Design and Synthesis of Artemisinin-Dipeptidyl Vinyl Phosphonate Hybrid Molecules as Novel Antimalarial Agents; Synthesis of Biologically Active Molecules and Studies Directed Towards Bioactives from *Vernonia arborea*

Thesis Submitted to AcSIR

For the Award of the Degree of

DOCTOR OF PHILOSOPHY

In

CHEMICAL SCIENCES

By

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November 2018

Dedicated to

My Beloved Family Members

THESIS CERTIFICATE

This is to certify that the work incorporated in this Ph.D. thesis entitled **"Design and Synthesis of Artemisinin-Dipeptidyl Vinyl Phosphonate Hybrid Molecules as Novel Antimalarial Agents; Synthesis of Biologically Active Molecules and Studies Directed Towards Bioactives from *Vernonia arborea*''** submitted by **Mr. Eswara Kumar Aratikatla** to Academy of Scientific and Innovative Research (**AcSIR**) in fulfilment of the requirements for the award of the Degree of **Doctor of Philosophy**, embodies original research work under my supervision. I further certify that this work has not been submitted to any other University or Institution in part or full for the award of any degree or diploma. Research material obtained from other sources has been duly acknowledged in the thesis. Any text, illustration, table etc., used in the thesis from other sources, have been duly cited and acknowledged.

Eswara Kumar Aratikatla
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Declaration by the Candidate

I hereby declare that the original research work embodied in this thesis entitled, **"Design and Synthesis of Artemisinin-Dipeptidyl Vinyl Phosphonate Hybrid Molecules as Novel Antimalarial Agents; Synthesis of Biologically Active Molecules and Studies Directed Towards Bioactives from *Vernonia arborea*"** submitted to the Academy of Scientific and Innovative Research (AcSIR) for the award of degree of Doctor of Philosophy (Ph.D.) is the outcome of experimental investigations carried out by me under the supervision of **Dr. Asish K. Bhattacharya**, Senior Scientist, Organic Chemistry Division, CSIR-National Chemical Laboratory, Pune. I affirm that the work incorporated is original and has not been submitted to any other academy, university or institute for the award of any degree or diploma.

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Eswara Kumar Aratikatla

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Abbreviations

AA	Amino acid
Ac	Acetyl
AcOH	Acetic acid
Ac_2O	Acetic anhydride
ACT	Artemisinin Combination Therapy
AD	Asymmetric dihydroxylation
ADP	Adenosine diphosphate
AED	Antiepileptic drug
ATP	Adenosine triphosphate
Anhyd.	Anhydrous
Aq.	Aqueous
atm.	Atmosphere
$BF_3 \cdot Et_2O$	Boron trifluoride-diethyletherate
Boc	*tert*-Butyloxycarbonyl
t-BuOH	*tert*-Butanol
^{n}BuLi	*n*-Butyl lithium
Bz	Benzoyl
°C	Degree Celsius
cat.	Catalytic
Cbz	Carboxybenzyl
CC_{50}	Cytotoxic concentration that is toxic to 50% cells
$CDCl_3$	Deuterated chloroform
CDI	Carbodiimide
$CHCl_3$	Chloroform
CH_3CN	Acetonitrile
Conc.	Concentrated
m-CPBA	*m*-Chloroperbezoic acid

Abbreviations

CQ	Chloroquine
DCM (CH_2Cl_2)	Dichloromethane
(*R*,*R*)-DACH	(1*R*,2*R*)-(+)-1,2-Diaminocyclohexane-*N*,*N*′-bis(2-diphenylphosphino-1-naphthoyl)
DBU	1,8-Diazabicyclo[5.4.0]undecene-7
DCC	*N*,*N*′-Dicyclohexylcarbodiimide
DEPT	Distortionless Enhancement by polarization Transfer
DHP	3,4-Dihydro-2*H*-pyran
$(DHQ)_2PHAL$	1,4-Bis(dihydroquinin-9-*O*-yl)phthalazine
DIAD	Diisopropylazocarboxylate
DIBAL-H	Diisobutylaluminium hydride
D-(-)-DIPT	(-)-Diisopropyl D-tartrate
DMAP	4-Dimethylaminopyridine
DMF	*N*, *N*′-Dimethylformamide
DMSO	Dimethyl sulfoxide
DMTMM	4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride
DPAP1	Dipeptidyl amino-peptidase 1
DPPA	Diphenylphosphoryl azide
DTPP	Diethoxytriphenylphosphorane
DYKAT	Dynamic Kinetic Asymmetric Transformation
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ee	Enantiomeric excess
ELSD	Evaporative light scattering detector
ESI-LCMS	Electrospray ionization-liquid chromatography-mass spectrometry
EtOAc	Ethyl acetate
EtOH	Ethanol
equiv.	Equivalent

Abbreviations

FAA	Functionalized amino acid
FACS	Fluorescence-activated cell sorting
FT-IR	Fourier-transform infrared spectroscopy
FP-2	Falcipain-2
µg	Microgram
h	hour (s)
HAP	Histo-aspartic protease
HEPES	*N*-2-Hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid
HKR	Hydrolytic kinetic resolution
HOBT	Hydroxybenzotriazole
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
HTS	High-throughput screening
Hz	Hertz
IBCF	Isobutyl chloroformate
IC_{50}	Half-maximal inhibitory concentration
IPA	Isopropyl alcohol
IR	Infra red
J	Coupling constant (in NMR)
KOH	Potassium hydroxide
LiOH	Lithium hydroxide
µM	Micromolar
Me	Methyl
MeI	Methyl iodide
MeOH	Methanol
mg	Milligram
MHz	Megahertz

Abbreviations

MIC	Minimum inhibitory concentration
min.	Minutes
mL	Millilitre (s)
mmol	Millimole (s)
m.p.	Melting point
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
m/z	Mass to charge ratio
$NaBH_4$	Sodium borohydride
NaH	Sodium hydride
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydoxide
NCE	New chemical entity
NMR	Nuclear Magnetic Resonance
nM	Nanomolar
NMM	*N*-Methyl morpholine
NOESY	Nuclear Overhauser Effect Spectroscopy
Pd	Palladium
Ph	Phenyl
PMP	*p*-methoxy phenyl
PPh_3	Triphenyl phosphine
ppm	Parts per million
PPTS	Pyridinium *p*-toluenesulfonate
PTSA	*para*-Toluenesulfonic acid
Py	Pyridine
rac	Racemic
R_f	Retention factor
RM	Reaction mixture

Abbreviations

RT (rt)	Room temperature
SAD	Sharpless asymmetric dihydroxylation
SAR	Structure-activity relationship
sd	standard deviation
SI	Selectivity index
SiO_2	Silicagel
$SOCl_2$	Thionyl chloride
SP	Sulfadoxine/pyrimethamine
TBAI ($^nBu_4NI$)	tetra-*n*-Butylammonium iodide
TCA-NCO	trichloroacetyl isocyanate
TCCA	Trichloroisocyanuric acid
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
tert	Tertiary
TFAA	Trifluoroacetic anhydride
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TOF mass analyzer	Time-of-flight mass analyzer
t_R	Retention time
*p*TsCl	*para*-Toluenesulfonyl chloride
UCB	Union Chimique Belge
UV	Ultraviolet
WHO	World Health Organization
XRD	X-ray diffraction

General remarks

- Independent compound and reference numbering have been used for each chapter as well as for sections of the chapters.

- All reagents and solvents were purchased from commercial suppliers and used as such without any further purification. Starting materials were obtained from commercial suppliers or prepared using known procedures.

- All the known compounds reported in literature were characterized by their NMR spectra.

- Solvents were distilled and dried following standard procedures. Petroleum ether used for column chromatography was of 60-80 °C boiling range.

- Column chromatographic separations were carried out on silica gel (100-200 or 230-400 mesh size).

- Flash chromatography was carried out by **CombiFlash®Rf *200i*** Isco Teledyne Inc., USA instrument using UV/ELSD detector, 230-400 mesh silica gel Redisep® column and appropriate solvent system.

- All reactions were monitored by TLC with 0.25 mm pre-coated E-Merck silica gel plates (60 F254) and TLC spots were made visible by exposing to UV light, Iodine adsorbed on silica gel or by immersion into an ethanolic solution of phosphomolybdic acid (PMA), *p*-anisaldehyde, ninhydrin or $KMnO_4$ followed by heating with a heat gun for ~15sec.

- NMR spectra were recorded on Bruker AV200 (200.13 MHz for ^{1}H NMR and 50.03 MHz for ^{13}C NMR), AV 400 (400.13 MHz for ^{1}H NMR and 100.03 MHz for ^{13}C NMR), Jeol-400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) and DRX 500 (500.13 MHz for ^{1}H NMR and 125.03 MHz for ^{13}C NMR) spectrometers.

- Chemical shifts (δ) have been expressed in ppm units relative to tetramethylsilane (TMS) as an internal standard and coupling constants (*J*) were measured in Hertz.

- The following abbreviations were used for ^{1}H NMR: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, brs = broad singlet, dd = doublet of doublet, dt = doublet of triplet, td = triplet of doublet and ddd = doublet of doublet of doublet.

- All the reported melting points are uncorrected and were recorded using Buchi melting point apparatus (Buchi B-540).

General remarks

- IR spectra were recorded on Shimadzu FT-IR-8300 spectrometer as thin films in chloroform using NaCl plates and absorptions were expressed in cm^{-1}.

- Optical rotations were recorded on a JASCO P-1020 polarimeter at 589 nm (sodium D-line). Specific rotations $[\alpha]_D$ are reported in deg/dm, and the concentration (c) is given in g/100 mL in the specific solvent.

- Structures and IUPAC nomenclature were generated using ChemBioDraw Ultra 14.0 software.

- Mass spectra were recorded on LC-MS/MS-TOF API QSTAR PULSAR spectrometer, samples introduced by fusion method using Electrospray Ionization Technique.

- High-resolution mass spectra (HRMS) (ESI) were recorded on an Orbitrap (quadrupole plus ion trap) and TOF mass analyzer.

- HPLC was performed with Shimadzu CLASS-VP or Agilent HPLC system (**UV** detection at 215/220/254 nm, **Chiral column:** Chiralpak-IA or IB (0.46 mmϕ X 250 mmL) or Chiralcel OD-H (0.46 cm X 25 cm), **Mobile phase:** EtOH in ^{n}hexane or Isopropyl alcohol (IPA) in ^{n}hexane, **Flow rate:** 1 mL/min or 0.5 mL/min).

Synopsis

Synopsis of the Thesis to be submitted to the Academy of Scientific and Innovative Research for Award of the Degree of Doctor of Philosophy in Chemistry	
Name of the Candidate	Eswara Kumar Aratikatla
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Research Supervisor	Dr. Asish K. Bhattacharya

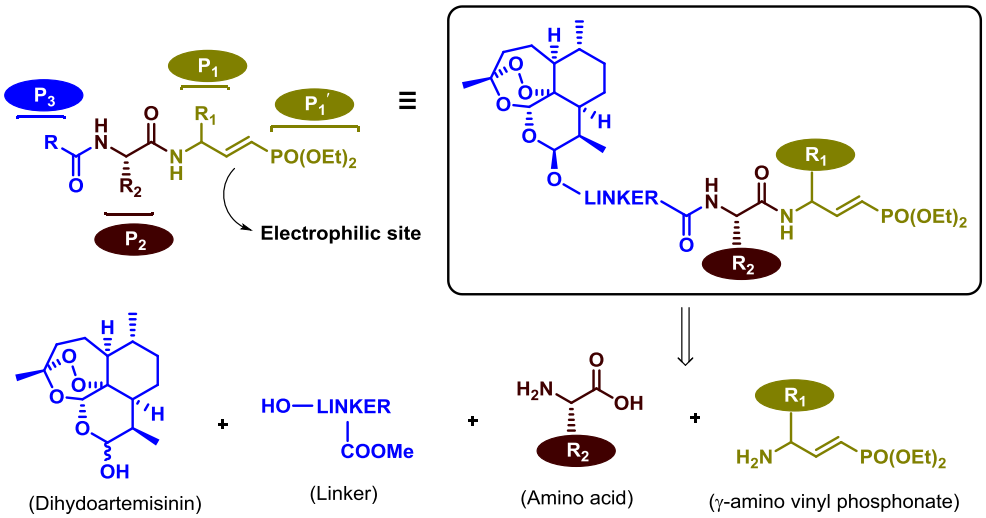
The thesis is divided into three chapters. Chapter **1** deals with the design and synthesis of artemisinin-based hybrid molecules as novel antimalarial agents targeting falcipain-2 enzyme. These hybrid molecules were assayed for parasiticidal activities against *P. falciparum* and exhibited better activities than the currently available drug artemisinin. Chapter **2** deals with the synthesis of syncarpamide, a norepinephrine alkaloid showing the antiplasmodial activity of 2.04 µM and 3.06 µM against *Plasmodium falciparum* D6 and W2 clones, respectively. Chiral pool synthesis of antiepileptic drug (*R*)-lacosamide from natural amino acid L-serine will also be discussed. Chapter **3** deals with the isolation of sesquiterpene lactones zaluzanin C and D. It also deals with the synthesis of analogs of zaluzanin D with new C-N bond formed using Michael addition reaction and analogs with new C-C bond formation using Heck reaction. The anticancer studies of synthesized analogs will also be discussed in this chapter.

Chapter 1: Design and synthesis of artemisinin-dipeptidyl vinyl phosphonate hybrid molecules as novel antimalarial agents

Malaria is caused by protozoan parasites of the genus *Plasmodium.* Development of resistance by the parasites against generally used antimalarial drugs, *e.g.* quinine, chloroquine, etc. further complicate its treatment. The situation was improved with the isolation of artemisinin, a natural sesquiterpene lactone endoperoxide from the Chinese plant *Artemisia annua*. However, artemisinin resistance has been observed in the *P. falciparum* strains isolated from patients treated with artemisinin from Cambodia, French Guiana, and Senegal.[1] The hemoglobin degradation by the malaria parasite is carried out by a variety of proteases present within parasite, and the inhibition of this process is considered to be a potential drug target for the development of new antimalarial agents.[2]

The cysteine proteases present in *P. falciparum, e.g.*, falcipain-2 and falcipain-3 are considered to be the ideal drug targets for the development of new antimalarial agents. Falcipain-2 inhibitors in principle would require hydrophobic interaction in P_1 and P_2 pockets and essentially electrophilic center at the active-site of the enzyme.[3] We visualized that these requirements could be ideally fulfilled by artemisinin-dipeptidyl vinyl phosphonate hybrid molecules.

As shown in the **Scheme 1**, artemisinin-dipeptidyl vinyl phosphonate hybrid molecules were synthesized by combining dihydroartemisinin with peptidyl vinyl phosphonate through suitable linkers by using etherification reaction (12 hybrid molecules were synthesized). A linker could be any compound that has one free hydroxyl group and some functionality which could be easily converted to free acid *e. g.* methyl ester.



Scheme 1. Design and synthesis of artemisinin-dipeptidyl vinyl phosphonate hybrid molecules.

Hybrid molecules **75**, **76**, and **81** exhibited parasiticidal activities against *P. falciparum in vitro* culture in **nM** range (**Figure 1**). These molecules were further assayed for their *in vitro* toxicity against A549 human cells and found to be non-toxic to the human cells.

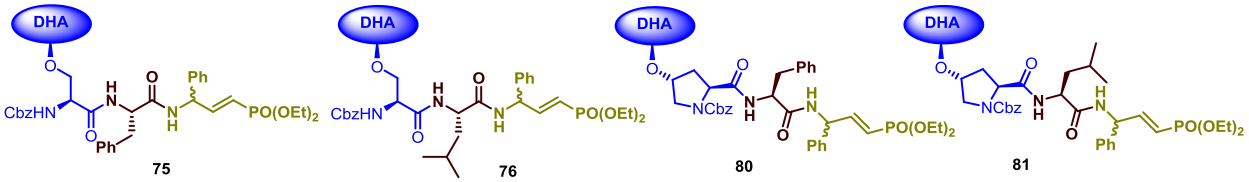


Figure 1. Synthesized artemisinin-dipeptidyl vinyl phosphonate hybrid molecules (where, DHA = dihydroartemisinin).

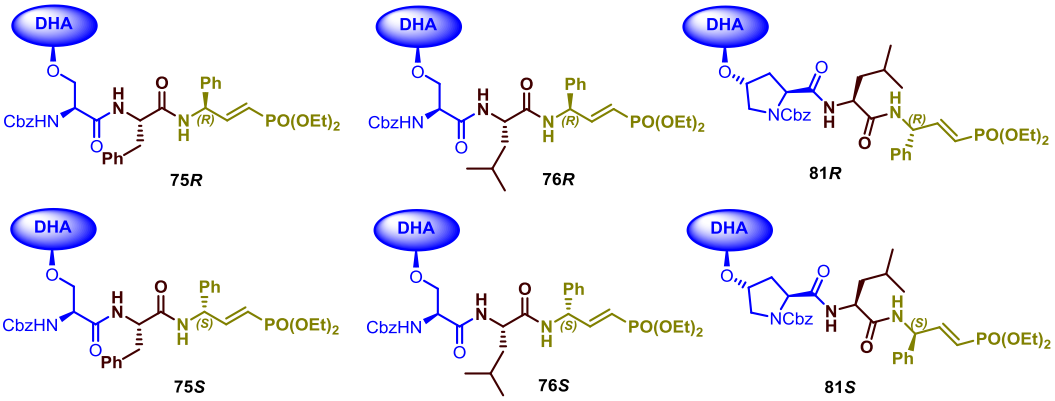


Figure 2. Synthesized diastereomers of hybrid molecules **75**, **76** and **81** (where, DHA = dihydroartemisinin).

Since the active molecules **75**, **76** and **81** were a mixture of two diastereomers, their individual diastereomers were also synthesized by asymmetric synthesis using chiral (*R*) or (*S*)-γ-amino vinyl phosphonate in the final amide coupling reactions (**Figure 2**).[4]

Design and synthesis of artemisinin-dipeptidyl vinyl sulfone hybrid molecules as novel antimalarial agents

Peptidyl vinyl sulfones are known potent irreversible inhibitors of falcipain-2 and 3. They act as Michael acceptors of the catalytic cysteine residue.[5] The excellent antimalarial activities of hybrid molecules **75**, **76** and **81** encouraged us to synthesize their sulfone variants **92**, **93** and **94** (and their diastereomers) as shown below in **Figure 3**. The hybrid molecules **92**, **93** and **94** (and their diastereomers) were synthesized by using *rac* or (*R*) or (*S*)-γ-amino vinyl sulfonate in the final amide coupling reaction.

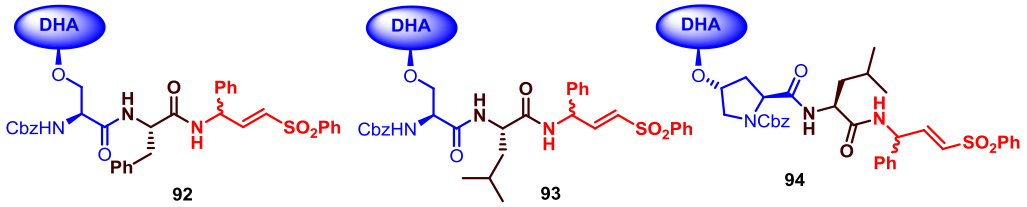


Figure 3. Synthesized artemisinin-dipeptidyl vinyl sulfonate hybrid molecules (where, DHA = dihydroartemisinin).

Chapter 2: Section A: Synthesis of syncarpamide, its analogs and study of their *in vitro* and *in vivo* antiplasmodial activities

Syncarpamide is a norepinephrine alkaloid isolated from the stems of *Zanthoxylum syncarpum*.[6] It shows antiplasmodial activities of 2.04 µM and 3.06 µM against *Plasmodium falciparum* D6 and W2 clones, respectively. Syncarapmide was synthesized using Sharpless asymmetric dihydroxylation (SAD) of 3,4-dimethoxy styrene. Further, a library of **58** analogs and oxy-analogs of syncarpamide were synthesized by taking different styrenes and carboxylic acids (**Figure 4**) and these analogs were assayed for their *in vitro* and *in vivo* antimalarial activities.[7]

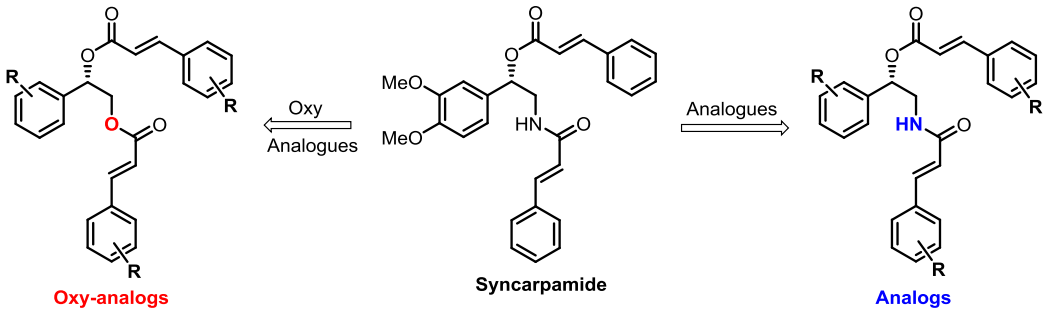
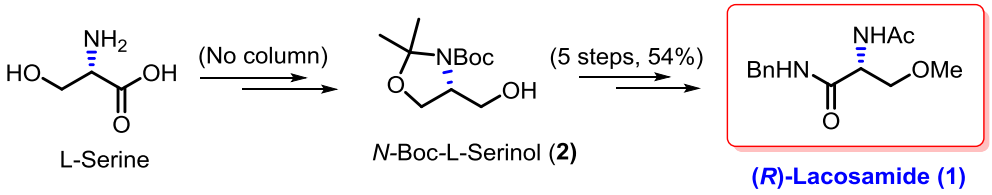


Figure 4. Structures of syncarpamide, analogs and oxy-analogs.

From SAR study, it was observed that electron donating groups (such as OMe groups) on both ester and amide side chains of syncarpamide increased its *in vitro* antiplasmodial activity.

Section B: Synthesis of antiepileptic drug (*R*)-lacosamide using chiral pool strategy

(*R*)-Lacosamide (Vimpat) is a drug used to treat epilepsy which is a neurological disorder that disturbs the normal activity of the brain cells. We thought of an efficient and straightforward synthesis of **1**, which could be achieved on an industrial scale at a much cheaper cost. The key starting material, *N*-Boc-*N*,*O*-isopropylidene-L-serinol **2** could easily be synthesized in gram scale from natural L-serine in four steps without the need of any purification of the intermediates. (*R*)-Lacosamide **1** was synthesized from **2** in 5 steps with 54% overall yield and 100% *ee* (**Scheme 2**).[8]



Scheme 2. Synthesis of (*R*)-lacosamide from L-serine.

Chapter 3: Isolation of sesquiterpene lactones zaluzanin C and D from *Vernonia arborea*, synthesis of analogues of zaluzanin D and their anticancer studies

We wished to undertake systematic chemical examination of *Vernonia arborea* (family Asteraceae), an endemic plant of the Western Ghats for its bioactive constituents. The genus Vernonia has been reported to a rich source of several bioactive secondary metabolites. We carried out extraction of *Vernonia arborea* leaves and undertook chemical examination of petroleum extract of the leaves. We isolated two sesquiterpene lactones from the petroleum extract which were identified as zaluzanin C and D using spectroscopic techniques.[9] Further, we confirmed the structure of zaluzanin D by its single crystal x-ray analysis as shown in below in **Figure 5**.

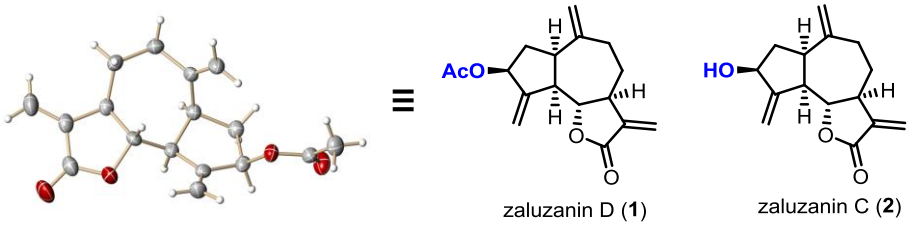
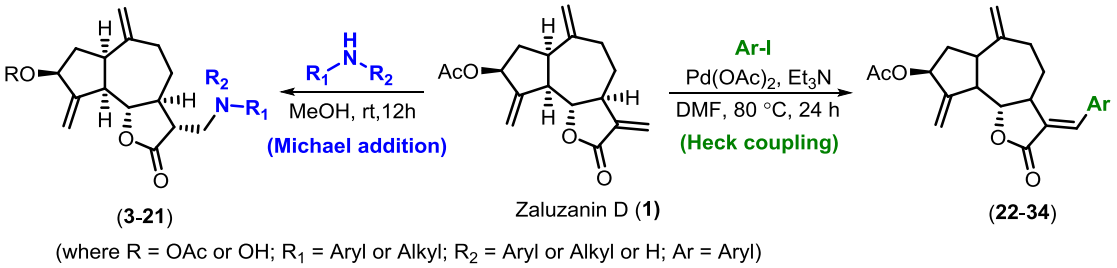


Figure 5. Structures of zaluzanin C and D.

Since the amino analogs of sesquiterpene lactones are known for their increased cytotoxicity,[10] we synthesized several C-N bond amino analogs of zaluzanin D using Michael addition by reacting with different aliphatic or aromatic amines. Further, C-C bond analogs of zaluzanin D were also synthesized using Heck coupling reaction by reacting with different aryl iodides as shown below in **Scheme 3**.[11] The synthesized analogues were assayed for their anticancer activities against human breast cancer cell lines, MCF7, MDA-MB-231 and MCF10A.



Scheme 3. Synthesis of analogs of zaluzanin D using Michael and Heck reactions.

Noteworthy Findings:

1) Design and synthesis of artemisinin-dipeptidyl vinyl phosphonate and sulfonate hybrid molecules as novel antimalarial agents.
2) Synthesis of norepinephrine alkaloid, (+)-syncarpamide and its analogs employing Sharpless asymmetric dihydroxylation (SAD) of 3,4-dimethoxy styrene.
3) Synthesis of antiepileptic drug (*R*)-lacosamide using chiral pool strategy from L-serine.
4) Isolation of sesquiterpene lactones zaluzanin C and D from *Vernonia arborea*, synthesis of C-N bond analogs of zaluzanin D using Michael addition reaction and C-C bond analogs of zaluzanin D using Heck reaction and anticancer studies of synthesized analogs.

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Chapter 1

Design and Synthesis of Artemisinin-Dipeptidyl Vinyl Phosphonate Hybrid Molecules as Novel Antimalarial Agents

Design and Synthesis of Artemisinin-Dipeptidyl Vinyl Phosphonate Hybrid Molecules as Novel Antimalarial Agents

1.1. INTRODUCTION

Malaria is a disease caused by the protozoan parasites of the genus *Plasmodium*, which is transmitted to humans by the bites of female *Anopheles* spp. mosquitoes. Species of *Plasmodium* named *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* are responsible for the spread of the disease in humans. Among them, *P. falciparum* is the most dangerous and is the cause of most of the deaths. The parasite *P. falciparum* once enters the human body, first attacks the liver where it multiplies and then invades the red blood cells. According to the latest World Malaria Report 2017, more than 216 million cases of malaria were reported leading to approximately 445,000 deaths worldwide in the year 2016.[1] In India alone 331 malaria deaths were reported in 2016, making it the highest in the entire Southeast Asia region. The worldwide distribution of malaria disease in various countries is shown in **Figure 1**.[2]

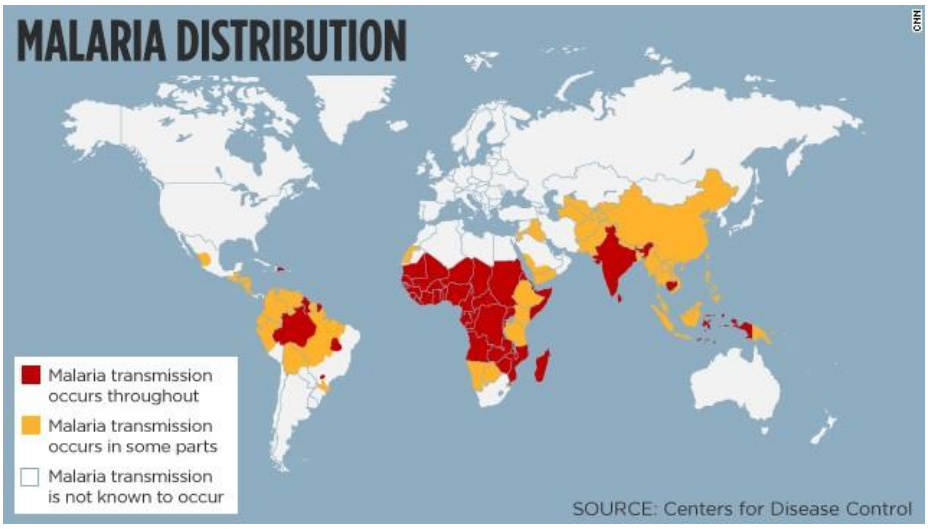


Figure 1. Worldwide distribution of malaria disease.[2]

The different antimalarial drugs used for the treatment of malaria include sulfadoxine, pyrimethamine, proguanil, quinine, chloroquine, primaquine, pamaquine, tafenoquine, mefloquine, atovaquone, bulaquine, tafenoquine, amodiaquine, pyronaridine, piperaquine,

halofantrine, lumefantrine, pyronaridine, tetracycline, doxycycline, artemisinin and its semisynthetic derivatives, such as artemether and artesunate. Some of the structures of these antimalarial drugs are shown in **Figure 2**.

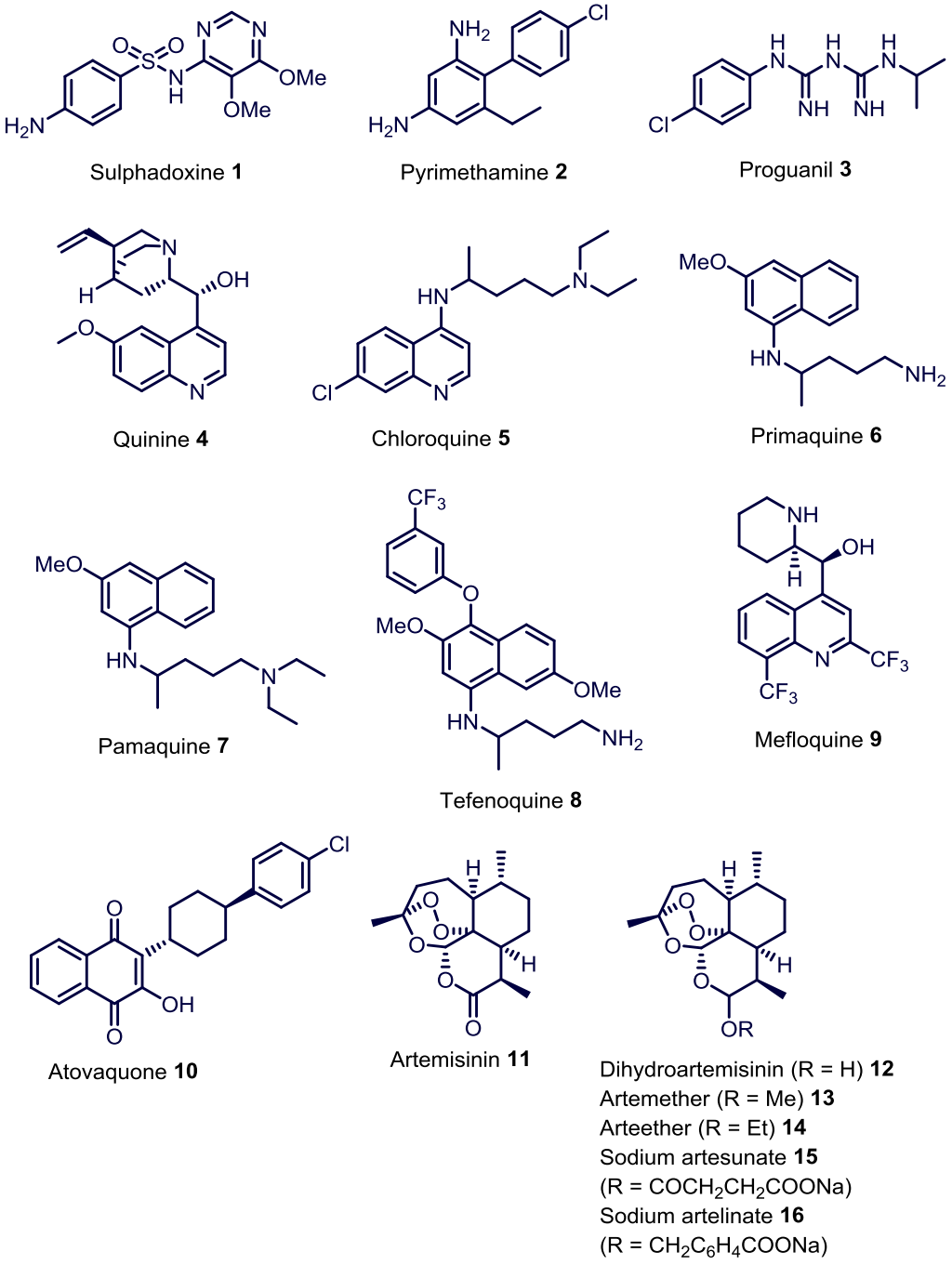


Figure 2. Structures of selected antimalarial drugs.

1.2. REVIEW OF LITERATURE

1.2.1. Artemisinin and its derivatives

Artemisinin **11** (qinghaosu) is a sesquiterpene lactone endoperoxide isolated from the Chinese medicinal plant *Artemisia annua* (sweet wormwood), a herb employed in the traditional Chinese system of medicine. It was discovered in 1972 by Prof. Youyou Tu, a Chinese scientist who shared half of the 2015 Nobel Prize in Medicine for her discovery of artemisinin.[3] The final structure of artemisinin **11** was established in 1977 by NMR and its single crystal X-ray studies.[4] It contains an unusual endoperoxide bridge, which is responsible for the mechanism of action of the drug (**Figure 2**).

It is superior in plasmocidal and blood schizontocidal activities compared to other class of antimalarial drugs such as chloroquine and quinine against malaria. Artemisinin **11** is found to be active at nanomolar concentrations *in vitro* against chloroquine-sensitive as well as chloroquine-resistant strains of *P. falciparum*. However, artemisinin has poor pharmacokinetic profile due to the poor solubility either in oil or water,[5a] high rate of parasite recrudescence after treatment,[5b] short-plasma half-life (3-5 h) and poor oral activity.[5c] This prompted many researchers to design and synthesize various derivatives which can overcome these shortcomings of artemisinin **11**. Some of these derivatives are shown in **Figure 2**. The carbonyl group of artemisinin **11** can easily be reduced to lactol, dihydroartemisinin **12** in high yields using sodium borohydride and MeOH at 0 °C. The dihydroartemisinin **12** was subsequently converted to a series of semi-synthetic analogs including the oil-soluble artemether **13** and arteether **14** and water-soluble sodium artesunate **15** and sodium artelinate **16**. These derivatives were found to be very potent antimalarial drugs effective against chloroquine-resistant strains of *P. falciparum*.[6] These artemisinin derivatives are exceptionally fast acting against intra-erythrocytic asexual blood-stage malarial parasite affecting up to 10000 fold reductions in parasite burden every 48 h.

1.2.2. Artemisinin Combination Therapy (ACT) [9,10]

The inherent disadvantage of the artemisinin derivatives (such as dihydroartemisinin **12**, artemether **13**, arteether **14**, artesunate **15** and sodium artelinate **16**) is their very short *in vivo* half-lives (typically ~1 h in humans).[7] Also, the World Health Organization

discouraged the use of single-drug artemisinin drugs (artemisinin monotherapy) to prevent malaria parasites from developing resistance to the drug.[8]

As a result, artemisinin derivatives are combined with longer half-life partner drugs, such as lumefantrine, amodiaquine, piperaquine, mefloquine, sulphadoxine-pyrimethamine or pyronaridine to remove the residual parasites (Artemisinin Combination Therapies).[9,10] These combinations help to prevent recrudescence (which can occur even after five days of artemisinin monotherapy), and hence they are currently recommended by WHO to slow the development of parasite resistance.

The ACTs recommended by the WHO are artemether/lumefantrine, artesunate/amodiaquine, artesunate/mefloquine, dihydroartemisinin/piperaquine, artesunate/pyronaridine and artesunate/sulfadoxine–pyrimethamine.

1.2.3. Antimalarial drug resistance[11-20]

The resistance in *P. falciparum* has emerged against all the available antimalarial drugs throughout the world.[11] The synthetic antimalarial drug chloroquine (CQ) was initially used in the 1950s to treat, prevent and eradicate malaria worldwide. However, resistance to CQ is widespread throughout the world which led to re-emergence of malaria and the spread of CQ-resistant parasite in Southeast Asia and South America.[12] Due to the resistance to CQ, CQ-resistant parasite was spread to Africa in 1980s and resulted in 2-3 fold increase in malaria-related deaths.[13] Hence, CQ was replaced with the two-drug combination, sulfadoxine/pyrimethamine (SP) as the first line of treatment for malaria. But resistance to SP is widespread in Asia, South America and also in Africa.[14] When the parasites developed resistance against these drugs, these were replaced by the more expensive mefloquine drug monotherapy. However, resistance soon developed for mefloquine in some areas of South East Asia, the Amazon region of South America and sporadically in Africa.[15] Since then use of two or more drugs has been widely practiced for treating uncomplicated *falciparum* malaria in all areas rather than using single antimalarial drug preferably with an artemisinin derivative as one of the partner drugs (Artemisinin Combination Therapies). [9,10]

Resistance to artemisinin and artemisinin combination therapy (ACT)[16-20]

Unfortunately over the last decade resistance against artemisinins has emerged in *P. falciparum* malaria and spread within Southeast Asia (Myanmar, Thailand, Cambodia, Vietnam, and Laos).[16-18] Clinically, resistance to artemisinins is defined as a slower rate of parasite clearance in patients treated with an artemisinin derivative or an ACT. Resistance to artemisinin was first reported as a 100-fold reduction in parasite clearance rate in Pailin, Western Cambodia in 2009.[19] It is known from the past decades that CQ-resistant *P. falciparum* strains spread to India from Southeast Asian countries through the northeastern states. Now, the artemisinin-resistant strain has been spreading from Southeast Asia to other parts of the world. If artemisinin-resistant malaria does spread to or emerge in India, the human health consequences will be immense. India is already experiencing declining efficacy of its currently recommended first-line ACT (artesunate-sulfadoxine-pyrimethamine) in the northeastern states of the country.[20] Due to the continuous emergence of *P. falciparum* resistance to all available antimalarial drugs, there is an urgent need to develop new antimalarial agents with a different mode of action.

1.2.4. Drug Target for Malaria: Falcipain-2 (FP-2)

Malaria parasite degrades most of the host cell hemoglobin which is essential to the survival of the parasite.[21] Degradation of hemoglobin by the malarial parasites produces "heme" and "globin fragments" which will be further degraded to small amino acids. These amino acids resulting from hemoglobin degradation are transferred to parasite proteins or utilized for energy metabolism of malarial parasites.[22] The release of heme is toxic to parasite itself and causes membrane damage due to its peroxidative properties[23] and hence parasites convert heme into an insoluble crystalline form called hemozoin (also called malaria pigment).[24a] The formation of hemozoin is essential to the survival of parasites. Antimalarial drugs, such as chloroquine and mefloquine are believed to kill malaria parasites by inhibiting hemozoin biocrystallization.[24b]

The actual process of hemoglobin degradation is carried out by different proteases present within the malaria parasite (**Figure 3**). Aspartic proteases named plasmepsins I, II and IV and histo-aspartic protease (HAP) participate in hemoglobin degradation[25] to release heme moiety and globin fragments followed by further degradation of globin fragments by

cysteine proteases named falcipains-2, -2', and -3.[26] However, the precise sequence of events particularly whether a plasmepsin or a falcipain initiates the degradation step is still unclear.[27] Further degradation of oligopeptides by a metalloprotease (falcilysin)[28] and dipeptidyl amino- peptidase 1 (DPAP1)[29] yields small peptides. Finally, small peptides are then pumped out of the food vacuole into the cytoplasm and an amino peptidase activity provides amino acids essential for parasites survival.[30] As shown in **Figure 3**, the degradation process follows an ordered pathway. Therefore, blocking the process or inhibiting any of these enzymes would be lethal to the parasite. It can be concluded from the literature that the inhibition of cysteine proteases (*i.e.,* falcipains) could be considered as an ideal drug target for the development of new antimalarial drugs.[31-40]

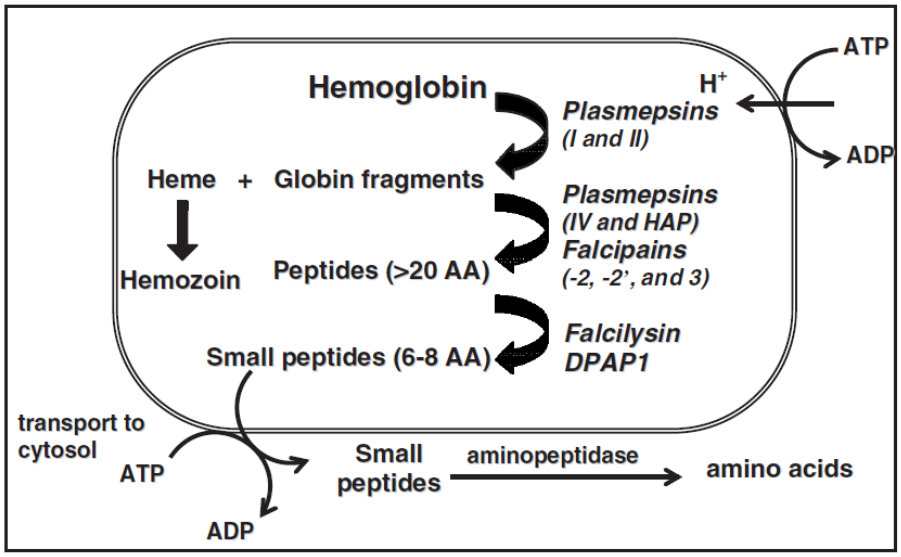


Figure 3. Hemoglobin degradation pathway in *Plasmodium* food vacuole.[40b]

Several falcipain-2 inhibitors have been synthesized in literature[32-40] and evaluated as potential antimalarial agents such as peptidyl fluromethyl ketones,[32] peptidyl vinyl sulfones,[33] peptidyl aldehydes,[34] peptidyl epoxides,[35] peptidomimetics based on a 1,4-benzodiazepine scaffold,[36] chalcones,[37] isoquinolines,[38] thiosemicarbazones,[39] peptidyl vinyl sulfonamide and sulfonate esters, vinyl ketones, amides, esters and nitriles, phenothiazines, pyridines and peptidyl aziridines (**Figure 4**).

However, the use of many falcipain-2 inhibitors (such as peptide-based FP-2 inhibitors) as therapeutic agents is limited due to some of the following properties of falcipain-2 inhibitors.

(i) Poor pharmacological profile; (ii) Poor absorption through cell membranes; (iii) Susceptibility to protease degradation; (iv) Limited bioavailability; (v) Problems with the formulation.

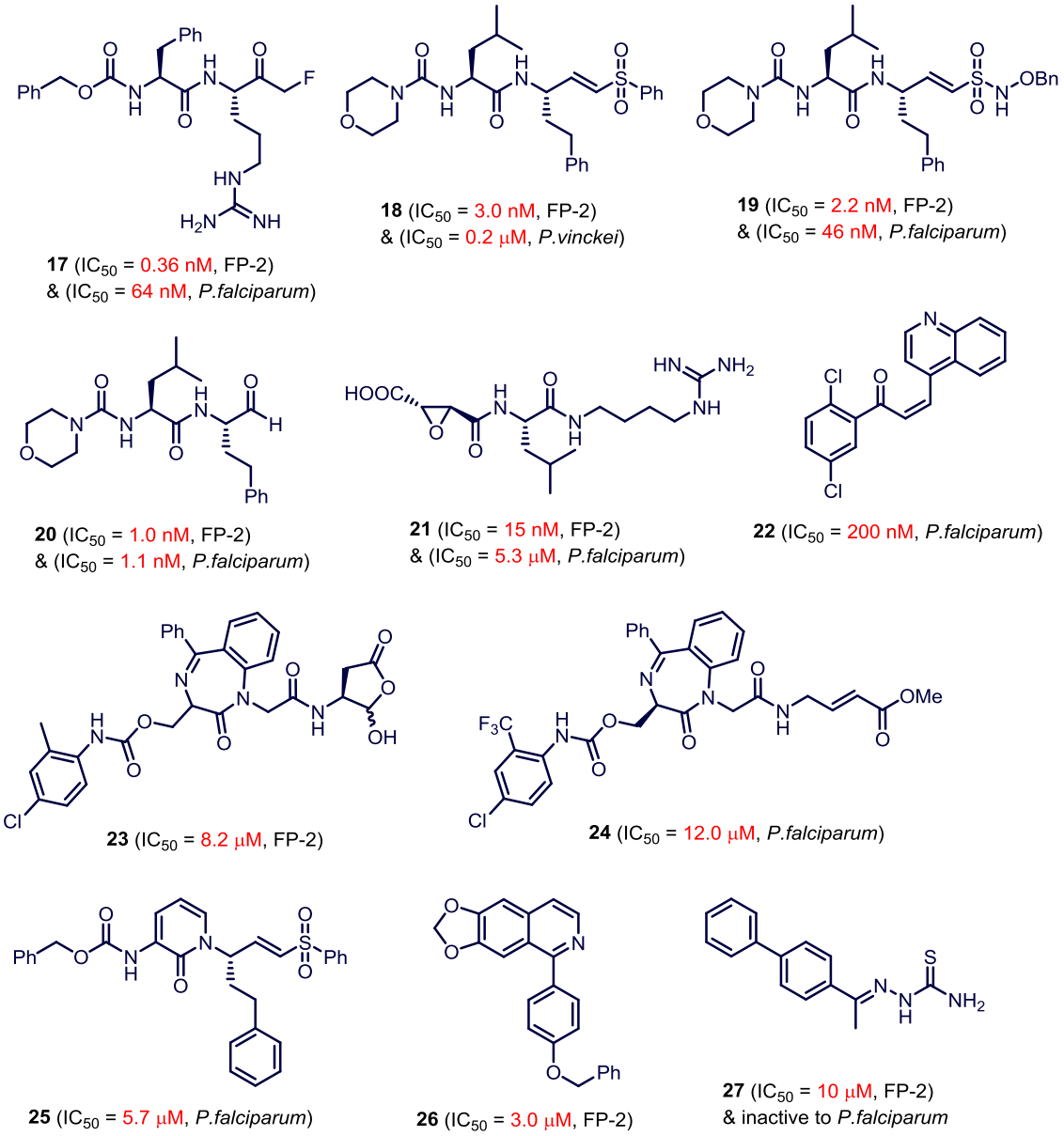


Figure 4. Some of the reported inhibitors of the falcipain-2 enzyme.

1.2.5. General inhibition mechanism of cysteine protease (FP-2) inhibitors

Falcipain-2 inhibitors in principle require hydrophobic interaction in P_1, P_2 pockets and an electrophilic (or) an alkylation centre, which behaves as a Michael acceptor (**Figure 5**).[31a,31i,31j,33a,40] FP-2 inhibitors such as vinyl sulfones inactivate the enzyme by irreversible addition of the thiol group of active site Cys42 to the electrophilic double bond of vinyl

sulfone moiety. The obtained negative charge at the α-carbon is eliminated *via* protonation by the histidinium residue[40b] (**Figure 6**).

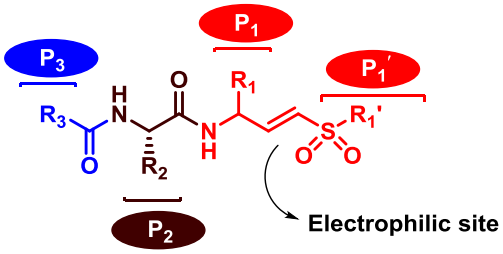


Figure 5. General structure of the peptidyl vinyl sulfones containing an amide linkage between the R_3 substituent and the peptide residue. R_1 and R_2 represent the side chains of the amino acids (P_1 and P_2).

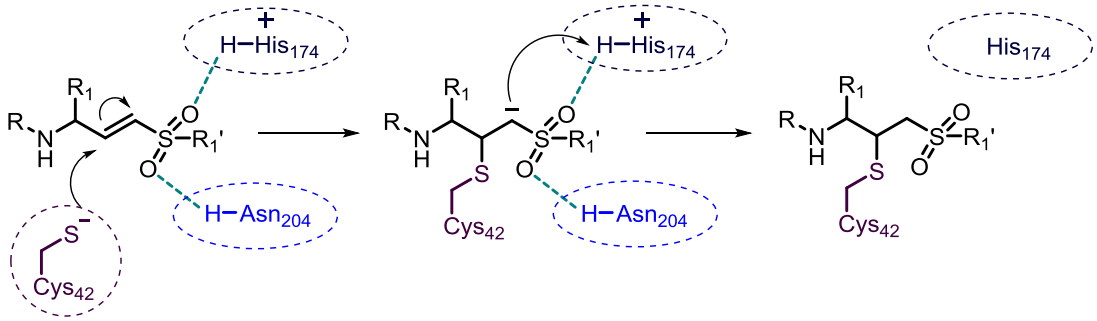


Figure 6. Mechanism of inhibition of the FP-2 enzyme by vinyl sulfones.

1.2.6. Hybrid molecules

Hybrid molecules can be defined as chemical entities with two or more distinct natural or unnatural moieties having a different biological functions and dual activity suggesting that a hybrid molecule acts as two distinct pharmacophores.[41] Synthesis of hybrid molecules[42] is a new promising approach in the development of lead molecules for the medicinal and pharmaceutical industry as the biological activities of hybrid molecules is superior to that of individual parent compounds. Another advantage of this concept over a combinatorial chemistry approach is that it may provide possibilities to generate a diverse group of new type of molecules for applications in biology. In the modern combinatorial chemistry[43] millions of new compounds can be synthesized in a relatively short period and then evaluated for their biological activities using high-throughput screening (HTS) method.[44] However, the success of such a random approach is limited due to the lack of new chemical entities (NCEs) with high diversity. Hence the synthesis of hybrid molecules can be considered as a new approach to achieve structural diversity in compounds with different

biological activities. The use of hybrid molecules also reduces the risk of treatment failure and the partner drug may be protected from the spread of resistance.

In fact, nature already used the hybrid molecule approach to synthesize several structurally diverse "natural hybrid molecules" or "naturally occurring hybrid molecules" through various metabolic pathways. For example, nature employed the shikimic acid pathway for the synthesis of natural hybrid molecule vitamin E (**28**) (**Figure 7**). Nature also synthesizes several interesting dimeric natural product hybrids. Vinblastine (**29**) and vincristine (**30**) are the examples for natural dimeric indole alkaloids used to treat some types of cancer. The dimeric hybrids, vinblastine and vincristine consist of vindoline and catharanthine monomeric alkaloid substructures where the individual monomers do not exhibit any pronounced or useful biological activities. Cephalostatin 1 (**31**) and blepharocalyxin D (**32**) are some other examples for dimeric natural product hybrids (**Figure 7**).

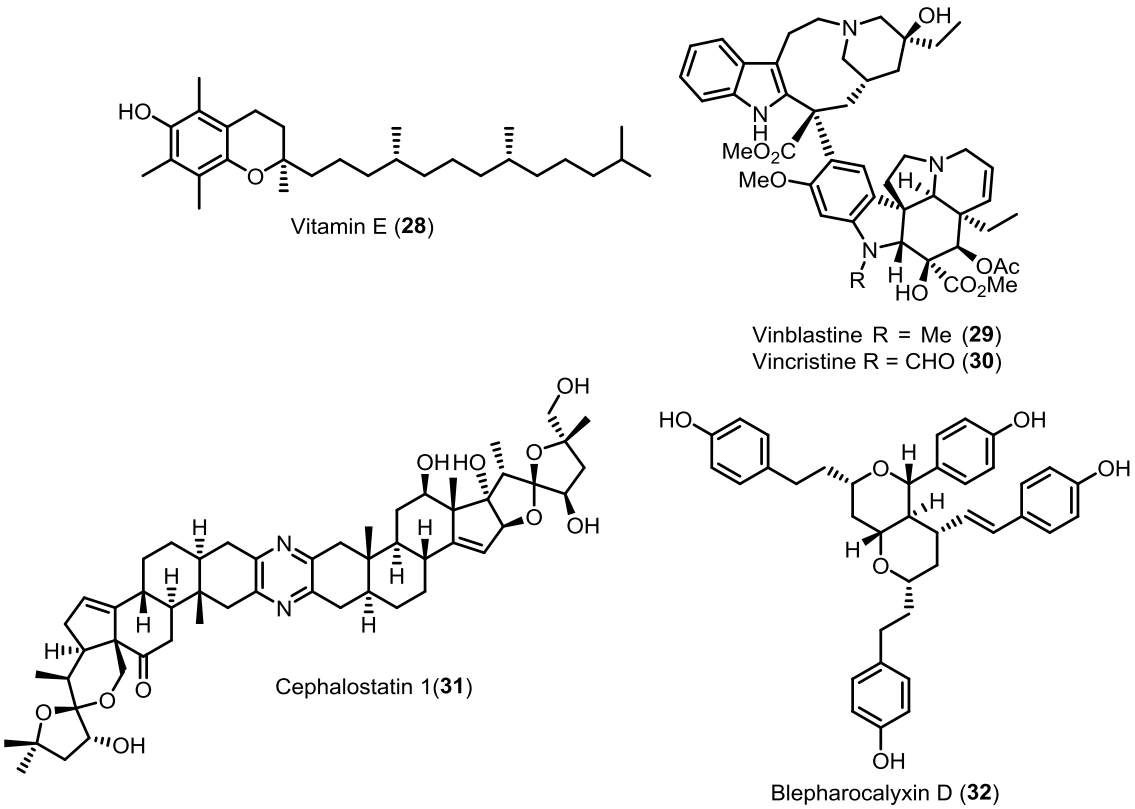


Figure 7. Examples for naturally occurring hybrid molecules.

Artificial or synthetic hybrid molecules can be synthesized by covalently joining the two individual natural or synthetic moieties through a suitable linker. Depending on the types of

linkers connecting the two individual moieties, synthetic hybrid molecules can be classified into four types (**Figure 8**).

(a) **Conjugate hybrids:** In conjugate hybrids, both the pharmacophores are joined through a metabolically stable linker which is not part of the either of the two molecules. *e.g.*, aminoquinoline-trioxane hybrid **33**.[41a]

(b) **Cleavage conjugate hybrids:** In this type, both the pharmacophores joined through a metabolically cleavable linker inside the biological system to release the two pharmacophore units that interact independently with different targets. *e.g.*, CF_3 artemisinin-mefloquine hybrid **34**.[45]

(c) **Fused hybrids:** In fused hybrids, the size of the linker is decreased/removed such that the framework of the pharmacophores is in contact. Pyrroloquinoline fused hybrid **35** was synthesized by the reaction of *o*-aminophenylpyrrole and imidazole-2-carboxaldehyde.[46] Similarly, fused hybrid **36** was synthesized by coupling L-DOPA and (*R*)-α-lipoic acid.[47]

(d) **Merged hybrids:** The two components are merged by taking advantage of the common pharmacophore in the structures which give rise to smaller and simpler hybrid molecules. Merged hybrid **37** was designed by taking the commonalities of the 4-aminoquinoline and 9-acridone.[48]

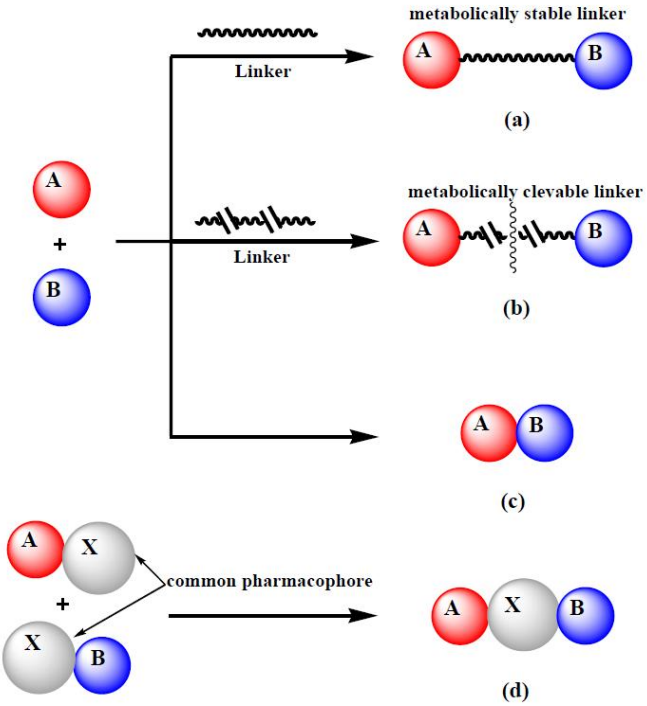


Figure 8. Classification of hybrid molecules (a) Conjugate hybrid, (b) Cleavage conjugate hybrid, (c) Fused hybrid, (d) Merged hybrid.

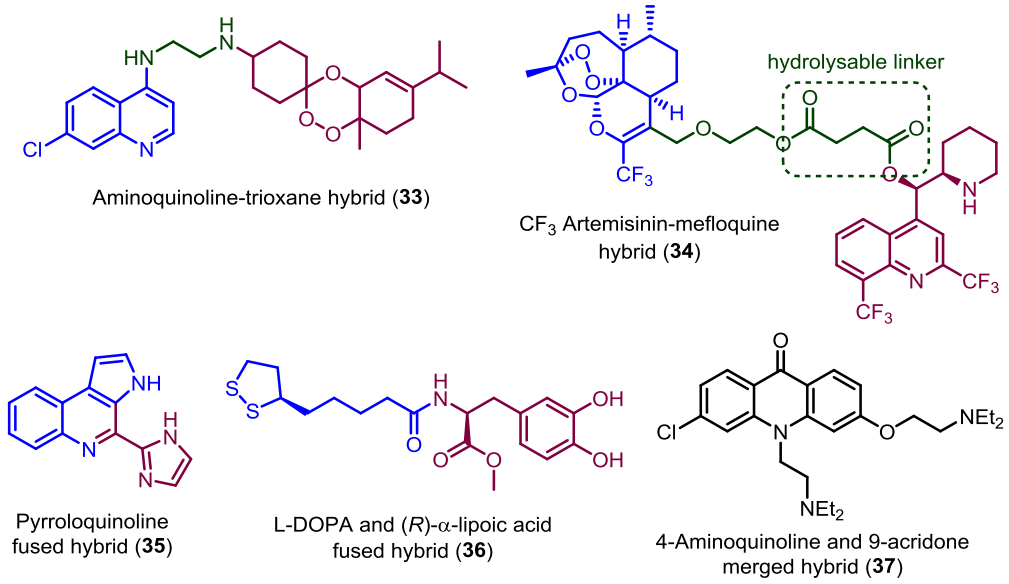


Figure 9. Examples of the various types of hybrid molecules.

The design of structurally diverse hybrid molecules has been receiving increasing attention in the field of medicinal chemistry from the past two decades.[42] The concept of hybrid molecules has been utilized by several research groups to develop a diverse class of compounds to treat several diseases such as cancer, AIDS, diabetes, tuberculosis and is now gaining momentum in the field of antimalarial drug discovery.[49]

Advantages of antimalarial hybrid drugs over currently used combination therapy for malaria treatment

In the treatment of malaria utilizing drug combination therapy, two or more agents are co-formulated into a single tablet termed as a multicomponent drug. Compared to the currently used combination therapy, hybrid drugs may be less expensive with a lower risk of drug-drug adverse interactions compared to multicomponent drugs. A hybrid drug possesses a single pharmacokinetic profile which becomes easy to predict and control[41,50] and hence superior to the standard combination therapy.[51] It is believed that hybrid drugs may be absorbed, distributed, metabolized and excreted at one single rate.[52] Also, pharmacokinetic properties can be controlled by the linkage moiety.[53] The use of hybrid molecules improves pharmacokinetic profile and activity against resistant strains compared to that of combination therapy but have the disadvantages of less flexible administration.

Walsh *et al.* synthesized an artemisinin-quinine hybrid **38** (**Figure 10)** by coupling dihydroartemisinin to the carboxylic acid derivative of quinine *via* an ester-linkage.[49a] The hybrid **38** exhibited potent *in vitro* activity against sensitive and resistance strains of *P. falciparum* than that of quinine and artemisinin alone.

The most important challenge when designing hybrid compounds is to overcome issues associated with drug resistance, pharmacokinetics, potency, solubility, metabolism, mode of administration and toxicity.[51] The fact is that only one synthetic aminoquinoline-trioxaquine hybrid molecule **41** (PA1103/SAR116242) has reached the clinical trials.[54] However, it was later abandoned in preclinical development. Hence the development of novel hybrid molecules with a different mode of action using different drugs or pharmacophores remains one of few remaining resources as there is a lack of effective treatment for malaria.

Several antimalarial hybrid molecules have been synthesized in literature[33d,49] by using different drugs or pharmacophores and some of the examples are shown in **Figure 10**.

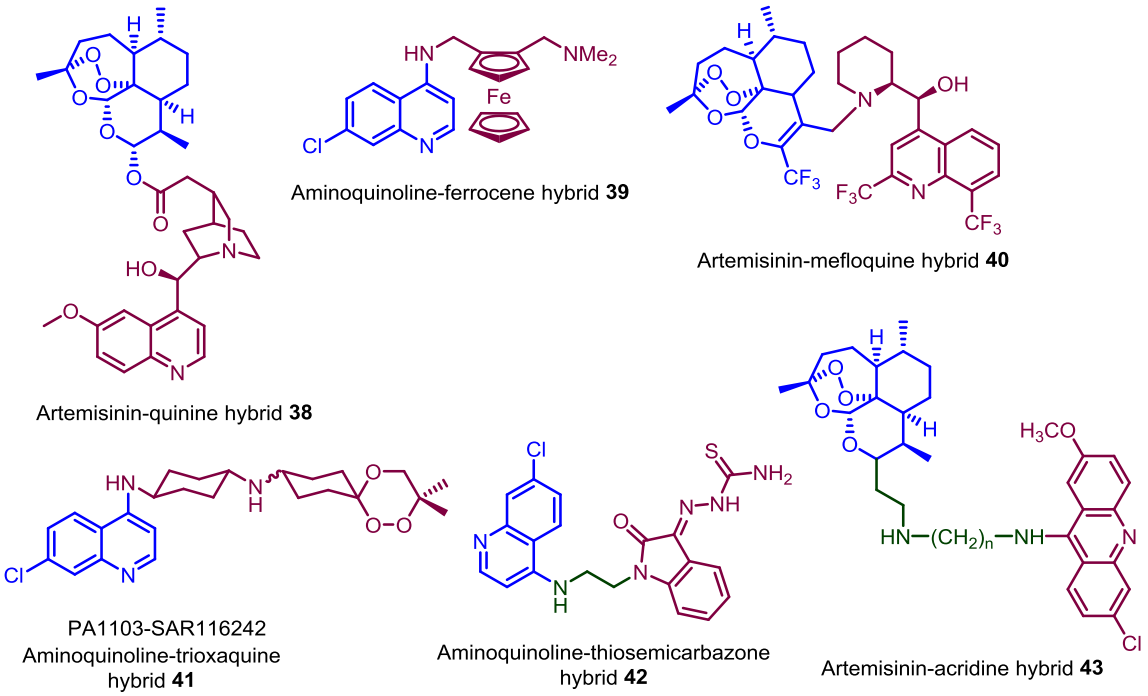


Figure 10. Some of the reported antimalarial hybrid molecules.

