity. After unsuccesfull attempts for crysatlization, we decided to derivatize triol **124**

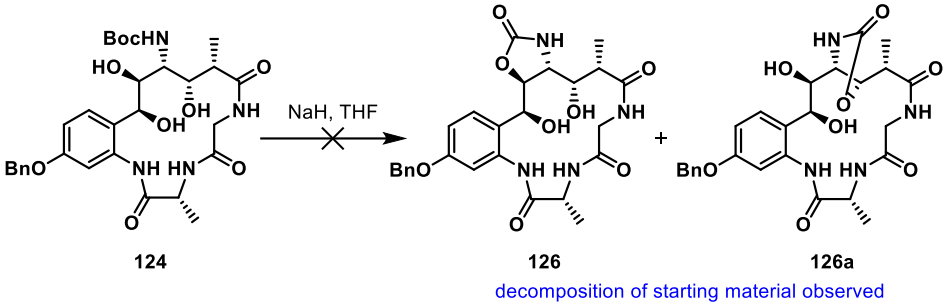


Scheme 1.19. Total synthesis of revised structure of solomonamide A

which was necessary to induce some conformational rigidity into the macrolide ring, which will be useful to do some NMR experiments otherwise it might be flexible in NMR studies.

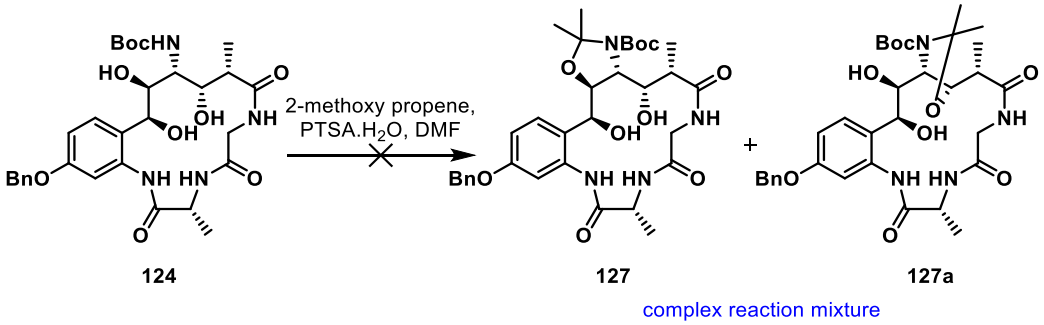
1.1.5.2.3. Attempts towards derivatization of triol (124)

1. **Carbamate synthesis:** We attempted synthesis of five-membered cyclic carbamate derivatives **126** and **126a** from triol **124** by treatment with 60% sodium hydride in anhydrous THF for 12 h. Unfortunately under this condition we did not observe formation of carbamates **126** and **126a** instead we observed decomposition of triol **124**.



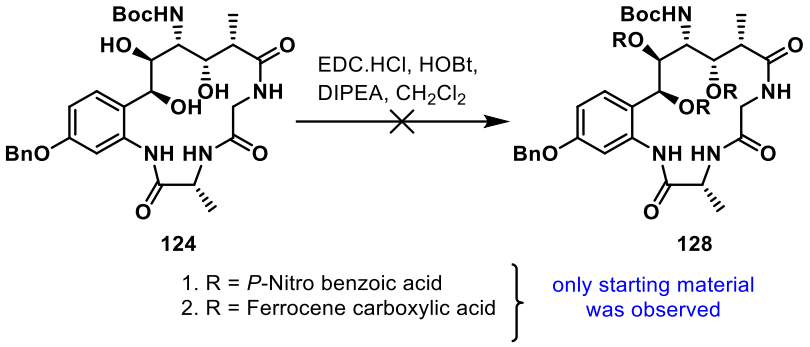
Scheme 1.20. Towards synthesis of Carbamates **126** and **126a**

2. **Acetonide synthesis:** After unsuccessful attempts to synthesize carbamate derivative next we focused on synthesis of acetonide derivative of triol **124**. For this transformation we used 2-methoxy propene in presence of catalytic amount of PTSA.H_2O in DMF but we did not observed desired formation of acetonides **127** and **127a** under this condition we observed complex reaction profile.



Scheme 1.21. Attempts towards synthesis of acetonide

3. **Ester formation:** After above failed trials next, we planned esterification reaction on triol to get crystalline material. We used 4-nitro benzoic acid and ferrocene carboxylic acid for coupling with triol **124** using EDC.HCl/DIPEA in DCM as solvent but we failed to get esters, instead we recovered starting material **124**. We also made a few attempts with corresponding acid chlorides but not successful.



Scheme 1.22. Attempts for esterification

1.1.6. Computational details

After all unsuccesfull attempts,we decided to rely on a QM/NMR approach to find out the absolute stereochemistry of C-5 configuration for solomonamide A. For that purpose, we established a collaboration with Prof. Giuseppe Bifulco, Department of Pharmacy, Italy. Briefly, this methodology is based on the comparison of the experimental NMR chemical shift data and the related values calculated at the density functional theory (DFT) for all the possible theoretical diastereoisomers of a natural product and intermediates.[40] Specifically, the isomer showing the lowest mean absolute error (MAE) values represents the solution mostly compatible with the experimental data. Accordingly, we compared the experimental ^{13}C chemical shift data of solomonamide A (**122**) with the related values calculated for C-5*R* and C-5*S* isomers (compounds **122a** and **122b**, respectively, see Figure 1.8), using the MPW1PW91/6-31g (d,p) level of theory. The predicted ^{13}C chemical shift

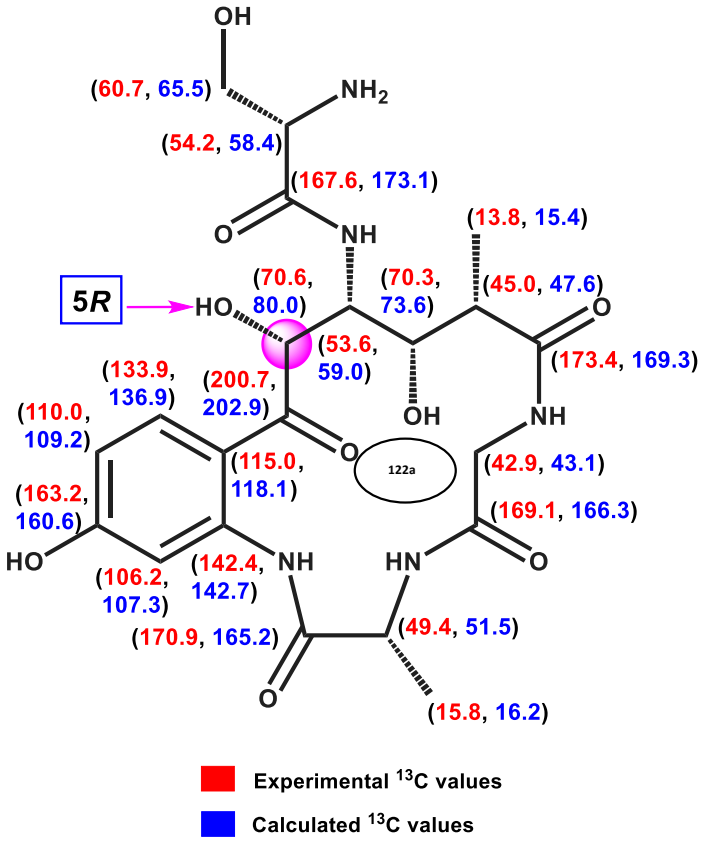


Figure 1.7. Comparison of predicted ^{13}C NMR values of **122a** with experimental values

data, compared with the set of experimental values, allowed us to assign *S* configuration at C-5 for **122**, since **122b** showed the lowest MAE value (See Table 4, Experimental section). In order to corroborate the proposed absolute configuration of **122**, we also considered its precursor **123** that differs from **122** for the absence of L-serine residue and must feature the same configuration at C-5, as reported in Scheme 1.21. Again, the related diastereoisomer of **123** featuring *S* configuration at C-5 of **123b**, was showing the higher similarity between experimental and calculated ^{13}C chemical shift data accordingly, the lower MAE values, compared to the related C-5 *R* isomer (see Table 5, Experimental section). Taken together, all these data allowed us to assign 2*S*, 3*S*, 4*R*, 5*S* absolute configuration for solomonamide A, **122** (Figure 1.7. and figure 1.8.).

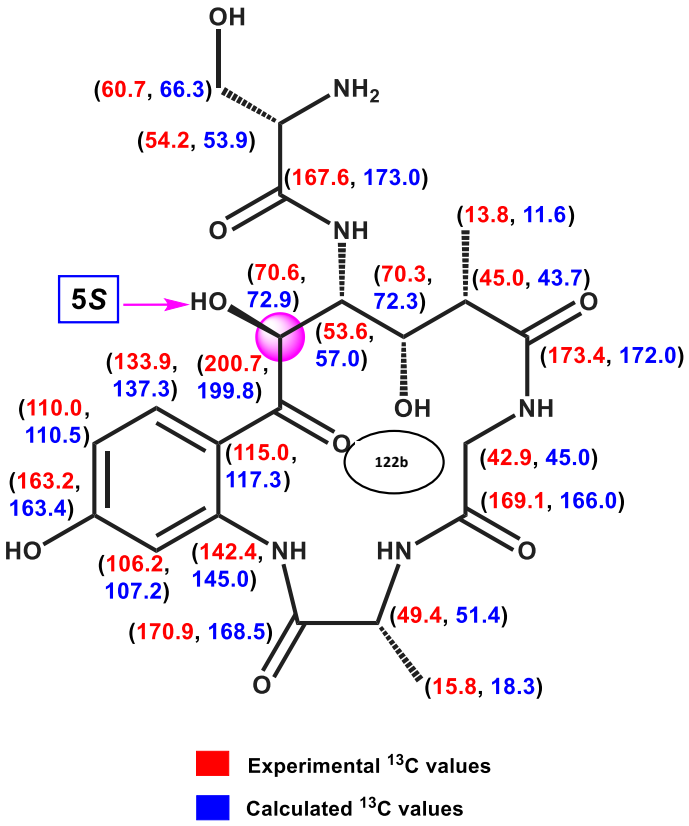


Figure 1.8. Comparison of predicted ^{13}C NMR values of **122b** with experimental values

1.1.7. Biological evaluation of solomonamide A

Further, we decided to evaluate anti-inflammatory potential of solomonamide A. For this activity, we established another collaboration with Dr. Manoj Kumar Barthwal research group, Central Drug Research Institute (CDRI), India. We have evaluated the synthesized solomonamide A in carrageenan-induced mouse paw edema model and reduction in inflammation was measured at three different concentration (0.03, 0.1 and 0.3 mg/kg; i.p.) in comparison with carrageenan alone. Dexamethasone (10 mg/kg, i.p.) was used as a reference anti-inflammatory drug. Solomonamide A (**122)** showed dose dependent anti-inflammatory effect at 4 h & 6 h. Synthesized solomonamide A at concentration of 0.1 and 0.03 mg/kg showed comparatively low inhibition at 4 h it showed around 48% and 44%,

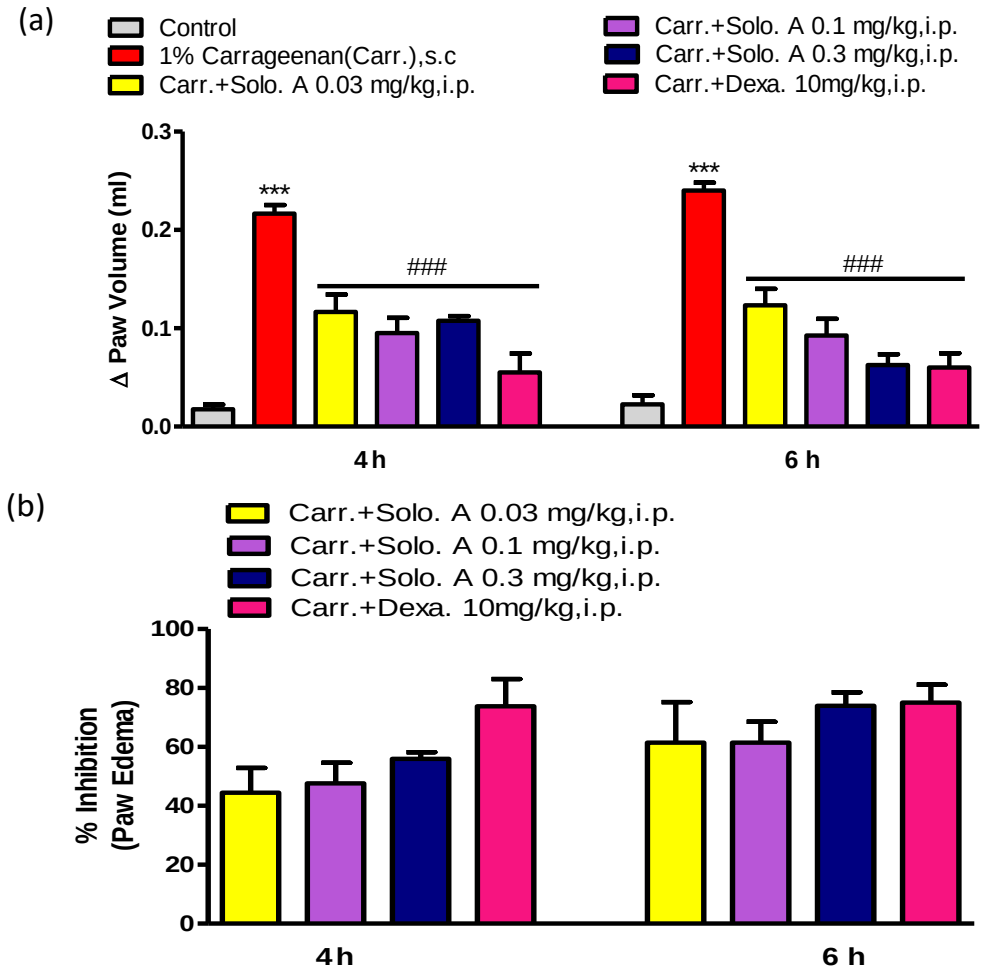
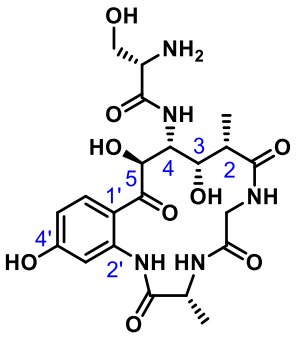


Figure 1.9. Anti-inflammatory activity of solomonamide with different concentrations of 0.03, 0.1 & 0.3 mg/kg, i.p.) and dexamethasone concentration of 10 mg/kg, i.p.) in carrageenan-induced paw edema model in mice (n = 6-10 animals in each group). 50 µL of saline or 1% of carrageenan in treatment group or saline in control group was given subcutaneously in mice hind paw. Results are shown in Mean±SEM (a) Paw volume in mL (b) Percent inhibition of paw edema.

respectively, at 6 h time interval and same concentration of solomonamide A showed inhibition of 61 and 60% under same concentration, respectively. Whereas at the concentration of (0.3 mg/kg) of solomonamide A showed 56% at 4 h while 74% inhibition at 6 h which was significantly higher as comparable to dexamethasone effect (~74% and

75% at 4 h and 6 h respectively). From this data, it is evident that synthesized solomonamide A shows potent anti-inflammatory activity which is very much in agreement with natural solomonamide A (Figure 1.9.).



Solomonamide A, 122
revised structure

Table 3. Comparison of ^{1}H and ^{13}C NMR chemical shift data of natural vs synthetic solomonamide A (**122**)

Comparison of ^{1}H NMR Values of Solomonamide A		Comparison of ^{13}C NMR Values of Solomonamide A			
Natural	Synthetic (**122**)	Residue		Natural	Synthetic (**122**)
0.98 (d, *J* = 7.1 Hz, 3H)	0.98 (d, *J* = 7.3 Hz Hz, H)	Glycine	Carbonyl	169.2	169.1
1.31 (d, *J* = 7.0 Hz, 3H)	1.32 (d, *J* = 6.7 Hz, 3H)		CH_2	42.8	42.9
2.34 (dq, *J* = 9.7, 7.1 Hz, 1H)	2.28(dq, *J* = 9.2, 7.3 Hz, 1H)	D-Alanine	Carbonyl	170.7	170.9
3.17 (ovl, 1H)	3.17 (t, *J* = 6.71 Hz, 1H)		CH	49.3	49.4
3.68 (dd, *J* = 11.4, 6.9 Hz, 1H)	3.68 (m, 1H)		Methyl	15.6	15.8

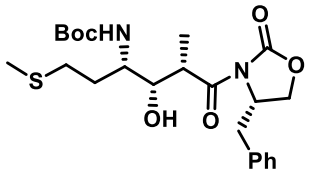
3.76 (dd, *J* = 15.3, 4.7 Hz, 1H)	3.77 (m, 1H)	L-Serine	Carbonyl	167.5	167.6
3.81 (dd, *J* = 11.4, 6.9 Hz, 1H)	3.81 (m, 1H)		CH	54.2	54.2
3.90 (dd, *J* = 15.3, 6.6 Hz, 1H)	3.98 (brs, 1H)		CH_2	60.7	60.7
4.02 (m, 1H)	4.00 (m, 1H)	AHMOA	1 Carbonyl	173.4	173.4
4.43 (quint, *J* = 7.6 Hz, 1H)	4.43 (quint, *J* = 7.3 Hz, 1H)		2 CH attached to CH_3	44.5	45.0
4.54 (t, *J* = 9.6, 1H)	4.53 (t, *J*=9.77 Hz, 1H)		3 CH attached to OH	70.3	70.3
4.75 (d, *J* =9.9 Hz, 1H)	4.76 (d, *J* = 9.16 Hz, 1H)		4 CH attached to NH	53.5	53.6
5.26 (brs, 1H)	5.32 (brs, 1H) OH Proton		5 CH attached to OH	70.5	70.6
5.39 brs	OH Proton		6 Keto carbonyl	201.1	200.7
5.53 (brs, 1H)	5.52 (brs, 2H)		7 Methyl	13.8	13.8
6.57 (dd, *J* = 8.8 Hz, 2.3, 1H)	6.58 (d, *J* = 8.5 Hz, 1H)	Aromatic	1’	115.2	115.0
7.49(brt, *J* = 5.5 Hz, 1H)	NH Proton		2’	141.8	142.4
7.86 (d, *J* = 8.7 Hz, 1H)	7.87 (d, *J* = 8.5 Hz, 1H)		3’	106.3	106.2
7.89 (d, *J* = 9.6 Hz, 1H)	7.89 (brs, 1H)		4’	162.8	163.2
8.02 (d, *J* = 2.3 Hz, 1H)	8.04 (brs, 1H)		5’	109.8	110.0
8.10 (brd, *J* = 4.3 Hz, 2H)	8.10 (brs, 2H)		6’	133.6	133.9
8.77 (d, *J* = 7.9 Hz, 1H)	8.75 (d, *J* = 7.93, 1H)				

1.1.8. Conclusions

We have accomplished the total synthesis of the revised structure of solomonamide B for the first time by changing the stereochemistry at C-3 and C-4 positions. It was proposed in literature that solomonamide A was biogenetically derived from solomonamide B, which makes the structural revision of solomonamide A inevitable. Accordingly, we have accomplished the total synthesis of the solomonamide A for the first time. During synthesis of solomonamides we used an interesting Evans aldol reaction, ligand free intramolecular Heck reaction, regioselective Wacker oxidation, Upjohn dihyroxylation and chemoselective benzylic oxidation. After completion of total synthesis of solomonamide A the fixing of stereochemistry at C5-hydroxy position was a challenging task which was accomplished with the help of DFT calculations, applying a quantum-mechanical (QM)/NMR combined approach. The revised structure was further supported by biological evaluation in anti-inflammatory mouse model.

1.1.9. Experimental section

***tert*-Butyl ((3*S*,4*S*,5*S*)-6-((*S*)-4-benzyl-2-oxooxazolidin-3-yl)-4-hydroxy-5-methyl-1-(methylthio)-6-oxohexan-3-yl)carbamate**



114 (major)

To a stirred solution of oxazolidinone **110** (7.0 g, 30.0 mmol) in CH_2Cl_2 (70 mL) was added Bu_2BOTf (33 mL, 33.0 mmol) and DIPEA (6.2 mL, 36.0 mmol) at 0 ^{0}C. After stirring for 1 h, the solution was cooled to -78 °C and maintained at same temprature for 30 min. A solution containing aldehyde **109** (7.7 g, 33.0 mmol) in CH_2Cl_2 (30 mL) was added dropwise and the solution was allowed to warm slowly to room temperature and stirred for

overnight. The reaction was quenched by the addition of aqueous ammonium chloride solution (10 mL) at 0 ^{0}C and then H_2O (30 mL) was added to the reaction mixture. The mixture was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic extracts were dried over anhydrous sodium sulphate, filtered, and concentrated in vacuo. The organic residue was purified by column chromatography (silica gel 230-400 mesh 15% ethyl acetate-pet ether) to afford **114** and **114a** with 7:3 Diastereomeric ratio with 65% yield.

Data for compound **114**

IR υ_{max}(film): 3439, 3019, 2980, 1781, 1693, 1502 cm^{-1}

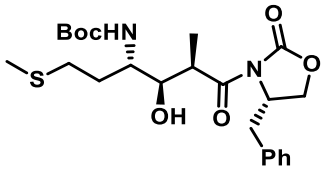
Specific rotation $[\alpha]_D^{26}$ = + 44.48 (*c* 0.95, CH_3OH)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35 - 7.21 (m, 5H), 4.78 (t, J = 10.0 Hz, 2H), 4.32 (t, J = 8.1 Hz, 1H), 4.14 (d, J = 8.8 Hz, 1H), 3.89 (brs,1H), 3.81 - 3.71 (m, 2H), 3.25 - 3.22 (m, 1H), 2.82 (dd, J = 9.5, 13.0 Hz, 1H), 2.60 - 2.50 (m, 2H), 2.47 - 2.46 (m, 1H), 2.12 (s, 3H), 1.86 (q, J = 7.5 Hz, 2H), 1.40 (s, 9H), 1.38 (d, *J* = 6.4 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 175.8, 156.3, 153.2, 135.4, 129.4, 128.9, 127.3, 79.6, 74.7, 66.4, 55.3, 51.8, 40.9, 38.1, 32.8, 30.8, 28.3, 15.6, 14.8

HRMS (ESI): calculated for $C_{23}H_{34}O_6N_2NaS$ $[M+Na]^+$: 489.2030, found 489.2027.

Data for tert-butyl ((3*S*,4*R*,5*R*)-6-((*S*)-4-benzyl-2- oxooxazolidin-3-yl)-4-hydroxy-5-methyl-1-(methylthio)-6-oxohexan-3-yl)carbamate



114a (minor)

IR υ_{max}(film): 3443, 3014, 2995, 1788, 1685, 1508 cm^{-1}

Specific rotation $[\alpha]_D^{26}$ = + 66.87 (*c* 0.59, CH_3OH)

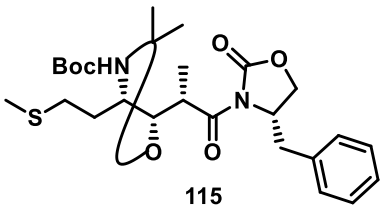
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33 - 7.17 (m, 5H), 4.67 - 4.65 (m, 2H), 4.23 - 4.15 (m, 2H), 3.93(s, 1H), 3.84 - 3.82 (m, 1H), 3.78 - 3.73 (m, 1H), 3.23 (d, J = 13.3 Hz, 1H), 20

2.78 - 2.73 (m, 1H), 2.57 - 2.50 (m, 2H), 2.10 - 2.08 (m, 1H), 2.07 (s, 3H), 1.65- 1.63 (m, 1H), 1.41 (s, 9H), 1.30 (d, *J* = 6.4 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 177.0, 155.7, 152.8, 135.0, 129.4, 128.9, 127.4, 79.7, 74.0, 66.2, 55.1, 51.8, 39.6, 37.7, 30.9, 30.6, 28.3, 15.6, 11.1

HRMS calculated for $C_{23}H_{34}O_6N_2NaS$ $[M+Na]^+$: 489.2030, found 489.2027.

***tert*-butyl (5*R*)-5-((*S*)-1-((*S*)-4-benzyl-2-oxooxazolidin-3-yl)-1-oxopropan-2-yl)-2,2-dimethyl-4-(2-(methylthio)ethyl)oxazolidine-3-carboxylate**



115

To a stirred solution of compound **114** (6.0 g, 12.87 mmol), and 2-methoxypropene (2.78 mL, 38.62 mmol) in dry DMF (20 mL) PTSA.H_2O (442 mg, 2.57 mmol) was added at 0 °C under argon atmosphere. The resulting solution was stirred at room temperature for 4 h. The reaction was then diluted with H_2O (5 mL) and extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with cold saturated $NaHCO_3$ solution (15 mL), H_2O (10 mL), brine (10 mL), and evaporated in vacuo. Purification by column chromatography (silica gel 100-200 mesh 10% ethyl acetate-pet ether) yielded compound **115** (5.5 g) as a pale yellow liquid.

Yield: 85%

IR υ_{max}(film): 3384, 3020, 2982, 1689, 1525, 1390 cm^{-1}

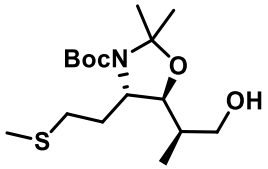
Specific rotation $[\alpha]_D^{26}$ = + 35.76 (*c* 0.43, CH_3OH)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37 - 7.21 (m, 5H), 4.69 (s, 1H), 4.30 (d, *J* = 8.1 Hz, 1H), 4.20 (d, *J* = 8.8 Hz, 1H), 4.11 (brs,1H), 3.85 - 3.83 (m, 2H), 3.23 - 3.20 (m, 1H),2.83 (dd, *J* = 9.5, 13.0 Hz, 1H), 2.78 - 2.67 (m, 2H), 2.45 - 2.10 (m, 3H), 1.92 - 1.91 (m, H), 1.65 - 1.61 (m, 6H), 1.51 (s, 9H), 1.38 (d, *J* = 6.4 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.1, 152.9, 152.0, 135.1, 129.5, 129.0, 127.4, 94.0, 81.2, 80.5, 66.2, 61.3, 55.0, 42.2, 37.7, 32.6, 30.4, 28.8, 28.4, 15.3, 14.7, 14.3.

HRMS (ESI): calculated for $C_{26}H_{38}O_8N_2S$ $[M+H]^+$: 507.6610, found 507.6614

***tert*-Butyl (4*R*,5*R*)-5-((*S*)-1-((S)-4-benzyl-2-oxooxazolidin-3-yl)-1-oxopropan-2-yl)-2,2- dimethyl-4-(2-(methylthio)ethyl)oxazolidine-3-carboxylate**



116

The acetonide protected (5.5 g, 10.8 mmol) compound **115** was taken in dry THF (30 mL) at 0 °C, $LiBH_4$ (0.70 g, 32.4 mmol) was added portion wise under argon atmosphere stirred at room temperature for 5 h. Excess $LiBH_4$ was decomposed with 1N HCl and extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with cold saturated brine (10 mL), dried over Na_2SO_4 and evaporated in vacuo. The organic residue was purified by column chromatography (silica gel 230-400 mesh 15% ethyl acetate – pet ether) to afford **116** as a oil.

Yield: 87% .

IR υ_{max}(film): 3384, 3020, 2982, 1689, 1525, 1390 cm^{-1}

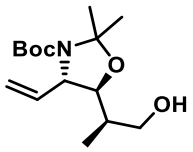
Specific rotation $[\alpha]_D^{26}$ = + 15.86 (*c* 0.51, CH_3OH)

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.91 - 3.89 (m, 2H), 3.62 (dd, *J* = 1.6, 5.3 Hz, 2H), 2.52 - 2.45 (m, 2H), 2.11 (s, 3H), 1.98 - 1.81 (m, 2H), 1.47 (s, 15H), 1.01 (d, *J* = 6.9 Hz, 3H); **^{13}C**

^{13}C NMR (100 MHz, $CDCl_3$): δ 152.0, 94.0, 81.2, 80.1, 66.0, 59.0, 38.4, 31.9, 30.4, 28.5, 27.5, 27.3, 15.6, 11.7;

HRMS (ESI): calculated for $C_{16}H_{31}O_4NNaS$ $[M+Na]^+$: 356.1866, found 356.1861

***tert*-Butyl (4S,5S)-5-((R)-1-hydroxypropan-2-yl)-2,2-dimethyl-4-vinyloxazolidine-3-carboxylate**



117

To a solution of compound **116** (3.0 g, 9.0 mmol) in CH_2Cl_2 (60 mL) Ozone was bubbled at -78 °C until the colour becomes blue, once the blue color appears oxygen was bubbled to remove excess ozone, then reaction mixture was allowed to warm to room temperature and stirred for 1 h. Concentrated the reaction mixture, here sulfur got oxidized to sulfoxide. The crude sulfoxide compound was taken in 1,2 dichloro benzene (30 mL), $CaCO_3$ (2.7 g, 27.0 mmol) was added and refluxed for 6 h. The crude reaction mixture was purified by column chromatography (silica gel 230-400 mesh 20% ethyl acetate - CH_2Cl_2) to afford **117.**

Yield: 32% for 2 steps

IR υ_{max}(film): 3401, 3020, 2934, 1688, 1529, 1390 cm^{-1}

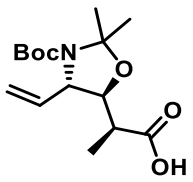
Specific rotation $[\alpha]_D^{26}$ = - 29.01 (*c* 0.63, CH_3OH)

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.72 - 5.64 (m, 2H), 5.18 - 5.16 (m, 1H), 4.04 (brs, 1H), 3.90 (dd, *J* = 3.3, 8.0 Hz, 1H), 3.65 (d, *J* = 5.0 Hz, 2H), 1.96 - 1.91 (m,1 H), 1.58 - 1.50 (brs, 6H), 1.43 (s, 9H), 1.00 (d, *J* = 7.0 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 152.1, 138.3, 117.0, 94.3, 80.7, 80.2, 66.3, 62.3, 45.1, 35.8, 29.7, 28.4, 26.2, 25.6, 10.8

HRMS (ESI): $C_{15}H_{27}O_4NNa$ $[M+Na]^+$: 308.1832, found 308.1831.

(*S*)-2-((4*S*,5*S*)-3-(tert-Butoxycarbonyl)-2,2-dimethyl-4-vinyloxazolidin-5-yl)propanoic acid



108

To a stirred solution of compound **117** (0.65 g, 2.2 mmol) in DMF (6 mL), Pyridinium dichromate (PDC) (2.54 g, 6.84 mmol) was added and stirred at room temperature for 4 h. To the reaction mixture water (5 mL) was added and extracted with ethyl acetate (20 mL x 2), combined the organic layers and washed with brine (5 mL) concentrated under reduced pressure. Purification by column chromatography (silica gel 100-200 mesh 30% ethyl acetate – per ether) afforded acid **108**.

Yield: 82%

IR υ_{max}(film): 3342, 3020, 2935, 1688, 1523, 1458 cm^{-1}

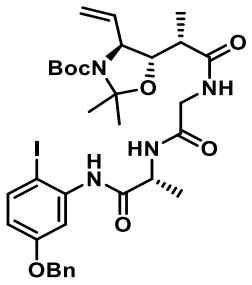
Specific rotation $[\alpha]_D^{26}$ = - 15.77 (*c* 0.38, CH_3OH)

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.75 - 5.72 (m, 1H), 5.18(d, *J* = 8.3 Hz, 2H), 4.10 - 4.07 (m, 2H), 2.72 - 2.67 (m, 1H), 1.59 (s, 3H), 1.53 (s, 3H), 1.43 (s, 9H), 1.29 (d, *J* = 6.8 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 179.1, 152.0, 137.4, 117.1, 94.6, 79.6, 63.4, 41.7, 28.4, 26.5, 26.3, 11.9

HRMS (ESI): $C_{15}H_{25}O_5NNa$ $[M+Na]^+$: 322.1625, found 322.1625.

***tert*-Butyl (4*S*,5*S*)-5-((*S*)-1-((2-(((*R*)-1-((5-(benzyloxy)-2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-1-oxopropan-2-yl)-2,2-dimethyl-4-vinyloxazolidine-3- carboxylate**



118

To a solution of **97** (665 mg, 1.2 mmol) in CH_2Cl_2 (5 mL), TFA (1.0 mL) was added at 0 °C and stirred at 25 °C for 3 h. After completion of the reaction (monitored by TLC), the reaction mixture was concentrated under reduced pressure to afford the amine as TFA salt. Compound **108** (300 mg, 1.0 mmol) was taken in dry DMF (5 mL), added above amine salt, then HATU (457 mg, 1.2 mmol), DIPEA (0.43 mL, 2.5 mmol) were added and the

resulting solution was stirred at ambient temperature for 16 h. Reaction mass was diluted with ethyl acetate (50 mL) washed with saturated solution of aq $NaHCO_3$ (5 mL), brine (5 mL) and then evaporated to dryness. Purification by column chromatography (silica gel 230-400 mesh 4% methanol - CH_2Cl_2) yielded compound **118** (00 mg, 57 %, for 2 steps) as an off white solid.

Yield: 57%

Melting point = 89- 90 °C

IR υ_{max}(film): 3021, 1666, 1586, 1518, 1420, 1215 cm^{-1}

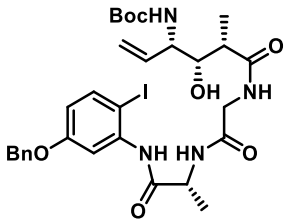
Specific rotation $[\alpha]_D^{26}$ = + 22.89 (*c* 0.21, CH_3OH)

^{1}H NMR (400 MHz, CD_3OD): δ 7.73 - 7.67 (m, 1H), 7.59 (brs, 1H), 7.42 - 7.20 (m, 7H), 6.72 - 6.65 (m, 1H), 5.89 (ddd, *J* = 5.4, 11.0, 16.9 Hz, 1H), 5.21 - 5.13 (m, 2H), 5.05 (s, 2H), 4.65 - 4.52 (m, 1H), 4.14 (brs, 1H), 4.04 - 4.00 (m, 2H), 3.85 (s, 1H), 3.81 - 3.76 (m, 1H), 3.05 - 2.92 (m, 2H), 2.55 - 2.50 (m, 1H), 1.54 - 1.43 (m, 9H), 1.38 (s, 9H), 1.24 (d, *J*= 6.8 Hz, 3H)

^{13}C NMR (100 MHz, CD_3OD): δ 179.0, 173.5, 173.2, 172.3, 170.3, 164.3, 161.1, 158.3, 140.7, 140.5, 138.8, 138.3, 129.7, 129.2, 128.8, 116.1, 115.5, 114.4, 112.5, 83.8, 82.2, 80.8, 75.9, 71.4, 71.3, 56.7, 51.3, 47.4, 45.1, 44.5, 40.3, 29.0, 17.5, 15.4.

HRMS (ESI): calculated for $C_{33}H_{43}O_7N_4NaI$ $[M+Na]^+$: 757.2069, found 757.2044.

***tert*-Butyl ((3*S*,4*S*,5*S*)-6-((2-(((*R*)-1-((5-(benzyloxy)-2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-4-hydroxy-5-methyl-6-oxohex-1-en-3-yl)carbamate**



107

To a solution of compound **118** (500 mg, 0.6 mmol) in methanol (4 mL), Camphorsulphonic acid (31 mg, 0.3 mmol) was added and stirred for 18 h. reaction was

monitored by TLC showed only 50% conversion. Concentrated the reaction mixture diluted with ethyl acetate (30 mL), washed with saturated solution of aq $NaHCO_3$ (5 mL), brine (5 mL) and then evaporated to dryness. Purification by column chromatography (silica gel 230-400 mesh 4 - 6 % methanol - CH_2Cl_2) yielded starting material **118** (240 mg) and compound **107** (190 mg, 40% yield, 77%, brsm) as an off white solid.

Yield: Isolated 41% and 82% brsm

Melting point = 92- 93 °C

IR υ_{max}(film): 3322, 3020, 1679, 1585, 1520 cm^{-1}

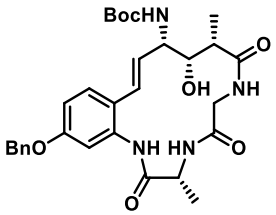
Specific rotation $[\alpha]_D^{26}$ = +12.13 (*c* 0.70, CH_3OH)

^{1}H NMR (400 MHz, CD_3OD): δ 7.69 (d, *J* = 8.6 Hz, 1H), 7.59 (s, 1H), 7.38 - 7.30 (m, 2H), 7.36 (t, *J* = 7.4 Hz, 2H), 7.33 - 7.28 (m, 1H), 6.67 (dd, *J* = 2.7, 8.8 Hz, 1H), 5.88 (ddd, *J* = 5.3, 10.7, 16.8 Hz, 1H), 5.21 - 5.13 (m, 2H), 5.06 (s, 2H), 4.63 (m, 1H), 4.14 (brs, 1H), 4.03 - 4.00 (d, *J* = 17.2 Hz, 1H), 3.84 - 3.35 (m, 2H), 2.53 - 2.50 (m, 1H), 1.49 (d, *J* = 7.2 Hz, 3H), 1.38 (s, 9H), 1.22 (d, *J* = 6.9 Hz, 3H)

^{13}C NMR (100 MHz, CD_3OD): δ 177.4, 171.7, 170.8, 159.6, 156.7, 139.0, 138.9, 137.3, 136.8, 128.2, 127.6, 127.2, 114.5, 114.1, 111.0, 80.7, 79.2, 74.4, 69.8, 55.2, 49.8, 43.6, 42.9, 27.5, 16.0, 13.8

HRMS (ESI): calculated for $C_{30}H_{39}O_7N_4NaI$ $[M+Na]^+$: 717.1756, found 717.1755.

***tert*-Butyl((3*R*,9*S*,10*R*,11*R*,*E*)-16-(benzyloxy)-10-hydroxy-3,9-dimethyl-2,5,8-trioxo2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)carbamate**



106

To a solution of compound **107** (120 mg, 0.17 mmol) in anhydrous acetonitrile (120 mL), $Pd(OAc)_2$ (5 mol%) and triethylamine (0.24 mL, 1.7 mmol) were added and heated at 75 °C for 12h. The reaction mixture was concentrated in vacuo. Purification by column chromatography (silica gel 230-400 mesh 4% methanol - CH_2Cl_2) yielded compound **106** as an off white solid.

Yield: 48%

Melting point = 136- 139 °C

IR υ_{max}(film): 3384, 3021, 2401, 1657, 1523, 1423cm^{-1}

Specific rotation $[\alpha]_D^{26}$ = + 9.28 (*c* 0.56, CH_3OH)

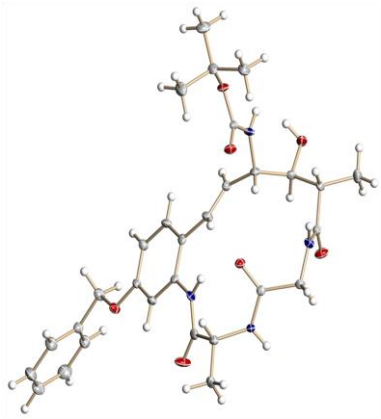
^{1}H NMR (500 MHz, CD_3OD): δ 7.46 - 7.42 (m, 3H), 7.36 (t, *J* = 6.7 Hz, 3H), 7.30 (d, *J* = 6.5 Hz, 1H), 6.99 (brs, 1H), 6.88 (d, *J* = 8.0 Hz, 1H), 6.61 (s, 1H), 5.96 (brs, 1H), 5.07 (s, 2H), 4.38 (d, *J* = 6.5 Hz, 2H), 4.08 (brs, 1H), 3.70 (brs, 1H), 3.65 - 3.60 (m, 1H), 2.60 (t, *J* = 7.1 Hz, 1H), 1.48 (d, *J* = 6.9 Hz, 3H), 1.45 (brs, 9H), 1.09 (d, *J* = 6.5 Hz, 3H)

^{13}C NMR (125 MHz, CD_3OD): δ 177.9, 174.1, 172.8, 160.1, 158.3, 138.6, 136.7, 129.7, 129.4, 129.1, 128.7, 128.4, 127.6, 126.6, 115.1, 113.8, 80.4, 77.5, 71.3, 59.5, 51.9, 46.8, 44.5, 28.9, 17.0, 15.0.

HRMS (ESI): calculated for $C_{30}H_{38}O_7N_4Na$ $[M+Na]^+$: 589.2633, found 589.2635.

X-ray Crystal Structure Details of compound 106

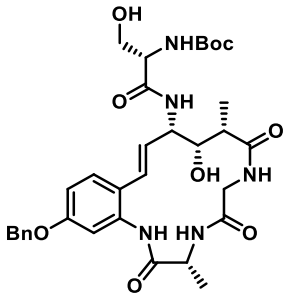
Single crystals of compound **106** were obtained from (ethyl acetate- hexane) by slow evaporation methd. X-ray intensity data were collected on a Bruker SMART APEX II CCD diffractometer with graphite-monochromatized (Mo Kα=0.71073 Å) radiation at temperatures 150(2) K respectively. The X-ray generator was operated at 50 kV and 30 mA. A preliminary set of cell constants and an orientation matrix were calculated from three sets of 12 frames (total 36 frames). Diffraction data were collected with a with a frame time of 25 sec keeping theθ and 2φ scan width of 0.5° and at different settings of ω sample-to-detector distance fixed at 5.00 cm. The X-ray data acquisition was monitored by APEX II program suite. All the data were corrected for Lorentz-polarization and absorption effects using SAINT and SADABS programs integrated in APEX II program package.



ORTEP diagram of compound **106**

The structures were solved by direct method and refined by full matrix least squares, based on F 2, using SHELX-97. Molecular diagrams were generated using XSHELL program integrated in SHELXTL package. Crystallographic data of **106** ($C_{30}H_{38}N_4O_7$): M = 566.64, Crystal dimensions 0.44 x 0.29 x 0.19 mm3 =β, monoclinic, space group C2, a = 28.205(7), b = 5.7022(12), c = 17.974(4) Å, 91.077(4)°, V = 2932.5(12) Å3 calcd = 1.383 gcm–3ρ, Z = 4,) = 0.092 mm–1α, μ (Mo-K , F(000) = , T = 150(2) K, 18488 reflections collected, 7297 unique reflections°max = 57.58θ1208, 2 (Rint=0.1314), 4170 observed (I (I)) reflections, 376 refined parameters, R value 0.0817,σ> 2 wR2 = 0.1554, (all data R = 0.1443, wR2 = 0.1807), S = 0.958, minimum and maximum transmission 0.961 and 0.983; maximum and minimum residual electron densities +0.44 and – 25 0.38 e Å-3. All the H-atoms were placed in geometrically idealized position and constrained to ride on their parent atoms. Crystallographic data for compound **106** deposited with the Cambridge Crystallographic Data Centre as supplementary publication no.CCDC 1478861

***tert*-Butyl ((S)-1-(((3*R*,9*S*,10*S*,11*S*,*E*)-16-(benzyloxy)-10-hydroxy-3,9-dimethyl-2,5,8-trioxo2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)amino)-3- hydroxy-1-oxopropan-2-yl)carbamate**



120

To a solution of compound **106** (95 mg, 0.17 mmol) in CH_2Cl_2 (5 mL) trifluoro acetic acid (1.0 mL) was added at 0 °C and the resulting suspension was stirred for 2 h at the same temperature. Reaction was monitored by TLC, and then concentrated. This residue was dissolved in dry DMF (3 mL), then HATU (74 mg, 0.19 mmol), DIPEA (96 µL, 0.44 mmol) and *N*-(tert-butoxycarbonyl)-L-serine **119** (40 mg, 0.19 mmol) was added. The resulting solution was stirred at ambient temperature for 16 h. Reaction mass was diluted with ethyl acetate (15 mL), washed with saturated solution of $NaHCO_3$ (5 mL), H_2O (5 mL). The organic layer was dried over Na_2SO_4 and the crude material obtained after removal of the solvent was purified by column chromatography (silica gel 230-400 mesh 4% methanol - CH_2Cl_2) to afford **120** as off white solid.

Yield: 67%

Melting point = 98-99 °C

Specific rotation $[\alpha]_D^{26}$ = + 16.77 (*c* 0.28, CH_3OH)

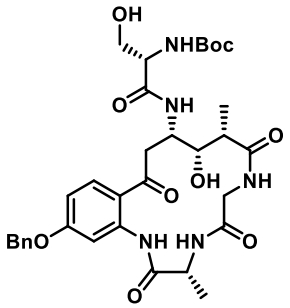
^{1}H NMR (400 MHz, CD_3OD): δ 7.46 - 7.30 (m, 6H), 6.95 - 6.88 (m, 2H), 6.64 (d, *J* = 8.7 Hz, 1H), 6.09 – 6.03 (m, 1H), 5.07 (s, 2H), 4.71 (d, *J* = 7.3 Hz, 1H), 4.36 - 4.32 (m, 1H), 4.16 - 4.14 (m, 1H), 4.02 (d, *J* = 14.7 Hz, 1H), 3.79 - 3.69 (m, 4H), 2.50 (dd, *J* = 7.1, 9.0 Hz, 1H), 1.48 (d, *J* = 7.1 Hz, 3H), 1.45 (s, 9H), 1.13 (d, *J* = 6.4 Hz, 3H)

^{13}C NMR (100 MHz, CD_3OD): δ 176.5, 172.9, 171.4, 171.3, 158.7, 137.0, 135.3, 128.3, 128.1, 127.5, 127.2, 126.1, 125.3, 113.7, 112.2, 79.6, 75.6, 69.7, 61.9, 56.9, 56.3, 50.5, 45.5, 42.5, 27.3, 15.4, 13.6

HRMS (ESI): calculated for $C_{33}H_{43}O_9N_5Na$ $[M+Na]^+$: 676.2953, found 676.2945.

***tert*-Butyl ((*S*)-1-(((3*R*,9*S*,10*S*,11*S*)-16-(benzyloxy)-10-hydroxy-3,9-dimethyl-2,5,8,13-tetraoxo-2,3,4,5,6,7,8,9,10,11,12,13-dodecahydro-1H-benzo[h][1,4,7]triazacyclo pentadecin11-yl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamate**

To a stirred solution of $PdCl_2$ (10 mol%), CuCl (9 mg, 0.09 mmol) in DMF-water (5 mL, 2:1) compound **120** (75 mg, 0.09 mmol) was added and heated at 65 °C under O_2 atmosphere for 8 h. The reaction mixture was diluted with ethyl acetate (20 mL) and washed water (10 mL) and brine (10 mL) organic layer was separated, dried over Na_2SO_4, concentrated



121

under reduced pressure. Purification by column chromatography (silica gel 230-400 mesh 6% methanol - CH_2Cl_2) yielded compound **121** (58 mg) as a white color solid.

Yield: 71%

Melting point = 258-260 °C

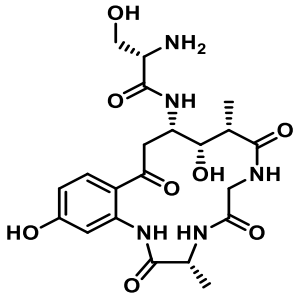
Specific rotation $[\alpha]_D^{26}$ = + 10.76 (*c* 0.13, CH_3OH)

^{1}H NMR (500 MHz, DMSO-d_6): δ 11.99 (brs, 1H), 8.80 (d, *J* = 6.1 Hz, 1H), 8.15 (s, 1H), 7.87 (d, *J* = 8.4 Hz, 1H), 7.47 - 7.27 (m, 7H), 7.28 (d, *J* = 7.1 Hz, 1H), 6.99 (d, *J* = 7.6 Hz, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 5.49 (brs, 1H), 5.21 (s, 2H), 5.09 (s, 1H), 4.82 (brs, 1H), 4.34 (d, *J* = 6.9 Hz, 1H), 4.18 - 4.10 (m, 27 1H), 3.91 - 3.81 (m, 2H), 3.79 (d, J = 15.3 Hz, 2H), 3.60 - 3.28 (m, 2H), 2.87(d, *J* = 17.1 Hz, 1H), 2.34 - 2.20 (m, 1H), 1.41 (s, 9H), 1.37 (d, *J* = 6.5 Hz, 3H), 1.02 (m, d, *J* = 6.8 Hz, 3H)

^{13}C NMR (125 MHz, DMSO-d_6): δ 201.6, 173.5, 171.3, 169.9, 169.3, 162.7, 155.3, 141.0, 136.3, 133.9, 128.5, 128.4, 128.6, 128.1, 127.7, 127.6, 117.4, 109.1, 105.8, 78.3, 72.2, 57.5, 49.9, 46.01, 43.1, 42.9, 28.2, 16.1, 13.8.

HRMS (ESI): calculated for $C_{33}H_{43}O_{10}N_5Na$ $[M+Na]^+$: 692.2902, found 692.2892.

(*S*)-2-Amino-N-((3*R*,9*S*,10*S*,11*S*)-10,16-dihydroxy-3,9-dimethyl-2,5,8,13-tetraoxo 2,3,4,5,6,7,8,9,10,11,12,13-dodecahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)-3- hydroxypropanamide



Revised Structure of Solomonamide B, **105**

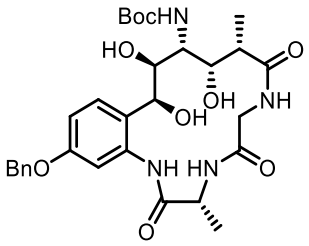
To a solution of compound **120** (9 mg, 0.013 mmol) in methanol (3 mL), 10% Pd/C (~ 3 mg) was added and stirred under H_2 atmosphere for 2 h. The reaction mixture was then filtered through silica gel column, concentrated to afford phenolic compound. The phenolic compound was dissolved in CH_2Cl_2 (3 mL), TFA (0.3 mL) was added at 0 °C and the resulting suspension was stirred for 2 h at the same temperature. Concentrated the reaction mixture and azeotroped with diethyl ether (3 mL x 3) and dried under vacuum to afford compound **105** (4.5 mg) as off white solid.

Yield: 70% for two steps

Specific rotation $[\alpha]_D^{26}$ = + 9.99 (*c* 0.23, CH_3OH)

HRMS (ESI): calculated for $C_{21}H_{30}O_8N_5$ $[M+H]^+$: 480.2089, found 480.2086.

***tert*-Butyl ((3*R*,9*S*,10*S*,11*R*)-16-(benzyloxy)-10,12,13-trihydroxy-3,9-dimethyl-2,5,8-trioxo-2,3,4,5,6,7,8,9,10,11,12,13-dodecahydro-1H-benzo[h][1,4,7]triazacyclopentadecin -11-yl)carbamate**



124

To a solution of compound **106** (175 mg, 0.308 mmol) in *t*-BuOH-H_2O (2 ml, 1:1), NMO (54.2 mg, 0.463 mmol) and OsO_4 (94 µL, 2.5 % solution in *t*-BuOH) was added at 0 °C and stirred for 6 h. After completion of reaction *t*-BuOH was removed under vacuum, reaction mixture was diluted with EtOAc (5 mL) washed with saturated sodium sulfite solution (4 mL) and brine (4 ml). Organic layer was separated, dried under vacuum. Purification by column chromatography (silica gel 230-400 mesh 4% methanol - CH_2Cl_2) yielded compound **124** (138 mg) as white solid as single diastereomer.

Yield: 74%

Melting point = 121-123 °C

IR υ_{max}(film): 3646, 3282, 2926, 1626, 1528, 1453 cm^{-1}

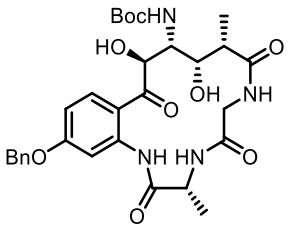
Specific rotation $[\alpha]_D^{22}$ = - 26.23 (*c* 0.81, CH_3OH)

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.89 (brs, 1H), 8.16 (brs, 1H), 7.45 - 7.31 (m, 7H), 6.87 - 6.85(d, *J* = 3.7 Hz, 1H), 5.07 (s, 2H), 5.03 - 5.01 (m, 1H), 4.71 (brs, 1H), 4.61 (d, *J* = 3.7 Hz, 1H), 4.54 (brs, 1H), 4.41 (brs, 1H), 4.10 (d, *J* = 6.71, 1H), 4.06 (d, *J* = 6.1 Hz, 1H), 3.97 (brs, 1H), 3.83 (d, *J* = 7.9 Hz, 1H), 3.71(d, *J* = 6.10 Hz, 1H), 3.63 - 3.59 (m, 2H), 3.26 (d, *J* = 12.2 Hz, 1H), 2.29 - 2.22 (m, 1H), 1.40 (s, 9H), 1.30 (d, *J* = 6.7 Hz, 3H), 0.98 (d, *J* = 6.1 Hz, 3H)

^{13}C NMR (100 MHz, CD_3OD): δ178.3, 172.2, 159.8, 158.3, 138.7, 129.6, 129.0, 128.7, 81.5, 78.1, 71.2, 63.4, 59.1, 45.4, 28.9, 19.0, 14.5

HRMS (ESI): calculated for $C_{30}H_{40}O_9N_4Na$ $[M+Na]^+$: 623.2679, found 623.2687.

***tert*-Butyl ((3*R*,9*S*,10*S*,11*R*)-16-(benzyloxy)-10,12-dihydroxy-3,9-dimethyl-2,5,8,13-tetraoxo-2,3,4,5,6,7,8,9,10,11,12,13-dodecahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)carbamate**



123

To a solution of compound **124** (0.110 g, 0.183 mmol) in dry CH_2Cl_2 at 0 °C solid $NaHCO_3$ (0.019 g, 0.219 mmol) was added followed by addition of Dess–Martin periodinane (0.077 g, 0.183 mmol) under nitrogen atmosphere. The resulting mixture was stirred at room temperature for 3h. After completion of the reaction (TLC analysis), saturated $NaHCO_3/Na_2SO_3$ solution (1:1, 20 mL) was added, the aqueous phase was separated and extracted with CH_2Cl_2 (2 × 20 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated. Purification of the residue by flash chromatography gave ketone **123** (74 mg) as white solid.

Yield: 67%

Melting point = 138-140 °C

IR υ_{max}(film): 3465, 3285, 2929, 1659, 1630, 1529, 1450 cm^{-1}

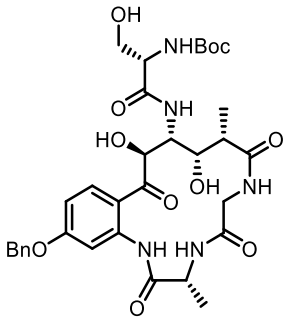
Specific rotation $[\alpha]_D^{22}$ = -7.12 (*c* 0.81, CH_3OH)

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.49 (brs, 1H), 8.72 (d, *J* = 7.3 Hz, 1H), 8.17 (brs, 1H), 7.98 (d, *J* = 7.9 Hz, 1H), 7.91 (d, *J* = 8.5 Hz, 1H), 7.70 (d, *J* = 7.3 Hz, 1H), 7.49 - 7.35 (m, 6H), 7.25 - 7.21 (m, 1H), 6.85 (d, *J* = 8.5 Hz, 1H), 5.60 (d, *J* = 9.8 Hz, 1H), 5.53 (d, *J* = 7.9 Hz, 1H), 5.21 (s, 2H), 5.01 (d, *J* = 6.7 Hz, 1H), 4.68 (t, *J* = 8.5 Hz, 1H), 4.43 (t, *J* = 7.0 Hz, 1H), 4.13 - 4.08 (m, 1H), 3.90- 3.85 (m, 1H), 3.80 - 3.77 (m, 1H), 3.15 (t, *J* = 8.2 Hz, 1H), 2.40 - 2.33(m, 1H), 1.41 (s, 9H), 1.31 (d, *J* = 7.3 Hz, 3H), 0.97 (d, *J* = 6.7 Hz, 3H)

^{13}C NMR (125 MHz, DMSO-d_6): δ 202.1, 173.8, 171.1, 169.3, 162.6, 156.0, 141.6, 136.3, 133.4, 128.5, 128.4, 128.0, 127.7, 127.5, 117.2, 108.9, 106.1, 77.9, 71.4, 70.6, 69.5, 54.7, 49.3, 44.5, 42.8, 28.2, 15.7, 13.8

HRMS (ESI): calculated for $C_{30}H_{39}O_9N_4$ [M+H]+ : 599.2706, found 599.2712.

***tert*-Butyl ((2*S*)-1-(((3*R*,9*S*,10*S*,11*R*)-16-(benzyloxy)-10,12-dihydroxy-3,9-dimethyl-2,5,8,13-tetraoxo-2,3,4,5,6,7,8,9,10,11,12,13-dodecahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamate**



125

To a solution of compound **123** (45 mg, 0.075 mmol) in CH_2Cl_2 (5mL) trifluoro acetic acid (1.0 mL) was added at 0 °C and the resulting suspension was stirred for 2 h at the same temperature. Reaction was monitored by TLC, and then concentrated. This residue was dissolved in dry DMF (3 mL), then HATU (35 mg, 0.091 mmol), DIPEA (27µL, 0.150 mmol) and *N*-(*tert*-Butoxycarbonyl)-L-serine (**119**) (18 mg, 0.091 mmol) was added. The resulting solution was stirred at ambient temperature for 16 h. Reaction mass was diluted with ethyl acetate (15 mL), washed with saturated solution of $NaHCO_3$ (5 mL), H_2O (5 mL), 1N HCl (5 mL). The organic layer was dried over Na_2SO_4 and the crude material obtained after removal of the solvent was purified by column chromatography (silica gel 230-400 mesh 5% methanol - CH_2Cl_2) to afford **125** (34 mg) as off white solid.

Yield: 65%

Melting point = 271-273 °C

IR υ_{max}(film): 3646, 3285, 2926, 1669, 1632, 1529, 1221 cm^{-1}

Specific rotation $[\alpha]_D^{22}$ = + 11.81 (*c* 0.23, CH_3OH)

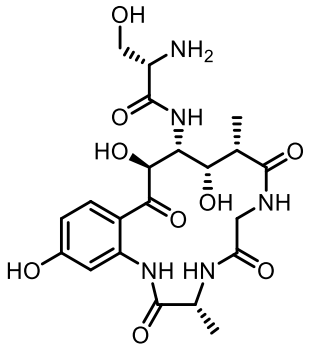
^{1}H NMR (500 MHz, DMSO-d_6): δ 11.49 (s, 1H), 8.73 (d, *J* = 7.6 Hz, 1H), 8.17 (d, *J* = 1.53 Hz, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 7.50 - 7.33 (m, 7H), 6.86 - 6.84 (m, 1H), 5.60 (d, *J* = 9.9 Hz, 1H), 5.54 (d, *J* = 8.0 Hz, 1H), 5.21 (s, 2H), 5.02 (d, *J* = 6.9 Hz, 1H), 4.68 (t, *J* = 8.8 Hz, 1H), 4.43 (t, *J* = 7.2 Hz, 1H), 4.10(t, *J* = 9.54 Hz, 1H), 3.86 (d, *J* = 6.1 Hz, 1H),

3.80 - 3.77 (m, 1H), 3.15 (t, *J* = 8.39 Hz, 1H), 2.39 - 2.34 (m, 1H), 1.41 (s, 9H), 1.31 (d, *J* = 6.9 Hz, 3H), 0.97 (d, *J* = 6.9 Hz, 3H);

^{13}C NMR (125 MHz, DMSO-d_6): δ 202.1, 173.8, 171.1, 169.3, 162.6, 156.0, 141.6, 136.3, 133.4, 128.5, 128.4, 128.0, 127.7, 127.5, 117.2, 108.9, 106.1, 77.9, 71.4, 70.6, 69.5, 69.1, 54.7, 49.3, 44.5, 42.8, 28.2, 15.7, 13.8

HRMS (ESI): calculated for $C_{33}H_{43}O_{11}N_5Na$ $[M+Na]^+$: 708.2842, found 708.2851.

(2*S*)-2-Amino-3-hydroxy-N-((3*R*,9*S*,10*S*,11*R*)-10,12,16-trihydroxy-3,9-dimethyl-2,5,8,13-tetraoxo-2,3,4,5,6,7,8,9,10,11,12,13-dodecahydro-1H-benzo[h][1,4,7] triazacyclopentadecin-11-yl)propanamide



Revised Structure of Solomonamide A,**122**

To a solution of compound **125** (15 mg, 0.021 mmol) in methanol (3 mL), 10% Pd/C (~ 3 mg) was added and stirred under H_2 atmosphere for 2 h. The reaction mixture was then filtered through silica gel column, concentrated to afford phenolic compound. The phenolic compound was dissolved in CH_2Cl_2 (3 mL), TFA (0.3 mL) was added at 0 °C and the resulting suspension was stirred for 2 h at the same temperature. Concentrated the reaction mixture and azeotroped with diethyl ether (3 mL x 3) and dried under vacuum to afford compound **122** (8.5 mg) as off white solid.

Yield: 78% for 2 steps

IR υ_{max}(film): 3439, 3067, 1669, 1578, 1529, 1221 cm^{-1}

Specific rotation $[\alpha]_D^{27}$ = +3.4 (*c* 0.52, CH_3OH)

HRMS (ESI): calculated for $C_{21}H_{30}O_9N_5$ $[M+H]^+$: 496.2036, found 496.2041.

1.1.9.1. Biological activity

1.1.9.1.1. Material and Method

Male Swiss albino mice weighing 30-35 g were divided into 6 groups. Animals received either a sub plantar injection of either 50 µl of saline (control) or 1% carrageenan in the left hind paw. One hour prior to the carrageenan injection, respective group of animals was administered with a single dose of either vehicle (PEG, i.p.) or solomonamide A revised (0.03, 0.1 and 0.3 mg/kg, i.p.) and dexamethasone (10 mg/kg, i.p.). Plethysmometer (Ugo Basile, Italy) was used to measure the paw volume, before and after sub plantar injection of carrageenan at 4 & 6 h. The increase in paw volume was calculated as the difference between the paw volume measured at 4 & 6 h and the basal paw volume. Percentage inhibition was calculated by using formula Vc-Vt/Vc X 100. Where, Vc and Vt represent the mean paw volume of carrageenan and solomonamide A treated animals respectively.

1.1.9.1.2. Statistical analysis

Results were expressed as Mean ± S.E.M. Statistical analysis was determined by one-way ANOVA followed by Tukey's test for multiple comparisons, using Graph Pad Prism software (Graph Pad Software Inc., San Diego, CA). Differences were considered statistically significant when $p<0.05$.$^{***}p<0.001$ control vs. carrageenan; $^{###}p<0.001$ carrageenan vs. Carrageenan + solomomnamide A or Carrageenan + Dexamethasone.

1.1.9.2. Computational details

The starting 3D chemical structures of compounds **122a**, **122b**, **123a**, and **123b** (Chart S1) were built with Maestro11.1. Optimizations of the starting 3D structures were performed using the OPLS force fieldand the Polak-Ribier conjugate gradient algorithm (PRCG, maximum derivative less than 0.001 kcal/mol)with Macro Model 11.5. For all the compounds, exhaustive conformational searches were performed at the empirical molecular mechanics (MM) level, combining Monte Carlo Multiple Minimum (MCMM) method (50000 steps) and Low Mode Conformational Search (LMCS) method (50000

steps), in order to allow a full exploration of the conformational space. Furthermore, the conformational search was also integrated with molecular dynamics simulations at 450, 600, 700, and 750 K, with a time step of 2.0 fs, an equilibration time of 0.1 ns, and a simulation time of 10 ns. For each investigated compound, all the conformers obtained from the above reported conformational search rounds were minimized (PRCG, maximum derivative less than 0.001 kcal/mol) and superimposed. Then, the "Redundant Conformer Elimination" module of Macromodel 11.5 was used to select non-redundant conformers, excluding those differing more than 21.0 kJ/mol (5.02 kcal/mol) from the most energetically favoured conformation and setting a 0.5 Å RMSD (root-mean-square deviation) minimum cut-off for saving structures.

All the subsequent QM calculations were performed using Gaussian 09 software. Specifically, all the conformers obtained by MM conformational search rounds were optimized at the QM level using the MPW1PW91 functional and the 6-31G(d) basis set.[ref] After the optimization of the geometries, the conformers were visually inspected in order to remove further redundant conformers. The subsequent computation of the ^{13}C NMR chemical shifts was performed on the selected conformers for the investigated compounds, using the MPW1PW91 functional and the 6-31G (d,p) basis set. Final ^{13}C NMR chemical shift sets of data for each of the diastereoisomers were extracted and computed considering the influence of each conformer on the total Boltzmann distribution taking into account the relative energies. Calibrations of calculated ^{13}C chemical shifts were performed following the multi-standard approach (MSTD) (Tables S1-S2, Supporting Information). In particular, sp^2 ^{13}C NMR chemical shifts (with the exception of carbonyl carbons) were computed using benzene as reference compound[ref,ref] while TMS was used for computing sp^3 ^{13}C chemical shift data.

Experimental and calculated ^{13}C NMR chemical shifts were compared (**122a**/**122b**, **123a**/**123b** diastereoisomeric pairs) computing the Δδ parameter (Tables S1–S2, Supporting Information):

$$\Delta\delta = |\delta_{exp} - \delta_{calc}|$$

where δ_{exp} (ppm) and δ_{calc} (ppm) are the ^{13}C experimental and calculated chemical shifts, respectively.

The mean absolute errors (MAEs) for all the considered diastereoisomers were computed using the following equation:

$$\mathrm{MAE} = \frac{\sum(\Delta\delta)}{n}$$

Defined as the summation (Σ) of the n computed absolute error values (Δδ), normalized to the number of chemical shifts considered (n) (Tables S1–S2, Supporting Information).

Table 4. ^{13}C experimental and calculated NMR chemical shifts for **122a** and **122b**, with [a] $|\Delta\delta|(^{13}C)$ and [c] MAE values. Chemical shift data here reported were produced using benzene as reference compound for sp^2 carbons, and tetramethylsilane (TMS) for sp^3 carbons.

Residue		δ_{exp} (^{13}C), ppm	δ_{calc} (^{13}C), ppm		$\lvert\Delta\delta\rvert$ (^{13}C), ppm[a]	
			1a	**1b**	**1a**	**1b**
Glycine	Carbonyl	169.1	166.3	166.0	2.84	3.11
	CH_2	42.9	43.1	45.0	0.17	2.09
D-Alanine	Carbonyl	170.9	165.2	168.5	5.73	2.42
	CH	49.4	51.5	51.4	2.14	2.01
	Methyl	15.8	16.2	18.3	0.39	2.54
L-Serine	Carbonyl	167.6	173.1	173.0	5.50	5.35
	CH	54.2	58.4	53.9	4.18	0.26
	CH_2	60.7	65.5	66.3	4.82	5.59
AHMOA	1 Carbonyl	173.4	169.3	172.0	4.09	1.42
	2 CH attached to CH_3	45.0	47.6	43.7	2.58	1.26
	3 CH attached to OH	70.3	73.6	72.3	3.32	2.01
	4 CH attached to NH	53.6	59.0	57.0	5.37	3.39
	5 CH attached to OH	70.6	80.0	72.9	9.37	2.30
	6 Keto carbonyl	200.7	202.9	199.8	2.15	0.87
	7 Methyl	13.8	15.4	11.6	1.60	2.24
Aromatic	1'	115.0	118.1[b]	117.3[b]	3.07	2.28
	2'	142.4	142.7[b]	145.0[b]	0.30	2.55
	3'	106.2	107.3[b]	107.2[b]	1.06	1.04
	4'	163.2	160.6[b]	163.4[b]	2.64	0.23
	5'	110.0	109.2[b]	110.5[b]	0.78	0.53
	6'	133.9	136.9[b]	137.3[b]	2.96	3.40
MAE, ppm[c]					3.10	**2.23**

[a] **$|\Delta\delta|(^{13}C)$** = $|\delta_{exp} - \delta_{calc}|$ (^{13}C), ppm: absolute differences for experimental versus calculated ^{13}C NMR chemical shifts

[b] ^{13}C chemical shifts calculated using benzene as reference compound; all the remaining values calculated using TMS as reference compound.

[c] **MAE** = $(\Sigma[|(\delta_{exp} - \delta_{calc})|])/n$, summation through n of the absolute error values (difference of the absolute values between corresponding experimental and ^{13}C chemical shifts), normalized to the number of the chemical shifts.

Table 5. ^{13}C experimental and calculated NMR chemical shifts for **123a** and **123b**, with [a]$|\Delta\delta|(^{13}C)$ and [c] MAE values. Chemical shift data here reported were produced using tetramethylsilane (TMS).

Residue		δ_{exp} (^{13}C), ppm	δ_{calc} (^{13}C), ppm		$\|\Delta\delta\|$ (^{13}C), ppm[a]	
			5a	5b	5a	5b
Glycine	Carbonyl	169.3	164.9	166.8	4.42	2.50
	CH_2	40.0	46.5	45.2	6.52	5.23
D-Alanine	Carbonyl	171.1	168.2	166.6	2.90	4.50
	CH	49.3	48.0	51.1	1.27	1.79
	Methyl	15.7	18.3	15.5	2.60	0.16
Boc	Carbonyl	156.0	151.8	153.8	4.17	2.19
	C	77.9	79.5	79.7	1.62	1.81
	CH_3	28.2	27.5	27.4	0.68	0.76
AHMOA	1 Carbonyl	173.8	170.1	170.9	3.67	2.88
	2 CH attached to CH_3	39.8	45.2	46.1	5.38	6.35
	3 CH attached to OH	69.5	71.3	72.0	1.81	2.45
	4 CH attached to NH	54.7	56.8	58.5	2.14	3.80
	5 CH attached to OH	70.5	77.7	70.5	7.25	0.03
	6 Keto carbonyl	202.1	198.0	199.8	4.1	2.30
	7 Methyl	13.8	14.7	15.6	0.85	1.82
Aromatic	1'					
	2'					
	3'					
	4'					
	5'					
	6'					

	CH_2				
	1"				
	2"				
	3"				
	4"				
	5"				
	6"				
MAE, ppm[b]				3.29	2.57

[a] **$\Delta\delta|(^{13}C)$** = $|\delta_{exp} - \delta_{calc}|$ (^{13}C), ppm: absolute differences for experimental versus calculated ^{13}C NMR chemical shifts

[b] **MAE** = $(\Sigma[|(\delta_{exp} - \delta_{calc})|])/n$, summation through n of the absolute error values (difference of the absolute values between corresponding experimental and ^{13}C chemical shifts), normalized to the number of the chemical shifts

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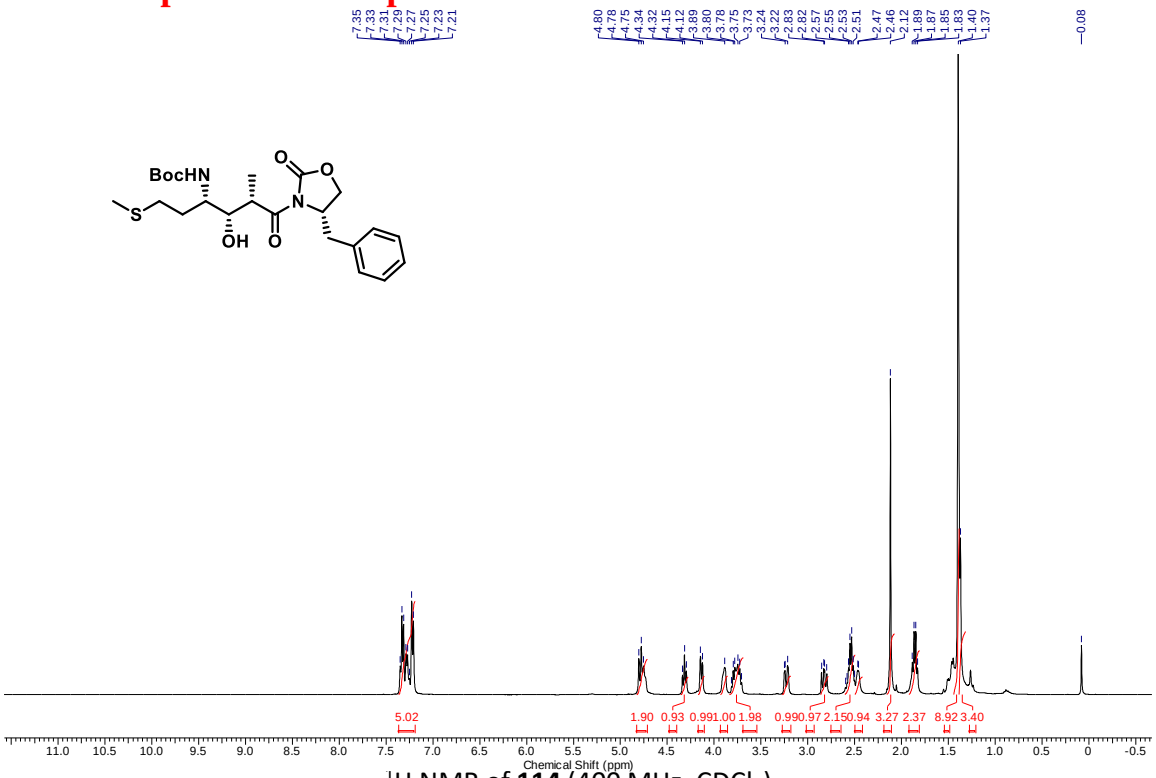
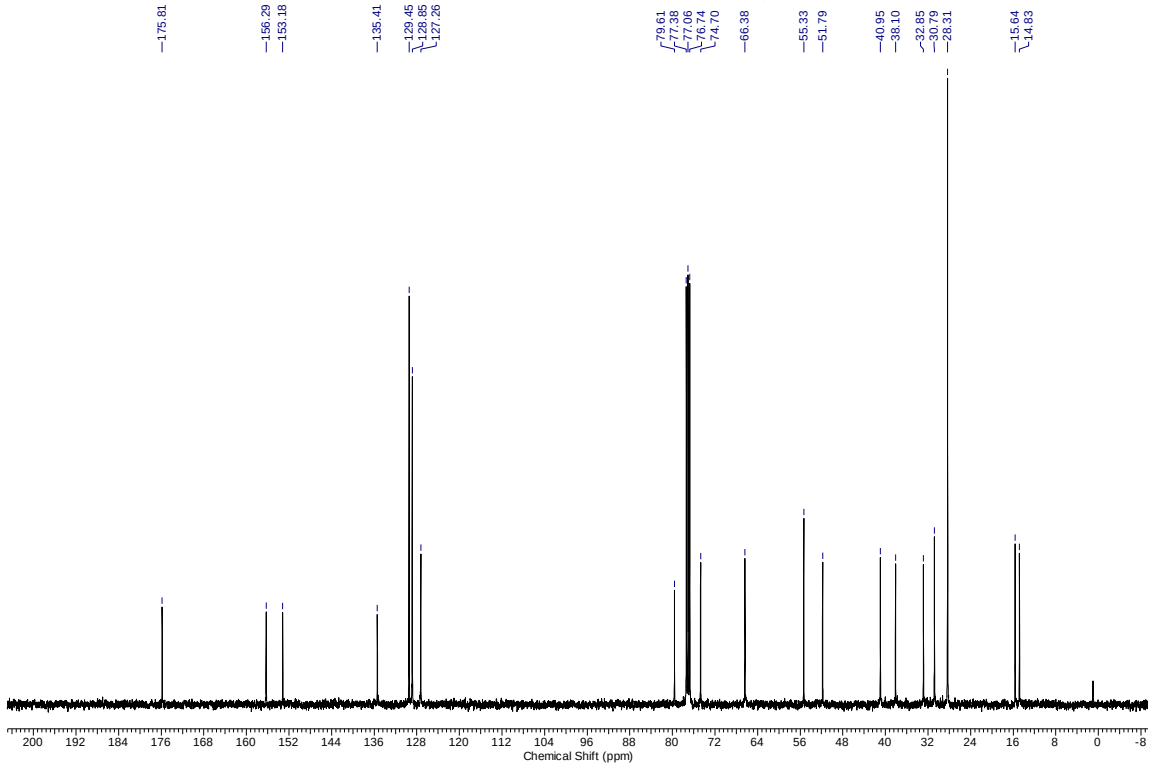
1.1.11. Copies of NMR spectra



^{1}H NMR of **114** (400 MHz, $CDCl_3$)



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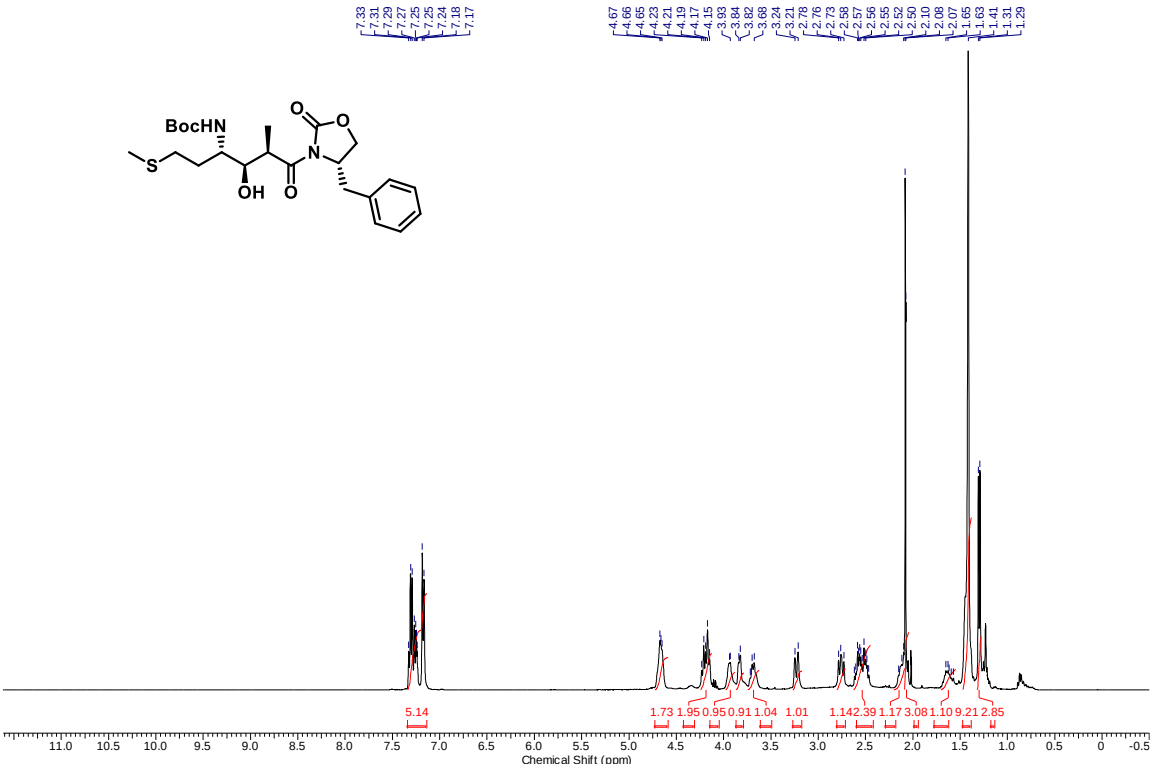

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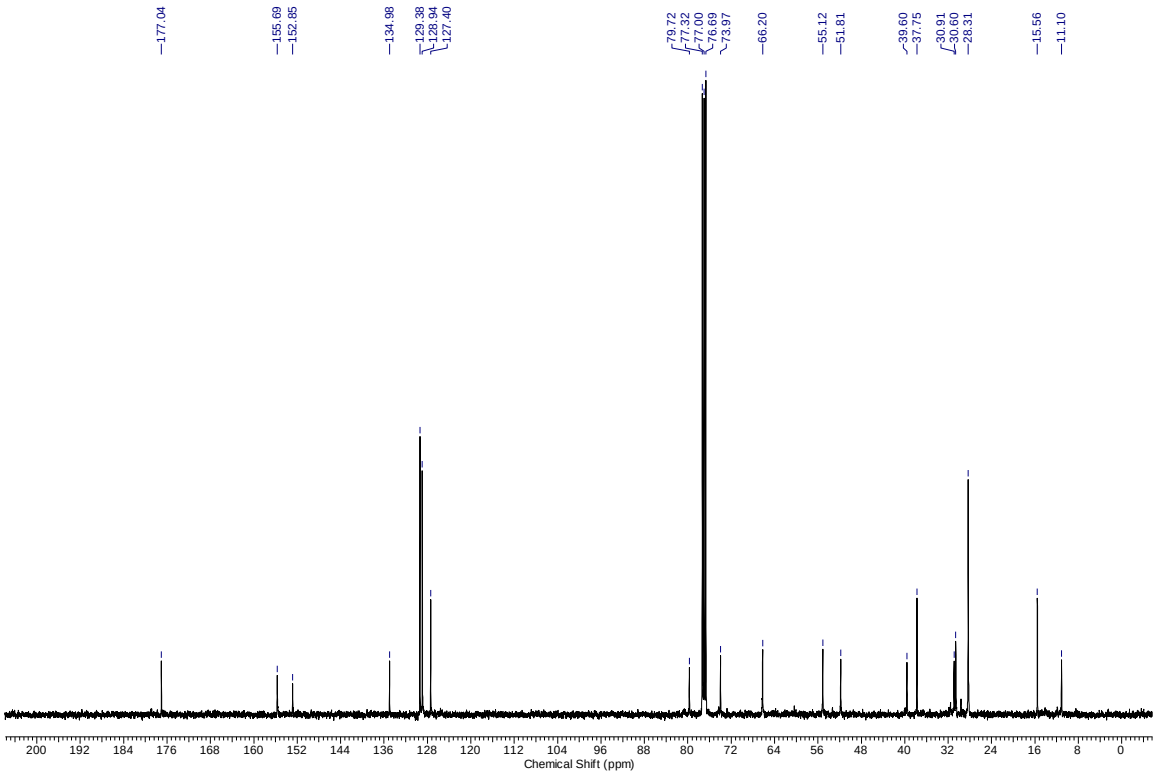

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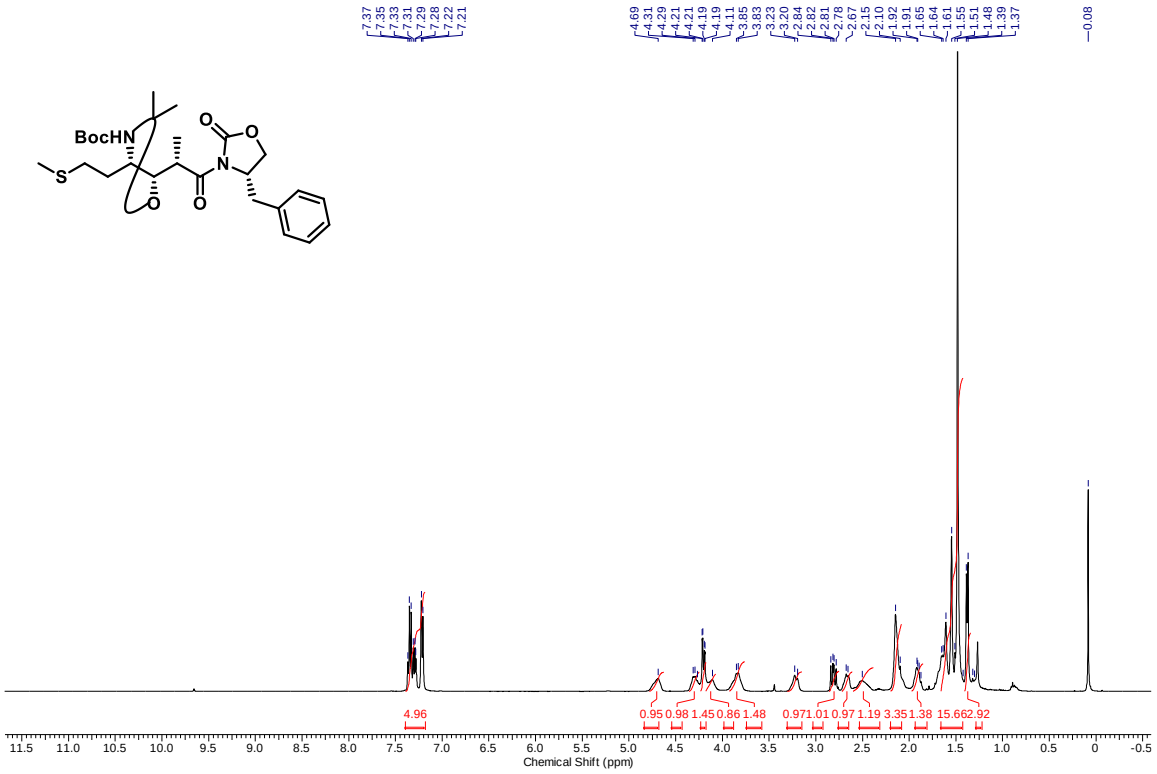

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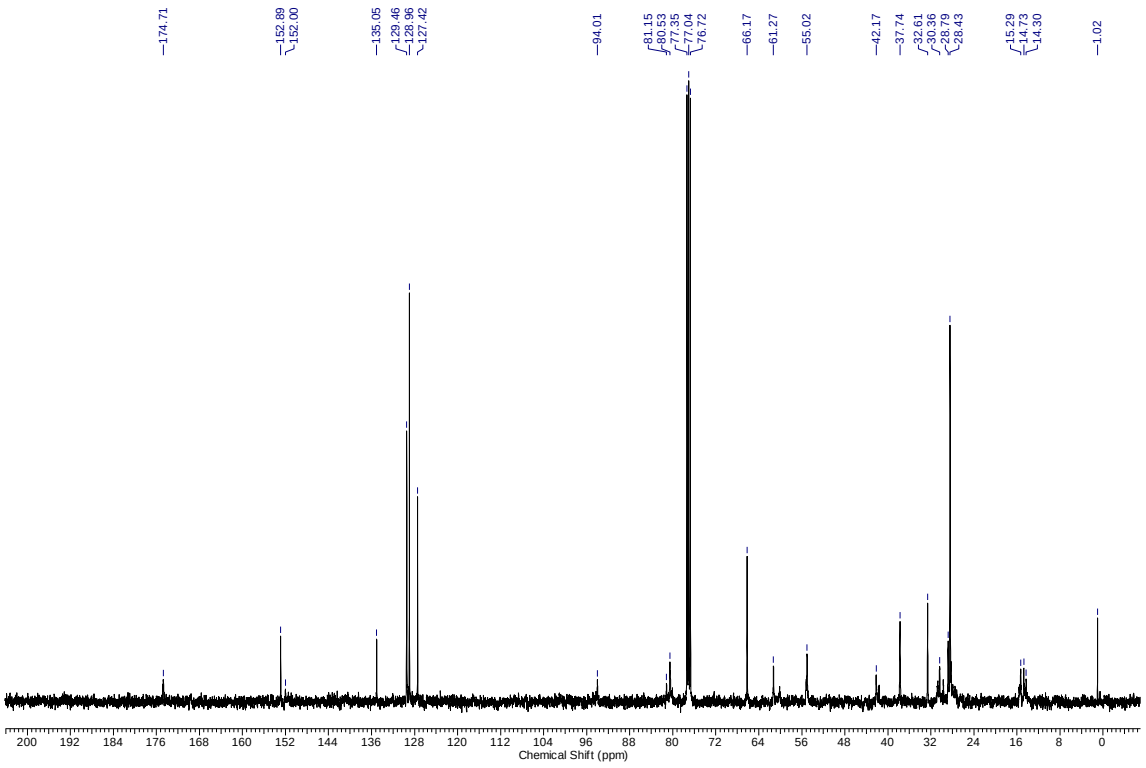

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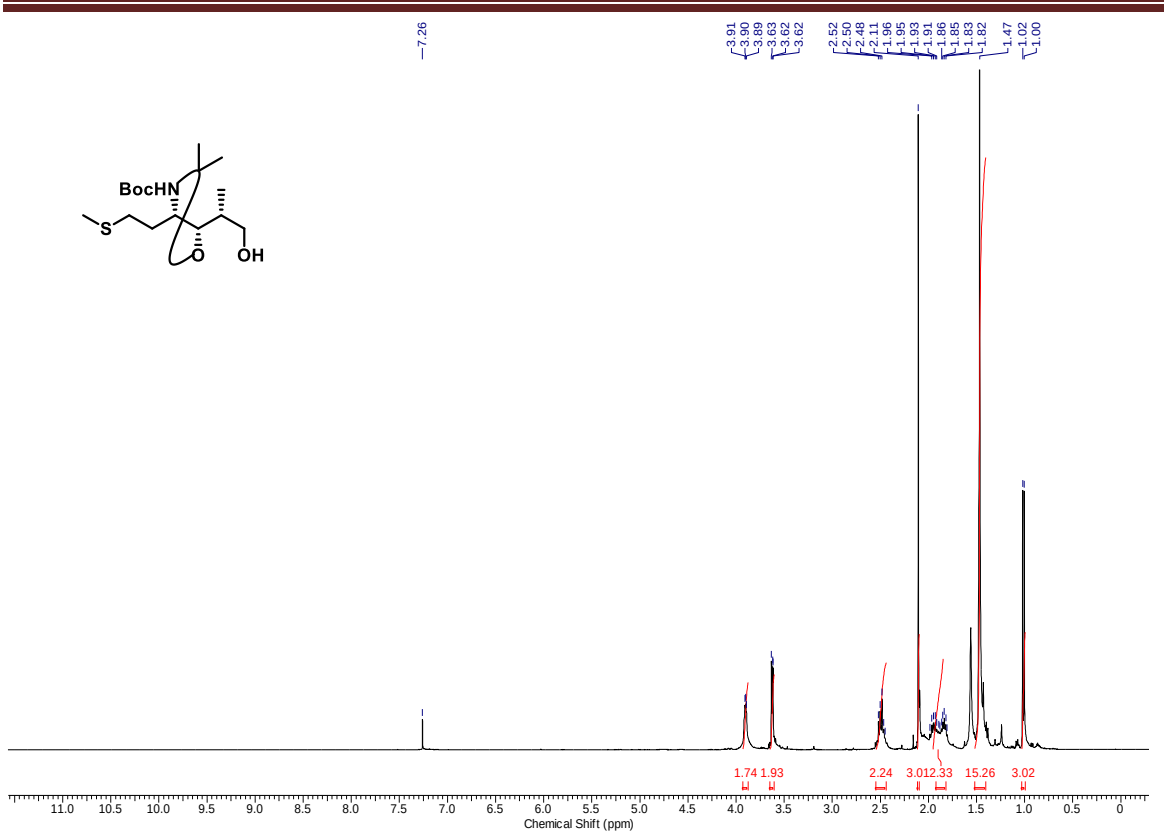

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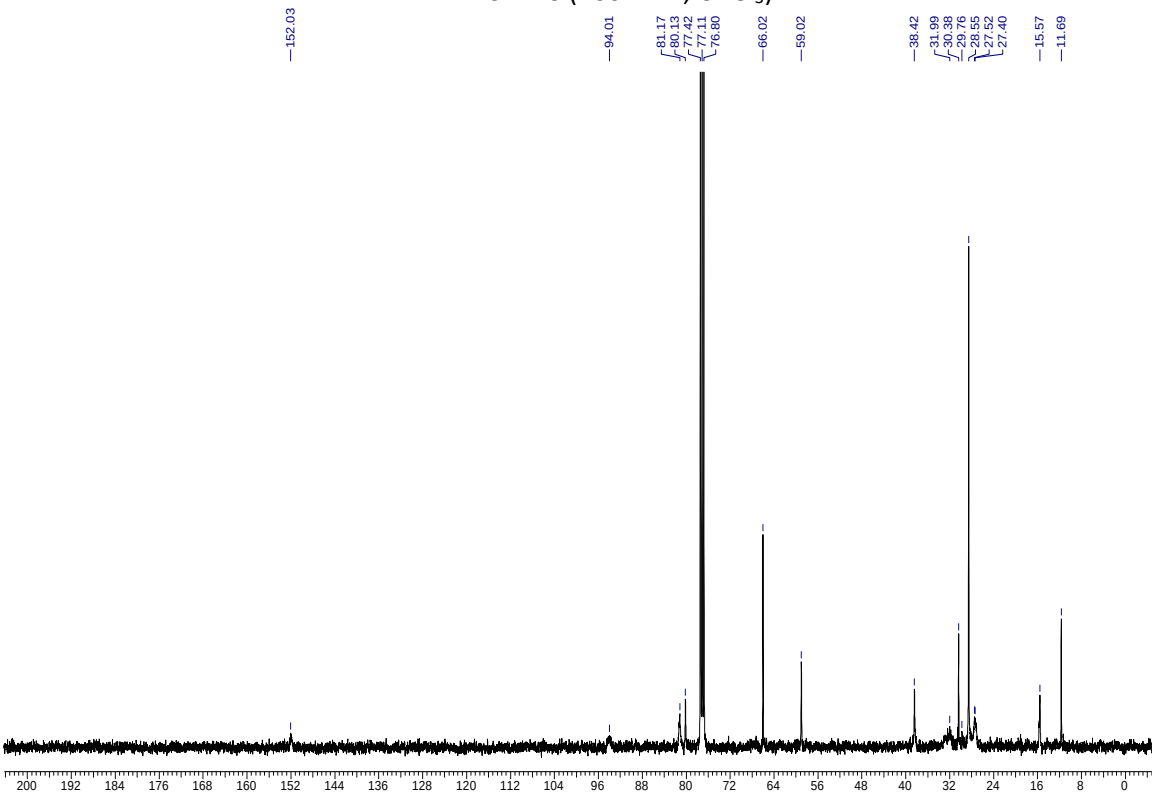

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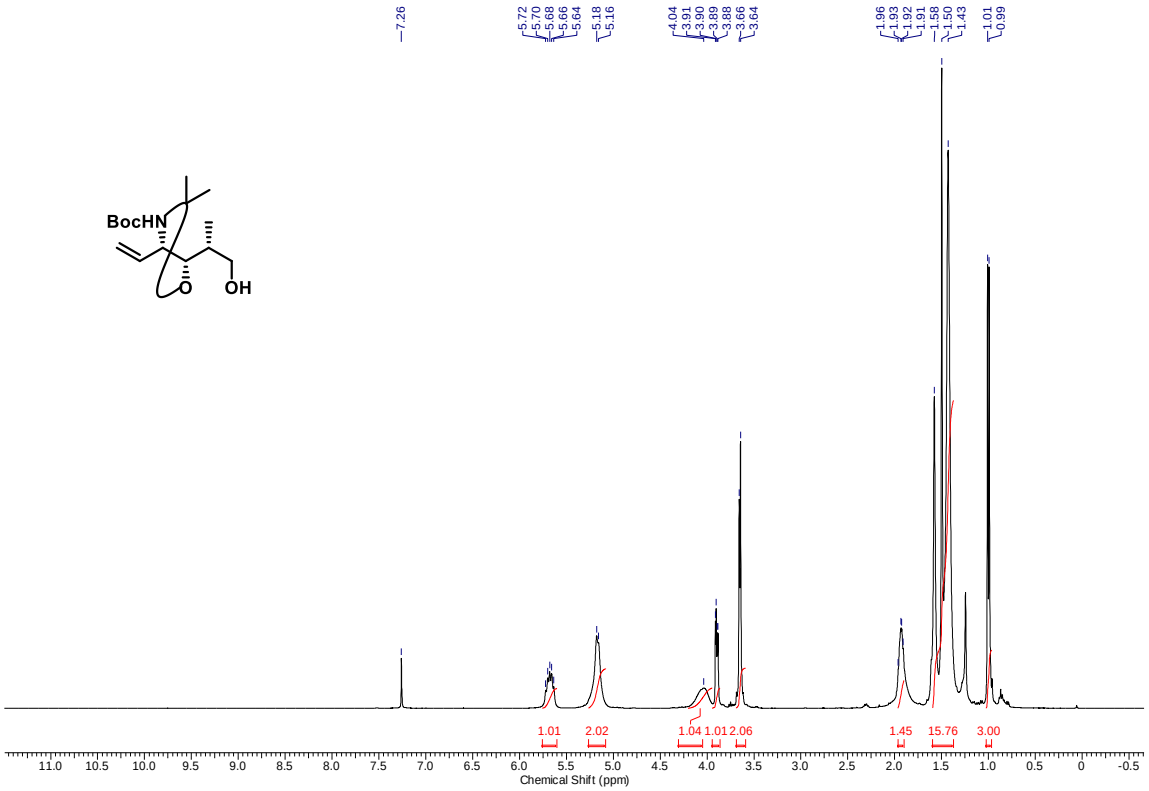

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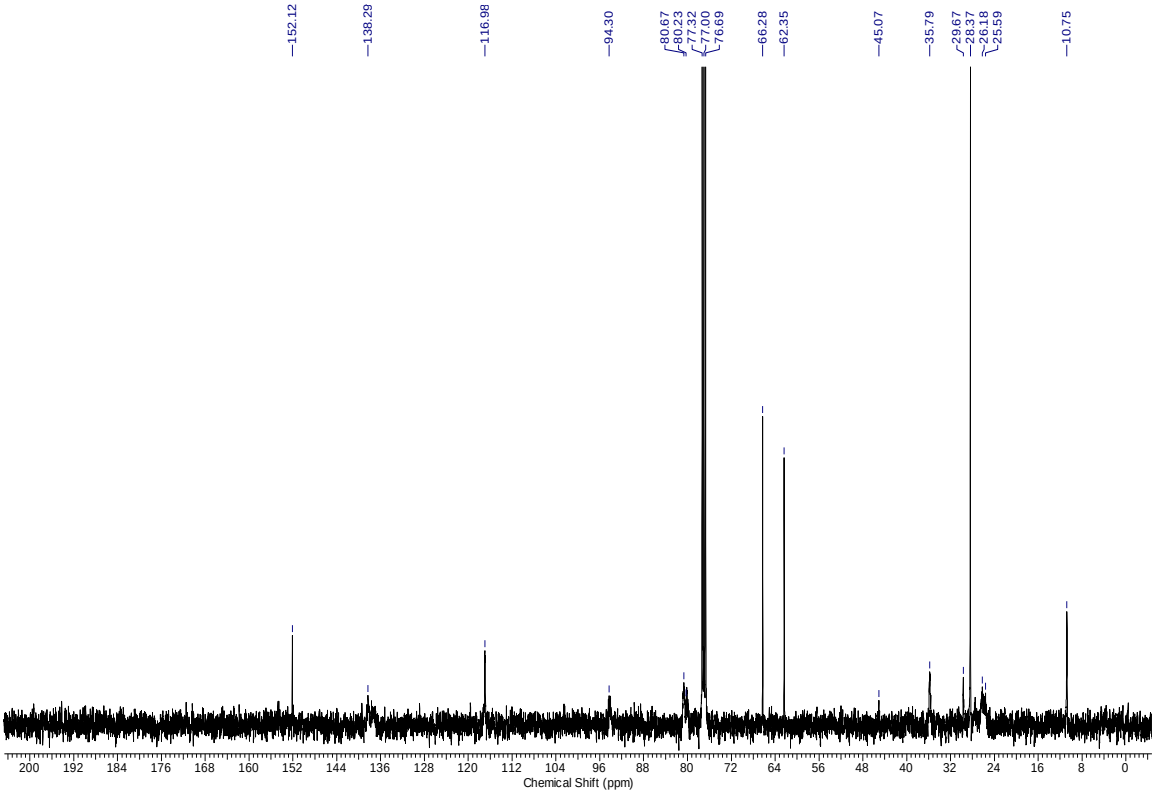
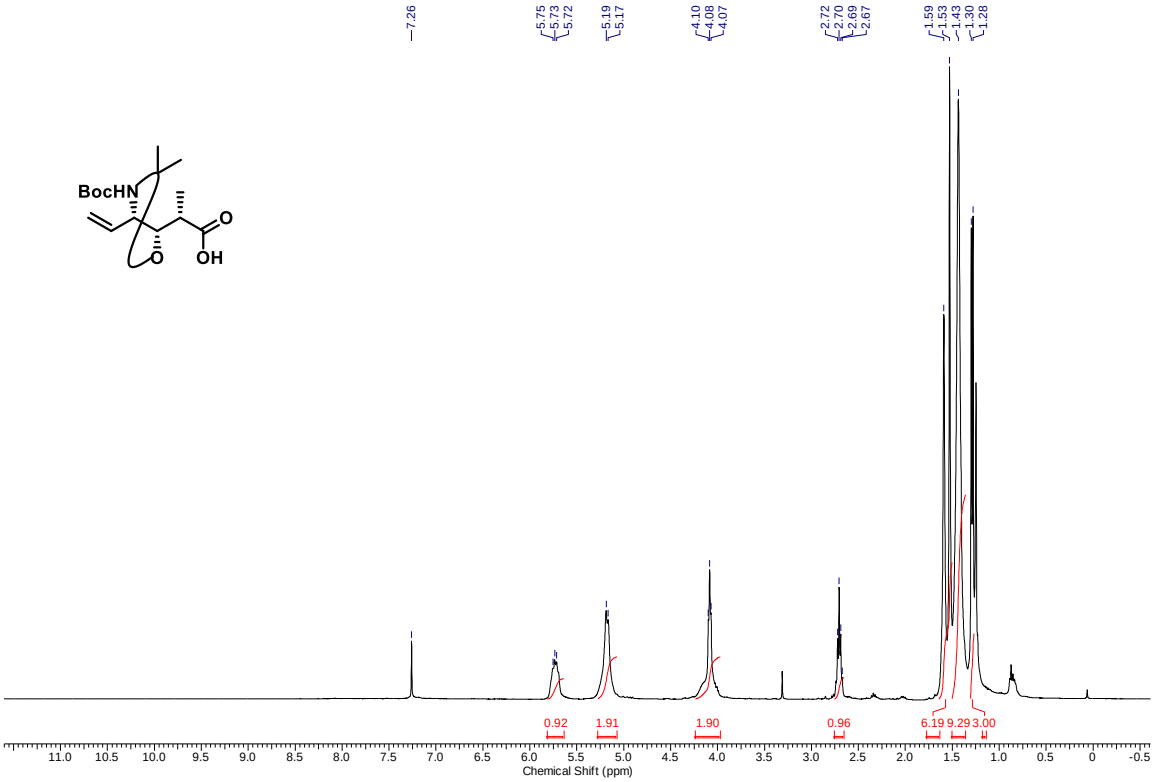


^{13}C NMR of **117** (100 MHz, $CDCl_3$)



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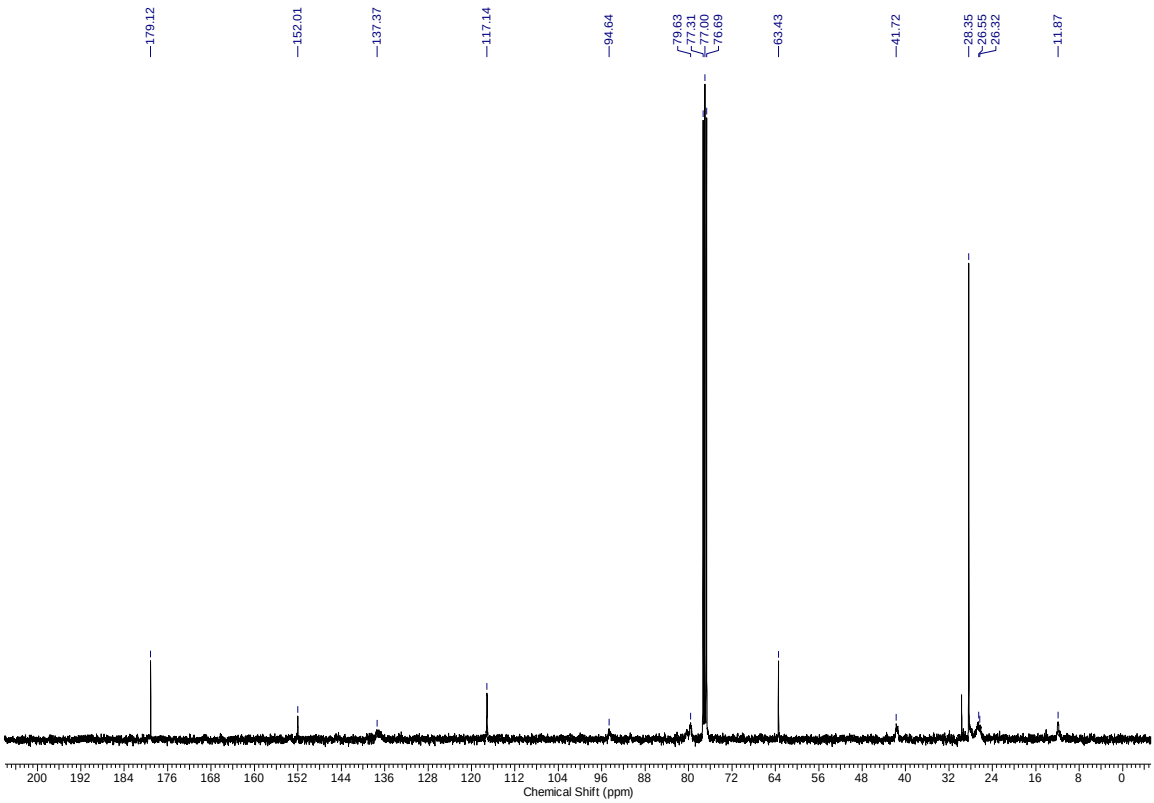
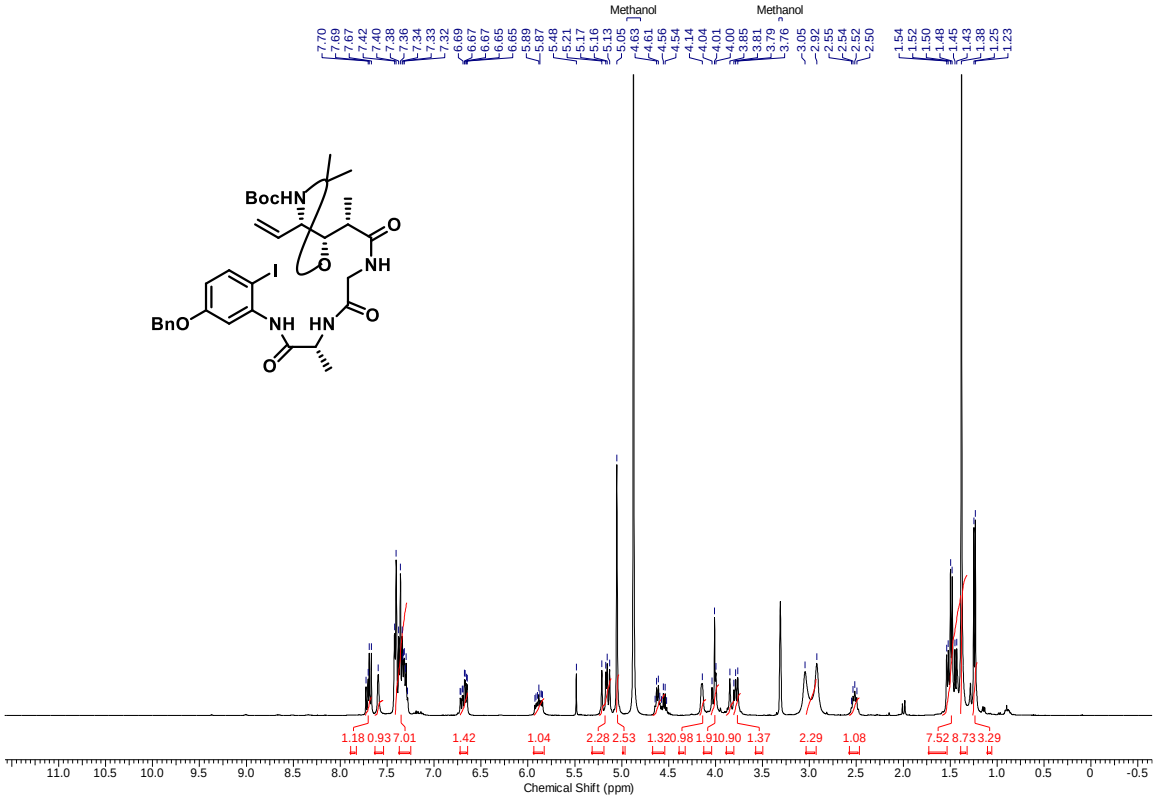
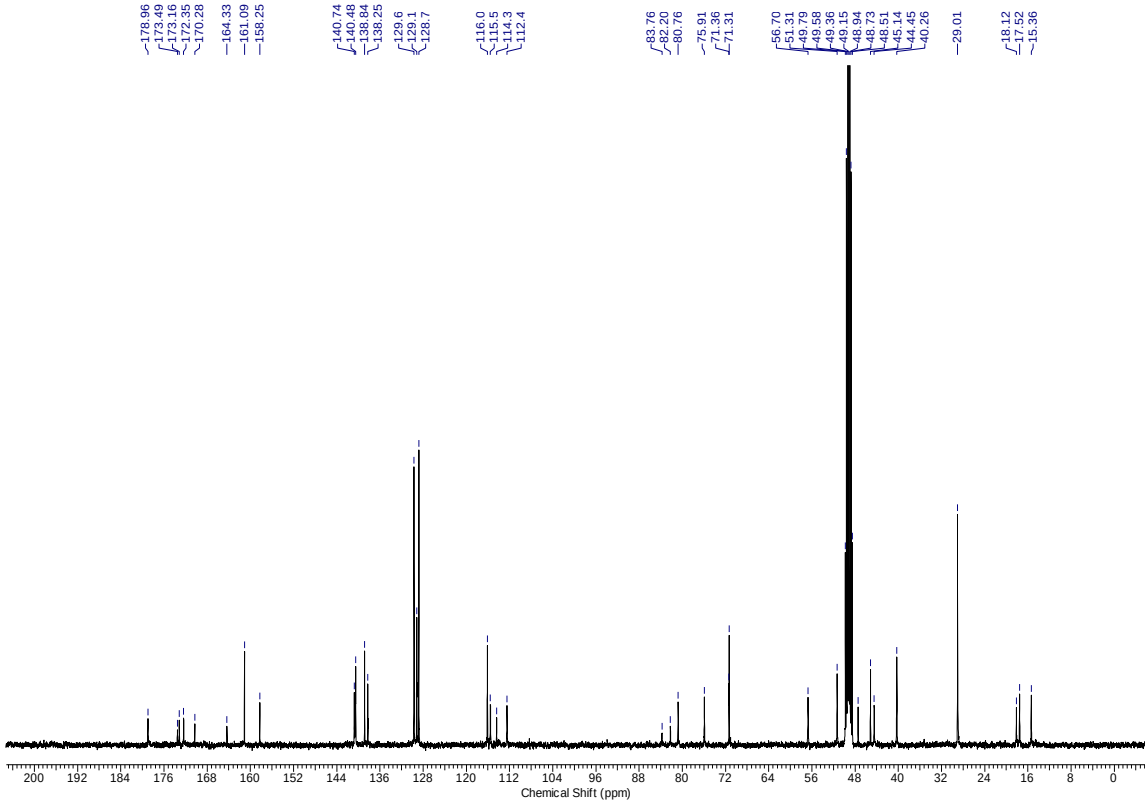


