

A Divergent, Stereoselective Synthesis of Neopeltolide-Inspired Macrocycles And Synthesis of Isoxazole-Derived Macrocyclic Toolbox

A Thesis
Submitted for Degree of
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In Chemistry

BY
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living god

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Declaration

I, hereby, declare that the matter embodied in this thesis is the result of research investigation carried-out by me at the Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Hyderabad, India, under the supervision of ***Professor Prabhat Arya***.

In keeping with the general practice of reporting scientific observations, due acknowledgements have been made wherever the work described is based on the findings of other investigators. Any omission, which might have occurred by oversight or error, is regretted.

Dr. Reddy's Institute of Life Sciences
University of Hyderabad
March 2017

Jagan Gaddam

Certificate

This is to certify that the thesis entitled “*A Divergent, Stereoselective Synthesis of Neopeltolide-Inspired Macrocycles And Synthesis of Isoxazole-Derived Macrocyclic Toolbox*” being submitted by **Mr. Jagan Gaddam** to the University of Hyderabad for the award of **Doctor of Philosophy in Chemistry** has been carried out by him under my supervision and the same has not been submitted elsewhere for a degree. I am satisfied that the thesis has reached to the standard of fulfilling the requirements of the regulations relating to the nature of the degree.

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I dedicate this thesis in memory of my Uncle, **Kaalla Yellaiah** and Aunty **Kaalla Laxmamma** to **Living God**. K. Yellaiah My Uncle was my best supporter, guardian and a greatest role model. He was an amazing guardian, mentor. Dear Uncle, wherever you are, you are gone but you will never be forgotten.

Dr. Reddy's Institute of Life Sciences
March 2017

Jagan Gaddam

Synopsis

This thesis entitled, *“A Divergent Stereoselective Synthesis of Neopeltolide-Inspired Macrocycles and Synthesis of Isoxazole-Based Macrocyclic Toolbox”* contains four chapters.

Chapter 1: Synthesis and Biological Evaluation of Electron Transport Chain {Complex-I, II, III, IV and V (ATP Synthase)} Inhibitors, Protein-Protein Interactions and the Importance of Macrocycles

In this chapter, We provided the detailed introduction to mitochondria ATP synthesis through the cellular respiration, which includes, glycolysis and crebs cycle, and oxidative phosphorylation. In oxidative phosphorylation, We discussed the importance of electron transport chain (ETC) complex structures and mechanism, followed by the synthesis of the ETC inhibitors. In addition, We also discussed the importance of protein-protein interactions as the emerging targets for drug discovery. Finally, We have also discussed the importance of macrocyclic compounds (natural products as well as synthetic compounds) in medicinal chemistry and their distinct structural features when compared to their equivalent acyclic small molecules in the context of finding small molecule modulators of protein-protein interactions.

Chapter 2: Stereoselective Synthesis of Neopeltolide-Inspired Macrocycles

2.1 Literature Work on the Synthesis of Neopeltolide and its Analogs

This section covers isolation, biological evaluation and the synthesis of neopeltolide and its analogs, reported over the years, by several leading research groups.

2.2 Stereoselective Synthesis of Neopeltolide-Inspired Macrocycles

Keeping in mind the key biological responses that are associated with neopeltolide, a marine natural product, our interest in this area was developing a novel synthesis method for obtaining a new type of scaffold, termed as neopeltolide-inspired compounds; our generic structure is shown in **Figure 1**. In our design approach, we were keen in retaining the 14-membered macrolide architecture, while bringing some of the diversity-based fragments in the design. For example, as shown in **Figure 1**, we wanted to incorporate the amino acid derivative (i.e. amino alcohol moiety) which would allow obtaining several analogs using the functional group R₁ as the diversity site. As in the case of neopeltolide, we also wanted

to have the pyran ring moiety embedded in our 14-membered ring design. All together, the presence of two diversity groups in our designed scaffold would then allow us obtaining several structurally related analogs for thoroughly exploring their biological application(s). These designed novel small molecules can be classified in two categories, and, they are outlined as follows in **Figure 1**.

(i) The tetrahydropyran macrocycles (**F1.1**)

(ii) The piperidine macrocycles (**F1.2**)

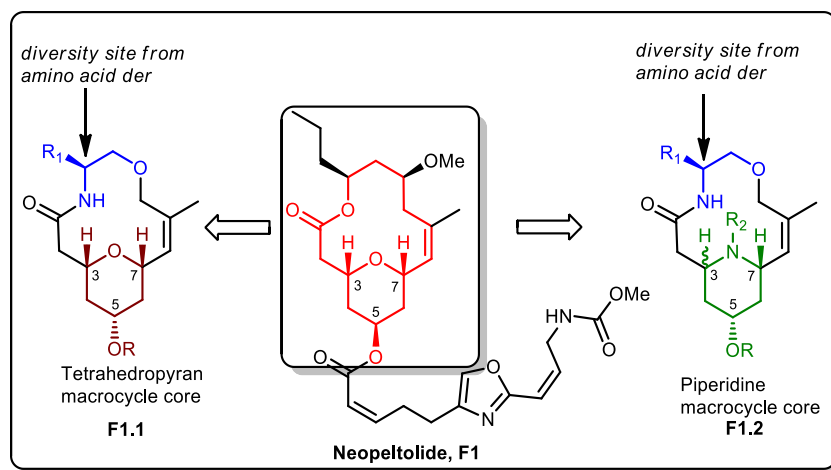


Figure 1: The Proposed Plan to Obtain Neopeltolide Macrocytic Core Like 14-Membered Macrocytic Compounds **F1.1** and **F1.2**

2.2.1 Section A: Synthesis of Tetrahydropyran Core-based Macrocytic Compounds

The retrosynthesis of macrocycle **F1.1** is shown in **Figure 2**. Macrocycle **F1.1** could be obtained by Ring Closing Metathesis (RCM) with Grubbs' 2nd generation catalyst. The corresponding *bis*-olefin was prepared by an amino acid coupling reaction of **F2.1**. Tri-substituted tetrahydropyran motif was synthesized from oxy-Michael addition precursor **F2.2** by a regio- and stereoselective oxy-Michael reaction, followed by the Wittig reaction. Finally, Michael addition precursor **F2.2** could be synthesized from the commercially available, cheap starting material **F2.3**. The key steps involved in our synthesis planning are shown in **Scheme 3**. Our synthesis of the well-stereodefined pyran moiety starts with D-Xylose, **2.1**. It was converted into compound **3.2** in two steps (i.e. bromine oxidation and acetylation) that are well-reported in the literature. Product **3.2** was then converted to **3.3** as the -OH carboxyl ester derivative, in further, two more steps, which was then easily transformed to **3.4**, in a simple series of steps. The primary hydroxyl group in compound **3.4** was then subjected to the leaving group formation; finally, this was then treated with the nucleophilic vinyl attack, giving compound **3.5**. It was then easily converted to compound

3.6 utilizing the carbon-chain extension by the metathesis approach, which is also well-documented in the literature.

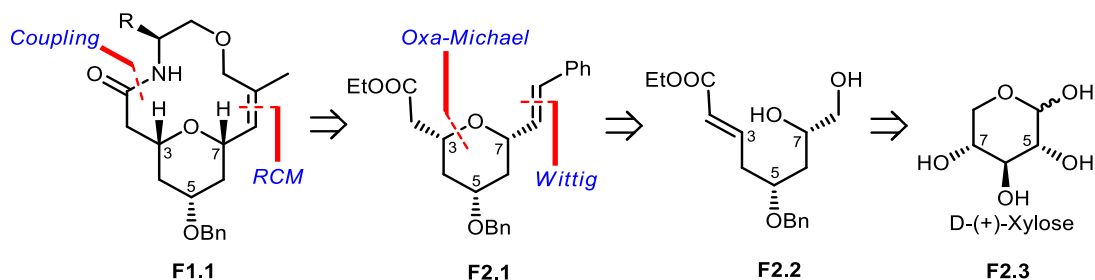
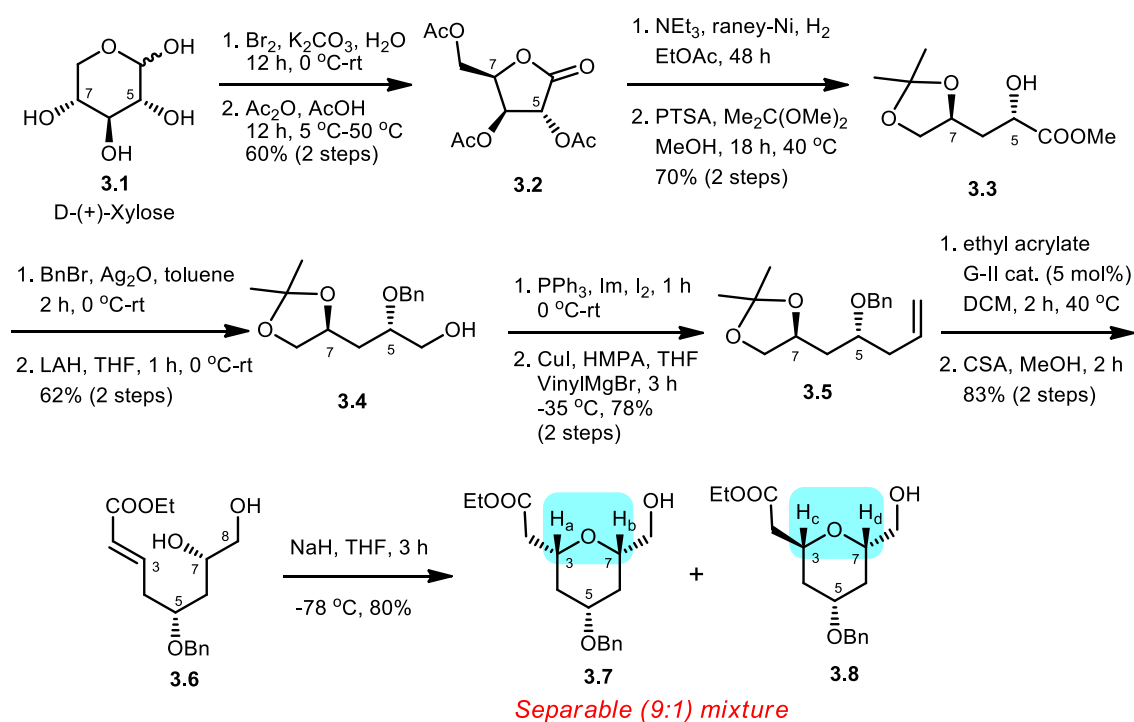


Figure 2: Our Key Disconnections Reaching the Desired Target Compounds

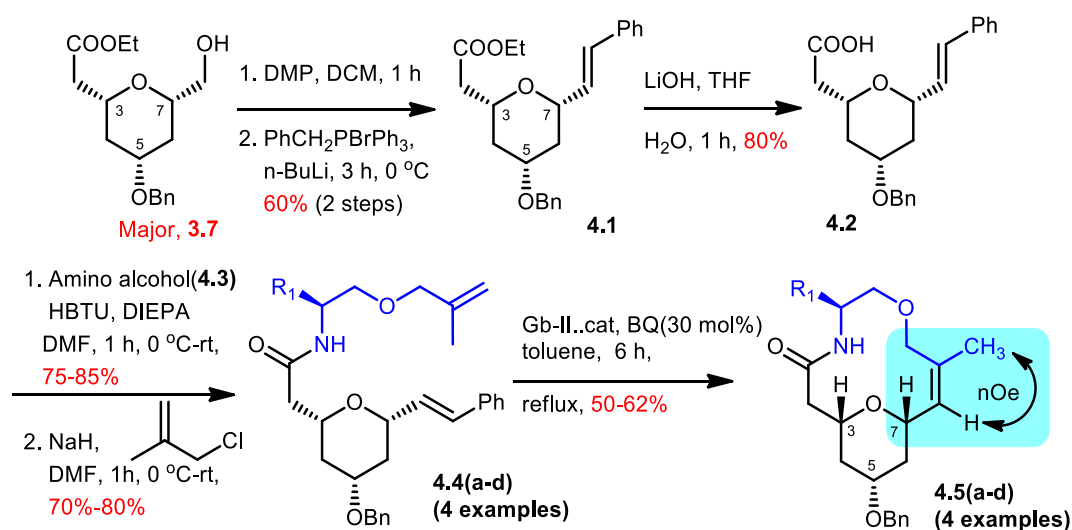
The next crucial steps were to examine the regio- and stereo-selective intra molecular Michael addition reaction under the reaction conditions (see **Scheme 3**). This reaction worked very-well and we observed two separable, cyclic pyrans as **3.7** (major) and **3.8** (minor) products.



Scheme 3: Regio- and Stereoselective Synthesis of the Desired Pyran Derivatives

These two diastereomers were easily separable and both of them were thoroughly subjected to the detailed structural analysis with ^1H -NMR, 1D nOe and 2D nOe studies. Finally, as shown in **Scheme 4**, the major pyran product (compound **3.7**) was subjected to the primary

hydroxyl group modification giving **4.1**, which then led the synthesis of the corresponding acid derivative, **4.2**. This upon coupling with 4 different amino alcohol derivatives



Scheme 4: Completing the Synthesis of 14-Membered Macrocylic Compounds

(compound **4.3**) then produced the key compound **4.4.a-d** as the crucial precursor leading to the final macrocyclic product **4.5a-d**. When subjected to the key macrocyclic ring closing metathesis reaction, all the four compounds **4.4a-d**, successfully produced the desired 14-membered macrocyclic ring products, **4.5a-d** in good yields. The products at every step were properly purified and thoroughly analyzed by ¹H and 2D NMR and MS studies. The geometry of the tri-substituted olefin was assigned by observing positive 2D nOe between C-9 methyl and C-8 hydrogen.

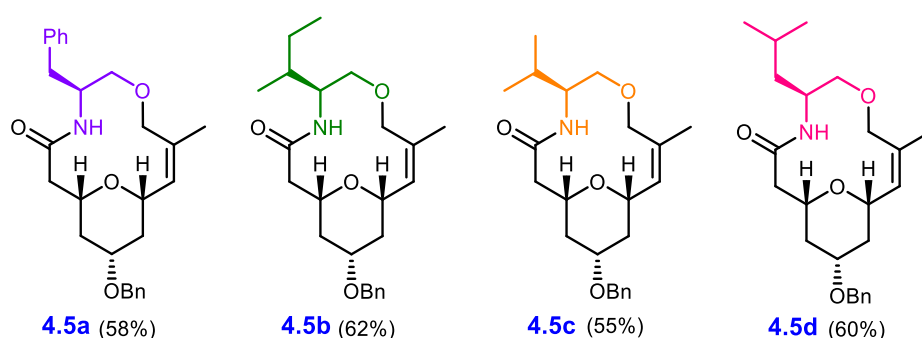


Figure 3: Four 14-Membered Macrocylic Compounds Synthesized

2.2.1 a Biological Evaluation

All the small molecule obtained from this project were then subjected for different biological studies with our various collaborators and outlined below is one case study example in the area of stem cells research.

Chemical Approaches to Trans -differentiate Human (from Healthy Donor) Mesenchymal Stem Cells to Neurons by Small Molecules (Note - this study was performed as a collaboration with Transcell Biologics, a stem cell-based biotech company in Hyderabad) Compounds were screened (10.0 μ M each) on donated (from healthy donor) cord tissue derived mesenchymal stem cells based predictive platform with the positive control as cells exposed to forskolin (note - the structure is not shown in this document) and the internal control as the cells exposed to known neuro-inductive medium. The phenotypic changes obtained with active compounds below. Further, the expression of bio-markers of neurons like nestin and agrin are qualitatively evaluated in the cells by Polymerase Chain Reaction (PCR).

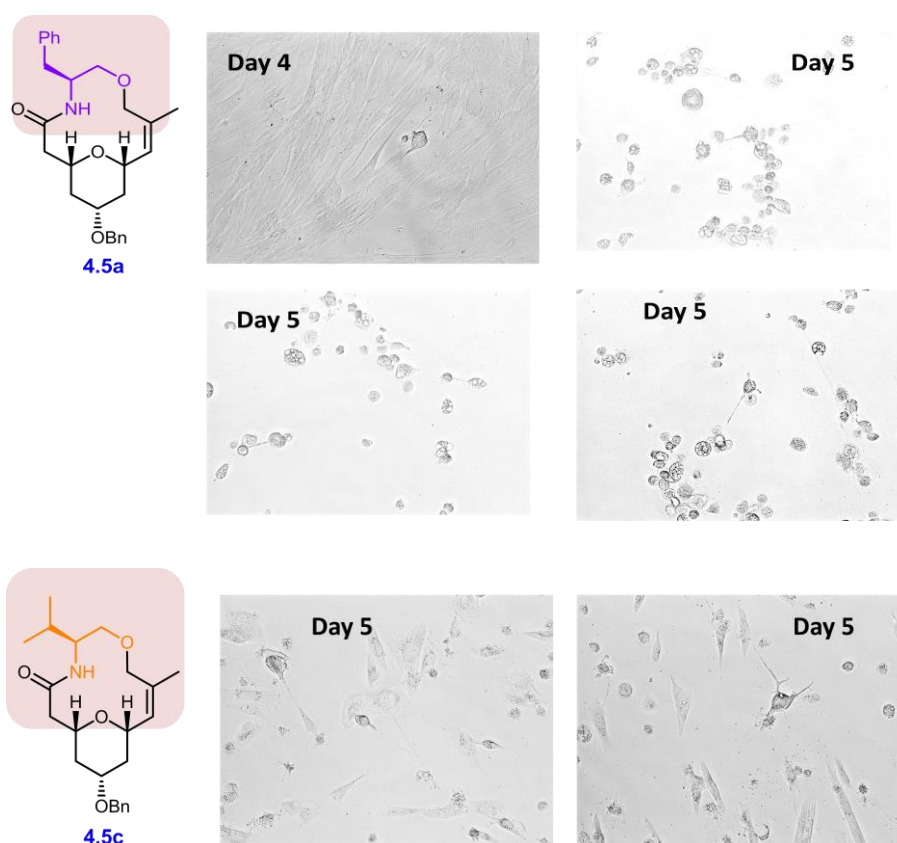


Figure 3a: The Direct Conversion of Human Mesenchymal Stem Cells to Neurons by Small Molecules

2.2.2 Section B: Synthesis of Piperidine Core Macrocycle

Macrocycle **F1.2** was accessed by Ring Closing Metathesis of *bis*-olefin which was obtained from tri-substituted piperidine **F4.2** in two steps (i.e. hydrolysis and coupling reaction). Compound **F4.2** could be prepared from stereoselective aza-Michael addition reaction of **F4.1**. This precursor could be prepared from L- Aspartic acid **F4.3** in a couple of steps.

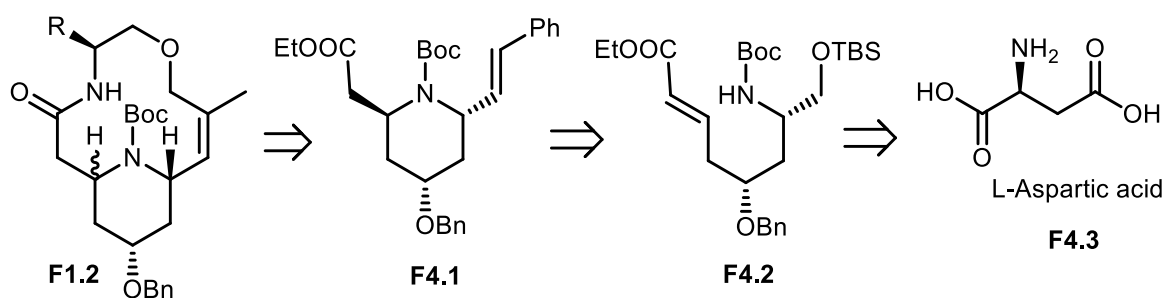
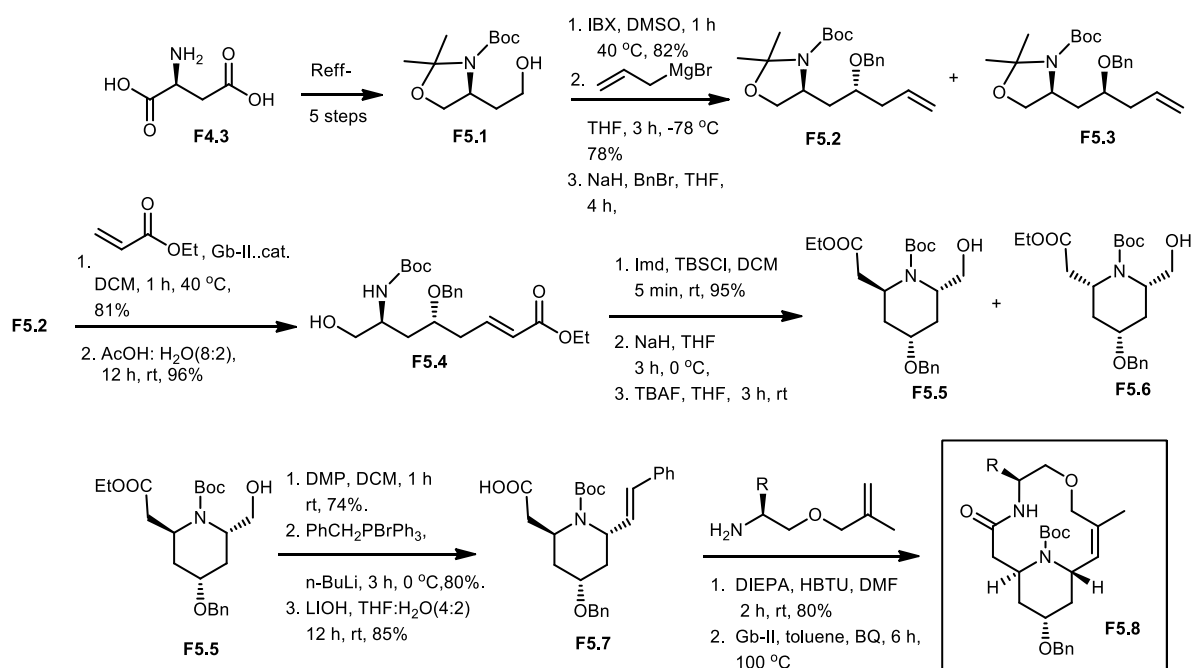


Figure 4: Our Key Disconnections Reaching the Desired Target Compound

Synthesis of Macrocycle **F1.2**

We started the synthesis tri-substituted piperidine **F4.2** with commercially available, cheap starting material (L-aspartic acid **F4.3** as shown in **Scheme 5**. It was converted into compound **5.1** in four steps in good yields. It was then converted into an aldehyde derivative followed by subsection to allylation reaction and this approach offered two diastereomers, separated by column chromatography.



Scheme 5: Synthesis of Piperidine Core Macrocycle

The allyl product, secondary -OH group was protected with benzyl bromide gives **5.2** (major) and **5.3** (minor). It was then easily subjected to the carbon-chain extension by the metathesis approach. Followed by the acetonide removal with p-TSA monohydrate for obtaining **5.4**, the next silyl protection of hydroxyl group was undertaken. It was followed by a crucial stereoselective intra molecular aza-Michael addition reaction under the

mentioned reaction conditions. This reaction worked very-well and we observed two separable, cyclic piperidine as **5.5** (major) and **5.6** (minor) products. The major piperidine product (compound **5.6**) was subjected to primary hydroxyl group modification, oxidation, Wittig reaction, and, hydrolysis giving **5.7**. This upon coupling with different amino alcohol derivatives produced the *bis*-allyl key compound as the crucial precursor to the final macrocyclic product. It was then subjected to the key macrocyclic ring closing metathesis reaction for completing the synthesis of **5.8**. *This work is still in progress*. In RCM reaction, we used 1,4 benzoquinone for avoiding the polymerization and, isomerization of the double bond products at high temperature (>100 °C). All the products in this synthesis program were properly purified and thoroughly analyzed at every step (except the last step) by ¹H and 2D NMR and MS studies.

2.3 Selected References

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Chapter 3: A Divergent Synthesis of Isoxazole-based Macrocyclic Toolbox

Given the importance of the isoxazole moiety as the "privileged scaffold", we were interested in utilizing this sub-structure in developing the design and synthesis of a new family of different types of macrocyclic compounds for exploring their biological application(s). For our first generation approach, as shown in **Figure 5**, we focused on the design and synthesis of compounds with 15-membered macrocyclic rings, having several attractive features. These unique features are: (i) the presence of an amino acid moiety

allow exploring the scope of a chiral functional group (ii) the embedded isoxazole moiety which is considered as the privileged substructure may provide the additional features while binding with the biological targets, and (iii) finally, the presence of two diversity sites located on the 15-membered macrocyclic ring would allow further the "chemical space exploration" by obtaining several structurally related analogs. In addition to these features, our modular design approach which allows connecting the amino acid moiety either from the aromatic ring side or the isoxazole moiety would also lead to obtaining two entirely different 15-membered rings.

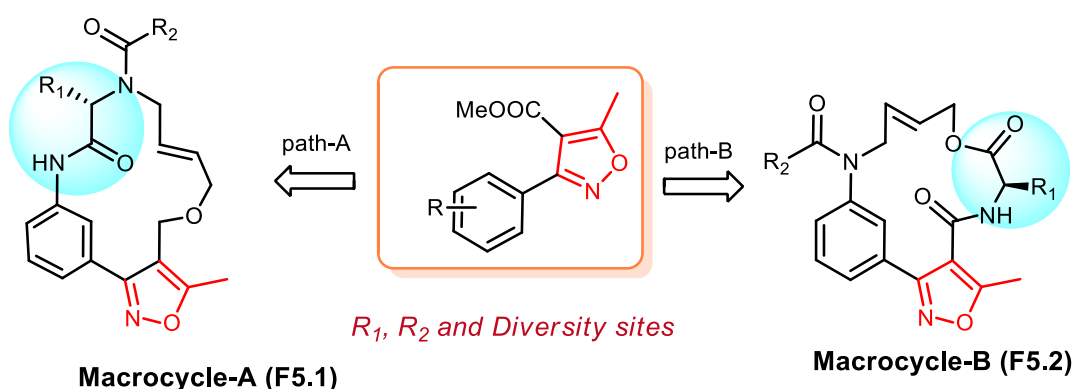


Figure 5: Our Diversity-based, Design Approach for Obtaining Two Different 15-Membered Macrocyclic Compounds Having an Embedded Isoxazole Moiety.

Our interest is in accessing these novel macrocyclic small molecules for several reasons, and some of these include: (i) given the macrocyclic nature of these compounds, they provide an excellent opportunity for accessing compounds that are different from the traditional types of compounds, generally belonging to the category of simple heterocyclic compounds; and (ii) these compounds are also attractive for exploring their value in undertaking biological targets, considered difficult reaching by conventional small molecules, for example, targets dealing with protein-protein interactions for covering a large surface area.¹ These compounds are sufficiently complex and could easily be taken further for developing the next level, medicinal chemistry studies, following the identification of interesting hit compounds.² A practical and relatively ease of the synthesis for these compounds is a key attractive factor in this design approach. These compounds are also considered close to a wide variety of bioactive natural products, thus their probability of interacting with the biological targets, may be higher, when compared to highly simpler heterocyclic small molecules.³

3.1 Retrosynthesis of Macrocycle A F5.1

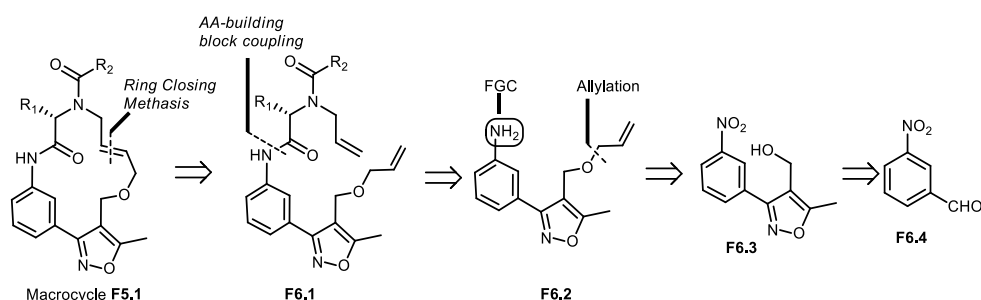
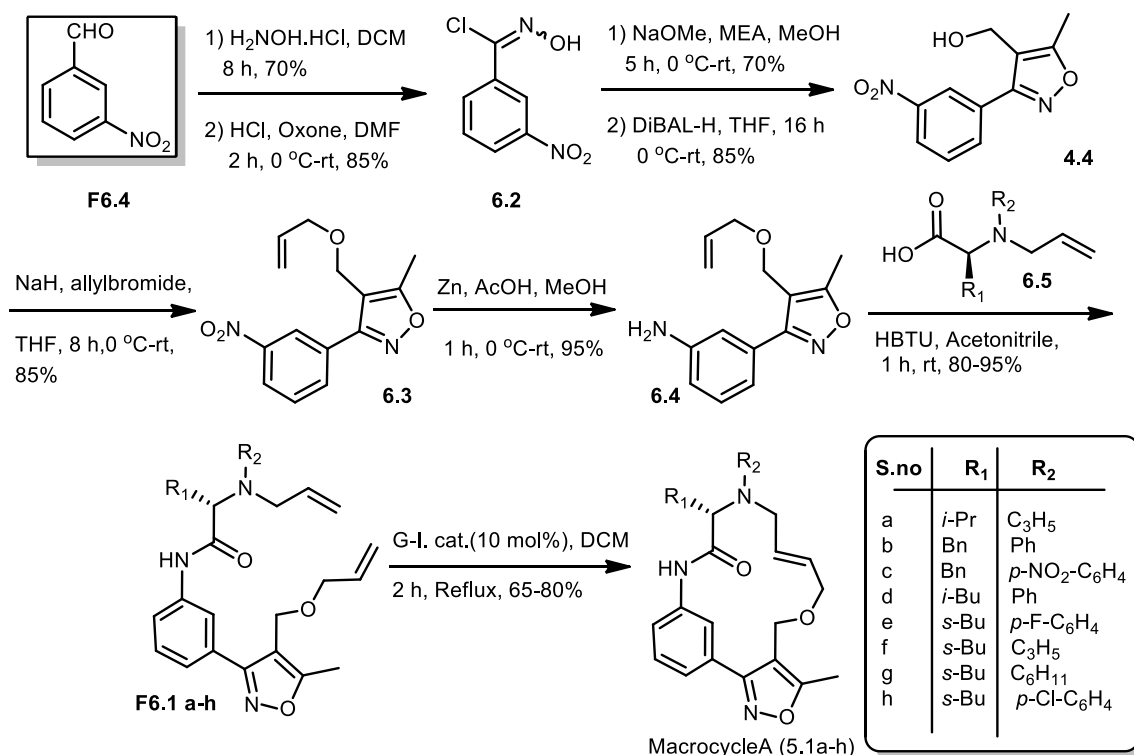


Figure 6: Retrosynthesis of Macrocycles A

The retrosynthetic analysis of macrocycle A is shown in **Figure 6**. The macrocyclization was planned by ring closing metathesis of bi-s-allylated compound **F6.1**. Compound **F6.1** was obtained from **F6.2** through amino acid building block coupling. **F6.2** was prepared from nitro group reduction and *O*-allylation of alcohol **F6.3**, which was planned to be synthesised from **F6.4** by chlorooxime preparation, (3+2) cycloaddition reaction followed by the corresponding ester reduction.



Scheme 6: Synthesis of Macrocycle-A Series Compounds

3.2 Synthesis of Macrocycle A (F5.1) Series

Our approach to the synthesis of isoxazole-derived macrocyclic compounds (**macrocycle A**) is shown in **Scheme 6**. The synthesis starts with *m*-nitrobenzaldehyde (**6.4**) and this was

then converted to the corresponding chlorooxime **6.1** in two steps aldoxime formation with $\text{NH}_2\text{OH}\cdot\text{HCl}$, followed by chlorooxime with oxone and HCl , in high yields. This was then utilized leading the synthesis of the isoxazole derivative **F4.4** by [3+2] cycloaddition with methyl acetoacetate (MEA) and sodium methoxide, followed by reduction with lithium borohydride. The primary hydroxyl group in compound **F4.4** was then allylated with allyl bromide, giving the product **6.3**, which was then subjected to nitro group reduction with Zn/AcOH . This approach led the synthesis of the amine derivative as compound **6.4**. The free amino group in **6.4** was coupled to 4 different *N*-allylated amino acid derivatives (**6.5**) Prepared from (s)-amino acid, with coupling reagent HBTU, giving the coupled product, **F6.1**, as the precursor to the Ring Closing Metathesis (RCM)-based macrocyclic ring formation. Using the first generation Grubbs' catalyst, the reaction worked-well and it produced the expected 15-membered ring macrocyclic compounds (**macrocycle A**, **5.1a-h**). Shown in **Figure 5**, are the eight compounds that were synthesized in diversity-oriented manner utilizing both the diversity sites, (i.e. R_1 and R_2) All the products at every step were thoroughly characterized by ^1H and ^{13}C NMR and HPLC-MS studies. The geometry of the olefin obtained from RCM reaction was assigned with ^1H NMR coupling constant values (coupling constant value is 16.0 Hz), with this result we conform the olefin geometry as *E*-olefin macrocycle, **F5.1**.

3.3 Retrosynthetic analysis of Macrocycle B (F5.2)

In retrosynthetic analysis macrocycle B, **F5.2** was planned through Ring closing reaction (RCM) of **F7.1** which could be prepared from amino acid building block coupling with

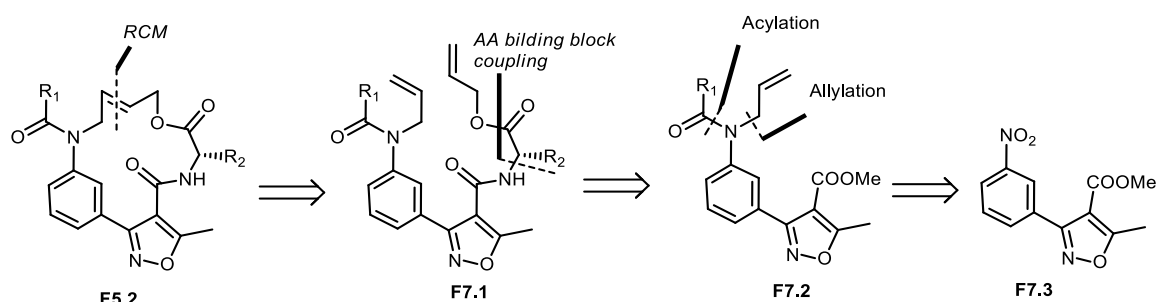
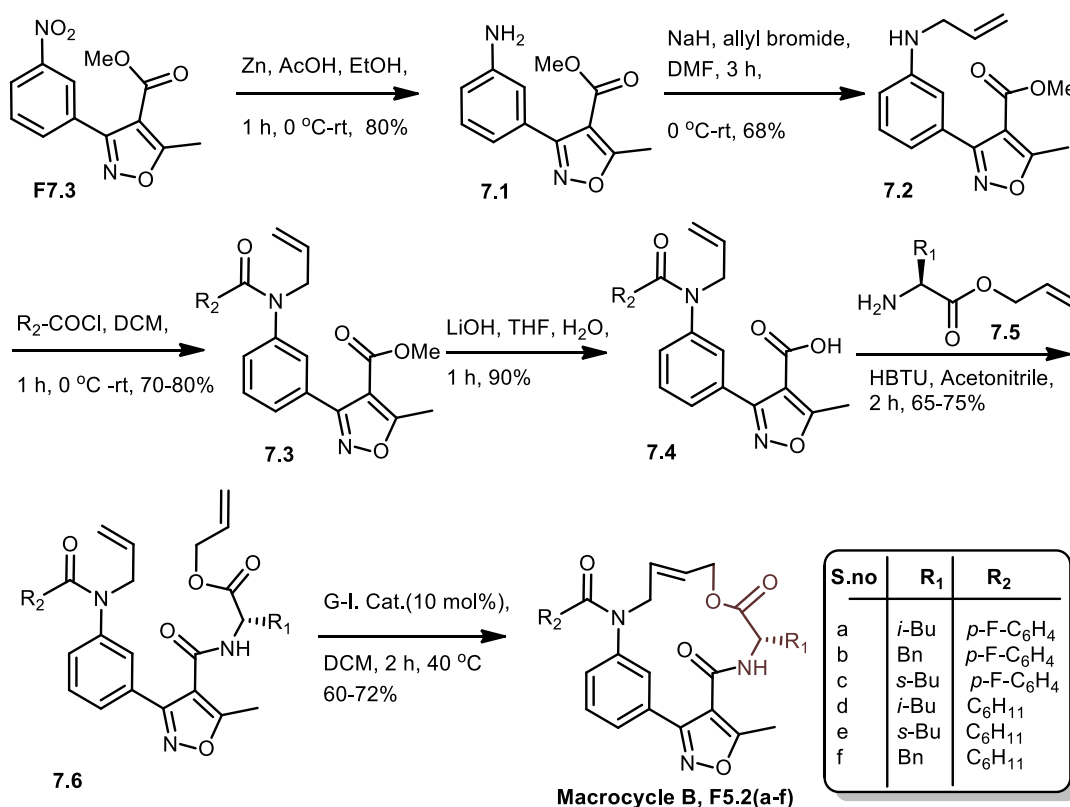


Figure 7: Retrosynthesis of Macrocycle B (F5.2)

F7.2. Compound **F7.2** could be obtained by allylation of free amine which could be resulted from the reduction of nitro compound **F7.3**

3.4 Synthesis of Macrocycle B (F5.2) Series

Our synthesis for the macrocycle B series is shown in **Scheme 7**. It was initiated with compound **F7.3** as the key starting material. This was subjected to nitro group reduction using zinc and acetic acid conditions giving the corresponding amine, as compound **7.1**. Following the mono-allylation which gave **7.2**, this compound was further amidated utilizing different acid chlorides, giving compound **7.3**. It was hydrolyzed, giving the free carboxylic acid (**7.4**), which was then coupled with different amino acid derivatives **7.6**, for obtaining compound **7.7**.



Scheme 7: Synthesis of Macrocycle B Series Compounds (**F 5.2**)

As a precursor for testing our key ring closing metathesis-based macrocyclic ring formation, **7.7** were then taken further for this step. The macrocyclic ring synthesis worked-well by using Grubbs' 1st generation catalyst (10 mol%). This approach was tried on six substrates, and in all six cases, the 15-membered ring was formed easily, and also with the single, *E*-olefin geometry across the double bond (see compound **F5.2a-h** structures). The structural information on all six products at every step was thoroughly obtained and each compound was purified by flash column chromatography and extensively analyzed by ¹H and ¹³C NMR and MS studies.

3.5 Selected References

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- (2) Aeluri, M.; Dasari, B.; Arya, P.: Divergent Approach to Building a Latrunculin Family Derived Hybrid Macrocyclic Toolbox. *Org. Lett.* **2015**, *17*, 472-475.
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- (5) Guduru, S. K. R.; Chamakuri, S.; Chandrasekar, G.; Kitambi, S. S.; Arya, P.: Tetra hydroquinoline-derived Macrocyclic Toolbox Identifies Novel Anti angiogenesis Agents and Inhibitors of an Early Embryo Development in Zebrafish Assays. *ACS Med. Chem. Lett.* **2013**, *4*, 666-670.

Chapter 4: Synthesis of Novel Pyrrolidine and Piperidine Scaffolds by Cycloaddition Reaction of Tert-butylsulfonamideimine/sulfinamideimine and Allenolate.

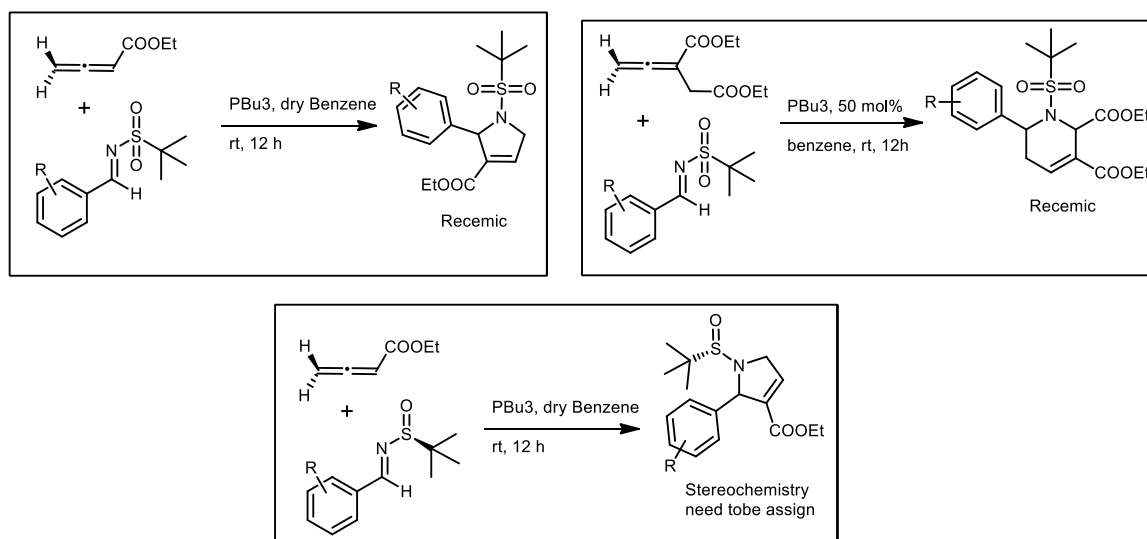


Figure 8: Synthesis of Pyrrolidine and Piperidine Analogs

The pyrrolidines and piperidine moiety are the significant component of various natural products, pharmaceuticals and functional materials. In this chapter, I discussed the synthesis

of new pyrrolidine and piperidine scaffolds and efforts to synthesis of chiral scaffolds using tert-butylsulfonamideimine/ sulfonamideimine as the key core and allenolate as the coupling partner and tri-butyl phosphine as a catalyst, in good yields.

Selected References

- (1) Xu, Z.; Lu, X. *Tetrahedron Lett.* **1997**, 38, 3461.
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1. 17-Membered Macrocyclic Ring Derived Toolbox: Identification of Small Molecule Inhibitor of Angiogenesis. Aeluri, M.; Gaddam, J.; Trinath, D. V. K. S.; Chandrasekar, G.; Kitambi, S. S.; Arya, P. *Eur. J. Org. Chem.* **2013**, 3955-3958.
2. Macrocyclic toolbox from Epothilone Fragment Identifies a Compound Showing Molecular Interactions with Actin and Novel Promoters of Apoptosis in Patent-derived Brain Tumor cells. Bhanudas Dasari, Temesgen Fufa, Madhu, Aeluri, Jagan Gaddam, Girdhar Singh Deora, Frank Gaunitz, Satish Srinivas Kitambi, and Prabhat Arya. *Asian. J. Org. Chem.* **2016**, 5, 976-980.
3. Geldanamycin-Inspired Compounds Induce Direct Trans-differentiation of Human Mesenchymal Stem Cells to Neurons. Srinivas Jogula, Anand Soorneedi, Jagan Gaddam, Srinivas Chakakuri, Girdhar Singh Deora, Ranjith Kumar Indarapu, Subhadra Dravida, and Prabhat Arya. *Eur. J. Med. Chem.* **2017**, reviewer's comments received (close to accepted)

Patents

- 1 A Stereoselective Synthesis of Neopeltolide-inspired Macrocyclic Compounds and their Application in the Conversion of Human Mesenchymal Stem Cells to Neurons. Dravida, S.; Kumar, R.; Soorneedi, A.; Gaddam, J.; Arya, P. **India and US provisional patent (submitted, 2016)**
- 2 Design and synthesis of heterocyclic-derived macrocyclic compounds and their application as the direct converters of human mesenchymal stem cells to neurons.

Subhadra Dravida, Ranjit Kumar, Anand Soorneedi, Jagan Gaddam, Madhu Aeluri and Prabhat Arya. **India and US provisional patent, submitted, 2017.**

Book Chapters

1. Ring Closing Metathesis-based Methods in Chemical Biology: Building A Natural Product-Inspired Macrocyclic Toolbox for Undertaking Protein-Protein Interactions. Gaddam, J.; Mallurwar, N.K.; Khatravath, M.; Konda, S.; Aeluri, M.; Mitra, P.; Arya, P. (*Wiley Publishers, 2017 in press*)

Seminars/Conferences Attended

2013	Presented a poster at DRILS, University of Hyderabad Campus, India
2014	Attended at the X-JNOST 2014 international conference, IIT, Madras, India
2015	Presented a poster at “Trend Setting Innovations in Chemical Sciences & Technology Applications in Pharmaceutical Industry” that was held at JNTUH, Hyderabad, India

Abbreviations

ER	:	Endoplasmic Reticulum
ATP	:	Adenosine Triphosphate
ADP	:	Adenosine Diphosphate
PGK	:	Phosphoglycerate Kinase
NADPH	:	Nicotinamide Adenine Dinucleotide Phosphate
FAD or FADH	:	Flavin Adenine Dinucleotide
FMN	:	Flavin Mono Nucleotide
ISP	:	Iron-Sulfur Protein
IMS	:	Inter Membrane Space
ROS	:	Reactive Oxygen Species
ETC	:	Electron Transport Chain
KCOS	:	Potassium channel openers
Q	:	Ubiquinone
QH ₂	:	Ubiquinol (dihydroxyquinone)
CoQ	:	Coenzyme Q
Ac	:	Acetyl
Ag ₂ O	:	Silver oxide
aq.	:	Aqueous
BnBr	:	Benzyl bromide
Boc	:	<i>t</i> -butoxy carbonyl
CH ₃ CN	:	Acetonitrile
CDCl ₃	:	Deuterated chloroform
CuI	:	Copper Iodide
DBU	:	1,8-Diazabicyclo-[5.4.0]-undec-7-ene
DCM	:	Dichloromethane
DMF	:	Dimethylformamide
DMAP	:	4-Dimethylaminopyridine
DMSO	:	Dimethylsulphoxide
EDCI	:	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EtOAc	:	Ethyl acetate
Et ₃ N or TEA	:	Triethylamine

EtO ₂ CCH ₂ PO(OEt) ₂	:	Triethyl phosphonoacetate
ES	:	Electro Spray
G-II	:	Grubbs 2 nd generation catalyst
H ₂	:	Hydrogen
HMPA	:	Hexamethyl phosphoramide
2N HCl	:	2 Normal Hydrochloric acid
h	:	Hour
Im	:	Imidazole
I ₂	:	Iodine
K ₂ CO ₃	:	Potassium carbonate
KOH	:	Potassium hydroxide
LiAlH ₄	:	Lithium aluminium hydride
Ms	:	Mesyl
NH ₄ Cl	:	Ammonium chloride
NaHCO ₃	:	Sodium bicarbonate
Na ₂ SO ₄	:	Sodium sulphate
NaH	:	Sodium hydride
NaBH ₄	:	Sodium borohydride
NMR	:	Nuclear magnetic resonance
Me	:	Methyl
MeOH	:	Methanol
Zn	:	Zinc
DIBAL	:	Diisobutylaluminium hydride
Py-BOP	:	Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate
MOMCl	:	methoxymethyl chloride
SAMP	:	(S)-(-)-1- Amino-2-(Methoxymethyl)pyrrolidine
CAN	:	Ceric ammonium nitrate
PDC	:	Pyridinium Dichromate
LDA	:	Lithium Diispropylamide
<i>p</i> TSA	:	<i>para</i> -toluene sulfonic acid
Pd/C	:	Palladium on Carbon
PPh ₃	:	Triphenylphosphene
rt	:	Room temperature

R _f	:	Retardation factor
TBDSCI	:	Tert-Butylchlorodimethylsilane
TBAF	:	Tetra-n-butylammonium fluoride
TBAI	:	Tetrabutylammonium iodide
TFA	:	Trifluoroacetic acid
THF	:	Tetrahydrofuran
TLC	:	Thin Layer Chromatography
TiCl ₄	:	Titanium tetrachloride
BQ	:	Benzoquinone
TMS	:	Trimethylsilyl

General Information

^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Varian 400 MHz NMR spectrometer at the frequency indicated. Where indicated, the NMR peak assignments were made using COSY experiments. All chemical shifts are quoted on the δ -scale and were referenced to the residual solvent as an internal standard. Combinations of the following abbreviations are used to describe NMR spectra: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet. Mass spectra and LC-MS were recorded using electron impact, chemical ionization or electrospray ionization techniques, on Agilent-6430 mass spectrometer. High-performance liquid chromatography was carried out on Agilent-1200 instrument using X-BRIDGE C-18 150 $\times$ 4.6mm 5 μ column. Thin layer chromatography (TLC) was carried out on aluminum sheets coated with silica gel 60F₂₅₄ (Merck, 1.05554) and the spots were visualized with UV light at 254 nm or alternatively by staining with aqueous basic potassium permanganate or ceric ammonium molybdate or ninhydrin. Flash column chromatography was performed using silica gel (Merck, 60A, 230-400 Mesh). Commercially available reagents were used as supplied and some of them were distilled before use. All reactions were performed in oven dried glassware. DMF, DCM, MeOH and THF were dried immediately prior to use according to standard procedures: dimethylformamide, dichloromethane was distilled under N₂ from CaH₂, methanol was distilled under N₂ over Mg and tetrahydrofuran was distilled under N₂ over Na. All solvents were removed by evaporation under reduced pressure.



Chapter 1

Synthesis and Biological Evaluation of Electron Transport Chain Complex (I, II, III, IV and ATP Synthase) Inhibitors, Protein-Protein Interactions and the Importance of Macrocycles



1.1 Introduction to Mitochondria

Mitochondria, a double membrane bounded organelles is found in eukaryotic cells,¹ called as the power house of the cell, as they produce most of the chemical energy for the cell survival in the form of ATP.² Mitochondria plays an important role in several cellular functions, such as, oxidative phosphorylation, signal transduction (for example, buffering and storage of intracellular calcium), apoptosis (i.e. programmed cell death), and Fe-S clusters synthesis.³ Mitochondria has four main components: outer membrane, inter-membrane space, inner membrane, and, matrix.⁴

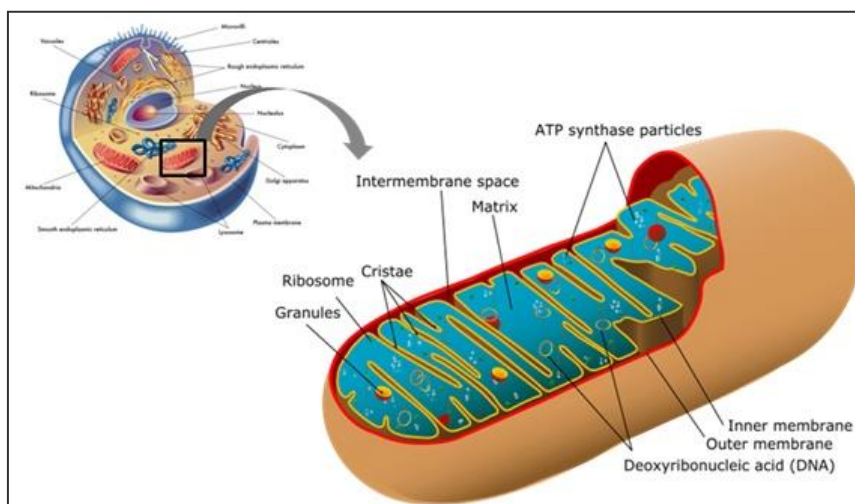


Figure 1: Structure of Mitochondria (taken from tutorvista.com)

1.1a Outer Membrane

The entire mitochondria is enclosed by the outer membrane. It contains a large number of proteins, generally called as porins,⁵ which play a central role in providing the permeability of molecules about 10 kilo daltons or less (the size of the smallest proteins), nutrient molecules, ions, ATP and ADP etc. This membrane is associated with endoplasmic reticulum (ER) membrane, plays an important role in ER-mitochondria calcium signaling, and in the transfer of lipids between the ER and mitochondria.⁶

1.1b Inner Membrane

The inner membrane of mitochondria is made-up of different poly-peptides, proteins and phospholipids. It is freely permeable to oxygen, carbon dioxide, and water. This membrane controls five functions.

1. Redox reactions of oxidative phosphorylation
2. Generation of ATP in the matrix by ATP synthase

3. Specific transport proteins, which regulate the passage of metabolites into and out of the matrix
4. Protein import machinery
5. Mitochondrial fusion and fission proteins

1.1c Intermembrane Space

It is the smallest sub-compartment of mitochondria, present between the outer membrane and inner membrane. However, it plays an important role in the modification of proteins, lipids, metal ions and transport, and in the regulation and assembly of the respiratory chain complexes. Moreover, it is involved in oxidative phosphorylation and also coordinates the key steps in programmed cell death.

1.1d Matrix

It is the space enclosed by the inner membrane, with 2/3 of proteins present in mitochondria. It contains enzymes that are responsible for the citric acid cycle reactions. The matrix also contains dissolved oxygen, water, carbon dioxide, and the recyclable intermediates that serve as the energy shuttles, and much more.

1.2 ATP (Adenosine Triphosphate)

ATP is an abbreviation for *adenosine triphosphate*, a complex small molecule that contains the nucleoside *adenosine* and a tail consisting of three phosphates. It was discovered by Karl Lohmann in 1929.⁶ In all the organisms from simplest bacteria to humans, it is the primary energy currency.⁵ The ATP plays an important role in many cell functions.

1. A major role of ATP is in the chemical work, supplying the needed energy to synthesize the multi-thousands of types of macromolecules that the cell needs to survive.
2. It is also used for the mechanical work, supplying the energy needed for muscle contraction in heart and skelton.
3. The shape of the protein chain depends on the usage of ATP. It is also used for switching off and on a chemical reaction and for sending the messages.

ATP Production

The concentration of ATP in the cell is generally 1-10 mM.⁵ ATP can be formed by the redox reaction of simple and complex carbohydrate, proteins and lipids. These are

the end products of digestion which further breaks down into smaller nutrients glucose, amino acids and small-and long-chain fatty acids. These three classes of small nutrients further gets converted to ATP, through the major metabolic biochemical cellular respiration pathway.⁷ The sole purpose of cellular respiration is the breakdown of glucose, fatty acid and amino acid into ATP. In the eukaryotic organism, the ATP can be produced by three main pathways:

1. Glycolysis
2. Beta Oxidation
3. Citric Acid Cycle and Oxidative Phosphorylation

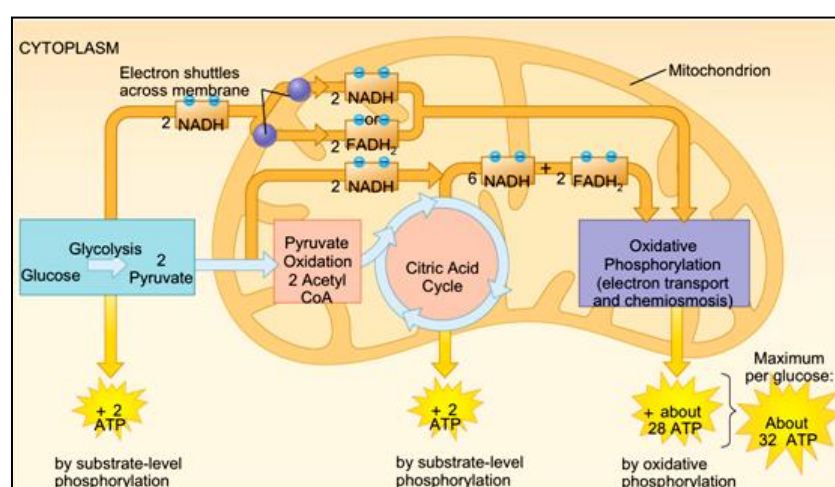


Figure 2: Cellular Respiration (taken from socratic.org)

The majority of the ATP can be produced by non-photosynthetic aerobic pathway, taking place in mitochondria, which can make up nearly 25% of the total volume of the typical cell.

1.2.1 Glycolysis

This is the first stage of cellular respiration; it is unique to glucose metabolism; taking place in the cytoplasm of the cell. Glucose and glycerol gets break down to pyruvate / pyruvic acid, through the series of biochemical reactions catalyzed by two enzymes, PGK and pyruvate kinase, thus giving two molecules of ATP. The glycolysis also generates two NADPH molecules. The reduced form of NADH, further act as the electron carrier to mitochondria, utilized in the electron transport chain to produce additional ATP molecules by the ATP synthase.⁸ The pyruvate generated as the end product in this process is further utilized for the Krebs cycle.

1.2.2 Beta Oxidation

Beta oxidation is the process by which fatty acids breakdown to acetyl-CoA in mitochondria. Normally 60% to 90% of the energy required for contraction of the heart muscles is derived from this beta oxidation only. Each round of this cyclic process reduces the length of the chain by two carbon atoms, and this process produces one each NADH and FADH molecules. These are then used as co-enzymes in the production of ATP by an oxidative phosphorylation. The dozens of ATP molecules can be generated, in a single acyl-chain beta oxidation.⁹

1.2.3 Citric Acid Cycle/Krebs Cycle

The glycolysis end product pyruvate, in the presence of oxygen, enters into mitochondria and forms acetyl-coA, during the pyruvate oxidation. The acetyl-CoA then enters into the citric acid cycle which is a circular series of enzymatic reaction taking place in the matrix/inner compartment of mitochondria. Two molecules of ATP and six molecules of NADH are then produced. Two molecules of FADH are also formed in this cycle, which are further utilized in the electron transport chain for the additional ATP production.¹

1.2.4 Oxidative Phosphorylation

It is a metabolic process, in which, the generation of ATP takes place as a result of an electron transport in a redox reaction. The energy released by this redox reaction is further utilized in forming the ATP. It is the major source of ATP in an aerobic organism, compared to glycolysis and the beta-oxidation pathways. Thirty ATP molecules are typically formed in the cell by glucose complete oxidation to carbon dioxide and water. Out of thirty, twenty six ATP molecules are formed from the oxidative phosphorylation only. In eukaryotic cells, these redox reactions are carried-out by a series of protein complexes, present in the inner membrane of mitochondria. Whereas, in prokaryotes, many different enzymes are present, using a variety of electron donors and acceptors. The oxidative phosphorylation happens only in two tightly coupled processes and these are:

- i) The electron transport chain: No ATP here, just lots of electrons and protons being transported.
- ii) Chemiosmosis: The flow of protons (H^+) in inter-membrane space drives the enzyme ATP synthase for making ATP.

1.2.4.1 The Electron Transport Chain

Here, the name itself implies that the transfer of electrons from the donor (NADH) and (FADH_2) to acceptor molecular oxygen takes place in a chain process, through a series of compounds which are present in the inner membrane.

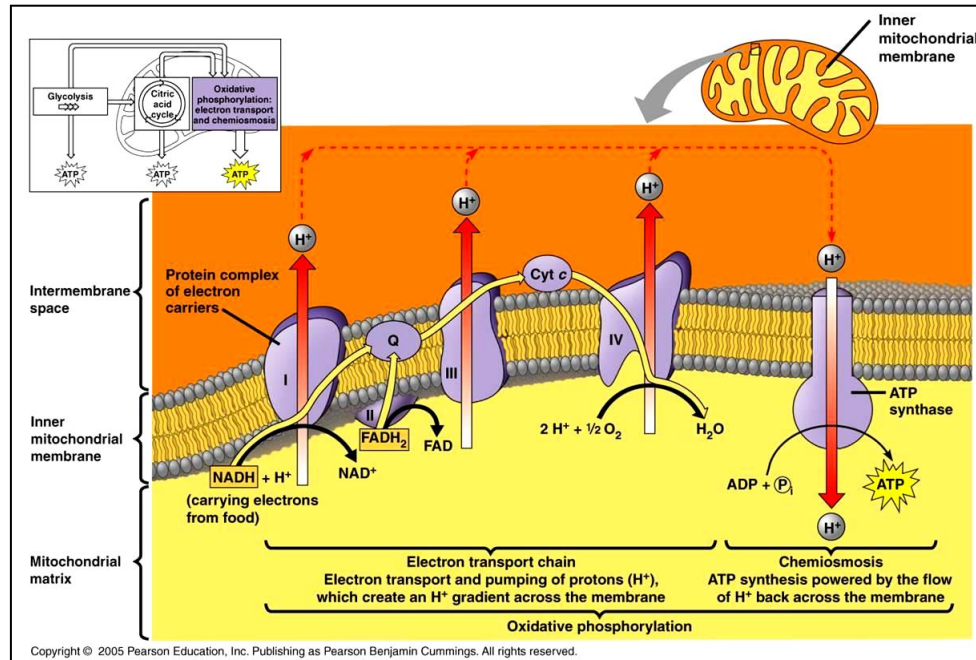


Figure 3: Oxidative Phosphorylation (picture taken from study blue.com)

Fixed to these reduction-oxidation reaction steps, it is a route in which the protons (H^+) present in the mitochondrial matrix are trans-located to the intermembrane space, thus leading the formation of the proton gradient across the space. The result of this process is that the redox energy of the NADH and FADH_2 is converted into proton gradient energy. The flow of the electron through the inner membrane is carried-out through several enzymes complexes, and these are: complex-I, complex-II, complex-III, complex-IV and complex-V (ATP synthase).

1.2.4.1a Complex-I (NADH-Dehydrogenase)

It is also known as NADH-Ubiquinone Oxidoreductase. It shows the L-shaped large complex structure, which is composed of NADH dehydrogenase with flavin mono nucleotide (FMN) as the cofactor, forty five proteins and non-heme-iron proteins with at least six iron-sulfur centers. The peripheral domain of the FMN cofactor accept 2e^- from the donor NADH, iron sulfur centers located at the hydrophilic peripheral domain forms a pathway for the transfer of electrons from FMN to coenzyme Q.

The initial electron transfer

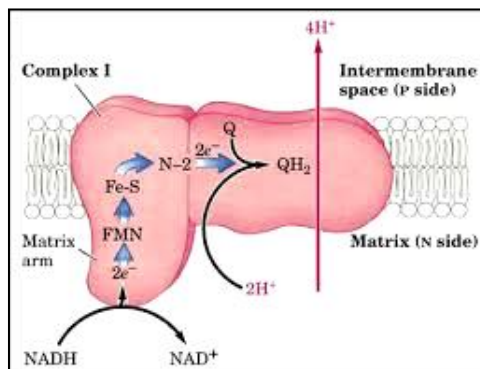
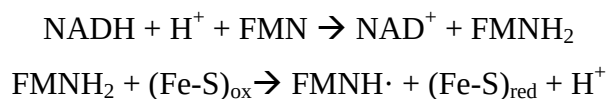


Figure 4: Structure of NADH-dehydrogenase (Complex-I) (picture taken from biochemden.com)

After Fe-S is re-oxidized by the transfer of the electron to the next iron-sulfur center in the pathway:

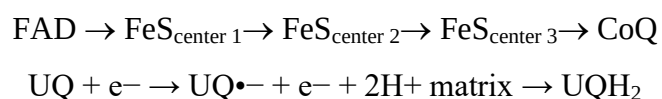


The electrons pass through a series of iron-sulfur centers, and finally, they are then transferred to coenzyme Q.

1.2.4.1b Complex-II (Succinate-Dehydrogenase)

It is also known as Succinate-ubiquinone Oxidoreductase and contains four protein subunits. Two hydrophilic sub-units are projected into the matrix and the other two hydrophobic subunits form a membrane anchor. One of the hydrophilic subunits contains the succinate-binding site and FAD. The FAD is the initial electron acceptor and is reduced into FADH_2 during the oxidation of succinate to fumarate. Another hydrophilic subunit contains Three Fe-S centers, which transfer electrons from FAD to ubiquinone. The above FADH_2 is further re oxidized to FAD by the transfer of electrons through these Three Fe-S centers to CoQ, to yield QH_2 . These QH_2 products may be re-oxidized via complex-III.

The Electron transfer pathway:



1.2.4.1c Complex-III (Cytochrome bc_1 Complex)

It is also known as the coenzyme Q-cytochrome c oxidoreductase. The electrons are transferred from coenzyme QH_2 to complex-III, QH_2 is the product of complexes-I and II, and is able to diffuse along and across the inner mitochondrial membrane. The two protons released into inter-membrane space by oxidation of QH_2 at UQH_2 oxidation site of complex-III and the electrons are transferred to the cytochrome c present in the inter membrane space.

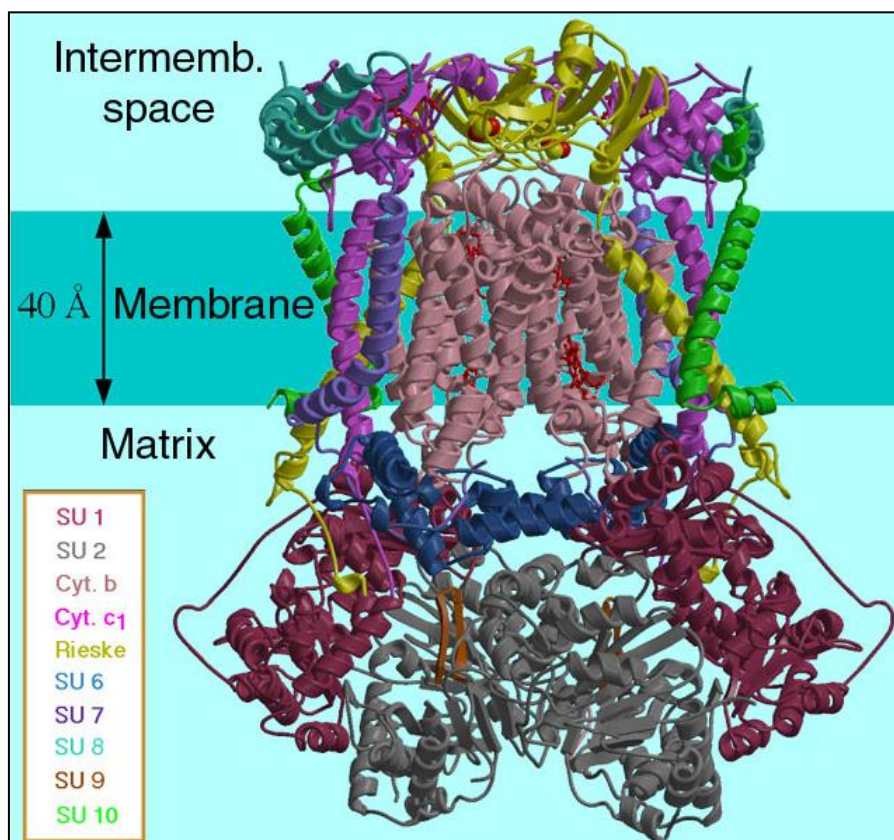


Figure 5: Structure of Complex-III (Cytochrome bc_1 Complex)

(Picture taken from <http://elcamino.cytbc1.net>)

Structure

The complex-III is present as the closely interacting dimeric form. Each bc_1 monomer contains eleven different protein subunits. It can be divided into three main regions: 1) The trans-membrane spinning region, 2) the matrix region, and 3) inter-membrane space region.

Matrix region

This region is elongated 75 Å from the trans-membrane inter-membrane space region and contains subunit 1, subunit 2, subunit 6, a part of the subunit 7 and the N-terminal

part of the (Fe-S protein) ISP, the C-terminal portion of cytochrome bc_1 , and a polypeptide of about fifty residues with the unassigned sequence, most likely to be a subunit nine. More than half of the molecular mass of complex-III is located in the matrix region of the molecule. Sub-units one and two are structurally similar, each unit contains two domains are roughly equal in size and having the identical folding topology.

The trans membrane helices region is spread over $40 \text{ \AA}$ thickness and consists thirteen helices, eight of which belongs to cytochrome b with the five remaining belonging one each, cytochrome c_1 , Fe-S protein and subunits seven, ten, and eleven.

Mechanism

The process by which electrons transfer from the QH_2 to cytochrome c is known as the Q cycle. This Q cycle proceeds in a bifurcated way. The electron transfer starts in complex-III from the ubiquinol oxidation, catalyzed at UQ-binding site Q_0 site, and is nearer to the inter-membrane space (IMS) in cytochrome b which is close to the heme b_L (Heme B unit). In the process, the globular domain of Fe-S protein is rotated toward this Q_0 site and this leads to the UQH_2 binding by bridging both proteins. The remarkable redox reaction then takes place.

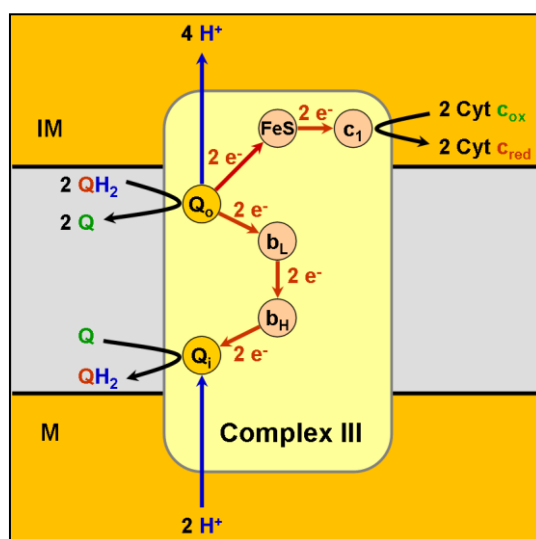
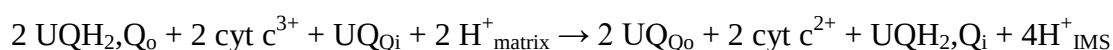


Figure 6: Schematic Illustration of Complex-III Reaction
(Picture taken from Wikipedia)

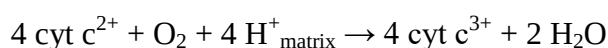
One of the UQH_2 electrons gets transferred to the Fe-S center (Figure 6) and second to the heme b_L . Two UQH_2 protons then get transferred into the inter-membrane space. The globular domain of the rieske (Fe-S) protein then turns towards the

cytochrome c_1 where the electrons are transferred from the Fe-S center to cytochrome c via cytochrome c_1 heme unit. In the second path (in **Figure 6**), the electrons on the heme b_L travel across the membrane to reduce primarily to heme b_H and next to an additional Q_i site, that can bind to UQ and reduce it back to its reduced form UQH_2 with two electrons received from UQH_2 oxidation and two protons from matrix. This whole catalytic cycle involves the following order of oxidation of QH_2 with the release of four electrons from two UQH_2 that travel along the chain in reducing the cytochrome c and UQ at Q_i . Hence, the full reaction is:



1.2.4.1d Complex-IV (Cytochrome C Oxidase)

It is the final complex of electron transport chain. The cytochrome c oxidase works as an electron carrier from reduced form of cytochrome c to molecular oxygen. This reduction requires four electrons from cytochrome c and four protons are taken from the matrix and one oxygen molecule. The overall reaction is:



During this reaction, the proton gradient and charge gradient across the intermembrane space are then consumed from the matrix and electrons are generated from the inner mitochondrial membrane (IMM).

Structure

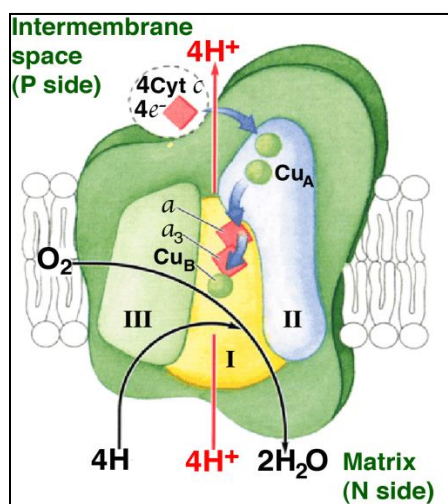


Figure 7: Structure of Cytochrome c Oxidase (Complex-IV) (taken from study blue.com)

Cytochrome *c* oxidase is a homo-dimer with each monomeric unit composed of thirteen different proteins. The main functional core of these complexes is made-up of three subunits (see in **Figure 7**). Two of this subunit I and II, catalyze the proton and electron transfer reaction, the subunit also provide a channel for oxygen diffusion into IMM.

Cytochrome *c* contains two important groups:

- 1) Two heme groups (heme *a* and heme *a*₃)
- 2) Three copper atoms (CuA/CuA and CuB)

Subunit-II has two α spinning helices and a hydrophilic globular domain. It also contains the bimetallic copper center, CuA. Subunit-I formed from 12 membrane-spinning α helices that are arranged in such a way to form three cavities. One of these cavities has a heme-*a* which is also linked between two histidines. A second cavity houses oxygen reduction site formed from heme *a*₃, together with histidine-linked copper, CuB. This site is often called as a binuclear center.

Mechanism

In the process of electron transport chain, electrons are transferred initially from the reduced cytochrome *c* to complex-IV bimetallic copper CuA/CuA. These electrons then move towards the heme-*a* then onto the heme *a*₃ followed by CuB. Here, CuB gets reduced. Then, the second electron from other reduced cytochrome *c* electron goes in the same pathway and reduces the heme *a*₃. Once the heme *a*₃ and CuB are in their fully reduced form, the oxygen diatomic molecule can bind to these, and further, abstract two electrons while forming the peroxide bond. In the process, two more reduced cytochrome *c* molecules are oxidized for transferring the additional two electrons to heme *a*₃ and CuB. Two protons are obtained from the matrix, forming heme *a*₃-OH and CuB-OH through breaking the peroxide bond. In the final step, two more protons are further abstracted from the matrix to oxidize the CuB and heme *a*₃ into their original state and this result in the formation of two water molecules.

1.2.4.1e Complex-V (ATP synthase)

ATP synthase is the final complex (enzyme) in oxidative phosphorylation. This enzyme utilizes the potential energy from the proton gradient across the inter membrane space for generating ATP from ADP and phosphate. Two to three protons are further required for an ATP generation.

Structure

ATP synthase contains mushrooms like head and stalk shape. It contains sixteen protein subunits and a bipartite structure, commonly as portion F_0 and portion F_1 .

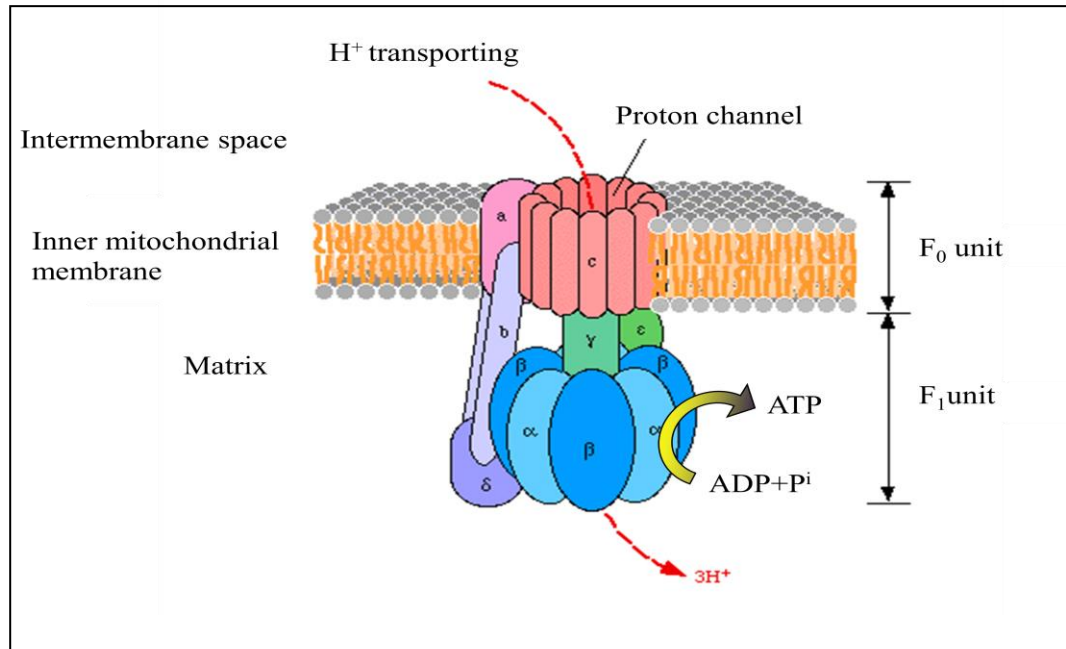


Figure 8: Complex-V (ATP synthase) (taken from www.Diapedia.org)

F_0 portion

This region is mostly hydrophobic and lies within the inner membrane. 10 to 14 C subunits are organized into the ring structure that acts as the proton channel. A single α subunit that binds to the outside of the C subunits ring structure, this subunit also helps connect F_0 to the F_1 portions and plays a role in the proton transport.

F_1 portion

This portion lies in the matrix of mitochondria and is hydrophilic in nature. It is made-up of five types of polypeptide chains- α , β , γ , ϵ , and δ . The 3 α and 3 β chains combine to form the hexameric ring structure that is responsible for catalyzing the ATP synthesis. The central stalk is formed by the γ , ϵ chains, it stimulates the synthesis and release of ATP molecules.

The F_0 portion is connected with F_1 portion through two connection points, one is γ , ϵ central stalk and the arm formed by a subunit, two β subunits, and the δ subunit.

The ATP synthesis consists of:

The Rotating region is made up of the C ring and γ ϵ stalk. Station region is made up of the remaining sub unit.

Mechanism

According to the Boyer and Walker, it works through the binding change, or flip-flop, mechanism theory. It is postulated that the synthesis of ATP depends on the conformational changes taking place in the β subunits of ATP synthase, generated by the rotation of the γ subunit.

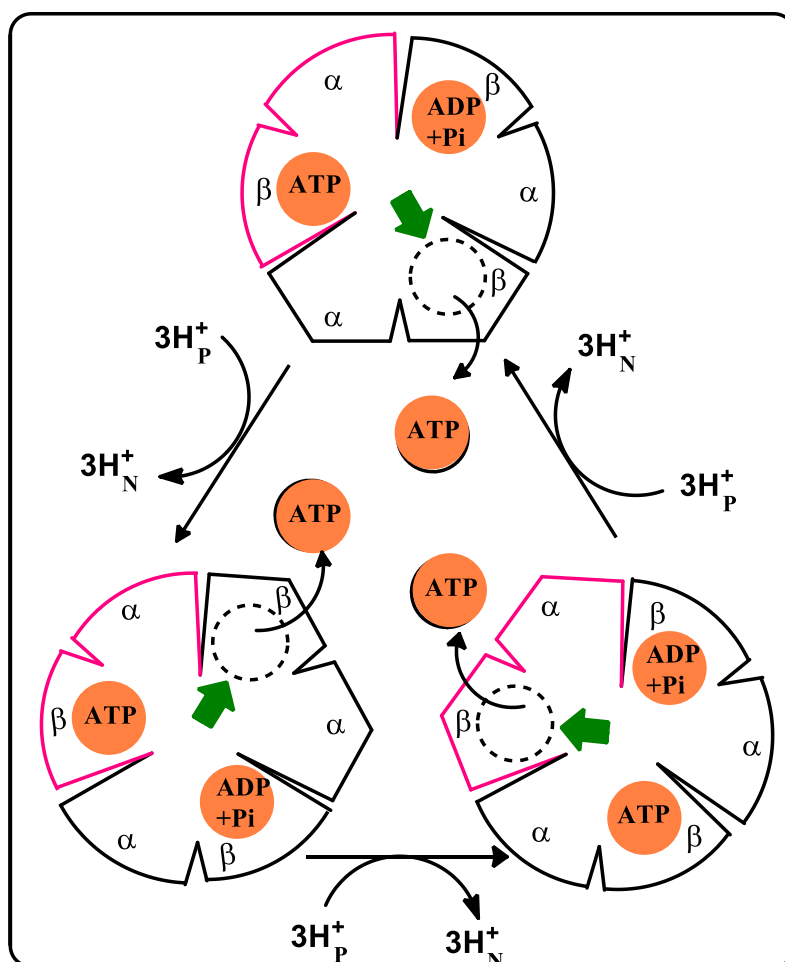


Figure 9: Binding Change Model for ATP Synthase (picture taken from biochemDen.com)

Three β subunits assume 3 different conformations and these are: 1) β -ADP, 2) β -ATP, and 3) β -empty.

First, the given subunit starts in the form of β -ADP conformation, which then binds ADP and Pi from the surrounding medium. Next, these subunit changes their conformation into the β -ATP form that tightly binds and stabilizes the ATP and this brings about the ready equilibration of ADP+Pi with the ATP on the enzyme surface. Finally, the subunit changes to the β -empty confirmation which has a very low affinity for ATP and the newly synthesized ATP leaves the enzyme surface. Another

round of catalysis begins when this subunit again assumes the β -ADP is formed and binds with ADP and Pi. The conformational changes central to this mechanism are driven by the passage of protons through the F_0 portion of ATP synthase. The diffusion of protons through the F_0 “pore” causes the cylinder of C subunits and the attached γ subunit to rotate about the long axis of γ , which is perpendicular to the plane of the membrane. With each rotation of 120° , γ comes into contact with a different β subunit, and the contact forces that β subunit into the β -empty conformation. These three subunits interact in such a way that when one assumes the β -empty conformation, its neighbor to one side must assume the β -ADP form, and the other neighbor the β -ATP form. Thus, one complete rotation of the γ subunit causes each β subunit to cycle through all three of its possible conformations, and for each rotation, three ATP are synthesized and released from the enzyme surface.

As I discussed above in detail, on how mitochondria carry-out very important biochemical functions that are essential for cells such as cell death and survival, homeostasis calcium, in addition to the ATP production. Due to these reasons, mitochondria have gained significant interest from the scientific community for obtaining a better understanding of its various functions in normal and disease states. This led to a growing interest in considering mitochondria as the potential drug target related to metabolic disorders and hyper-proliferative activity. Also, un-similar conditions in the reduction/oxidation of the tumor and non-tumor cells may be beneficial for obtaining the selective cytotoxic and anti-colony genetic effects on tumor cell populations. Thus, it was shown that pro-oxidant drugs, including trisenox¹⁰ and elesclomol¹¹ have therapeutic benefits in the treatment of cancer. The results obtained with Bz-423 in mouse expressing the potential for mitochondria-targeted drugs for controlling the disorders of the immune function.

1.3 Why Choosing Mitochondria as the Target

It is now well-known that mitochondria are a major source for Reactive Oxygen Species (ROS) is that super oxide and H_2O_2 generated as by the product of Electron Transport Chain (ETC) reactions. The cellular oxidative stress level is also revealed by this ROS, over-releasing of ROS causing severe damage to the macromolecule of the cell. However, when formed in a controlled amount, ROS may also play important signaling roles in different reduction/oxidation-dependent processes, including cell proliferation, apoptosis, and hypoxia.¹² In addition to this, mitochondria are very

active players in cellular calcium homeostasis.¹² Mitochondrial Ca^{2+} accumulation regulates various functions of aerobic metabolism and cell death machinery. Finally, mutations in mitochondrial DNA are responsible for different mitochondrial metabolic disorders and are thought to contribute to aging by promoting the apoptosis. Thus, because of their pivotal role in regulating cell life and death, mitochondria represent an attractive target for mitochondrial gene therapy as well as for developing drugs treating either degenerative or hyper proliferative diseases.

1.4 Mitochondrial Diseases

Mitochondrial dysfunction triggers the cell death signaling cascade, and thus, results in organ failure and disease. Through several studies related to various disorders and diseases, it was shown that most were sharing the common features of the disturbances of mitochondrial ROS, ATP or Ca^{2+} metabolism.

Table 1.1: Mitochondrial Diseases

Cardiovascular Diseases	Neurodegenerative Diseases	Chronic autoimmune inflammatory diseases	Metabolic diseases	Cancer
Atherosclerosis	Alzheimer's	Rheumatoid arthritis	Diabetes	Hepatocellular Carcinoma
Ischemia/reperfusion injury	Parkinson's		Obesity	Adenocarcinoma
Heart failure	Huntington's			Breast cancer
Stroke	Amyotrophic lateral sclerosis			Prostate cancer
Congestive heart failure	Friedreich's ataxia			

Some of these include, cardiovascular diseases, aging and neurodegenerative diseases, Huntington's disease, chronic autoimmune inflammatory diseases, metabolic diseases

as well as ionizing radiation injury. In the above **table (1.1)**, a few examples are highlighted.

1.5 Strategies for Targeting Mitochondria

Small molecule or biologics as drugs can act on mitochondria through various pathways. Many of these mechanisms are argued in more detail in the following sections, and a detailed discussion on this topic would immensely encroach the purpose of this chapter. In addition to this, several attractive current approaches include OXPHOS uncoupling, mitochondrial Ca^{2+} modulation, ETC inhibition and toxicology studies -cells, drugs and environment control of oxidative stress through an increase or decrease of mitochondrial ROS accumulation.

1.5.1 Targeting Transporters and Channels in Mitochondria

It is well-recognized that the totality of the mitochondrial membrane is crucial for the overall mitochondrial function(s). Not only are the inner and outer membranes targeted by drugs, but, in addition, many of the ion channels, proteins, and transporters embedded within the lipid membrane are also targeted. Among the main drug targets are:

1. Lipophilic cations targeting the IMM (e.g., rhodamine-123)
2. Carnitine palmitoyl transferase- 1 (CPT-1) inhibitors (e.g., oxfenicine, perhexiline, and etomoxir)
3. B-cell lymphoma 2 (Bcl-2) protein inhibitors (e.g., Fenretinide)
4. IMM potassium channel regulators (e.g., glibenclamide and diazoxide)

1.5.2 Mitochondrial KATP Channels Target for Therapy

Potassium channel openers (KCOs) are agents, discovered in the early 1980s, and they act by stimulating ion flux through K^+ channels. It seems that mitochondria are particularly very important targets for KCOs because the interaction of these compounds with mitochondria appears to mediate the cardio-protection of KCOs. The protective role of mitochondrial ion channels was recently summarized¹³ and mitochondrial targets for anti-ischemic drugs were also described recently. Many small molecule drugs such as diazoxide, nicorandil, and cromakalim have been identified as KCOs.¹⁴

1.5.3 Targeting the Mitochondrial Electron Transport Chain (ETC)

The ETC is the major source of Reactive Oxygen Species (ROS), and complex-I and complex-III have been identified as main superoxide-generating sites.¹⁵ Whereas, the low levels of ROS generation are necessary for maintaining the cellular signaling processes sustaining the higher levels of ROS may predispose to cancerogenesis in contrast, a sudden and substantial increase in ROS levels generally commit the cells to apoptosis.¹⁶

1.5.3a Complex-I Inhibitors

I discussed the complex-I structure and its mechanism of action in section 1.2.4.1a. It is one of the major sources of superoxide within the ETC. More than 60 classes of inhibitors are known today and most of them are specific for an UbQ binding region.¹⁷ For example, piericidins (A1) (**F10.1**) and rotenone (**F10.2**) are the apoptosis inducers. They inhibit the transfer of electrons from iron-sulfur centers in complex-I to ubiquinone. This interferes with the NADH during the creation of usable cellular energy (ATP), thus leading the formation of ROS which can damage mitochondria. Tamoxifen (**F10.3**) is a well-established anti-cancer drug. It inhibits complex-I at flavin site, which increases the cellular hydrogen peroxide production.

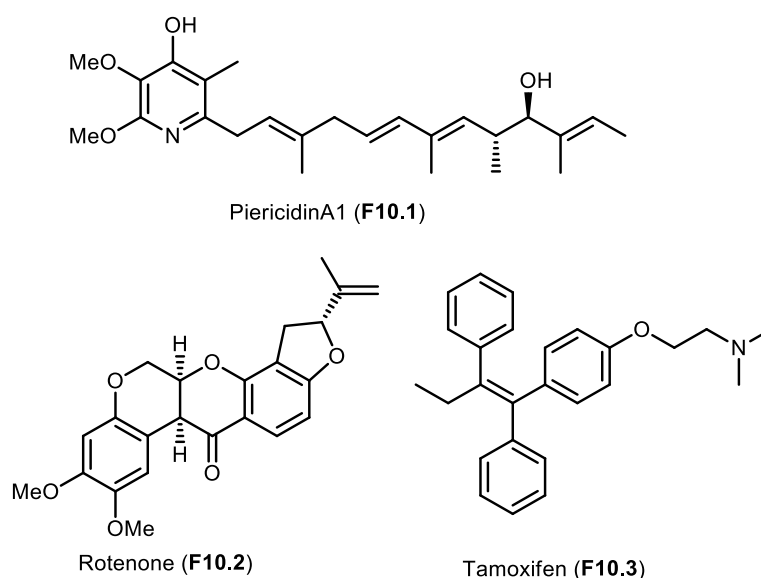


Figure10: Complex-I Inhibitors

1.5.3b Complex-II: Inhibitors

The structure and mechanism of complex-II were already discussed in section 1.2.4.1b. Complex-II inhibitors can be classified according to their mode of action; inhibitors like, funiculosin, antimycin and the hydroxyquinoline-N-oxides block the Q_D site. Other inhibitors such as 1, 2 hydroxy-1-4, benzoquinone derivatives, and monoamine oxidase block the Q_P site. The inhibition of Complex-II with thenoyl trifluoroacetone (**F11.1**) binds at the quinone reduction site in complex-II, thus preventing ubiquinone from binding. This process slows the cell cycle progression and increases the ROS production and glutathione oxidation into generation of superoxide. The amide, ester and ether-linked analogs of tocopheryl succinate (TOS) (**F11.2**)— α Vitamin E derivatives function as the potential as an anti-cancer agent. It competes with ubiquinone in binding to Q sites on the complex. It has been shown to inhibit tumor cell growth and in inducing apoptosis in animal xenograft models.⁴

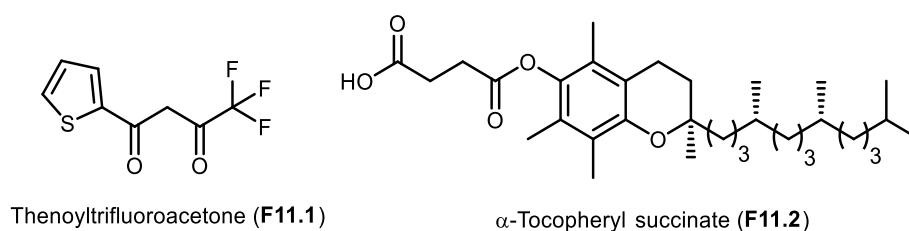


Figure11: Complex-II Inhibitors

1.5.3c Complex-III Inhibitors

I discussed the structure and mechanism of ETC for this complex in section 1.2.4.1c. This complex-III is considered as the main source for superoxide radicals due to the leakage of electrons, either by inhibition of UbQ-binding sites or by increasing the mitochondrial membrane potential. This complex-III contains two UbQ binding sites namely Q_o and Q_i participated in the Q cycle. Antimycin A (**F12.1**) is the Q_i site inhibitor and it specifically binds to the Q_i site of the complex-III leading to the ROS production at Q_o .¹⁸ In doing so, antimycin A can induce apoptosis and kills cancer cells very effectively.¹⁹ Stigmatellin (**F12.2**) also inhibits the complex-III in a different manner. It is the Q_i site inhibitor and function by mainly preventing the transfer of electrons from Fe_2S_2 complex to cytochrome c_1 to bc_1 complex of the complex-III. In this manner, it acts as a strong oxidant in forming the reduced Fe_2S_2 complex and oxygen radicals. Myxothiazol (**F12.3**) is a potent inhibitor of complex-

III from quinol antagonists that includes the oudemansins and strobilurines, The methoxy acrylate group common amongst these inhibitors

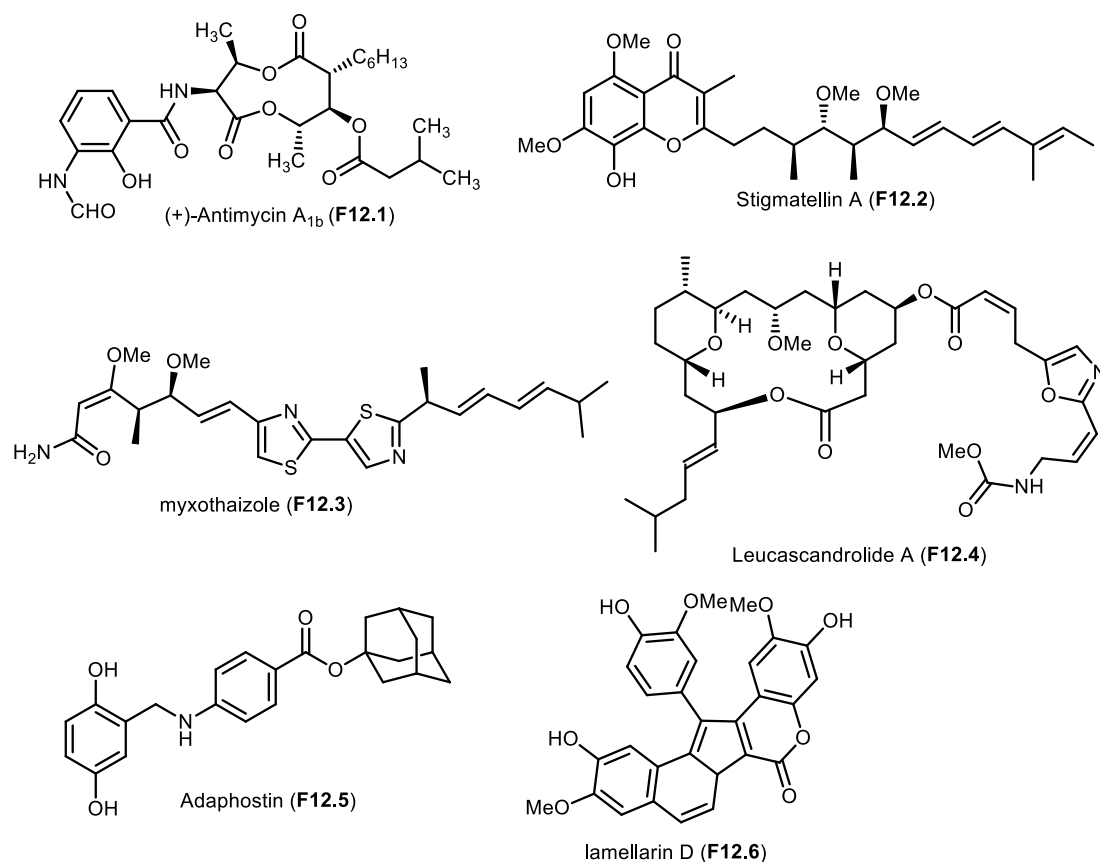


Figure 12: Complex-III Inhibitors

resembles the structure of ubiquinone such that their binding to the Qo site of Complex-III blocks ubiquinol oxidation.²⁰ Their structures resemble the structure of ubiquinone such that they are binding to the Qo site of complex-III in blocking the ubiquinol oxidation. Leucascandrolide A (F12.4) is a highly potent inhibitor of complex-III and it stops the formation of ATP, resulting in cell death. Adaphostin (F12.5) is a promising natural product that induces the ROS production and apoptosis by targeting the Qi site of the complex-III.²¹

1.5.3d Complex-IV Inhibitors

I discussed the structure and function of this complex-IV in section 1.2.4.1d. Complex-IV exists mainly in three conformational states, namely fully oxidized, partially reduced, and finally, as the fully reduced form. Each inhibitor has a high affinity to a different state.²² The functional groups/reagents such as cyanide, carbon monoxide, and azide etc, all binds to complex-IV. In this process, they competitively

inhibit the binding of oxygen at the active site and produce the majority of ATP which is utilized by the cell.

1.5.3e Complex-V (F_1F_0 -ATPase) Inhibitors

As I discussed in section 1.2.4.1e about the structure and function of complex-V. It performs the terminal step of an oxidative phosphorylation and in disrupting the energy metabolism of the cell. Over the years, numerous compounds are identified for their ability to inhibiting the ATP synthesis.

Oligomycin (**F13.1**) is a model inhibitor of the F_0 subunit of ATP synthase, which is necessary for the oxidative phosphorylation of ADP to ATP. The oxidative phosphorylation synthesis by oligomycin A significantly reduces the electron flow through the electron transport chain; however, the electron flow is not stopped

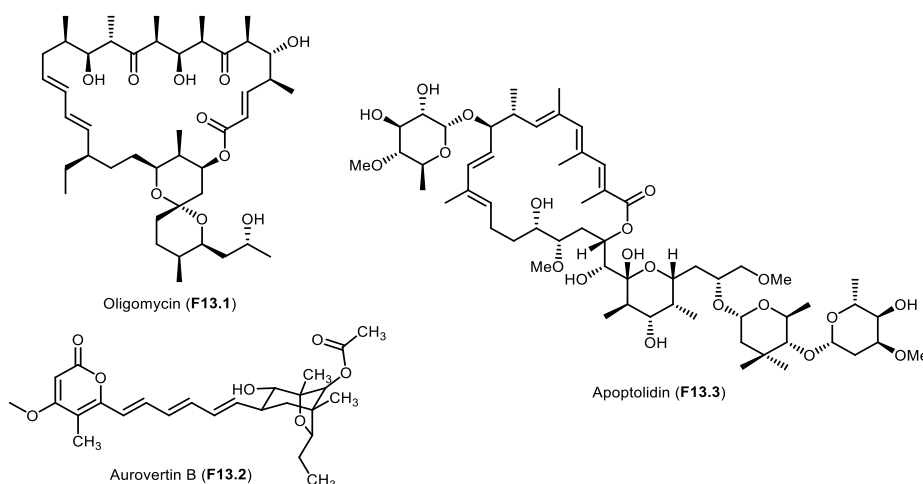


Figure 13: Complex-V Inhibitors

completely due to a process known as proton leak or mitochondrial uncoupling.¹ This process is due to the facilitated diffusion of protons into the mitochondrial matrix through an uncoupling protein such as thermogenin, or UCP1. Aurovertin (**F13.2**) is an effective inhibitor of F_1 subunit of ATP synthase. Aurovertin specially bind to two equivalent sites situated between the nucleotide binding and C-terminal domains of the βE -and βTP -subunit of F_1 subunit. Apoptolidin (**F13.3**) a novel cancer cell apoptosis inducer, and it display a considerable selectivity only for the subset of cancer-derived cell lines like E1A and E1A/E1B19K transformed these cells to apoptosis with considerable potency.²³

1.6.1 Synthesis of Complex-I Inhibitor

1.6.1. a Apiericidin-A1

Piericidins are potent biologically active natural products and belong to the family of antibiotics produced by various *Streptomyces* strains. Up to now, more than twenty different piericidins are isolated, and all these, share some similar characteristics in their structural arrangements. As of today, the isolation of novel piericidins remains an active field and multiple biological targets besides the long-known inhibitory effects of electron transport chain and it has attracted the attention of researchers from various disciplines. Piericidin A1 (**F10.1**) is the proto-typical member of this family and it was isolated from *Streptomyces mobaraensis* and *S. pactam* by Takahashi and co-workers in 1963. The originally attributed configuration of this family was not established until the first total synthesis of piericidin A1 and B1 was published in 2005 by Boger and Schnermann. It is a potent inhibitor of complex-I (K_i 0.6 1.0 μm) in the mitochondrial electron transport chain sequence, in which protein NADH: Ubiquinone Oxidoreductase (or NADH dehydrogenase) is responsible for the oxidation of NADH to NAD^+ using coenzyme Q10 (ubiquinone, 2) as the hydride acceptor. Initially, in 1977, the first synthesis of the piericidin structures was reported by Schmidtchen and Rapoport. In 2005, the Boger group reported the first total synthesis of piericidin A1. After this, in 2006, Keaton and Phillips completed the synthesis of natural 7-demethylpiericidin A1 containing the same aromatic motif. In 2009, Lipshutz and co-workers synthesized piericidin A1 in 11 steps and with an overall yield of 8% for the longest linear sequence. The crucial step for this successful strategy was the Mukaiyama aldol reaction developed by Kobayashi and co-workers.²⁴

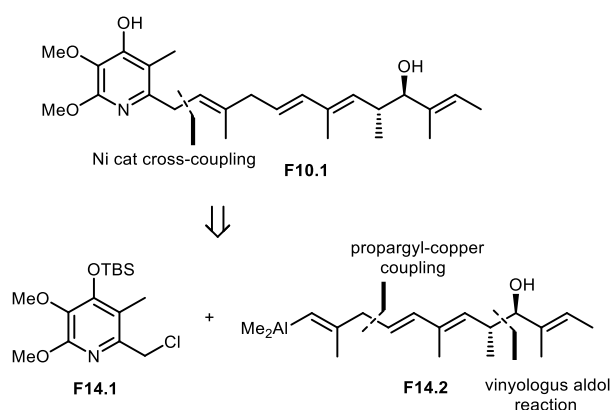
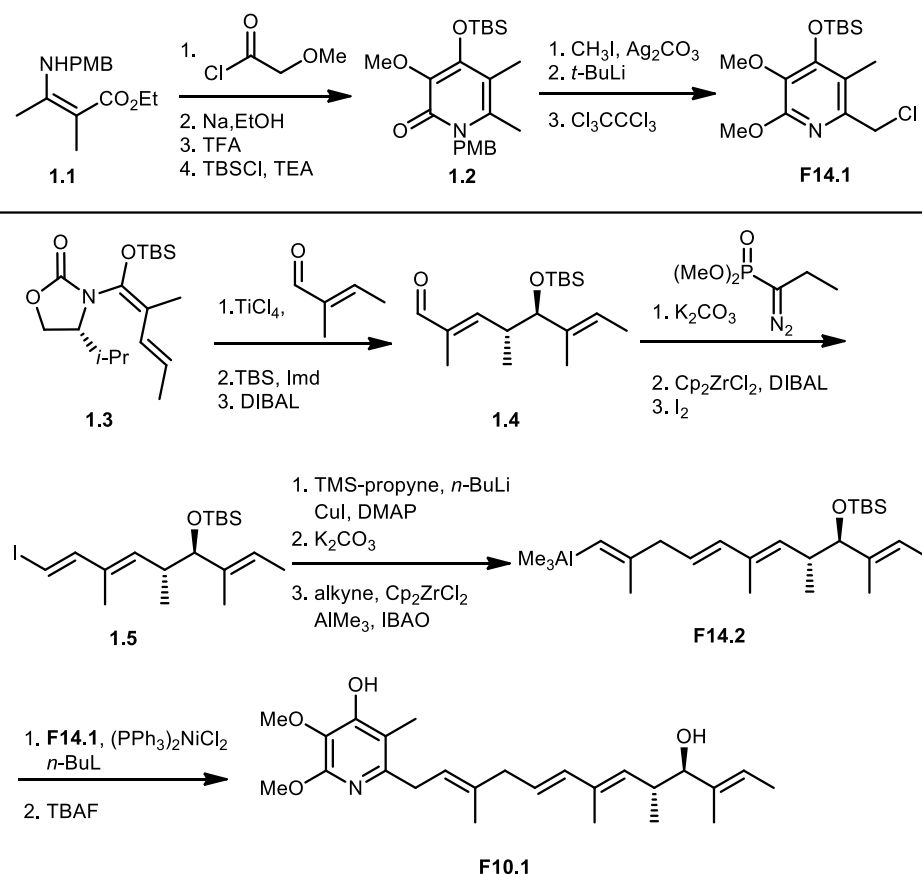


Figure 14: Retrosynthesis of Piericidin A1 by Lipshutz and Co-workers

The team synthesized **F10.1**, by using a one-pot Ni-catalyzed coupling of the chloromethylated pyridine (**F14.1**) and vinylalane (**F14.2**) generated *in situ* via the modified carbo-alumination. Compound **F14.1** was then obtained from the N-p-methoxybenzyl-3-aminotiglate (**1.1**) in seven steps.



Scheme 1: Synthesis of **F14.1**, **F14.2** and Piericidin A1

The synthesis was started by performing acylation with methoxyacetyl chloride on compound **1.1**, followed by the base-promoted Dieckmann cyclization giving, 2-pyridone. This upon treatment with TFA under heating conditions followed by the selective O-silylation, gave **1.2**. Compound **1.2** was then subjected to alkylative aromatization (CH₃I, Ag₂CO₃) and further followed by *ortho* methyl chlorination with *t*-BuLi /Cl₃CCl₃, giving the chloromethylated picoline (**F14.1**). Synthesis of **F14.2** was started by operating a key vinylogous Mukaiyama aldol reaction, promoted by TiCl₄ between **1.3** and tiglic aldehyde. Followed by silylation with TBSCl and removal of the chiral auxiliary with DIBAL, led the synthesis of **1.4**. Alkynylation of **1.4** with Bestmann-Ohira diazophosphonate then produced enyne derivative. This was followed by hydrozirconation with *in situ* prepared Schwartz's reagent followed by I₂

quench, gave the vinyl iodide (**1.5**). The propargyl coupling of **1.5** with TMS propyne then provided an unstable enyne which was immediately converted into stable terminal acetylene. This was then subjected to crucial carbo-alumination of terminal alkyne, giving product **F14.2**. It was finally subjected to Ni(0) catalyzed coupling reaction with **F14.1**, followed by the subsequent removal of silyl group, which completed the synthesis of piericidin A1.

1.6.2 Synthesis of Complex-III Inhibitors

1.6.2a Antimycin A and Related Analogs

Antimycins are a family of natural products produced by various *Streptomyces* species and they share a common structural skeleton. A nine-membered dilactone ring is substituted with one alkyl (C-7), one acyloxy (C-8), two methyl moieties (C-4 and C-9), and an amide linkage (C-3) connecting to a 3' formamido salicylic acid. So far, more than forty antimycin type compounds varying in the alkyl and acyl chains are isolated and reported since the first isolation in 1949 by Strong and co workers.²⁵ The antimycin A family (AAs) is a potent electron transport chain inhibitor through binding to cytochrome *bc*₁ complex. The highly potent biological activity and the difficulty in procuring sufficient quantities of samples led to significant interest from the synthetic community. Several groups accomplished the total synthesis of AAs. Kinoshita and co-workers²⁶ Wasserman and co-workers,²⁷ Chakraborty and co-workers,²⁸ and Tsunoda and co-workers.²⁹ Herein, I am going to discuss the effort of Batey and co-workers in total synthesis of one of antimycin A family member, antimycin A_{1b}. The researchers utilized practically robust and scalable organo-trifluoroborate-based allylation and crotylation methods. This total synthesis constitutes to be one of shortest syntheses of a member of the antimycin A family, involving a linear sequence of 12 steps (19 steps overall) and 18% overall yield. Antimycin A_{1b} was could be obtained by an amidation reaction between **2.6** and **15.1** the synthesis of **2.6** was achieved from **15.2** which was obtained from **15.6** and **15.7** in a few steps.

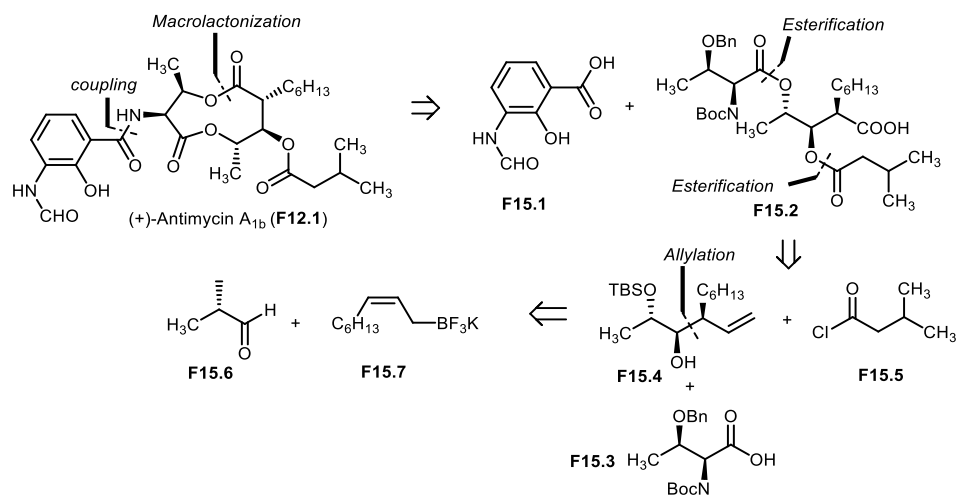
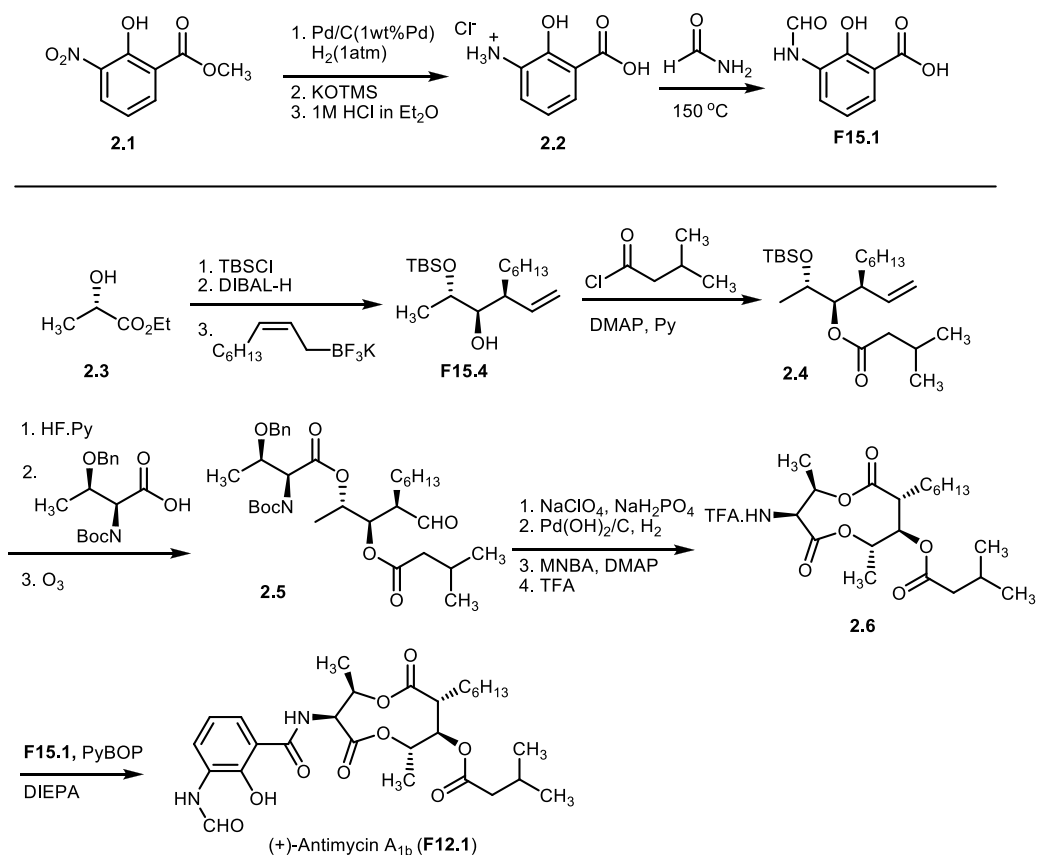


Figure15: Retrosynthesis of Antimycin A_{1b} by Batey and Co-workers

Here, I am going to cover the efforts of Batey and co-workers. The total synthesis of antimycin A_{1b} (F12.1) was started with the synthesis of unprotected substituted salicylic acid fragment F15.1, which was obtained from methyl 2-hydroxy-3-nitrobenzoate in four steps.



Scheme 2: Synthesis of Antimycin A_{1b}

Another coupling partner **2.6** acid core was obtained from ethyl lactate **2.3**. Compound **2.3**, was then subjected to silyl protection followed by DIBAL reaction giving an aldehyde, which upon subjection to diastereoselective montmorillonite K10-promoted allylation reaction with substituted potassium allyltrifluoroborate salt, gave **F15.4** with high diastereoselectivity. Compound **15.4**, was treated with acid chloride to obtain **2.4**, which was then subjected to de-silylation with HF.py followed by coupling with the threonine acid moiety, giving the ester derivative. The terminal double bond was then subjected to ozonolysis, which led the synthesis of aldehyde **2.5**. The Pinnick oxidation of this aldehyde gave the acid derivative which was followed by debenzilation, macrolactanization with MNBA and *N*-Boc removal with TFA, leading the synthesis of **2.6**. Finally, applying the coupling reaction between **2.6** and **F15.1** with Py-BOP, the synthesis of antimycin A_{1b} (**F12.1**) was completed.

1.6.2b Stigmatellin A

Stigmatellin A was isolated by Hofle and co-workers in 1984 from gliding bacterium *Stigmatella aurantiaca*.³⁰ It was one of the most potent inhibitors of the electronic transport chain in mitochondria and chloroplast.³¹

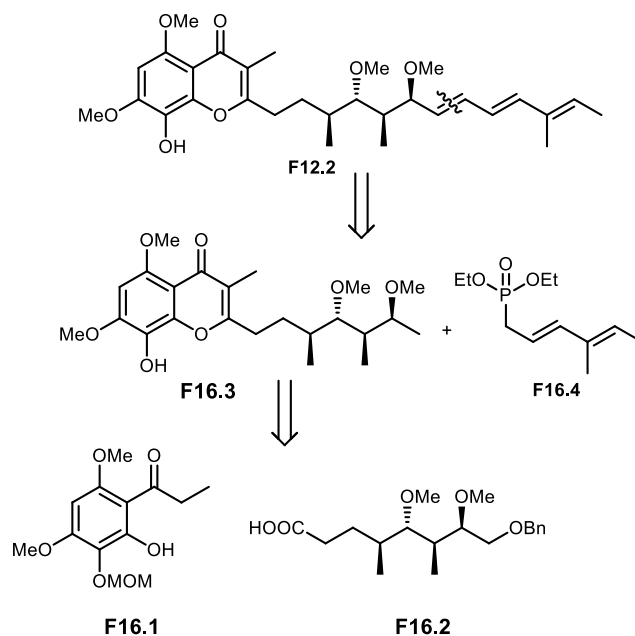
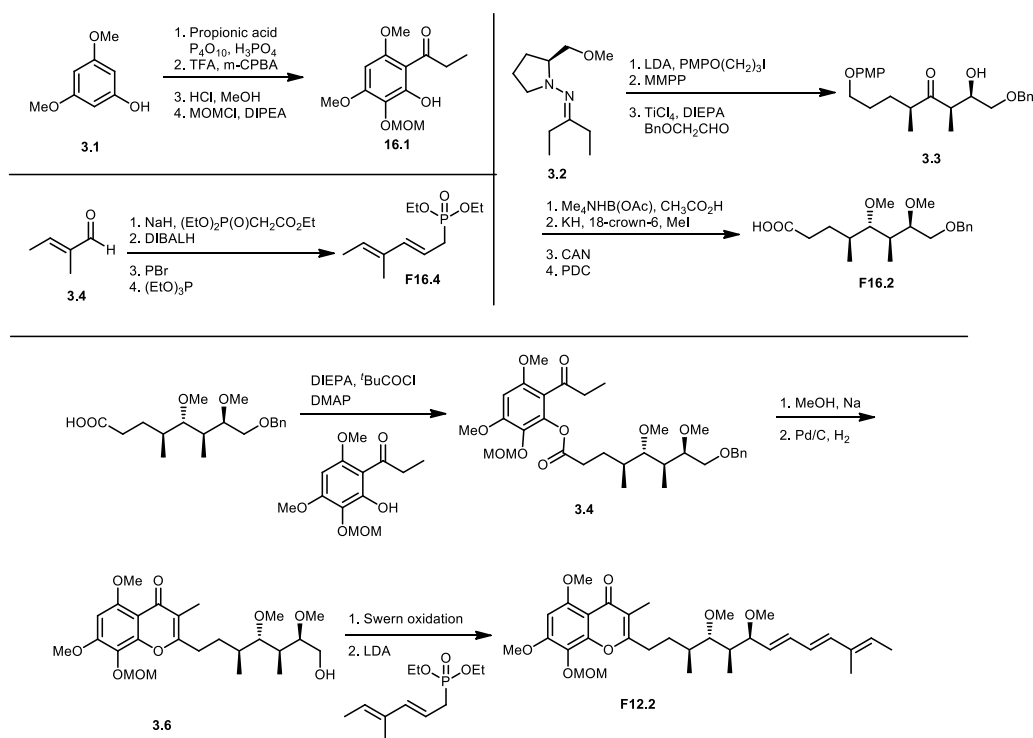


Figure16: Retrosynthetic analysis of Stigmatellin

Its importance in elucidating the mode of action of these two vitally fundamental processes have been considerable. The point of attack is on the one hand of the cytochrome *bc*₁-segment of the respiratory chain and on the other hand the reducing

side of photosystem-II and the cytochrome b6/f-complex.³² The structure of stigmatellin A was finally elucidated from proton ^1H NMR, ^{13}C -NMR, IR, MS, UV and C,H,N analysis data. During the past few years, a few groups reported the synthesis of stigmatellin A. In the following section, I outlined the first total synthesis of stigmatellin A (**F12.2**) by Enders and co-workers, which was reported in 2000. This is the first diastereo- and enantio-selective total synthesis of stigmatellin A.³³ The retrosynthetic analysis of this approach is shown in **Scheme 16**. Compound **F12.2** was obtained from **F16.3** and **F16.4** by using Horner-Wordsworth-Emmons reaction according to the procedure published by Nicolaou and co-workers. **F16.4** was obtained from **F16.1** and **F16.2** by applying Baker-Vankataraman rearrangement. First, the researchers started the synthesis of an aromatic ketone fragment **F16.1** from dimethoxy phenol (**3.1**).



