Total Synthesis and Biological Evaluation of Marine Natural Products Palmyrolide A, Nitrosporeusines and their Analogues

Thesis Submitted to AcSIR

For the Award of the Degree of

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In

CHEMICAL SCIENCES

By

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Under the guidance of
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Every challenging work needs self efforts as well as guidance of elders especially those who are really close to our heart…

I dedicate all my efforts to my beloved uncle Dr. Venkat Palle and my loving parents

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Thesis Certificate

This is to certify that the work incorporated in this Ph.D. thesis entitled **"Total Synthesis and Biological Evaluation of Marine Natural Products Palmyrolide A, Nitrosporeusines and their Analogues"** submitted by **Mr. Satish Chandra Philkhana** 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 and Biological Evaluation of Marine Natural Products Palmyrolide A, Nitrosporeusines and their Analogues"** 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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I wish to thank the great scientific community whose achievements are constant source of inspiration for me.

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

Satish Chandra Philkhana

General remarks

All reagents, starting materials, and solvents were obtained from commercial suppliers and used as such without further purification. Solvents were dried using standard protocols or dried using Mbraun (MB SPS-800) instrument. Reactions were carried out in oven-dried glassware under a positive pressure of argon unless otherwise mentioned with magnetic stirring. Air sensitive reagents and solutions were transferred *via* syringe or cannula and were introduced to the apparatus *via* rubber septa. The progress of reactions was monitored by thin layer chromatography (TLC) with 0.25 mm pre-coated silica gel plates (60 F254). Visualization was accomplished with either UV light, Iodine adsorbed on silica gel or by immersion in ethanolic solution of phosphomolybdic acid (PMA), *p*-anisaldehyde or $KMnO_4$ followed by heating with a heat gun for ~15 sec. Column chromatography was performed on silica gel (100-200 or 230-400 mesh size). All the melting points are uncorrected and 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 and 2D NMR analysis were obtained using a 200 MHz, 400 MHz, 500 or 700 MHz spectrometer. Coupling constants were measured in Hertz. All chemical shifts are quoted in ppm, relative to TMS, using the residual solvent peak as a reference standard. The following abbreviations are used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. HRMS (ESI) were recorded on ORBITRAP mass analyser (Q Exactive). Infrared (IR) spectra were recorded on a FT-IR spectrometer as thin films in chloroform using NaCl plates. Optical rotations were recorded on a P-2000 polarimeter at 589 nm (sodium D-line). Chemical nomenclature (IUPAC) and structures were generated using Chem Bio Draw Ultra. All microwave reactions were carried out in anton-paar monowave 300 instrument.

Abbrevations

Ac	Acetyl
AcOH	Acetic acid
Ac_2O	Acetic anhydride
ACN	Acetonitrile
Bn	Benzyl
Boc	*tert*-Butoxy carbonyl
brsm	based on recovery of starting material
brs	broad singlet
BuLi	Butyl lithium
BAIB	Bis-acetoxy iodobenzene
^{t}BuOH	*tert*-butanol
Cat.	Catalytic
$CDCl_3$	Deuterated chloroform
CBA	Cytokine Bead Assay
CNS	Central nervous system
CBS	Corey-Bakshi-Shibata
cm^{-1}	1/centimeter
CuI	Copper Iodide
CI	Confidence interval
Cs_2CO_3	Caesium carbonate
$CuSO_4$	Copper Sulfate
°C	degree celcius
DABCO	1,4-Diazabicyclo[2.2.2]octane
DBU	1,8-Diazabicyclo[5.4.0]undecene-7
DCM	Dichloromethane
DCC	N,N'-Dicyclohexylcarbodiimide
DCE	1,2-Dichloroethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIAD	Diisopropyl azodicarboxylate
DIBAL-H	Diisobutylaluminiumhydride
DIPEA	N,N-Diisopropylethylamine

Abbrevations

DMF	*N*, *N'*-Dimethylformamide
DMEDA	*N*, *N'*-Dimethylethylenediamine
DMAP	*N*,*N*-Dimethylaminopyridine
DMSO-*d6*	deuterated Dimethyl sulfoxide
DRG	Dorsal root ganglion
DFT	Density Functional Theory
ee	Enantiomeric excess
equiv.	Equivalents
EtOH	Ethanol
Et	Ethyl
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
Et_3N	Triethylamine
$FeSO_4$	Ferrous sulfate
G-II	Grubbs second generation catalyst
g	gram
Hz	Hertz
HRMS	High resolution mass spectroscopy
HMPA	Hexamethylphosphoramide
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High pressure liquid chromatography
HCl	Hydrchloric acid
H_2	Hydrogen gas
Hsp90	Heat shock protein-90
HWE	Horner-Wadsworth-Emmons
Im	Imidazole
IR	Infrared
i.e.	that is
IC_{50}	Half maximal inhibitory concentration
IL	Interleukin
I_2	Iodine

Abbrevations

in vitro	outside a living organism
in vivo	inside a living organism
J	coupling constant
KHMDS	Potassium bis(trimethylsilyl)amide
K_2CO_3	Potassium carbonate
LiOH	Lithium hydroxide
Me	Methyl
MeOH	Methanol
mg	Milligram
min	Minutes
mL	Millilitre
µmol	Micromolar
mmol	Millimole
m/z	mass to charge ratio
m-CPBA	*meta*-Chloroperoxybenzoic acid
mp	Melting point
MS	Molecular seives
MsCl	Methanesulfonyl chloride
Me	Methyl
MeI	Methyl iodide
MPLC	Medium Pressure Liquid Chromatograohy
$NaBH_4$	Sodiumborohydride
NaH	Sodium hydride
NaHMDS	Sodium bis(trimethylsilyl)amide
$NaIO_4$	Sodium meta-periodate
NaOH	Sodium hydroxide
NA	no activity
NBS	*N*-Bromosuccinimide
NO	nitric oxide or nitrogen oxide
NF-κB	Nuclear factor-kappa B
NMR	Nuclear Magnetic Resonance

Abbrevations

ORTEP	Oak Ridge Thermal-Ellipsoid Plot
OsO_4	Osmium tetraoxide
PivCl	Pivaloyl chloride
Ph	Phenyl
Py	Pyridine
PCC	Pyridinium Chlorochromate
PEG	Polyethylene glycol
Pd	Palladium
PBE	Perdew–Burke–Ernzerhof
ROS	Reactive oxygen species
RCM	Ring closing metathesis
SeO_2	Selenium Dioxide
SAR	Structure Activity Relationship
SBFI	Sodium benzofuran isophthalate
TBAI	Tetra-*n*-butylammonium iodide
TBAF	Tetra-*n*-butylammonium fluoride
TBSCl	*tert*-Butyldimethyl silyl chloride
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TEA	Triethyl amine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TPP	Triphenylphosphine
TsCl	*p*-Toluenesulfonyl chloride
TLC	Thin Layer Chromatography
TS	transition state
TNF	Tumor necrosis factor
US-FDA	United States- Food and Drug Administration
VGSC	Voltage gated sodium channels
VRT	Veratridine
vs	versus

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	**Satish Chandra Philkhana**
Degree Enrolment No. & Date	**Ph. D in Chemical Sciences (10CC11A26032); August 2011**
Title of the Thesis	**Total Synthesis and Biological Evaluation of Marine Natural Products Palmyrolide A, Nitrosporeusines and their Analogues**
Research Supervisor	**Dr. D. Srinivasa Reddy**

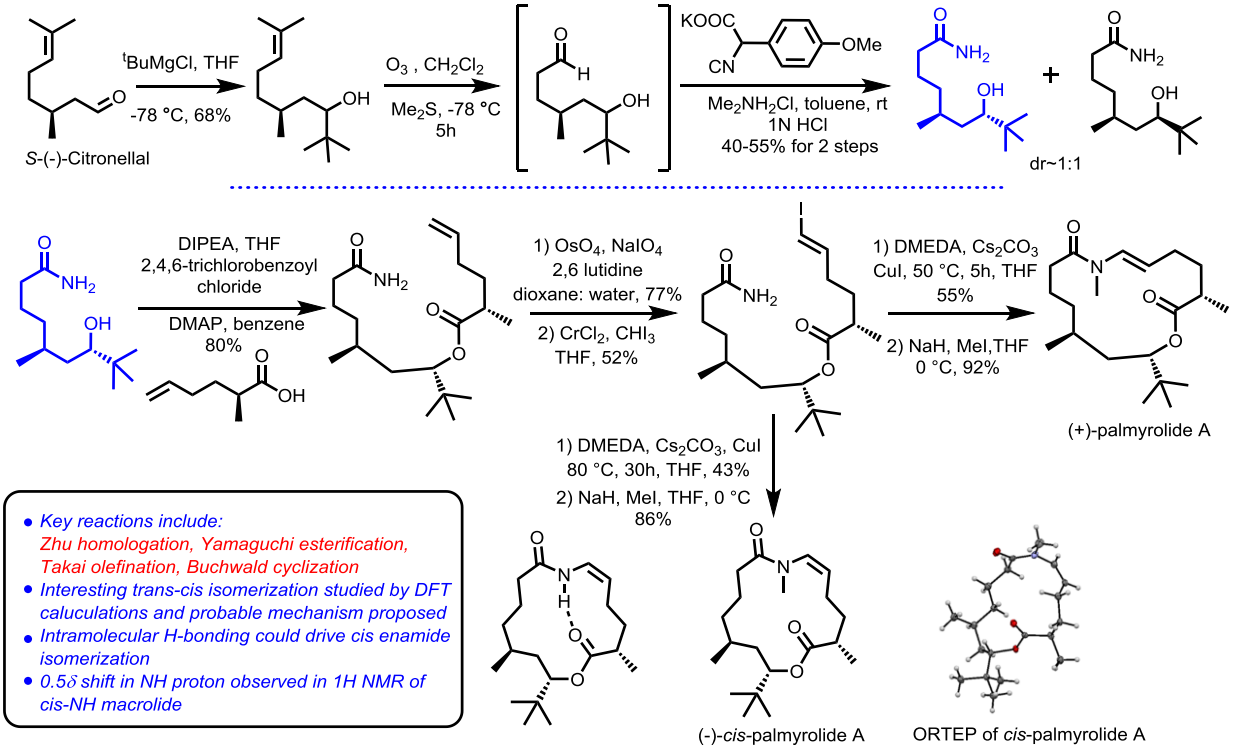
The thesis is divided into two chapters. **Chapter 1** introduces to marine and enamide containing natural products, then describes the total synthesis of palmyrolide A and *cis*-palmyrolide A. The next part describes the design and synthesis of various analogues of palmyrolide A, their biological evaluation towards voltage gated sodium channel (VGSC) inhibition and structure activity studies. **Chapter 2** includes the total synthesis of a new class of marine natural compounds nitrosporeusines, followed by analogue synthesis and their biological evaluation using anti-inflammatory assays.

Chapter 1: Synthesis and biological evaluation of Palmyrolide A and its analogues

Marine environment is a rich source of biologically active compounds with vast structural diversity and medicinal properties. Over past decades, many enamide-containing macrolides such as laingolide, laingolide A, laingolide B and madangolide having unique structural core, have been isolated from marine sources. Belonging to this class and most interesting one is palmyrolide A, isolated by Gerwick and coworkers in 2010 from marine cyanobacterial assemblage in Northern Pacific Ocean. It posess a *trans*-N-methyl enamide moiety and a *tert*-butyl adjacent to lactone moiety encumbered in a fifteen membered ring, which is rarely found in natural product literature. Initial screening of palmyrolide A revealed that it has potent sodium and calcium channel blocking abilities with IC_{50} of 5.2 μM and IC_{50} of 3.7μM respectively. It is also found to have less toxicity and has potential in treating various neurological disorders by modulating the voltage gated channels. It's exciting biological and chemical profile attracted many research groups around the world and to date five total synthesis are reported including one from us.

At start of the project we envisioned total synthesis of palmyrolide A, synthesis of close analogues and their biological evaluation towards VGSC inhibition. Towards this, a chiral pool strategy was employed involving an interesting homologation reaction to achieve key intermediate in shortest possible way. The synthesis of (+)-palmyrolide A started with *tert*-butyl Grignard addition on (*S*)-citronellal to yield alkenol which was further subjected to ozonolysis to get desired aldehyde. The aldehyde was then homologated to hydroxyl amide using a protocol developed by Zhu and coworkers in which, the use of isocyanate based reagent homologates aldehyde to its corresponding amide. Accordingly, this Zhu's reaction gave hydroxyl amides in 1:1 diastereomeric ratio with overall 40-55% yield. The required hydroxyl amide on being subjected to Yamaguchi esterification conditions followed by oxidative cleavage and Takai olefination gave desired vinyl iodide precursor for macrocyclization.

After a few attempts, modified Buchwald conditions were applied to achieve final macrocyclization. During these attempts under forced conditions, the intramolecular Buchwald reaction resulted in the formation of unexpected *cis*-enamide. Detailed analysis using DFT studies revealed that hydrogen bonding is key for stabilization of *cis*-enamide over its *trans* congener. Later macrocyclization was carried out under controlled Buchwald conditions to have palmyrolide A natural product, which was in complete agreement with the literature data. After successful total synthesis, the next target was to synthesize various analogues around this palmyrolide A scaffold. Accordingly, a modified approach was



Scheme 1: Synthesis of (+)-palmyrolide A and *cis*-palmyrolide A

designed involving Yamaguchi coupling and ring closing metathesis as key steps and 18 new compounds were synthesized. In collaboration with Prof. Murray (Creighton University, USA), all the compounds were screened for their ability to antagonize voltage gated sodium channels and four analogues were found to be comparable in activity to palmyrolide A. Some valuable conclusions were drawn from structure activity relation (SAR) studies as compiled in figure 1.

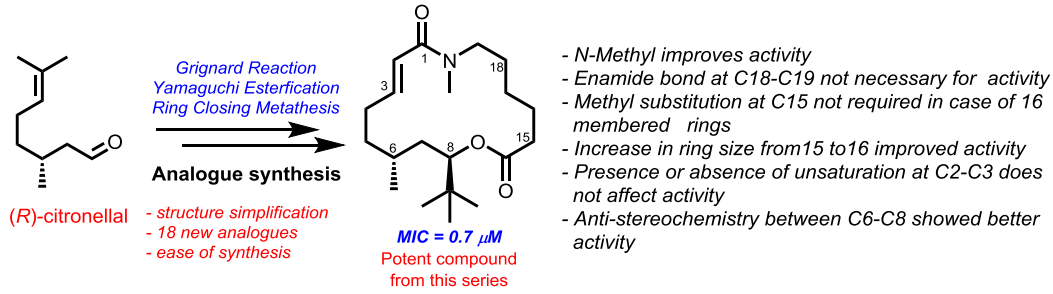
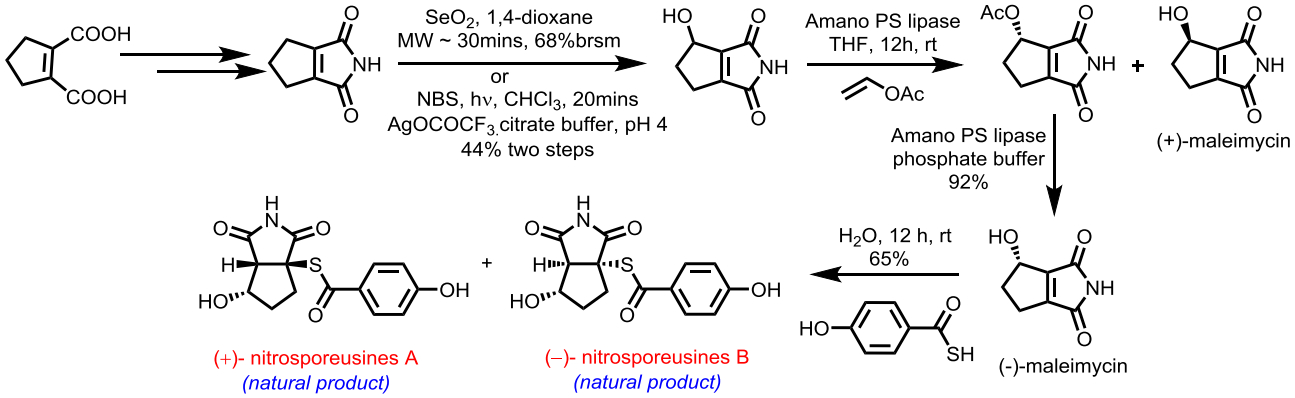


Figure 1: Synthesis of palmyrolide A analogues with key reactions involved and SAR conclusions

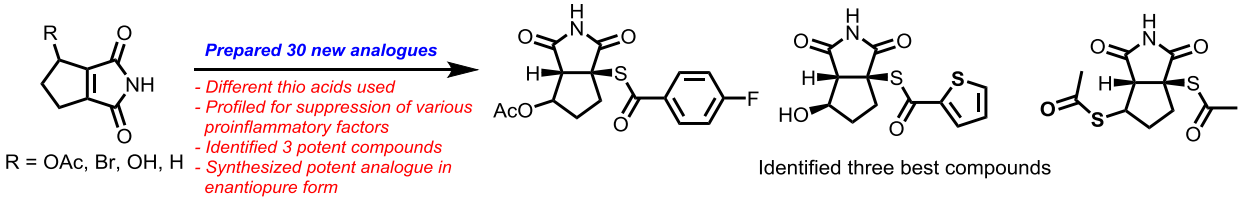
Chapter 2: Synthesis and biological evaluation of new anti-inflammatory agents Nitrosporeusines and their analogues.

Nitrosporeusines A and B are two new marine natural products with unprecedented skeleton containing benzenecarbo-thiocyclopenta[c]pyrrole-1,3-dione isolated by Lin and co-workers in 2013. Initial studies on these novel compounds from Arctic Chukchi sea showed promising inhibitory activity against the influenza WSN virus (H1N1) in MDCK cells. According to some recent Chinese patents, nitrosporeusine A is also found to exhibit multiple medical applications especially in treating acute renal failure, rhinitis, renal fibrosis, oral ulcers and chronic heart failure. The interesting biological features coupled with the potential of these new carbon skeletons in medicinal chemistry as anti-inflammatory agents, attracted our attention to initiate a program on nitrosporeusines. The planned synthesis began with synthesizing known



Scheme 2: Synthesis of Nitrosporeusines A and B

maleimide derivative in good quantities, which on allylic oxidation resulted in racemic maleimycin. Amano lipase mediated enzymatic kinetic resolution on maleimycin using vinyl acetate gave both maleimycins in enantiopure forms. Michael addition of (-)-maleimycin with 4-hydroxybenzothioic *S*-acid in water gave desired natural products (+)-nitrosporeusine A and (-)-nitrosporeusine B in optically pure forms. We next explored the possibility of synthesis of a library of compounds around this scaffold to obtain new analogues with improved activities. Accordingly, ~30 analogues were synthesized and profiled for their anti-inflammatory potential in various *in vitro* and *in vivo* assays with the help of Prof. Anirban Basu, NBRC. Now, we have identified three compounds with very good potency in modulating various inflammatory markers. Further work on identification of active enantiomer of potent compound is under progress.



Scheme 4: Nitrosporeusine analogues synthesized and potent compounds identified

Noteworthy Findings:

1. Synthesized (+)-palmyrolide A and its (-)-*cis*-palmyrolide A using a nine step sequence with Zhu's oxidative homologation as key reaction.
2. 19 new analogues of palmyrolide A were synthesized using a slightly modified approach and 4 analogues are found to have comparable activity to that of palmyrolide A in VGSC inhibition.
3. First chemical synthesis of nitrosporeusines A and B in both racemic and enantiopure forms was achieved and 30 new analogues have been synthesized.
4. All the analogues were profiled for their anti-inflammatory potential and three compounds are identified for further analysis.

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1. **Philkhana, S. C.**; Seetharamsingh B.; Dangat, Y. B.; Vanka, K.; Reddy, D. S. *Chem. Commun*. **2013**, *49*, 3342.
2. **Philkhana, S. C.**; Dhasaiyan, P.; Prasad, B. L. V; Reddy , D. S. *RSC Adv*. **2014**, *4*, 30923
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4. **Philkhana, S. C.**; Jachak, G. R.; Gunjal, V. B.; Reddy, D. S. **WO 2016051425** Benzenecarbothioccyclopenta[c] pyrrole-1,3-dione compounds and process for synthesis thereof

Contents

Chapter 2. Synthesis and biological evaluation of new anti-inflammatory agents Nitrosporeusines and their analogues

Chapter 1

Synthesis and Biological Evaluation of Palmyrolide A and its Analogues

Chapter 1

Synthesis and Biological Evaluation of Palmyrolide A and its Analogues

1.1 Introduction

Since ancient times, natural products are rich sources of medicines for treatment of various diseases and illnesses of humans. They are very well crafted by the nature so as to interact with many specific targets within biological systems. Compared to totally synthetic molecules, natural products are perceived to have more "drug-likeness and biological friendliness"[1] as many secondary metabolites from natural sources are elaborated in living systems. With their diverse structures and the intricate carbon skeletons, natural products are often validated starting points for many drug discovery programs.[2] Over the years, due to advancements in deeper understanding of disease biology, there is shift from nature's pharmacy to purely synthetic drugs. However, the major health challenges thrown by the complex chiral world could only be tackled by natural products, which offer the unique structural and chemical diversity along with novel medicinal properties. To date, they are still the source or inspiration for the majority of US-FDA approved drugs (Figure 1.1). In fact, the influence of natural products on drug discovery is so huge that there are many reports and reviews documented in literature.[1] The relatively minor class of natural products (~3%) are macrocycles, which contain cyclic framework of 12 or more atoms.[3] The most common naturally occurring macrocycles are 14-, 15- and 16- membered frameworks with largest containing 50+ atoms.[3c] According to a recent report, almost 100 macrocycles are either marketed drugs or in various stages of clinical trials. The most impressive part is that one third of macrocyclic drugs are oral drugs.[3] The conformational restrictions and relatively large surface areas of macrocycles impart them with higher target binding and improved oral bioavailability, thus making them an ideal candidates for developing drugs. In addition, macrocycles offer balanced rigidity and flexibility for specific binding to the desired targets.[4,5] Because of these features, currently, macrocycles are gaining momentum in the field of medicinal chemistry, particularly from industry. There are several macrocyclic marketed drugs[5b,6] like erythromycin, azithromycin, rifampicin (as antibiotics), amphotericin B (as

antifungal) and ixabepilone, peloruside A (as anticancer agents) (see Figure 1.1). There are different classes that macrocycles can be divided into: peptidic and nonpeptidic natural products, nonnatural or synthetic peptide macrocycles and synthetic macrocycles.

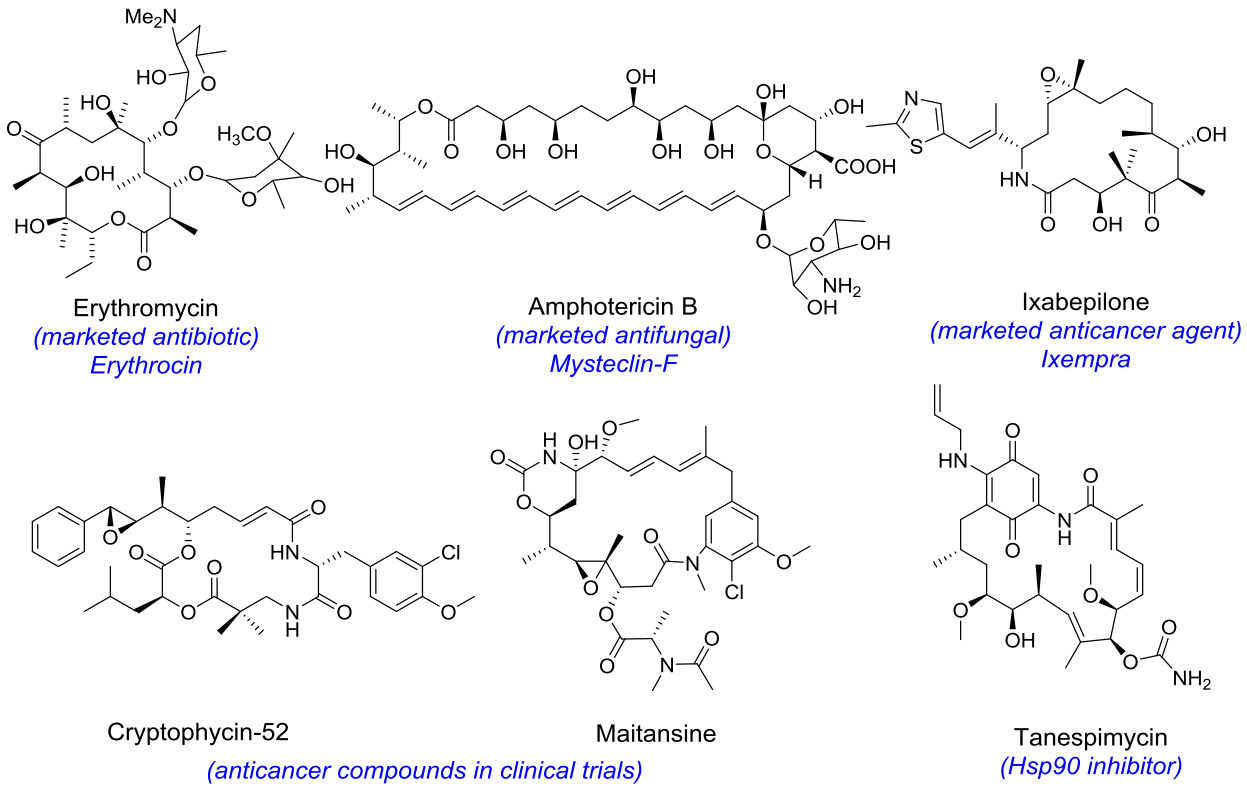
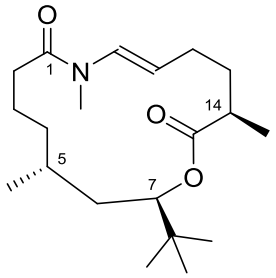


Figure 1.1: Structures of selected marketed drugs with macrocyclic structure

Our group has continued interest in all these classes of macrocyclic natural products where our focus was on synthesis of natural products as well as their analogues towards identifying lead molecules for various diseases. Accordingly, we have been working on macrocyclic targets such as solomonamides (anti-inflammatory),[7a] gliomasolides (anti-cancer),[7b] and cyanolide A (anti-parasitic).[7c] Along these lines, we planned and carried out our studies on natural product - palmyrolide A, a new class of sodium channel blocker which is potentially useful in developing neuroprotective agents (Figure 1.2). (–)-Palmyrolide A was isolated by Gerwick and coworkers in 2010 from marine assemblage consisting of *Leptolyngbya* and *Oscillatoria* species at Palmyra Atoll in the Northern Pacific Ocean.[8] It consists of a unique skeleton which is very rarely found in marocycle literature, a *trans*-*N*-methyl enamide moiety and a lactone moiety adjacent to tertiary butyl group, all in a 15-membered ring. The structure and relative configurations in

palmyrolide A were established by Gerwick's group through extensive NMR studies and Murata *J*-based configurational analysis.



(-)-Palmyrolide A
(*sodium and calcium channel blocker*)
present target

Figure 1.2: Structure of Palmyrolide A

Initial biological studies on (–)-palmyrolide A have shown that it possess voltage-gated sodium channel blocking ability in Neuro-2a cells with an IC_{50} of 5.2 μM.[8] It also showed significant suppression of spontaneous calcium oscillations in murine cerebrocortical neurons, with an IC_{50} of 3.7 μM. The significance of this ion channel blocking ability could be understood from the fact that all movement of ions across our biological membranes are highly selective & tightly regulated by ion channels (Na^+, K^+, Ca^{2+}, Cl^-) and any dysfunction of these ion channels contribute to various diseases associated with renal disorders, endocrine disorders, bone diseases, neurological disorders, cardiac arrhythmia *etc*[9]. During the propagation of an action potential through nerve cells, all the ion channels work in conjunction and have very specific roles.

- The voltage gated sodium channels (VGSC) initiates action potential,
- Voltage gated calcium channels (VGCC) initiate processes like muscle contraction, synaptic transmission, neurotransmitter and hormonal release in response to membrane depolarization,
- Voltage gated potassium channels (VGKC) terminate action potential and return the membrane potential to its resting value.

So, ion channels initiate action potentials in neurons and other excitable cells, and they are responsible for propagation of action potentials along nerves (axons), muscle fibers and the neuronal somato-dendritic compartment.[10] Apart from electrical signal transduction these ion channels also participate in many other functions like chemical signalling, *trans*-epithelial transport, regulation of cytoplasmic or vesicular ion concentration and pH, and regulation of cell

volume. Out of all these channels, studies on voltage gated sodium channels (VGSCs) are of significant interest due their close association with many CNS disorders. They are membrane protein complexes forming a pore which controls the flow of sodium ions inward and outward cell's plasma membranes, thus regulating the cellular excitability and physiological processes associated.[11] Mutation causing functional changes in these ion channels results in altered regulation, which may be cause for hyperexcitability of neurons leading to inherited forms of epilepsy, chronic pain, and other syndromes.[10b] VGSCs are made up of one α-subunit (has conducting channels) and two auxiliary β-subunits that mediate the linkage of the α-subunit to the plasma membrane. There are nine α-subunits $Na_v1.1$ to $Na_v1.9$ identified and functionally expressed. Any mutations in genes of them could result in various diseases related to Central nervous system (CNS), Heart, Dorsal root ganglion (DRG), Skeletal Muscles etc (Shown in Table 1.1).

Table 1.1: The inherited diseases when mutation occurs in gene of that VGSC type.

VGSC type	Gene	Primary tissue	Inherited disease
$Na_v1.1$	SCN1A	CNS	Epilepsy
$Na_v1.2$	SCN2A	CNS	Epilepsy
Nav1.3	SCN3A	CNS	
Nav1.4	SCN4A	Skeletal Muscle	Paralysis
Nav1.5	SCN5A	Heart	Arrythmia
Nav1.6	SCN8A	DRG	
Nav1.7	SCN9A	DRG and CNS	Pain
Nav1.8	SCN10A	DRG	
Nav1.9	SCN11A	DRG	

There has been considerable research done in VGSC's in recent years to identify new sodium channel blockers[12] and there are many new drugs either in market or in various phases of human clinical trials (Figure 1.3). However significant contributions were made by Hoyet's group[12a] and Guliani's group[12b] where two classes Benzodiazepinones and Diarylimidazoles were studied in detail and potent compounds which are better than marketed drugs were identified figure 1. 3. Although the exact mechanism of action of palmyrolide A in blocking ion channels (sodium & calcium) is not known, the unique structure different from other known scaffolds and recent interest in macrocycles based drug discovery attracted us towards this target.

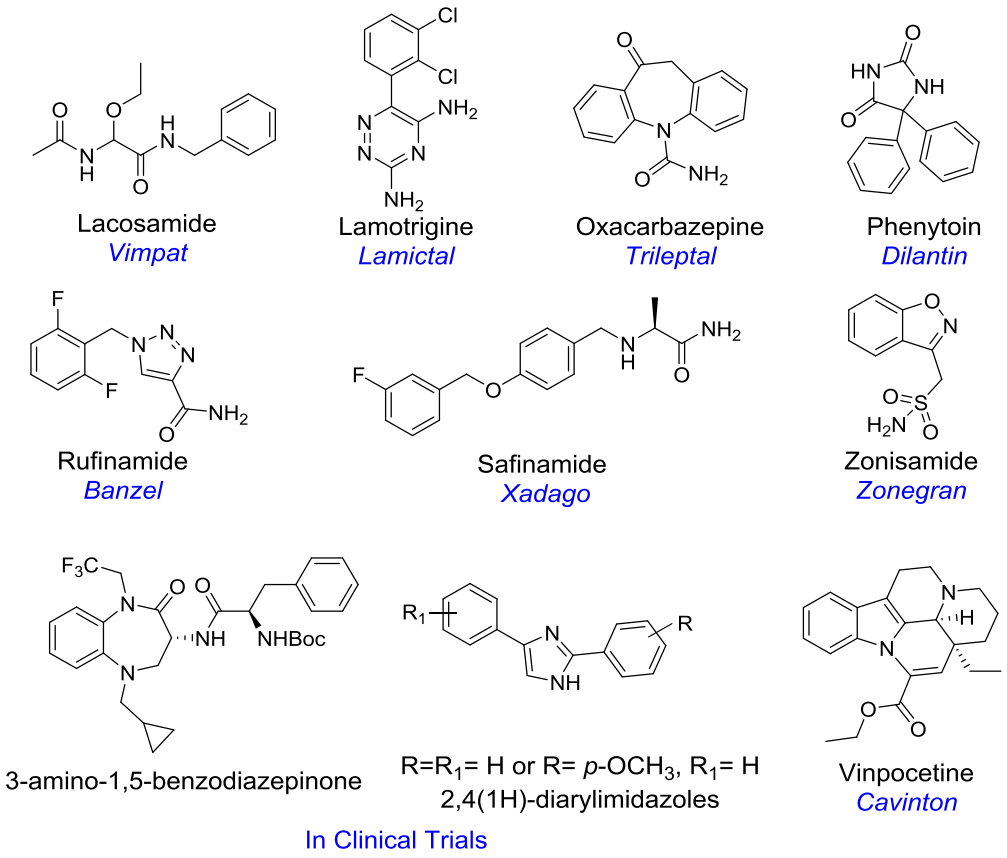


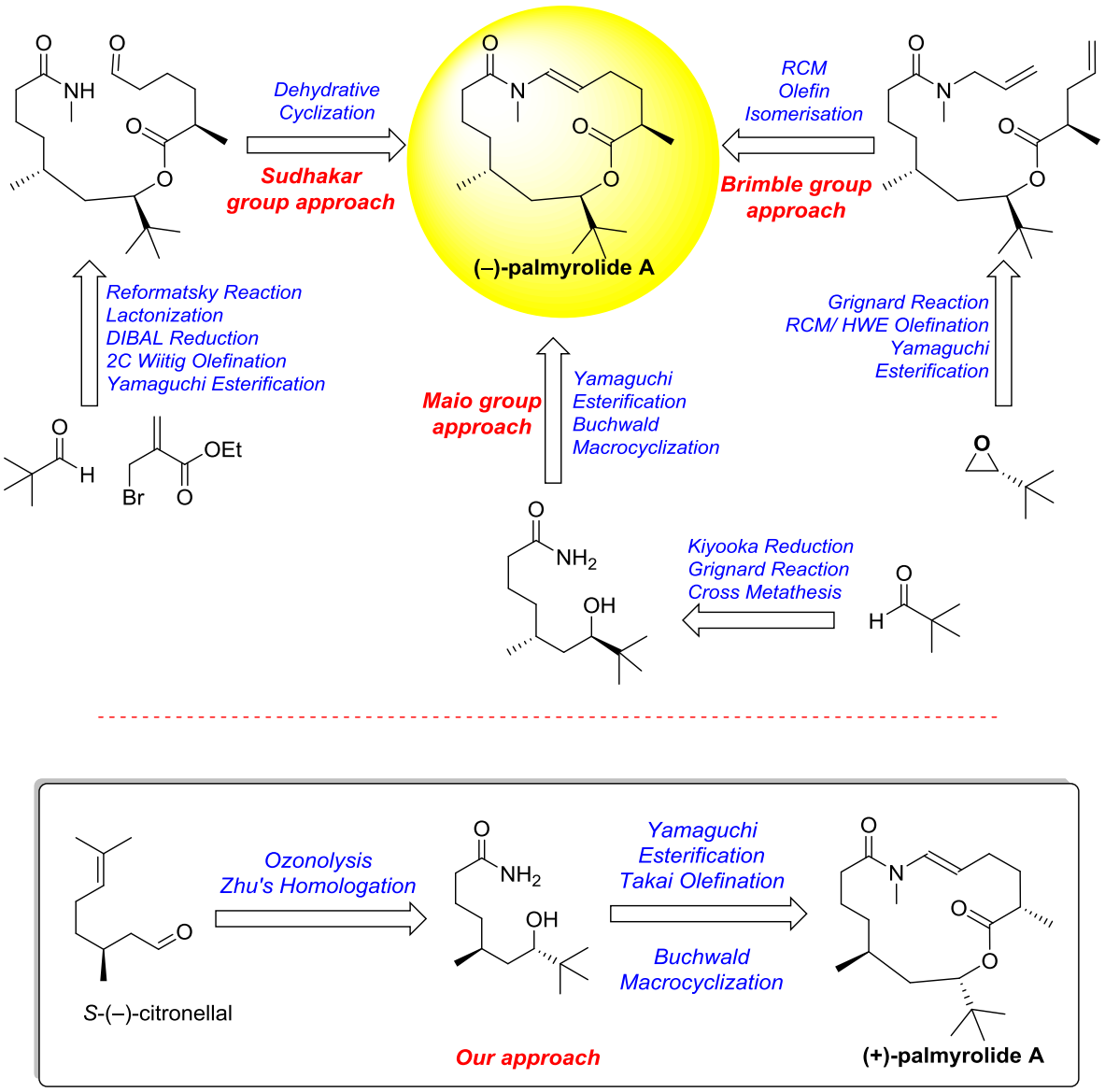
Figure 1.3: Sodium channel blockers in market or in clinical trials

We thought to synthesize and explore its activity with respect to its potential in blocking VGSCs specifically. The synthesis of analogues around this new potential scaffold could also help in developing agents for treating various neurological disorders associated with these channels.[9,10b,13] In addition, unlike many macrocyclic natural products, the present target palmyrolide A has molecular weight 337 (<500), cLogP = 4.75 (<5), H bond donors = 0 (<5), H bond acceptors = 4 (<10), thus showing no violation of Lipinski's rule of five[14] which makes it more interesting for medicinal chemists. Due to interesting structural and biological profile of palmyrolide A, coupled with features of a good medicinal chemistry starting point, this target also attracted the attention of many other research groups around the world.

1.2 Synthetic approaches toward palmyrolide A

To date five total synthesis (including our group) of palmyrolide A have been documented in the literature including our synthesis[15-19] (Scheme 1.1). Before the discussion of

our work, synthetic efforts from other research groups are discussed briefly in following sections.

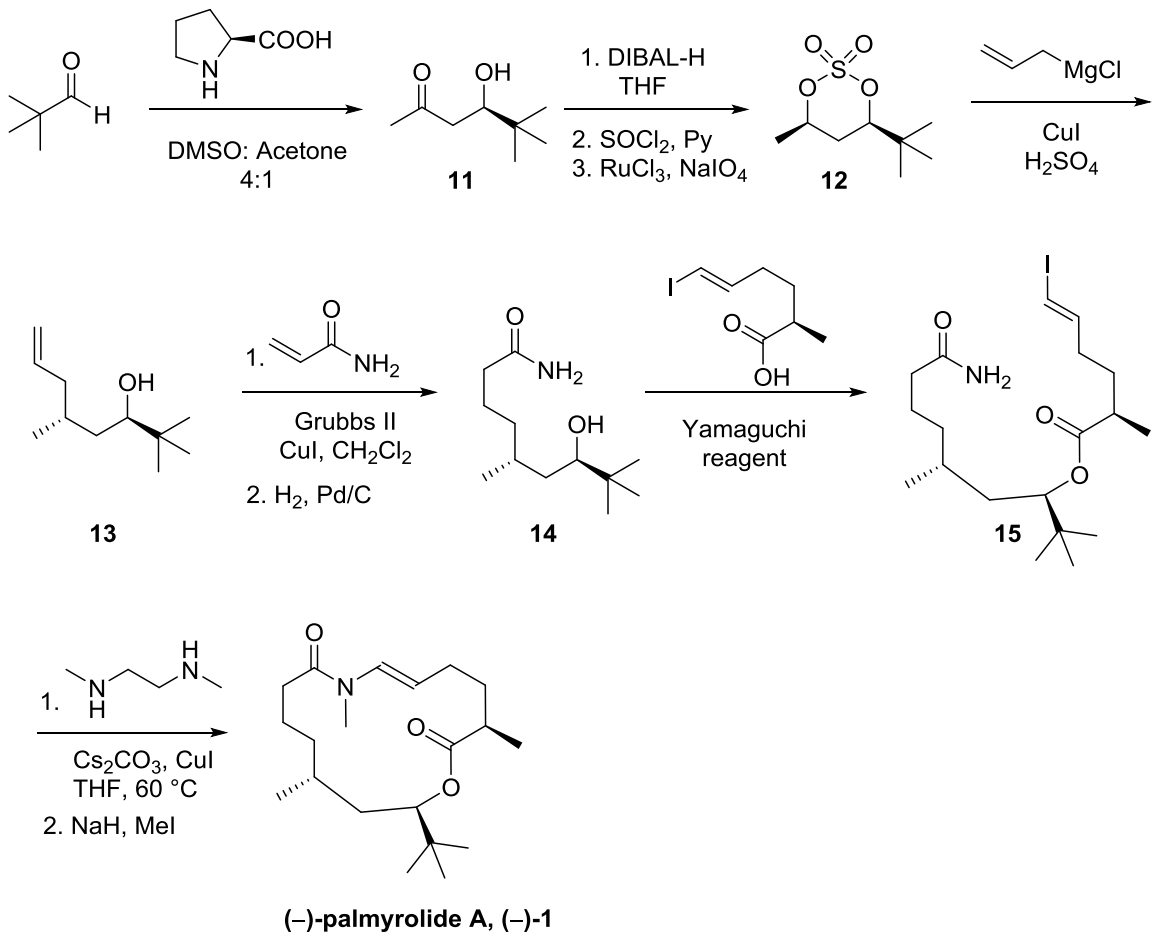


Scheme 1.1 Summary of all approaches toward palmyrolide A (key steps highlighted)

1.2.1 Maio's synthesis

The first synthesis was reported by Maio and coworkers,[15] in which they have utilized an interesting Buchwald-type cyclization to construct the enamide macrocycle. They also revised

the initially assigned relative configurations between the *tert*-butyl and methyl groups, there by establishing the correct structure of (–)-palmyrolide A (Scheme 1.2).



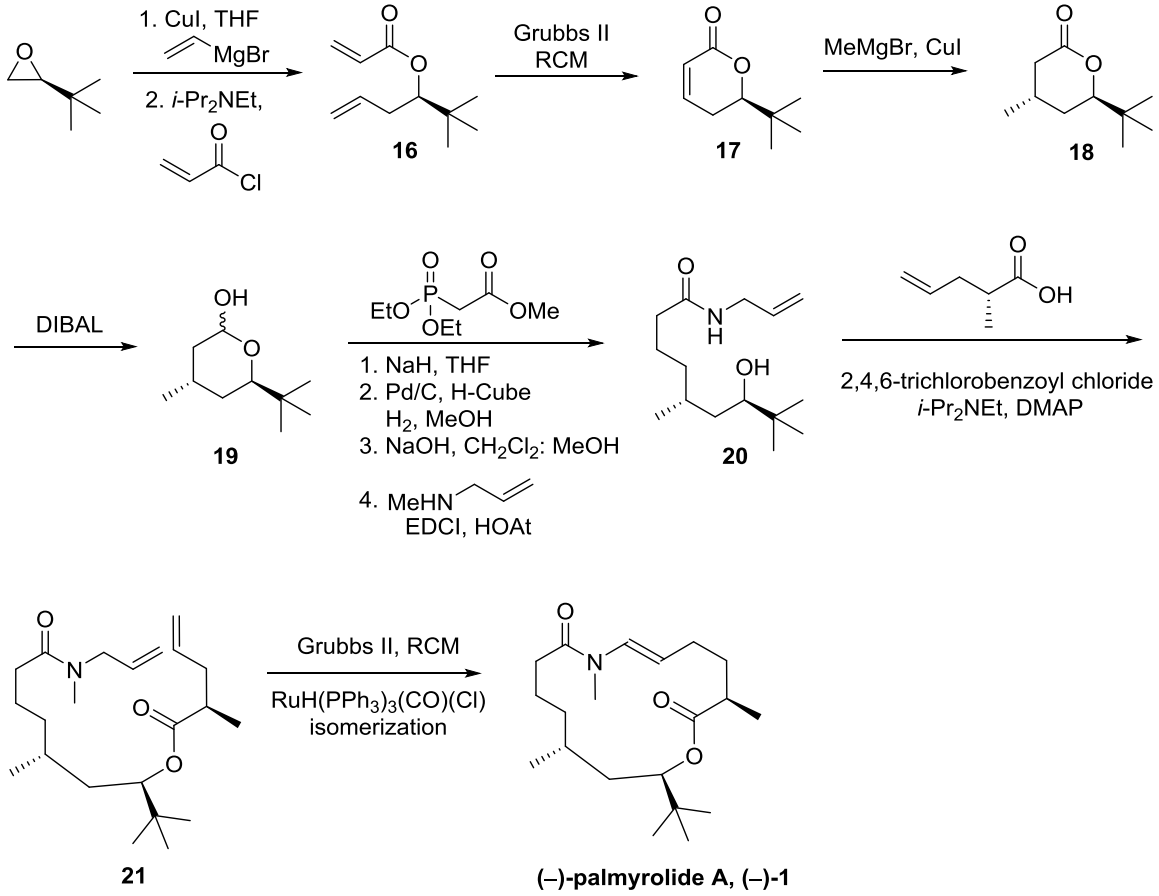
Scheme 1.2: Maio's synthesis of palmyrolide A

Maio's synthesis started with organo-catalysed asymmetric aldol condensation of trimethylacetaldehyde and acetone in presence of L-proline which selectively gave hydroxyalcohol **11** with *tert*-butyl stereochemistry as *R*. This was followed by a stereoselective Kiyooka *syn* reduction with DIBAL-H which selectively gave *syn* diol with good diastereoselectivity. Treatment of *syn*-diol with thionyl chloride in pyridine followed by oxidation using RuO_4, converted it into *syn*-cyclic sulfate **12**. The next few steps involved nucleophilic ring opening using mixed organometallic reagent to get **13**, cross metathesis of **13** with acryl amide and hydrogenation of resultant double bond gave the key intermediate of the synthesis hydroxyl-amide (**14**). Yamaguchi esterification with required acid gave the advanced vinyl iodide intermediate **15** which on Buchwald conditions afforded (–)-palmyrolide A. They

have extended this strategy to synthesize all other isomers of palmyrolide A and studied their biological activities as well (SAR will be discussed in the later part of this chapter).

1.2.2 Brimble's synthesis

Brimble and coworkers achieved synthesis of (–)-palmyrolide A using an interesting strategy involving a sequential ring closing metathesis/olefin isomerization reaction for final

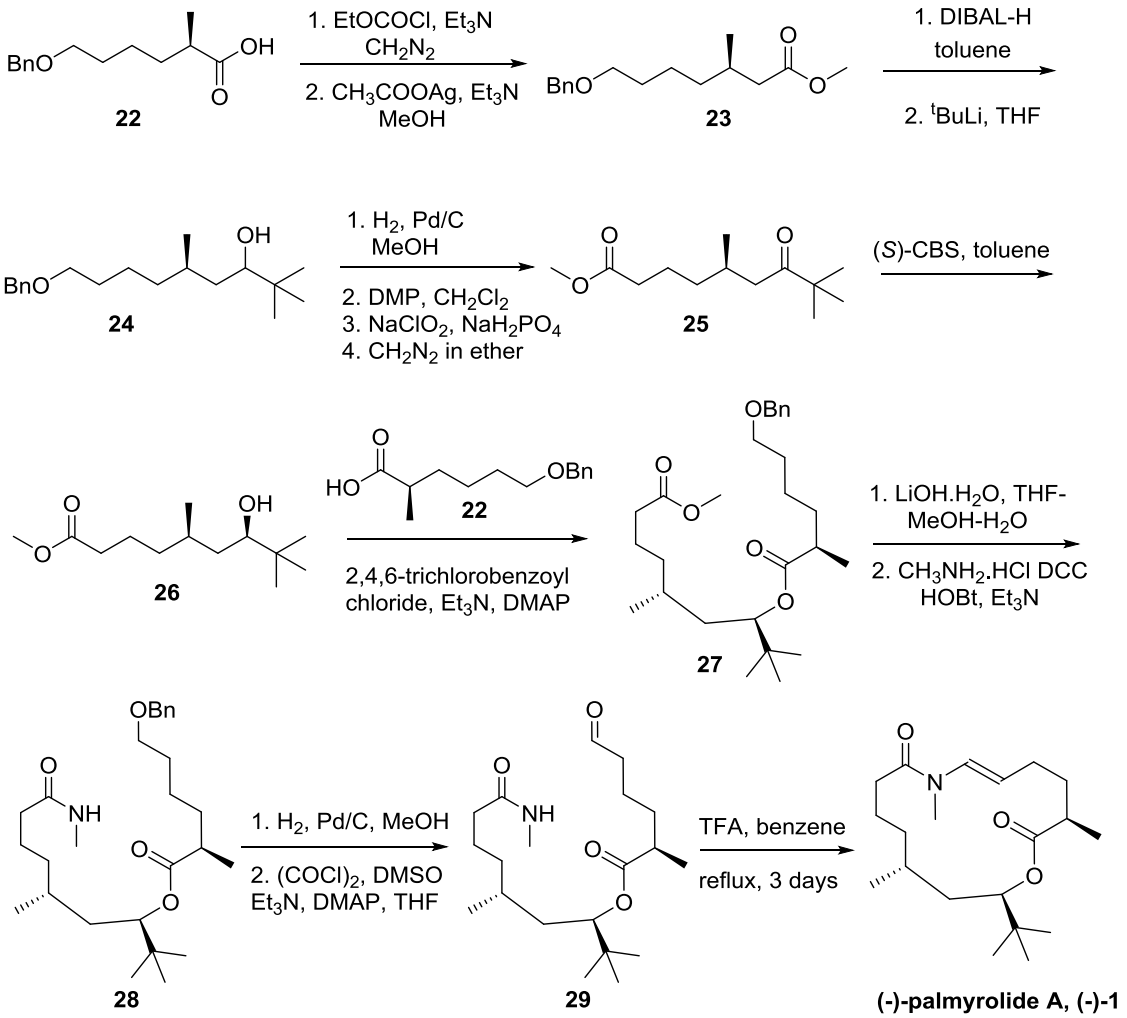


Scheme 1.3: Brimble's synthesis of palmyrolide A

macrocyclization.[16] Her synthesis commenced with addition of vinylmagnesium bromide on known epoxide followed by coupling with acroyl chloride to get the required diene **16**. Grubbs II mediated ring closing metathesis (RCM) of **16** gave dihydropyranone **17**, which upon addition of methylmagnesium bromide in presence of CuI resulted in anti-alkylated product **18** as a single diastereomer (Scheme 1.3). Reduction of lactone (**18**) under DIBAL conditions gave them mixture of lactols (**19**) which were then subjected to a sequence of four reactions involving Wittig reaction, hydrogenation of double bond, ester hydrolysis and coupling with *N*-

methylprop-2-en-1-amine to afford the desired hydroxyl-amide fragment **20** in good yields. Stitching of (*R*)-2-methylpent-4-enoic acid with **20** using Yamaguchi conditions resulted in required RCM precursor **21**. The RCM was achieved with Grubbs II catalyst and gave desired marcocycle. The final step involved a double bond isomerization on this *N*-allylated tertiaryamide using carbonylchlorohydridotris(triphenylphosphine)-ruthenium(II), which gave (–)-palmyrolide A in good yields (Scheme 1.3).

1.2.3 Sudhakar's synthesis



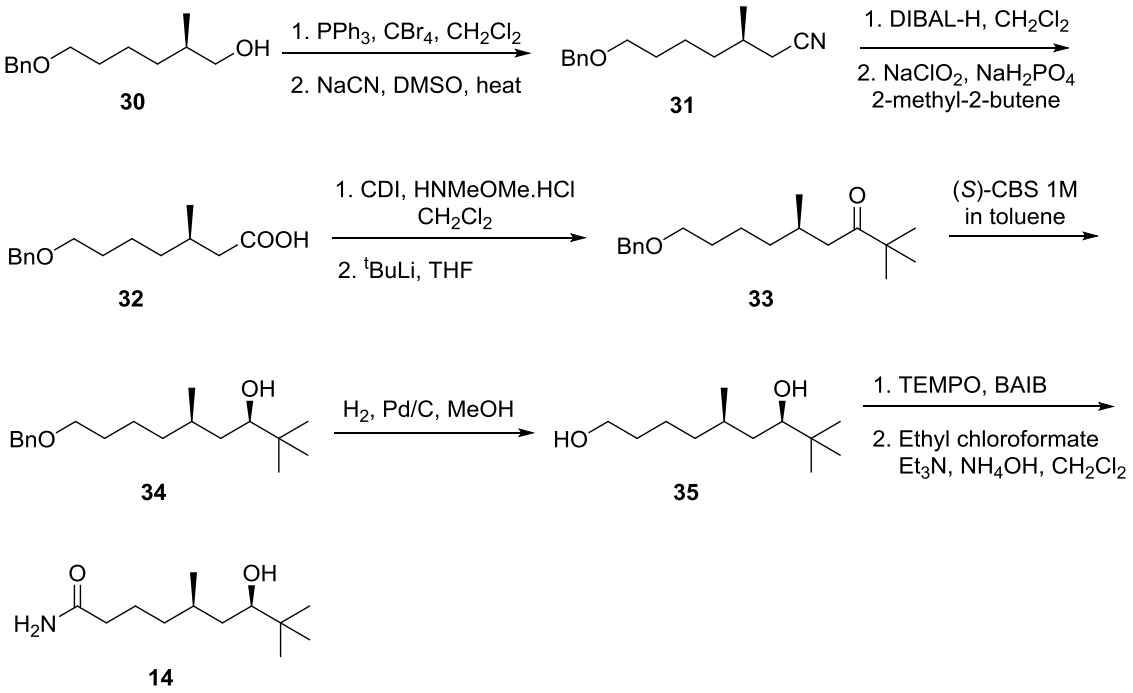
Scheme 1.4: Sudhakar's synthesis of palmyrolide A

Sudhakar's group approach comprised of synthesis of key fragment **26** from common intermediate **22** which itself is obtained from commercially available 1,6-hexanediol in few

synthetic operations. Acid **22** was homologated to produce methyl ester **23** which was then reduced to aldehyde followed by *tert*-BuLi addition to give **24** (Scheme 1.4). The alcohol **24** was then hydrogenated and oxidized to keto-acid. Here, esterification of keto-acid followed by stereoselective ketone reduction using (*S*)-CBS gave the key fragment **26**. The Yamaguchi esterification of fragments **26** and **22** gave ester **27**, and conversion of ester in **27** to amide gave the intermediate **28**. Benzyl deprotection followed by oxidation to aldehyde resulted in the macrocylization precursor **29**. Here, natural product (–)-palmyrolide A had been synthesized by relying on interesting intramolecular dehydrative cyclization to assemble aldehyde and secondary amide to achieve the desired enamide moiety (Scheme 1.4)[18].

1.2.4 Yadav and Srihari's synthesis

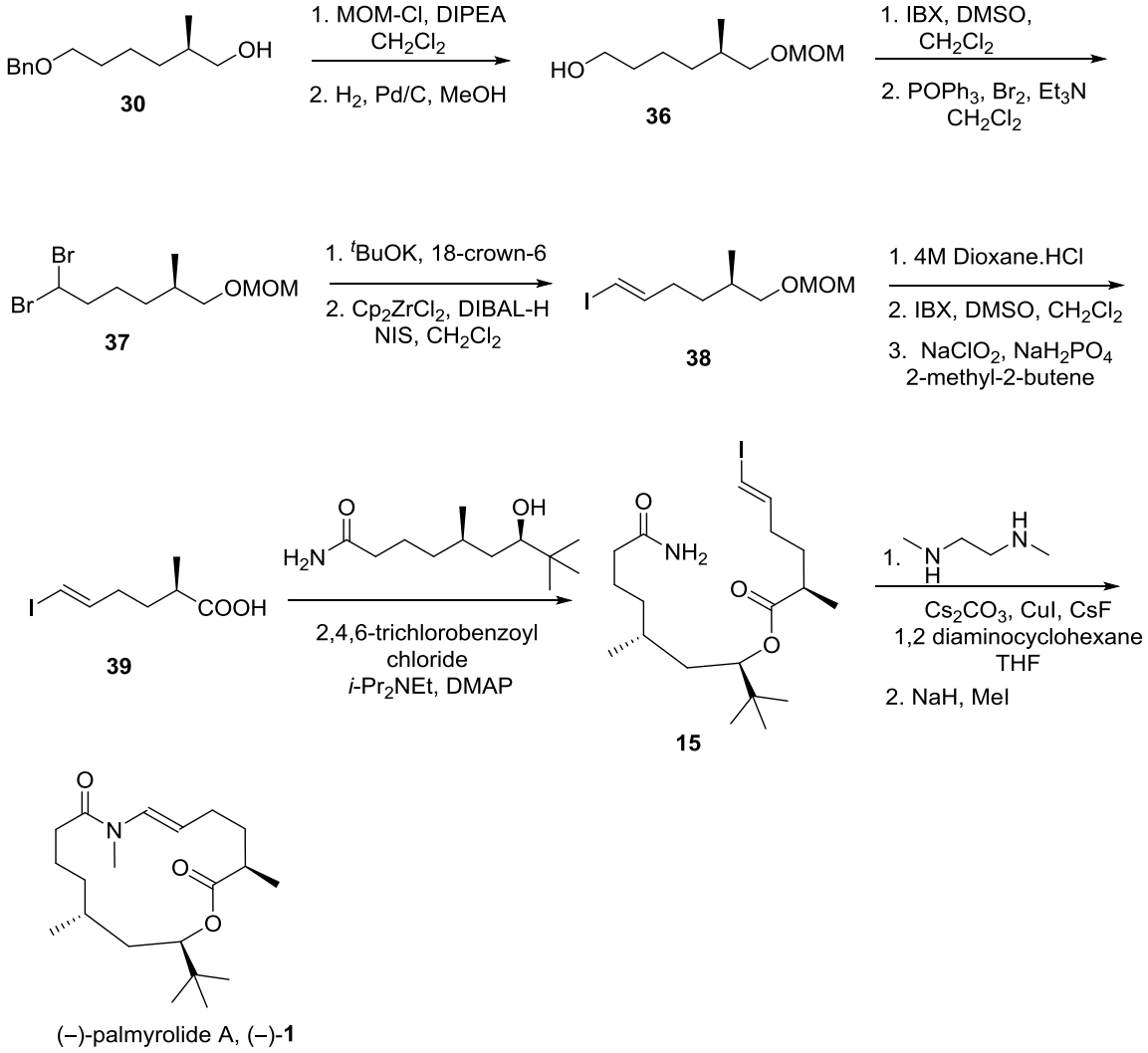
Very recently, there is another synthesis reported by Yadav and Srihari's group[19] which starts from intermediate **30** which could be derived from acid **22** previously used by Sudhakar *et.al.*. But, the approach differs in synthesis of the key hydroxyl-amide fragment.



Scheme 1.5: Yadav and Srihari's synthesis of palmyrolide A

The alcohol **30** was converted to acid through a sequence of four steps involving bromination, cyanation, DIBAL reduction and Pinnick oxidation (Scheme 1.5). The acid (**32**) thus obtained

was treated with N-hydroxylamine hydrochloride and *tert*-butyl lithium to afford required ketone **33**. Here again CBS mediated reduction of carbonyl group gave the desired *tert*-butyl alcohol **34** with required stereochemistry. Benzyl deprotection in **34** gave diol **35**, which was directly oxidized to acid by exploiting TEMPO/BAIB conditions (Scheme 1.5).



Scheme 1.6: Yadav & Srihari's synthesis of palmyrolide A

The crude acid was then converted to respective amide **14** in presence of aqueous ammonia (Scheme 1.5). The other fragment for coupling was again synthesized from **30**, in which the key step involved was the formation of vinyl iodide from corresponding alkyne using zirconocene dichloride (Cp_2ZrCl_2). The vinyl iodide was then converted to corresponding acid **39** and then coupled to alcohol **14** to get the vinyl iodide precursor **15**. This intermediate was subjected to slightly modified Buchwald conditions followed by Maio and our research group and obtained (–)-palmyrolide A (Scheme 1.6).

Looking at all synthetic approaches, one can clearly observe that key to synthesis is achieving the hydroxyl-amide fragment in best possible way. Maio's group employed a seven step strategy involving asymmetric aldol reaction and cross methathesis to achieve this key fragment. His was the first synthesis published in 2012 which was later followed by Brimble's approach. She reported a nine step synthetic route to *N*-allylated hydroxyl-amide which includes ring closing metathesis and Grignard additions as key steps. Even though both synthetic approaches were versatile, they include more number of steps. During the same time we were simultaneously working on synthesis of palmyrolide A, where we achieved the key hydroxyl fragment in just three steps using an interesting homologation reaction (discussed in following sections). Sudhakar's group also developed a new way in which they achieved the hydroxyl-ester fragment in nine steps, which was converted to amide at later stage. The recent approach by Yadav and Srihari group also highlight the synthesis through hydroxyl-amide fragment.

1.3 Present work:

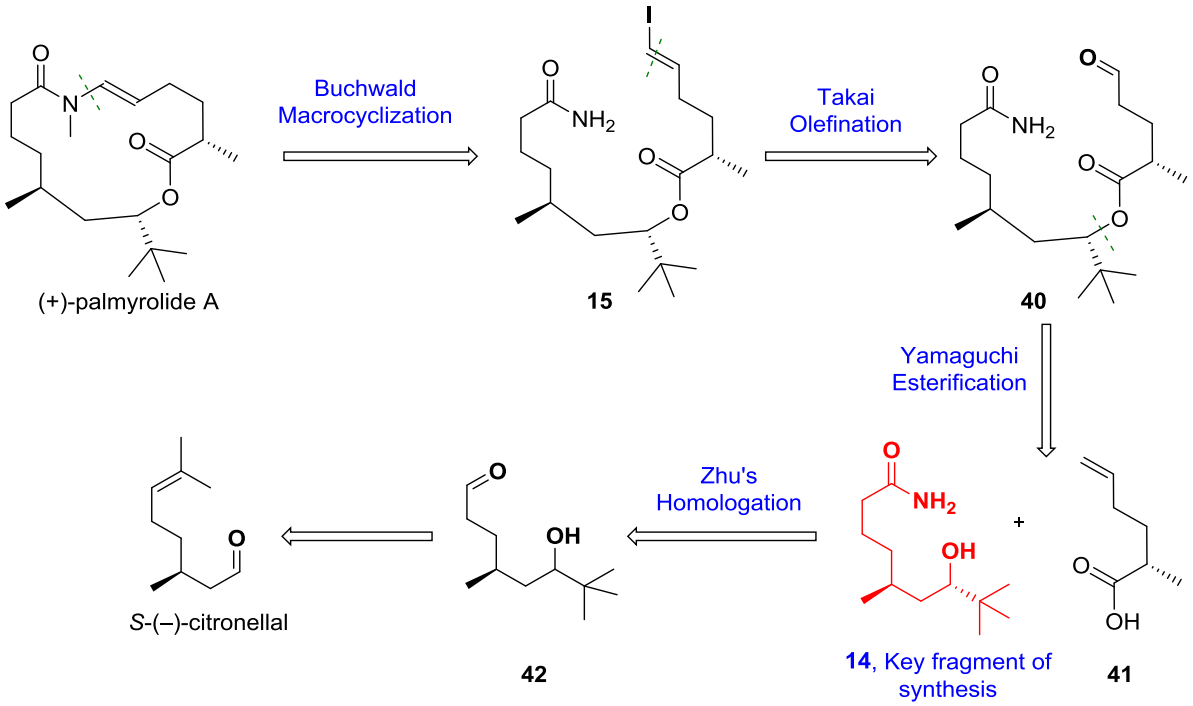
Our work mainly focused on total synthesis, analogue synthesis, and biological evaluation of this exciting natural product palmyrolide A. We have accomplished the total synthesis of (+)-palmyrolide A,[17] (+)-**1** and its unnatural isomer *cis*-palmyrolide A (**7**), by employing Zhu's oxidative homologation as a key step. Later, we explored the unusual *trans* to *cis* isomerization of enamide moiety (observed during the synthesis) using DFT studies. We then diverted our efforts towards synthesis of a library of analogues around this palmyrolide A macrocyle and test for their biological activity (potential to antagonize veratridine stimulated sodium influx). The IC_{50} values obtained for all the analogues enabled us to draw some useful conclusions about the structure activity relations in palmyrolide A scaffold. This work has been discussed in detail in following sections.

1.3.1 Total synthesis of (+)-palmyrolide A and (–)-*cis*-palmyrolide A

1.3.1.1 Retrosynthesis

From the structure of palmyrolide A, we envisioned that *N*-methyl enamide and lactone moieties are two key functional groups to be installed which are challenging because of instability and steric hindrance, respectively. The final macrocylization could be achieved via enamide formation using Buchwald coupling conditions on vinyl iodide (**15)**. This vinyl iodide

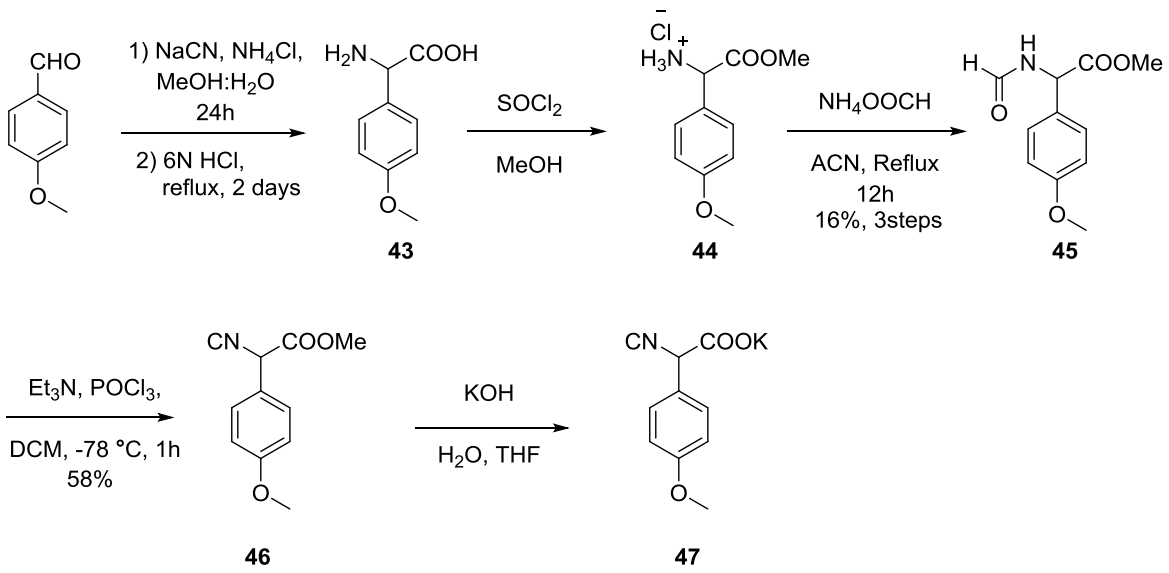
intermediate was planned from aldehyde **40** through Takai olefination. Oxidative osmylation could yield above desired aldehyde from corresponding olefin which in turn could be obtained from Yamaguchi esterification of hydroxyl-amide **14** and (*S*)-2-methylhexenoic acid **41** (Scheme 1.7). The acid **41** could be synthesized from cyclohexanone in few synthetic operations.[20] Here the key to synthesis would be obtaining the hydroxyl-amide fragment (**14**) in efficient and shortest way. Towards that we planned to obtain **14** from aldehyde **42** using an interesting homologation strategy developed by Zhu and co-workers.[21] The aldehyde **42** could be synthesized in two steps starting from citronellal. One of our group strategies is to exploit chiral pool approaches to synthesize natural products and here we thought to employ either *S* or *R* citronellal, which installs the C5 methyl center in palmyrolide A. Prior to start of our synthetic route to palmyrolide A, there had been first synthesis reported by Maio's group, in which the hydroxyl-amide (**14**) was obtained in nine synthetic steps. So here, we envisioned to obtain this desired key intermediate **14** in just three steps using a new and interesting approach.



Scheme 1.7: Retrosynthesis of (+)-palmyrolide A

1.3.1.2 Synthesis of Zhu's homologation reagent

Before starting our planned synthesis, we wanted to prepare potassium 2-isocyano-2-(4-methoxyphenyl)acetate, the reagent required for our key transformation in which an aldehyde gets homologated to amide (Zhu's homologation).



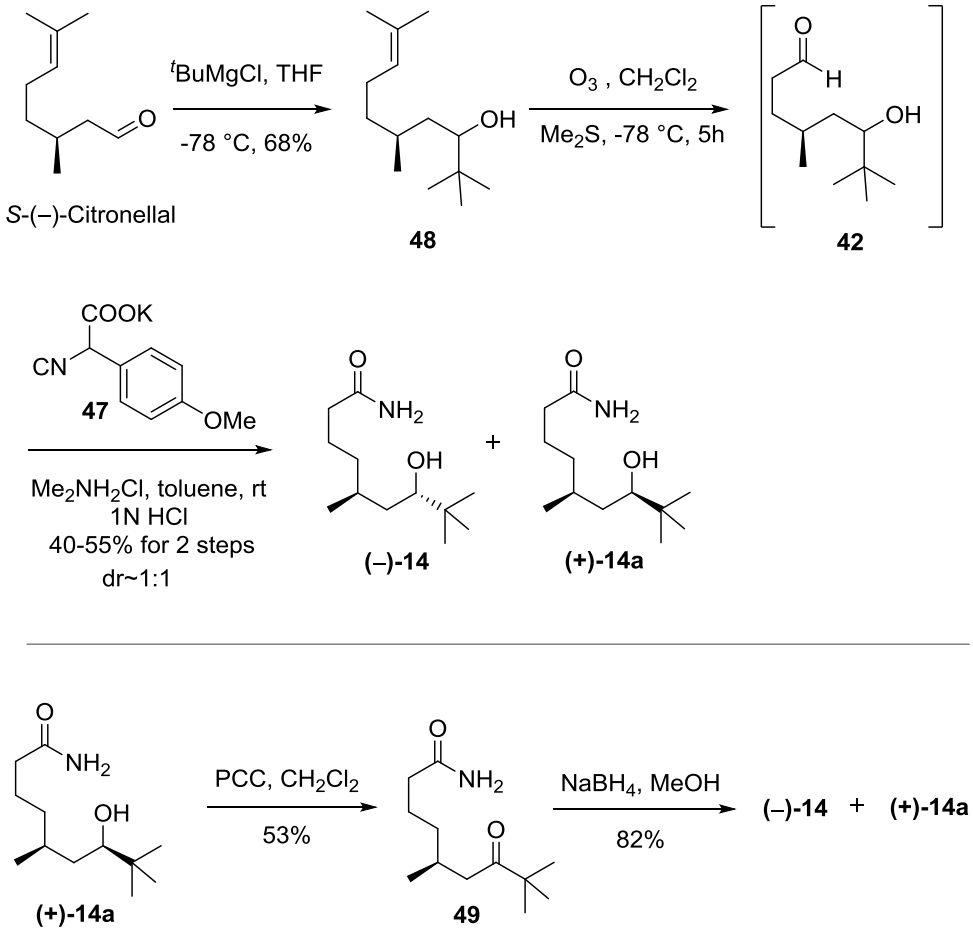
Scheme 1.8: Synthesis of Zhu's homologation reagent

We have prepared the desired Zhu's reagent by following the procedures described by his group in the literature.[21] The cyanation on *p*-methoxyanisaldehyde followed by hydrolysis to get **43**, which was then esterified in presence of thionyl chloride to get ammonium salt **44**. Treatment of **44** with ammonium formate in acetonitrile under reflux conditions resulted in *N*-formyl intermediate, **45**, which was then dehydrated using $POCl_3$/Et_3N to have **46** (Scheme 1.8). The isocyanoester (**46**) thus formed was stored under refrigeration and freshly hydrolysed to **47** when required (detailed procedure discussed in experimental section).

1.3.1.3 Synthesis of palmyrolide A

After having reagent **46** in hand, we embarked on the total synthesis of palmyrolide A. Here, we initially focussed on synthesis of four isomers of the key intermediate **14** and (*R*) and (*S*)-2-methylhexenoic acid (**41**).

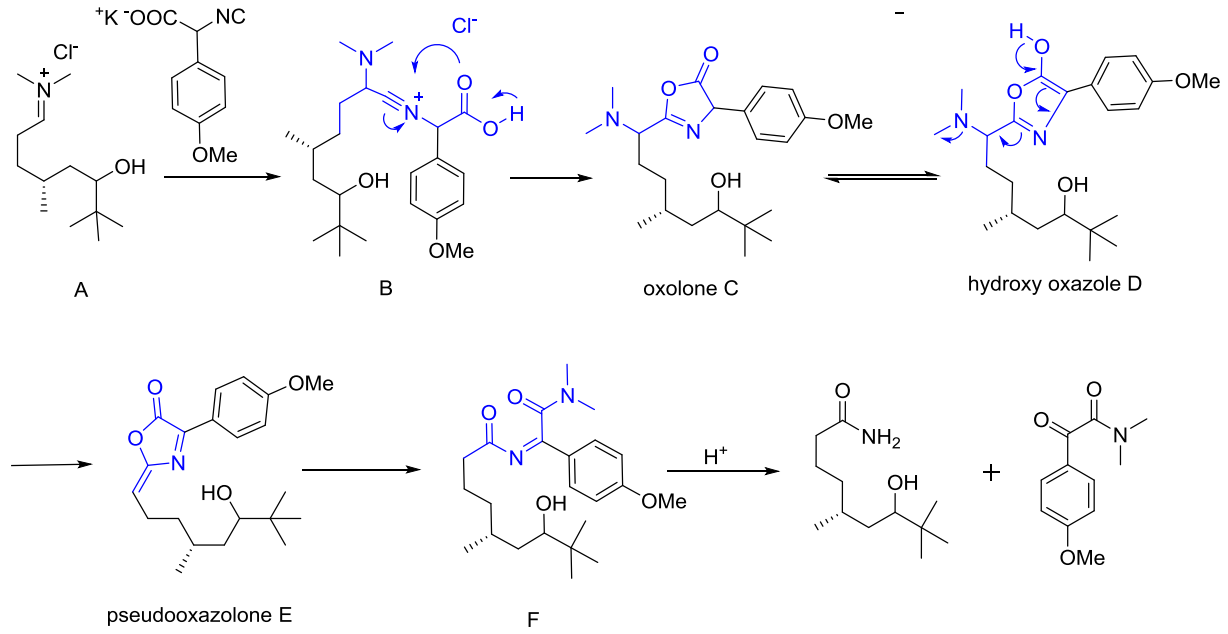
Grignard reaction on (*S*)–citronellal gave *tert*-butyl alcohol **48** as a ~1:1 mixture of diastereomers. The methyl group present on citronellal has no influence on diastereoselectivity of the reaction. The formation of *tert*-butyl alcohol **48** was confirmed by characteristic peak at δ 0.88 (s, 9H) corresponding to *tert*-butyl group and appearance of peak at δ 3.28 (d, J = 12.0 Hz, 1H) corresponding to proton attached to newly formed hydroxyl group. The two diastereomers formed were inseparable at this stage.



Scheme 1.9: Synthesis of key fragment of (+)-palmyrolide A

So, we have made few attempts to separate the diastereomers by derivatising them to their corresponding 4-nitro-benzoyl esters. However, these esters of two diastereomers could not be seen as separate compounds on TLC under different solvent systems. We then subjected the mixture of alcohols **48** as such to ozonolysis to obtain aldehyde **42**. The aldehyde formation could be clearly seen in the IR spectrum which showed characteristic peak at1723 cm^{-1} which is absent in alcohol **48** (IR: 3349, 2964, 2874, 1670, 1512 cm^{-1}). The aldehyde **42** was then homologated to amide using a one pot strategy developed by Zhu and co-workers. Compound **42** on reacting with freshly prepared potassium 2-isocyano-2-(4-methoxyphenyl)acetate (**47**) in presence of dimethylamine hydrochloride and toluene as solvent gave acylimino-amide intermediate **F**, which on acidification gave desired homologated amides (–)-**14** and (+)-**14a**. Mechanistically,[21] this reaction starts with condensation of dimethylamine hydrochloride with aldehyde **42** to form iminium ion (**A**) which then triggers the intramolecular nucleophilic attack

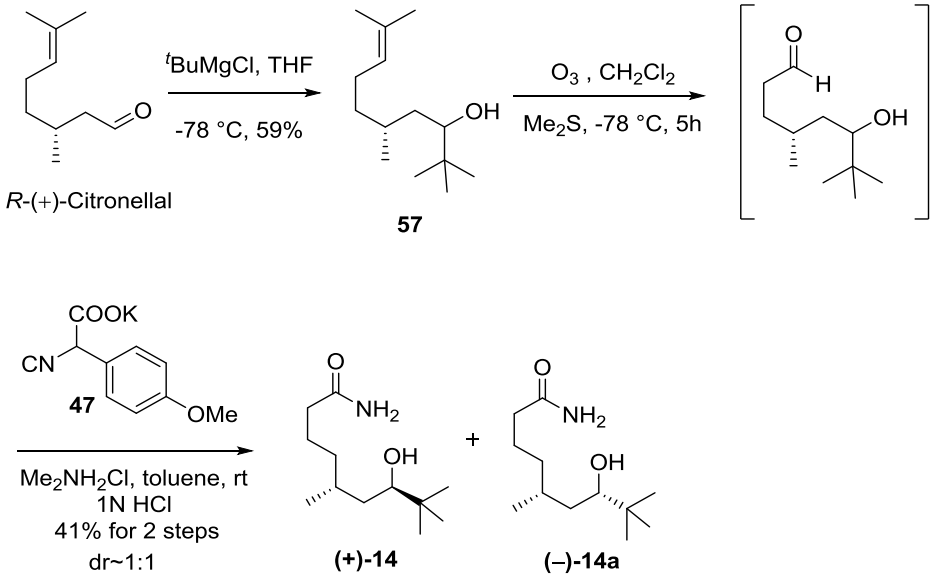
by isonitrile group (Scheme 1.10). The resulting isonitrilium intermediate **B** undergoes intramolecular addition of carbonyl group thus forming oxazolone (**C**) which exists in equilibrium with hydroxy oxazole (**D**). The shift of equilibrium here decides the course of reaction further. If there are no additional stabilizing groups, the oxazolone intermediate gives dipeptide by reacting with amine. But, presence of aryl groups enhances the acidity of proton α to carbonyl group and offers extra stabilization through conjugation, resulting in shift of equilibrium towards hydroxy oxazole **D**. Then, 1,6 ammonium ion elimination in **D** takes place with assistance of hydroxyl group and ends up in formation of pseudooxazolone **E** (Scheme 1.10).



Scheme 1.10: Mechanism of Zhu's homologation

A second molecule of dimethylamine hydrochloride then opens up the pseudooxazolone ring and the forms acylimino-amide **F**, which when treated with 1N HCl gets hydrolysed to give hydroxyl-amides (–)-**14** and (+)-**14a** in 1:1 diastereomeric ratio. The formation of (–)-**14** was confirmed by appearance of signals in ^{1}H NMR spectrum at δ 5.90 (bs, 1H), 5.79 (bs, 1H) corresponding to amide protons and 2.20 (t, J = 7.2 Hz, 2H) corresponding to protons attached to newly inserted carbon. The disappearance of certain ^{1}H NMR signals in **14** when compared to **48** (olefin at δ 5.10 (t, J = 7.0 Hz, 1H), dimethyls at 1.67 (s, 3H), 1.60 (s, 3H)) also confirms the formation of desired amides. Similarly, the signals in ^{13}C NMR also supported the formation of **14** with appearance of amide carbonyl at δ 176.3 and disappearance of olefin carbons. The

homologation of carbon chain has been confirmed by number of signals in ^{13}C NMR as well. It was further confirmed by HRMS, which showed a peak at 238.1774 corresponding to molecular formula $C_{12}H_{25}NO_2Na$ $[M+Na]^+$, with calculated value 238.1778. Both the amides thus obtained were completely characterized and compared with those reported by Maio's group as well.[16] The Zhu's one carbon homologation has good substrate scope and is a mechanistically interesting reaction. Even though it was developed in 2005, its potential was not explored previously in natural products synthesis. We were the first to show its application through this synthesis. Due to complex mechanistic transformations and large number of intermediates, the homologation reaction gave moderate yields. However, we could cleanly separate the two diastereomers formed by column chromatography using 80% EtOAc/Hexane and then with 3% MeOH/CH_2Cl_2. We could scale up the reaction to gram scale and later optimised its purification on MPLC (Combiflash) for scale up and good recovery. We have also put efforts in conversion of the unrequired isomer to required isomer (–)-**14** one through oxidation reduction sequence.

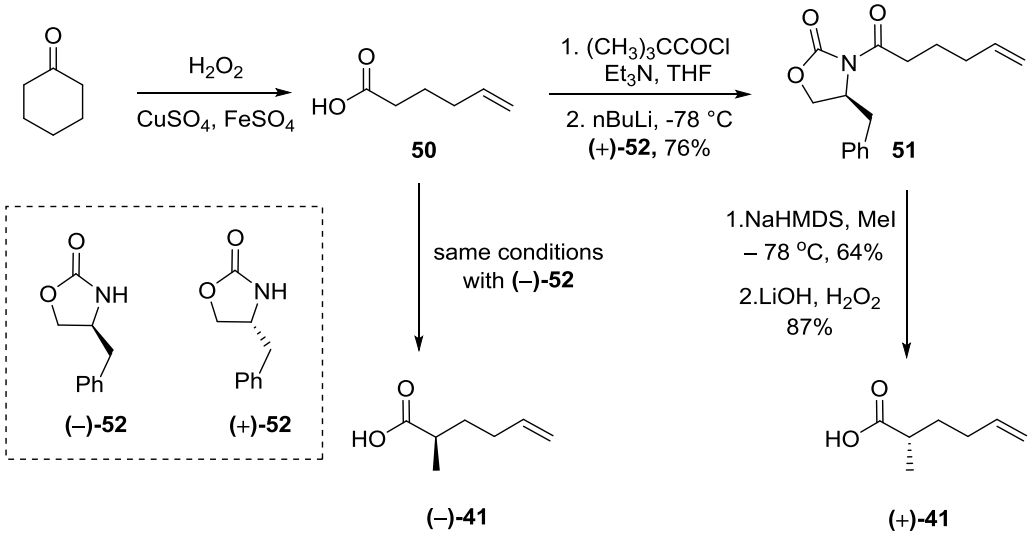


Scheme 1.11: Synthesis of isomers of **14**

The hydroxyl-amide (+)-**14a** was oxidised using pyridinium chlorochromate (PCC) to form ketone **49**, which was confirmed by disappearance of proton attached to hydroxyl group at δ 3.28 and appearance of peaks at 2.28-2.41 (m, 2–H) corresponding to protons adjacent to ketone carbonyl. The appearance of characteristic ketone peak at δ 216.0 in ^{13}C NMR also confirms the ketone formation. We tried selective reduction of ketones using different reducing agents (DIBAL, R-CBS, K-selectride) however desired selectivity was not achieved and yields were

low (details in experimental section). So we relied on simple sodium borohydride reduction and obtained the both alcohols which we separated again. We have also prepared another set of amides (+)-**14** and (–)-**14a** starting from *R*-citronellal using same protocol as above (Scheme 1.11). All the spectral data were identical to that of (–)-**14** and (+)-**14a**, except for the rotation values which are having opposite sign similar magnitude. The alcohol **57** prepared during this synthesis was later used during preparation of analogues.

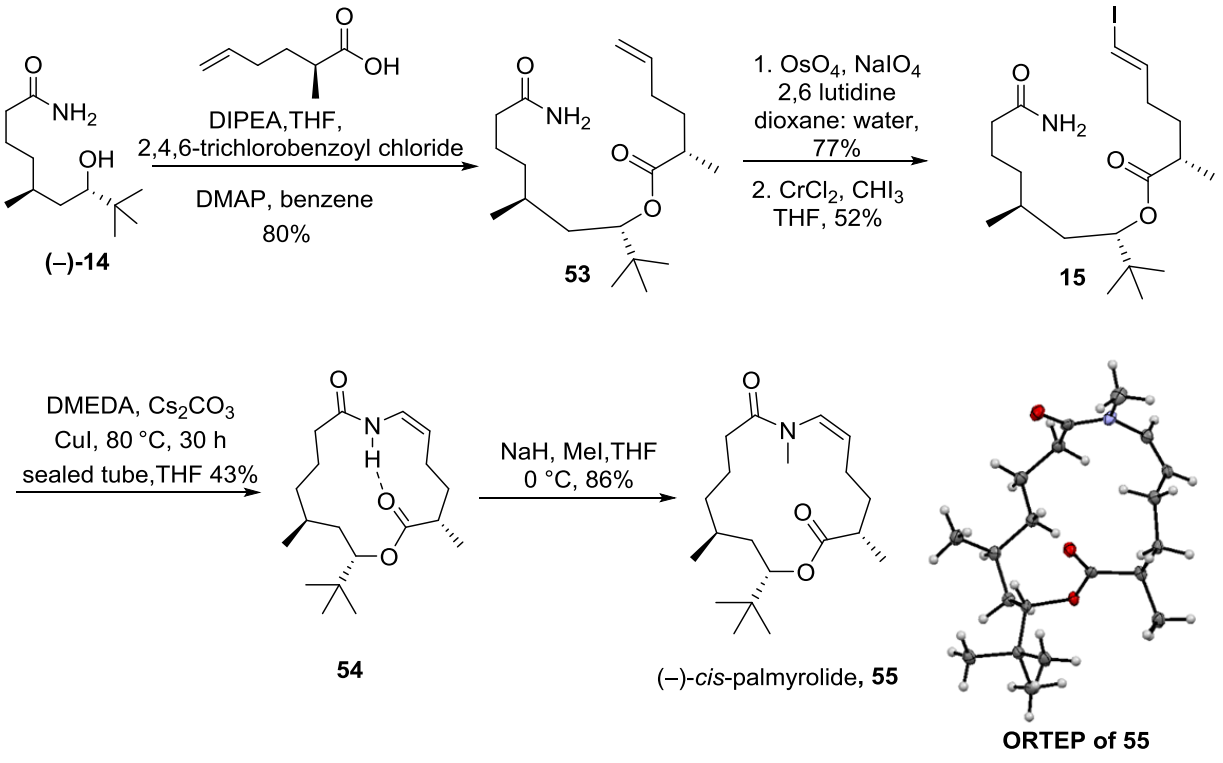
After successful synthesis of all four key fragments (–)-**14**, (+)-**14a,** (+)-**14** and (–)-**14a** , we next synthesized (*S*)-2-methyl hex-5-enoic acid (**41**) using known literature procedures.[20] Its synthesis started with oxidative cleavage of cyclohexanone to 5-hexenoic acid[20b] using $FeSO_4/CuSO_4$ in presence of H_2O_2. Pivaloyl chloride mediated auxiliary coupling to acid using (*S*)-(+)-4-phenyl-2-oxazolidinone followed by selective methylation on **51** gave the intermediate with requisite stereochemistry. Hydrolysis using $LiOH/H_2O_2$ gave the desired acid (+)-**41** in good overall yields. Following similar reaction sequences, with use of (*R*)-(−)-4-phenyl-2-oxazolidinone we synthesized (*R*)-2-methylhex-5-enoic acid (–)-**41** (Scheme 1.12). After synthesizing acids with both stereochemistries, we went forward with (*S*)-2-methyl hexenoic acid towards synthesizing (+)-palmyrolide A.



Scheme 1.12: Synthesis of key coupling fragments

Yamaguchi conditions [22] were employed for coupling, where (*S*)-2-methyl hex-5-enoic acid **41** was treated with 2,4,6-trichlorobenzoyl chloride and DIPEA resulting in a mixed anhydride which was then treated with the alcohol (–)-**14** in presence of DMAP to yield the ester **53**

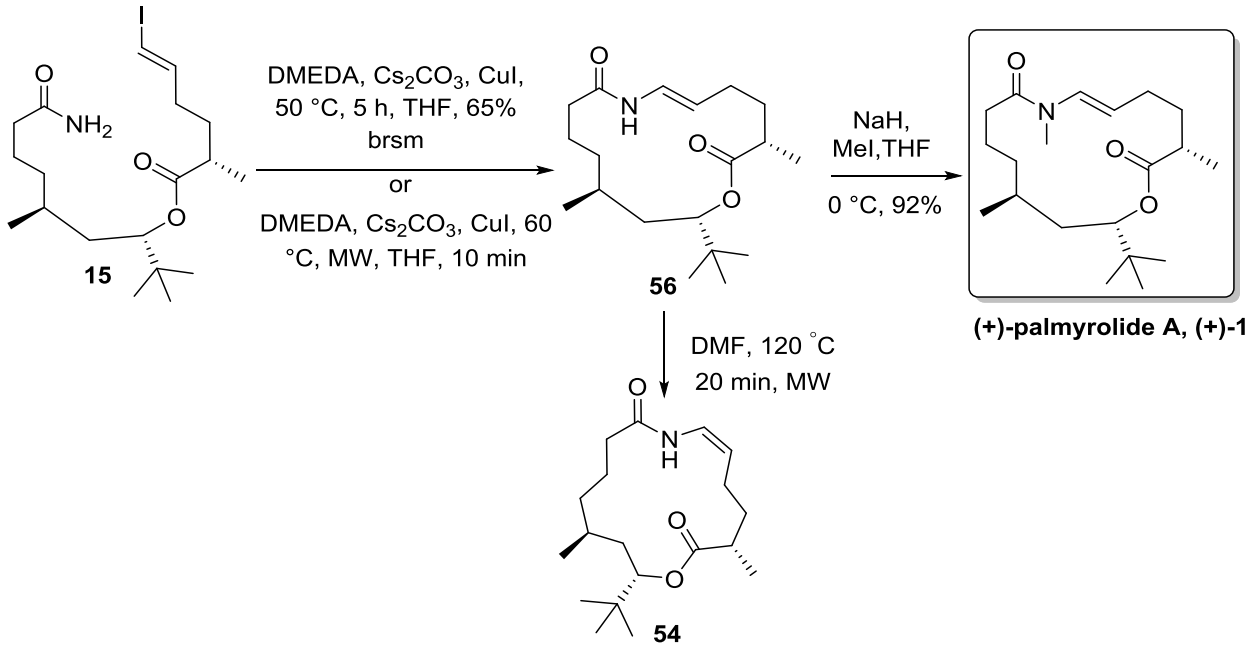
(Scheme 1.13). The formation of **53** was indicated by the presence of signals in ^{1}H NMR at δ 4.74-4.77 (m, 1 H) corresponds to proton attached to *tert*-butyl group, and δ 5.69-5.79 (m, 1H), 4.92-4.99 (m, 2H) corresponds to terminal olefin, signals at δ 1.13 (d, J = 6.8 Hz, 3H), 0.86 (d, overlapped, 3H) correspond to methyl groups attached. The ^{13}C NMR also confirmed the formation of **53** with characteristic ester/amide peaks and olefin peaks at δ 176.8, 175.9, 137.9, 115.1. It was further confirmed by HRMS, which showed a peak at 326.2688 corresponding to molecular formula $C_{19}H_{36}NO_3$ $[M+H]^+$ with calculated value 326.2690. Oxidative cleavage[23] of double bond present in **53** using $OsO_4/NaIO_4$ gave aldehyde which was confirmed by the disappearance of olefin signals and appearance of aldehyde proton in ^{1}H NMR at δ 9.77 (t, J = 1.26 Hz, 1H). It was again confirmed by HRMS, which showed a peak at 328.2482 corresponding to molecular formula $C_{18}H_{34}NO_4$ $[M+H]^+$, with calculated value 328.2479. Without extensive characterization, the obtained aldehyde was immediately subjected to Takai olefination ($CHI_3/CrCl_2$)[24] to get macrocyclization precursor having vinyl iodide moiety (Scheme 1.13).



Scheme 1.13: Synthesis of (-)-*cis*-palmyrolide

This chromium mediated vinylation of aldehyde resulted in *trans*-vinyl iodide which is confirmed by the coupling constants in proton NMR spectra. The signals at δ 6.44-6.51 (m, 1H), 6.02 (d, 1H) correspond to olefin protons with a coupling constant of 14Hz, clearly indicate presence of *trans*-stereochemistry of vinyl iodide. The ^{13}C NMR signals also show the reappearance of olefin signals (δ 145.5, 78.9) along with presence of amide and ester signals at δ 176.5, 175.8. The olefin peak at δ 78.9 is characteristic peak corresponding to iodo attached carbon which confirmed the formation of vinyl iodide. After complete characterization, we subjected **15** to Buchwald macrocyclization conditions (CuI/ DMEDA/ Cs_2CO_3)[25] previously developed and used by Maio's group in their synthesis of palmyrolide A. Incomplete consumption of starting material prompted us to increase the temperature (>80 °C) and prolong the reaction for longer times (>30 h). We expected the formation of (+)-palmyorlide A having *trans*-enamide bond, but surprisingly we have isolated *cis*-enamide **54** with *cis* stereochemistry exclusively (Scheme 1.13). The formation of *cis*-enamide could be clearly seen in ^{1}H NMR of **54**, δ 6.59 (t, *J* = 8.04 Hz, 1H), 4.97 (q, *J* = 8.00 Hz, 1H) corresponds to olefin protons with a characteristic *cis* coupling constants. It was surprising because we have started with *E*-olefin in the acyclic precursor and ended up with *Z*-olefin in the macrocycle. The peaks at 1.21 (d, *J* = 8.00 Hz, 3H), 0.89 (s, 9H), 0.85 (d, *J* = 6.27 Hz, 3H) corresponds to methyl and *tert*-Butyl groups in macrocycle. ^{13}C NMR spectra show the presence of olefin carbons at δ 122.8 and 113.0 which implies the vinyl iodide moiety is absent. Signals at δ 177.4, 172.1 in ^{13}C NMR shows that ester and amide groups are intact after reaction. We then methylated **54** to furnish *cis*-palmyrolide A (**55**) and observed similar *cis* coupling constant values at δ 6.11 (d, *J* = 7.5 Hz, 1H), 5.36-5.41 (m, 1H). Further, we crystallised *cis*-palmyrolide A using ethyl acetate/ dichloromethane mixture and obtained single crystal X-ray which confirmed its *cis* stereochemistry without any ambiguity. Now to have *trans*-enamide present in natural product, we have to either perform the reaction under milder conditions or go for another strategy involving dehydrative cyclization[26] of aldehyde and amide. We first resorted to former where we decreased the temperature to 50 °C and stirred for only 5 hours. Even though there is unreacted starting material, we quenched the reaction and thus could isolate *trans*-enamide macrocycle, which existed as rotamers at room temperature. So, without complete characterization it was sugbjected to methylation (NaH/MeI) to get pure (+)-palmyrolide A, (+)-**1** (Scheme 1.14). The ^{1}H NMR showed peaks at δ 6.47 (d, *J* = 13.7 Hz, 1H), 5.28 (dt, *J* = 7.02, 13.7 Hz, 1H) indicating

the presence of olefin protons with coupling constant of 13.7 Hz, which is characteristic of *trans* olefin.



Scheme 1.14: Synthesis of (+)-palmyrolide A

It was further confirmed by comparing with all the spectral data reported in literature.[8] After successful synthesis of (+)-palmyrolide A, we became interested in unusual formation of *cis*-enamide. We believe that *cis* macrocycle is formed through *trans* to *cis* isomerisation as we could not find any literature report which shows formation of *cis*-enamide when *trans*-vinyl iodide is employed for Buchwald conditions. Towards confirming this hypothesis, we repeated the same experiments under microwave irradiation and monitored the reaction with thin layer chromatography where *trans*-enamide formation was observed first then got converted to *cis*-enamide on continuous irradiation and increase of temperatures. We have also observed that the *trans*-enamide macrocycle exists as rotamers at room temperature where as *cis*-enamide doesn't (this observation was based NMR spectra). The ^{1}H NMR analysis of *cis* and *trans*-enamide shows that the NH proton in *cis*-enamide is deshielded by δ 0.5 ppm compared to *trans* isomer. To account for all these observations we took help of Dr. Kumar Vanka, physical chemist at NCL-Pune who explained this through density functional theory (DFT) and investigated using full quantum chemical calculations.

1.3.1.4 DFT studies on *trans* to *cis* isomerisation of enamide

The main task in the DFT studies was to find out the feasible intermediates possible in the conversion from *trans* to *cis*-enamide in palmyrolide A macrocycle. Considering the functional groups and electronics involved, we expected the transformation to proceed *via* intermediates a, b, c with TS1 to TS4 as transition states. Accordingly, the calculations have been performed using the Turbomole 6.0 suite of programs.[27] The PBE functional[28] and the TZVP basis set[29] have been employed.

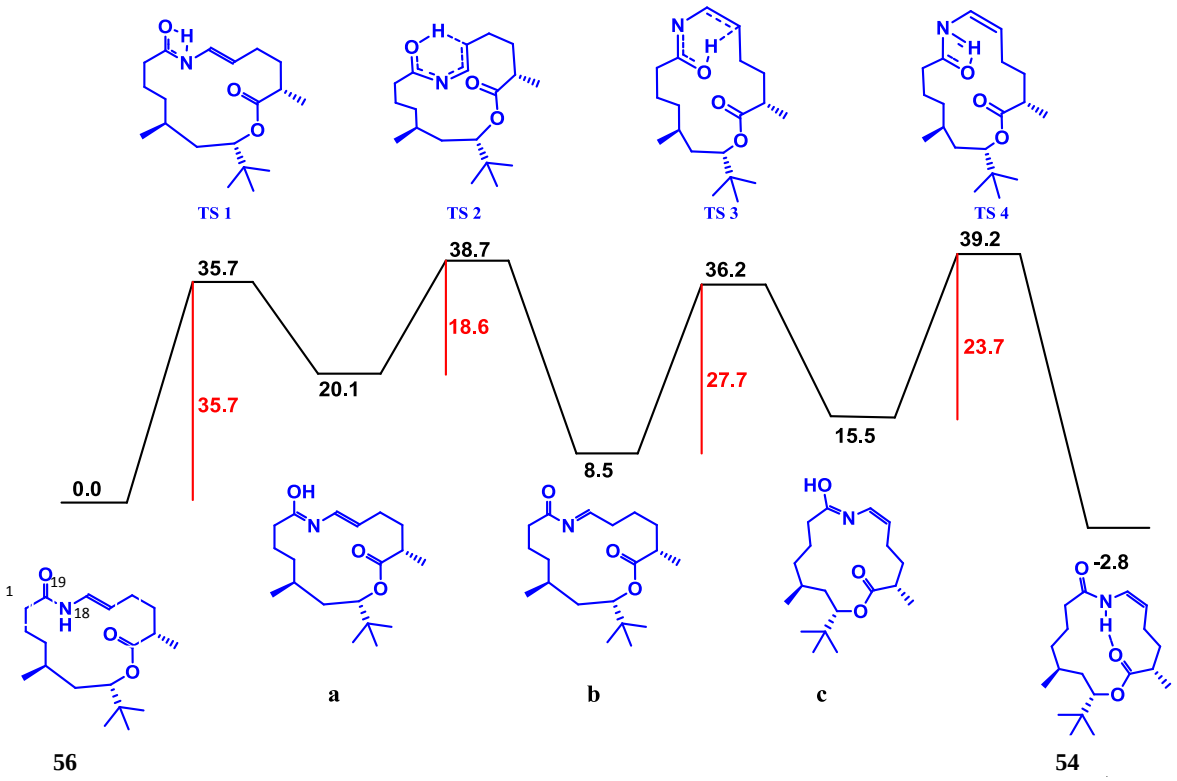


Figure 1.4: The energy profile along with mechanism for *trans* to *cis*-enamide conversion

The free energy profile for the *trans–cis* conversion is shown in figure 1.4. The results from energy caluculations indicate that the conversion involves a series of steps, beginning with the 'enol' type tautomerism in **56**, where a transfer of proton from the N–H group[30] to the adjoining carbonyl group happens, *via* TS1 transition state. And forms intermediate **a**. Here, it is possible for an intermolecular transfer of proton to C18 olefinic carbon *via* six membered transition state TS2 to form second intermediate **b**. Now, the C18-C19 bond is free to rotate and a reprotonation of double bond could occur from either side resulting in formation of *cis* and *trans* double bonds, *i.e* formation of **b** and **c**. Once intermediate **c** forms, it rearranges to **54** *via* TS4 (Figure 1.4). All

these transition states have an energy barrier in the range of 35-39 kcal, which are feasible at elevated temperatures. The close proximity of carbonyl and NH groups in *cis*-enamide made us to explore the possible internal hydrogen bonding between them. Accordingly, when calculations were performed, it is observed that there is an extra stabilization being offered by the H-bonding and *cis*-enamide macrocyle is 2.8 kcal/mol more stable than *trans*-enamide **56**.

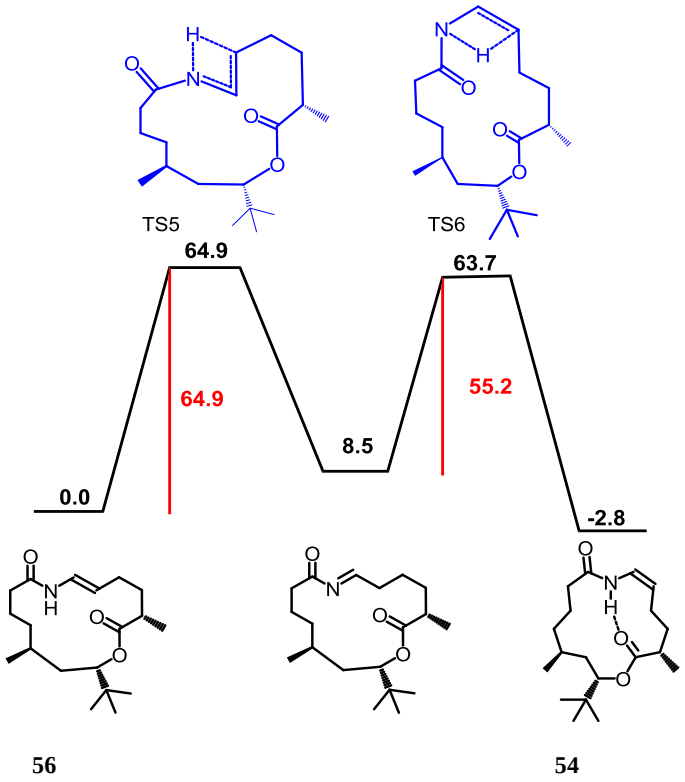
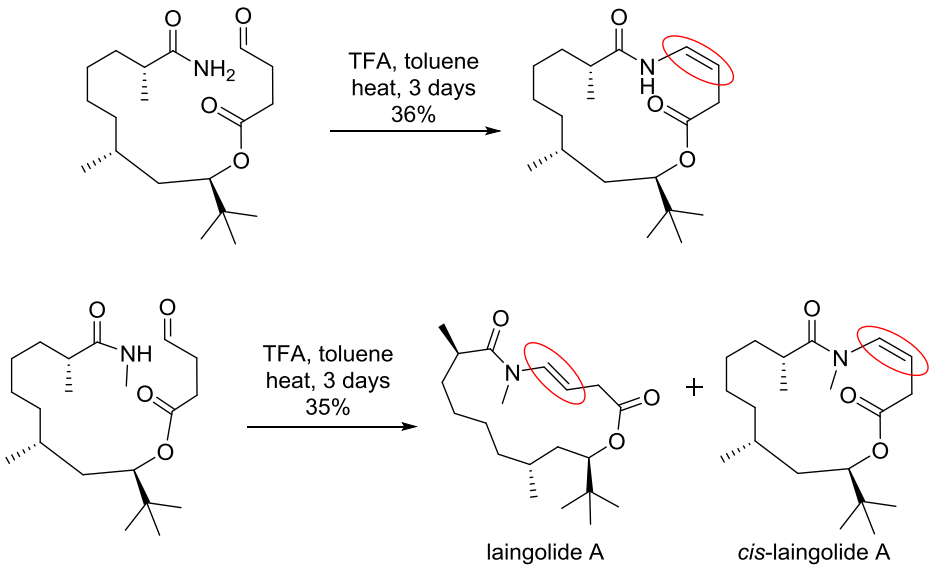


Figure 1.5: Energy profile for alternate possibility

The intramolecular hydrogen bond in the *cis* isomer **(54)**, is indicated by short O–H distance (2.27 Å). The reason for earlier observation of N–H proton shift (0.5 ppm) towards the deshielding region in the proton NMR spectrum of **54** could now be attributed to this intramolecular hydrogen bonding effect. The alternate possibility, involving the direct transfer of the proton from the nitrogen to olefin carbon was also explored and found to have a prohibitively high barrier of 64.9 kcal mol^{-1} (Figure 1.5). The possibility of an intermolecular pathway was also considered and found to be unfavourable. Moreover, the possibility of an explicit solvent molecule participating and influencing the barrier height was also considered, but the results indicate that the presence of the solvent molecule has no effect on the transition state. The height of the barrier provides an explanation as to why the *trans–cis* conversion occurs at higher

temperatures, but not at room temperature. To recheck and confirm the role of NH proton in mechanism, we subjected the (+)-palmyrolide A, having *N*-methyl, to similar heating conditions and observed that no isomerisation of enamide bond taking place in absence of NH. With all the above observations and results we proposed the possible mechanism for observed *trans* to *cis* isomerisation as shown in figure 1.4. After we documented all these results in our publication[17], there was a similar independent observation (*trans* to *cis* isomerization) reported by Phansavath and co workers during their synthesis of laingolide A,[31] a related macrocyclic natural product containing enamide moiety. In their synthesis, they used dehydrative cyclization of amide and aldehyde as tool to construct enamide macrocycle. They observed exclusively *cis* isomer formation when primary amides are employed (Scheme 1.15). When same reaction was repeated with secondary amides, formation of both *cis* and *trans* enamides was observed.



Scheme 1.15: Observations by Phansavath's group on enamide isomerization

In either conditions followed by us and Phansavath, it is expected that *trans*-enamide should form according to known literature reports.[15, 18, 25] But we observed some contrary results and based on them, it is possible to draw some useful conclusions as follows.

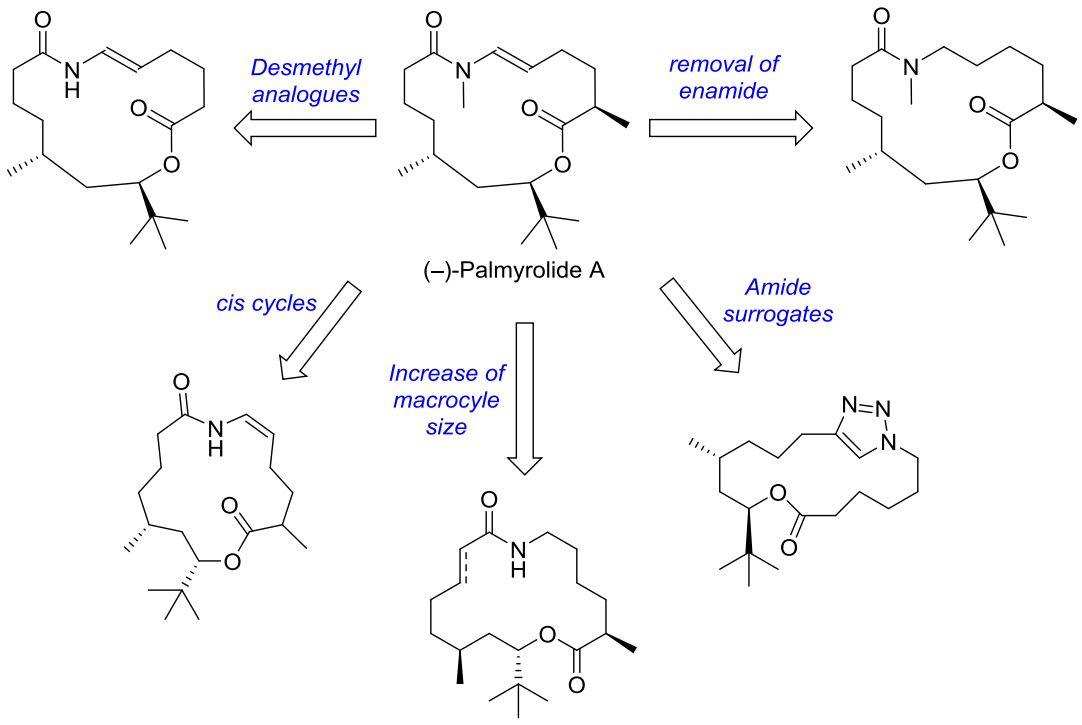
1. The formation of *cis*-enamide is independent of type of reaction, whether Buchwald coupling or dehydrative condensation.
2. Probably, in both reaction conditions first *trans*-enamide is formed which then isomerizes to *cis*-enamide.

3. The reactions were carried out at higher temperature and longer times which could be necessary for isomerization to take place.
4. When primary amide was employed, in both coupling conditions (Buchwald & dehydrative) initially exclusive *cis*-enamide formation was observed. (*Later we controlled the reaction temperature and time to get access to trans-enamide*)
5. The proton attached to amide (NH) is necessary for transformation (*trans* to *cis)* to take place as observed by us.

After successfully documenting the synthesis of (+)-palmyrolide A and *cis*-palmyrolide A along with studies on *trans* to *cis* isomerisation,[17] we next diverted our efforts towards design and synthesis of analogues around this interesting scaffold to identify potential sodium channel blockers based on palmyrolide macrocyclic scaffold.

1.3.2 Synthesis of analogues of palmyrolide A

When we planned new analogues on palmyrolide A macrocycle, we narrowed our focus into structure simplification and understanding of systematic SAR (Scheme 1.16).

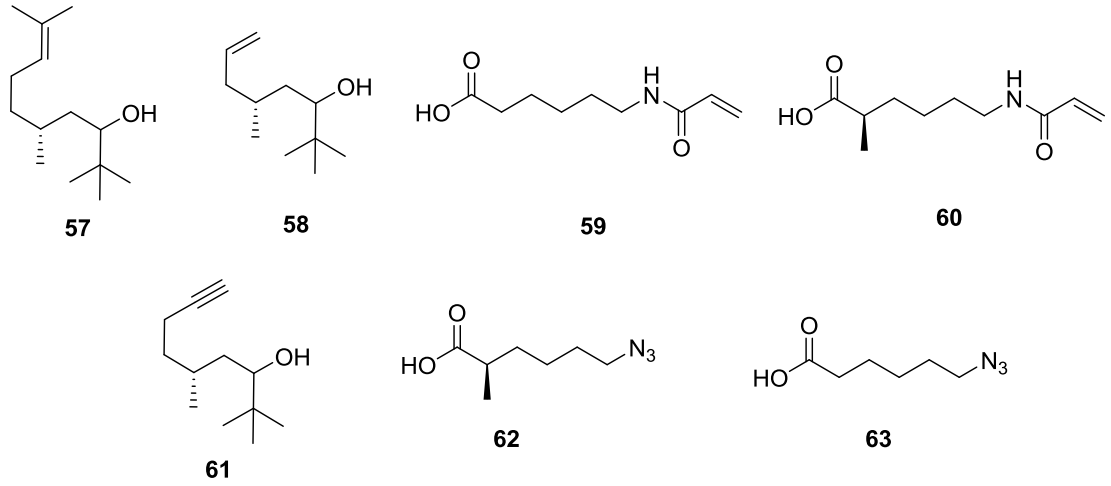


Scheme 1.16: Planned analogues over palmyrolide A macrocycle

Accordingly, we chose to prepare analogues based on following aspects (i) increase in macrocycle size, (ii) removal of enamide moiety, (iii) removal of C14 methyl group and (iv) introduction of amide surrogates. The access to *cis*-enamides encouraged us to initially plan for demethylated *cis* and *trans* palmyrolides. Apart from that, we wondered whether enamide bond would be really contributing towards activity.