1.3. PRESENT WORK

Objective

As part of our ongoing research on synthesizing novel antimalarial agents having a different mode of action, we wished to synthesize antimalarial hybrid molecules targeting falcipain-2 protease enzyme. Although several peptidyl vinyl sulfones as FP-2 enzyme inhibitors have been reported,[33] vinyl phosphonates as FP-2 inhibitors have not yet been extensively studied. Ettari *et al.* in 2008 synthesized benzodiazepine-based vinyl and allyl phosphonates as FP-2 inhibitors.[55a] Bhattacharya *et al.* had reported peptidyl vinyl phosphonates as cysteine protease inhibitors.[55b]

Therefore, the present work describes the design and synthesis of artemisinin-peptidyl vinyl phosphonate hybrid molecules as novel antimalarial agents targeting falcipain-2 protease enzyme. Further, the synthesized hybrids are assayed for their *in vitro* and *in vivo* antimalarial activities against falcipain-2 and various strains of *P. falciparum*.

1.4. RESULTS AND DISCUSSION

1.4.1. Design of artemisinin-dipeptidyl vinyl phosphonate hybrid molecules

Since falcipain-2 inhibitors in principle would require the hydrophobic interaction in P_1 and P_2 pockets and essentially electrophilic center at the active site of the enzyme, [31a,31i,31j,33a,40] we visualized that these requirements could be ideally fulfilled by artemisinin-peptidyl vinyl phosphonate hybrid molecules as shown in **Figure 11**. The lactol group (the cyclic equivalent of a hemiacetal or ketal) present in dihydroartemisinin allowed us to design the hybrid molecules by combing it with other suitable molecules through the linkers.

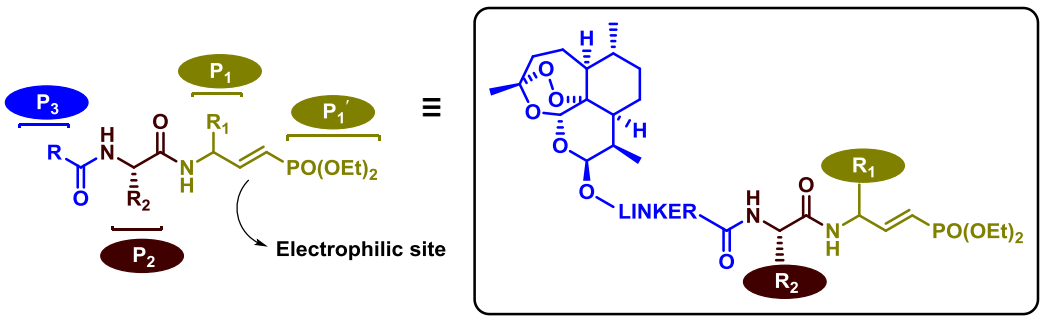


Figure 11. Design of artemisinin-dipeptidyl vinyl phosphonate hybrid molecules.

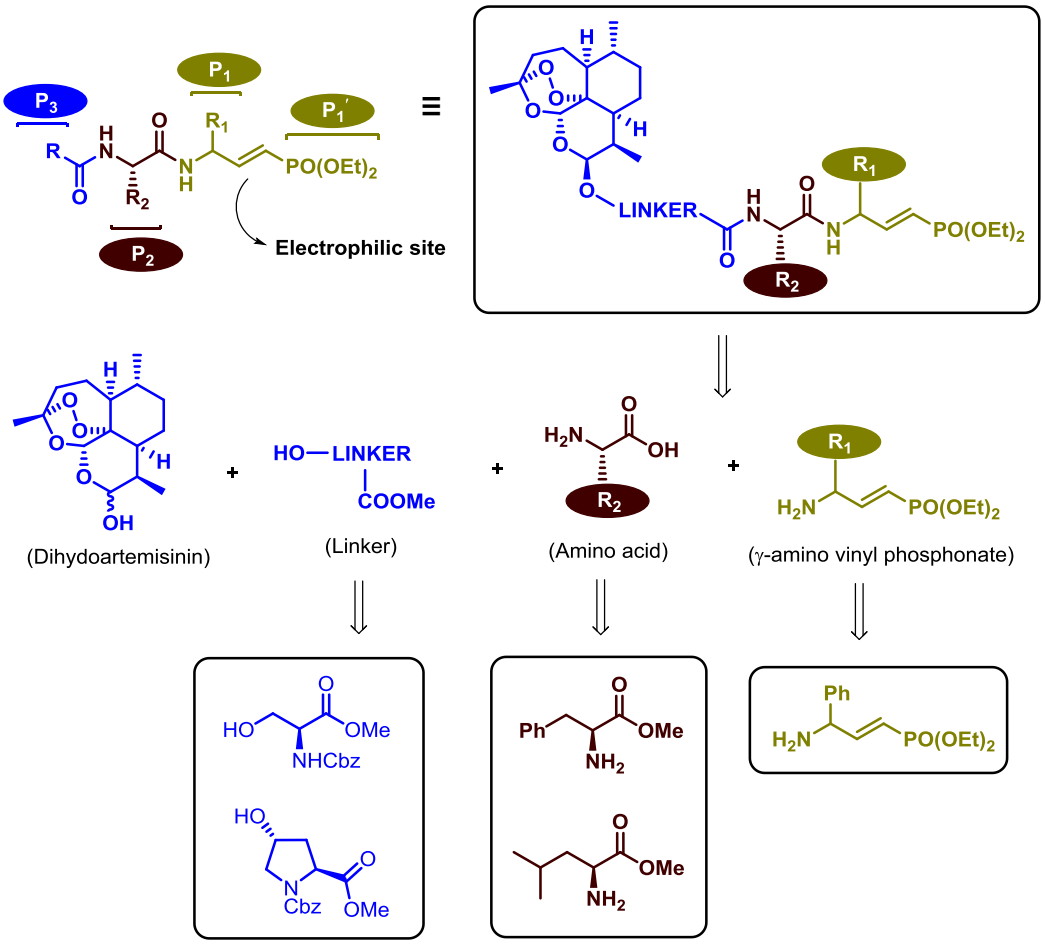
Retrosynthesis

As shown in **Scheme 1**, we envisioned artemisinin-dipeptidyl vinyl phosphonate hybrid molecules could be synthesized by the following steps:

Step 1: Attaching dihydroartemisinin with an amino acid based linker using an etherification reaction to obtain artemisinin monopeptide. A linker could be any compound that has one free hydroxyl group and some functionality which could be easily converted to free acid *e. g.* methyl ester.

Step 2: Coupling of the free acid of the obtained artemisinin monopeptide with amino acid methyl ester using DCC and HOBT to obtain artemisinin dipeptide.

Step 3: Coupling of the free acid of the obtained artemisinin dipeptide with γ-amino vinyl phosphonate using DCC and HOBT to obtain artemisinin dipeptidyl hybrid molecule (or artemisinin-dipeptidyl vinyl phosphonate hybrid molecule).



Scheme 1. Retrosynthesis of artemisinin-dipeptidyl vinyl phosphonate hybrid molecules.

In **Scheme 1**, R_1 represents the substituent of γ-amino vinyl phosphonate required for P_1 site and phosphonate group of γ-amino vinyl phosphonate was chosen for P_1' site required for falcipain-2 inhibitors, R_2 represents the side chain of amino acid required in P_2 site, and artemisinin attached linker (product obtained from **Step 1**) comprises P_3 site required for falcipain-2 inhibitors.

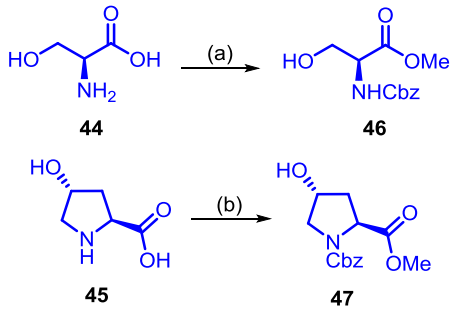
N-Cbz protected L-serine, and *trans*-4-hydroxy L-proline methyl esters were selected as linkers. Both the linkers possessed one hydroxyl group and one methyl ester group, which can be later converted into a free carboxylic acid. The study of cysteine protease inhibitors revealed that phenylalanine and leucine were found to be the best among all the amino acid residues at R_2 (See section **1.2.4.** and **1.2.5.**) and hence, phenylalanine and leucine methyl esters were chosen for the amide coupling to exert an effective binding in the P_2 pocket. Finally, γ-phenyl-γ-amino vinyl phosphonate was chosen for the final amide coupling.

1.4.2. Synthesis of artemisinin-peptidyl vinyl phosphonate hybrid molecules

As depicted in **Scheme 1**, the synthesis of hybrid molecules requires dihydroartemisinin, linkers, amino acid methyl esters and γ-phenyl-γ-amino vinyl phosphonate. The linear synthesis using these four units would provide the required hybrid molecules. Dihydroartemisinin was synthesized by the reduction of artemisinin with $NaBH_4$ and MeOH following the reported literature procedure.[56]

1.4.2.1. Synthesis of linkers

The linkers *N*-Cbz protected L-serine **46** and *trans*-4-hydroxy L-proline methyl ester **47** were synthesized from their corresponding free amino acids L-serine **44** and *trans*-4-hydroxy L-proline **45**, respectively by following the literature procedures[57] (**Scheme 2**).

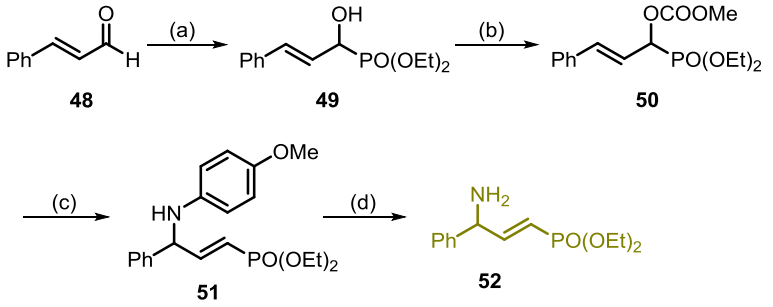


Scheme 2. Synthesis of linkers **46** and **47**. *Reagents and conditions:* (a) (i) $SOCl_2$, MeOH, 25 °C, 24 h, 98%; (ii) CbzCl, $NaHCO_3$, DCM, 97%; (b) (i) $SOCl_2$, MeOH, 45 °C, 18 h; (ii) CbzCl, $NaHCO_3$, dioxane/H_2O (1:1), 25 °C, 21 h, 100%.

1.4.2.2. Synthesis of γ-phenyl-γ-amino vinyl phosphonate 52

1.4.2.2.1. Synthesis of 52 using Tsuji-Trost reaction

Bhattacharya *et al*.[55b] reported the first synthesis of γ-phenyl-γ-amino vinyl phosphonate **52** starting from cinnamaldehyde **48** employing Tsuji-Trost reaction as a key step (**Scheme 3**). Cinnamaldehyde **48** was treated with diethyl phosphite to afford hydroxy phosphonate **49** in 77% yield. The hydroxyl group of **49** was converted to carbonate derivative **50** which was then subjected to Tsuji-Trost reaction conditions with *p*-anisidine to afford compound **51**. Final deprotection of *p*-methoxy phenyl (PMP) group was achieved by treating with trichloroisocyanuric acid (TCCA) in acidic medium to furnish **52** as its hydrochloride salt.



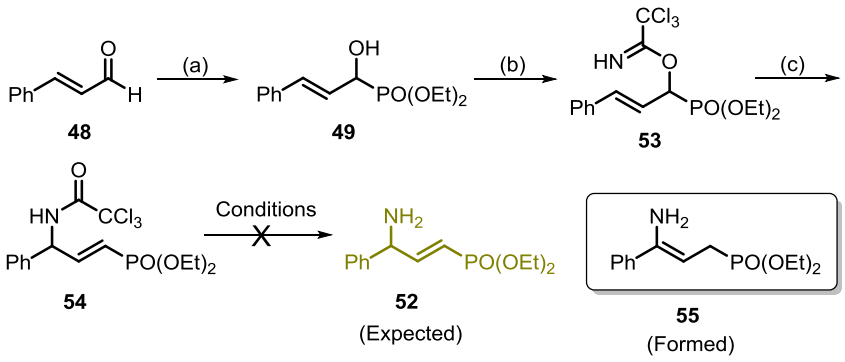
Scheme 3. Synthesis of (±)-γ-amino vinyl phosphonate **52** using Tsuji-Trost reaction.[55b] *Reagents and conditions:* (a) Diethyl phosphite, Et_3N, DCM, 0 °C to rt, 3 h, 77%; (b) MeOCOCl, Py, MeCN, 0 °C to rt, 12 h, 65%; (c) *p*-anisidine, $Pd(OAc)_2$, PPh_3, THF, rt, 1 h, 71%; (d) TCCA, MeCN/H_2O (1:1), 1M aq H_2SO_4, 12 h, rt, 21%.

The low yield in final deprotection step and use of $Pd(OAc)_2$ catalyst in Tsuji-Trost reaction allowed us to search for an alternate route for the synthesis of vinyl phosphonate **52**. Moreover, it is difficult to synthesize the enantiomers of **52** (*i.e.* (*R*)-**52** and (*S*)-**52**) with high enantioselectivities employing the asymmetric version of Tsuji-Trost reaction.

1.4.2.2.2. Synthesis of 52 using Overman rearrangement

The Overman rearrangement is the conversion of allylic alcohols into allylic amines involving the rearrangement of an allylic trichloroacetimidate to an allylic trichloroacetamide through an imidate intermediate.[58] Since Overman rearrangement yields allylic trichloroacetamides, we thought of deprotection of allylic trichloroacetamide **54**

would give the required allylic amine **52**. As shown in **Scheme 4**, allylic alcohol (hydroxy phosphonate) **49** was treated with trichloroacetonitrile under basic conditions at −35 °C to yield allylic trichloroacetimidate **53** in 98% yield. Imidate **53** underwent rearrangement on refluxing with toluene to furnish allylic trichloroacetamide **54** in 92% yield with the exclusive formation of the *E*-double bond. Several conditions were employed (acidic and basic conditions, see **Table 1**) to deprotect the trichloroacetamide group, which resulted in the formation of free amine **55** with unwanted isomerization of the double bond.



Scheme 4. *Reagents and conditions:* (a) Diethyl phosphite, Et_3N, DCM, 0 °C to rt, 3 h, 77%; (b) CCl_3CN, DBU, DCM, −35 °C, 30 min, 98%; (c) toluene, reflux, 24 h, 92%.

Table 1. Different conditions used for the deprotection of allylic trichloroacetamide **54**.

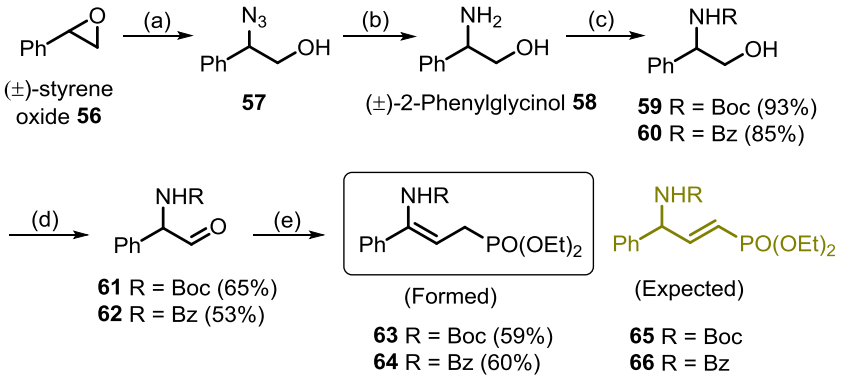
S. No	Conditions	Product formed
1	AcOH, H_2O, 80 °C, 12 h	**55**
2	30 % NaOH, EtOH, 80 °C, 3 h	**Complex mixture**
3	3N KOH, EtOH, rt, 24 h	**55**
4	30 % NH_3 in MeOH, rt, 16 h	**55**

1.4.2.2.3. Synthesis of 52 from 2-phenylglycinol (58)

In another approach, the synthesis of **52** was planned from 2-phenylglycinol **58** keeping in mind the vinyl phosphonate moiety can be introduced *via* Wittig olefination of an α-amino aldehyde with tetraethyl methylenediphosphonate.

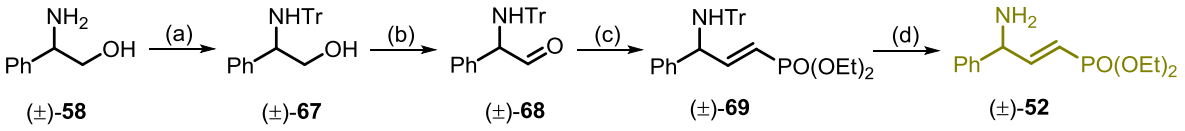
As shown in **Scheme 5**, the synthesis commenced from (±)-styrene oxide **56**. Ring opening of epoxide **56** with sodium azide in H_2O at 60 °C yielded azido alcohol **57** in 91% yield.[59] α-Azido alcohol **57** was subjected to hydrogenation under Pd/C to yield 2-phenylglycinol **58** in

99% crude yield which was used directly for the next step. Compound **58** was treated with $(Boc)_2O$ and BzCl to furnish Boc and Bz protected amino alcohols **59** and **60**, respectively. Compounds **59** and **60** were oxidized with Dess-Martin periodinane to yield the corresponding α-amino aldehydes **61** and **62**, respectively.[60] Unfortunately, Wittig olefination of both the aldehydes **61** and **62** with tetraethyl methylenediphosphonate and NaH in dry THF resulted in the unexpected double bond isomerized Wittig products **63** and **64** (enamides), respectively.



Scheme 5. *Reagents and conditions:* (a) NaN_3, H_2O, 60 °C, 3.5 h, 91%; (b) H_2, Pd/C, rt, 12 h, 99%; (c) $(Boc)_2O$ (or) BzCl, 0 °C, 3 h; (d) Dess-Martin periodinane, 3 h, rt; (e) tetraethyl methylenediphosphonate, NaH, THF, 0 °C to rt, 3 h.

To our delight when the trityl protecting group was introduced in α-amino aldehyde and performed the same Wittig olefination, isomerization of the double bond was not observed. α-Amino alcohol **58** was protected with trityl chloride to afford trityl protected amino alcohol **67** in 63% yield. Compound **67** on oxidation with Dess-Martin periodinane furnished α-amino aldehyde **68** followed by immediate Wittig olefination with tetraethyl methylene diphosphonate yielded olefin **69** in 60% over two steps without any isomerization of the formed double bond (confirmed by ^1H and ^{13}C NMR). The final deprotection of the trityl group with TFA in DCM yielded required amine (±)-**52** in 95% yield (**Scheme 6**). It should be mentioned that the amine (±)-**52** is found to be unstable and decomposes slowly at room temperature. Hence, it should be prepared and used just before the coupling reaction.



Scheme 6. Synthesis of (±)-γ-amino vinyl phosphonate **52**. *Reagents and conditions:* (a) trityl chloride, Et_3N, 25 °C, 12 h, 63%; (b) Dess-Martin periodinane, DCM, 0 °C to 25 °C, 30 min; (c) tetraethyl methylenediphosphonate, NaH, THF, 0 °C to 25 °C, 3 h, 60% over two steps; (d) TFA, DCM, 25 °C, 30 min, 95%.

Synthesis of artemisinin-peptidyl vinyl phosphonate hybrid molecules

After successfully synthesizing γ-phenyl-γ-amino vinyl phosphonate **52**, we planned to synthesize the hybrid molecules as designed previously. As sown in **Figure 12**, *"dihydroartemisinin—linker"* was allowed to react independently with vinyl phosphonate **52** and amino acid methyl ester to furnish *"dihydroartemisinin—linker—vinyl phosphonate"* hybrid molecule of type-1 and *"dihydroartemisinin—linker—amino acid"*, respectively. Further coupling of *"dihydroartemisinin—linker—amino acid"* with vinyl phosphonate **52** furnished *"dihydroartemisinin—linker—amino acid—vinyl phosphonate"* hybrid molecule of type-2.

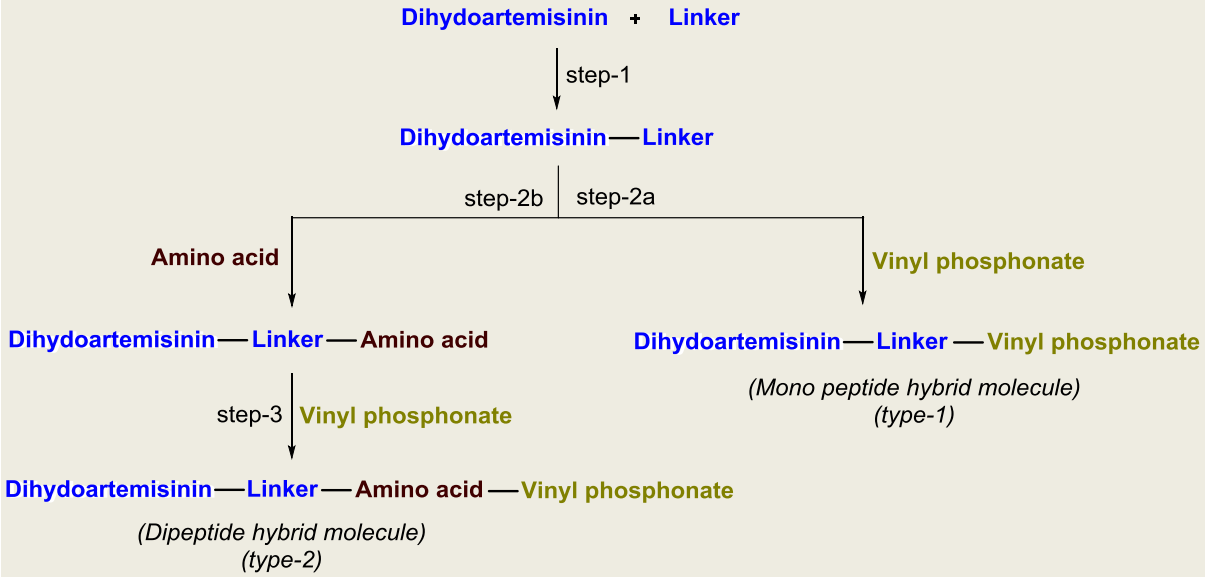
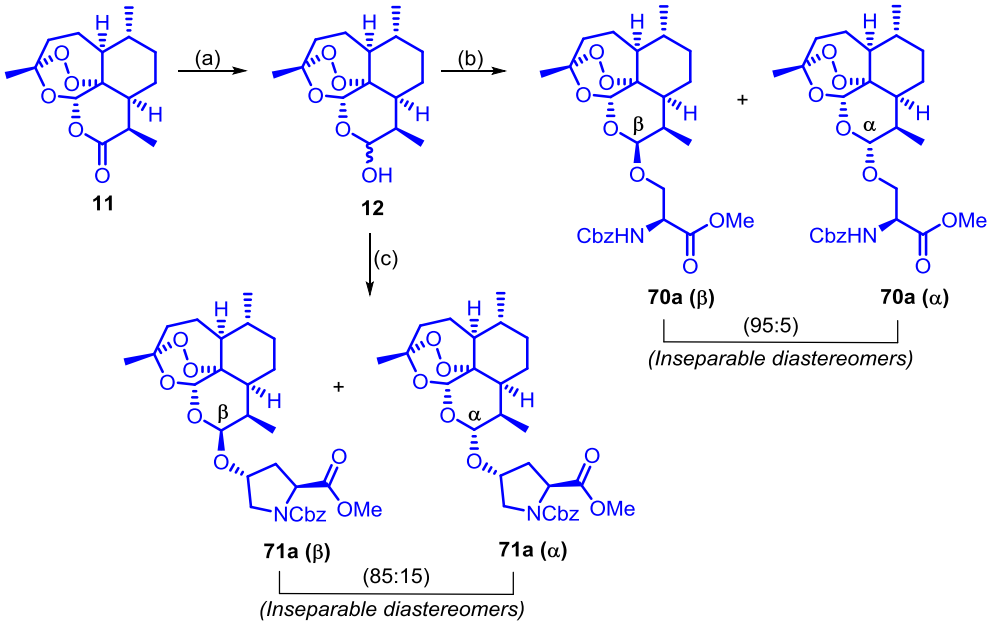


Figure 12. Schematic representation of the synthesis of hybrid molecules.

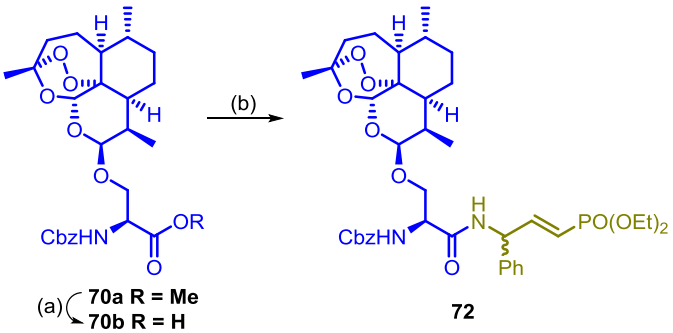
As shown in **Scheme 7**, reduction of artemisinin **11** was carried out with $NaBH_4$ and MeOH at 0 °C to afford dihydroartemisinin **12**.[56] The linkers **46** and **47** when reacted with dihydroartemisinin **12** in the presence of Lewis acid ($BF_3 \cdot Et_2O$) in dichloromethane at 0 °C furnished artemisinin derivatives **70a** and **71a**, respectively.

Compounds **70a** and **71a** were found to be an inseparable mixture of two diastereomers by column chromatography. Chiral HPLC analysis of **70a** and **71a** revealed that β-diastereomer is major than that of α-diastereomer. Compound **70a** was obtained in 95:5 (β:α) ratio and compound **71a** was obtained in 85:15 (β:α) ratio (See chiral HPLC data **Section 1.9**).



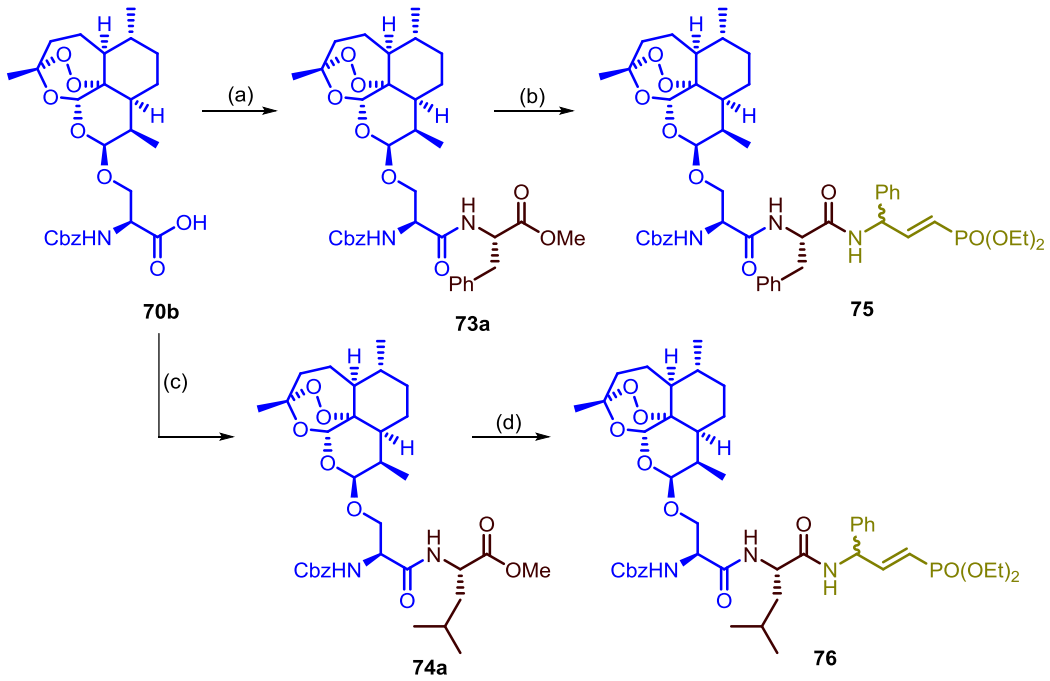
Scheme 7. Synthesis of artemisinin attached linkers **70a** and **71a**. *Reagents and conditions:* (a) $NaBH_4$, MeOH, 0 °C, 2 h, 84% (b) **46**, $BF_3{\cdot}Et_2O$, DCM, 0 °C to 25 °C, 3 h, 55%; (c) **47**, $BF_3{\cdot}Et_2O$, DCM, 0 °C to 25 °C, 3 h, 67%.

The linker attached artemisinin **70a** was then subjected to alkaline hydrolysis using 2M aq LiOH to yield the free acid **70b** which was used without any further purification for the next amide coupling reaction with racemic γ-amino vinyl phosphonate (±)-**52** under DCC and HOBT condition to furnish artemisinin-peptidyl vinyl phosphonate hybrid molecule **72** as an inseparable mixture of diastereomers (**Scheme 8**).



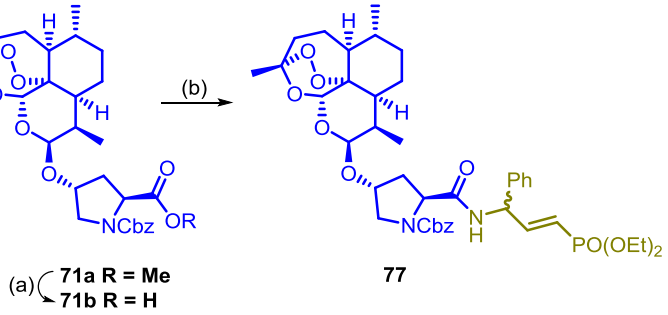
Scheme 8. Synthesis of hybrid molecule **72**. *Reagents and conditions:* (a) 2M aq LiOH, THF, 25 °C, 2 h; (b) (±)-**52**, DCC, HOBT, THF, 0 °C to 25 °C, 2 h, 79%.

Free acid **70b** obtained from the alkaline hydrolysis of **70a** was subjected to peptide coupling reactions with L-phenylalanine methyl ester and L-leucine methyl ester to afford dipeptides **73a** and **74a**, respectively which were hydrolyzed to obtain free acids of dipeptides **73b** and **74b**, respectively followed by peptide coupling reaction with (±)-**52** furnished artemisinin-dipeptidyl vinyl phosphonate hybrid molecules **75** and **76**, respectively as an inseparable mixture of diastereomers (**Scheme 9**).



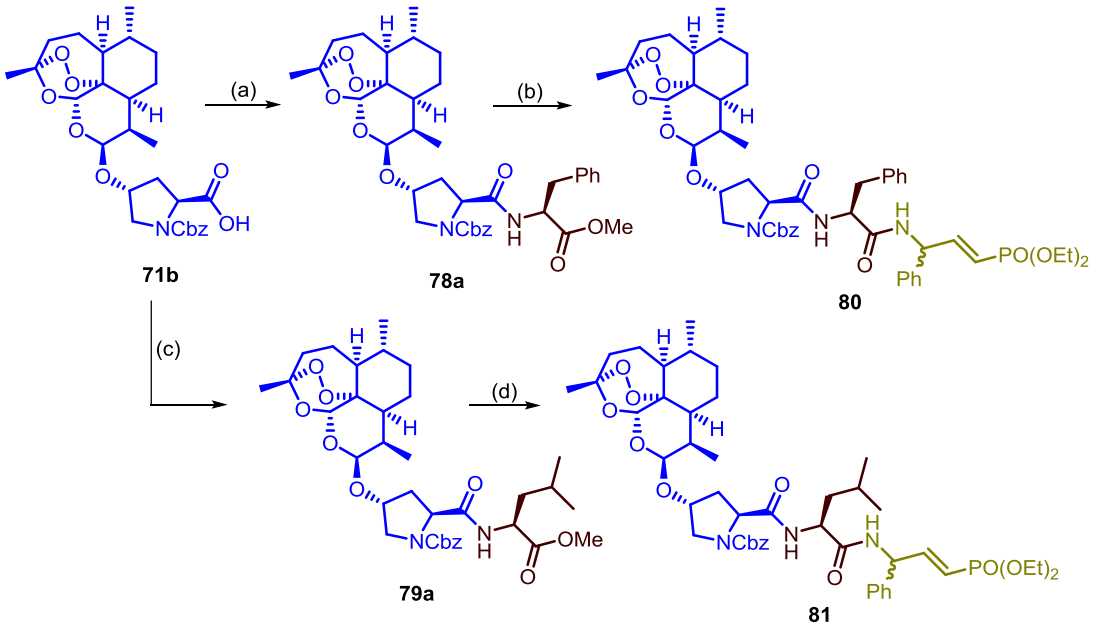
Scheme 9. Synthesis of hybrid molecules **75** and **76**. *Reagents and conditions:* (a) L-phenylalanine methyl ester, DCC, HOBT, THF, 0 °C to 25 °C, 2 h, 63%; (b) (i) 2M aq LiOH, THF, 25 °C, 2 h; (ii) (±)-**52**, DCC, HOBT, THF, 0 °C to 25 °C, 2 h, 74%; (c) L-leucine methyl ester, DCC, HOBT, THF, 0 °C to 25 °C, 2 h, 67%; (d) (i) 2M aq LiOH, THF, 25 °C, 2 h; (ii) (±)-**52**, DCC, HOBT, THF, 0 °C to 25 °C, 2 h, 51%.

Similarly, the linker attached artemisinin compound **71a** was subjected to alkaline hydrolysis using 2M aq LiOH to yield the free acid **71b** which was subjected to coupling reaction with racemic γ-amino vinyl phosphonate (±)-**52** under DCC and HOBT condition to furnish the hybrid molecule **77** as an inseparable mixture of diastereomers (**Scheme 10**).



Scheme 10. Synthesis of hybrid molecule **77**. *Reagents and conditions:* (a) 2M aq LiOH, THF, 25 °C, 2 h; (b) (±)-**52**, DCC, HOBT, THF, 0 °C to 25 °C, 2 h, 73%.

Free acid **71b** obtained from the alkaline hydrolysis of **71a** was subjected to peptide coupling reactions with L-phenylalanine methyl ester and L-leucine methyl ester to afford dipeptides **78a** and **79a**, respectively which were hydrolyzed to obtain free acids of dipeptides **78b** and **79b**, respectively followed by immediate peptide coupling reaction with (±)-**52** furnished artemisinin-dipeptidyl vinyl phosphonate hybrid molecules **80** and **81**, respectively as an inseparable mixture of diastereomers (**Scheme 11**).



Scheme 11. Synthesis of hybrid molecules **80** and **81**. *Reagents and conditions:* (a) L-phenylalanine methyl ester, DCC, HOBT, THF, 0 °C to 25 °C, 2 h, 70%; (b) (i) 2M aq LiOH, THF, 25 °C, 2 h; (ii) (±)-**52**, DCC, HOBT, THF, 0 °C to 25 °C, 2h, 48%; (c) L-leucine methyl ester, DCC, HOBT, THF, 0 °C to 25 °C, 2 h, 95%; (d) (i) 2M aq LiOH, THF, 25 °C, 2 h; (ii) (±)-**52**, DCC, HOBT, THF, 0 °C to 25 °C, 2 h, 44%.

Since **71a** is an 85:15 inseparable mixture of **71a (β):71a (α)**, hybrid molecules **77**, **80** and **81** were obtained as an inseparable mixture of four diastereomers. For example, chiral HPLC of hybrid molecule **81** showed 4 peaks corresponding to the four diastereomers as shown in **Figure 13**. Peaks **(a)** and **(b)** correspond to diastereomers formed from **71a (β)** and **71a (α)** with enantiomer (*S*)-**52** of (±)-**52**, respectively**.** Peaks **(c)** and **(d)** correspond to diastereomers formed from **71a (β)** and **71a (α)** with enantiomer (*R*)-**52** of (±)-**52**, respectively (See **Section 1.9**).

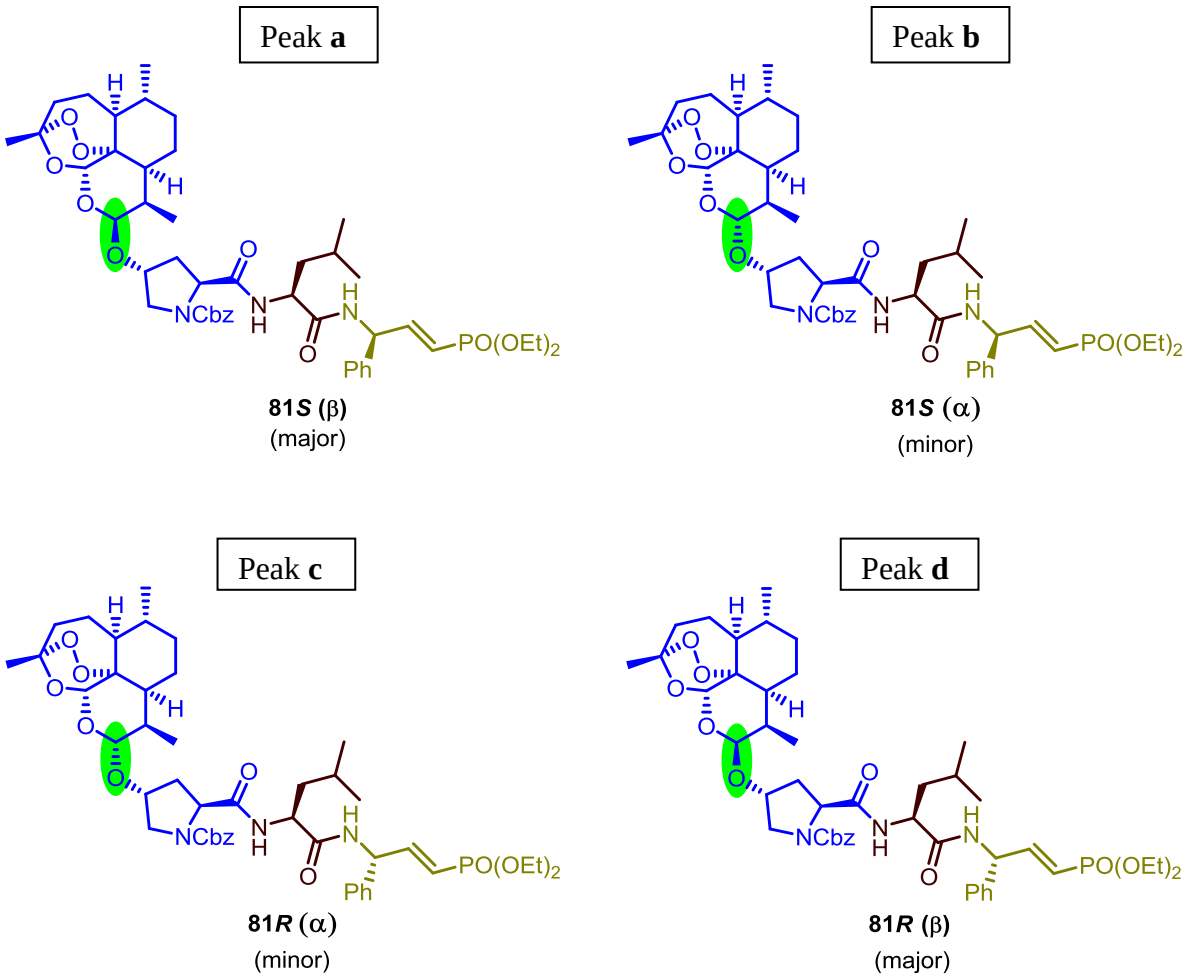


Figure 13. Structures of four inseparable diastereomers of hybrid molecule **81**.

1.4.3. Antimalarial activity of synthesized artemisinin-peptidyl vinyl phosphonate hybrid molecules 72, 75, 76, 77, 80 and 81

All the synthesized artemisinin-dipeptidyl vinyl phosphonate hybrid molecules (**72, 75, 76, 77, 80** and **81**) were assayed for their inhibition activity against falcipain-2 protease enzyme. The inhibitions of falcipain-2 enzyme by all the hybrid molecules are expressed in IC_{50} values and are summarized in **Table 2**.

Among all six synthesized hybrid molecules, dipeptide hybrid molecules **75**, **76** and **81** exhibited falcipain-2 enzyme inhibition in µM range whereas mono peptide hybrid molecules **72** and **77** were found to be inactive against falcpain-2 enzyme. The dipeptide hybrid molecule **75** having phenylalanine residue in P_2 pocket and serine in P_3 pocket showed IC_{50} value of 5.90 µM against FP-2 enzyme. The dipeptide hybrid molecule **76** having leucine residue in P_2 pocket and serine in P_3 pocket showed IC_{50} value of 5.62 µM against FP-2. The dipeptide hybrid molecule **81** having leucine residue in P_2 pocket and hydroxyproline in P_3 pocket showed IC_{50} value of 5.62 µM against FP-2 whereas the dipeptide hybrid molecule **80** having phenylalanine residue in P_2 pocket and hydroxyproline in P_3 pocket was found to be inactive against FP-2. The inactivity of mono peptide hybrid molecules **72** and **77** against FP-2 may be attributed due to the complete absence of P_2 pocket residue.

Table 2. Inhibition of falcipain-2 activity by compounds.

Compound	IC_{50} (µM)	K_i (µM)
72	>100	NA
75	**5.90 ± 0.45**	**3.49 ± 0.2668**
76	**5.62 ± 0.30**	**3.32 ± 0.177**
77	>100	NA
80	>100	NA
81	**7.67 ± 0.35**	**4.54 ± 0.206**

Further, all the synthesized artemisinin-peptidyl vinyl phosphonate hybrid molecules (**72, 75, 76, 77, 80** and **81**) were assayed for their antiplasmodial activities against different strains such as chloroquine-sensitive (3D7), chloroquine-pyrimethamine resistant (7G8) and chloroquine-pyrimethamine-mefloquine resistant (Dd2) strains of *P. falciparum* (**Table 3**).

Among these, hybrids **75**, **76** and **81** were found to exhibit very effective parasiticidal activities *in vitro* and *in vivo*. The parasiticidal efficacies of the hybrids **75**, **76** and **81** were higher than the existing antimalarial agent, artemisinin.

The instant dual-targeting artemisinin-dipeptidyl vinyl phosphonate hybrids **75**, **76** and **81** were found to be several-fold more potent than artemisinin alone against various strains of parasites (**Table 3**). Also, the hybrid molecules effectively killed *P. falciparum* strains that are resistant to commonly used antimalarial compounds such as chloroquine, pyrimethamine and mefloquine (**Table 3**).

The antiplasmodial activity data in **Table 3** suggests that the hybrid molecules **75**, **76** and **81** exhibited potent antiplasmodial activities in the nM range against all the strains of *P. falciparum*, being more active than artemisinin. The hybrid **75** displayed EC_{50} values of 2.7, 0.47 and 1.9 nM against 3D7, 7G8 and Dd2 strains of *P. falciparum*, respectively. The hybrid **76** showed EC_{50} values of 3.3, 0.23 and 3.5 nM against 3D7, 7G8 and Dd2 strains of *P. falciparum*, respectively. The hybrid **81** showed EC_{50} values of 2.57, 1.2 and 5.5 nM against 3D7, 7G8 and Dd2 strains of *P. falciparum*, respectively.

Table 3. Parasiticidal activity of selected compounds on *P. falciparum in vitro* culture.

Compound	EC_{50} on *P. falciparum* 3D7 (nM)	EC_{50} on *P. falciparum* 7G8* (nM)	EC_{50} on *P. falciparum* Dd2** (nM)
75	2.7	0.47	1.9
76	3.3	0.23	3.5
81	2.57	1.2	5.5
Artemisinin	~27	~18	~15

*Chloroquine and Pyrimethamine resistant
**Chloroquine, Pyrimethamine and Mefloquine resistant

The artemisinin-dipeptidyl vinyl phosphonate hybrids **75**, **76** and **81** showed excellent *in vivo* antimalarial efficacies (**Table 4**). It was observed that the treatment with these compounds (12.5 mg/kg of body weight) completely cleared the parasites in infected mice and 100% protection was observed in treated group of mice as compared to untreated

control where all mice died after 10-15 days of infection. Moreover, the survival days of mice after treatment with the hybrids **75**, **76** and **81** were found to be >60 days (**Table 4**).

Table 4. Protection of mice against malaria after treatment with selected compounds.

Compound (4 doses of 12.5mg/Kg of body weight)	Complete Protection - Mice with complete parasite clearance (% of treated mice)	Survival after treatment (Days)
75	80%	>60
76	100%	>60
81	100%	>60
Artemisinin	0%	15-25
Control (solvent alone)	0%	12-15

The hybrids **75**, **76** and **81** were further assayed for their *in vitro* toxicity on mammalian cell culture (A549 human cells) and were found to be non-toxic to the human cells. The EC_{50} values of these compounds on A549 human cells are shown in **Table 5**.

Table 5. *In vitro* toxicity of selected compounds on mammalian cell culture.

Compound	EC_{50} on A549 human cells (nM)
75	14600 ± 174.8
76	3816 ± 160.6
81	1282 ± 156.1

As shown in **Figure 14**, the effect of selected hybrid compounds **75**, **76** and **81** on the morphology and development of *P. falciparum* through its asexual stage was studied. The ring stage parasites were treated with the selected compounds or solvent alone as control.

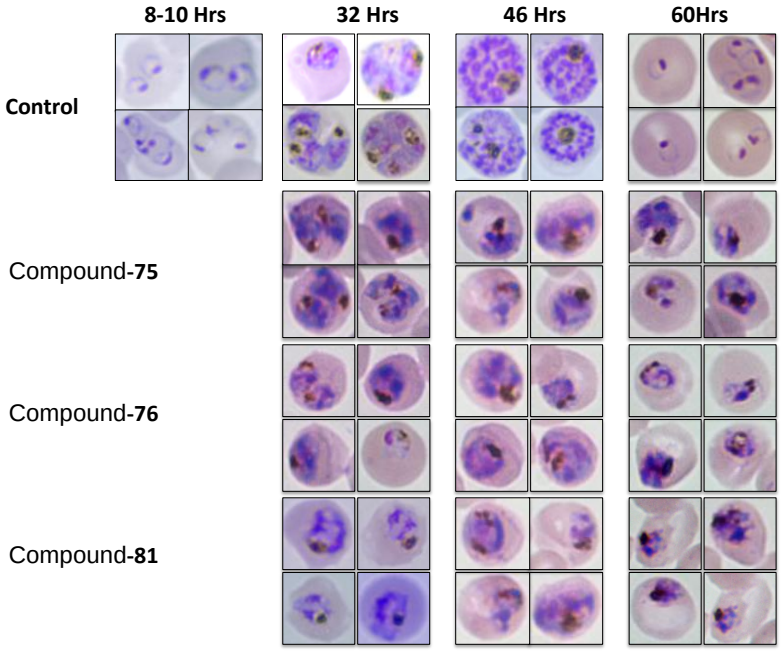


Figure 14. Effect of compounds **75**, **76** and **81** on *P. falciparum* morphology.

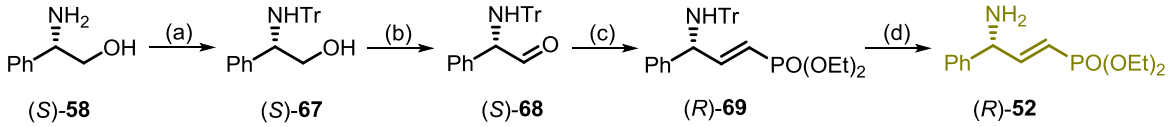
1.4.4. Asymmetric synthesis (diastereoselective synthesis) of artemisinin-dipeptidyl vinyl phosphonate hybrid molecules 75, 76 and 81 (75*R*&75*S*, 76*R*&76*S* and 81*R*&81*S*)

Since hybrid molecules **75, 76** and **81** are mixtures of diastereomers and exhibited potent antimalarial activities against falcipain-2 enzyme, hence we were interested in synthesizing the individual diastereomers of **75**, **76** and **81** to study the role of stereochemistry on the antimalarial activity. We opined that the synthetic strategy outlined in **Scheme 9** and **Scheme 11** could be utilized for the diastereoselective synthesis of hybrid molecules **75**, **76** and **81** using chiral (*R*) or (*S*)-γ-amino vinyl phosphonate (*i.e.* (*R*)-**52** or (*S*)-**52**) in the final amide coupling reaction.

1.4.4.1. Asymmetric synthesis of γ-amino vinyl phosphonate 52

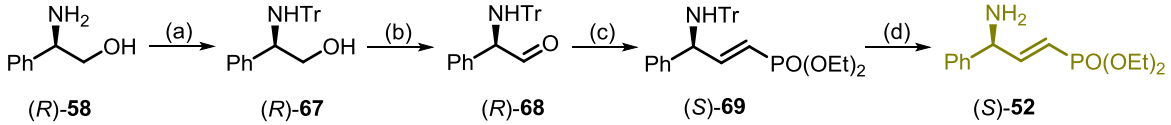
The enantiomers of **52** were synthesized using the racemic strategy described in **Scheme 6**. (*S*)-2-Phenylglycinol ((*S*)-**58**) was protected with trityl chloride to furnish trityl protected amino alcohol (*S*)-**67** which on oxidation with Dess-Martin periodinane furnished α-amino aldehyde (*S*)-**68** followed by immediate Wittig olefination with tetraethyl methylene diphosphonate yielded trityl protected olefin (*R*)-**69** (100% *ee*) without any racemization

during the oxidation and Wittig steps (confirmed by chiral HPLC). The final deprotection of the trityl group with TFA in DCM afforded amine (*R*)-**52** in 95% yield (**Scheme 12**).



Scheme 12. Synthesis of (*R*)-γ-amino vinyl phosphonate **52**. *Reagents and conditions:* (a) trityl chloride, Et_3N, 25 °C, 12 h, 60%; (b) Dess-Martin periodinane, DCM, 0 °C to 25 °C, 30 min; (c) tetraethyl methylenediphosphonate, NaH, THF, 0 °C to 25 °C, 3 h, 60% over two steps; (d) TFA, DCM, 25 °C, 30 min, 95%.

Similarly, trityl protection of (*R*)-2-phenylglycinol ((*R*)-**58**) yielded (*R*)-**67**, which on oxidation with Dess-Martin periodinane furnished α-amino aldehyde (*R*)-**68** followed by immediate Wittig olefination with tetraethyl methylenediphosphonate produced (*S*)-**69** in 100% *ee* (chiral HPLC) without any racemization. The final deprotection of the trityl group with TFA in DCM yielded amine (*S*)-**52** in 95% yield (**Scheme 13**).



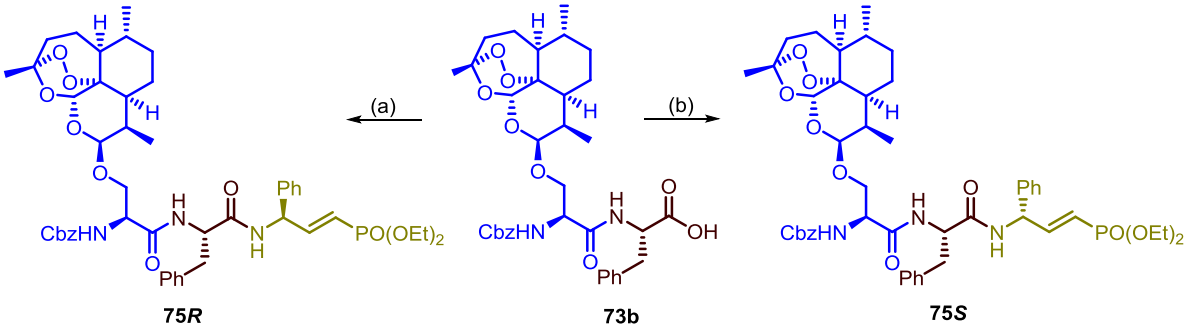
Scheme 13. Synthesis of (*S*)-γ-amino vinyl phosphonate **52**. *Reagents and conditions:* (a) trityl chloride, Et_3N, 25 °C, 12 h, 63%; (b) Dess-Martin periodinane, DCM, 0 °C to 25 °C, 30 min; (c) tetraethyl methylenediphosphonate, NaH, THF, 0 °C to 25 °C, 3 h, 64% over two steps; (d) TFA, DCM, 25 °C, 30 min, 95%.

Similar to the racemic amine (±)-**52,** the chiral amines (*R*)-**52** and (*S*)-**52** are also found to be unstable, racemize and decompose slowly at room temperature. Hence, they should be prepared and used just before the coupling reaction.

Asymmetric synthesis of hybrid molecules 75, 76 and 81

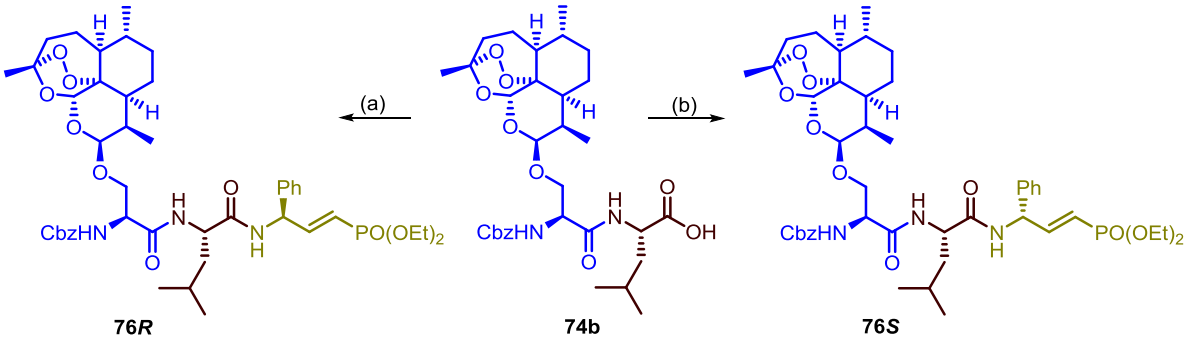
After the successful synthesis of (*R*)-**52** or (*S*)-**52**, we targeted the asymmetric synthesis of potent antimalarial hybrid molecules **75**, **76** and **81**.

The diastereomers of hybrid molecule **75** (*i.e.*, **75*R*** and **75*S***) were synthesized by coupling **73b** with (*R*)-**52** and (*S*)-**52**, respectively (**Scheme 14**).



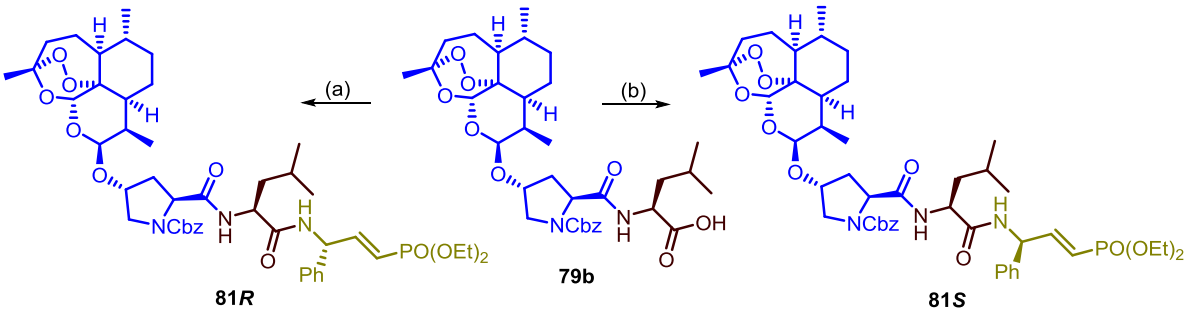
Scheme 14. Synthesis of hybrid molecules **75*R*** and **75*S***. *Reagents and conditions:* (a) (*R*)-**52**, DCC, HOBT, THF, 0 °C to 25 °C, 2 h, 58%; (b) (*S*)-**52**, DCC, HOBT, THF, 0 °C to 25 °C, 2 h, 54%.

The diastereomers of hybrid molecule **76** (*i.e.*, **76*R*** and **76*S***) were also synthesized by coupling **74b** with (*R*)-**52** and (*S*)-**52**, respectively (**Scheme 15**).



Scheme 15. Synthesis of hybrid molecules **76*R*** and **76*S***. *Reagents and conditions:* (a) (*R*)-**52**, DCC, HOBT, THF, 0 °C to 25 °C, 2 h, 57%; (b) (*S*)-**52**, DCC, HOBT, THF, 0 °C to 25 °C, 2 h, 62%.

Similarly, the diastereomers of hybrid molecule **81** (*i.e.*, **81*R*** and **81*S***) were synthesized by coupling **79b** with (*R*)-**52** and (*S*)-**52**, respectively (**Scheme 16**).



Scheme 16. Synthesis of hybrid molecules **81*R*** and **81*S***. *Reagents and conditions:* (a) (*R*)-**52**, DCC, HOBT, THF, 0 °C to 25 °C, 2 h, 75%; (b) (*S*)-**52**, DCC, HOBT, THF, 0 °C to 25 °C, 2 h, 51%.

1.4.5. Antimalarial activity of diastereomers of artemisinin-dipeptidyl vinyl phosphonate hybrid molecules 75, 76 and 81 (75*R*&75*S*, 76*R*&76*S* and 81*R*&81*S*)

All the synthesized diastereomers of hybrid molecules **75**, **76** and **81** (*i.e.*, **75*R* & 75*S*, 76*R* & 76*S*** and **81*R* & 81*S***) were assayed for their efficacy against falcipain-2 enzyme. The inhibition of falcipain-2 activity of all the synthesized diastereomers are summarized in **Table 6**.

It is evident from the **Table 6** that the diastereomer **75*R*** synthesized from (*R*)-γ-phenyl-γ-amino vinyl phosphonate was found to be more active against FP-2 than the corresponding diastereomer **75*S*** synthesized from (*S*)-γ-phenyl-γ-amino vinyl phosphonate. Similarly, diastereomers **76*R*** and **81*R*** were found to show more FP-2 inhibition activities than the corresponding diastereomers **76*S*** and **81*S***, respectively.

Table 6. Inhibition of falcipain-2 activity by compounds.

Compound	IC_{50} (μM)	K_i (μM)
75*R*	3.78 ± 0.52	2.78
75*S*	13.46 ± 6.74	9.92
76*R*	3.38 ± 0.47	2.49
76*S*	9.38 ± 1.97	6.91
81*R*	3.53 ± 0.25	2.60
81*S*	>10	NA

Further, all the diastereomers **75*R* & 75*S*, 76*R* & 76*S*** and **81*R* & 81*S*** were assayed for their antiplasmodial activities against 3D7 strain of *P. falciparum* (**Table 7**). All the diastereomers showed potent antiplasmodial activities in nM range compared to the existing antimalarial agent, artemisinin. Also, the diastereomers **75*R***, **76*R*** and **81*R*** were found to be more active than the corresponding diastereomers **75*S***, **76*S*** and **81*S***. The *in vitro* antiplasmodial activities of all the diastereomers on 3D7 strain of *P. falciparum* are summarized in **Table 7**.

The diastereomer **75*R*** exhibited EC_{50} value of 0.95 nM against the 3D7 strain of *P. falciparum* whereas **75*S*** exhibited EC_{50} value of 2.03 nM against the 3D7 strain of *P. falciparum.* The diastereomer **76*R*** showed EC_{50} value of 10.90 nM against the 3D7 strain whereas **76*S*** showed EC_{50} value of 16.73 nM against the 3D7 strain. Similarly, the diastereomer **81*R*** exhibited EC_{50} value of 1.04 nM against the 3D7 strain whereas **81*S*** exhibited EC_{50} value of 6.12 nM against the 3D7 strain.

Table 7. Parasiticidal activity of selected compounds on *P. falciparum in vitro* culture.

Compound	EC_{50} on *P. falciparum* 3D7 nM (SD)
75*R*	0.95 ± 0.49
75*S*	2.03 ± 3.10
76*R*	10.90 ± 39.6
76*S*	16.73 ± 12.40
81*R*	1.04 ± 0.53
81*S*	6.12 ± 23.58
Artemisinin	~27

In conclusion, the hybrids **75*R*** and **81*R*** could be considered as the ideal compounds for the further development of novel antimalarial drugs with a different mode of action.

1.4.6. Plausible mechanism of action of all the synthesized hybrid molecules 75, 76, 81 and their diastereomers 75*R* & 75*S*, 76*R* & 76*S* and 81*R* & 81*S*

The hybrids are believed to exhibit the dual mode of action of both the artemisinin and the peptidyl vinyl phosphonate units. Artemisinin works by the generation of carbon free radicals through its heme-mediated decomposition. The generated free radicals alkylate parasite proteins resulting in the death of parasites. Whereas peptidyl vinyl phosphonate works by inhibiting falcipain-2 proteases present in *P. falciparum* malaria parasite. Falcipain-2 proteases play a vital role in degrading the host hemoglobin into small amino acids. These amino acids resulting from hemoglobin degradation are transferred to parasite proteins or utilized for energy metabolism of the malarial parasites. Thus, inhibiting the

falcipain-2 proteases in *P. falciparum* makes the parasites unable to degrade the host hemoglobin resulting in the parasite death.

1.4.7. Synthesis of artemisinin-dipeptidyl vinyl sulfone hybrid molecules

As discussed in **Section 1.2.4.** and **1.2.5.**, peptidyl vinyl sulfones are well-known inhibitors of the falcipain-2 enzyme.[33] In extension to the synthesis and antimalarial studies of artemisinin-dipeptidyl vinyl phosphonate hybrid molecules as falcipain-2 enzyme inhibitors, we further aimed to synthesize the sulfone variants (*i.e.*, artemisinin-dipeptidyl vinyl sulfone hybrid molecules). We wished to synthesize dipeptidyl vinyl sulfone analogs of the most active artemisinin-dipeptidyl vinyl phosphonate hybrid molecules **75**, **76** and **81** to understand the complete structure-activity relationship (SAR) of the hybrid molecules (**Figure 15**). The sulfone hybrid molecules could be synthesized by coupling γ-amino vinyl sulfone **80** in the final amide coupling reaction.