Scheme 3: Synthesis of Stigmatellin A

The first step was the diacylation with propionic acid, followed by Baeyer-Villiger conversion of the ketone to ester. Hydrolysis of an ester gave the free hydroxyl group, which upon protection with MOMCl led the synthesis of compound **16.1** quantitatively and completely chemoselective. The synthesis of the central portion **15.2** was started with alkylation of SAMP-hydrazone (**S**) with iodide prepared from 1

chloropropan-3-ol in two steps with a good asymmetric induction. It was oxidatively cleaved giving the corresponding ketone. This ketone was then subjected to TiCl_4 mediated *syn* aldol reaction giving **3.3**. It was further followed by *anti*-selective reduction of the keto group according to the procedure developed by Evans and co-workers, giving the diol. It was then subjected to di-ether formation with potassium hydride and methyl iodide. Next, *p*-methoxyphenyl removal with CAN, followed by the PDC oxidation, led the synthesis of acid (**F16.2**) quantitatively. The third fragment diene (**F16.4**) was synthesized from tiglaldehyde (**3.4**), which was subjected to Horner-Wadsworth-Emmons olefin reaction. The selective ester reduction to alcohol, hydroxyl to bromide conversion followed by phosphonate formation then led the synthesis of **F16.4**. At the end, these three fragments were coupled with each other, primarily the ester formation between **16.1** and **16.2** giving **3.4**. This upon subjection to Baker-Venkataraman rearrangement followed by Swern oxidation and Horner-Wadsworth-Emmons reaction finally gave **F12.2** with good yield.

1.6.2c Leucascandrolide A

Leucascandrolide A (**F12.4**), a marine natural product isolated by Pietra and co-workers in 1989 from a new genus of calcareous sponges called *L.caveolata*. It shows potent cytotoxicity against several cancer cell lines, like human KB tumor cell lines and P388 cell lines. As well as, it is a potent antifungal inhibitor against *Candida albicans*. Presently, the only reliable source for the compound **F12.4** is the chemical synthesis. First total synthesis of leucascandrolide A was reported by Leighton group in 2000. after that, several total syntheses were reported by Kozmin, Paterson and Panek. In addition to that, many formal syntheses were also reported by Carreira, Crimmins, Wipf and Williams, and including, one from the Leighton group.

The retrosynthesis of Leucascandrolide A by Rychnovsky and Co-workers was shown in **Figure 17**. Leucascandrolide A could be obtained from cesium carboxylate side chain (**F17.2**) by the displacement of the macrolactone secondary bromide obtained from **F17.1**. A late stage titanium tetrabromide-promoted Mukaiyama Aldol Prins Reaction (MAP) led the cyclization of aldehyde (**F17.3**) and one of the homoallylic enol ethers (**F17.4**) provided the desired 2,4,6-cis-tetrahydropyran (**F17.1**).

Compound **F17.3** could be prepared from silyl enol ether (**F17.6**) addition to acetoxy ether (**F17.5**), which was obtained from **F17.7** in a few steps.

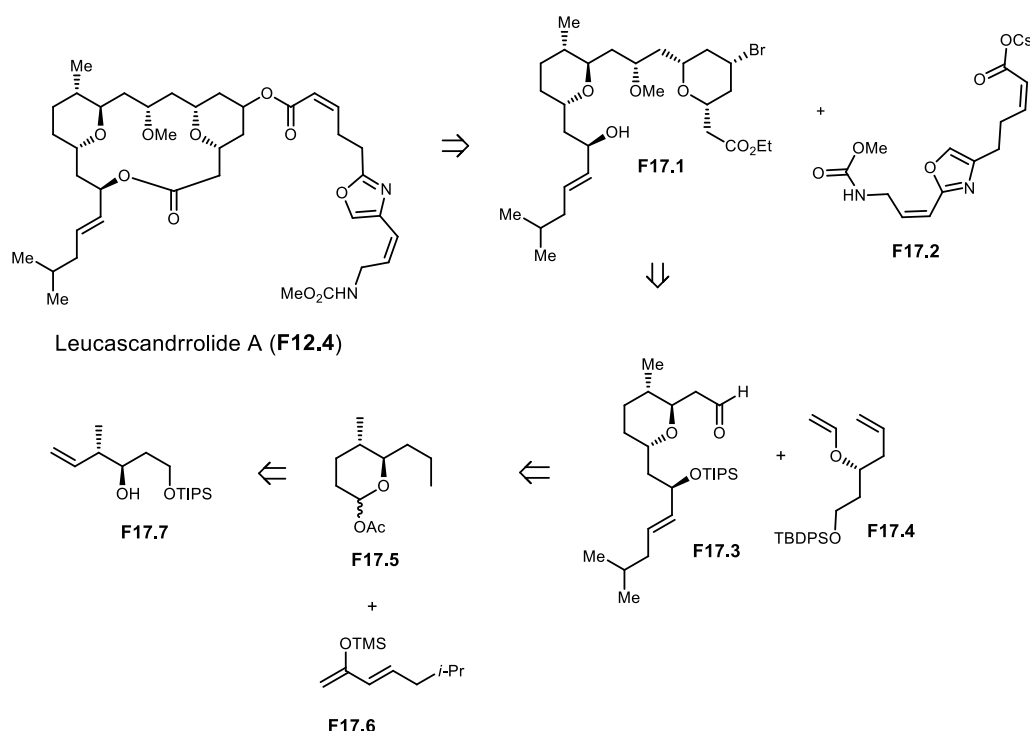
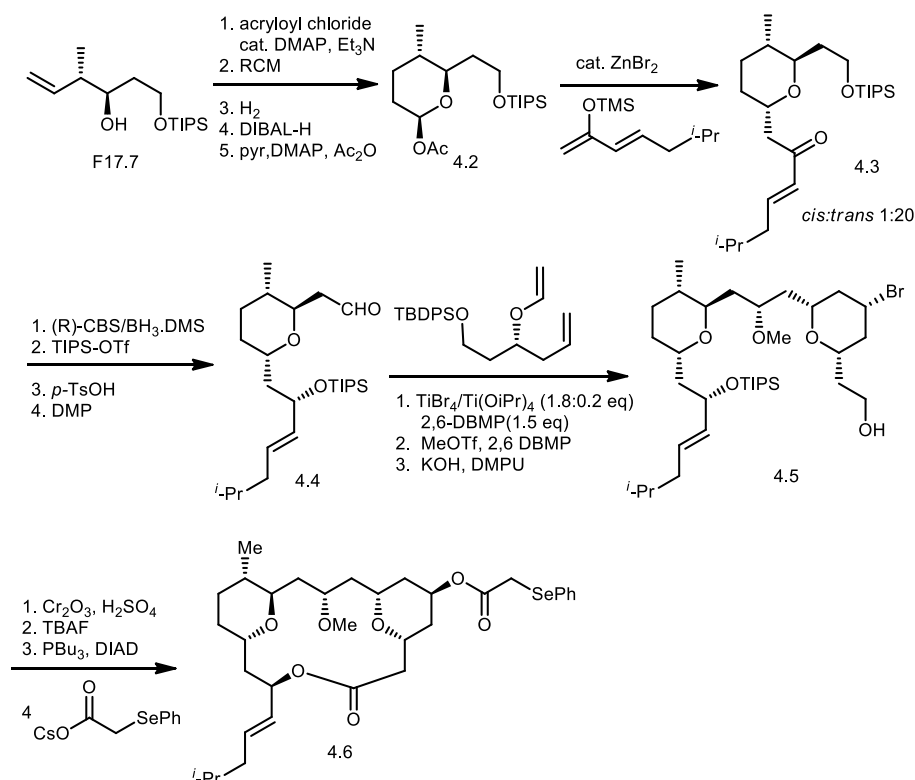


Figure17: Second Generation Retrosynthesis of Leucascandrolide A by Rychnovsky and Co-workers

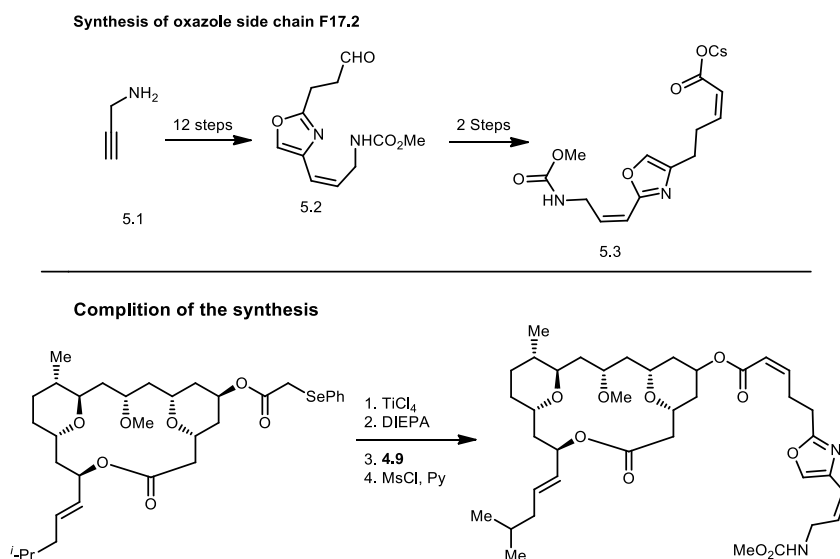
Synthesis of 18 membered macrocyclic bromide was obtained from homoallylic alcohol (**F17.7**).³⁴ Initially, it was subjected to acylation for obtaining an acrylate, which was then subjected to tandem one-pot Ring Closing Metathesis (RCM) by portion wise addition of Grubbs' 2nd generation catalyst and hydrogenation afforded the reduced product. Subsequent reductive acetylation gave **4.2** with 15:1 *cis:trans* isomers. This was then coupled with silyl enol (**F17.6**) ether in the presence of catalytic zinc bromide to give the trans THP (**4.3**) with a high diastereoselectivity (20:1). The regio- and diastereo-selective reduction of the ketone (**4.3**) with (*R*)-CBS gave selectively, a single diastereomer. Followed by the TIPS protection and selective deprotection of primary TIPS ether with mild *p*-TsOH, the corresponding alcohol was obtained; this, followed by oxidation gave aldehyde **4.4**. The key MAP reaction was performed between aldehyde **4.4** and silyl enol ether in different conditions like variation in Lewis acid and base in different equivalents. Finally, it was found that $\text{TiBr}_4/\text{Ti}(\text{OiPr})_4$ (1.8:0.2) and 2,6-DBMP (1.5) base gave good diastereoselective

compound with good yields. It was then subjected to methylation, followed by TBDPS deprotection under basic conditions completing the synthesis of **4.5**.



Scheme 4: Synthesis of **4.6**

Compound **4.5** was oxidized with DMP followed by Kraus oxidation, provided the corresponding acid in one pot. The *O*-TIPS removal with TBAF led the alcohol and which is upon subject to Mitsunobu reaction, gave the macrolactone. Finally, it was treated with cesium-2-(phenylselenanyl) acetate, giving compound **4.6**.



Scheme 5: Completion of Synthesis

The synthesis was started from propargyl amine **5.1**, based on Leighten and co-workers reported procedure in 12 steps, compound **5.2** was obtained. Following the Kozmin's approach, in 2 steps compound **5.3** was synthesized. To complete the synthesis of leucascandrolide A, the researchers further attached the oxazole side chain **5.3** to **4.6** by using *syn* aldol reaction followed by a stereospecific dehydroselenation resulted in **F12.4**. The total synthesis of leucascandrolide A was completed in 17 steps with an overall yield of 4.3%. This approach also demonstrated that the MAP reaction was highly efficient and diastereoselective for the preparation of the tetrahydropyranyl bromide.

1.6.3 Complex-V Inhibitors

1.6.3. a Oligomycin C

Oligomycins belongs to the category of highly functionalized macrolide antibiotics and their structures contain the hydroxyl groups, lactone and spiro moieties and double bonds. In this section, I am discussing the oligomycin C, its structural features which are similar to the rutamycin B, but differ by a single methyl group at the C-26 position of the spiroketal. The first oligomycin was isolated in 1954³⁵ from the strain of *Streptomyces*, *diastatochromogènes* consisted of variable proportions of three major components A, B, and C. Oligomycins are reported to inhibit oxidative phosphorylation in mitochondria by preventing the synthesis of ATP³⁶ These are involved in the decoupling of Fo and F1 factors, which are responsible for the proton transfer through the inner mitochondrial membrane. The structures of oligomycin A, B, and C were assigned on the basis of degradation products and 1-H NMR and 13-C NMR correlation experiments. Panek and co-workers reported the first asymmetric total synthesis of oligomycin C in 1998, and later, in 2000 the same group reported another synthesis. In this section, I am going to discuss the efforts of Panek group for developing the total synthesis of oligomycin C.

The retrosynthetic analysis of oligomycin is shown in **Figure 18**. Oligomycin C could be prepared from the spiroketal fragment (**F18.1**) and polypropionate fragment (**F18.2**) using the Suzuki cross coupling reaction employed by Evans³⁷ and White³⁸ in their synthesis of rutamycin B, and macrolactanization approaches. The spiroketal fragment (**F18.1**) was prepared from an open chain fragment **F18.3**, which was obtained from the left-hand subunit **F18.4** and right-hand subunit **F18.5** by lithiated

hydrazone cross coupling reaction. In fragment **F18.2**, out of nine chiral centers six were introduced by an asymmetric crotylation strategy.

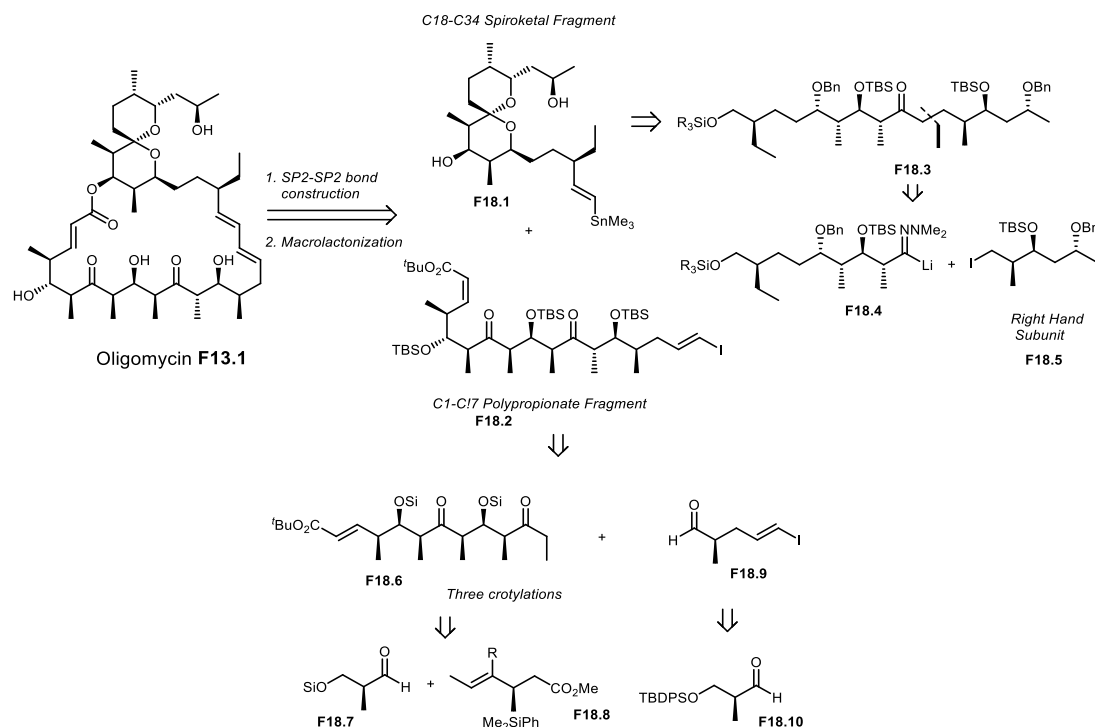
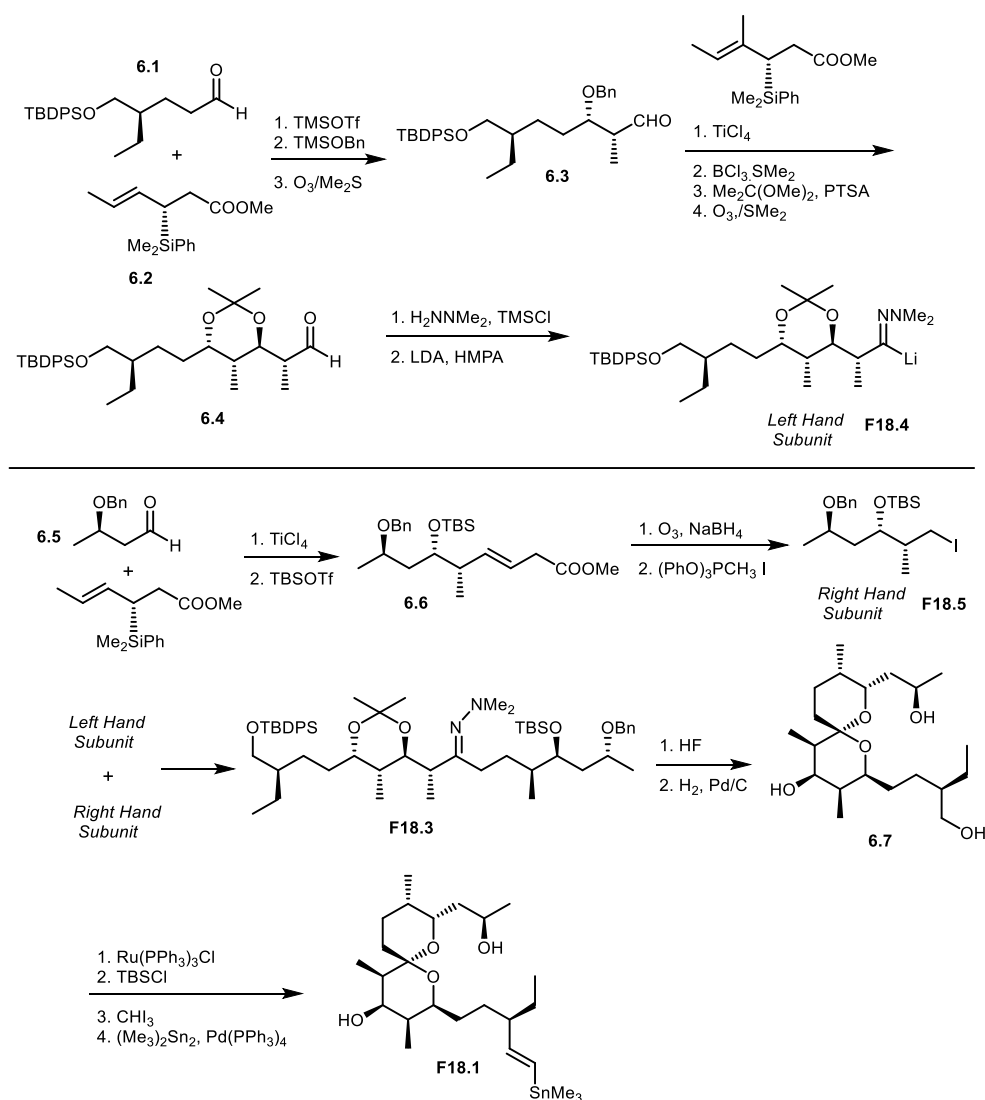


Figure 18: Retrosynthetic Analysis of Oligomycin C

Polypropionate fragment was obtained by employing a double-stereo differentiating *anti* aldol reaction with Felkin selectivity. Compound **F18.6** was prepared by three crotylation reactions with **F18.7** and **F18.8**. The fragment **F18.9** was prepared from α -methyl aldehyde **F18.10** using Kakai's protocol.

Synthesis of spiroketal fragment is discussed in **Scheme 6**. Spiroketal synthesis was started with the first synthesis of left-hand fragment then synthesis of right-hand fragment, finally coupling and cyclization strategies. Synthesis of left-hand fragment with LDA to obtain the subunit **F18.4**, this was the intermediate in the next step alkylation. After achieving the synthesis of **F18.4**, the next objective was aimed at synthesis of a right-hand fragment, starting from the chiral fragment which was obtained from a chiral aldehyde, (**6.1**). This aldehyde was prepared from ethyl diethyl malonate and further undergoes the *in situ* acetal formation followed by an asymmetric crotylation gives the *syn* stereochemistry of the benzyl protected homoallylic alcohol with (*syn*:*anti* >6:1) diastereoselectivity. Oxidation of the olefin under ozonolysis condition then leads to aldehyde (**6.3**). This was then subjected to TiCl₄ promoted double-stereo-differentiating crotylation reaction using the methyl-

substituted silane and this approach led to a single diastereomer with *anti* stereoselectivity at the newly generated chiral centers. Followed by the benzyl group deprotection and ketalization with dimethoxy propane, finally, oxidation of double bond with ozone provided aldehyde (**6.4**). It was then converted to N,N-Dimethylhydrazone, and further, treated with LDA for obtaining the left-hand subunit, **F18.4**. This was used as the key intermediate in the next step alkylation. After obtaining **F18.4**, the next objective was aimed at the synthesis of the right-hand fragment of spiroketal, which is shown in **Scheme 6**. The synthesis was started initially with TiCl₄ promoted crotylation of aldehyde (**6.5**) with **6.2**, followed by

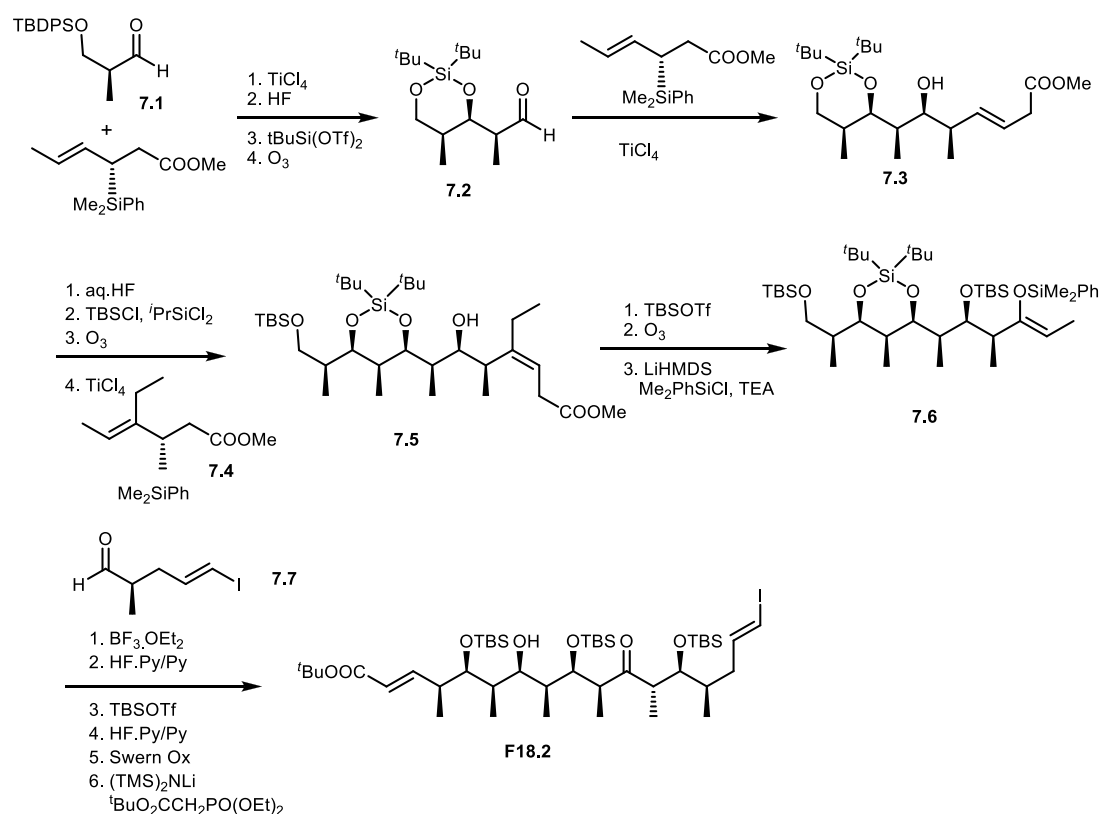


Scheme 6: Synthesis of Spiroketal Fragment

silyl protection, giving **6.6**. This compound **6.6** was further oxidized with ozone and then reduced with sodium boro hydride giving the alcohol. It was then converted into

iodide for obtaining the right-hand fragment of spiroketal (**F18.5**). Keeping these two spiroketal fragments in hand, the next step was performed as the alkylation reaction, seeking the coupled product, **F18.3**. Finally, the deprotection of two silyl groups and cyclization with HF, followed by debenzoylation by hydrogenolysis led accessing the spiroketal fragment of Oligomycin C.

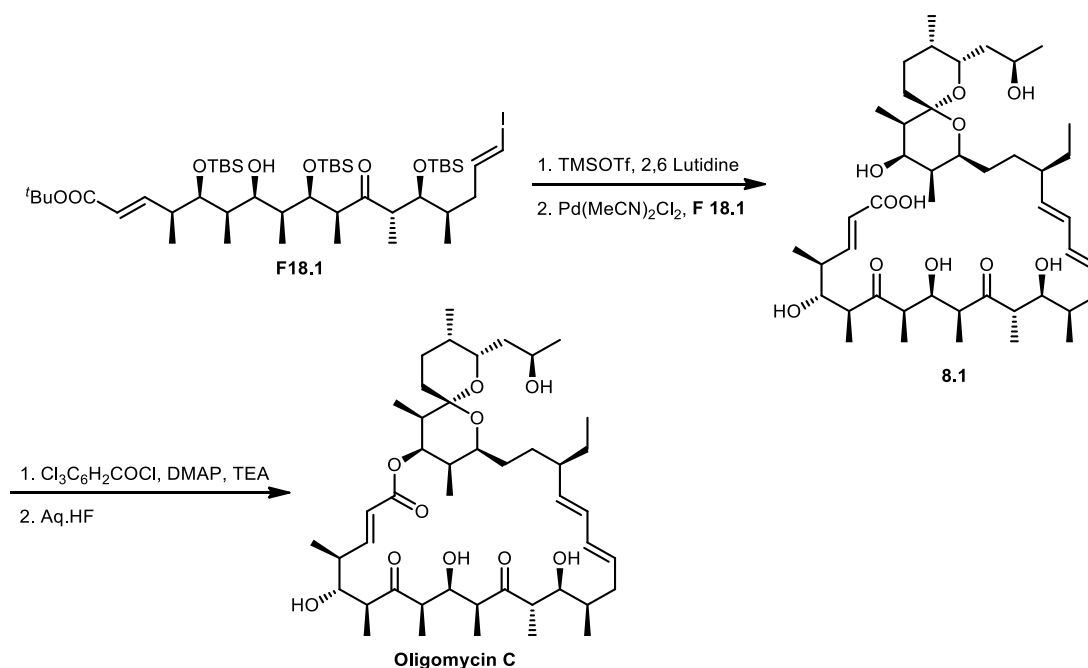
The detailed synthesis of polypropionate fragment is shown in **Scheme 7**. It was initiated with the reaction of a chiral aldehyde (**7.1**) with crotylsilane in presence of TiCl_4 and this approach offered the homoallylic alcohol, followed by silyl deprotection with HF, and cyclic silyl group formation between 1, 3 diol groups. Finally, oxidation of the olefin with ozone resulted in aldehyde (**7.2**).



Scheme 7: Synthesis of Polypropionate Fragment

Condensation of the aldehyde again with crotylsilane was promoted by TiCl_4 giving (**7.3**) with a good diastereoselectivity *syn:anti* (30:1). Compound (**7.3**) was further converted into aldehyde with the following sequence reactions: silyl removal, primary alcohol silyl protection, cyclic silyl protection between 1,3 diols, and finally, oxidation. This aldehyde was subjected to crotylsilane (third time) **7.4** condensation in the presence of TiCl_4 , giving **7.5** with 20:1 *syn:anti* diastereoselectivity. Protection of secondary alcohol with TBSCl, followed by cleavage of the olefin with ozone gave

ketone. This was further converted into Z-O-silyl enol ether (**7.6**). Compound **7.6** was then subjected to Mukaiyama aldol reaction with aldehyde (**7.7**) in the presence of $\text{BF}_3 \cdot \text{OEt}_2$ at -78°C , giving *anti* aldol product with a good diastereoselectivity (dr = 7:1). This aldol product was further converted into **F18.2** through the following steps which included, (i) a selective removal of C5-C7 silylene protecting group, (ii) selective silyl protection at C5 and C13 hydroxyl groups, (iii) selective deprotection of primary hydroxyl silyl protecting group, (iv) Swern oxidation, and, finally, (v) (E)-selective Horner-Emmons reaction, giving **F18.2** with a good yield. Prior to actual fragment coupling experiments, the tert-butyl ester of **F18.1** was converted to the corresponding carboxylic acid, by treatment with TMSOTf in the presence of 2,6-lutidine. Coupling of this acid polypropionate fragment with **F18.2** using Pd (CH_3CN) $_2\text{Cl}_2$ catalyzed Stille approach gave the protected seco acid fragment (**8.1**). It was then subjected to Yamaguchi macrolactonization, followed silyl group deprotection and this completed the synthesis of oligomycin C.



Scheme 8: Completion of Oligomycin C Synthesis

1.7 Introduction to Protein-Protein Interactions

Physical interactions between two or more similar or dissimilar proteins by a biochemical event (electrostatic forces) are called as protein-protein interaction (PPI).

³⁹ Protein-protein interactions plays an important role within the cellular machinery for specific functions, like, signal transduction, translation, transcription, replication,

various controls in Cell Cycle etc.⁴⁰ Owing to the diversity of protein–protein interactions, there is an essential need for careful investigation of the nature of the protein interfaces. The protein interface residues are a determinant of the stability and specificity of protein–protein interactions. The length of the protein interface decides whether the complex will be transient or obligatory, these protein-protein interactions are regulated by environmental condition such as p^H , temperature and ionic strength etc. based on the stability of the protein complex (there are two types: permanent stable complex and temporary stable complex)^{41, 42} The temporary protein complex interfaces have unique properties for each interacting pair of proteins whereas the permanent stable protein complex interfaces have similar properties on their surfaces as their formation is considered to be a continuation of protein folding. The prediction of protein–protein interaction is very crucial in drug discovery. Unlike enzymes, protein-protein interactions have multiple smaller interacting regions called as “hotspots” that comprise the subset of residues that contribute the bulk of the binding free energy.⁴³ Functional genomics studies provided information that there are 3,000–10,000 disease-modifying proteins but the pharmaceutical industry has explored only 400 proteins for the therapeutic development and most of these belong into a few families, such as enzymes, the G protein–coupled receptors, ion channels, nuclear hormone receptors, and other targets.⁴⁴ In general, protein-protein interactions involve shallow surfaces cover a relatively large surface area. PPIs are largely driven by hydrophobic interfaces.⁴⁵ The hydrogen bonds, electrostatic interactions,⁴⁶ and the covalent bonds are considered important for PPIs.

Many pathological and physiological cellular processes depend on PPIs which can be influenced by external compounds.⁴⁷ The present modern drug discovery approaches mainly involve three steps (1) identification of the prospective drug target, (2) investigating its properties, and finally, (3) designing of a the corresponding ligand.⁴⁸ Therefore, the knowledge of protein–protein interaction can be useful in designing modulators that can target the protein complex involved in various diseases. But a number of factors can contribute to the challenge of identifying small molecules that inhibit such interactions. These include the general lack of small-molecule starting points for drug design, the typical flatness of the interface, and the difficulty of distinguishing real from false binding, and the size and character of typical small-molecule libraries. However, much of these problems can be solved through an advancement of molecular biology and computational modeling techniques.

1.8 Protein–Protein Interactions as Drug Targets

Druggability is defined as modulating the function of a target protein by small-molecules with high affinity.⁴⁹ Most of the proteins involved in disease pathways exert their functions through interactions with other proteins, and they lack obvious druggable pockets for small molecules. Although protein–protein interactions (PPIs) are important in many cellular functions and disease pathways, targeting protein–protein interaction with small molecule is a challenging task.⁵⁰ The main reasons are that protein–protein interface hot spots are unevenly distributed across the protein surfaces, and secondly, the shape of protein–protein recognition sites which are mostly flat unlike protein–ligand recognition sites which have deeper cleft.⁵⁰ The druggability issue with protein–protein contacts has greatly been solved with the growing computational modeling methods,^{51,52} X-ray crystallography techniques and the advancement of fragment based drug discovery.⁵² In general, small molecules that affect protein–protein interactions function through three distinct mechanisms: (i) orthosteric inhibition: in this approach, there is a competition between orthosteric inhibitor and the partner protein for the same binding site on target protein. orthosteric inhibitors bind to the target protein prior to the partner protein for the same binding site, thus directly inhibiting the formation of macromolecular complexes.⁵³

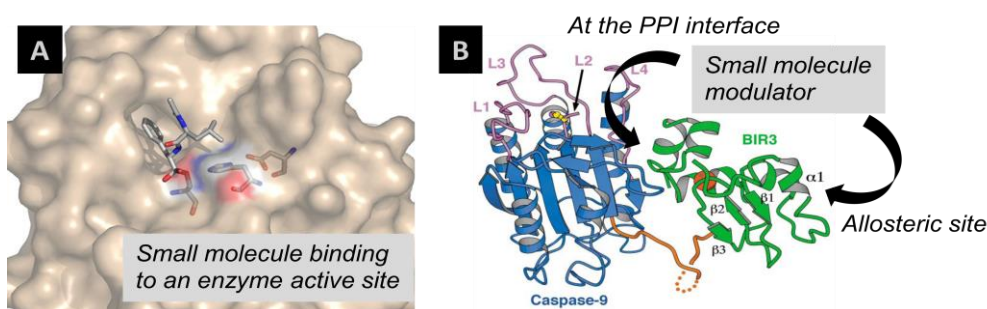


Figure 19: (A) Small Molecule Binding to an Enzyme Active Site; (B) Small Molecule Modulators of Protein-Protein Interactions via Interface or an Allosteric Site.⁵⁷ (note: this figure is taken from a review article from our group, *Chem. Rev.* **2014**, *114*, 4640–4694)

(ii) In this approach, small-molecule ligands (allosteric inhibitors) bind to the target protein at the sites distinct from the partner protein binding site (used for macromolecular complex). The binding of an allosteric inhibitor on target protein

changes the dynamic properties or static conformation of the target protein(s) and with this, partner protein cannot form the macromolecular complex with the target protein.⁵⁴ (iii) The third category is interfacial binders: In this approach, ligands and proteins form a ternary complex. An interfacial inhibitor binds to a pocket at the macromolecular interface, the ligand and proteins which sometimes is only transiently formed in a transition state, and locks the complex into a non-productive arrangement.^{55,56}

1.9 Introduction to Macrocycles

Macrocycles are the molecule that contains 12 or more atom with the cyclic framework.⁵⁸ The molecular weight of macrocyclic compounds is around 500-2000 Da. Naturally occurring macrocycles acquire 50+ atoms in the largest ring. However, recent analysis suggested that 14, 16, and 18 member frameworks are commonly occurring macrocyclic scaffolds.⁵⁸ Macrocycles have a structure that affords a degree of conformational pre-organization due to restricted rotation. There are different classes that macrocycles could fall into, including peptidic and non-peptidic natural products, non-natural (synthetic) peptides and non-natural (synthetic) macrocycles. Their unique features allow several applications as the inhibitors of challenging targets, such as, proteins with flat feature less surfaces or protein-protein interactions that are hardly tractable by classical small-molecule drugs.⁵⁹ For the range of therapeutic targets, macrocyclic compounds could be isolated from natural sources, and, several natural products have proven their application as therapeutics.⁶⁰ For many targets, however, no natural product macrocycles are found, and, this creates the need for developing synthetic macrocycles by mimicking or inspired by natural products. To achieve this goal, we need a better understanding of the structures and binding modes of macrocycles, and must have efficient strategies and tools for developing the synthesis plans.⁶¹

One of the key objectives of the macrocyclic natural products/natural products-inspired small molecules is to discover novel compounds as the modulators of PPIs, and this then leads to building the diversity-based organic synthesis program. This aims at populating the chemical space having a set of compounds obtained from the inspiration of natural products.⁶² The early days of the high-throughput chemistry arena was more focused on accessing a large number of compounds by utilizing rather simple chemistry. However, this paradigm shift from the enzyme inhibition to

PPI is also challenging the chemistry community in developing novel methods allowing us populate the chemical space with compounds having (i) a wide variety of 3-dimensional scaffolds with a few chiral centers, (ii) a few diversity points, (iii) an acceptable pharma-related complexity, and (iv) acceptable to medicinal chemistry to be synthesized in a relatively short period.

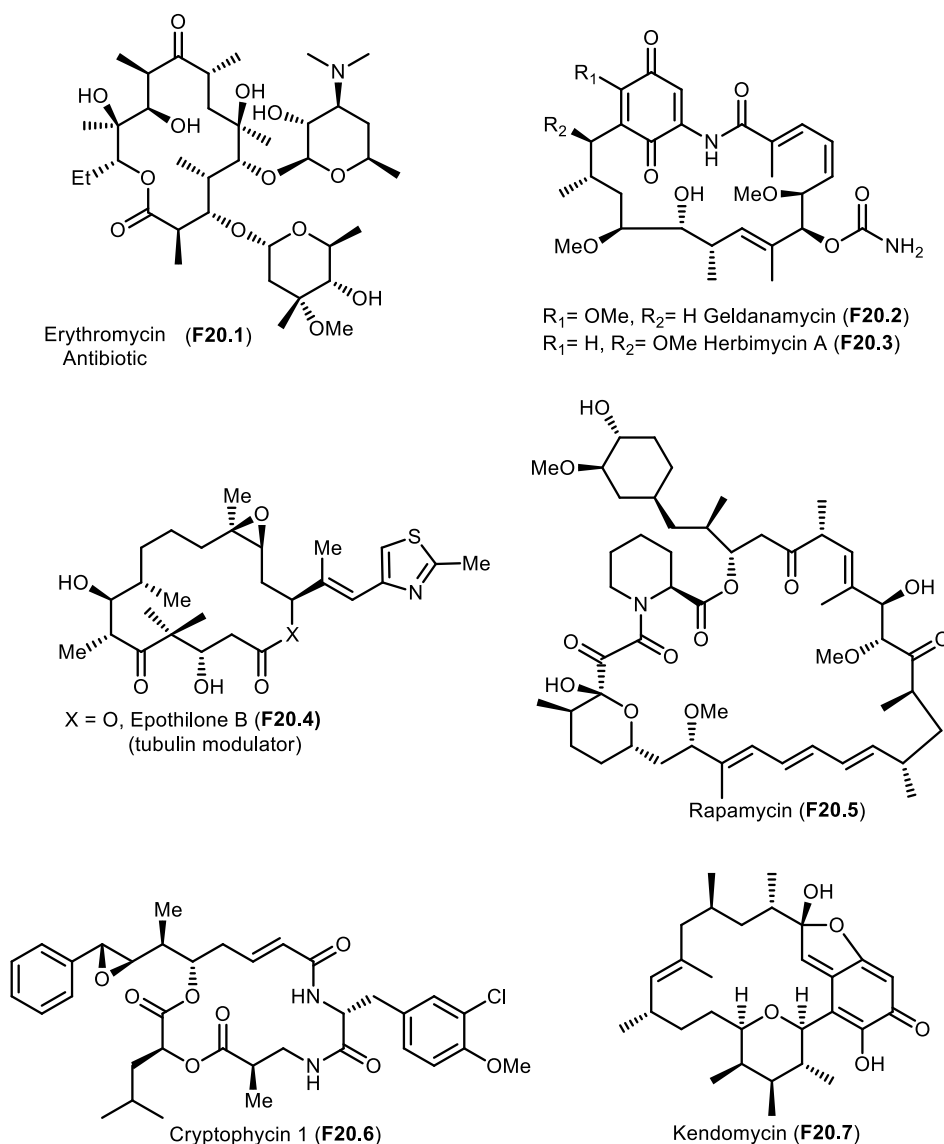


Figure 20: Biologically Active Macrocyclic Natural Products

Around 68 macrocycle drugs are currently used in the market.⁶³ Out of these 68 drugs 34 are used for the treatment of bacterial infection. While 10 are used for the treatment of different cancers and remaining the remaining 28 are applied in gynecological and cardiovascular, immunological therapeutic areas, as well as in a range of indications, including anesthesiology and pain.⁶⁴ Majority of these macrocycles are natural products or directly derived from natural products. In **(Figure**

20), I am showing the structures of biologically active macrocyclic natural products erythromycin (**F20.1**) was the first bioactive macrolidic agent to become an anti-infective drug. geldanamycin (**F20.2**),⁶⁵ herbimycin A (**F20.3**),⁶⁶ epothilone (**F20.4**),⁶⁷ rapamycin (**F20.5**), Cryptophycin (**F20.6**),⁶⁸ kendomycin (**F20.7**).⁶⁹ Some druggable macrocyclic molecules are also synthesized from biologically active natural products. Examples in this category are shown in (**Figure 21**). Ixabepilone (**F21.1**) (Xemptra), a natural product analog of epothilone B is a microtubulin stabilizer.^{67a} 17-Allylamino-geldanamycin, is a natural product analog (**F21.2**) (benzoquinone ansamycin) and a HSP90 inhibitor. Temsirolimus (Torisel)⁷⁰ (**F21.3**) is a natural product analog of rapamycin.

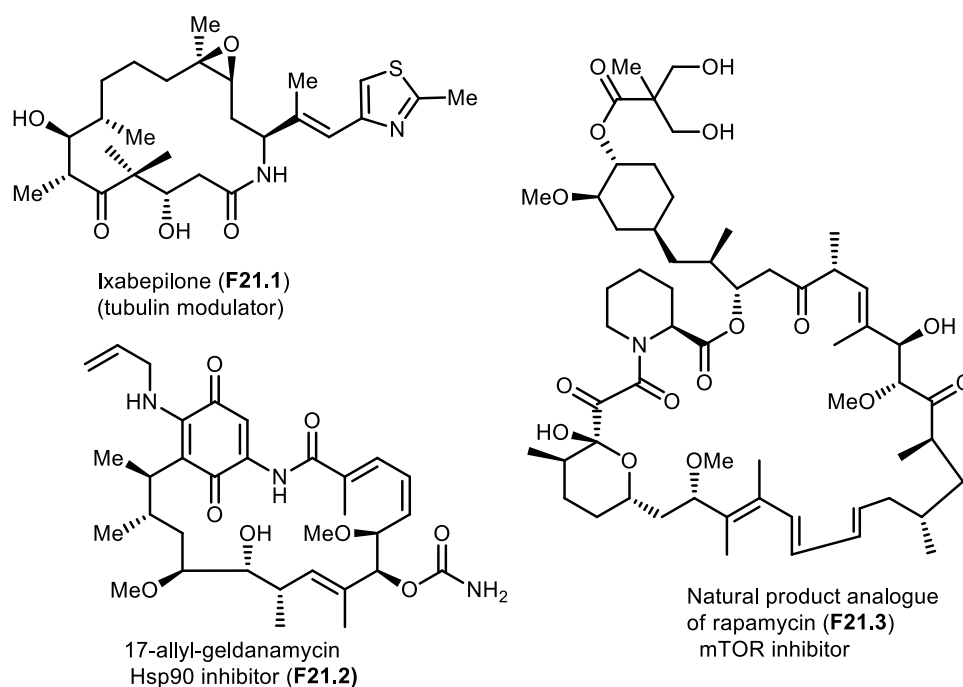


Figure 21: Biologically Active Natural Product Derived Macrocycles

In recent years, combinatorial approaches like Diversity-Oriented Synthesis (DOS) for obtaining synthetic macrocycles is increasingly considered as a source of searching macrocycle drugs.⁷¹

Conventional metrics that are used for classical small-molecule drugs such as those of Lipinski's 'Rule of Five' cannot be applied to macrocycles.⁶⁴ Macrocycles are found to have a smaller binding energy per heavy atom (potential ligand efficiency) compared to small-molecule drugs. In (**Figure 22**), I am showing some synthetic macrocyclic molecules which are in different stages of clinical trials and development. Macrocycle pacritinib (SB1518) (**F22.1**) is a JAK2/FLT3 kinase inhibitor, now in

advanced Phase II trials. The CDK2/JAK2/FLT3 inhibitor SB1317 (**F22.2**) in Phase I trial, and other one is a synthetic cyclic peptide, cilengitide (**F22.3**) in Phase III investigations for the treatment of glioblastoma, and the compound Mpan-CDK inhibitor is proposed as the development candidate.

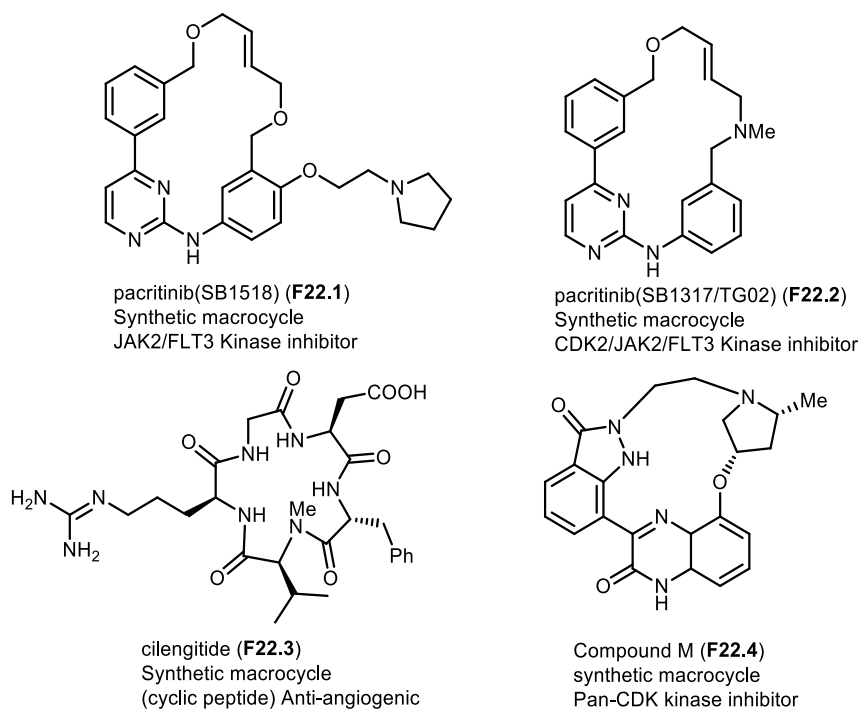


Figure 22: Purely Synthetic Macrocyclic Molecules in Clinical Trails

1.10 Acyclic Molecules v/s Macrocycles

Based on the literature reports, there are many examples which show the power of making macrocyclic rings. For protein-protein interactions, there are several reviews majorly focused on the importance of macrocycles.⁷² Natural product-inspired synthetic macrocycles are showing a good track record in the drug discovery area. From the chemistry point of view, macrocycles can offer stereochemical complexity and diverse functionality in a conformationally restricted manner. For example, some of the macrocyclic compounds have more potency than their equivalent acyclic structure. More than that, macrocyclic compounds can also demonstrate several favorable drug-like properties, which include an improved membrane penetration, enhanced metabolic stability, good solubility, good oral bioavailability, and an increased lipophilicity with desirable pharmacokinetic and pharmacodynamic properties.^{73,71,60} (**Figure 23**): Macrocycle (**F23.2**) Show Better Affinity than It's Acyclic Compound **F23.1**⁷⁴ in the Inhibition CHK1.

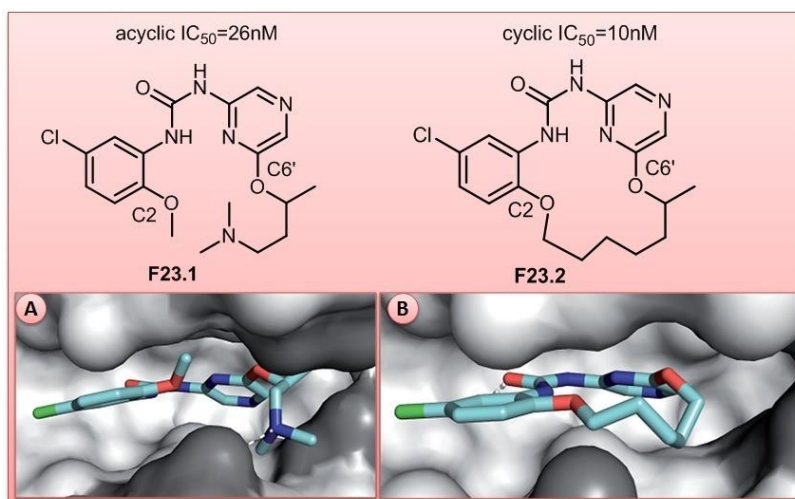
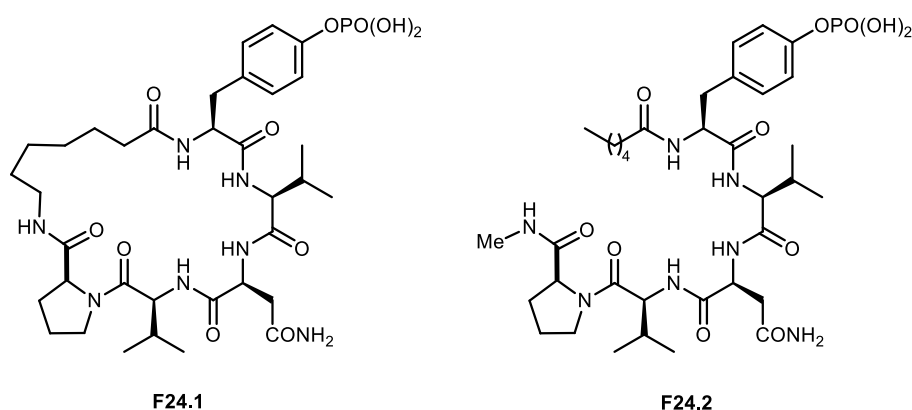


Figure 23: Acyclic and Macrocytic CHK1 Inhibitors. X-ray Co-Crystal Structure of Inhibitors in CHK1: (A) Acyclic inhibitor (B) Macrocytic inhibitor.⁷⁵ (note: this figure is taken from *Future Med. Chem.* **2012**, 1412)



Shown in (Figure 24), macrocycle (F24.1) is 2-fold greater affinity than its linear acyclic Compound (F24.2)⁷⁶ for stabilizing the biologically active conformation of Grb2 SH2 peptides.

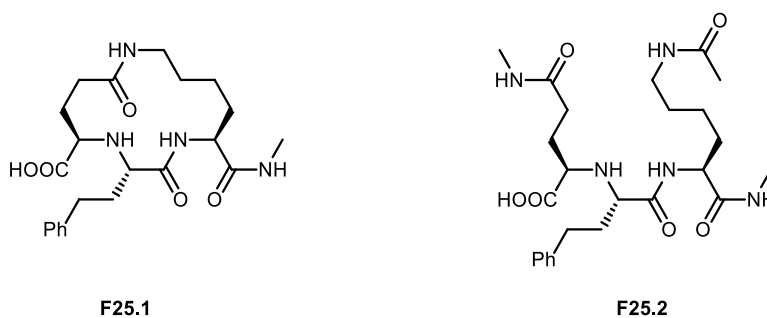


Figure 25: Macrocycle (**F25.1**) is 17-fold More Affinity than it's Acyclic Compound (**F25.2**)⁷⁴ in the Inhibition of MMP-8 which Belongs to the Family of MMPs needed for the Maintenance of the Extracellular Matrix.

Before going to discussion about the neopeltolide in detailed in next chapter here I am discussing about the biology, how it inhibit ATP formation. Neopeltolide is a marine natural product isolated by write and coworkers from the deep water lithisted sponge Neopeltide. Neopeltolide act as the Mitochondrial ATP synthesis inhibitor. As we discussed in the above sections oxidative phosphorylation takes place in inner membrane of mitochondria. It involves two steps electrons transportation chain and chemiosmosis. Four complexes are involved in electrons transportation, neopeltolide attack on the cytochrome bc_1 complex (complex-III) for stopping the electron transportation to the next level. Obviously it leads to the inhibition of the ATP synthesis (**Figure 26**).

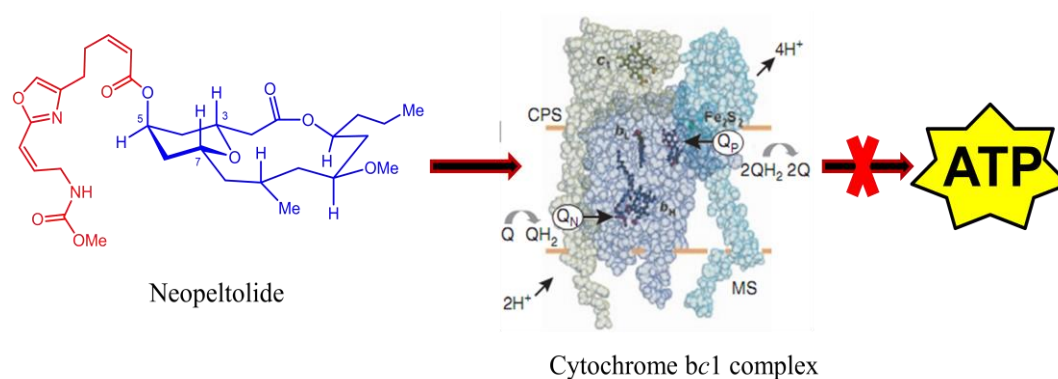


Figure 26: ATP synthesis Inhibition by Neopeltolide

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Chapter 2

A Divergent Stereoselective Synthesis of Neopeltolide-Inspired Macrocycles



2.1 Introduction

This chapter reviews the current literature pertaining to the isolation, structural determination, biological evaluation and synthetic efforts of a few leading groups. Initially, isolation and identification of neopeltolide is discussed, followed by both the total synthesis and analogs synthetic work available up to date. Following this, I am discussing our synthetic route for making neopeltolide-inspired macrocycles.

2.2 Extraction and Isolation

Neopeltolide, a novel biologically active marine-derived complex macrolide compound, introduced to the world by Write and co-workers in 2007.¹ It was isolated from the deep water lithistid sponge of the family neopeltidae taxonomic description and was most closely matched with the genus *Deodalopelta*. By using the Johnson-sea link human-occupied submersible, two samples were collected from *Deodalopelta* sponge near the north Jamaican coast. From 105.0 g of sponge, by exhaustive extraction with ethanol, concentration under reduced pressure got crude residue 3.2 g. The crude was partitioned between n-butanol and water, after concentration the butanol fraction was directly used as slurry to the column in a mixture of heptane-ethyl acetate. the fraction containing the neopeltolide was further purified by HPLC using VydacC18 protein and peptide column.¹

2.3 Structural Determination

With the help of ¹³C-NMR and HRMS data, the molecular formula was proposed for neopeltolide as C₃₁H₄₆O₉N₂ [(M + H⁺) *m/z* observed 591.32692, calcd 591.32816, Δ=1.2 mmu)], indicating the presence of 10 degrees of unsaturation. ¹³C-NMR values also revealed two hetero-substituted sp²-hybridized carbons [δ C 161.9 (C), 159.6-(C)], two ester carbonyls (δ C 173.0, 166.9) and six additional olefin carbons (δ C 150.0 (CH), 142.3 (C), 139.2 (CH), 135.9 (CH), 121.7 (CH), 115.7 (CH)) accounted for seven of the degrees of unsaturation, suggesting that neopeltolide is a tricyclic compound. The planar carbon skeleton was established from the analysis of 1D and 2D-NMR data, where 1D double pulsed field gradient spin echo (DPFGSE) NOE experiments proved instrumental in the regions of heavy overlap. These results indicated that neopeltolide contains a 14-membered macrolactone bearing a 2,4,6-trisubstituted tetrahydropyran ring, which served as an anchor for an oxazole-containing side chain. The six stereocenters configuration was assigned on the bases of analysis of coupling constant, COSY, 2D-

NOESY spectrum and a few 1D-DPGSE NOE experiments. The observed NOESY experiments strongly support the diaxial coupling between H-3 and H-7. However, the H-5 proton was assigned as equatorial. Due to weak coupling with H-3 and H-7 protons, further, the relative stereochemistry of H-9, H-11, and H-13 was assigned as the same side of H-3, H-7 protons, by observing enhanced NOE between H3-H7, H7-H9, H9-H11, and H11-H13 respectively.

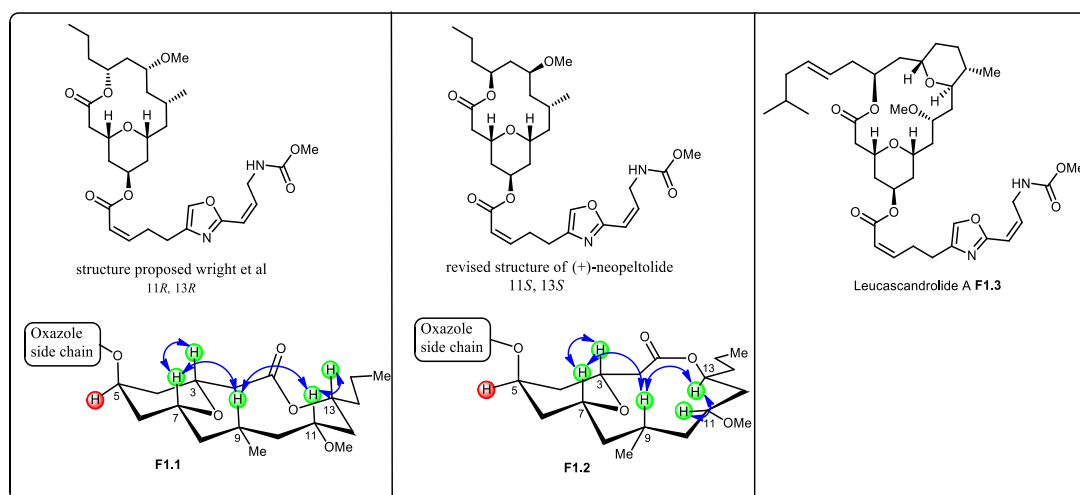


Figure 1: The proposed (F1.1) and revised (F1.2) structures of neopeltolide. Key NOE interaction data from within Neopeltolide's framework used to define the relative stereochemistry by Wright and co-workers.¹ and the structural revision by Scheidt² are presented in blue. The oxazole-containing side chain is not included for clarity purposes. (F1.3) shows the structure of leucascandriolide A.

Wright and co-workers assigned the relative configuration of neopeltolide with the small amount, due to the lack of material, they were not able to assign the absolute configuration. This was later, assigned by the chemical synthesis, which ultimately overturned the proposed C11 and C13 assignment. Further, Panek and co-workers provided an explanation for the NOSEY studies which was used as the heavy support for the originally proposed configuration despite the incorrect configuration. Their hypothesis centered on the region of heavy overlap in the proton spectrum of neopeltolide, which was coupled with NOE enhancements between H7-H9 and H9-H11, suggesting a *cis* relationship. In their work, Youngsaye and co-workers proposed that the aforementioned NOE enhancements were uncertain due to the crowded aliphatic region of the C9 methine. Within the buried region of the C9 proton ($\delta = 1.38$ ppm), are the overlapping protons from the neighboring C8 methylene ($\delta=1.36$ ppm) and C12

methylene groups ($\delta = 1.28$ ppm), respectively (**Figure 1.2**). Considering the close proximity of these three protons, it was suggested that the initially reported NOE enhancements between H7-H9 and H9-H11 were actually occurring between H7-H8 and H11-H12. Under this alternative interpretation, it was concluded that no information existed in relation to the C9 stereochemistry the heavy overlap of the C8 proton ($\delta = 1.36$ ppm, m), C9 proton ($\delta = 1.38$ ppm, m), and C12 proton ($\delta = 1.28$ ppm, m) region, was noted as indicated in blue color.

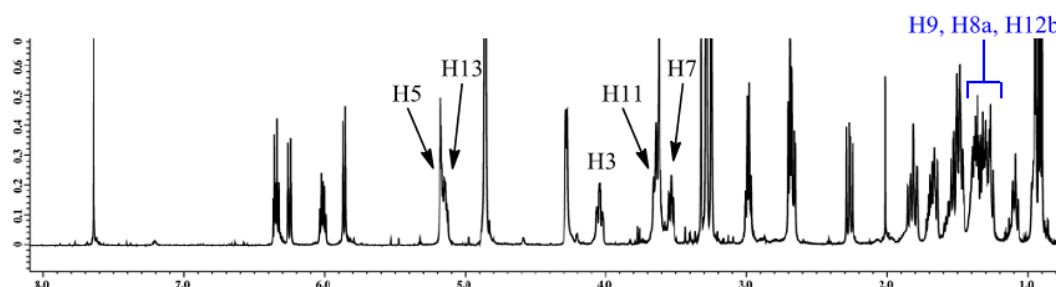


Figure 2: The ^1H NMR Spectrum of the Originally-Isolated Neopeltolide Sample by the Wright group. 600 MHz CD_3OD .

To elaborate his hypothesis, Youngsaye team rationalized that the stereocenters of natural neopeltolide were segregated into three regions based on the spectroscopic analysis and observations, and these are: (1) a C11,C13-*cis* segment, (2) a C3,C5-*trans*-C5,C7-*trans* tri substituted tetrahydropyran, and (3) an excluding enantiomeric pairings, there exists four stereoisomers of the three segregated regions, one of which should the natural isomer be of (+)-neopeltolide, and would possess the absolute stereochemistry (**Figure 2**).

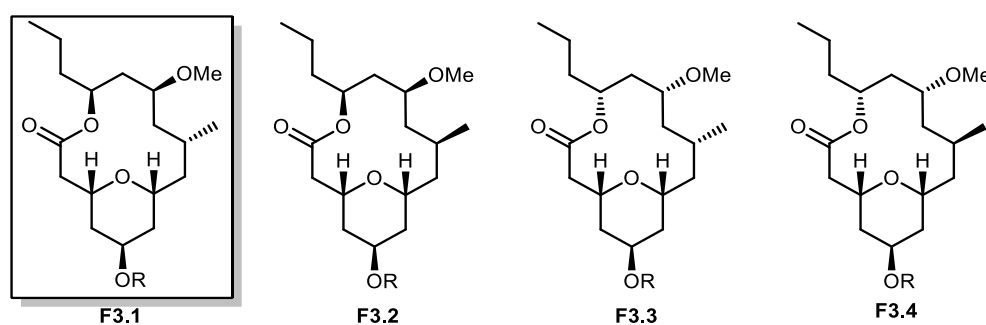


Figure 3: Summary of Four Candidate Stereoisomers of (+)-Neopeltolide Based on the Three Segregated Regions of Stereocenters.

The originally proposed structure of neopeltolide by Wright and co-workers (**F3.3**) represents one the four stereoisomers, and the remaining three were prepared synthetically by the Panek group for validating their hypothesis.³ The successful synthesis of all three stereoisomers (**F3.2**, **F3.3**, and **F3.4**) identified an isomer (**F3.1**) as the natural (+)-neopeltolide, based on the comparison of spectroscopic data.

2.4 Biological Evaluation

With the extracted small amount of neopeltolide, Wright and co-workers carried-out several biological experiments. It acts as a potent antifungal agent towards the *Candida albicans* pathogen (MIC value of 0.625 µg/mL) and also as a potent *in vitro* cytotoxic agent against the growth of several cancer cell lines.

Cancer cell	IC ₅₀ (nM)
A549 human lung adenocarcinoma	1.2
NCI/ADR-RES ovarian sarcoma	5.1
388 murine leukemia	0.56

2.5 Mode of Action

Preliminary investigations into the mechanism of action for neopeltolide were carried-out in conjunction with an analog of the structurally homologous leucascandrolide-A as shown in (**F1.3**).⁴ Neopeltolide shares several common structural features with leucascandrolide A, including a 2,4,6- tri substituted tetrahydropyran (THP) subunit, with an identical oxazole-containing, highly unsaturated side chain (see, **Figure 1**). Thus, one can assume similar biological targets for these two compounds, as neopeltolide may be considered a simplified analog of leucascandrolide A.⁵ Using flow cytometric methods, Wright and co-workers reported that neopeltolide causes a block of the cell cycle at G1 at the doses of 100.0 nM in the A549 lung adenocarcinoma cell line.¹ The Kozmin group substantiated this finding in their publication that highlights the finding of neopeltolide inhibiting cell proliferation.^{4c,6} Kozmin and co-workers suggested that neopeltolide does not act via interaction with tubulin or actin, but rather inhibits complex-III of the electron transport chain, thus inducing apoptosis. Cancerous cells are particularly vulnerable to disruption of oxidative phosphorylation due to an increased dependency on glycolysis, known as the Warburg effect,⁷ making neopeltolide a promising lead compound. Although it remains uncertain whether the inhibition of

cytochrome bc_1 fully accounts for the potent anti proliferative activity of neopeltolide, the results provide a molecular basis for the further experimentation required for complete elucidation. Additionally, following the hydrolysis of the oxazole-containing side chain ester linkage, further, evaluation of leucascandrolide A, and (+)-neopeltolide by extension, it was demonstrated that the macrocyclic domain is solely responsible for the cytotoxicity.³² Moreover, the oxazole-containing unsaturated side chain was reported responsible for the antifungal properties, suggesting that the bioactivity of neopeltolide functions under the same conditions.

2.6 Literature Synthetic Work

Neopeltolide has the potential biological activity profile and a complex structure with the macrolide ring. Because of the limited supply from the natural source, it has gained significant interest from the organic synthetic community. More than fifteen total and formal synthesis have been reported, few groups also prepared neopeltolide analogs for examining the potency of the molecules. The first two syntheses of the neopeltolide were reported Panek and co-workers in **2007** and another one by Scheidt⁸ group, the same year with revised configuration (S) at C11 and C13 stereocenters. This revision gives the absolute stereochemistry (+)-neopeltolide, and also, inverted the relative stereochemistry proposed by Wright and co-workers¹ by spectroscopic analysis, with the comparison of the prepared diastereomers of neopeltolide.

2.6.1 Total Synthesis of Neopeltolide by Panek and Co-workers

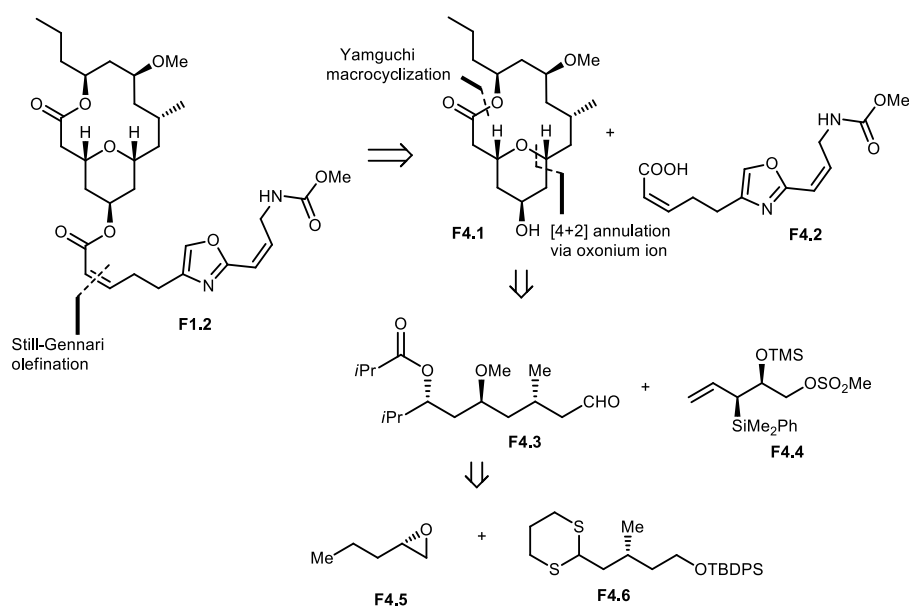
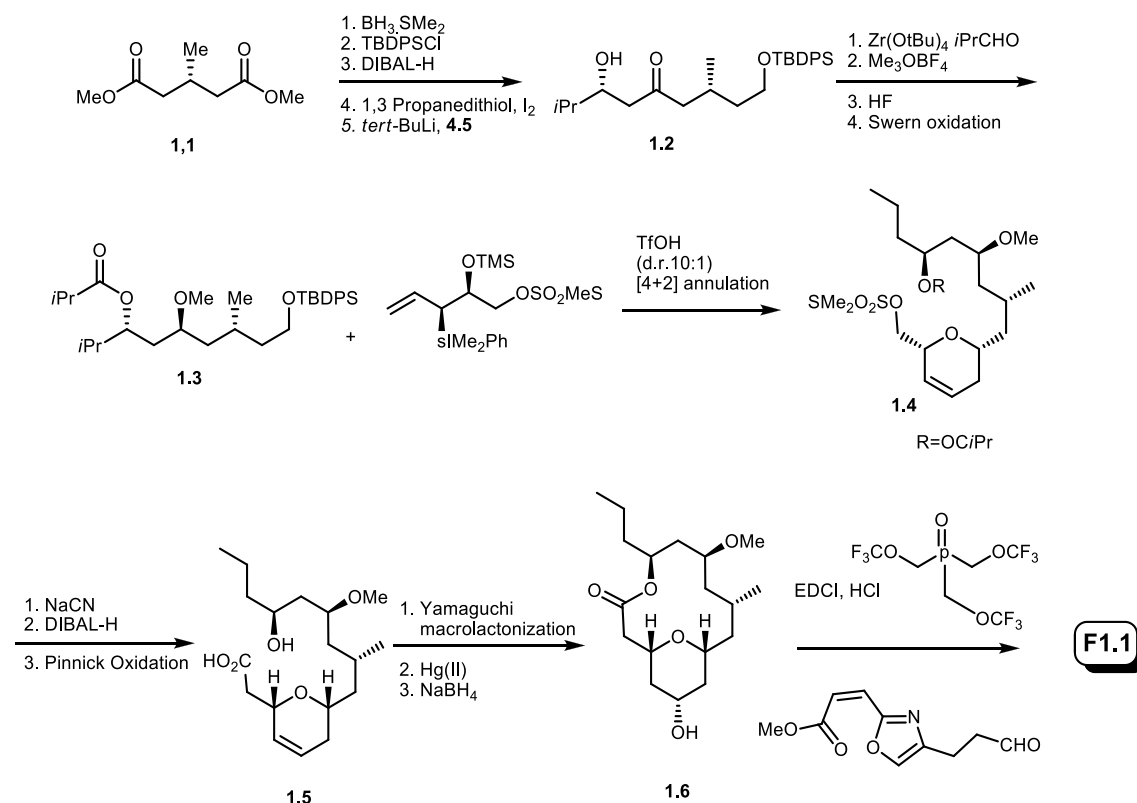


Figure 4: Retrosynthetic Analysis of James Panek Group (2007)

In this section, I am going to discuss the work from Panek group for the synthesis and the revision of stereochemistry of neopeltolide at C11 and C13 carbons. Initially, the researchers started the synthesis of the original neopeltolide as the proposed structure by Wright and co-workers. In this study, they obtained a compound with several spectroscopic discrepancies from the natural product data. In order to identify the absolute configuration of neopeltolide, Panek and co-workers also undertook the synthesis of other diastereomers.

The neopeltolide (**F1.2**) could be obtained from macrolide (**F4.1**) and oxazole side chain (**F4.2**) by Still-Gennari olefination. The 14 membered macrolactone (**F4.1**) was achieved by installation of the tetrahydropyran unit, which was formed by using a [4+2] annulation strategy with allyl silane (**F4.4**) while macrocyclization was achieved through Yamaguchi esterification. Allyl-silane (**F4.4**) was readily prepared in a six-steps sequence from commercially available 3-propyn-1-ol.⁹ The intermediate (**F4.3**) was synthesized from the enantioenriched epoxide (**F4.5**)¹⁰ and dithiane (**F4.6**) Finally, the oxazole (**F4.2**) was assembled as previously reported by the same group.



Scheme 1: The Panek Synthesis of Neopeltolide

Macrolactone core synthesis (**Scheme 1**) was initiated with the preparation of C7-C11 fragment from the commercially available methyl (R)-(+)-3-methyl glutarate.¹¹ First, the

chemoselective reduction of acid with $\text{BH}_3\cdot\text{DMS}$, followed by TBDPS protection of alcohol and hydride reduction with DIBAL, hydride reduction was followed by an iodine-catalyzed dithio acetalization of the resulting aldehyde for affording the dithiane (**F4.6**), which was coupled with epoxide (**F4.5**), giving **1.2**. The removal of dithioketal group then led the synthesis of ketone, which upon modified Evans-Taischenko¹² reduction provided the *anti* stereoselective product at C11 and C13 stereocenters with (d.r. 14:1). The DIBAL-H then gave aldehyde and the subsequent oxidation provided the carboxylic acid (**1.5**).¹³ The seco acid (**1.5**) was subjected to Yamaguchi macro lactanization for forming the macrolide. Selective oxymercuration of pyran olefin gave the selective axial C5 alcohol⁹ which upon acylation with bis (2,2,2-trifluoroethyl) phosphonoacetic acid¹⁴ then led the synthesis of phosphonoacetate. It was then treated with KHMDS for deprotonation followed by reaction with side chain aldehyde, and this approach successfully provided a mixture (7:1) of neopeltolide (**F1.2**). In this synthesis, the longest linear sequence required is 19 steps with an overall yield of 1.3%.

2.6.2 Synthesis of Neopeltolide by Hoveyda and Co-workers

Neopeltolide is a complex molecule with efficient anti-proliferative activity, therefore from the date of isolation, many groups worked on the total synthesis. Scheidt (2007), Kozmin (2008),¹⁵ Fuwa (2008),¹⁶ Paterson (2008),¹⁷ Floreancig (2009),¹⁸ Roulland,¹⁹ Sasaki (2010),²⁰ Yang, (2011)²¹ and Ghosh (2013)²² completed the total and formal synthesis of neopeltolide. Here, I am discussing the synthetic work reported by Hoveyda and co-workers. The key strategy involved in this synthesis is the olefin metathesis reactions promoted by molybdenum (Mo), Tungsten (W) and Ruthenium (Ru) complexes.²³ First, I will discuss the retrosynthesis of **F1.2** as shown in (**Figure 5**). Neopeltolide was obtained from the coupling reaction between **F5.1** and **F5.2**, 14-membered macrolactone (**F5.1**), which was obtained from diene (**F5.3**) by an efficient catalytic macrocyclic ring closing metathesis (MRCM), followed by hydrogenation.²⁴ The diene (**F5.3**) was formed by the coupling of enantiomerically pure acid (**F5.4**) and secondary alcohol (**F5.5**) The acid fragment (**F5.4**) was generated by performing a catalytic enantioselective Ring Opening Cross Metathesis (ROCM) on unsaturated oxabicyclic (**F5.6**). The oxazole diene side chain (**F5.2**) was prepared by implementing the *Z*-selective Cross Metathesis (CM) reaction as the key reaction. Compound **F5.2** was then accessed through direct *Z*-selective CM from **F5.7** which was obtained by the functional group conversion using **F5.8**. It was obtained through the cross coupling

reaction of **F5.9** and **F5.10**. Finally, **F5.9** was obtained by Z-selective CM between **F5.11** and **F5.12**, respectively. First, I will discuss the synthesis of macrocyclic fragment (**F5.1**). The synthesis was started with an enantioselective conjugate borylation²⁵ of the unsaturated Weinreb amide (**2.1**) followed by the oxidative work up, giving the hydroxy amide.

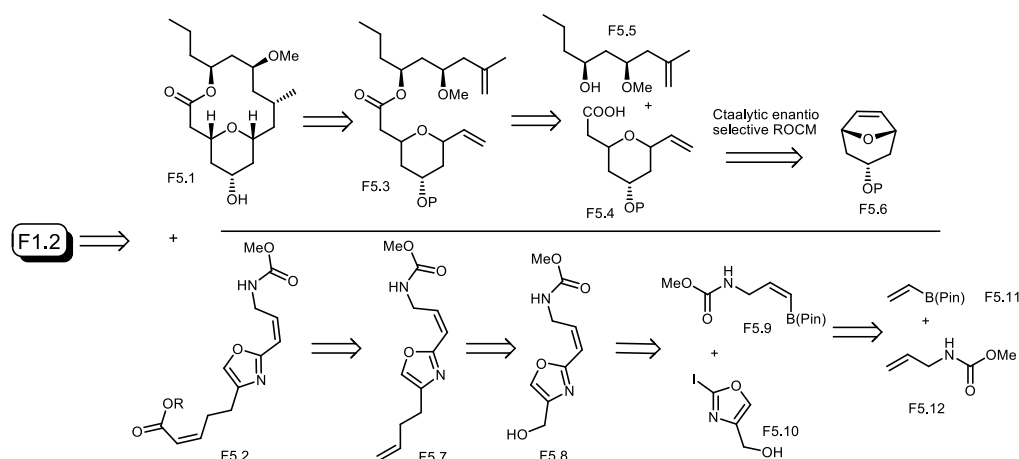
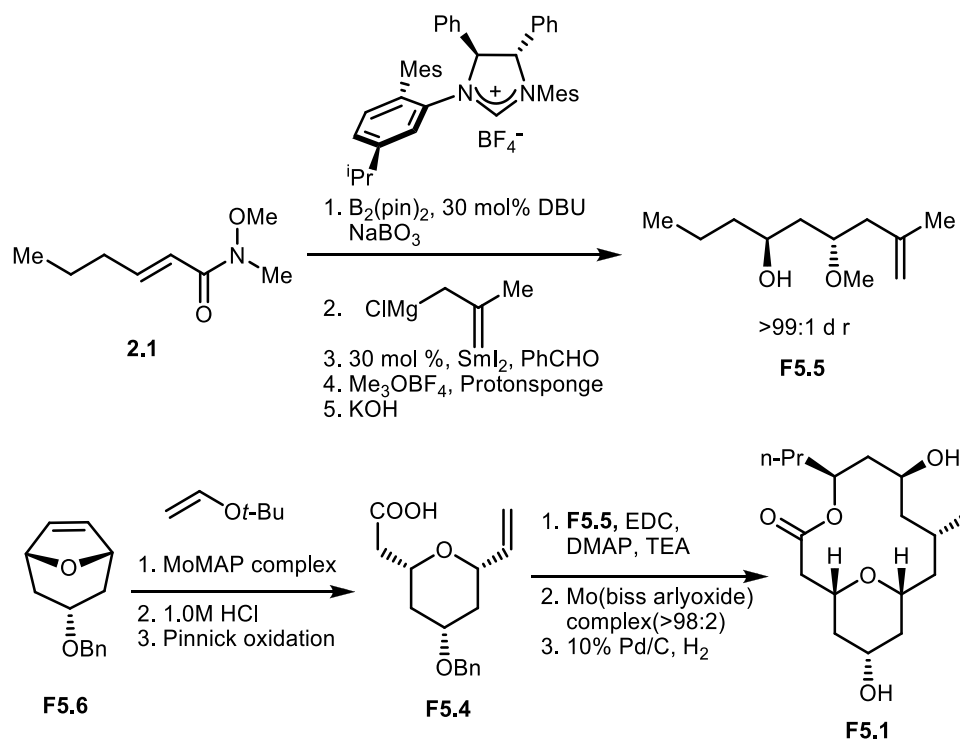


Figure 5: Retrosynthetic of Neopeltolide by Hoveyda and co-workers

Next, it was treated with 2-methylallyl magnesium chloride, followed by Tischenko-type reduction with SmI_2 and this approach generated the *anti* mono benzoylated product with (d.r. 99:1) which was subjected to O-methylation and debenzoylation, giving **F5.5**.



Scheme 2: Synthesis of Macrolactone

The synthesis of pyran acid moiety (**F5.4**) began with desymmetrizing ring-opening/cross metathesis cascade of meso-oxabicyclo alkene (**F5.6**), with *n*-butyl vinyl ether by the action of the chiral molybdenum mono aryl oxide pyrrolide catalyst (MoMAP) (**structure 1**), as shown in (**Figure 6**), affording the 2,6-*cis*-substituted tetrahydropyran.

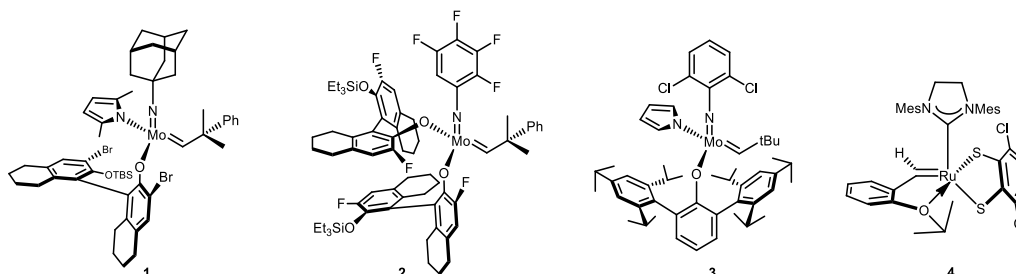
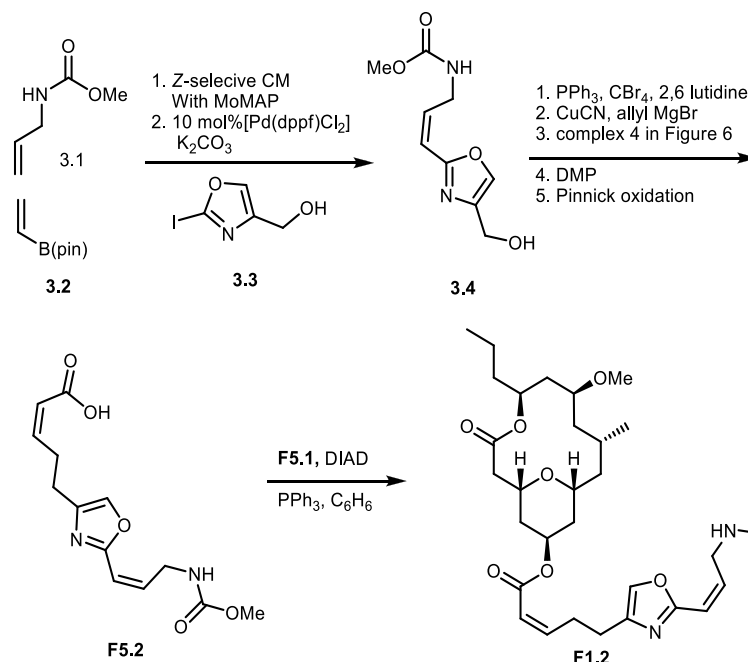


Figure 6: Olefin Metathesis Catalyst

This was then followed by the hydrolysis of the enol ether moiety, Pinnick oxidation of the resultant aldehyde gave the carboxylic acid (**F5.4**). Coupling of the secondary alcohol (**F5.5**) and **F5.4**, then gave the ester. The ring-closing metathesis of diene was performed under the influence of the chiral molybdenum bis (aryloxide) complex (**structure 2**) in (**Figure 6**), furnishing the macrocycle.²⁶



Scheme 3: Synthesis of Oxazole Side Chain and Completion of Synthesis

The trisubstituted olefin was subjected to diastereoselective hydrogenation and de-benzylation, giving the saturated alcohol (**F5.1**).²⁰ Next, a short and stereoselective

synthesis of oxazole diene side chain fragment was planned. Here, the researchers applied the stereoselective CM reaction between vinyl-B (pin)²⁷ (**F5.11**) and allyl carbamate (**F5.12**) catalyzed by MoMA catalyst (**structure 1** in Figure 6), giving the CM product with (98:2 /Z:E). This was followed by catalytic cross coupling with (**F5.10**) catalyzed by phosphine Pd complex, and this approach delivered Z-alkene with (>98:2/ Z:E) selectivity for the synthesis of (**3.4**).²⁸ The primary alcohol was converted to bromide followed by the treated with allyl magnesium bromide, giving the terminal alkene. Here again, the researchers performed the Z-selective olefin cross-metathesis catalyzed by ruthenium (Ru) complex (**structure 4** in Figure 6) with Z-alkene diol, provided the Z-selective product (98:2/Z:E). This was then followed by oxidation of primary alcohol pinnick oxidation afforded (**F5.2**)²⁹ with good yield. At the end, the coupling of the macrocyclic (**F5.1**) and linear diene (**F5.2**) fragments furnished (+)-neopeltolide in 74% yield. This synthesis involves 28 steps including the preparation of oxabicyclic substrate (**F5.6**). We can say that this is the most efficient method over previously reported synthesis.

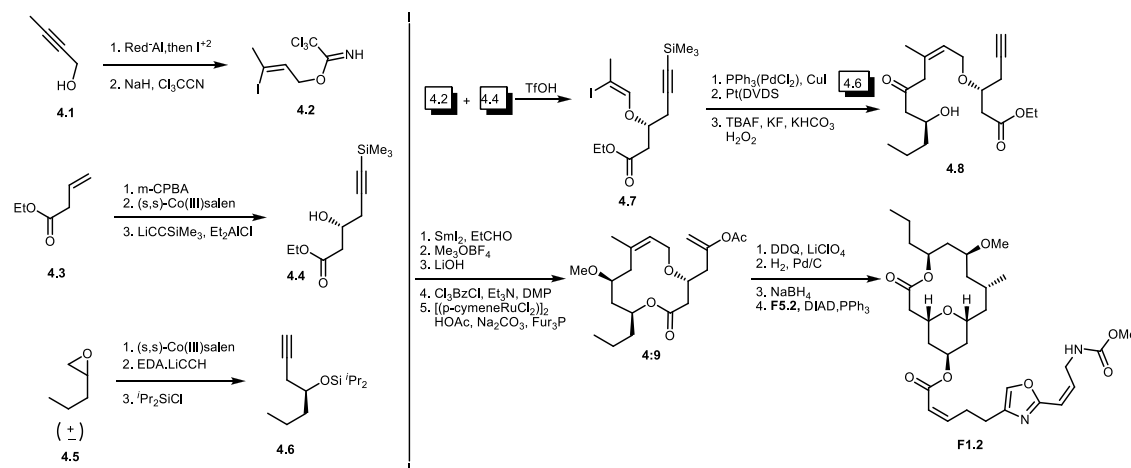
2.7 Synthesis of Neopeltolide Analogs

Kozmin and co-workers disclosed that cytochrome *bc*₁ complex is a cellular target of two different natural products namely neopeltolide and leucascandrolide-A differing in the macrolactone part.^{4c} It appears that there is a tolerance to simplifying the structure of macrolactone. These structurally simplified synthetic studies caused moderate to a large drop in the biological activity. Several groups worked on the synthesis of neopeltolide analogs for checking the potency of the analogs. In 2008, Maier and co-workers modified the oxazole side chain. In 2009 Schidt group, modified the side chain with benzoyl group, and n-octanoyl group. In 2013, Fuwa and co-workers also prepared neopeltolide analogs by modifying the oxazole side chain length, double bond geometry, and an aromatic ring. A few researchers also modified the macrolactone moiety.

2.7.1 Total Synthesis of Neopeltolide and Analogs by Florence and Co -Workers

Florence and co-workers contributed significantly towards the total synthesis of neopeltolide and for obtaining analogs in 2010 and 2013. The key step involved in their synthesis was the DDQ-mediated oxidative cyclization reaction¹⁸ for installing the

tetrahydropyran moiety embedded in 14-membered macrolactone. The synthesis of **4.2** proceeded through 2-butyne-1-ol (**4.1**) hydroalumination with Red-Al,³⁰ followed by quenching with iodine. The desired trichloroacetimidates was formed by treating the iodoallylic alcohol with Cl_3CCN . The next fragment **4.4** was prepared from ethyl 3 butenoate (**4.3**) further treated with *m*-CPBA, followed by the hydrolytic kinetic resolution³¹ with Jacobsen's (S,S) catalyst- reacted with lithium trimethylsilyl acetylide and di-ethyl alumenium chloride (Et_2AlCl)³² giving the third fragment (**4.6**).

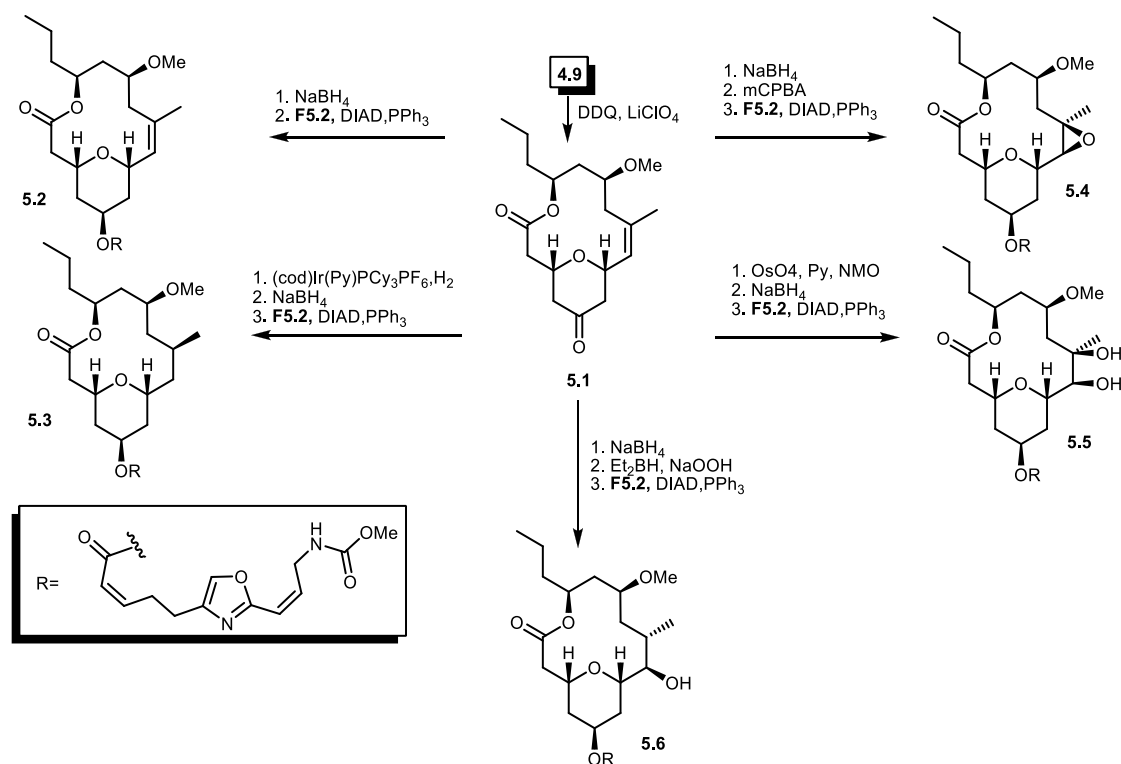


Scheme 4: Total Synthesis of Neopeltolide

The next attempt was the TfOH promoted etherification reaction between **4.2** and **4.4**, giving **4.7**. This was then subjected to Sonogashira coupling with **4.6**. The regioselective alkyne hydration proceeded through an intramolecular hydrosilylation with $\text{Pt}(\text{DVDS})$ ³³ followed by reaction with Bu_4NF , KF , KHCO_3 , and an aqueous H_2O_2 , offered compound **4.8** with 11:1 mixture of the conjugated isomer. These conditions also led to concomitant cleavage of the alkynylsilane group. The Evans-Tischenko reduction,¹² selective methylation of alcohol followed by hydrolysis of the ester and Yamaguchi macrolactanization,³⁴ gave the corresponding lactone. It was then exposed to HOAc and $[(p\text{-cymene})\text{RuCl}_2]_2$ ³⁵ and this provided the cyclized product (**4.9**). In this approach, the key reaction was conducted at this point on **4.9** by using an oxidative cyclization protocol with DDQ , LiClO_4 , giving a single diastereomer. It was then followed by C8-C9 olefin reduction and the carbonyl reduction, leading to the synthesis of **F5.1**, which upon Mitsunobu coupling with **F5.2**, delivered neopeltolide (**F1.2**).

After the total synthesis of neopeltolide, Florence and co-workers turned their focus towards the synthesis of neopeltolide analogs. The researchers prepared two types of analogs by changing the macrolactone core structure and other one is by modification in

the oxazole side chain. The presence of unsaturation between C8 and C9 creates a good opportunity for macrolactone core modified analogs synthesis. In (**Scheme 5**), I discussed the synthesis of different analogs from intermediate **5.1** obtained from **4.9**, in one step. A simple reduction of C5 carbonyl group with NaBH₄, followed by Mitsunobu coupling with oxazole acid fragment (**F5.2**), delivered **5.2**.

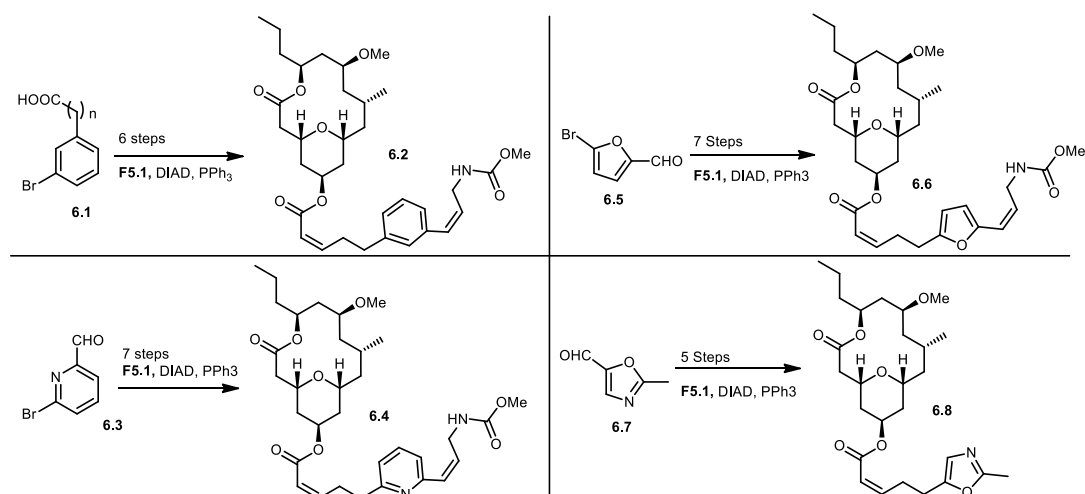


Scheme 5: Floreancig's Synthesis of Neopeltolide Analogs

The Crabtree's catalyst controlled hydrogenation of **5.1** from the convex face with low diastereo-selectivity (1.4:1), reduction of double bond with same condition followed by Mitsunobu coupling with acid, gave C9-*epi*-neopeltolide (**5.3**).³⁶ The dihydroxyl groups introduced by carrying-out Osmylation following same reactions, then gave **5.5** in reasonable yields. Finally, the monohydroxy derivative **5.6** prepared from **5.1**, through C5 reduction, hydroboration with BH₃·THF followed by NaOOH.

As we discussed above, a few groups worked on the modification of oxazole side chain by changing the length of side chain, or by changing the geometry of the alkenes, or by changing the oxazole with other aromatic groups. In (**scheme 6**), I discussed about Florence and co-workers' analogs synthesis. The researchers worked on the aromatic group replacement and on the length of the side chain. The benzene ring containing side chain analogs were prepared started from *m*-bromohydrocinnamic acid (**6.1**) in six steps including key reactions Sonogashira coupling³⁷ and selective *cis* reduction with P2-

Nickel,³⁸ Still–Gennari protocol³⁹ for obtaining the acid coupling partner. Finally, the Mitsunobu coupling reaction between alcohol and acid led the synthesis of an analog, (**6.2**). The pyridine containing side chain was prepared from 6-bromo-2-pyridinecarboxaldehyde (**6.3**) in seven steps including key reaction, such as Horner–Wadsworth–Emmons,⁴⁰ Sonogashira conditions, Still–Gennari protocol for obtaining the acid, which upon Mitsunobu coupling with alcohol provided **6.4**.



Scheme 6: Synthesis of Oxazole Side Chain Modified Analogs by Florence and Co-Workers

They also selected some five membered heterocyclic rings for replacing the oxazole moiety. The synthesis of these analogs were commenced with Horner–Wadsworth–Emmons as an extension of 5-bromofurfural (**6.5**) followed by a few steps, including Sonogashira coupling and Still–Genanari olefination and the hydrolysis of ester giving acid, which was coupled with alcohol, for completing the synthesis of **6.6**.

2.8. Natural Products with 14-Membered Macrolides

Containing the Pyran Moiety

In literature, several natural products are reported containing 14-membered macrolide embedded with the tetrahydropyran moiety.^{41,42} Many of these marine macrolides demonstrate potent cell growth inhibition properties and other biological activities. Here, we are showing some natural products, such as aspergillides-A (**F7.1**), B (**F7.2**) and C (**F7.3**) are new novel 14-membered macrolides, isolated from marine-derived fungus, *Aspergillus ostianus* strain 01F313 and it shows significant cytotoxic activity against

mouse lymphocytic leukemia cells (L1210) with LD50 values of 2.1, 71.0, and 2.0 $\mu\text{g/mL}$, respectively. The following structures bear a 14-membered macrolactone ring incorporated with 2, 6 *trans*-tri-substituted pyran moiety and *E*- olefin geometry at C-8 and C-9 carbons.

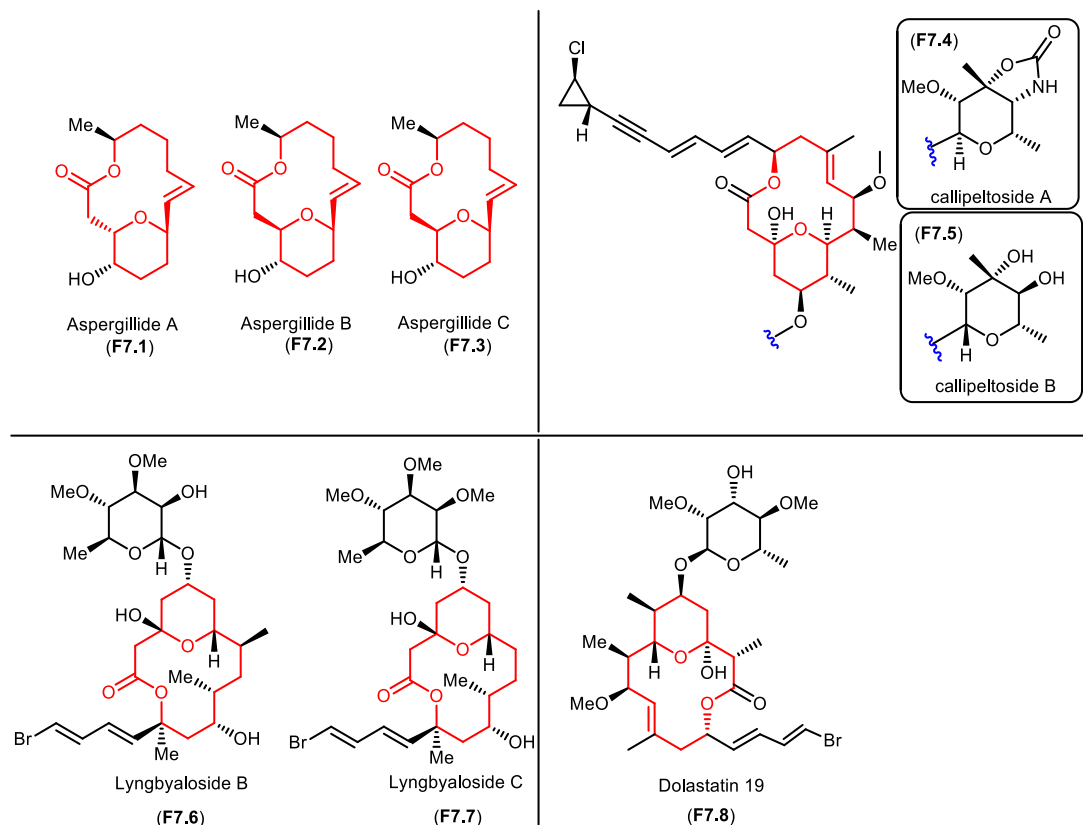


Figure7: Biologically Active Natural Products with 14-Membered Macrolactone Incorporating with the Substituted Pyran Motif

Here, we are showing another natural product, Callipeltoside, which was isolated by Minale and co workers, Callipeltosides A (F7.4), B(F7.5) from the Lithistida marine sponge *callipelta* sp.⁴³ This molecule plays an important role in exhibiting the cytotoxic activity against human broncho-pulmonary NSCLC-N6 and P388 cell lines. The structural features include the 14-membered macrolactone incorporating the tetrasubstituted pyran motif. Along with this, an unusual *trans*-configured chlorocyclopropane ring conjugated to a di-ene-yne unit. Lyngbyaloside B (F7.6), C (F7.7) are marine macrolide glycosides isolated from marine cyanobacterium *lyngby*-sp by Moore and co-workers. These natural products exhibit anti-cancer activity against several cancer cell lines.

Dolastatin-19 (**F7.8**) is a polyketide 14-membered macrolide isolated in 2004 by Pettit and co-workers from the sea hare *Dolabellaauricularia*, collected in the Gulf of California. The structural features include tetrasubstituted pyran motif appended with a bromine-substituted (E,E)-diene and a 2,4-di-O-methyl-L-rhamnopyranoside. The initial biological screening indicated significant cancer cell growth inhibitory activity (GI₅₀ values of 0.72 mg/mL and 0.76 mg/mL for breast MCF-7 and colon KM20L2 cell lines, respectively).

2.9. Our Design

Due to the important biological activity reported by this natural product, several researchers embarked the total synthesis efforts for neopeltolide, and shown in (**Schemes 1, 2 and 3**), are the two examples.^{21-22, 44} In addition to this, serious synthetic studies were also undertaken by several leading research groups for obtaining various structural analogs in this family of natural products and some of these efforts are shown in (**Scheme 4**). The unique features of neopeltolide (i.e. pyran moiety embedded in 14-membered ring macrolide) are also commonly found in several other biologically active natural products, and some of the selected examples are shown in (**Figure 7**). Keeping in mind the key biological responses that are associated with neopeltolide, the marine natural product, our interest in this area was developing a novel synthesis method for obtaining a new type of scaffold, termed as *neopeltolide-inspired compounds* and our generic structure is shown in (**Figure 8**). In our design approach, we were keen in retaining the 14-membered macrolide architecture, while bringing some of the diversity-based fragments in our design. For example, as shown in (**Figure 9**), we wanted to incorporate the amino acid derivative (i.e. amino alcohol moiety) which would allow us obtaining several analogs using the functional group R₁ as the diversity site. As in the case of neopeltolide, we also wanted to have the pyran ring moiety embedded in our 14-membered ring design. All together, the presence of two diversity groups in our design scaffold would allow us obtaining several structurally related analogs to thoroughly explore their biological applications. These designed novel small molecules can be classified in two categories, and, they are outlined as follows in (**Figure 8**).

- (i) Tetrahydropyran-derived Macrocycles (**F8.1**)
- (ii) Piperidine-derived Macrocycles (**F8.2**)

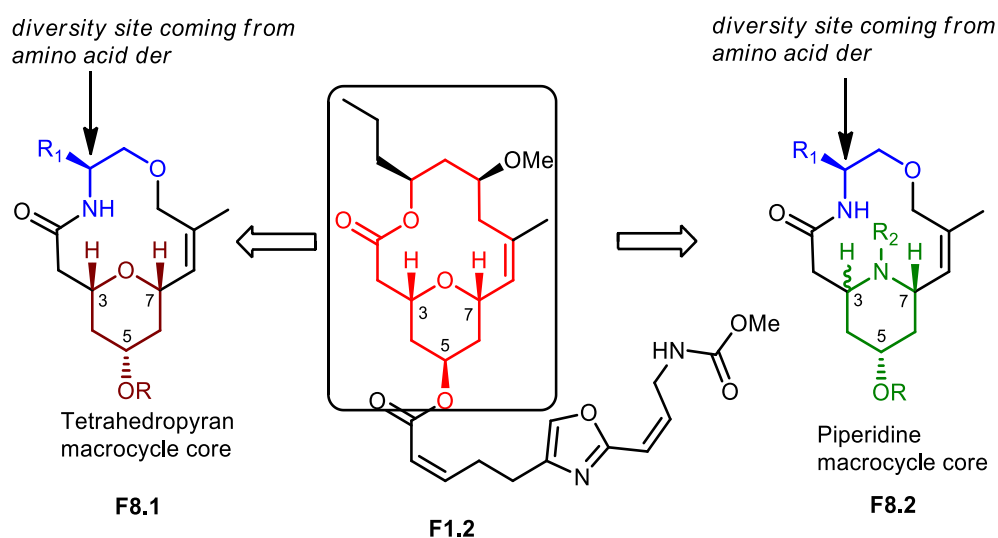


Figure 8: The Proposed Plan to Obtain Neopeltolide Macrocyclic Core like 14-Membered Macrocyclic Compounds (**F8.1**) and (**F8.2**)

i) Tetrahydropyran-based Macrocycles

2.10 Retrosynthesis of Tetrahydropyran-derived Macrocycle

The retrosynthesis of macrocycle (**F8.1**) is shown in (**Figure 9**). Macrocycle (**F8.1**) could be obtained by Ring Closing Metathesis (RCM) with Grubbs' 2nd generation catalyst from the corresponding *bis*-olefin. This could be obtained from amino acid coupling reaction with **F9.1**.

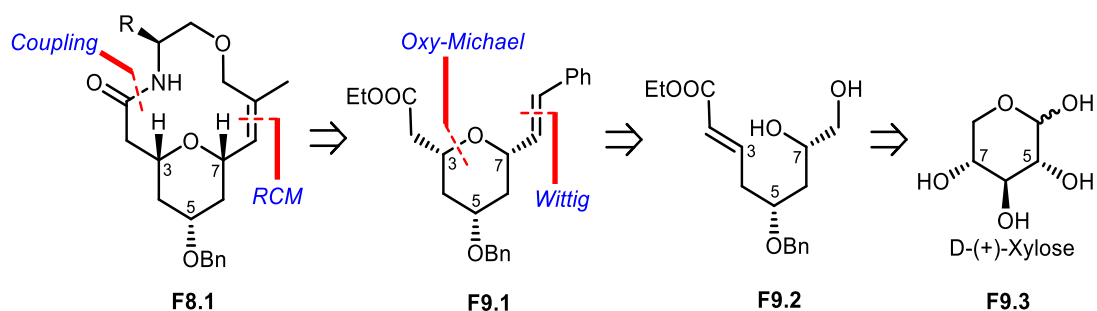


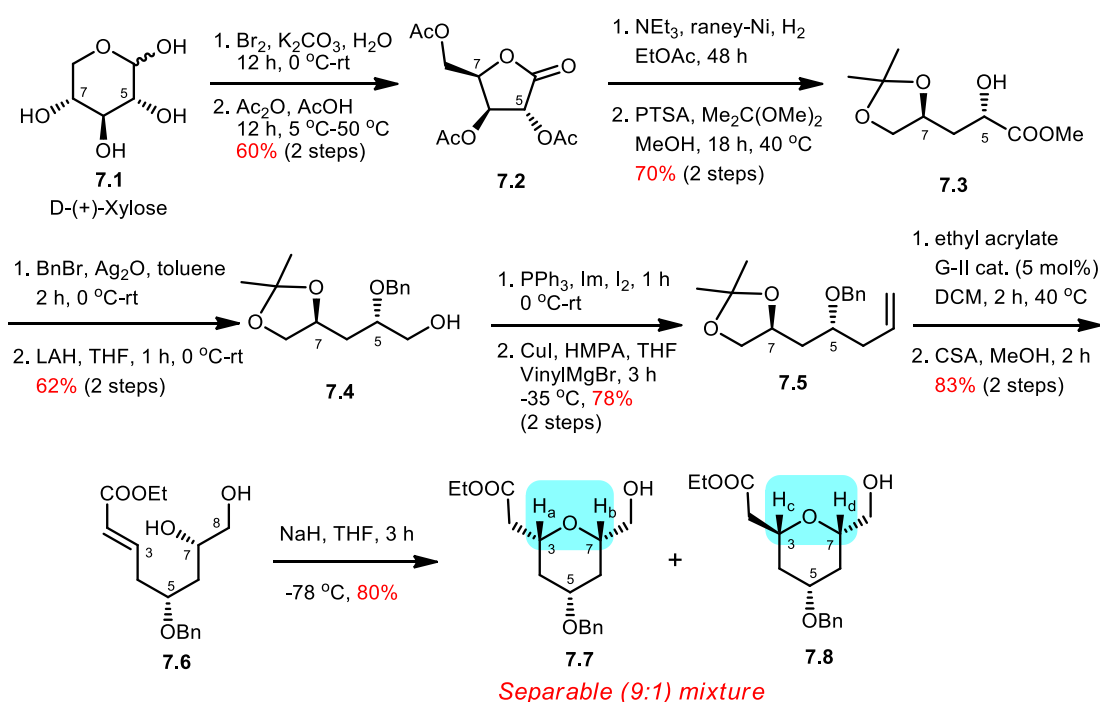
Figure 9: Our Key Disconnections Reaching the Desired Target Compound

The trisubstituted tetrahydropyran motif could be synthesized from Oxy-Michael addition precursor (**F9.2**) by a regio- and stereoselective Oxy-Michael reaction, followed by the Wittig reaction. Finally, the Michael addition precursor (**F9.2**) can be synthesized from commercially available cheap starting material **F9.3**. The key steps involved in our synthesis planning are shown in (**Scheme 7**). For developing the synthesis of the needed

pyran intermediate, we utilized D-xylose as the cheap source of commercially available starting material.

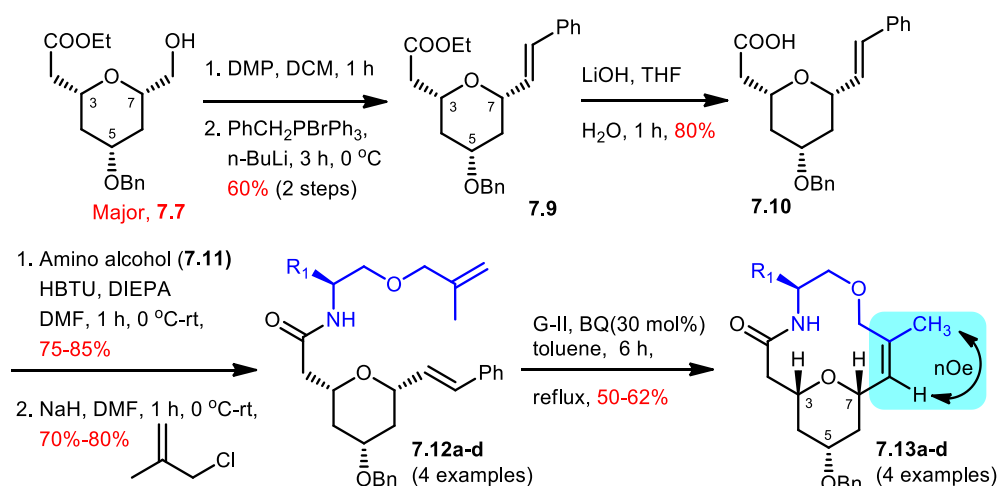
2.11 Synthesis of Macrocycle (F8.1)

Our synthesis of well-stereodefined pyran moiety that starts with D-xylose (**7.1**) and is shown in (**Scheme 7**). Compound **7.1** converted into compound **7.2** in two steps. bromine oxidation and acetylation that are well-reported in the literature.⁴⁵ Product **7.2** was then converted to **7.3** as the -OH carboxyl ester derivative in further two more steps, which was easily transformed to **7.4** in a simple series of steps. The primary hydroxyl group in compound **7.4** was then subjected to the leaving group (for example, iodo group) formation and this was then treated to nucleophilic vinyl attack giving compound **7.5**.



Scheme 7: Regio- and Stereoselective Synthesis of the Desired Pyran Derivatives

All the new compounds throughout this series were thoroughly characterized by ¹H and ²D NMR and MS studies. Having the synthesis of the product **7.5** developed, it was then easily converted to compound **7.6** utilizing the carbon-chain extension by the metathesis approach, which is also well-documented in the literature. The next crucial steps were to examine the regio and stereoselective intramolecular Michael addition reaction under the mentioned reaction conditions (see **Scheme 7**). This reaction worked very-well and we observed two separable, cyclic pyrans as **7.7** (major) and **7.8** (minor) products.



Scheme 7: Completing the Synthesis of 14-Membered Macroyclic Compounds

Structural Analysis

In this section, the details of the stereochemical assignments are provided as follows. Based on 2D nOe analysis, we assigned the stereochemistry of C-3 carbon in compounds **7.7** and **7.8**. In compound **7.7**, the **H_a** proton showed nOe with **H_b** which indicated that it is having a H-3 and H-7 as axial-axial *cis*-orientation, whereas in compound **7.8**, there is no nOe between **H_a** and **H_b**, indicating this diastereomer as the 3,7 axial-equatorial *trans*-isomer (see **Scheme 7**). Finally, as shown in (**Scheme 7**), the major pyran product (compound **7.7**) was subjected to the primary hydroxyl group modification, giving **7.9**, which led the synthesis of the corresponding acid derivative, **7.10**. This upon coupling with 4 different amino alcohol derivatives (compound **11**) then produced the key compound **7.12(a-d)** as the crucial precursor to the final macrocyclic product **7.13(a-d)**. When subjected to the key macrocyclic ring closing metathesis reaction, all the four compounds **7.12(a-d)**, successfully produced the desired 14-membered macrocyclic ring products, **7.13(a-d)** in good yields.