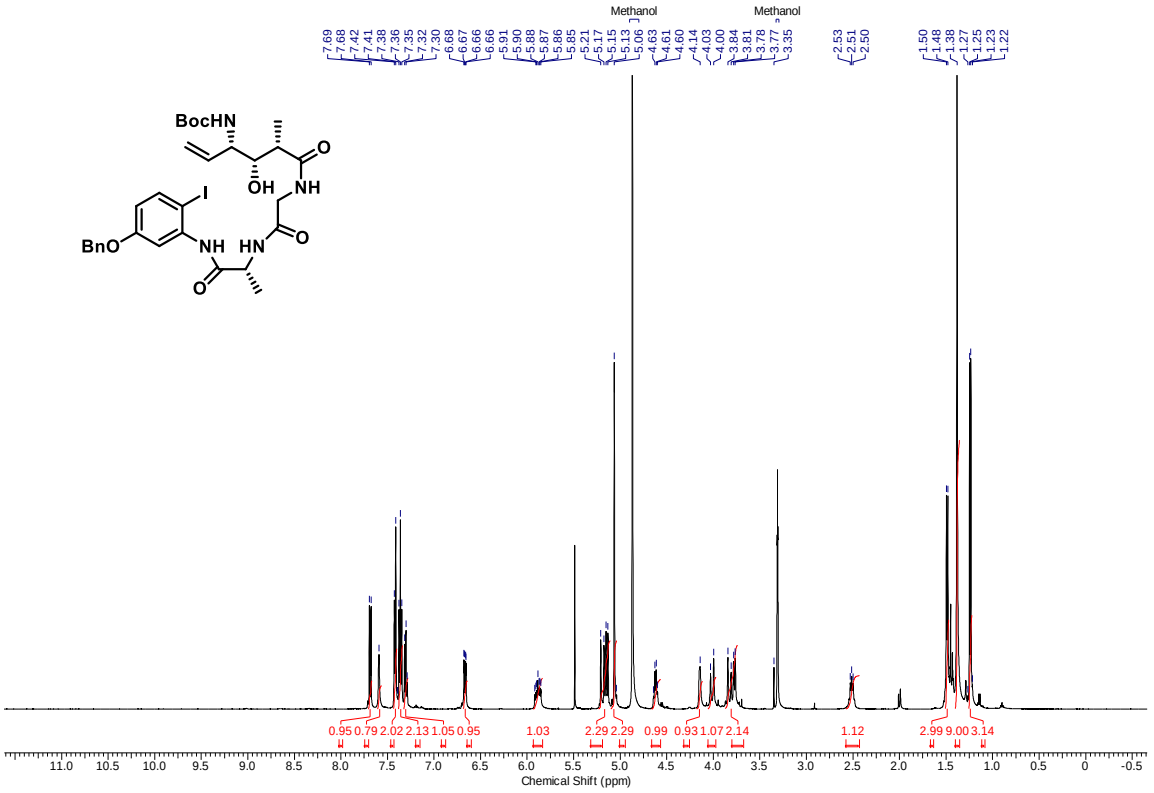
^{13}C NMR of **108** (100 MHz, $CDCl_3$)



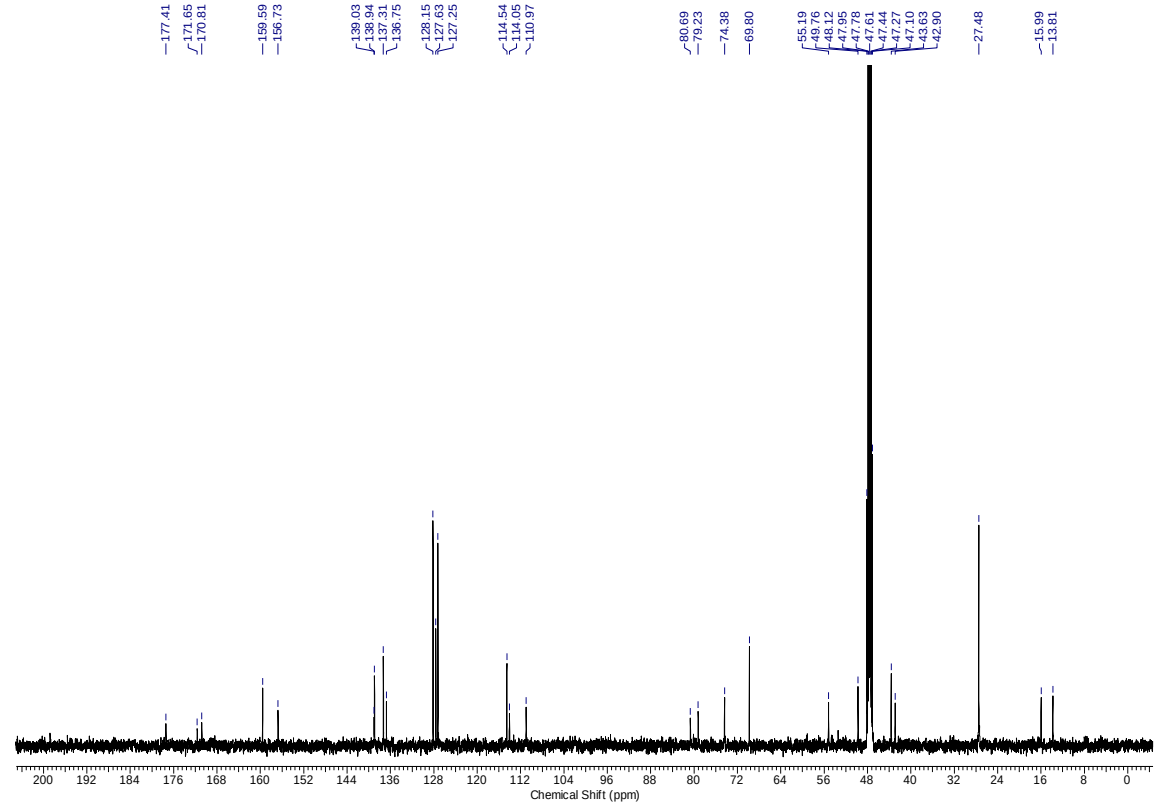
^{1}H NMR of **118** (400 MHz, CD_3OD)



^{13}C NMR of **118** (100 MHz, CD_3OD)



^{1}H NMR of **107** (400 MHz, CD_3OD)



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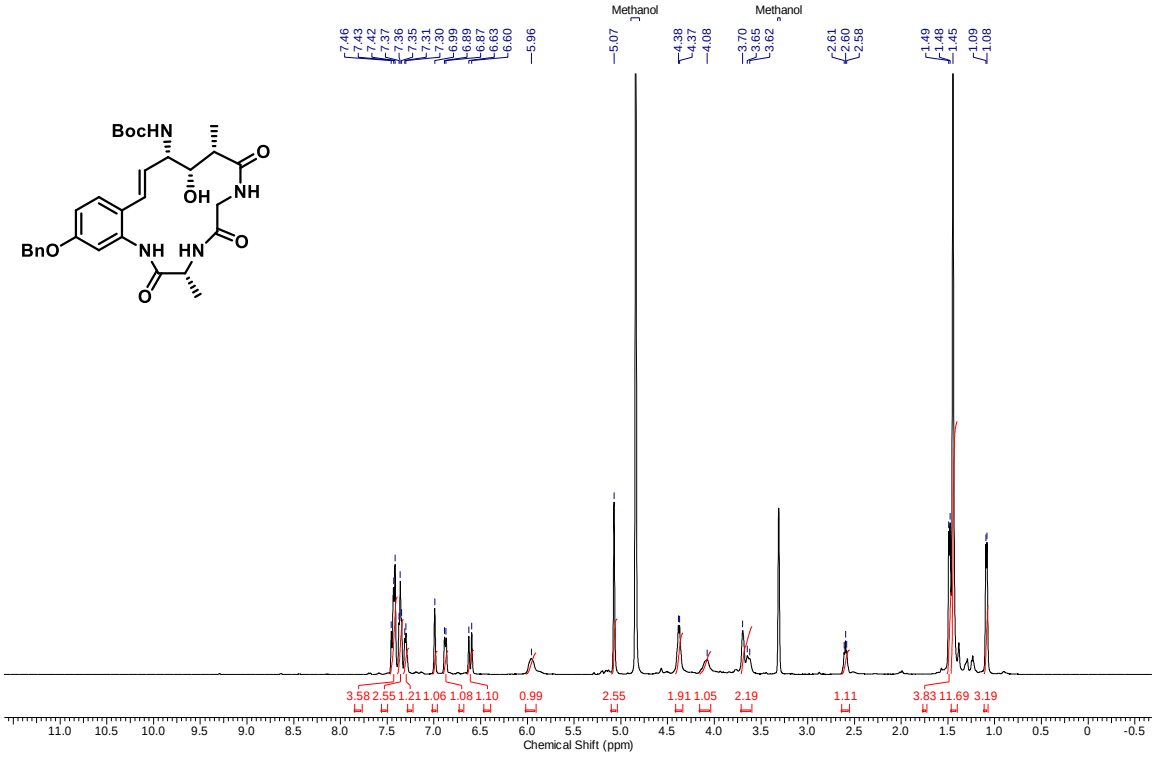
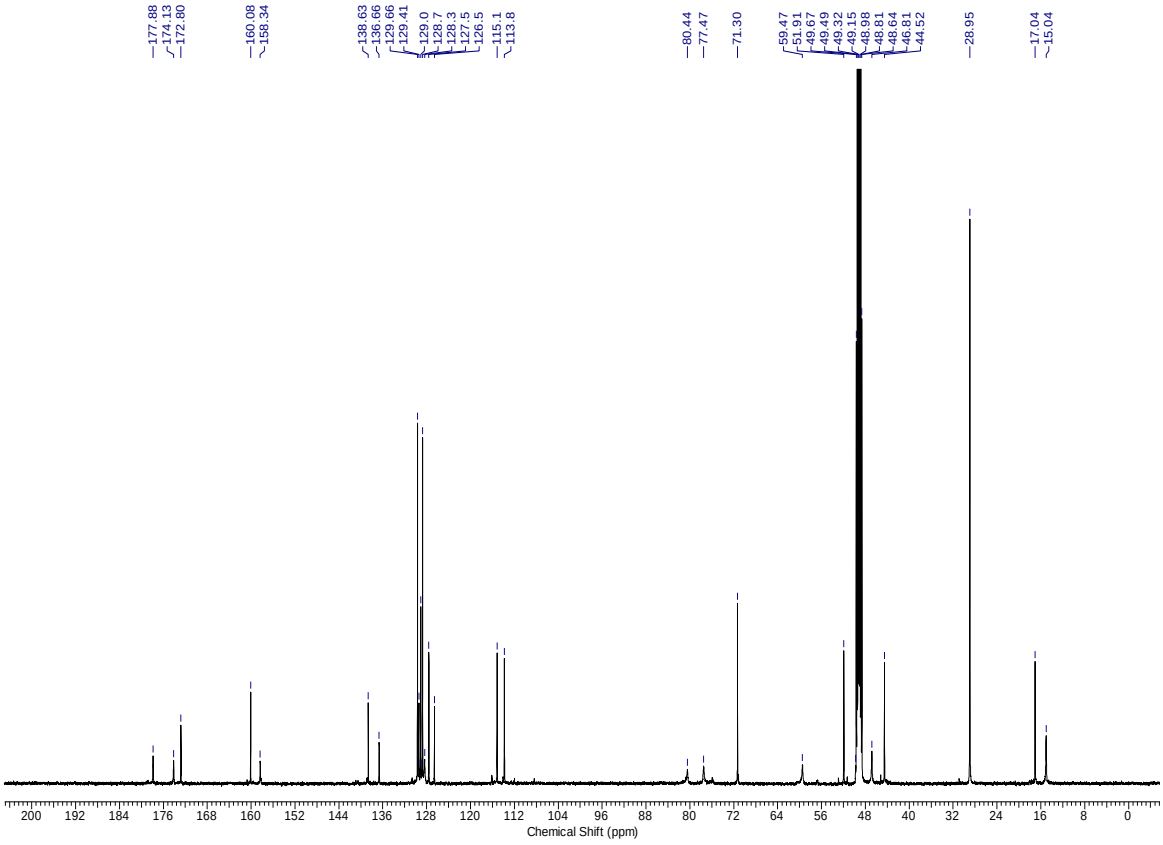
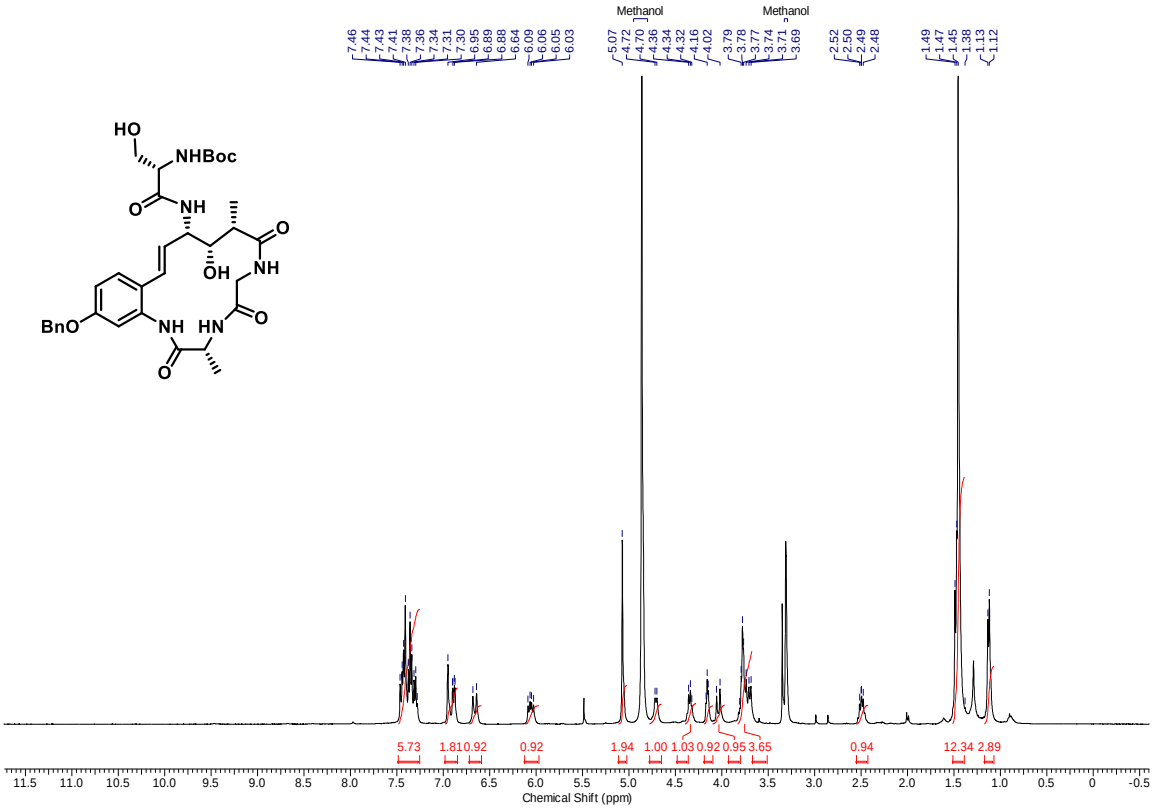


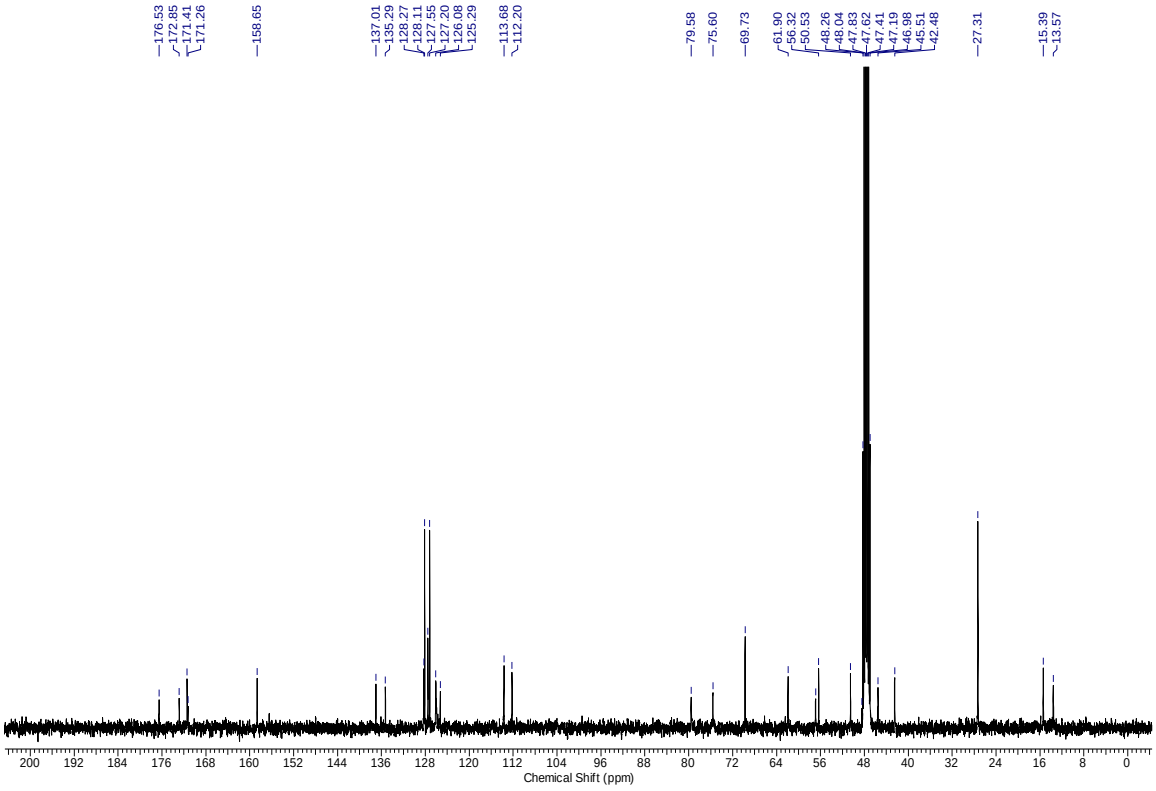
^{1}H NMR of **106** (500 MHz, CD_3OD)



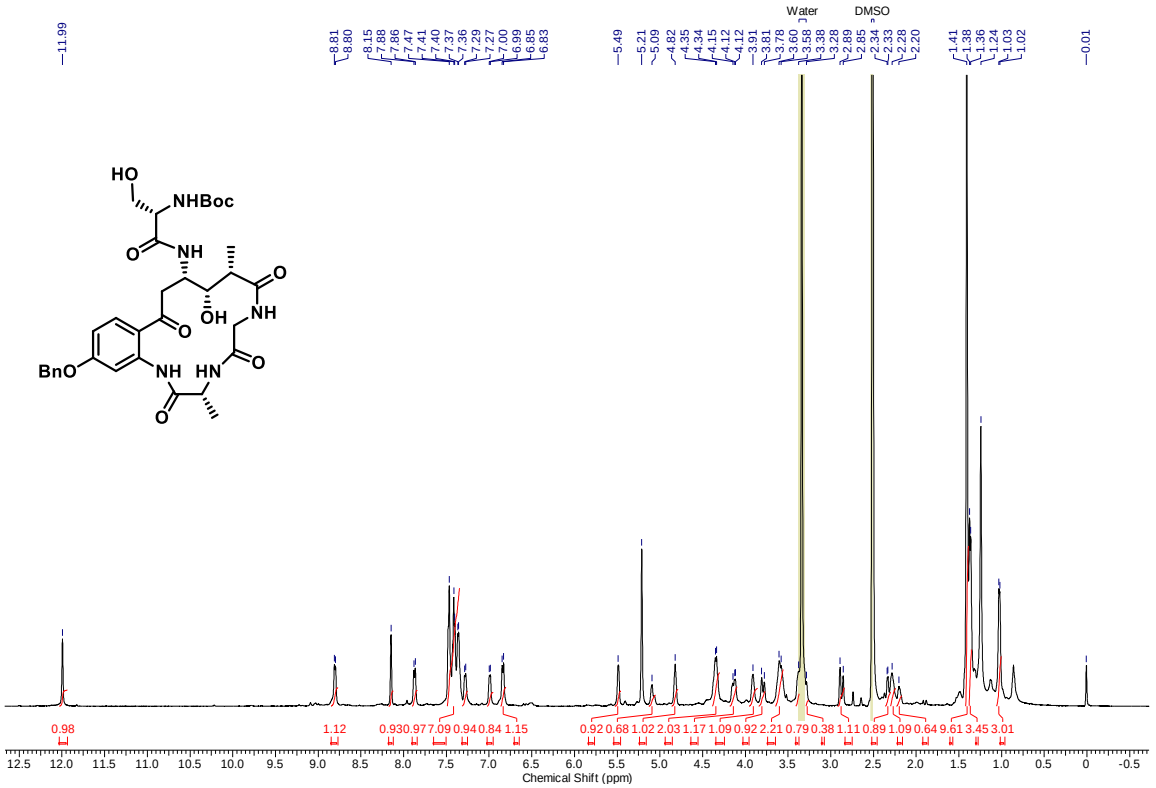
^{13}C NMR of **106** (125 MHz, CD_3OD)



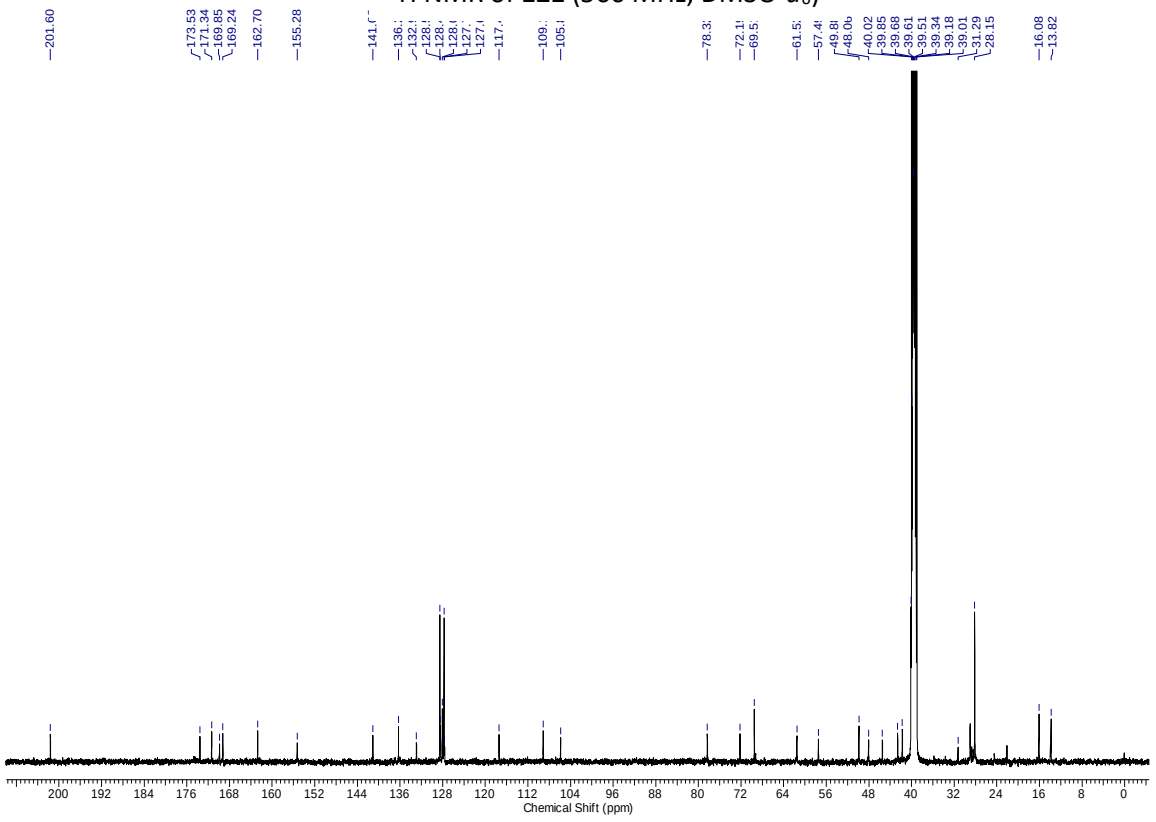
^{1}H NMR of **120** (400 MHz, CD_3OD)



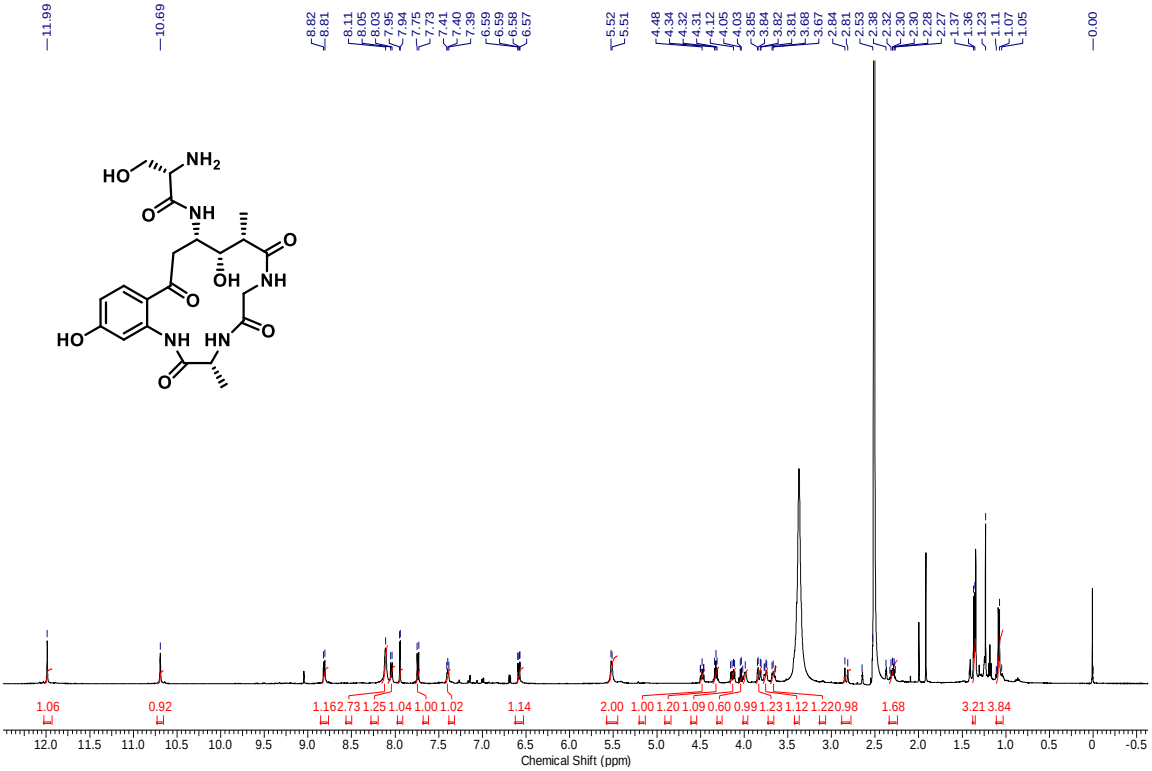
^{13}C NMR of **120** (100 MHz, CD_3OD)



^{1}H NMR of **121** (500 MHz, DMSO-d_6)



^{13}C NMR of **121** (125 MHz, DMSO-d_6)



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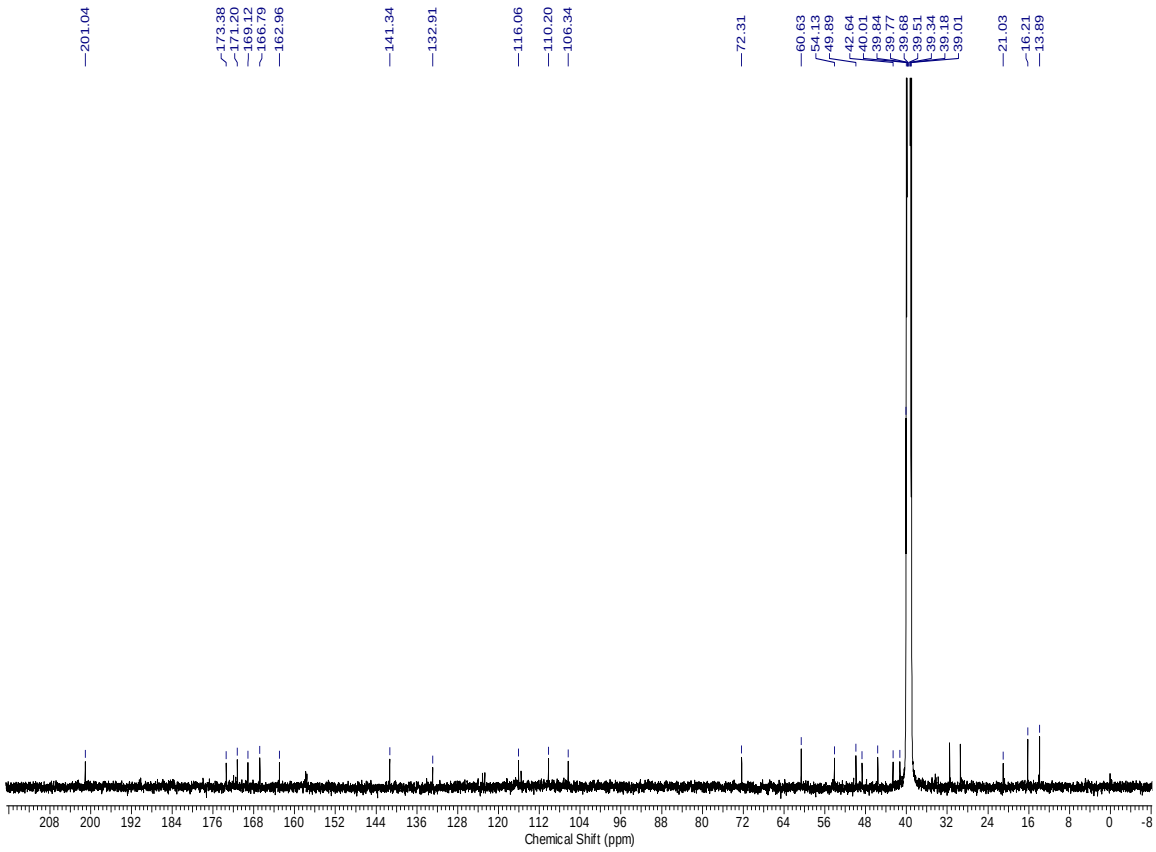
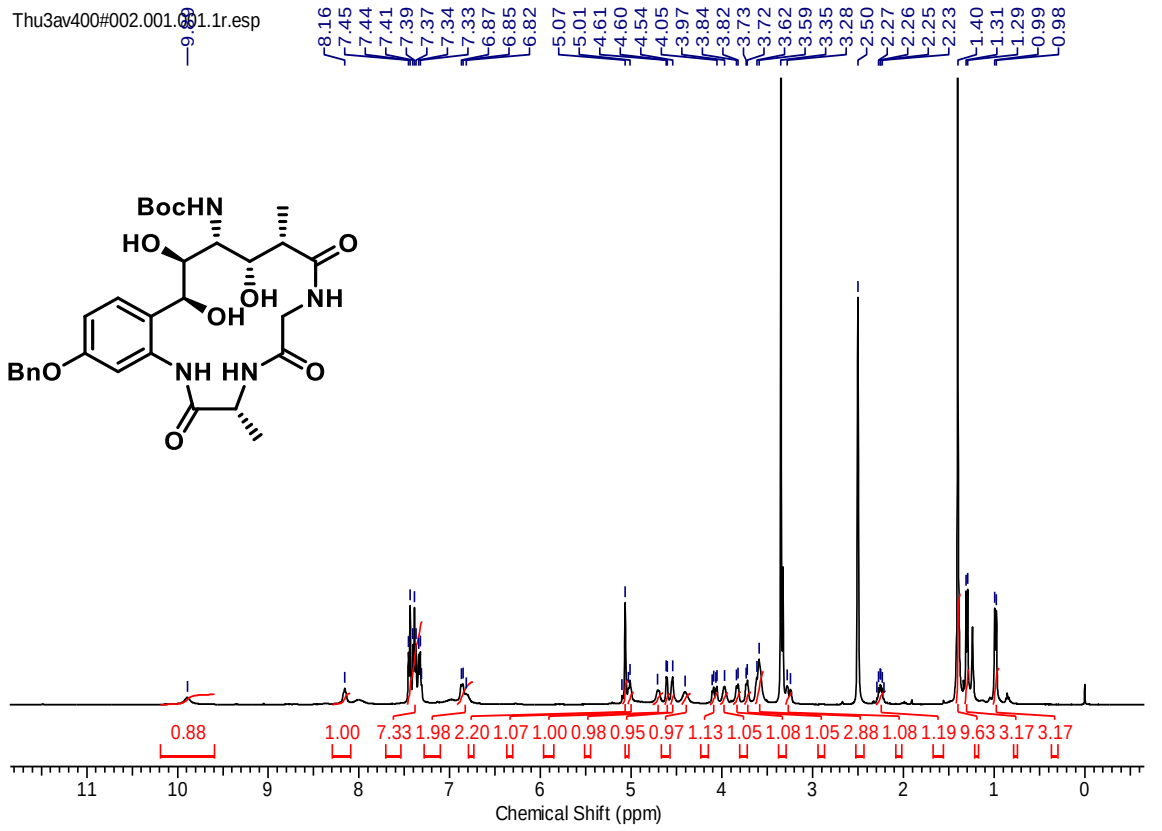
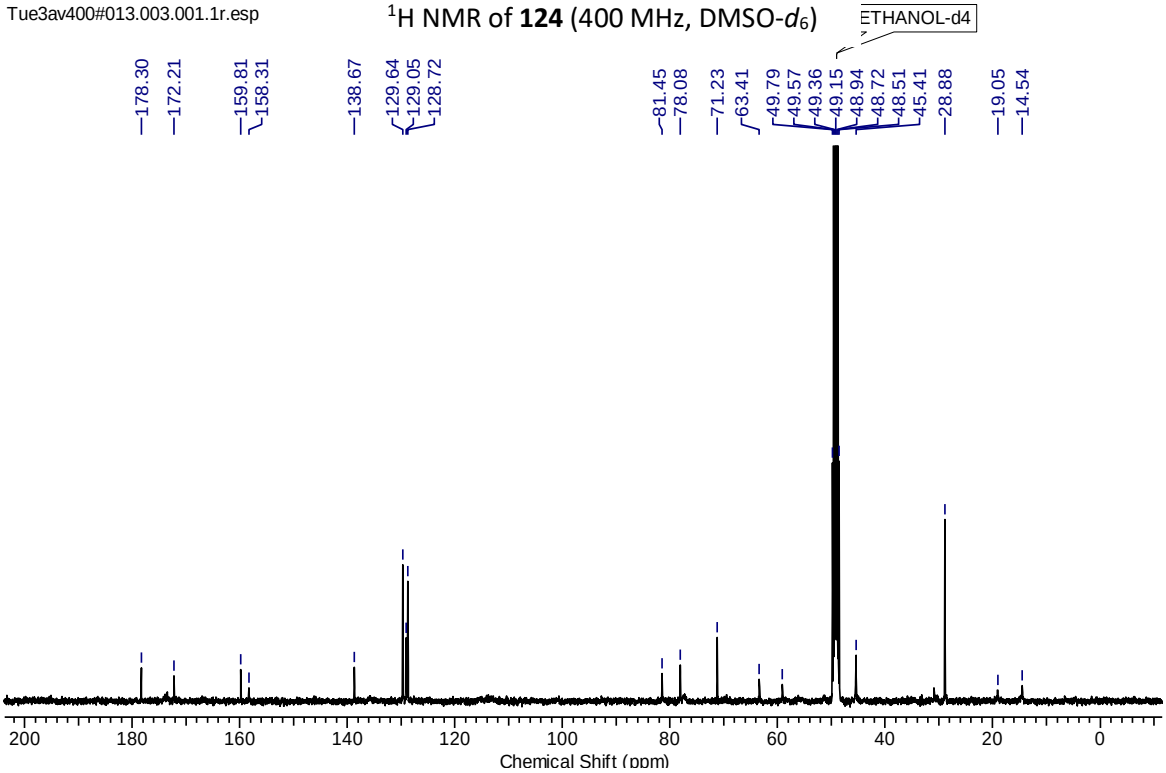


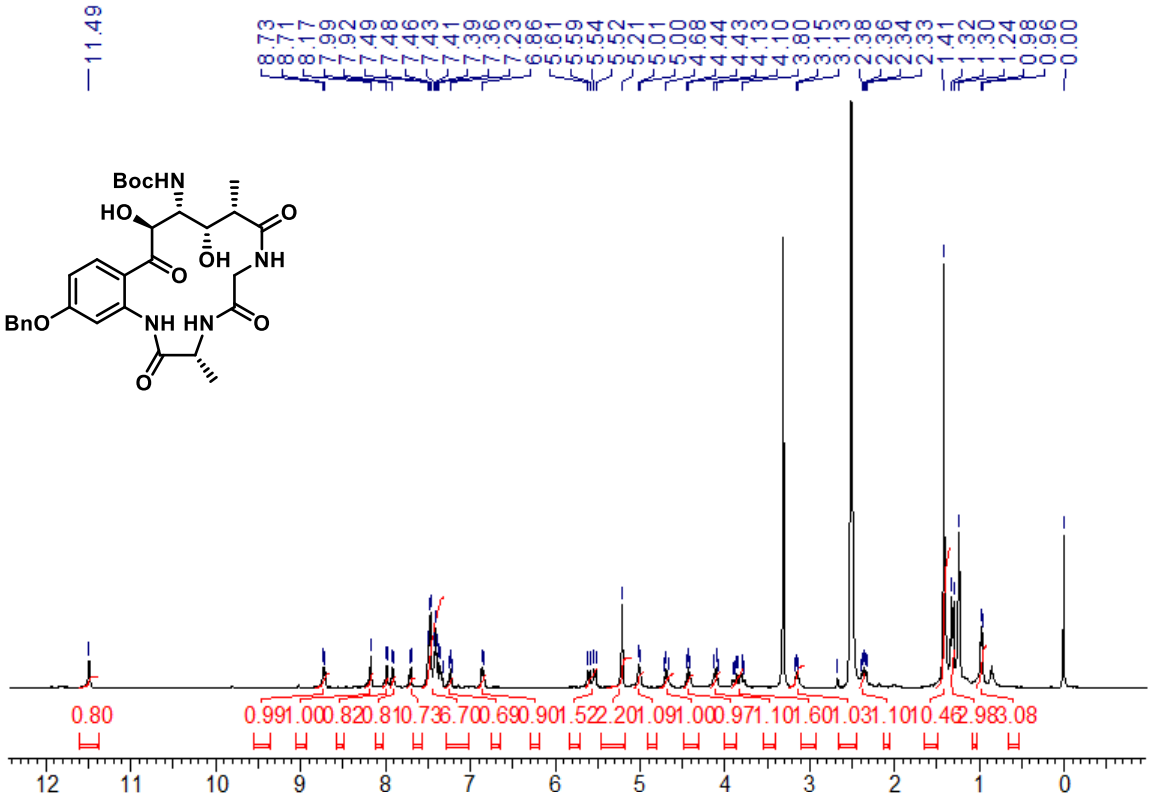
^{13}C NMR of **105** (125 MHz, DMSO-d_6)



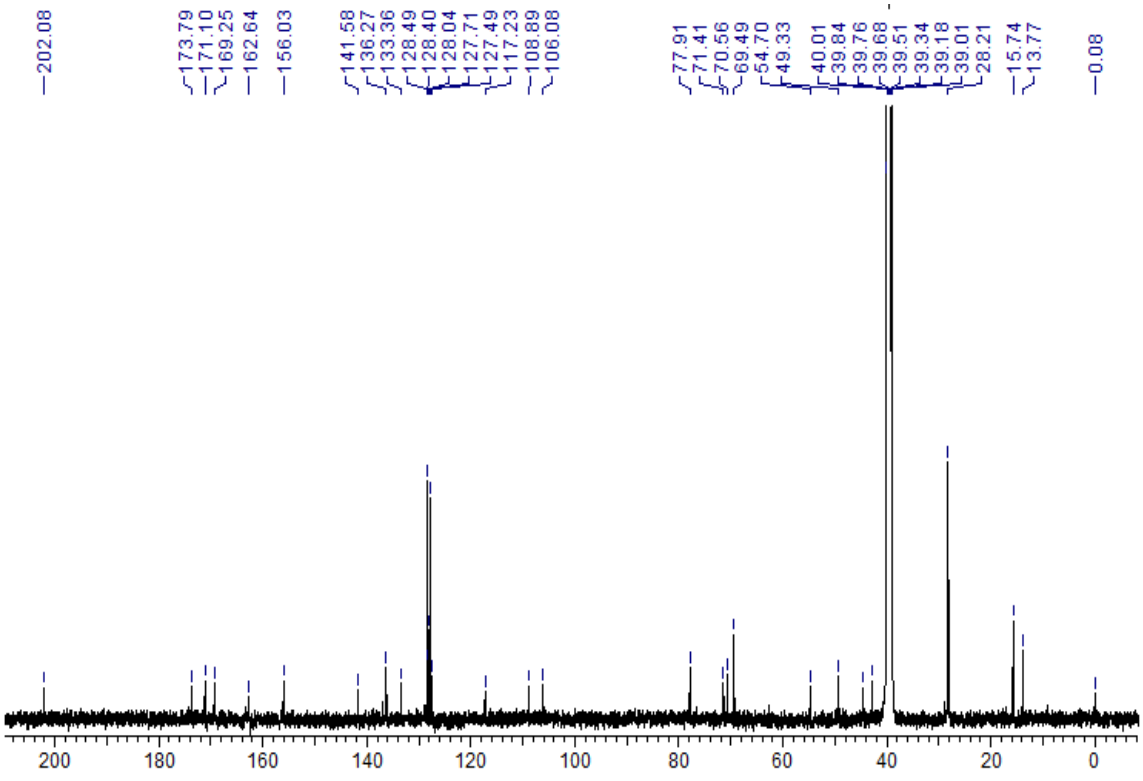
^{1}H NMR of **124** (400 MHz, DMSO-d_6)



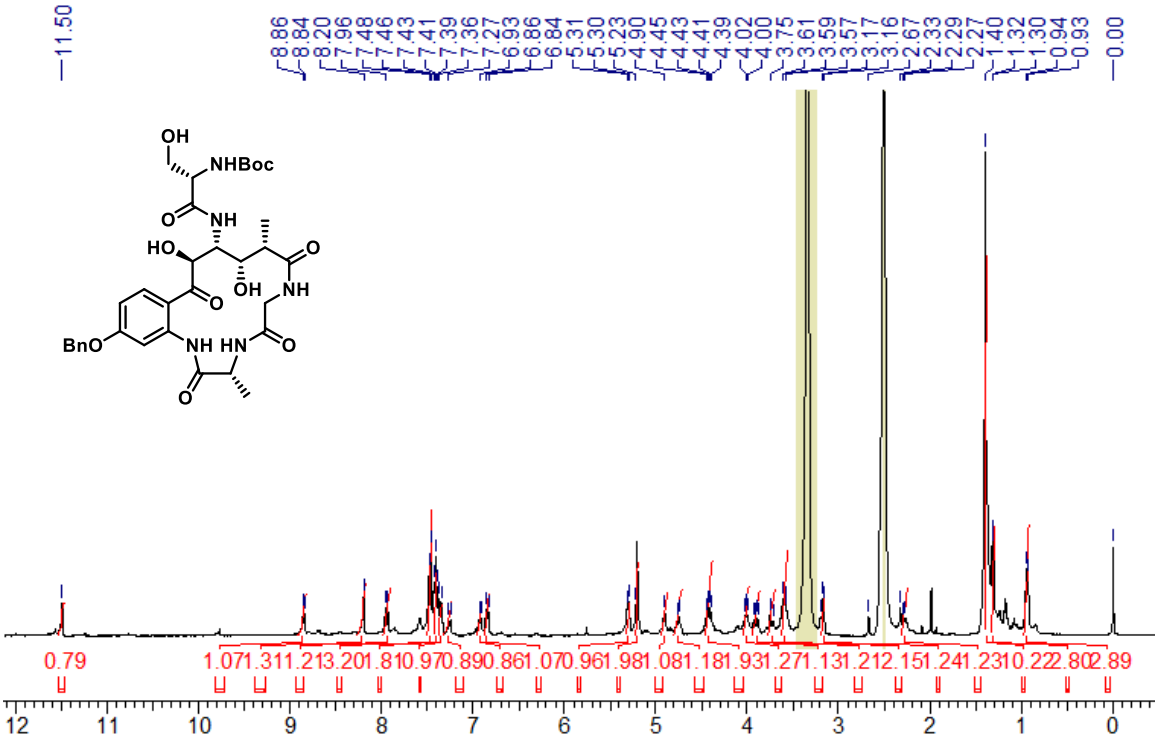
^{13}C NMR of **124** (100 MHz, DMSO-d_6)



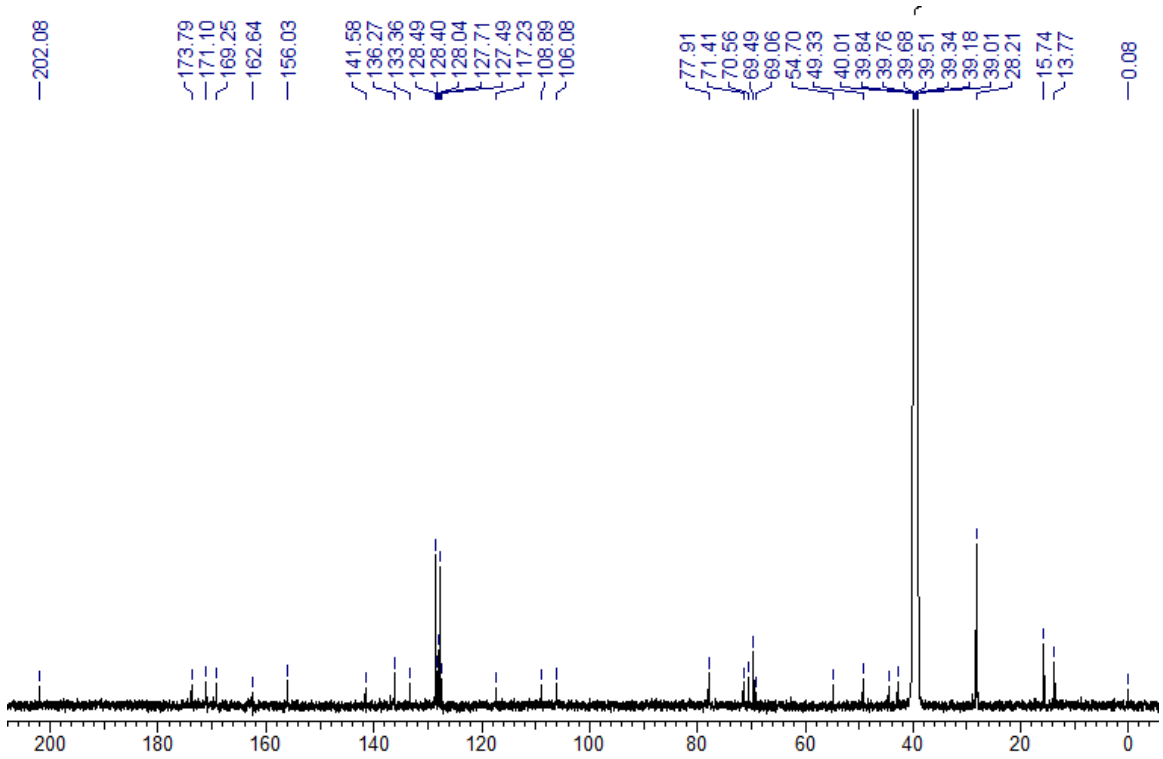
^{1}H NMR of **123** (400 MHz, DMSO-d_6)



^{13}C NMR of **123** (125 MHz, DMSO-d_6)



^{1}H NMR of **125** (500 MHz, DMSO-d_6)



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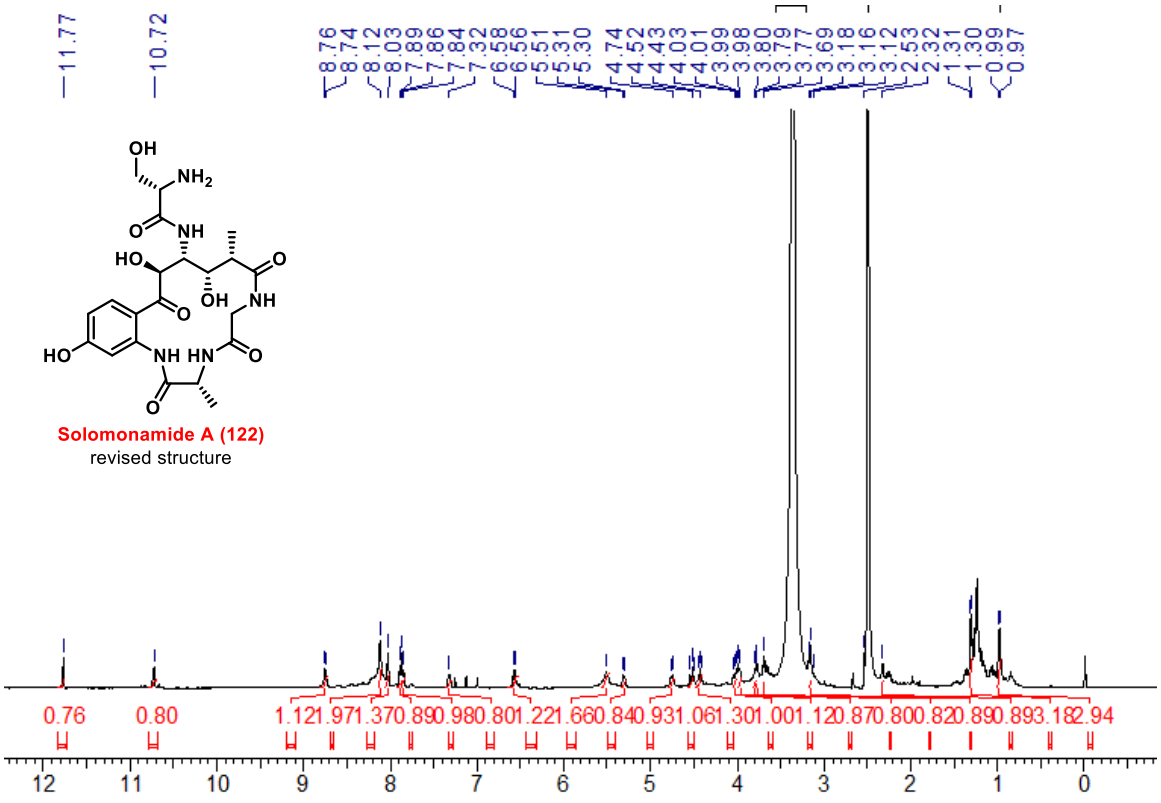
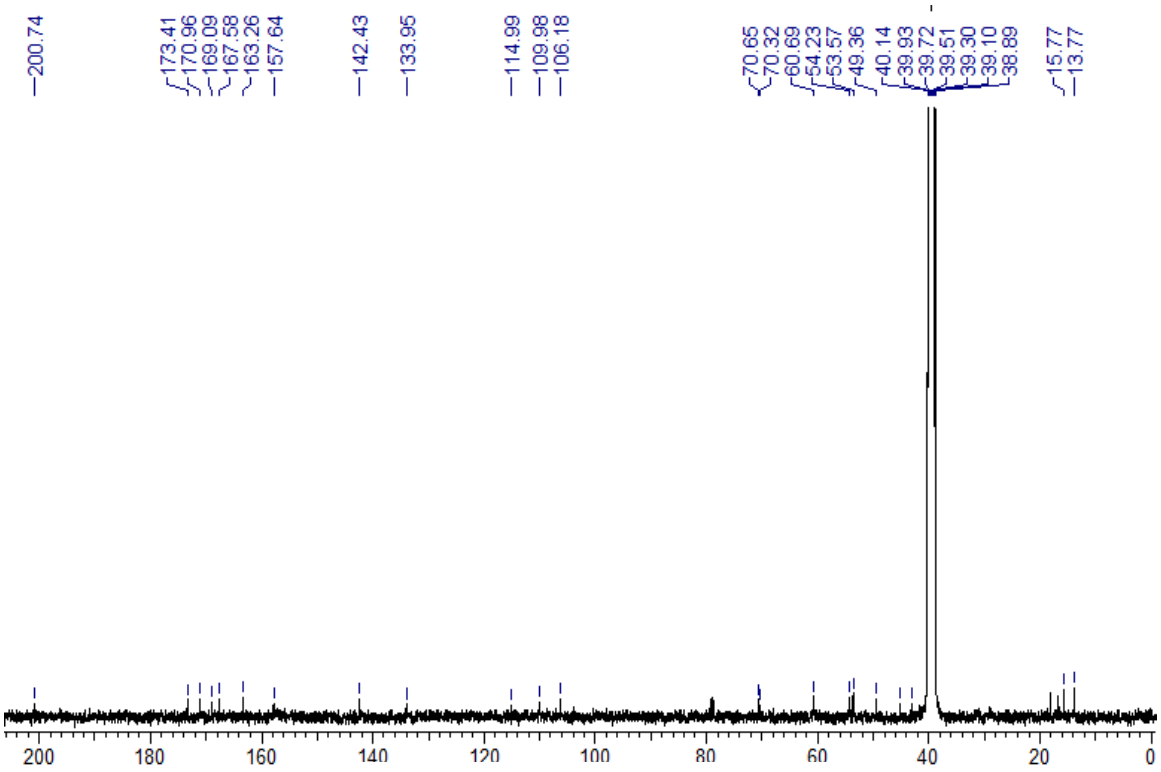


Solomonamide A (122)
revised structure

^{1}H NMR of **122** (500 MHz, DMSO-d_6)



^{13}C NMR of **122** (125 MHz, DMSO-d_6)

Section II Synthesis of solomonamide analogues and their biological evaluations

1.2.1. Planned SAR around solomonamide scaffold

Successful documentation of structural revision of solomonamides[1, 2] and their interesting biological profile encourage us to plan and systematically synthesize a library of analogues around the scaffold to identify potential lead compound(s) towards the development of anti-inflammatory agents. Initially, our aim was to obtain a simplified structure with desirable properties. The total synthesis route developed was amenable for the synthesis of structurally close analogues of the natural products. Our efforts were dedicated on structure simplification and understanding the Structure Activity Relationships (SAR). Planned structural modifications are depicted below (Figure 2.1.).

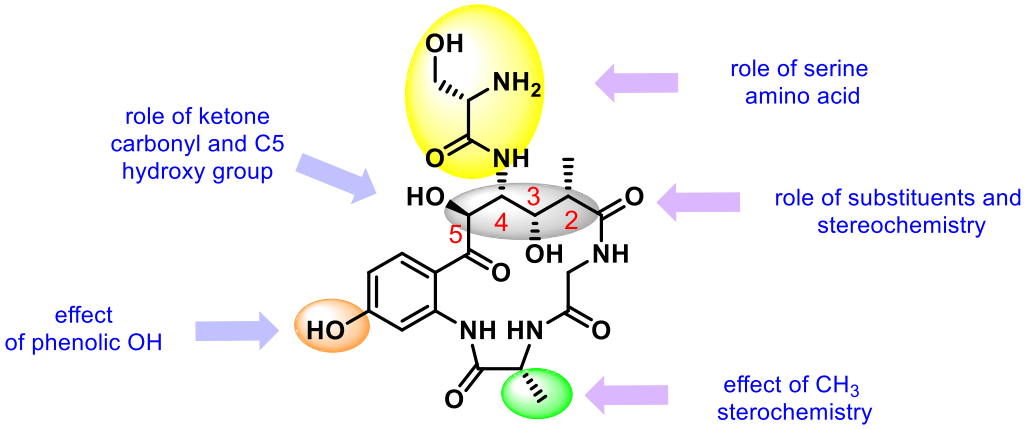


Figure 2.1. Planned SAR around the solomonamide scaffold

Accordingly, we chose to prepare analogues based on the following aspects:

a) Change in stereochemistry of the non-peptide portion

b) Change of alanine stereochemistry

c) Importance of phenolic hydroxyl group

d) Effect of removal of C2 methyl and C3 hydroxyl group

e) Removal of C3 hydroxyl and C4 amine group

f) Understanding the role of pendant serine amino acid

g) Role of ketone carbonyl and C5 hydroxyl group

l.2.2. Key fragments for analogue synthesis

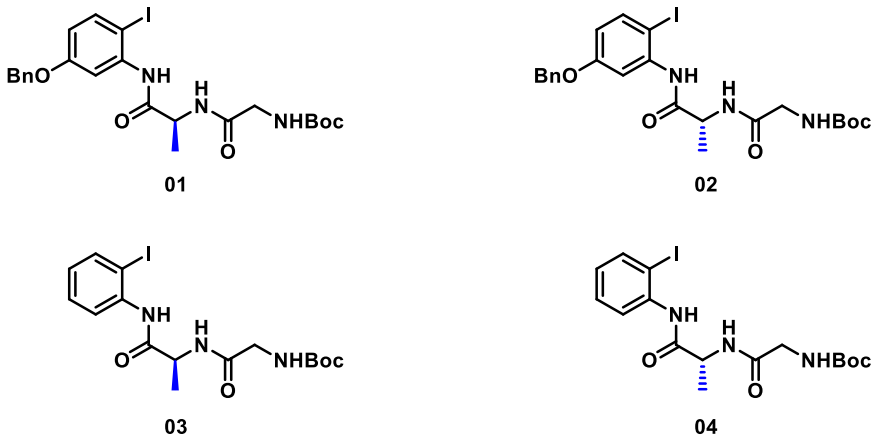


Figure 2.2. Planned dipeptides

As per the plan, we need to synthesize key building blocks. Initially, we planned to make selected dipeptides to study the effect of alanine stereochemistry and role of substitution on biological potential. Accordingly, we designed dipeptides namely **01**, **02**, **03** and **04** among these dipeptides, **02** was made previously during the synthesis of natural products (Figure 2.2.). For the non-peptide acids we designed different combinations.

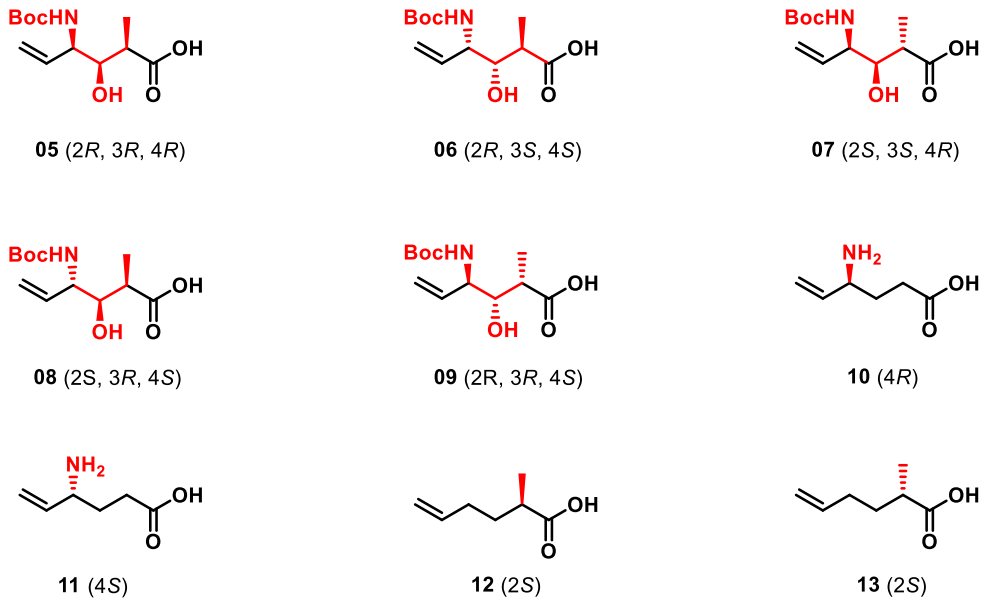
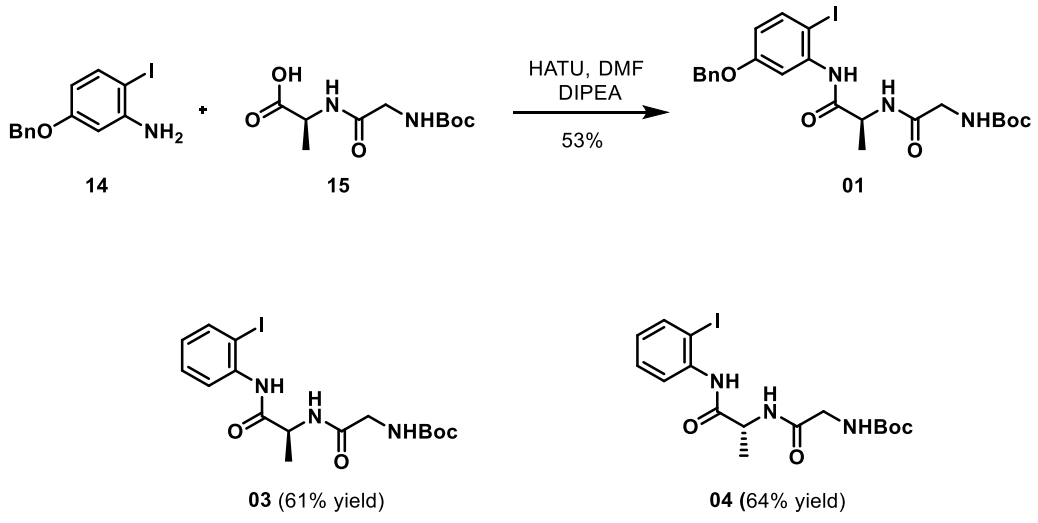


Figure 2.3. Designed non-peptide acids

Compounds **05-09** having three stereocenters by changing their stereochemistry to visualize the role of absolute stereochemistry on biological potential. In addition, we also planned few analogues with or without stereocenter on non-peptide portion to simplify structure of the natural products. Accordingly, we designed synthesis of non-peptide acids having only NH (**10** and **11**) as well as only CH_3 (**12** and **13**) stereocenter in both enantiomeric forms (Figure 2.3.).

1.2.2.1. Synthesis of dipeptides

Synthesis of dipeptide **01** commenced with aniline derivative **14**[3] which on coupling with Boc-Gly-L-ala-OH **15** using known protocol[4] gave the desired dipeptide **01** with 53% yield. Formation of dipeptide **01** was indicated by the presence of polar spot on TLC compared to that of aniline derivative **14**. Total number of protons in ^{1}H NMR confirmed the formation of **01**. Presence of characteristic peaks corresponding to two amide carbonyl at δ 171.5, 169.9 ppm and carbamate carbonyl at δ 159.2 in ^{13}C NMR further confirmed the formation of compound **01**. In addition, HRMS supported the presence of **01** and showed exact mass value at *m/z* 576.0953 corresponding to $C_{23}H_{28}O_5N_3NaI$. Following the



Scheme 2.1. Synthesis of dipeptides

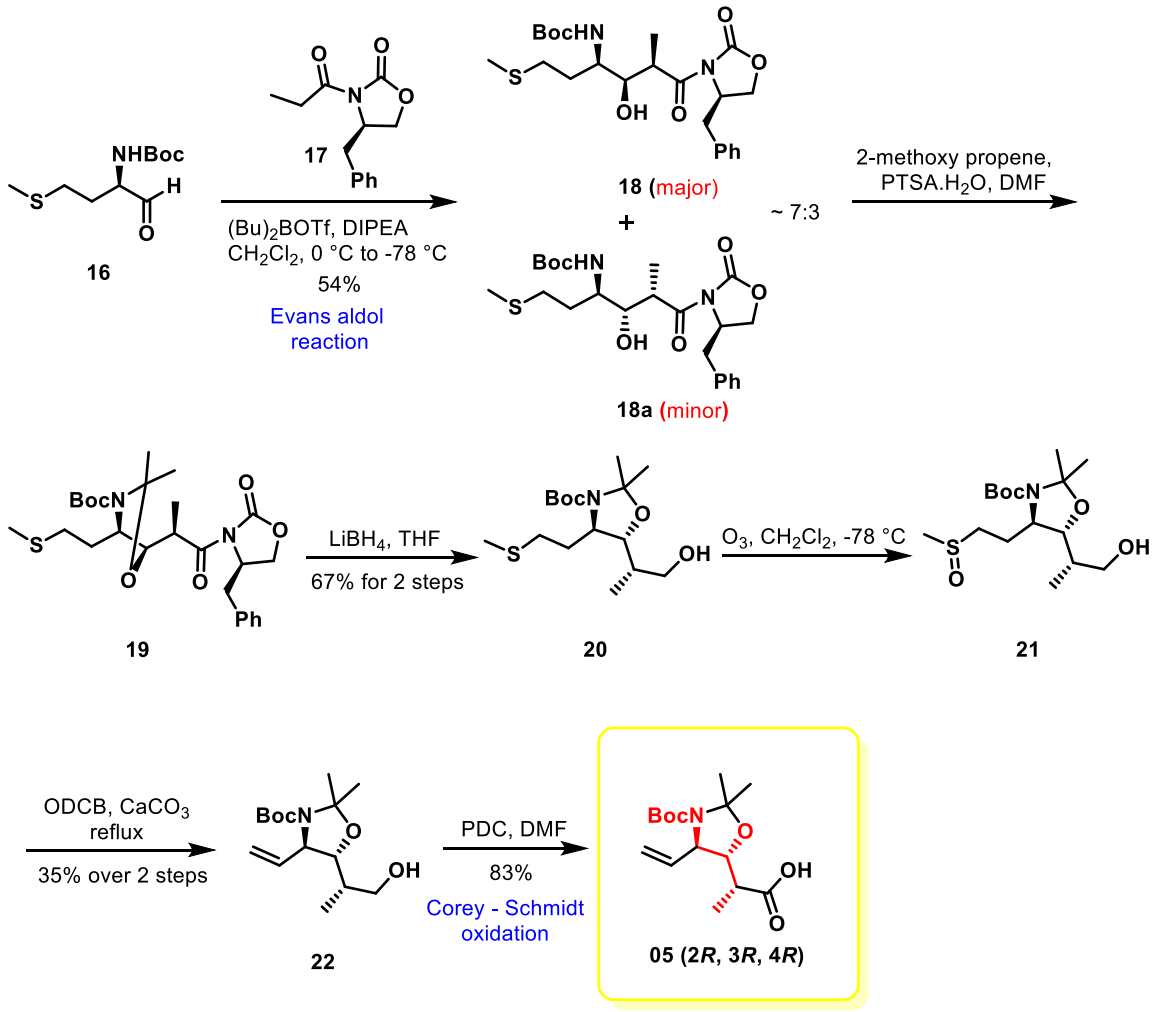
same synthetic sequence using 2-iodo aniline, Boc-Gly-L-Ala-OH (**15**) and its enantiomer Boc-Gly-D-Ala-OH we have synthesized dipeptides **03** and **04** with 61% and 64% yield respectively. ^{1}H NMR, ^{13}C NMR and HRMS analysis supported the formation of desired dipeptides **03** and **04** (Scheme 2.1.). After successful synthesis of dipeptides in gram scale, we next focused on the synthesis of non-peptide acids.

1.2.2.2. Synthesis of non-peptide acids with three stereocenters

It is documented in the literature on several occasions that stereochemistry plays a pivotal role in medicinal chemistry and drug discovery.[5] To examine the role of stereochemistry on the potency of solomonamide analogues, we planned to synthesize all possible stereoisomers of natural products. However, in the non-peptide portion of solomonamide B, there are three contiguous stereocenters (non-peptide portion) so total eight stereoisomers are possible. Among them, three acids with 2*S*, 3*R*, 4*R*; 2*S*, 3*S*, 4*S* and 2*R*, 3*S*, 4*R* stereochemistry were previously synthesized in our lab (see previous section). To synthesize the remaining five possible stereoisomers (**05**-**09**) involves remodelling of the synthetic pathways. To prepare these fragments we relied on well-established protocols such as Brown crotylation, Evans aldol reaction as well as crotylation reaction.

Initially, we targeted synthesis of the non-peptide acid **05** having 2*R*, 3*R*, 4*R* stereochemistry from D-methionine aldehyde **16** using Evans aldol reaction as a key step. Accordingly, aldehyde **16** on Evans aldol reaction[6] using boron (*Z*)-enolate derived from (*R*)-4-benzyloxazolidin-2-one derivative **17** and dibutyl boron triflate in presence of DIPEA in DCM resulted in the formation of compounds **18** and **18a** with 7:3 diastereomeric ratio in 54% yield. Although the diasteroselectivity was not great, we were able to separate the two diastereomers cleanly using simple silica gel column chromatography. The structure of corresponding aldol products were confirmed by comparing NMR spectral data along with their enantiomers which were discussed previously, during synthesis of revised structure of solomonamide B.[1] Amino alcohol **18** was protected as acetonide using 2-methoxy propene in presence of catalytic amount of PTSA.H_2O in DMF resulting in the formation of **19**. Crude acetonide **19** was treated with

$LiBH_4$ in THF to remove the auxiliary, [7] which originated alcohol **20** with a yield of 67% over two steps. Disappearance of signals in ^1H NMR at δ 7.35-7.21 (m, 5H) corresponding to aromatic proton and appearance of an additional peak at δ 3.91-3.89 (m, 2H) assured reduction of compound **19**. In ^{13}C NMR spectrum, peaks corresponding to carbonyls at δ 175.8 and 153.2 ppm disappeared and appearance of new peak at δ 80.1 ppm confirmed

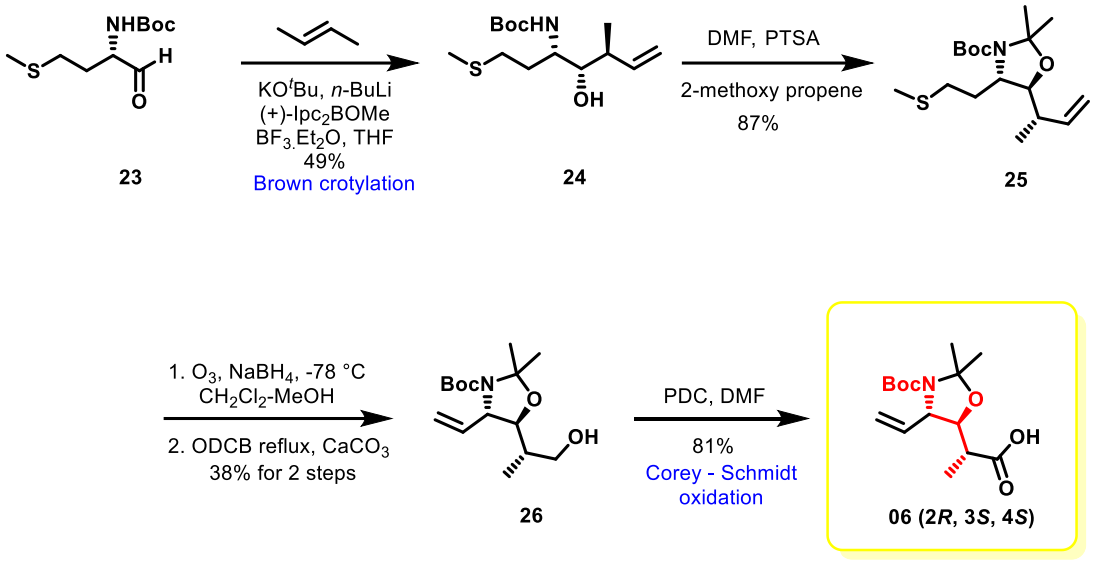


Scheme 2.2. Synthesis of non-peptide acid **05** with 2*R*, 3*R*, 4*R* configuration

the reduction to alcohol. Obtained alcohol **20** on ozonolysis in DCM produced a sufoxide intermediate **21** which on removal of solvent followed by pyrolytic elimination by

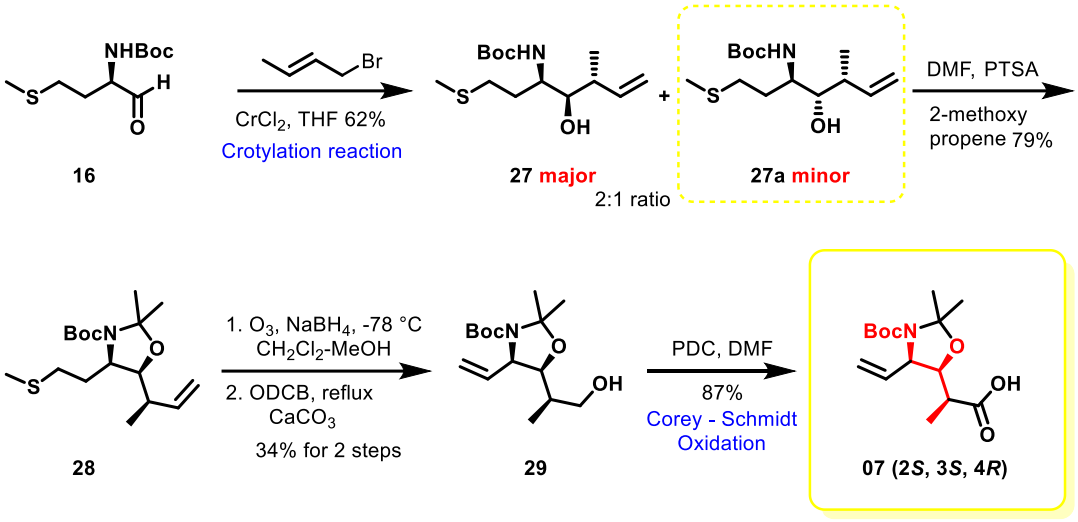
refluxing in 1, 2-dichloro benzene in presence of $CaCO_3$ afforded alkenol **22** in 35% yield over 2 steps.[8] Primary alcohol in compound **22** was oxidized directly to acid **05** (2*R*, 3*R*, 4*R*) using Corey Schmidt protocol (PDC in DMF)[9] with a yield of 83% (Scheme 2.2.). The formation of carboxylic acid **05** was monitored by TLC and it is more polar than alkenol **22** which stipulated formation of same. The disappearance of peak in ^{1}H NMR at δ 3.91-3.89 (m, 2H) confirmed the oxidation of primary alcohol while in ^{13}C NMR peak corresponding to CH_2 at δ 80.1 ppm attached to hydroxy group disappeared and appearance of new peak corresponding to acid carbonyl at δ 179.1 ppm confirmed the formation of acid **05**. All the spectral data of synthesized non-peptide acid **05** was in complete agreement with its enantiomer with (2*S*, 3*S*, 4*S*) which was synthesized previously during stereochemical revision of solomonamide B.[1]

Subsequently, we outlined the synthesis of non-amino acid fragment **06** having 2*R*, 3*S*, 4*S* stereochemistry from L-methionine aldehyde **23** using Brown crotylation. Accordingly, aldehyde **23** on treatment with (+)-Ipc_2BOMe, *trans* 2-butene and $BF_3.Et_2O$ in presence of base KO^{t}Bu and *n*-BuLi in anhydrous THF at -78 °C obtained crotylated product **24** with 49% yield.[10] Amino alcohol **24** thus obtained was protected as acetonide resulted in the formation of **25** with a yield of 87%. Compound **25** on reductive ozonolysis in DCM and methanol in presence of $NaBH_4$ provided the sufoxide intermediate.[11] During ozonolysis reaction, sulphur was oxidised to sufoxide and the olefin was converted to alcohol simultaneously in a single operation. Crude sufoxide was forwarded for pyrolytic elimination in presence of $CaCO_3$ by refluxing in 1, 2-dichloro benzene which afforded alkenol **26** in 38% yield over 2 steps. Primary alcohol in compound **26** was then subjected to Corey Schmidt oxidation providing acid **06** with 81% yield (Scheme 2.3.). Formation of acid **06** was confirmed by appearance of peak in ^{1}H NMR at δ 5.80-5.72 (m, 1H), 5.20 (d, 2H) which corresponds to characteristic terminal olefins and the peaks at δ 178.9, 151.9 in ^{13}C NMR corresponds to acid and carbamate carbonyls. Additionaly, structure of corresponding acid **06** was asserted by comparing spectral details with its enantiomer (2*S*, 3*R*, 4*R*) which was reported earlier.[1]



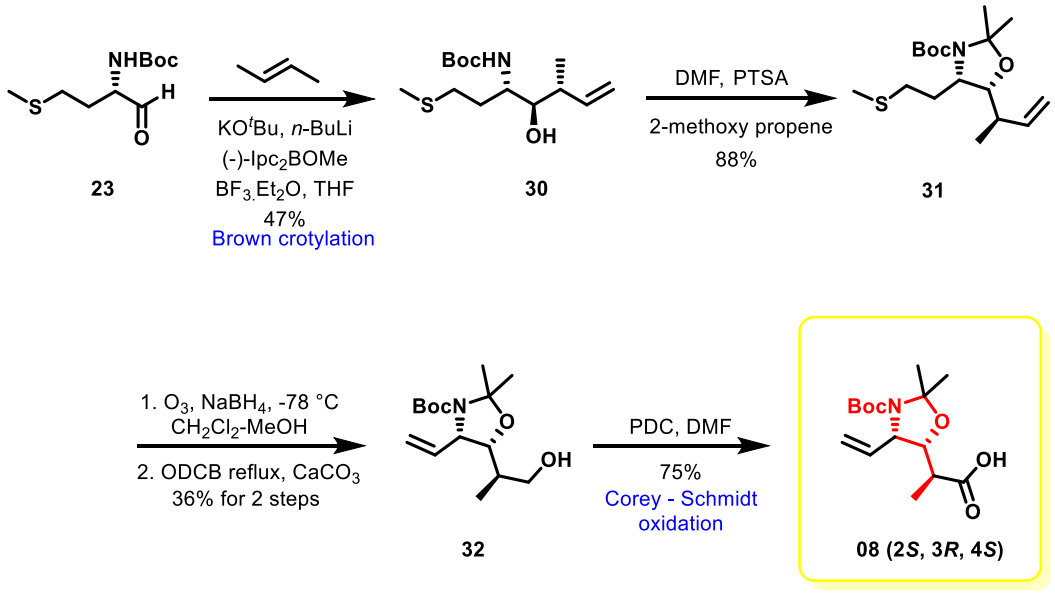
Scheme 2.3. Synthesis of non-amino acid fragment **06** with 2*R*, 3*S*, 4*S* configuration

After the synthesis of acid **05** and **06**, we turned our attention towards synthesis of acid **07** with 2*S*, 3*S*, 4*R* stereochemistry from D-methionine aldehyde **16** using crotylation reaction as the key step. Thus, aldehyde **16** underwent crotylation reaction using freshly activated $CrCl_2$ and *trans*-crotyl bromide in THF gave a 2:1 ratio of diastereomers **27** and **27a** in 62% yield. All the spectral details of major diastereomer **27** was in complete agreement with its structure reported earlier.[1] Stereochemistry of compound **27a** was assigned on the basis of the literature precedence[11] which was reported during synthesis of deoxy-solomonamide B. Thus, minor diastereomer **27a** having (2*S*, 3*S*, 4*R*) was utilized for analogue synthesis. The amino alcohol **27a** was converted to acid **07** through intermediacy of acetonide **28** and alcohol **29** following the similar synthetic sequence as in the previous scheme 2.3. Formation of acid **07** was confirmed by presence of peak at δ 5.75 - 5.66 (m, 1H), 5.36-5.18 (m, 2H) ppm corresponding to terminal olefin and acetonoide methyl's at δ 1.42 (s, 6H). In addition, formation of **07** was further confirmed with HRMS which showed peak at 322.1615 corresponding to molecular formula $C_{15}H_{25}O_5NNa$ $[M+H]^+$ with calculated value at 322.1625.



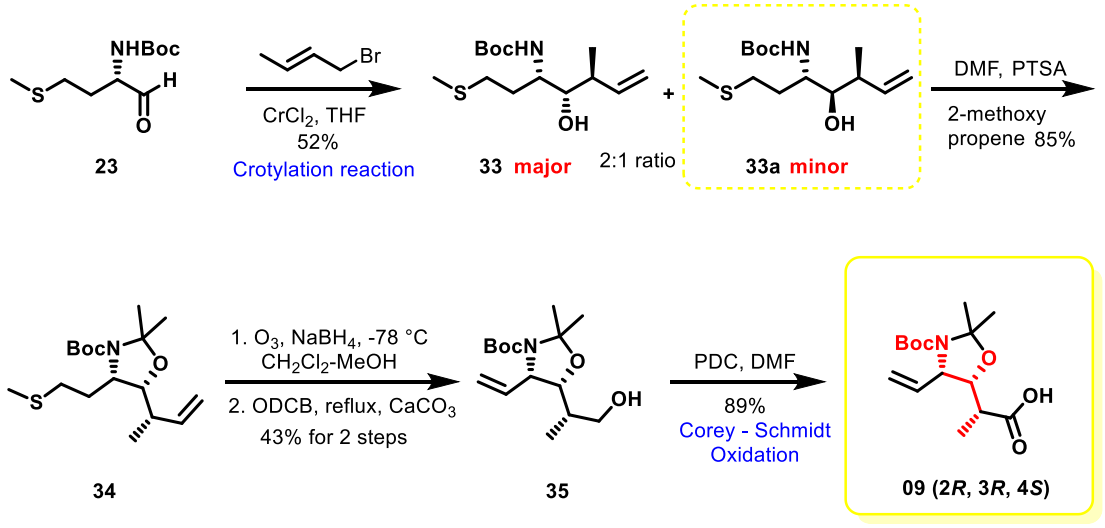
Scheme 2.4. Synthesis of non-amino acid fragment **07** with 2*S*, 3*S*, 4*R* configuration

Our next task was to synthesize the acid **08** having 2*S*, 3*R*, 4*S* stereochemistry from L-methionine aldehyde **23** for which we relied on Brown crotylation. Accordingly, aldehyde **23** on Brown crotylation using (-)-Ipc$_2$BOMe, *trans*-2-butene, BF$_3$.Et$_2$O in presence of base KO^{t}Bu and *n*-BuLi in anhydrous THF afforded crotylated product **30** with



Scheme 2.5. Synthesis of non-amino acid **08** with 2*S*, 3*R*, 4*S* configuration

47% yield.[10] After which, compound **30** was converted to key non-amino acid **08** through the intermediacy of **31** and **32** by following similar synthetic sequence as mentioned in the previous scheme 2.4. Formation of acid **08** was confirmed by presence of characteristic acid carbonyl peak at δ 179.9 in ^{13}C NMR spectra. In addition, formation of **08** was further confirmed with HRMS which showed peak at 322.1616 corresponding to molecular formula $C_{15}H_{25}O_5NNa$ $[M+H]^+$ with calculated value at 322.1625.



Scheme 2.6. Synthesis of non-amino acid fragment **09** with 2*R*, 3*R*, 4*S* configuration

Subsequently, synthesis of non-peptide acid **09** having 2*R*, 3*R*, 4*S* stereochemistry was envisioned from L-methionine aldehyde **23** using crotylation reaction. The aldehyde **23** underwent crotylation reaction using freshly activated $CrCl_2$, *trans*-crotyl bromide in THF gave a 2:1 ratio of diastereomers **33** and **33a** in 52% yield.[12] All the spectral details of major diastereomer **33** was in complete agreement with its enantiomer which was previously synthesized using Brown crotylation from our group.[1] The stereochemistry of minor diastereomer **33a** was assigned through comparison with its enantiomer **27a**. The required diastereomer **33a (**minor) was utilized for acid synthesis where the amino alcohol in compound **33a** was protected as acetonide using 2-methoxy propene in presence of catalytic amount of PTSA.H_2O in DMF gave acetonide **34** in 85% yield. Compound **34** on

reductive ozonolysis in DCM and methanol in presence of $NaBH_4$ resulted in the formation of sufoxide which was forwarded for pyrolytic elimination in presence of $CaCO_3$ in 1, 2-dichloro benzene under reflux condition to afford alkenol **35** in 43% yield for 2 steps. Primary alcohol in compound **35** was then oxidized to acid **09** using PDC in DMF with 89% yield[9] (Scheme 2.6.). All spectral data of synthesized acid **09** (2*R*, 3*R*, 4*S*) was in complete agreement with its enantiomer **07** (2*S*, 3*S*, 4*R).*

Thus, we accomplished the synthesis of non-peptide acids from **05** to **09** having three contiguous chiral centres in good quantities.

1.2.2.3. Synthesis of non-peptide acids with only NH stereocenter

After successful synthesis of acids **05** to **09** having three chiral centers, we next focused on synthesizing the non-peptide acid **10** and **11** having only NH as stereocenter which is useful for understanding the role of remaining two stereocenters (CH_3 and OH) of non-peptide acid fragment. While we are working on making these fragments, we realized that the compounds **10** and **11** after removal of Boc group is nothing but vigabatrin (**36**), a well-known marketed drug, which is used for the treatment of epilepsy.[13] However, biochemical mechanisms responsible for epilepsy, are not fully understood, it is known that GABA is a major inhibitory neurotransmitter present in the mammalian central nervous system (CNS) that prevents seizures.[14] The GABA-aminotransferase (GABA-T) is an important enzyme in catabolic process, which degrades GABA to succinic semi aldehyde.[15] The 4-amino-5-hexenoic acid known as vigabatrin (**36**) which is an anticonvulsant drug sold under the brand name Sabril (Figure 2.4.).[13] It is one of the most

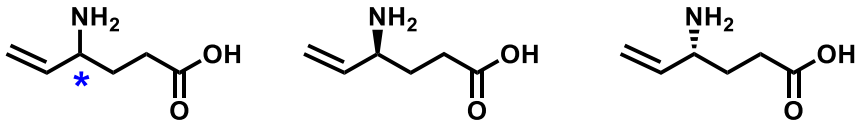
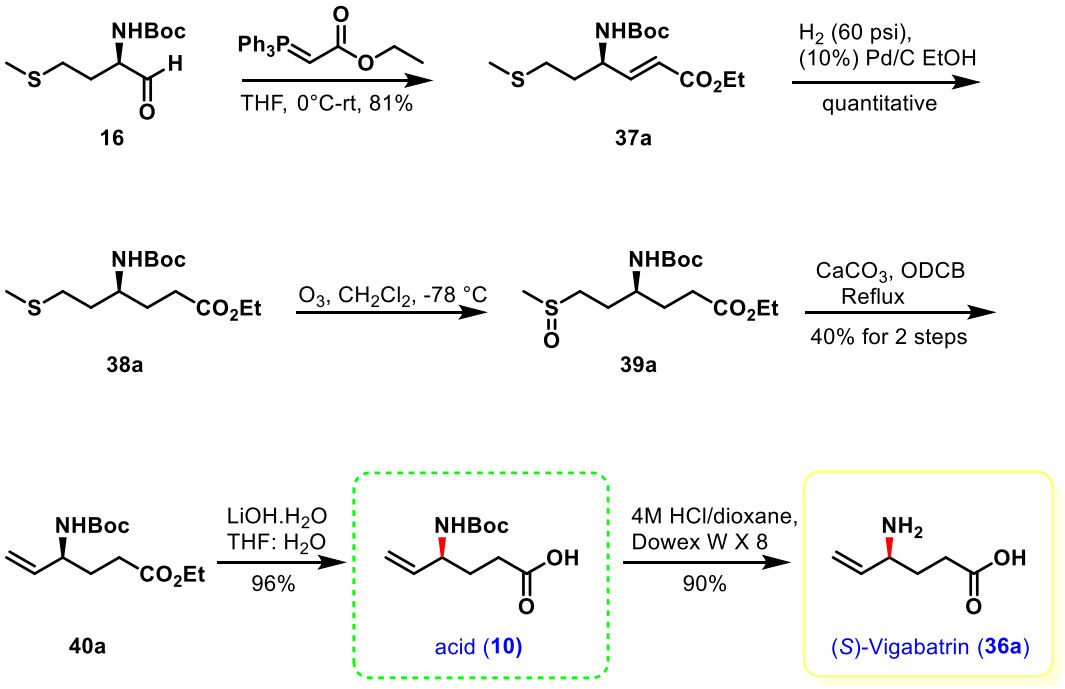


Figure 2.4. Structure of drug vigabatrin

effective and selective inhibitors of GABA-T available in the market. Pharmacological properties of vigabatrin have been well documented in literature which demonstrate its absolute configuration to be highly decisive for the concerned biological activity of the drug. Although, the marketed drug is avilable in racemic form however, (*S*)-vigabatrin (**36a**) is the pharmacologically active enantiomer.[16] Since stereochemistry is a key important fact in the efficiency and safety of a drug molecule, synthesis of chirally pure drugs is presently an area of major interest in the domain of medicinal chemistry and drug discovery. Hence the asymmetric synthesis of vigabatrin holds a critical standpoint in this context. A few methods are reported for the racemic as well as chiral synthesis of this anti-convulsive drug some of them having certain disadvantages like more number of reaction steps and expensive catalysts coupled with low enantiomeric purity.[17] In this connection, we outlined a plan to access vigabatrin (**36**) starting from methionine which is readily available in both enantiomeric forms. Accordingly, the synthesis of (*S*)-vigabatrin **36a** commenced with a known D-methionine aldehyde **16** which on two carbon Wittig olefination reaction with ethyl 2-(triphenyl-l5-phosphaneylidene) acetate in CH_2Cl_2 gave the desired α,β-unsaturated ester **37a** in 81% yield.[18] Synthesized ester **37a** showed very prominent charring in $KMnO_4$ stain as well as UV activity on TLC which indicated the formation of same. The formation of **37a** was confirmed by ^{1}H NMR spectra where presence of peak at δ 6.80 (dd, *J* = 4.9, 15.9 Hz, 1H), 5.89 (d, *J* = 15.3 Hz, 1H) corresponds to proton attached to olefin and a peak at δ 2.05(s, 3H) corresponding to methylthio group. The ^{13}C NMR spectra clearly shows ester carbonyl at δ 166.1 ppm and characteristic olefin carbon at δ 147.5, 121.0 ppm which supported the formation of Wittig homologated product **37a**. In addition, the HRMS analysis showed a peak at m/z 304.1571 corresponding to a molecular formula $C_{14}H_{26}O_4NS$ $[M+H]^+$ further confirming the formation of ester **37a**. To our delight, compound **37a** upon hydrogenation using catalytic amount of 10% Pd/C in ethanol under 60 psi pressure of hydrogen observed the formation of ester **38a** with quantitative yield. The synthesized ester **38a** after hydrogenation is UV inactive which suggests the hydrogenation of ester. In ^{1}H NMR absence of olefinic peak at δ 6.80 (dd, *J* = 4.9, 15.9 Hz, 1H), 5.89 (d, *J* = 15.3 Hz, 1H) confirms formation of **38a**. Additionally, in ^{13}C NMR absence of peaks at δ 147.5, 121.0 ppm and appearance of new peaks at δ 31.0,

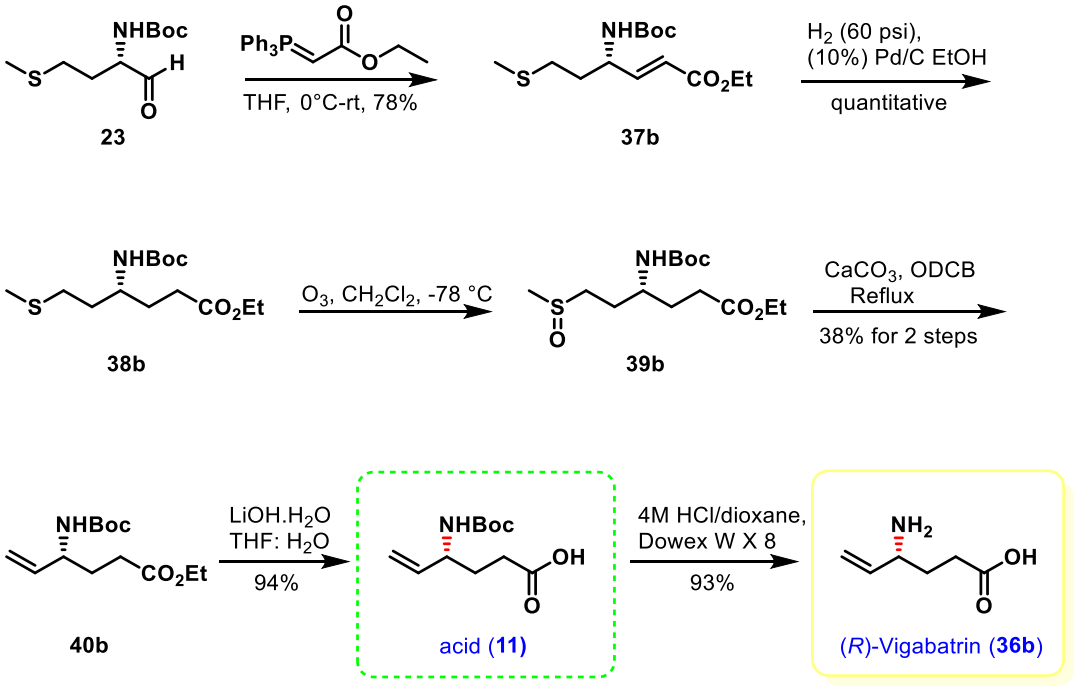
30.3 ppm further confirmed the structure **38a**. Additionally, the HRMS (ESI) analysis showed a peak at m/z 306.1721 corresponding to saturated ester **38a**. It was reported in the literature that reduction of α,β-unsaturated ester having methylthio group is difficult because of its interference in catalytic hydrogenation reaction or poisoning of catalyst.[19] However, it is worth highlighting that we have performed hydrogenation on a gram scale in single batch operation without any difficulties. Next, we have utilized ozonolysis reaction to convert sulphur to sufoxide in CH_2Cl_2 at -78˚C thereafter solvent was removed



Scheme 2.7. Synthesis of (*S*)-vigabatrin

and crude sufoxide (**39a**) thus obtained was subjected for pyrolytic elimination in the presence of optimized condition which resulted in the formation of alkenol **40a** with a yield of 40% over two steps. Authenticity of compound **40a** was confirmed by ^{1}H NMR spectra where the peaks at δ 5.74 (ddd, *J* = 5.7, 10.6, 16.9 Hz, 1H), 5.19-5.10 (m, 2H) corresponds to terminal olefin and a peak at δ 2.09 (s, 3H) corresponding to methyl thio group were absent. In ^{13}C NMR spectra, two characteristic peaks at δ 138.3, 115.0 ppm further

confirms the formation of terminal olefin. In addition, the HRMS analysis showed a peak at m/z 280.1513 corresponding to a molecular formula $C_{13}H_{23}O_4NNa$ $[M+Na]^+$ depicting the formation of **40a**. Essentially following the same synthetic sequence, we have prepared corresponding enantiomer **40b** starting from **23b**. The enantiopurity of compound **40a** and its enantiomer **40b** was verified by using chiral HPLC method which was found to be >98%.[20] Having an ester **40a** in hand, we hydrolyzed it using $LiOH.H_2O$ in THF:H_2O mixture providing a non-peptide acid fragment **10** with a 96% yield in gram scale. Hydrolysis of ester was indicated by presence of polar spot on TLC as compared to

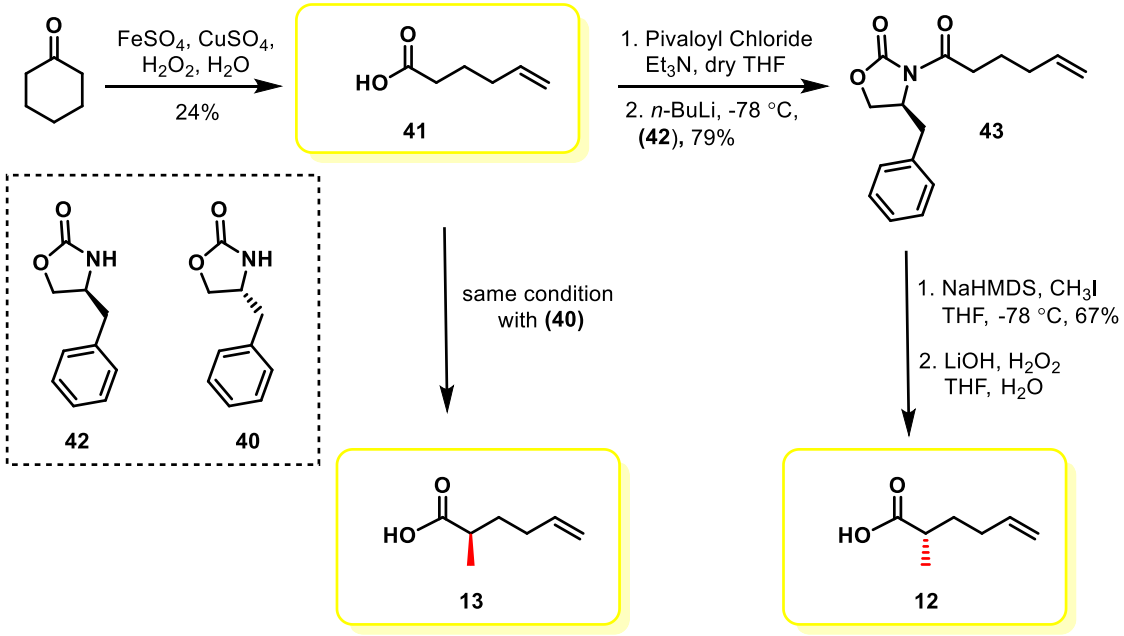


Scheme 2.8. Route for synthesis of (*R*)-vigabatrin

ester **40a**. We recorded ^1H NMR spectra in DMSO-d_6 solvent and observed peak at δ 11.99 (brs, 1H) ppm corresponding to acid proton and disappearance of peaks at δ 4.13 (q, J = 7.0 Hz, 2H), 1.26 (t, J = 7.2 Hz, 3H) confirming the hydrolysis of ester. Disappearance of ethyl ester peak at δ 60.5, 14.2 ppm in ^{13}C NMR, and new characteristic peak at δ 174.1 corresponding to acid further confirmed the structure of acid **10**. Finally, Boc deprotection

using 4N HCl in dioxane followed by passing through Dowex resin resulted in (*S*)-vigabatrin (**36a**) with a yield of 90% for 2 steps (Scheme 2.7.). All the spectral data of synthesized (*S*)-vigabatrin (**36a**) was in complete agreement with reported data.[17] After the successful synthesis of (*S*)-vigabatrin (**36a**), synthesis of its enantiomer (*R*)-vigabatrin (**36b**) was taken up. Accordingly, gram scale synthesis of (*R*)-vigabatrin (**36b**) as well as non-peptide acid **11** was achieved by repeating the same synthetic protocol used for synthesis of **36a** (Scheme 2.8.). In short, we have accomplished gram scale synthesis of drug vigabatrin in both enantiomeric forms.

1.2.2.4. Synthesis of non-peptide acids with and without CH_3 stereocenter



Scheme 2.9. Synthesis of non-peptide acids with and without CH_3 stereocenter

Subsequently, we focused on synthesis of acid fragment with or without methyl substitution. Synthesis of the (*S*)-2-methyl hex-5-enoic acid (**12**) started with an oxidative cleavage of cyclohexanone to 5-hexenoic acid (**41**)[21] using $FeSO_4/CuSO_4$ in presence of H_2O_2. Pivaloyl chloride mediated auxiliary coupling to acid using (*S*)-(+)-4-phenyl-2-

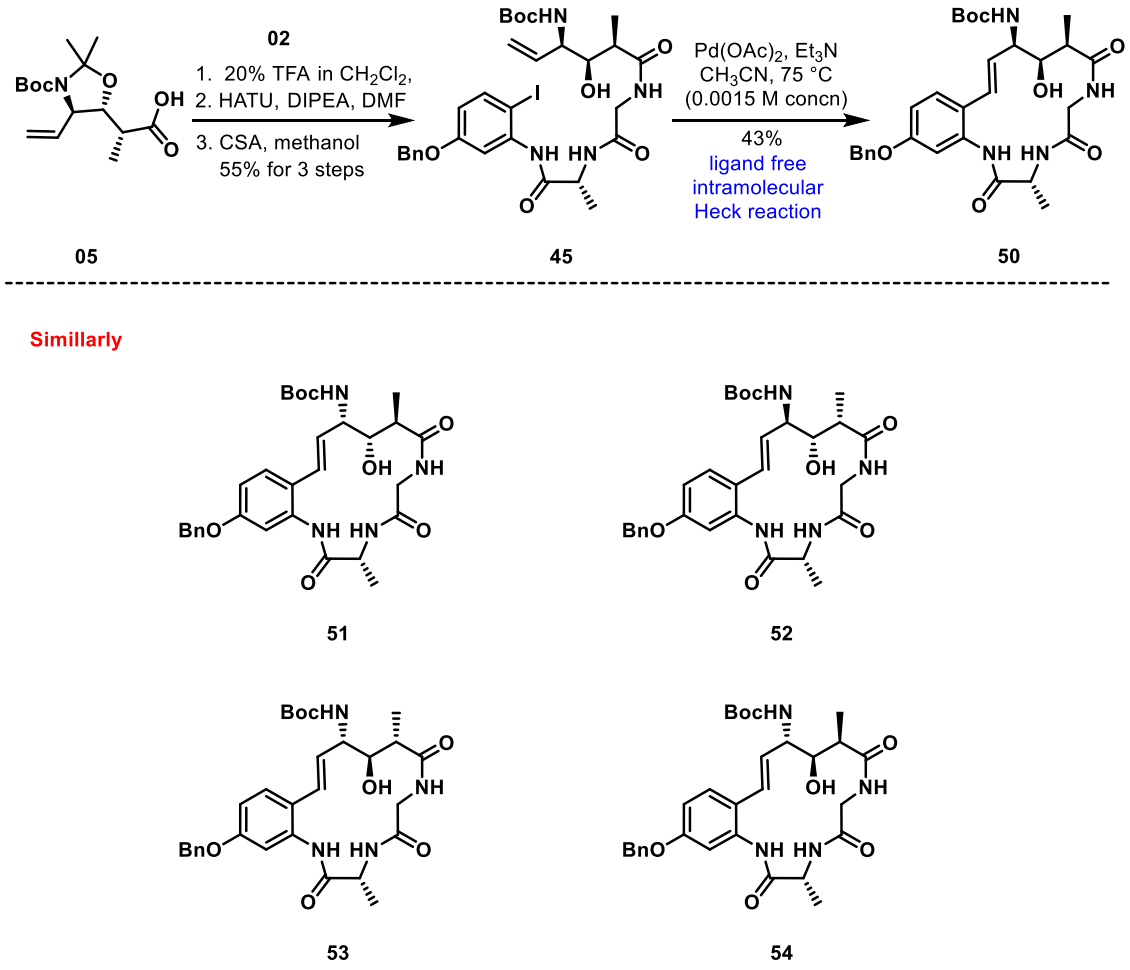
oxazolidinone **42** followed by selective methylation on **43** gave the intermediate with requisite stereochemistry. Hydrolysis using $LiOH/H_2O_2$ gave the desired acid **12** in overall good yield. Following similar reaction sequences, by using (*R*)-(−)-4-phenyl-2-oxazolidinone **44** and acid (**41**) resulted in the formation of (*R*)-2-methylhex-5-enoic acid (**13)** in good yield (Scheme 2.9.). All the spectral data of synthesized acids **12** and **13** are in complete agreement with reported data.[21]

1.2.3. Synthesis of macrocycles with three stereocenters

After having all the desired key fragment in hand we planned the synthesis of macrocycles using them. Accordingly, Boc deprotection of dipeptide **02** using 20% TFA in DCM followed by coupling with non-peptide acid **05** (2*S*, 3*S*, 4*S*) employing standard coupling protocol (HATU-DIPEA in DMF) furnished the required coupling product.[1] Formation of coupling product was indicated by presence of polar spot on TLC as compared to that of acid **05**. After coupling, we took the coupling product forwarded for next step without further characterization for chemoselective deprotection of acetonide in presence of Boc group using catalytic amount of camphor sulphonic acid (CSA) in methanol afforded macrocyclic precursor **45** with moderate yield. Formation of macrocyclic precursor **45** was confirmed by ^{1}H NMR spectra where peak corresponding to terminal olefin appeared at δ 5.88 (ddd, *J* = 5.3, 10.7, 16.8 Hz, 1H), 5.21-5.13 (m, 2H) ppm. In addition, formation of **45** was further confirmed with HRMS which showed peak at 717.1758 corresponding to molecular formula $C_{30}H_{39}O_7N_4INa[M+Na]^+$ with calculated value at 717.1756. Further, we performed ligand free intramolecular Heck cyclization of **45** ($Pd(OAc)_2$, Et_3N) in acetonitrile at 75 °C to furnish the macrocycle **50** with 43% yield.[22] Formation of macrocycle **50** was concluded as the characteristic terminal olefin peaks at δ 5.88 (ddd, *J* = 5.3, 10.7, 16.8 Hz, 1H), 5.21 - 5.13 (m, 2H) disappeared in ^{1}H NMR spectra. Besides, the presence of 1, 2 substituted olefin peak at δ 6.65 - 6.64 (m, 1H), 6.21 (dd, *J* = 3.1, 16.0 Hz, 1H) confirmed the formation of macrocycle **50** with *trans* geometry around double bond having 16.8 Hz coupling constant. In addition to this, HRMS analysis showed a peak

at 589.2635 corresponding to molecular formula $C_{30}H_{38}O_7N_4Na[M+Na]^+$ with calculated value at 589.2633.