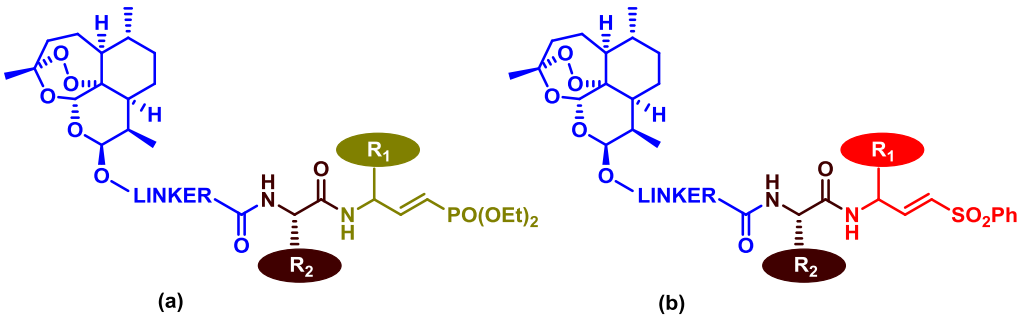
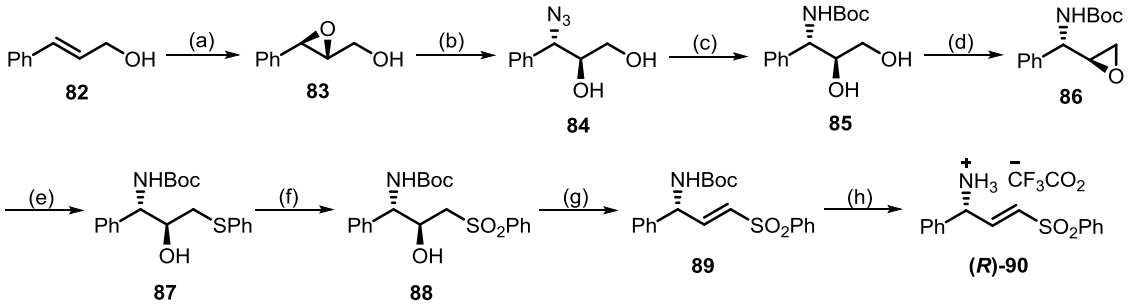


Figure 15. General structures of (a) artemisinin-dipeptidyl vinyl phosphonate hybrid molecule; (b) artemisinin-dipeptidyl vinyl sulfone hybrid molecule.

1.4.7.1. Racemic and enantioselective syntheses of γ-amino vinyl sulfone 90

The enantioselective synthesis of γ-aryl-γ-amino vinyl sulfones employing Wittig olefination of the corresponding stereochemically labile α-amino-α-aryl aldehydes is a challenging task. Pico´ *et al.* reported the asymmetric synthesis of γ-amino vinyl sulfone **90** starting from Sharpless asymmetric epoxidation of cinnamyl alcohol.[61] As shown in **Scheme 17**, Sharpless asymmetric epoxidation of cinnamyl alcohol **82** yielded epoxy alcohol **83**. Ring opening of epoxide **83** with NaN_3 and $LiClO_4$ followed by protection with Boc_2O furnished Boc-amino diol **85**. Boc-amino diol **85** was subjected to Mitsunobu cyclization conditions with triphenylphosphine and diisopropyl azodicarboxylate to afford epoxide **86**. Ring opening of the epoxide **86** with thiophenol afforded hydroxy sulfide **87** which on oxidation with *m*-

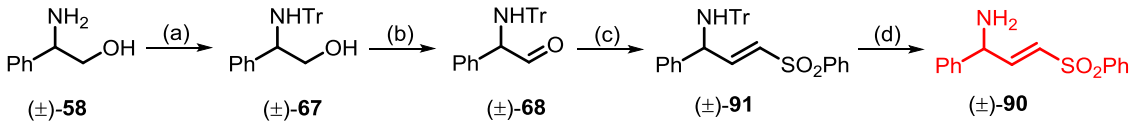
CPBA yielded hydroxy sulfone **88**. Dehydration of hydroxy sulfone **88** was achieved using water-soluble carbodiimide morpho-CDI in the presence of catalytic amounts of copper (II) chloride to furnish Boc-protected vinyl sulfone **89**. The final deprotection of **89** with 40% TFA in CH_2Cl_2 afforded the final γ-phenyl-γ-amino vinyl sulfone **90** as a TFA salt in quantitative yield.



Scheme 17. Reported[61] synthesis of γ-phenyl-γ-amino vinyl sulfone (*R*)-**90**. *Reagents and conditions:* (a) cat. $Ti(OiPr)_4$, cat. D-(-)-DIPT, ^tBuOOH, CH_2Cl_2, −20 °C, 80-90%; (b) NaN_3, $LiClO_4$, CH_3CN, reflux, 94%; (c) H_2, cat. 10% Pd/C, Boc_2O, EtOAc, rt, 82%; (d) PPh_3, DIAD, $CHCl_3$, reflux, 86%; (e) thiophenol, Et_3N, CH_3OH, reflux, 100%; (f) *m*-CPBA, CH_2Cl_2, rt, 100%; (g) morpho-CDI, cat. $CuCl_2$, CH_3CN, reflux, 100%; (h) 40% TFA in CH_2Cl_2, rt (quant).

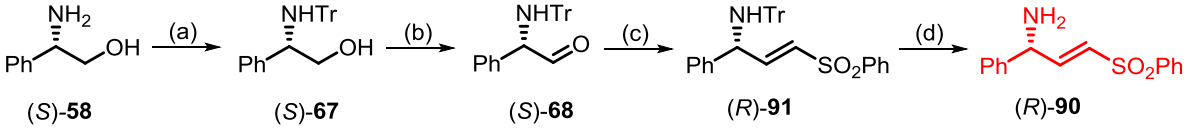
We have designed a simple and novel approach for the synthesis of γ-phenyl-γ-amino vinyl sulfone **90** as compared to Pico´ *et al.*[61] synthesis. Compound **90** was synthesized employing Wittig olefination of the trityl protected α-amino-α-phenyl aldehyde **68** using the similar strategy used for the synthesis of γ-phenyl-γ-amino vinyl phosphonate **52** (please see **Scheme 6**) without any possible racemization of α-amino-α-phenyl aldehyde **68** during the Wittig olefination step.

As shown in **Scheme 18**, trityl protected α-amino-α-phenyl aldehyde **68** obtained from Dess-Martin oxidation of **67** was subjected to the Wittig olefination condition with diethyl ((phenylsulfonyl)methyl)phosphonate and NaH to afford trityl protected vinyl sulfone **91**. Diethyl ((phenylsulfonyl)methyl)phosphonate was synthesized using the reported literature procedure.[62] Final trityl deprotection of **91** with TFA in DCM yielded γ-phenyl-γ-amino vinyl sulfone **90** in quantitative yield.

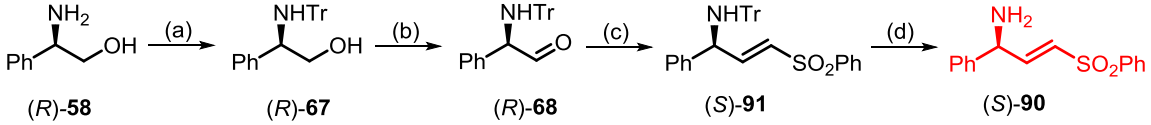


Scheme 18. Synthesis of (±)-γ-amino vinyl sulfone **90**. *Reagents and conditions:* (a) trityl chloride, Et_3N, 25 °C, 12 h, 63%; (b) Dess-Martin periodinane, DCM, 0 °C to 25 °C, 30 min; (c) diethyl ((phenylsulfonyl)methyl)phosphonate, NaH, THF, 0 °C to 25 °C, 3 h, 74% over two steps; (d) TFA, DCM, 25 °C, 30 min, quant.

By using this methodology, the enantiomers of γ-phenyl-γ-amino vinyl sulfone (*R*)-**90** and (*S*)-**90** were synthesized as shown in **Scheme 19** and **Scheme 20**, respectively. The structure of the trityl-protected compound (*S*)-**91** was further confirmed by its single crystal XRD (**Figure 16** & **17**).



Scheme 19. Synthesis of (*R*)-γ-amino vinyl sulfone **90**. *Reagents and conditions:* (a) trityl chloride, Et_3N, 25 °C, 12 h, 60%; (b) Dess-Martin periodinane, DCM, 0 °C to 25 °C, 30 min; (c) diethyl ((phenylsulfonyl)methyl)phosphonate, NaH, THF, 0 °C to 25 °C, 3 h, 71% over two steps; (d) TFA, DCM, 25 °C, 30 min, quant.



Scheme 20. Synthesis of (*S*)-γ-amino vinyl sulfone **90**. *Reagents and conditions:* (a) trityl chloride, Et_3N, 25 °C, 12 h, 63%; (b) Dess-Martin periodinane, DCM, 0 °C to 25 °C, 30 min; (c) diethyl ((phenylsulfonyl)methyl)phosphonate, NaH, THF, 0 °C to 25 °C, 3 h, 54% over two steps; (d) TFA, DCM, 25 °C, 30 min, quant.

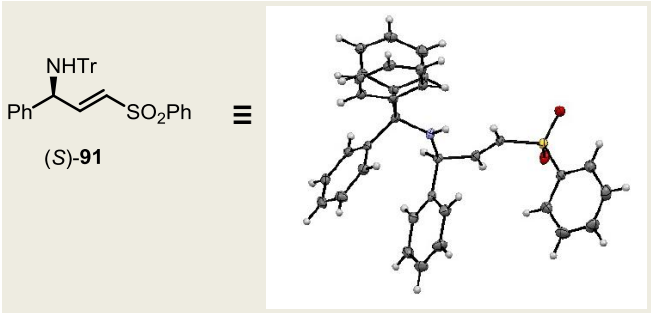


Figure 16. Single crystal XRD of compound (*S*)-**91**.

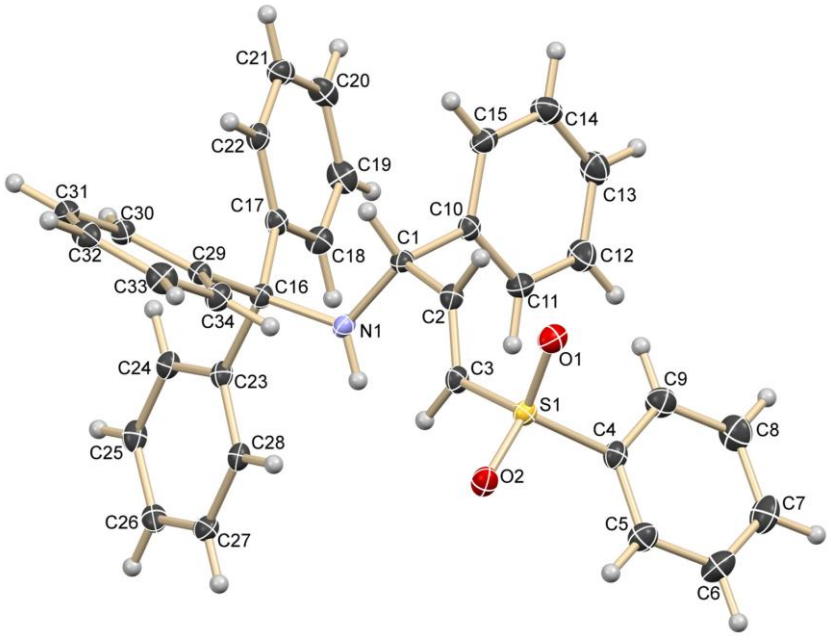
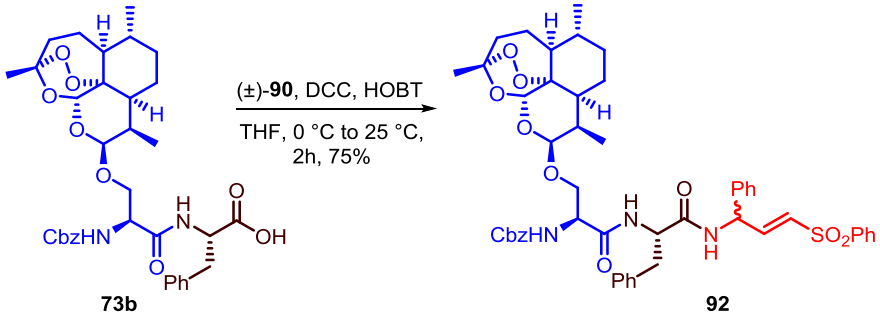


Figure 17. ORTEP diagram of compound (*S*)-**91** (CCDC 1882103).

Synthesis of artemisinin-dipeptidyl vinyl sulfone hybrid molecules 92, 93 and 94

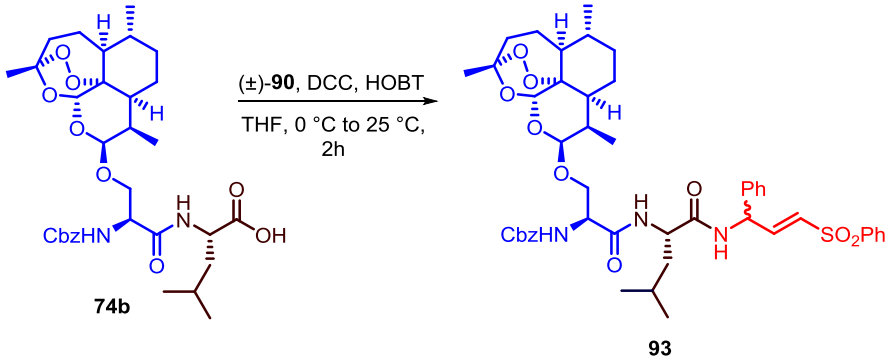
After the successful synthesis of racemic and chiral γ-phenyl-γ-amino vinyl sulfone **90**, we further aimed to synthesize the sulfone analogs of the most active artemisinin-dipeptidyl vinyl phosphonate hybrid molecules **75**, **76** and **81**.

Free carboxylic acid **73b** was subjected to amide coupling with racemic γ-phenyl-γ-amino vinyl sulfone (±)-**90** to furnish hybrid molecule **92** as an inseparable mixture of diastereomers (**Scheme 21**).



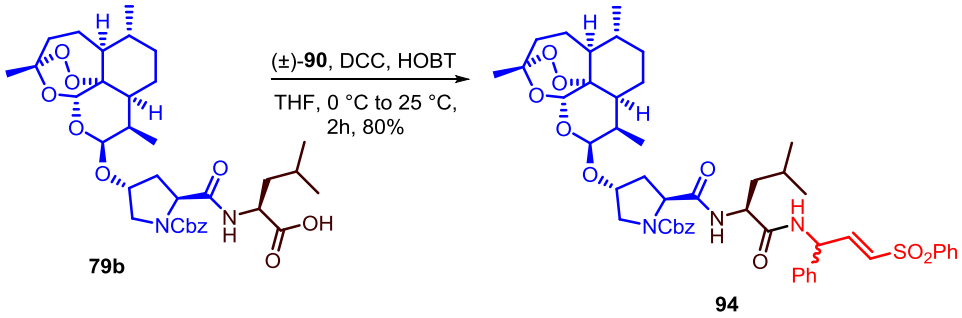
Scheme 21. Synthesis of artemisinin-dipeptidyl vinyl sulfone hybrid molecule **92**.

Free carboxylic acid **74b** was subjected to amide coupling with racemic γ-phenyl-γ-amino vinyl sulfone (±)-**90** to furnish hybrid molecule **93** as an inseparable mixture of diastereomers (**Scheme 22**).



Scheme 22. Synthesis of artemisinin-dipeptidyl vinyl sulfone hybrid molecule **93**.

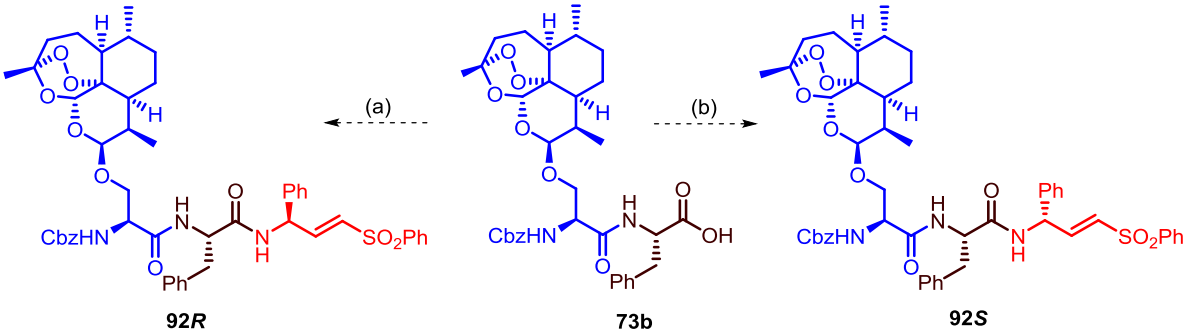
Similarly, free carboxylic acid **79b** was subjected to amide coupling with racemic γ-phenyl-γ-amino vinyl sulfone (±)-**90** to furnish hybrid molecule **94** as an inseparable mixture of diastereomers (**Scheme 23**).



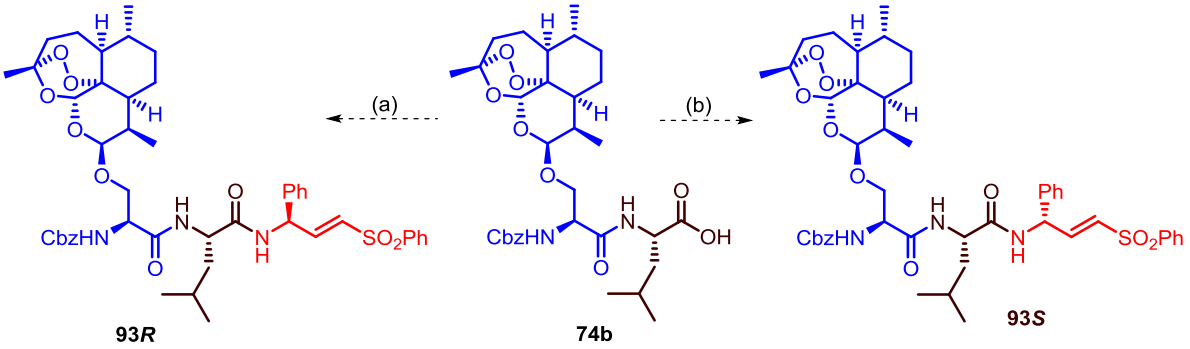
Scheme 23. Synthesis of artemisinin-dipeptidyl vinyl sulfone hybrid molecule **94**.

1.4.8. Asymmetric synthesis of artemisinin-dipeptidyl vinyl sulfone hybrid molecules 92, 93 and 94

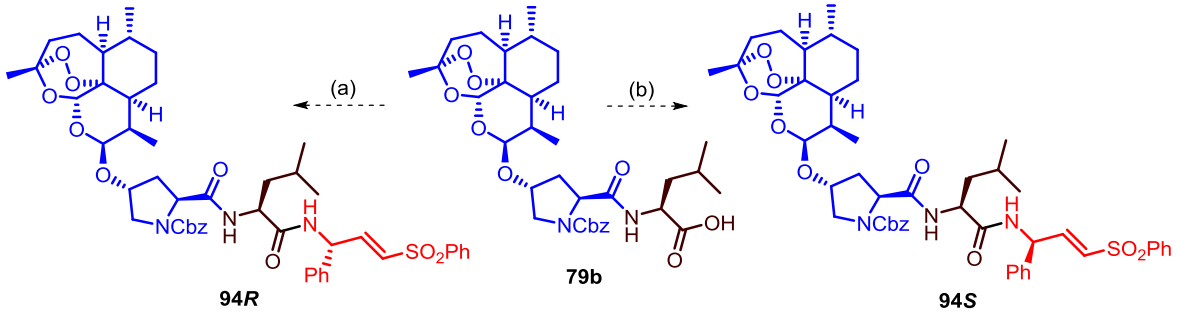
After completing the synthesis of racemic hybrid molecules **92**, **93** and **94**, we were interested in accomplishing the diastereoselective synthesis of hybrid molecules **92, 93** and **94** using similar synthetic strategy. Since we have already synthesized chiral γ-phenyl-γ-amino vinyl sulfones (*R*)-**90** and (*S*)-**90**, synthesis of diastereomers of hybrid molecules **92, 93** and **94** could be achieved by coupling of compound **73b**, **74b** and **79b** with chiral γ-phenyl-γ-amino vinyl sulfones (*R*)-**90** and (*S*)-**90** using our earlier amide coupling method (please see **Section 1.4.4.**). The synthesis of diastereomers of hybrid molecules **92, 93** and **94** is at present underway in our laboratory **(Scheme 24**, **25** and **26**, respectively).



Scheme 24. Proposed synthesis of chiral hybrid molecules **92*R*** and **92*S***. *Reagents and conditions:* (a) (*R*)-**90**, DCC, HOBT, THF, 0 °C to 25 °C, 2 h; (b) (*S*)-**90**, DCC, HOBT, THF, 0 °C to 25 °C, 2 h.



Scheme 25. Proposed synthesis of chiral hybrid molecules **93*R*** and **93*S***. *Reagents and conditions:* (a) (*R*)-**90**, DCC, HOBT, THF, 0 °C to 25 °C, 2 h; (b) (*S*)-**90**, DCC, HOBT, THF, 0 °C to 25 °C, 2 h.



Scheme 26. Proposed synthesis of chiral hybrid molecules **94*R*** and **94*S***. *Reagents and conditions:* (a) (*R*)-**90**, DCC, HOBT, THF, 0 °C to 25 °C, 2 h; (b) (*S*)-**90**, DCC, HOBT, THF, 0 °C to 25 °C, 2 h.

Further, all the synthesized sulfone hybrid molecules will be assayed for their inhibition against falcipain-2 enzyme *in vitro* and *in vivo* and further assayed for their antimalarial activities against different strains of *P. falciparum*.

1.5. CONCLUSION

In conclusion, novel artemisinin-peptidyl vinyl phosphonate antimalarial hybrid molecules (**72, 75, 76, 77, 80** and **81**) have been designed and synthesized targeting falcipain-2 enzyme of *P.falciparum*. The amide coupling of *"dihydroartemisinin—linker"* with freshly prepared γ-phenyl-γ-amino vinyl phosphonate (±)-**52** furnished *"dihydroartemisinin—linker—vinyl phosphonate"* hybrid molecules **72** and **77** of type-1. Similarly, the amide coupling of *"dihydroartemisinin—linker—amino acid"* with (±)-**52** furnished *"dihydroartemisinin—linker—amino acid—vinyl phosphonate"* hybrid molecules **75**, **76**, **80** and **81** of type-2.

Among all the six synthesized hybrid molecules (**72, 75, 76, 77, 80** and **81**), dipeptide hybrid molecules **75**, **76** and **81** exhibited falcipain-2 enzyme inhibition in µM range whereas mono peptide hybrid molecules **72** and **77** were found to be inactive against falcpain-2 enzyme. The inactivity of mono peptide hybrid molecules **72** and **77** against FP-2 may be due to the complete absence of P_2 pocket residue required for a falcpain-2 inhibitor. Further, the hybrids **75**, **76** and **81** were found to be several-fold more potent in nM range than artemisinin alone against various strains (3D7, 7G8 and Dd2) of *P. falciparum*. The hybrid molecules **75**, **76** and **81** effectively killed *P. falciparum* strains that are resistant to commonly used antimalarial compounds such as chloroquine, pyrimethamine and mefloquine. These hybrids also showed excellent *in vivo* antimalarial efficacies. It was observed that the treatment with these compounds (12.5 mg/kg of body weight) completely cleared the parasites in infected mice and 100% protection was observed in treated group of mice as compared to untreated control where all mice died after 10-15 days of infection. Moreover, the survival days of mice after treatment with the hybrids **75**, **76** and **81** were found to be >60 days. Also, the hybrids **75**, **76** and **81** were assayed for their *in vitro* toxicity on mammalian cell culture (A549 human cells) and were found to be non-toxic to the human cells.

The diastereomers of most potent antimalarial hybrid molecules **75**, **76** and **81** (*i.e.*, **75*R*** & **75*S***, **76*R*** & **76*S*** and **81*R*** & **81*S***) were successfully synthesized by using chiral γ-phenyl-γ-amino vinyl phosphonate (*R*)-**52** or (*S*)-**52** in the final amide coupling reaction.

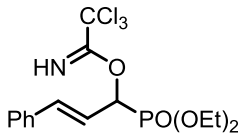
The diastereomers **75*R***, **76*R*** and **81*R*** were found to be more active than the corresponding diastereomers **75*S***, **76*S*** and **81*S*** against FP-2 enzyme and *P. falciparum*. Among all the

diastereomers **75*R*** and **81*R*** showed superior *in vitro* antiplasmodial activities with EC_{50} values of 0.95 and 1.04 nM, respectively against 3D7 strain of *P. falciparum.* Hence, the hybrids **75*R*** and **81*R*** could be considered as the ideal compounds for the further development of novel antimalarial drugs with a different mode of action.

In order to understand the complete structure-activity relationship (SAR), we further synthesized the sulfone variants of most potent antimalarial phosphonate hybrid molecules (**75**, **76** and **81**) by the amide coupling of *"dihydroartemisinin—linker—amino acid"* with γ-phenyl-γ-amino vinyl sulfone (±)-**90** to obtain *"dihydroartemisinin—linker—amino acid—vinyl sulfone"* hybrid molecules **92**, **93** and **94**. The synthesis of diastereomers of these sulfone hybrid molecules **92**, **93** and **94** (*i.e.*, **92*R*** & **92*S***, **93*R*** & **93*S*** and **94*R*** & **94*S***) using chiral γ-phenyl-γ-amino vinyl sulfone (*R*)-**90** or (*S*)-**90** is currently underway in our laboratory.

1.6. EXPERIMENTAL SECTION

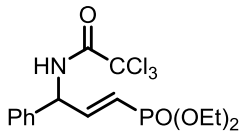
(*E*)-1-(Diethoxyphosphoryl)-3-phenylallyl 2,2,2-trichloroacetimidate (53)



53

To a stirred solution of hydroxy phosphonate **49** (1.0 equiv.) and CCl_3CN (3.0 equiv.) in dry DCM was added a catalytic amount of DBU (0.5 equiv.) under Ar at −35 °C. Stirring was continued at this temperature for 30 min. After completion (monitored by TLC), solvent and volatile compounds were removed on a rotator evaporator with external cooling (10 °C). The residue was then immediately purified by flash chromatography on silica gel to afford pure trichloroacetimidate **53** as pale yellow syrup. R_f = 0.35 (EtOAc-petroleum ether, 2:3); **^{1}H NMR (200 MHz, $CDCl_3$):** δ 8.59 (s, 1H), 7.49-7.21 (m, 5H), 6.86 (dd, J = 16.0, 4.0 Hz, 1H), 6.43-6.22 (m, 1H), 6.06 (dd, J = 14.0, 6.9 Hz, 1H), 4.37-4.09 (m, 4H), 1.37-1.30 (m, 6H); **^{13}C NMR (50 MHz, $CDCl_3$):** δ 161.4, 161.2, 135.8, 135.0, 134.8, 129.0, 128.6, 128.4, 127.2, 126.9, 123.4, 119.6, 119.5, 114.1, 91.0, 75.6, 72.2, 63.8, 63.7, 63.5, 63.4, 62.2, 16.5, 16.4; **ESI-LCMS:** *m/z* 437.9 $(M+Na)^+$.

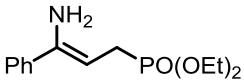
Diethyl (*E*)-(3-phenyl-3-(2,2,2-trichloroacetamido)prop-1-en-1-yl)phosphonate (54)



54

A solution of trichloroacetimidate **53** in dry toluene was refluxed under Ar for 24 h. After complete disappearance of starting material (TLC), the solvent was removed in vacuo and the residue was subjected to flash chromatography on silica gel to afford trichloroacetamide **54** as a colorless crystalline solid. R_f = 0.35 (EtOAc-petroleum ether, 1:1); **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.57-7.46 (m, 1H), 7.44-7.29 (m, 5H), 6.95 (t, *J* = 17.9 Hz, 1H), 5.89 (t, *J* = 17.7 Hz, 1H), 5.69 (brs, 1H), 4.15-3.95 (m, 4H), 1.38-1.18 (m, 6H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 161.3, 148.6, 148.5, 137.2, 129.3, 128.8, 127.3, 119.8, 118.3, 92.4, 62.2, 62.1, 57.2, 57.0, 16.4, 16.3, 16.3; **ESI-LCMS:** *m/z* 437.9 $(M+Na)^+$.

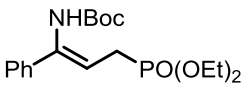
Diethyl (*Z*)-(3-amino-3-phenylallyl)phosphonate (55)



55

Trichloroacetamide **54** (150 mg, 0.361 mmol, 1.0 equiv.) was dissolved in 3.5 mL of EtOH and was added 3N KOH solution (670 µL). The whole mixture was stirred for 24 h at rt. After the completion of the reaction, the reaction mixture was extracted with EtOAc (3 X 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to give the crude product which was further purified by flash chromatography on silica gel to give pure amine **55** as colorless syrup (97 mg, 100%). R_f = 0.42 (EtOAc-petroleum ether, 1:1); **^{1}H NMR (500 MHz, $CDCl_3$):** δ 10.04 (brs, 1H), 7.49-7.43 (m, 2H), 7.42-7.33 (m, 3H), 5.81-5.73 (m, 1H), 4.21-4.11 (m, 4H), 2.81 (d, *J* = 8.0 Hz, 1H), 2.76 (d, *J* = 8.0 Hz, 1H), 1.35 (t, 6H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 160.7, 139.4, 139.3, 135.8, 135.7, 128.8, 128.6, 125.7, 125.7, 112.4, 112.3, 92.8, 62.8, 62.8, 26.4, 25.3, 16.5, 16.4; **ESI-LCMS:** *m/z* 292.9 $(M+Na)^+$.

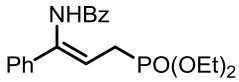
***tert*-Butyl (*Z*)-(3-(diethoxyphosphoryl)-1-phenylprop-1-en-1-yl)carbamate (63)**



63

In a round bottom flask, tetraethyl methylenediphosphonate (7.37 mmol, 1.7 equiv.) was taken up in dry THF (8 mL). The reaction mixture was cooled to 0 °C in an ice bath. NaH (6.50 mmol, 1.5 equiv.) was added to the reaction mixture. The solution was stirred at 0 °C for 15 min. *N*-Boc protected α-amino aldehyde **61** (4.33 mmol, 1.0 equiv.) was taken up in dry THF (7 mL) and was added to the reaction mixture. The reaction mixture was then warmed to rt and stirred for 3 h. After completion of reaction (TLC), the reaction mixture was concentrated in vacuo. The residue was dissolved in water, extracted with DCM (3X80 mL). The combined organic layers were washed with water, brine and concentrated to give the crude product which was purified by column chromatography on a silica gel column with ethyl acetate: petroleum ether as eluant to give pure olefin **63** as pale yellow syrup. $\boldsymbol{R_f}$ = 0.31 (EtOAc-petroleum ether, 2:3); **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.45-7.40 (m, 2H), 7.37-7.28 (m, 3H), 5.43-5.33 (m, 1H), 4.21-4.08 (m, 4H), 2.81 (d, *J* = 8.0 Hz, 1H), 2.76 (d, *J* = 8.0 Hz, 1H), 1.48-1.25 (m, 15H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 153.5, 140.1, 128.4, 128.2, 128.1, 126.1, 108.4, 80.2, 62.4, 62.4, 28.1, 27.7, 26.7, 25.5, 16.5, 16.4; **ESI-LCMS:** *m/z* 370.0 $(M+H)^+$.

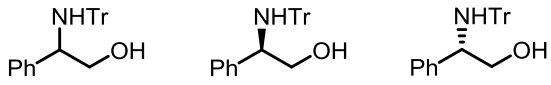
Diethyl (*Z*)-(3-benzamido-3-phenylallyl)phosphonate (64)



64

Same Wittig procedure as described for **63** was followed for the synthesis of olefin **64** using *N*-Bz protected α-amino aldehyde **62** and tetraethyl methylenediphosphonate as starting materials. $\boldsymbol{R_f}$ = 0.15 (EtOAc-petroleum ether, 2:3); **^{1}H NMR (200 MHz, $CDCl_3$):** δ 9.95 (brs, 1H), 8.17-7.99 (m, 2H), 7.63-7.39 (m, 5H), 7.39-7.21 (m, 3H), 5.60 (q, *J* = 7.8 Hz, 1H), 4.26-4.01 (m, 4H), 2.84 (d, *J* = 7.8 Hz, 1H), 2.73 (d, *J* = 7.8 Hz, 1H), 1.39-1.26 (m, 6H); **ESI-LCMS:** *m/z* 374.0 $(M+H)^+$.

General procedure for the synthesis of 2-phenyl-2-(tritylamino)ethan-1-ol ((±)-67 or (*R*)-67 or (*S*)-67)

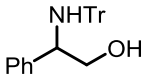


(±)-**67** (*R*)-**67** (*S*)-**67**

To a mixture of 2-phenylglycinol (±)-**58** or (*R*)-**58** or (*S*)-**58** (1.0 g, 1.0 equiv.) and triphenylmethyl chloride (2.03 g, 1.0 equiv.) in dichloromethane (25 mL) was added

triethylamine (0.74 g, 1.0 equiv.). The resulting mixture was stirred at 25 °C for 12 h. The mixture was diluted with ethyl acetate (75 mL) and washed with water and brine. The ethyl acetate fraction was dried (Na_2SO_4), filtered and concentrated. The solid was purified by chromatography on a silica gel column with ethyl acetate: petroleum ether as eluant to furnish the pure product (±)-**67** or (*R*)-**67** or (*S*)-**67**, respectively as colorless viscous syrup or foaming solid.

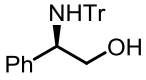
2-Phenyl-2-(tritylamino)ethan-1-ol ((±)-67)



(±)-**67**

Colorless viscous syrup; $\boldsymbol{R_f}$ = 0.43 (EtOAc-petroleum ether, 1:4).

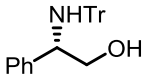
(*R*)-2-phenyl-2-(tritylamino)ethan-1-ol ((*R*)-67)



(*R*)-**67**

Colorless viscous syrup; $\boldsymbol{R_f}$ = 0.43 (EtOAc-petroleum ether, 1:4); $\boldsymbol{[\alpha]_D^{24}}$ -97.3 (*c* 1.0, $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.58-7.45 (m, 6H), 7.33-7.12 (m, 14H), 3.80 (t, *J* = 4.6 Hz, 1H), 3.21 (dd, *J* = 10.7, 3.8 Hz, 1H), 2.78 (dd, *J* = 10.7, 5.3 Hz, 1H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 146.6, 143.6, 129.0, 128.4, 128.0, 127.9, 127.2, 126.7, 126.5, 71.9, 67.0, 58.6.

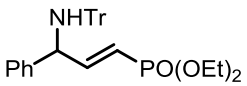
(*S*)-2-phenyl-2-(tritylamino)ethan-1-ol ((*S*)-67)



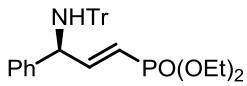
(*S*)-**67**

Colorless viscous syrup; $\boldsymbol{R_f}$ = 0.43 (EtOAc-petroleum ether, 1:4); $\boldsymbol{[\alpha]_D^{24}}$ +96.7 (*c* 1.0, $CHCl_3$).

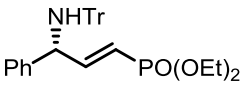
General procedure for the synthesis of diethyl (*E*)-(3-phenyl-3-(tritylamino)prop-1-en-1-yl)phosphonate ((±)-69 or (*R*)-69 or (*S*)-69)



(±)-**69**



(*S*)-**69**



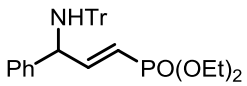
(*R*)-**69**

2-Phenyl-2-(tritylamino)ethan-1-ol (±)-**67** or (*R*)-**67** or (*S*)-**67** (1.32 mmol, 1.0 equiv.) was dissolved in DCM (10 mL) and cooled to 0 °C. To this cold solution was added Dess-Martin

periodinane (1.98 mmol, 1.5 equiv.) portion wise over 10 min and then stirred at 0 °C for 10 min. The reaction mixture was allowed to slowly warm to 25 °C and stirred for 30 min. After completion of the reaction (TLC), the reaction mixture was diluted with DCM. The reaction mixture was placed in an ice-water bath and a 1:1 mixture of saturated aqueous $NaHCO_3$ solution and saturated $NaHSO_3$ solution (4 mL) was added and the cooling bath was removed and the mixture was stirred at 25 °C until the formation of two clear layers was observed. The reaction mixture was transferred to a separatory funnel containing a saturated aqueous $NaHCO_3$ solution (20 mL). The aqueous layer was extracted with ethyl acetate (3 x 25 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated to give crude aldehyde (±)-**68** or (*R*)-**68** or (*S*)-**68**, respectively as colorless foaming solid. The crude aldehyde residue was used immediately for the next step without any further purification.

Tetraethyl methylenediphosphonate (2.24 mmol, 1.7 equiv.) was taken up in dry THF (4 mL) and was cooled to 0 °C in an ice bath. NaH (60 % dispersion in mineral oil, 1.98 mmol, 1.5 equiv.) was added to the reaction mixture in portion wise over a period of 5-10 min. The solution was stirred at 0 °C for 15 min. Crude aldehyde (±)-**68** or (*R*)-**68** or (*S*)-**68** was taken in dry THF (4 mL) and was added to the reaction mixture. The reaction mixture was then warmed to 25 °C and stirred for 3 h. After completion of the reaction (TLC), the reaction mixture was concentrated in vacuo. The residue was dissolved in water, extracted with DCM (3X20 mL). The combined organic layers were washed with water, brine and concentrated to give crude product which was purified either by recrystallization from EtOAc-Petroleum ether mixture or column chromatography on a silica gel column with ethyl acetate: petroleum ether as eluant to give pure olefin (±)-**69** or (*S*)-**69** or (*R*)-**69**, respectively as colorless solid.

Diethyl (*E*)-(3-phenyl-3-(tritylamino)prop-1-en-1-yl)phosphonate ((±)-69)

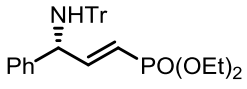


(±)-**69**

Colorless solid; **m.p.:** 187-188 °C; R_f = 0.28 (EtOAc-petroleum ether, 1:1); **^{31}P NMR (162 MHz, $CDCl_3$):** δ 19.09; **ESI-LCMS:** *m/z* 534.2 $(M+Na)^+$; **HPLC:** Chiralpak-IA (0.46 mm ϕ X 250 mmL), 3% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R =

9.0 min for (*R*)-isomer and t_R = 10.0 min for (*S*)-isomer; **HRMS (ESI):** *m/z* for $C_{32}H_{34}O_3NNaP$ $(M+Na)^+$: calcd 534.2169, found 534.2160.

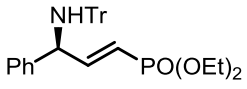
Diethyl (*R*,*E*)-(3-phenyl-3-(tritylamino)prop-1-en-1-yl)phosphonate ((*R*)-69)



(*R*)-**69**

Colorless solid; **m.p.:** 115-117 °C; R_f = 0.28 (EtOAc-petroleum ether, 1:1); $[\alpha]_D^{23}$ -43.8 (*c* 1.0, $CHCl_3$); **^{31}P NMR (162 MHz, $CDCl_3$):** δ 19.09; **ESI-LCMS:** *m/z* 534.0 $(M+Na)^+$; **HPLC:** *ee* 100% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 3% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 9.0 min for (*R*)-isomer].

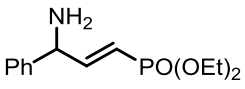
Diethyl (*S*,*E*)-(3-phenyl-3-(tritylamino)prop-1-en-1-yl)phosphonate ((*S*)-69)



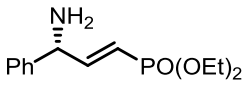
(*S*)-**69**

Colorless solid; **m.p.:** 115-117 °C; R_f = 0.28 (EtOAc-petroleum ether, 1:1); $[\alpha]_D^{23}$ +41.0 (*c* 1.0, $CHCl_3$); **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.51-7.41 (m, 6H), 7.24-7.18 (m, 6H), 7.18-7.10 (m, 6H), 6.99-6.92 (m, 2H), 6.56-6.44 (m, 1H), 5.73-5.62 (m, 1H), 4.31-4.25 (m, 1H), 4.03-3.87 (m, 4H), 1.32-1.21 (m, 6H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 155.5, 155.4, 146.1, 142.3, 128.9, 128.4, 127.8, 127.0, 126.8, 126.6, 115.7, 114.2, 71.9, 61.7, 61.6, 61.6, 61.6, 60.5, 60.3, 16.4, 16.4, 16.3, 16.3; **^{31}P NMR (162 MHz, $CDCl_3$):** δ 19.09; **ESI-LCMS:** *m/z* 534.0 $(M+Na)^+$; **HPLC:** *ee* 100% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 3% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 10.0 min for (*S*)-isomer].

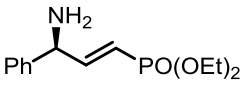
General procedure for the synthesis of diethyl (*E*)-(3-amino-3-phenylprop-1-en-1-yl)phosphonate ((±)-52 or (*R*)-52 or (*S*)-52)



(±)-**52**



(*R*)-**52**



(*S*)-**52**

Trityl protected amine (±)-**69** or (*R*)-**69** or (*S*)-**69** (0.5 g, 1.0 equiv.) was dissolved in DCM and trifluoroacetic acid (150 µL, 3.0 eq) was added at 25 °C and reaction mixture was stirred for 30 min. After completion of the reaction (TLC), DCM was removed under reduced pressure. Water (10 mL) was added to the residue and the aqueous layer was washed with diethyl ether (3X20 mL). The remaining aqueous layer was basified with a saturated

aqueous solution of $NaHCO_3$ until pH 9, after which it was extracted with DCM (3X20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give crude amine (±)-**52** or (*R*)-**52** or (*S*)-**52**, respectively as pale yellow syrup in quantitative yield, which was used for the final coupling reaction without any further purification. R_f = 0.54 (MeOH-DCM, 1:9); **^{1}H NMR (200 MHz, $CDCl_3$):** δ 7.51-7.16 (m, 5H), 7.08-6.76 (m, 1H), 6.10-5.83 (m, 1H), 4.74-4.59 (m, 1H), 4.21-3.93 (m, 4H), 1.43-1.18 (m, 6H); **^{13}C NMR (50 MHz, $CDCl_3$):** δ 155.0, 154.9, 142.3, 128.8, 127.7, 127.5, 126.8, 125.9, 117.4, 113.7, 61.8, 61.7, 58.3, 57.8, 16.4, 16.3; **ESI-LCMS:** *m/z* 292.0 $(M+Na)^+$.

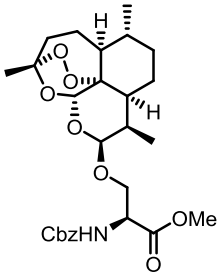
General procedure for hydrolysis of methyl ester (70a or 71a or 73a or 74a or 78a or 79a) to the corresponding carboxylic acid (70b or 71b or 73b or 74b or 78b or 79b)

Methyl ester (**70a** or **71a** or **73a** or **74a** or **78a** or **79a**) (1.5 mmol) was dissolved in THF (17 mL) and a solution of 2M aq. LiOH (7 mL) was added at 25 °C with stirring and stirred for 2 h at 25 °C. After completion of the reaction (TLC), THF was evaporated and the remaining aqueous layer was neutralized with acetic acid. The compound was extracted with ethyl acetate (3x20 mL), dried over Na_2SO_4 and concentrated under vacuum to obtain the corresponding crude carboxylic acid (**70b** or **71b** or **73b** or **74b** or **78b** or **79b**), which was used as such for the subsequent steps.

General procedure for peptide coupling reaction

L-Phenylalanine methyl ester or L-leucine methyl ester or γ-amino vinyl phosphonate (±)-**52** or (*R*)-**52** or (*S*)-**52** (0.5 mmol), HOBt (0.5 mmol) and free acid obtained from the ester hydrolysis **70b** or **71b** or **73b** or **74b** or **78b** or **79b** (0.5 mmol) were dissolved in dry THF (5 mL) and the resulting solution was stirred in an ice-cooled water bath then DCC (0.6 mmol) was added. Stirring was continued for 1 h at 0 °C and then an additional 1 h at 25 °C. After completion of reaction (TLC), the solid which precipitated was removed by filtration and the solvent was evaporated in vacuum. After evaporation of the solvent, the resulting crude product was dissolved in EtOAc and washed with a saturated aqueous $NaHCO_3$ solution (3X20 mL). Finally, the crude peptide derivative was purified by chromatography on a silica gel column to furnish the corresponding peptide.

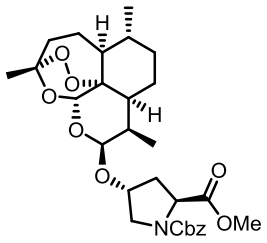
Methyl *N*-((benzyloxy)carbonyl)-*O*-((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyl decahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)-*L*-serinate (70a)



70a

To a solution of dihydroartemisinin **12** (0.46 g, 1.0 equiv.) and alcohol **46** (0.50 g, 1.2 equiv.) in DCM (7 mL), $BF_3{\cdot}Et_2O$ (40 µL) was added. The resulting mixture was stirred at 25 °C for 3 h and then washed with aqueous $NaHCO_3$ (10 mL) followed by brine (10 mL). The organic layers were dried over (Na_2SO_4) and concentrated in vacuum to afford the crude product which was purified by column chromatography (silica gel) using ethyl acetate: petroleum ether (1:9) as eluant to furnish the pure product **70a** (0.47 g, 55%) as colorless solid; $\boldsymbol{R_f}$ = 0.42 (EtOAc-petroleum ether, 3:7); $\boldsymbol{[\alpha]_D^{20}}$ = +57.76 (*c* 1.0, $CHCl_3$); **IR ($CHCl_3$):** 3439, 3019, 2955, 2876, 1723, 1698, 1505, 1455, 1377, 1215, 1028 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.44-7.30 (m, 5H), 5.73 (d, *J* = 8.4 Hz, 1H), 5.39 (s, 1H), 5.21-5.10 (m, 2H), 4.76 (d, *J* = 3.1 Hz, 1H), 4.63-4.54 (m, 1H), 4.08 (dd, *J* = 10.3, 2.3 Hz, 1H), 3.93 (dd, *J* = 10.3, 3.4 Hz, 1 H), 3.77 (s, 3H), 2.69-2.58 (m, 1H), 2.38 (dt, *J* = 13.9, 3.8 Hz, 1H), 2.10-1.99 (m, 1H), 1.94-1.83 (m, 1H), 1.78-1.69 (m, 1H), 1.69-1.45 (m, 5H), 1.43 (s, 3H), 1.39-1.18 (m, 2H), 0.95 (d, *J* = 6.5 Hz, 3H), 0.84 (d, *J* = 7.6 Hz, 3H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 170.7, 155.8, 136.3, 128.5, 128.2, 128.1, 104.2, 103.0, 88.0, 80.9, 69.9, 67.1, 54.5, 52.5, 44.2, 37.4, 36.4, 34.5, 30.7, 26.1, 24.6, 24.4, 20.3, 12.8; **ESI-LCMS:** *m/z* 542.8 $(M+Na)^+$; **HRMS (ESI):** *m/z* for $C_{27}H_{37}O_9NNa$ $(M+Na)^+$: calcd 542.2361, found 542.2355; **HPLC:** Chiralpak-IB (0.46 mm ϕ X 250 mmL), 10% IPA in hexane, flow rate 1.0 mL min^{-1}, UV detection at 210 nm, t_R = 10.7 min for **70a (β)** and t_R = 13.2 min for **70a (α)**.

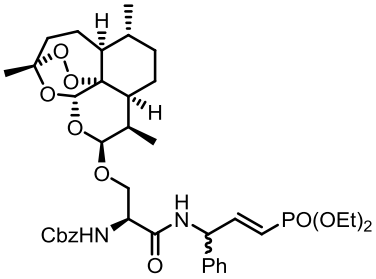
1-Benzyl-2-methyl(2*S*,4*R*)-4-(((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyldeca-hydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)pyrrolidine-1,2-di carboxylate (71a)



71a

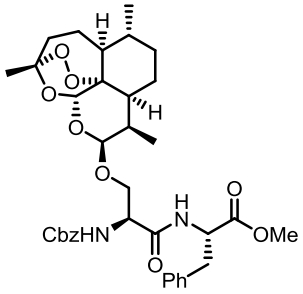
To a solution of dihydroartemisinin **12** (2.72 g, 1.0 equiv.) and alcohol **47** (6.7 g, 1.2 equiv.) in DCM (60 mL) was added $BF_3{\cdot}Et_2O$ (240 µL) at rt. The resulting mixture was stirred at room temperature for 3 h and then washed with aqueous sodium bicarbonate (30 mL) followed by brine (30 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo to furnish the crude product which was purified by column chromatography (silica gel) using petroleum ether-ethyl acetate (9:1) as eluant to afford the pure product **71a** as colorless syrup (3.51 g, 67%). $\boldsymbol{R_f}$ = 0.34 (EtOAc-petroleum ether, 3:7); **IR ($CHCl_3$):** 3436, 3019, 2956, 2876, 1747, 1704, 1605, 1455, 1422, 1358, 1215 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.43-7.25 (m, 5H), 5.40 (s, 1H), 5.34-5.31 (m, 1H), 5.25-5.01 (m, 2H), 4.89-4.80 (m, 1H), 4.59-4.37 (m, 2H), 3.83-3.75 (m, 2H), 3.72-3.56 (m, 3H), 2.68-2.58 (m, 1H), 2.44-2.31 (m, 2H), 2.26-2.14 (m, 1H), 2.13-2.01 (m, 1H), 1.95-1.86 (m, 1H), 1.79-1.47 (m, 5H), 1.44 (s, 3H), 1.38-1.19 (m, 3H), 0.98 (d, J = 6.1 Hz, 3H), 0.89-0.73 (m, 3H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 173.0, 172.8, 155.0, 154.4, 136.5, 128.5, 128.4, 128.1, 128.0, 127.9, 127.9, 127.7, 104.2, 100.8, 100.7, 98.5, 91.1, 88.2, 80.9, 75.2, 75.0, 74.1, 67.3, 67.1, 58.3, 58.1, 57.8, 57.7, 53.0, 52.5, 52.4, 52.2, 51.8, 51.6, 51.5, 45.2, 44.2, 37.9, 37.5, 37.4, 37.0, 36.4, 36.2, 35.3, 34.5, 34.2, 32.4, 30.5, 26.1, 25.9, 24.6, 24.5, 22.2, 20.3, 12.7, 12.6; **ESI-LCMS:** m/z 568.1 $(M+Na)^+$; **HRMS (ESI):** m/z for $C_{29}H_{39}O_9NNa$ $(M+Na)^+$: calcd 568.2517, found 568.2513; **HPLC:** Chiralpak-IB (0.46 mm ϕ X 250 mmL), 10% IPA in hexane, flow rate 1.0 mL min^{-1}, UV detection at 210 nm, t_R = 12.7 min for **71a (β)** and t_R = 17.0 min for **71a (α)**.

Benzyl ((2*S*)-1-(((*E*)-3-(diethoxyphosphoryl)-1-phenylallyl)amino)-1-oxo-3-(((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)propan-2-yl)carbamate (72)



Pale yellow syrup; $\boldsymbol{R_f}$ = 0.34 (MeOH-DCM, 1:19); **IR ($CHCl_3$):** 3428, 3019, 2929, 1687, 1601, 1496, 1450, 1394, 1216, 1029 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.39-7.14 (m, 11H), 6.91-6.75 (m, 1H), 5.89-5.76 (m, 1H), 5.76-5.68 (m, 2H), 5.32 (d, *J* = 7.6 Hz, 1H), 5.28 (s, 1H), 5.14-5.03 (m, 2H), 4.80-4.69 (m, 1H), 4.43 (brs, 1H), 4.12-3.91 (m, 5H), 2.60-2.50 (m, 1H), 2.38-2.25 (m, 1H), 2.06-1.90 (m, 3H), 1.87-1.75 (m, 1H), 1.53-1.42 (m, 2H), 1.39-1.35 (m, 4H), 1.31-1.22 (m, 7H), 1.21-1.13 (m, 2H), 0.90-0.85 (m, 3H), 0.76-0.66 (m, 3H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 168.9, 168.9, 156.2, 150.2, 138.5, 129.1, 128.7, 128.7, 128.4, 128.3, 128.3, 128.2, 127.4, 127.3, 118.8, 116.9, 104.3, 104.2, 103.1, 102.9, 88.0, 80.9, 80.9, 69.4, 69.0, 67.3, 62.1, 55.4, 55.2, 53.5, 52.5, 52.4, 44.2, 37.4, 36.4, 34.5, 30.8, 29.8, 26.1, 24.7, 24.5, 20.4, 17.7, 16.4, 12.9, 12.9; **^{31}P NMR (202 MHz, $CDCl_3$):** δ 17.61; **ESI-LCMS:** *m/z* 779.1 $(M+Na)^+$; **HRMS (ESI):** *m/z* for $C_{39}H_{53}O_{11}N_2NaP$ $(M+Na)^+$: calcd 779.3279, found 779.3270; **HPLC:** Chiralpak-IB (0.46 mm ϕ X 250 mmL), 10% IPA in hexane, flow rate 1.0 mL min^{-1}, UV detection at 210 nm, t_R = 15.1 min and t_R = 17.6 min.

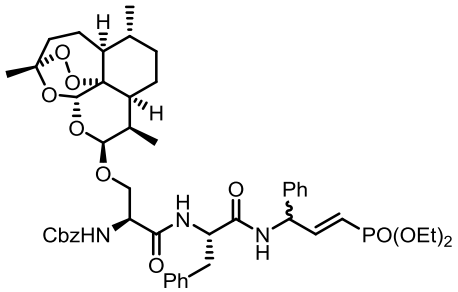
Methyl *N*-((benzyloxy)carbonyl)-*O*-((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyl deca-hydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)-*L*-seryl-*L*-phenyl-alaninate (73a)



Colorless solid; $\boldsymbol{R_f}$ = 0.40 (EtOAc-petroleum ether, 2:3); **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.45-7.03 (m, 10H), 5.57 (brs, 1H), 5.46-5.30 (m, 1H), 5.26-5.07 (m, 2H), 4.98-4.74 (m, 2H), 4.63-4.27 (m, 1H), 3.99 (dd, *J* = 10.4, 6.1 Hz, 1H), 3.87-3.59 (m, 4H), 3.27-3.02 (m, 2H), 2.74-2.55 (m, 1H), 2.37 (dt, *J* = 14.0, 3.7 Hz, 1H), 2.09-1.97 (m, 1H), 1.96-1.82 (m,

1H), 1.71 (brs, 2H), 1.68-1.60 (m, 1H), 1.59-1.50 (m, 2H), 1.43 (s, 3H), 1.38-1.20 (m, 3H), 0.98-0.91 (m, 3H), 0.88-0.78 (m, 3 H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 171.4, 169.7, 169.2, 135.6, 129.3, 129.2, 128.6, 128.6, 128.5, 128.2, 128.1, 127.2, 126.9, 104.2, 103.0, 101.0, 91.1, 87.9, 80.9, 68.9, 67.2, 67.0, 54.7, 53.4, 52.5, 52.3, 52.2, 51.5, 45.2, 44.2, 37.9, 37.8, 37.4, 37.2, 36.4, 36.2, 34.6, 34.2, 30.7, 26.1, 25.8, 24.6, 24.4, 22.1, 20.3, 12.8, 12.4; **ESI-LCMS:** *m/z* 689.1 $(M+Na)^+$; **HRMS (ESI):** *m/z* for $C_{36}H_{46}O_{10}N_2Na$ $(M+Na)^+$: calcd 689.3045, found 689.3029.

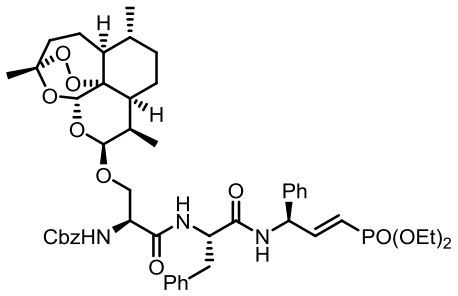
Benzyl ((2*S*)-1-(((2*S*)-1-(((*E*)-3-(diethoxyphosphoryl)-1-phenylallyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-(((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyl decahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)propan-2-yl) carbamate (75)



Colorless solid; R_f = 0.40 (MeOH-DCM, 1:19); $[\alpha]_D^{24}$ +41.5 (*c* 1.03, $CHCl_3$); **IR ($CHCl_3$):** 3422, 2106, 1643, 1217, 1027, 977, 759, 666 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$):** 7.40-7.26 (m, 9H), 7.23-7.15 (m, 4H), 7.15-7.09 (m, 2H), 7.08-6.99 (m, 2H), 6.88-6.72 (m, 2H), 5.85-5.59 (m, 3H), 5.45-5.34 (m, 1H), 5.16-5.05 (m, 1H), 5.04-4.96 (m, 1H), 4.85-4.64 (m, 2H), 4.34-4.22 (m, 1H), 4.13-3.98 (m, 4H), 3.98-3.81 (m, 2H), 3.27-3.12 (m, 1H), 3.03-2.91 (m, 1H), 2.68-2.57 (m, 1H), 2.43-2.29 (m, 1H), 2.10-1.99 (m, 2H), 1.94-1.83 (m, 1H), 1.76-1.66 (m, 1H), 1.66-1.51 (m, 2H), 1.47-1.39 (m, 4H), 1.36-1.24 (m, 8H), 0.97-0.90 (m, 3H), 0.88-0.78 (m, 3H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 169.6, 169.5, 169.3, 156.4, 150.2, 138.6, 138.4, 135.9, 135.7, 129.2, 128.9, 128.8, 128.8, 128.6, 128.6, 128.4, 128.3, 128.1, 128.0, 127.5, 127.3, 127.2, 127.1, 118.4, 116.9, 104.3, 103.3, 101.9, 87.9, 80.8, 80.8, 69.1, 69.0, 67.4, 67.4, 62.0, 61.9, 55.9, 55.7, 55.3, 55.0, 54.2, 52.4, 52.4, 44.1, 44.1, 38.0, 37.4, 36.3, 34.6, 30.7, 26.0, 24.7, 24.6, 20.3, 20.3, 16.3, 12.9; **^{31}P NMR (162 MHz, $CDCl_3$):** δ 17.76; **ESI-LCMS:** *m/z* 926.1 $(M+Na)^+$; **HRMS** (ESI): *m/z* calcd for $C_{48}H_{62}O_{12}N_3NaP$ $[M + Na]^+$ 926.3963; found: 926.3947; **HPLC:** Chiralpak-IA (0.46 mm ϕ X 250 mmL), 18% IPA in

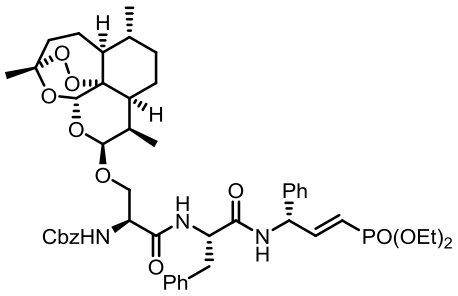
hexane, flow rate 1.0 mL min^{-1}, UV detection at 210 nm, t_R = 15.3 min for **75*R*** diastereomer and t_R = 19.3 min for **75*S*** diastereomer.

Benzyl ((*S*)-1-(((*S*)-1-(((*R*,*E*)-3-(diethoxyphosphoryl)-1-phenylallyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-(((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyl decahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)propan-2-yl) carbamate (75*R*)



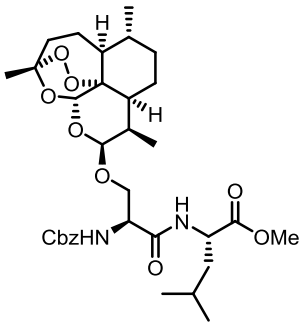
Colorless solid; R_f = 0.38 (MeOH-DCM, 1:19); **$[\alpha]_D^{24}$** +61.7 (*c* 1.01, $CHCl_3$); **IR ($CHCl_3$):** 3418, 3020, 1668, 1505, 1216, 1027, 770, 670 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.40-7.03 (m, 15H), 6.83-6.69 (m, 2H), 5.76-5.63 (m, 2H), 5.33 (s, 1H), 4.94 (d, *J* = 11.9 Hz, 1H), 4.81-4.70 (m, 2H), 4.67 (d, *J* = 6.0 Hz, 1H), 4.24-4.17 (m, 1H), 4.08-3.96 (m, 4H), 3.91-3.78 (m, 2H), 3.23-3.11 (m, 1H), 2.96-2.89 (m, 1H), 2.64-2.54 (m, 1H), 2.40-2.27 (m, 1H), 2.05-1.94 (m, 2H), 1.89-1.79 (m, 1H), 1.72-1.63 (m, 1H), 1.61-1.51 (m, 1H), 1.45-1.37 (m, 4H), 1.33-1.18 (m, 8H), 0.97-0.87 (m, 3H), 0.85-0.75 (m, 3H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 169.5, 169.2, 156.5, 150.2, 138.7, 135.9, 135.8, 129.4, 129.3, 129.2, 129.1, 128.9, 128.7, 128.7, 128.6, 128.6, 128.5, 128.3, 128.2, 128.1, 127.8, 127.6, 127.3, 118.6, 116.8, 104.3, 104.2, 103.4, 88.0, 80.9, 69.0, 67.5, 62.0, 62.0, 61.9, 56.0, 55.2, 55.0, 54.2, 52.5, 44.2, 44.1, 37.9, 37.5, 37.4, 36.4, 34.6, 30.8, 26.1, 24.7, 24.7, 20.4, 16.5, 16.4, 12.9; **^{31}P NMR (162 MHz, $CDCl_3$):** δ 17.77; **ESI-LCMS:** *m*/*z* 926.1 $(M+Na)^+$; **HRMS** (ESI): *m*/*z* calcd for $C_{48}H_{62}O_{12}N_3NaP$ $[M + Na]^+$ 926.3963; found: 926.3945; **HPLC:** *de* 100% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 18% IPA in hexane, flow rate 1.0 mL min^{-1}, UV detection at 210 nm, t_R = 15.4 min for **75*R*** diastereomer].