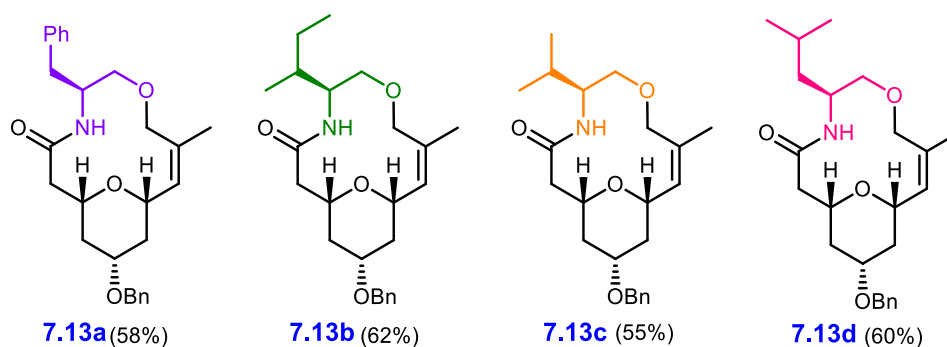


Figure 10: Four 14-Membered Macrocyclic Compounds Synthesized

In our ring closing metathesis approach, we also utilized 1,4- benzoquinone for avoiding the polymerization and, isomerization of the double bond (note - this may occur in ring closing metathesis reactions at high temperatures (>100 °C)). All the products at every step were properly purified and thoroughly analyzed by ¹H and ²D NMR and MS studies. The geometry of the tri-substituted olefin was assigned by observing the positive 2D nOe between C-9 Methyl and C-8 hydrogen.

2.12 Biological Evaluation

All the small molecule obtained from this project were then subjected for different biological studies with our various collaborators and outlined below is one case study example in the area of stem cells research.

Chemical Approaches to Trans-differentiate Human (from Healthy Donor) Mesenchymal Stem Cells to Neurons by Small Molecules (Note - this study was performed as a collaboration with Transcell Biologics, a stem cell-based biotech company in Hyderabad):

Neopeltolide-inspired acyclic and macrocyclic compounds as the set of small molecules were screened on donated (from healthy donor) cord tissue derived mesenchymal stem cells based predictive platform (as disclosed in Indian Patent application no. 201643028709 dated August 23, 2016) with the positive control as cells exposed to forskolin (note - the structure is not shown in this document) and the internal control as the cells exposed to known neuro-inductive medium (Promocell).⁴⁶ All these compounds were tested at 10.0 μM concentration. The phenotypic changes obtained with active compounds (**Figure 12-18**) are captured. They were documented under the phase contrast inverted microscope as the representative small molecule-induced neurogenesis on the proprietary predictive MSC platform. Further, the expression of bio-markers of neurons like nestin and agrin are qualitatively evaluated in the cells harvested from the wells exposed to one of the hit compounds (**7.13a**) (**Figures 12 and 13**) by Polymerase Chain Reaction (PCR).⁴⁷ As a follow-up of this, we are taking this project in the following two directions, and, these are:

1. To carry-out the full scale genomic studies on the initial mesenchymal stem cells and the converted neurons for obtaining the complete picture on the landscape of various genes regulation involved in both type of cells. This knowledge, in the long-run will be helpful in identifying the novel targets that are affected by our novel small molecules, playing an important role in converting the stem cells to neurons.

2. To examine the scope of some of our active compounds as the neuroprotective or neurogenesis agents in various neuro-related disorders in collaboration with Steve Haggarty, MGH Boston and Amitabha Majumdar, NCCS Pune.

This work of directly trans-differentiating human mesenchymal stem cells to neurons by small molecules is an exciting direction for us and an initial patent (note - Indian, patent which will be followed by the US initial patent protection).

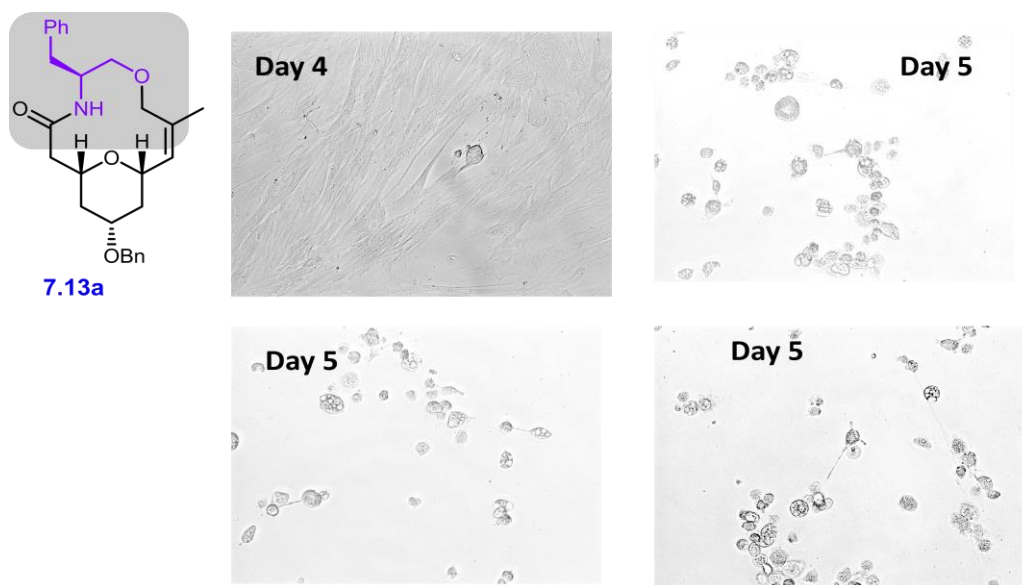


Figure 12: The Direct Conversion of Human Mesenchymel Stem Cells to Neurons by Neopeltolide-inspired, Macrocyclic Compound, **7.13a**

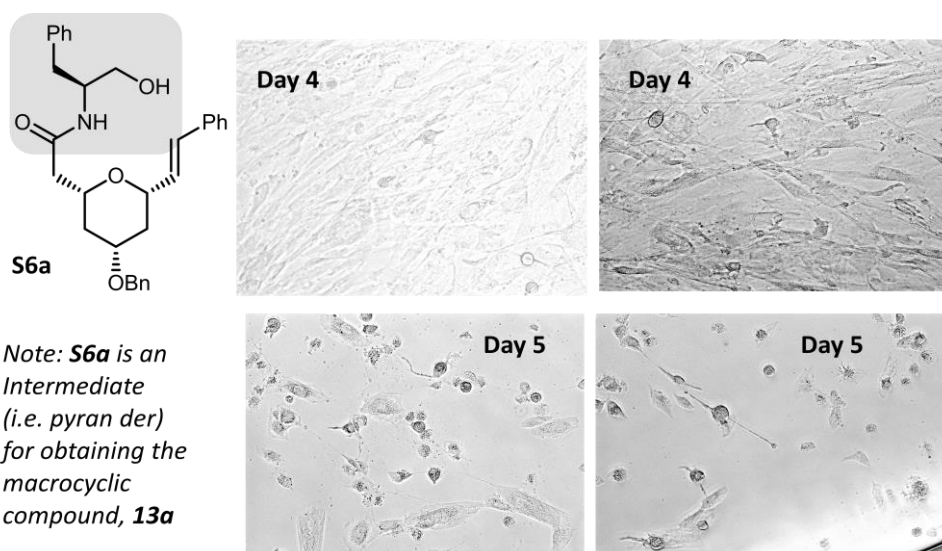


Figure 13: The Direct Conversion of Human Mesenchymal Stem Cells to Neurons by **S6a**

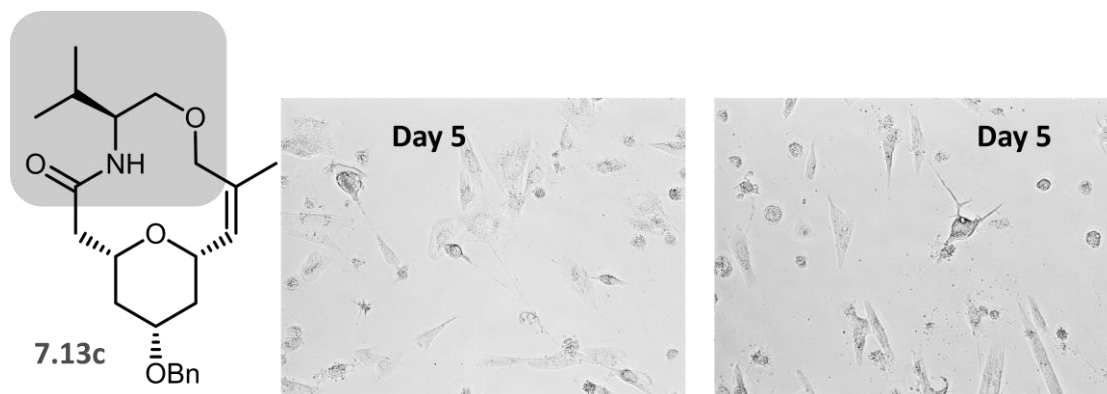


Figure 14: The Direct Conversion of Human Mesenchymal Stem Cells to Neurons by another analog as the neopeltolide-inspired macrocyclic compound, **7.13c**

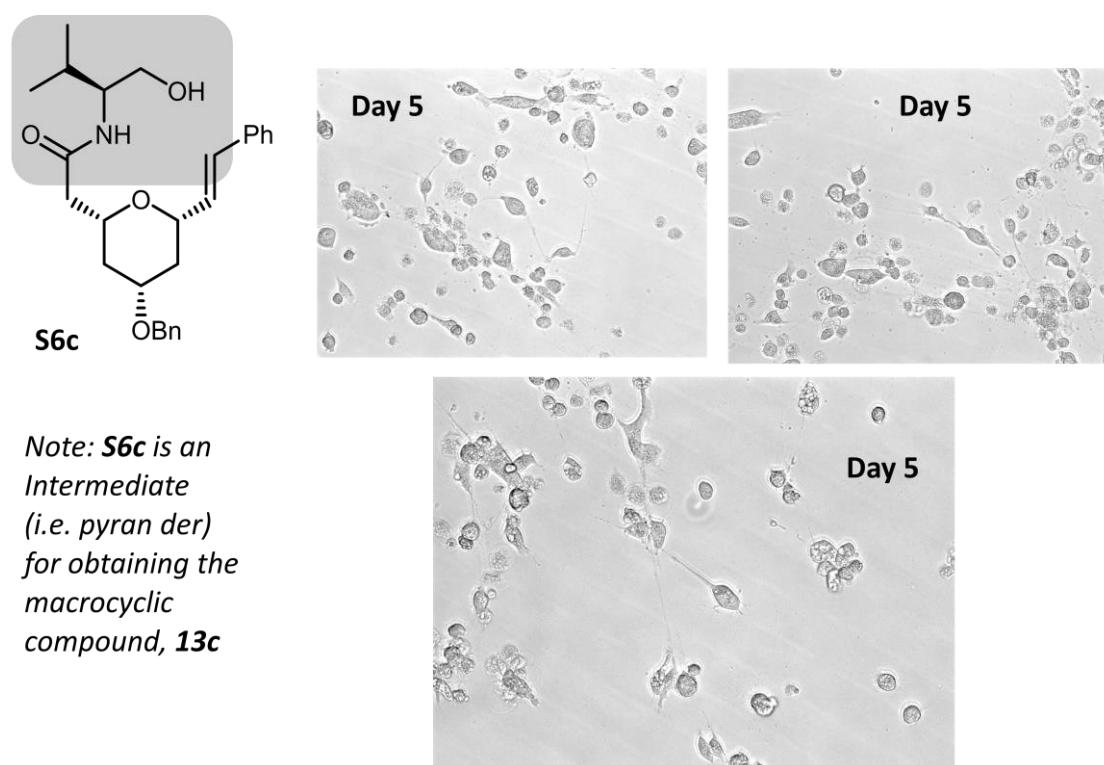


Figure 15: The Direct Conversion of Human Mesenchymal Stem Cells to Neurons by **S6c**.

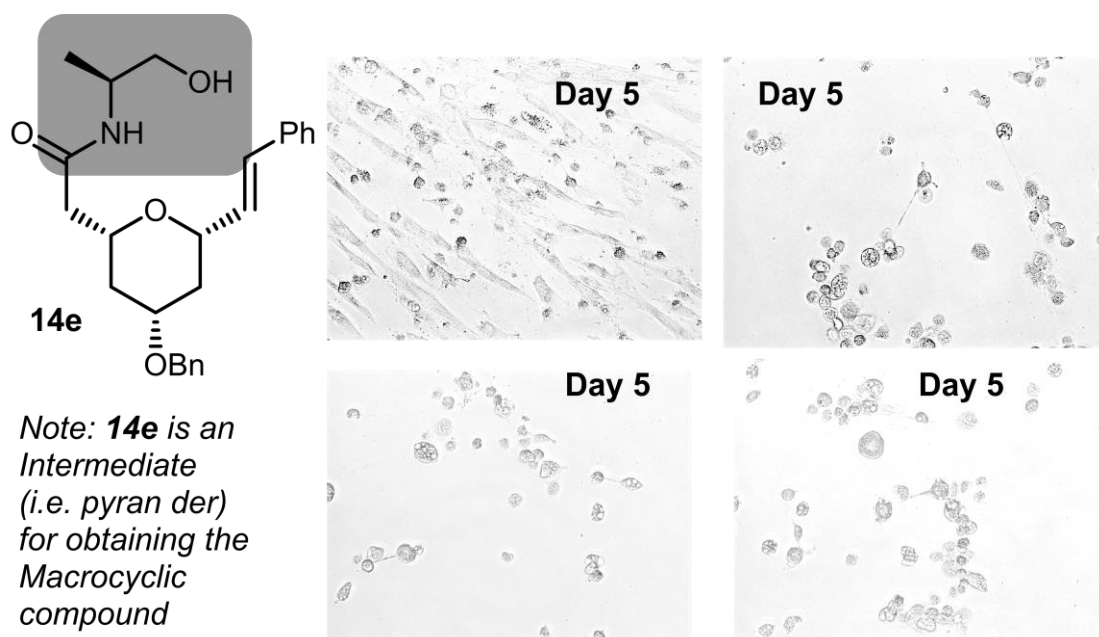


Figure 16: The Direct Conversion of Human Mesenchymal Stem Cells to Neurons by 7.14e

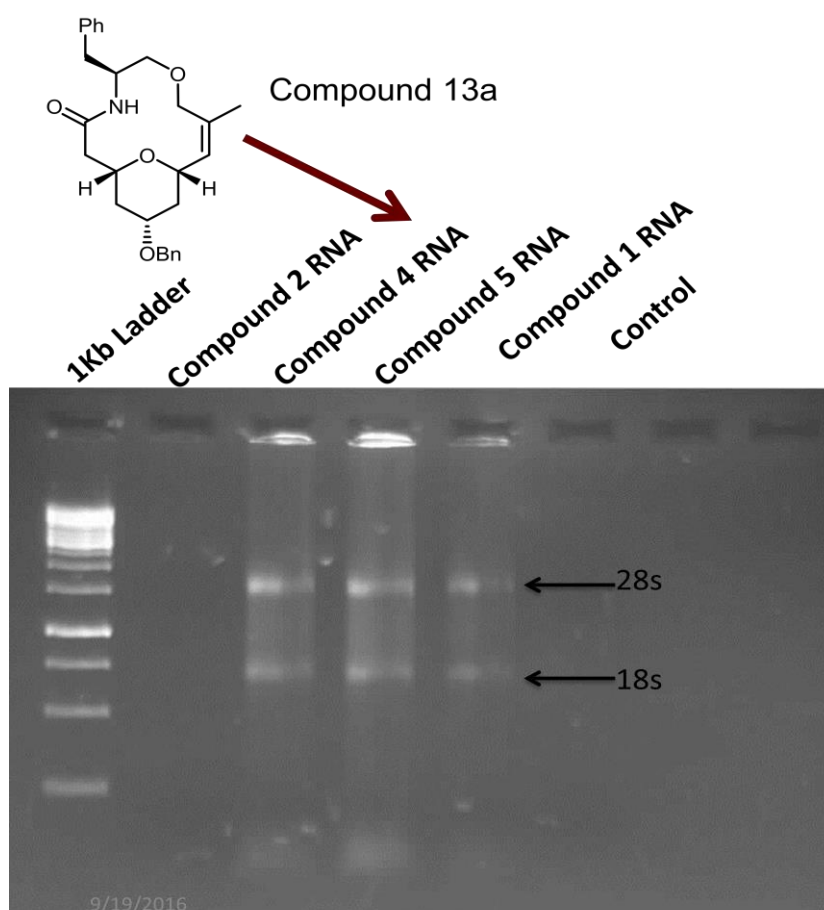


Figure 17: Towards the Identification of Biomarkers that are Associated with Neuronal cells.

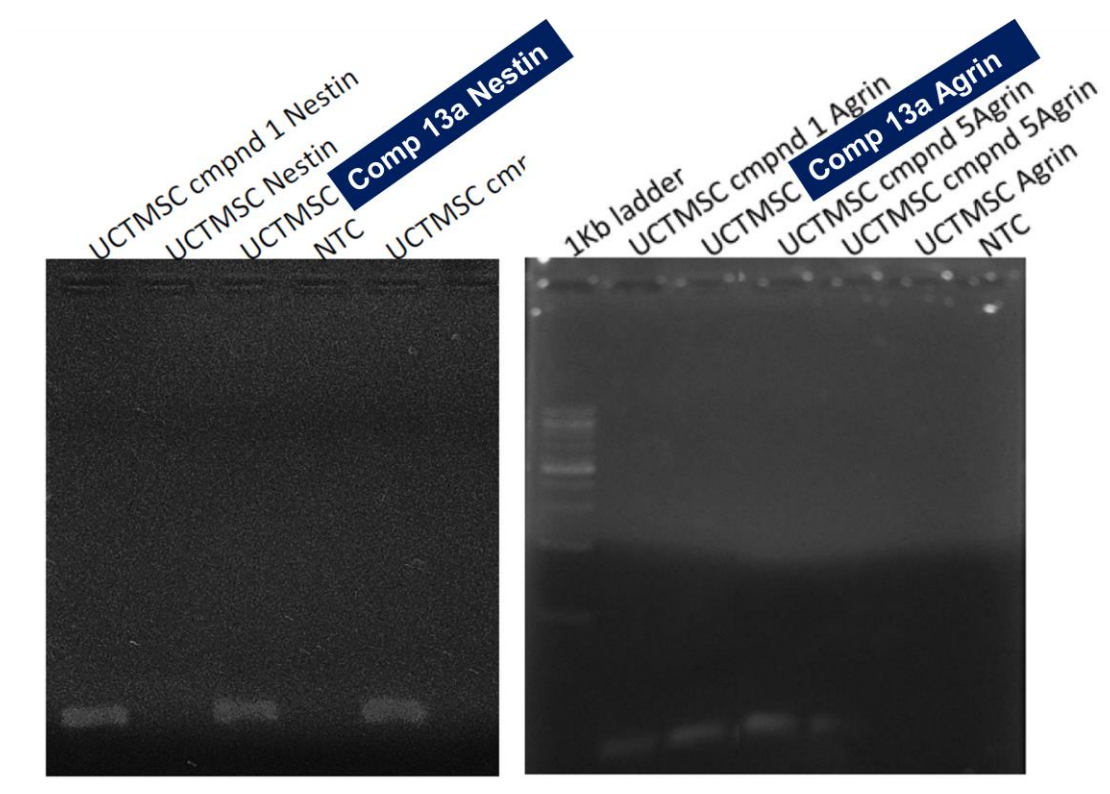


Figure 18: The identification of two key biomarkers (i.e. Nestin and Agrin) that are associated with neuronal cells obtained after the treatment of mesenchymal stem cells with compound **13a**.

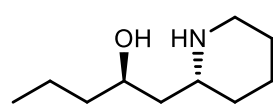
ii) Piperidine Derived Macrocycles (F8.2)

As per our design molecules that are shown in (Figure 8), we started the synthesis of (F8.2) Macrocyclic compounds. In this section, first, I discuss about the importance of piperidine containing natural products in different biological areas. Followed by this, the retrosynthesis and synthesis and conclusions are outlined.

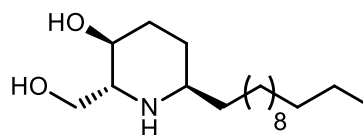
2.13 Biologically Active Natural Products with Piperidine

Core

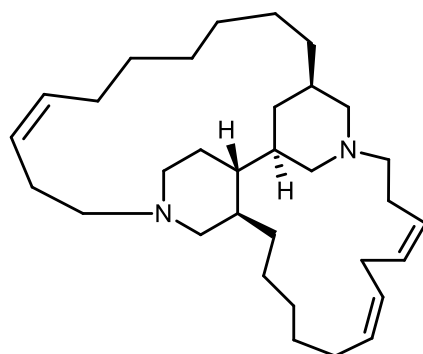
Piperidine is a privileged heterocyclic core present in many biologically natural products. Piperidine derivatives are also be as important synthons due to their adaptability in the direction of chemical transformations to several useful synthetic intermediates. The derivatives of piperidine are largely employed in the area of therapeutics and pharmaceuticals. Here, we are showing a few examples in (Figure 19).



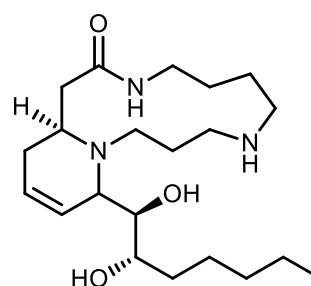
Halosaline
F19.1



(+) Desoxoprosopinine
F19.2



Haliclonacyclamine F
F19.3



(+) Cannabisativine
F19.4

Figure 19: Biologically Active Natural Products with Piperidine as the Scaffold

Halosalime (F19.1), a naturally occurring alkaloid with a piperidine ring. It was isolated in 1967 from *Haloxylon Salicornicum*.⁴⁸ It is used as a medicine for the treatment of eye sores and sight improvement of the eye. Desoxoprosophylline (F19.2) a piperidin-3-ols containing molecule, isolated from the African mimosa *Prosopis africana Taub*.⁴⁹

Showing potent glycosidase inhibitory activity,^{50, 51, 52} (+) cannabissativine is an unusual and challenging alkaloid, isolated from the roots of the common marijuana plant *cannabis sativa* L.⁵³

Based on the above observations and interesting biological activities, we were interested in incorporating the substituted piperidine ring into the 14 membered macrolactone core of the neopeltolide (**F1**) for obtaining piperidine ring containing macrocyclic compounds, see **F8.2**.

2.14 Retrosynthesis of (F8.2)

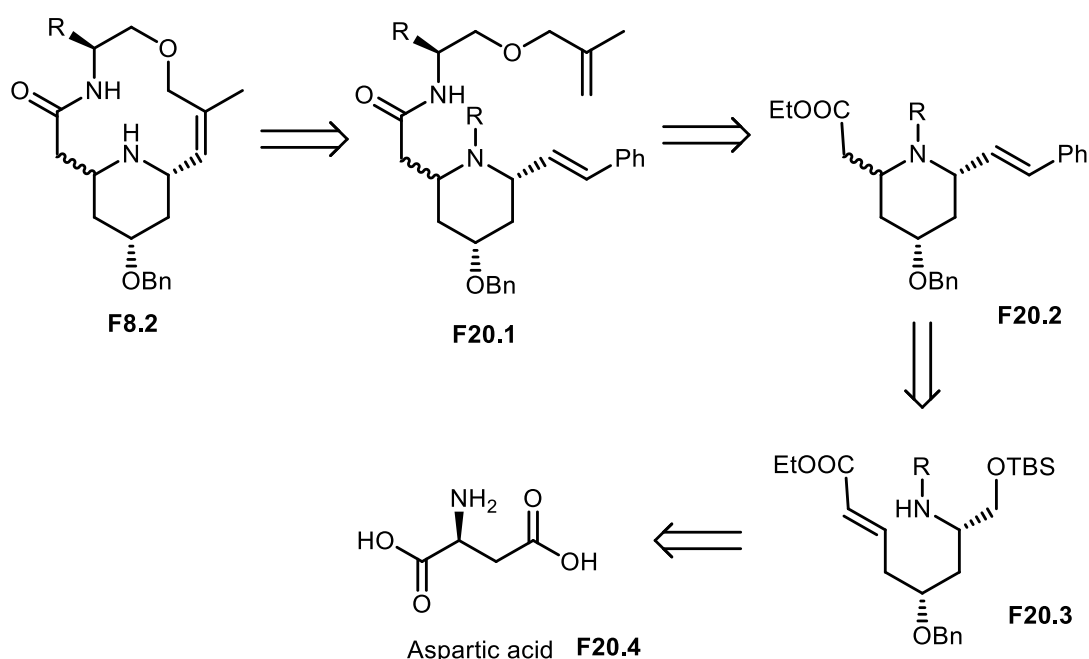


Figure 20: Retrosynthetic Analysis of Macrocycle (**F8.2**)

Macrocycle (**F8.2**) was accessed by ring closing metathesis of *bis* olefin (**F20.1**). This could be obtained from trisubstituted piperidine (**F20.2**) in two steps and these are: hydrolysis and coupling reaction. Compound **F20.2** can prepare from stereoselective aza-Michael addition reaction of **F20.3**. This precursor could be prepared from L-aspartic acid in a couple of steps.

2.15 Synthesis of Macrocycle (F8.2)

We started the synthesis trisubstituted piperidine (**F20.2**) with the commercially available cheap starting material (L)-aspartic acid (**F20.4**), as shown in (**Scheme 8**). It was converted into compound **F21.1** in four steps with good yields.⁵⁴ This compound

was then subjected to oxidation with IBX followed by allylation reaction led the synthesis of two diastereomer which were separable by flash column chromatography. Relative stereochemistry of the new chiral center was assigned after aza-Michael addition reaction. This allyl product secondary hydroxyl group was then protected with benzyl bromide, giving two products, **F21.2** (major) and **F21.3** (minor). All the new compounds throughout this series were thoroughly characterized by ^1H and 2D NMR and MS studies. Having the synthesis of product **F21.2** developed, it was then easily subjected to utilizing the carbon-chain extension by the metathesis approach.

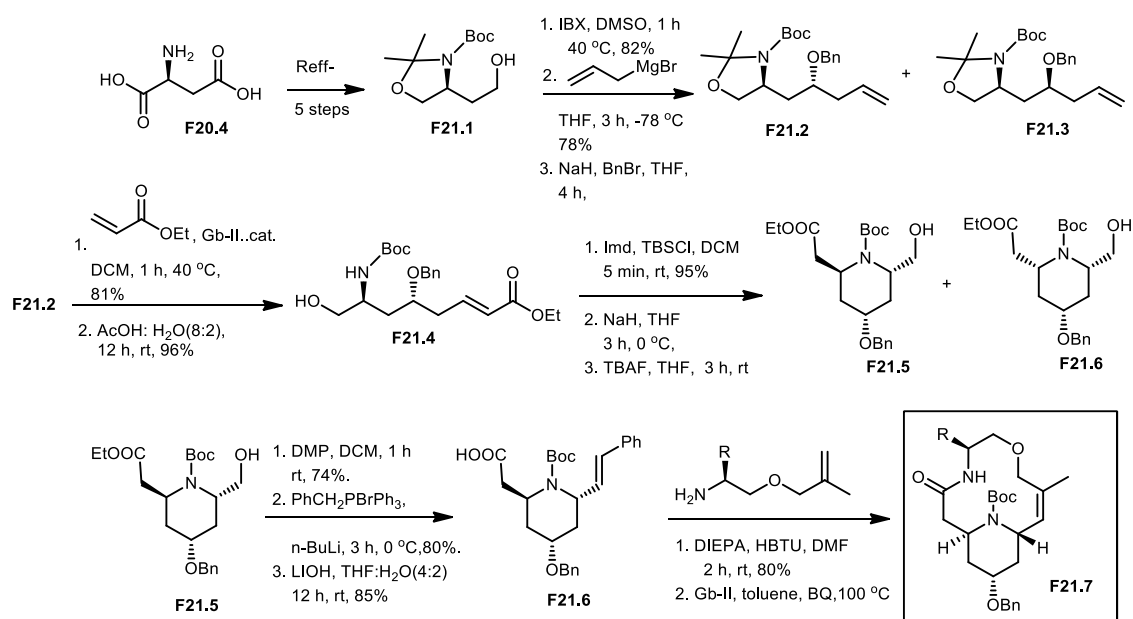


Figure 21: Synthesis of Piperidine core Macrocycle

Followed by the actinide removal with AcOH: H_2O (8:2), we obtained compound **F21.4**. The silyl protection of the hydroxyl group followed by a crucial stereoselective intramolecular aza-Michael addition reaction under the mentioned reaction conditions was then carried-out (see **Figure 21**). This reaction worked very-well and we observed two separable, cyclic piperidine derivatives as **F21.5** (major) and **F21.6** (minor) products.

Structural Analysis

The details of the stereochemical assignments are provided as follows. Based on 2D nOe analysis, we assigned the stereochemistry of C3 carbon in compounds (**F21.5**) and (**F21.6**). In compound (**F21.5**), H_a proton not showing **nOe** with H_b which indicated that it is having a 3,7 *trans*-isomer, and C5 (H_c) and C7 (H_b) hydrogens are showing positive nOe, indicating that the two hydrogens are *cis* orientation. Whereas, in

compound (**F21.6**), there is **nOe** between H_a and H_b , indicating that this diastereomer as the 3,7 *cis*-isomer. Finally, as shown in (**Figure 22**), the major piperidine product (compound **F21.6**) was subjected to the primary hydroxyl group modifications, oxidation, Wittig reaction, and hydrolysis, finally giving **F21.6**.

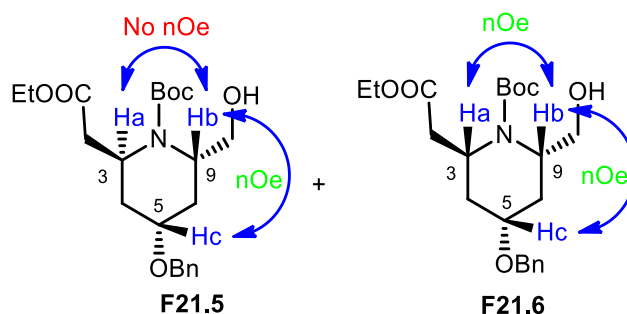


Figure 22: Structural Analysis of Piperidine Ring

This upon coupling with different amino alcohol derivatives produced the *bis*-allyl key compound as the crucial precursor to the final macrocyclic product. It was then subjected to the key macrocyclic ring closing metathesis reaction for completing the synthesis of **F21.7**. *This work is still in progress*. In RCM reaction conditions, we used 1,4 benzoquinone for avoiding the polymerization and, isomerization of the double bond products **F21.7**.at high temperature (>100 °C). All the products in this synthesis program were properly purified and thoroughly analyzed at every step (except the last step) by ^1H and 2D NMR and MS studies.

2.16 Conclusions

- To summarize, we succeeded in developing a practical and scalable synthesis of *cis* 3,7 substituted tetrahydropyran which is present in neopeltolide as well as several other bioactive natural products.

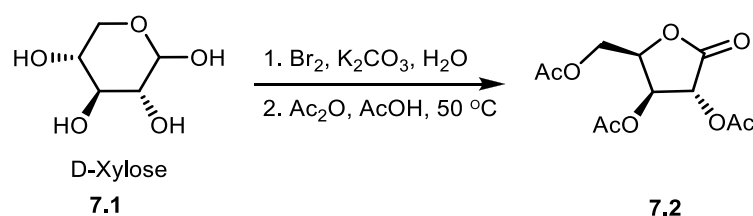
We also synthesized tri-substituted piperidine moiety that is commonly present in different biologically active natural Products.

- The privileged scaffold **7.7** was further utilized in obtaining different types of 14-membered macrocyclic rings.
- The incorporation of an amino acid moiety in all macrocyclic rings allow introducing a chiral diversity site for further obtaining several analogs having a variation in the chiral side chain present within the macrocyclic ring skeleton.

➤ The key step involved in our synthesis was an oxy-Michael addition, aza-Michael addition and Ring Closing Metathesis. An advantage of our approach is the use of a cheap chiral starting material, D-(+) xylose. and L-aspartic acid. In collaboration with Transcell Biologics Hyderabad, a few novel small molecules that were capable of directly trans-differentiating human mesenchymal stem cells to neurons were identified. This study was later validated by the specific identification of certain biomarkers that are unique to neuronal cells. Further, work is ongoing in the genomic level studies that would provide the detailed landscape of all the genes differing in both type of cell leading to further validation of this novel finding.

2.17 Experimental Section

(2R, 3S, 4R)-2-(acetoxymethyl)-5-oxotetrahydrofuran-3, 4-diyl diacetate (7.2)



Bromine oxidation: An oven dried 500 mL round bottom flask was equipped with magnetic stirrer and thermometer and charged with of D- Xylose (60.0 g, 400 mmol) (7.1) and water (160 mL). Potassium carbonate (68 g, 492 mmol) was added portion wise to the reaction mixture, keeping the temperature below 10 °C. After the mixture cooled to 5 °C, bromine (12 mL, 440 mmol) was added dropwise over 90 minutes, keeping the temperature below 10 °C. The resulted solution was stirred for 30 minutes, stirred at room temperature overnight. The reaction was quenched by dropwise addition of 98% (10 mL) formic acid. The orange colour was turned to the colourless solution. The reaction mixture was concentrated at 50 °C on a rotary evaporator. Acetic acid (40 mL) was added to the reaction mixture, concentrated at the same temperature to remove any residual water.

Acetylation: The residual white semi-solid is transferred to a 500 mL, three-necked, round-bottomed flask with the aid of warm acetic acid (80 mL). The flask is equipped with a magnetic stirrer, thermometer, and an addition funnel. After the suspension is warmed to 50 °C, acetic anhydride (360 mL, 1900 mmol) was added dropwise over 120 min, keeping the temperature between 50– 60 °C. After the mixture was stirred at that

temperature overnight and then cooled to room temperature, water (400 mL) was added. The mixture was extracted with dichloromethane (400 mL) (CH_2Cl_2). The organic layer was washed with water (400 mL). The combined aqueous layers are back-extracted with CH_2Cl_2 (200 mL). The combined organic layers are placed in a 1-L Erlenmeyer flask. After the solution was cooled with an ice-water bath, 2 N sodium hydroxide (400 mL) was added and the mixture was stirred for 30 minutes. The aqueous layer was separated and back-extracted with CH_2Cl_2 (100 mL). The combined organic layers are washed with brine (400 mL), dried over sodium sulfate, and concentrated under reduced pressure. The residue (108 g) was suspended in ethyl acetate (100 mL). To the stirred suspension, hexane (140 mL) was added dropwise. After stirring at room temperature for 1 hour, the mixture was cooled with an ice-water bath for 30 minutes. The precipitate was filtered and washed with a cold 1:2 ethyl acetate/hexane mixture to get **7.2** (66 g) as light beige crystals.

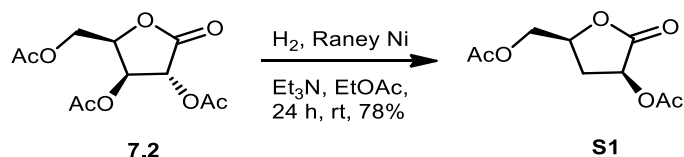
Molecular Formula: $\text{C}_{11}\text{H}_{14}\text{O}_8$

$R_f = 0.2$ (30% ethyl acetate/hexanes)

LRMS: (ES+) $m/z = 275.07$ [M+1]

Yield: 60%

(2S, 4S)-4-acetoxy-5-oxotetrahydrofuran-2-yl) methyl acetate (S1)



A 2 L, bench-top, the high-pressure reactor was charged with (**7.2**) (70 g, 255 mmol), Raney nickel (7.12 g), and ethyl acetate (800 mL). After that Triethylamine (53.4 mL, 510 mmol) was added. The pressure in the bomb was immediately raised to 100 psi with hydrogen. The mixture was stirred vigorously at room temperature for 24 hours. The pressure was released, and the bomb was flushed with nitrogen. The catalyst was removed by filtration and washed with ethyl acetate. The combined filtrate and washes are washed with water (5x100 mL). The aqueous layer was back extracted with ethyl acetate (180 mL). The combined organic layers then washed with 1 N Hydrochloric acid (500 mL). The aqueous layer was back extracted with ethyl acetate (200 mL). The combined organic layers were washed with saturated sodium bicarbonate solution and brine solution (500 mL), the organic layer dried over sodium sulfate. The solvent was

removed to give crude product. It was purified by column chromatography(30% of Ethyl acetate and hexane) to get **S1** (42.7 g) as white solid.

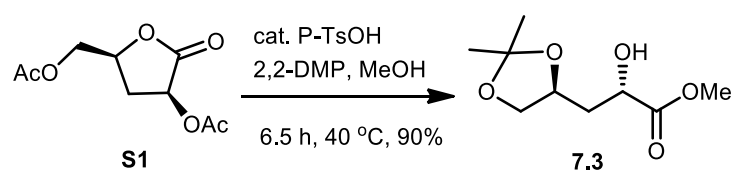
Molecular Formula: C₉H₁₂O₆

R_f = 0.2 (30% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 217.06 [M+1]

Yield: 78%

(S)-Methyl3-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-hydroxy propanoate (7.3)



A 1L, round bottomed reaction flask equipped with a magnetic stirrer, reflux condenser and charged with (35 g, 162 mmol) of the diacetate (**S1**), p-toluenesulfonic acid monohydrate (3 g, 16.2 mmol), and methanol (30 mL). After the mixture was refluxed for 3 hours cooled to room temperature, (120 mL, 907 mmol) of 2,2-dimethoxypropane was added. After 3 hours of stirring, sodium acetate powder, (2.65 g, 32.5 mmol) was added and the mixture was stirred for 20 minutes to quench the reaction. Ethyl acetate (350 mL) and saturated sodium bicarbonate solution (350 mL) are added to the reaction mixture. The organic layer was removed and the aqueous layer was back-extracted twice with ethyl acetate (2x 200mL). The combined organic layers were washed with brine (150 mL), dried over sodium sulfate, and concentrated to afford 37 g of colourless oil. It was purified by column chromatography, using 20% of ethyl acetate and hexane to get **7.3** (29.7 g), as colourless oil.

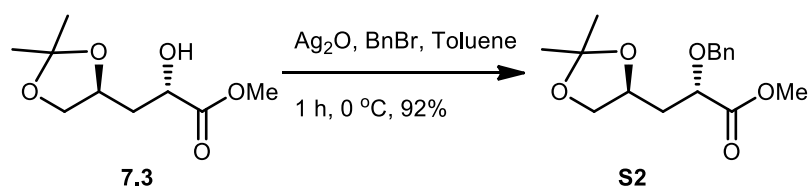
Molecular Formula: C₉H₁₆O₅

R_f = 0.1 (20% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 205.1[M+1]

Yield: 90%

(S)-Methyl2-(benzyloxy)-3-((S)-2,2-dimethyl-1,3-dioxolan-4yl) propanoate (S2)



An oven dried (500 mL) round-bottomed flask was equipped with a magnetic stirring bar, alcohol (**7.3**) (27 g, 132 mmol), and dry toluene (250 mL). (61.3 g, 264 mmol) of silver oxide was added at 0-5 °C, the resulted suspension was stirred at same temperature in an ice bath, (33.9 g, 198.5 mmol) of benzyl bromide was added over ca.10 minutes. The ice bath was removed, and the mixture was allowed to stir for 2 hours at room temperature. The suspension was filtered through Celite, the filter cake was washed with (2x150 mL) toluene, and the combined filtrates were evaporated under reduced pressure to obtain crude product. Flash column chromatography eluted with 10% ethyl acetate/hexane (1:9) afforded the title compound **S2** (30.0 g) as colourless oil.

Molecular Formula: C₁₆H₂₂O₅

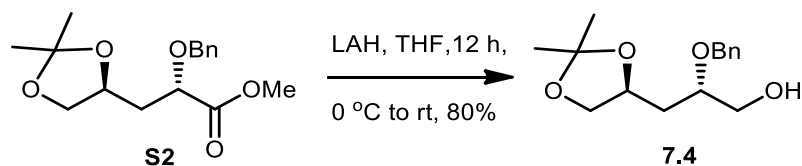
R_f = 0.3 (10% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 295.1[M+1]

Yield: 77%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.33-7.27 (m, 5H), 4.72 (d, *J* = 11.32 Hz, 1H), 4.44 (d, *J* = 11.34 Hz, 1H), 4.27 (qd, *J* = 8.02, 6.70 Hz, 1H), 4.18 (dd, *J* = 10.29, 3.16 Hz, 1H), 4.07-4.02 (m, 1H), 3.74 (d, *J* = 2.98 Hz, 3H), 3.55 (dd, *J* = 8.09, 7.01 Hz, 1H), 2.08-2.00 (m, 1H), 1.95-1.86 (m, 1H), 1.39 (s, 3H), 1.34 (s, 3H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 173.0, 137.3, 128.4, 128.1, 108.7, 75.5, 72.8, 72.6, 69.5, 52.0, 37.3, 27.0, 25.7.

(S)-2-(benzyloxy)-3-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)propan-1-ol (7.4)



To a solution of ester (**S2**) (30 g, 102 mmol) in THF (300 mL) at 0 °C was added lithium aluminum hydride (7.75 g, 204 mmol) and the reaction mixture was stirred for 2 hours. After this time the reaction mixture was quenched by careful addition of 2N sodium hydroxide, ethyl acetate was added and stirred 3 hours. The mixture was filtered through celite pad and washed with ethyl acetate, the combined organic layer was dried over sodium sulfate, concentrated, purified by column chromatography. Eluted with 30% ethyl acetate and hexane gives desired compound **7.4** (21.7 g), as a pale yellow colour liquid.

Molecular Formula: C₁₅H₂₂O₄

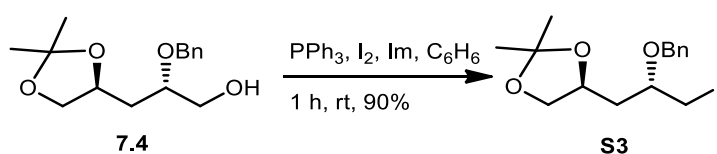
$R_f = 0.2$ (30% ethyl acetate/hexanes)

LRMS: (ES+) $m/z = 267.1[M+1]$

Yield: 80%

^1H NMR (CDCl_3 , 400 MHz) δ ppm: 7.35-7.27 (m, 5H), 4.61 (q, $J = 11.66$ Hz, 2H), 4.27-4.18 (m, 1H), 4.05 (dd, $J = 7.99, 5.94$ Hz, 1H), 3.77 (dd, $J = 11.55, 3.79$ Hz, 1H), 3.73-3.66 (m, 1H), 3.55 (dd, $J = 11.26, 3.95$ Hz, 1H), 3.51 (t, $J = 7.78$ Hz, 1H), 2.49 (s, 1H), 1.91-1.82 (m, 1H), 1.76-1.67 (m, 1H), 1.32 (s, 3H), 1.27 (s, 3H); **^{13}C NMR** (CDCl_3 , 100MHz) δ ppm: 128.5, 128.0, 108.7, 74.9, 72.9, 71.8, 69.7, 39.8, 27.1, 25.9, 10.6.

(S)-4-((S)-2-(benzyloxy)-3-iodopropyl)-2,2-dimethyl-1,3-dioxolane (S3)



To a cool solution of alcohol **7.4** (10 g, 37.5 mmol) in anhydrous benzene (150 mL), triphenylphosphine (13.7 g, 52.6 mmol) was added under nitrogen atmosphere and stirred for 5 min. Then iodine (7.161 g, 56.3 mmol) and imidazole (5.1 g, 75.1 mmol) were added. Reaction mixture cooled to room temperature, Stirred for 2 hours. After completion of the reaction (monitored by TLC) iodine was quenched by saturated solution of sodium thio sulfate, extracted with ethyl acetate, the organic layer was dried over sodium sulfate, concentrated at reduced pressure and purified by column chromatography with 5% ethyl acetate and hexane to get desired product **S3** (12.2 g, 90%) as light yellow oil.

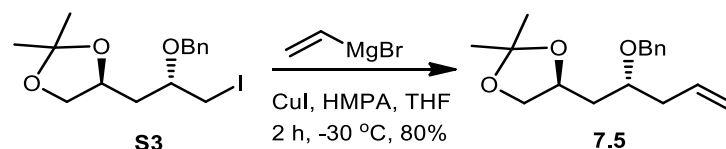
Molecular Formula: $\text{C}_{15}\text{H}_{21}\text{IO}_3$

LRMS: (ES+) $m/z = 377.06[M+1]$

$R_f = 0.2$ (5% ethyl acetate/hexanes)

^1H NMR (CDCl_3 , 400 MHz) δ ppm: 7.40-7.28 (m, 5H), 4.69 (dd, $J = 11.24$, 1H), 4.50-4.45 (m, 1H), 4.30-4.22 (m, 1H), 4.04 (dd, $J = 8.06, 5.96$ Hz, 1H), 3.56-3.50 (m, 2H), 3.37-3.33 (m, 2H), 1.88 (ddd, $J = 13.90, 8.56, 3.30$ Hz, 1H), 1.79 (tdd, $J = 13.92, 9.22, 4.57$ Hz, 1H), 1.41 (s, 3H), 1.35 (s, 3H); **^{13}C NMR** (CDCl_3 , 100MHz) δ ppm: 138.2, 128.5, 127.8, 108.8, 77.4, 73.2, 72.1, 69.8, 64.3, 36.0, 27.0, 25.8.

(S)-4-((R)-2-(benzyloxy)pent-4-enyl)-2,2-dimethyl-1,3-dioxolane (7.5)



To a stirred solution of compound **S3** (10 g, 26.5 mmol) in dry THF (100 mL) was added CuI (2.5 g, 13.2 mmol) and HMPA (4.62 mL, 26.59 mmol) at 23 °C. Then the reaction mixture temperature was cooled to -35 °C. (79.5 mL, 79.5 mmol) of vinyl magnesium bromide was drop wise. After stirring reaction mixture 3 hours at same temperature was quenched with saturated NH_4Cl solution, NaOH and extracted with ethyl acetate (3×100 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography (5% ethyl acetate/Hexane) to provide **7.5** (6.23 g), as colorless oil.

Molecular Formula: $\text{C}_{17}\text{H}_{24}\text{O}_3$

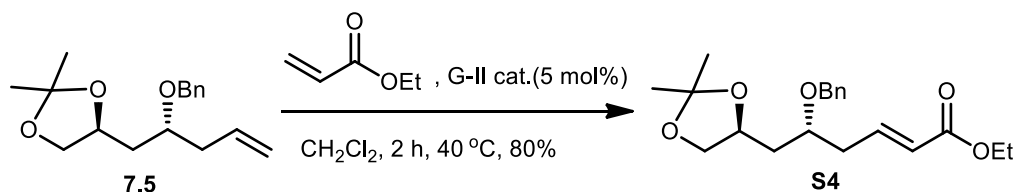
LRMS: (ES⁺) $m/z = 277.12[\text{M}+1]$

$R_f = 0.3$ (5% ethyl acetate/hexanes)

Yield: 85%

^1H NMR (CDCl_3 , 400 MHz) δ ppm: 7.34 (t, $J = 4.90$ Hz, 4H), 7.29 (ddd, $J = 7.04, 5.33, 2.27$ Hz, 1H), 5.91-5.79 (m, 1H), 5.16-5.08 (m, 2H), 4.66 (dd, $J = 11.37, 4.17$ Hz, 1H), 4.48 (d, $J = 11.29$ Hz, 1H), 4.30-4.22 (m, 1H), 4.04 (dd, $J = 8.01, 5.90$ Hz, 1H), 3.70 (dt, $J = 9.49, 9.16, 5.38$ Hz, 1H), 3.52 (t, $J = 7.82$ Hz, 1H), 2.44-2.31 (m, 2H), 1.80 (ddd, $J = 14.03, 7.71, 3.07$ Hz, 1H), 1.75-1.66 (m, 1H), 1.41 (s, 3H), 1.36 (s, 3H); **^{13}C NMR** (CDCl_3 , 100MHz) δ ppm: 138.5, 134.1, 128.3, 127.8, 127.5, 117.5, 108.3, 75.9, 73.5, 71.4, 70.0, 38.6, 27.0, 25.8.

(R,E)-ethyl5-(benzyloxy)-6-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)hex-2-enoate (S4)



To the above olefin compound **7.5** (0.5 g, 1.81mmol) was taken in a dry round bottom flask, equipped with magnetic stirrer, dichloromethane (5 mL), Ethyl acrylate (3.62 mmol), and (5 mol %) grubs II catalyst were added. Resulted reaction mixture was

refluxed at 40 °C temperature for about 2 hours, after completion of the reaction monitored by TLC, cooled to room temperature and concentrated on rotavapor. Obtained crude was purified by column chromatography method using 10% ethyl acetate /hexane to get pure product **S4** (0.504 g) as a pale yellow liquid.

Molecular Formula: C₂₀H₂₈O₅

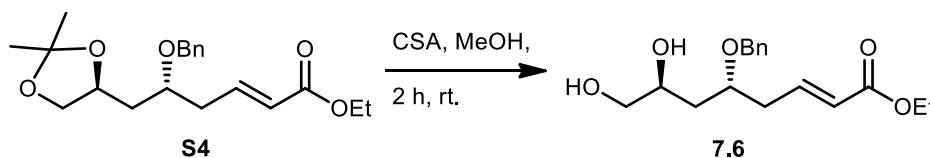
R_f = 0.3(10% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 349.2[M+1]

Yield: 80%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.37-7.26 (m, 5H), 7.01-6.91 (m, 1H), 5.91-5.86 (m, 1H), 4.63-4.59 (m, 1H), 4.50-4.46 (m, 1H), 4.25-4.20 (m, 1H), 4.18-4.15 (q, *J* = 7.2 Hz, 2H), 4.02 (dd, *J* = 8.03, 5.90 Hz, 1H), 3.80-3.74 (m, 1H), 3.49 (t, *J* = 7.81 Hz, 1H), 2.52-2.46 (m, 2H), 1.72 (t, *J* = 6.41 Hz, 2H), 1.38 (s, 3H), 1.33 (s, 3H), 1.28 (t, *J* = 7.13 Hz, 3H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 161.4, 139.6, 133.3, 123.7, 123.0, 119.2, 103.8, 70.5, 68.5, 67.0, 65.1, 55.5, 34.2, 32.4, 22.2, 21.0, 9.4.

(5R,7S,E)-ethyl5-(benzyloxy)-7, 8-dihydroxyoct-2-enoate (7.6)



To a solution of compound **S4** (5 g, 14.3 mmol) in methanol, was added camphor-10 sulfonic acid (247 mg, 1.43 mmol) in one portion at 0 °C under nitrogen atmosphere. Resulted reaction mixture was warmed to room temperature and stirred at the same temperature for 1 hour. It was quenched with a saturated aqueous solution of sodium hydrogen bicarbonate (20 mL) methanol was removed in vacuum and extracted with ethyl acetate (3x50 mL). Dried over anhydrous sodium sulfate and concentrated in vacuum to get crude product, purification was done by column chromatography, eluted with 40% ethyl acetate and hexane, offered **7.6** (3.76 g) as colour less liquid.

Molecular Formula: C₁₇H₂₄O₅

R_f = 0.1 (40 % ethyl acetate/hexanes)

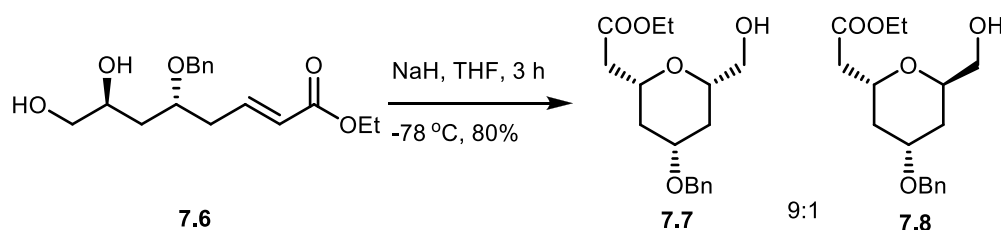
LRMS: (ES+) *m/z* = 309.16[M+1]

Yield: 85%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.35-7.25 (m, 5H), 6.94 (td, *J* = 15.27, 7.47 Hz, 1H), 5.92-5.86 (m, 1H), 4.64 (dd, *J* = 11.44, 2.91 Hz, 1H), 4.51 (dd, *J* = 11.38, 8.15 Hz,

1H), 4.19 (q, $J = 7.14$ Hz, 2H), 3.95 (ddd, $J = 12.55, 6.60, 3.09$ Hz, 1H), 3.90-3.84 (m, 1H), 3.60 (dd, $J = 11.09, 3.14$ Hz, 1H), 3.46-3.39 (m, 1H), 2.71 (dd, $J = 1.93, 1.01$ Hz, 1H), 2.52 (dq, $J = 8.15, 7.77, 4.16$ Hz, 2H), 2.21-2.08 (m, 1H), 1.69-1.63 (m, 1H), 1.58 (ddd, $J = 14.57, 8.35, 3.08$ Hz, 1H), 1.29 (t, $J = 7.15$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100MHz) δ ppm: 166.2, 144.2, 137.7, 128.5, 128.4, 127.9, 127.9, 124.0, 74.9, 71.6, 68.8, 66.8, 36.9, 36.6, 14.2.

Ethyl 2-((2R, 4R, 6S)-4-(benzyloxy)-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl) acetate (7.7)



To the suspension of NaH (160 mg, 4 mmol) in dry THF (20 mL) at -78 $^\circ\text{C}$ was added slowly compound **7.6** (308 mg, 1 mmol) in THF (3 mL). The reaction was stirred for 3 hours, after which TLC showed complete conversion. Then solid ice pieces were added to quench the reaction and then the THF layer was separated and the aqueous layer was extracted with ether (3x20 mL). The combined organic layers were washed with water, brine, dried over anhydrous sodium sulfate and concentrated under vacuum to yield the crude product, which was purified by column chromatography with 30% ethyl acetate/hexane to get the pure product **7.7** (252 mg) as a colour less liquid.

Molecular Formula: $\text{C}_{17}\text{H}_{24}\text{O}_5$

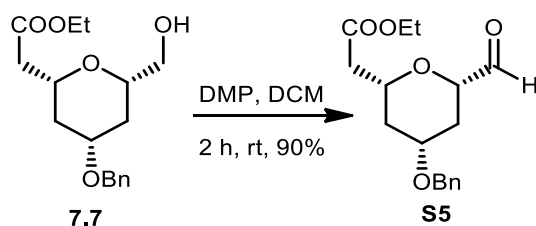
$R_f = 0.2$ (30% ethyl acetate/hexanes)

LRMS: (ES+) $m/z = 309.16[\text{M}+1]$

Yield: 80%

^1H NMR (CDCl_3 , 400 MHz) δ ppm: 7.37-7.26 (m, 5H), 4.56 (s, 2H), 4.14 (q, $J = 7.12$ Hz, 2H), 3.83-3.78 (m, 1H), 3.68-3.61 (m, 1H), 3.57-3.55 (m, 2H), 3.52-3.44 (m, 1H), 2.60 (dd, $J = 15.36, 7.61$ Hz, 1H), 2.44 (dd, $J = 15.34, 5.45$ Hz, 1H), 2.18 (s, 2H), 2.15-2.09 (m, 1H), 1.99-1.93 (m, 1H), 1.31 (dd, $J = 11.36, 4.58$ Hz, 1H), 1.25 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100MHz) δ ppm: 171.5, 138.2, 128.4, 127.6, 127.4, 71.7, 70.6, 70.2, 64.4, 63.6, 60.8, 39.0, 34.6, 30.7, 14.2.

Ethyl 2-((2R, 4R, 6S)-4-(benzyloxy)-6-formyltetrahydro-2H-pyran-2-yl) acetate (S5)



To a stirred solution of **7.7** (250 mg, 0.81 mmol) in dry CH_2Cl_2 (3 mL) at 0 °C, Dess-Martin periodinane (692 mg, 1.63 mmol) was added under N_2 atmosphere. After 2 hours, reaction quenched with sat. aq. solution of sodium thio sulfate (5 mL) and sodium bicarbonate, extracted with CH_2Cl_2 (2x5 mL), combined organic layer dried over anhydrous sodium sulfate, concentrated to get the crude aldehyde, which was purified by flash column chromatography (20% ethyl acetate and hexanes) gave **S5**, (223 mg) as colour les liquid.

Molecular Formula: $\text{C}_{17}\text{H}_{22}\text{O}_5$

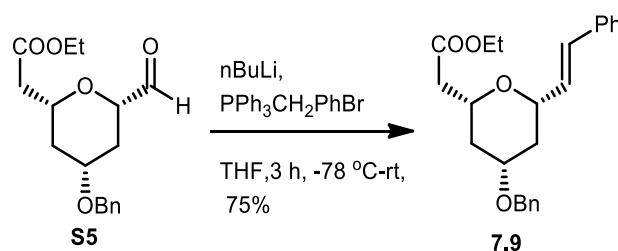
$R_f = 0.2$ (20% ethyl acetate/hexanes)

LRMS: (ES+) $m/z = 307.12[\text{M}+1]$

Yield: 90%

^1H NMR (CDCl_3 , 400 MHz) δ ppm: 9.62 (s, 1H), 7.38-7.27 (m, 5H), 4.64-4.53 (m, 2H), 4.17 (q, $J = 7.10$ Hz, 2H), 3.94-3.86 (m, 1H), 3.82 (dd, $J = 12.24, 2.08$ Hz, 1H), 3.65 (tt, $J = 10.93, 4.47$ Hz, 1H), 2.73-2.65 (m, 1H), 2.54-2.45 (m, 1H), 2.31 (tdd, $J = 12.55, 4.39, 2.19$ Hz, 1H), 2.18-2.10 (m, 1H), 1.40-1.28 (m, 2H), 1.25 (dd, $J = 7.17, 3.74$ Hz, 3H); **^{13}C NMR** (CDCl_3 , 100MHz) δ ppm: 200.7, 170.6, 138.0, 128.4, 127.7, 127.5, 79.8, 73.4, 72.6, 69.8, 60.6, 40.8, 37.5, 32.2, 14.1.

Ethyl 2-((2R, 4R, 6S)-4-(benzyloxy)-6-styryltetrahydro-2H-pyran-2-yl) acetate (7.9)



Benzyl triphenyl phosphonium bromide (424 mg, 0.98 mmol) was dissolved in freshly distilled THF (3 mL) and n-BuLi (0.261 mL, 0.65 mmol, 2.5 M in hexanes) was added

to the above solution at 0 °C. After 20 minutes additional stirring at room temperature, the reaction temperature was lowered to -78 °C and the crude aldehyde **S5** (200 mg, 0.65 mmol) in THF (2 mL) was added to the above solution and stirred for 30 minutes. After 2 hours, the reaction mixture was warmed to room temperature, quenched with ethyl acetate (10 mL) and a saturated aqueous sodium bicarbonate solution (10 mL). This mixture was extracted with ethyl acetate (3 X 10 mL), washed with brine (10 mL), dried over sodium sulfate, filtered, concentrated under reduced pressure and purified through column chromatography (10% ethyl acetate in hexanes) to give **7.9** (186 mg) as a pale-yellow oil.

Molecular Formula: C₂₄H₂₈O₄

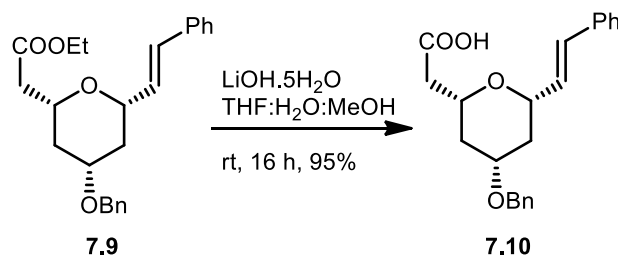
R_f = 0.2 (10% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 381.20 [M+1]

Yield: 75%

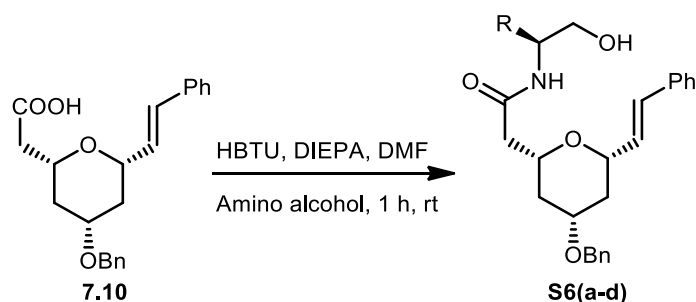
¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.41-7.25 (m, 10H), 6.63 (d, *J* = 11.50 Hz, 1H), 5.68 (dd, *J* = 11.47, 8.78 Hz, 1H), 4.55 (s, 2H), 4.22-4.13 (m, 3H), 3.96-3.87 (m, 1H), 3.63-3.55 (m, 1H), 2.69 (dd, *J* = 15.48, 8.10 Hz, 1H), 2.48 (dd, *J* = 15.46, 4.18 Hz, 1H), 2.15-2.07 (m, 2H), 1.52-1.44 (m, 1H), 1.39-1.34 (m, 1H), 1.25 (t, *J* = 7.2 Hz, 3H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 171.1, 138.3, 136.4, 133.0, 130.9, 128.8, 128.4, 128.3, 127.6, 127.5, 127.4, 73.8, 72.0, 71.9, 69.6, 60.5, 41.3, 38.0, 37.4, 14.2.

2-((2R, 4R,6S)-4-(benzyloxy)-6-styryltetrahydro-2H-pyran-2-yl)acetic acid (**7.10**)



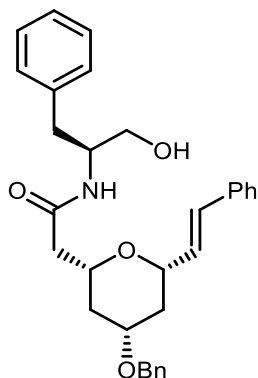
Compound **7.9** (150 mg, 0.39 mmol) 5 mL (4:1) THF: H₂O, then LiOH·H₂O (81 mg, 1.97 mmol) was added and stirred for 16 hours at room temperature. Then acidified to pH 2 with 2 N HCl and extracted with ethyl acetate (2x5 mL), the combined organic layer dried over Na₂SO₄, concentrated to get the crude acid **7.10** (132 mg, 95%), directly used in the next step without further purification.

Coupling Reaction



Oven dried round bottom flask was equipped with magnetic bar and above crude Acid **7.10** (50 mg 0.142 mmol), (2 mL) of DMF, were added HBTU (1.1eq) and DIEPA (0.5 eq), Amino alcohol (2 eq) at cool condition stirred at same temperature for 1 hour, reaction mixture was quenched with water and extracted with ethyl acetate (5 mL), dried over anhydrous sodium sulfate concentrated under reduced pressure, crude product was purified by column chromatography with 40% ethyl acetate and hexane to get pure product.

2-((2R,4R,6S)-4-(benzyloxy)-6-styryltetrahydro-2H-pyran-2-yl)-N-((S)-1-hydroxy-3-phenylpropan-2-yl)acetamide (S6-a)



Molecular Formula: C₃₁H₃₅NO

R_f = 0.1 (40% ethyl acetate/hexanes)

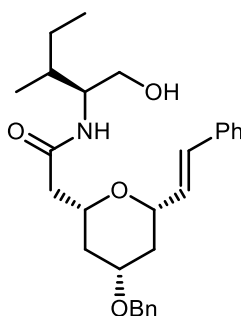
LRMS: (ES⁺) *m/z* = 486.3[M+1]

Yield: 80%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.33-7.18 (m, 10H), 7.13-7.05 (m, 3H), 6.97 (dd, *J* = 6.71, 2.67 Hz, 2H), 6.53 (d, *J* = 11.65 Hz, 1H), 6.40 (d, *J* = 7.58 Hz, 1H), 5.57 (dd, *J* = 11.59, 8.69 Hz, 1H), 4.46 (d, *J* = 6.87 Hz, 2H), 4.14 (t, *J* = 9.55 Hz, 1H), 4.10-4.01 (m, 1H), 3.65-3.42 (m, 4H), 2.84-2.75 (m, 1H), 2.71 (dd, *J* = 12.26, 4.76 Hz, 1H), 2.63 (dd, *J* = 13.72, 7.14 Hz, 1H), 2.32 (dd, *J* = 10.30, 5.94 Hz, 1H), 2.08-2.01 (m, 1H),

2.00-1.93 (m, 1H), 1.38 (dd, $J = 14.37, 7.26$ Hz, 1H), 1.30-1.15 (m, 2H); ^{13}C NMR (CDCl_3 , 100MHz) δ ppm: 171.2, 138.3, 137.6, 136.3, 132.5, 130.9, 129.2, 129.1, 128.8, 128.6, 128.5, 128.5, 127.7, 127.6, 126.5, 126.4, 73.6, 72.3, 69.8, 64.1, 53.0, 43.3, 37.9, 37.4, 37.0.

2-((2R,4R,6S)-4-(benzyloxy)-6-styryltetrahydro-2H-pyran-2-yl)-N-((2S,3R)-1-hydroxy-3-methylpentan-2-yl)acetamide (S6-b)



Molecular Formula: $\text{C}_{28}\text{H}_{37}\text{NO}_4$

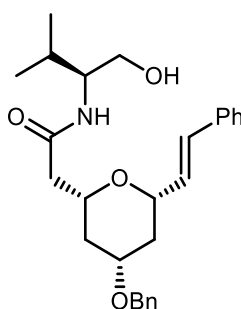
$R_f = 0.1$ (40% ethyl acetate/hexanes)

LRMS: (ES^+) $m/z = 452.27[\text{M}+1]$

Yield: 75%

^1H NMR (CDCl_3 , 400 MHz) δ ppm: 7.32-7.19 (m, 8H), 7.14 (d, $J = 7.16$ Hz, 2H), 6.51 (d, $J = 11.74$ Hz, 1H), 6.40 (d, $J = 7.28$ Hz, 1H), 5.58 (dd, $J = 11.60, 8.76$ Hz, 1H), 4.49 (s, 2H), 4.20 (t, $J = 9.58$ Hz, 1H), 3.76-3.50 (m, 5H), 2.40 (d, $J = 2.94$ Hz, 1H), 2.39 (s, 1H), 2.09-1.97 (m, 2H), 1.50-1.27 (m, 5H), 1.04-0.90 (m, 1H), 0.75 (d, $J = 6.84$ Hz, 3H), 0.70 (t, $J = 7.35$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100MHz): 171.9, 138.2, 136.2, 132.1, 130.8, 128.7, 128.4, 128.4, 127.7, 127.6, 127.5, 73.6, 72.4, 72.4, 69.8, 64.4, 56.7, 43.4, 37.9, 37.4, 35.8, 25.4, 15.4, 11.4.

2-((2R,4R,6S)-4-(benzyloxy)-6-styryltetrahydro-2H-pyran-2-yl)-N-((S)-1-hydroxy-3-methylbutan-2-yl)acetamide (S6-c)



Molecular Formula: $\text{C}_{27}\text{H}_{35}\text{NO}_4$

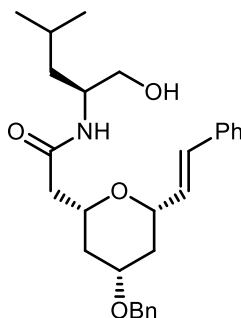
$R_f = 0.1$ (40% ethyl acetate/hexanes)

LRMS: (ES+) $m/z = 438.26[M+1]$

Yield: 78%

$^1\text{H NMR}$ (CDCl_3 , 400 MHz) δ ppm: 7.30-7.17 (m, 8H), 7.13 (d, $J = 7.18$ Hz, 2H), 6.51 (d, $J = 11.55$ Hz, 1H), 6.43 (d, $J = 7.38$ Hz, 1H), 5.58 (dd, $J = 11.60, 8.81$ Hz, 1H), 4.48 (s, 2H), 4.20 (t, $J = 9.59$ Hz, 1H), 3.74-3.67 (m, 1H), 3.63-3.49 (m, 4H), 3.11-2.92 (m, 1H), 2.09-1.97 (m, 2H), 1.72 (dt, $J = 13.68, 13.43, 6.95$ Hz, 1H), 1.46-1.36 (m, 1H), 1.35-1.25 (m, 1H), 1.03 (dd, $J = 19.22, 6.76$ Hz, 1H), 0.82 (dd, $J = 15.74, 6.85$ Hz, 1H), 0.76 (d, $J = 7.6$ Hz, 3H), 0.73 (d, $J = 7.6$ Hz, 3H); $^{13}\text{C NMR}$ (CDCl_3 , 100MHz) δ ppm: 171.9, 138.2, 136.2, 132.2, 130.8, 128.7, 128.5, 128.4, 127.7, 127.6, 127.5, 73.6, 72.5, 72.4, 69.8, 64.4, 57.5, 43.4, 37.9, 37.4, 28.9, 19.4 18.4.

2-((2R,4R,6S)-4-(benzyloxy)-6-styryltetrahydro-2H-pyran-2-yl)-N-((S)-1-hydroxy-4-methylpentan-2-yl) acetamide (S6-d)



Molecular Formula: $\text{C}_{28}\text{H}_{37}\text{NO}_4$

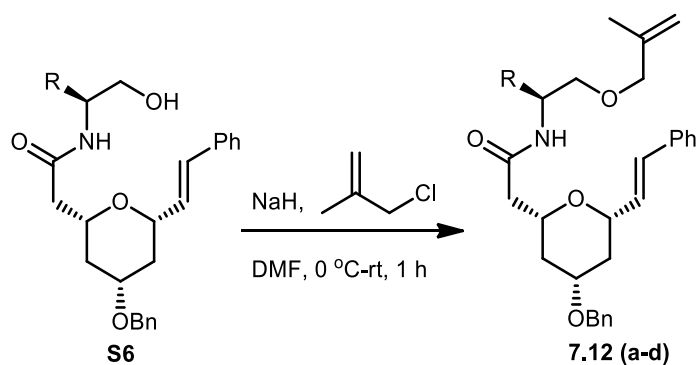
$R_f = 0.1$ (40% ethyl acetate/hexanes)

LRMS: (ES+) $m/z = 452.2[M+1]$

Yield: 70%

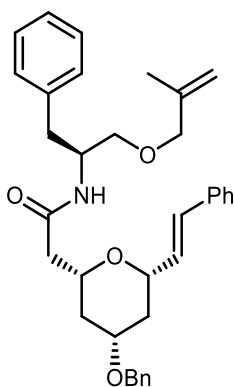
$^1\text{H NMR}$ (CDCl_3 , 400 MHz) δ ppm: 7.31-7.19 (m, 8H), 7.16 (d, $J = 7.26$ Hz, 2H), 6.52 (d, $J = 11.57$ Hz, 1H), 6.17 (d, $J = 7.44$ Hz, 1H), 5.57 (dd, $J = 11.61, 8.73$ Hz, 1H), 4.48 (s, 2H), 4.18 (t, $J = 9.78$ Hz, 1H), 3.93-3.85 (m, 1H), 3.78-3.69 (m, 1H), 3.58 (dd, $J = 11.04, 3.36$ Hz, 1H), 3.55-3.49 (m, 1H), 3.43 (dd, $J = 11.03, 6.22$ Hz, 1H), 2.37 (d, $J = 6.37$ Hz, 2H), 2.08-1.08 (m, 2H), 1.52-1.27 (m, 4H), 0.78 (dd, $J = 14.51, 7.62$ Hz, 2H), 0.71 (d, $J = 6.4$ Hz, 3H), 0.69 (d, $J = 6.8$ Hz, 3H); $^{13}\text{C NMR}$ (CDCl_3 , 100MHz) δ ppm:

Allylation



To the stirred solution of alcohol **S6** (1eq) in dry DMF (2 mL) at 0 °C were added NaH (1.5 eq) stirred for 5 minutes then 3-chloro-2-methylprop-1-ene (1.2 eq), resulted solution was stirred for 2 hours at room temperature. reaction mixture was quenched with water (5 mL) extracted with ethyl acetate, dried over anhydrous sodium sulfate, evaporated the solvent, Purification of crude compound by flash chromatography over silica gel with 20% ethyl acetate/hexane afforded the biss allyl product compounds (**7.12**).

2-((2R,4R,6S)-4-(benzyloxy)-6-styryltetrahydro-2H-pyran-2-yl)-N-((S)-1-(2-methylallyloxy)-3-phenylpropan-2-yl)acetamide (7.12-a**)**



Molecular Formula: C₃₅H₄₁NO₄

R_f = 0.2 (20% ethyl acetate/hexanes)

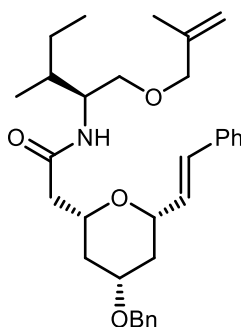
LRMS: (ES⁺) *m/z* = 540.30[M+1]

Yield: 90%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.40-7.26 (m, 10H), 7.20-7.13 (m, 3H), 7.11-7.06 (m, 2H), 6.60 (d, *J* = 11.61 Hz, 1H), 6.49 (d, *J* = 8.71 Hz, 1H), 5.67 (dd, *J* = 11.58, 8.74 Hz, 1H), 4.95 (s, 1H), 4.88(s, 1H), 4.55 (s, 2H), 4.33-4.27 (m, 1H), 4.23 (t, *J* = 9.51 Hz, 1H), 3.85 (q, *J* = 12.50 Hz, 2H), 3.75-3.69 (m, 1H), 3.63-3.54 (m, 1H), 3.34-3.26 (m,

2H), 2.85-2.74 (m, 2H), 2.40 (dd, $J = 10.72, 5.94$ Hz, 1H), 2.38 (dd, $J = 14.8, 4.0$, 1H), 2.15-2.09 (m, 1H), 2.08-2.02 (m, 1H), 1.74 (s, 3H), 1.47 (dd, $J = 23.61, 11.25$ Hz, 1H), 1.37-1.33 (m, 1H); ^{13}C NMR (CDCl_3 , 100MHz) δ ppm: 169.9, 142.0, 138.3, 138.1, 136.4, 132.4, 131.0, 129.4, 128.8, 128.4, 128.3, 127.6, 127.6, 127.5, 126.2, 112.2, 75.1, 73.7, 72.3, 72.3, 69.7, 50.1, 43.5, 38.0, 37.5, 37.4, 29.6, 19.5.

2-((2R,4R,6S)-4-(benzyloxy)-6-styryltetrahydro-2H-pyran-2-yl)-N-((2S,3R)-3-methyl-1-(2-methylallyloxy)pentan-2-yl) acetamide (7.12-b)



Molecular Formula: $\text{C}_{32}\text{H}_{43}\text{NO}_4$

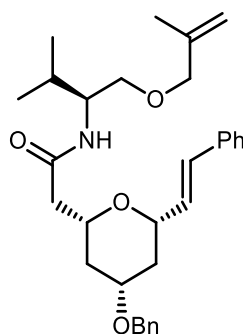
$R_f = 0.2$ (20% ethyl acetate/hexanes)

LRMS: (ES^+) $m/z = 506.32[\text{M}+1]$

Yield: 75%

^1H NMR (CDCl_3 , 400 MHz) δ ppm: 7.37-7.18 (m, 10H), 6.56 (d, $J = 11.59$ Hz, 1H), 6.36 (d, $J = 9.23$ Hz, 1H), 5.65 (dd, $J = 11.58, 8.82$ Hz, 1H), 4.93(s, 1H), 4.87 (s, 1H), 4.55 (s, 2H), 4.25 (t, $J = 9.68$ Hz, 1H), 3.89-3.81 (m, 3H), 3.65-3.55 (m, 1H), 3.50 (dd, $J = 9.72, 3.92$ Hz, 1H), 3.37 (dd, $J = 9.71, 3.92$ Hz, 1H), 2.43 (d, $J = 5.95$ Hz, 2H), 2.12-2.05 (m, 2H), 1.71 (s, 3H), 1.64-1.57 (m, 1H), 1.50-1.41 (m, 2H), 1.40-1.31 (m, 1H), 1.01-0.92 (m, 1H), 0.82 (d, $J = 6.78$ Hz, 3H), 0.74 (t, $J = 7.38$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100MHz) δ ppm: 170.1, 142.1, 138.3, 136.3, 131.9, 131.1, 128.7, 128.4, 128.3, 127.6, 127.6, 127.4, 112.2, 75.1, 73.7, 72.5, 72.3, 69.7, 69.7, 53.0, 43.7, 38.0, 37.4, 35.7, 25.4, 19.5, 15.4, 11.3.

2-((2R,4R,6S)-4-(benzyloxy)-6-styryltetrahydro-2H-pyran-2-yl)-N-((S)-3-methyl-1-(2-methylallyloxy)butan-2-yl)acetamide (7.12-c)



Molecular Formula: C₃₁H₄₁NO₄

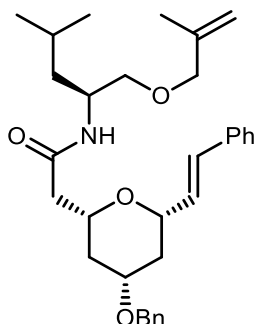
R_f = 0.2 (20% ethyl acetate/hexanes)

LRMS: (ES⁺) *m/z* = 492.30[M+1]

Yield: 80%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.35-7.20 (m, 10H), 6.56 (d, *J* = 11.60 Hz, 1H), 6.37 (d, *J* = 9.22 Hz, 1H), 5.65 (dd, *J* = 11.59, 8.87 Hz, 1H), 4.93 (s, 1H), 4.87 (s, 1H), 4.55 (s, 2H), 4.26 (t, *J* = 9.60 Hz, 1H), 3.89-3.76 (m, 3H), 3.64-3.55 (m, 1H), 3.50 (dd, *J* = 9.68, 3.84 Hz, 1H), 3.35 (dd, *J* = 9.69, 4.22 Hz, 1H), 2.44 (d, *J* = 2.82 Hz, 1H), 2.13-2.04 (m, 2H), 1.89-1.80 (m, 1H), 1.71 (s, 3H), 1.61 (s, 2H), 1.46 (dd, *J* = 23.90, 11.26 Hz, 1H), 1.39-1.31 (dd, *J* = 23.6, 11.6 Hz, 1H), 0.84 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 6.8 Hz, 3H); **¹³C NMR** (CDCl₃, 100 MHz) δ ppm: 170.1, 142.1, 138.3, 136.3, 131.9, 131.1, 128.7, 128.4, 128.3, 127.6, 127.4, 112.1, 110.0, 75.1, 73.7, 72.5, 72.3, 69.9, 69.7, 54.0, 43.7, 38.0, 37.4, 29.2, 19.5, 18.8.

2-((2R,4R,6S)-4-(benzyloxy)-6-styryltetrahydro-2H-pyran-2-yl)-N-((S)-4-methyl-1-(2-methylallyloxy)pentan-2-yl)acetamide (7.12-d)



Molecular Formula: C₃₂H₄₃NO₄

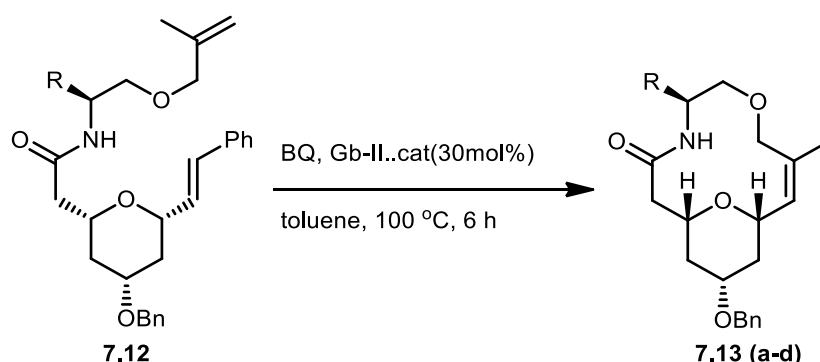
R_f = 0.2 (20% ethyl acetate/hexanes)

LRMS: (ES⁺) *m/z* = 506.32[M+1]

Yield: 73%

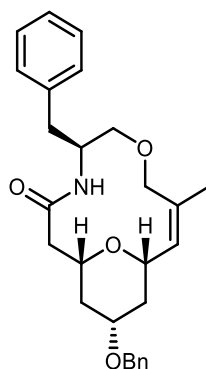
^1H NMR (CDCl_3 , 400 MHz) δ ppm: 7.23-7.10 (m, 10H), 6.51 (d, J = 11.67 Hz, 1H), 6.12 (d, J = 9.23 Hz, 1H), 5.58 (dd, J = 11.54, 8.84 Hz, 1H), 4.86 (s, 1H), 4.81 (s, 1H), 4.47 (s, 2H), 4.17 (t, J = 9.74 Hz, 1H), 4.12-4.03 (m, 1H), 3.84-3.73 (m, 3H), 3.52 (t, J = 4.52 Hz, 1H), 3.31 (t, J = 3.33 Hz, 2H), 2.35 (d, J = 1.22 Hz, 1H), 2.05-1.98 (m, 2H), 1.63 (s, 3H), 1.49-1.43 (m, 1H), 1.41-1.33 (m, 1H), 1.31-1.24 (m, 3H), 0.79 (dd, J = 11.07, 6.66 Hz, 1H), 0.71 (t, J = 6.59 Hz, 6H); **^{13}C NMR** (CDCl_3 , 100MHz) δ ppm: 169.9, 142.1, 138.3, 136.3, 132.1, 131.0, 128.7, 128.4, 128.4, 127.6, 127.6, 127.4, 112.1, 73.7, 72.4, 72.3, 71.9, 69.7, 47.1, 43.7, 41.0, 38.0, 37.4, 24.7, 22.9, 22.3, 19.4.

Ring Closing Metathesis Reaction



To a solution of above diene **7.12** (1 eq) and 1,4-benzoquinone (0.63 eq) in degassed toluene (5.2 mL) at 100 °C was added a solution of G-II.cat (0.3 eq) in degassed toluene (2.3 mL) over a period of 6 hours. The resultant solution was stirred at 100 °C for 16 hours, cooled to room temperature. The reaction mixture was concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel with 20-30% ethyl acetate/hexanes gave macro cycles (**7.13a-d**).

(1R,5S,11S,13R,Z)-5-benzyl-13-hydroxy-9-methyl-7, 15-dioxo-4-azabicyclo [9.3.1] pentadec-9-en-3-one (7.13-a)



Molecular Formula: C₂₇H₃₃NO₄

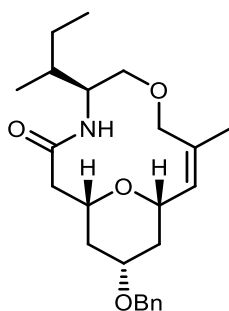
R_f = 0.2 (30% ethyl acetate/hexanes)

LRMS: (ES⁺) *m/z* = 436.2[M+1]

Yield: 58%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.33-7.28 (m, 4H), 7.31-7.26 (m, 3H), 7.20 (t, *J* = 6.75 Hz, 3H), 6.40-6.35 (m, 1H), 5.47 (dd, *J* = 5.46, 1.39 Hz, 1H), 4.57 (d, *J* = 2.46 Hz, 2H), 4.04-3.98 (m, 1H), 3.96 (d, *J* = 8.63 Hz, 1H), 3.84 (d, *J* = 8.60 Hz, 1H), 3.76-3.68 (m, 1H), 3.65 (d, *J* = 1.73 Hz, 2H), 3.31-3.25 (m, 1H), 2.87-2.80 (m, 1H), 2.36 (d, *J* = 3.20 Hz, 1H), 2.28 (d, *J* = 9.65 Hz, 1H), 2.15-2.09 (m, 1H), 2.08-2.03 (m, 1H), 1.80 (s, 3H), 1.49-1.42 (m, 2H), 1.41-1.35 (m, 2H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 172.2, 140.0, 138.7, 138.3, 129.3, 129.2, 128.4, 128.3, 127.6, 127.6, 126.3, 74.2, 73.4, 71.9, 71.8, 70.0, 69.7, 54.2, 44.0, 38.0, 37.0, 36.5, 23.0.

(1R,5S,11S,13R,Z)-13-(benzyloxy)-5-sec-butyl-9-methyl-7,15-dioxa-4-azabicyclo [9.3.1] pentadec-9-en-3-one (7.13-b)



Molecular Formula: C₂₄H₃₅NO₄

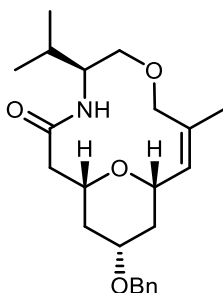
R_f = 0.2 (30% ethyl acetate/hexanes)

LRMS: (ES⁺) *m/z* = 402.26[M+1]

Yield: 62%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.31-7.20 (m, 5H), 6.26 (d, *J* = 6.47 Hz, 1H), 5.43 (dd, *J* = 5.84, 1.39 Hz, 1H), 4.50 (d, *J* = 4.22 Hz, 2H), 3.94 (dd, *J* = 10.79, 5.70 Hz, 1H), 3.85 (dd, *J* = 25.47, 8.91 Hz, 2H), 3.63 (dd, *J* = 10.33, 4.27 Hz, 2H), 3.58-3.51 (m, 2H), 3.32-3.29 (m, 1H), 2.38 (dd, *J* = 14.02, 3.55 Hz, 1H), 2.24 (dd, *J* = 14.04, 8.97 Hz, 1H), 2.08-1.94 (m, 3H), 1.75 (s, 3H), 1.46-1.44 (m, 1H), 1.41-1.33 (m, 2H), 1.09-1.02 (m, 1H), 0.82-0.78 (m, 6H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 171.5, 139.3, 138.3, 129.2, 128.4, 127.6, 127.6, 74.2, 73.2, 72.2, 71.5, 69.7, 68.4, 55.9, 43.6, 38.0, 36.8, 34.5, 26.0, 23.5, 15.3, 11.1.

**(1R,5S,11S,13R,Z)-13-(benzyloxy)-5-isopropyl-9-methyl-7,15-dioxabicyclo
[9.3.1]pentadec-9-en-3-one(7.13-c)**



Molecular Formula: C₂₃H₃₃NO₄

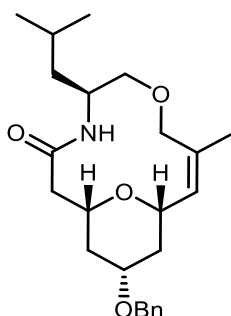
R_f = 0.2 (30% ethyl acetate/hexanes)

LRMS: (ES⁺) *m/z* = 388.24[M+1]

Yield: 55%

¹H NMR (CDCl₃, 400 MHz): 7.30-7.20 (m, 5H), 6.39-6.27 (m, 1H), 5.43 (d, *J* = 2.01 Hz, 1H), 4.50 (d, *J* = 2.33 Hz, 2H), 3.98-3.90 (m, 1H), 3.91 (d, *J* = 8.80 Hz, 1H), 3.81 (d, *J* = 8.60 Hz, 1H), 3.53-3.68 (m, 4H), 3.25-3.16 (m, 1H), 2.48-2.36 (m, 1H), 2.33-2.22 (m, 1H), 2.19-2.08 (m, 1H), 2.03 (s, 2H), 1.78 (s, 3H), 1.44-1.32 (m, 2H), 0.84 (dd, *J* = 15.40, 8.82 Hz, 6H) ; ¹³C NMR (CDCl₃, 100MHz) δ ppm: 172.0, 139.3, 138.3, 129.2, 128.4, 127.6, 127.6, 74.2, 73.2, 72.3, 71.7, 69.7, 68.7, 57.9, 38.0, 36.9, 29.7, 28.5, 23.4, 19.4.

**(1R,5S,11S,13R,Z)-13-(benzyloxy)-5-isobutyl-9-methyl-7,15-dioxabicyclo
[9.3.1]pentadec-9-en-3-one(7.13-d)**



Molecular Formula: C₂₄H₃₅NO₄

R_f = 0.2 (30% ethyl acetate/hexanes)

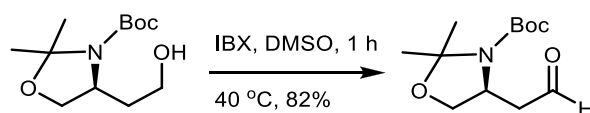
LRMS: (ES⁺) *m/z* = 402.26[M+1]

Yield: 60%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.35-7.27 (m, 5H), 6.21 (d, J = 5.80 Hz, 1H), 5.52-5.45 (m, 1H), 4.57 (d, J = 4.20 Hz, 2H), 4.04-3.98 (m, 1H), 3.98 (d, J = 8.61 Hz, 1H), 3.86 (d, J = 8.61 Hz, 1H), 3.74-3.68 (m, 1H), 3.65-3.62 (m, 2H), 3.51-3.45 (m, 1H), 2.43-2.37 (m, 1H), 2.30-2.23 (m, 1H), 2.14-2.05 (m, 2H), 1.94-1.85 (m, 1H), 1.82 (s, 3H), 1.70-1.61 (m, 2H), 1.50-1.44 (m, 1H), 1.39-1.33 (m, 1H), 1.22-1.14 (m, 1H), 0.91(d, J = 6.5 Hz, 3H), 0.89 (d, J = 6.4Hz, 3H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 171.9, 140.0, 138.3, 129.2, 128.4, 127.6, 127.6, 74.3, 73.5, 71.9, 71.0, 69.7, 51.4, 44.2, 39.0, 38.0, 37.1, 25.0, 23.1, 23.0, 22.1.

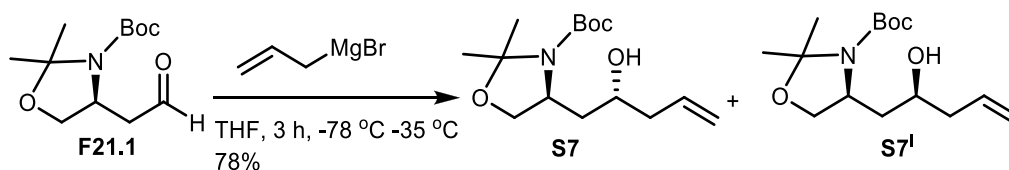
2.17 Experimental Section for Piperidine core containing Macrocycles Synthesis

(S)-tert-butyl 2,2-dimethyl-4-(2-oxoethyl)oxazolidine-3-carboxylate



An oven dried round bottom flask was equipped with alcohol **F21.1** (10g, 40.8 mmol), 2-iodoxybenzoic acid (IBX) (17.31g, 61.2mmol) and DMSO (100 mL), the resulted reaction mixture was subjected to 40 °C and stirred for 1 hour, after completion of the reaction monitored by TLC quenched with Sodium thiosulfate and sodium bi carbonate, the compound was extracted with ethyl acetate The combined organic layers were dried over anhydride sodium sulfate and concentrated under reduced pressure. The crude residue was purified by column chromatography (15% ethyl acetate/ hexane) to provide **F21.1** (8.3 g, 82%) as pale yellow colour oil.

(S)-tert-butyl 4-((R)-2-hydroxypent-4-en-1-yl)-2,2-dimethyloxazolidine-3-carboxylate (**S7**, **S7^I**)



An oven dried round bottom flask was filled with aldehyde **F21.1** (10 g, 41.1 mmol) in dry THF (100 mL) it was cooled to -78 °C, allyl magnesium bromide (123.45 mmol) was added to the aldehyde and. Then the reaction mixture temperature was cooled to -35 °C. After stirring reaction mixture 3 hours at same temperature was quenched with citric acid solution, and extracted with ethyl acetate (3×100 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography (20% ethyl acetate/ Hexane) to provide **S7**, **S7^I** (**9:6**). 9.1 g yield as colorless oil.

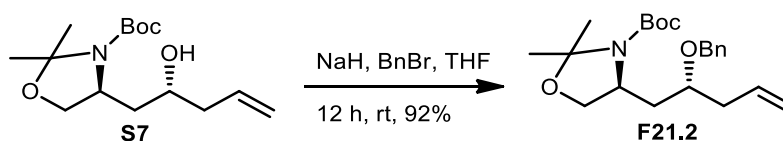
Molecular Formula: C₁₅H₂₇NO₄

LRMS: (ES+) $m/z = 286.2[M+1]$

$R_f = 0.3$ (20% ethyl acetate/hexanes)

Yield: 78%

(S)-tert-butyl 4-((R)-2-(benzyloxy)pent-4-en-1-yl)-2,2-dimethyloxazolidine-3-carboxylate (F21.2)



Major Diastereomer **S7** (8 g, 28.07 mmol) was taken in to round bottom flask; solvent dry THF was added and cooled to 0 °C. Sodium Hydride was added and stirred for 15 minutes, followed by Benzyl Bromide addition at same temperature. Resulted reaction mixture was stirred for overnight. 10 volumes of water was added to the reaction mixture and the compound was extracted with ethyl acetate, the combined organic reaction mass was washed with brine, organic fraction was dried over sodium sulfate, concentrated on rotavapor to obtained crude product it was purified by column chromatography (10% ethyl acetate/hexanes) to get pure product **F21.2**, (9.68 g) yield as colour less oil.

Molecular Formula: C₂₂H₃₃NO₄

$R_f = 0.3$ (10% ethyl acetate/hexanes)

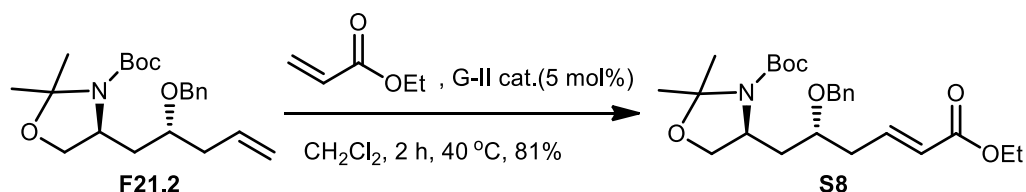
LRMS: (ES+) $m/z = 376.2[M+1]$

Yield: 92%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.32-7.20 (m, 5H), 5.85-5.70 (m, 1H), 5.10-4.98 (m, 2H), 4.55 (d, $J = 9.85$ Hz, 1H), 4.44-4.34 (m, 1H), 4.14-3.94 (m, 1H), 3.77-3.72 (m,

1H), 3.67 (d, $J = 8.72$ Hz, 1H), 3.42 (s, 1H), 2.38-2.26 (m, 2H), 1.86 (s, 1H), 1.51 (d, $J = 16.49$ Hz, 3H), 1.39 (d, $J = 11.19$ Hz, 13H); ^{13}C NMR (CDCl_3 , 100MHz) δ ppm: 156.3, 138.0, 134.3, 133.9, 128.5, 128.3, 117.9, 75.8, 71.4, 68.4, 56.7, 38.3, 38.1, 30.9, 28.5, 28.4.

(S)-tert-butyl 4-((R,E)-2-(benzyloxy)-6-ethoxy-6-oxohex-4-en-1-yl)-2,2-dimethyl oxazolidine-3-carboxylate (S8)



To the above olefin compound **F21.2** (0.5 g 1.33 mmol) was taken in a dry round bottom flask, equipped with magnetic stirrer, 5mL of dichloromethane, Ethyl acrylate (2 mmol), and (5 mol%) grubs II catalyst were added. Resulted reaction mixture was refluxed at 40 °C temperature for about 2 hours, after completion of the reaction monitored by TLC, cooled to room temperature and concentrated on rotavapor. Obtained crude was purified by column chromatography method (10% ethyl acetate/hexanes) to get pure product **S8** (0.48 g) as a colour less oil.

Molecular Formula: $\text{C}_{25}\text{H}_{37}\text{NO}_6$

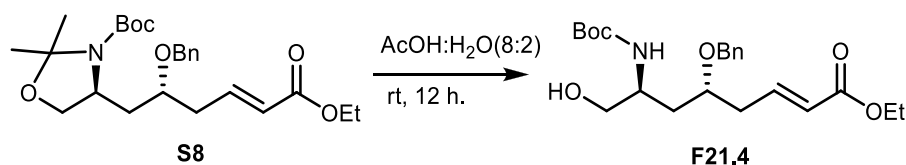
$R_f = 0.3$ (10% ethyl acetate/hexanes)

LRMS: (ES+) $m/z = 448.2$ [M+1]

Yield: 81%

^1H NMR (CDCl_3 , 400 MHz) δ ppm: 7.36-7.28 (m, 5H), 6.99-6.92 (m, 1H), 5.90 (d, $J = 15.65$ Hz, 1H), 4.60 (d, $J = 11.15$ Hz, 1H), 4.45 (d, $J = 11.15$ Hz, 1H), 4.19 (q, $J = 7.15$ Hz, 2H), 3.76-3.74 (m, 1H), 3.71-3.65 (m, 1H), 3.60 (dd, $J = 11.17, 3.74$ Hz, 1H), 3.54 (dd, $J = 11.16, 5.08$ Hz, 1H), 2.63-2.45 (m, 3H), 2.17 (s, 6H), 1.75-1.69 (m, 2H), 1.43 (s, 9H), 1.29 (t, $J = 7.24$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100MHz) δ ppm: 206.9, 166.1, 143.9, 137.5, 128.6, 128.5, 128.1, 128.1, 128.0, 124.2, 110.0, 79.8, 75.7, 71.1, 66.1, 60.3, 51.3, 36.3, 35.8, 30.9, 28.3, 14.2.

(5R,7S,E)-ethyl 5-(benzyloxy)-7-((tert-butoxycarbonyl)amino)-8-hydroxyoct-2-enoate (F21.4)



To the above cross metathesis compound **S8** (0.5 g, 1.11 mmol), were added (8:2) acetic acid and water at cool condition the resulted reaction mixture was stirred at room temperature for about 12 hours. After completion of reaction monitored by TLC, solvent was removed and it was quenched with a saturated aqueous solution of sodium hydrogen bicarbonate (5 mL) extracted with ethyl acetate (3x20 mL). Dried over anhydrous sodium sulfate and concentrated in vacuum to get crude product, purification was done by column chromatography, eluted with 40% ethyl acetate and hexane offered **F21.4**, (0.364 g) as colour less liquid.

Molecular Formula: C₂₂H₃₃NO₆

R_f = 0.1 (40 % ethyl acetate/hexanes)

LRMS: (ES⁺) *m/z* = 408.2[M+1]

Yield: 80%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.37-7.26 (m, 5H), 6.95 (td, *J* = 15.18, 7.40 Hz, 1H), 5.89 (d, *J* = 15.66 Hz, 1H), 5.12 (s, 1H), 4.58 (d, *J* = 11.19 Hz, 1H), 4.44 (d, *J* = 11.18 Hz, 1H), 4.18 (q, *J* = 7.13 Hz, 2H), 3.77-3.63 (m, 2H), 3.61-3.49 (m, 2H), 2.76 (s, 1H), 2.62-2.52 (m, 1H), 2.52-2.43 (m, 1H), 1.72 (dd, *J* = 8.93, 6.26 Hz, 2H), 1.42 (s, 9H), 1.28 (t, *J* = 7.13 Hz, 3H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 166.2, 144.1, 137.5, 128.5, 128.0, 127.9, 124.1, 71.1, 60.3, 51.2, 36.3, 35.7, 28.3, 28.3, 14.2.

(5R,7S,E)-ethyl5-(benzyloxy)-7-((tert-butoxycarbonyl)amino)-8-((tert butyl dimethyl silyl)oxy)oct-2-enoate (S9)



To the compound **F21.4** (0.5 g, 1.23 mmol) in dry DCM (5mL) were added imidazole (0.167 g, 2.4 mmol), TBSCl (0.22 g, 1.84 mmol), at 5 °C. The reaction was continued to stir at room temperature for 5 minutes. The completion of the reaction was monitored by TLC. Reaction was quenched with the ammonium chloride and water. Extracted with

DCM (20 mL). The combined organic layers were washed with brine and dried over anhydrous sodium sulfate. Purified by column chromatography, eluted with 5% ethyl acetate and hexane to get pure product **S9** (0.588 g) as a colour less liquid.

Molecular Formula: C₂₈H₄₇NO₆Si

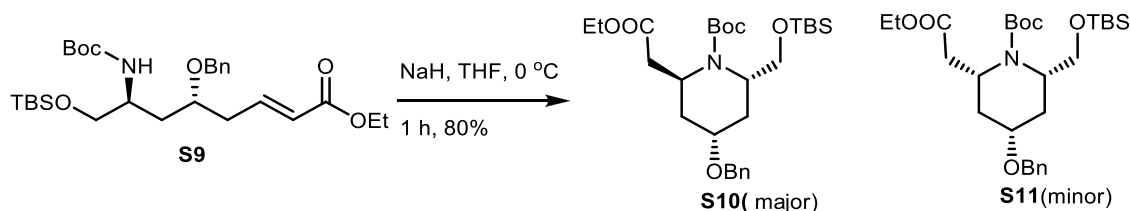
R_f = 0.2 (10% ethyl acetate/hexanes)

LRMS: (ES⁺) *m/z* = 522.3[M+1]

Yield: 92%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.36-7.24 (m, 5H), 7.02-6.91 (m, 1H), 5.89 (d, *J* = 15.67 Hz, 1H), 4.77 (d, *J* = 7.19 Hz, 1H), 4.56 (d, *J* = 11.43 Hz, 1H), 4.45 (d, *J* = 11.43 Hz, 1H), 4.17 (q, *J* = 7.12 Hz, 2H), 3.72 (s, 1H), 3.62-3.54 (m, 1H), 3.54-3.44 (m, 2H), 2.60-2.44 (m, 2H), 1.72 (t, *J* = 6.61 Hz, 2H), 1.42 (s, 9H), 1.28 (t, *J* = 7.12 Hz, 3H), 0.86 (s, 9H), 0.01 (d, *J* = 2.81 Hz, 6H); **¹³C NMR** (CDCl₃, 100MHz); δ ppm: 166.2, 155.5, 144.7, 138.1, 128.5, 128.4, 128.3, 127.7, 123.9, 79.0, 79.0, 75.2, 71.9, 71.8, 65.3, 60.2, 49.0, 37.2, 36.8, 28.4, 28.4, 25.9, 25.6, 18.3, 14.3, -3.6, -5.4.

(2S,4R,6S)-tert-butyl-4-(benzyloxy)-2-(((tert-butyldimethylsilyl)oxy)methyl)-6-(2-ethoxy-2-oxoethyl)piperidine-1-carboxylate (S10, S11)



To a stirred solution of **S9** (0.5 g, 0.959 mmol) in THF (5 mL) was added NaH (44 mg, 1.9 mmol) at 0 °C, and the mixture was stirred at same temperature for 1 hour. After being quenched with addition of cold aqueous NH₄Cl, the resulting mixture was extracted with ethyl acetate. The extracts were washed successively with water, brine, dried and concentrated. The residue was purified by chromatography on silica gel with 5% of ethyl acetate and hexane, give a diastereomeric mixture of (2:1) products **S10** and **S11** (416 mg) as a colourless liquid.

Molecular Formula: C₂₈H₄₇NO₆Si

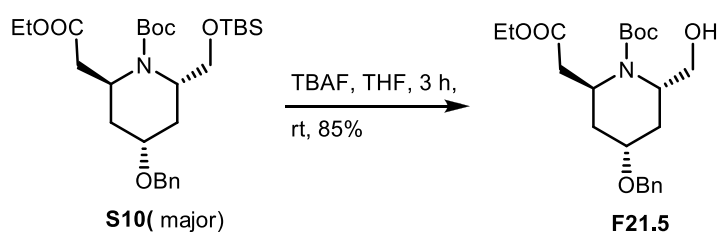
R_f = 0.4 (10% ethyl acetate/hexanes)

LRMS: (ES⁺) *m/z* = 522.3[M+1]

Yield: 80%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.33 (d, *J* = 4.43 Hz, 4H), 7.31-7.26 (m, 1H), 4.72 (d, *J* = 2.05 Hz, 1H), 4.55 (s, 2H), 4.36-4.27 (m, 1H), 4.13 (q, *J* = 7.14 Hz, 2H), 3.84 (dd, *J* = 15.52, 6.78 Hz, 1H), 3.51 (dd, *J* = 9.80, 6.06 Hz, 2H), 2.46 (d, *J* = 6.61 Hz, 2H), 2.37-2.30 (m, 1H), 2.05 (d, *J* = 12.97 Hz, 1H), 1.63-1.54 (m, 1H), 1.50 (dd, *J* = 12.66, 7.25 Hz, 1H), 1.45 (s, 9H), 1.26 (t, *J* = 7.14 Hz, 3H), 0.89 (s, 9H), 0.05 (s, 6H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 171.1, 154.6, 138.5, 128.4, 128.4, 127.6, 80.1, 70.0, 68.4, 64.4, 60.5, 34.0, 30.8, 28.4, 28.3, 25.9, 25.6, 18.2, 14.2, -5.4, -5.5.

(2S,4R,6S)-tert-butyl4-(benzyloxy)-2-(2-ethoxy-2-oxoethyl)-6-(hydroxymethyl) piperidine-1-carboxylate (F21.5)



To a stirred solution of **S10** (0.4 g, 0.767 mmol) in THF (8 mL), was added TBAF (0.3 g, 1.15 mmol). Resulted solution was stirred for 3 hours, reaction quenched by adding (10 mL) of saturated NaHCO₃ aqueous solution and extracted with ethyl acetate (15 mL) combined organic layers were dried over anhydrous sodium sulfate, concentrated and purified by column chromatography with 30% ethyl acetate and hexane to get pure compound **F21.5**, (265 mg) as a colour liquid.

Molecular Formula: C₂₂H₃₃NO₆

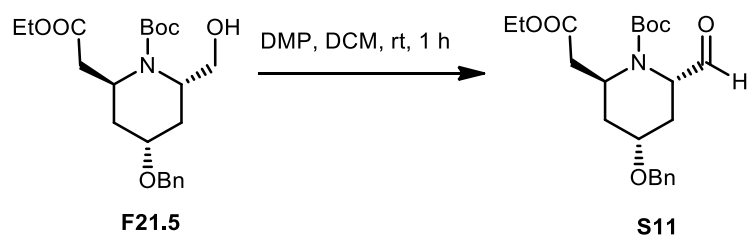
R_f = 0.2 (30% ethyl acetate/hexanes)

LRMS: (ES⁺) *m/z* = 408.23[M+1]

Yield: 85%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.37-7.25 (m, 5H), 4.81 (dd, *J* = 13.33, 6.63 Hz, 1H), 4.58-4.47 (m, 3H), 4.20-4.07 (m, 2H), 3.82-3.74 (m, 1H), 3.62 (s, 1H), 3.60 (s, 1H), 2.53 (dd, *J* = 13.63, 7.73 Hz, 1H), 2.44 (dd, *J* = 13.67, 7.69 Hz, 1H), 2.07 (d, *J* = 13.21 Hz, 1H), 2.04-1.97 (m, 1H), 1.71-1.63 (m, 1H), 1.63-1.55 (m, 1H), 1.45 (s, 9H), 1.28 (t, *J* = 7.15 Hz, 3H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 155.8, 138.2, 128.4, 127.7, 127.6, 80.5, 70.2, 68.5, 65.5, 61.1, 52.6, 48.0, 40.6, 34.7, 31.5, 28.3, 28.2, 14.1 1.0.

(2S,4R,6S)-tert-butyl4-(benzyloxy)-2-(2-ethoxy-2-oxoethyl)-6-formylPiperidine-1-carboxylate (S11)



To a stirred solution of **F21.5** (250 mg, 0.614 mmol) in 3 mL of dry DCM at 0 °C, Dess-Martin periodinane (390 mg, 0.921 mmol) was added under N₂ atmosphere. After 2 hours, reaction quenched with 5 mL of sat. aq. solution of sodium thio sulfate and sodium bicarbonate, extracted with DCM (2x5 mL), combined organic layer dried over anhydrous sodium sulfate, concentrated to get the crude aldehyde, which was purified by flash column chromatography with 20% ethyl acetate and hexanes gave **S11**, (199 mg) as colour les liquid.

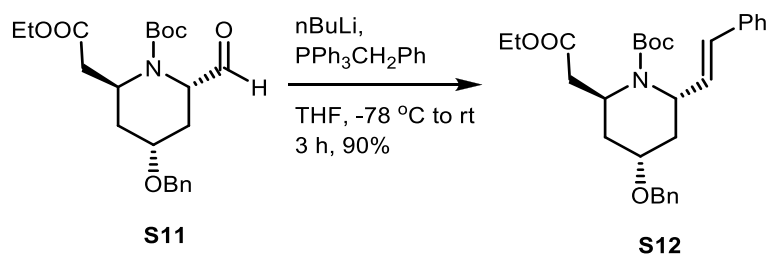
Molecular Formula: C₂₂H₃₁NO₆

R_f = 0.2 (20% ethyl acetate/hexanes)

LRMS: (ES⁺) *m/z* = 406.22[M+1]

Yield: 80%

(2S,4R,6S)-tert-butyl 4-(benzyloxy)-2-(2-ethoxy-2-oxoethyl)-6-((E)-styryl)Piperidine-1-carboxylate (S12)



Benzyl triphenyl phosphonium bromide (481 mg, 1.1 mmol) was dissolved in freshly distilled THF (3 mL) and n-BuLi (0.22 mL, 0.65 mmol, 2.5 M in hexanes) was added to the above solution at 0 °C. After 20 minutes of additional stirring at room temperature, the reaction temperature was lowered to -78 °C and the crude aldehyde **S5** (150 mg, 0.370 mmol) in THF (2 mL) was added to the above solution and stirred for 30 minutes. After 2 hours, the reaction mixture was warmed to room temperature, quenched with ethyl acetate (10 mL) and a saturated aqueous sodium bicarbonate solution (10 mL). This mixture was extracted with ethyl acetate (3 X 10 mL), washed with brine (10 mL), dried over sodium sulfate, filtered, concentrated under reduced pressure and purified through

column chromatography (10% ethyl acetate in hexanes) to give **S12** (160 mg) as a pale-yellow oil.

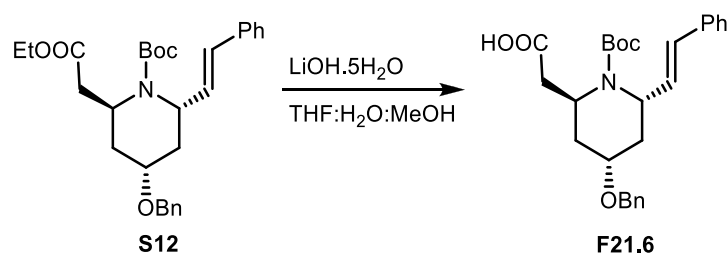
Molecular Formula: C₂₉H₃₇O₅

R_f = 0.2 (10% ethyl acetate/hexanes)

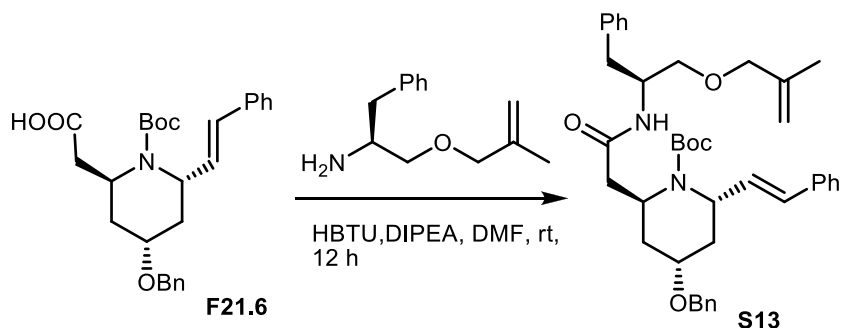
LRMS: (ES⁺) *m/z* = 480.2 [M+1]

Yield: 90%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.38-7.27 (m, 9H), 7.25-7.22 (m, 1H), 6.42 (d, *J* = 11.58 Hz, 1H), 5.70 (dd, *J* = 11.81, 9.53 Hz, 1H), 5.50-5.44 (m, 1H), 4.85-4.77 (m, 1H), 4.52 (s, 2H), 4.20-4.10 (m, 2H), 3.90-3.81 (m, 1H), 2.72-2.28 (m, 2H), 2.24-2.17 (m, 1H), 2.17-2.10 (m, 1H), 1.79-1.71 (m, 1H), 1.71-1.63 (m, 1H), 1.27 (t, *J* = 6.8 Hz, 3H), 1.21 (s, 9H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 171.0, 155.6, 154.7, 138.3, 136.4, 132.7, 129.5, 128.7, 128.4, 127.6, 127.2, 126.4, 80.1, 70.1, 68.9, 60.6, 49.4, 40.1, 39.0, 36.5, 34.0, 28.0, 14.2.



Compound **S12** (100 mg, 0.208 mmol) 5 mL (4:1:0.5) THF: H₂O: MeOH then LiOH·H₂O (43 mg, 1.04 mmol) were added and stirred for 16 hours at room temperature. Then acidified to pH 2 with 2 N HCl and extracted with ethyl acetate (2x5 mL), the combined organic layer dried over Na₂SO₄, concentrated to get the crude acid **F21.6** (84 mg, 90%), directly used in the next step without further purification.



Oven dried round bottom flask was equipped with magnetic bar and above crude Acid **F21.6** (50 mg 0.11mmol), 2 mL of DMF, were added HBTU (1.1eq) and DIEPA

(0.5eq), Aminoacid building block (2eq) at cool condition stirred at same temperature for 1h, reaction mixture was quenched with water and extracted with ethyl acetate (5mL), dried over anhydrous sodium sulfate concentrated under reduced pressure, crude product was purified by column chromatography with 40% ethyl acetate and hexane to get **S13** pure product.

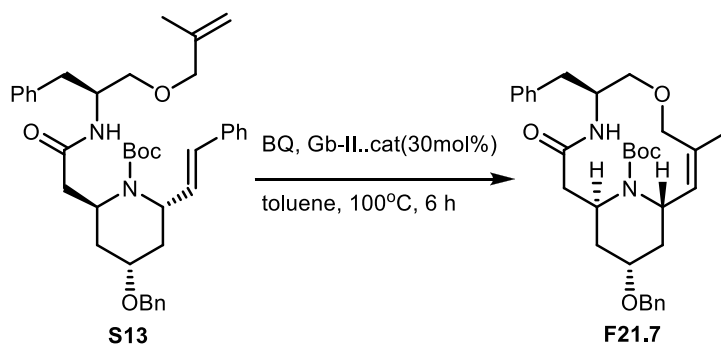
Molecular Formula: C₂₉H₅₀N₂O₅

R_f = 0.2 (10% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 639.3 [M+1]

Yield: 73%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.28-7.26 (m, 3H), 7.25-7.24 (m, 2H), 7.22-7.20 (m, 3H), 7.19-7.14 (m, 7H), 6.67-6.55 (m, 1H), 6.34 (d, *J* = 10.8 Hz, 1H), 5.55 (t, *J* = 10.8 Hz, 1H), 5.36-5.30 (m, 1H), 4.91 (s, 1H), 4.82 (s, 1H), 4.51(d, *J* = 11.2 Hz, 1H), 4.45(d, *J* = 11.2 Hz, 1H), 4.32-4.25 (m, 2H), 3.86-3.77 (m, 3H), 3.32 (d, *J* = 2.71 Hz, 2H), 2.91-2.76 (m, 3H), 2.48-2.41 (m, 2H), 2.30-2.25 (m, 1H), 2.13-2.01 (m, 3H), 1.58-1.52 (m, 1H), 1.40-1.35 (m, 1H), 1.10 (s, 9H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 166.8, 155.4, 142.0, 138.4, 138.0, 137.4, 136.3, 132.2, 129.6, 129.4, 128.7, 128.4, 128.4, 127.6, 127.5, 127.2, 126.4, 112.3, 80.4, 75.1, 70.2, 68.8, 50.1, 49.6, 37.6, 36.6, 33.7, 29.7, 28.0, 24.8, 22.7, 19.5.