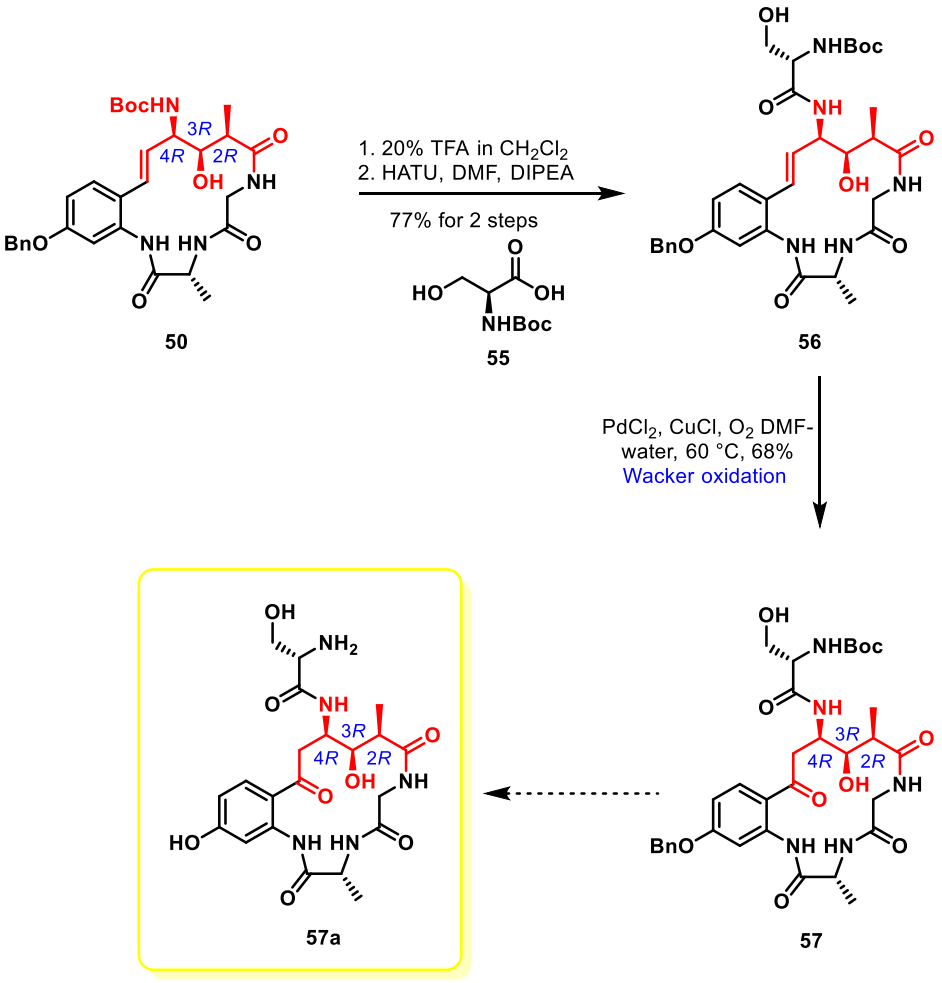
Following same synthetic sequence using non-peptide acid **06**, **07**, **08**, **09** and dipeptide **02** we have synthesized macrocyclic precursors **46**, **47**, **48**, **49** and using them we have synthesized macrocycles from **51-54** in moderate yield. Formation of all macrocycles were confirmed by using ^{1}H NMR, ^{13}C NMR as well as HRMS analysis (Scheme 2.10.).



Scheme 2.10. Synthesis of macrocycles with three chiral centers on non-peptide portion

1.2.4. Synthesis of analogues from macrocycles with three chiral centers

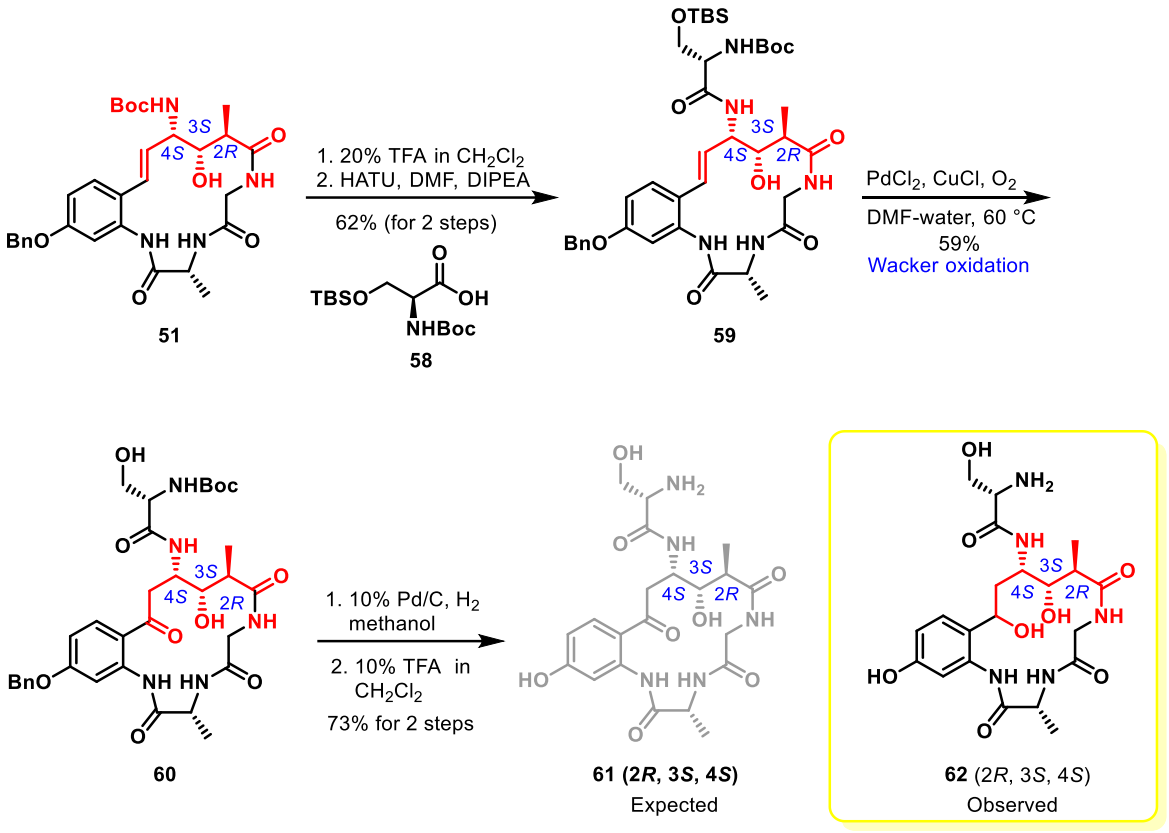
After successful synthesis of macrocycles from **50-54** with three chiral centres on non-peptide portion further, we planned for coupling of serine on synthesized macrocycles followed by installation of ketone moiety to explore its role in biological activity. Thus, macrocycle **50** (2*R*, 3*R*, 4*R*) on *N*-Boc deprotection using 20% TFA in DCM followed by coupling with BocNH-L-ser-OH (**55)** using standard coupling protocol resulted in the formation of serine coupled product **56** with 77% yield for 2 steps.[23] Formation of coupled product was indicated by TLC; serine coupled compound **56** is more polar than



Scheme 2.11. Synthesis of compound **57**

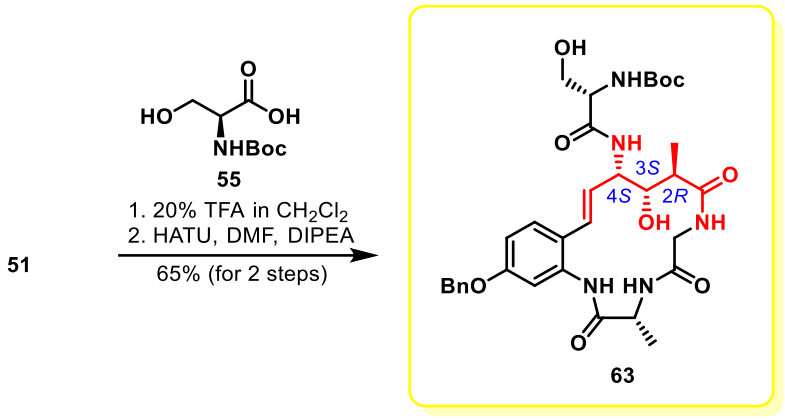
macrocycle **50**. The presence of certain ^{1}H NMR signals in **56** corresponding to serine CH_2 4.63 (d, *J* = 6.8 Hz, 1H), 4.31 (t, *J* = 4.6 Hz, 1H) and total number of protons also confirms the formation of **56**. Besides, ^{13}C NMR also confirmeded the formation of **56** with appearance of four amide carbonyl peak at δ 175.9, 172.6, 172.0, 171.1 ppm and carbonyl carbamate peak at δ 158.6 ppm. It was further supported by HRMS, which showed a peak corresponding to molecular formula of **56.** Serine coupled compound was subjected to Wacker oxidation under optimized condition to give benzylic ketone **57** exclusively in 68% yield.[1, 24] Formation of ketone **57** was indicated by TLC as it was more polar than serine coupled compound also it showed prominent charring in 2, 4- DNP which stipulated formation of same. Formation of ketone **57** was confirmed by disappearance of internal olefin peaks at δ 6.65-6.64 (m, 1H) and 6.21 (dd, *J* = 3.1, 16.0 Hz, 1H) in ^{1}H NMR and the appearance of ketone carbonyl at δ 201.5 ppm in ^{13}C NMR. In addition, formation of ketone **57** was further confirmed with HRMS which showed peak at 692.2892 corresponding to molecular formula $C_{33}H_{43}O_{10}N_5Na[M+Na]^+$ with calculated value at 692.2902 (Scheme 2.11.). However, we could not perform the deprotection of benzyl and Boc group to get **57a** due to the scarcity of material.

As per the plan, macrocycle **51** (2*R*, 3*S*, 4*S*) was then utilized for further analogue synthesis. Macrocycle **51** on treatment with 20% TFA in DCM followed by coupling with the serine derivative (**58**) using standard coupling condition (HATU, DIPEA, DMF) resulted in the formation of desired serine coupled compound **59** in 62% yield for 2 steps.[23] The presence of peaks at δ 0.09 (s, 6H) and 0.91 (s, 9H) ppm in ^{1}H NMR which corresponds to TBS group of serine in addition appearance of signal at δ -6.67 and 24.9 ppm in ^{13}C NMR further confirms serine coupling. Compound **59**, was subjected for Wacker oxidation under optimized condition afforded benzylic ketone **60** having free alcohol (we observed TBS deprotection in the same pot) in 59% yield.[1] Obtained compound on TLC showed prominent 2,4-DNP charring which indicates formation of ketone **60**. In addition, IR showed a characteristic signal at 1710 cm^{-1} corresponds to the ketone carbonyl. The absence of signals in the ^{1}H NMR spectrum at δ 0.09 (s, 6H) and 0.91 (s, 9H) confirmed TBS deprotection during Wacker oxidation and peak at δ 6.67 (d, *J* = 15.6 Hz, 1H), 5.97 (dd, *J* = 10.2, 15.4 Hz, 1H) ppm corresponding to olefins disappeared suggesting



Scheme 2.12. Synthesis of compound **61**

the formation of ketone **60**. In ^{13}C NMR spectrum, presence of peak at δ 201.4 corresponding to ketone confirmed the formation of benzylic ketone **60**. In addition, formation of **60** was further confirmed with HRMS which showed peak at 692.2878 corresponding to molecular formula of same. Ketone **60** was forwarded for debenzylation reaction using hydrogenation and the reaction was monitored by TLC. The formed phenol was forwarded without characterization for Boc deprotection using 10% TFA in DCM at 0 °C to afford **61** with 73% yield for 2 steps (Scheme 2.11.). However, to our surprise, in ^{13}C NMR of **61** we did not observe any peak at δ 201.4 corresponding to ketone inspite we observed additional peak corresponding to alcohol which indicated that during hydrogenation step, reduction of benzylic ketone took place. It was further confirmed by HRMS which showed peak corresponding to reduction product **62** (Scheme 2.12.).

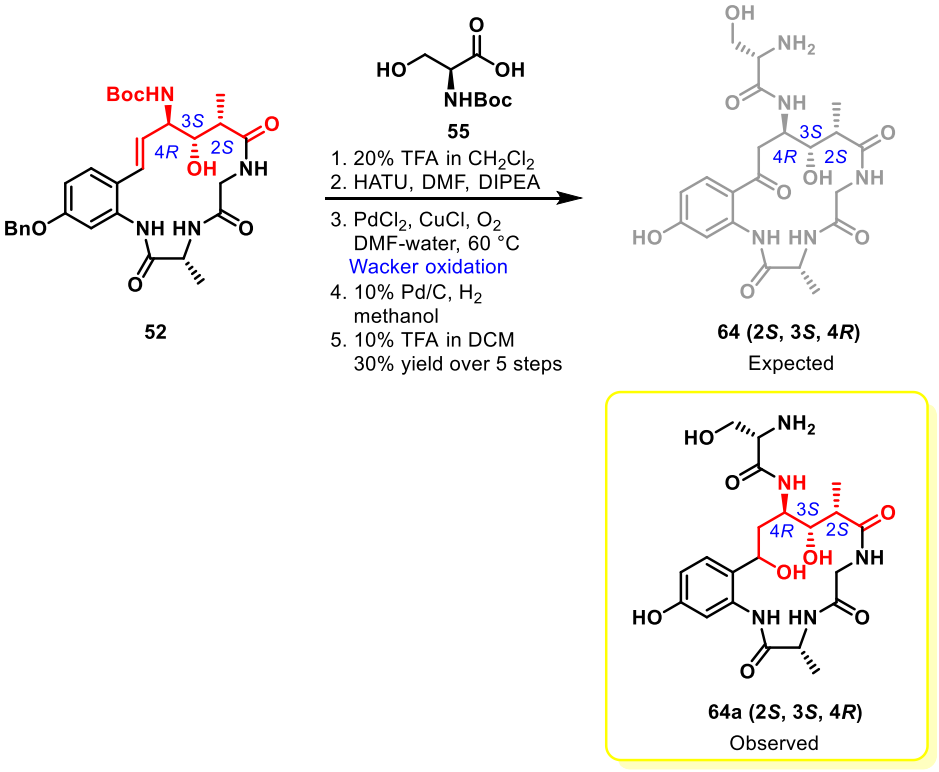


Scheme 2.13. Synthesis of compound **63**

We also performed the coupling of serine without TBS protecting group for which macrocycle **51** after Boc deprotection using 20% TFA in DCM followed by coupling with BocNH-L-ser-OH (**55**) using HATU, DIPEA in DMF afforded serine coupled compound **63** with 65% yield. [23] Formation of product **63** was indicated by presence of polar spot on TLC as compared to macrocycle **51.** Appearance of peak at δ 4.65 (t, *J* = 9.2 Hz, 1H) and 4.39 (q, *J* = 7.2 Hz, 1H) in ^{1}H NMR corresponds to serine CH_2 as well as appearance of signal at δ 1.47 (s, 9H) corresponding to Boc group confirms the formation of **63** (Scheme 2.13.).

After synthesis of analogues utilizing macrocycles **50** and **51**, we planned to synthesize analogues using macrocycle **52** having (2*S*, 3*S*, 4*R*) stereochemistry. Accordingly, BocNH-L-ser-OH (**55)** was coupled (HATU-DIPEA, DIPEA) with the free amine prepared from macrocycle **52** by Boc deprotection using 20% TFA in DCM affording serine coupled compound which was utilized for Wacker oxidation under optimized condition without further characterization which furnished benzylic ketone.[24] Formation of ketone were confirmed by TLC charring in 2,4-DNP stain. Ketone was forwarded for next step without further characterization. Finally for removal of protecting groups (OBn and Boc) we used hydrogenation followed by treatment with 10% TFA resulted in the formation of undesired compound **64a** instead of desired **64** with 30% yield over 5 steps. Similar to compound **61** we did not observe ketone peak in ^{13}C NMR of **64a** besides, we observed three peaks corresponding to alcohol at δ 83.4, 75.5 and 60.7 ppm

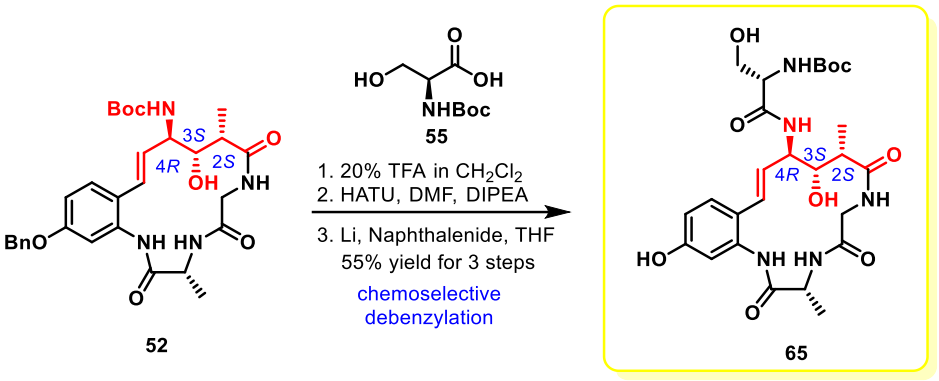
confirming that during hydrogenation reduction of benzylic ketone took place. Further confirmation was done by HRMS which showed peak corresponding to the product **64a** (Scheme 2.14.). Interesting to know that the reduction of carbonyl group was also very stereoselective, probably because of rigid conformation through internal H-bonding network.



Scheme 2.14. Synthesis of compound **64**

Next, macrocycle with free phenolic functionality was synthesized as shown in Scheme 2.15. Thus, BocNH-L-ser-OH (**55**)[23] was coupled with the free amine prepared from macrocycle **52** to afford serine coupled compound, which was utilized for the next step without further characterization. Chemoselectively benzyl group was deprotected using freshly prepared Li/Naphthalenide in THF at -40 °C afforded the desired product **65** with 55% yield over 3 steps.[25] Formation of phenol derivative **65** was indicated by presence of polar spot on TLC as compared to macrocycle **52**. Formation of **65** was confirmed by presence of only three protons in aromatic region and absence of aromatic peaks

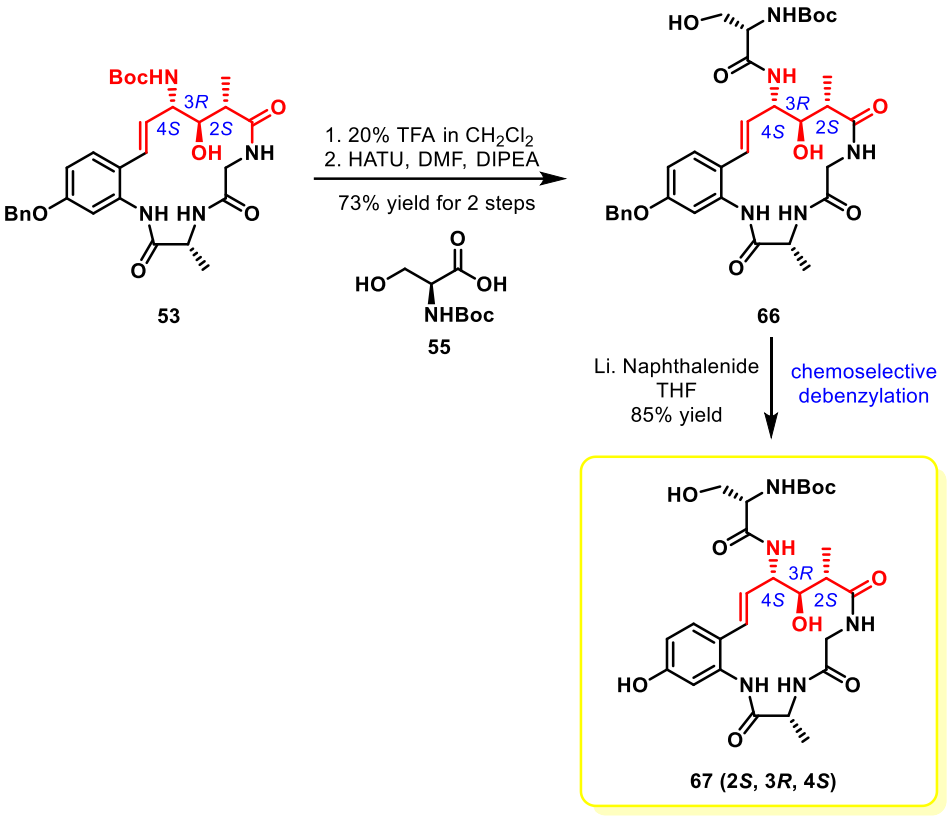
corresponding to benzyl group in ^{1}H NMR. Formation of **65** was further confirmed by presence of only six peaks in ^{13}C NMR corresponding to aromatic carbons at δ 156.5, 127.0, 125.3, 125.1, 123.3 and 113.4 ppm.



Scheme 2.15. Synthesis of compound **65**

Further set of analogues were synthesized using the macrocycle **53** having 2*S*, 3*R*, 4*S* stereochemistry. Boc deprotection of **53** using 20% TFA in DCM followed by coupling with BocNH-L-ser-OH (**55)** using optimized condition (HATU, DIPEA in DMF) afforded the serine coupled compound **66** with 73% yield over 2 steps.[23] Formation of serine coupled compound **66** was indicated by presence of polar spot on TLC as compared to macrocycle **53**. Appearance of peak at δ 4.61 (d, *J* = 5.1, 8.1 Hz, 1H) and 4.50-4.47 (m, 1H) in ^{1}H NMR which corresponds to serine CH_2 as well as appearance of signal at δ 1.45 (s, 9H) corresponding to Boc group confirms the formation of **66**. In addition, formation of **66** was further confirmed with HRMS which showed peak at 676.2919 corresponding to molecular formula $C_{33}H_{43}O_9N_5Na[M+Na]^+$ with calculated value at 676.2953 (Scheme 2.16.). Next, task was to remove benzyl group chemoselectively in presence of double bond. For this transformation, we treated serine coupled compound **66** with Li/Naphthalenide in THF at -40 °C to afford phenol **67** with 85% yield.[25] Formation of phenol **67** was indicated by presence of polar spot on TLC as compared to **66**. The peaks corresponding to OBn group disappeared and presence of only three aromatic peaks in ^{1}H NMR spectrum confirmed the deprotection of OBn group. Presence of only 6 peaks in ^{13}C NMR in aromatic region further confirmed formation of phenol **67**. In addition, formation of **67** was further confirmed with

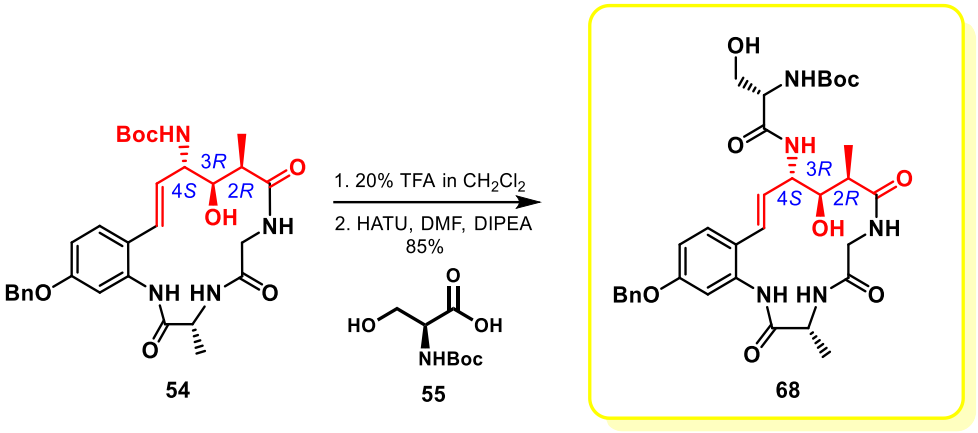
HRMS which showed peak at 564.2651 corresponding to molecular formula $C_{26}H_{38}O_9N_5[M+H]^+$ with calculated value at 564.2664. However we could not perform Wacker oxidation and Boc deprotection steps on compound **67** due to the unavailability of sufficient material (Scheme 2.16.).



Scheme 2.16. Synthesis of compound **67**

The macrocycle **54** with 2*R*, 3*R*, 4*S* stereochemistry in non-peptide portion was used for synthesis of additional analogues. In this context, coupling of BocNH-L-ser-OH (**55)** using (HATU-DIPEA in DMF) with the free amine prepared from macrocycle **54** by Boc deprotection using 20% TFA in DCM afforded serine coupled compound **68** with 85% yield.[23] Formation of serine coupled compound **68** was indicated by presence of polar spot on TLC as compared to macrocycle **54**. Appearance of peak at δ 4.61 (d, *J* = 4.4, 8.3 Hz, 1H) and 4.52 (d, *J* = 7.3 Hz, 1H) in ^{1}H NMR which corresponds to serine CH_2 confirmed the formation of **68**. In addition, formation of **68** was further confirmed with HRMS which

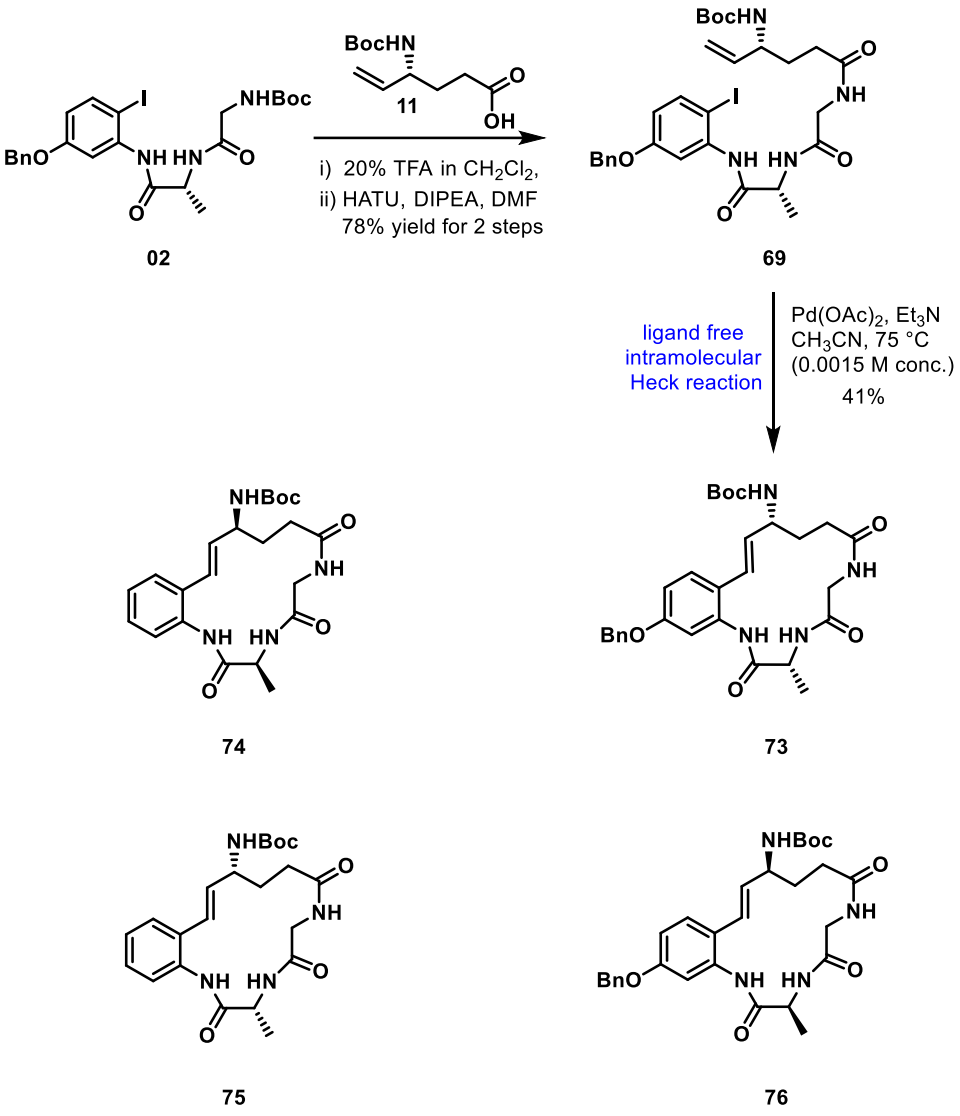
showed peak at 676.2922 corresponding to molecular formula $C_{33}H_{43}O_9N_5Na$ $[M+Na]^+$ with calculated value at 676.2953 (Scheme 2.17.).



Scheme 2.17. Synthesis of compound **68**

1.2.5. Synthesis of macrocycles with only NH chiral center

After synthesis of analogues having three stereocenter on non-peptide portion we turned our attention to synthesize analogues having only NH as chiral center to reduce complexity for synthesis on non-peptide fragment and also to understand the role of remaining stereocenters (OH and CH_3) on biological activity. Synthesis of macrocycle possessing only NH stereocenter began with dipeptide **02** on Boc deprotection using 20% TFA in DCM followed by coupling with non-peptide acid **11** using standard protocol resulted in the formation of macrocyclic precursor **69** with a yield of 78% for 2 steps. Formation of coupling product **69** was indicated by presence of polar spot on TLC as compared to that of acid **11**. Formation of macrocyclic precursor **69** was confirmed by ^1H NMR spectra where peak corresponding to Boc group and alanine CH_3 appeared at δ 1.37 (s, 12H). In ^{13}C NMR presence of three amide carbonyl at δ 172.7, 171.4, 169.5 ppm and carbamate carbonyl peak at δ 159.2 ppm further confirmed the formation of same. In addition, the HRMS analysis showed peak at 687.1620 corresponding with the molecular formula $C_{29}H_{39}O_6N_4INa[M+Na]^+$ further confirmed formation of **69**. After successful



Scheme 2.18. Synthesis of macrocycle with NH stereocenter

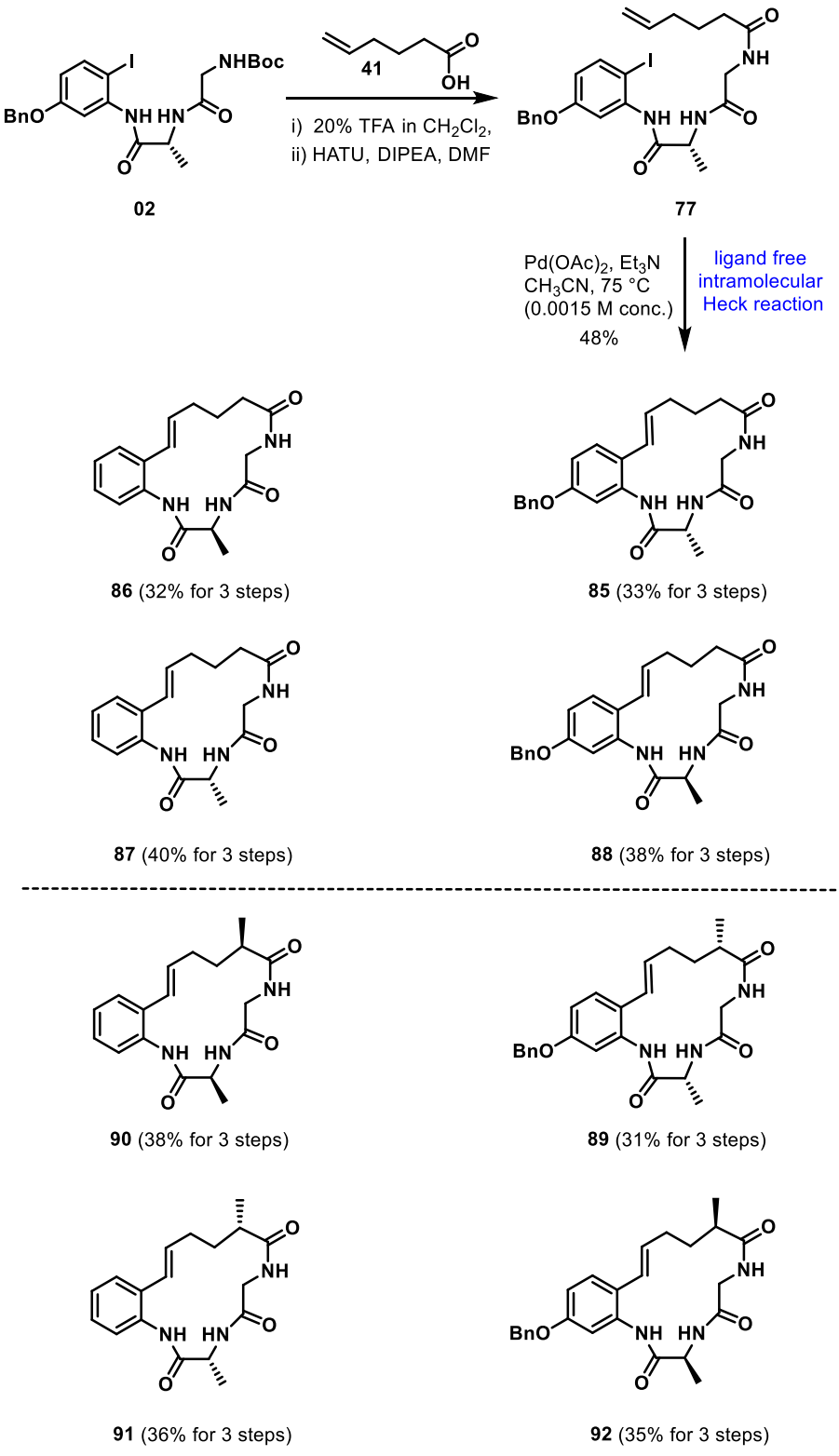
synthesis of macrocyclic precursor **69** we performed ligand free intramolecular Heck cyclization ($Pd(OAc)_2$, Et_3N) in acetonitrile at 75 °C to furnish the macrocycle **73** with 41% yield. Formation of macrocycle **73** was confirmed by presence of internal olefin with *trans* geometry of double bond having characteristic 16.0 Hz coupling constant. In addition, the HRMS analysis showed peak at 559.2516 corresponding to the molecular

formula $C_{29}H_{36}O_6N_4Na$ $[M+Na]^+$ further confirmed the formation of macrocycle **73** (Scheme 2.19.). Following the same synthetic sequence we have synthesized macrocyclic precursors **70**, **71**, **72** and converted them to macrocycle **74**, **75** and **76**. All the macrocycles were fully characterized with the help of spectroscopic techniques (^1H, ^{13}C NMR, IR, and HRMS).

1.2.6. Synthesis of macrocycles with and without CH_3 stereocenter

Due to our keen interest to understand SAR in detail, we planned to synthesize analogues using achiral non-peptide acid **41** (5-hexenoic acid) and using acids **12** and **13** with CH_3 chiral center. Accordingly, we have coupled acid **41** with dipeptide **02** after Boc deprotection using 20% TFA in DCM followed by coupling using HATU, DIPEA in DMF which afforded macrocyclic precursor **77** with 68% yield for two steps. The conformation of coupling product **77** was done by ^{1}HNMR spectra where the presence of characteristic terminal olefin pattern at δ 5.98-5.69 (m, 1H), 5.03-4.93 (m, 2H) in product, as well as presence of aromatic peaks and total number of proton counts confirmed the same. In addition, the HRMS analysis showed peak at 444.1881 corresponding to a molecular formula $C_{24}H_{27}O_4N_3Na[M+Na]^+$ further confirming the formation of macrocycle **85** (Scheme 2.21.). By repeating same synthetic sequence we have synthesized macrocyclic precursors **78**, **79**, **80**, **81**, **82**, **83**, **84** and converted them to macrocycles namely **86, 87, 88, 89, 90, 91, 92** using intramolecular Heck reaction. Structure of macrocycles were confirmed by using ^{1}H NMR, ^{13}C NMR as well as HRMS analysis.

Thus, we have prepared more than 30 macrocyclic analogues of solomonamides with complete characterization. To understand their biological potential and study their structure - activity relationships (SARs), we established a collaboration between our research group and Dr. Manoj Kumar Barthwal from CSIR-Central Drug Research Institute, Lucknow. Details are discussed in following sections.



Scheme 2.20. Synthesis of macrocyclic precursor without stereocenter

1.2.7. Biological evaluation of solomonamide analogues using anti-inflammatory assay

After generating a library of solomonamide analogues with various structural features, we carried out biological evaluations of the selected compounds using animal experiments. Anti-inflammatory potential was measured through the percentage paw edema inhibition in carrageenan-induced mouse (paw edema) model.[26, 27] We have used Dexamethasone (10 mg/kg, i.p.) as a standard reference anti-inflammatory drug[28] (Figure 2.5.). The results obtained for all the synthesized compounds have been captured in Table 2.1. Initially, we did a quantitative measurement of inflammation reduction for all the analogues at 1 mg/kg dose (4 h and 6 h); i.p. in comparison with carrageenan where we observed that standard drug Dexamethasone at concentration of 10 mg/kg showed 78.8 and 79.8% inhibition at 4 h and 6 h respectively. We have chosen 10-fold lower dose of our test compounds when compared with dexamethasone because of the high potency of the natural solomonamide A reported by Zampella's group.[28] Our results shows that the solomonamide A (**NDS-101359**) with revised structure, proposed solomonamide B (**NDS-101357**) and revised solomonamide B (**NDS- 101358**) showed >76% inhibition at both time points (4 h and 6 h). Five out of the tested compounds namely **NDS-101357** - **NDS-101359**, **NDS-101365** and **NDS-101366** showed >76% paw edema inhibition at 4 h and 6 h. However, compounds **NDS-101364**, **NDS-101336**, **NDS-101341**, **NDS-101342**, **NDS-101343**, **NDS-101348**, **NDS-101349**, **NDS-101351** and demonstrated a moderate inhibition of >50% at 4 h and 6 h duration. Whereas, remaining analogues **NDS-101360**, **NDS-101361**, **NDS-101363**, **NDS-101362**, **NDS-101334**, **NDS-101335**, **NDS-101337**, **NDS-101338**, **NDS-101339**, **NDS-101340**, **NDS-101344**-**NDS-101347**, **NDS-101350**, **NDS-101352** and **NDS-101353** showed even less inhibition compared to the other congeners. All the compounds are tested as salt of TFA and numbers are given as with NDS as sequence.

Table 2.1. Percent inhibition (paw edema) at 1 mg/kg

Compound Code	% Inhibition (Paw edema) at 1 mg/kg	
	4 h	6 h
NDS-101357	76.68 ± 2.74	81.15 ± 2.09
NDS-101358	78.05 ± 3.3	79.51 ± 3.67
NDS-101359	78.60 ± 2.01	81.56 ± 3.13
NDS-101360	43.42 ± 5,1	53.89 ± 1.02
NDS-101361	45.47 ± 5.4	44.67 ± 5.9
NDS-101362	41,36 ± 5,4	46.72 ± 1.67
NDS-101363	48.57 ± 4.89	48.77 ± 5.9
NDS-101364	61.93 ± 3.51	68.24 ± 4.24
NDS-101365	78.74 ± 2.47	78.83 ± 5.01
NDS-101366	75. 31 ± 2.12	76.09 ± 4.29
NDS-101534	47.9 ± 2.69	52.87 ± 5.4
NDS-101535	45.83 ± 3.4	49.89 ± 8.6
NDS-101536	58.33 ± 3.8	70.29 ± 4.5
NDS-101537	34.38 ± 4.3	40.57 ± 2.6
NDS-101538	43.57 ± 2.7	60.1 ± 5.4
NDS-101539	41.67 ± 4.5	56.97 ± 5.2
NDS-101540	42.71 ± 3.1	53.89 ± 4.8
NDS-101541	52.08 ± 2.7	66.19 ± 1.9
NDS-101542	57.29 ± 3.5	68.24 ± 5.9
NDS-101543	50.1 ± 4.2	54.92 ± 1.7
NDS-101544	29.17 ± 4.5	42.62 ± 3.7
NDS-101545	39.58 ± 6.5	40.57 ± 4.3
NDS-101546	29.17 ± 4.8	42.62 ± 6.1
NDS-101547	38.54 ± 4.2	41.6 ± 4.2
NDS-101548	65.28 ± 2.8	72.68 ± 1.4

NDS-101549	61.46 ± 3.9	56.9 ± 2.1
NDS-101550	28.13 ± 6.8	45.7 ± 4.2
NDS-101551	65.63 ± 2.6	78.48 ± 5.4
NDS-101552	52.08 ± 2.6	48.7 ± 3.9
NDS-101553	46.88 ± 1.9	56.97 ± 3.5
Dexamethasone (10 mg/kg)	78.05 ± 4.05	79.1 ± 2.4

Based on this preliminary results we decided to evaluate the five active compounds namely **NDS-101357**, **NDS-101358**, **NDS-101359**, **NDS-101365** and **NDS-101366** (showing >76% paw edema inhibition) at further lower doses (0.03, 0.1, 0.3 and 1 mg/kg; i.p.) and the results of the same are depicted in table 2.2. This exercise will also help us in arriving at dose response of selected compounds. We observed 75% and 80% inhibition at 0.3 mg/kg for **NDS-101357** after 4 h and 6 h, respectively, whereas **NDS-101365** showed 72% and 78% inhibition at the same time span. **NDS-101358**, **NDS-101359**, **NDS-101366** showed 54%, 63%, 59% inhibition at 4 h and 58%, 73%, 58% inhibition at 6 h. Besides **NDS-101357** showed 68% and 62% inhibition at 4 h whereas at 6 h it showed an inhibition of 64% and 56% at 0.1 and 0.03 mg/kg dose. **NDS-101365** showed 69% and 61% inhibition at 0.1 and 0.03 mg/kg (4 h) and an inhibition of 62% and 58% at 6 h. Comparatively low inhibition were observed in the case of compound **NDS-101358**, **NDS-101359** and **NDS-101366** at a similar lower concentration of 0.1 and 0.03 mg/kg. The detailed graphs of active compounds (**NDS-101357**- **NDS-101359**, **NDS-101365** and **NDS-101366**) are shown in figure 2.6.

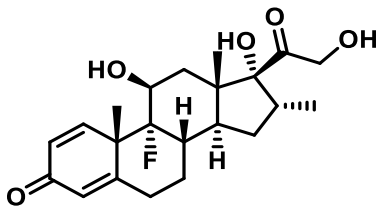


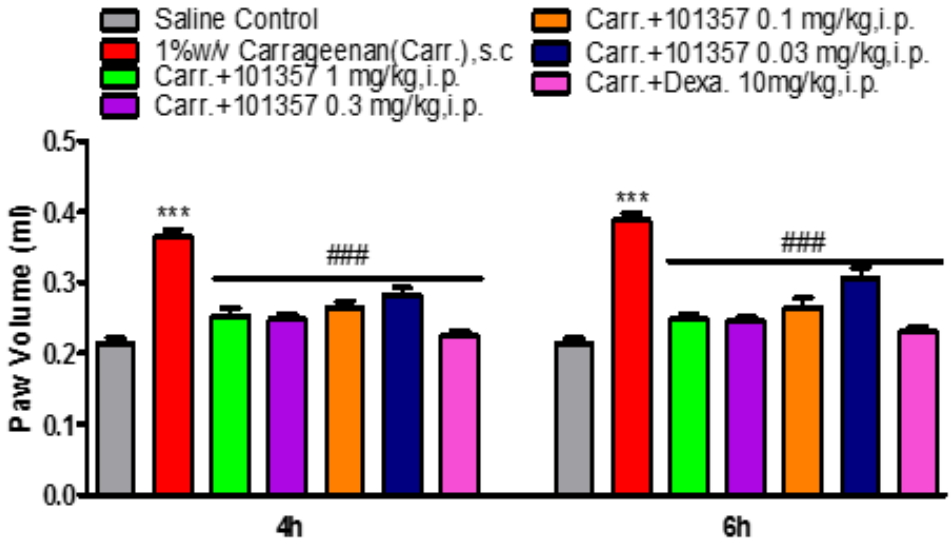
Figure 2.5. Structure of drug dexamethasone

Table 2.2. Percent inhibition (paw edema) at 1, 0.3, 0.1 and 0.03 mg/kg

Compound Code	% Inhibition (Paw edema) – 1 mg/kg		% Inhibition (Paw edema) – 0.3 mg/kg		% Inhibition (Paw edema) – 0.1 mg/kg		% Inhibition (Paw edema) – 0.03 mg/kg	
	4 h	6 h	4 h	6 h	4 h	6 h	4 h	6 h
NDS-101357	76.68 ± 2.78	81.15 ± 2.9	75.01 ± 1.7	80.21 ± 1.9	68.79 ± 6.2	64.58 ± 5.5	62.50 ± 4.8	56.94 ± 5.5
NDS-101358	78.05 ± 3.3	79.51 ± 3.6	54.17 ± 6.5	58.33 ± 2.4	51.04 ± 5.9	50.01 ± 3.4	47.22 ± 3.67	47.22 ± 5.0
NDS-101359	78.60 ± 2.0	81.56 ± 3.1	63.1 ± 2.2	73.96 ± 4.6	55.95 ± 3.5	61.46 ± 7.1	47.62 ± 4.7	48.61 ± 6.9
NDS-101365	78.74 ± 2.47	78.83 ± 5.0	78.92 ± 3.6	78.25 ± 3.9	69.79 ± 6.8	62.5 ± 5.6	61. 11 ± 6.0	58.02 ± 4.8
NDS-101366	75.31 ± 2.1	76.09 ± 4.2	59.38 ± 5.2	58.33 ± 4.5	42.71 ± 2.6	57.29 ± 2.6	40.28 ± 6.0	53.21 ± 4.8
Dexamethasone (10 mg/kg)	78.05 ± 4.0	79.1 ± 2.4						

NDS-101357:

a)



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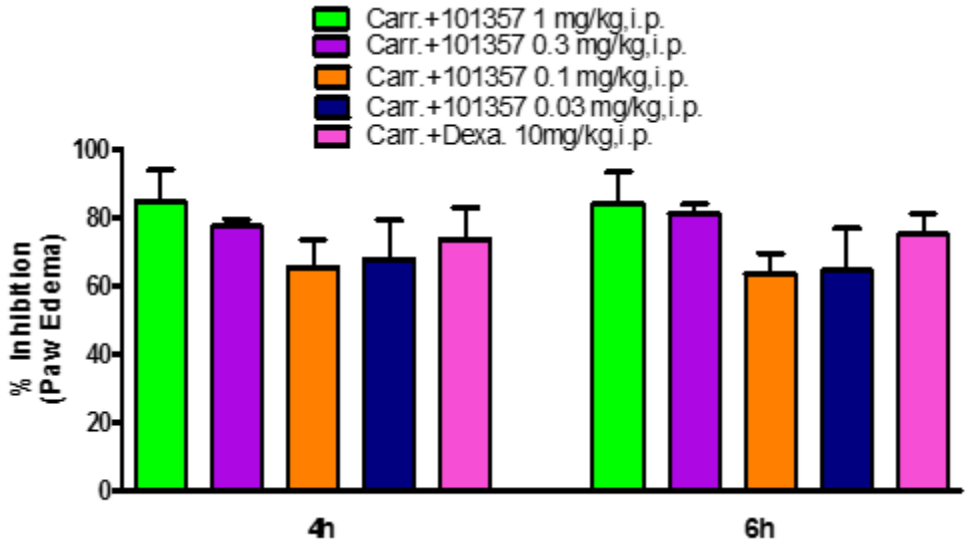
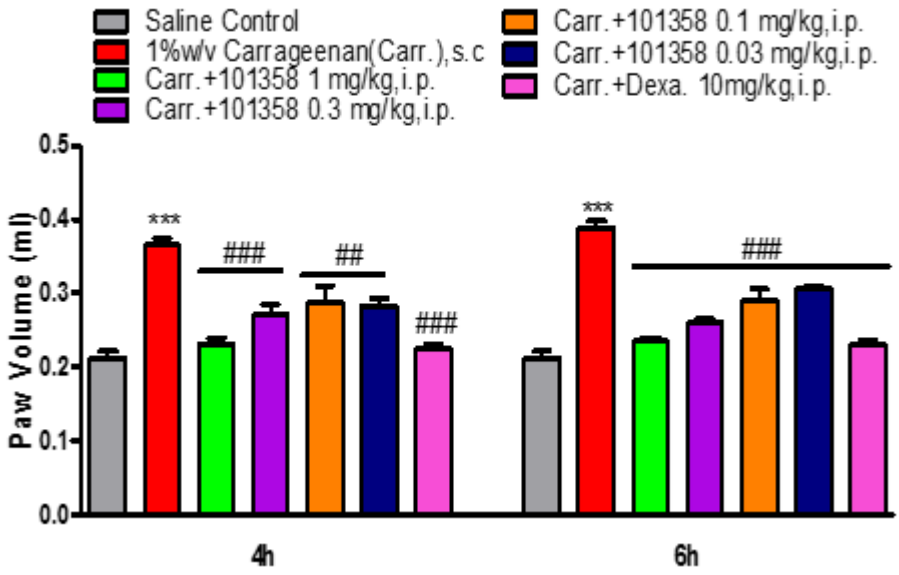


NDS-101358:

a)



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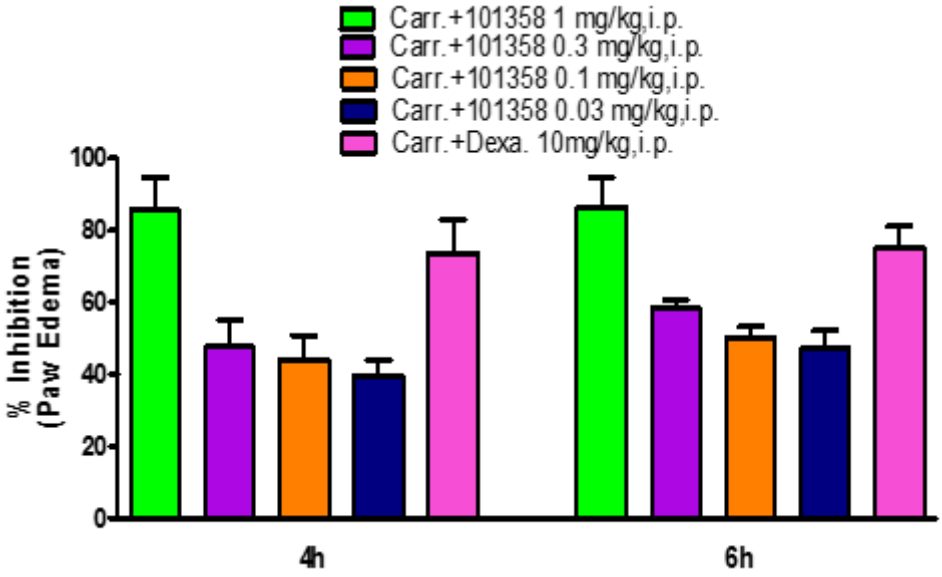
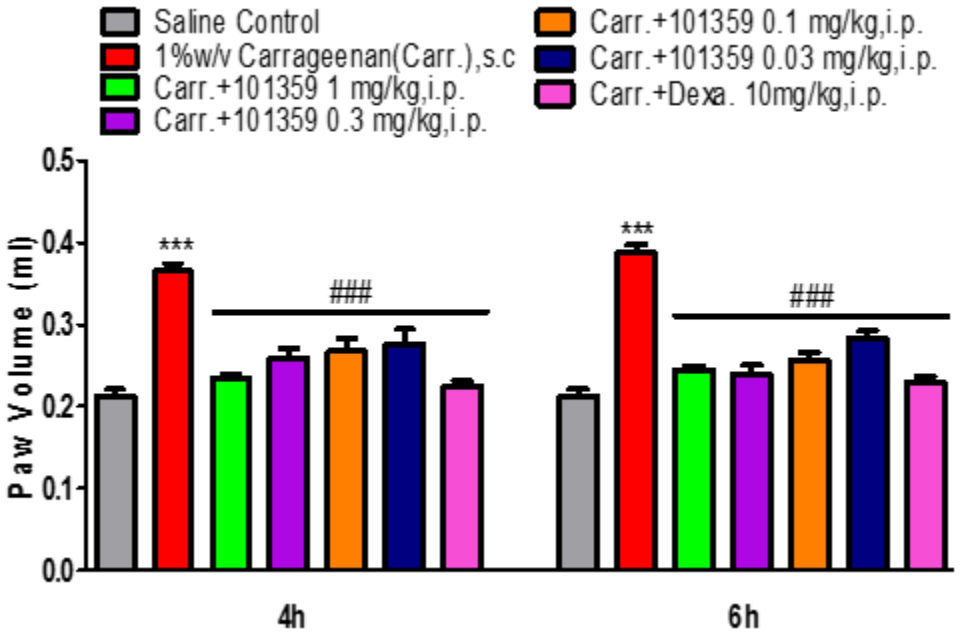


NDS-101359:

a)



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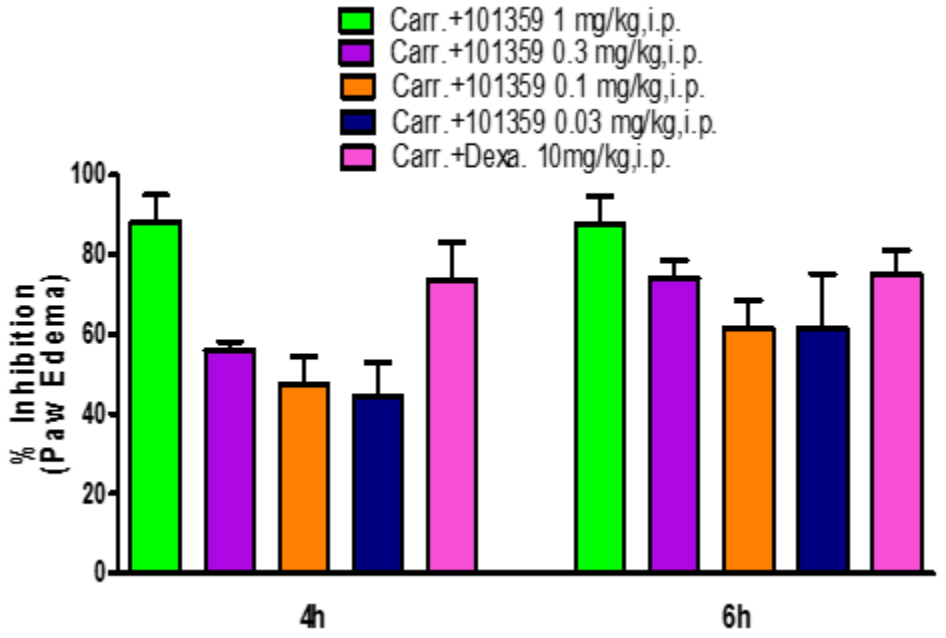

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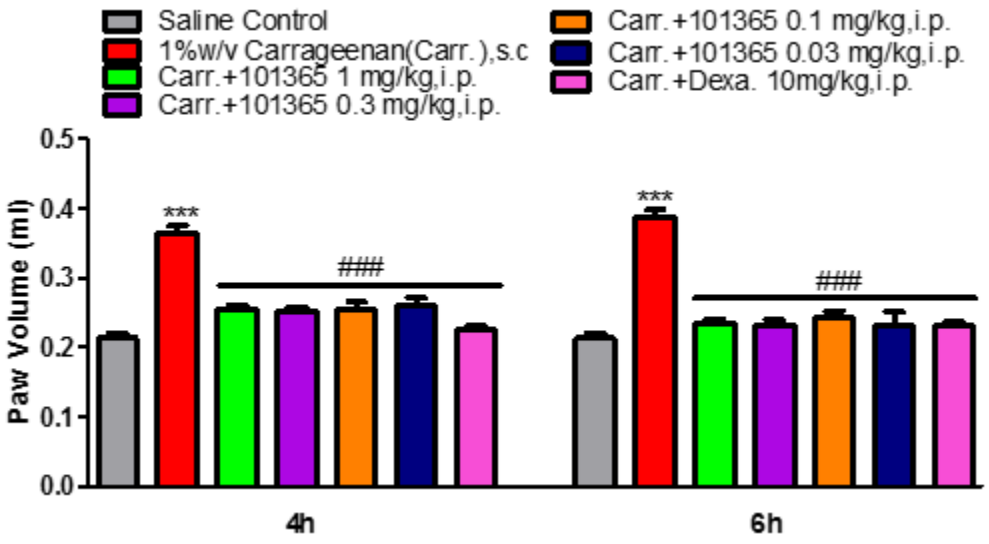
a)



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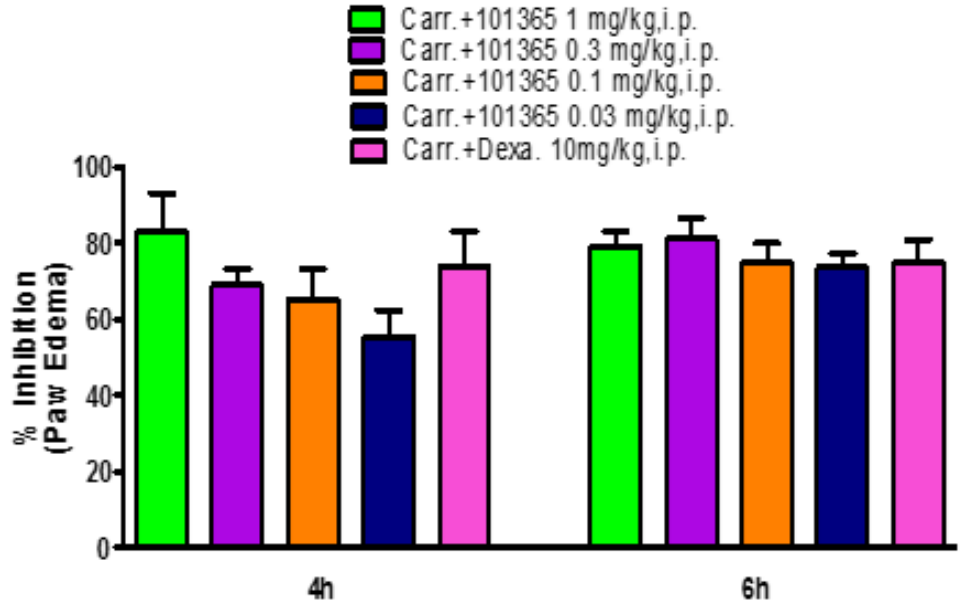
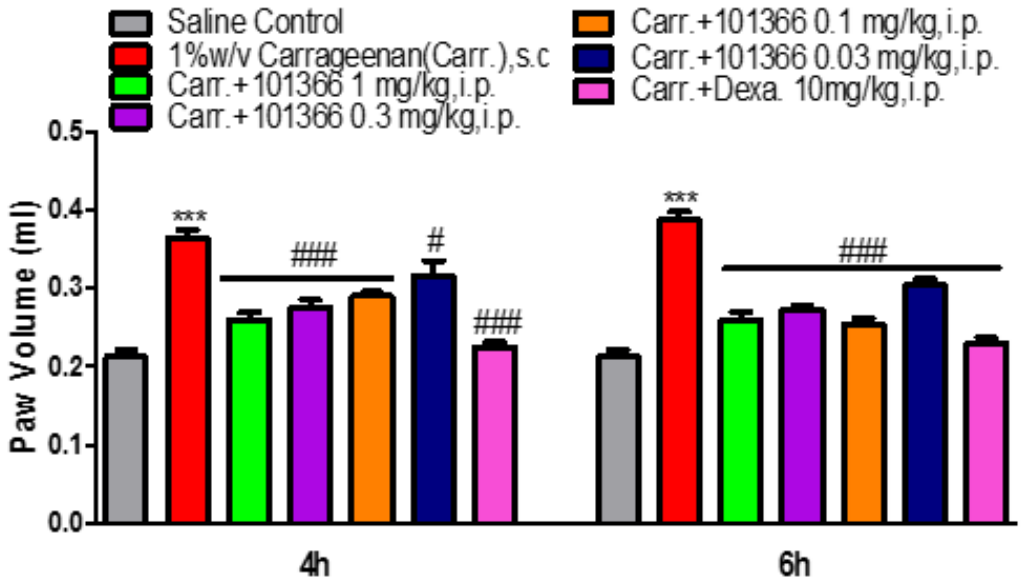


NDS-101366:

a)



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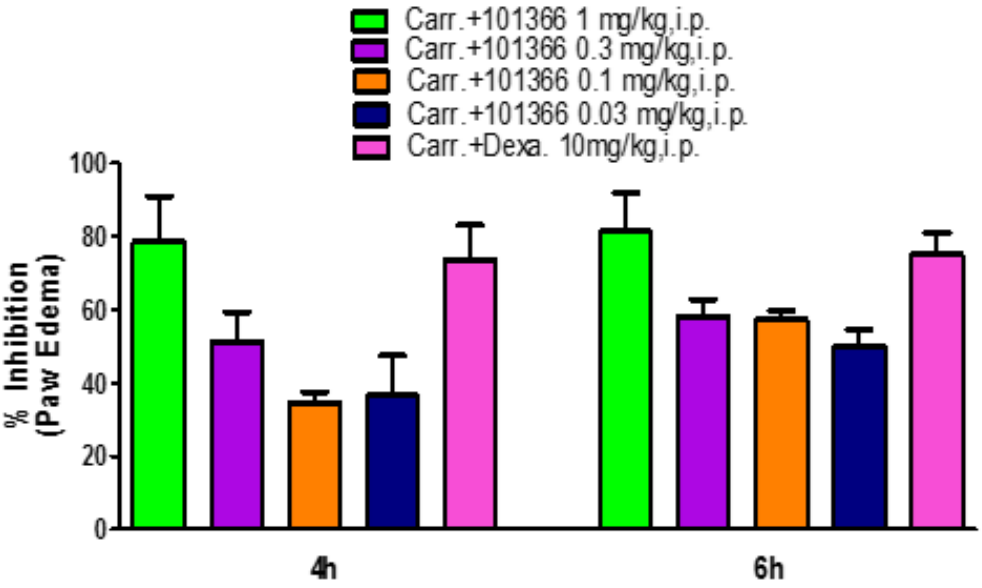


Figure 2.6. Anti-inflammatory activity of NDS-101357, NDS-101358, NDS-101359, NDS-101365 and NDS-101366 (at 0.03, 0.1, 0.3 and 1 mg/kg^{-1}, i.p.) and dexamethasone (10 mg/kg^{-1}, i.p.) in the carrageenan-induced paw edema in mice model (n = 6 -10 animals per group). 50 µL of saline or 1% carrageenan in the treatment group or saline in the control

group was administered subcutaneously in the mice hind paw. The results are shown as mean ± SEM: (a) paw volume in mL and (b) % inhibition in paw edema.

1.2.8. Structure activity relationship studies

The values (percentage of inhibition) obtained for all the compounds have been tabulated and the structures of all the tested compounds are compiled (Figure 2.7.). The percent inhibition values (reduction in paw edema) obtained for solomonamide analogues permitted us to draw some valuable conclusions. Although data from animal experiments, in particular without plasma concentrations and rate of metabolism etc. it may not provide exact structure activity relationships. Accordingly, we tried to make some conclusions based on the initial results from animal experiments. From results we observed that revised solomonamide A (**NDS-101359**), proposed solomonamide B (**NDS-101357**), and revised solomonamide B (**NDS-101358**) showed comparable activity. These observations inevitably proves that presence of extra hydroxyl group (C5-OH) in solomonamide A (**NDS-101359**) is not necessary for activity. However, **NDS-101361** having all the functionality of natural product showed (45% and 44% inhibition at 4 h and 6 h) which is very less as compared to natural product it proves that stereochemistry of non-peptide portion is playing important role. In case of **NDS-101360** and **NDS-101552** having benzyl and Boc protection we observed less inhibition (at 4 h and 6 h) it indicates that free phenol and amine group are key factors for activity. While comparison of **NDS-101365** (without OBn protection) and **NDS-101360** and **NDS-101552** (with OBn and Boc protection) we found that **NDS-101365** showed promising activity which is comparable to the natural product. This further confirms the necessity of phenolic hydroxyl group for activity. Whereas, in case of serine coupled compounds namely **NDS-101550**, **NDS-101551** and **NDS-101553** we observed **NDS-101551** to be superior among the trio. Further for understanding the role of stereochemistry of non-peptide portion we evaluated biological potential of macrocycles such as **NDS-101362**, **NDS-101544**, **NDS-101545**, **NDS-101546**, **NDS-101547**, **NDS-101548** and **NDS-101549**. Out of the tested macrocycles **NDS-101548** showed the highest inhibition of >65% in both the time points whereas **NDS-101549**

showed 61% and 56% inhibition at 4 h and 6 h. All the remaining macrocycles did not exhibit promising inhibition in identical dose and time scale. These results indicated that a stereochemistry of 2*S*, 3*S*, 4*R* in **NDS-101548** and 2*S*, 3*R*, 4*R* in **NDS-101549** in the non-peptide portion are the key detrimental factor for potency. In case of macrocycles having only NH and CH_3 stereocenter in the non-peptide portion showed moderate activity as compared to natural product. Out of the eight tested compounds **NDS-101366** having only CH_3 stereocenter showed promising activity and came out to be a simplified analogue of the natural product. In addition, we also observed that different stereochemistry of CH_3 and NH functionality on the macrocycles did not happen to be an influential factor for a proper bio-potency. Moving from macrocycles with chiral centers on non-peptide portion to macrocycles with an achiral non-peptide portion, did not lead to much differences in activity. In conclusion, our objective of simplifying the structure had yielded encouraging results with compound **NDS-101365** being the most potent which showed similar activity with natural product solomonamide A at lower doses (Figure 2.7.).

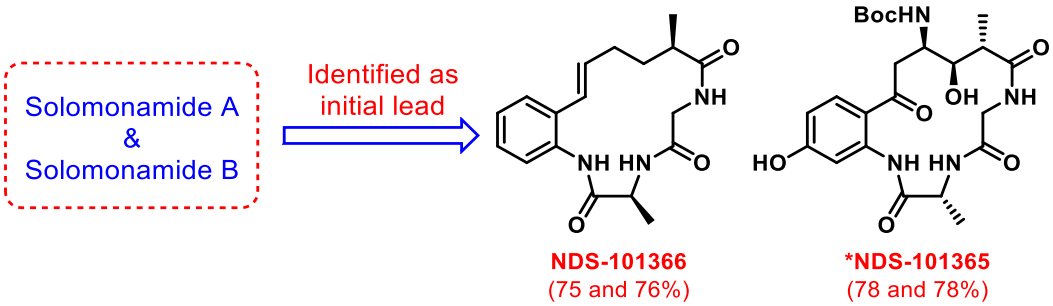


Figure 2.7. Structure of initial identified lead molecule based on paw edema assay

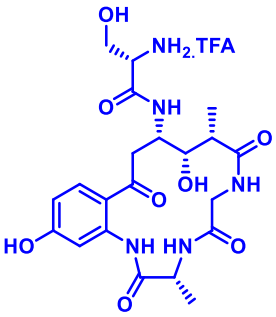
Based on structure activity relationships (SAR) we arrived at following conclusions

a) Stereochemistry of three stereocenter 2*S*, 3*R*, 4*R* in **NDS-101357** and 2*S*, 3*S*, 4*S* in **NDS-101358** in the non-peptide portion of macrocycle are playing important role in the activity.

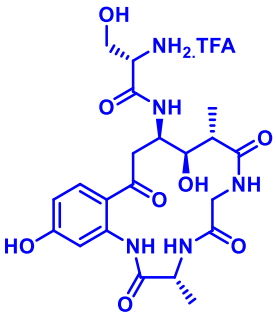
b) Change of CH_3 and NH stereochemistry in the non-peptide portion are not affecting any activity.

c) Change in stereochemistry of alanine showed similar potency.

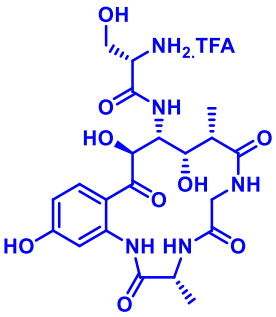
d) Free phenolic hydroxy is necessary for activity (**NDS-101365**).



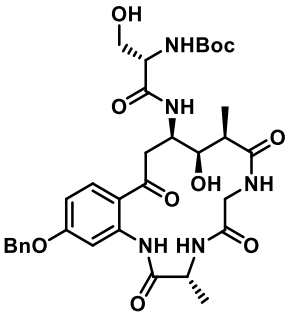
NDS-101357
(76, 81)



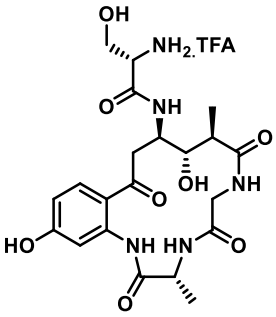
***NDS-101358**
(78, 79)



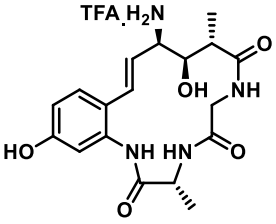
NDS-101359
(78, 81)



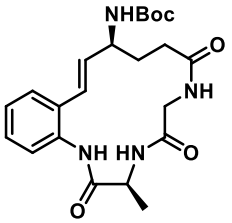
NDS-101360
(43, 53)



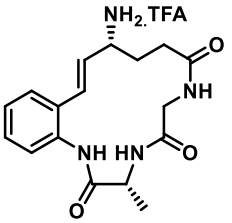
***NDS-101361**
(45, 44)



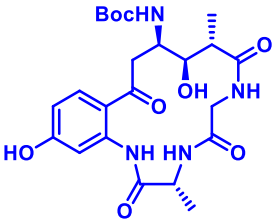
***NDS-101362**
(41, 46)



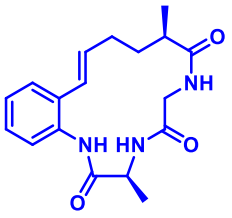
NDS-101363
(48, 48)



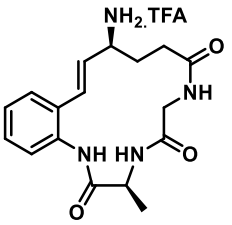
NDS-101364
(61, 68)



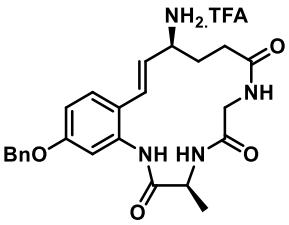
***NDS-101365**
(78, 78)



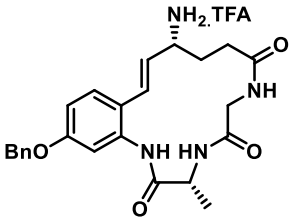
NDS-101366
(75, 76)



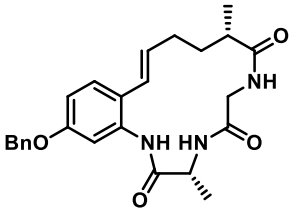
NDS-101534
(47, 52)



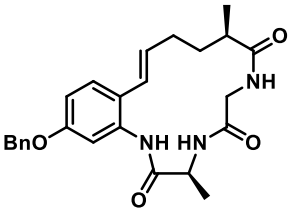
NDS-101535
(45, 49)



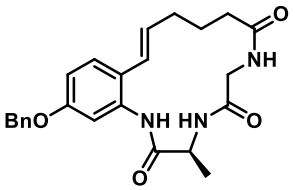
NDS-101536
(58, 70)



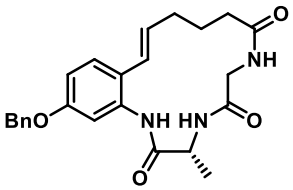
NDS-101537
(34, 40)



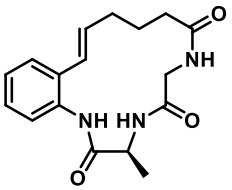
NDS-101538
(43, 60)



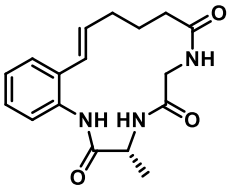
NDS-101539
(41, 56)



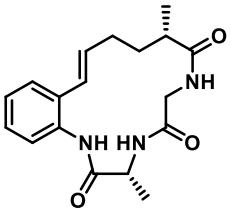
NDS-101540
(42, 53)



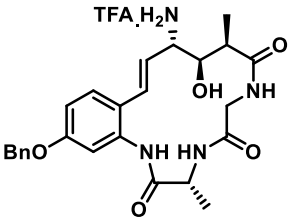
NDS-101541
(52, 66)



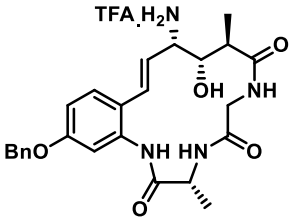
NDS-101542
(57, 68)



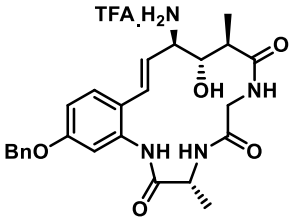
NDS-101543
(50, 54)



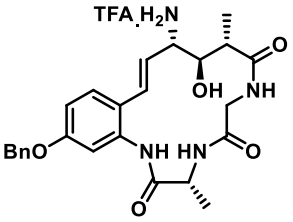
NDS-101544
(29, 42)



NDS-101545
(39, 40)



***NDS-101546**
(29, 42)



NDS-101547
(38, 41)

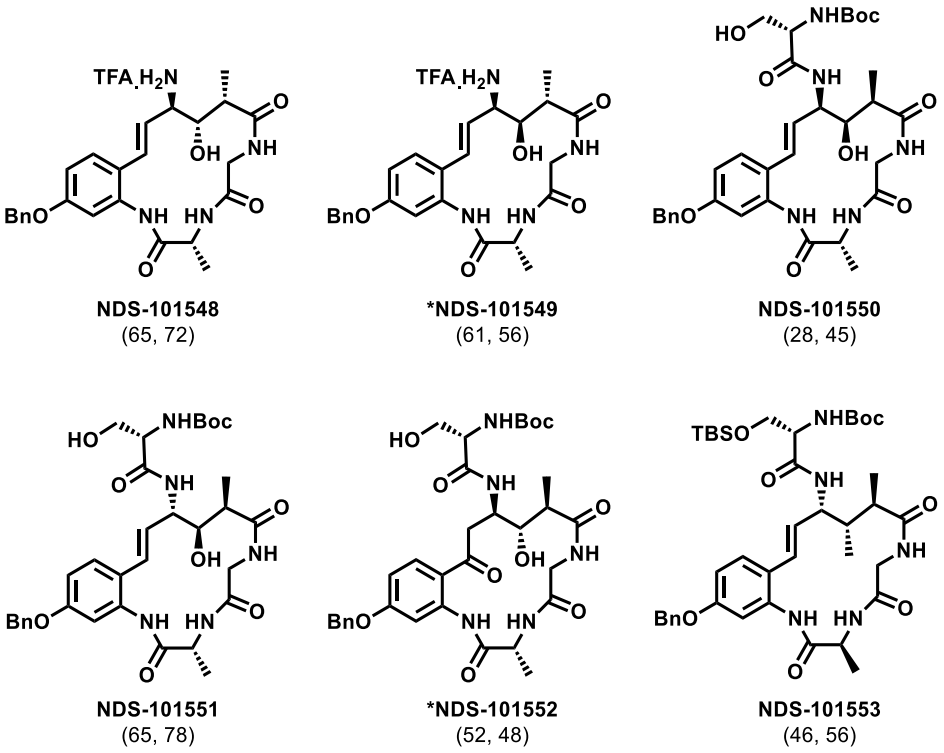


Figure 2.7. Percent inhibition (paw edema) values of selected compounds at 4 h and 6 h. * Compounds were synthesized previously from our group and data are given in thesis of Dr. K. Kashinath of our group

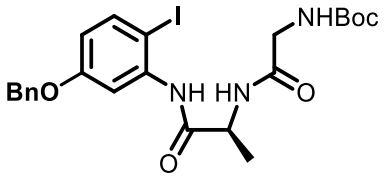
1.2.9. Conclusions

The total syntheses and structural revision of solomonamide A and solomonamide B were further extended to access a series of macrocyclic analogues. Accordingly, new analogues were synthesized using well established Brown crotylation, Evans aldol reaction, crotylation, Wacker oxidation and ligand free intramolecular Heck reactions as key steps. During analogues synthesis we have developed a novel enantiospecific route to access both the *R* and *S* isomers of drug vigabatrin from methionine. A two carbon homologation through Wittig olefination, hydrogenation followed by pyrolytic elimination are key features of the vigabatrin synthesis. Overall, during the course of this project we have synthesized more than 30 macrocyclic compounds based solomonamide scaffold.

Among them, selected macrocycles were evaluated for their anti-inflammatory potential. Analysed the structure-activity relationship of all the compounds based on their anti-inflammatory potential. Out of the tested compounds, three of them were found to be equipotent to that of the natural product at 1 mg/kg. Further, we tested those three compounds at lower doses and found that compounds **NDS-101365** and **NDS-101366** are superior. Among the two compounds **NDS-101365** is very close to the natural product solomonamide with four chiral centres whereas **NDS-101366** is the simplified compound. At present, we have selected these two compounds based on their anti-inflammatory potential and ease of synthesis. Further profiling of selected compounds including scale up, measurement of inflammatory markers and mechanism of action are ongoing work on this project.

1.2.10. Experimental Section

***tert*-Butyl (S)-(2-((1-((5-(benzyloxy)-2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)carbamate**



01

To a mixture of 5-(benzyloxy)-2-iodoaniline **14** (10.0 g, 30.0 mmol), Boc-Gly-L-Ala-OH 7.56 g, 40 mmol) in DMF (50 mL). HATU (17 g, 46 mmol), Hünig's base (10 mL, 60 mmol) were added at 0 °C and stirred for 10 h at room temperature, the reaction mixture was diluted with ethyl acetate (2 X 300 mL) and washed with 2N HCl (2 X 150 mL) and sat. $NaHCO_3$ solution (2 X 200 mL) organic layer was separated, dried over Na_2SO_4, concentrated under reduced pressure. Purification by column chromatography (silica gel 100-200 mesh 35% ethyl acetate - pet ether) yielded compound **01**.

Yield: 53%

Specific rotation: $[\alpha]_D^{30}$ = - 28.84 (*c* 0.47, $CHCl_3$)

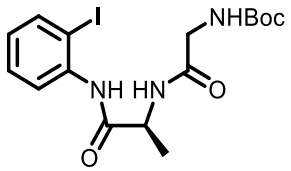
IR υ_{max}(film): 3380, 3015, 1684, 1422 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.27 (brs, 1H), 8.18 (d, *J* = 6.7 Hz, 1H), 7.72 (d, *J* = 8.5 Hz, 1H), 7.46 - 7.31 (m, 6H), 6.99 (brs, 1H), 6.72 (dd, *J* = 2.4, 8.5 Hz, 1H), 5.08 (s, 2H), 4.64 - 4.40 (m, 1H), 3.64 (d, *J* = 6.1 Hz, 2H), 1.37 (brs, 12H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 171.5, 169.9, 159.2, 156.3, 140.1, 139.5, 137.1, 128.9, 128.4, 128.1, 114.7, 113.1, 78.6, 69.9, 49.2, 43.7, 28.7, 18.5

HRMS (ESI): calculated for $C_{23}H_{28}O_5N_3NaI$ $[M+Na]^+$: 576.0966, found 576.0953.

***tert*-Butyl (*S*)-(2-((1-((2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)carbamate**



03

Compound **03** was synthesized by following similar procedure for the synthesis of **01**

Yield: 61%

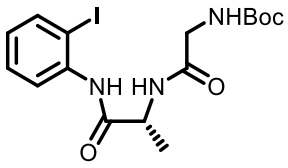
IR υ_{max}(film): 3305, 3008, 1689, 1534 cm^{-1}

^{1}H NMR (100 MHz, DMSO-d_6): δ 9.38 (brs, 1H), 8.15 (d, *J* = 6.7 Hz, 1H), 7.87 (d, *J* = 7.3 Hz, 1H), 7.50 - 7.47 (m, 1H), 7.38 (t, *J* = 7.6 Hz, 1H), 6.98 (t, *J* = 7.0 Hz, 2H), 4.51 - 4.47 (m, 1H), 3.63 (d, *J* = 5.5 Hz, 2H), 1.37 (brs, 12H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 171.5, 169.8, 156.3, 139.4, 129.2, 128.0, 126.8, 78.6, 49.2, 43.7, 28.6, 18.5

HRMS (ESI): calculated for $C_{16}H_{22}O_4N_3NaI$ $[M+Na]^+$: 470.0547, found 470.0537.

***tert*-Butyl (*R*)-(2-((1-((2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)carbamate**



04

Compound **04** was synthesized by following similar procedure for the synthesis of **01**

Yield: 64%

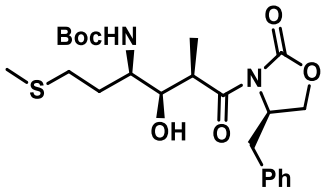
IR υ_{max}(film): 3305, 3008, 1689, 1534 cm^{-1}

^{1}H NMR (100 MHz, DMSO-d_6): δ 9.37 (s, 1H), 8.15 (d, *J* = 6.7 Hz, 1H), 7.87 (d, *J* = 7.9 Hz, 1H), 7.49 (d, *J* = 7.9 Hz, 1H), 7.38 (t, *J* = 7.3 Hz, 1H), 7.04 - 6.90 (m, 2H), 4.60 - 4.36 (m, 1H), 3.63 (d, *J* = 6.1 Hz, 2H), 1.37 (s, 12H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 171.5, 169.8, 156.3, 139.4, 129.2, 128.0, 126.9, 96.2, 78.6, 49.1, 43.7, 40.6, 40.4, 40.2, 40.0, 39.8, 39.6, 39.4, 28.6, 18.6

HRMS (ESI): calculated for $C_{16}H_{22}O_4N_3NaI$ $[M+Na]^+$: 470.0547, found 470.0536.

***tert*-Butyl ((3*R*,4*R*,5*R*)-6-((*R*)-4-benzyl-2-oxooxazolidin-3-yl)-4-hydroxy-5-methyl-1-(methylthio)-6-oxohexan-3-yl)carbamate**



18 (major)

To a stirred solution of oxazolidinone **17** (4.6 g, 20.0 mmol) in CH_2Cl_2 (50 mL) was added Bu_2BOTf (22 mL, 22.0 mmol) and DIPEA (4.12 mL, 24.0 mmol) at 0 ^{0}C. After stirring for 1 h, the solution was cooled to –78 °C and maintained at -78 °C for 30 min. A solution containing aldehyde **16** (5.1 g, 22.0 mmol) in CH_2Cl_2 (40 mL) was added dropwise and the solution was allowed to warm slowly to room temperature and stirred for overnight. The reaction was quenched by the addition of aqueous ammonium chloride solution (25 mL) at 0 ^{0}C and then H_2O (50 mL) was added to the reaction mixture. The mixture was extracted

with CH_2Cl_2 (3 x 60 mL). The combined organic extracts were dried over anhydrous sodium sulphate, filtered, and concentrated in vacuo. The organic residue was purified by column chromatography (silica gel 230-400 mesh 15% ethyl acetate- pet ether) to afford **18** and **18**a with 7:3 Diastereomeric ratio.

Yield: 54%.

Data for **18 (**major diastereomer)

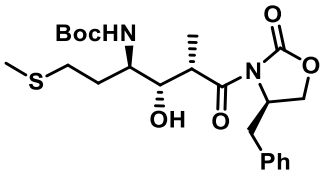
Specific rotation: $[\alpha]_D^{30}$ = - 40.06 (*c* 0.85, CH_3OH)

IR υ_{max}(film): 3424, 3012, 2968, 1771, 1683, 1502 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35 - 7.21 (m, 5H), 4.78 (t, *J* = 10.0 Hz, 2H), 4.32 (t, *J* = 8.1 Hz, 1H), 4.14 (d, *J* = 8.8 Hz, 1H), 3.89 (brs,1H), 3.81 - 3.71 (m, 2H), 3.25 - 3.22 (m, 1H), 2.82 (dd, *J* = 9.5, 13.0 Hz, 1H), 2.60 - 2.50 (m, 2H), 2.47 - 2.46 (m, 1H), 2.12 (s, 3H), 1.86 (q, *J* = 7.5 Hz, 2H), 1.40 (s, 9H), 1.38 (d, *J* = 6.4 Hz, 3H)

HRMS (ESI): calculated for $C_{23}H_{35}O_6N_2S$ $[M+H]^+$: 467.2210, found 467.2211.

***tert*-Butyl ((3R,4S,5S)-6-((R)-4-benzyl-2-oxooxazolidin-3-yl)-4-hydroxy-5-methyl-1-(methylthio)-6-oxohexan-3-yl)carbamate**



18a (minor)

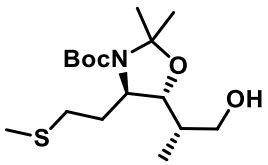
Data for **18a (**minor diastereomer)

Specific rotation: $[\alpha]_D^{30}$ = + 68.04 (c 0.87, CH_3OH)

IR υ_{max}(film): 3438, 3057, 2995, 1795, 1680 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 - 7.16 (m, 5H), 4.66 - 4.64 (m, 2H), 4.22 - 4.14 (m, 2H), 3.92 (s, 1H), 3.83 - 3.81 (m, 1H), 3.77 - 3.72 (m, 1H), 3.22 (d, *J* = 13.3 Hz, 1H), 2.77 - 2.72 (m, 1H), 2.56 - 2.49 (m, 2H), 2.09 - 2.07 (m, 1H), 2.06 (s, 3H), 1.64- 1.62 (m, 1H), 1.40 (s, 9H), 1.29 (d, *J* = 6.4 Hz, 3H)

***tert*-Butyl (4*R*,5*R*)-5-((*S*)-1-hydroxypropan-2-yl)-2,2-dimethyl-4-(2-(methylthio)ethyl)oxazolidine-3-carboxylate**



20

To a stirred solution of compound **18** (9.0 g, 19.305 mmol), and 2-methoxypropene (4.17 mL, 57.93 mmol) in dry DMF (50 mL) PTSA.H_2O (663 mg, 3.85 mmol) was added at 0 °C under argon atmosphere. The resulting solution was stirred at room temperature for 4 h. The reaction was then diluted with H_2O (25 mL) and extracted with EtOAc (2 x 90 mL). The combined organic layers were washed with cold saturated $NaHCO_3$ solution (15 mL), H_2O (10 mL), brine (10 mL), and evaporated in vacuo. The crude acetonide protected (8.25 g, 16.4 mmol) compound was taken in dry THF (30 mL) at 0 °C, $LiBH_4$ (1.05 g, 48.6 mmol) was added portion wise under argon atmosphere stirred at room temperature for 5 h. Excess $LiBH_4$ was decomposed with 1N HCl and extracted with EtOAc (2 x 80 mL). The combined organic layers were washed with cold saturated brine (30 mL), dried over Na_2SO_4 and evaporated in vacuo. The organic residue was purified by column chromatography (silica gel 230-400 mesh 15% ethyl acetate – pet ether) to afford **20** as a oil.

Yield: 67% for 2 steps.

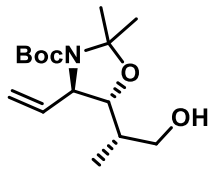
Specific rotation: $[\alpha]_D^{30}$ = -17.59 (*c* 1.2, CH_3OH)

IR υ_{max}(film): 3390, 3011, 2980, 1684, 1529, 1390 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.94 - 3.84 (m, 2H), 3.61 (dd, *J* = 1.6, 5.3 Hz, 2H), 2.53 - 2.44 (m, 2H), 2.09 (s, 3H), 1.99 - 1.79 (m, 4H), 1.46 (s, 15H), 1.00 (d, *J* = 6.9 Hz, 3H)

HRMS (ESI): calculated for $C_{16}H_{32}O_4NS$ $[M+H]^+$: 334.2047, found 334.2038.

***tert*-Butyl (4*R*, 5R)-5-((*S*)-1-hydroxypropan-2-yl)-2,2-dimethyl-4-vinyloxazolidine-3-carboxylate**



22

To a solution of compound **20** (6.0 g, 13.5 mmol) in CH_2Cl_2-MeOH(1:1, 90 mL) Ozone was bubbled at –78 °C until the colour becomes blue, once the blue color appears oxygen was bubbled to remove excess ozone, then reaction mixture was allowed to room temperature and stirred for 8-10 h. Concentrated the reaction mixture, here sulfur also got oxidized to sulfoxide. The crude sulfoxide compound was taken in 1,2 dichloro benzene (50 mL) $CaCO_3$ (5.54 g, 55.0 mmol) was added and refluxed for 6 h. The crude reaction mixture was purified by column chromatography (silica gel 230-400 mesh 30% ethylacetate - CH_2Cl_2) to afford

22 as colorless liquid.

Yield: 35% for 2 steps

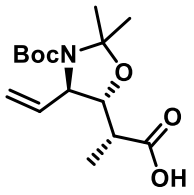
Specific rotation: $[\alpha]_D^{30}$ = + 26.12 (*c* 0.87, CH_3OH)

IR υ_{max}(film): 3418, 3006, 2943, 1680, 1538, 1390 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.72 - 5.64 (m, 2H), 5.18 - 5.16 (m, 1H), 4.04 (brs, 1H), 3.90 (dd, *J* = 3.3, 8.0 Hz, 1H), 3.65 (d, *J* = 5.0 Hz, 2H), 1.96 - 1.91 (m,1 H), 1.58 - 1.50 (brs, 6H), 1.43 (s, 9H), 1.00 (d, *J* = 7.0 Hz, 3H)

HRMS (ESI): calculated for $C_{15}H_{27}O_4NNa$ $[M+Na]^+$ 308.1832, found 308.1833.

(*R*)-2-((4*R*,5*R*)-3-(tert-Butoxycarbonyl)-2,2-dimethyl-4-vinyloxazolidin-5-yl)propanoic acid



05

To a stirred solution of compound **22** (1 g, 3.4 mmol) in DMF (6 mL), Pyridinium dichromate (PDC) (5.28 g, 14.0 mmol) was added and stirred at room temperature for 4 h. To the reaction mixture water (15 mL) was added and extracted with diethyl ether (60 mL X 2), combined the organic layers and washed with brine (5 mL) concentrated under reduced pressure. Purification by column chromatography (silica gel 100-200 mesh 30% ethyl acetate – CH_2Cl_2) yielded compound **05** as a colorless liquid.

Yield: 83%

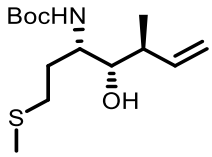
Specific rotation: $[\alpha]_D^{30}$ = + 17.16 (*c* 0.42, CH_3OH)

IR υ_{max}(film): 3348, 3015, 2917, 1676, 1533, 1451 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.75 - 5.72 (m, 1H), 5.18(d, *J* = 8.3 Hz, 2H), 4.10 - 4.07(m, 2H), 2.72 - 2.67 (m, 1H), 1.59 (s, 3H), 1.53 (s, 3H), 1.43 (s, 9H), 1.29 (d, *J* = 6.8 Hz, 3H)

HRMS (ESI): calculated for $C_{15}H_{25}O_5NNa$ $[M+Na]^+$: 322.1625, found 322.1628.

***tert*-Butyl ((3*S*,4*S*,5*S*)-4-hydroxy-5-methyl-1-(methylthio)hept-6-en-3-yl)carbamate**



24

(*E*)-2-butene (1.5 mL, 8.5 mmol, 2 equiv) was condensed into flask containing KO^{t}Bu (1.2 g, 10.75 mmol, 1.25 equiv) in THF (20 mL) cooled to –78 °C. After careful addition of n-BuLi (6.7 mL, 1.6 M in hexanes, 10.7 mmol, 1.25 equiv) over 30 min (maintaining internal temperature below –70 °C), the reaction mixture was warmed to –50 °C for 15 min. The mixture was cooled to –78 °C once again, and a solution of (+)-B-methoxy diisopinocampheyl borane (13 mL, 1M in THF, 13 mmol, 1.45 equiv) was added slowly over 20 min. Next, $BF_3.Et_2O$ (2.1 mL, 14.6 mmol, 1.7 equiv) was added at –78 °C over 20 minutes, then the solution of reagent was treated with a solution of aldehyde **23** (2.2 g, 8.5 mmol) in THF (15 mL).The reaction mixture was stirred at –78 °C for 12 h, then warmed to –15 °C. A mixture of 3N NaOH (4 mL) and 30% hydrogen peroxide (2 mL) was added

dropwise to the reaction. After being heated to reflux for 1 h, the mixture was cooled to 0 °C. Concentrated the reaction mixture, diethyl ether (35 mL) was added and washed with water (15 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 and the crude material obtained after removal of the solvent was purified by column chromatography (silica gel 230-400 mesh 10% ethylacetate - petether) to afford **24** as colorless liquid as a single diastereomer.

Yield: 49%

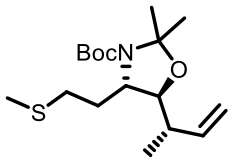
Specific rotation: $[\alpha]_D^{26}$ = - 8.78 (*c* 0.75, $CHCl_3$)

IR υ_{max}(film): 3443, 3015, 2964, 1715, 1508 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.78 - 5.60 (m, 1H), 5.21 - 5.14 (m, 2H), 4.83 (brs, 1H), 3.88 (brs, 1H), 3.24(d, *J* = 8.5 Hz, 1H), 2.56 (t, *J* = 7.5 Hz, 2H), 2.25 - 2.17 (m, 1H), 2.11 (s, 3H), 1.94 - 1.78 (m,2H), 1.45 (brs, 9H), 1.04 (d, *J* = 5.4 Hz, 3H)

HRMS (ESI): calculated for $C_{14}H_{27}O_3NNaS$ $[M+Na]^+$: 312.1604, found 312.1596.

***tert*-Butyl (4*S*, 5S)-5-((*S*)-but-3-en-2-yl)-2,2-dimethyl-4-(2-(methylthio)ethyl)oxazolidine-3-carboxylate**



25

To a stirred solution of compound **24** (3.5 g, 12.1 mmol), and 2-methoxypropene (2.91 mL, 30.25 mmol) in dry DMF (20 mL) PTSA.H_2O (47 mg, 2.4 mmol) was added at 0 °C under argon atmosphere. The resulting solution was stirred at room temperature for 4 h. The reaction was then diluted with H_2O (15 mL) and extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with cold saturated $NaHCO_3$ solution (25 mL), H_2O (20 mL), brine (20 mL), and evaporated in vacuo. Purification by column chromatography (silica gel 100-200 mesh 10% ethyl acetate-pet ether) yielded compound **25** as a pale yellow color liquid.

Yield: 87%

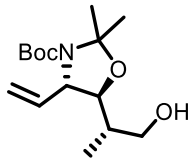
Specific rotation: $[\alpha]_D^{26}$ = + 9.83 (*c* 0.5, $CHCl_3$)

IR υ_{max}(film): 3025, 2967, 1698, 1567 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.90 - 5.75 (m, 1H), 5.11 - 4.96 (m, 2H), 3.88 (brs, 1H), 3.62 (dd, *J* = 3.7, 7.8 Hz, 1H), 2.53 - 2.28 (m, 3H), 2.10 (s, 3H), 1.92 (dd, *J* = 6.6, 7.3 Hz, 2H), 1.55 (brs, 3H), 1.46 (s, 12H), 1.02 (d, *J* = 6.8 Hz, 3H)

HRMS (ESI): calculated for $C_{17}H_{31}O_3NNaS$ $[M+Na]^+$: 352.1917, found 352.1909.

***tert*-Butyl (4*S*,5*S*)-5-((*S*)-1-hydroxypropan-2-yl)-2,2-dimethyl-4-vinyloxazolidine-3-carboxylate**



26

Compound **26** was synthesized from **25** by following similar procedure for the synthesis of **22**

Yield: 38% for 2 steps

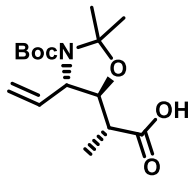
Specific rotation: $[\alpha]_D^{26}$ = -13.38 (*c* 0.68, $CHCl_3$)

IR υ_{max}(film): 3023, 2976, 1689, 1580 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.76 - 5.57 (m, 1H), 5.14 (d, *J* = 10.0 Hz, 2H), 4.06 (brs, 1H), 3.71 (t, *J* = 6.8 Hz, 1H), 3.66 - 3.57 (m, 2H), 2.56 (brs, 1H), 1.97 - 1.83 (m, 1H), 1.57 (s, 3H), 1.46 (s, 3H), 1.39 (brs, 9H), 0.93 (d, *J* = 6.8 Hz, 3H)

HRMS (ESI): calculated for $C_{15}H_{27}O_4NNa$ $[M+Na]^+$: 308.1832, found 308.1823.

(*R*)-2-((4*S*, 5S)-3-(*tert*-Butoxycarbonyl)-2,2-dimethyl-4-vinyloxazolidin-5-yl)propanoic acid



06

Compound **06** was synthesized from **26** by following similar procedure for the synthesis of **05**

Yield: 81%

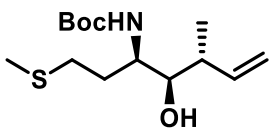
Specific rotation: $[\alpha]_D^{26}$ = - 28.91 (*c* 1.20, $CHCl_3$)

IR υ_{max}(film): 3315, 3021, 1667, 1538 cm^{-1}

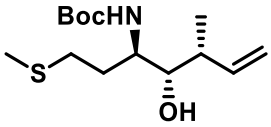
^{1}H NMR (400 MHz, $CDCl_3$): δ 5.80 - 5.72 (m, 1H), 5.20 (d, *J* = 10.0 Hz, 2H), 4.28 - 4.12 (m, 1H), 4.03 (dd, *J* = 5.3, 7.7 Hz, 1H), 2.80 - 2.76 (m, 1H), 1.62 (s, 3H), 1.53 (s, 3H), 1.45 (brs, 9H), 1.25 (d, *J* = 7.1 Hz, 3H)

HRMS (ESI): calculated for $C_{15}H_{25}O_5NNa$ $[M+Na]^+$: 322.1625, found 322.1617.

***tert*-Butyl ((3R,4S,5R)-4-hydroxy-5-methyl-1-(methylthio)hept-6-en-3-yl)carbamate**



27 (major)



27a (minor)

Anhydrous chromium (II) chloride (4.75 g, 38.6 mmol) was transferred into a round bottomed flask under argon atmosphere and heated up to 200 °C for 40 min under high vaccum. D- Methionine N-Boc aldehyde **16** (3.0 g, 12.8 mmol) in THF (40 mL) was added at 0 °C followed by *trans* crotyl bromide (2.8 mL, 25.6 mmol) and the reaction mixture was stirred at room temperature for 8 h. Reaction mass was quenched with saturated aq. NH_4Cl (20 mL) and extracted with Et_2O (4 x 100 mL). The combined organic layer was dried over anhydrous Na_2SO_4. The crude material obtained after removal of solvent was purified by column chromatography (silica gel 100-200 mesh, 1:15 to 1:10 ethyl acetate - pet ether) to afford **27** and **27a** respectively (~2:1 ratio, 62%).

All the spectral data of **27a** are in complete agreement with literature data reported from our group during synthesis of solomonamide B.

Data of **27a**

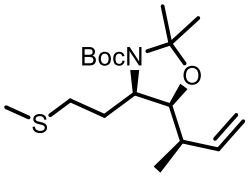
IR υ_{max} (film): 3443, 2957, 2859, 1716, 1473 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$): δ 5.79 - 5.65 (m, 1H), 5.19- 5.11 (m, 2H), 4.94 (brs, 1H), 3.85 (brs, 1H), 3.39 (dd, *J* = 3.5, 8.1 Hz, 1H), 2.65- 2.54 (m, 2H), 2.31 - 2.24 (m, 1H), 2.12 (s, 3H), 1.86 - 1.63 (m, 2H), 1.45 (s, 9H), 1.06 (d, *J* = 6.8 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.9, 140.6, 117.1, 79.4, 77.3, 77.0, 76.7, 51.5, 41.8, 31.1, 29.7, 28.4, 16.3, 15.7

HRMS (ESI): calculated for $C_{14}H_{27}O_3NNaS$ $[M+Na]^+$: 312.1604, found 312.1595.

***tert*-Butyl (5*R*)-5-((*R*)-but-3-en-2-yl)-2,2-dimethyl-4-(2-(methylthio)ethyl)oxazolidine-3-carboxylate**



28

Compound **28** was synthesized from aldehyde **16** by following similar procedure for the synthesis of **25**

Yield: 79%

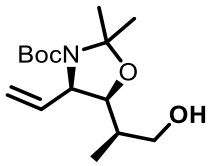
IR υ_{max}(film**)**: 3028, 2974, 1691, 1579 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.86 - 5.70 (m, 1H), 5.05 - 4.88 (m, 2H), 4.05 - 3.93 (m, 1H), 3.63 - 3.52 (m, 1H), 2.58 - 2.37 (m, 2H), 2.27 (td, *J* = 6.7, 10.1 Hz, 1H), 2.01 (d, *J* = 2.9 Hz, 3H), 1.84 - 1.61 (m, 2H), 1.49 - 1.40 (m, 6H), 1.37 (s, 9H), 0.90 (t, *J* = 7.0 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 152.8, 151.8, 140.5, 114.2, 93.1, 92.6, 80.6, 80.3, 79.9, 79.7, 57.5, 36.3, 31.2, 31.1, 29.8, 29.6, 28.4, 28.3, 28.1, 27.2, 24.9, 23.4, 16.4, 16.3, 15.5

HRMS (ESI): calculated for $C_{17}H_{31}O_3NNaS$ $[M+Na]^+$: 352.1917, found 352.1908.

***tert*-Butyl (5*R*)-5-((*R*)-1-hydroxypropan-2-yl)-2,2-dimethyl-4-vinyloxazolidine-3-carboxylate**



29

Compound **29** was synthesized from **28** by following similar procedure for the synthesis of **22**

Yield: 34% over 2 steps

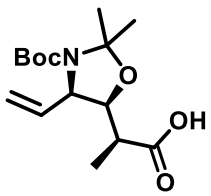
IR υ_{max}(film): 3018, 2984, 1678, 1579 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.78 - 5.61 (m, 1H), 5.39 - 5.12 (m, 2H), 4.19 (dd, *J* = 4.9, 8.8 Hz, 1H), 3.86 (dd, *J* = 4.8, 10.1 Hz, 1H), 3.71 - 3.62 (m, 1H), 3.60 (brs, 1H), 2.78 (brs, 1H), 1.94 (td, *J* = 3.2, 6.7 Hz, 1H), 1.81 (brs, 1H), 1.66 - 1.53 (m, 6H), 1.53 - 1.38 (m, 9H), 0.81 (d, *J* = 6.6 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 151.6, 132.8, 132.2, 119.0, 118.4, 93.7, 93.3, 81.8, 81.6, 80.3, 79.7, 67.8, 62.7, 62.5, 34.8, 28.4, 28.0, 27.2, 25.1, 24.0, 12.3

HRMS (ESI): calculated for $C_{15}H_{27}O_4NNa$ $[M+Na]^+$: 308.1832, found 308.1824.

(2*S*)-2-((5*R*)-3-(tert-butoxycarbonyl)-2,2-dimethyl-4-vinyloxazolidin-5-yl)propanoic acid



07

Compound **07** was synthesized from **29** by following similar procedure for the synthesis of **05**

Yield: 87%

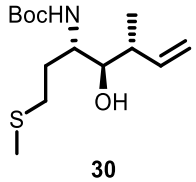
IR υ_{max}(film): 3020, 2994, 1660, 1648, 1569 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.75 - 5.66 (m, 1H), 5.36 - 5.18 (m, 2H), 4.20 - 4.13 (m, 2H), 2.61 - 2.59 (m, 1H), 2.06 (t, *J* = 6.6 Hz, 1H), 1.61 - 1.45 (m, 9H), 1.42 (s, 6H), 1.15 (d, *J* = 6.8 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 178.8, 178.6, 132.3, 131.6, 119.9, 119.2, 93.9, 93.4, 80.5, 79.8, 62.0, 61.7, 39.6, 32.0, 29.7, 28.4, 27.9, 27.1, 24.8, 23.8, 20.9, 13.1

HRMS (ESI): calculated for $C_{15}H_{25}O_5NNa$ $[M+Na]^+$: 322.1625, found 322.1615.

***tert*-Butyl ((3*S*,4*R*,5*R*)-4-hydroxy-5-methyl-1-(methylthio)hept-6-en-3-yl)carbamate**



30

(E)-2-butene (6 mL, 34 mmol, 2 equiv.) was condensed into flask containing KO^{t}Bu (5.8 gm, 51.4 mmol, 1.5 equiv) in THF (20 mL) chilled to –78 °C. After careful addition of *n*-BuLi (32.2 mL, 1.6 M in hexanes, 51.4 mmol, 1.5 equiv) over 1 hour (maintaining internal temperature below –70 °C), the reaction mixture was warmed to –50 °C for 15 min. The mixture was chilled to –78 °C once more, and a solution of (-)-B-methoxy diisopinocampheyl borane (16.2 gm in 50 mL THF, 51.4 mmol) was added slowly over 30 min. Next, BF_3•Et_2O (7.6 mL, 61.8 mmol, 1.8 equiv) was added at –78 °C over 30 minutes, then the solution of reagent was treated with a solution of compound **23** (8.0 g, 34.2 mmol) in THF (25 mL). The reaction mixture was stirred at –78 °C for 12 h, then warmed to –15 °C. A mixture of 3N NaOH (15.3 mL) and 30% hydrogen peroxide (6.1 mL) was added dropwise to the reaction. After being heated to reflux for 1 h, the mixture was cooled to 0 °C. Concentrated the reaction mixture, diethyl ether (50 mL) was added and washed with water (20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 and the crude material obtained after removal of the solvent was purified by column chromatography (silica gel 230-400 mesh 12% ethylacetate - petether) to afford **30** (4.8 gm, 47%) as colorless liquid as a single diastereomer

Yield: 47%

Specific rotation: $[\alpha]_D^{30}$ = - 28.79 (*c* 0.95, $CHCl_3$)

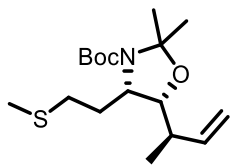
IR υ_{max}(film): 3450, 3018, 2977, 1705, 1530 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.74 (td, *J* = 8.93, 17.85 Hz, 1H), 5.17 - 5.13 (m, 2H), 4.94 (d, *J* = 9.29 Hz, 1H), 3.85 (t, *J* = 9.90 Hz, 1H), 3.38 (d, *J* = 5.38 Hz, 1H), 2.63 - 2.60 (m, 1H), 2.55 - 2.53 (m, 1H), 2.18 - 2.16 (m, 1H), 2.12 (s, 3H), 1.73 - 1.75 (m, 2H), 1.44 (s, 9H), 1.06 (d, *J* = 6.60 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.9, 140.6, 117.1, 79.4, 76.7, 51.4, 41.8, 31.0, 28.4, 16.3, 15.7;

HRMS (ESI): calculated for $C_{14}H_{27}O_3NSNa$ $[M+Na]^+$: 312.1604, found 312.1595.

***tert*-Butyl (4*S*,5*R*)-5-((*R*)-but-3-en-2-yl)-2,2-dimethyl-4-(2-(methylthio)ethyl)oxaz-olidine-3-carboxylate**



31

Compound **31** was synthesized from **30** by following similar procedure used for the synthesis of **25**.

Yield: 88%

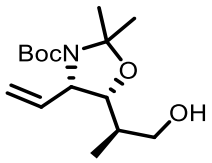
IR υ_{max}(film): 3020, 2978, 1684, 1597 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.93 - 5.63 (m, 1H), 5.15 - 4.85 (m, 2H), 3.74 - 3.55 (m, 1H), 2.63 - 2.43 (m, 2H), 2.38 - 2.26(m, 1H), 2.07 (s, 3H), 1.79 (brs, 1H), 1.77 - 1.67 (m, 1H), 1.55 - 1.45 (m, 6H), 1.43 (s, 9H), 0.96 (t, *J* = 6.85 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): (mixture of rotamers) δ 152.9, 151.9, 140.6, 139.7, 115.4, 114.3, 93.2, 92.7, 80.7, 80.4, 80.0, 79.8, 77.4, 77.1, 76.8, 57.6, 41.4, 36.4, 31.2, 31.2, 30.3, 29.9, 29.6, 28.5, 28.4, 28.1, 27.3, 24.9, 23.5, 17.0, 16.5, 16.4, 15.6

HRMS (ESI): calculated for $C_{17}H_{31}O_3NSNa$ $[M+Na]^+$: 352.1917, found 352.1907.

***tert*-Butyl (4*S*,5*R*)-5-((*R*)-1-hydroxypropan-2-yl)-2,2-dimethyl-4-vinyloxazolidine-3-carboxylate**



32

Compound **32** was synthesized from **31** by following similar procedure used for the synthesis of **26**.

Yield: 36% yield for 2 steps

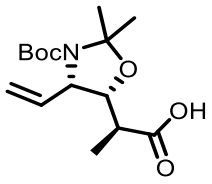
Specific rotation: $[\alpha]_D^{30}$ = - 46.79 (*c* 1.5, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.74 (m, 1H), 5.04 - 5.33 (m, 2H), 3.99 - 4.22 (m, 1H), 3.74 - 3.89 (m, 1H), 3.56 - 3.69 (m, 1H), 3.45 - 3.56 (m, 1H), 2.83 (brs, 1H), 1.86 (brs, 1H), 1.46 - 1.60 (m, 6H), 1.42 - 1.37 (m, 9H), 0.76 (d, *J* = 6.36 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 151.6, 132.9, 132.2, 119.0, 118.4, 93.6, 93.2, 81.5, 81.3, 80.3, 79.6, 67.5, 62.7, 62.4, 34.8, 34.7, 28.4, 28.0, 27.2, 25.1, 24.0, 12.3

HRMS (ESI): calculated for $C_{15}H_{27}O_4NNa$ $[M+Na]^+$: 308.1832, found 308.1824.

(*S*)-2-((4*S*,5*R*)-3-(tert-butoxycarbonyl)-2,2-dimethyl-4-vinyloxazolidin-5-yl)propanoic acid



08

Compound **08** was synthesized from **32** by following similar procedure used for the synthesis of **05**.