Benzyl ((*S*)-1-(((*S*)-1-(((*S*,*E*)-3-(diethoxyphosphoryl)-1-phenylallyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-(((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyl decahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)propan-2-yl) carbamate (75*S*)



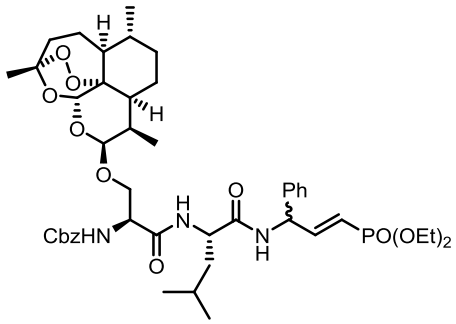
Colorless solid; R_f = 0.38 (MeOH-DCM, 1:19); $[\alpha]_D^{24}$ +25.0 (*c* 1.03, $CHCl_3$); **IR ($CHCl_3$):** 3424, 3020, 2095, 1641, 1215, 1027, 756, 668 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.45-7.25 (m, 9H), 7.24-7.01 (m, 7H), 7.01-6.89 (m, 2H), 6.88-6.75 (m, 1H), 5.78-5.68 (m, 2H), 5.43-5.38 (m, 1H), 5.18-5.06 (m, 1H), 5.05-4.93 (m, 1H), 4.79 (d, *J* = 3.4 Hz, 1H), 4.76-4.65 (m, 1H), 4.30 (td, *J* = 4.8, 6.8 Hz, 1H), 4.12-4.00 (m, 4H), 3.99-3.94 (m, 1H), 3.92-3.85 (m, 1H), 3.26-3.13 (m, 1H), 3.03-2.91 (m, 1H), 2.69-2.58 (m, 1H), 2.44-2.31 (m, 1H), 2.10-2.00 (m, 1H), 1.93-1.85 (m, 1H), 1.78-1.68 (m, 1H), 1.66-1.53 (m, 2H), 1.50-1.40 (m, 4H), 1.35-1.25 (m, 8H), 0.95 (d, *J* = 6.5 Hz, 3H), 0.85 (d, *J* = 7.2 Hz, 3H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 169.5, 169.2, 156.4, 150.1, 138.5, 135.9, 129.2, 128.9, 128.8, 128.6, 128.3, 128.1, 127.3, 127.2, 118.6, 117.1, 104.2, 103.3, 88.0, 80.8, 69.1, 67.5, 62.0, 61.9, 55.7, 55.3, 55.1, 54.4, 52.4, 44.1, 38.0, 37.4, 36.3, 34.6, 30.7, 26.1, 24.7, 24.6, 22.7, 20.3, 16.4, 12.9; **^{31}P NMR (162 MHz, $CDCl_3$):** δ 17.65; **ESI-LCMS:** *m/z* 926.1 $(M+Na)^+$; **HRMS** (ESI): *m/z* calcd for $C_{48}H_{62}O_{12}N_3NaP$ $[M + Na]^+$ 926.3963; found: 926.3950; **HPLC:** *de* 100% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 18% IPA in hexane, flow rate 1.0 mL min^{-1}, UV detection at 210 nm, t_R = 19.1 min for **75*S*** diastereomer].

Methyl *N*-((benzyloxy)carbonyl)-*O*-((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyl decahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)-*L*-seryl-*L*-leucinate (74a)



Colorless syrup; R_f = 0.30 (EtOAc-petroleum ether, 3:7); **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.39-7.26 (m, 5H), 6.67-6.58 (m, 1H), 5.41 (s, 1H), 5.11 (s, 2H), 4.82 (brs, 1H), 4.64-4.54 (m, 1H), 4.38 (brs, 1H), 3.96 (dd, *J* = 10.7, 6.1 Hz, 1H), 3.85 (dd, *J* = 10.7, 4.6 Hz, 1H), 3.73-3.68 (m, 4H), 2.68-2.56 (m, 1H), 2.41-2.27 (m, 1H), 2.06-1.95 (m, 2H), 1.92-1.78 (m, 2 H), 1.72-1.42 (m, 10H), 1.40 (s, 3H), 1.31-1.18 (m, 3H), 0.91 (brs, 3H), 0.90 (brs, 6H), 0.85 (d, 3 H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 173.8, 173.0, 169.9, 169.5, 156.1, 136.1, 128.6, 128.3, 128.2, 128.1, 104.3, 103.2, 88.0, 80.9, 69.3, 67.3, 54.8, 52.5, 52.4, 50.9, 50.8, 44.2, 41.8, 41.6, 37.3, 36.4, 34.6, 30.8, 29.8, 26.1, 24.9, 24.7, 24.6, 23.2, 22.9, 22.8, 22.1, 22.0, 20.4, 13.1, 12.9; **ESI-LCMS:** *m/z* 655.1 $(M+Na)^+$; **HRMS (ESI):** *m/z* for $C_{33}H_{48}O_{10}N_2Na$ $(M+Na)^+$: calcd 655.3201, found 655.3190.

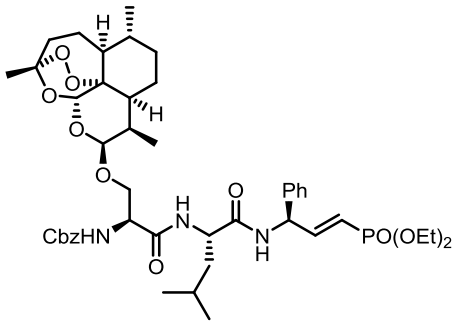
Benzyl ((2*S*)-1-(((2*S*)-1-(((*E*)-3-(diethoxyphosphoryl)-1-phenylallyl)amino)-4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-(((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyl decahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)propan-2-yl) carbamate (76)



Colorless solid; R_f = 0.40 (MeOH-DCM, 1:19); **$[\alpha]_D^{25}$** +47.7 (*c* 1.02, $CHCl_3$); **IR ($CHCl_3$):** 3422, 2112, 1645, 1217, 1025, 976, 873, 762, 666 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.46-7.22 (m, 10H), 7.00-6.76 (m, 2H), 5.96-5.68 (m, 3H), 5.48-5.3 (m, 1H), 5.22-4.97 (m, 2H), 4.78 (brs, 1H), 4.60-4.45 (m, 1H), 4.40-4.25 (m, 1H), 4.17-3.79 (m, 6H), 2.63 (brs, 1H), 2.44-2.30 (m, 1H), 2.24-2.10 (m, 2H), 2.09-1.96 (m, 1H), 1.95-1.81 (m, 1H), 1.79-1.65 (m, 2H), 1.64-1.54 (m, 2H), 1.53-1.45 (m, 2H), 1.42 (s, 3H), 1.37-1.21 (m, 8H), 0.97-0.82 (m, 12H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 170.9, 170.7, 169.7, 156.4, 150.5, 138.7, 138.7, 136.0, 135.8, 128.9, 128.6, 128.3, 128.3, 128.1, 128.0, 127.5, 127.3, 118.7, 118.6, 116.9, 116.7, 104.2, 103.3, 88.0, 87.9, 80.8, 80.8, 69.2, 69.1, 67.4, 67.4, 67.2, 62.0, 62.0, 55.6, 55.5, 55.3, 55.1, 54.9, 52.4, 52.4, 52.1, 51.9, 44.1, 44.0, 40.8, 40.6, 37.3, 36.3, 34.5, 34.4, 30.7, 30.7, 26.0, 24.8, 24.7, 24.6, 24.5, 22.9, 22.0, 22.0, 20.3, 20.3, 16.4, 16.3, 16.3, 12.8;

^{31}P NMR (162 MHz, $CDCl_3$): δ 17.68; **ESI-LCMS:** *m/z* 892.2 $(M+Na)^+$; **HRMS** (ESI): *m/z* calcd for $C_{45}H_{64}O_{12}N_3NaP$ $[M + Na]^+$ 892.4120; found: 892.4106; **HPLC:** Chiralpak-IB (0.46 mm ϕ X 250 mmL), 10% IPA in hexane, flow rate 0.8 mL min^{-1}, UV detection at 215 nm, t_R = 11.4 min for **76*R*** diastereomer and t_R = 14.2 min for **76*S*** diastereomer.

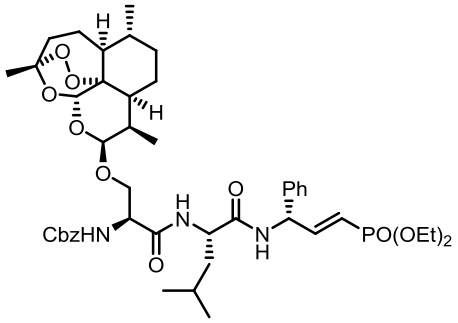
Benzyl ((*S*)-1-(((*S*)-1-(((*R*,*E*)-3-(diethoxyphosphoryl)-1-phenylallyl)amino)-4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-(((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyl decahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)propan-2-yl) carbamate (76*R*)



Colorless solid; R_f = 0.38 (MeOH-DCM, 1:19); $[\alpha]_D^{25}$ +68.9 (*c* 1.03, $CHCl_3$); **IR ($CHCl_3$):** 3421, 3021, 2402, 2095, 1654, 1508, 1216, 1028, 976, 767, 669 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.39-7.32 (m, 5H), 7.32-7.26 (m, 5H), 6.97-6.83 (m, 1H), 6.62 (d, *J* = 8.0 Hz, 1H), 5.87 (t, *J* = 17.9 Hz, 1H), 5.76 (brs, 2H), 5.32 (s, 1H), 5.11-4.97 (m, 2H), 4.83-4.75 (m, 1H), 4.58-4.49 (m, 1H), 4.35-4.25 (m, 1H), 4.13-4.01 (m, 4H), 3.90-3.80 (m, 2H), 2.68-2.59 (m, 1H), 2.37 (dt, *J* = 13.9, 3.8 Hz, 1H), 2.09-1.98 (m, 2H), 1.93-1.82 (m, 1H), 1.80-1.66 (m, 2H), 1.65-1.55 (m, 3H), 1.54-1.45 (m, 3H), 1.44-1.40 (m, 3H), 1.35-1.28 (m, 6H), 1.29-1.19 (m, 2H), 0.96-0.82 (m, 12H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 170.6, 169.7, 156.4, 150.5, 150.4, 138.7, 135.8, 129.0, 128.8, 128.6, 128.4, 128.1, 127.5, 127.3, 118.5, 117.0, 104.2, 103.3, 87.9, 80.8, 69.0, 67.4, 62.0, 55.5, 55.1, 55.0, 52.4, 51.9, 44.0, 40.6, 37.3, 36.3, 34.4, 30.7, 26.0, 24.8, 24.6, 22.9, 22.0, 20.3, 16.3, 12.8; **^{31}P NMR (162 MHz, $CDCl_3$):** δ 17.71; **ESI-LCMS:** *m/z* 892.2 $(M+Na)^+$; **HRMS** (ESI): *m/z* calcd for $C_{45}H_{64}O_{12}N_3NaP$ $[M + Na]^+$ 892.4120; found: 892.4100; **HPLC:** *de* 86% [Chiralpak-IB (0.46 mm ϕ X 250 mmL), 10% IPA in hexane, flow rate 0.8 mL min^{-1}, UV detection at 215 nm, t_R = 11.5 min for **76*R*** diastereomer].

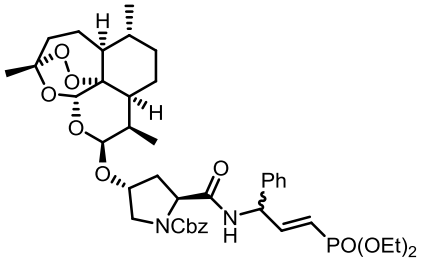
Benzyl ((*S*)-1-(((*S*)-1-(((*S*,*E*)-3-(diethoxyphosphoryl)-1-phenylallyl)amino)-4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-(((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyl

decahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)propan-2-yl) carbamate (76*S*)



Colorless solid; R_f = 0.40 (MeOH-DCM, 1:19); $[\alpha]_D^{24}$ +26.4 (*c* 1.01, $CHCl_3$); **IR ($CHCl_3$):** 3417, 1639, 1218, 1030, 769, 672 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.42-7.28 (m, 10H), 6.99-6.79 (m, 2H), 5.94-5.71 (m, 3H), 5.42 (s, 1H), 5.21-5.08 (m, 2H), 4.85-4.76 (m, 1H), 4.56-4.47 (m, 1H), 4.39-4.31 (m, 1H), 4.13-3.89 (m, 6H), 2.69-2.58 (m, 1H), 2.38 (dt, *J* = 13.9, 3.4 Hz, 1H), 2.14-1.97 (m, 2H), 1.96-1.83 (m, 1H), 1.80-1.66 (m, 2H), 1.65-1.46 (m, 5H), 1.43 (s, 3H), 1.34-1.25 (m, 8H), 0.94 (d, *J* = 6.1 Hz, 3H), 0.90-0.87 (m, 6H), 0.87-0.84 (m, 3H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 170.8, 169.7, 150.4, 138.8, 136.0, 129.0, 128.6, 128.3, 128.2, 127.3, 118.5, 117.1, 104.2, 103.3, 88.0, 80.8, 69.1, 67.5, 62.0, 55.5, 55.3, 55.1, 52.4, 52.1, 44.1, 40.9, 37.3, 36.3, 34.5, 30.7, 26.0, 24.8, 24.6, 22.9, 21.9, 20.3, 16.3, 12.8; **^{31}P NMR (162 MHz, $CDCl_3$):** δ 17.65; **ESI-LCMS:** *m/z* 892.2 $(M+Na)^+$; **HRMS** (ESI): *m/z* calcd for $C_{45}H_{64}O_{12}N_3NaP$ $[M + Na]^+$ 892.4120; found: 892.4108; **HPLC:** *de* 83% [Chiralpak-IB (0.46 mm ϕ X 250 mmL), 10% IPA in hexane, flow rate 0.8 mL min^{-1}, UV detection at 215 nm, t_R = 15.0 min for **76*S*** diastereomer].

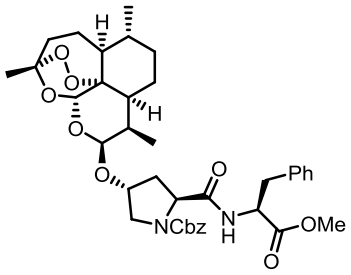
Benzyl (2*S*,4*R*)-2-(((*E*)-3-(diethoxyphosphoryl)-1-phenylallyl)carbamoyl)-4-(((3*R*,5a*S*, 6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)pyrrolidine-1-carboxylate (77)



Colorless syrup; R_f = 0.34 (MeOH-DCM, 1:19); **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.41-7.06 (m, 10H), 6.93-6.76 (m, 1H), 6.03-5.75 (m, 1H), 5.74-5.63 (m, 1H), 5.39-5.31 (m, 1H),

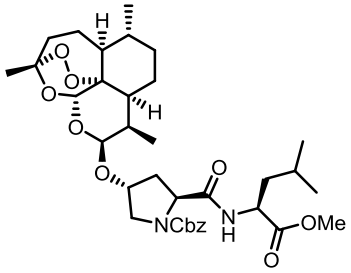
5.22-5.02 (m, 2H), 4.84-4.71 (m, 1H), 4.54-4.34 (m, 2H), 4.15-3.95 (m, 4H), 3.83-3.61 (m, 1H), 3.43-3.23 (m, 1H), 2.62-2.51 (m, 1H), 2.50-2.39 (m, 1H), 2.38-2.28 (m, 1H), 2.21-2.08 (m, 1H), 2.07-1.93 (m, 2H), 1.91-1.80 (m, 2H), 1.69-1.51 (m, 3H), 1.42-1.37 (m, 3H), 1.34-1.19 (m, 9H), 0.97-0.89 (m, 3H), 0.66 (d, 3H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 170.4, 156.8, 150.7, 150.4, 138.5, 136.0, 129.1, 128.7, 128.4, 128.3, 128.2, 127.5, 127.3, 118.4, 116.7, 114.2, 104.3, 100.0, 91.2, 88.2, 81.0, 74.6, 67.9, 62.1, 59.7, 55.4, 52.5, 51.7, 45.2, 44.2, 37.5, 37.4, 36.4, 36.3, 35.5, 34.5, 30.5, 29.8, 26.2, 26.0, 24.7, 24.5, 22.3, 20.4, 16.5, 16.5, 16.4, 12.7; **^{31}P NMR (162 MHz, $CDCl_3$):** δ 18.02; **ESI-LCMS:** *m/z* 805.2 $(M+Na)^+$; **HRMS (ESI):** *m/z* for $C_{41}H_{55}O_{11}N_2NaP$ $(M+Na)^+$: calcd 805.3436, found 805.3423.

Benzyl (2*S*,4*R*)-2-(((*S*)-1-methoxy-1-oxo-3-phenylpropan-2-yl)carbamoyl)-4-(((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)pyrrolidine-1-carboxylate (78a)



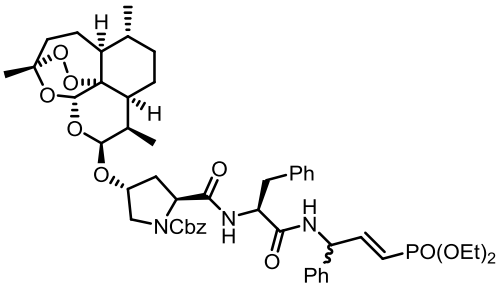
Colorless syrup; **$[\alpha]_D^{20}$** +30.8 (*c* 0.5, $CHCl_3$); **^{1}H NMR (200 MHz, $CDCl_3$):** δ 7.34-7.09 (m, 10H), 5.35 (s, 1H), 5.12 (s, 2H), 4.86-4.76 (m, 2H), 4.41-4.39 (m, 2H), 3.71 (s, 3H), 3.85-3.52 (m, 2H), 3.29-2.98 (m, 2H), 2.61-2.53 (m, 1H), 2.34-2.20 (m, 3H), 1.96-1.85 (m, 3H), 1.62-1.57 (m, 4H), 1.41 (s, 3H), 1.28-1.12 (m, 3H), 0.94 (d, 3H), 0.70 (d, 3H); **^{13}C NMR (50 MHz, $CDCl_3$):** δ 171.7, 170.8, 156.3, 136.2, 135.8, 129.3, 128.5, 128.4, 128.1, 128.0, 127.0, 104.2, 100.0, 88.0, 80.9, 74.4, 67.5, 59.3, 53.3, 52.4, 52.3, 51.5, 44.2, 37.8, 37.4, 36.3, 35.5, 34.5, 30.4, 26.1, 24.6, 24.4, 20.3, 12.6; **ESI-LCMS:** *m/z* 715.5 $(M+Na)^+$.

Benzyl (2*S*,4*R*)-2-(((*S*)-1-methoxy-4-methyl-1-oxopentan-2-yl)carbamoyl)-4-(((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)pyrrolidine-1-carboxylate (79a)



Colorless solid; R_f = 0.40 (EtOAc-petroleum ether, 1:1); **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.60-7.23 (m, 5H), 5.49-5.28 (m, 1H), 5.27-5.02 (m, 2H), 4.90-4.74 (m, 1H), 4.66-4.36 (m, 3H), 3.86-3.61 (m, 4H), 3.52-3.34 (m, 1H), 2.66-2.46 (m, 1H), 2.37 (dt, J = 13.7, 3.7 Hz, 1H), 2.25-2.14 (m, 1H), 2.11-1.98 (m, 1H), 1.96-1.84 (m, 1H), 1.80-1.72 (m, 1H), 1.71-1.57 (m, 5H), 1.43 (s, 3H), 1.36-1.19 (m, 3H), 0.97 (d, 3H), 0.95-0.88 (m, 6H), 0.73 (d, 3H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 173.2, 170.8, 156.5, 136.4, 136.2, 128.5, 128.5, 128.2, 128.0, 127.6, 104.2, 100.1, 98.7, 91.1, 88.1, 80.9, 80.2, 75.8, 74.5, 73.7, 67.5, 67.4, 60.1, 59.4, 58.7, 53.0, 52.5, 52.2, 51.6, 51.0, 50.5, 45.2, 44.2, 41.4, 38.3, 37.5, 37.4, 36.4, 36.3, 35.4, 34.5, 34.2, 32.5, 30.4, 26.1, 25.9, 24.8, 24.7, 24.6, 24.5, 22.7, 22.2, 22.0, 20.3, 12.6, 12.5; **ESI-LCMS:** *m/z* 681.1 $(M+Na)^+$; **HRMS (ESI):** *m/z* for $C_{35}H_{50}O_{10}N_2Na$ $(M+Na)^+$: calcd 681.3358, found 681.3335.

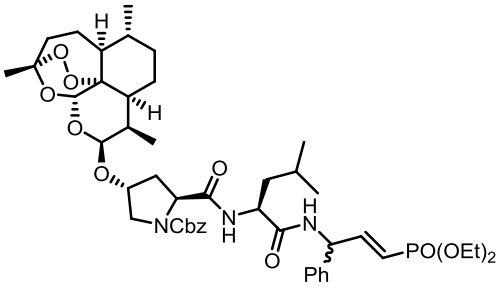
Benzyl (2*S*,4*R*)-2-(((2*S*)-1-(((*E*)-3-(diethoxyphosphoryl)-1-phenylallyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)-4-(((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyl decahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)pyrrolidine-1-carboxylate (80)



Colorless syrup; **IR ($CHCl_3$):** 3409, 3368, 3018, 2957, 2876, 1682, 1497, 1455, 1416, 1358, 1216, 1030 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$):** δ 7.17-7.30 (m, 15H), 6.75-6.94 (m, 1H), 5.71-5.93 (m, 2H), 4.80-5.30 (m, 2H), 4.73-4.90 (m, 2H), 4.27-4.39 (m, 2H), 3.96-4.13 (m, 2H), 3.57-4.13 (m, 2H), 3.13-3.33 (m, 2H), 2.27-2.57 (m, 3H), 1.85-1.99 (m, 3H), 1.49-1.70 (m, 3H), 1.42 (s, 3H), 1.22-1.32 (m, 10H), 0.94 (d, 3H), 0.63 (d, 3H); **^{13}C NMR (50 MHz,**

$CDCl_3$): δ 171.4, 169.9, 156.2, 151.5, 138.4, 136.8, 136.1, 129.2, 128.8, 128.7, 128.6, 128.5, 128.0, 127.9, 127.4, 126.9, 117.1, 104.2, 99.9, 88.1, 80.9, 73.9, 67.7, 62.1, 60.6, 55.3, 53.8, 52.4, 51.8, 44.1, 37.4, 36.9, 36.8, 36.3, 34.4, 30.4, 26.1, 24.6, 24.4, 20.3, 16.3, 16.2, 12.6; **^{31}P NMR (162 MHz, $CDCl_3$):** δ 18.21; **ESI-LCMS:** *m/z* 953.2 $(M+Na)^+$.

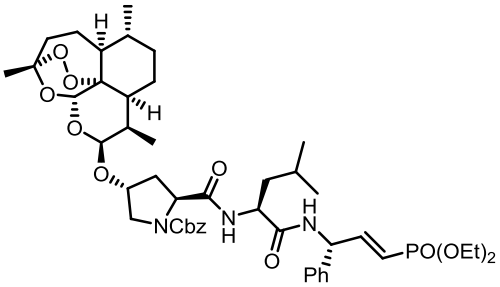
Benzyl (2*S*,4*R*)-2-(((2*S*)-1-(((*E*)-3-(diethoxyphosphoryl)-1-phenylallyl)amino)-4-methyl-1-oxopentan-2-yl)carbamoyl)-4-(((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyl decahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)pyrrolidine-1-carboxylate (81)



Colorless solid; R_f = 0.32 (MeOH-DCM, 1:19); **$[\alpha]_D^{26}$** +9.0 (*c* 1.01, $CHCl_3$); **IR ($CHCl_3$):** 3419, 3018, 2962, 2402, 1683, 1519, 1418, 1353, 1217, 1101, 1029, 989, 876, 768, 668 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.52-7.24 (m, 10H), 7.03-6.82 (m, 1H), 6.45 (d, *J* = 7.3 Hz, 1H), 6.03-5.65 (m, 2H), 5.46-5.29 (m, 1H), 5.28-5.07 (m, 1H), 5.06-4.72 (m, 2H), 4.62-4.29 (m, 3H), 4.20-3.95 (m, 4H), 3.82-3.61 (m, 1H), 3.41 (t, *J* = 11.6 Hz, 1H), 2.60 (brs, 1H), 2.44-2.29 (m, 2H), 2.17-1.99 (m, 4H), 1.96-1.76 (m, 2H), 1.72-1.53 (m, 5H), 1.52-1.40 (m, 5H), 1.39-1.21 (m, 9H), 0.97 (d, 3H), 0.94-0.85 (m, 6H), 0.76-0.62 (m, 3H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 171.4, 171.0, 156.5, 150.8, 150.5, 150.5, 138.8, 138.7, 128.9, 128.8, 128.6, 128.1, 127.8, 127.3, 119.9, 118.7, 118.4, 116.6, 116.6, 116.6, 104.2, 100.0, 91.1, 88.1, 80.9, 80.3, 74.1, 74.0, 68.0, 67.8, 61.9, 60.6, 60.3, 55.4, 55.1, 54.9, 52.5, 52.1, 51.9, 51.6, 45.2, 44.1, 40.1, 37.5, 37.4, 36.7, 36.3, 34.5, 34.2, 30.4, 26.1, 25.9, 25.1, 24.9, 24.6, 24.5, 23.0, 22.2, 21.6, 21.5, 20.3, 16.4, 16.3, 12.6, 12.6; **^{31}P NMR (202 MHz, $CDCl_3$):** δ 18.01; **ESI-LCMS:** *m/z* 918.1 $(M+Na)^+$; **HRMS** (ESI): *m/z* calcd for $C_{47}H_{66}O_{12}N_3NaP$ $[M + Na]^+$ 918.4276; found: 918.4255; **HPLC:** Chiralpak-IA (0.46 mm ϕ X 250 mmL), 20% IPA in hexane, flow rate 1.0 mL min^{-1}, UV detection at 215 nm, t_R = 11.8 min for **81*S* (β)** and t_R = 14.8 min for **81*S* (α)**, and t_R = 20.6 min for **81*R* (α)** and t_R = 31.2 min for **81*R* (β)**.

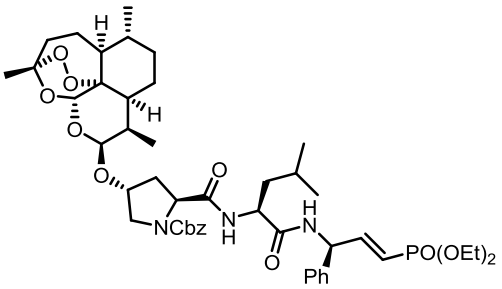
Benzyl (2*S*,4*R*)-2-(((*S*)-1-(((*R*,*E*)-3-(diethoxyphosphoryl)-1-phenylallyl)amino)-4-methyl-1-oxopentan-2-yl)carbamoyl)-4-(((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyl

decahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)pyrrolidine-1-carboxylate (81*R*)



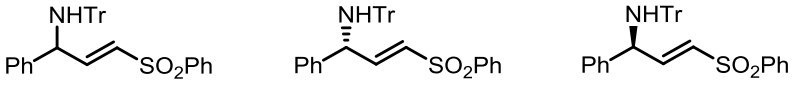
Colorless solid; R_f = 0.30 (MeOH-DCM, 1:19); **[α]$_D^{26}$** +28.7 (*c* 1.00, $CHCl_3$); **IR ($CHCl_3$):** 3420, 3339, 3018, 2963, 2878, 2402, 1684, 1516, 1418, 1352, 1216, 1099, 1029, 989, 876, 771, 669 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.44-7.22 (m, 10H), 6.92 (t, *J* = 18.3 Hz, 1H), 6.50-6.33 (m, 1H), 5.92 (t, *J* = 17.5 Hz, 1H), 5.80 (brs, 1H), 5.42-5.28 (m, 1H), 5.05-4.93 (m, 1H), 4.90-4.71 (m, 2H), 4.49 (brs, 2H), 4.41-4.26 (m, 1H), 4.14-4.00 (m, 4H), 3.94-3.59 (m, 1H), 3.44-3.33 (m, 1H), 2.65-2.54 (m, 1H), 2.43-2.26 (m, 2H), 2.18-1.78 (m, 5H), 1.71-1.48 (m, 5H), 1.47-1.40 (m, 4H), 1.36-1.20 (m, 9H), 0.97 (brs, 3H), 0.94-0.87 (m, 6H), 0.67 (brs, 3H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 171.3, 171.0, 156.5, 150.5, 138.8, 135.7, 128.8, 128.6, 128.5, 128.4, 128.1, 127.8, 127.2, 118.3, 116.8, 104.2, 99.9, 91.2, 88.1, 80.9, 74.0, 67.8, 61.9, 60.6, 55.0, 54.9, 52.5, 51.9, 51.6, 45.2, 44.1, 40.1, 37.5, 36.7, 36.3, 34.4, 34.1, 30.4, 26.1, 25.9, 25.1, 24.6, 24.4, 23.0, 21.5, 20.3, 16.4, 16.3, 12.6; **^{31}P NMR (162 MHz, $CDCl_3$):** δ 18.05; **ESI-LCMS:** *m/z* 918.1 $(M+Na)^+$; **HRMS** (ESI): *m/z* calcd for $C_{47}H_{66}O_{12}N_3NaP$ $[M + Na]^+$ 918.4276; found: 918.4257; **HPLC:** Chiralpak-IA (0.46 mm ϕ X 250 mmL), 20% IPA in hexane, flow rate 1.0 mL min^{-1}, UV detection at 215 nm, t_R = 20.1 min for **81*R* (α)** and t_R = 29.6 min for **81*R* (β)**.

Benzyl (2*S*,4*R*)-2-(((*S*)-1-(((*S*,*E*)-3-(diethoxyphosphoryl)-1-phenylallyl)amino)-4-methyl-1-oxopentan-2-yl)carbamoyl)-4-(((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyl decahydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)pyrrolidine-1-carboxylate (81*S*)



Colorless solid; R_f = 0.32 (MeOH-DCM, 1:19); $[\alpha]_D^{26}$ -14.5 (*c* 1.04, $CHCl_3$); **IR ($CHCl_3$):** 3420, 3019, 2964, 2402, 1682, 1519, 1419, 1353, 1216, 1099, 1029, 989, 876, 769, 670 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.47-7.34 (m, 7H), 7.33-7.28 (m, 3H), 6.93 (t, *J* = 18.3 Hz, 1H), 6.79-6.64 (m, 1H), 5.92-5.68 (m, 2H), 5.40 (brs, 1H), 5.30-5.09 (m, 2H), 4.80 (brs, 1H), 4.62-4.33 (m, 3H), 4.20-3.96 (m, 4H), 3.95-3.62 (m, 1H), 3.44 (d, *J* = 10.7 Hz, 1H), 2.61 (brs, 1H), 2.50-2.14 (m, 3H), 2.12-1.75 (m, 4H), 1.74-1.53 (m, 5H), 1.53-1.38 (m, 5H), 1.37-1.19 (m, 9H), 0.98 (d, 3H), 0.95-0.91 (m, 3H), 0.90-0.86 (m, 3H), 0.71 (d, 3H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 171.3, 171.0, 156.7, 150.8, 138.7, 136.1, 128.9, 128.6, 128.4, 128.2, 128.0, 127.8, 127.3, 118.5, 117.1, 104.2, 99.9, 91.2, 88.1, 80.9, 74.1, 68.0, 61.9, 60.4, 55.4, 55.2, 52.5, 52.1, 51.9, 51.6, 44.2, 40.1, 37.5, 36.3, 34.5, 30.4, 26.1, 25.9, 25.0, 24.6, 24.5, 23.0, 22.2, 21.6, 20.3, 16.3, 12.6; **^{31}P NMR (162 MHz, $CDCl_3$):** δ 17.96; **ESI-LCMS:** *m/z* 918.2 $(M+Na)^+$; **HRMS** (ESI): *m/z* calcd for $C_{47}H_{66}O_{12}N_3NaP$ $[M + Na]^+$ 918.4276; found: 918.4258; **HPLC:** Chiralpak-IA (0.46 mm ϕ X 250 mmL), 20% IPA in hexane, flow rate 1.0 mL min^{-1}, UV detection at 215 nm, t_R = 11.3 min for **81*S* (β)** and t_R = 14.1 min for **81*S* (α)**.

General procedure for the synthesis of (*E*)-1-phenyl-3-(phenylsulfonyl)-*N*-tritylprop-2-en-1-amine ((±)-91 or (*R*)-91 or (*S*)-91)



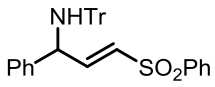
(±)-**91** (*R*)-**91** (*S*)-**91**

2-Phenyl-2-(tritylamino)ethan-1-ol (±)-**67** or (*R*)-**67** or (*S*)-**67** (1.32 mmol, 1.0 equiv.) was dissolved in DCM (10 mL) and cooled to 0 °C. To this cold solution was added Dess-Martin periodinane (1.98 mmol, 1.5 equiv.) portion wise over 10 min and then stirred at 0 °C for 10 min. The reaction mixture was allowed to slowly warm to 25 °C and stirred for 30 min. After completion of the reaction (TLC), the reaction mixture was diluted with DCM. The reaction mixture was placed in an ice-water bath and a 1:1 mixture of saturated aqueous

$NaHCO_3$ solution and saturated $NaHSO_3$ solution (4 mL) was added and the cooling bath was removed and the mixture was stirred at 25 °C until the formation of two clear layers was observed. The reaction mixture was transferred to a separatory funnel containing a saturated aqueous $NaHCO_3$ solution (20 mL). The aqueous layer was extracted with ethyl acetate (3X25 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated to give crude aldehyde (±)-**68** or (*R*)-**68** or (*S*)-**68**, respectively as colorless foaming solid. The crude aldehyde residue was used immediately for the next step without any further purification.

Diethyl ((phenylsulfonyl)methyl)phosphonate (2.24 mmol, 1.7 equiv.) was taken up in dry THF (4 mL) and was cooled to 0 °C in an ice bath. NaH (60 % dispersion in mineral oil, 1.98 mmol, 1.5 equiv.) was added to the reaction mixture in portion wise over a period of 5-10 min. The solution was stirred at 0 °C for 15 min. Crude aldehyde (±)-**68** or (*R*)-**68** or (*S*)-**68** was taken in dry THF (4 mL) and was added to the reaction mixture. The reaction mixture was then warmed to 25 °C and stirred for 3 h. After completion of the reaction (TLC), reaction mixture was concentrated in vacuo. The residue was dissolved in water, extracted with DCM (3X20 mL). The combined organic layers were washed with water, brine and concentrated to give crude product which was purified either by recrystallization from EtOAc-Petroleum ether mixture or column chromatography on a silica gel column with ethyl acetate: petroleum ether as eluant to give pure olefin (±)-**91** or (*S*)-**91** or (*R*)-**91**, respectively as colorless solid.

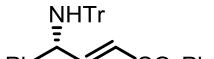
(*E*)-1-phenyl-3-(phenylsulfonyl)-*N*-tritylprop-2-en-1-amine (91)



(±)-**91**

Colorless solid; **m.p.:** 168-169 °C; R_f = 0.40 (EtOAc-petroleum ether, 1:4); **^{1}H NMR (200 MHz, $CDCl_3$):** δ 7.74-7.44 (m, 5H), 7.43-7.30 (m, 6H), 7.24-7.07 (m, 12H), 7.05-6.92 (m, 2H), 6.67 (dd, *J* = 14.9, 6.2 Hz, 1H), 6.13 (d, *J* = 14.9 Hz, 1H), 4.26 (t, *J* = 5.3 Hz, 1H), 2.45 (d, *J* = 5.7 Hz, 1H); **^{13}C NMR (50 MHz, $CDCl_3$):** δ 149.1, 145.8, 141.3, 140.6, 133.2, 129.2, 128.8, 127.9, 127.7, 127.4, 127.1, 126.8, 72.0, 59.0; **ESI-LCMS:** *m/z* 538.0 $(M+Na)^+$; **HPLC:** Chiralpak-IA (0.46 mm ϕ X 250 mmL), 5% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 11.3 min for (*R*)-isomer and t_R = 12.8 min for (*S*)-isomer.

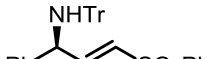
(*R*,*E*)-1-phenyl-3-(phenylsulfonyl)-*N*-tritylprop-2-en-1-amine ((*R*)-91)



(*R*)-**91**

Colorless solid; **m.p.:** 163-165 °C; $\boldsymbol{R_f}$ = 0.40 (EtOAc-petroleum ether, 1:4); $\boldsymbol{[\alpha]_D^{25}}$ -52.3 (*c* 1.0, $CHCl_3$); **ESI-LCMS:** *m/z* 538.0 $(M+Na)^+$; **HPLC:** *ee* 100% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 5% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 11.3 min for (*R*)-isomer].

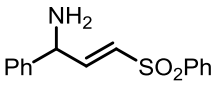
(*S*,*E*)-1-phenyl-3-(phenylsulfonyl)-*N*-tritylprop-2-en-1-amine ((*S*)-91)



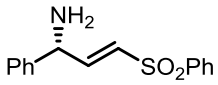
(*S*)-**91**

Colorless solid; **m.p.:** 165-166 °C; $\boldsymbol{R_f}$ = 0.40 (EtOAc-petroleum ether, 1:4); $\boldsymbol{[\alpha]_D^{25}}$ +59.0 (*c* 1.03, $CHCl_3$); **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.72-7.67 (m, 2H), 7.64 (t, *J* = 7.4 Hz, 1H), 7.56-7.50 (m, 2H), 7.44-7.38 (m, 6H), 7.26-7.12 (m, 12H), 7.06-7.00 (m, 2H), 6.71 (dd, *J* = 14.9, 6.5 Hz, 1H), 6.17 (dd, *J* = 14.9, 1.1 Hz, 1H), 4.31 (d, *J* = 6.1 Hz, 1H), 2.49 (brs, 1H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 149.0, 145.8, 141.3, 140.6, 133.2, 129.2, 128.9, 128.8, 128.7, 127.9, 127.7, 127.4, 127.0, 126.7, 72.0, 59.0; **ESI-LCMS:** *m/z* 538.0 $(M+Na)^+$; **HRMS (ESI):** *m/z* for $C_{34}H_{29}O_2NNaS$ $(M+Na)^+$: calcd 538.1811, found 538.1805; **HPLC:** *ee* 99.2% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 5% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 12.6 min for (*S*)-isomer].

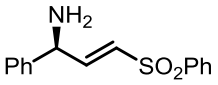
General procedure for the synthesis of (*E*)-1-phenyl-3-(phenylsulfonyl)prop-2-en-1-amine ((±)-90 or (*R*)-90 or (*S*)-90)



(±)-**90**



(*R*)-**90**

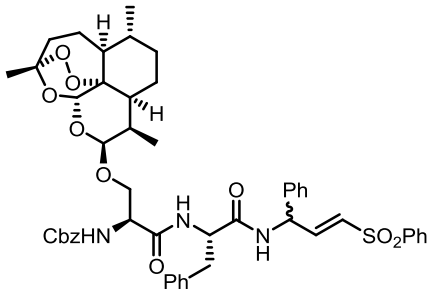


(*S*)-**90**

Trityl protected amine (±)-**91** or (*R*)-**91** or (*S*)-**91** (1.0 g, 1.94 mmol, 1.0 equiv.) was dissolved in DCM (30 mL) and trifluoroacetic acid (300 µL, 2.0 equiv.) was added at 25 °C and reaction mixture was stirred for 30 min. After completion of the reaction (TLC), DCM was removed under reduced pressure. Water (15 mL) was added to the residue and the aqueous layer was washed with diethyl ether (3X20 mL). The remaining aqueous layer was basified with a saturated aqueous solution of $NaHCO_3$ until pH 9, after which it was

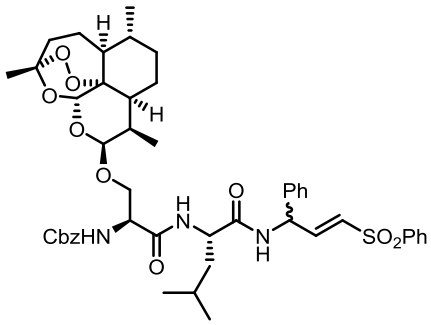
extracted with DCM (3X20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give crude amine (±)-**90** or (*R*)-**90** or (*S*)-**90**, respectively as colorless solid in quantitative yield, which was used for the final coupling reaction without any further purification. R_f = 0.42 (MeOH-DCM, 1:19); **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.92-7.81 (m, 2H), 7.66-7.57 (m, 1H), 7.56-7.48 (m, 2H), 7.39-7.18 (m, 5H), 7.09 (dd, *J* = 15.3, 4.6 Hz, 1H), 6.65 (dd, *J* = 14.5, 1.5 Hz, 1H), 4.76-4.67 (m, 1H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 148.7, 141.5, 140.4, 133.5, 129.7, 129.4, 129.1, 128.2, 127.8, 126.9, 56.4; **ESI-LCMS:** *m/z* 296.0 $(M+Na)^+$.

Benzyl ((2*S*)-1-oxo-1-(((2*S*)-1-oxo-3-phenyl-1-(((*E*)-1-phenyl-3-(phenylsulfonyl)allyl) amino)propan-2-yl)amino)-3-(((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyldeca hydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)propan-2-yl) carbamate (92)



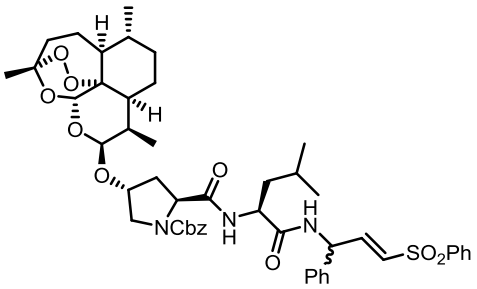
Colorless solid; R_f = 0.37 (EtOAc-petroleum ether, 1:1); **^{1}H NMR (400 MHz, $CDCl_3$):** δ 8.00-7.80 (m, 2H), 7.72-7.48 (m, 3H), 7.46-7.23 (m, 8H), 7.23-6.99 (m, 7H), 6.99-6.82 (m, 1H), 6.80-6.62 (m, 1H), 6.56-6.31 (m, 1H), 5.93-5.78 (m, 2H), 5.49-5.33 (m, 1H), 5.16-4.88 (m, 1H), 4.83-4.56 (m, 2H), 4.28-4.08 (m, 1H), 3.97-3.77 (m, 1H), 3.32-3.07 (m, 1H), 2.99-2.82 (m, 1H), 2.72-2.56 (m, 1H), 2.48-2.27 (m, 1H), 2.11-1.80 (m, 4H), 1.79-1.68 (m, 1H), 1.67-1.51 (m, 2H), 1.47-1.40 (m, 3 H), 1.37-1.17 (m, 5H), 1.01-0.92 (m, 3H), 0.89-0.80 (m, 3H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 169.6, 169.5, 169.3, 156.6, 145.1, 144.5, 140.2, 137.9, 135.7, 135.6, 135.5, 133.6, 133.4, 131.3, 129.4, 129.3, 129.3, 129.1, 129.1, 129.0, 128.9, 128.6, 128.6, 128.5, 128.4, 128.1, 127.7, 127.7, 127.4, 127.4, 127.3, 127.2, 104.3, 103.4, 88.0, 80.8, 69.1, 69.0, 67.7, 67.5, 56.3, 56.0, 54.1, 53.3, 52.3, 44.0, 37.5, 36.3, 34.5, 34.1, 30.7, 29.7, 26.0, 24.7, 24.6, 23.2, 22.7, 20.3, 14.1, 12.9; **ESI-LCMS:** *m/z* 930.1 $(M+Na)^+$; **HRMS** (ESI): *m/z* calcd for $C_{50}H_{57}O_{11}N_3NaS$ $[M + Na]^+$ 930.3606; found: 930.3594.

Benzyl ((2*S*)-1-(((2*S*)-4-methyl-1-oxo-1-(((*E*)-1-phenyl-3-(phenylsulfonyl)allyl)amino) pentan-2-yl)amino)-1-oxo-3-(((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyldeca hydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)propan-2-yl) carbamate (93)



Colorless solid; R_f = 0.46 (EtOAc-petroleum ether, 1:1); **^{1}H NMR (400 MHz, $CDCl_3$):** δ 8.03-7.82 (m, 2H), 7.73-7.47 (m, 3H), 7.42-7.32 (m, 6H), 7.31-7.22 (m, 3H), 7.14 (td, *J* = 15.1, 4.0 Hz, 1H), 6.64-6.41 (m, 2H), 5.91-5.77 (m, 1H), 5.72 (brs, 1H), 5.47-5.37 (m, 1H), 5.25-5.15 (m, 1H), 5.08-4.94 (m, 1H), 4.85-4.71 (m, 1H), 4.58-4.33 (m, 1H), 4.33-4.17 (m, 1H), 4.14-3.94 (m, 1H), 3.93-3.72 (m, 1H), 2.65 (brs, 1H), 2.47-2.30 (m, 1H), 2.11-1.96 (m, 1H), 1.96-1.85 (m, 1H), 1.84-1.67 (m, 5H), 1.66-1.47 (m, 4H), 1.45-1.39 (m, 3H), 1.37-1.19 (m, 2H), 1.09-0.80 (m, 12H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 170.6, 169.9, 156.2, 144.5, 140.1, 137.8, 135.9, 133.6, 133.5, 131.4, 129.4, 129.3, 129.2, 129.1, 128.6, 128.5, 128.4, 128.1, 127.7, 127.4, 127.3, 126.2, 104.3, 103.5, 88.0, 88.0, 80.8, 69.1, 67.7, 67.6, 55.7, 53.5, 53.3, 52.4, 52.2, 44.1, 40.1, 37.3, 36.3, 34.5, 34.4, 30.7, 26.0, 24.9, 24.6, 24.6, 22.9, 21.8, 20.3, 12.8; **ESI-LCMS:** *m/z* 896.1 $(M+Na)^+$; **HRMS (ESI):** *m/z* for $C_{47}H_{59}O_{11}N_3NaS$ $(M+Na)^+$: calcd 896.3763, found 896.3752.

Benzyl (2*S*,4*R*)-2-(((2*S*)-4-methyl-1-oxo-1-(((*E*)-1-phenyl-3-(phenylsulfonyl)allyl)amino) pentan-2-yl)carbamoyl)-4-(((3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyldeca hydro-12*H*-3,12-epoxy[1,2]dioxepino[4,3-*i*]isochromen-10-yl)oxy)pyrrolidine-1-carboxylate (94)



Colorless solid; R_f = 0.47 (MeOH-DCM, 1:19); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 171.6, 171.5, 171.1, 156.9, 156.6, 145.0, 144.7, 140.3, 140.2, 138.1, 138.0, 136.0, 135.7, 134.1, 133.6, 133.4, 131.4, 129.4, 129.2, 129.0, 128.7, 128.4, 128.2, 128.1, 128.0, 127.8, 127.3, 126.3, 104.4, 100.1, 91.3, 88.2, 81.0, 74.2, 74.0, 68.2, 68.0, 60.8, 60.6, 53.5, 53.4, 52.5, 52.2, 51.7, 44.2, 40.4, 39.9, 39.7, 37.6, 37.5, 36.9, 36.8, 36.4, 34.7, 34.5, 31.7, 30.5, 30.2, 29.1, 27.0, 26.2, 26.0, 25.4, 25.1, 25.1, 24.7, 24.6, 23.1, 22.7, 21.6, 21.5, 20.8, 20.4, 14.2, 12.7, 11.5; **ESI-LCMS:** *m/z* 922.1 $(M+Na)^+$; **HRMS (ESI):** *m/z* for $C_{49}H_{61}O_{11}N_3NaS$ $(M+Na)^+$: calcd 922.3919, found 922.3907.

Antimalarial activity protocol:

All the *Plasmodium falciparum* strains used in the present study (3D7, 7G8 and Dd2) were obtained from Malaria Research and Reference Reagent Resource Center (MR4).

(a) Expression and purification of recombinant falcpain-2 enzyme:

Recombinant falcipain-2 was prepared according to the method described by Shenai *et al.*[63] and Kumar *et al.*[64] with slight modification. Briefly, *Escherichia coli* M15 containing pQE30-FP-2 plasmid were grown to mid-log phase and induced with isopropyl-1-thio-β-D-galactopyranoside (IPTG, 0.5 mM) for 5 h at 37 °C. Cells were harvested, washed with ice-cold 100 mM Tris-Cl, 10 mM EDTA, pH 7.4, sonicated (12 cycles of 10 s each, with cooling for 10 s between the cycles), and centrifuged at 15,000 rpm for 45 min at 4 °C. The pellet was washed twice with 2.5 M urea, 20 mM Tris-Cl, 2.5% Triton X-100, pH 8.0; centrifuged at 15,000 rpm for 45 min at 4 °C; and solubilized in 6 M guanidine HCl, 20 mM Tris-Cl, 250 mM NaCl, 20 mM imidazole, pH 8.0 (5 mL/g of inclusion body pellet) at RT for 60 min with gentle stirring. Insoluble material was separated by centrifuging at 15,000 rpm for 60 min at 4 °C. For the purification of the recombinant protein, the supernatant was incubated overnight at 4 °C with a nickel-nitrilotriacetic acid (Ni-NTA) resin. The resin was loaded on a column and washed with 10 bed volumes each of 6 M guanidine HCl, 20 mM

Tris-Cl, 250 mM NaCl, pH 8.0; 8 M urea, 20 mM Tris-Cl, 500 mM NaCl, pH 8.0; and 8 M urea, 20 mM Tris-Cl, 30 mM imidazole, pH 8.0. Bound protein was eluted with 8 M urea, 20 mM Tris-Cl, 1 M imidazole, pH 8.0 and quantified by the Bicinchoninic acid assay. For the refolding, the fractions containing falcipain-2 protein were pooled in the ice-cold refolding buffer: 100 mM diluted in Tris-Cl, 1 mM EDTA, 20% glycerol, 250 mM L-arginine, 1 mM GSH, 1 mM GSSG, pH 8.0 was added in a 100 fold dilution. The mixture was incubated with moderate stirring at 4 °C for 24 h, and concentrated to 25 mL using a stirred cell with a 10-kDa cut-off membrane (Pellicon XL device, Millipore) at 4 °C. The sample was then filtered using a 0.22-mm syringe filter. The purified and concentrated protein was quantified using bicinchoninic acid assay.

(b) Fluorometric assay for falcipain-2 activity:

For screening of falcipain-2 inhibitors, a 96-well plate fluorometric assay was developed following a protocol described by Kumar *et al.*[64] Briefly, reaction was set up in a 200 mL reaction mixture containing 100 mM NaOAc, 10 mM DTT, 6 mg enzyme and different concentrations of inhibitors, pH 5.5. 10 mM of fluorogenic substrate benzyloxycarbonyl-Phe-Arg-7-amino-4-methylcoumarin hydrochloride (ZFR-AMC) was added, and the release of 7-amino-4-methylcoumarin (AMC) was monitored (excitation 355 nm; emission 460 nm) over 30 min at RT in Perkin Elmer Victor3 multilabel counter. Activities were compared as fluorescence released over time in the assay without or with different concentration of each compound tested. The IC_{50} values were calculated from curve fittings by software Workout V 2.5. K_i values were derived from the Cheng-Prusoff equation relating both parameters when substrate concentration and K_M are known:

$$K_i = \frac{IC_{50}}{1+\left(\frac{[S]}{K_M}\right)}$$

(c) Expression and activity of heme detoxification protein (HDP)

Briefly, cultures of *E. coli* M15 cells containing plasmid pHDP (HDP gene cloned in pQE expression vector) were grown to mid-log phase, induced with IPTG (1 mM) for 4 h at 37 °C, and harvested by centrifugation at 4,000 × g for 20 min. The total cell pellet was resuspended in wash buffer (50 mM Tris·HCl at pH 7.5, 20 mM EDTA) containing 0.5

mg/mL lysozyme and incubated for 1 h at room temperature with intermittent shaking, and then with vigorous shaking for an additional 30 min. The washed cell pellet was lysed after adding wash buffer containing 0.5 M NaCl and 2.5% Triton X-100. The inclusion bodies were pelleted by centrifugation at 13,000 rpm for 50 min at 4 °C, resuspended in wash buffer containing 1% Triton X-100 using a sonicator, pelleted again, and then washed four times in wash buffer without Triton X-100. The inclusion bodies were solubilized for 30 min in 50 mM CAPS buffer at pH 11.0 containing 1.5% N-lauryl sarcosine and 0.3 M NaCl and centrifuged at 10,000 × g for 30 min. The protein was purified from the supernatant using a His-Trap, a high-performance nickel affinity column (GE Health Care) by an imidazole gradient in 50 mM CAPS at pH 11.0 containing 0.3% N-lauryl sarcosine and 0.3 M NaCl. Protein-containing fractions were pooled and dialyzed against 25 mM CAPS buffer (pH 11.0) containing 135 mM NaCl. The activity of the protein was assessed by its ability to convert heme to hemozoin (Hz).[65]

(d) *In vitro* hemozoin formation assay

The *in vitro* hemozoin formation assay were carried out in 1 mL reaction buffered with 500 mM sodium acetate at pH 5.2 with 5 mM reduced glutathione containing heme (300 µM) and 0.5 µM recombinant HDP, the reaction was carried out at 37 °C for 3h. Subsequently, free heme in the reaction was removed by repeated washing of the pellet with 2.5% SDS and 0.1M sodium bicarbonate (pH 9.1) followed by repeated washing with distilled water until no soluble heme was visible in the supernatant. The Hz pellet was resuspended in 1 mL of 0.1 N NaOH, and absorbance was measured at 400 nm, heme concentration was calculated from the standard curve. Heme stock (10mM) was prepared by dissolving 3.3 mg of hemin (Sigma) in 500 µl of 1 M NaOH, which was used to make dilutions ranging from 50 µm-600 µm to plot the standard curve. A reaction containing buffered heme alone was used as negative control. To assess the effect of different compounds on hemozoin formation, the assay was performed in the presence of the compounds at various concentrations.

(e) *P. falciparum* growth inhibition assay

P. falciparum strain 3D7, Dd2 and 7G8 were cultured in RPMI media (Invitrogen) supplemented with 0.5% albumax and 4% haematocrit using a protocol described previously.[66] Cultures were synchronized by repeated sorbitol treatment following a protocol

described previously.[67] Each growth inhibition assay was performed in triplicate and the experiment was repeated twice. Each well contained 0.5 mL of complete media [RPMI (invitrogen) with 0.5% albumax], 4% haematocrit and the parasitaemia adjusted to ~1%; the compound added to the parasite cultures to desired final concentrations (0-100 μM) and same amount of solvent (DMSO) was added to the control wells. The cultures were allowed to grow further for 48 h. Parasite growth was assessed by DNA fluorescent dye-binding assay using SYBR green (Sigma) following Smilkstein *et al.* procedure.[68]

(f) *In vitro* toxicity assay on CHO cells

The cytotoxicity assay was carried out using the CHO cells proliferation test. CHO cells were seeded in triplicates at 100 μL aliquots (1×10^4 cells per well) with DMEM medium in Nunclon flat bottom 96-well plates and were allowed to grow for 24 h. Subsequently, the medium was replaced by a test medium containing each inhibitor (0-500 μM), or DMSO as a control. The CHO cells were allowed to grow for further 24 h, and then 10 μL of the WST-1 reagent was added and plates were incubated for 30 min. The plates were read at 450 nm absorption and 630 nm reference wavelengths using a TECAN GENios Pro microplate reader. The percentage growth was calculated by comparing with the control set. The EC_{50} values for each compound were calculated from the growth inhibition curve.

(g) *In vivo* parasiticidal assays with *P. berghei* mice malaria model

Five groups of female BALB/c mice, 6-8 weeks of age (four mice/group) were used for testing the *in vivo* parasiticidal and protective efficacy of different drug treatments. Mice were injected IP with 1×10^6 *P. berghei* infected erythrocytes. The drug treatment was initiated 5 days after inducing parasitemia; mice in a group were given 4 doses of a compound at 12.5 mg/Kg of body weight. Control mice were given solvent alone. The parasitemia was assessed every day in thin smears of Giemsa stained RBCs from the tail blood of each mouse, by counting parasitized erythrocytes in more than 10,000 erythrocytes. Time of death of the mice in each group was also monitored.