1.3.2.1 Synthesis of key components towards preparation of analogues

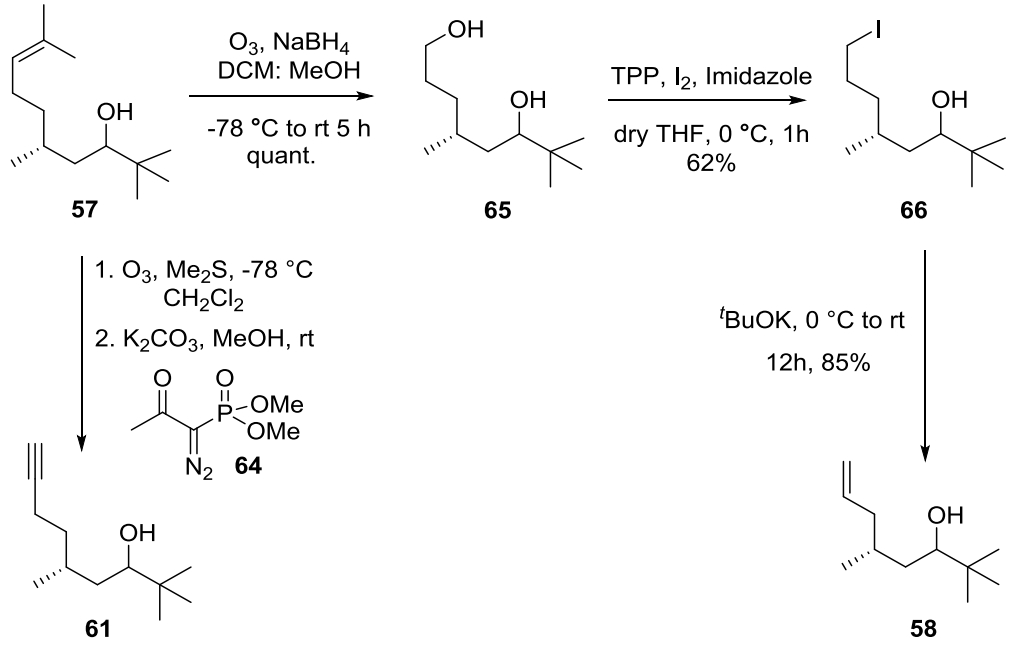
After careful analysis, we have planned fragments **57** to **63** as necessary fragments for synthesis of all analogues (Scheme 1.17).



Scheme 1.17: Required fragments for analogue synthesis

Acids **59**, **60** and **63** are the important fragments where the acid group in them has been exploited and coupled with different alcohols **57**, **58** & **61**. The fragment **58** is planned to mainly obtain fifteen membered analogues with or without C15 methyl group. Similarly to synthesize triazole analogues we require fragment **61**. The synthesis of fragments **57** and **63** started with *tert*-Butyl Grignard reaction on (*R*)-citronellal which gave alcohol **57**. Here, ozonolysis under different conditions gave either alcohol or aldehyde as products. After the initial purging of ozone gas at -78 °C, quenching of ozonide with dimethyl sulphide gave aldehyde, whereas addition of sodium borohydride ($NaBH_4$) resulted in diol intermediate **65**.[32] The aldehyde obtained was immediately subjected to Ohira-Bestman conditions[33] (K_2CO_3/MeOH) to obtain alkyne **61**. The formation of **61** was confirmed by disappearance of dimethyl and olefin signals and appearance of alkyne attached proton in ^{1}H NMR at δ 1.95 - 1.93 (m, 1H). The ^{13}C NMR also shows characteristic

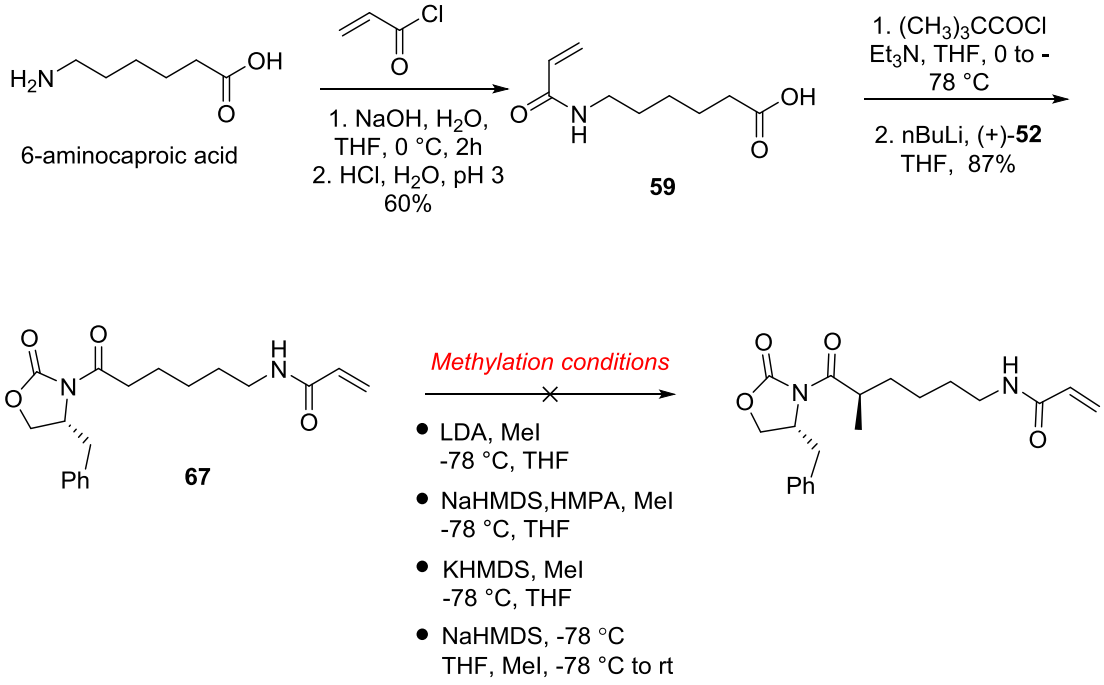
alkyne carbons at δ 84.8, 84.7 which further confirms its formation. On the other hand, the diol formation **65** was confirmed by similar disappearance of olefin and dimethyl peaks in ^{1}H NMR of **65** along with appearance of peak at δ 3.60 - 3.57 (m, 2H), which corresponds to -CH_2 protons attached to alcohol.



Scheme 1.18: Synthesis of fragment **58** and **61**

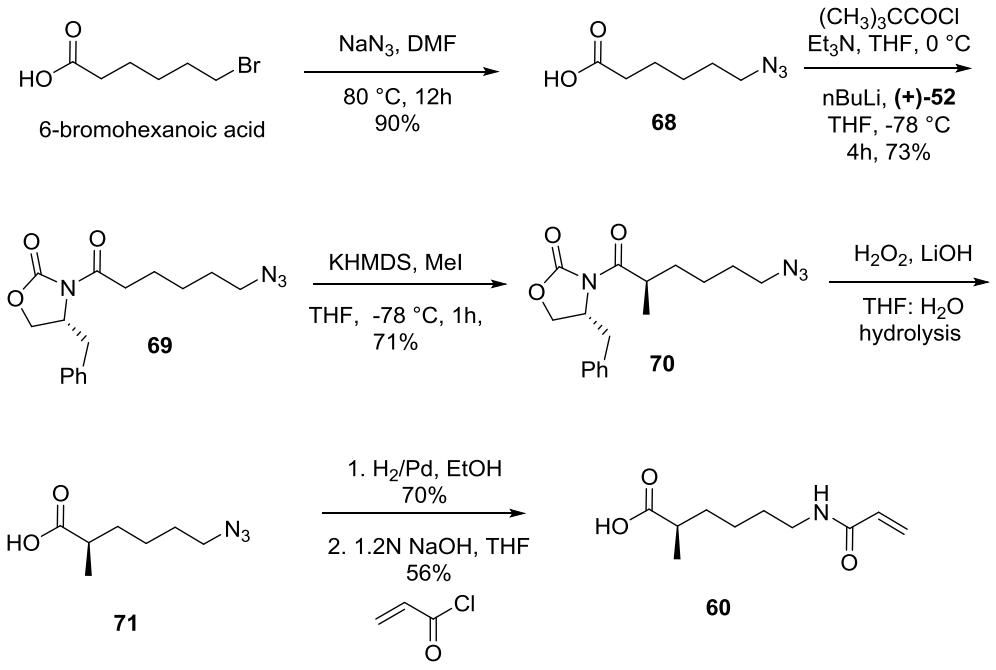
Compound **65** was subjected to iodination TPP/I_2 to get **66** which was then subjected to dehydrohalogenation[32] using potassium *tert*-butoxide to give alkenol **58** in 85% yield (Scheme 1.18). The formation of **58** is clearly evident in its ^{1}H NMR, where δ 5.85-5.67 (m, 1H), 5.04-4.94 (m, 2H) corresponds to the terminal olefin pattern. Due to low boiling nature of **58**, we did not characterise it completely and as such forwarded for further reactions. The compounds **59** and **60** were planned to be prepared from 6-aminocaproic acid, which on coupling with acroyl chloride followed by acidification gave desired acid fragment **59**. The appearance of terminal olefin pattern again in ^{1}H NMR of **59** and comparison with literature values,[34] confirmed the formation of **59**. Coupling of acid **59** with (*R*)-4-benzyl-2-oxazolidinone gave **67**, which was then subjected to methylation under various conditions to obtain **60** (Scheme 1.19). However, in spite of several efforts we always ended up with complex reaction mixtures and C2 methylated acid **60** could not be obtained (Scheme 1.19). Later we found in literature[35] that when auxiliary mediated methylations are performed on amines containing electron withdrawing groups, there is

formation of unwanted cyclised products. So, we revised our strategy and planned **60** *via* selective methylation on known azide **68**.



Scheme 1.19: Synthesis of olefinic acid **59**

Accordingly, we followed a reported synthetic sequence[35] where 6-bromo hexanoic acid was converted to its corresponding azide **68** in presence of NaN_3/DMF and then coupled with (*R*)-4-benzyl-2-oxazolidinone to get intermediate **69**. Stereoselective methylation on **69** went smoothly using KHMDS/MeI at – 78 °C in 1h to get **70** which on hydrolysis using $LiOH/H_2O_2$ gave azido-acid **71**. The formation of **71** was confirmed by its 1H NMR δ 3.28 (t, *J* = 6.7 Hz, 2H) corresponds to protons attached to azide and 1.19 (d, *J* = 6.9 Hz, 3 H) corresponds to newly formed methyl group. Further the specific rotation $[\alpha]_D^{25.0}$= – 12.5 obtained for **71** also exactly matches with the literature reports[35] thereby confirming the stereochemsitry of the methyl group on compound **71**. The azide in **71** was then subjected to hydrogenation to obtain amine, which was then coupled to acroyl chloride to obtain the fragment **60**. The formation of **60** was confirmed by appearance of characterstic α,β-unsaturated olefin and methyl peaks in ^{1}H NMR, δ 6.48 - 6.05 (m, 2H), 5.71-5.58 (m, 1H) corresponds to olefin protons and 1.16 (d, *J* = 6.9 Hz, 3 H) corresponds to methyl group. In ^{13}C NMR the appearance of olefin signals at δ 130.8, 126.5, and amide signals at δ 181.7, further confirmed the formation of **60** (Scheme 1.20).



Scheme 1.20: Synthesis of olefinic acid **60**

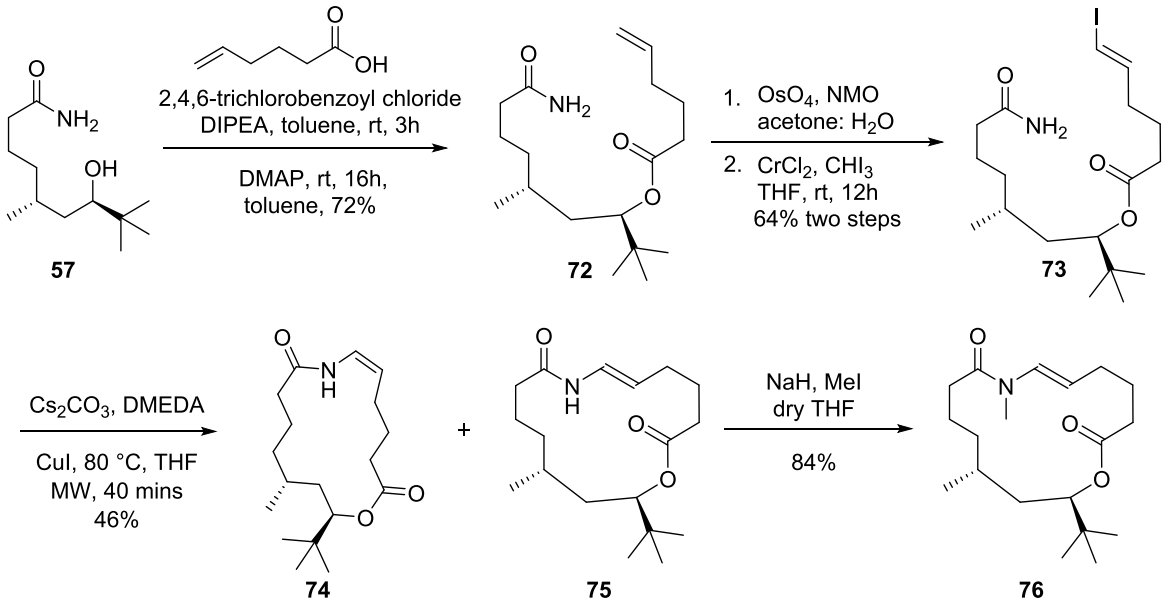
After synthesizing all the required fragments for analogues, we came across a new report published by Maio's group describing the structure activity relationship studies on palmyrolide A[36]. This publication provided valuable insights into the structural features contributing to voltage-gated sodium channel (VGSC) blocking activity.

According to that report,

1. Both the enantiomers of palmyrolide A showed similar activity.
2. The contribution of unstable enamide bond in blocking VGSC proved to be negligible
3. The relative *anti* stereochemistry between C5 and C7, probably, which gives a definite orientation to macrocycle was shown to be essential for activity.
4. Acyclic analogues were inactive

The report also indirectly suggests that the C15 methyl contribution to VGSC activity is not very significant. So, before synthesizing analogues with the planned fragments, we first thought we will access desmethyl *cis* and *trans*-palmyrolide A. The synthetic route developed by us earlier during total synthesis of palmyrolide A (Scheme 1.13 & 1.14) was amenable for analogue synthesis and accordingly we synthesized first three analogues of palmyrolide A as shown in scheme 1.21. The hydroxyl-amide (+)-**14** was coupled to 5-hexenoic acid using previously optimised Yamaguchi conditions to get

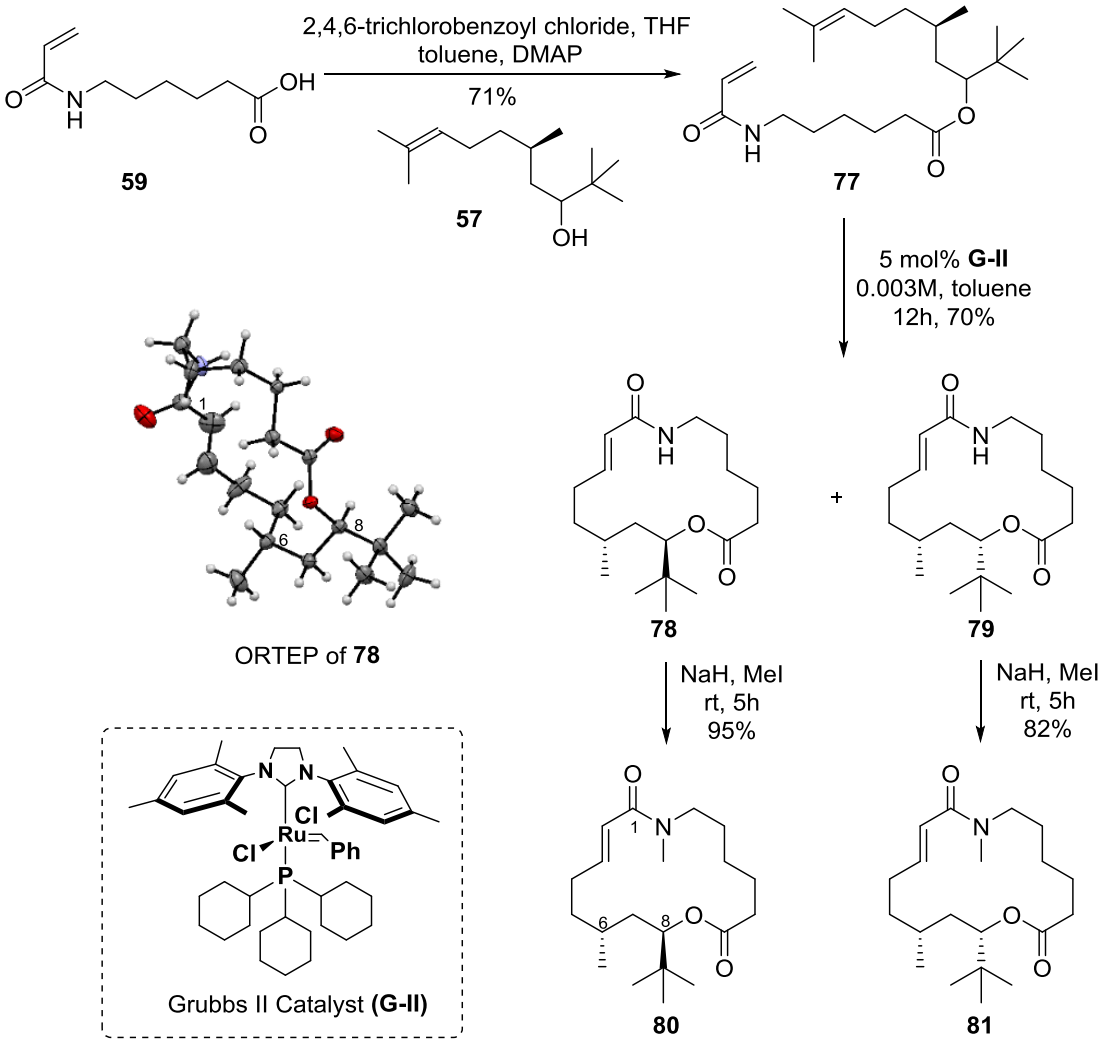
olefinic ester **72**. The characteristic peaks in ^{1}H NMR at δ 5.95 (1H), 5.72 (1H) corresponds to amide protons, peak at 5.89 - 5.77 (m, 1H) and 5.09-4.97 (m, 2H) correspond to terminal olefin peaks, whereas peak at 4.84-4.77 (m, 1H) correspond to proton adjacent to *tert*-butyl group. These observations confirm the formation of **72** in similar manner to **53**. The olefin in **72** was then subjected to oxidative cleavage using OsO_4/ $NaIO_4$, followed by Takai olefination to yield vinyl iodide intermediate **73**. Again the confirmation of **73** is achieved by comparing the ^{1}H and ^{13}C NMR spectra with that of **15**.



Scheme 1.21: Synthesis of desmethyl palmyrolide A analogues

For final macrocyclization, we slightly modified earlier conditions and employed microwave assisted Buchwald conditions (CuI/DMEDA/80°C/40 mins) to get **74** and **75**. Similar *trans* to *cis* transformations were again observed during this macrocyclization thereby giving us access to desired *cis* and *trans* macrocycles. The *cis* stereochemistry in **74** was confirmed by coupling constant observed in ^{1}HNMR with δ 6.86 (dd, *J* = 10.9, 8.9 Hz, 2H), 4.83 (d, *J* = 10.4 Hz, 1H). The macrocycle **75,** which exists as rotamers, was then methylated using NaH/ MeI to give desmethyl palmyrolide A, **76**. The formation of **76** could be confirmed by comparing the spectra with palmyrolide A. The ^{1}H NMR of **76** shows δ 6.62 (d, *J* = 13.7 Hz, 1H), 4.97 - 4.83 (m, 2H) corresponding to olefin and proton adjacent to *tert*-butyl group and δ 3.06 (s, 3H) corresponding to *N*-methyl protons which

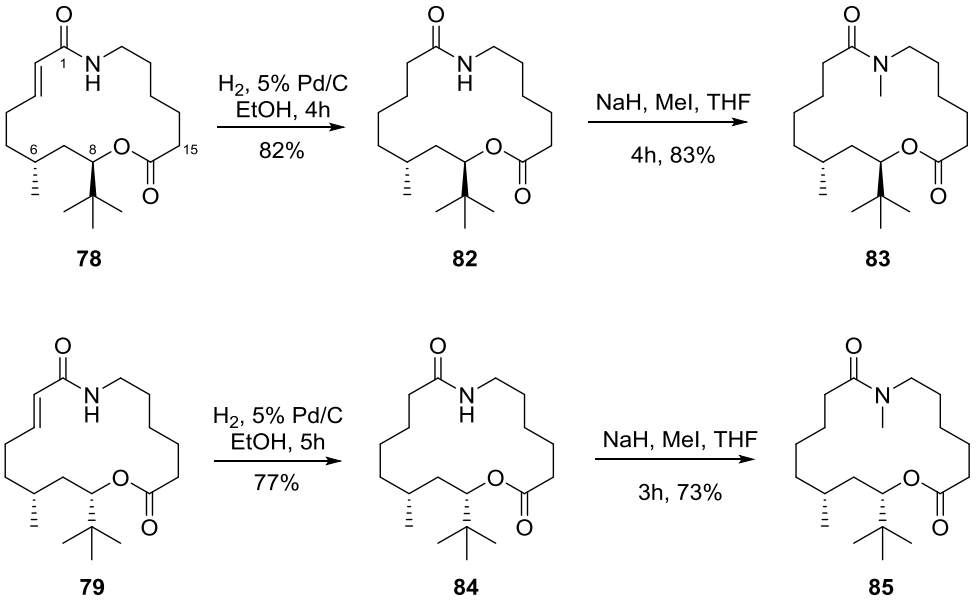
clearly confirms the formation of **76**. It is also confirmed by HRMS which showed peak at 346.2350 corresponding to molecular formula $C_{19}H_{33}O_3NNa$ $[M+Na]^+$ with calculated value 346.2353. We next focused on synthesis of macrocycles with an increase in size of ring. We designed a modified route, involving Yamaguchi coupling and ring closing methathesis (RCM) for synthesis of fifteen and sixteen membered analogues of palmyrolide A.



Scheme 1.22: Synthesis of sixteen membered palmyrolide A analogues

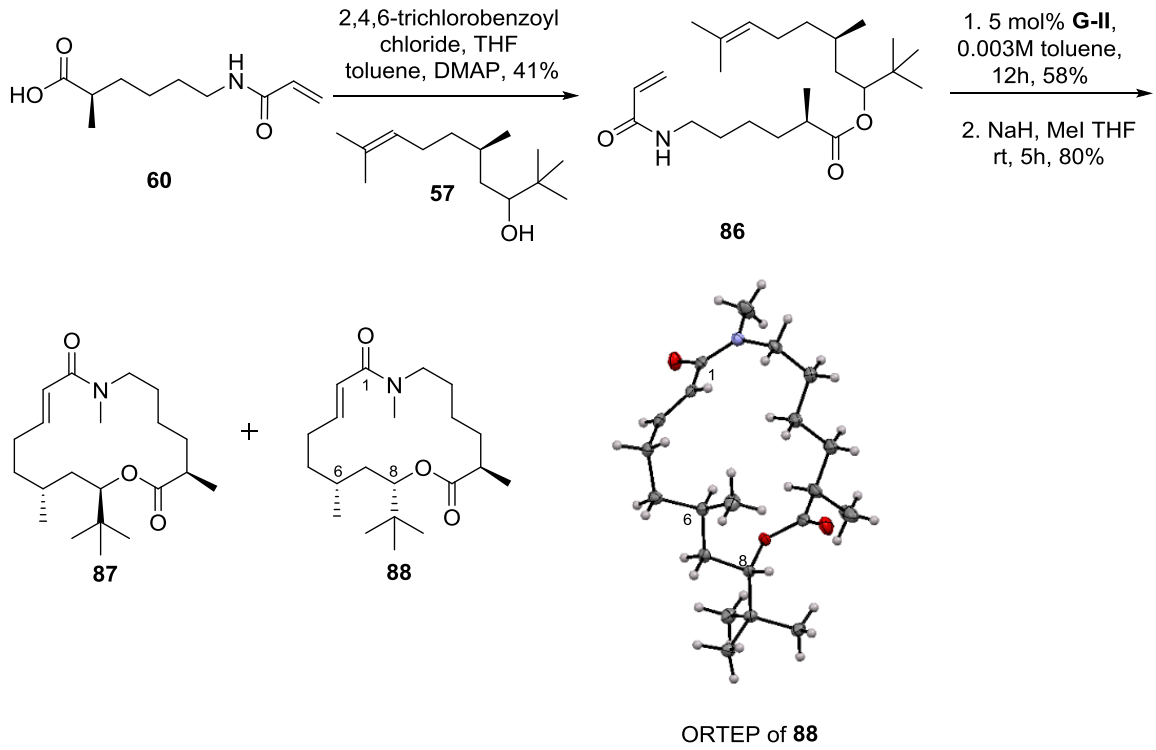
In our plan, we reasoned out that the increase in size of macrocycle may vary the structural features (macrocycle conformations) and thus could alter the factors contributing to the sodium channel blocking activity. Yamaguchi esterification of carboxylic acid **59** and alcohol **57** which gave **77** in 71% yield. The formation of **77** was confirmed by ^{1}H NMR where δ 6.34-6.01 (m,

2H), 5.64 (dd, *J* = 1.7, 9.9 Hz, 2H) corresponds to four olefin protons and 4.89-4.83 (m, 1H) corresponds to characteristic proton adjacent to *tert*-butyl group. The diastereomeric mixture of diene **77** was then subjected to RCM[37] at 0.003M dilution in toluene using Grubbs second generation catalyst (**G-II**) to yield desired macrocycles 7**8** and **79** in 70% yield (Scheme 1.22). The two diastereomers were separated using column chromatography and characterized using all spectral techniques. The disappearance of signals corresponding to four olefin protons and dimethyl groups along with appearance of two new olefinic proton signals δ 6.33 - 6.25 (m, 1H) and 5.81 (d, *J* = 16.1 Hz, 1H) in ^{1}H NMR of **78** clearly indicates that RCM has taken place and the desired macrocyle was formed.The geometry of newly formed double bond was assigned as *trans* based on proton coupling constants of olefin. Similar observation in ^{1}H NMR of **79** confirms its formation. However, at this stage we were not sure of the absolute stereochemistry of either macrocycle and it was difficult to assign with certainty using NMR spectroscopy due to flexible nature of ring. After a few efforts, we crystallized one of the compounds (**78**) in EtOAc/CH_2Cl_2 and the single crystal X-ray structure analysis of **78** revealed that the relative stereochemistry between methyl and tertiary butyl groups to be *anti*. As we know the absolute stereochemistry of C6 methyl group (coming from *R*-citronellal), we could assign the absolute stereochemistry of **78** as 6*R*,8*R*. This on corollary explained the relative stereochemistry of **79** to be *syn* and its absolute stereochemistry as 6*R*,8*S*. Then we planned to explore macrocycles **78** and **79** further to understand more about the structural features contributing to activity such as presence of unsaturation and *N*-Methyl group in macrolide. Thus, the macrocycles **78** and **79** were methylated using NaH/MeI to get two new macrocycles **80** and **81** respectively (Scheme 1.23). The appearance of methyl singlets at around δ 2.9 and disappearance of NH proton signals in ^{1}H NMR's of **80** and **81** indicates that methylation happened. Hydrogenation of compounds **78** and **79** using H_2/Pd in EtOH gave macrocycles **82** and **84** respectively, which on further methylations using MeI/NaH gave macrocycles **83** and **85** (Scheme 1.20). The macrolides **82** and **84** were confirmed with the disappearance of peaks corresponding to olefin protons. With access to eight new macrocycles, we next targeted C15 methylated sixteen membered analogues using similar strategy as followed above. Towards achieving these methylated macrocyles we subjected acid **60** and alcohol **57** to optimized Yamaguchi conditions, which resulted in a diastereomeric mixture of diene **86.**



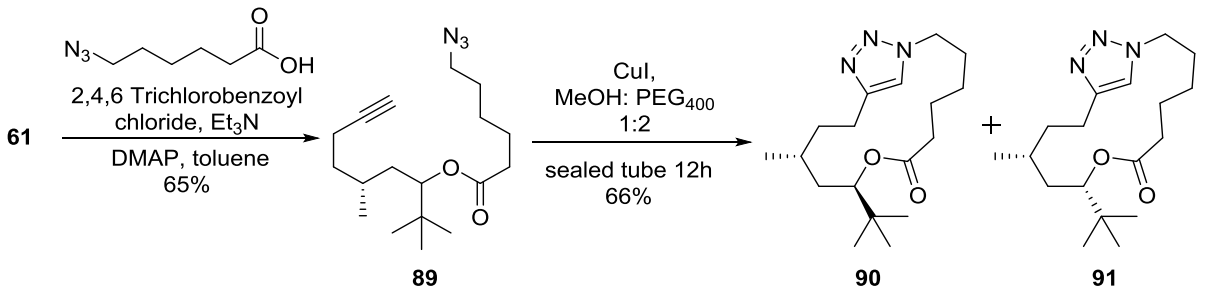
Scheme 1.23 Synthesis of saturated analogues

Here again the formation of diene is confirmed by characteristic appearance of four olefin protons δ 6.33 - 6.02 (m, 2H), 5.62 (dd, *J* = 1.8, 9.9 Hz, 1H), 5.09 - 5.06 (m, 1H) and proton adjacent to *tert*-butyl group 4.87 - 4.82 (m, 1H) in ^{1}H NMR, which is similar to that observed for diene **77**. Ring closing metathesis on diene **86** at high dilutions (0.003M) using **G-II**, followed by *N*-Methylation gave macrocycles **87** and **88**. Both the compounds are cleanly separated by column chromatography. The formation of compound **87** was confirmed by analyzing ^{1}HNMR which showed presence of only two olefin peaks at δ 6.76-6.69 (m, 1H), 6.25-6.21 (m, 1 H) and a proton adjacent to *tert*-butyl group at 4.91 (d, *J* = 11.0 Hz, 1H), along with methyl doublets at 1.15 and 0.93, and *N*-methyl at 2.95 (s, 3H). The ^{13}C NMR of **87** showed peaks corresponding to ester and amide at δ 175.0, 169.0 and two olefinic carbons at δ 141.6, 124.8 thus confirming macrocyle. It is further confirmed by HRMS which showed peak at 374.2670 corresponding to molecular formula $C_{21}H_{37}O_3NNa$ $[M+Na]^+$ with caluculated value 374.2666. The ultimate confirmation came when one of the compound (**88**) was crystallized in EtOAc/Hexanes. The single crystal X-ray structure obtained for **88** enabled us to assign the absolute configurations of methyl and *tert*-butyl groups as 6*R*,8*S*,*15R* and hence its diastereomer **87** has 6*R*,8*R*,*15R* configuration (Scheme 1.24). Compound **88** also showed similar ^{1}H and ^{13}C NMR pattern to that of **87**.



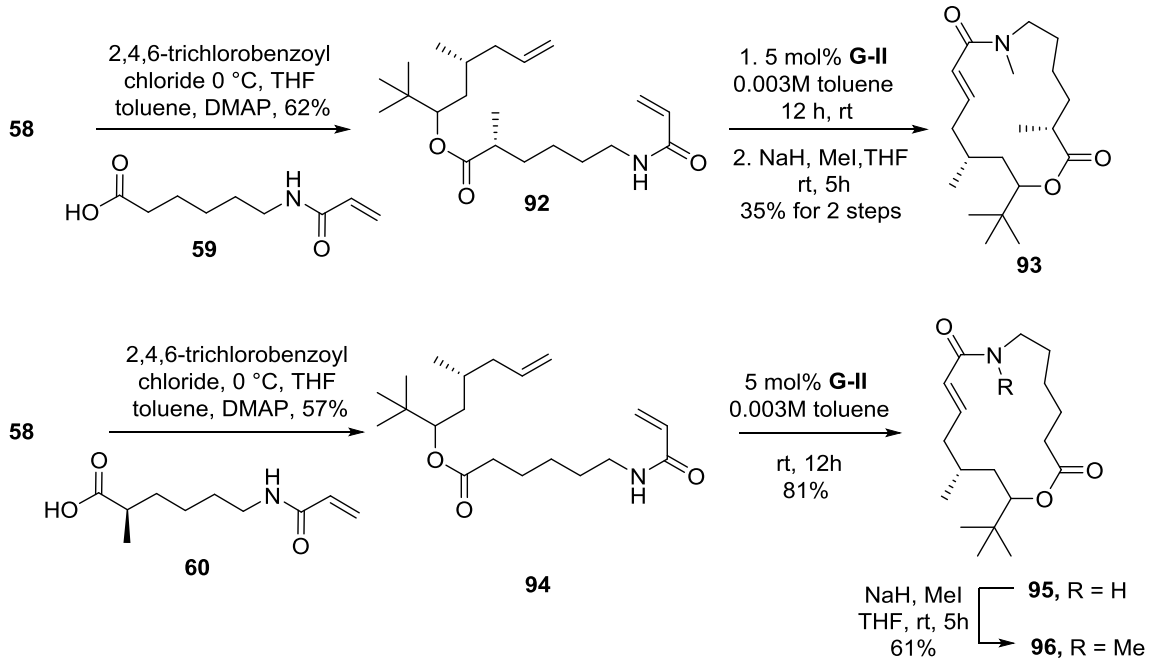
Scheme 1.24: Synthesis of palmyrolide A analogues

Next we turned our attention towards amide surrogates. The strategy of using five membered heterocycles as amide surrogates has been practiced in medicinal chemistry to improve the druggability of a lead compound.[38] Here, we planned triazole heterocycle as a replacement for enamide functionality. Compound **61** on Yamaguchi esterification with 6-azidohexanoic acid (**63**) gave the required precursor of click macrocyclization, **89** in 65% yield. Confirmation of **89** came from ^{1}H NMR where two characteristic peaks corresponding to alkyne proton and proton adjacent to *tert*-butyl group appeared at δ 1.91-1.89 (m, 1H) and δ 4.85-4.81 (m, 1H) respectively. The ^{13}C NMR also confirms the formation of **89** with characteristic peaks at δ 173.3, 173.1, 84.5. After few attempts, intramolecular click macroccyclization[39] on **89** proceeded smoothly in a sealed tube with catalytic amount of CuI in 1:2 MeOH:PEG400 at 80 °C resulting in desired triazole macrocycles **90** and **91** with an overall yield of 66% (Scheme 1.25). The two diastereomers are separated by column chromatography and were obtained in 2:1 diastereomeric ratio. However we could not crystallize either of the compounds and hence could not assign absolute stereochemistry of tertiary butyl group in **90** or **91**.



Scheme 1.25: Synthesis of triazole analogues

The triazole formation was confirmed by ^{1}H NMR with appearance of characteristic aromatic proton at δ 7.53 (s, 1 H). The ^{13}C NMR showed only one peak at δ 174.4 corresponding to ester which shows disappearance of amide carbonyl. In addition there are two new peaks at δ 146.3, 123.6 corresponding to olefin carbons which clearly indicate triazole formation. The triazole **90** was further confirmed by HRMS which showed peak at 322.2484 corresponding to molecular formula $C_{18}H_{32}O_2N_3$ $[M+H]^+$ with calculated value 322.2490. Similar characterization was done for **91** and confirmed its structure.



Scheme 1.26: Synthesis of fifteen membered palmyrolide A analogues

The simple strategy developed for analogue synthesis had enabled us to access macrocycles with much ease, so we thought to extend the strategy further to synthesize a few fifteen membered

analogues of palmyrolide A as well. Now the compounds **58** and **59** are subjected to a reaction sequence involving Yamaguchi coupling/RCM/*N*-Methylation to yield the desired macrocycles **93** as an inseparable mixture of diastereomers. The structural confirmation came from ^{1}H NMR which showed only two peaks corresponding to olefin protons at δ 6.75-6.34 (m, 1H), 6.24-6.14 (m, 1H) along with characteristic proton adjacent to *tert*-butyl group at 4.92-4.85 (m, 1 H). The methyl doublets at δ 1.16 and 0.94 and *N*-methyl at δ 3.01 also confirms the formation of macrocycle **93**. The formation of **93** was further confirmed with HRMS which showed peak at 338.2683 corresponding to molecular formula $C_{20}H_{36}O_3N$ $[M+H]^+$ with calculated value at 338.2690. Following the same synthetic operations, macrocycles **95** and **96** were prepared as a mixture of diastereomers starting from alcohol **58** and carboxylic acid **60** (Scheme 1.26). As we could not separate the diastereomers of **93**, **95** and **96** despite a few efforts, we planned to screen the macrocycles as such for sodium channel blocking ability.

After the synthesis analogues, we next carried out biological evaluation of all the synthesized compounds and studied their structure activity relationships. Towards that we took help from Prof. Thomas Murray and Dr. Suneet Mehrotra of Creigton University, who have analyzed compounds for inhibition of veratridine induced sodium influx.

1.3.3 Biological evaluation of palmyrolide analogues towards blocking Voltage Gated Sodium Channels

As discussed earlier voltage-gated sodium channels are critical elements of action potential initiation and propagation in excitable cells because they are responsible for the initial depolarization of the membrane. When the cell membrane is depolarized by a few millivolts, sodium channels activate and inactivate within milliseconds. Influx of sodium ions through the integral membrane proteins comprising the channel depolarizes the membrane further and initiates the rising phase of the action potential.[40] To detect an interaction of (–)-palmyrolide A and its analogues with VGSCs, we examined the ability of these compounds to antagonize veratridine-stimulated Na^+ influx in murine primary neuronal cultures. Veratridine is a partial agonist at neurotoxin site 2 on the VGSC α subunit. Murine cerebrocortical neurons were loaded with the Na^+-binding benzofuran isophthalate (SBFI) dye and tested for the ability of (–)-palmyrolide A and its analogues to block veratridine-induced elevation of neuronal

$[Na^+]_I$.[41,42]All the neuronal cultures were exposed to veratridine (VRT) at a 3µM concentration which increases sodium influx as reflected in the increase in SBFI fluorescence ratios (340:380).

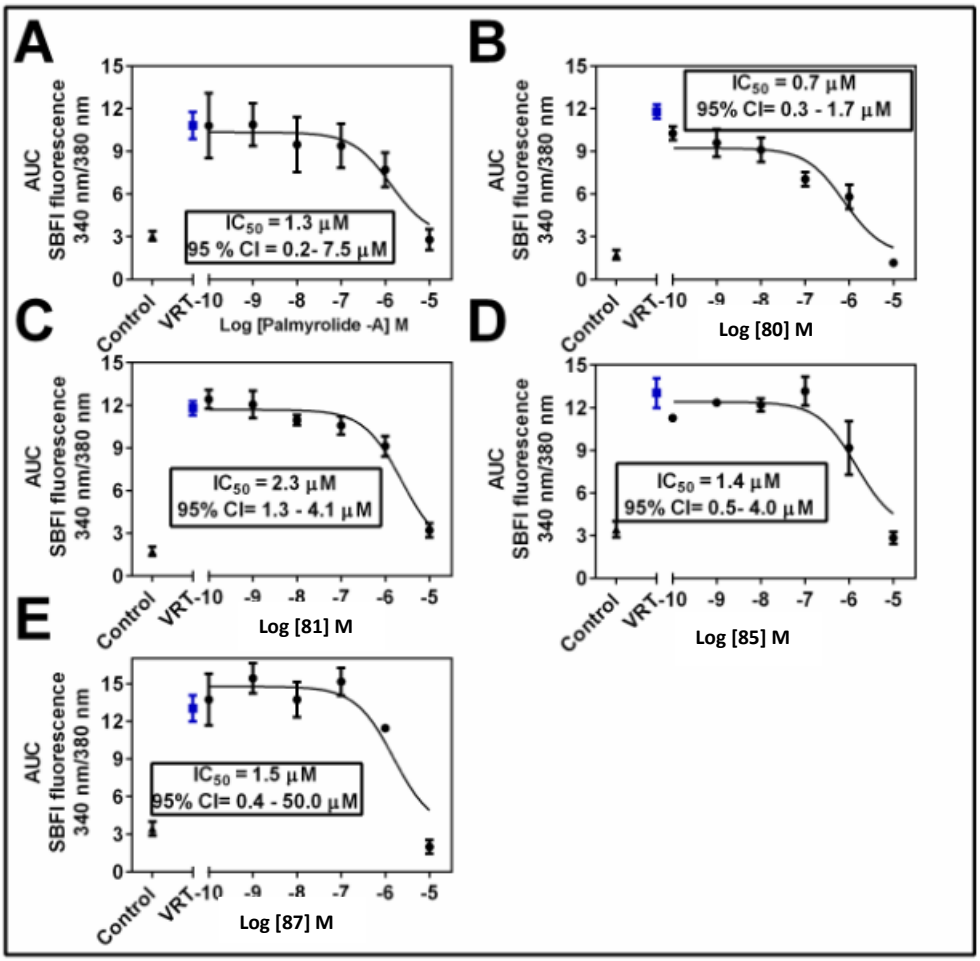


Figure 1.6: Concentration-response analysis graphs of (–)-palmyrolide A and four active analogues where sodium influx was monitored with SBFI (340/380) responses to veratridine in the absence and presence of compounds. The compounds shown are (A) (–)-palmyrolide A (**1**); (B) (14*R*,16*R*,E)-16-(*tert*-butyl)-8,14-dimethyl-1-oxa-8-azacyclohexadec-10-ene-2,9-dione (**80**); (C) (14*R*,16*S*,E)-16-(*tert*-butyl)-8,14-dimethyl-1-oxa-8-azacyclohexadec-10-ene-2,9-dione (**81**); (D) (14*R*,16*S*)-16-(*tert*-butyl)-8,14-dimethyl-1-oxa-8-azacyclohexadecane-2,9-dione (**85**); (E) (3*R*,14*R*,16*R*,E)-16-(*tert*-butyl)-3,8,14-trimethyl-1-oxa-8-azacyclohexadec-10-ene-2,9-dione(**87**). The effect of different concentrations (0.1 – 10,000 nM) of palmyrolide A and analogues on VRT stimulated sodium influx was determined and nonlinear regression analysis of resulting concentration-response data was performed (detailed graphs in experimental section).

Table 1.2: Estimated IC_{50} values (with 95% CIs) for (–)-palmyrolide A and its analogues. Values represent mean of 3-4 representative experiments performed each with 2-3 replicates. *NA= no activity at a test concentration of 10μM.

Compound	IC_{50} (μM)	95% CI (μM)	% Maximum effect
Palmyrolide A,**(–)-1**	1.3	0.2-7.5	1.00
96	17.3	5.3-56	0.5
76	NA*	NA*	NA*
93	5.5	1.8-15	0.8
81	2.3	1.3-4.1	1.1
79	NA*	NA*	NA*
80	0.7	0.3-1.7	1.1
87	1.5	0.4-50	1.4
85	1.4	0.5-4	1.04
74	NA*	NA*	NA*
91	NA*	NA*	NA*
88	5.0	1.5-15	1.04
55	6.0	1.7-20	0.92
90	7.9	2.8-22	1.09
75	NA*	NA*	NA*
95	NA*	NA*	NA*
83	3.0	0.3-26	0.87
78	5.4	0.3-37	0.54
82	3.3	0.3-34	0.54
84	6.7	1.3-35	0.47

The graphs A to E in figure 1.6 shows plot of SBFI fluorescence vs concentration of given compound. When there is high sodium influx, we observe high SBFI fluorescence and upon addition of (–)-palmyrolide A the graphs shows decrease in SBFI indicating inhibition of sodium influx. From this analysis (–)-palmyrolide A was shown to have an IC_{50} of 1.3μM with

95% confidence interval of 0.2-7.5 µM. Similar analysis of all synthesized analogues were performed and corresponding IC_{50} values calculated and shown in table 1.2. The detailed graphs of some active compounds (**80**, **81**, **85**, **87**) were shown in figure 1.6 where a 3-parameter logistic fit of the palmyrolide A analogues concentration-response inhibition of veratridine is shown for each compound tested which were repeated in 3-4 experiments.

From IC_{50} values of all the analogues synthesized, it has been observed that compound 8**0** showed a two-fold more potent inhibition than natural palmyrolide A with an IC_{50} of 0.7µM whereas compounds **74**, **75**, **76**, **79**, **91** and **95** showed no inhibition of veratridine-stimulated Na^+ influx. On further analysis two new compounds with sixteen membered skeletons **85** (1.4 µM) and **87** (1.5 µM) showed comparable activities with respect to (–)-palmyrolide A. The values obtained for all the synthesized analogues have been tabulated and systematic structure activity relationship studies were carried out.

1.3.4 Structure activity relationship studies

The values obtained for all the synthesized analogues have been tabulated and systematic structure activity relationship studies were carried out. The IC_{50} values obtained for palmyrolide A analogues were promising and permitted us to draw some valuable conclusions. Our objective of simplifying the structure had yielded encouraging results with compound **80** being the most potent compound (IC_{50}= 0.7 μM), which is indeed somewhat more potent than the natural product (–)-palmyrolide A (IC_{50}= 1.3 μM). As expected from previous report,[21] the enamide bond contribution to activity was negligible, which can be seen while comparing activity of (–)-palmyrolide A with analogues **80**, **85** and **87**. All these compounds do not have an enamide moiety, but they showed activity comparable to natural product. In case of fifteen membered macrocycles, the removal of C14 methyl group in palmyrolide A had been detrimental to activity as there was no activity observed in cases of **74**, **75** and **76**. Similar observations could be made when comparing the activities of fifteen membered analogues **93**, **95** and **96**. Compound **93** showed 5.5 μM activity whereas compound **95**, having neither C14 methyl nor *N*-methyl groups showed no activity at all. On the other hand compound **96**, deprived of C14 methyl showed a three-fold decrease in activity compared to **93** again highlighting the importance of this methyl group. Compounds **90** and **91**, containing triazole moieties did not prove to be very useful as there is a five-fold decrease in activity compared to palmyrolide A.

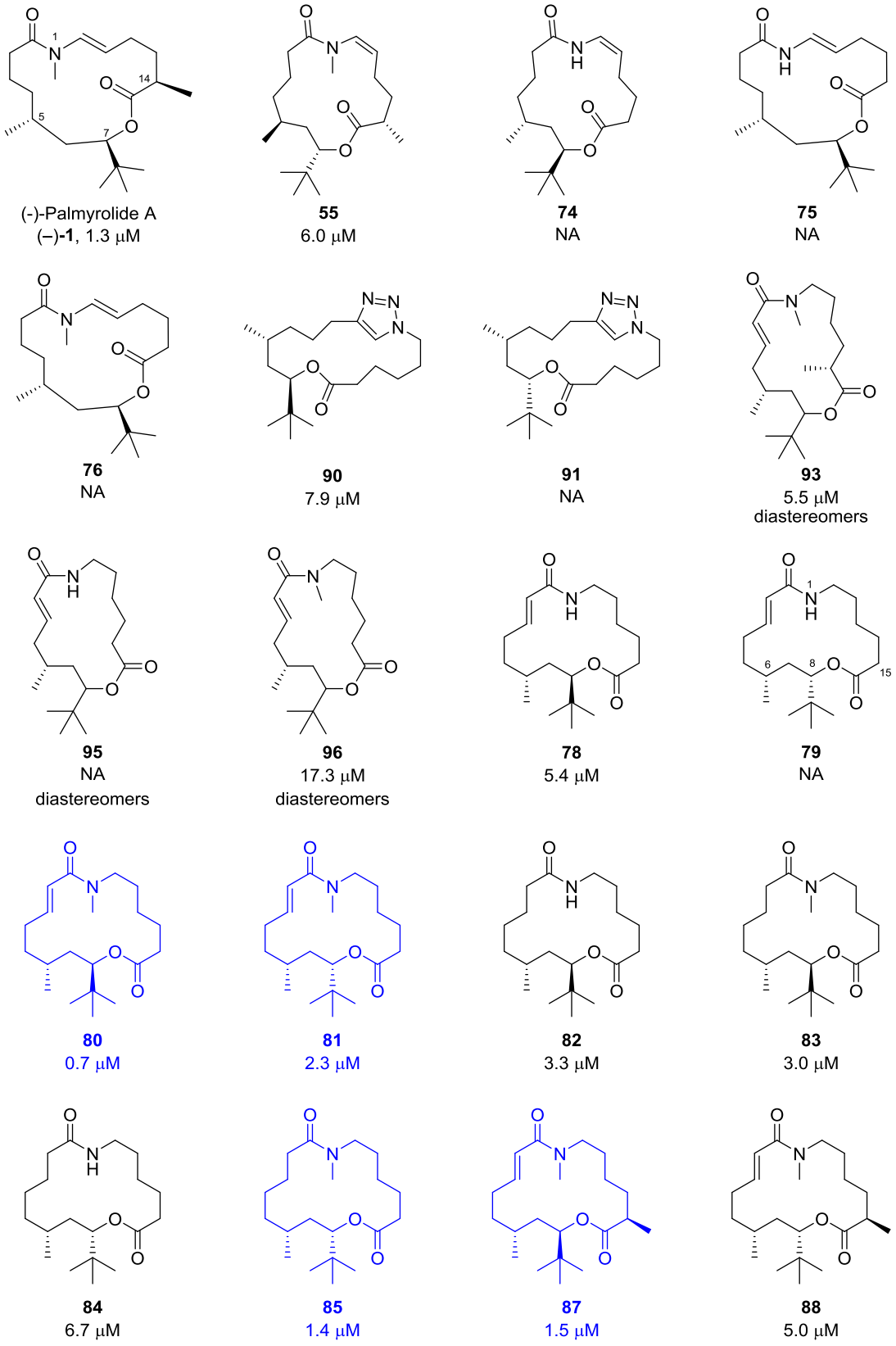


Figure 1.7: IC_{50} values for all synthesized analogues of palmyrolide A

Our strategy of increasing the size of macrocycle had been proven to be beneficial with most of the sixteen membered macrocycles synthesized showing better activity compared to corresponding fifteen membered macrocycles (which is palmyrolide A ring size).

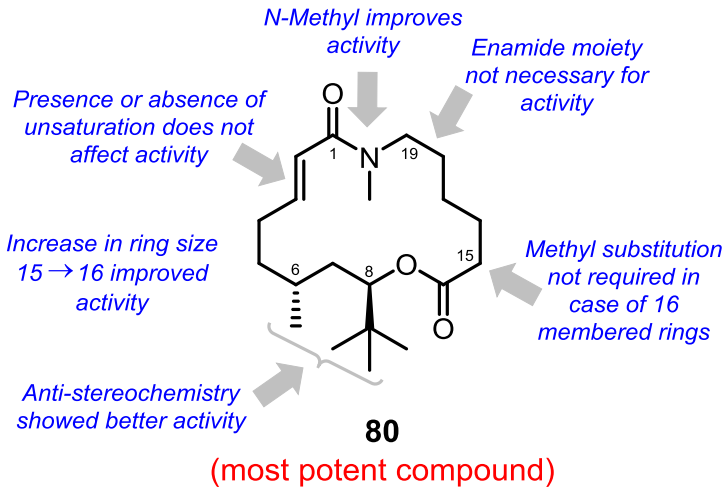


Figure 1.8: Summary of structure activity relationship studies

Moving from fifteen to sixteen membered macrocycles, keeping the methyl and *tert*-butyl groups intact, striking improvement in activity was observed. Firstly, in case of sixteen membered rings the contribution of C15 methyl group to activity was negligible, which is evident from comparisons of palmyrolide A (1.3 μM) with compounds **80** (0.7 μM), **87** (1.5 μM), and **85** (1.4 μM). However, the presence of *N*-methyl group is important for VGSC inhibitory activity as the compounds **80**, **81**, **83**, **85** showed better activities compared to either of **78**, **79**, 8**2**, **84**. The presence or absence of unsaturation in macrocycle did not influence the activity of macrocycle. On further analysis of all analogues synthesized by us and those previously by Maio's group we could propose that *anti*-stereochemistry between methyl and *tert*-butyl groups could be contributing significantly to VGSC inhibition. The compounds **78**, **80**, **83**, **82** and **87**, all having *anti*- relation between C6 methyl and C8 *tert*-butyl groups showed $IC_{50} < 5$ μM, with most potent compound being **80** with an IC_{50} of 0.7 μM (two fold better active than (–)-palmyrolide A. All the SAR findings are captured in Figure 1.8 for a ready reference.

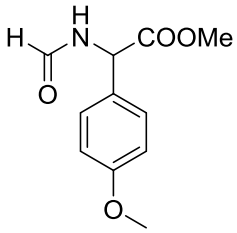
1.4 Conclusions

We have achieved the total synthesis of (+)-palmyrolide A and its *cis* isomer using an interesting Zhu's homologation strategy in total of nine steps. The key fragment

(challenging and significant portion) of palmyrolide A has been prepared in gram scale with all possible enantiomers. During the synthetic endeavour we observed a new *trans* to *cis* isomerisation in enamide macrocyle taking place and studied it in detail using DFT studies. The synthesis of palmyrolide A was further extended to access a series of macrocycles containing fifteen and sixteen membered rings. The analogue synthesis was planned and executed on the concept of structure simplification and ease of synthesis, and accordingly a new simple and scalable approach involving Yamaguchi esterification and ring closing metathesis as key steps was followed. We have made 19 analogues and all of them were screened for their ability to antagonize veratridine-stimulated Na+ influx in murine primary neuronal cultures. Out of them, four compounds were found to be of comparable potency to the natural product and compound **80** was the most potent with an IC_{50} of 0.7 μM. We also analyzed the structure-activity relationships of all compounds and characterized the essential elements for blockade of voltage-gated sodium channels based on palmyrolide scaffold. Optimization of present series can lead to druggable candidates which may ultimately be useful for treating various disorders influenced by voltage-gated sodium channels.

1.5 Experimental procedures

Methyl 2-formamido-2-(4-methoxyphenyl)acetate (45)



A mixture of sodium cyanide (3.6 g, 73.45 mmol) and solid ammonium chloride (3.9 g, 73.45 mmol) were added with 35 mL of water and stirred for ten minutes at room temperature. A solution of 4-methoxy anisaldehyde (10 g, 73.45 mmol) in methanol (35 mL) was then added to above reaction mixture and allowed to stir for 24 hours. Water (20 mL) was added and then excess methanol was evaporated under reduced pressure. The aqueous layer thus obtained was added with CH_2Cl_2 (50 mL) and extracted twice. The combined organic layer was washed with

brine (20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product obtained was redissolved in 6N HCl (50 mL) and refluxed for two days. The reaction mixture was the evaporated to dryness and white solid obtained is treated with 1:1 Isopropanol/water (~70 mL). The precipitated ammonium chloride was filtered off and the filtrate thus obtained was concentrated in vacuo to obtain crude Amino(carboxy)(4-methoxyphenyl)methanide (**43**).

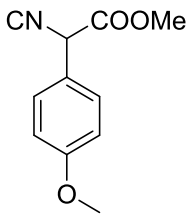
- Sodium cyanide is to be handled carefully with full protection to hands, body and face.
- The reaction is to be carried only in fume hood.
- The aqueous layer was carefully transferred to tub containing saturated solution of $KMnO_4$ which quenches the traces of sodium cyanide if left any.

The crude compound **43** (11g, 0.06 mmol) was taken in methanol (90 mL) and added with thionly chloride (9.2 mL, 0.012 mmol) dropwise at 0 °C over a period of 15 minutes. The reaction was allowed to stir at room temperature for 10h. Excess thionyl chloride was removed *in vacuo* and the obtained salt of methyl 2-amino-2-(4-methoxyphenyl)acetate **44** (6.6 g) was subjected to *N*-Formylation.

To a stirred solution of the above obtained amino ester hydrochloride salt (6.6 g, 28.49 mmol) in dry acetonitrile (30 mL) was added anhydrous ammonium formate (3.8 g, 59.82 mmol). The resulting reaction mixture was refluxed for 12 h. The solvent was then evaporated and the crude mixture was diluted with water (50 mL), extracted with ethyl acetate (2 x 20 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography over silica gel (30-40% EtOAc/hexanes) afforded methyl 2-formamido-2-(4-methoxyphenyl)acetate (**45**) as a orange solid (2.6 g, 16% for three steps).

^{1}H NMR (200 MHz, $CDCl_3$) δ 8.37 (1H, s), 7.44 (2H, d, *J* = 8.7 Hz), 7.04 (2H, d, *J* = 8.7 Hz), 6.77 (1H, brd, *J* = 7.0 Hz), 5.75 (1H, d, *J* = 7.0 Hz), 3.89 (3H, s), 3.95 (3H, s).

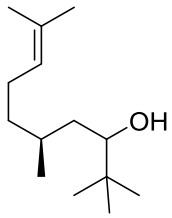
Methyl 2-isocyano-2-(4-methoxyphenyl)acetate (46)



To a solution of the formamide **45** (3.7 g, 16.5 mmol, 1.0 eq.) and triethylamine (11.5 mL, 82.87 mmol) in dry THF (50 mL) at -78 °C under argon atmosphere was added dropwise phosphoryl chloride (1.91 mL, 19.89 mmol). Stirring was continuted for 1h at 0 °C. The reaction mixture was added with cold water (50.0 mL) and extracted with ethyl acetate (2 x 30 mL). The combined organic layer was washed with water (2 × 30 mL), dried with anhydrous sodium sulphate and evaporated under vaccum. Purification by flash chromatography over silica gel (10-15% EtOAc/hexanes) afforded 2-isocyano-2-(4-methoxyphenyl)acetate **(46)** as oil (1.96 g, 58 %).

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.39 (2H, d, *J* = 6.4 Hz), 6.94 (2H, d, *J* = 6.4 Hz), 5.31 (1H, s), 3.82 (3H, s), 3.79 (3H, s).

(5*S*)-2,2,5,9-Tetramethyldec-8-en-3-ol (48)



tert-Butylmagnesium chloride (2.0 M in THF 12.9 mL, 25.97 mmol) was added dropwise to a solution of commercial *S*-citronellal (2.00 g, 12.98 mmol) in dry THF (20 mL) at –78 °C. The reaction mixture was stirred at same temperature for 2 h. The reaction was warmed to 0 °C and saturated aqueous ammonium chloride (50 mL) was added slowly. The aqueous layer was extracted with EtOAc (3 x 20 mL).The combined organic extracts were washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography over silica gel (1-5% EtOAc/hexanes) to afford alcohol **3** (1.83 g, 68% yield, ~ 1:1 diasteromeric mixture) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.10 (t, *J* = 7.0 Hz, 1 H), 3.28 (d, *J* = 12.0 Hz, 1 H), 2.08 -1.85 (m, 2 H), 1.67 (s, 3 H), 1.60 (s, 3 H), 1.51 -1.43 (m, 0.58 H), 1.36 -1.17 (m, 4 H), 1.09 - 1.00 (m, 0.58 H), 0.94 (d, *J* = 6.7 Hz, 1.5 H), 0.89 (d, overlapped, 1.5 H), 0.88 (s, 9 H);
^{13}C NMR(400 MHz, $CDCl_3$) δ 131.3, 125.0, 124.9, 77.7, 77.3, 39.4, 39.0, 38.5, 35.7, 35.0, 34.9, 29.8, 29.3, 25.9, 25.8, 25.7, 25.7, 25.4, 21.0, 18.9, 17.8;

IRυ_{max}(film) 3349, 2964, 2874, 1670, 1512 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{14}H_{29}O$ $[M+H]^+$ 213.2213, found 213.2211.

(5*S*,7*S*)-7-Hydroxy-5,8,8-trimethylnonanamide (–)-14 and (5*S*,7*R*)-7-hydroxy-5,8,8-trimethylnonanamide (+)-14a'

This reaction was carried out in three steps where step 1 and 2 are carried out simultaneously.

Step 1:A stream of ozone was bubbled through a cold (–78 °C) solution of the alcohol **48** (0.3 g, 1.41 mmol) in CH_2Cl_2 (10 mL) until the distinctive blue color of ozone was clearly observed. Ozonolysis was then terminated, and excess ozone was displaced by passing a stream of nitrogen through the solution for 5–10 min, and then neat Me_2S (0.2 mL, 2.82 mmol) was added dropwise. The resulting reaction mixture was allowed to warm to room temperature and stirred for 8 h. The reaction mixture was concentrated *in vacuo.* The crude product obtained was filtered by short silica gel column chromatography to get aldehyde (290 mg) as colorless oil. It was forwarded for next step without taking any extensive characterization.

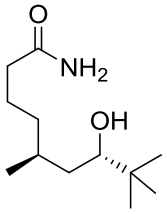
IRυ_{max}(film) 3446,2954, 1723,1458 cm^{-1}.

Step 2: The homologation reagent 2-isocyano-2-(4-methoxyphenyl) acetate **(47)** was freshly prepared before reaction. The ester **46** (0.45 g, 2.19 mmol) in THF (20 mL) and water (5 mL) was added potassium hydroxide (820 mg, 14.6 mmol) and the resulting mixture was stirred at room temperature for 5 h. The solvent was then removed in vacuo and the resulting salt **47** (0.48 g) was used as such without further purification.

Step 3: Dimethylamine hydrochloride (137 mg, 1.69 mmol) was added to a solution of above aldehyde taken in dry toluene (2 mL) and stirred at room temperature for 10 minutes. The resultant suspension was transferred via syringe to a solution of freshly prepared potassium 2-isocyano-2-(4-methoxyphenyl) acetate **47** (485 mg, 2.12 mmol) and dimethylamine hydrochloride (137 mg, 1.69 mmol) in dry toluene (6.0 mL). Then it was allowed to stir at room temperature for 24 h. The reaction mixture was then evaporated to dryness. The crude product obtained was dissolved in EtOAc (5 mL), washed with water (3.0 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The resulting light yellow oil was dissolved in THF (4 mL), treated with 1N HCl (10.6 mL, 7.05 mmol), and the reaction mixture was allowed to stir at room temperature for 5 h. The reaction mixture was basified with aqueous saturated $NaHCO_3$ (about 15 mL, pH~10), and extracted with

EtOAc (3 x 20 mL). The combined organic extracts were washed with brine (5.0 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* and subjected to flash chromatography over silica gel (1.5% MeOH/CH_2Cl_2) afforded two separable alcohols (–)-**14** (85 mg, 28%) as a colorless oil and (+)-**14** (80mg, 26% yield) as a white solid respectively.

Data for compound (–)-**14**,



Specific rotation $[\alpha]_D^{25.3}$= – 41.5 (*c* = 1.0, $CHCl_3$);

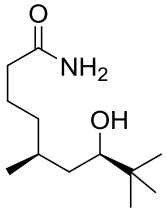
1H NMR (400 MHz, $CDCl_3$) δ 5.90 (bs, 1 H), 5.79 (bs, 1 H), 3.26 (dd, *J* = 1.7, 10.3 Hz, 1 H), 2.20 (t, *J* = 7.2 Hz, 2 H), 1.78 - 1.46 (m, 5 H), 1.36 - 1.29 (m, 1 H), 1.25 - 1.18 (m, 1 H), 0.99-1.08 (m, 1 H), 0.92 (d, *J* = 6.5 Hz, 3 H), 0.86 (s, 9 H);

^{13}C NMR(100 MHz, $CDCl_3$) δ 176.3, 77.4, 39.1, 36.0, 35.0, 34.6, 29.4, 26.0, 23.0, 21.0;

IR υ_{max}(film): 3353, 3196, 2953, 1667, 1463, 1403 cm^{-1};

HRMS (ESI):*m*/*z* calculated for $C_{12}H_{25}NO_2Na$ $[M+Na]^+$238.1778, found 238.1774.

Data for compound (+)-**14a**,



Melting point = 95 - 97°C;

Specific rotation $[\alpha]_D^{26.5}$ = + 26.9 (*c* = 0.43, $CHCl_3$);

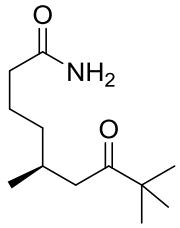
1H NMR (400 MHz, $CDCl_3$) δ 5.61 (bs, 2 H), 3.28 (dd, *J* = 2.0, 10.5 Hz,1 H), 2.22 (dt, *J* = 2.0, 8.0 Hz, 2 H), 1.72 -1.63 (m, 3 H), 1.35 -1.15 (m, 5 H), 0.90 (d, overlapped, 3 H), 0.88 (s, 9 H);

^{13}C NMR(100 MHz, $CDCl_3$) δ 176.03, 77.49, 38.7, 37.8, 36.1, 35.0, 29.4, 25.8, 23.0, 19.1;

IR υ_{max}(film): 3356, 2953, 1655, 1512, 1459, 1406 cm^{-1};

HRMS (ESI):*m*/*z* calculated for $C_{12}H_{26}NO_2$ $[M+H]^+$ 216.1958, found 216.1953

(*S*)-5,8,8-Trimethyl-7-oxononanamide (49)



A solution of alcohol (+)-**14a** (50.0 mg, 0.24mmol) in dry CH_2Cl_2 (5 mL) at 0 °C was treated with PCC (80 mg, 0.37 mmol) and stirred at room temperature for 2 h. The reaction mixture was filtered through celite pad, and the pad was washed thoroughly with diethyl ether (15 mL). Combined washings were concentrated *in vacuo*. Purification by flash chromatography over silica gel (50-80% EtOAc/hexanes) afforded keto compound **49** (26.0 mg, 53% yield) as white solid.

Melting Point = 64-67 °C;

Specific rotation $[\alpha]_D^{25.0}$= – 4.6 (*c* = 0.35, $CHCl_3$);

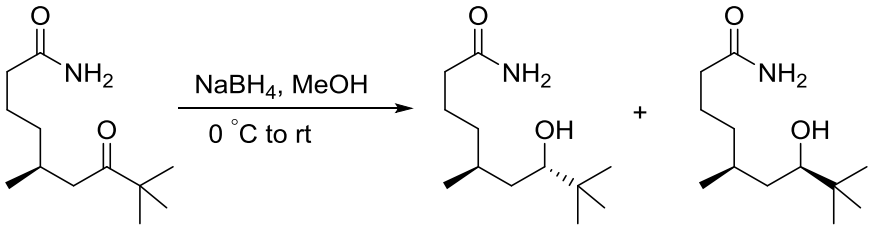
1H NMR (400 MHz, $CDCl_3$) δ 5.96 (bs, 1 H), 5.83 (bs, 1 H), 2.41-2.28 (m, 2 H), 2.18 (t, *J* = 7.2 Hz, 2 H), 2.06-1.98 (m, 1 H), 1.69-1.51 (m, 2 H), 1.31-1.21 (m, 1 H), 1.18-1.07 (m, overlapped,1 H), 1.07 (s, 9 H), 0.83 (d, *J* = 6.5 Hz, 3 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 216.0, 176.1, 44.26, 43.8, 36.1, 35.9, 28.1, 26.3, 23.0, 19.8;

IRυ_{max}(film) 3359, 3193, 2952, 1698, 1661, 1633 cm^{-1};

HRMS (ESI) *m*/*z* calculated for $C_{12}H_{24}NO_2$ $[M+H]^+$ 214.1802, found 214.1799.

Reduction of compound 49 to alcohols (–)-14 and (+)-14a

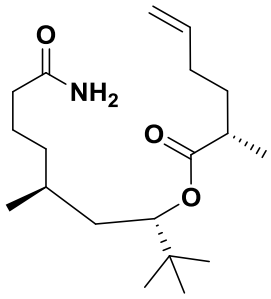


Condition	Solvent	Result
R-CBS (0.3eq),	THF	Ketone **49** + mixture of alcohols

BH_3-DMS (3eq.)		No selectivity
R-CBS (1 eq), BH_3-DMS (3eq.)	Toluene	Ketone **49** + mixture of alcohols No selectivity
R-CBS (1eq), BH_3-DMS (10eq.)	THF	No selectivity
K-selectride (1eq-3eq.),	THF	No reaction
DIBAL-H (1-10eq)	Toulene	No reaction
$NaBH_4$ (2eq)	THF + MeOH	No selectivity

A solution of keto compound **49** (20 mg, 0.10 mmol) in MeOH: THF (3.0 mL) at 0 °C was treated with $NaBH_4$ (8 mg, 0.2 mmol) and stirred at room temperature for 1 h. The reaction mixture concentrated *in vacuo*. Purification by flash chromatography over silica gel (50-80% EtOAc/hexanes) afforded alcohols (-)-**14** and (+)-**14a** (16.5 mg, 82% yield, 1:1 mixture of diastereomers).

(*S*)-(3*S*,5*S*)-9-Amino-2,2,5-trimethyl-9-oxononan-3-yl 2-methylhex-5-enoate (53)



To a solution of known acid (+)-**41** (100 mg, 0.78 mmol) in benzene (3.0 mL), was added 2,4,6-trichlorobenzylchloride (0.17 mL, 1.12 mmol), followed by Hunigs base (0.16 mL, 0.95 mmol), alcohol (–)-**14** (120 mg, 0.56 mmol) and DMAP (170 mg, 1.40 mmol). The reaction mixture was stirred at room temperature for 12 h before being diluted with EtOAc (10 mL). Then the organic layer was washed with water (3 x 5 mL), brine (5.0 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography over silica gel (50-60% EtOAc/hexanes) afforded **53** (146 mg, 80% yield) as a colorless oil.

Specific rotation $[\alpha]_D^{25.3}$= – 26.44 (*c* = 1.01, $CHCl_3$);

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.10 (bs, 1 H), 5.96 (bs, 1 H), 5.79-5.69 (m, 1 H), 4.99-4.92 (m, 2 H), 4.77-4.74 (m, 1 H), 2.47-2.39 (m, 1 H), 2.16-2.01 (m, 4 H), 1.81-1.67 (m, 2 H), 1.46- 1.24 (m, 6 H), 1.13 (d, *J* = 6.8 Hz, 3 H), 1.04-0.90 (m, 1 H), 0.86 (d, overlapped, 3 H), 0.84 (s, 9 H);

^{13}C NMR(100 MHz, $CDCl_3$) δ 176.8, 175.9, 137.9, 115.1, 78.6, 39.5, 37.6, 35.4, 34.5, 34.5, 32.8, 31.5, 28.8, 26.0, 22.7, 20.9, 17.4;

IRυ_{max}(film) 3349, 3197, 2961, 2872, 1729, 1668 cm^{-1};

HRMS (ESI) *m*/*z* calculated for $C_{19}H_{36}NO_3$ $[M+H]^+$326.2690, found 326.2688.

(*S*,*E*)-(3*S*,5*S*)-9-Amino-2,2,5-trimethyl-9-oxononan-3-yl 6-iodo-2-methylhex-5-enoate (15)

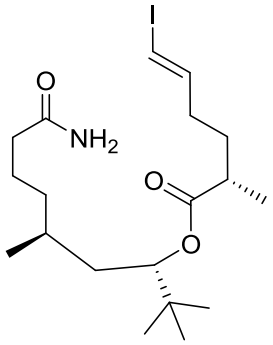
A solution of olefin **53** (90 mg, 0.276 mmol) in dioxane-water (4 mL, 3:1) was treated with 2,6-lutidine (65 µL, 0.55 mmol), followed by OsO_4 (2.5 % solution in ^{t}BuOH, 30 µL, 0.03 mmol) and $NaIO_4$ (234 mg, 1.10 mmol). The reaction mixture was stirred at room temperature and monitored by TLC. When starting material was completely consumed, water (5 mL) and EtOAc (5 mL) were added and the aqueous layer was extracted with EtOAc (3 x 5 mL). The combined organic extracts were washed with saturated aqueous solution of $Na_2S_2O_3$ (5 mL), brine (5 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Crude was partly purified by short silica gel column chromatography to get aldehyde (70 mg, 77% yield) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (t, *J* = 1.26 Hz, 1 H), 5.88 (bs, 1 H), 5.34 (bs, 1 H), 4.80 (dd, *J* = 9.0, 2.5 Hz, 1 H), 2.56-2.45 (m, 3 H), 2.25-2.11 (m, 2 H), 2.03-1.94 (m, 1 H), 1.82-1.71 (m, 2 H), 1.56-1.45 (m, 2 H), 1.43-1.38 (m, 2 H), 1.20 (d, *J* = 7.0 Hz, 3 H), 1.19-0.93 (m, 2 H), 0.90 (d, overlapped, 3 H), 0.87 (s, 9 H);

HRMS (ESI) *m*/*z* calculated for $C_{18}H_{34}NO_4$ $[M+H]^+$328.2479, found 328.2482.

To a slurry of anhydrous $CrCl_2$ (134 mg, 1.10 mmol) in dry THF (2.0 mL) at 0 °C, was added dropwise a solution of above aldehyde (60.0 mg, 0.18 mmol) and CHI_3 (144 mg, 0.36 mmol) in dry THF (3.0 mL). The reaction mixture was stirred at 0 °C for 10 h and then quenched with water (5.0mL). The mixture was extracted with Diethyl ether (5 x 8mL) and the combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash

chromatography over silica gel (60-80% EtOAc/hexanes) afforded **15** (43 mg, 53% yield) as a colorless oil.



Specific rotation $[\alpha]_D^{25.3}$= – 23.29 (*c* = 1.01, $CHCl_3$);

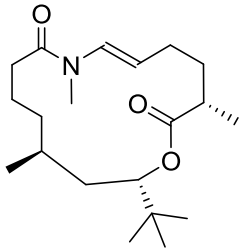
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.51-6.44 (m, 1 H), 6.02 (d, *J* = 14.5 Hz, 1 H), 5.93 (bs, 1 H), 5.52 (bs, 1 H), 4.78 (dd, *J* = 3.4, 8.6 Hz, 1 H), 2.47-2.40 (m, 1 H), 2.23-2.11 (m, 2 H), 2.10- 2.04 (m, 2 H), 1.85-1.71 (m, 3 H), 1.54-1.43 (m, 3 H), 1.42-1.33 (m, 2 H),1.16 (d, *J* = 6.1 Hz, 3 H), 1.08-1.00 (m, 1 H), 0.89 (d, overlapped, 3 H), 0.86 (s, 9 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 176.5, 175.8, 145.5, 78.9, 75.5, 39.4, 37.7, 35.6, 34.6, 33.9, 32.2, 29.0, 26.1, 22.8, 21.0, 17.6;

IRυ_{max}(film): 3349, 3197, 2960, 2871, 1725, 1669 cm^{-1};

HRMS (ESI):*m*/*z* calculated for $C_{19}H_{34}INO_4Na$ $[M+Na]^+$474.1472, found 474.1476.

(3*S*,13*S*,15*S*,*E*)-15-(*tert*-Butyl)-3,8,13-Trimethyl-1-oxa-8-azacyclopentadec-6-ene-2,9-dione (+)1



A mixture of amide **15** (38mg, 0.084 mmol), copper iodide (8.0mg, 0.042 mmol), and cesium carbonate (52 mg, 0.159 mmol) were suspended in dry THF (5.0mL). N,N′ Dimethylethylenediamine (34 μL, 0.30 mmol) was added, and the reaction flask was degassed by bubbling dry argon gas for 10 min. The septum was quickly removed and replaced with a Teflon stopper. The contents of the flask were then heated at 50 °C for 10h.The flask was allowed to

cool to room temperature, diluted with EtOAc (5.0 mL) and filtered through a short plug of silica gel. The crude N-H macrolide was then concentrated in vacuo and purified by flash column chromatography over silica gel (20-25% EtOAc/hexane) to afford enamide **56** (15 mg, 65% brsm) as colorless oil, which was used in the next step without extensive characterization.

Enamide **56** (8 mg, 0.024 mmol) was dissolved in dry THF (1.5 mL), cooled to 0 °C, and treated with sodium hydride (60% dispersion, 3 mg, 0.072 mmol). The cooling bath was removed, and the flask was allowed to warm to room temperature and stir for 20 min. Iodomethane (0.1 mL, 1.61 mmol) was then added. After 30 min, the reaction mixture was diluted with EtOAc (5 mL) and quenched with water. The phases were separated, and the aqueous phase was extracted with EtOAc (3 x 3 mL). The combined organic extracts were then dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography over silica gel (15-20% EtOAc/hexanes) to afford (+)-palmyrolide A, (+)**-1,** (8 mg, 95%) as a colorless oil.

Specific rotation $[\alpha]_D^{25}$= + 24.55 (*c* = 0.49, $CHCl_3$).

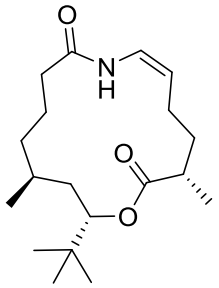
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.47 (d, *J* = 13.7 Hz, 1 H), 5.28 (dt, *J* = 7.02, 13.7 Hz, 1 H), 4.88 (dd, *J* = 2.0, 9.76 Hz, 1 H), 3.05 (s, 3 H), 2.49-2.46 (m, 1 H), 2.42-2.34 (m, 2 H), 2.33-2.25 (m, 2 H), 1.84-1.72 (m, 3 H), 1.69-1.64 (m, 2 H), 1.51-1.45 (m, 1 H), 1.40-1.31 (m, 2 H), 1.21 (d, *J* = 7.02 Hz, 3 H), 1.10-1.03 (m, 1 H), 0.90 (d, *J* = 6.70 Hz, 3 H), 0.87 (s, 9 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5, 173.08, 130.8, 117.5, 77.0, 39.0, 35.9, 35.4, 34.7, 34.6, 33.0, 31.9, 29.5, 27.1, 26.2, 24.5, 20.8, 16.9;

IRυ_{max}(film) 2959, 2930, 1726, 1650 cm^{-1};

HRMS (ESI) *m*/*z* calculated for $C_{20}H_{35}NO_3Na$ $[M+Na]^+$360.2509, found 360.2503.

(3S,13S,15S,Z)-15-(*tert*-Butyl)-3,13-dimethyl-1-oxa-8-azacyclopentadec-6-ene-2,9-dione (54)



A mixture of amide **15** (33 mg, 0.073 mmol), copper iodide (6.9 mg, 0.09 mmol), and cesium carbonate (45 mg, 0.138 mmol) were suspended in dry THF (5 mL). *N, N′*-Dimethylethylenediamine (30μL, 0.31 mmol) was added, and the reaction flask was degassed by bubbling dry argon gas for 10 minutes. The septum was quickly removed and replaced with a teflon stopper. The contents of the flask were then heated at 80 °C for 24-30h. The flask was allowed to cool to room temperature, diluted with EtOAc (5 mL) and filtered through a short plug of silica gel. The crude N-H macrolide was then concentrated in vacuo and purified by flash column chromatography (20-25% EtOAc/hexane) to afford *cis*-enamide **54** (10 mg, 43%) as a colorless oil.

Specific rotation $[\alpha]_D^{25.7} = -72.6$ (*c* = 0.24, $CHCl_3$);

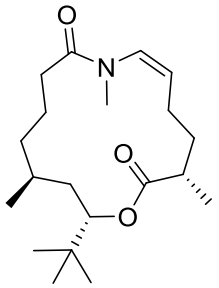
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, *J* = 8.0 Hz, 1 H), 6.59 (t, *J* = 8.04 Hz, 1 H), 4.97 (q, *J* = 8.00 Hz, 1 H), 4.90 (d, *J* = 8.00 Hz, 1 H), 2.57-2.47 (m, 2 H), 2.43- 2.37 (m, 1 H), 2.12-2.06 (m, 1 H), 2.01-1.94 (m, 1 H), 1.88-1.67 (m, 4 H), 1.53-1.28 (m, 4 H), 1.21 (d, *J* = 8.00 Hz, 3 H), 1.13-1.05 (m, 1 H), 0.89 (s, 9 H), 0.85 (d, *J* = 6.27 Hz, 3 H);

^{13}C NMR(100 MHz, $CDCl_3$) δ 177.4, 172.1, 122.8, 113.0, 79.1, 39.9, 38.6, 37.3, 36.9, 34.8, 34.4, 28.2, 26.2, 26.0, 23.5, 22.2, 20.2, 17.3;

IRυ_{max}(film) 3361, 3019, 2967, 1658, 1501, 1215 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{19}H_{33}NO_3Na$ $[M+Na]^+$ 346.2353, found 346.2350.

(3*S*,13*S*,15*S*,*Z*)-15-(*tert*-butyl)-3,8,13-trimethyl-1-oxa-8-azacyclopentadec-6-ene-2,9-dione (55)



Enamide (10mg, 0.03 mmol) was dissolved in dry THF (1.0 mL), cooled to 0 °C, and treated with sodium hydride (60% dispersion, 6.0 mg, 0.15 mmol). The cooling bath was removed, and the flask was allowed to warm to room temperature and stir for 20 min. Iodomethane (0.1 mL,

1.61 mmol) was then added. After 3 h, the reaction mixture was diluted with EtOAc (5.0 mL) and quenched with water. The phases were separated, and the aqueous phase was extracted with EtOAc (3 x 3.0 mL). The combined organic layers were then dried over Na_2SO4, filtered, and concentrated. The crude product was purified by flash column chromatography over silica gel (15-20% EtOAc/hexanes) to afford *cis*-(–)-Palmyrolide A (**55)** (9.0 mg, 86%) of as a white solid.

Specific rotation $[\alpha]_D^{25.8}$= – 6.65 (*c* = 0.497, $CHCl_3$);

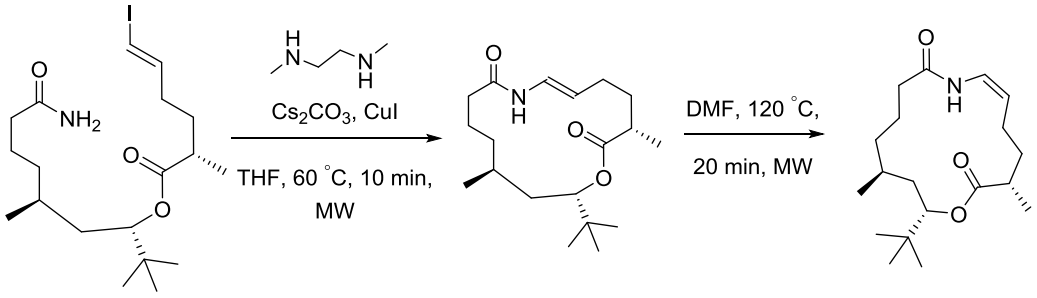
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.11 (d, *J* = 7.5 Hz, 1 H), 5.41- 5.36 (m, 1 H), 4.87 (d, *J* = 10.54 Hz, 1 H), 2.99 (s, 3 H), 2.48 -2.29 (m, 3 H), 2.11-1.91(m, 3 H), 1.72-1.65 (m, 1 H), 1.51 -1.29 (m, 6 H), 1.20 (d, *J* = 7.03 Hz, 3 H), 0.94 - 0.89 (m, 1 H), 0.89 (d, *J* = 6.0 Hz, 3 H), 0.85 (s, 9 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 172.5, 130.2, 130.0, 76.7, 40.8, 37.7, 35.3, 35.2, 33.6, 33.3, 31.9, 28.2, 26.1, 24.4, 22.3, 19.7, 17.4;

IRυ_{max}(film) 3019, 2966, 1715, 1635, 1522, 1215 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{35}NO_3Na$ $[M+Na]^+$ 360.2509, found 360.2505.

Microwave assisted macrocylization:

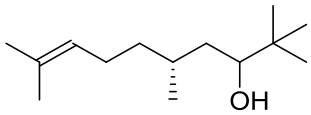


A mixture of amide **15** (8.0 mg, 0.017 mmol), copper iodide (3.3 mg, 0.017 mmol), and caesium carbonate (15 mg, 0.046 mmol) was suspended in dry THF (3.0 mL). N,N′ Dimethylethylenediamine (10 μL, 0.069 mmol) was added, and the reaction flask was degassed by bubbling dry argon gas for 10 min and then heated in a microwave reactor at 60 °C for 10-12 min, The flask was allowed to cool to room temperature, diluted with EtOAc (5.0 mL) and filtered through a short plug of silica gel. The crude N-H macrolide was then concentrated in vacuo and purified by flash column chromatography over silica gel (20-25% EtOAc/hexane) to afford enamide **56** (3.0mg, 70% brsm) as colorless oil.

Conversion of *trans*-enamide to *cis*-enamide *via* microwave heating:

The *trans*-enamide **56** (3 mg), obtained in above reaction was taken in dry DMF (2 mL), and heated in microwave reactor at 120 °C for 20 min, the *trans*-enamide was converted to *cis*-enamide **54** (compared on TLC with authentic *cis*-enamide sample).

(5*R*)-2,2,5,9-Tetramethyldec-8-en-3-ol (57)



tert-Butylmagnesium chloride (2.0 M in THF, 21 mL, 42.14 mmol) was added dropwise to a solution of commercial *R*-citronellal (5 g, 32.41 mmol) in dry THF (40 mL) at –78 °C and stirred at same temperature for 2 h. Then the reaction mixture was brought to -20 °C and quenched with saturated aqueous ammonium chloride (50 mL) and extracted with EtOAc (4 x 30 mL). The combined organic extracts were washed with brine (30 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to obtain crude product which was purified by flash chromatography over silica gel (1-5% EtOAc/Petroleum ether) to afford alcohol **57** (4.1 g, 59%, ~ 1:1 diasteromeric mixture) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.11-5.08 (m, 1 H), 3.30-3.27 (m, 1 H), 2.03-1.95 (m, 2 H), 1.67 (s, 3 H), 1.59 (s, 3 H), 1.48 (br. s., 1 H), 1.44-1.16 (m, 5 H), 1.00-0.85 (m, 12 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 131.1, 131.1, 124.9, 124.9, 77.6, 77.2, 39.3, 38.8, 38.4, 35.6, 34.9, 34.8, 29.7, 29.2, 26.7, 25.7, 25.7, 25.6, 25.4, 25.3, 20.9, 18.8, 17.6;

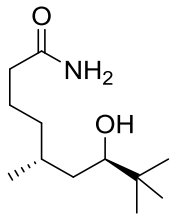
IR$_{\upsilon max}$(film) ν 3349, 2964, 2874, 1670, 1512 cm^{-1}.

(5*R*,7*R*)-7-Hydroxy-5,8,8-trimethylnonanamide (+)-14 and (5*R*,7*S*)-7-hydroxy-5,8,8-trimethylnonanamide (–)-14a

A stream of ozone was bubbled through a cold (–78 °C) solution of the alcohol **57** (1.5 g, 7.05 mmol) in CH_2Cl_2 (20 mL) until the distinctive blue color of ozone was clearly observed. Ozonolysis was then terminated, and excess ozone was displaced by passing a stream of oxygen through the solution for 5-10 min, and then neat Me_2S (1.2 mL, 14.10 mmol) was added dropwise. The resulting reaction mixture was allowed to warm to room temperature and stirred for 5 h. The reaction mixture was concentrated *in vacuo*. The crude product obtained was filtered by short silica gel column to get aldehyde as colorless

oil. It was forwarded for next step immediately without taking any extensive characterization. Dimethylamine hydrochloride (0.74 g, 9.10 mmol) was added to a solution of above aldehyde (7.05 mmol) taken in dry toluene (20 mL) and stirred at room temperature for 10 minutes. The resultant suspension was transferred via syringe to a solution of freshly prepared potassium 2-isocyano-2-(4-methoxyphenyl) acetate (3.2 g, 14.10 mmol) (prepared freshly as described earlier) and dimethylamine hydrochloride (0.70 g, 9.10 mmol) in dry toluene (10 mL) and it was allowed to stir at room temperature for 6h. Additionally, dimethylamine hydrochloride (0.30 g) and Et_3N (0.1 mL). The reaction mixture was then evaporated to dryness and the crude product obtained was dissolved in EtOAc (25 mL), washed with water (15 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The resulting light yellow oil was dissolved in THF (15 mL), treated with 1N HCl (35 mL, 35.37 mmol), and strirred at room temperature for 5 h. The reaction mixture was then basified with aqueous saturated $NaHCO_3$ (about 40-50 mL, pH~10), and extracted with EtOAc (4 x 20 mL). The combined organic extracts were washed with brine (15 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* and subjected to flash chromatography over silica gel (1-1.5% MeOH/CH_2Cl_2) to afford two separable alcohols (+)-**14** as a colorless oil and (–)-**14a** as a white solid respectively with an overall yield of 41%.

Data for Compound (+)-**14:**



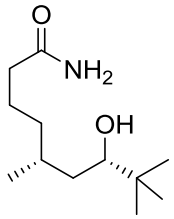
Specific rotation $[\alpha]_D^{25.0}$= + 49.2 (*c* = 1.2, $CHCl_3$);

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.96 (br. s., 1 H), 5.88 (br. s., 1 H), 3.27 - 3.24 (m, 1 H), 2.21-2.17 (m, 2 H), 1.71-1.46 (m, 4 H), 1.36-1.24 (m, 2 H), 1.36-1.16 (m, 1 H), 1.08 - 0.96 (m, 1 H), 0.92 (d, *J* = 6.5 Hz, 3 H), 0.86 (s, 9 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 176.3, 77.4, 39.0, 35.9, 34.9, 34.5, 29.3, 25.7, 22.8, 20.9;

IRυ_{max}(film) ν 3408, 2986, 1673, 1518, 1382, 1218 cm^{-1}.

Data for Compound (–)-**14a:**



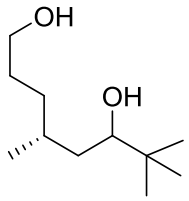
Specific rotation $[\alpha]_D^{25.0} = -21.3$ (c = 1.1, $CHCl_3$);

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.60 (br. s., 2 H), 3.27 (d, J = 10.3 Hz, 1 H), 2.21 (t, J = 7.3 Hz, 2 H), 1.70-1.65 (m, 3 H), 1.34-1.17 (m, 5 H), 0.92-0.87 (m, 12 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 175.8, 77.3, 38.6, 37.7, 36.0, 34.9, 29.3, 25.7, 22.8, 19.0.

IRυ_{max} (film) ν 3356, 2983, 1672, 1512, 1406 cm^{-1}.

(4*R*)-4,7,7-Trimethyloctane-1,6-diol (65)

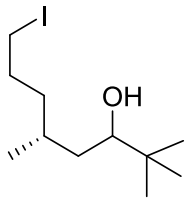


A solution of alcohol **57** (0.33 g, 1.50 mmol) in dichloromethane (10 mL) and methanol (1:1, 10 mL) was cooled to -78 °C and then bubbled with ozone from ozone generator for 20 minutes until the solution turned to pale blue in color. Oxygen was then bubbled through the solution at −78 °C until the blue color disappeared. Solid sodium borohydride (0.3 g, 7.70 mmol) was added at −78 °C, and the reaction mixture was allowed to warm to room temperature and stirred for 5h. After concentration under reduced pressure, the residue was partitioned between saturated aqueous sodium hydrogen carbonate (20 mL) and ether (30 mL). The organic layer was washed with brine (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Purification over silica gel chromatography (10-15% EtOAc/Petroleum ether) gave (4*R*)-4,7,7-trimethyloctane-1,6-diol **65** (0.3 g, 90%) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.60-3.57 (m, 2 H), 3.26 (t, J = 11.25 Hz, 1 H), 2.82 (br. s., 1 H), 2.28 (br. s., 1 H), 1.65-1.16 (m, 7 H), 0.92-0.85 (m, 12 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 77.4, 77.1, 62.8, 62.6, 39.1, 38.7, 34.9, 34.8, 34.2, 30.9, 30.0, 29.7, 29.3, 29.2, 25.7, 20.9, 19.1.

(5*R*)-8-Iodo-2,2,5-trimethyloctan-3-ol (66)

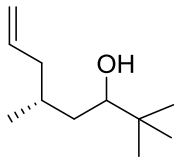


Iodine (2.80 g, 11.1 mmol) was added portionwise to a solution of triphenylphosphine (2.90 g, 11.1 mmol), imidazole (1.51 g, 22.30 mmol) and (4*R*)-4,7,7-trimethyloctane-1,6-diol (1.40 g, 7.40 mmol) in dichloromethane (30 mL) at 0 °C, and then reaction was stirred at room temperature for 2 h. Saturated aqueous sodium sulfite (20 mL) and dichloromethane (10 mL) were added, and the mixture was partitioned between water and dichloromethane. The organic layer was washed with brine (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure to get crude compound which on silica gel chromatography eluting with 5-8% EtOAc/Hexanes) gave (5*R*)-8-iodo-2,2,5-trimethyloctan-3-ol (**66**) (1.5 g, 67%) as a colorless oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ 3.33 - 3.25 (m, 1H), 3.25 - 3.16 (m, 2 H), 1.92 - 1.71 (m, 3 H), 1.40 - 1.17 (m, 5 H), 0.97 - 0.90 (m, 12 H).

^{13}C NMR (50 MHz, $CDCl_3$) δ 77.4, 77.1, 39.2, 39.0, 38.7, 36.3, 35.0, 34.8, 31.3, 30.9, 29.2, 29.0, 25.7, 21.0, 18.9, 7.6, 7.5.

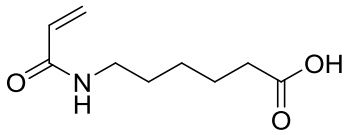
(5*R*)-2,2,5-trimethyloct-7-en-3-ol (58)



The iodo-compound **66** (1.50 g, 5.28 mmol) was dissolved in diethyl ether (20 mL) and potassium *tert*-butoxide (1.77 g, 15.84 mmol) was added portionwise at 0 °C, and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was partitioned between cold water (15 mL) and diethyl ether (20 mL). The organic layer was washed with brine (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure at low temperature to yield a colorless oil of 0.85g identified as (5*R*)-2,2,5-trimethyloct-7-en-3-ol (**58**). Without purification, this alkene was used for further reactions.

^{1}H NMR (200 MHz, $CDCl_3$) δ 5.85-5.67 (m, 1 H), 5.04-4.94 (m, 2 H), 3.32-3.25 (m, 1 H), 2.24-1.70 (m, 4 H), 1.42-1.34 (m, 1H), 0.98-0.87 (m, 12 H).

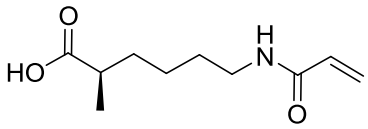
6-Acrylamidohexanoic Acid (59)



3.87 mL (45.7 mmol) of acryloyl chloride in 10 mL of THF were added dropwise into a solution of 5 g (38.1 mmol) of 6-aminocaproic acid in 30 mL of 1.27 N aqueous sodium hydroxide at 0 °C. After 2 h of stirring at same temperature, the solution was acidified to pH 3 with 1N hydrochloric acid. Afterward, the mixture was poured on ice water and extracted with ethyl acetate. The organic phase was washed with water and dried over sodium sulphate. The solvent was then removed via rotary evaporation. The crude product was purified by recrystalization in hexane/ethyl acetate to get 4.1g of acid **59** as white powder in 58% yield. The ^{1}H NMR values were compared to literature reports.[34]

^{1}H NMR (200 MHz, DMSO-d_6) δ 12.05 (s, 1 H), 8.09 (br. s., 1 H), 6.30 - 6.02 (m, 2 H), 5.58 (dd, *J* = 2.78, 9.60 Hz, 1 H), 3.13 (q, *J* = 6.61 Hz, 2 H), 2.26 - 2.18 (m, 2 H), 1.56 - 1.23 (m, 6 H).

(*R*)-6-Acrylamido-2-methylhexanoic acid (60)



Acid **71** is prepared through known literature procedures.[35]

Specific rotation $[\alpha]_D^{25.0}$= – 12.5 (*c* = 1.3, $CHCl_3$).

^{1}H NMR (200 MHz, $CDCl_3$) δ 3.28 (t, *J* = 6.7 Hz, 2 H), 2.49 - 2.42 (m, 1 H), 1.71 - 1.38 (m, 6 H), 1.19 (d, *J* = 6.9 Hz, 3 H).

To a solution of acid **71** (0.4 g, 2.34 mmol) in EtOH was added 10% wt/wt palladium on carbon (10 mg). The atmosphere in the flask was replaced with hydrogen, and the reaction mixture was allowed to stir at room temperature for 12 h before being filtered through a short plug of celite to afford desired amine (0.23 g, 70%). This amine was

forwarded for next step without any purification or characterization. The amine obtained above (0.23 g, 1.60 mmol) was taken in 1.2N NaOH (1.5 mL) and cooled to 0 °C, and was slowly added with a solution of acroyl chloride (0.14 mL, 1.74 mmol) in dry THF for over ten minutes. After 3 hours at room temperature, the reaction mixture was diluted with ethyl acetate (5 mL) and organic phase was separated. The aqueous layer was acidified to pH 3 using 2N HCl, poured on ice water and extracted again with ethyl acetate (3 x 8 mL). The organic phase was washed with water (10 mL), brine (10 mL) and dried over Na_2SO_4. The solvent was then removed via rotary evaporation. The desired acid (**60**) was obtained in 56% yield as thick colorless liquid and was sufficiently pure from NMR data.

Specific rotation $[\alpha]_D^{25} = -13.7$ (c = 7.1, $CHCl_3$);

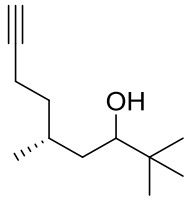
^{1}H NMR (200 MHz, $CDCl_3$) δ 8.14 (br. s., 1 H), 6.48 - 6.05 (m, 2 H), 5.71 - 5.58 (m, 1 H), 3.38 - 3.26 (m, 2 H), 2.49 - 2.36 (m, 1 H), 1.70 - 1.26 (m, 6 H), 1.16 (d, J = 6.9 Hz, 3 H);

^{13}C NMR (50 MHz, $CDCl_3$) δ 181.7, 166.0, 130.8, 126.5, 39.4, 39.3, 33.1, 29.2, 24.5, 17.0;

IR υ_{max} (film) ν 3455, 2983, 1721, 1636, 1610 cm^{-1};

HRMS (ESI): Calculated for $C_{10}H_{17}O_3NNa$ $[M+Na]^+$: 222.1101, found 222.1099.

(5*R*)-2,2,5-Trimethylnon-8-yn-3-ol (61)



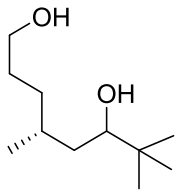
A solution of alcohol **57** (0.28 g, 1.30 mmol) in CH_2Cl_2 (15 mL) was cooled to 0 °C and purged with ozone gas until the color of solution turns pale blue. Oxygen was then allowed to pass for few minutes till the solution again becomes colorless. Me_2S (0.3 mL, 3.90 mmol) was added and warmed to room temperature over a period of 5 hours. The reaction mixture was then concentrated *in vacuo* and dried. The crude aldehyde thus obtained (1.30 mmol) was redissolved in dry MeOH (5 mL) and added drop wise to a solution containing Ohirabestman reagent (0.51 g, 2.60 mmol) and K_2CO_3 (0.36 g, 2.60

mmol) in MeOH. The reaction was allowed to stir for 12 hours at room temperature. The reaction mixture was evaporated in *vacuo* to remove excess of MeOH and redissolved in CH_2Cl_2 (10 mL). The organic layer was washed with water (10 mL), brine (10 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to obtain crude alkyne which was purified by flash column chromatography over silica gel (5-10% EtOAc/hexane) to afford alkyne **61** as a colorless liquid (0.1 g, 43% for two steps).

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.32 - 3.29 (m, 1H), 2.27 - 2.18 (m, 2 H), 1.95 - 1.93 (m, 1 H), 1.80- 1.73 (m, 2 H), 1.59 - 1.20 (m, 4 H), 0.98 - 0.89 (m, 12 H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 84.8, 84.7, 77.4, 77.4, 68.2, 68.1, 38.9, 38.4, 36.7, 34.9, 34.8, 34.4, 29.4, 28.9, 25.7, 25.6, 20.5, 18.6, 16.2, 16.2.

(4*R*)-4,7,7-Trimethyloctane-1,6-diol (65)

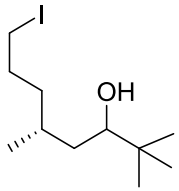


A solution of alcohol **57** (0.33 g, 1.50 mmol) in dichloromethane and methanol (1:1, 18 mL) was cooled to -78 °C and then bubbled with ozone from ozone generator for 20 minutes until the solution turned to pale blue in color. Oxygen was then bubbled through the solution at −78 °C until the blue color disappeared. Solid sodium borohydride (0.3 g, 7.70 mmol) was added at −78 °C, and the reaction mixture was allowed to warm to room temperature and stirred for 5 h. After concentration under reduced pressure, the residue was partitioned between saturated aqueous sodium hydrogen carbonate (20 mL) and ether (30 mL). The organic layer was washed with brine (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Purification over silica gel chromatography (10-15% EtOAc/Petroleum ether) gave (4*R*)-4,7,7-trimethyloctane-1,6-diol **65** (0.3 g, 90%) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.60 - 3.57 (m, 2 H), 3.26 (t, *J* = 11.25 Hz, 1 H), 2.82 (br. s., 1 H), 2.28 (br. s., 1 H), 1.65 - 1.16 (m, 7 H), 0.92 - 0.85 (m, 12 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 77.4, 77.1, 62.8, 62.6, 39.1, 38.7, 34.9, 34.8, 34.2, 30.9, 30.0, 29.7, 29.3, 29.2, 25.7, 20.9, 19.1.

(5*R*)-8-Iodo-2,2,5-trimethyloctan-3-ol (66)

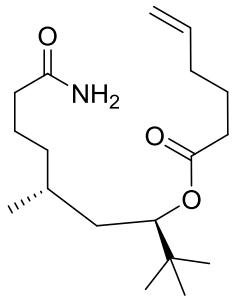


Iodine (2.80 g, 11.1 mmol) was added portionwise to a solution of triphenylphosphine (2.90 g, 11.1 mmol), imidazole (1.51 g, 22.30 mmol) and (4*R*)-4,7,7-trimethyloctane-1,6-diol (1.40 g, 7.40 mmol) in dichloromethane (30 mL) at 0 °C, and then reaction was stirred at room temperature for 2 h. Saturated aqueous sodium sulfite (20 mL) and dichloromethane (10 mL) were added, and the mixture was partitioned between water and dichloromethane. The organic layer was washed with brine (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure to get crude compound which on silica gel chromatography eluting with 5-8% EtOAc/ Petroleum ether) gave (5*R*)-8-iodo-2,2,5-trimethyloctan-3-ol (**66**) (1.5 g, 67%) as a colorless oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ 3.33 - 3.25 (m, 1H), 3.25 - 3.16 (m, 2 H), 1.92 - 1.71 (m, 3 H), 1.40 - 1.17 (m, 5 H), 0.97 - 0.90 (m, 12 H).

^{13}C NMR (50 MHz, $CDCl_3$) δ 77.4, 77.1, 39.2, 39.0, 38.7, 36.3, 35.0, 34.8, 31.3, 30.9, 29.2, 29.0, 25.7, 21.0, 18.9, 7.6, 7.5.

(3*R*,5*R*)-9-Amino-2,2,5-trimethyl-9-oxononan-3-yl hex-5-enoate (72)



Hex-5-enoic acid is prepared using literature procedures.[20b]

To a solution of hex-5-enoic acid (0.25 g, 2.20 mmol) in toluene (7 mL), was added 2,4,6-trichlorobenzylchloride (0.34 mL, 2.20 mmol), followed by N,N-Diisopropylethylamine (0.7 mL, 4.10 mmol) and reaction mixture was allowed to stir for 3 hours at room

temperature. Alcohol (+)-**14** (0.24 g, 1.10 mmol) and DMAP (0.27 g, 2.20 mmol) were taken in toluene (4 mL) and added to above reaction mixture and stirred at room temperature for 12h before being diluted with EtOAc (10 mL). Then the organic layer was washed with saturated $NaHCO_3$ (2 x 10 mL), brine (15 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography over silica gel (70 - 80% EtOAc/Petroleum ether) afforded **72** (0.26 g, 72%) as a colorless oil.

Specific rotation $[\alpha]_D^{25.3}$= + 38.4 (*c* = 1.1, $CHCl_3$).

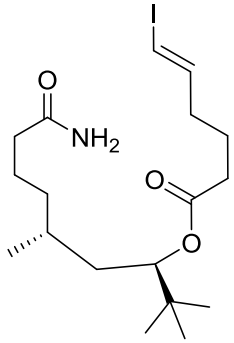
^{1}H NMR (200 MHz, $CDCl_3$) δ 5.95 (m, 1 H), 5.85-5.65 (m, 2 H), 5.09-4.97 (m, 2 H), 4.84-4.77 (m, 1 H), 2.38-2.29 (m, 2 H), 2.23-2.04 (m, 4 H), 1.81-1.65 (m, 3 H), 1.57 - 1.25 (m, 5 H), 1.12-1.02 (m, 1 H), 0.92-0.87 (m, 12 H).

^{13}C NMR (50 MHz, $CDCl_3$) δ 175.9, 173.8, 137.7, 115.4, 78.8, 37.4, 35.6, 34.7, 34.6, 33.9, 33.2, 29.0, 25.9, 24.3, 22.7, 20.8.

IRυ_{max} (film) ν 3487, 3357, 2963, 1719, 1672, 1377, 1221 cm^{-1}.

HRMS (ESI): Calculated for $C_{18}H_{34}O_3N$ $[M+H]^+$: 312.2533, found 312.2527.

(3*R*,5*R*)-9-Amino-2,2,5-trimethyl-9-oxononan-3-yl (*E*)-6-iodohex-5-enoate (73)



A solution of olefin **72** (0.10 g, 0.32 mmol) in acetone-water (8 mL, 3:1) was treated with *N*-Methylmorpholine N-oxide (75 mg, 0.64 mmol), followed by OsO_4 (2.5% solution in *t*-BuOH, 30 μL, 0.03 mmol) and $NaIO_4$ (0.34 g, 1.60 mmol). The reaction mixture was stirred at room temperature for 6 hours. Water (5 mL) and EtOAc (5 mL) were added and the aqueous layer was extracted with EtOAc (3 x 5 mL). The combined organic extracts were washed with saturated aqueous solution of $Na_2S_2O_3$ (10 mL), brine (10 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Crude compound obtained was partly

purified by very short silica gel bed to get aldehyde which was immediately used for next step.

To a slurry of anhydrous $CrCl_2$ (0.23 g, 1.90 mmol) in dry THF (5 mL) at 0 °C, was added dropwise a solution of above aldehyde (0.11 g, 0.32 mmol) and CHI_3 (0.25 g, 0.6 mmol) in dry THF (4 mL). The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 12 h, it was then diluted with Et_2O (10 mL) and continued stirring for further 30 minutes. Water (5 mL) was added and the mixture was extracted with Et_2O (5 x 8 mL) and the combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography over silica gel (80-90% EtOAc/Petroleum ether) afforded **73** (90 mg, 64% for two steps) as a colorless oil. **Specific rotation** $[\alpha]_D^{25.3}$= – 36.4 (*c* = 1.35, $CHCl_3$);

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.51 - 6.43 (m, 1 H), 6.04 - 5.98 (m, 2 H), 5.74 (br. s., 1 H), 4.80 - 4.76 (m, 1 H), 2.38 - 2.28 (m, 2 H), 2.23 - 2.06 (m, 4 H), 1.78 - 1.70 (m, 3 H), 1.53 - 1.23 (m, 5 H), 0.99 - 1.01 (m, 1 H), 0.88 - 0.85 (m, 12 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 176.0, 173.4, 145.3, 79.0, 75.6, 37.3, 35.7, 35.4, 34.6, 34.1, 33.6, 29.1, 25.9, 23.7, 22.7, 20.8;

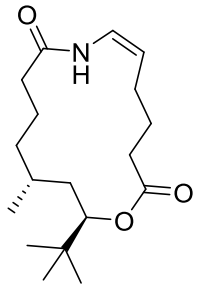
IRυ_{max} (film) ν 3349, 3197, 2960, 2871, 1725, 1669 cm^{-1};

HRMS (ESI): Calculated for $C_{18}H_{33}O_3NI$ $[M+H]^+$: 438.1500, found 438.1497.

(13*R*,15*R*,*Z*)-15-(*tert*-Butyl)-13-methyl-1-oxa-8-azacyclopentadec-6-ene-2,9-dione (74) and (13*R*,15*R*,*E*)-15-(*tert*-butyl)-13-methyl-1-oxa-8-azacyclopentadec-6-ene-2,9-dione (75)

A mixture of amide **73** (80 mg, 0.18 mmol), copper iodide (18 mg, 0.09 mmol), and caesium carbonate (0.11 g, 0.36 mmol) was suspended in dry THF (4 mL). N,N′ dimethylethylenediamine (10 μL, 0.09 mmol) was added, and the reaction flask was degassed by bubbling dry argon gas for 10 min and then irradiated in a microwave reactor at 80 °C for 40 min. The flask was allowed to cool to room temperature, diluted with EtOAc (5 mL) and filtered through a short plug of silica gel. The crude N-H macrolides were then concentrated *in vacuo* and purified by flash column chromatography over silica gel (20-25% EtOAc/hexane) to afford *cis*- enamide (+)-**74** (11 mg) and *trans*-enamide

(+)-**75** (15 mg) with 46% overall yield and recovered starting material **73** (26 mg). Compound **74:**



Specific rotation $[\alpha]_D^{27.1}$= + 14.9 (*c* = 0.51, $CHCl_3$);

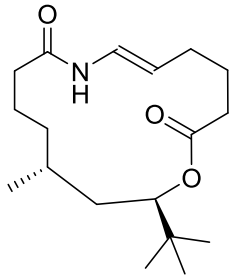
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (br. s, 1 H), 7.45 (br. s, 1 H), 6.86 (dd, *J* = 10.9, 8.9 Hz, 2 H), 4.83 (d, *J* = 10.4 Hz, 1 H), 4.60 (ddd, *J* = 5.8, 8.9, 12.1 Hz, 1 H), 2.83 - 2.79 (m, 1 H), 2.52 - 2.48 (m, 2 H), 2.37 - 2.32 (m, 1 H), 2.11 - 1.95 (m, 3 H), 1.86 - 1.83 (m, 1 H), 1.60 - 1.56 (m, 2 H), 1.46 - 1.39 (m, 2 H), 1.23 - 1.18 (m, 1 H), 1.08 - 1.04 (m, 2 H), 0.91 - 0.82 (m, 12 H);

^{13}C NMR (125 MHz, $CDCl_3$) δ 175.8, 171.2, 123.9, 107.9, 78.3, 38.7, 38.0, 37.4, 34.5, 28.8, 27.1, 25.9, 22.8, 22.0, 21.1, 20.4;

IRυ_{max} (film) ν 3361, 2967, 1658, 1501, 1215 cm^{-1};

HRMS (ESI): Calculated for $C_{18}H_{31}O_3NNa$ $[M+Na]^+$: 332.2196, found 332.2189.

Compound **75** exists as rotamers**.**



Specific rotation $[\alpha]_D^{26.0}$ = + 59.9 (*c* = 0.06, $CHCl_3$);

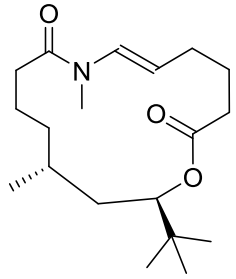
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 - 7.20 (m, 1 H), 6.57 - 6.28 (m, 1 H), 5.12 - 5.08 (m, 1 H), 5.02 - 4.67 (m, 1 H), 2.50 - 2.38 (m, 4 H), 2.13 - 2.08 (m, 2 H), 1.91 - 1.84 (m, 2 H), 1.68 - 1.50 (m, 5 H), 1.27 - 1.20 (m, 2 H), 0.93 - 0.82 (m, 12 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 170.7, 125.4, 121.1, 114.5, 111.5, 78.7, 76.7, 39.9, 37.6, 37.4, 36.2, 35.5, 34.1, 32.5, 30.8, 30.6, 29.2, 27.5, 27.4, 26.1, 25.9, 24.3, 21.8, 21.7, 21.0, 20.9, 20.0;

IRυ_{max} (film) ν 3353, 2959, 1726, 1650 cm^{-1};

HRMS (ESI): Calculated for $C_{18}H_{31}O_3NNa$ $[M+Na]^+$: 332.2196, found 332.2190.