To a solution of above diene **S13** (1eq) and 1,4-benzoquinone (0.63eq) in degassed toluene (5.2 mL) at 100 °C was added a solution of G-II (0.3eq) in degassed toluene (2.3 mL) over a period of 6 h. The resultant solution was stirred at 100 °C for 16 h, cooled to room temperature. The reaction mixture was concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel with 20-30% ethyl acetate/hexanes to get (**F21.7**). But we didn't get our desired product.

2.18 References

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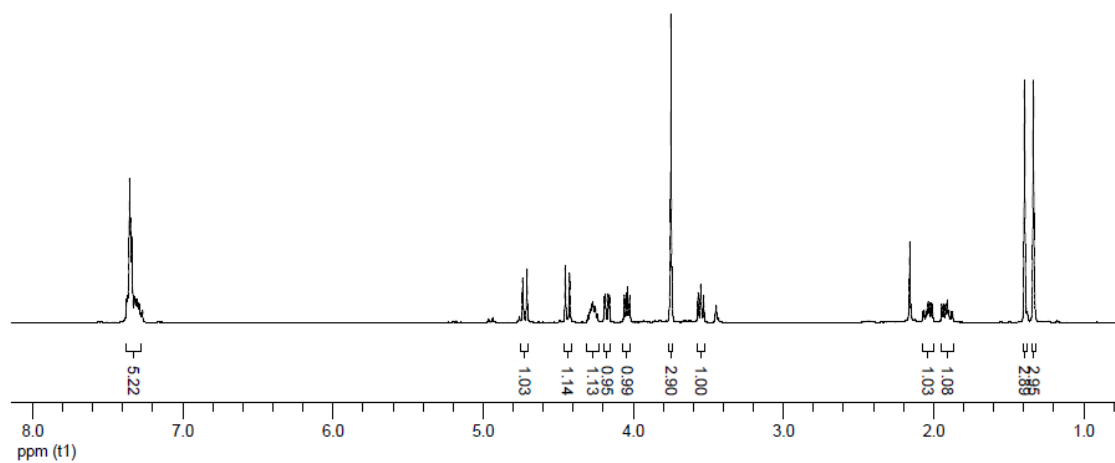
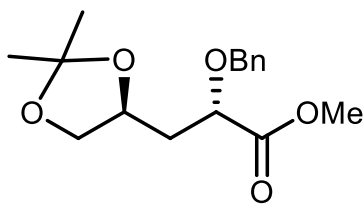
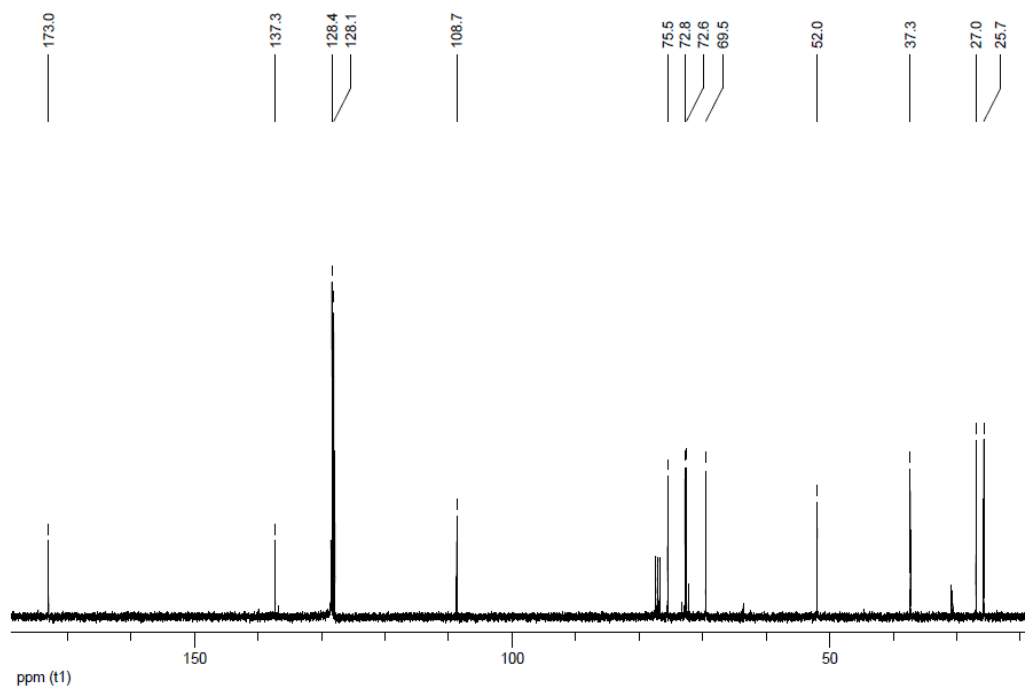
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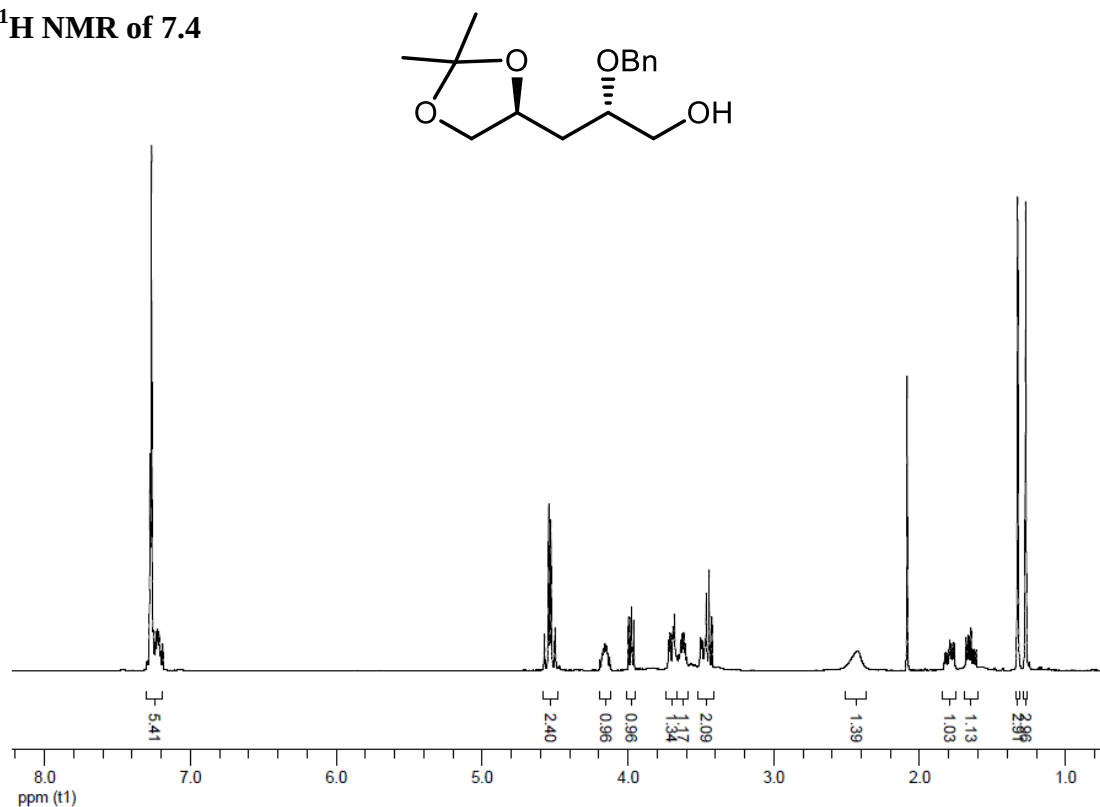
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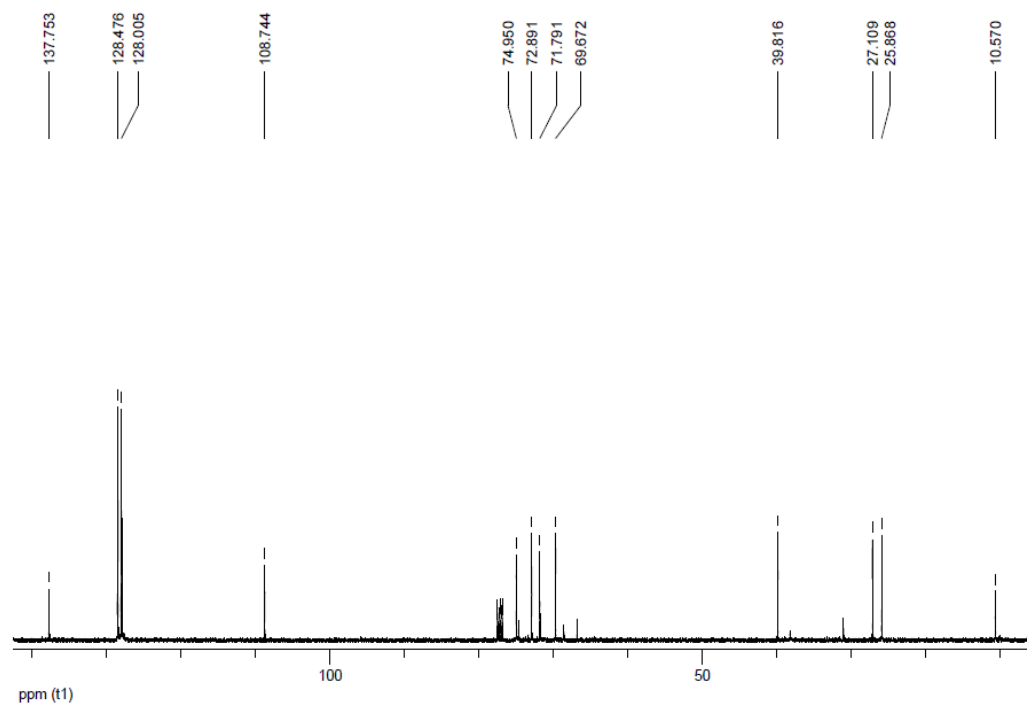
2.19 Spectral Data

 ^1H NMR of S2 ^{13}C NMR of S2

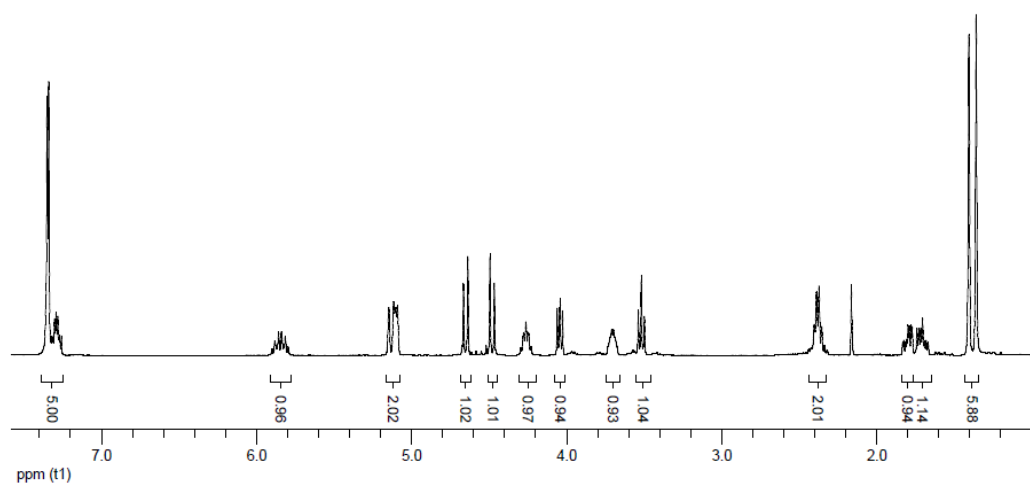
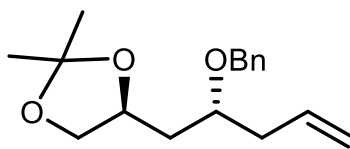
¹H NMR of 7.4



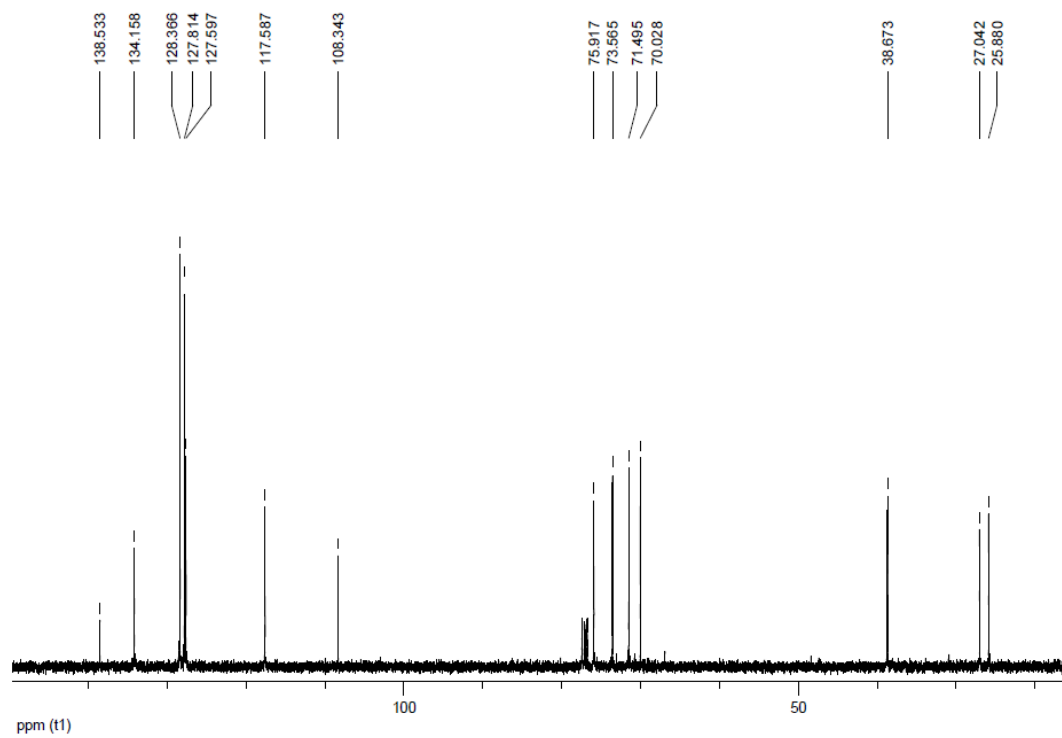
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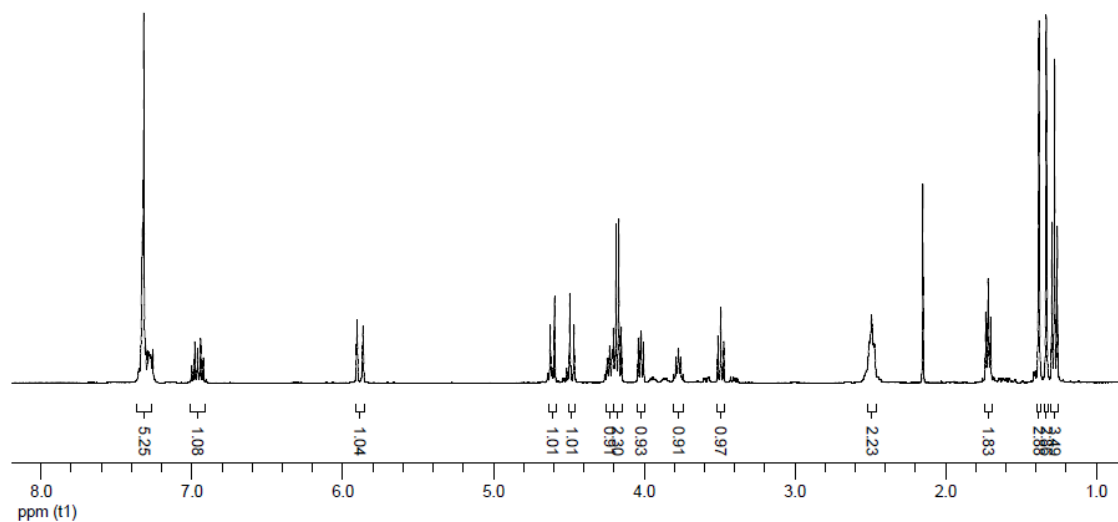
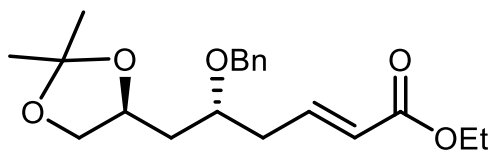
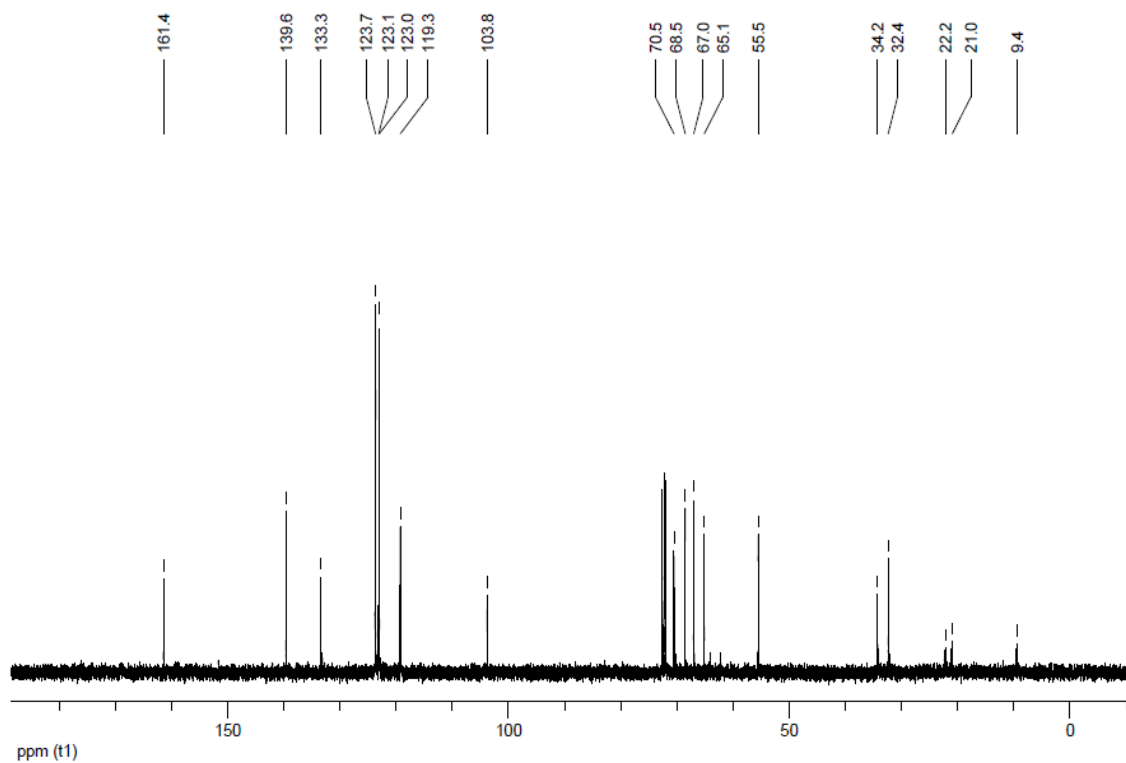


¹H NMR of 7.5

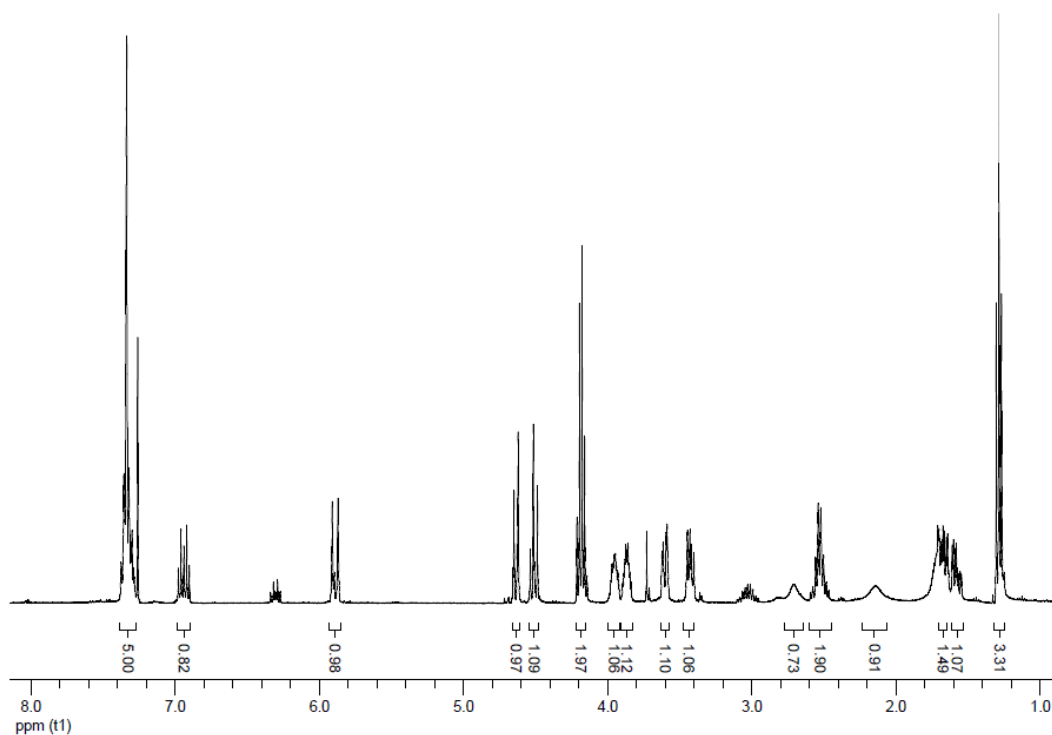
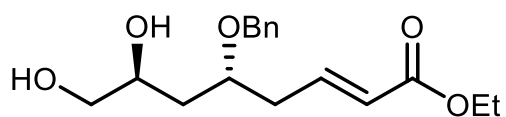


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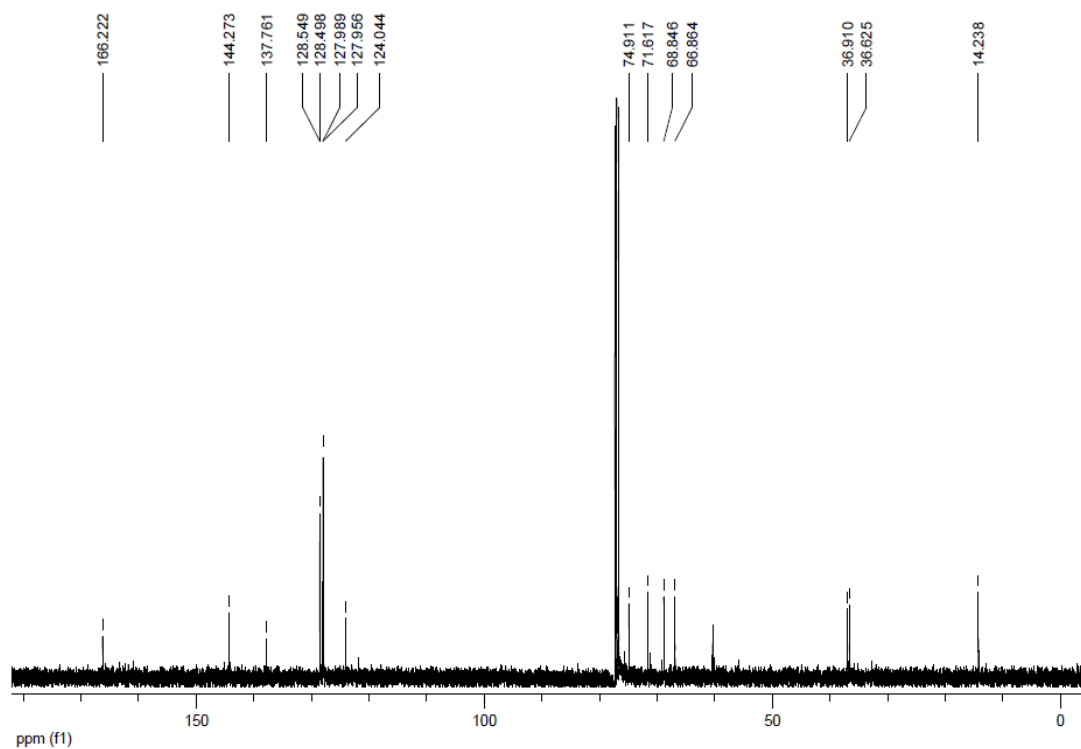


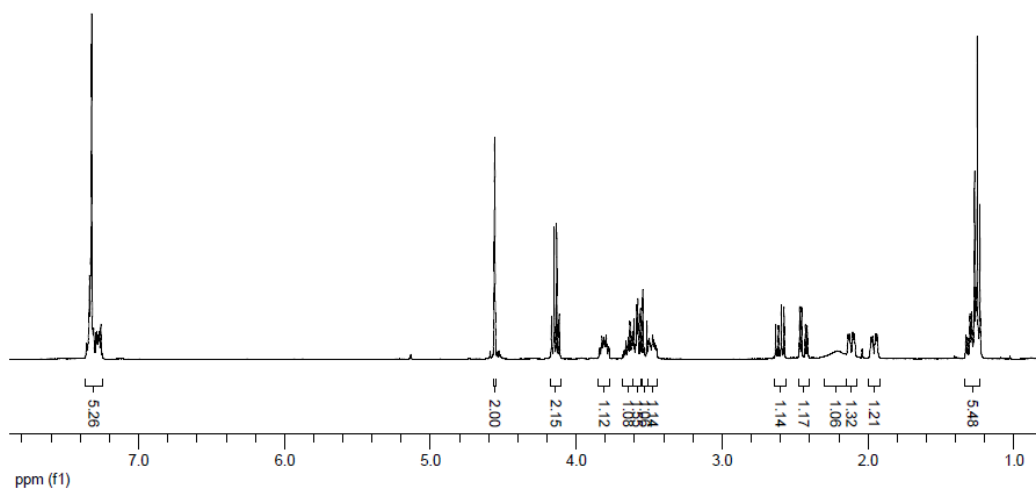
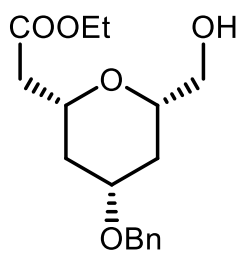
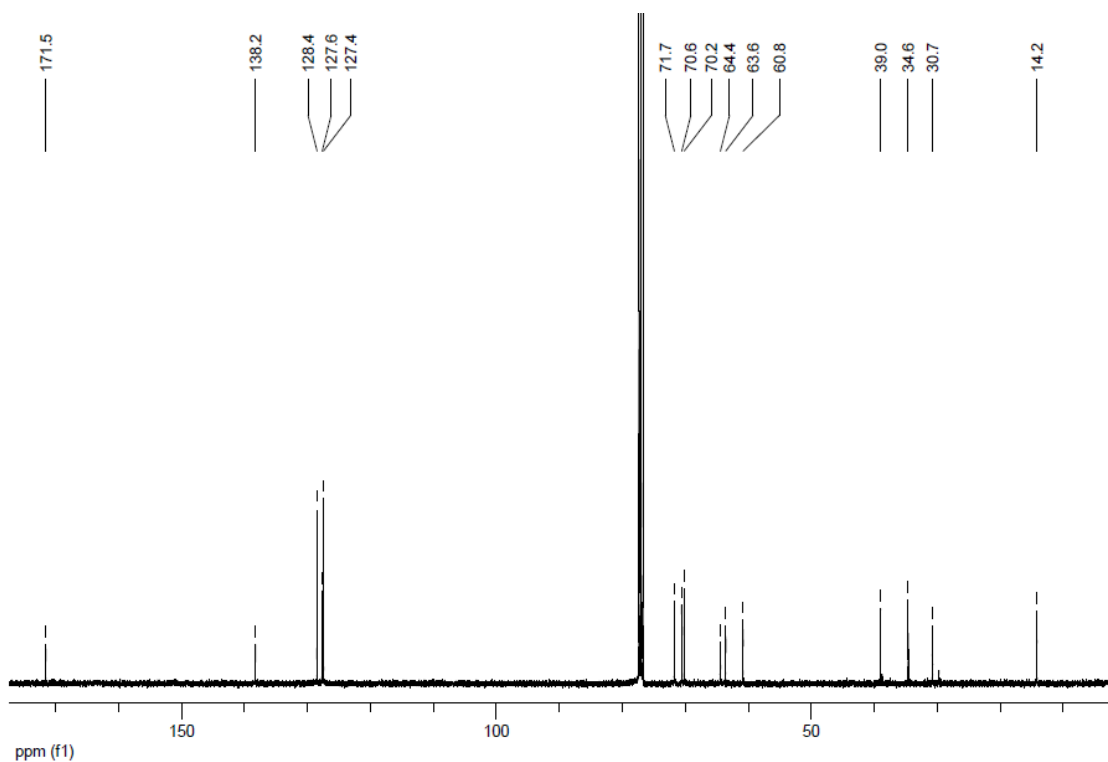
^1H NMR of S4 **^{13}C NMR of S4**

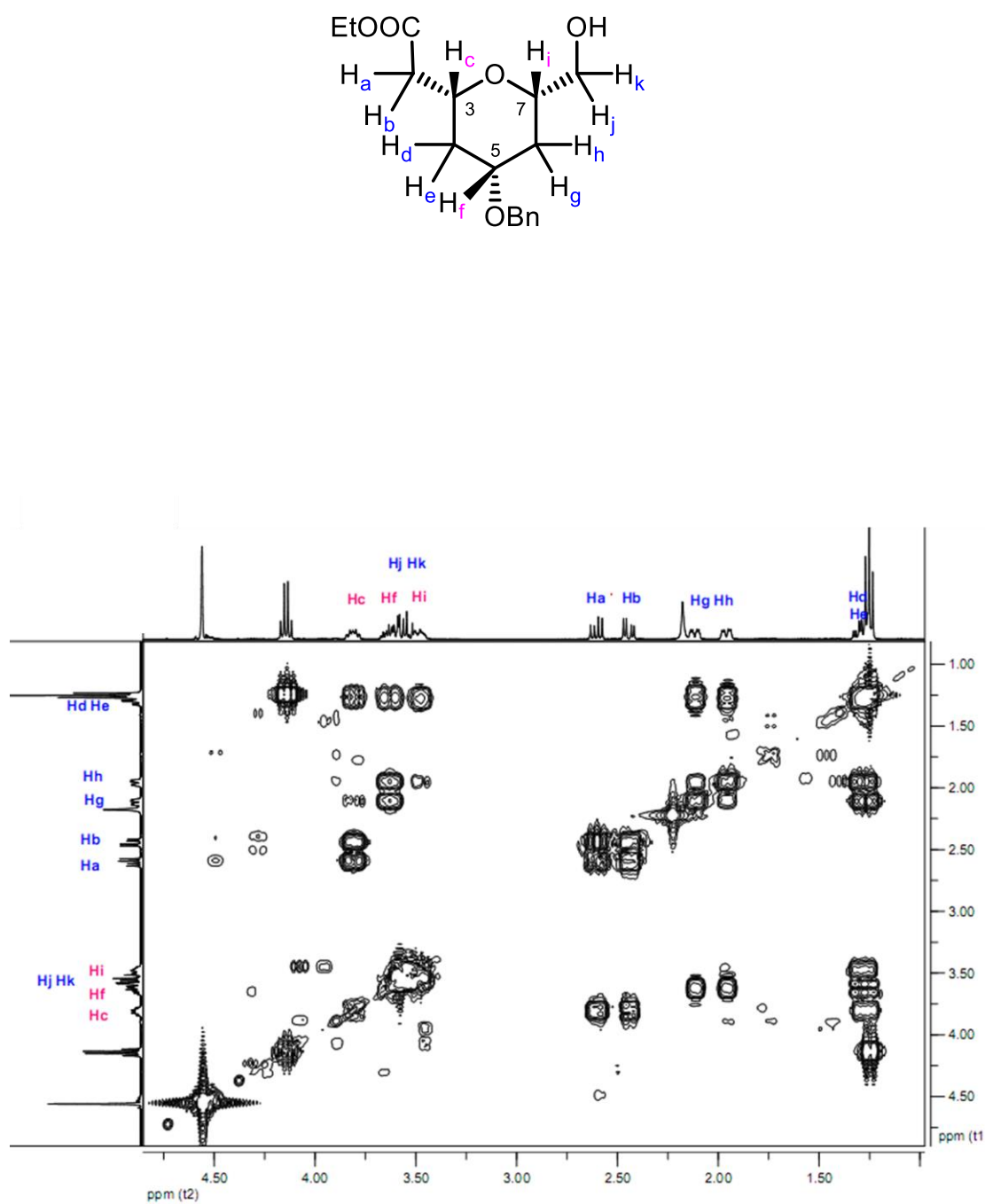
¹H NMR of 7.6



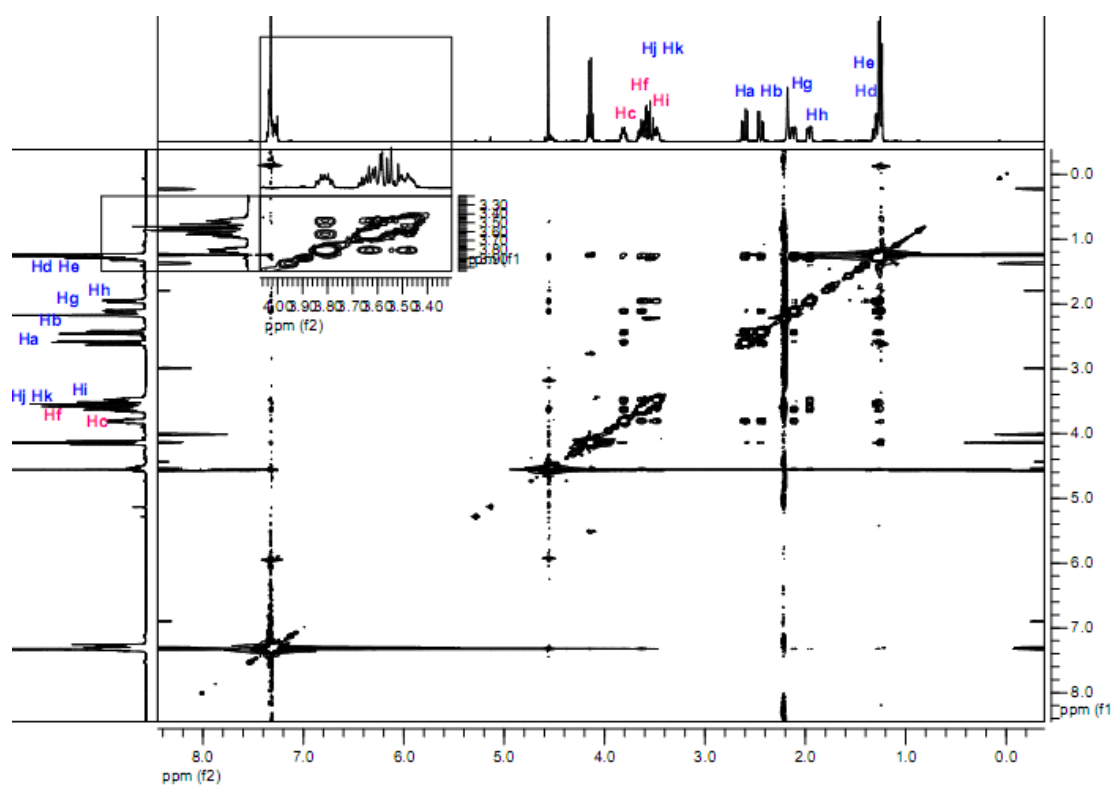
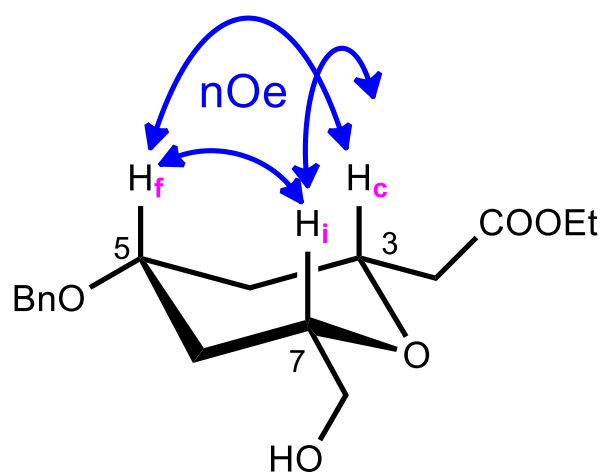
¹³C NMR of 7.6



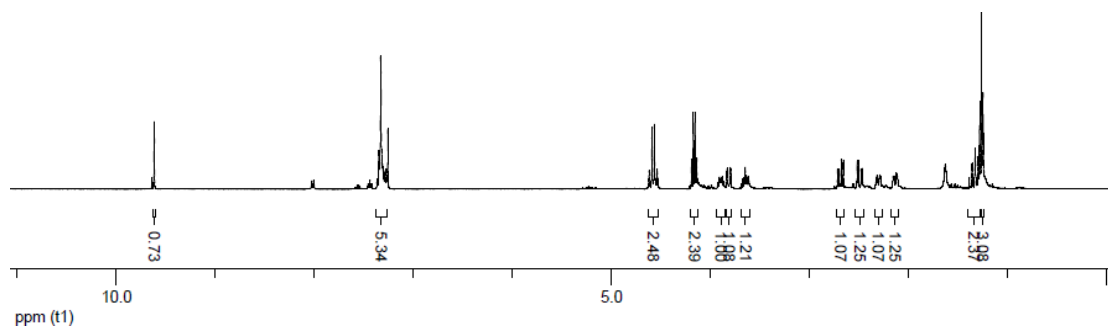
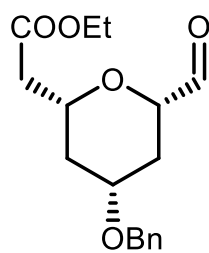
^1H NMR of 7.7 **^{13}C NMR of 7.7**

^1H - ^1H COSY of the Major Isomer


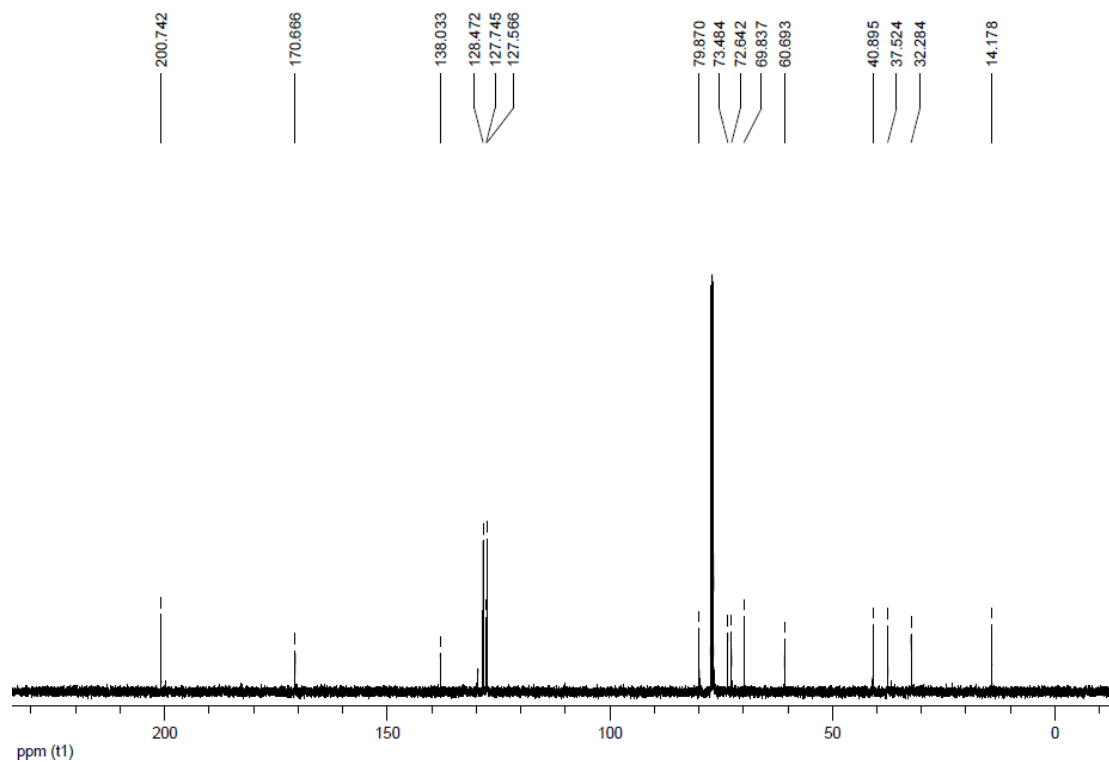
2D-NOESY of the Major Isomer

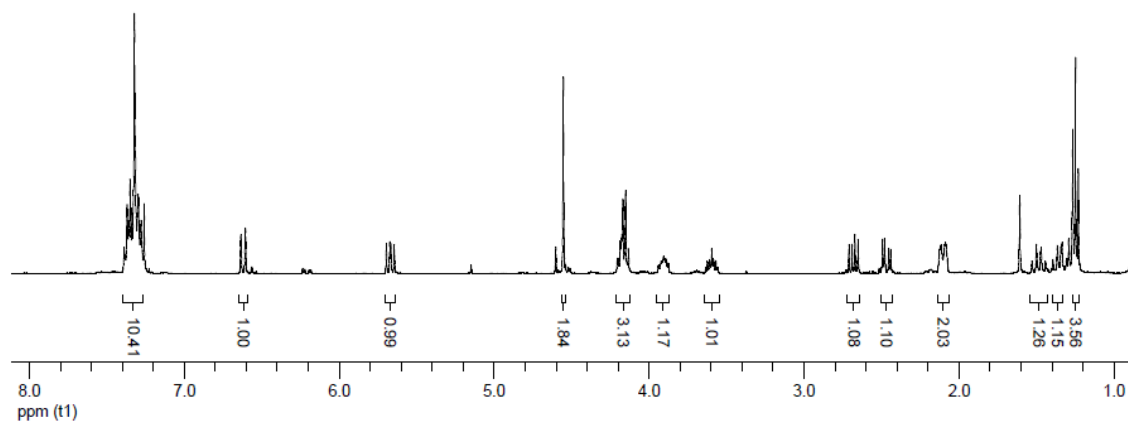
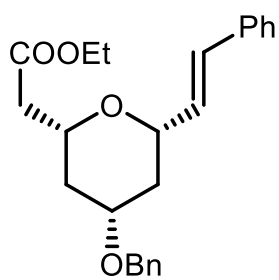
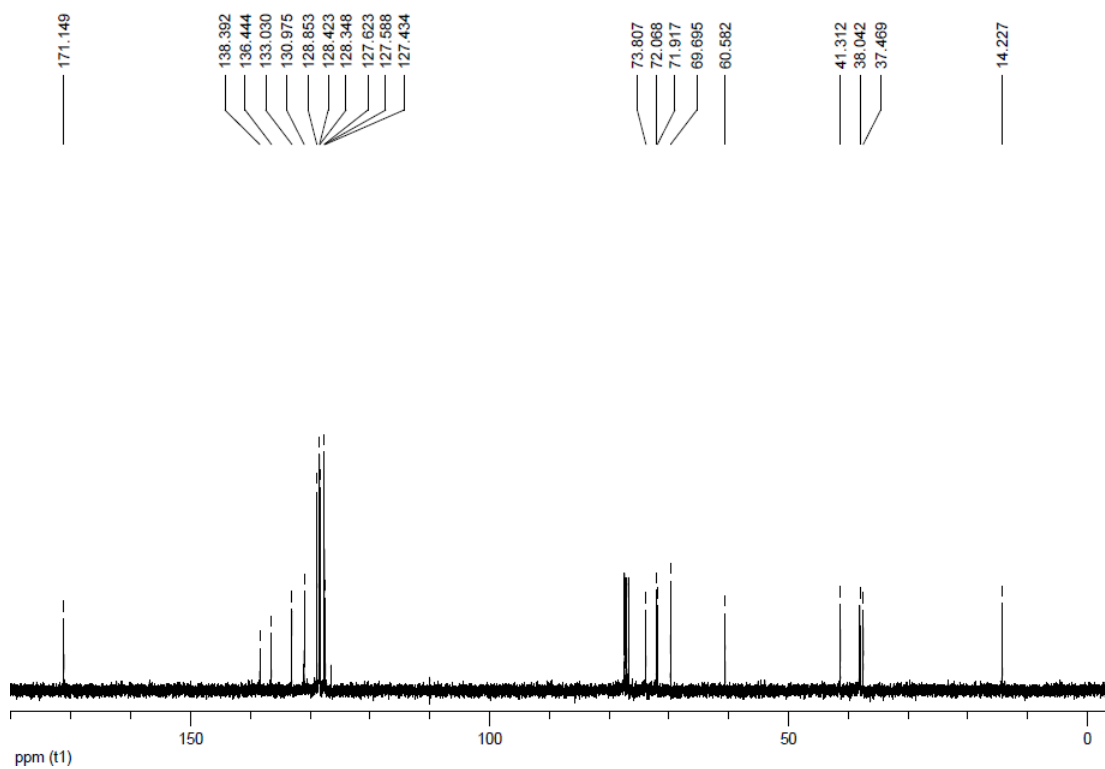


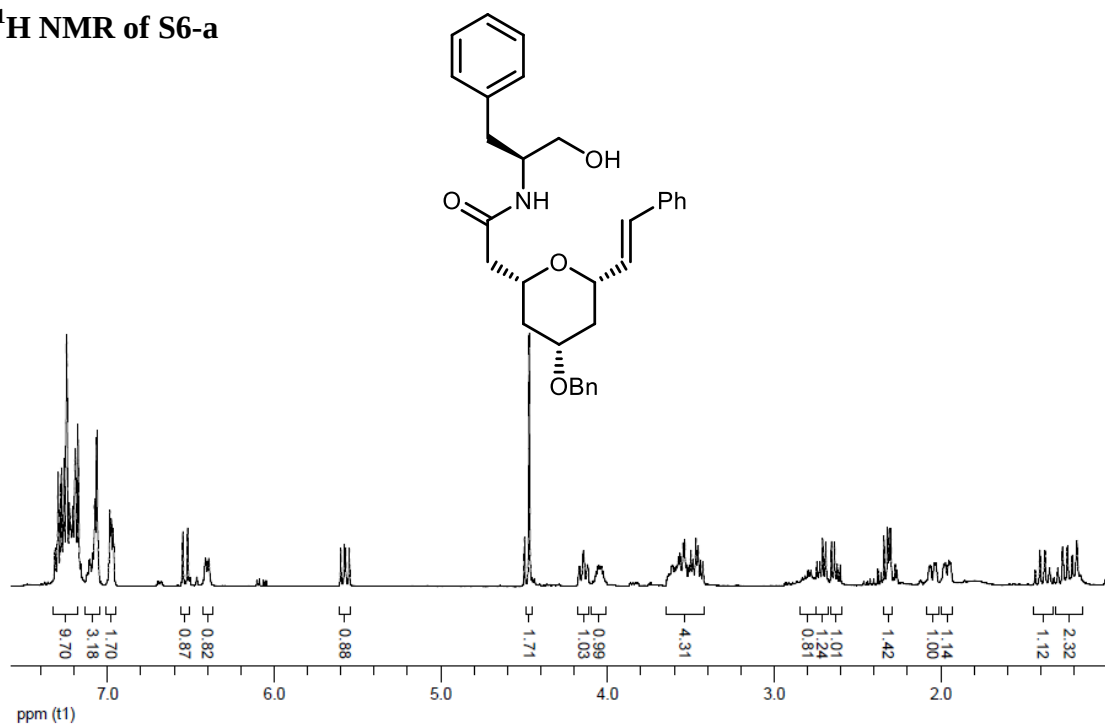
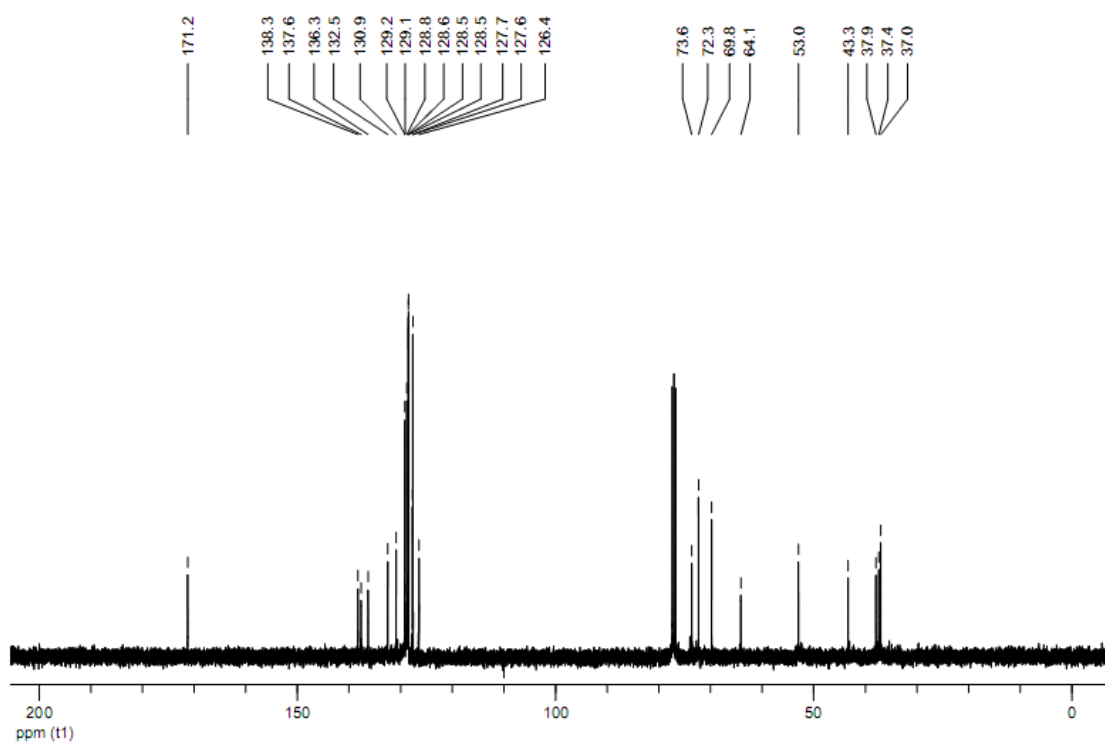
¹H NMR of S5



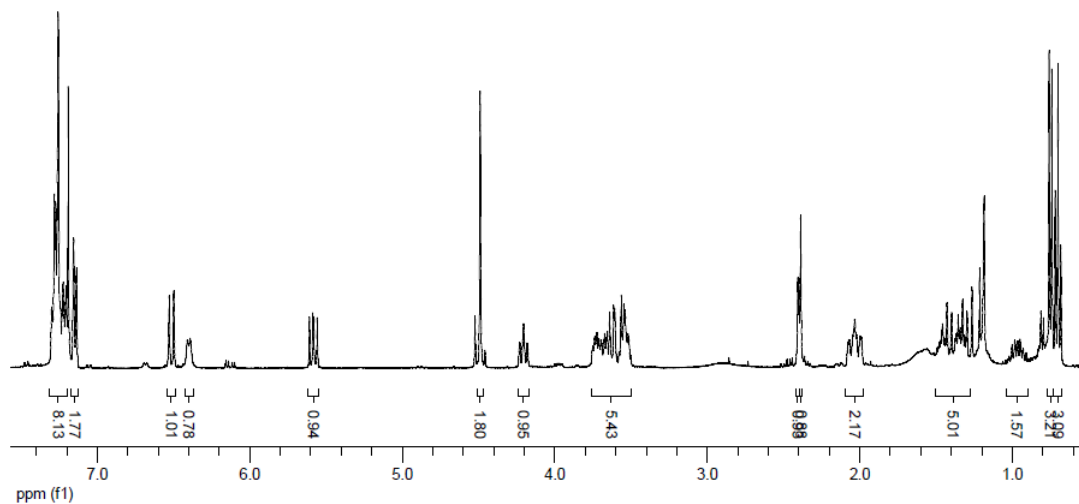
¹³C NMR of S5



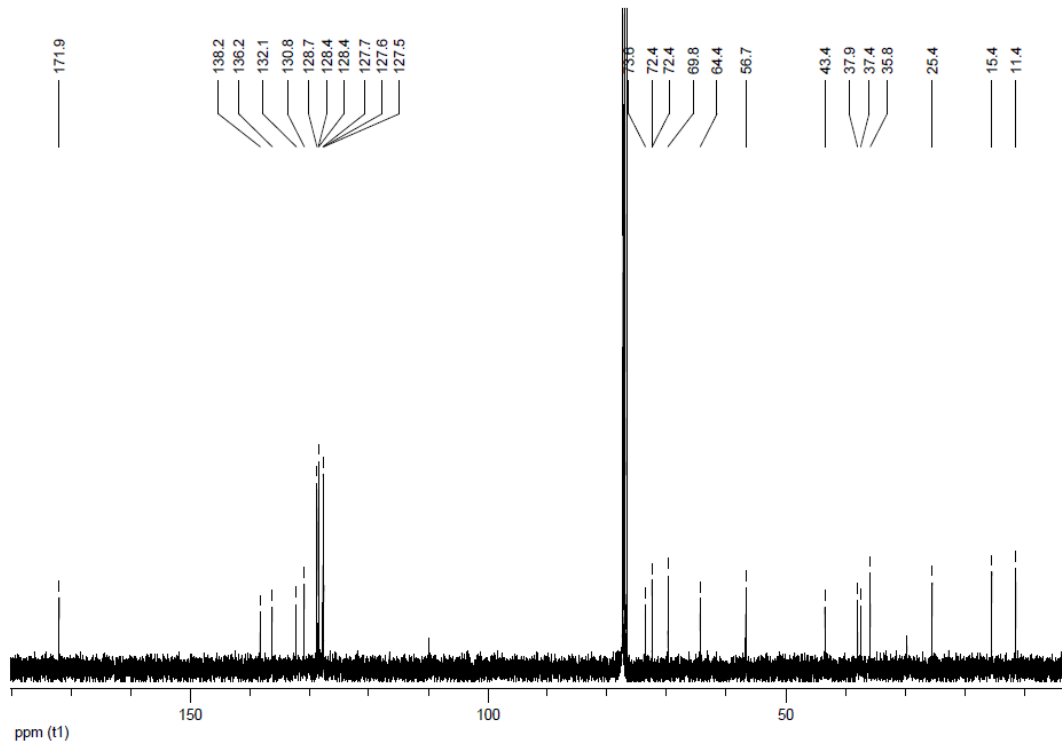
^1H NMR of 7.9 **^{13}C NMR of 7.9**

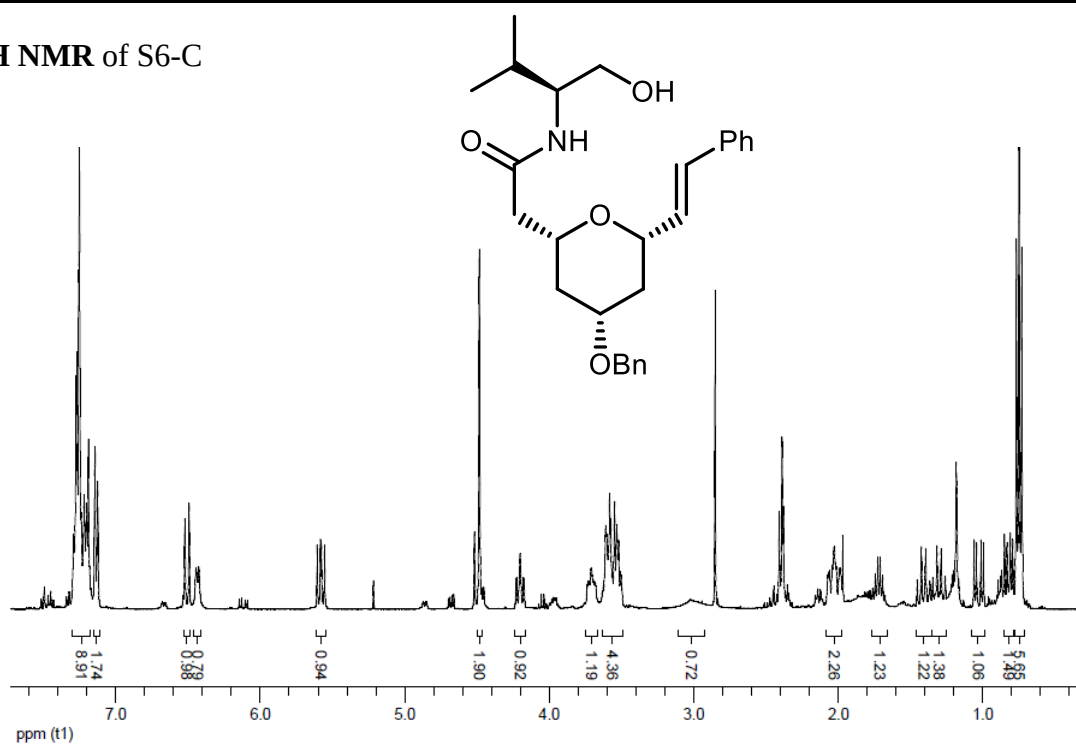
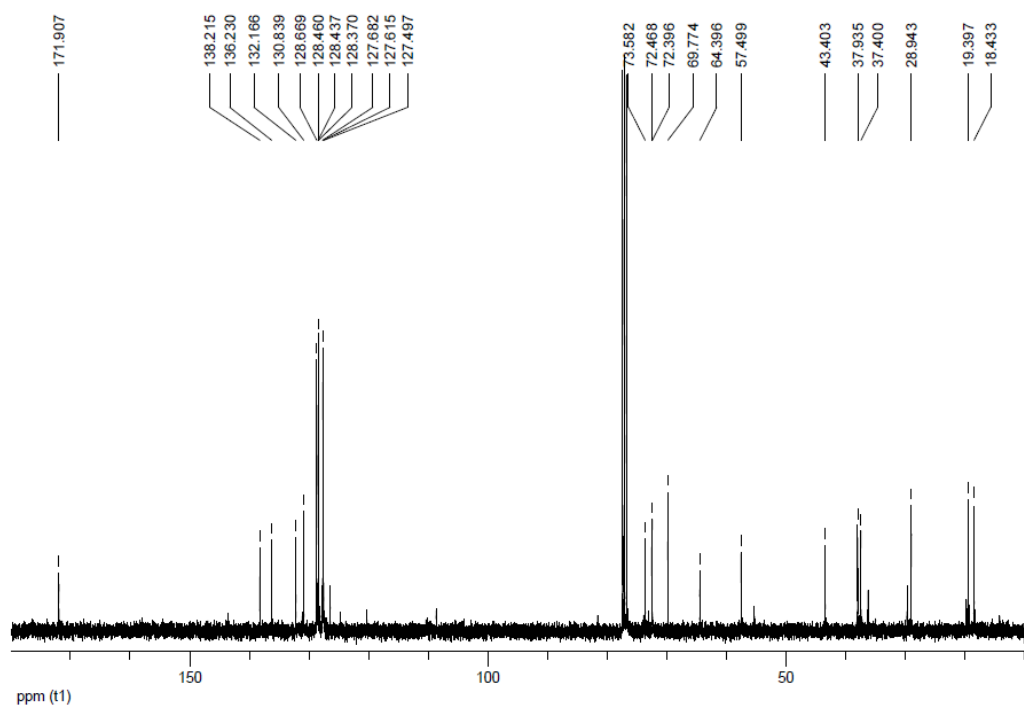
¹H NMR of S6-a

¹³C NMR of S6-a


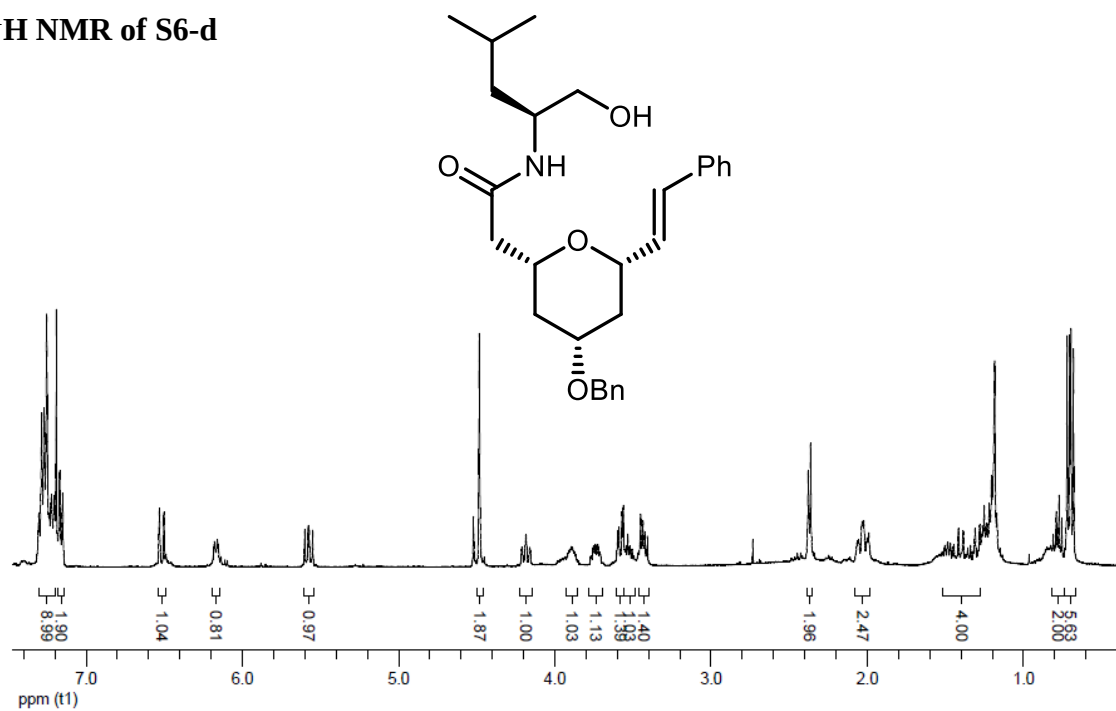
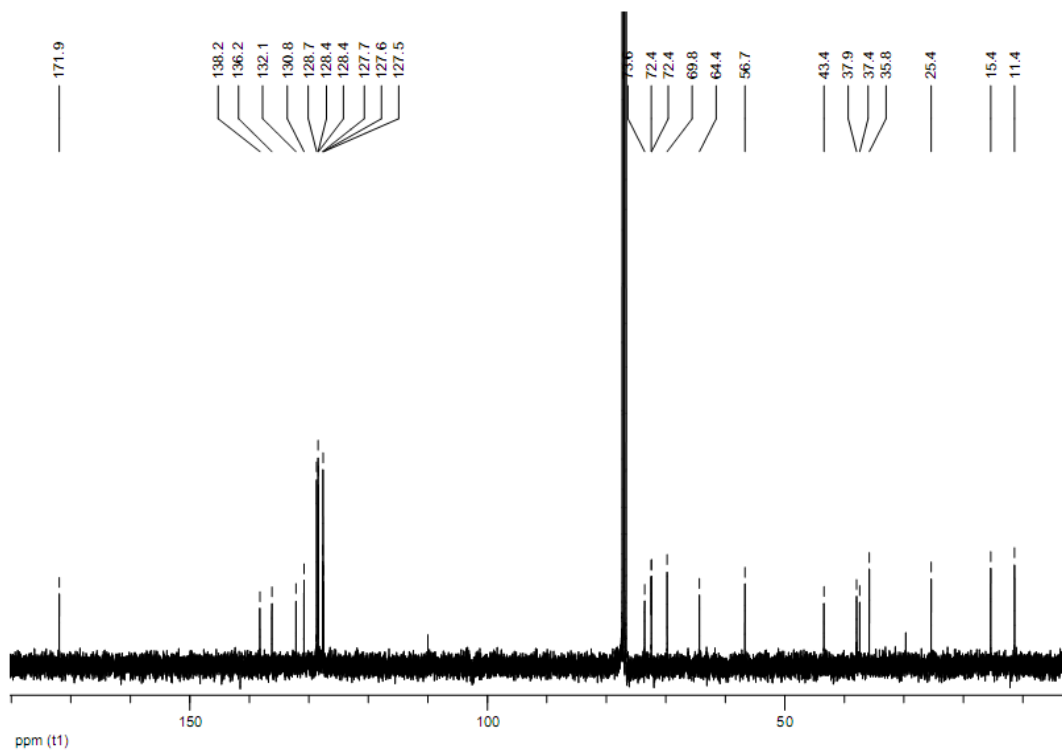
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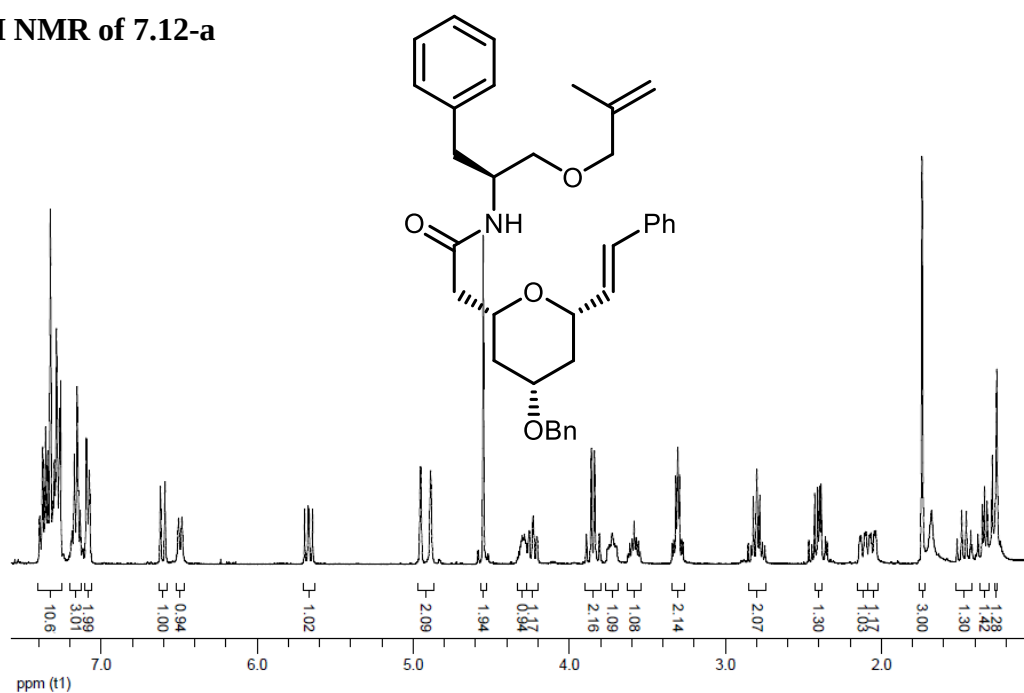
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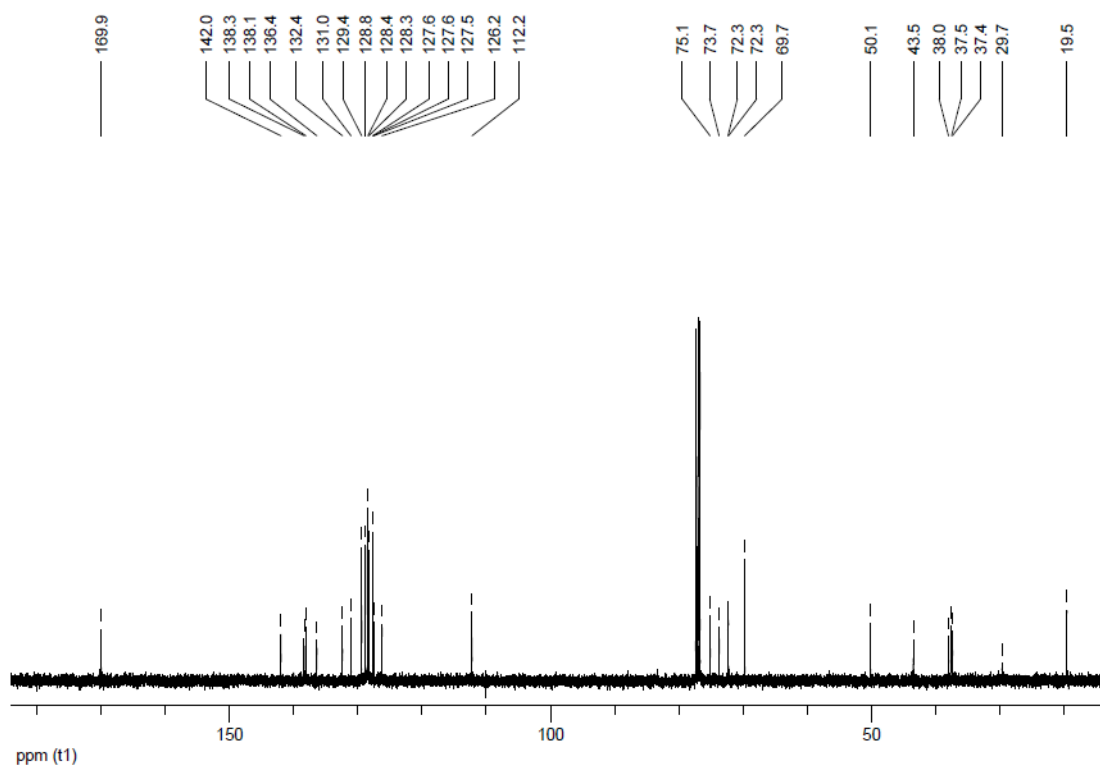
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¹³C NMR of S6-C


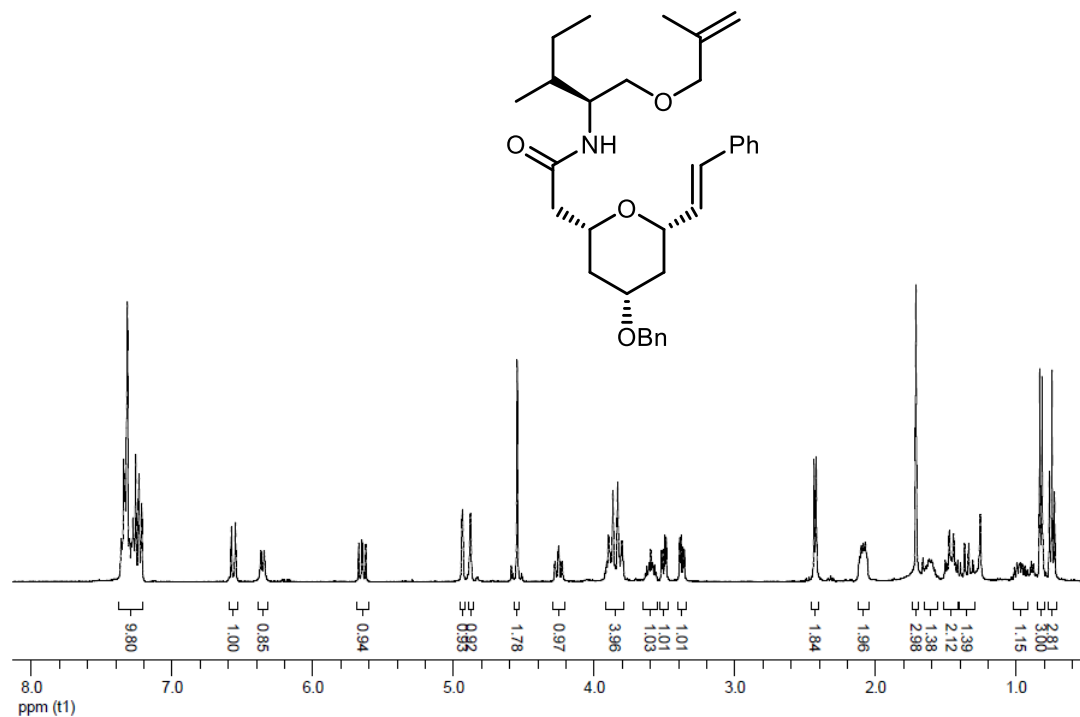
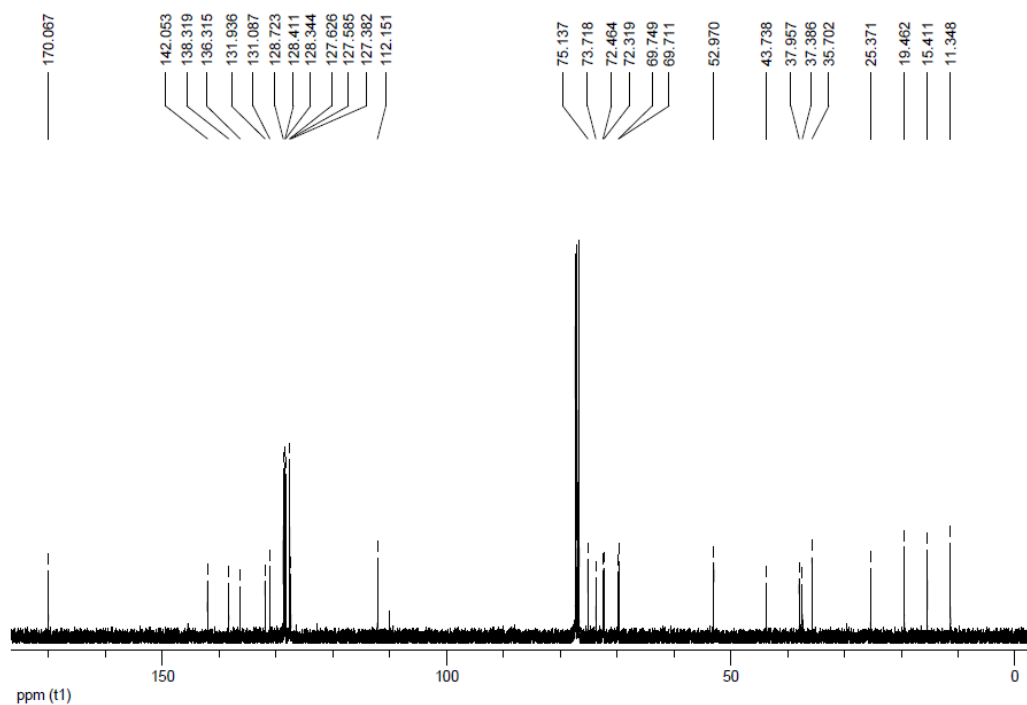
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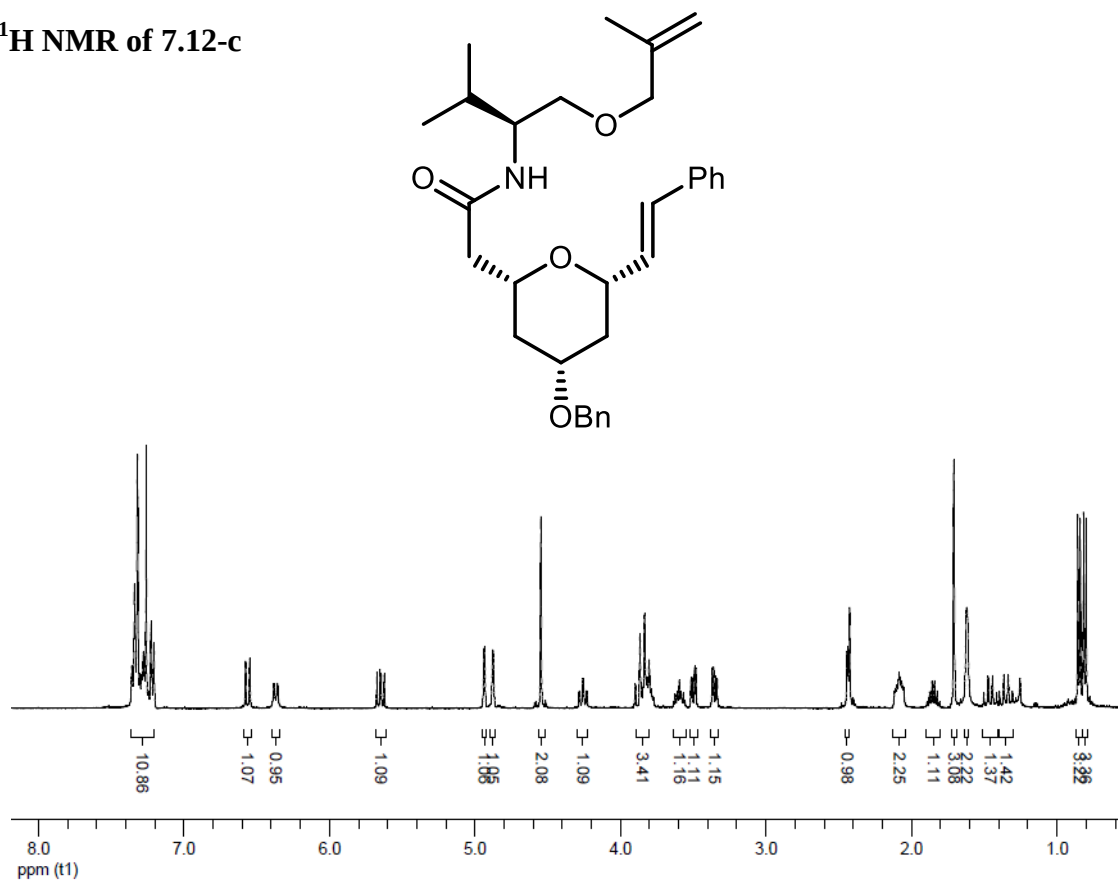
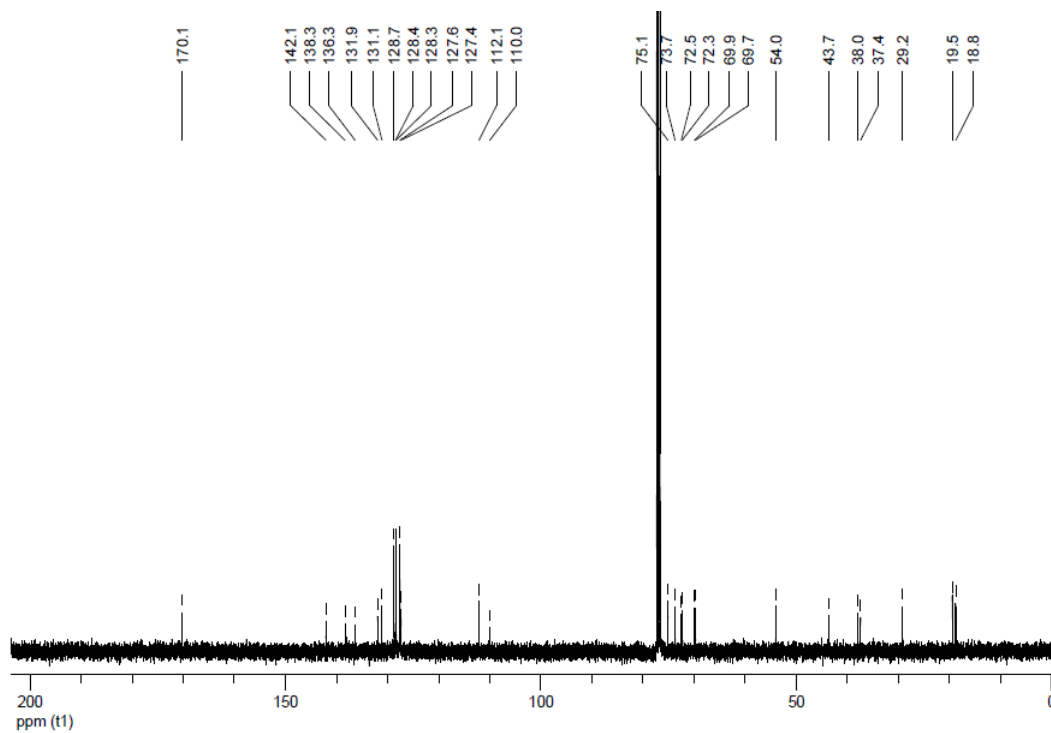
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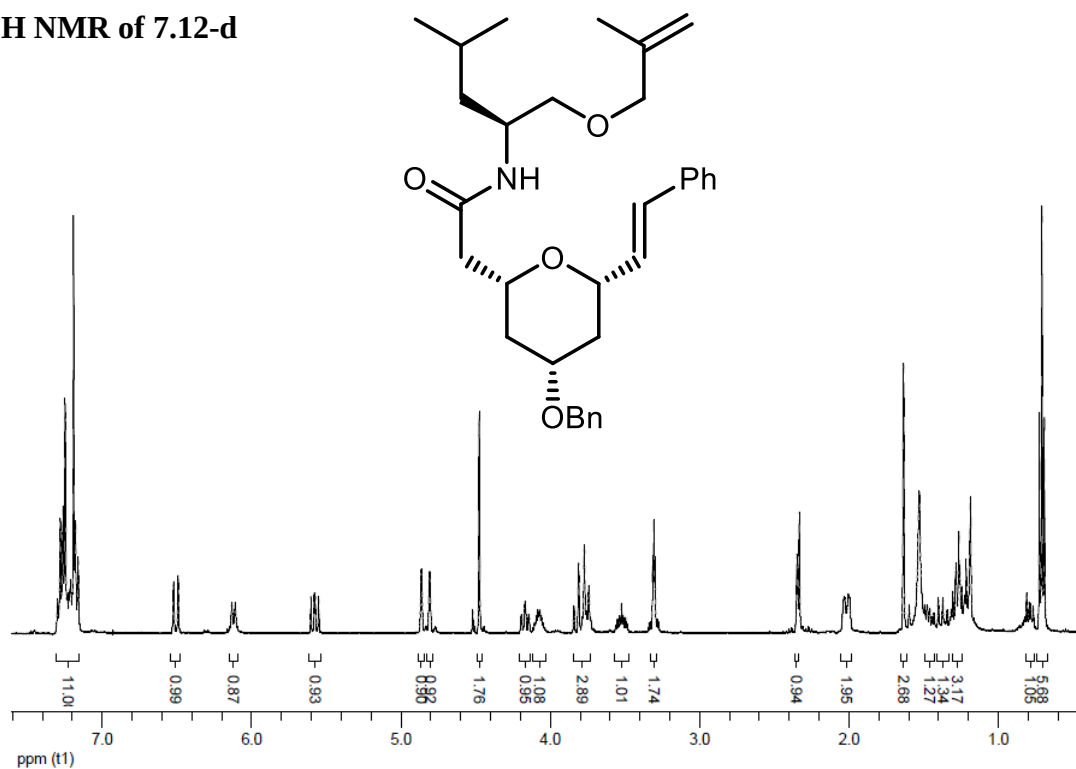
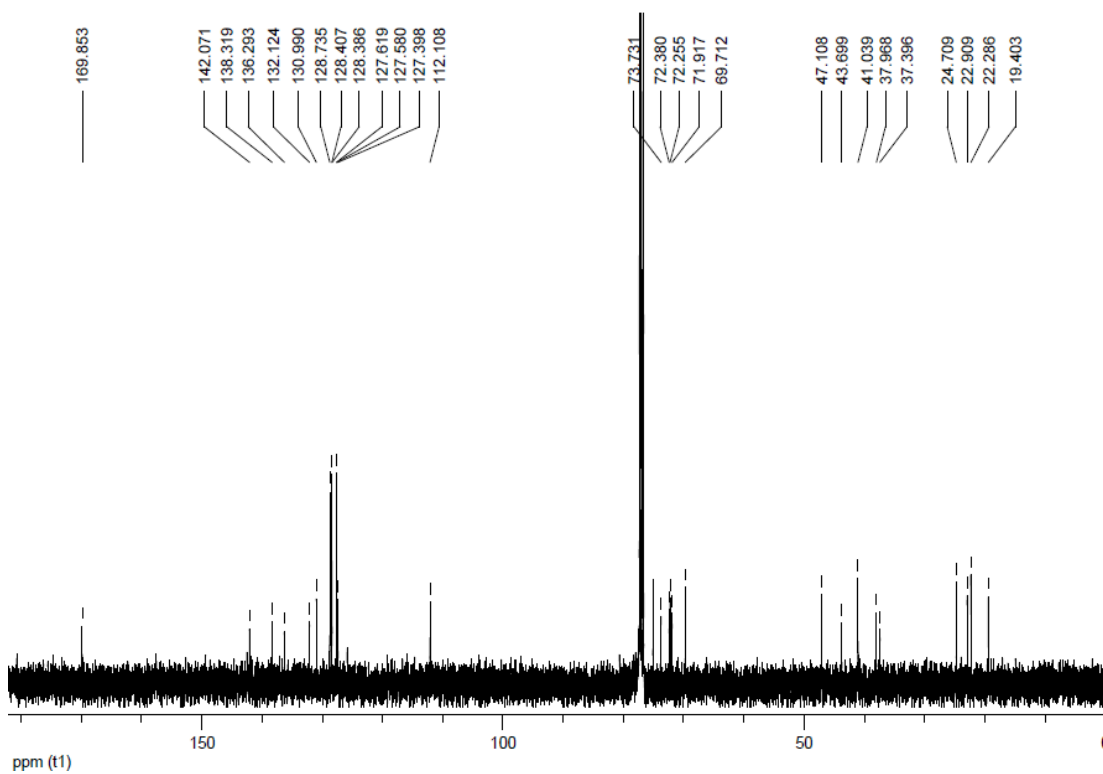


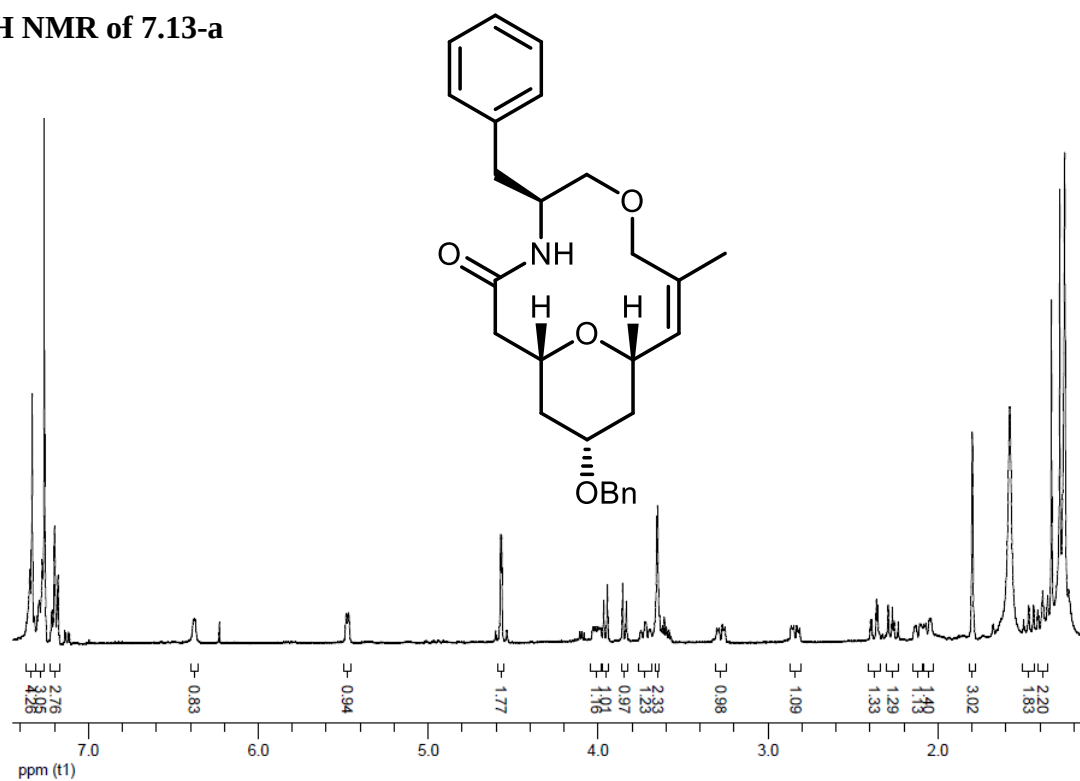
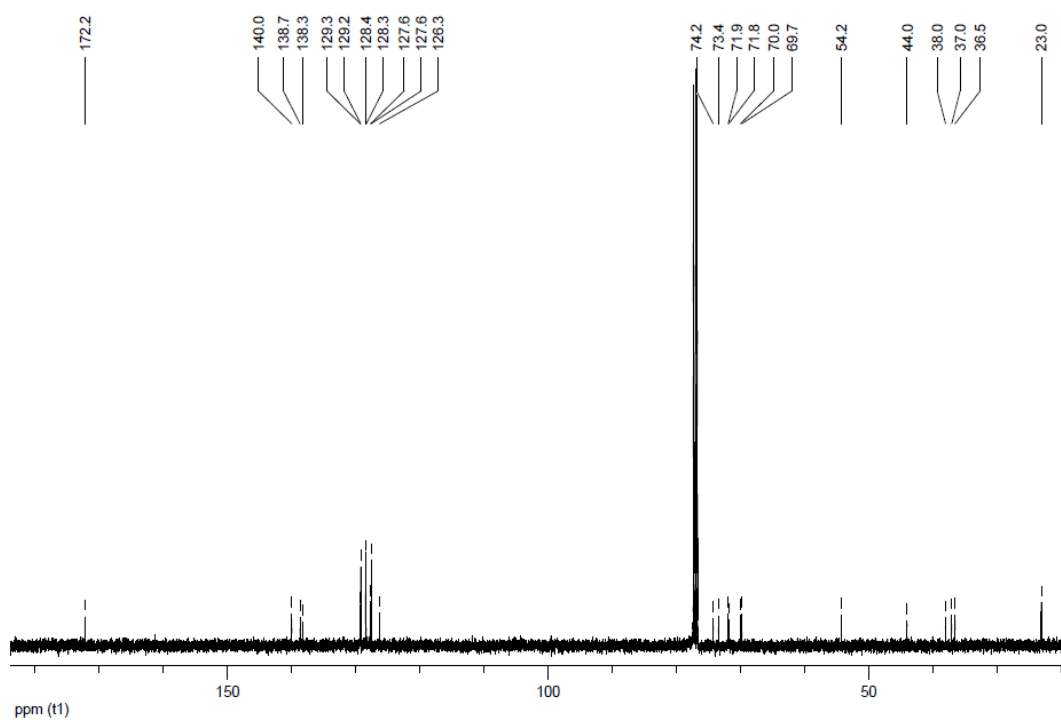
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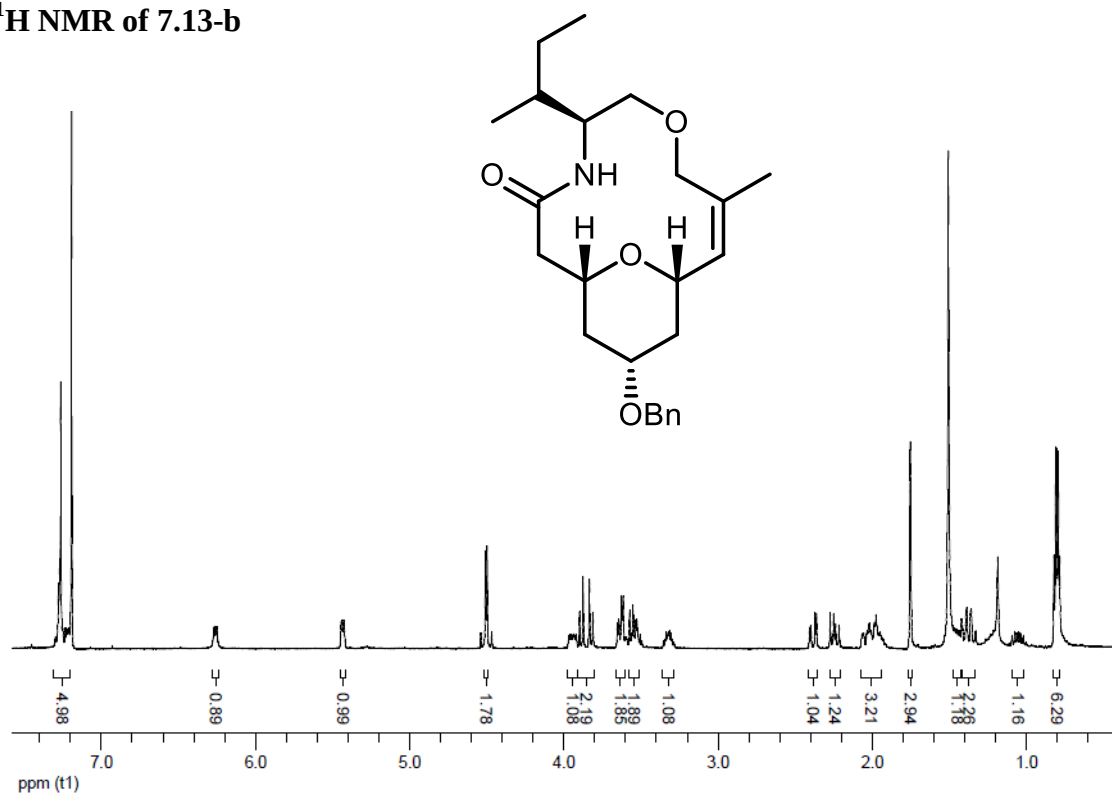
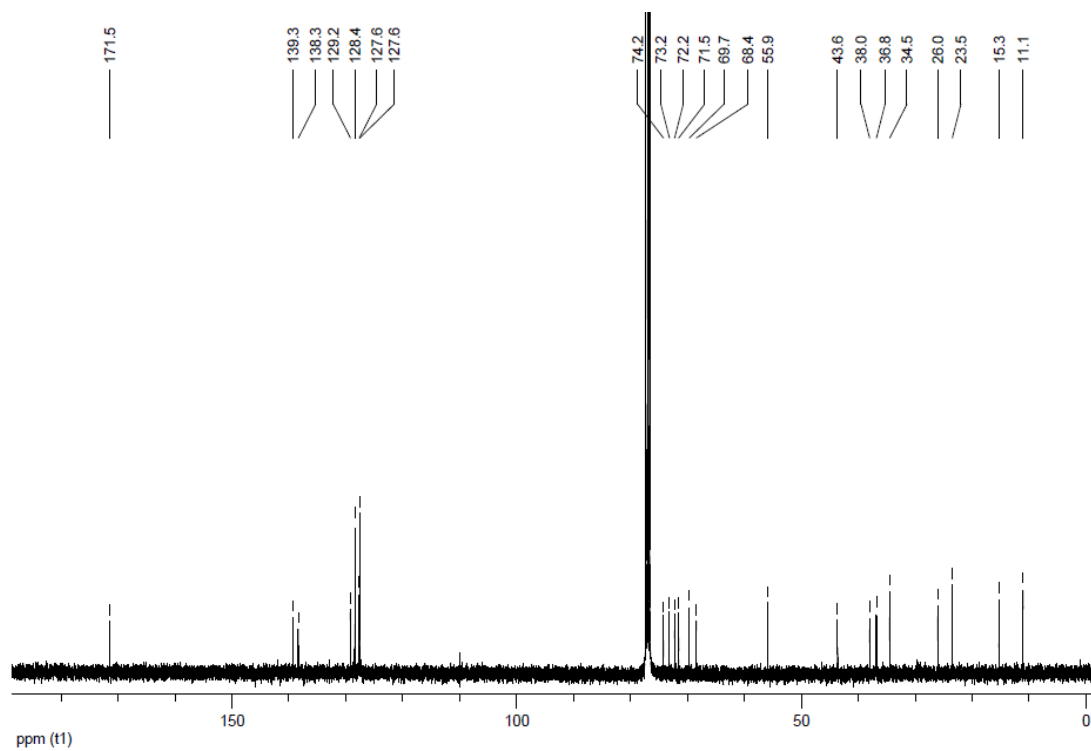


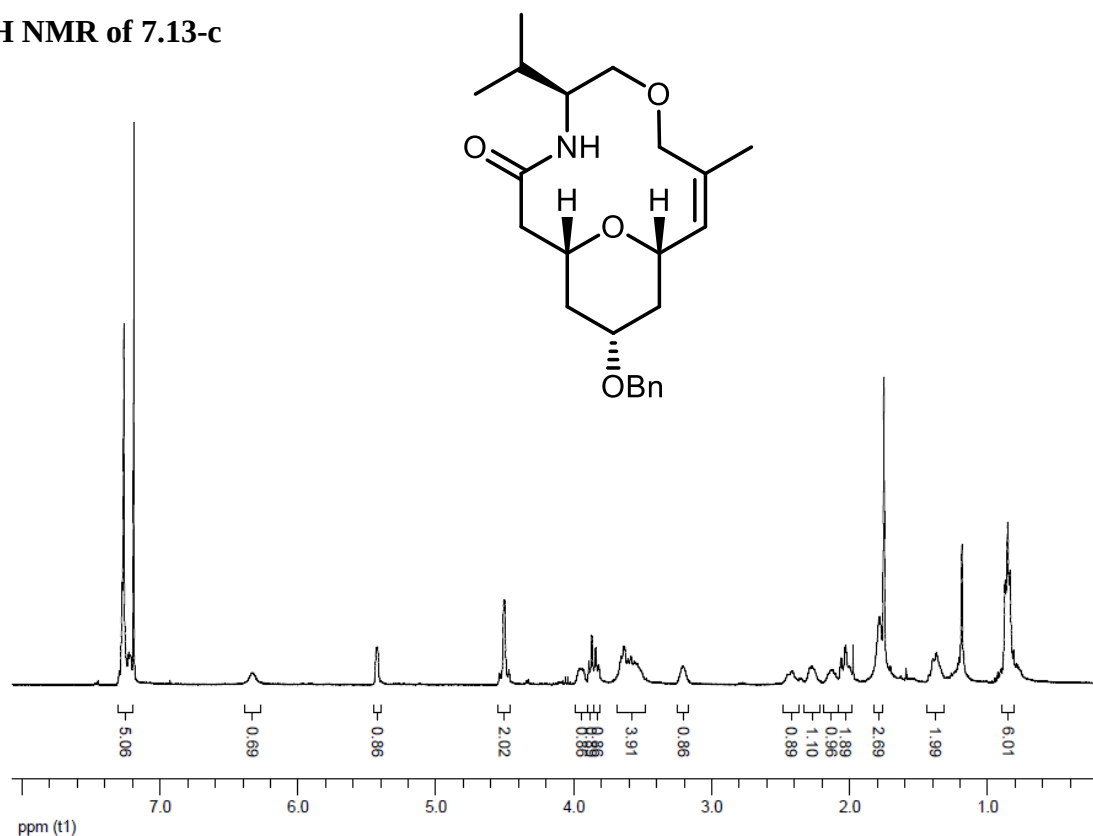
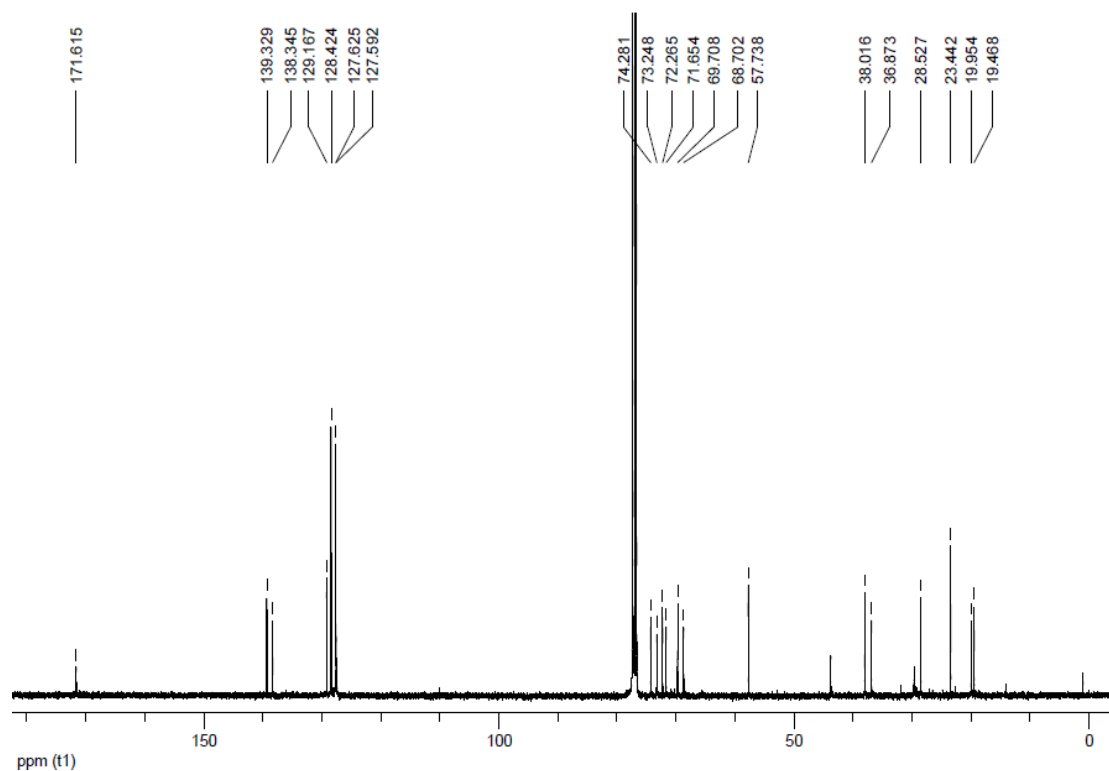
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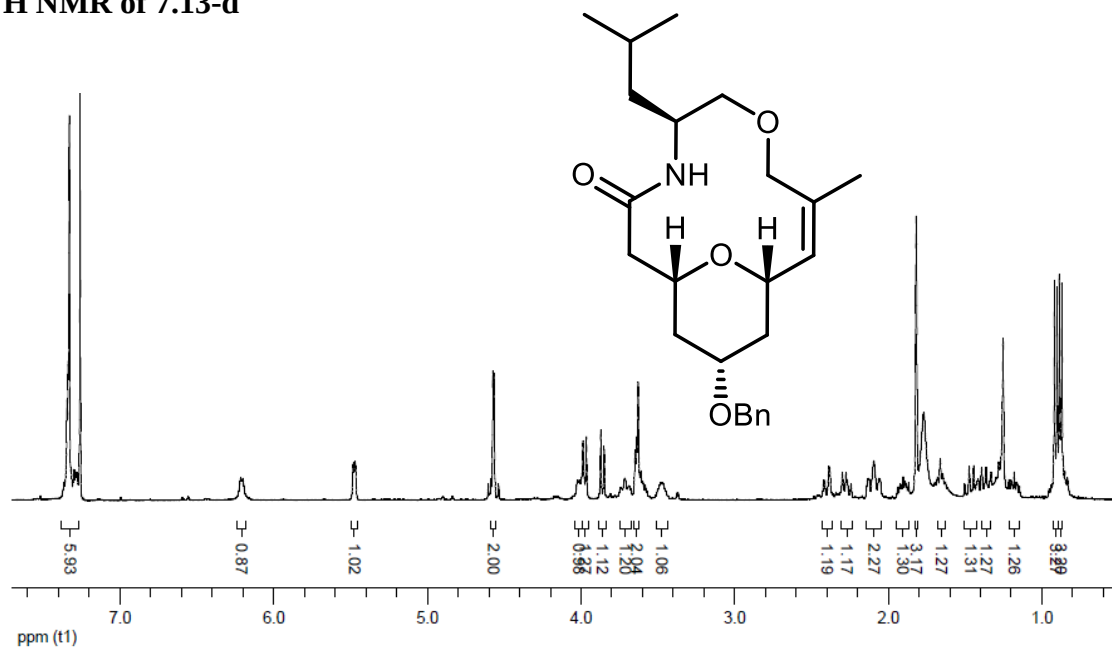
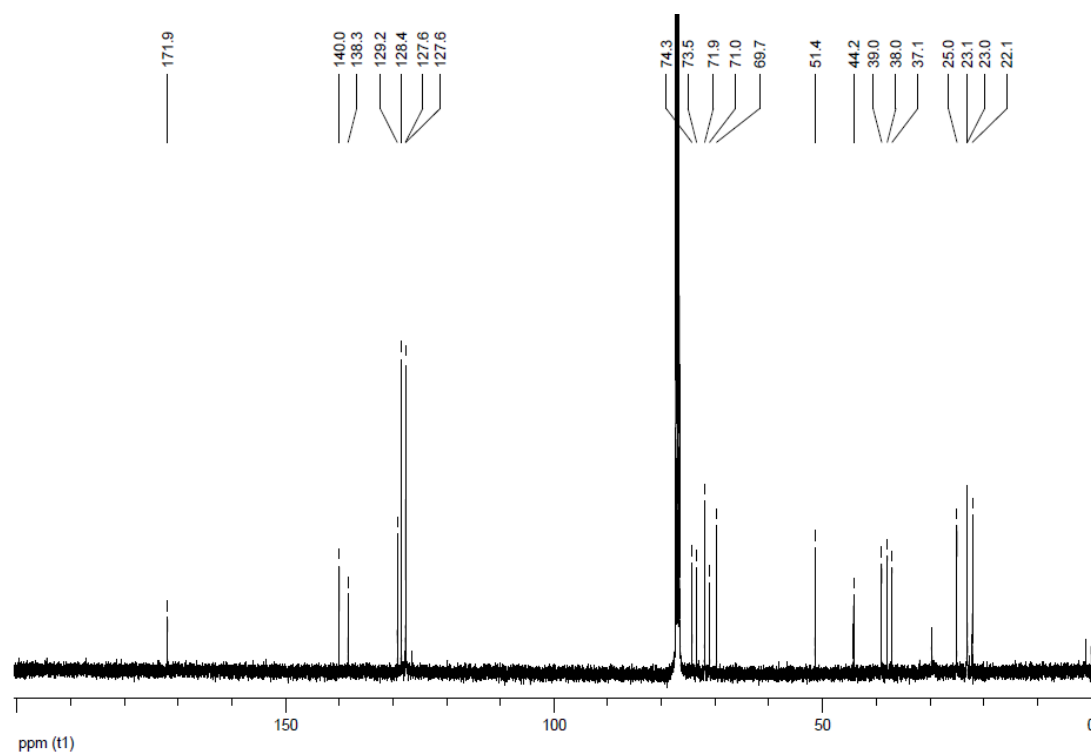
¹H NMR of 7.12-c

¹³C NMR of 7.12.c


^1H NMR of 7.12-d **^{13}C NMR of 7.12-d**

¹H NMR of 7.13-a

¹³C NMR of 7.13-a


^1H NMR of 7.13-b **^{13}C NMR of 7.13-b**

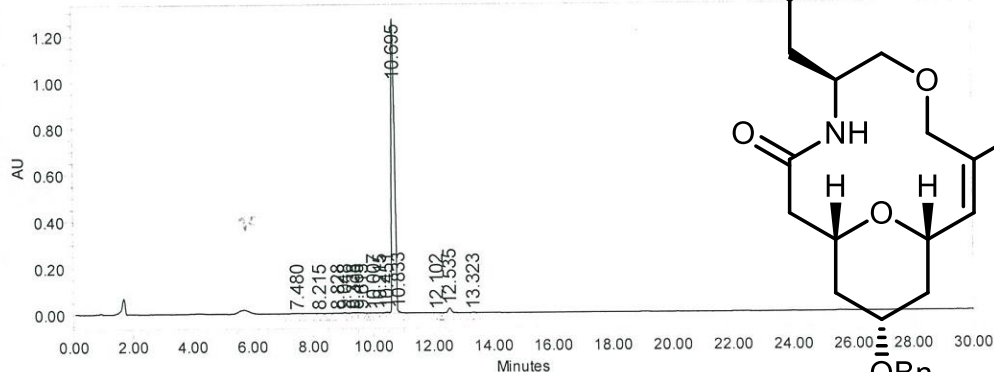
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¹³C NMR of 7.13-c


^1H NMR of 7.13-d **^{13}C NMR of 7.13-d**

HPLC

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Injection Volume:	20.00 ul	Proc. Chnl. Descr.:	PDA MaxPlot (190.0 nm to 800.0
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Date Processed:	9/12/2016 10:03:22 AM IST		

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 T/%B: 0/20, 3/20, 10/95, 23/95, 25/20, 30/20
 Flow: 1.0 ml /min, Diluent: ACN: WATER (80:20)



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4	9.048	12270	0.13	1597
5	9.338	16003	0.17	2244
6	9.463	17118	0.18	2124
7	9.615	8467	0.09	1158
8	10.007	3911	0.04	521
9	10.215	7178	0.07	759
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	RT	Area	% Area	Height
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12	10.833	30370	0.32	6938
13	12.102	2751	0.03	358
14	12.535	152964	1.59	20406
15	13.323	3070	0.03	520

Analysed by *M. Anil*
11/09/16

Checked by *S. Ali*
12/09/16

Reported by User: System
 Report Method: CPRI@DRILS
 Report Method ID: 2465
 Page: 1 of 1

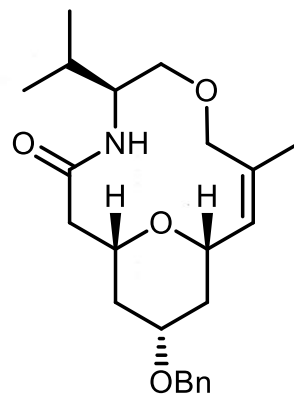
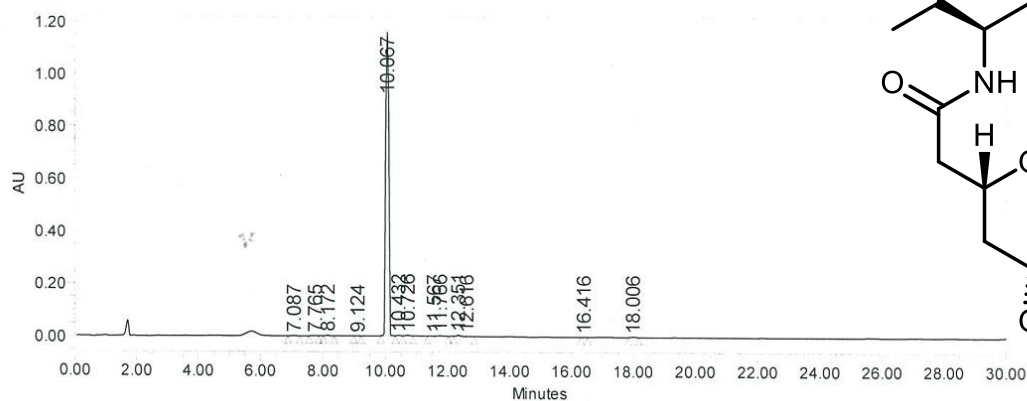
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HPLC

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Date Processed:	9/12/2016 10:07:13 AM IST		

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4	9.124	2850	0.03	362
5	10.067	8090993	98.08	1154793
6	10.432	9736	0.12	1447
7	10.726	20448	0.25	2392
8	11.567	5000	0.06	599
9	11.766	24480	0.30	1779
10	12.351	40452	0.49	5118