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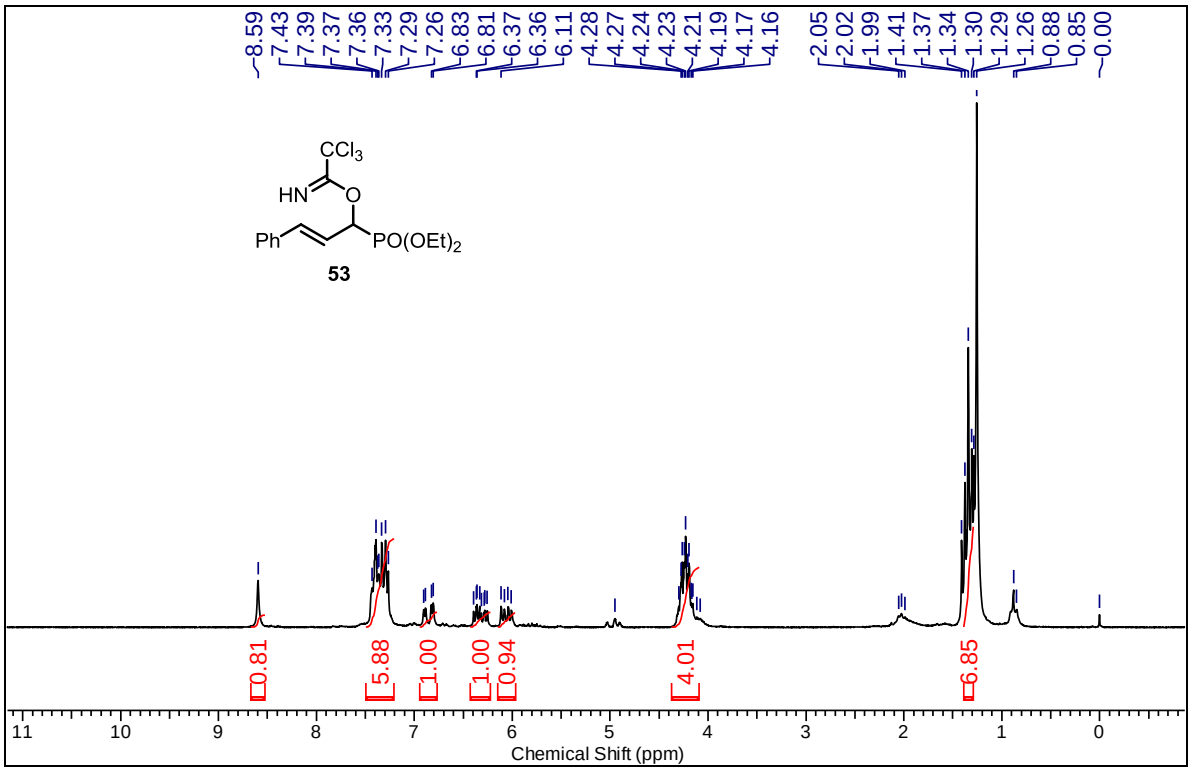
1.8. SPECTRA

^{1}H NMR of Compound 53 (200 MHz, $CDCl_3$)



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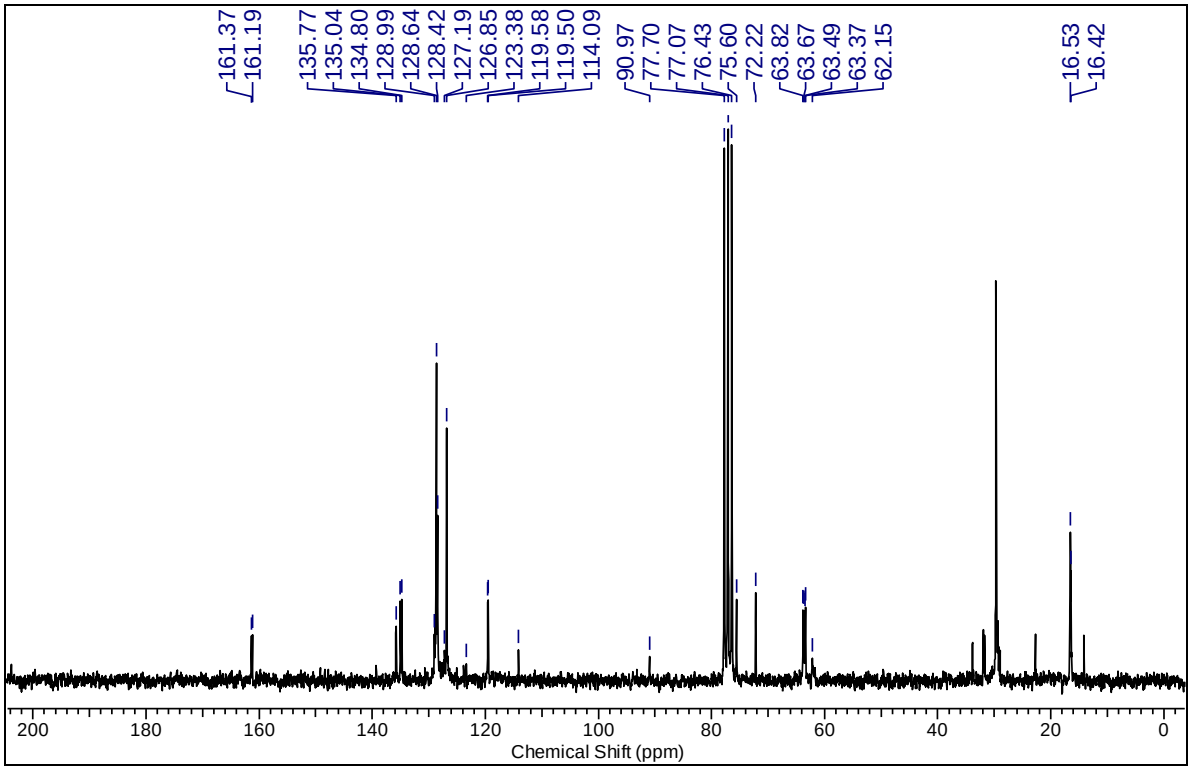

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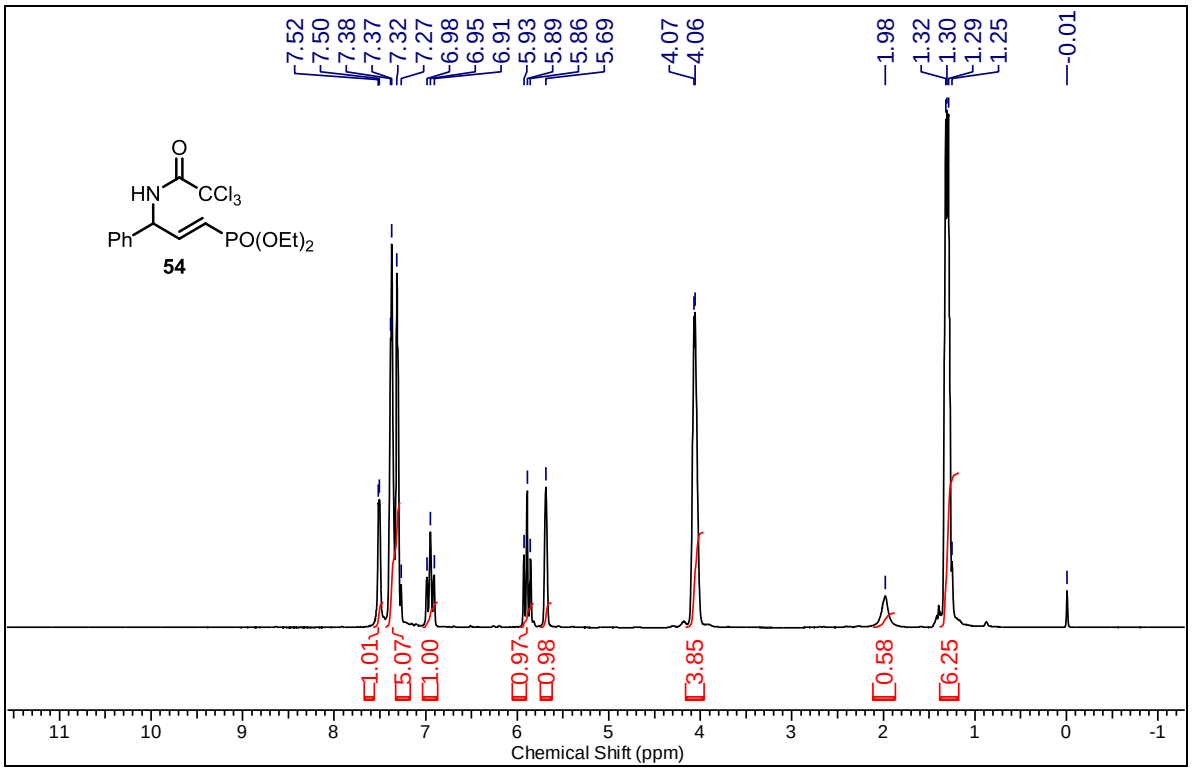

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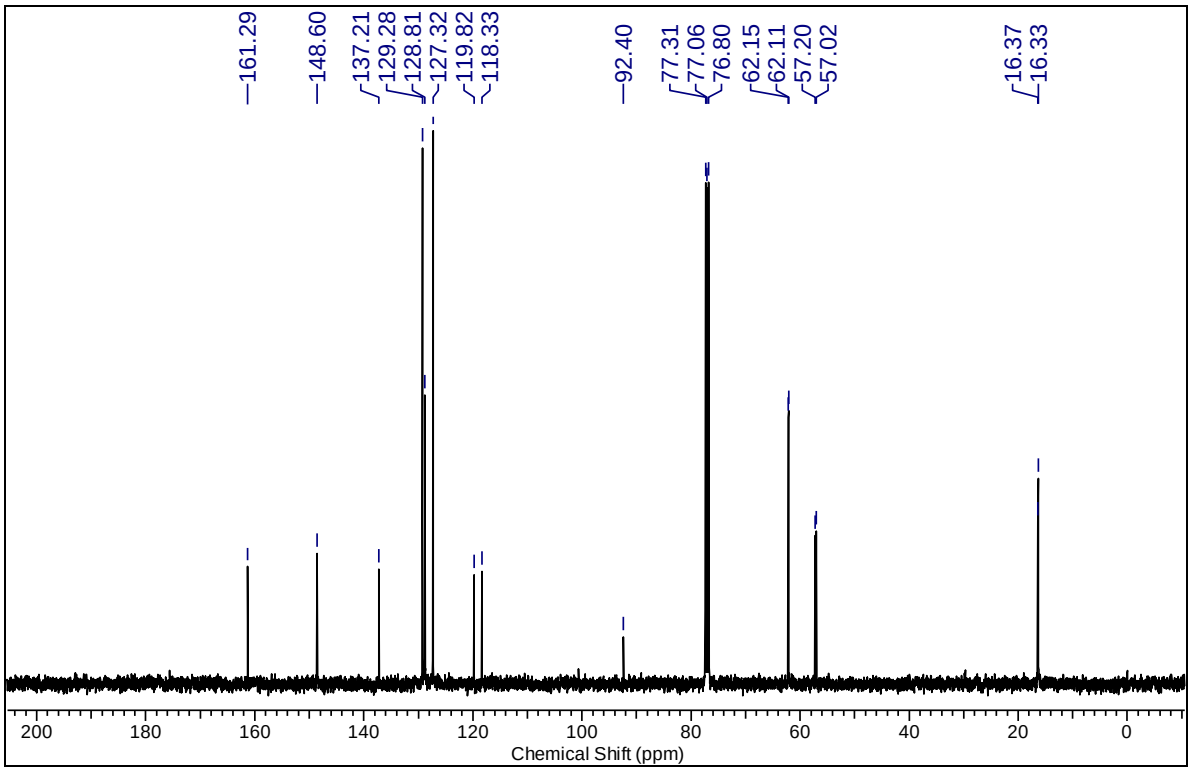

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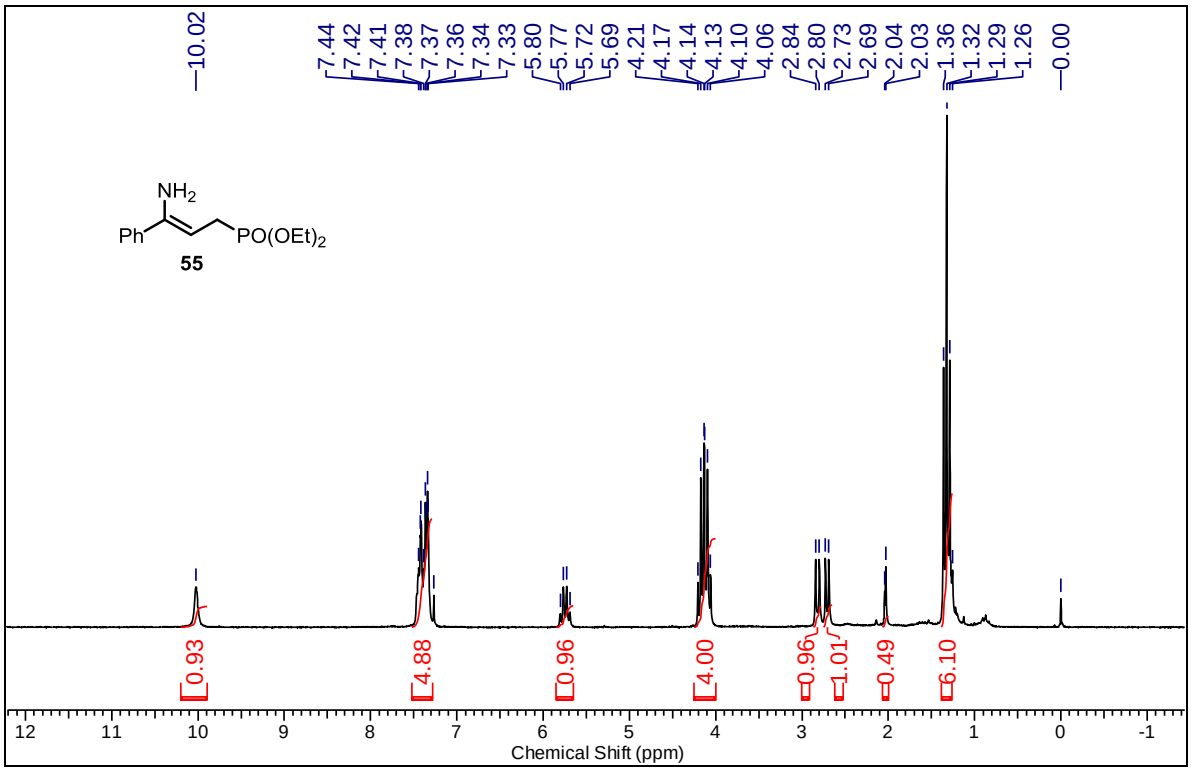

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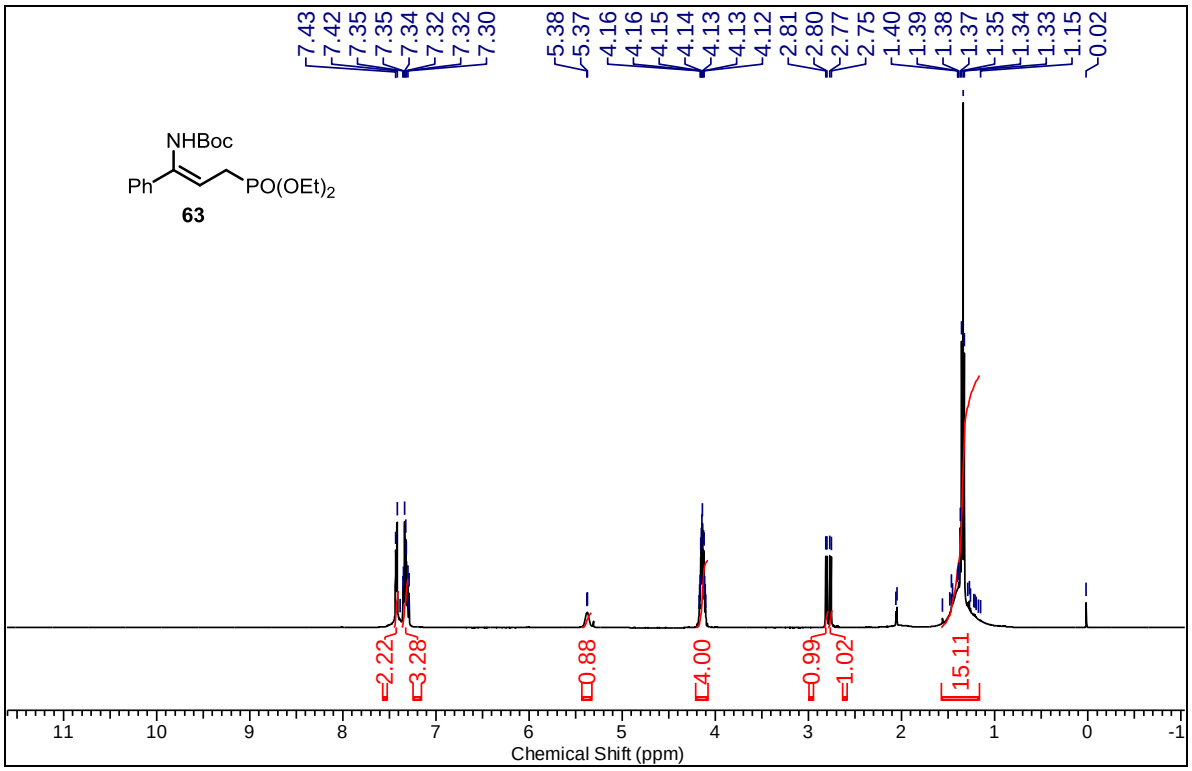

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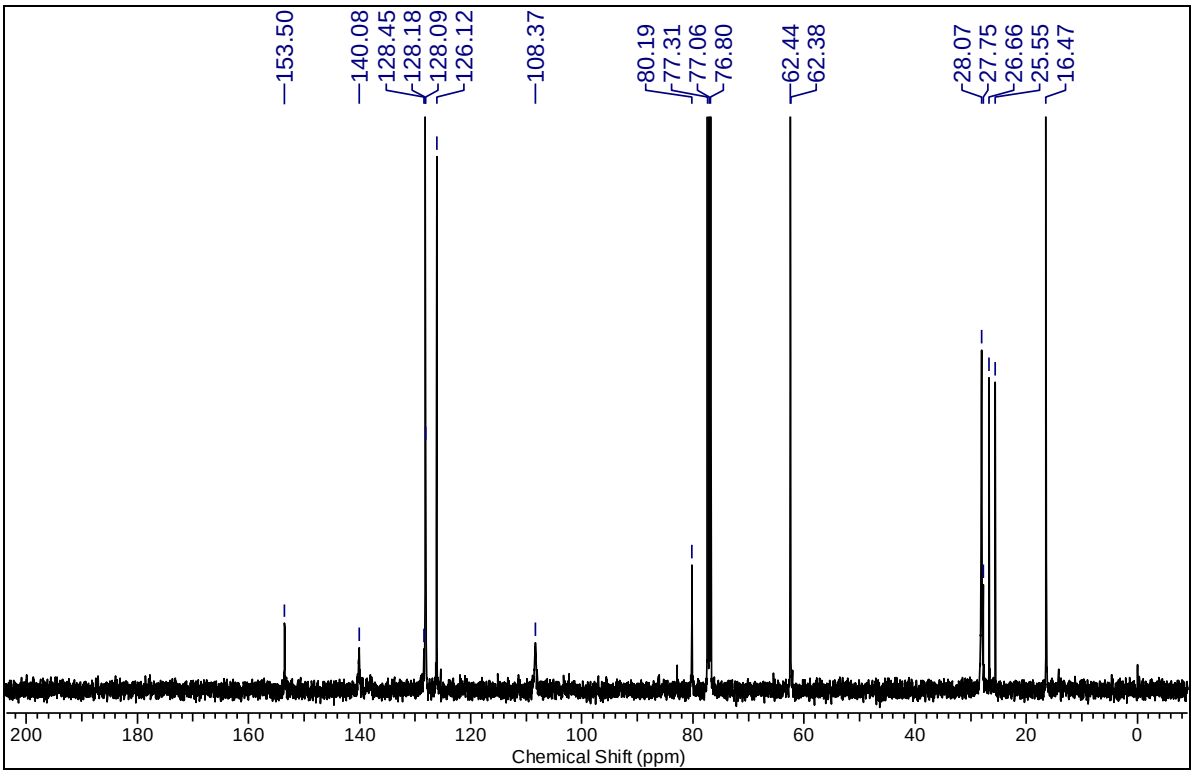

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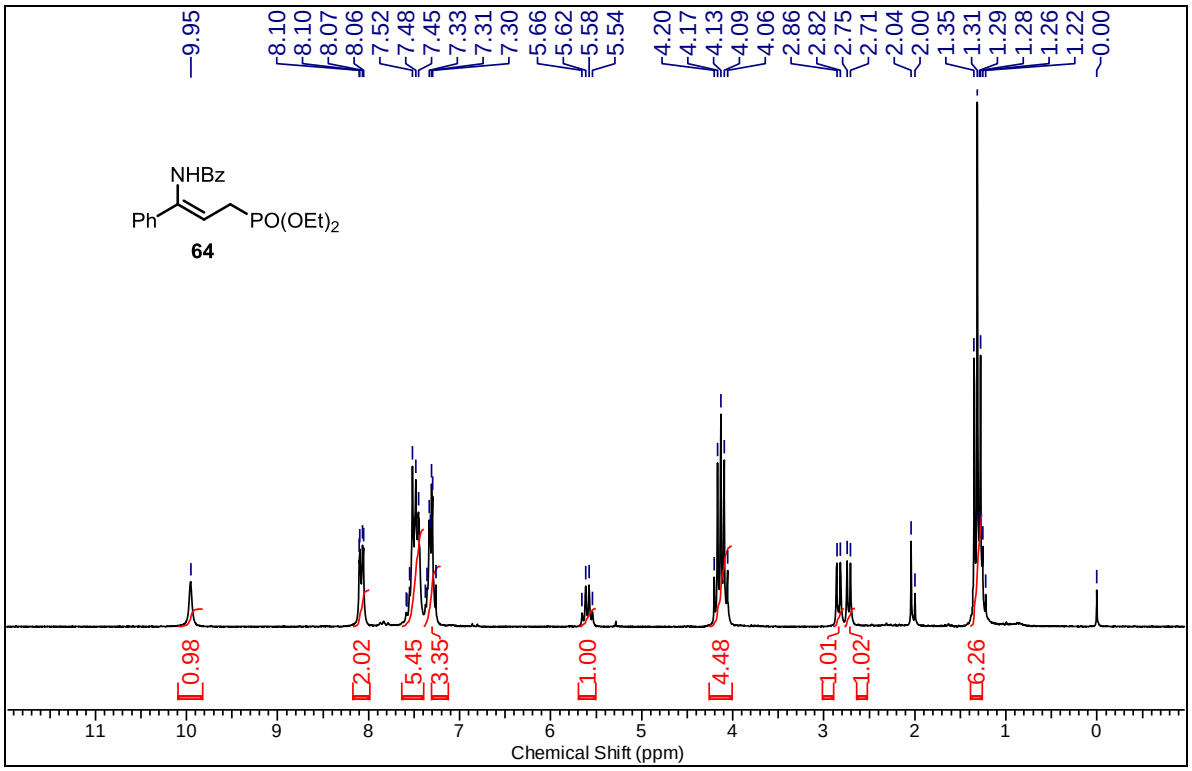

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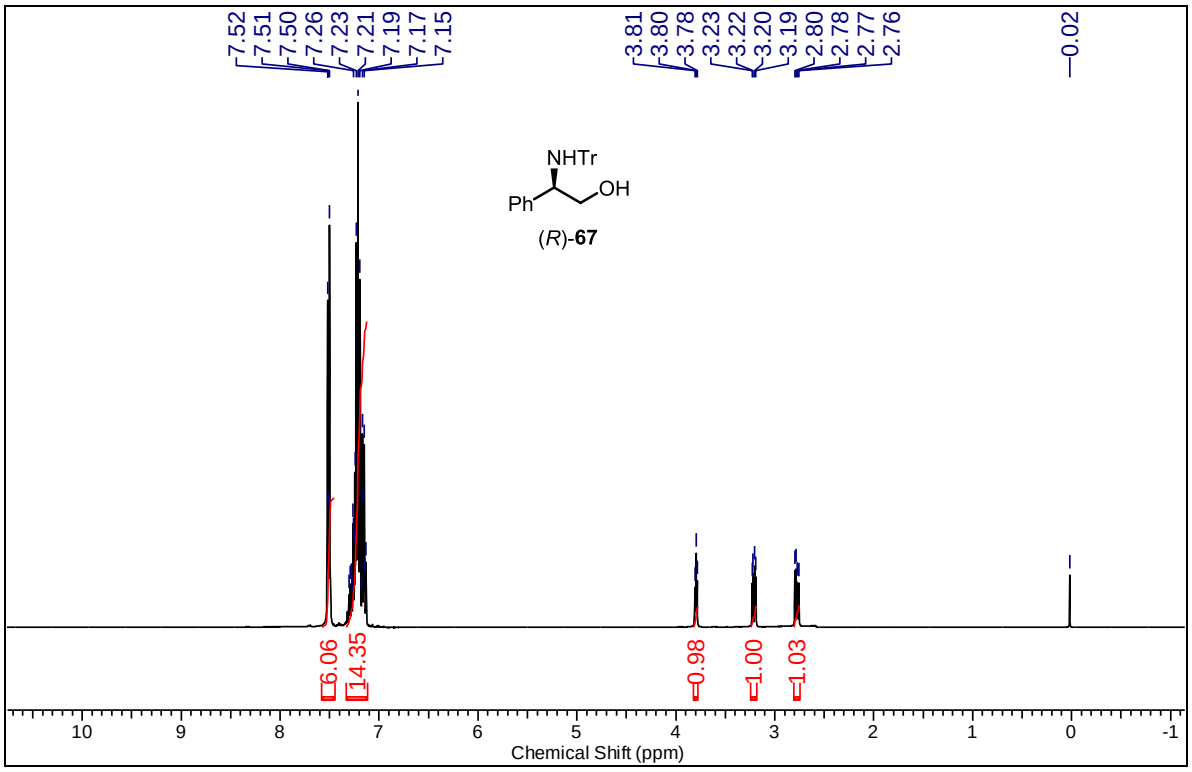

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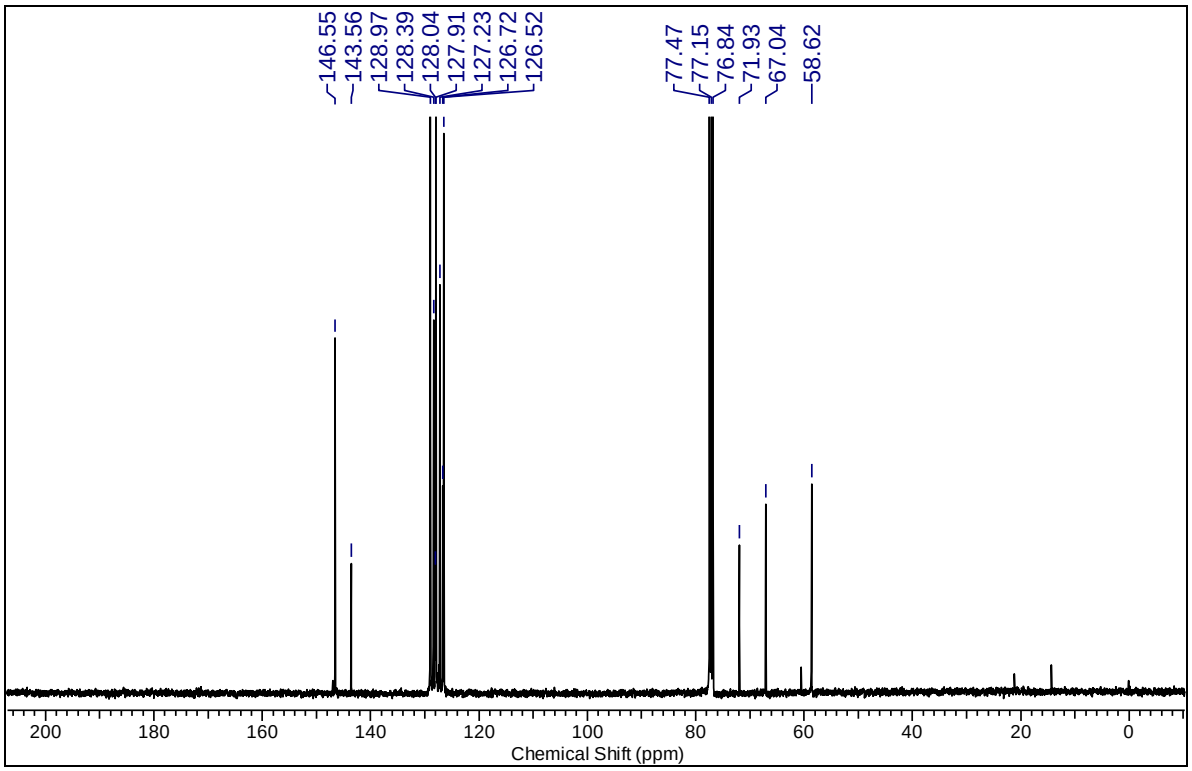

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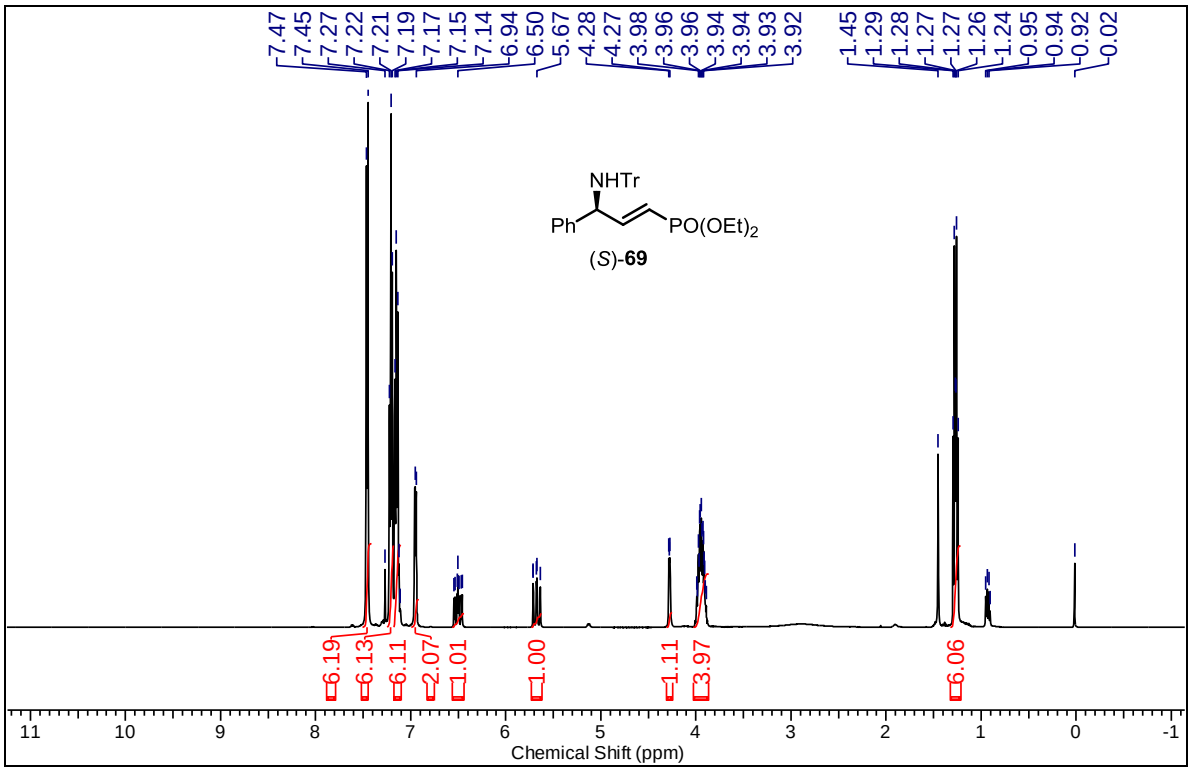

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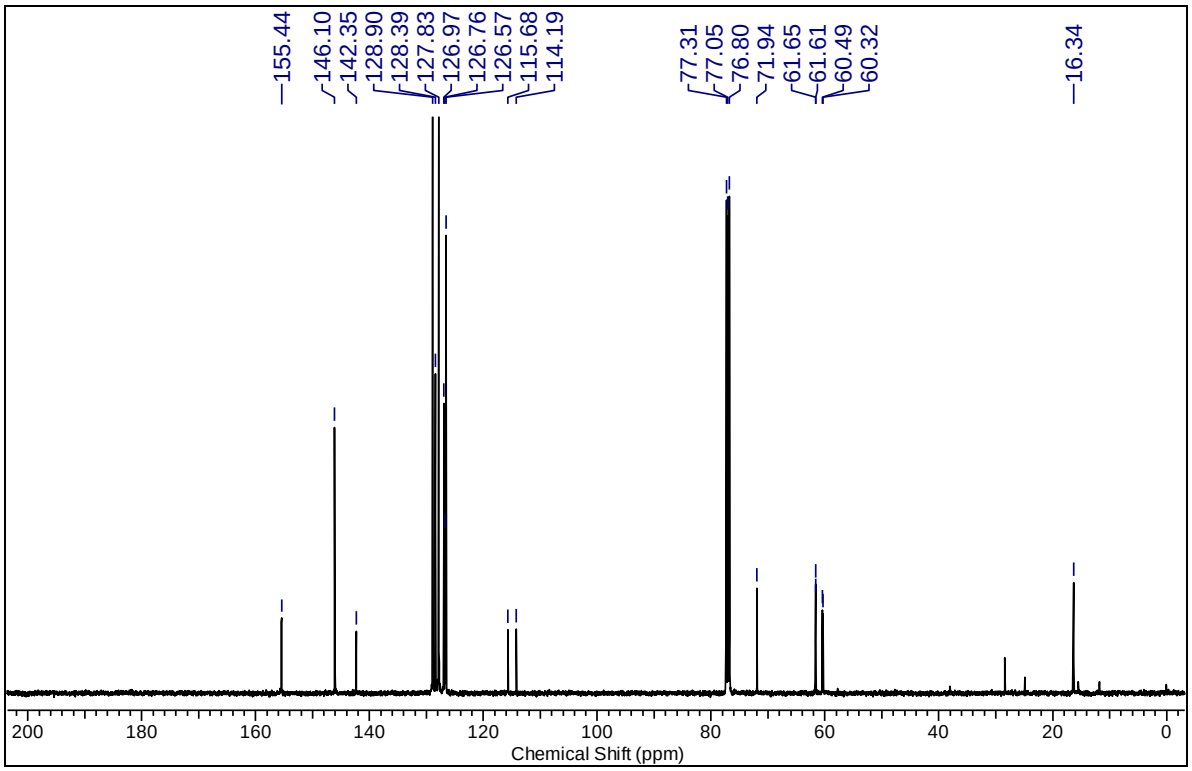

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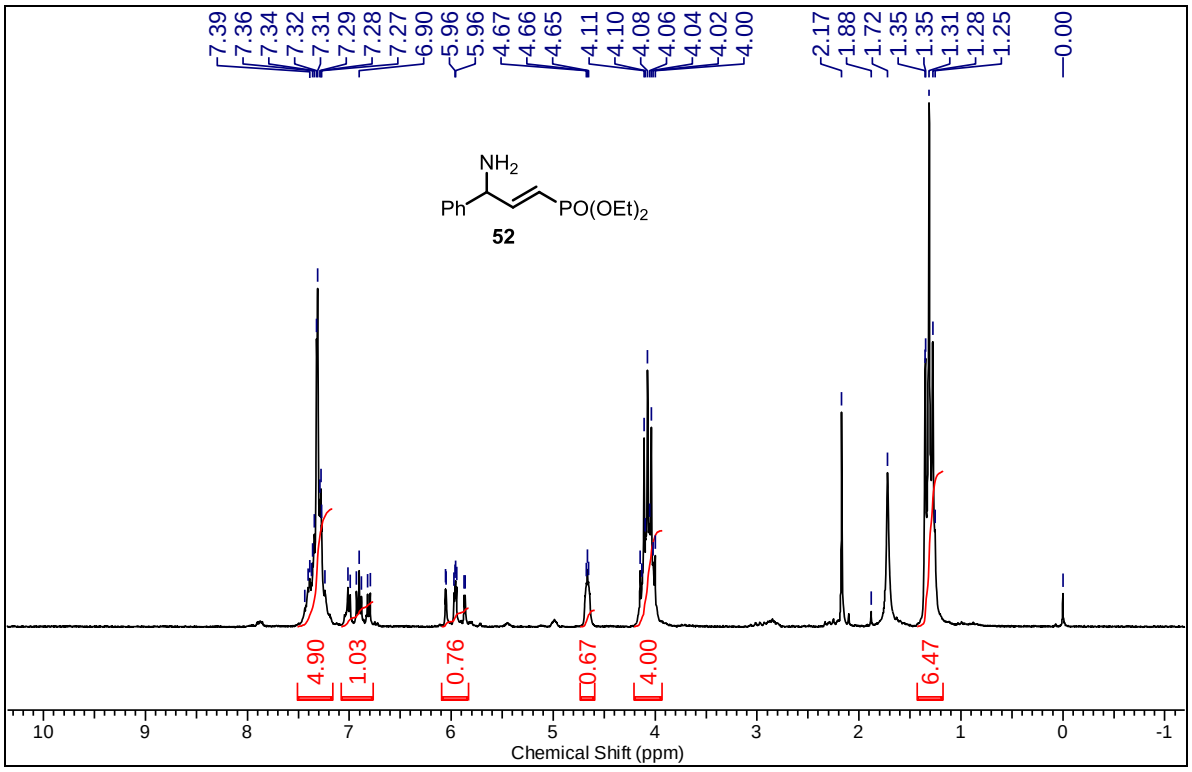

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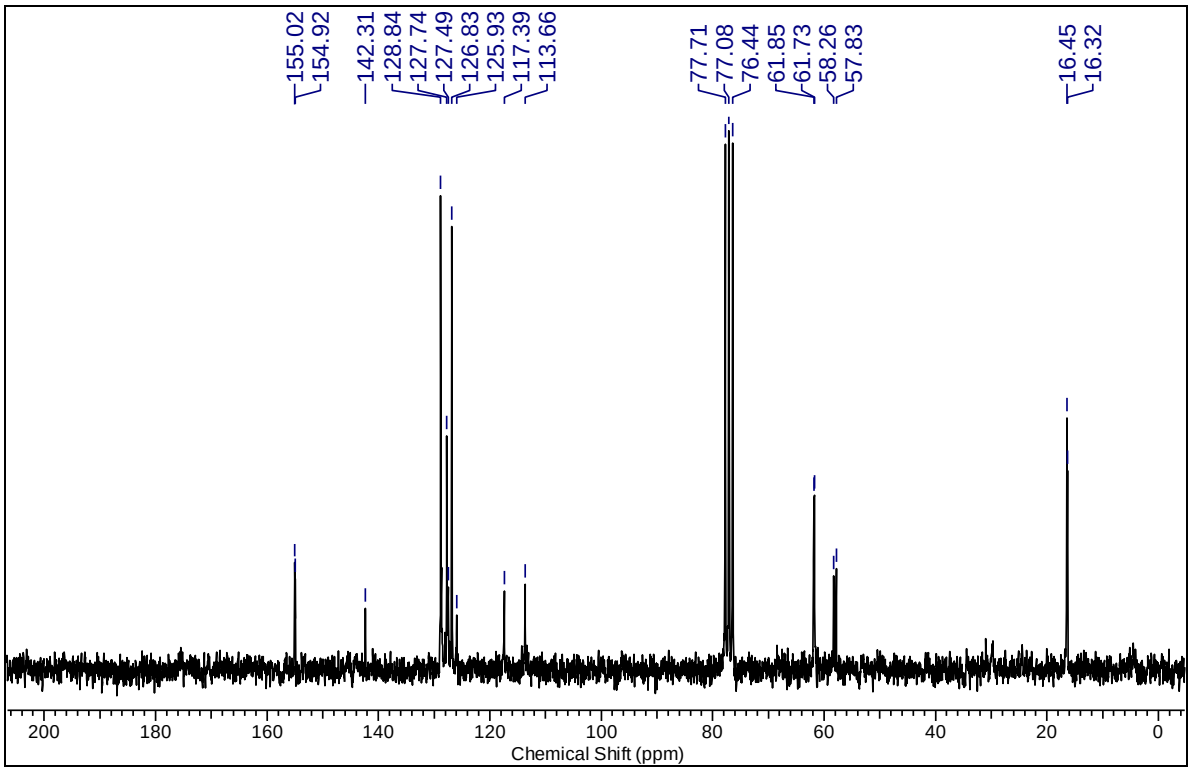

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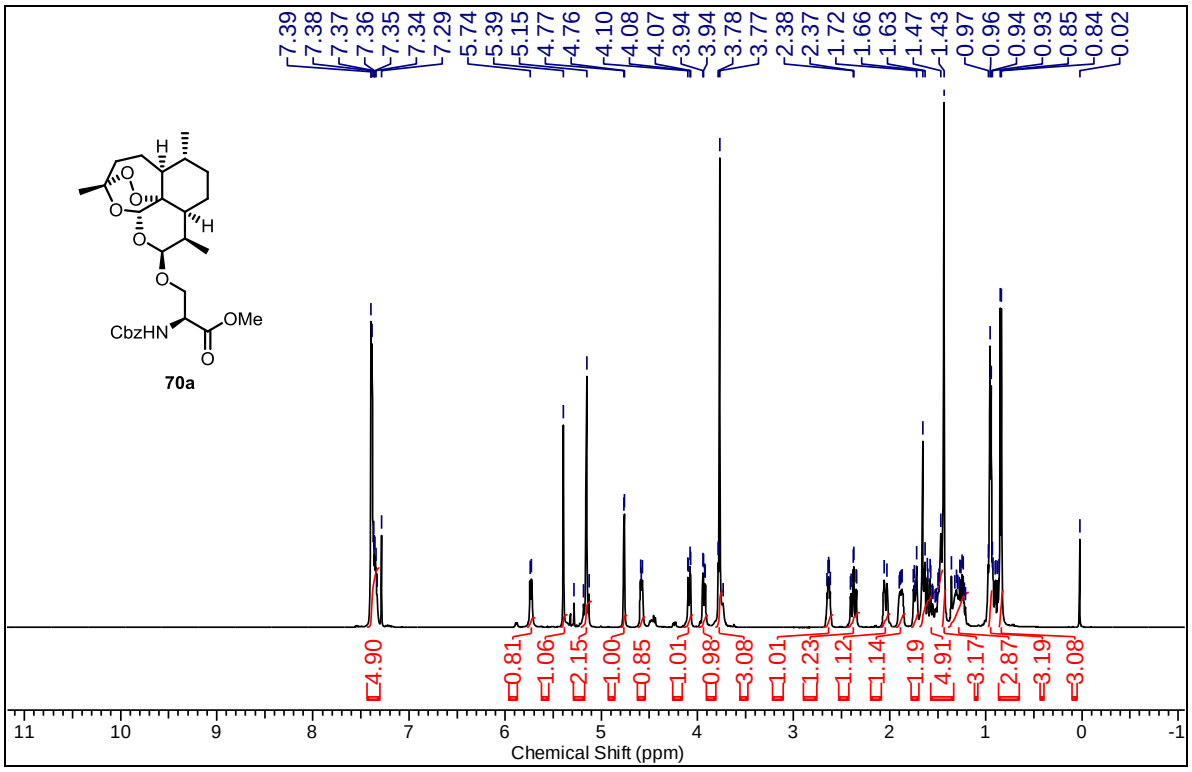

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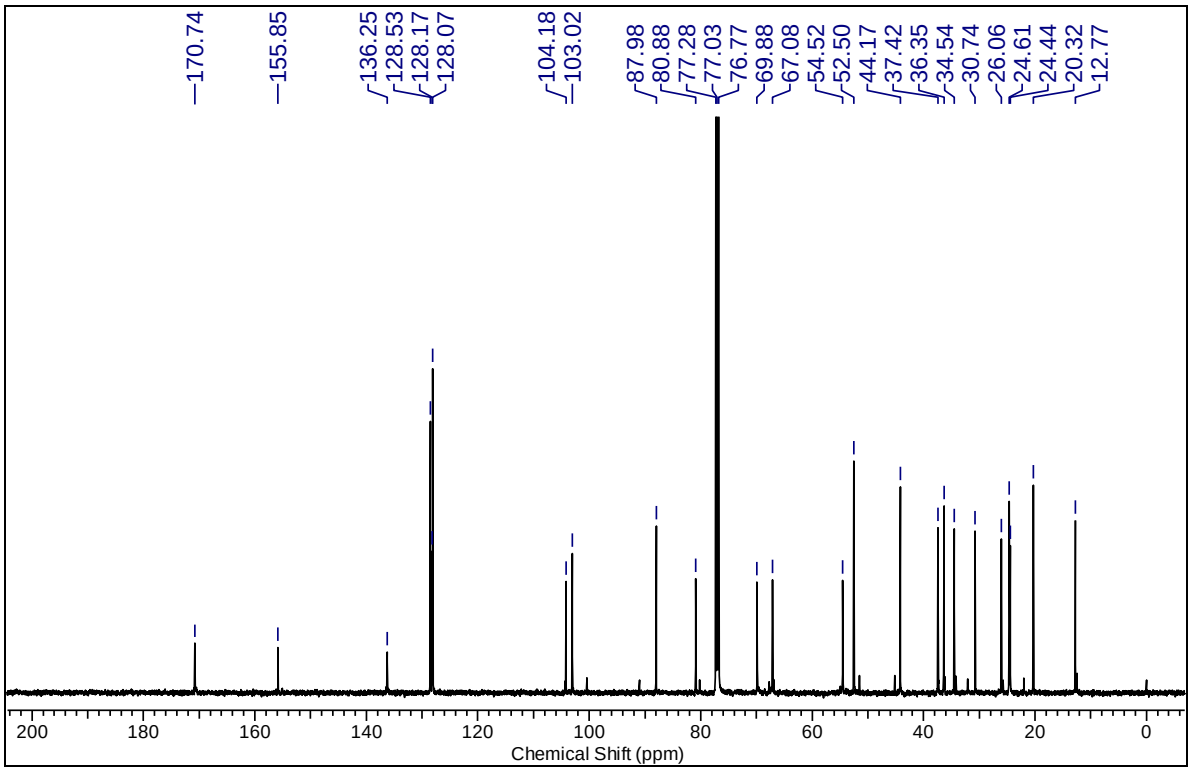

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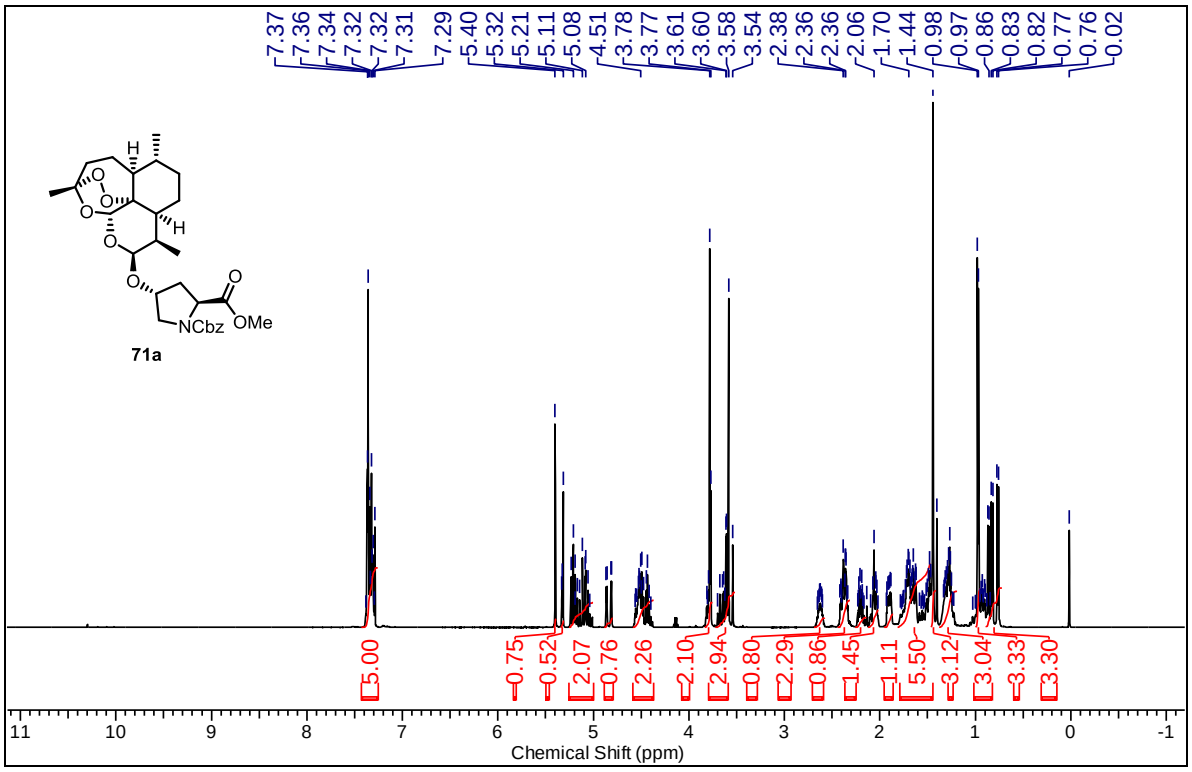

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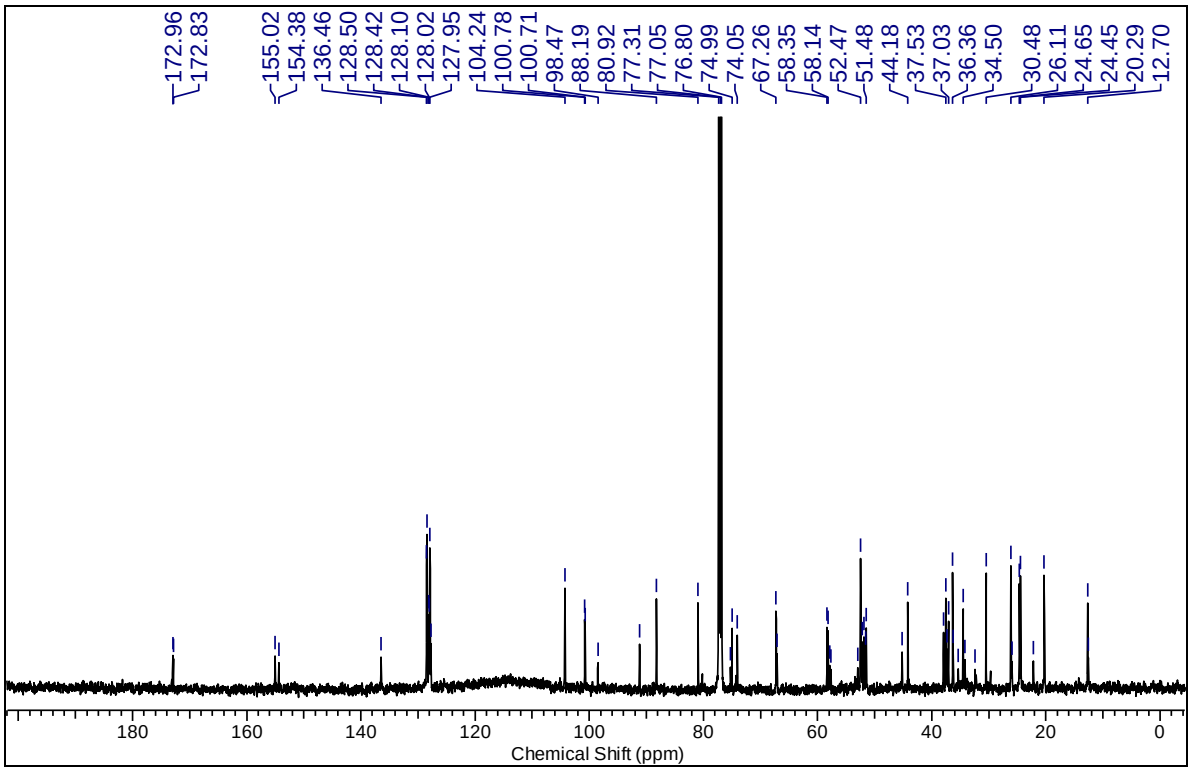

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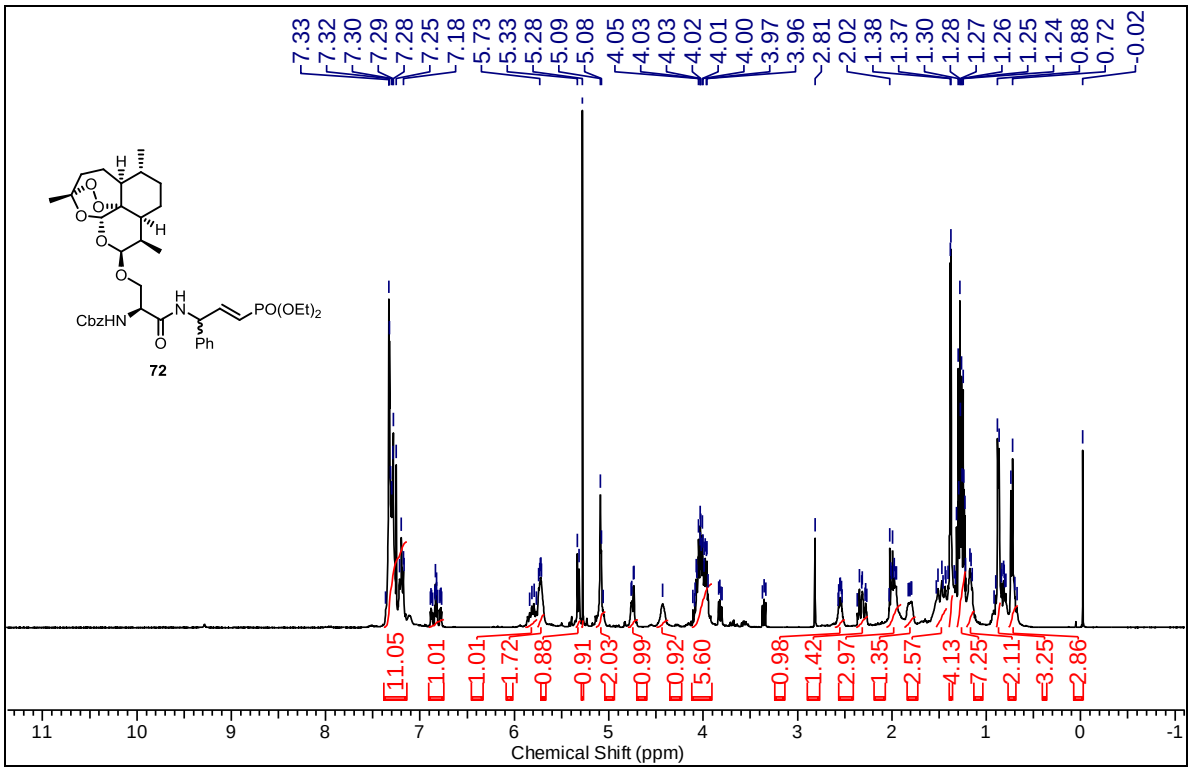

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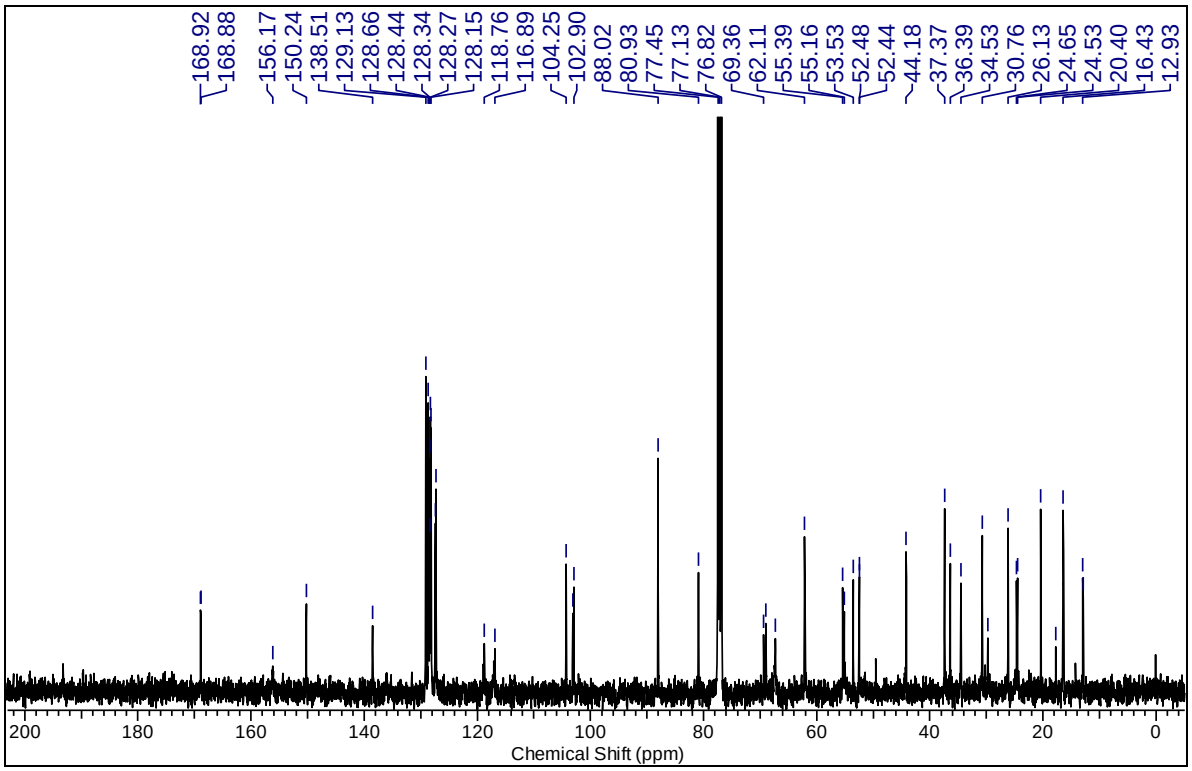
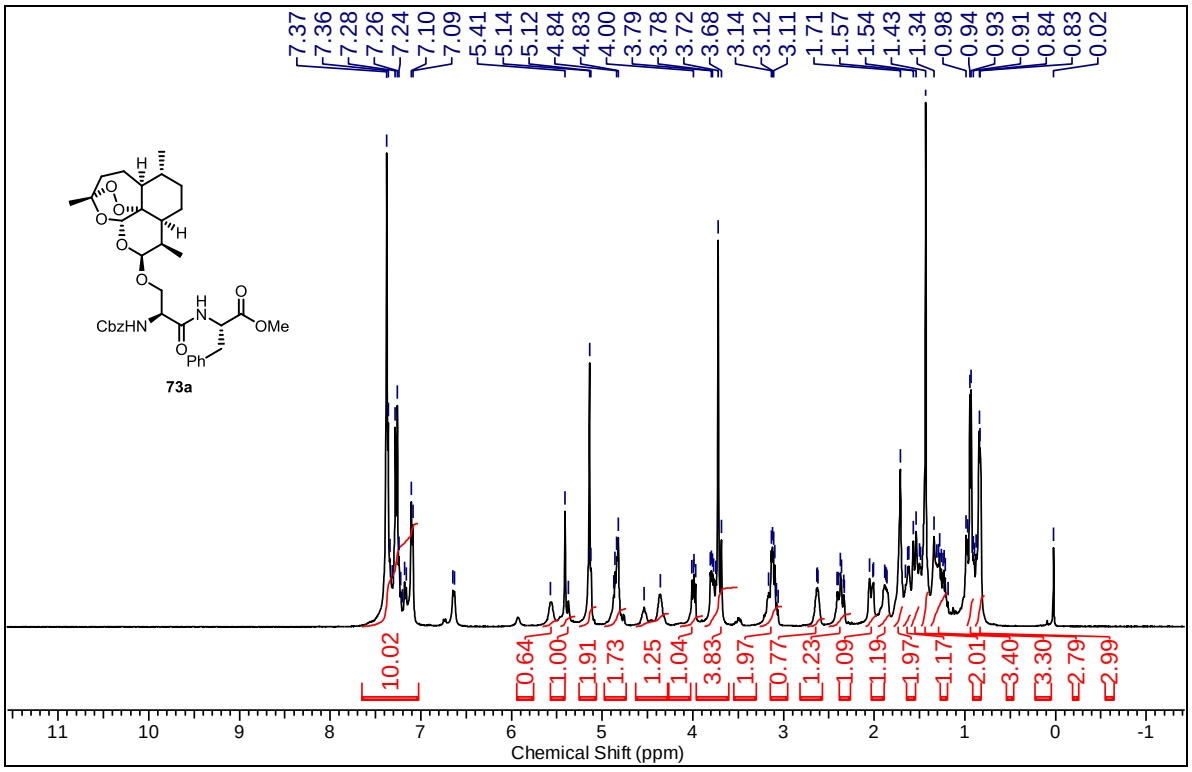
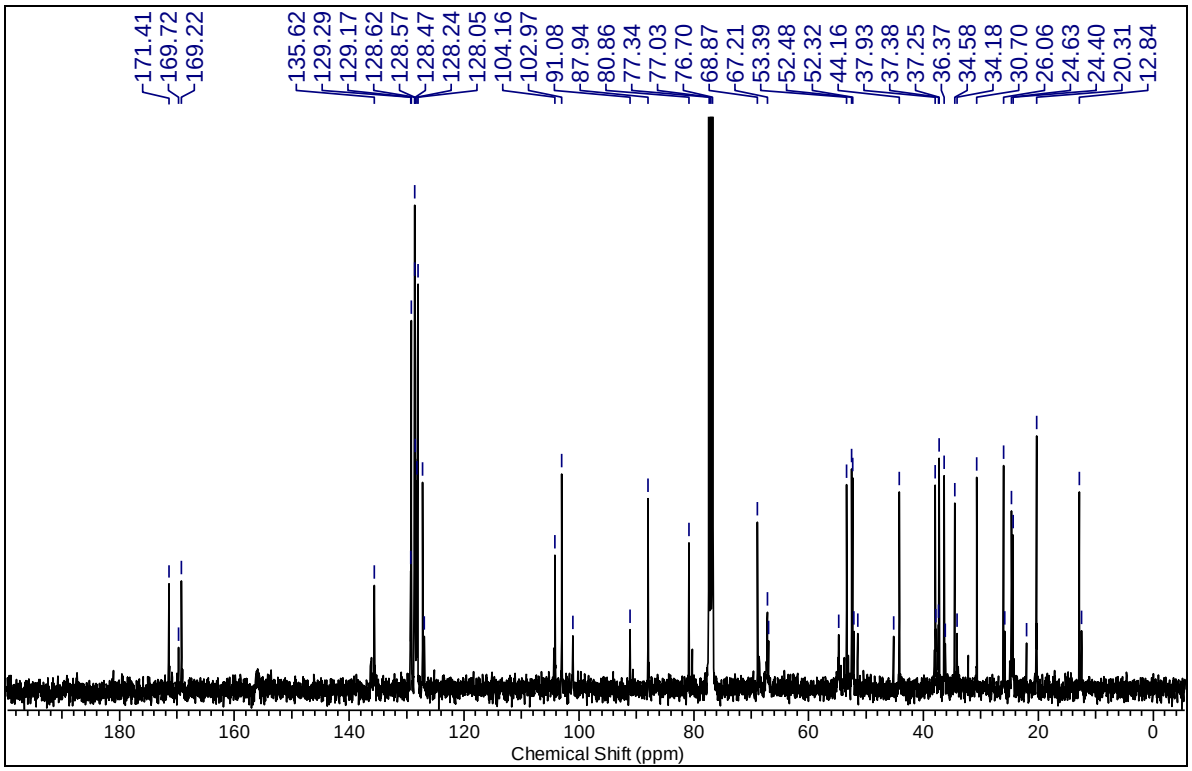


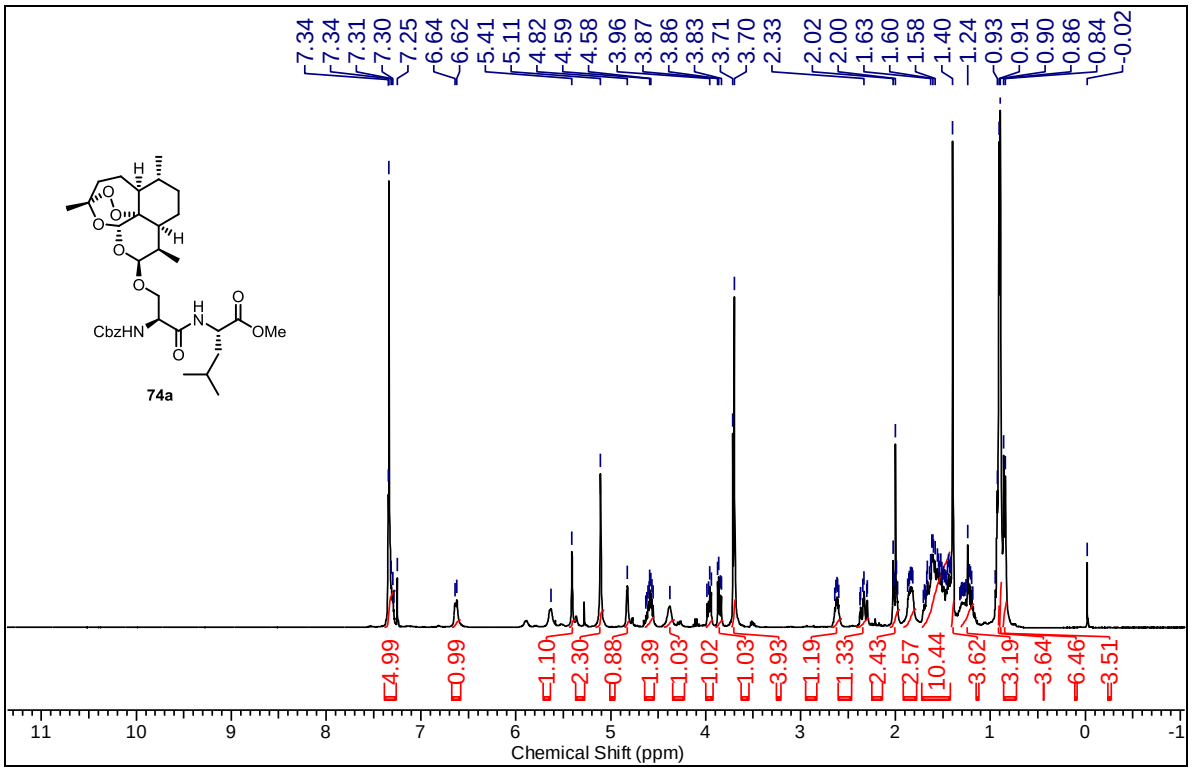
^{1}H NMR of Compound 73a (400 MHz, $CDCl_3$)



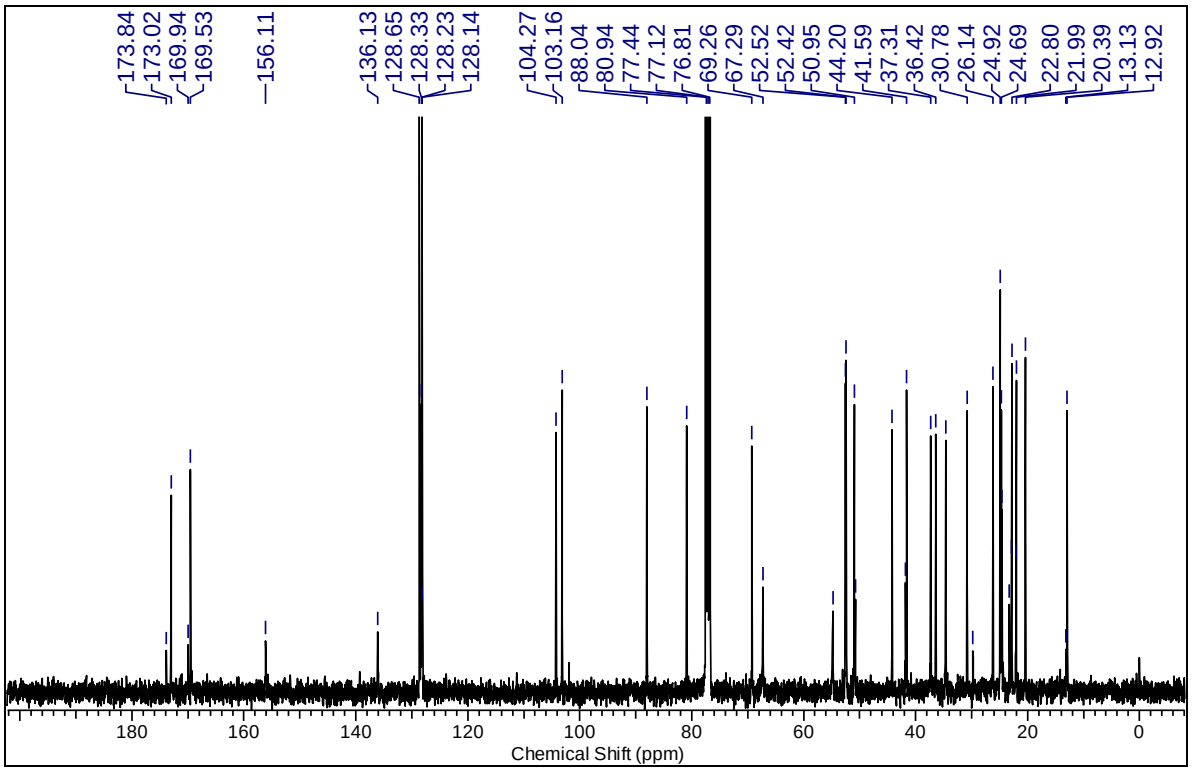
^{13}C NMR of Compound 73a (100 MHz, $CDCl_3$)