(13*R*,15*R*,*E*)-15-(*tert*-Butyl)-8,13-dimethyl-1-oxa-8-azacyclopentadec-6-ene-2,9-dione (76)



The trans-enamide **75** (9 mg, 0.03 mmol) was dissolved in dry THF (3 mL), cooled to 0 °C, and treated with sodium hydride (60% dispersion, 5 mg, 0.11 mmol). The cooling bath was removed, and the flask was allowed to warm to room temperature and stir for 20 min. Iodomethane (14 μL, 0.23 mmol) was then added. After 30 min, the reaction mixture was diluted with EtOAc (5.0 mL) and added with saturated aqueous NH_4Cl. The phases were separated, and the aqueous phase was extracted with EtOAc (3 x 4 mL). The combined organic extracts were then dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography over silica gel (15-20% EtOAc/Petroleum ether) to afford **76** (8 mg, 84%) as colorless oil.

Specific rotation $[\alpha]_D^{26.5}$ = + 31.0 (*c* = 0.64, $CHCl_3$);

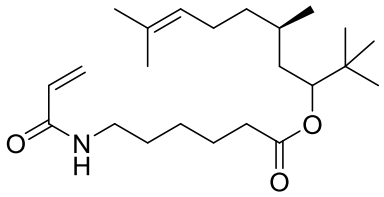
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.62 (d, *J* = 13.7 Hz, 1 H), 4.97 - 4.83 (m, 2 H), 3.06 (s, 3 H), 2.60 - 2.52 (m, 2 H), 2.39 - 2.35 (m, 3 H), 2.23 - 2.18 (m, 1 H), 2.04 - 1.76 (m, 2 H), 1.57 - 1.32 (m, 7 H), 0.91 - 0.85 (m, 12 H);

^{13}C NMR (100 MHz, $CDCl_3$)# δ 173.8, 173.5, 130.5, 110.3, 79.1, 43.5, 37.4, 36.3, 34.6, 34.3, 29.0, 26.2, 25.9, 24.5, 22.9, 21.6, 20.8;

HRMS (ESI): Calculated for $C_{19}H_{33}O_3NNa$ $[M+Na]^+$: 346.2353, found 346.2350.

There is slight decomposition of compound observed when recording ^{13}C NMR in $CDCl_3$.

(5*R*)-2,2,5,9-Tetramethyldec-8-en-3-yl 6-acrylamidohexanoate (77)



To a solution of acid **59** (1.16 g, 6.20 mmol) in THF (10 mL), was added triethylamine (1.7 mL, 0.70 mmol), followed by 2,4,6-trichlorobenzoylchloride (0.1 mL, 6.29 mmol) dropwise at 0 °C and then stirred at room temperature for 3 h. The reaction mixture was evaporated under reduced pressure and redissolved in dry toluene (10 mL). A solution of alcohol **57** (0.50 g, 2.7 mmol) and DMAP (0.67 g, 5.40 mmol) in toluene (2mL) was then added to above reaction mixture and further stirred at room temperature for 16 h. The reaction mixture was then diluted with EtOAc (25 mL) and organic layer was washed with 20 mL of saturated sodium bicarbonate solution ($NaHCO_3$), followed by water (10 mL), brine (10 mL) and dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography over silica gel (20-30% EtOAc/Petroleum ether) afforded desired compound **77** as slight yellow liquid (0.64 g, 71%). Compound **77** is isolated and characterized as a mixture of diastereomers.

^{1}H NMR (200 MHz, $CDCl_3$) δ 6.34 - 6.01 (m, 2 H), 5.64 (dd, *J* = 1.7, 9.9 Hz, 2 H), 5.08 (br. s., 1 H), 4.89 - 4.83 (m, 1 H), 3.35 (q, *J* = 6.8 Hz, 2 H), 2.33 (t, *J* = 7.3 Hz, 2 H), 2.05 - 1.95 (m, 2 H), 1.68 (m, 4 H), 1.61 (br. s., 6 H), 1.48 - 1.15 (m, 7 H), 0.91 - 0.88 (m, 12 H).

^{13}C NMR (50 MHz, $CDCl_3$) δ 173.5, 173.3, 165.6, 131.1, 131.1, 131.0, 126.1, 124.8, 124.7, 78.6, 78.5, 39.3, 38.2, 37.1, 36.8, 35.5, 34.7, 34.5, 34.3, 34.3, 29.4, 29.1, 29.0, 26.5, 26.0, 25.7, 25.5, 25.2, 24.5, 20.6, 19.1, 17.7, 17.6.

IRυ_{max} (film) ν 3287, 2960, 1728, 1663, 1551, 1233 cm^{-1}.

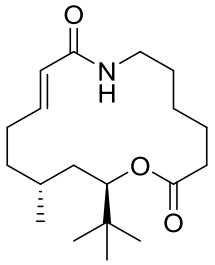
HRMS (ESI) Calculated for $C_{23}H_{41}O_3NNa$ $[M+Na]^+$: 402.2979, found 402.2974.

(14*R*,16*R*,*E*)-16-(*tert*-Butyl)-14-methyl-1-oxa-8-azacyclohexadec-10-ene-2,9-dione (78) and (14*R*,16*S*,*E*)-16-(*tert*-butyl)-14-methyl-1-oxa-8-azacyclohexadec-10-ene-2,9-dione (79)

A solution of diene **77** (0.64 g, 1.5 mmol) in toluene (0.5 L, 0.003M) was degassed for 15 minutes and added with 5 mol% of Grubbs IInd generation catalyst (**G-II**)(67 mg) and

stirred at room temperature for 12 hours. The reaction was then concentrated under reduced pressure to remove excess toluene and filtered through a short pad of silica to give a thick black residue. Further careful purification by silica gel chromatography (50-60% EtOAc/Petroleum ether) allowed the separation of two diastereomers and afforded desired macrocycles **18** and **19** in approxiomately 1:1 ratio with a combined yield of 70%.

Compound **18** (0.19 g) as a white crystalline solid.



Melting point 121.2 °C;

Specific rotation $[\alpha]_D^{27.1}$= + 77.8 (*c* = 0.47, $CHCl_3$);

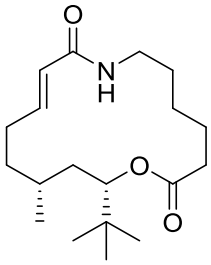
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.78 - 6.61 (m, 1 H), 6.33 - 6.25 (m, 1 H), 5.81 (d, *J* = 16.1 Hz, 1H), 4.84 (d, *J* = 11.0 Hz, 1 H), 3.87 - 3.82 (m, 1 H), 2.98 - 2.92 (m, 1 H), 2.43 - 2.19 (m, 4 H), 1.87 - 1.74 (m, 3 H), 1.45 - 1.15 (m, 8 H), 0.84 - 0.80 (m, 12 H);

^{13}C NMR (100 MHz , $CDCl_3$) δ 175.3, 167.6, 140.5, 126.7, 79.0, 37.9, 36.8, 34.5, 33.0, 32.9, 28.3, 26.3, 25.9, 25.8, 24.8, 20.8, 19.9;

IRυ_{max} (film) ν 3387, 3020, 1710, 1619, 1217 cm^{-1};

HRMS (ESI): Calculated for $C_{19}H_{34}O_3N$ $[M+H]^+$: 324.2533, found 324.2533.

Compound **79** (0.18 g) is a white solid



Melting point 146.1-149.5 °C;

Specific rotation $[\alpha]_D^{26.7}$ = – 28.8 (*c* = 0.43, $CHCl_3$);

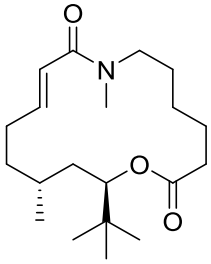
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.64 - 6.56 (m, 1 H), 5.73 (d, *J* = 15.4 Hz, 1 H), 5.57 (br. s., 1 H), 4.83 (d, *J* = 11.2 Hz, 1 H), 3.74 - 3.71 (m, 1 H), 3.09 - 3.12 (m, 1 H), 2.30 - 2.20 (m, 4 H), 1.73 - 1.13 (m, 11 H), 0.85 (br. s, 12 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 166.6, 143.7, 125.3, 78.2, 39.5, 37.0, 35.8, 34.5, 34.0, 28.8, 28.6, 27.0, 25.9, 25.6, 24.8, 17.4;

IRυ_{max} (film) ν 3388, 3368, 3018, 1719, 1612, 1218 cm^{-1};

HRMS (ESI): Calculated for $C_{19}H_{34}O_3N$ $[M+H]^+$: 324.2533, found 324.2534.

(14*R*,16*R*,*E*)-16-(*tert*-Butyl)-8,14-dimethyl-1-oxa-8-azacyclohexadec-10-ene-2,9-dione (80)



A solution of amide **78** (0.15 g, 0.46 mmol) was dissolved in dry THF (3 mL), cooled to 0 °C, and treated with sodium hydride (60% dispersion, 91 mg, 2.30 mmol). The cooling bath was removed, and the flask was allowed to warm to room temperature and stirred for 20 min. The reaction mixture was cooled again to 0 °C and Iodomethane (0.3 mL. 4.60 mmol) was added dropwise. After 30 min at 0 °C, the reaction mixture was stirred at room temperature for 4 hours and then diluted with EtOAc (10 mL) and quenched with saturated ammonium chloride solution (10 mL). The phases were separated, and the aqueous phase was extracted with EtOAc (2 x 10 mL). The combined organic extracts were then dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography over silica gel (18-20% EtOAc/CH_2Cl_2) afforded desired macrocycle **80** (0.15 g, 95%).

Specific rotation $[\alpha]_D^{26.8}$ = + 37.2 (*c* = 2.5, $CHCl_3$);

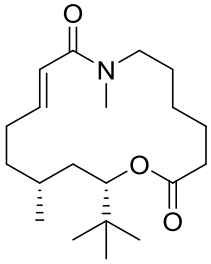
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.71 - 6.63 (m, 1 H), 6.20 (d, *J* = 15.1 Hz, 1 H), 4.89 (d, *J* = 11.2 Hz, 1 H), 3.57 - 3.50 (m, 1 H), 3.26 - 3.20 (m, 1 H), 2.91 (s, 3 H), 2.40 - 2.33 (m, 1 H), 2.27 - 2.15 (m, 2 H), 2.03 (br. s., 1 H), 1.69 - 1.43 (m, 7 H), 1.31 - 1.19 (m, 3 H), 1.09 - 1.02 (m, 1 H), 0.92 (d, *J* = 6.3 Hz, 3 H), 0.84 (s, 9 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 168.1, 143.4, 122.8, 77.1, 49.5, 37.2, 34.8, 33.5, 33.4, 33.1, 29.6, 27.4, 27.3, 26.0, 25.6, 23.5, 19.5;

IRυ_{max} (film) ν 3020, 1721, 1654, 1216 cm^{-1};

HRMS (ESI): Calculated for $C_{20}H_{35}O_3NNa$ $[M+Na]^+$: 360.2509, found 360.2503.

(14*R*,16*S*,*E*)-16-(*tert*-Butyl)-8,14-dimethyl-1-oxa-8-azacyclohexadec-10-ene-2,9-dione (81)



Compound **81** was prepared from **79** following procedure similar to that followed for **80**. Purification over silica gel chromatography (18-20% EtOAc/CH_2Cl_2) afforded **81** (0.16 g, 82%) as a light brown solid;

Melting point 80.1-85.3°C;

Specific rotation $[\alpha]_D^{26.4}$= – 46.5 (*c* = 1.3, $CHCl_3$);

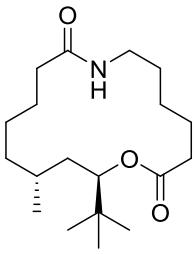
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76 - 6.69 (m, 1 H), 6.25 - 6.22 (m, 1 H), 4.92 (d, *J* = 12.0 Hz, 1 H), 3.69 - 3.65 (m, 1 H), 3.21 - 3.17 (m, 1 H) 2.95 (s, 3 H), 2.38 - 2.21 (m, 4 H), 1.65 - 1.38 (m, 6 H), 1.32 - 1.06 (m, 5 H), 0.92 - 0.86 (m, 12 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 167.3, 145.4, 121.4, 77.7, 49.4, 37.3, 36.0, 34.5, 33.8, 33.1, 29.2, 28.0, 26.3, 25.9, 25.7, 24.8, 17.1;

IRυ_{max} (film) ν 3022, 2928, 1716, 1597, 1417, 1218 cm^{-1};

HRMS (ESI): Calculated for $C_{20}H_{35}O_3NNa$ $[M+Na]^+$: 360.2509, found 360.2502.

(14*R*,16*R*)-16-(*tert*-Butyl)-14-methyl-1-oxa-8-azacyclohexadecane-2,9-dione (82)



To a solution of **78** (0.12 g, 0.37 mmol) in absolute EtOH (10 mL) was added 10% wt/wt palladium on carbon (15 mg). The atmosphere in the flask was replaced with hydrogen, and the reaction mixture was allowed to stir at room temperature for 4 h before being filtered through a celite pad. After rinsing several times with EtOH, the filtrate was concentrated and purified via column chromatography (20-30% EtOAc/CH_2Cl_2) to afford compound **82** (0.10 g, 82%) as amorphous solid.

Melting point 98.2-100.6 °C;

Specific rotation $[\alpha]_D^{27.0}$ = + 81.4 (*c* = 1.05, $CHCl_3$);

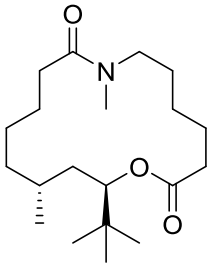
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.45 (br. s., 1 H), 5.03 (dd, *J* = 1.6, 12.1 Hz, 1 H), 3.85 - 3.78 (m, 1 H), 2.96 - 2.89 (m, 1 H), 2.50 - 2.44 (m, 1 H), 2.33 - 2.25 (m, 2 H), 2.19 - 2.14 (m, 1 H), 1.90 - 1.71 (m, 4 H), 1.61 - 1.35 (m, 7 H), 1.26 - 1.10 (m, 4 H), 0.88-0.86 (m, 12 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 173.5, 77.9, 37.5, 36.7, 35.2, 34.4, 33.2, 32.5, 29.5, 26.3, 26.0, 25.6, 25.5, 24.4, 20.8, 20.7;

IRυ_{max} (film) ν 3685, 3021, 1716, 1598, 1217 cm^{-1};

HRMS (ESI): Calculated for $C_{19}H_{36}O_3N$ $[M+H]^+$: 326.2690, found 326.2686.

(14*R*,16*R*)-16-(*tert*-Butyl)-8,14-dimethyl-1-oxa-8-azacyclohexadecane-2,9-dione (83)



Compound **83** was prepared from **82** following procedure similar to that followed for **80**. Purification over silica gel chromatography (10-15% EtOAc/CH_2Cl_2) afforded **83** (31 mg, 83%) as a white solid and appear in nmr as a mixture of rotational isomers.

Melting point 68.9 - 70.7 °C.

Specific rotation $[\alpha]_D^{25.9}$ = + 88.3 (*c* = 0.5, $CHCl_3$);

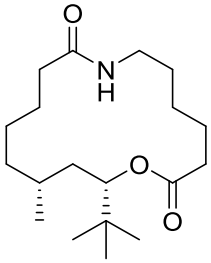
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.99 - 4.93 (m, 1 H), 4.42 (br. s., 1 H), 2.98 - 2.87 (m, 3 H), 2.45 - 2.25 (m, 3 H), 2.30 - 2.13 (m, 2 H), 1.63 - 1.43 (m, 10 H), 1.22 - 1.14 (m, 5 H), 0.88 - 0.83 (m, 12 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 173.3, 77.0, 49.4, 45.4, 37.0, 37.0, 34.9, 34.7, 34.6, 34.5, 33.8, 33.5, 33.3, 32.9, 32.5, 29.4, 29.0, 27.1, 27.1, 26.8, 26.6, 26.0, 25.9, 25.1, 25.1, 24.9, 24.3, 22.8, 20.9, 19.7;

IRυ_{max} (film) ν 3021, 1716, 1611, 1217 cm^{-1};

HRMS (ESI): Calculated for $C_{20}H_{38}O_3N$ $[M+H]^+$: 340.2846, found 340.2843.

(14*R*,16*S*)-16-(*tert*-Butyl)-14-methyl-1-oxa-8-azacyclohexadecane-2,9-dione (84)



To a solution of **79** (68 mg, 0.21 mmol) in absolute EtOH (5 mL) was added 10% palladium on carbon (5 mg). The atmosphere in the flask was replaced with hydrogen, and the reaction mixture was allowed to stir at room temperature for 5 h before being filtered through a short plug of silica. After rinsing several times with EtOH, the filtrate was concentrated and purified via column chromatography (20-30% EtOAc/CH_2Cl_2) to afford compound **84** (53 mg, 77%) as a white solid.

Melting point 123.8-128.6 °C.

Specific rotation $[\alpha]_D^{26.9}$= – 22.1 (*c* = 0.36, $CHCl_3$);

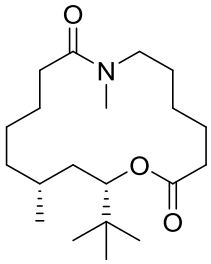
^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.65 (br. s., 1 H), 4.77 (d, *J* = 10.0 Hz, 1 H), 3.41 - 3.39 (m, 1 H), 3.26 - 3.22 (m, 1 H), 2.36 - 2.34 (m, 2 H), 2.19 - 2.14 (m, 2 H), 1.69 - 1.47 (m, 8 H), 1.37 - 1.14 (m, 7 H), 0.92 (d, *J* = 4.4 Hz, 3 H), 0.89 (br. s., 9 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 173.2, 79.5, 39.4, 37.0, 36.3, 36.3, 34.7, 34.5, 30.6, 29.3, 27.2, 26.3, 25.8, 25.3, 25.2, 20.3;

IRυ_{max} (film) ν 3685, 3021, 1716, 1599, 1217 cm^{-1};

HRMS (ESI): Calculated for $C_{19}H_{36}O_3N$ $[M+H]^+$: 326.2690, found 326.2687.

(14*R*,16*S*)-16-(*tert*-Butyl)-8,14-dimethyl-1-oxa-8-azacyclohexadecane-2,9-dione (85)



Compound **85** was prepared from **84** following procedure similar to that followed for 8**0**. Purification over silica gel chromatography (10-15% EtOAc/CH_2Cl_2) afforded **85** (37 mg, 73%) as white solid and appear in nmr as a mixture of rotational isomers.

Specific rotation $[\alpha]_D^{26.3}$= – 9.38 (*c* = 0.65, $CHCl_3$).

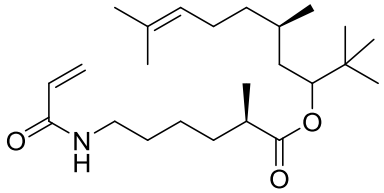
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.91 - 4.75 (m, 1 H), 3.41 - 3.38 (m, 3 H), 2.98 - 2.88 (m, 3 H), 2.40 - 2.33 (m, 2 H), 2.28 - 2.20 (m, 1 H), 1.64 - 1.50 (m, 5 H), 1.45 - 1.15 (m, 10 H), 0.94 - 0.83 (m, 12 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 173.2, 79.5, 78.7, 50.1, 47.0, 45.8, 37.1, 36.6, 36.4, 35.7, 34.5, 33.7, 33.6, 32.5, 32.0, 30.3, 28.1, 27.5, 27.3, 26.1, 25.9, 25.8, 25.7, 25.3, 25.2, 24.8, 24.5, 20.5, 19.0, 8.7.

IRυ_{max} (film) ν 2988, 1716, 1598, 1217 cm^{-1}.

HRMS (ESI): Calculated for $C_{20}H_{37}O_3NNa$ $[M+Na]^+$: 362.2666, found 362.2661.

(5*R*)-2,2,5,9-Tetramethyldec-8-en-3-yl (2*R*)-6-acrylamido-2-methylhexanoate (86)



Compound **86** was prepared from **60** and **57** following similar procedure to that followed for compound **77**. The silica gel column chromatography (20-30% EtOAc/ Petroleum ether) of **86** afforded colorless liquid as a mixture of diastereomers (0.11 g, 41%).

^{1}H NMR (200 MHz, $CDCl_3$) δ 6.33 - 6.02 (m, 2 H), 5.86 (br. s., 1 H), 5.62 (dd, *J* = 1.8, 9.9 Hz, 1 H), 5.09 - 5.06 (m, 1 H), 4.87 - 4.82 (m, 1 H), 3.36 - 3.28 (m, 2 H), 2.45 - 2.38 (m, 1 H), 1.97 - 1.74 (m, 3 H), 1.67 (s, 3 H), 1.59 (s, 3 H), 1.52 - 1.23 (m, 10 H), 1.19 - 1.14 (m, 3 H), 0.92 - 0.79 (m, 12 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 176.3, 165.6, 131.0, 131.0, 126.0, 124.7, 124.6, 78.3, 78.2, 40.0, 39.9, 39.3, 38.2, 37.3, 36.9, 35.4, 34.6, 33.1, 33.0, 29.3, 29.2, 29.0, 26.0, 25.7, 25.5, 25.2, 24.6, 24.6, 20.7, 19.0, 17.6, 17.6, 17.5;

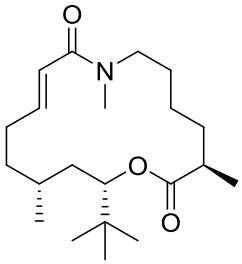
IRυ_{max} (film) ν 3287, 1728, 1663, 1541, 1239 cm^{-1};

HRMS (ESI): Calculated for $C_{24}H_{43}O_3NNa$ $[M+Na]^+$: 416.3135, found 416.3131.

(3*R*,14*R*,16*S*,*E*)-16-(*tert*-Butyl)-3,8,14-trimethyl-1-oxa-8-azacyclohexadec-10-ene-2,9-dione (88) and (3*R*,14*R*,16*R*,*E*)-16-(*tert*-butyl)-3,8,14-trimethyl-1-oxa-8-azacyclohexadec-10-ene-2,9-dione (87)

Compound **87** and **88** were prepared from **86** by using similar ring closing metathesis procedure followed for compounds **78, 79**. The silica gel column chromatography (30-40% EtOAc/ Petroleum ether) of crude residue obtained afforded macrolide as a mixture of diastereomers (57 mg, 58%). The mixture was then subjected to *N*-methylation procedure similar to the one followed for compound 8**0** and obtained compounds **87** and **88**. Careful silica gel chromatography (30-40% EtOAc/Petroleum ether) of crude compound yielded **87** and **88** as two separate diastereomers (48 mg, 80%).

Compound **88** (22 mg) is a crystalline solid.



Melting point 100.3 °C.

Specific rotation $[\alpha]_D^{27.1}$= – 18.9 (*c* = 0.5, $CHCl_3$);

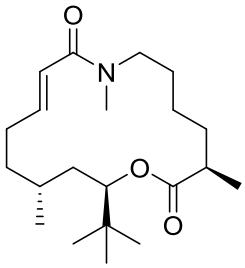
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76 - 6.69 (m, 1 H), 6.25 - 6.21 (m, 1 H), 4.91 (d, *J* = 11.0 Hz, 1 H), 3.73 - 3.67 (m, 1 H), 3.17 - 3.13 (m, 1 H), 2.95 (s, 3 H), 2.39 - 2.21 (m, 3 H), 1.77 - 1.40 (m, 9 H), 1.22 - 1.18 (m, 2 H), 1.15 (d, *J* = 6.8 Hz, 3 H), 0.93 - 0.82 (m, 12 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 167.3, 145.4, 121.4, 77.2, 49.5, 40.4, 37.3, 36.0, 34.6, 34.5, 33.0, 29.2, 28.3, 26.1, 26.0, 25.0, 19.4, 16.9;

IRυ_{max} (film) ν 3019, 2966, 1714, 1610, 1468, 1217 cm^{-1};

HRMS (ESI): Calculated for $C_{21}H_{37}O_3NNa$ $[M+Na]^+$: 374.2666, found 374.2670.

Compound **87** (26 mg) is a crystalline solid.



Melting point 80.1 °C.

Specific rotation $[\alpha]_D^{26.0} = -5.97$ (*c* = 0.72, $CHCl_3$).

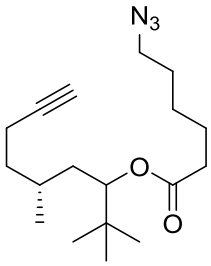
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.40 - 6.32 (m, 1 H), 6.18 - 6.14 (d, *J* = 15.4 Hz, 2 H), 4.90 (d, *J* = 11.0 Hz, 1 H), 3.49 - 3.46 (m, 1 H), 3.27 - 3.23 (m, 1 H), 2.90 (s, 3 H), 2.52 - 2.48 (m, 1 H), 2.33 - 2.18 (m, 2 H), 1.94 - 1.86 (m, 1 H), 1.72 - 1.28 (m, 8 H), 1.18 (d, *J* = 7.1 Hz, 3 H), 1.06 - 0.97 (m, 1 H), 0.91 - 0.84 (m, 12 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 175.0, 169.0, 141.6, 124.8, 76.9, 49.6, 41.2, 37.9, 34.8, 33.4, 32.8, 32.1, 29.7, 27.4, 26.6, 26.0, 23.7, 19.5, 17.6.

IRυ_{max} (film) ν 3020, 2965, 1712, 1611, 1215 cm^{-1}.

HRMS (ESI): Calculated for $C_{21}H_{37}O_3NNa$ $[M+Na]^+$: 374.2666, found 374.2659.

(5*R*)-2,2,5-Trimethylnon-8-yn-3-yl 6-azidohexanoate (89)



Compound **89** was prepared from alkyne **61** and 6-azidohexanoic acid following similar procedure to that followed for compound **77**. The silica gel column chromatography (20-30% EtOAc/Petroleum ether) of **89** afforded colorless oil as a mixture of diastereomers (0.11 mg, 65%).

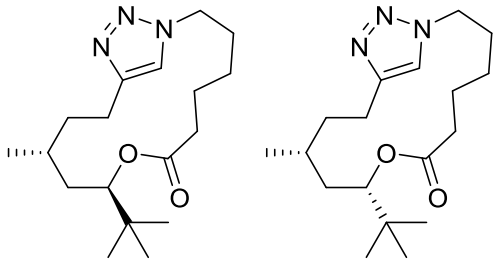
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.85 - 4.81 (m, 1H), 3.25 (t, *J* = 6.85 Hz, 2H), 2.34 - 2.29 (m, 2H), 2.20 - 2.10 (m, 2H), 1.91 - 1.89 (m, 1H), 1.75 - 1.57 (m, 4H), 1.47 - 1.36 (m, 5H), 1.24 - 1.18 (m, 2H), 0.89 - 0.86 (m, 12H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 173.1, 84.5, 78.2, 78.1, 68.2, 51.2, 36.9, 36.5, 34.7, 34.5, 34.3, 34.1, 29.1, 28.6, 26.3, 25.9, 25.9, 24.5, 20.1, 18.6, 16.1, 16.0.

IRυ_{max} (film) ν 3021, 2873, 2433, 1720, 1575, 1216 cm^{-1}.

HRMS (ESI) Calculated for $C_{18}H_{31}O_2N_3Na$ $[M+Na]^+$: 344.2308, found 344.2306.

Synthesis of Triazoles 90 and 91:



90 **91**

To a sealed tube equipped with a stirring bar was added **89** (50 mg, 0.15 mmol), polyethylene glycol 400 (3.3 mL) and methanol (1.6 mL). The mixture was stirred for 30 seconds to make homogeneous mixture of two solvents. Triethylamine (0.16 mL, 1.24 mmol) and CuI (6 mg, 0.031 mmol, 20 mol%) were added to the reaction mixture. The tube was closed and heated at 60 °C for 17 hours (no precaution was taken to remove air or moisture). The reaction was then cooled back to room temperature and concentrated to remove excess methanol. Water (4 mL) was added and aqueous layer was extracted with EtOAc (2 x 5 mL), concentrated to obtain crude compound which was loaded directly on a silica column. Purification by chromatography (50-60% EtOAc/hexane) afforded the two diastereomers* in 2:1 ratio (33 mg, 66%).

Data for Compound **90** (21mg).

Specific rotation $[\alpha]_D^{25.3}$= + 43.6 (*c* = 0.19, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 1 H), 5.00 (dd, *J* = 0.98, 11.74 Hz, 1 H), 4.58 (td, *J* = 4.28, 13.69 Hz, 1 H), 4.26 (ddd, *J* = 3.18, 11.43, 14.00 Hz, 1 H), 3.00 - 2.96 (m, 1 H), 2.86 - 2.81 (m, 1 H), 2.45 (ddd, *J* = 2.32, 5.87, 15.77 Hz, 1 H), 2.31 - 2.22 (m, 1 H), 2.18

- 2.06 (m, 2 H), 1.98 - 1.92 (m, 1 H), 1.77 - 1.70 (m, 2 H), 1.52 - 1.27 (m, 5 H), 1.18 - 1.12 (m, 1 H), 0.91 - 0.86 (m, 12 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 146.3, 123.6, 77.9, 47.8, 37.8, 34.5, 33.7, 32.7, 28.9, 25.9, 25.3, 24.0, 22.2, 21.9, 19.7.

IRυ_{max} (film) ν 3346, 3022, 1575, 1217 cm^{-1}

HRMS (ESI): Calculated for $C_{18}H_{32}O_2N_3$ $[M+H]^+$: 322.2490, found 322.2484.

Data for Compound **91** (12mg):

Specific rotation $[\alpha]_D^{25.3}$= – 2.3 (*c* = 0.16, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (s, 1 H), 4.82 (d, *J* = 10.5 Hz, 1 H), 4.44 - 4.40 (m, 2 H), 2.87 - 2.81 (m, 2 H), 2.26 - 2.21 (m, 2 H), 1.90 - 1.85 (m, 2 H), 1.68 - 1.43 (m, 8 H), 1.17 - 1.08 (m, 1 H), 0.91 - 0.82 (m, 12 H).

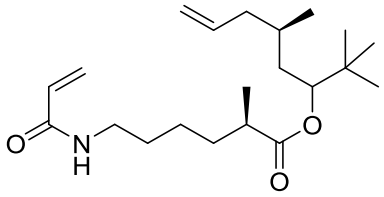
^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 147.0, 121.4, 78.5, 49.8, 37.2, 37.1, 34.5, 33.9, 29.7, 29.5, 25.9, 25.6, 24.0, 22.2, 17.6.

IRυ_{max} (film) ν 3346, 3021, 1575, 1217 cm^{-1}

HRMS (ESI): Calculated for $C_{18}H_{32}O_2N_3$ $[M+H]^+$: 322.2490, found 322.2488.

*The two isomers were cleanly separated however the absolute stereochemistry could not be assigned to them.

(5*R*)-2,2,5-Trimethyloct-7-en-3-yl (2*R*)-6-acrylamido-2-methylhexanoate (92)



Compound **92** was prepared from acid **60** and alkenol **58** following similar procedure to that followed for compound **77**. The silica gel column chromatography (40-50% EtOAc/Petroleum ether) of crude product afforded colorless oil **92** as a mixture of diastereomers (0.19 g, 62%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.25 - 6.06 (m, 3 H), 5.72 - 5.67 (m, 1 H), 5.69 (dd, *J* = 9.5, 17.4 Hz, 1 H), 4.99 - 4.93 (m, 2 H), 4.83 - 4.80 (m, 1 H), 3.32 - 3.25 (m, 2 H), 2.42 -

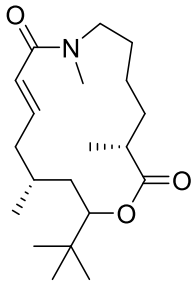
2.39 (m, 1 H), 2.20 - 2.18 (m, 1 H), 1.98 - 1.91 (m, 1 H), 1.70 - 1.66 (m, 1 H), 1.56 - 1.48 (m, 2 H), 1.41 - 1.23 (m, 6 H), 1.16 - 1.13 (m, 3 H), 0.89 - 0.82 (m, 12 H);

^{13}C NMR (125 MHz, $CDCl_3$) δ 176.5, 176.5, 165.6, 137.1, 136.6, 131.0, 126.1, 116.2, 115.9, 78.2, 78.1, 42.4, 40.0, 39.9, 39.4, 39.3, 36.5, 36.4, 36.4, 34.6, 34.6, 33.1, 33.1, 29.4, 29.4, 29.3, 29.2, 26.0, 24.6, 24.6, 20.6, 18.9, 17.5;

IRυ_{max} (film) ν 3268, 1730, 1625, 1551, 1230 cm^{-1};

HRMS (ESI): Calculated for $C_{21}H_{37}O_3NNa$ $[M+Na]^+$: 374.2666, found 374.2661.

(3*R*,13*R*,*E*)-15-(*tert*-Butyl)-3,8,13-trimethyl-1-oxa-8-azacyclopentadec-10-ene-2,9-dione (93)



A solution of **92** (50 mg, 0.14 mmol) in toluene (46 mL, 0.003M) was degassed for 15 minutes and added with 5 mol% of Grubbs' IInd generation catalyst (**G-II**) (6 mg, 0.007 mmol) and stirred at room temperature for 12 hours. The reaction was then concentrated under reduced pressure to remove excess toluene and purified by flash chromatography over silica gel (50-60% EtOAc/Petroleum ether) to afford the desired macrocycles as a mixture of diastereomers. The mixture (20 mg, 0.06 mmol) was as such taken in dry THF (3 mL), cooled to 0 °C and added with sodium hydride (60% dispersion in oil, 7.4 mg, 0.31 mmol). The cooling bath was then removed, and the flask was allowed to warm to room temperature and stirred for 20 min. The reaction mixture was cooled again to 0 °C and Iodomethane (30 μL, 0.61 mmol) was then added dropwise. After 30 min, it was diluted with EtOAc (5 mL) and quenched with saturated aqueous ammonium chloride (8 mL). The phases were separated, and the aqueous phase was extracted with EtOAc (3 x 5 mL). The combined organic extracts were then dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography over silica gel (60-70% EtOAc/Petroleum ether) afforded desired macrocycles as inseparable mixture of diastereomers** **93** (17 mg, 35% for two steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.75 - 6.34 (m, 1 H), 6.24 - 6.14 (m, 1 H), 4.92 - 4.85 (m, 1 H), 3.75 - 3.10 (m, 2 H), 3.01 (s, 3 H), 2.34 - 2.21 (m, 3 H), 1.93 - 1.34 (m, 9 H), 1.17 - 1.14 (m, 3 H), 0.94 - 0.87 (m, 12 H);

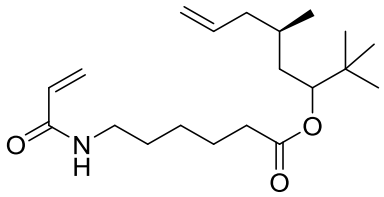
^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 175.1, 169.0, 167.3, 145.4, 141.7, 124.8, 121.4, 77.3, 76.9, 49.5, 41.2, 40.4, 37.9, 37.3, 36.0, 34.8, 34.6, 34.5, 33.4, 33.0, 32.8, 29.7, 29.2, 28.3, 27.4, 26.7, 26.0, 26.0, 25.0, 23.7, 19.5, 19.4, 17.6, 16.9.

IRυ_{max} (film) ν 3019, 2961, 1714, 1612, 1478, 1216 cm^{-1}.

HRMS (ESI): Calculated for $C_{20}H_{36}O_3N$ $[M+H]^+$: 338.2690, found 338.2683.

**Diastereomers could not be separated on column chromatography inspite of best efforts.

(5*R*)-2,2,5-Trimethyloct-7-en-3-yl 6-acrylamidohexanoate (94)



To a solution of acid **59** (2.01 g, 10.80 mmol) in toluene (15 mL), was added 2,4,6-trichlorobenzylchloride (1.70 mL, 10.8 mmol), followed by triethylamine (2.80 mL, 20.1 mmol) and reaction mixture was allowed to stir for 3 hours. Alcohol **58** (0.85 g, 5.40 mmol) and DMAP (1.31 g, 10.80 mmol) were taken in toluene and added to above reaction mixture dropwise and stirred at room temperature for 12 h before being diluted with EtOAc (20 mL). Then the organic layer was washed with saturated $NaHCO_3$ (2 x 10 mL), brine (15 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography over silica gel (50-60% EtOAc/Petroleum ether) afforded **94** (0.96 g, 57%) as a colorless oil.

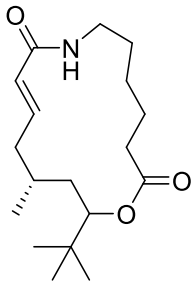
^{1}H NMR (200 MHz, $CDCl_3$) δ 6.32 - 6.01(m, 2 H), 5.86 (br. s., 1 H), 5.58 - 5.53 (m, 2 H), 5.03 - 4.95 (m, 2 H), 4.87 - 4.81 (m, 1 H), 3.38 - 3.28 (m, 2 H), 2.36 - 2.28 (m, 2 H), 2.04 - 1.89 (m, 2 H), 1.73 - 1.17 (m, 9 H), 0.91 - 0.86 (m, 12 H).

^{13}C NMR (50 MHz, $CDCl_3$) δ 173.6, 165.7, 137.1, 136.6, 131.0, 126.0, 116.2, 115.9, 78.6, 78.4, 42.4, 39.5, 39.3, 36.4, 36.2, 34.7, 34.6, 34.3, 29.5, 29.4, 29.1, 26.5, 25.9, 24.6, 20.6, 18.9.

IRυ_{max} (film) ν 3268, 1730, 1625, 1551, 1230 cm^{-1}.

HRMS (ESI): Calculated for $C_{20}H_{36}O_3N$ $[M+H]^+$: 338.2689, found 338.2681.

(13*R*,*E*)-15-(*tert*-Butyl)-13-methyl-1-oxa-8-azacyclopentadec-10-ene-2,9-dione (95):



Compound **95** was prepared from **94** following similar procedure to that followed for compound **78** to afford it as a mixture of inseparable diastereomers (0.37 g, 81%).

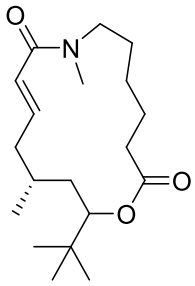
^{1}H NMR (200 MHz, $CDCl_3$) δ 6.84 - 6.69 (m, 1 H), 6.59 - 6.53 (m, 1 H), 5.89 - 5.77 (m, 1 H), 4.95 - 4.81 (m, 1 H), 3.42 - 3.09 (m, 2 H), 2.37 - 2.28 (m, 2 H), 2.10 - 2.03 (m, 1 H), 1.83 - 1.18 (m, 10 H), 0.97 - 0.87 (m, 12 H).

^{13}C NMR (50 MHz, $CDCl_3$) δ 173.5, 166.3, 143.1, 142.5, 125.3, 124.8, 78.3, 40.5, 40.3, 39.5, 39.3, 37.2, 37.1, 37.0, 36.8, 34.6, 34.5, 33.8, 30.3, 30.2, 29.6, 29.4, 26.4, 26.3, 25.9, 25.0, 24.3, 20.6, 19.5, 19.3.

IRυ_{max} (film) ν 3056, 2962, 1714, 1610, 1468, 1217 cm^{-1}.

HRMS (ESI) Calculated for $C_{18}H_{32}O_3N$ $[M+H]^+$: 310.2377, found 310.2374.

(*13R,E*)-15-(*tert*-Butyl)-8,13-dimethyl-1-oxa-8-azacyclopentadec-10-ene-2,9-dione (96)



Compound **96** was prepared from **95** following procedure similar to that followed for compound **80** to afford crude residue which on purification over silica gel chromatography yielded **91** as semi solid (52 mg, 61%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89 - 6.77 (m, 1H), 6.24 - 6.17 (m, 1 H), 4.87 - 4.82 (m, 1 H), 3.35 - 3.30 (m, 2 H), 3.04 - 2.94 (m, 3 H), 2.31 - 2.28 (m, 2 H), 2.02 - 2.11 (m, 2 H), 1.13 - 1.65 (m, 9 H), 0.85 - 0.94 (m, 12 H).

^{13}C NMR (50 MHz, $CDCl_3$) δ 173.5, 173.2, 166.6, 166.3, 144.3, 144.0, 121.9, 121.7, 121.4, 78.4, 78.1, 49.8, 47.9, 40.6, 36.5, 35.5, 34.6, 34.2, 33.9, 30.0, 29.9, 28.7, 26.9, 26.3, 25.9, 24.9, 20.7, 19.2.

IRυ_{max} (film) ν 3022, 1720, 1605, 1217 cm^{-1}.

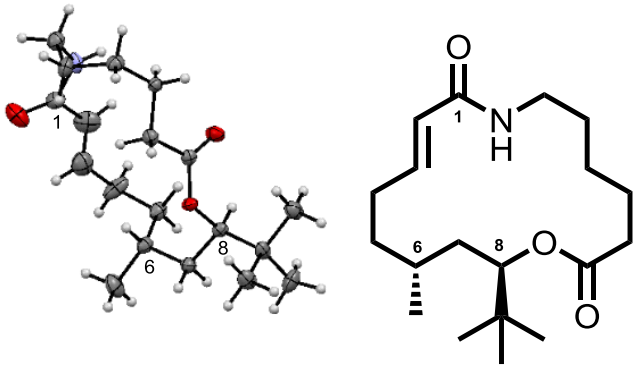
HRMS (ESI): Calculated for $C_{19}H_{33}O_3NNa$ $[M+Na]^+$: 346.2352, found 346.2343.

Single X-ray Crystal Structures of 78 and 88

Single crystals of compound **78** and **88** were obtained from Ethyl acetate/ Hexanes solvent system. X-ray intensity data were collected on a SMART APEX II DUO diffractometer with graphite-monochromatized (Mo Kα=0.71073 Å) radiation at 296 K. ORTEPs were generated using Mercury program. All the H-atoms were located in the difference Fourier and refined isotropically.

1.6 Crystal structure data

Compound 78:



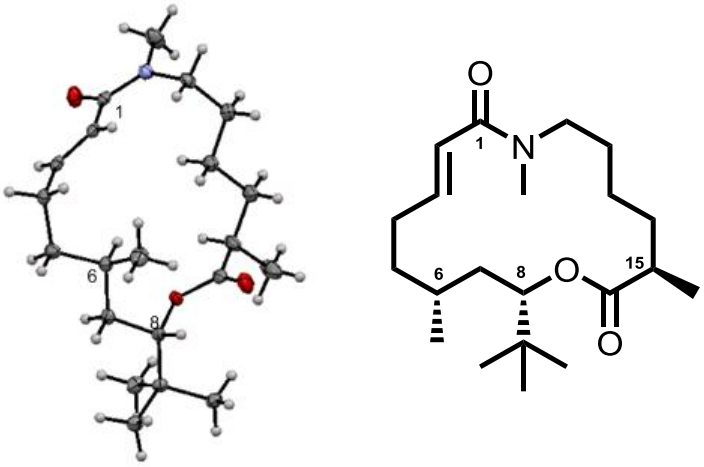
ORTEP of Compound **78 (**50% probability for the thermal ellipsoids)

Crystallographic data for compound **78** deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. **CCDC1454803**.

Crystallographic data for 78 ($C_{19}H_{33}O_3N$): *M* =323.2, Crystal dimensions 0.450 x 0.360 x 0.180 mm^3, monoclinic, space group *P* 2_1,*a* = 11.998(3), *b* = 8.740(2), *c* = 18.751(5) Å, *α* =

90.00°,β= 104.393(6)°, γ =90.00°, V = 1904.6 (8) Å^3, Z = 4, ρ_{calcd} = 1.128gcm^{-3}, μ (Mo-K$_\alpha$) = 0.075mm^{-1}, *F(000)* = 712.0, 2θ_{max} = 56.68°, T = 296 K, 36143 reflections collected, 9449unique, 5015 observed ($I > 2\sigma$ (I)) reflections, 424 refined parameters, R value 0.0701, $wR2$ = 0.0.1336, (all data R = 0.1626, $wR2$ = 0.1757), S = 0.987, minimum and maximum transmission 0.967 and 0.987; maximum and minimum residual electron densities 0.674 and -0.316 eÅ^{-3}.

Compound 88:

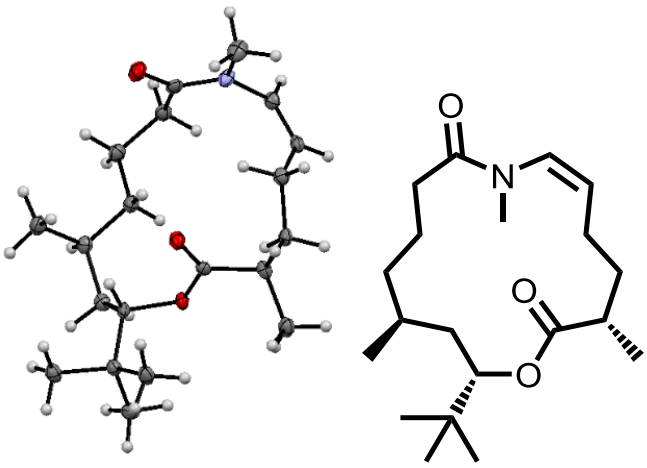


ORTEP of Compound **30 (**50% probability for the thermal ellipsoids)

Crystallographic data for compound **88** deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. **CCDC1454804**.

Crystallographic data for 88 ($C_{21}H_{37}O_3N$): M = 337.2, Crystal dimensions 0.460 x 0.320 x 0.110 mm^3, Orthorhombic, space group $P\ 2_12_12_1$,a = 5.615 (3), b = 10.654 (5), c = 35.955(17) Å, α = 90.00°, β= 90.00°, γ = 90.00°, V = 2151.0 (18) Å^3, Z = 4, ρ_{calcd} = 1.085gcm^{-3}, μ (Mo-K$_\alpha$) = 0.071 mm^{-1}, *F(000)* = 776.0, 2θ_{max} = 52°, T = 296 K, 19159 reflections collected, 4182 unique, 3406 observed ($I > 2\sigma$ (I)) reflections, 232 refined parameters, R value 0.0445, $wR2$ = 0.0607, (all data R = 0.0617, $wR2$ = 0.0628), S = 1.618, minimum and maximum transmission 0.968 and 0.992; maximum and minimum residual electron densities 0.166 and -0.201 e.Å^{-3}

Compound 55:

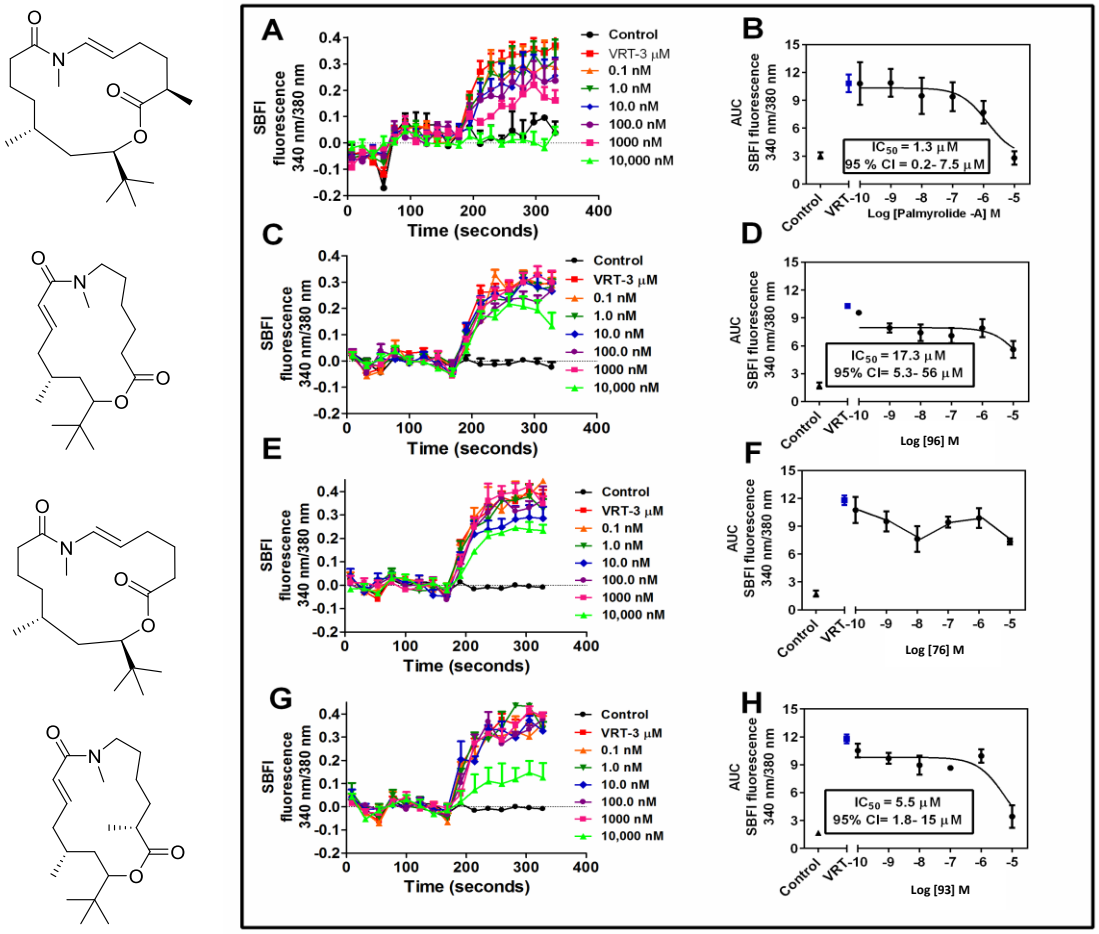


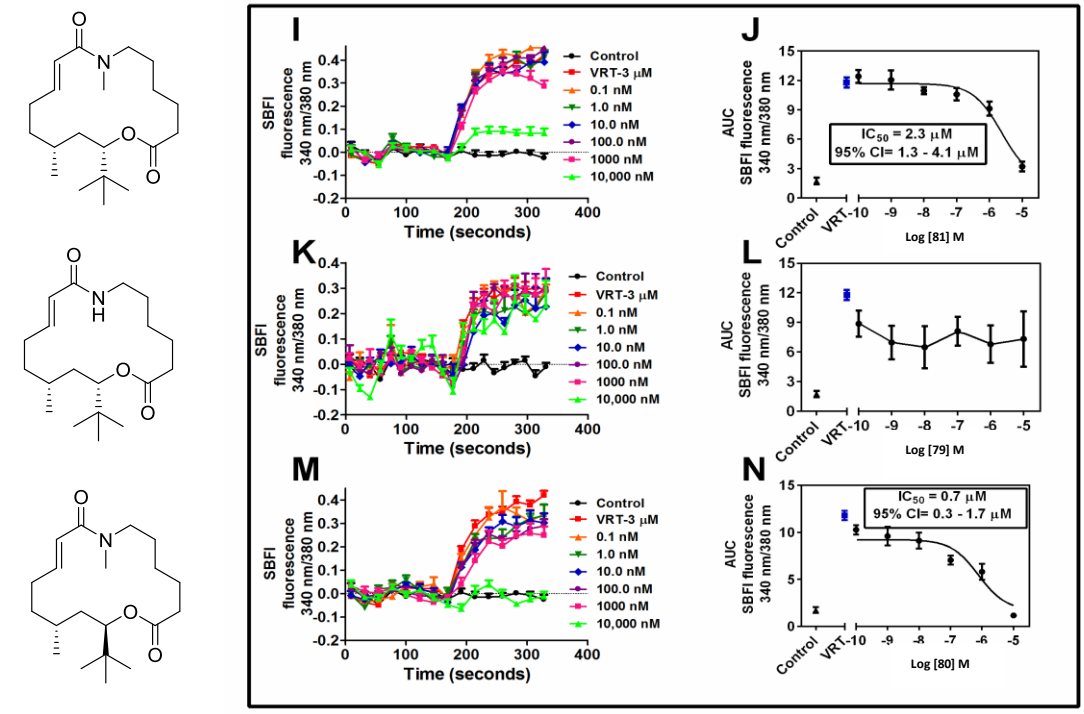
ORTEP of Compound **55** (50% probability for the thermal ellipsoids)

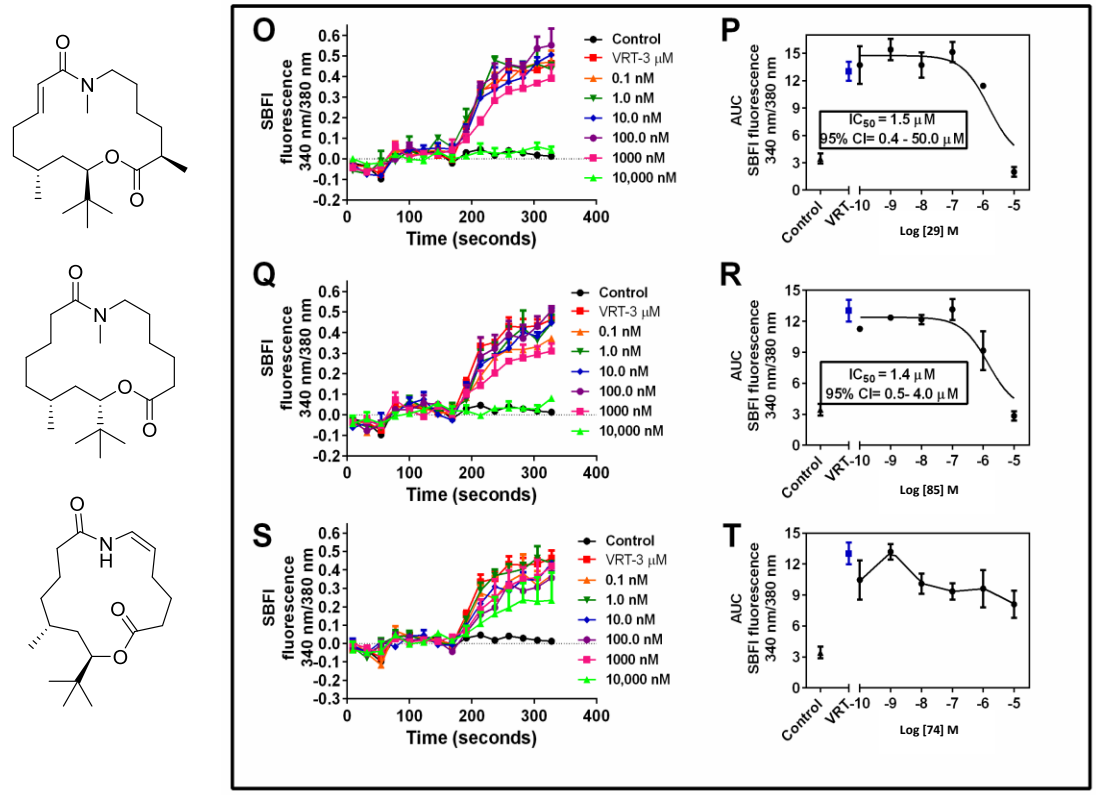
Crystallographic data for compound **55** deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. **CCDC920726.**

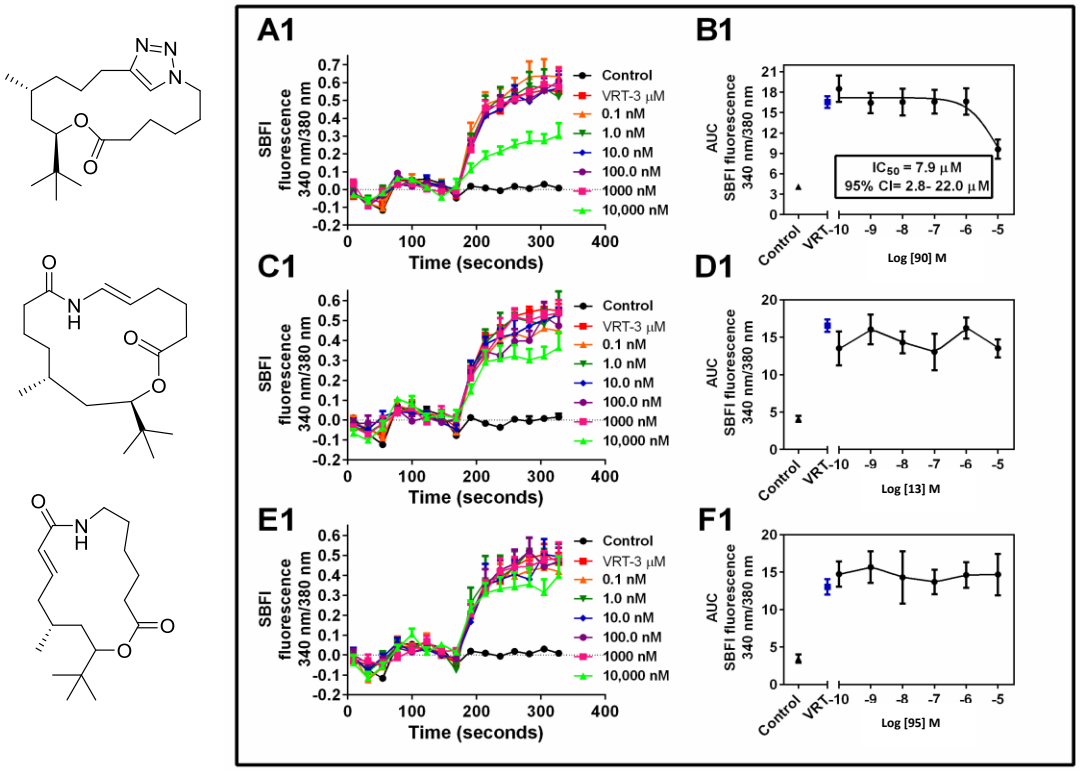
Inhibition of VRT induced sodium influx for all syntheiszed analogues:

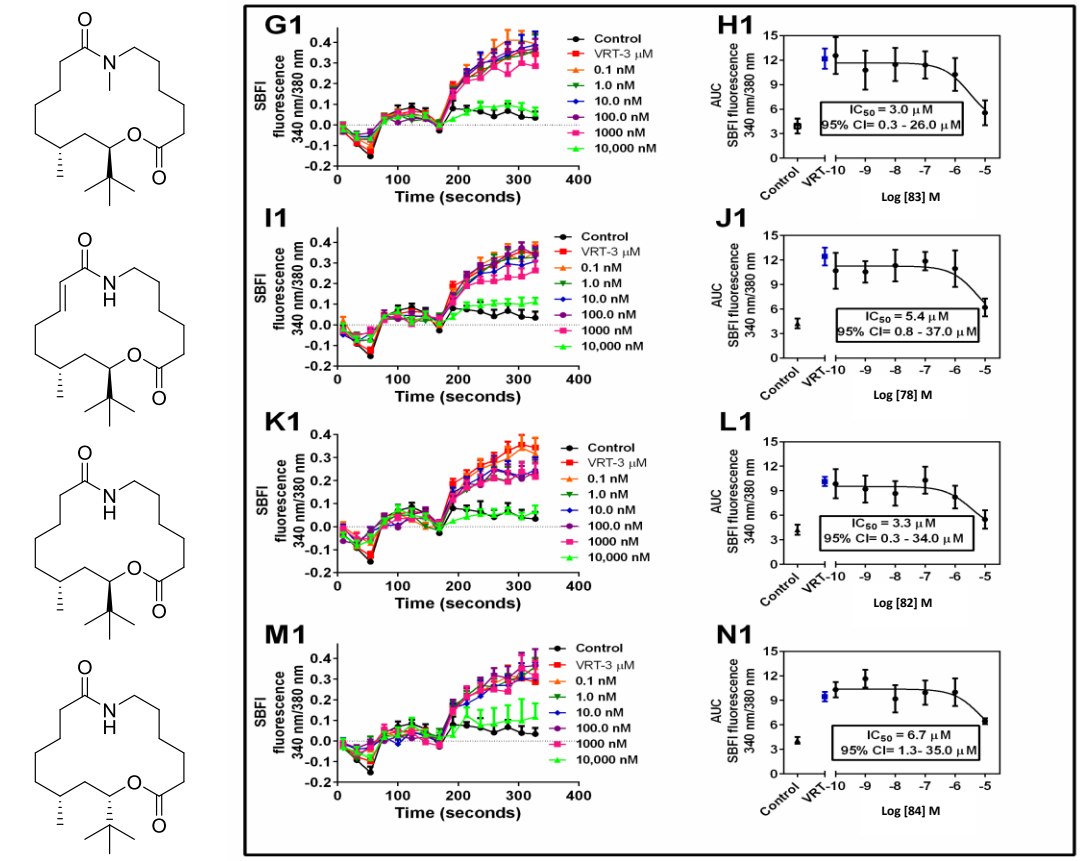
Time and concentration-response analysis of (−)-palmyrolide A, **(-)-1** and its analogues where sodium influx was monitored with SBFI (340/380) responses to veratridine in the absence and presence of each analogue.


A
B
C
D
E
F
G
H
SBFI fluorescence 340 nm/380 nm
Time (seconds)
Control
VRT-3 μM
0.1 nM
1.0 nM
10.0 nM
100.0 nM
1000 nM
10,000 nM
AUC SBFI fluorescence 340 nm/380 nm
IC50 = 1.3 μM
95 % CI = 0.2- 7.5 μM
Log [Palmyrolide -A] M
IC50 = 17.3 μM
95% CI= 5.3- 56 μM
Log [96] M
Log [76] M
IC50 = 5.5 μM
95% CI= 1.8- 15 μM
Log [93] M


I
J
K
L
M
N
SBFI fluorescence 340 nm/380 nm
Time (seconds)
Control
VRT-3 μM
0.1 nM
1.0 nM
10.0 nM
100.0 nM
1000 nM
10,000 nM
AUC SBFI fluorescence 340 nm/380 nm
IC50 = 2.3 μM
95% CI= 1.3 - 4.1 μM
Log [81] M
Log [79] M
IC50 = 0.7 μM
95% CI= 0.3 - 1.7 μM
Log [80] M


O
SBFI fluorescence 340 nm/380 nm
Time (seconds)
Control
VRT-3 μM
0.1 nM
1.0 nM
10.0 nM
100.0 nM
1000 nM
10,000 nM
P
AUC SBFI fluorescence 340 nm/380 nm
IC50 = 1.5 μM
95% CI= 0.4 - 50.0 μM
Log [29] M
Q
IC50 = 1.4 μM
95% CI= 0.5- 4.0 μM
Log [85] M
S
Log [74] M
U
Log [34] M
W
IC50 = 5.0 μM
95% CI= 1.5 - 15.0 μM
Log [88] M
Y
IC50 = 6.0 μM
95% CI= 1.7 - 20.0 μM
Log [55] M


A1
SBFI fluorescence 340 nm/380 nm
Time (seconds)
Control
VRT-3 μM
0.1 nM
1.0 nM
10.0 nM
100.0 nM
1000 nM
10,000 nM
B1
AUC SBFI fluorescence 340 nm/380 nm
IC50 = 7.9 μM
95% CI= 2.8- 22.0 μM
Log [90] M
C1
Log [13] M
D1
E1
Log [95] M
F1


G1
SBFI fluorescence 340 nm/380 nm
Time (seconds)
Control
VRT-3 μM
0.1 nM
1.0 nM
10.0 nM
100.0 nM
1000 nM
10,000 nM
H1
AUC SBFI fluorescence 340 nm/380 nm
IC50 = 3.0 μM
95% CI= 0.3 - 26.0 μM
Log [83] M
I1
J1
IC50 = 5.4 μM
95% CI= 0.8 - 37.0 μM
Log [78] M
K1
L1
IC50 = 3.3 μM
95% CI= 0.3 - 34.0 μM
Log [82] M
M1
N1
IC50 = 6.7 μM
95% CI= 1.3- 35.0 μM
Log [84] M

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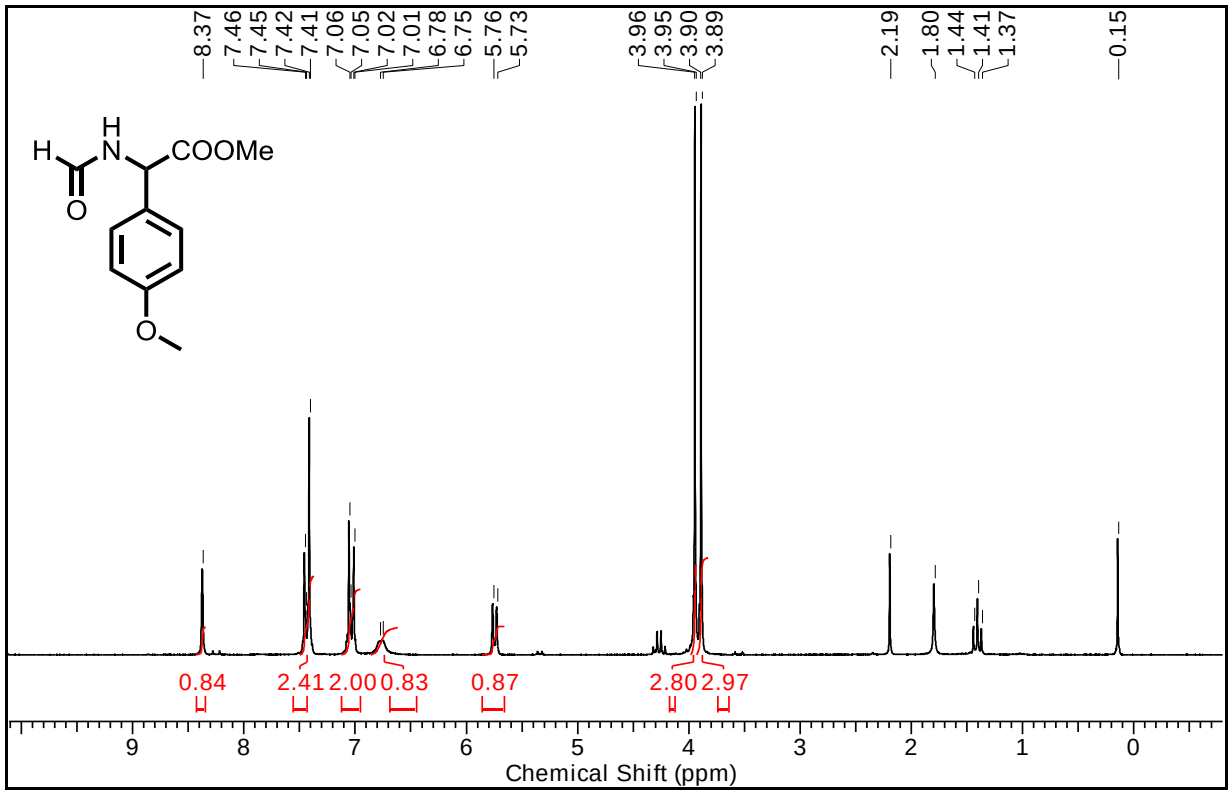
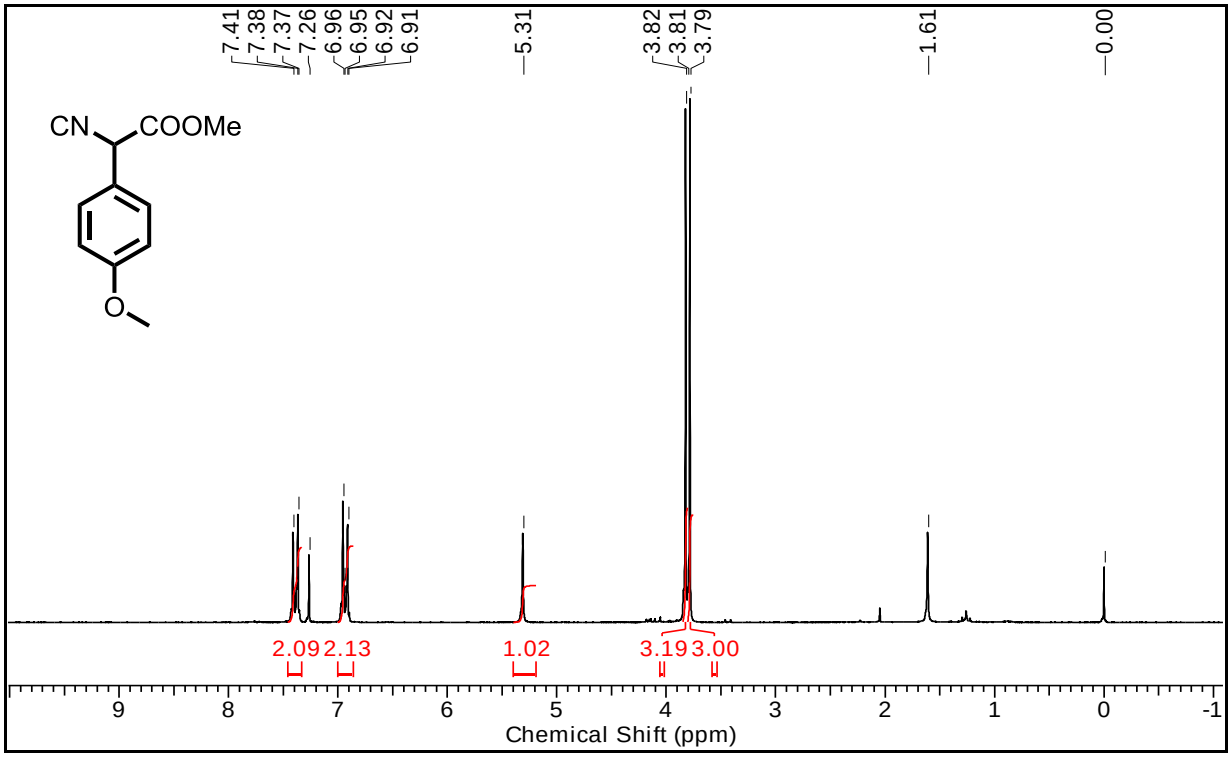
1.8 Copies of ^{1}H and ^{13}C spectra

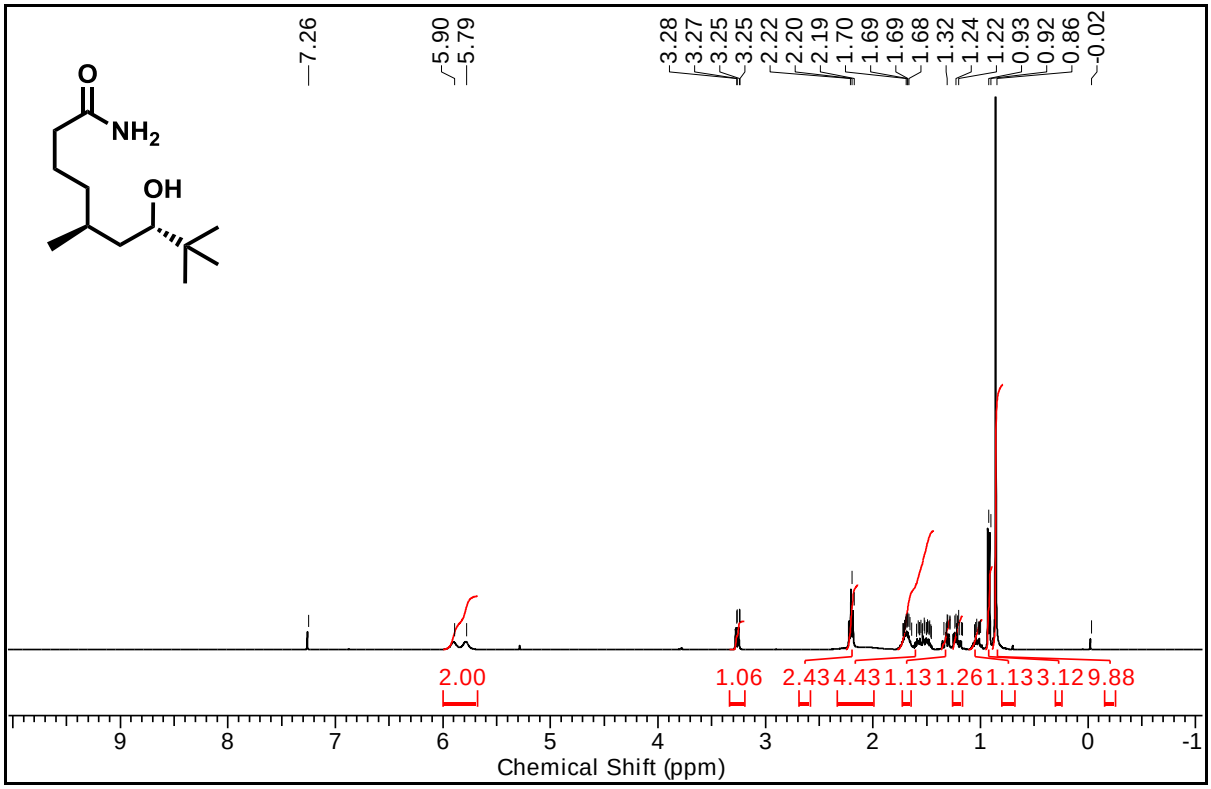
^{1}H NMR of Compound 45 at 200 MHz in $CDCl_3$



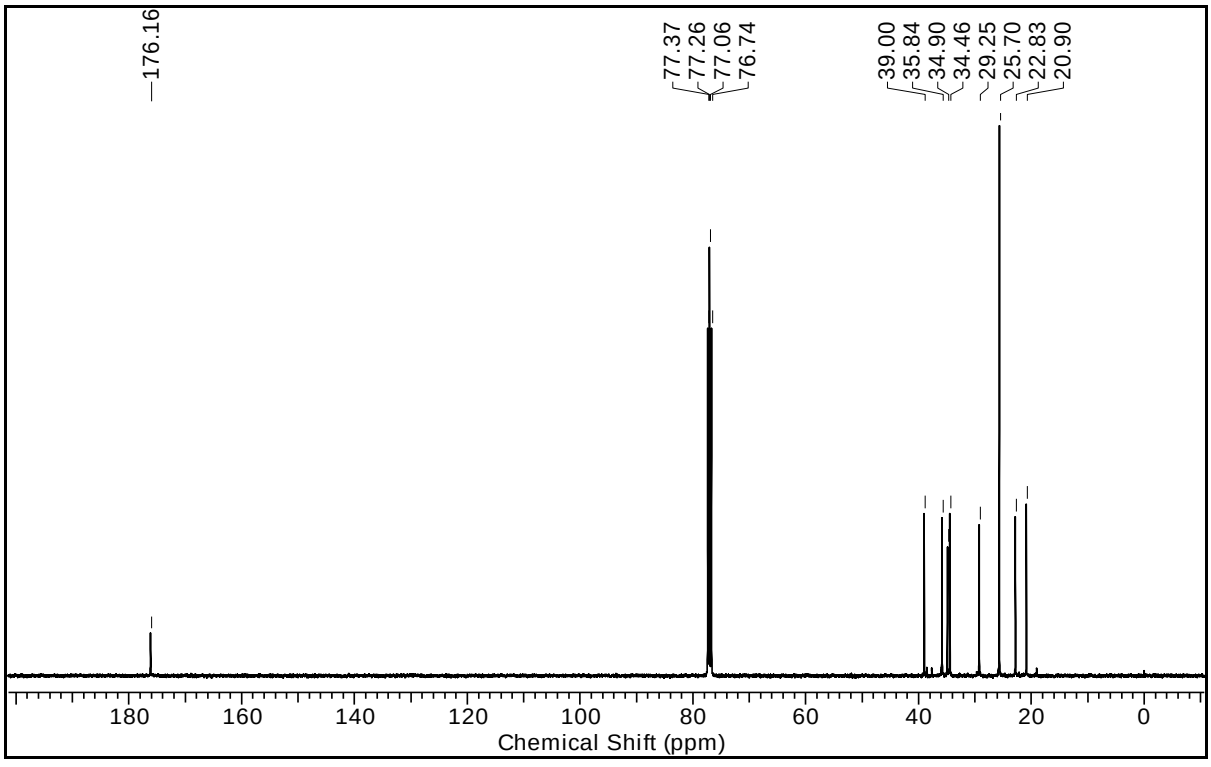
^{1}H NMR of Compound 46 at 200 MHz in $CDCl_3$



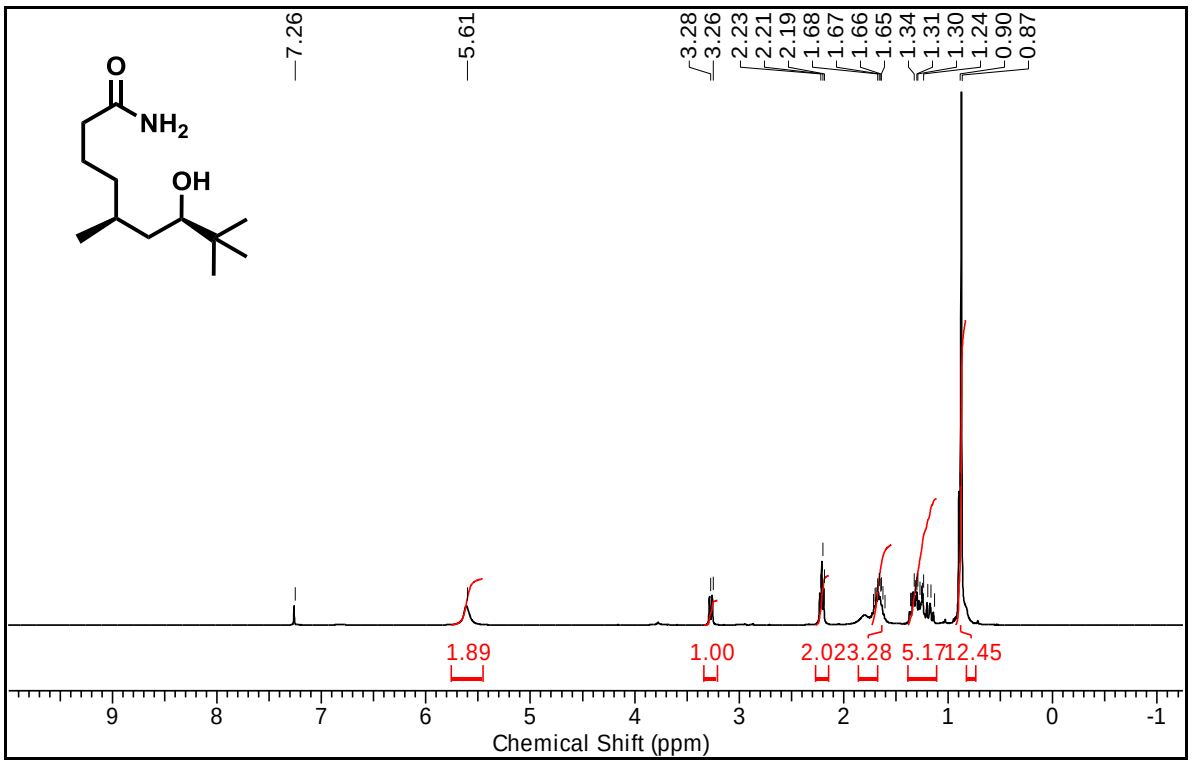
^{1}H NMR of Compound (-)-14 at 400 MHz in $CDCl_3$



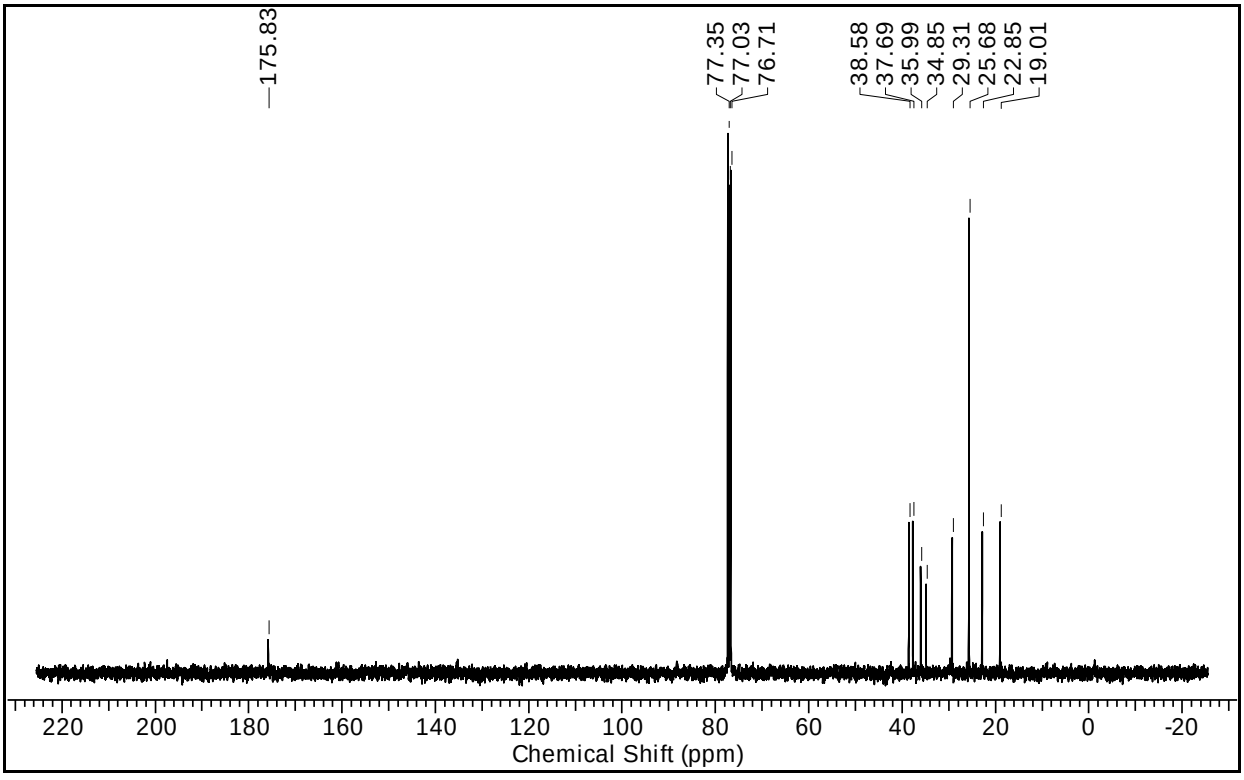
^{13}C NMR of Compound (-)-14 at 100 MHz in $CDCl_3$



^{1}H NMR of Compound (+)-14a at 400 MHz in $CDCl_3$



^{13}C NMR of Compound (+)-14a at 100 MHz in $CDCl_3$



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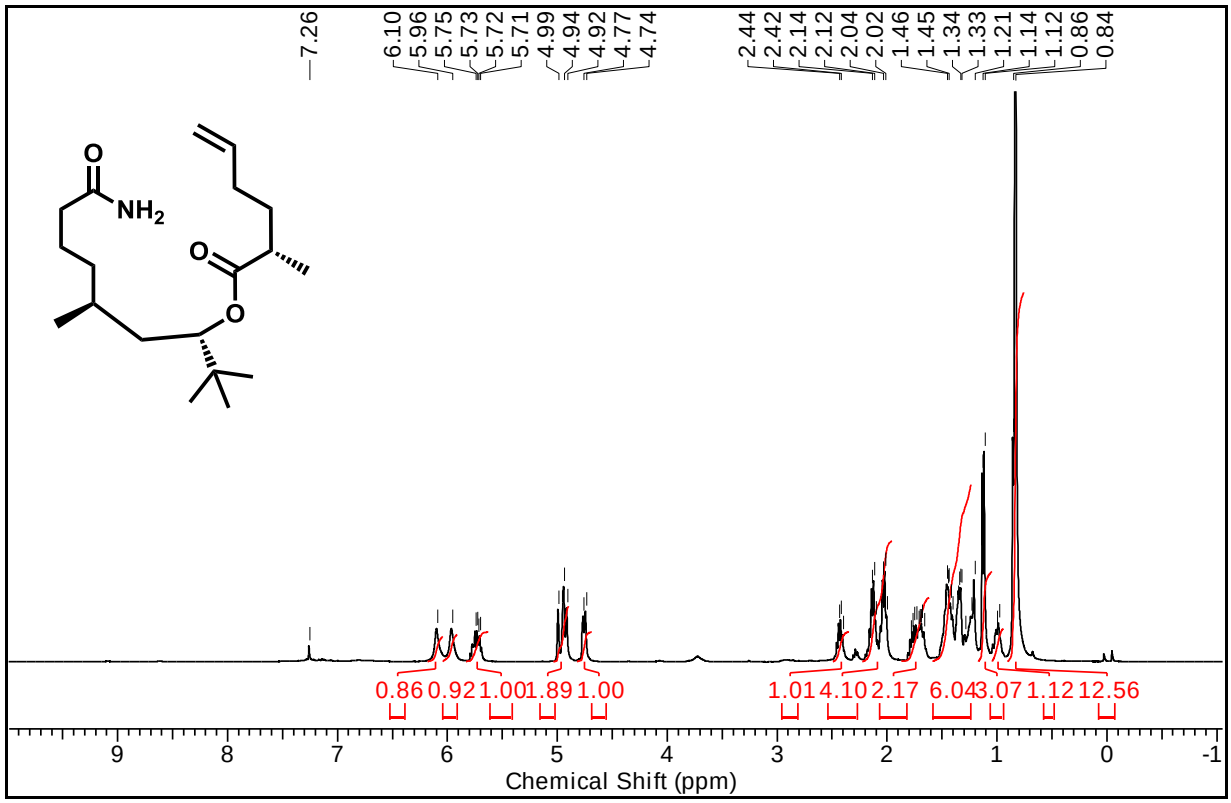

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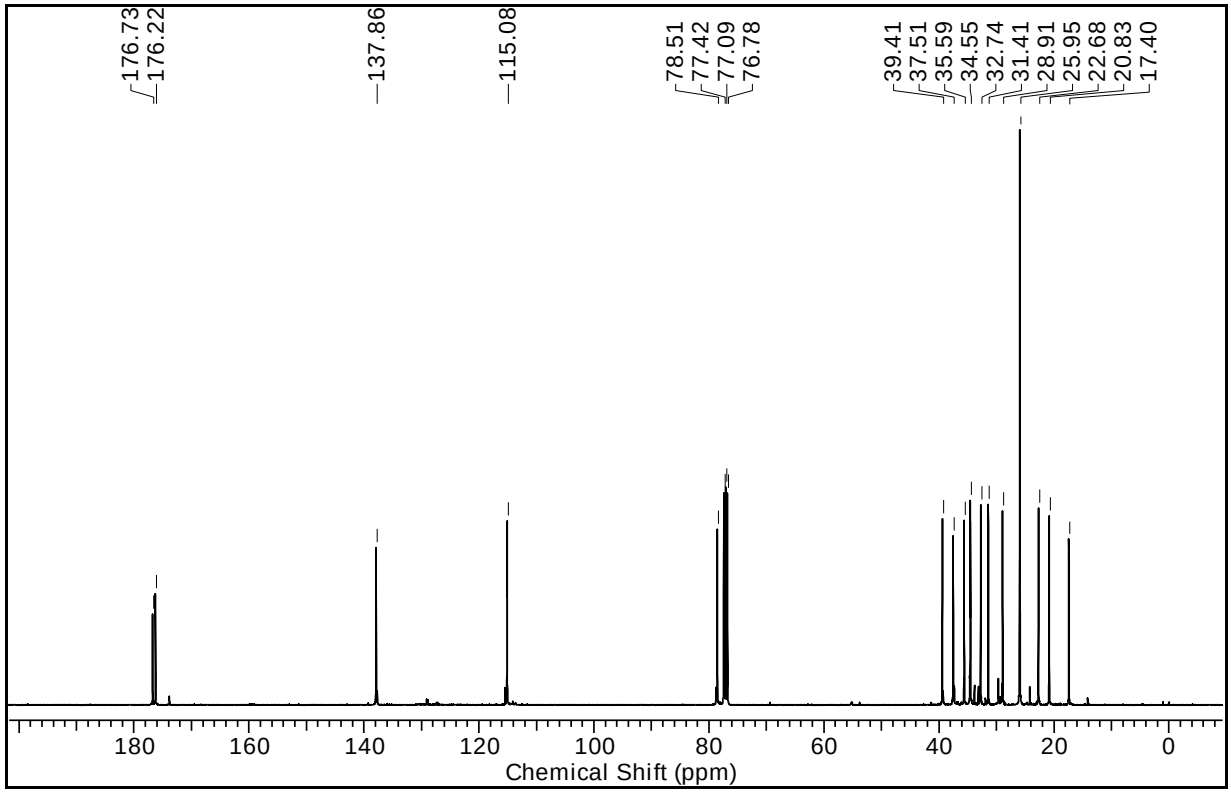

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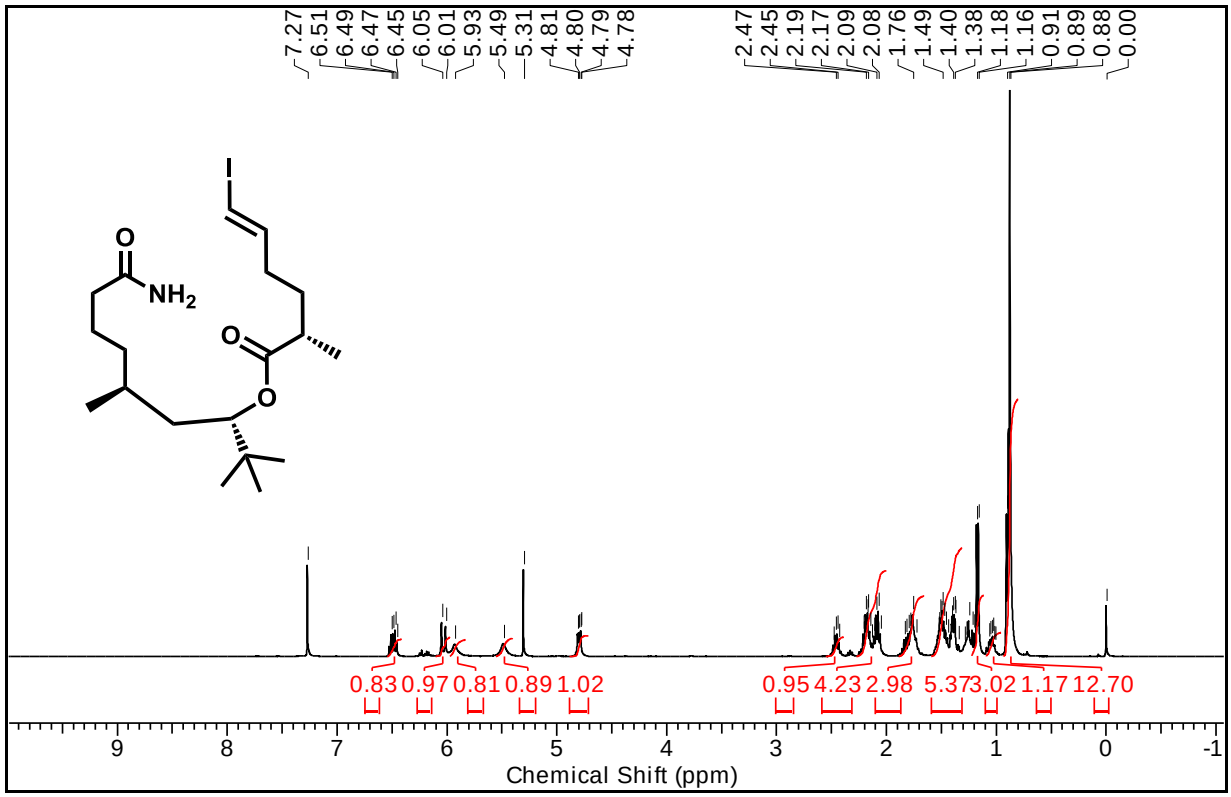

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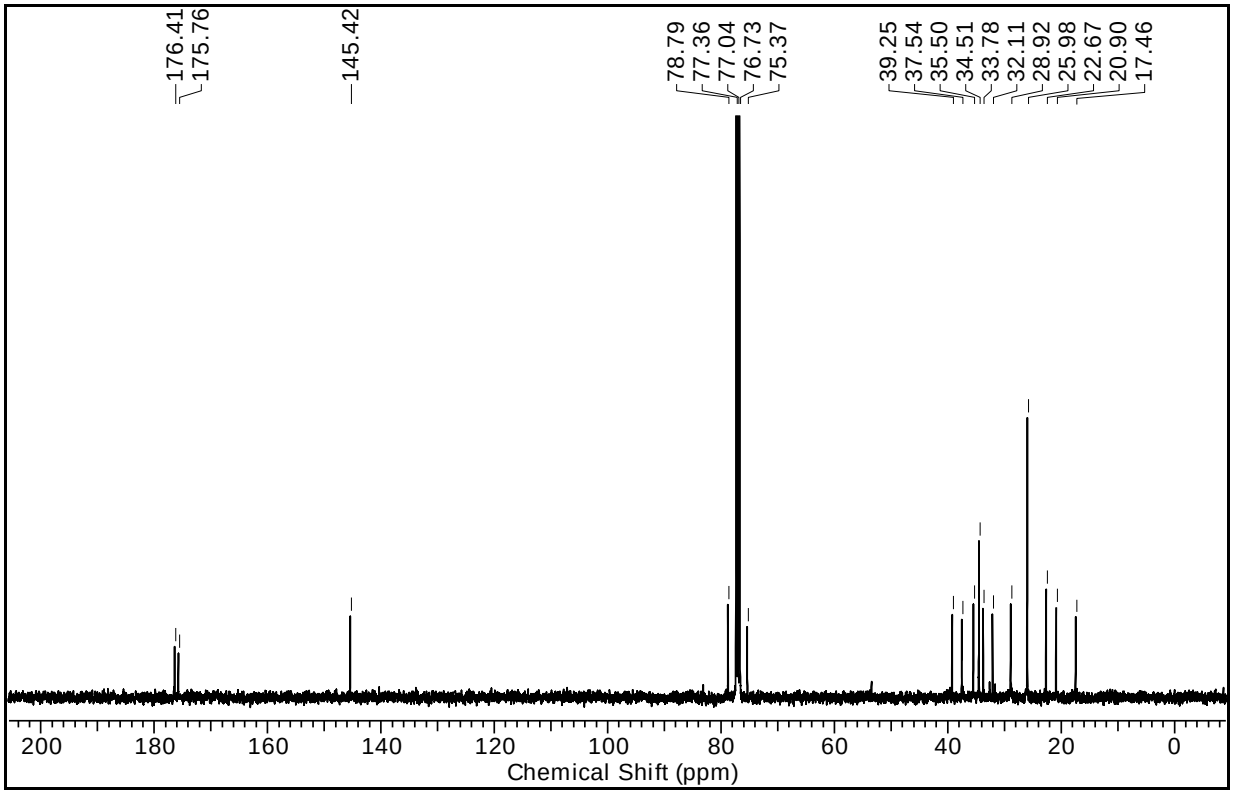

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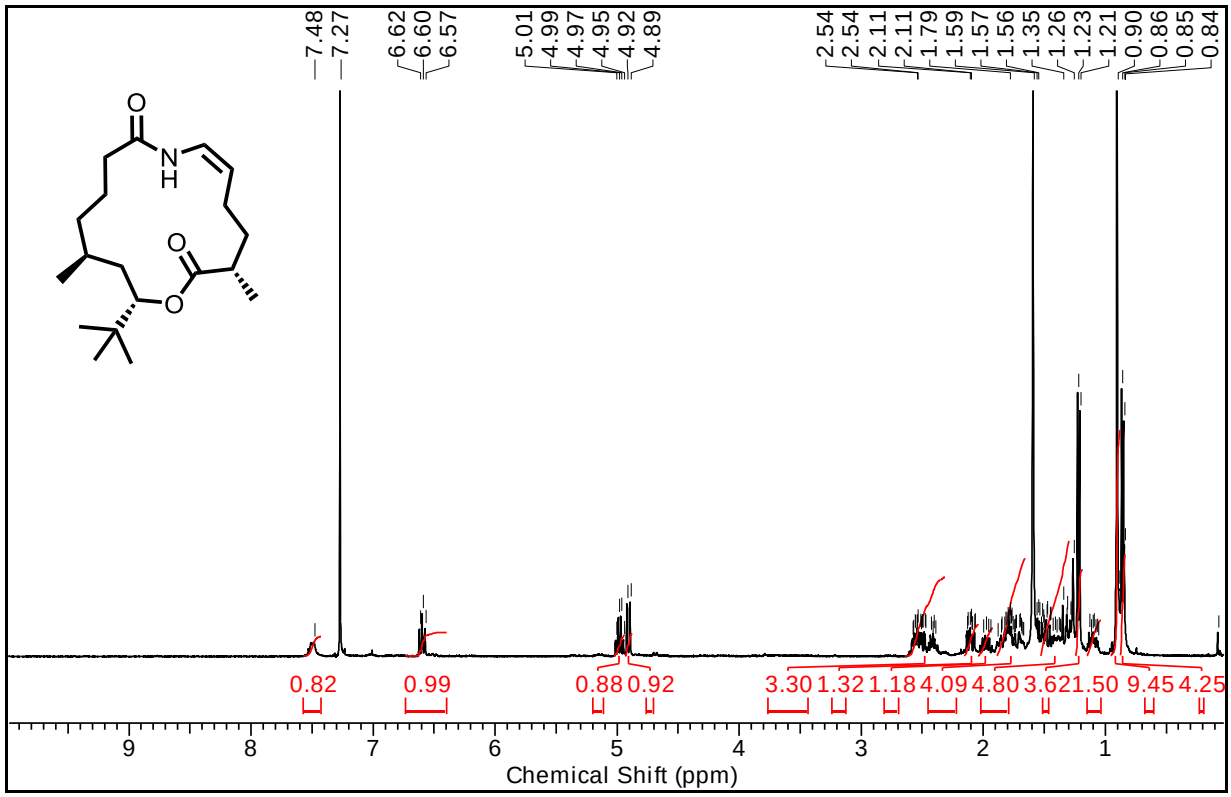

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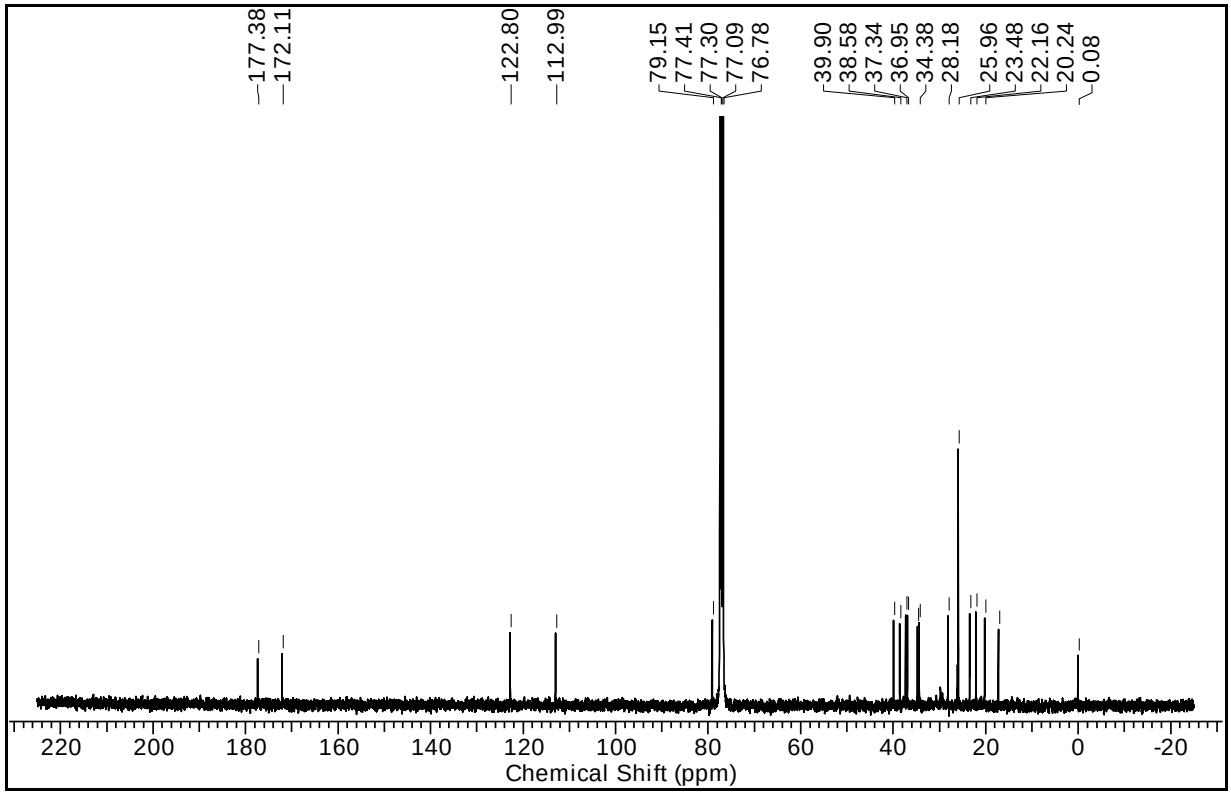
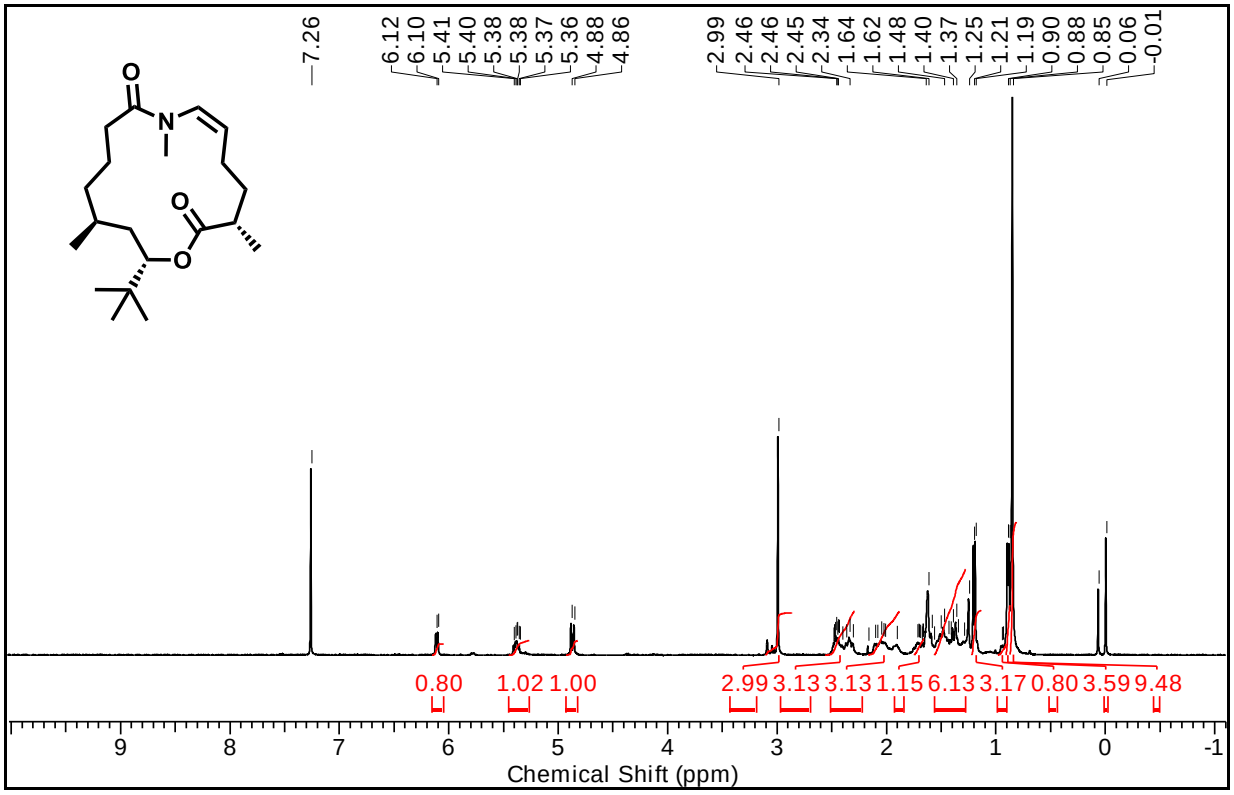


^{1}H NMR of Compound (-)-55 at 400 MHz in $CDCl_3$



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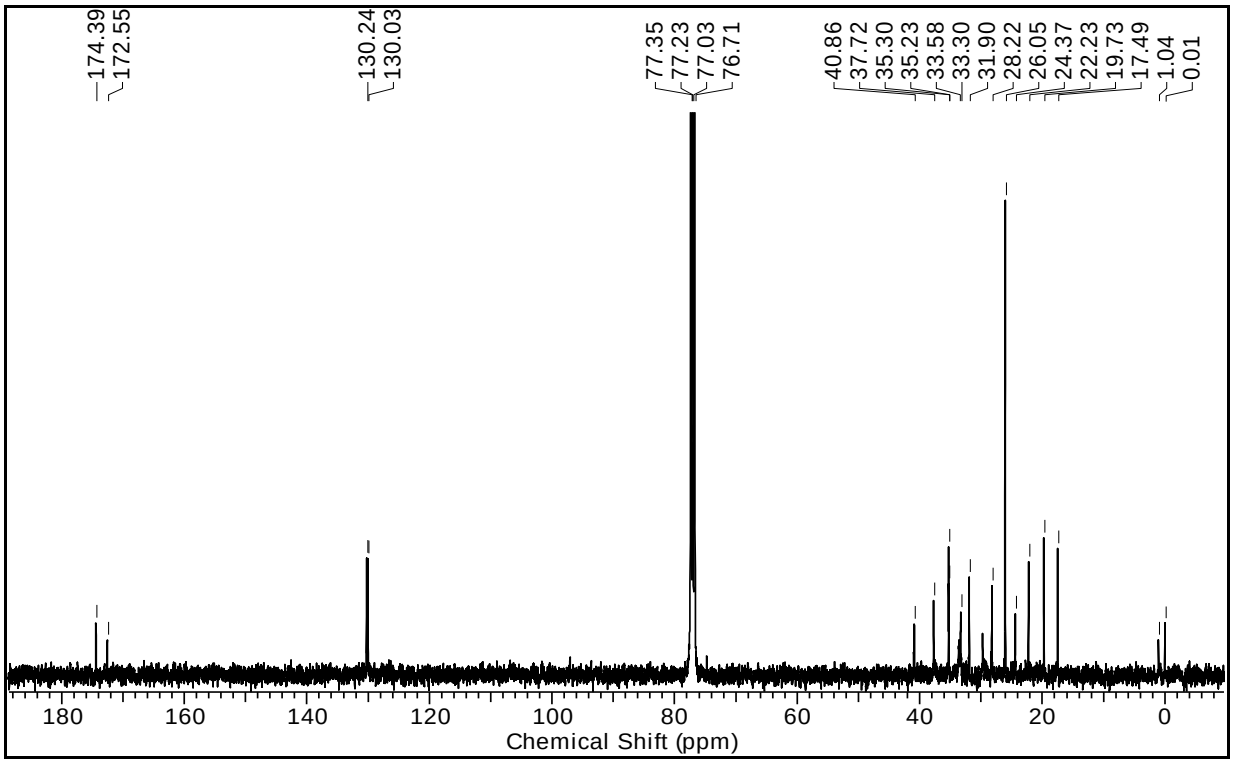
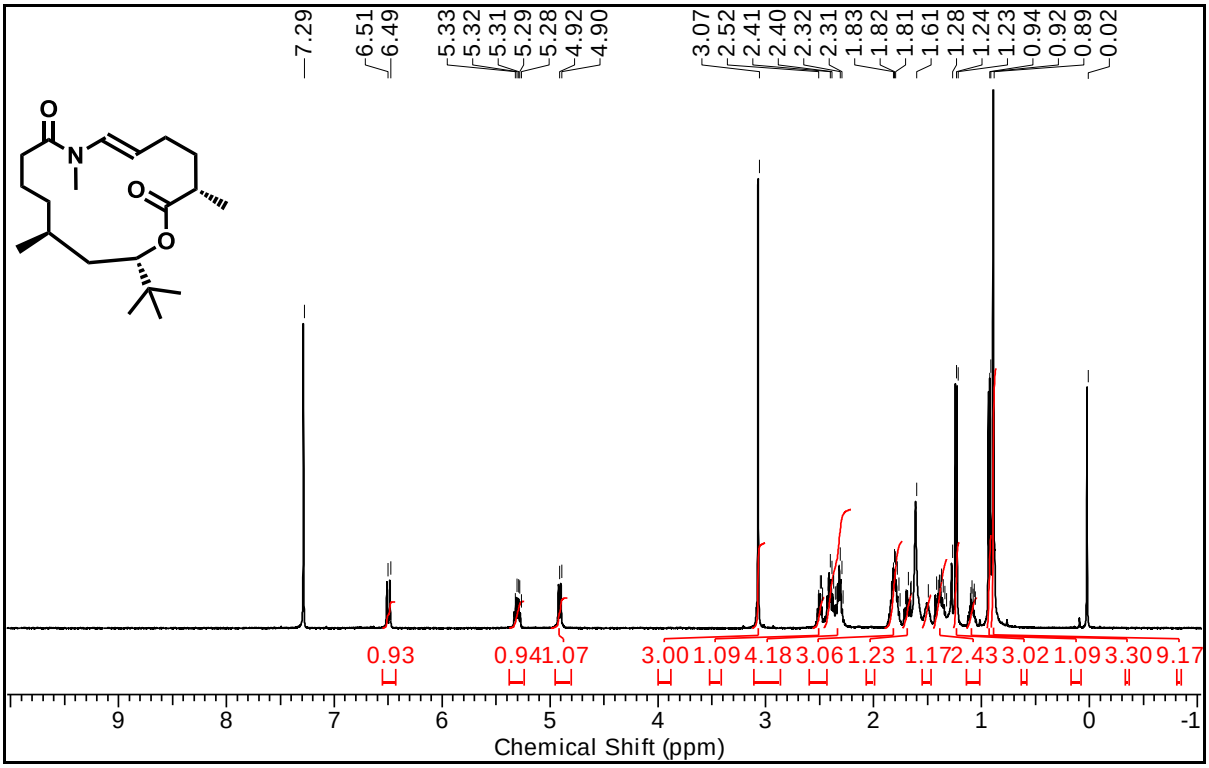
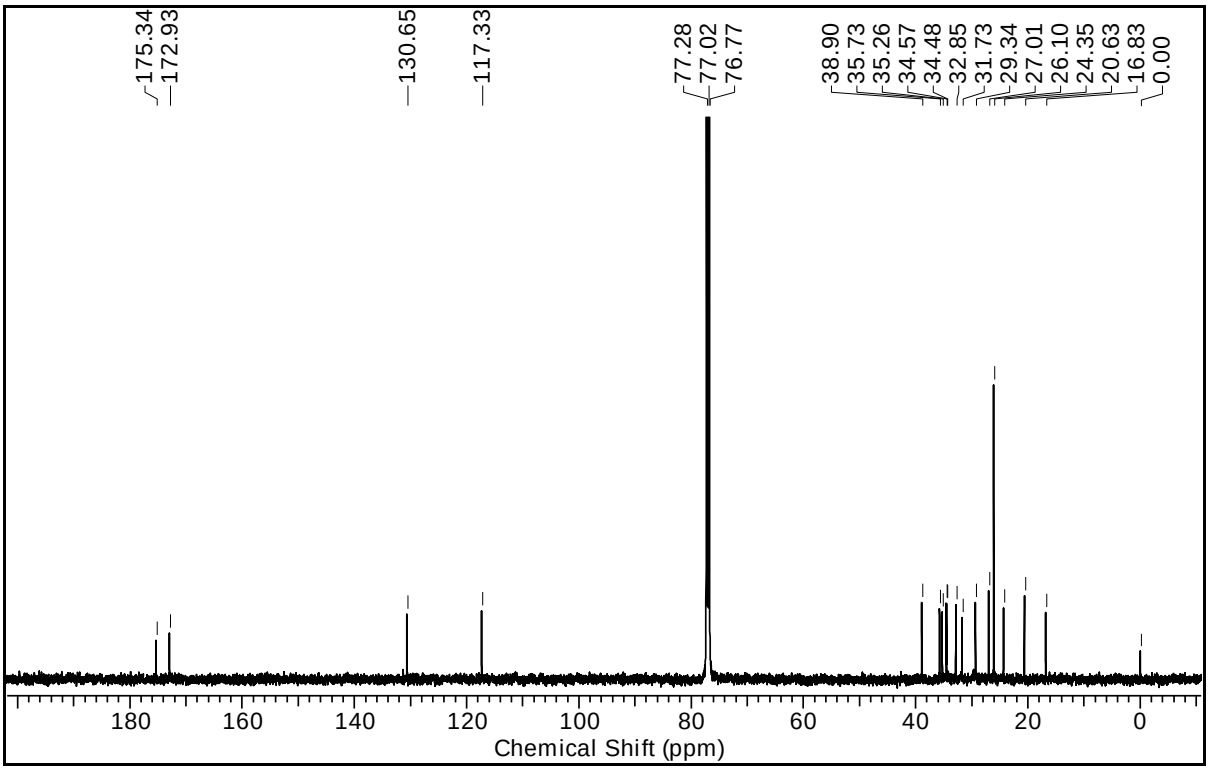