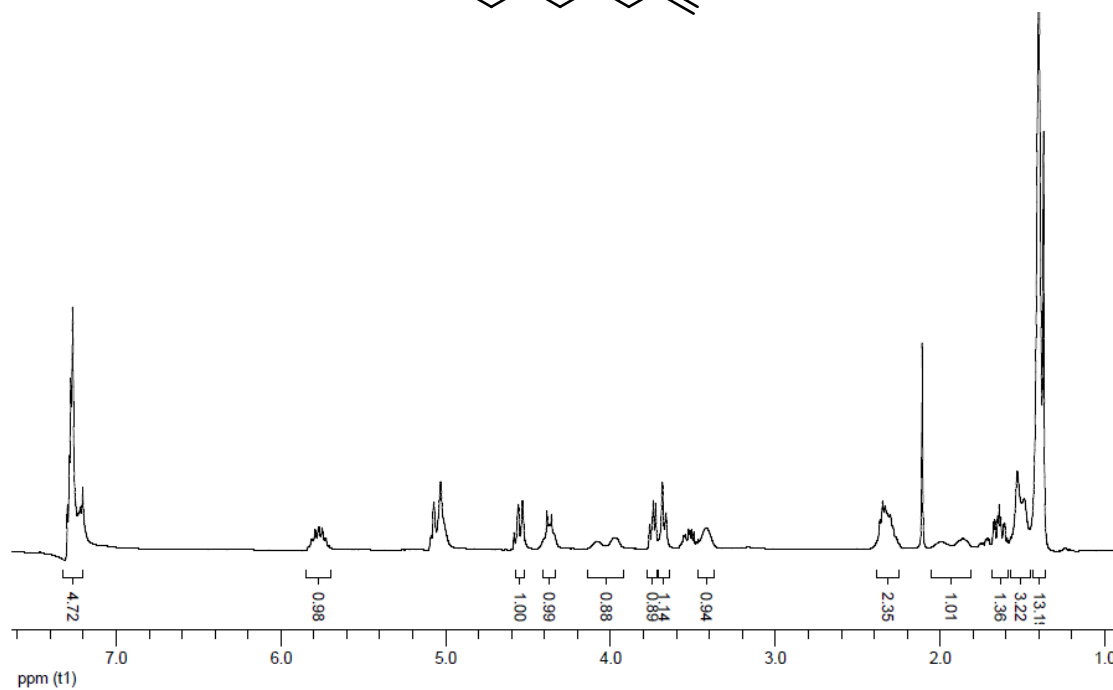
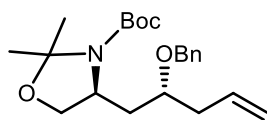
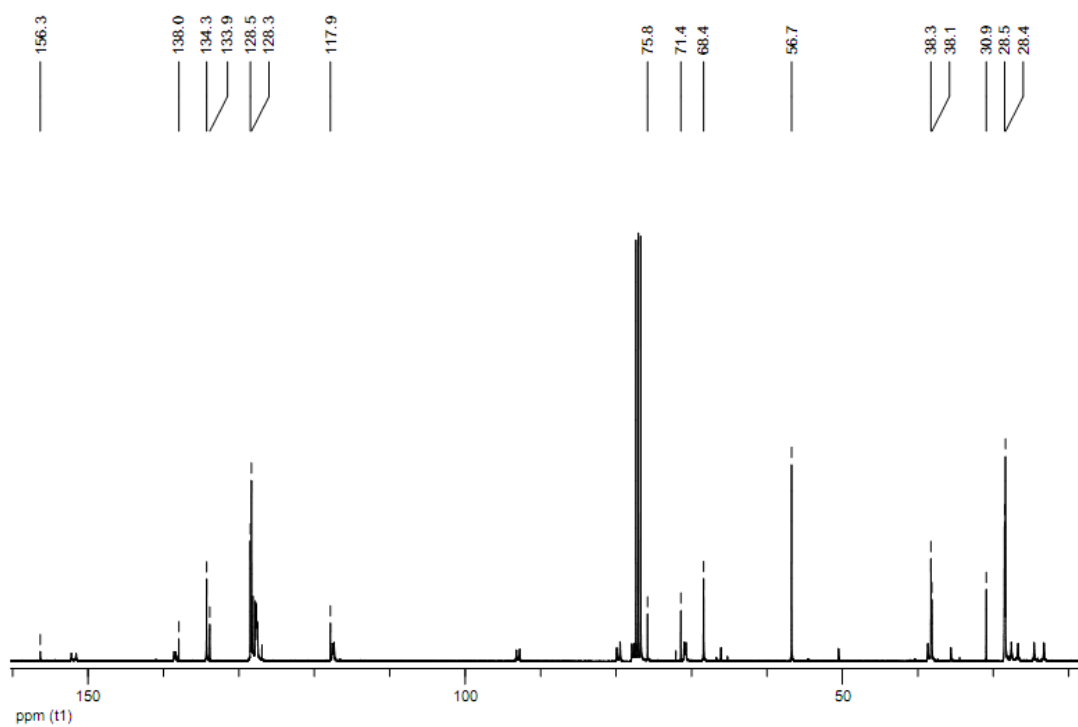
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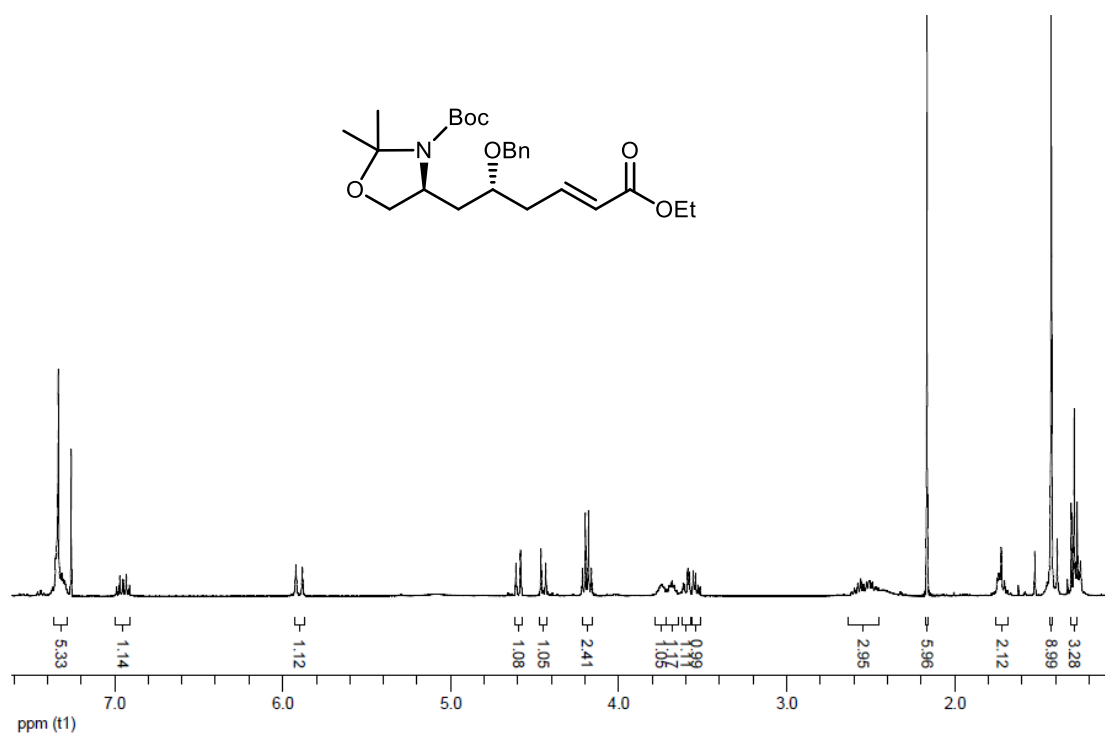
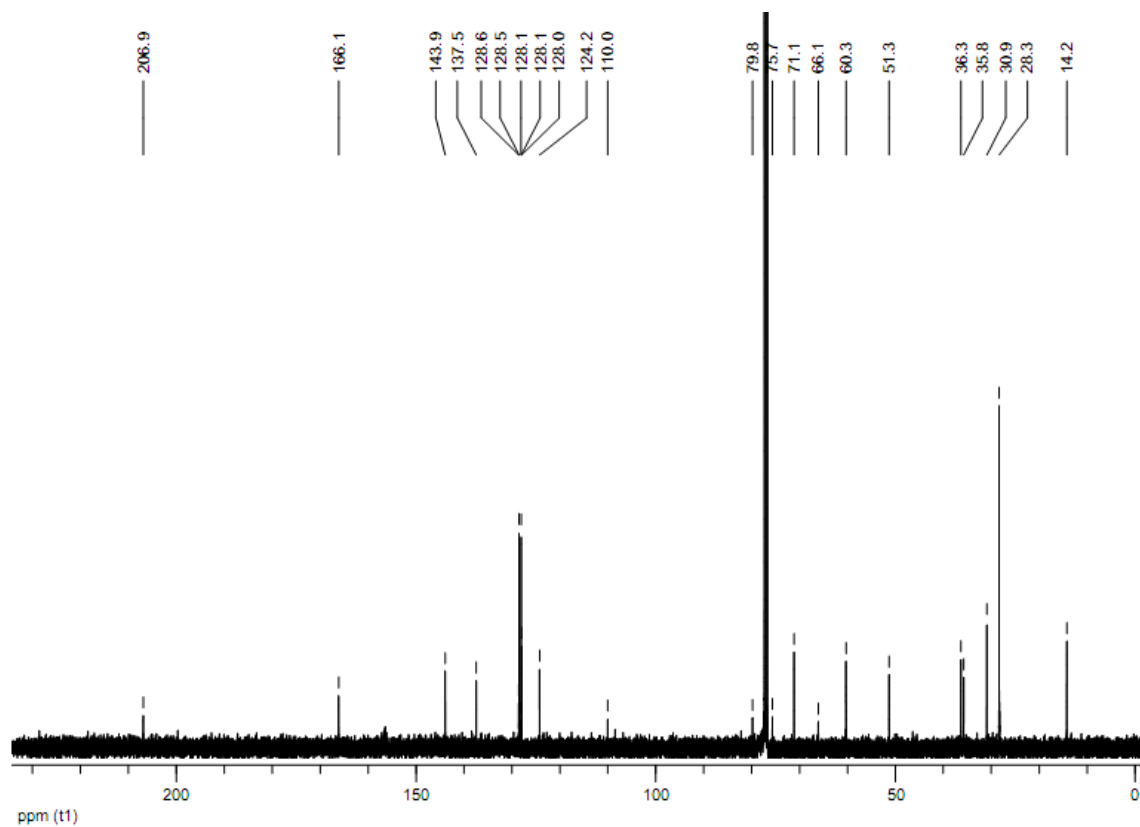
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12/09/16

Checked by *ABU*
12/09/16

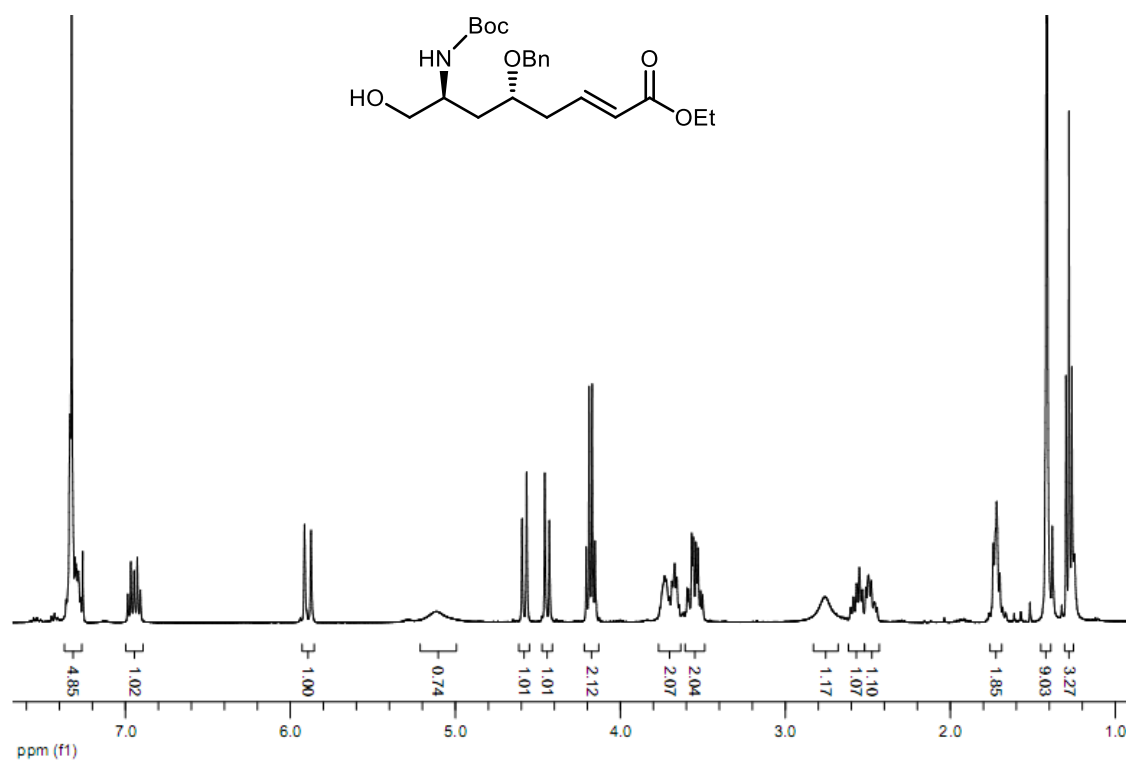
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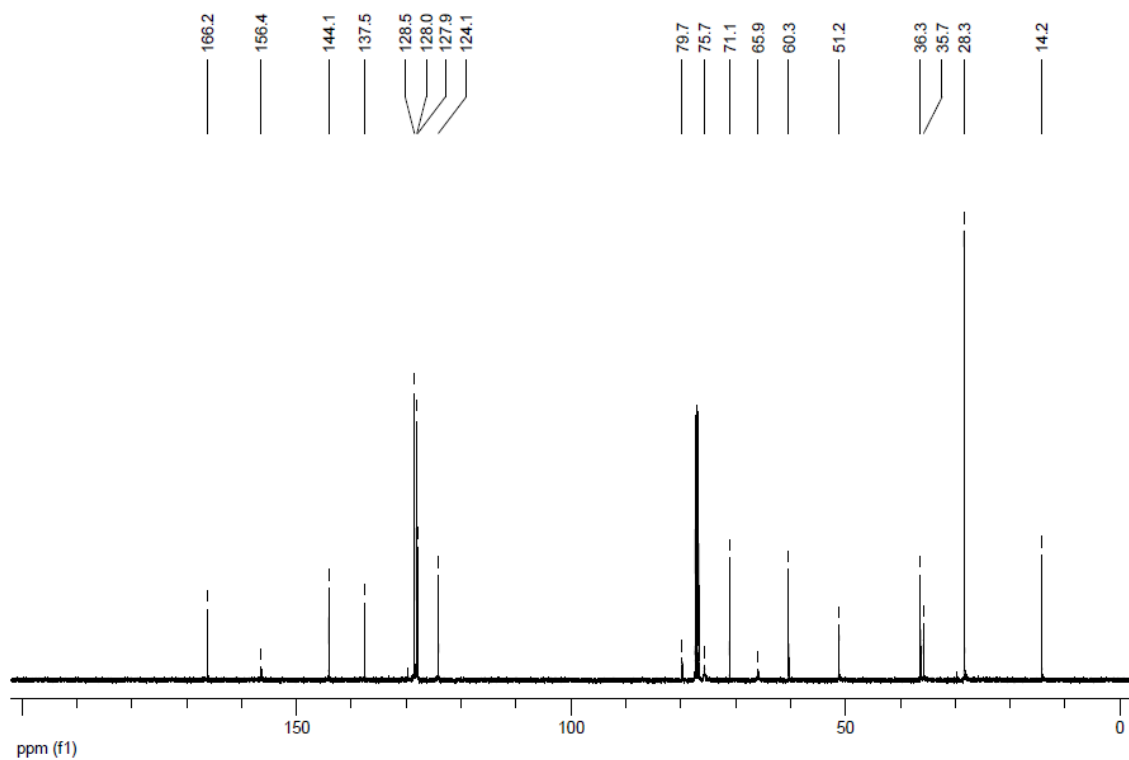
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¹³C NMR of 21.2


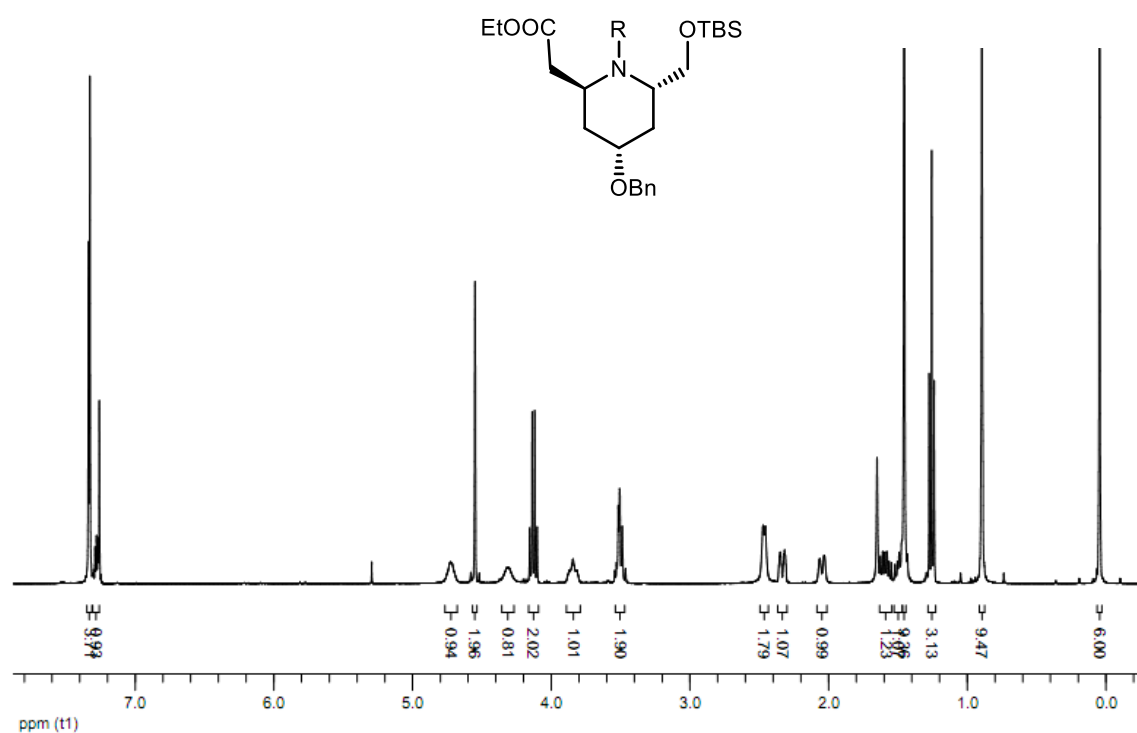
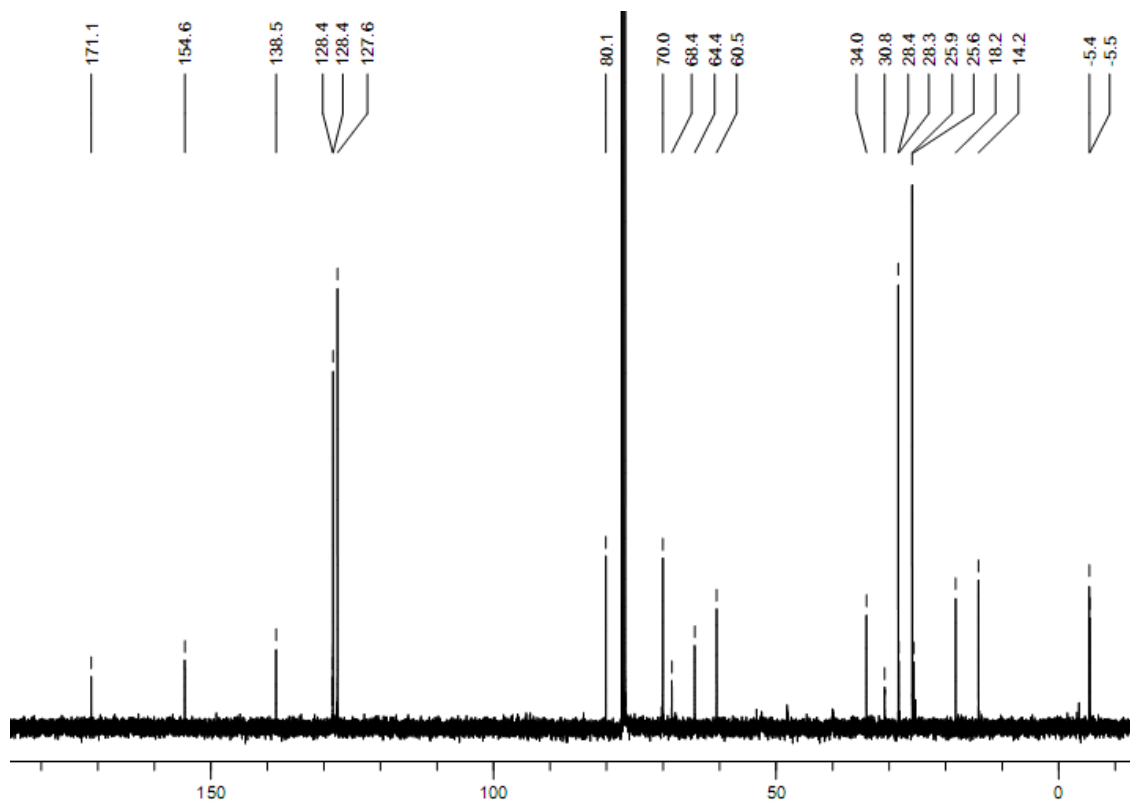
^1H NMR of S8 **^{13}C NMR of S8**

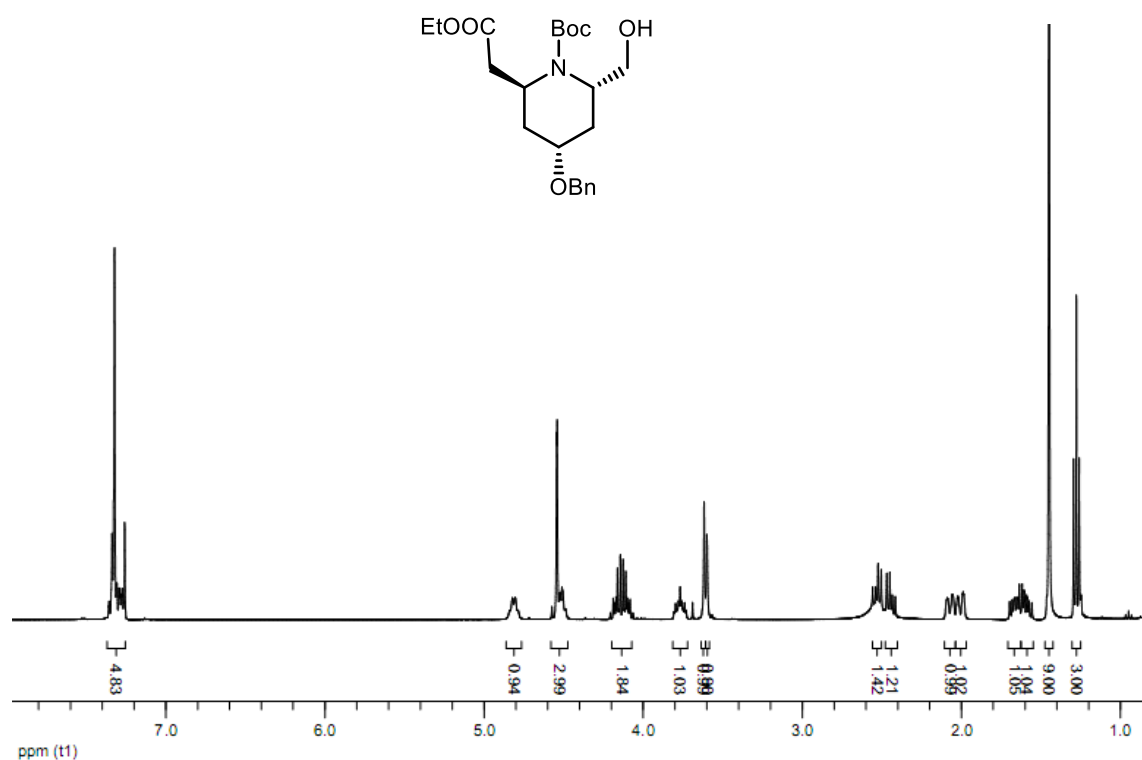
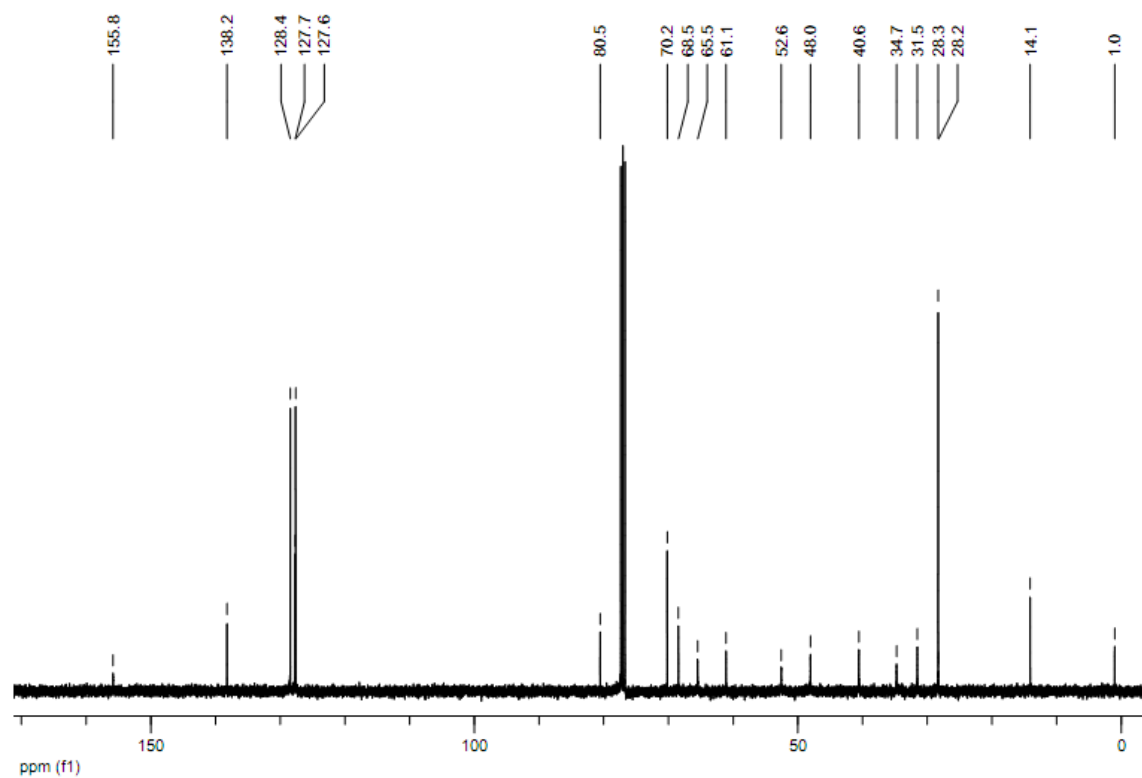
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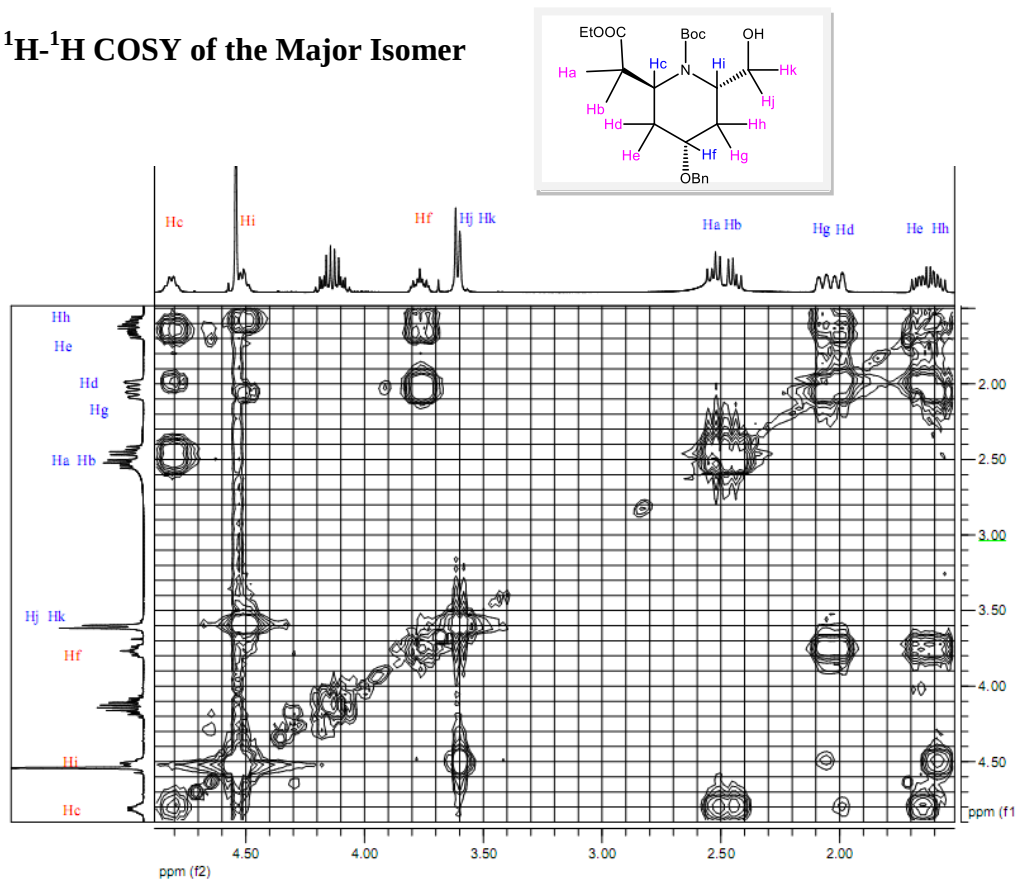
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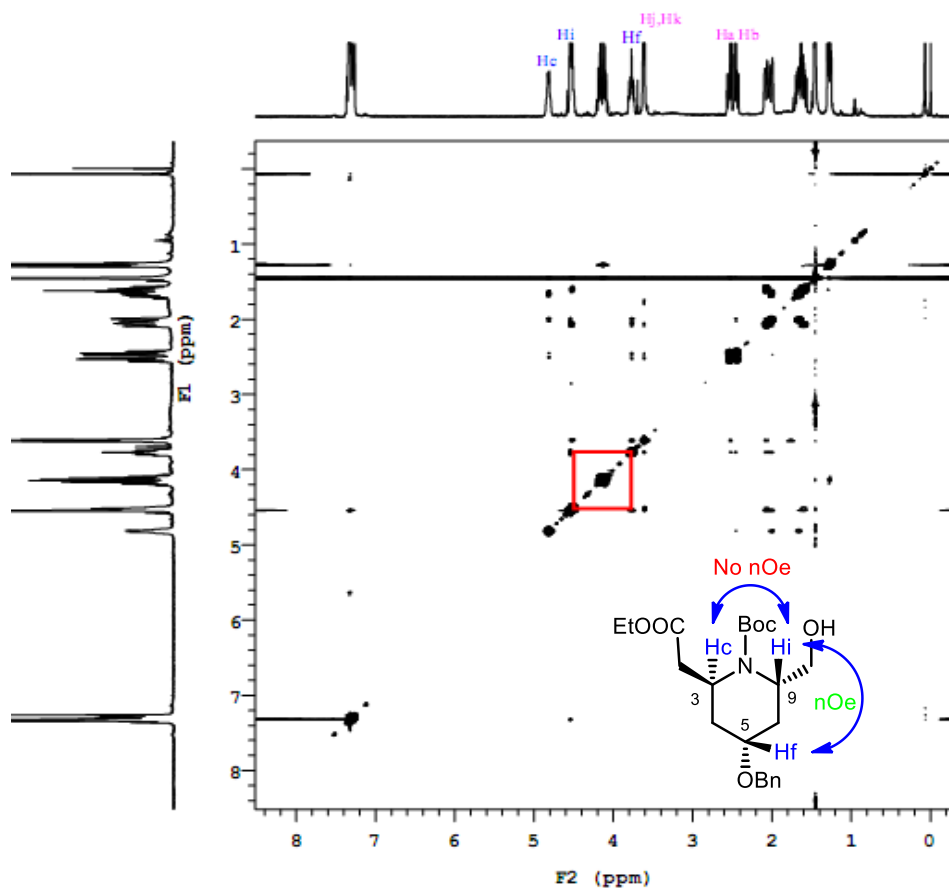
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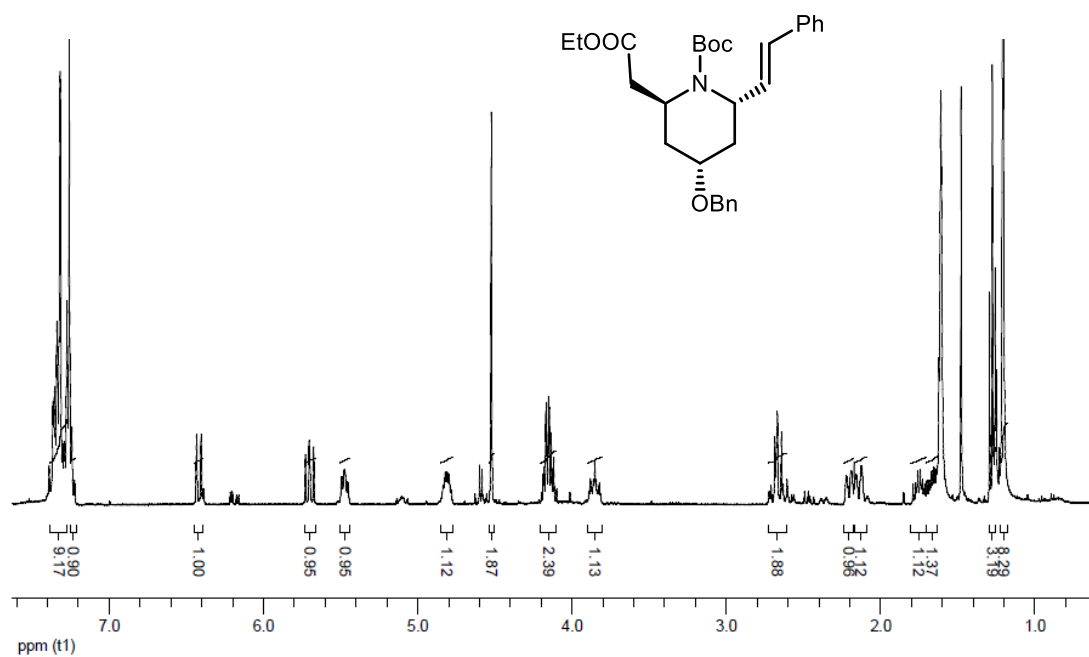
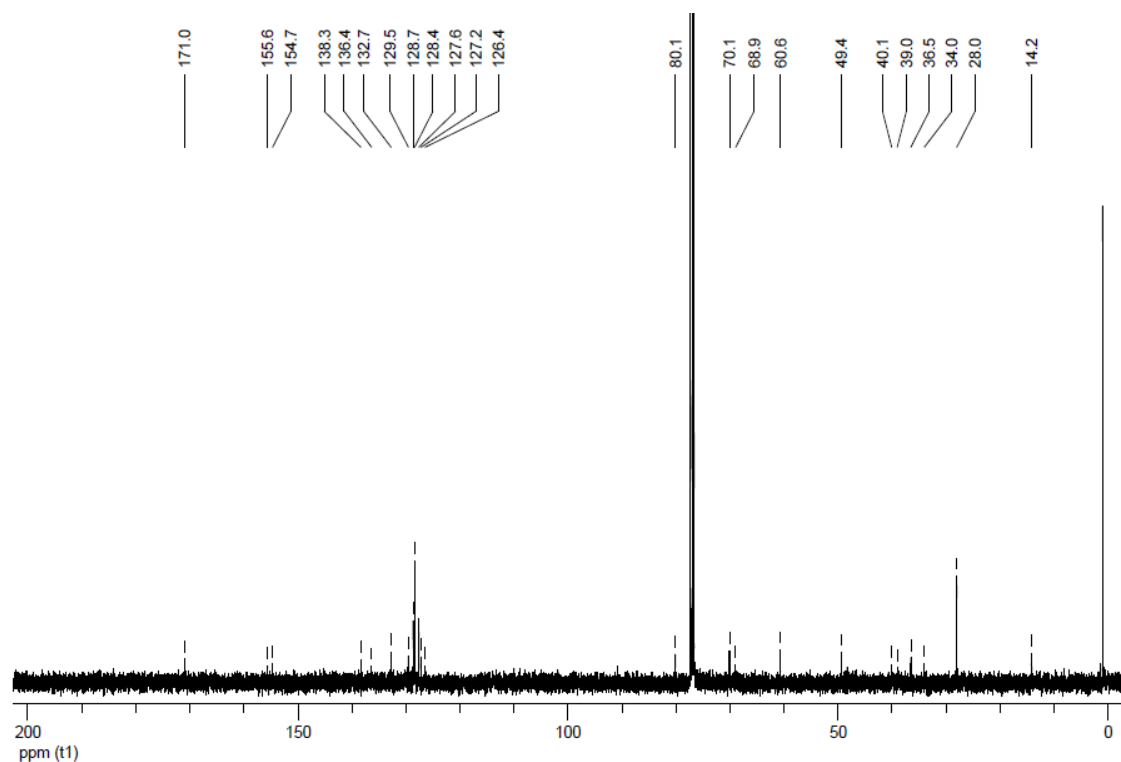
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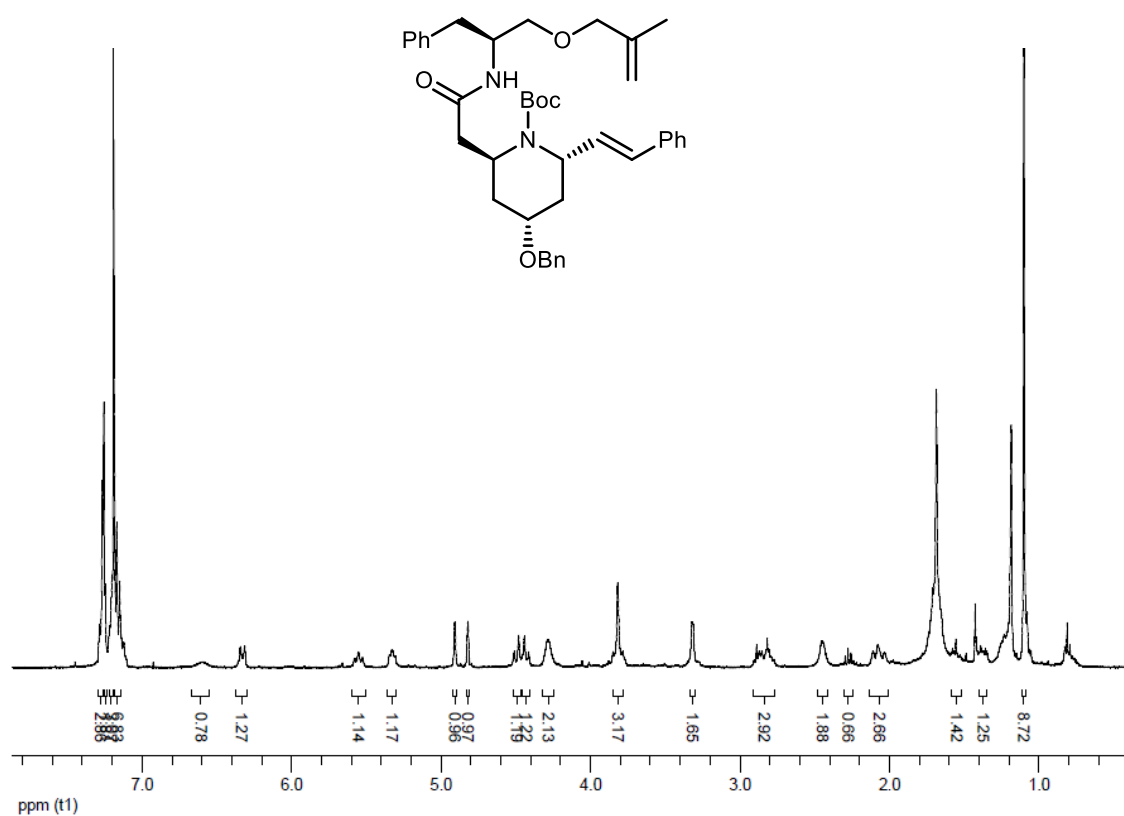
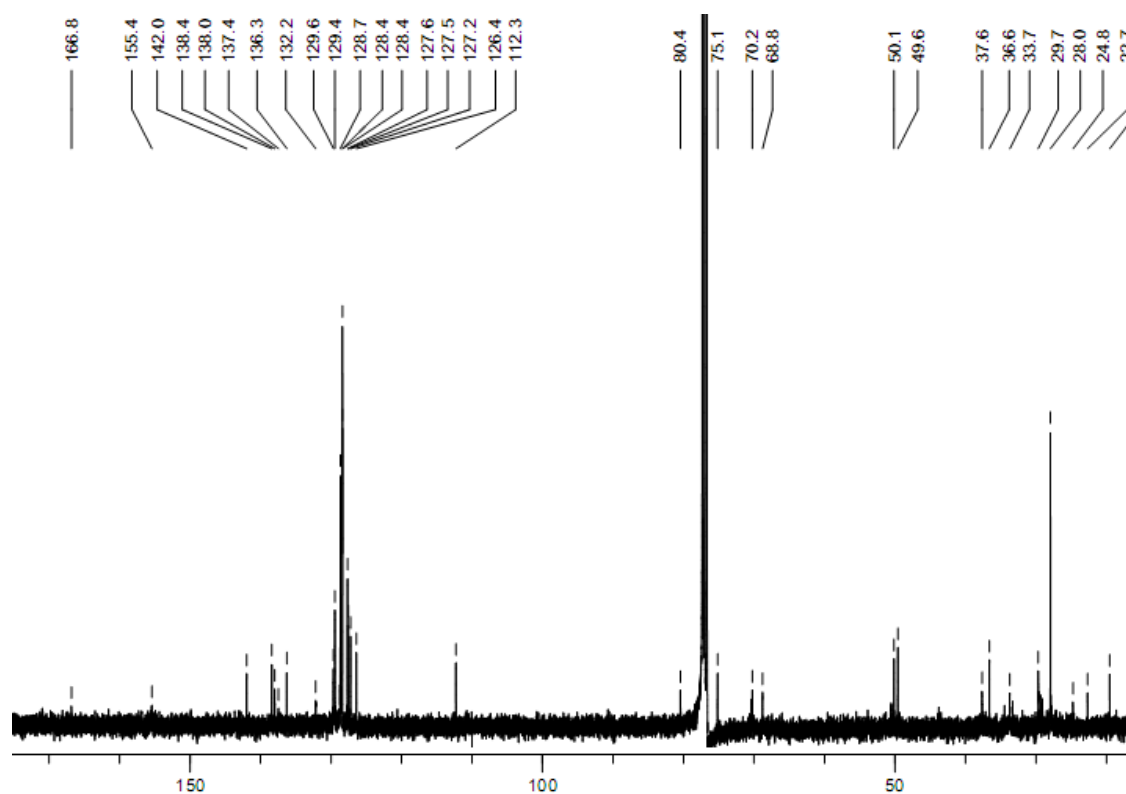
^1H - ^1H COSY of the Major Isomer



2D-NOESY of the Major Isomer



^1H NMR of S12 **^{13}C NMR of S12**

¹H NMR of S13

¹³C NMR of S13




Chapter 3

A Divergent Synthesis of Isoxazole-based Macrocyclic Toolbox



3.1 Introduction

Isoxazole is a five membered ring with oxygen, nitrogen atoms at the 1, 2 positions. Isoxazoles have an excellent track record in the broad drug discovery arena: their chemistry is closely connected with Ludwig Claisen, who recognized the cyclic structure of 3- methyl-5-phenyl isoxazole, first time in 1888. Dunstan and Dymond were the first to synthesize the isoxazole ring.¹ This isoxazole heterocyclic ring is present in some natural products as some examples with different biological activities are shown in (Figure 1).

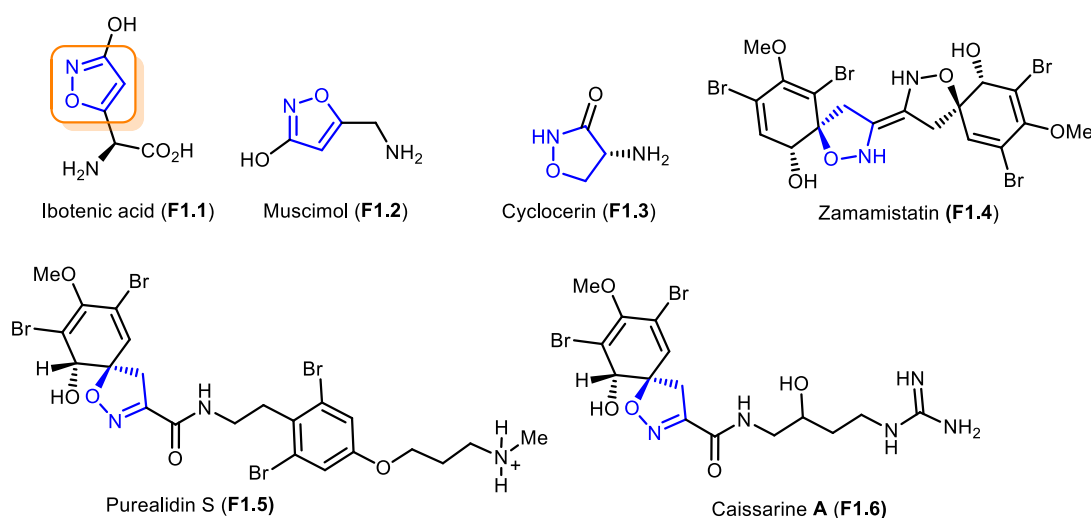


Figure 1: Biologically Active Natural Products with Isoxazole Motif

3.2 Biologically Active Natural Products with Isoxazole Scaffold

Ibotenic acid (F1.1), which is also known as ibotenate, extracted from naturally occurring mushroom like species *Amanita muscaria*, typically found in the temperate and boreal regions of the northern hemisphere. It acts as a non-selective glutamate receptor agonist.^{2,3} The next example is a natural product, muscimol (F1.2) and this compound is well-known for GABA agonist activity.⁴ Muscimol plays a key role in activating the receptor for the brain's principal inhibitory neurotransmitter, GABA. As in the case of GABA, muscimol also appear to binding the same site on the GABA_A receptor complex, and this is just contrast to other GABAergic drugs, for example, barbiturates and benzodiazepines, that are known for binding to bind to the separate regulatory locations.^{5,6} It is well-known that GABA_A receptors are widely distributed in the brain, thus the interactions of muscimol alter the neuronal activity in multiple

regions, including the cerebral cortex, hippocampus, and cerebellum.⁷ It is also known in the literature that, in patients with Huntington's disease and chronic schizophrenia, the oral doses of muscimol causes an increase in both, prolactin and the growth hormone.^{8,9} Cyclocerine (4-amino-3-isoxazolidinone) (**F1.3**), an amino acid derivative, possesses an unusual structure, produced by the bacteria, it is an effective antibiotic against *Mycobacterium tuberculosis* and one of the important medicines referred by World Health Organization (WHO) as an essential medicine. Zamamistatin (**F1.4**), is a dibromotyrosin derivative, and exhibits good antibacterial activity against *Rhodospirillum bacterium sp.* It was isolated from the *Okinawan sponge sp.* Other new bromotyrosine alkaloid, purealidin-S,¹⁰ (**F1.5**) was isolated from the Fijian marine sponge *Druinella sp* by Jaspars and co-workers in 2002. It shows potent cytotoxicity. Caissarine-A (**F1.6**), a dibromo tyrosin derivative alkaloid, isolated from the Brazilian Endemic Marine Sponge *Aplysina caissara*.¹¹

3.3 Isoxazole-derived Biologically Active Compounds

The substituted isoxazole are also considered to be important synthons due to their adaptability in the direction of chemical transformations to useful synthetic intermediates. The derivatives of isoxazole are largely employed in the area of therapeutics and pharmaceuticals, such as, antibacterial, insecticidal, antibiotic, antifungal, antitumour, antituberculosis, and anticancer, ulcerogenic, and HIV-inhibitory activities. A few derivatives are used in the market, such as COX-2 inhibitor-based anti-inflammatory drugs. Some derivatives such as sulfamethoxazole, sulfisoxazole, cycloserine, oxacillin, and acivicin have been in commercial use for many years. Cycloserine is a well-known antibiotic drug for antitubercular and antibacterial activities and in treatment of leprosy. Acivicin is an antileishmania and antitumour drug, while Isoxaflutole is used as the herbicidal drug. Shown in (**Figure 2**) are several examples of small bioactive small molecules having this sub-structure and a brief description of selected examples is provided below. The first example in (**Figure 2**), **F2.1** shows several compounds that belong to the category of β -lactam antibiotics. The notable examples in this category are oxacillin, cloxacillin, dicloxacillin, flucloxacillin. Oxacillin belongs to the family of β -lactam antibiotics and is known for its penicillinase-resistant activity.^{12,13} Structurally, oxacillin is very similar to methicillin (note - the structure of methicillin is not shown here), and has replaced methicillin, in clinical use. Due to its resistant effects to penicillinase

enzymes, oxacillin is also widely used clinically in the United States for treating the penicillin-resistant *Staphylococcus aureus*.

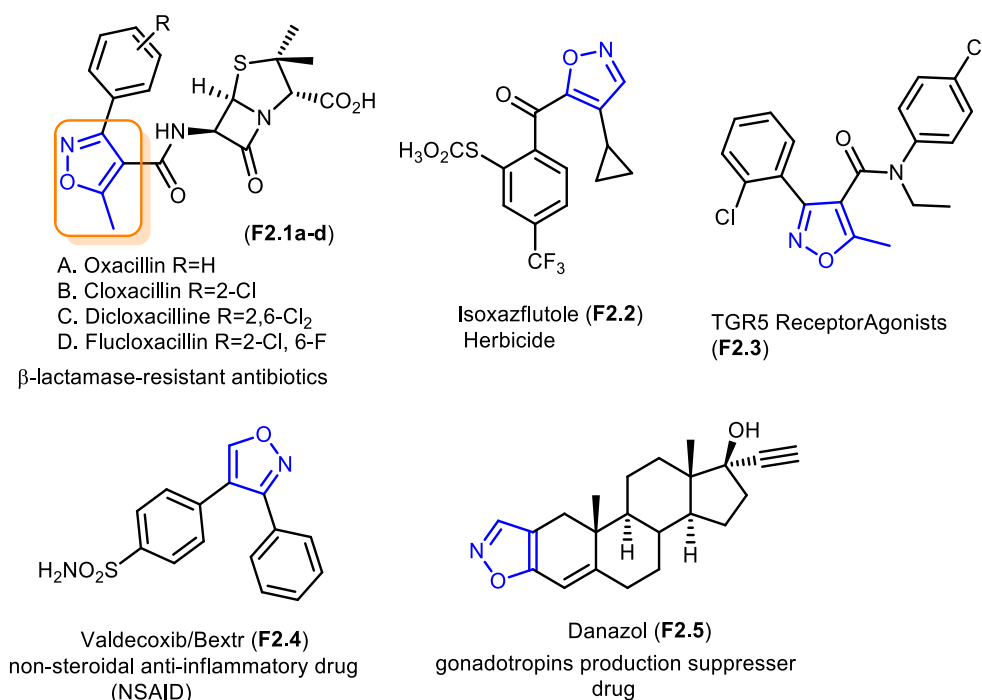


Figure 2: Several Examples of Bioactive Compounds having the Isoxazole Moiety

The second example **F2.2** shown in (**Figure 2**) is isoxaflutole, which is a herbicide.¹⁴ Another example **F2.3**, shown in (**Figure 2**), is a synthetic compound which was identified as the TGR5 receptor agonist.^{15,16} A number of compounds in this family were synthesized by the researchers and a high-throughput screen identified potent small molecule agonists of the human TGR5 G-protein coupled receptor.¹⁶ Further, modifications to this structure led the identification of potent compounds which demonstrated an improved GLP-1 secretion *in vivo* via an intra-colonic dose co-administered with the glucose challenge in a canine model. These novel compounds can be highly useful in metabolic disorders, such as type II diabetes and its associated complications. Valdecoxib/Bextron (**F2.4**) steroidal anti-inflammatory drug (NSAID). Finally, danazol (**F2.5**) gonadotropins as produced as the suppresser drug.

3.4 Our Design for Obtaining Macrocyclic Compounds Having an Embedded Isoxazole Moiety

Given the importance of the isoxazole moiety as the "privileged scaffold", we were interested in utilizing this substructure in developing the design and synthesis of a new family of different types of macrocyclic compounds for exploring their biological

application(s). For our first generation approach, as shown in **(Figure 3)**, we focused on the design and synthesis of compounds with 15-membered macrocyclic rings having several attractive features. These unique features are: (i) the presence of an amino acid moiety within the macrocyclic ring allow exploring the scope of a chiral functional group which will be obtained from the natural or unnatural amino acid. (ii) the embedded isoxazole moiety which is considered as the privileged substructure may provide the additional features while binding with the biological targets, and (iii) finally, the presence of two diversity sites located on the 15-membered macrocyclic ring would allow further the "chemical space exploration" by obtaining several structurally related analogs. In addition to these features, our modular design approach which allows connecting the amino acid moiety either from the aromatic ring side or the isoxazole moiety would also lead to obtaining two entirely different 15-membered rings.

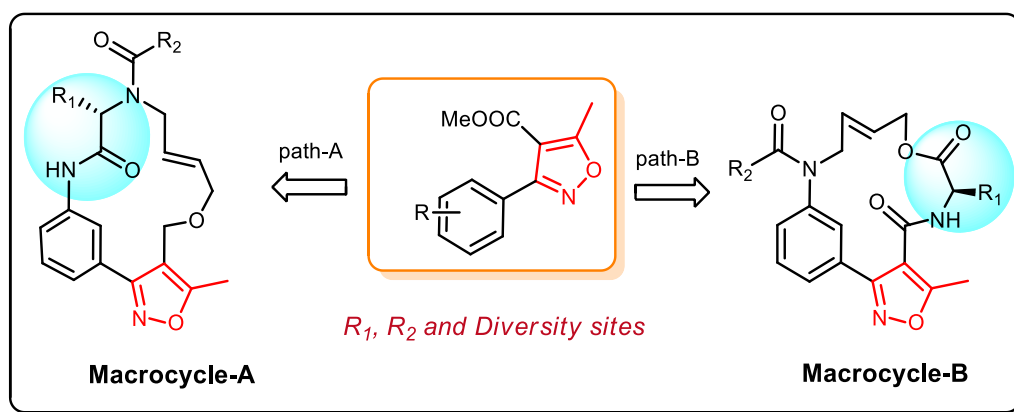


Figure 3: Our Diversity-based, Design Approach for Obtaining Two Different 15-Membered Macrocyclic Compounds having an Embedded Isoxazole Moiety.

Our interest in accessing these novel macrocyclic small molecules for several reasons, and some of these include: (i) given the macrocyclic nature of these compounds, they provide an excellent opportunity for accessing compounds that are different from the traditional types of compounds, generally belonging to the category of simple heterocyclic compounds, and (ii) these compounds are also attractive for exploring their value in undertaking biological targets considered difficult, for example, targets dealing with protein-protein interactions for covering a large surface area.¹⁷ These compounds are sufficiently complex and could easily be taken further for developing the next level, medicinal chemistry studies, following the identification of interesting hit compounds.¹⁸ A practical and relatively ease of the synthesis for these compounds

is a key attractive factor in this design approach. These compounds are also considered close to a wide variety of bioactive natural products, thus their probability of interacting with the biological targets, may be higher, when compared to highly simpler heterocyclic small molecules.¹⁹

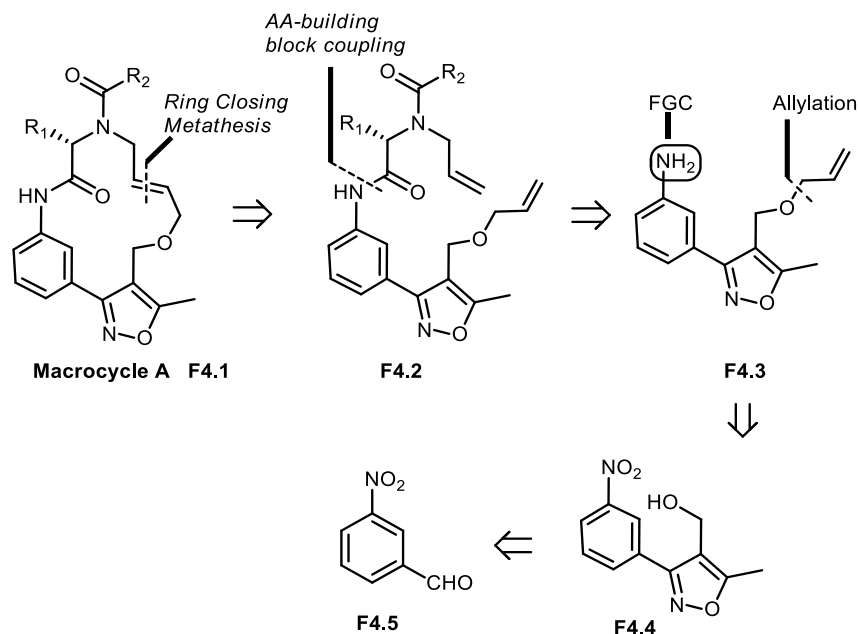


Figure 4: Retrosynthesis of Macrocycles A

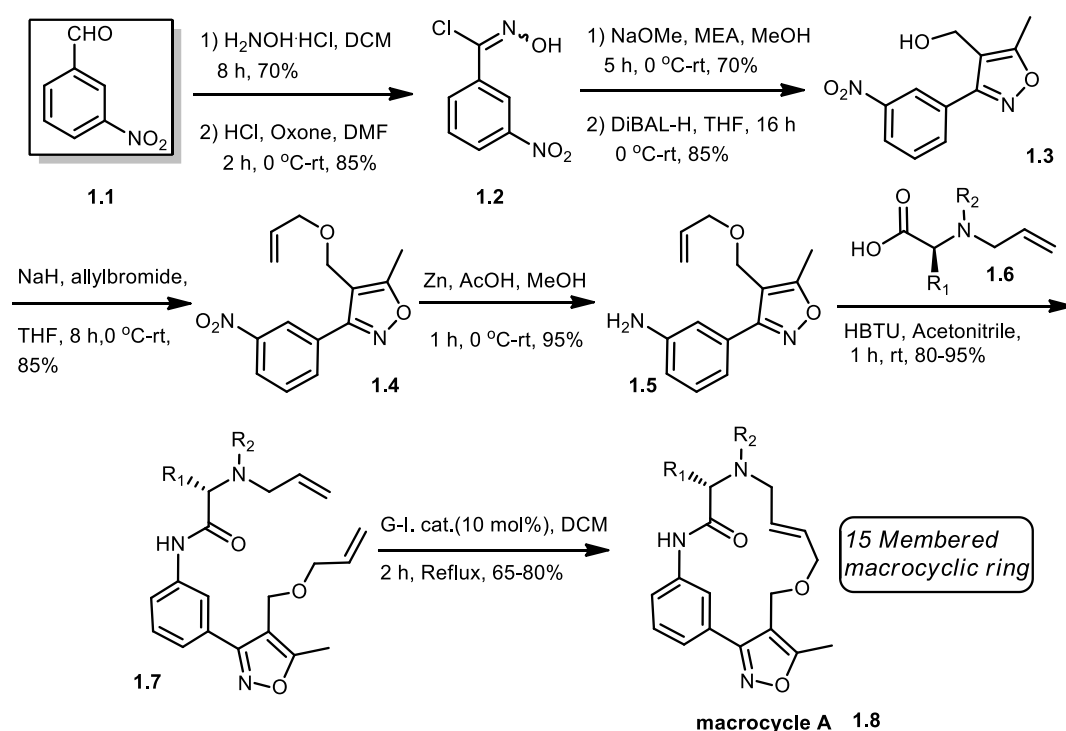
3.5 Retrosynthesis of Macrocycles A

The retrosynthetic analysis of macrocycle A is shown in (**Figure 4**). Macrocyclization was carried-out by ring closing metathesis of bis-allylated compound (**F4.2**). It was obtained from **F4.3** through the aminoacid building block coupling. **F4.3** was prepared from the nitro group reduction and O-allylation of alcohol (**F4.4**). Which would be synthesized from **F4.5** by chlorooxime preparation, (3+2) cycloaddition reaction followed by the ester group reduction. Our approach to the synthesis of isoxazole-derived macrocyclic compounds (**macrocycle A**) is shown in (**Scheme 1**).

3.6 Synthesis of Macrocycle A Series

The synthesis starts with *m*-nitrobenzaldehyde (**1.1**) and this was then converted to the corresponding chlorooxime (**1.2**) in two steps aldoxime formation with $\text{NH}_2\text{OH} \cdot \text{HCl}$ followed by chlorooxime with oxone and HCl, in high yields. This was utilized leading the synthesis of the isoxazole derivative **1.3** by (3+2) cycloaddition with Methyl acetoacetate (MEA) and sodium methoxide, followed by reduction with Diisobutylaluminium hydride. The primary hydroxyl group in compound **1.3** was then

allylated with allyl bromide, giving the product **1.4**, which was then subjected to nitro group reduction with Zn/AcOH. This approach led the synthesis of the amine derivative as compound **1.5**. The free amino group in **1.5** coupled to four different *N*-allylated amino acid derivatives **1.6** prepared from (s)-amino acid, with coupling reagent HBTU, giving the coupled product **1.7**, as the precursor to the Ring closing metathesis (RCM)-based macrocyclic ring formation. Using the first generation Grubbs' catalyst (for the RCM), the reaction worked-well, and it produced the expected 15-membered ring macrocyclic compounds (**macrocycle A**, **1.8**). Shown in (figure 5), are the eight compounds that were synthesized in diversity-oriented manner.



Scheme 1: Synthesis of Macrocycle A Series Compounds

Utilizing both the diversity sites, (i.e. R_1 and R_2) the successful implementation of this approach by utilizing various amino acids further nicely validated the scope of this methodology. All the products at every step were thoroughly purified flash column chromatography approach and systematically characterized by ^1H and ^{13}C NMR and HPLC-MS studies. The geometry of the olefin obtained from RCM reaction was assigned with ^1H NMR coupling constant values (coupling constant value is 16Hz) with this result, we confirm the olefin geometry as *E*- olefin macrocycle (**1.8**).

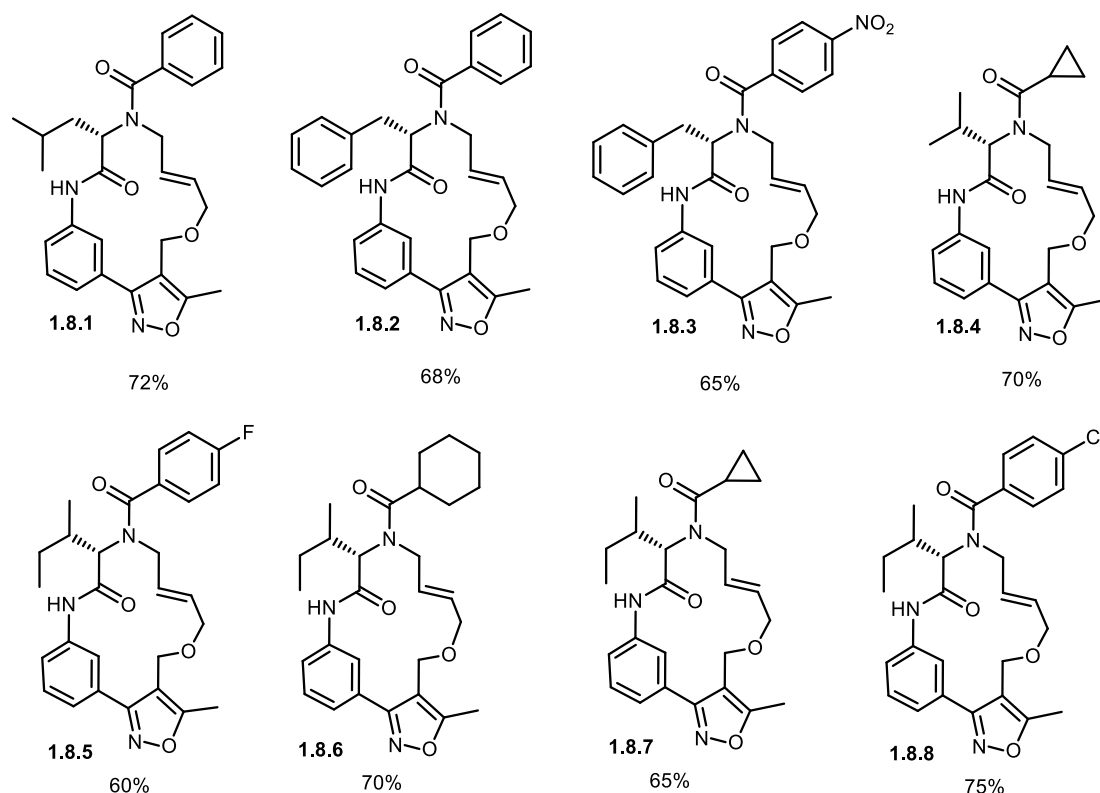


Figure 5: Structural Information of 8 Compounds Synthesized by this Approach

3.7 Retrosynthetic Analysis of Macrocycle B

In retrosynthetic analysis, macrocycle B (**F6.1**) was accessed through Ring closing reaction (RCM) of **F6.2** and this could be prepared from amino acid building block coupling with **F6.3**. Compound **F6.3** was obtained by an allylation of the free amine coming from the reduction of the nitro compound **F4.5**.

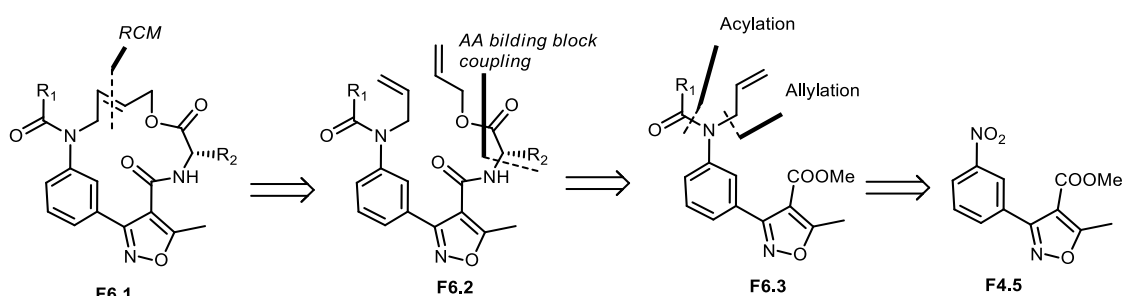
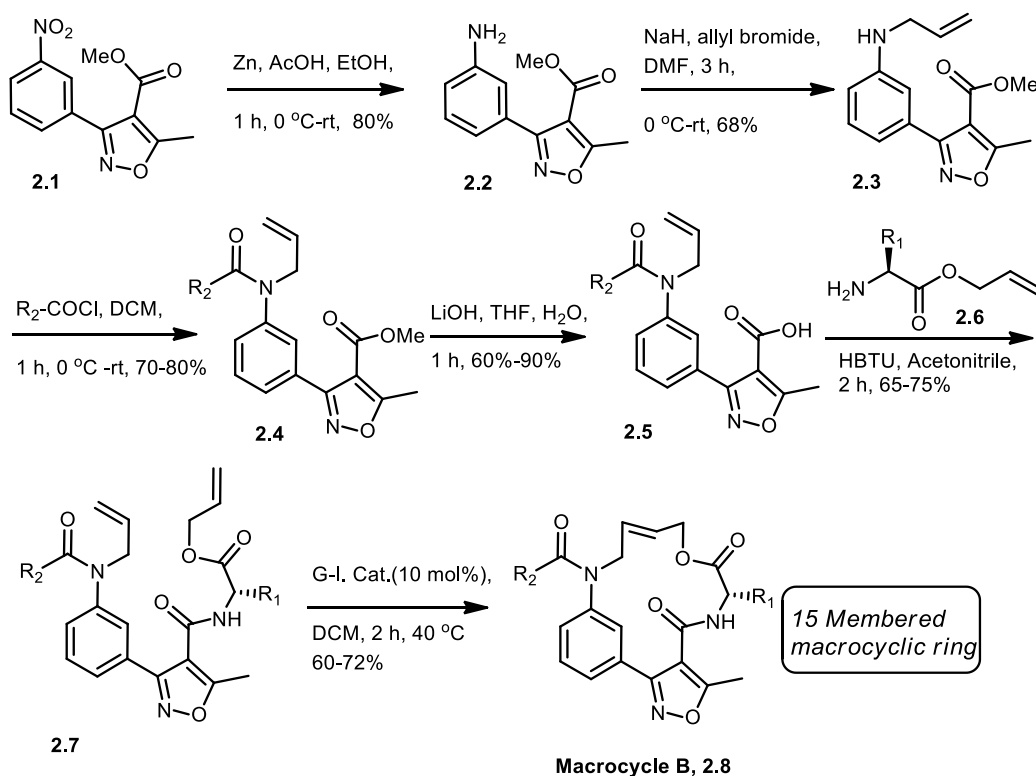


Figure 6: Retrosynthesis of Macrocycle-B

3.8 Synthesis of Macrocycle B Series

Our synthesis for the **macrocycle B** series is shown in **Scheme 2**. It was initiated with compound **2.1** as the key starting material. This was subjected to nitro group reduction using Zinc and Acetic acid conditions giving the corresponding amine.

Followed by the mono-allylation give allyl product (**2.3**), this compound was further amidated utilizing different acid chlorides, giving compound **2.4**.



Scheme 2: Synthesis of Macrocycle B Series Compounds (**2.8**)

It was hydrolyzed, giving the free carboxylic acid (**2.5**), which was then coupled with different amino acid derivatives (**2.6**), for obtaining compound **2.7**. As a precursor for testing our key ring closing metathesis-based macrocyclic ring formation, **2.7** were then taken further for this step.

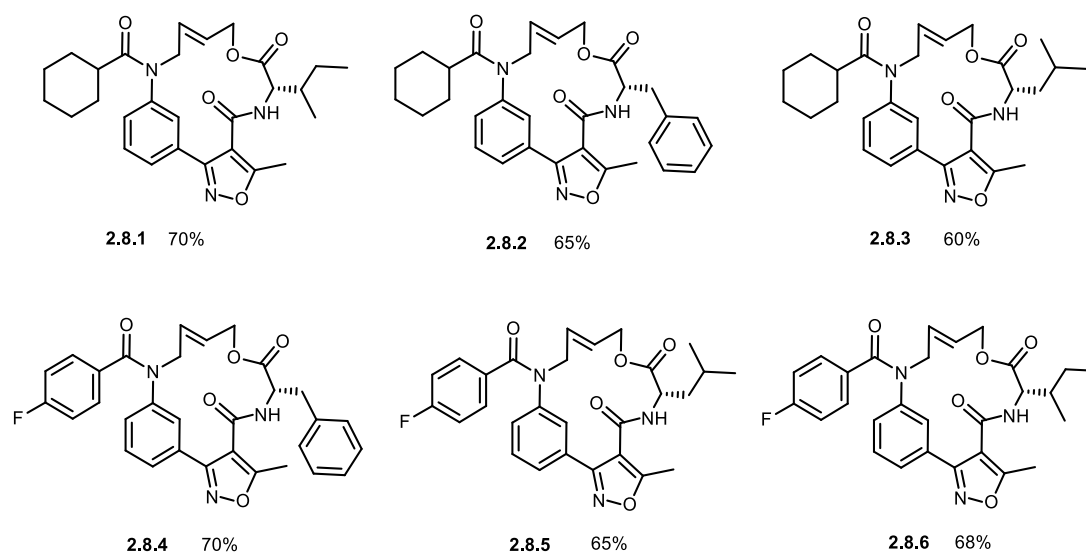


Figure 7: Synthesis of Macrocycle B Series Compounds

The macrocyclic ring synthesis worked-well by using Grubbs' 1st generation catalyst (10 mol %). This approach was tried on six substrates, and in all six cases, the 15-membered ring was formed easily, and also with the single, *E*-olefin geometry across the double bond (see **compound 2.8 structure**). The structural information on all six compounds is provided in (**Figure 6**). All the products at every step were thoroughly purified by flash column chromatography and extensively analyzed by ^1H and ^{13}C NMR and MS studies.

3.9 Biological Application using Isoxazole-derived Macrocyclic and Acyclic Compounds

The biological finding of utilizing novel isoxazole compounds for the neuro-regeneration on human (healthy donor /patient derived mesenchymal stem cells (MSCs) in collaboration with Transcell Biologics Hyderabad, researchers:

Isoxazole-derived compounds as the set of small molecules were screened on donated (from healthy donor) cord tissue derived MSCs' predictive platform as disclosed in Indian Patent application no. 201643028709 (dated August 23, 2016) with the positive control as cells exposed to Forskolin (note - the structure is not shown in this document) and the internal control as the cells exposed to known neuro-inductive medium (Promocell). The phenotypic changes obtained with active compounds are shown in (**Figure 8 and 9**). They were documented under the phase contrast inverted microscope of the representative the small molecule-induced neurogenesis on the proprietary predictive MSC platform.

This collaborative work is being submitted for obtaining an Indian patent and following this, we will be filing the US patent.

.

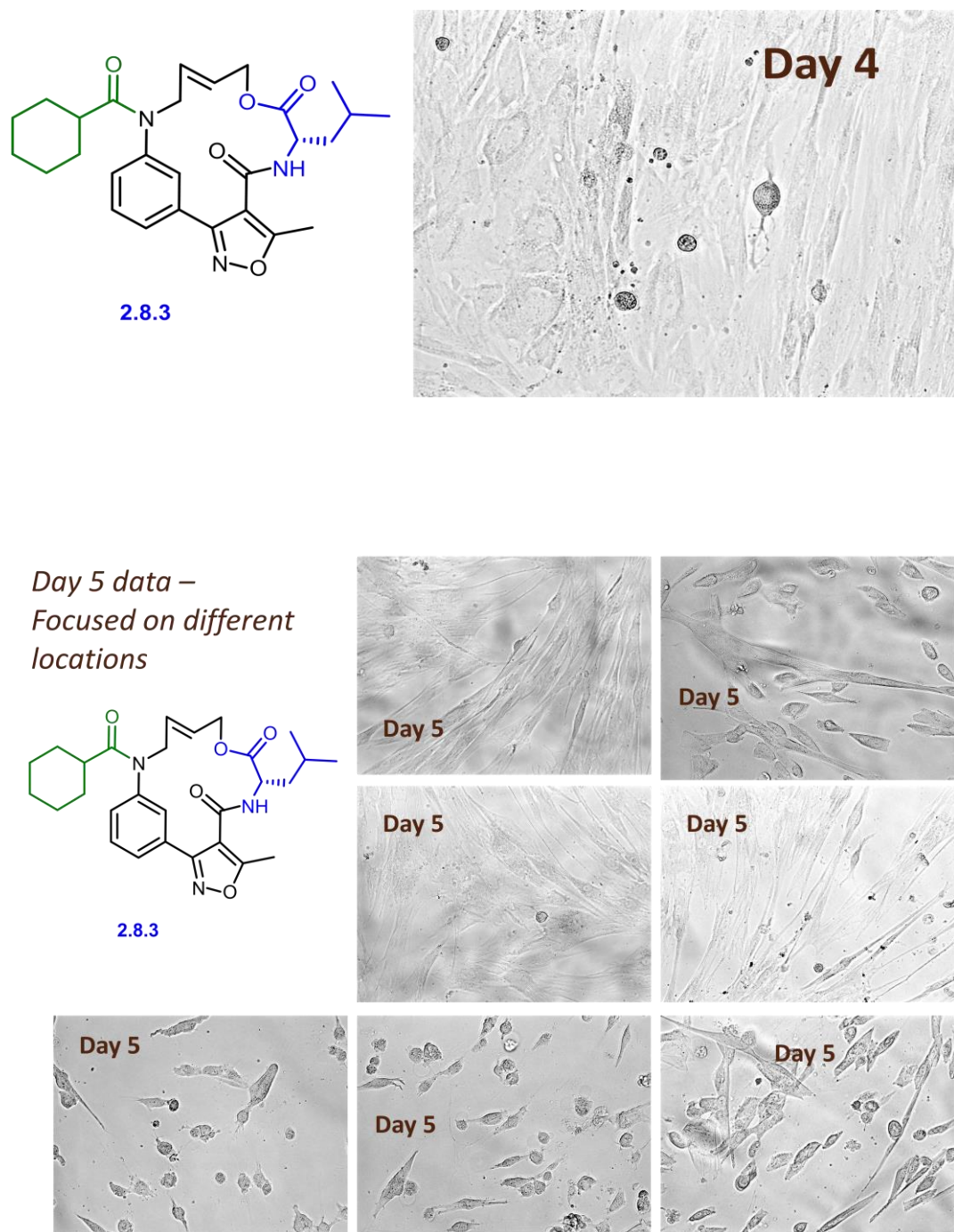


Figure 8: The Direct Conversion of Human Mesenchymal Stem Cells to Neurons by a Isoxazole-derived Macrocyclic compound, **2.8.3**

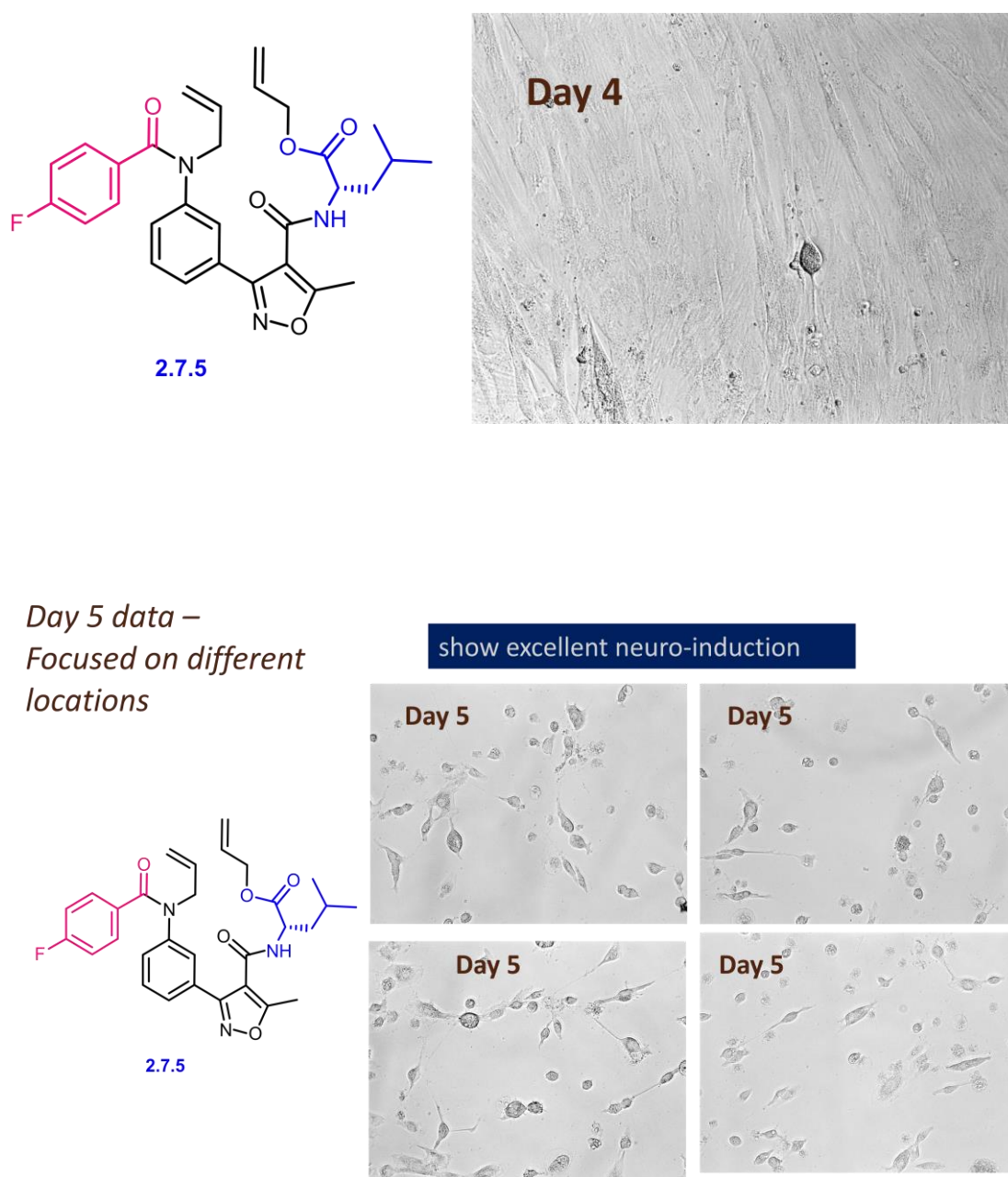
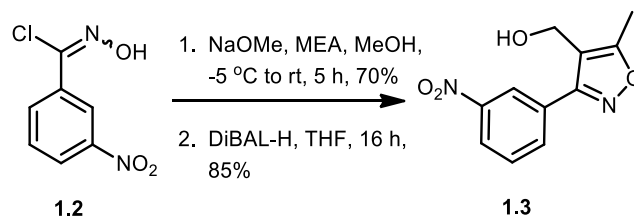


Figure 9: The Direct Conversion of Human Mesenchymal Stem Cells to Neurons by a Isoxazole-derived Precursor to a Macrocylic Compound, **2.7.5**

3.10 Experimental section

(5-methyl-3-(3-nitrophenyl) isoxazol-4-yl) methanol (1.3)



N-hydroxy-3-nitrobenzimidoyl chloride **1.2** (50.0 g, 251 mmol) and methyl acetoacetate (43.7 g, 376 mmol) were dissolved in methanol (1.1 L). The resultant solution was stirred for 30 minutes while the reaction mixture was cooled to -5 °C then, sodium methoxide (40.5 g, 750 mmol) was slowly added thereto. The resultant product was warmed up to room temperature, stirred for 4.5 hours, and vacuum-evaporated to remove methanol. Then, ethyl acetate (400 mL) was added. The resultant product was washed with water (500 mL) and saturated sodium chloride aqueous solution (400 mL), respectively, dried with anhydrous sodium sulfate, and vacuum-evaporated. Through column chromatography, a purified yellow solid required compound **S1**, (45.9 g) was obtained.

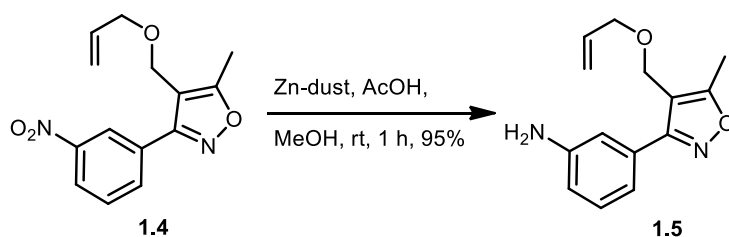
Molecular Formula: C₁₂H₁₀N₂O₅

R_f = 0.3 (20% ethyl acetate/hexane s)

LRMS: (ESI+) *m/z* = 263.06 [M+1]

Yield = 70%

To a solution of above ester **S1** (20 g, 76.3 mmol) in dry THF (250 mL) at 0 °C and under N₂ atmosphere, was added a 1.5 M solution of Diisobutylaluminium hydride in toluene (152 mL, 229 mmol) slowly. The resultant solution was allowed to stir at room temperature over night then was re cooled to 0 °C. And Rochelle's salt (200 mL) was added slowly followed by ethyl acetate (300 mL). An additional 250 mL of Rochelle's salt and ethyl acetate (500 mL) were added and the mixture was stirred at 0 °C For approximately 20 minutes and then at ambient temperature for approximately 4 hours. The mixture was filtered. Two layers were separated and the aqueous layer extracted with ethyl acetate. The combined organic layer dried over anhydrous sodium sulfate concentrated, purified by column chromatography to offered alcohol **1.3** as liquid (15.2 g, 85%).

3-(4-((allyloxy) methyl)-5-methylisoxazol-3-yl) aniline (1.5)

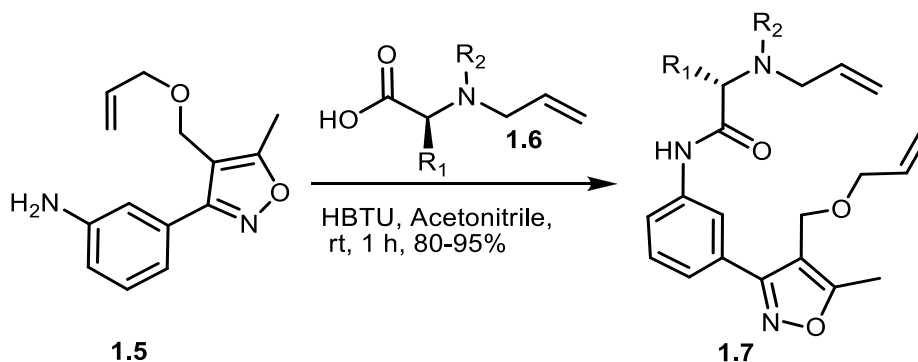
To a suspension of above 4-((allyl oxy) methyl)-5-methyl-3-(3-nitrophenyl) isoxazole compound **1.4** (14 g, 51.1 mmol) in methanol (100 mL), Zinc dust (66.5 g, 1021 mmol) Acetic acid (14.5 mL, 255 mmol) were added at 0 °C slowly and allowed to stir the reaction mixture for 1 hour. After completion of the reaction, mixture was passed through celite and concentrated, to leave a crude oil, which was purified by column chromatography (30% ethyl acetate/ hexane) to give the pure compound **1.5** (11.8 g) as pale yellow oil.

Molecular Formula: C₁₄H₁₆N₂O₂

R_f = 0.2 (30% ethyl acetate /hexane)

LRMS: (ESI+) *m/z* = 245.12[M+1]

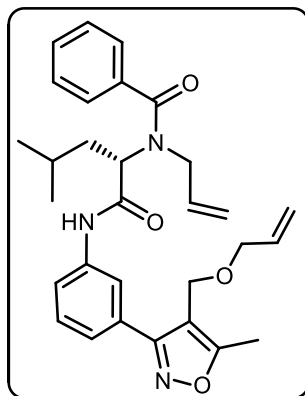
Yield = 95%

Coupling Reaction (1.7)

To a suspension of 3-(4-((allyloxy) methyl)-5-methylisoxazol-3-yl) aniline (**1.5**) (1 eq) in Acetonitrile (5 mL), Amino acid building block (**1.6**) (1.5 eq), HBTU (1.5 eq) were added at room temperature and allowed to stirred for 1 hour. After completion of the reaction monitored by TLC, quenched with sodium bicarbonate solution (5 mL) concentrated, and extracted with ethyl acetate (2x10 mL). Combined organic layer were washed with brine, dried over anhydrous sodium sulfate, filtered and

concentrated to leave a crude compound, which was purified by column chromatography to give the pure compound.

N-allyl-N-(1-(3-(4-(allyloxymethyl)-5-methylisoxazol-3-yl) phenyl amino)-4 methyl -1-oxopentan-2-yl) benzamide (1.7.1)



Molecular Formula: C₃₀H₃₅N₃O₄

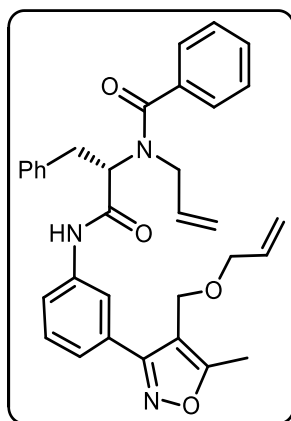
R_f = 0.3 (30% ethyl acetate /hexane)

LRMS: (ESI+) *m/z* = 502.26[M+1]

Yield = 87 %

¹H NMR (CDCl₃, 400 MHz) δ ppm: 9.39-9.31 (m, 1H), 7.88 (s, 1H), 7.76 (d, *J* = 8.00 Hz, 1H), 7.53 (d, *J*=7.6 Hz, 1H), 7.44 (m, 6H), 6.02-5.90 (m, 1H), 5.74-5.60 (m, 1H), 5.30 (dd, *J* = 17.25, 1.51 Hz, 1H), 5.20 (dd, *J* = 10.36, 1.13 Hz, 1H), 5.10-5.03 (m, 1H), 5.01-4.95 (m, 1H), 4.37 (s, 2H), 4.06 (d, *J* = 5.70 Hz, 2H), 3.93-3.84 (m, 1H), 2.49 (s, 3H), 2.06-1.92 (m, 2H), 1.77-1.65 (m, 1H), 1.61 (s, 2H), 1.03 (d, *J* = 7.35 Hz, 6H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 169.0, 162.3, 135.5, 134.3, 133.1, 133.0, 130.3, 129.9, 129.4, 128.5, 128.5, 127.1, 124.0, 121.0, 119.7, 118.6, 117.8, 117.6, 110.5, 71.0, 60.6, 50.7, 36.7, 25.0, 22.8, 22.3, 11.3.

N-allyl-N-(1-(3-(4-(allyloxymethyl)-5-methylisoxazol-3-yl) Phenyl amino)-1-oxo-3 phenylpropan-2-yl) benzamide(1.7.2)



Molecular Formula: C₃₃H₃₃N₃O₄

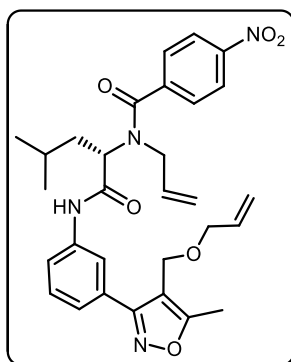
R_f = 0.3 (30% ethyl acetate /hexane)

LRMS: (ESI+) *m/z* = 536.25 [M+1]

Yield = 85 %

¹H NMR (CDCl₃, 400 MHz) δ ppm: 9.67 (s, 1H), 7.90 (s, 1H), 7.77 (d, *J* = 7.79 Hz, 1H), 7.55 (d, *J* = 7.62 Hz, 1H), 7.47-7.36 (m, 2H), 7.35-7.26 (m, 7H), 7.15 (d, *J* = 6.88 Hz, 2H), 6.02-5.90 (m, 1H), 5.53-5.36 (m, 1H), 5.30 (dd, *J* = 17.23, 1.34 Hz, 1H), 5.21 (d, *J* = 10.35 Hz, 1H), 5.09-4.98 (m, 2H), 5.0-4.9 (m, , 3H), 4.37 (s, 2H), 4.06 (d, *J* = 5.70 Hz, 2H), 3.8-3.7 (m, 2H), 3.6-3.5 (d, *J* = 10.74 Hz, 1H), 3.42 (dd, *J* = 14.06, 6.04 Hz, 1H), 2.48 (s, 3H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 169.0, 162.4, 138.4, 137.0, 135.3, 134.4, 132.6, 130.4, 129.9, 129.4, 129.2, 128.6, 128.4, 127.0, 126.9, 124.2, 121.2, 119.8, 119.0, 117.7, 110.6, 71.1, 63.2, 60.6, 52.5, 34.2, 11.3.

N-allyl-N-(1-(3-(4-(allyloxymethyl)-5-methylisoxazol-3-yl) phenylamino)-1-oxo-3-phenylpropan-2-yl)-4-nitrobenzamide (1.7.3)



Molecular Formula: C₃₃H₃₂N₄O₆

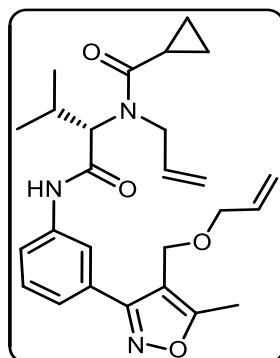
$R_f = 0.3$ (30% ethyl acetate /hexane)

LRMS: (ESI+) $m/z = 581.23$ [M+1]

Yield = 80 %

^1H NMR (CDCl_3 , 400 MHz) δ ppm: 9.28 (s, 1H), 8.16 (d, $J = 8.33$ Hz, 2H), 7.84 (s, 1H), 7.78 (d, $J = 7.68$ Hz, 1H), 7.56 (d, $J = 7.55$ Hz, 1H), 7.43 (t, $J = 7.92$ Hz, 1H), 7.36-7.29 (m, 5H), 7.27 (d, $J = 9.77$ Hz, 2H), 6.03-5.91 (m, 1H), 5.53-5.38 (m, 1H), 5.32 (dd, $J = 17.22, 1.29$ Hz, 1H), 5.22 (d, $J = 10.36$ Hz, 1H), 5.13 (d, $J = 7.18$ Hz, 1H), 5.04 (d, $J = 10.15$ Hz, 1H), 4.93 (d, $J = 17.20$ Hz, 1H), 4.37 (s, 2H), 4.07 (d, $J = 5.63$ Hz, 2H), 3.88 (dd, $J = 16.46, 5.14$ Hz, 1H), 3.68 (dd, $J = 16.64, 5.42$ Hz, 1H), 3.53-3.39 (m, 2H), 2.50 (d, $J = 4.03$ Hz, 3H); **^{13}C NMR** (CDCl_3 , 100MHz) δ ppm: 172.1, 169.1, 168.2, 162.3, 148.5, 141.4, 138.2, 136.6, 134.3, 132.3, 129.9, 129.5, 129.2, 128.8, 128.0, 127.2, 124.4, 123.7, 121.3, 119.8, 119.1, 117.7, 110.5, 71.0, 62.1, 60.6, 51.3, 34.4, 11.3.

N-allyl-N-(1-(3-(4-(allyloxymethyl)-5-methylisoxazol-3-yl) phenyl amino)-3-methyl-1-oxobutan-2-yl)cyclopropanecarboxamide (1.7.4)



Molecular Formula: $\text{C}_{26}\text{H}_{33}\text{N}_3\text{O}_4$

$R_f = 0.3$ (30% ethyl acetate /hexane)

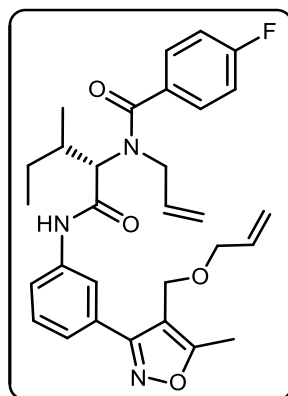
LRMS: (ESI+) $m/z = 452.3$ [M+1]

Yield = 75 %

^1H NMR (CDCl_3 , 400 MHz) δ ppm: 9.08-8.88 (m, 1H), 7.82 (s, 1H), 7.71 (d, $J = 8.01$ Hz, 1H), 7.51 (d, $J = 7.69$ Hz, 1H), 7.40 (t, $J = 7.91$ Hz, 1H), 6.02-5.90 (m, 1H), 5.90-5.78 (m, 1H), 5.30 (dd, $J = 17.24, 1.28$ Hz, 1H), 5.24-5.11 (m, 3H), 4.35 (s, 2H), 4.25 (dd, $J = 17.6, 5.68$ Hz, 1H), 4.12 (dd, $J = 17.2, 4.91$ Hz, 1H), 4.05 (d, $J = 6.4$ Hz, 2H), 2.48 (s, 3H), 2.38-2.28 (m, 1H), 1.80-1.72 (m, 1H), 1.60-1.40 (m, 1H), 1.166-1.04 (m, 2H), 1.01-0.98 (m, 5H), 0.99-0.79 (m, 5H); **^{13}C NMR** (CDCl_3 , 100MHz) δ ppm:

176.4, 169.0, 168.9, 162.4, 138.4, 134.3, 133.6, 129.8, 129.3, 124.0, 121.0, 119.7, 117.7, 117.3, 110.5, 71.0, 60.6, 29.7, 26.3, 19.9, 19.0, 12.4, 11.3, 8.7

N-allyl-N-((2S,3R)-1-(3-(4-(allyloxymethyl)-5-methylisoxazol-3-yl) Phenyl amino)-3-methyl-1-oxopentan-2-yl)-4-fluorobenzamide (1.7.5)



Molecular Formula: C₃₀H₃₄FN₃O₄

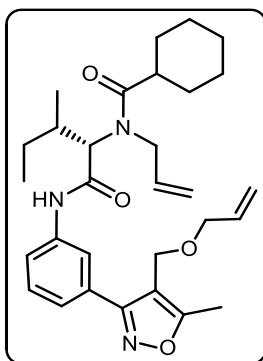
R_f = 0.3(30% ethyl acetate /hexane)

LRMS: (ESI+) *m/z* = 520.25 [M+1]

Yield = 85 %

¹H NMR (CDCl₃, 400 MHz) δ ppm: 9.75-9.43 (m, 1H), 7.90 (s, 1H), 7.78 (t, *J* = 7.86 Hz, 1H), 7.53 (d, *J* = 7.69 Hz, 1H), 7.48-7.44 (m, 2H), 7.20 (t, *J* = 8.0 Hz 1H), 7.10 (t, *J* = 8.45 Hz, 2H), 5.99-5.92 (m, 1H), 5.72-5.58 (m, 1H), 5.29 (dd, *J* = 17.25, 1.06 Hz, 1H), 5.20 (d, *J* = 10.38 Hz, 1H), 5.03 (d, *J* = 9.93 Hz, 1H), 4.93 (d, *J* = 16.98 Hz, 1H), 4.36 (s, 2H), 4.33-4.18 (m, 1H), 4.06 (d, *J* = 5.71 Hz, 2H), 3.99 (d, *J* = 5.43 Hz, 1H), 3.94-3.84 (m, 1H), 2.65-2.55 (m, 1H), 2.49 (s, 3H), 1.70-1.56 (m, 1H), 1.2-1.1 (m, 1H), 1.0-0.9 (m, 6H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 169.0, 162.4, 138.5, 138.5, 134.3, 132.6, 131.9, 129.9, 129.4, 129.4, 129.3, 124.1, 121.1, 121.1, 119.7, 117.6, 115.8, 115.5, 110.5, 71.1, 60.6, 32.0, 26.4, 25.1, 15.9, 15.3, 11.3, 10.4.

N-allyl-N-((2S,3R)-1-(3-(4-(allyloxymethyl)-5-methylisoxazol-3-yl) phenyl amino)-3-methyl-1-oxopentan-2-yl) cyclohexane carboxamide (1.7.6)



Molecular Formula: C₃₀H₄₁N₃O₄

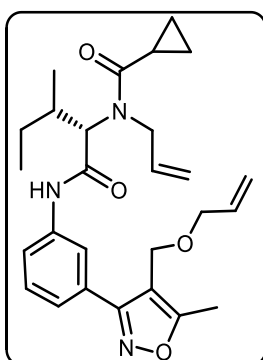
R_f = 0.3 (30% ethyl acetate /hexane)

LRMS: (ESI+) *m/z* = 508.3 [M+1]

Yield: 80%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 8.88-8.68 (m, 1H), 7.84-7.82 (m, 1H), 7.69-7.65 (m, 1H), 7.52-7.49 (m, 1H), 7.39 (t, *J* = 7.91 Hz, 1H), 6.01-5.90 (m, 1H), 5.81-5.70 (m, 1H), 5.32(dd, *J* = 10.00, 1.30 Hz, 1H), 5.21 (dd, *J* = 10.39, 1.34 Hz, 1H), 5.14 (d, *J* = 9.74 Hz, 2H), 5.11 (s, 1H), 4.55-4.47 (m, 1H), 4.35 (s, 2H), 4.04 (d, *J* = 5.74 Hz, 2H), 4.02 (d, *J* = 5.96 Hz, 1H), 3.99-3.94 (m, 1H), 2.48 (s, 3H), 2.39-2.28 (m, 1H), 1.82-1.71 (m, 4H), 1.59-1.45 (m, 3H), 1.44-1.34 (m, 1H), 1.17-1.03 (m, 1H), 1.00-0.81 (m, 9H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 179.3, 168.9, 162.3, 138.4, 134.4, 134.1, 133.9, 129.9, 129.3, 124.1, 121.0, 119.7, 117.5, 116.9, 110.5, 71.0, 60.6, 53.3, 41.8, 31.9, 30.1, 29.0, 26.4, 25.9, 25.5, 24.6, 15.9, 14.1, 11.2, 10.2.

N-allyl-N-((2S,3R)-1-(3-(4-(allyloxymethyl)-5-methylisoxazol-3-yl) phenyl amino)-3-methyl-1-oxopentane-2-yl) cyclopropane carboxamide (1.7.7)



Molecular Formula: C₂₇H₃₅N₃O₄

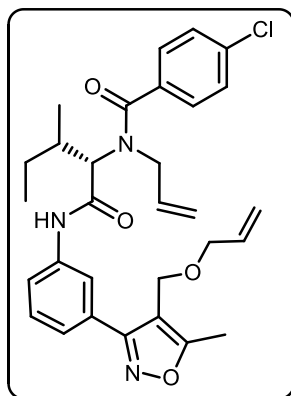
R_f = 0.3 (30% ethyl acetate /hexane)

LRMS: (ESI+) *m/z* = 466.26[M+1]

Yield = 82 %

¹H NMR (CDCl₃, 400 MHz) δ ppm: 9.07-8.74 (m, 1H), 7.82 (s, 1H), 7.69 (d, *J* = 8.05 Hz, 1H), 7.51 (d, *J* = 7.70 Hz, 1H), 7.39 (t, *J* = 7.90 Hz, 1H), 6.01-5.92 (m, 1H), 5.88-5.77 (m, 1H), 5.33-5.26 (m, 1H), 5.24-5.13 (m, 3H), 4.34 (s, 2H), 4.30-4.21 (m, 1H), 4.04-4.14 (m, 1H), 4.04 (d, *J* = 5.73 Hz, 2H), 2.48 (s, 3H), 2.32 (s, 1H), 1.80-1.72 (m, 1H), 1.64-1.40 (m, 1H), 1.02-0.90 (m, 5H), 0.90-0.80 (m, 5H), 1.17-1.02 (m, 2H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 176.4, 168.9, 162.3, 138.4, 134.3, 133.7, 129.8, 129.3, 124.0, 121.0, 121.0, 119.6, 117.6, 117.2, 110.4, 71.0, 60.5, 53.4, 32.0, 26.3, 24.8, 15.8, 12.3, 11.2, 11.2, 10.4, 8.6.

N-allyl-N-((2S,3R)-1-(3-(4-(allyloxymethyl)-5-methylisoxazol-3-yl) phenyl amino)-3-methyl-1-oxopentan-2-yl)-4-chlorobenzamide(1.7.8)



Molecular Formula: C₃₀H₃₄ClN₃O₄

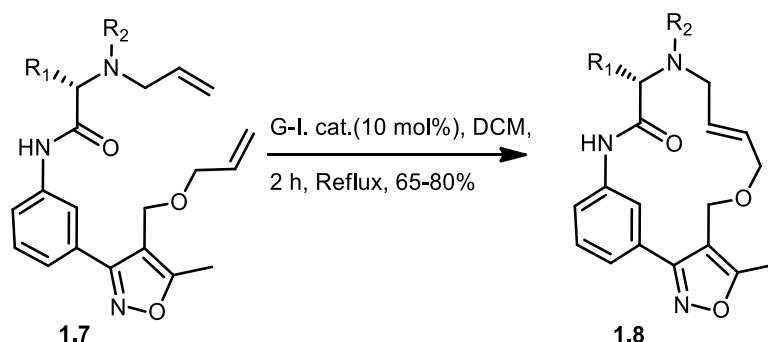
R_f = 0.3 (30% ethyl acetate /hexane)

LRMS: (ESI+) *m/z* = 536.22 [M+1]

Yield = 80 %

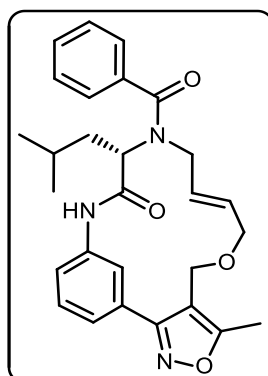
¹H NMR (CDCl₃, 400 MHz) δ ppm: 9.54 (s, 1H), 7.90 (s, 1H), 7.75 (d, *J* = 6.14 Hz, 1H), 7.53 (d, *J* = 7.67 Hz, 1H), 7.42 (d, *J* = 7.97 Hz, 1H), 7.39 (d, *J* = 11.96 Hz, 4H), 6.00-5.90 (m, 1H), 5.70-5.57 (m, 1H), 5.28 (dd, *J* = 17.2, 1.2, 1H), 5.20 (d, *J* = 10.33 Hz, 1H), 5.02 (d, *J* = 9.99 Hz, 1H), 4.92 (d, *J* = 17.02 Hz, 1H), 4.35 (s, 2H), 4.05 (d, *J* = 5.68 Hz, 2H), 4.02-3.85 (m, 2H), 2.62-2.52 (m, 1H), 2.48 (s, 3H), 1.69-1.55 (m, 1H), 1.32-1.22 (m, 3H), 1.07-0.92 (m, 6H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 173.4, 169.0, 168.7, 162.4, 138.5, 136.4, 134.3, 132.7, 132.6, 129.9, 129.4, 128.8, 128.6, 124.2, 121.2, 119.8, 119.0, 117.7, 110.5, 71.0, 60.6, 32.2, 29.7, 26.4, 25.1, 15.9, 11.3, 10.5.

RCM Reaction



To above bis allyl compound **1.7** (1eq) was taken in dry dichloromethane (50 mL) under nitrogen atmosphere and Grubb's 1st generation catalyst (0.1eq) was added and the reaction mixture was heated to 40 °C for 2 hours. After completion of the reaction, monitored by TLC, reaction mixture was concentrated and purified by column chromatography to give desired pure product **1.8**.

1.8.1



Molecular Formula: C₂₈H₃₁N₃O₄

R_f = 0.2 (30% ethyl acetate /hexane)

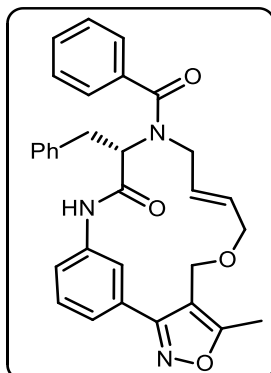
LRMS: (ESI+) *m/z* = 474.23 [M+1]

Yield = 75 %

¹H NMR (CDCl₃, 400 MHz) δ ppm: 8.73-8.43 (m, 1H), 8.38-8.22 (m, 1H), 7.73-7.39 (m, 8H), 6.56-6.18 (m, 1H), 5.75-5.25 (m, 2H), 4.74-4.45 (m, 1H), 4.39 (d, *J* = 10.64 Hz, 1H), 4.35-4.22 (m, 1H), 4.17 (s, 1H), 4.04-3.81 (m, 2H), 2.52-2.41 (m, 3H), 1.97-1.83 (m, 1H), 1.69 (d, *J* = 9.76 Hz, 2H), 1.10-0.80 (m, 6H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 162.6, 137.2, 137.1, 136.8, 135.3, 131.2, 130.3, 129.2, 128.6, 128.4,

127.1, 126.1, 124.6, 123.7, 119.7, 117.0, 110.5, 70.4, 29.6, 24.8, 23.3, 22.6, 21.9, 14.1, 11.0.

1.8.2



Molecular Formula: C₃₁H₂₉N₃O₄

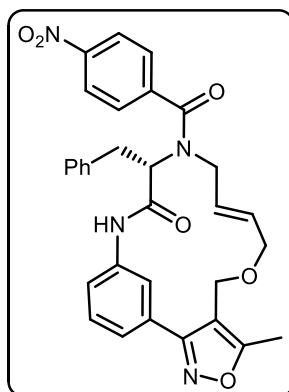
R_f = 0.2 (30% ethyl acetate /hexane)

LRMS: (ESI+) *m/z* = 508.2 [M+1]

Yield = 65 %

¹H NMR (CDCl₃, 400 MHz) δ ppm: 8.79-8.43 (m, 1H), 8.32-8.22 (m, 1H), 7.68-7.60 (m, 1H), 7.54-7.37 (m, 1H), 7.38-7.14 (m, 8H), 6.98 (dd, *J* = 21.98, 7.06 Hz, 2H), 6.85 (d, *J* = 7.16 Hz, 1H), 6.49-6.20 (m, 1H), 5.75-5.45 (m, 2H), 4.93-4.58 (m, 1H), 4.40 (t, *J* = 10.8 Hz, 1H), 4.30(t, *J*=10.8 Hz, 1H) 4.12-3.89 (m, 3H), 3.66-3.58 (m, 1H), 3.48-3.25 (m, 1H), 2.47 (d, *J* = 21.67 Hz, 3H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 174.2, 169.2, 168.3, 162.6, 136.9, 136.5, 135.1, 130.8, 130.2, 130.1, 129.8, 129.3, 129.0, 128.7, 128.5, 126.9, 126.8, 124.9, 123.7, 120.2, 110.6, 70.5, 67.3, 62.1, 47.0, 33.3, 29.6.

1.8.3



Molecular Formula: C₃₁H₂₈N₄O₆

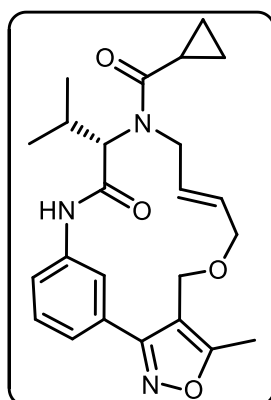
$R_f = 0.2$ (30% Ethyl acetate /Hexane)

LRMS: (ESI+) $m/z = 553.20$ [M+1]

Yield = 70 %

^1H NMR (CDCl_3 , 400 MHz) δ ppm: 8.40 (d, $J = 23.31$ Hz, 1H), 8.28-8.20 (m, 1H), 8.15 (d, $J = 8.54$ Hz, 1H), 8.05 (d, $J = 8.65$ Hz, 1H), 7.63 (t, $J = 6.4$ Hz, 1H), 7.56-7.41 (m, 1H), 7.41-7.27 (m, 4H), 7.16 (dd, $J = 23.31, 8.30$ Hz, 1H), 7.09 (d, $J = 8.52$ Hz, 1H), 6.97 (d, $J = 7.20$ Hz, 1H), 6.89 (d, $J = 8.25$ Hz, 1H), 6.52-6.24 (m, 1H), 5.83-5.42 (m, 2H), 4.72-4.59 (m, 1H), 4.40 (dd, $J = 18.32, 9.46$ Hz, 1H), 4.35-4.27 (m, 1H), 4.19-4.10 (m, 1H), 4.05-3.92 (m, 2H), 3.74-3.61 (m, 1H), 3.47-3.31 (m, 1H), 2.52-2.44 (m, 3H); **^{13}C NMR** (CDCl_3 , 100MHz) δ ppm: 172.0, 168.5, 148.5, 141.1, 137.1, 136.6, 136.5, 129.4, 129.0, 128.8, 127.9, 127.1, 126.3, 125.1, 124.9, 123.9, 123.6, 120.3, 110.5, 110.3, 110.0, 70.4, 67.5, 62.1, 60.5, 33.3, 11.1.

1.8.4



Molecular Formula: $\text{C}_{24}\text{H}_{29}\text{N}_3\text{O}_4$

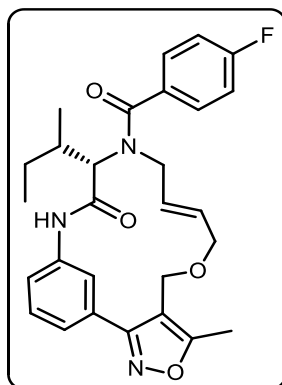
$R_f = 0.2$ (30% Ethyl acetate /Hexane)

LRMS: (ESI+) $m/z = 424.21$ [M+1]

Yield = 63 %

^1H NMR (CDCl_3 , 400 MHz) δ ppm: 8.24 (s, 1H), 8.07 (s, 1H), 7.64 (d, $J = 7.79$ Hz, 1H), 7.37 (t, $J = 7.87$ Hz, 1H), 7.00 (d, $J = 7.97$ Hz, 1H), 5.86-5.76 (m, 2H), 4.92-4.84 (m, 1H), 4.61 (d, $J = 10.76$ Hz, 1H), 4.32 (d, $J = 17.2$, 1H), 4.18-4.04 (m, 3H), 3.99 (dd, $J = 11.73, 3.59$ Hz, 1H), 2.45 (s, 3H), 1.76-1.70 (m, 1H), 1.27-1.24 (m, 1H), 1.10-0.98 (m, 5H), 0.89-0.80 (m, 5H); **^{13}C NMR** (CDCl_3 , 100MHz) δ ppm: 175.7, 168.2, 161.5, 136.9, 129.8, 129.1, 127.9, 124.5, 123.1, 122.6, 110.7, 71.0, 61.1, 29.7, 25.7, 19.8, 18.1, 18.1, 12.0, 11.1, 8.9, 8.8.

1.8.5



Molecular Formula: $C_{28}H_{30}FN_3O_4$

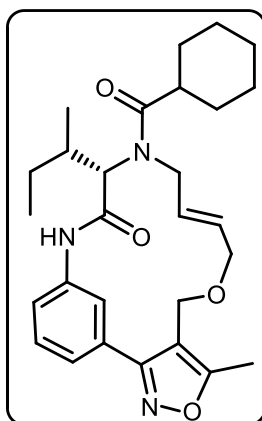
R_f = 0.2 (30% Ethyl acetate /Hexane)

LRMS: (ESI+) m/z = 492.22[M+1]

Yield = 68 %

^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 8.65-8.38 (m, 1H), 8.31 (s, 1H), 7.64 (d, J = 7.35 Hz, 1H), 7.48-7.36 (m, 3H), 7.18-7.02 (m, 3H), 5.67-5.64 (m, 1H), 5.47-5.35 (m, 1H), 5.02-4.79 (m, 1H), 4.55-4.46 (m, 1H), 4.19-4.04 (m, 2H), 4.01-3.80 (m, 3H), 2.44 (s, 3H), 1.68-1.52 (m, 1H), 1.30-1.23 (m, 2H), 1.01 (m, 6H); **^{13}C NMR** ($CDCl_3$, 100MHz) δ ppm: 173.3, 168.3, 168.2, 162.4, 162.2, 137.0, 136.9, 131.7, 129.9, 129.4, 129.3, 129.2, 124.7, 115.9, 115.7, 110.7, 70.6, 61.7, 31.1, 29.7, 26.7, 16.0, 11.0.

1.8.6



Molecular Formula: $C_{28}H_{37}N_3O_4$

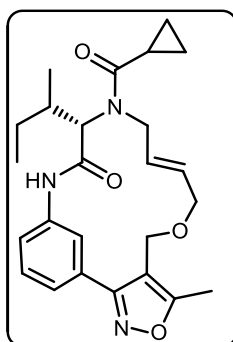
R_f = 0.2 (30% Ethyl acetate /Hexane)

LRMS: (ESI+) m/z = 480.28 [M+1]

Yield = 80 %

¹H NMR (CDCl₃, 400 MHz) δ ppm: 8.23 (s, 1H), 8.17-8.07 (m, 1H), 7.65 (d, *J* = 7.78 Hz, 1H), 7.37 (t, *J* = 7.85 Hz, 1H), 7.02 (d, *J* = 7.69 Hz, 1H), 5.74 (d, *J* = 2.54 Hz, 2H), 5.02-4.92 (m, 1H), 4.60 (d, *J* = 10.69 Hz, 1H), 4.09 (s, 4H), 3.98-3.20 (m, 1H), 2.45 (s, 3H), 2.33-2.15 (m, 1H), 1.84-1.71 (m, 4H), 1.70-1.57 (m, 3H), 1.52-1.33 (m, 2H), 1.29-1.18 (m, 3H), 1.16-1.01 (m, 1H), 1.00-0.93 (m, 3H), 0.87 (t, *J* = 7.38 Hz, 2H), 0.80 (d, *J* = 6.72 Hz, 1H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 178.5, 168.2, 161.4, 136.8, 130.8, 129.7, 129.1, 127.0, 124.4, 123.1, 122.6, 110.6, 70.9, 61.2, 53.4, 41.6, 31.4, 30.6, 28.7, 26.5, 26.1, 25.6, 25.3, 15.7, 11.4, 11.0, 10.1.

1.8.7



Molecular Formula: C₂₅H₃₁N₃O₄

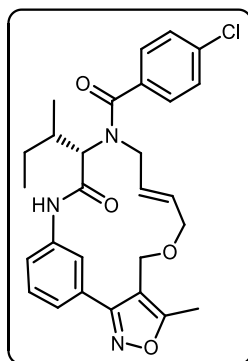
R_f = 0.2 (30% Ethyl acetate /Hexane)

LRMS: (ESI+) *m/z* = 438.23[M+1]

Yield = 70 %

¹H NMR (CDCl₃, 400 MHz) δ ppm: 8.25 (s, 1H), 8.11-8.02 (m, 1H), 7.65 (d, *J* = 7.85 Hz, 1H), 7.37 (d, *J* = 7.86 Hz, 1H), 7.00 (d, *J* = 8.00 Hz, 1H), 5.81 (s, 2H), 5.02-4.92 (m, 1H), 4.61 (d, *J* = 10.40 Hz, 1H), 4.38-4.28 (m, 1H), 4.19-4.05 (m, 3H), 4.02-3.95 (m, 1H), 2.45 (s, 3H), 1.76-1.70 (m, 1H), 1.56-1.48 (m, 1H), 1.08-1.03 (m, 3H), 0.99-0.94 (m, 3H), 0.92-0.86 (m, 3H), 0.85-0.80 (m, 3H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 175.7, 168.2, 136.9, 136.9, 129.8, 129.1, 127.7, 124.4, 123.0, 122.4, 122.4, 110.7, 71.0, 61.1, 44.6, 31.5, 29.7, 24.2, 22.7, 15.8, 12.0, 11.4, 11.1, 10.4, 8.9.

1.8.8



Molecular Formula: C₂₈H₃₀ClN₃O₄

R_f = 0.2 (30% Ethyl acetate /Hexane)

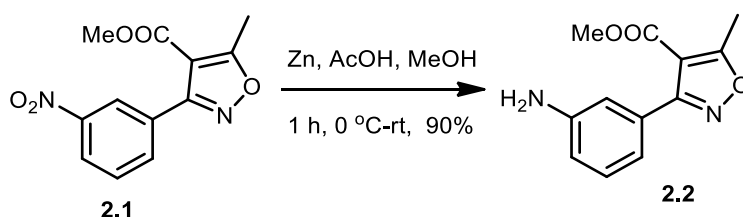
LRMS: (ESI+) *m/z* = 509.19 [M+1]

Yield = 67 %

¹H NMR (CDCl₃, 400 MHz) δ ppm: 8.60-8.40 (m, 1H), 8.30 (s, 1H), 7.63 (d, *J* = 7.45 Hz, 1H), 7.45-7.33 (m, 5H), 7.03 (s, 1H), 5.74-5.64(m, 1H), 5.44-5.34(m, 1H), 4.5-4.43 (m, 1H), 4.12-4.05 (m, 2H), 4.00-3.81 (m, 3H), 2.43 (s, 3H), 2.00-1.88 (m, 1H), 1.65-1.50 (m, 1H), 1.26 (dd, *J* = 10.73, 3.55 Hz, 2H), 1.05 (d, *J* = 6.15 Hz, 2H), 0.99 (dd, *J* = 13.27, 6.46 Hz, 4H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 173.1, 168.2, 162.1, 136.9, 136.4, 136.3, 134.1, 134.1, 129.9, 129.2, 128.9, 128.5, 128.5, 124.7, 110.7, 70.7, 61.7, 31.1, 26.6, 24.5, 21.0, 15.9, 14.2, 11.4, 11.0, 10.1.