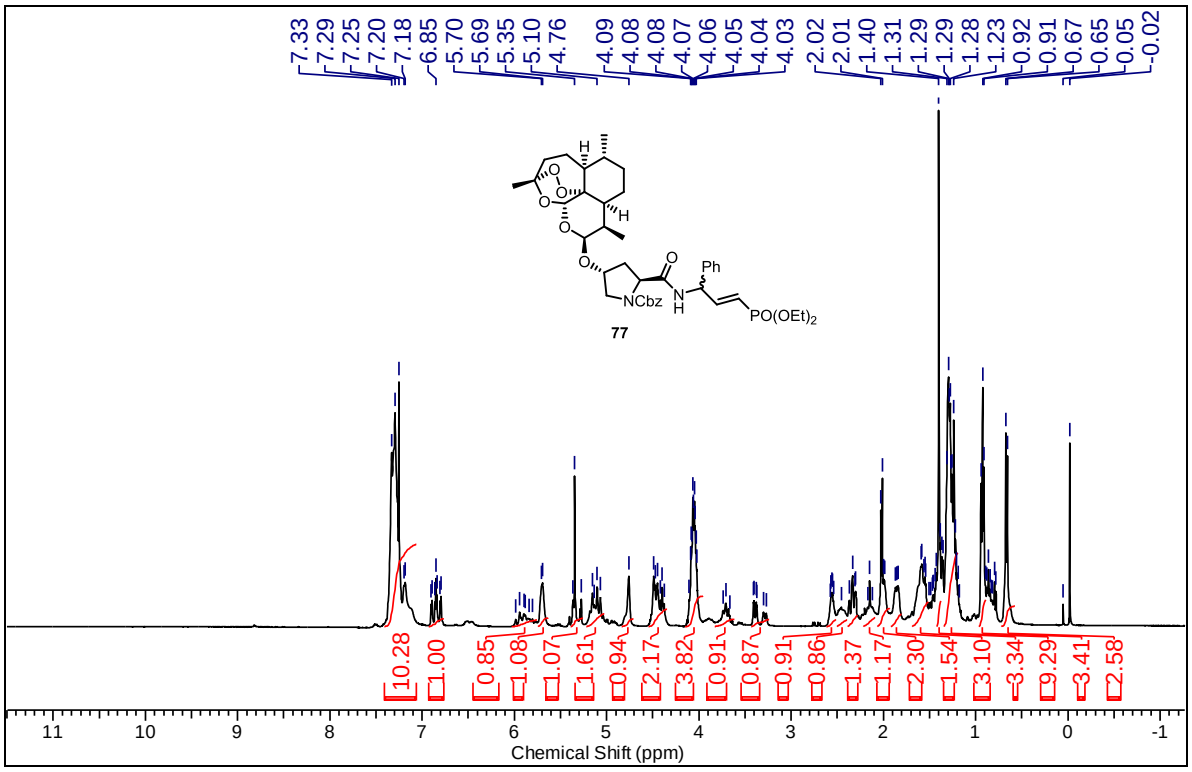
^{1}H NMR of Compound 74a (400 MHz, $CDCl_3$)



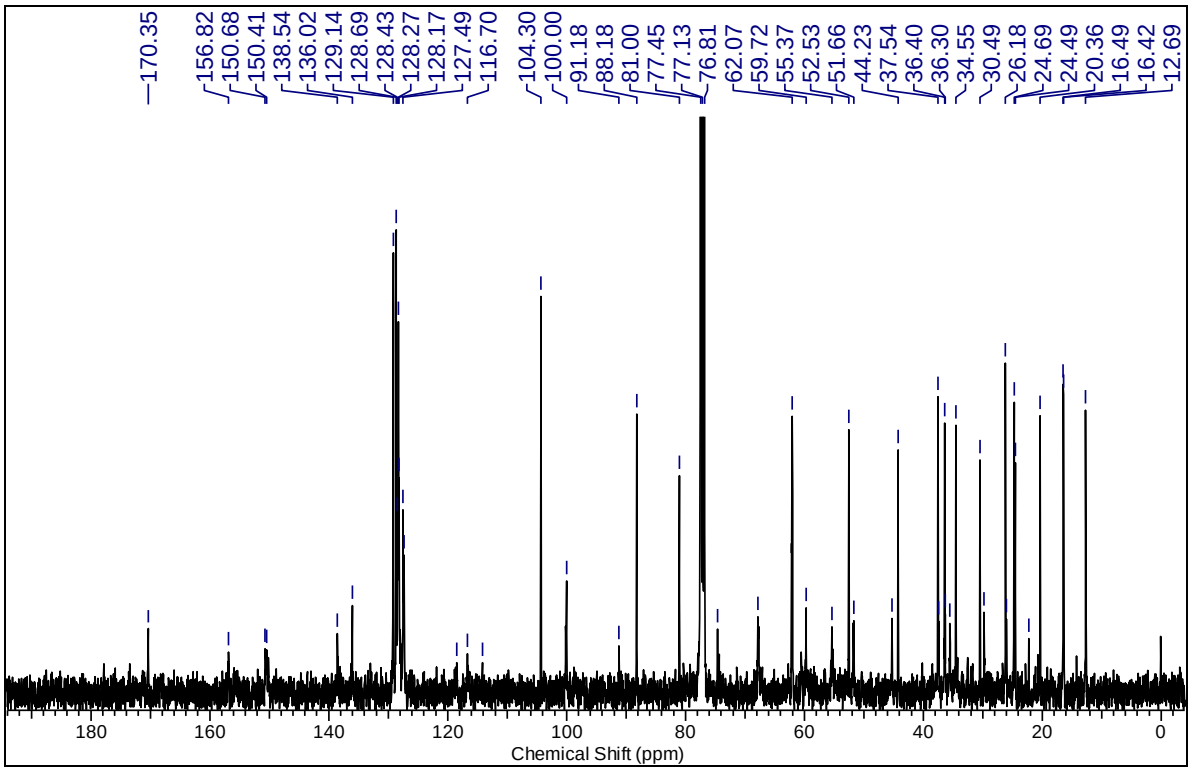
^{13}C NMR of Compound 74a (100 MHz, $CDCl_3$)



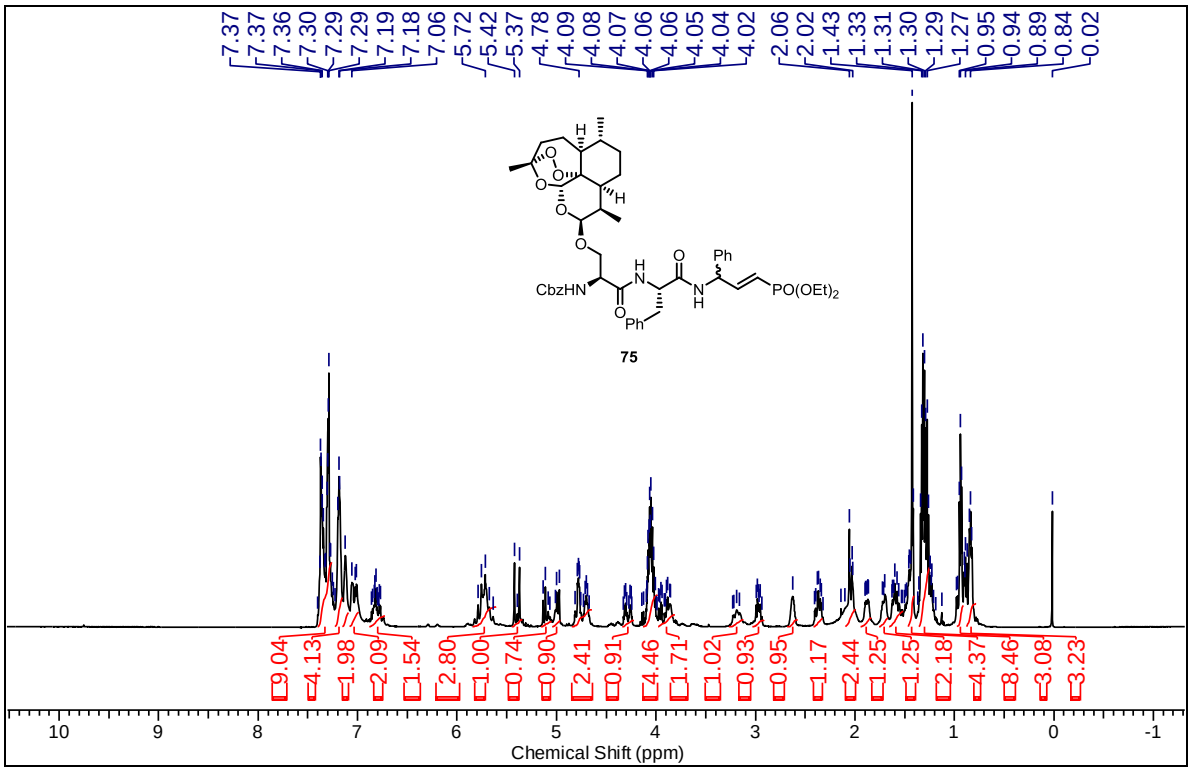
^{1}H NMR of Compound 77 (400 MHz, $CDCl_3$)



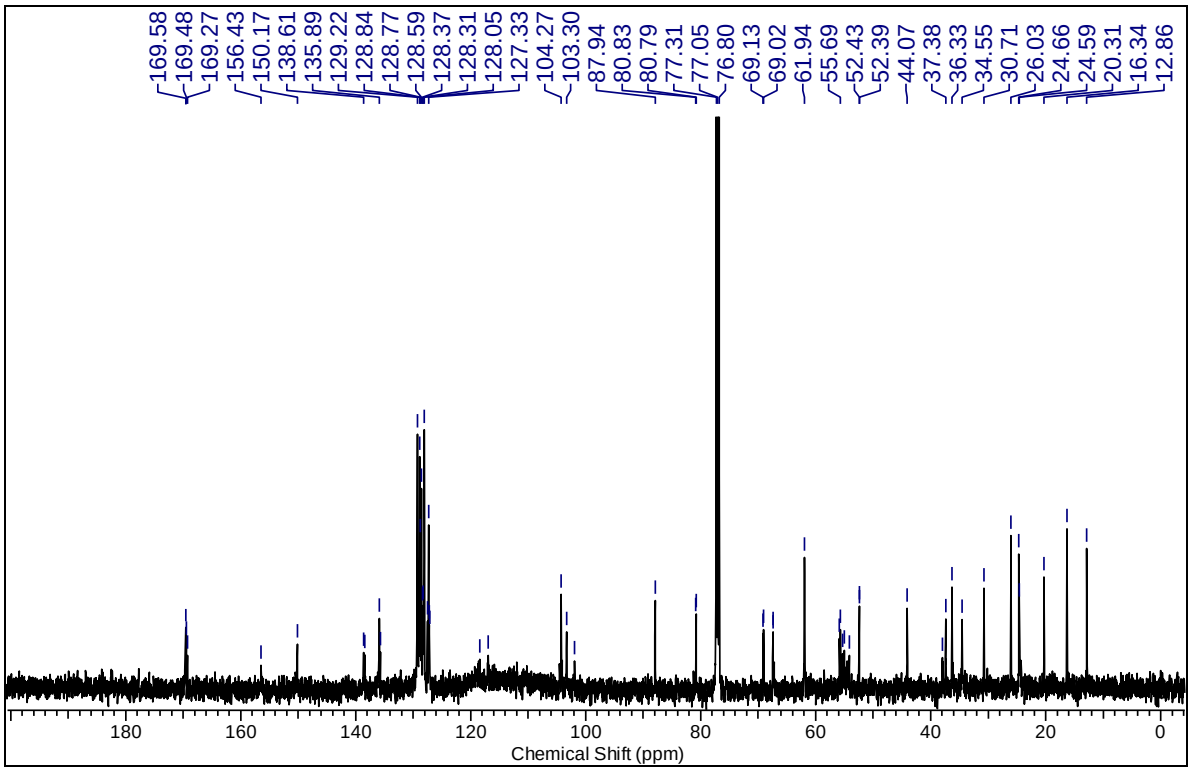
^{13}C NMR of Compound 77 (100 MHz, $CDCl_3$)



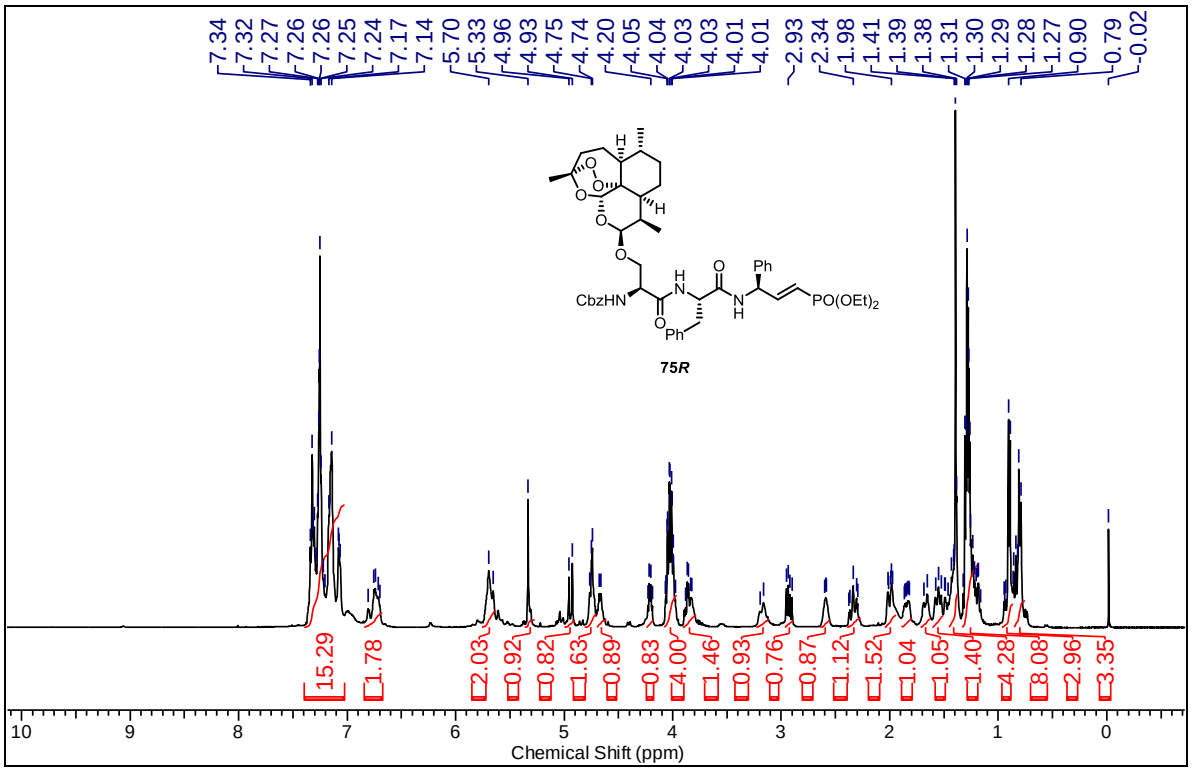
^{1}H NMR of Compound 75 (500 MHz, $CDCl_3$)



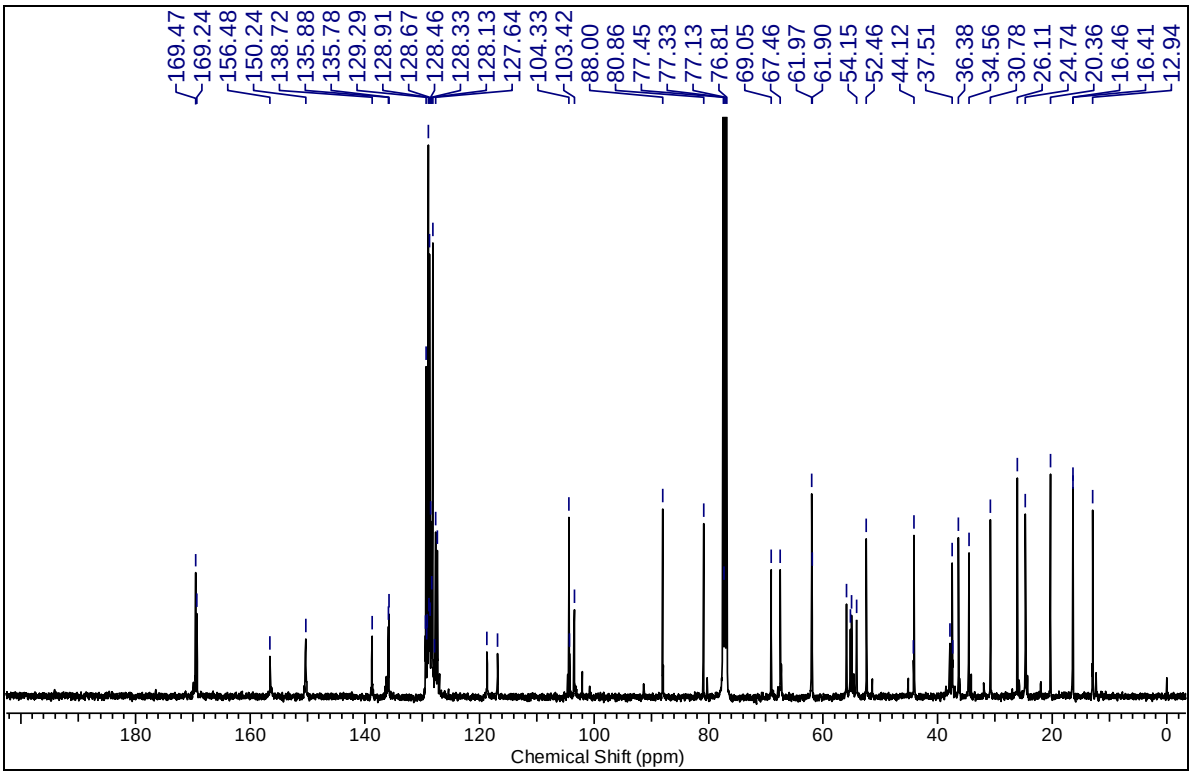
^{13}C NMR of Compound 75 (125 MHz, $CDCl_3$)



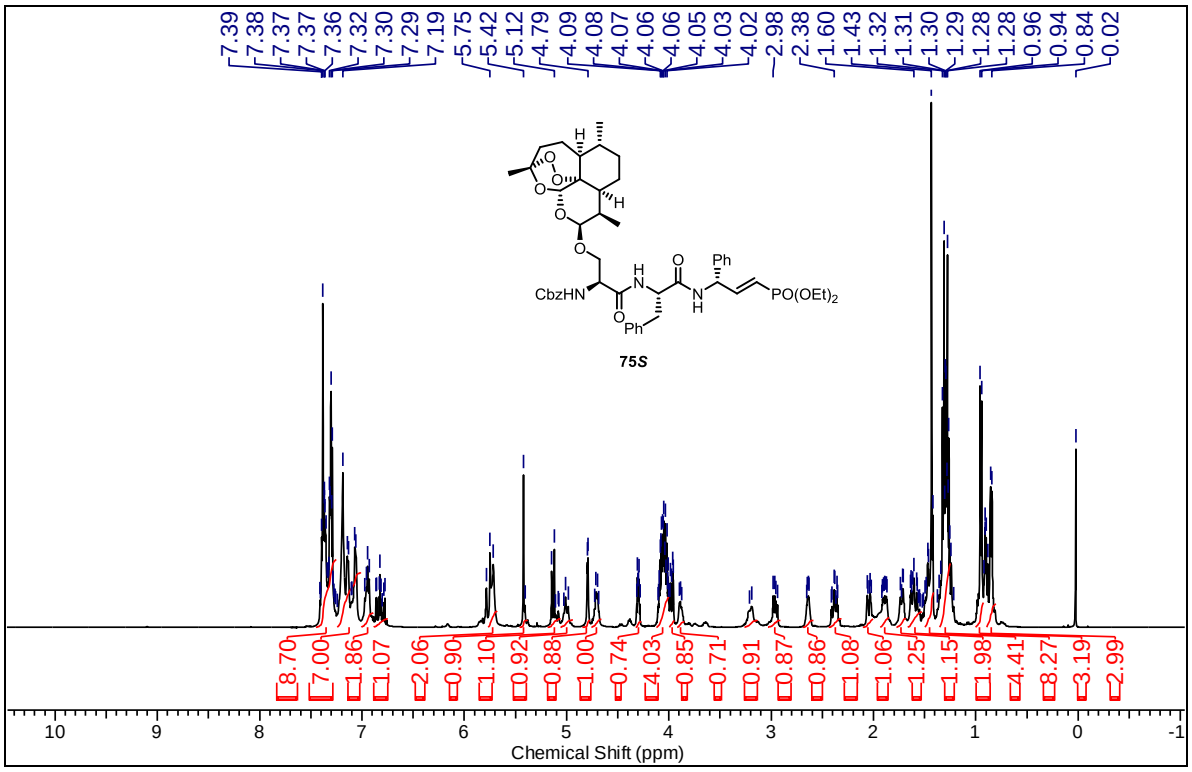
^{1}H NMR of Compound 75*R* (400 MHz, $CDCl_3$)



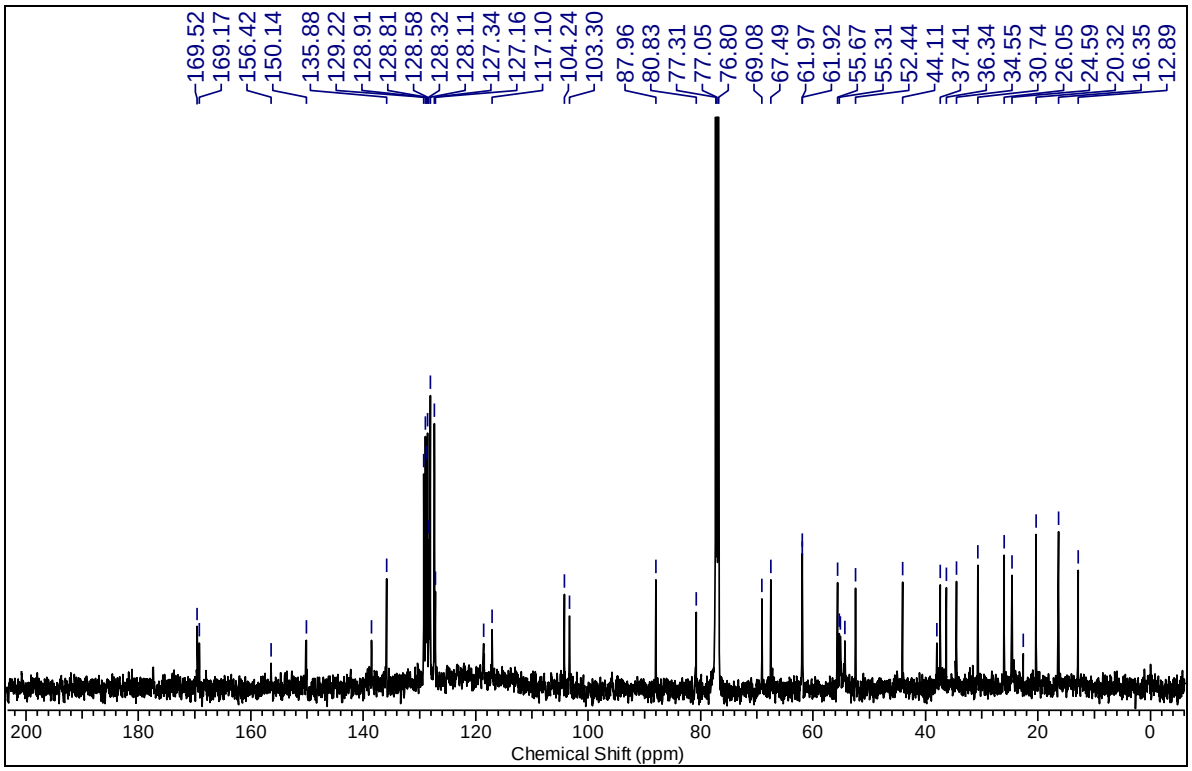
^{13}C NMR of Compound 75*R* (100 MHz, $CDCl_3$)



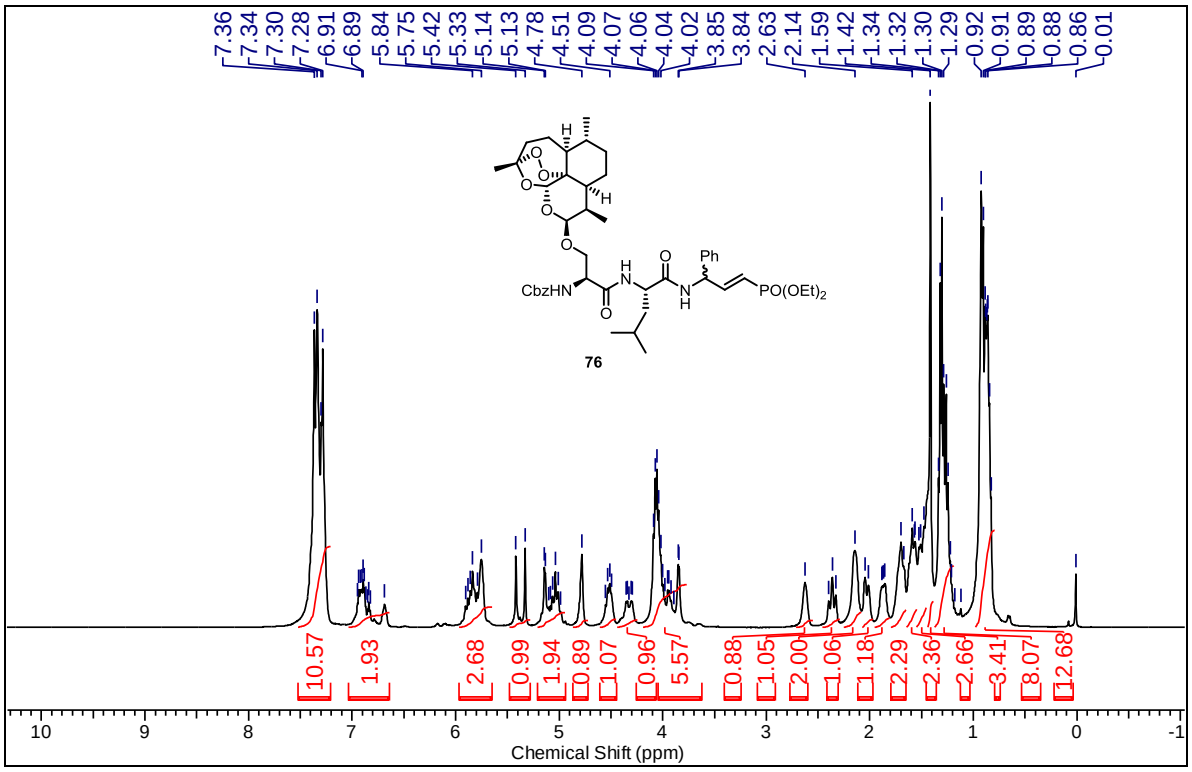
^{1}H NMR of Compound 75*S* (500 MHz, $CDCl_3$)



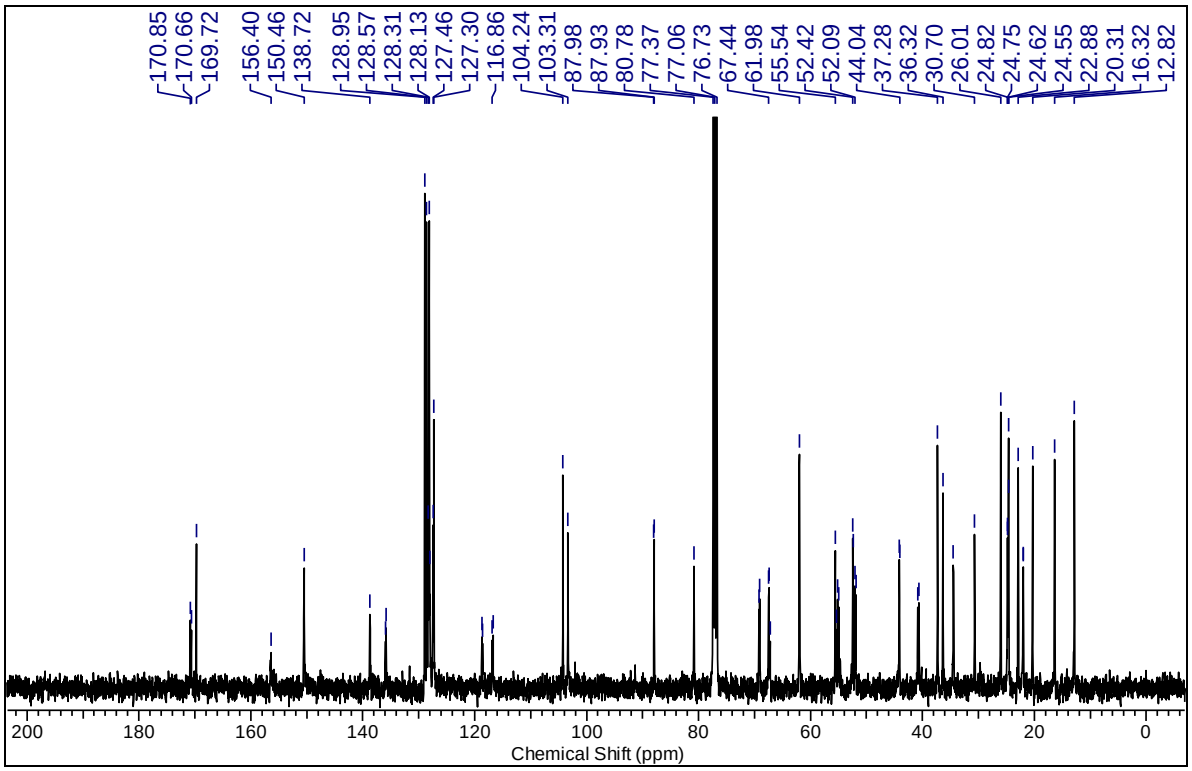
^{13}C NMR of Compound 75*S* (125 MHz, $CDCl_3$)



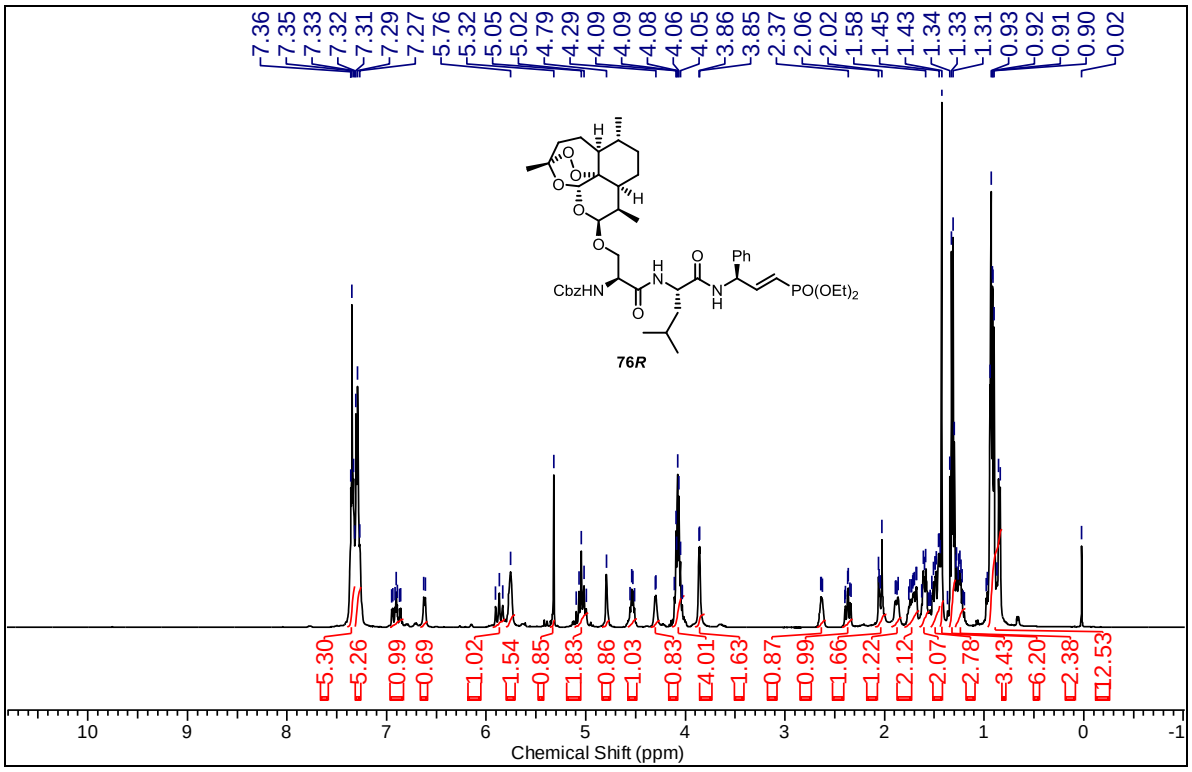
^{1}H NMR of Compound 76 (400 MHz, $CDCl_3$)



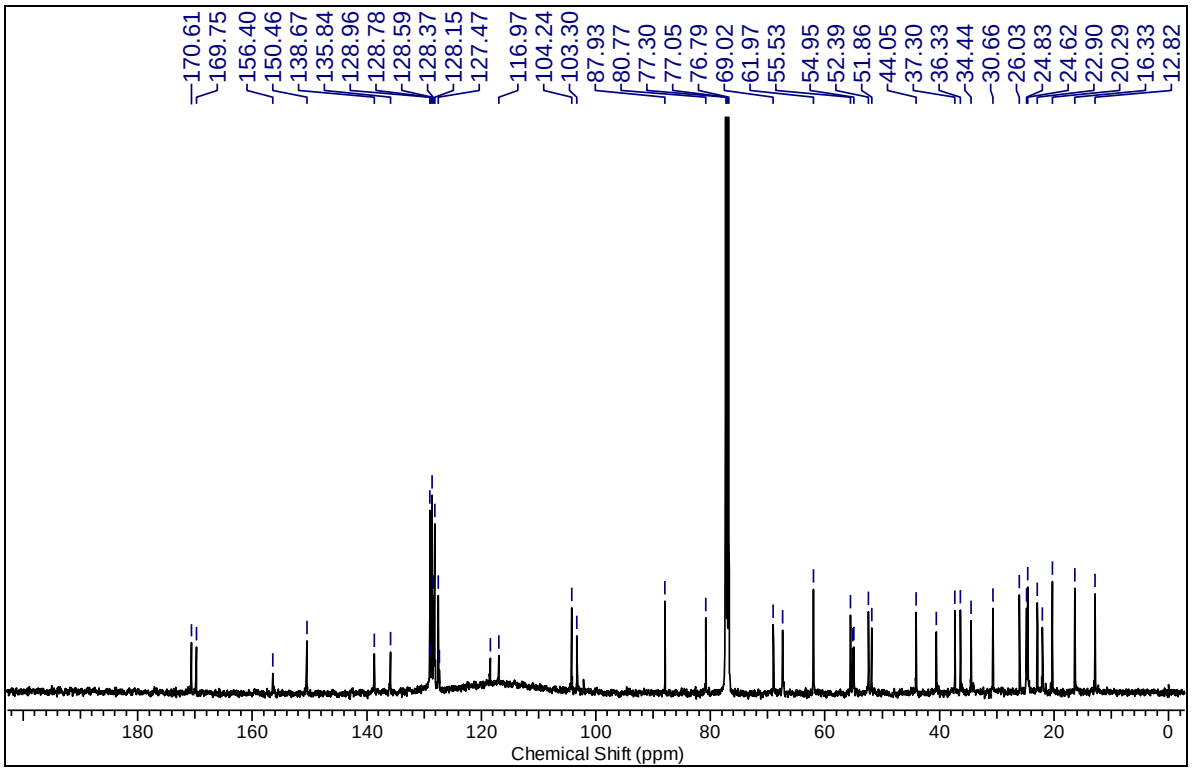
^{13}C NMR of Compound 76 (100 MHz, $CDCl_3$)



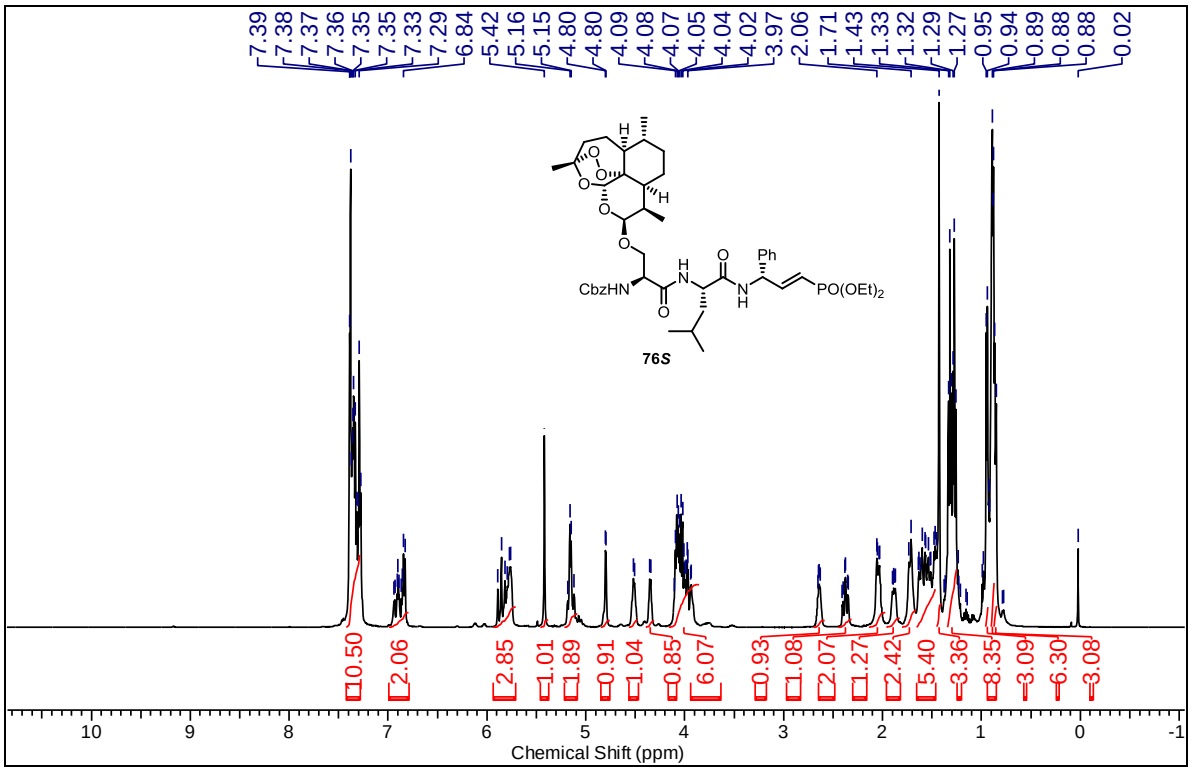
^{1}H NMR of Compound 76*R* (500 MHz, $CDCl_3$)



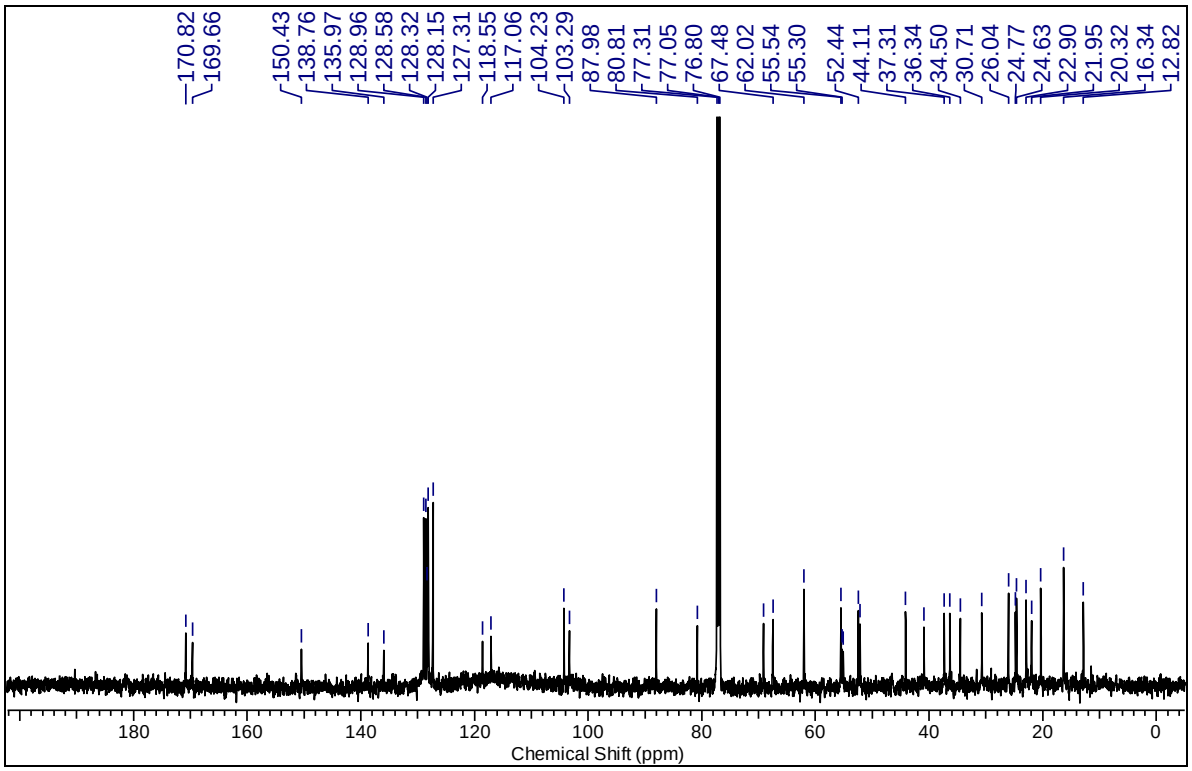
^{13}C NMR of Compound 76*R* (125 MHz, $CDCl_3$)



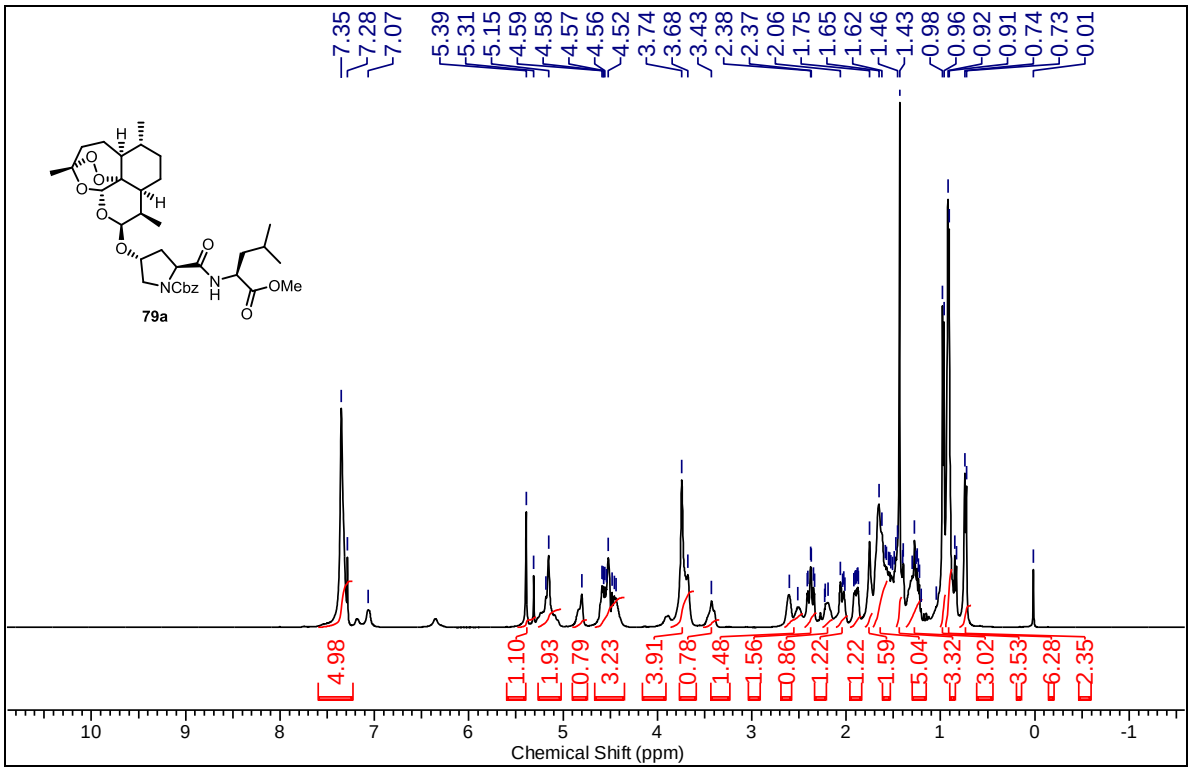
^{1}H NMR of Compound 76*S* (500 MHz, $CDCl_3$)



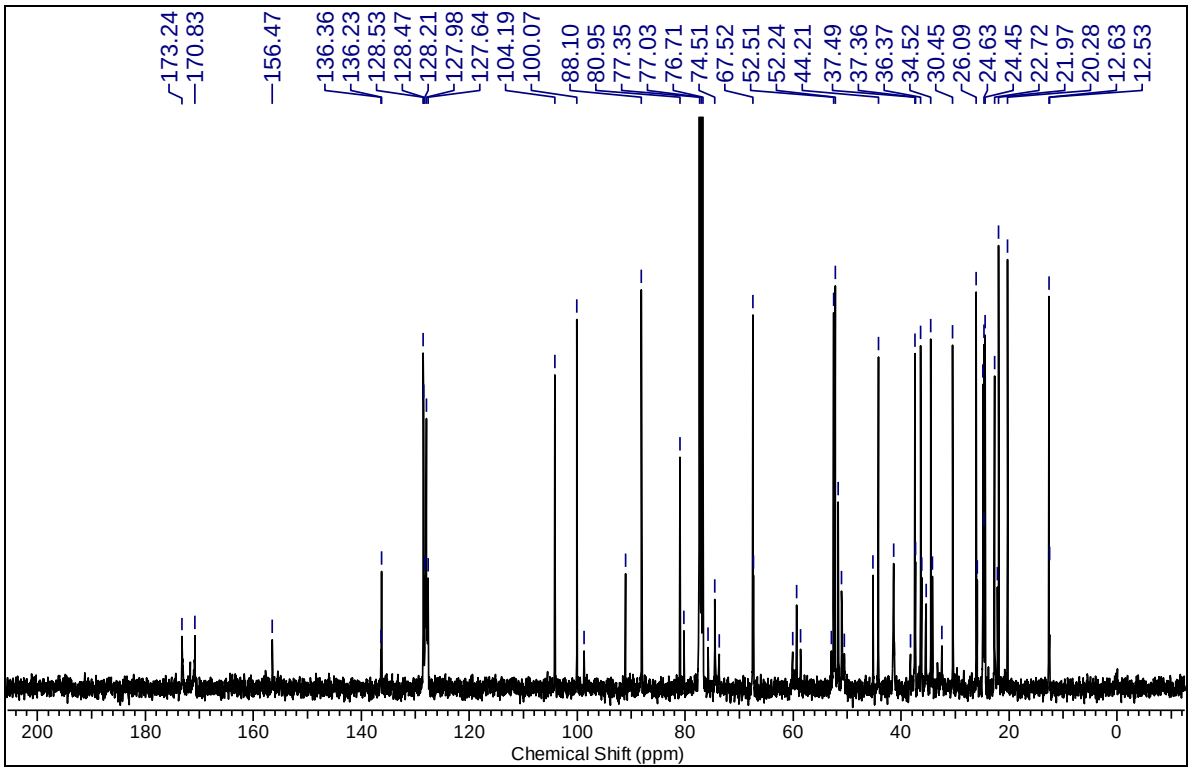
^{13}C NMR of Compound 76*S* (125 MHz, $CDCl_3$)



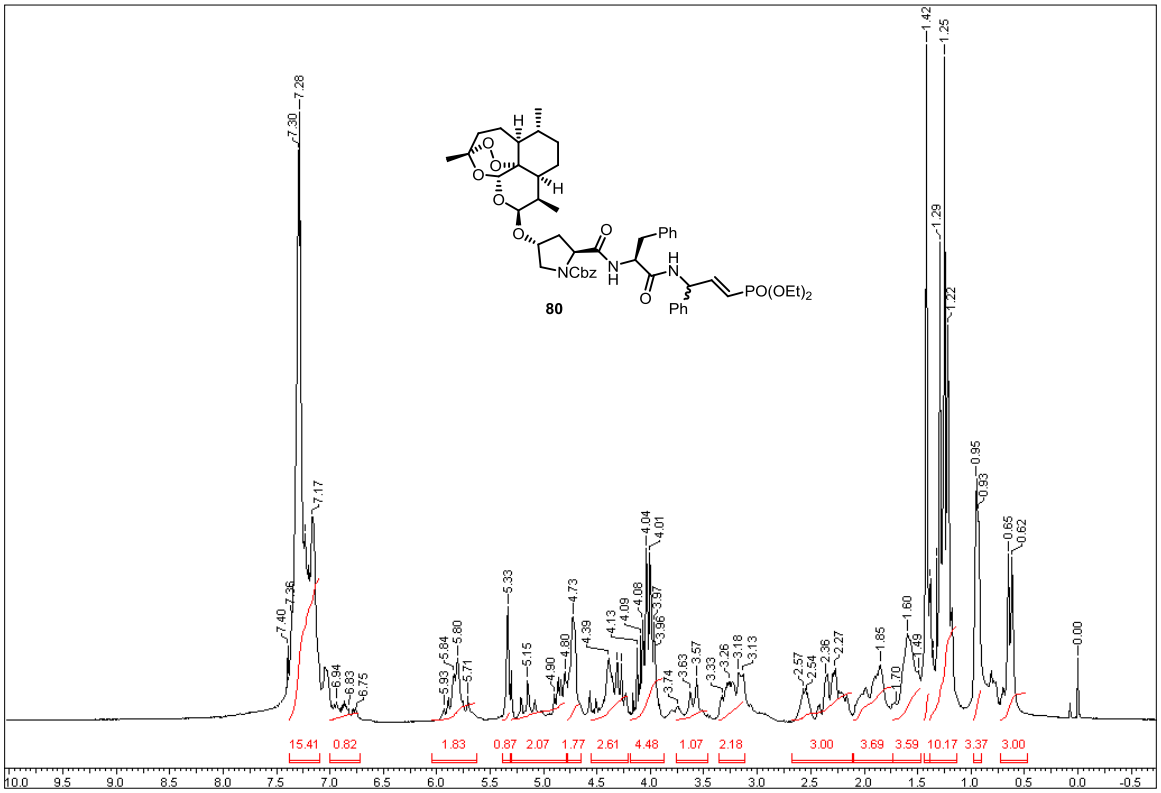
^{1}H NMR of Compound 79a (400 MHz, $CDCl_3$)



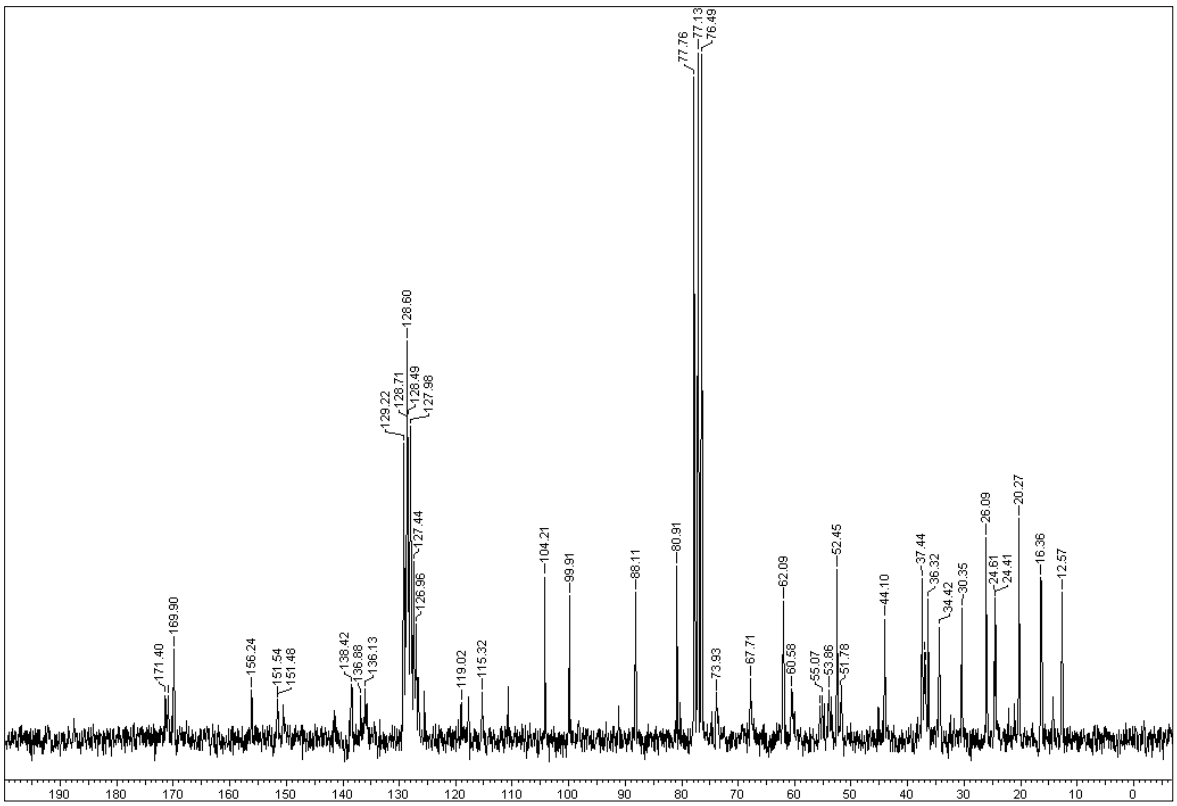
^{13}C NMR of Compound 79a (100 MHz, $CDCl_3$)



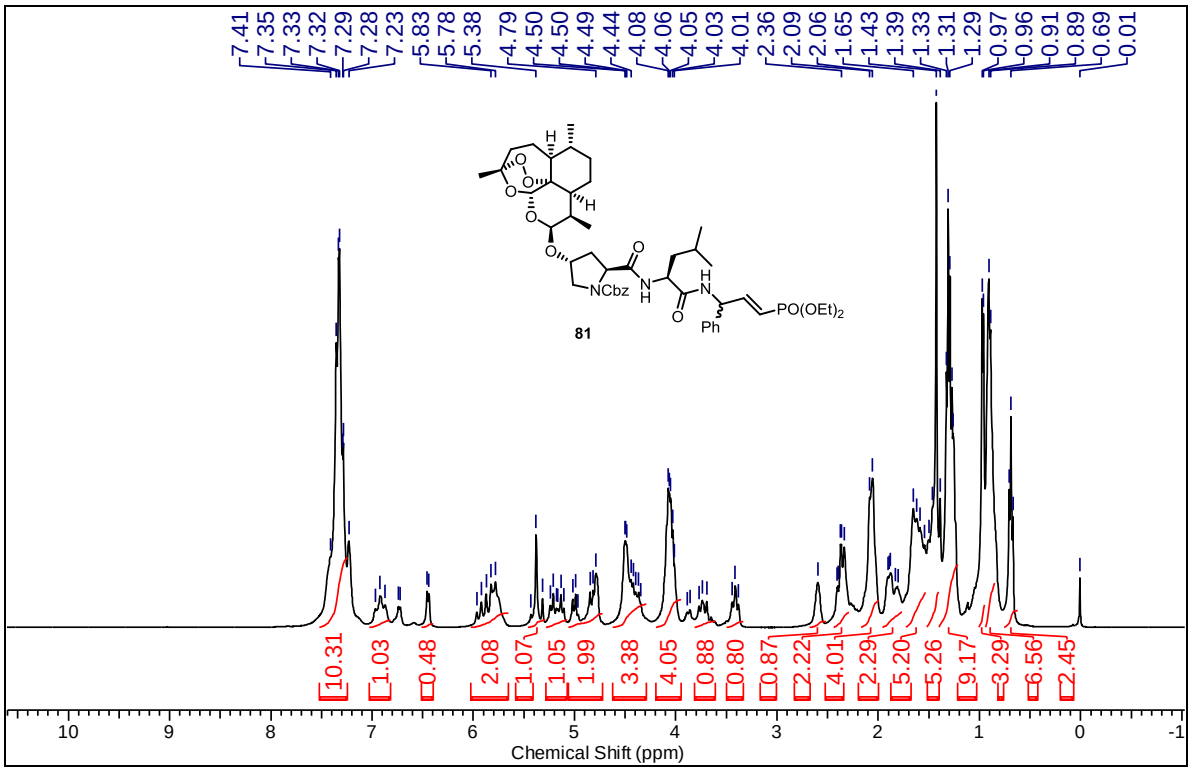
^{1}H NMR of Compound 80 (200 MHz, $CDCl_3$)



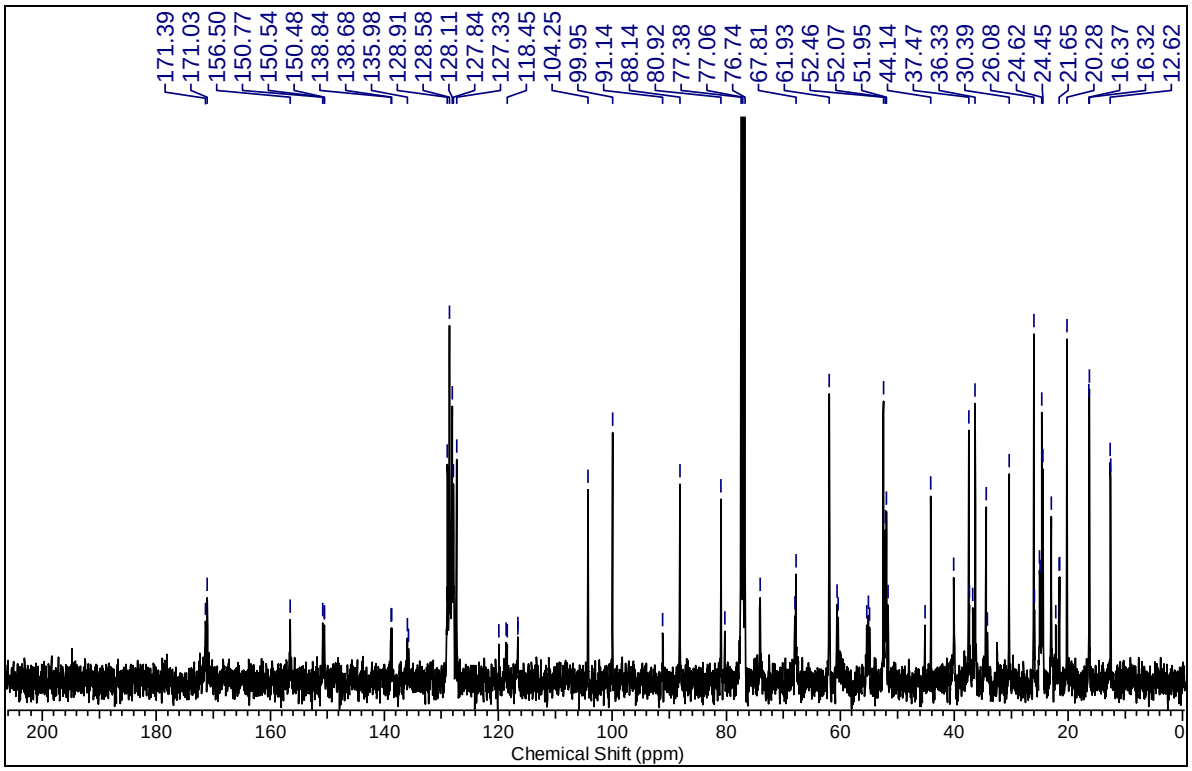
^{13}C NMR of Compound 80 (50 MHz, $CDCl_3$)



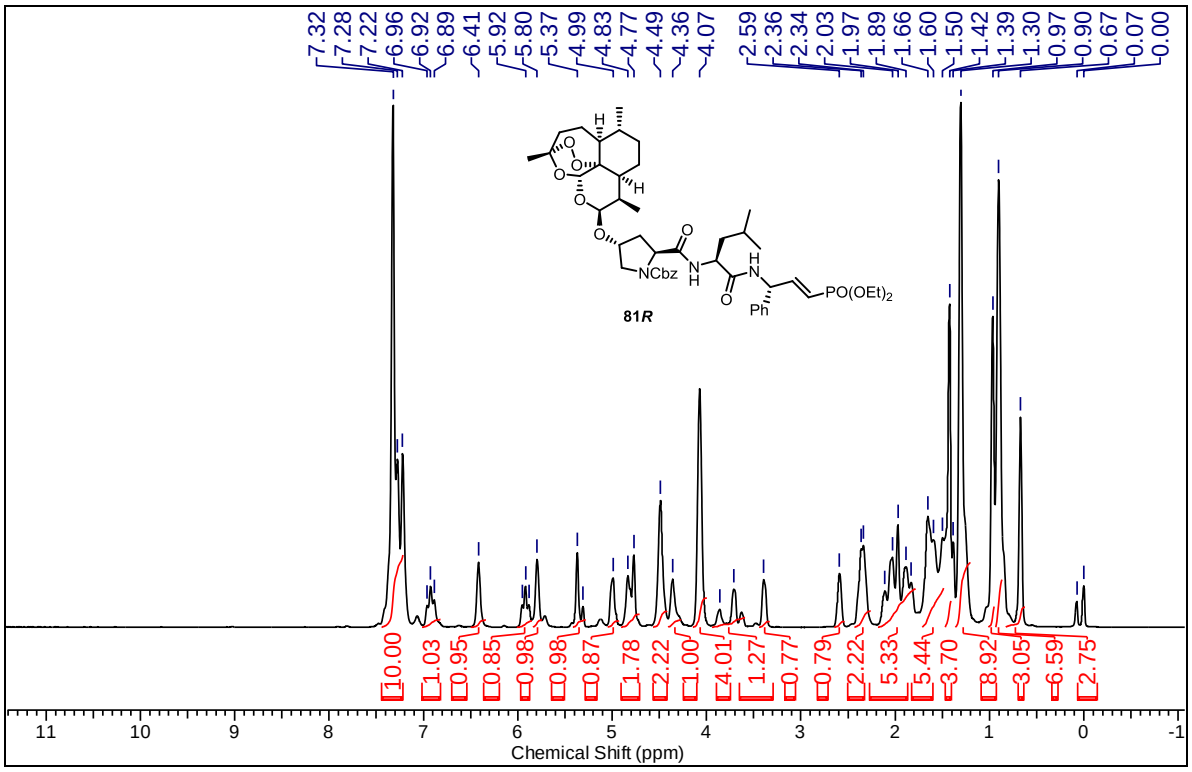
^{1}H NMR of Compound 81 (400 MHz, $CDCl_3$)