^{1}H NMR of Compound (+)-1 at 500 MHz in $CDCl_3$



^{13}C NMR of Compound (+)-1 at 125 MHz in $CDCl_3$



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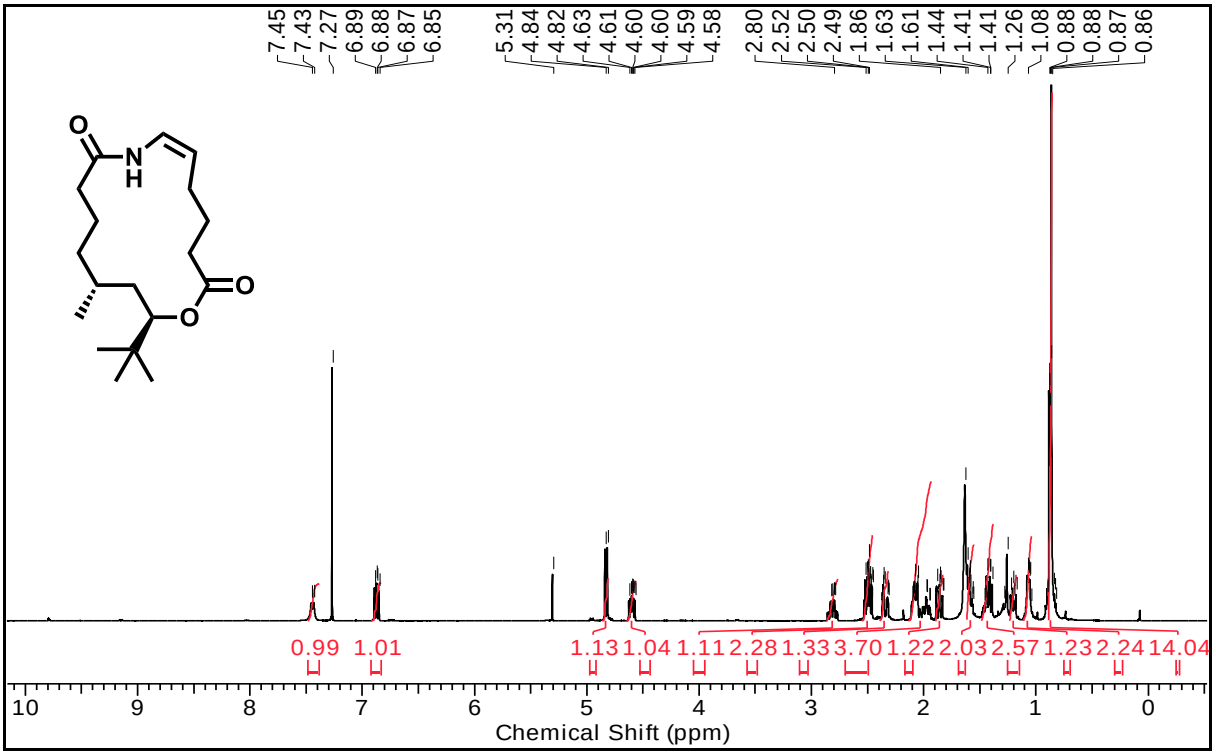

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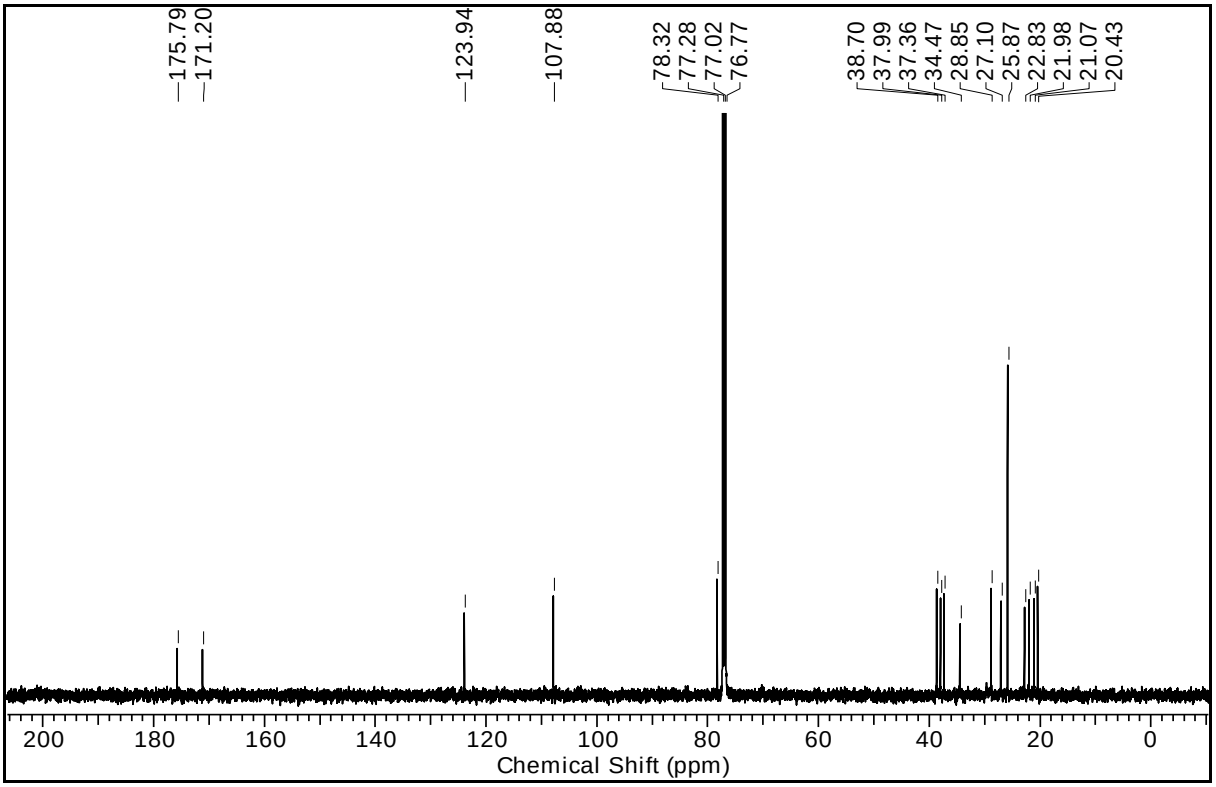
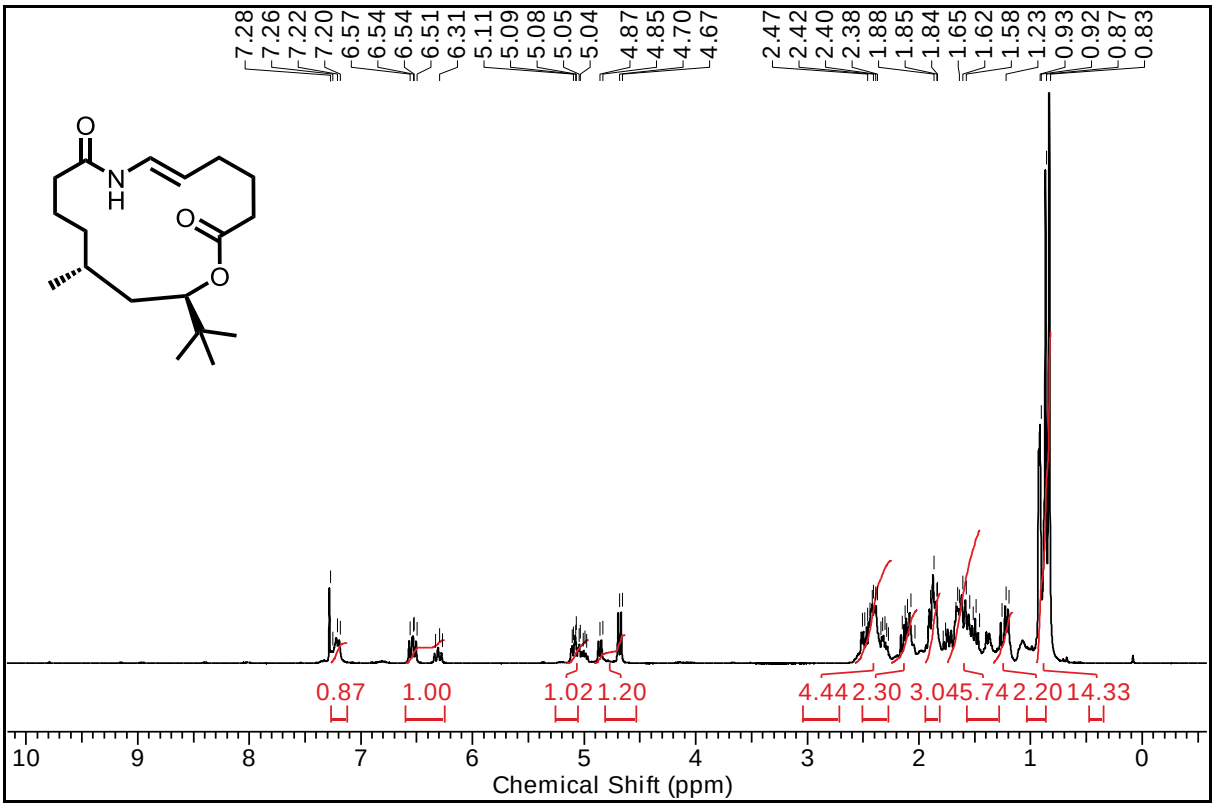
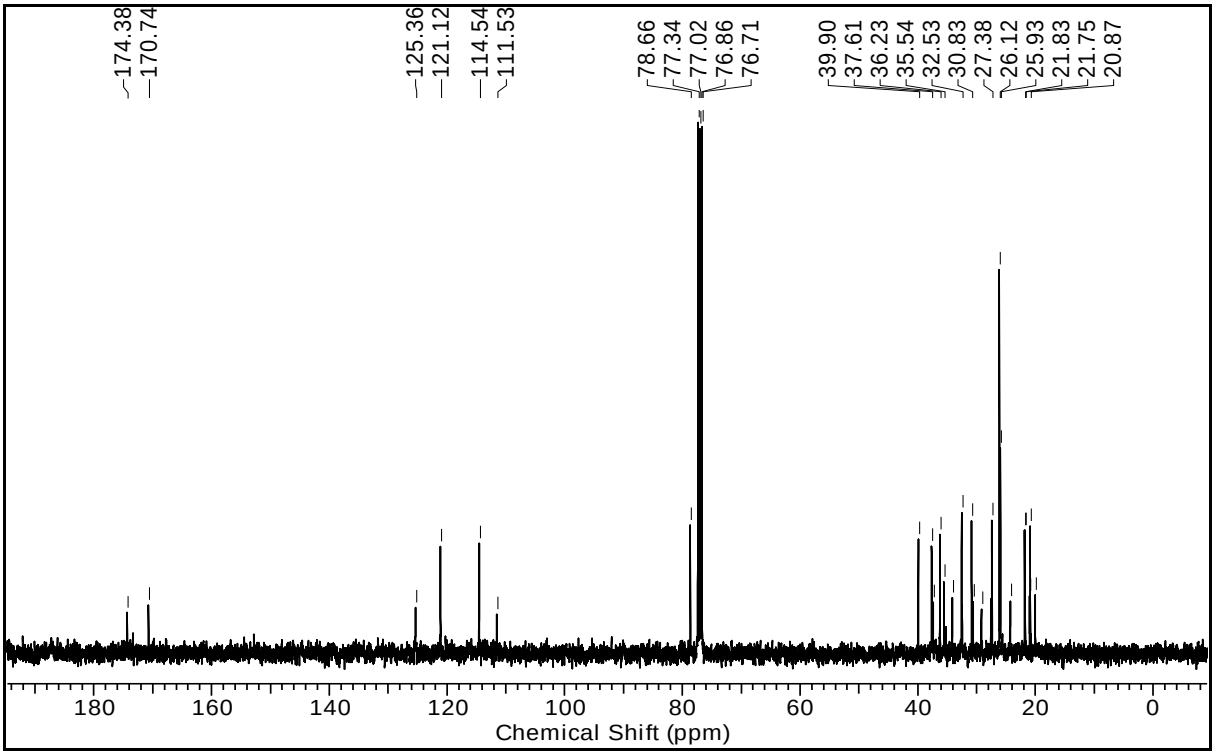


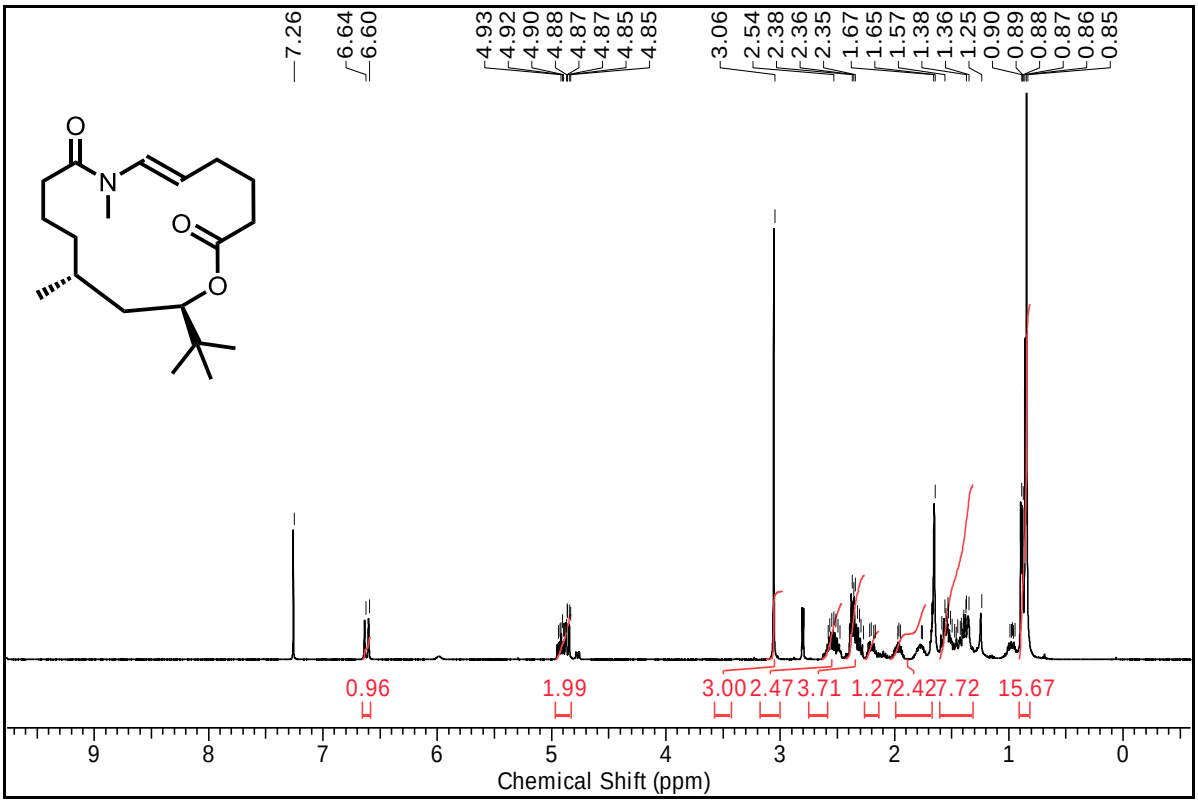
^{1}H NMR of Compound 75 at 400 MHz in $CDCl_3$



^{13}C NMR of Compound 75 at 100 MHz in $CDCl_3$



^{1}H NMR of Compound 76 at 400 MHz in $CDCl_3$



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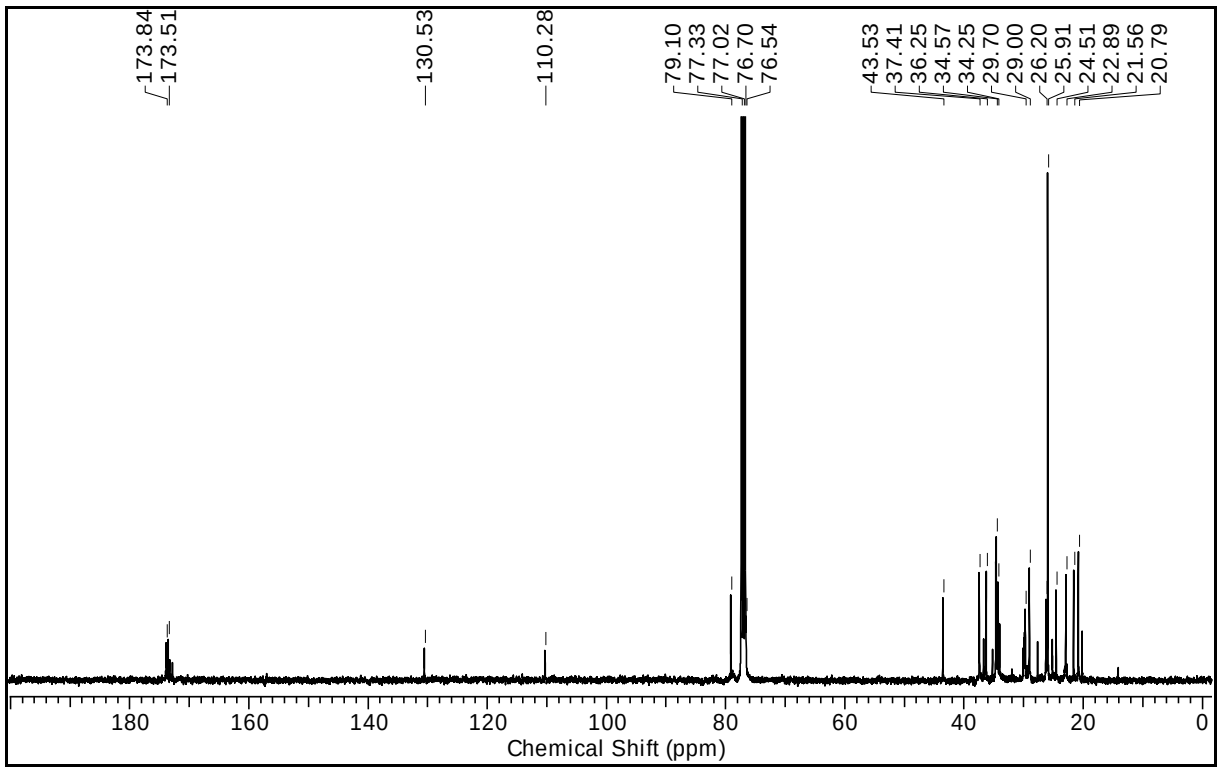
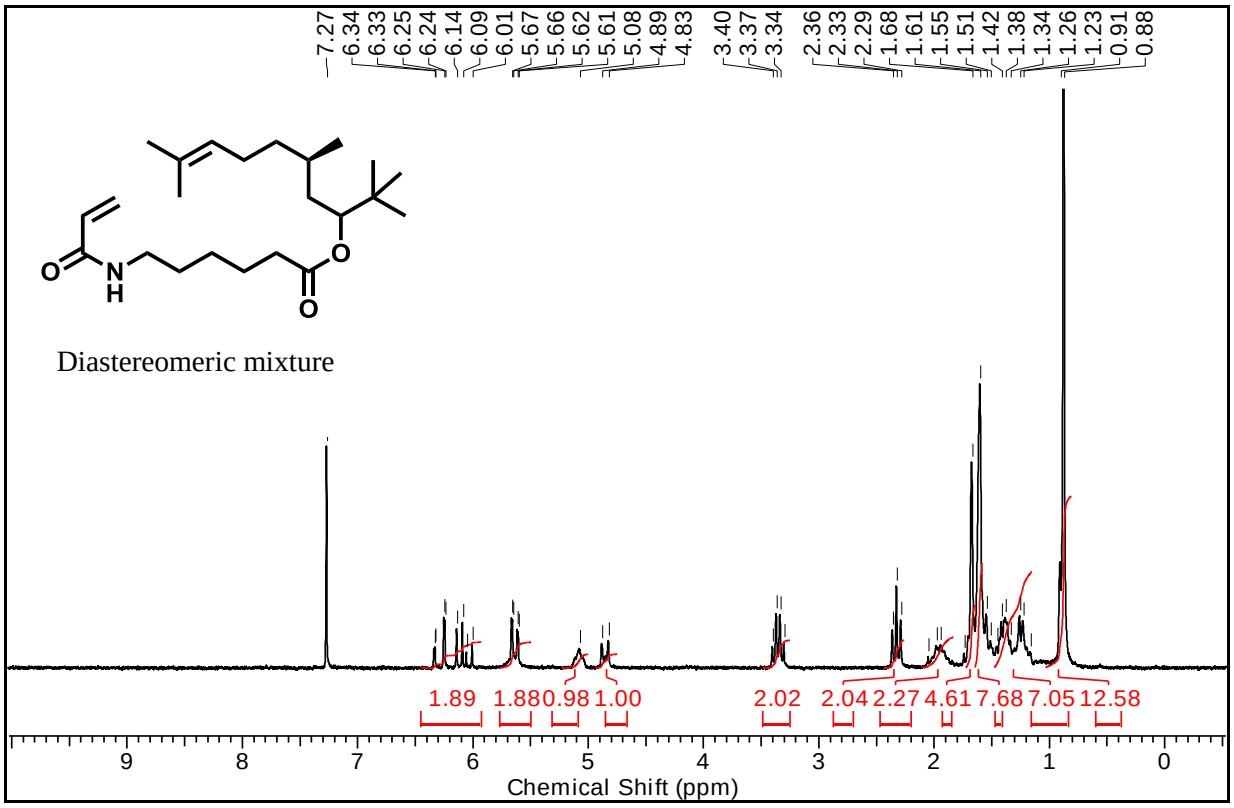


^{1}H NMR of Compound 77 at 200 MHz in $CDCl_3$



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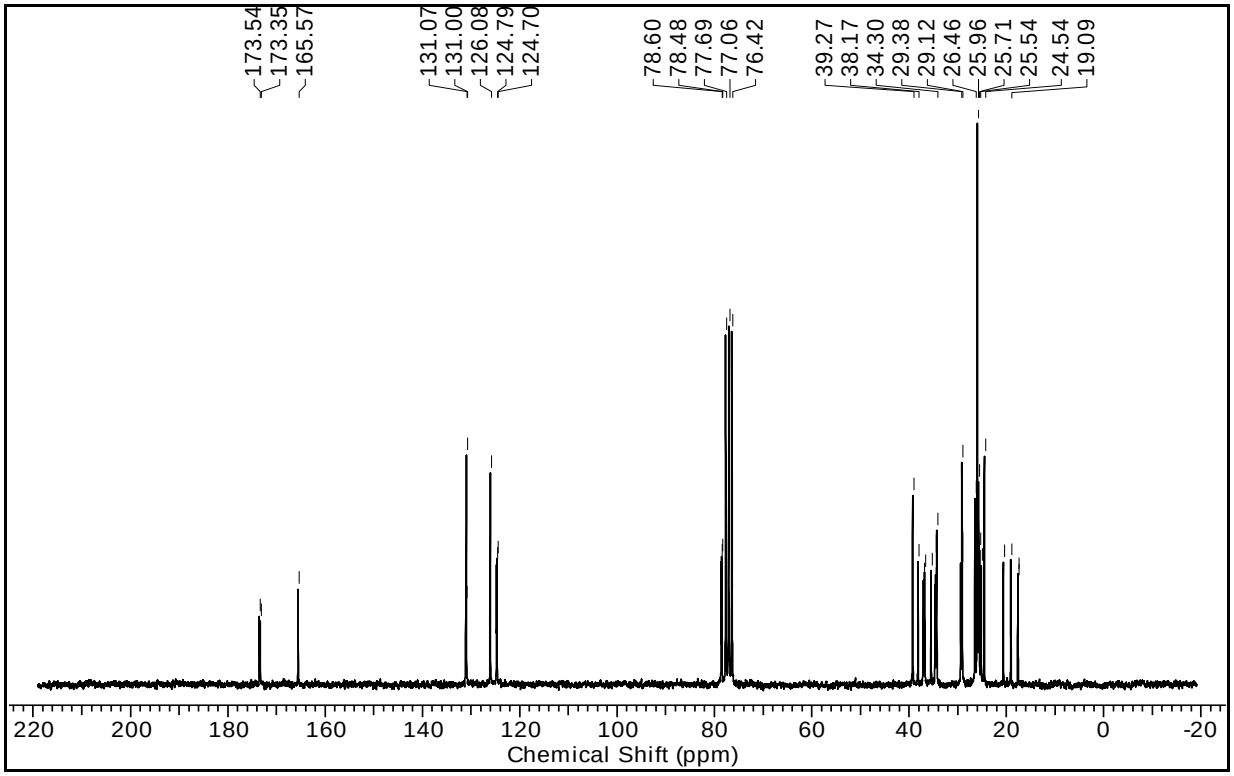
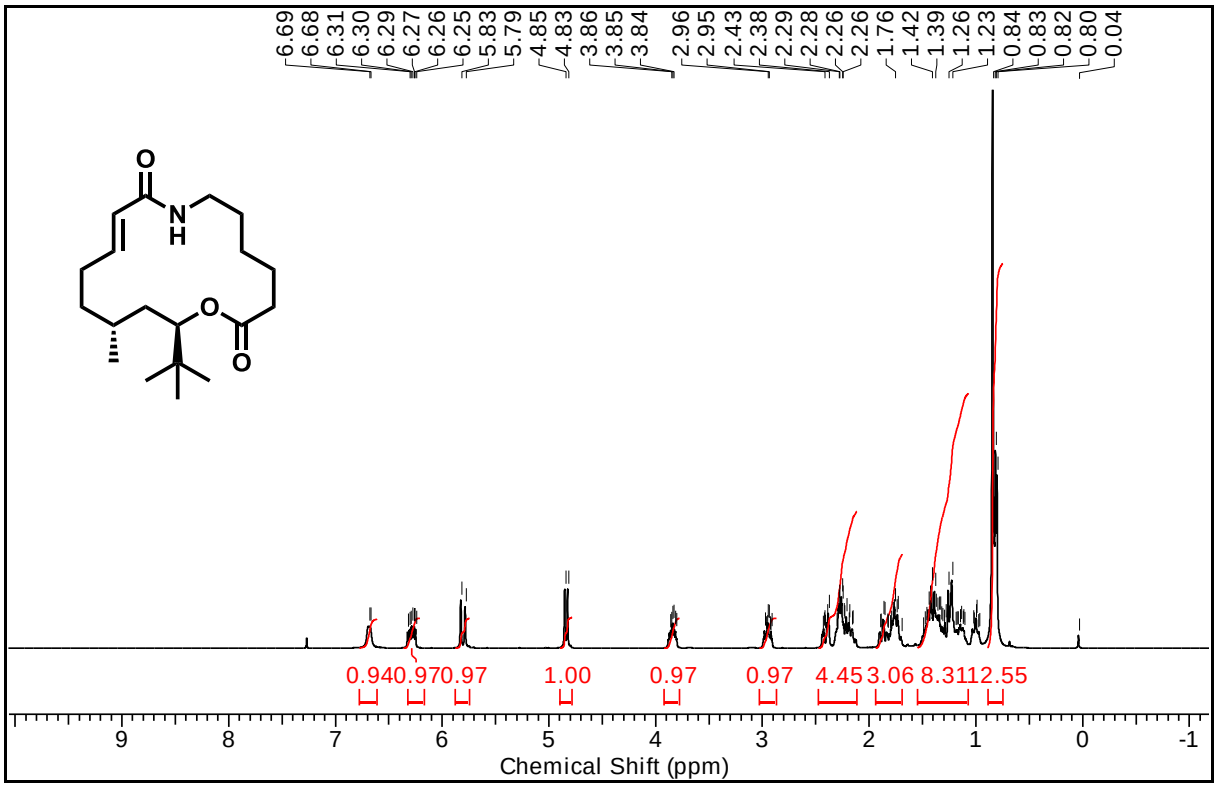


^{1}H NMR of Compound 78 at 400 MHz in $CDCl_3$



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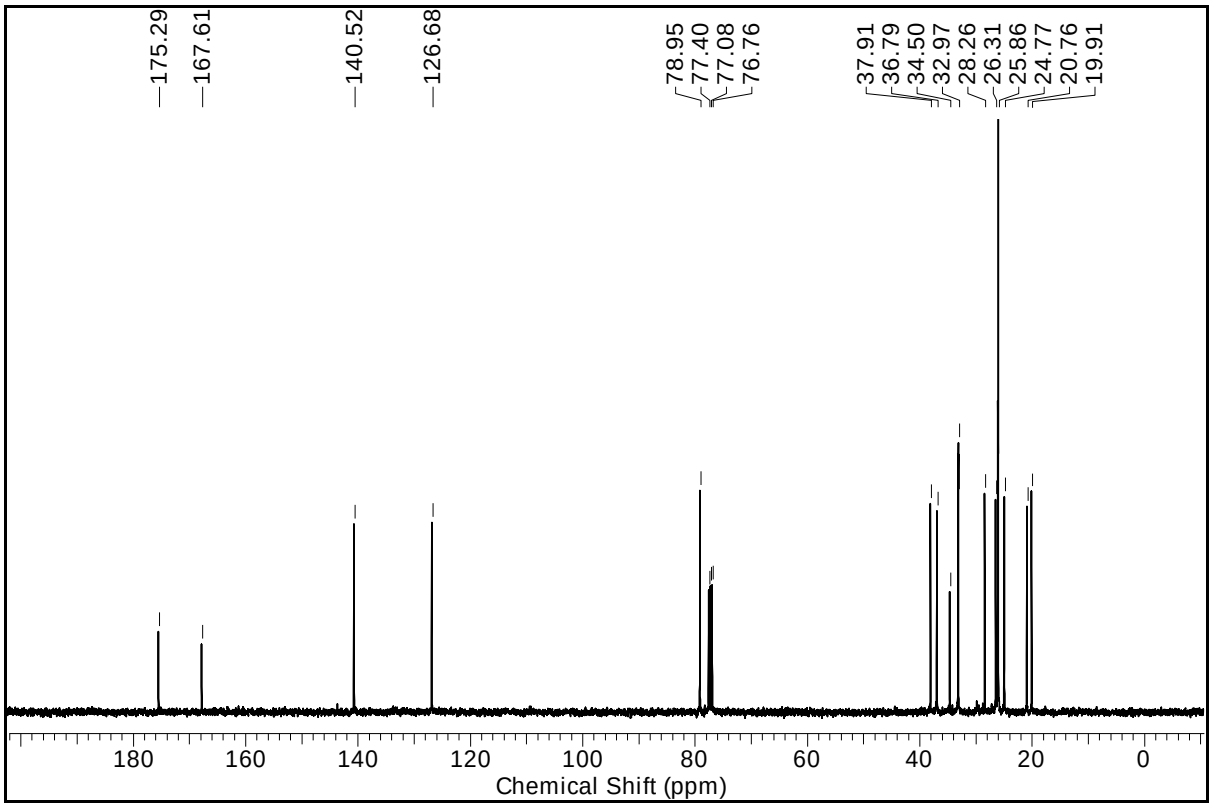

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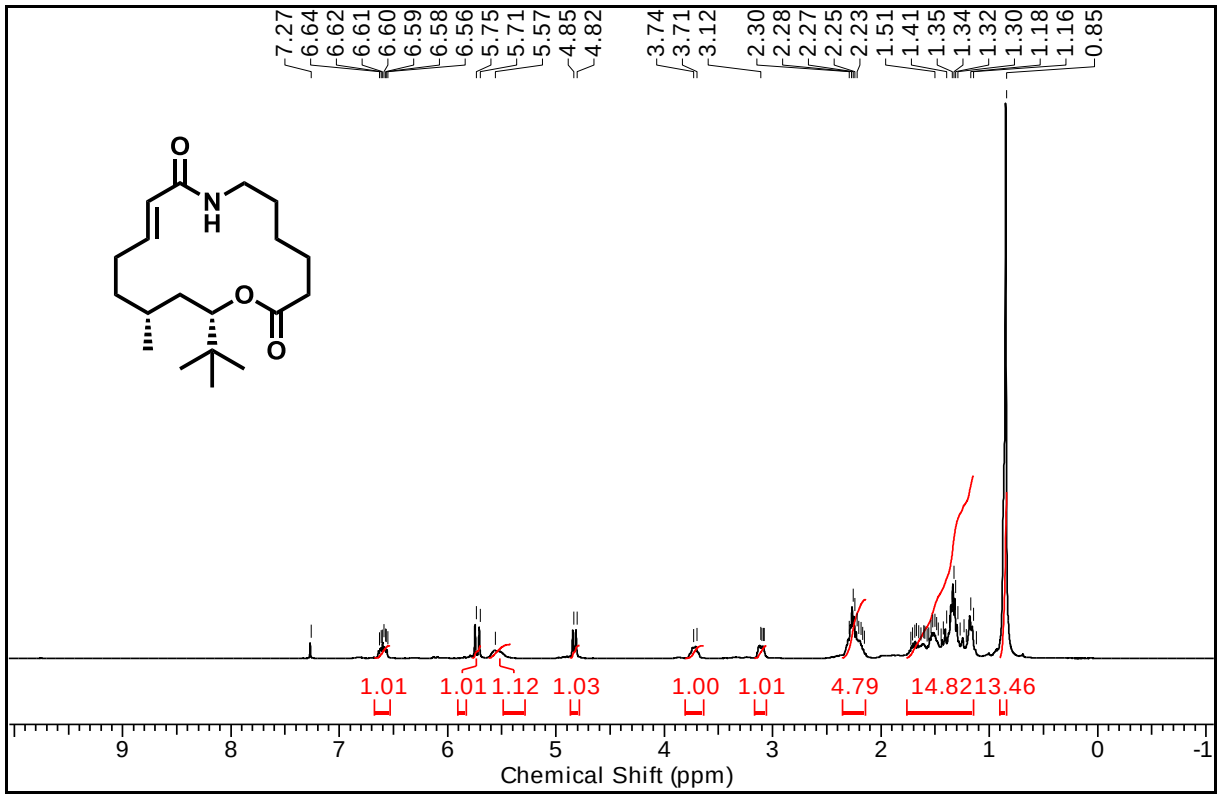
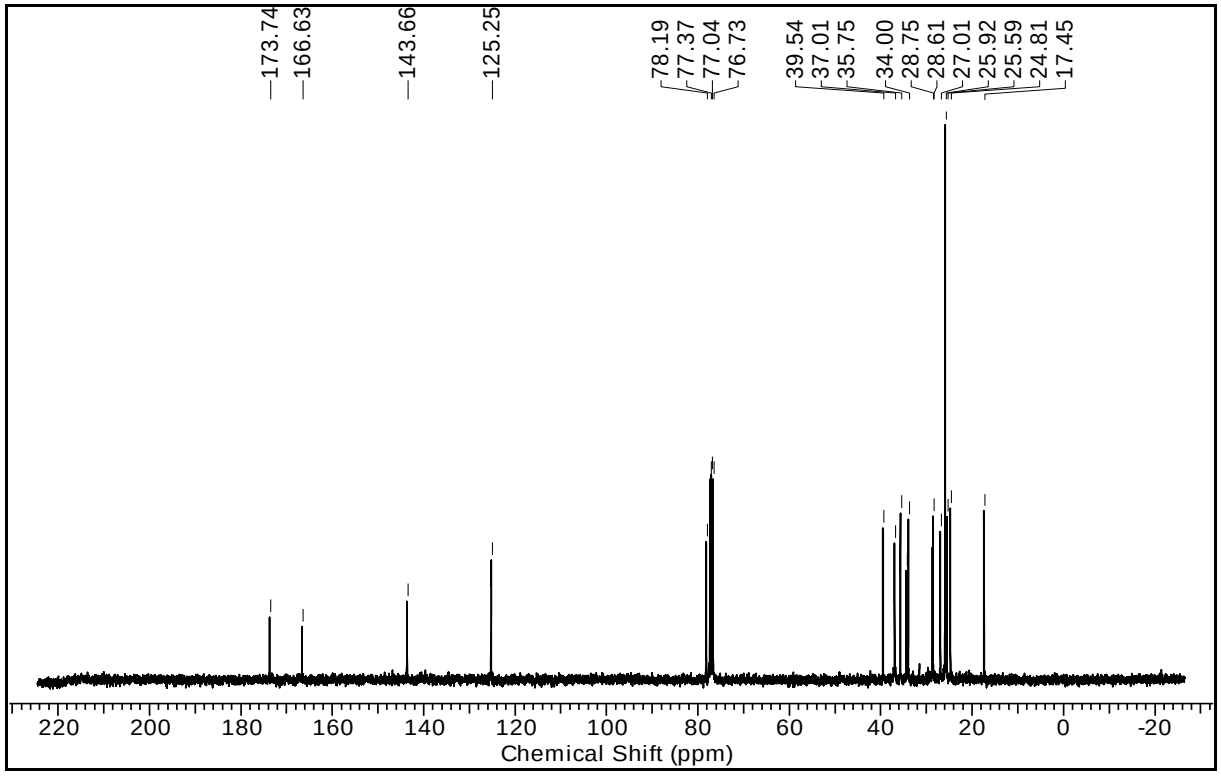
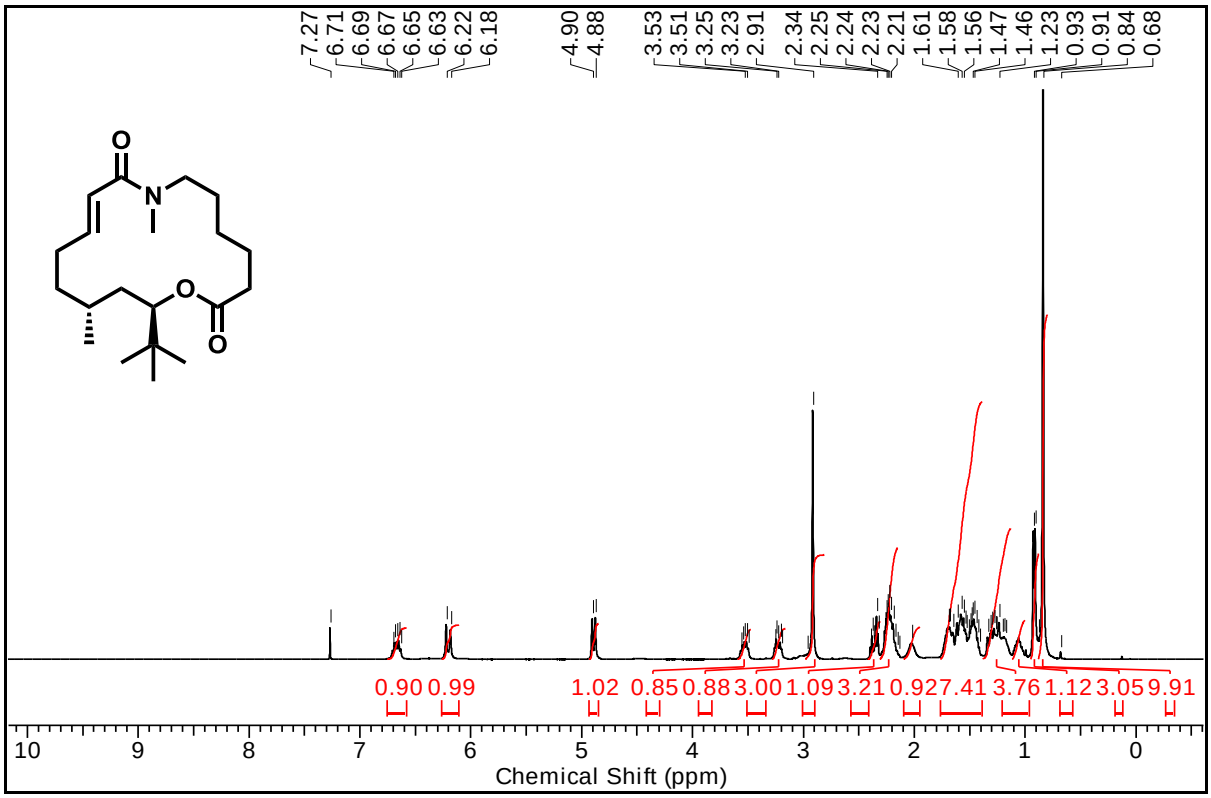


^{13}C NMR of Compound 79 at 100 MHz in $CDCl_3$



^{1}H NMR of Compound 80 at 400 MHz in $CDCl_3$



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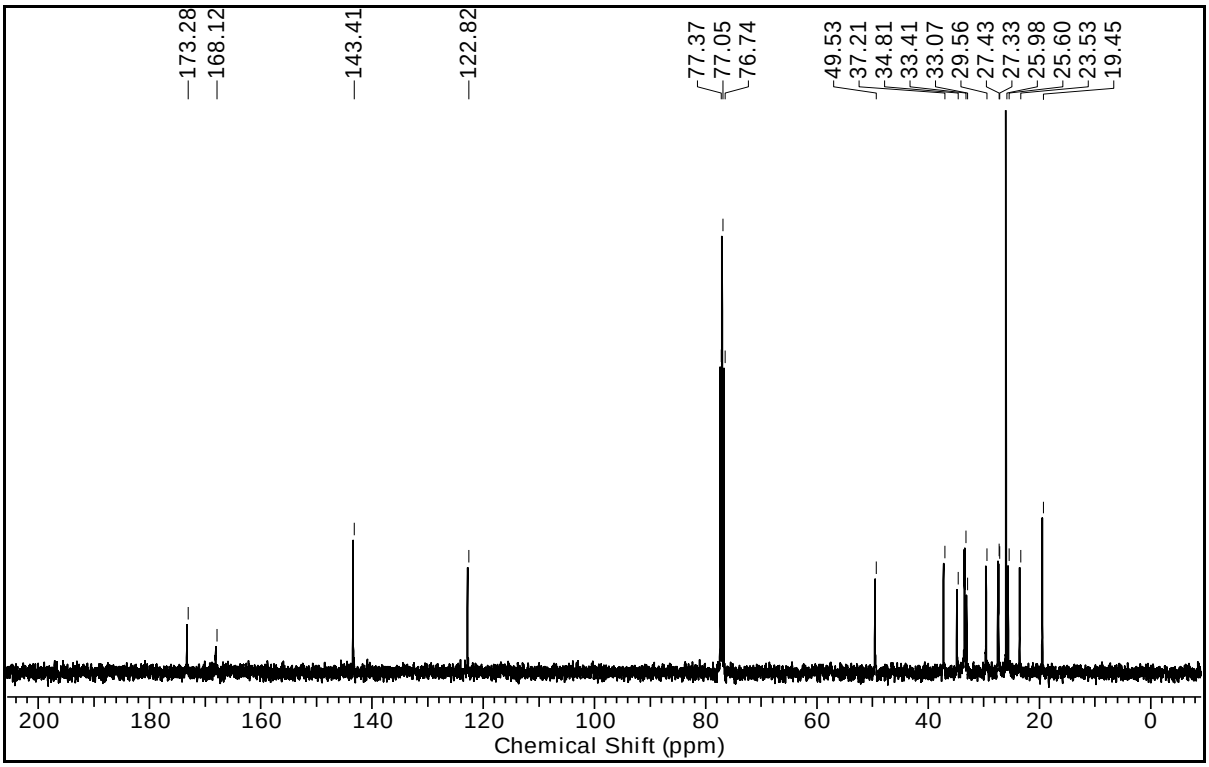

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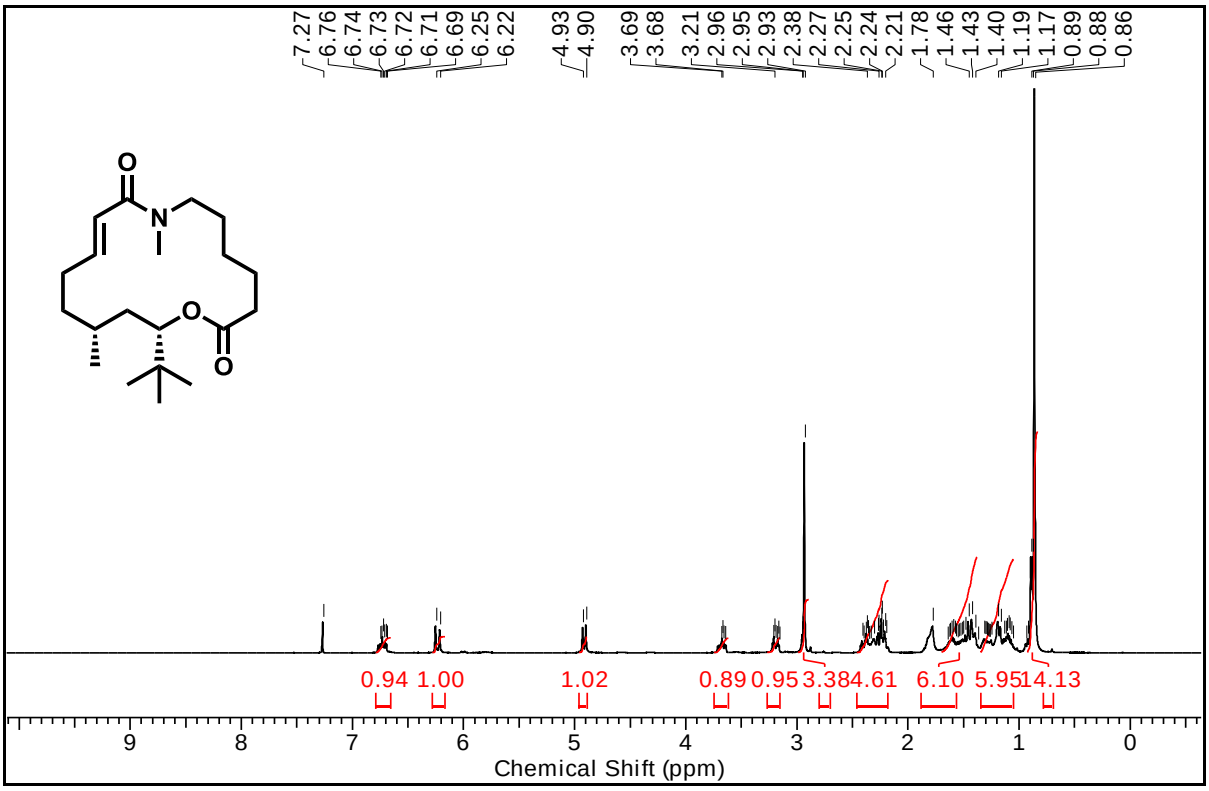

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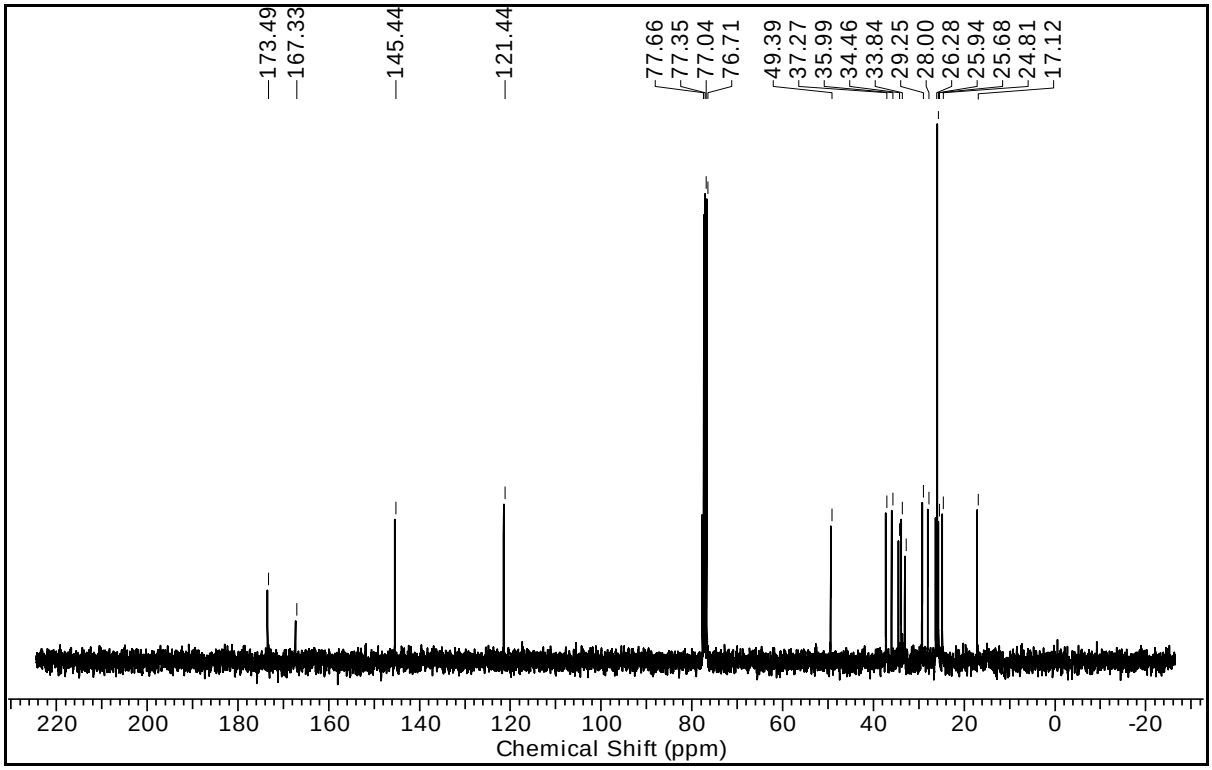

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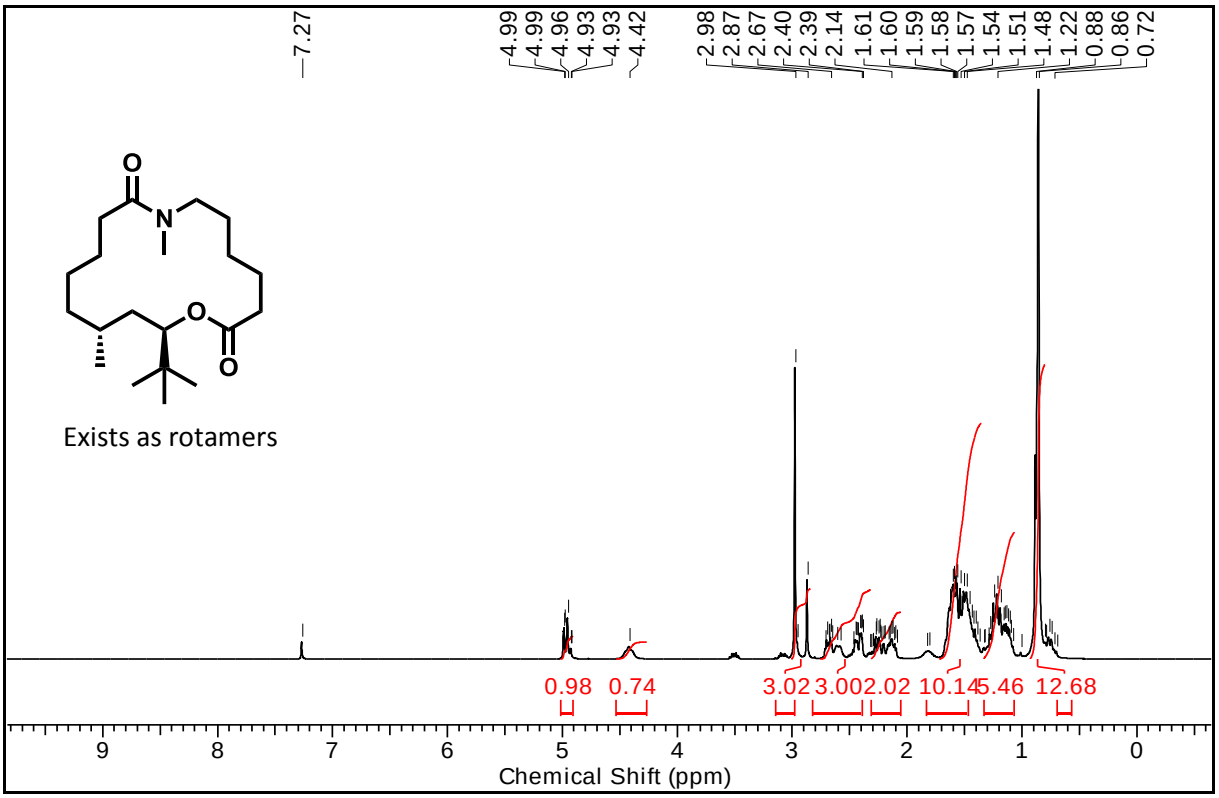

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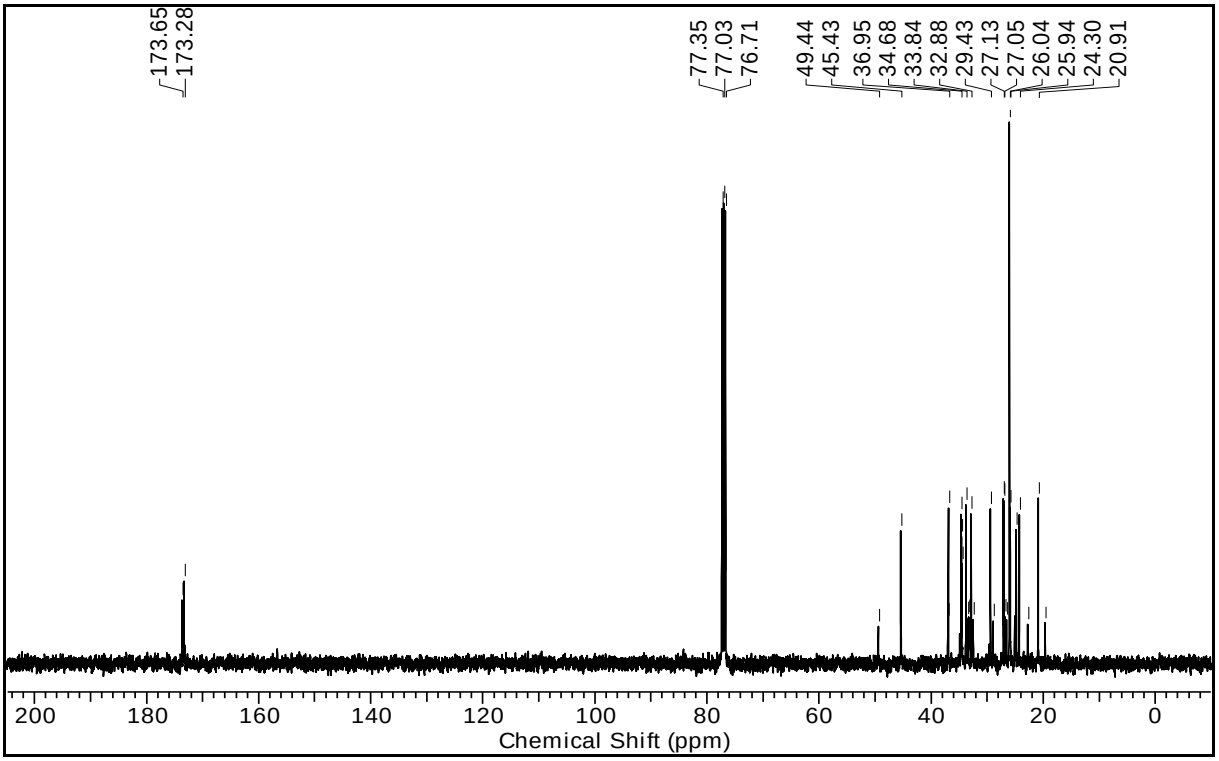
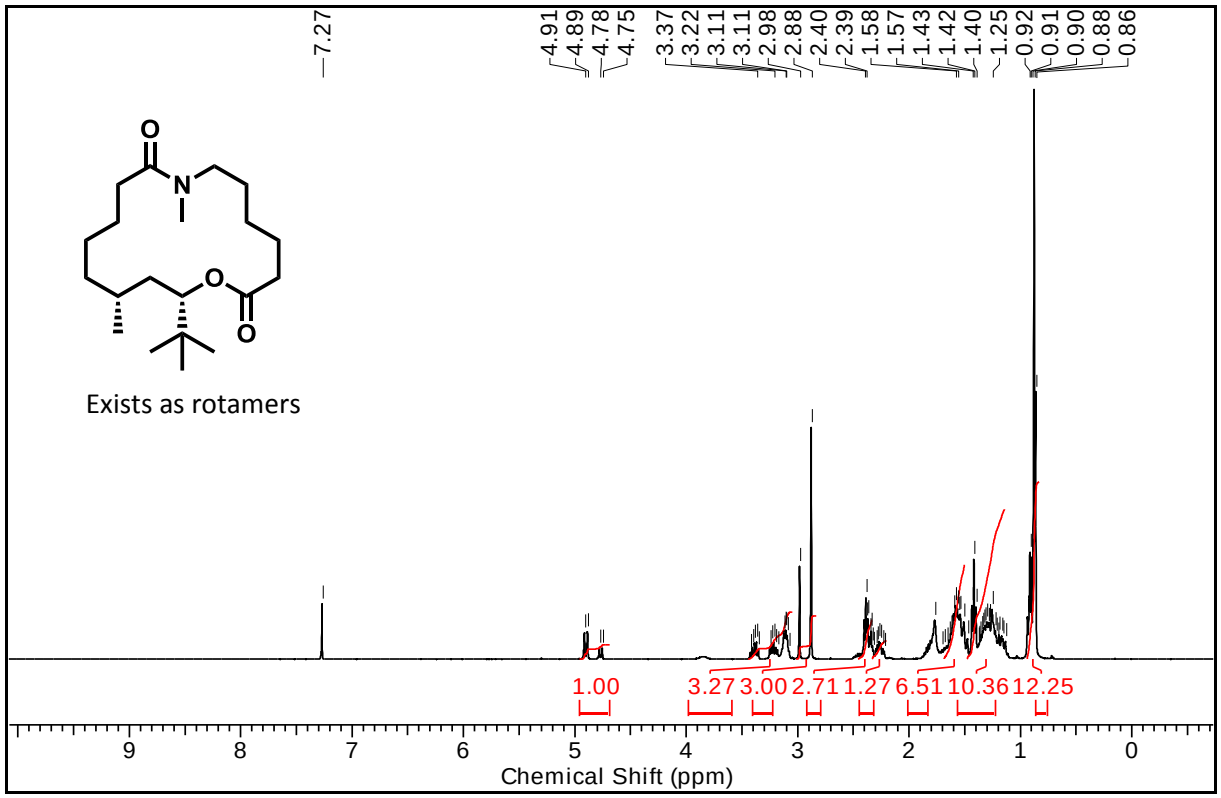


^{1}H NMR of Compound 85 at 400 MHz in $CDCl_3$



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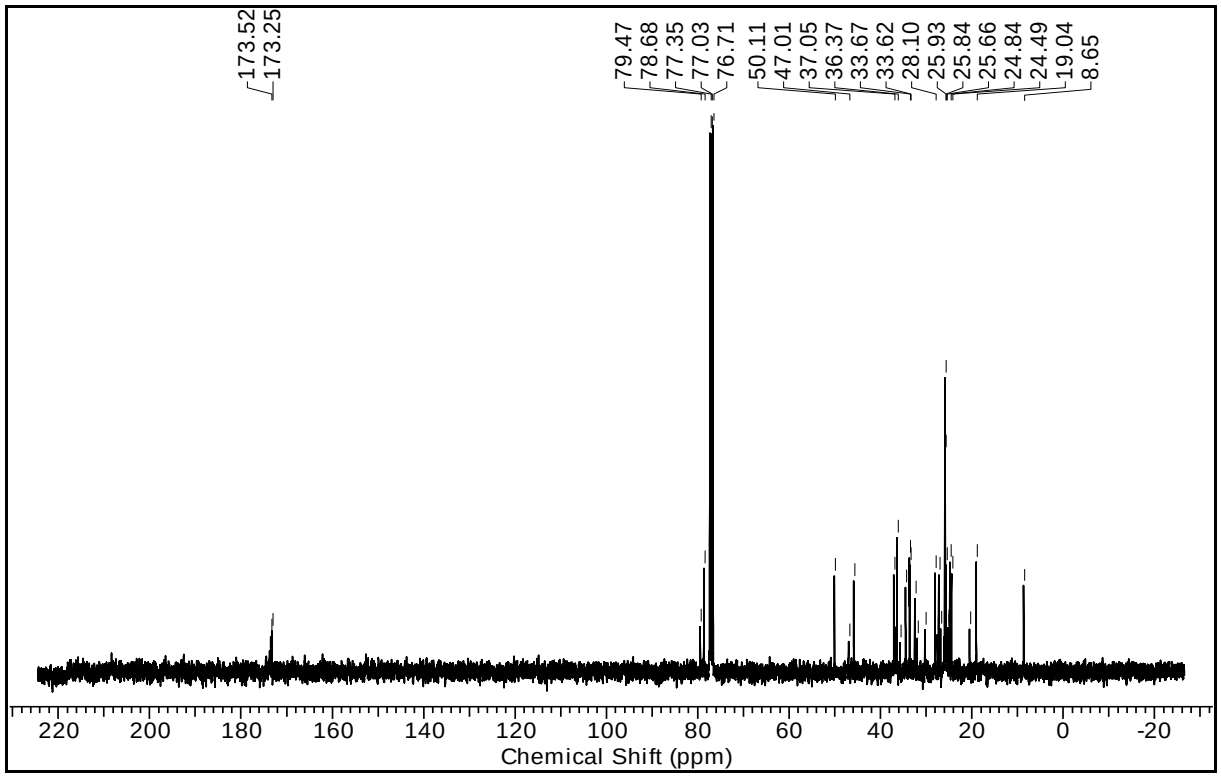
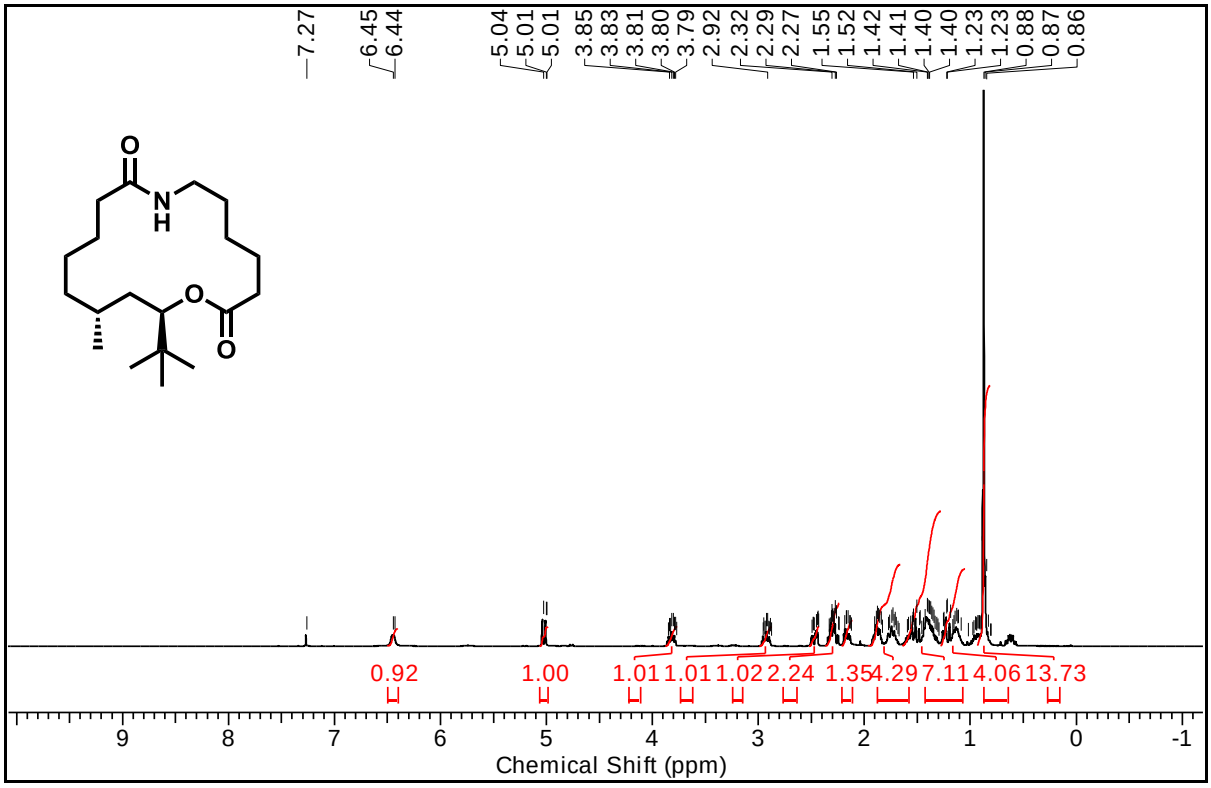


^{1}H NMR of Compound 82 at 400 MHz in $CDCl_3$



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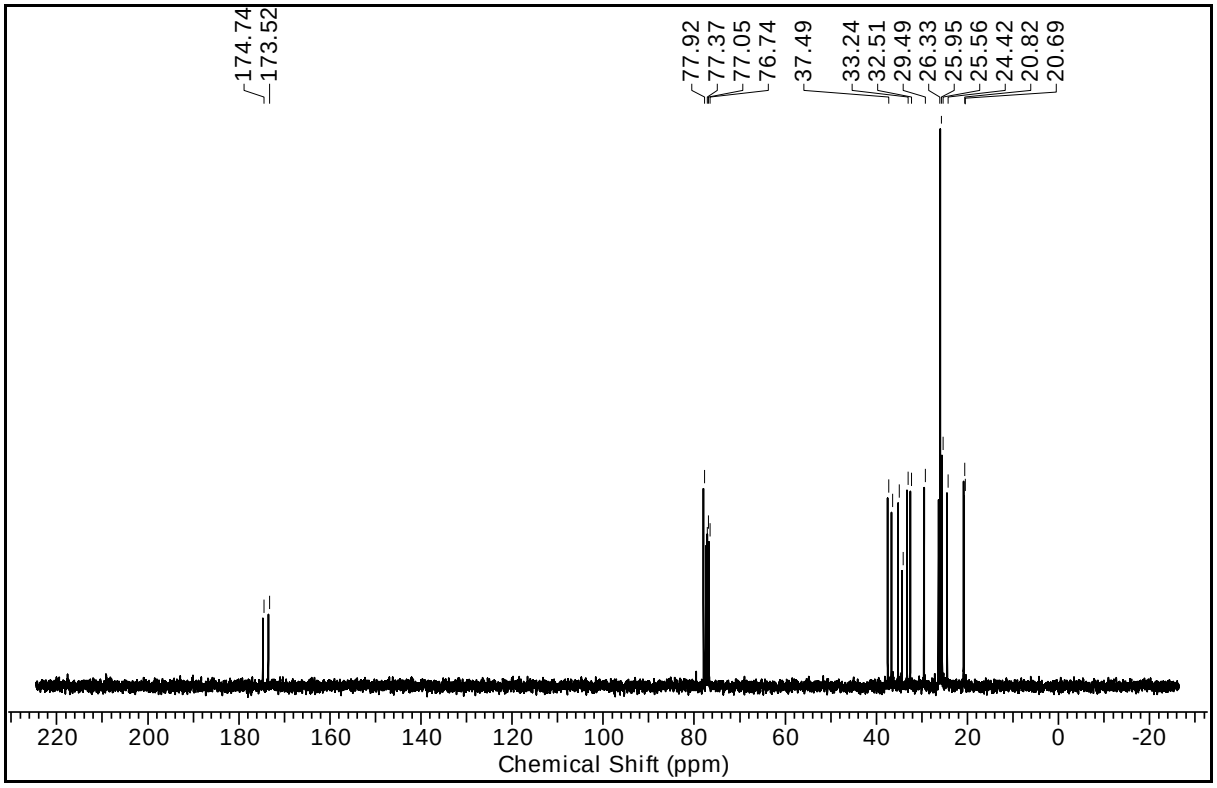
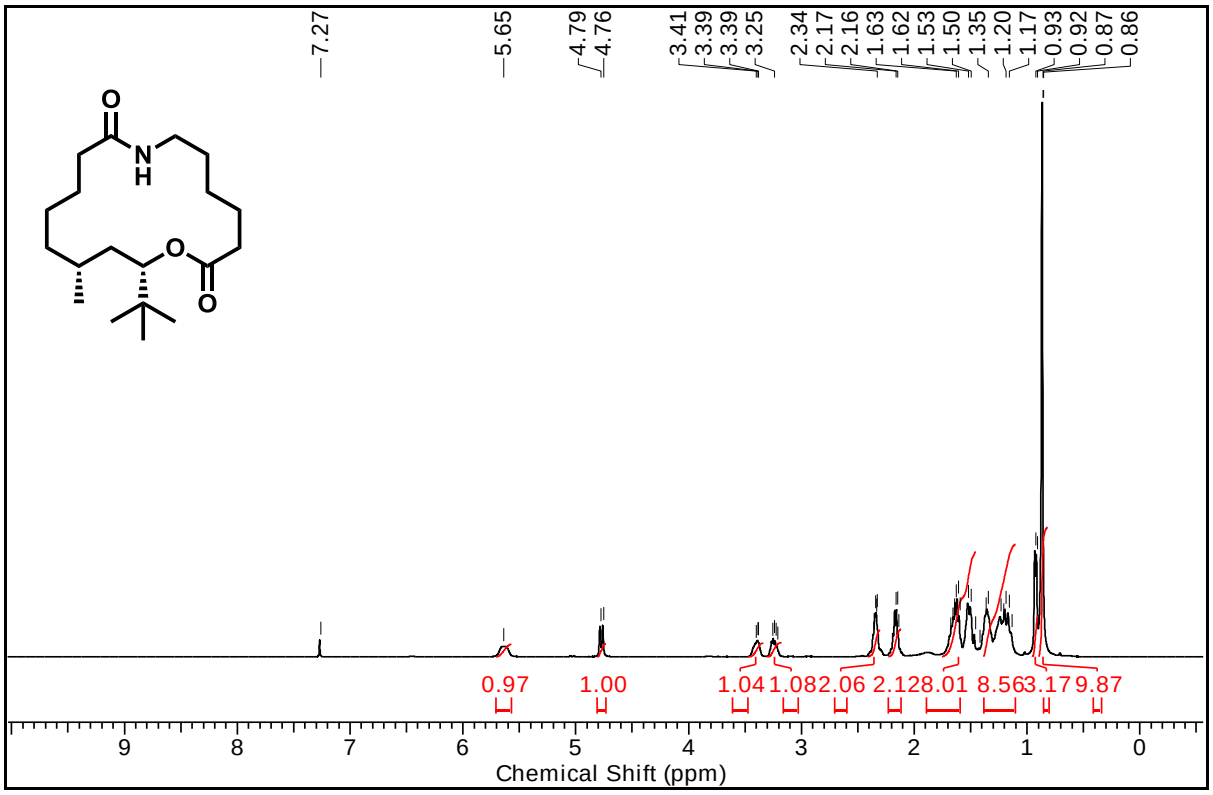


^{1}H NMR of Compound 84 at 400 MHz in $CDCl_3$



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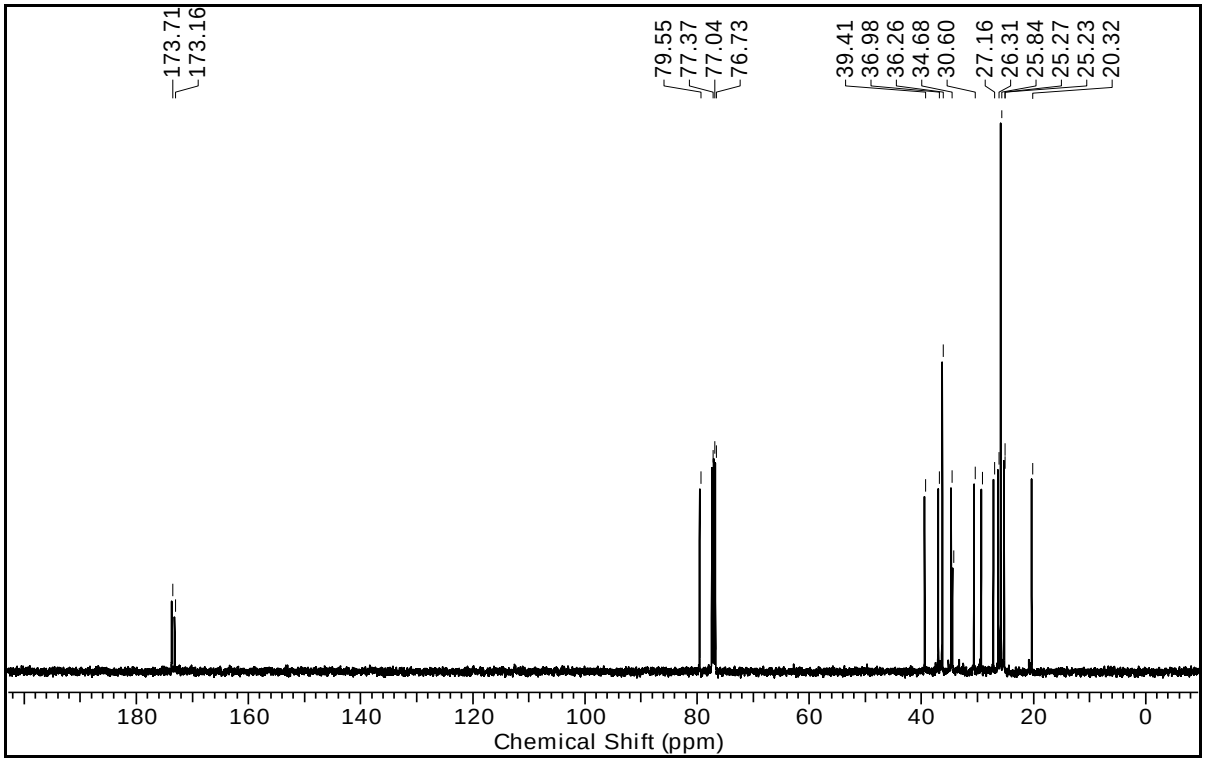
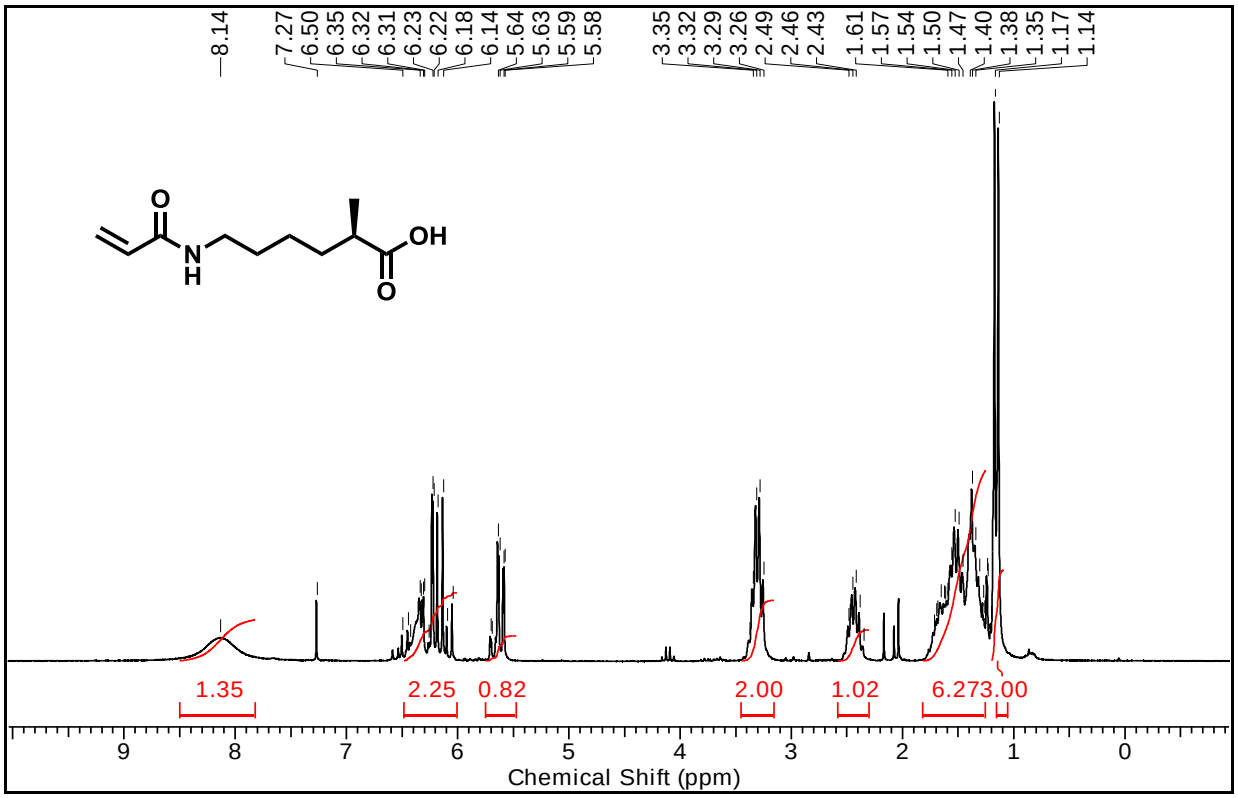


^{1}H NMR of Compound 60 at 200 MHz in $CDCl_3$



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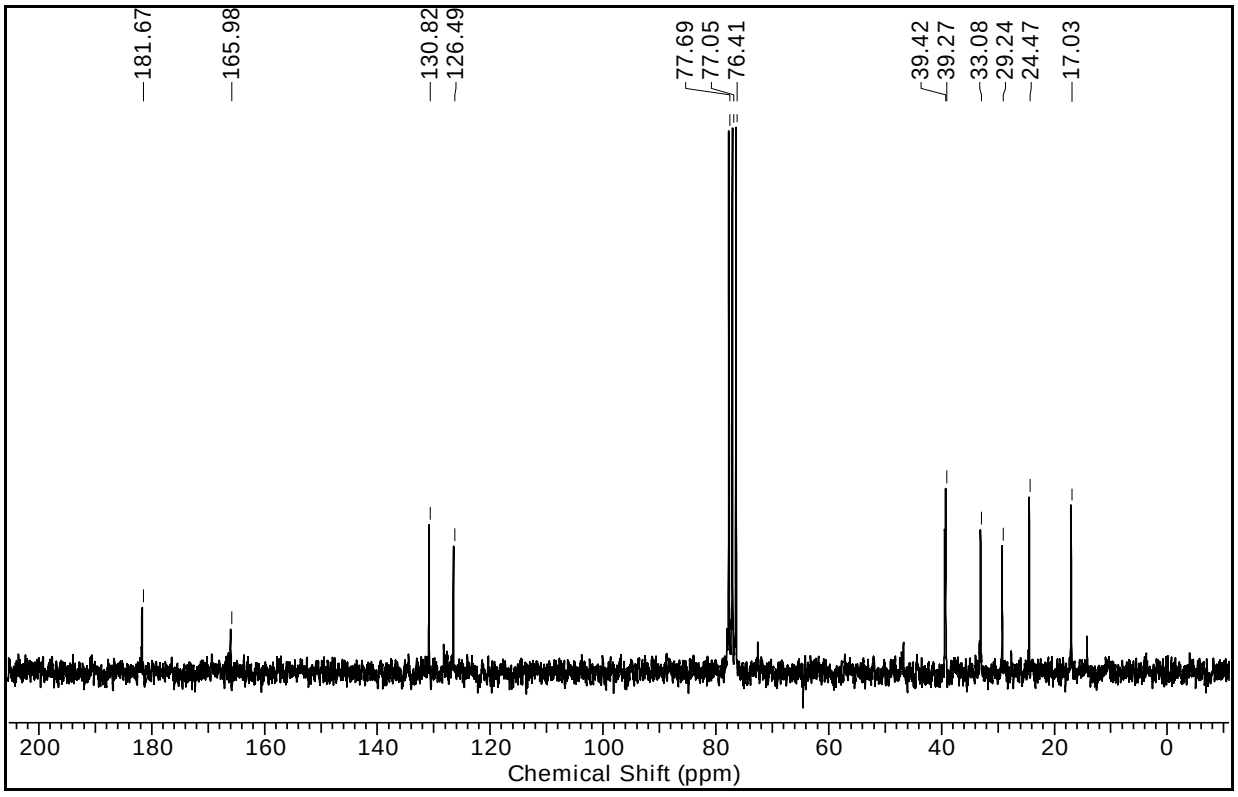
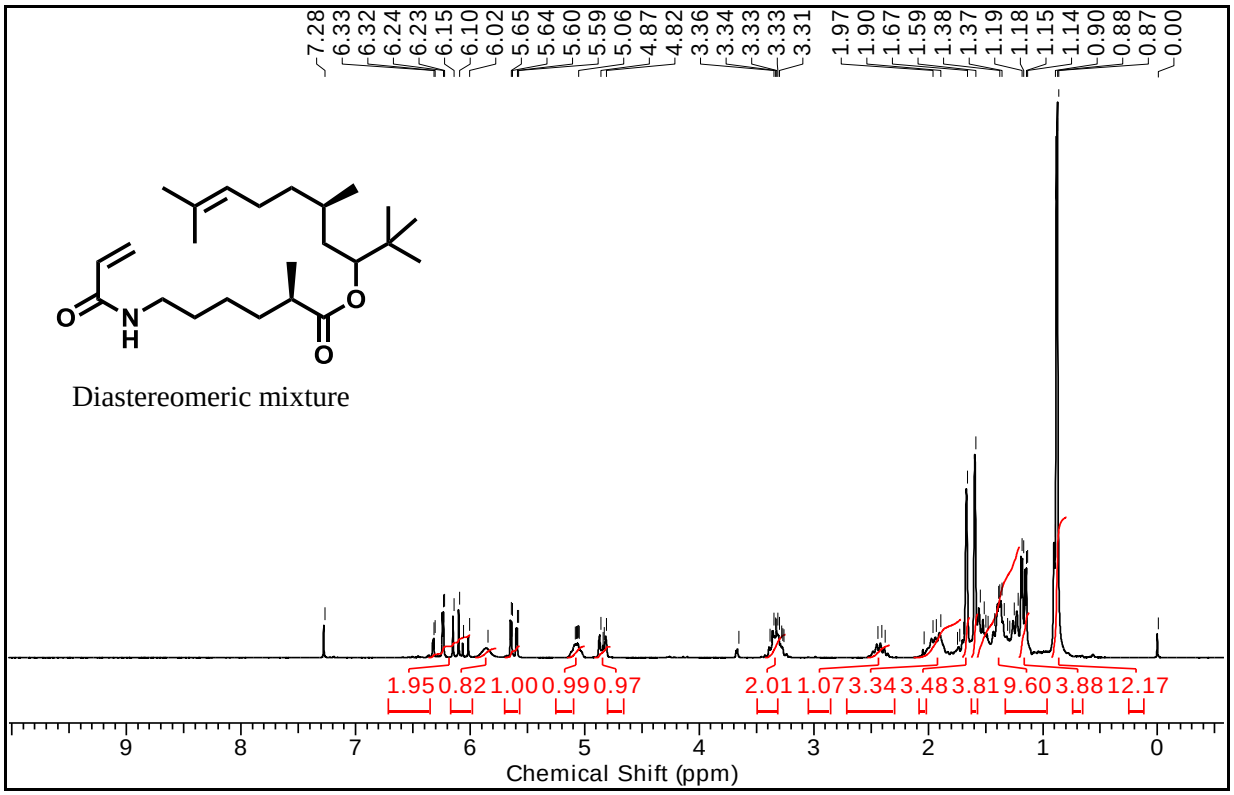
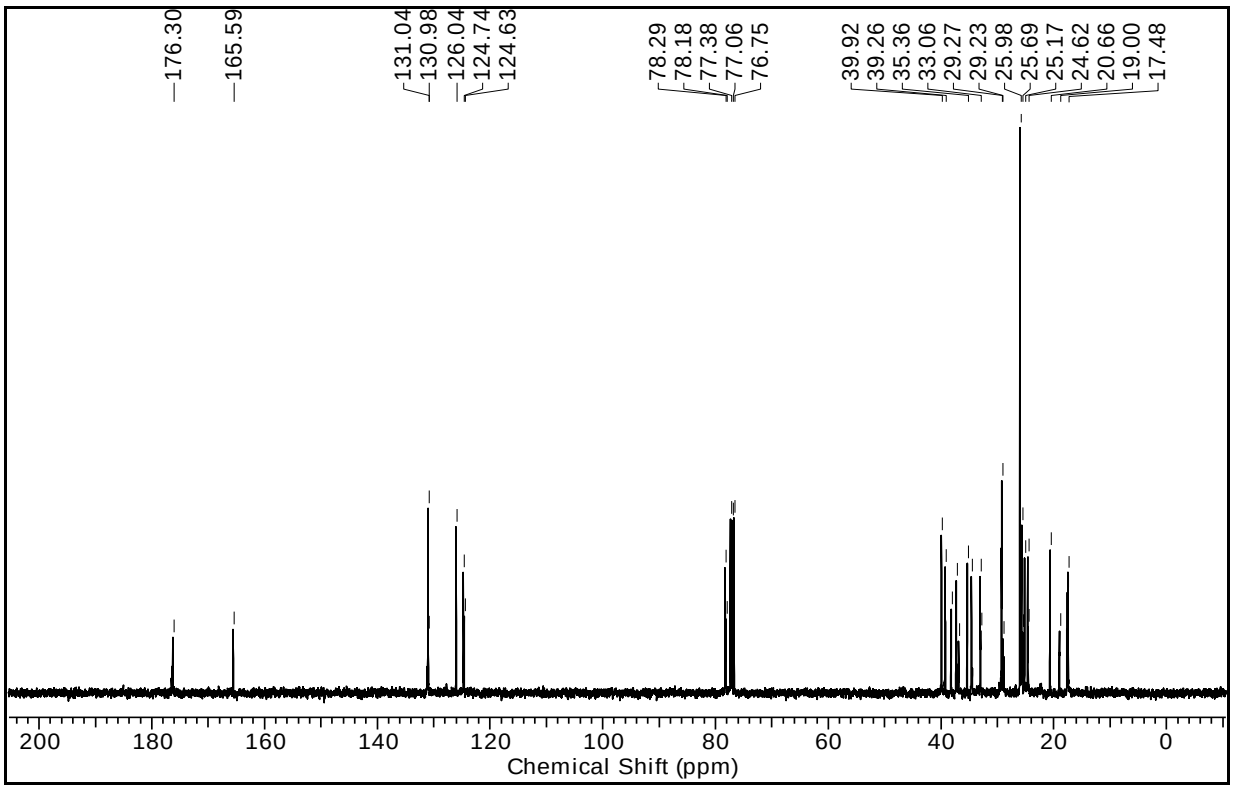


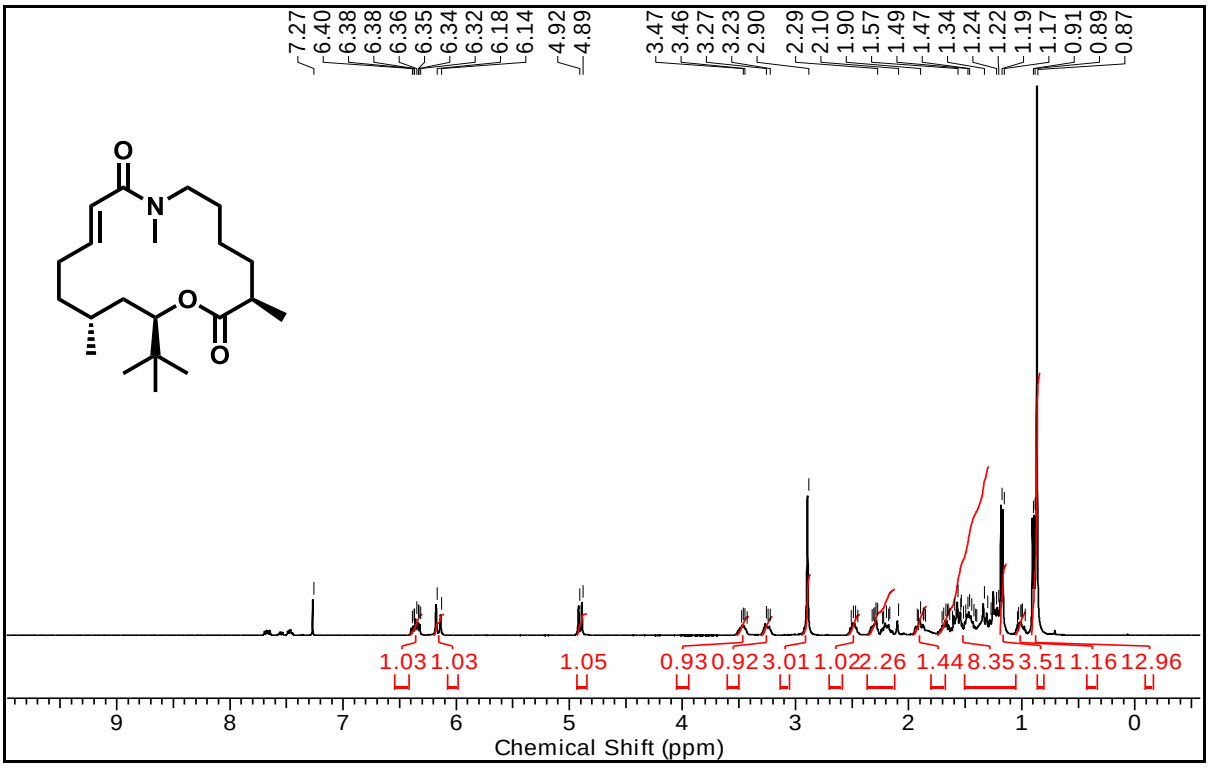
^{1}H NMR of Compound 86 at 200 MHz in $CDCl_3$



^{13}C NMR of Compound 86 at 100 MHz in $CDCl_3$



^{1}H NMR of Compound 87 at 400 MHz in $CDCl_3$



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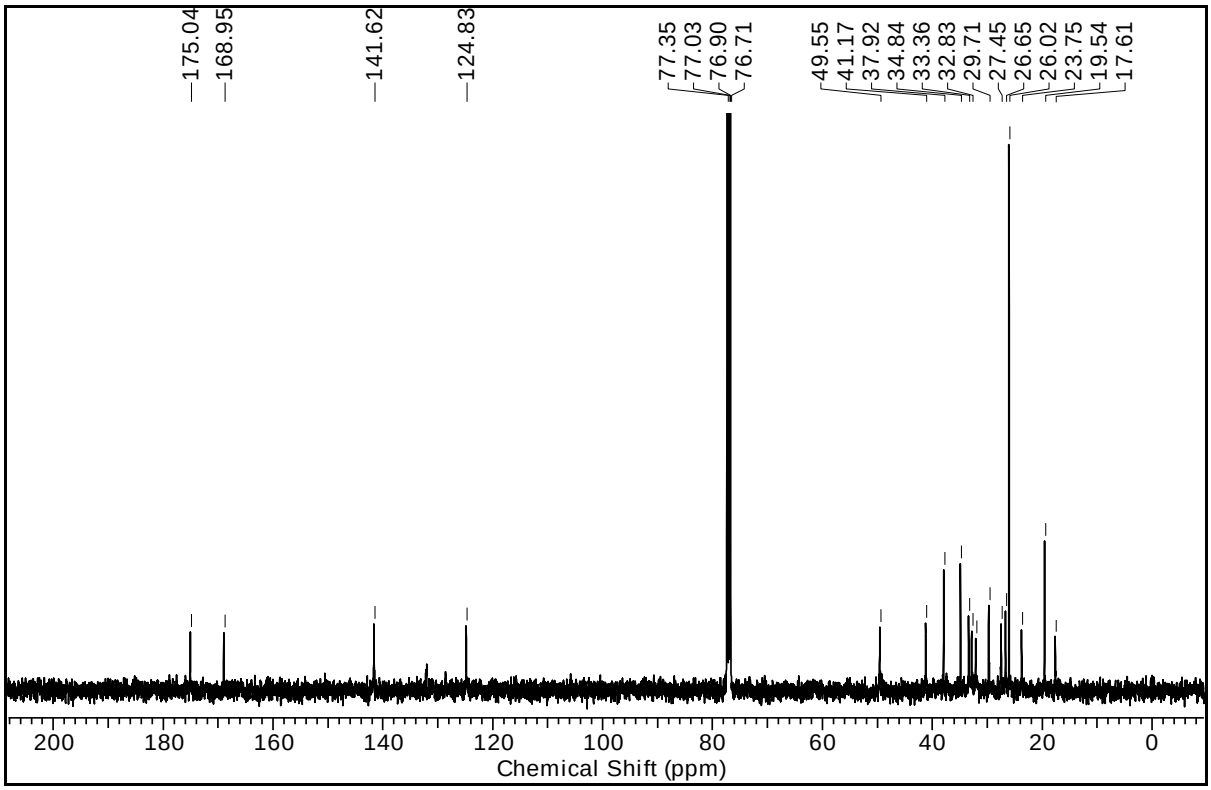
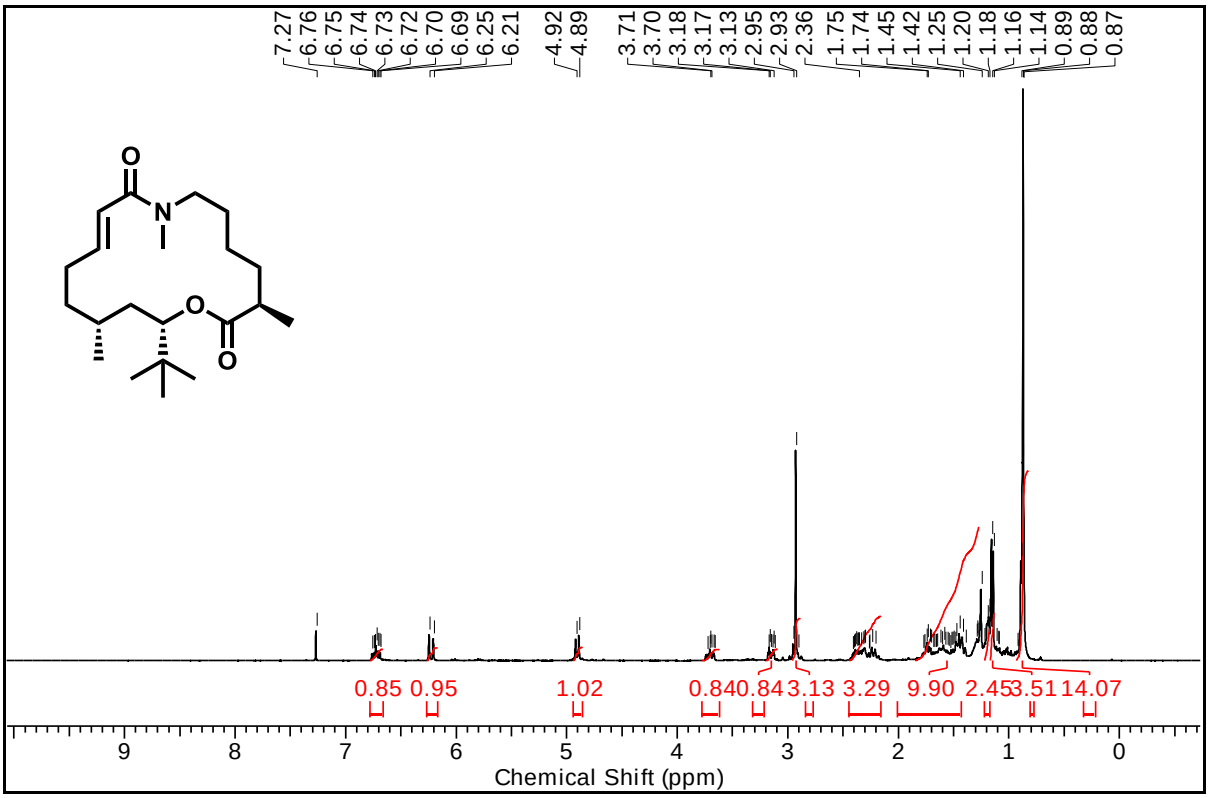


^{1}H NMR of Compound 88 at 400 MHz in $CDCl_3$



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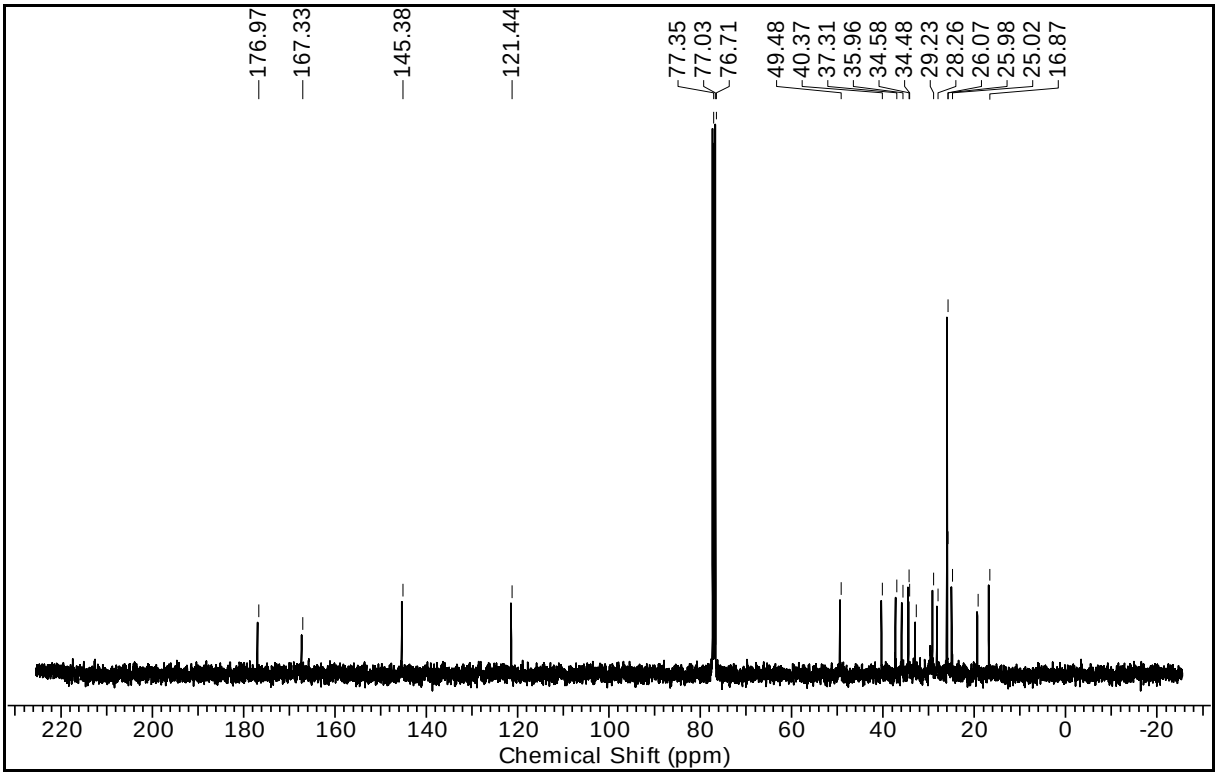
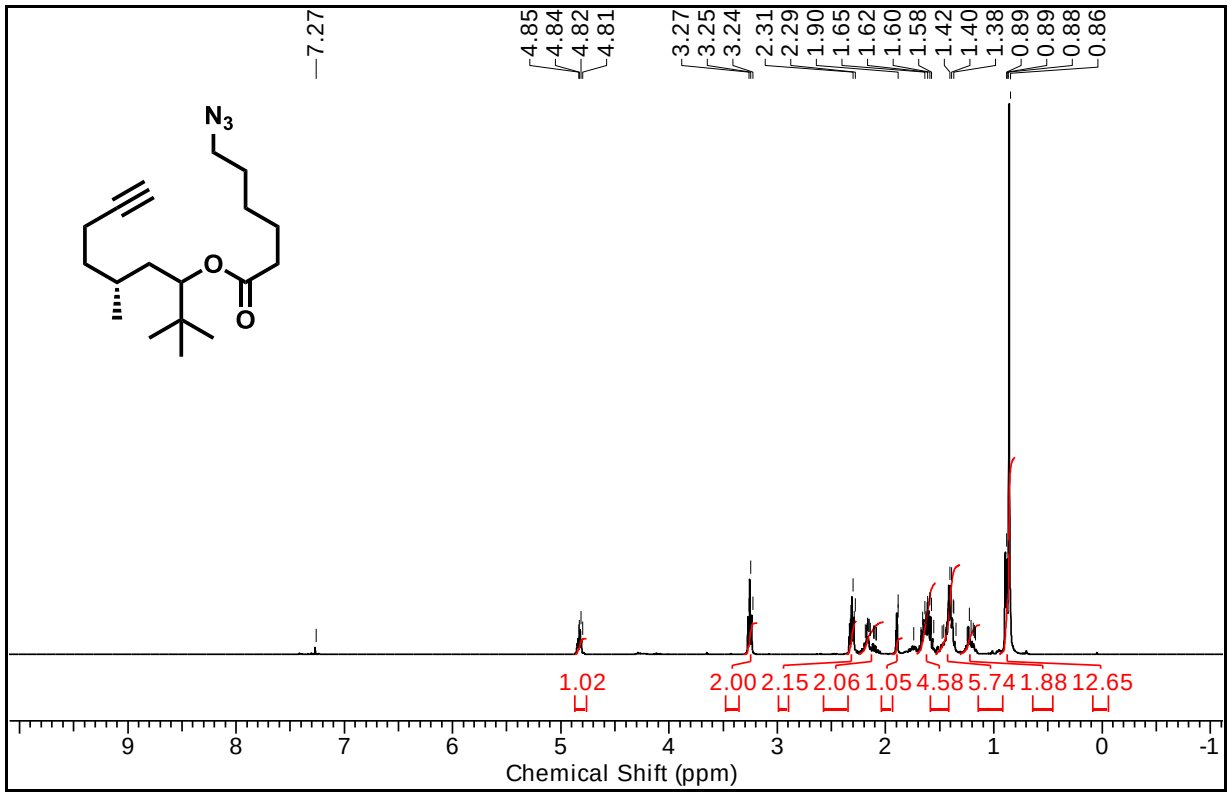
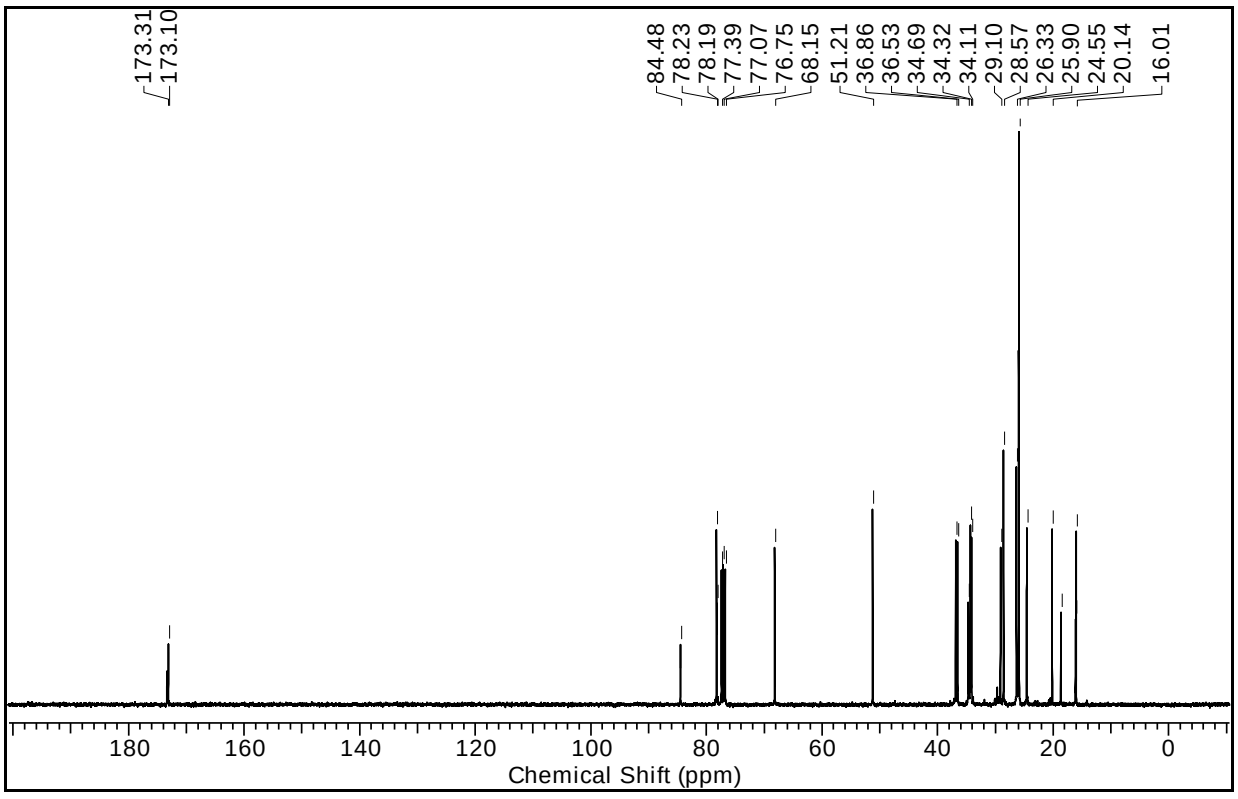


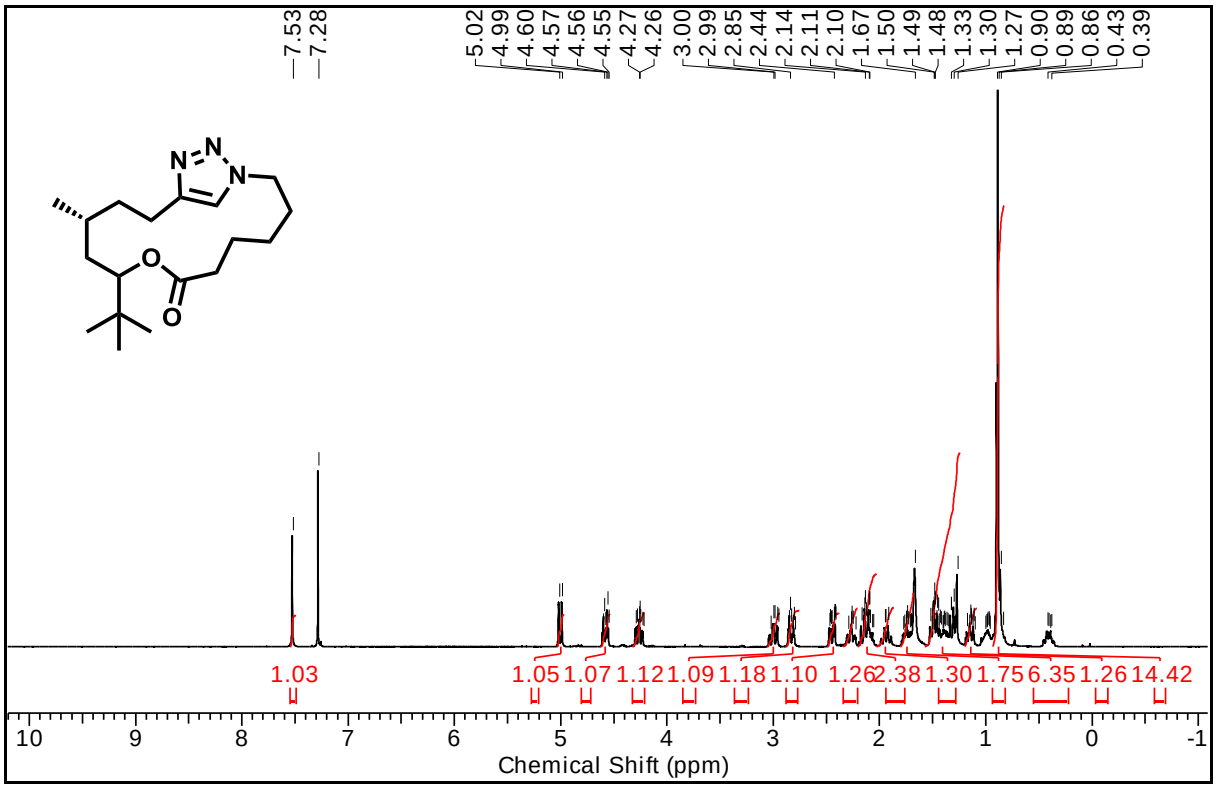
^{1}H NMR of Compound 89 at 400 MHz in $CDCl_3$