Experimental Section for Macrocycle-B

Methyl 3-(3-aminophenyl)-5-methylisoxazole-4-carboxylate (2.2)



To a suspension of above methyl 5-methyl-3-(3-nitrophenyl) isoxazole-4-carboxylate compound **2.1** (10 g, 38.1 mmol) in Methanol (100 mL), Zinc dust (49.8 g, 762 mmol), Acetic acid (10.8 mL, 190.5 mmol) was added at 0 °C slowly and allowed to stir the reaction mixture for 1 hour. After completion of the reaction mixture was passed through celite and concentrated, to leave a crude oil, which was purified by column chromatography (40% ethyl acetate/ hexane) to give the pure compound **2.2** (7.96 g) as colour less oil.

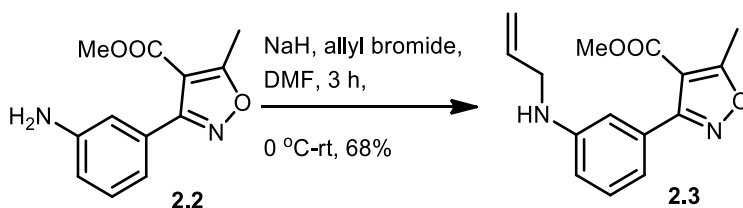
Molecular Formula: C₁₂H₁₂N₂O₃

R_f = 0.2 (40% Ethyl acetate /Hexane)

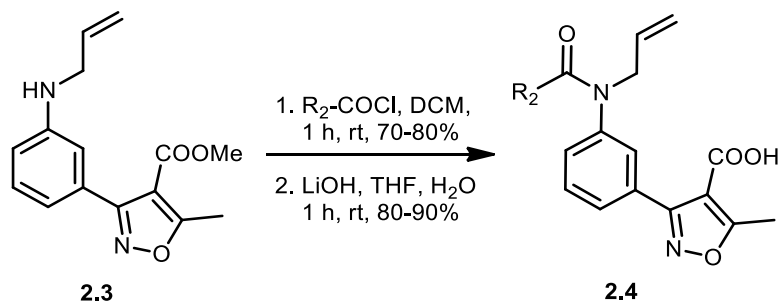
LRMS: (ES⁺) *m/z* = 233.3 [M+1]

Yield = 90%

Methyl 3-(3-(allylamino) phenyl)-5-methylisoxazole-4-carboxylate (2.3)

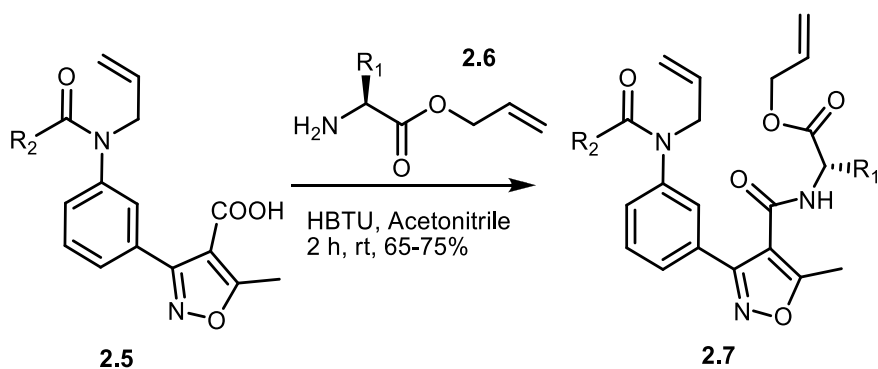


To the solution of compound **2.2** methyl 3-(3-aminophenyl)-5-methylisoxazole-4-carboxylate (7 g, 30.1 mmol) in dry DMF (20 mL) at 0 °C, NaH (1.08 g, 45.2 mmol) followed by allyl bromide (3.13 mL, 36.2 mmol) were added under N₂ atmosphere. Then reaction was stirred for 2 hours at room temperature. After completion of the reaction, reaction mixture was quenched with ammonium chloride solution (20 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were subsequently washed with brine (20 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated in vacuo and purified by flash chromatography using 20% ethyl acetate/Hexane to yield compound **2.3**, (5.58 g) as solid.

Molecular Formula: C₁₅H₁₆N₂O₃**R_f** = 0.3 (20% Ethyl acetate /Hexane)**LRMS:** (ES⁺) *m/z* =273.1 [M+1]**Yield** = 68%

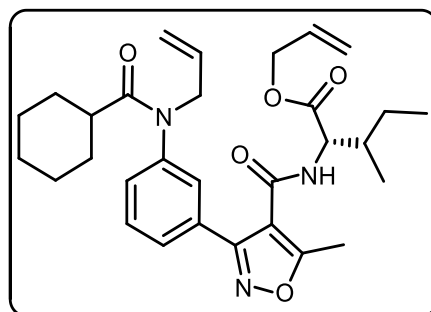
To a suspension of compound **2.3** (1 mmol) in DCM (10 mL), acid chloride (1.5 mmol) was added at 0 °C and allowed to stir for 1 hour. After completion of the reaction, reaction mixture was quenched with sodium bicarbonate solution (5 mL), and extracted with ethyl acetate (3X20 mL). Combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to leave crude product and which was purified by column chromatography to give pure amide **2.4**.

To the above ester compound **2.4** (1 mmol) in THF:H₂O (2:1) (10 mL) was added lithium hydroxide (5 mmol) at room temperature, after 1 hour the reaction was completed monitored by TLC and base was neutralized with aq. 10% HCL. The reaction mixture was diluted with ethyl acetate and water. The organic layer was separated and dried over anhydrous sodium sulfate, concentrated to rota vapour to get crude acid **2.5**.



To the above Acid compound **2.5** (100 mg, 0.4 mmol) in Acetonitrile (5 mL), Amino acid building block (0.612 mmol), HBTU (0.81 mmol) were added at room temperature and allowed to stirred for 2 hours. After completion of the reaction monitored by TLC, quenched with sodium bicarbonate solution (5 mL) concentrated, and extracted with ethyl acetate (2x10 mL). Combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to leave a crude compound, which was purified by column chromatography to give the pure compounds **2.7.(1-6)**.

(2R,3S)-allyl 2-(3-(3-(N-allylcyclohexanecarboxamido)phenyl)-5-methylisoxazole-4-carboxamido)-3-methylpentanoate (2.7.1)



Molecular Formula: C₃₀H₃₉N₃O₅

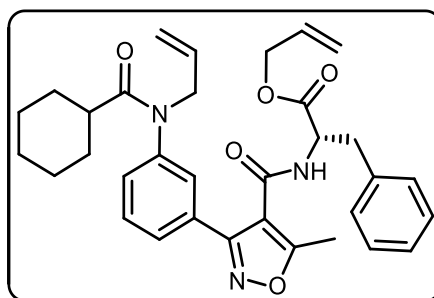
R_f = 0.3 (30% Ethyl acetate /Hexane)

LRMS: (ES+) *m/z* =522.3 [M+1]

Yield = 70%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.63 (d, *J* = 7.32 Hz, 1H), 7.55-7.49 (m, 2H), 7.29 (d, *J* = 7.56 Hz, 1H), 5.99 (d, *J* = 8.23 Hz, 1H), 5.93-5.79 (m, 2H), 5.32 (dd, *J* = 17.19, 1.24 Hz, 1H), 5.26 (d, *J* = 10.42 Hz, 1H), 5.08 (t, *J* = 14.30 Hz, 2H), 4.67 (dd, *J* = 8.31, 4.35 Hz, 1H), 4.60 (t, *J* = 6.42 Hz, 2H), 4.35-4.24 (m, 2H), 2.71 (s, 3H), 2.24-2.14 (m, 1H), 1.92-1.82 (m, 1H), 1.66 (d, *J* = 9.12 Hz, 4H), 1.55 (dd, *J* = 16.23, 7.54 Hz, 3H), 1.30-1.25 (dd, *J* = 10.38, 1.96 Hz, 1H), 1.18 (d, *J* = 12.84 Hz, 1H), 1.01-0.89 (m, 3H), 0.89-0.79 (m, 6H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 171.1, 161.2, 159.5, 143.6, 133.2, 133.1, 131.3, 130.1, 130.0, 128.5, 128.3, 128.3, 119.2, 117.8, 111.2, 66.0, 56.5, 52.2, 41.7, 37.7, 29.5, 29.5, 25.6, 25.4, 25.4, 25.1, 15.6, 13.0, 11.6.

(2S)-allyl 2-benzyl-4-(3-(3-(1-cyclohexyl-1-oxopent-4-en-2-yl) phenyl)-5-methyl isoxazol-4-yl)-4-oxobutanoate (2.7.2)



Molecular Formula: C₃₃H₃₇N₃O₅

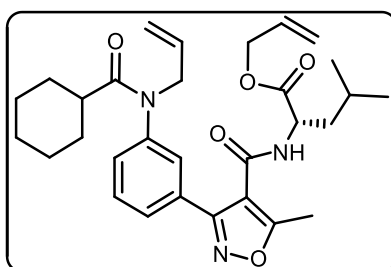
R_f = 0.3 (30% Ethyl acetate /Hexane)

LRMS: (ES+) *m/z* = 556. 2 [M+1]

Yield: 68%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.48-7.39 (m, 2H), 7.34 (t, *J* = 7.73 Hz, 1H), 7.25 (d, *J* = 5.15 Hz, 4H), 7.01 (s, 2H), 6.01 (d, *J* = 7.05 Hz, 1H), 5.98-5.78 (m, 2H), 5.31 (d, *J* = 17.29 Hz, 1H), 5.26 (d, *J* = 10.45 Hz, 1H), 5.11-5.02 (m, 2H), 4.94-4.88 (m, 1H), 4.60 (d, *J* = 5.71 Hz, 2H), 4.24 (d, *J* = 5.22 Hz, 2H), 3.24 (dd, *J* = 14.05, 4.8 Hz, 1H), 3.08 (dd, *J* = 13.60, 6.0 Hz, 1H), 2.57 (s, 3H), 2.23-2.12 (m, 1H), 1.63 (s, 4H), 1.57-1.47 (m, 3H), 1.19-1.11 (m, 1H), 0.99 (s, 2H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 175.8, 172.5, 170.7, 161.0, 159.7, 143.4, 135.5, 133.2, 131.2, 129.9, 129.4, 129.1, 128.7, 128.3, 128.0, 128.0, 127.3, 119.4, 117.7, 111.0, 66.3, 53.4, 52.2, 41.6, 37.3, 29.5, 25.6, 25.3, 12.7.

(R)-allyl 2-(3-(3-(N-allylcyclohexanecarboxamido) phenyl)-5-methylisoxazole-4-carboxamido)-4-methylpentanoate (2.7.3)



Molecular Formula: C₃₀H₃₉N₃O₅

R_f = 0.3 (30% Ethyl acetate /Hexane)

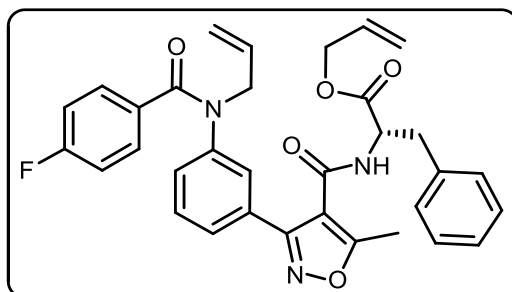
LRMS: (ES+) *m/z* = 522.2 [M+1]

Yield: 82%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.63 (d, *J* = 6.98 Hz, 1H), 7.53-7.48 (m, 2H), 7.28 (d, *J* = 7.67 Hz, 1H), 5.94-5.78 (m, 3H), 5.32 (d, *J* = 17.23 Hz, 1H), 5.25 (d, *J*

= 10.38 Hz, 1H), 5.13-5.01 (d, J = 10.57 Hz, 2H), 4.72-4.62 (m, 1H), 4.60 (d, J = 5.26 Hz, 2H), 4.28 (t, J = 7.01 Hz, 2H), 2.70 (s, 3H), 2.24-2.12 (m, 1H), 1.91-1.71 (m, 1H), 1.70-1.63 (m, 4H), 1.59-1.47 (m, 4H), 1.41 (m, 1H), 1.21-1.10 (m, 1H), 1.04-0.97 (m, 2H), 0.90 (dd, J = 11.29, 6.36 Hz, 6H); ^{13}C NMR (CDCl_3 , 100MHz) δ ppm: 175.8, 173.3, 172.2, 161.3, 159.5, 143.5, 133.1, 131.4, 130.1, 129.9, 128.5, 128.2, 119.0, 117.8, 111.1, 110.0, 66.1, 52.2, 50.9, 41.7, 41.3, 29.5, 29.4, 25.6, 25.4, 25.0, 22.7, 21.9, 12.9

(R)-allyl 2-(3-(3-(N-allyl-4-fluorobenzamido) phenyl)-5-methylisoxazole-4-carbox amido)-3-phenylpropanoate (2.7.4)



Molecular Formula: $\text{C}_{33}\text{H}_{30}\text{FN}_3\text{O}_5$

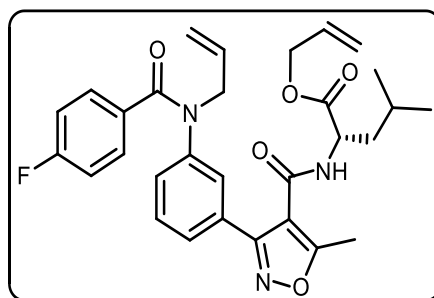
R_f = 0.3 (30% Ethyl acetate /Hexane)

LRMS: (ES+) m/z = 568.2 [M+1]

Yield: 93%

^1H NMR (CDCl_3 , 400 MHz) δ ppm: 7.47 (s, 1H), 7.35-7.32 (m, 2H), 7.25-7.22 (d, J = 2.60 Hz, 4H), 7.13 (t, J = 7.85 Hz, 1H), 7.03-6.96 (m, 3H), 6.85 (t, J = 8.67 Hz, 2H), 6.01-5.81 (m, 3H), 5.32 (dd, J = 17.20, 1.29 Hz, 1H), 5.26 (d, J = 9.20 Hz, 1H), 5.24-5.15 (m, 2H), 4.92 (q, J = 6.61 Hz, 1H), 4.61 (d, J = 5.87 Hz, 2H), 4.50 (d, J = 5.83 Hz, 2H), 3.22 (dd, J = 14.09, 5.71 Hz, 1H), 3.4 (dd, J = 14.09, 5.71 Hz, 1H), 2.56 (s, 3H); ^{13}C NMR (CDCl_3 , 100MHz) δ ppm: 173.2, 170.8, 169.0, 164.6, 162.1, 161.0, 159.6, 144.2, 135.5, 132.8, 131.2, 131.2, 129.6, 129.5, 129.1, 128.7, 127.3, 127.0, 126.9, 119.4, 117.9, 115.1, 114.9, 110.9, 66.3, 53.4, 53.3, 37.3, 12.7.

(R)-allyl 2-(3-(3-(N-allyl-4-fluorobenzamido) phenyl)-5-methylisoxazole-4-carbox amido) - 4-methylpentanoate (2.7.5)



Molecular Formula: C₃₀H₃₂FN₃O₅

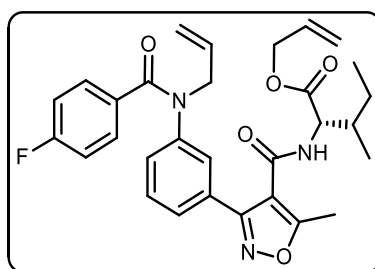
R_f = 0.3 (30% Ethyl acetate /Hexane)

LRMS: (ES+) *m/z* =534.2[M+1]

Yield: 88%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.53 (s, 1H), 7.44 (d, *J* = 7.70 Hz, 1H), 7.36 (dd, *J* = 8.43, 5.46 Hz, 2H), 7.30 (d, *J* = 7.60 Hz, 1H), 7.02 (d, *J* = 7.79 Hz, 1H), 6.87 (t, *J* = 8.58 Hz, 2H), 5.92-5.80 (m, *J* = 3H), 5.32 (d, *J* = 17.17 Hz, 1H), 5.21 (dd, *J* = 29.75, 10.93 Hz, 3H), 4.72-4.63 (m, 1H), 4.61 (s, 2H), 4.54 (d, *J* = 5.60 Hz, 2H), 2.68 (s, 3H), 1.64-1.47 (m, 2H), 1.38 (s, 1H), 0.90 (dd, *J* = 14.36, 6.31 Hz, 6H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 173.4, 172.2, 169.1, 162.1, 161.3, 159.4, 144.3, 132.7, 131.5, 131.5, 131.3, 131.2, 131.1, 129.8, 129.5, 129.3, 127.1, 119.1, 118.1, 115.2, 115.0, 111.1, 66.1, 53.3, 50.9, 41.2, 25.0, 22.7, 21.8, 12.8.

(2R,3S)-allyl 2-(3-(4-fluorobenzamido)phenyl)-5-methylisoxazole-4-carbox amido)-3-methylpentanoate (2.7.6)



Molecular Formula: C₃₀H₃₂FN₃O₅

R_f = 0.3(30% Ethyl acetate /Hexane)

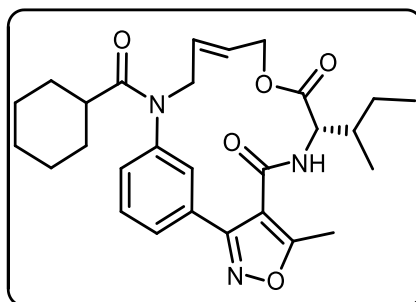
LRMS: (ES+) *m/z* =534.2 [M+1]

Yield: 82%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.51 (s, 1H), 7.44 (d, *J* = 7.69 Hz, 1H), 7.36 (dd, *J* = 8.50, 5.45 Hz, 2H), 7.30-7.25 (m, 1H), 7.03 (d, *J* = 7.78 Hz, 1H), 6.87 (t, *J* = 8.61

Hz, 2H), 6.03-5.83 (m, 3H), 5.36-5.15 (m, 4H), 4.66 (dd, $J = 8.24, 4.46$ Hz, 1H), 4.64-4.58 (m, 2H), 4.54 (d, $J = 5.71$ Hz, 2H), 2.69 (s, 3H), 1.84 (s, 1H), 1.25 (d, $J = 7.61$ Hz, 1H), 0.91 (dd, $J = 8.14, 5.48$ Hz, 1H), 0.83 (q, $J = 6.81$ Hz, 6H); ^{13}C NMR (CDCl₃, 100MHz) δ ppm: 173.4, 172.2, 169.1, 162.1, 161.3, 159.4, 144.3, 132.7, 131.5, 131.5, 131.3, 131.2, 131.1, 129.8, 129.5, 129.3, 127.1, 119.1, 118.1, 115.2, 115.0, 111.1, 66.1, 53.3, 50.9, 41.2, 25.0, 22.7, 21.8, 12.8.

2.8.1



Molecular Formula: C₂₈H₃₅N₃O₅

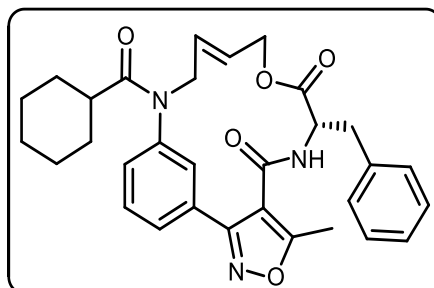
R_f = 0.2 (30% Ethyl acetate /Hexane)

LRMS: (ES+) $m/z = 494.2[M+1]$

Yield: 70%

^1H NMR (CDCl₃, 400 MHz) δ ppm: 7.66 (d, $J = 7.67$ Hz, 1H), 7.55 (t, $J = 7.80$ Hz, 1H), 7.28 (d, $J = 7.87$ Hz, 1H), 7.19 (s, 1H), 6.00 (d, $J = 6.19$ Hz, 1H), 5.78-5.67 (m, 1H), 5.64-5.52 (m, 1H), 4.67-4.59 (m, 1H), 4.50 (s, 1H), 4.42 (dd, $J = 11.6, 4.72$ Hz, 1H), 2.70 (s, 3H), 2.16-2.06 (m, 1H), 1.93-1.80 (m, 1H), 1.71-1.44 (m, 7H), 1.35-1.10 (m, 7H), 0.94 (d, $J = 6.89$ Hz, 6H); ^{13}C NMR (CDCl₃, 100MHz) δ ppm: 175.9, 161.6, 147.0, 142.5, 130.4, 129.7, 129.6, 129.5, 128.2, 127.4, 124.4, 124.0, 119.1, 111.7, 64.3, 57.5, 49.6, 41.7, 31.4, 30.2, 29.7, 29.4, 25.5, 25.4, 22.7, 15.6, 13.0, 11.2

2.8.2



Molecular Formula: C₃₁H₃₃N₃O₅

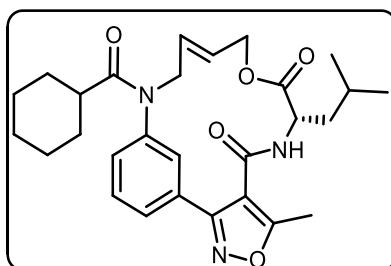
R_f = 0.2 (30% Ethyl acetate /Hexane)

LRMS: (ES+) *m/z* = 528.2[M+1]

Yield: 65%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.52 (d, *J* = 7.62 Hz, 1H), 7.36 (t, *J* = 8.03 Hz, 1H), 7.32-7.24 (m, 3H), 7.18-7.08 (m, 4H), 5.75 (d, *J* = 7.25 Hz, 1H), 5.72-5.62 (m, 1H), 5.55-5.45 (m, 1H), 4.78-4.69 (m, 1H), 4.50 (s, 2H), 3.2 (dd, *J* = 14.0, 5.2 Hz, 1H), 3.04 (dd, *J* = 13.8, 5.4 Hz, 1H), 2.22 (s, 3H), 2.06-1.98 (m, 1H), 1.56-1.36 (m, 8H), 1.16-1.00 (m, 2H), 0.94-0.81 (m, 2H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 175.9, 170.7, 161.3, 142.4, 135.8, 130.3, 129.6, 129.5, 129.2, 129.4, 129.1, 128.9, 128.1, 127.5, 126.9, 111.5, 77.3, 64.7, 54.3, 49.7, 41.6, 36.9, 29.4, 29.3, 25.5, 25.3, 25.3, 12.3.

2.8.3



Molecular Formula: C₂₈H₃₅N₃O₅

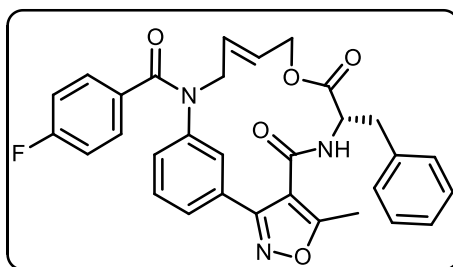
R_f = 0.2 (30% Ethyl acetate /Hexane)

LRMS: (ES+) *m/z* = 494.2 [M+1]

Yield: 60%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.70 (d, *J* = 7.62 Hz, 1H), 7.53 (t, *J* = 7.80 Hz, 1H), 7.28 (s, 1H), 7.22-7.17 (m, 1H), 5.95 (d, *J* = 7.60 Hz, 1H), 5.83-5.73 (m, 1H), 5.69-5.58 (m, 1H), 4.70-4.60 (m, 1H), 4.61-4.53 (m, 1H), 4.50 (dd, *J* = 12.45, 4.82 Hz, 1H), 2.67 (s, 3H), 2.12-2.00 (m, 1H), 1.75 (s, 2H), 1.70-1.41 (m, 10H), 1.01-0.94 (m, 6H), 1.19-1.09 (m, 1H), 0.94-0.89 (m, 1H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 175.9, 175.8, 161.8, 142.6, 135.5, 130.3, 129.7, 129.6, 129.3, 128.2, 127.0, 115.9, 111.8, 109.2, 64.7, 51.9, 49.8, 41.6, 40.5, 29.4, 29.3, 25.5, 25.3, 25.1, 22.7, 22.0, 12.9.

2.8.4



Molecular Formula: C₃₁H₂₆FN₃O₅

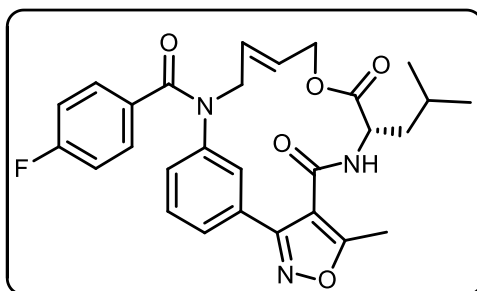
R_f = 0.2 (30% Ethyl acetate /Hexane)

LRMS: (ES+) *m/z* =540.1 [M+1]

Yield: 70%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.41 (d, *J* = 7.70 Hz, 1H), 7.35-7.27 (tm, 5H), 7.22 (t, *J* = 7.88 Hz, 1H), 7.20-7.13 (m, 3H), 7.04 (d, *J* = 7.96 Hz, 1H), 6.84 (t, *J* = 8.59 Hz, 2H), 5.92-5.85 (s, 1H), 5.78 (d, *J* = 7.71 Hz, 1H), 5.74-5.58, (m, 1H), 4.83 (q, *J* = 5.93 Hz, 1H), 4.67, 4.65 (d, *J* = 5.11 Hz, 1H), 4.64-4.54 (m, 2H), 4.45-4.36 (m, 1H), 3.22 (dd, *J* = 14.03, 5.68 Hz, 1H), 3.07 (dd, *J* = 14.03, 5.68 Hz, 1H), 2.29 (s, 3H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 171.6, 170.5, 169.1, 164.6, 162.1, 161.3, 160.1, 143.4, 135.8, 131.3, 131.2, 131.1, 131.1, 131.0, 129.7, 129.3, 129.2, 129.1, 128.9, 128.2, 127.4, 127.1, 127.0, 115.2, 114.9, 111.3, 64.6, 54.2, 50.7, 37.0, 12.4.

2.8.5



Molecular Formula: C₂₈H₂₈FN₃O₅

R_f = 0.2 (30% Ethyl acetate /Hexane)

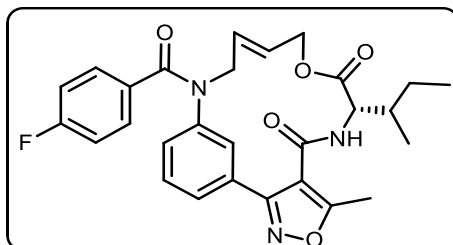
LRMS: (ES+) *m/z* =506.2 [M+1]

Yield: 65%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.52 (d, *J* = 7.68 Hz, 1H), 7.35-7.28 (m, 3H), 7.16 (s, 1H), 7.07 (d, *J* = 7.90 Hz, 1H), 6.85 (t, *J* = 8.58 Hz, 2H), 5.89 (t, *J* = 10.96

Hz, 2H), 5.76 (s, 1H), 4.49 (s, 1H), 4.68-4.56 (m, 4H), 2.65 (s, 3H), 1.70-1.60 (m, 4H), 1.57 (d, $J = 8.12$ Hz, 1H), 0.97 (t, $J = 6.73$ Hz, 6H); ^{13}C NMR (CDCl_3 , 100MHz) δ ppm: 171.8, 171.3, 169.1, 164.5, 162.1, 161.7, 160.2, 143.5, 131.3, 131.2, 131.1, 131.1, 130.7, 129.7, 129.4, 129.3, 128.1, 127.1, 115.2, 115.0, 111.5, 64.6, 51.9, 50.8, 40.6, 25.1, 22.6, 22.0, 12.9.

2.8.6



Molecular Formula: $\text{C}_{28}\text{H}_{28}\text{FN}_3\text{O}_5$

R_f = 0.2 (30% Ethyl acetate /Hexane)

LRMS: (ES+) $m/z = 506.2[\text{M}+1]$

Yield: 68%

^1H NMR (CDCl_3 , 400 MHz) δ ppm: 7.48 (d, $J = 7.67$ Hz, 1H), 7.34 (dd, $J = 14.78$, 8.57 Hz, 3H), 7.11 (d, $J = 7.58$ Hz, 2H), 6.86 (t, $J = 8.59$ Hz, 2H), 5.92-5.87 (m, 1H), 5.85 (d, $J = 16.22$ Hz, 1H), 5.70 (s, 1H), 4.78-4.69 (m, 1H), 4.60 (dd, $J = 11.99$, 6.97 Hz, 1H), 4.50 (td, $J = 9.31$, 6.50 Hz, 3H), 2.67 (s, 3H), 1.90-1.81 (m, 1H), 1.46 (s, 1H), 1.18 (d, $J = 7.55$ Hz, 1H), 0.92 (t, $J = 6.26$ Hz, 6H); ^{13}C NMR (CDCl_3 , 100MHz) δ ppm: 172.2, 170.5, 169.2, 164.6, 162.1, 161.5, 159.9, 143.5, 131.2, 131.2, 131.1, 131.0, 129.8, 129.4, 128.4, 127.6, 127.1, 115.3, 115.0, 111.4, 64.3, 57.5, 50.5, 37.1, 25.4, 15.6, 13.0, 11.2.

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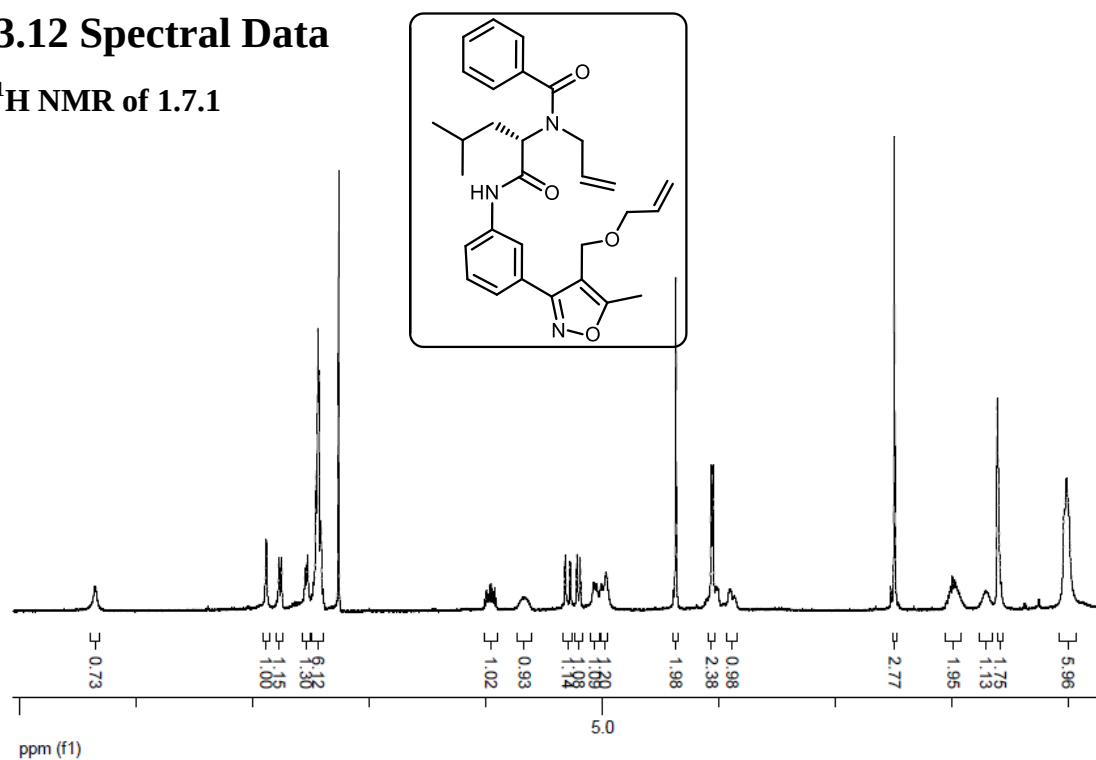
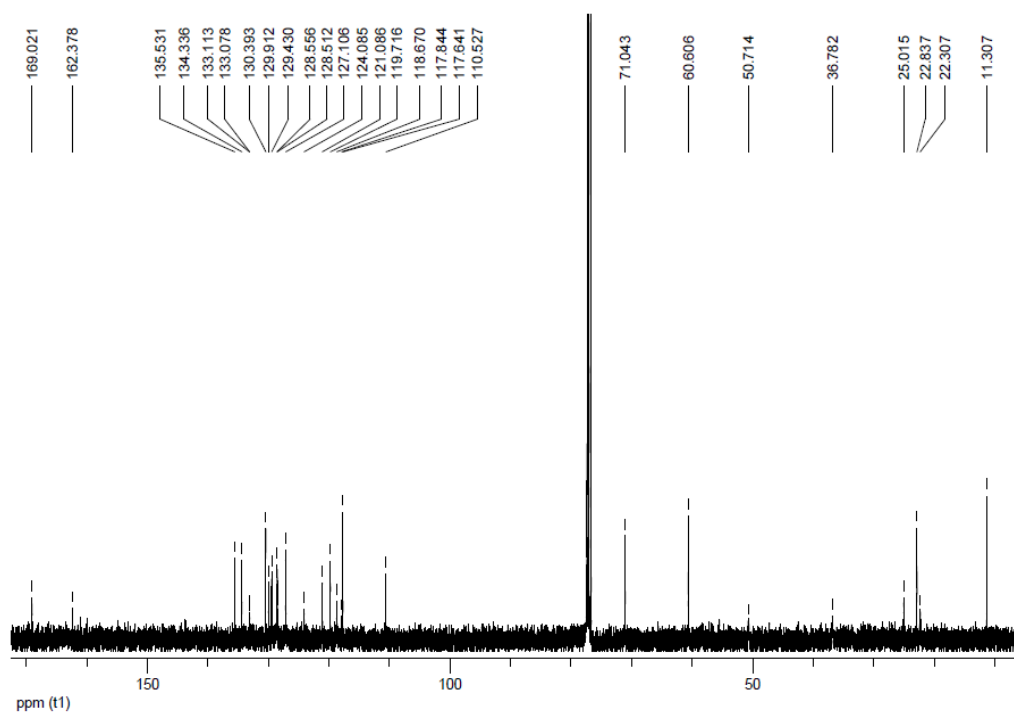
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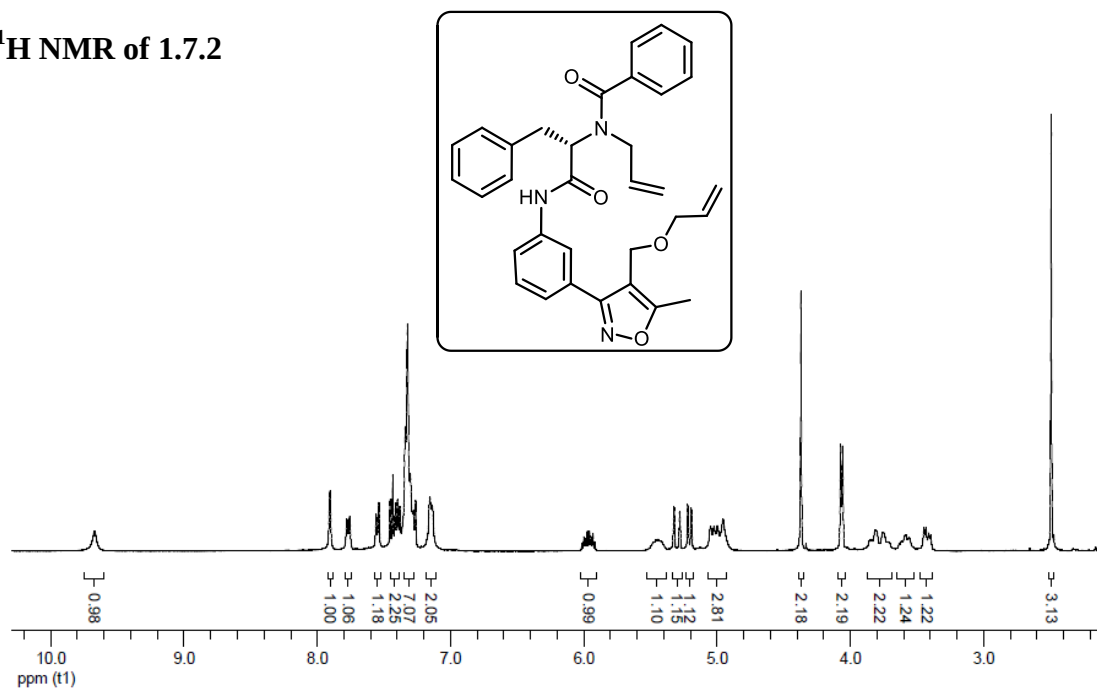
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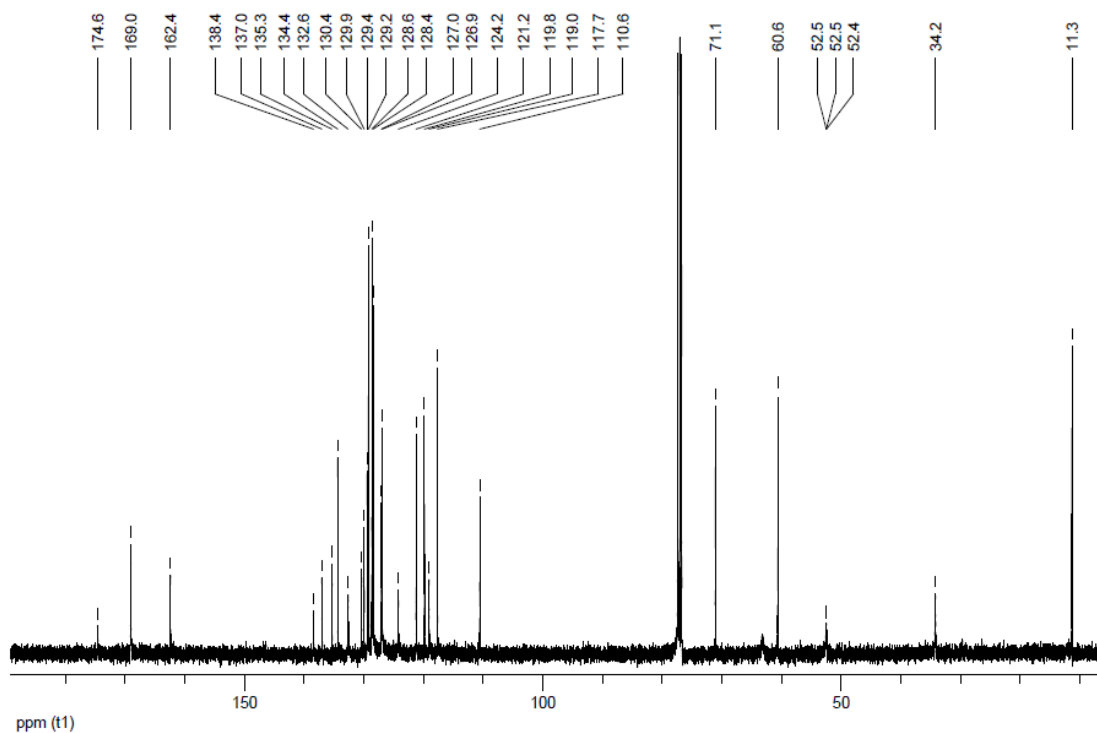
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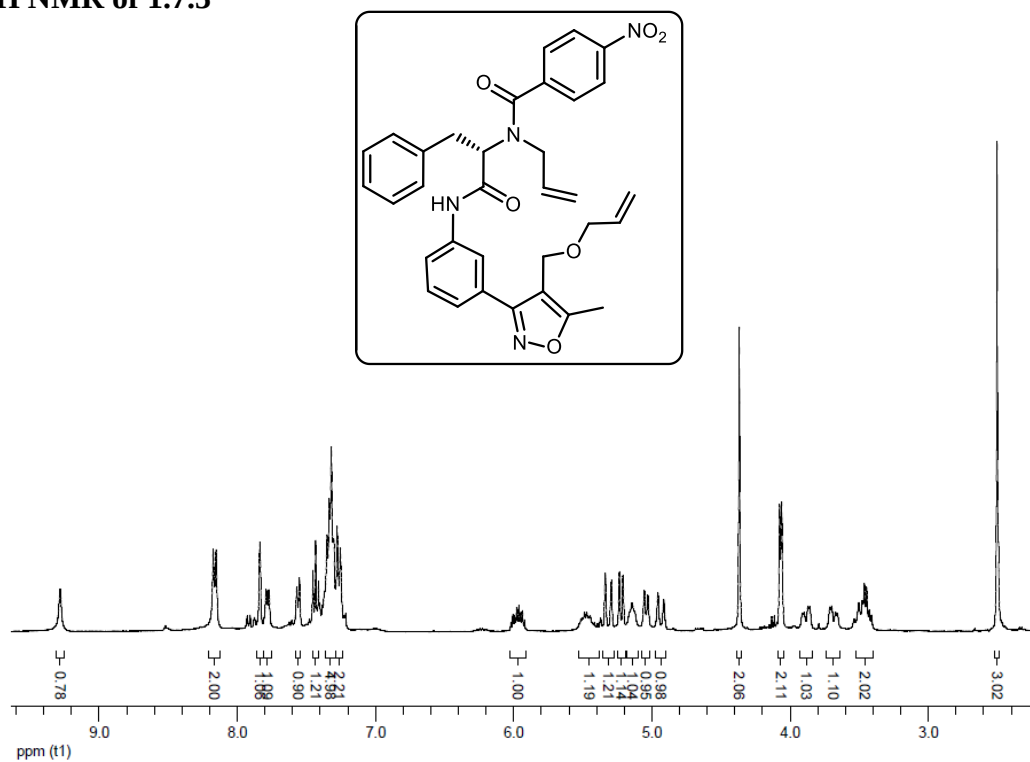
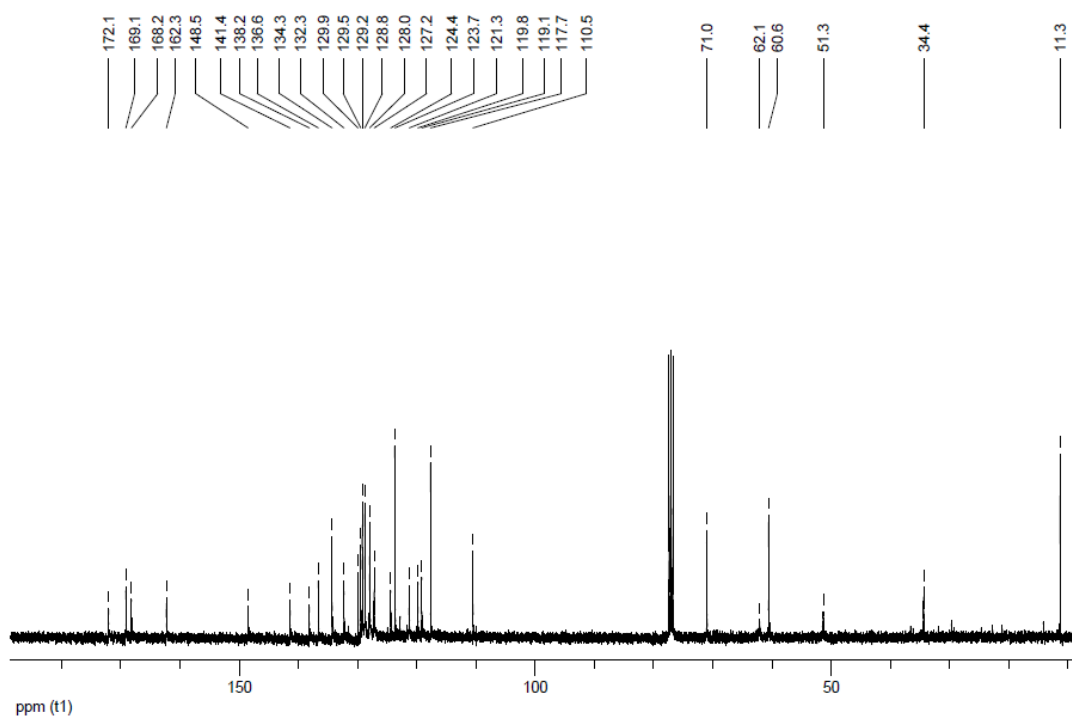
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^1H NMR of 1.7.2

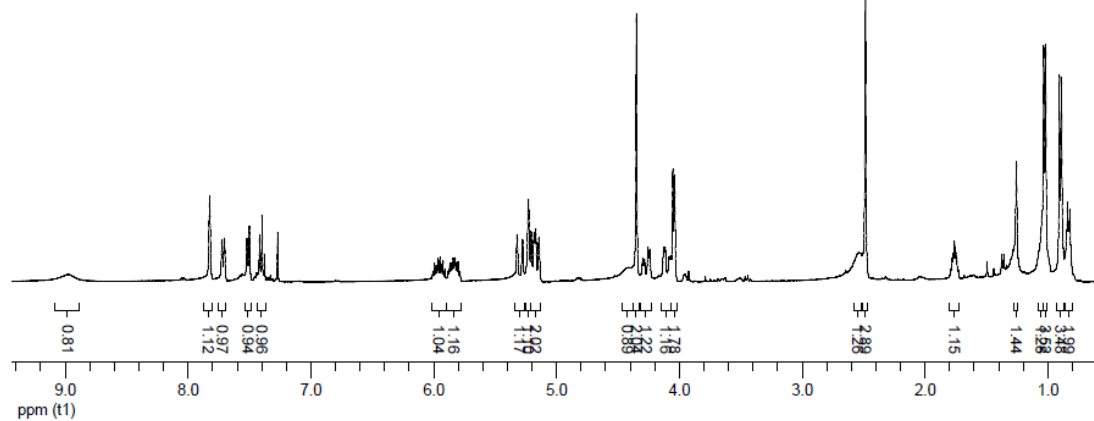
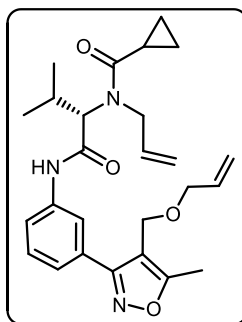


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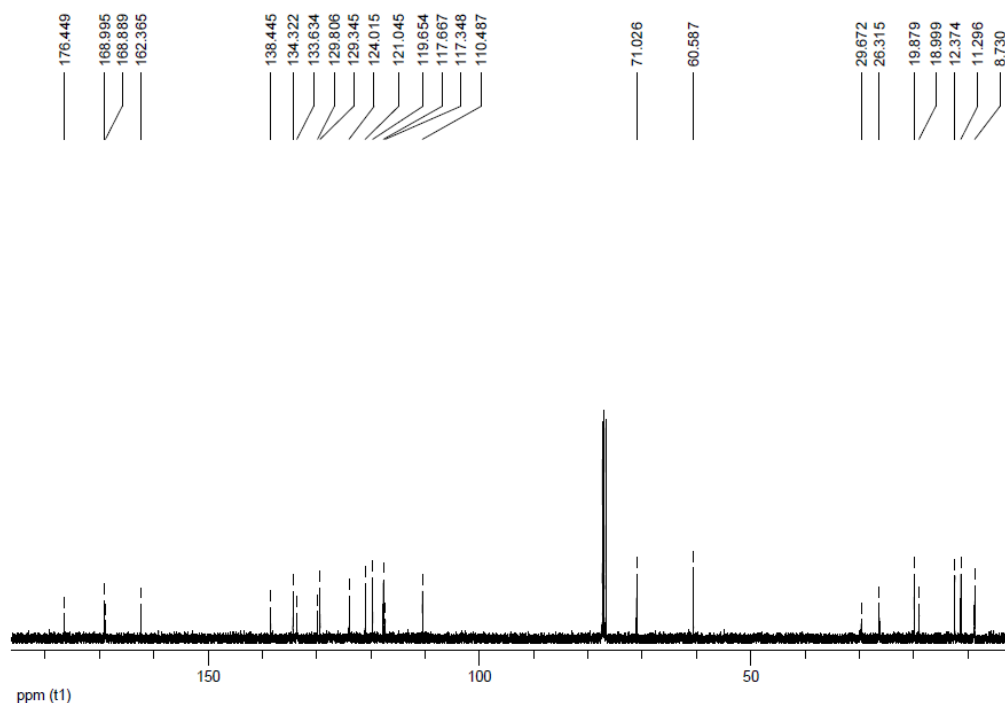


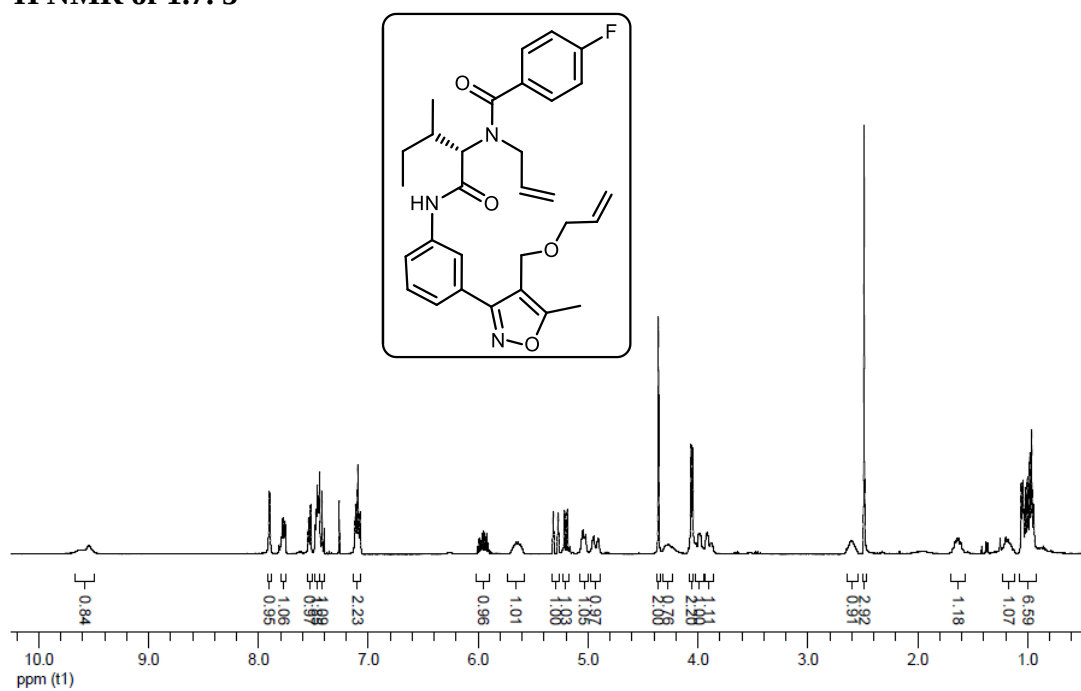
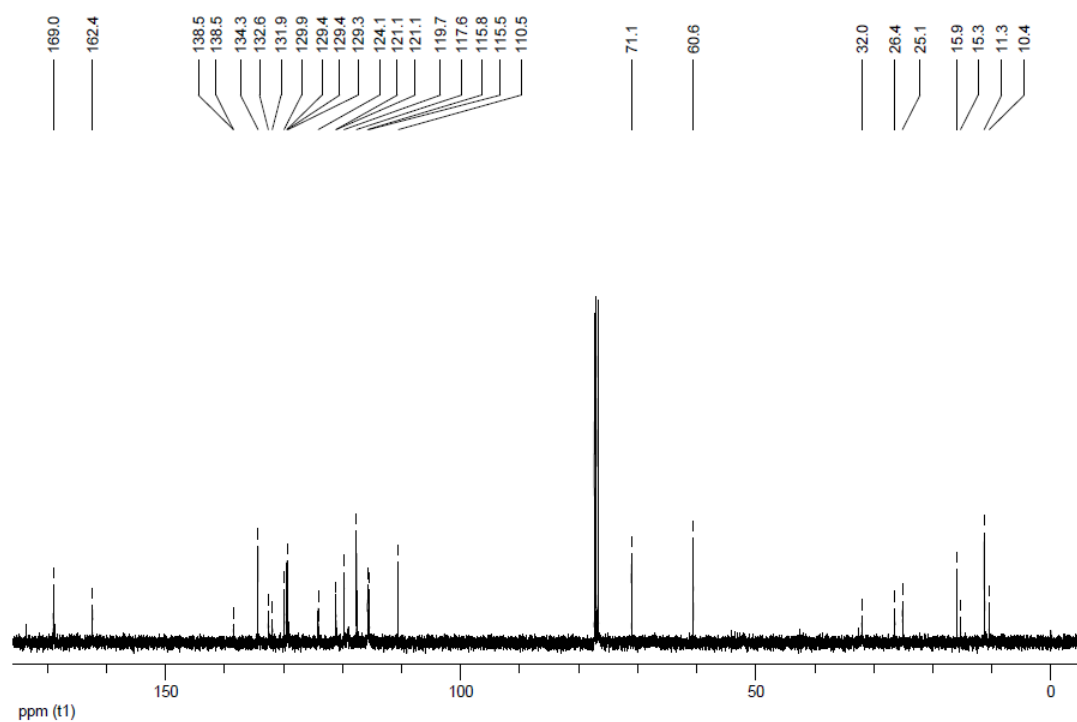
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¹H NMR of 1.7.4

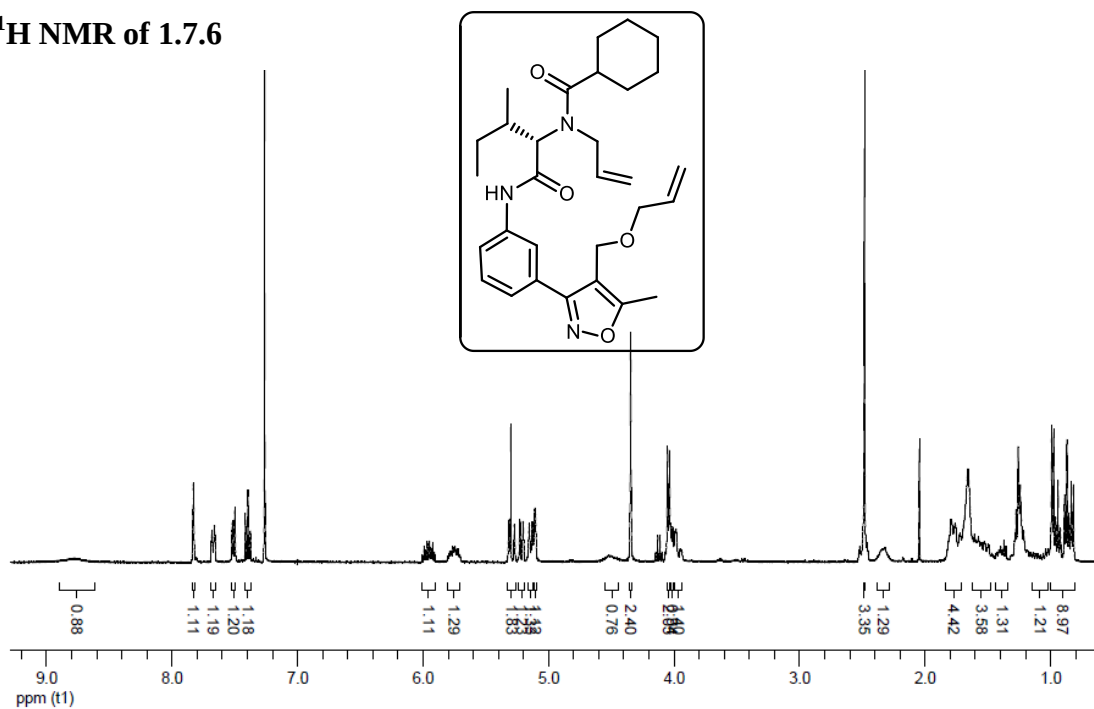


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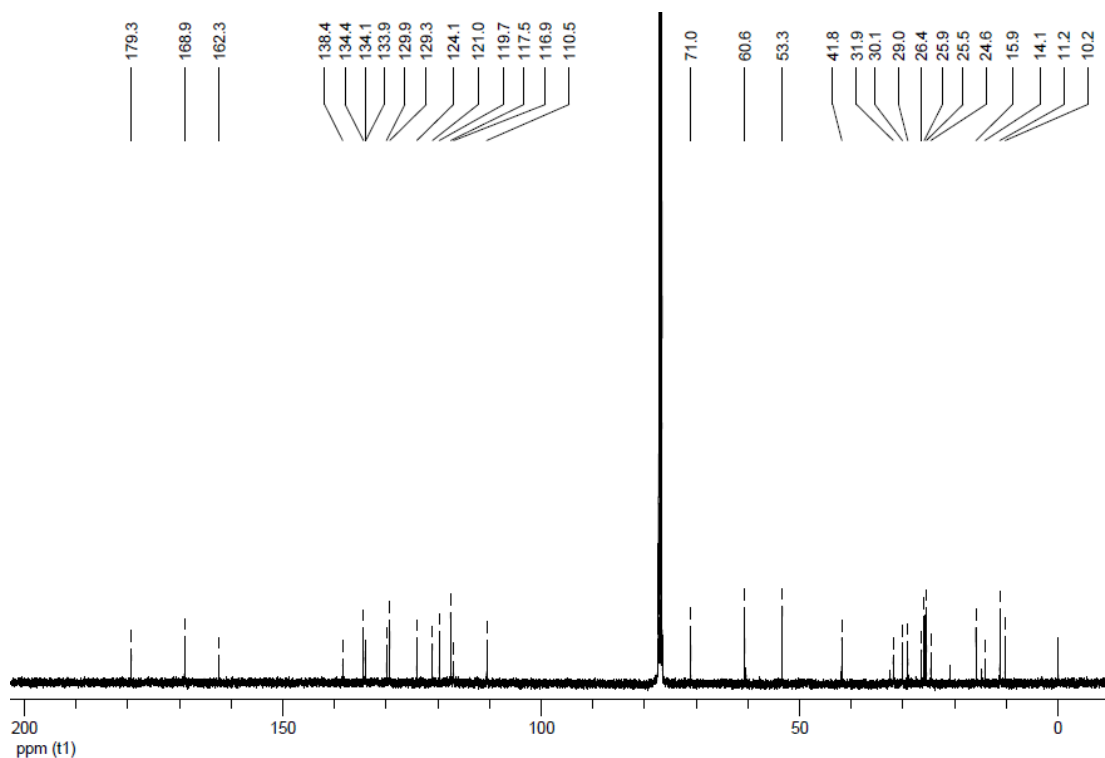


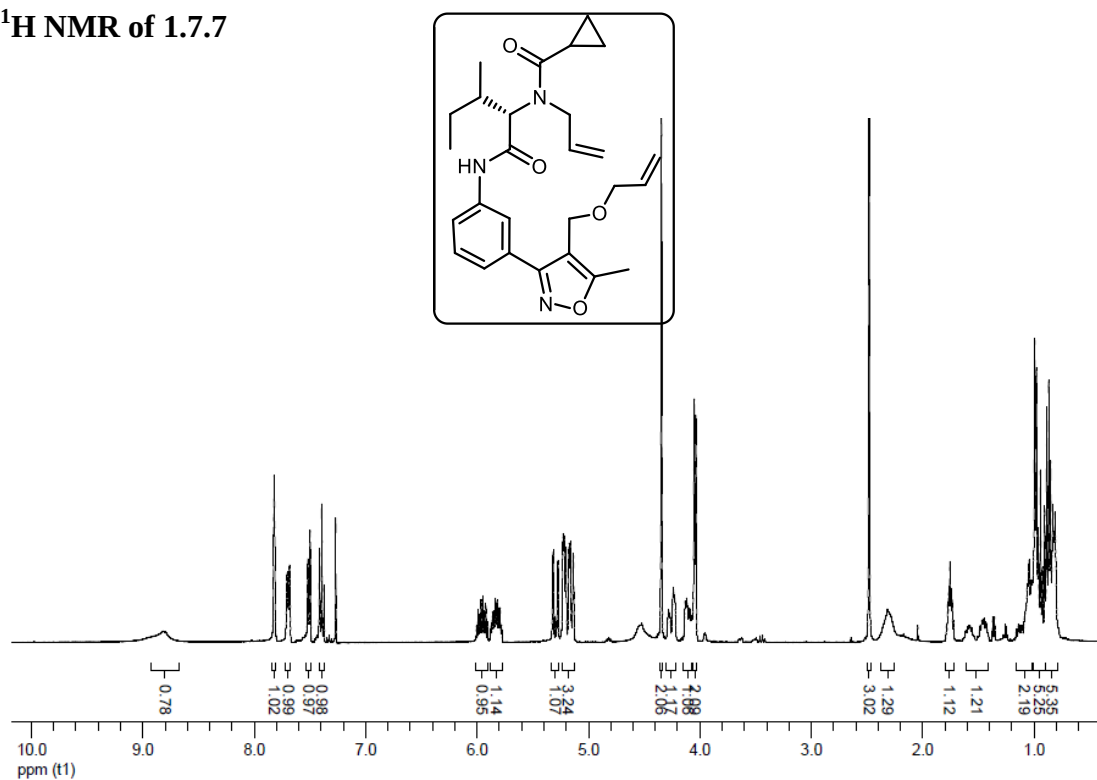
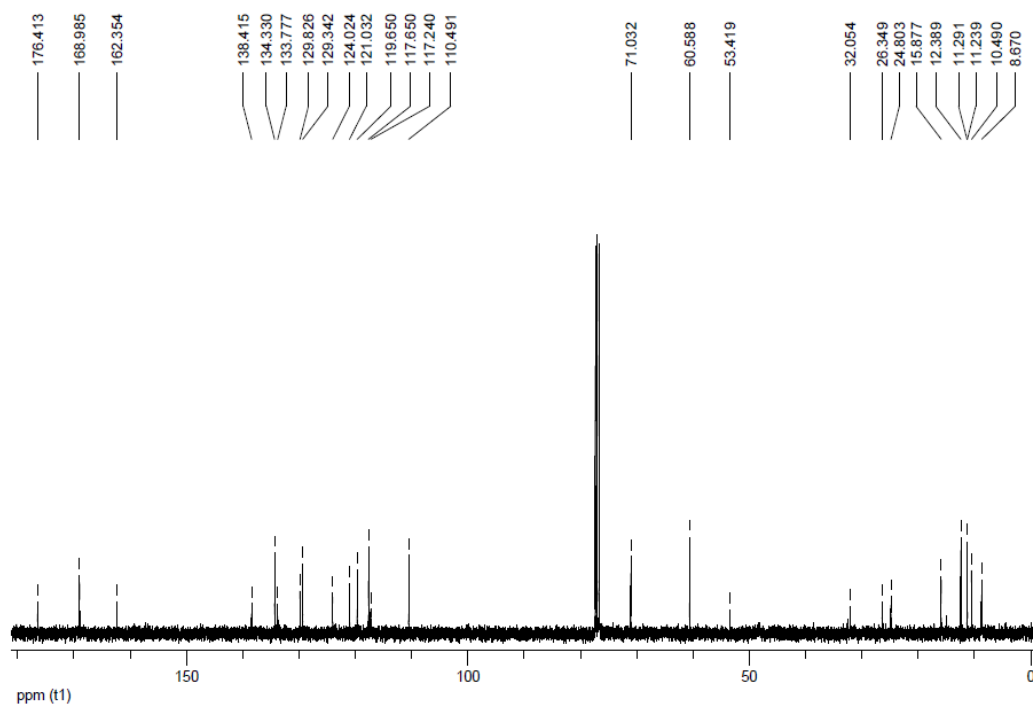
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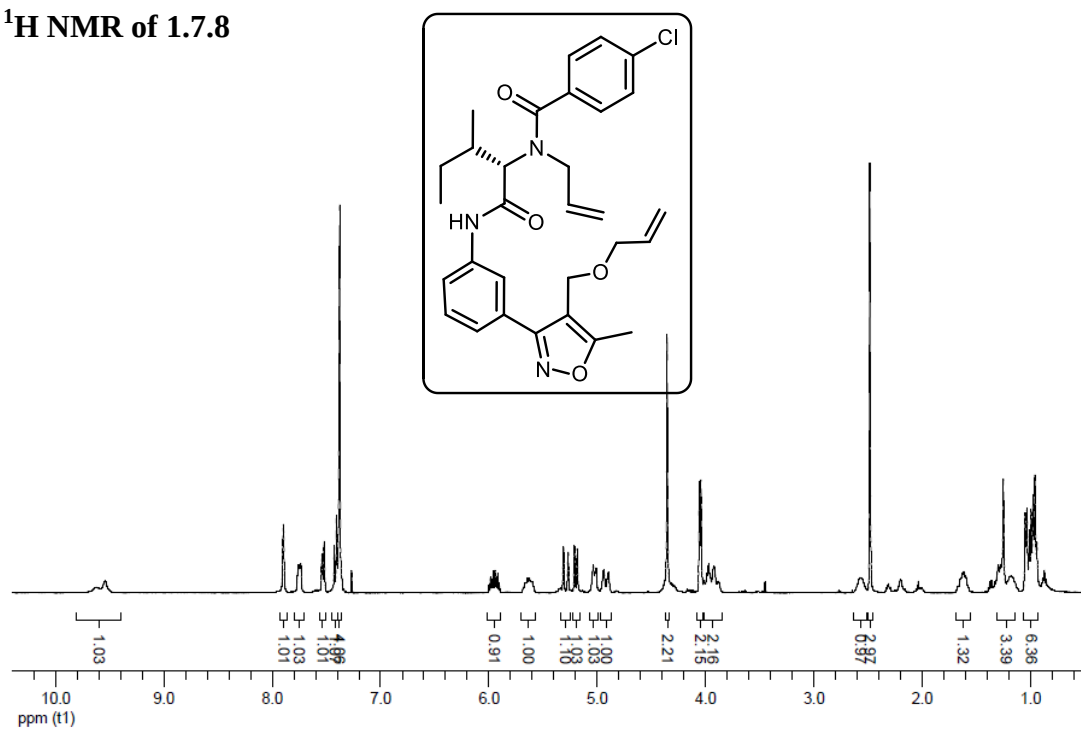


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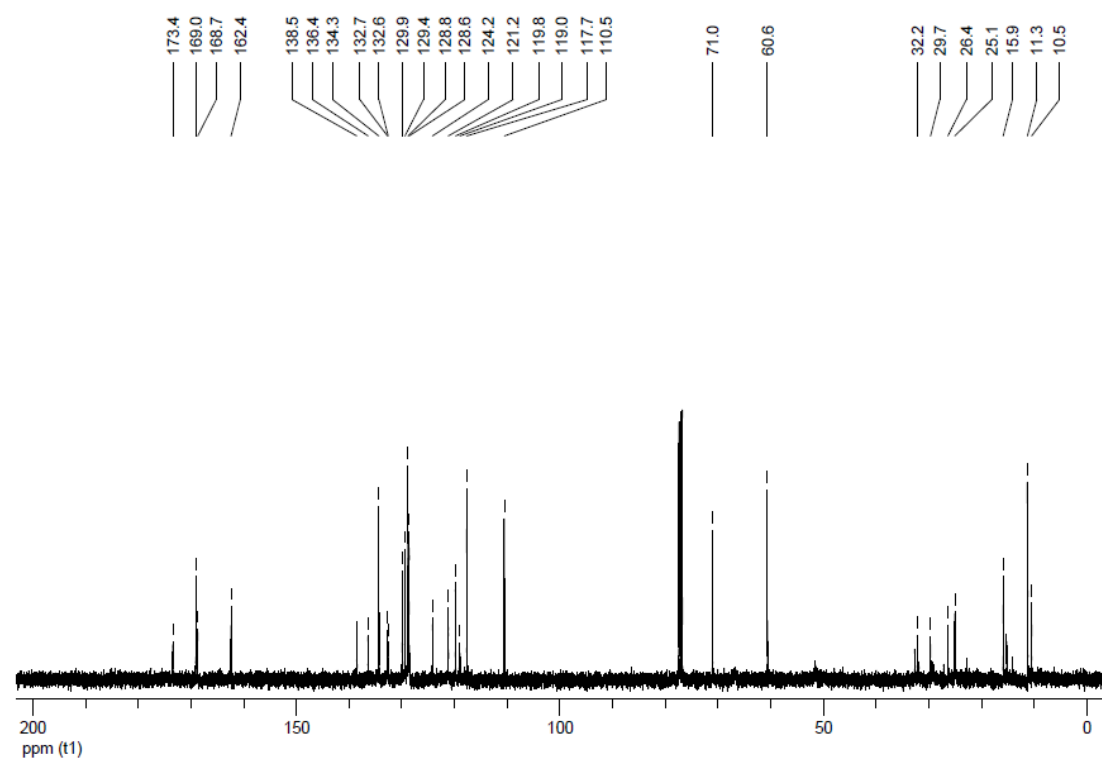


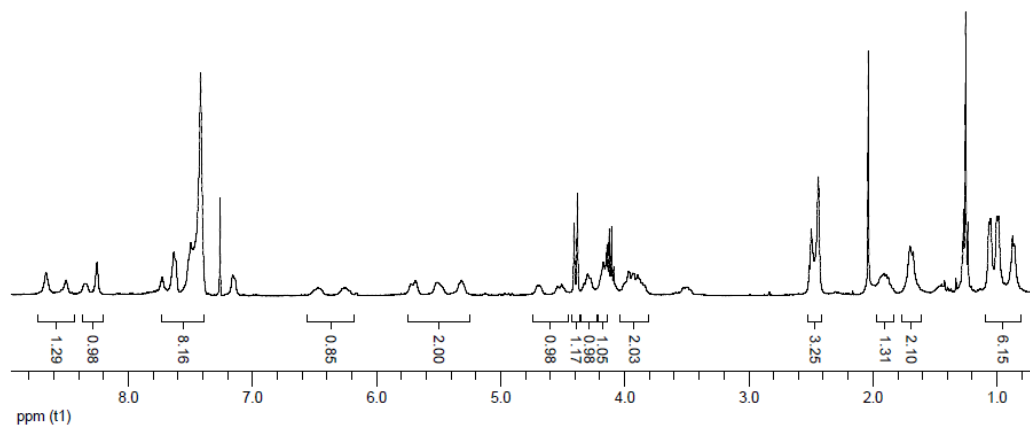
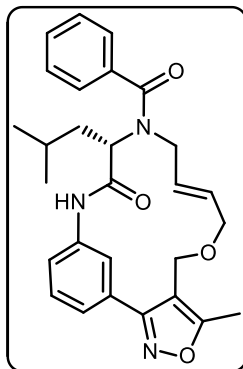
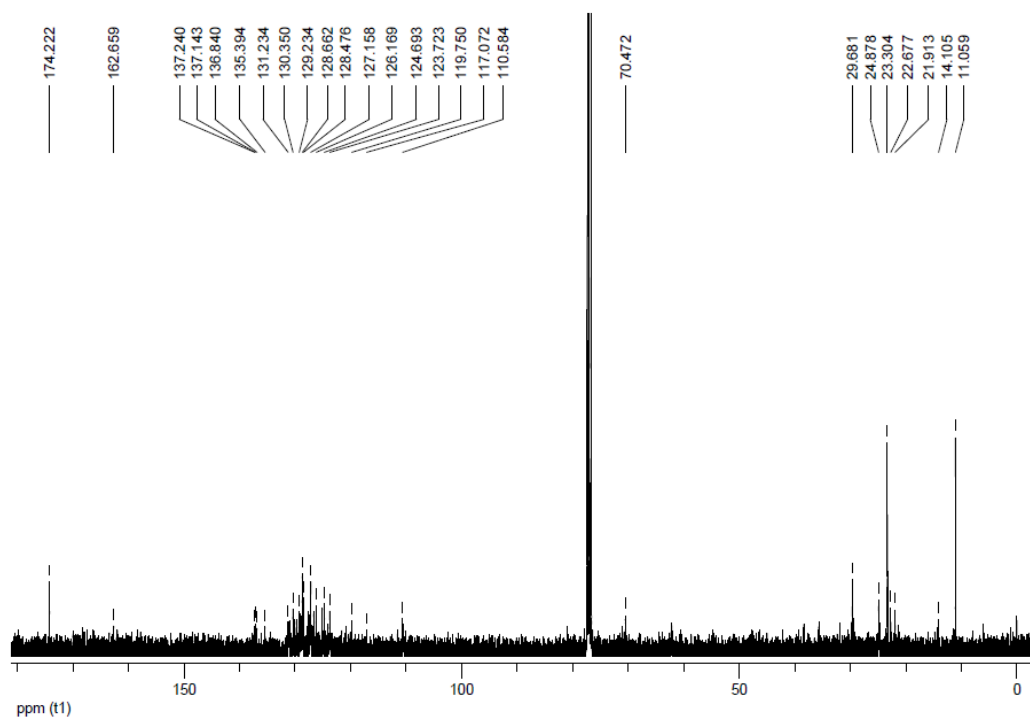
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^1H NMR of 1.7.8

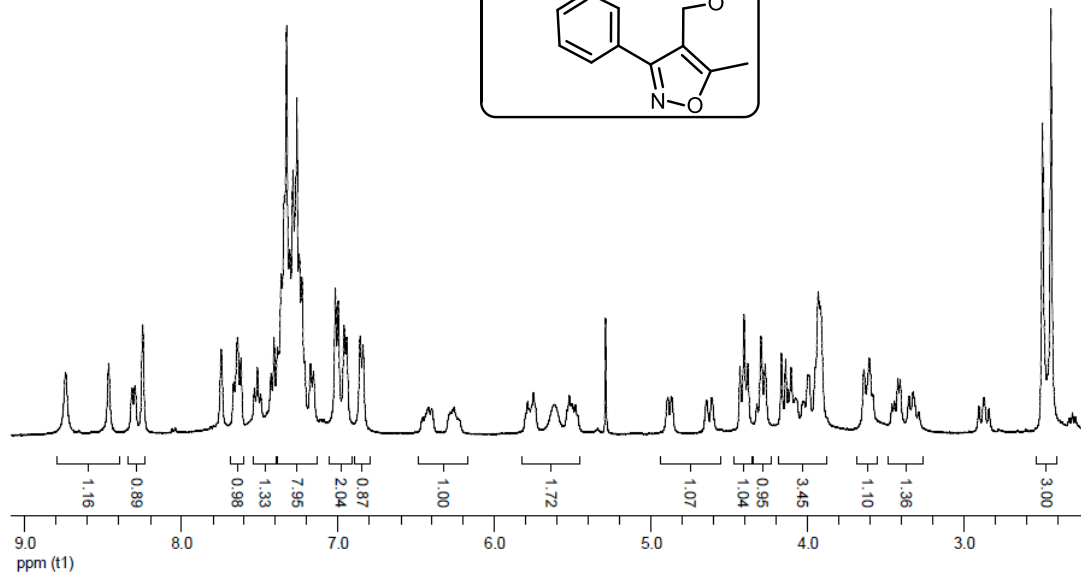
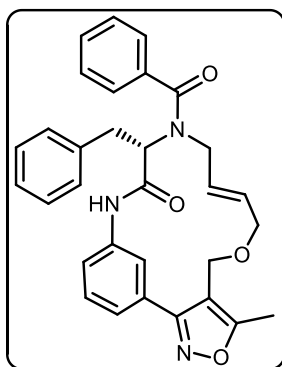


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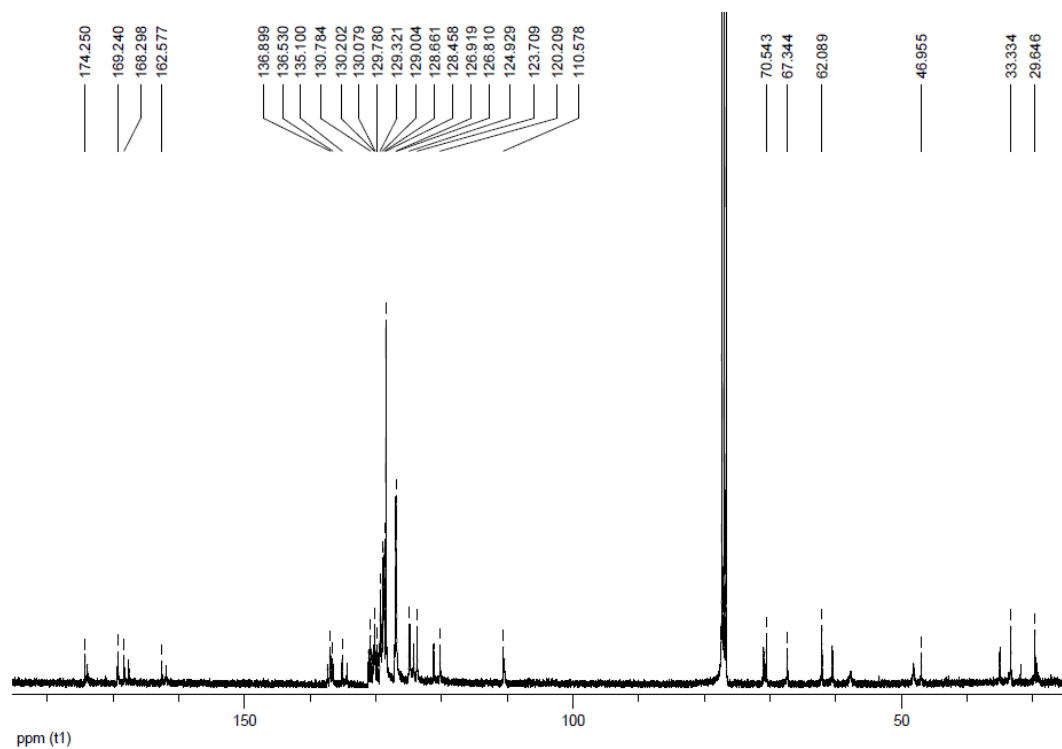


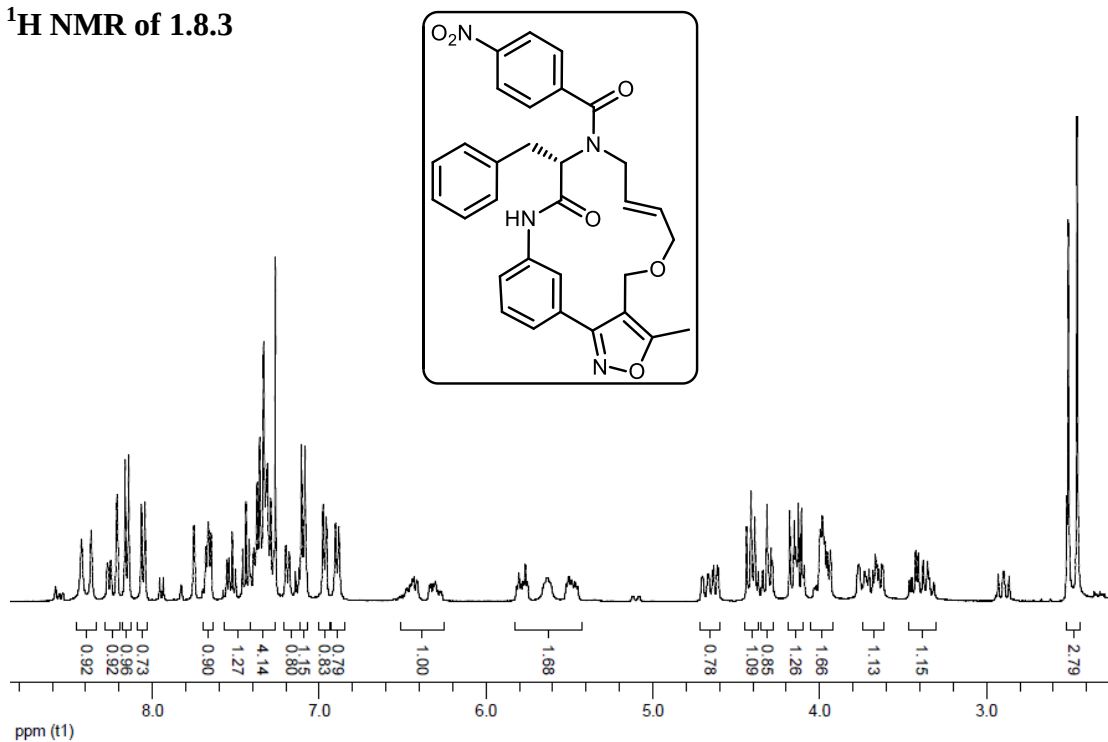
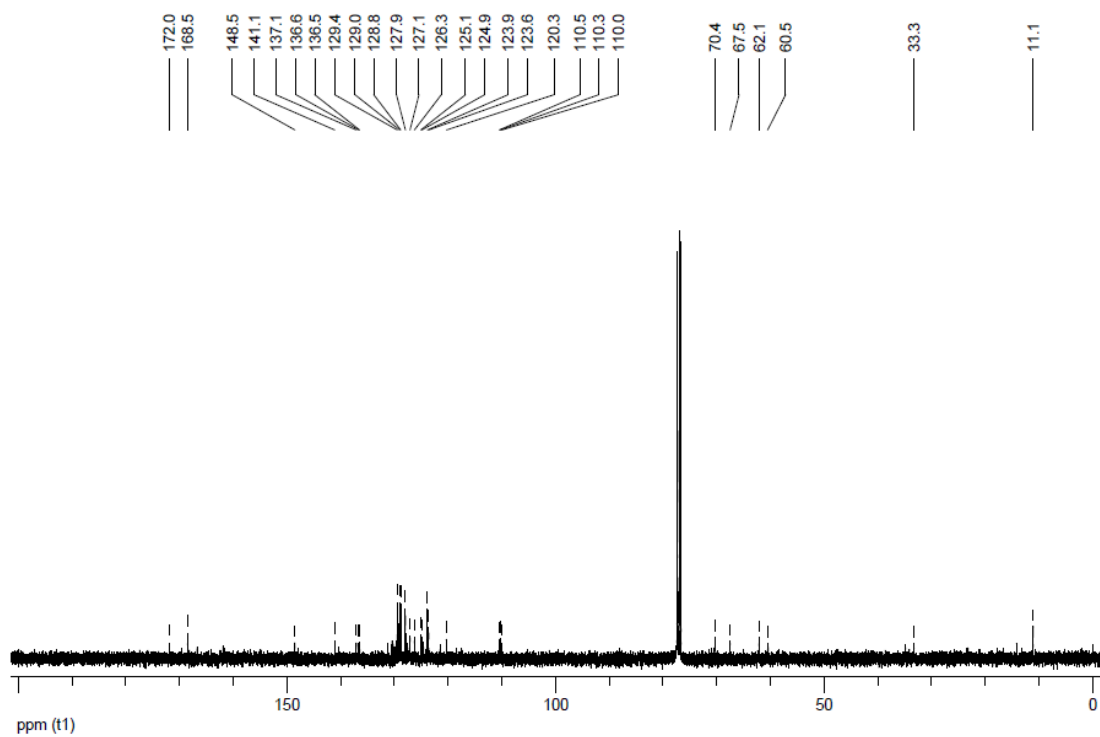
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¹H NMR of 1.8.2

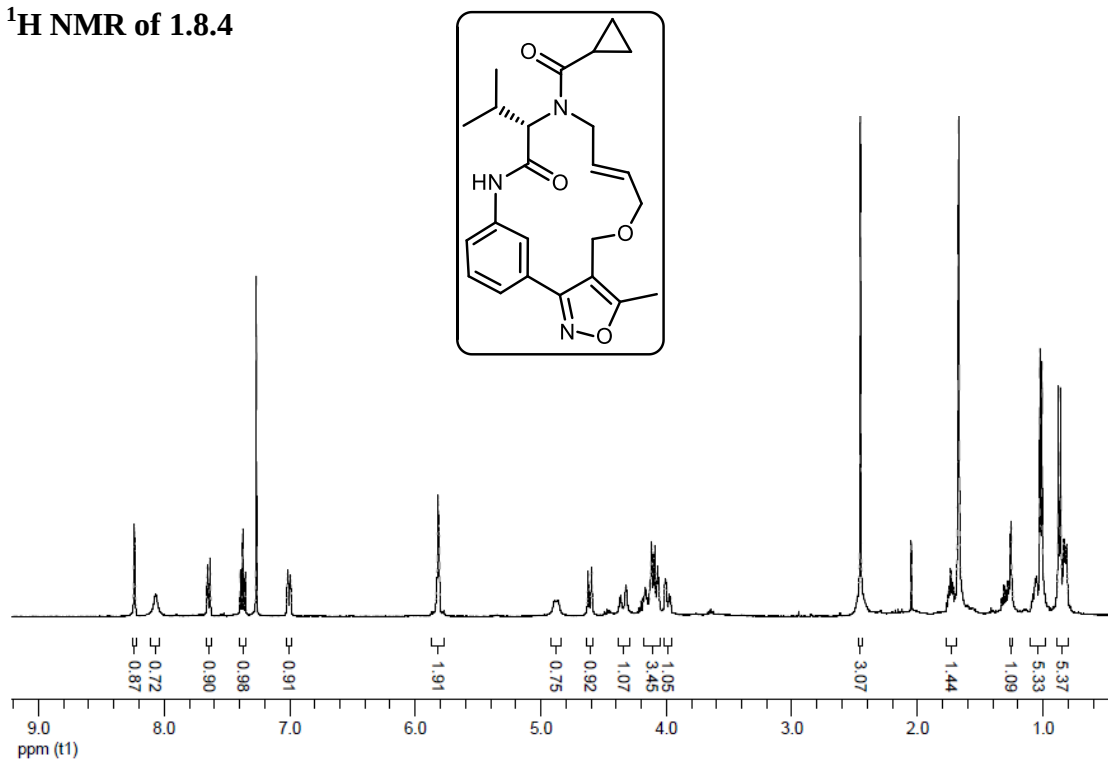


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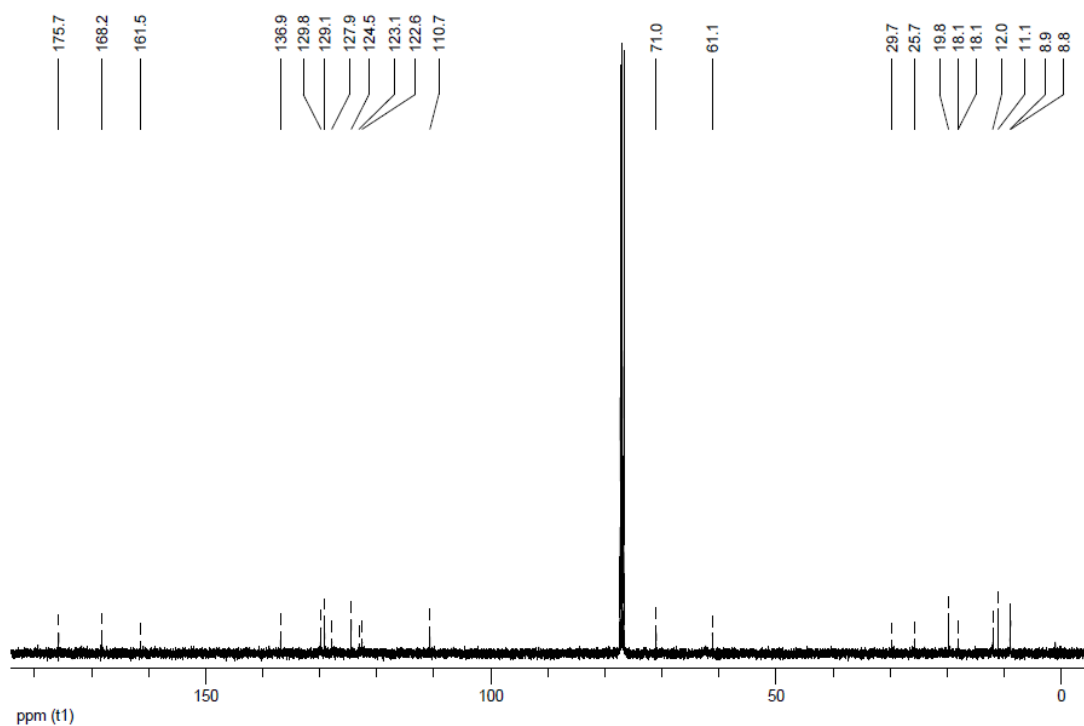


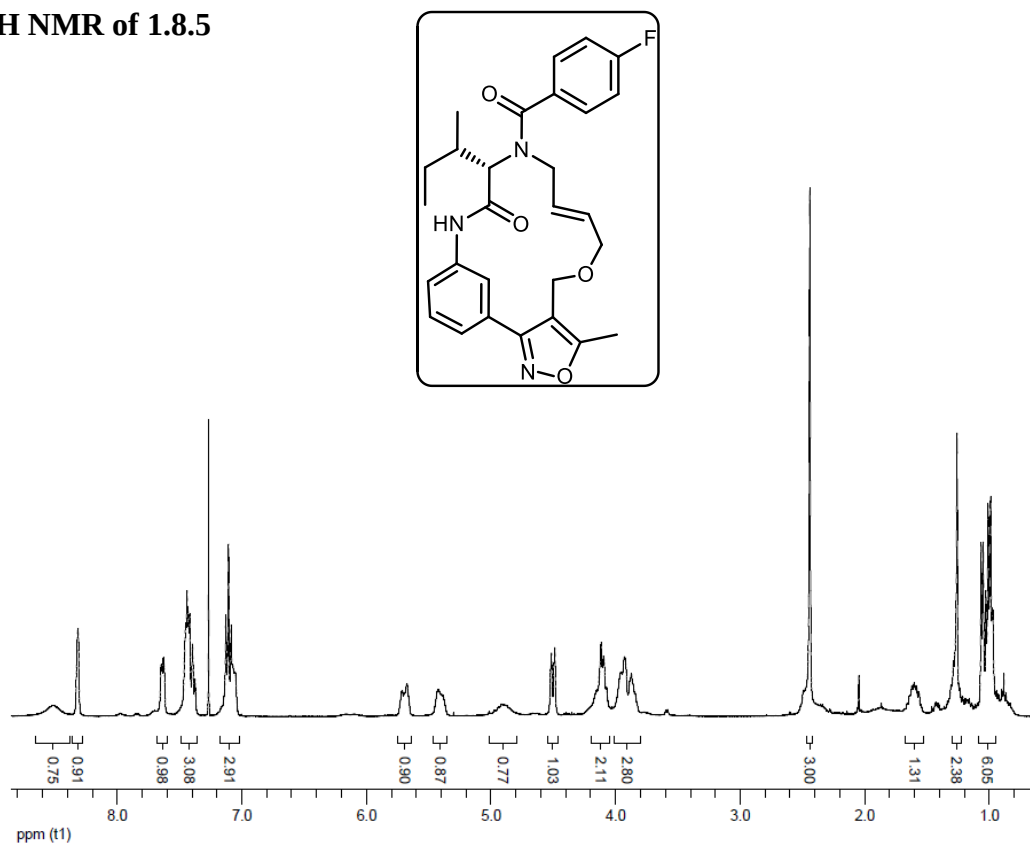
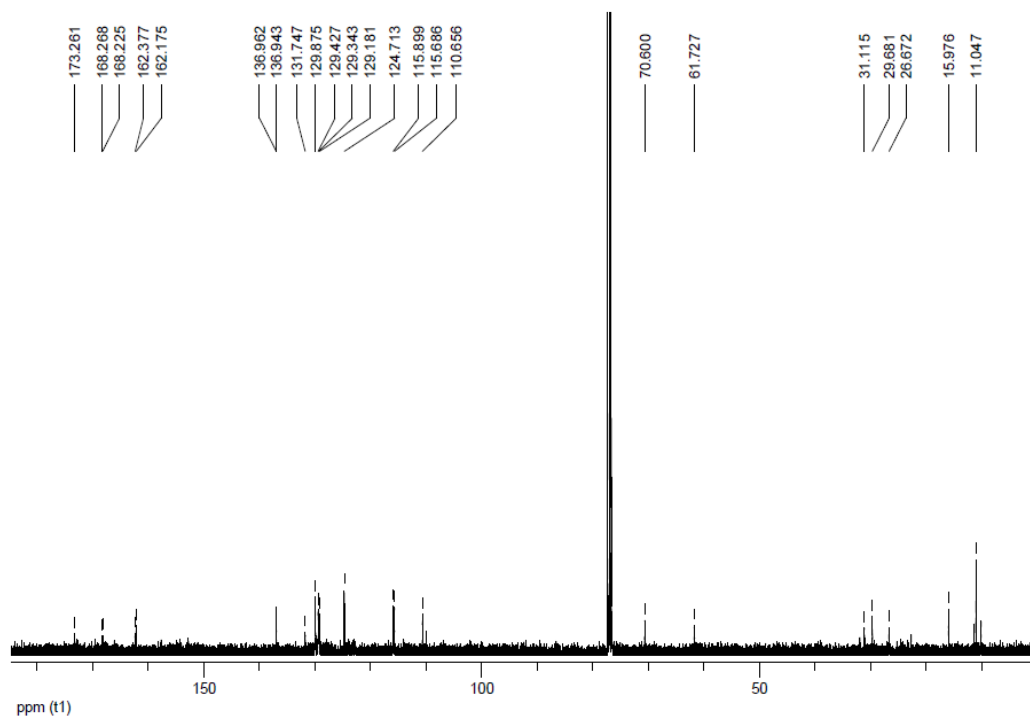
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¹H NMR of 1.8.4

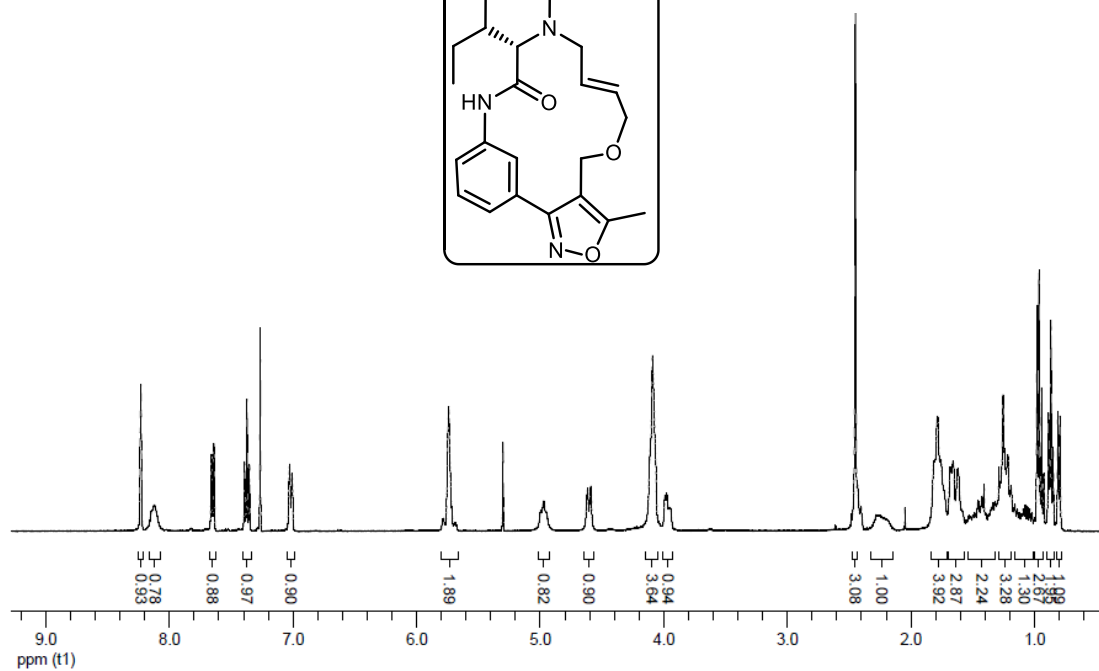
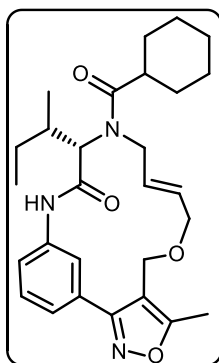


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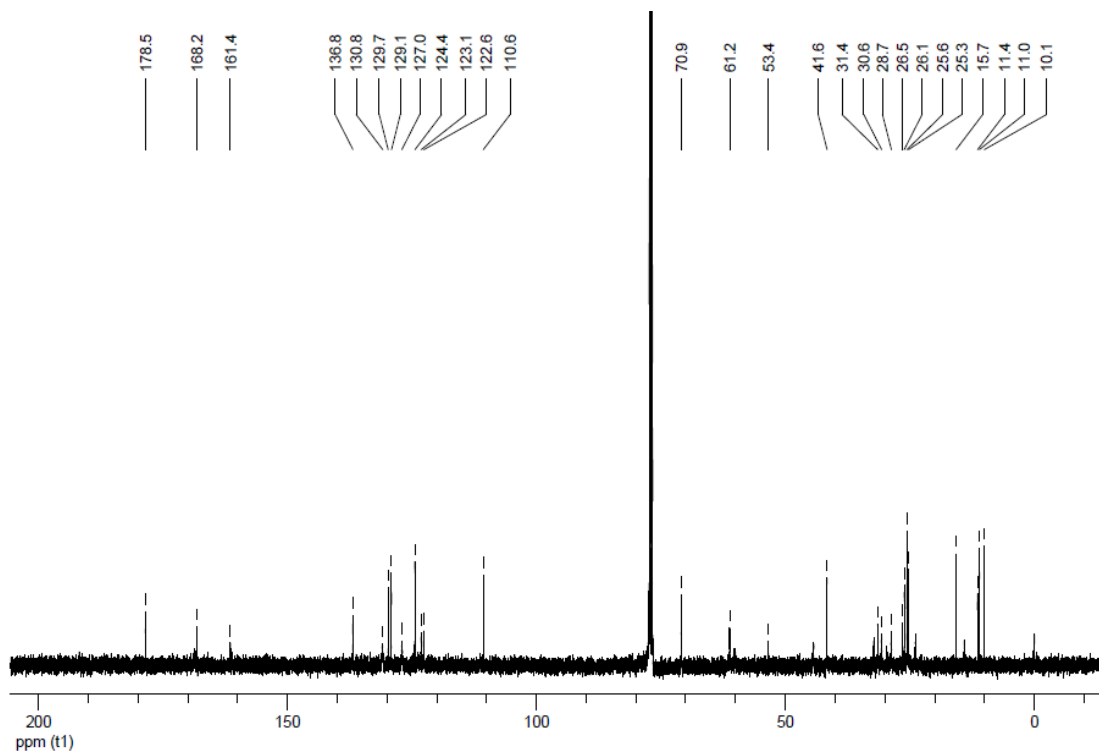


^1H NMR of 1.8.5 **^{13}C NMR of 1.8.5**

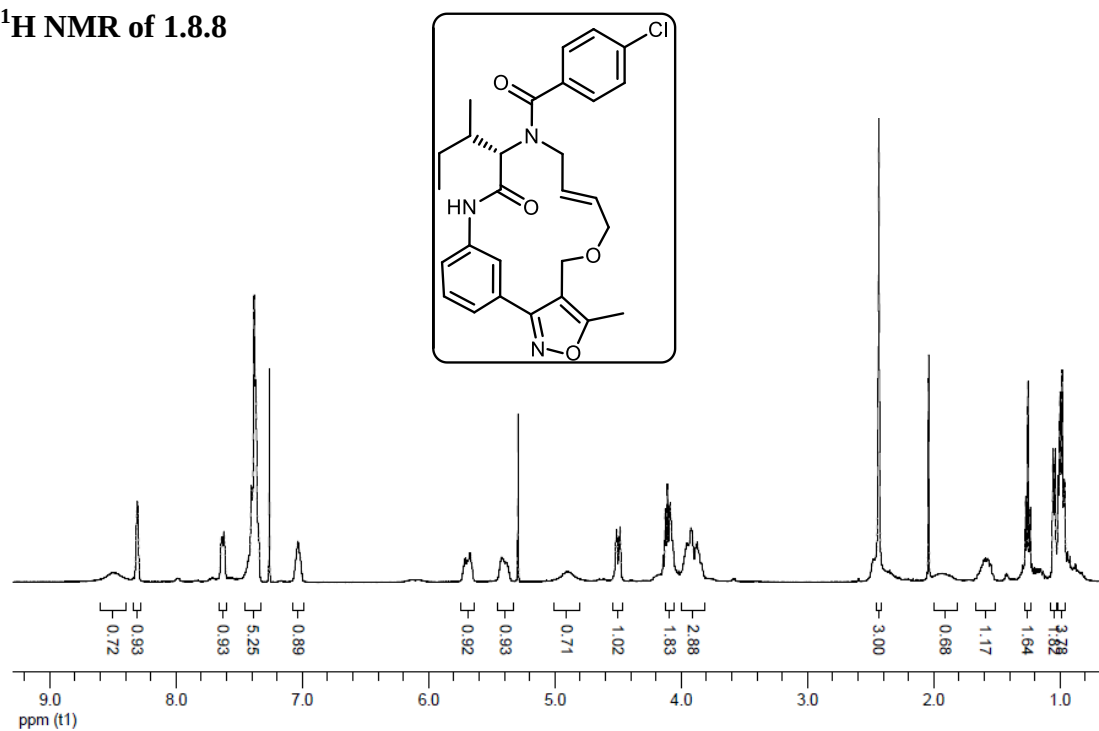
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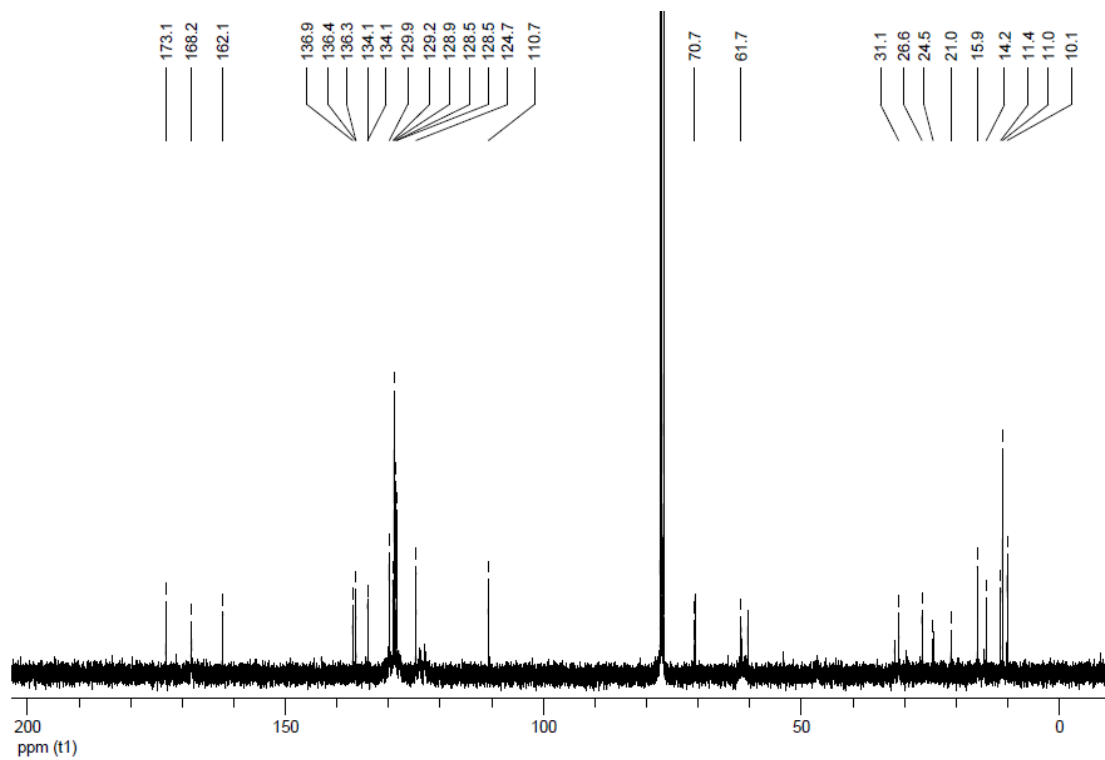
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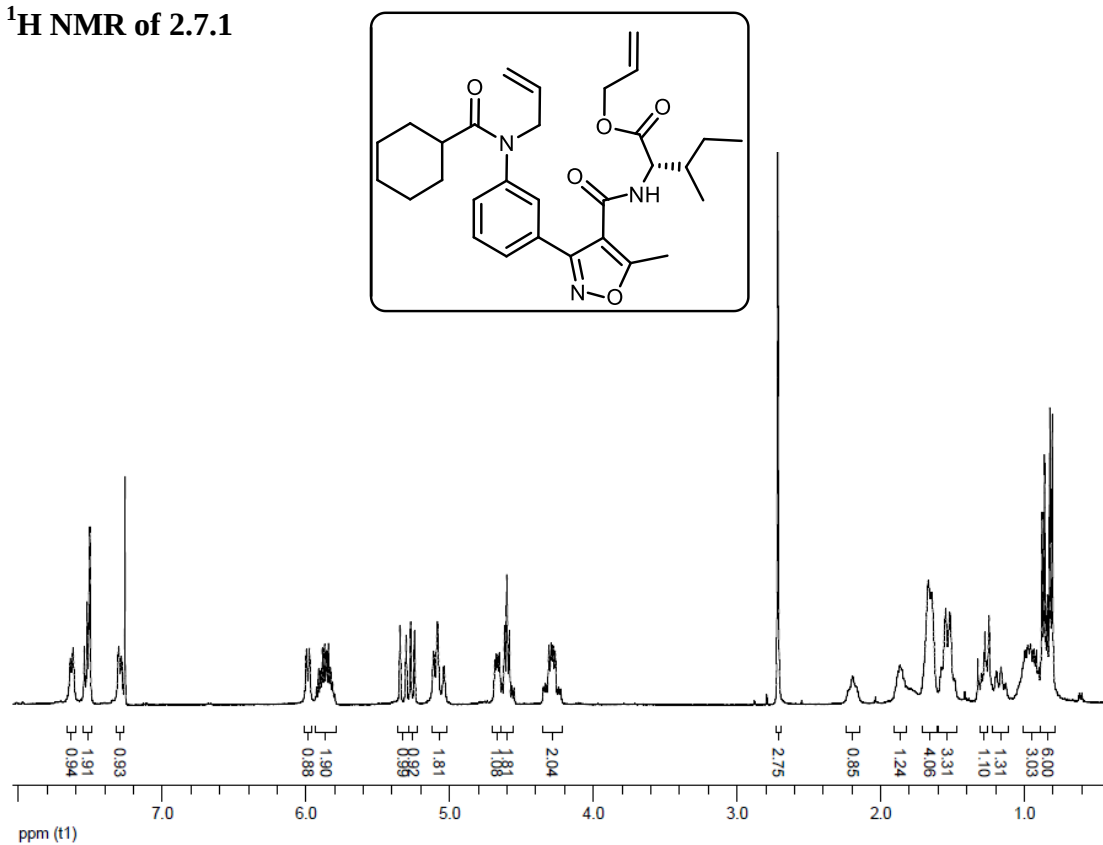
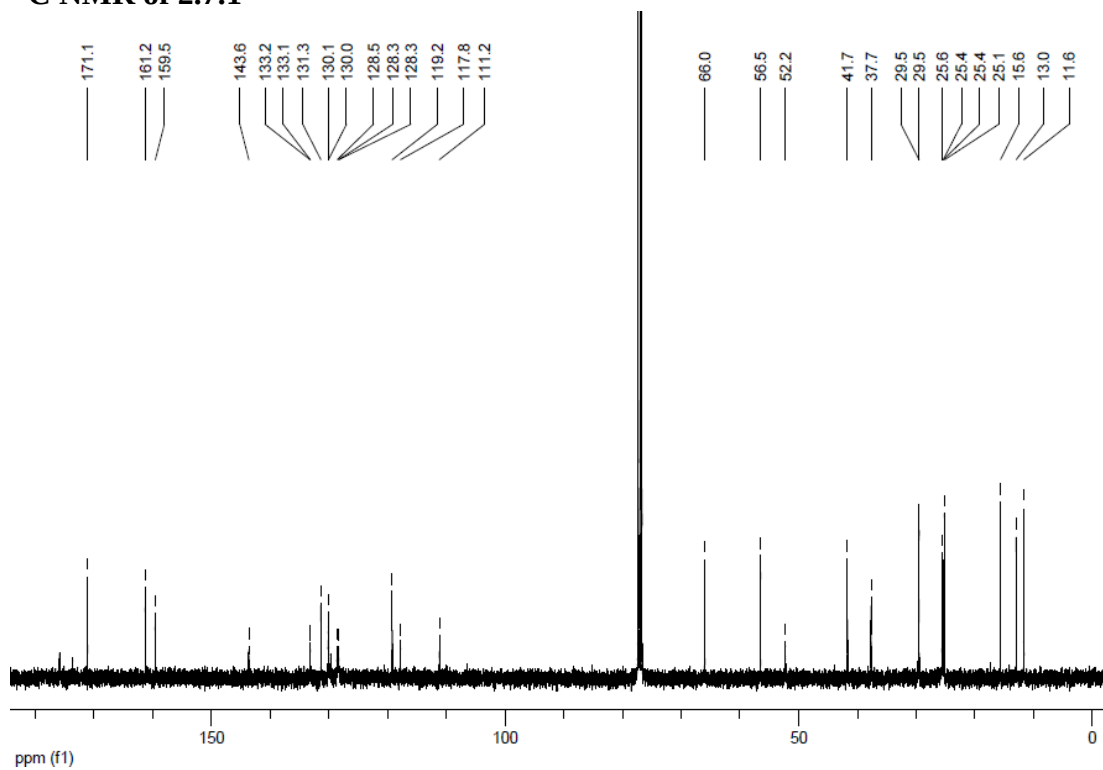


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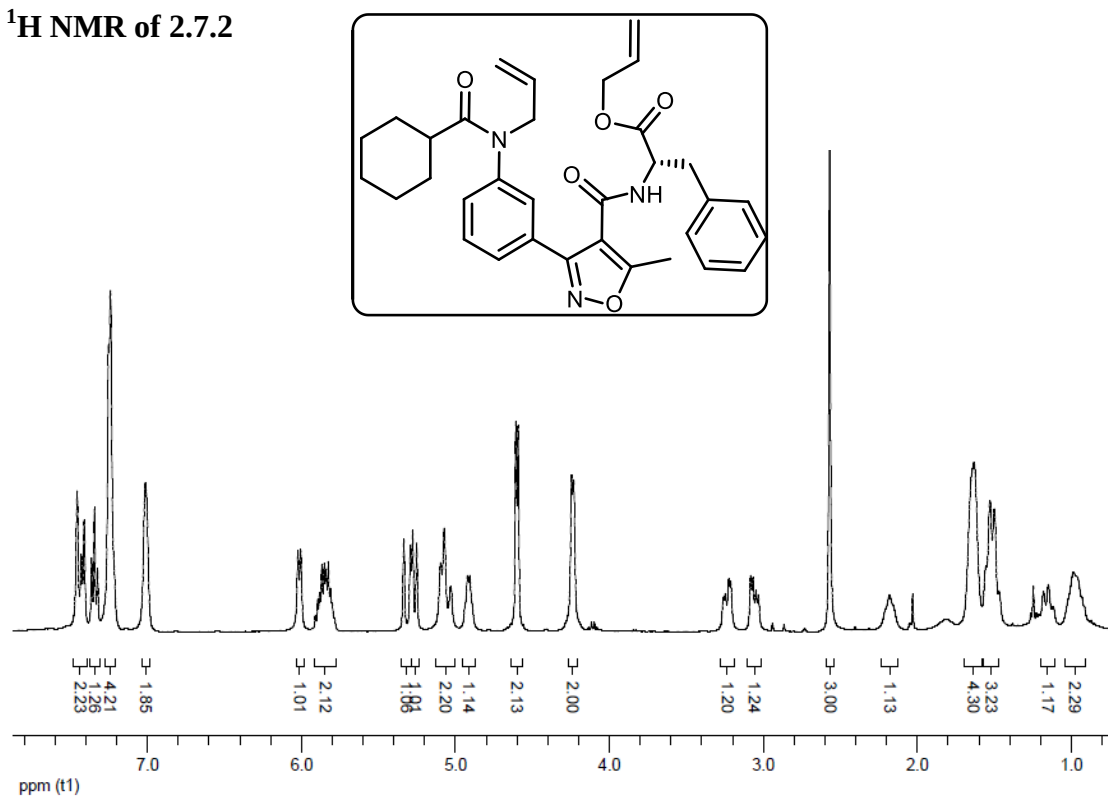


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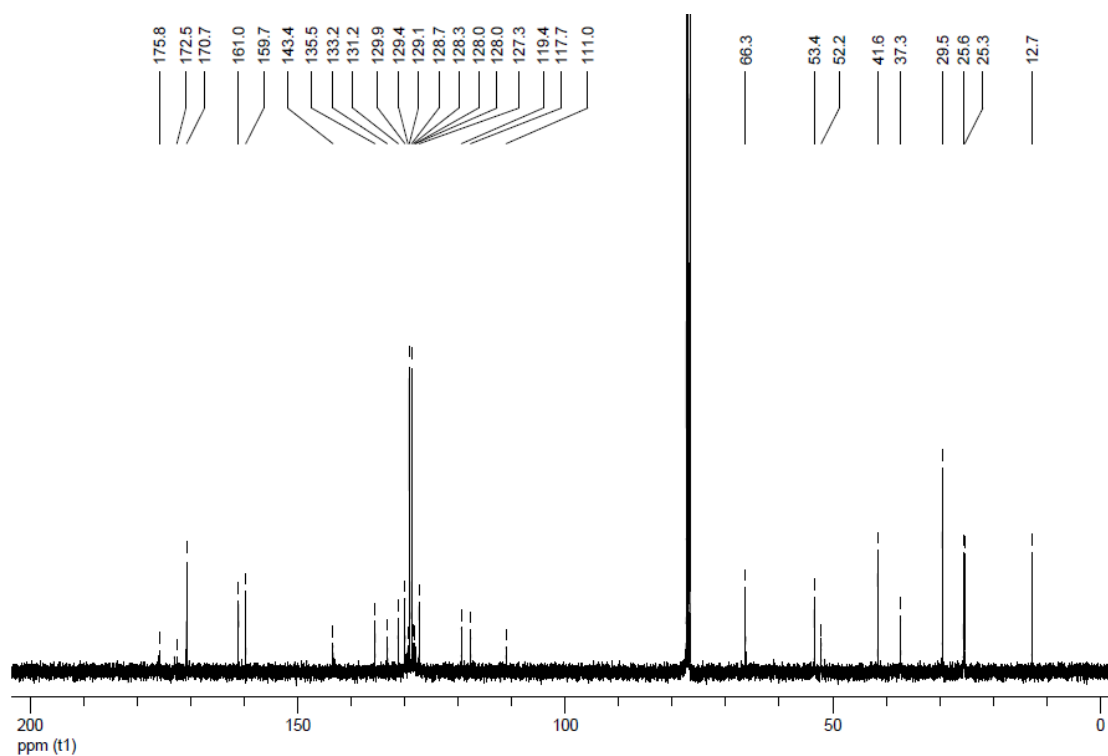