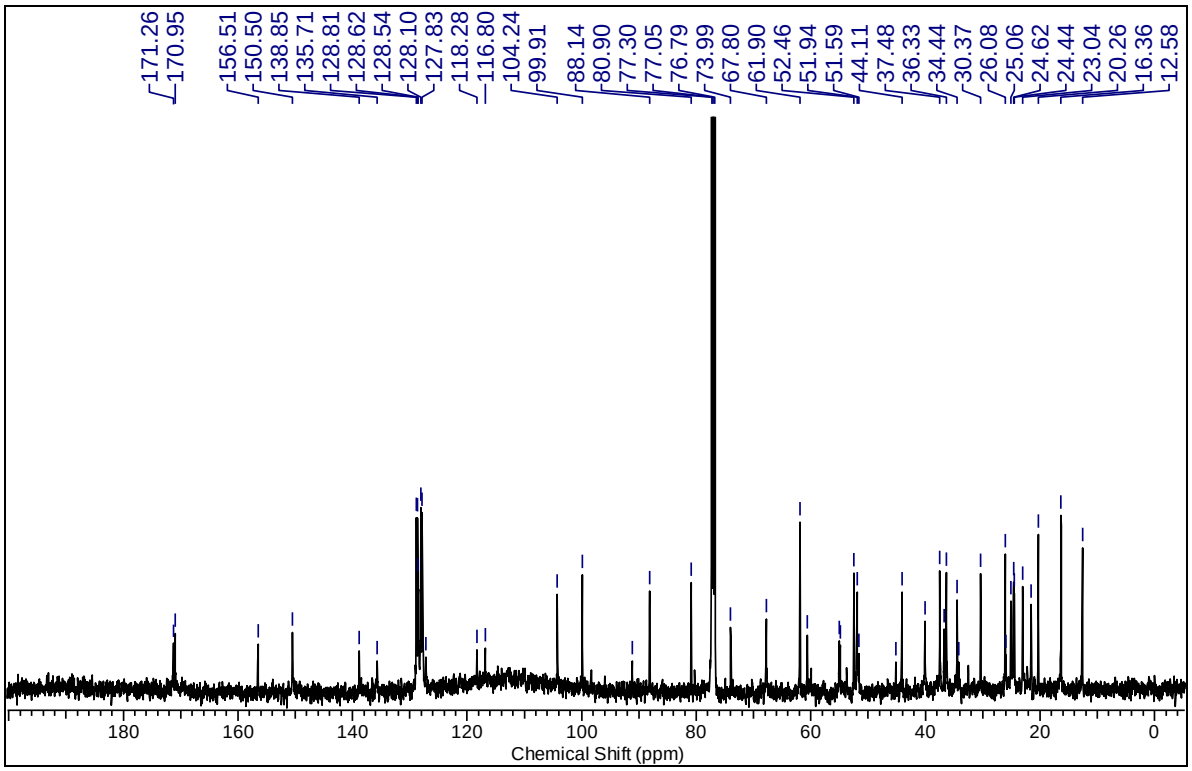
^{13}C NMR of Compound 81 (100 MHz, $CDCl_3$)



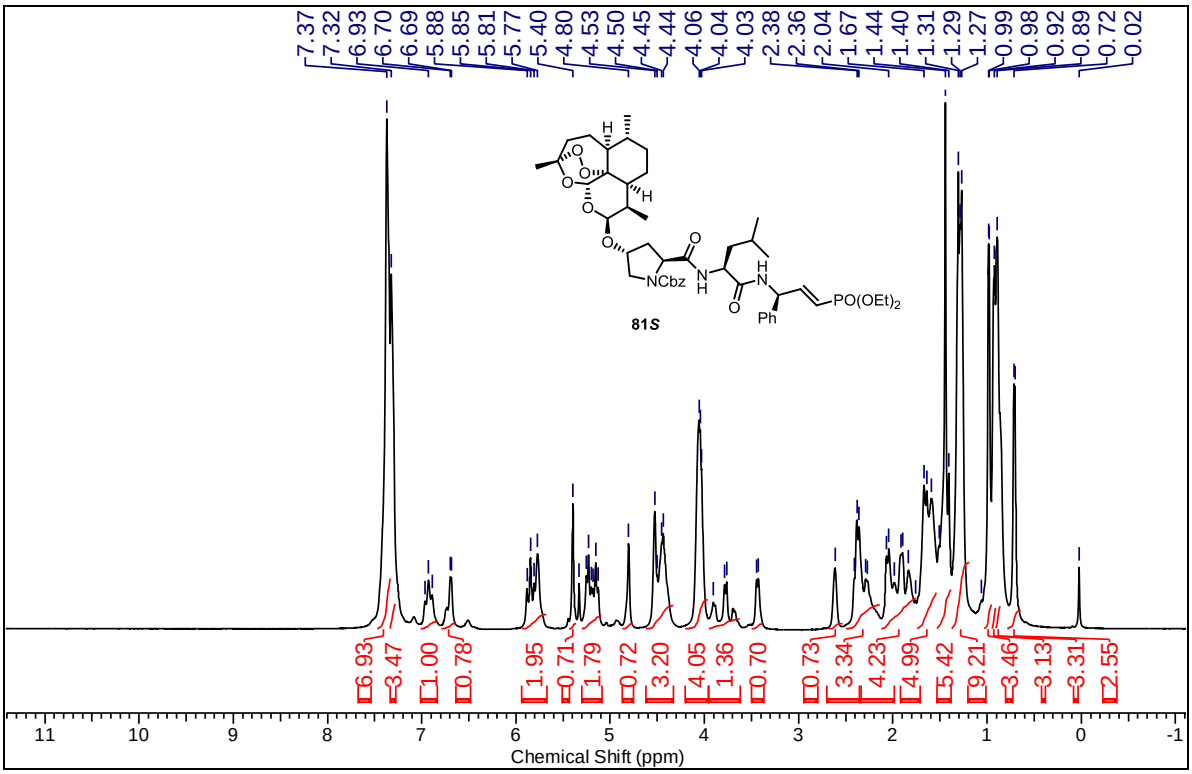
^{1}H NMR of Compound 81*R* (500 MHz, $CDCl_3$)



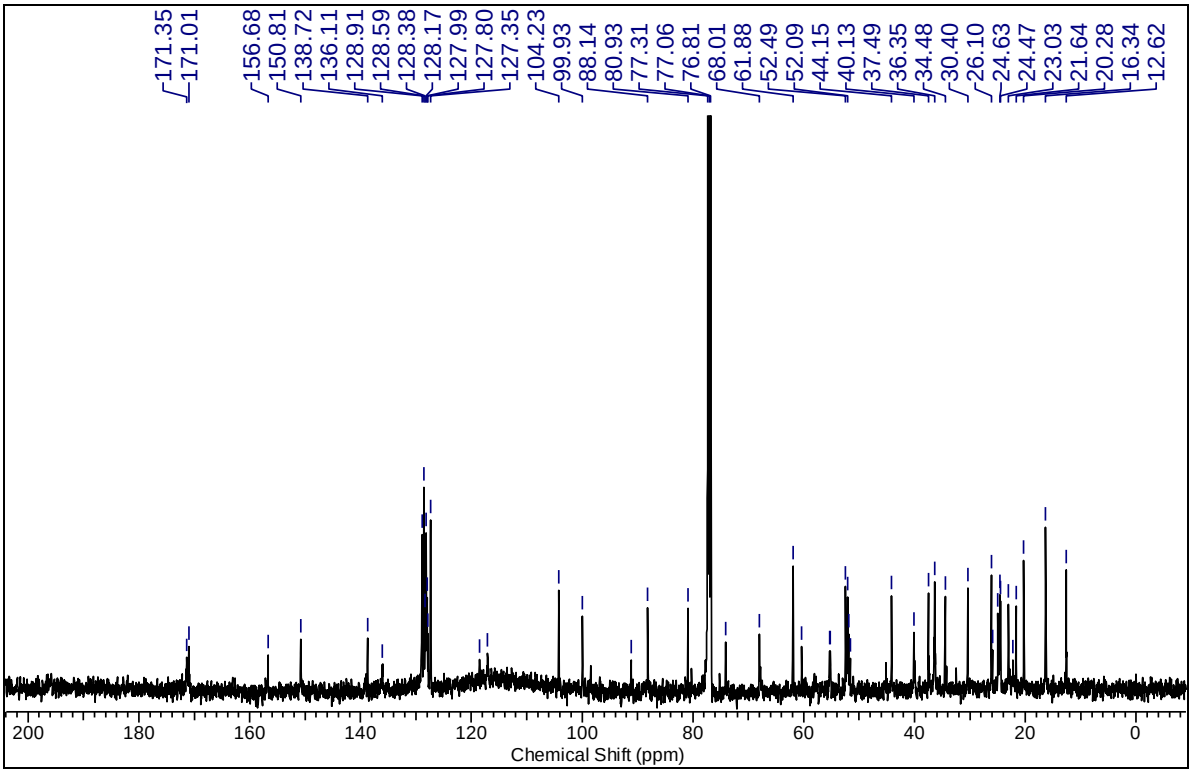
^{13}C NMR of Compound 81*R* (125 MHz, $CDCl_3$)



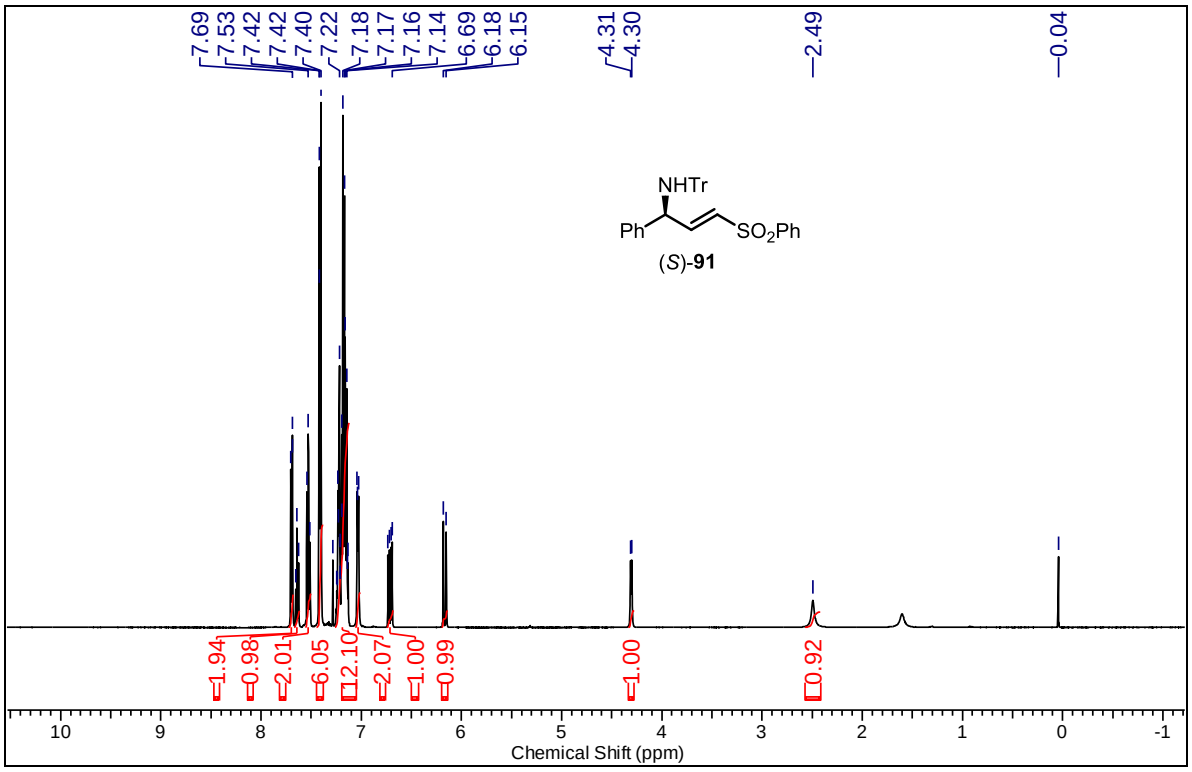
^{1}H NMR of Compound 81*S* (500 MHz, $CDCl_3$)



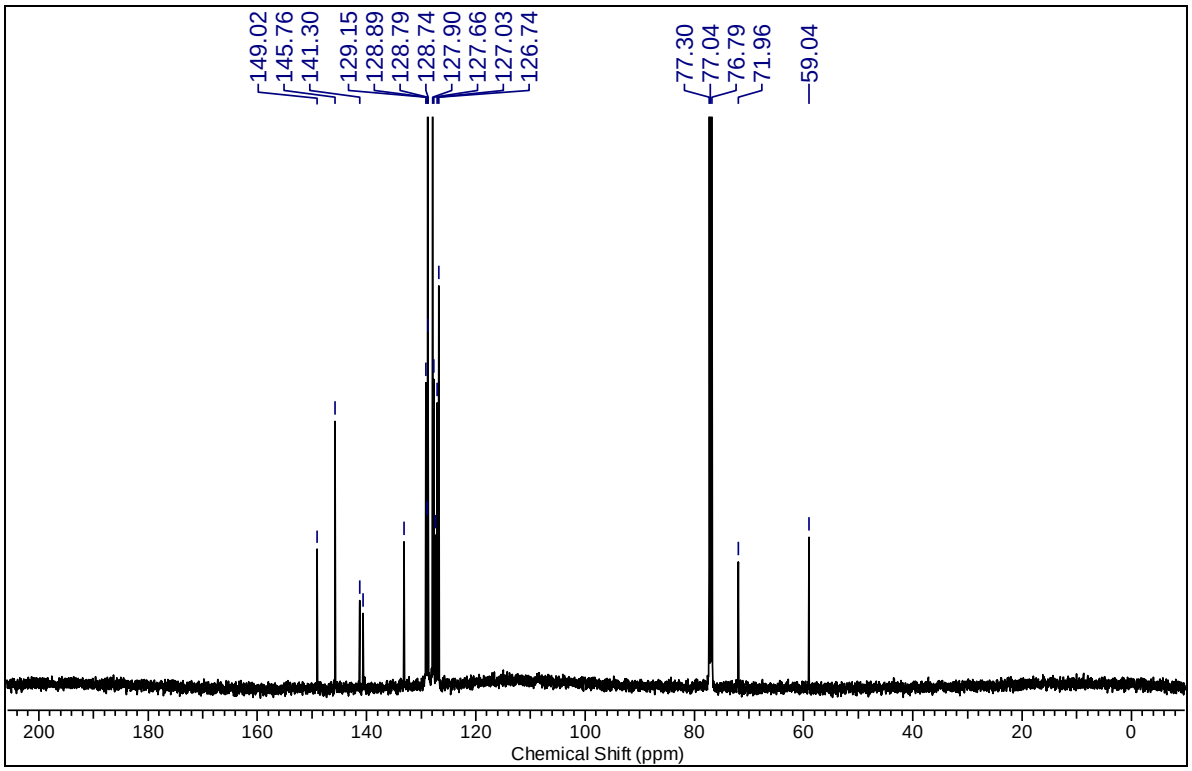
^{13}C NMR of Compound 81*S* (125 MHz, $CDCl_3$)



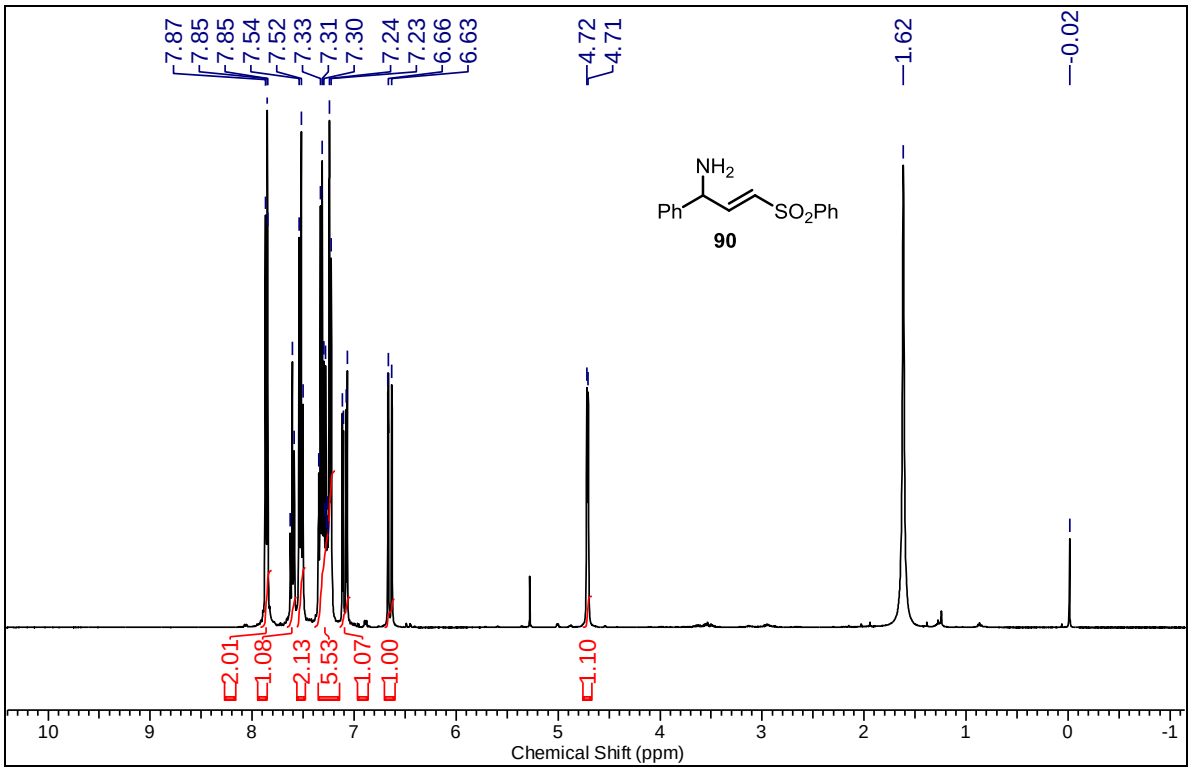
^{1}H NMR of Compound (*S*)-91 (500 MHz, $CDCl_3$)



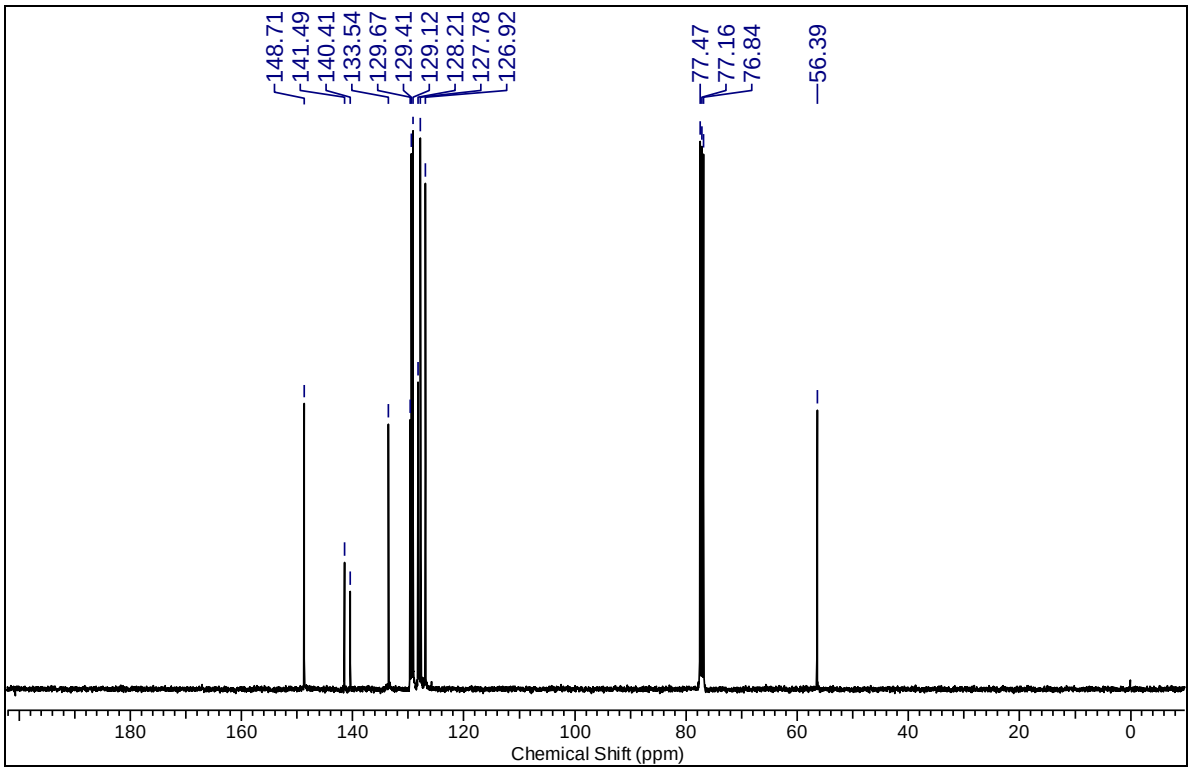
^{13}C NMR of Compound (*S*)-91 (125 MHz, $CDCl_3$)



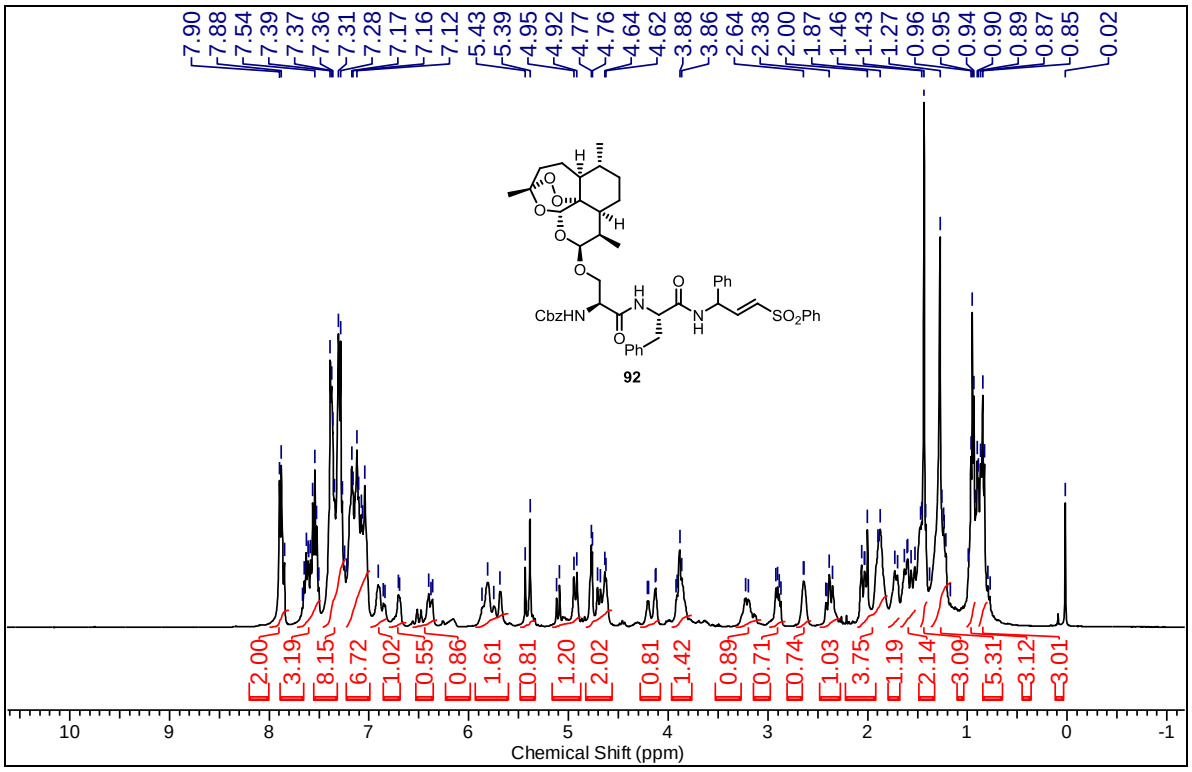
^{1}H NMR of Compound 90 (400 MHz, $CDCl_3$)



^{13}C NMR of Compound 90 (100 MHz, $CDCl_3$)

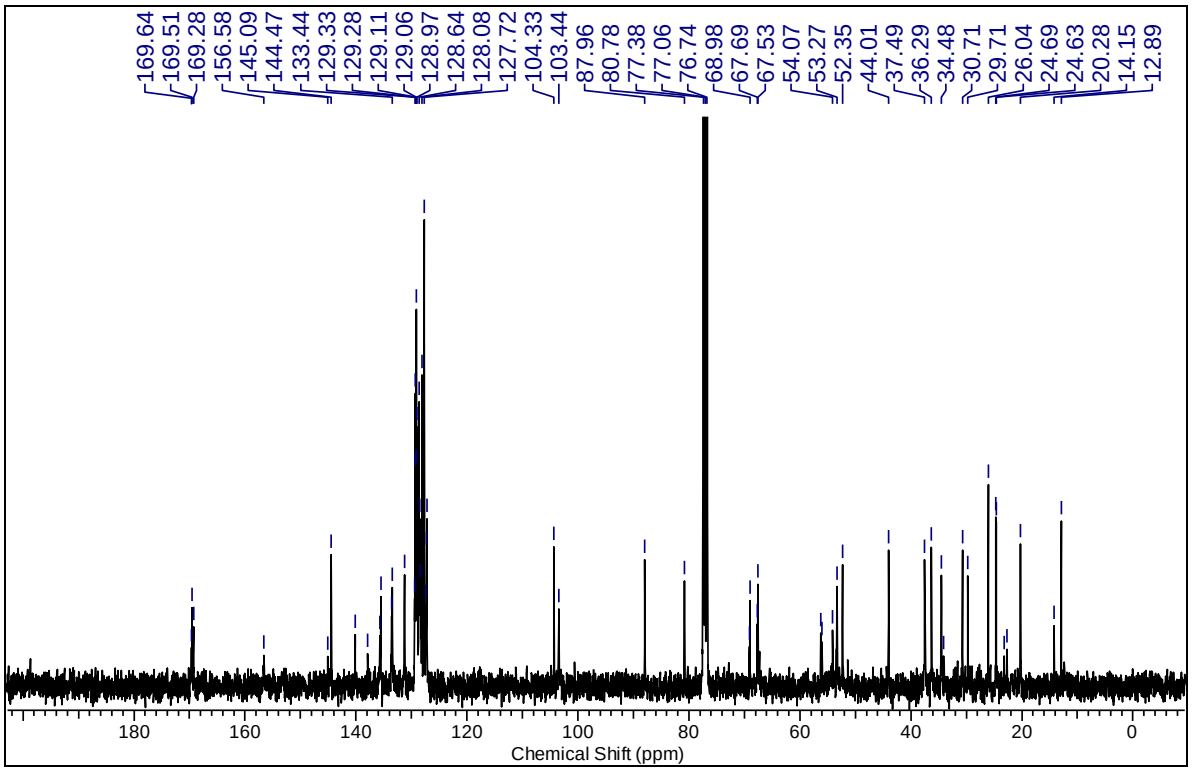


^{1}H NMR of Compound 92 (400 MHz, $CDCl_3$)

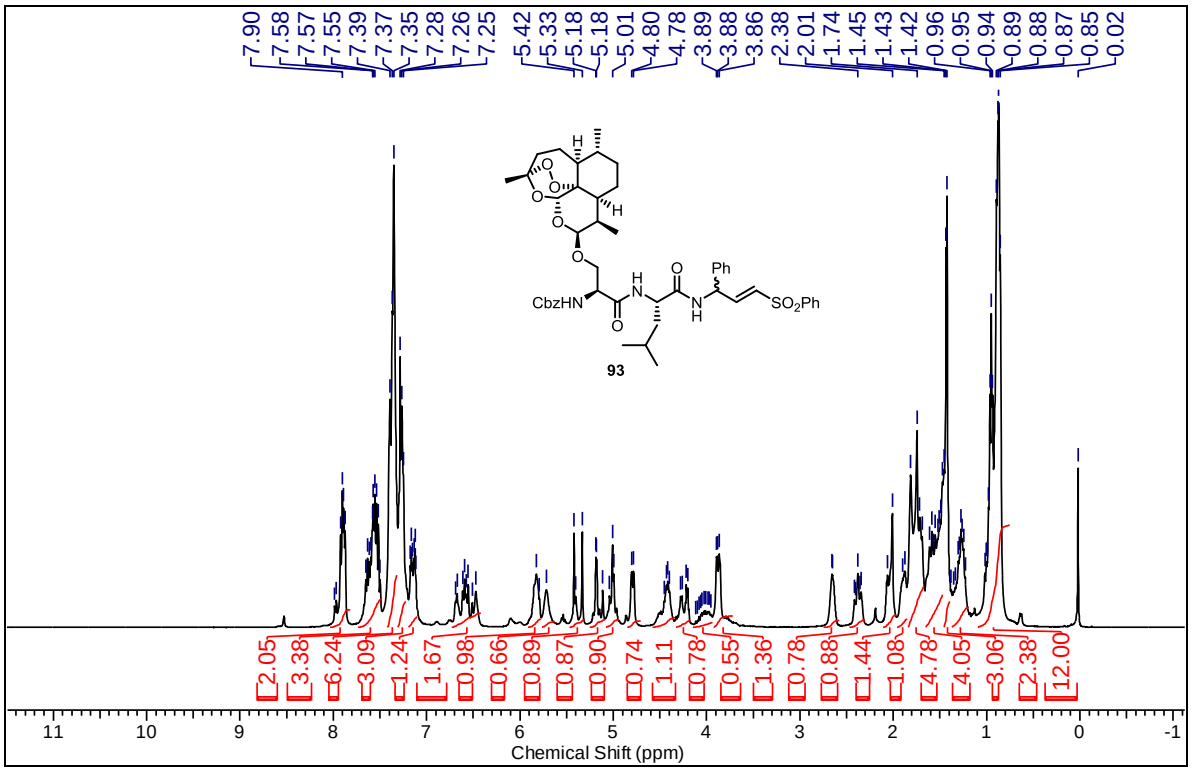


92

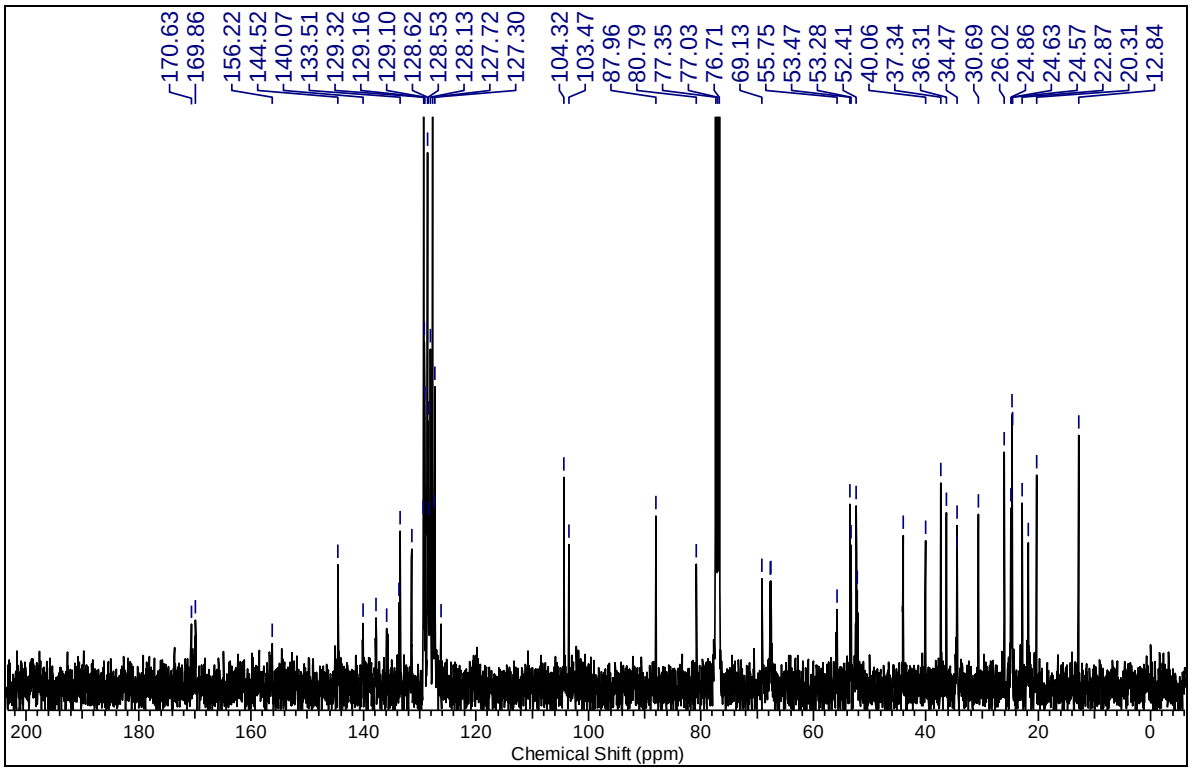
^{13}C NMR of Compound 92 (100 MHz, $CDCl_3$)



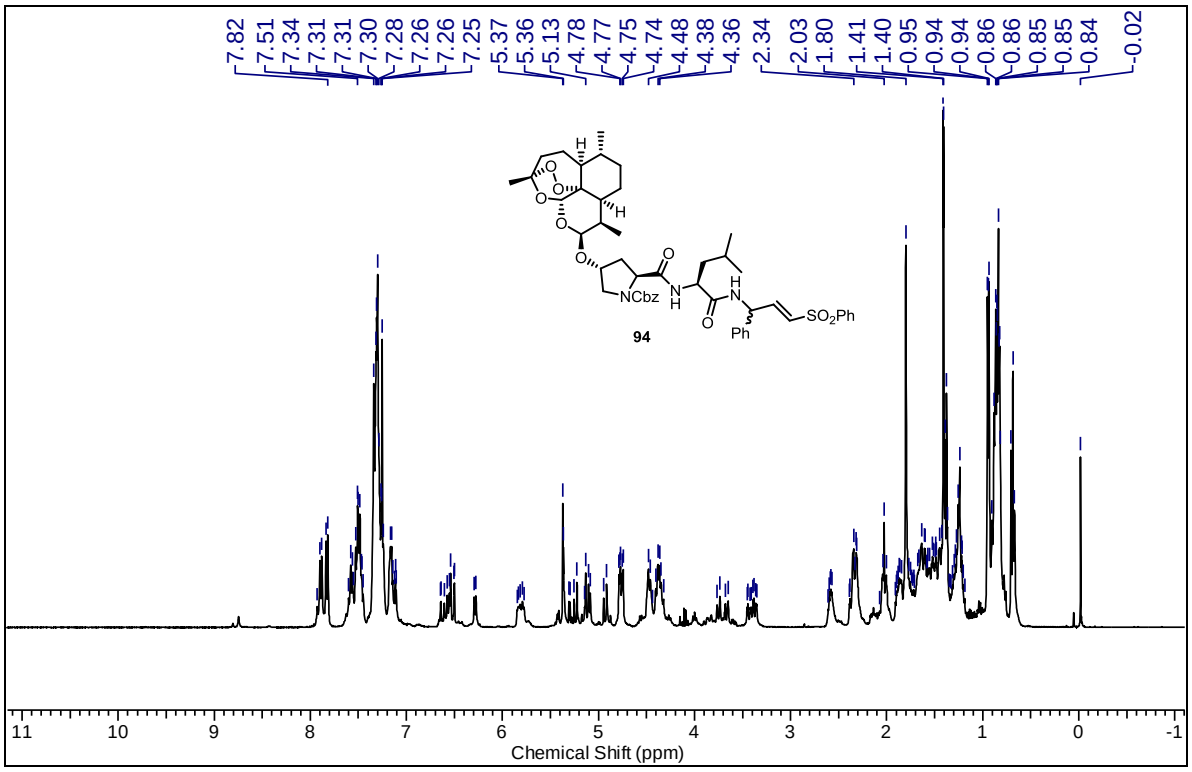
^{1}H NMR of Compound 93 (400 MHz, $CDCl_3$)



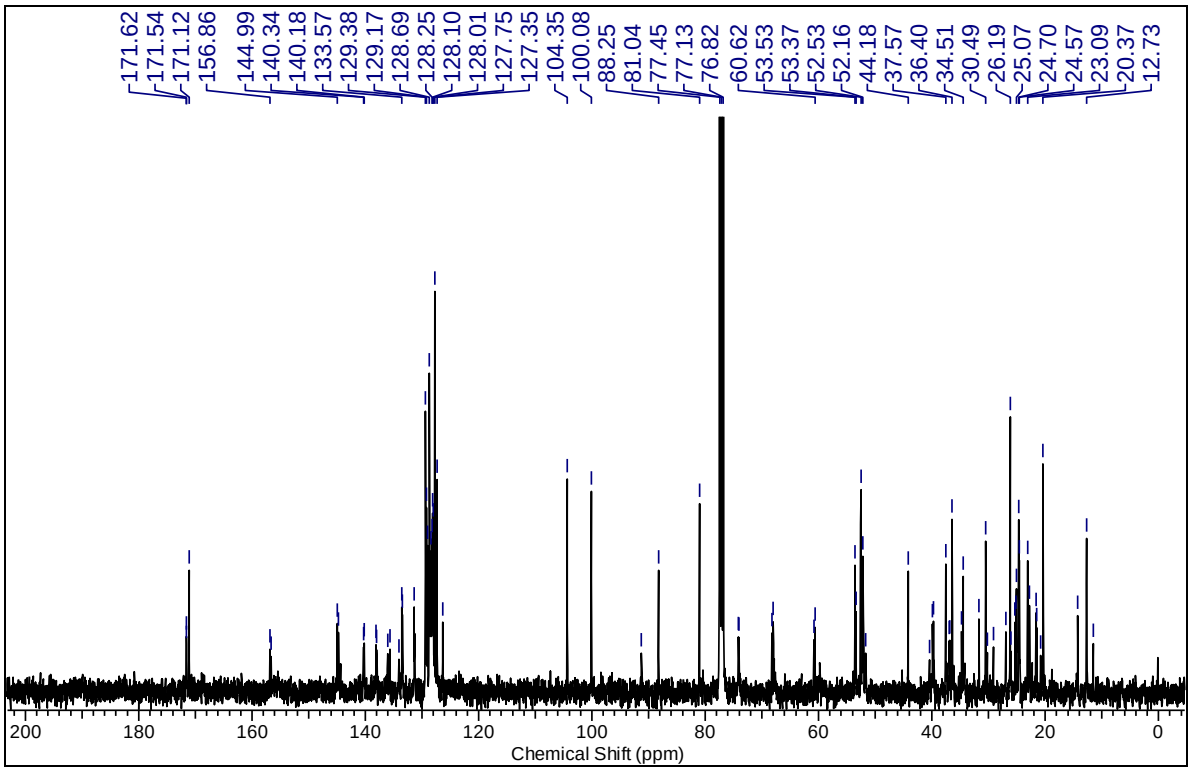
^{13}C NMR of Compound 93 (100 MHz, $CDCl_3$)



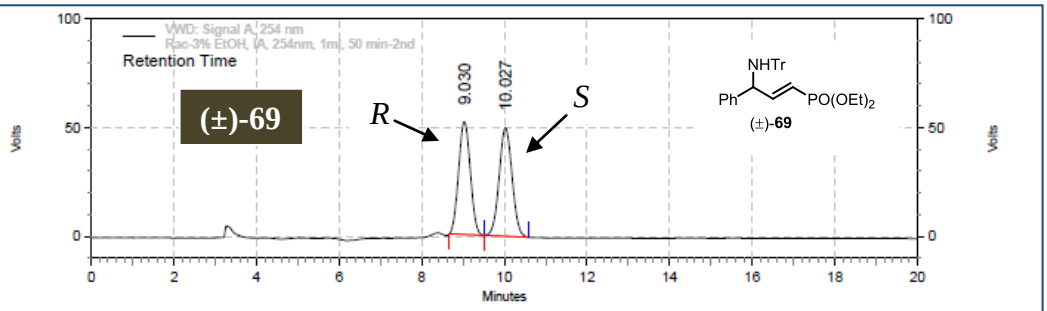
^{1}H NMR of Compound 94 (400 MHz, $CDCl_3$)



^{13}C NMR of Compound 94 (100 MHz, $CDCl_3$)

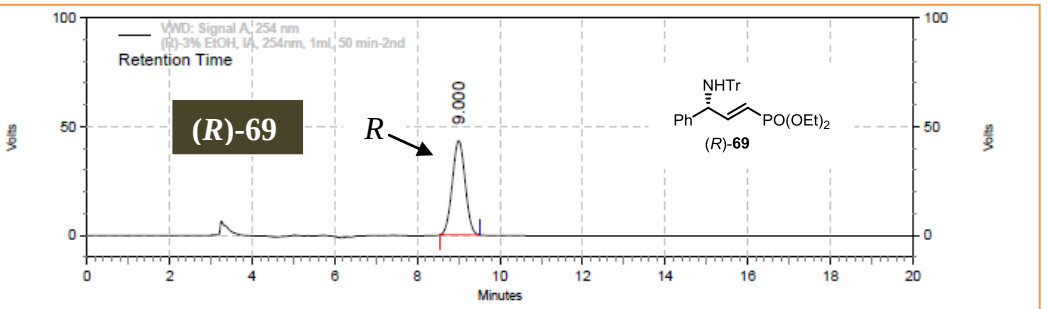


1.9. HPLC Chromatograms of (±)-69, (*R*)-69 and (*S*)-69
Chiralpak-IA, Hexane:EtOH (97:3), flow 1.0 mL/min, det.: UV 254 nm



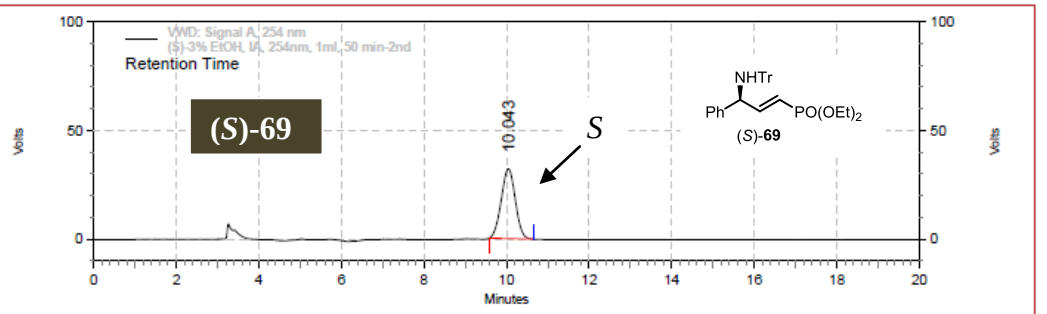
VWD: Signal A, 254 nm Results

Retention Time	Area	Area %
9.030	17112553	48.47
10.027	18189264	51.53
Totals	35301817	100.00



VWD: Signal A, 254 nm Results

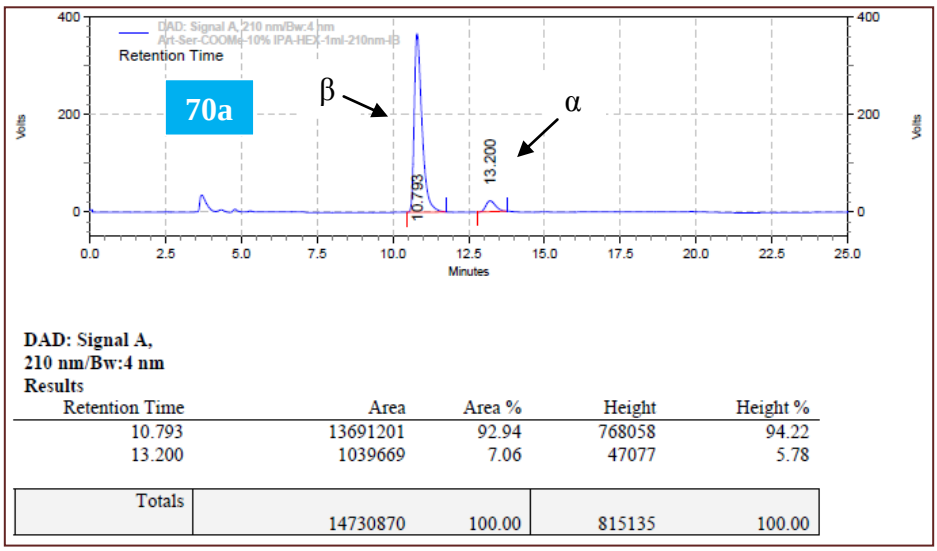
Retention Time	Area	Area %
9.000	15669743	100.00
Totals	15669743	100.00



VWD: Signal A, 254 nm Results

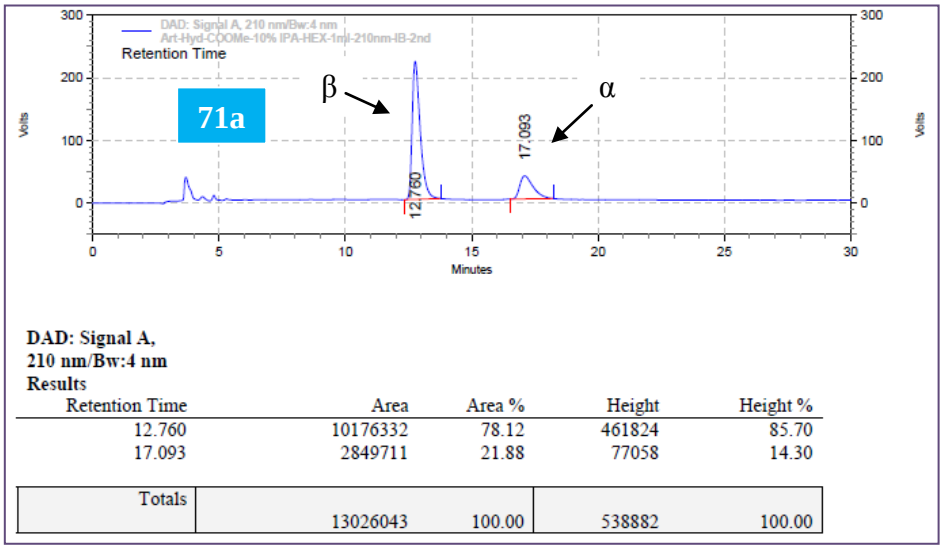
Retention Time	Area	Area %
10.043	12711697	100.00
Totals	12711697	100.00

HPLC Chromatograms of 70a and 71a
Chiralpak-IB, Hexane:IPA (90:10), flow 1.0 mL/min, det.: UV 210 nm



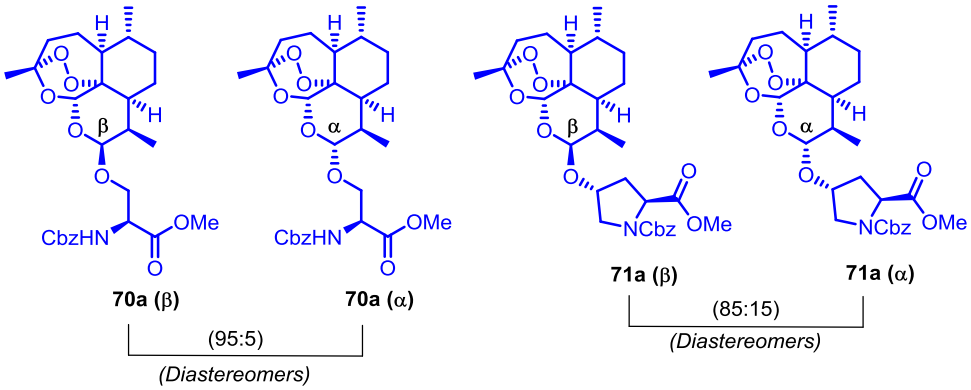
DAD: Signal A,
210 nm/Bw:4 nm
Results

Retention Time	Area	Area %	Height	Height %
10.793	13691201	92.94	768058	94.22
13.200	1039669	7.06	47077	5.78
Totals	14730870	100.00	815135	100.00

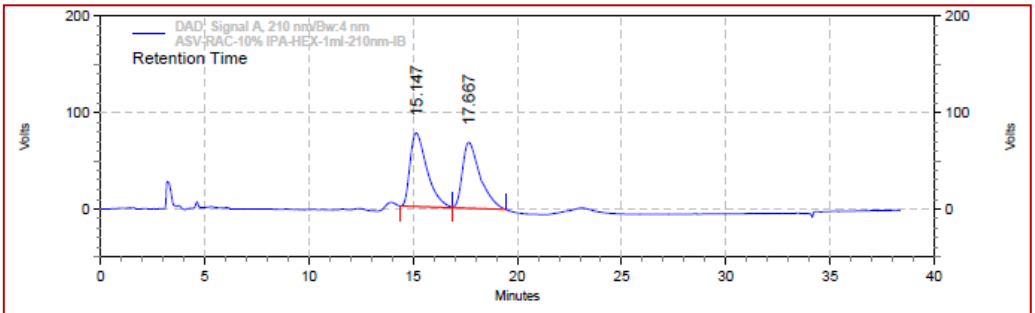


DAD: Signal A,
210 nm/Bw:4 nm
Results

Retention Time	Area	Area %	Height	Height %
12.760	10176332	78.12	461824	85.70
17.093	2849711	21.88	77058	14.30
Totals	13026043	100.00	538882	100.00

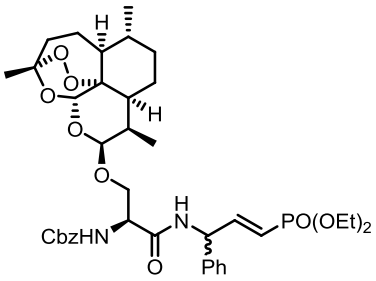


HPLC Chromatogram of 72
Chiralpak-IB, Hexane:IPA (90:10), flow 1.0 mL/min, det.: UV 210 nm



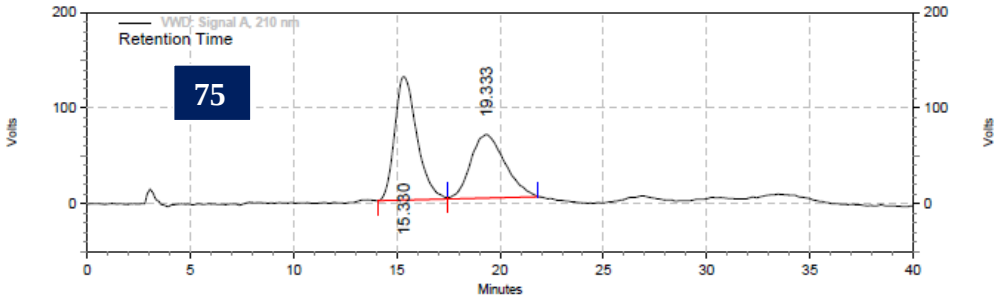
DAD: Signal A,
210 nm/Bw:4 nm
Results

Retention Time	Area	Area %	Height	Height %
15.147	9018993	51.63	159446	52.75
17.667	8449064	48.37	142828	47.25
Totals	17468057	100.00	302274	100.00



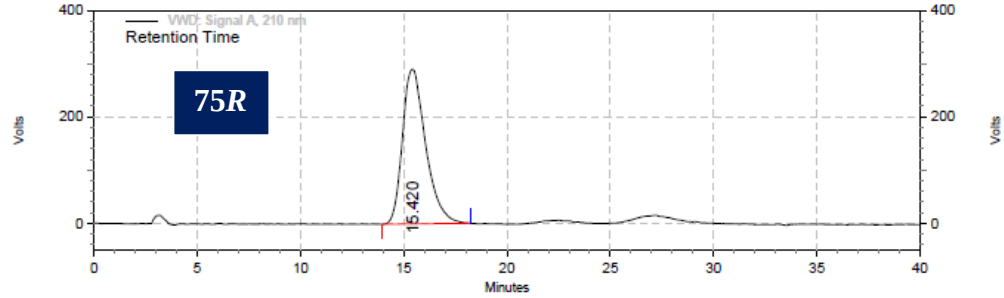
72

HPLC Chromatograms of Hybrd Molecules 75, 75*R* and 75*S*
Chiralpak-IA, Hexane:IPA (82:18), flow 1.0 mL/min, det.: UV 210 nm



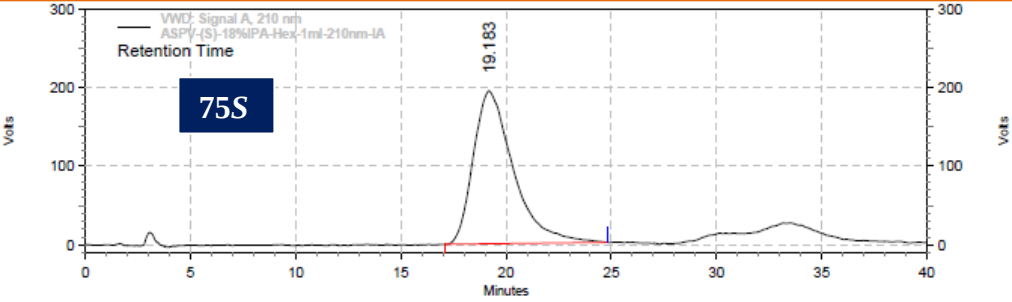
VWD: Signal A, 210 nm Results

Retention Time	Area	Area %
15.330	162146985	55.70
19.333	128978787	44.30
Totals		
	291125772	100.00



VWD: Signal A, 210 nm Results

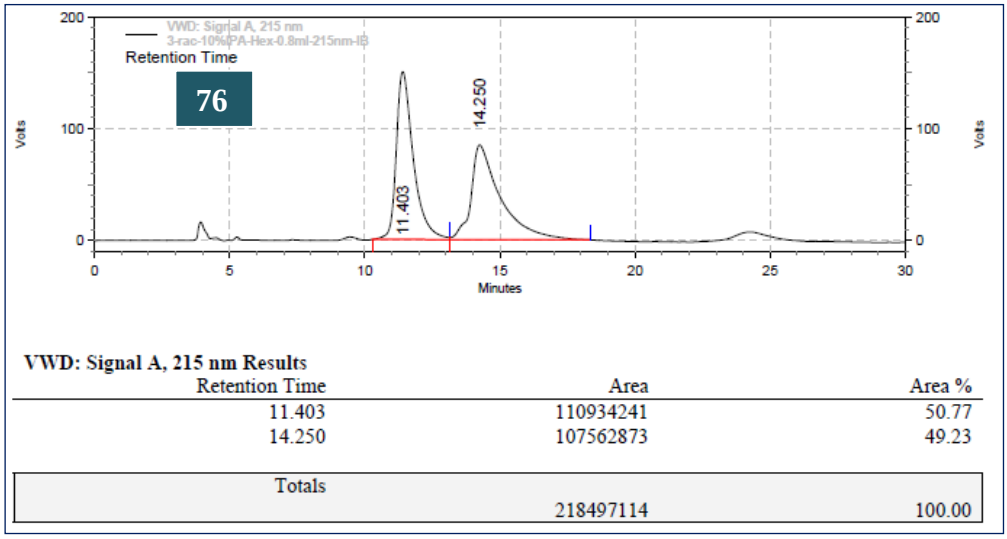
Retention Time	Area	Area %
15.420	380790525	100.00
Totals		
	380790525	100.00



VWD: Signal A, 210 nm Results

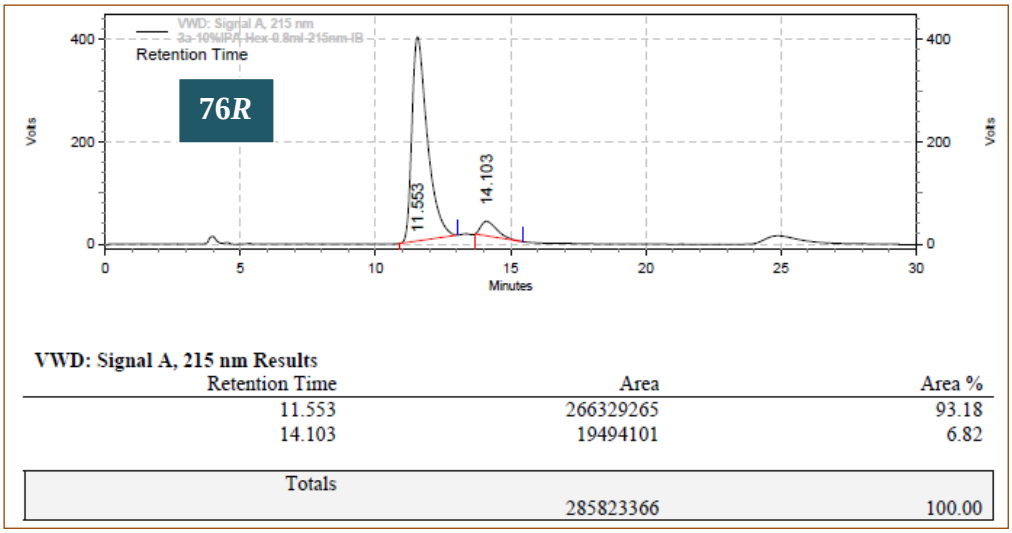
Retention Time	Area	Area %
19.183	430677551	100.00
Totals		
	430677551	100.00

HPLC Chromatograms of Hybrd Molecules 76, 76*R* and 76*S*
Chiralpak-IB, Hexane:IPA (90:10), flow 0.8 mL/min, det.: UV 215 nm



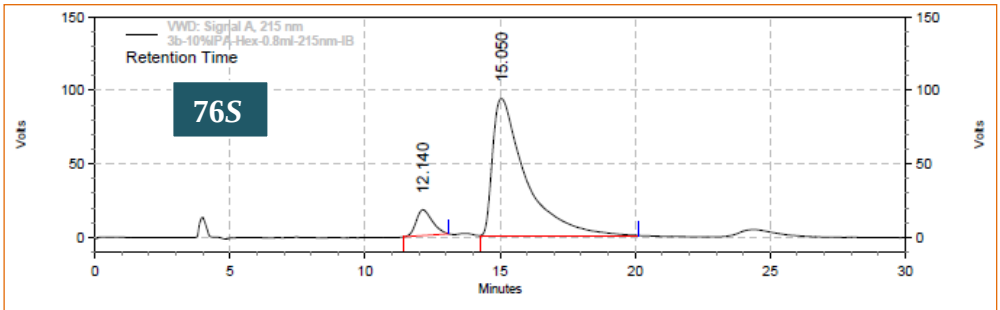
VWD: Signal A, 215 nm Results

Retention Time	Area	Area %
11.403	110934241	50.77
14.250	107562873	49.23
Totals	218497114	100.00



VWD: Signal A, 215 nm Results

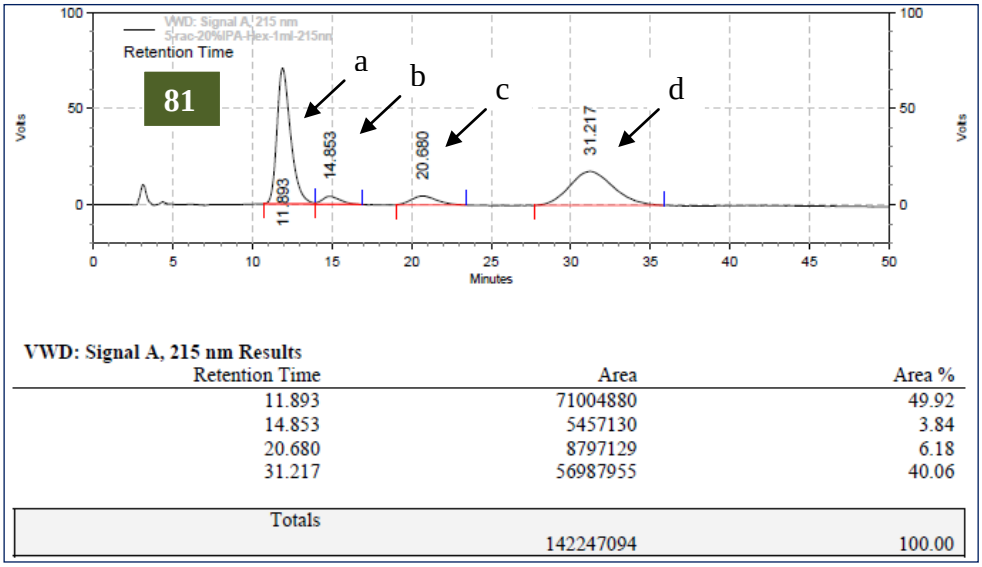
Retention Time	Area	Area %
11.553	266329265	93.18
14.103	19494101	6.82
Totals	285823366	100.00



VWD: Signal A, 215 nm Results

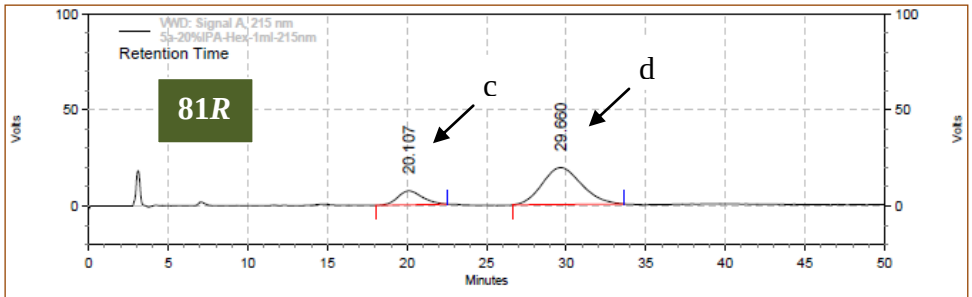
Retention Time	Area	Area %
12.140	12163465	8.41
15.050	132446203	91.59
Totals	144609668	100.00

HPLC Chromatograms of Hybrd Molecules 81, 81*R* and 81*S*
Chiralpak-IA, Hexane:IPA (80:20), flow 1.0 mL/min, det.: UV 215 nm



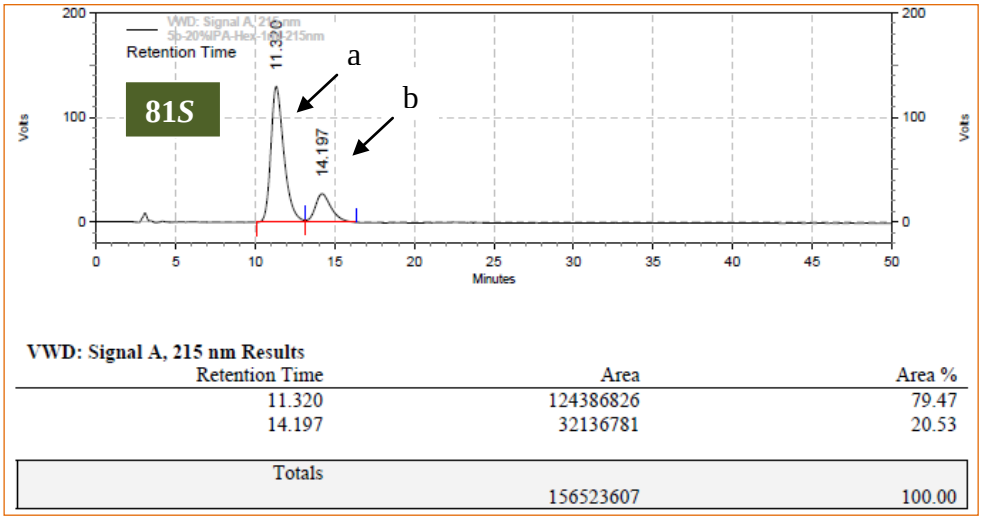
VWD: Signal A, 215 nm Results

Retention Time	Area	Area %
11.893	71004880	49.92
14.853	5457130	3.84
20.680	8797129	6.18
31.217	56987955	40.06
Totals	142247094	100.00



VWD: Signal A, 215 nm Results

Retention Time	Area	Area %
20.107	13101285	19.10
29.660	55504507	80.90
Totals	68605792	100.00



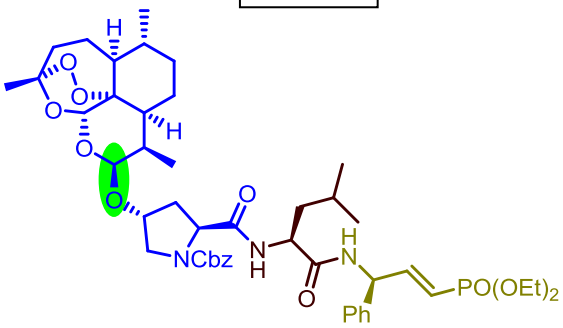
VWD: Signal A, 215 nm Results

Retention Time	Area	Area %
11.320	124386826	79.47
14.197	32136781	20.53
Totals	156523607	100.00

In the above HPLC chromatograms, peak **a** corresponds to β diastereomer (epimer) of dihydroartemisinin formed from the (*S*)-γ-phenyl-γ-amino vinyl phosphonate (*i.e.* (*S*)-**52**) and peak **b** corresponds to α diastereomer (epimer) of dihydroartemisinin formed from the same (*S*)-γ-phenyl-γ-amino vinyl phosphonate (*i.e.* (*S*)-**52**).