^{13}C NMR of Compound 86 at 100 MHz in $CDCl_3$



^{1}H NMR of Compound 90 at 400 MHz in $CDCl_3$



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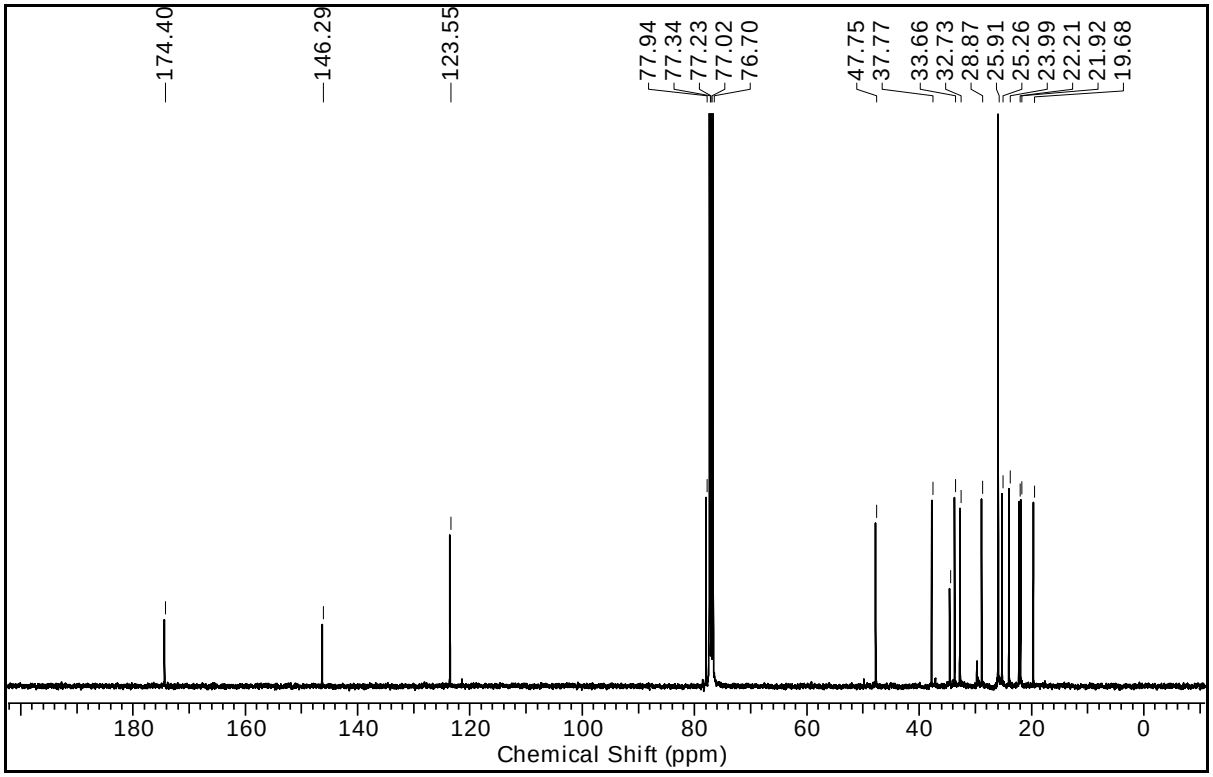

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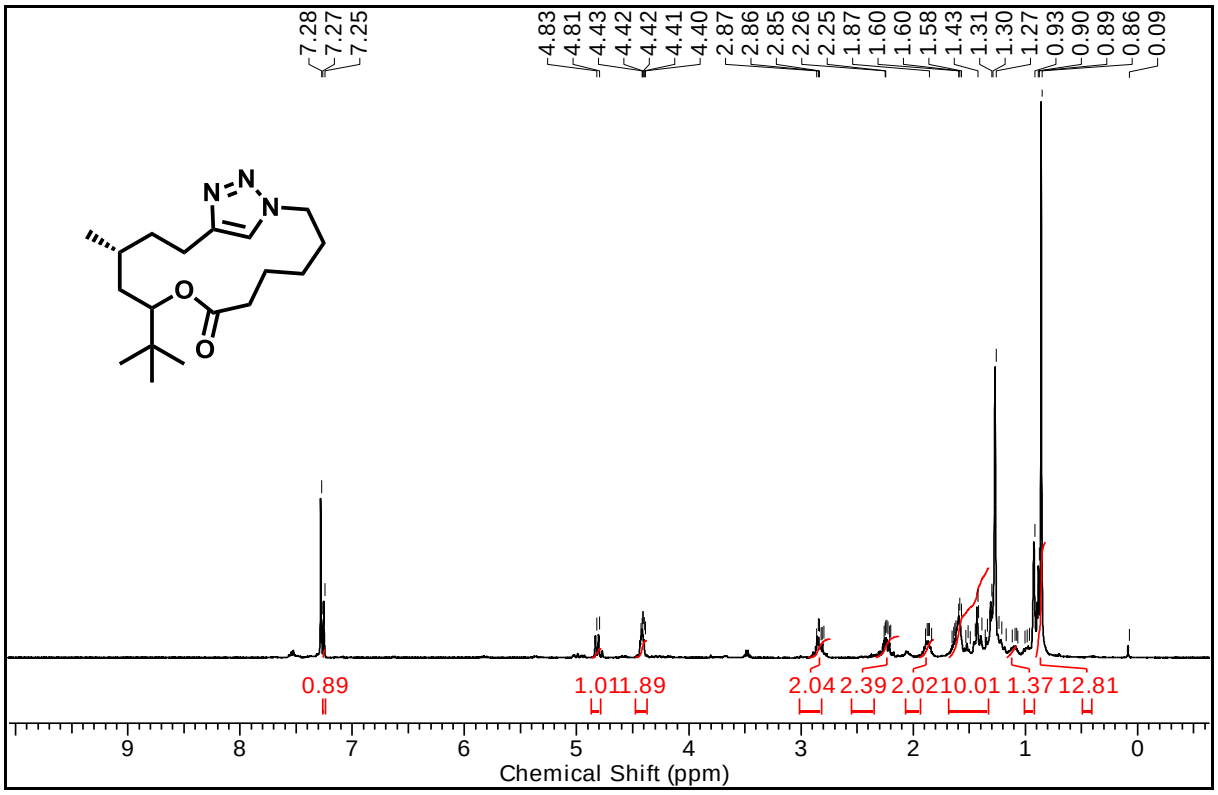

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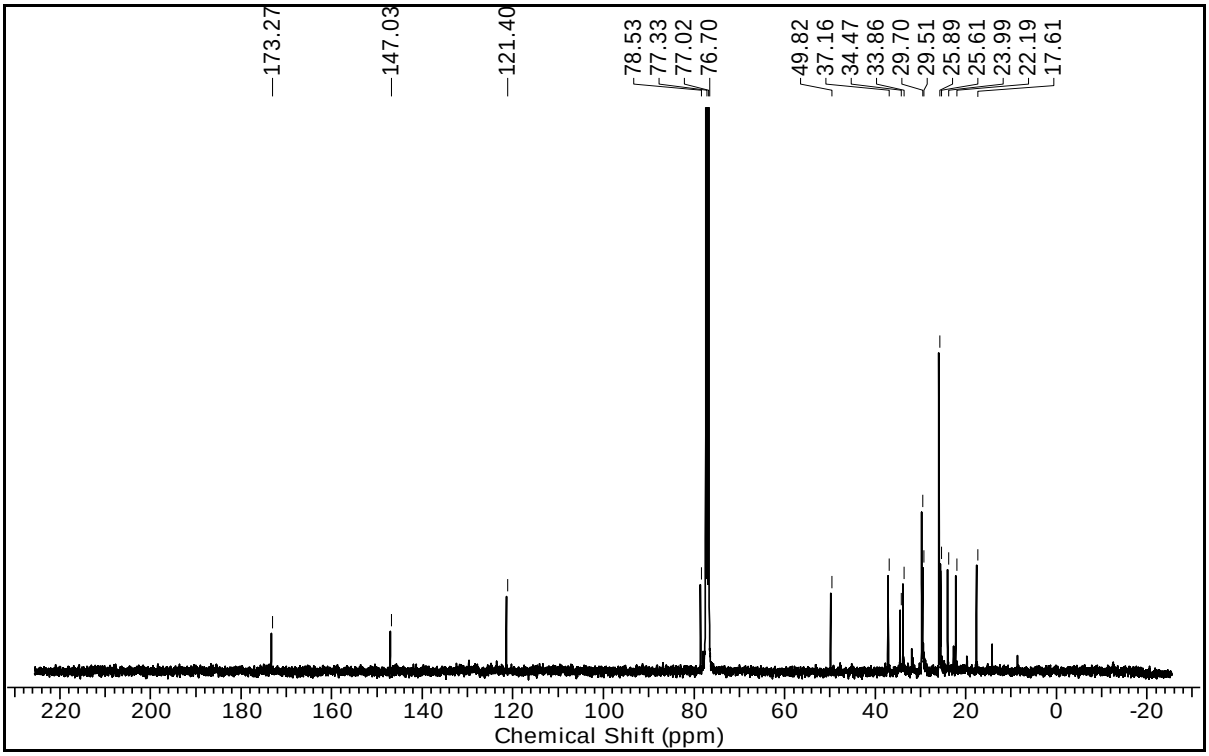

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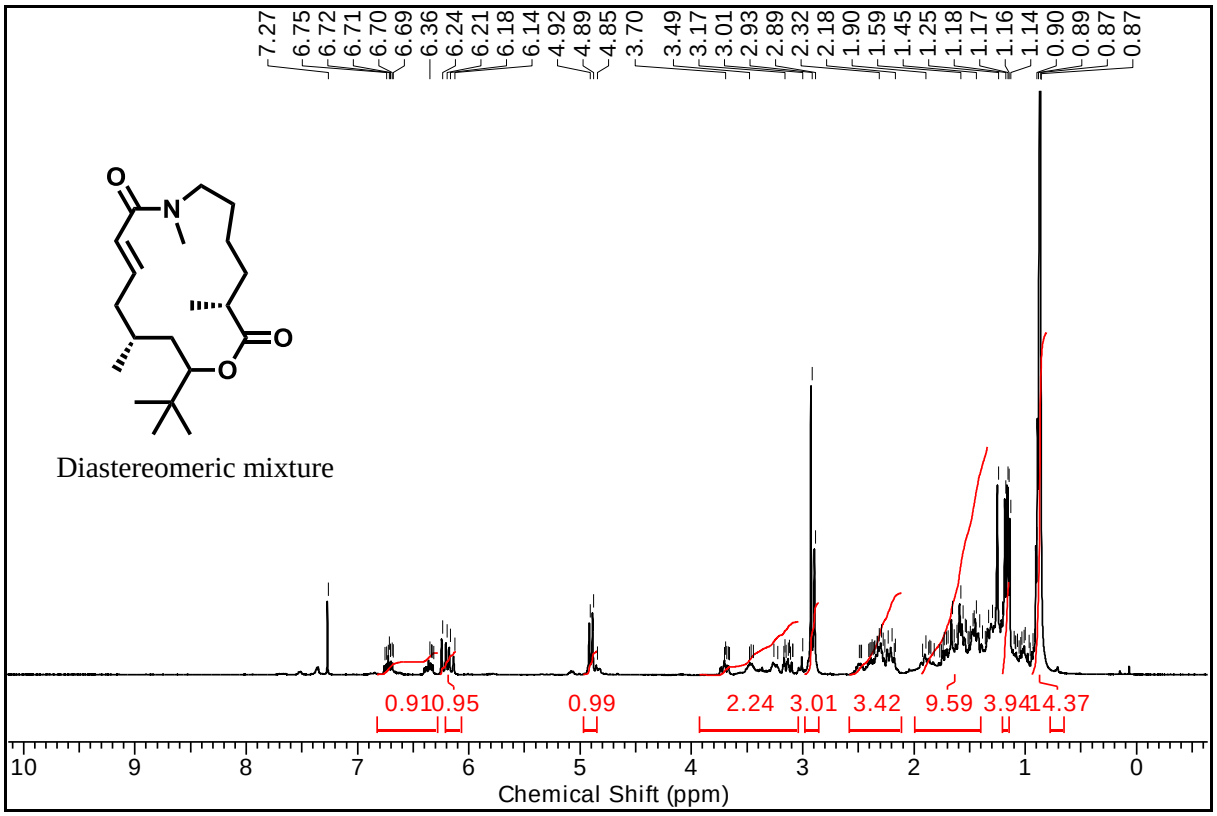

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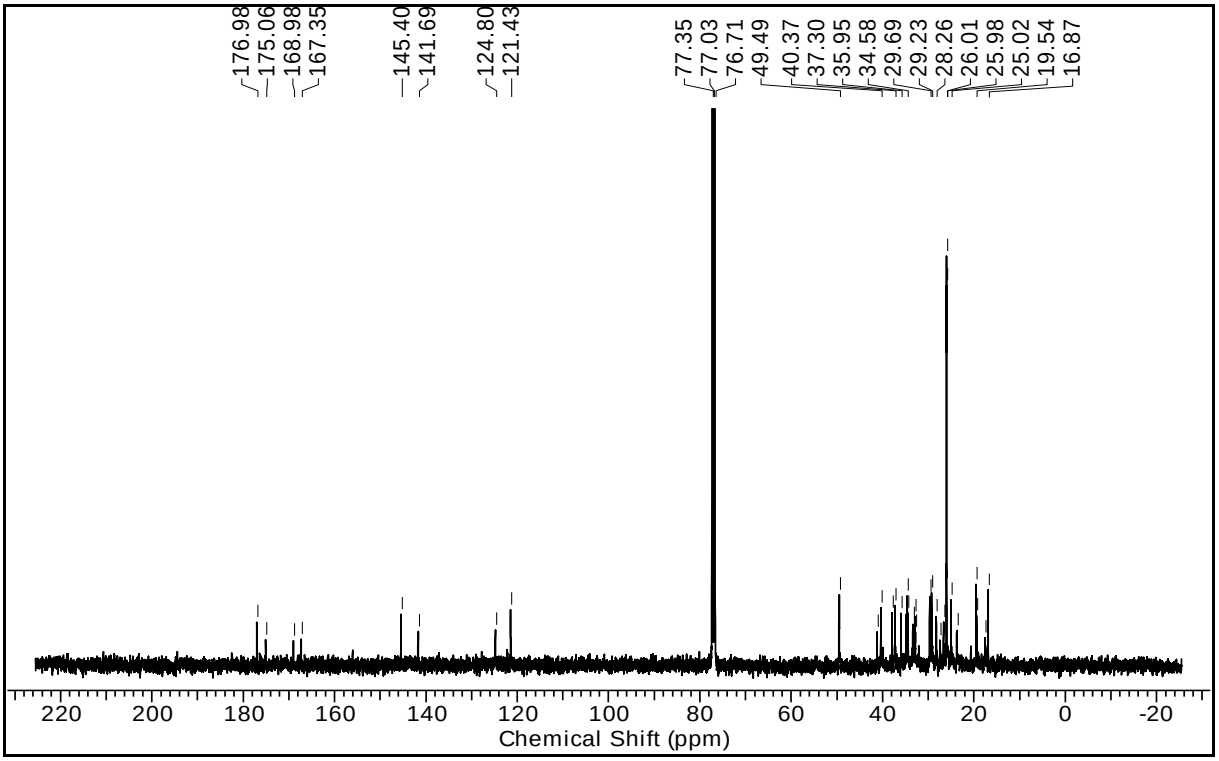
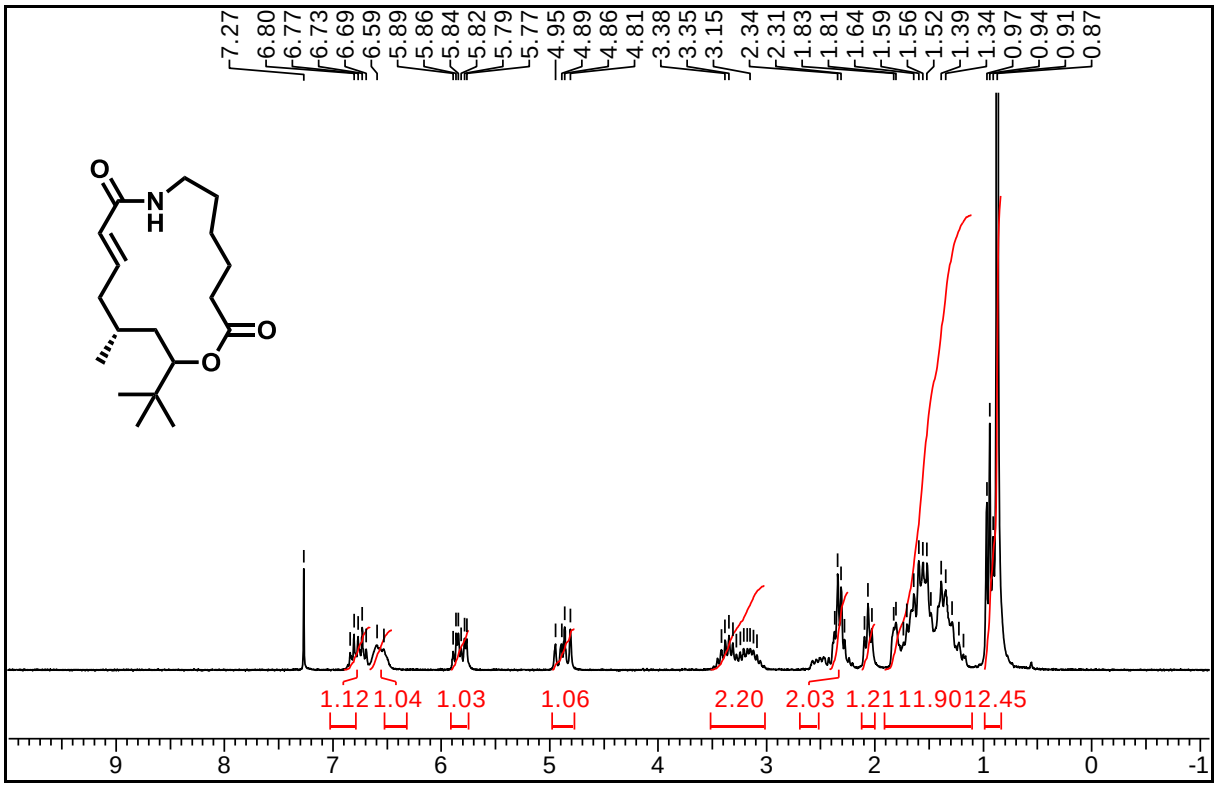
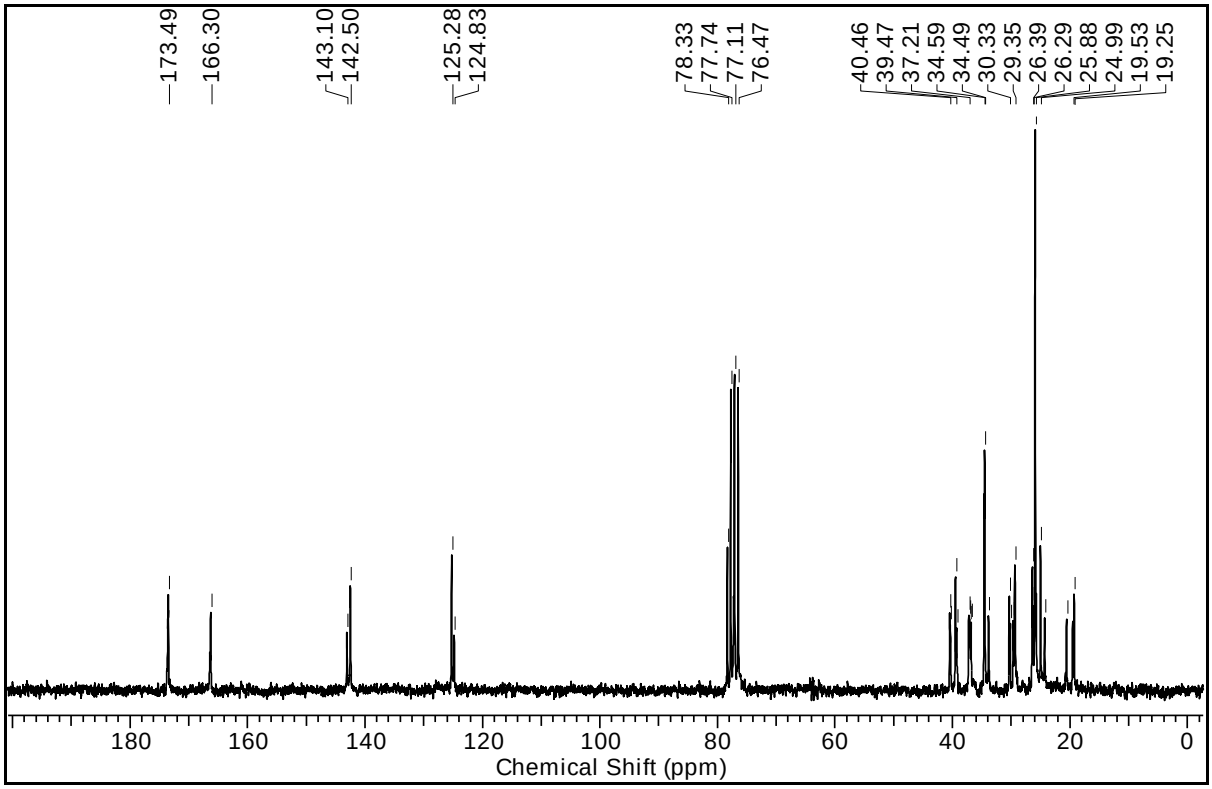


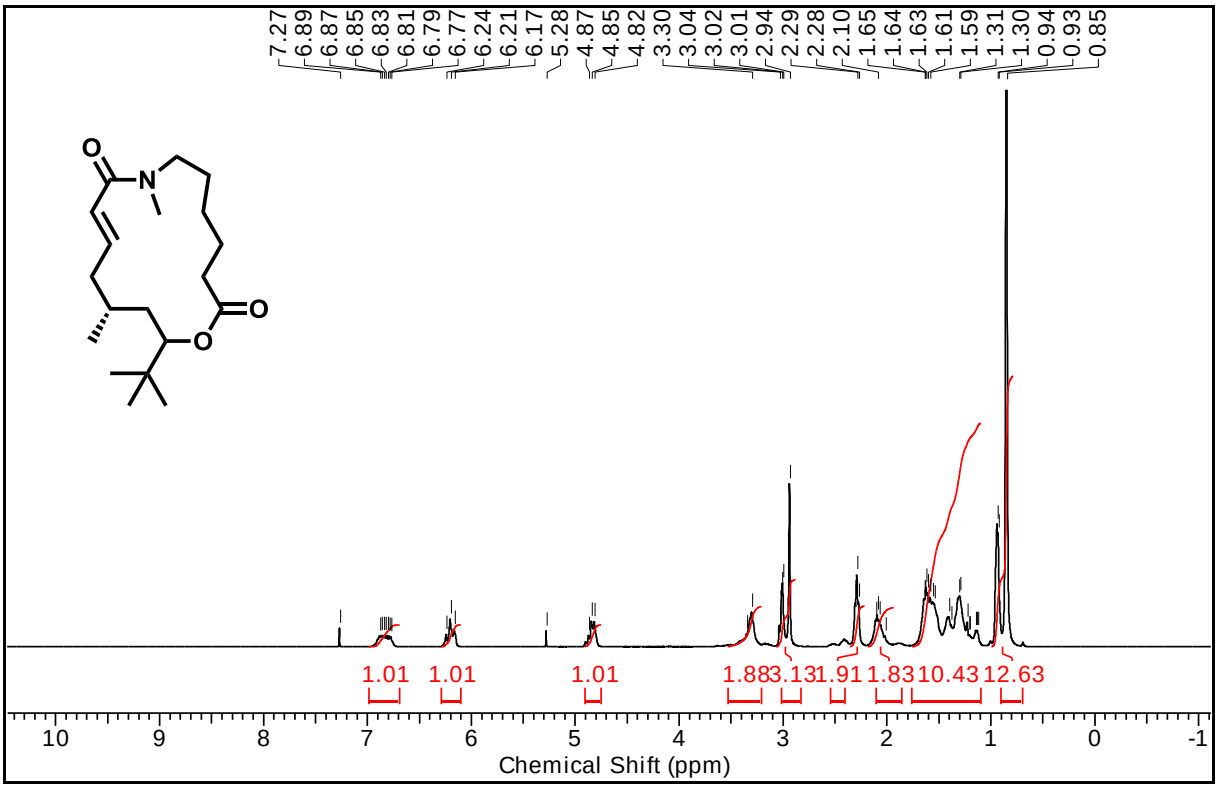
^{1}H NMR of Compound 95 at 200 MHz in $CDCl_3$



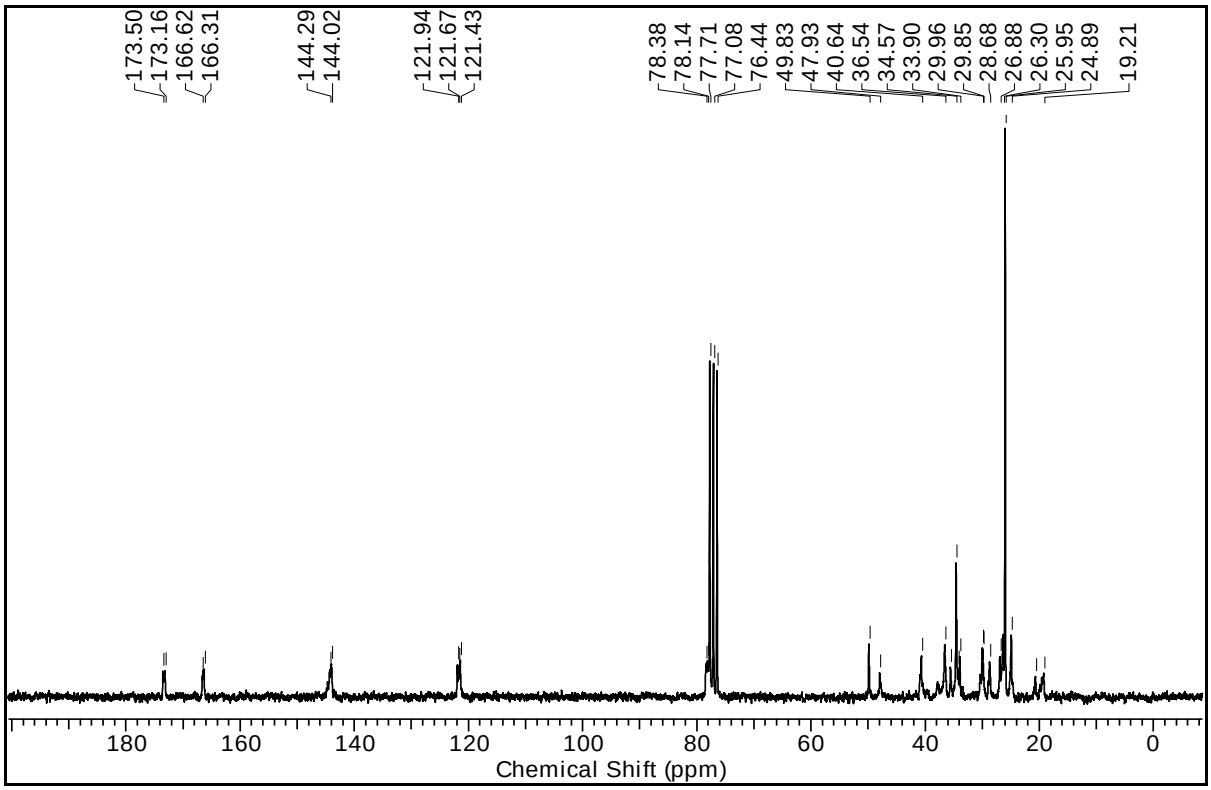
^{13}C NMR of Compound 95 at 50 MHz in $CDCl_3$



^{1}H NMR of Compound 96 at 400 MHz in $CDCl_3$



^{13}C NMR of Compound 96 at 50 MHz in $CDCl_3$



Chapter 2

Synthesis and Biological Evaluation of New Anti-inflammatory Agents Nitrosporeusines and their Analogues

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Synthesis and Biological Evaluation of New Anti-inflammatory Agents Nitrosporeusines and their Analogues

2.1 Introduction

2.1.1 Introduction to Inflammation

Inflammation is an immune response in body initiated because of pathogen invasion or tissue injury, and is intrinsically a beneficial process which restores the physiological balance by eliminating the factors harmful to body. There are mainly three major elements of our immune system (Complement, Phagocytes, Lymphocytes) which work synergistically during an immune response and are very well coordinated by various inflammatory mediators or cytokines.[1] During an inflammatory response, all the mediators, such as pro-inflammatory cytokines (e.g., interleukin-1β (IL-1β), IL-6, IL-12, and the chemokine IL-8), tumor necrosis factors (e.g., TNF-α and TNF-β), interferons (e.g., IFN-γ), eicosanoids (e.g., prostaglandins and leukotrienes) and vasoactive amines (e.g., histamine) are released.[2] After the complete neutralization of inflammatory stimuli *via* phagocytosis, the response has to subside and inflammation should resolve to normal state with all the macrophages and lymphocytes returning to pre-inflammatory numbers and types.

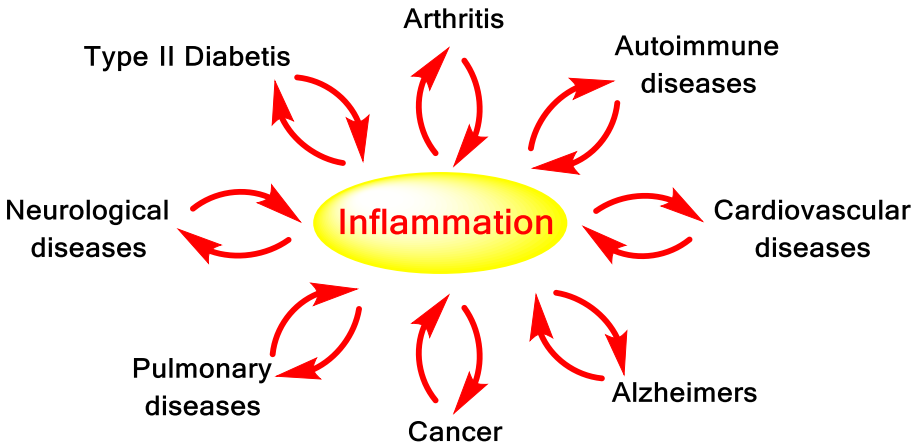


Figure 2.1: Inflammation and associated human diseases

If this inflammatory process is not properly phased out there can be continuous over expression of various pro-inflammatory factors such as reactive oxygen species (ROS), cytokines (IL-1β, IL-6, and TNF-α) leading to tissue damage and cause serious inflammatory diseases.[3] Hence all these factors play important roles in inflammatory processes and also are useful biomarkers for inflammation.[4] Apart from all these triggering factors there are certain autoimmune responses in which body mistakes normal healthy tissues for foreign bodies and carry out inflammatory attack which leads to chronic inflammation and autoimmune diseases. Sometimes, as in case of asthma there is also possibility that inflammatory response is worse than actual irritant or foreign bodies. Inflammation is classified as either acute or chronic, depending on whether it involves a short response or a prolonged one, respectively.[5] In spite of the fact that inflammation is primarily a protective response, the chronic and uncontrolled inflammation becomes detrimental to tissues.[6] There are also inferences of the chronic inflammation in the pathogeneses of arthritis, cancer, cardiovascular, autoimmune as well as viral infections which made it a serious medical issue.[7] Therefore, research has been directed in recent years to develop safer and potent anti-inflammatory drugs to attenuate the severity of inflammation.[7,8] As the human immune system is a complex process involving many factors and can go awry many times, it is very challenging to develop novel efficient chemical entities for treating inflammation.[9] As a part of our continuous interest[10] in search of biologically active natural molecules, in particular for the development of anti-inflammatory agents, we have come across a novel class of compounds with benzenecarbothioccyclopenta[c]pyrrole-1,3-dione scaffold – nitrosporeusines. Nitrosporeusines A (**1**) and B (**2**) are two new marine natural products having unique skeleton isolated by Lin and co-workers from Arctic Chucki sea.[11]

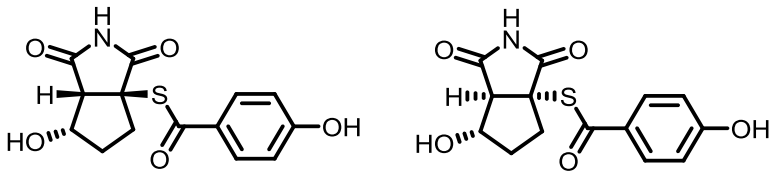


Figure 2.2: Natural products nitrosporeusines A (**1**) and B (**2**)

The sediments contain actinomycete *S. nitrosporeus* CQT14 which has major component as PKC inhibitor staurosporine and minor ones being nitrosporeusines. The structure of both compounds was established through detailed NMR and single crystal X ray studies. Before discussing our

work on this natural products, we would like to highlight the biological importance of this scaffolds which made us to choose this target.

2.1.2 Biological activity of nitrosporeusine A & B and its importance

Initial biological evaluation of nitropsoreusine A (**1**) and B (**2**) revealed that both compounds have promising potential in treating influenza virus strains A/WSN/33(H1N1).[11] Nitrosporeusine A and B were studied by Lin and co-workers for their antiviral activity using Madin-Darby Canine Kidney Epithelial Cells (MDCK). These cell lines were first obtained in 1958 from kidney of normal female adult Cocker Spaniel and they support wide range of animal viruses for study. In initial biological evaluation of nitrosporeusines, MDCK cells are propagated with influenza virus strains A/WSN/33(H1N1) and tested with nitroposreusines A and B which showed 18% and 30% inhibition respectively at 50 µM concentration with marketed drug Oseltamivir (Osv-P) as standard for comparison (Table 2.1). The cytotoxicity assay results also show that at 300 µM, nitrosporeusines A and B showed no inhibitory activity on uninfected MDCK cells.

Table 2.1: Comparison of nitrosporeusines with marketed drug Oseltamivir phosphate against Influenza virus strains A/WSN/33(H1N1) propagated in MDCK cells[11]

Compound	Uninfected cells	Infected cells inhibition rate (%) at 50 µM
Oseltamivir phosphate (positive control)	-	54.5 ± 1.3
Nitrosporeusine A	No effect (at 300µM)	18.6 ± 4.9
Nitrosporeusine B	No effect (at 300µM)	30.9± 5.5

To further study the antiviral activity of nitrosporeusine B, a viral plaque formation study was performed on it. The MDCK cells were incubated with test compound and adsorbed with virus. They were then overlaid with agarose and viral plaques formed were counted after 48h. The more the viral plaque formation, more is the production of viral progeny. Nitrosporeusine B (**2**) exhibited dose-dependent reduction with EC_{50} value of 112.7 ±4.4 µM, where EC_{50} value of Osv-P was 67.0 ±1.6 µM.

Following these results and publication, there were five Chinese patents filed by Chen and co workers in which they claimed to have carried out *in vivo* studies in mouse models with nitrosporeusine A (**1**) as test compound. According to these patents and data available with them, nitrosporeusine A has exceptional activity in treating wide range of diseases such as rhinitis, oral ulcer, chronic heart failure, acute renal failure and renal fibrosis.[12]

Allergic rhinitis is irritation and inflammation of the mucous membrane inside the nose which happens when our immune system overreacts to allergens in air. Chen's group has conducted *in vivo* experiments on rats with nitrosporeusine A, where inhibition of ovalbumin-induced allergic rhinitis was observed through sneezing / scratching nose response and compared to existing drug Terfenadine. In another study, nitrosporeusine A was explored for their potential in treatment and prevention of renal fibrosis**.** Renal fibrosis is the inevitable consequence of an excessive accumulation of extracellular matrix that occurs in virtually every type of chronic kidney disease. Nitrosporeusine A can significantly reduce renal interstitial fibrosis in mice as it is observed to decrease fibronectin (FN) and Hyperkalemic periodic paralysis HYPP levels at 50 mg/kg concentration. The results were comparable to marketed drug Benazepril.

Oral ulceration is a common condition which is often characterised by loss of mucosal membrane in mouth. Chen and co workers showed that application of nitrosporesuine A at an optimum dose of 20 mg for 4 times could significantly reduce the ulcer area in six days.

Another application of nitrosporeusine A was found to be in treatment of acute renal failure (ARF). It is an abrupt loss of kidney function that develops within 7 days. Blood urine nitrogen (BUN) is a marker for how well one's kidneys are working. When kidneys do not work properly there is a higher BUN level observed in blood. On similar lines kidneys also maintain blood creatinine levels by disposing creatinine, a waste produced during muscle metabolism, through urine. Its measure is also an indicator for kidneys health, where elevated creatinine levels signify kidney disease or impaired functioning. Chen and co-workers reported that there is significant reduction in levels creatinine and blood urine nitrogen when administered with higher doses of nitrosporeusine A in mouse models. It had also been showed that nitrosporeusine A could be used in treatment or prevention of chronic heart failure and results compared to drug captopril.

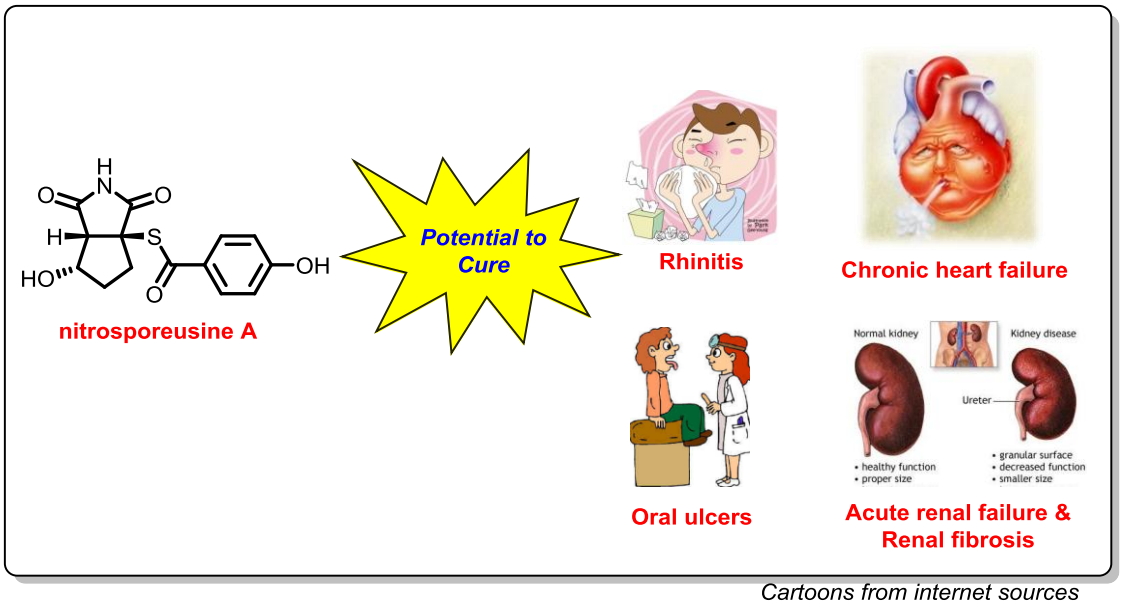


Figure 2.3: Potential applications of nitrosporeusine A (**1**)

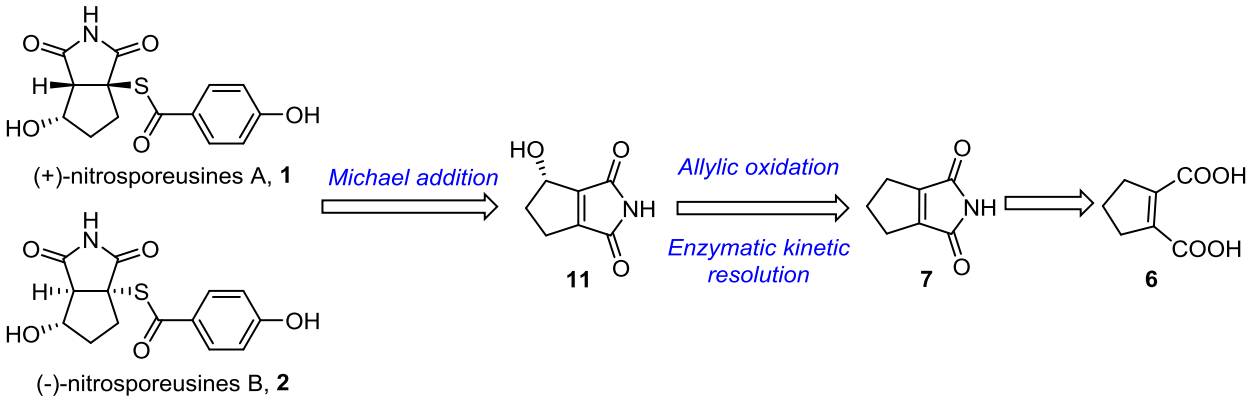
These biological activity results reported for nitrosporeusines, in particular with compound **1** are exceptional and attracted our attention. The claims from Chinese patents using animal experiments are very impressive, therefore, we planned for a complete study over this novel scaffold. Due to close association of many microbial and viral infections with inflammation,[13] we envisioned to synthesize and study the nitrosporeusines in detail towards its anti-inflammatory potential. Accordingly, we first planned the total synthesis of nitrosporeusines A & B using a simple and flexible route which could be extended to synthesize a library of close analogues as well.

2.2 Synthesis of nitrosporeusines A and B

2.2.1 Retrosynthesis

Nitrosporeusines A and B share a common structural core with difference only in relative stereochemistry between hydroxyl group and ring junction. So, both the natural products are envisioned to be obtained through Michael addition on hydroxyl intermediate (**11**) which in turn could be obtained from maleimide (**7**) through allylic oxidation and resolution (Scheme 2.1). The maleimide (**7**) could be prepared from cyclopent-1-ene-1,2-dicarboxylic acid in a few synthetic operations starting from 2-oxocyclopentane-1-carboxylic acid. While we were planning for the synthesis, we chose a strategy which would be amenable for synthesis of both natural products,

as well as close analogues. We planned to exploit the key maleimide intermediate to make several building blocks for analogues.

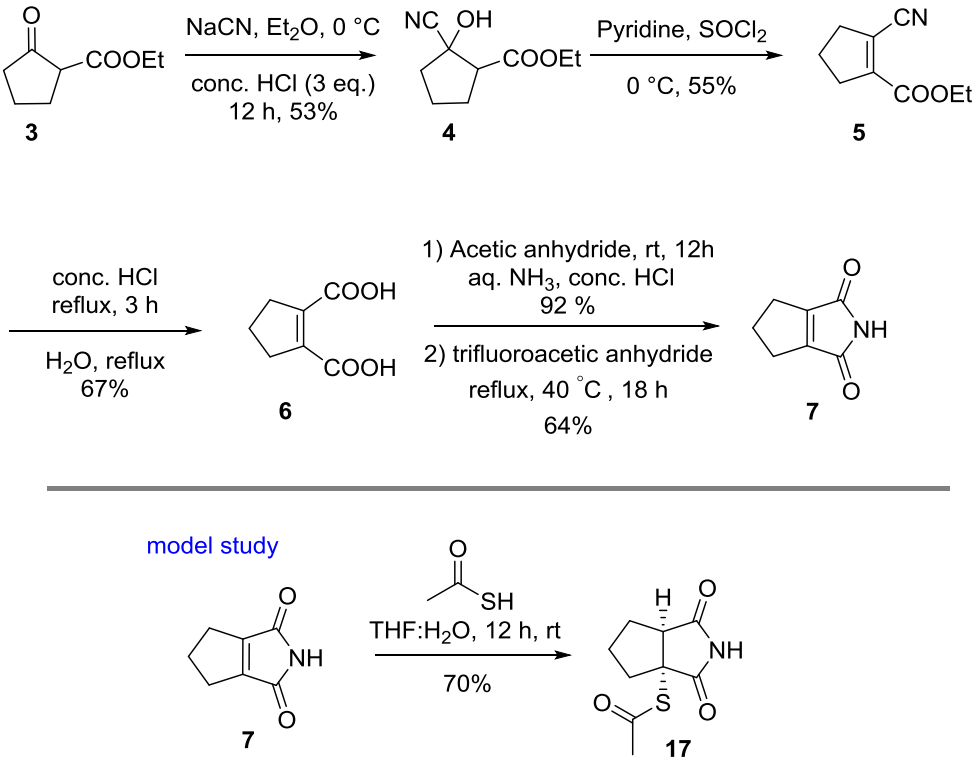


Scheme 2.1: Approach towards nitrosporeusines and their analogues

2.2.2 Total synthesis of nitrosporeusines A & B

We started with an aim to prepare maleimide (**7**) in good quantities, which enable us to synthesize natural products as well as analogues. By following the literature procedures,[14] commercially available ethyl 2-oxocyclopentane-1-carboxylate was subjected to cyanation using NaCN/HCl which gave cyanohydrin **4**, which was then eliminated with pyridine/$SOCl_2$ to yield intermediate **5**. The formation of **5** was confirmed from presence of characteristic peaks in ^{13}C NMR at regions δ 114.3 corresponding to cyano carbon, δ 121.3 and 149.2 corresponding to olefin carbons along with a presence of ester carbonyl at δ 162.2. The cyanide group was then hydrolysed by refluxing in conc. HCl to afford cyclopent-1-ene-1,2-dicarboxylic acid (**6**). Its formation was confirmed with disappearance of characteristic cyano carbon at δ 114.3 along with appearance of single olefinic peak for two carbons in ^{13}C NMR spectra which also explains the symmetric nature of compound **6**. Thus obtained dicarboxylic acid was treated with acetic anhydride/NH_3 and then subjected to intramolecular condensation using trifluoroacetic anhydride resulting in desired maleimide (**7**) in good yields (Scheme 2.2). The formation of **7** was confirmed by comparing the spectral values obtained with those reported in literature where the characteristic peaks of carbons at δ 154.0 (olefin), 167.9 (amide carbonyl) were observed in ^{13}C

NMR spectra. After having desired intermediate 7 in hand we first planned to explore the feasibility of Michael addition on this maleimide moiety.

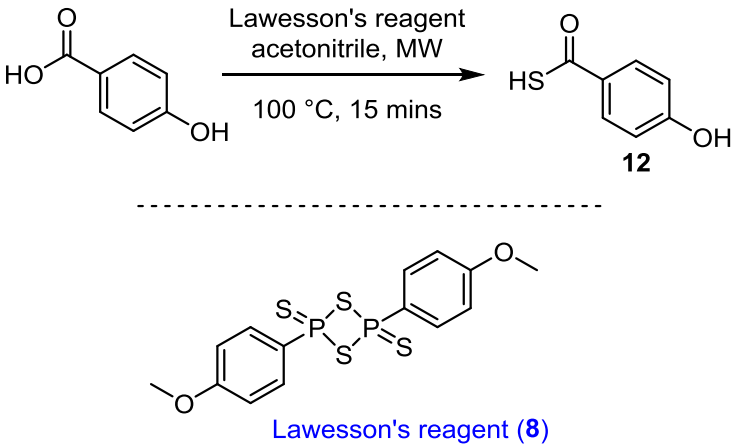


Scheme 2.2: Synthesis of key maleimide and model study

Accordingly, 5,6-dihydrocyclopenta[*c*]pyrrole-1,3(2*H*,4*H*)-dione **7** was subjected to Michael reaction with commercially available thioacetic acid.[15] As expected, the reaction went smoothly to yield the desired Michael adduct **17** and a more pleasing outcome was that the reaction could be carried out in water/THF at room temperature (Scheme 2.2). The formation of **17** was confirmed by ^{1}H NMR spectra where peak at δ 3.27-3.24 (d, J = 8.8 Hz, 1H) corresponds to proton attached to imide carbonyl and a peak at δ 2.34 (s, 3H) corresponding to methyl singlet of thio-ester group. The ^{13}C NMR spectra clearly shows thio-ester carbonyl at δ 196.3 and characteristic tertiary carbon attached to sulphur at δ 59.5 which confirms Michael addition of thioacetic acid to **7**.

After scaling up the reaction and making sufficient quantities of compound **7**, firstly we proceeded for synthesis of nitrosporeusines A and B in racemic forms. Towards that we subjected the maleimide (**7**) to microwave mediated allylic oxidation using selenium dioxide,[16]

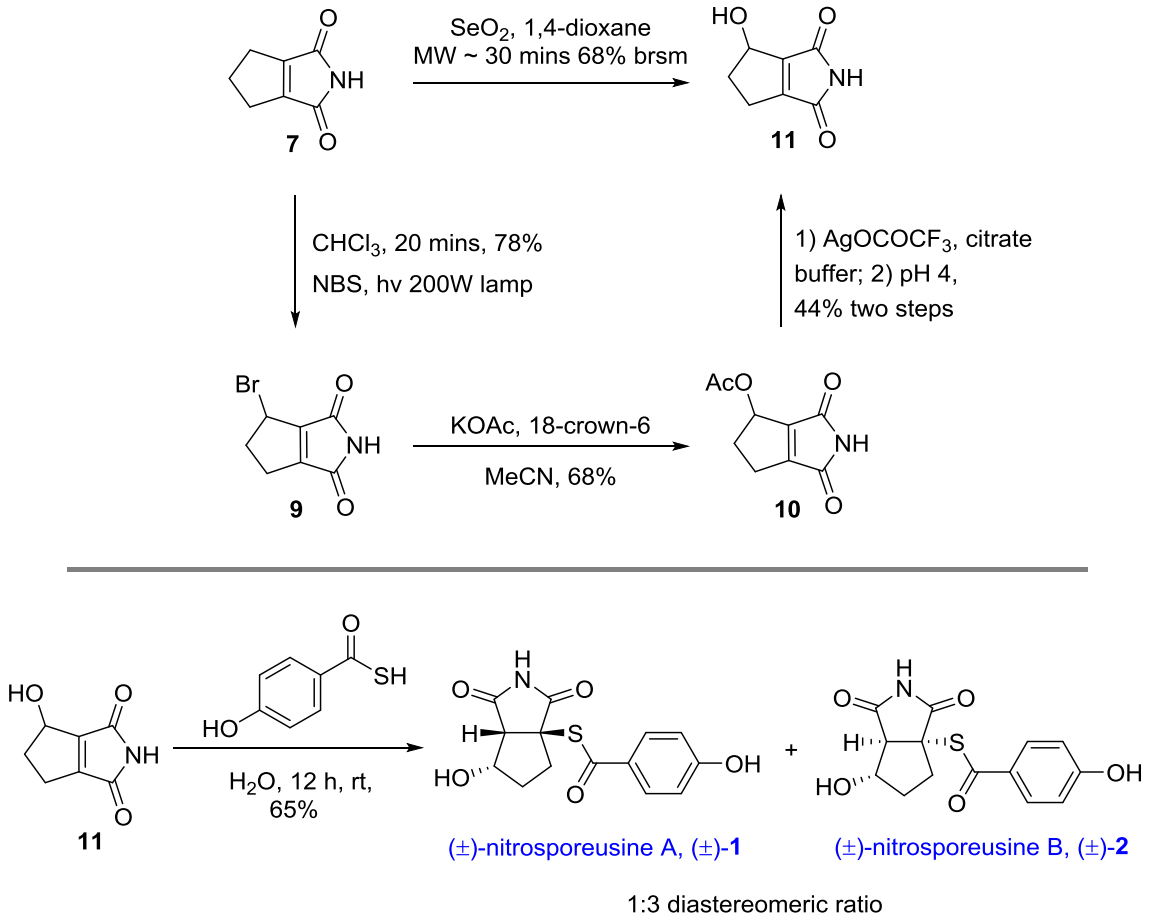
and after few optimizations we could obtain desired allylic alcohol (**11**) in moderate yields. (Scheme 2.4). The formation of **11** was confirmed by the de-symmetrisation observed in ^{1}H NMR spectra when compared with that of compound **7**. The ^{1}H NMR in D_2O shows appearance of the peak at δ 5.55 corresponding to proton at allylic position linked to hydroxyl group, which confirmed the allylic oxidation product **11**. Alternately the same alcohol **11** was obtained by allylic bromination (NBS/hυ/$CHCl_3$) followed by acetylation and hydrolysis protocol with better yields (Scheme 2.4). The compound **11** is a racemic form of natural product maleimycin, which itself is a biologically active molecule.[17] Having the required racemic maleimycin in hand we next prepared required thio-acid required for Michael addition. The 4-hydroxybenzothioic *S*-acid was obtained from corresponding acid using Lawesson's reagent. Even though there are many reagents known for conversion of acid to thio-acids, we preferred Lawesson's reagent due to its ready availability and ease of handling. Lawesson's reagent could also be prepared easily and safely from phosphorus pentasulfide by refluxing in anisole. Another advantage of using it is that it reacts in nearly equimolar proportions with a wide range of carbonyl compounds to give, in most cases, almost quantitative yields and easily purifiable products.



Scheme 2.3: Preparation of 4-hydroxybenzothioic *S*-acid

For the preparation of desired thio-acid, we subjected commercially available 4-hydroxybenzoic acid along with solid Lawesson's reagent and dissolved in acetonitrile which was then irradiated under microwave conditions (100 ºC/ 15 mins)[18] to get 4-hydroxybenzothioic *S*-acid (**12**) (Scheme 2.3). This thio-acid prepared dimerises very fast, so it was immediately used for next step (Michael addition) with maleimycin (**11**) using only water as solvent. Pleasingly,

the reaction proceeded very cleanly to produce both nitrosporeusines A and B in 1:3 diastereomeric ratio respectively (Scheme 2.4).



Scheme 2.4: Synthesis of racemic nitrosporeusines A and B

Although distereoselectivity was poor, the pleasing outcome of the reaction was the formation both natural products in same reaction. Both the diastereomers were cleanly separated using silica gel column chromatography and completely characterized with all spectral techniques (^{1}H, ^{13}C, IR, HRMS) which was then compared to those reported in literature[11] (Table 2.2). Although, the distereoselectivity was poor, we tried to give a possible explanation for the observed diastereoselectivity(~1:3) in the products formed. Firstly, the Michael addition of any nucleophile to compound **7** is expected to give exclusively *cis* fused ring - as *trans* fused five-five systems (diquinane) are not favourable to exist. Moreover, the incoming nucleophile (thio-acid) adds to planar maleimide (**7**) from either direction (β or α) with equal probability thus forming products in 1:1 ratio. On similar lines, the compound **11** also possesses a planar

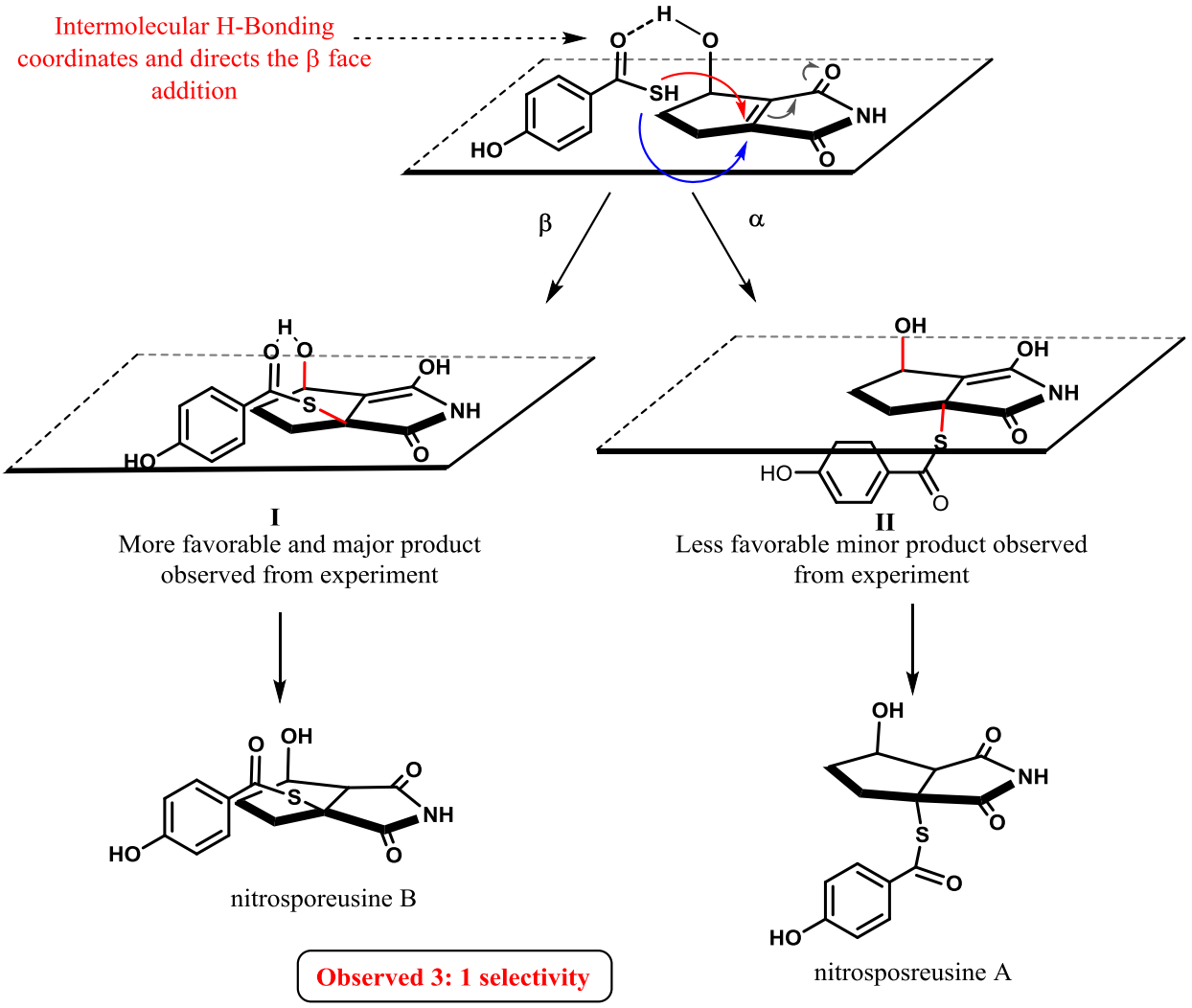
geometry with hydroxy group lying either above or below the plane of molecule. But when **11** was subjected to Michael addition, this hydroxyl group present at C6 position directs the incoming nucleophile and determines the relative stereochemistry to be formed with respect to ring junction.

Table 2.2: Comparison of spectral data of isolated and synthesized nitrosporeusines A & B

Nitrosporeusine A in DMSO d6				**Nitrosporeusine B in DMSO d6**			
^{1}H NMR		^{13}C NMR		^{1}H NMR		^{13}C NMR	
Reported	Obtained	Reported	Obtained	Reported	Obtained	Reported	Obtained
11.32, s, 1H	11.28, s, 1H	179.3	179.15	11.55, s, 1H	11.57, s, 1H	179.4	179.3
2.23, m, 1H	2.25, m, 1H	59.2	59.24	2.21, m, 1H 2.17, m, 1H	2.17 – 2.12, m, 2H	58.2	58.16
1.89, m, 1H	1.92 – 1.89, m, 2H	35.0	35.06			33.8	33.79
1.89, m, 1H		32.8	32.84	1.79, m, 1H	1.79 – 1.78, m, 1H	32.4	32.46
1.64, m, 1H	1.71 – 1.69, m, 1H	72.3	72.34	1.56, m, 1H	1.59 – 1.56, m, 1H	74.5	74.55
4.46, dt(7.3, 6.7), 1H	4.47, br s, 1H	60.2	60.22	4.38, brt, 1H	4.39, brt, 1H	64.0	63.96
3.25, d, (7.3) 1H	3.26, d, 1H	175.3	175.22	3.10, br s, 1H	3.11, br s, 1H	176.9	176.89
7.73, d(8.8), 2H	7.74, d(8.8Hz), 2H	190.1	190.04	7.76, d(7.6), 2H	7.76, d(8.8Hz), 2H	190.5	190.48
6.87, d(8.8), 2H	6.87, d (8.8Hz), 2H	127.0	127.01	6.88, d(7.6), 2H	6.88, d (8.8Hz), 2H	126.8	126.82
5.25, br s, 1H	5.28, br s, 1H	130.0	130.02	5.25, br s, 1H	5.27, br s, 1H	130.1	130.05
10.77, s, 1H	10.64, s, 1H	116.2	116.20	10.68, s, 1H	10.65, s, 1H	116.2	116.24
		163.7	163.67			163.8	163.73

So when 4-hydroxybenzothioic *S*-acid is added to **11**, there are two possible ways Michael addition could happen – α face attack or β face attack as shown in scheme 2.5. Here looking at

the structural features of **11**, there is possibility of only hydrogen bonding playing major role in determining the stereochemical outcome of reaction. The possibly strong intermolecular hydrogen bonding between the C6-hydroxyl group and incoming thio-acid carbonyl would facilitate the β face addition of thio-acid more than α face addition. Thus, the all *syn* product nitrosporeusine B is formed in lager ratio. On the other hand, the unfavourable α face attack led to products with *anti* relation with nitrosporeusine A forming in minor quantities. Similar hydrogen bonding mediated Anti-aza-Michael addition reactions were reported in literature[19] which helped us in proposing this probable mechanism.



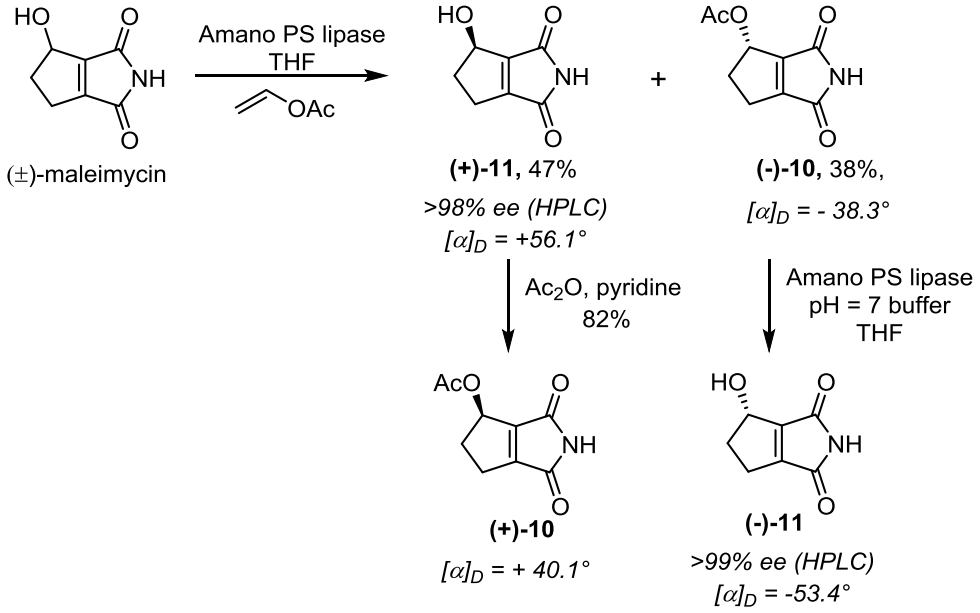
Scheme 2.5: Probable explanation for observed selectivity

The successful racemic synthesis of natural products with a simple and efficient route encouraged us to synthesize enantiopure natural products as well. Towards this, we needed compound **11** in enantiopure form which was not reported earlier in the literature. Here, we decided to explore enzymatic kinetic resolution, a well documented tool,[20] for resolution of alcohols. There are various enzymes which could carry out this resolution, so we initially screened three enzymes where alcohol **11** was subjected to different acetylation conditions (see Table 2.3) in which one of the alcohols would selectively get acetylated and give enantiopure compounds (Scheme 2.6).

Table 2.3: Conditions of enzyme catalyzed acetylation

S. No.	Conditions	Observation
1	*Candida antartica* lipase B, THF, rt 24h	10% conversion
2	*Candida rugusa* lipase, THF, rt 24h	17% conversion
3	0.5 wt% *Amano PS* lipase, THF, 12h	30%, 87% ee
4	**1.5 wt%. *Amano PS* lipase, dry THF, 4h**	**47%, 99% ee**

The vinyl acetate mediated selective acetylation using lipases *Candida rugusa* and *Candida antartica* were not successful, as we ended up with incomplete conversion. Thus after a few attempts, we observed upto 30% conversion using *Amano PS* lipase.



Scheme 2.6: Synthesis of enantiopure maleimycins

Hence we increased the equivalents and alcohol **11** was resolved using vinyl acetate in presence of *Amano PS* lipase in dry THF to give acetate (-)-**10** and corresponding pure alcohol (+)-**11** with > 98% *ee*. The optical purity of **11** was also confirmed by HPLC[21] and optical rotation values. The specific rotation of compound **11**, the natural product maleimycin, is reported to be +23.1 in water, however when we tried to record the same in water we could not get a constant value. So, we recorded the specific rotation of above obtained **11** in methanol where we observed a value of +56.1. We then synthesized (-)-**11** and (+)-**10** from (-)-**10** and (+)-**11**, respectively, where compound (+)-**11** on acetylation with pyridine/Ac_2O gave (+)-**10.** On the other hand base mediated hydrolysis of (-)-**10** to get (-)-**11** led to complex reaction mixtures (probably opening of imide moiety). So, we again relied on mild conditions where lipase mediated hydrolysis was carried out using *Amano PS* lipase at pH 7 and obtained (-)-**11**. The optical rotation data obtained for both the acetates and alcohols were in opposite sign with same magnitude (Scheme 2.6).

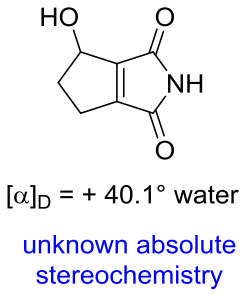
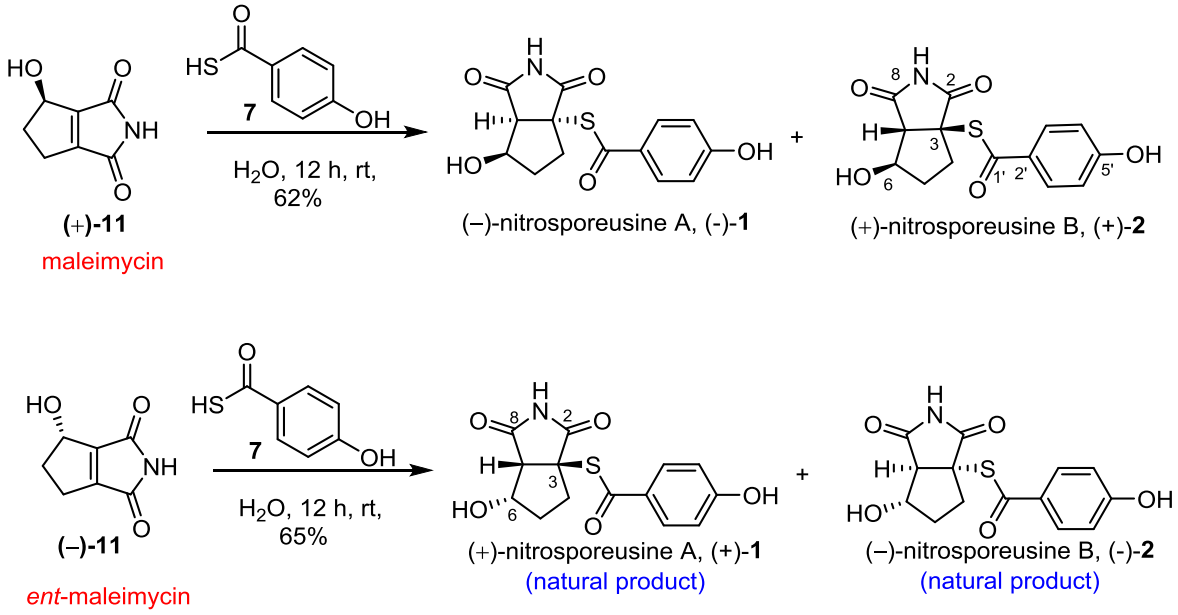


Figure 2.4: Structure of maleimycin

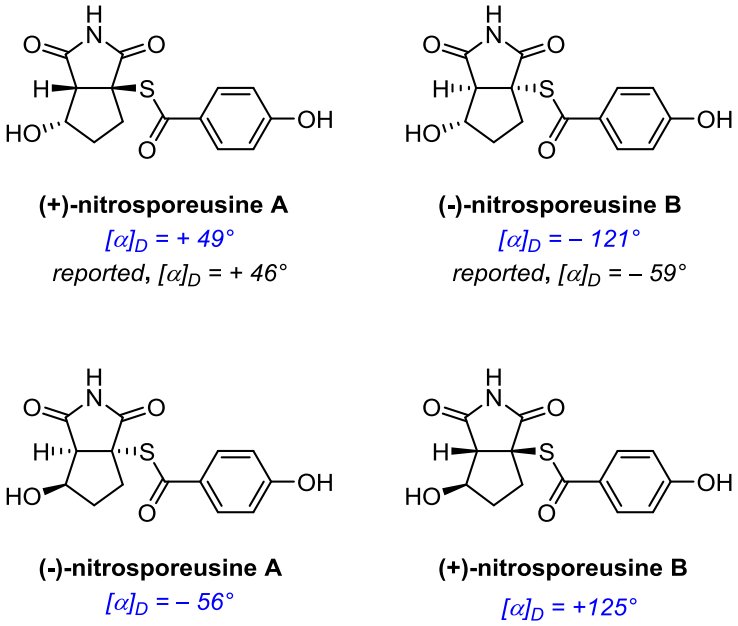
Here it is interesting to note that (+)-**11** is a natural product maleimycin which was reported to have shown interesting antibacterial activities like inhibition of *E. coli*, *S. aureus* and *M. phlei* and anticancer activities in leukemia L-1210 cells.[17] Even though specific rotation of naturally occurring **11** is reported, its absolute configuration has not been assigned yet. Having (+)-**11** in hand, we then subjected it to Michael addition conditions using 4-hydroxybenzothioic *S*-acid (**12**) to furnish the target compounds. As per the earlier raecemic synthesis we obtained similar results with nitrosporeusines A and B in 1:3 ratio. The spectral data pertaining to ^{1}H, ^{13}C and HRMS was in perfect agreement with above racemic nitrosporeusines and literature values. But, we obtained optical rotation value opposite to that of natural products. To our surprise, the naturally occurring (+)-maleimycin ((+)-**11**) did not yield natural nitrosporeusines (+)-**1** and (-)-**2**, instead the other enantiomers (-)-**1** and (+)-**2** were obtained (Scheme 2.7). Hence, we treated the unnatural maleimycin ((-)-**11**) under same conditions with thio-acid **12** to obtain (+)-

nitrosporeusine A and (-)-nitrosporeusine B whose spectral data is in complete agreement with those reported in literature.[11]



Scheme 2.7: Synthesis of enantiopure nitrosporeusines A and B

However there is discrepancy in the optical rotation value obtained for nitrosporeusine B (Scheme 2.8). We observed a value of -121° instead of -59° as reported in literature. We got same consistent value with opposite sign for other isomer of nitrosporeusine B which confirmed our obtained rotation (took multiple times with pure samples and confirmed).

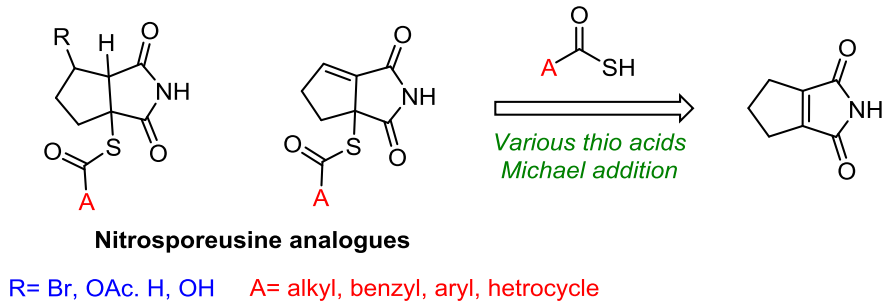


Scheme 2.8: Optical rotation values for all nitrosporeusines

As we synthesized natural products nitrosporeusine A & B from maleimycin (-)-**11** we thought to indirectly assign the absolute stereochemistry of naturally occurring maleimycin (+)-**11**. The absolute configuration of the hydroxyl group in the two natural products is known, so we could assign the absolute stereochemistry of (-)-maleimycin as *S*-configuration and that of (+)-maleimycin as *R*-configuration, which were previously unknown. Although nothing much is known about the biogenesis of nitrosporeusines, based on our findings, it can be surmised that natural (+)-maleimycin may not be the biogenetic precursor for the nitrosporeusines A and B.

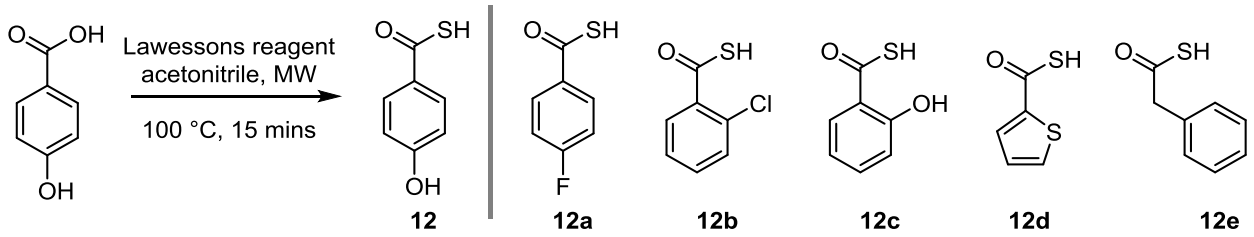
2.2.3 Synthesis of nitrosporeusine analogues

After documenting all the results obtained,[22] we next planned to expanded the scope of these interesting natural products by synthesizing a series of analogues around the nitrosporeusine scaffold and subjected them for various anti-inflammatory studies. To obtain close analogues of nitrosporeusines we exploited the maleimide intermediate (**7**) by synthesizing various Michael acceptors **9**, **10**, **11** in good quantities (Scheme 2.9).



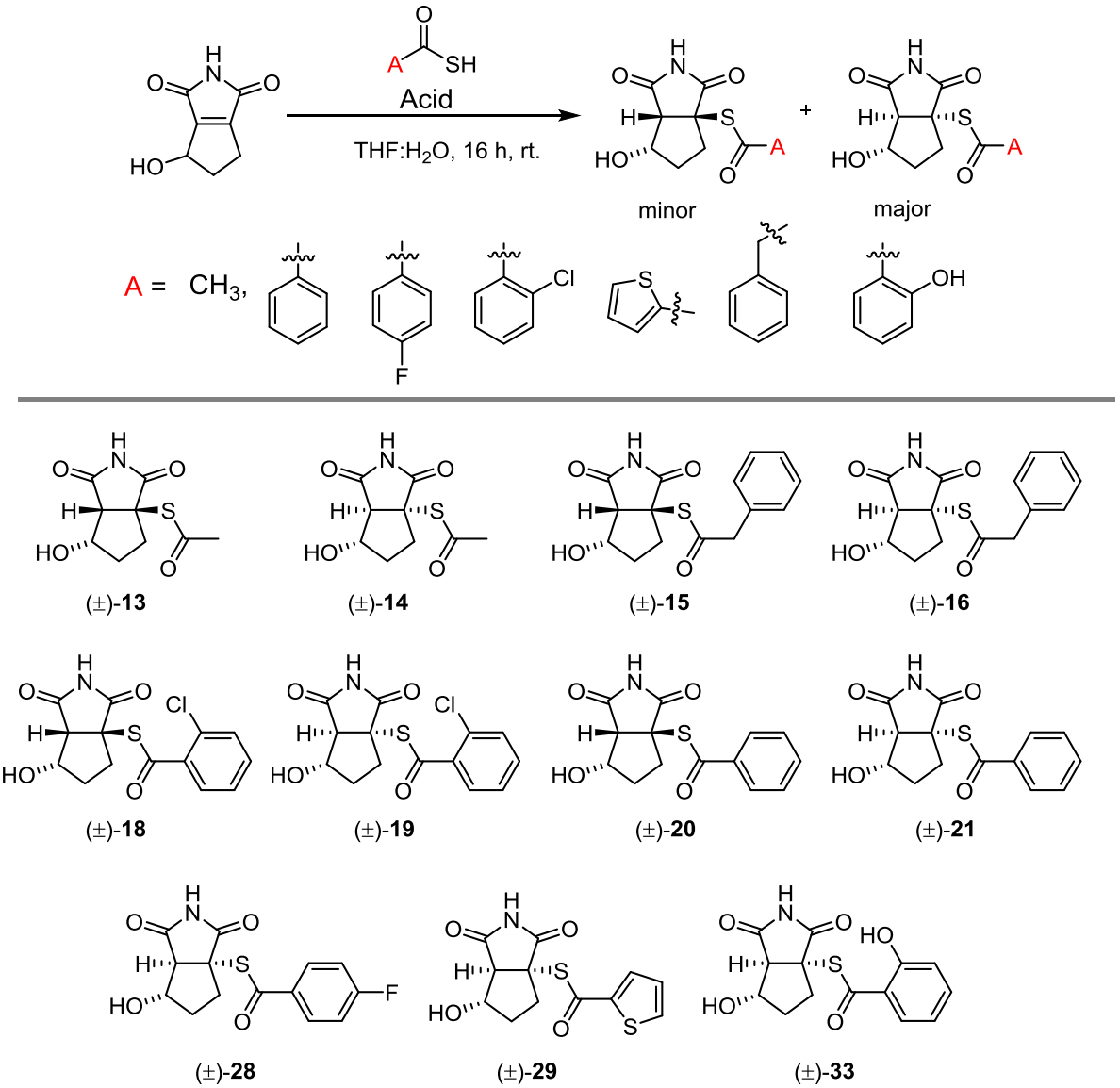
Scheme 2.9: Plan for nitrosporeusine analogues

Towards the preparation of different thio-acids, we relied on our earlier method where we used Lawesson's reagent under microwave conditions (Scheme 2.10) to convert carboxylic acids to corresponding thio-acids.[18] These thio-carboxylic acids are found to dimerise immediately even when stored at 4 °C, so we freshly prepared them when required and partially purified them over short silica column before use. We started analogue synthesis with Michael reaction of various thio-acids and maleimycin (±)-**11** (Scheme 2.11). The addition of thioacetic acid, thio-benzoic acid, 2-chlorobenzothioic *S*-acid and 2-phenylethanethioic *S*-acid to **11** yielded two diastereomers in each case, with major one being the *'syn'* diastereomer with respect to hydroxyl group and ring junction (approx 1:1.5).



Scheme 2.10: Synthesis of thio-acids from corresponding carboxylic acids

However in case of 4-fluorobenzothioic *S*-acid, 2-hydroxybenzothioic *S*-acid and thiophene-2-carbothioic *S*-acid, the ratio of diastereomers was heavily favoured the *'syn'* isomer and very less amounts of other diastereomer was seen.



Scheme 2.11: Synthesis of hydroxy nitrosporeusine analogues

As hypothesized earlier the observed diastereoselectivity could be due to hydrogen bonding between thio-ester carbonyl and hydroxyl group, the extent and strength of this hydrogen bonding depends on many factors. Here, the electronegativity of substituents present on aromatic ring can probably explain the observed selectivity. The formation of these thio-ester products were confirmed by disappearance of olefin peaks in their ^{13}C NMR along with appearance of characteristic peak in ^{1}H NMR at around δ 3.3 which corresponds to C7 proton adjacent to imide carbonyl and thio-ester moiety.

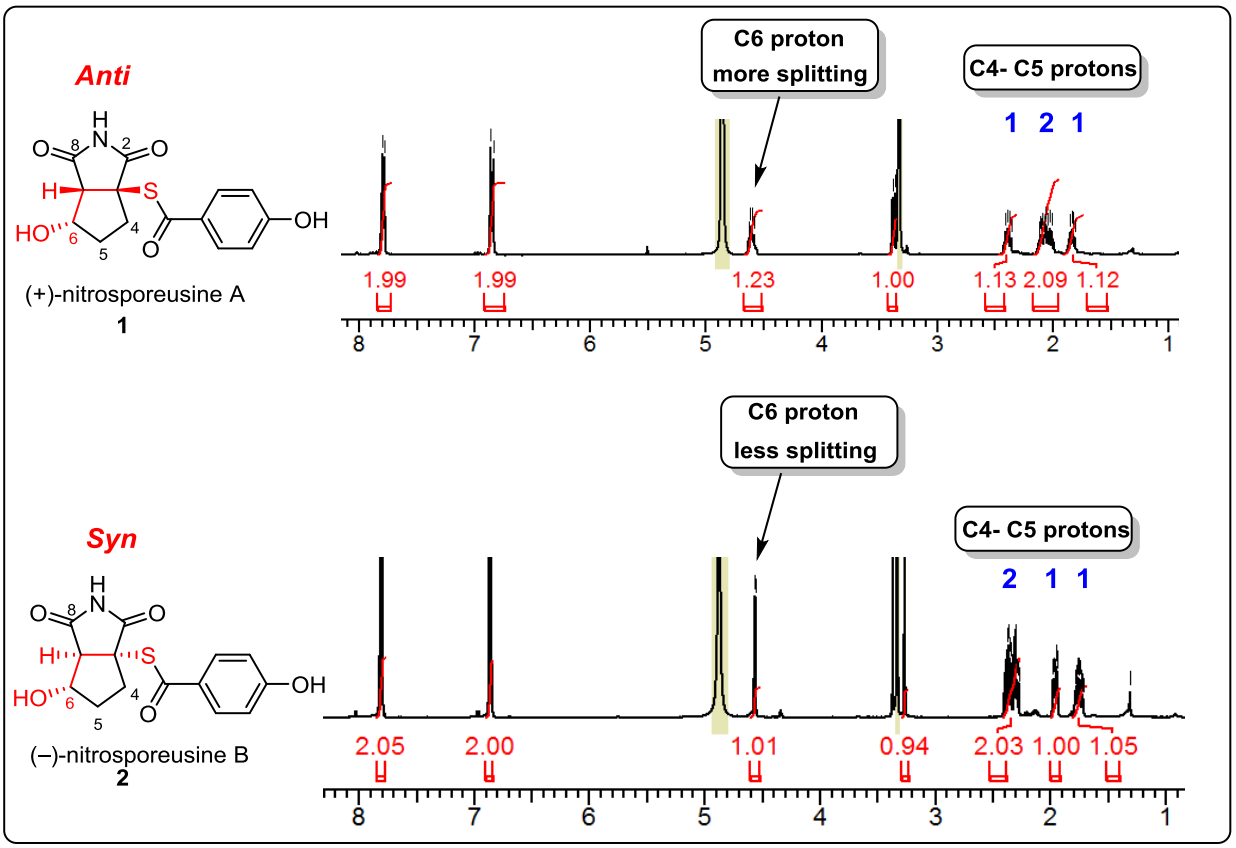
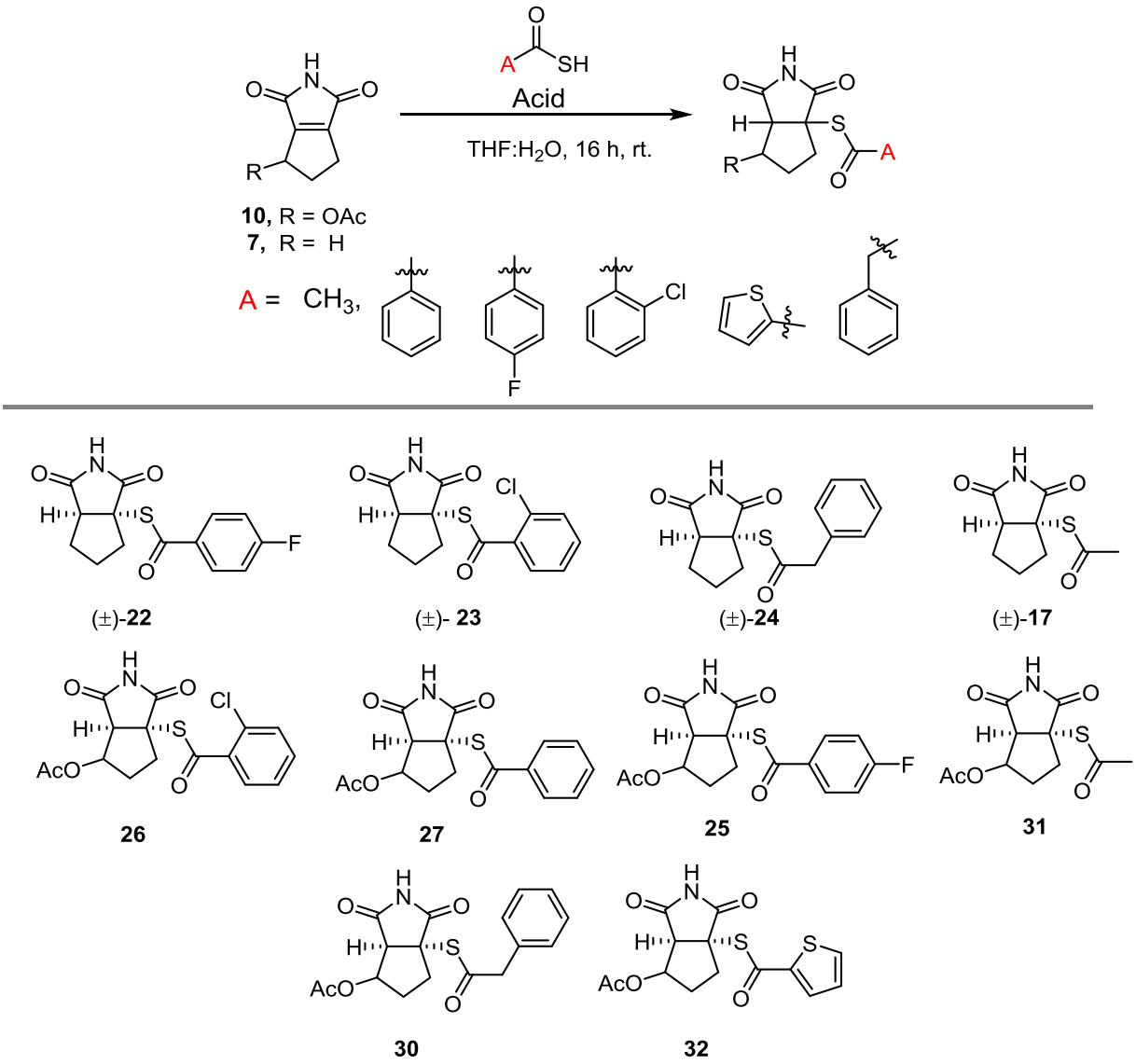


Figure 2.5: NMR pattern observed for C6, C4, C5 protons in nitrosporeusines

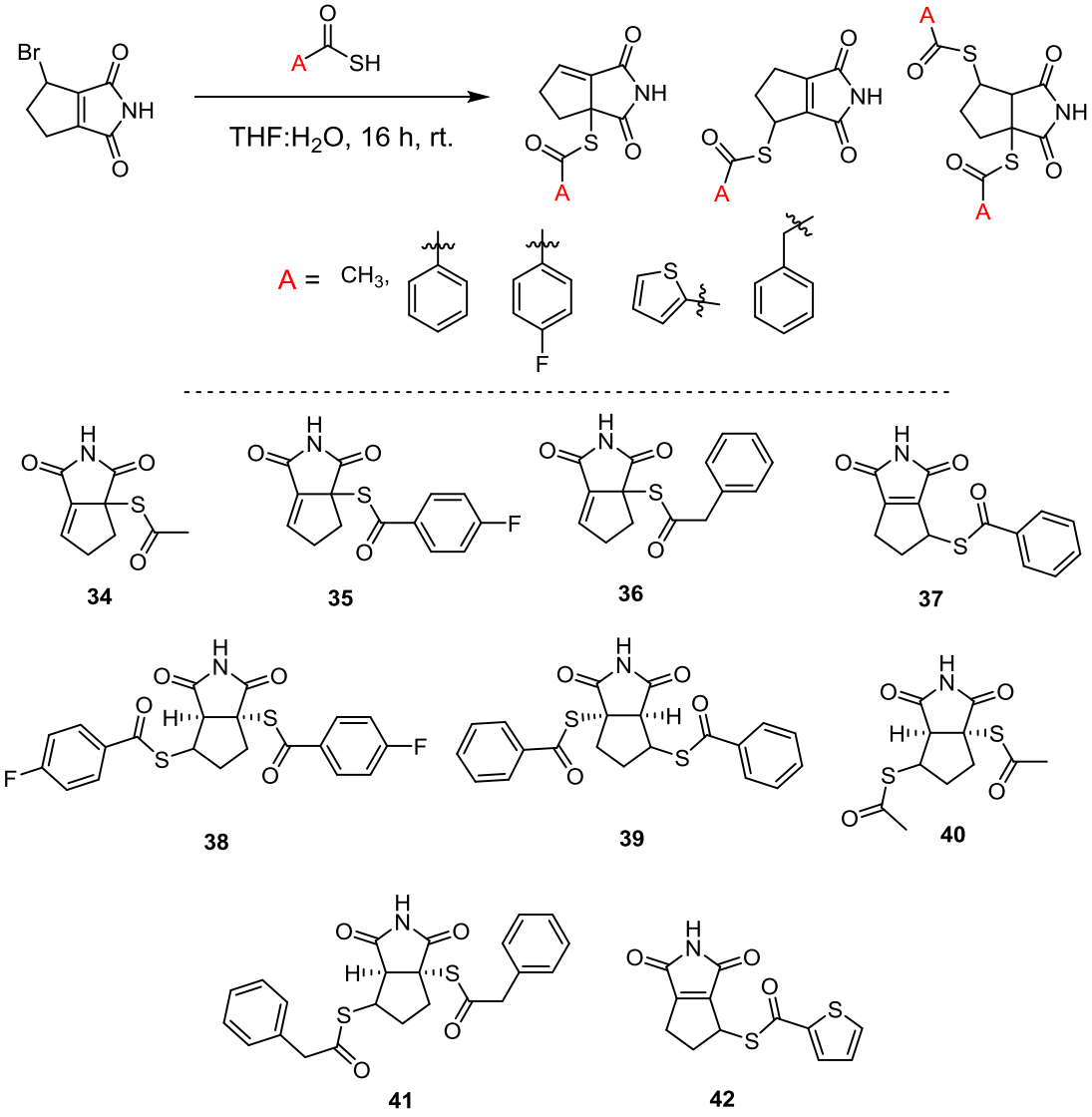
The stereochemistry of these products was assigned based on the ^{1}H NMR patterns observed in natural products nitrosporeusine A and B. In the ^{1}H NMR spectrum the splitting pattern of the C6 proton which couples with both the adjacent protons at C5 and C7, explains the *syn* or *anti* relation between hydroxyl group and ring junction. In both the natural products the extent of coupling between C6 and C5 protons is more or less the same; but in case of nitrosporeusine A, owing to the *syn* relationship between the C6 and C7 protons, the C6 proton couples with the C7

proton to a larger extent as compared to nitrosporeusine B, where the concerned protons (C6 and C7) are *anti* to each other. As a result, C6 proton of nitrosporeusine A shows a greater extent of splitting than nitrosporeusine B (Figure 2.5). Apart from this we can also observe the pattern of C4 – C5 protons which is different in both natural products. Nitrosporeusine B having hydroxyl group in *'syn'* relation with ring junction shows C4 and C5 protons at δ 2.22-2.17 (m, 2H), 1.83-1.78 (m, 1H), 1.59-1.56 (m, 1H) where as nitrosporeusine A having hydroxyl group *'anti'* to ring junction shows 2.42-2.40 (m, 1H), 2.12-2.05 (m, 2H), 1.87-1.85 (m, 1H) (Scheme 2.10). Similar pattern of C6, C4 and C5 protons were observed in ^{1}H NMR spectras of diastereomers obtained



Scheme 2.12: Synthesis of nitrosporeusine analogues

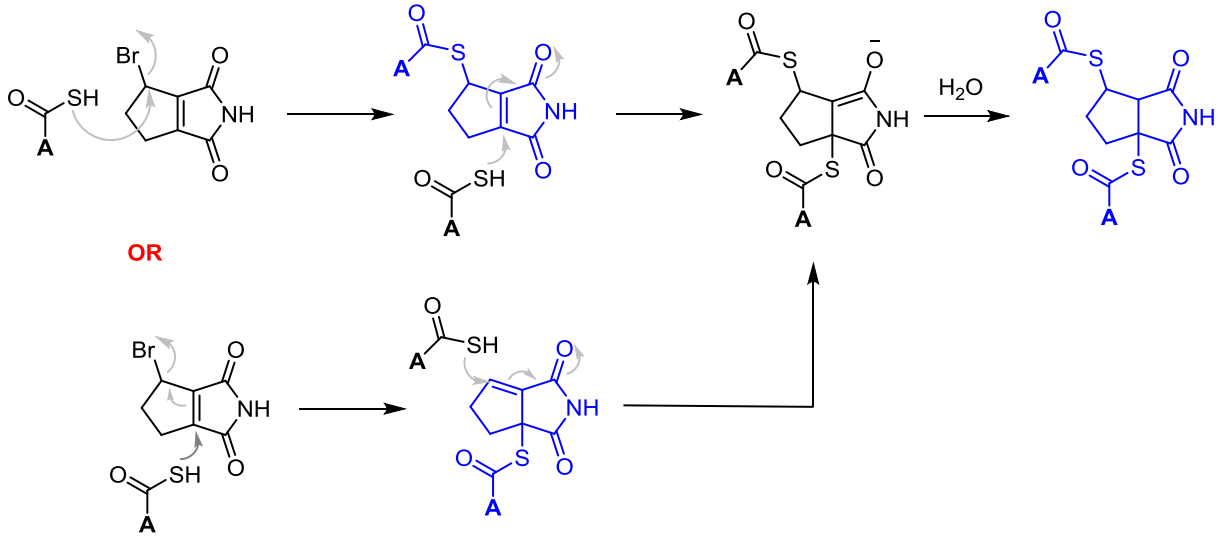
from **11** and different thio-acids, which enabled us to assign the relative stereochemistry between hydroxyl group and ring junction in them. In total we synthesized eleven new analogues **13-16**, **18-21** and **28**, **29**, **33** from racemic maleimycin (Scheme 2.11).We next subjected intermediate **7** to Michael addition with freshly prepared 2-chlorobenzothioic *S*-acid, 2-phenylethanethioic *S*-acid and 4-fluorobenzothioic *S*-acid to get corresponding nitrosporeusine analogues **22-24** as shown in scheme 12. These compounds were all racemic and are fully characterised by all spectral techniques (mp, ^{1}H, ^{13}C, HRMS). Here again the thio-ester formation is confirmed by ^{1}H NMR which shows appearance of peak at δ 3.3 corresponding to C7 proton. Further we synthesized a series of analogues around compound **10** using similar thio-acids (Scheme 2.12).



Scheme 2.13: Synthesis of disubstituted and unsaturated nitrosporeusine analogues

However the diastereomers obtained in each case could not be separated in spite of several efforts. We prepared six new analogues **25-27** and **30-32** from **10** and all compounds are fully characterised.

The synthesis of analogues over compound **9** was then explored where Michael addition using thio-acids gave entirely new and interesting compounds. Instead of simple addition adducts, we observed Michael addition followed by elimination to give olefin analogues which further underwent second Michael addition with thio-acids giving rise to di-substituted analogues. Accordingly, when compound **9** was subjected to Michael addition using 4-fluorobenzothioic *S*-acid, it resulted in compound **35** (Scheme 2.13). Probably, Michael addition followed by dehydrobromination resulted in compound **35** which is an interesting intermediate for generating a variety of compounds around this skeleton. For example, the newly generated double bond potentially can be used for addition of various nucleophiles, epoxidation followed by opening with different nucleophiles, dihydoxylation, allylic oxidation *etc*. Similar olefin analogues were obtained when compound **9** reacts with thioacetic acid, 2-phenylethanethioic *S*-acid. But when the same reaction was performed on **9** using thiobenzoic acid, it resulted in product **37** in good yields, which is the result of substitution of bromine.



Scheme 2.14: Probable mechanism for dimer formation

Compound **37** is also an interesting intermediate towards the library creation. Similar product **42** was obtained when Michael addition performed using thiophene-2-carbothioic *S*-acid. During all

these reactions we also isolated compounds **38**, **39**, **40**, **41** in minor quantities. These compounds were then prepared exclusively by prolonging the reaction time and addition of more equivalents of respective thio-acids. Following this approach we could prepare total nine compounds **34** –**42** and a probabale mechanism for formation of all analogues was shown in scheme 2.14. In total by using different Michael acceptors and thio-acids we prepared 32 new compounds which were well characterized and considered for biological screening. The inflammatory studies on all compounds were planned in a systematic manner where first cytotoxicity and NO inhibition studies were to be carried out. Based on the results we thought to select few compounds which would be analysed for their inflammatory potential with different pro-inflammatory factors. To carry these biological studies we collaborated with Dr. Anirban Basu's research group, NBRC-Gurgaon who helped us with all assays and experiments related to biology.

2.3 Anti-inflammatory activity of nitrosporeusines

For a systematic evaluation of biological activity of different compounds it is necessary that one uses proper biological tools for evaluation of the compounds efficacy. Biomarkers generally refer to a measurable indicator of some biological state or condition. Before discussion on evaluation of the syntheisized analogues for their inflammatory potential, following section included with a brief overview on various inflammatory markers used for analysis of compounds.

2.3.1 Inflammatory markers for biological study

Nuclear factor-κβ:

The inflammatory response is often associated with expression of certain genes which are closely regulated by a family of transcription factors called nuclear factor-κβ, which is present in cytoplasm of all cells. They play a central role in the inflammatory response by regulating the expression of various genes encoding pro-inflammatory cytokines, adhesion molecules, chemokines, growth factors, and inducible enzymes such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). As they are directly related to inflammation, they are expected and found to be involved in the onset and progression of many chronic inflammatory diseases.[23,24] Moreover, some recent studies are directed towards studying the role NF-κβ in cancer development as well.[23b] The importance of NF-κβ in many interrelated processes of

inflammation renders it as a useful tool for understanding and development of various anti-inflammatory compounds.

Nitrogen Oxide:

In human body, nitrogen oxide (NO) is the smallest molecular mediator synthesized from amino acid L-arginine by a family of NOS enzymes through 'L-arginine–NO pathway'. NO has been beneficial for body and is mainly involved in regulation of host defense mechanisms, vascular tone, neurotransmissions, apoptosis, acute and chronic inflammations. Under normal physiological conditions NO contributes to anti-inflammatory effect but under certain abnormal conditions, there is an over production of nitric oxide making it pro-inflammatory mediator and induces inflammation. The role of nitrogen oxide in inflammatory process is being extensively studied[24,25] and NO is known to involve in pathogenesis of various inflammatory disorders of the joint, gut and lungs.[24,25] The NO production is carried out mainly by three enzymes neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). Synthesis of NO mediated by nNOS and eNOS happens in response to increase in intracellular calcium levels or due to shear stress and these processes are tightly regulated. However, the third enzyme iNOS is least dependent on calcium levels and tend to produce higher concentrations of NO in cells for longer times. So, the study of inhibition of this inducible NOS (iNOS) is considered as an important marker in anti-inflammatory studies.

Reactive oxygen species (ROS):

Reactive oxygen species (ROS) are generally referred to as partially reduced metabolites of oxygen possessing strong oxidizing capabilities. At low physiological concentrations, they act as signalling molecules by mediating complex signalling functions that regulate cell growth, differentiation, adhesion of cells toward other cells, senescence, and apoptosis. During inflammation, at high concentration they are helpful in killing the invading pathogens but if unchecked by sophisticated antioxidant mechanisms they show deleterious effects on human cells and are responsible for progression of many inflammatory diseases.[26]

COX enzymes:

The study of anti-inflammatory agents is always supported with the study on inhibition of prostaglandin-endoperoxide synthase (PTGS) enzyme often referred to as cyclooxygenase-2 or COX 2.[27] Prostaglandins are known to participate as pro-inflammatory as well as in resolution of

inflammation, where the former is well established. Traditionally, COX 1 and COX 2, the two isoforms of cyclooxygenase enzyme are targets for various NSAIDs but later it was realized that there is increased expression of COX 2 in inflamed tissues or due to induced inflammation. This provided a rational in which COX 2 enzymes were extensively studied for development of new inflammatory agents.

2.3.2 Evaluation of NO inhibition and Cytotoxicity

Firstly all compounds were evaluated for their inhibition of NO production in lipopolysaccaride (LPS) stimulated RAW264.7 cells and nitrite levels, a strong metabolite of NO, were measured in culture media using Griess reagent.[28] During inflammation, large amount of NO are produced and in turn amplify inflammatory response to multiple fold. The primary results indicated that almost all compounds evidently decreased NO level in LPS treated cell as shown in Table 2.4. Simultaneously, cytotoxicity of all compounds were assessed using MTT assay and IC_{50} was calculated (Table 2.4). The compounds showing effective NO inhibitory activity with low cytotoxicity were selected based on the selectivity index (cytotoxicity IC_{50}/NO inhibition IC_{50}). Compounds **25**, **29** and **40** were selected for further study, since they exhibited superior selectivity indices (≥300) among all the derivatives.

Table 2.4: Cytotoxicity and LPS induced NO inhibitory activity in RAW 264.7 cells

	Cytotoxicity (A)		NO inhibition (B)		
Com-pound	IC_{50} (µM)	95% confidence interval (µM)	IC_{50} (µM)	95% confidence interval (µM)	Selectivity index (A/B)
(-)-1	616.1	513.7-802.9	42	31.2-56.3	14.66905
(+)-1	1015.6	856-1281.4	71.8	60-84.6	14.14485
(±)-2	433.8	307.4-592.6	225.3	182.8-289.4	1.925433
(+)-2	2914.9	2307-3449.4	126.8	98.3-146.7	22.98817
(-)-2	1383.9	1045.3-1536.8	140.5	118.1-157.3	9.849822
(-)-11	216.7	103.8-354.7	95.9	79.4-116.3	2.259645
(+)-11	319.2	284.3-367.8	121.3	96.3-152.8	2.631492168
(-)-10	128	98.4-137.7	866.9	682.3-993.5	0.147653

13	558.5	472.6-637.2	147.9	112.5-186.9	3.7762
14	856.7	622.4-983.3	832.8	664.7-1004.7	1.028698
15	834.5	727.4-967.9	132	107.7-165.2	6.32197
16	773.7	682.8-867.8	142.8	116.4-204.7	5.418067
17	674.4	493.7-819.6	59.2	46.8-76.9	11.39189
18	667.7	512.3-788.6	127.3	106.3-146.8	5.24509
19	717.1	604.6-833.6	178.3	119.7-213.4	4.021873
20	479.4	294.5-601	72.4	49.8-87.4	6.621547
21	17063	15948-18666	65.2	47.8-78.1	261.7025
22	383.6	305.1-459.8	389.8	327.5-466.1	0.984094
23	834.5	773.8-923.1	59.8	43.6-77.5	13.95485
24	425.6	301.4-556.2	56.9	33.5-83.6	7.479789
25	**12135.6**	**11853-12738**	**34.3**	**24-43.2**	**353.8076**
26	9076.1	8113-10263	101.4	74.1-121.8	89.50789
27	1912	1628.4-2249.9	64.3	52.9-93.8	29.73561
28	473.7	355.6-513.9	318	271.2-380	1.489623
29	**10872.1**	**9332.1-11382**	**36.6**	**24.5-46.1**	**297.0519**
30	1607.3	1283.5-1983.5	68.2	44.7-87.6	23.56745
34	184.5	109.2-228.5	20.5	15.6-26.1	9
36	93.07	84.7-109.3	30.6	18.9-43.7	3.041503
38	210.6	183.2-237.4	98.3	83.6-123.6	2.142421
40	**10007.6**	**9438.3-10837.8**	**19.8**	**14.5-25.4**	**505.4343**
41	710.8	525.6-984.4	57.5	42.8-76.1	12.36174
42	26.7	13.5-42.6	65	48.6-74.8	0.410769

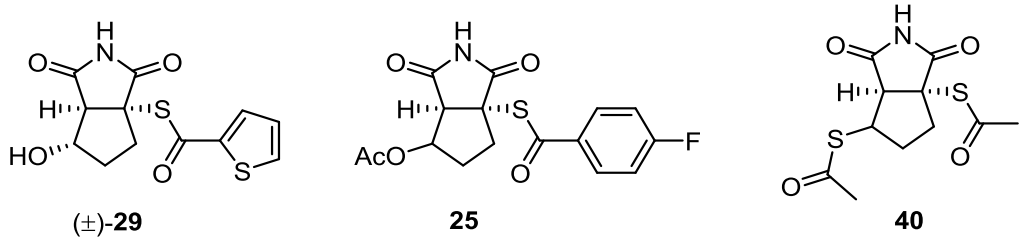


Figure 2.6: Three selected compounds based on cytotoxicity and NO inhibition potential

2.3.3 Studies of selected compounds with respect to various inflammatory markers

Further, the anti-inflammatory potency of compounds **25**, **29** and **40** were determined by measuring the level of intracellular reactive oxygen species (ROS) in LPS treated RAW 264.7 cells. ROS generation is a key marker for inflammation and is reported in several cases as a triggering factor for apoptosis.[29] As depicted in Figure 2.7, increase of ROS in LPS treatment was reduced significantly in presence of all these three compounds in a dose dependent manner as analysed by mean fluorescence intensity (MFI).

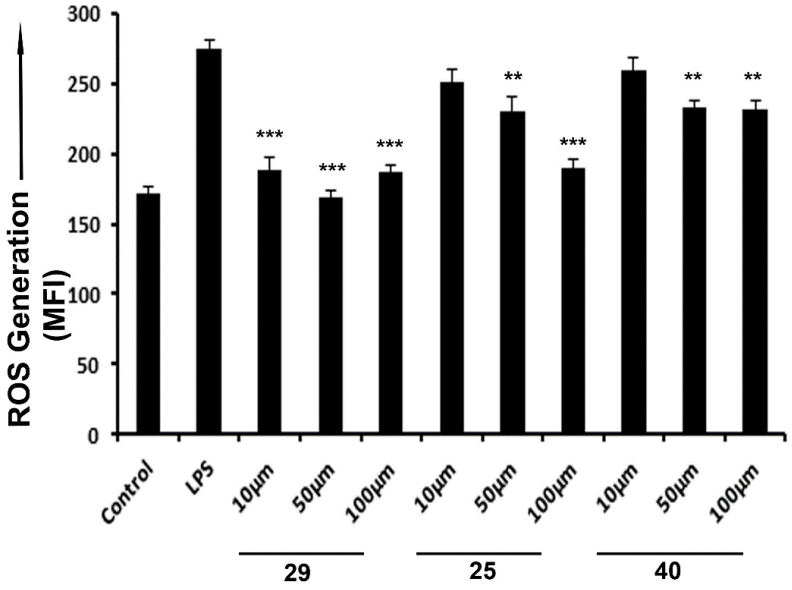


Figure 2.7: Effects of compounds on ROS level in LPS stimulated RAW 264.7 cells. The plot represents the mean fluorescence intensity (MFI) of ROS generation in presence of three selected compounds. Data represent mean ± SD of three independent experiments. $^{**}p<0.01$ and $^{***}p<0.001$in comparison to LPS-treated values.

During the inflammatory process, large quantities of the inflammatory mediators are produced by the inducible isoforms of iNOS and COX-2.[30] Thus the levels of iNOS and COX-2 were checked by immunoblotting in response to drugs treatment in LPS administered cells. It is found that 50µM of all three selected compounds are efficient in reducing iNOS and COX-2 levels compared to LPS (Figure 2.8A & 2.8B).

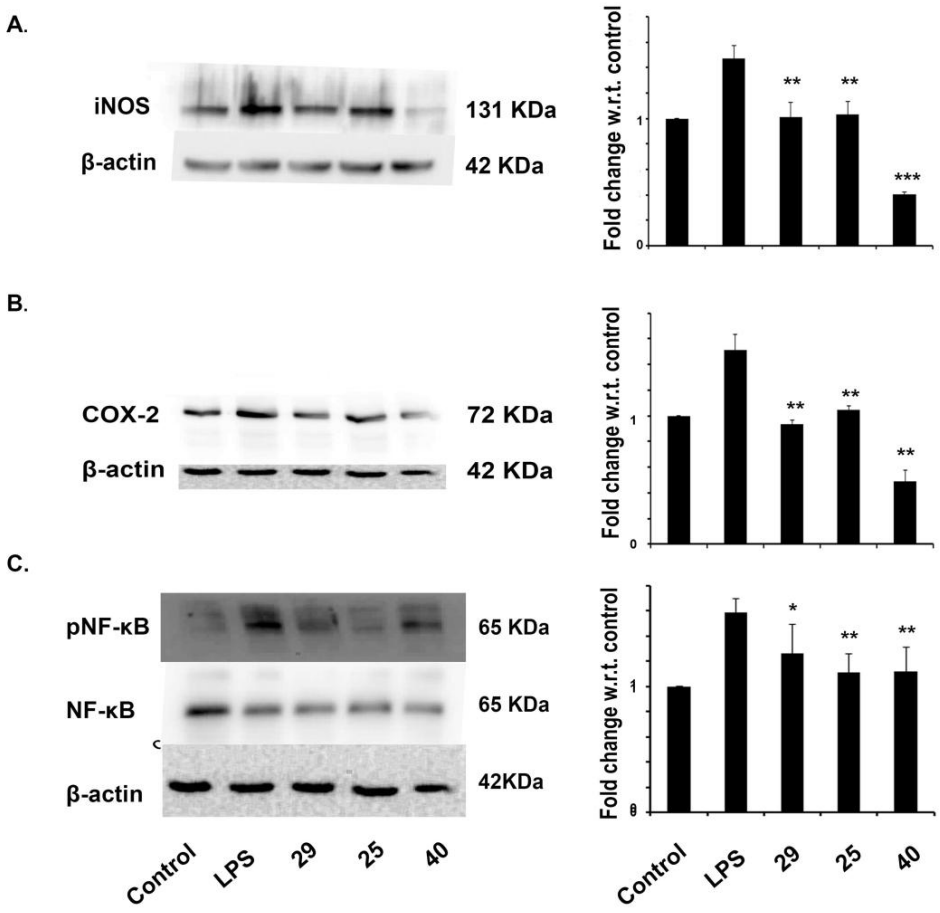


Figure 2.8: Protein isolated from RAW 264.7 cells of control, LPS and LPS+drug conditions were analysed by immunoblot. The graphs represent the densitometric quantification of protein bands- iNOS(A), COX-2 (B) and phospho-NFκB. (C) normalised to β-actin. Values represent mean ± SD from 3 independent experiments. *p<0.05, **p<0.01 and ***p<0.001 in comparison to LPS treated values.

As discussed earlier, NF-κB activation is an important marker of LPS-induced inflammation, the influence of these compounds on NF-κB activation is assessed. In response to inflammatory stimuli, IκB is phosphorylated and degraded, and NF-κB is released and translocated into the nucleus,[31] where it binds promoter region of many inflammatory genes, including iNOS, COX-2, and TNF-α and augments their expression.[32] The results showed that **25**, **29** and **40** potentially decreased LPS induced NF-κB activation as analysed phosphorylated NF-κB level (pNF-κB) by western blot (Figure 2.8C).

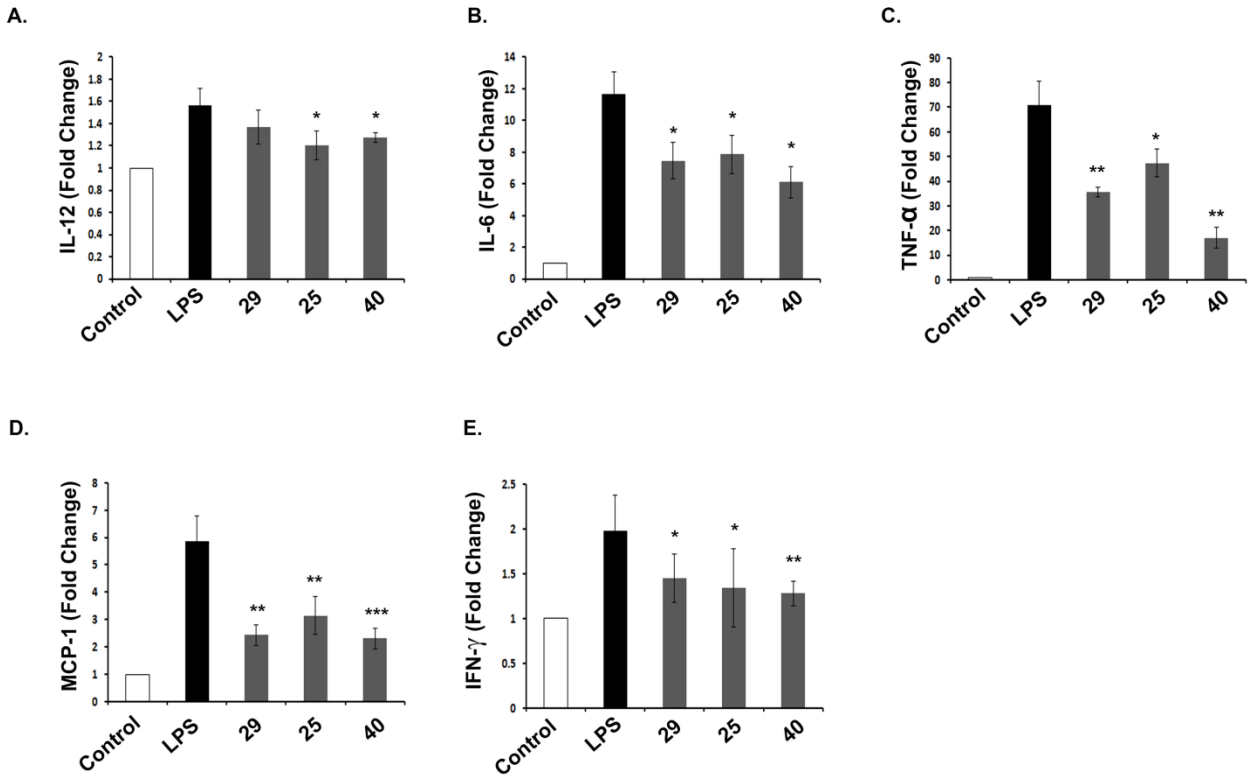


Figure 2.9: Cytokine bead assay (CBA) analysis of protein extract isolated from RAW 264.7 cells treated with LPS along with compounds. Addition of compounds (50 μM) revealed substantial decrease in the levels of IL-12 (A), IL-6 (B), TNF-α(C),MCP-1 (D) and IFN-γ (E) compared to LPS treated cells. Data represent mean ± SD of three independent experiments. **p*<0.05,***p*<0.01 and ****p*<0.001in comparison to LPS treated values.