Yield: 75%

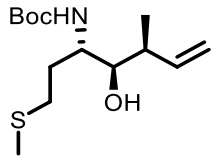
IR υ_{max}(film): 3040, 2939, 1635, 1567 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.73 - 5.65 (m, 1H), 5.33 - 5.16 (m, 2H), 4.35 - 4.15 (m, 2H), 2.57 (brs, 1H), 1.59 - 1.41 (m, 15H), 1.12 (d, *J* = 5.9 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$): (mixture of rotamers) δ 179.9, 151.8, 151.6, 132.4, 131.7, 119.7, 119.0, 93.7, 93.3, 80.5, 61.9, 61.6, 40.0, 29.7, 28.4, 27.9, 27.1, 24.8, 23.8, 13.3

HRMS (ESI): calculated for $C_{15}H_{25}O_5NNa$ $[M+Na]^+$: 322.1625, found 322.1616.

***tert*-Butyl ((3S,4R,5S)-4-hydroxy-5-methyl-1-(methylthio)hept-6-en-3-yl)carbamate**



33a

Compound **33a** was synthesized from **23** by following similar procedure used for the synthesis of **27'**.

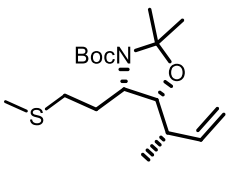
Yield: 52%

IR υ_{max}(film): 3021, 2934, 1641, 1569 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$): δ 5.79 - 5.65 (m, 1H), 5.19 - 5.11 (m, 2H), 4.94 (brs, 1H), 3.85 (brs, 1H), 3.39 (dd, *J* = 3.5, 8.1 Hz, 1H), 2.65- 2.54 (m, 2H), 2.31 - 2.24 (m, 1H), 2.12 (s, 3H), 1.86 - 1.63 (m, 2H), 1.45 (s, 9H), 1.06 (d, *J* = 6.8 Hz, 3H)

HRMS (ESI): calculated for $C_{14}H_{27}O_3NSNa$ $[M+Na]^+$: 312.1604, found 312.1596.

***tert*-Butyl (4*S*,5*R*)-5-((S)-but-3-en-2-yl)-2,2-dimethyl-4-(2-(methylthio)ethyl)oxazolidine-3-carboxylate**



34

Compound **34** was synthesized from **33'** by following similar procedure used for the synthesis of **25**.

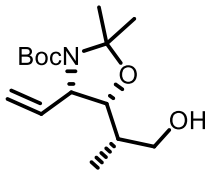
Yield: 85%

IR υ_{max}(film): 3021, 2954, 1638, 1571, 1151 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.86 - 5.70 (m, 1H), 5.05 - 4.88 (m, 2H), 4.05 - 3.93 (m, 1H), 3.63 - 3.52 (m, 1H), 2.58 - 2.37 (m, 2H), 2.27 (td, *J* = 6.7, 10.1 Hz, 1H), 2.01 (d, *J* = 2.9 Hz, 3H), 1.84 - 1.61 (m, 2H), 1.49 - 1.40 (m, 6H), 1.37 (s, 9H), 0.90 (t, *J* = 7.0 Hz, 3H)

HRMS (ESI): calculated for $C_{17}H_{31}O_3NSNa$ $[M+Na]^+$: 352.1917, found 352.1907.

***tert*-Butyl (4*S*,5*R*)-5-((*S*)-1-hydroxypropan-2-yl)-2,2-dimethyl-4-vinyloxazolidine-3-carboxylate**



35

Compound **35** was synthesized from **34** by following similar procedure used for the synthesis of **22**.

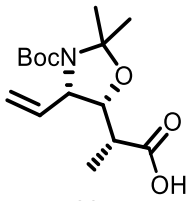
Yield: 43% for 2 steps

IR υ_{max}(film): 3038, 2934, 1634, 1180 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.80 - 5.60 (m, 1H), 5.35 - 5.11 (m, 2H), 4.17 (dd, *J* = 4.9, 8.3 Hz, 1H), 3.84 (dd, *J* = 4.6, 10.0 Hz, 1H), 3.69 - 3.61 (m, 1H), 3.61 - 3.52 (m, 1H), 2.01 - 1.83 (m, 1H), 1.63 - 1.51 (m, 6H), 1.47 (s, 4H), 1.41 (s, 5H), 0.79 (d, *J* = 6.4 Hz, 2H)

HRMS (ESI): calculated for $C_{15}H_{27}O_4NNa$ $[M+Na]^+$: 308.1832, found 308.1825.

(*R*)-2-((4*S*,5*R*)-3-(tert-butoxycarbonyl)-2,2-dimethyl-4-vinyloxazolidin-5-yl)propanoic acid



09

Compound **09** was synthesized from **35** by following similar procedure used for the synthesis of **05**.

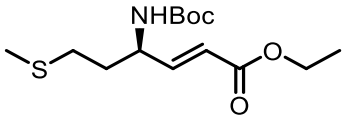
Yield: 89%

IR υ_{max}(film): 3033, 2945, 1651, 1581, 1156 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.75 - 5.66 (m, 1H), 5.36 - 5.18 (m, 2H), 4.20 - 4.11 (m, 2H), 2.61 - 2.59 (m, 1H), 1.62 - 1.53 (m, 6H), 1.48 - 1.42 (s, 9H), 1.15 (d, *J* = 6.8 Hz, 3H)

HRMS (ESI): calculated for $C_{15}H_{25}O_5NNa$ $[M+Na]^+$: 322.1625, found 322.1616.

Ethyl (*R*,*E*)-4-((tert-butoxycarbonyl)amino)-6-(methylthio)hex-2-enoate



37a

To a solution of compound **16** (15.0 g, 64.29 mmol) in anhydrous dichloromethane (250 mL) at 0°C under the nitrogen atmosphere (ethyl 2-(triphenyl-l5-phosphaneylidene)propanoate) Wittig ylide (40.31 g, 115.72 mmol) was added in portion wise. The progress of reaction was monitored by TLC. After the completion of reaction (8 h) CH_2Cl_2 was evaporated and product was purified by column chromatography (silica gel 230-400 mesh 8% ethyl acetate - pet ether) to afford compound **37a** as an oily liquid (15.7 g).

Yield: 81%.

IR υ_{max}(film): 3345, 2978, 2918, 1690, 1515, 1366 cm^{-1}

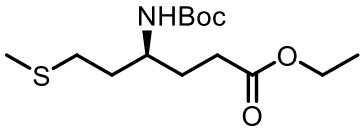
Specific rotation: $[\alpha]_D^{22}$ = + 5.23 (*c* 0.6, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.80 (dd, *J* = 4.9, 15.9 Hz, 1H), 5.89 (d, *J* = 15.3 Hz, 1H), 4.87 (brs, 1H), 4.39 (brs, 1H), 4.15 (q, *J* = 7.3 Hz, 2H), 2.54 - 2.49 (m, 2H), 2.05 (s, 3H), 1.84 - 1.74 (m, 2H), 1.39 (s, 9H), 1.24 (t, *J* = 7.0 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.1, 155.0, 147.5, 121.0, 79.7, 60.5, 60.4, 50.7, 33.8, 30.2, 28.2, 15.4, 14.1;

HRMS (ESI): calculated for $C_{14}H_{26}O_4NS$ $[M+H]^+$: 304.1577, found 304.1571.

Ethyl (*S*)-4-((tert-butoxycarbonyl)amino)-6-(methylthio)hexanoate



38a

To a solution of compound **37a** (15.5 g, 51.08 mmol) in ethanol (70 mL), 10% Pd/C (~ 300 mg) was added and stirred in reactor with 60 psi pressure of H_2 atmosphere for 8 h. The reaction mixture was then filtered through a pad of celite, concentrated to afford saturated ester compound **38a** as a colorless liquid with quantitative yield.

IR υ_{max}(film): 3351, 2977, 2919, 1711, 1687, 1516, 1447 cm^{-1}

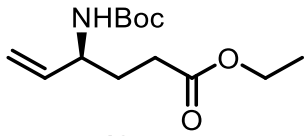
Specific rotation: $[\alpha]_D^{22}$ = + 16.18 (*c* 0.67, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.40 (d, *J* = 8.5 Hz, 1H), 4.12 (q, *J* = 6.7 Hz, 2H), 3.72 - 3.66 (m, 1H), 2.56 - 2.48 (m, 2H), 2.37 (t, *J* = 7.3 Hz, 2H), 2.09 (s, 3H), 1.87 - 1.81 (m, 1H), 1.78 - 1.73 (m, 1H), 1.68 - 1.65 (m, 2H), 1.42 (brs, 9H), 1.25 (t, *J* = 7.0 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$):δ 173.5, 155.6, 79.2, 60.5, 49.9, 35.4, 31.0, 30.6, 30.3, 28.3, 15.6, 14.2

HRMS (ESI): calculated for $C_{14}H_{28}O_4NS$ $[M+H]^+$: 306.1734, found 306.1721.

Ethyl (*S*)-4-((tert-butoxycarbonyl)amino)hex-5-enoate



40a

To a solution of compound **38a** (15.3 g, 50.09 mmol) in CH_2Cl_2 (250 mL) ozone was bubbled at -78 °C until the color becames blue, once the blue color appeared oxygen was bubbled to remove excess ozone, then reaction mixture was allowed to warm to room temperature and stirred for 1 h. After concentration under vacuo crude sulfoxide compound was taken in 1,2 dichloro benzene (500 mL), followed by addition of $CaCO_3$ (5 g, 50.09 mmol) and refluxed for 6 h. The crude reaction mixture was purified by column chromatography (silica gel 230-400 mesh 10% ethyl acetate – pet ether) to afford **40a** as a colourless powder (5.20 g, 40% yield).

Yield: 40% for 2 steps.

Melting point = 61 - 63 °C

IR υ_{max}(film): 3557, 2979, 2933, 1711, 1692, 1513, 1366 cm^{-1}

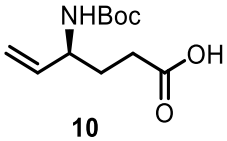
Specific rotation: $[\alpha]_D^{22}$ = + 13.5 (*c* 0.95, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.74 (ddd, *J* = 5.7, 10.6, 16.9 Hz, 1H), 5.19 - 5.10 (m, 2H), 4.56 (brs, 1H), 4.13 (q, *J* = 7.0 Hz, 3H), 2.36 (t, *J* = 7.6 Hz, 2H), 1.91 - 1.87 (m, 1H), 1.80 - 1.76 (m, 1H), 1.43 (s, 9H), 1.25 (t, *J* = 7.2 Hz, 3H);

^{13}C NMR (125 MHz, $CDCl_3$): δ 173.3, 155.3, 138.2, 115.0, 79.4, 60.5, 52.4, 30.8, 29.9, 28.3, 14.2;

HRMS (ESI): calculated for $C_{13}H_{23}O_4NNa$ $[M+H]^+$: 280.1519, found 280.1513.

(*S*)-4-((tert-Butoxycarbonyl)amino)hex-5-enoic acid



10

To a solution of compound **40a** (4.00 g, 15.54 mmol) in ethanol (30 mL) was added LiOH.H_2O (1.27 g, 31.08 mmol) in H_2O (30 mL) drop wise at 0 °C and stirred for 5 h at room temperature. Reaction mass was evaporated to dryness, diluted with H_2O (50 mL), washed with diethyl ether (50 mL), acidified with citric acid (20% solution). The suspension thus formed was extracted with EtOAc (3 x 70 mL), washed with H_2O (50 mL), brine (50 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **10** as a yellow solid (3.36 g, 96% yield).

Yield: 96%

IR υ_{max}(film): 3325, 2979, 2932, 1702, 1512, 1393cm^{-1}

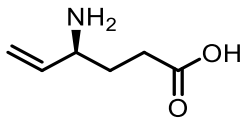
Specific rotation: $[\alpha]_D^{22}$ = + 13.5 (*c* 0.95, $CHCl_3$)

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.99 (brs, 1H), 6.87 (d, *J* = 7.9 Hz, 1H), 5.71 (ddd, *J* = 6.1, 10.4, 17.1 Hz, 1H), 5.07- 4.99 (m, 2H), 3.91 (brs, 1 H), 2.19 (t, *J* = 7.3 Hz, 2H), 1.69 - 1.56 (m, 2H), 1.37 (s, 9H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 174.1, 155.1, 139.3, 114.1, 77.6, 51.9, 30.3, 29.4, 28.2

HRMS (ESI): calculated for $C_{13}H_{24}O_4N$ $[M+H]^+$: 130.0861, found 130.0863.

(*S*)-4-Aminohex-5-enoic acid



(*S*)-Vigabatrin (36a)

To a solution of compound **10** (300 mg, 1.31 mmol), 4N HCl in dioxane (5 mL) was added and stirred at room temperature for 2 h. The solvent was then removed in vacuo, and the residue was washed with diethyl ether (10 mL) to give a yellow colorled solid, which happened to be the hydrochloride salt. ^{1}H NMR (200 MHz, D_2O): δ = 5.80 (m, 1H), 5.47 - 5.36 (m, 2H), 3.82 (m, 1H), 2.46 (m, 2H), 2.08 (m, 1H), 1.94 (m, 1H). The compound was introduced in water (1 mL) solution was passed through a column of Dowex 50WX8 ion exchange resin (4 g, 200–400 mesh, H^+ form). The column was eluted with H_2O until the eluent pH was neutral. Further elution with 2N aq. NH_4OH, and removal of the latter

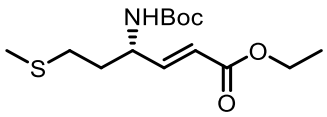
in vacuo, afforded (*S*)-vigabatrin (**36a**) (151 mg) as a colorless solid. The spectroscopic data for this compound was in full agreement with that reported.

Yield: 90% for 2 steps

Melting point = 173 - 175 °C

Specific rotation: $[\alpha]_D^{22}$ = + 14.2 (*c* 0.94, H_2O)

Ethyl (*S*,*E*)-4-((tert-butoxycarbonyl)amino)-6-(methylthio)hex-2-enoate



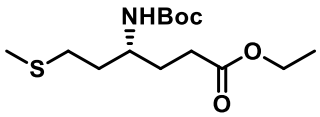
37b

Compound **37b** was synthesized from **23** by following similar procedure for the synthesis of compound **37a**.

Specific rotation: $[\alpha]_D^{22}$ = + 6.03 (*c* 0.81, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.80 (dd, *J* = 4.9, 15.9 Hz, 1H), 5.89 (d, *J* = 15.3 Hz, 1H), 4.87 (brs, 1H), 4.39 (brs, 1H), 4.15 (q, *J* = 7.3 Hz, 2H), 2.54 - 2.49 (m, 2H), 2.05 (s, 3H), 1.84 - 1.74 (m, 2H), 1.39 (s, 9H), 1.24 (t, *J* = 7.0 Hz, 3H)

Ethyl (*R*)-4-((tert-butoxycarbonyl)amino)-6-(methylthio)hexanoate



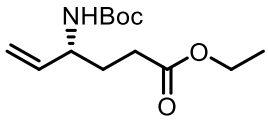
38b

Compound **38b** was synthesized from **37b** by following similar procedure for the synthesis of compound **38a**.

Specific rotation: $[\alpha]_D^{22}$ = - 15.37 (*c* 0.57, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ 4.40 (d, *J* = 8.8 Hz, 1H), 4.12 (q, *J* = 7.2 Hz, 2H), 3.72 - 3.59 (m, 1H), 2.54 - 2.49 (m, 2H), 2.37 (t, *J* = 7.6 Hz, 2H), 2.09 (s, 3H), 1.87 - 1.83 (m, 1H), 1.78 - 1.74 (m, 1H), 1.69 - 1.65 (m, 2H), 1.42 (s, 9H), 1.27 - 1.20 (m, 3H)

Ethyl (*R*)-4-((tert-butoxycarbonyl)amino)hex-5-enoate



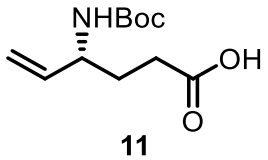
40b

Compound **40b** was synthesized from **38b** by following similar procedure for the synthesis of compound **40a**.

Specific rotation: $[\alpha]_D^{22}$ = +12.6 (*c* 0.82, $CHCl_3$)

^{1}H NMR (200 MHz, $CDCl_3$): δ 5.74 (ddd, *J* = 5.7, 10.6, 16.9 Hz, 1H), 5.22 - 5.09 (m, 2H), 4.52 (brs, 1H), 4.13 (q, *J* = 7.0 Hz, 3H), 2.37 (t, *J* = 7.6 Hz, 2H), 1.91 - 1.87 (m, 1H), 1.80 - 1.76 (m, 1H), 1.44 (s, 9H), 1.26 (t, *J* = 7.2 Hz, 3H).

(*R*)-4-((tert-Butoxycarbonyl)amino)hex-5-enoic acid

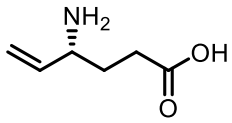


11

Compound **11** was synthesized from **40b** by following similar procedure for the synthesis of compound **40a**.

^{1}H NMR (200 MHz, DMSO-d_6): δ 12.0 (brs, 1H), 6.88 (d, *J* = 7.9 Hz, 1H), 5.71 (ddd, *J* = 6.1, 10.4, 17.1 Hz, 1H), 5.09 - 4.98 (m, 2H), 3.91 (brs, 1H), 2.20 (t, *J* = 7.3 Hz, 2H), 1.69 - 1.52 (m, 2H), 1.38 (s, 9H).

(*R*)-4-Aminohex-5-enoic acid



(*R*)-Vigabatrin (36b)

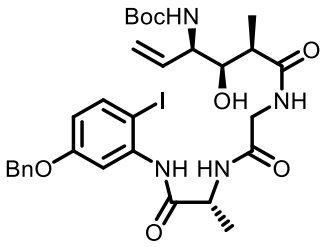
Compound **36b** was synthesized from **11** by following similar procedure for the synthesis of compound **36a**.

Melting point = 170 - 173 °C

Specific rotation: $[\alpha]_D^{22}$ = - 12.81 (*c* 0.56, H_2O)

^{1}H NMR (200 MHz, D_2O): δ 5.80 (m, 1H), 5.47 - 5.36 (m, 2H), 3.82 (m, 1H), 2.46 (m, 2H), 2.08 (m, 1H), 1.94 (m, 1H).

***tert*-Butyl ((3*R*,4*R*,5*R*)-6-((2-(((*R*)-1-((5-(benzyloxy)-2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-4-hydroxy-5-methyl-6-oxohex-1-en-3-yl)carbamate**



45

To a solution of **02** (997 mg, 1.8 mmol) in CH_2Cl_2 (10 mL), TFA (2.0 mL) was added at 0 °C and stirred at 25 °C for 3 h. After completion of the reaction (monitored by TLC), the reaction mixture was concentrated under reduced pressure to afford the amine as TFA salt. Compound **05** (450 mg, 1.5 mmol) was taken in dry DMF (10 mL), added above amine salt, then HATU (685 mg, 1.8 mmol), DIPEA (0.65 mL, 3.7 mmol) were added and the resulting solution was stirred at ambient temperature for 16 h. Reaction mass was diluted with ethyl acetate (50 mL) washed with saturated solution of aq $NaHCO_3$ (5 mL), brine (5

mL) and then evaporated to dryness. Purification by column chromatography (silica gel 230-400 mesh 4% methanol - CH_2Cl_2) yielded coupled compound which was dissolved in methanol (4 mL), catalytic amount of Camphorsulphonic acid was added and stirred for 18 h. reaction was monitored by TLC showed only 50% conversion. Concentrated the reaction mixture diluted with ethyl acetate (40 mL), washed with saturated solution of aq $NaHCO_3$ (10 mL), brine (10 mL) and then evaporated to dryness. Purification by column chromatography (silica gel 230-400 mesh 4-6 % methanol - CH_2Cl_2) yielded compound **45**.

Yield: 55% for 3 steps

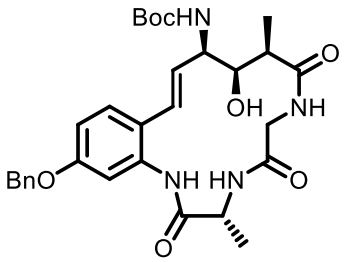
IR υ_{max}(film): 3038, 2967, 1657, 1582 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD): δ 7.69 (d, *J* = 8.3 Hz, 1H), 7.41 (d, *J* = 6.8 Hz, 2H), 7.36 (t, *J* = 6.8 Hz, 3H), 7.33 - 7.30 (m, 1H), 6.69 - 6.67 (m, 1H), 5.82 - 5.78 (m, 1H), 5.16 (d, *J* = 17.6 Hz, 1H), 5.04 (s, 2H), 4.62 - 4.56 (m, 1H), 4.20 (brs, 1H), 4.10 - 4.07 (m, 1H), 3.99 (d, *J* = 16.6 Hz, 1H), 3.85 (d, *J* = 16.6 Hz, 1H), 3.74 (d, *J* = 7.3 Hz, 1H), 2.53 - 2.50 (m, 1H), 1.52 (d, *J* = 7.3 Hz, 3H), 1.46 (brs, 9H), 1.22 (d, *J* = 7.3 Hz, 3H)

^{13}C NMR (100 MHz , CD_3OD): δ 177.3, 176.7, 171.9, 170.6, 159.5, 156.7, 139.2, 139.1, 137.0, 136.7, 128.2, 127.6, 127.3, 114.6, 112.5, 112.3, 79.1, 74.1, 69.8, 60.2, 55.1, 49.7, 43.5, 42.7, 29.4, 27.5, 16.3, 13.7

HRMS (ESI): calculated for $C_{30}H_{39}O_7N_4NaI$ $[M+Na]^+$: 717.1756, found 717.1758.

***tert*-Butyl ((3*R*,9*R*,10*R*,11*R*,*E*)-16-(benzyloxy)-10-hydroxy-3,9-dimethyl-2,5,8-trioxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)carbamate**



50

To a solution of compound **45** (240 mg, 0.34 mmol) in anhydrous acetonitrile (240 mL), $Pd(OAc)_2$ (5 mol%) and triethylamine (0.48 mL, 3.4 mmol) were added and heated at 75 °C for 12 h. The reaction mixture was concentrated in vaccuo. Purification by column chromatography (silica gel 230-400 mesh 4% methanol - CH_2Cl_2) yielded compound **50.**

Yield: 43%

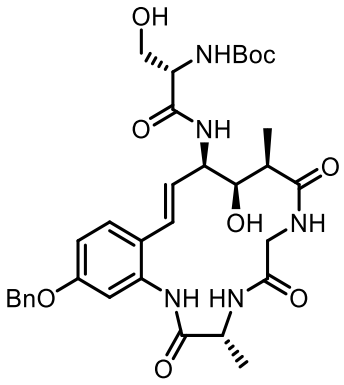
IR υ_{max}(film): 3384, 3021, 2401, 1657, 1523, 1423 cm^{-1}

^{1}H NMR (500 MHz, CD_3OD): δ 7.44 (d, *J* = 7.2 Hz, 3H), 7.37 (t, *J* = 8.0 Hz, 3H), 7.34 - 7.31 (m, 2H), 6.84 (dd, *J* = 2.5, 8.6 Hz, 1H), 6.65 - 6.64 (m, 1H), 6.21 (dd, *J* = 3.1, 16.0 Hz, 1H), 5.10 (s, 2H), 4.60 - 4.55 (m, 2H), 4.26 (d, *J* = 13.7 Hz, 1H), 3.87 (dd, *J* = 2.9, 10.1 Hz, 1H), 3.49 (d, *J* = 14.1 Hz, 1H), 2.25 (dd, *J* = 6.7, 10.1 Hz, 1H), 1.50 - 1.48 (m, 12H), 1.22 (d, *J* = 6.9 Hz, 3H)

^{13}C NMR (125 MHz, CD_3OD): δ 175.5, 171.7, 171.5, 158.5, 156.8, 137.1, 135.6, 128.1, 127.5, 127.5, 127.2, 126.2, 124.7, 123.9, 112.2, 110.2, 78.8, 74.6, 69.6, 57.5, 57.4, 50.0, 45.0, 42.2, 27.5, 15.4, 14.2

HRMS (ESI): calculated for $C_{30}H_{38}O_7N_4Na$ $[M+Na]^+$: 589.2633, found 589.2635.

***tert*-Butyl ((S)-1-(((3R,9R,10R,11R,E)-16-(benzyloxy)-10-hydroxy-3,9-dimethyl-2,5,8-trioxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamate**



56

To a solution of compound **50** (90 mg, 0.158 mmol) in CH_2Cl_2 (10 mL) trifluoro acetic acid (2.0 mL) was added at 0 °C and the resulting suspension was stirred for 2 h at the same temperature. Reaction was monitored by TLC, and then concentrated. This residue was dissolved in dry DMF (10 mL), then HATU (120 mg, 0.32 mmol), DIPEA (82 µL, 0.46 mmol) and *N*-(tert-butoxycarbonyl)-O-(tert-butyldimethylsilyl)-L serine **55** (56 mg, 0.174 mmol) was added. The resulting solution was stirred at ambient temperature for 16 h. Reaction mass was diluted with ethyl acetate (45 mL), washed with saturated solution of $NaHCO_3$ (15 mL), H_2O (15 mL). The organic layer was dried over Na_2SO_4 and the crude material obtained after removal of the solvent was purified by column chromatography (silica gel 230-400 mesh 4% methanol - CH_2Cl_2) to afford **56.**

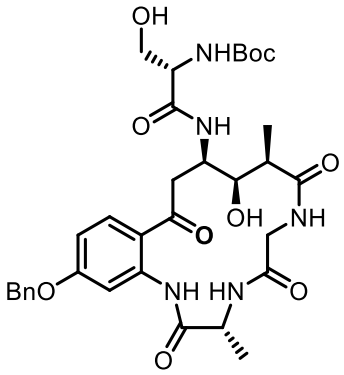
Yield: 77% for 2 steps

IR υ_{max}(film): 3048, 2411, 1584, 1518, 1481 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.46 - 7.41 (m, 2H), 7.40 - 7.30 (m, 4H), 6.88 - 6.79 (m, 1H), 6.66 (d, *J* = 16.1 Hz, 1H), 6.20 (d, *J* = 16.1 Hz, 1H), 5.10 (s, 2H), 4.63 (d, *J* = 6.8 Hz, 1H), 4.31 (t, *J* = 4.6 Hz, 1H), 3.95 - 3.87 (m, 2H), 3.80 (dd, *J* = 5.1, 11.5 Hz, 1H), 3.59 (d, *J* = 14.2 Hz, 1H), 2.32 - 2.21 (m, 1H), 1.52 (d, *J* = 7.3 Hz, 3H), 1.45 (s, 9 H), 1.24 (d, *J* = 6.8 Hz, 3H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 175.9, 172.6, 172.0, 171.1, 158.6, 156.5, 137.1, 135.7, 128.1, 127.5, 127.2, 125.3, 124.9, 112.2, 79.5, 73.7, 69.6, 62.0, 57.4, 56.2, 50.2, 27.3, 16.0, 14.0.

***tert*-Butyl ((*S*)-1-(((3*R*,9*R*,10*R*,11*R*)-16-(benzyloxy)-10-hydroxy-3,9-dimethyl-2,5,8,13-tetraoxo-2,3,4,5,6,7,8,9,10,11,12,13-dodecahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamate**



57 (NDS-101360)

To a stirred solution of $PdCl_2$ (10 mol%), CuCl (4 mg, 0.04 mmol) in DMF-water (3 mL, 2:1) compound **56** (34 mg, 0.04 mmol) was added and heated at 65 °C under O2 atmosphere for 8 h. The reaction mixture was diluted with ethyl acetate (10 mL) and washed water (10 mL) and brine (10 mL) organic layer was separated, dried over Na_2SO_4, concentrated under reduced pressure. Purification by column chromatography (silica gel 230-400 mesh 6% methanol - CH_2Cl_2) yielded compound **57**.

Yield: 68%

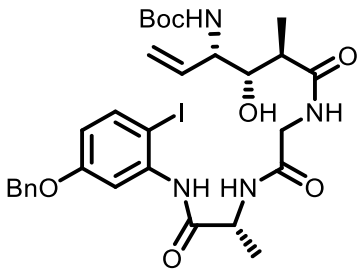
IR υ_{max}(film): 3454, 3015, 2965, 1710, 1541, 1431 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 12.50 (s, 1H), 9.18 (d, *J* = 5.9 Hz, 1H), 8.26 (brs, 1H), 7.95 (d, *J* = 9.3 Hz, 1H), 7.47 - 7.46 (m, 2H), 7.42 - 7.30 (m, 2H), 7.35 (d, *J* = 7.3 Hz, 1H), 7.16 (d, *J* = 6.8 Hz, 1H), 6.92 (d, *J* = 7.8 Hz, 1H), 6.84 (d, *J* = 7.8 Hz, 1H), 6.61 (d, *J* = 6.4 Hz, 1H), 5.54 (d, *J* = 5.4 Hz, 1H), 5.21 (brs, 2H), 4.84 (t, *J* = 5.6 Hz, 1H), 4.60 (dd, *J* = 6.6, 14.9 Hz, 1H), 4.38 (brs, 1H), 4.17 - 4.15 (m, 1H), 3.95 (brs, 1H), 3.59 (d, *J* = 4.9 Hz, 1H), 3.54 - 3.45 (m, 3H), 3.28 (d, *J* = 9.3 Hz, 2H), 2.93 (d, *J* = 17.1 Hz, 1H), 2.32 (brs, 2H), 1.41 - 1.39 (m, 12H), 1.10 (d, *J* = 7.3 Hz, 3H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 201.5, 173.9, 172.6, 170.3, 170.2, 163.6, 142.6, 136.7, 134.1, 129.0, 128.5, 128.3, 116.0, 109.7, 105.8, 78.8, 73.1, 70.0, 61.8, 57.1, 51.4, 47.3, 46.8, 43.1, 42.2, 41.8, 28.6, 17.3, 14.2.

HRMS (ESI): calculated for $C_{33}H_{43}O_{10}N_5Na$ $[M+Na]^+$: 692.2902, found 692.2892.

***tert*-Butyl ((3*S*,4*S*,5*R*)-6-((2-(((*R*)-1-((5-(benzyloxy)-2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-4-hydroxy-5-methyl-6-oxohex-1-en-3-yl)carbamate**



46

Compound **46** was synthesized from dipeptide **02** and acid **06** by following similar procedure for the synthesis of compound **35**.

Yield: 58% for 3 steps

Specific rotation: $[\alpha]_D^{27}$ = - 32.18 (*c* 1.40, $CHCl_3$)

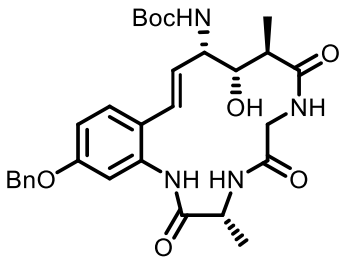
IR υ_{max}(film): 3021, 2931, 1647, 1576 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD): δ 7.76 - 7.65 (m, 1H), 7.46 - 7.26 (m, 7H), 6.69 (dd, *J* = 2.8, 8.7 Hz, 1H), 5.89 (dt, *J* = 5.1, 11.1 Hz, 1H), 5.25 - 5.11 (m, 3H), 5.05 (brs, 2H), 4.61 - 4.50 (m, 1H), 4.25 (brs, 1H), 4.11 - 4.01 (m, 1H), 3.85 (d, *J* = 16.9 Hz, 1H), 3.80 - 3.66 (m, 2H), 3.41 - 3.23 (m, 1H), 2.63 - 2.48 (m, 1H), 1.49 (d, *J* = 7.1 Hz, 3H), 1.44 (s, 9H), 1.14 (d, *J* = 6.8 Hz, 3H)

^{13}C NMR (100 MHz, CD_3OD): δ 178.6, 173.5, 172.0, 161.1, 140.6, 138.5, 138.2, 129.7, 129.2, 128.8, 116.3, 116.2, 114.1, 83.7,80.6, 76.7, 71.4, 55.5, 51.2, 45.3, 43.8, 28.9, 18.1, 14.5

HRMS (ESI): calculated for $C_{30}H_{39}O_7N_4INa$ $[M+Na]^+$: 717.1756, found 717.1743.

***tert*-Butyl ((3*R*,9*R*,10*S*,11*S*,*E*)-16-(benzyloxy)-10-hydroxy-3,9-dimethyl-2,5,8-trioxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)carbamate**



51

Compound **51** was synthesized from dipeptide **46** by following similar procedure for the synthesis of compound **50**.

Yield: 51%

Specific rotation: $[\alpha]_D^{27}$ = - 26.19 (*c* 0.85, $CHCl_3$)

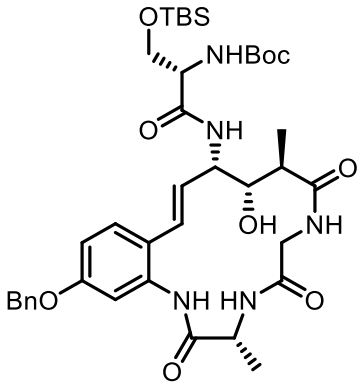
IR υ_{max}(film): 3341, 3035, 2415, 1684, 1535 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD): δ 7.41 (d, *J* = 7.6 Hz, 3H), 7.36 (t, *J* = 7.3 Hz, 3H), 7.30 (d, *J* = 7.3 Hz, 1H), 7.00 (d, *J* = 2.4 Hz, 1H), 6.87 (dd, *J* = 2.3, 8.7 Hz, 1H), 6.63 (d, *J* = 15.7 Hz, 1 H), 5.79 (brs, 1H), 5.06 (s, 2H), 4.37 - 4.230 (m, 2H), 3.88 (d, *J* = 13.9 Hz, 1H), 3.68 (d, *J* = 13.9 Hz, 2H), 2.27 (dd, *J* = 2.6, 7.2 Hz, 1H), 1.54 (d, *J* = 7.6 Hz, 3H), 1.44 (s, 12H), 1.24 (d, *J* = 7.3 Hz, 3H)

^{13}C NMR (100 MHz, CD_3OD): δ 179.9, 174.1, 173.3, 160.0, 158.5, 138.6, 136.3, 131.3, 129.6, 129.1, 128.7, 127.8, 126.1, 115.2, 114.5, 71.2, 71.1, 52.1, 49.8, 46.0, 45.5, 30.9, 29.0, 17.1, 14.6

HRMS (ESI): calculated for $C_{30}H_{38}O_7N_4Na$ $[M+Na]^+$: 589.2633, found 589.2623.

***tert*-Butyl ((*S*)-1-((3*R*,9*R*,10*R*,11*S*,*E*)-16-(benzyloxy)-10-hydroxy-3,9-dimethyl-2,5,8-trioxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)-3-((tert-butyldimethylsilyl)oxy)-1-oxopropan-2-yl)carbamate**



59

Compound **59** was synthesized from **52** and **58** by following similar procedure for the synthesis of compound **56**.

Yield: 62% for 2 steps

Specific rotation: $[\alpha]_D^{27}$ = + 21.37 (*c* 0.56, $CHCl_3$)

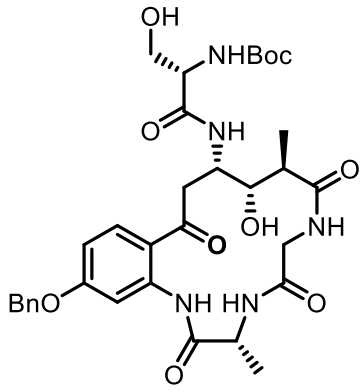
IR υ_{max}(film): 3028, 2414, 1532, 1578, 1484 cm^{-1}

^{1}H NMR (500 MHz, CD_3OD): δ 7.45 - 7.32 (m, 6H), 7.03 (d, *J* = 2.1 Hz, 1H), 6.89 (dd, *J* = 2.4, 8.5 Hz, 1H), 6.67 (d, *J* = 15.6 Hz, 1H), 5.97 (dd, *J* = 10.2, 15.4 Hz, 1H), 5.11 (s, 2H), 4.60 (t, *J* = 9.3 Hz, 1H), 4.38 (q, *J* = 7.5 Hz, 1H), 4.19 (t, *J* = 4.7 Hz, 1H), 3.93 - 3.86 (m, 4H), 3.71 (d, *J* = 14.0 Hz, 1H), 2.34 - 2.31 (m, 1H), 1.57 (d, *J* = 7.3 Hz, 3H), 1.49 (s, 9H), 1.26 (d, *J* = 7.3 Hz, 3H), 0.91 (s, 9H), 0.09 (d, *J* = 4.0 Hz, 6H)

^{13}C NMR (125 MHz, CD_3OD): δ 178.2, 172.5, 171.9, 171.5, 158.6, 156.5, 137.0, 134.8, 130.3, 128.1, 127.5, 127.2, 126.4, 124.5, 124.1, 113.6, 113.0, 79.6, 74.5, 69.7, 63.3, 60.5, 56.8, 50.6, 44.5, 43.9, 27.3, 25.0, 17.8, 15.6, -6.7, -6.8

HRMS (ESI): calculated for $C_{39}H_{57}O_9N_5NaSi$ $[M+Na]^+$: 790.3818, found 790.3822.

***tert*-Butyl((*S*)-1-((3*R*,9*R*,10*R*,11*S*)-16-(benzyloxy)-10-hydroxy-3,9-dimethyl-2,5,8,13-tetraoxo-2,3,4,5,6,7,8,9,10,11,12,13-dodecahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)-3-hydroxy-1-oxopropan-2-yl)carbamate**



60

Compound **60** was synthesized from **59** by following similar procedure for the synthesis of compound **57**.

Yield: 59%

Specific rotation: $[\alpha]_D^{27}$ = + 17.84 (*c* 0.47, $CHCl_3$)

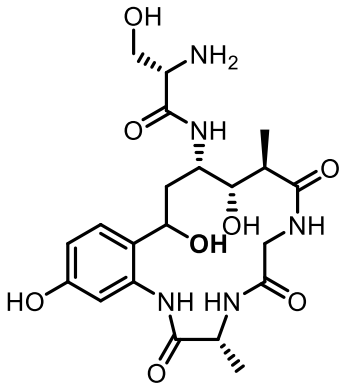
IR υ_{max}(film) 3454, 3015, 2965, 1710, 1541, 1431 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 12.28 (s, 1H), 8.94 - 8.90 (m, 2H), 8.25 (d, *J* = 2.4 Hz, 1H), 7.89 - 7.82 (m, 2H), 7.71 (d, *J* = 8.6 Hz, 1H), 7.48 - 7.39 (m, 10H), 7.26 (d, *J* = 7.6 Hz, 1H), 7.07 - 6.92 (m, 1H), 6.83 - 6.80 (m, 1H), 5.42 - 5.31 (m, 1H), 5.20 (s, 2H), 5.109 - 5.07 (m, 1H), 4.85 (t, *J* = 5.7 Hz, 1H), 4.40 - 4.16 (m, 3H), 3.63 - 3.61 (m, 3H), 3.26 (dd, *J* = 11.4, 18.0 Hz, 1H), 3.05 - 2.86 (m, 1H), 2.62 (dd, *J* = 3.2, 6.8 Hz, 1H), 1.40 (s, 9H), 1.38 (d, *J* = 7.1 Hz, 3H), 0.89 (d, *J* = 6.6 Hz, 3H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 201.4, 172.8, 171.8, 171.2, 169.8, 169.4, 163.3, 155.8, 142.3, 137.4, 136.7, 136.0, 129.0,128.9, 128.5, 128.2, 128.1, 116.6, 109.4, 105.8, 78.7, 71.6, 69.9, 69.8, 62.1, 57.9, 50.7, 45.5, 43.0, 42.5, 28.6, 17.1, 15.9

HRMS (ESI): calculated for $C_{33}H_{43}O_{10}N_5Na$ $[M+Na]^+$: 692.2902, found 692.2878.

(3*R*,9*R*,10*R*,11*S*)-11-(L-seryl)-10,13,16-trihydroxy-3,9-dimethyl-3,4,6,7,10,11,12,13-octahydro-1H-benzo[h][1,4,7]triazacyclopentadecine-2,5,8(9H)-trione



62

To a solution of compound **60** (18 mg, 0.026 mmol) in methanol (5 mL), 10% Pd/C (~ 5 mg) was added and stirred under H_2 atmosphere for 2 h. The reaction mixture was then filtered through silica gel column, concentrated to afford phenolic compound. The phenolic compound was dissolved in CH_2Cl_2 (3 mL), TFA (0.3 mL) was added at 0 °C and the resulting suspension was stirred for 2 h at the same temperature. Concentrated the reaction mixture and azeotroped with diethyl ether (3 mL x 3) and dried under vacuum to afford compound **62**.

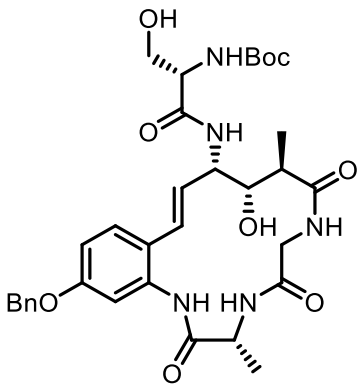
Yield: 73% for 2 steps

IR υ_{max}(film): 3461, 3029, 2965, 1601, 1431 cm^{-1}

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.21 (s, 1H), 8.62 (s, 1H), 8.45 (d, *J* = 9.5 Hz, 1H), 8.13 - 8.10 (m, 3H), 8.04 (d, *J* = 7.9 Hz, 1H), 7.70 (d, *J* = 2.4 Hz, 1H), 7.02 (d, *J* = 8.5 Hz, 1H), 6.48 (dd, *J* = 2.4, 8.5 Hz, 1H), 4.69 - 4.64 (m, 2H), 4.21 - 4.19 (m, 1H), 4.02 (d, *J* = 7.0 Hz, 1H), 3.87 - 3.80 (m, 4H), 3.74 (dd, *J* = 4.0, 11.0 Hz, 2H), 2.82 (dd, *J* = 5.3, 7.8 Hz, 1H), 2.47 - 2.45 (m, 1H), 2.03 - 12.01(m, 1H), 1.37 (d, *J* = 7.0 Hz, 3H), 0.96 (d, *J* = 6.7 Hz, 3H)

^{13}C NMR (125 MHz, DMSO-d_6): δ 175.3, 170.8, 170.5, 166.8, 157.7, 139.1, 118.8, 110.2, 108.8, 84.5, 76.4, 61.0, 54.7, 50.0, 48.5, 44.7, 41.1, 37.6, 16.8, 13.0.

***tert*-Butyl ((*S*)-1-((3*R*,9*R*,10*R*,11*S*,*E*)-16-(benzyloxy)-10-hydroxy-3,9-dimethyl-2,5,8-trioxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)-3-hydroxy-1-oxopropan-2-yl)carbamate**



63

Compound **63** was synthesized from macrocycle **51** and acid **55** by following similar procedure for the synthesis of compound **56**.

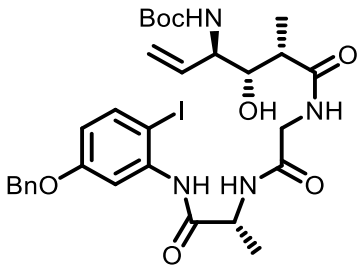
Yield: 65% for 2 steps

IR υ_{max}(film): 3021, 2407, 1527, 1578, 1470 cm^{-1}

^{1}H NMR (500 MHz, CD_3OD): δ 7.44 (d, *J* = 8.0 Hz, 3H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.34 - 7.27 (m, 1H), 7.05 - 7.00 (m, 1H), 6.89 (dd, *J* = 2.7, 8.8 Hz, 1H), 6.68 (d, *J* = 15.6 Hz, 1H), 5.94 (dd, *J* = 10.1, 15.4 Hz, 1H), 5.13 - 5.07 (m, 2H), 4.65 (t, *J* = 9.2 Hz, 1H), 4.39(q, *J* = 7.2 Hz, 1H), 4.17 (brs, 1H), 3.91 (d, *J* = 13.7 Hz, 1H), 3.84 (d, *J* = 7.6 Hz, 1H), 3.79 (d, *J* = 4.6 Hz, 2H), 3.76 - 3.69 (m, 1H), 2.36 - 2.33 (m, 1H), 1.56 (d, *J* = 7.6 Hz, 3H), 1.47 (s, 9H), 1.27 (d, *J* = 7.2 Hz, 3H)

^{13}C NMR (125 MHz, CD_3OD): δ 178.2, 172.5, 171.9, 171.7, 158.6, 156.6, 137.1, 134.8, 130.3, 128.1, 127.5, 127.2, 126.4, 124.5,124.2, 115.2, 113.7, 113.0, 79.6, 74.5, 69.7, 62.0, 60.2, 56.9, 50.6, 44.4, 43.9, 27.3, 15.5, 15.4.

***tert*-Butyl ((3*R*,4*S*,5*S*)-6-((2-(((*R*)-1-((5-(benzyloxy)-2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-4-hydroxy-5-methyl-6-oxohex-1-en-3-yl)carbamate**



47

Compound **47** was synthesized from dipeptide **02** and acid **07** by following similar procedure for the synthesis of compound **45**.

Yield: 45% for 3 steps

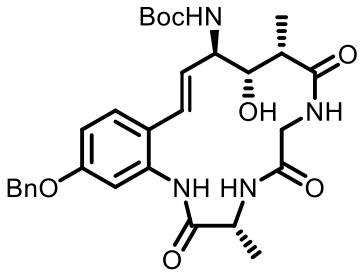
IR υ_{max}(film): 3021, 2931, 1647, 1576 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD): δ 7.71 (d, *J* = 8.8 Hz, 1H), 7.43 - 7.39 (m, 2H), 7.37 (t, *J* = 7.1 Hz, 3H), 7.34 - 7.29 (m, 1H), 6.71 - 6.68 (m, 1H), 5.94 (ddd, *J* = 7.3, 10.1, 17.3 Hz, 1H), 5.28 - 5.20 (m, 2H), 5.06 (s, 2H), 4.57 (q, *J* = 7.0 Hz, 1H), 4.17 - 4.10 (m, 1H), 4.09 - 4.04 (m, 1H), 3.87 - 3.83 (m, 1H), 3.73 - 3.71 (m, 1H), 2.54 (d, *J* = 7.3 Hz, 1H) 1.51 (d, *J* = 7.1 Hz, 3H), 1.44 (s, 9H), 1.17 (d, *J* = 6.6 Hz, 3H)

^{13}C NMR (100 MHz, CD_3OD): δ 175.5, 170.5, 169.0, 158.1, 137.6, 137.6, 135.2, 132.3, 126.9, 126.6, 126.1, 125.8, 115.0, 113.2, 111.0, 77.5, 74.1, 68.3, 53.8, 48.2, 41.7, 40.9, 25.9, 14.9, 11.5

HRMS (ESI): calculated for $C_{30}H_{39}O_7N_4INa$ $[M+Na]^+$: 717.1756, found 717.1716.

***tert*-Butyl ((3*R*,9*S*,10*S*,11*R*,*E*)-16-(benzyloxy)-10-hydroxy-3,9-dimethyl-2,5,8-trioxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)carbamate**



52

Compound **52** was synthesized from **47** by following similar procedure for the synthesis of compound **50**.

Yield: 49%

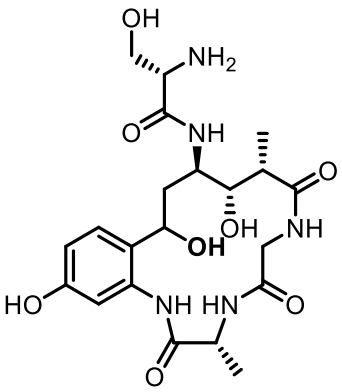
IR υ_{max}(film): 3384, 3021, 2401, 1657, 1523, 1423 cm^{-1}

^{1}H NMR (500 MHz, CD_3OD): δ 7.42 (d, *J* = 7.3 Hz, 3H), 7.36 (t, *J* = 8.1 Hz, 4H), 7.34 - 7.32 (m, 1H), 7.29 - 7.23 (m, 1H), 6.84 (dd, *J* = 2.4, 8.5 Hz, 1H), 6.56 (d, *J* = 15.6 Hz, 1H), 5.94 (dd, *J* = 5.3, 15.7 Hz, 1H), 5.07 (s, 2H), 4.63 - 4.62 (m, 1H), 4.48 (d, *J* = 7.0 Hz, 1H), 3.96 (d, *J* = 14.0 Hz, 1H), 3.85 - 3.70 (m, 2H), 2.49 - 2.46 (m, 1H), 1.50 (d, *J* = 7.0 Hz, 3H), 1.45 (s, 9H), 1.26 (d, *J* = 7.3 Hz, 3H)

^{13}C NMR (125 MHz, CD_3OD): δ 177.4, 171.9, 171.7, 158.5, 137.1, 135.1, 128.1, 127.5, 127.2, 125.9, 124.5, 112.9, 111.4, 78.8, 73.6, 69.7, 56.5, 50.3, 43.3, 42.8, 27.4, 15.3, 14.7

HRMS (ESI): calculated for $C_{30}H_{38}O_8N_4Na$ $[M+Na]^+$: 589.2633, found 589.2620.

(2*S*)-2-amino-3-hydroxy-N-((3*R*,9*S*,10*S*,11*R*)-10,13,16-trihydroxy-3,9-dimethyl-2,5,8-trioxo-2,3,4,5,6,7,8,9,10,11,12,13-dodecahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)propanamide



64a

Compound **64a** was synthesized from macrocycle **52** by following similar procedure for the synthesis of compound **62**.

Yield: 30% for 5 steps

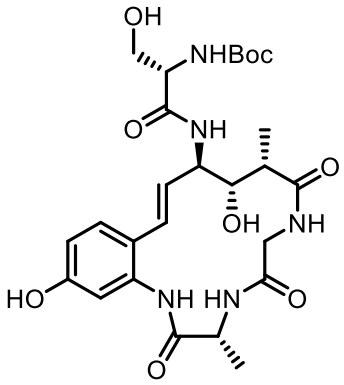
IR υ_{max}(film): 3510, 3024, 2967, 1541, 1484 cm^{-1}

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.61 (brs, 1H), 9.10 (s, 1H), 8.67 (d, *J* = 7.6 Hz, 1H), 8.28 - 8.21 (m, 1H), 8.20 (d, *J* = 8.5 Hz, 1H), 8.12 - 8.10 (m, 4H), 7.53 (d, *J* = 2.1 Hz, 1H), 7.04 - 7.01 (m, 1H), 6.50 (dd, *J* = 2.4, 8.5 Hz, 1H), 5.19 (t, *J* = 7.2 Hz, 1H), 4.48 - 4.36 (m, 1H), 4.35 (td, *J* = 7.3, 14.6 Hz, 1H), 3.97 - 3.92 (m, 3H), 3.82 (dd, *J* = 6.7, 10.1 Hz, 3H), 2.60 (dd, *J* = 6.6, 10.5 Hz, 2H), 2.30 - 2.28 (m, 1H), 1.32 (d, *J* = 7.3 Hz, 3H), 0.99 (d, *J* = 6.7 Hz, 3H)

^{13}C NMR (125 MHz, DMSO-d_6): δ 174.6, 170.9, 166.7, 157.6, 138.4, 127.4, 121.0, 110.7, 109.9, 83.4, 75.5, 60.7, 54.7, 53.8, 49.3, 45.3, 36.4, 31.2, 18.4, 13.9

***tert*-Butyl ((*S*)-1-(((3*R*,9*S*,10*S*,11*R*,*E*)-10,16-dihydroxy-3,9-dimethyl-2,5,8-trioxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamate**

To a solution of compound **52** (45 mg, 0.079 mmol) in CH_2Cl_2 (5mL) trifluoro acetic acid (1.0 mL) was added at 0 °C and the resulting suspension was stirred for 2 h at the same temperature. Reaction was monitored by TLC, and then concentrated. This residue was dissolved in dry DMF (3 mL), then HATU (60 mg, 0.16 mmol), DIPEA (41µL, 0.23mmol) and N-(tert-butoxycarbonyl)-O-(tert-butyldimethylsilyl)-L-serine **55** (28 mg,0.087 mmol)



65

was added. The resulting solution was stirred at ambient temperature for16h. Reaction mass was diluted with ethylacetate (15 mL), washed with saturated solution of $NaHCO_3$ (5 mL), H_2O (5 mL). The organic layer was dried over Na_2SO_4 and the crude material

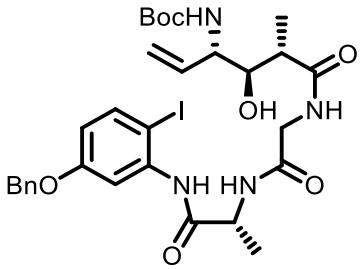
obtained after removal of the solvent was forwarded for next step without further purification for benzyl deprotection. Thus Li-Napthalenide (3 mL, 0.17 M in THF) was added to a stirred solution of crude serine coupled product at – 40 °C and stirred for 3 h at same temperature reaction was quenched with saturated NH_4Cl and reaction mass was diluted with ethylacetate (10 mL),washed with H_2O (5 mL) brine (5 mL). The organic layer was dried over Na_2SO_4 and the crude material obtained after removal of the solvent was purified by column chromatography (silica gel 230-400 mesh 7% methanol - CH_2Cl_2) to afford **65** (25 mg, 55% for 3 steps) as off white solid.

IR υ_{max}(film): 3445, 3018, 2967, 1534, 1422 cm^{-1}

^{1}H NMR (500 MHz, CD_3OD): δ 7.29 (d, *J* = 8.4 Hz, 1H), 7.01 (brs, 1H), 6.69 - 6.62 (m, 2H), 5.99 - 5.95 (m, 1H), 5.18 (brs, 1H), 4.78 - 4.49 (m, 2H), 4.41 (t, *J* = 5.5 Hz, 1 H), 4.04 (dd, *J* = 2.7, 6.5 Hz, 1H), 3.85 - 3.80 (m, 3H), 3.76 (td, *J* = 5.2, 10.6 Hz, 1H), 2.46 (dd, *J* = 2.5, 7.1 Hz, 1H), 1.55 (d, *J* = 7.2 Hz, 3H), 1.46 (s, 9H), 1.26 (d, *J* = 7.2 Hz, 3H)

^{13}C NMR (125 MHz, CD_3OD): δ 177.8, 172.9, 172.8, 171.8, 157.2, 156.5, 127.0, 125.3, 125.1, 123.3, 113.4, 79.4, 73.2, 61.8, 57.1, 53.7, 50.6, 27.3, 15.2, 14.8

***tert*-Butyl ((3*S*,4*R*,5*S*)-6-((2-(((*R*)-1-((5-(benzyloxy)-2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-4-hydroxy-5-methyl-6-oxohex-1-en-3-yl)carbamate**



48

Compound **48** was synthesized from dipeptide **02** and acid **08** by following similar procedure for the synthesis of compound **45**.

Yield: 48% for 3 steps

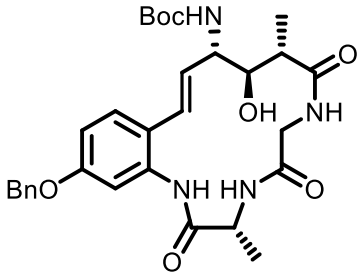
IR υ_{max}(film): 3011, 2978, 1654, 1568 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD): δ 7.70 (d, *J* = 8.8 Hz, 1H), 7.42 (d, *J* = 7.3 Hz, 3H), 7.36 (t, *J* = 7.3 Hz, 2H), 7.34 - 7.28 (m, 1H), 6.68 (dd, *J* = 2.4, 8.8 Hz, 1H), 5.97 - 5.93 (m, 1H), 5.28 - 5.20 (m, 2H), 5.06 (s, 2H), 4.57 - 4.53 (m, 1H), 4.16 (brs, 1H), 4.07 (d, *J* = 17.1 Hz, 1 H), 3.82 (d, *J* = 17.1 Hz, 1 H), 3.67 (brs, 1H), 2.55 - 2.52 (m, 1H), 1.49 (d, *J* = 7.3 Hz, 3H), 1.43 (s, 9H), 1.17 (d, *J* = 6.8 Hz, 3H)

^{13}C NMR (100 MHz, CD_3OD): δ 176.9, 171.9, 170.5, 159.6, 156.2, 139.1, 139.1, 136.7, 133.9, 128.2, 127.6, 127.3, 116.5, 114.5, 112.2, 81.8, 79.1, 75.6, 69.8, 55.4, 49.7, 43.1, 42.3, 27.4, 16.4, 13.1

HRMS (ESI): calculated for $C_{30}H_{39}O_7N_4INa$ $[M+Na]^+$: 717.1756, found 717.1718.

***tert*-Butyl ((3*R*,9*S*,10*R*,11*S*,*E*)-16-(benzyloxy)-10-hydroxy-3,9-dimethyl-2,5,8-trioxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)carbamate**



53

Compound **53** was synthesized from **48** by following similar procedure for the synthesis of compound **50**.

Yield: 50%

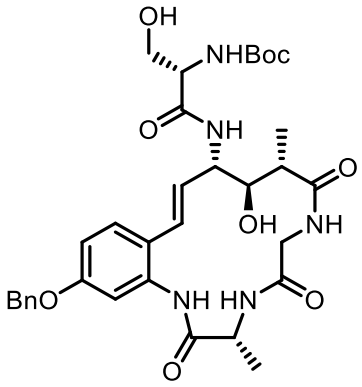
IR υ_{max}(film): 3374, 3051, 2438, 1627, 1520, 1422 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD): δ 7.44 - 7.42 (m, 2H), 7.37 (t, *J* = 7.3 Hz, 2H), 7.35 - 7.29 (m, 2H), 7.17 - 7.16 (m, 1H), 6.88 - 6.85 (m, 1H), 6.54 (d, *J* = 16.1 Hz, 1H), 5.92 (dd, *J* = 8.8, 15.7 Hz, 1H), 5.08 (s, 2H), 4.53 (q, *J* = 7.0 Hz, 1H), 4.26 (dd, *J* = 4.4, 8.8 Hz, 1H), 3.88 (d, *J* = 8.8 Hz, 2H), 3.75 - 3.74 (m, 1H), 2.74 (d, *J* = 6.8 Hz, 1H), 1.47 (s, 12H), 1.25 (d, 7.1 Hz, 3H)

^{13}C NMR (100 MHz, CD_3OD): δ 175.9, 171.9, 170.9, 158.3, 156.1, 137.0, 134.7, 130.3, 128.6, 128.1, 127.5, 127.2, 126.7, 124.7, 112.9, 111.7, 78.9, 77.0, 69.7, 58.8, 49.9, 44.3, 42.8, 27.4, 16.1, 15.3

HRMS (ESI): calculated for $C_{30}H_{38}O_7N_4Na$ $[M+Na]^+$: 589.2633, found 589.2618.

***tert*-Butyl ((*S*)-1-(((3*R*,9*S*,10*R*,11*S*,*E*)-16-(benzyloxy)-10-hydroxy-3,9-dimethyl-2,5,8-trioxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamate**



66

Compound **66** was synthesized from macrocycle **53** and serine acid **55** by following similar procedure for the synthesis of compound **56**.

Yield: 73% for 2 steps

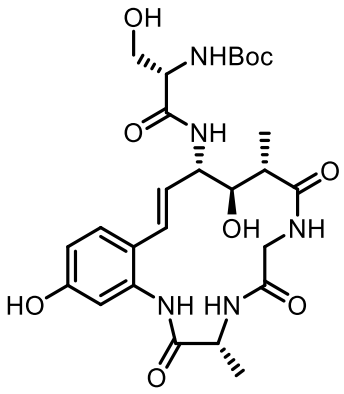
IR υ_{max}(film): 3058, 2438, 1521, 1487 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD): δ 7.43 - 7.41 (m, 2H), 7.38 - 7.36 (m, 2H), 7.32 - 7.30 (m, 2H), 7.12 (brs, 1H), 6.86 (dd, *J* = 2.4, 8.3 Hz, 1H), 6.57 (d, *J* = 16.1 Hz, 1H), 5.98 (dd, *J* = 8.8, 15.7 Hz, 1H), 5.07 (s, 2H), 4.61 (dd, *J* = 5.1, 8.1 Hz, 1H), 4.50 - 4.47 (m, 1H), 4.14 (brs, 1H), 3.94 - 3.84 (m, 2H), 3.75 (brs, 3H), 2.73 (d, *J* = 5.9 Hz, 1H), 1.46 - 1.44 (m, 12H), 1.31 (d, *J* = 6.8 Hz, 3H)

^{13}C NMR (100 MHz, CD_3OD): δ 178.5, 174.4, 173.4, 160.9, 139.4, 137.3, 133.2, 130.7, 130.5, 130.0, 129.6, 127.9, 127.1, 115.5, 114.2, 81.9, 78.5, 72.1, 64.4, 60.2, 59.1, 52.5, 46.5, 45.2, 29.7, 18.4, 17.6

HRMS (ESI): calculated for $C_{33}H_{43}O_9N_5Na$ $[M+Na]^+$: 676.2953, found 676.2919.

***tert*-Butyl ((*S*)-1-(((3*R*,9*S*,10*R*,11*S*,*E*)-10,16-dihydroxy-3,9-dimethyl-2,5,8-trioxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamate**



67

Compound **67** was synthesized from **66** by following similar procedure for the synthesis of compound **65**.

Yield: 53%

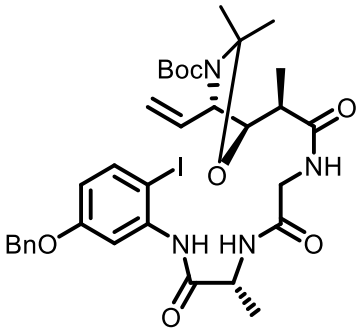
IR υ_{max}(film): 3421, 3041, 2978, 1534, 1434 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD): δ 7.25 (d, *J* = 8.3 Hz, 1H), 6.87 (brs, 1H), 6.69 - 6.60 (m, 1H), 6.55 (d, *J* = 16.1 Hz, 1H), 5.93 (dd, *J* = 7.6, 15.4 Hz, 1H), 4.62 (brs, 1H), 4.47 (d, *J* = 6.8 Hz, 1H), 4.13 (brs, 1H), 3.96 (d, *J* = 16.1 Hz, 1H), 3.84 (d, *J* = 16.1 Hz, 1H), 3.75 - 3.72 (m, 3H), 2.71 (brs, 1H), 1.44 (brs, 12H), 1.30 (d, *J* = 7.3 Hz, 3H)

^{13}C NMR (100 MHz, CD_3OD): δ 172.0, 171.1, 171.1, 171.0, 157.2, 156.4, 134.8, 131.2, 128.2, 124.4, 123.4, 113.5, 112.2, 79.5, 75.9, 62.0, 57.7, 56.7, 50.1, 44.0, 42.7, 27.3, 16.0, 15.1

HRMS (ESI): calculated for $C_{26}H_{38}O_9N_5$ $[M+H]^+$: 564.2664, found 564.2651.

***tert*-Butyl (5*R*)-5-((*R*)-1-((2-(((*R*)-1-((5-(benzyloxy)-2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-1-oxopropan-2-yl)-2,2-dimethyl-4-vinyloxazolidine-3-carboxylate**



49a

Compound **49a** was synthesized from dipeptide **02** and acid **09** by following similar procedure for the synthesis of compound **45**.

Yield: 73% over 2 steps

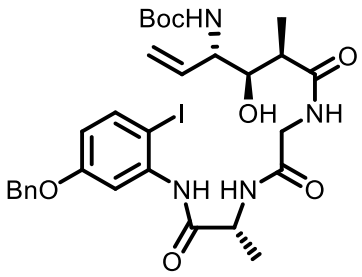
IR υ_{max}(film): 3405, 3047, 2919, 1561, 1449 cm^{-1}

^{1}H NMR (500 MHz, CD_3OD): δ 7.70 (d, *J* = 8.8 Hz, 1H), 7.44 - 7.41 (m, 3H), 7.36 (t, *J* = 7.4 Hz, 2H), 7.32 - 7.29 (m, 1H), 6.69 (d, *J* = 8.8 Hz, 1H), 5.78 - 5.74 (m, 1H), 5.35 - 5.23 (m, 2H), 5.06 (s, 2H), 4.60 - 4.56 (m, 1 H), 4.20 - 4.17 (m, 2H), 3.97 - 3.90 (m, 2H), 2.57 - 2.52 (m, 1H), 1.58 (d, *J* = 5.7 Hz, 3H), 1.50 (d, *J* = 7.2 Hz, 5H), 1.46 (s, 3H), 1.42 (s,6H), 1.36 (s, 1H), 1.30 (brs, 2H), 1.08 (d, *J* = 6.9 Hz, 2H)

^{13}C NMR (125 MHz, CD_3OD): δ 175.7, 171.7, 170.2, 170.0, 159.6, 151.9, 139.2, 139.1, 136.8, 132.5, 131.8, 128.2, 127.6, 127.3, 118.6, 118.3, 114.6, 112.3, 93.4, 93.2, 82.0, 81.9, 80.5, 79.7, 77.9, 77.9, 69.9, 62.0, 61.6, 49.7, 42.3, 42.0, 40.8, 40.2, 27.3, 26.2, 26.1, 22.7, 22.6, 16.7, 16.5, 12.3, 12.2

HRMS (ESI): calculated for $C_{33}H_{43}O_7N_4INa$ $[M+Na]^+$: 757.2069, found 757.2031.

***tert*-Butyl ((3*S*,4*R*,5*R*)-6-((2-(((*R*)-1-((5-(benzyloxy)-2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-4-hydroxy-5-methyl-6-oxohex-1-en-3-yl)carbamate**



49

Compound **49** was synthesized from **49** by following similar procedure for the synthesis of compound **45**.

Yield: 85 % BRSM

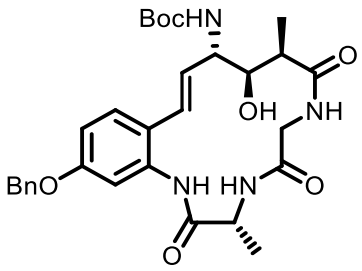
IR υ_{max}(film): 3021, 2931, 1658, 1579 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD): δ 7.70 (d, *J* = 8.8 Hz, 1H), 7.42 (d, *J* = 7.3 Hz, 3H), 7.36 (t, *J* = 7.3 Hz, 2H), 7.33 - 7.30 (m, 1H), 6.69 (dd, *J* = 2.9, 8.8 Hz, 1 H), 5.97 - 5.90 (m, 1H), 5.28 - 5.20 (m, 2H), 5.06 (s, 2H), 4.56 (q, *J* = 7.3 Hz, 1 H), 4.16 - 4.09 (m, 1H), 4.07 - 4.04 (m, 1H), 3.83 (d, *J* = 17.1 Hz, 1H), 3.67 (brs, 1 H), 3.20 - 3.12 (m, 1H), 2.53 (t, *J* = 7.2 Hz, 1H), 1.49 (d, *J* = 7.1 Hz, 3H), 1.43 (s, 9H), 1.17 (d, *J* = 6.8 Hz, 3H)

^{13}C NMR (100 MHz, CD_3OD): δ 175.4, 170.4, 169.0, 158.1, 137.6, 137.6, 135.2, 132.3, 126.6, 126.1, 125.7, 115.0, 113.0, 110.6, 80.3, 77.6, 74.1, 68.3, 53.9, 48.2, 41.6, 40.8, 25.9, 14.8, 11.6

HRMS (ESI): calculated for $C_{30}H_{39}O_7N_4INa$ $[M+Na]^+$: 717.1756, found 717.1720.

***tert*-Butyl ((3*R*,9*R*,10*R*,11*S*,*E*)-16-(benzyloxy)-10-hydroxy-3,9-dimethyl-2,5,8-trioxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)carbamate**



54

Compound **54** was synthesized from **49** by following similar procedure for the synthesis of compound **50**.

Yield: 46%

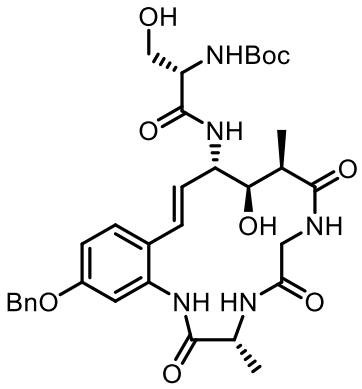
IR υ_{max}(film): 3380, 3011, 2424, 1675, 1523 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD): δ 7.45 - 7.40 (m, 3H), 7.38 (t, *J* = 7.3 Hz, 3H), 7.32 (d, *J* = 8.3 Hz, 2H), 7.18 (brs, 1H), 6.89 (d, *J* = 6.8 Hz, 1H), 6.55 (d, *J* = 16.1 Hz, 1H), 5.92 (dd, *J* = 8.8, 15.7 Hz, 1H), 5.07 (m, 2H), 4.54 (d, *J* = 7.3 Hz, 1H), 4.25 (dd, *J* = 4.4, 8.8 Hz, 1H), 3.89 (d, *J* = 9.3 Hz, 1H), 3.75 (brs, 1H), 2.74 (d, *J* = 5.9 Hz, 1H), 1.47 - 1.44 (m, 12H), 1.35 (d, *J* = 7.3 Hz, 3H)

^{13}C NMR (100 MHz, CD_3OD): δ 174.4, 170.4, 169.4, 156.8, 154.6, 135.5, 133.2, 128.7, 127.1, 126.6, 126.0, 125.7, 125.1, 123.2, 111.4, 110.1, 75.5, 68.2, 48.4, 46.7, 42.8, 41.3, 25.9, 14.5, 13.8

HRMS (ESI): calculated for $C_{30}H_{38}O_7N_4Na$ $[M+Na]^+$: 589.2633, found 589.2618.

***tert*-Butyl ((*S*)-1-(((3*R*,9*R*,10*R*,11*S*,*E*)-16-(benzyloxy)-10-hydroxy-3,9-dimethyl-2,5,8-trioxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-11-yl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamate**



68

Compound **68** was synthesized from macrocycle **54** and serine acid **55** by following similar procedure for the synthesis of compound **56**.

Yield: 85% over two steps

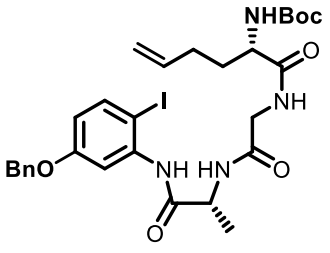
IR υ_{max}(film): 3033, 2419, 1519, 1584, 1471 cm^{-1}

^{1}H NMR (400 MHz, CD_3OD): δ 7.45 - 7.43 (m, 2H), 7.38 (t, *J* = 7.3 Hz, 2H), 7.34 - 7.31 (m, 2H), 7.19 - 7.17 (m, 1H), 6.88 (dd, *J* = 2.0, 8.3 Hz, 1H), 6.59 (d, *J* = 16.1 Hz, 1H), 6.03 (d, *J* = 8.8 Hz, 1H), 5.09 (s, 2H), 4.61 (dd, *J* = 4.4, 8.3 Hz, 1H), 4.52 (d, *J* = 7.3 Hz, 1H), 4.15 - 4.11 (m, 1H), 3.90 (s, 2H), 3.77 (d, *J* = 5.4 Hz, 3 H), 2.78 - 2.73 (m, 1H), 1.51 - 1.42 (m, 12H), 1.33 (d, *J* = 7.3 Hz, 3H)

^{13}C NMR (100 MHz, CD_3OD): δ 176.0, 171.9, 171.1, 170.1, 158.4, 137.0, 134.8, 130.7, 128.5, 128.1, 127.5, 127.2, 125.6, 124.6, 113.0, 79.5, 76.3, 69.7, 61.9, 57.9, 56.6, 50.0, 44.2, 42.8, 27.3, 16.0, 13.1.

HRMS (ESI): calculated for $C_{33}H_{43}O_9N_5Na$ $[M+Na]^+$: 676.2953, found 676.2922.

***tert*-Butyl ((S)-1-((2-(((R)-1-((5-(benzyloxy)-2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-1-oxohex-5-en-2-yl)carbamate**



69

Compound **69** was synthesized from dipeptide **02** and acid **11** by following similar procedure for the synthesis of compound **45**.

Yield: 78% yield for 2 steps

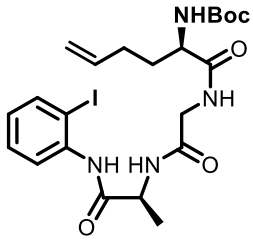
IR υ_{max}(film): 3035, 2988, 1551, 1339 cm^{-1}

^{1}H NMR (200 MHz, DMSO-d_6): δ 9.26 (d, *J* = 4.9 Hz, 1H), 8.27 (d, *J* = 7.3 Hz, 1H), 8.07 (t, *J* = 5.2 Hz, 1H), 7.72 (d, *J* = 8.5 Hz, 1H), 7.46 - 7.30 (m, 6H), 6.89 (d, *J* = 7.3 Hz, 1H), 6.75 - 6.69 (m, 1H), 5.77 - 5.63 (m, 1H), 5.08 (s, 2H), 5.02 - 4.97 (m, 2H), 4.48 (t, *J*= 7.3 Hz, 1H), 3.90 (brs, 1H), 3.79 (brs, 2H), 2.15 (t, *J* = 7.3 Hz, 2H), 1.73 - 1.52 (m, 2H), 1.37 (s, 12H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 172.7, 171.4, 169.5, 159.2, 155.5, 140.0, 139.8, 139.4, 137.0, 128.8, 128.3, 128.1, 114.5, 113.0, 112.9, 78.1, 69.8, 52.6, 49.3, 42.4, 32.3, 30.6, 28.6, 18.2

HRMS (ESI): calculated for $C_{29}H_{37}O_6N_4NaI$ $[M+Na]^+$: 687.1650, found 687.1620.

***tert*-Butyl ((R)-1-((2-(((S)-1-((2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-1-oxohex-5-en-2-yl)carbamate**



70

Compound **70** was synthesized from dipeptide **03** and acid **10** by following similar procedure for the synthesis of compound **45**.

Yield: 71% yield for 2 steps

IR υ_{max}(film): 3115, 2960, 1633, 1248 cm^{-1}

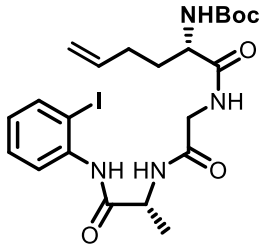
^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 9.55 (brs, 1H), 8.23 (d, *J* = 7.2 Hz, 1H), 8.06 (t, *J* = 5.5 Hz, 1H), 7.86 (d, *J* = 7.6 Hz, 1H), 7.50 (t, *J* = 7.6 Hz, 1H), 7.38 (t, *J* = 7.4 Hz, 1H), 6.97 (t, *J* = 7.4 Hz, 1H), 6.87 (d, *J* = 7.6 Hz, 1H), 5.79 - 5.64 (m, 1 H), 5.08 - 4.99 (m, 2H), 4.48

(t, *J* = 7.1 Hz, 1H), 3.89 (brs, 1H), 3.82 - 3.67 (m, 2H), 2.15 (t, *J* = 7.6 Hz, 2H), 1.68 - 1.59 (m, 2H), 1.37 (s, 12H)

^{13}C NMR (125 MHz, DMSO-d_6): δ 172.8, 171.5, 169.6, 155.6, 139.9, 139.4, 129.2, 127.9, 126.6, 114.5, 96.0, 78.1, 60.1, 52.8, 49.3, 42.5, 32.4, 30.7, 28.7, 18.4

HRMS (ESI): calculated for $C_{22}H_{31}O_5N_4NaI$ $[M+Na]^+$: 581.1231, found 581.1223.

***tert*-Butyl ((S)-1-((2-(((R)-1-((2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-1-oxohex-5-en-2-yl)carbamate**



71

Compound **71** was synthesized from dipeptide **04** and acid **11** by following similar procedure for the synthesis of compound **45**.

Yield: 74% yield for 2 steps

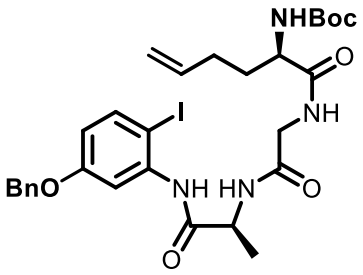
IR υ_{max}(film): 3451, 3017, 2988,1534, 1035 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.33 (brs, 1H), 8.22 (d, *J* = 7.3 Hz, 1H), 8.06 (t, *J* = 5.5 Hz, 1H), 7.85 (d, *J* = 7.9 Hz, 1H), 7.53 - 7.50 (m, 1H), 7.37 (t, *J* = 7.3 Hz, 1H), 6.96 (t, *J* = 7.3 Hz, 1H), 6.87 (d, *J* = 7.9 Hz, 1H), 5.70 (brs, 1H), 5.08 - 4.93 (m, 2H), 4.47 (t, *J* = 7.0 Hz, 1H), 3.88 (brs, 1 H), 3.87 - 3.67 (m, 2H), 2.18 - 2.04 (m, 2H), 1.66 - 1.58 (m, 2H), 1.36 (brs, 12H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 172.7, 171.5, 169.5, 155.5, 139.9, 139.3, 129.2, 127.9, 126.8, 126.7, 114.5, 96.1, 78.0, 52.7, 49.2, 42.4, 32.3, 30.7, 28.7, 18.4

HRMS (ESI): calculated for $C_{22}H_{31}O_5N_4NaI$ $[M+Na]^+$: 581.1231, found 581.1220.

***tert*-Butyl ((R)-1-((2-(((S)-1-((5-(benzyloxy)-2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-1-oxohex-5-en-2-yl)carbamate**



72

Compound **72** was synthesized from dipeptide **01** and acid **10** by following similar procedure for the synthesis of compound **45**.

Yield: 71% yield for 2 steps

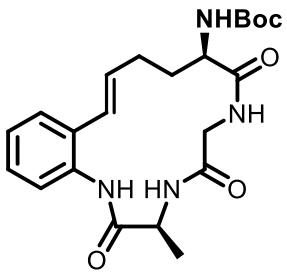
IR υ_{max}(film): 3041, 2986, 1551, 1341 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.23 (d, *J* = 4.9 Hz, 1H), 8.27 (d, *J* = 7.3 Hz, 1H), 8.06 (t, *J* = 5.2 Hz, 1H), 7.72 (d, *J* = 8.5 Hz, 1H), 7.45 - 7.39 (m, 6H), 6.89 (d, *J* = 7.3 Hz, 1H), 6.73 - 6.71 (m, 1H), 5.74 - 5.70 (m, 1H), 5.08 (s, 2H), 5.04–5.01 (m, 2H), 4.48 (t, *J*= 7.3 Hz, 1H), 3.90 (brs, 1H), 3.79 (brs, 2H), 2.16 (t, *J* = 7.3 Hz, 2H), 1.69 - 1.59 (m, 2H), 1.38 (s, 12H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 172.8, 171.5, 169.6, 159.2, 155.5, 140.1, 139.9, 139.5, 137.1, 128.9, 128.4, 128.1, 114.5, 113.0, 112.9, 78.1, 69.9, 52.7, 49.3, 42.5, 32.3, 30.7, 28.7, 18.3

HRMS (ESI): calculated for $C_{29}H_{37}O_6N_4NaI$ $[M+Na]^+$: 687.1650, found 687.1628.

***tert*-Butyl ((3S,9R,E)-3-methyl-2,5,8-trioxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-9-yl)carbamate**



74

Compound **74** was synthesized from **70** by following similar procedure for the synthesis of compound **50**.

Yield: 49%

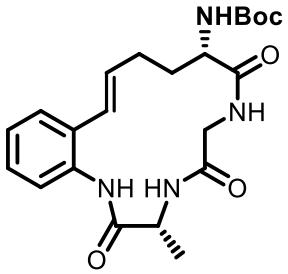
IR υ_{max}(film): 3405, 2867, 1534, 1422 cm^{-1}

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.93 (s, 1H), 8.64 (d, *J* = 6.1 Hz, 1H), 8.35 (d, *J* = 6.5 Hz, 1H), 7.48 (d, *J* = 7.6 Hz, 1H), 7.41 (d, *J* = 7.6 Hz, 1H), 7.27 (t, *J* = 7.2 Hz, 1H), 7.21 (t, *J* = 7.4 Hz, 1H), 6.93 (brs, 1 H), 6.44 (d, *J* = 15.6 Hz, 1H), 5.80 (dd, 9.8, 15.3 Hz, 1H), 4.25 (t, *J* = 6.9 Hz, 1H), 4.14 - 4.05 (m, 1H), 3.97 (dd, *J* = 8.8, 13.4 Hz, 1H), 3.53 (d, *J* = 11.8 Hz, 1H), 2.28 - 2.21 (m, 1H), 2.04 (d, *J* = 10.3 Hz, 2H), 1.59 - 1.56 (m, 1H), 1.38 (m, 12H)

^{13}C NMR (125 MHz, DMSO-d_6): δ 172.1, 171.2, 170.4, 154.9, 134.8, 132.0, 131.7, 127.8, 127.5, 127.0, 126.3, 126.0, 78.0, 50.3, 43.4, 32.3, 29.8, 28.7, 17.3

HRMS (ESI): calculated for $C_{22}H_{30}O_5N_4Na$ $[M+Na]^+$: 453.2108, found 453.2097.

***tert*-Butyl ((3R,9S,E)-3-methyl-2,5,8-trioxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[h][1,4,7]triazacyclopentadecin-9-yl)carbamate**



75

Compound **75** was synthesized from **71** by following similar procedure for the synthesis of compound **50**.

Yield: 43%

IR υ_{max}(film): 3438, 3018, 2986, 1564, 1422 cm^{-1}

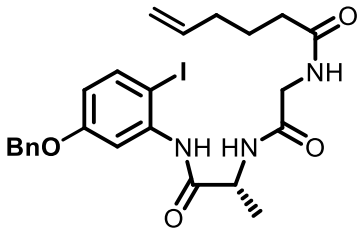
^{1}H NMR (400 MHz, DMSO-d_6): δ 8.92 (s, 1H), 8.64 (d, *J* = 6.1 Hz, 1H), 8.34 (d, *J* = 6.1 Hz, 1H), 7.47 (d, *J* = 7.9 Hz, 1H), 7.40 (d, *J* = 7.9 Hz, 1H), 7.30 - 7.15 (m, 2H), 6.92 (brs,

1H), 6.43 (d, *J* = 15.9 Hz, 1H), 5.80 (dd, *J* = 9.8, 15.3 Hz, 1H), 4.24 (t, *J* = 6.7 Hz, 1H), 4.07 (brs, 1H), 3.96 (dd, *J* = 8.9, 13.1 Hz, 1H), 3.52 (d, *J* = 12.8 Hz, 1H), 2.28 - 2.21 (m, 1H), 2.03 (d, *J* = 9.8 Hz, 2H), 1.59 - 1.56 (m, 1H), 1.38 (s, 12H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 171.2, 170.4, 154.9, 134.8, 132.0, 131.6, 127.8, 127.0, 126.3, 126.0, 78.0, 50.3, 43.4, 32.2, 29.8, 28.7, 17.3

HRMS (ESI): calculated for $C_{22}H_{30}O_5N_4Na$ $[M+Na]^+$: 453.2108, found 453.2099.

(*R*)-*N*-(2-((1-((5-(benzyloxy)-2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)hex-5-enamide



77

Compound **77** was synthesized from dipeptide **02** and acid **41** by following similar procedure for the synthesis of compound **45**.

Yield: 68% over 2 steps

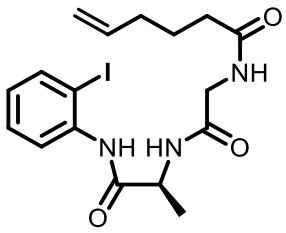
IR υ_{max}(film): 3014, 2978, 1554, 1481 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.26 (s, 1H), 8.25 (d, *J* = 6.7 Hz, 1H), 8.06 (brs, 1H), 7.72 (d, *J* = 8.5 Hz, 1H), 7.45 - 7.28 (m, 6H), 6.94 - 6.64 (m, 1H), 5.98 - 5.69 (m, 1H), 5.08 (s, 2H), 5.03 - 4.93 (m, 2H), 4.48 (t, *J* = 7.0 Hz, 1H), 3.78 (d, *J* = 1.5, 6.3 Hz, 2H), 2.14 (t, *J* = 7.3 Hz, 2H), 2.01(m, 2H), 1.60 - 1.56 (m, 2H), 1.37 (d, *J* = 7.3 Hz, 3H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 172.9, 171.5, 169.6, 159.2, 140.1, 139.5, 138.8, 137.1, 128.9, 128.4, 128.1, 115.5, 114.7, 113.1, 84.1, 69.9, 49.3, 42.4, 40.6, 40.4, 40.2, 40.0, 39.8, 39.6, 39.4, 35.0, 33.2, 24.9, 18.4

HRMS (ESI): calculated for $C_{24}H_{28}O_4N_3INa$ $[M+Na]^+$: 572.1017, found 572.1002.

(*S*)-*N*-(2-((1-((2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)hex-5-enamide



78

Compound **78** was synthesized from dipeptide **03** and acid **41** by following similar procedure for the synthesis of compound **45**.

Yield: 65% over 2 steps

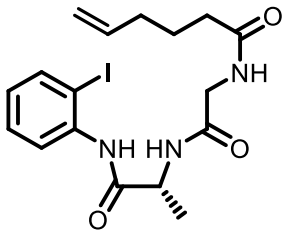
IR υ_{max}(film): 3014, 2978, 1554, 1481 cm^{-1}

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.39 (s, 1H), 8.23 (d, *J* = 6.9 Hz, 1H), 8.07 (t, *J* = 5.3 Hz, 1H), 7.88 (d, *J* = 7.6 Hz, 1H), 7.49 (d, *J* = 7.6 Hz, 1H), 7.40 (t, *J* = 7.6 Hz, 1H), 7.01 - 6.98 (m, 1H), 5.82 - 5.77 (m, 1H), 5.03 - 4.95 (m, 2H), 4.49 (t, *J* = 6.9 Hz, 1H), 3.78 (dd, *J* = 2.7, 5.7 Hz, 2H), 2.15 (t, *J* = 7.6 Hz, 2H), 2.02 (q, *J* = 7.6 Hz, 2H), 1.59 (quin, *J* = 7.4 Hz, 2H), 1.38 (d, *J* = 6.9 Hz, 3H)

^{13}C NMR (125 MHz, DMSO-d_6): δ 172.9, 171.5, 169.5, 139.4, 138.8, 129.2, 128.0, 126.8, 115.5, 96.2, 49.2, 42.4, 38.7, 35.0, 33.1, 24.9, 18.5

HRMS (ESI): calculated for $C_{17}H_{22}O_3N_3INa$ $[M+Na]^+$: 466.0598, found 466.0584.

(*R*)-*N*-(2-((1-((2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)hex-5-enamide



79

Compound **79** was synthesized from dipeptide **04** and acid **41** by following similar procedure for the synthesis of compound **45**.

Yield: 73% over 2 steps

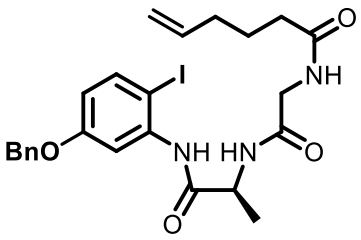
IR υ_{max}(film): 3014, 2978, 1554, 1481 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.40 (s, 1H), 8.23 (d, *J* = 7.6 Hz, 1H), 8.08 (t, *J* = 6.1 Hz, 1H), 7.88 - 7.86 (m, 1H), 7.47 (dd, *J* = 1.5, 7.6 Hz, 1H), 7.40 - 7.37 (m, 1H), 6.98 (dt, *J* = 1.5, 7.6 Hz, 1H), 5.79 - 5.78 (m, 1H), 5.03 - 4.94 (m, 2H), 4.48 (t, *J* = 7.2 Hz, 1H), 3.77 (dd, *J* = 1.9, 5.7 Hz, 2H), 2.14 (t, *J* = 7.2 Hz, 2H), 2.01 (q, *J* = 7.1 Hz, 2H), 1.58 (quin, *J* = 7.4 Hz, 2H), 1.37 (d, *J* = 6.9 Hz, 3H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 173.0, 171.6, 169.6, 139.4, 138.8, 129.3, 128.1, 127.0, 115.6, 96.4, 49.2, 42.4, 39.4, 35.1, 33.2, 24.9, 18.6

HRMS (ESI): calculated for $C_{17}H_{22}O_3N_3INa$ $[M+Na]^+$: 466.0598, found 466.0586.

(*S*)-*N*-(2-((1-((5-(benzyloxy)-2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)hex-5-enamide



80

Compound **80** was synthesized from dipeptide **01** and acid **41** by following similar procedure for the synthesis of compound **45**.

Yield: 72% over 2 steps

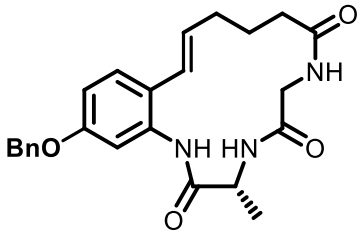
IR υ_{max}(film): 3014, 2978, 1554, 1480 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.28 (s, 1H), 8.27 (d, *J* = 7.6 Hz, 1H), 8.08 (t, *J* = 5.7 Hz, 1H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.44 - 7.28 (m, 6H), 6.72 (dd, *J* = 3.1, 8.4 Hz, 1H), 5.78 (d, *J* = 6.9 Hz, 1H), 5.08 (s, 2H), 5.03 - 4.93 (m, 2H), 4.48 (t, *J* = 7.2 Hz, 1H), 3.78 (dd, *J* = 1.5, 6.1 Hz, 2H), 2.14 (t, *J* = 7.6 Hz, 2H), 2.01 (q, *J* = 6.9 Hz, 2H), 1.60 - 1.56 (m, 2H), 1.36 (d, *J* = 6.9 Hz, 3H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 172.9, 171.6, 169.6, 159.3, 140.2, 139.6, 138.8, 137.1, 129.0, 128.5, 128.2, 115.6, 114.7, 113.2, 84.3, 70.0, 49.3, 42.4, 35.1, 33.2, 24.9, 18.5

HRMS (ESI): calculated for $C_{24}H_{28}O_4N_3INa$ $[M+Na]^+$: 572.1017, found 572.1002.

(*R*,E)-16-(benzyloxy)-3-methyl-3,4,6,7,10,11-hexahydro-1H-benzo[h][1,4,7]triazacyclopentadecine-2,5,8(9H)-trione



85

Compound **85** was synthesized from **77** by following similar procedure for the synthesis of compound **50**.

Yield: 48%

Specific rotation: $[\alpha]_D^{30}$ = - 8.71 (*c* 0.75, CH_3OH)

IR υ_{max}(film): 3444, 3007, 2961, 1559, 1440 cm^{-1}

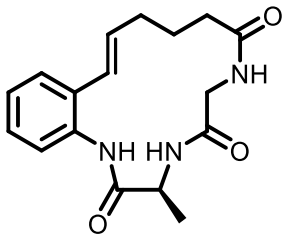
^{1}H NMR (400 MHz, DMSO-d_6): δ 8.78 (s, 1H), 8.70 (d, *J* = 6.7 Hz, 1H), 8.24 - 8.16 (m, 1H), 7.45 - 7.37 (m, 5H), 7.33 - 7.21 (m, 2H), 6.84 (dd, *J* = 2.4, 8.5 Hz, 1H), 6.36 (d, *J* = 15.3 Hz, 1H), 5.90 - 5.88 (m, 1H), 5.09 (s, 2H), 4.29 - 4.26 (m, 1H), 3.85 (dd, *J* = 7.0, 13.1

Hz, 1H), 3.67 (dd, *J* = 3.7, 13.4 Hz, 1H), 3.15 - 3.09 (m, 1H), 2.23 - 2.11 (m, 3H), 1.81 (brs, 1H), 1.63 - 1.60 (m, 1H), 1.36 (d, *J* = 7.3 Hz, 3H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 171.9, 171.0, 170.1, 157.7, 137.5, 135.5, 129.7, 128.9, 128.3, 128.0, 127.0, 126.9, 124.9, 112.8, 112.0, 69.7, 50.2, 46.3, 43.4, 33.3, 31.2, 23.2, 17.1

HRMS (ESI): calculated for $C_{24}H_{27}O_4N_3Na$ $[M+Na]^+$: 444.1894, found 444.1881.

(*S*,E)-3-methyl-3,4,6,7,10,11-hexahydro-1H-benzo[h][1,4,7]triazacyclopentadecine-2,5,8(9H)-trione



86

Compound **86** was synthesized from **78** by following similar procedure for the synthesis of compound **50**.

Yield: 49%

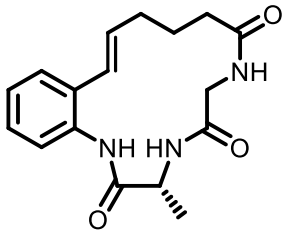
IR υ_{max}(film): 3444, 3017, 2964, 1559, 1445 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.81 (s, 1H), 8.70 (d, *J* = 6.1 Hz, 1H), 8.25 (brs, 1H), 7.46 (dd, *J* = 1.5, 7.6 Hz, 1H), 7.40 - 7.35 (m, 1H), 7.20 - 7.16 (m, 2H), 6.42 (d, *J* = 16.0 Hz, 1H), 6.03 (d, *J* = 8.6 Hz 1H), 4.36 - 4.22 (m, 1H), 3.86 (dd, *J* = 7.6, 13.0 Hz, 1H), 3.63 (dd, *J* = 3.8, 13.7 Hz, 1H), 2.21 - 2.06 (m, 4H), 1.36 (d, *J* = 7.2 Hz, 3H), 1.28 (d, *J* = m, 2H)

^{13}C NMR (125 MHz, DMSO-d_6) : δ 172.0, 171.1, 170.1, 134.4, 132.5, 131.6, 127.5, 127.4, 126.5, 126.2, 126.0, 50.2, 43.4, 33.4, 31.2, 23.1, 17.2

HRMS (ESI): calculated for $C_{17}H_{21}O_3N_3Na$ $[M+Na]^+$: 338.1475, found 338.1466.

(*R*,E)-3-methyl-3,4,6,7,10,11-hexahydro-1H-benzo[h][1,4,7]triazacyclopentadecine-2,5,8(9H)-trione



87

Compound **87** was synthesized from **79** by following similar procedure for the synthesis of compound **50**.

Yield: 54%

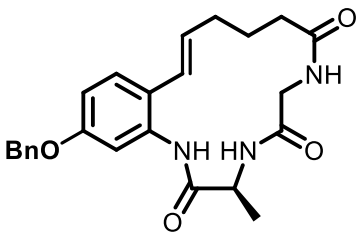
IR υ_{max}(film): 3444, 3017, 2964, 1559, 1445 cm^{-1}

^{1}H NMR (200 MHz, DMSO-d_6): δ 8.80 (s, 1 H), 8.70 (d, *J* = 6.2 Hz, 1H), 8.23 (brs, 1H), 7.51 - 7.39 (m, 2H), 7.26 - 7.15 (m, 2H), 6.43 (d, *J* = 15.5 Hz, 1H), 6.03 (dd, *J* = 8.6, 15.2 Hz, 1H), 4.31 - 4.20 (m, 1H), 3.86 (dd, *J* = 7.3, 13.1 Hz, 1H), 3.63 (dd, *J* = 4.0, 13.5 Hz, 1H), 2.21 - 2.06 (m, 4H), 1.36 (d, *J* = 7.3 Hz, 3H), 1.29 (m, 2H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 171.1, 170.2, 134.5, 132.6, 131.6, 127.6, 127.5, 126.7, 126.3, 126.1, 50.3, 43.5, 33.5, 29.6, 23.2, 17.2, 14.5

HRMS (ESI): calculated for $C_{17}H_{21}O_3N_3Na$ $[M+Na]^+$: 338.1475, found 338.1467.

(*S*,E)-16-(benzyloxy)-3-methyl-3,4,6,7,10,11-hexahydro-1H-benzo[h][1,4,7]triazacyclopentadecine-2,5,8(9H)-trione



88

Compound **88** was synthesized from **80** by following similar procedure for the synthesis of compound **50**.

Yield: 52%

Specific rotation: $[\alpha]_D^{30}$ = + 7.98 (*c* 1.20, CH_3OH)

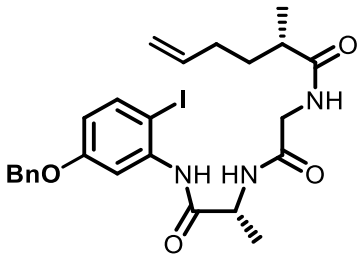
IR υ_{max}(film): 3444, 3007, 2961, 1559, 1440 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.78 (s, 1H), 8.70 (d, *J* = 6.7 Hz, 1H), 8.24 - 8.16 (m, 1H), 7.45 - 7.37 (m, 5H), 7.33 - 7.21 (m, 2H), 6.84 (dd, *J* = 2.4, 8.5 Hz, 1H), 6.36 (d, *J* = 15.3 Hz, 1H), 5.90 - 5.88 (m, 1H), 5.09 (s, 2H), 4.29 - 4.26 (m, 1H), 3.85 (dd, *J* = 7.0, 13.1 Hz, 1H), 3.67 (dd, *J* = 3.7, 13.4 Hz, 1H), 3.15 - 3.09 (m, 1H), 2.23 - 2.11 (m, 3H), 1.81 (brs, 1H), 1.63 - 1.60 (m, 1H), 1.36 (d, *J* = 7.3 Hz, 3H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 171.9, 171.0, 170.1, 157.7, 137.5, 135.5, 129.7, 128.9, 128.3, 128.0, 127.0, 126.9, 124.9, 112.8, 112.0, 69.7, 50.2, 46.3, 43.4, 33.3, 31.2, 23.2, 17.1

HRMS (ESI): calculated for $C_{24}H_{27}O_4N_3Na$ $[M+Na]^+$: 444.1894, found 444.1881.

(*S*)-*N*-(2-(((*R*)-1-((5-(benzyloxy)-2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)-2-methylhex-5-enamide



81

Compound **81** was synthesized from dipeptide **02** and acid **13** by following similar procedure for the synthesis of compound **45**.

Yield: 65% for 2 steps

IR υ_{max}(film): 3453, 3124, 2981, 1558, 1527 cm^{-1}

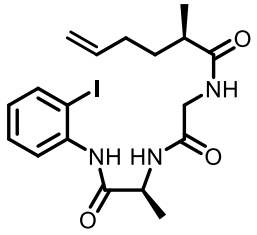
^{1}H NMR (500 MHz, DMSO-d_6): δ 9.27 (s, 1H), 8.20 (d, *J* = 7.2 Hz, 1H), 8.04 (t, *J* = 4.6 Hz, 1H), 7.72 (d, *J* = 8.8 Hz, 1H), 7.44 - 7.29 (m, 6H), 6.72 (dd, *J* = 2.7, 8.8 Hz, 1H), 5.87

- 5.66 (m, 1H), 5.08 (s, 2H), 5.00 (d, *J* = 17.2 Hz, 1H), 4.92 (d, *J* = 9.9 Hz, 1H), 4.48 (quin, *J* = 6.9 Hz, 1H), 3.81 - 3.76 (m, 2H), 2.35 - 2.33 (m, 1H), 2.01 - 1.98 (m, 2H), 1.59 - 1.48 (m, 2H), 1.37 (d, *J* = 6.9 Hz, 3H), 1.33 - 1.31 (m, 1H), 1.00 (d, *J* = 6.9 Hz, 3H)

^{13}C NMR (125 MHz, DMSO-d_6): δ 176.3, 171.5, 169.6, 159.3, 140.1, 139.5, 139.0, 137.1, 128.9, 128.4, 128.1, 115.2, 114.7, 113.1, 70.0, 49.3, 42.4, 39.5, 33.4, 31.5, 18.5, 18.2

HRMS (ESI): calculated for $C_{25}H_{30}O_4N_3NaI$ $[M+Na]^+$: 586.1173, found 586.1163.

(*R*)-N-(2-(((S)-1-((2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)-2-methylhex-5-enamide



82

Compound **82** was synthesized from dipeptide **03** and acid **12** by following similar procedure for the synthesis of compound **45**.

Yield: 74% for 2 steps

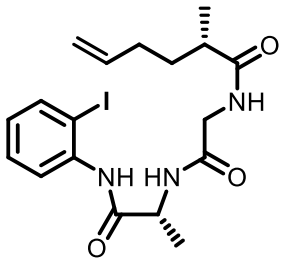
IR υ_{max}(film): 3453, 3124, 2981, 1558, 1527cm^{-1}

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.39 (brs, 1H), 8.16 (brs, 1H), 8.05 (brs, 1H), 7.87 (br s, 1 H), 7.47 (brs, 1H), 7.38 (brs, 1H), 6.98 (brs, 1H), 5.78 (brs, 1H), 5.13 - 4.95 (m, 1 H), 4.93 (brs, 1 H), 4.48 (brs, 1H), 3.77 (brs, 2H), 2.34 (brs, 2 H), 1.98 (br. s., 2 H), 1.59 (brs, 1H), 1.37 (brs, 3H), 1.00 (brs, 3H)

^{13}C NMR (125 MHz, DMSO-d_6): δ 176.5, 169.6, 139.5, 139.0, 129.3, 128.1, 126.9, 115.3, 111.6, 49.3, 42.4, 33.4, 31.5, 18.6, 18.3

HRMS (ESI): calculated for $C_{18}H_{24}O_3N_3NaI$ $[M+Na]^+$: 480.0755, found 480.0744.

(*S*)-N-(2-(((R)-1-((2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)-2-methylhex-5-enamide



83

Compound **83** was synthesized from dipeptide **04** and acid **13** by following similar procedure for the synthesis of compound **45**.

Yield: 69% for 2 steps

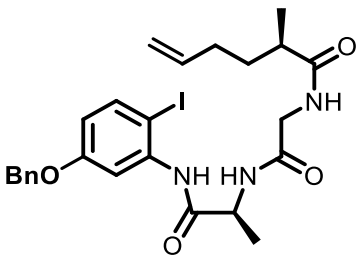
IR υ_{max}(film): 3453, 3124, 2981, 1558, 1527 cm^{-1}

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.39 (s, 1H), 8.17 (d, *J* = 6.9 Hz, 1H), 8.04 (t, *J* = 5.3 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.38 (t, *J* = 7.4 Hz, 1H), 6.98 (t, *J* = 7.4 Hz, 1H), 5.78 (ddd, *J* = 3.4, 6.7, 17.0 Hz, 1H), 5.00 (d, *J* = 16.8 Hz, 1H), 4.93 (d, *J* = 10.3 Hz, 1H), 4.49 (t, *J* = 7.1 Hz, 1H), 3.82 - 3.75 (m, 2H), 2.39 - 2.24 (m, 1H), 1.99 (q, *J* = 6.9 Hz, 2H), 1.60 (dd, *J* = 7.8, 13.2 Hz, 1H), 1.37 (d, *J* = 7.2 Hz, 3H), 1.35 - 1.32 (m, 1H), 1.00 (d, *J* = 6.5 Hz, 3H)

^{13}C NMR (125 MHz, DMSO-d_6): δ 176.3, 171.5, 169.5, 139.4, 139.0, 129.2, 128.0, 126.8, 115.2, 49.2, 42.4, 40.6, 40.4, 40.2, 40.1, 39.9, 39.7, 39.5, 39.5, 33.4, 31.5, 18.6, 18.2

HRMS (ESI): calculated for $C_{18}H_{24}O_3N_3NaI$ $[M+Na]^+$: 480.0755, found 480.0750.

(*R*)-N-(2-(((*S*)-1-((5-(benzyloxy)-2-iodophenyl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)-2-methylhex-5-enamide



84

Compound **84** was synthesized from dipeptide **01** and acid **12** by following similar procedure for the synthesis of compound **45**.

Yield: 71% for 2 steps

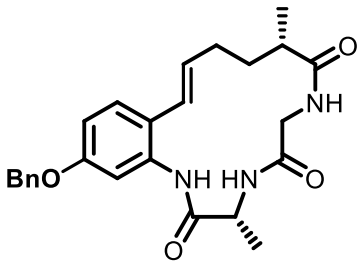
IR υ_{max}(film): 3445, 3115, 2979, 1558, 1522 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.30 (brs, 1H), 8.22 (d, *J* = 6.7 Hz, 1H), 8.07 (brs, 1H), 7.73 (d, *J* = 8.5 Hz, 1H), 7.45 - 7.37 (m, 7H), 6.72 (dd, *J* = 2.1, 8.9 Hz, 1H), 5.79 - 5.77 (m, 1H), 5.08 (s, 2H), 5.02 - 4.91 (m, 2H), 4.51 - 4.47 (m, 1H), 2.35 - 2.33 (m, 1H), 1.98 (d, *J* = 6.7 Hz, 2H), 1.61 - 1.57 (m, 21H), 1.37 (d, *J* = 6.7 Hz, 3H), 1.34 - 1.30 (m, 1H), 1.01 (d, *J* = 6.7 Hz, 3H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 176.3, 171.5, 169.6, 159.2, 140.1, 139.5, 138.9, 137.1, 128.9, 128.4, 128.1, 115.2, 114.7, 113.2, 69.9, 49.3, 42.3, 39.5, 33.4, 31.5, 18.5, 18.2

HRMS (ESI): calculated for $C_{25}H_{30}O_4N_3NaI$ $[M+Na]^+$: 586.1173, found 586.1160.

(3*R*,9*S*,E)-16-(benzyloxy)-3,9-dimethyl-3,4,6,7,10,11-hexahydro-1H-benzo[h][1,4,7]triazacyclopentadecine-2,5,8(9H)-trione



89

Compound **89** was synthesized from **81** by following similar procedure for the synthesis of compound **50**.

Yield: 48%

IR υ_{max}(film): 3445, 3018, 2977, 1534, 1435 cm^{-1}

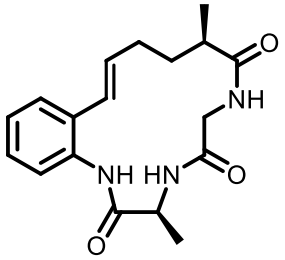
^{1}H NMR (400 MHz, DMSO-d_6): δ 8.88 (s, 1H), 8.73 (d, *J* = 5.5 Hz, 1H), 8.16 (d, *J* = 9.2 Hz, 1H), 7.44 - 7.33 (m, 6H), 7.06 (brs, 1H), 6.86 (d, *J* = 8.5 Hz, 1H), 6.32 (d, *J* = 15.3 Hz,

1H), 5.92 - 5.86 (m, 1H), 5.10 (s, 2H), 4.21 - 4.15 (m, 2H), 2.32 - 2.17 (m, 3H), 2.15 - 1.91 (m, 2H), 1.70 (t, *J* = 12.2 Hz, 1H), 1.39 (d, *J* = 7.5 Hz, 3H), 1.00 (d, *J* = 6.2 Hz, 3H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 175.2, 171.2, 170.1, 157.6, 137.5, 135.4, 129.2, 128.9, 128.3, 128.0, 126.9, 126.7, 125.7, 113.1, 69.7, 50.6, 43.0, 32.7, 32.0, 31.3, 29.9, 29.5, 19.3, 17.1

HRMS (ESI): calculated for $C_{25}H_{29}O_4N_3Na$ $[M+Na]^+$: 458.2050, found 458.2047.

(3*S*,9*R*,E)-3,9-dimethyl-3,4,6,7,10,11-hexahydro-1H-benzo[h][1,4,7] triazacyclopentadecine-2,5,8(9H)-trione



90

Compound **90** was synthesized from **82** by following similar procedure for the synthesis of compound **50**.

Yield: 51%

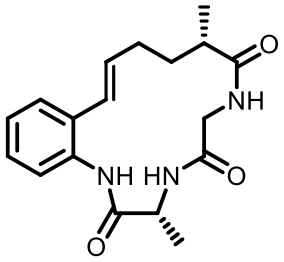
IR υ_{max}(film): 3445, 3018, 2977, 1534, 1435 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.84 (d, *J* = 7.3 Hz, 1H), 8.69 (s, 1H), 8.28 (d, *J* = 6.1 Hz, 1H), 7.60 (d, *J* = 7.9 Hz, 1H), 7.47 (d, *J* = 7.3 Hz, 1H), 7.24 - 7.13 (m, 2H), 6.52 (d, *J* = 15.3 Hz, 1 H), 6.08–6.04 (m, 1H), 4.42 - 4.38 (m, 1H), 4.21 (dd, *J* = 7.9, 13.4 Hz, 1H), 3.34 - 3.27 (m, 1H), 2.36 (dt, *J* = 3.7, 7.0 Hz, 1H), 2.24 - 2.21 (m, 2H), 1.66 – 1.40 (m, 2H), 1.57 - 1.45 (m, 1H), 1.34 (d, *J* = 7.3 Hz, 3H), 0.99 (s, 3H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 175.5, 170.6, 170.0, 134.6, 133.1, 131.5, 127.5, 126.2, 126.1, 125.7, 125.0, 49.6, 43.9, 32.6, 31.1, 29.5, 19.5, 16.7

HRMS (ESI): calculated for $C_{18}H_{23}O_3N_3Na$ $[M+Na]^+$: 352.1632, found 352.1625.