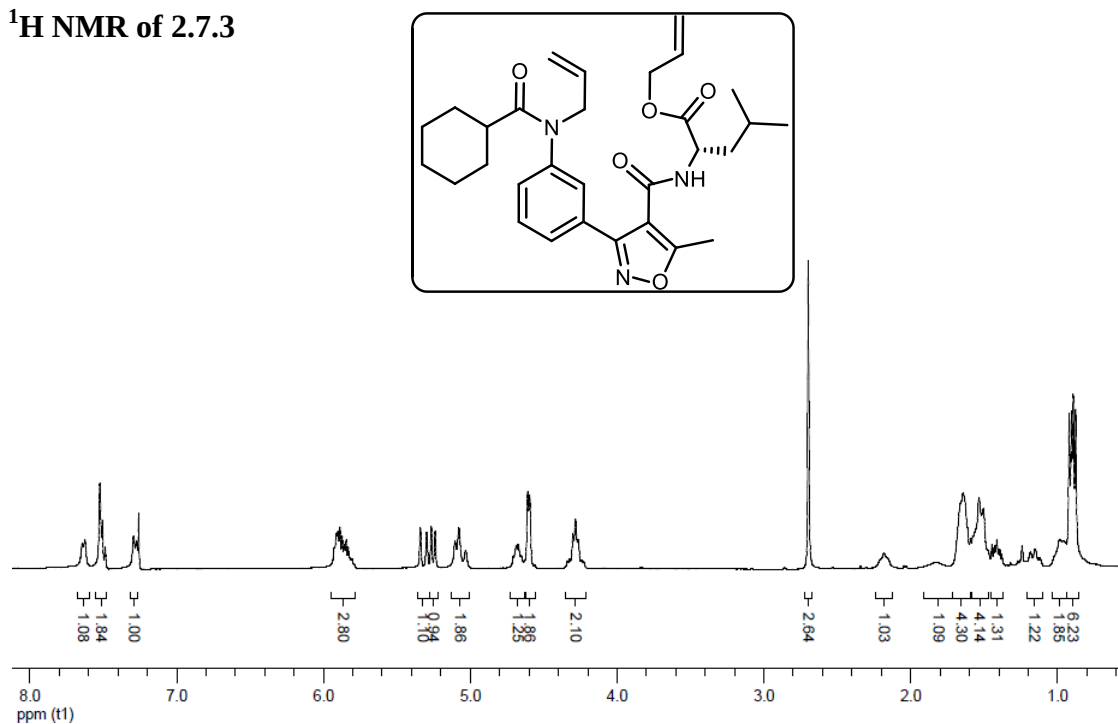
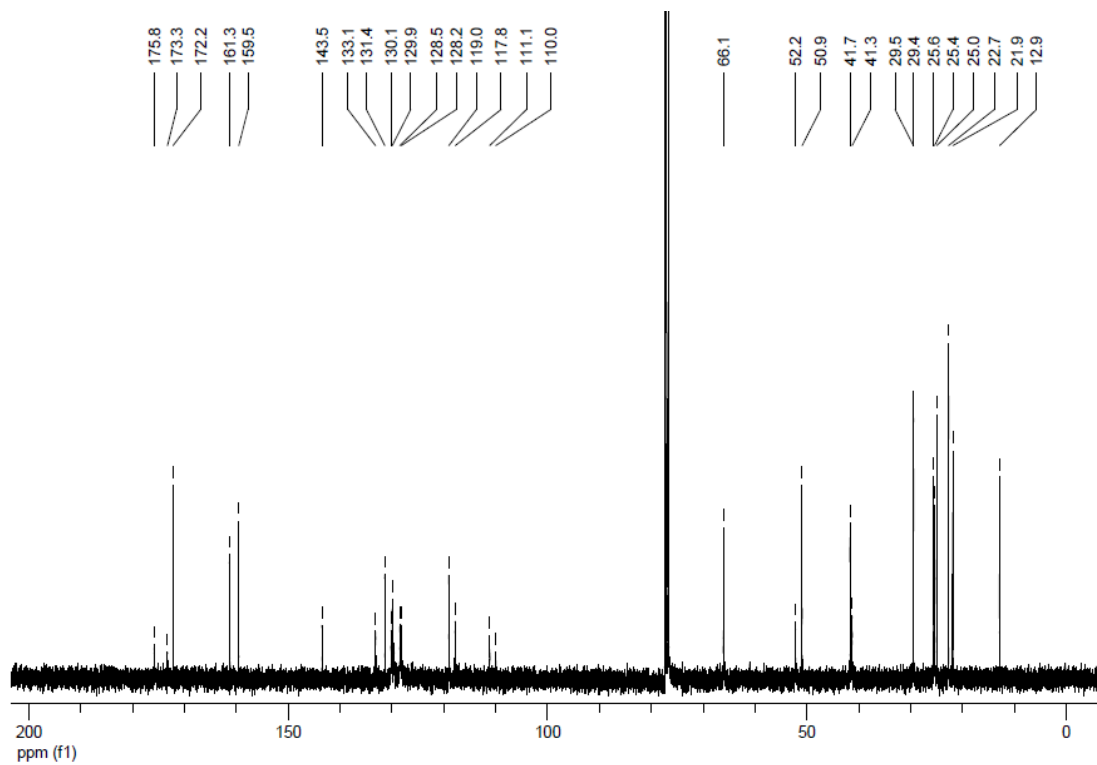
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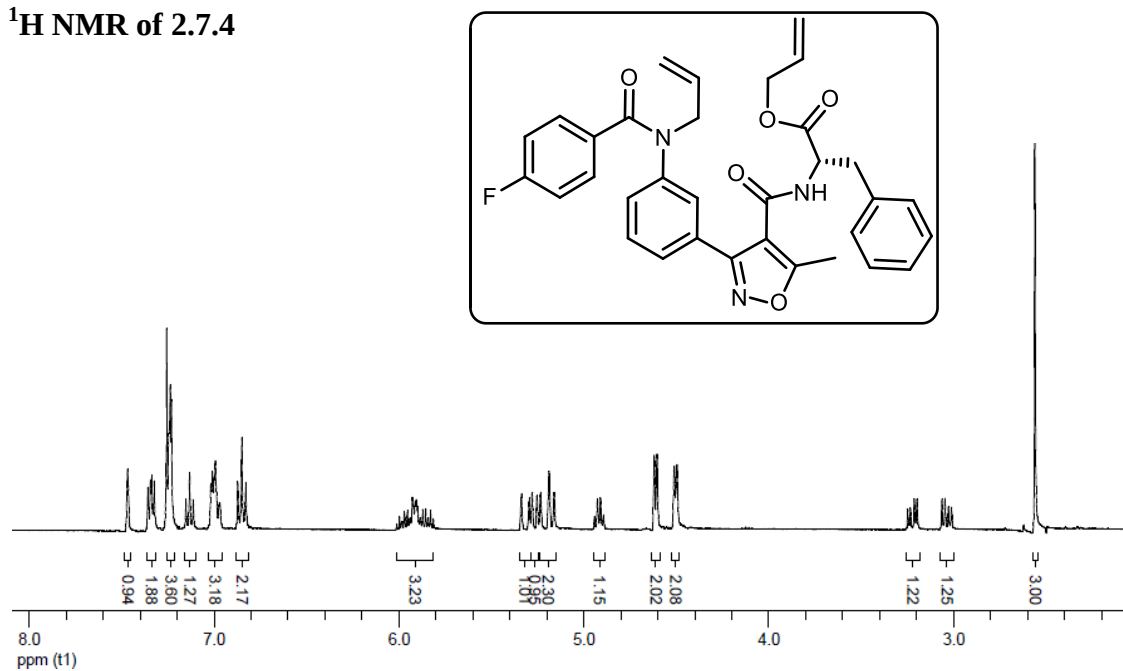


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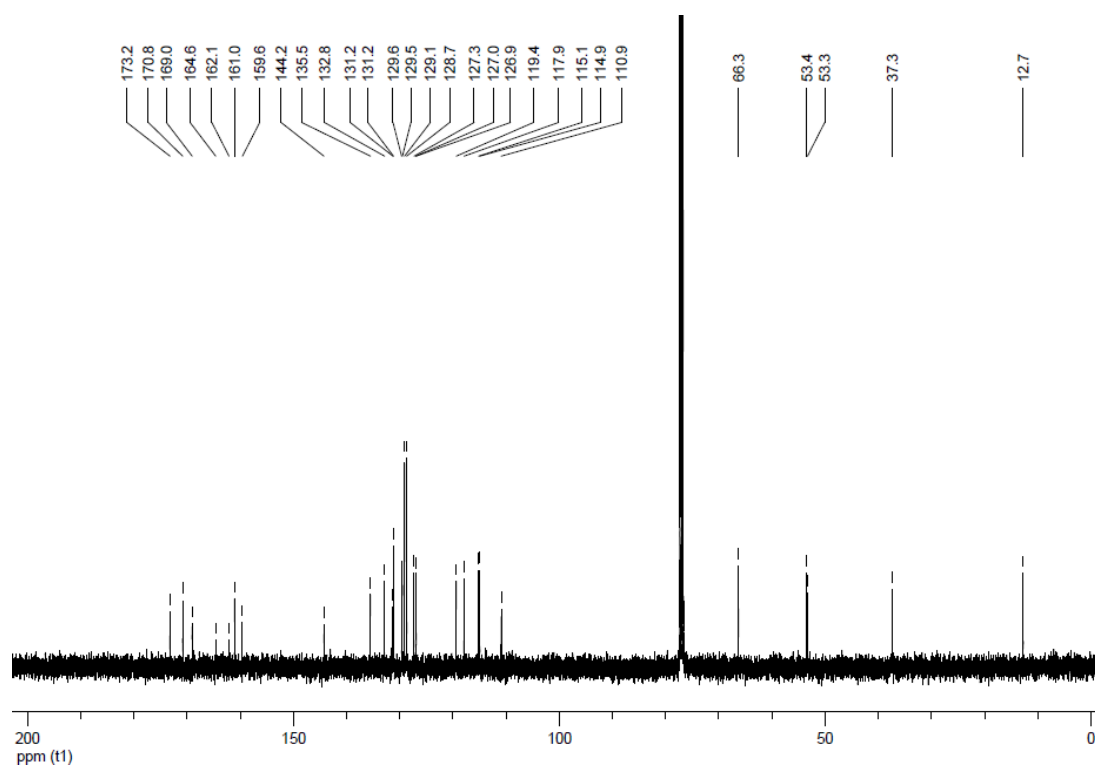


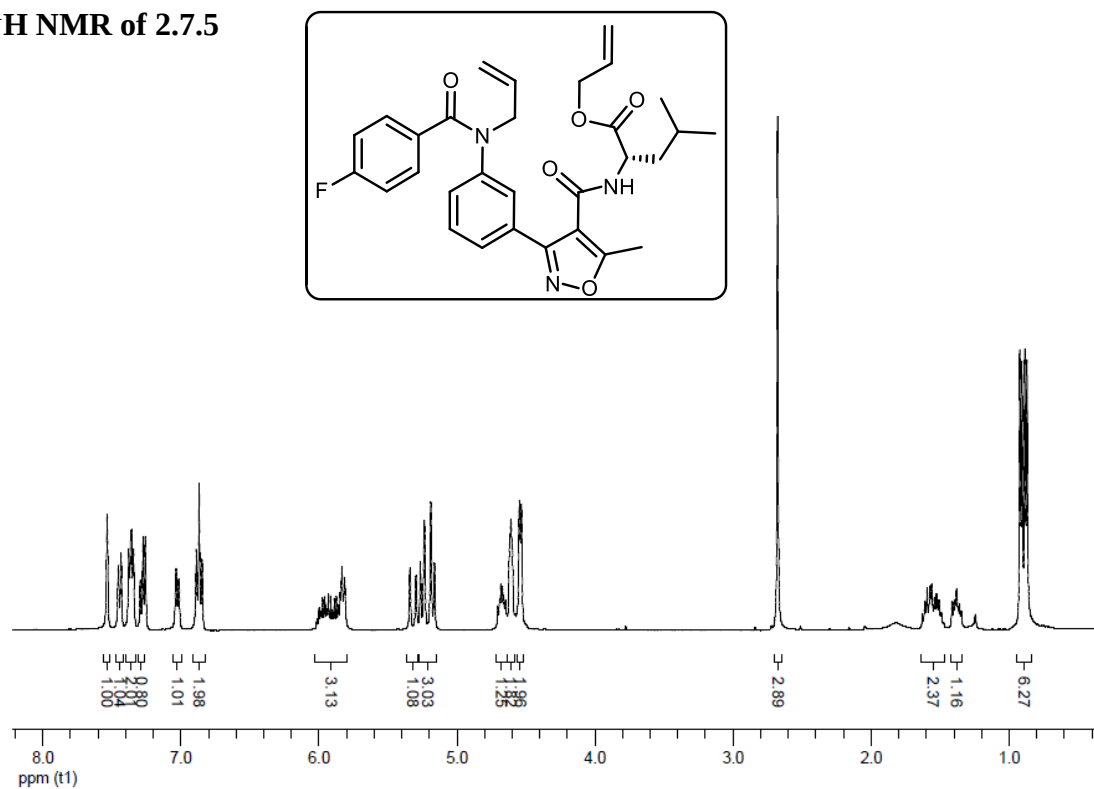
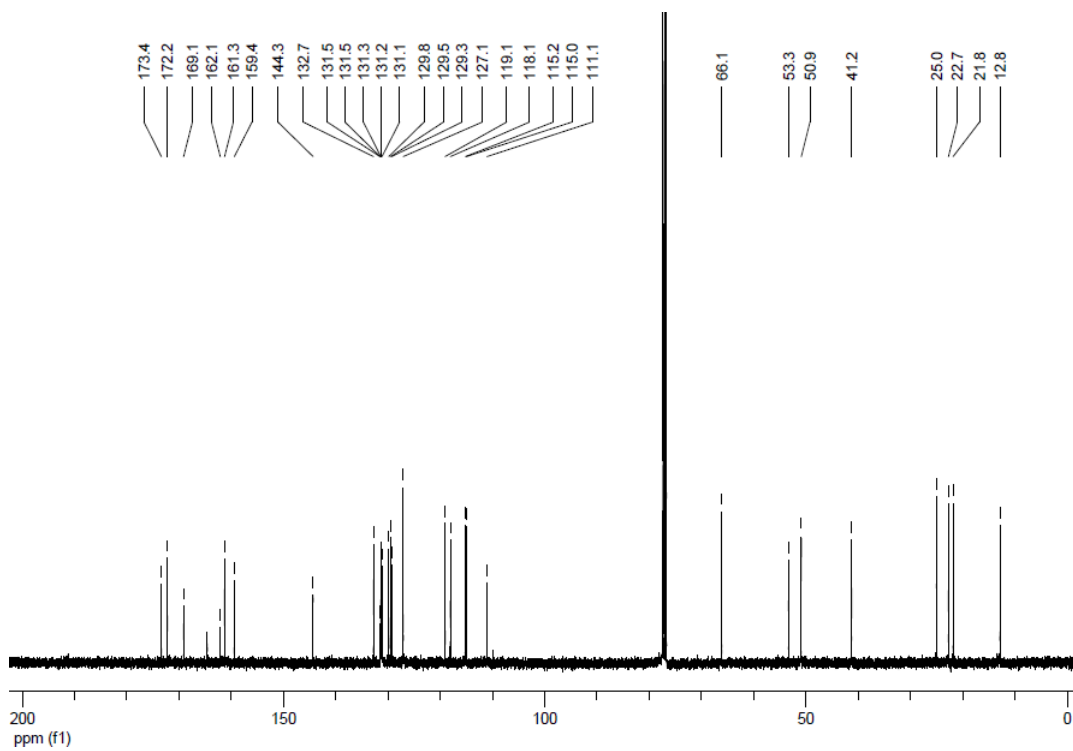
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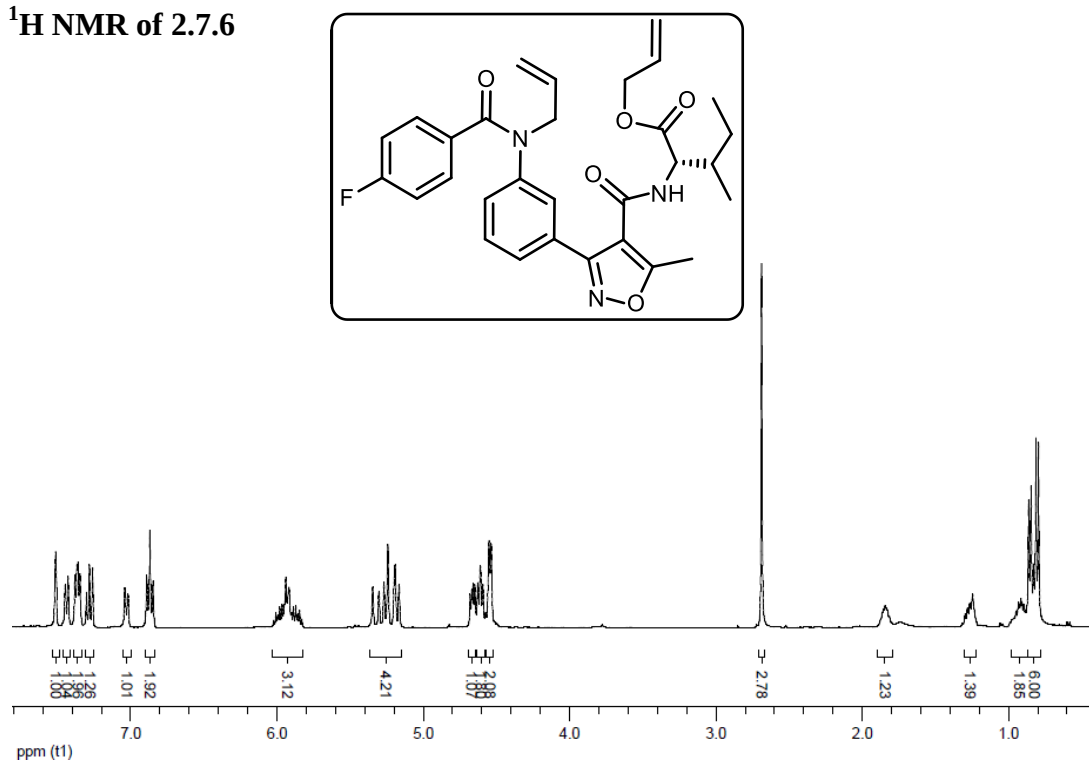


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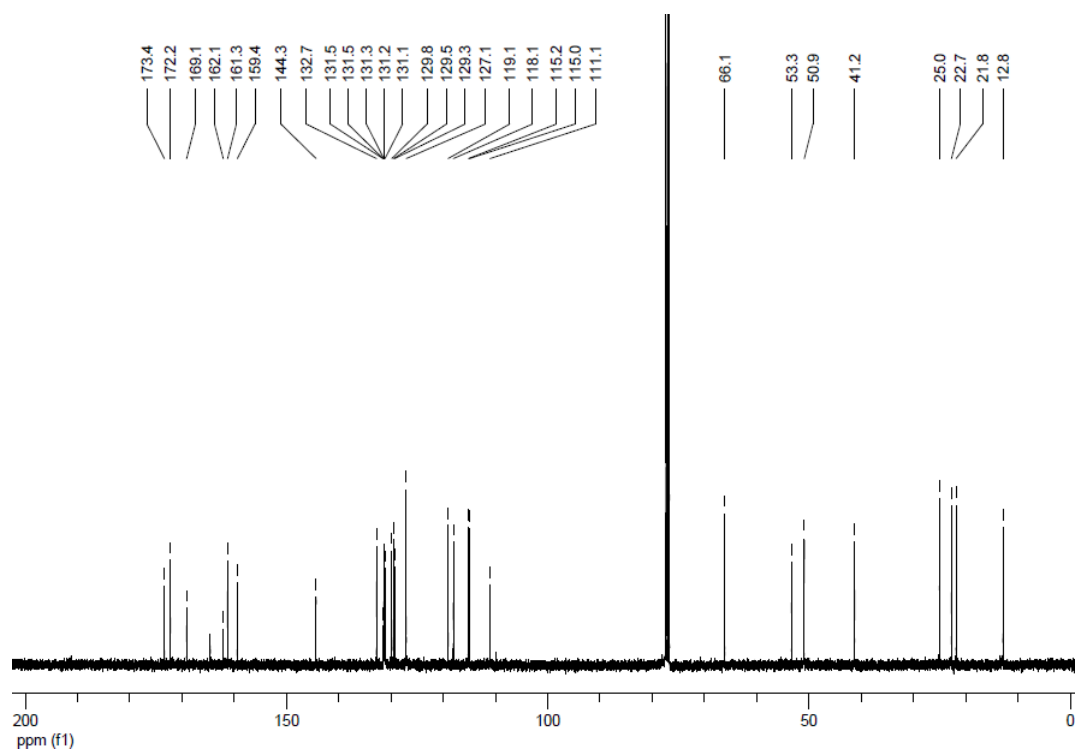


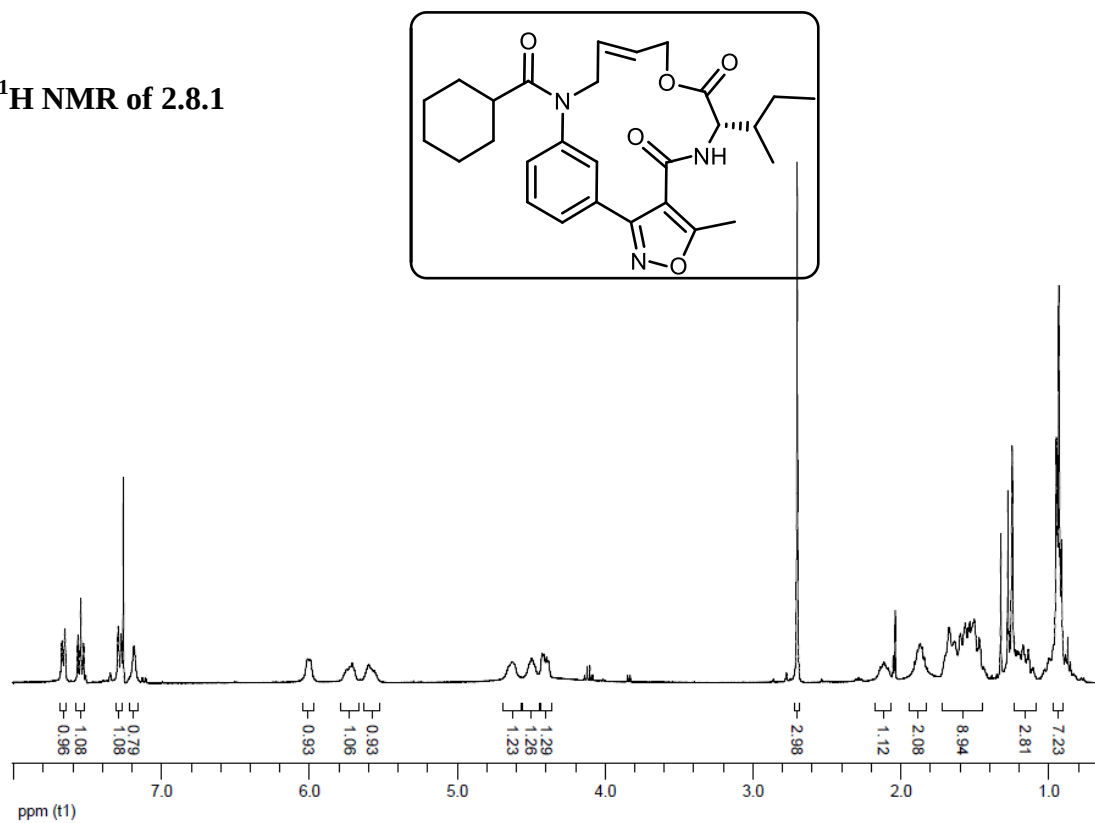
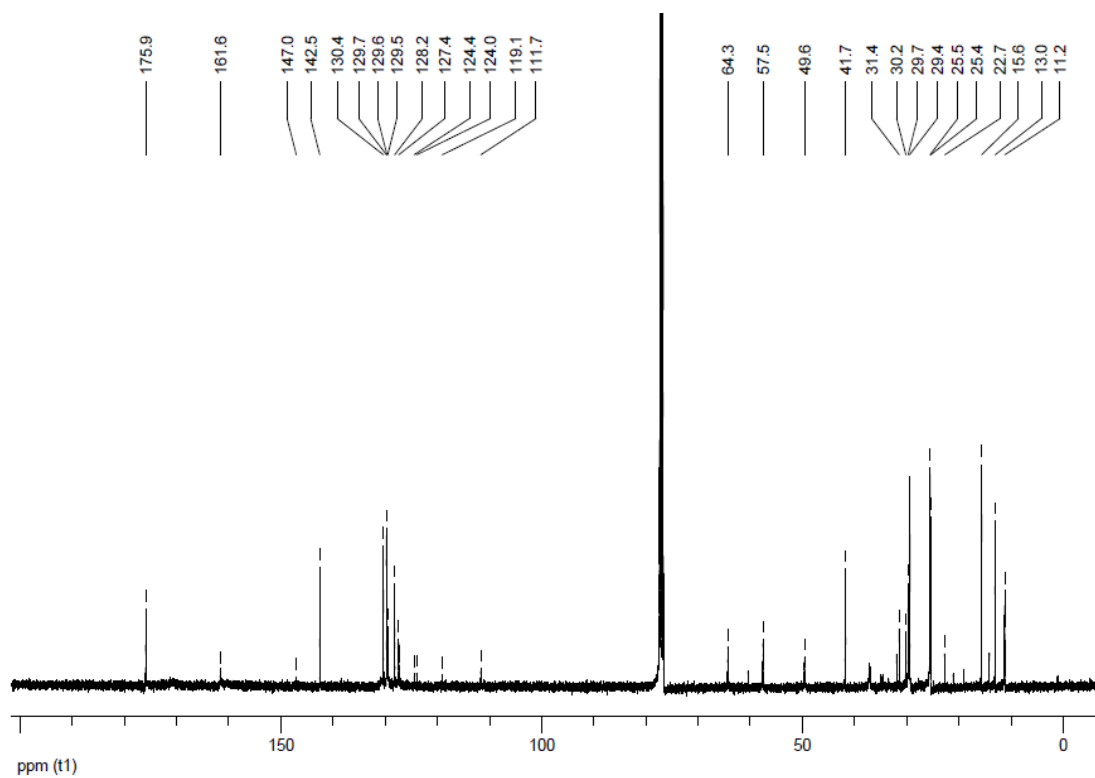
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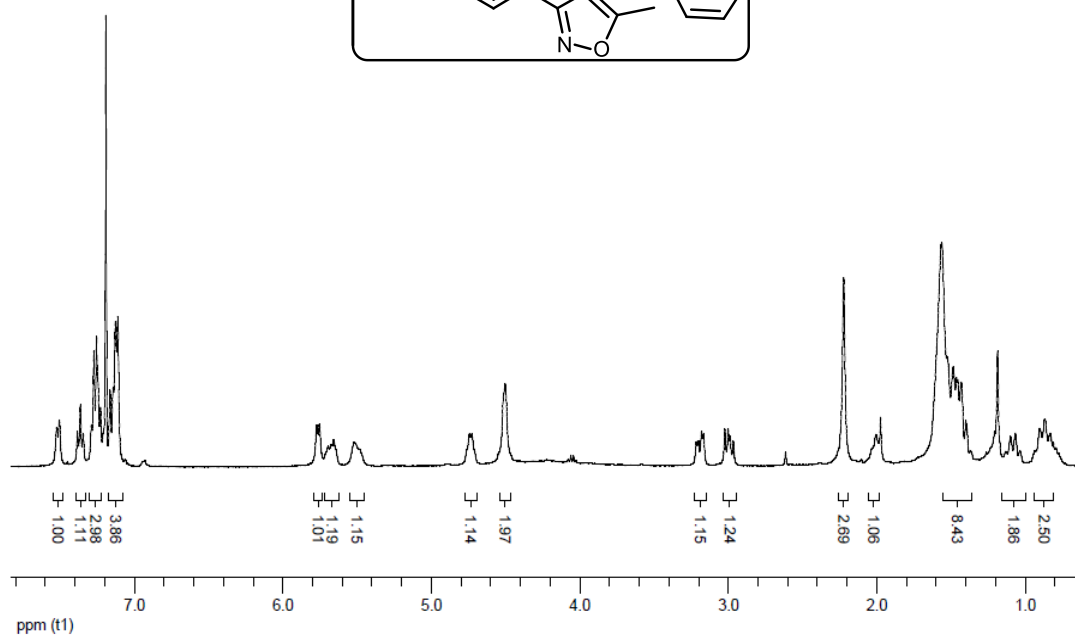
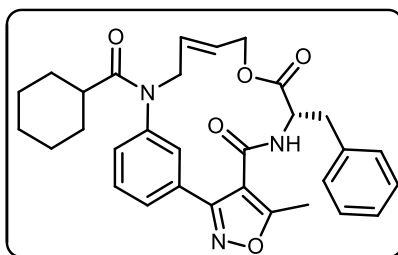


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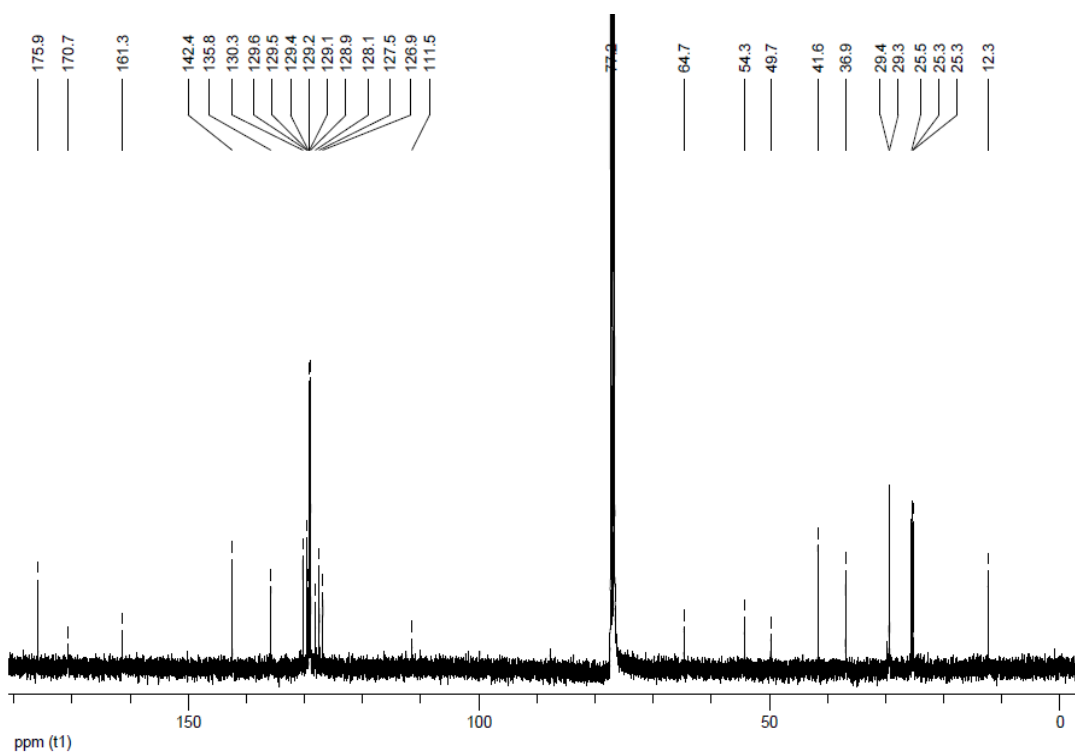


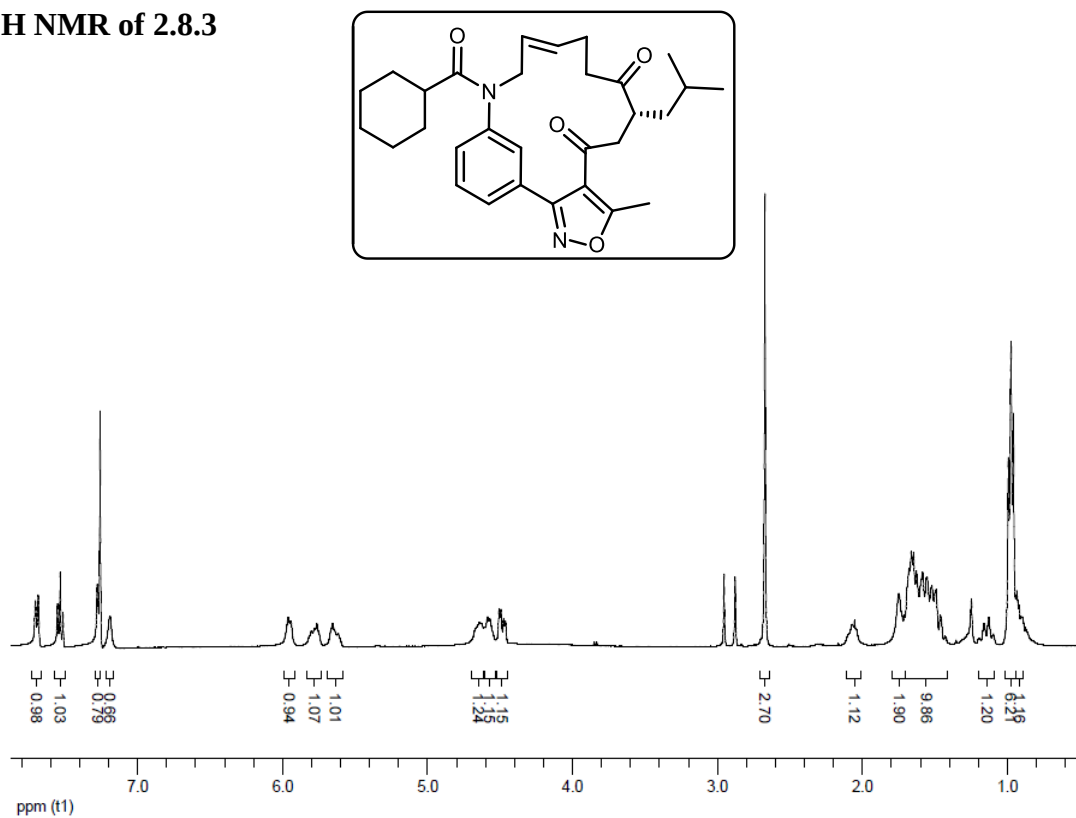
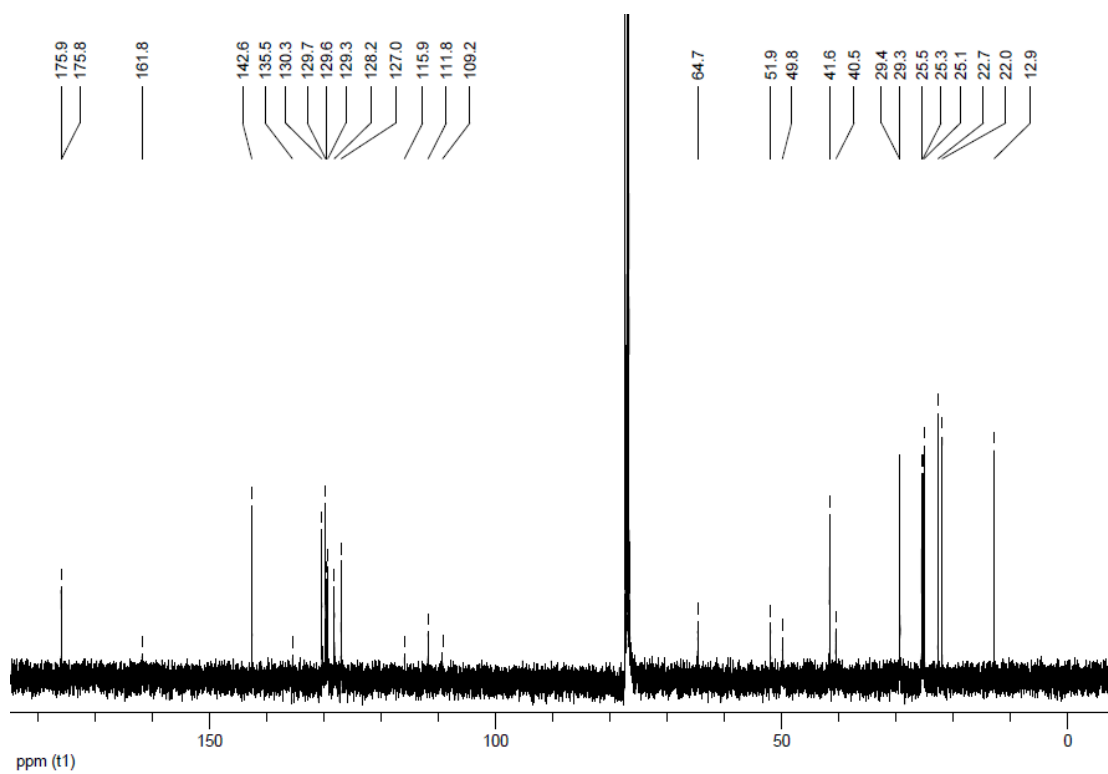
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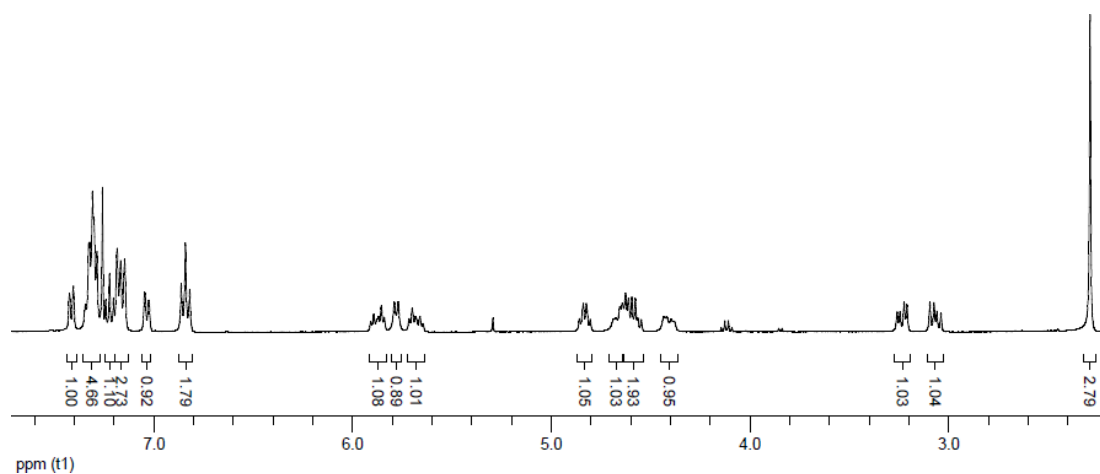
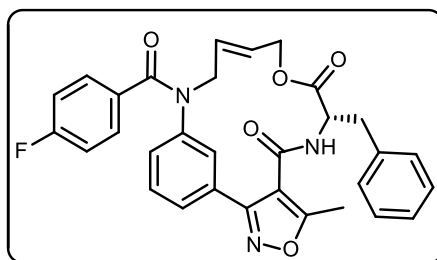


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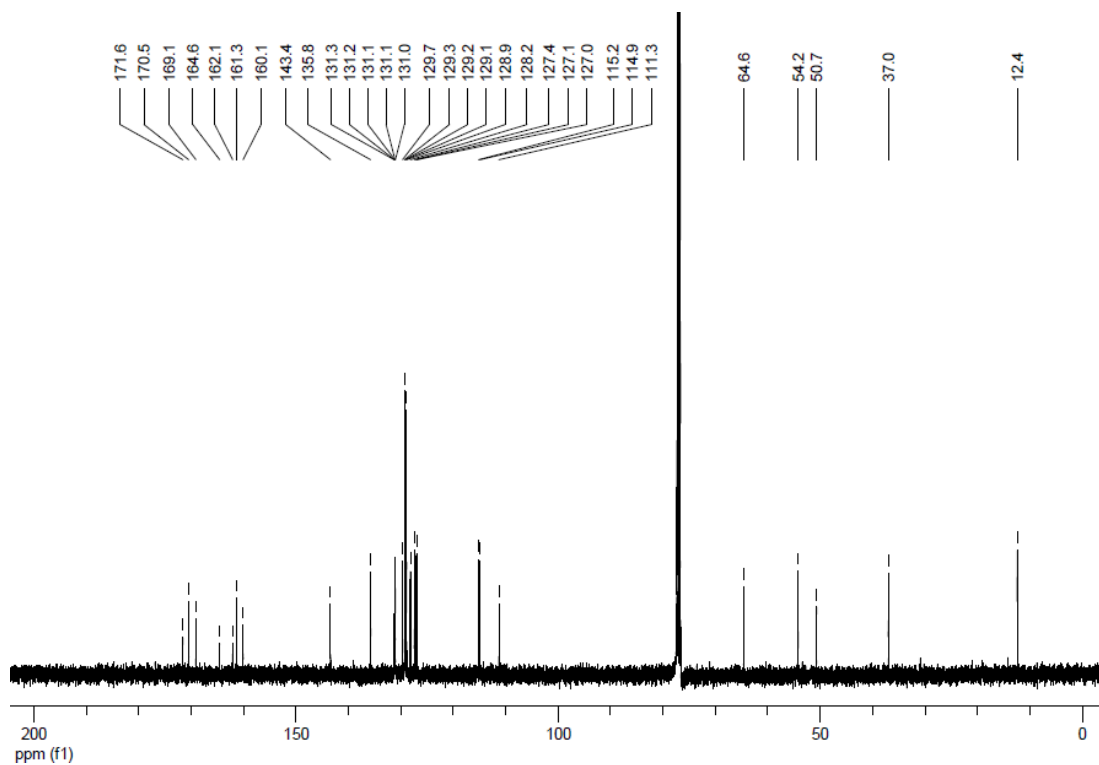


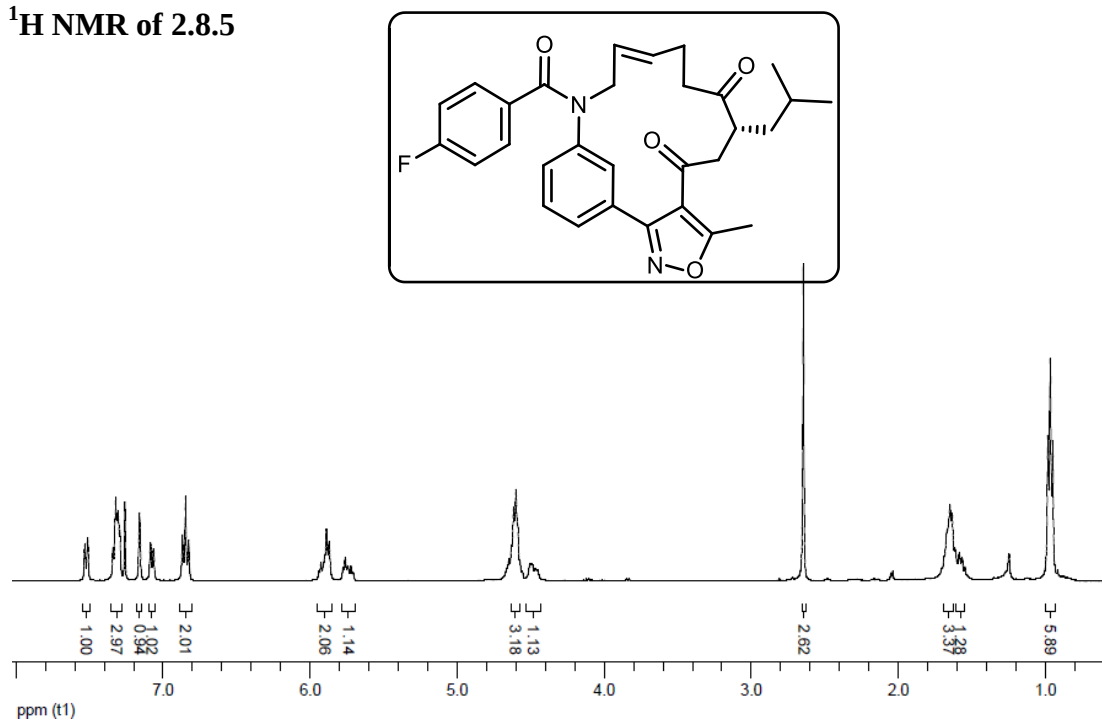
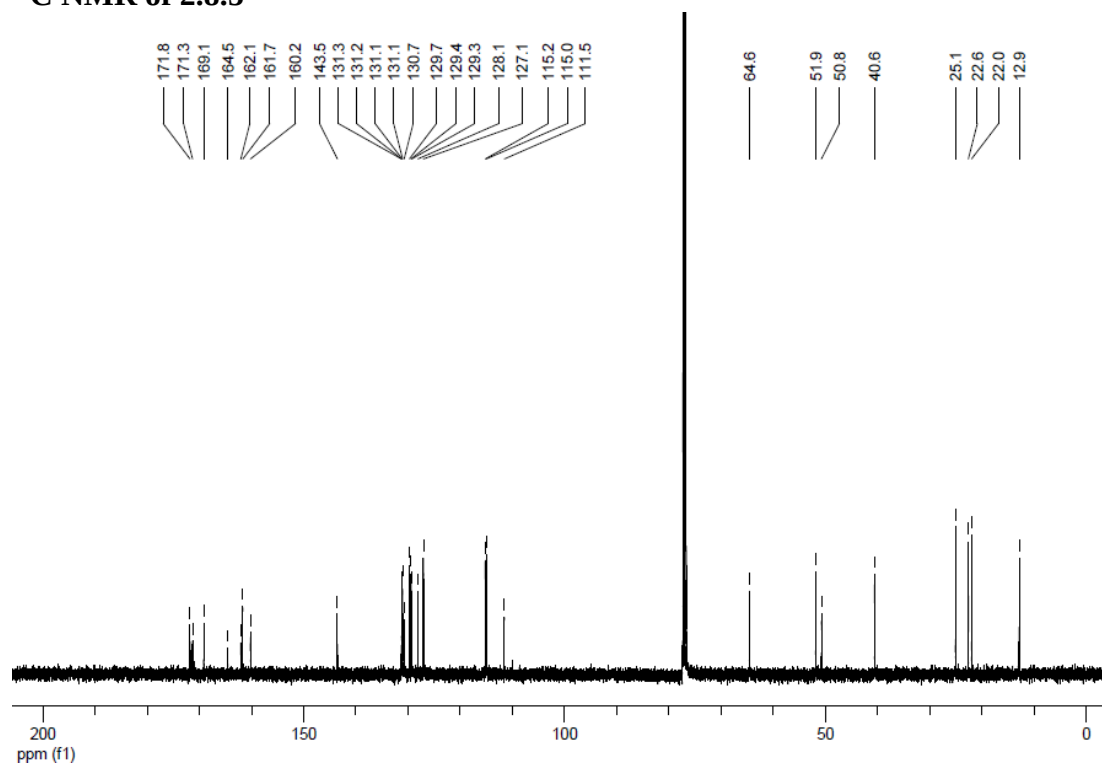
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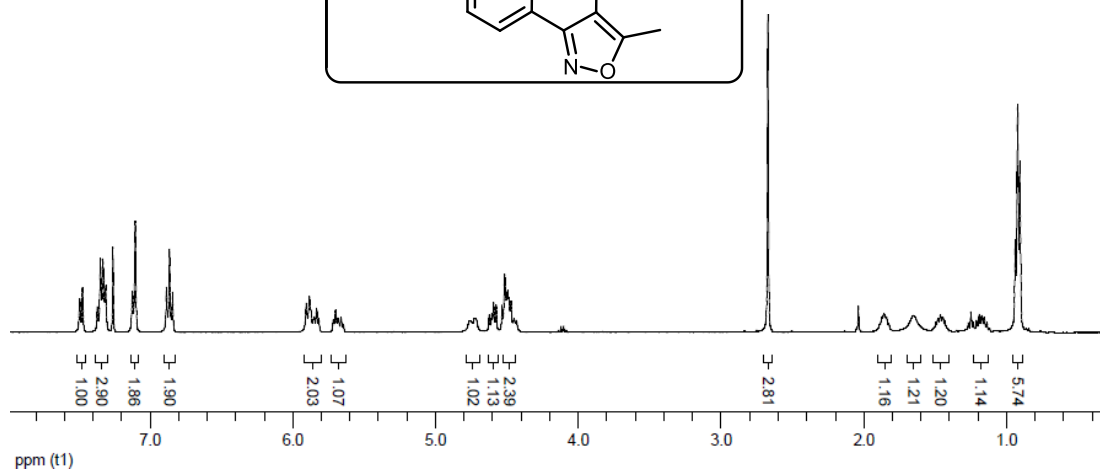
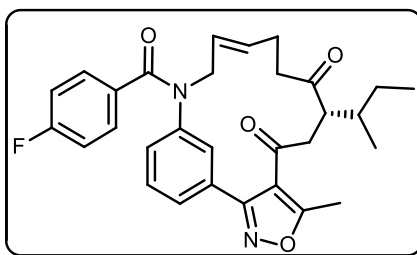


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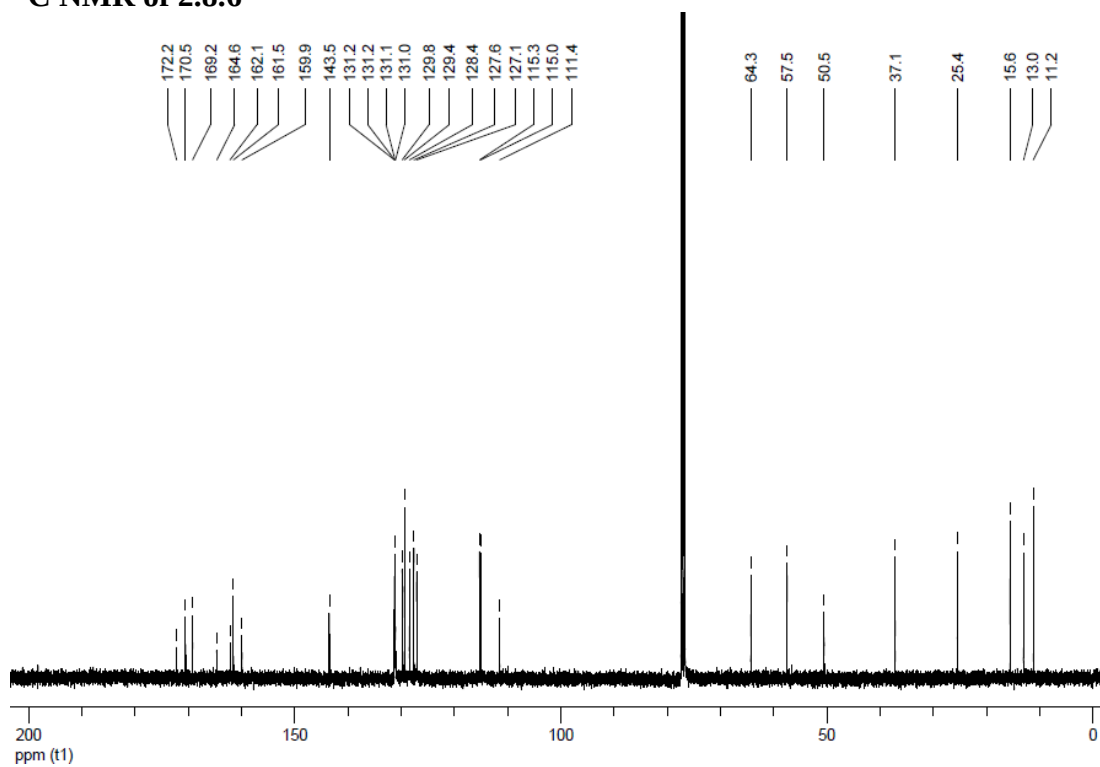


^1H NMR of 2.8.5 **^{13}C NMR of 2.8.5**

^1H NMR of 2.8.6



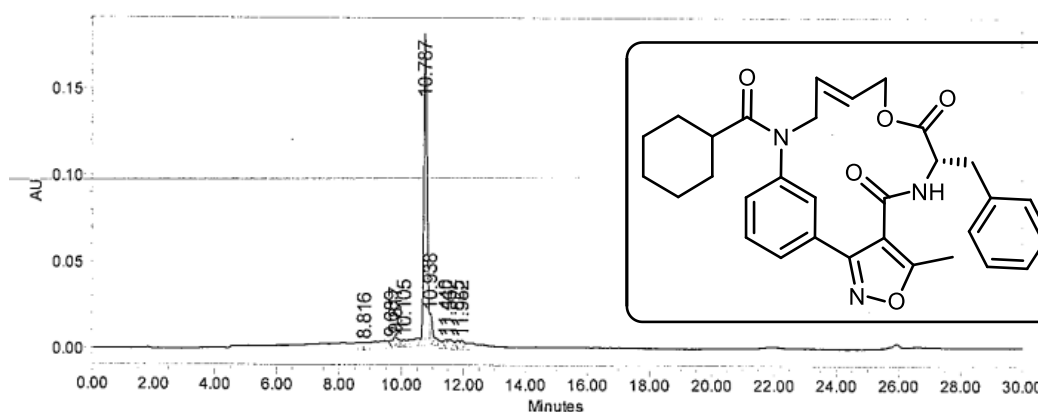
^{13}C NMR of 2.8.6



HPLC Data

SAMPLE INFORMATION			
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A R Number:	CM16D008	Acq. Method Set:	MEDCHEM
Vial:	7	Processing Method:	MED CHEM
Injection #:	1	Channel Name:	254.0nm
Injection Volume:	10.00 uI	Proc. Chnl. Descr.:	PDA 254.0 nm
Run Time:	30.0 Minutes		
Date Acquired:	4/7/2016 12:59:05 AM IST		
Date Processed:	4/7/2016 11:57:25 AM IST		

Column: X Bridge C18 150*4.6mm 5µm
 Mobile phase: A) 0.1% HCOOH in water B) ACN
 T/%B: 0/20, 3/20, 10/95, 23/95, 25/20, 30/20
 Flow: 1.0 ml/min, Diluent: ACN: WATER (80:20)



	RT	Area	% Area	Height
1	8.816	672	0.05	132
2	9.689	1003	0.07	184
3	9.817	11054	0.80	1749
4	10.105	1474	0.11	313
5	10.787	1250654	90.72	175923
6	10.938	78763	5.71	14617
7	11.440	6584	0.48	1101
8	11.562	11942	0.87	1217
9	11.825	7261	0.53	1191
10	11.952	9214	0.67	1347

Analysed by *M. Anli*
07/04/16

Checked by *[Signature]*
07/04/16

Reported by User: System
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 Report Method ID: 5383
 Page: 1 of 1

Project Name: APRIL_2016
 Date Printed:
 4/7/2016
 12:10:36 PM Asia/Calcutta

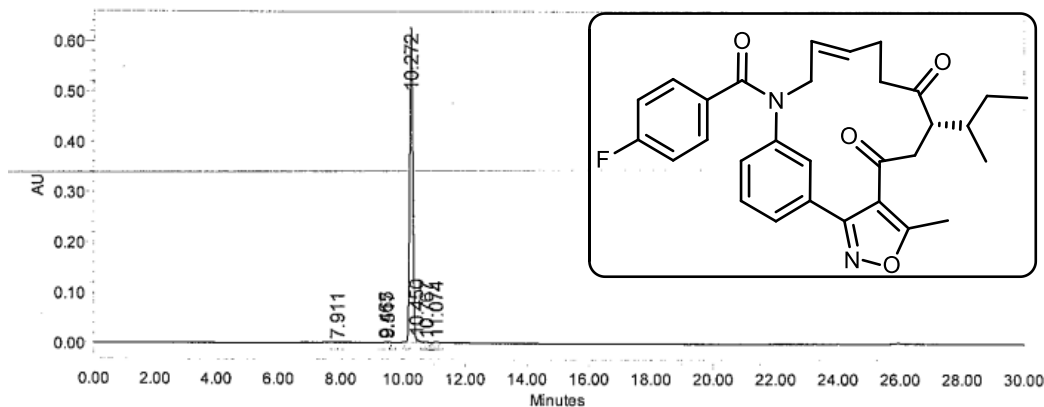
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Injection #: 1
Injection Volume: 10.00 µl
Run Time: 30.0 Minutes

Sample Set Name: 06042016 seq
Acq. Method Set: MEDCHEM
Processing Method: MEDCHEM PRO
Channel Name: 254.0nm
Proc. Chnl. Descr.: PDA 254.0 nm

Date Acquired: 4/7/2016 1:34:54 AM IST
Date Processed: 4/7/2016 12:04:05 PM IST

Column: X Bridge C18 150*4.6mm 5µm
Mobile phase: A) 0.1% HCOOH in water B) ACN
T/%B: 0/20, 3/20, 10/95, 23/95, 25/20, 30/20
Flow: 1.0 ml/min, Diluent: ACN: WATER (80:20)



	RT	Area	% Area	Height
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2	9.465	3582	0.08	533
3	9.517	2319	0.05	407
4	10.272	4347791	98.91	626542
5	10.450	26064	0.59	4634
6	10.767	3128	0.07	490
7	11.074	11835	0.27	1621

Analysed by M. Anil
07/04/16

Checked by [Signature]

Reported by User: System
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Report Method ID: 5383
Page: 1 of 1

Project Name: APRIL_2016
Date Printed: 4/7/2016
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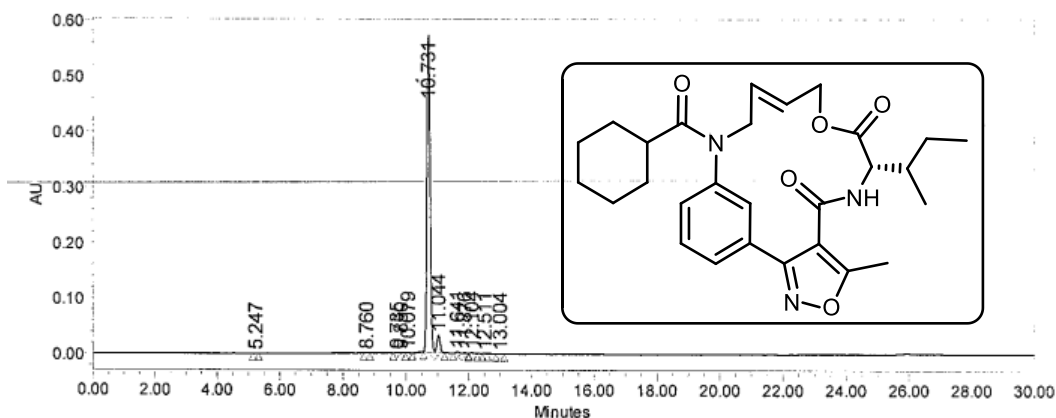
SAMPLE INFORMATION

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 Run Time: 30.0 Minutes

Sample Set Name: 06042016 seq
 Acq. Method Set: MEDCHEM
 Processing Method: MED CHEM
 Channel Name: 254.0nm@2
 Proc. Chnl. Descr.: PDA 254.0 nm

Date Acquired: 4/7/2016 12:23:20 AM IST
 Date Processed: 4/7/2016 11:47:27 AM IST

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 T/%B: 0/20, 3/20, 10/95, 23/95, 25/20, 30/20
 Flow: 1.0 ml/min, Diluent: ACN: WATER (80:20)



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4	9.850	931	0.02	159
5	10.079	1586	0.04	250
6	10.731	4012906	93.64	569438
7	11.044	220840	5.15	31107
8	11.641	18100	0.42	2465
9	11.876	7098	0.17	979
10	12.104	11857	0.28	1642

	RT	Area	% Area	Height
11	12.511	1895	0.04	355
12	13.004	1663	0.04	208

Analysed by *M. Anthe*
 07/04/16

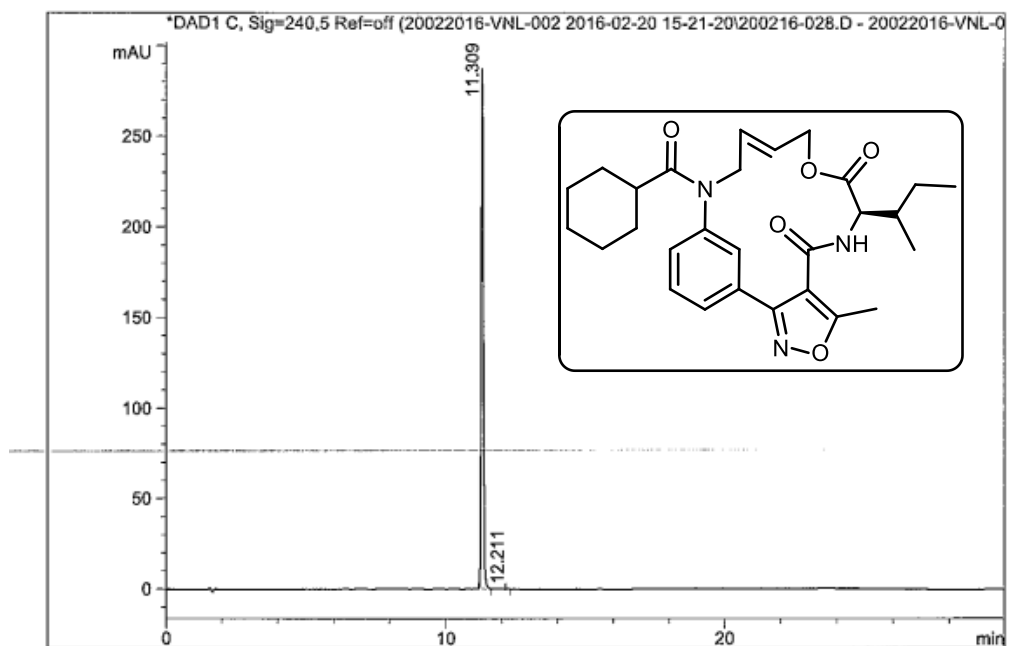
Checked by

Reported by User: System
 Report Method: CPRI@DRILS
 Report Method ID: 5383
 Page: 1 of 1

Project Name: APRIL_2016
 Date Printed: 4/7/2016
 12:11:16 PM Asia/Calcutta

CPRI @ DRILS
HPLC ANALYSIS REPORT

Inj Date : Sun, 21. Feb. 2016 Acq Operator: RADHA
Sample Name : DRILS-GJ-NPD-518 Vial 28
A R Number : CM16B015 -> 20.000
Acq. Method : D:\chem32\1\DATA\20022016-VNL-002 2016-02-20 15-21-2->
Analysis Method : D:\CHEM32\1\METHODS\MEDCHEM.M
Method Info : Column : X-BRIDGE C-18 150*4.6mm 5 µm
Mobile phase: A) 0.1%FA in Water B) ACN
T/%B:0/20,3/20,12/95,23/95,25/20,30/20
Flow:1.0 ml/min, Diluent: ACN:Water(80:20)



Signal 1: DAD1 C, Sig=240,5 Ref=off

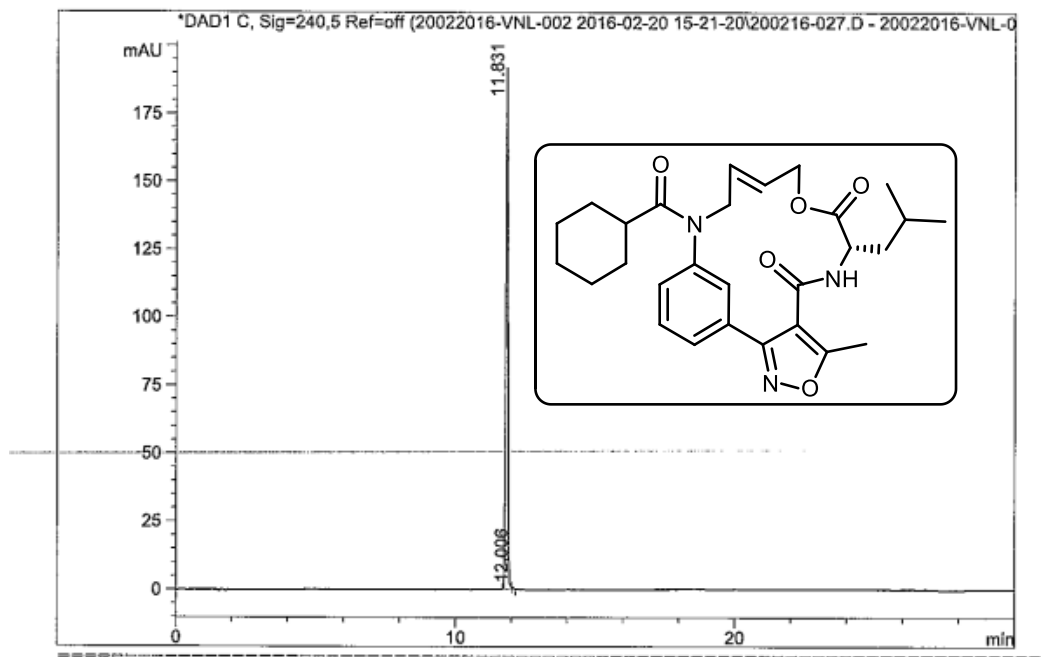
Peak #	RT [min]	Area	Area %
1	11.309	1440.340	99.872
2	12.211	1.847	0.128

Analysed by : *At*
22/02/16

Checked by : *f6*
22/02/16

CPRI @ DRILS
HPLC ANALYSIS REPORT

Inj Date : Sun, 21. Feb. 2016 Acq Operator: RADHA
Sample Name : DRILS-GJ-NPD-516 Vial 27
A R Number : CM16B014 -> 20.000
Acq. Method : D:\chem32\1\DATA\20022016-VNL-002 2016-02-20 15-21-2->
Analysis Method : D:\CHEM32\1\METHODS\MEDCHEM.M
Method Info : Column : X-BRIDGE C-18 150*4.6mm 5 µm
Mobile phase: A) 0.1%FA in Water B) ACN
T/%B:0/20,3/20,12/95,23/95,25/20,30/20
Flow:1.0 ml/min, Diluent: ACN:Water(80:20)



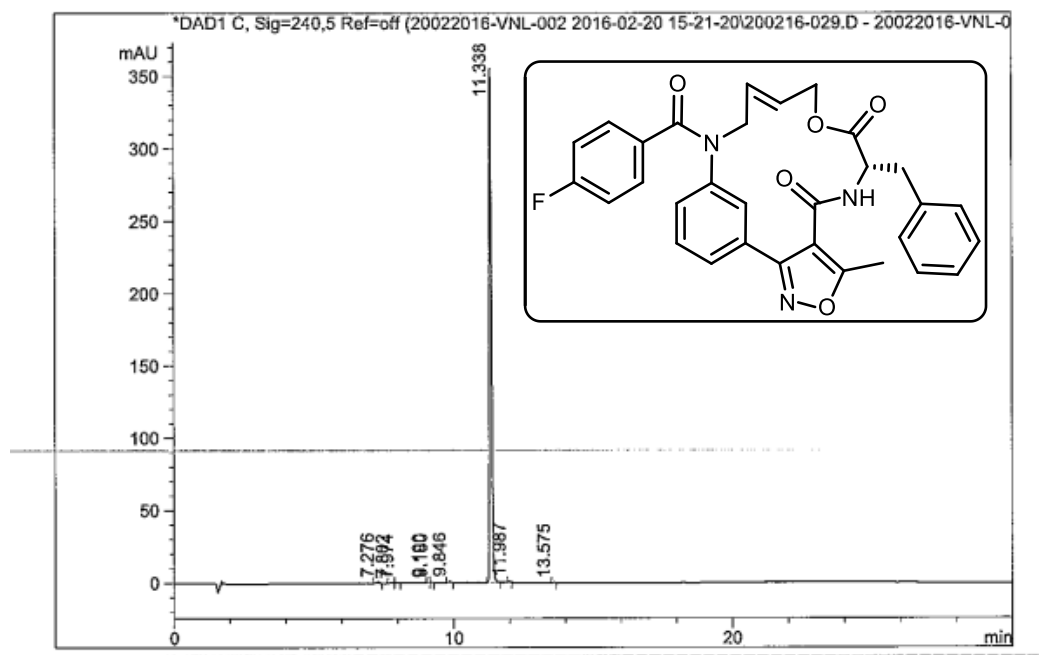
Signal 1: DAD1 C, Sig=240,5 Ref=off

Peak #	RT [min]	Area	Area %
1	11.831	969.644	99.738
2	12.006	2.547	0.262

Analysed by : *At*
22/02/16Checked by : *fb*
22/02/16

CPRI @ DRILS
HPLC ANALYSIS REPORT

Inj Date : Sun, 21. Feb. 2016 Acq Operator: RADHA
Sample Name : DRILS-GJ-NPD-511 Vial 29
A R Number : CM16B008 -> 20.000
Acq. Method : D:\chem32\1\DATA\20022016-VNL-002 2016-02-20 15-21-2->
Analysis Method : D:\CHEM32\1\METHODS\MEDCHEM.M
Method Info : Column : X-BRIDGE C-18 150*4.6mm 5 µm
Mobile phase: A) 0.1%FA in Water B) ACN
T/%B:0/20,3/20,12/95,23/95,25/20,30/20
Flow:1.0 ml/min, Diluent: ACN:Water(80:20)



Signal 1: DAD1 C, Sig=240,5 Ref=off

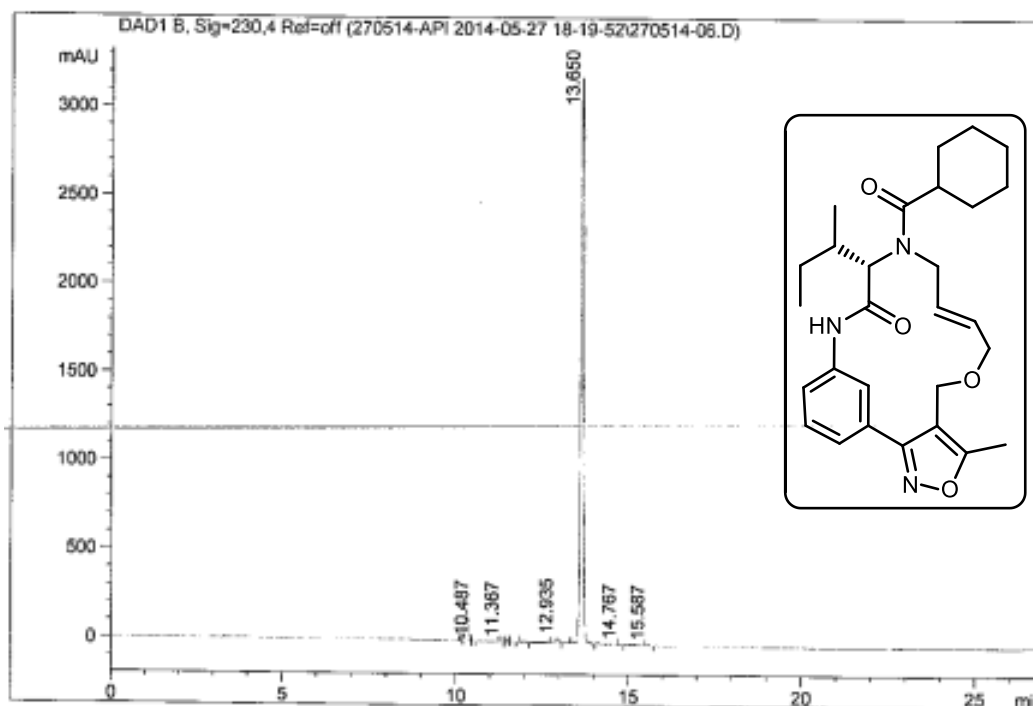
Peak #	RT [min]	Area	Area %
1	7.276	5.655	0.318
2	7.802	1.167	0.066
3	7.974	1.208	0.068
4	9.100	1.494	0.084
5	9.180	2.282	0.128
6	9.846	4.789	0.269
7	11.338	1757.435	98.751
8	11.987	3.536	0.199
9	13.575	2.104	0.118

Analysed by : *At*
22/02/16

Checked by : *fb*
22/02/16

CPRI @ DRILS
HPLC ANALYSIS REPORT

Inj Date : Tue, 27. May. 2014 Acq Operator: SHASHIDHAR
 Sample Name : DRILS-GJ-NO2-022 RCM Vial 16
 A.R Number : CM14E011 ->Inj. Vol. : 5µL
 Acq. Method : D:\chem32\1\DATA\270514-API 2014-05-27 18-19-52\API ->
 Analysis Method : D:\CHEM32_002\1\METHODS\API DTV.M
 Method Info : Column: X-Terra C-18 250*4.6mm, 5µm
 Mobile phase: A) 5mm Ammonium Acetate in water B) ACN
 T/%B: 0/20, 3/20, 12/95, 23/95, 25/20, 30/20
 Flow: 1.0ml/min Diluent: ACN:WATER (50:50)

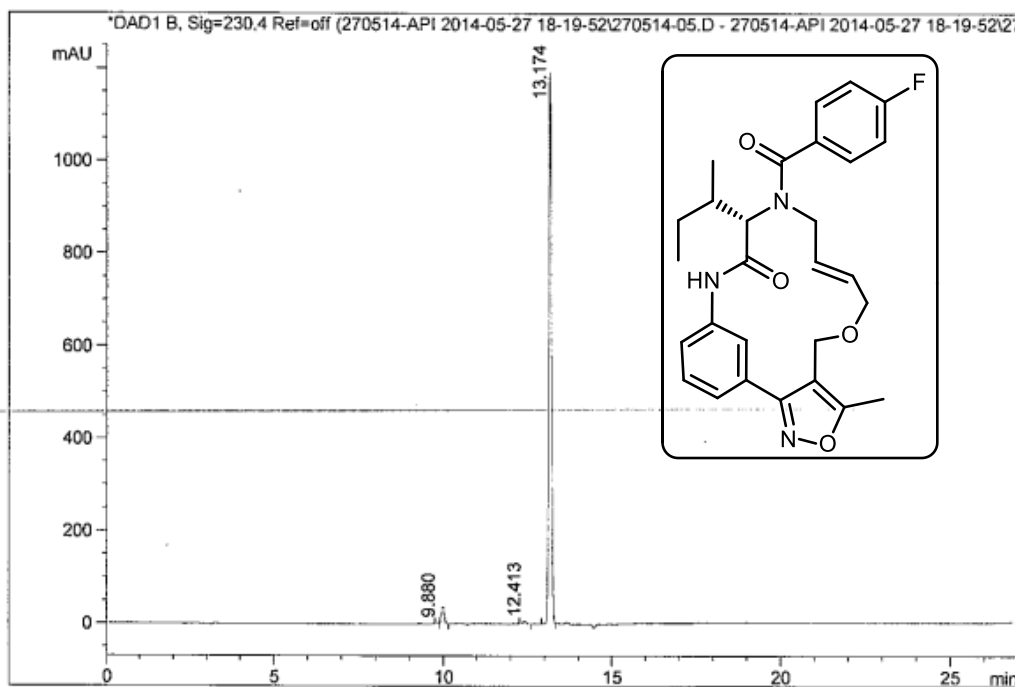


Signal 1: DAD1 B, Sig=230,4 Ref=off

Peak #	RT [min]	Area	Area %
1	10.220	22.345	0.107
2	10.323	963.104	4.617
3	10.487	9.009	0.043
4	11.367	5.579	0.027
5	11.491	37.314	0.179
6	11.580	22.574	0.108
7	11.976	33.290	0.160
8	12.935	159.973	0.767
9	13.650	19581.109	93.866
10	14.767	11.536	0.055
11	15.587	14.906	0.071

CPRI @ DRILS
HPLC ANALYSIS REPORT

Inj Date : Tue, 27. May. 2014 Acq Operator: SHASHIDHAR
Sample Name : DRILS-GJ-NO2-019 RCM Vial 15
A.R Number : C14E010 ->Inj. Vol. : 5µL
Acq. Method : D:\Chem32\1\DATA\270514-API 2014-05-27 18-19-52\API ->
Analysis Method : D:\CHEM32_002\1\METHODS\API DTV.M
Method Info : Column: X-Terra C-18 250*4.6mm, 5µm
Mobile phase: A) 5mm Ammonium Acetate in water B) ACN
T/%B:0/20,3/20,12/95,23/95,25/20,30/20
Flow:1.0ml/min Diluent: ACN:WATER(50:50)



Signal 1: DAD1 B, Sig=230,4 Ref=off

Peak #	RT [min]	Area	Area %
1	9.880	8.321	0.132
2	9.995	185.823	2.939
3	12.413	35.219	0.557
4	13.174	6093.417	96.372

Analysed by :

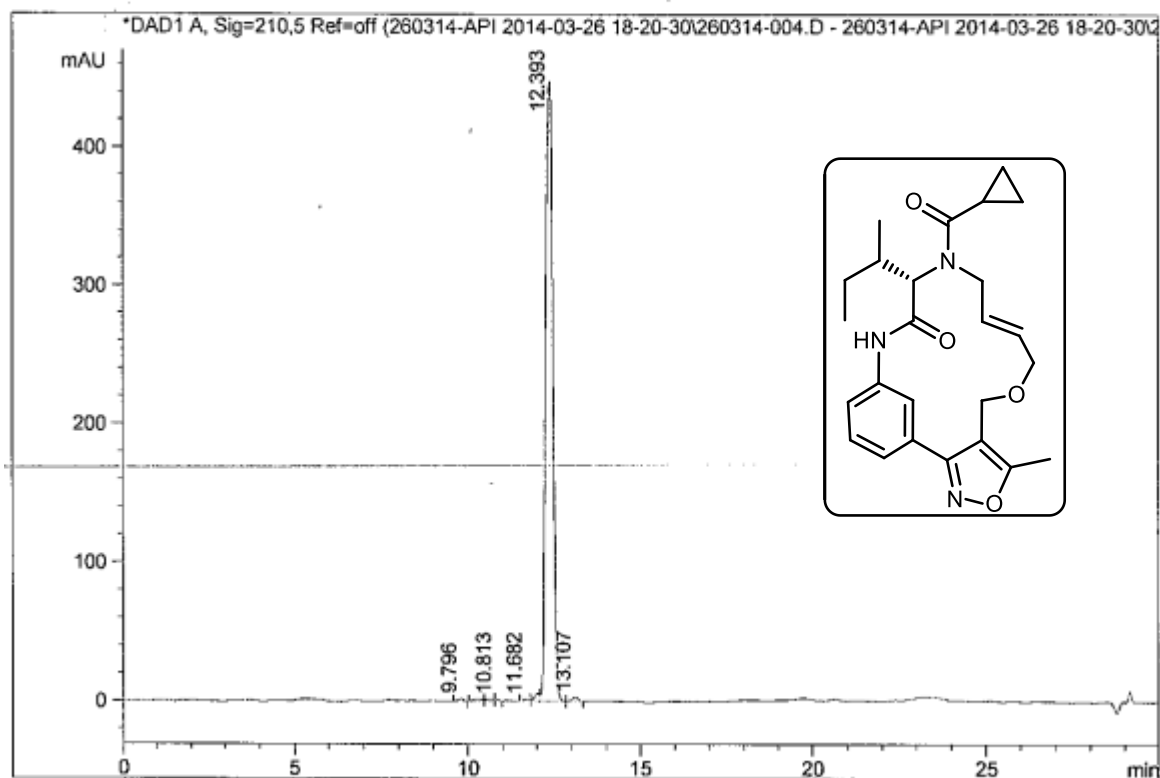
Dr. S. S.
28/05/14

Checked by :

Dr. S. S.
28/5/14

CPRI @ DRILS
HPLC ANALYSIS REPORT

		Seq Line	:	4
Injection Date	:	Wed, 26. Mar. 2014	Location	:
				Vial 6
Sample Name	:	ILS-GJ-HC-NO2-21-RCM	Inj. No.	:
				1
Acq Operator	:	RADHA	Inj. Vol.	:
				5 µl
Acq. Method	:	D:\chem32\1\DATA\260314-API 2014-03-26 18-20-30\API ->		
Analysis Method	:	D:\CHEM32_002\1\METHODS\API CAN NEW.M		
Method Info	:	Column: X Terra C-18 250*4.6mm 5µ		
		Mobile phase : A) 0.1% TFA in water, B) ACN		
		T/B%:0/40,15/65,18/90,25/90,27/40,30/40		
		Flow:1.0 ml/min Diluent: ACN:WATER(80:20)		



Signal 1: DAD1 A, Sig=210,5 Ref=off

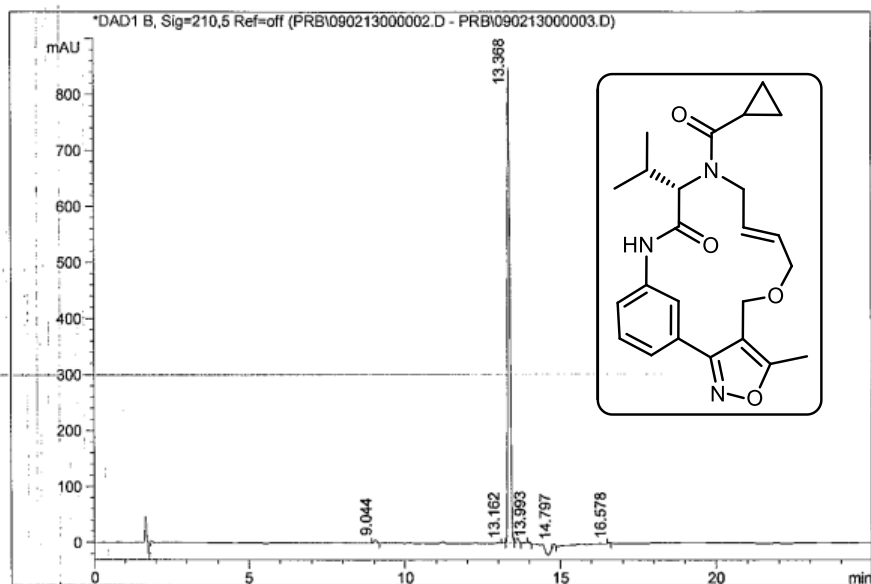
Peak #	RT [min]	Area	Area %
1	9.796	16.789	0.256
2	10.339	26.271	0.400
3	10.682	19.689	0.300
4	10.813	8.042	0.123
5	11.682	14.257	0.217
6	12.013	52.831	0.805
7	12.393	6391.258	97.392
8	13.107	33.267	0.507

M 2708/14

COSMIC DISCOVERIES @ ILS
HPLC ANALYSIS REPORT

Injection Date : Sat, 9. Feb. 2013
Sample Name : ILS-GJ-HC-N02-016
Acq Operator : VARMA
Acq. Method : D:\CHEM32\1\METHODS\AMD PRB.M
Analysis Method : D:\CHEM32\1\METHODS\AMD PRB.M
Method Info : Column : X-Bridge C-18 150*4.6mm, 5µm
Mobile phase: A) 0.1% HCOOH in water , B) ACN
T/B%: 0/10, 5/10, 15/95, 20/95, 22/10, 25/10
Flow: 1.0 ml/min Diluent: ACN:Water(90:10) CTemp: 25°C

Seq Line : 0
Location : Vial 3
Inj. No. : 0
Inj. Vol. : 1 µl



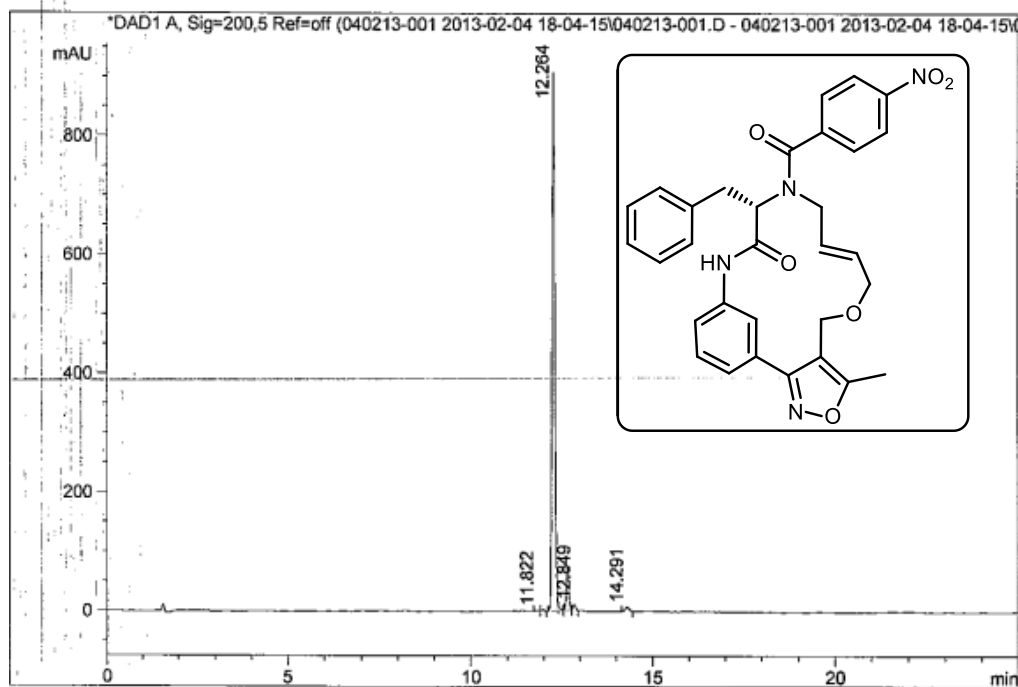
Signal 1: DAD1 B, Sig=210,5 Ref=off

Peak #	RT [min]	Area	Area %
1	9.044	37.508	0.828
2	13.162	5.714	0.126
3	13.368	4393.624	97.031
4	13.627	44.733	0.988
5	13.993	11.371	0.251
6	14.797	30.321	0.670
7	16.578	4.774	0.105

*Patent
11/02/13*

COSMIC DISCOVERIES @ ILS
HPLC ANALYSIS REPORT

Injection Date : Mon, 4. Feb. 2013
 Sample Name : ILS-GJ-HC-PH-NO2-RCM
 Acq Operator : VARMA
 Acq Method : D:\Chem32\1\DATA\040213-001 2013-02-04 18-04-15\AMD ->
 Analysis Method : D:\CHEM32\1\METHODS\AMD PRB.M
 Method Info : Column : X-Bridge C-18 150*4.6mm, 5µm
 Mobile phase: A) 0.1% HCOOH in water , B) ACN
 T/B%: 0/20, 3/20, 13/95, 20/95, 22/20, 25/20
 Flow: 1.0 ml/min Diluent: ACN:Water(90:10) CTemp: 25°C



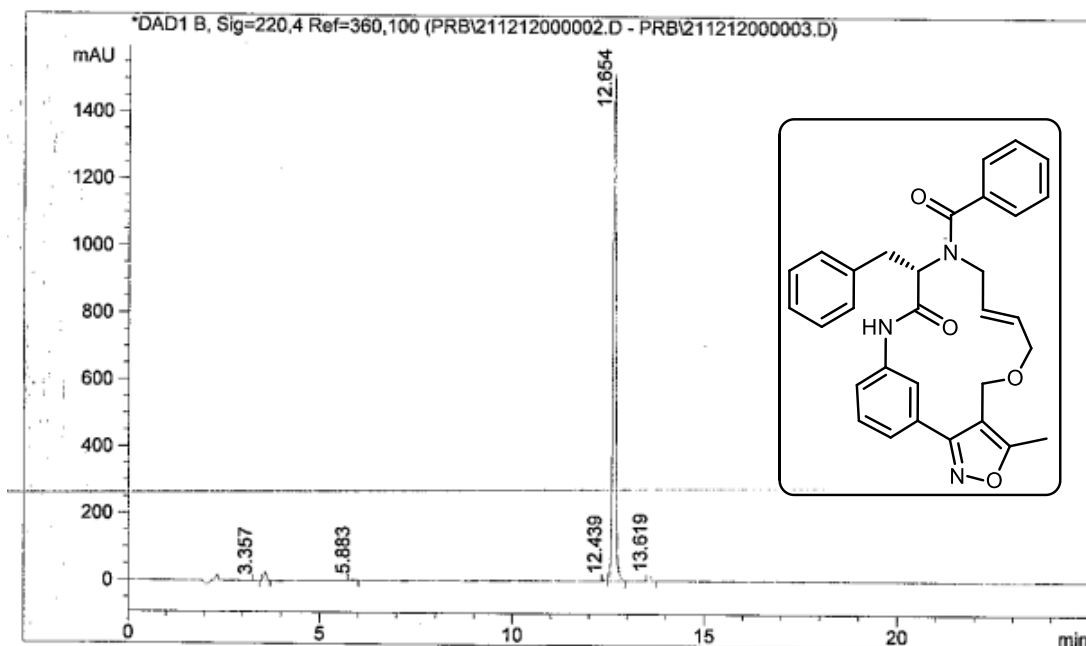
Signal 1: DAD1 A, Sig=200,5 Ref=off

Peak #	RT [min]	Area	Area %
1	11.822	6.291	0.121
2	11.987	10.832	0.208
3	12.264	4717.676	90.393
4	12.406	18.477	0.354
5	12.656	361.805	6.932
6	12.849	54.094	1.036
7	14.291	49.870	0.956

*Peaks
04/02/13*

COSMIC DISCOVERIES @ ILS
HPLC ANALYSIS REPORT

Injection Date	: Fri, 21. Dec. 2012	Seq Line	: 0
Sample Name	: ILS-GJ-HO-Ph-Ph-RCM	Location	: Vial 2
Acq Operator	: VARMA	Inj. No.	: 0
Acq. Method	: D:\CHEM32\1\METHODS\DA.M	Inj. Vol.	: 5 µl
Analysis Method	: D:\CHEM32\1\METHODS\DA.M		
Method Info	: Column: Eclipse Plus C-18 250*4.6mm 5µm		
	Mobile phase: A) 0.1% HCOOH in Water B) ACN		
	T/%B: 0/50, 5/50, 10/75, 15/90, 20/90, 22/50, 25/50		
	Flow: 1.0ml/min, Diluent: ACN		



Signal 1: DAD1 B, Sig=220,4 Ref=360,100

Peak #	RT [min]	Area	Area %
1	3.357	5.360	0.065
2	3.603	108.061	1.318
3	5.883	14.909	0.182
4	12.439	30.634	0.374
5	12.654	7908.211	96.433
6	12.781	74.219	0.905
7	13.619	59.365	0.724



Chapter 4

*Synthesis of Novel Pyrrolidine and Piperidine
Scaffolds by Cycloaddition Reaction of Tert-butyl
sulfonamideImine /sulfinamideimine and
Allenoate*



In this minor chapter, I discuss the synthesis of novel aryl substituted *N*-tertiary-butylsulfonyl pyrrolidine and piperidine derivatives using [3+2] and [4+2]-dipolar cyclo-addition reactions of substituted aryl sulfonamides and allenoate in the presence of phosphine catalyst. At the end, some efforts towards an asymmetric synthesis of pyrrolidine and piperidines using chiral *N*-tertiarybutylsulfinamide imines are also described.

4.1 Introduction

Pyrrolidine and piperidines are the most important five and six member N-heterocyclic rings commonly found in many natural products and biologically active synthetic small molecules. Here, I discuss few examples for each type of heterocyclic compound.

4.2 Biologically Active Molecules Containing the Pyrrolidine Ring

In **Figure 1**, shown are some of the bioactive natural products possessing pyrrolidine ring. Kainic acid (**F1.1**) is a marine natural product isolated in 1953 from some seaweed *alga Digenea simplex* by Takemoto and co-workers.¹ It is a potent neuro-excitatory acid compound that acts by activating the kainate receptors for glutamate, which is the principal excitatory neurotransmitter in the central nervous system.²

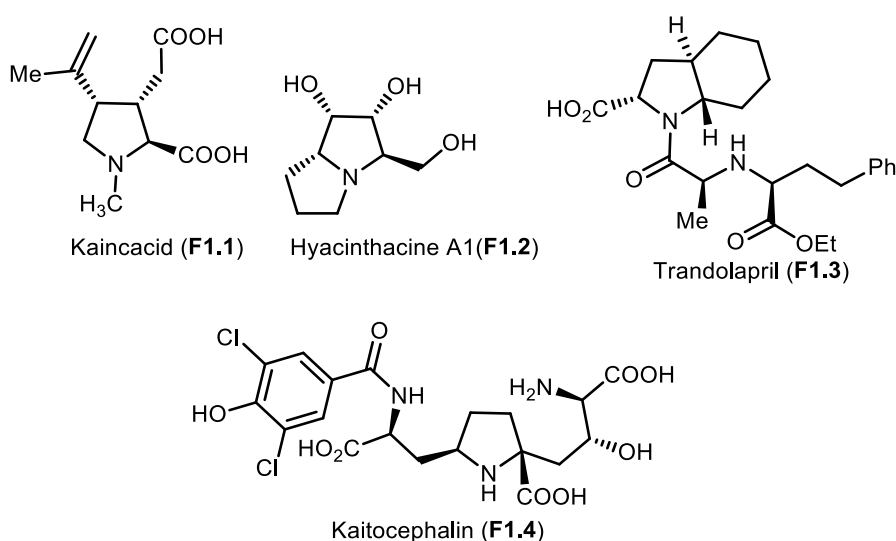


Figure 1: Examples of Natural Products Containing Pyrrolidine Ring

Kainic acid acts as an agonist for kainate receptors, a type of ionotropic glutamate receptor. The natural product hyacinthacine A1 (**F1.2**),³ isolated in less than 0.005% yield from the bulbs of *Muscariarmemiacum*, and it acts as the glycosidase inhibitor. Trandolapril (**F1.3**) is an inhibitor of angiotensin converting enzyme (ACE) used treating the high blood pressure. It is a pro-drug, that gets de-esterified to trandolaprilat. This drug is marketed by Abbott.⁴ Kaitocephalin (**F1.4**) is the first AMPA/KA antagonist, was isolated from *Eupenicillium shearii*. This compound showed the protection from the kainite toxicity at 500 μ M with EC₅₀ values being 0.68 and 2.4 μ M, respectively.⁵

4.3 Biologically Active Molecules Containing Piperidine

Ring

Dienomycin-C (**F2.1**), an alkaloid natural product isolated from the *Streptomyces* strain MC67-C1 by Umezawa and co-workers. It exhibits the antibiotic activity against some strains of *mycobacteria*.⁶ Solenopsin A (**F2.2**)⁷ is an alkaloid natural Product known to inhibit the angiogenesis via the PI3 kinase cell signaling pathway. Solenopsin-A is shown to have the cytotoxic, necrotic, insecticidal, hemolytic, antibacterial, antifungal, and anti-HIV properties.

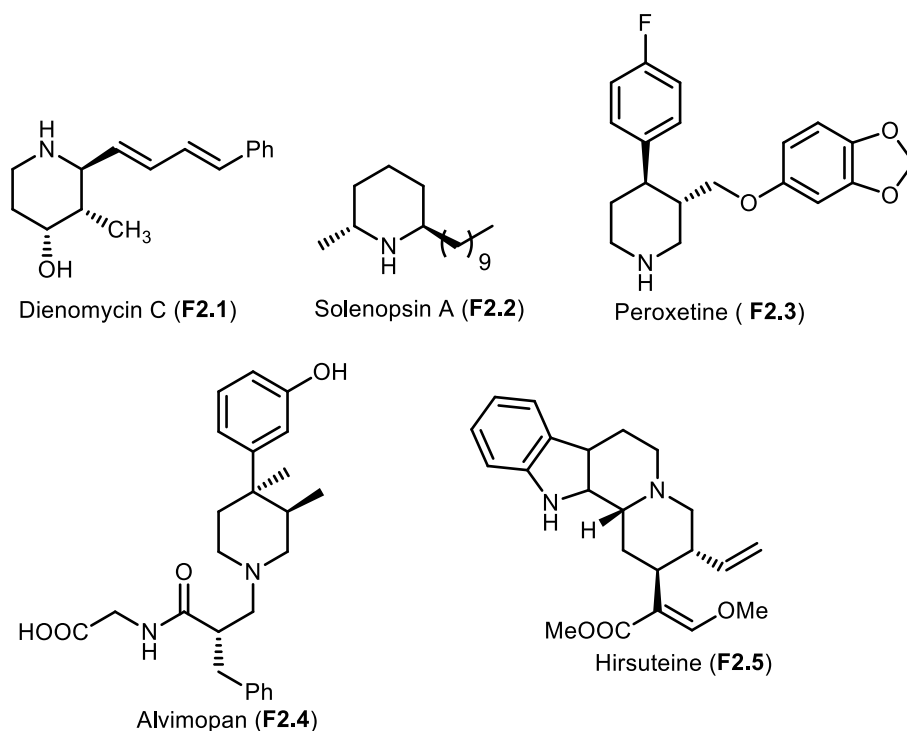
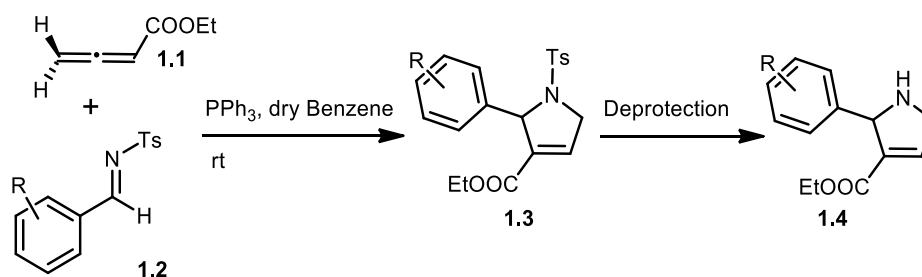


Figure 2: Biologically Active Piperidine Molecules.

Paroxetine (**F2.3**), is an anti-depressant of the selective serotonin re-uptake inhibitor (SSRI) class. It is also known by the trade names, such as Paxil and Seroxat among others, and is used to treat major depressive disorders, social anxiety disorder, obsessive-compulsive disorder, panic disorder, generalized anxiety disorder, premenstrual dysphoric disorder, and posttraumatic stress disorder. It has also been used in the treatment of hot flashes and night sweats associated with menopause.⁸ Alvimopan (**F2.4**) is a drug acting as μ -opioid antagonist and it is marketed by the trade name with Entereg.⁹ Hirsutine (**F2.5**) is an alkaloid known to act as an antagonist of nicotinic receptor.¹⁰

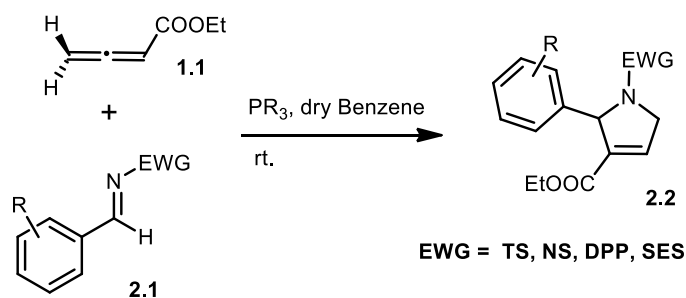
4.4 Precedents on Synthesis of Substituted Pyrrolidine

In 1997, Lu and co-workers demonstrated a novel Approach for preparing *N*-heterocycles via phosphine catalyzed [3+2]-cycloaddition reaction of allenates with *N*-tosylimine (**Scheme 1**).¹¹



Scheme 1: Lu and Co-workers Synthesis of Pyrrolidine in 1997

In 1998, the same group extended their work for optimizing the efficient reaction conditions in performing [3+2]-cycloaddition reaction¹² by modifying the imines and phosphine catalyst (see **Scheme 2**).



Scheme 2: Lu and Co-workers Synthesis of Pyrrolidine in 1998

This study revealed that, the diphenylphosphinyl (DPP) imines gives very low yield due to the low electrophilicity of the carbon atom of the corresponding imines. The

imine with *p*-nitrobenzenesulfonyl (NS) group gives the moderate yields due to the lower stability of the imine. Finally, the tosyl and β -trimethyl silyl ethane sulfonyl (SES) were found to be stable and high yielding imines for the cycloaddition reaction when compared to other imines.¹¹ Also, it was identified that the *tri*-butyl phosphine was the best catalyst and benzene was the best solvent for the cycloaddition reaction. In 2003, Kwon and co-workers described a novel method for the synthesis of functionalized piperidines via the PBU_3 -catalyzed [4+2] annulations of imines with allenates.¹³

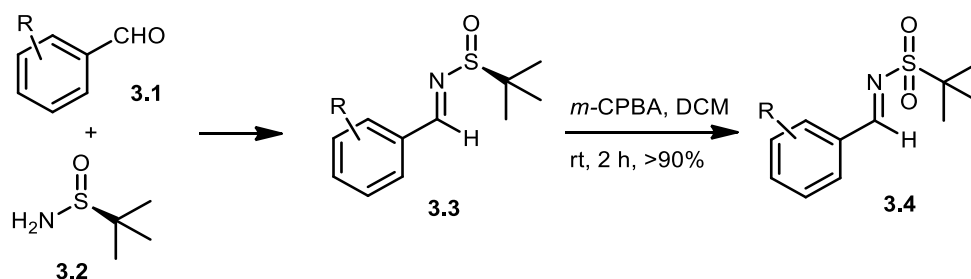
4.5 Present Work

Although several methods are reported in literature for the synthesis of substituted pyrrolidene and piperidines, however, the diversity-oriented synthetic approach for the quickly generation of a library of molecules around this class is always desirable. Here, I describe the [3+2] and [4+2]-cycloaddition reactions with the allenate and *tert*-butylsulfonamide or sulfonamideimines, which are stable in nature. This further allow us performing the asymmetric cycloaddition reaction using the commercially available, chiral *tert*-butylsulfonamides.

4.6 Results & Discussion

4.6.1 Preparation of Starting Material

The *tert*-butylsulfonamide imines are well known in literature and used in the synthesis of chiral amines. The key starting material (S,E)-*N*-benzylidene-2-methylpropane-2-sulfonamides (**3.3**) was obtained following the literature protocol, which upon oxidation with *m*-CPBA offers *tert*-butylsulfonamideimine (**3.4**) (**Scheme 3**).

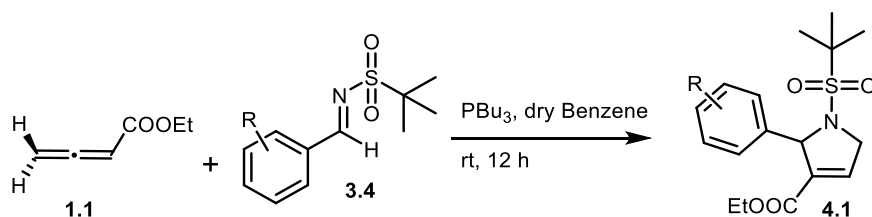


Scheme 3: Synthesis of Tertiary-butyl sulfonamideimine

The other coupling partner ethyl buta-2,3-dienoate (**1.1**) and diethyl 2-vinylidenesuccinate were prepared, according to the literature protocol, reported by Kwon and co-workers.¹⁴

4.6.2 Initial Results and Optimization of Reaction Conditions for the Achiral Synthesis Method

I subjected the ethyl 2,3 butadienoate (**1.1**) and sulfonamideimine (**3.4**) to known [3+2]-cycloaddition conditions using PBu₃ as the catalyst in benzene solvent. I also examined the effects of the solvent and temperature on this reaction, and these results are summarized below (see **Table 4.1**). It was observed that, the original reaction conditions gave much better yield when compared other non-polar solvents, at higher temperatures, the yields are dropped (**Table 4.1**). With this confirmation, I carried-out all our cycloaddition reactions following the original protocol known in the literature. All [3+2] cycloaddition reactions are performed using (50 mol%) PBu₃ in benzene as solvent at 30 °C, and the obtained derivatives using different tert-butyl sulfonamide imines and 2,3-butadiene are listed in **Table 4.2**. The compounds containing electron withdrawing groups (R= NO₂, CF₃, COOEt) gave good yields (compounds **4.1** (a-d), **Table 4.2**). The compounds containing electron donating groups (R = Cl, Br, OMe) gave moderate yields (compounds **4.1**(e-i), **Table 4.2**). These results also conclude that electron withdrawing groups favors the reaction by increasing the electrophilic character at carbon of the corresponding imine. The bulkier ter-butyl substitution also has no impact on the yield of the reaction and well tolerated with the reaction conditions.



Scheme 4: Synthesis of Substituted pyrrolidine

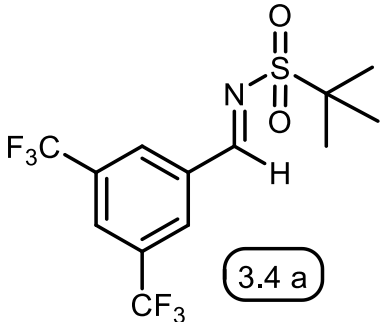
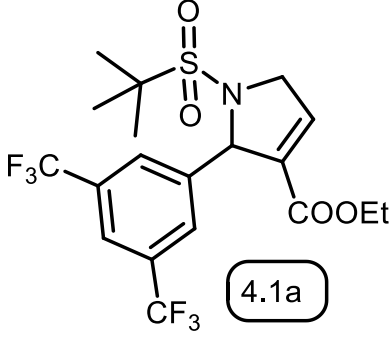
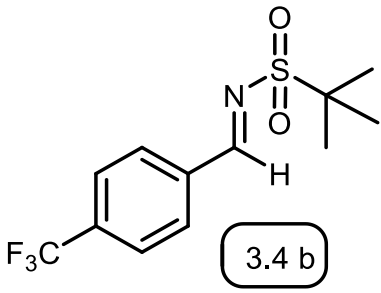
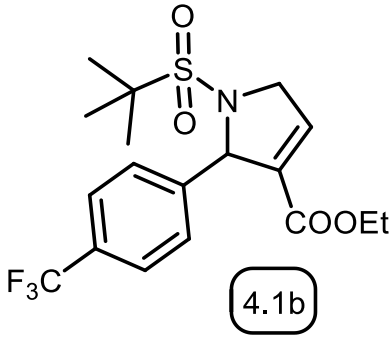
Table 4.1. Optimization of Reaction Conditions

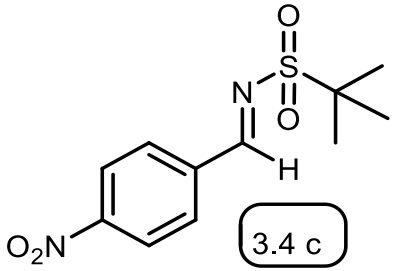
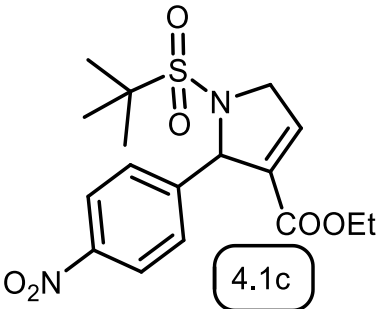
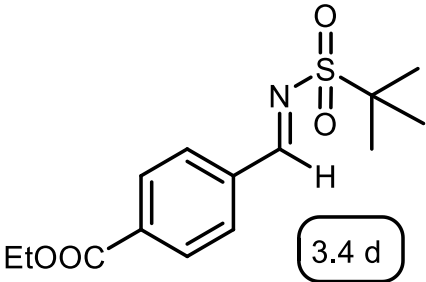
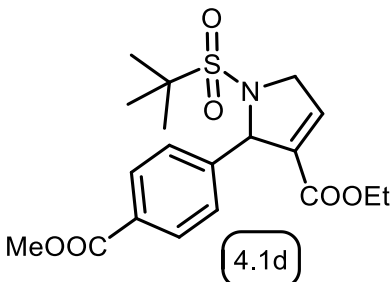
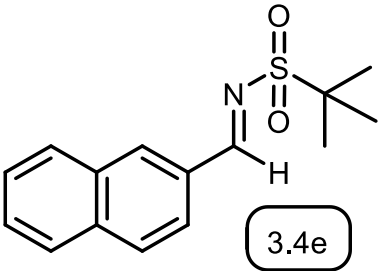
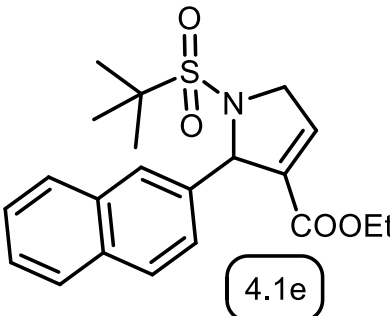
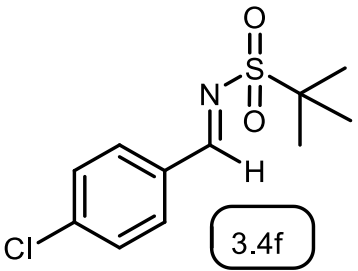
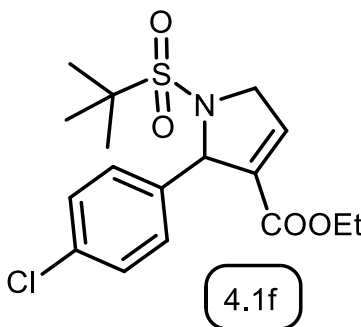
Entry	Solvent	Temp (°C)	Time (h)	Yield ^b (%)
1	Benzene	30	12h	87

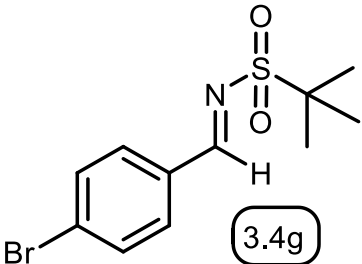
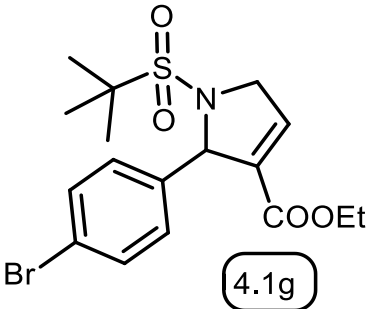
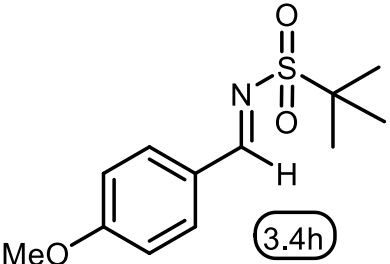
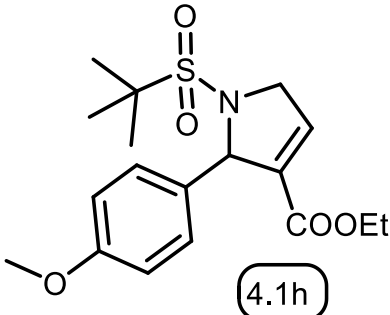
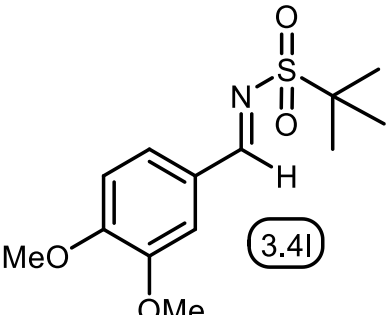
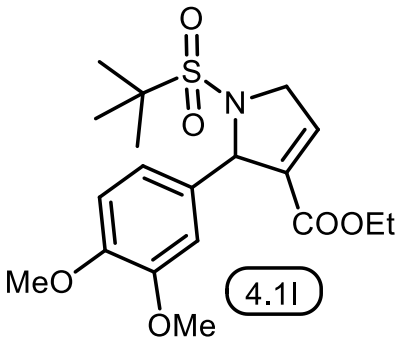
2	Toluene	30	12h	62
3	Xylene	30	12h	50
4	Mesitylene	30	12h	55
5	Heptane	30	12h	47
6	n-Petane	30	12h	55
7	Benzene	80	12h	32
8	Toluene	100	12h	10

^aAll reactions were performed using the 2,3 Butadienoate **1.1** (1 mmol), Aryl sulfonamide (1.3 mmol), Benzene (3.0 mL) under inert condition. ^bIsolated yield

Table 4.2. List of Pyrrolidine Scaffolds

Entry	substituted Aryl sulfonamide	Allenoate	Products
1	 <chem>CC(C)(C)S(=O)(=O)N=Cc1cc(C(F)(F)F)cc(C(F)(F)F)c1</chem> 3.4 a	 1.1	 <chem>CCOC(=O)C1=CC=C(C1N(S(=O)(=O)C(C)(C)C)Cc2cc(C(F)(F)F)cc(C(F)(F)F)c2)C2=CC=CC=C2C(F)(F)F</chem> 4.1a
2	 <chem>CC(C)(C)S(=O)(=O)N=Cc1ccc(C(F)(F)F)cc1</chem> 3.4 b	 1.1	 <chem>CCOC(=O)C1=CC=C(C1N(S(=O)(=O)C(C)(C)C)Cc2ccc(C(F)(F)F)cc2)C2=CC=CC=C2</chem> 4.1b

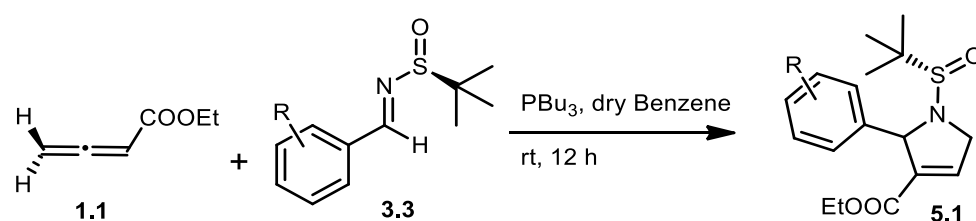
3		1.1	
4		1.1	
5		1.1	
6		1.1	

7	 3.4g	1.1	 4.1g
8	 3.4h	1.1	 4.1h
9	 3.4l	1.1	 4.1l

4.7 Efforts Toward the Asymmetric Synthesis of Pyrrolidine using [3+2] Cycloaddition

Having well-optimized reaction conditions for the synthesis of the racemic version of substituted pyrrolidines, I started the optimization of an asymmetric version. Our initial study was started with 2,3 butadienoate (**1.1**) and chiral sulfonamide imine (**3.3**) and PBU_3 as the catalyst in benzene solvent at room temperature, the results are listed below (see **Table 4.4**). I observed that, when R is a strong electron withdrawing group like nitro, or bis-trifluoromethane, the reaction proceeded and gave the moderate yields. In case, where R was poor electron withdrawing group like $-\text{COOEt}$

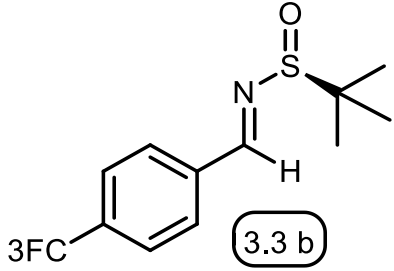
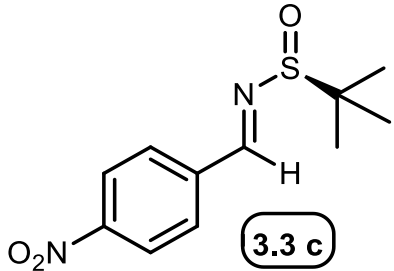
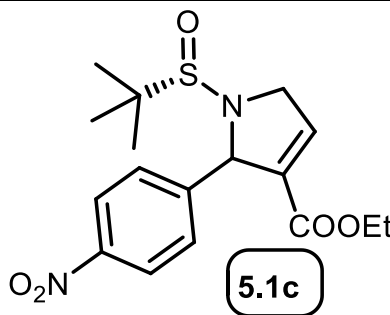
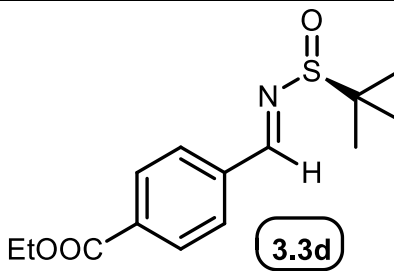
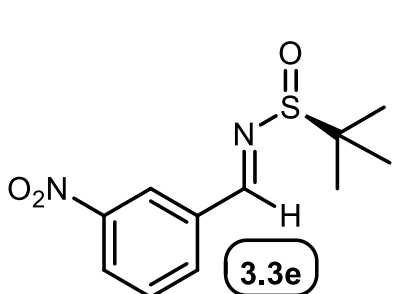
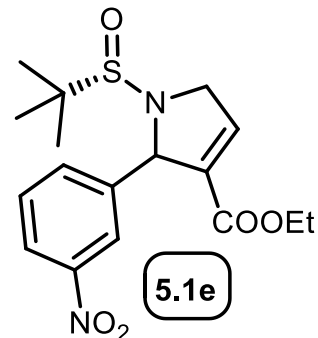
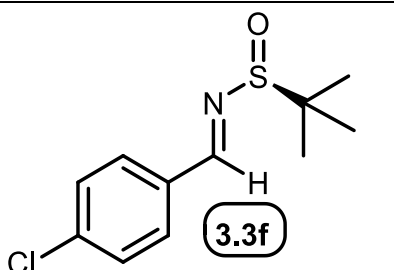
and mono-trifluoro methane or electron releasing groups, no formation of the product was observed. In these cases, the increase in the temperature as well as catalyst loading did not show any effect, however, the increase in temperature led the formation of multiple side products (not isolated). From these results, it was concluded that the electrophilicity of the imine carbon of the corresponding sulfonamide imine was not sufficient for attack by the nucleophile generated in the reaction mixture with PBU_3 and allenolate. Next, I started looking at the ways to increasing the electrophilic character of imine carbon. In literature, it was known that, it could be done using Lewis acid catalyst.¹⁵ Following this, I used different Lewis acid catalysts and this information is listed in **Table 4.5**. However, no product was observed. This may be due to neutralization of phosphine catalyst with the Lewis acid. I have not taken any chiral HPLC for the cyclic compounds **5.1**.