Under the pathogenic attack in our body, activated macrophages release numerous pro-inflammatory cytokine and inflammatory mediators.[33] Hence, the macrophage cell line provides an excellent model for drug screening and evaluation of potential inhibitors of the inflammatory response. LPS treatment can induce inflammation, resulting in the extreme production of numerous pro-inflammatory mediators including IL-12, TNF-α, IFN-γ, MCP-1 and IL-6. However, the anti-inflammatory activity of compounds **25**, **29** and **40** were evaluated by measuring the expression of these inflammatory cytokines in LPS and compound treated RAW264.7 cells by Cytokine bead assay (CBA). As evidenced from figure 2.9, it was found that 50 μM dose of all these drugs effectively suppressed pro-inflammatory mediators. *In vivo* testing is often employed over *in vitro* because it is better suited for observing the overall effects of an experiment on a living subject. Therefore, we next investigated anti-inflammatory activity of

compounds *in vivo* using serum samples collected intracardially from mouse. We measured levels of pro-inflammatory cytokines by CBA and based on the results, compound **25** was found very promising in *in vivo* though other two compounds were also effective in reducing cytokines level when compared with LPS treated group (Figure 2.10).

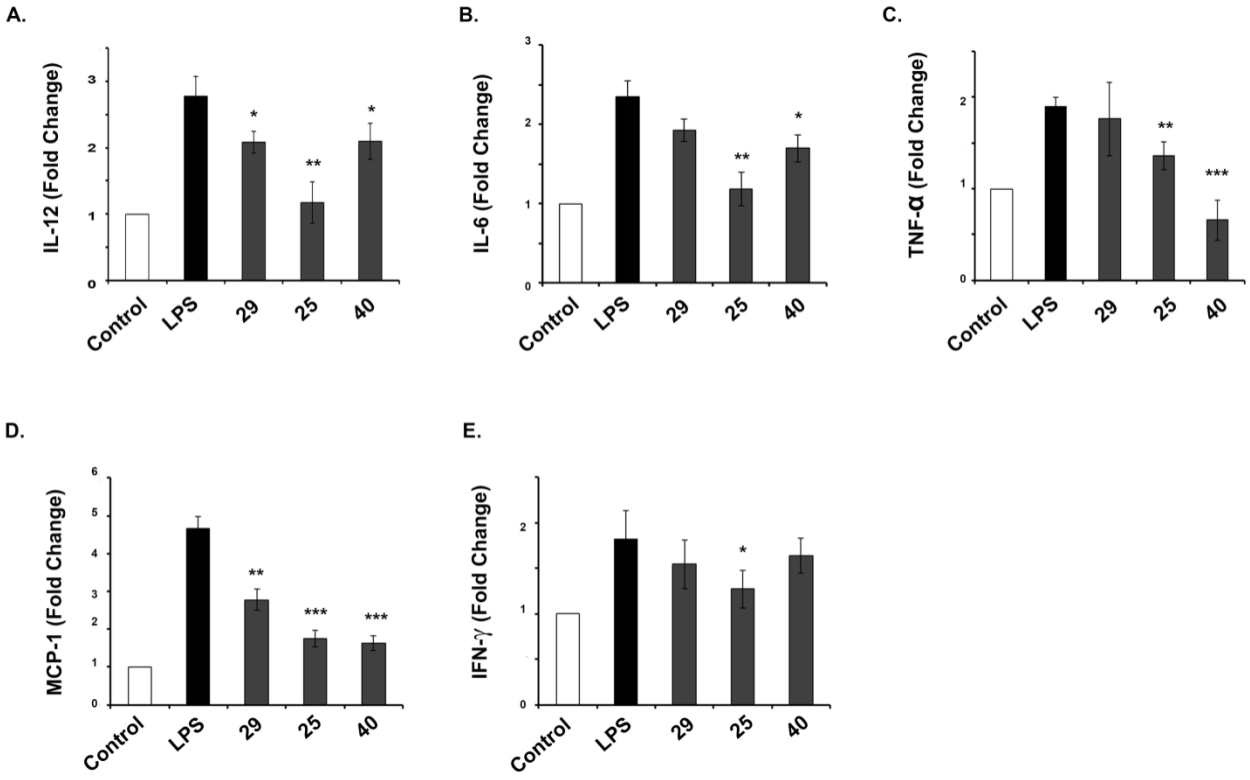
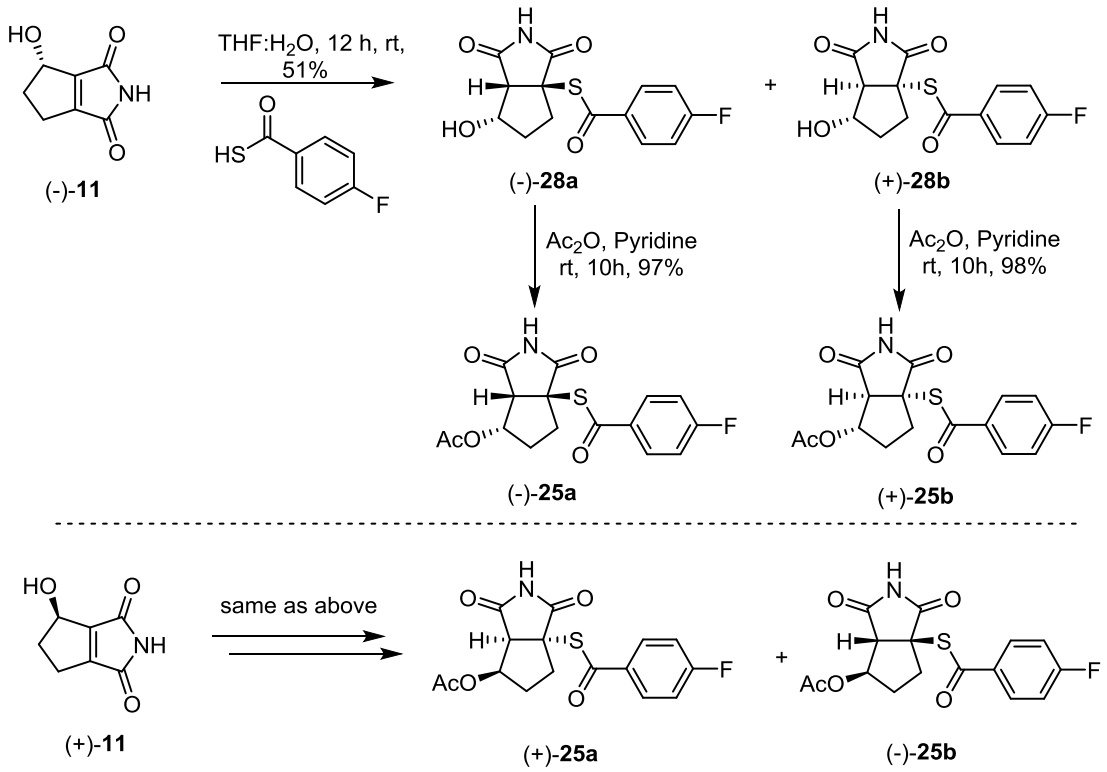


Figure 2.10: Mouse serum samples were used to study anti-inflammatory activity of drugs *in vivo*. There were significant reductions in the levels of IL-12 (A), IL-6 (B), TNF-α(C), MCP-1 (D), and IFN-γ (E) in compounds (10 mg/kg body weight) treated serum samples compared to only LPS injected mice. Values represent mean ± SD of serum samples collected from five mice. **p*<0.05,***p*<0.01 and ****p*<0.001 in comparison to LPS treated values.

2.3.4 Synthesis of enantiopure isomers of active compound 25

After all the studies done at NBRC for identifying potential of these compounds in various *in vitro* and *in vivo* assays, compound **25** was identified to be showing most potent anti-inflammatory activity among them. Compound **25** having three chiral centres was initially prepared by us as a mixture of diastereomers and as such we profiled for its activity. Therefore now we set out to prepare the enantiopure forms of **25** to further understand contribution of

spatial arrangements to anti-inflammatory activity. Accordingly, we prepared enantiopure compounds (-)-**11** and (+)-**11** in gram scale following similar enzymatic resolution method. Compound (-)-**11**, on subjection to previously optimized Michael addition conditions gave desired adducts (-)-**28a** and (+)-**28b** in 1:3 diastereomeric ratio (Scheme 2.7). Both the compounds were then carefully separated using silica gel chromatography and characterized. The compound (+)-**28b** was then acetylated using Ac_2O/pyridine conditions to obtain enantiopure acetate (+)-**25b**. Similarly, (-)-**28a** was converted to acetate (-)-**25a**. Using similar reactions with (+)-**11** as starting material, acetate compounds (+)-**25a** and (-)-**25b** have been obtained in enantiopure forms (Scheme 2.15). The formation of acetate (+)-**25a** was confirmed by appearance of peaks in ^{1}H NMR at δ 5.47 - 5.43 (m, 1H) corresponding to proton attached acetate group and δ 2.13 (s, 3H) corresponding to methyl group in acetate. The presence of characteristic ring junction proton at δ 3.75 and aromatic protons also supports the formation of compound (+)-**25a**. Further, the formation (+)-**25a** was confirmed from ^{13}C NMR where four peaks corresponding to carbonyl carbons at δ 190.0, 176.8, 171.8, 170.3 appeared along with characteristic carbon attached to thio-ester group at δ 58.7.



Scheme 2.15: Synthesis of active enantiopure analogues of nitrosporeusines

All the other enantiomers (+)-**25b**, (-)-**25b**, (-)-**25a** also showed similar peaks in their ^{1}H and ^{13}C spectra. Moreover the optical rotations obtained for them confirmed the enantiopurity of each isomer. With all the four isomers of **25** in hand, we again assessed them through *in vitro* and *in vivo* assays to identify the potent lead (best compound) among them.

2.3.5 Study of anti-inflammatory activity of diastereomers of compound 25

Similar to analysis done for **25**, **29**, **41**, the anti-inflammatory efficiency of isomers of **25** was determined by measuring the NO level in culture supernatants as well as intracellular ROS in LPS treated RAW 264.7 cell. Although marked reduction in NO and ROS level were observed in all enantiomer treated cells, (-)-**25a** showed most potential inhibition among them (Figure 2.11).

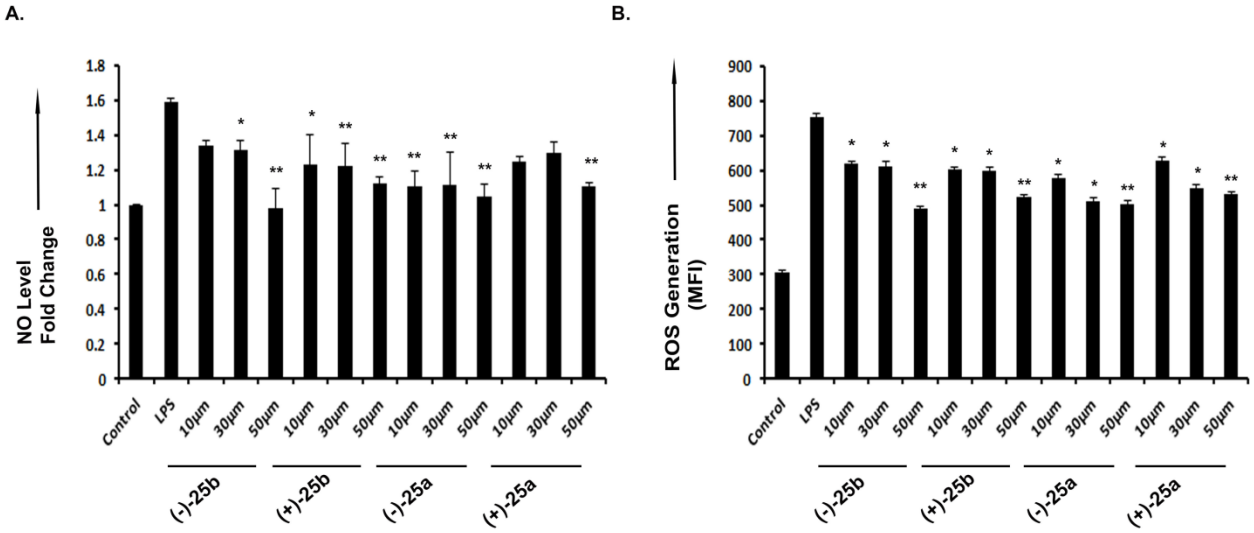


Figure 2.11: Effects of enantiomers on NO and ROS level in LPS stimulated RAW 264.7 cells. The plot represents the fold change of NO (A) and mean fluorescence intensity (MFI) of ROS generation (B) in presence of four enantiomers. Data represent mean ± SD of three independent experiments. *p<0.05 and **p<0.01 in comparison to LPS-treated values.

Next, we checked the levels of iNOS, COX-2 and pNF-κB by immunoblotting in response to isomers treatment in LPS treated cells. Among them, enantiomer (-)-**25a** most significantly attenuated NF-κB activation and a comparable reductions in iNOS and COX-2 expression were observed (Figure 2.12).

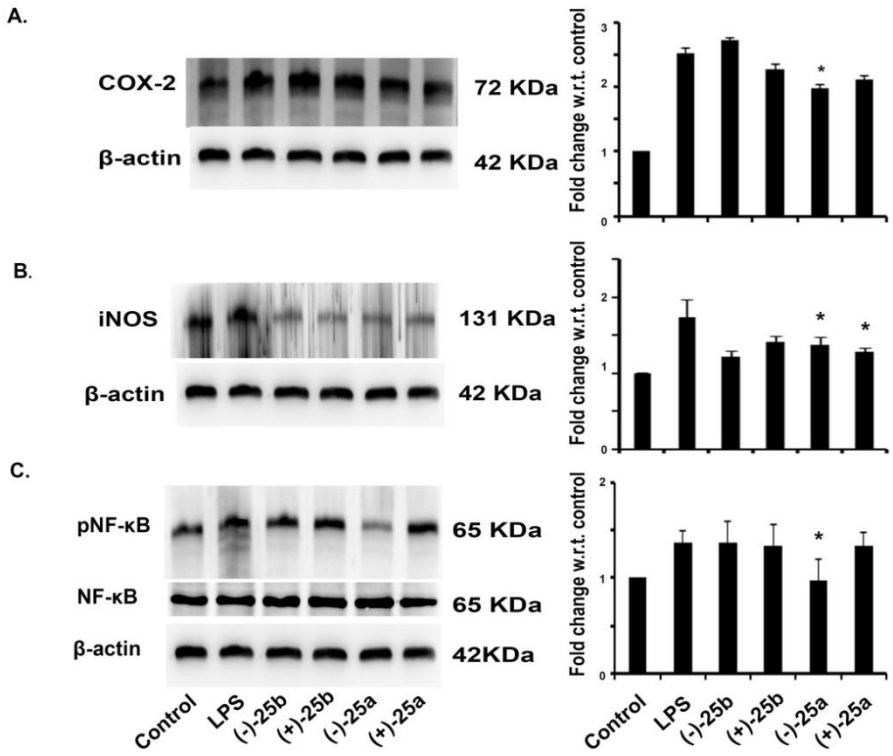


Figure 2.12: Protein isolated from RAW 264.7 cells of control, LPS and LPS + enantiomer(30 μM) conditions were analysed by immunoblot. The graphs represent the densitometric quantification of protein bands- COX-2 (A), iNOS (B) and phospho-NF-κB (C) normalised to β-actin. Values represent mean ± SD from 3 independent experiments. **p*<0.05 in comparison to LPS treated values.

Even though all the enantiomers of **25** showed good activity, enantiomer (-)-**25a** in comparison to others, most significantly reduced the expressions of pro-inflammatory cytokines in LPS treated cells as assessed through CBA analysis (Figure 2.13). From all the above biological studies it may be concluded that compound (-)-**25a** showed potent anti-inflammatory activity among all the analogues and its structure and stereochemistry closely relates to nitrosporeusine A. Incidentally, nitrosporeusine A showed significant activity in various animal models for treatment of different diseases (as discussed in section 2.1.2).

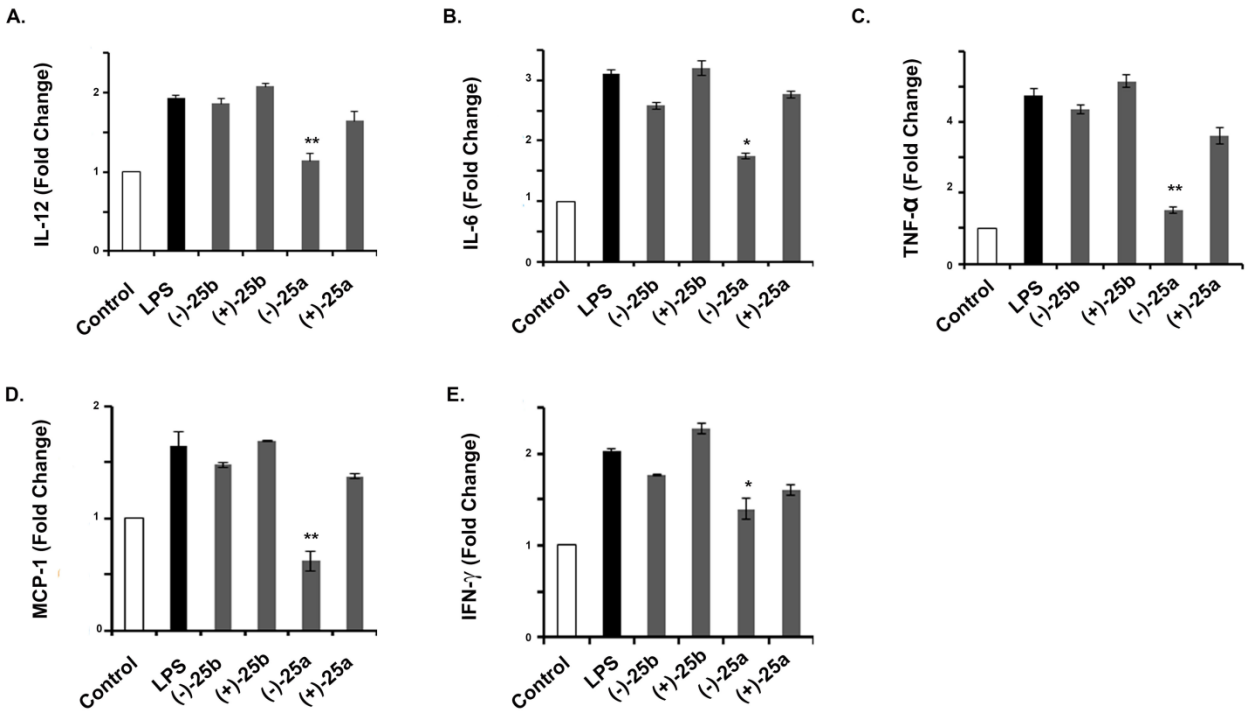


Figure 2.13: CBA analysis of protein extract isolated from RAW 264.7 cells treated with LPS along with enantiomers. Addition of compounds (30 µM) showed substantial decrease in the levels of IL-12 (A), IL-6 (B), TNF-α(C), MCP-1 (D) and IFN-γ (E) compared to LPS treated cells. Data represent mean ± SD of three independent experiments. *p<0.05,**p<0.01 in comparison to LPS treated values.

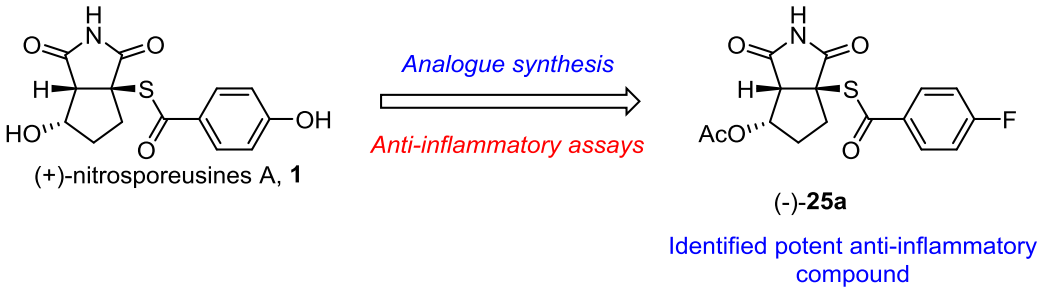


Figure 2.14: Identification of potent anti-inflammatory agent

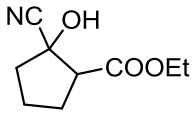
2.4 Conclusions

The intriguing structure and exceptional biological activity reported for nitrosporeusines A and B by Lin and Chen groups has encouraged us to plan and execute a medicinal chemistry program around nitrosporeusine scaffold. Here we achieved first synthesis of nitrosporeusines A

and B in both racemic and enantiopure forms using short and simple route with key steps involving allylic oxidation (SeO_2) and Michael addition (in water). We synthesized four isomers of the natural products in enantiopure forms by employing gram scale kinetic enzymatic resolution method using *Amano PS* lipase. Enroute, we could also synthesize the enantiopure maleimycin and assigned its absolute stereochemistry which was hitherto unknown. We then diverted our efforts towards synthesis of 31 close analogues of nitrosporeusines. With the help of biology groups, all the synthesized compounds were profiled for their anti-inflammatory potential in different assays. The initial screening with respect to NO inhibition and cytotoxicity had shown that three of the compounds are promising and further studies were carried out on them. Accordingly, compounds **25**, **29** and **40** have been studied in detail with various inflammatory markers and **25** showed the best anti-inflammatory potential. The four enantiomers of compound **25** were further prepared in enantiopure forms and evaluated for their anti-inflammatory activity using different assays. Out of all compounds, enantiomer (-)-**25a** showed superior activity and was recognized as the potential lead for further optimization.

2.5 Experimental procedures

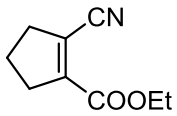
Ethyl 2-cyano-2-hydroxcyclopentanecarboxylate (4)



To a stirred solution of commercially available ethyl 2-oxocyclopentane-1-carboxylate **3** (10 g, 65.35 mmol) in 100 mL of diethyl ether was added NaCN (9.60 g, 196.07 mmol) and reaction mixture was cooled to 0 °C. Maintaining the same temperature, concentrated HCl (17.04 mL, 196.07 mmol) was added drop-wise during 1h under inert atmosphere. After stirring for additional 12h at room temperature, 50 mL of water was added to the reaction mixture and it was extracted three times with diethyl ether (3 x 50 mL). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4 and was concentrated under vacuum to give crude cyanohydrin. Purification by column chromatography with elution of 7% EtOAc/petroleum ether gave product **4** in 53% yield with recovery of starting material.

Note: The material used for sodium cyanide was quenched with saturated aqueous solution of potassium permanganate.

Ethyl 2-cyanocyclopent-1-enecarboxylate (5)

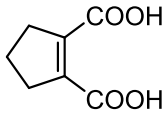


Compound **4** (2.6 g, 14.2 mmol) was dissolved in cold pyridine and was added $SOCl_2$ (1.54 mL, 21.3 mmol) during 1h drop-wise at 0 ^oC and the reaction mixture was stirred for 12h at room temperature. Ice was added, followed addition of CH_2Cl_2 (25 mL) and stirred for additional 45 minutes (this will reduced the dark-black mass formation in reaction mixture during extraction). The reaction mixture was extracted with CH_2Cl_2 (4 x 10mL) and the combined organic layers were washed with brine (20 mL) and dried over anhydrous Na_2SO_4. Organic layer was then concentrated *in vacuo* and crude residue obtained was purified by column chromatography. Compound **5** was eluted with 2.5% EtOAc/petroleum ether with yield of 26% and rest of mass was mixture with other isomer. Isomer conversion was carried out with help of DBU (1,8-Diozabicyloundec-7ene) with 53% conversion to **5** with an overall yield of 60%.

^{1}H NMR (200 MHz, $CDCl_3$) δ 4.26 (q, *J* = 8Hz, 2H), 2.76 (q, *J* = 6 Hz, 4H), 1.96 - 2.12 (m, 2H), 1.32 (t, *J* = 8Hz, 3H)

^{13}C NMR (50 MHz, $CDCl_3$) δ 162.2, 149.2, 121.3, 114.3, 61.5, 36.5, 33.3, 22.1, 13.9

Cyclopent-1-ene-1,2-dicarboxylic acid (6)



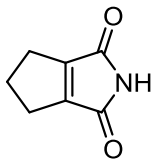
A solution of compound **5** (0.6 g, 3.63 mmol) in conc. HCl (1 mL) was brought to reflux for 2.5 h until a clear solution is observed. Upon addition of 1 mL of H_2O, there was precipitate formation which was further refluxed for 10h until again a clear solution is obtained. Concentrate the solution *in vacuo* to compomd **6** as brown solid with the yield of 67%. The obtained values were compared to literature reports.

Melting point: 153 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.27 (br s, 2H), 2.83 – 2.75 (m, 4H), 1.96 – 1.90 (m, 2H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 167, 142, 34, 22

5, 6-Dihydrocyclopenta[*c*]pyrrole-1, 3(2*H*,4*H*)-dione (7)



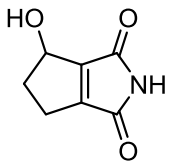
Under inert conditions, a solution of compound **6 (**0.38 g, 2.43 mmol**)** in Ac_2O (3.79 mL) was stirred of 12h at room temperature. The above reaction mixture was added with 3.79 mL of aq. NH_3 and stirred for additional 30 minutes, followed by drop-wise addition of conc. HCl until a white precipitate is obtained (pH < 3). The precipitate obtained was filtered off using Buchner funnel to get a white solid which was then treated with trifluoroacetic anhydride and brought to reflux for 18 h (45 °C). The reaction mixture was evaporated to dryness and the solid product obtained was crystallised in chloroform /hexane to obtain compound **7** as light brown crystals with yield of 64%.

Melting point 188 °C.

^{1}H NMR (200 MHz, $CDCl_3$) δ 6.95 (br s, 1H), 2.71 – 2.64 (m, 4H), 2.52 – 2.41 (m, 2H);

^{13}C NMR (100 MHz $CDCl_3$) δ 167.9, 154.0, 27.6, 26.4;

4-Hydroxy-5, 6-dihydroclopenta[*c*]pyrrole-1, 3 (2*H*, 4*H*)-dione (11)



To a solution of compound **7** (0.10 g, 0.73 mmol) in dry 1, 4-dioxane (1.5 mL) was added SeO_2 (0.32 g, 2.91 mmol) and was subjected to microwave irradiation in closed vessel at 110 °C for 30 minutes (Antonpaar monowave 300 instrument). The reaction mixture was then evaporated to dryness and the crude mixture obtained was purified over column chromatography (50 - 70% EtOAc/petroleum ether) to obtain product **11** (30 mg) in 61% yield (brsm). Unreacted starting material was recovered (54 mg).

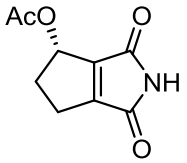
^{1}H NMR (400 MHz, D_2O) δ 5.56-5.53 (m, 1H), 3.18 (m, 2H), 3.12-2.99 (m, 1H), 2.64-2.60 (m, 1H)

^{13}C NMR (100 MHz, D_2O) δ 169.7, 169.0, 152.9, 152.0, 69.8, 37.3, 24.0.

(*S*)-4-Hydroxy-5,6-dihydrocyclopenta[*c*]pyrrole-1,3(2*H*,4*H*)-dione ((+)-11)

To a solution of alcohol **6** (1.12 g, 7.32 mmol) in dry THF was added Amano PS lipase (1.5 g) followed by addition of vinyl acetate (3.3 mL, 36.6 mmol) and stirred at room temperature for 10h. The reaction was monitored by chiral HPLC analysis (Chiralpak IB column,) and upon 50% conversion, the reaction mixture was filtered through celite bed, concentrated and was added with 10 mL of water. The aqueous layer was extracted thrice with EtOAc (3x10 mL) and combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to obtain crude mixture of alcohol and acetate which on chromatographic separation yielded (*R*)-1,3-dioxo-1,2,3,4,5,6-hexahydrocyclopenta[*c*]pyrrol-4-yl **(–)-9** (0.538 g) in 38% yield as white solid and (+)-(*S*)-4-hydroxy-5,6-dihydrocyclopenta[*c*]pyrrole-1,3(2*H*,4*H*)-dione **(+)-6** (0.518 g) with 98% *ee* in 47% yield as white solid. HPLC conditions: Chiralpak IB column, Petroleum ether/2-propanol = 95: 5, flow rate = 1 mL/min, 230nm UV detector, t_1 = 46.2 min (minor) and t_2 = 51.9 min (major).

Compound **(–)-10**

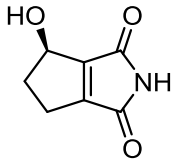


Specific rotation $[\alpha]_D^{26}$= – 38.3 (*c* 0.77, $CHCl_3$),

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.43 (br s, 1H), 5.96-5.91 (m, 1H), 2.92-2.64 (m, 3H), 2.32-2.27 (m, 1H), 2.02 (s, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 165.7, 165.6, 164.8, 159.2, 149.8, 72.0, 36.1, 25.1, 20.9.

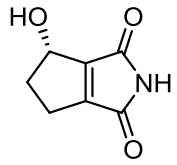
Compound **(+)-11**



Specific rotation $[\alpha]_D^{26}$= +56.1 (*c* 1.07, MeOH),

^{1}H NMR (200 MHz, D_2O) δ 5.21-5.10 (m, 1 H), 2.91-2.67 (m, 2H), 2.65-2.47 (m, 1H), 2.31-2.13 (m, 1H).

(*R*)-4-Hydroxy-5,6-dihydrocyclopenta[*c*]pyrrole-1,3(2*H*,4*H*)-dione ((–)11)



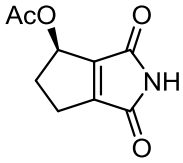
To a solution of acetate **(–)-10** (0.10 g, 0.50 mmol) in acetone was added Amano PS lipase (0.08 g) and phosphate buffer (10 mL) of pH 7 and warmed at 40 °C for 3h. The reaction mixture was then concentrated *in vacuo* to remove acetone and extracted twice with EtOAc (2x4 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated to obtain crude residue which was subjected to chromatographic separation to yield (–)-**11** (0.072 g) as white solid with 99% *ee* in 92% yield.

Specific rotation $[\alpha]_D^{25} = -53.4$ (*c* 1.34, MeOH),

^{1}H NMR (200 MHz, D_2O) δ 5.22-5.08 (m, 1H), 2.92-2.66 (m, 2H), 2.65-2.48 (m, 1H), 2.31-2.13 (m, 1H);

HPLC condition: Chiralpak IB column, Petroleum ether/2-propanol = 95:5, 1 mL/min, 230 nm UV detector, t_1 = 46.2 min (major) and t_2 = 51.9 min (minor).

(*R*)-1,3-Dioxo-1,2,3,4,5,6-hexahydrocyclopenta[*c*]pyrrol-4-yl acetate ((+)-10)



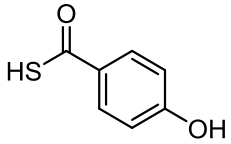
To a solution of alcohol (+)-**11** (0.10 g, 0.65 mmol) in dry CH_2Cl_2 (3.0 mL) was added pyridine (0.10 mL, 1.30 mmol) and Ac_2O (0.066 mL, 0.65 mmol). The reaction mixture was stirred at room temperature for 10h. Then the reaction mixture was diluted with water (3.0 mL) and extracted with CH_2Cl_2 (3x3 mL). The combined organic layer was washed with 1N HCl and then with brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to obtain crude residue which was subjected to chromatographic separation (30% EtOAc/Petroleum ether) to yield **(+)-10** (0.105 g) as white solid with 82% yield.

Specific rotation $[\alpha]_D^{26} = +40.1$ (*c* 0.54, $CHCl_3$),

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.43 (br s, 1H), 5.96-5.91 (m, 1H), 2.92-2.64 (m, 3H), 2.32-2.27 (m, 1H), 2.02 (s, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 165.7, 165.6, 164.8, 159.2, 149.8, 72.0, 36.1, 25.1, 20.9.

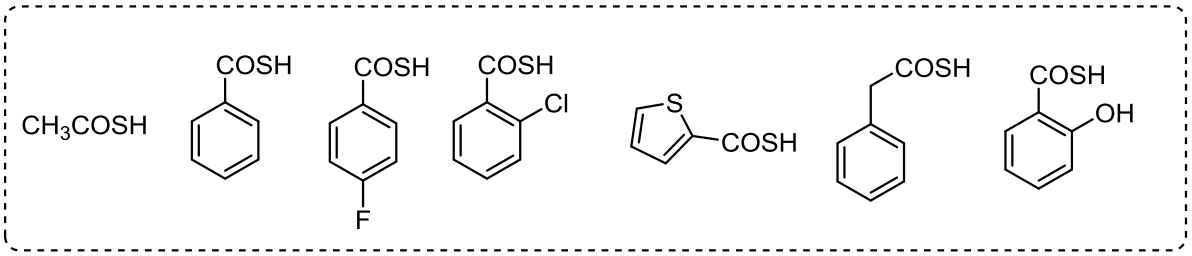
4-Hydroxybenzothioic *S*-acid (12)



To a solution of 4-hydorxy benzoic acid (0.50 g, 3.62 mmol) in dry acetonitrile (5.0 mL) was added Lawesson's reagent (0.73 g, 1.81 mmol) and was subjected to microwave irradiation in closed vessel at 100 °C for 15 min. (Antonpaar monowave 300 instrument). The reaction mixture was evaporated to dryness and the crude residue obtained was washed several times with 1N HCl, then with brine solution and dried over anhydrous Na_2SO_4. The combined organic layers were concentrated *in vacuo* and purified by silica gel column chromatography with elution of 20-30% EtOAc/Petroleum ether to obtain product **12** (300 mg) in 53% yield.

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.85 (d, *J*= 8.0 Hz, 2H), 6.88 (d, *J*= 8.0 Hz, 2H), 3.58 (br s, 1H);

** Following similar procedure, the following thioacids are prepared, which are coupled with requisite substrates to generate a library of nitrosporeusine analogues.



***S*-((3*R**,6*R**,6*R**)-6-Hydroxy-1,3-dioxohexahydrocyclopenta[c]pyrrol-3a(1*H*)-yl) 4-hydroxybenzothioate (Racemic nitrosporeusine A) and**

***S*-((3a*S**,6*R**,6a*R**)-6-Hydroxy-1,3-dioxohexahydrocyclopenta[c]pyrrol-3a(1*H*)-yl) 4-hydroxybenzothioate (Racemic nitrosporeusine B)**

A round-bottomed flask equipped with a magnetic stirrer, was charged with p-hydroxy thiobenzoic acid **12** (0.053 g, 0.34 mmol), compound **11** (0.048 g, 0.31 mmol), and water (2.0 mL). The reaction mixture was stirred vigorously at room temperature for 10h. The reaction mixture was then diluted with EtOAc and extracted thrice (3 x 5 mL). The combined organic layer was washed with saturated aqueous $NaHCO_3$ followed by brine solution and dried over anhydrous Na_2SO_4. The crude compound obtained was subjected to column chromatography (60-70% EtOAc/Petroleum ether) to obtain a 3:1 diastereomeric mixture of compound **2** (45 mg) and compound **1** (16 mg) as white solids in 63% overall yield.

(±)-nitrosporeusine B (**2)**

IR (neat) ν_{max} 3535, 2924, 1706, 1649, 1206 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d6) δ 11.57 (s, 1H), 10.65 (s, 1H), 7.74 (d, *J*= 8.0 Hz, 2H), 6.88 (d, *J*= 8.0 Hz, 2H), 5.26 (br s, 1H), 4.38-4.39 (m, 1H), 3.11 (br s, 1H), 2.22-2.17 (m, 2H), 1.83-1.78 (m, 1H), 1.59-1.56 (m, 1H);

^{13}C NMR (100 MHz, DMSO-d6) δ 190.4, 179.3, 176.9, 163.7, 130.0, 126.8, 116.2, 74.5, 63.9, 58.1, 33.8, 32.4;

HRMS (ESI) *m/z* calculated for $C_{14}H_{13}NO_5S$ $[M+Na]^+$ 330.0407 found 330.0392.

(±)-nitrosporeusine A (**1):**

IR (neat) ν_{max} 3744, 2925, 1705, 1649, 1532 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d6) δ 11.28 (br s, 1H), 7.74 (d, *J*= 8.0 Hz, 2H), 6.85 (d, *J*= 8.0 Hz, 2H), 5.31-5.29 (m, 1H), 4.47 (br s, 1H), 3.25 (d, *J*= 7.3 Hz, 1H), 2.28-2.20 (m, 2H), 1.92-1.90 (m, 2H), 1.71-1.69 (m, 1H);

^{13}C NMR (100 MHz, DMSO-d6) δ 190.0, 179.1, 175.2, 163.6, 130.0, 127.0, 116.2, 72.3, 60.2, 59.2, 35.0, 32.8;

HRMS (ESI) *m/z* calculated for $C_{14}H_{13}NO_5S$ $[M+Na]^+$ 330.0407 found 330.0400.

Procedure A: General procedure for synthesis of analogues through Michael addition

In a round-bottomed flask equipped with a magnetic stirrer, thioacid (1.1 eq.), unsaturated compound (1 eq.), and THF:water (1:1) were charged. The reaction mixture was stirred vigorously at room temperature for 10 to 12h, then it was diluted with EtOAc and extracted

twice (2x3 mL). The combined organic layer was washed with saturated aqueous $NaHCO_3$ solution followed by brine solution and concentrated *in vacuo* to obtain a crude mixture which was purified by column chromatography (silica gel; EtOAc/Petroleum ether) to obtain the desired nitrosporeusine analogues.

#All the products are isolated in 55-71% overall yield.

Procedure B: General procedure for synthesis of thio-acids

To a solution of substituted benzoic acid (1 eq.) in dry acetonitrile was added Lawesson's reagent (0.5 eq.) and was subjected to microwave irradiation in closed vessel at 100 °C for 15 minutes (Antonpaar monowave 300 instrument). The reaction mixture was evaporated to dryness and the crude residue obtained was diluted with EtOAc and washed several times with 1N HCl, then with brine solution and dried over anhydrous Na_2SO_4. The combined organic layers were concentrated in vacuo and purified by silica gel column chromatography with elution of 20-30% EtOAc/Petroleum ether.

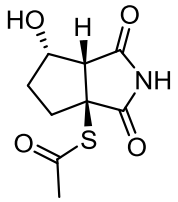
* **All thio-acids are prepared freshly before reaction and used immediately for reactions**

Procedure C: General procedure for acetylation

To a solution of alcohol (1 eq.) in pyridine as solvent was added 1.5 eq. of acetic anhydride and 0.1 eq. of Dimethylaminopyridine (DMAP) and stirred at room temperature for 6-8 hours. The reaction was diluted with ethylacetate and slowly added with 1N HCl until pH turns acidic. The layers were separated and organic layer was washed with water, brine and dried over anhydrous Na_2SO_4. The crude reaction reaction mixture obtained upon concentrating in vacuo was purified over column chromatography (silica gel; 5-10% EtOAc/CH_2Cl_2) to obtained the desired acetate compounds.

***S*-((3a*S**,6*S**,6a*R**)-6-Hydroxy-1,3-dioxohexahydrocyclopenta[*c*]pyrrol-3a(1*H*)-yl) ethanethioate (13) and *S*-((3a*R**,6*S**,6a*S**)-6-Hydroxy-1,3-dioxohexahydrocyclopenta [*c*]pyrrol-3a(1*H*)-yl) ethanethioate (14)**

Following general procedure A alcohol **11** has been treated with thioacetic acid to get diastereomers **13** and **14** in approximately 1:1.2 ratio. Compound **13** (20 mg) obtained as white solid.



IRυ_{max} (film) 3809, 1708, 1692, 1515 cm^{-1};

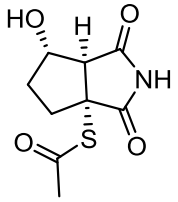
^{1}H NMR (400 MHz, CD_3OD) δ 4.60-4.56 (m, 1H), 3.34-3.30 (d, 1H), 2.33 (s, 3H), 2.31-2.28 (m, 1H) 2.02-1.89 (m, 1H), 1.88-1.81 (m, 1H), 1.79-1.77 (m, 1H);

^{13}C NMR (100 MHz, CD_3OD) δ 198.0, 181.0, 177.1, 73.9, 61.0, 60.7, 35.2, 33.4, 29.8;

HRMS (ESI) *m/z* calculated for $C_9H_{11}NO_4S$ $[M+Na]^+$ 252.0301, found 252.0299.

RT_{HPLC} 5.538 min, purity >90%, method-A.

Compound **14** (23 mg) obtained as white solid.



Melting Point 120-129 °C;

IRυ_{max} (film) 3809, 1708, 1692, 1515 cm^{-1};

^{1}H NMR (400 MHz, CD_3OD): δ 4.53-4.51 (m, 1H), 3.17-3.15 (d, 1H), 2.31 (s, 3H), 2.23-2.19 (m, 2H), 1.89-1.88 (m, 1H), 1.70-1.65 (m, 1H);

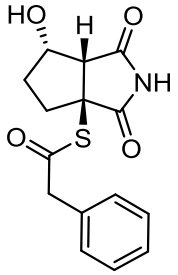
^{13}C NMR (100 MHz, CD_3OD): δ 196.9, 179.6, 176.7, 74.8, 63.6, 58.3, 32.9, 31.7, 28.0;

HRMS (ESI): *m/z* calculated for $C_9H_{11}NO_4S$ $[M+Na]^+$ 252.0301, found 252.0299.

_S_-((3a_S_*,6_S_*,6a_R_*)-6-Hydroxy-1,3-dioxohexahydrocyclopenta[_c_]pyrrol-3a(1_H_)-yl) 2-phenylethanethioate (15) & _S_-((3a_R_*,6_S_*,6a_S_*)-6-Hydroxy-1,3-dioxohexahydrocyclo penta[_c_]pyrrol-3a(1_H_)-yl) 2-phenylethanethioate (16)

Following general procedure A, alcohol **11** has been treated with freshly prepared 2-phenylethanethioic *S*-acid to get diastereomers **15** and **16** in approximately 1:2 ratio.

Compound **15** (25 mg) obtained as yellow solid.



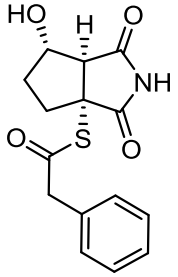
^{1}H NMR (400 MHz, CD_3OD) δ 7.36 -7.28 (m, 5H), 4.55-4.53 (m, 1H), 3.84 (s, 2H), 3.25-3.23 (d, *J* = 7.6 Hz, 1H), 2.31-2.27 (m, 1H), 1.99-1.87 (m, 1H), 1.85-1.75 (m, 2H);

^{13}C NMR (125 MHz, CD_3OD) δ 199.9, 181.0, 177.1, 134.5, 131.0, 129.9, 128.8, 73.9, 61.0, 50.3, 49.7, 35.2, 33.5;

HRMS (ESI) *m/z* calculated for $C_{15}H_{15}NO_4S$ $[M+Na]^+$ 328.0614, found 328.0601.

RT_{HPLC} = 6.10 min, purity >95%, 25:75 H_2O/ MeOH.

Compound **16** (45 mg) obtained as white solid.



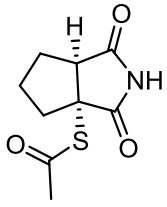
^{1}H NMR (500 MHz, CD_3OD) δ 7.35-7.30 (m, 5H), 4.52-4.51 (m, 1H), 3.86 (s, 2H), 3.13 (s, 1H), 2.21-2.18 (m, 2H), 1.91-1.87 (m, 1H), 1.69-1.66 (m, 1H);

^{13}C NMR (125 MHz, CD_3OD) δ 198.9, 179.6, 176.7, 133.0, 129.5, 128.3, 127.2, 74.8, 63.6, 58.4, 48.6, 33.0, 31.7;

HRMS (ESI) *m/z* calculated for $C_{15}H_{15}NO_4S$ $[M+Na]^+$ 328.0614, found 328.0601.

RT_{HPLC} = 5.72 min, purity >93%, 25:75 H_2O/ MeOH.

***S*-((3a*R**,6a*S**)-1,3-dioxohexahydrocyclopenta[c]pyrrol-3a(1*H*)-yl) ethanethioate (17)**



Following general procedure A, compound **3** has been treated with commercially available thioacetic acid to get **17** (71 mg) as white solid,

Melting point 120-122 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (br s, 1H), 3.27-3.24 (d, *J* = 8.8 Hz, 1H), 2.34 (s, 3H), 2.32-2.24 (m, 2H), 1.83-1.54 (m, 4H);

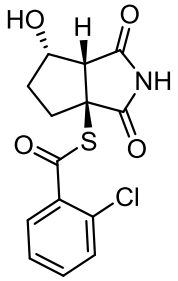
^{13}C NMR (100 MHz, $CDCl_3$) δ 196.3, 178.2, 178.2, 59.5, 54.7, 36.2, 29.7, 29.6, 24.3;

HRMS (ESI) *m/z* calculated for $C_9H_{11}NO_3S$ $[M+Na]^+$ 236.0348, found 236.0352.

_S_-((3a_S_*,6_S_*,6a_R_*)-6-Hydroxy-1,3-dioxohexahydrocyclopenta[_c_]pyrrol-3a(1_H_)-yl) 2-chlorobenzothioate (18) & _S_-((3aR*,6_S_*,6a_S_*)-6-Hydroxy-1,3-dioxohexahydrocyclopenta [_c_]pyrrol-3a(1_H_)-yl) 2-chlorobenzothioate (19)

Following general procedure A, alcohol **11** has been treated with freshly prepared 2-chlorobenzothioic *S*-acid to get diastereomers **18** and **19** in approximately 1:3 ratio.

Compound **18** (26 mg) obtained as white solid.



Melting point 173-174 °C;

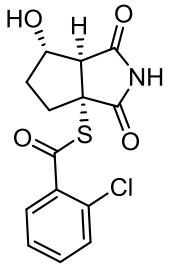
IRυ_{max} (film) 3743, 2925, 2320, 1707, 1515 cm^{-1};

^{1}H NMR (400 MHz, CD_3OD) δ 7.72-7.69 (m, 1H), 7.55-7.53 (m, 2H), 7.45-7.43 (m, 1H), 4.67-4.63 (m, 1H), 3.46 (d, *J* = 7.6 Hz, 1H), 2.43-2.39 (m, 1H), 2.08-1.99 (m, 2H), 1.86-1.83 (m, 1H);

^{13}C NMR (100 MHz, CD_3OD) δ 196.0, 183.0, 179.3, 139.7, 136.9, 134.6, 134.3, 133.0, 130.9, 76.3, 64.0, 63.3, 37.7, 35.9;

HRMS (ESI) *m/z* calculated for $C_{14}H_{12}NO_4ClS$ $[M+Na]^+$ 348.0068, found 348.0061.

Compound **19** (82 mg) obtained as white solid



Melting point 154-158 °C;

IRυ_{max} (film) 3743, 2925, 2320, 1707, 1515 cm^{-1};

^{1}H NMR (200 MHz, CD_3OD) δ 7.78-7.76 (m, 1H), 7.58-7.50 (m, 3H), 4.63-4.60 (td, *J* = 3.7, 1.2 Hz, 1H), 3.34-3.32 (m, 1H), 2.39-2.31 (m, 2H), 1.97-1.95 (m, 1H), 1.82-1.80 (m, 1H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 192.6, 178.3, 176.1, 135.0, 133.2, 131.3, 131.1, 129.9, 126.9, 75.4, 63.7, 59.2, 33.2, 32.5;

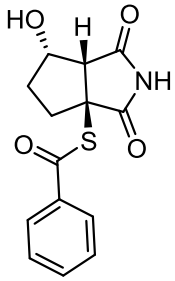
HRMS (ESI) *m/z* calculated for $C_{14}H_{12}NO_4ClS$ $[M+Na]^+$ 348.0068, found 348.0061.

RT_{HPLC} = 6.70 min, purity >99%, 40:60 H_2O/ MeOH.

_S_-((3a_S_*,6_S_*,6a_R_*)-6-Hydroxy-1,3-dioxohexahydrocyclopenta[_c_]pyrrol-3a(1_H_)-yl) benzothioate (20) & _S_-((3a_R_*,6_S_*,6a_S_*)-6-Hydroxy-1,3-dioxohexahydrocyclopenta[_c_] pyrrol-3a(1_H_)-yl) benzothioate (21)

Following general procedure A, alcohol **11** has been treated with commercially available Thiobenzoic acid to get diastereomers **20** and **21** in approximately 1:2 ratio.

Compound **20** (26 mg) as white solid.



Melting point 185-186 ºC;

IRυ_{max} (film) 3743, 2927, 2320, 1741, 1706, 1531 cm^{-1};

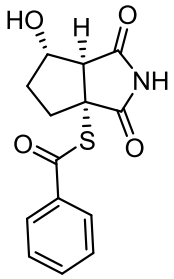
^{1}H NMR (400 MHz, CD_3OD) δ 7.91-7.89 (dd, *J* = 8.4, 1.1 Hz, 2H), 7.66-7.64 (m, 1H), 7.53-7.49 (m, 2H), 4.62-4.59 (m, 1H), 3.42 (d, *J* = 7.6 Hz, 1H), 2.42-2.39 (dd, *J* = 13.2, 7.1 Hz, 1H), 2.08-2.02 (m, 2H), 1.84-1.82 (m, 1H);

^{13}C NMR (100 MHz, CD_3OD) δ 193.6, 181.1, 177.2, 137.4, 135.6, 130.3, 128.4, 73.9, 61.1, 60.6, 35.3, 33.7;

HRMS (ESI) *m/z* calculated for $C_{14}H_{13}NO_4S$ $[M+Na]^+$ 314.0457, found 314.0456.

RT_{HPLC} = 6.02 min, purity >90%, 25:75 H_2O/ MeOH.

Compound **21** (47 mg) as white solid.



Melting point 180-182 ºC;

IRυ_{max} (film) 3743, 2927, 2320, 1741, 1706, 1531 cm^{-1};

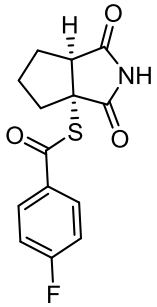
^{1}H NMR (400 MHz, CD_3OD) δ 7.94-7.91 (m, 2H), 7.69-7.66 (m, 1H), 7.54-7.51 (m, 2H), 4.59-4.58 (m, 1H), 3.34-3.42 (m, 1H), 2.40-2.33 (m, 2H), 1.96-1.95 (m, 1H), 1.78-1.77 (m, 1H);

^{13}C NMR (100 MHz, CD_3OD) δ 191.1, 178.1, 175.27, 134.21, 132.54, 127.23, 125.36, 73.36, 62.28, 56.75, 31.75, 30.32;

HRMS (ESI) *m/z* calculated for $C_{14}H_{13}NO_4S$ $[M+Na]^+$ 314.0457, found 314.0456.

RT_{HPLC} = 10.8 min, purity >88%, 40:60 H_2O/ MeOH.

_S_-((3a_R_*,6a_S_*)-1,3-Dioxohexahydrocyclopenta[c]pyrrol-3a(1_H_)-yl) 4-fluorobenzothioate (22)



Following general procedure A, compound **7** has been treated with freshly prepared 4-fluorobenzothioic *S*-acid to get **22** (40 mg) as white solid.

Melting point 186-187 °C.

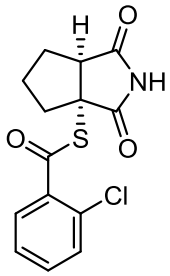
IRυ_{max} (film) 3744, 2922, 1770, 1647 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (br s, 1H) 7.96-7.89 (m, 2H), 7.18-7.08 (m, 2H), 3.39-3.35 (m, 1H), 2.48-2.28 (m, 2H), 2.09-1.91 (m, 2H), 1.64-1.59 (m, 2H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 190.6, 178.3, 178.2, 167.6, 165.1, 131.9, 130.1, 130.0, 116.1, 116.0, 59.5, 55.0, 36.5, 29.8, 24.5;

HRMS (ESI) *m/z* calculated for $C_{14}H_{12}NO_3FS$ $[M+Na]^+$ 316.0414, found 316.0408.

_S_-((3a_R_*,6a_S_*)-1,3-Dioxohexahydrocyclopenta[c]pyrrol-3a(1_H_)-yl) 2-chlorobenzothioate (23)



Following general procedure A, compound **7** has been treated with freshly prepared 2-chlorobenzothioic *S*-acid to get **23** (31 mg) as white solid.

Melting point 188-190 °C.

IRυ_{max} (film) 3229, 1707, 1675, 1547 cm^{-1};

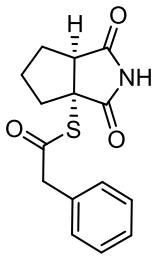
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (br s, 1H), 7.72-7.70 (d, *J* = 7.8 Hz, 1H), 7.44-7.43 (m, 2H), 7.35-7.33 (m, 1H), 3.44-3.42 (d, *J* = 8.8 Hz, 1H), 2.41-2.38 (m, 1H), 2.32-2.30 (m, 1H), 1.95-1.93 (m, 1H), 1.92-1.90 (m, 2H), 1.63-1.61 (m, 1H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 191.9, 178.1, 177.9, 135.3, 133.1, 131.4, 131.1, 129.7, 126.7, 60.3, 54.8, 36.4, 29.8, 24.5;

HRMS (ESI) *m/z* calculated for $C_{14}H_{12}NO_3ClS$ $[M+Na]^+$ 332.0109, found 332.0119.

RT_{HPLC} = 7.8 min, purity >92%, 25:75 H_2O/ MeOH.

_S_-((3a_R_*,6a_S_*)-1,3-dioxohexahydrocyclopenta[c]pyrrol-3a(1_H_)-yl) 2-phenylethanethioate (24)



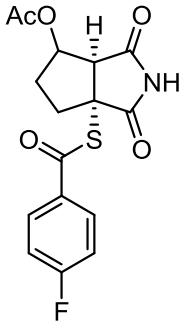
Following general procedure A, compound **7** has been treated with freshly prepared 2-phenylethanethioic *S*-acid to get **24** (80 mg) as sticky liquid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (br. s., 1H), 7.38-7.18 (m, 5H), 3.82 (s, 4 H), 3.21 (d, *J* = 8.8 Hz, 1 H), 2.33-2.22 (m, 2H), 2.00 - 1.77 (m, 3 H), 1.91 - 1.70 (m, 1H), 1.55 (dd, *J* = 6.1, 12.0 Hz, 1H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 198.3, 178.1, 178.1, 132.4, 129.7, 128.9, 127.8, 59.6, 54.8, 49.5, 36.3, 29.8, 24.3;

HRMS (ESI): *m/z* calculated for $C_{15}H_{15}NO_3S$ $[M+Na]^+$ 312.0665, found 312.0657.

6a-((4-Fluorobenzoyl)thio)-1,3-dioxooctahydrocyclopenta[*c*]pyrrol-4-yl acetate (25)



Following general procedure A, compound **10** has been treated with freshly prepared 4-fluorobenzothioic *S*-acid to get inseparable mixture of diastereomers of compound **25** (45 mg) as white solid.

Melting point 118-119 °C;

IRυ_{max} (film) 3743, 3057, 2925, 1707, 1649, 1513 cm^{-1};

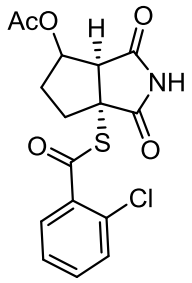
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58-8.40 (br s, 1H), 8.00-7.93 (m, 2H), 7.23-7.14 (m, 2H), 5.58-5.43 (m, 1H), 3.79-3.55 (m, 1H), 2.51-2.32 (m, 2H), 2.20-2.11 (m, 4H), 1.96-1.87 (m, 1H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 190.0, 177.7, 177.6, 174.0, 169.9, 169.4, 165.4, 131.7, 130.2, 130.1, 116.2, 116.0, 73.6, 60.9, 58.6, 58.3, 55.3, 33.6, 31.8, 30.4, 29.9, 29.6, 21.0, 20.8;

HRMS (ESI): *m/z* calculated for $C_{16}H_{14}NO_5SF$ $[M+Na]^+$ 374.0456, found 374.0469.

RT_{HPLC} = 6.96 min, purity >90%, 25:75 H_2O/ MeOH.

6a-((2-Chlorobenzoyl)thio)-1,3-dioxooctahydrocyclopenta[*c*]pyrrol-4-yl acetate (26)



Following general procedure A, compound **10** has been treated with freshly prepared 2-chlorobenzothioic *S*-acid to get inseparable mixture of diastereomers of compound **26** (35 mg) obtained as white solid.

Melting point 90-91 °C;

IRυ_{max} (film) 3744, 1771, 1707, 1547, 1626 cm^{-1};

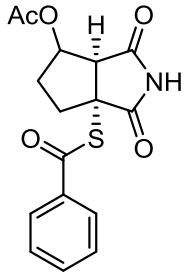
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.77 (br s, 1H), 7.74-7.73 (d, *J* = 7.6 Hz, 1H), 7.47-7.37 (m, 2H), 7.36-7.33 (m, 1H), 5.55-5.54 (m, 1H), 3.57-3.56 (m, 1H), 2.46-2.43 (m, 1H), 2.26-2.24 (m, 1H), 2.16-2.13 (dd, *J* = 6.6, 4.7 Hz, 1H), 2.11 (s, 3H), 2.09-1.91 (m, 1H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 191.9, 177.2, 173.6, 169.9, 134.9, 133.3, 131.6, 131.3, 129.9, 126.9, 73.6, 60.8, 59.0, 55.2, 33.4, 31.7, 30.4, 30.0, 21.0, 20.9;

HRMS (ESI): *m/z* calculated for $C_{16}H_{14}NO_5ClS$ $[M+Na]^+$ 390.0173, found 390.0158.

RT_{HPLC} = 6.98 min, purity >96%, 25:75 H_2O/ MeOH .

6a-(Benzoylthio)-1,3-dioxooctahydrocyclopenta[*c*]pyrrol-4-yl acetate (27)



Following general procedure A, compound **10** has been treated with freshly prepared Thiobenzoic acid to get inseparable mixture of diastereomers of compound **27** (25 mg) obtained as white solid.

IRυ_{max} (film) 3743, 2922, 1737, 1707, 1675, 1546, 1208 cm^{-1};

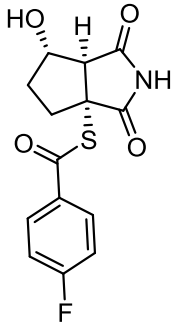
^{1}H NMR (400 MHz, $CDCl_3$): δ 9.05 (br s, 1H), 7.92-7.86 (m, 2H), 7.64-7.60 (m, 1H), 7.49-7.45 (m, 2H), 5.55-5.44 (m, 1H), 3.76-3.54(s, 1H), 2.49-2.47 (m, 1H), 2.29-2.14 (dt, *J* = 13.4, 6.9 Hz, 1H), 2.10 (s, 3H), 2.11-2.06 (s, 1H), 1.96-1.93 (m, 2H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 192.2, 177.7, 174.0, 169.9, 135.3, 134.4, 128.9, 127.5, 73.7, 61.0, 58.2, 55.4, 33.6, 31.8, 30.4, 30.1, 29.7, 21.1, 20.8;

HRMS (ESI): *m/z* calculated for $C_{16}H_{15}NO_5S$ $[M+Na]^+$ 356.0553 found 356.0563.

RT_{HPLC} = 6.77 min, purity >98%, 25:75 H_2O/ MeOH.

_S_-((3a_R_*,6_S_*,6a_S_*)-6-Hydroxy-1,3-dioxohexahydrocyclopenta[_c_]pyrrol-3a(1_H_)-yl) 4-fluorobenzothioate (28)



Following general procedure A, alcohol **11** has been treated with freshly prepared 4-fluorobenzothioic *S*-acid to get **28** (50 mg) as brown solid.

Melting point 184-186 °C;

IRυ_{max} (film) 3314, 2934, 1798, 1024 cm^{-1};

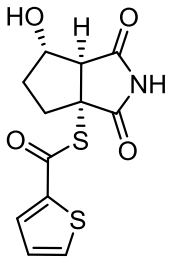
^{1}H NMR (400 MHz, CD_3OD) δ 8.02-7.98 (m, 2H), 7.29-7.24 (m, 2H), 4.59-4.58 (m, 1H), 3.34-3.28 (m, 1H), 2.40-2.33 (m, 2H), 1.98-1.95 (m, 1H), 1.84-1.69 (m, 1H);

^{13}C NMR (100 MHz, CD_3OD) δ 191.1, 179.5, 176.7, 167.6, 132.2, 132.2, 129.8, 129.7, 115.8, 115.6, 74.8, 63.7, 58.3, 33.2, 31.8;

HRMS (ESI) *m/z* calculated for $C_{14}H_{12}NO_4SF$ $[M+Na]^+$ 332.0363 found 332.0361.

RT_{HPLC} = 6.17 min, purity >94%, 40:60 H_2O/ MeOH.

_S_-((3a_R_*,6_S_*,6a_S_*)-6-Hydroxy-1,3-dioxohexahydrocyclopenta[_c_]pyrrol-3a(1_H_)-yl) thiophene-2-carbothioate (29)



Following general procedure A, alcohol **11** has been treated with freshly prepared thiophene-2-carbothioic *S*-acid to get **20** (70 mg) as white solid.

IRυ_{max} (film) 3808, 1741, 1706, 1693, 1515 cm^{-1};

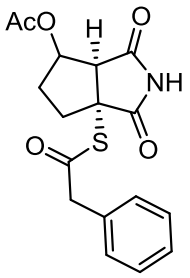
^{1}H NMR (400 MHz, CD_3OD) δ 7.91-7.87 (m, 2H), 7.23-7.20 (dd, *J* = 4.8, 4.0 Hz, 1H), 4.58 - 4.57 (m, 1H), 3.31-3.30(d, 1H), 2.36-2.30 (m, 2H), 1.95-1.94 (m, 1H), 1.76-1.73 (m, 1H);

^{13}C NMR (100 MHz, CD_3OD) δ 182.8, 177.9, 175.1, 138.5, 132.7, 130.7, 126.7, 73.4, 62.3, 56.9, 31.7, 30.3;

HRMS (ESI) *m/z* calculated for $C_{12}H_{11}NO_4S_2$ $[M+Na]^+$ 320.0002, found 320.0008.

RT_{HPLC} = 6.96 min, purity >91%, 40:60 H_2O/ MeOH.

1,3-Dioxo-6a-((2-phenylacetyl)thio)octahydrocyclopenta[_c_]pyrrol-4-yl acetate (30)



Following general procedure A, compound **10** has been treated with freshly prepared 2-phenylethanethioic *S*-acid to get inseparable mixture of diastereomers of compound **30** (45 mg) as white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61-8.53 (br s, 1H), 7.31-7.17 (m, 5H), 5.39-5.23 (m, 1H), 3.72 (s, 2H), 3.51-3.27 (m, 1H), 2.28-2.25 (m, 1H), 2.15 (s, 3H), 2.01-1.94 (m, 1H), 1.70-1.68 (m, 2H);

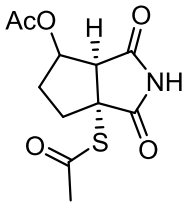
^{13}C NMR (100 MHz, $CDCl_3$)

δ 198.4, 197.9, 177.6, 173.7, 172.6, 170.5, 169.8, 132.2, 132.0, 129.8, 129.7, 128.8, 127.8, 73.6, 60.7, 58.7, 58.3, 55.1, 49.5, 49.3, 33.4, 31.5, 30.3, 29.8, 29.7, 21.0, 20.8;

HRMS (ESI) *m/z* calculated for $C_{17}H_{17}NO_5S$ $[M+Na]^+$ 370.0720, found 370.0717.

RT_{HPLC} = 6.72 min, purity >98%, 25:75 H_2O/ MeOH.

6a-(Acetylthio)-1,3-dioxooctahydrocyclopenta[*c*]pyrrol-4-yl acetate (31)



Following general procedure A, compound **10** has been treated with thioacetic acid to get inseparable mixture of diastereomers of compound **31** (60 mg) as white solid.

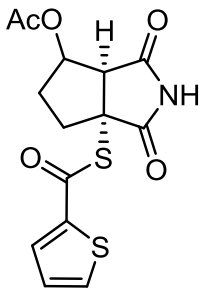
^{1}H NMR (200 MHz, $CDCl_3$) δ 8.45 (br s, 1 H), 5.53 - 5.35 (m, 1H), 3.67-3.43 (m, 1H), 2.39-2.37 (m, 4H), 2.35-2.09 (m, 4H), 1.93-1.82 (m, 2H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 196.6, 196.0, 177.8, 177.7, 174.0, 172.8, 170.5, 169.9, 73.6, 60.7, 58.7, 58.3, 55.1, 33.3, 3I.4, 30.3, 29.9, 29.7, 29.6, 29.5, 21.0, 20.8;

HRMS (ESI) *m/z* calculated for $C_{11}H_{13}NO_5S$ $[M+Na]^+$ 294.0407, found 294.0402.

RT_{HPLC} = 5.03 min, purity >90%, 20:80 H_2O/ MeOH.

1,3-Dioxo-6a-((thiophene-2-carbonyl)thio)octahydrocyclopenta[*c*]pyrrol-4-yl acetate (32)



Following general procedure A, compound **10** has been treated with freshly prepared thiophene-2-carbothioic *S*-acid to get inseparable mixture of diastereomers of compound **32** (49 mg) as red color solid.

Melting point 140-141 °C;

IRυ_{max} (film) 3830, 2922, 2853, 1737, 1707, 1646, 1514, 1210 cm^{-1};

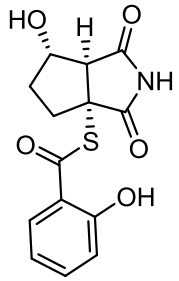
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (br s, 1H), 7.80-7.69 (d, *J* = 3.9 Hz, 1H), 7.70-7.69 (d, *J* = 4.9 Hz, 1H), 7.15-7.12 (m, 1H), 5.53-5.52 (d, *J* = 3.9 Hz, 1H), 3.56-3.49 (m, 1H), 2.49-2.43 (m, 1H), 2.26-2.24 (m, 1H), 2.11-2.09 (m, 4H), 2.07-2.06 (m, 1H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 183.8, 177.3, 173.6, 169.8, 140.0, 134.4, 134.3, 132.5, 128.3, 61.0, 58.5, 55.6, 33.6, 30.4, 30.1, 29.7, 21.1;

HRMS (ESI) *m/z* calculated for $C_{14}H_{13}NO_5S_2$ $[M+H]^+$ 340.0302, found 340.0308.

RT_{HPLC} = 5.58 min, purity >95%, 20:80 H_2O/ MeOH.

(*S*-((3a*R,6*S**,6a*S**)-6-Hydroxy-1,3-dioxohexahydrocyclopenta[*c*]pyrrol-3a(1*H*)-yl) 2-hydroxybenzothioate (33)**



Following general procedure A, alcohol **11** has been treated with freshly prepared 2-hydroxybenzothioic *S*-acid to get **33** (23 mg) as white solid.

Melting point 213-216 °C;

IRυ_{max} (film) 2935, 2827, 1823, 1448, 1023 cm^{-1};

^{1}H NMR (400 MHz, CD_3OD) δ 7.85-7.84 (m, 1H), 7.55-7.51 (m, 1H), 6.98-6.95 (m, 2H), 4.59 (m, 1H), 3.34-3.32 (d, 1H), 2.41-2.33 (m, 2H), 2.00-1.96 (m, 1H), 1.78-1.75 (m, 1H);

^{13}C NMR (100 MHz, CD_3OD) δ 196.0, 179.6, 176.6, 158.9, 136.1, 128.8, 119.7, 119.3, 117.6, 75.0, 63.7, 58.26, 33.1, 31.7;

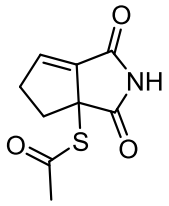
HRMS (ESI) *m/z* calculated for $C_{14}H_{13}NO_5S$ $[M+Na]^+$ 330.0407, found 330.0405.

RT_{HPLC} = 4.96 min, purity >97%, 40:60 H_2O/ MeOH.

_S_-(1,3-Dioxo-2,3,4,5-tetrahydrocyclopenta[_c_]pyrrol-3a(1_H_)-yl)ethanethioate (34) & S,S'-(1,3-Dioxohexahydrocyclopenta[_c_]pyrrole-3a,6(1_H_)-diyl)diethanethioate (40)

Following general procedure A, compound **9** has been treated with Thioacetic acid to get compounds **34** and **40.**

Compound **34** (40 mg) as white solid.



Melting point 170-172 °C;

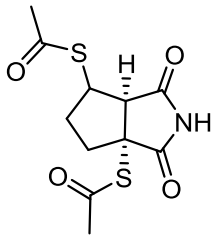
IRυ_{max} (film) 3744, 2924, 2854, 1707, 1515, 1462 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (br s, 1H), 6.85 (dd, *J* = 4.2, 2.0 Hz, 1H), 3.16-3.13 (m, 1H), 2.80-2.75 (m, 1H), 2.48-2.37 (m, 2H), 2.32 (s, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 194.5, 173.1, 163.1, 140.4, 140.3, 63.8, 36.8, 35.9, 30.4;

HRMS (ESI): *m/z* calculated for $C_9H_9NO_3S$ $[M+Na]^+$ 234.0195, found 234.0194.

Compound **40** (33 mg) as mixture of diastereomers.



^{1}H NMR (200 MHz, $CDCl_3$) δ 8.8 (br s, 1H), 4.37-4.35 (m, 1H), 3.27 (s, 1H), 2.34 (s, 3H), 2.32 (s, 3H), 2.10-1.95 (m, 4H);

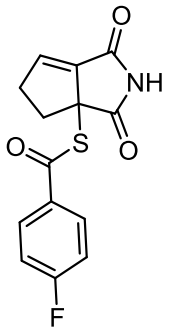
^{13}C NMR (100 MHz, $CDCl_3$) δ 196.3, 193.3, 177.22, 174.7, 60.2, 58.7, 46.3, 34.1, 31.6, 30.7, 29.6

HRMS (ESI) *m/z* calculated for $C_{11}H_{13}NO_4S_2$ $[M+Na]^+$ 310.0178, found 310.0171.

RT_{HPLC} = 6.78 min, 6.42min purity >95%, 40:60 H_2O/ MeOH (diastereomers)

***S*-(1,3-Dioxo-2,3,4,5-tetrahydrocyclopenta[*c*]pyrrol-3a(1*H*)-yl) 4-fluorobenzothioate (35) & *S*,*S'*-(1,3-Dioxohexahydrocyclopenta[*c*]pyrrole-3a,6(1*H*)-diyl) bis(4-fluorobenzothioate) (38):** Following general procedure A, compound **9** has been treated with freshly prepared 4-fluorobenzothioic *S*-acid to get compounds **35** and **38**.

Compound **35** (35 mg) obtained as white solid.



Melting point 191-192 °C;

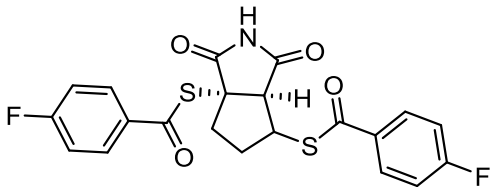
IRυ_{max} (film) 3159, 2979, 1714, 1661, 1594, 1200 cm^{-1};

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.97-7.89 (m, 3H), 7.17-7.09 (m, 2H), 6.92-6.89 (dd, *J* =4.0, 2.0 Hz, 1H), 3.23-3.14 (m, 1H), 2.87-2.85 (m, 1H), 2.59-2.56 (m, 2H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 188.9. 173.1, 167.3, 165.3, 163.3, 140.5, 132.4, 130.2, 130.1, 116.1, 115.9, 63.6, 37.2, 36.0;

HRMS (ESI) *m/z* calculated for $C_{14}H_{10}NO_3FS$ $[M+Na]^+$ 314.0258, found 314.0250. \

Compound **38** (35 mg) obtained as sticky liquid.



^{1}H NMR (200 MHz, $CDCl_3$) δ 8.35 (br s, 1H), 7.98-7.92 (m, 4H), 7.19-7.09 (m, 4H), 4.62-4.60 (m, 1H), 3.53-3.52 (t, *J* = 1.4 Hz, 1H), 2.50-2.29 (m, 1H), 2.29-2.01 (m, 4H);

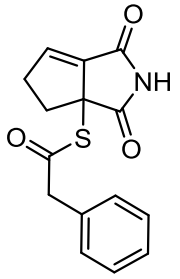
^{13}C NMR (100 MHz, $CDCl_3$) δ 190.5, 188.4, 177.2, 174.8, 167.8, 167.4, 165.2, 164.9, 132.7, 131.7, 131.6, 130.3, 130.2, 130.0, 129.9, 116.2, 116.1, 115.8, 60.7, 58.7, 46.7, 34.6, 31.5, 29.7;

HRMS (ESI) *m/z* calculated for $C_{21}H_{15}NO_4F_2S_2$ $[M+Na]^+$ 470.0285, found 470.0293.

_S_-(1,3-Dioxo-2,3,4,5-tetrahydrocyclopenta[_c_]pyrrol-3a(1_H_)-yl)2-phenylethanethioate (36) and _S,S'_-(1,3-dioxohexahydrocyclopenta[c]pyrrole-3a,6(1_H_)-diyl) bis(2-phenylethanethioate) (41)

Following general procedure A, compound **9** has been treated with freshly prepared 2-phenylethanethioic *S*-acid to get compounds **36** (30 mg) as white solid, and **41** as sticky liquid.

Compound **36**:



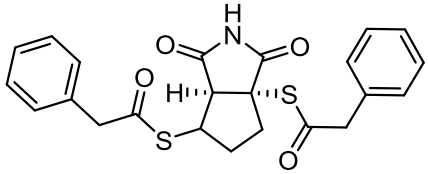
Melting point 155-158 °C;

IRυ_{max} (film) 3200, 2923, 1764, 1693, 1267 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.86 (br s, 1H) 7.36-7.24 (m, 5H), 6.84-6.82 (m, 1H), 3.81 (s, 2H), 3.14-3.00 (m, 1H), 2.82-2.67 (m, 1H), 2.47-2.36 (m, 2H);

^{13}C NMR (125 MHz, $CDCl_3$) δ 196.1, 173.2, 163.4, 140.5, 140.4, 132.3, 129.8, 128.8, 127.8, 63.8, 50.2, 36.8, 35.9;

HRMS (ESI) *m/z* calculated for $C_{15}H_{13}NO_3S$ $[M+Na]^+$ 310.0508, found 310.0501.

Compound **41**:



^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (br. s., 1H), 7.39-7.23 (m, 10H), 4.31 (br. s., 1H), 3.82 (d, *J* = 6.1 Hz, 4H), 3.21 (s, 1H), 2.30 - 2.28 (m, 1H), 2.09-2.00 (m, 2H), 1.91 - 1.87 (m, 1H);

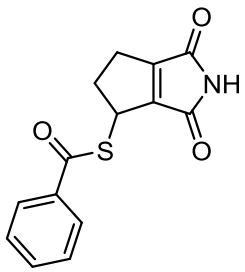
^{13}C NMR (100 MHz, $CDCl_3$) δ 198.3, 195.5, 174.7, 132.9, 132.2, 129.8, 129.6, 128.9, 128.8, 127.9, 127.7, 77.4, 77.1, 76.8, 60.2, 58.7, 50.5, 49.3, 46.5, 34.2, 31.5;

HRMS (ESI): *m/z* calculated for $C_{23}H_{21}NO_4S_2$ $[M+H]^+$ 440.0985, found 440.0981.

RT_{HPLC} 22.26 min, purity >95%, 15:85 H2O/ MeOH.

_S_-(1,3-Dioxo-1,2,3,4,5,6-hexahydrocyclopenta[_c_]pyrrol-4-yl) benzothioate (37) & _S,S'_-(1,3-Dioxohexahydrocyclopenta[_c_]pyrrole-3a,6(1_H_)-diyl) dibenzothioate (39)

Following general procedure A, compound **9** has been treated with Thiobenzoic acid to get compounds **37** and **39**. Compound **37** (30 mg) obtained as white solid.



Melting point 149-151 °C;

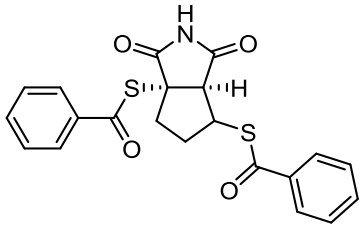
IRυ_{max} (film) 3806, 2922, 1707, 1676, 1532 cm^{-1};

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.94 (dd, *J* = 8.4, 1.3 Hz, 2H), 7.64-7.43 (m, 3H), 7.23 (br s, 1H), 5.09-5.02 (m, 1H), 3.28-3.21 (m, 1H), 2.8-2.80 (m, 2H), 2.61-2.50 (m, 1H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 190.2, 165.5, 164.7, 156.5, 151.4, 136.2, 133.9, 128.8, 127.4, 41.5, 38.3, 25.7;

HRMS (ESI) *m/z* calculated for $C_{14}H_{11}NO_3S$ $[M+Na]^+$ 296.0352, found 296.0345.

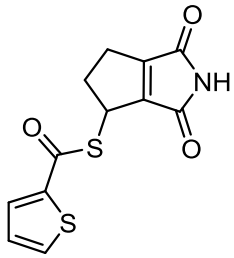
Compound **39** (35 mg) obtained as sticky liquid.



^{1}H NMR (200 MHz, $CDCl_3$) δ 8.45 (br s, 1H), 7.98-7.87 (m, 4H), 7.50-7.47 (m, 2H), 7.46-7.41 (dd, *J* = 7.5, 1.7 Hz, 4H), 4.63-4.61 (m, 1H), 3.53 (s, 1H), 2.51-2.14 (m, 4H**)**;

^{13}C NMR (100 MHz, $CDCl_3$) δ 192.1, 190.0, 177.4, 174.9, 136.4, 135.3, 134.4, 133.8, 128.9, 128.7, 127.6, 127.5, 60.7, 58.6, 46.6, 34.6, 31.6.

_S_-(1,3-Dioxo-1,2,3,4,5,6-hexahydrocyclopenta[_c_]pyrrol-4-yl)thiophene-2-carbothioate(42)

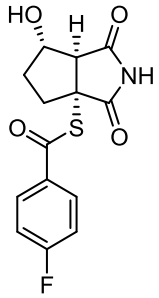


Following general procedure A, compound **9** has been treated with freshly prepared thiophene-2-carbothioic *S*-acid to get compounds **42** (38 mg) as white solid and traces of dimerised product.
Melting point 138-139 °C;
IRυ_{max} (film) 2921, 2853, 1709, 1647, 1461 cm^{-1};
^{1}H NMR (200 MHz, $CDCl_3$) δ 7.79 (d, *J* = 3.9 Hz, 1H), 7.69-7.65 (m, 1H), 7.16-7.11 (m, 2H), 5.03-4.96 (m, 1H), 3.30-3.16 (m, 1H), 2.85-2.79 (m, 2H), 2.76-2.51 (m, 1H);
^{13}C NMR (100 MHz, $CDCl_3$): δ 182.1, 165.5, 164.8, 156.6, 151.1, 141.0, 133.5, 131.8, 128.1, 41.7, 38.3, 25.8;
HRMS (ESI): *m/z* calculated for $C_{12}H_9NO_3S_2$ $[M+Na]^+$ 301.9916, found 301.9910.

_S_-((3a_R_,6_S_,6a_S_)-6-Hydroxy-1,3-dioxohexahydrocyclopenta[c]pyrrol-3a(1_H_)-yl) 4-fluorobenzothioate ((+)-28b) and _S_-((3aS,6S,6aR)-6-hydroxy-1,3-dioxohexahydrocyclopenta[c]pyrrol-3a(1_H_)-yl) 4-fluoro benzothioate ((-)-28a)

Following general procedure A, compound (–)-**11** has been treated with freshly prepared 4-fluorobenzothioic *S*-acid to get compounds (+)-**28b** (58 mg) and (–)-**28a (**13 mg**)** as white solids in 6:1 diastereomeric ratio with 51% overall yield, which were carefully separated using silica gel column chromatography.

Compound (+)-**28b**



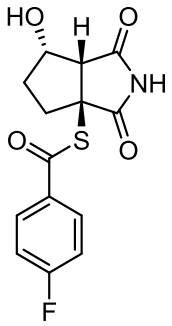
Specific rotation $[\alpha]_D^{30.2}$= +111.4 (*c* = 0.93, MeOH);

^{1}H NMR (400 MHz, CD_3OD) δ 7.98 (dd, *J* = 5.2, 8.9 Hz, 2H), 7.24 (t, *J* = 8.5 Hz, 2H), 4.56 (d, J = 3.1 Hz, 1H), 3.27 (s, 1H), 2.49 - 2.23 (m, 2H), 1.94 (br. s., 1H);

^{13}C NMR (101MHz, CD_3OD) δ 191.1, 179.5, 176.7, 167.6, 165.1, 132.2, 132.2, 129.8, 129.7, 115.9, 115.6, 74.8, 63.7, 58.3, 33.2, 31.8;

HRMS (ESI): *m/z* calculated for $C_{14}H_{12}NO_4SF$ $[M+Na]^+$ 332.0363 found 332.0367.

Compound (–)-**28a:**



(-)-**28a**

Specific rotation $[\alpha]_D^{25.5}$= –32.3 (*c* = 0.85, MeOH);

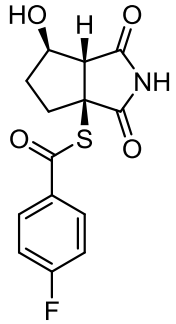
^{1}H NMR (400MHz, CD_3OD) δ 8.03 - 7.97 (m, 2 H), 7.28-7.24 (m, 2 H), 4.63-4.60 (m, 1H), 3.42-3.36 (m, 1H), 2.43-2.40 (m, 1H), 2.22 - 1.94 (m, 2H), 1.87-1.82 (m, 1H);

HRMS (ESI): *m/z* calculated for $C_{14}H_{12}NO_4SF$ $[M+Na]^+$ 332.0363 found 332.0365.

_S_-((3a_S_,6_R_,6a_R_)-6-Hydroxy-1,3-dioxohexahydrocyclopenta[c]pyrrol-3a(1_H_)-yl) 4-fluorobenzothioate ((-)-28b) & S-((3a_R_,6_R_,6a_S_)-6-hydroxy-1,3-dioxohexahydrocyclopenta[c]pyrrol-3a(1_H_)-yl) 4-fluorobenzothioate

Following general procedure A, compound (+)-**11** has been treated with freshly prepared 4-fluorobenzothioic *S*-acid to get compounds (–)-**28b** (63 mg) and (+)-**28a** (14 mg) as white solids in approx 6:1 diastereomeric ratio which were carefully separated using silica gel column chromatography.

Compound ((–)-**28b**):

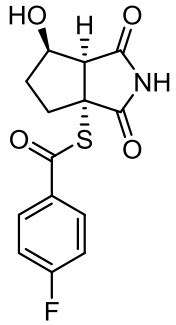


Specific rotation $[\alpha]_D^{30.2}$= – 138.4 (*c* = 0.42, MeOH);

^{1}H NMR (400 MHz, CD_3OD) δ 7.97 (dd, *J* = 5.2, 8.9 Hz, 2 H), 7.24 (t, *J* = 8.9 Hz, 2H), 4.62 - 4.49 (m, 1 H), 3.27 (s, 1 H), 2.39 - 2.30 (m, 2 H), 1.97 – 1.92 (m, 1 H); 1.78 - 1.72 (m, 1H);

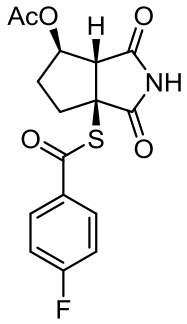
^{13}C NMR (100 MHz, CD_3OD) δ 192.4, 168.9, 166.4, 133.5, 133.4, 131.1, 131.0, 117.2, 116.9, 76.1, 65.0, 59.7, 34.5, 33.1.

Compound ((+)-**28a**):



^{1}H NMR (400MHz, CD_3OD) δ 8.03 - 7.97 (m, 2 H), 7.28 - 7.24 (m, 2 H), 4.63 - 4.60 (m, 1H), 3.42 - 3.36 (m, 1H), 2.43 - 2.40 (m, 1H), 2.22 - 1.94 (m, 2 H), 1.87 - 1.82 (m, 1H).

(3a*R*,4*R*,6a*S*)-6a-((4-Fluorobenzoyl)thio)-1,3-dioxooctahydrocyclopenta[c]pyrrol-4-yl acetate ((-)-25b)



Following general procedure C, compound (–)-**25b** (45 mg) was obtained as white solid in 97% yield from (–)-**28b**.

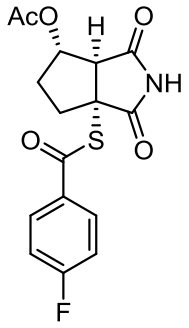
Specific rotation $[\alpha]_D^{25}$= -141.7 (*c* = 0.42, $CHCl_3$);

^{1}H NMR (400MHz, $CDCl_3$) δ = 8.81 (br. s., 1H), 7.92 (dd, *J* = 5.5, 7.9 Hz, 2 H), 7.13 (t, *J* = 8.2 Hz, 2H), 5.52 (d, *J* = 3.1 Hz, 1H), 3.51 (s, 1H), 2.49- 2.44 (m, 1H), 2.32 - 2.24 (m, 1 H), 2.19 - 2.10 (m, 1H), 2.06 (s, 3H), 1.92 - 1.86 (m, 2H);

^{13}C NMR (100MHz, $CDCl_3$) δ 190.6, 177.4, 173.8, 169.9, 167.7, 165.2, 131.7, 130.2, 130.1, 116.2, 116.0, 61.0, 58.3, 33.6, 30.4, 21.1.

RT_{HPLC} = 5.72 min, purity >99%, 15:85 H_2O/ MeOH.

(3a*S*,4*S*,6a*R*)-6a-((4-Fluorobenzoyl)thio)-1,3-dioxooctahydrocyclopenta[c]pyrrol-4-yl acetate ((+)-25b)



Following general procedure C, compound (+)-**25b** (46 mg) was obtained as white solid in 98% yield from (+)-**28b**.

Specific rotation $[\alpha]_D^{25}$= +124.8 (*c* = 0.42, $CHCl_3$);

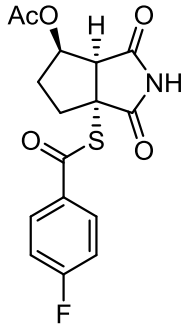
^{1}H NMR (400MHz, $CDCl_3$) δ 8.98 - 8.92 (m, 1H), 7.94 (dd, *J* = 5.5, 7.9 Hz, 2H), 7.15 (t, *J* = 8.5 Hz, 2H), 5.54 (d, *J* = 3.1 Hz, 1H), 3.53 (s, 1H), 2.48 (dd, *J* = 7.3, 13.4 Hz, 1H), 2.34 - 2.26 (m, 1H), 2.16 - 2.12 (m, 1H), 2.08 (s, 3H), 1.96 - 1.86 (m, 2H);

^{13}C NMR (100MHz, $CDCl_3$) δ 190.6, 177.5, 173.9, 169.9, 167.7, 165.2, 131.7, 130.2, 130.1, 116.2, 116.0, 77.3, 61.0, 58.3, 33.6, 30.4, 21.1.

HRMS (ESI): *m/z* calculated for $C_{16}H_{14}NO_5SF$ $[M+Na]^+$ 374.0469 found 374.0472.

RT_{HPLC} = 6.24 min, purity >99%, 15:85 H_2O/ MeOH.

(3a*S*,4*R*,6a*R*)-6a-((4-Fluorobenzoyl)thio)-1,3-dioxooctahydrocyclopenta[c]pyrrol-4-yl acetate ((+)-25a)



Following general procedure C, compound (+)-**25a** (10 mg) was obtained as white solid in 98% yield from (+)-**28a**.

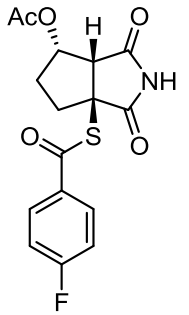
Specific rotation $[\alpha]_D^{25}$= +72.8 (*c* = 0.75, $CHCl_3$);

^{1}H NMR (500 MHz , $CDCl_3$) δ 8.07 (br. s., 1H), 7.93 - 7.91 (m, 2H), 7.16 (t, *J* = 8.2 Hz, 2H), 5.47 - 5.43 (m, 1H), 3.75 (d, *J* = 8.3 Hz, 1H), 2.53 - 2.50 (m, 1H), 2.27 -2.23 (m, 1H), 2.13 (s, 3H), 2.09 - 1.93 (m, 2H);

^{13}C NMR (125 MHz, $CDCl_3$) δ 190.0, 176.8, 171.8, 170.3, 167.5, 165.2, 131.7, 130.2, 130.1, 116.3, 116.1, 73.6, 58.7, 55.5, 31.8, 30.0, 29.7, 20.8.

RT_{HPLC} = 8.30 min, purity >98%, 40:60 H_2O/ MeOH.

(3aR,4S,6aS)-6a-((4-Fluorobenzoyl)thio)-1,3-dioxooctahydrocyclopenta[c]pyrrol-4-yl acetate ((–)-25a)



Following general procedure C, compound (–)-**25a** (13 mg) was obtained as white solid in 90% yield from (–)-**28a**.

Specific rotation $[\alpha]_D^{25.3}$ = –79.6 (*c* = 0.42, $CHCl_3$);

^{1}H NMR (400 MHz , $CDCl_3$) δ 8.43 (br. s., 1H), 7.93-7.87 (m, 2H), 7.15-7.11 (m, 2H), 5.44-5.39 (m, 1H), 3.73-3.71 (m, 1H), 2.52-2.49 (m, 1H), 2.27-2.23 (m, 1H), 2.13 (s, 3H), 2.09-1.93 (m, 2H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 190.0, 177.1, 172.1, 170.3, 167.5, 165.2, 131.7, 130.2, 130.1, 116.3, 116.1, 73.6, 58.7, 55.5, 31.8, 30.0, 20.8;

HRMS (ESI): *m/z* calculated for $C_{16}H_{14}NO_5SF$ $[M+Na]^+$ 374.0469 found 374.0476.

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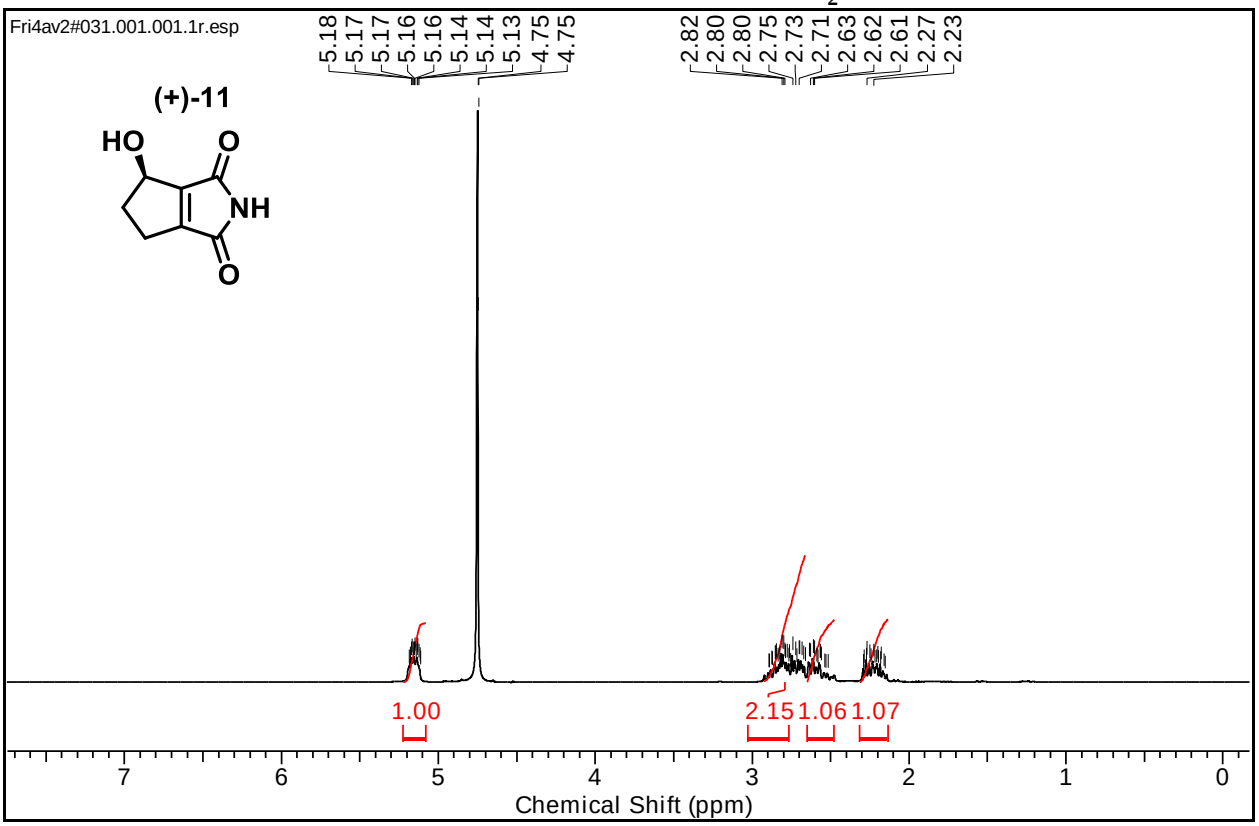
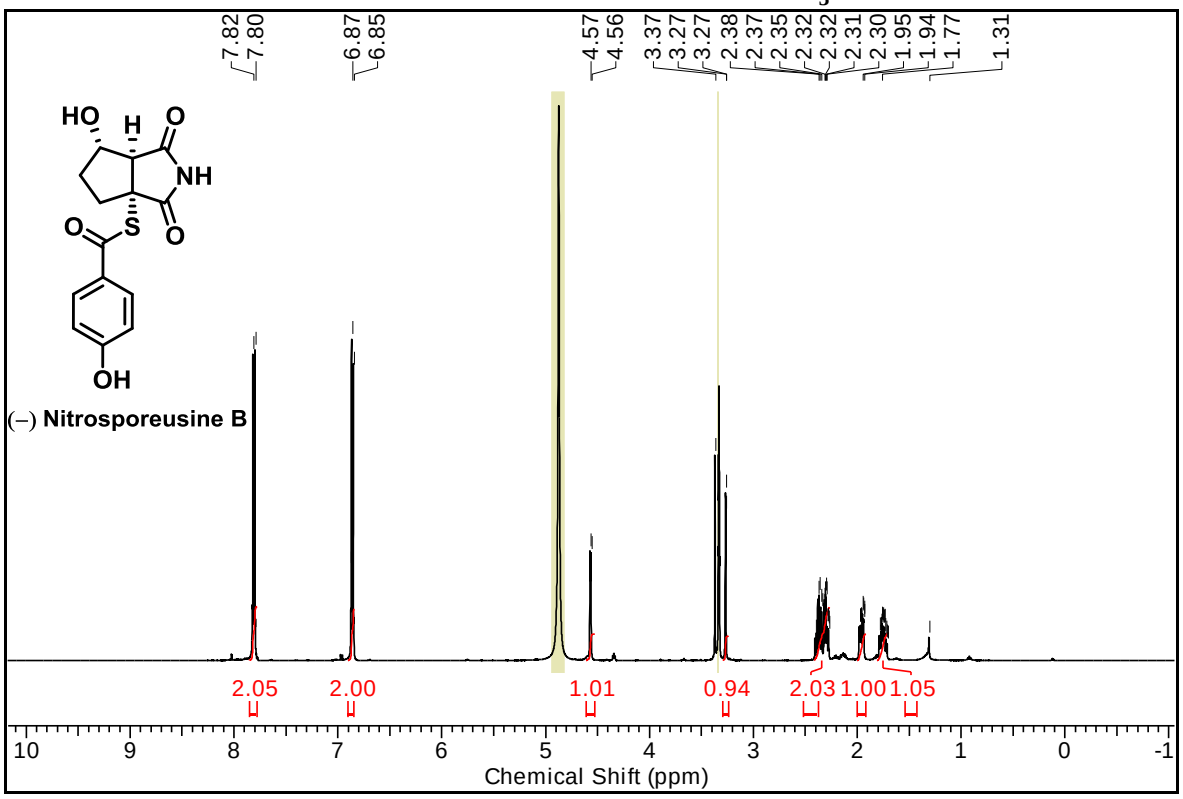
2.7 Copies of ^{1}H and ^{13}C NMR spectra

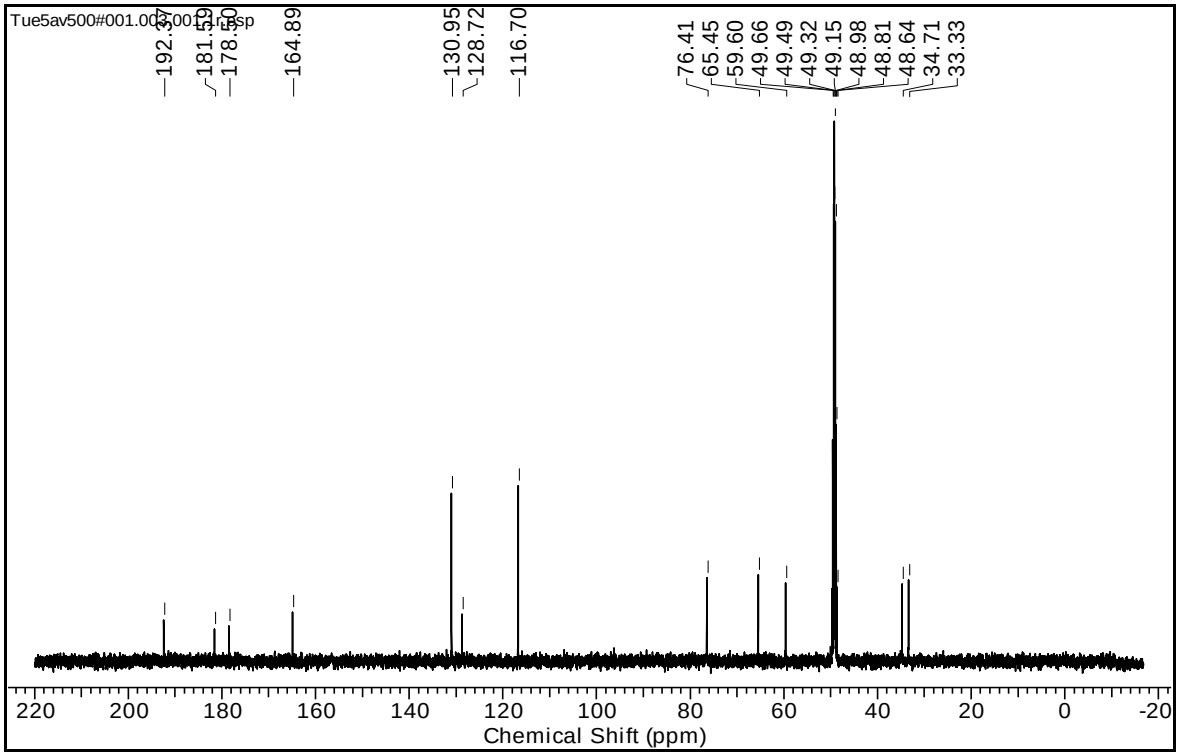
^{1}H NMR Spectrum of compound (+)-11 in D_2O at 200 MHz



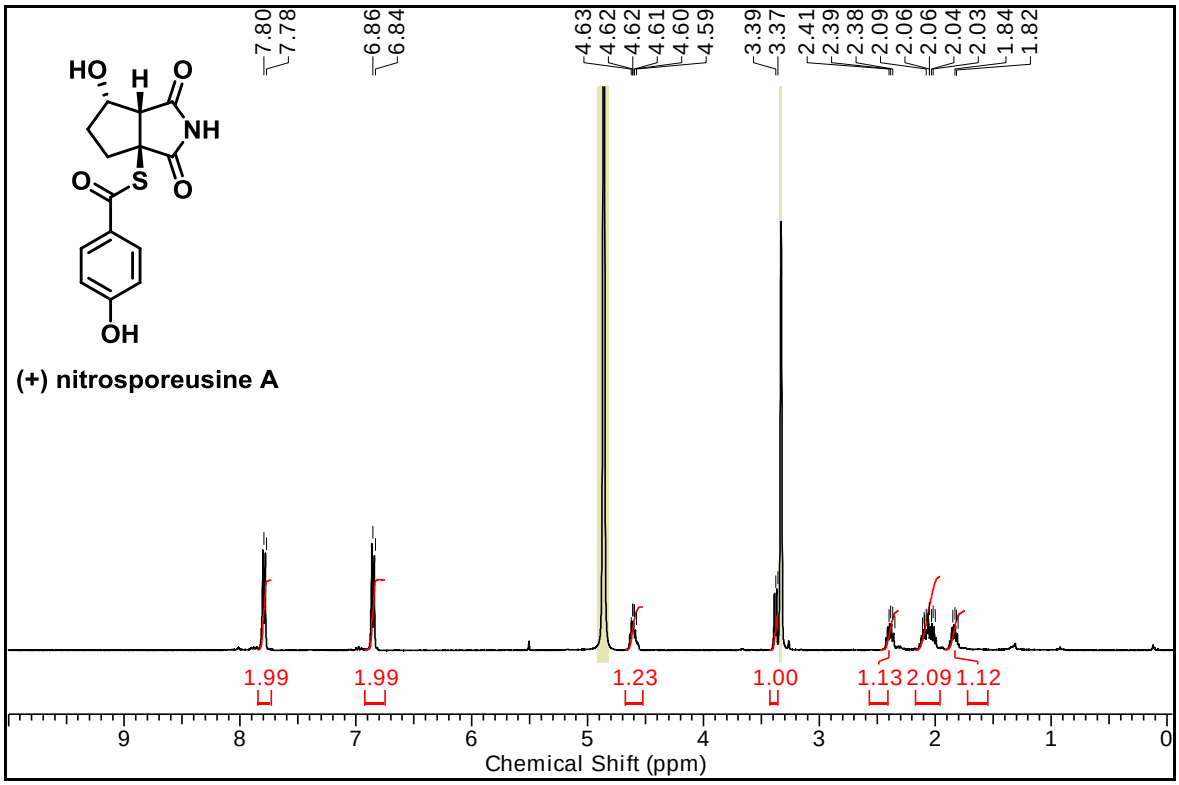
^{1}H NMR Spectrum of (−)-nitrosporeusine B in CD_3OD at 500 MHz



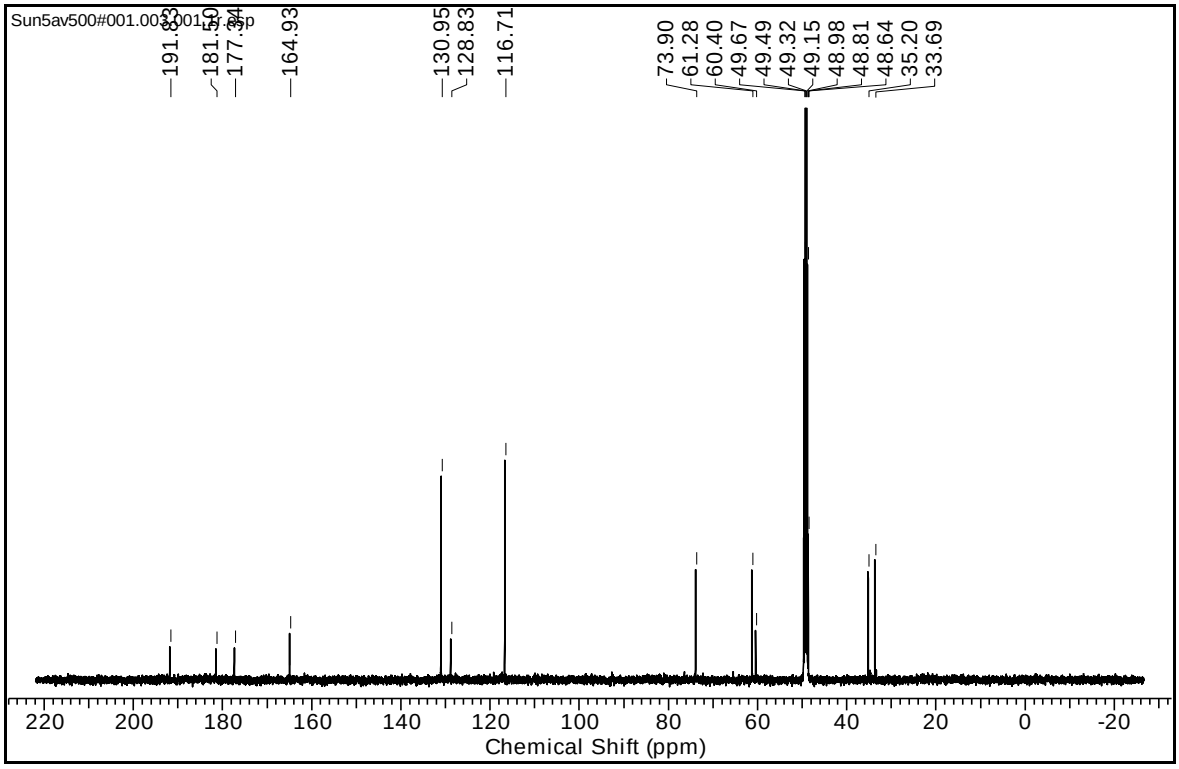
^{13}C NMR Spectrum of (−)-nitrosporeusine B in CD_3OD at 125 MHz



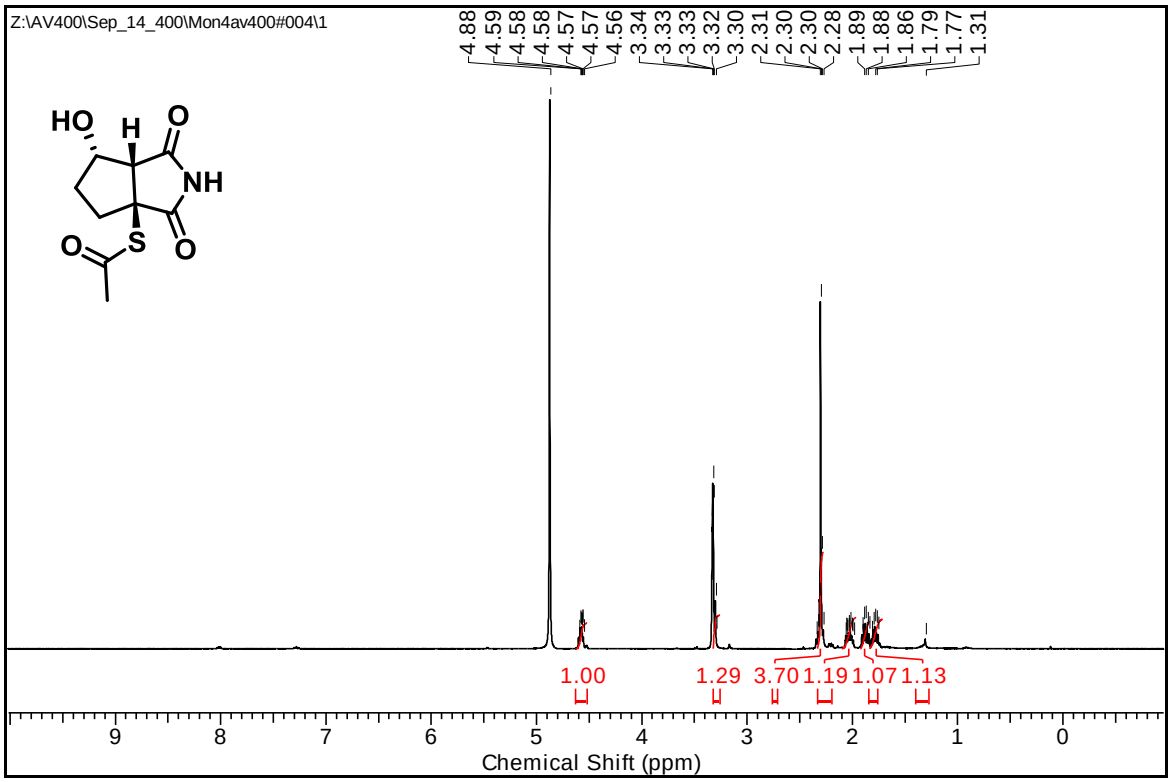
^{1}H NMR Spectrum of (+)-nitrosporeusine A in CD_3OD at 400 MHz



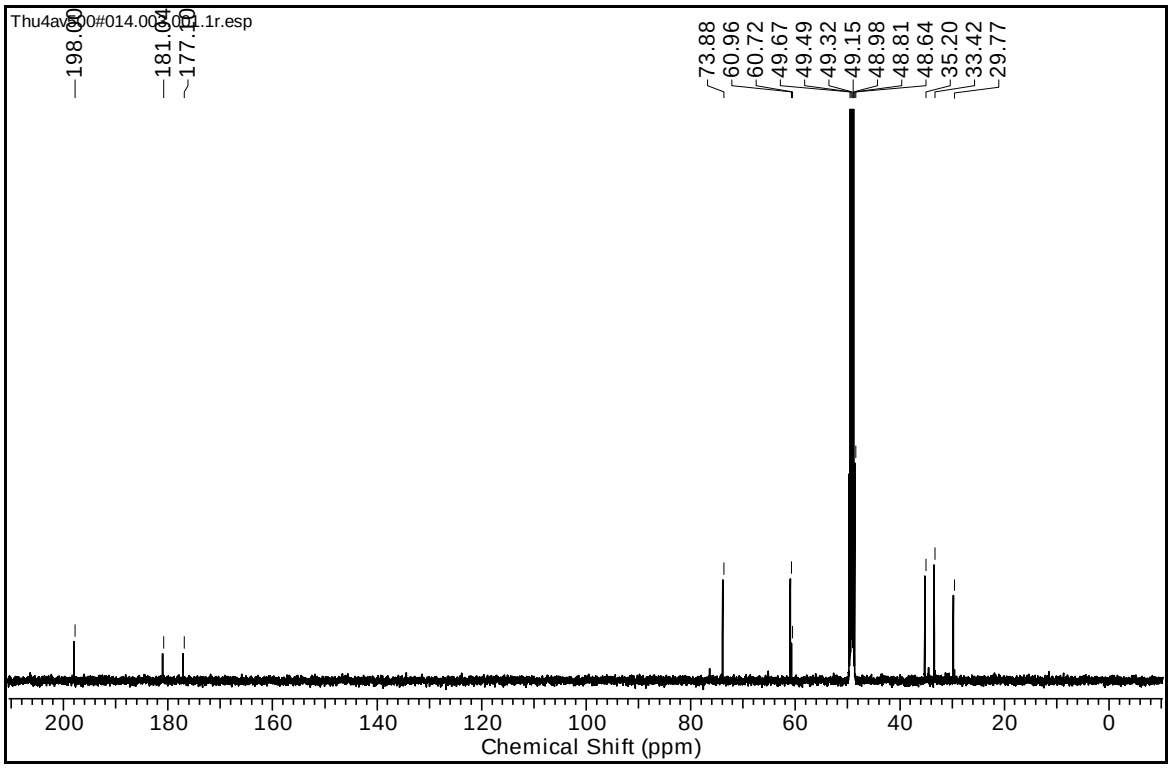
^{13}C NMR Spectrum of (+)-nitrosporeusine A in CD_3OD at 125 MHz