(3*R*,9*S*,*E*)-3,9-dimethyl-3,4,6,7,10,11-hexahydro-1H-benzo[h][1,4,7]triazacyclopentadecine-2,5,8(9H)-trione



91

Compound **91** was synthesized from **83** by following similar procedure for the synthesis of compound **50**.

Yield: 53%

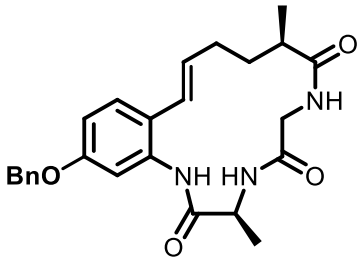
IR υ_{max}(film): 3445, 3018, 2977, 1534, 1435 cm^{-1}

^{1}H NMR (200 MHz, DMSO-d_6): δ 8.85 (d, *J* = 7.6 Hz, 1H), 8.73 - 8.60 (m, 1H), 8.29 (d, *J* = 6.1 Hz, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.47 (d, *J* = 7.3 Hz, 1H), 7.25 - 7.13 (m, 2H), 6.52 (d, *J* = 15.3 Hz, 1H), 6.07 (dd, *J* = 7.1, 15.2 Hz, 1H), 4.42 - 4.36 (m, 1H), 4.21 (dd, *J* = 7.9, 13.6 Hz, 1H), 3.34 - 3.29 (m, 1H), 2.35 - 2.23 (m, 3H), 1.74 - 1.47 (m, 2 H), 1.33 (d, *J* = 7.1 Hz, 3H), 0.98 (d, *J* = 6.7 Hz, 3H)

^{13}C NMR (125 MHz, DMSO-d_6): δ 175.6, 170.6, 170.1, 134.6, 133.1, 131.5, 127.5, 126.1, 125.7, 124.9, 49.6, 43.9, 32.6, 31.1, 19.5, 16.7

HRMS (ESI): calculated for $C_{18}H_{23}O_3N_3Na[M+Na]^+$: 352.1632, found 352.1623.

(3*S*,9*R*,*E*)-16-(benzyloxy)-3,9-dimethyl-3,4,6,7,10,11-hexahydro-1H-benzo[h][1,4,7]triazacyclopentadecine-2,5,8(9H)-trione



92

Compound **92** was synthesized from **84** by following similar procedure for the synthesis of compound **50**.

Yield: 49%

IR υ_{max}(film): 3445, 3018, 2977, 1534, 1435 cm^{-1}

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.88 (s, 1H), 8.73 (d, *J* = 5.5 Hz, 1H), 8.16 (d, *J* = 9.2 Hz, 1H), 7.46 - 7.33 (m, 6H), 7.06 (brs, 1H), 6.86 (d, *J* = 8.5 Hz, 1H), 6.32 (d, *J* = 15.3 Hz, 1H), 5.92 - 5.89 (m, 1H), 5.10 (s, 2H), 4.21 - 4.16 (m, 2H), 2.34 - 2.17 (m, 3H), 2.15 - 1.91 (m, 2H), 1.70 (t, *J* = 12.2 Hz, 1H), 1.39 (d, *J* = 7.3 Hz, 3H), 1.00 (d, *J* = 6.1 Hz, 3H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 175.2, 171.2, 170.1, 157.6, 137.5, 135.4, 129.2, 128.9, 128.3, 128.0, 126.9, 126.7, 125.7, 113.1, 69.7, 50.6, 43.0, 32.7, 32.0, 31.3, 29.9, 29.5, 19.3, 17.1

HRMS (ESI): calculated for $C_{25}H_{29}O_4N_3Na$ $[M+Na]^+$: 458.2050, found 458.2048.

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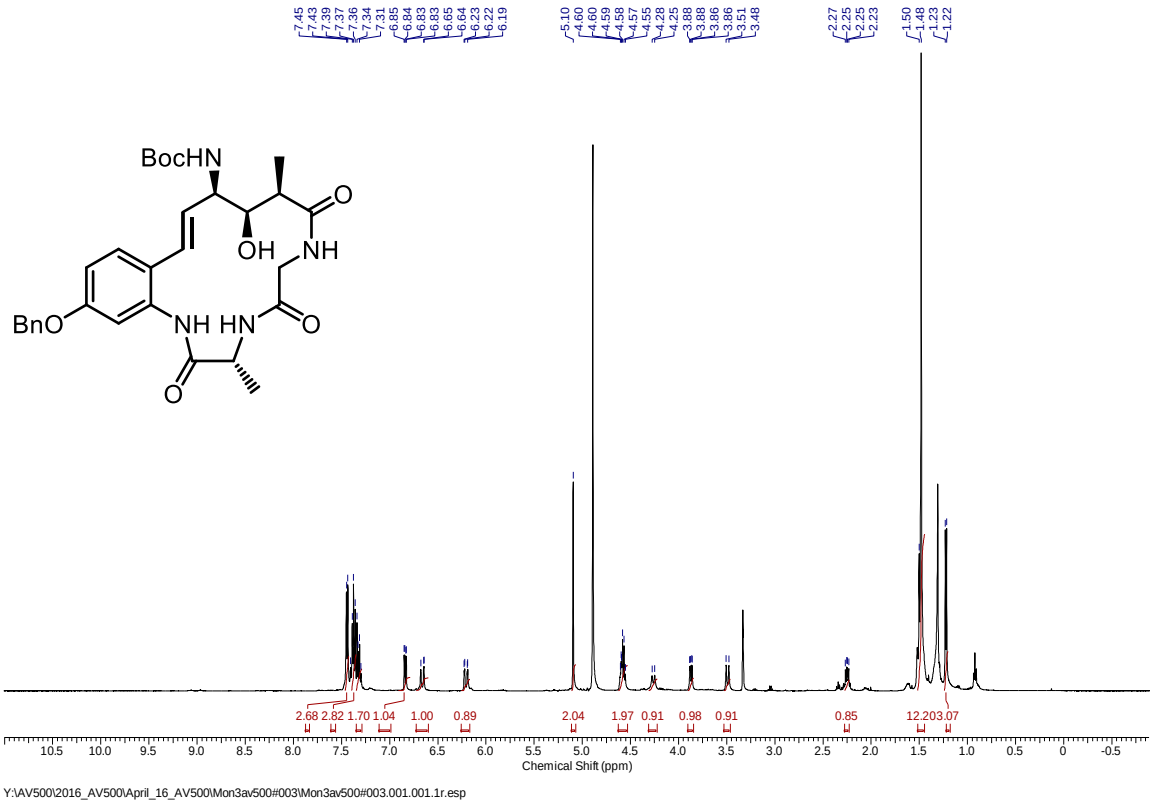
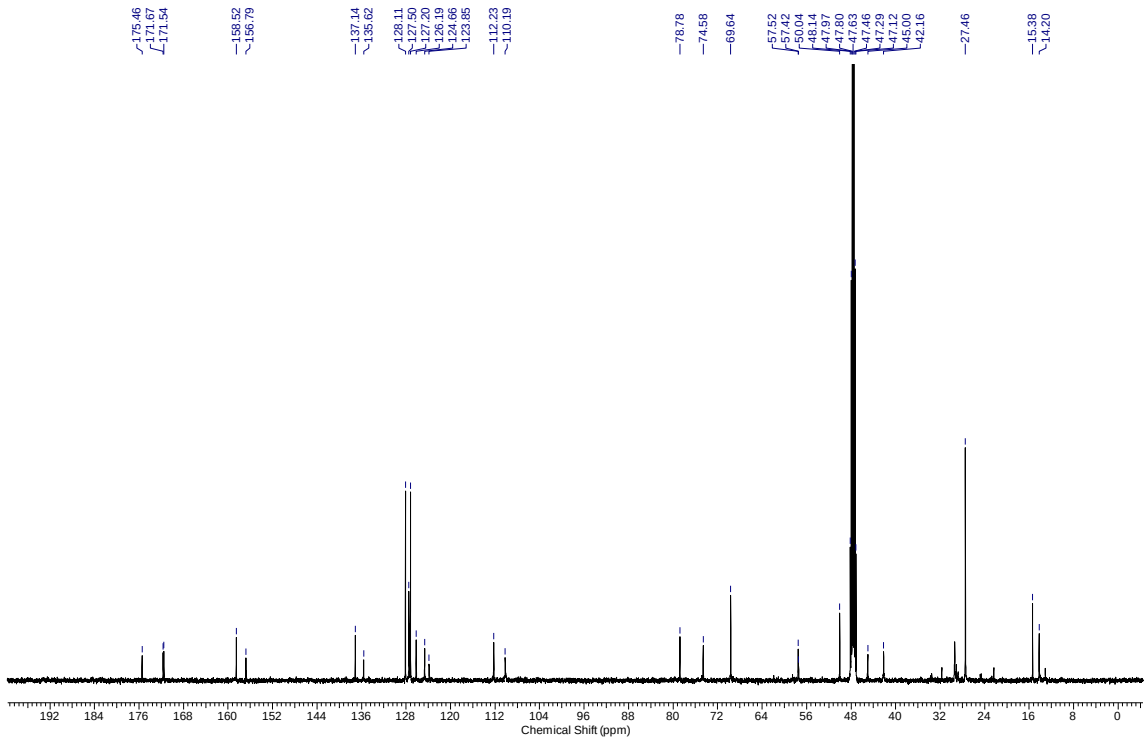
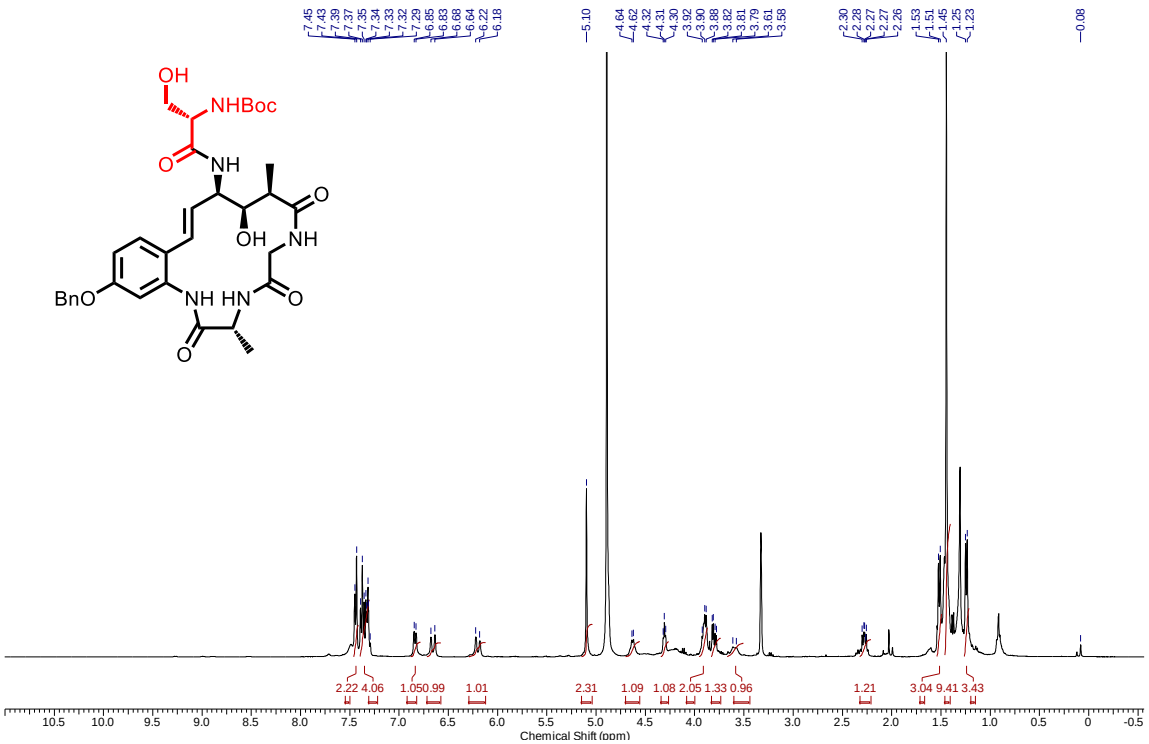
1.2.12. Copies of NMR spectra



^{1}H NMR of **50** (500 MHz, CD_3OD)



^{13}C NMR of **50** (125 MHz, CD_3OD)



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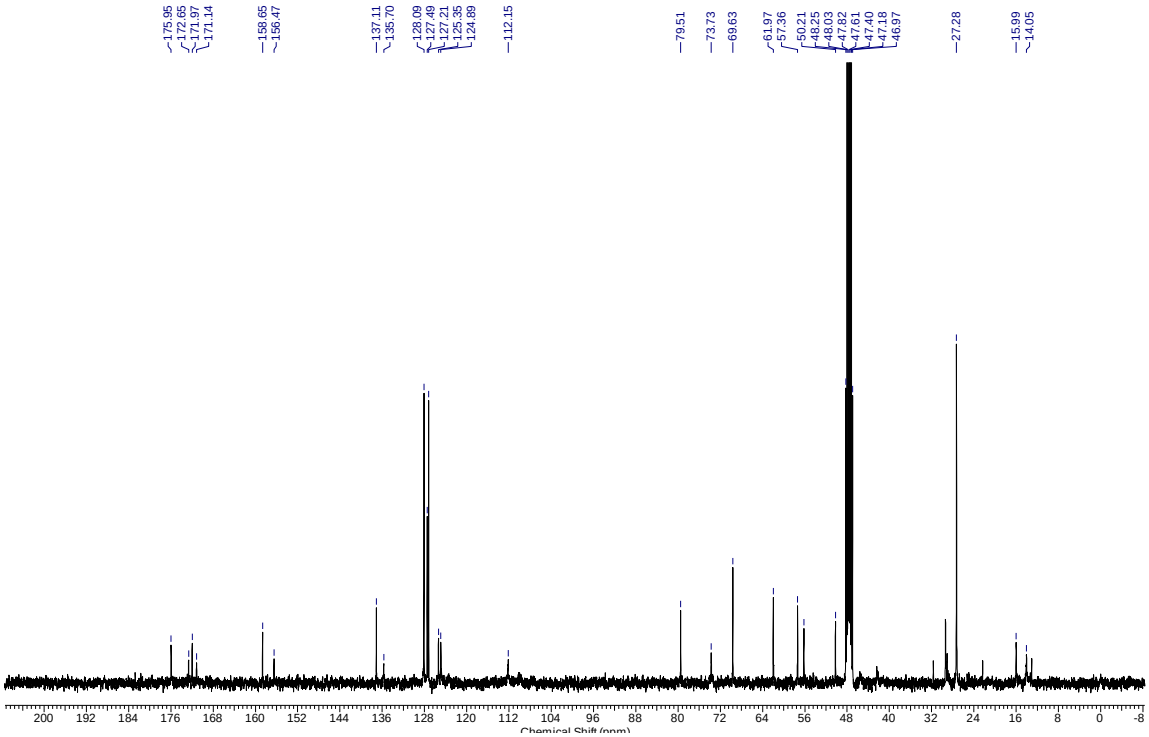

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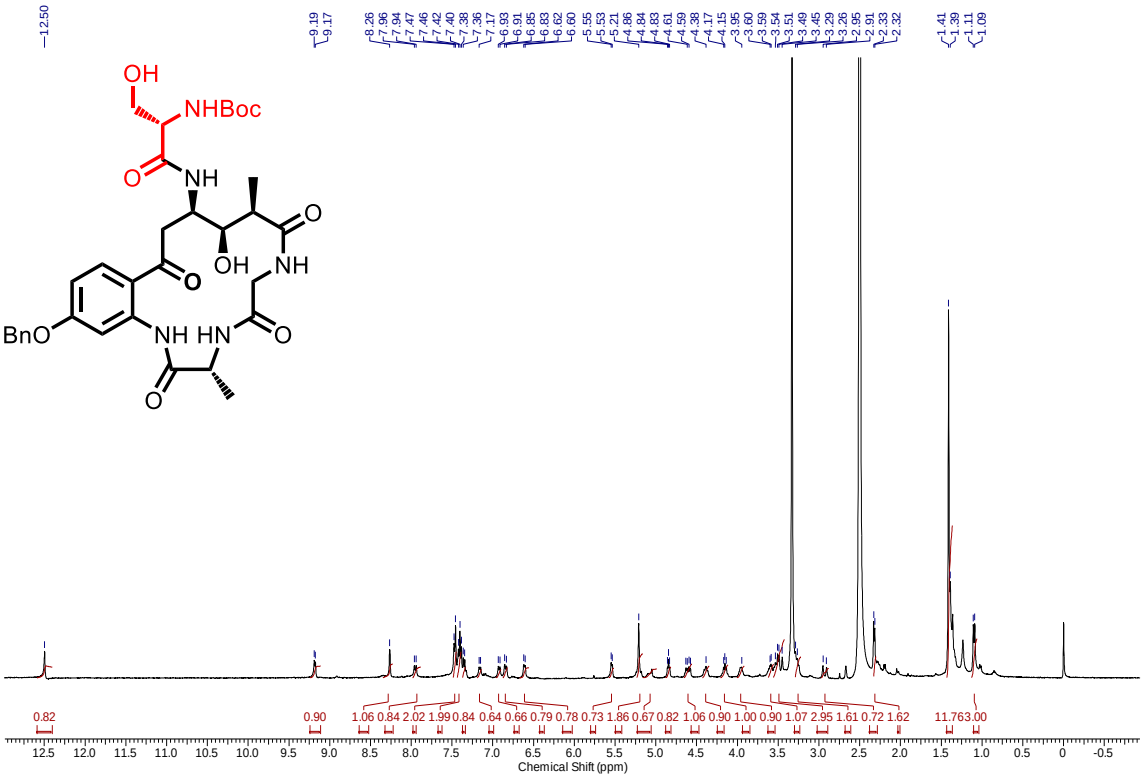
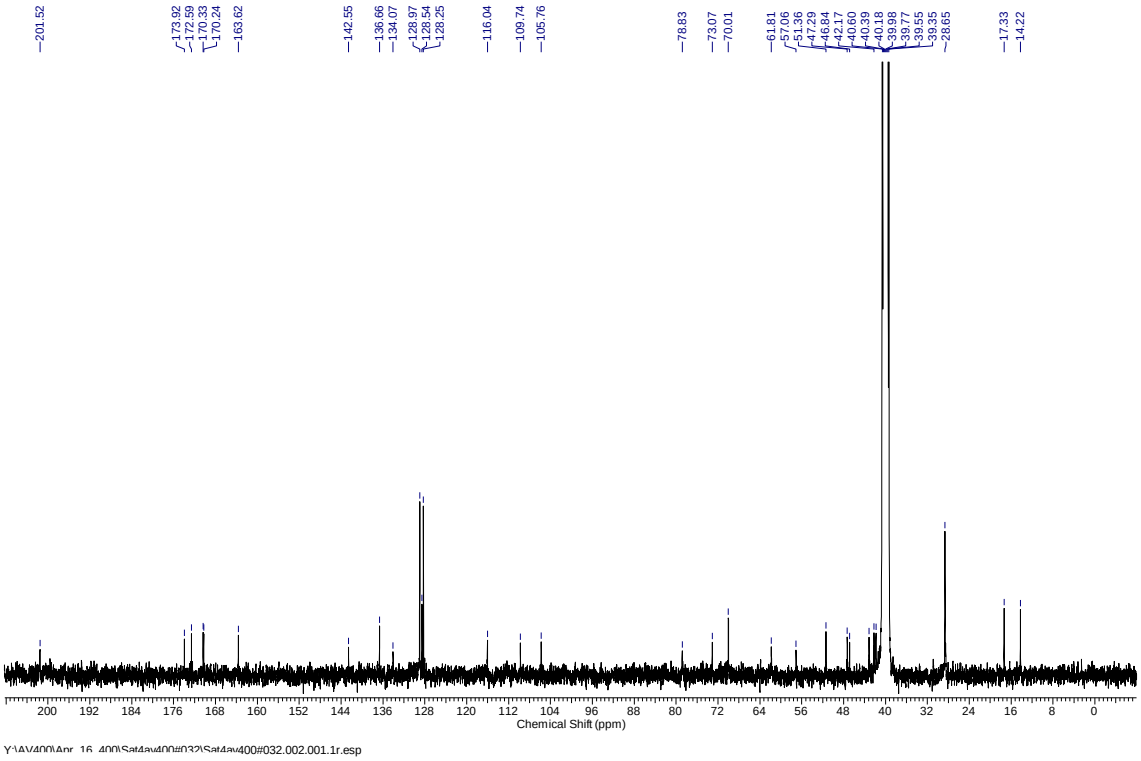
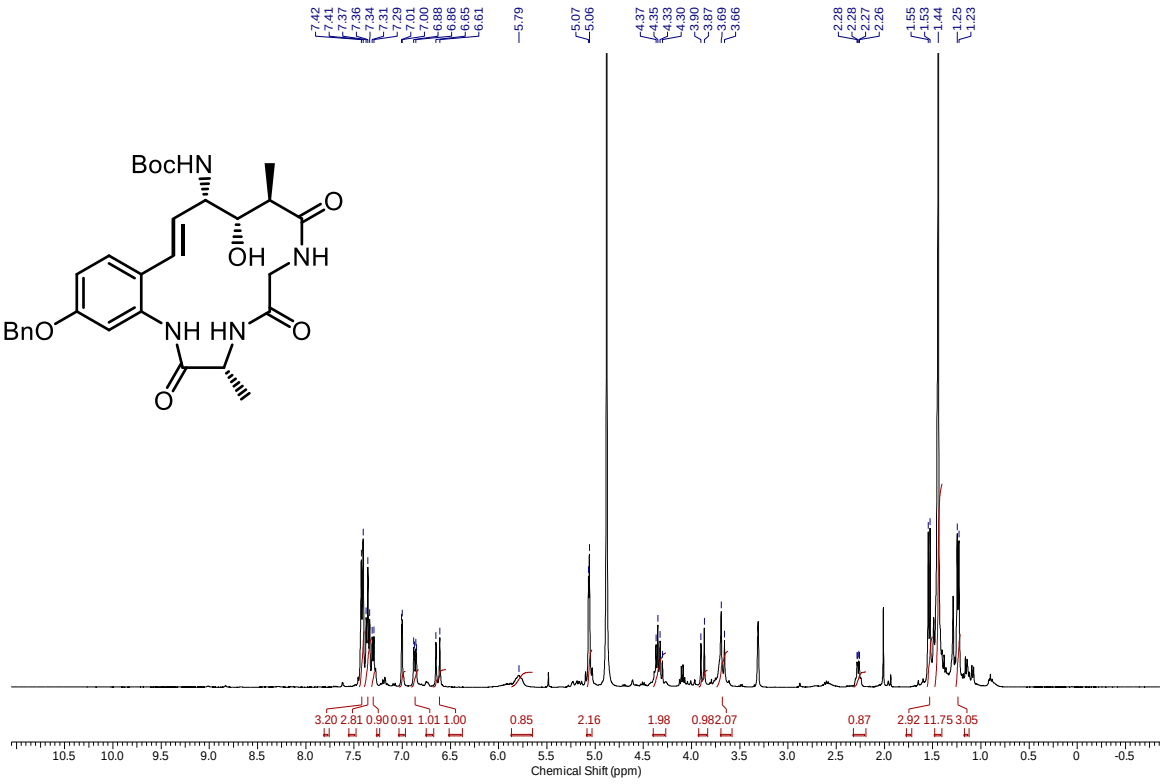


^{1}H NMR of **57** (400 MHz, DMSO-d_6)



^{13}C NMR of **57** (100 MHz, DMSO-d_6)



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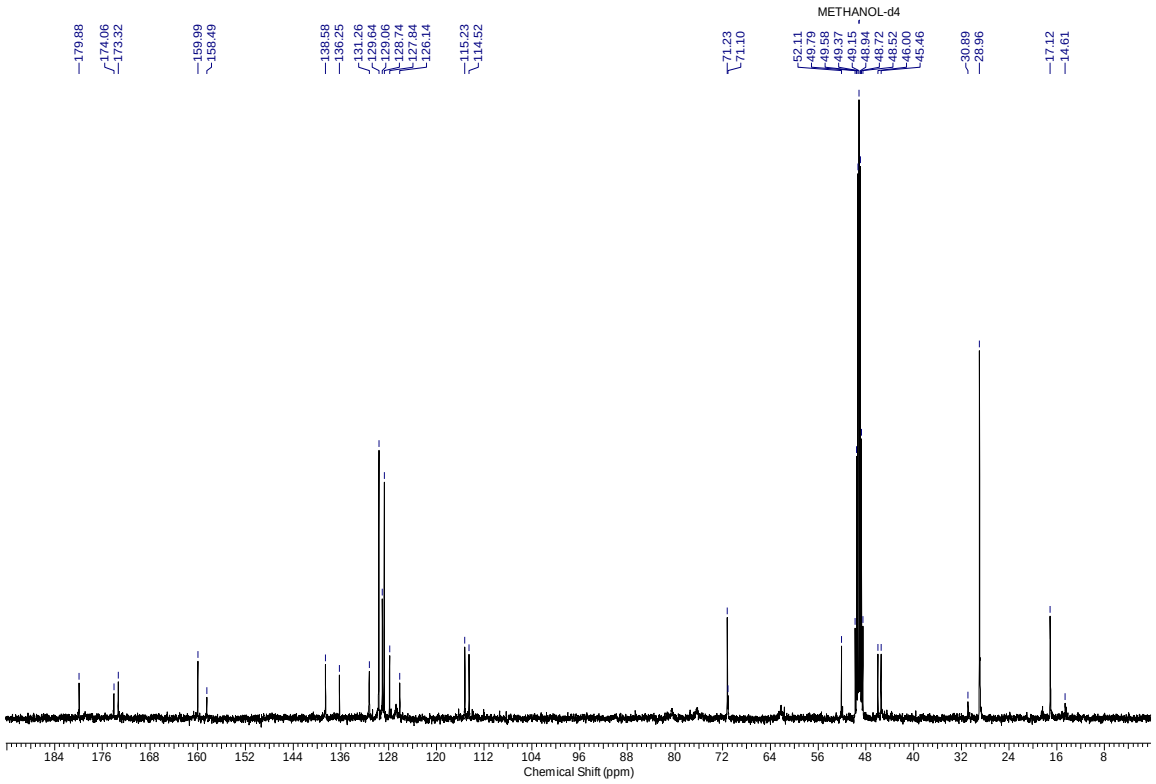
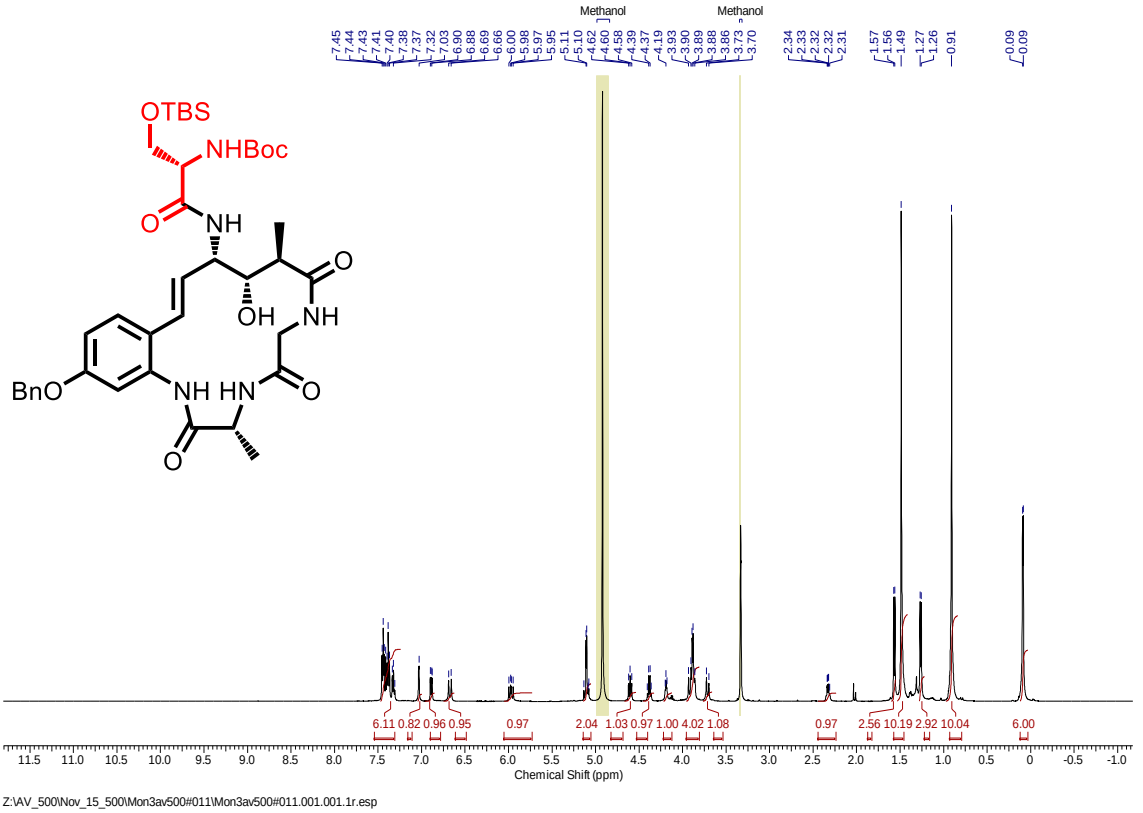
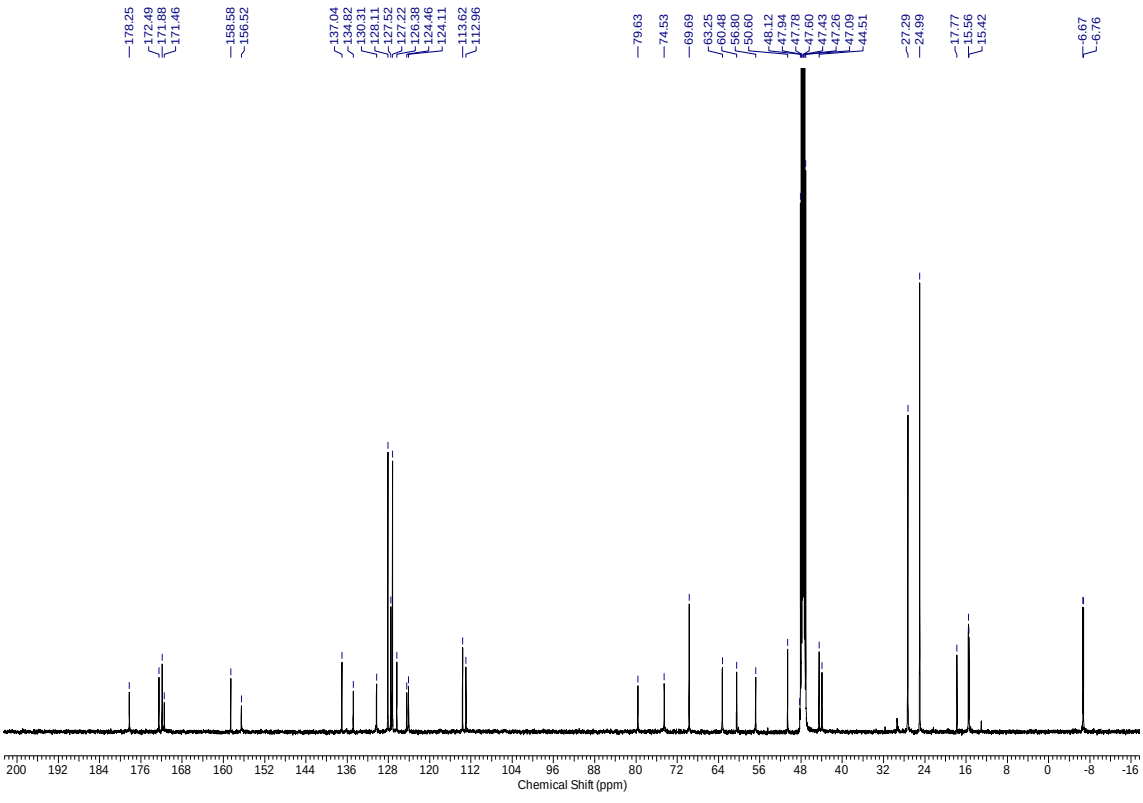
^{1}H NMR of **51** (400 MHz, CD_3OD)



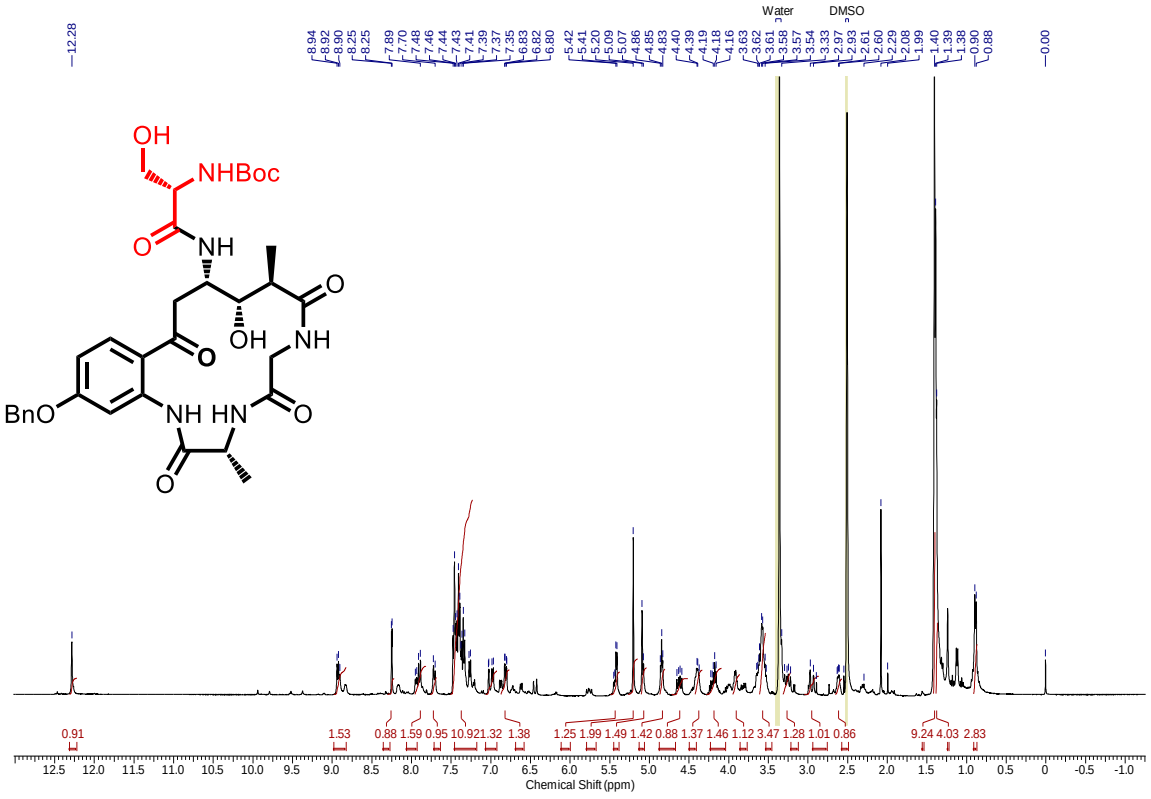
^{13}C NMR of **51** (100 MHz, CD_3OD)



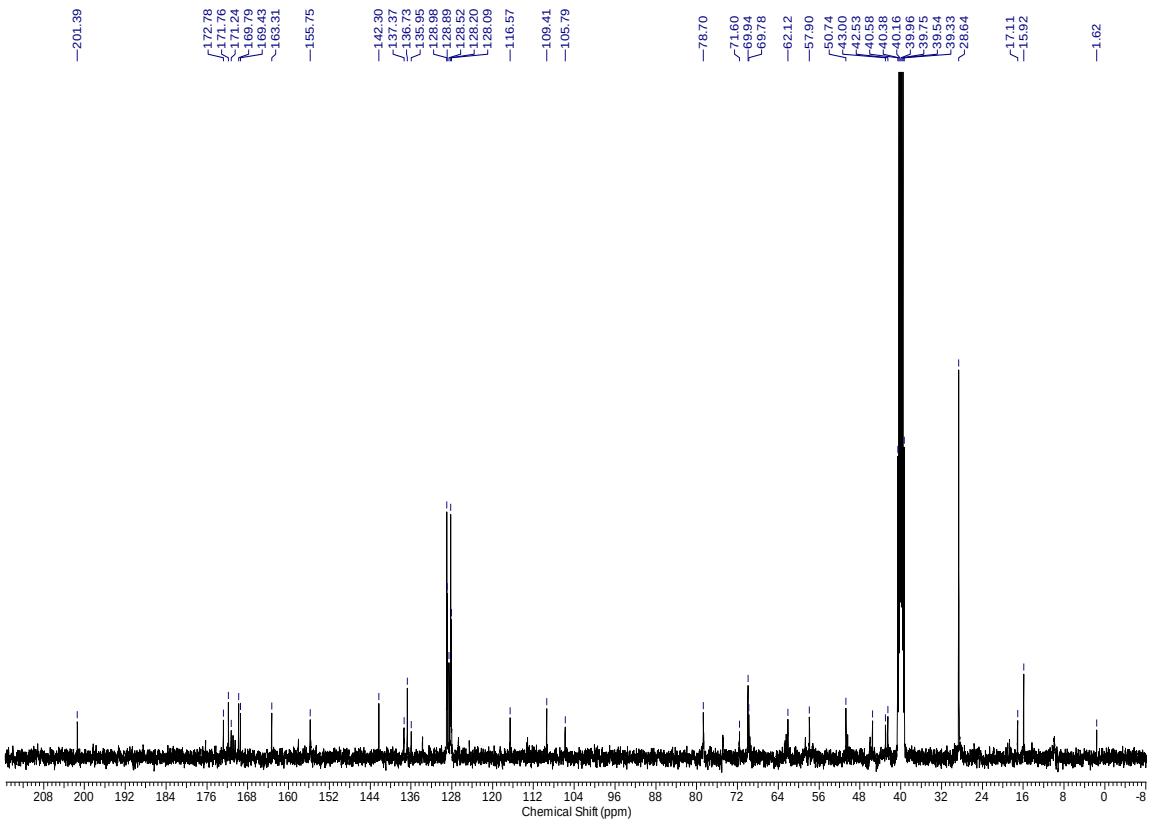
^{1}H NMR of **59** (500 MHz, CD_3OD)



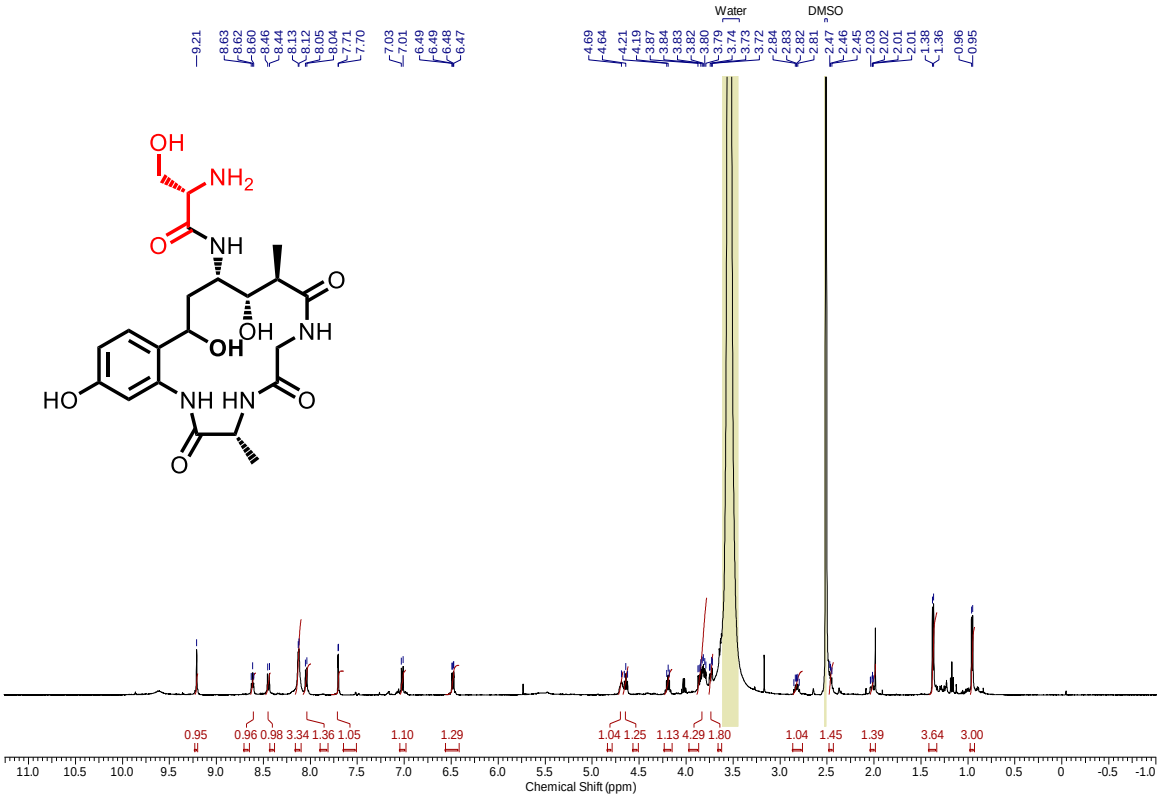
^{13}C NMR of **59** (125 MHz, CD_3OD)



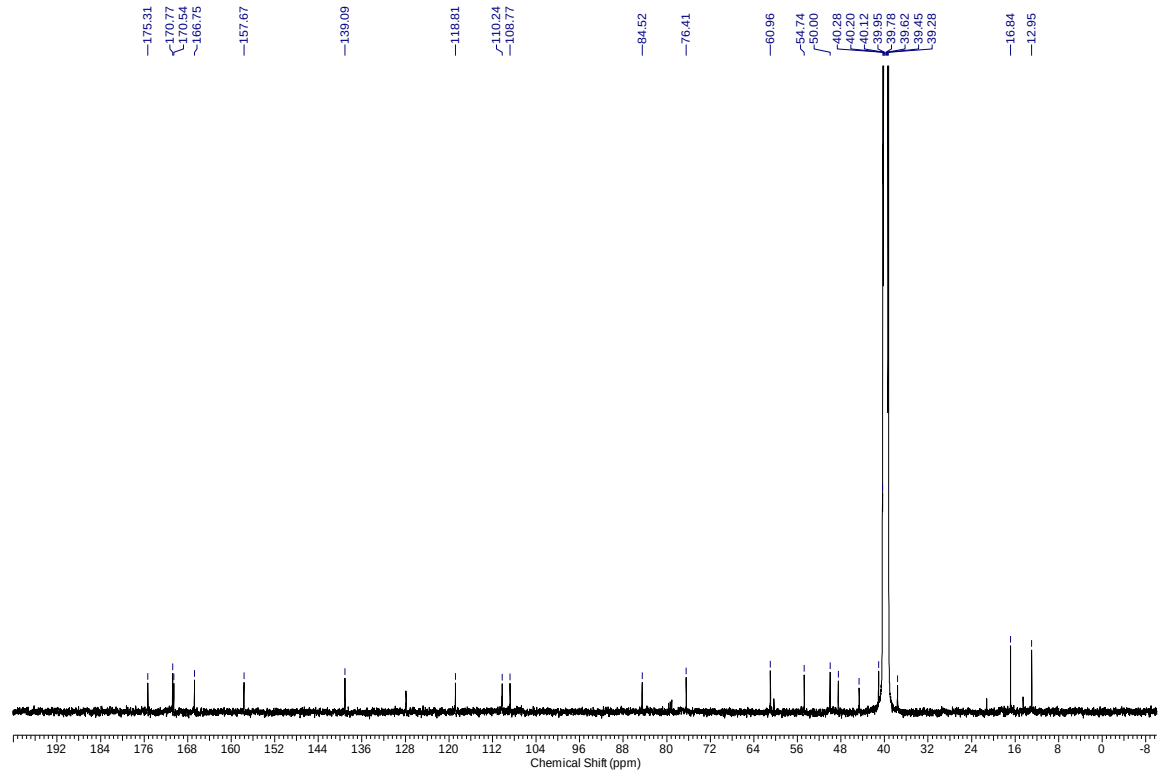
^{1}H NMR of **60** (400 MHz, DMSO-d_6)



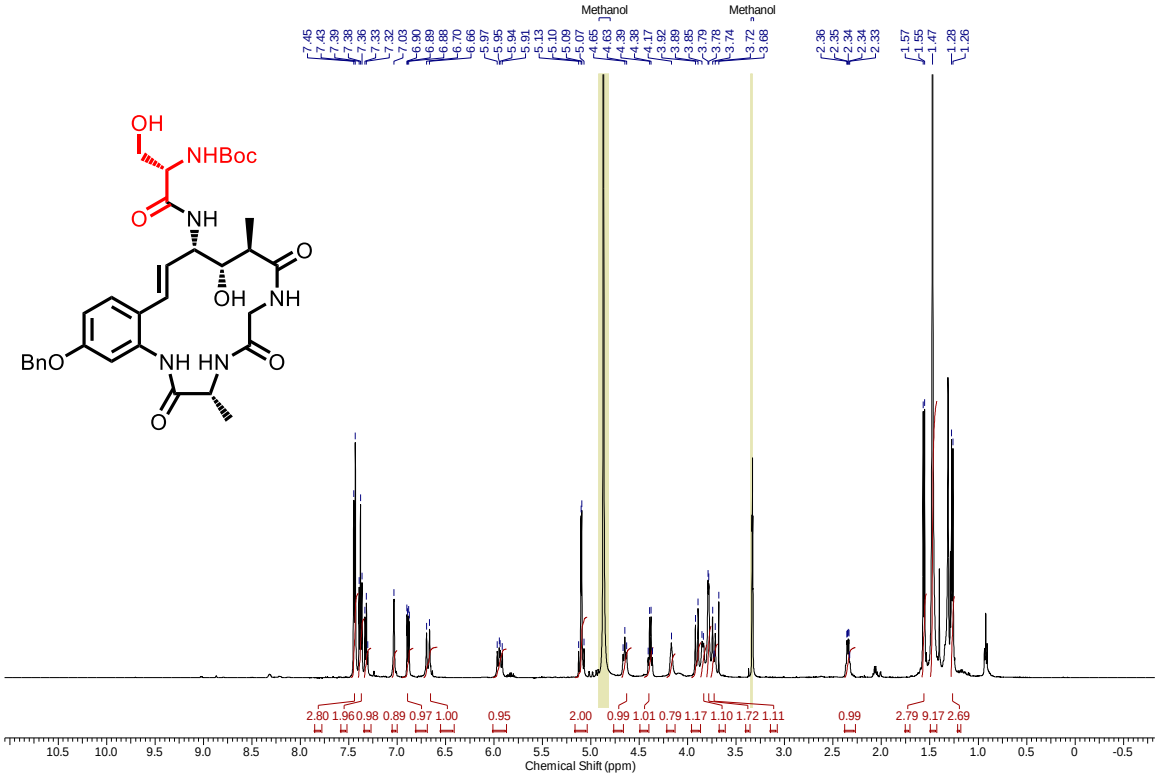
^{13}C NMR of **60** (100 MHz, DMSO-d_6)



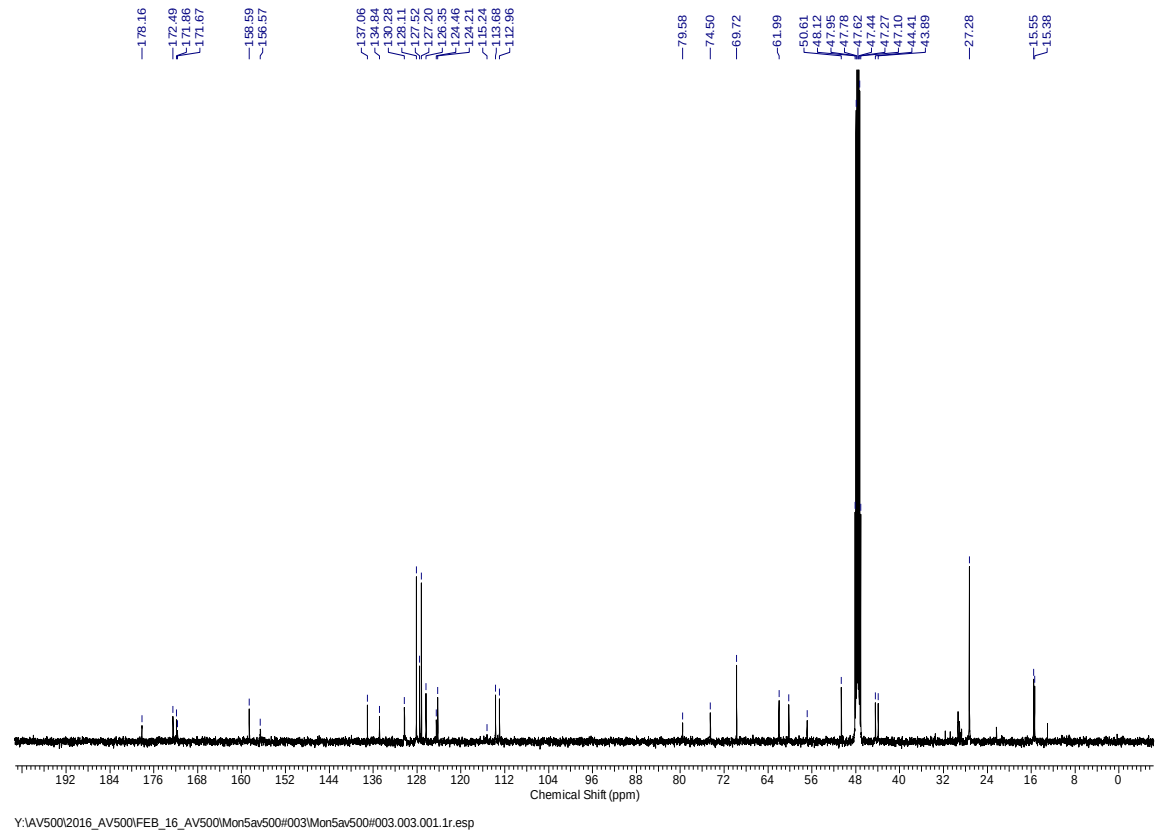
^{1}H NMR of **62** (500 MHz, DMSO-d_6)



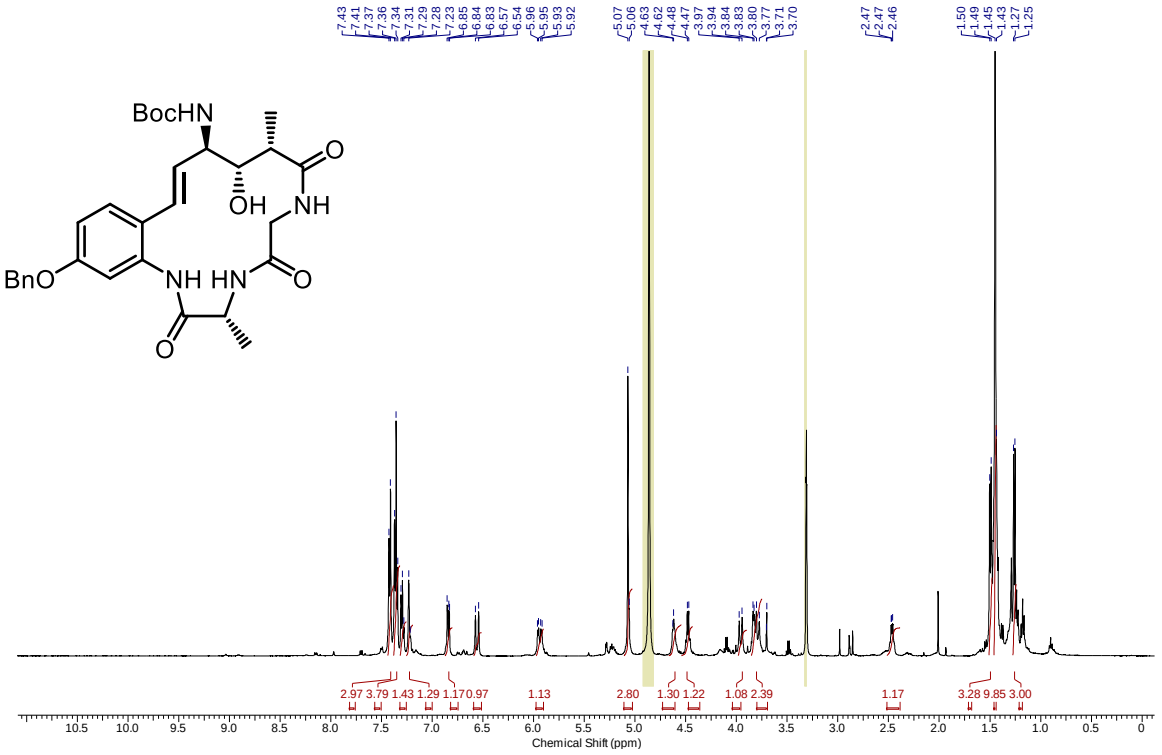
^{13}C NMR of **62** (125 MHz, DMSO-d_6)



^{1}H NMR of **63** (500 MHz, CD_3OD)



^{13}C NMR of **63** (125 MHz, CD_3OD)



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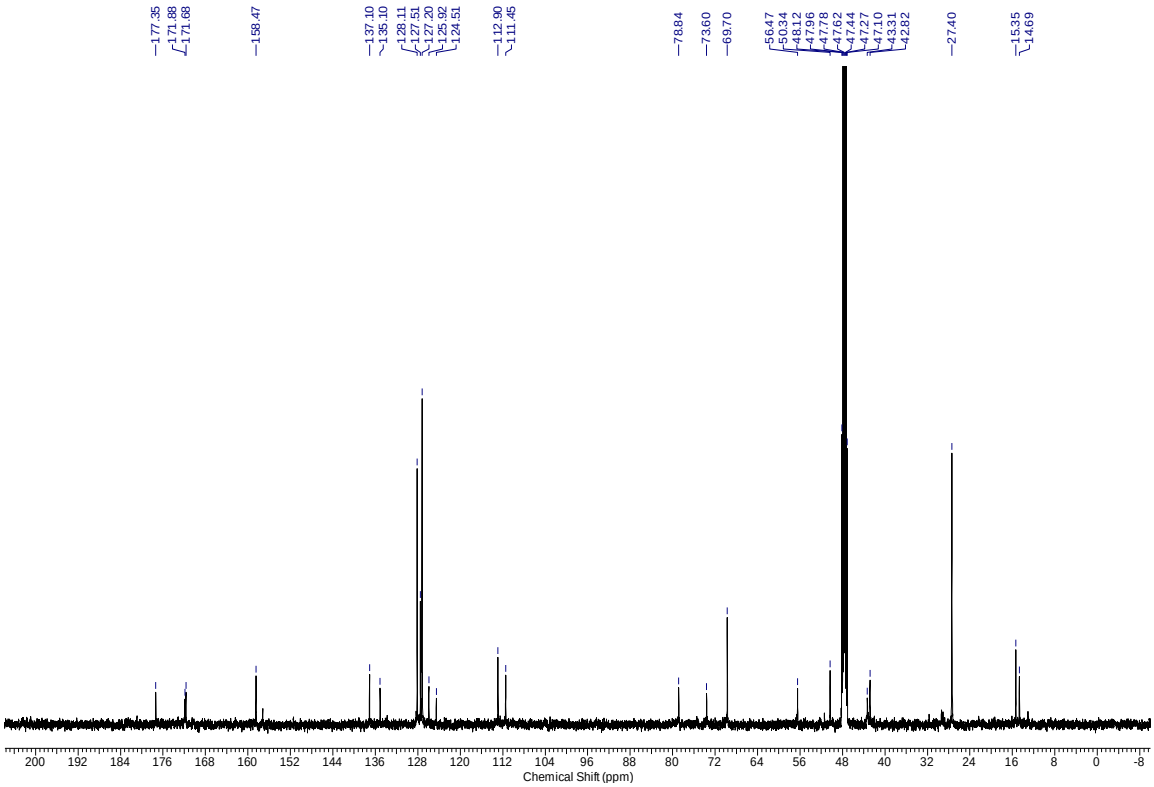
^{1}H NMR of **52** (500 MHz, CD_3OD)



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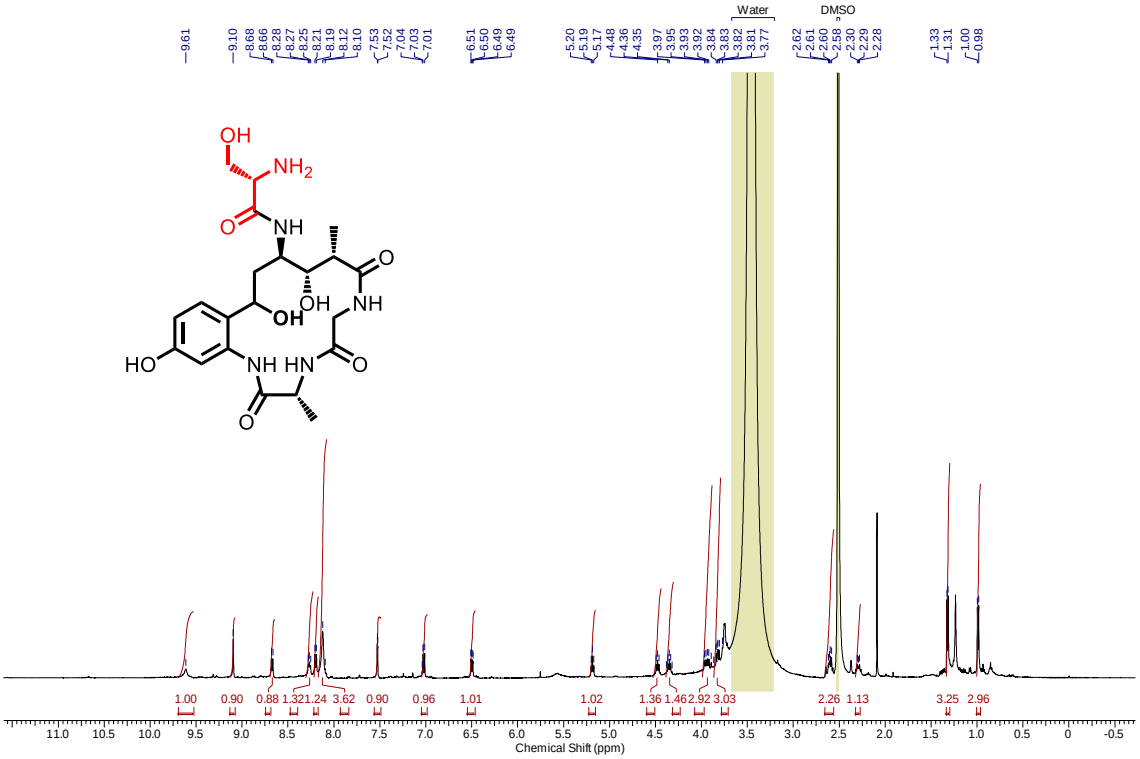
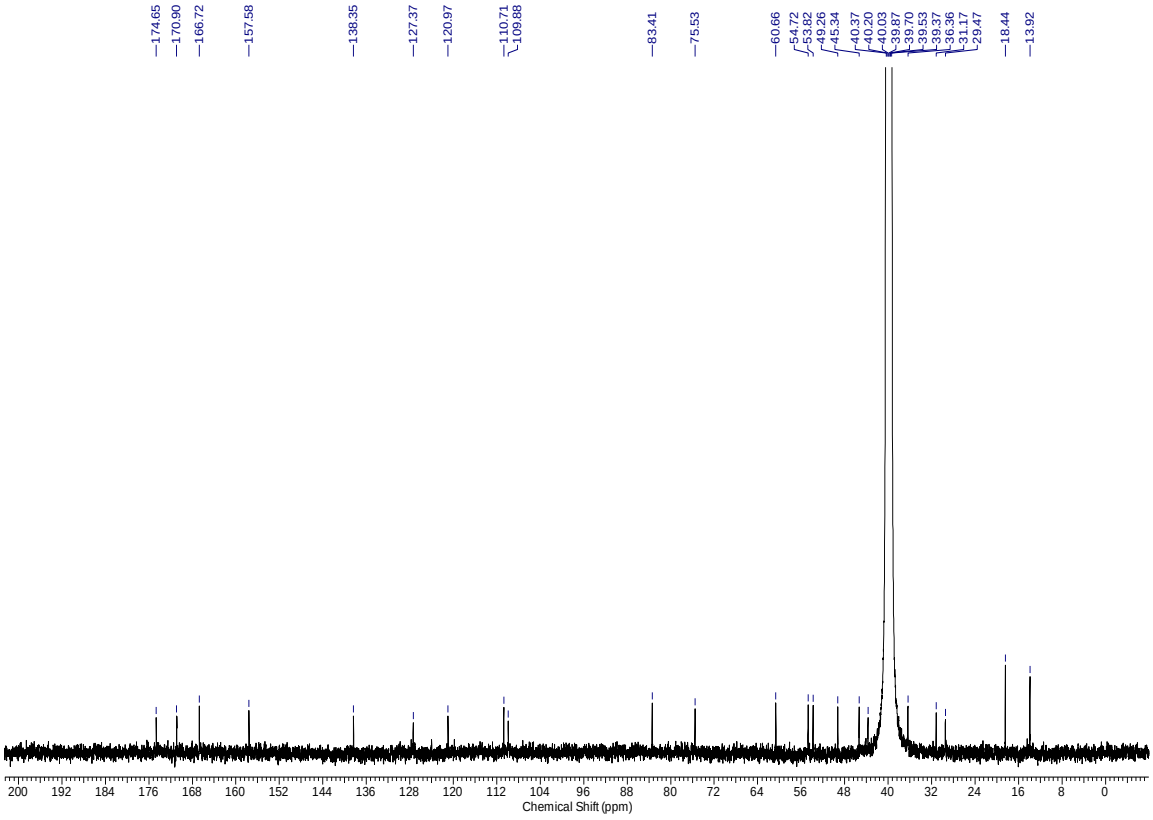
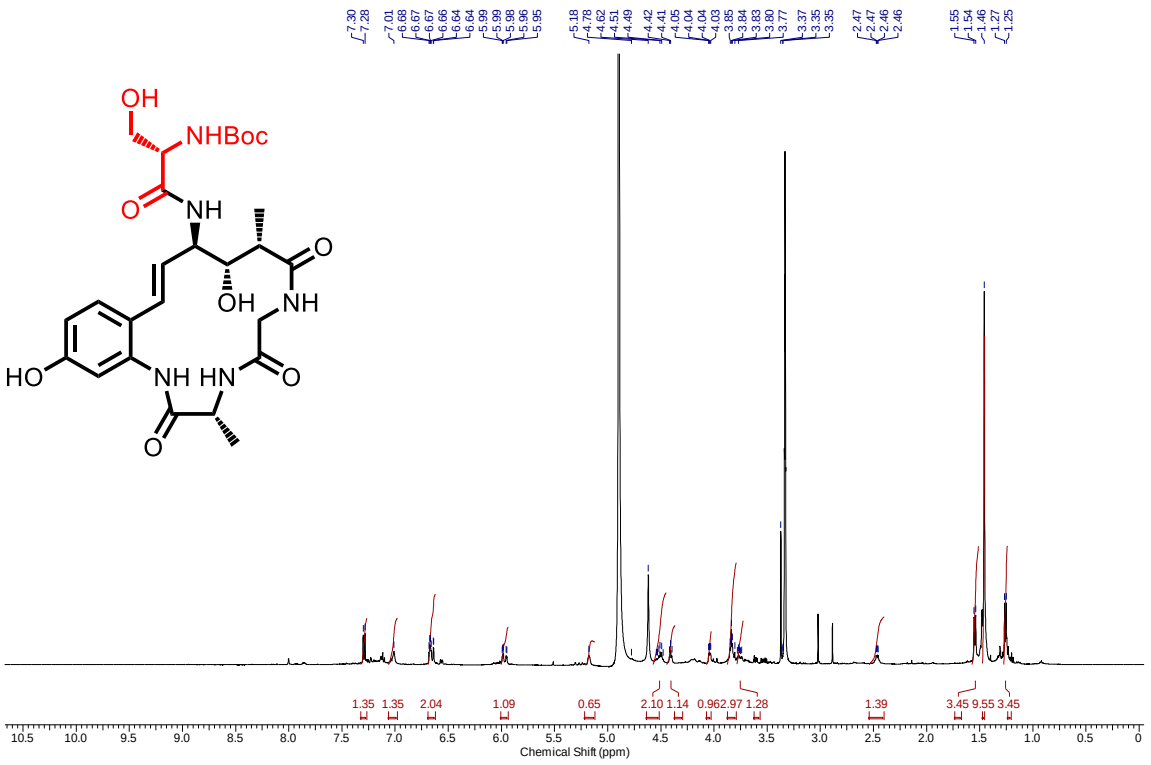
^{13}C NMR of **52** (125 MHz, CD_3OD)



^{1}H NMR of **64** (500 MHz, DMSO-d_6)



^{13}C NMR of **64** (125 MHz, DMSO-d_6)



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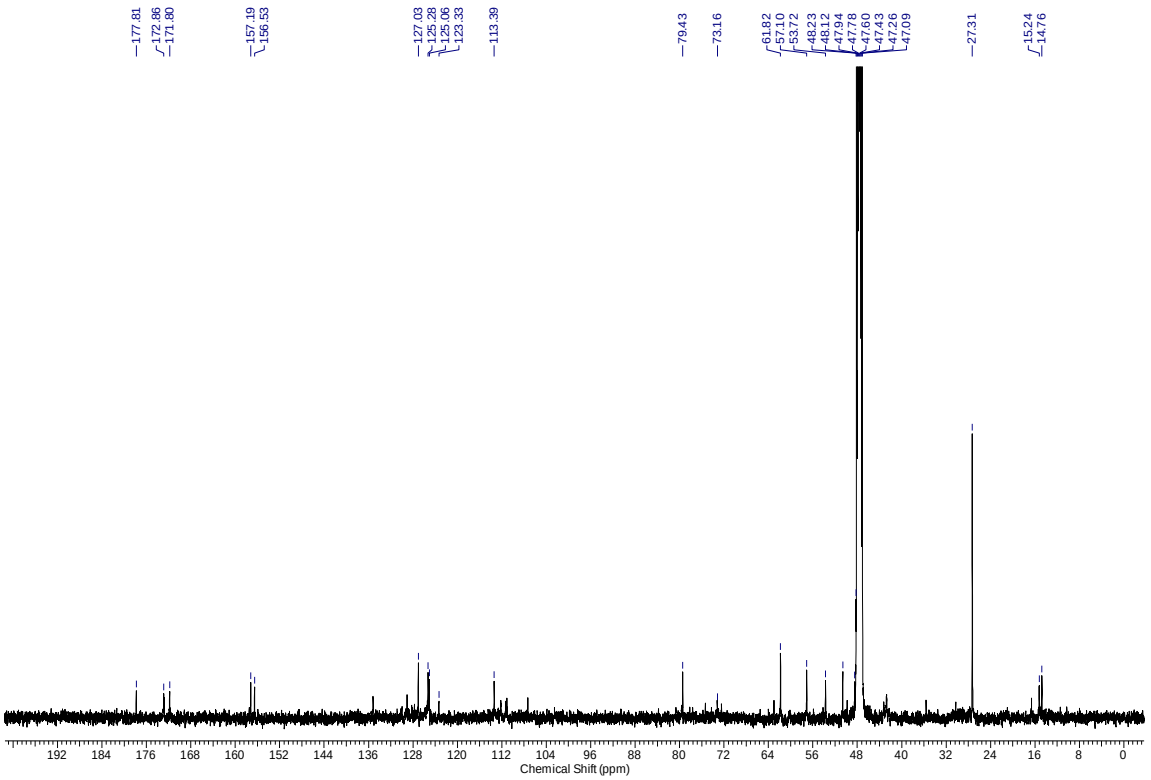
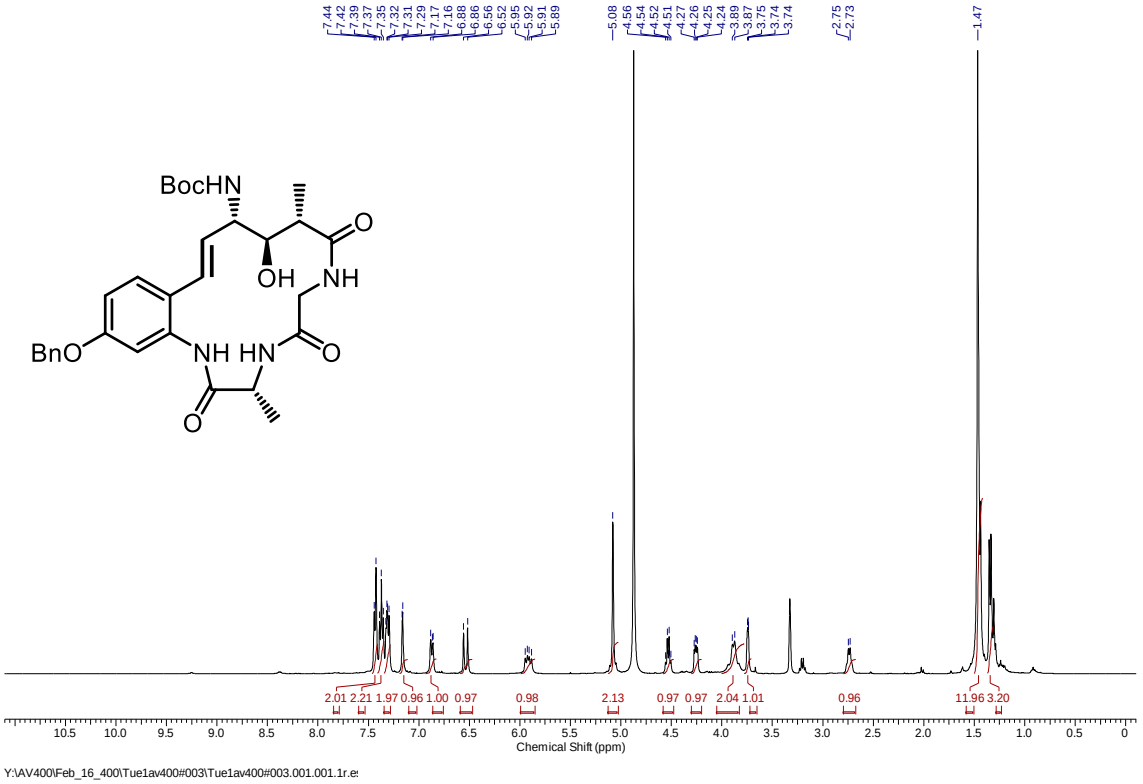
^{1}H NMR of **65** (500 MHz, CD_3OD)

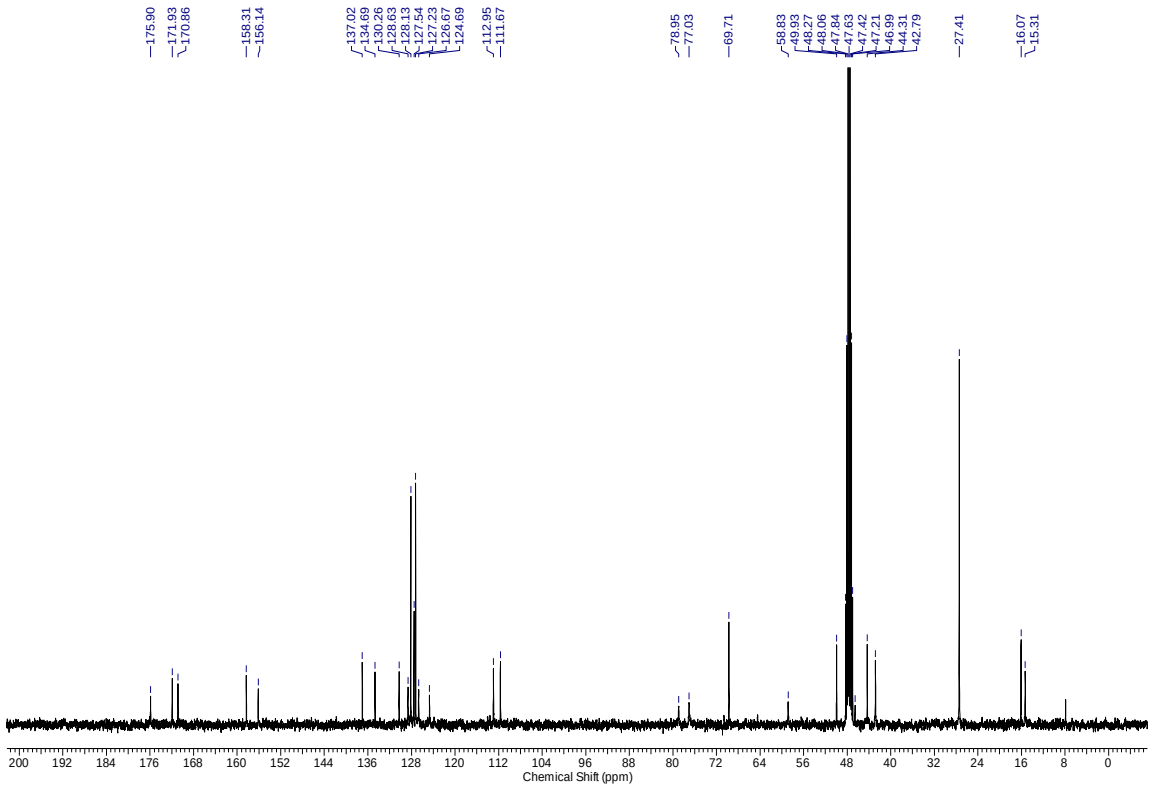


Y:\AV500\2016_AV500\FEB_16_AV500\Sat4av500#021\Sat4av500#

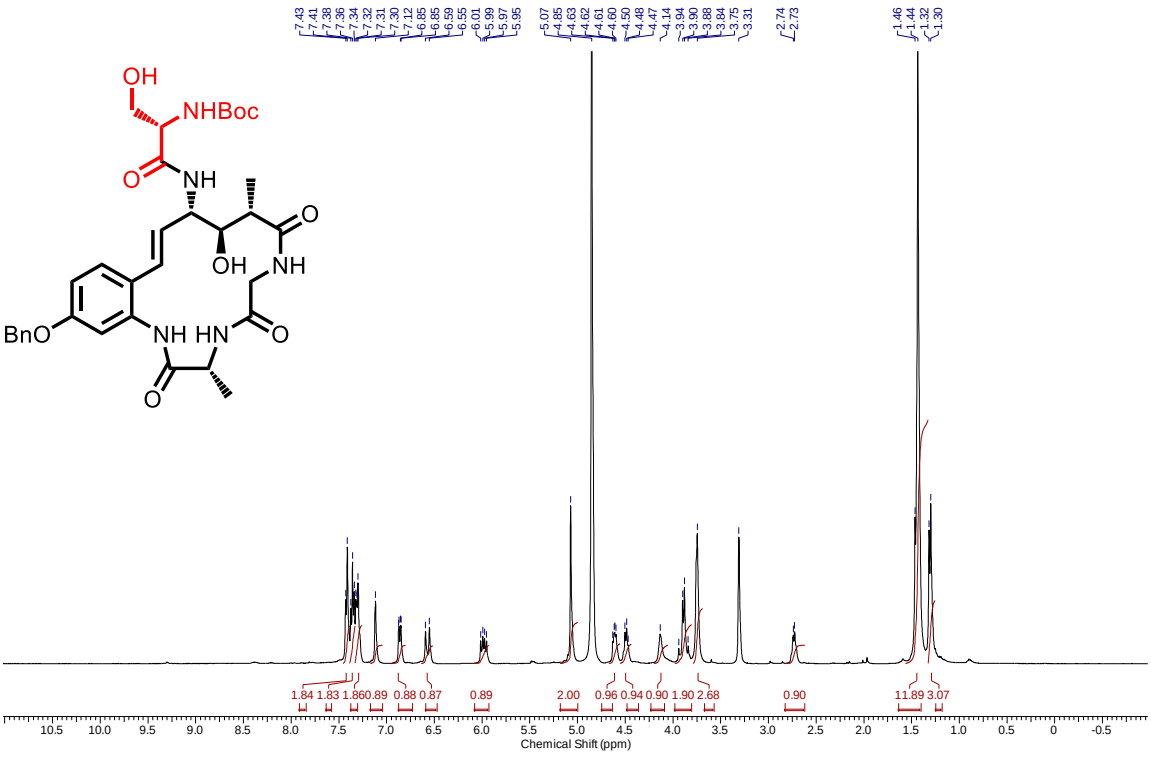
^{13}C NMR of **65** (125 MHz, CD_3OD)



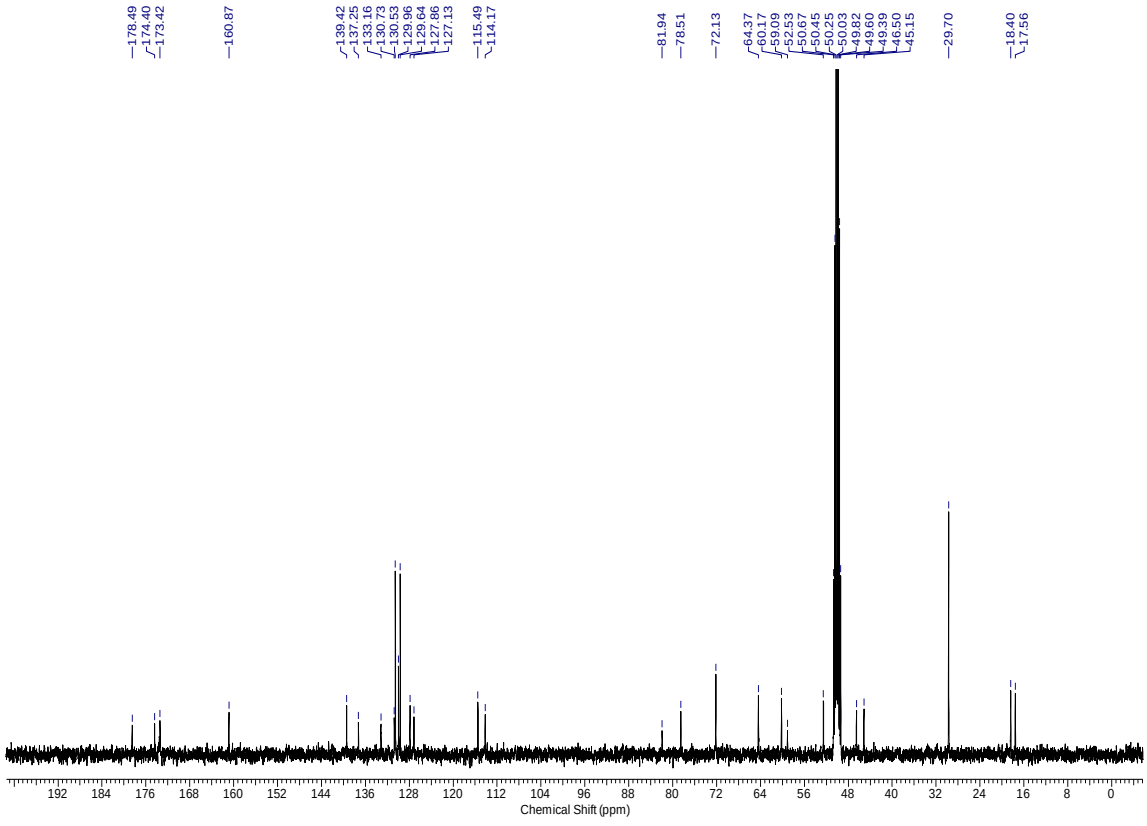
^{1}H NMR of **53** (400 MHz, CD_3OD)



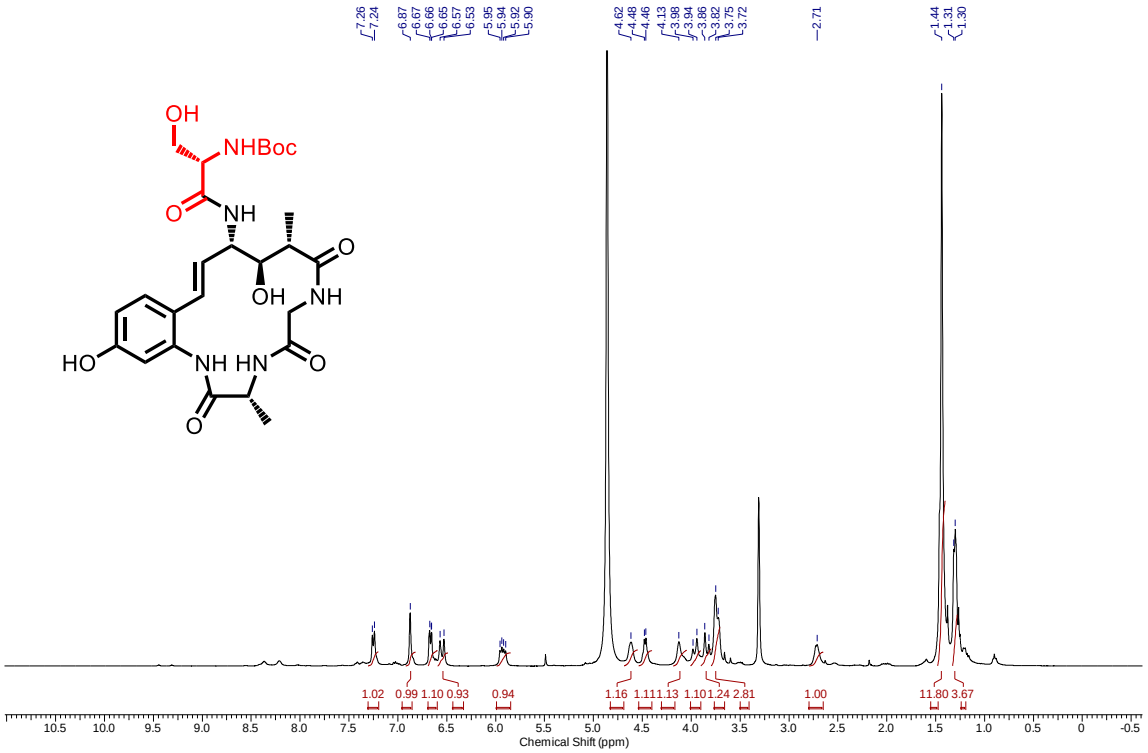
^{13}C NMR of **53** (100 MHz, CD_3OD)



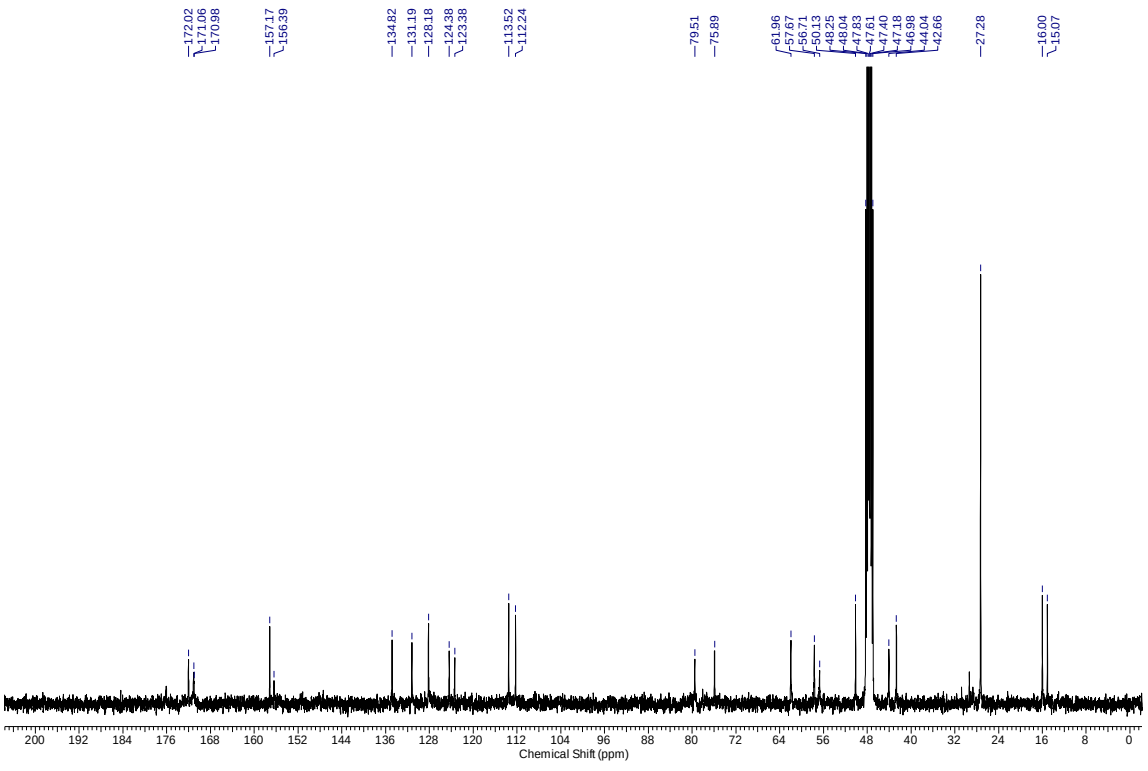
^{1}H NMR of **66** (400 MHz, CD_3OD)



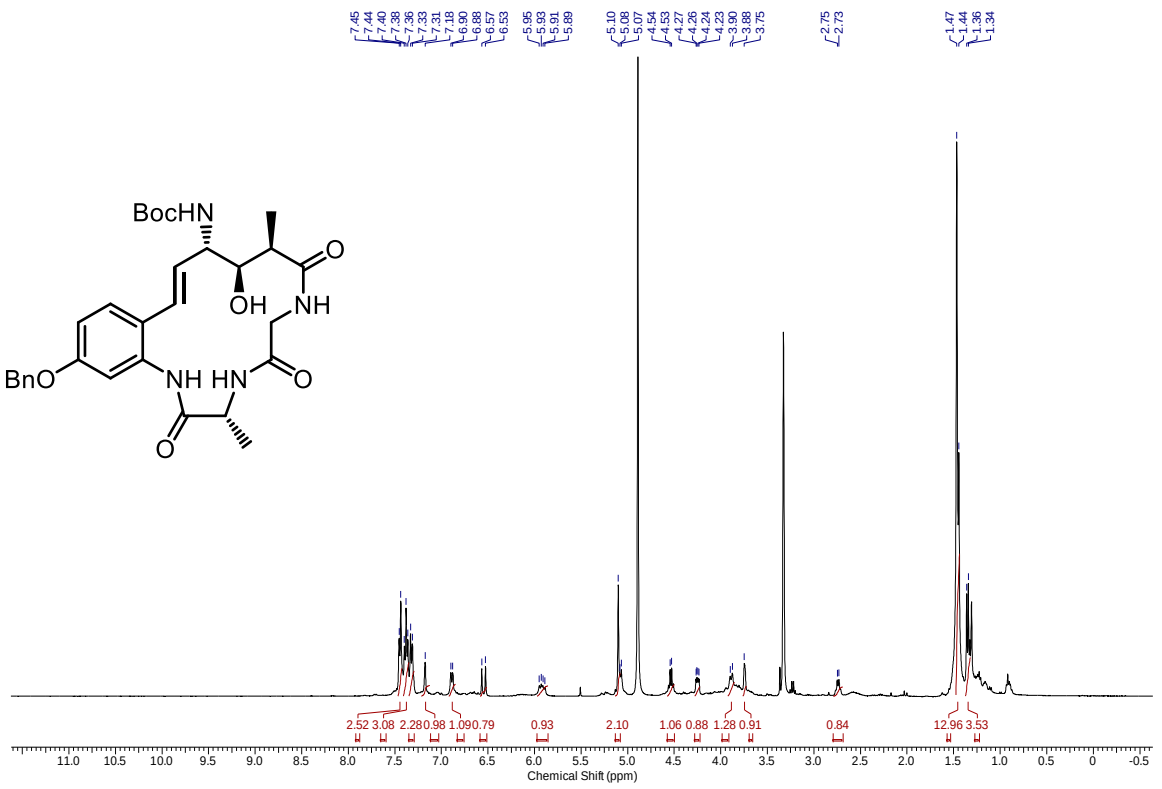
^{13}C NMR of **66** (100 MHz, CD_3OD)



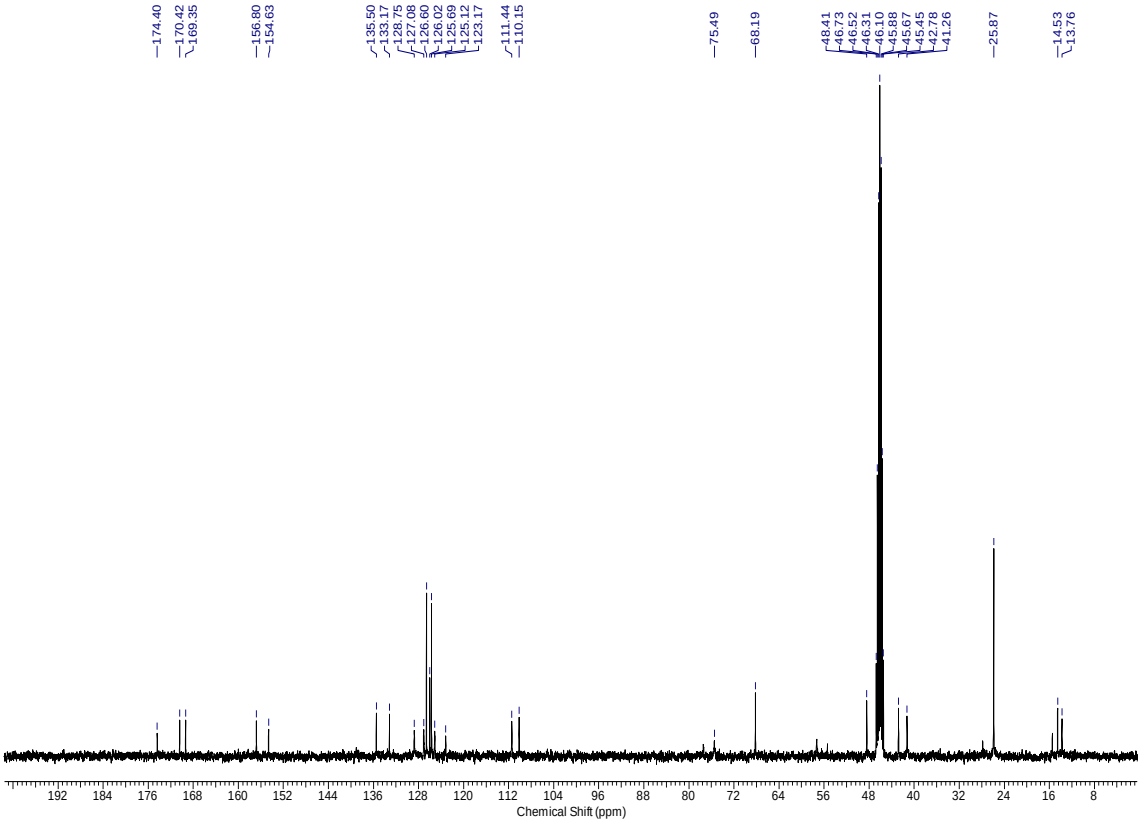
^{1}H NMR of **67** (400 MHz, CD_3OD)



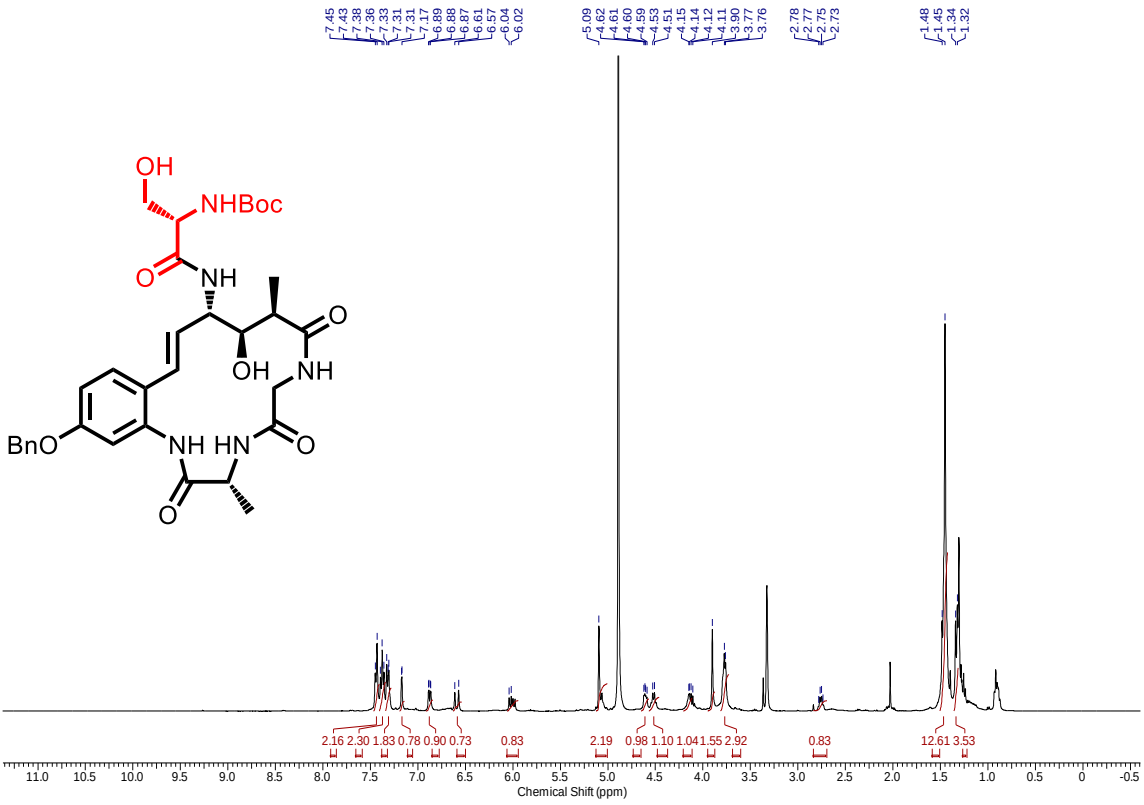
^{13}C NMR of **67** (100 MHz, CD_3OD)



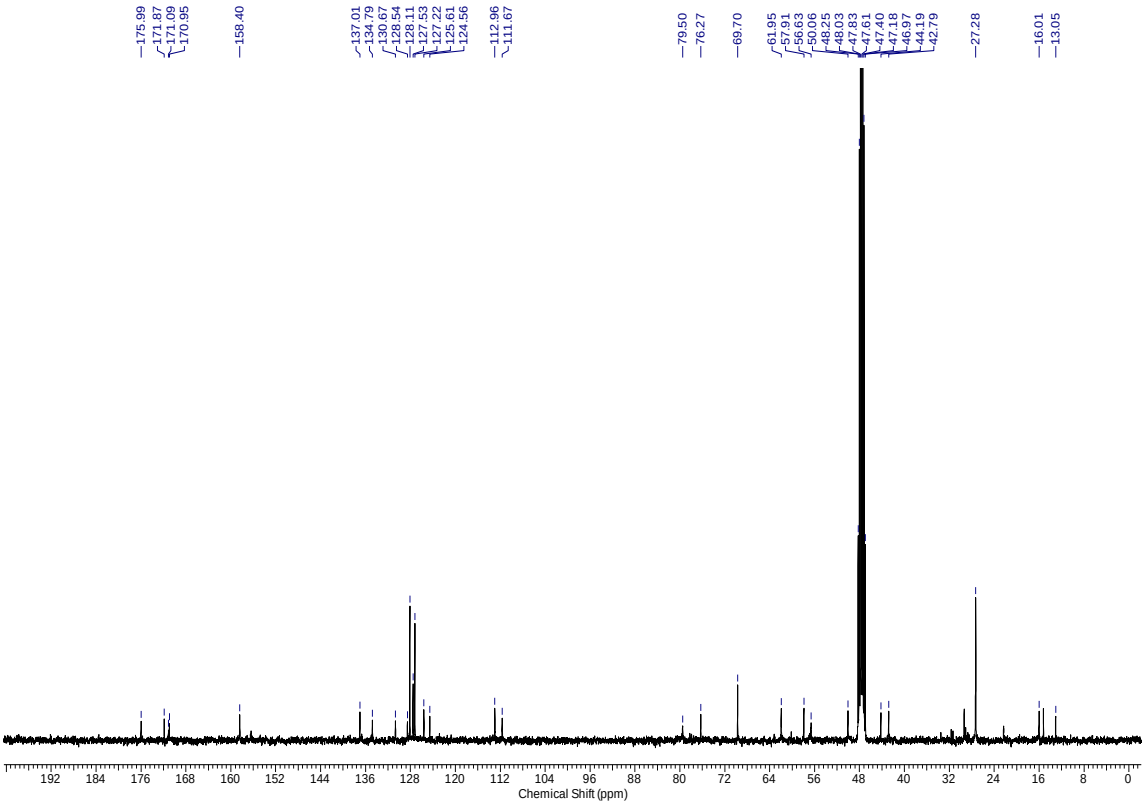
^{1}H NMR of **54** (400 MHz, CD_3OD)



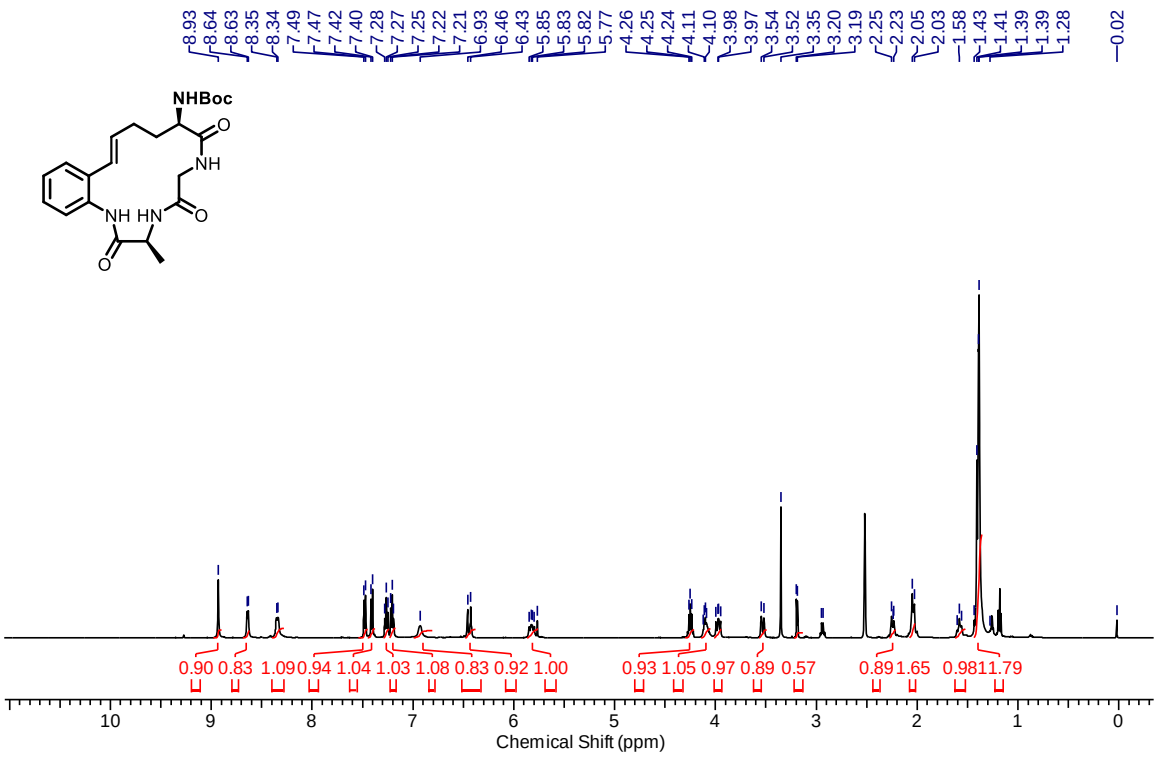
^{13}C NMR of **54** (100 MHz, CD_3OD)



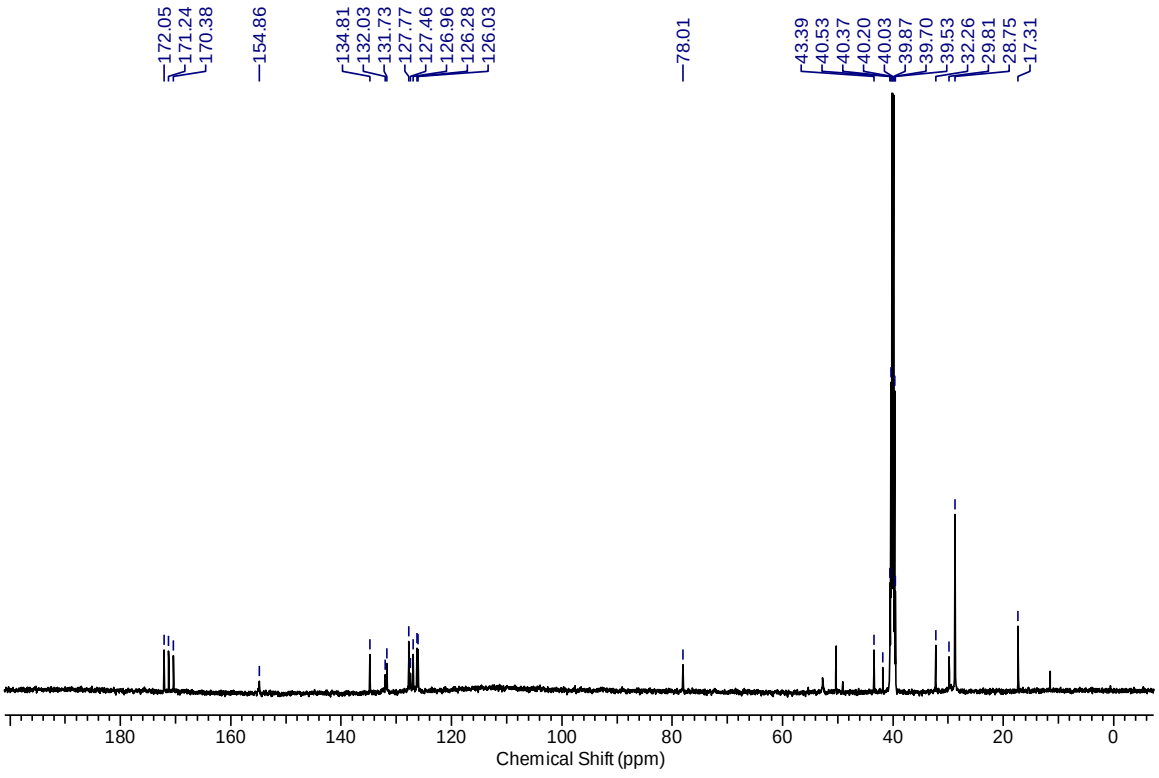
^{1}H NMR of **68** (400 MHz, CD_3OD)



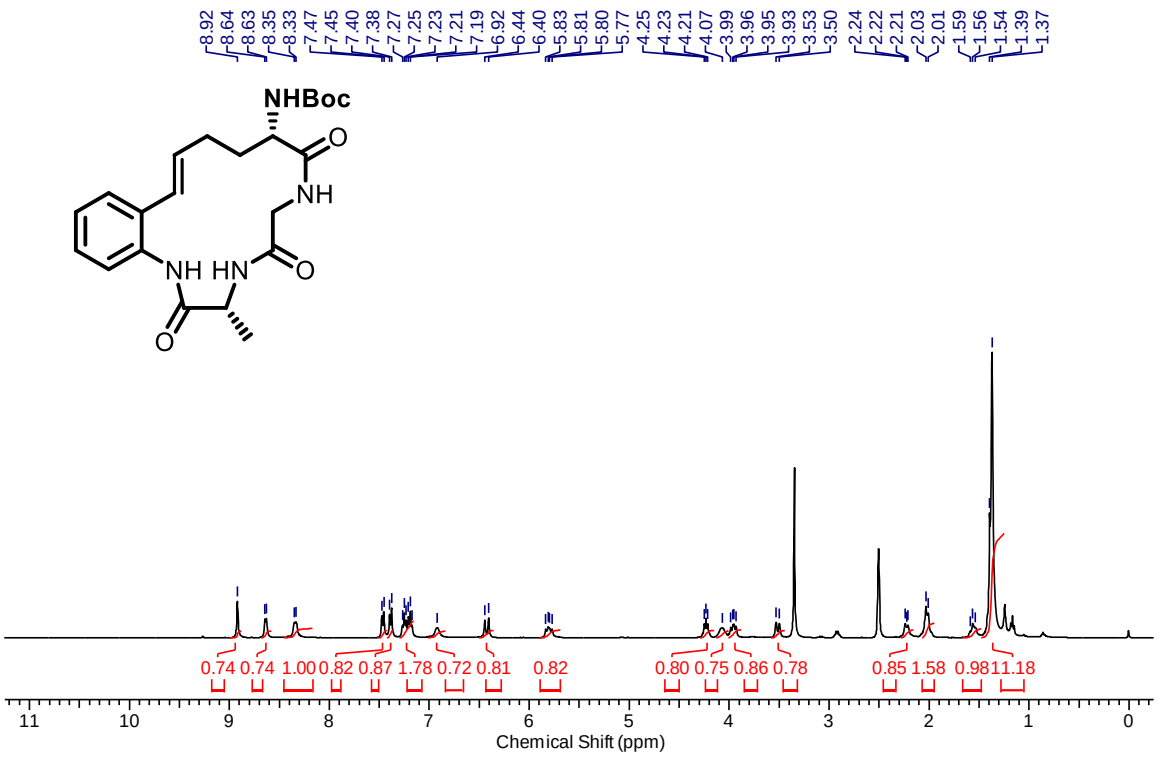
^{13}C NMR of **68** (100 MHz, CD_3OD)



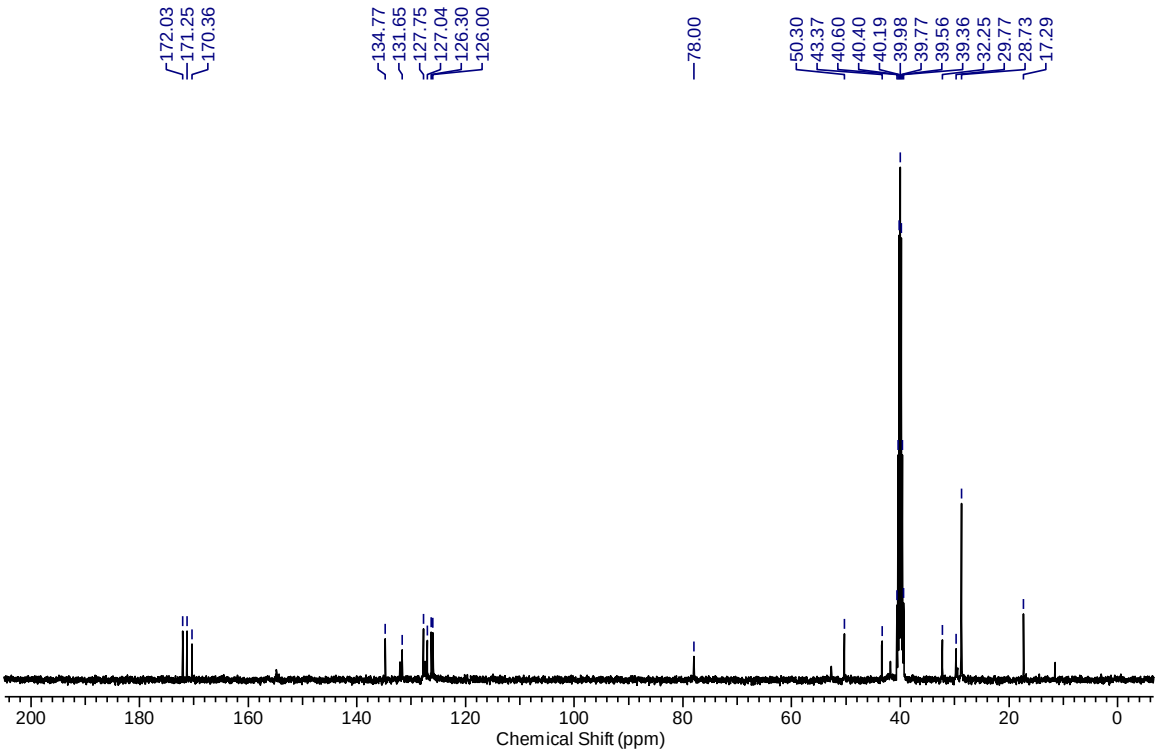
^{1}H NMR of **74** (500 MHz, DMSO-d_6)