Scheme 5: Synthesis of Substituted pyrrolidine

Table 4.4: Reaction with Different Sulfinamideimines

Entry	Substituted Aryl sulfinamide	Allenolate	Products
1	 3.3 a	 1.1	 5.1a

2	 3.3 b	1.1	No Product
3	 3.3 c	1.1	 5.1c
4	 3.3d	1.1	No Product
5	 3.3e	1.1	 5.1e
6	 3.3f	1.1	No Product

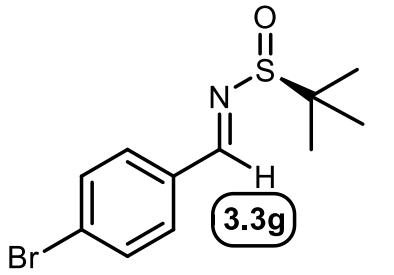
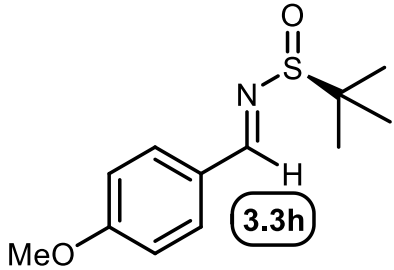
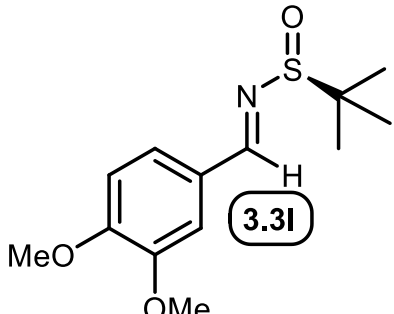
7		1.1	No Product
8		1.1	No Product
9		1.1	No Product

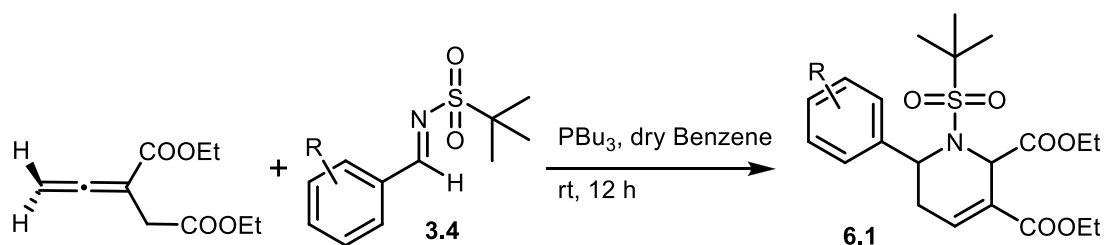
Table 4.5. Optimization of Reaction Conditions with Lewis Acids

Entry	Lewis Acid	Temp (°C)	Time (h)	Yield ^b (%)
1	BF ₃ .OEt ₂	30	12	0
2	AlCl ₃	30	12	0
3	FeCl ₃	30	12	0
4	ZnCl ₂	30	12	0
5	Ti(OiPr)	30	12	0
6	InBr ₂	30	12	0
7	ZnBr ₂	30	12	0

^aAll reactions were performed using the 2,3 butadienoate **1.1** (1eq), Aryl sulfinamide (1.3 mmol), in Benzene (3.0 mL) under inert condition. ^bIsolated yield

4.8 Synthesis of Substituted Piperidene by [4+2] Cyclo addition Reaction

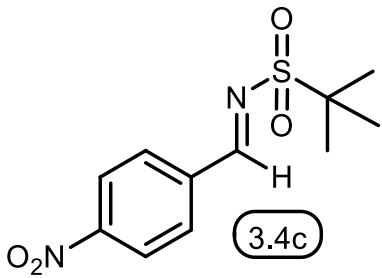
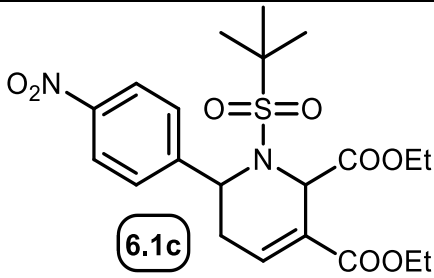
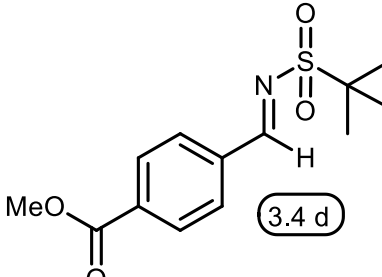
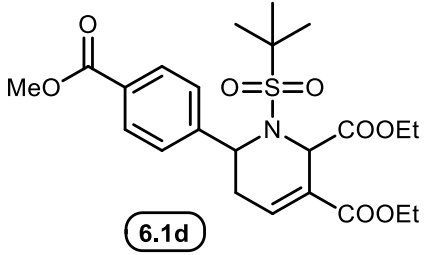
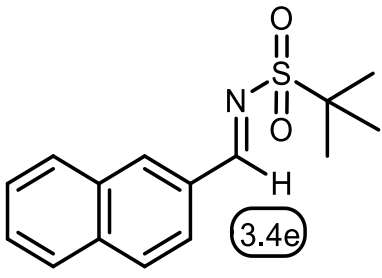
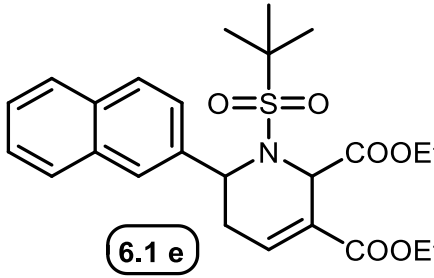
I discussed in section 4.3, substituted piperidines are commonly found in many bioactive natural products and synthetic small molecules drugs. With this information, keeping in mind is obtaining good results in pyrrolidines synthesis, I planned the synthesis of the piperidene derivatives. For this study, I utilized the same reaction conditions but the allenote (1.1) was changed with diethyl 2-vinylidenesuccinate. Here, I used different ter-butyl sulfonamide imines as the electron withdrawing group, moderate electron withdrawing group and electron donating group substituted imines. As a result I got moderate yields with a little variation in the percentages of yields.



Scheme 6: Synthesis of Piperidine derivatives

Table 4.3: Piperidine Derivatives

Entry	Substituted Aryl sulfonamide	Allenoate	Products
1	(3.4 a)	4.2	(6.1a)

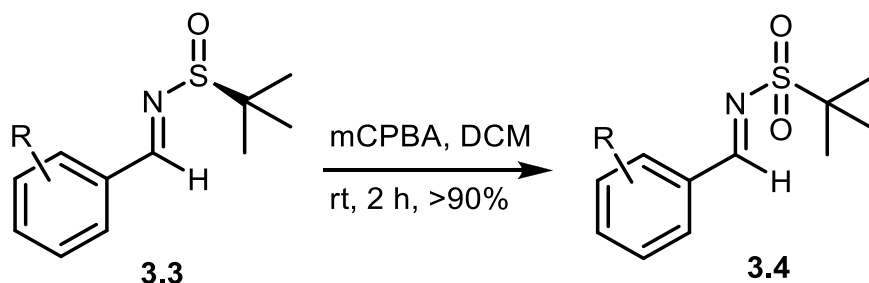
2	 <chem>CC(C)(C)S(=O)(=O)N=Cc1ccc([N+](=O)[O-])cc1</chem> 3.4c	4.2	 <chem>CCOC(=O)C1=CC(=CC=C1N(S(=O)(=O)C(C)(C)C)C2=CC=C([N+](=O)[O-])C=C2)C(=O)OCC</chem> 6.1c
3	 <chem>CC(C)(C)S(=O)(=O)N=Cc1ccc(C(=O)OC)cc1</chem> 3.4 d	4.2	 <chem>CCOC(=O)C1=CC(=CC=C1N(S(=O)(=O)C(C)(C)C)C2=CC=C(C(=O)OC)C=C2)C(=O)OCC</chem> 6.1d
4	 <chem>CC(C)(C)S(=O)(=O)N=Cc1ccc2ccccc2c1</chem> 3.4e	4.2	 <chem>CCOC(=O)C1=CC(=CC=C1N(S(=O)(=O)C(C)(C)C)C2=CC3=CC=CC=C3C=C2)C(=O)OCC</chem> 6.1 e

4.9 Conclusion

I have successfully used the tert-butylsulfonamide imine as a good dienophile for [3+2] and [4+2] cycloaddition reactions to prepare *N*-tertiarybutylsulfonamide pyrrolidines and piperidines. In addition to this, I also tried developing asymmetric version of this method using chiral *N*-tert-butylsufinamide imines. However, it appears this reaction is limited to strong electron withdrawing substituted sulfonamide imines.

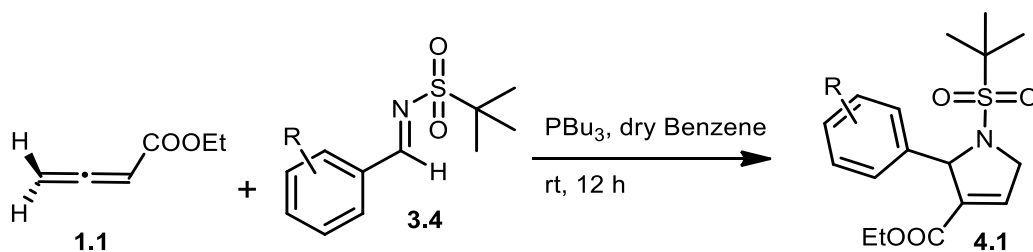
4.10 Experimental Section

(E)-N-benzylidene-2-methylpropane-2-sulfonamide



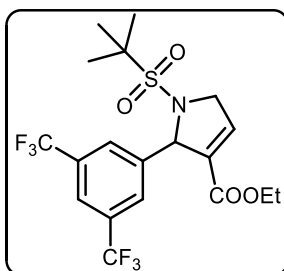
To a solution of the corresponding sulfenylidene **3.3** (0.4 mmol) in CH_2Cl_2 (2 mL) dry m-CPBA (0.6 mmol) was added at room temperature in one portion. When the reaction was completed, the reaction was diluted with 4 mL of CH_2Cl_2 , washed with 3x5 mL of saturated solution of NaHCO_3 . Finally, the organic layer was dried over anhydrous Na_2SO_4 and the solvent evaporated, obtaining the corresponding pure sulfonylimines **3.4** in a crude form, which was purified by column chromatography (30% ethyl acetate and hexane) to get pure product.

Ethyl 1-(tert-butylsulfonyl)-2- substituted phenyl-2,5-dihydro-1H-pyrrole-3 carboxylate



To a mixture of tributyl phosphine (30 mg, 0.1 mmol) and N-tertiarybutylimine **3.4** (1.0 mmol) in benzene (3 mL) was added a solution of methyl 2,3-butadienoate (**1.1**) (110 mg, 1.1 mmol) in benzene (1 mL). The mixture was then stirred at room temperature under Nitrogen and monitored by TLC. After the reaction was complete, the reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silicagel to give **4.1**.

Ethyl 2-(3,5-bis(trifluoromethyl)phenyl)-1-(tert-butylsulfonyl)-2,5-dihydro-1H-pyrrole-3-carboxylat (4.1a)



Molecular Formula: C₁₉H₂₁F₆NO₄S

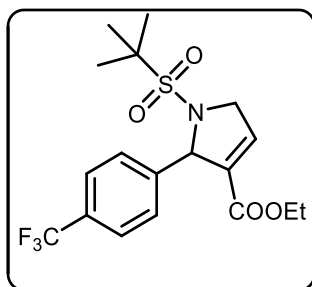
LRMS: (ES+) $m/z = 474.1[M+1]$

$R_f = 0.2$ (30% ethyl acetate/hexanes)

Yield: 87%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.81 (s, 1H), 7.76 (s, 2H), 7.00 (d, $J = 1.80$ Hz, 1H), 6.20 (d, $J = 5.71$ Hz, 1H), 4.89 (td, $J = 17.31, 2.32$ Hz, 1H), 4.35 (ddd, $J = 17.28, 5.72, 1.63$ Hz, 1H), 4.14-4.02 (m, 2H), 1.13 (t, $J = 7.14$ Hz, 3H), 1.08 (s, 9H);
¹³C NMR (CDCl₃, 100MHz) δ ppm: 161.3, 144.0, 137.6, 134.3, 132.0, 131.6, 128.2, 128.2, 128.2, 68.4, 61.2, 61.0, 57.3, 24.1, 13.8.

Ethyl-1-(tert-butylsulfonyl)-2-(4-(trifluoromethyl)phenyl)-2,5-dihydro-1H-pyrrole -3-carboxylate (4.1b)



Molecular Formula: C₁₈H₂₂F₃NO₄S

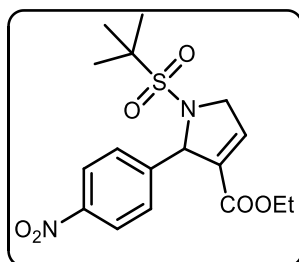
LRMS: (ES+) $m/z = 406.1[M+1]$

$R_f = 0.2$ (30% ethyl acetate/hexanes)

Yield: 82%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.59 (d, *J* = 8.11 Hz, 2H), 7.44 (d, *J* = 8.10 Hz, 2H), 6.95 (d, *J* = 1.69 Hz, 1H), 6.12 (d, *J* = 5.56 Hz, 1H), 4.88 (td, *J* = 17.17, 2.05 Hz, 1H), 4.32 (ddd, *J* = 17.29, 5.65, 1.50 Hz, 1H), 4.12-4.01 (m, 2H), 1.13 (t, *J* = 7.13 Hz, 3H), 1.07 (s, 9H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 172.2, 161.6, 145.0, 140.6, 136.7, 135.1, 128.3, 125.5, 125.4, 68.7, 60.9, 60.9, 57.2, 24.2, 24.0, 13.9

Ethyl 1-(tert-butylsulfonyl)-2-(4-nitrophenyl)-2,5-dihydro-1H-pyrrole-3-carboxylate (4.1c)



Molecular Formula: C₁₇H₂₂N₂O₆S

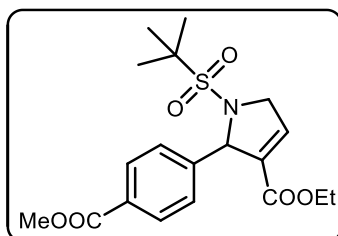
LRMS: (ES⁺) *m/z* = 383.1[M+1]

R_f = 0.2 (30% ethyl acetate/hexanes)

Yield: 75%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 8.20 (d, *J* = 8.69 Hz, 2H), 7.50 (d, *J* = 8.68 Hz, 2H), 6.97 (d, *J* = 1.71 Hz, 1H), 6.17 (d, *J* = 5.58 Hz, 1H), 4.89 (td, *J* = 17.24, 2.10 Hz, 1H), 4.34 (ddd, *J* = 17.29, 5.62, 1.50 Hz, 1H), 4.13-3.99 (m, 2H), 1.14 (t, *J* = 7.13 Hz, 3H), 1.09 (s, 9H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 161.4, 148.2, 147.6, 137.1, 134.7, 128.9, 123.8, 68.4, 61.1, 60.9, 57.4, 29.7, 24.1, 13.9.

Ethyl 1-(tert-butylsulfonyl)-2-(4-(methoxycarbonyl)phenyl)-2,5-dihydro-1H-pyrrole-3-carboxylate (4.1d)



Molecular Formula: C₁₉H₂₅NO₆S

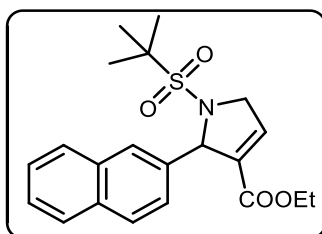
LRMS: (ES⁺) *m/z* = 396.1[M+1]

R_f = 0.2 (30% ethyl acetate/hexanes)

Yield: 70%

$^1\text{H NMR}$ (CDCl_3 , 400 MHz) δ ppm: 8.00 (d, J = 8.15 Hz, 2H), 7.38 (d, J = 8.20 Hz, 2H), 6.93 (s, 1H), 6.11 (d, J = 5.52 Hz, 1H), 4.88 (d, J = 17.21 Hz, 1H), 4.31 (ddd, J = 16.87, 5.58, 1.46 Hz, 1H), 4.09-3.99 (m, 2H), 3.90 (s, 3H), 1.10 (t, J = 7.13 Hz, 3H), 1.05 (s, 9H); $^{13}\text{C NMR}$ (CDCl_3 , 100MHz) δ ppm: 166.8, 161.7, 146.0, 136.5, 135.2, 129.9, 129.8, 128.0, 128.0, 68.9, , 60.9, 60.8, 57.3, 52.2, 24.0, 13.9.

Ethyl 1-(tert-butylsulfonyl)-2-(naphthalen-2-yl)-2,5-dihydro-1H-pyrrole-3-carboxylate (4.1e)



Molecular Formula: $\text{C}_{21}\text{H}_{25}\text{NO}_4\text{S}$

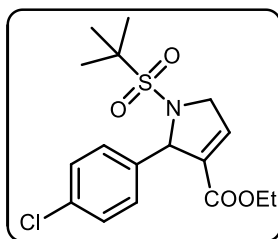
LRMS: (ES^+) m/z = 388.2[M+1]

R_f = 0.2 (30% ethyl acetate/hexanes)

Yield: 75%

$^1\text{H NMR}$ (CDCl_3 , 400 MHz) δ ppm : 7.85-7.79 (m, 4H), 7.51-7.44 (m, 2H), 7.36 (dd, J = 8.39, 1.55 Hz, 1H), 6.96 (d, J = 1.54 Hz, 1H), 6.25 (d, J = 5.52 Hz, 1H), 4.96-4.89 (m, 1H), 4.37 (ddd, J = 17.16, 5.57, 1.59 Hz, 1H), 4.05-3.97 (m, 2H), 1.08 (t, J = 7.12 Hz, 3H), 1.02 (s, 9H); $^{13}\text{C NMR}$ (CDCl_3 , 100MHz) δ ppm: 162.0, 138.1, 136.1, 135.7, 133.1, 133.1, 128.4, 128.2, 127.6, 126.2, 126.1, 125.0, 69.5, 60.8, 60.8, 57.2, 24.1, 13.9.

Ethyl 1-(tert-butylsulfonyl)-2-(3-chlorophenyl)-2,5-dihydro-1H-pyrrole-3-carboxylate (4.1f)



Molecular Formula: C₁₇H₂₂ClNO₄S

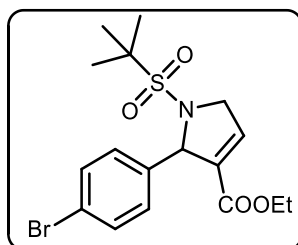
LRMS: (ES+) m/z = 373.09[M+1]

R_f = 0.2 (30% ethyl acetate/hexanes)

Yield: 70%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.32-7.23 (m, 5H), 6.95 (d, J = 1.91 Hz, 1H), 6.07 (d, J = 5.58 Hz, 1H), 4.89 (td, J = 17.12, 2.13 Hz, 1H), 4.32 (ddd, J = 16.98, 5.58, 1.56 Hz, 1H), 4.16-4.05 (m, 2H), 1.17 (t, J = 7.14 Hz, 3H), 1.12 (s, 9H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 161.7, 143.1, 136.5, 135.2, 134.3, 129.7, 128.3, 127.9, 126.4, 68.8, 60.9, 60.9, 57.1, 24.1, 13.9.

Ethyl 2-(4-bromophenyl)-1-(tert-butylsulfonyl)-2,5-dihydro-1H-pyrrole-3-carboxylate (4.1g)



Molecular Formula: C₁₇H₂₂ BrNO₄S

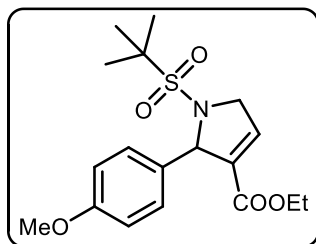
LRMS: (ES+) m/z = 417.01[M+1]

R_f = 0.2(30% ethyl acetate/hexanes)

Yield: 70%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.45 (d, J = 8.40 Hz, 2H), 7.19 (d, J = 8.41 Hz, 2H), 6.91 (d, J = 1.98 Hz, 1H), 6.04 (d, J = 5.60 Hz, 1H), 4.86 (td, J = 17.12, 2.18 Hz, 1H), 4.28 (ddd, J = 17.17, 5.62, 1.72 Hz, 1H), 4.11-4.02 (m, 2H), 1.14 (t, J = 5.69 Hz, 3H), 1.08 (s, 9H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 161.7, 140.1, 136.3, 135.3, 131.6, 129.7, 122.1, 68.7, 60.9, 60.8, 57.1, 24.1, 13.9

ethyl 1-(tert-butylsulfonyl)-2-(4-methoxyphenyl)-2,5-dihydro-1H-pyrrole-3-carboxylate (4.1h)



Molecular Formula: C₁₈H₂₅NO₅S

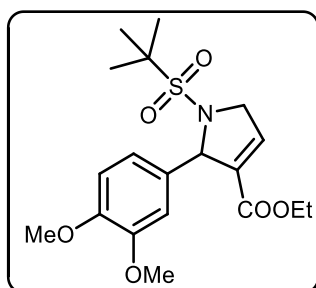
LRMS: (ES⁺) m/z = 368.1[M+1]

R_f = 0.2 (30% ethyl acetate/hexanes)

Yield: 68%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.23 (d, J = 8.64 Hz, 2H), 6.88 (d, J = 1.35 Hz, 1H), 6.85 (d, J = 8.65 Hz, 2H), 6.04 (d, J = 5.52 Hz, 1H), 4.89-4.82 (m, 1H), 4.28 (ddd, J = 17.09, 5.59, 1.59 Hz, 1H), 4.13-4.02 (m, 2H), 3.81 (s, 3H), 1.15 (t, J = 7.13 Hz, 3H), 1.08 (s, 9H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 157.3, 154.5, 131.0, 130.8, 128.4, 124.4, 109.0, 64.1, 56.0, 56.0, 52.0, 50.5, 19.3, 19.3, 19.3, 19.3, 9.2

Ethyl 1-(tert-butylsulfonyl)-2-(3,4-dimethoxyphenyl)-2,5-dihydro-1H-pyrrole-3-carboxylate (4.1i)



Molecular Formula: C₁₉H₂₇NO₆S

LRMS: (ES⁺) m/z = 398.1[M+1]

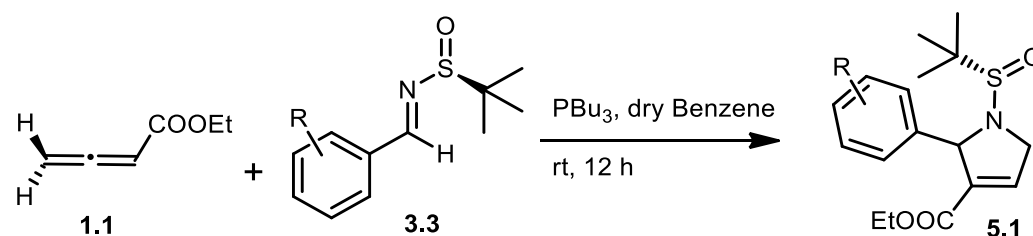
R_f = 0.2 (30% ethyl acetate/hexanes)

Yield: 65%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 6.88 (d, J = 1.56 Hz, 1H), 6.87-6.78 (m, 3H), 6.02 (d, J = 5.55 Hz, 1H), 4.85 (td, J = 17.03, 2.06 Hz, 1H), 4.25 (ddd, J = 17.17, 5.54, 1.70 Hz, 1H), 4.12-4.02 (m, 2H), 3.86 (d, J = 2.45 Hz, 6H), 1.15 (t, J = 7.12 Hz, 4H), 1.07 (s, 9H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 162.0, 148.8, 135.7, 135.6, 133.6, 120.2, 111.2, 110.9, 69.1, 60.8, 60.8, 56.8, 56.0, 55.8, 24.1, 14.0.

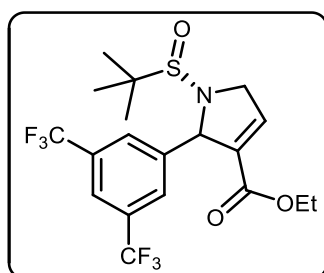
Experimental Section for Asymmetric Method

Ethyl 1-((S)-tert-butylsulfinyl)-2-phenyl-2,5-dihydro-1H-pyrrole-3-carboxylate



To a mixture of tributyl phosphine (30 mg, 0.1 mmol) and N-tertarybutylimine (**3.3**) (1.0 mmol) in benzene (5 mL) was added a solution of methyl 2,3-butadienoate (**1.1**) (110 mg, 1.1 mmol) in benzene (5 mL). The mixture was then stirred at room temperature under Nitrogen and monitored by TLC. After the reaction was complete, the reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silicagel to give **5.1**

ethyl 2-(3,5-bis(trifluoromethyl)phenyl)-1-((S)-tert-butylsulfinyl)-2,5-dihydro-1H-pyrrole-3-carboxylate (**5.1a**)



Molecular Formula: $\text{C}_{19}\text{H}_{21}\text{F}_6\text{NO}_3\text{S}$

LRMS: (ES⁺) $m/z = 458.1[\text{M}+1]$

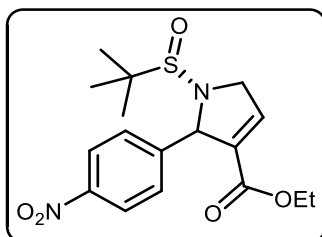
$R_f = 0.25$ (30% ethyl acetate/hexanes)

Yield: 75%

^1H NMR (CDCl_3 , 400 MHz) δ ppm: 7.80 (s, 1H), 7.76 (s, 2H), 6.99 (s, 1H), 5.83 (dd, $J = 2.78, 2.25$ Hz, 1H), 4.85 (d, $J = 17.32$ Hz, 1H), 4.09 (dd, $J = 12.68, 7.08$ Hz, 2H), 3.95-3.88 (m, 1H), 1.17 (t, $J = 7.15$ Hz, 3H), 1.06 (s, 9H); **^{13}C NMR** (CDCl_3 ,

100MHz) δ ppm: 161.7, 144.5, 139.9, 135.0, 131.8, 131.4, 128.0, 128.0, 121.8, 74.2, 61.1, 57.7, 48.2, 23.2, 13.9

ethyl 1-((S)-tert-butylsulfinyl)-2-(4-nitrophenyl)-2,5-dihydro-1H-pyrrole-3-carboxylate (5.1c)



Molecular Formula: C₁₇H₁₉N₂O₅S

LRMS: (ES⁺) m/z = 367.1[M+1]

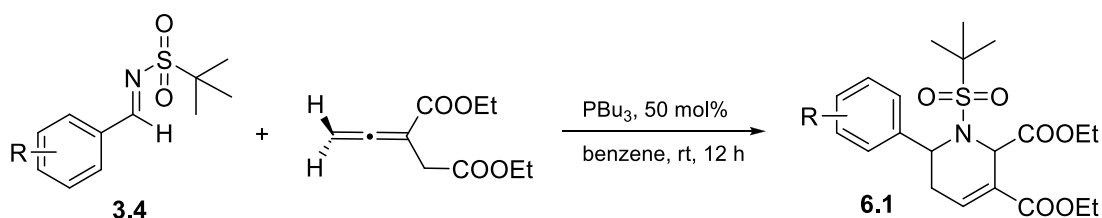
R_f = 0.25 (30% ethyl acetate/hexanes)

Yield: 70%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 8.21 (s, 1H), 8.19 (s, 1H), 7.48 (s, 1H), 7.45 (s, 1H), 6.97 (d, J = 2.14 Hz, 1H), 5.79 (d, J = 5.56 Hz, 1H), 4.85 (d, J = 17.38 Hz, 1H), 4.07 (dd, J = 9.77, 7.14 Hz, 2H), 3.92-3.84 (m, 1H), 1.16 (t, J = 7.13 Hz, 4H), 1.03 (s, 9H)

Experimental Section for Substituted Piperidine Preparation

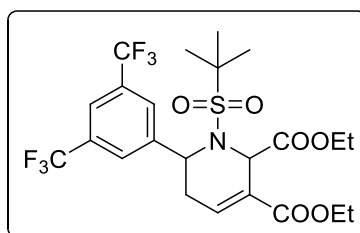
diethyl1-(tert-butylsulfonyl)-6-substituted phenyl-1,2,5,6-tetrahydropyridine-2,3 dicarboxylate



To a mixture of tributyl phosphine (30 mg, 0.1 mmol) and N-tertarybutylimine **3.4** (1.0mmol) in benzene (5mL) was added a solution of methyl diethyl 2-vinylidenesuccinate (110 mg, 1.1 mmol) in benzene (5 mL). The mixture was then

stirred at room temperature under Nitrogen and monitored by TLC. After the reaction was complete, the reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silicagel to give Piperidene (**6.1**).

(2R,6R)-diethyl 6-(3,5-bis(trifluoromethyl)phenyl)-1-(tert-butylsulfonyl)-1,2,5,6-tetrahydropyridine-2,3-dicarboxylate (6.1a**)**



Molecular Formula: C₂₃H₂₇F₆NO₆S

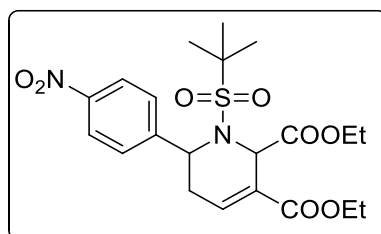
LRMS: (ES+) m/z = 560.1[M+1]

R_f = 0.2(30% ethyl acetate/hexanes)

Yield: 74%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.88 (s, 2H), 7.79 (s, 1H), 7.38-7.32 (m, 1H), 5.39 (d, J = 6.94 Hz, 1H), 4.25 (q, J = 7.01 Hz, 2H), 3.51 (d, J = 7.11 Hz, 1H), 3.44-3.34 (m, 1H), 3.14-3.03 (m, 1H), 2.87-2.76 (m, 1H), 1.52 (s, 9H), 1.31 (d, J = 7.12 Hz, 3H), 0.97 (t, J = 7.14 Hz, 3H), 5.35 (s, 1H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 167.9, 164.2, 140.7, 140.0, 138.8, 136.9, 135.2, 135.2, 131.7, 131.3, 129.6, 129.2, 63.1, 61.7, 61.2, 54.1, 53.2, 27.8, 24.6, 14.1, 13.3.

diethyl 1-(tert-butylsulfonyl)-6-(4-chlorophenyl)-1,2,5,6-tetrahydropyridine-2,3-dicarboxylate (6.1c**)**



Molecular Formula: C₂₁H₂₈N₂O₈S

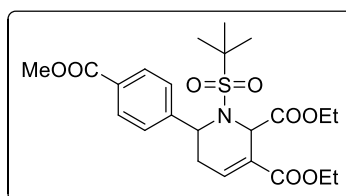
LRMS: (ES+) m/z = 469.1 [M+1]

R_f = 0.2(30% ethyl acetate/hexanes)

Yield: 57%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 8.14 (d, *J* = 8.81 Hz, 2H), 7.62 (d, *J* = 8.72 Hz, 2H), 7.36-7.32 (m, 1H), 5.36 (d, *J* = 8.64 Hz, 2H), 4.30-4.19 (m, 2H), 3.58-3.48 (m, 1H), 3.38 (dd, *J* = 10.77, 7.19 Hz, 1H), 3.12-3.02 (m, 1H), 2.82 (dd, *J* = 19.86, 5.00 Hz, 1H), 1.52 (s, 9H), 1.29 (t, *J* = 7.2 Hz, 3H), 0.99 (t, *J* = 7.16 Hz, 3H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 168.1, 164.4, 147.5, 145.0, 135.5, 130.1, 129.5, 123.2, 63.0, 61.6, 61.2, 54.1, 53.2, 28.0, 24.7, 14.1, 13.5.

diethyl 1-(tert-butylsulfonyl)-6-(4-(methoxycarbonyl)phenyl)-1,2,5,6-tetrahydro pyridine-2,3-dicarboxylate (6.1d)



Molecular Formula: C₂₃H₃₁NO₈S

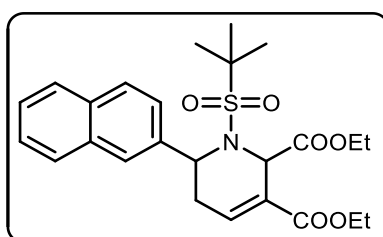
LRMS: (ES+) *m/z* = 482.18[M+1]

R_f = 0.2(30% ethyl acetate/hexanes)

Yield: 70%

¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.94 (d, *J* = 8.31 Hz, 2H), 7.48 (d, *J* = 8.28 Hz, 2H), 7.36-7.34 (m, 1H) 5.33 (d, *J* = 7.36 Hz, 2H), 4.30-4.16 (m, 2H), 3.89 (s, 3H), 3.51-3.41 (m, 1H), 3.32-3.22 (m, 1H), 3.10-2.99 (m, 1H), 2.81 (dd, *J* = 19.99, 5.19 Hz, 1H), 1.53 (s, 9H), 1.28 (t, *J* = 7.15 Hz, 4H), 0.95 (t, *J* = 7.15 Hz, 3H); **¹³C NMR** (CDCl₃, 100MHz) δ ppm: 166.8, 161.7, 146.0, 136.5, 135.2, 129.9, 129.8, 128.0, 128.0, 68.9, 68.9, 60.9, 60.8, 57.3, 52.2, 52.2, 24.0, 13.9.

(2R,6R)-diethyl 1-(tert-butylsulfonyl)-6-(naphthalen-2-yl)-1,2,5,6-tetrahydro pyridine-2,3-dicarboxylate (6.1e)



Molecular Formula: C₂₅H₃₁NO₆S

LRMS: (ES+) *m/z* = 474.1

R_f = 0.2 (30% ethyl acetate/hexanes)

Yield: 72%

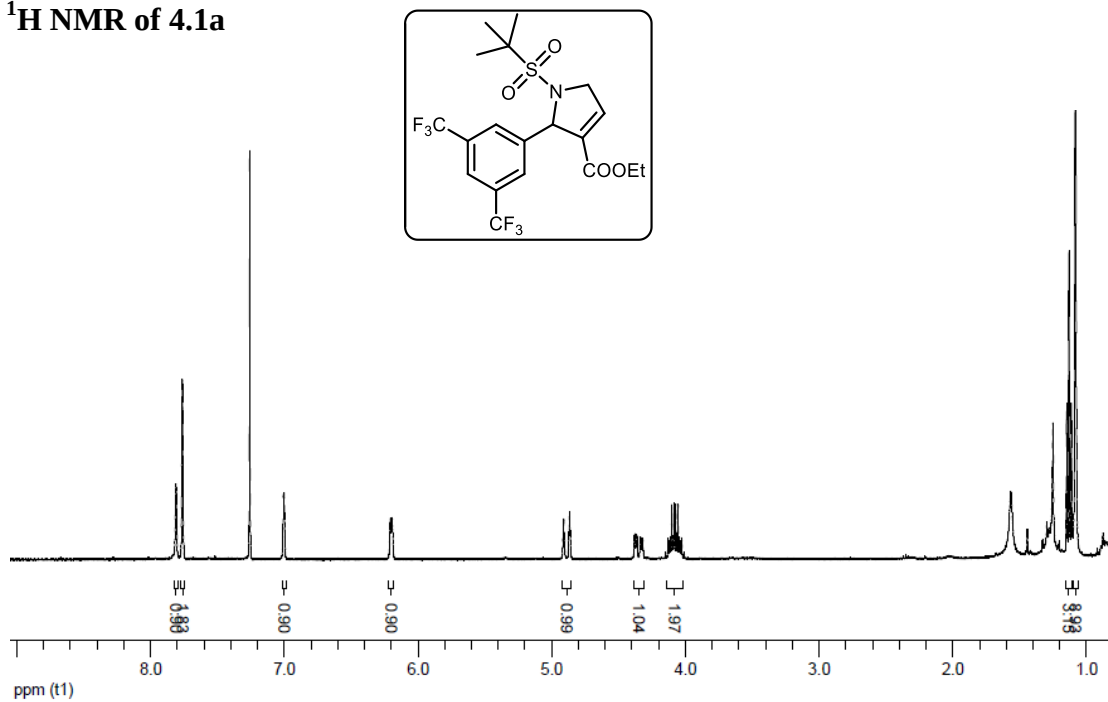
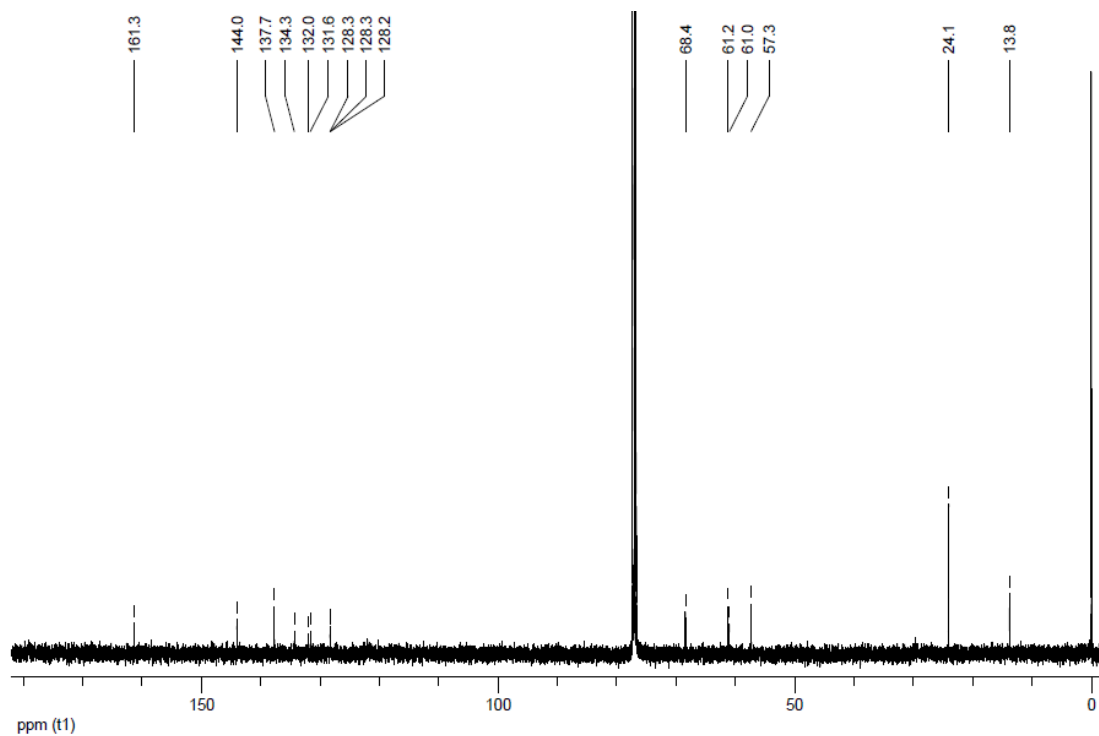
¹H NMR (CDCl₃, 400 MHz) δ ppm: 7.78-7.59 (m, 3H), 7.70 (s, 1H), 7.61 (dd, *J* = 8.51, 1.22 Hz, 1H), 7.49-7.42 (m, 3H), 5.46 (d, *J* = 7.16 Hz, 1H), 5.32 (s, 1H), 4.24 (dddd, *J* = 17.93, 10.82, 7.12, 3.72 Hz, 2H), 3.17-3.09 (m, 1H), 3.02-2.93 (m, 2H), 2.88-2.81 (m, 1H), 1.59 (s, 9H), 1.29 (t, *J* = 7.13 Hz, 3H), 0.67 (t, *J* = 7.15 Hz, 3H);
¹³C NMR (CDCl₃, 100MHz) δ ppm: 168.3, 164.8, 140.3, 139.2, 136.7, 134.8, 132.8, 132.6, 129.1, 128.2, 127.8, 127.4, 126.6, 126.2, 62.9, 61.2, 61.0, 54.1, 54.0, 28.3, 24.7, 14.2, 13.1

4.11 Selected Reference

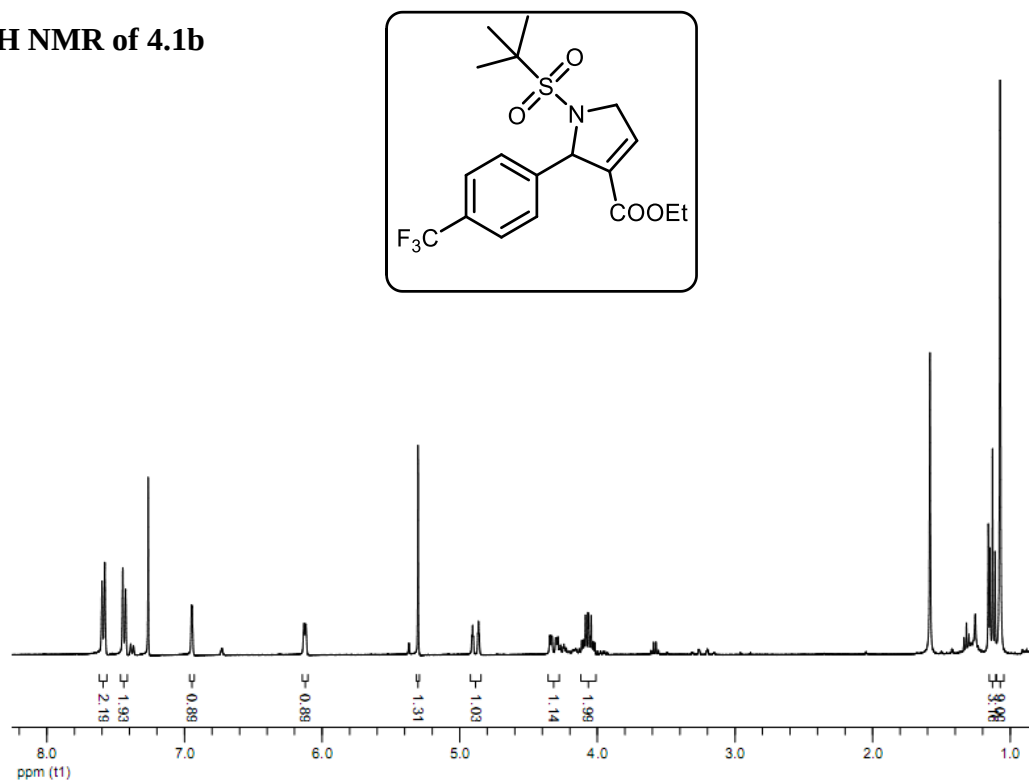
- (1) Murkami, s.; Takemoto, T.; Shimizu, Z. *Yakugaku Zasshi-Journal of the Pharmaceutical Society of Japan* **1953**, *73*, 1026.
- (2) Moloney, M. G. *Nat. Prod. Rep.* **2002**, *19*, 597.
- (3) Asano, N.; Kuroi, H.; Ikeda, K.; Kizu, H.; Kameda, Y.; Kato, A.; Adachi, I.; Watson, A. A.; Nash, R. J.; Fleet, G. W. *Tetrahedron: Asymmetry*. **2000**, *11*, 1.
- (4) Wiseman, L. R.; McTavish, D. *Drugs* **1994**, *48*, 71.
- (5) Shin-ya, K.; Kim, J.-S.; Furihata, K.; Hayakawa, Y.; Seto, H. *Tetrahedron Lett.* **1997**, *38*, 7079.
- (6) Umezawa, S.; Tsuchiya, T.; Tatsuta, K.; Horiuchi, Y.; Usui, T.; UMEZAWA, H.; HAMADA, M.; YAGI, A. *J. Antibiot.* **1970**, *23*, 20.
- (7) MacConnell, J.; Blum, M.; Fales, H. *Tetrahedron*. **1971**, *27*, 1129.
- (8) Gunasekara, N. S.; Noble, S.; Benfield, P. *Drugs*. **1998**, *55*, 85.
- (9) Reddy, B. R.; Reddy, K. S.; Dubey, M. K.; Kumari, Y. B.; Bandichhor, R. *Org. Process Res. Dev.* **2013**, *18*, 163.
- (10) Hudlicky, T.; Kutchan, T. M.; Wilson, S. R.; Mao, D. T. *J. Am. Chem. Soc.* **1980**, *102*, 6351.
- (11) Xu, Z.; Lu, X. *Tetrahedron Lett.* **1997**, *38*, 3461.
- (12) Xu, Z.; Lu, X. *J. Org. Chem.* **1998**, *63*, 5031.
- (13) Zhu, X.-F.; Lan, J.; Kwon, O. *J. Am. Chem. Soc.* **2003**, *125*, 4716.

- (14) Zhu, X.-F.; Henry, C. E.; Wang, J.; Dudding, T.; Kwon, O. *Org. Lett.* **2005**, 7, 1387.
- (15) Davis, F. A.; McCoull, W. *J. Org. Chem.* **1999**, 64, 3396.

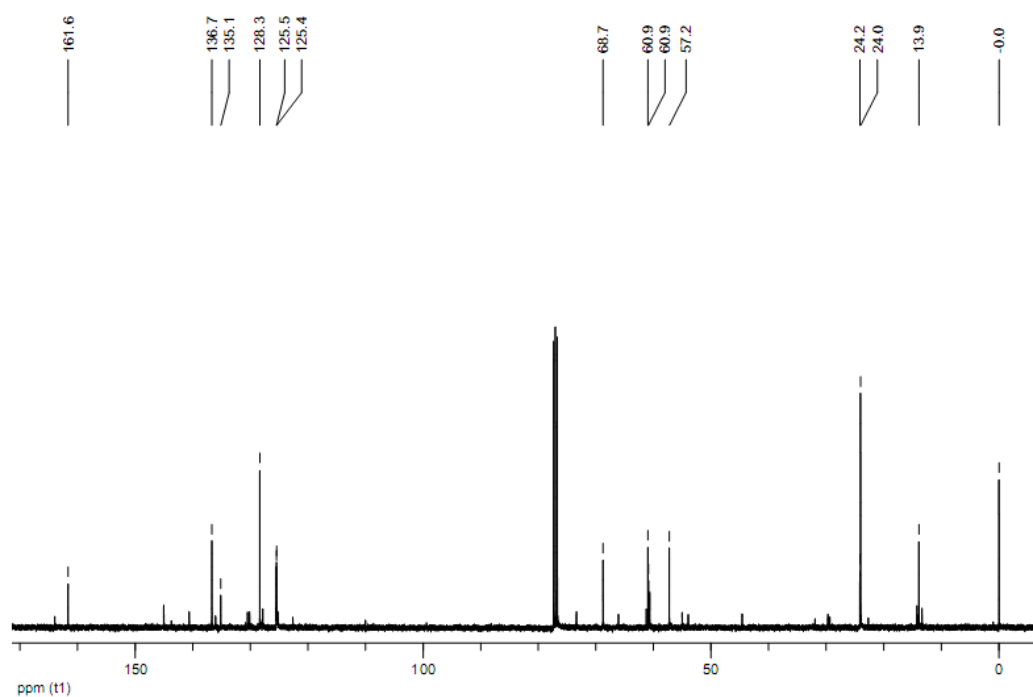
4.12 Spectral Data

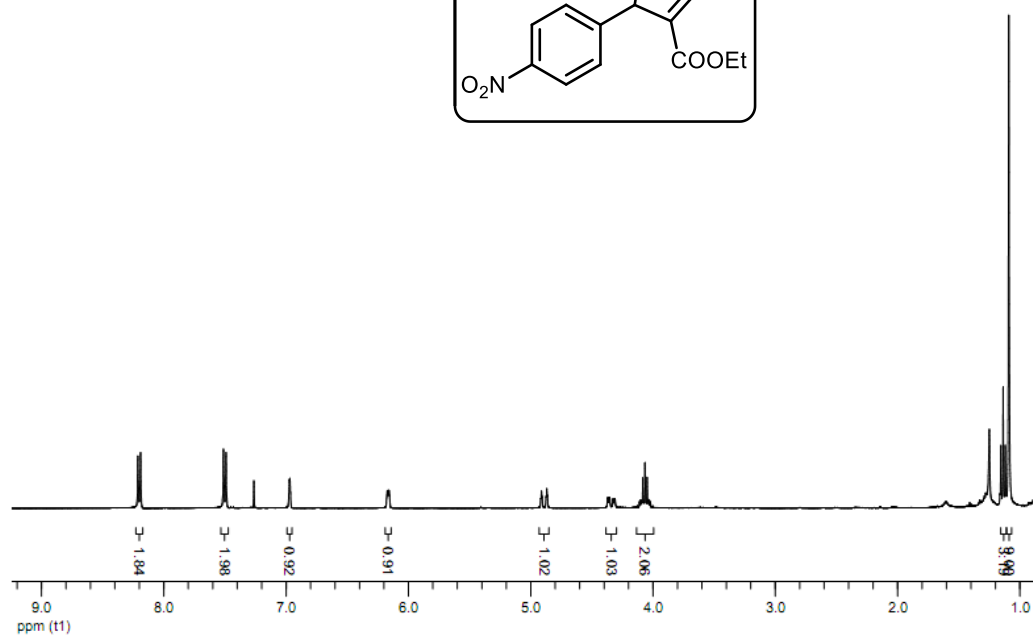
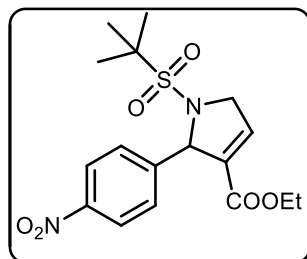
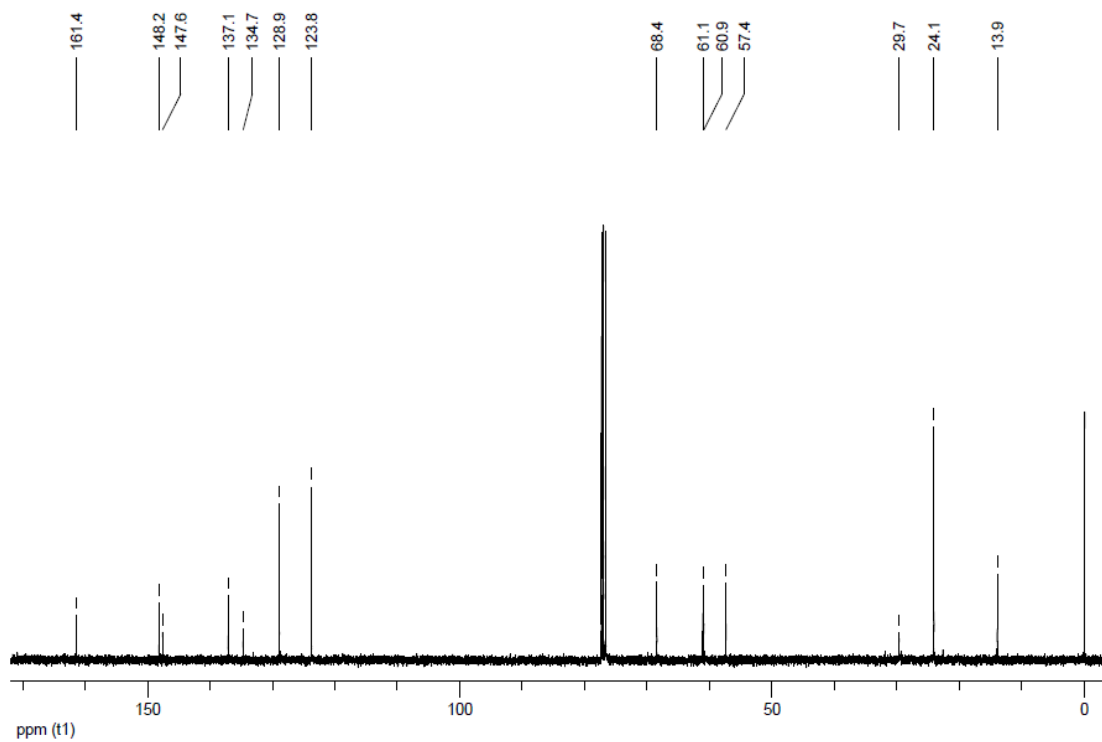
 ^1H NMR of 4.1a ^{13}C NMR of 4.1a

¹H NMR of 4.1b

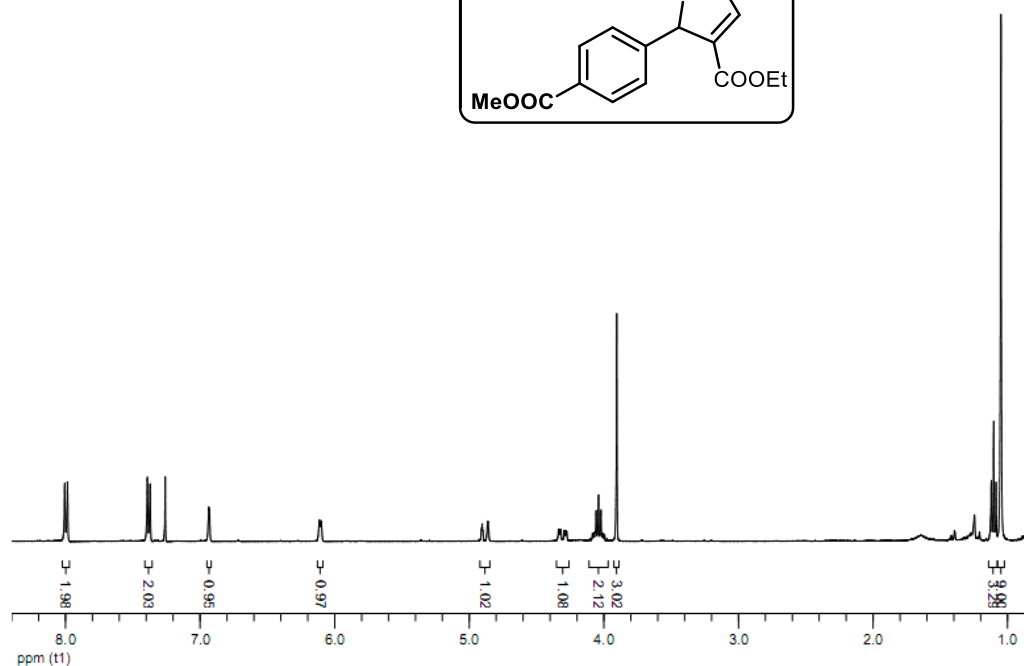
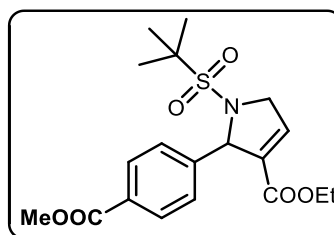


¹³C NMR of 4.1b

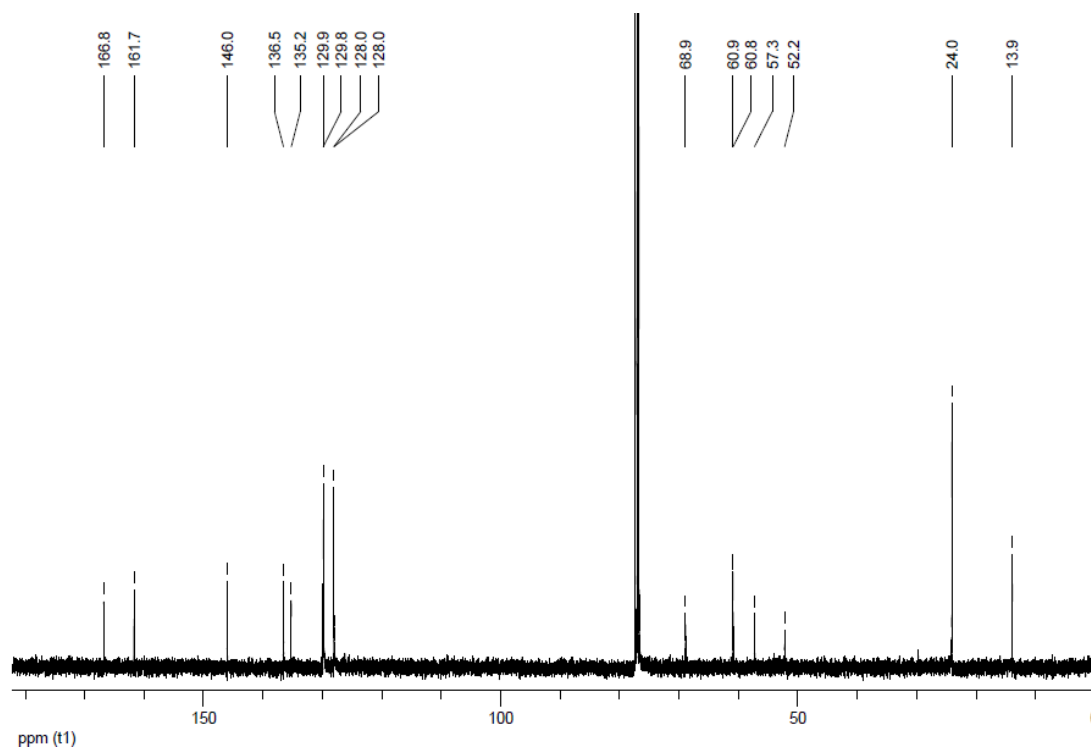


^1H NMR of 4.1c **^{13}C NMR of 4.1c**

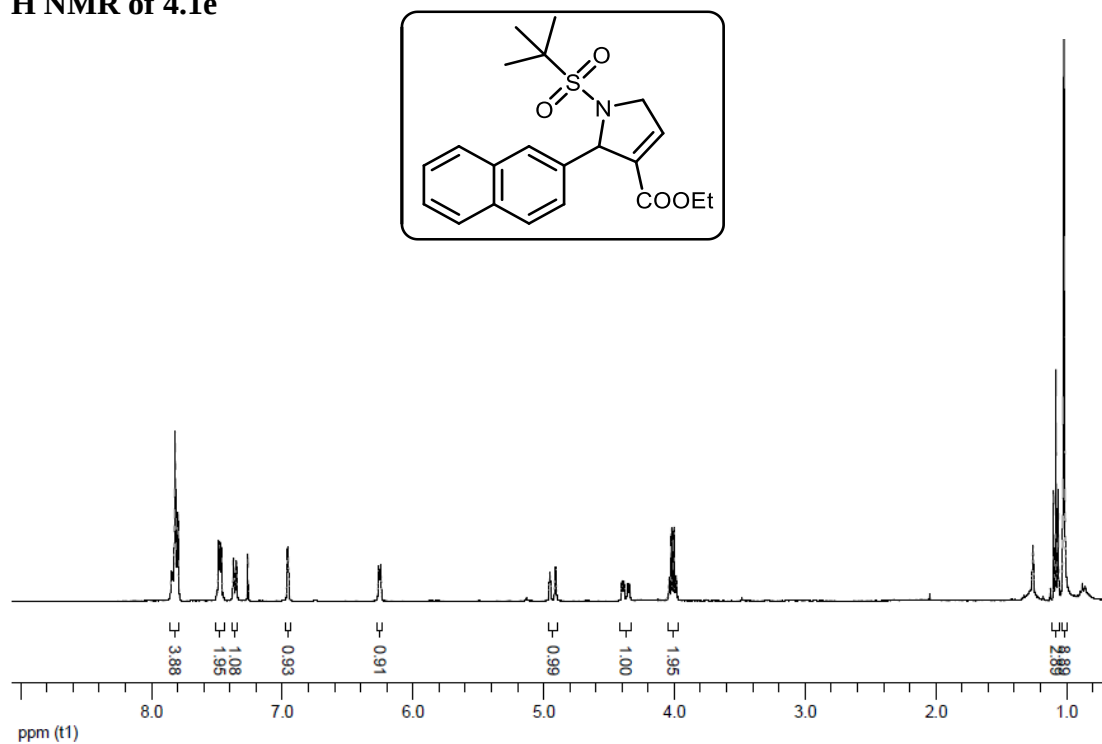
¹H NMR of 4.1d



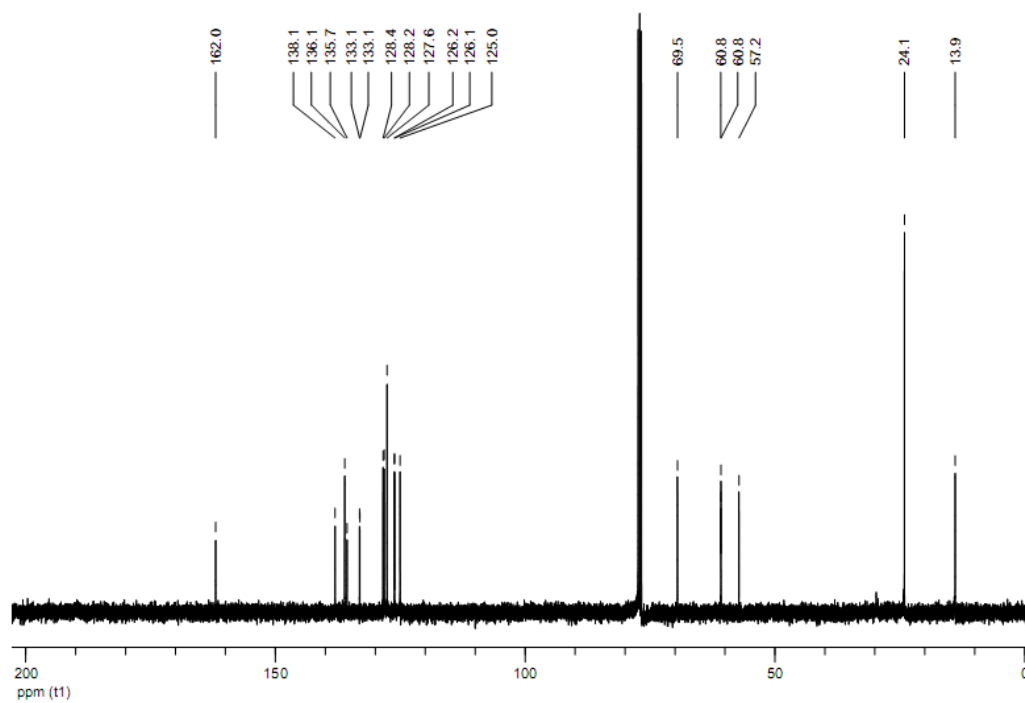
¹³C NMR of 4.1d



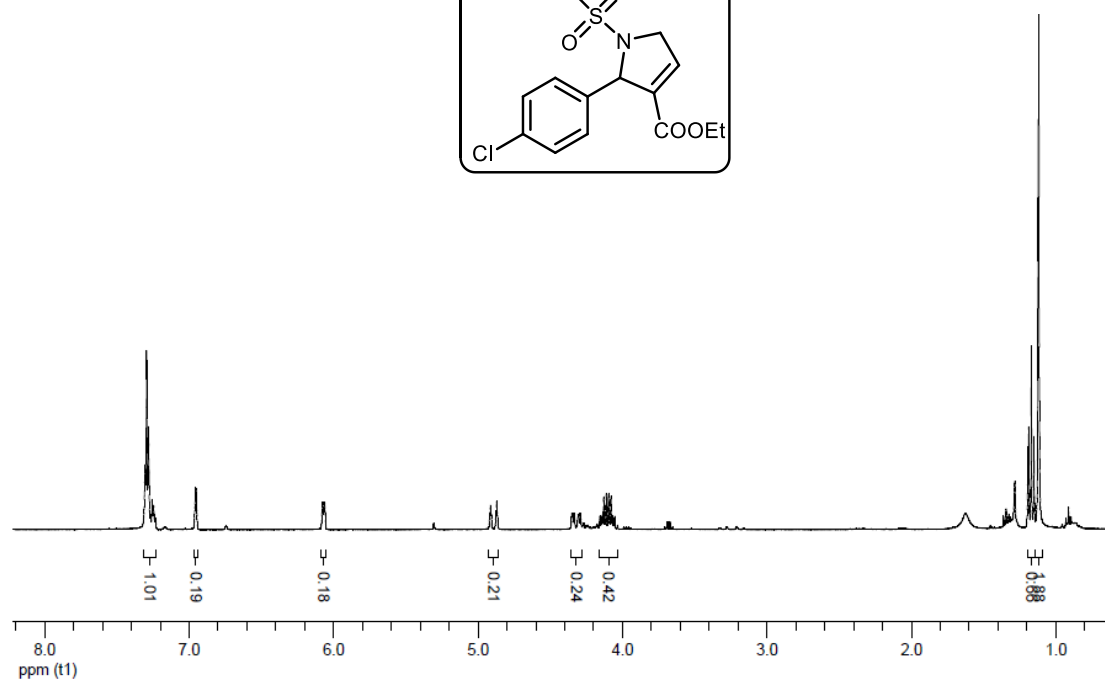
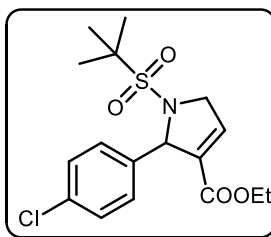
¹H NMR of 4.1e



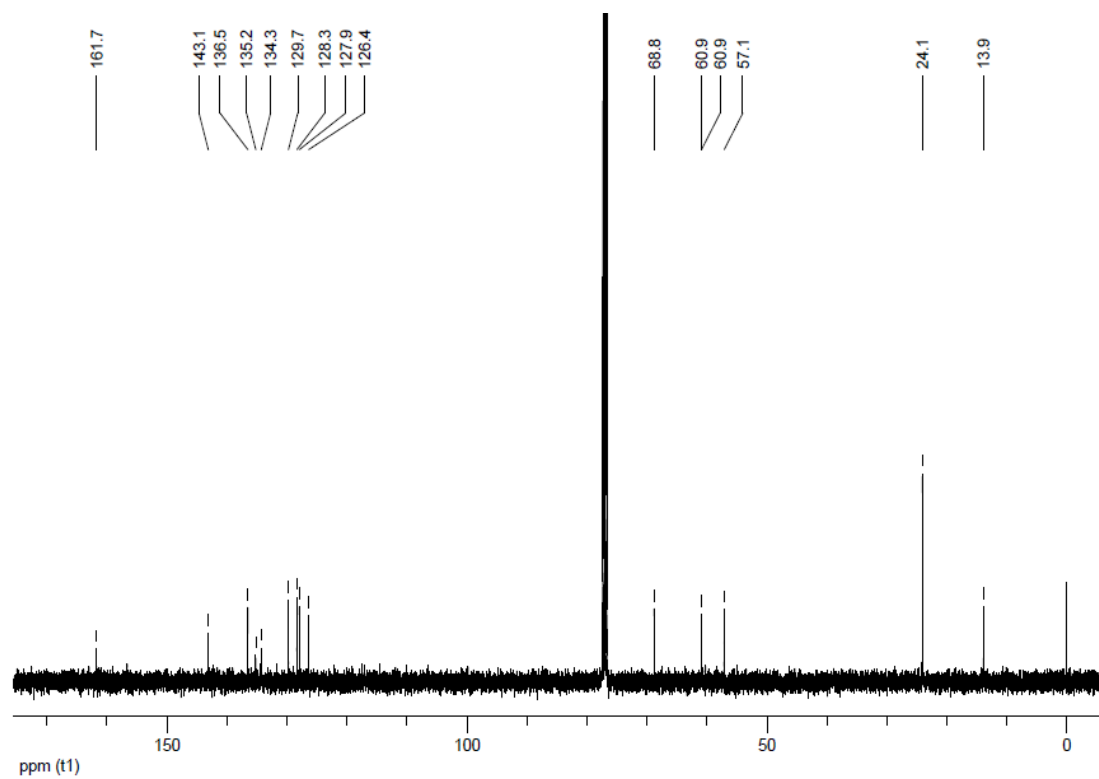
¹³C NMR of 4.1e

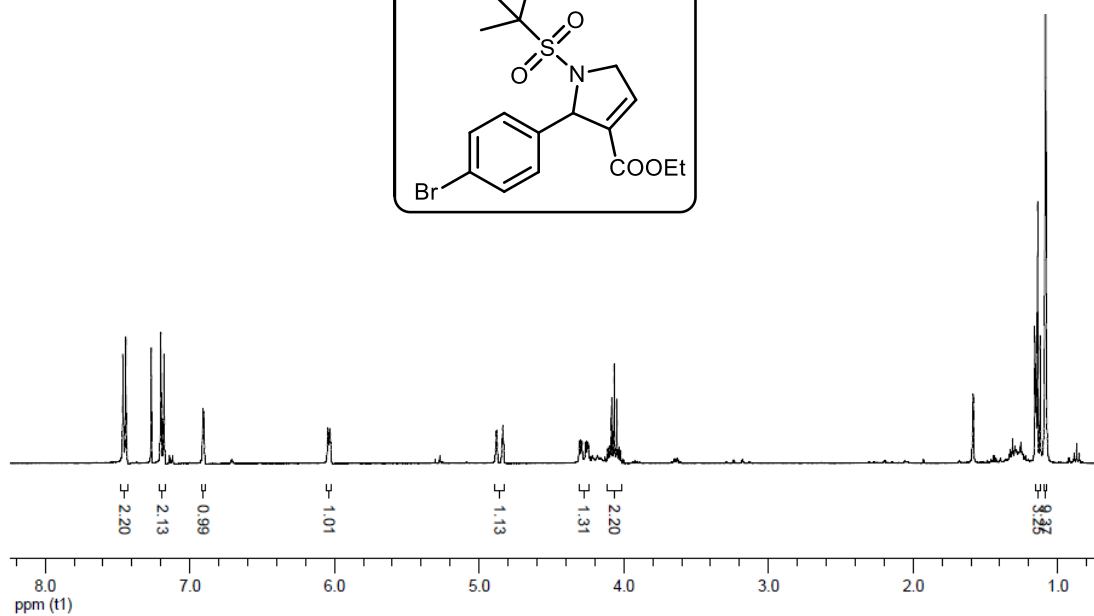
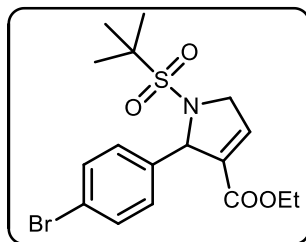
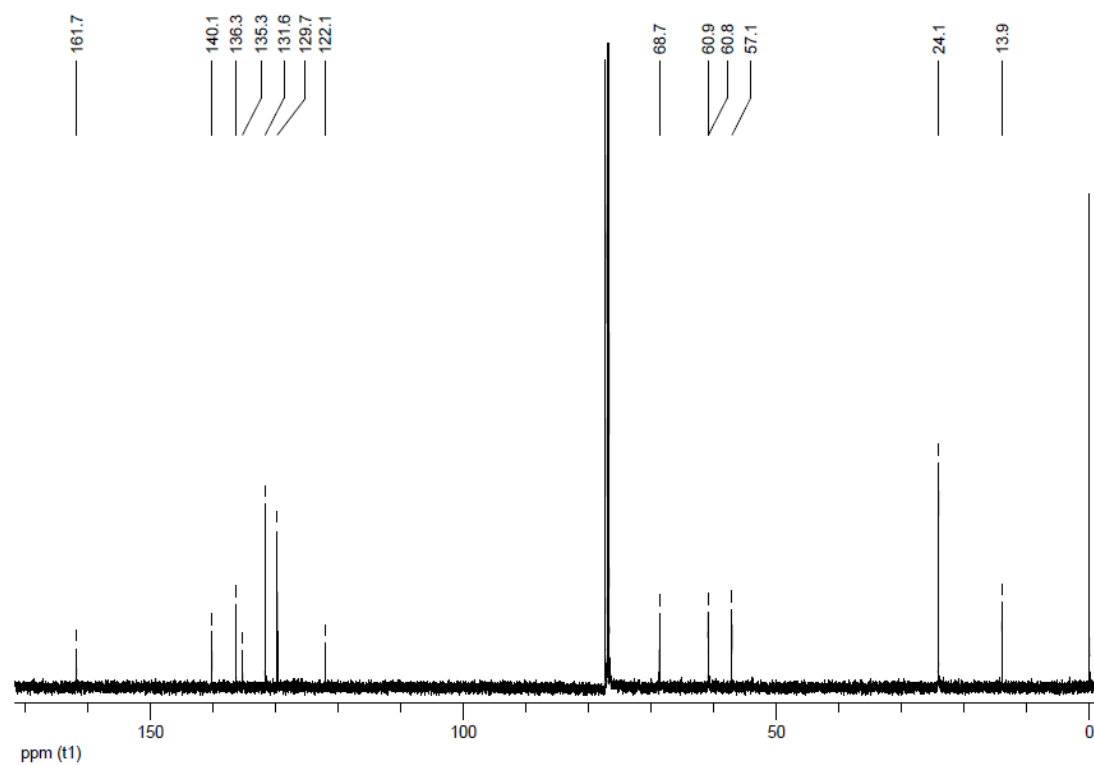


^1H NMR of 4.1f

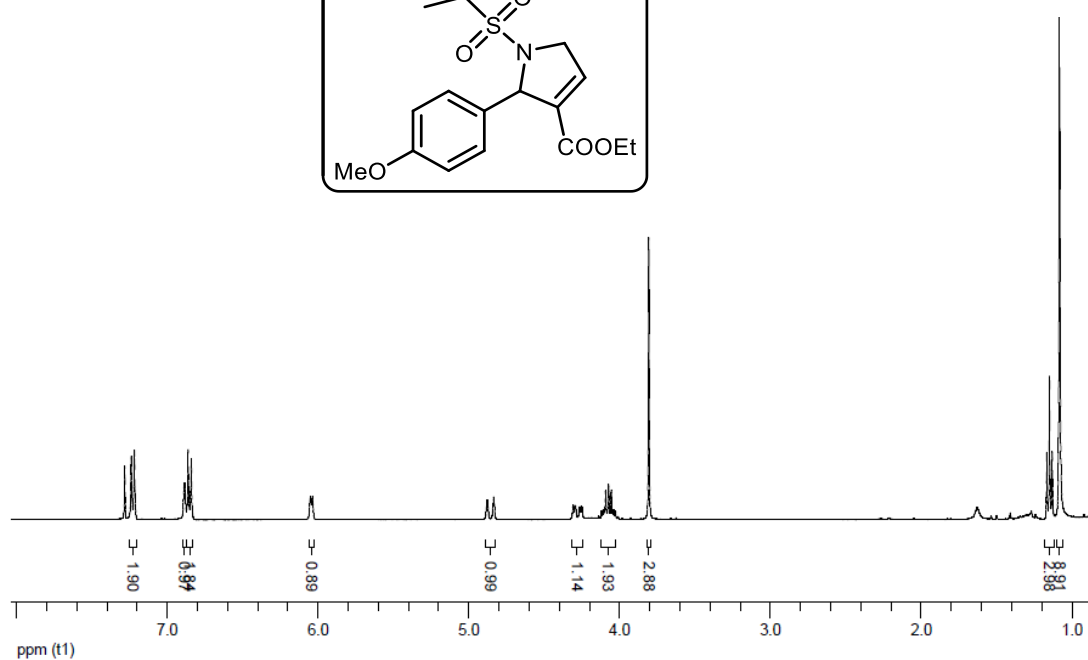
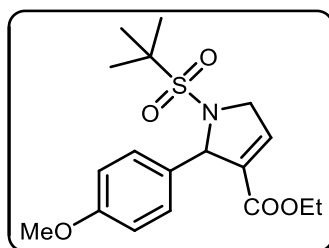


^{13}C NMR of 4.1f

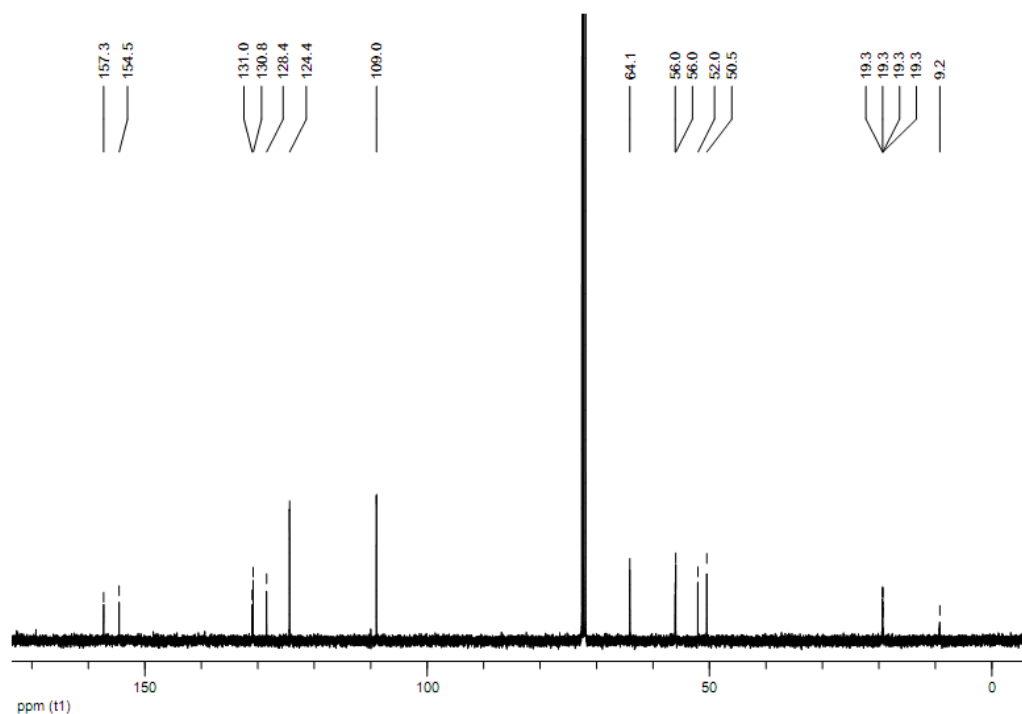


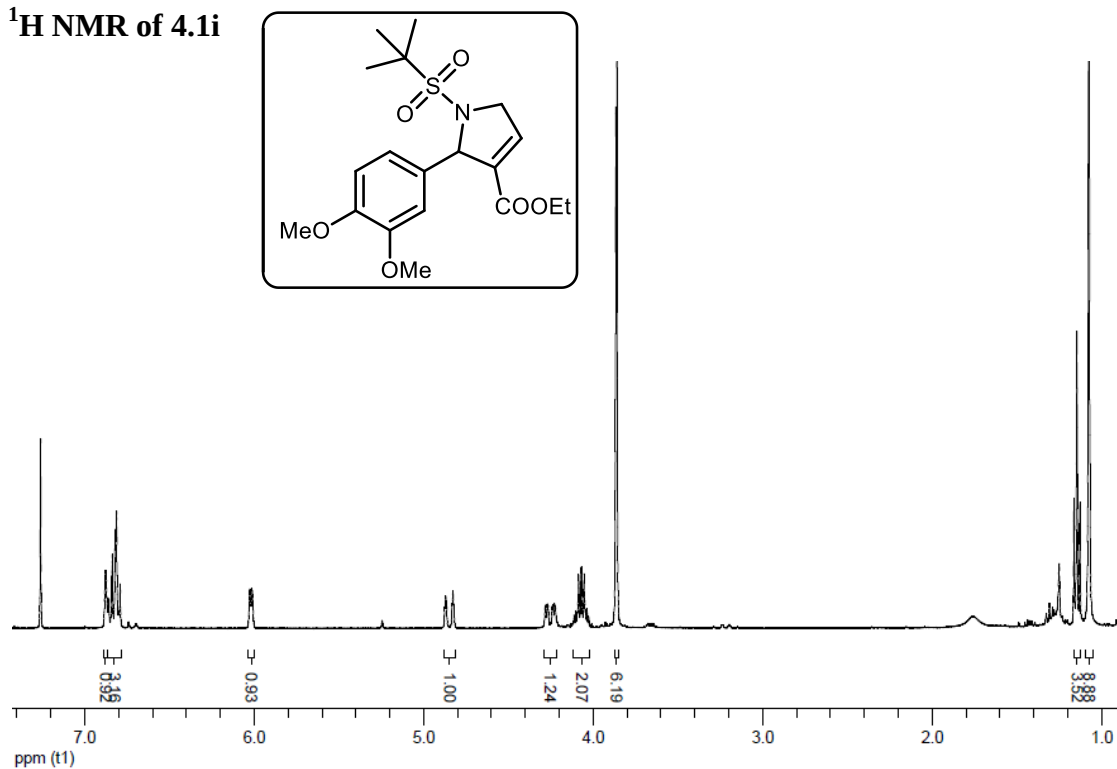
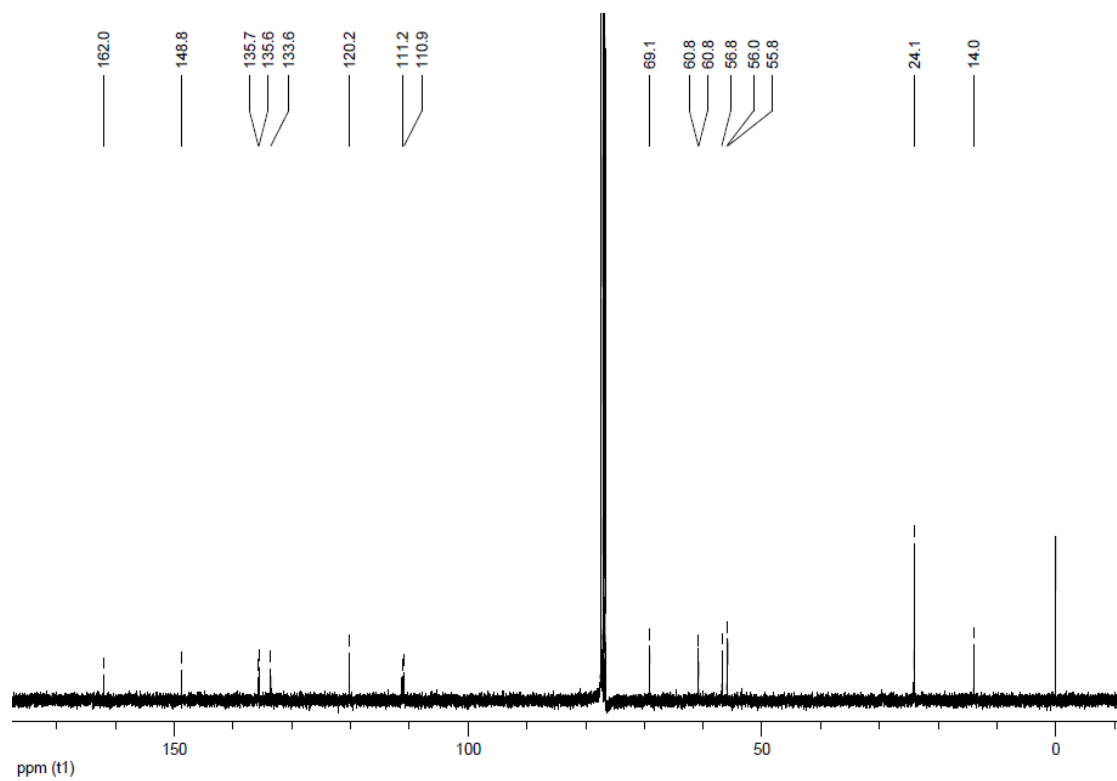
^1H NMR of 4.1g **^{13}C NMR of 4.1g**

¹H NMR of 4.1h

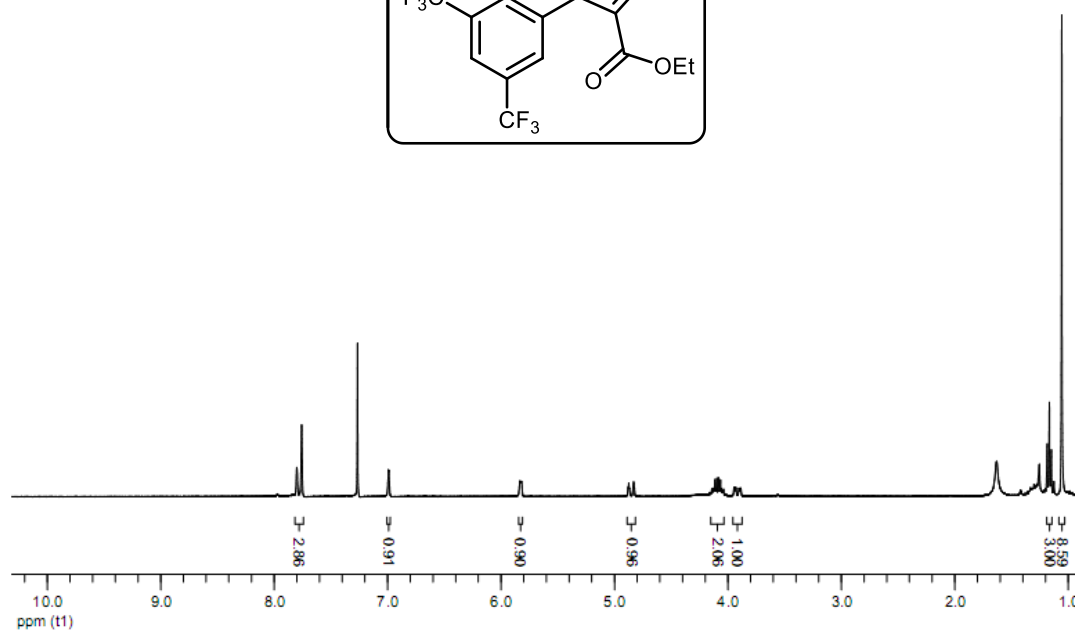
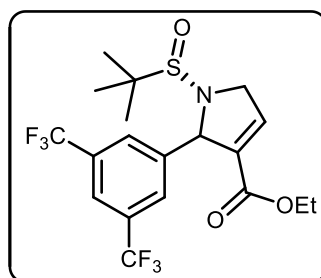


¹³C NMR of 4.1h

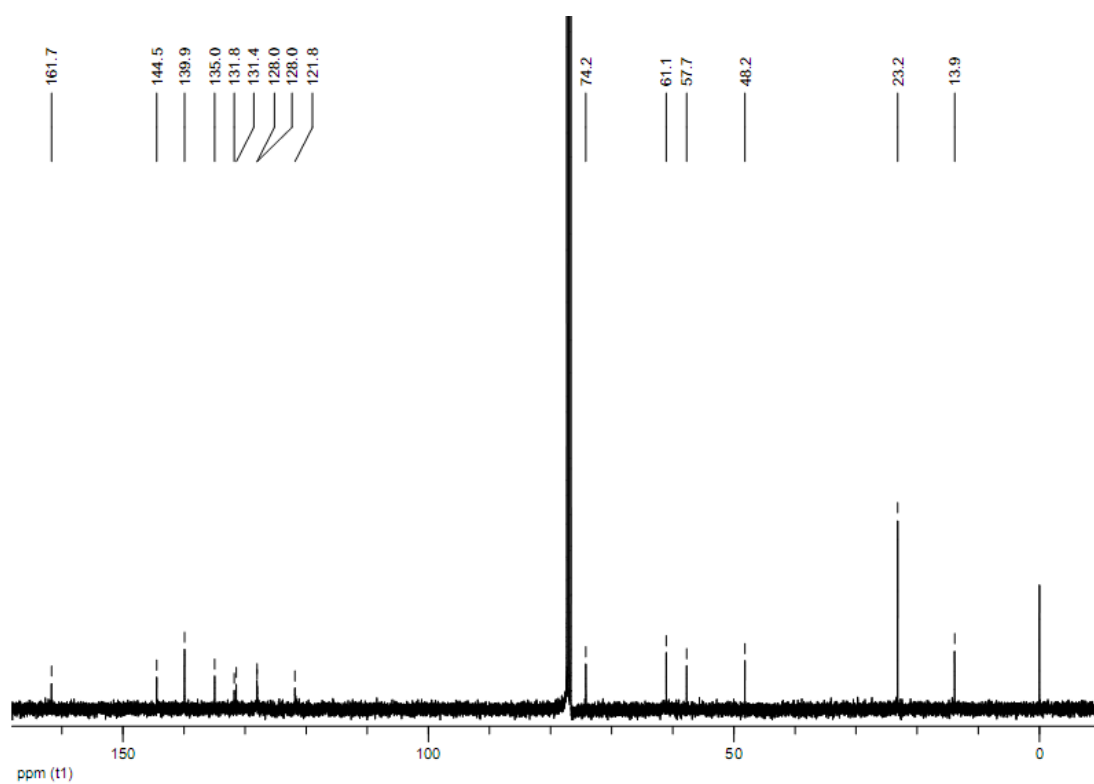


^1H NMR of 4.1i **^{13}C NMR of 4.1i**

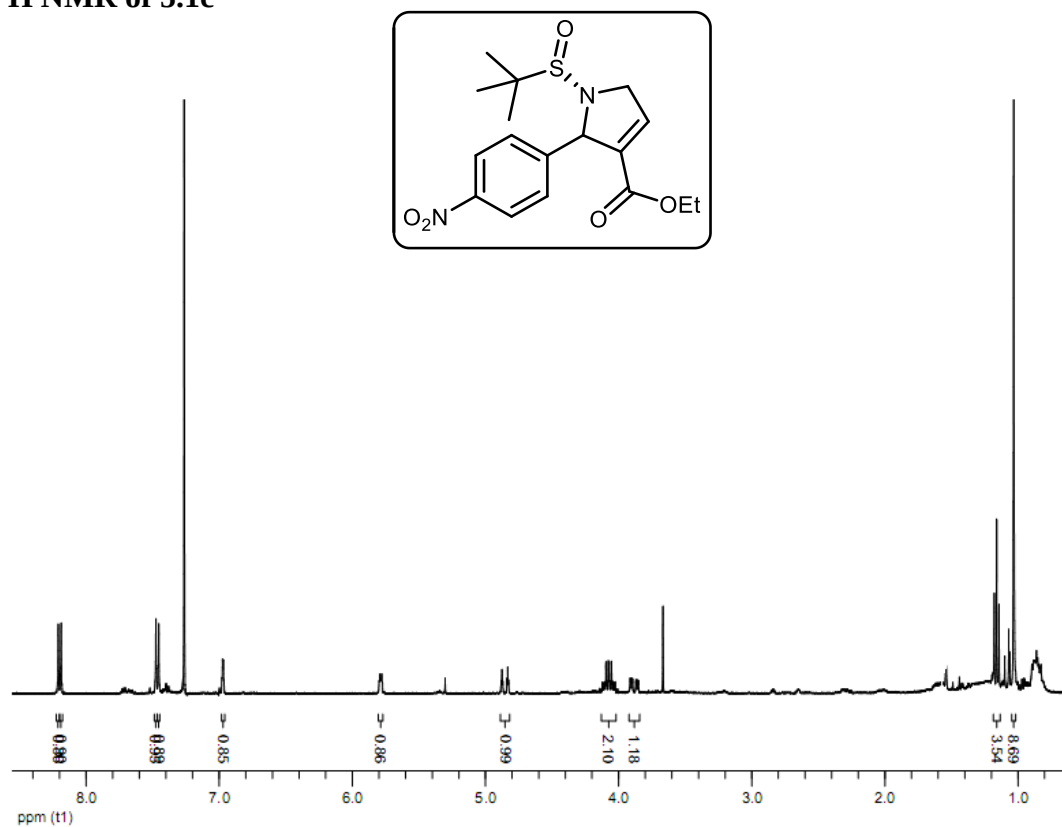
¹H NMR of 5.1a



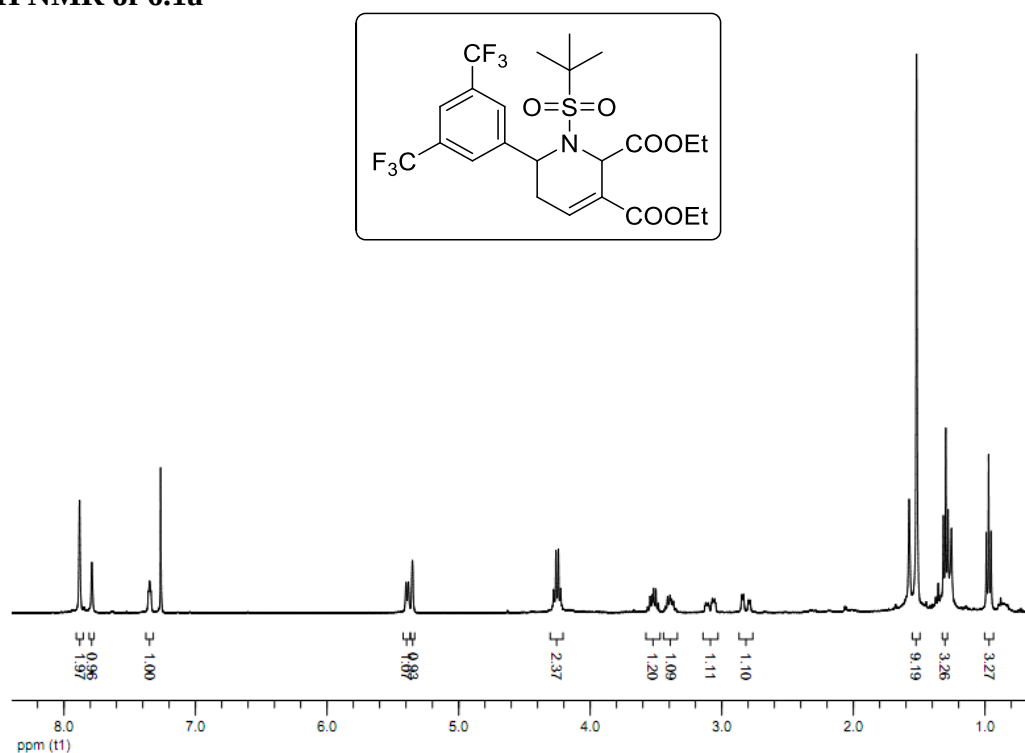
¹³C NMR of 5.1a



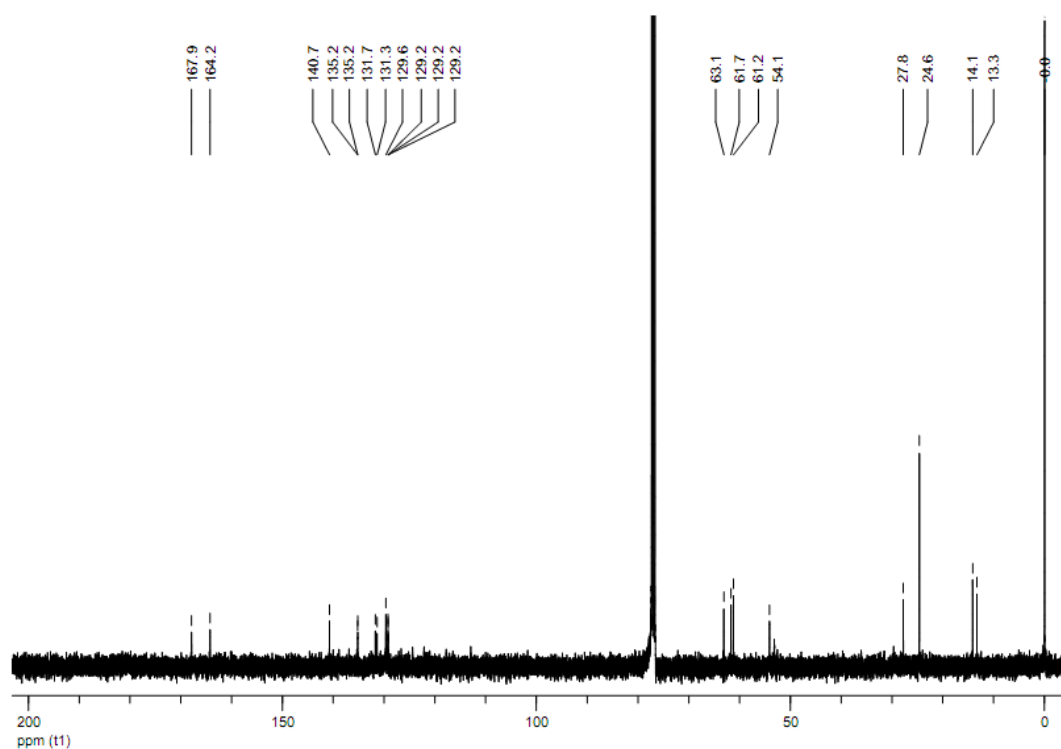
¹H NMR of 5.1c

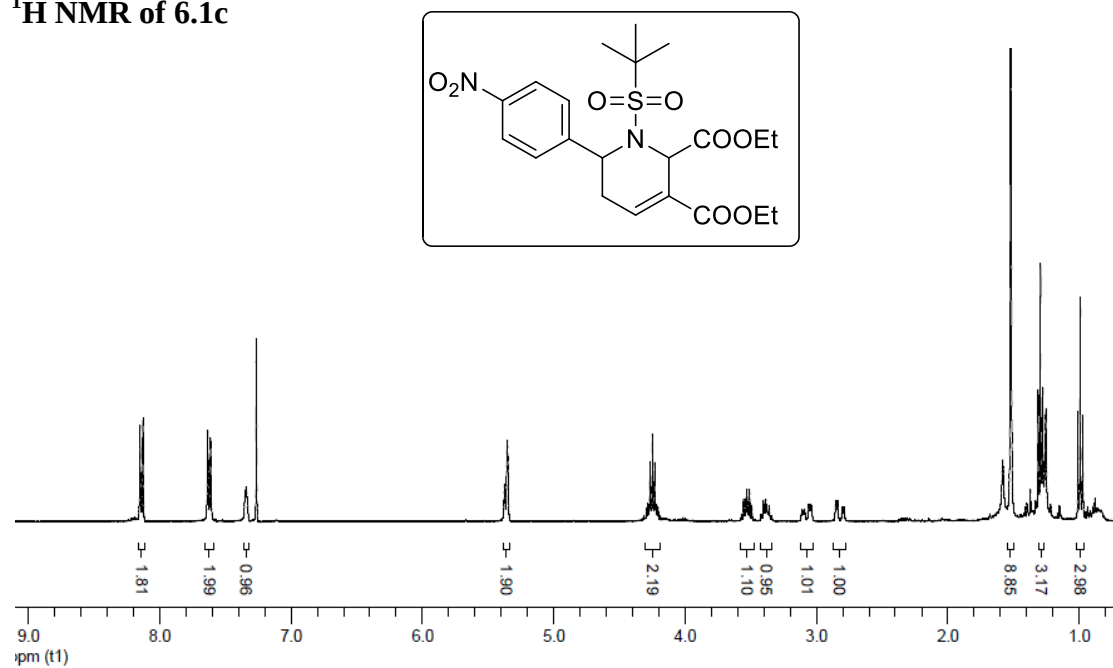
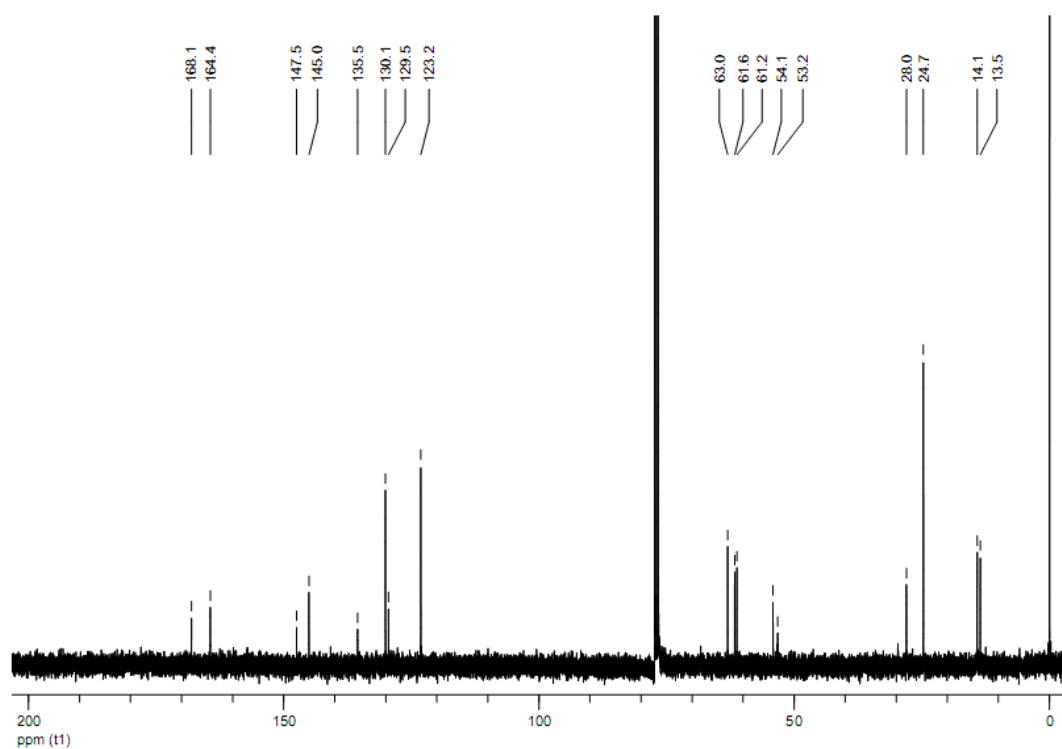


¹H NMR of 6.1a

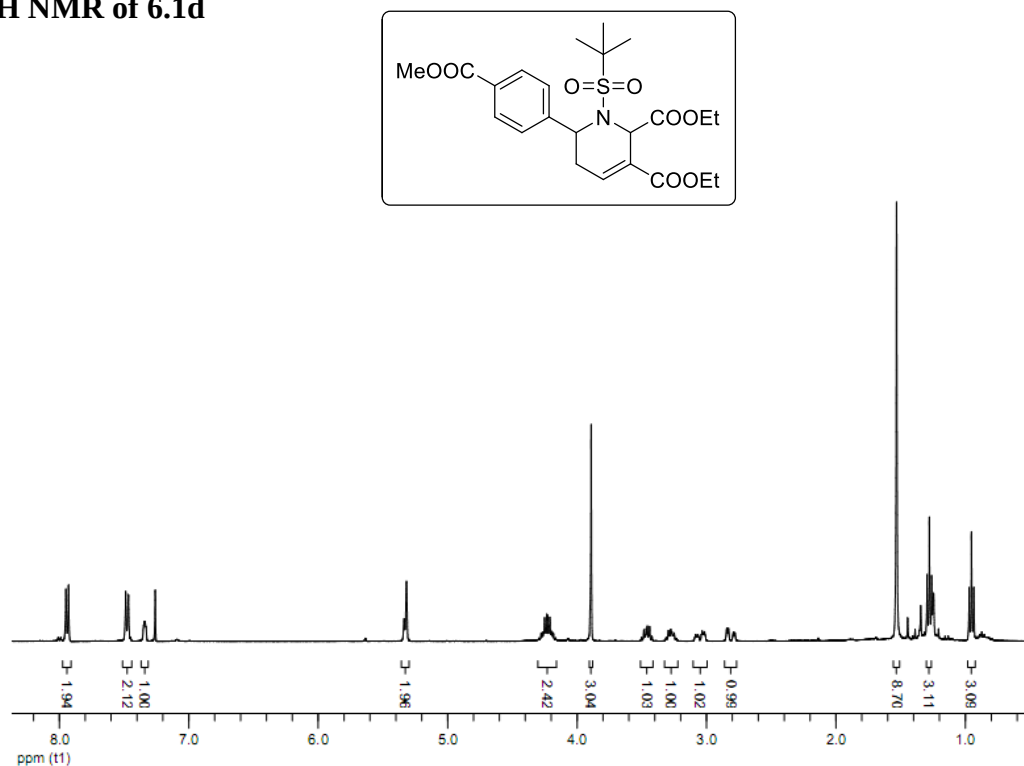


¹³C NMR of 6.1a

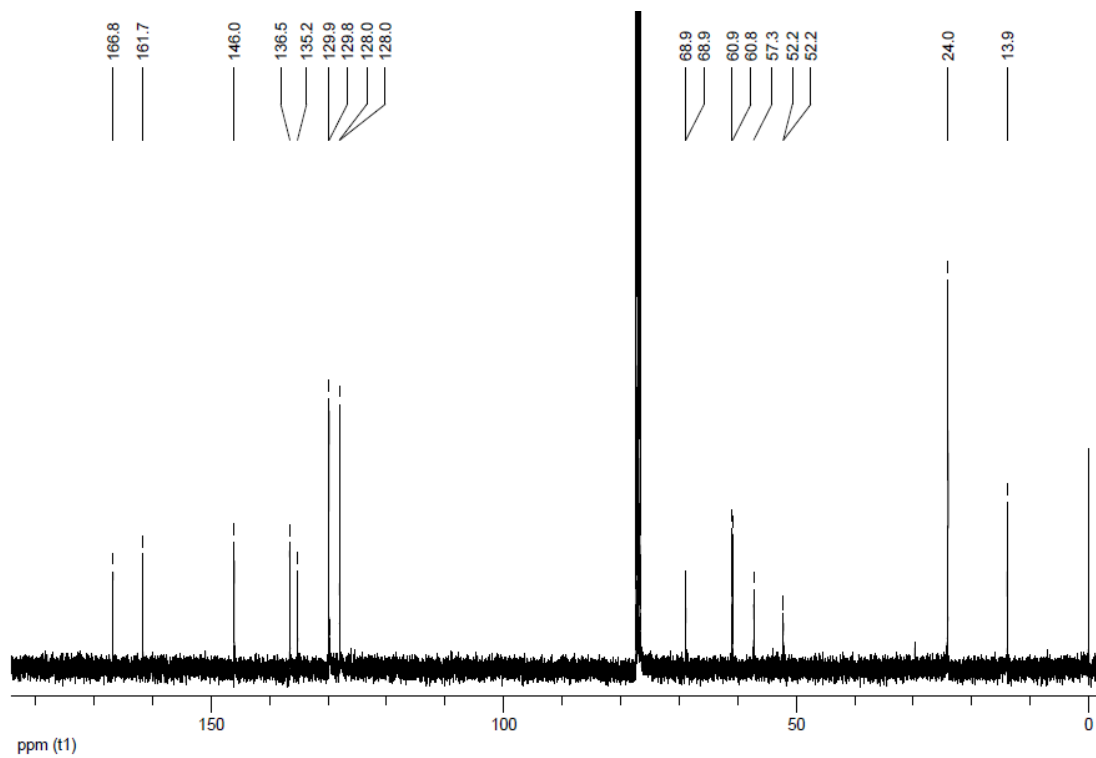


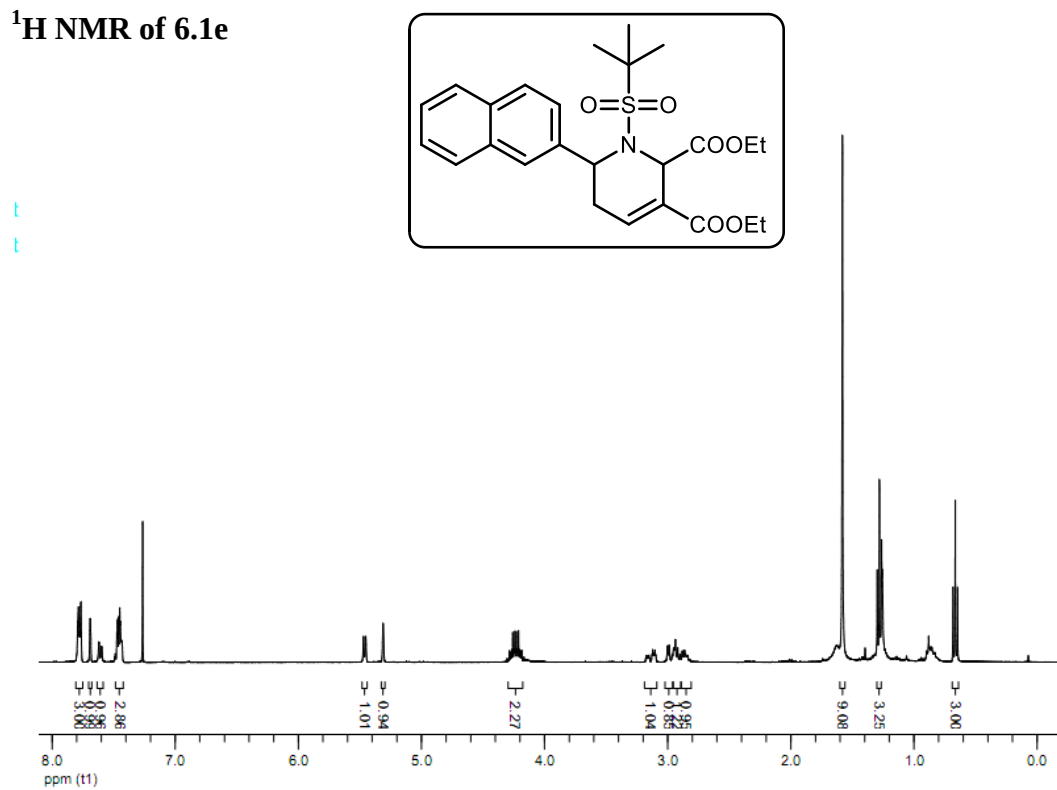
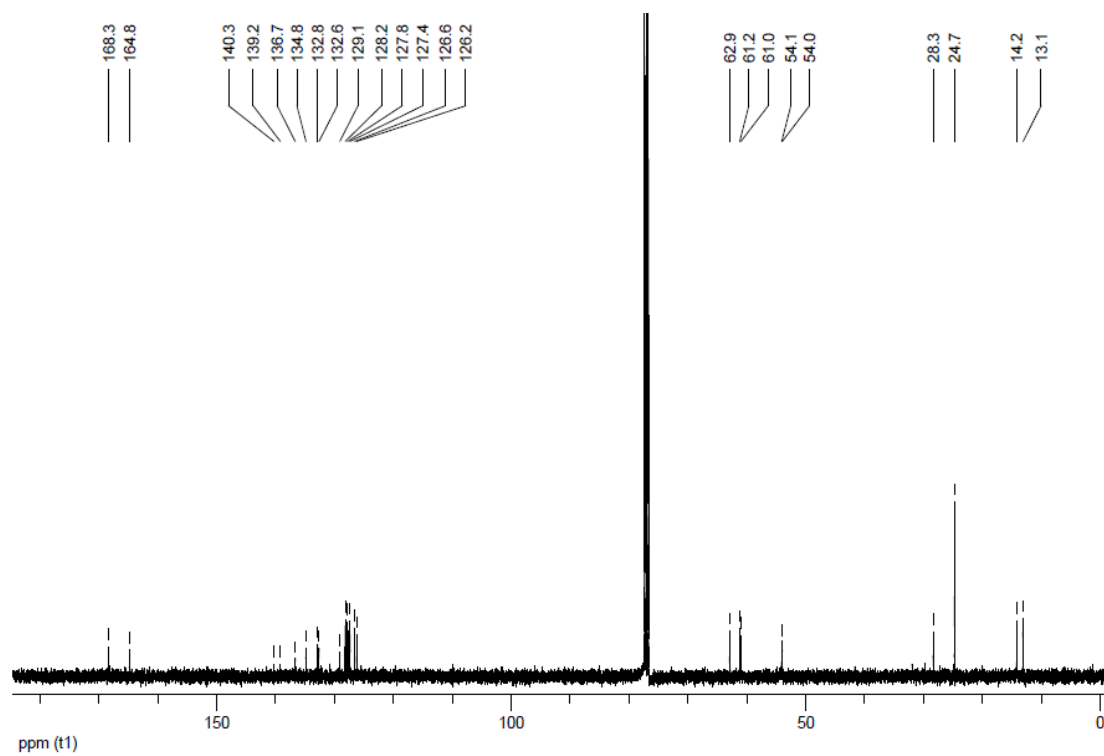
^1H NMR of 6.1c **^{13}C NMR of 6.1c**

¹H NMR of 6.1d



¹³C NMR of 6.1d



^1H NMR of 6.1e **^{13}C NMR of 6.1e**

Peer Reviewed Publications

1. 17-Membered Macrocyclic Ring Derived Toolbox: Identification of Small Molecule Inhibitor of Angiogenesis. Aeluri, M.; Gaddam, J.; Trinath, D. V. K. S.; Chandrasekar, G.; Kitambi, S. S.; Arya, P. *Eur. J. Org. Chem.* **2013**, 3955-3958.
2. Macrocyclic toolbox from Epothilone Fragment Identifies a Compound Showing Molecular Interactions with Actin and Novel Promoters of Apoptosis in Patent-derived Brain Tumor cells. Bhanudas Dasari, Temesgen Fufa, Madhu, Aeluri, Jagan Gaddam, Girdhar Singh Deora, Frank Gaunitz, Satish Srinivas Kitambi, and Prabhat Arya. *Asian. J. Org. Chem.* **2016**, 5, 976-980.
3. Geldanamycin-Inspired Compounds Induce Direct Trans-differentiation of Human Mesenchymal Stem Cells to Neurons. Srinivas Jogula, Anand Soorneedi, Jagan Gaddam, Srinivas Chamakuri, Girdhar Singh Deora, Ranjith Kumar Indarapu, Subhadra Dravida, and Prabhat Arya. *Eur. J. Med. Chem.* **2017**, reviewer's comments received (close to accepted)

Patents

- 1 A Stereoselective Synthesis of Neopeltolide-inspired Macrocyclic Compounds and their Application in the Conversion of Human Mesenchymal Stem Cells to Neurons. Dravida, S.; Kumar, R.; Soorneedi, A.; Gaddam, J.; Arya, P. **India and US provisional patent (submitted, 2016)**
- 2 Design and synthesis of heterocyclic-derived macrocyclic compounds and their application as the direct converters of human mesenchymal stem cells to neurons. Subhadra Dravida, Ranjit Kumar, Anand Soorneedi, Jagan Gaddam, Madhu Aeluri and Prabhat Arya. **India and US provisional patent, submitted, 2017.**

Book Chapters

Ring Closing Metathesis-based Methods in Chemical Biology: Building A Natural Product-Inspired Macrocyclic Toolbox for Undertaking Protein-Protein Interactions. Gaddam, J.; Mallurwar, N.K.; Khatravath, M.; Konda, S.; Aeluri, M.; Mitra, P.; Arya, P. (Wiley Publishers, 2017 in press)

Seminars/Conferences Attended

- 2013 Presented a poster at DRILS, University of Hyderabad Campus, India
- 2014 Attended at the **X-JNOST** 2014 international conference, IIT, Madras, India
- 2015 Presented a poster at “Trend Setting Innovations in Chemical Sciences & Technology Applications in Pharmaceutical Industry” that was held at JNTUH, Hyderabad, India