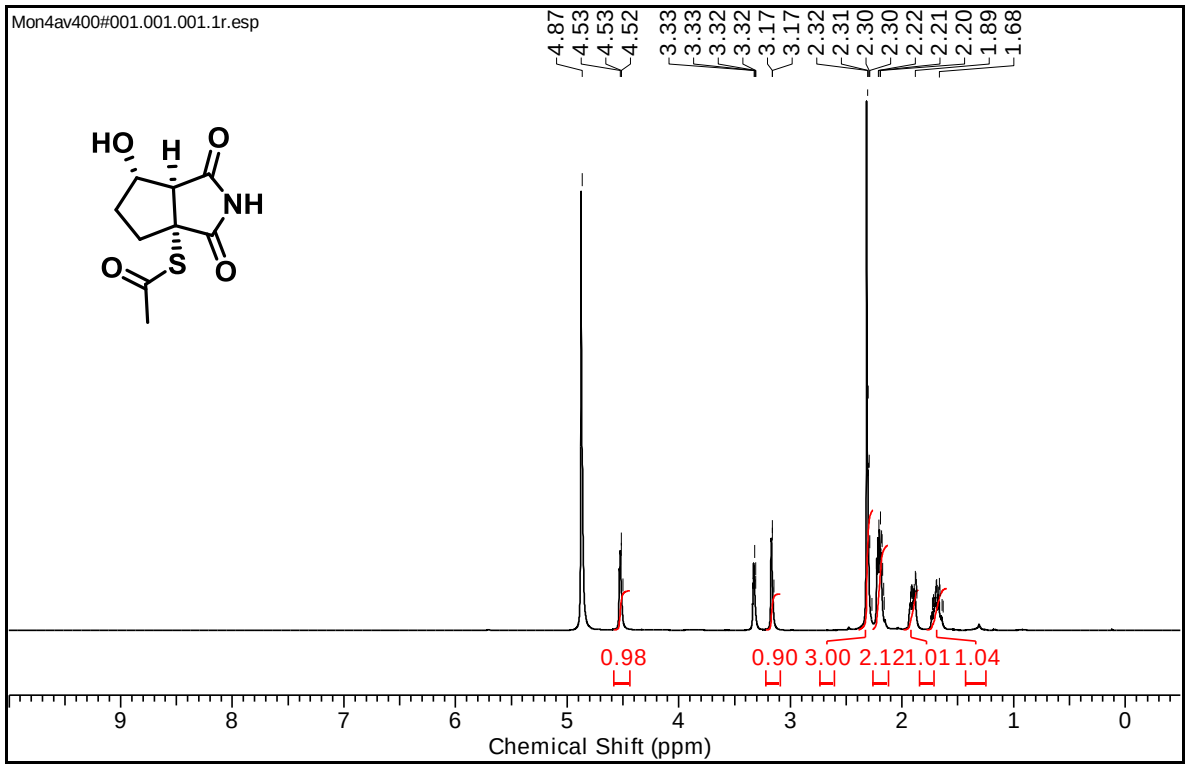
^{1}H NMR Spectrum of compound (±)-13 in CD_3OD at 400 MHz



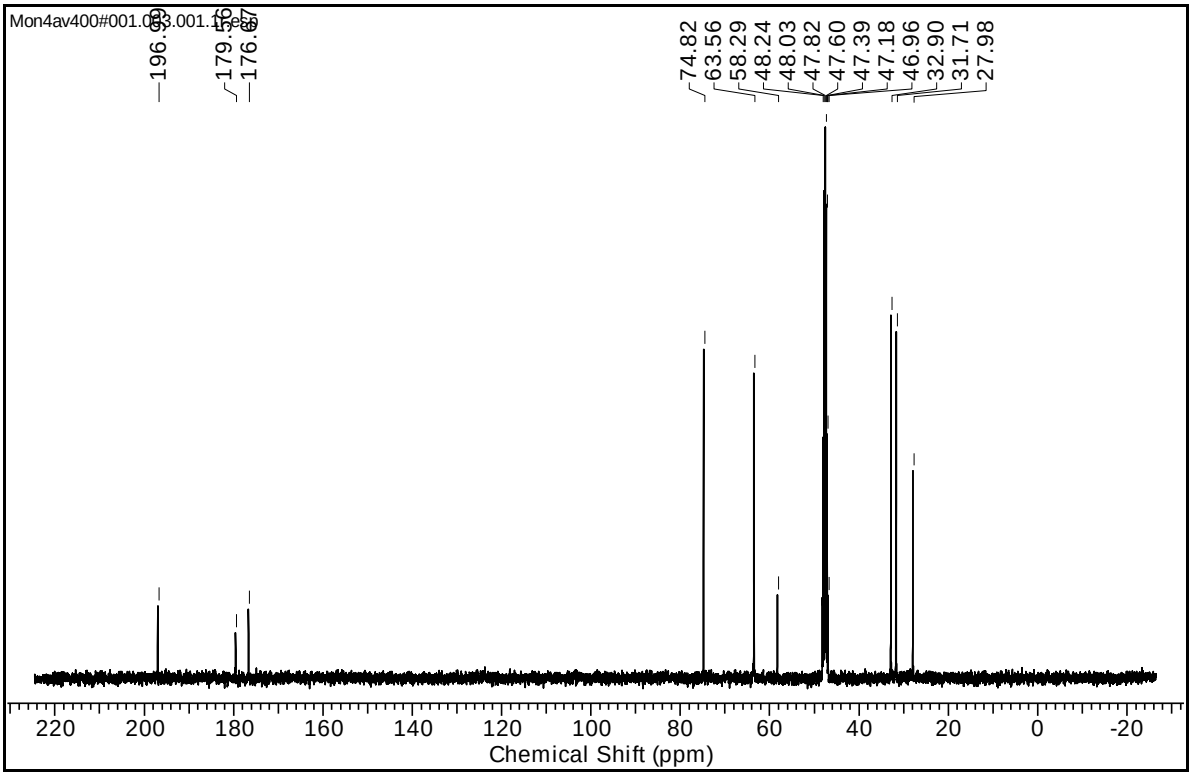
^{13}C NMR Spectrum of compound (±)-13 in CD_3OD at 125 MHz



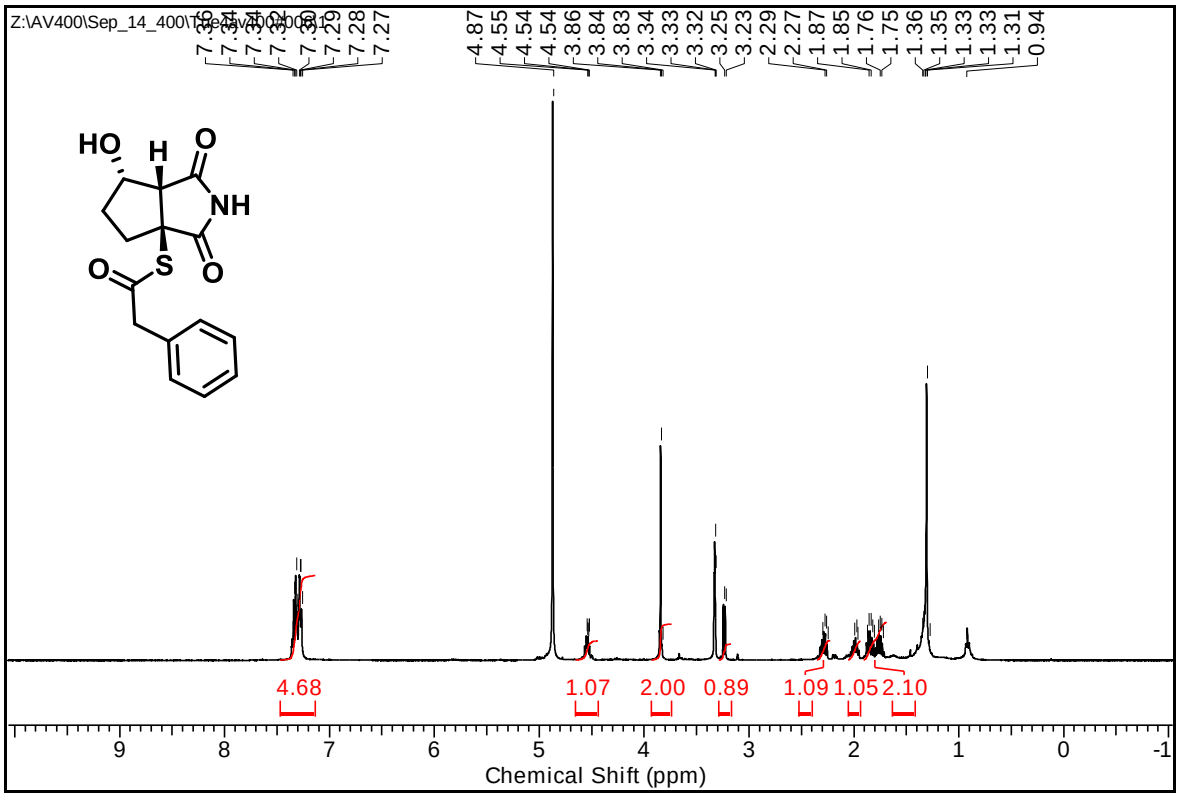
^{1}H NMR Spectrum of compound (±)-14 in CD_3OD at 400 MHz



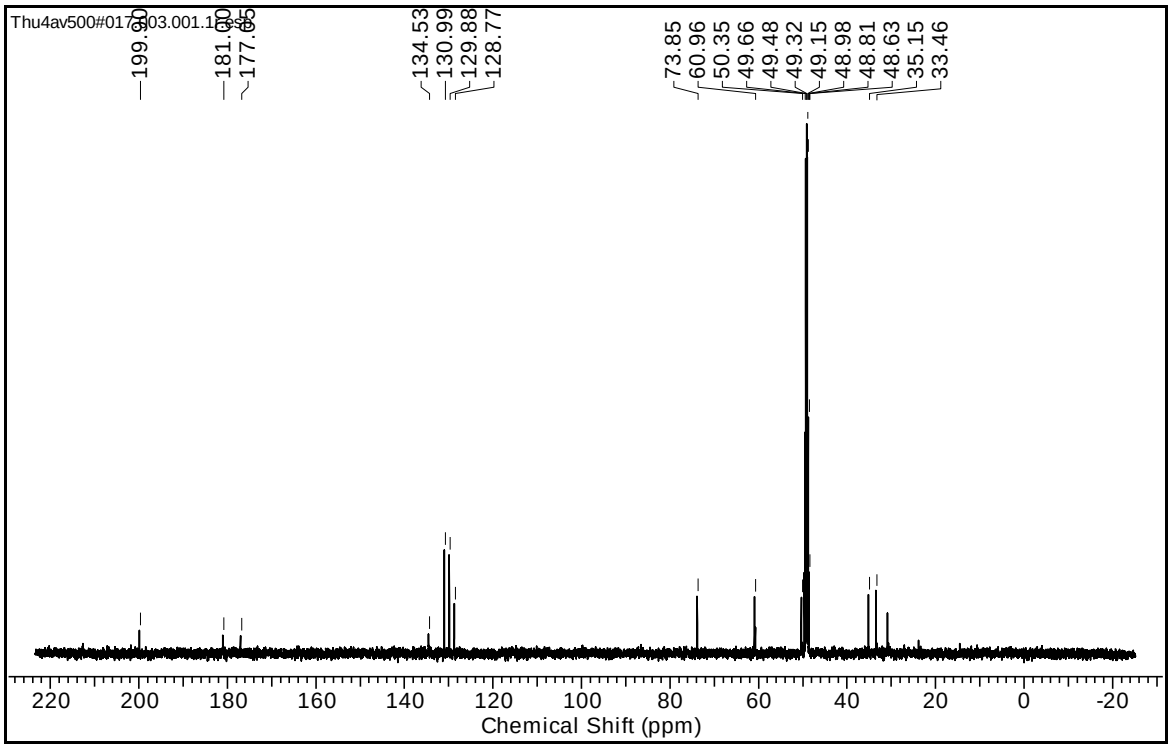
^{13}C NMR Spectrum of compound (±)-14 in CD_3OD at 100 MHz



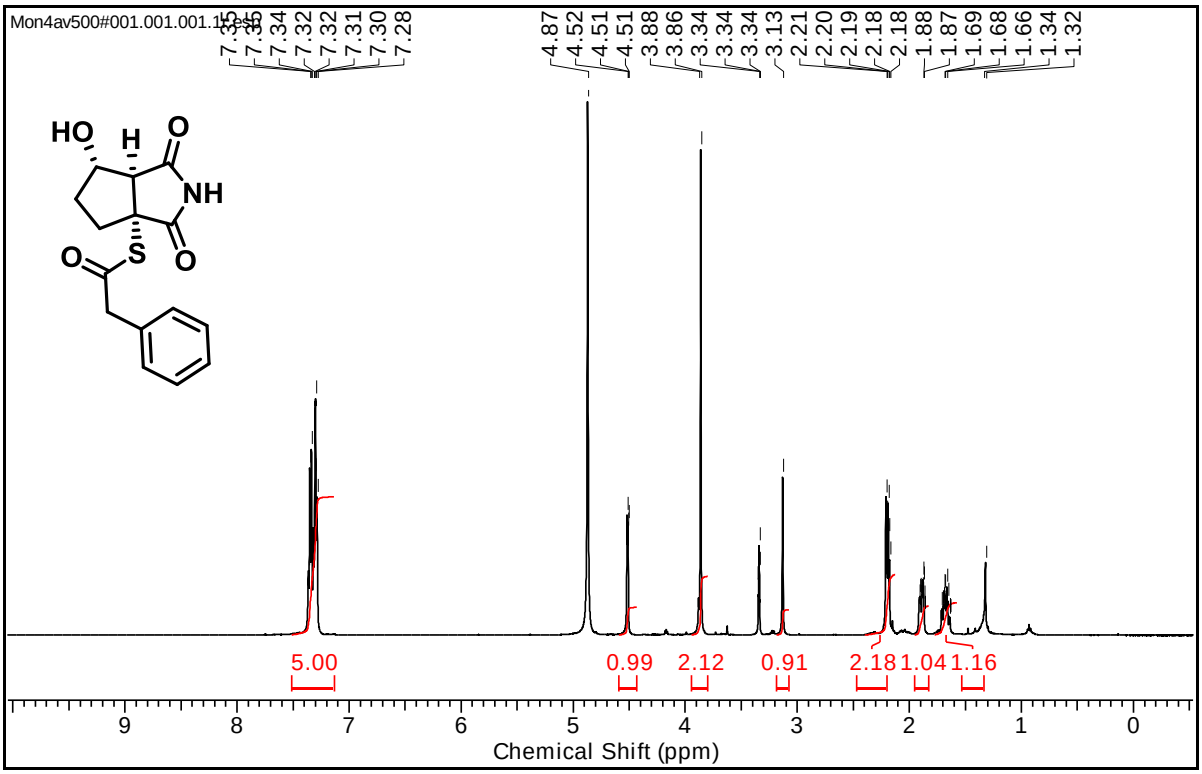
^{1}H NMR Spectrum of compound (±)15 in CD_3OD at 400 MHz



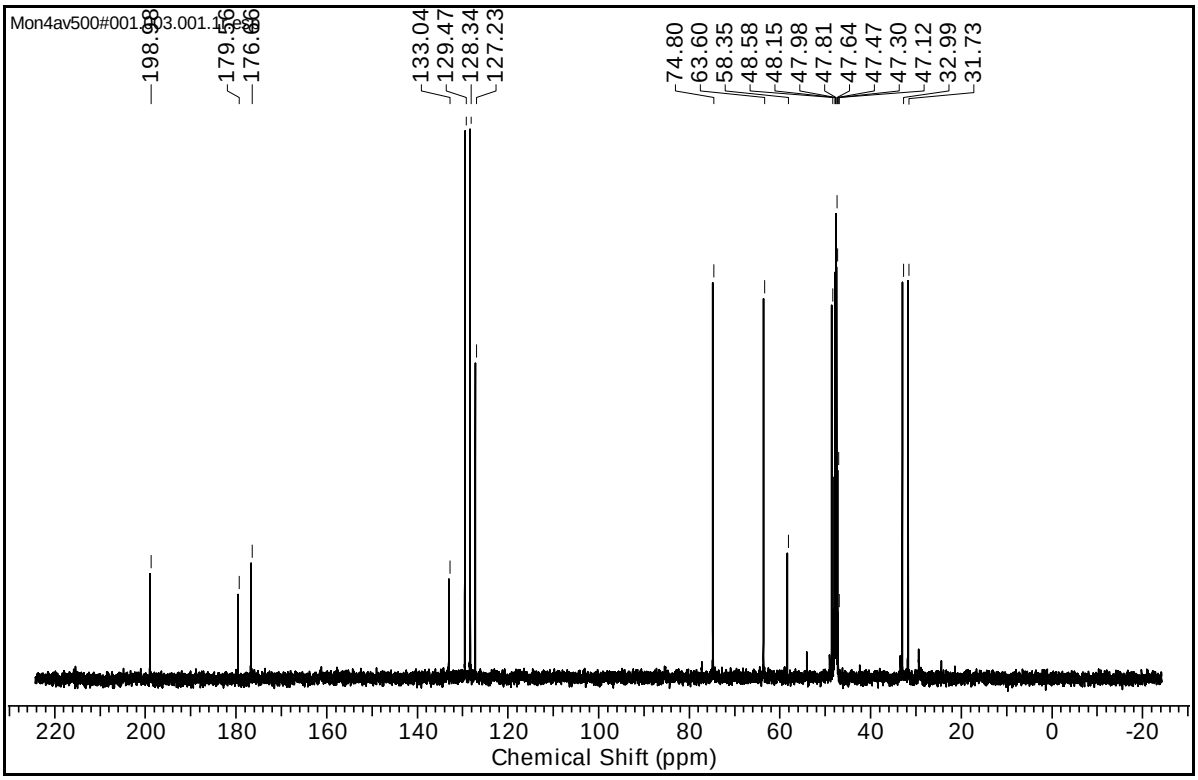
^{13}C NMR Spectrum of compound (±)-15 in CD_3OD at 125 MHz



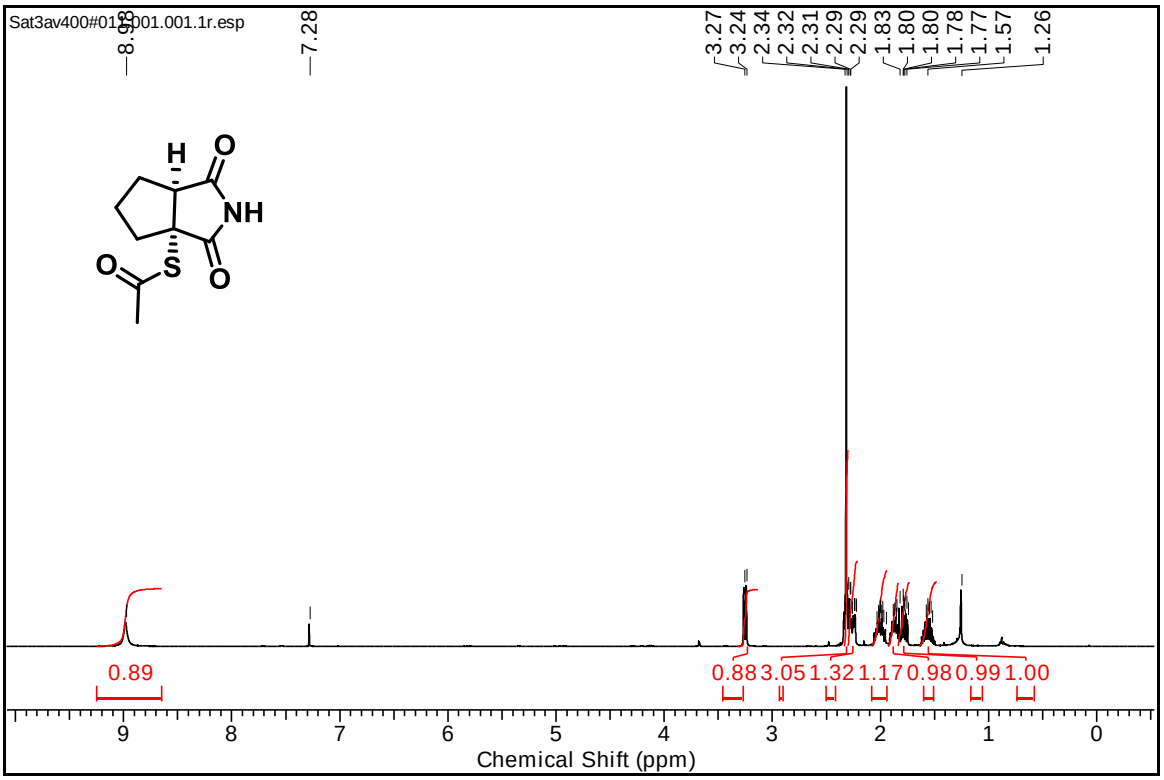
^{1}H NMR Spectrum of compound (±)-16 in CD_3OD at 500 MHz



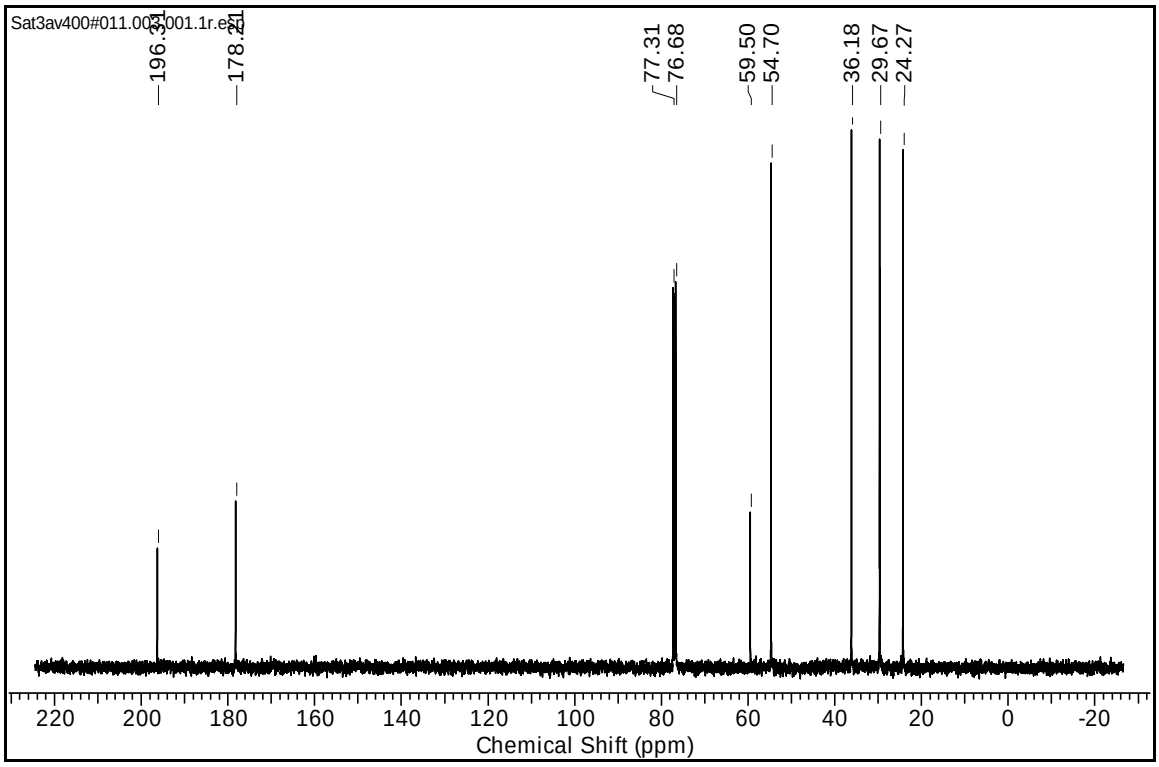
^{13}C NMR Spectrum of compound (±)-16 in CD_3OD at 125 MHz



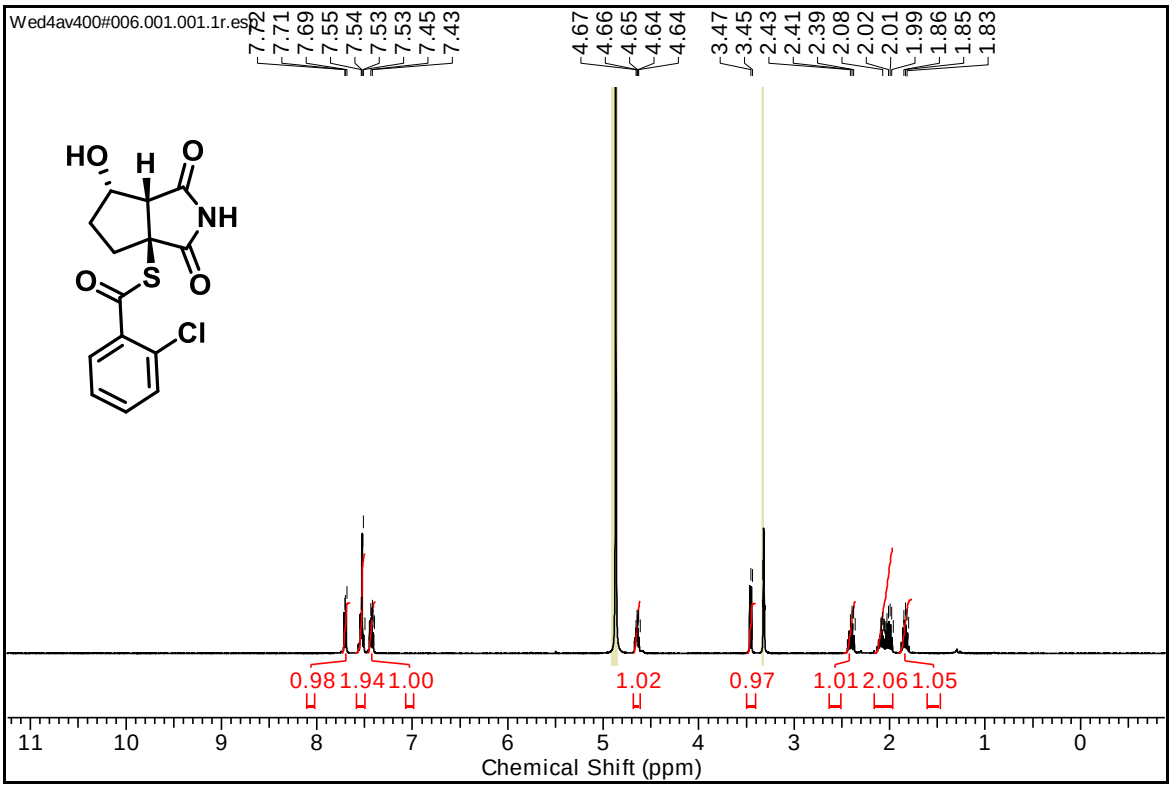
^{1}H NMR Spectrum of compound (±)-17 in $CDCl_3$ at 400 MHz



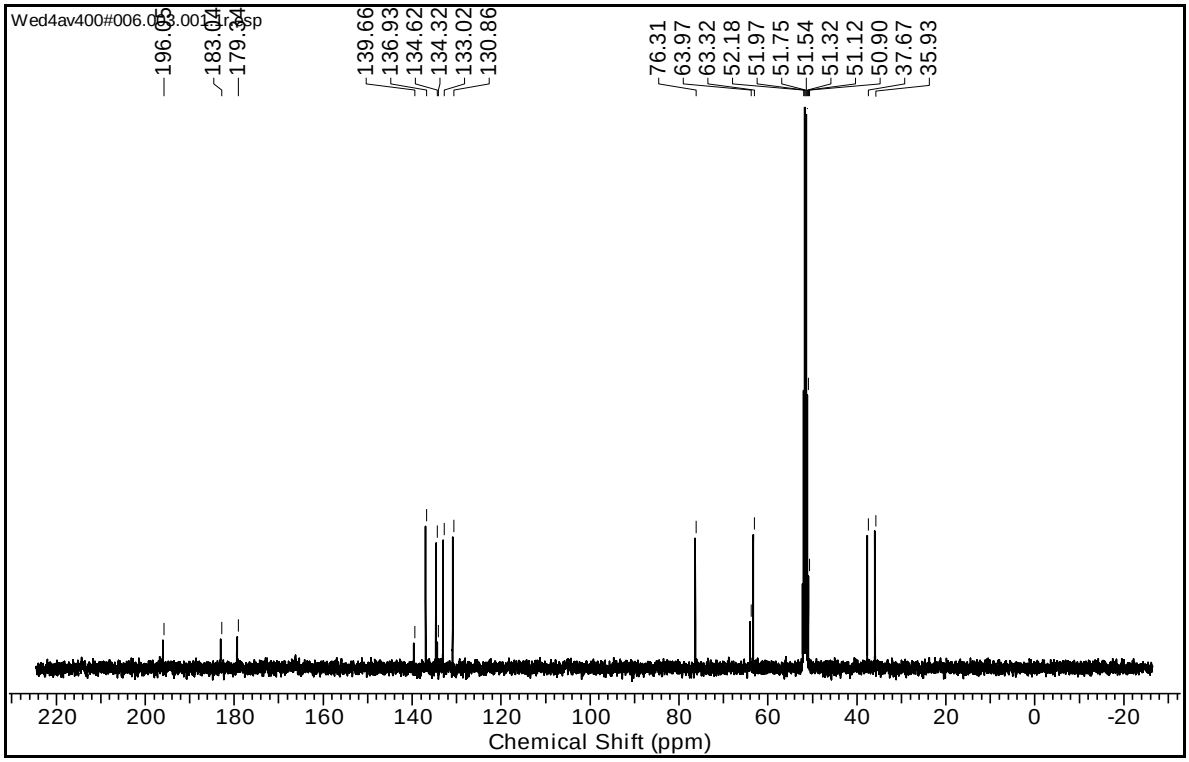
^{13}C NMR Spectrum of compound (±)-17 in $CDCl_3$ at 100 MHz



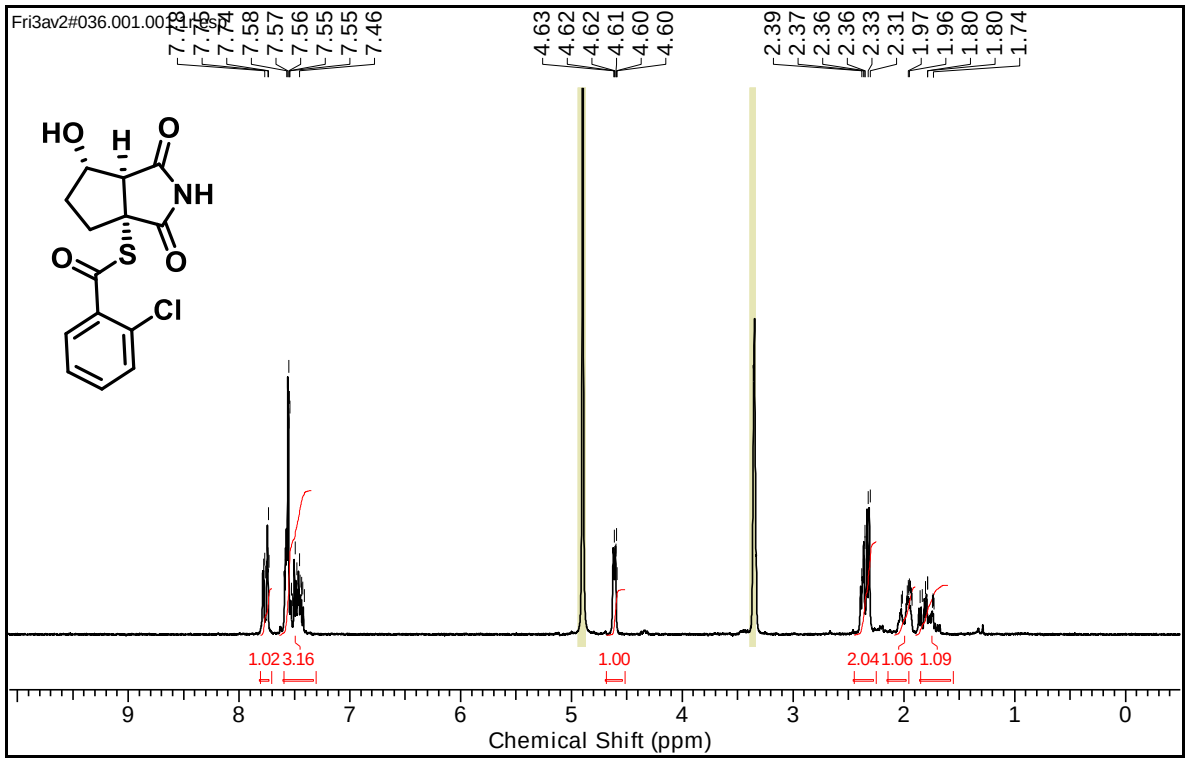
^{1}H NMR Spectrum of compound (±)-18 in CD_3OD at 400 MHz



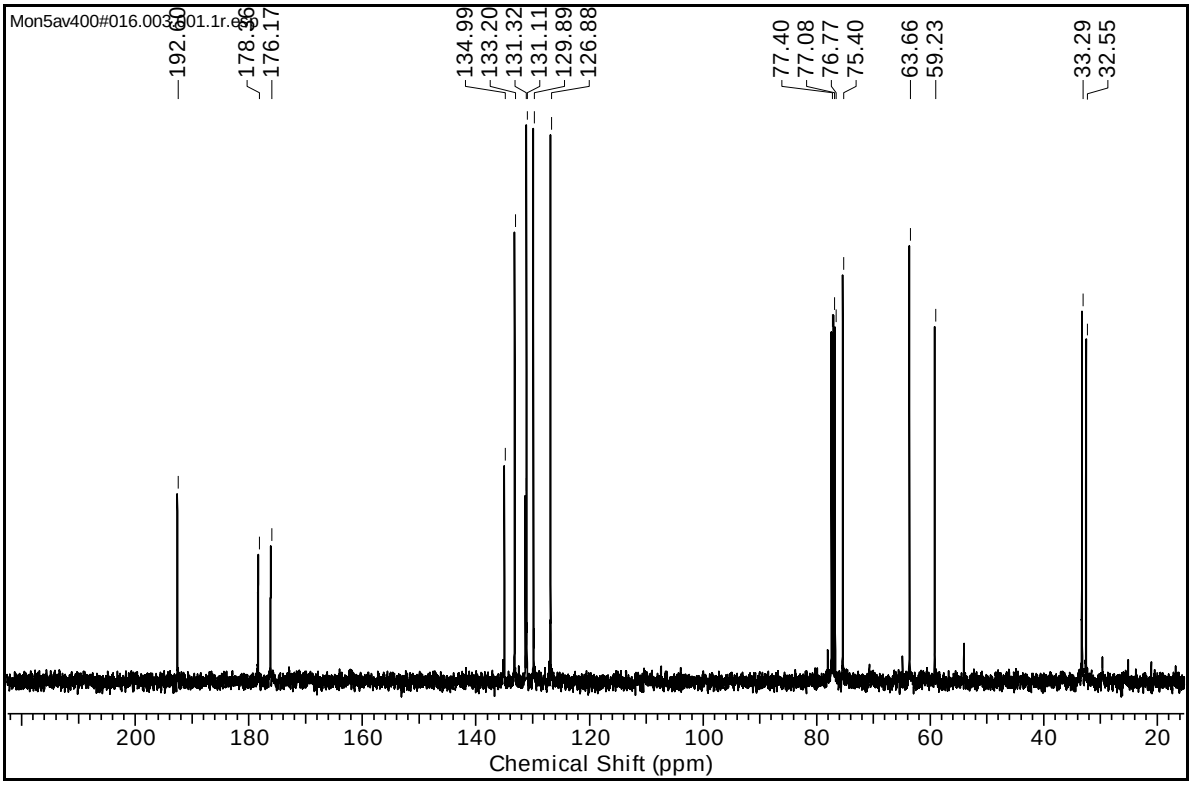
^{13}C NMR Spectrum of compound (±)-18 in CD_3OD at 100 MHz



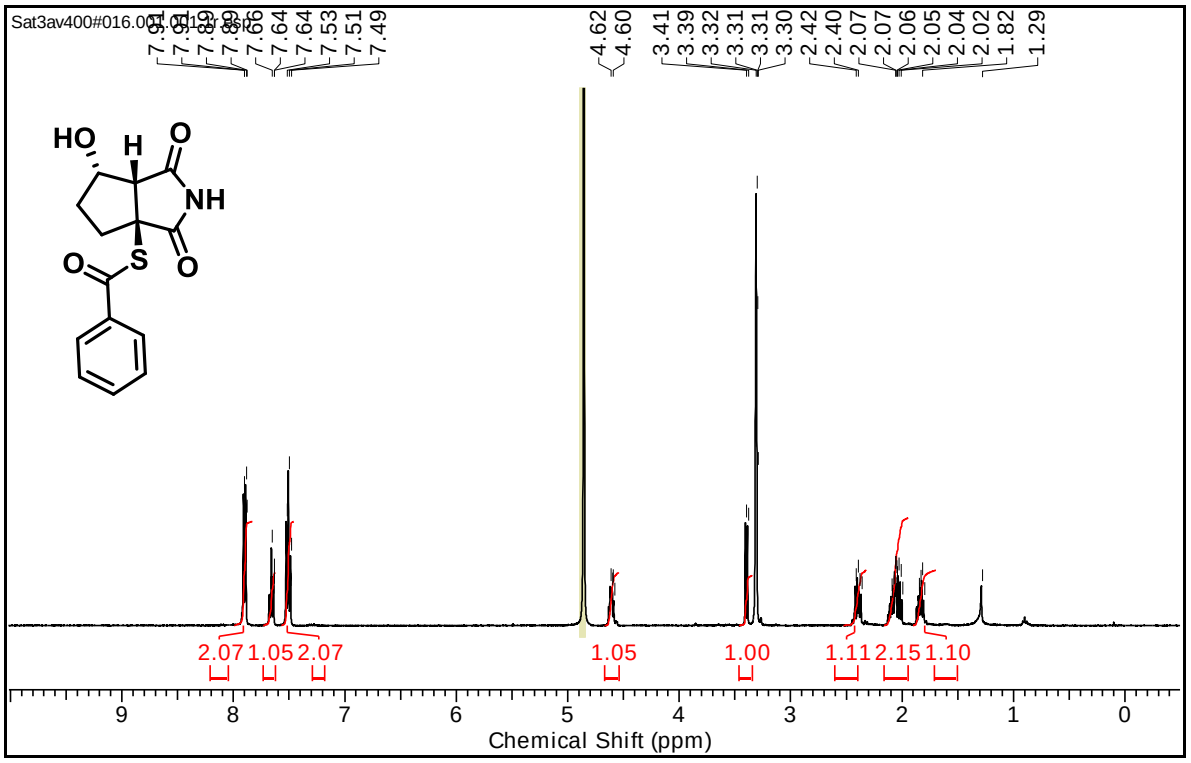
^{1}H NMR Spectrum of compound (±)-19 in CD_3OD at 200 MHz



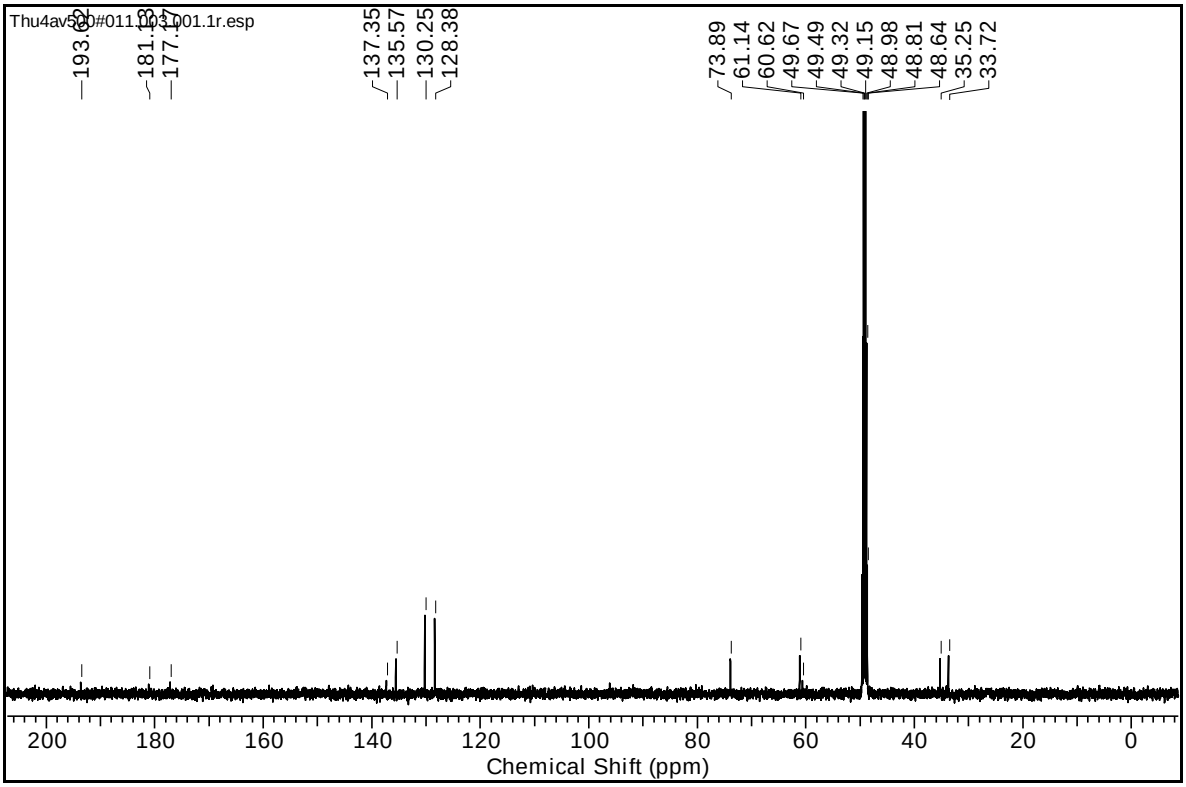
^{13}C NMR Spectrum of compound (±)-19 in CD_3OD at 100 MHz



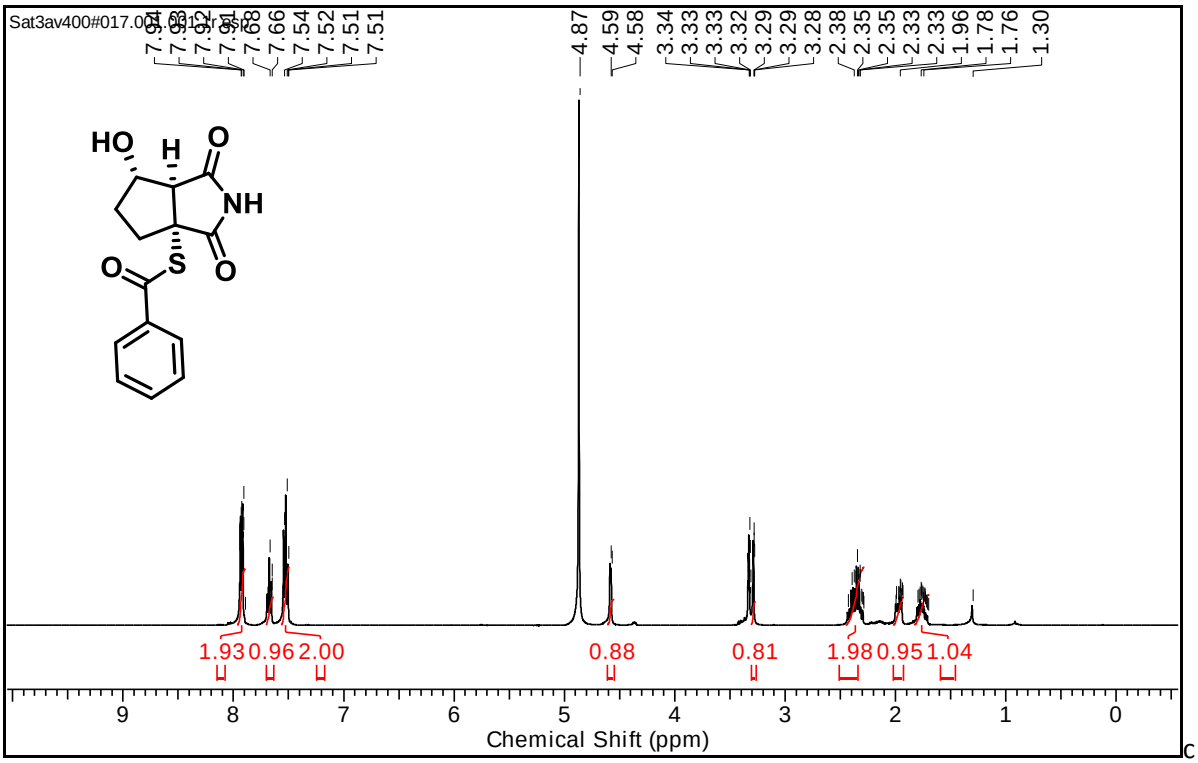
^{1}H NMR Spectrum of compound (±)-20 in CD_3OD at 400 MHz



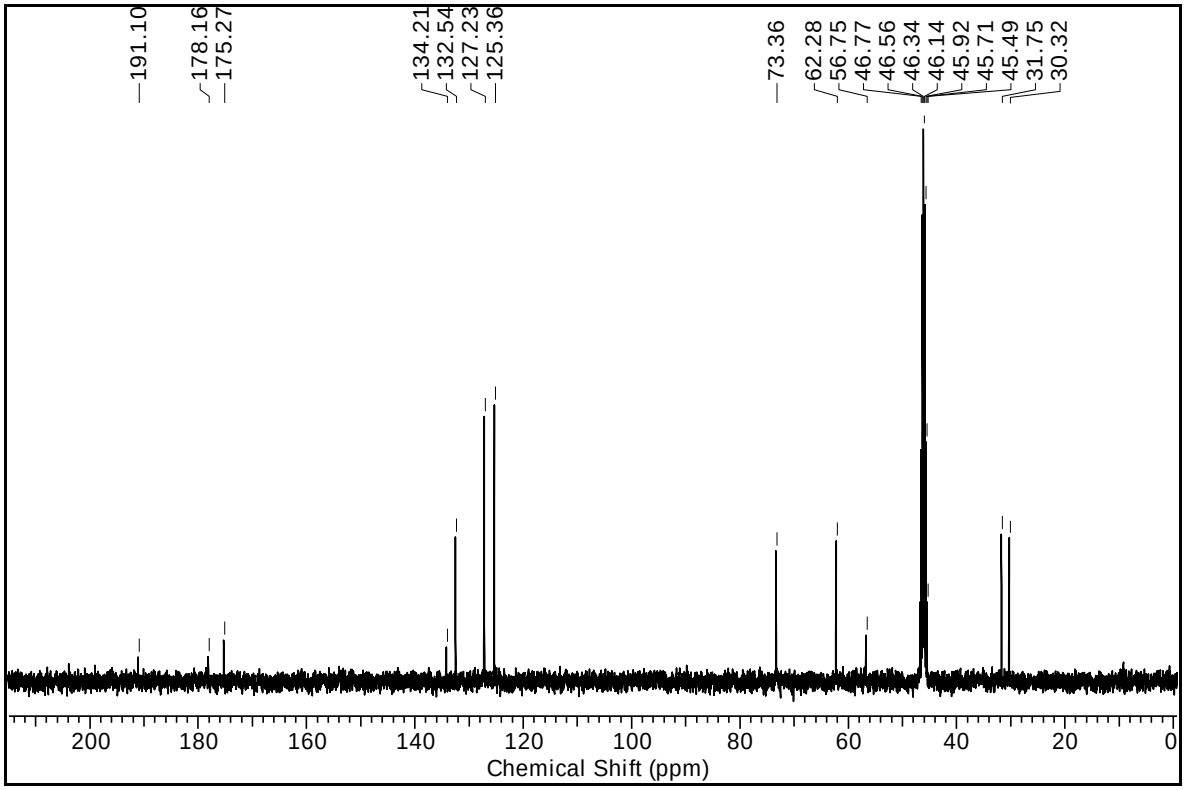
^{13}C NMR Spectrum of compound (±)-20 in CD_3OD at 125 MHz



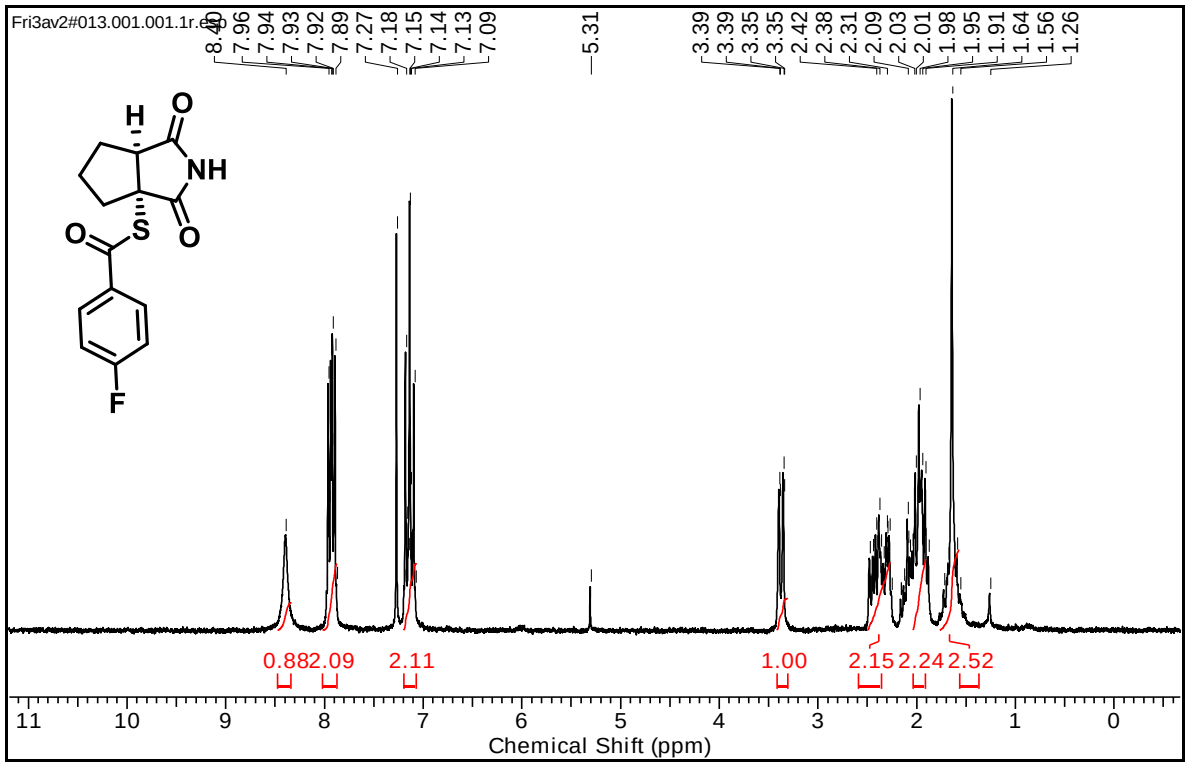
^{1}H NMR Spectrum of compound (±)-21 in CD_3OD at 400 MHz



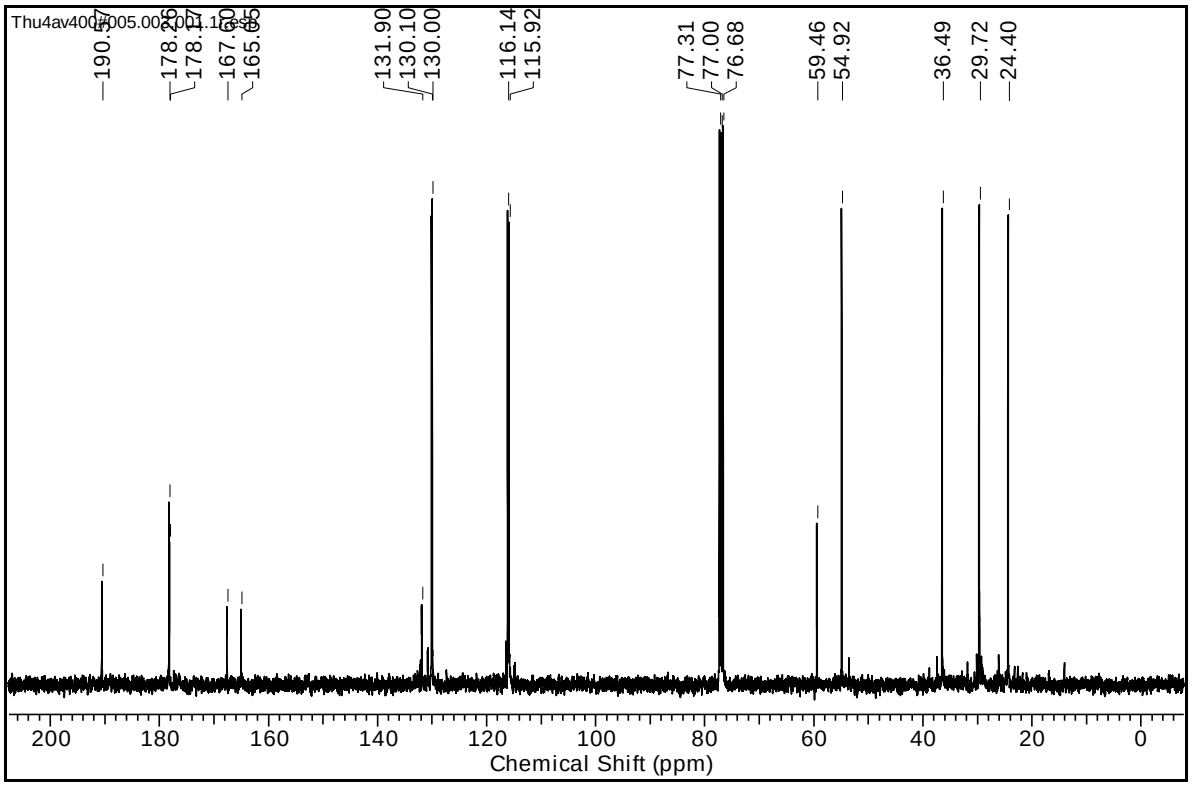
^{13}C NMR Spectrum of compound (±)-21 in CD_3OD at 100 MHz



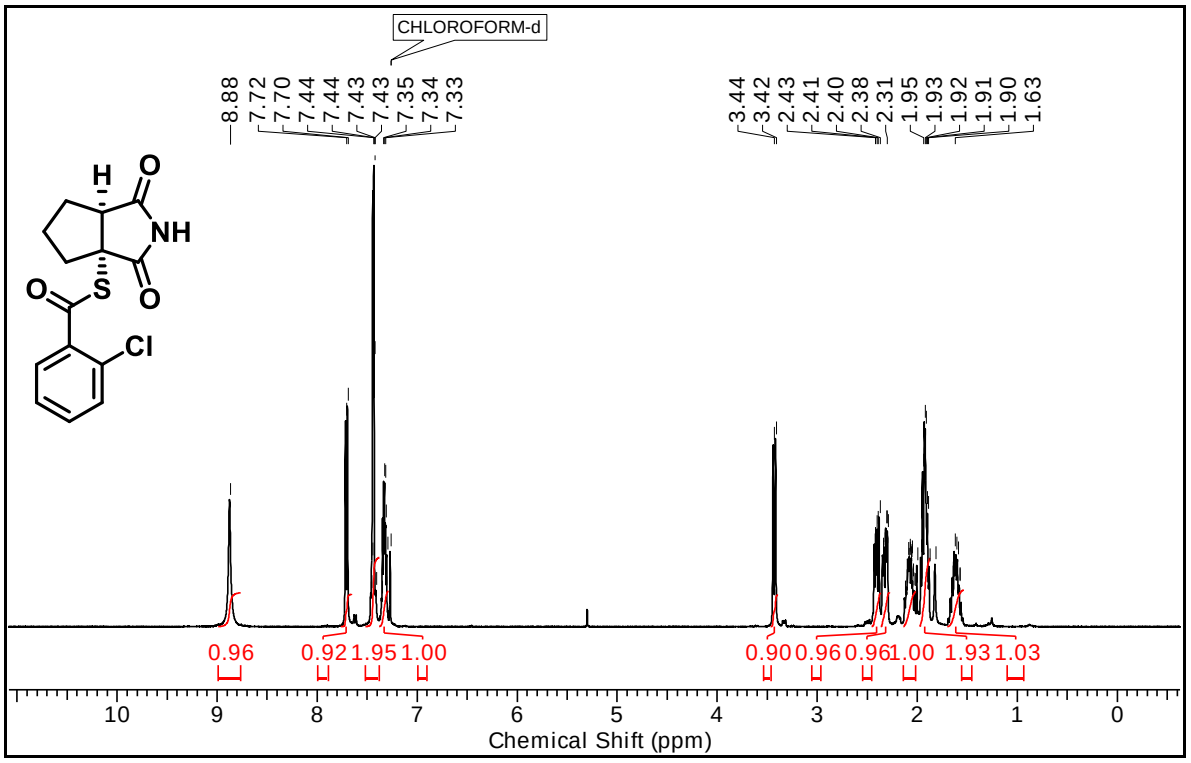
^{1}H NMR Spectrum of compound (±)-22 in $CDCl_3$ at 200 MHz



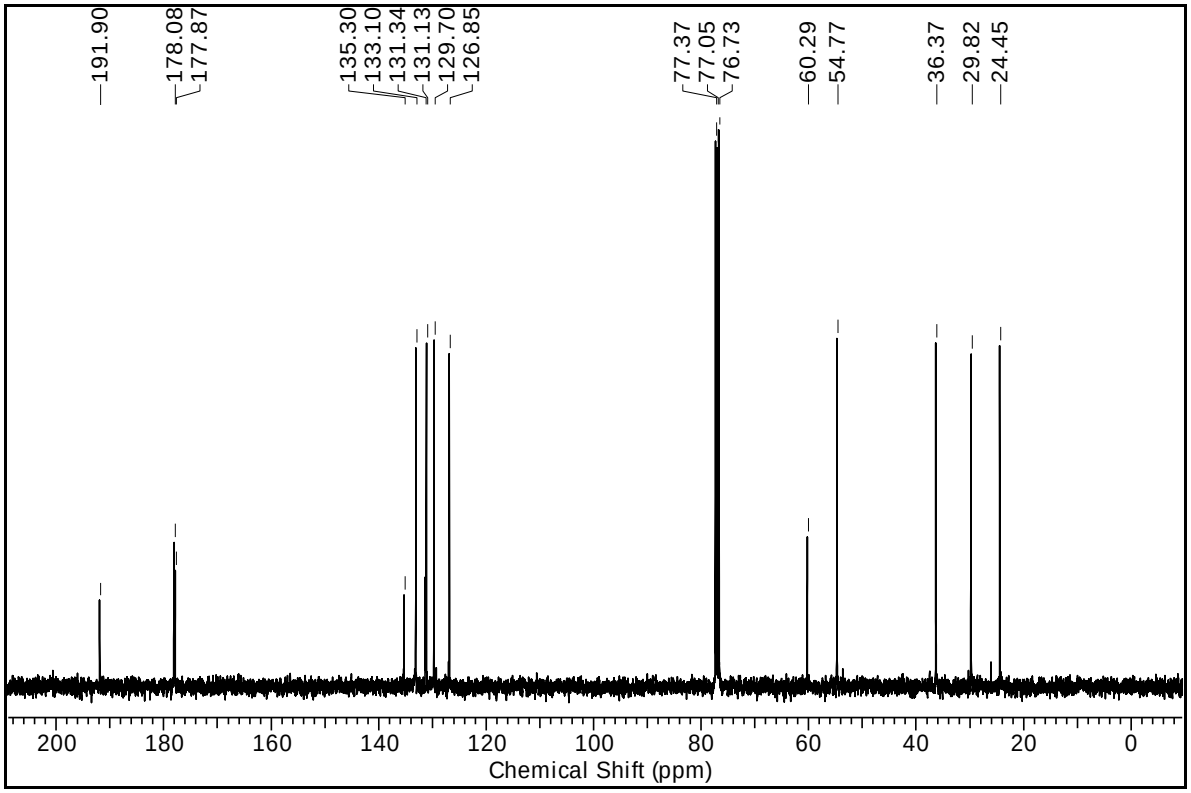
^{13}C NMR Spectrum of compound 22 in $CDCl_3$ at 100 MHz



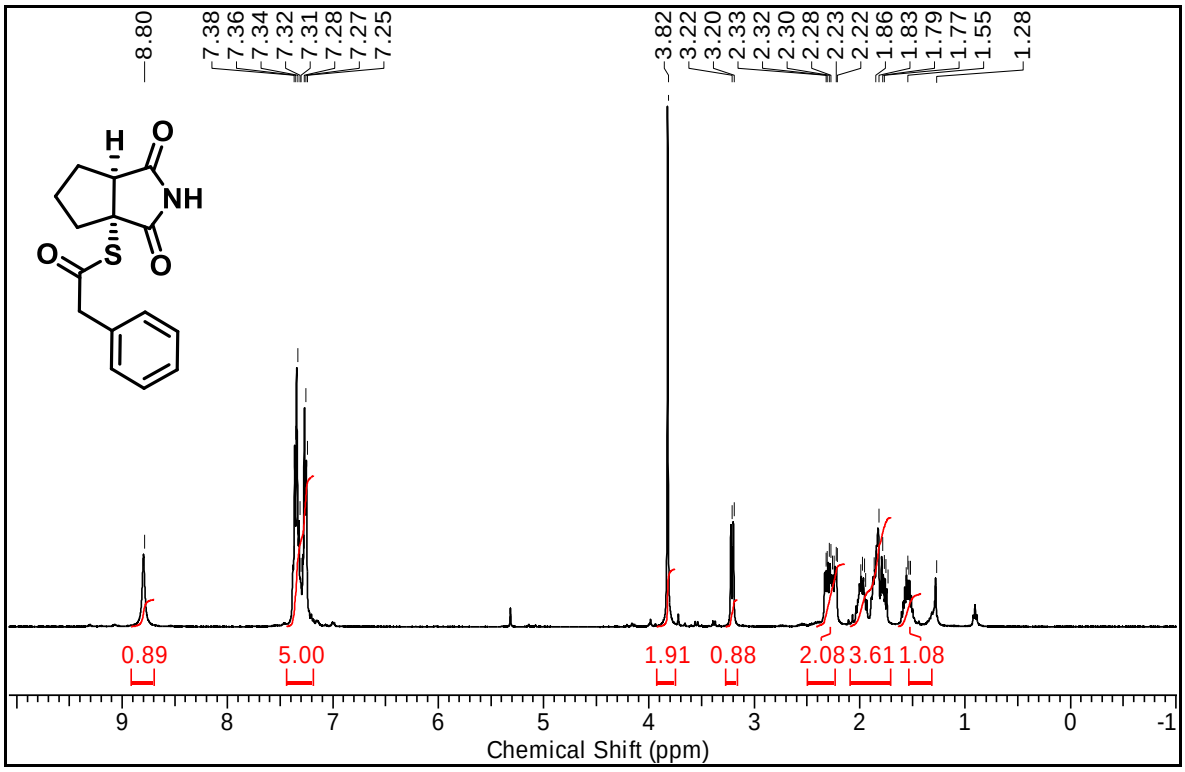
^{1}H NMR Spectrum of compound (±)-23 in $CDCl_3$ at 400 MHz



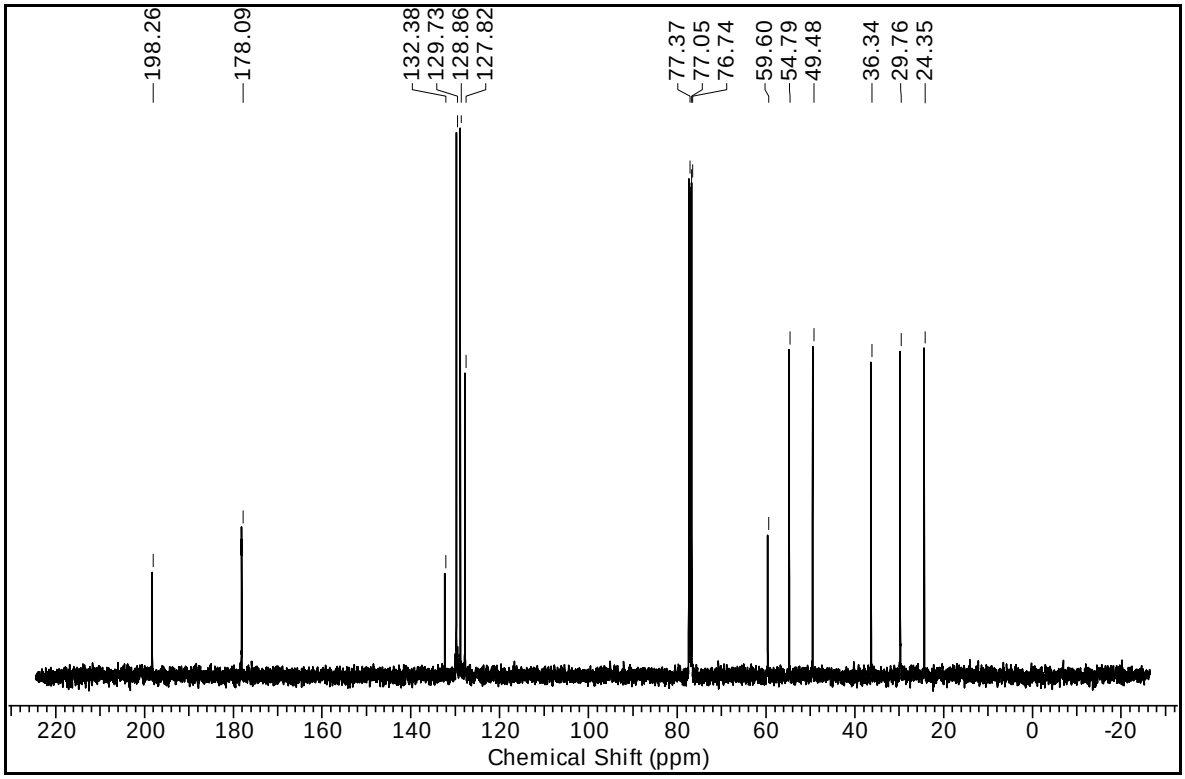
^{13}C NMR Spectrum of compound 23 in $CDCl_3$ at 100 MHz



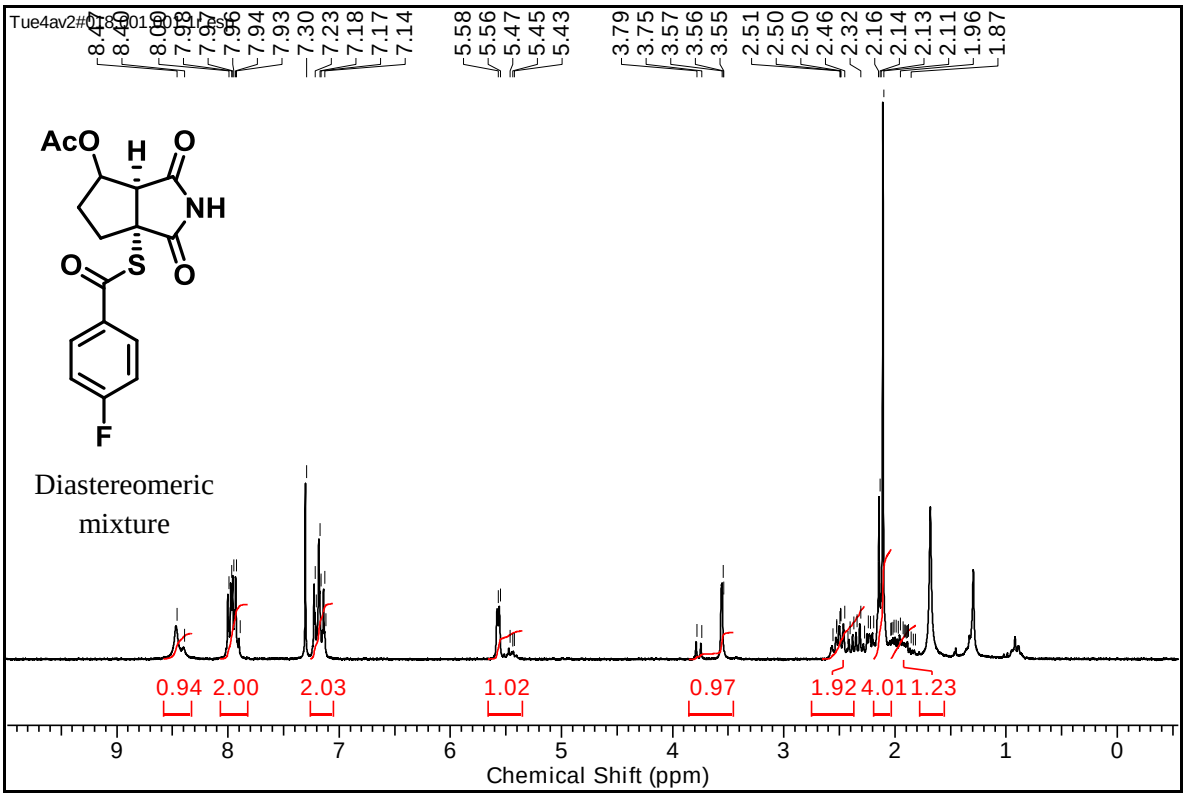
^{1}H NMR Spectrum of compound (±)-24 in $CDCl_3$ at 200 MHz



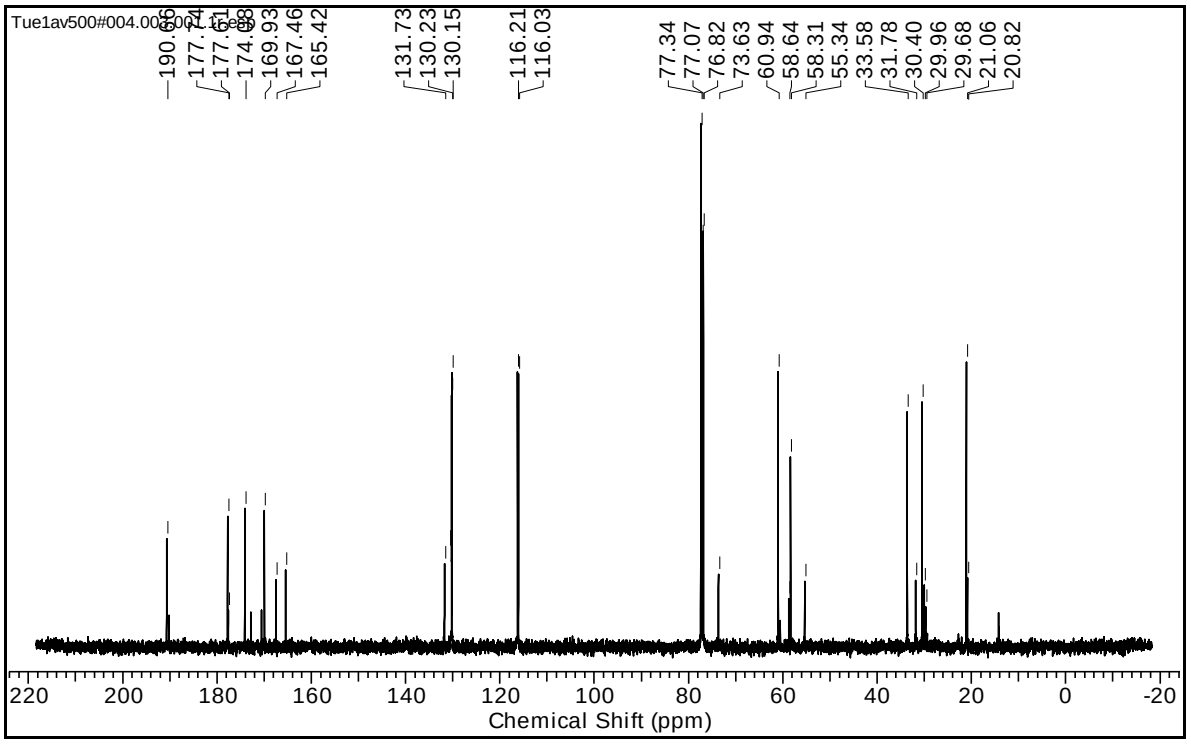
^{13}C NMR Spectrum of compound 24 in $CDCl_3$ at 100 MHz



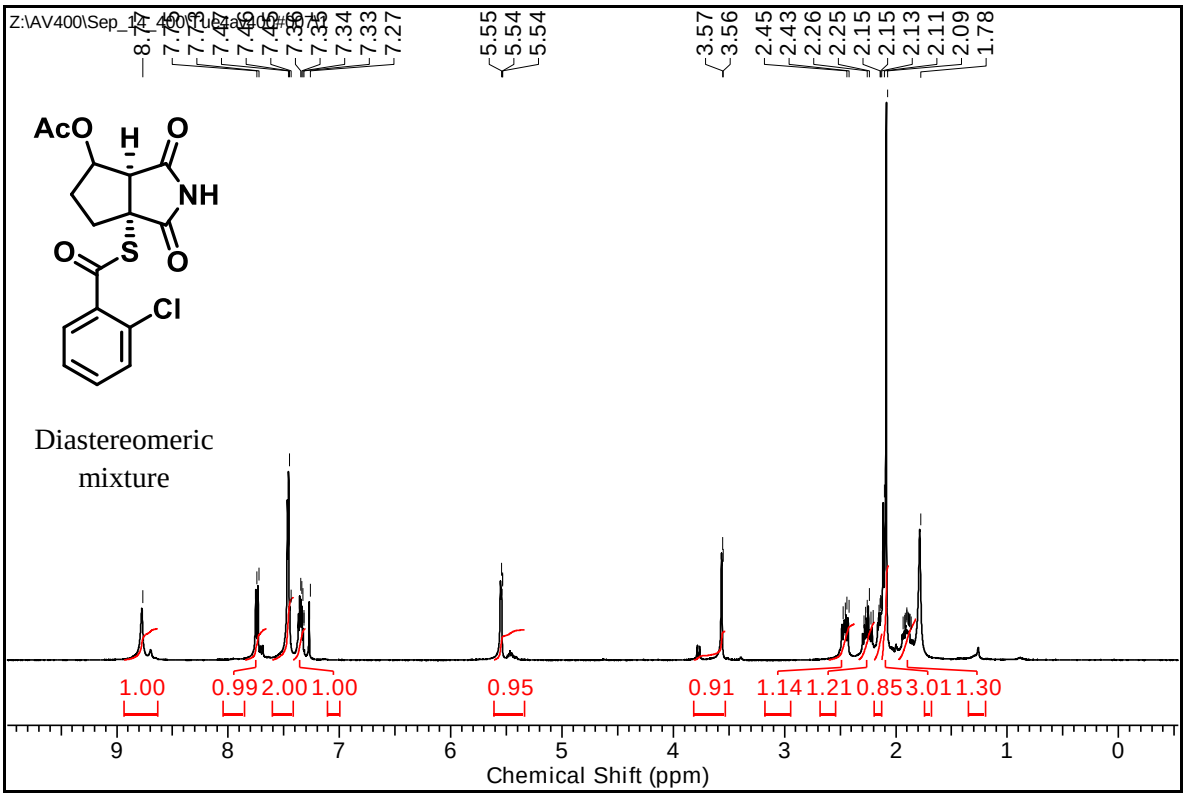
^{1}H NMR Spectrum of compound 25 in $CDCl_3$ at 200 MHz



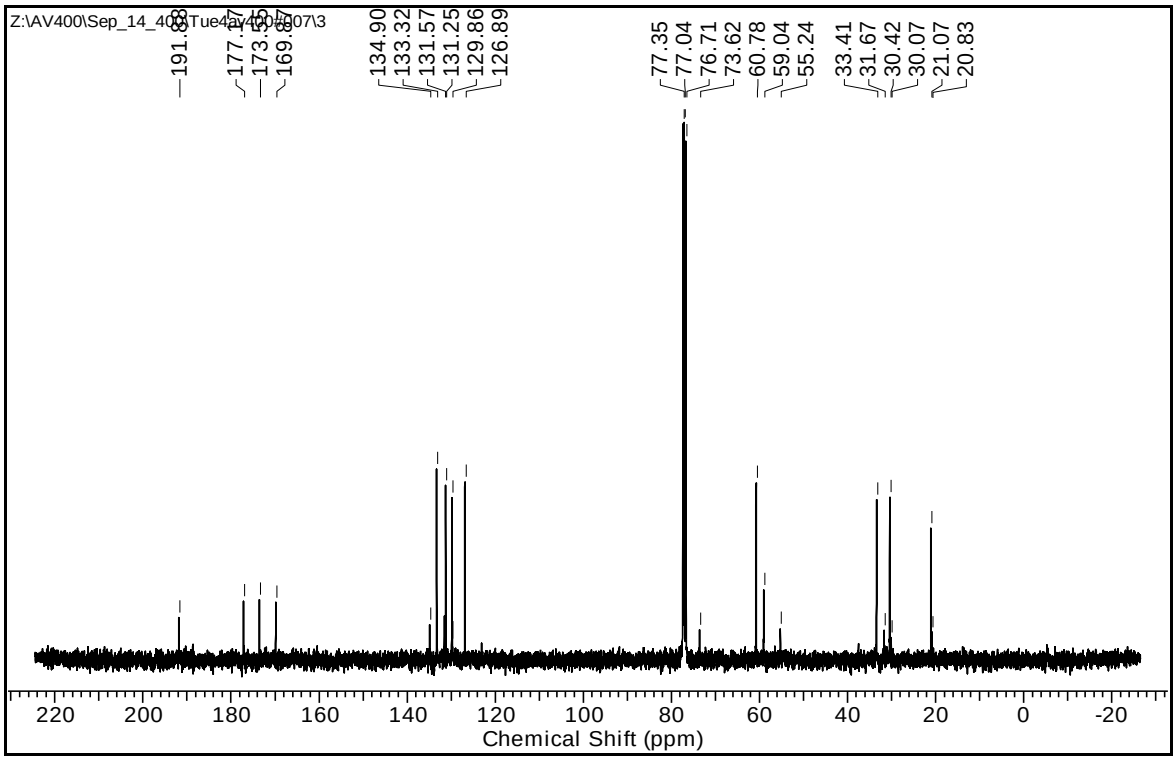
^{13}C NMR Spectrum of compound 25 in $CDCl_3$ at 125 MHz



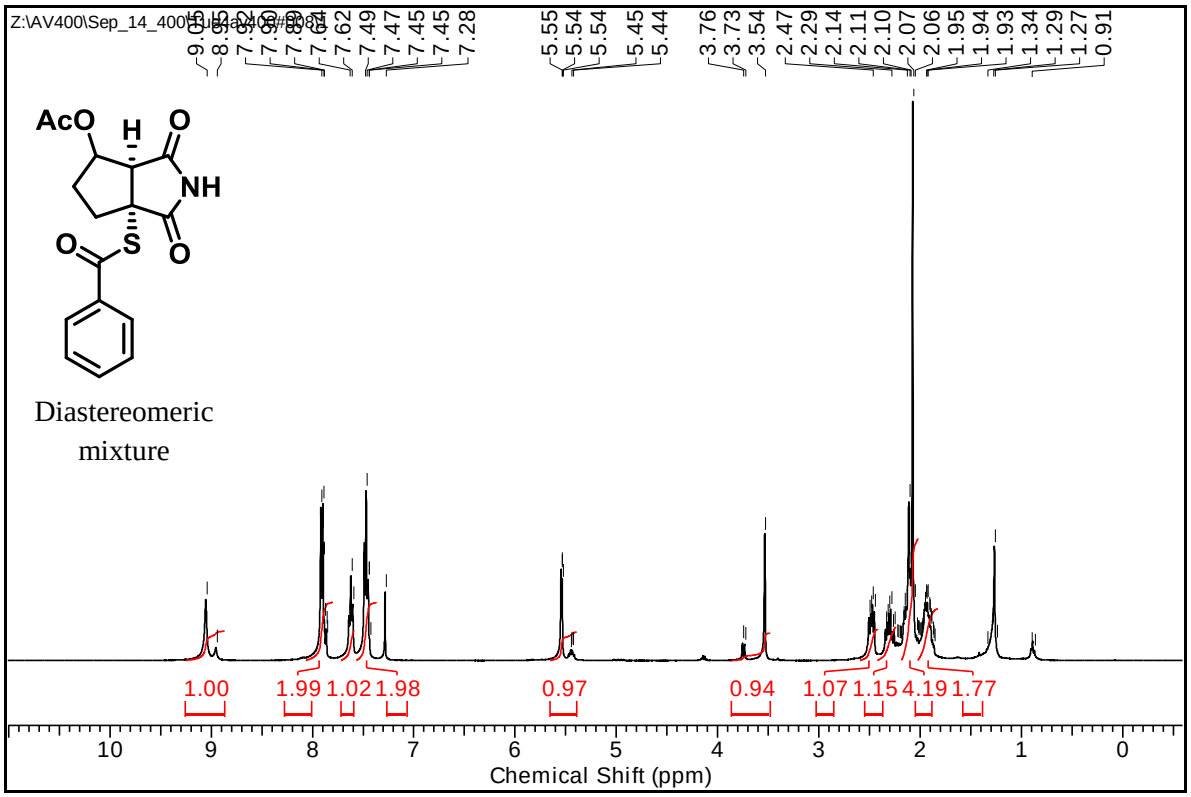
^{1}H NMR Spectrum of compound 26 in $CDCl_3$ at 400 MHz



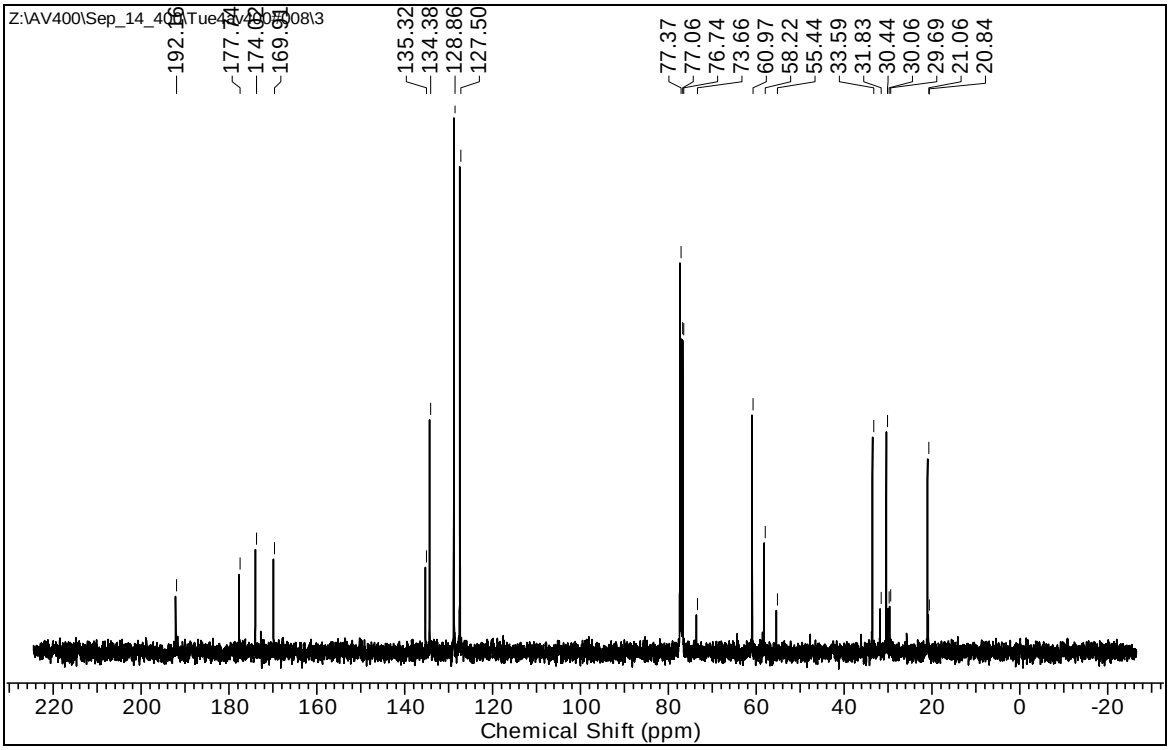
^{13}C NMR Spectrum of compound 26 in $CDCl_3$ at 100 MHz



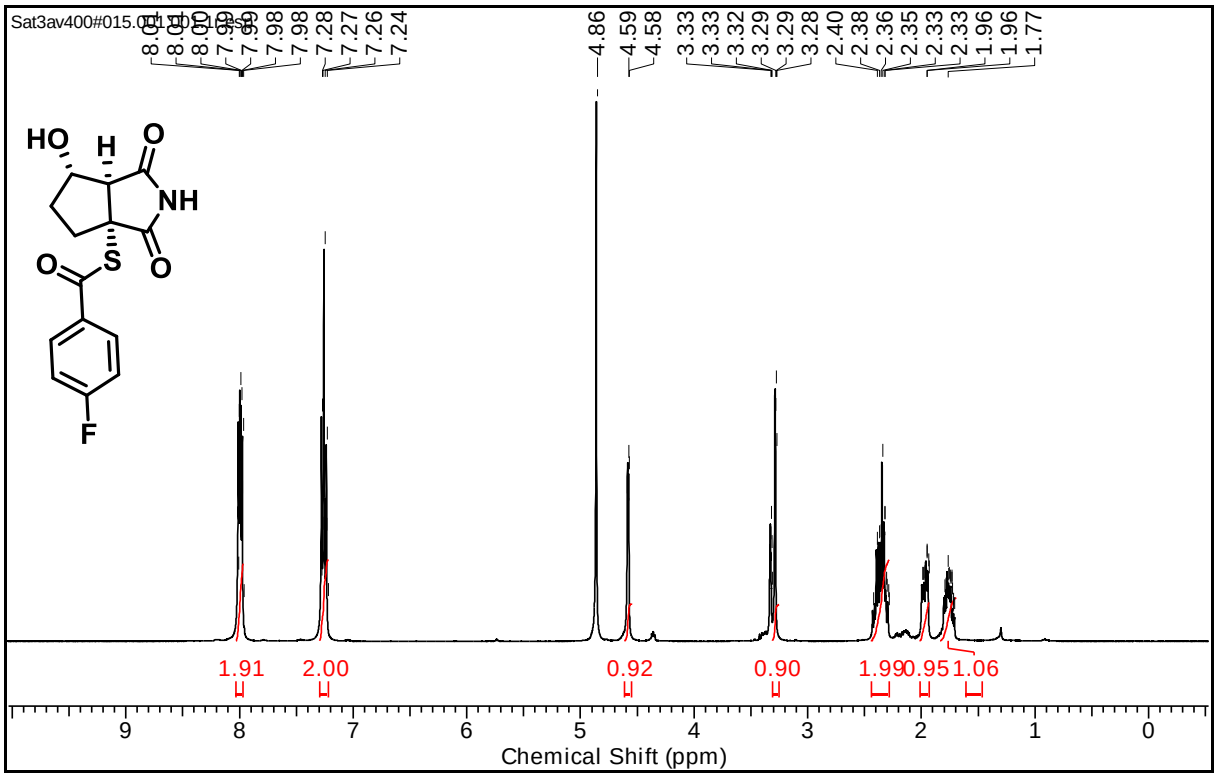
^{1}H NMR Spectrum of compound 27 in $CDCl_3$ at 400 MHz



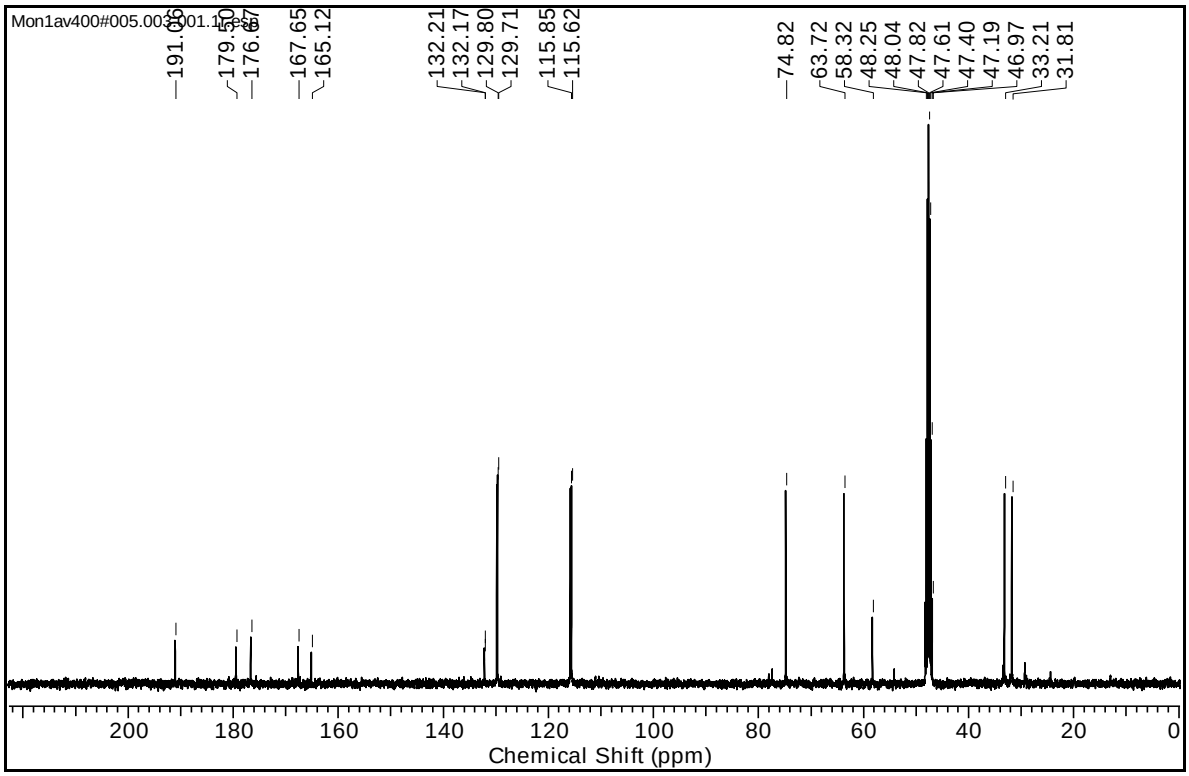
^{13}C NMR Spectrum of compound 27 in $CDCl_3$ at 100 MHz



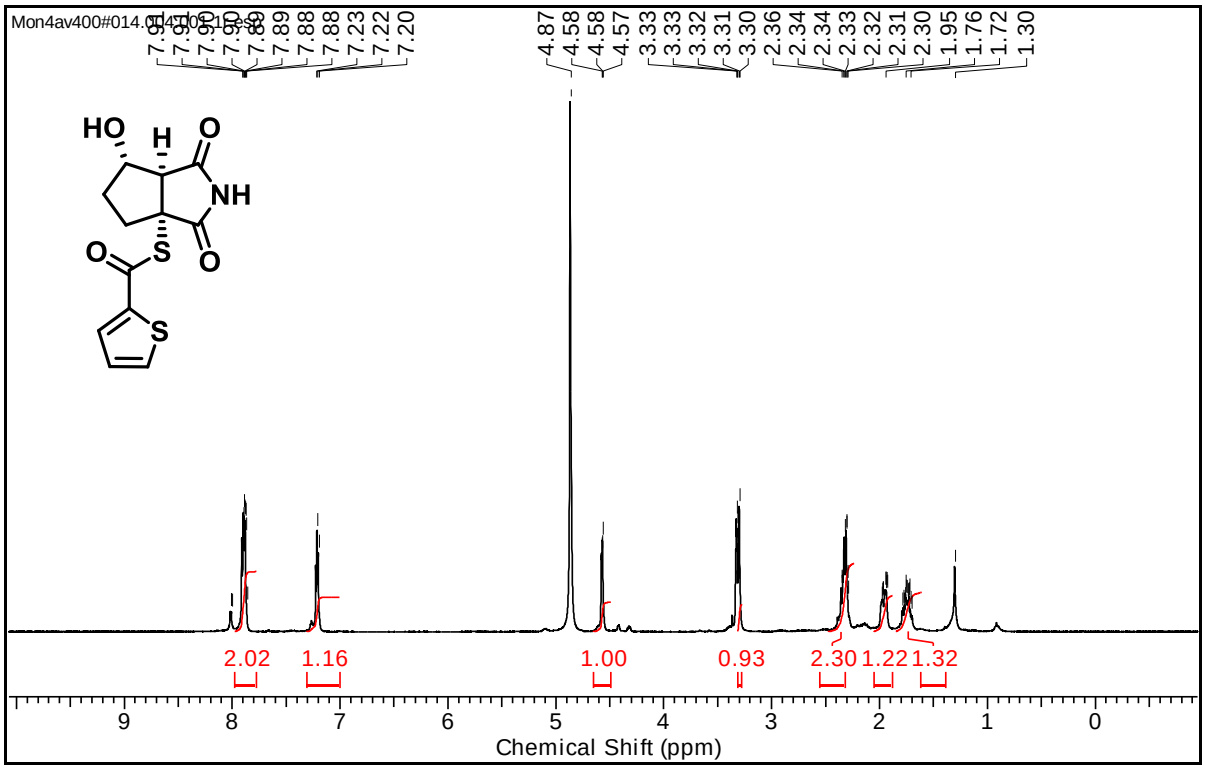
^{1}H NMR Spectrum of compound (±)-28 in CD_3OD at 400 MHz



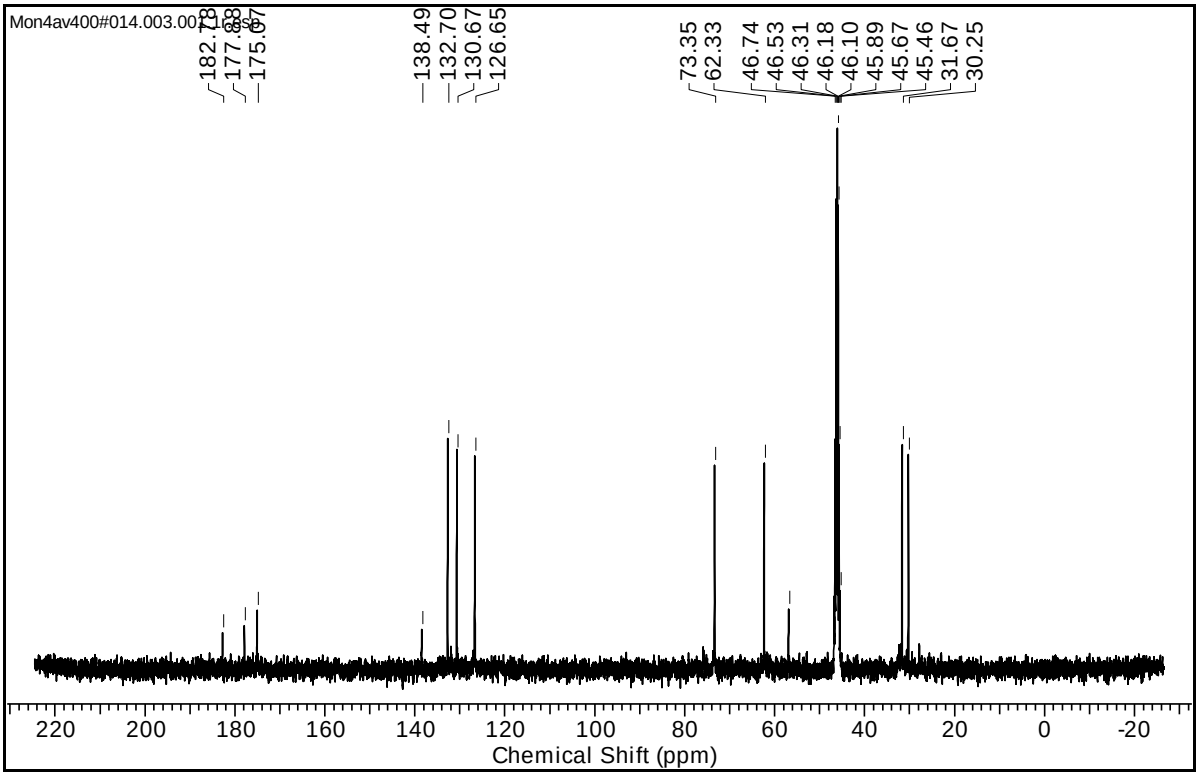
^{13}C NMR Spectrum of compound (±)-28 in CD_3OD at 100 MHz



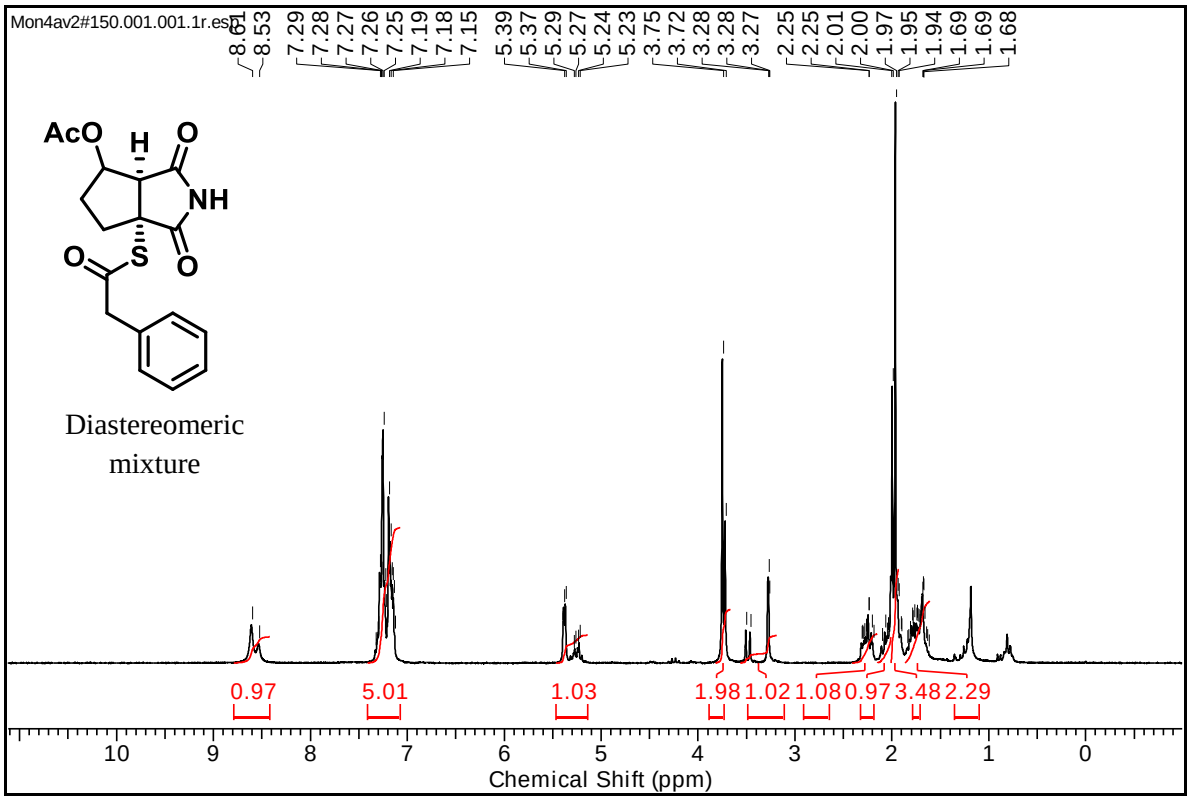
^{1}H NMR Spectrum of compound (±)-29 in CD_3OD at 400 MHz



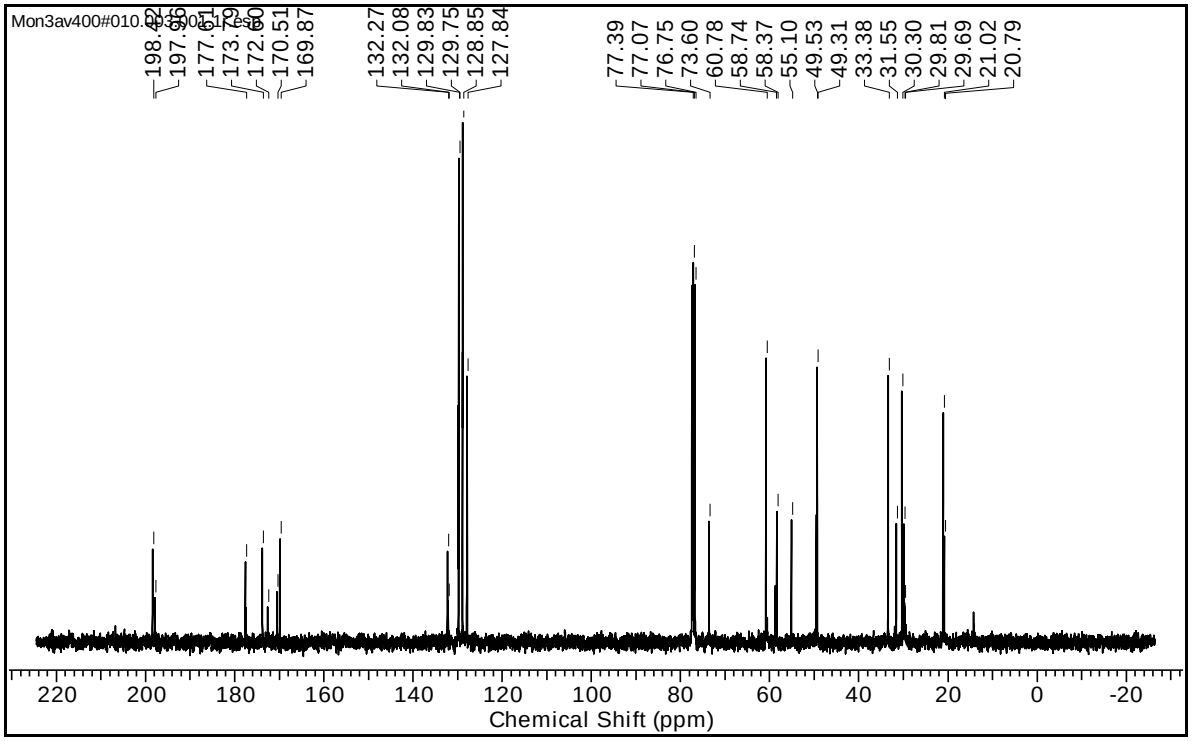
^{13}C NMR Spectrum of compound (±)-29 in CD_3OD at 100 MHz



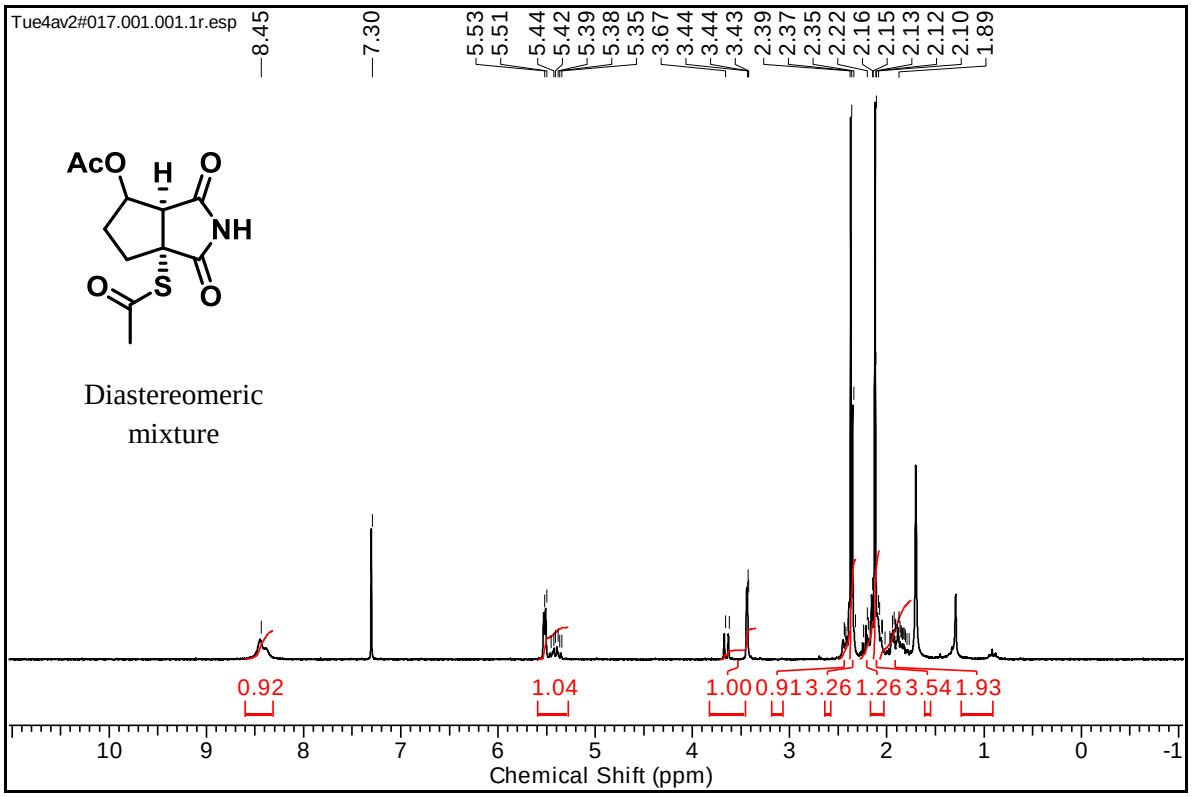
^{1}H NMR Spectrum of compound 30 in $CDCl_3$ at 200 MHz



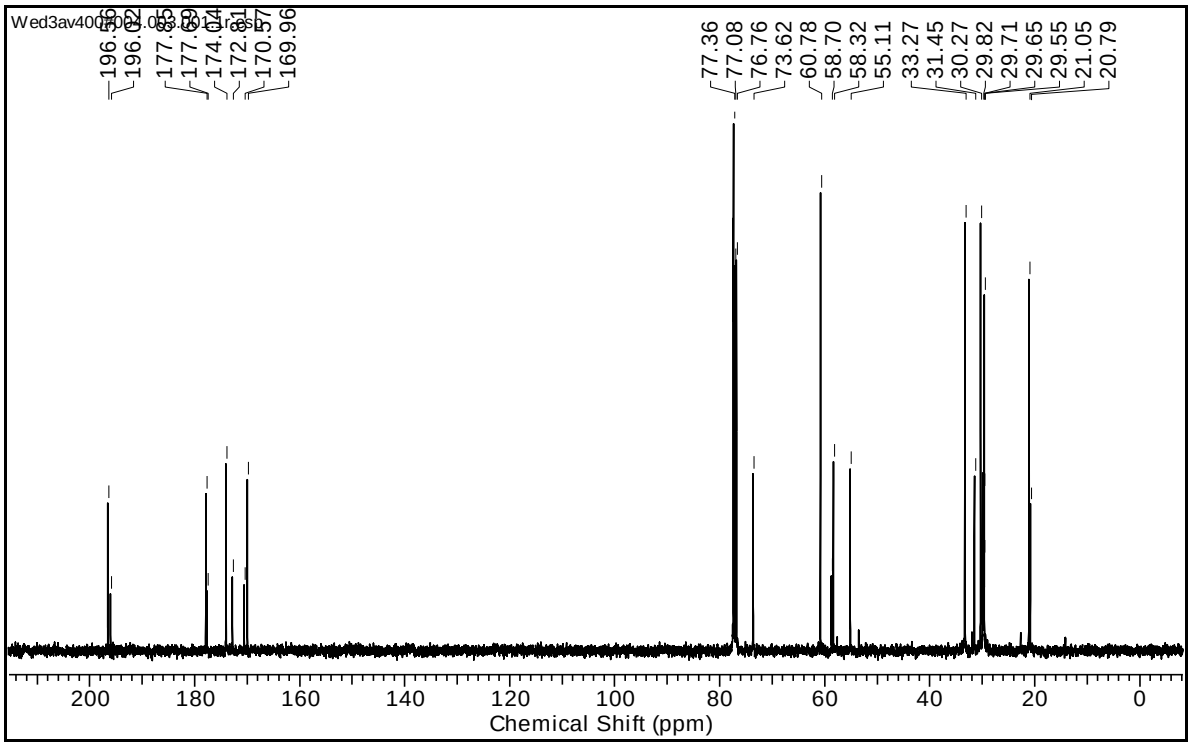
^{13}C NMR Spectrum of compound 30 in $CDCl_3$ at 100 MHz



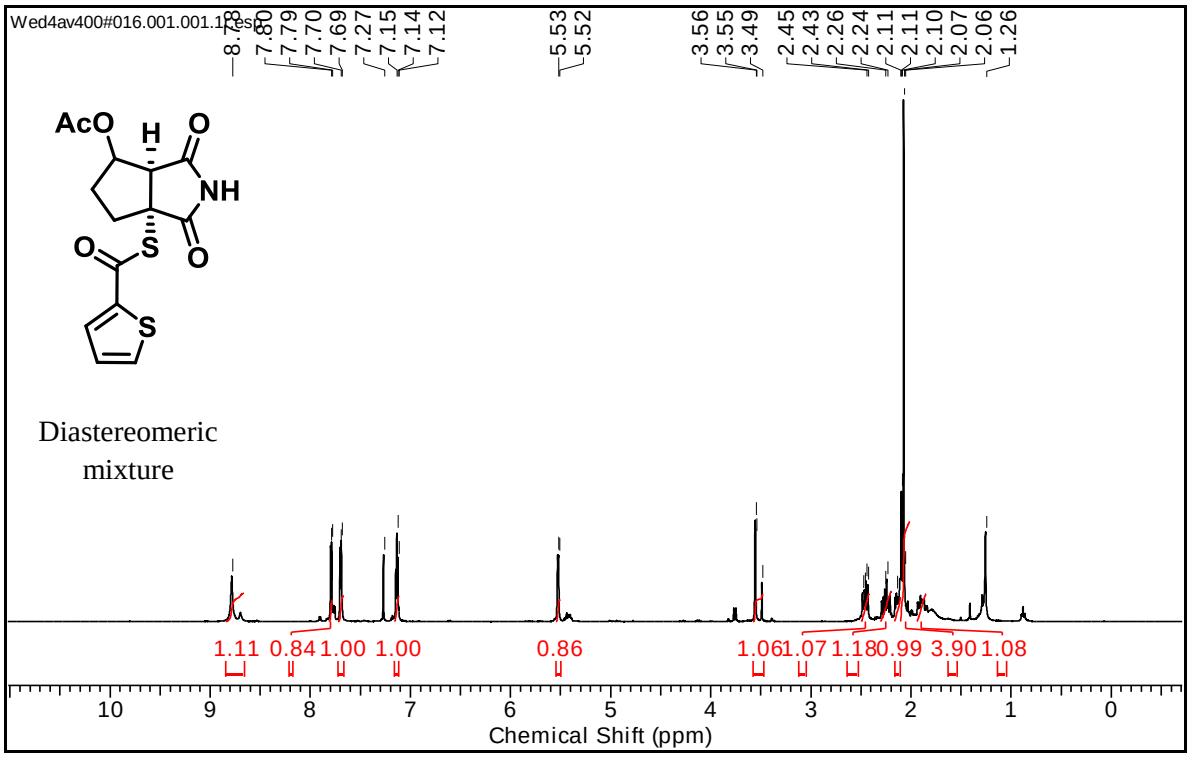
^{1}H NMR Spectrum of compound 31 in $CDCl_3$ at 200 MHz