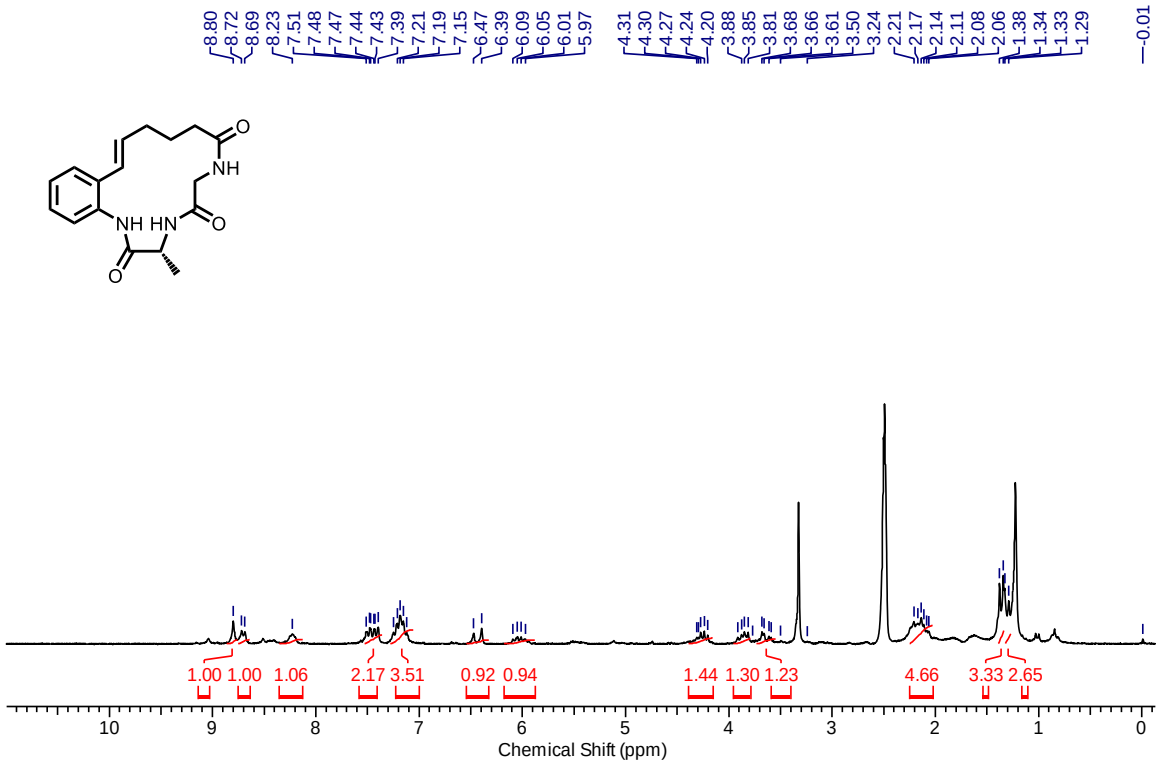
^{13}C NMR of **74** (125 MHz, DMSO-d_6)



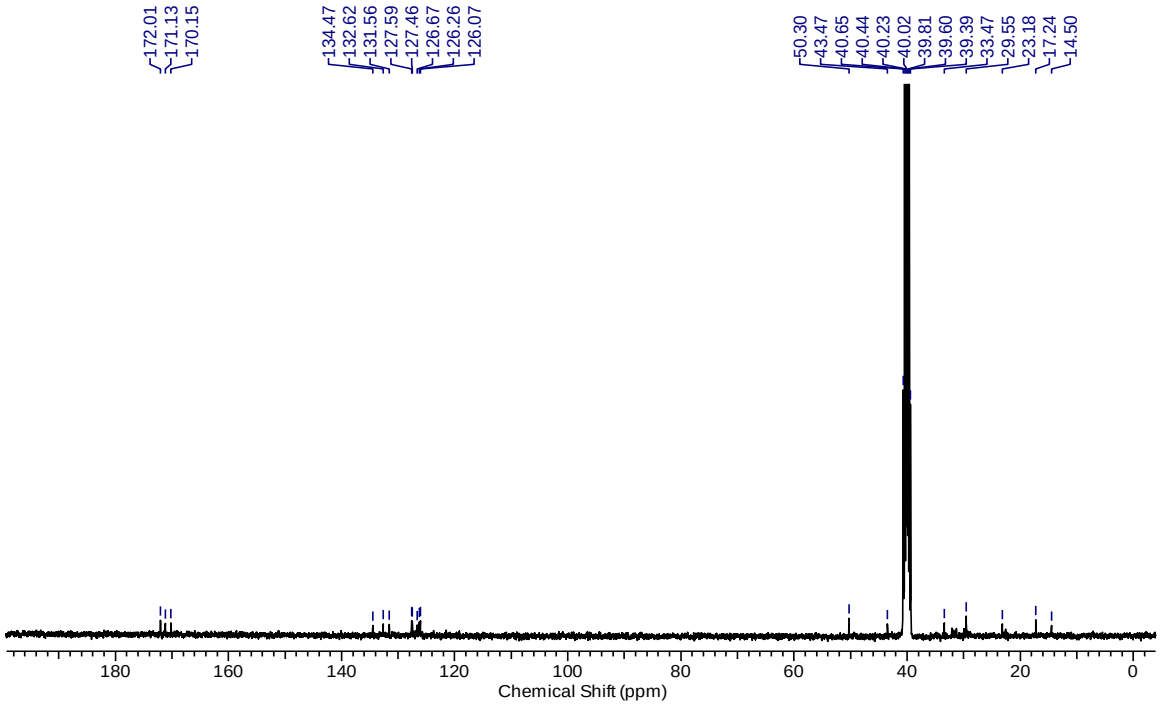
^{1}H NMR of **75** (400 MHz, DMSO-d_6)



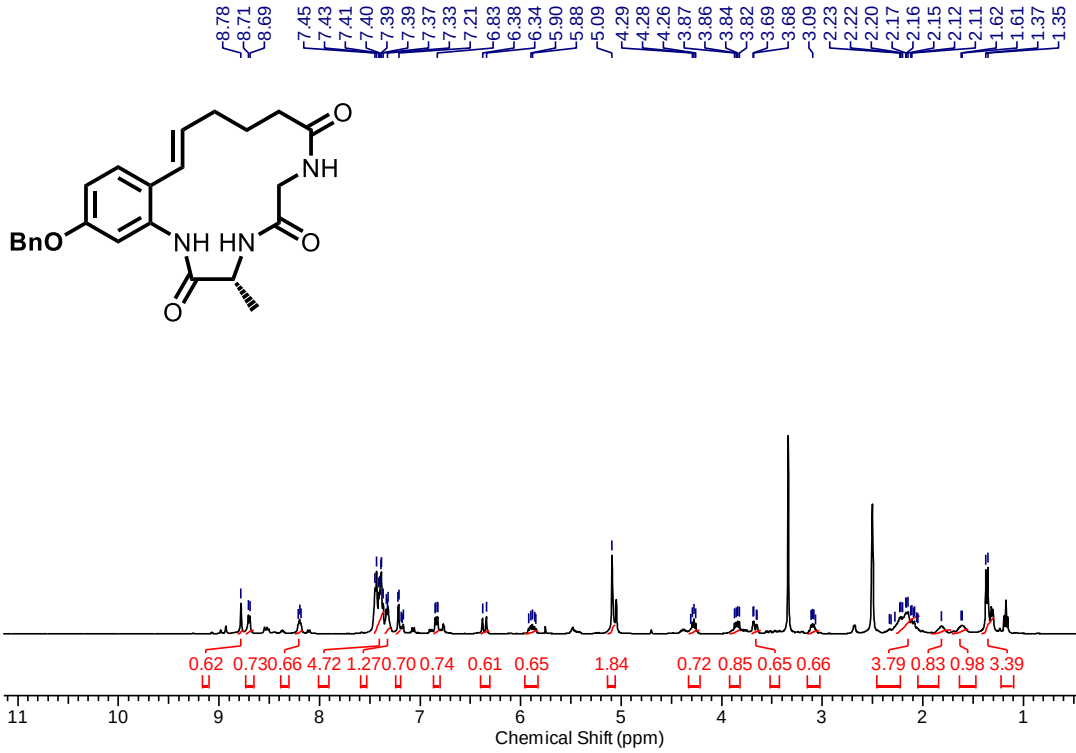
^{13}C NMR of **75** (100 MHz, DMSO-d_6)



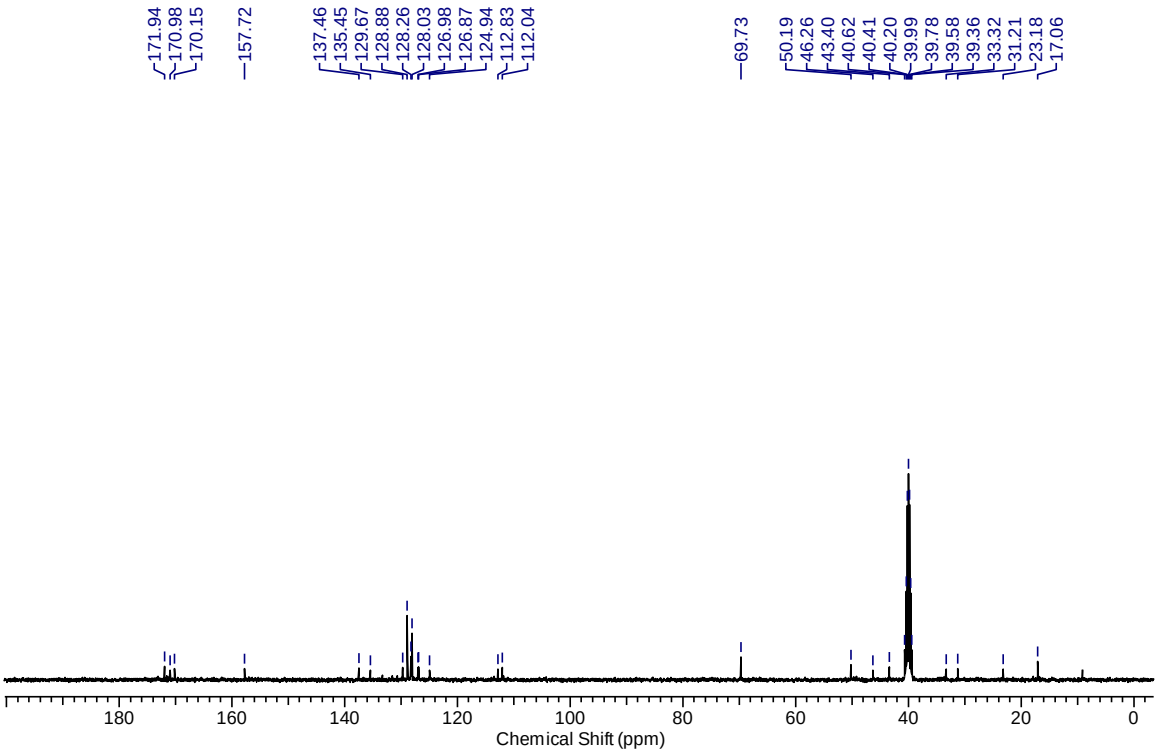
^{1}H NMR of **86** (200 MHz, DMSO-d_6)



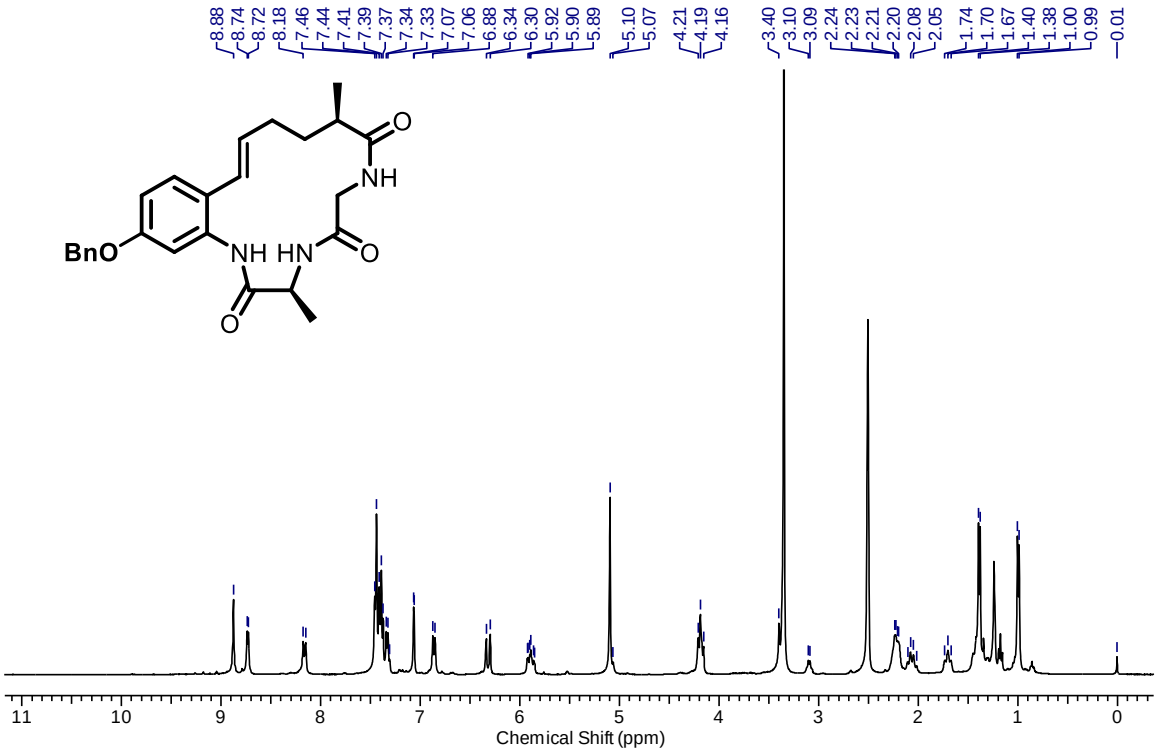
^{13}C NMR of **86** (100 MHz, DMSO-d_6)



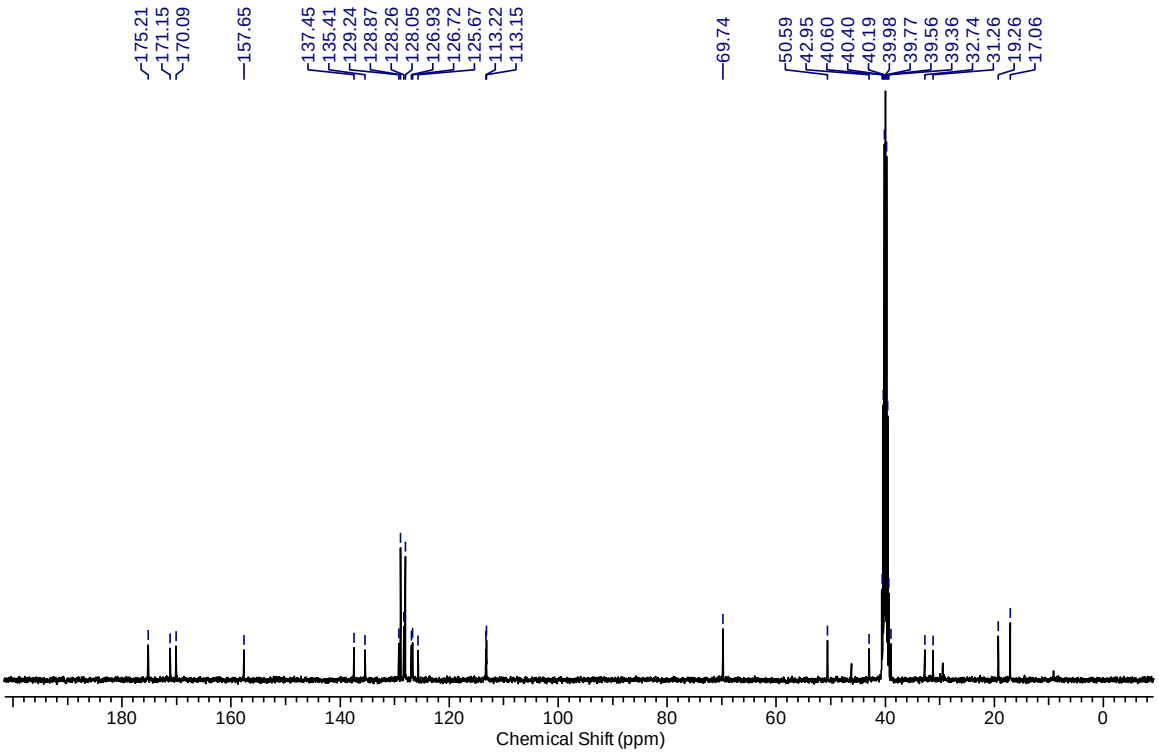
^{1}H NMR of **85** (400 MHz, DMSO-d_6)



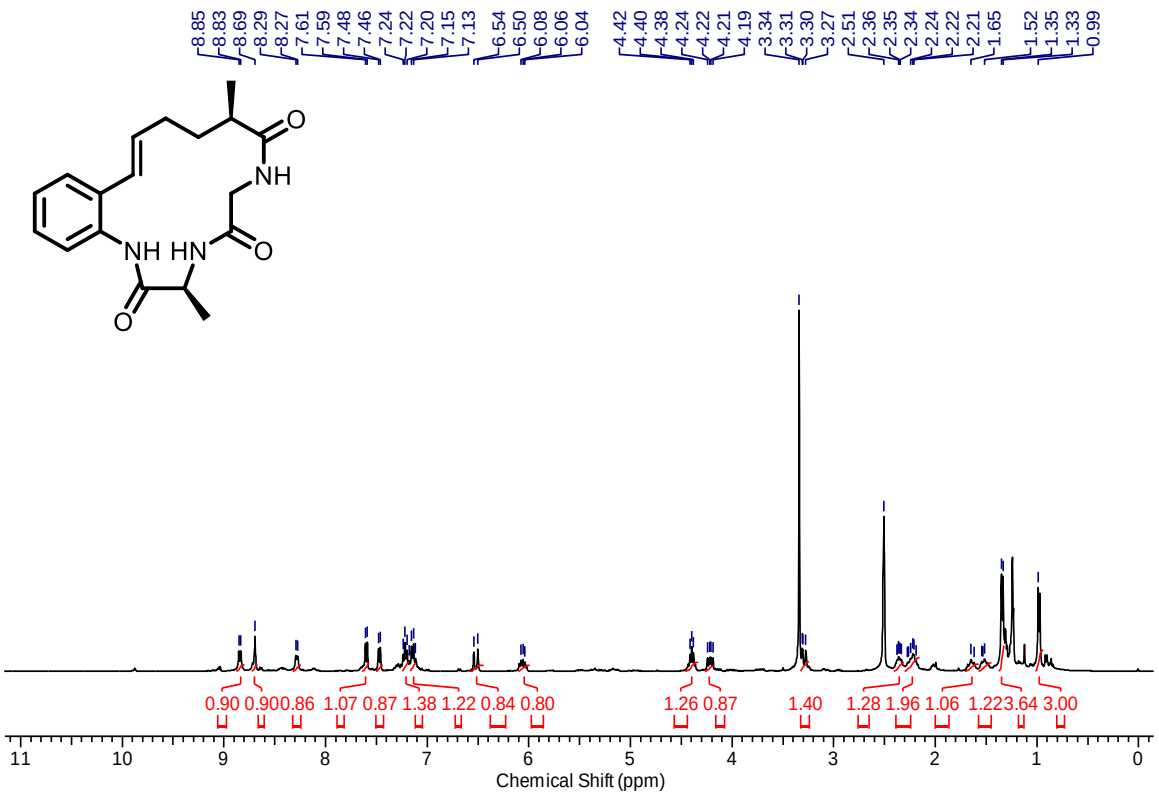
^{13}C NMR of **85** (100 MHz, DMSO-d_6)



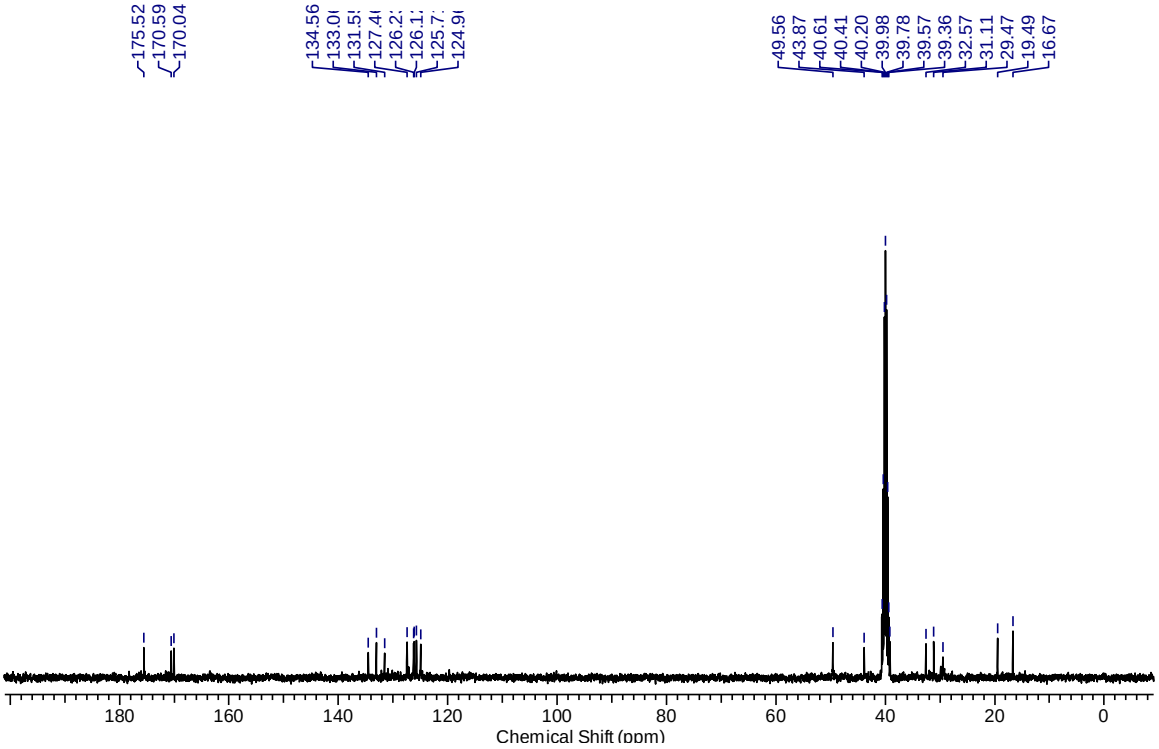
^{1}H NMR of **92** (400 MHz, DMSO-d_6)



^{13}C NMR of **92** (100 MHz, DMSO-d_6)



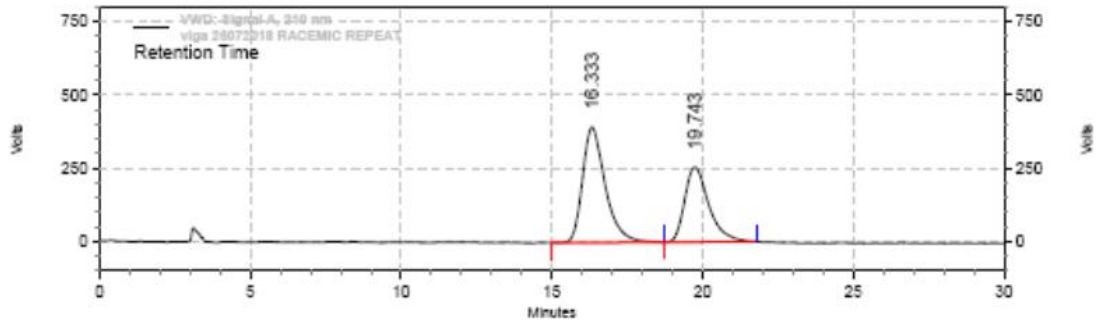
^{1}H NMR of **90** (200 MHz, DMSO-d_6)



^{13}C NMR of **90** (125 MHz, DMSO-d_6)

1.2.13. HPLC spectra

Compound: Racemic Vigabatrin
Acquired: 7/26/2018 10:45:57 PM Printed: 8/1/2018 8:51:08 PM

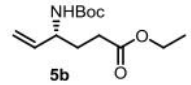


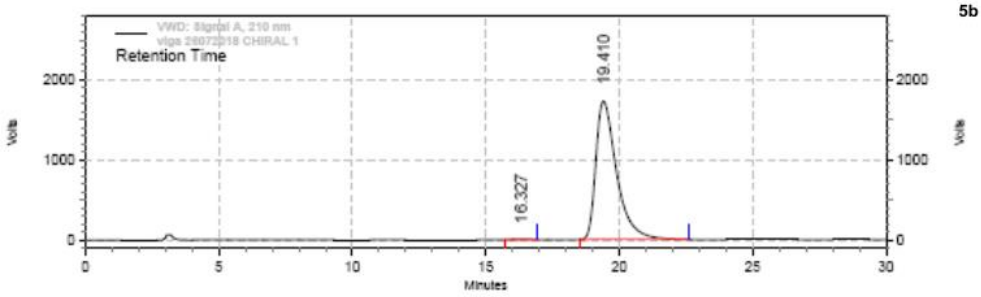
VWD: Signal A, 210 nm Results

Retention Time	Area	Area %
16.333	346664382	59.22
19.743	238767559	40.78
Totals	585431941	100.00

Column : CHIRALPAK AD-H
Eluent System : 95 : 05 (HEXANE:IPA)
Flow rate: 0.6 ml/min
Injection vol..: 20µl
Wavelength: 210 nm
Sample Conc.: 2 mg/ ml

Compound: (*R*)-Vigabatrin
Acquired: 7/26/2018 9:43:19 PM Printed: 8/1/2018 8:55:59 PM

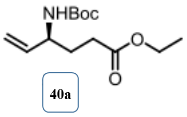




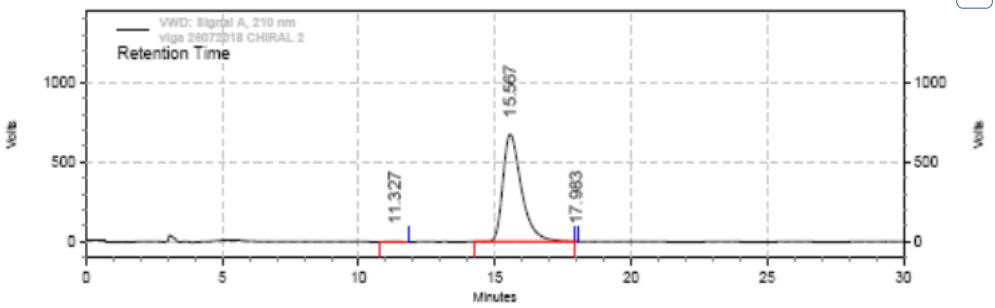
VWD: Signal A, 210 nm Results

Retention Time	Area	Area %
16.327	3972630	0.25
19.410	1569564493	99.75
Totals	1573537123	100.00

Column : CHIRALPAK AD-H
Eluent System : 95 : 05 (HEXANE:IPA)
Flow rate: 0.6 ml/min
Injection vol..: 20µl
Wavelength: 210 nm
Sample Conc.: 4 mg/ ml



Compound: (*S*)-Vigabatrin
Acquired: 7/26/2018 10:14:46 PM Printed: 8/1/2018 8:59:22 PM



VWD: Signal A, 210 nm Results

Retention Time	Area	Area %
11.327	456681	0.09
15.567	536786901	99.91
17.983	23563	0.00
Totals		
	537267145	100.00

Column : CHIRALPAK AD-H
Eluent System : 95 : 05 (HEXANE:IPA)
Flow rate: 0.6 ml/min
Injection vol.: 20µl
Wavelength: 210 nm
Sample Conc.: 1 mg/ ml

S17

Chapter 2

Design, Synthesis and Biological Evaluation of Silicon Incorporated Morpholine Antifungals

2.1. Introduction

2.1.1. Brief history of fungicides

Fungicides are the agents derived from chemicals or biologicals which are used to inhibit the growth of fungi or diseases associated with them. Additionally, fungicides play a crucial role in maintaining the yield and quality of the crop. Thus, a proper knowledge of fungicides and their use in agriculture leads to the increase in the overall yield of crops and storage life of harvested material. These fungicides are extensively being used in agriculture, horticulture and floriculture sectors which are producing vegetables, fruits, oilseed, grains, cereals, flowers and other crops. In addition to these, they also happen to be equally useful in fighting fungal infections in animals. From recent reports it is quite evident that fungicides' market is estimated to be valued at USD 14.49 Billion in 2016 and projected to grow to USD 19.17 Billion by the year 2022.[1] This report suggests that fungicidal market growth can be attributed to increased crop loss due to fungal diseases, rise in food security concerns for the growing population along with the change in farming practices from traditional to conventional methods. Hence, fungicides can be viewed as a promising business opportunity in the years to come. Whereas slow development of anti-fungal drugs can be attributed to the fact that fungi are eukaryotic, closer to human hosts which complicates the search for antifungal targets. However, modern developments and tools has helped in the understanding of the mechanism of action of several antifungal agents.[2, 3] Further efforts are also being devoted towards the development of new and novel antifungals with reducing toxicity, enhanced bioavailability and improved antifungal spectrum against resistant fungus as well. Though various anti-fungal drugs are presently available in the market for addressing issues related to human health and crop protection observation of developed resistance by the fungal pathogens against these present-line treatment are worth considering and necessitates more efforts.[4 – 6]

2.1.2. Desirable properties of fungicides

An ideal antifungal should possess the following characteristics (a) broad spectrum of activity towards a different yeast and fungi (b) fungicidal in action rather than fungistatic (c) specific fungal target should be selective with respect to host targets (d) orally available, having minimal side effects or toxicities. It is clear that developing an antifungal drug that satisfies above criteria is a challenging task and requires the proper knowledge of biological targets that are unique and specific to fungi.

2.2. Therapeutic targets of antifungal agents

The fungal cell wall and cell membrane are the attractive therapeutic anti-fungal targets because of unique fungal structure.[7] Details are provided below as they are very relevant to present thesis work.

2.2.1 Cell membrane

Unlike mammalian cells, fungal cell wall constitutes membrane lipid ergosterol which is present in major amount along with chitin. Besides, in eukaryotic cell wall, sterols play crucial role in determining membrane fluidity, permeability, controls the organization and structure of cell membranes.[8, 9] They serve as precursors for hormones essential for parasitic growth development and regulation of parasitic life function, thus making it a classic target for anti-parasitic agents.[10, 11] Sterols are classified into three main types depending on the type of sources as cholesterol is present in animals while phytosterol in plants and ergosterol is present in the fungal cell wall.[3] Vertebrates, plants and fungi follows same biogenetic pathway for sterols which starting at acetyl-CoA up to squalene. Ergosterol is the most common type of sterol present in fungal and protozoal membranes. Based on this and the fact that the squalene segment from lanosterol to ergosterol, are specific for fungi, biosynthetic pathway of the same is considered to be crucial in the research and development of new antifungal compounds.[10,11] This difference in sterol composition was exploited as one of the target for antifungal drug discovery, which

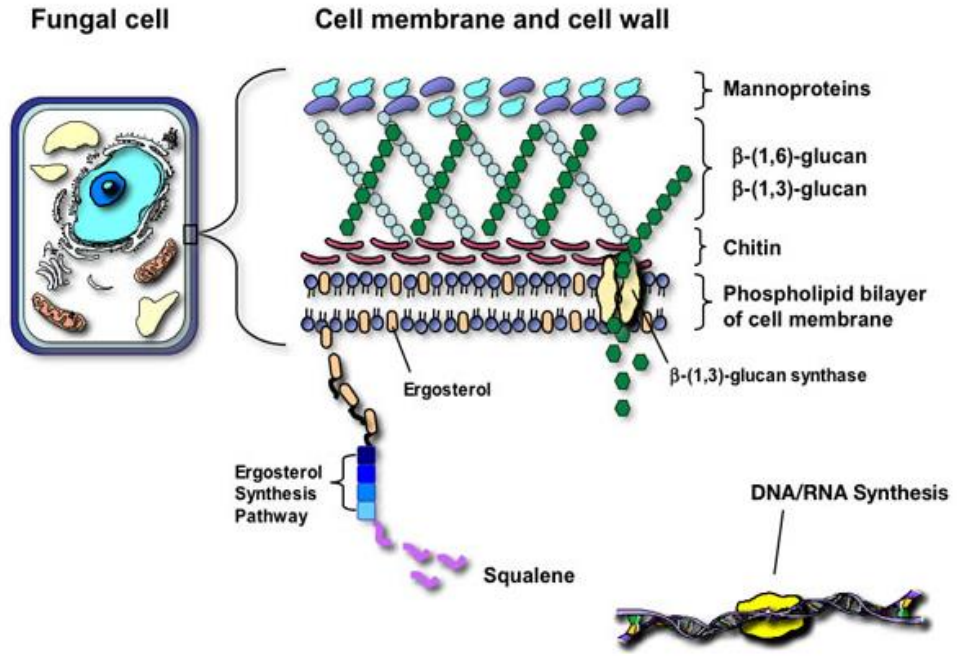


Figure 2.1. Fungal cell wall and cell membrane with its components

(Image taken from: http://www.biologydiscussion.com/fungi/structure-of-fungal-cell-with-diagram-fungi/63013)

resulted in several classes of antifungal agents currently available for the treating various fungal infections.[12] For instance, currently marketed antifungals, which are inhibiting ergosterol synthesis through selective inhibition of different enzymes involved in the biosynthesis pathway can be divided in to three classes of compounds such as polyenes, azoles and amines.

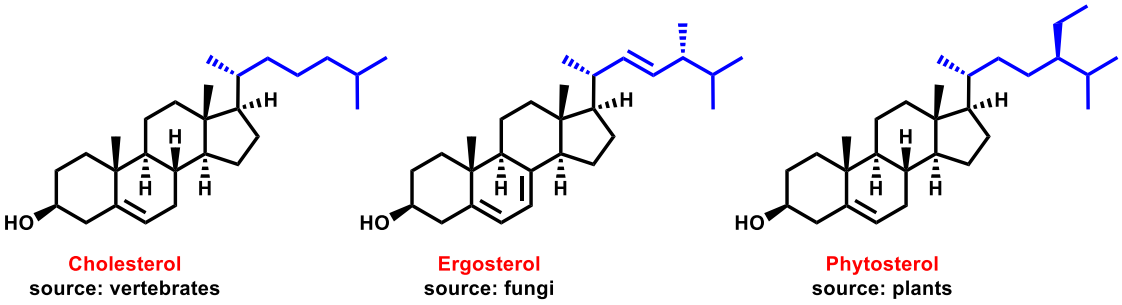


Figure 2.2. Structure of sterols; cholesterol, ergosterol and phytosterol

2.2.1.1. Polyene antifungal agents

The polyene class of compounds possesses macrocyclic skeleton having multiple conjugated double bonds with polyhdroxy groups in one side on the ring which are opposite to the conjugated alkenes system. These special arrangements make polyene class of compounds amphiphilic.[13] Polyne class of compound binds with ergosterol which is present in the cell membrane of fungas resulting in the alteration of the transition temperature of cell causes disruption of cell membrane, increased permeability

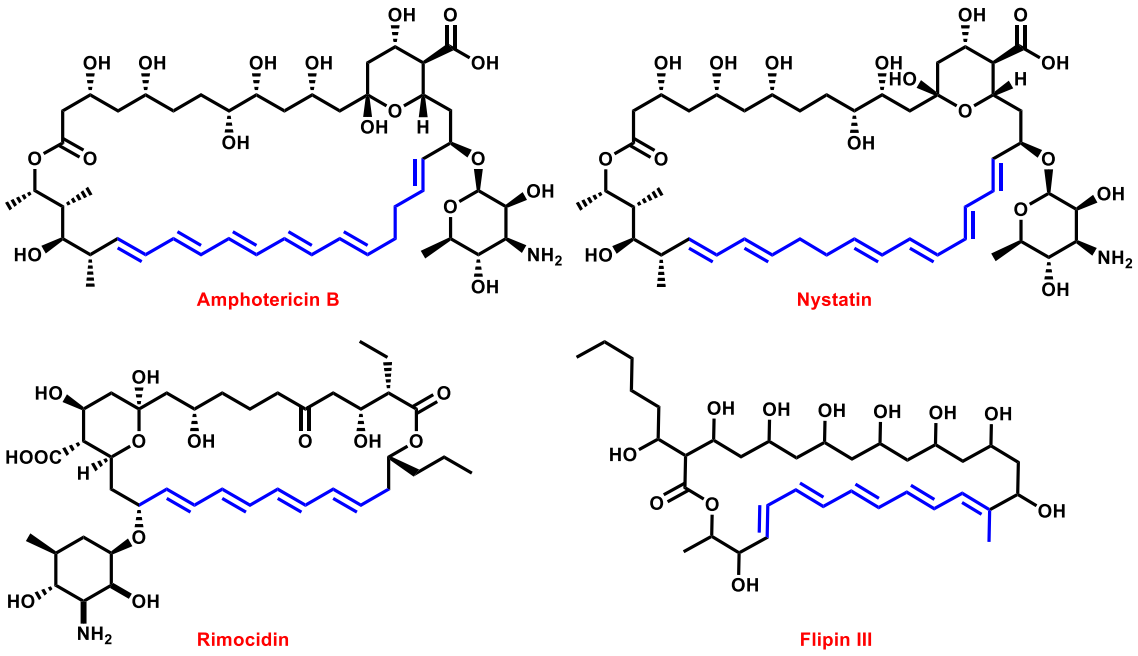


Figure 2.3. Selected polyene antifungal agents

of cells and leakage of cytoplasmic contents which results in death of cells.[14,15] There are several marketed compounds from this class such as amphotericin B, natamycin, rimocidin, filipin III, nystatin and candicine (Figure 2.3.) which are highly selective towards ergosterol than its mammalian counterpart cholesterol. Thus making them selective towards fungal species.

2.2.1.2. Azoles

Azoles are the entirely synthetic class of compounds and one of the most rapidly emerging groups of antifungals.[16, 17] They are subdivided into two types namely imidazoles and triazoles on the basis of number of nitrogens present in the ring. Most of the compounds from this class of antifungal exhibits broad-spectrum activity against fungi *via* the formation of a stoichiometric complex with the heme iron of P-450 through azole nitrogen.[18] Oxiconazole, sulconazole, isoconazole, clotrimazole, butoconazole, tioconazole and bifonazole (Figure 2.4.) from imidazole class are used as topical antifungal agents, they interact with cell membrane which results in death of cell.[19] While the triazoles like fluconazole, itraconazole, isavuconazole, ravuconazole, terconazole, posaconazole and voriconazole are relatively new compared to imidazole and they are found to exhibit low toxicity and increased beneficial effects.

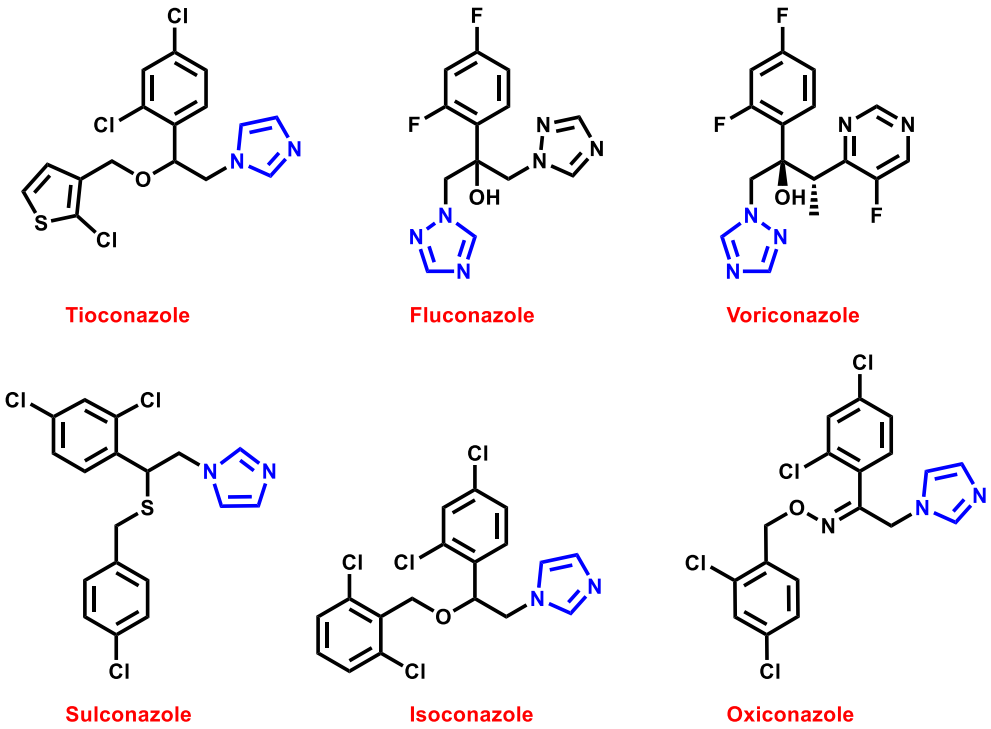


Figure 2.4. Selected azole containing antifungal agents

2.2.1.3. Amine (allylamine and morpholine antifungal agents)

Amines are class of compounds which include allylamines and morpholine antifungal agents. Allylamines indirectly affects fatty acid and biosynthesis of phospholipid, which results in change of squalene as well as ergosterol levels in cellular membranes, which disturbs membrane-bound enzymes eventualy leading to ergosterol depletion and ultimately cell death.[20, 21] Marketed compounds from this class are naftifine, terbinafine, tolnaftate and butenafine used as antifungal agents.[21]

The morpholine class of compounds are entirely synthetic. From this class of compounds only amorolfine is used for human clinical application (nail infections), [22] the rest being agricultural fungicides like fenpropimorph and fenpropidin. They act on the ergosterol pathway by inhibiting two enzymes, Δ^{14} reductase and Δ^{7}-Δ^{8} isomerase.[22, 23] However, the antifungal morpholines such as fenpropimorph, fenpropidin also inhibits ergosterol biosynthesis in fungal cells by inhibiting biosynthesis of sterol reductase or isomerase.[24]

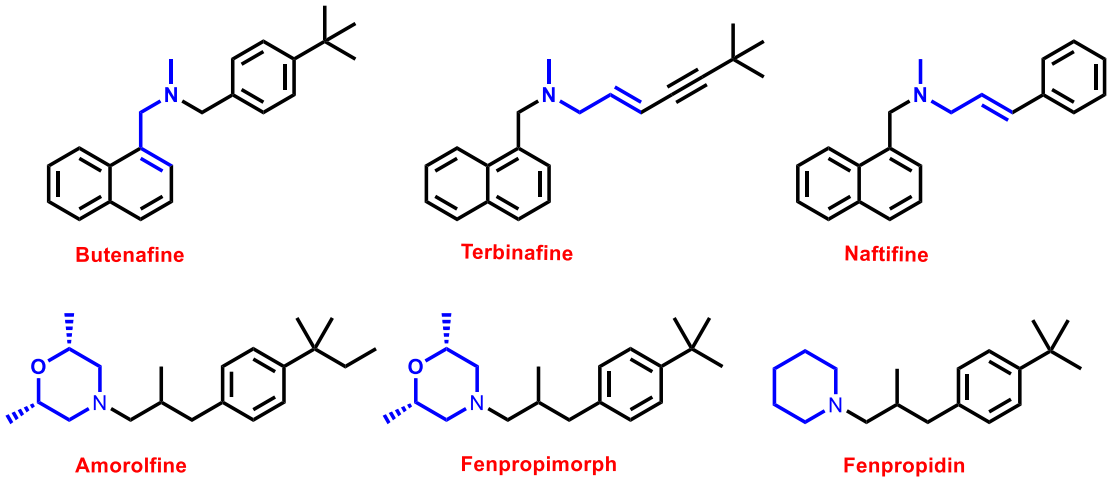


Figure 2.5. Allylamines and morpholine containing antifungal agents

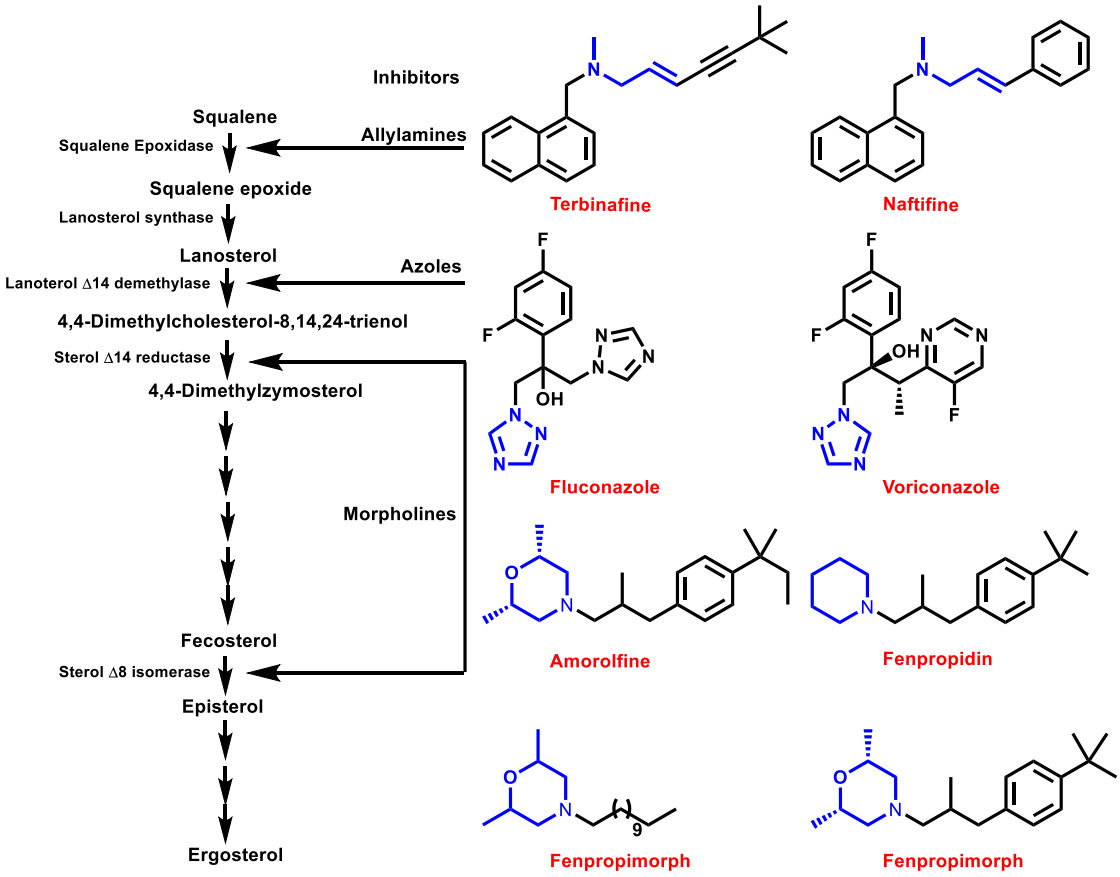


Figure 2.6. Ergosterol biosynthetic pathway and known inhibitors of the pathway enzymes

2.3. Silicon in drug discovery

Bioisosteres are substituents or groups with identical, chemical or physical properties which makes broadly similar biological potential in a chemical compound.[25-27] The utility of bioisosteres is to get better potency, enhanced selectivity, altered physical properties, modulated metabolism and reduced toxicity.[28, 29] Thus, substitution of carbon by silicon in biologically active compounds has been an attractive idea for many synthetic chemists.[25-27, 30] Replacement of carbon with silicon which is generally known as 'silicon switch' has been there for some time in the area of medicinal chemistry and it

is a growing field.[31-34] The metabolism of silicon-containing molecules sometimes differ from their carbon analogues and also no unusual toxicities related to silicon were reported in the literature yet. Silicon has been explored as an isostere of carbon in the several bioactive molecules.[31-34]

Following are the similarities and differences between silicon and carbon which are useful in drug discovery.

- *Carbon-Silicon bond length:* Bond length of Si-C is 1.87 Å and which is larger (20%) as compared to that of C-C bond length (1.54 Å).[35] This difference in bond length may attribute to its shape, size and conformation of the molecule and also their interaction with the particular enzyme or receptor.
- *Lipophilicity:* Generally silicon containing compounds are more lipophilic than carbon containing compounds and this increase in lipophilicity of molecules after incorporating silicon will be useful in increasing the tissue penetration of drugs.[36]
- *Bonding preferences*: Silicon and carbon have different bonding preferences because of the availability of 3d orbitals in silicon over carbon. In contrast to carbon, silicon does not favour double and triple bond formation.[37]
- *Electropositivity*: Silicon is more electropositive than carbon which leads to difference in bond polarity of silanols than the corresponding carbinols. Thus, differences allows silanols to strongly interact with pharmacophore and increase the potency of the concerned compound.

Owing to these promising facts about silicon switch, many groups are working on utility of silicon in drug discovery[38-44] including our own group. In this section of the thesis, selected silicon containing compounds from the literature are discussed and highlighted the potential of same in drug discovery as well as in agrochemicals.

2.3.1. Organosilanes having wide spectrum of biological activities

Although there is no silicon-containing compound in the market till date but few of them entered in human clinical trials (Figure 2.7.). To the best of our knowledge, nine compounds are in clinical trials at different phases, suggesting that silicon incorporation in drugs is a beneficial strategy does not cause any toxicity. In the literature, it was reported that after incorporation of silicon in molecule an increase in lipophilicity was

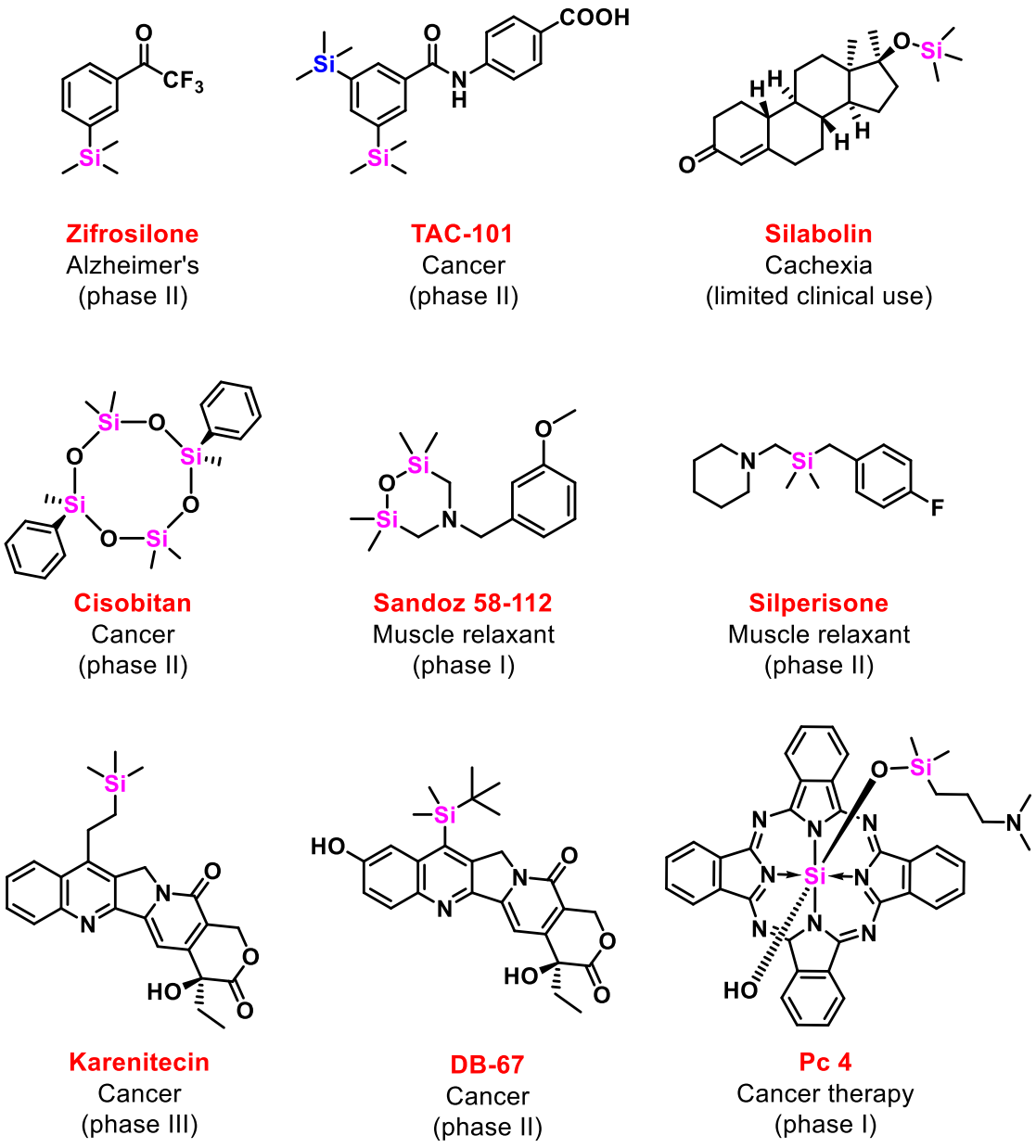


Figure 2.7. Organosilanes which entered human clinical trials

observed. This property of silicon was utilized in the synthesis of stable camptothecin analogues which is used as anticancer agent to inhibit the enzyme DNA topoisomerase I.[45] Other example are silatecans (DB-67) clinical candidate developed by Tigen pharmaceuticals, which is analogue of the camptothecin possesses a lipophilic silyl group, and both being familiar examples of bioactive silicon containing compounds because of their success in clinical trials (phase II).[46-49] The compound DB-67 penetrates the blood-brain barrier more effectively than camptothecin and hence was tested for cancers of the CNS showing promising results. Taiho pharmaceuticals developed an orally active agonist which is a clinical candidate entitled TAC101[50] it entered in phase II clinical trials for the treatment of lung cancer. Another example of the silicon-containing compound includes zifrosilone[51] (phase II clinical trials) which is found to exhibit activity against Alzheimer's disease while cisobitan[52] and Pc-4 are in phase-II and phase-I clinical trials for the treatment of cancer. Silperisone in phase-I clinical trial for the treatment of muscle relaxant. Along with these, there are many silicon-containing

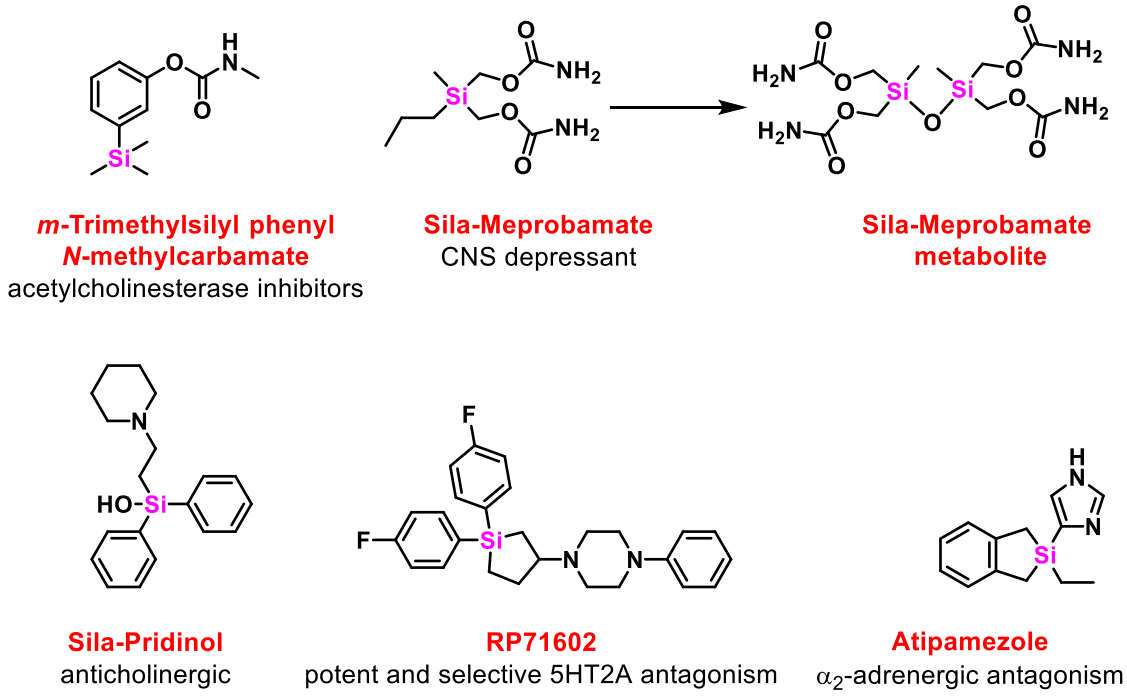


Figure 2.8. Organosilanes compounds having various biological activities

compounds are reported for potent biological activity. For instance, sila-meprobamate is found to exhibit central nervous system depressant activity (CNS depressant) and sila-pridinol shows anti-cholinergic activity five times better than its carbon analogue whereas, sila-atipamezole acts as α_2-adrenergic antagonism. Thus, silicon incorporation demonstrated beneficial effects when it is used strategically to address certain problems.

2.3.2. Organosilanes containing marketed fungicides as agrochemicals

Along with medicinal chemistry and drug discovery domain, silicon also plays an important role in agrochemicals (Figure 2.9.). Incorporation of silicon in agrochemicals and its success in variety of crops confirm the benefits of organosilicon in agrochemicals. A few selected cases are discussed below.

Flusilazole: Marketed as systemic fungicide and a member of the triazole class.[53] It is rapidly absorbed by the plant and its systemic action was translocated in the whole plant, resulting in the inhibition of fungus growth and results in effective control of the powdery mildew in crops. Flusilazole is known to inhibit the ergosterol biosynthesis, which is a structural key component of fungal cell walls thus proving its antifungal property.

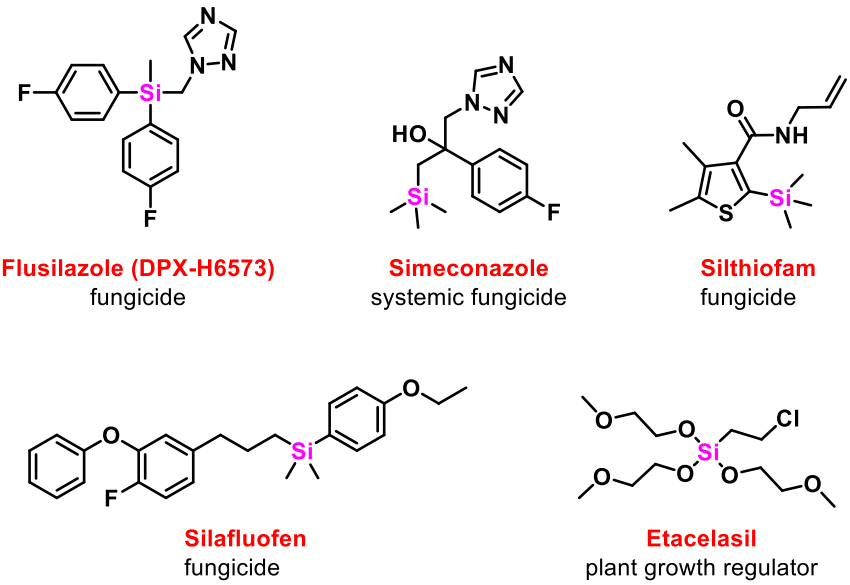


Figure 2.9. Marketed organosilanes which are used as agrochemicals

Silthiofam: Developed in 1989, through a random screening process at Monsanto against *Gaeumannomyces graminis*. Silthiofam is used for seed treatment and is termed as a fungistat, since it protects the cereal, wheat and barley plant from the effects of the fungus outlined above, leading to fungal death. It acts by inhibiting ATP production in the mitochondria.[54]

Etacelasil: Used as plant growth modifiers. However, they act by self-decomposing in the concerned plants to release the plant growth hormone ethylene.

Silafluofen: Belongs to a class of pyrethroid insecticides. This class of compounds possess various environmental problems. To address these issues, in 1984 additional structural modifications were done in pyrethroids which resulted in the invention of silafluofen insecticide.[55] Silafluofen is new and novel compound which is quite different in structure from the basic pyrethrins. After incorporation of silicon it was found that silafluofen acts on the neuroaxonal sodium channels which are different than pyrethroid class of compounds. In short, silafluofen have many advantages over pyrethroid class of compounds which includes its potent insecticidal activity, low mammalian toxicity, novel mode of action and stability in the alkaline soil.

Simeconazole: Belong to the group of azoles and it was introduced in 2001 by Sankyo fungicide. It is a chiral compound and marketed as racemic mixture.[56] Simeconazole is used as broad spectrum fungicide and found to exhibit its concerned mode of action through sterol demethylation inhibition.[56]

Considering the importance of silicon in drug discovery process and agrochemicals, our research group at CSIR-National Chemical Laboratory initiated the medicinal chemistry projects to incorporate silicon and to modulate the properties of molecules towards the identification of lead compounds.

2.3.3. Silicon incorporation to increase brain penetration

Our group successfully demonstrated the advantage of silicon by its incorporation in to the marketed drug called linezolid (a well-known antibiotic).[57] Other related molecule called sutezolid is in clinical trials for treatment of tuberculosis having thio-morpholine

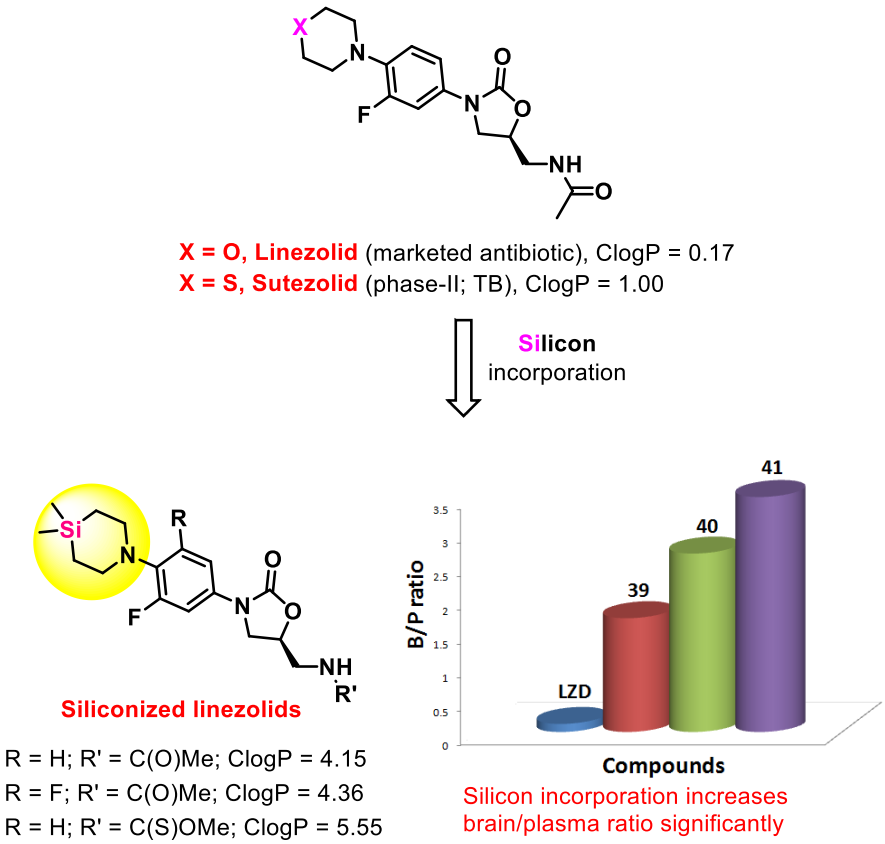


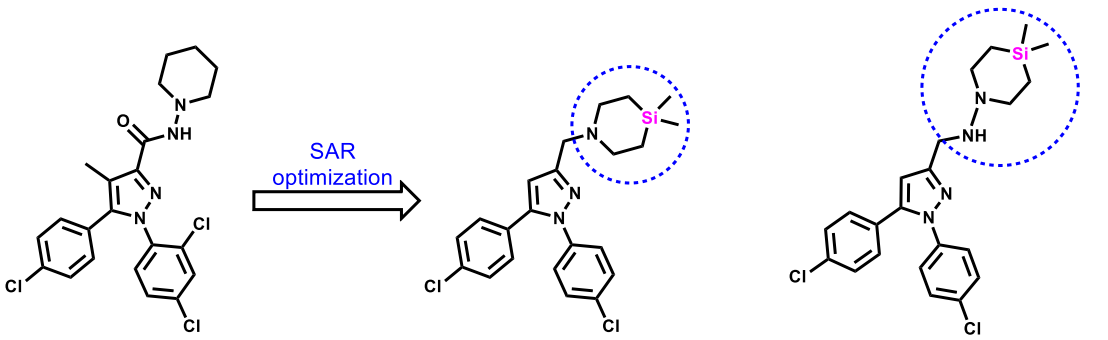
Figure 2.10. Silicon incorporation in linezolid drug

ring. To make use of their antibacterial potential and proven safety profiles in humans, our group incorporated silicon in these scaffolds towards the treatment of brain infections. The brain endothelial cells have tight junctions of capillaries termed as blood brain barrier (BBB) which allows only selected compounds to enter in the tissue of brain over the others.[58] Which is why, the infections of the brain that do occur are often difficult to treat. However, literature precedence's suggest that silicon incorporation into a molecule increases its lipophilicity, leading to an ultimate increase in the availability of a drug in brain tissue. Accordingly, it was planned and synthesized silicon analogs of linezolid having morpholine ring (Figure 2.10.).[59] Several compounds were synthesized around linezolid and sutezolid by incorporating silicon in place of oxygen/sulphur atom of the ring. From the results of the synthesized compounds revealed that incorporation of silicon increased brain penetration. One of the synthesized compound showed 29-times better brain/plasma ratio as compared to the marketed drug linezolid. Thus, a systematic

study showed that incorporation of silicon led to a dramatic increase in lipophilicity (increases brain penetration).

2.3.4. Silicon substitution in repurposing of drug

Our group also successfully demonstrated the advantage of silicon incorporation approach in repurposing the anti-obesity drug rimonabant as antitubercular agent.[60] Structural similarity of rimonabant with MmpL3 inhibitor like BM212 which is an antitubercular agent prompted us to evaluate rimonabant and its siliconized analogues against H37Rv (Figure 2.11.). A systematic study of synthesized rimonabant analogues showed that incorporation of silicon resulted in a dramatic enhancement in anti-TB activity. The increased potency of these siliconized compounds could ultimately be attributed to the increased lipophilicity of the concerned analogues. Interestingly, compounds with additional nitrogen atom in the linker such as NDS100546 showed improved metabolic stability. With this background, we have initiated the projects for the development of novel antifungal agents, in particular using the concept of silicon incorporation approach in morpholine antifungals. Details are discussed in following sections.



Rimonabant
CB1 receptor blocker
for treating obesity
MIC: 25 μg/mL (Mtb, H37Rv)

NDS 100529
for Tuberculosis
MIC: 31 ng/mL (Mtb, H37Rv)
% plasma stability (human): 100
% metabolic stability in HLM: 20.3

NDS 100546
for Tuberculosis
MIC: 0.39 μg/mL (Mtb, H37Rv)
% plasma stability (human): 100
% metabolic stability in HLM: 93.1

Figure 2.11. Repurposing of anti-obesity drug

2.4. Present work

2.4.1. Incorporation of silicon in amorolfine, fenpropimorph and fenpropidin

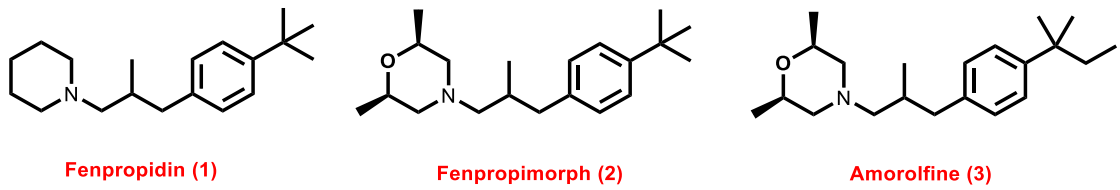


Figure 2.12. Structure of morpholine class of fungicides

There is a necessity for bringing new antifungal to the market because of development of resistance against available antifungal drugs and also they possess certain drawbacks such as less clinical efficacy and some are associated with side effects.[61, 62] Similarly, fungal infections are major concern in agriculture causing crop loss with consequential heavy economic burden worldwide. In the market, there are several classes of fungicides available for crop protection, however, they do suffer from drawbacks, necessitating to explore new antifungal agents. Fenpropidin and fenpropimorph are marketed fungicides from morpholine class which are used to control a range of diseases in cereals,wheat and barley such as powdery mildew, brown and yellow rusts, leaf spots. Amorolfine is used as an anti-fungal or a topical antimycotic, that is used to cure fungal infections of foot and nails. It is marketed as loceryl, locetar, curanail and odenil, and is commonly available in the form of a nail lacquer, containing 5% amorolfine hydrochloride as the active ingredient. Specifically, we have rationalized our design around amorolfine, fenpropidin and fenpropimorph which are closely related antifungals (Figure 2.12.).

2.4.2. Design and synthesis of silamorpholine analogues as antifungal agents

The metabolism of pharmaceutical drugs is one of important aspect of pharmacology and medicine. Metabolism determines and regulates the duration and intensity of a compound's and its pharmacologic action. In this context, Roberts *et al*. (in

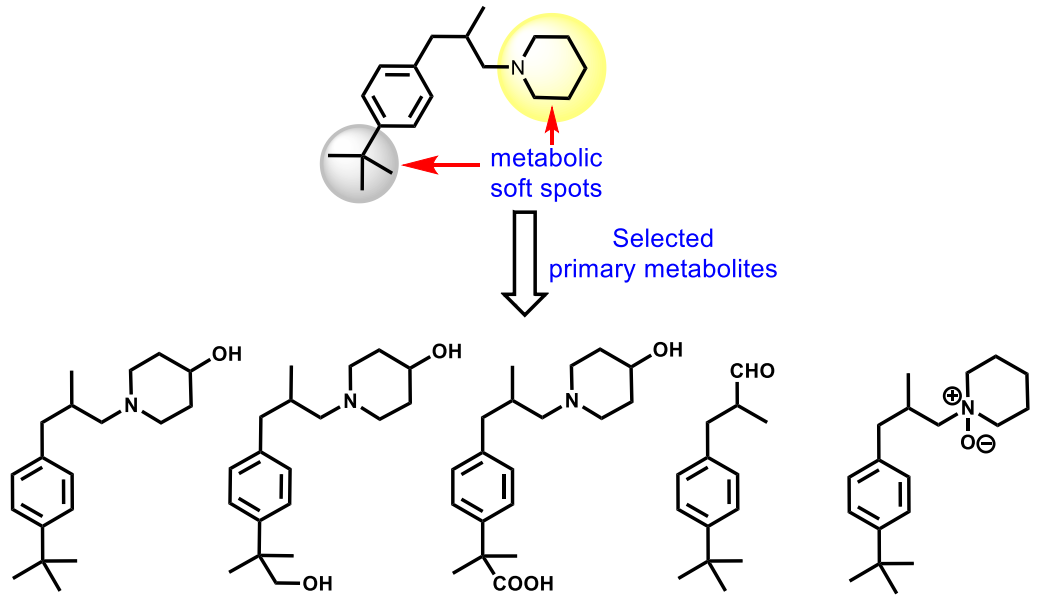


Figure 2.13. Selected metabolites of morpholine antifungals

1984) studied the metabolism of fenpropidin in wheat plants and concluded that generally metabolism of fenpropidin takes place at piperidine and aromatic *t*-butyl group through hydroxylation of the piperidine ring and/or hydroxylation and simultaneous oxidation of the methyls of aromatic *t*-butyl group (Figure 2.13.).[64] Amorolfine which is closely related to fenpropidin also undergoes oxidation at *iso*-pentyl group present at *P*-position of the aromatic ring. From metabolic study of this class of compounds we hypothesized that silicon incorporation at these metabolic soft spots in morpholine antifungals would help to overcome their drawbacks and give more potent and stable compounds (Figure 2.13. and 2.14.).

To find out the effect of silicon incorporation on lipophilicity we calculated the lipophilicity of selected silicon analogues which are expected to be more lipophilic compared with fenpropidin, fenpropimorph, and amorolfine. Based on calculated cLogP values, incorporation of one silicon atom showed only slight increase in lipophilicity (cLogP = 6.837), but two silicon atoms incorporation (cLogP = 9.44) resulted in large

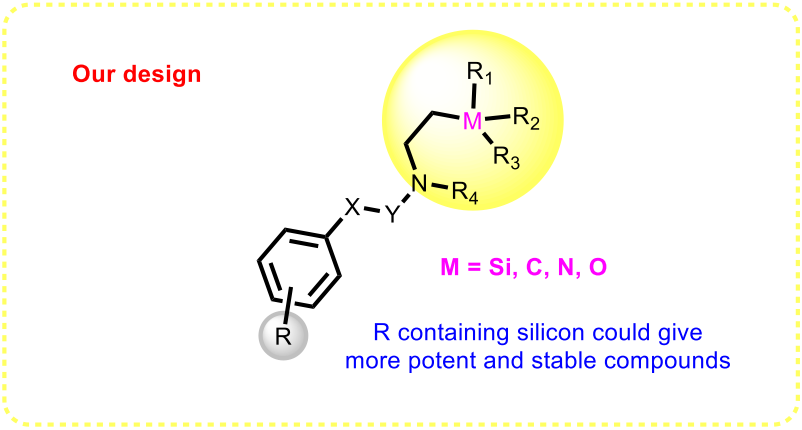
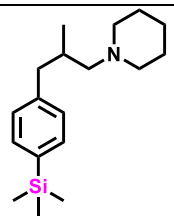
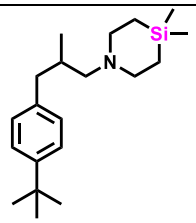
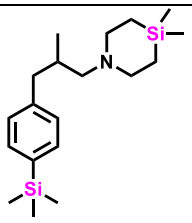
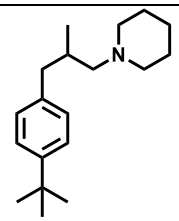
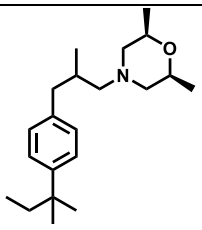
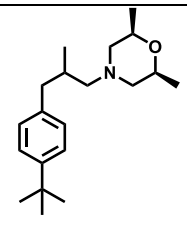


Figure 2.14. Our designs

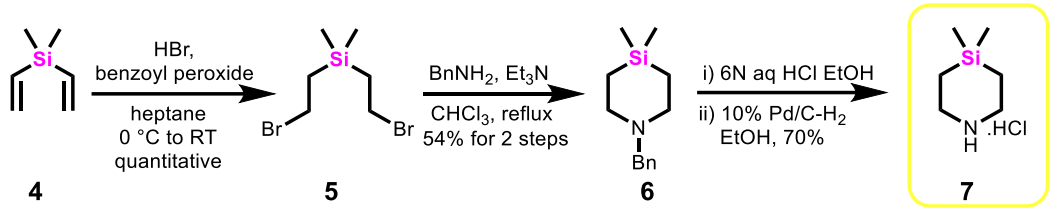
increase in lipophilicity with respect to fenpropidin (cLogP = 6.082) see Table 2.1. However, incorporation of silicon into heterocycle (piperidine ring) has more effect when compared to silicon incorporation in the aromatic ring. In case of heterocycle after silicon incorporation it showed cLogP = 8.685 while silicon incorporation in aromatic ring showed cLogP = 6.082. Sometimes, high lipophilicity can be detrimental to the druggable properties such as solubility. This undesired increase in lipophilicity can be counter

Table 2.1. Calculated clogP values of designed sila analogues

Structure	clogP	Structure	clogP	Structure	clogP
	6.837		8.685		9.440
 Fenpropidin	6.082	 Amorolfine	6.435	 Fenpropimorph	5.906

balanced by the addition of polar groups to the molecule, for example, morpholine containing silicon analogue showed lower cLogP values with respect to all three fungicides fenpropidin, amorolfine, and fenpropimorph. In general, siliconized compounds are more lipophilic with respect to their counter parts of carbon analogues. However, metabolism of silicon containing compounds is little known or under explored. Probably, enzymes responsible for metabolism may not recognize siliconized compounds, hence they are expected to have minimum problems related to metabolism. Therefore it is expected that our present designs with silicon incorporation may address particular issues with existing drugs or clinical compounds

2.4.3. Sila-piperidine synthesis

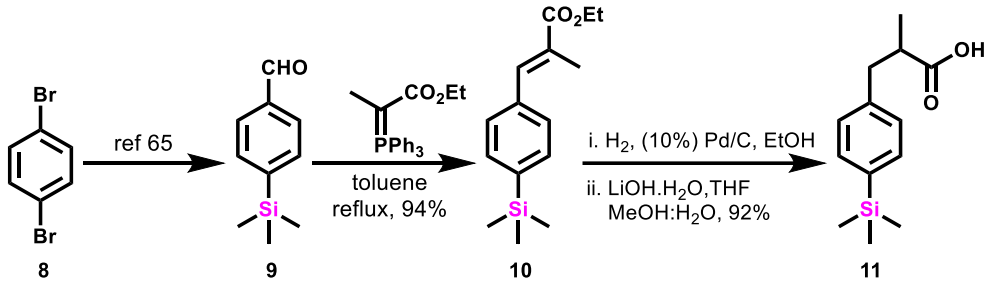


Scheme 2.1. Route for synthesis of sila-piperidine

The 4, 4-dimethyl silapiperidine (**7**) was prepared by following the improved procedures developed in our group earlier.[59] Commercially available dimethyl divinyl silane (**4**) on addition of freshly prepared HBr gas in presence of benzoyl peroxide in heptane gave the dibromo derivative (**5**) in quantitative yield. This dibromo compound on cyclization with benzyl amine (TEA, $CHCl_3$) under reflux gave the benzylated silapiperidine (**6**) in moderate yield. Compound **6** was transformed into its hydrochloride salt using 6 N aq. HCl and debenzylated under an atmosphere of hydrogen to give the required silapiperidine (**7**) as its HCl salt in a gram scale. The NMR was compared with the reported values and found to be identical[59] (Scheme 2.1.)

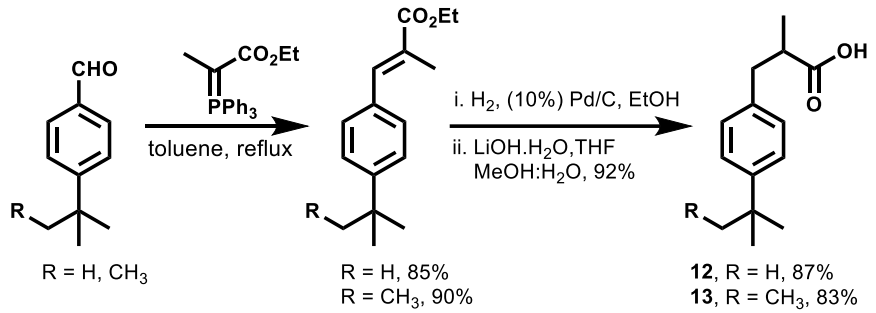
2.4.4. Synthesis of acids

After having the required silapiperidine **7** in hand, next, we focussed on the synthesis of silicon containing acid (**11**). The synthesis of sila-acid started with, aldehyde **9** which was prepared using known protocol from 1,4-dibromobenzene **8**[65] in two steps. After having aldehyde **9** in hand, we have performed Wittig homologation reaction using the ylide ethyl-2-(triphenyl phosphanylidene) propanoate [66] in dry toluene under reflux for 12 h resulted in the formation of ethyl 2-methyl-3-(4-(trimethylsilyl)phenyl) acrylate **10** with a yield of 94%. Formation of ester **10** was indicated in ^{1}H NMR by appearance of an olefinic proton at δ 7.69 ppm as a singlet in addition methyl present on olefin appeared at δ 2.14 ppm as a singlet. The presence of characteristic peaks in ^{13}C NMR at regions δ 14.3 ppm corresponding to olefin methyl along with the presence of peak corresponding to ester carbonyl at δ 188.7 ppm confirms the formation of **10**. The formation of ester



Scheme 2.2. Synthesis of sila-acid **11**

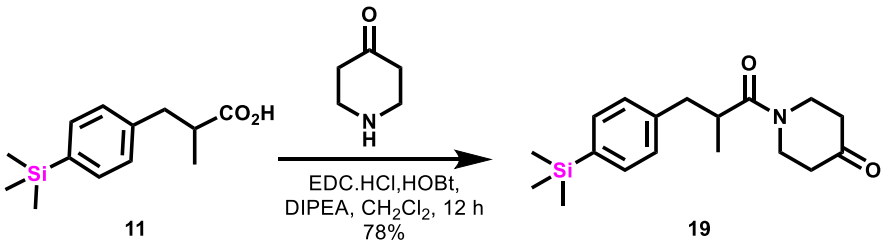
further confirmed by HRMS analysis which showed peak at 263.1462 corresponding to the molecular formula $C_{15}H_{23}O_2Si$ $[M+H]^+$ thus confirming ester (**10**) (Scheme 2.2). Hydrogenation of compound **10** was achieved using catalytic 10% Pd/C in ethanol under the blanket of hydrogen gave saturated ester. Hydrogenation of ester was indicated by TLC (difference using $KMnO_4$ stain). Crude ester used for hydrolysis in THF-MeOH-H_2O using LiOH.H_2O to give carboxylic acid **11**. In ^{1}H NMR, the disappearance of the olefinic proton at δ 7.69 ppm (singlet) which was present in α,β-unsaturated ester **10** additionally shift of methyl protons from δ 2.14 ppm (singlet) to δ 1.20 ppm (doublet) confirmed the hydrogenation of double bond. In ^{13}C NMR, the characteristic acid carbonyl was observed at δ 182.3 ppm while the disappearance of olefinic carbons at δ



Scheme 2.3. Synthesis of the acids **12** and **13**

141.0, 133.3 ppm and disappearance of peak corresponding to ethyl ester further confirms the hydrogenation and hydrolysis of **10**. The HRMS analysis showed peak at 259.1123 matched with the molecular formula $C_{13}H_{20}O_2Si$ $[M+Na]^+$ thus further confirm structure 2-Methyl-3-(4-(trimethylsilyl)phenyl)propanoic acid **11**. Repeating similar synthetic sequence, we have synthesized carboxylic acid derivatives **12** and **13**,[67, 68] (without silicon) from 4-*tert*-butylbenzaldehyde and 4-*neo*-pentyl benzaldehyde respectively which were used for the analogues synthesis (Scheme 2.3.).

2.4.5. Synthesis of various analogues with amide functionality



Scheme 2.4. Synthesis of amide **19**

After having required acids and amines in hand, we planned to synthesize various amides using them. Thus, sila-acid **11** which on coupling with 4-piperidone using standard coupling condition (EDC.HCl and HOBt) in presence of DIPEA in dichloromethane at room temperature furnished amide **19** with 78% yield. Formation of ketoamide **19** was indicated by the presence of non-polar spot on TLC compared to that of acid **11**. Besides activity of compound **19** in 2,4-DNP on TLC further indicated the formation of same

(Scheme 2.4.). Presence of two IR peaks at 1719, 1637 cm^{-1} corresponding to ketone and amide carbonyl indicated formation of ketoamide **19**. Total number of proton counts in ^{1}H NMR and presence of characteristic peaks of amide carbonyl at δ 174.9 ppm and ketone carbonyl at δ 206.8 ppm in ^{13}C NMR confirmed the formation of ketoamide **19**. In HRMS analysis observed a mass peak at *m/z* 340.1699 corresponding to $C_{18}H_{27}O_2NSi$ which further confirm formation of ketoamide **19**. Using optimized protocol in hand we have synthesized amides from **14-25** in good yield. Formation of desired amides were confirmed by using IR, ^{1}H NMR, ^{13}C NMR and further confirmed by HRMS analysis.

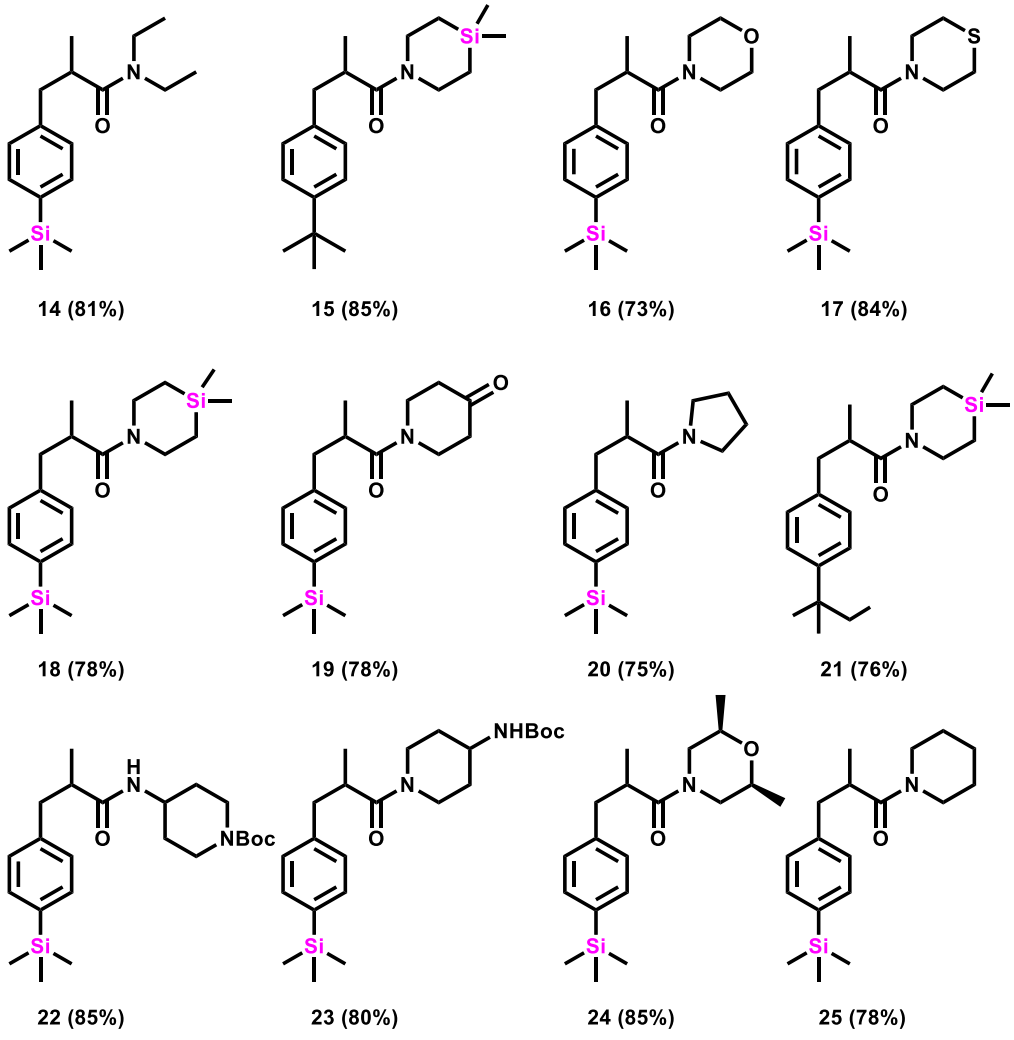
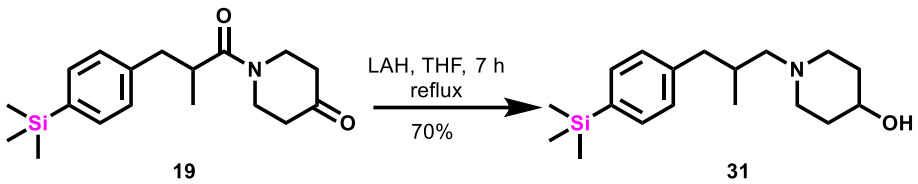


Figure 2.15. Synthesized amides with silicon incorporation

2.4.6. Synthesis of various analogues of amines



19 31

Scheme 2.5. Synthesis of amine **31**

After successful synthesis of amides (**14-25**), we planned to convert them to the corresponding amines (**26-37**). Thus, ketoamide **19** on treatment with lithium aluminium hydride in anhydrous THF under reflux condition for 7 h furnished aminol **31** in 70% yield (Scheme 2.5.).[69] On TLC, the formation of amino alcohol **31** was indicated by the presence of polar spot as compared to starting material. Besides inactivity of compound **31** in 2, 4-DNP further suggests the reduction of ketone in same pot. Disappearance of IR peak from 1719, 1637 cm^{-1} corresponding to ketone and amide carbonyl indicates formation of amino alcohol **31**. The characteristic peaks of amide carbonyl at δ 174.9 ppm and ketone carbonyl at δ 206.8 ppm are disappeared in ^{13}C NMR confirms the formation of aminol **31**. The HRMS analysis showed a mass peak at *m/z* 306.2248 corresponding to $C_{18}H_{32}ONSi$ which supported the formation of same. Using optimized protocol in hand, we have synthesized different amines from **26-37** in good yield (Figure 2.16.). Formation of desired amines were confirmed by using ^{1}H NMR, ^{13}C NMR and further confirmed by HRMS analysis.

Subsequently, we planned to synthesize amine **39** having open chain sila-amine derivative to study its effect on biological potential. In this context carboxylic acid **12** on coupling with open chain sila-amine using optimized condition furnished amide **38** with the yield of 85%. However, the reduction of amide **38** to give corresponding amine **39** under optimized condition was not successful (Scheme 2.6.).

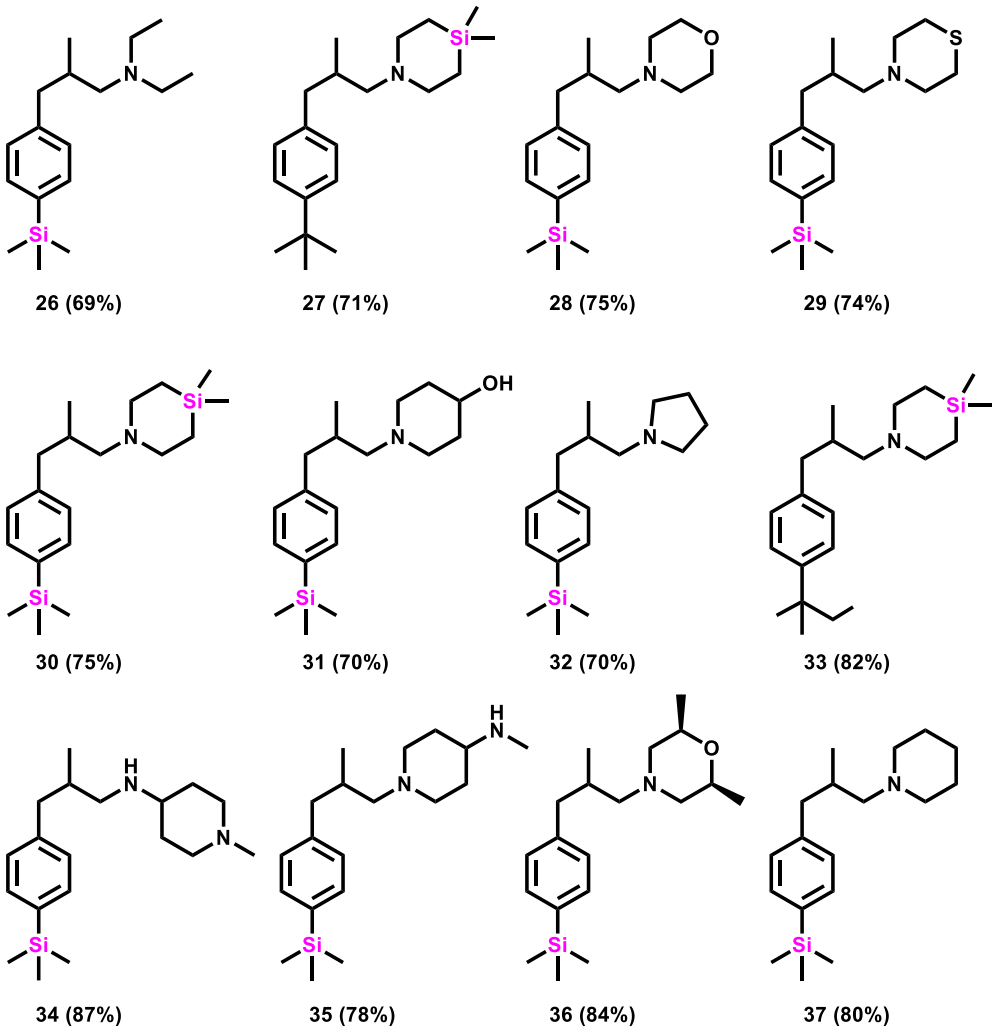
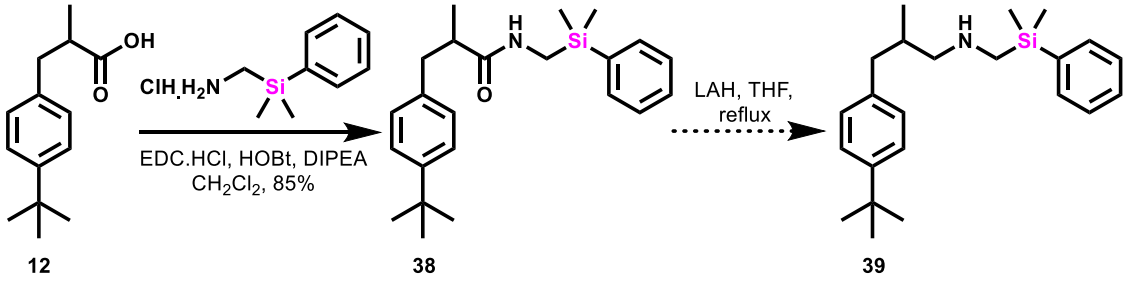


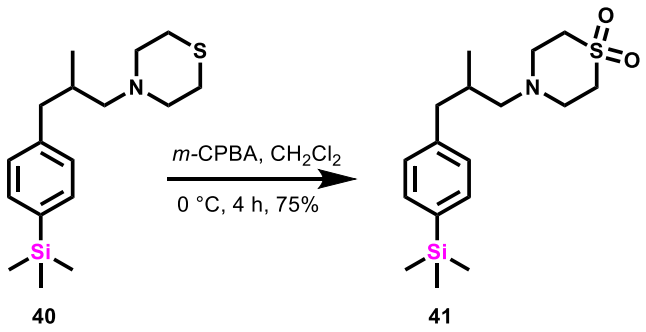
Figure 2.16. Synthesized amines with silicon incorporation



Scheme 2.6. Towards synthesis of amine **39**

2.4.7. Synthesis of sulfone derivative

The sulfone derivative **41** was prepared from **40** by the transformation of sulphur to sulfone by using *m*-CPBA in CH_2Cl_2 as solvent with yield of 75%.[70] On TLC, we observed the formation of polar spot as compared to starting material **40** which indicated the formation of sulfone (**41**). In ^{1}H NMR, the methylenes attached to sulphur are deshielded in sulfone **41** in addition characteristic sulphur attached to methylene carbon at δ 41.3, 32.5 ppm in ^{13}C NMR deshielded to some extent and appeared at δ 46.2 and 42.9 ppm, respectively, confirmed the formation of same. The HRMS analysis showed a mass peak at *m/z* 340.1760 corresponding to $C_{17}H_{30}O_2NSSi$ which supported formation of sulfone **41** (Scheme 2.7.). Thus, we have synthesized 15 sila-amine derivatives and they are well characterized using various analytical tools. Complete details about the



Scheme 2.7. Synthesis of sulfone (**41**)

procedures and characterization data are available in the experimental section. At this stage, we have evaluated their biological potential of both amides and amines in collaboration with Dr. M. V. Deshpande's research group at Biochemical Sciences Division, CSIR-NCL. Details are discussed in the following sections.

2.5. Biological studies

2.5.1. Antifungal potential towards human pathogenic fungi

All the synthesized sila-analogues were evaluated for their antifungal potential towards various human pathogenic fungi such as *Candida albicans* (ATCC 24433), *Candida*

albicans (ATCC 10231), *Candida glabrata* (NCYC 388), *Candida tropicalis* (ATCC 750), *Cryptococcus neoformans* (ATCC 34664) and *Aspergillus niger* (ATCC 10578) by Clinical Laboratory Standards Institute's (CLSI) broth microdilution assay (CLSI document M27-A3 and CLSI M38-A2)[71, 72] in Dr. Mukund V. Deshpande's laboratory. The assay protocol followed is described here. Appropriate amount of compounds were dissolved in DMSO to get 100X final strength. The stock solution was then diluted 1:50 in RPMI 1640 medium and 200 µL from this was added to the first row of a 96-well microtitre plate. The compounds were sequentially diluted two time in successive wells to get final concentration in the range of 2-256 µg/mL. Yeast cells (~$2x10^3$ cfu/mL), recently grown overnight in RPMI 1640 medium, were suspended in the medium and inoculated (100 µL) in the wells of the microtitre plate. For *Aspergilius niger*, $2x10^4$ spores/mL were added. The microtitre plates were incubated for 24-36 h for yeasts and 48 h for filamentous fungi. After incubation, the absorbance was measured at 530 nm by using microtitre plate reader (Epoch, Biotech Instruments) to assess the cell growth. The results obtained from the assays are discussed below.

All the amides (**14-25**) showed no antifungal effect against human fungal pathogens even at higher concentrations (MIC ≥ 256 µg/mL) tested. Further, we evaluated potential of sila-amines **26-37** against *Candida albicans* (ATCC 24433), *Candida albicans* (ATCC 10231), *Candida glabrata* (NCYC 388), *Candida tropicalis* (ATCC 750), *Cryptococcus neoformans* (ATCC 34664), and *Aspergillus niger* (ATCC 10578). Out of the tested amines, compound **41** was found to be inactive in tested stains. While compound **27**, **33**, **29**, **30**, **31**, **35**, **34**, **26** and **32** are showing moderate activity. The sila fenpropimorph analogue **36** was the most potent compound from this series followed by sila fenpropidin analogues **28** and **37** (Table 2.2. - 2.4.). It is worth highlighting that silicon incorporation on aromatic ring seems to be more effective compared to silicon incorporation in heterocycle. Compound **36** having better antifungal potential than marketed fluconazole, morpholine, fenpropimorph and fenpropidin against all tested pathogens.

Table 2.2. Antifungal potential against human fungal pathogens IC_{50} in µg/mL

Compound	*Candida albicans* ATCC 24433	*Candia albicans* ATCC 10231	*Cryptococcus neoformans* ATCC 34664	*Candida glabrata* NCYC 388	*Candida tropicalis* ATCC 750	*Aspergillus niger* ATCC 10578
37	0.12	0.11	0.0625	0.035	1	41.24
27	1.85	0.31	0.875	1.52	16	13.82
33	1.68	0.28	1.4	1.47	13.8	0.16
28	0.74	0.26	0.125	0.144	0.5	105.93
29	0.245	0.5	0.80	0.25	0.25	1
30	1.25	0.45	1.98	1.75	44.8	0.5
31	0.396	0.19	0.93	0.196	0.198	0.25
35	1.55	1	3.77	1.63	5.87	1.25
34	5.13	1.59	6.9	5.92	1.50	4.5
26	3.75	0.57	1.5	1.85	4.85	64
32	0.97	0.31	0.25	0.319	2.76	50.08
36	0.043	0.25	0.079	0.017	0.164	0.5
Amides	-	-	-	-	-	-
Fenpropidin	0.19	0.27	0.22	0.1	0.125	16
Fenpropimorph	0.21	0.833	0.25	0.25	0.125	1
Amorolfine	0.028	0.1	0.031	0.038	0.173	0.125
Fluconazole	0.73	4.21	4.6	16	64	256

Table 2.3. Antifungal potential against human fungal pathogens MIC in μg/mL

Compound	*Candida albicans* ATCC 24433	*Candia albicans* ATCC 10231	*Cryptococcus neoformans* ATCC 34664	*Candida glabrata* NCYC 388	*Candida tropicalis* ATCC 750	*Aspergillus niger* ATCC 10578
37	0.25	0.25	0.125	0.125	4	64
27	4	0.5	2	4	64	32
33	4	0.5	2	4	64	0.5
28	1	0.5	0.5	0.5	8	128
29	1	1	2	0.5	2	2
30	4	1	8	8	64	2
31	1	0.25	2	0.5	4	0.5

35	4	2	8	2	16	2
34	8	2	16	8	64	8
26	8	2	4	4	16	256
32	2	0.5	1	1	16	64
36	0.125	0.5	0.125	0.125	0.5	2
Amides	256	256	256	256	256	256
Fenpropidin	0.25	1	1	0.5	256	32
Fenpropimorph	0.5	2	1	1	256	4
Amorolfine	1	0.25	0.125	0.125	256	0.5
Fluconazole	2	8	32	128	256	256

Table 2.4. Antifungal potential against human fungal pathogens MFC in μg/mL

Compound	*Candida albicans* ATCC 24433	*Candia albicans* ATCC 10231	*Cryptococcus neoformans* ATCC 34664	*Candida glabrata* NCYC 388	*Candida tropicalis* ATCC 750	*Aspergillus niger* ATCC 10578
37	2	32	8	8	64	128
27	8	0.5	4	8	64	64
33	16	64	4	4	256	64
28	8	8	2	16	16	256
29	8	2	64	8	256	256
30	8	4	64	16	256	256
31	2	4	16	8	256	256
35	8	2	8	4	16	64
34	8	2	16	16	256	256
26	32	4	32	256	256	256
32	8	2	4	32	128	256
36	0.5	1	2	2	64	256
Amides	256	256	256	256	256	256
Fenpropidin	0.5	2	32	16	256	128
Fenpropimorph	4	2	64	128	256	256
Amorolfine	8	32	64	32	256	256
Fluconazole	4	16	256	256	256	256

From the observed activity of synthesized sila-compounds, it is clear that compound **30** having silicon in morpholine ring as well as on aromatic ring showed moderate activity thus it confirms incorporation of two silicon decreases the activity. Compound **31**, **35**, **34** are moderately active may be because of presence of polar hydroxyl and N-CH_3 groups on piperidine ring. While **26** and **32** having diethyl amine (open chain) and pyrrolidine (five membered) showed less activity which might be because of conformational change in the ring. Moderate activity of the compounds **27** and **33** can be explained due to lack of silicon in aromatic ring. Compound **41** was inactive,

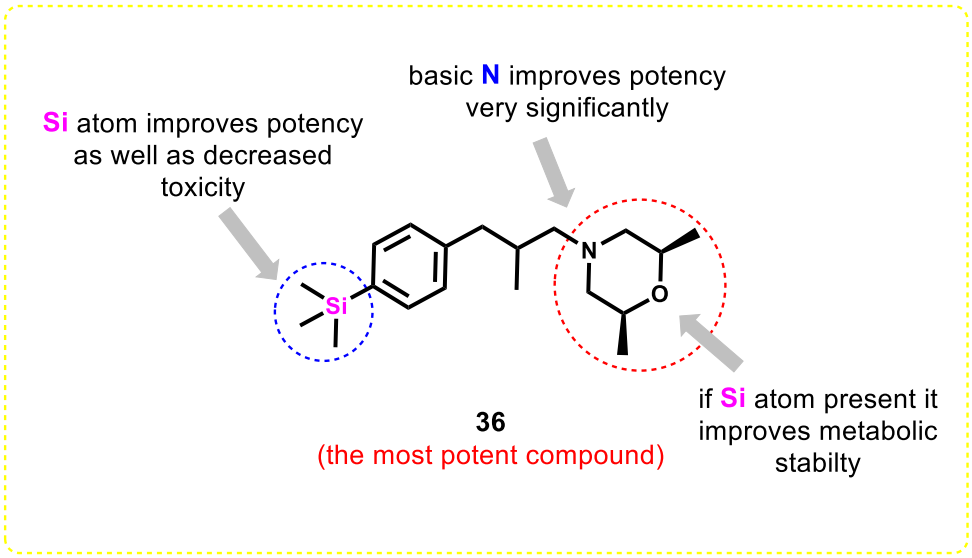


Figure 2.17. SAR conclusions of the current series

probably because of the presence of polar sulfone group on six membered ring. The compounds **28** and **37** having morpholine and piperidine ring with silicon in aromatic ring showed same potency as that of the marketed fungicide. While compound **36** having additional *cis* methyls on morpholine ring showing more fungicidal activity than **28** and **37** as well as marketed fungicides confirm that *cis* methyls on morpholine ring and silicon in aromatic ring is necessary for better activity. Along with this, the observed inactivity of amides (**14-25**) suggesting that the basic tertiary nitrogen is essential for the activity (Figure 2.17.).

2.5.2. Cytotoxicity study

To determine the cytotoxicity of synthesized compounds we carried out, hemolysis assay[73] using RBCs and MTT assay[73] using human embryonic kidney cells (HEK293).

Table 2.5. Cytotoxic effect of the selected compounds

Compound	% Hemolysis at		IC_{50} for HEK 293 cells μg/mL (mM)
	512 μg/mL	256 μg/mL	
37	8.18±0.80	ND	62.1±2.8 (0.214)
27	13.77±0.75	ND	221.8±6.4 (0.700)
29	15.15±0.26	ND	118.4±1.6 (0.386)
30	30.01±0.62	ND	71.9±1.3 (0.216)
31	90.34±3.21	16.50±1.90	187.31±4.8 (0.614)
35	83.45±3.07	22.65±0.79	9.81±1.1 (0.030)
34	100±1.01	62.97±2.76	103.2±2.5(0.323)
36	**7.53±0.26**	**ND**	**64±1.8(0.200)**
Fenpropidin	26.33±1.8	ND	45.4±2.2 (0.166)
Fenpropimorph	19.22±1.44	ND	62.8±3.1 (0.207)
Amorolfine	-	ND	103.33±5.8 (0.324)

At the concentration of 128 μg/mL, we did not observed RBC hemolysis whereas at a concentration of 256 μg/mL we observed partial hemolysis in case of amine **31**, **34**, and **35**. As this tested concentration (256 μg/mL) was many folds higher than the MICs of those compounds against different fungal pathogens, they may be considered as safe. In the case of potent compound **36**, we observed very less hemolysis (~7%) at concentration of 512 μg/mL. The concentrations of the compounds required for 50% reduction in the growth of HEK293 cells were also far higher than the MICs against fungi. Among the all tested compounds for cytotoxicity, only compound **34** was found to be toxic against HEK293 cells with IC_{50} of 9.81 μg/mL (Table 2.5.).

2.5.3. Mode of action

The fungicides belonging to the morpholine class work through inhibiting two enzymes called sterol Δ^{14} reductase and sterol Δ^{7}-Δ^{8} isomerase which are present in biosynthetic pathway of ergosterol, resulting in ergosterol depletion and accumulation of ignosterol and lichesterol (Figure 2.17.).[74, 75] To understand whether the synthesized sila-analogues act in a similar way as that of morpholine fungicides, *Candida albicans* (ATCC 24433)

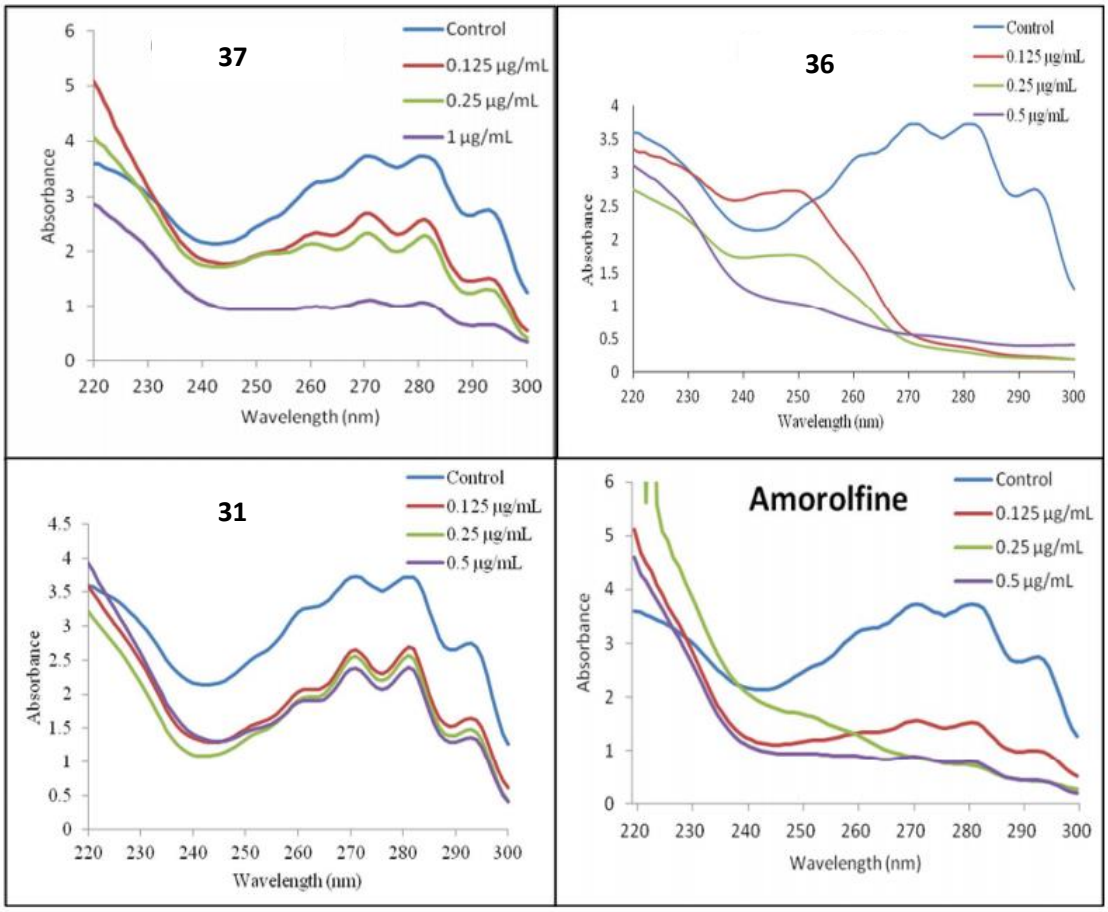


Figure 2.18. Spectrophotometric sterol measurement in *Candida albicans* (ATCC 24433) cells treated with various concentrations of sila-morpholines **37**, **31**, **36** and amorolfine

cells were grown in presence of selected compounds **37**, **31**, **36** and standard compound amorolfine at three different doses particularly at 0.125 µg/mL, 0.25 µg/mL and 0.5

µg/mL. The cellular sterols were extracted and measured using spectrophotometer. Ergosterol and the intermediate 24 (28) dehydroergosterol (24(28)DHE) in a sample shows characteristic four-peaked curve in a spectrometric scan between the wavelengths of 230-300 nm.[76] We observed decrease in the reduction of absorbance in case of compounds **37**, **31**, and **36** indicating that decrease in the ergosterol content in *Candida* cells (Figure 2.18.). Quantification of cellular sterols in samples of **36** and amorolfine treatment results confirmed the decrease in ergosterol content and concentration of ignosterol and/or lichesterol were increased. Here the quantification of sterols was measured with the help of GC-MS (Table 2.7.). The accumulation of these intermediates was similar to that of standard compound amorolfine treated cells, which

Table 2.6. Spectrophotometric sterol analysis of compound **36** and amorolfine

	Control	**Compound 36 0.125 µg/mL**	**Compound 36 0.25 µg/mL**	**Amorolfine 0.125 µg/mL**	**Amorolfine 0.25 µg/mL**
squalene	07.17	21.52	13.82	22.04	17.08
ergosterol	73.34	15.52	11.12	30.16	06.13
cholesta-8,24-dien-3-ol, 4-methyl-(3a′,4a′)	07.19	05.36	27.78	12.26	12.03
ergosta-5,8-dien-3-ol, (3a′)	05.15	N.D.*	N.D.	N.D.	N.D.
cholesta-5,24-dien-3-ol	03.58	15.34	00.87	00.44	N.D.
ignosterol	N.D.	07.23	42.29	N.D.	11.11
lichesterol	N.D.	29.84	N.D.	08.26	32.13
ergostenol	N.D.	05.19	04.12	11.56	05.71
ergosta-7,22-dien-3-ol,(3a′,22E)	N.D.	N.D.	N.D.	15.28	15.81
other sterols	03.57	N.D.	N.D.	N.D.	N.D.

suggests that synthesized sila analogues used in present study also work through same mode of action, i.e., inhibition of sterol reductase and sterol isomerase. More accumulation of lichesterol (32.13%) with respect to ignosterol (11.11%) for amorolfine indicated stronger action on isomerise enzyme than reductase, whereas, in the case of compound **36**, accumulation of ignosterol (42.29%) can be attributed to the major effect on sterol reductase at higher concentration (Table 2.6.). Depletion of ergosterol compromises membrane integrity, affects membrane protein functions due to membrane instability, and leads to the cessation of growth.

2.5.4. Metabolic stability

Metabolism is the most important phenomenon to be considered during the lead optimization studies in medicinal chemistry and drug discovery. The drug before enters the systemic circulation it enters into the liver, the microsomes which are present in the liver try to metabolize the drug/foreign substances by adding polar groups so that it can be easily removed from the body. The higher the rate of metabolism, the lower the concentration of active drug that reaches the systemic circulation. To balance this, higher dose has to be administered in order to achieve the desired pharmacological action. In the body, the most important enzymes that are responsible for the metabolism of the drug are Cytochrome P450 and Flavin containing monooxygenases. The *in vitro* metabolic stability is generally determined using liver microsomes because liver is rich in Cytochrome P450 enzyme. The percent microsomal stability of selected compounds was evaluated after 30 min incubation with mouse as well as human liver microsomes. All the tested compounds did not show good stability in mouse liver microsomes. However, compound **28** showed 5.1% metabolic stability while compounds **30, 36** showed 17.3% and 6.2% metabolic stability, it confirms that compounds **28**, **30** and **36** are relatively more stable than fenpropidin (1.1%) in mouse liver microsome (Table 2.7.). However, in human liver microsome, compound **30** showed a better stability of 44.5% than fenpropidin (34.45%) whereas, compound **36** (27.3%) was found to be slightly inferior to fenpropidin. Thus, it was found that after incorporation of silicon in piperidine ring the metabolic stability was improved.

Table 2.7. Percent metabolic stability of the compounds in mouse and liver microsome

Compounds	Persent metabolic stability	
	mouse	human
28	5.1	26.0
30	17.3	44.5
36	6.2	27.3
fenpropidin	1.1	34.4

Data obtained from Incozen therapeutics, Hyderabad

2.5.5. Antifungal activity against plant pathogenic fungi

After evaluation of sila-analogues in human fungal cells we planned to evaluate its antifungal potential in plant fungal pathogens like *Aspergillis niger, Aspergillis fumigates, Fusarium oxysporum, Aspergillis flavus, Ustilgo maeydis, drechslera oryzea, Claviceps purpurea, Alternaria soalni. In vitro* antifungal assays were performed by broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI, formerly NCCL) methods M-27-A3 and M-38-A2. Briefly, each compound stock was prepared in DMSO at a concentration of 12800 µg/mL. For the assay, compound stocks were serially diluted two-fold in a microtiter plate and 4 µL of this was used for assay to get a final concentration in the range of 256-2 µg/mL. Spores of the filamentous fungi ($2x10^4$ spores/mL) and yeast cells freshly grown in YPG broth in logarithmic phase ($2x10^3$ cfu/mL) were suspended in the RPMI 1640 medium and 196 µL from these were inoculated in the wells of the plate. The microtitre plate was incubated for 48-72 h. From the synthesized sila-amines **36** and **31** exhibited potent antifungal activity against all plant pathogenic fungi. The sila-amine **31** showed an MIC 1 µg/mL against *Aspergillis niger* which is comparable to fenpropimorph. While for other stains compounds **31** and **36**

Table 2.8. Antifungal potency towards different plant pathogenic fungi (MIC in µg/mL)

Compound	*Aspergillus niger*	*A. fumigatus*	*Fusarium oxyporum*	*A. flavus*	*Ustilago maeydis*	*Drechslera oryzae*	*Claviceps purpurea*	*Alternaria solani*
29	2	16	16	16	16	16	16	16
41	256	256	256	256	256	256	256	256
36	2	16	16	16	16	16	16	16
30	1	64	64	64	64	64	8	32
34	16	16	16	16	16	16	16	16
35	4	16	16	16	4	16	16	16
Amides	256	256	256	256	256	256	256	256
31	1	16	16	16	16	16	16	16
14	1	64	64	64	32	64	8	16
33	1	16	16	16	16	16	8	16
Fenpropimorph	1	16	16	16	16	16	4	16
Fenpropidin	0.5	64	64	64	64	64	32	32
28	N.D.							
32								
26								

showed comparable activity with fenpropimorph and showing superior activity than that of fenpropidin for all plant pathogenic fungi (all the MIC value are in µg/mL). However, all the amides did not show any activity against plant pathogenic fungi up to 256 µg/mL similar trend was observed for amides in human fungal pathogens. Amides inactivity suggested that basic nitrogen is essential for activity. However, among the tested compounds, amine**31** and **36** showed comparable activity with marketed fungicides such as fenpropimorph and fenpropidin. While amines **26**, **28, 41** and **32** are completely inactive in this assay. Sila-amines **29**, **30**, **34**, **35**, **14** and **33** showed moderate activity

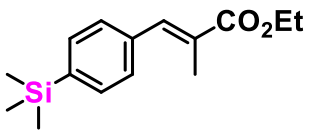
(Table 2.8.). It again proves that incorporation of silicon in the compounds **31** and **36** showed an increase in potency against plant fungal pathogens.

2.6. Conclusions

In conclusion, we have designed and synthesized several silicon analogues of marketed antifungals fenpropidin, fenpropimorph and amorolfine. Out of the synthesized compounds, **26-37** exhibited potent antifungal activity towards different human and plant fungal pathogens. Accordingly, eight compounds were evaluated for their cytotoxicity and it was found that compound **27** (14%), **29** (15%), **36** (7%) and **37** (8%) showed less hemolysis than fenpropidin (26%) and fenpropimorph (19%) even at 512 μg/mL. However at concentration of 125 μg/mL none of the compounds showed hemolysis. From all tested compounds, **36** showed better antifungal activity than fenpropidin, fenpropimorph, and amorolfine against different yeast and filamentous human pathogens as well as plant pathogens. From *in vitro* metabolic stability data in human and mouse liver microsome, it was found that after incorporation of silicon in the piperidine ring metabolic stability was improved, in particular compound **30** is more stable than fenpropidin in mouse and human liver microsome. We also confirmed that the synthesized sila analogues act similar to morpholine antifungals by targeting sterol reductase and sterol isomerase of the ergosterol synthesis pathway and cause depletion of ergosterol. At present compound **36** seems to be the best based on its antifungal activity and heamolysis assays. However, metabolic stability needs to be further improved. Further optimization of this series is in progress.

2.7. Experimental section

Ethyl (*E*)-2-methyl-3-(4-(trimethylsilyl)phenyl)acrylate (10)



10

To a solution of compound **9** (0.50 gm, 2.80 mmol) in anhydrous toluene (15 mL) was added ethyl 2-(triphenyl phosphanylidene)propanoate (1.22 gm, 3.37 mmol) and refluxed for 12 h. The reaction mixture was then evaporated to dryness and the crude mixture obtained was purified over silica gel column chromatography (1-2% EtOAc:Petroleum ether) to obtain product **10.**

Yield: 88%

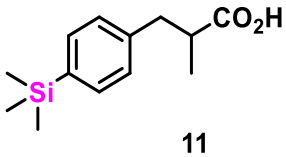
IR ν_{max} (thin film, $CHCl_3$):3020, 2966, 1669, 1632, 1260 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): d 7.69 (s, 1H), 7.57 (d, J = 7.9 Hz, 2H), 7.40 (d, J = 7.9 Hz, 2H), 4.32 - 4.26 (m, 2H), 2.14 (s, 3H), 1.37 (t, J = 7.7 Hz, 3H), 0.30 (s, 9H).

^{13}C NMR (100MHz, $CDCl_3$): d 188.7, 141.0, 138.6, 136.3, 133.3, 128.9, 128.8, 61.0, 14.4, 14.2, -1.1.

HRMS (ESI): Calculated for $C_{15}H_{23}O_2Si[M+H]^+$: 263.1462, found 263.1462.

2-Methyl-3-(4-(trimethylsilyl)phenyl)propanoic acid (11)



11

To a degassed solution of the unsaturated ester **10** (500 mg, 1.91 mmol) in EtOH (10 mL) was added 10% Pd/C (40 mg) and the mixture was stirred under hydrogen balloon pressure at room temperature for 3 h. The catalyst was filtered off and the filtrate

obtained was concentrated in *vaccuo* to afford ester (480 mg, 95%) as colorless oil. Crude product obtained was forwarded for next step without purification.

To a solution of above obtained ester in THF:MeOH (3:2, 5 mL), was added LiOH (139 mg, 3.40 mmol, in 3 mL water) and stirred for 3 h at room temperature. Solvent was then removed under reduced pressure and the residue obtained was acidified with 1 N HCl (pH ~3) and extracted with ethyl acetate (2 x 30 mL). The combined organic layer was washed with brine solution, dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure to afford **11**

Yield: 96%

IR υ_{max}(thin film, $CHCl_3$): 3510, 1702, 1635, 1382, 1259 cm^{-1}

^{1}H NMR (400MHz, $CDCl_3$): δ 7.46 (d, *J* = 7.8 Hz, 2H), 7.20(d, *J* = 7.8 Hz, 2H), 3.13 - 3.08 (m, 1H), 2.80 - 2.78 (m, 1H), 2.69 - 2.64 (m, 1H), 1.20 (d, *J* = 6.9 Hz, 3H), 0.27 (s, 9H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 182.3, 139.7, 138.3, 133.6, 128.5, 41.1, 39.3, 16.6, -1.0

HRMS (ESI): Calculated for $C_{13}H_{20}O_2NaSi[M+Na]^+$: 259.1125, found 259.1123.

General procedure for synthesis of amide and amine analogues

The generalised procedure for the synthesis of amides and amines are given below. Purity of products was determined by reverse phase HPLC analysis using Agilent technologies 1200 series; column: ZORBAX Eclipse XBD-C-18 (4.6 x 250 mm, 5 µ). Flow rate 1.00 mL/min, UV 254 nm; using mobile phases,

Method A: 90/10 CH_3OH/H_2O for 20 min;

Method B: 95/05 CH_3OH/H_2O for 20 min.

A. Procedure for amide coupling

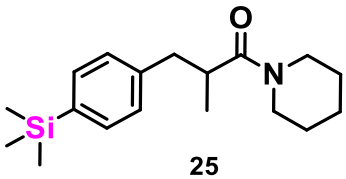
To a stirred solution of amine (1.1 mmol) and acid (1 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0℃ was added EDC.HCl (1.2 mmol), HOBt (1.2 mmol) and DIPEA (1.5 mmol) and the reaction mixture was stirred for 14 h at room temperature. After completion of the reaction (monitored by TLC), the reaction mixture was diluted with CH_2Cl_2 (15 mL), and then washed with 1N HCl (15 mL X 2), saturated aq. $NaHCO_3$ solution (15 x 2 mL) followed by brine wash and dried

over anhydrous Na_2SO_4. The crude material obtained after removal of solvent was purified by silica gel column chromatography using (EtOAc: Petroleum ether).

B. Procedure for preparation of amines

To a ice-cooled stirred solution of lithium aluminium hydride (5 mmol) in 15.0 mL of anhydrous THF under argon, was added dropwise with a solution of amide (1 mmol) in anhydrous THF (2 mL). The resulting reaction mixture was refluxed for 7 h, and then cooled to 0 °C and quenched with saturated aqueous sodium sulphate and diluted with EtOAc and stirred for 30 min. Then reaction mixture was filtered through a small pad of Celite, washed organic layer with water and brine, dried over Na_2SO_4, and concentrated in *vaccuo* to obtain desired amine.

2-Methyl-1-(piperidin-1-yl)-3-(4-(trimethylsilyl)phenyl)propan-1-one (25)



25

The compound **25** was synthesized from sila-acid **11**and piperidine by following general procedure A, as a colorless liquid.

Yield: 78%.

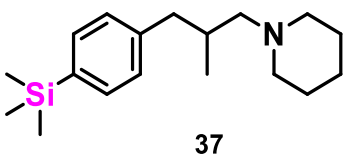
IR ν_{max} (thin film, $CHCl_3$): 3019, 1629, 1438, 1217, 1088 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 (d, *J* = 8.1 Hz, 2H), 7.18 (d, *J* = 7.8 Hz, 2H), 3.73 - 3.69 (m, 1H), 3.30 - 3.28 (m, 3H), 3.01 - 2.98 (m, 2H), 2.69 - 2.64 (m, 1H), 1.55 - 1.50 (m, 3H), 1.39 - 1.38 (m, 2H), 1.17 (d, *J* = 6.9 Hz, 3H), 1.10 - 0.97 (m, 1H), 0.25 (s, 9H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.1, 140.9, 137.8, 133.4, 128.6, 46.6, 42.9, 40.6, 37.3, 26.3, 25.6, 24.6, 17.9, -1.1

HRMS (ESI): Calculated for $C_{18}H_{29}ONSi[M+H]^+$: 304.2091, found 304.2088.

1-(2-Methyl-3-(4-(trimethylsilyl)phenyl)propyl)piperidine (37)



37

The compound **37** was synthesized from **25** by following general procedure B, as a colorless liquid

Yield: 80% brsm.

IR υ_{max}(thin film, $CHCl_3$): 3091, 2863, 1620, 1441, 1022 cm^{-1}

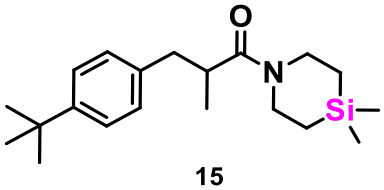
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (d, *J* = 7.8 Hz, 2H), 7.17 (d, *J* = 7.8 Hz, 2H), 2.84 (dd, *J* = 4.6, 13.4 Hz, 1H), 2.34 (dd, *J* = 5.7, 9.9 Hz, 4H), 2.31 - 2.25 (m, 1H), 2.22 - 2.13 (m, 1H), 2.13 - 2.04 (m, 1H), 2.04 - 1.92 (m, 1H), 1.59 (quin, *J* = 5.6 Hz, 4H), 1.45 - 1.43 (m, 2H), 0.85 (d, *J* = 6.6 Hz, 3H), 0.27 (s, 9H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 142.1, 137.0, 133.1, 128.8, 65.9, 55.0, 41.4, 32.4, 26.1, 24.7, 18.1, -1.0

HRMS (ESI): Calculated for $C_{18}H_{32}NSi[M+H]^+$: 290.2299, found 290.2298

HPLC analysis: 92.40% t_R = 11.14 min (method A).

3-(4-(*tert*-Butyl)phenyl)-1-(4,4-dimethyl-1,4-azasilinan-1-yl)-2-methylpropan-1-one (15)



15

The compound **15** was synthesized from coupling of carboxylic acid **12** and sila amine **7** by following general procedure A, as a yellow ccolored liquid

Yield: 85%.

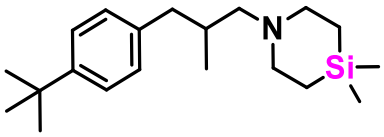
IR υ_{max}(thin film, $CHCl_3$): 3108, 2964, 1618, 1513, 1217, 1166 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.13 (d, *J* = 7.8 Hz, 2H), 7.00 (d, *J* = 7.8 Hz, 2H), 3.93 - 3.89 (m, 1H), 3.51 - 3.47 (m, 1H), 3.22 - 3.15 (m, 2H), 2.92 - 2.82 (m, 2H), 2.47 - 2.44 (m, 1H), 1.16 (s, 9H), 1.04 (d, *J* = 6.9 Hz, 3H), 0.56 - 0.54 (m, 2H), 0.43 - 0.42 (m, 1H), 0.21- 0.18 (m , 1H), -0.07 (s, 3H), -0.18 (s, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.7, 148.7, 137.4, 128.7, 125.0, 45.0, 42.2, 40.0, 37.9, 34.2, 31.2, 18.3, 15.1, 13.8, -2.6, -3.6

HRMS (ESI): m/z calculated for $C_{22}H_{23}ON_5Cl_3Na$ $[M+H]^+$ 478.0963; found, 478.0959

1-(3-(4-(*tert*-Butyl)phenyl)-2-methylpropyl)-4,4-dimethyl-1,4-azasilinane (27)



27

The compound **27** was synthesized from **15** by following general procedure B, as a colourless liquid

Yield: 71%.

IRυ_{max}(thin film, $CHCl_3$): 2963, 1248, 1109 cm^{-1}

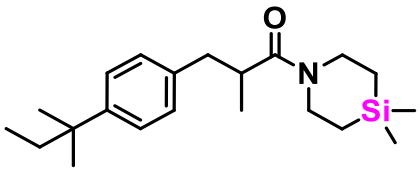
^{1}H NMR (200 MHz, $CDCl_3$): δ 7.32 (d, *J* = 8.5 Hz, 2H), 7.10 (d, *J* = 8.4 Hz, 2H), 3.49-3.38 (m, 2H), 3.16 (dt, *J* = 7.6, 13.2 Hz, 2H), 2.94 - 2.84 (m, 1H), 2.57 - 2.46 (m, 2H), 2.17 (dd, *J* = 6.4, 9.0 Hz, 1H), 1.34 (d, *J* = 6.6 Hz, 3H), 1.33 - 1.31 (m, 1H), 1.30 (s, 9H), 1.23 - 1.11 (m, 1H), 1.11 - 0.94 (m, 1H), 0.91 - 0.69 (m, 1H), 0.56 - 0.30 (m, 1H), 0.15 (s, 3H), 0.01 (s, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 149.7, 135.8, 128.8, 125.6, 57.1, 53.4, 49.3, 41.4, 34.5, 31.4, 20.0, 8.4, 7.8, -3.2, -3.8

HRMS (ESI): Calculated for $C_{20}H_{36}NSi[M+H]^+$: 318.2612, found 318.2609

HPLC analysis: 93.14% t_R = 13.7 min (method A).

1-(4,4-Dimethyl-1,4-azasilinan-1-yl)-2-methyl-3-(4-(tert-pentyl)phenyl)propan-1-one (21)



21

The compound **21** was synthesized from carboxylic acid **13** and silapiperidine **7** by following general procedure A, as a colorless liquid

Yield: 76%.

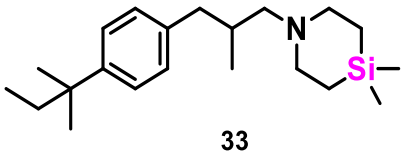
IR υ_{max}(thin film, $CHCl_3$): 3019, 1629, 1438, 1217, 1088 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.22 (d, *J* = 8.3 Hz, 2H), 7.13(d, *J* = 8.3 Hz, 2H), 3.97 (td, *J* = 6.1, 12.9 Hz, 1H), 3.57 - 3.56 (m, 1H), 3.41 - 3.37 (m, 2H), 3.04 - 2.96 (m, 2H), 2.62 (dd, *J* = 5.7, 12.8 Hz, 1H), 1.62 (q, *J* = 7.3 Hz, 3H), 1.26 (s, 6H), 1.17 (d, *J* = 6.6 Hz, 3H), 0.73 - 0.63 (m, 5H), 0.63 - 0.55 (m, 1H), 0.39 (ddd, *J* = 4.8, 9.7, 14.4 Hz, 1H), 0.07 (s, 3H), -0.02 (s, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.8, 147.2, 137.4, 128.7, 125.8, 45.0, 42.3, 40.1, 38.0, 37.6, 36.9, 28.4, 18.4, 15.3, 13.9, 9.2, -2.6, -3.4

HRMS (ESI): Calculated for $C_{21}H_{36}NOSi[M+H]^+$: 346.2488, found 346.2486.

4,4-Dimethyl-1-(2-methyl-3-(4-(tert-pentyl)phenyl)propyl)-1,4-azasilinane (33)



33

The compound **33** was synthesized from **21** by following general procedure B, as a liquid

Yield: 82%.

IRυ_{max}(thin film, $CHCl_3$): 2956, 1453, 1251 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.26 (d, *J* = 8.34 Hz, 2H), 7.11(d, *J* = 8.34 Hz, 2H), 3.50 - 3.44 (m, 2H), 3.18 - 3.12 (m, 2H), 2.79 - 2.76 (m, 1H), 2.69 - 2.45 (m, 3H), 2.27 - 2.10 (m, 1H), 1.64 - 1.57 (m, 2H), 1.35 (d, *J* = 6.6 Hz, 3H), 1.26 (s, 6H), 1.01 - 0.96 (m, 1H),

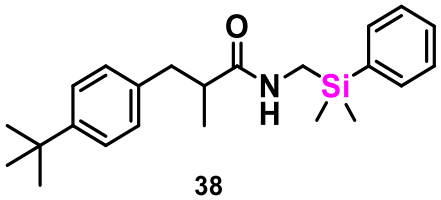
0.89 - 0.81 (m, 2H), 0.65 (t, *J* = 7.5 Hz, 3H), 0.44 – 0.39 (m, 1H), 0.16 (s, 3H), 0.01(s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 148.1, 135.7, 128.8, 126.4, 57.3, 53.5, 49.5, 41.4, 37.7, 36.9, 31.5, 30.4, 29.8, 28.5, 28.5, 20.1, 9.2, 8.5, 7.9, -3.3, -3.9.

HRMS (ESI): Calculated for $C_{21}H_{38}NSi[M+H]^+$: 332.2768, found 332.2766

HPLC analysis: 92.88% t_R = 7.53 min (method A).

3-(4-(*tert*-Butyl)phenyl)-N-((dimethyl(phenyl)silyl)methyl)-2-methylpropanamide (38)



38

The compound **38** was synthesized from coupling of carboxylic acid **12** and sila amine by following general procedure A, as a colorless liquid

Yield: 85%.

IR υ_{max}(thin film, $CHCl_3$): 3061, 3019, 2960, 1620, 1526, 1423 cm^{-1}

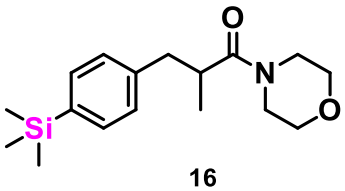
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41 - 7.33 (m, 5H), 7.29 (d, *J* = 8.2 Hz, 2H), 7.07 (d, *J* = 8.2 Hz, 2H), 4.93 (brs, 1H), 2.98 - 2.84 (m, 3H), 2.62 - 2.57 (m, 1H), 2.34 - 2.32 (m, 1H), 1.30 (s, 9H), 1.12 (d, *J* = 6.9 Hz, 3H), 0.24 (s, 3H), 0.18 (s, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 175.7, 149.1, 137.0, 136.6, 133.8, 129.6, 128.6, 128.2, 125.4, 44.0, 40.0, 34.4, 31.5, 28.5, 18.1, -4.1, -4.2

HRMS (ESI): Calculated for $C_{23}H_{34}ONSi[M+H]^+$: 368.6080, found 368.6082

HPLC analysis 92.34% t_R = 4.433 min (method A).

2-Methyl-1-morpholino-3-(4-(trimethylsilyl)phenyl)propan-1-one (16)



16

The compound **16** was synthesized from sila acid **11** and morpholine by following general procedure A, as a colorless liquid.

Yield: 73%.

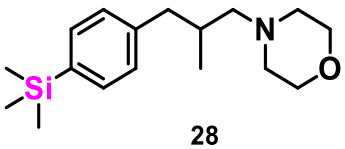
IRυ_{max}(thin film, $CHCl_3$): 3091, 2863, 1620, 1441, 1022 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44(d, *J* = 8.1 Hz, 2H), 7.17 (d, *J* = 7.8 Hz, 2H), 3.72 - 3.70 (m, 1H), 3.61 - 3.57 (m, 1H), 3.46 - 3.36 (m, 3H), 3.35 - 3.26 (m, 1H), 3.24 - 3.17 (m, 1H), 2.98 - 2.94 (m, 3H), 2.70 - 2.69 (m, 1H), 1.18 (d, *J* = 6.4 Hz, 3H), 0.25 (s, 9H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.4, 140.5, 138.3, 133.4, 128.5, 66.8, 66.4, 46.0, 42.1, 40.7, 37.2, 18.0, -1.1

HRMS (ESI): Calculated for $C_{17}H_{28}O_2NSi[M+H]^+$: 306.1884, found 306.1881.

4-(2-Methyl-3-(4-(trimethylsilyl)phenyl)propyl)morpholine (28)



28

The compound **28** was synthesized from **16** by following general procedure B, as a colorless liquid

Yield: 75%.

IRυ_{max}(thin film, $CHCl_3$): 3017, 2860, 1637, 1452, 1386, 1252, 1049 cm^{-1}

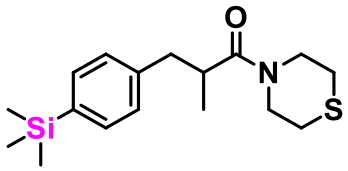
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (d, *J* = 7.8 Hz, 2H), 7.15 (d, *J* = 7.8 Hz, 2H), 3.72 (t, *J* = 4.6 Hz, 4H), 2.82 (dd, *J* = 4.6, 13.4 Hz, 1H), 2.44 - 2.40 (m, 3H), 2.32 (dd, *J* = 8.8, 13.4 Hz, 1H), 2.23 (dd, *J* = 7.6, 12.0 Hz, 1H), 2.14 (dd, *J* = 7.1, 12.0 Hz, 1H), 2.04 - 1.93 (m, 1H), 1.89 - 1.85 (m, 1H), 0.87 (d, *J* = 6.6 Hz, 3H), 0.27 (s, 9H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 141.6, 137.2, 133.1, 128.8, 67.1, 65.3, 54.0, 41.1, 32.0, 18.0, -1.0

HRMS (ESI): Calculated for $C_{17}H_{30}ONSi[M+H]^+$: 292.2091, found 292.2090

HPLC analysis 93.98% t_R = 6.70 min (method A).

2-Methyl-1-thiomorpholino-3-(4-(trimethylsilyl)phenyl)propan-1-one (17)



17

The compound **17** was synthesized from sila-acid **11**and thiomorpholine by following general procedure A, as a colorless liquid.

Yield: 84%.

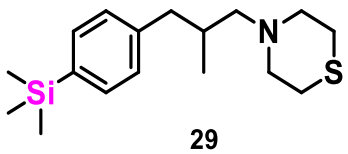
IRυ_{max}(thin film, $CHCl_3$): 3019, 1629, 1438, 1217, 1088 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (d, *J* = 7.8 Hz, 2H), 7.17 (d, *J* = 7.8 Hz, 2H), 4.18 - 4.12 (m, 1H), 3.64 - 3.61 (m, 1H), 3.53 - 3.50 (m, 2H), 3.00 - 2.94 (m, 2H), 2.68 - 2.66 (m, 1H), 2.47 (t, *J* = 5.3 Hz, 2H), 2.43 - 2.27 (m, 1H), 1.90 - 1.88 (m, 1H), 1.18 (d, *J* = 6.6 Hz, 3H), 0.26 (s, 9H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.5, 140.6, 138.3, 133.5, 128.6, 48.3, 44.6, 40.8, 37.7, 27.5, 27.3, 18.2, -1.1.

HRMS (ESI): Calculated for $C_{17}H_{28}ONSSi[M+H]^+$: 322.1583, found 322.1584

4-(2-Methyl-3-(4-(trimethylsilyl)phenyl)propyl)thiomorpholine (29)



29

The compound **29** was synthesized from **17** by following general procedure B, as a colorless liquid.

Yield: 75%.

IRυ_{max}(thin film, $CHCl_3$): 3020, 2956, 1453, 1251 cm^{-1}

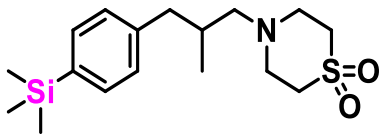
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (d, *J* = 7.8 Hz, 2H), 7.17 (d, *J* = 7.8 Hz, 2H), 2.82 (dd, *J* = 5.0, 13.3 Hz, 1H), 2.73 - 2.68 (m, 8H), 2.33 (dd, *J* = 8.2, 13.3 Hz, 1H), 2.24 - 2.22 (m, 1H), 2.19 - 2.17 (m, 1H), 2.05 - 2.00 (m, 1H), 0.88 (d, *J* = 6.9 Hz, 3H), 0.30 (s, 9H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 141.8, 137.3, 133.3, 128.8, 65.7, 55.6, 41.3, 32.5, 28.2, 18.2, -0.9

HRMS (ESI): Calculated for $C_{17}H_{30}SNSi$ $[M+H]^+$: 308.1863, found 308.1863

HPLC analysis (Method A): 91.56% t_R = 9.91 min

4-(2-Methyl-3-(4-(trimethylsilyl)phenyl)propyl)thiomorpholine 1,1-dioxide (41)



41

To a solution of **40** (80 mg 0.260 mmol) in anhydrous CH_2Cl_2 (20 ml) was added m-CPBA (118.4 mg 0.780 mmol) in portion wise at 0^0C. Reaction mixture was allowed to stirred at room temperature for 4 h (monitored by TLC), diluted with DCM and excess of m-CPBA was quenched by adding aqueous sodium sulphite and washed with sat. $NaHCO_3$ (10 mL) and brine (5 mL), dried over Na_2SO_4. The crude material obtained after removal of solvent was purified by column chromatography (50 - 70% EtOAc: Petroleum ether) to afford off white solid **41**

Yield: 75%.

IR υ_{max}(thin film, $CHCl_3$): 2958, 2400, 2360, 1424, 1250 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (d, *J* = 7.8 Hz, 2H), 7.16 (d, *J* = 7.8 Hz, 2H), 4.33 - 4.22 (m, 2H), 3.80 - 3.74 (m, 1H), 3.51 - 3.40 (m, 1H), 3.34 - 3.20 (m, 3H), 3.19 (dd, *J* =

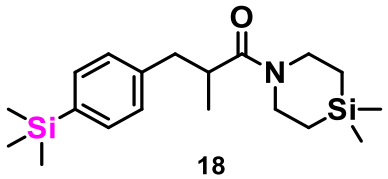
6.4, 12.8 Hz, 1H), 2.82 - 2.70 (m, 2H), 2.68 - 2.66 (m, 2H), 2.49- 2.47 (m, 1H), 1.26 (d, *J* = 6.4 Hz, 3H), 0.25 (s, 9H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 139.5, 139.0, 133.8, 128.7, 64.3, 62.9, 46.2, 42.9, 31.0, 22.0, -1.0

HRMS (ESI): Calculated for $C_{17}H_{30}O_2NSSi[M+H]^+$: 340.1761

HPLC analysis (Method A): 94.96% t_R = 5.57 min

1-(4,4-Dimethyl-1,4-azasilinan-1-yl)-2-methyl-3-(4-(trimethylsilyl)phenyl)propan-1 one (18)



18

The compound **18** was synthesized from sila-acid **11** and sila-amine **7** by following general procedure A, as a colourless viscous liquid.

Yield: 78%.

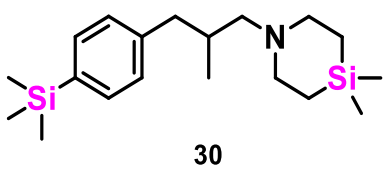
IRυ_{max}(thin film, $CHCl_3$): 3021, 1707, 1411, 1250, 1109 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.42 (d, *J* = 7.9 Hz, 2H), 7.21(d, *J* = 7.9 Hz, 2H), 4.18 - 4.06 (m, 1H), 3.62 - 3.55 (m, 1H), 3.37 - 2.99 (m, 4H), 2.96 – 2.59 (m, 1H), 1.18 (d, *J* = 6.8 Hz, 3H), 0.69 - 0.64 (m, 2H), 0.54 - 0.52 (m, 1H), 0.28 - 0.26 (m, 1H), 0.24 (s, 9H), 0.06 (s, 3H), -0.08 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.6, 141.3, 137.7, 133.3, 128.7, 45.2, 42.4, 40.7, 38.1, 18.5, 15.2, 13.9, -1.0, -2.4, -3.5

HRMS (ESI): Calculated for $C_{19}H_{34}ONSi_2$ $[M+H]^+$: 348.6490, found 348.6491.

4,4-Dimethyl-1-(2-methyl-3-(4-(trimethylsilyl)phenyl)propyl)-1,4-azasilinane (30)



30

The compound **30** was synthesized from **18** by following general procedure B, as a colourless liquid.

Yield: 75%.

IR ν_{max} (thin film, $CHCl_3$): 3021, 1599, 1410, 1250, 910 cm^{-1}

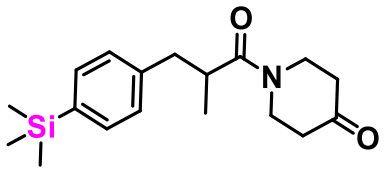
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (d, *J* = 7.8 Hz, 2H), 7.17 (d, *J* = 7.8 Hz, 2H), 2.83 (dd, *J* = 4.8, 13.3 Hz, 1H), 2.73 - 2.69 (m, 4H), 2.28 - 2.24 (m, 2H), 2.20 - 2.19 (m, 1H), 1.93 - 1.92 (m, 1H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.75 - 0.73 (t, *J* = 6.3, 12.4 Hz, 4H), 0.27 (s, 9H), 0.05 (s, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 142.2, 137.0, 133.1, 128.8, 64.0, 52.7, 41.4, 33.4, 30.3, 18.2, 13.5, -1.0, -2.9

HRMS (ESI): Calculated for $C_{19}H_{36}Si_2N[M+H]^+$: 334.2381,found 334.2382

HPLC analysis (Method A): Purity 93.02% t_R = 15.347 min

1-(2-Methyl-3-(4-(trimethylsilyl)phenyl)propanoyl)piperidin-4-one (19)



19

The compound **19** was synthesized from sila-acid **11** and 4-pyridoneby following general procedure A, as a yellow colored liquid.

Yield: 78%

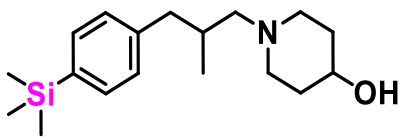
IR ν_{max} (thin film, $CHCl_3$) : 3017, 1719, 1637, 1247, 1104 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 (d, *J* = 7.3 Hz, 2H), 7.19 (d, *J* = 7.3 Hz, 2H), 4.29 - 4.24 (m, 1H), 3.60 - 3.56 (m, 1H), 3.42 - 3.35 (m, 2H), 3.09 - 3.03 (m, 1H), 3.00 - 2.97 (m, 1H), 2.75 - 2.71 (m, 1H), 2.32 - 2.23 (m, 2H), 2.12 - 2.08 (m, 1H), 1.45 - 1.40(m, 1H), 1.25 (d, *J* = 5.5 Hz, 3H), 0.22 (s, 9H).

^{13}CNMR (100 MHz, $CDCl_3$): δ 206.8, 175.0, 140.7, 138.8, 133.6, 128.6, 44.2, 41.2, 41.1, 40.9, 38.0, 18.5, –1.1.

HRMS (ESI): Calculated for $C_{18}H_{27}NSiO_2Na[M+Na]^+$: 340.1703, found 340.1699

1-(2-Methyl-3-(4-(trimethylsilyl)phenyl)propyl)piperidin-4-ol (31)



31

The compound **31**was synthesized from **19** by following general procedure B, as a yellow coloured liquid

Yield: 70%

IR ν_{max} (thin film, $CHCl_3$) : 3391, 3017, 2951, 1454, 1381 cm^{-1}

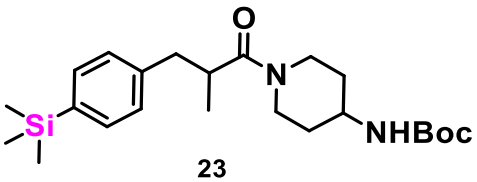
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 (d, *J* = 7.8 Hz, 2H), 7.19 (d, *J* = 7.8 Hz, 2H), 3.71 - 3.67 (m, 2H), 2.82 - 2.75 (m, 2H), 2.31 (dd, *J* = 8.6, 13.4 Hz, 1H), 2.18 - 2.15 (m, 1H), 2.13 - 2.06 (m, 2H), 1.91 - 1.88 (m, 4H), 1.87- 1.86 (m, 3H), 0.91 (d, *J* = 6.7, 3H), 0.28 (s, 9H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 141.8, 137.1, 133.2, 128.7, 68.1, 64.8, 51.5, 41.3, 34.6, 32.7, 18.1, -1.0.

HRMS (ESI): Calculated for $C_{18}H_{32}NOSi[M+H]^+$: 306.2248, found 306.2245

HPLC analysis (Method A): Purity 96.51% t_R = 4.70 min

***tert*-Butyl(1-(2-methyl-3-(4-(trimethylsilyl)phenyl)propanoyl)piperidin-4-yl)carbamate (23)**



23

The compound **23** was synthesized from sila-acid **11** and *tert*-butyl piperidin-4-ylcarbamate by following general procedure A, as a colourless solid**.**

Yield: 80%

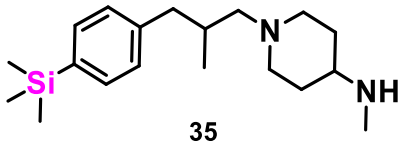
IRυ_{max}(thin film, $CHCl_3$): 3022, 2358, 1604, 1363, 1216 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41 (d, *J* = 7.3 Hz, 2H), 7.14 (d, *J* = 7.3 Hz, 2H), 4.93 (d, *J* = 7.8 Hz, 1H), 3.92 - 3.77 (m, 3H), 2.86 - 2.67 (m, 4H), 2.37 - 2.35 (m, 1H), 1.76 - 1.73 (m, 1H), 1.62 - 1.58 (m, 2H), 1.54 - 1.50 (m, 1H), 1.42 (s, 9H), 1.19 (d, *J* = 6.9 Hz, 3H), 0.23 (s, 9H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.7, 154.7, 140.5, 138.3, 133.5, 128.4, 79.7, 46.2, 44.1, 40.8, 31.8, 28.5, 17.8, -1.0.

HRMS (ESI): Calculated for $C_{23}H_{39}N_2O_3Si[M+H]^+$: 418.2652, found 418.2653.

***N*-methyl-1-(2-methyl-3-(4-(trimethylsilyl)phenyl)propyl)piperidin-4-amine (35)**



35

The compound **35** was synthesized from **23** by following general procedure B, as a Brown coloured Liquid.

Yield: 78%

IR ν_{max} (thin film, $CHCl_3$) : 3371, 2927, 2857, 1591, 1455, 1249 cm^{-1}

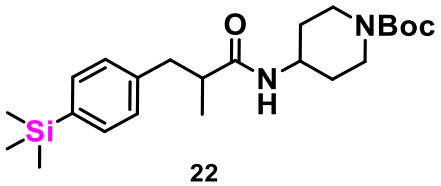
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 (d, *J* = 7.5 Hz, 2H), 7.16 (d, *J* = 7.5 Hz, 2H), 3.26 - 3.16 (m, 2H), 2.92 - 2.80 (m, 3H), 2.51 (s, 3H), 2.33 - 2.13 (m, 3H), 1.98 - 1.95 (m, 5H), 1.57- 1.50 (m, 2H), 0.86 (d, *J* = 6.5 Hz, 3H), 0.27 (s, 9H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 141.8, 137.1, 133.1, 128.7, 64.8, 56.8, 52.7, 41.3, 32.6, 32.5, 31.4, 18.1, -1.0

HRMS (ESI): Calculated for $C_{19}H_{35}N_2Si[M+H]^+$: 319.2564, found 319.2563

HPLC analysis (Method A): Purity 91.09% t_R = 6.98 min

***tert*-Butyl 4-(2-methyl-3-(4-(trimethylsilyl)phenyl)propanamido)piperidine-1-carboxylate (22)**



22

The compound **22** was synthesized from sila-acid **11** and *tert*-butyl 4-aminopiperidine-1-carboxylateby following general procedure A, as a white solid.

Yield: 85%

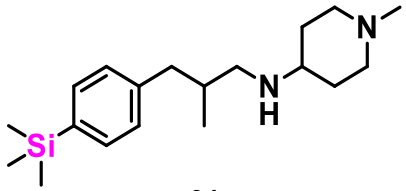
IR υ_{max}(thin film, $CHCl_3$): 3385, 3021, 1607, 1367, 1216 cm^{-1}

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.43 (d, *J* = 7.9 Hz, 2H), 7.16 (d, *J* = 7.9 Hz, 2H), 4.97 - 4.94 (m, 1H), 3.90 - 3.89 (m, 1H), 3.82 - 3.79 (m, 2H), 2.88 - 2.85 (m, 3H), 2.71 (dd, *J* = 6.0, 13.3 Hz, 1H), 2.38 (td, *J* = 6.5, 9.3 Hz, 1H), 1.64 - 1.62 (m, 1H), 1.53 - 1.52 (m, 1H), 1.47 - 1.44 (m, 1H), 1.44 (s, 9H), 1.20 (d, *J* = 7.0 Hz, 3H), 0.25 (s, 9H)

^{13}C NMR (125 MHz, $CDCl_3$): δ 174.7, 154.6, 140.4, 138.2, 133.5, 128.4, 79.6, 46.1, 44.0, 40.7, 31.8, 28.4, 17.7, -1.1

HRMS (ESI): Calculated for $C_{23}H_{39}N_2O_3Si[M+H]^+$: 419.2652, found 419.2652

1-Methyl-N-(2-methyl-3-(4-(trimethylsilyl)phenyl)propyl)piperidin-4-amine (34)



34

The compound **34** was synthesized from **22** by following general procedure B, as a colourless liquid

Yield: 87%.

IR ν_{max} (thin film, $CHCl_3$) : 3385, 2900, 2857, 1403, 1249 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 (d, *J* = 7.6 Hz, 2 H), 7.16 (d, *J* = 7.8 Hz, 2 H), 2.80 (d, *J* = 11.5 Hz, 2 H), 2.70 (dd, *J* = 6.1, 13.4 Hz, 1 H), 2.58 (dd, *J* = 6.0, 11.6 Hz, 1 H),

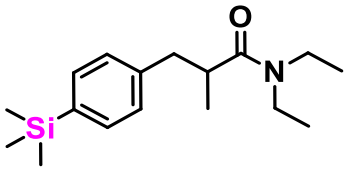
2.47 (dd, *J* = 7.1, 11.5 Hz, 1 H), 2.42 - 2.38 (m, 1 H), 2.26 (s, 3 H), 2.02 - 1.94 (m, 3 H), 1.82 (dt, *J* = 3.1, 6.4 Hz, 2 H), 1.38 - 1.32 (m, 2 H), 0.90 (d, *J* = 6.6 Hz, 3 H), 0.26(s, 9H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 141.6, 137.3, 133.3, 128.6, 54.7, 53.0, 46.3, 41.6, 35.5, 32.9, 32.8, 18.2, -1.0

HRMS (ESI): Calculated for $C_{19}H_{35}N_2Si[M+H]^+$: 319.2564, found 319.2563

HPLC analysis (Method A): Purity 95.89% t_R = 4.820 min

***N*,*N*-Diethyl-2-methyl-3-(4-(trimethylsilyl)phenyl)propanamide (14)**



14

The title compound was synthesized from silacarboxylic acid **11** and diethyl amine by following general procedure A.

Yield: 81%.

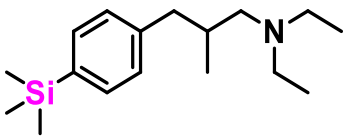
IR ν_{max} (thin film, $CHCl_3$) : 3010, 2980, 1626, 1449, 1381, 1217 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 (d, *J* = 8.1 Hz, 2H), 7.17 (d, *J* = 8.1 Hz, 2H), 3.42 (dd, *J* = 7.1, 13.4 Hz, 1H), 3.23 (dd, *J* = 6.8, 13.4 Hz, 1H), 3.17 - 2.96 (m, 3H), 2.92 - 2.81 (m, 1H), 2.64 (dd, *J* = 6.5, 13.1 Hz, 1H), 1.17(d, *J* = 6.7 Hz , 3H), 1.05 - 0.99 (m, 6H), 0.25 (s, 9H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 175.2, 140.9, 137.8, 133.3, 128.5, 41.7, 40.7, 40.4, 37.9, 18.1, 14.6, 13.0, -1.1

HRMS (ESI): Calculated for $C_{17}H_{30}ONSi[M+H]^+$: 292.2091, found 292.2086.

***N*,*N*-Diethyl-2-methyl-3-(4-(trimethylsilyl)phenyl)propan-1-amine (26)**



26

The compound **26** was synthesized from **14** by following general procedure B, as a liquid

Yield: 69%.

IR ν_{max} (thin film, $CHCl_3$) : 2965, 1425, 1252, 1216 cm^{-1}

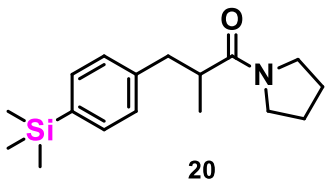
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 (d, *J* = 8.0 Hz, 2H), 7.17 (d, *J* = 8.0 Hz, 2H),, 2.87 (dd, *J* = 4.6, 13.4 Hz, 1H), 2.52 (q, *J* = 7.1 Hz, 4H), 2.29 - 2.19 (m, 3H), 1.90 - 1.85 (m, 1H), 1.01 (t, *J* = 7.1 Hz, 6H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.27 (s, 9H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 163.6, 148.3, 142.4, 137.9, 134.9, 133.8, 129.9, 129.3, 129.0, 127.9, 126.3, 110.4, 39.0, 36.1

HRMS (ESI): Calculated for $C_{17}H_{32}NSi$ $[M+H]^+$: 278.2299, found 278.2296

HPLC analysis (Method A): Purity: 91.06% t_R = 12.74 min.

2-Methyl-1-(pyrrolidin-1-yl)-3-(4-(trimethylsilyl) phenyl) propan-1-one (20)



20

The compound **20** was synthesized from sila-acid **11** and pyrrolidine by following general procedure A, as a Colorless liquid

Yield: 75%

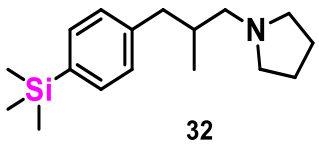
IR ν_{max} (thin film, $CHCl_3$) : 3019, 1629, 1438, 1217, 1088 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 (d, *J* = 7.8 Hz, 2H), 7.18 (d, *J* = 7.8 Hz, 2H), 3.42 - 3.40 (m, 2H), 3.28 (td, *J* = 6.7, 9.8 Hz, 1H), 2.98 - 2.95 (m, 2H), 2.79 - 2.77 (m, 1H), 2.67 - 2.64 (m, 1H), 1.78 - 1.62 (m, 4H), 1.17 (d, *J* = 6.8 Hz, 3H), 0.25 (s, 9H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.4, 140.8, 137.9, 133.3, 128.5, 46.3, 45.7, 40.5, 40.3, 26.0, 24.2, 17.3, -1.1

HRMS (ESI): Calculated for $C_{17}H_{28}ONSi[M+H]^+$: 290.1935, found 290.1933.

1-(2-Methyl-3-(4-(trimethylsilyl) phenyl) propyl)pyrrolidine (32)



32

The compound **32** was synthesized from **20** by following general procedure B, as a liquid

Yield: 70%.

IR ν_{max} (thin film, $CHCl_3$) : 3011, 1605, 1393, 1250, 1217, 1109 cm^{-1}

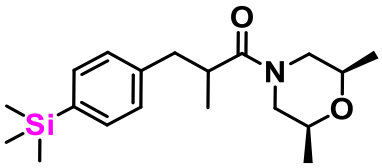
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (d, *J* = 7.8 Hz, 2H), 7.18 (d, *J* = 7.8 Hz, 2H), 3.77 (t, *J* = 6.7 Hz, 1H), 2.87 (dd, *J* = 4.8, 13.3 Hz, 1H), 2.52- 2.51 (m, 4H), 2.34 - 2.37 (m, 1H), 2.35 - 2.28 (m, 1H), 2.08- 1.90 (m, 1H), 1.88 (td, *J* = 3.2, 6.7 Hz, 1H), 1.83 - 1.80 (m, 3H), 0.90 (d, *J* = 6.6 Hz, 3H), 0.28 (s, 9H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 139.1, 138.7, 133.8, 128.6, 61.6, 55.7, 53.8, 41.5, 32.9, 23.4, 19.2, -1.0

HRMS (ESI): Calculated for $C_{17}H_{30}NSi[M+H]^+$: 276.2142, found 276.2138

HPLC analysis (Method A): Purity 91.03% t_R = 7.76 min

1-((2*S*,6*R*)-2,6-dimethylmorpholino)-2-methyl-3-(4-(trimethylsilyl)phenyl)propan-1-one (24)



24

The compound **24** was synthesized from sila-acid **11** and (2*S*,6*R*)-2,6-dimethyl morpholine by following general procedure A, as a Colorless liquid (74 mg, 85%) as rotamers having 2 :1 ratio.

Yield: 85%.

IR ν_{max} (thin film, $CHCl_3$) : 3019, 1629, 1438, 1217, 1088 cm^{-1}

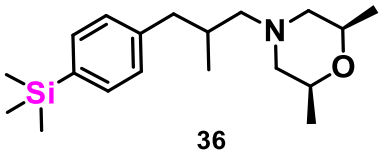
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (d, *J* = 7.8 Hz, 2H), 7.23 - 7.02 (m, 2H), 4.48- 4.44 (m, 1H), 3.50 - 3.47 (m, 2H), 3.39 (td, *J* = 2.3, 13.0 Hz, 1H), 3.24 - 3.12 (m, 2H), 2.99 -

2.95 (m, 2H), 2.77 - 2.55 (m, 2H), 2.34 - 2.28 (m, 1H), 2.24 - 2.18 (m, 1H), 1.21 - 1.19(m, 4H), 1.15 - 1.11 (m, 3H), 0.94 (d, *J* = 6.1 Hz, 2H), 0.25 (s, 9H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.3, 173.9, 140.5, 138.3, 133.4, 128.6, 128.5, 71.9, 71.7, 71.2, 51.3, 50.9, 47.1, 47.0, 41.2, 40.2, 37.5, 37.4, 18.7, 18.5, 18.3, 18.0, -1.08, -1.12.

HRMS (ESI): Calculated for $C_{19}H_{32}NSiO_2$ $[M+H]^+$: 334.2197, found 334.2195.

(2*S*,6*R*)-2,6-dimethyl-4-(2-methyl-3-(4-trimethylsilyl)phenyl)propyl)morpholine (36)



36

The compound **36** was synthesized from **24** by following general procedure B, as a liquid

Yield: 84%.

IR ν_{max} (thin film, $CHCl_3$) : 1597, 1450, 1389, 1146, 1074 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47 (d, *J* = 7.8 Hz, 2H), 7.19 (d, *J* = 7.8 Hz, 2H), 3.74 - 3.68 (m, 2H), 2.82 (dd, *J* = 4.9, 13.4 Hz, 1H), 2.72 (tdd, *J* = 2.0, 11.2, 19.3 Hz, 2H), 2.33 (dd, *J* = 8.6, 13.4 Hz, 1H), 2.25 - 2.17 (m, 1H), 2.17 - 2.09 (m, 1H), 2.07 - 1.95 (m, 1H), 1.77 - 1.65 (m, 2H), 1.18 (dd, *J* = 1.6, 6.2 Hz, 6H), 0.88 (d, *J* = 6.6 Hz, 3H), 0.28 (s, 9H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 141.7, 137.2, 133.2, 128.7, 71.7, 65.0, 60.0, 59.8, 41.3, 32.0, 19.2, 18.0, -1.0

HRMS (ESI): Calculated for $C_{19}H_{34}NOSi[M+H]^+$: 320.2404, found 320.2404

HPLC analysis (Method B): Purity 90.00% t_R = 8.96 min (method B).

2.8. References

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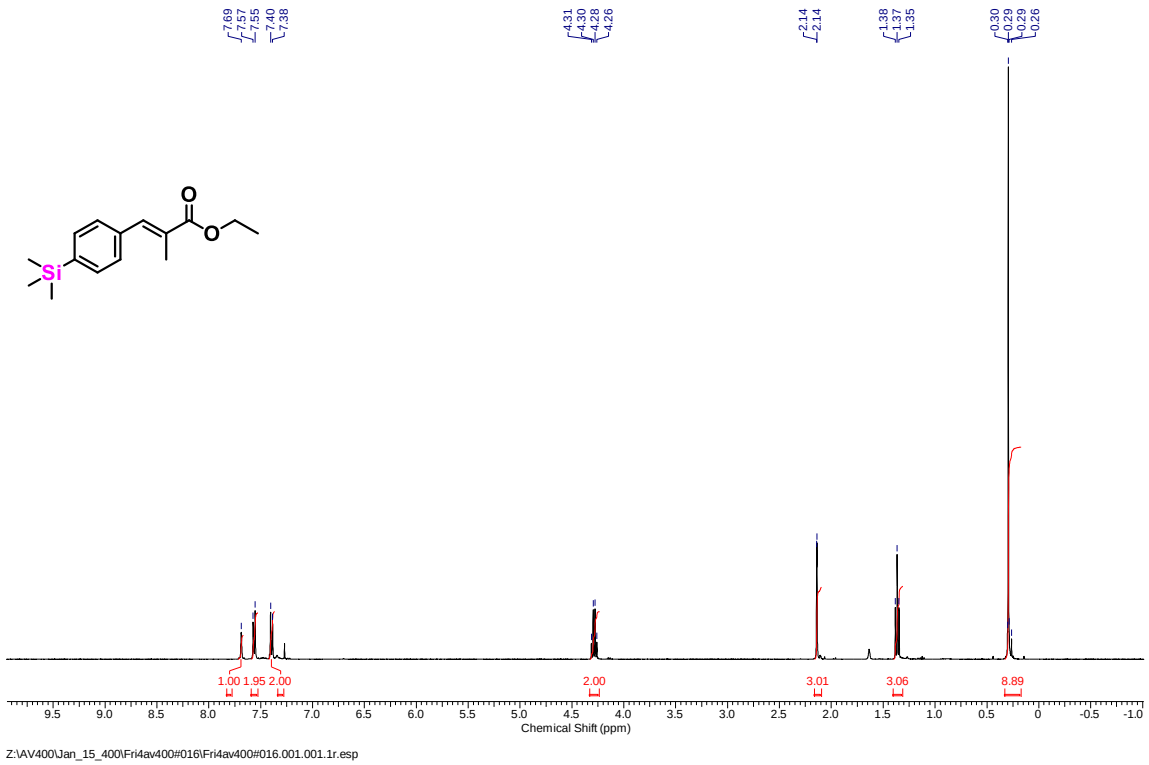
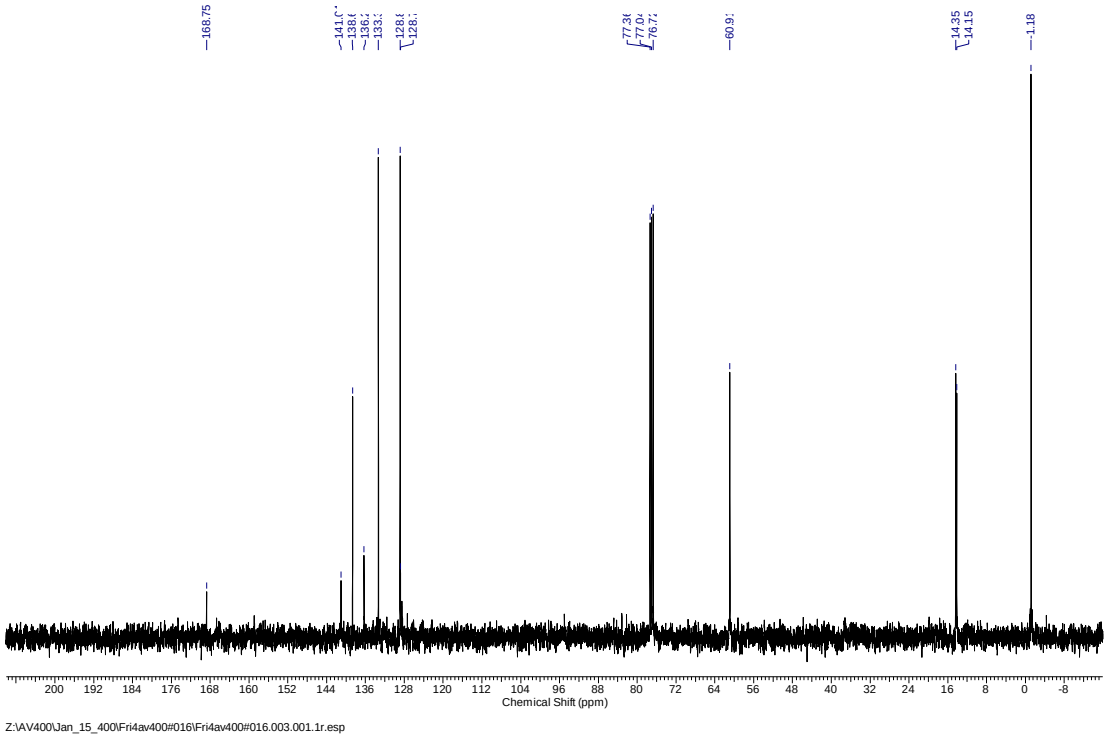
2.9. Copies of NMR Spectra



^{1}H NMR of **10** (400 MHz, $CDCl_3$)



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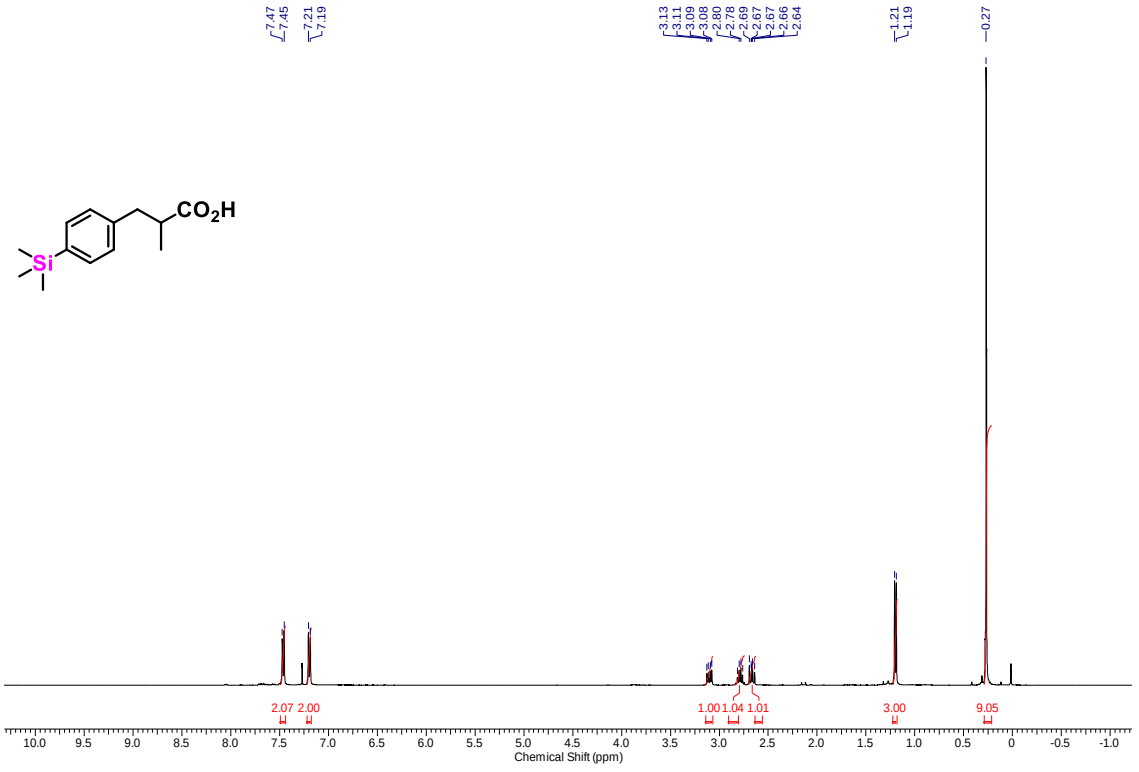

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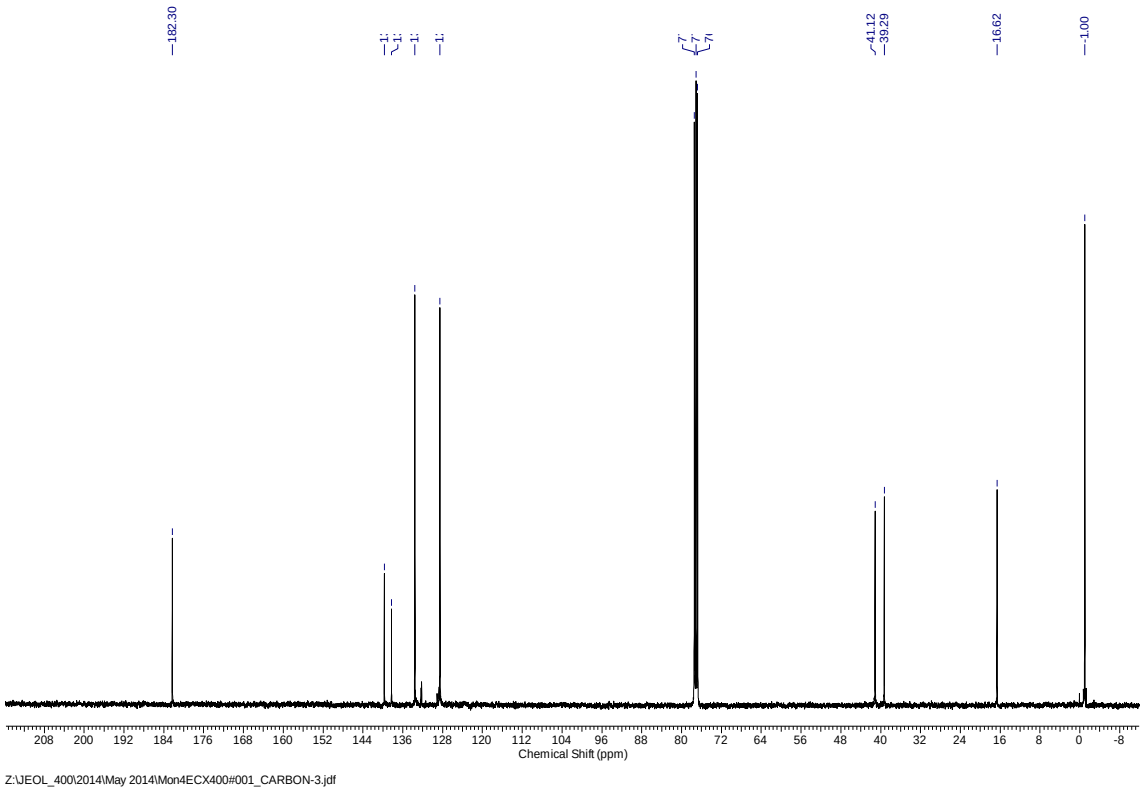

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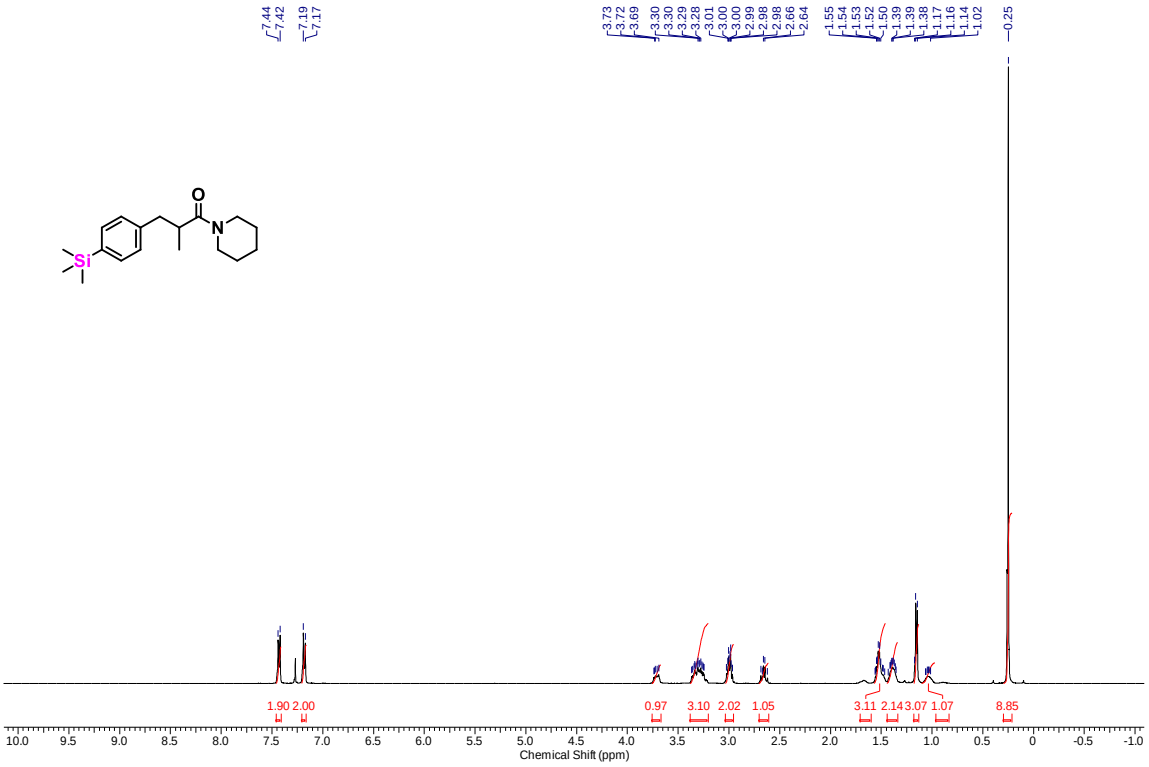

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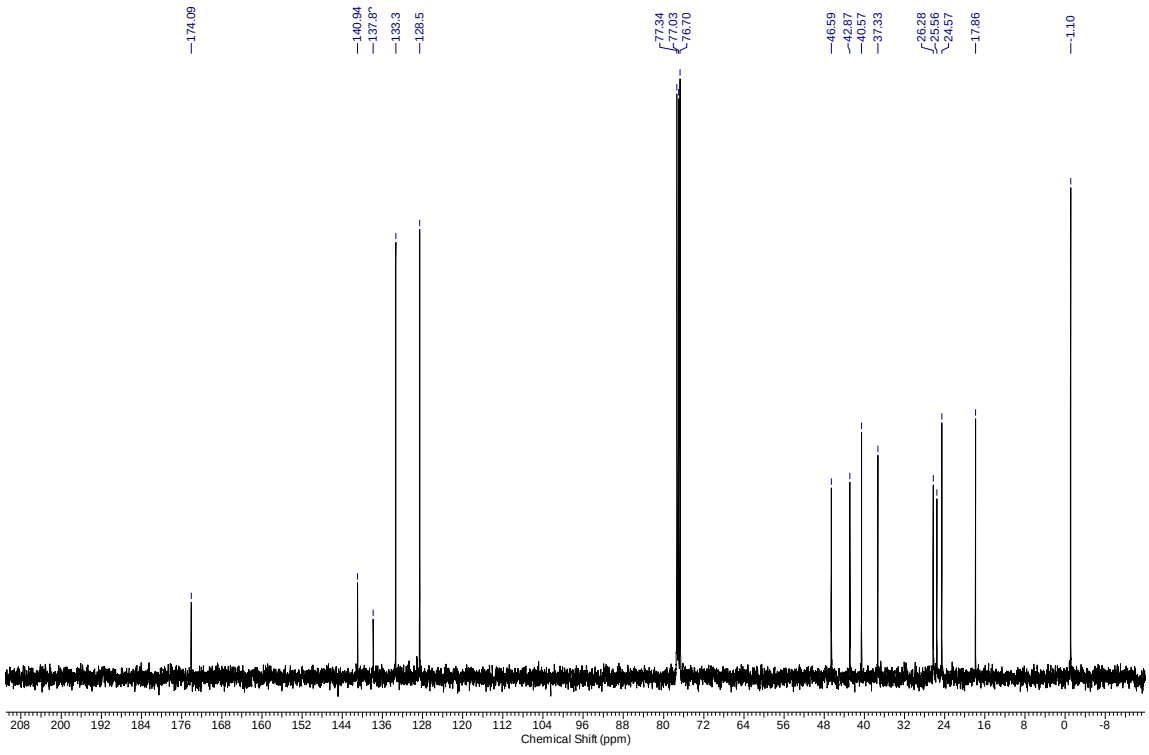
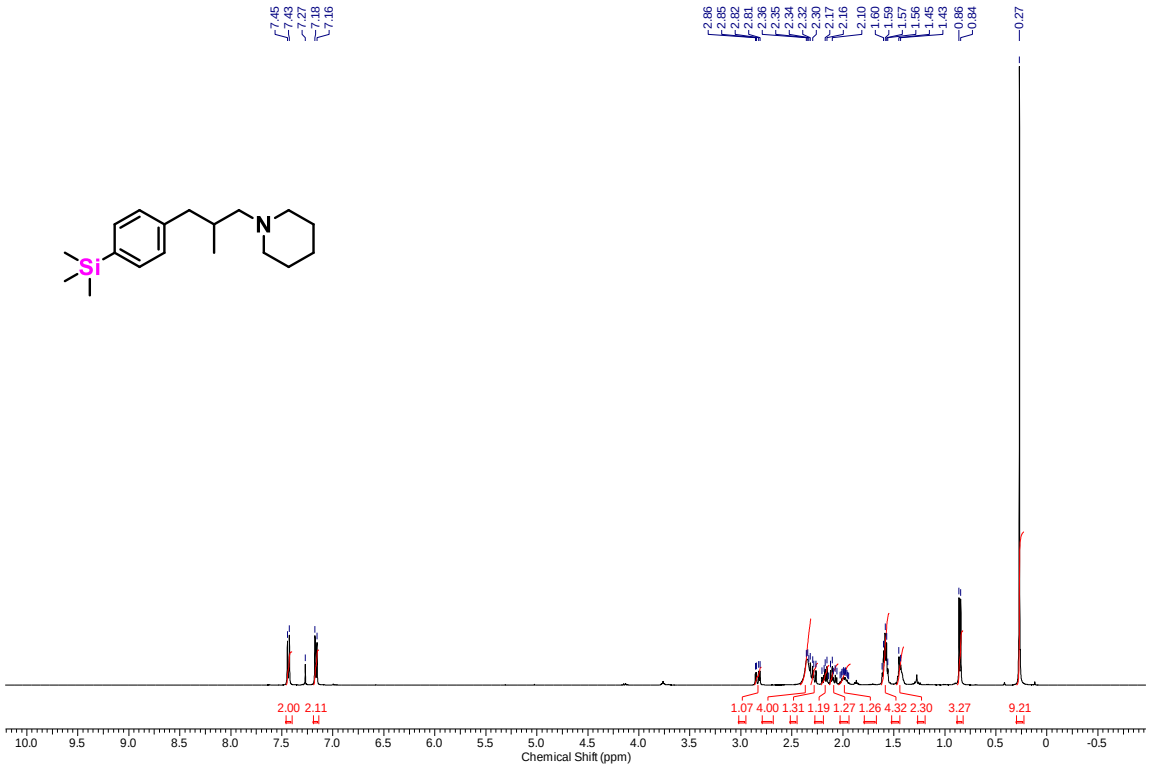
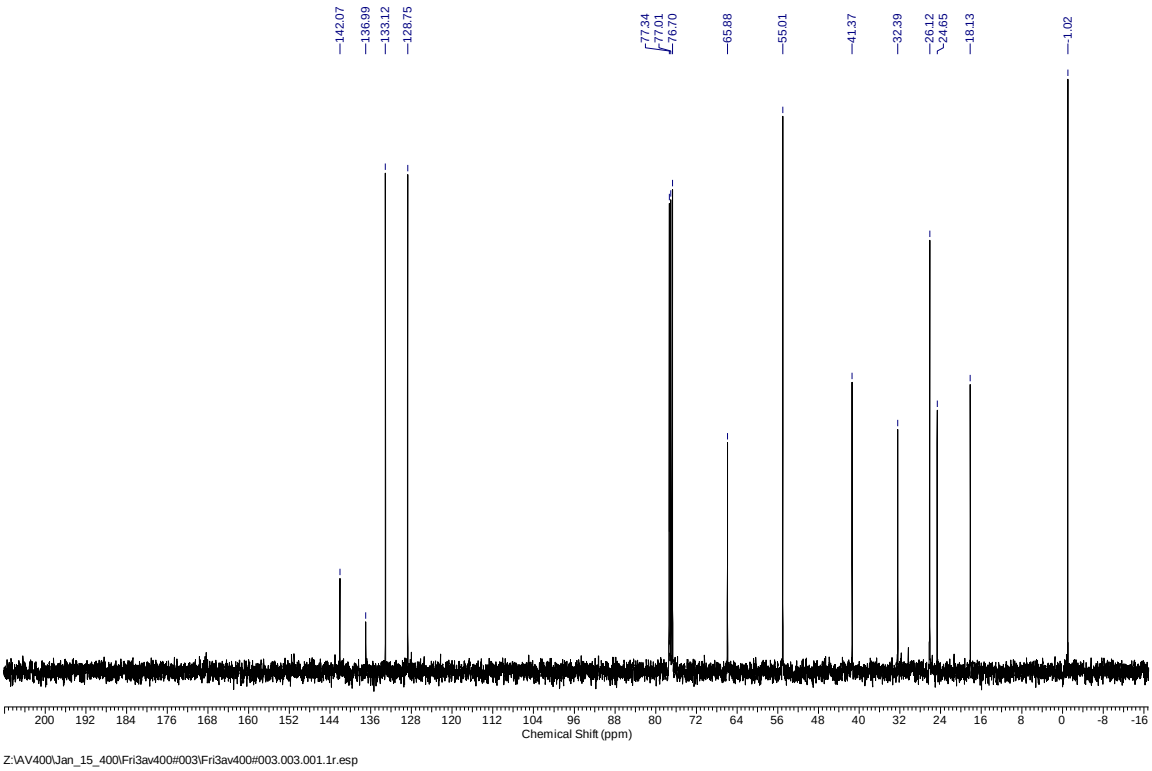


^{13}C NMR of **25** (100 MHz, $CDCl_3$)



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



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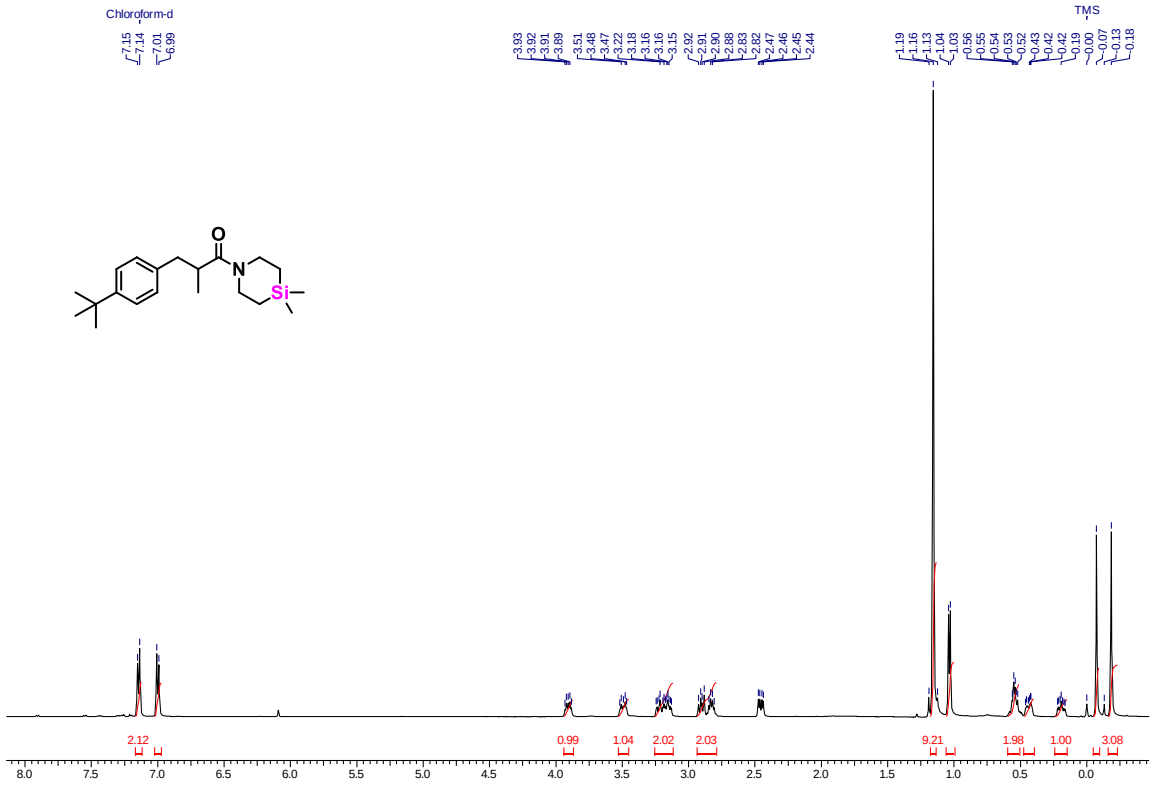

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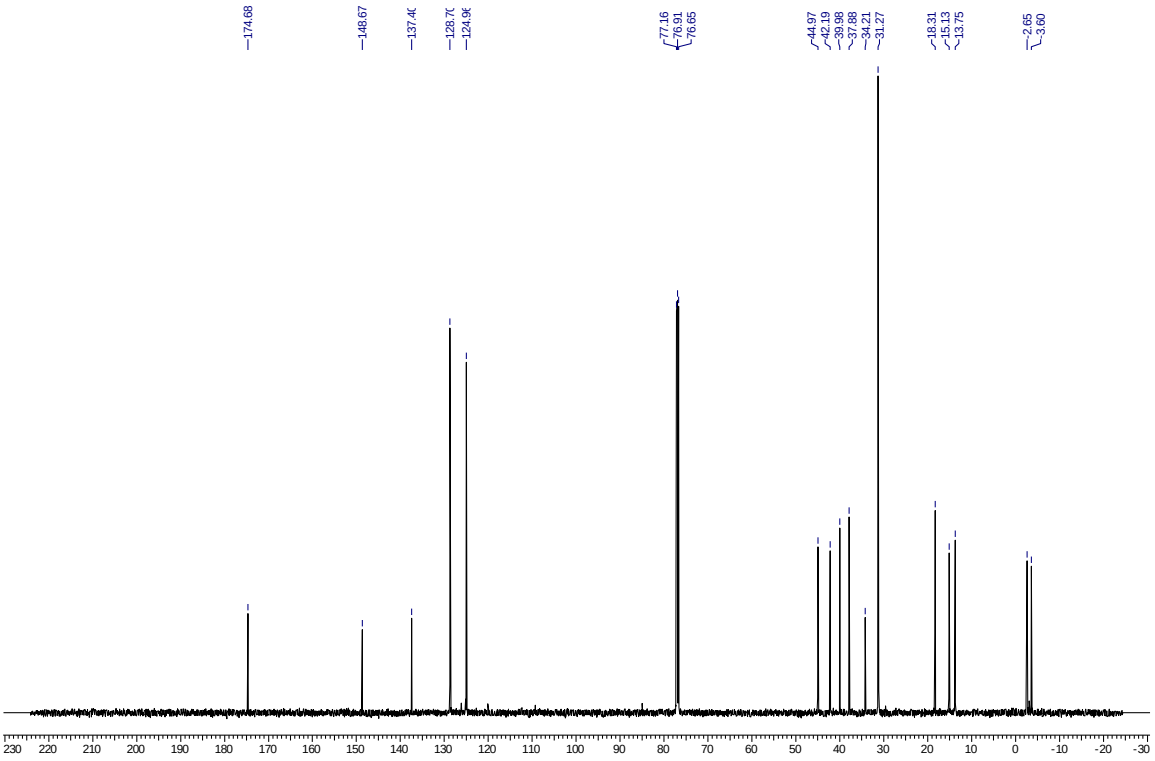
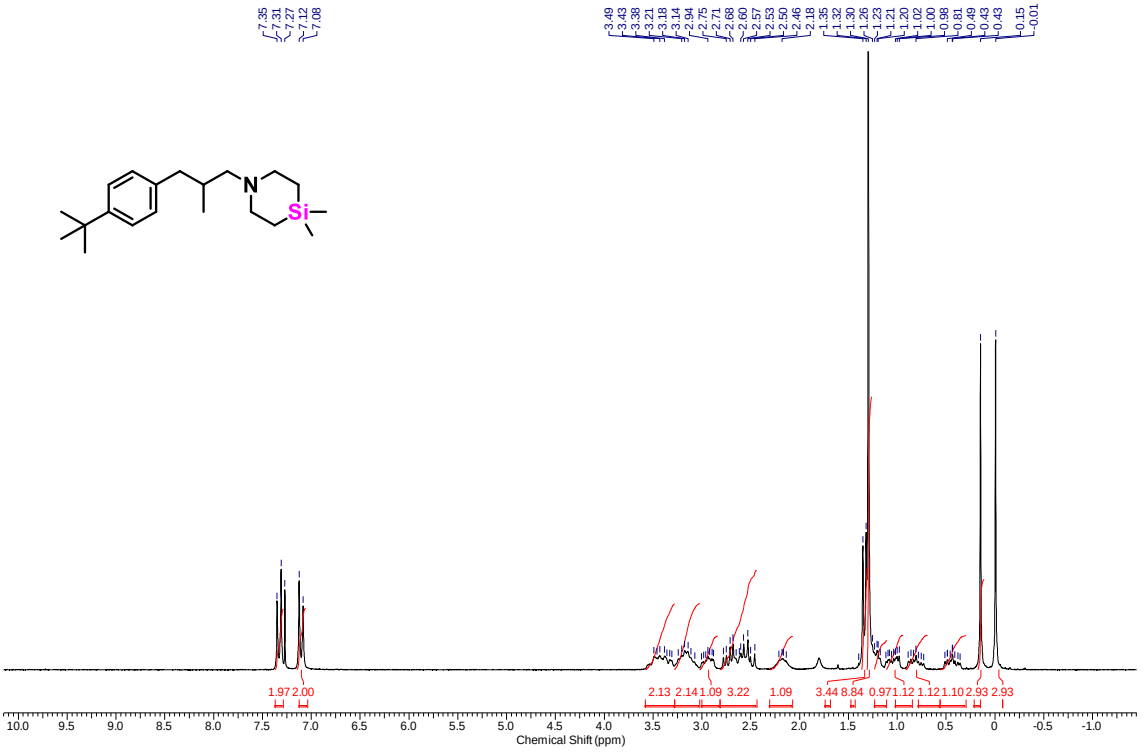
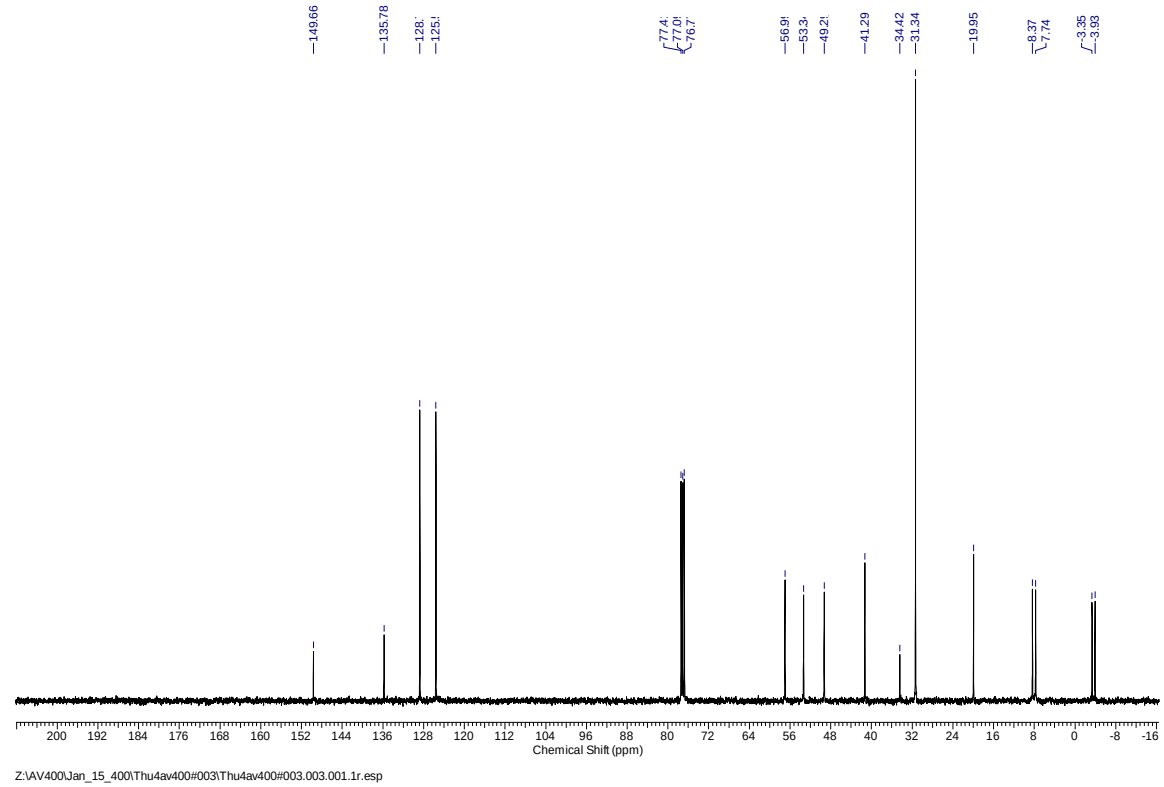


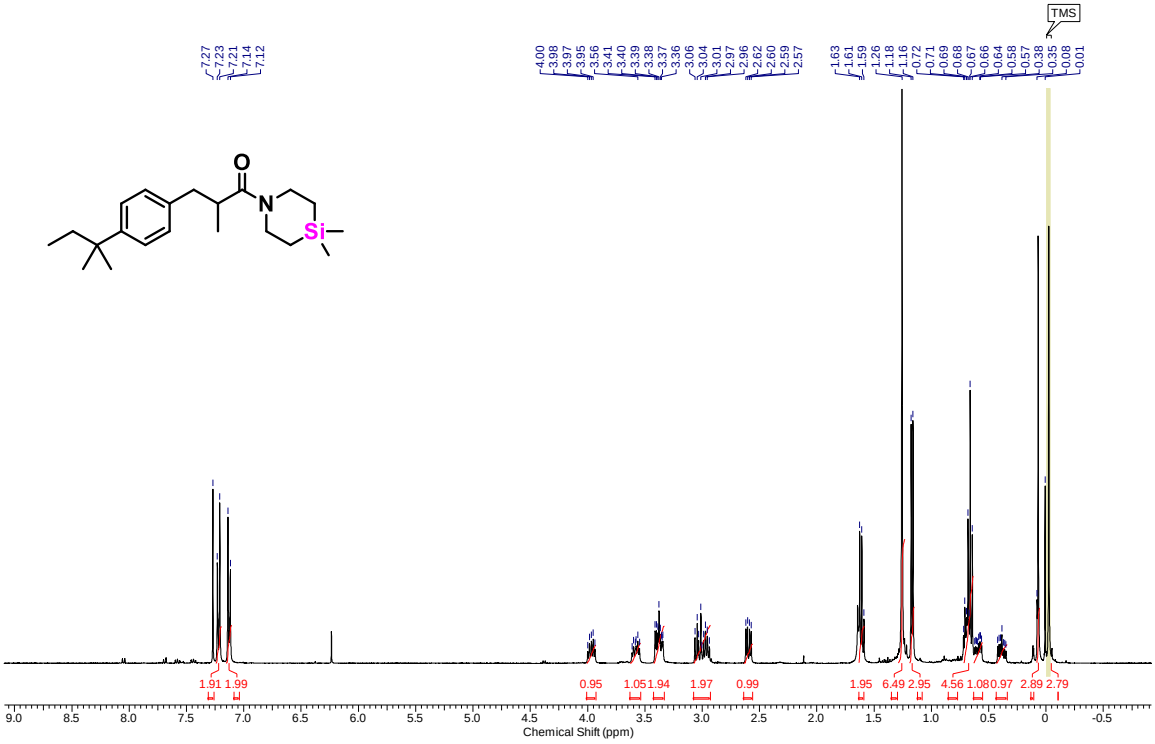
^{13}C NMR of **15** (100 MHz, $CDCl_3$)



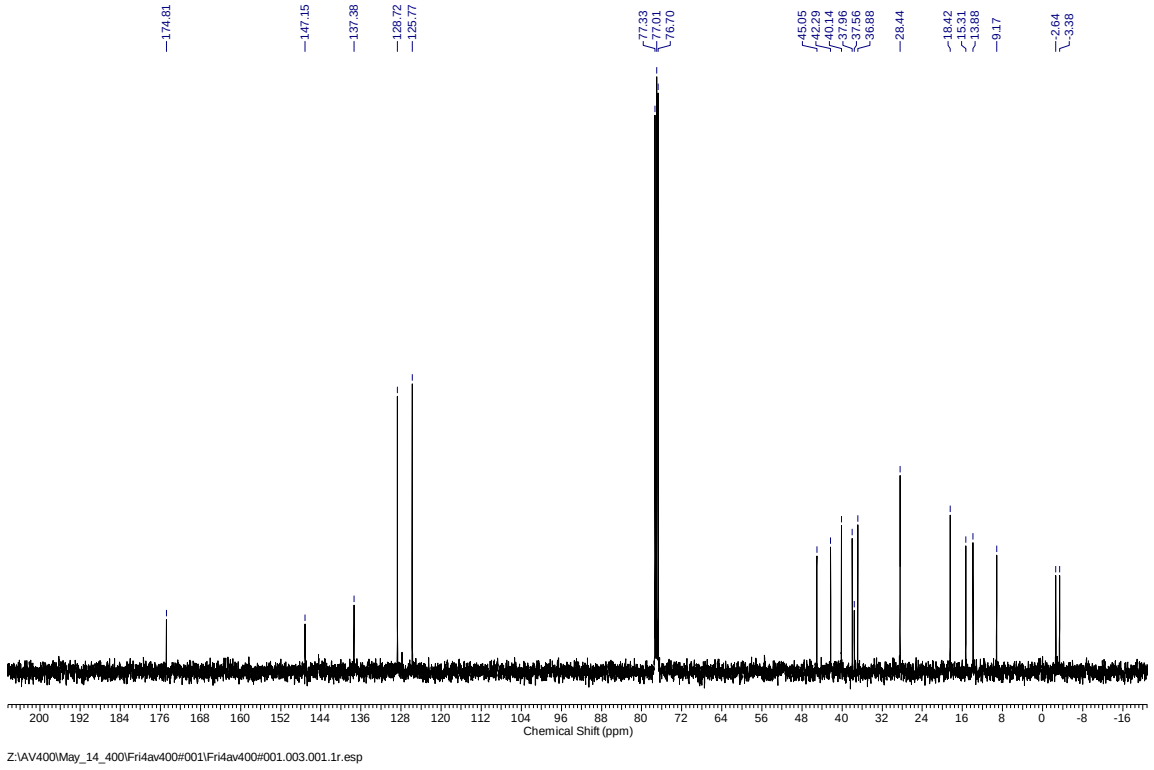
^{1}H NMR of **27** (200 MHz, $CDCl_3$)



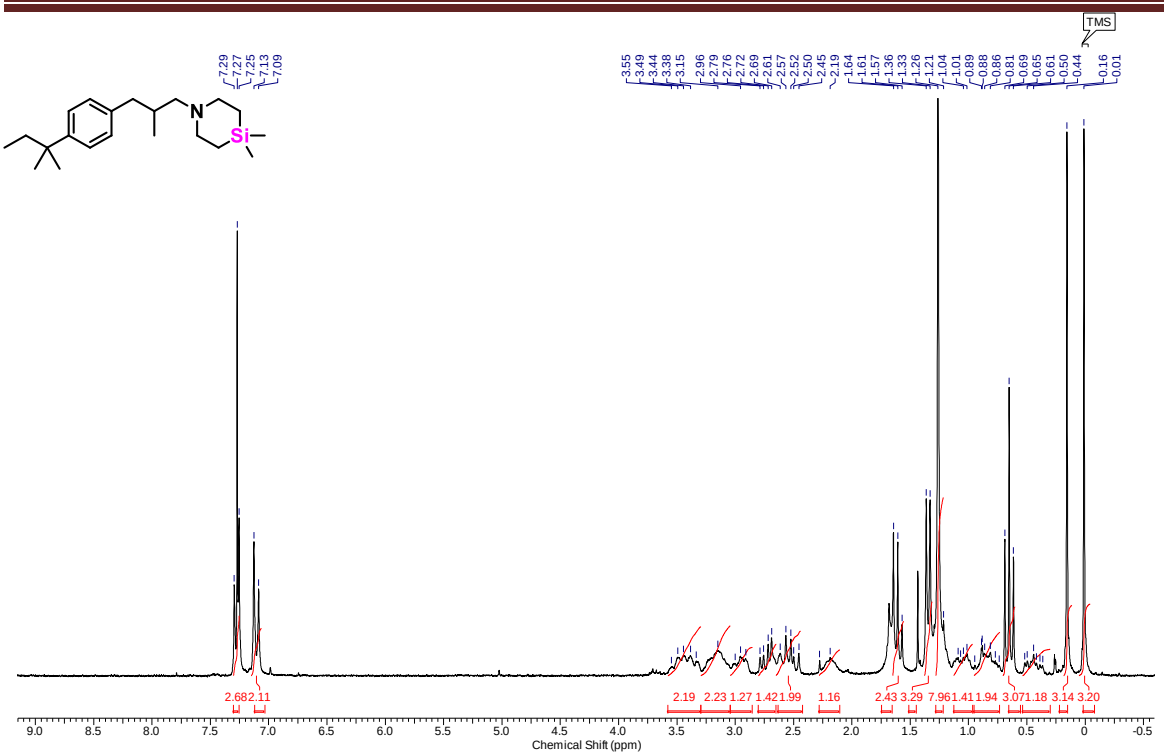
^{13}C NMR of **27** (100 MHz, $CDCl_3$)



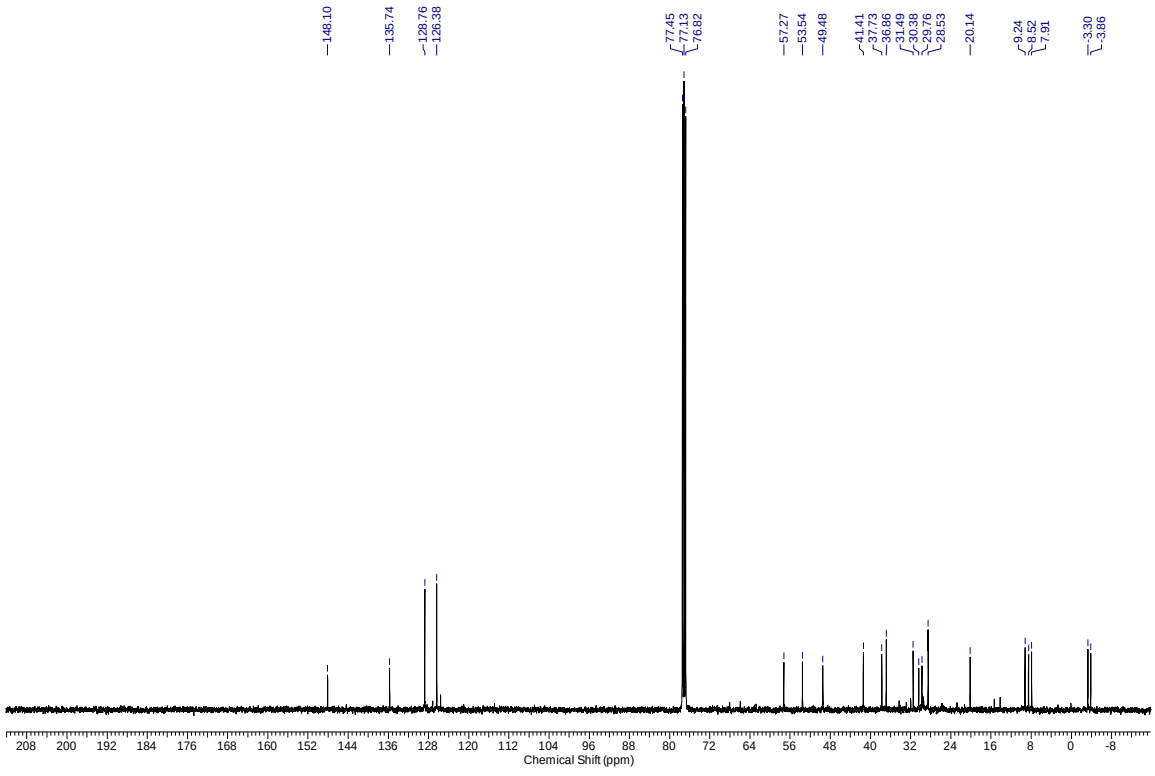
^{1}H NMR of **21** (200 MHz, $CDCl_3$)



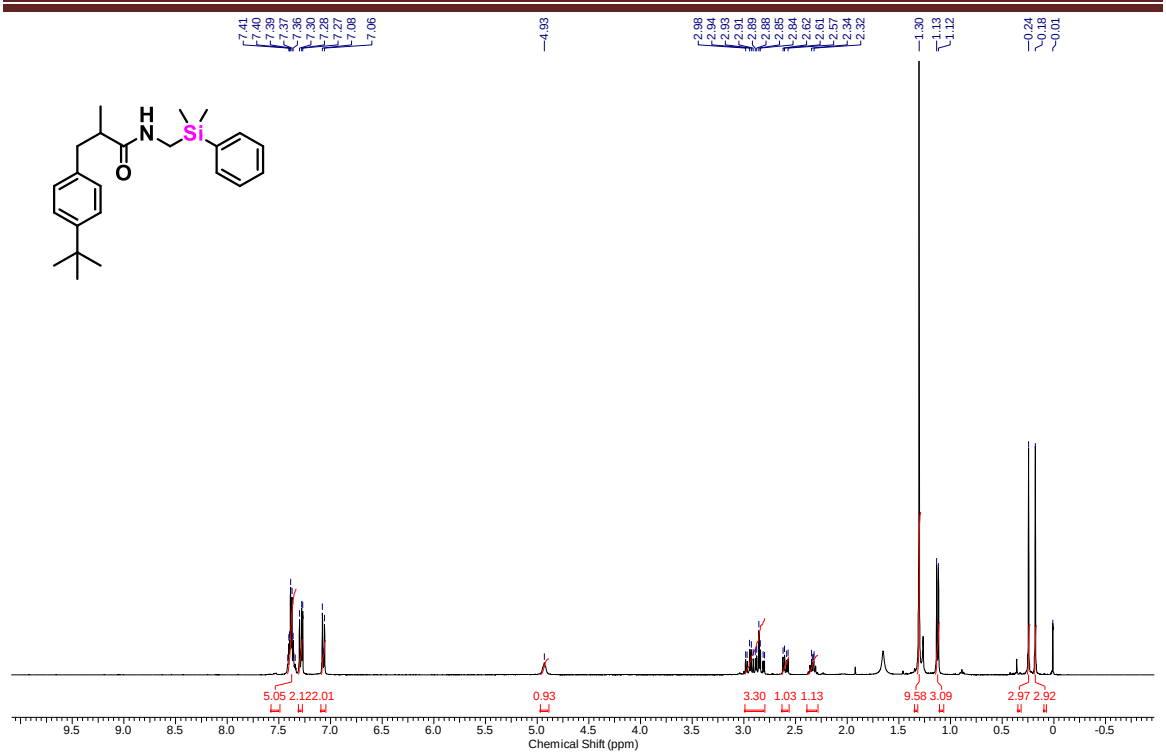
^{13}C NMR of **21** (100 MHz, $CDCl_3$)



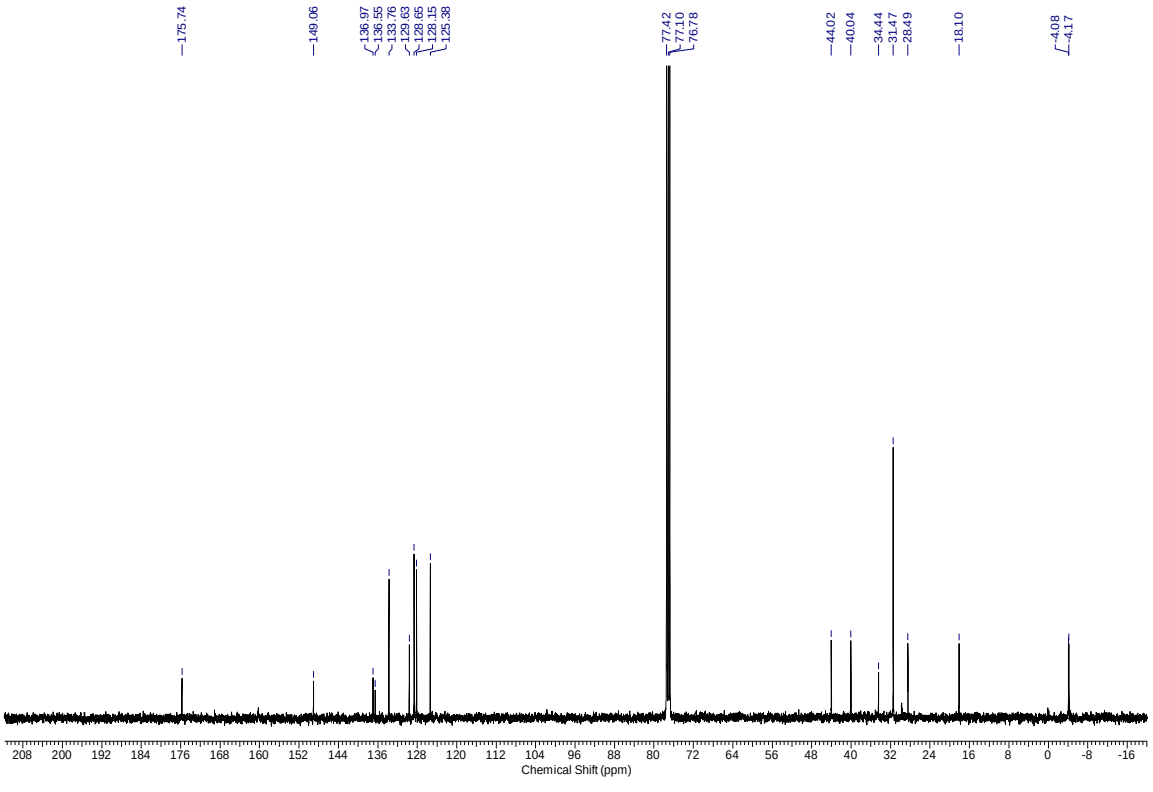
^{1}H NMR of **33** (200 MHz, $CDCl_3$)



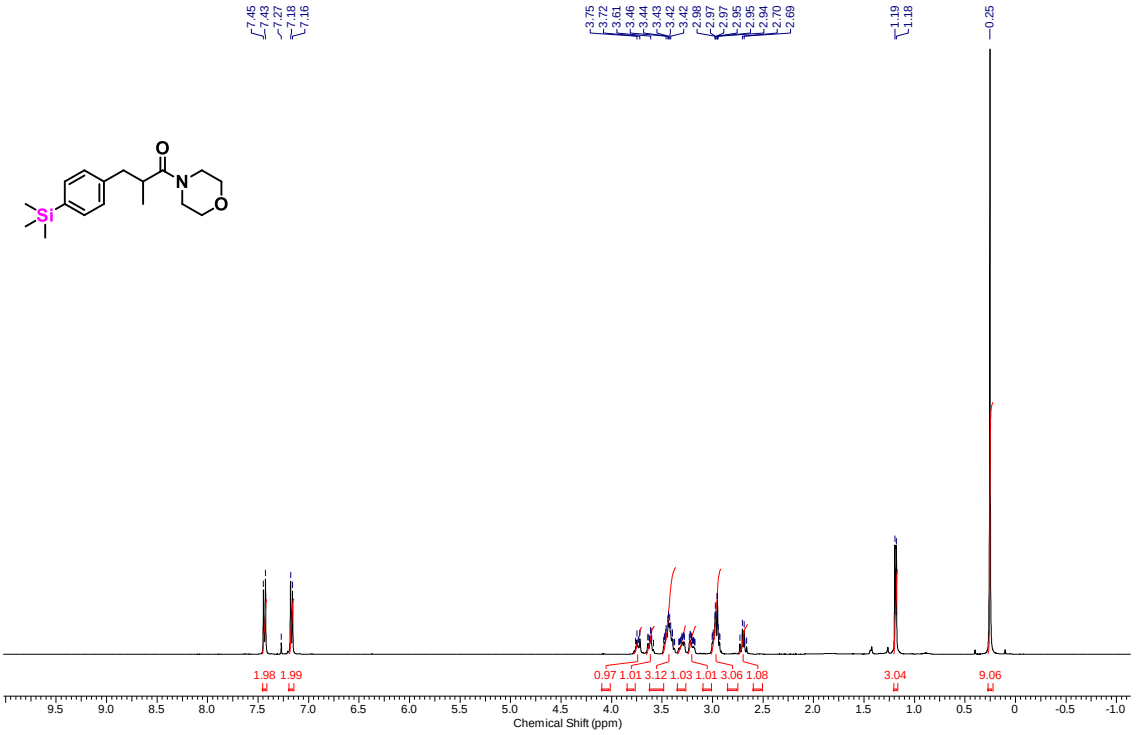
^{13}C NMR of **33** (100 MHz, $CDCl_3$)