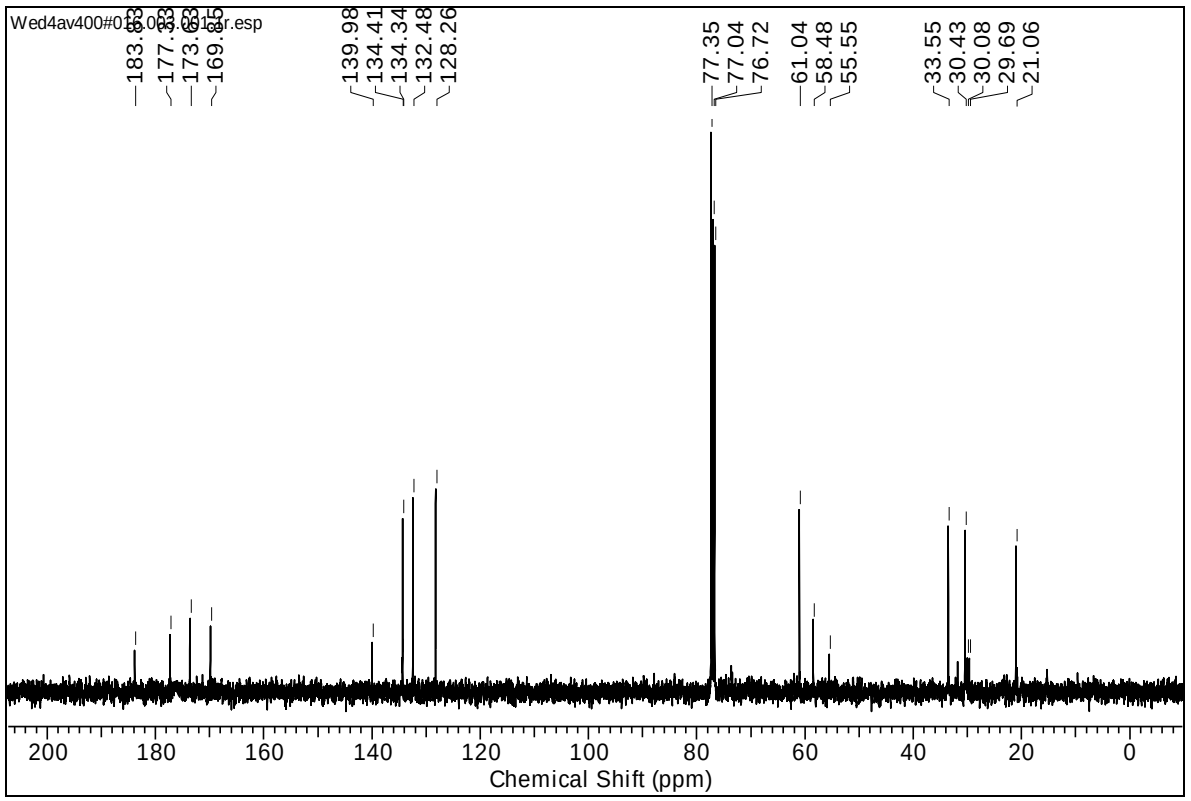
^{13}C NMR Spectrum of compound 31 in $CDCl_3$ at 100 MHz



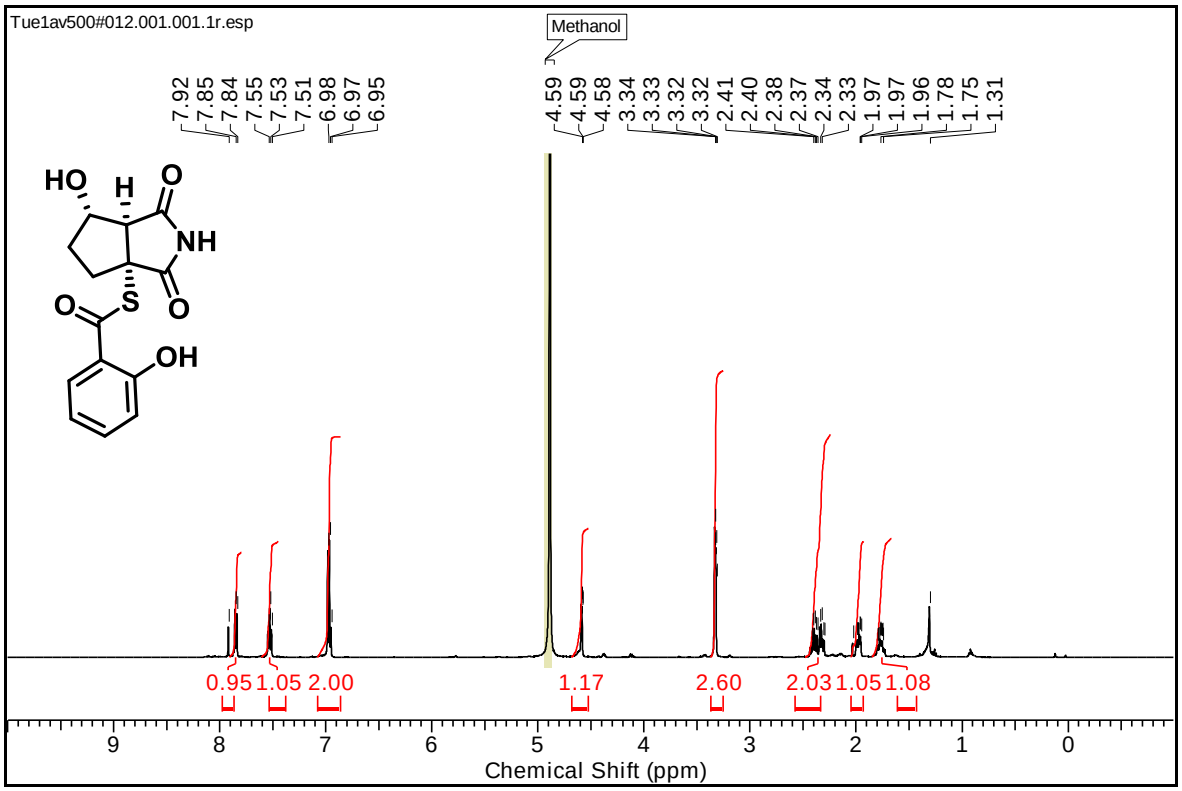
^{1}H NMR Spectrum of compound 32 in $CDCl_3$ at 400 MHz



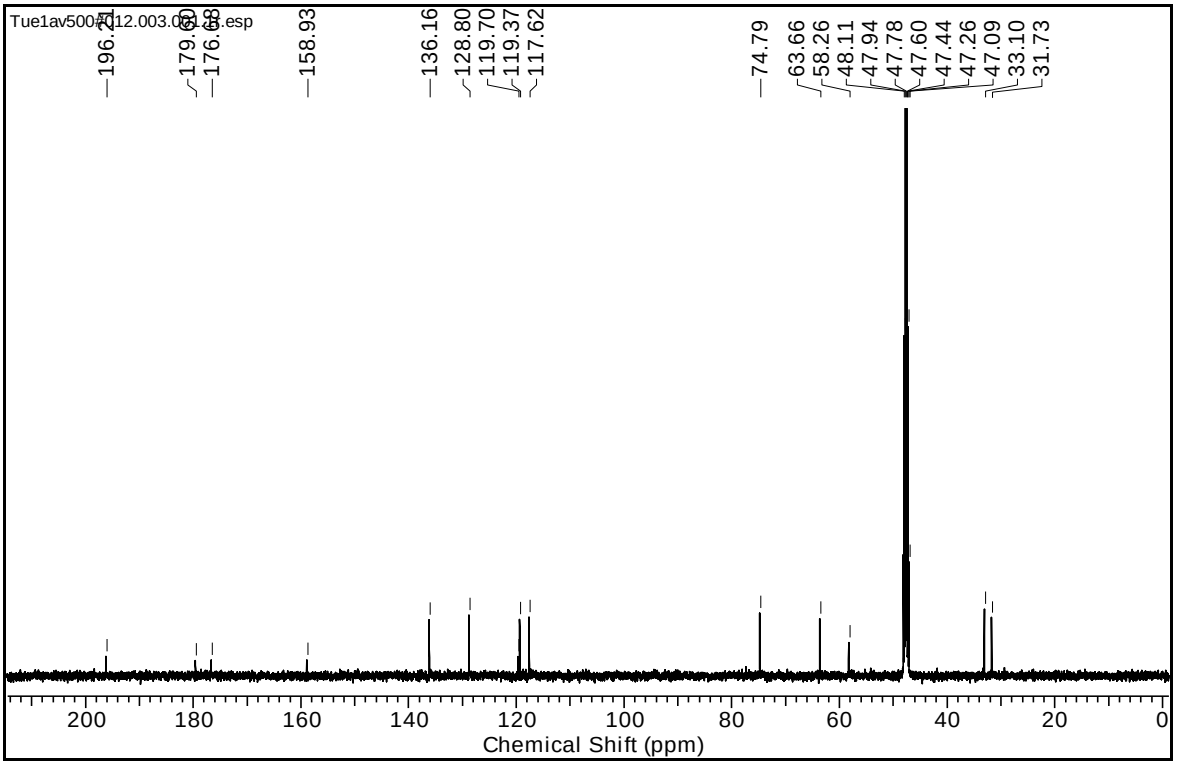
^{13}C NMR Spectrum of compound 32 in $CDCl_3$ at 100 MHz



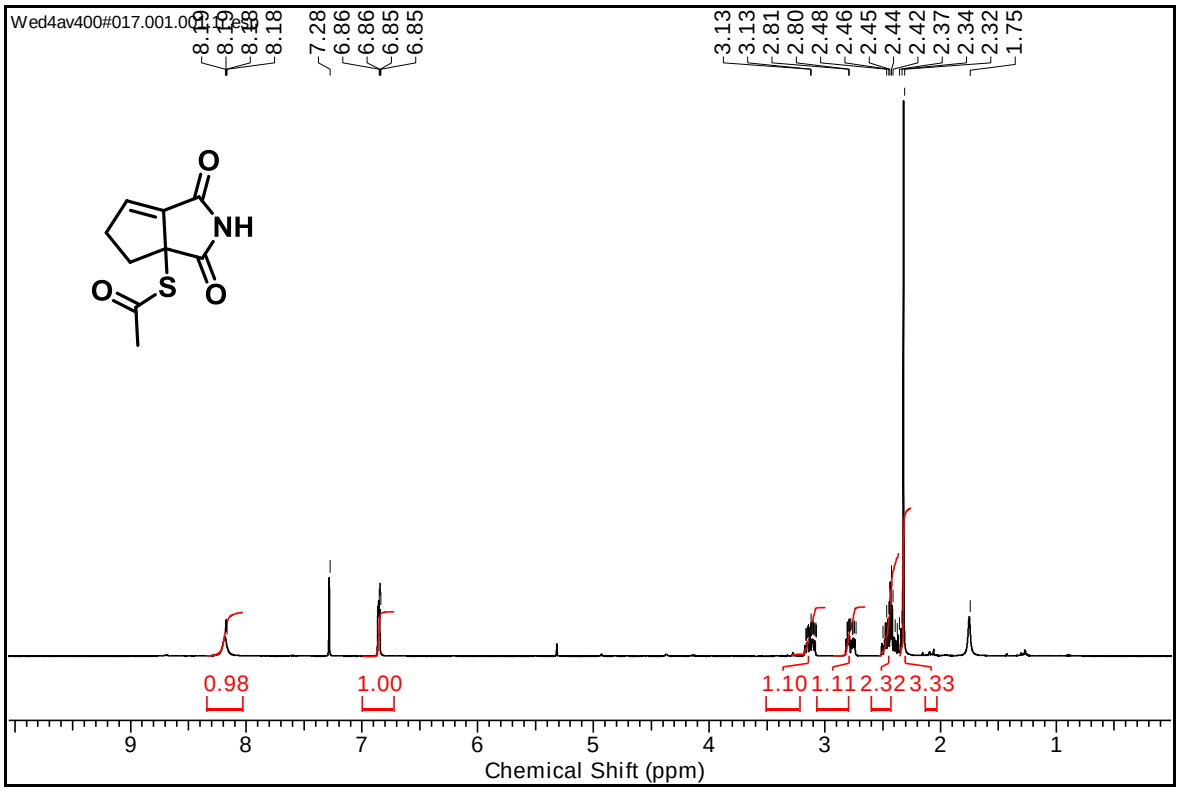
^{1}H NMR Spectrum of compound (±)-33 in CD_3OD at 500 MHz



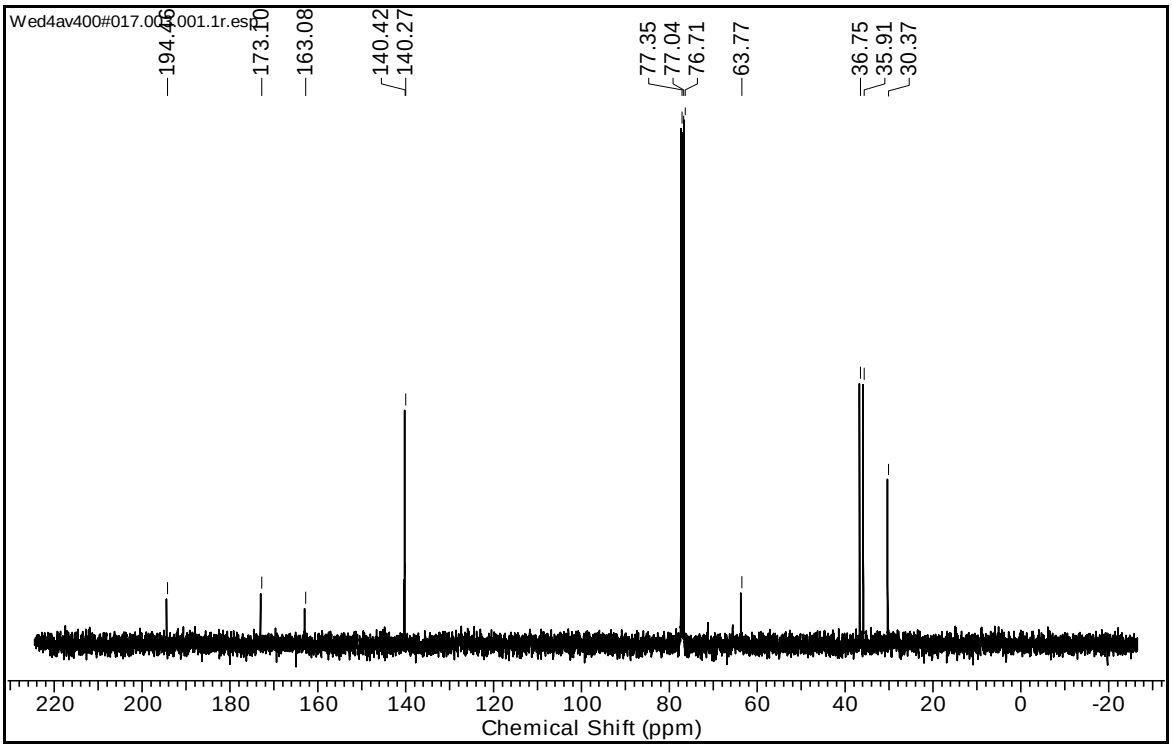
^{13}C NMR Spectrum of compound (±)-33 in CD_3OD at 125 MHz



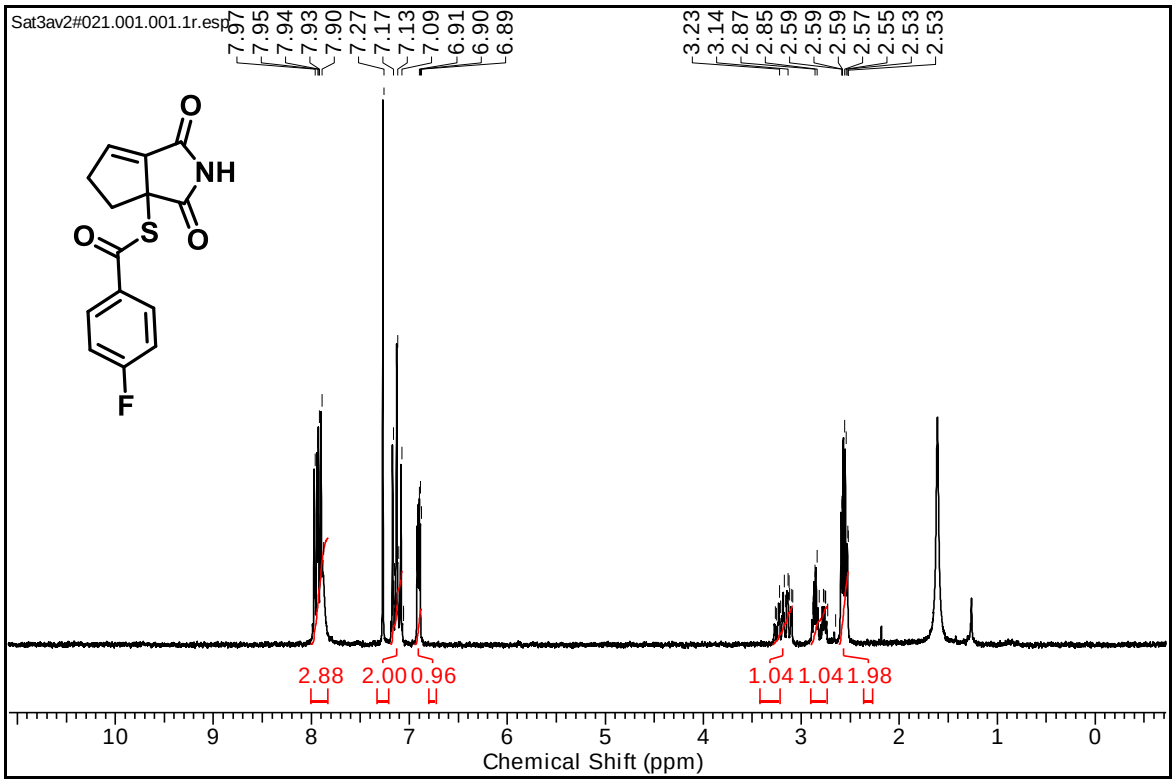
^{1}H NMR Spectrum of compound 34 in $CDCl_3$ at 400 MHz



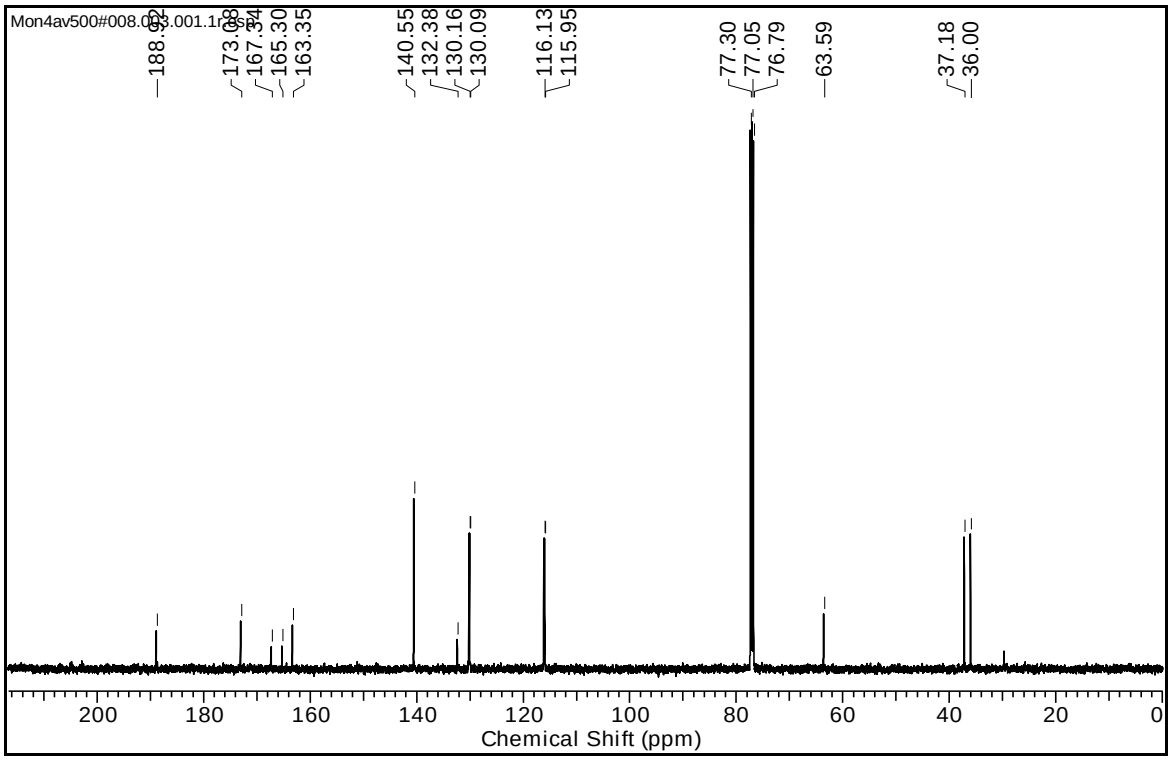
^{13}C NMR Spectrum of compound 34 in $CDCl_3$ at 100 MHz



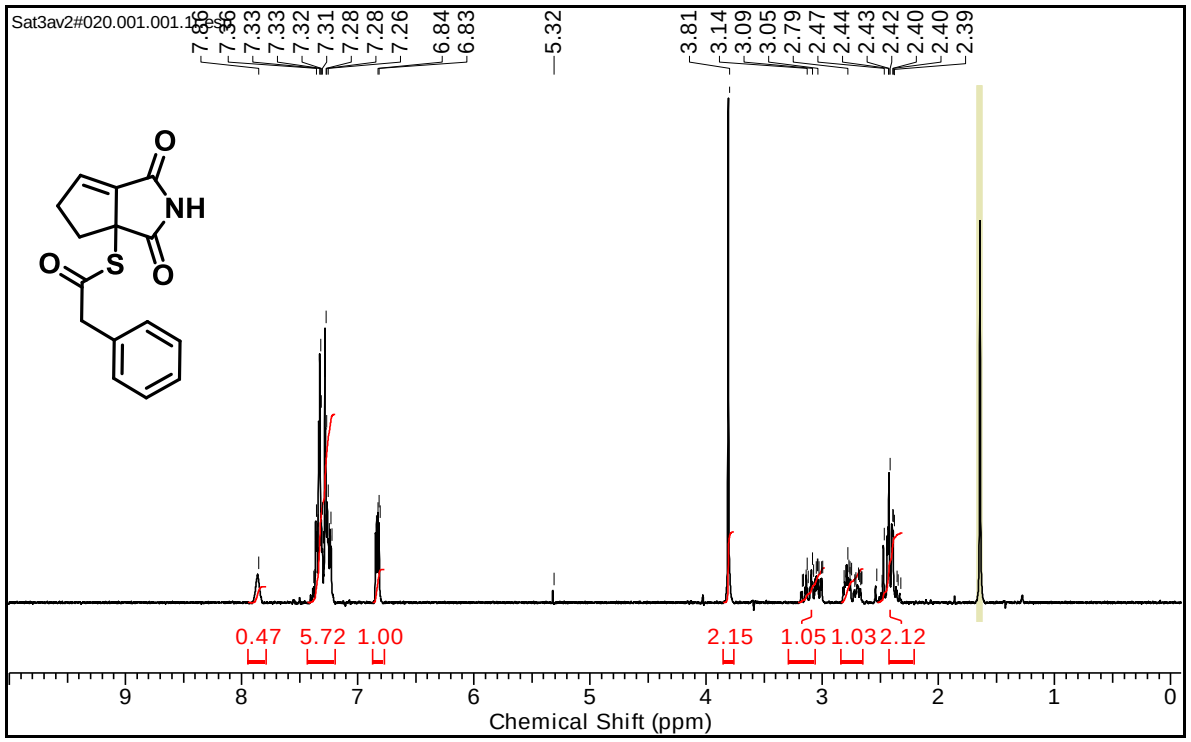
^{1}H NMR Spectrum of compound 35 in $CDCl_3$ at 200 MHz



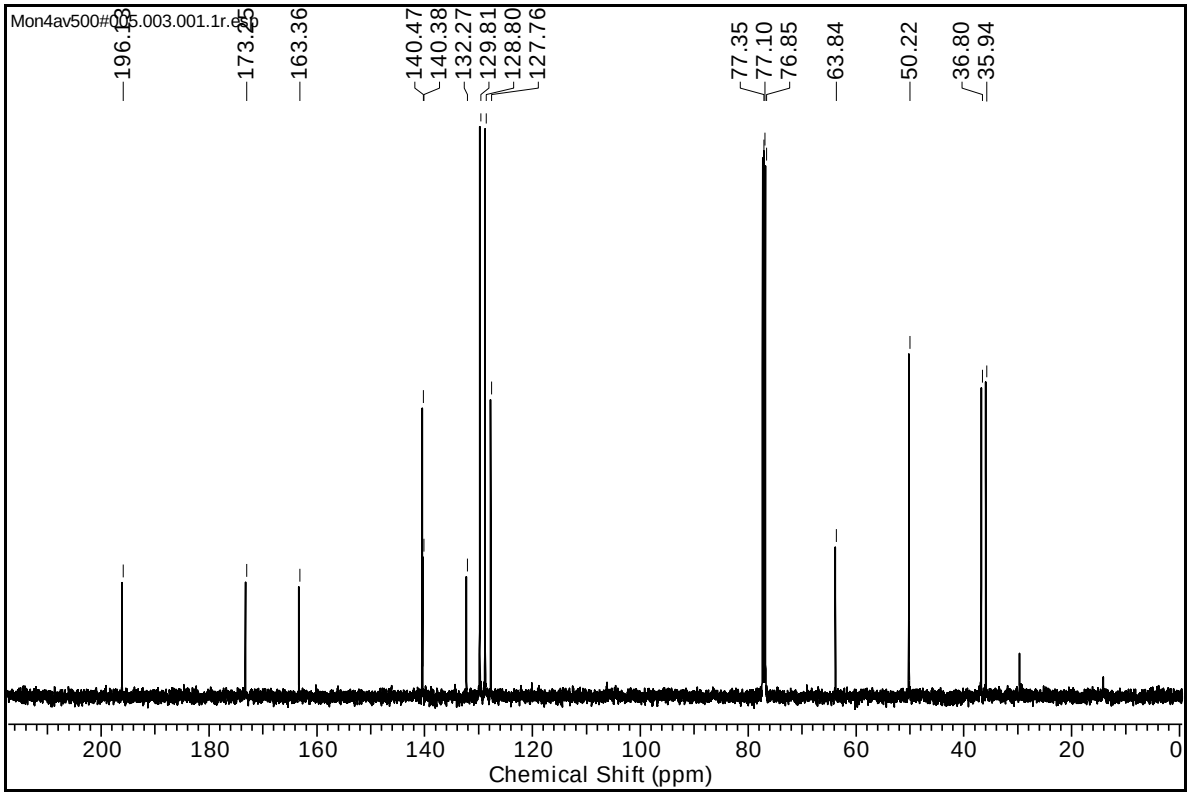
^{13}C NMR Spectrum of compound 35 in $CDCl_3$ at 125 MHz



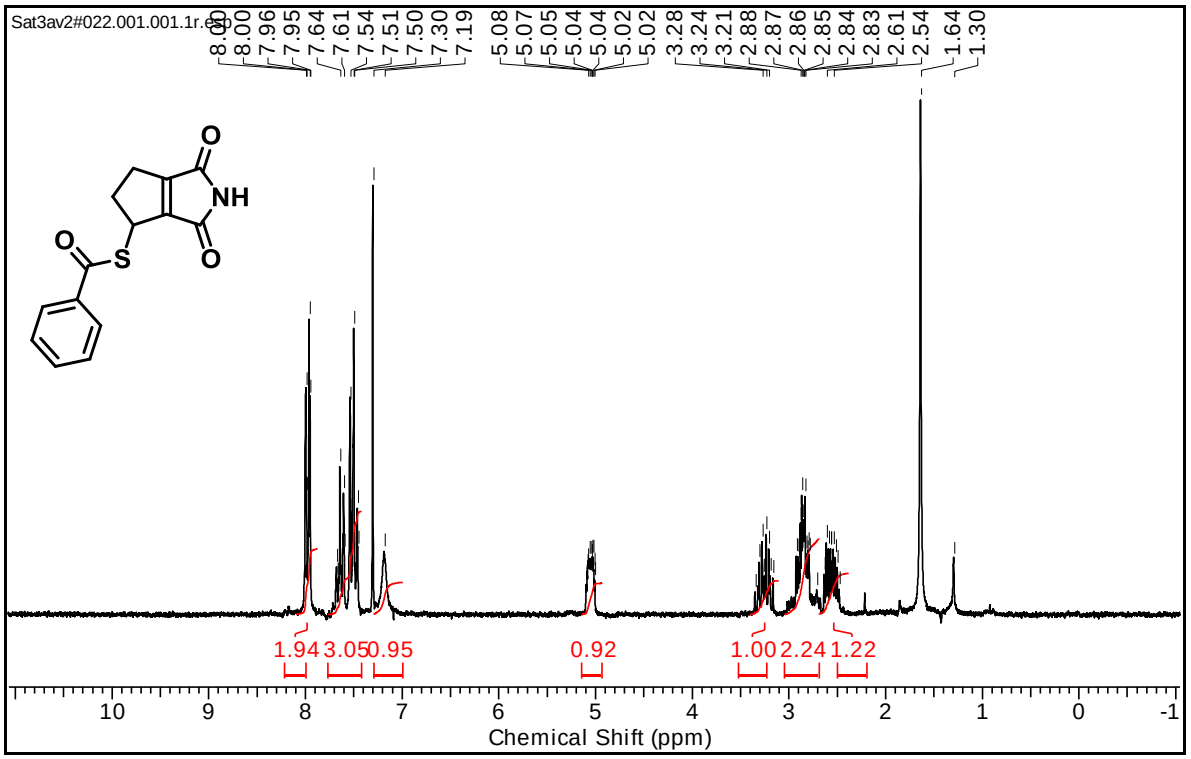
^{1}H NMR Spectrum of compound 36 in $CDCl_3$ at 200 MHz



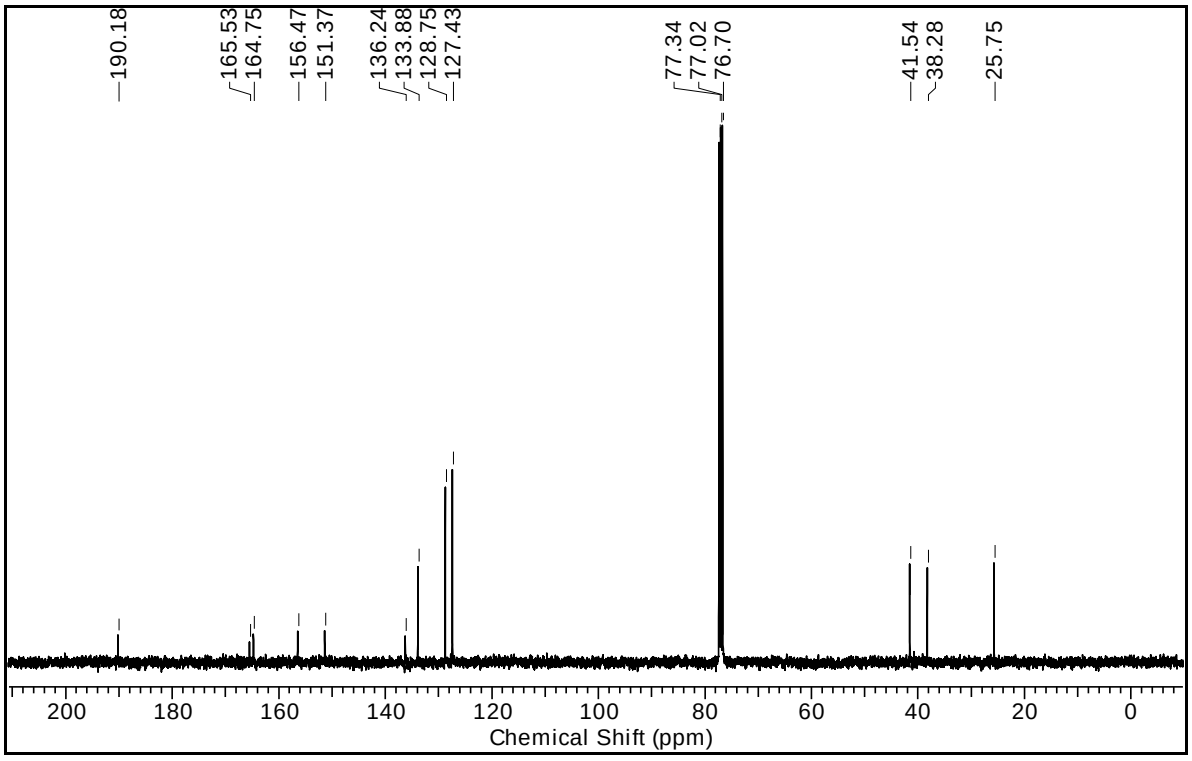
^{13}C NMR Spectrum of compound 36 in $CDCl_3$ at 125 MHz



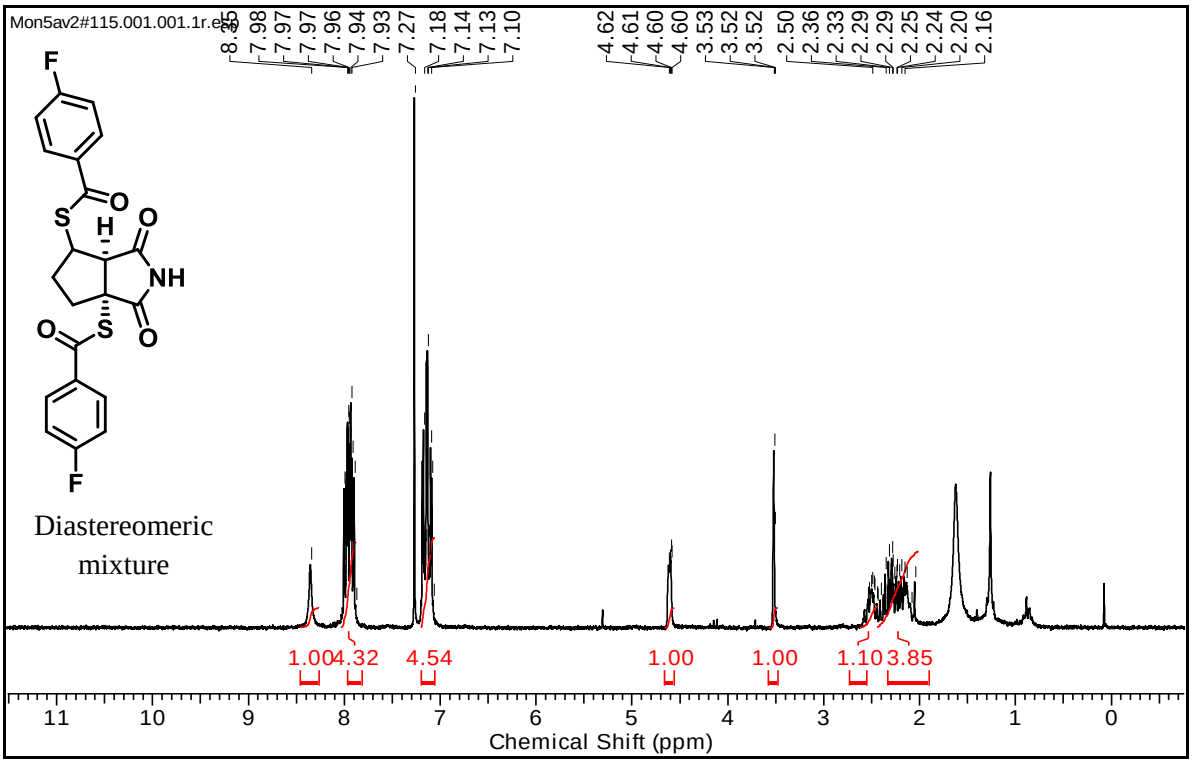
^{1}H NMR Spectrum of compound 37 in $CDCl_3$ at 200 MHz



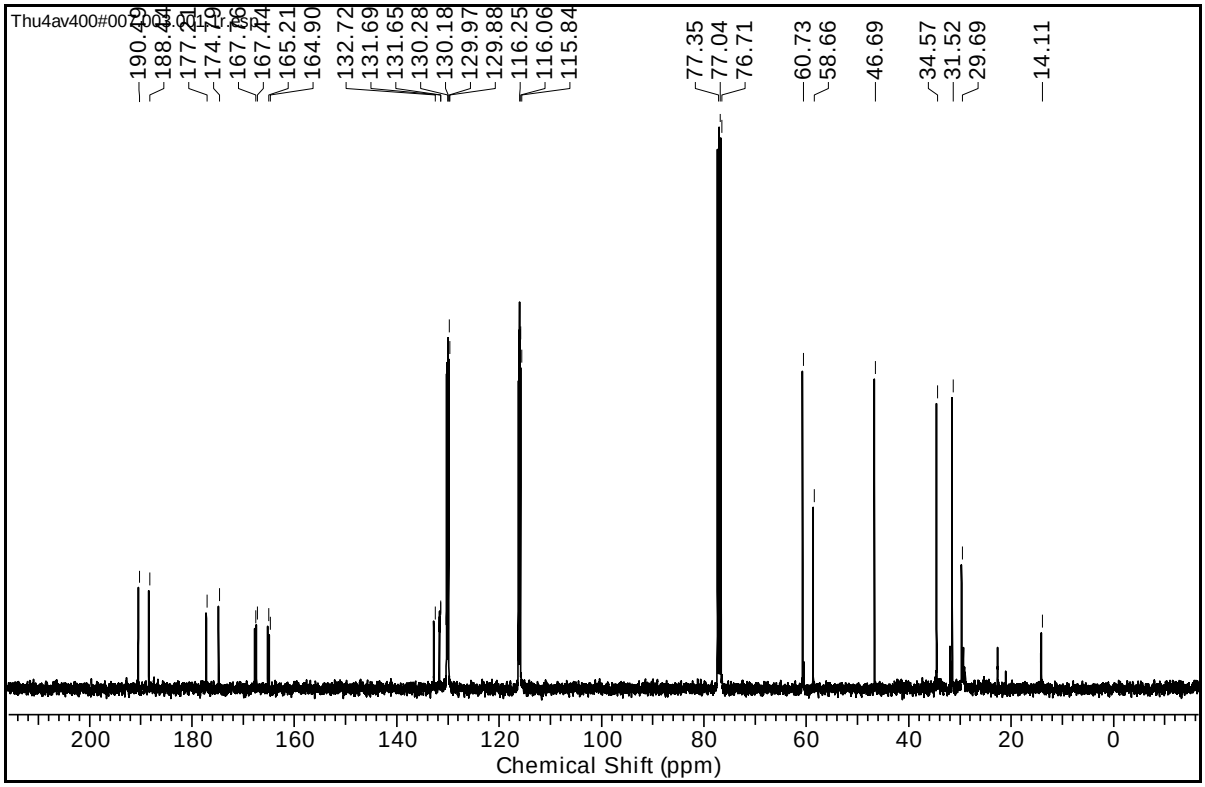
^{13}C NMR Spectrum of compound 37 in $CDCl_3$ at 100 MHz



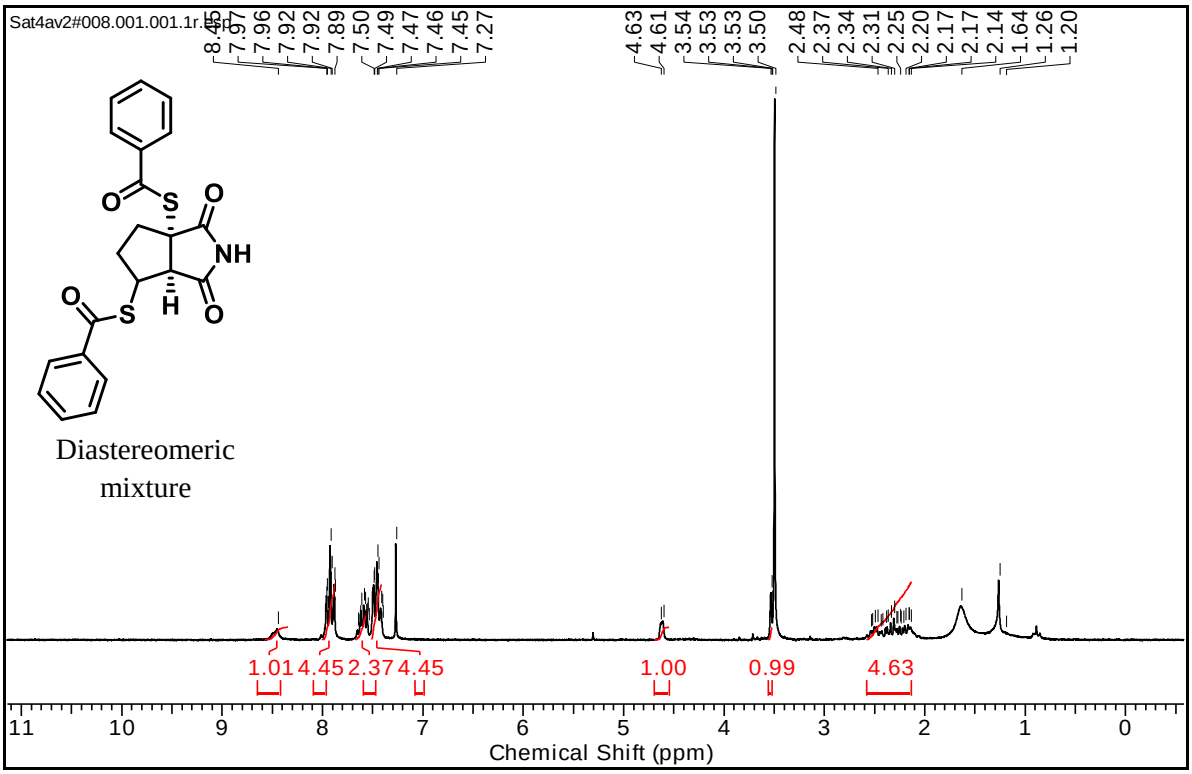
^{1}H NMR Spectrum of compound 38 in $CDCl_3$ at 200 MHz



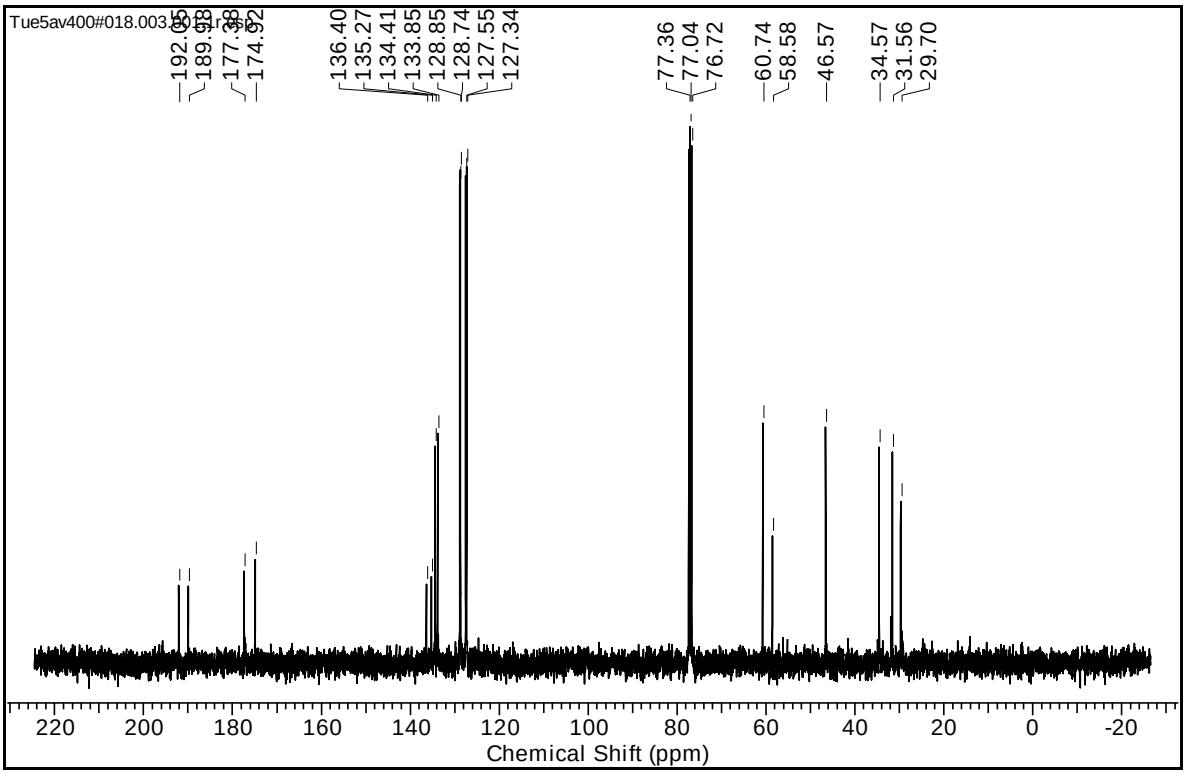
^{13}C NMR Spectrum of compound 38 in $CDCl_3$ at 100 MHz



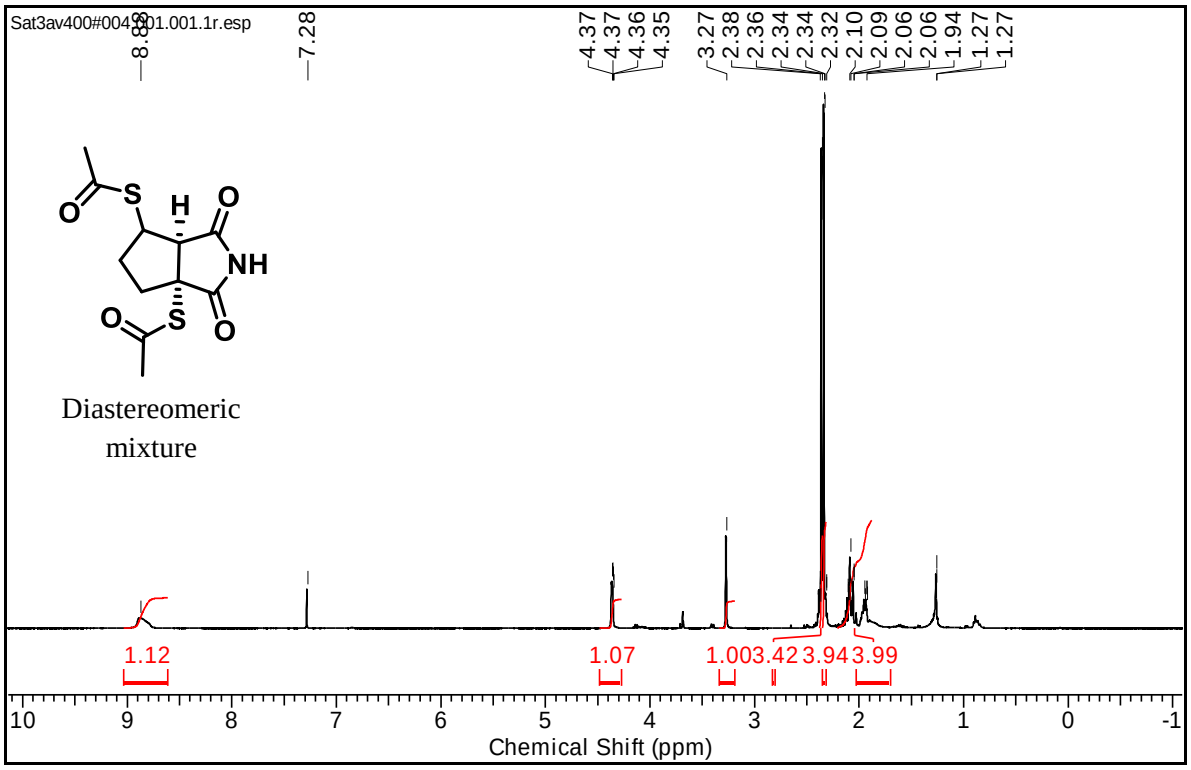
^{1}H NMR Spectrum of compound 39 in $CDCl_3$ at 200 MHz



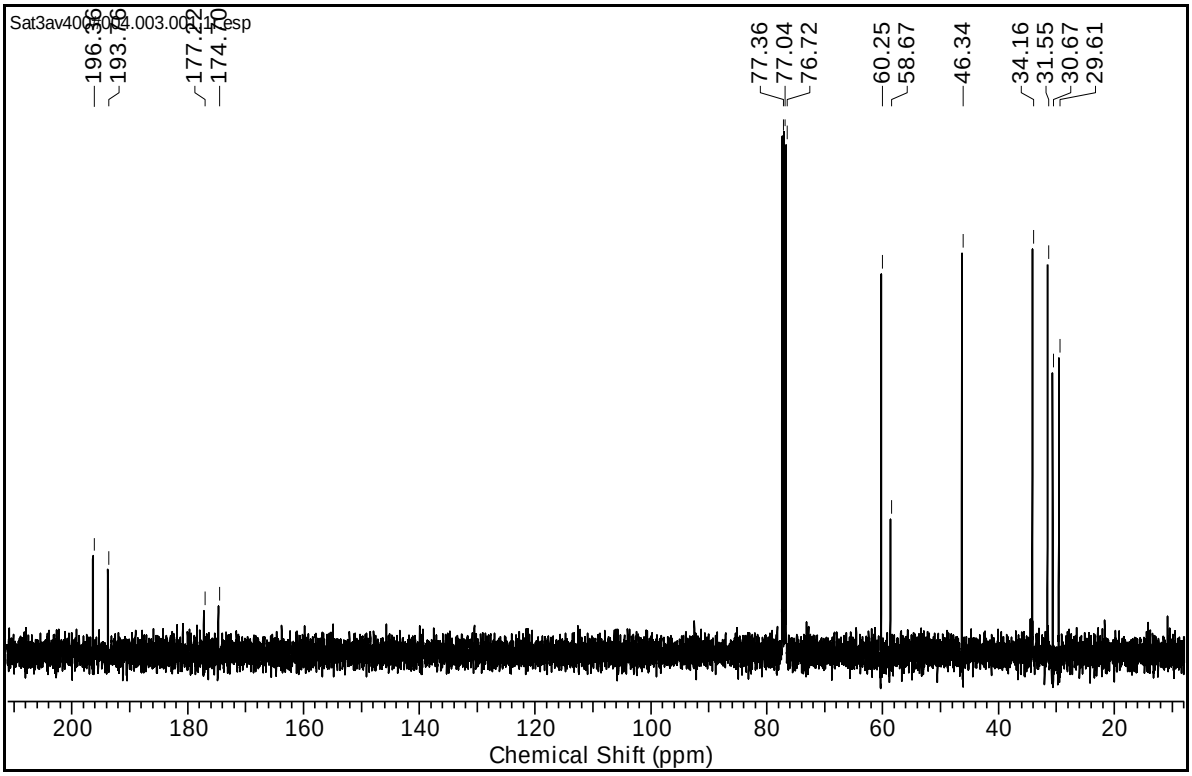
^{13}C NMR Spectrum of compound 39 in $CDCl_3$ at 100 MHz



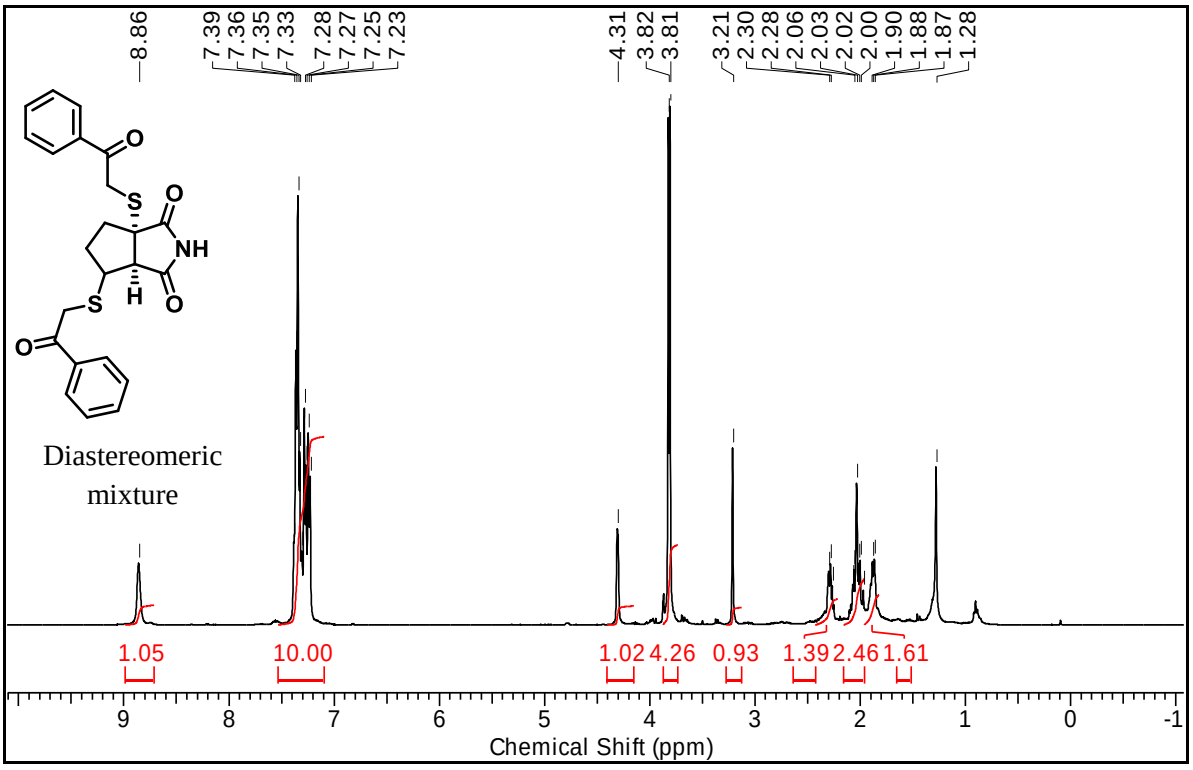
^{1}H NMR Spectrum of compound 40 in $CDCl_3$ at 400 MHz



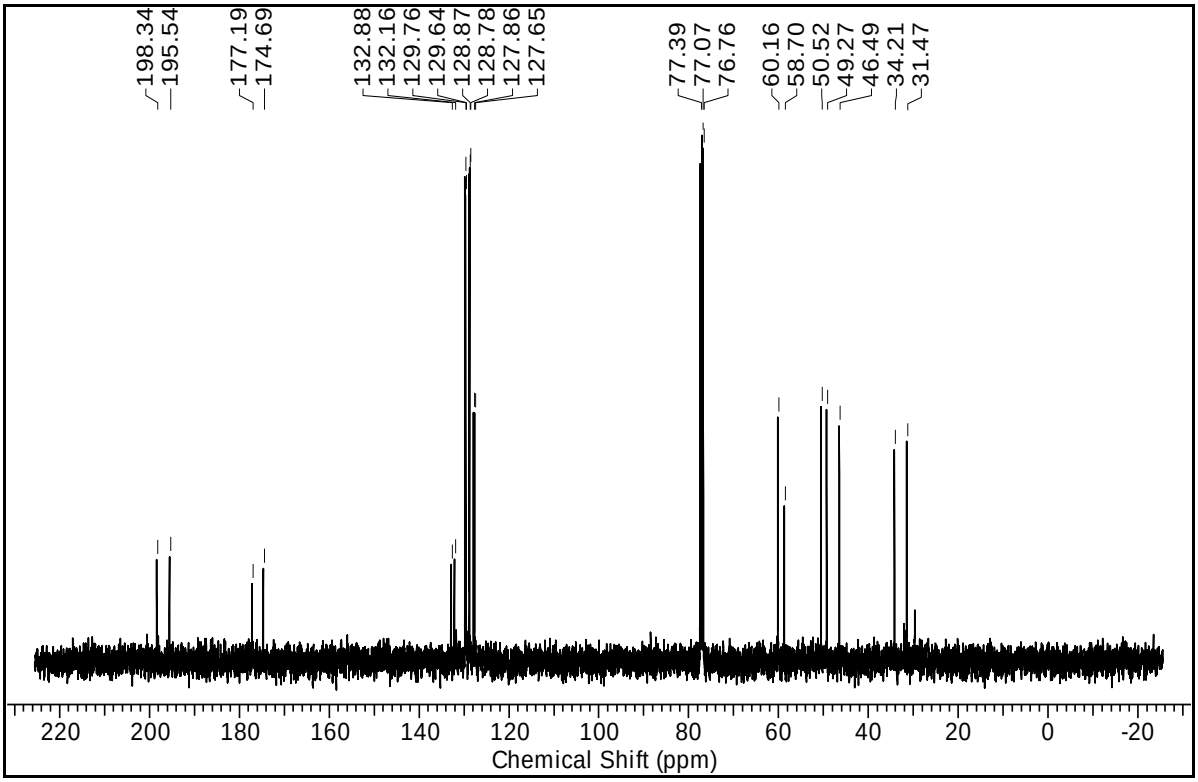
^{13}C NMR Spectrum of compound 40 in $CDCl_3$ at 100 MHz



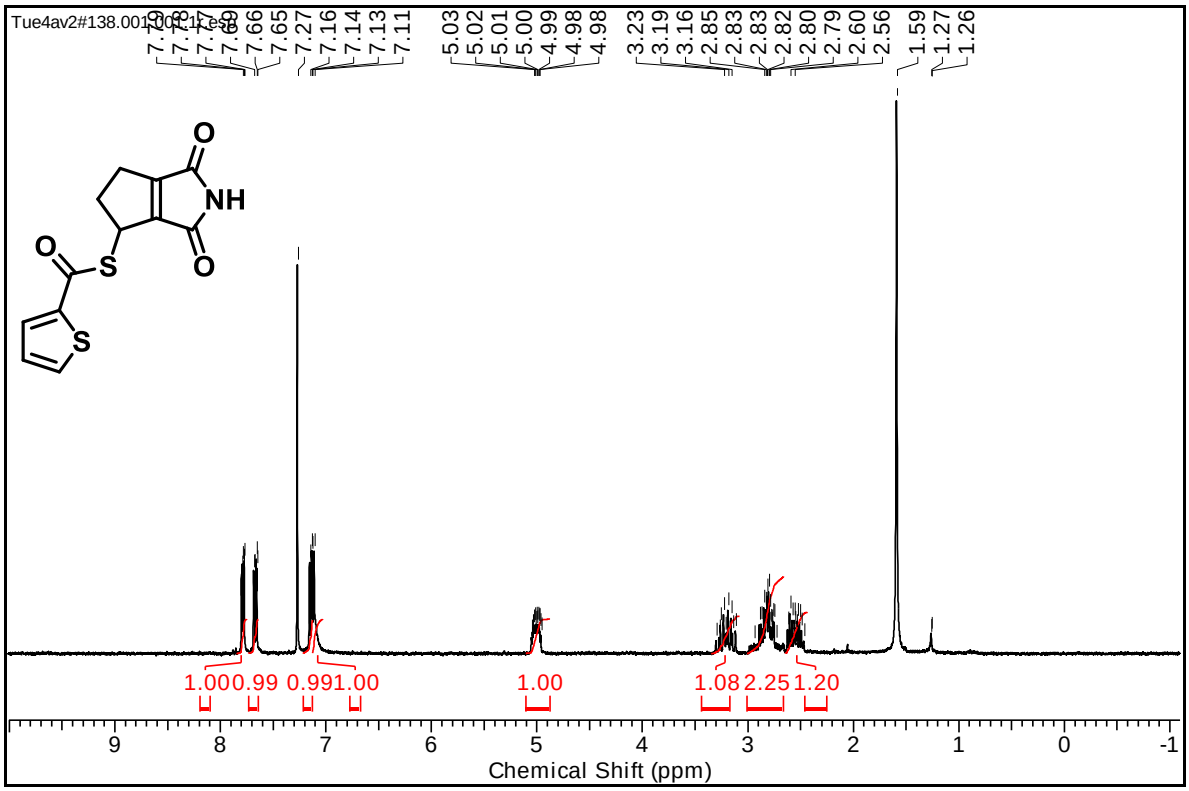
^{1}H NMR Spectrum of compound 41 in $CDCl_3$ at 400 MHz



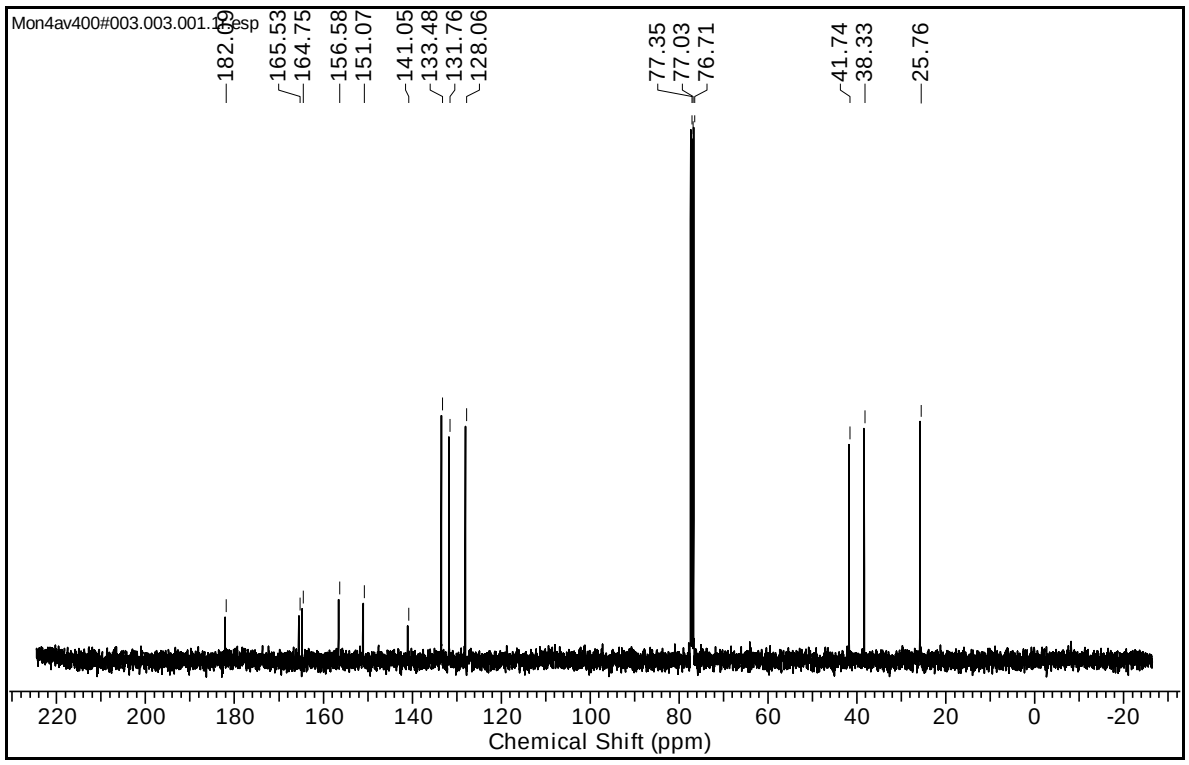
^{13}C NMR Spectrum of compound 41 in $CDCl_3$ at 100 MHz



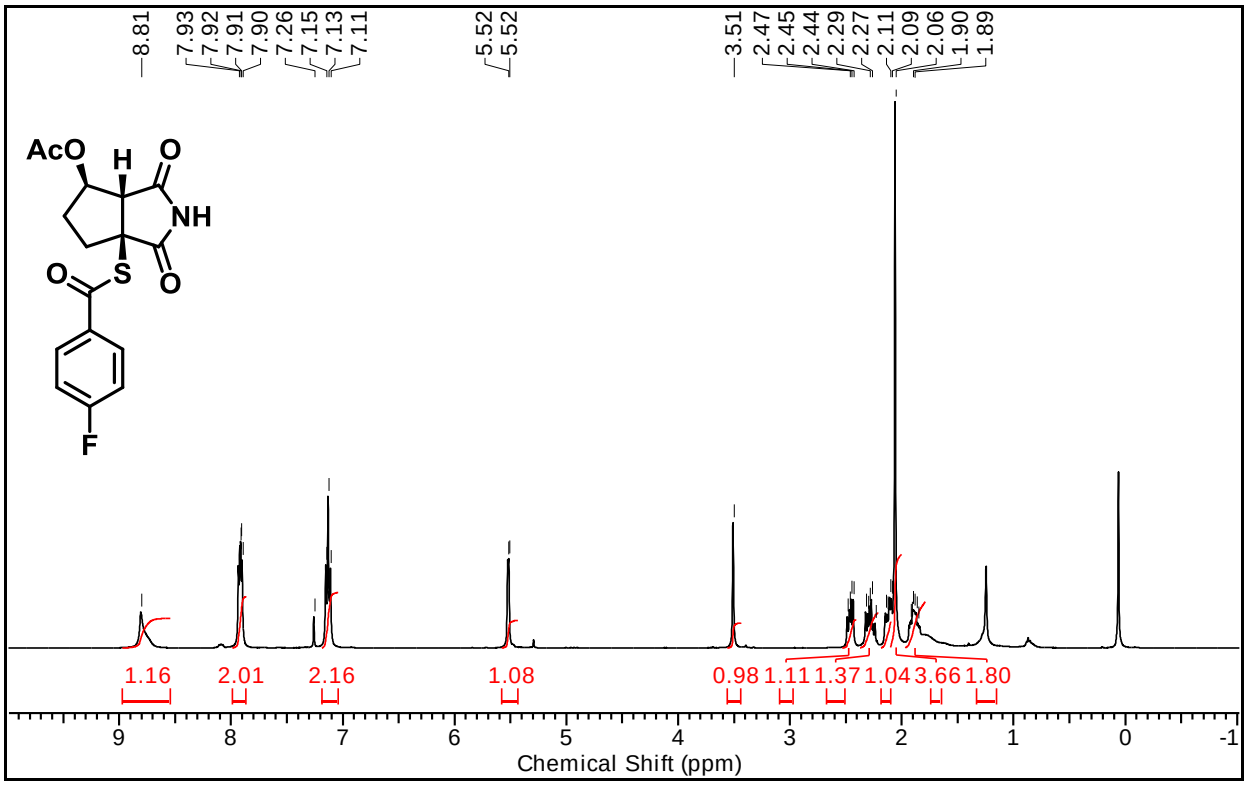
^{1}H NMR Spectrum of compound 42 in $CDCl_3$ at 200 MHz



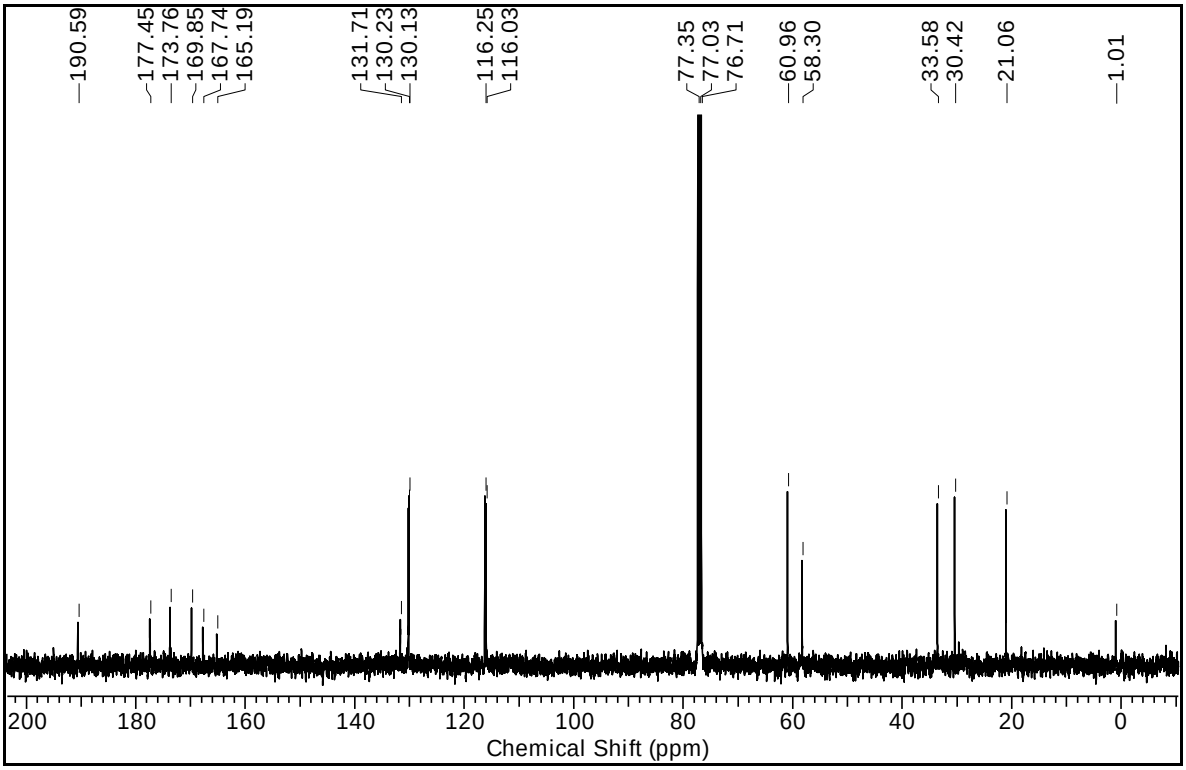
^{13}C NMR Spectrum of compound 42 in $CDCl_3$ at 100 MHz



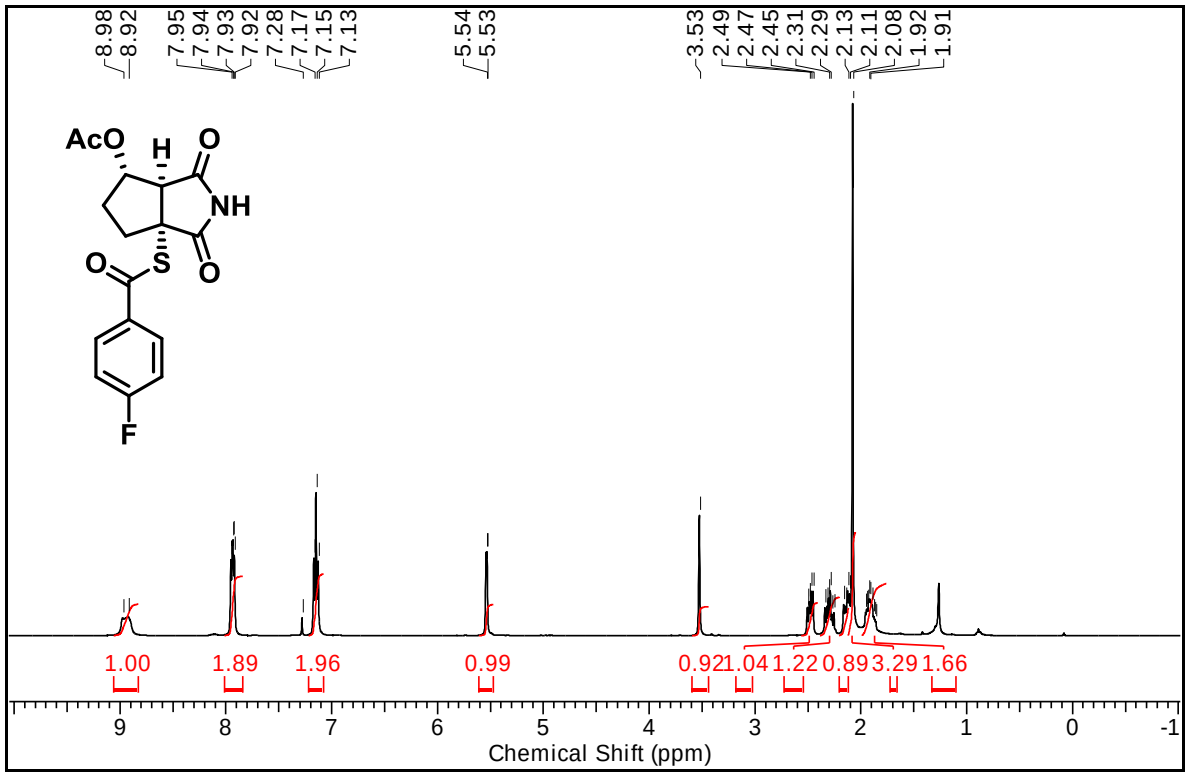
^{1}H NMR Spectrum of compound (-)-25b in $CDCl_3$ at 400 MHz



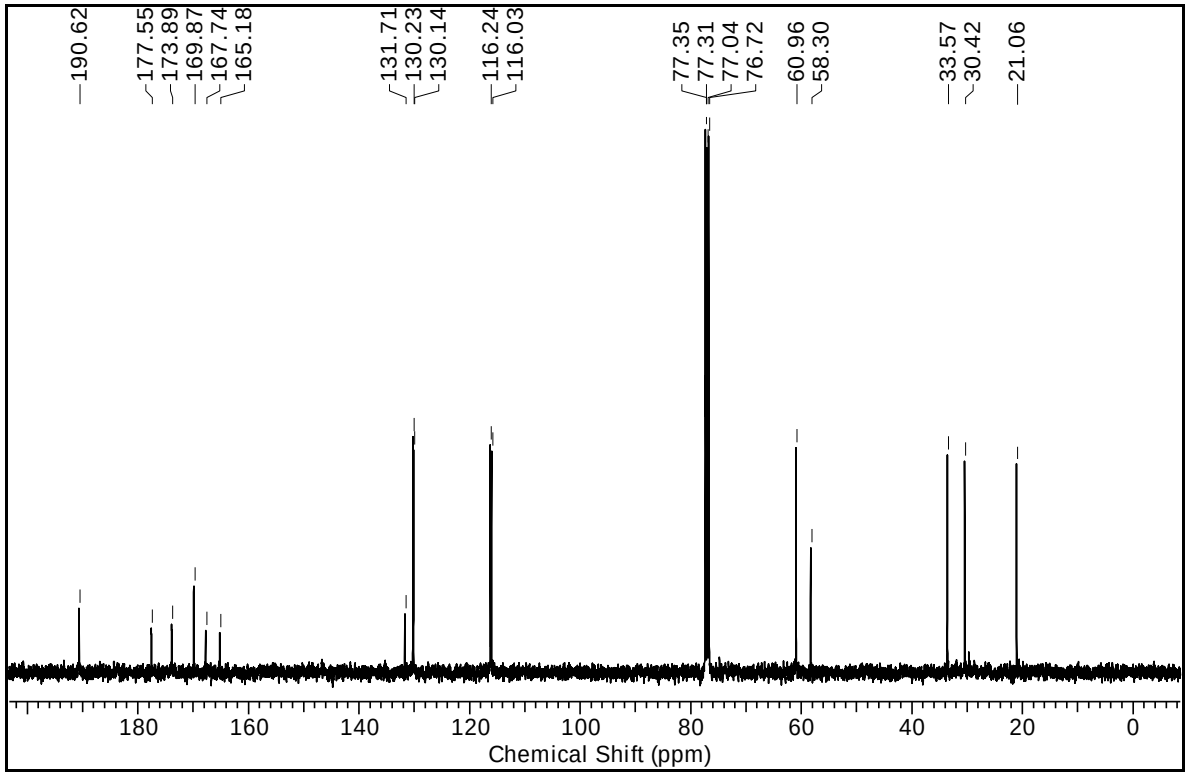
^{13}C NMR Spectrum of compound (-)-25b in $CDCl_3$ at 100 MHz



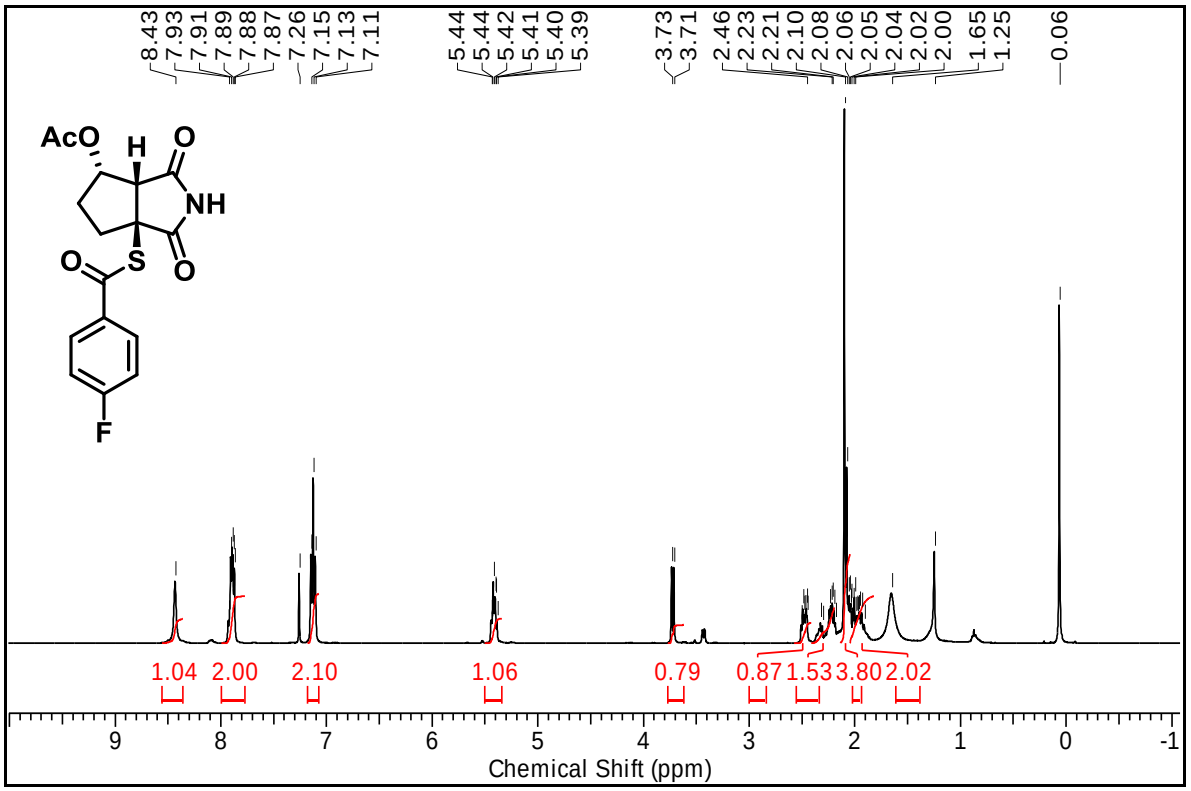
^{1}H NMR Spectrum of compound (+)-25b in $CDCl_3$ at 400 MHz



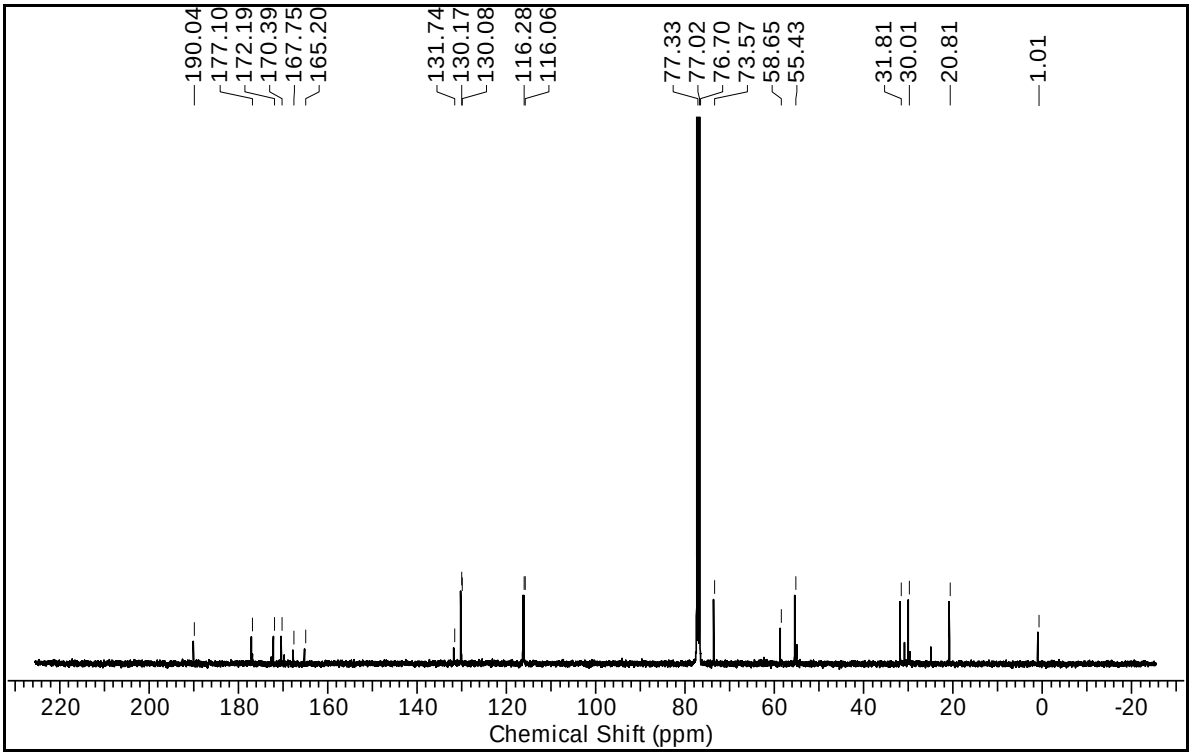
^{13}C NMR Spectrum of compound (+)-25b in $CDCl_3$ at 100 MHz



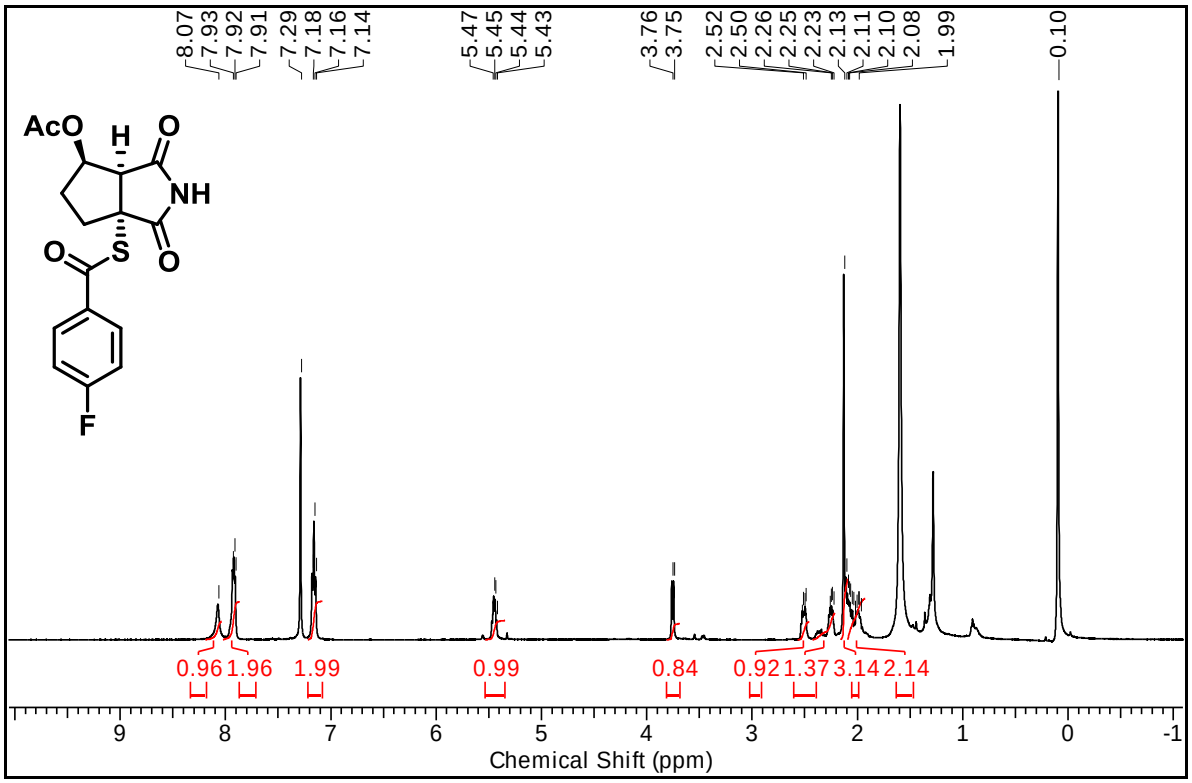
^{1}H NMR Spectrum of compound (–)-25a in $CDCl_3$ at 500 MHz



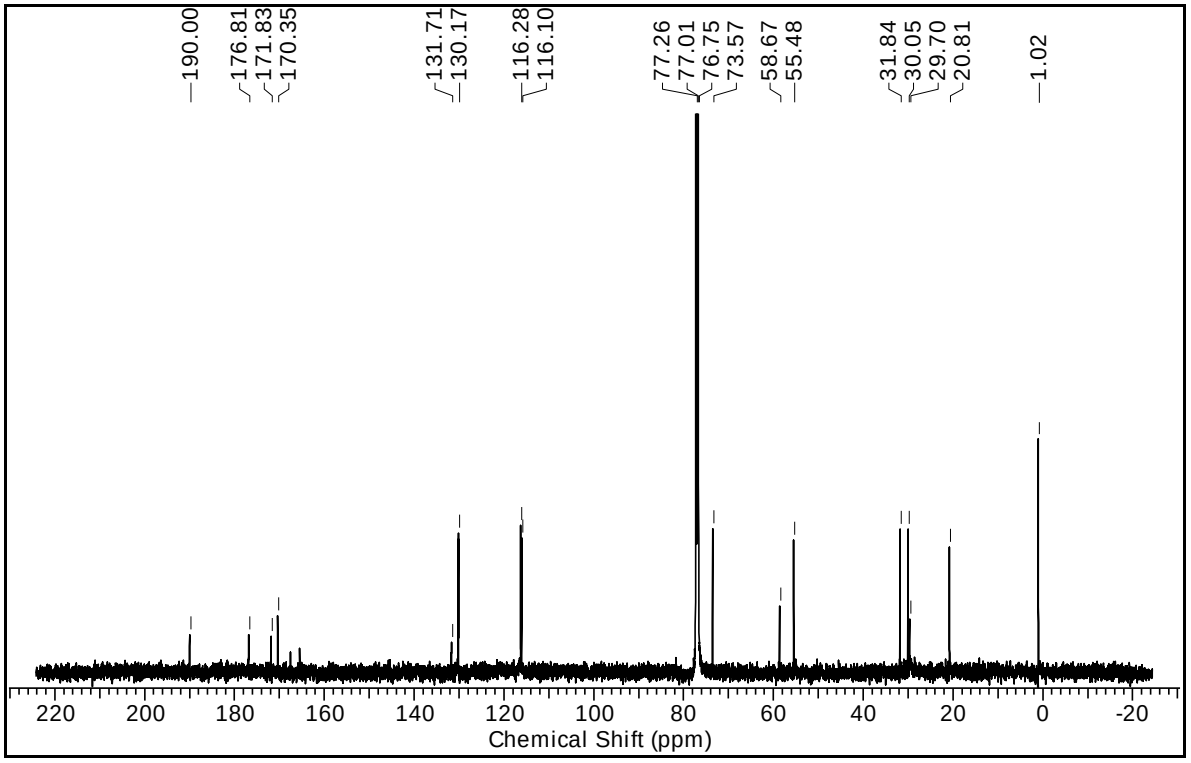
^{13}C NMR Spectrum of compound (–)-25a in $CDCl_3$ at 125 MHz



^{1}H NMR Spectrum of compound (+)-25a in $CDCl_3$ at 500 MHz



^{13}C NMR Spectrum of compound (+)-25a in $CDCl_3$ at 125 MHz



2.8 Copies of HPLC reports of compound **11**

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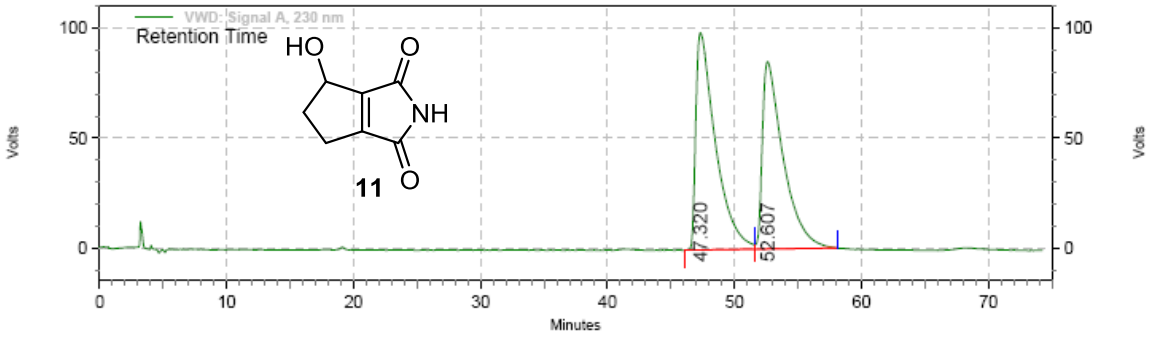

VWD: Signal A, 230 nm Results

Retention Time	Area	Area %	Height	Height %
47.320	177984022	52.27	1650872	53.70
52.607	162535868	47.73	1423406	46.30
Totals				
	340519890	100.00	3074278	100.00

Column : Chiral pak IB
Eluent System : 95 : 5 (PE:IPA)
Flow rate: 1mL/min
Injection vol. 20ul

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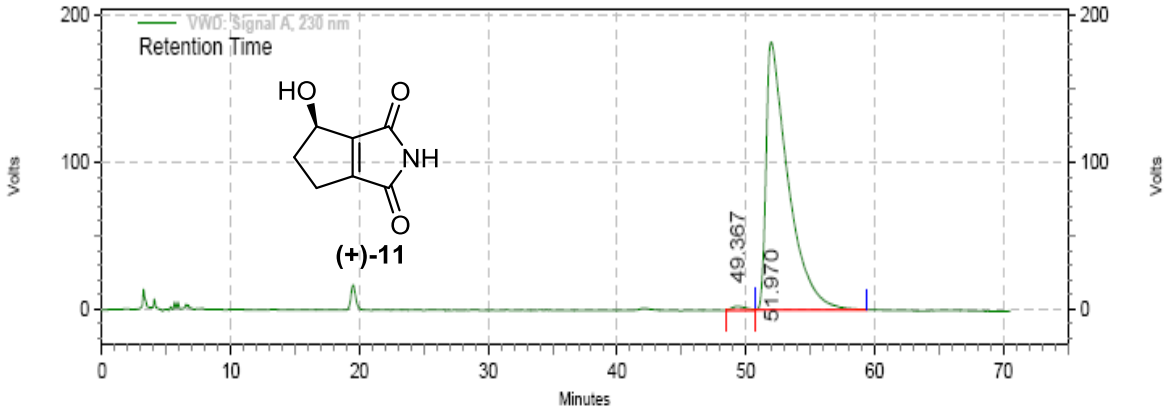

VWD: Signal A,
230 nm Results

Retention Time	Area	Area %	Height	Height %
49.367	3176746	0.87	41381	1.34
51.970	364007459	99.13	3057060	98.66
Totals				
	367184205	100.00	3098441	100.00

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Eluent System : 95 : 5 (PE:IPA)
Flow rate: 1mL/min
Injection vol. 20ul

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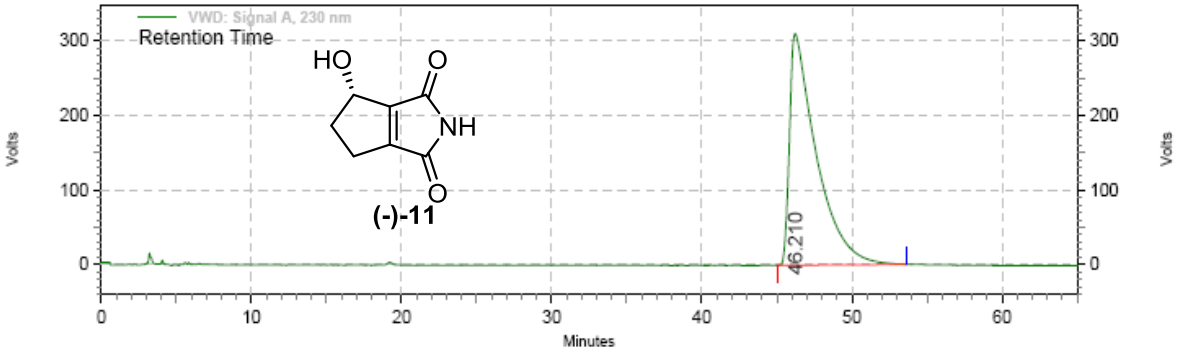

VWD: Signal A, 230 nm Results

Retention Time	Area	Area %	Height	Height %
46.210	643294249	100.00	5203057	100.00
Totals				
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Column : Chiral pak IB
Eluent System : 95 : 5 (PE:IPA)
Flow rate: 1mL/min
Injection vol. 20ul

Publications and patents

1. Synthesis of palmyrolide A and its *cis*-isomer and mechanistic insight into *trans–cis* isomerisation of the enamide macrocycle **Philkhana, S. C.**; Seetharamsingh B.; Dangat, Y. B.; Vanka, K.; Reddy, D. S. *Chem. Commun*. **2013**, *49*, 3342.

2. Access to harmonine, a chemical weapon of ladybird beetles **Philkhana, S. C.**; Dhasaiyan, P.; Prasad, B. L. V; Reddy, D. S. *RSC Adv.* **2014**, *4,* 30923

3. First synthesis of nitrosporeusines, alkaloids with multiple biological activities. **Philkhana, S. C.**; Jachak, G. R.; Gunjal, V. B.; Dhage, N. M.; Bansode A. H.; Reddy, D. S. *Tetrahedron Lett.* **2015**, *56*, 1252.

4. Synthesis and biological evaluation of palmyrolide A macrocycles as sodium channel blockers towards neuroprotection. **Philkhana, S. C.**; Mehrotra, S.; Murray, T. F.; Reddy, D. S. *Org. Biomol. Chem.* **2016**, *14,* 8457.

5. Identification of new anti-inflammatory agents through synthesis of marine natural products nitrosporeusines and their analogues. **Philkhana, S. C.**; Jachak, G. R.; Verma, A.; Hazra, B.; Basu, A.; Reddy, D. S. *Manuscript under preparation.*

6. Total synthesis of fregenedadiol. **Philkhana, S. C.**; Reddy, D. S. *Manuscript under preparation.*

7. Benzenecarbothioccyclopenta[c] pyrrole-1,3-dione compounds and process for synthesis thereof. **Philkhana, S. C**.; Jachak, G. R.; Gunjal, V. B.; Reddy, D. S. **WO 2016051425**

8. Synthesis of Harmonine analogs and there of. **Philkhana, S. C.**; Dhasaiyan, P.; Prasad, B. L. V; Reddy, D. S. Provisional patent filed at CSIR, New Delhi #1845/DEL/2014.

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Synthesis of palmyrolide A and its *cis*-isomer and mechanistic insight into *trans–cis* isomerisation of the enamide macrocycle†

Satish Chandra Philkhana,[a] B. Seetharamsingh,[a] Yuvraj B. Dangat,[b] Kumar Vanka[b] and D. Srinivasa Reddy*[a]

Concise and protecting-group free synthesis of *ent*-palmyrolide A and (−)-*cis*-palmyrolide A were achieved starting from commercially available (*S*)-citronellal. The key fragment of palmyrolide A, "(5*S*,7*S*)-7-hydroxy-5,8,8-trimethylnonanamide", which makes up the most challenging part of the target molecule, was prepared in just three steps. A plausible mechanism for the *trans–cis* isomerization of the double bond in the macrocycle has been investigated.

(−)-Palmyrolide A (**1**), a neuroactive macrocyclic compound, was isolated from the group of cyanobacteria comprised of *Leptolyngbya* and *Oscillatoria* species from Palmyra Atoll, south of Hawaii, by Gerwick's group.[1] Compound **1** was found to be a potent inhibitor of calcium ion oscillations in murine cerebrocortical neurons with an IC_{50} value of 3.7 μM. Based on other biological assay results, the same compound or its analogues have the potential to act as voltage-gated sodium channel (VGSC) antagonists or inhibitors.[2] We became interested in this target due to its interesting biological activity, intriguing molecular structure, with the presence of the rare *N*-methyl-*trans*-enamide moiety and the *t*-butyl group, and some uncertainty in the configurations. Our initial goal was to make all the four isomers with respect to C5 and C7 centers. Accordingly, we have designed the synthesis starting from citronellal, which is commercially available in both (*R*)- and (*S*)-forms. When we started working on this project, Maio's group reported the synthesis of palmyrolide A and its possible isomers in an elegant manner.[3] By this effort, the relative and absolute configuration of the natural product was established without any ambiguity. The second total synthesis was documented by the group of Brimble in a clever manner by using a sequential ring closing metathesis–olefin isomerization reaction.[4] Very recently, third synthesis was reported by Sudhakar *et al.*[5]

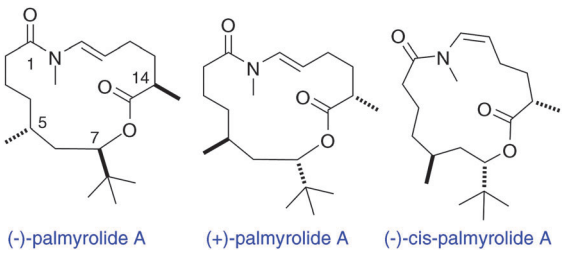

Fig. 1 Structures of target compounds.

After seeing the detailed contribution from Maio's group, we have restricted our focus to the synthesis of the target molecule with established stereochemistry (Fig. 1).

Our synthesis commenced with a reaction of commercially available *t*-BuMgCl on (*S*)-citronellal to obtain an ~1:1 diastereomeric mixture of alcohols **3** in 68% isolated yield. The inseparable mixture of alcohols was subjected to ozonolysis followed by Zhu's oxidative one-carbon homologation[6] of the resulting crude aldehyde to provide the desired primary amides **4** and **5** in moderate yields. To our knowledge, this mechanistically interesting reaction developed by Zhu *et al.* was applied in natural product synthesis for the first time. Both the compounds were cleanly separated using silica gel column chromatography and they were distinguished by comparing the NMR data with those of Maio's intermediates.[3] The undesired isomer for the present purpose was converted to the desired isomer through oxidation (PCC) and reduction ($NaBH_4$) cycles (Scheme 1).[7] It is noteworthy to mention that ketone-like **6** could be reduced selectively using chiral CBS reagents.[8] Thus, the key fragment **4** (5*S*,7*S*-7-hydroxy-5,8,8-trimethylnonanamide), which makes up the significant portion of the challenging part of the target molecule, can be prepared on the gram scale in just three steps starting from commercially available citronellal.

The secondary alcohol present in compound **4** was esterified with known carboxylic acid **7** (ref. 9) using Yamaguchi esterification[10] to obtain acyclic ester **8** in 80% yield. The aldehyde prepared from olefin **8** was subjected to Takai reaction ($CrCl_2$–CHI_3)[11] to furnish Maio's advanced intermediate **9** in 53% yield. Previously, compound **9** was transformed to (+)-palmyrolide A (**1**) using the modified

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[b] *Division of Physical Chemistry, CSIR-National Chemical Laboratory, Dr. HomiBhabha Road, Pune, 411008, India*

† Electronic supplementary information (ESI) available: Characterization data, NMR spectra and detailed experimental procedures. CCDC 920726 and 920727. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3cc40541a

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Access to harmonine, a chemical weapon of ladybird beetles†

Satish Chandra Philkhana,[a] Prabhu Dhasaiyan,[b] B. L. V. Prasad*[b] and D. Srinivasa Reddy*[a]

The synthesis of harmonine, a defense alkaloid from the harlequin ladybird is reported by three different routes. The preparation of several new analogs with the same molecular weight and the decoration of gold nanoparticles with harmonines are also part of the present communication.

The harlequin ladybird, also known as a ladybird beetle (*Harmonia axyridis*), has been introduced as a biological control agent against harmful pests that damage the crops in several countries in Europe and North America.[1] These Asian ladybirds are good eaters of other insects, for example, each beetle consumes roughly 200 aphids per day. Hence, it became popular and is considered as a green way to fight pests and has been commercially available for several decades.[1–3] Wiesner's group in Germany recently discovered that a defense alkaloid harmonine **1**, also known as (17*R*,9*Z*)-1,17-diaminooctadec-9-ene, is the principal compound with a broad spectrum of biological activities from the haemolymph of this species.[4] Compound **1** displayed very interesting biological activities against a variety of pathogens (see ESI† for the detailed biological activities). However, in recent times, it became a global problem because of the successful invading potential of the Asian ladybirds, where they outperform native ladybird species.[1,2] Sometimes, they can enter into houses in hundreds, leading to nuisance and allergies. They also affect the fruit industry by altering the quality of products through contamination.[3] These obnoxious Asian ladybirds can be made useful by utilizing harmonine as a lead compound towards discovery of new drugs. We initiated a medicinal chemistry program based on this chemotype and our intention is to make the target compound in good quantity and generate a focused library of compounds around this molecule, to understand SAR and ultimately find a lead compound. Although harmonine was isolated and synthesized[5] two decades ago, very recently, it was in the news[6a] and was highlighted on many internet blogs.[6b] The design and synthesis of harmonine and its analogues using three different approaches are described here.

Retrosynthetically, the target alkaloid harmonine was envisioned in two different strategies as shown in Fig. 1. The classical Wittig reaction and the modern *Z*-selective cross metathesis were chosen as key steps in our approach 1 and 2, respectively. Accordingly, the desired intermediates **A–D** are planned as the key building blocks which could be prepared from readily available starting materials. The only chiral center present in the harmonine can be introduced by use of amino acid D-alanine.

Our efforts to access harmonine began with the preparation of all the key intermediates **A–D** as described in Scheme 1. The reaction of known aldehyde **2** (ref. 7) and an ylide **B** generated from triphenylphosphoniumbromide **3** (ref. 8) resulted in olefin which on hydrogenation produced the saturated alcohol **4** in 68% yield over two steps. The Swern oxidation of alcohol **3** gave the key intermediate **A**, which on one-carbon Wittig homologation resulted in compound **C**. Compound **D** was prepared as per the published procedures[9] and it was further transformed to

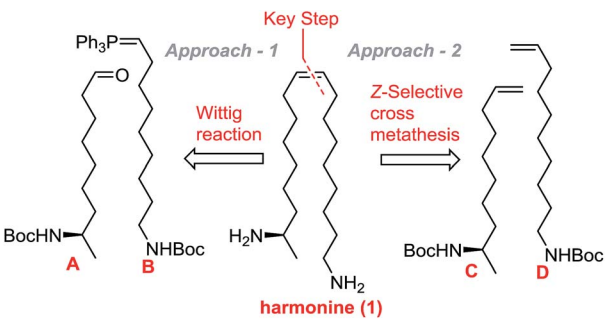

Fig. 1 Planned strategies to access harmonine (**1**).

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First synthesis of nitrosporeusines, alkaloids with multiple biological activities

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ABSTRACT

Synthesis of nitrosporeusines A and B, thioester-bearing alkaloids from the Arctic *Streptomyces nitrosporeus* with exceptional biological activity is disclosed for the first time. In addition, we have prepared another biologically important natural product, maleimycin, in optically pure form using a gram-scale enzymatic resolution method.

One of the dreadful diseases rapidly spreading across the globe is influenza commonly referred to as 'the flu'. Influenza spreads around the world in mostly seasonal epidemics, with an annual attack rate estimated at 5–10% in adults and 20–30% in children. This accounts to about 3–5 million cases of severe illness and about 250,000–500,000 deaths per annum.[1] The currently existing drugs in the market to treat influenza viruses are increasingly becoming ineffective due to constant resistance being developed by viruses[2] and hence the discovery of new inhibitors with a novel mode of action is necessary.[3] The natural products have also been used for the identification of anti-viral compounds with novel scaffolds so as to overcome the menace of drug resistance.[4] One such natural product family, with very good inhibitory activities against the H1N1 virus, is the nitrosporeusines, reported by Lin and co-workers,[5] attracted our attention (Fig. 1). According to this Letter, chemical examination from the sediments of the Arctic Chukchi Sea actinomycete *Streptomyces nitrosporeus* resulted in the isolation of two alkaloids, named as nitrosporeusines A (**1**) and B (**2**), with an unprecedented skeleton containing benzenecarbo-thiocyclopenta[*c*]pyrrole-1,3-dione. Both the compounds **1** and **2** are reported to have shown very impressive multiple biological activities (Fig. 1).[5,6] In the light of the extensive and exceptional

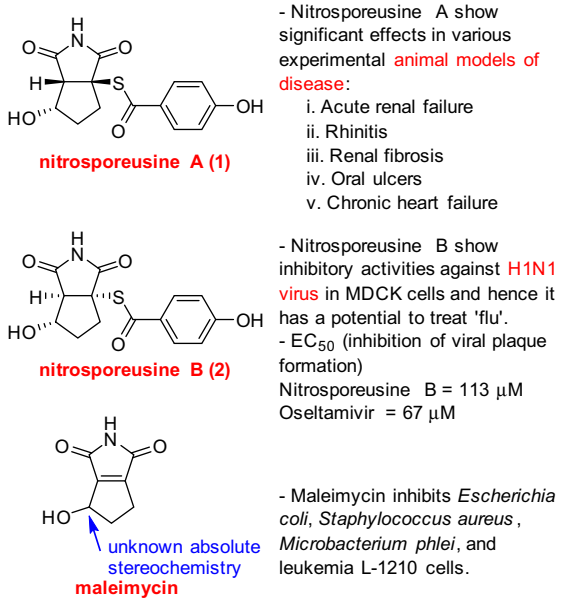

Figure 1. Structures of natural products along with their biological activities.

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Synthesis and biological evaluation of palmyrolide A macrocycles as sodium channel blockers towards neuroprotection†

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Palmyrolide A is a neuroprotective macrolide isolated by Gerwick and coworkers in 2010. This natural product is known to suppress neuronal spontaneous calcium ion oscillations through its voltage-gated sodium channel blocking ability which is of significant interest in CNS drug discovery. Herein, we give a detailed account on total synthesis of (+)-palmyrolide A and synthesis of a focused library of macrocycles around the scaffold, followed by their biological evaluation. Use of the chiral pool approach, Zhu's oxidative homologation, access to unnatural *cis*-palmyrolide A, preparation of 18 new analogues and identification of macrolides with improved sodium channel blocking activity are the important features of the present paper. As a measure of potency as voltage-gated sodium channel blockers, all the synthesized analogues were profiled for their ability to inhibit the veratridine-stimulated Na^+ influx in murine primary neuronal cultures. Four macrocycles were found to be more potent or comparable to that of the natural product (–)-palmyrolide A. The most potent compound from this series **20** was structurally simplified and readily accessible in good quantities for further biological profiling.

Introduction

Natural products have been rich sources of human medicine for a long time as they continue to inspire and serve as starting points for various drug discovery programs.[1] The relatively minor class of natural products (~3%) are macrocycles containing 12 or more membered rings.[2] According to a recent report, almost 100 macrocycles are either marketed drugs or in clinical trials and the most impressive part is that one third of them are oral drugs.[2] The conformational restrictions and relatively large surface areas of macrocycles impart them with higher target binding and improved oral bioavailability, thus making them ideal candidates for developing drugs. In addition, macrocycles offer a balanced rigidity and flexibility for specific binding to the desired targets.[3,4] Because of these features, macrocycles are gaining momentum in the field of medicinal chemistry and there are several macrocyclic marketed drugs[5,4b] like erythromycin, azithromycin, and rifampicin (as antibiotics), amphotericin B (as antifungal) and ixabepilone and peloruside A (as anticancer agents) (see Fig. 1). Our group has continued interest in this exciting class of macrocyclic natural products towards the identification of lead molecules and accordingly, we have been working on various targets such as solomonamides (anti-inflammatory),[6a] gliomasolides (anti-cancer),[6b] and cyanolide A (anti-parasitic).[6c]

Along these lines, we have initiated a program on the natural product palmyrolide A towards identifying a new class of macrocyclic neuroprotective agents. (–)-Palmyrolide A was isolated by Gerwick and coworkers in 2010 from a marine assemblage consisting of *Leptolyngbya* and *Oscillatoria* species at Palmyra Atoll in the Northern Pacific Ocean.[7] It consists of a unique skeleton which is very rarely found in the marocycle literature, a *trans-N*-methyl enamide moiety and a lactone moiety adjacent to the tertiary butyl group, all in a 15 membered ring. The structure and relative configurations in palmyrolide A were established by Gerwick's group through extensive NMR studies and Murata *J*-based configurational analysis. Initial biological studies on (–)-palmyrolide A have shown that it possesses voltage-gated sodium channel blocking ability in Neuro-2a cells with an IC_{50} of 5.2 μM.[7] They also showed a significant

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†Electronic supplementary information (ESI) available: Copies of ^1H and ^{13}C NMR spectra of all new compounds and copies of ^1H NMR spectra of all known compounds. X-ray crystallographic data for **7**, **18** & **30** (CIF). Time-concentration response curves of compounds **7**, **12–14**, **18–25**, **29–30**, **33–34**, **38–41**. CCDC 920726, 1454803 and 1454804. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c6ob01372d

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(54) Title: BENZENECARBOTHIOCCYCLOPENTA[C] PYRROLE- 1,3-DIONE COMPOUNDS AND PROCESS FOR SYNTHESIS THEREOF

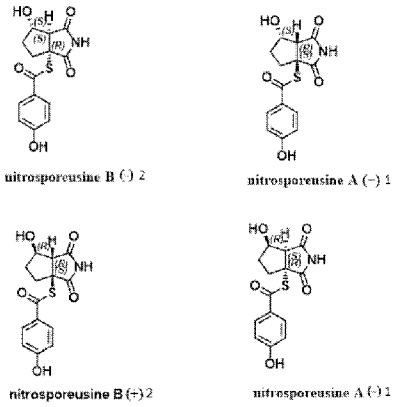
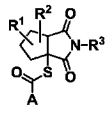


Fig. 1



(I)

(57) Abstract: The present invention relates to a novel analogues of benzene - carbothioccyclopenta[c]pyrrole-l,3-dione of formula (I) useful for treating various viral infections and process for synthesis thereof. The present invention provides a novel process for synthesis of nitrosporeusinesA(l) and B(2). More particularly, the present invention provides a synthetic route for synthesis of nitrosporeusinesA(l) and B(2). Said process is simple, industrially scalable, cost effective and eco-friendly.

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