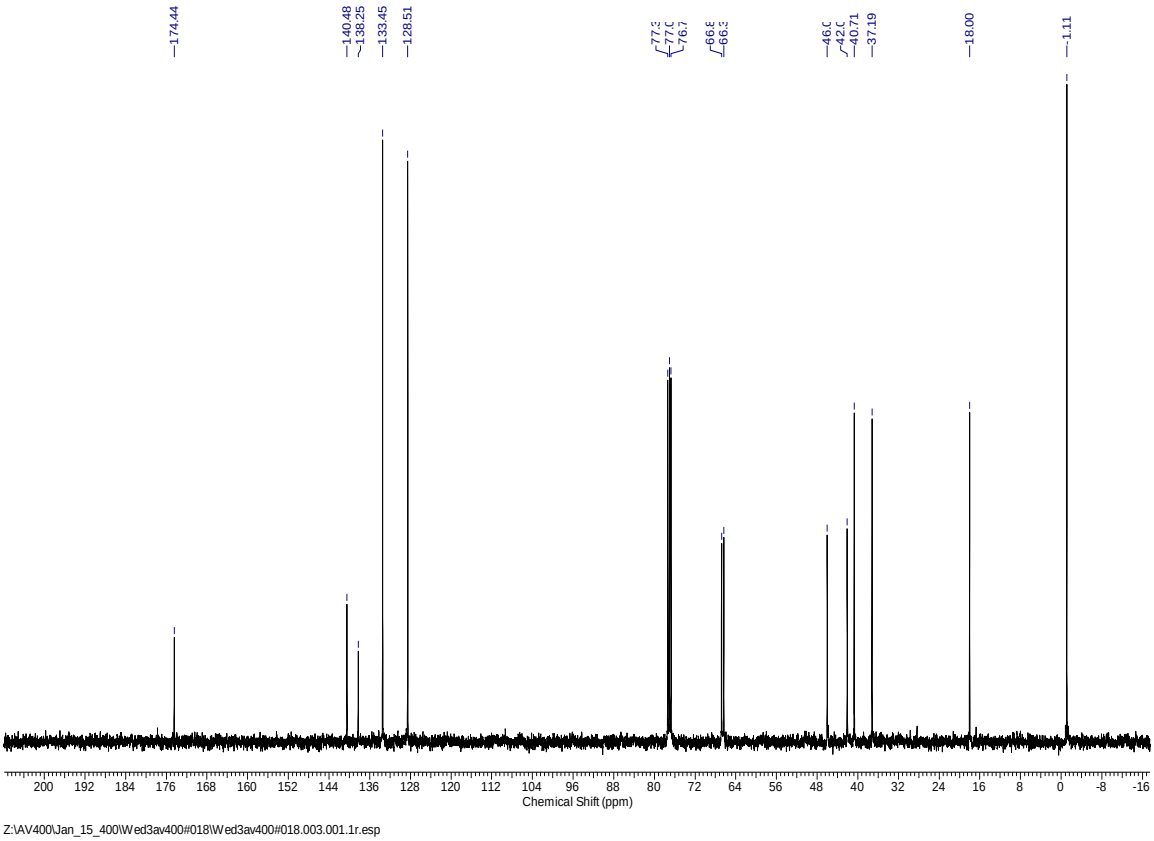
^{1}H NMR of **38** (400 MHz, $CDCl_3$)



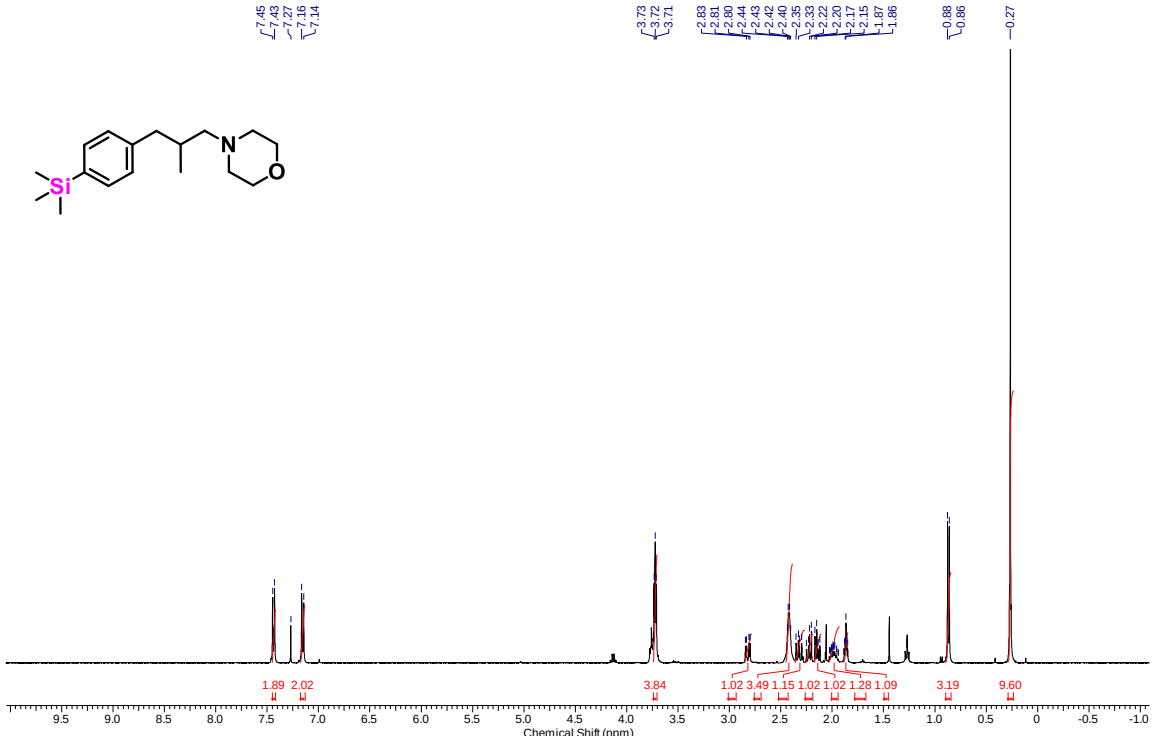
^{13}C NMR of **38** (100 MHz, $CDCl_3$)



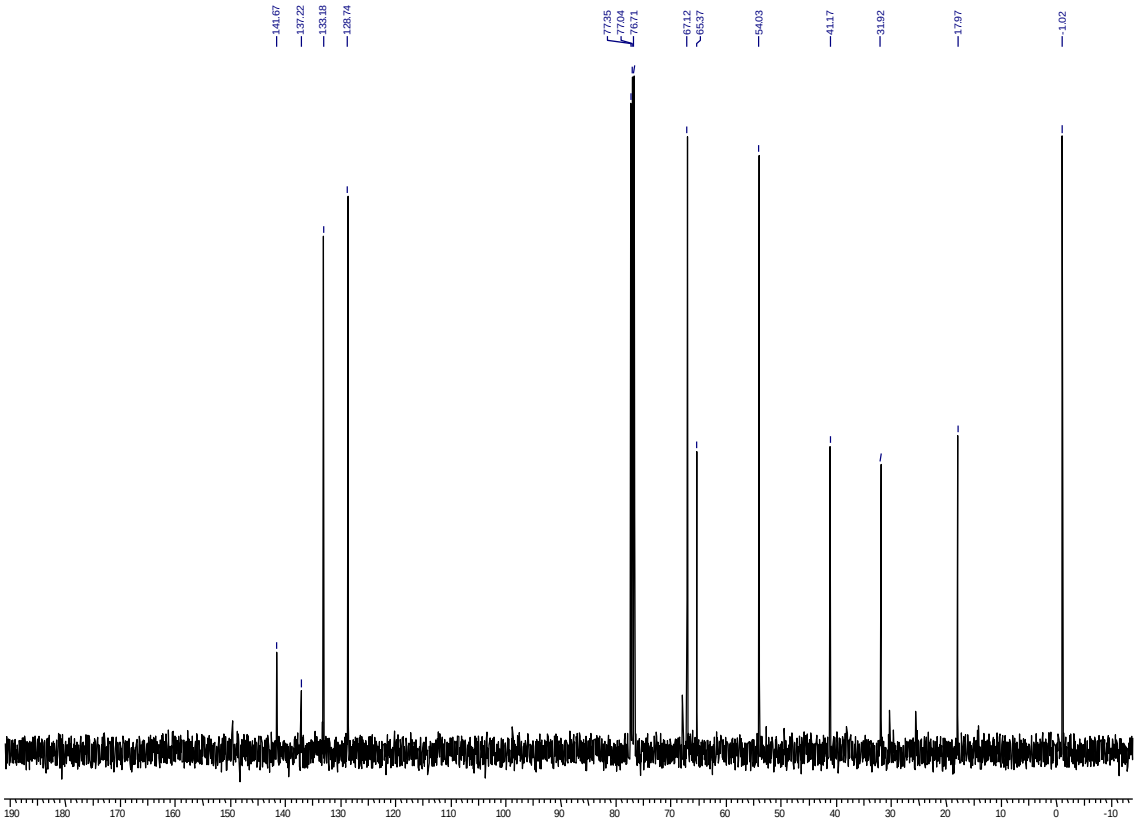
^{1}H NMR of **16** (400 MHz, $CDCl_3$)



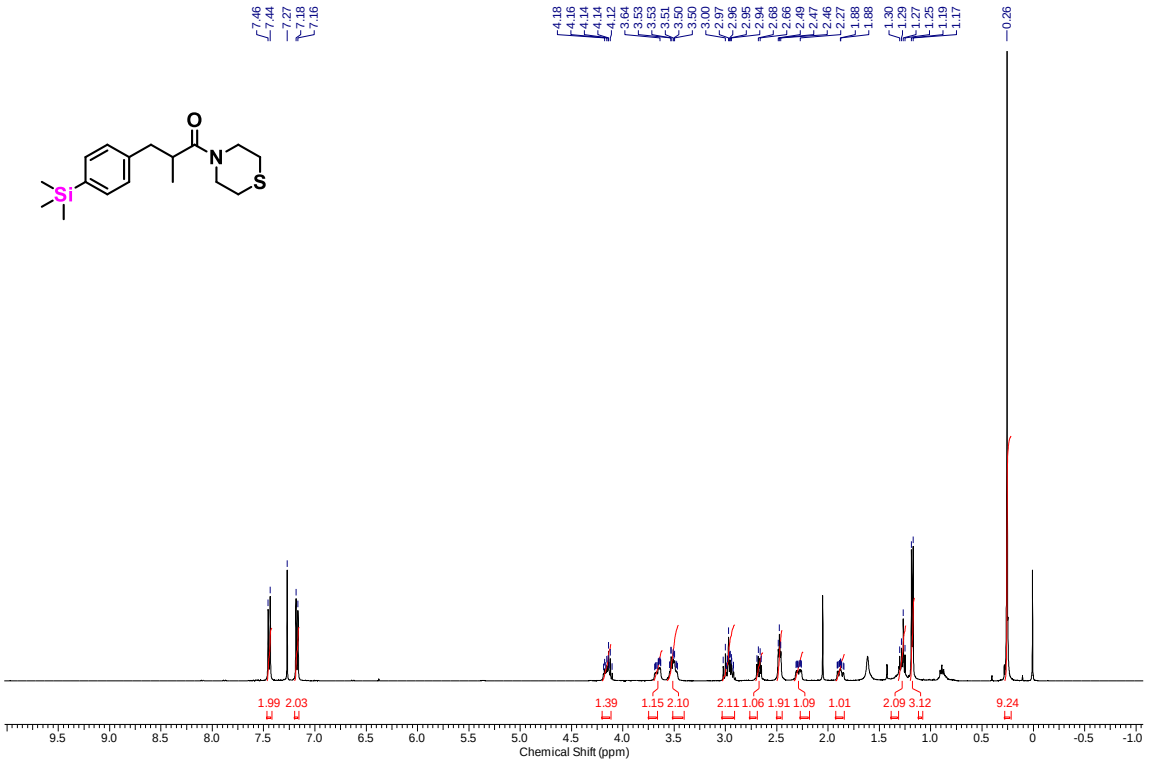
^{13}C NMR of **16** (100 MHz, $CDCl_3$)



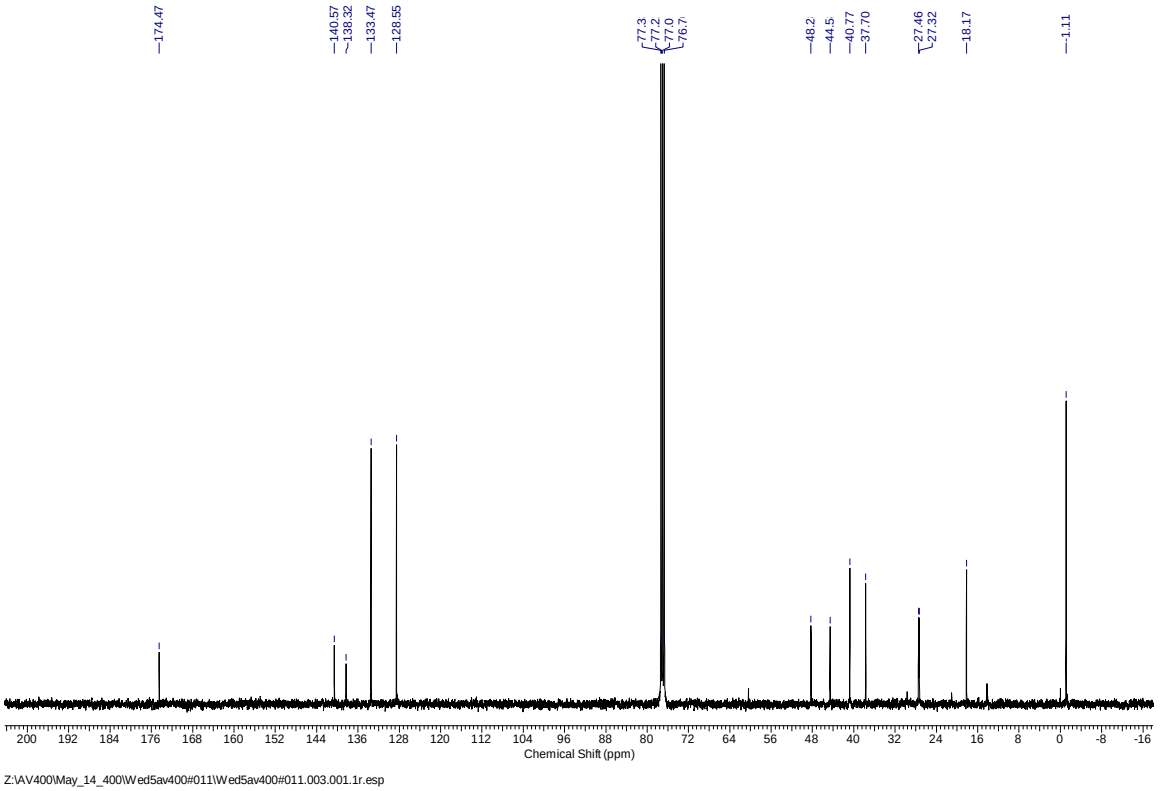
^{1}H NMR of **28** (400 MHz, $CDCl_3$)



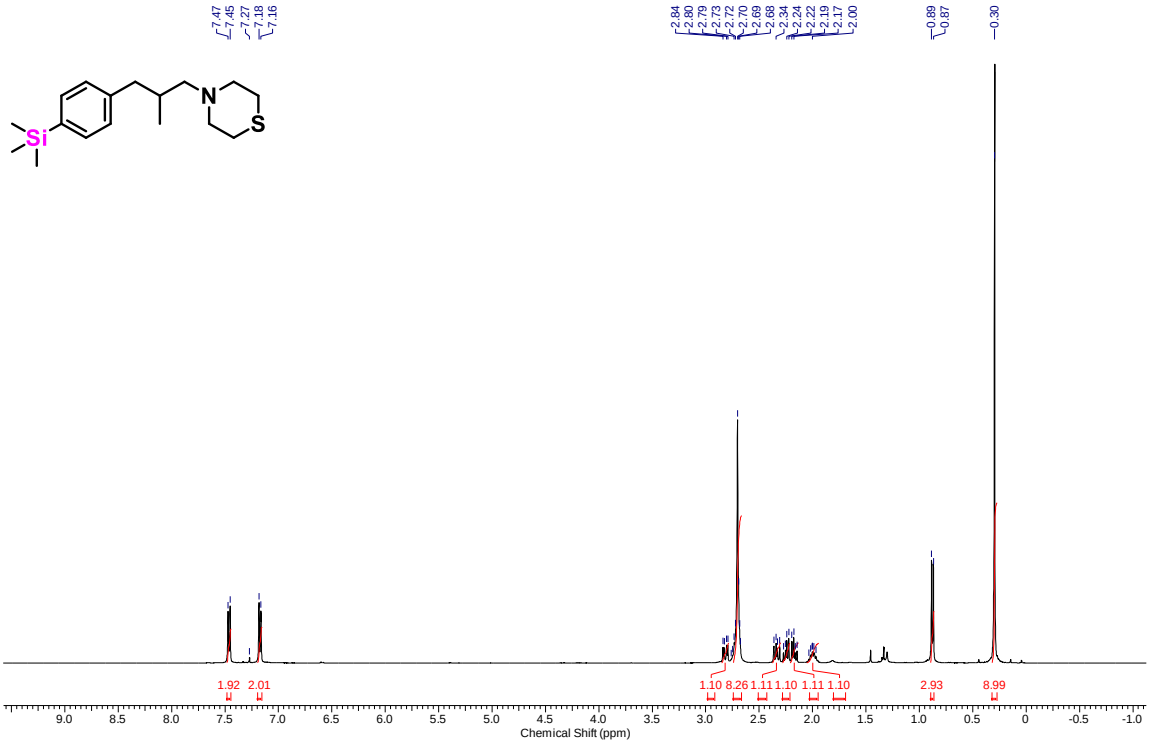
^{13}C NMR of **28** (100 MHz, $CDCl_3$)



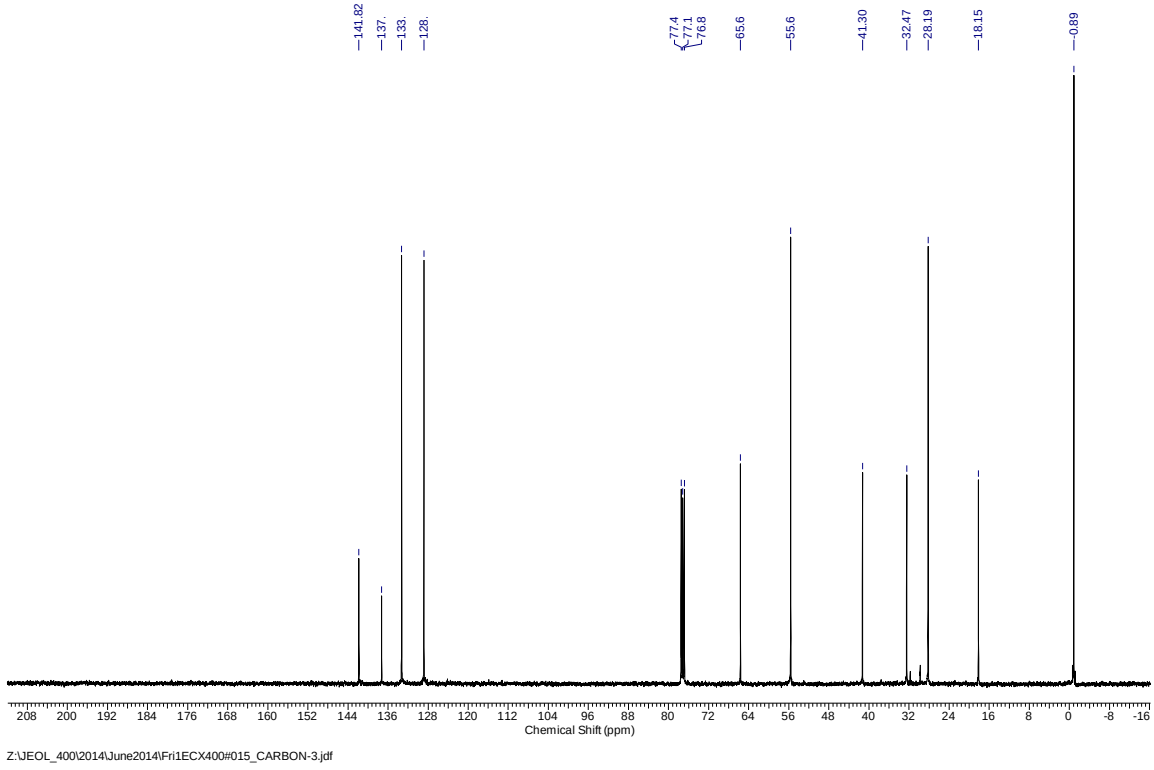
^{1}H NMR of **17** (400 MHz, $CDCl_3$)



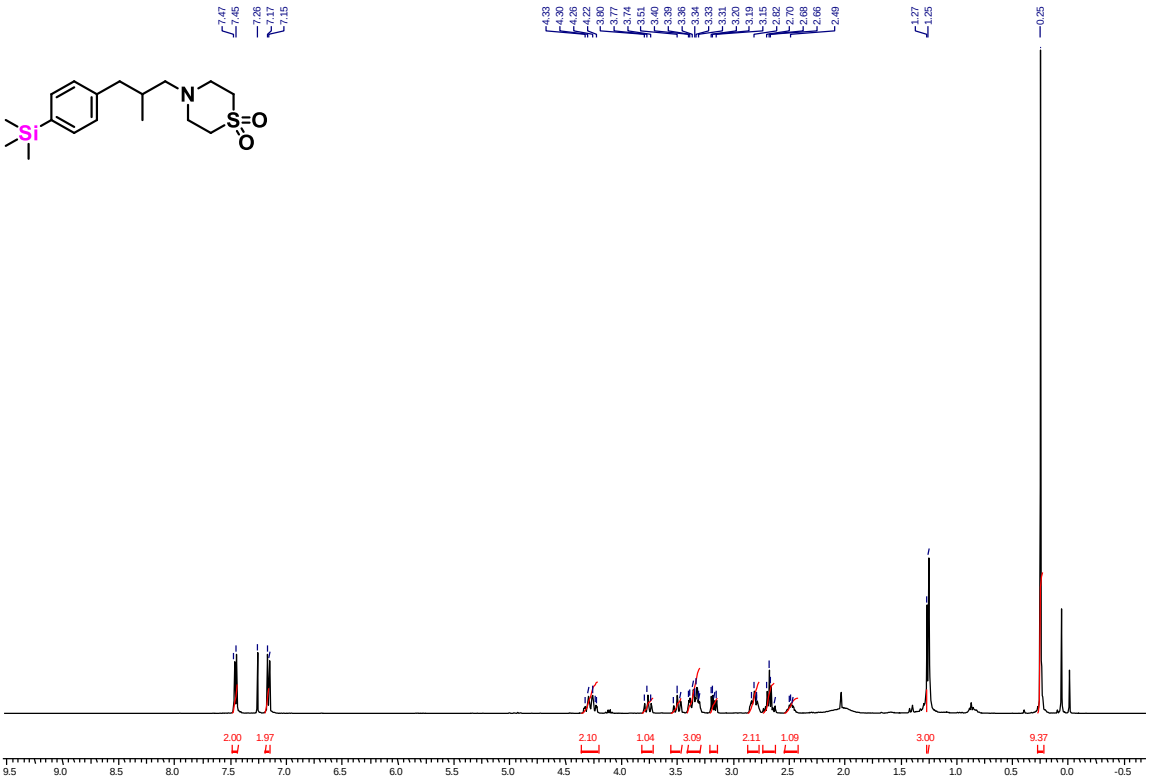
^{13}C NMR of **17** (100 MHz, $CDCl_3$)



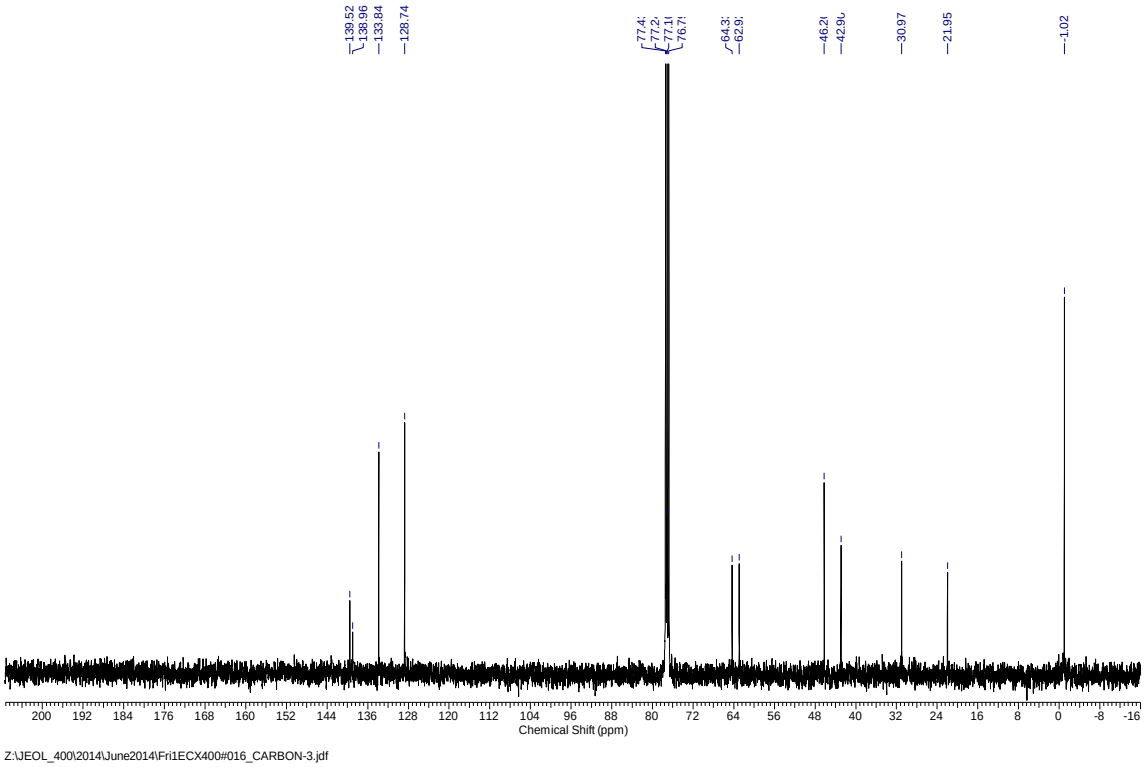
^{1}H NMR of **29** (400 MHz, $CDCl_3$)



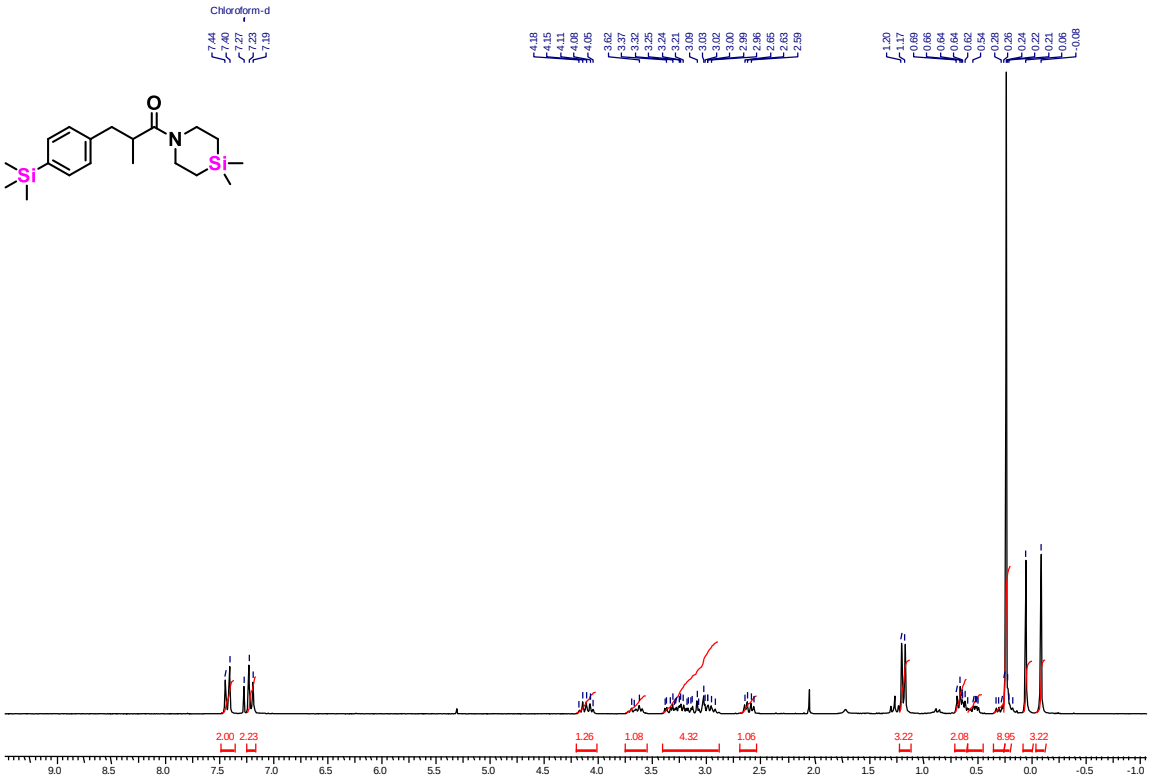
^{13}C NMR of **29** (100 MHz, $CDCl_3$)



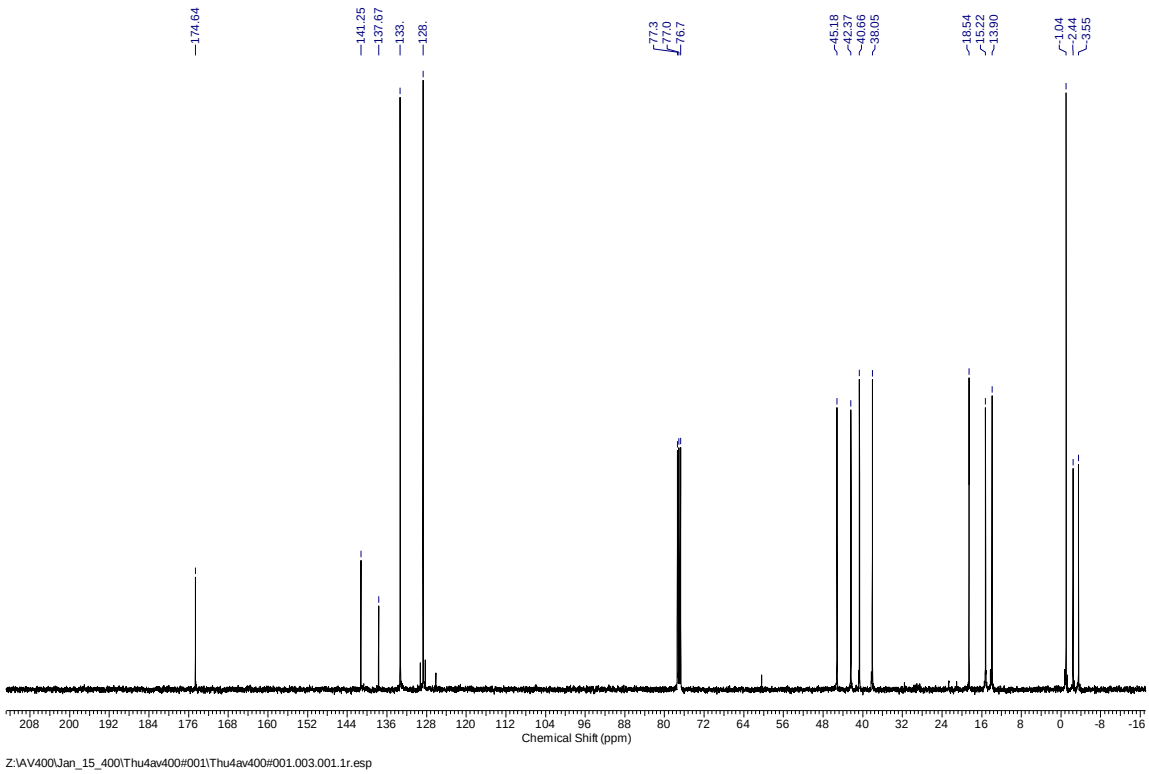
^{1}H NMR of **41** (400 MHz, $CDCl_3$)



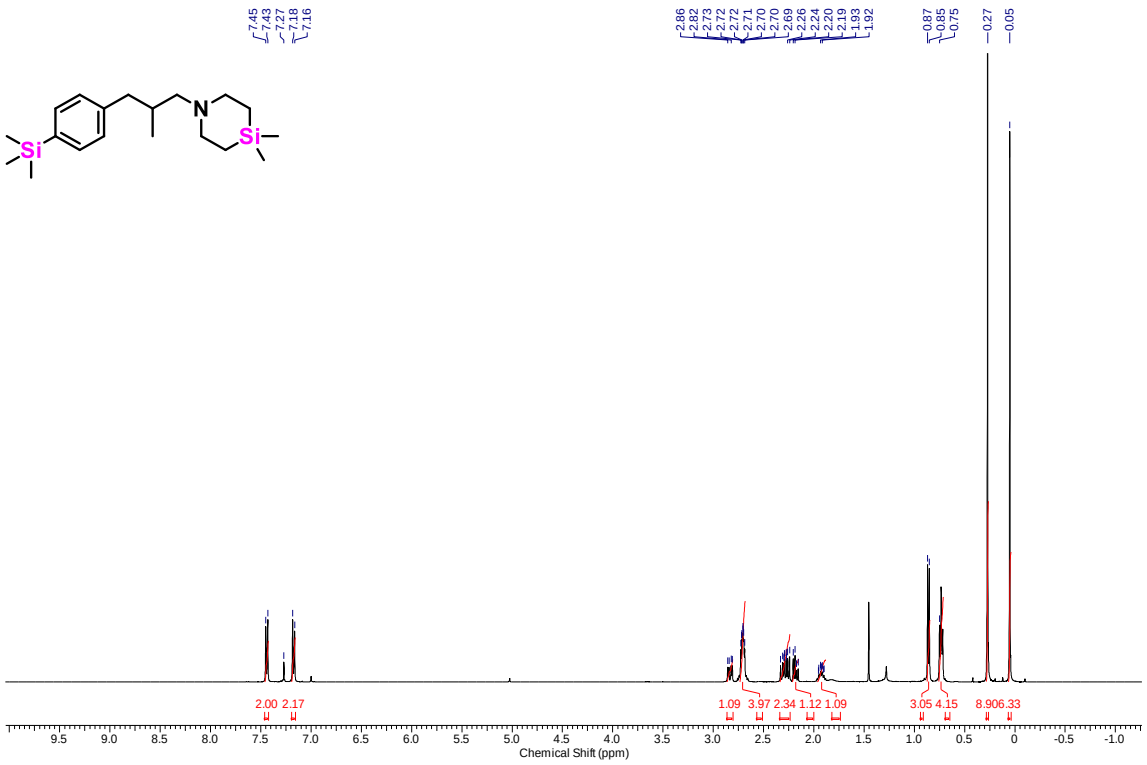
^{13}C NMR of **41** (100 MHz, $CDCl_3$)



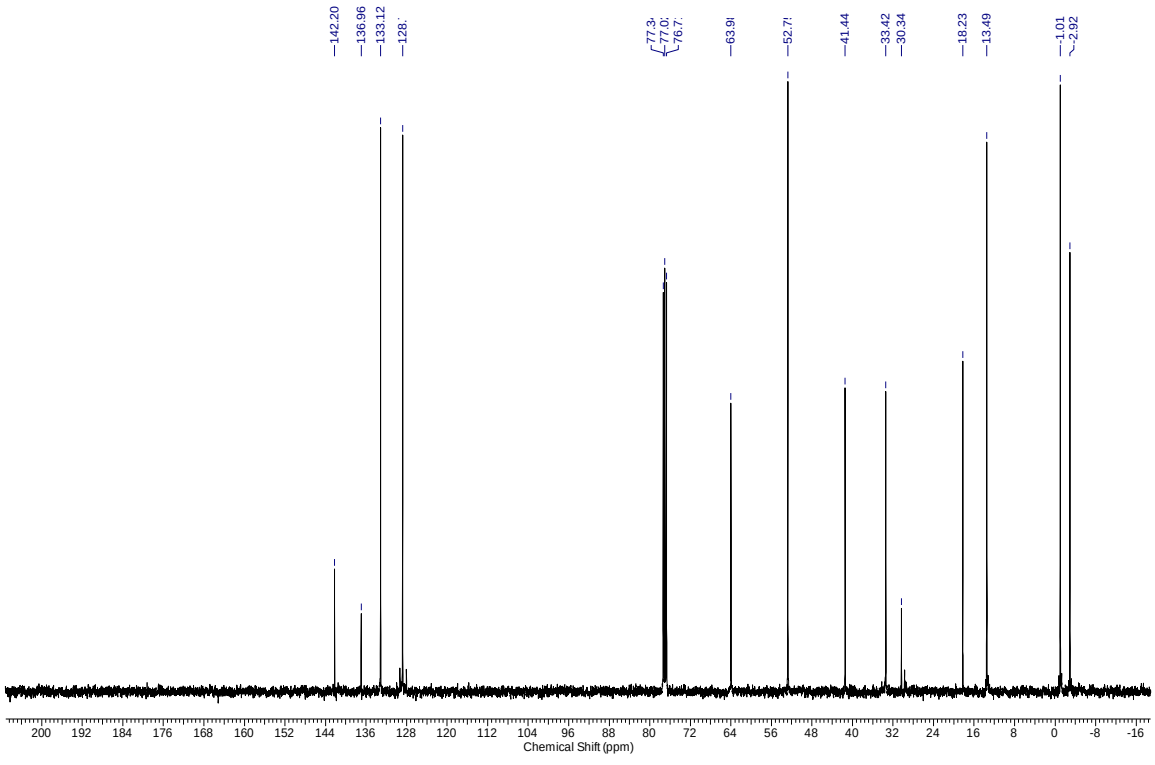
^{1}H NMR of **18** (400 MHz, $CDCl_3$)



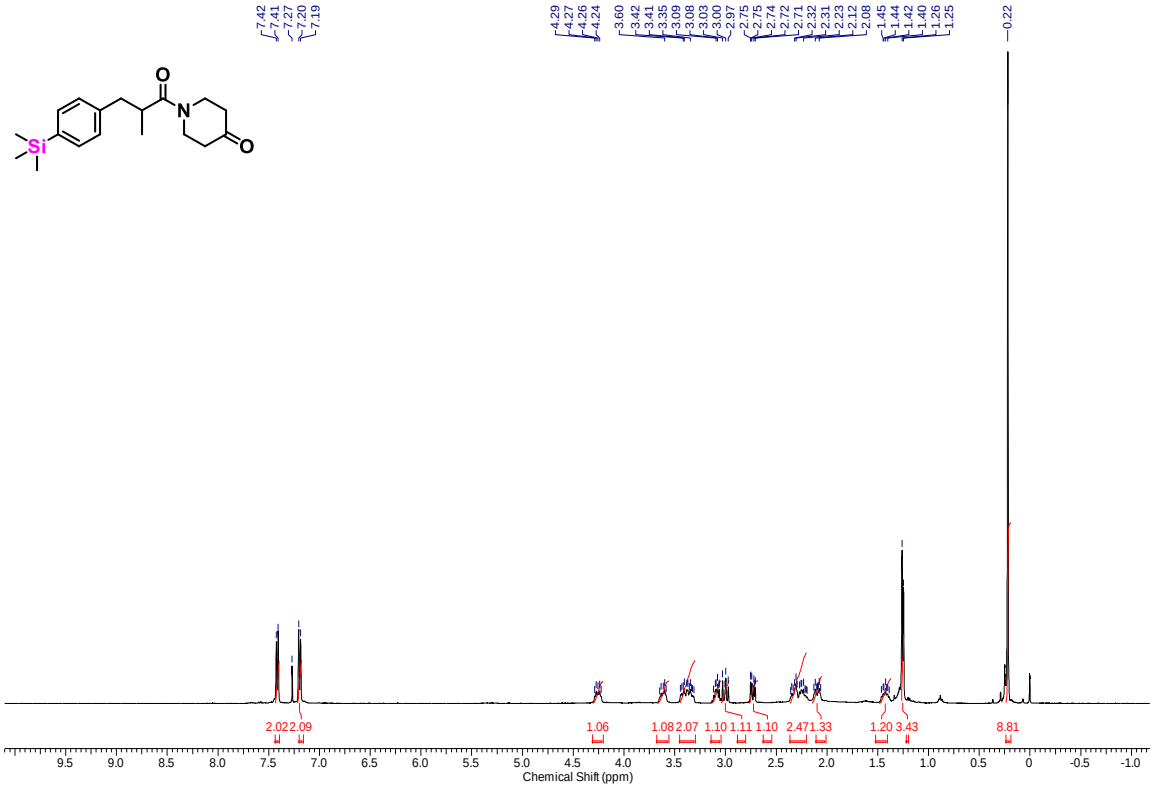
^{13}C NMR of **18** (100 MHz, $CDCl_3$)



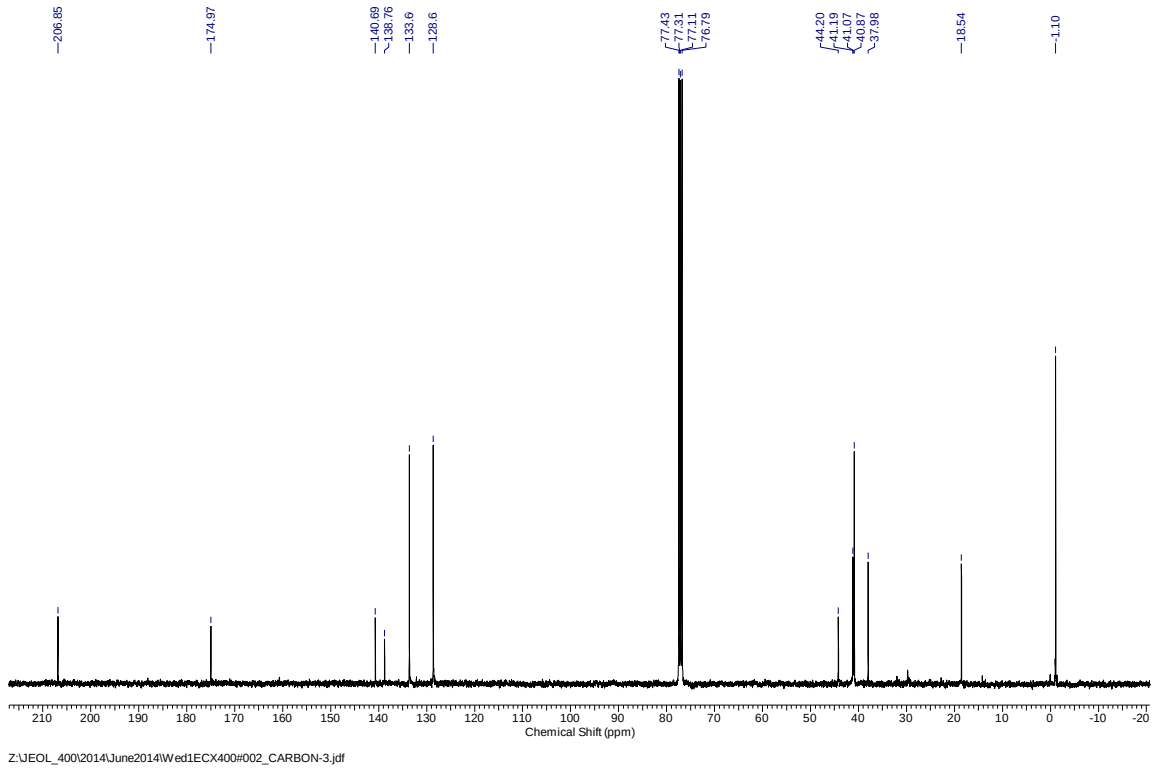
^{1}H NMR of **30** (400 MHz, $CDCl_3$)



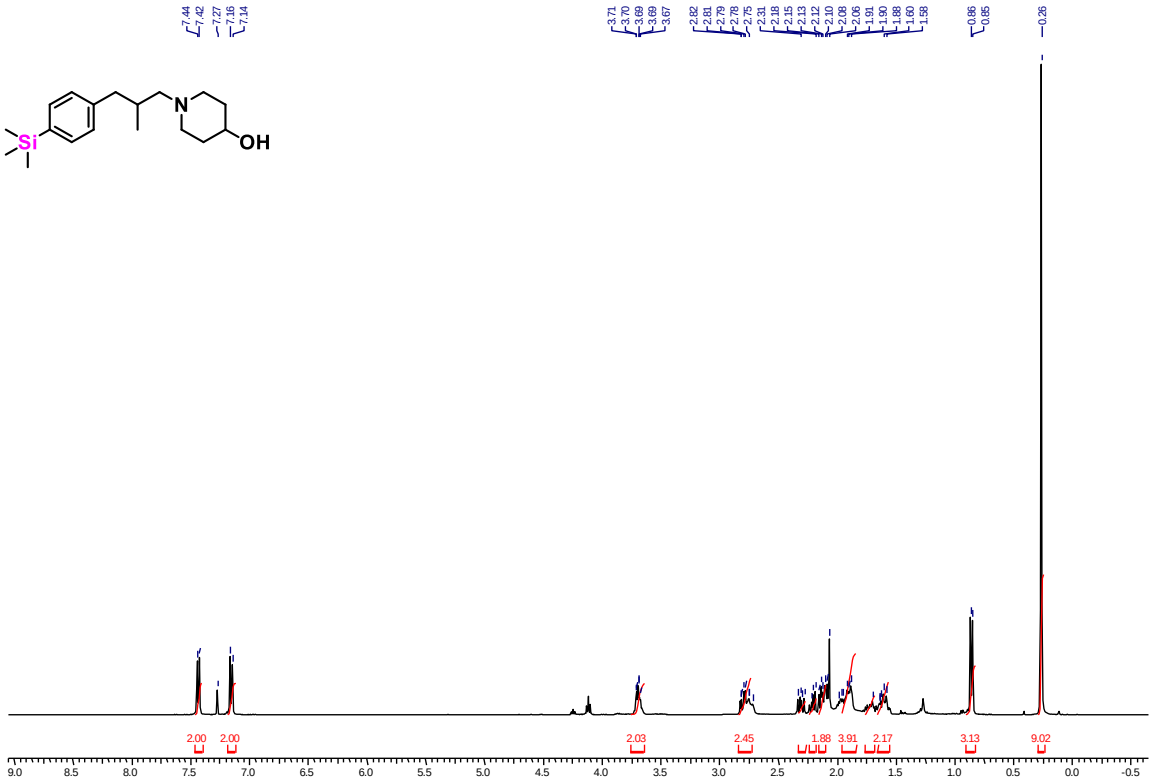
^{13}C NMR of **30** (100 MHz, $CDCl_3$)



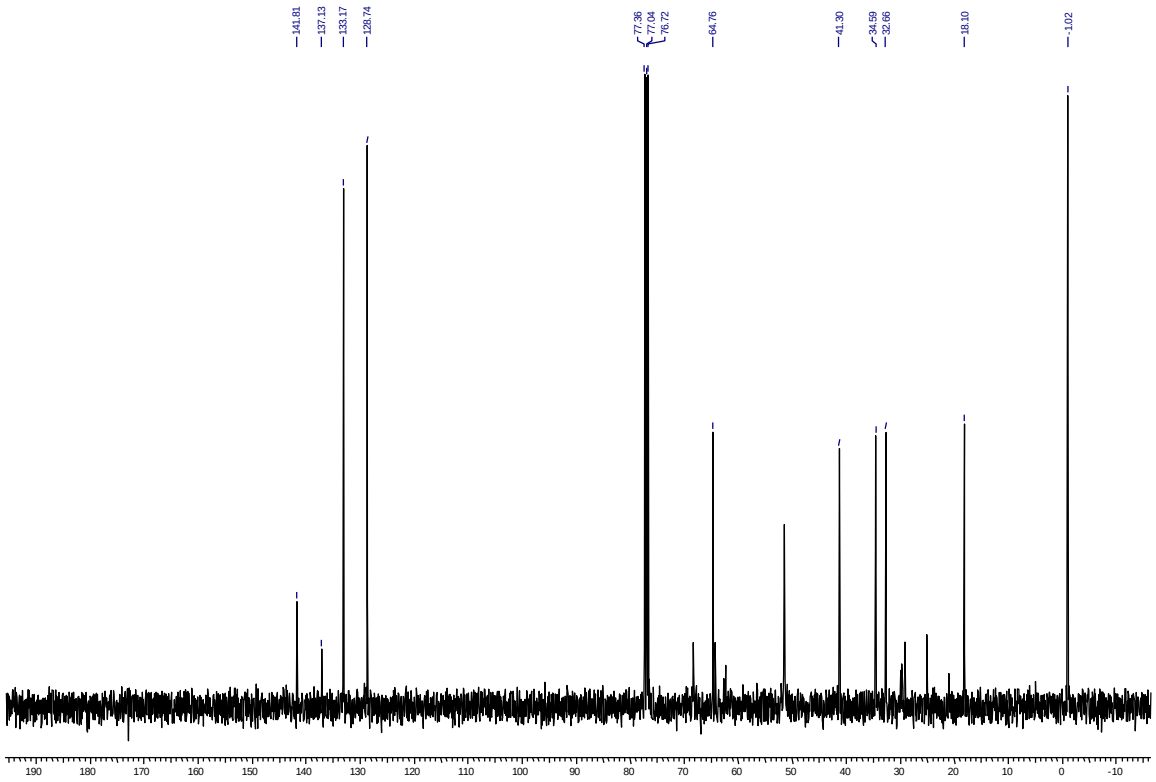
^{1}H NMR of **19** (400 MHz, $CDCl_3$)



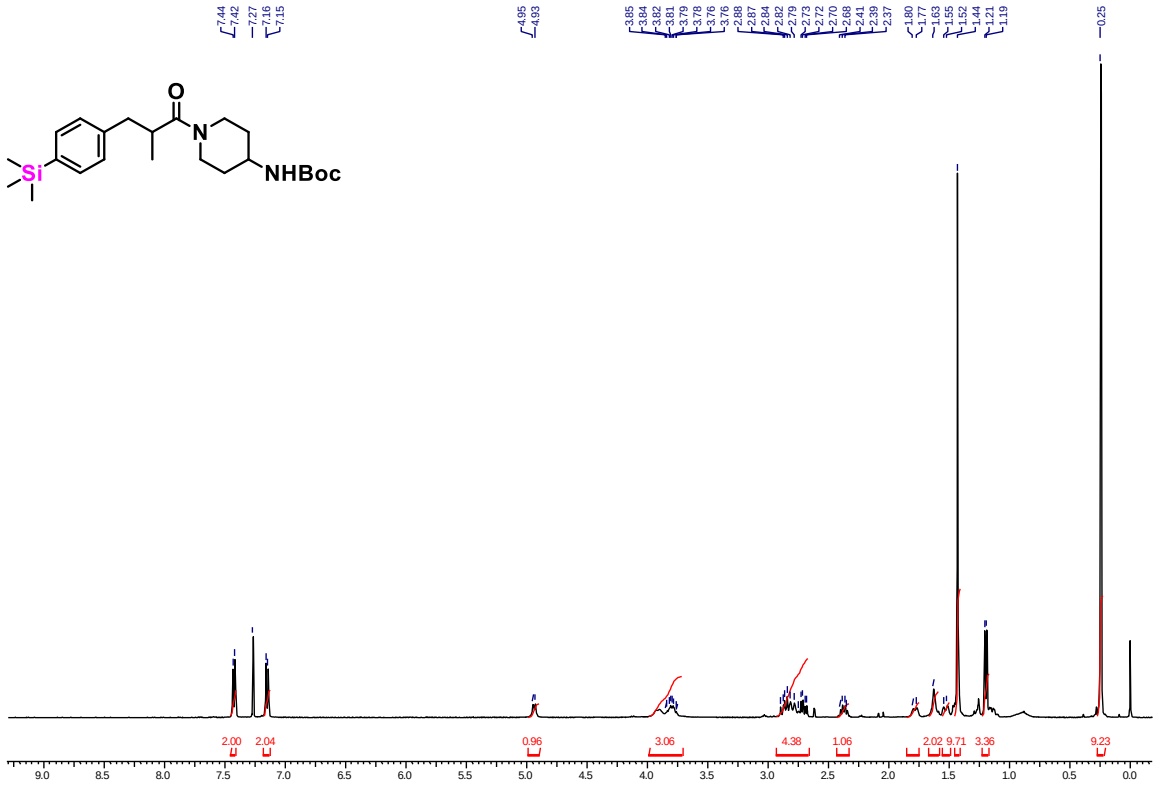
^{13}C NMR of **19** (100 MHz, $CDCl_3$)



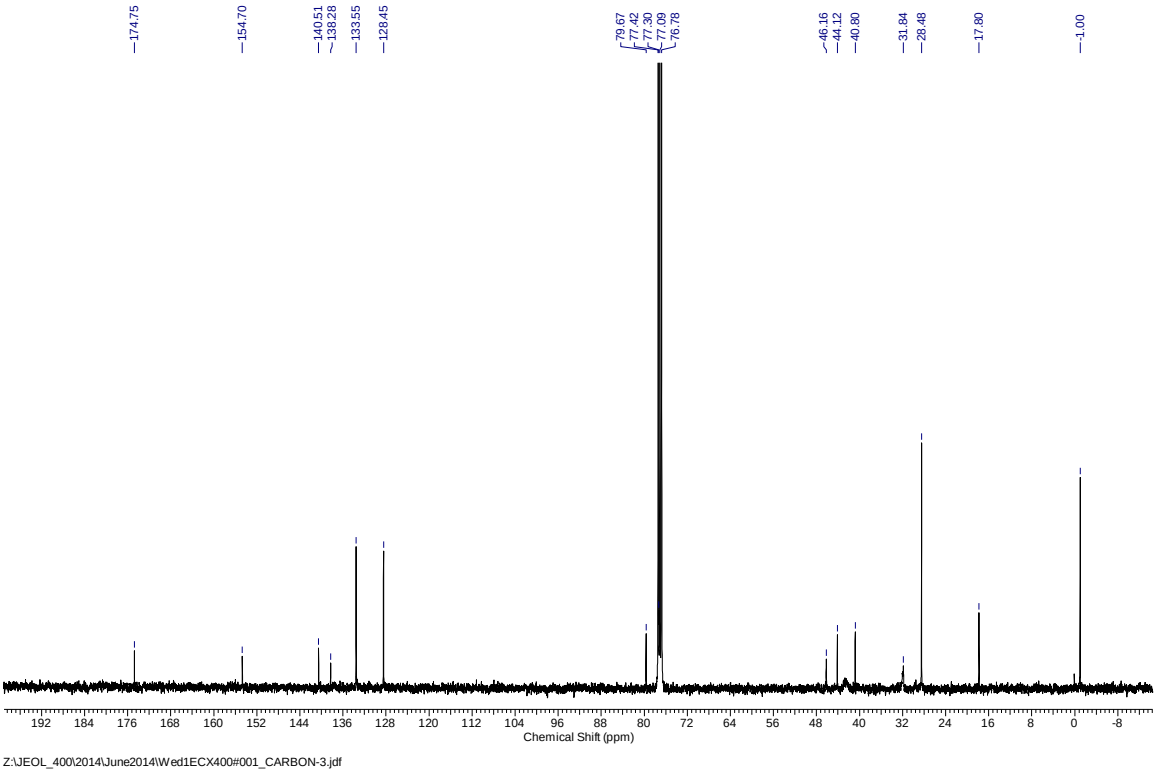
^{1}H NMR of **31** (400 MHz, $CDCl_3$)



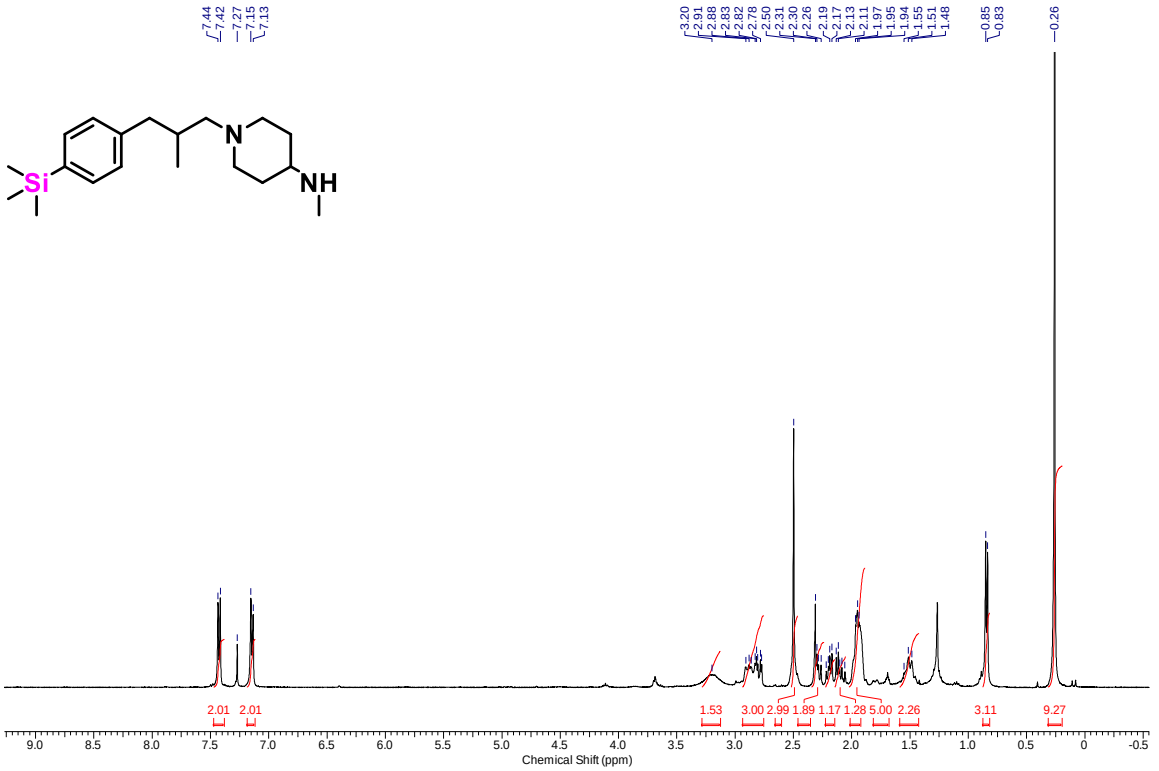
^{13}C NMR of **31** (100 MHz, $CDCl_3$)



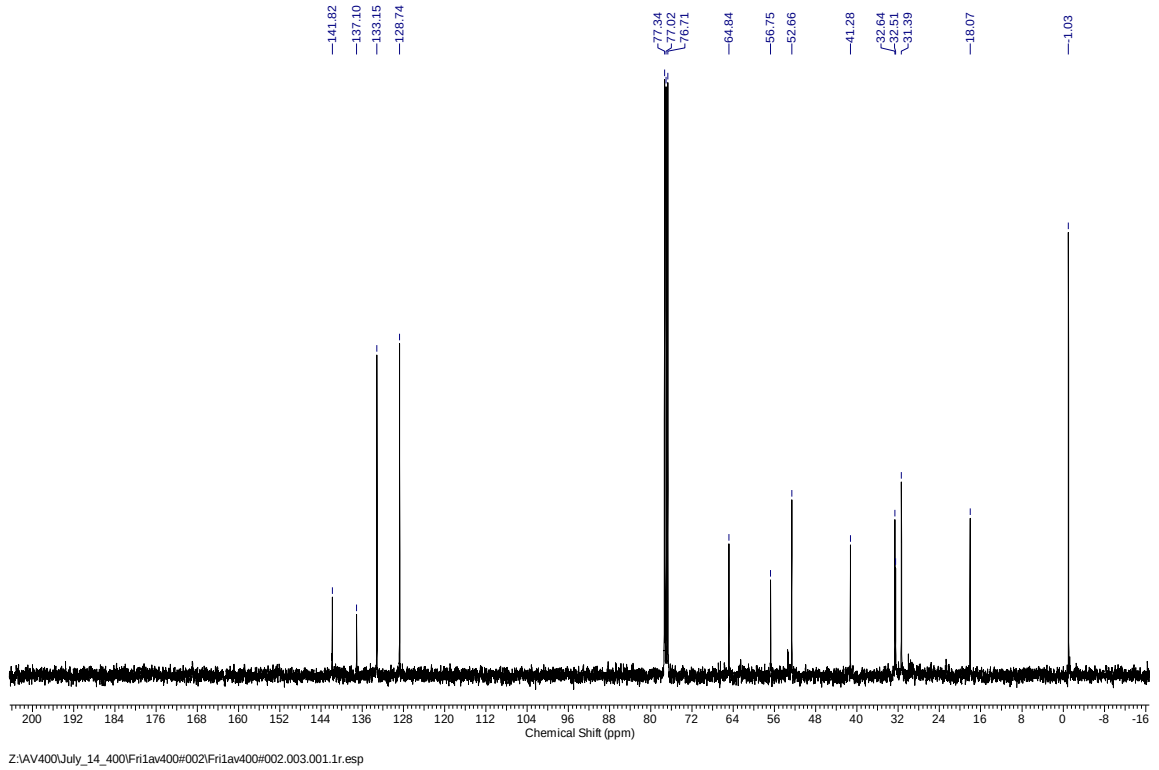
^{1}H NMR of **23** (400 MHz, $CDCl_3$)



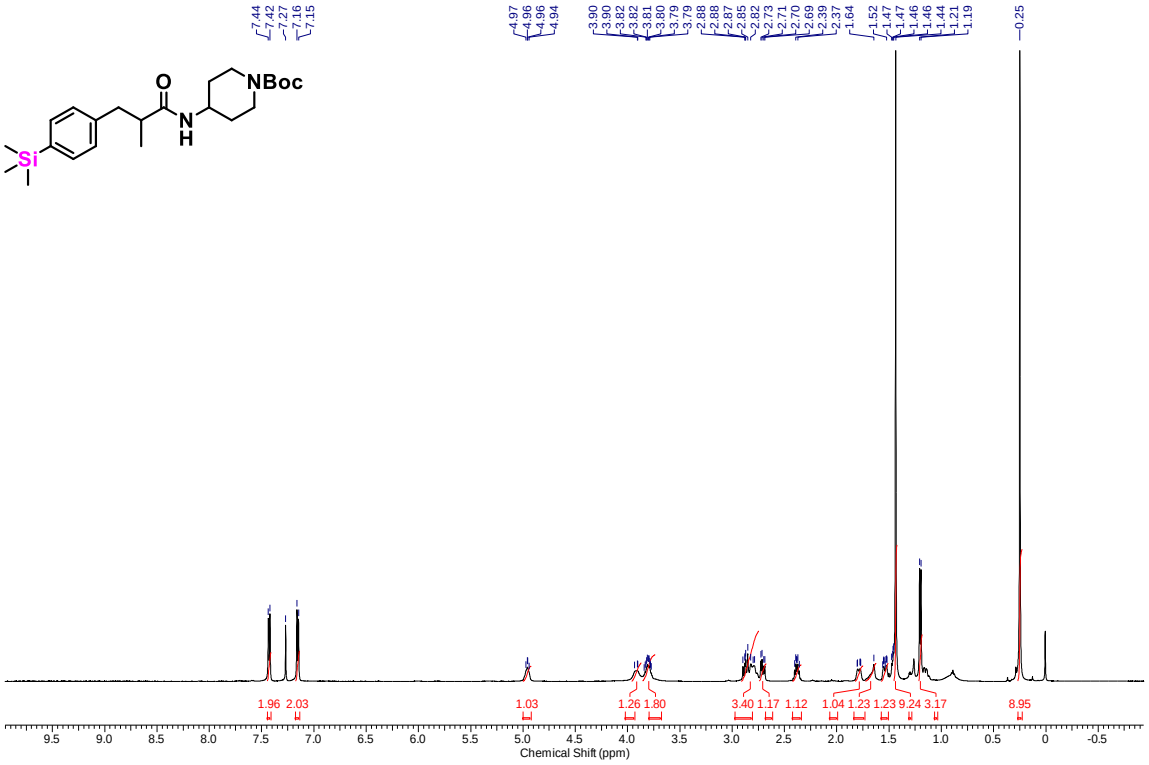
^{13}C NMR of **23** (100 MHz, $CDCl_3$)



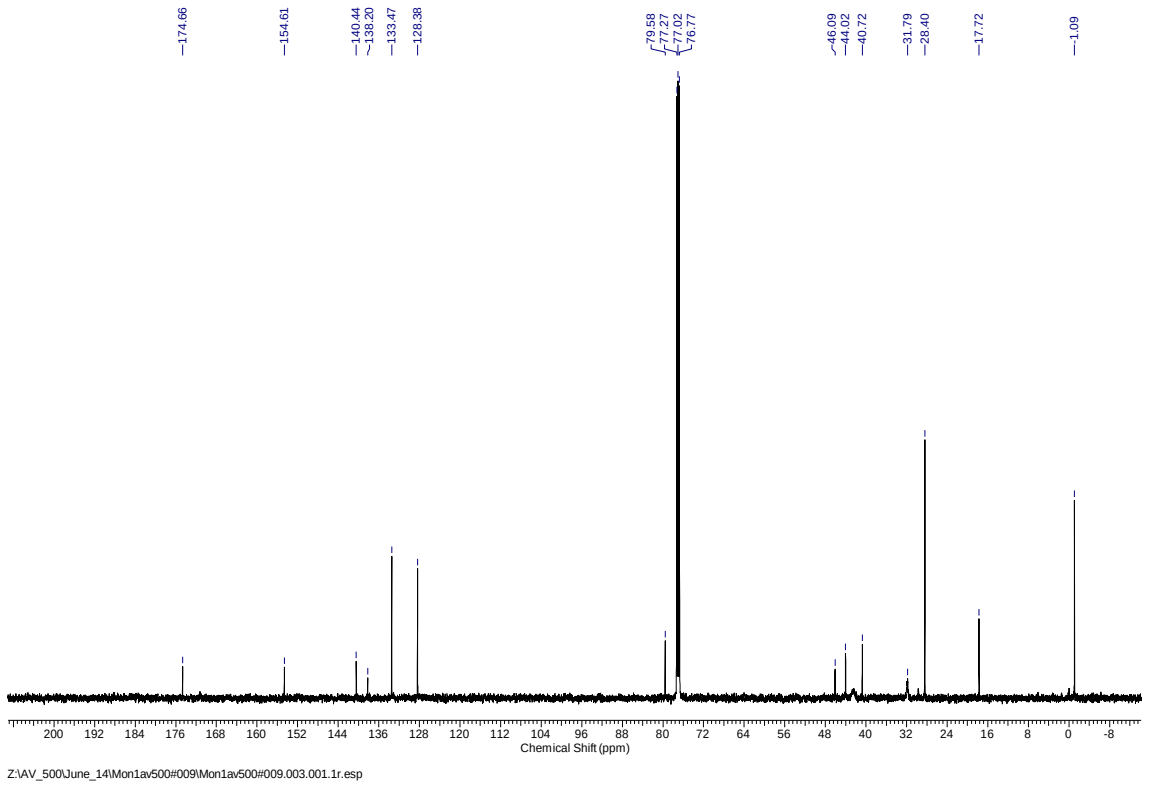
^{1}H NMR of **35** (400 MHz, $CDCl_3$)



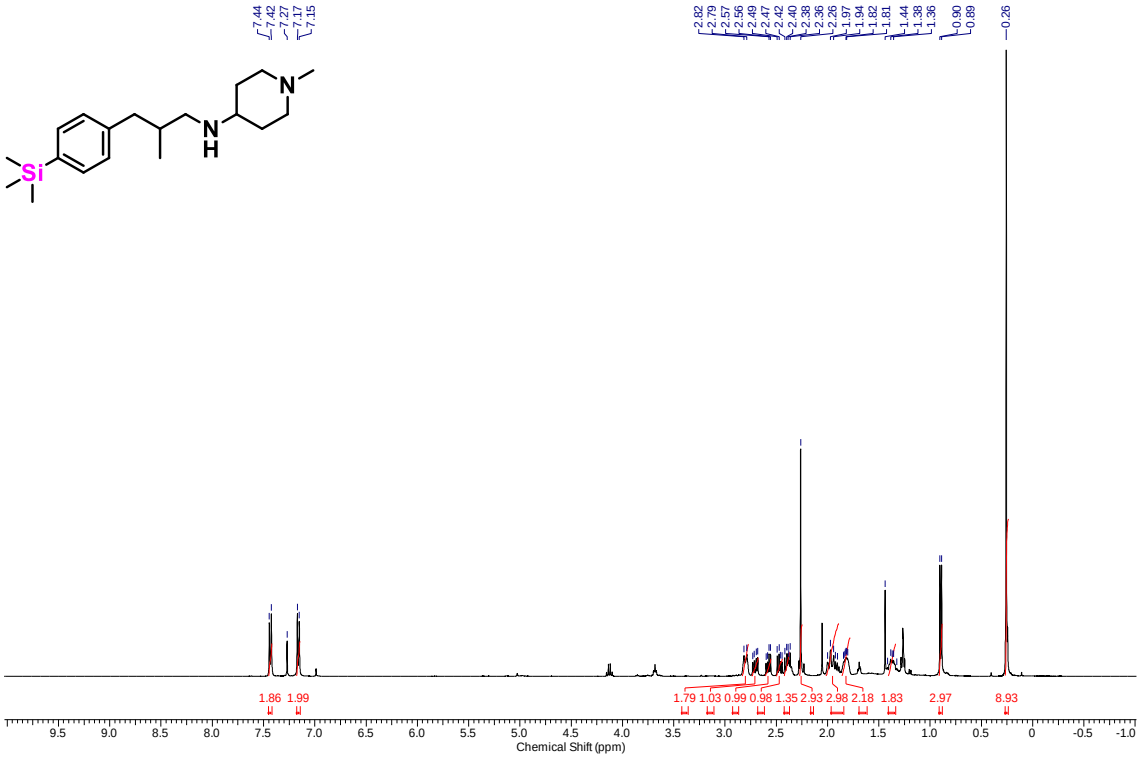
^{13}C NMR of **35** (100 MHz, $CDCl_3$)



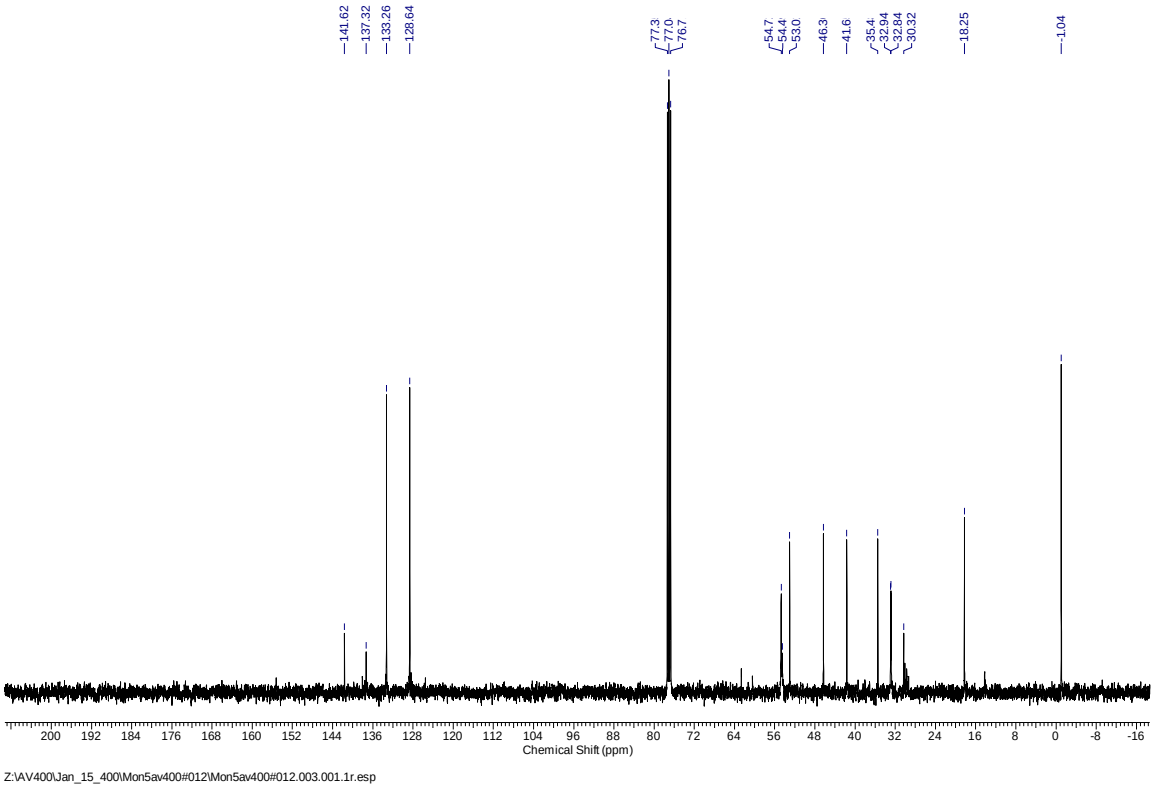
^{1}H NMR of **22** (500 MHz, $CDCl_3$)



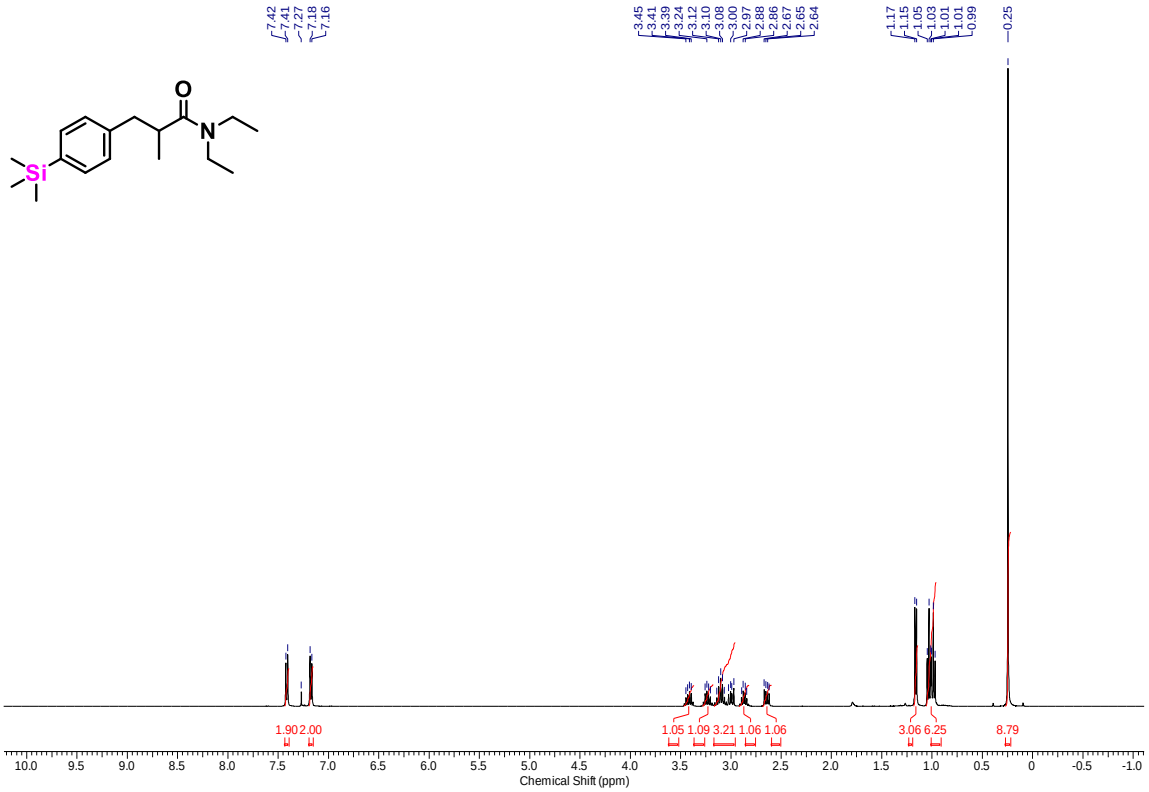
^{13}C NMR of **22** (125 MHz, $CDCl_3$)



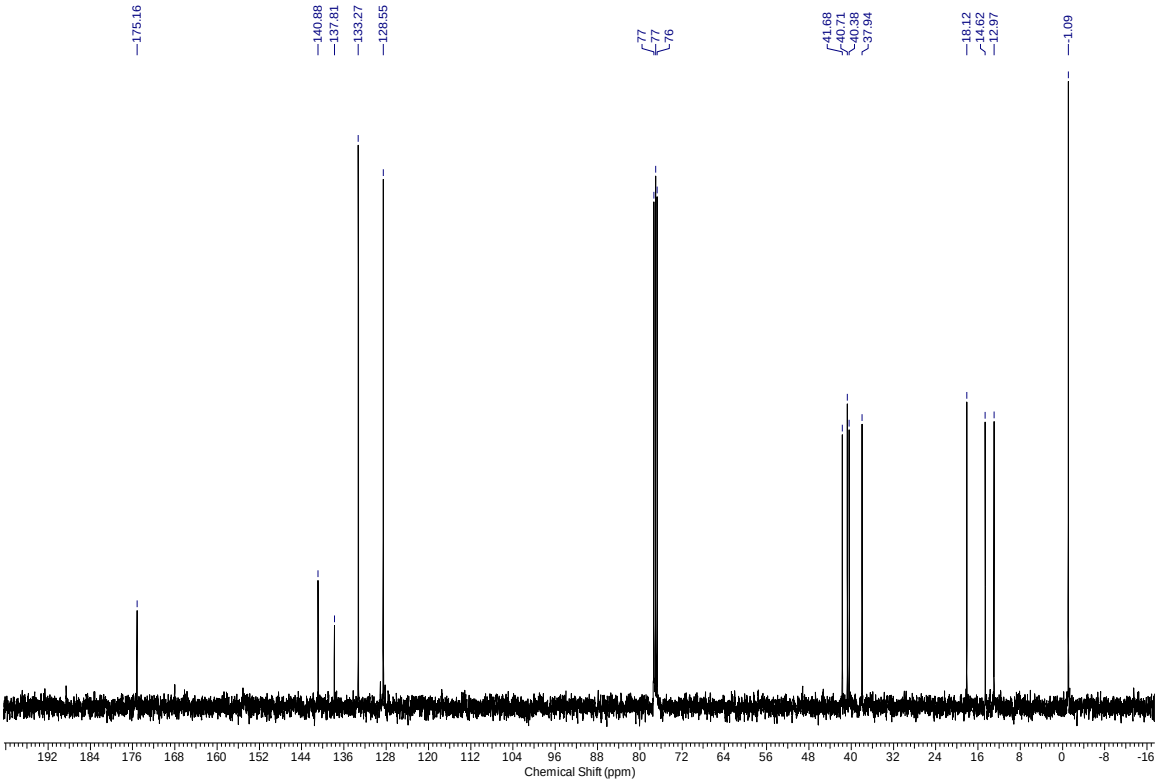
^{1}H NMR of **34** (400 MHz, $CDCl_3$)



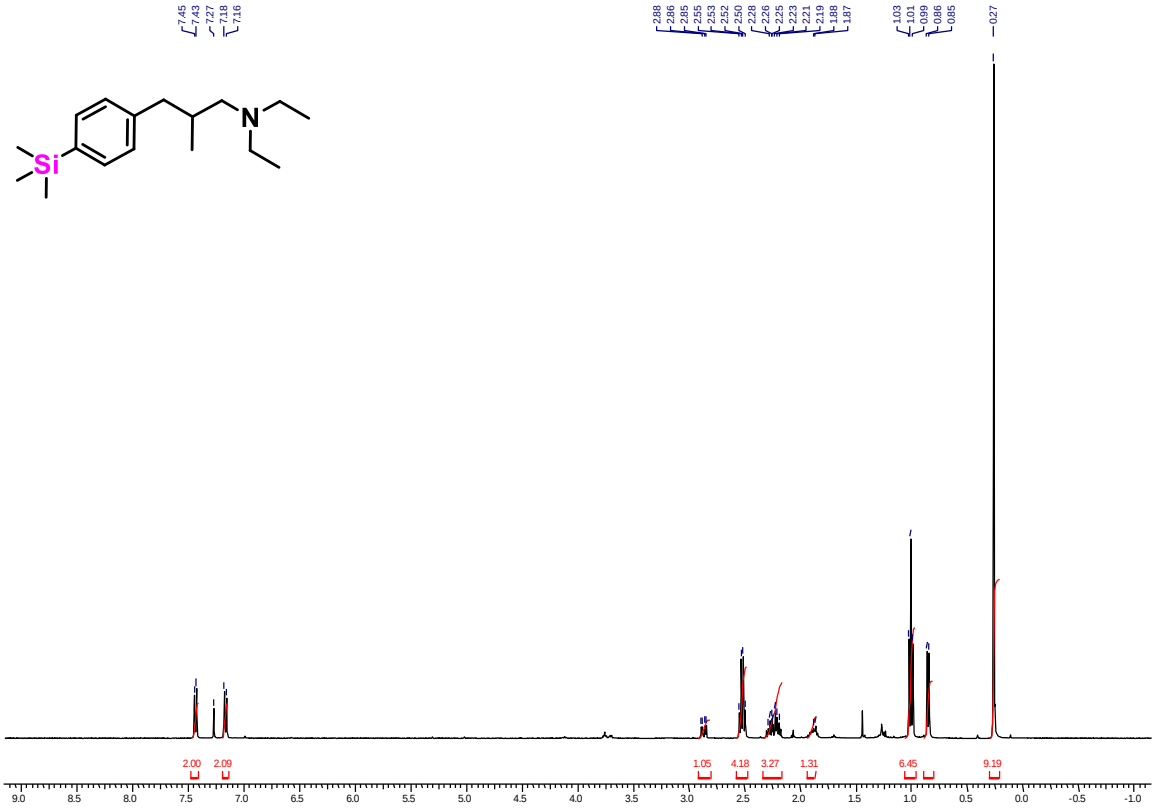
^{13}C NMR of **34** (100 MHz, $CDCl_3$)



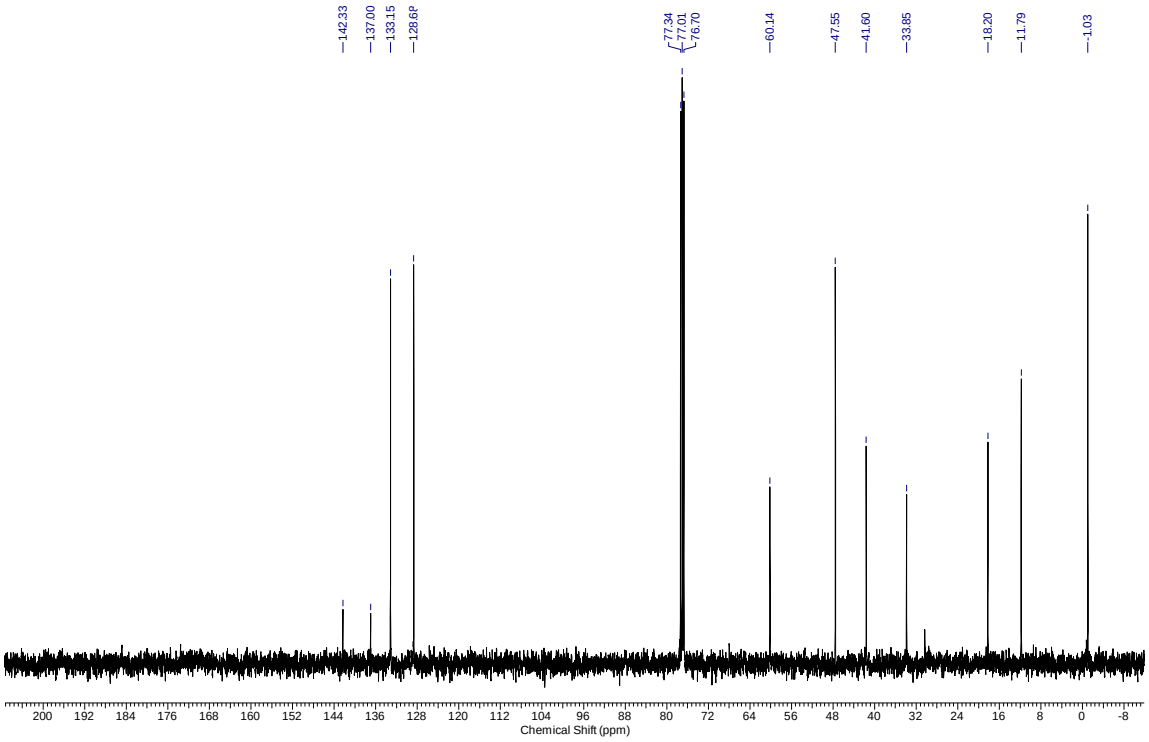
^{1}H NMR of **14** (400 MHz, $CDCl_3$)



^{13}C NMR of **14** (100 MHz, $CDCl_3$)



^{1}H NMR of **26** (400 MHz, $CDCl_3$)



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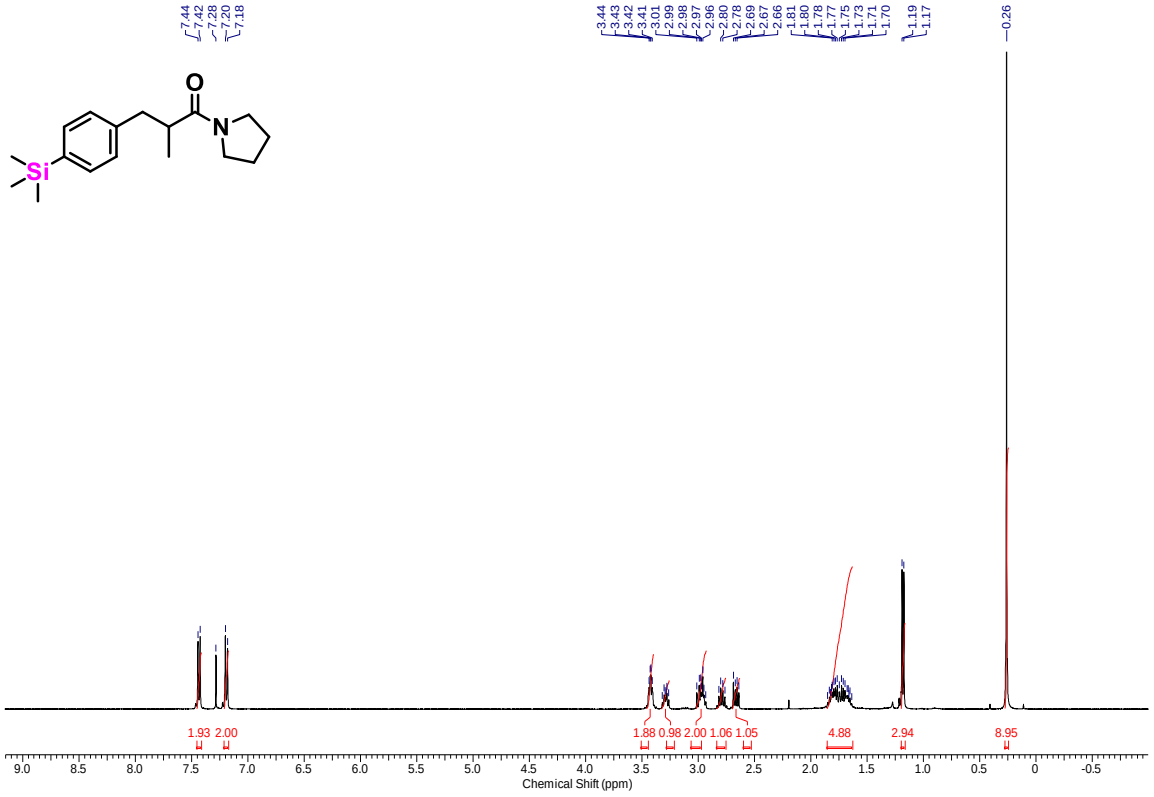
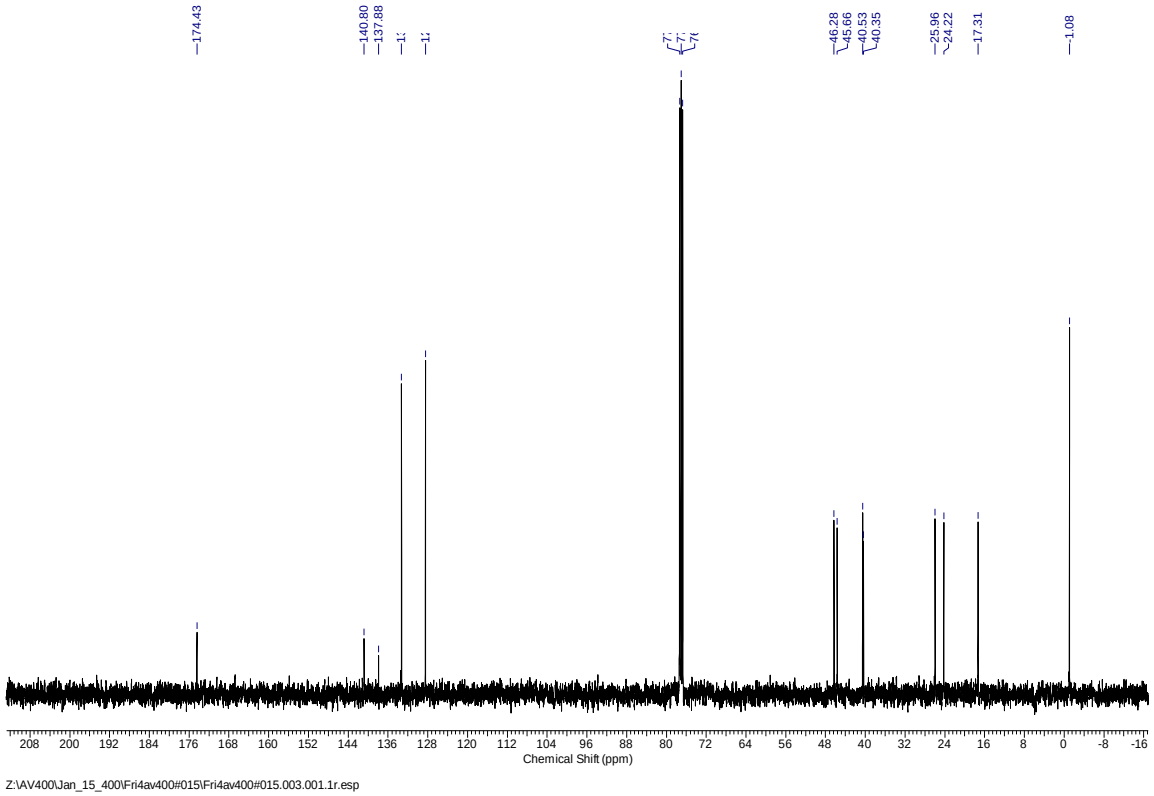
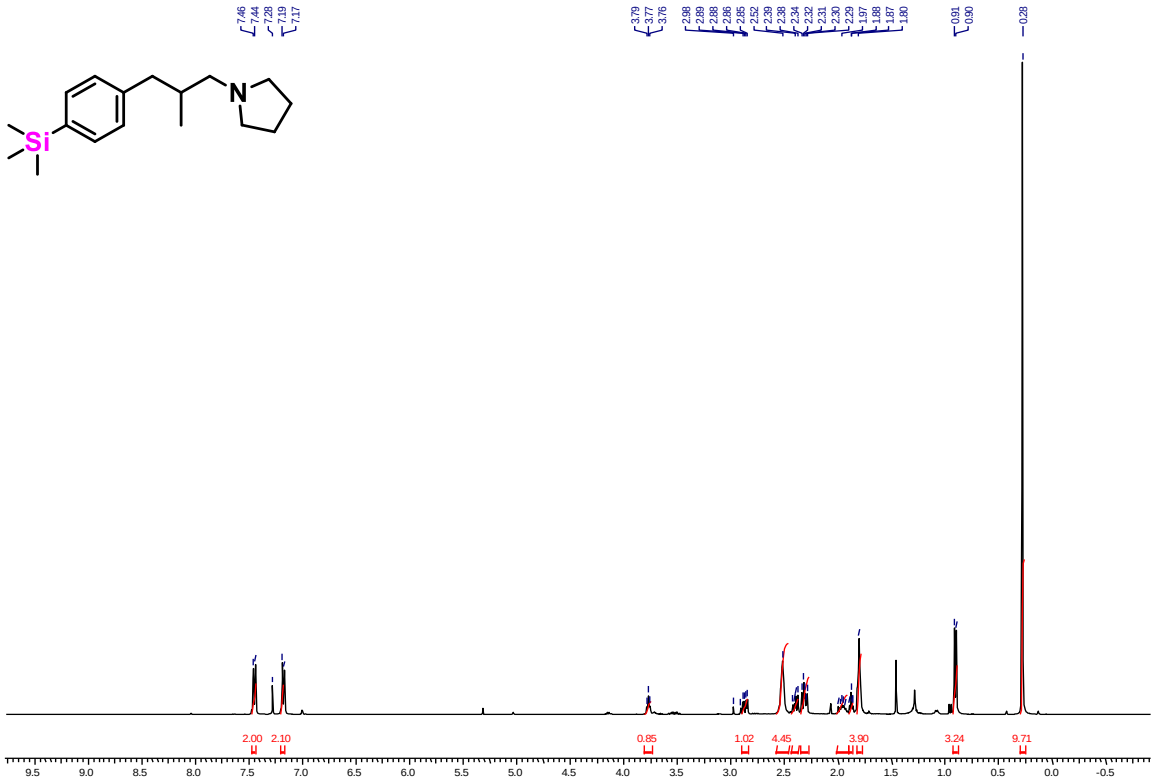
^{13}C NMR of **26** (100 MHz, $CDCl_3$)



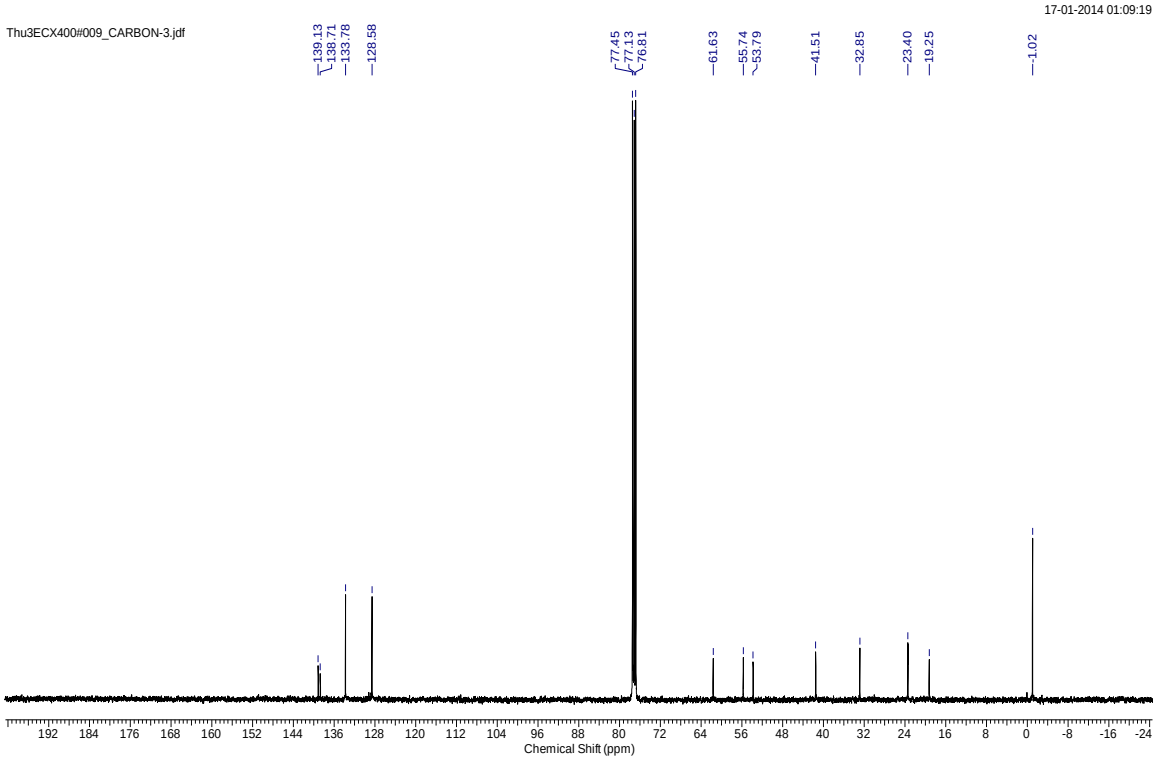
^{1}H NMR of **20** (400 MHz, $CDCl_3$)



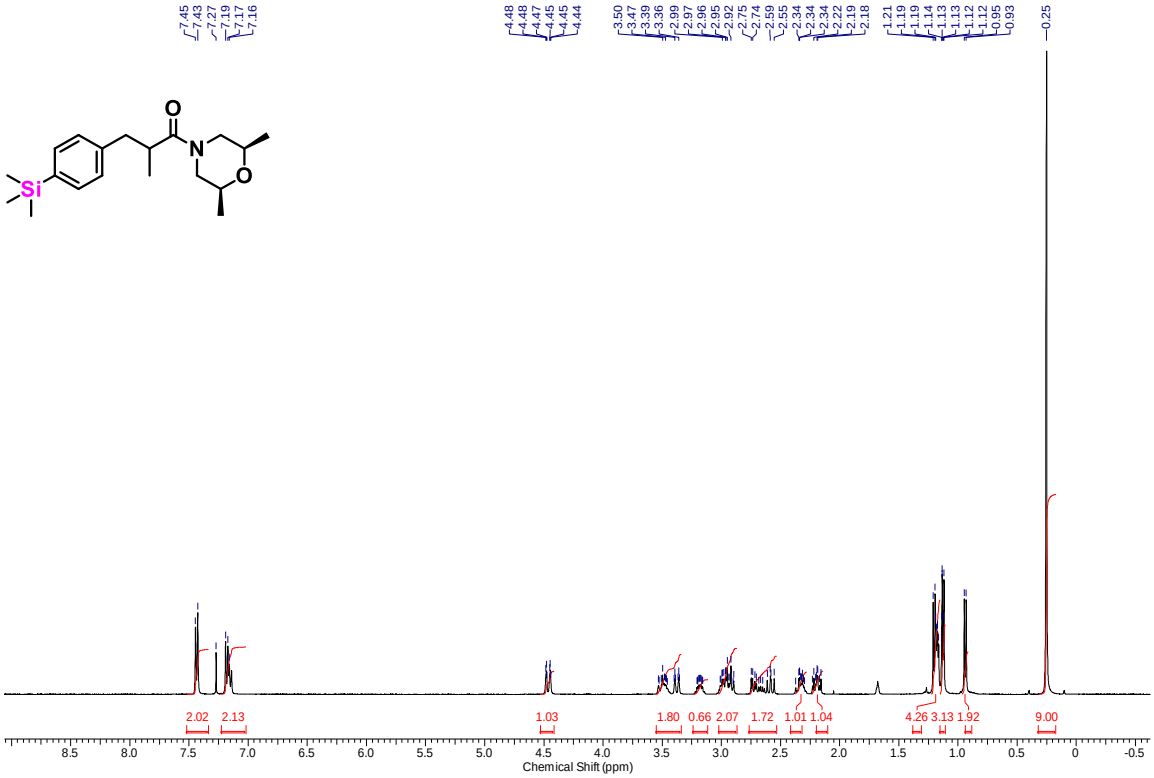
^{13}C NMR of **20** (100 MHz, $CDCl_3$)



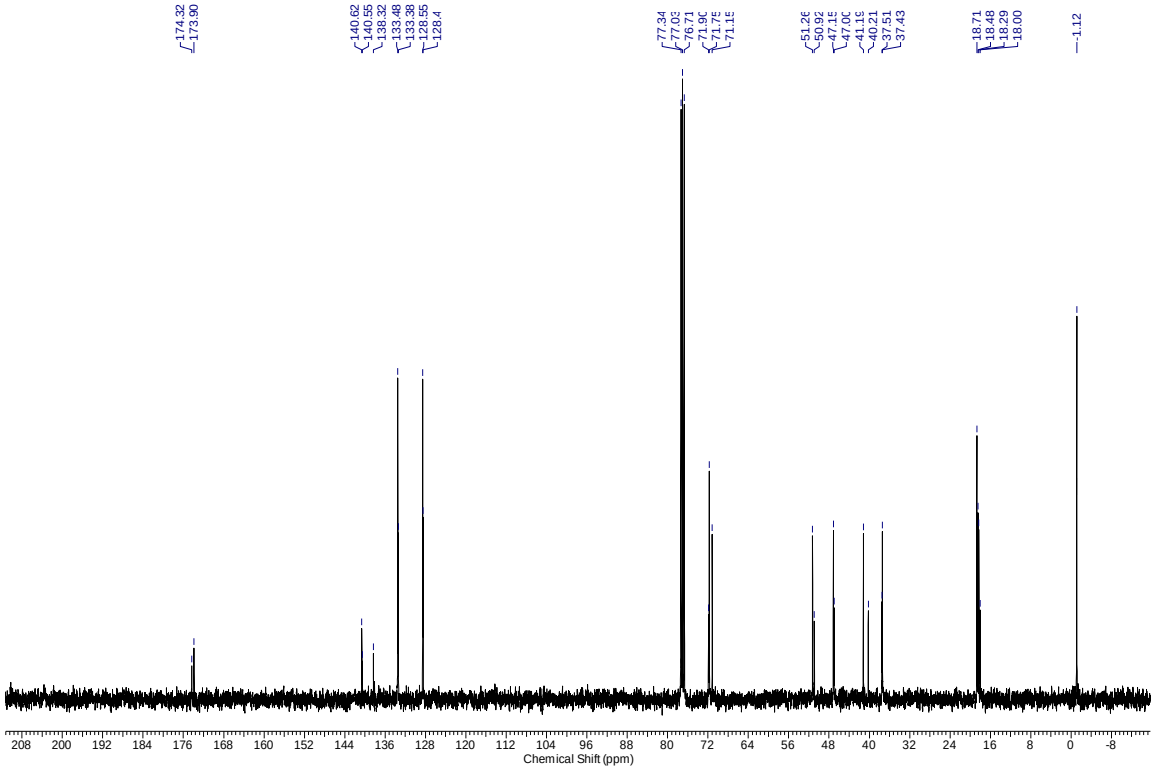
^{1}H NMR of **32** (400 MHz, $CDCl_3$)



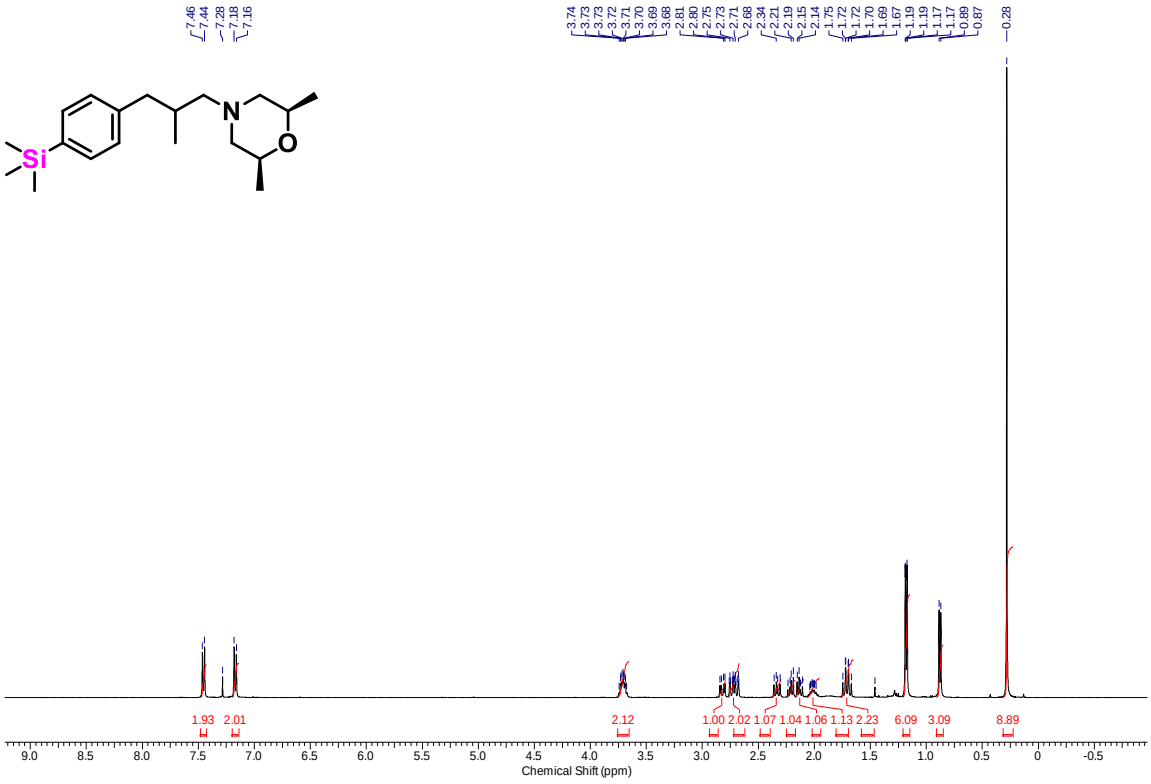
^{13}C NMR of **32** (100 MHz, $CDCl_3$)



^{1}H NMR of **24** (400 MHz, $CDCl_3$)



^{13}C NMR of **24** (100 MHz, $CDCl_3$)



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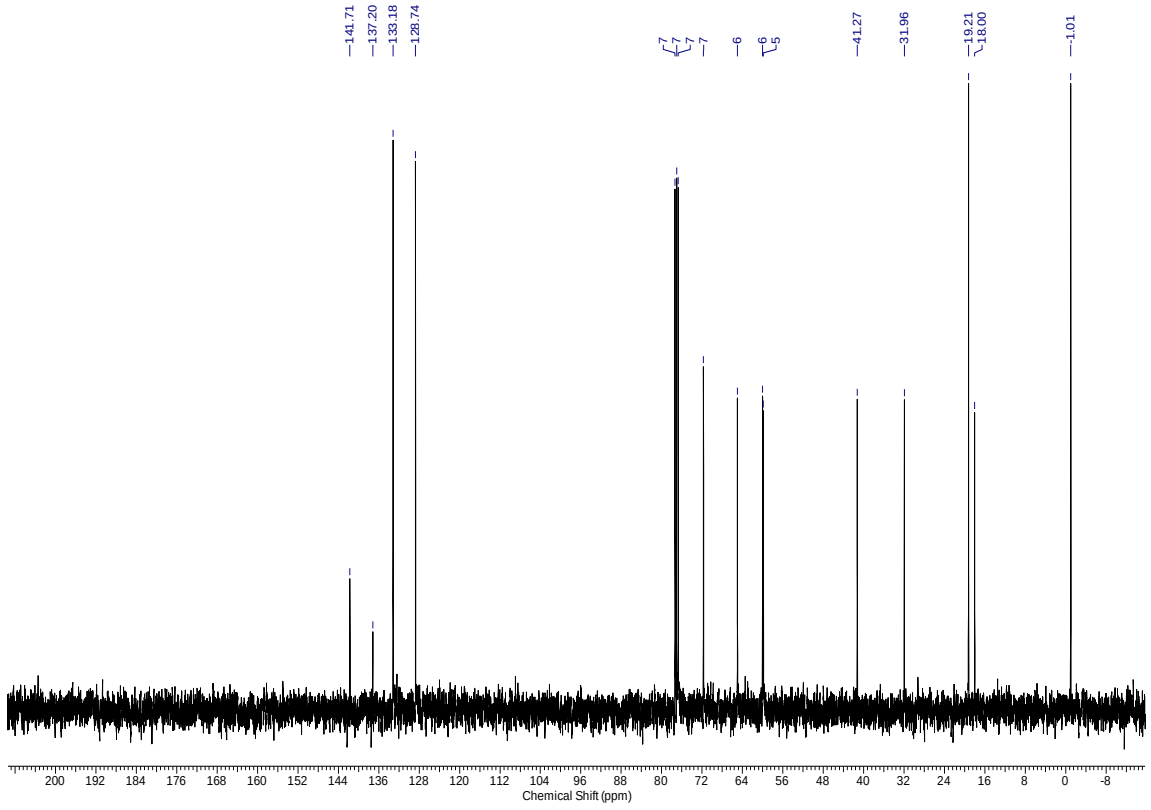
^{1}H NMR of **36** (400 MHz, $CDCl_3$)



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^{13}C NMR of **36** (100 MHz, $CDCl_3$)

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15. Vasudevan, N.; **Jachak, G. R**.; Reddy, D. S. WO2016181414A1 "Process for the synthesis of ivacaftor and related compounds"