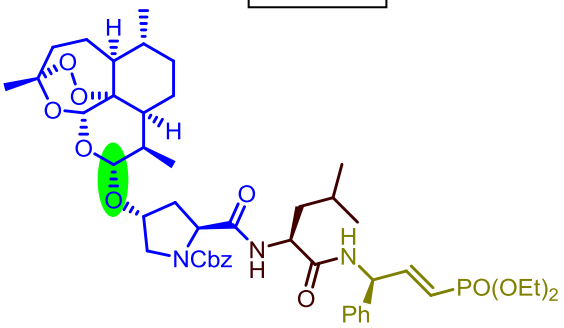
Similarly, peak **c** corresponds to α diastereomer of dihydroartemisinin formed from the (*R*)-γ-phenyl-γ-amino vinyl phosphonate (*i.e.* (*R*)-**52**) and peak **d** corresponds to β diastereomer of dihydroartemisinin formed from the same (*R*)-γ-phenyl-γ-amino vinyl phosphonate (*i.e.* (*R*)-**52**).

Peak **a**



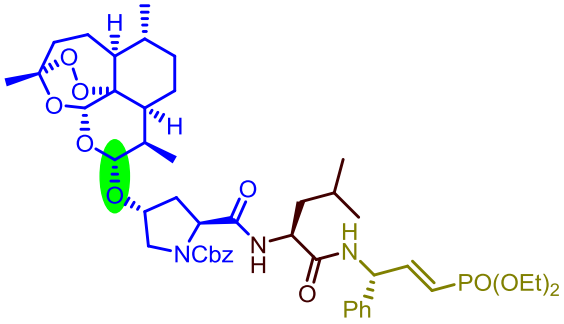
81*S* (β)
(major)

Peak **b**



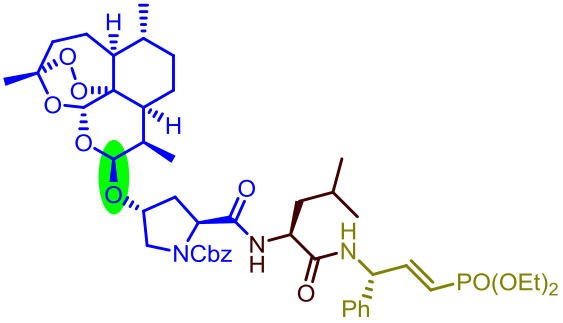
81*S* (α)
(minor)

Peak **c**



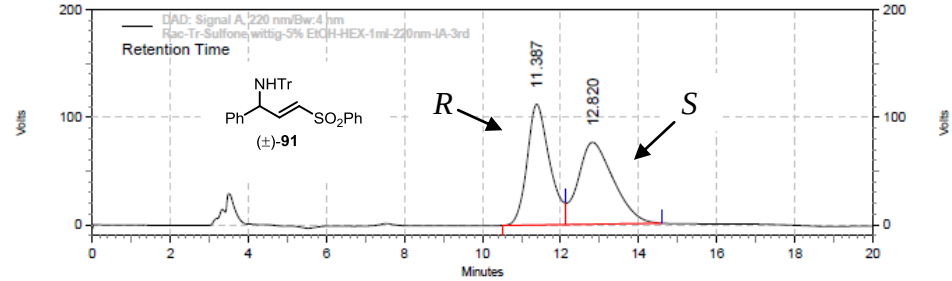
81*R* (α)
(minor)

Peak **d**



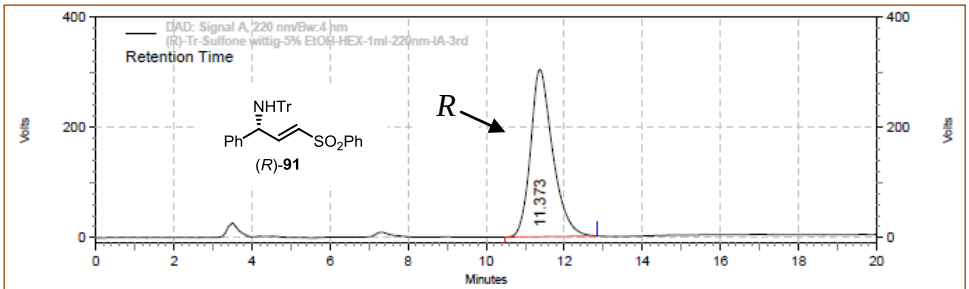
81*R* (β)
(major)

HPLC Chromatograms of (±)-91, (*R*)-91 and (*S*)-91
Chiralpak-IA, Hexane:EtOH (95:5), flow 1.0 mL/min, det.: UV 220 nm



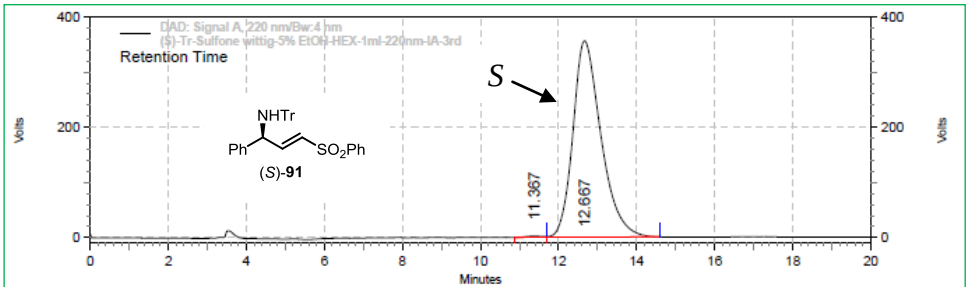
DAD: Signal A, 220 nm/Bw:4 nm
Results

Retention Time	Area	Area %
11.387	9464848	48.59
12.820	10014659	51.41
Totals	19479507	100.00



DAD: Signal A, 220 nm/Bw:4 nm
Results

Retention Time	Area	Area %
11.373	25056146	100.00
Totals	25056146	100.00



DAD: Signal A, 220 nm/Bw:4 nm
Results

Retention Time	Area	Area %
11.367	145151	0.39
12.667	37284235	99.61
Totals	37429386	100.00

Chapter 2

Synthesis of Biologically Active Molecules

2.1. SECTION A

Synthesis of syncarpamide, its analogs and study of their *in vitro* and *in vivo* antiplasmodial activities

2.1.1. INTRODUCTION

The genus *Zanthoxylum* is the largest in the family Rutaceae and contains more than 200 species. Plants belonging to the genus *Zanthoxylum* contain a variety of biologically active secondary metabolites such as alkaloids, coumarins, and lignans exhibiting febrifuge, sudorific, and diuretic properties.[1-10] The bark of *Zanthoxylum integrifoliolum* has been used in the traditional Chinese system of medicine by Ya-Mei aborigines in Lanyu island in Taiwan as a remedy for snakebite and dyspepsia and as an aromatic tonic in fever.[2-3] In Sri Lanka, the genus *Zanthoxylum* is represented by three species, *viz*., *Z. rhetza*, *Z. caudatum* and *Z. tetraspermum.*[4] Among these, *Z. tetraspermum* (*Sinhala*-katukeena) is used in folklore medicine for the treatment of rheumatism and some forms of diarrhea.[5] Antimalarial compounds containing α, β-unsaturated carbonyl moiety have been isolated from the root bark of *Z. gilletii*.[6] Sticks prepared from the bark of *Z. gilletii* are used for preventing toothache.[7] *Z. liebmanniaum* is used in Mexican traditional medicine for treating stomach aches, amoebiasis, intestinal parasites and as a local anesthetic agent.[8]

Zanthoxylum syncarpum Tul. is an evergreen tree occurring in both North and South America.[9] Although several chemical constituents from *Zanthoxylum* species have been reported[3-8] in the past however, not many secondary metabolites have been isolated from *Z. syncarpum*.[9,10] A (+)-norepinephrine derivative, syncarpamide (**1**) was isolated from the stems of *Zanthoxylum syncarpum*.[10a,b] Syncarpamide (**1**) exhibited antiplasmodial activities against *P. falciparum* with reported IC_{50} values of 2.04 µM and 3.06 µM against D6 and W2 clones, respectively.

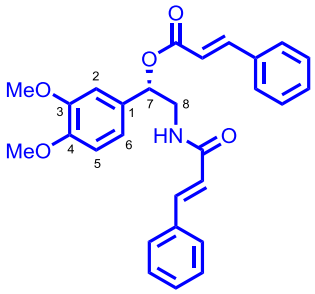


Figure 1. Structure of Syncarpamide **1**.

2.1.2. MALARIA-REVIEW OF LITERATURE

Malaria is caused by the protozoan parasites of the genus *Plasmodium*. Several antimalarial natural products from plant sources were reported in the literature for the treatment of malaria.[11] *Plasmodium* parasite developed resistance to the all available antimalarial drugs including artemisinin.[12] Few examples for natural antimalarial compounds are shown in **Figure 2**. Resistance to currently available antimalarial agents prompted several research groups worldwide to look for new naturally occurring secondary metabolites from plants as antimalarial compounds.

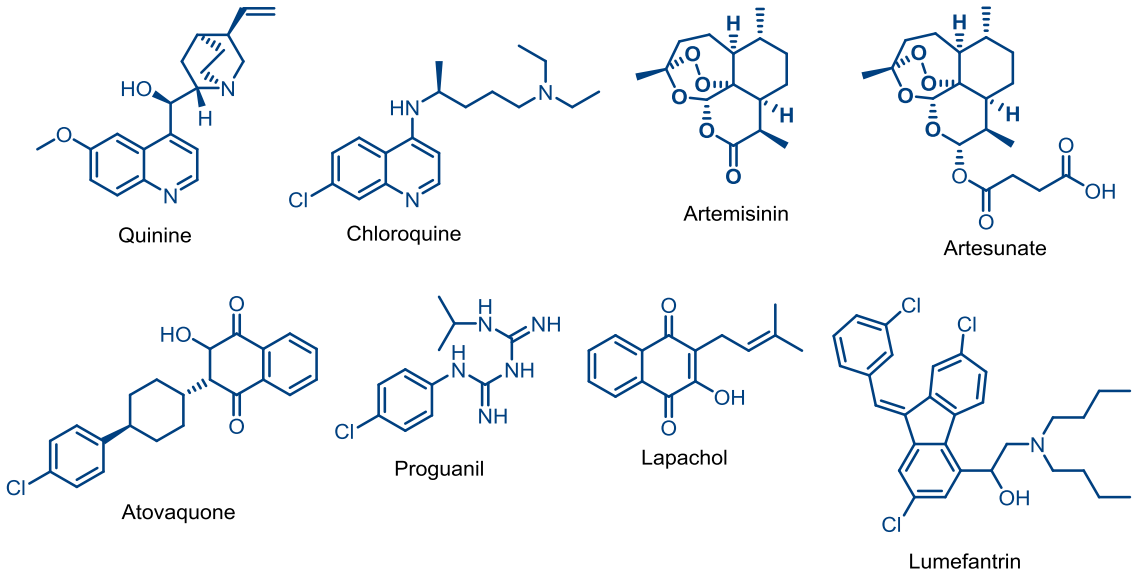


Figure 2. Some examples of natural antimalarial compounds

2.1.3. PRESENT WORK

Objective

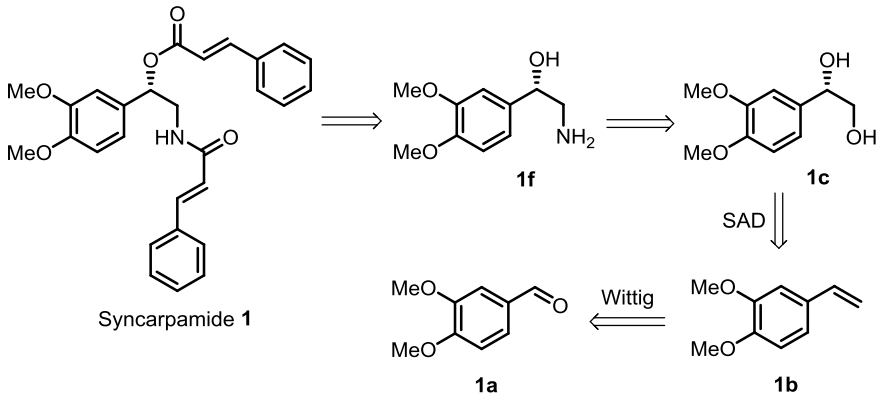
As a part of our ongoing research program on the development of novel antimalarial agents having a different mode of action, we targeted the first asymmetric synthesis of natural

product syncarpamide **1**. Further, we wished to synthesize several analogs by varying the substituents on the aromatic ring and also by varying the ester and amide chains of the parent natural product, syncarpamide. The stereogenic center present in the syncarpamide and its analogs was introduced using Sharpless asymmetric dihydroxylation (SAD) as a key step.

2.1.4. RESULTS AND DISCUSSION

2.1.4.1. Retrosynthesis

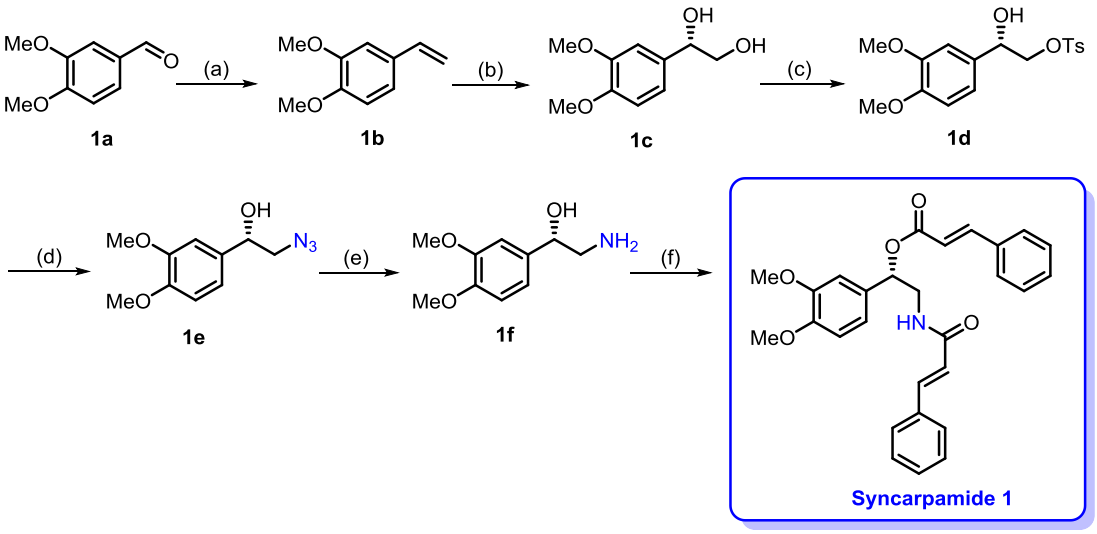
We envisioned that the natural product **1** could be obtained by one-pot esterification and amidation of the amino alcohol **1f**, which in turn could be obtained by the chiral diol **1c**. Chiral diol **1c** could be synthesized following the Sharpless asymmetric dihydroxylation (SAD) of 3,4-dimethoxystyrene **1b** followed by functional group manipulations (**Scheme 1**).



Scheme 1. Retrosynthesis of syncarpamide **1**.

2.1.4.2. Synthesis of syncarpamide 1

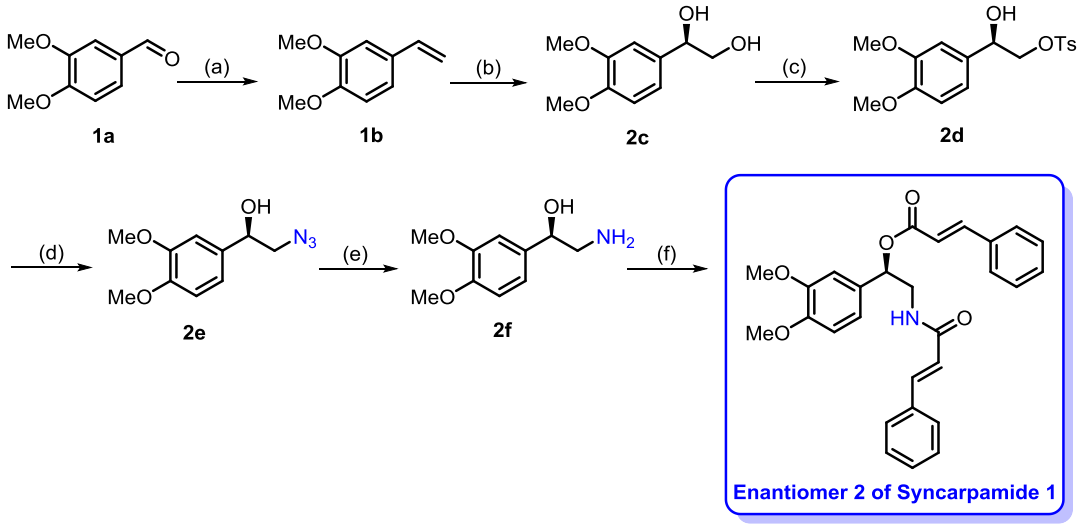
As illustrated in **Scheme 2**, the synthesis of syncarpamide **1** started from commercially available 3,4-dimethoxybenzaldehyde **1a**. Wittig olefination of **1a** with methyltriphenyl phosphonium bromide gave monosubstituted alkene **1b** in 98% yield, which on Sharpless asymmetric dihydroxylation (SAD) with AD-mix-α furnished chiral diol **1c** in 94% yield. Tosylation of diol **1c** with tosyl chloride furnished **1d** in 87% yield which was treated with sodium azide at 80-90 °C to give azido alcohol **1e** in 69% yield. Reduction of azido alcohol **1e** under Pd/C condition furnished amino alcohol **1f** in quantitative yield. Finally, one-pot esterification and amidation of amino alcohol **1f** with cinnamic acid under DCC, DMAP conditions yielded syncarpamide **1** in 75% yield (40% overall yield).[13]



Scheme 2. Synthesis of Syncarpamide **1** employing Sharpless asymmetric dihydroxylation. *Reagents and conditions*: (a) *n*-BuLi, $Ph_3P^+MeBr^-$, THF, −78 °C to rt, 4 h, 98%; (b) AD-mix-α, *t*-BuOH:H_2O (1:1), 0 °C, 12 h, 94%; (c) TsCl, Py, DCM, 0 °C, 2 h, 87%; (d) NaN_3, TBAI, DMF, 80 °C, 2 h, 69%; (e) H_2, Pd/C, MeOH, 4 h, rt, 98%; (f) Cinnamic acid, DCC, DMAP, DCM, 12 h, rt, 75%.

2.1.4.3. Synthesis of the enantiomer of syncarpamide (2)

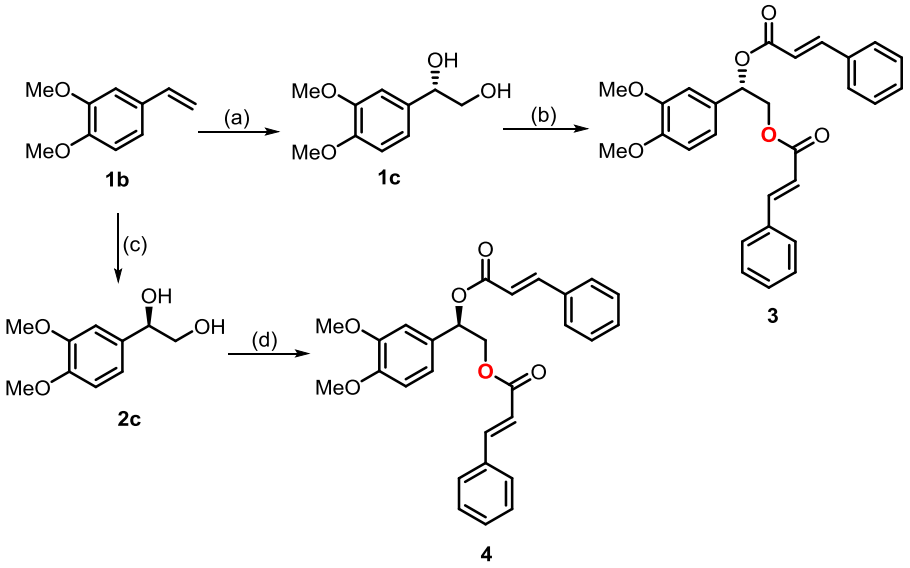
The role of chiral center on the antiplasmodial activity of syncarpamide **1** was studied by carrying out the synthesis of its enantiomer **2**. As shown in **Scheme 3**, an enantiomer of syncarpamide was synthesized following the similar strategy by using AD-Mix-β in the Sharpless asymmetric dihydroxylation to prepare the chiral diol **2c**. Final coupling of the amino alcohol **2f** with cinnamic acid under the earlier used DCC/DMAP conditions furnished the enantiomer **2** of syncarpamide **1**.



Scheme 3. Synthesis of the enantiomer of syncarpamide **2**. *Reagents and conditions*: (a) *n*-BuLi, $Ph_3P^+MeBr^-$, THF, −78 °C to rt, 4 h, 98%; (b) AD-mix-β, *t*-BuOH:H_2O (1:1), 0 °C, 12 h, 96%; (c) TsCl, Py, DCM, 0 °C, 2 h, 86%; (d) NaN_3, TBAI, DMF, 80 °C, 2 h, 60%; (e) H_2, Pd/C, MeOH, 4 h, rt, 98%; (f) Cinnamic acid, DCC, DMAP, DCM, 12 h, rt, 75%.

2.1.4.4. Synthesis of oxy-analogs of syncarpamide (3 and 4)

We then focused on synthesizing oxy-analogs of syncarpamide (*i.e.*, where nitrogen in syncarpamide is replaced by oxygen). Chiral diols **1c** and **2c** were obtained by the Sharpless asymmetric dihydroxylation of 3,4-dimethoxystyrene **1b** with AD-Mix-α and AD-Mix-β, respectively which were subjected directly to the coupling reaction with cinnamic acid under DCC and DMAP conditions to furnish the oxy-analogs of syncarpamide (**3** and **4**) (**Scheme 4**).



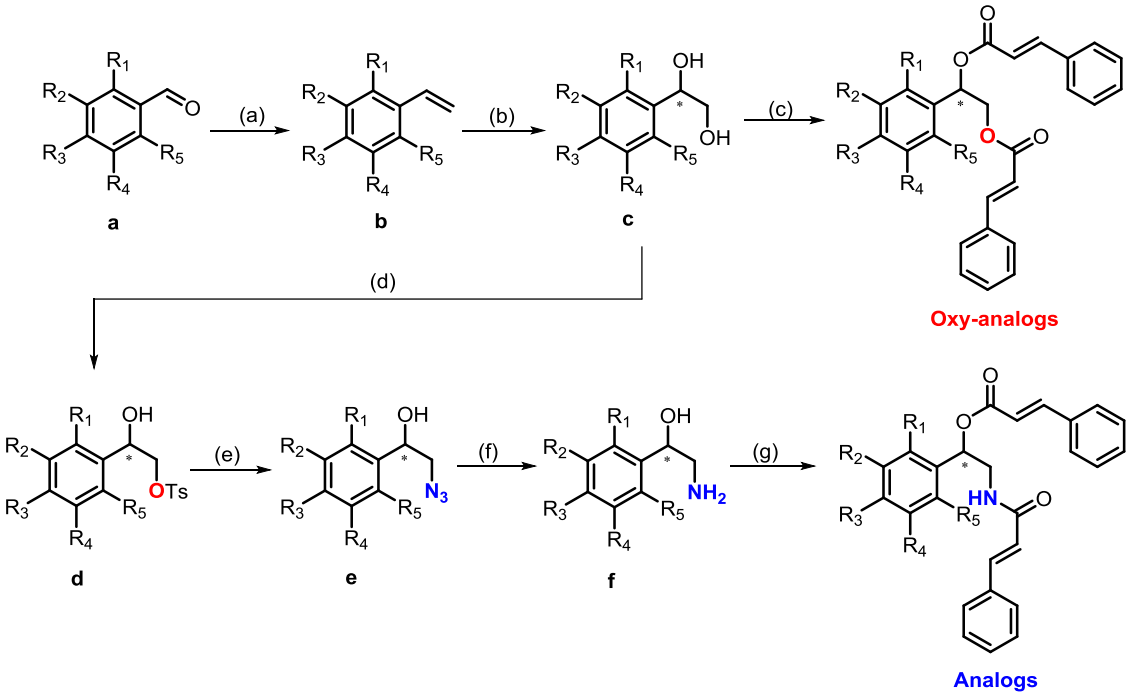
Scheme 4. Synthesis of oxy-analogs of syncarpamide (**3** and **4**). *Reagents and conditions*: (a) AD-mix-α, *t*-BuOH:H_2O (1:1), 0 °C, 12 h, 94%; (b) Cinnamic acid, DCC, DMAP, DCM, 12 h, rt, 98%; (c) AD-mix-β, *t*-BuOH:H_2O (1:1), 0 °C, 12 h, 96%; (d) Cinnamic acid, DCC, DMAP, DCM, 12 h, rt, 98%.

2.1.4.5. Synthesis of analogs and oxy-analogs of syncarpamide using various substituted aromatic aldehydes (5-38)

Following the strategy mentioned above, several analogs and oxy-analogs of syncarpamide (**5-38**) were synthesized by varying different substituents on aromatic aldehyde. Different substituted aromatic aldehydes were reacted with one-carbon Wittig salt, methyltriphenylphosphonium bromide under strongly basic conditions (^{n}BuLi or NaH or

$NaNH_2$) to furnish the corresponding styrenes **b**.[14] Substituted styrenes **b** on Sharpless asymmetric dihydroxylation[15] with AD-mix-α or AD-mix-β afforded the *S*- or *R*-diols **c**,[16] respectively with high enantiomeric excess (*ee*). Substituted chiral diols **c** on reaction with cinnamic acid under DCC, DMAP condition furnished the desired oxy-analogs of syncarpamide (**Scheme 5**) with high enantiomeric excess (*ee*) as determined by chiral HPLC analysis.

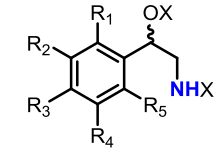
Monotosylation of the substituted chiral diols **c** with tosyl chloride furnished monotosylated compounds **d**.[17] Monotosylated compounds **d** when treated with NaN_3 in DMF at 80 °C furnished 1,2-azido alcohols.[18] Subsequent reduction of azido alcohols with 10% Pd/C furnished the desired 1,2-amino alcohols **e**.[19] 1,2-Amino alcohols **e** were treated with cinnamic acid under DCC, DMAP condition to furnish the desired analogs of syncarpamide (**Scheme 5**) with high enantiomeric excess (*ee*) as confirmed by chiral HPLC analysis. All the analogs and oxy-analogs of syncarpamide synthesized using various substituted aromatic aldehydes are depicted in **Table 1**.



Scheme 5. Synthesis of analogs and oxy-analogs of syncarpamide **1** by using various substituted aldehydes. *Reagents and conditions:* (a) Methyltriphenylphophonium bromide, base (^{n}BuLi or NaH or $NaNH_2$), THF, 85-99%; (b) AD-mix-α (or) AD-mix-β, *t*-BuOH:H_2O (1:1), 0 °C, 12 h, 80-98%; (c) Cinnamic acid, DCC, DMAP, DCM, rt, 12-16 h, 75-98%; (d) TsCl, Py, DCM, 0 °C, 3-4 h, 75-90%; (e) NaN_3, ^{n}Bu_4NI, DMF, 80-90 °C, 3-4 h, 80-95%; (f)

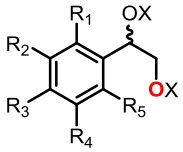
H_2, 10% Pd/C, MeOH, rt, 12 h, 99%; (g) Cinnamic acid, DCC, DMAP, DCM, rt, 12-16 h, 75-98%.

Table 1. Analogs and oxy-analogs of syncarpamide synthesized using various substituted aromatic aldehydes (**5-38**).



Analogs of syncarpamide

X = cinnamoyl

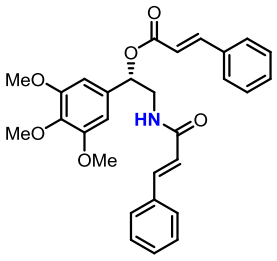


Oxy-analogs of syncarpamide

X = cinnamoyl

Analogs		Oxy-analogues		R-Groups				
S-Config.	*R*-Config.	*S*-Config.	*R*-Config.	R_1	R_2	R_3	R_4	R_5
5	**6**	**7**	**8**	H	OMe	OMe	OMe	H
9	**10**	**11**	**12**	OMe	H	H	H	H
13	**14**	**15**	**16**	H	OMe	H	H	H
17	**18**	**19**	**20**	H	H	OMe	H	H
21	**22**	**23**	**24**	OMe	OMe	H	H	H
25	**26**	**27**	**28**	H	H	H	H	H
29	**30**	**31**	**32**	H	H	Me	H	H
33	**34**	**35**	**36**	H	-OCH_2O-		H	H
n.s.	n.s.	**37**	**38**	-OCH_2O-		H	H	H

n.s.: Not synthesized



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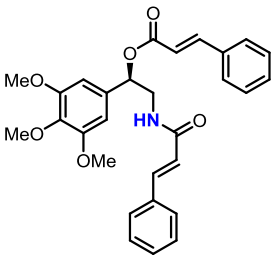

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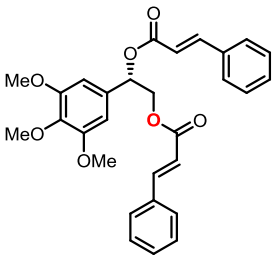

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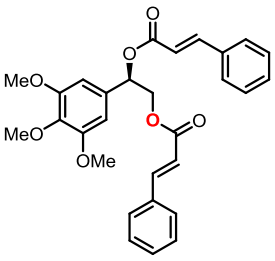

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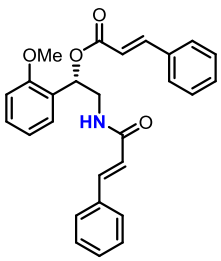

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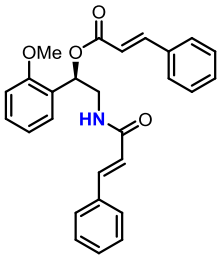

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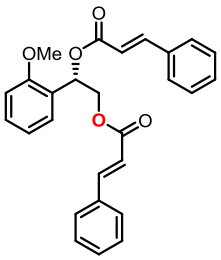

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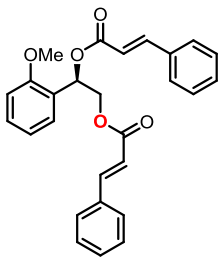

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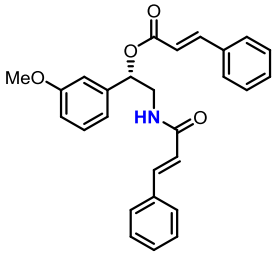

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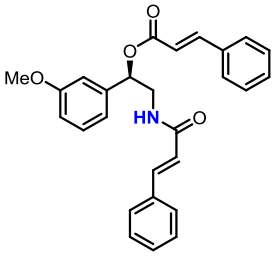

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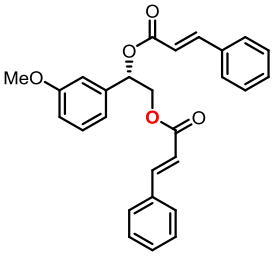

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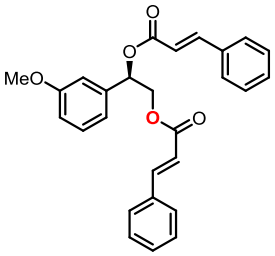

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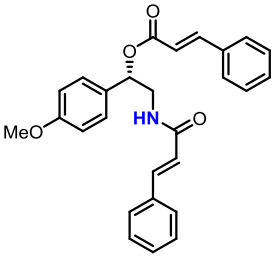

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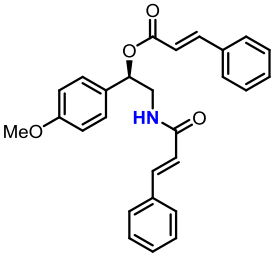

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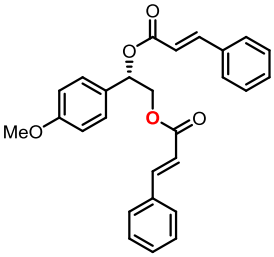

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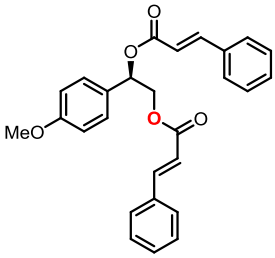

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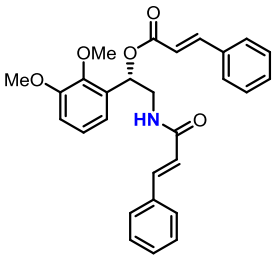

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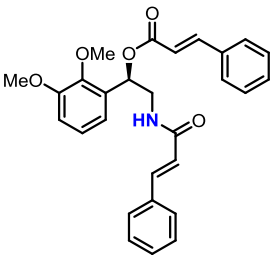

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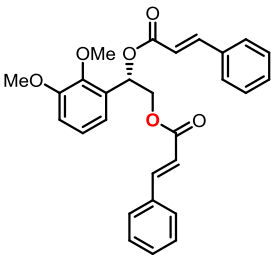

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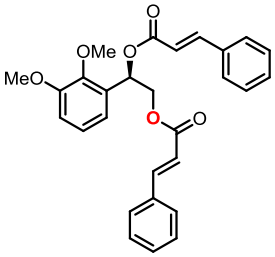

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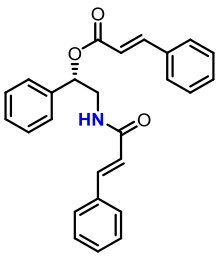

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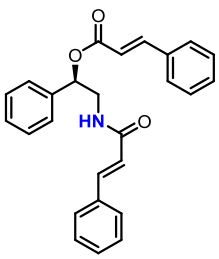

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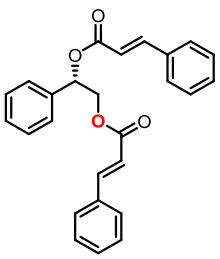

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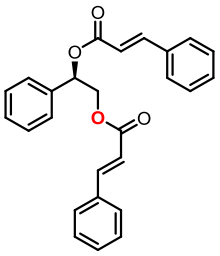

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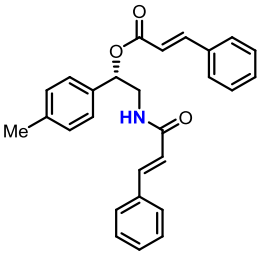

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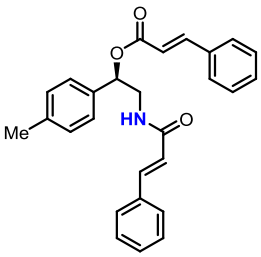

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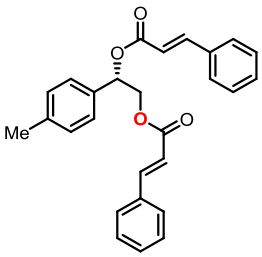

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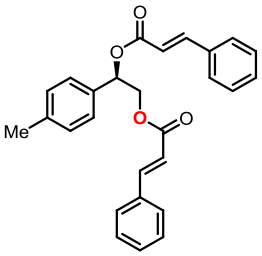

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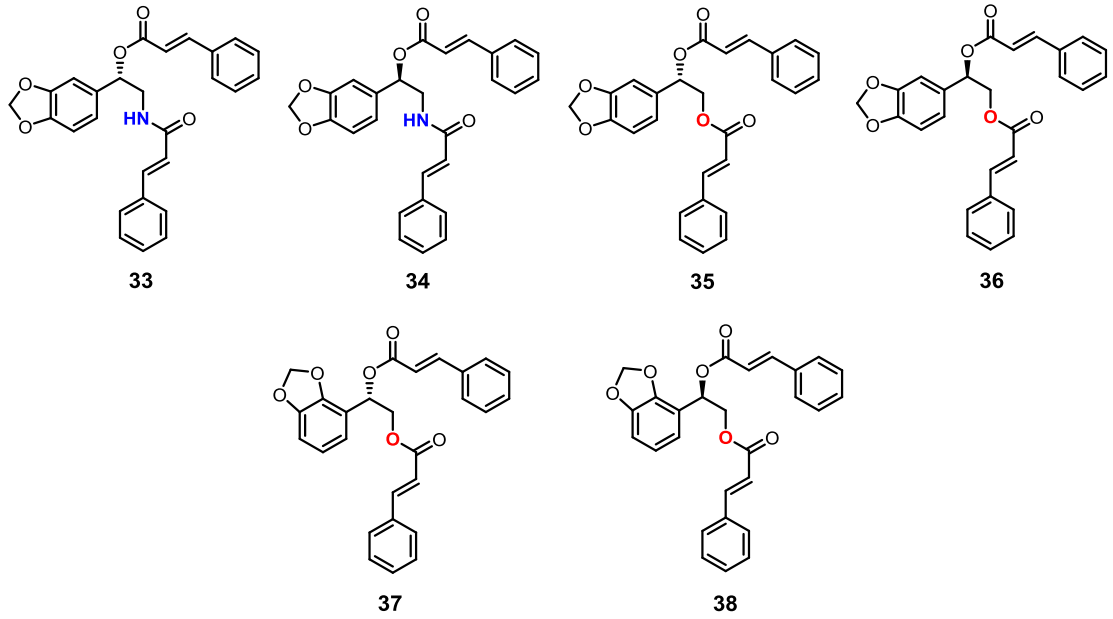
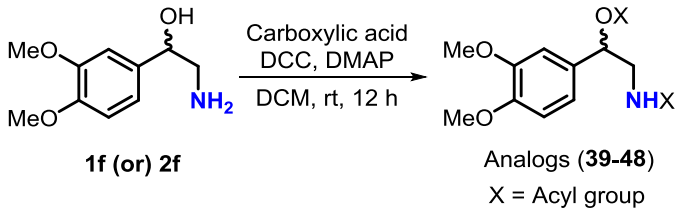


Figure 3. Structures of analogs and oxy-analogs of syncarpamide (**5-38**).

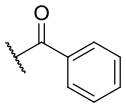
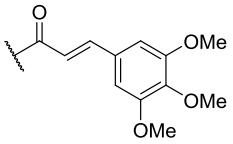
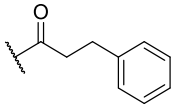
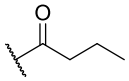
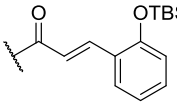
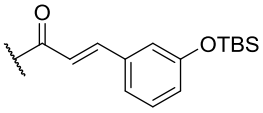
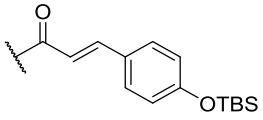
2.1.4.6. Synthesis of analogs of syncarpamide using various carboxylic acid chains (39-48)

A second variation in the analog synthesis of syncarpamide **1** was thought of varying different aliphatic or aromatic carboxylic acid chains (**Scheme 6**, **Table-2)**. Amino alcohol **1f** ((*S*)-configuration) or **2f** ((*R*)-configuration) synthesized from 3,4-dimethoxy benzaldehyde was coupled with aliphatic or aromatic carboxylic acid under DCC and DMAP condition to yield analogs **39-48**. **Table-2** depicts the synthesized analogs of syncarpamide using various aliphatic or aromatic carboxylic acids.

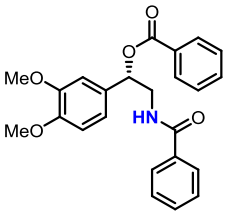


Scheme 6. Synthesis of analogs of syncarpamide using various carboxylic acids

Table 2. Analogs of syncarpamide synthesized using various carboxylic acids (**39-48**).

Analogs		X
S-Configuration	*R*-Configuration	
39	**40**	
41	**42**	
43	**44**	
45	n.s.	
46	n.s.	
47	n.s.	
48	n.s.	

n.s.: not synthesized



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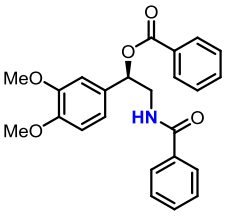

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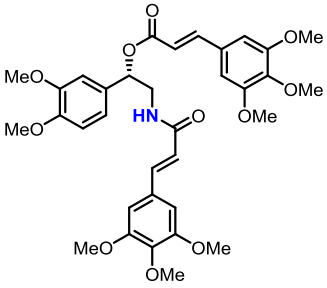

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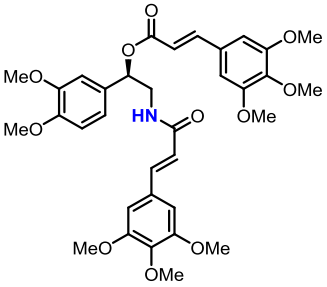

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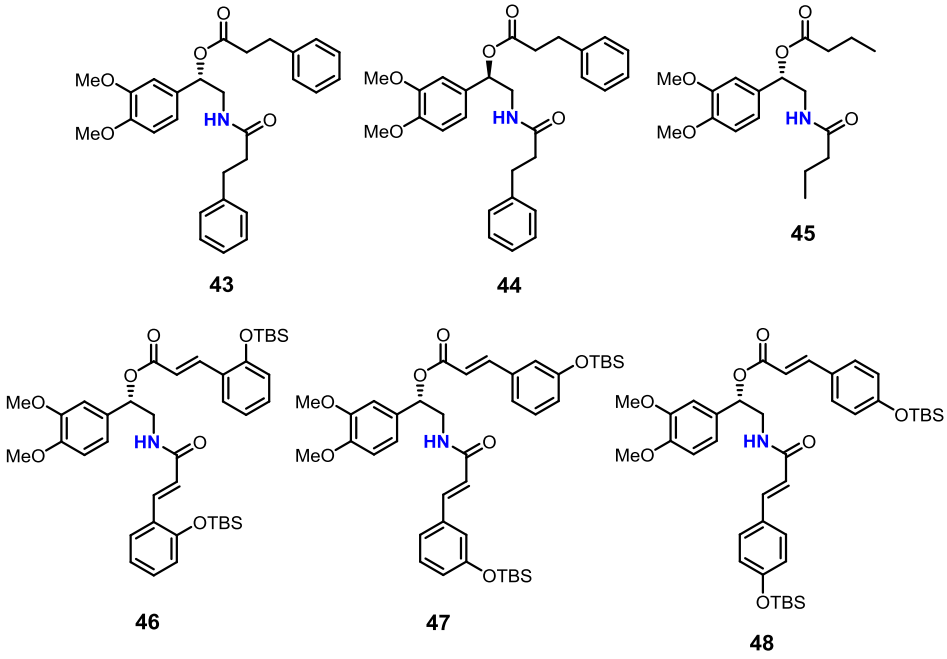
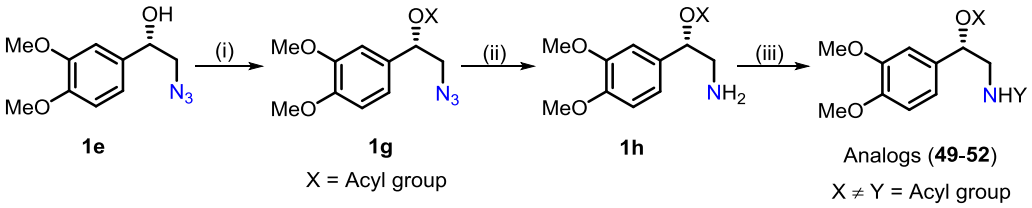


Figure 4. Structures of analogs **(39-48)**

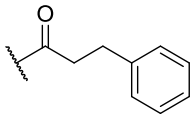
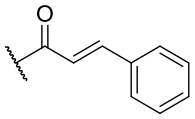
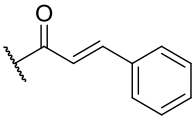
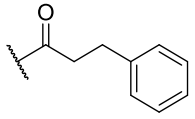
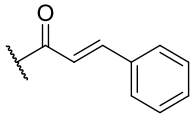
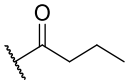
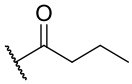
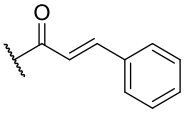
2.1.4.7. Synthesis of analogs of syncarpamide using various carboxylic acid chains (49-52) (where the ester and amide side chains are different)

In the third variation, we thought of synthesizing analogs of syncarpamide where the ester and amide side chains are different as shown in **Scheme 7**. Azido alcohol **1e** was coupled with aliphatic or aromatic carboxylic acid to furnish **1g**, which on reduction with either 10% Pd/C or PPh_3 furnished compound **1h**. Compound **1h** was further coupled with various aliphatic or aromatic acids to furnish the desired analogs **49-52** (**Table 3**).



Scheme 7. Synthesis of analogs of syncarpamide using various carboxylic acids. *Reagents and conditions:* (i) Carboxylic acid, DCC, DMAP, DCM, rt, 12-16 h, 74-80%; (ii) H_2, 10% Pd/C, MeOH, rt, 12 h (or) PPh_3, THF, rt, 12 h, 90-95%; (iii) Carboxylic acid, DCC, DMAP, DCM, rt, 12-16 h, 70-88%.

Table 3. Analogs of syncarpamide synthesized using various carboxylic acids (**49**-**52**).

Analogue	X	Y
S-Configuration		
49		
50		
51		
52		

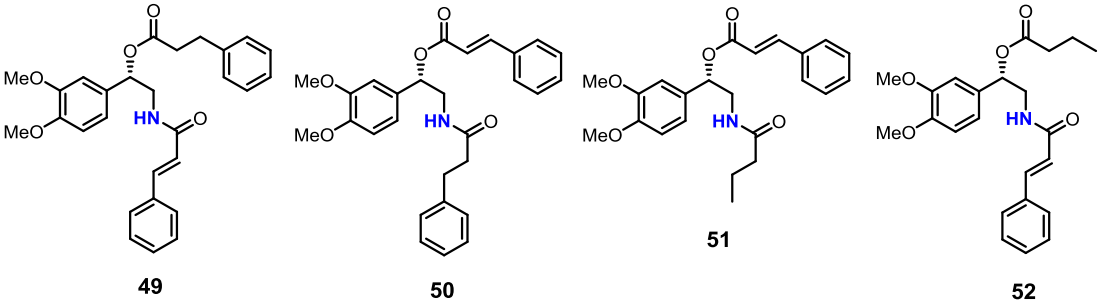
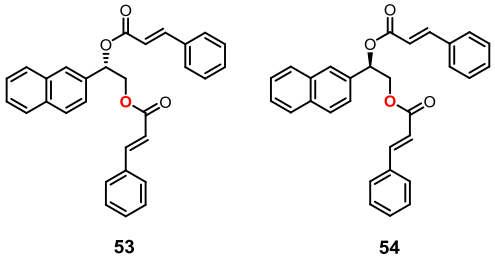


Figure 5. Structures of analogs (**49**-**52**).

2.1.4.8. Synthesis of other analogs of syncarpamide (53-58)

Following the strategy mentioned above, some other interesting analogs were synthesized (**53**-**57**). Analogs **53** and **54** were synthesized starting from 2-naphthaldehyde. Further, some more interesting analogs (**55**-**57**) have also been synthesized (**Figure 6**).



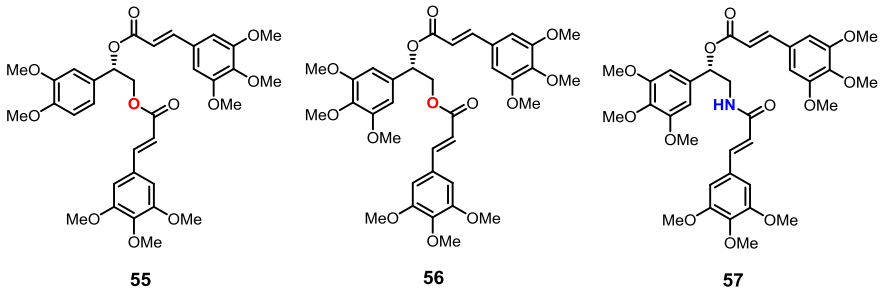
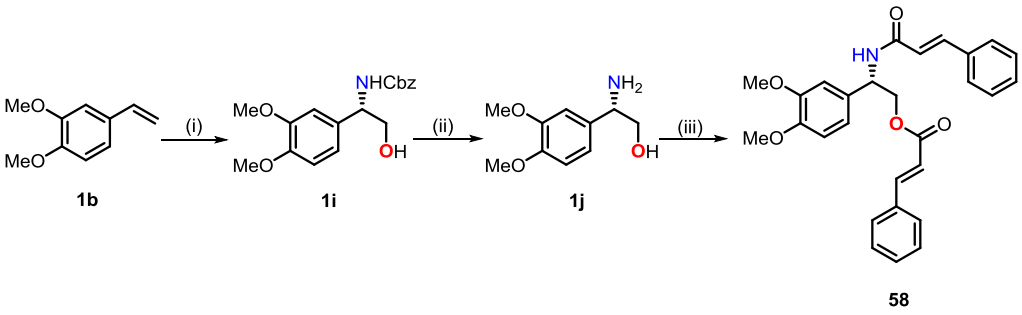


Figure 6. Further synthesized analogs of syncarpamide, **53**-**57**.

Synthesis of regioisomer of syncarpamide (58)

To study the structure-activity relationship (SAR), we thought of synthesizing a regioisomer **58** of syncarpamide where positions of nitrogen and oxygen atoms in syncarpamide are interchanged. Synthesis of regioisomer **58** started from 3,4-dimethoxy benzaldehyde **1b**. Sharpless amino hydroxylation[20] of **1b** furnished compound **1i**, which on Cbz deprotection with 10% Pd/C furnished the desired amino alcohol **1j**. Final coupling of **1j** with cinnamic acid under the DCC/DMAP conditions furnished the regioisomer **58** of syncarpamide (**Scheme 8**).



Scheme 8. Synthesis of regioisomer of syncarpamide *via* Sharpless aminohydroxylation strategy. *Reagents and conditions:* (i) Benzyl carbamate, NaOH, K_2OsO_4. $2H_2O$, $(DHQ)_2PHAL$, *t*-BuOCl, *n*-PrOH, 75%; (ii) H_2, 10% Pd/C, MeOH, rt, 2 h, 99%; (iii) cinnamic acid, DCC, DMAP, DCM, rt, 12-16 h, 60%.

2.1.5. BIOLOGICAL STUDIES

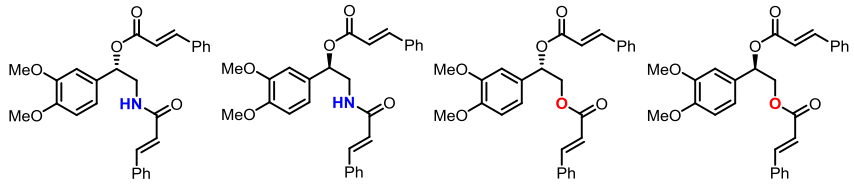
2.1.5.1. *In vitro* antiplasmodial activities

Several analogs of syncarpamide were synthesized by varying substituents on the aromatic ring of syncarpamide by changing the stereocenter at C7 carbon and by varying different acid/amide side chains. All these synthesized analogs were assayed for antiplasmodial activity against *P. falciparum* chloroquine sensitive (CQ) strain 3D7 and chloroquine

resistant strain K1 with chloroquine (CQ) as a reference drug.[21,22] Also, the effect of regioisomer **58** of syncarpamide **1** on antiplasmodial activity was studied. Cytotoxicity of all the synthesized molecules was carried out using Vero cell line (C1008; Monkey kidney fibroblast).[23]

2.1.5.1.1. *In vitro* antiplasmodial activity of syncarpamide 1, analogs and oxy-analogs of syncarpamide with different substituents on aromatic ring (2-38)

Syncarpamide **1** having *S*-configuration at C7 carbon showed IC_{50} values of 3.90 and 2.56 µM against 3D7 and K1 strains of *P. falciparum*, respectively and CC_{50} value of 80.66 µM against Vero cell line (C1008; Monkey kidney fibroblast) whereas enantiomer of syncarpamide, *i.e.* **2** having *R*-configuration at C7 carbon was found to be less active than **1** with IC_{50} values >10.0 µM against 3D7 and K1 strains of *P. falciparum*, respectively and CC_{50} value of 66.67 µM against Vero cell line (**Table 4**). The oxy-analogs of syncarpamide, *i.e.* (*S*)-**3** and (*R*)-**4** also showed less antiplasmodial activities than syncarpamide **1**. Compound (*R*)-**4** showed cytotoxicity with a CC_{50} value of 5.84 µM against Vero cell line.



Trimethoxyl substituted analog of syncarpamide (*S*)-**5** showed IC_{50} values of 6.69 and 3.64 µM against 3D7 and K1 strains of *P. falciparum*, respectively with a CC_{50} value of 66.74 µM. Its enantiomer, (*R*)-**6** was less active and showed cytotoxicity with a CC_{50} value of 5.08 µM. Corresponding oxy-analog (*S*)-**7** showed IC_{50} values of 3.37 and 3.47 µM against 3D7 and K1 strains, respectively and CC_{50} value of 6.44 µM which indicates its cytotoxic nature.

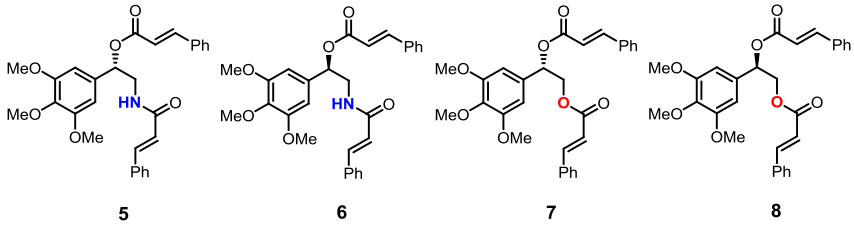


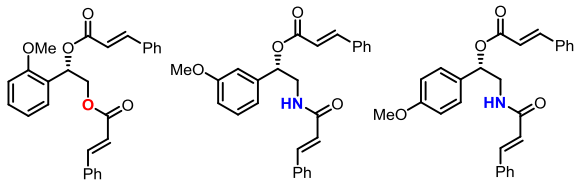
Table 4. *In vitro* antiplasmodial activity of syncarpamide **1**, analogs and oxy-analogs of syncarpamide with different substituents on aromatic ring (**2-38**).

Analogs	IC_{50} in µM (mean ± sd) against		CC_{50} in µM (mean ± sd)	SI (CC_{50}/IC_{50}) against
	3D7 *P. falciparum*	K1 *P. falciparum*	Vero cell line	3D7 *P. falciparum*
1	3.90 ± 0.4	2.56 ± 0.24	80.66 ± 2.35	20.68
2	>10.0	>10.0	66.67 ± 4.56	<6.68
3	>10.0	>10.0	24.29 ± 1.45	<2.4
4	>10.0	>10.0	5.87 ± 0.56	nd
5	6.69 ± 0.31	3.64 ± 0.64	66.74 ± 4.32	9.98
6	>10.0	>10.0	5.08 ± 0.4	nd
7	3.37 ± 26	3.47 ± 0.33	6.44 ± 0.86	1.91
8	>10.0	>10.0	121.81 ± 10.2	12.18
9	7.69 ± 0.35	>10.0	77.81 ± 8.21	10.12
10	>10.0	>10.0	117.47 ± 9.33	11.75
11	3.8 ± 0.6	3.23 ± 0.43	14.04 ± 2.2	3.69
12	>10.0	>10.0	51.69 ± 4.3	5.1
13	6.39 ± 0.09	4.27 ± 0.36	147.72 ± 8.55	23.12
14	>10	>10	62.73 ± 4.7	<6.27
15	>10	>10	61.89 ± 5.4	<6.19
16	>10	>10	22.31 ± 1.2	<2.23
17	6.82 ± 0.37	7.26 ± 0.14	153.0 ± 11.23	22.43
18	>10	>10	>200	~20
19	>10	>10	33.49 ± 4.6	<3.35
20	>10	>10	23.27 ± 3.2	<2.33
21	6.41 ± 0.70	2.71 ± 0.30	>200	>31.20
22	>10	>10	>200	~20
23	5.79 ± 0.36	>10	28.43 ± 3.2	4.91
24	>10	>10	82.74 ± 6.5	<8.27
25	3.85 ± 0.54	>10.0	31.69 ± 4.11	8.23

26	4.72 ± 0.68	>10.0	63.88 ± 5.36	13.53
27	>10	>10	39.15 ± 6.33	<3.92
28	>10	>10	30.33 ± 3.23	<3.03
29	6.77 ± 0.56	>10.0	140.67 ± 5.32	20.78
30	6.97 ± 1.1	>10.0	49.36 ± 4.3	7.08
31	>10	>10	>200	~20
32	>10	>10	>200	~20
33	5.99 ± 0.18	3.99 ± 1.04	189.61 ± 21	31.65
34	>10	>10	>200	~20
35	5.95 ± 0.23	>10	31.79 ± 3.5	5.34
36	>10	>10	56.64 ± 5.5	<5.66
37	>10.0	>10.0	>200	~20
38	7.42 ± 1.5	>10.0	>200	>26.95
CQ	0.005 ± 0.0	0.25 ± 0.020	75 ± 15	15000
Podophyllotoxin	nd	nd	5.74 ± 0.65	-

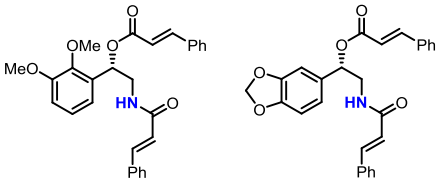
sd values for >10.0 were '0'; nd = not done; sd = standard deviation; SI = selectivity index.

Compounds (**9**-**20**) are mono methoxyl substituted analogs of syncarpamide. The oxy-analog (*S*)-**11** having methoxy group at the C2 position of aromatic ring showed IC_{50} values of 3.80 (3D7), 3.23 µM (K1) with cytotoxicity value (CC_{50}) of 14.04 µM. Compound (*S*)-**13** having methoxy group at C3 position showed IC_{50} values of 6.39 and 4.27 µM against 3D7 and K1 strains, respectively and CC_{50} value of 147.72 µM. Compound (*S*)-**17** with a methoxy group at C4 position exhibited IC_{50} values of 6.82 (3D7) and 7.26 µM (K1) with a CC_{50} value of 153.0 µM. The analogs (*S*)-**13** and (*S*)-**17** could be considered as one methoxyl deficient parent molecule syncarpamide and hence their antiplasmodial activities may be attributed due to the presence of one methoxy group either in C3 or C4 positions whereas parent molecule syncarpamide contains two methoxy groups in C3 and C4 positions. Compounds **10**, **12**, **14**, **15**, **16**, **18**, **19** and **20** were found to possess less antiplasmodial activities.



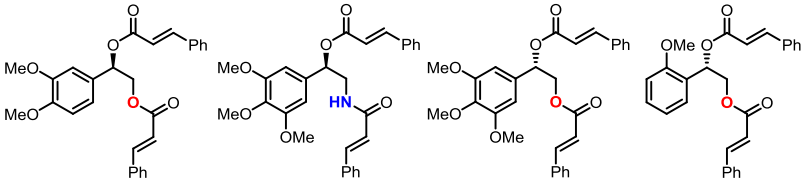
11 13 17

2,3-Dimethoxy substituted analog of syncarpamide compound (*S*)-**21** showed IC_{50} values of 6.41 and 2.71 µM against 3D7 and K1 strains, respectively with a CC_{50} value >200 µM. 3,4-(Methylenedioxy) substituted analog (*S*)-**33** showed IC_{50} values of 5.99 (3D7) and 3.99 µM (K1) with a CC_{50} value of 189.61 µM. The antiplasmodial activity of (*S*)-**33** may be due to its structural similarity with parent compound syncarpamide, which has two methoxy groups in C3 and C4 positions. Compounds (*S*)-**23**, (*S*)-**25**, (*R*)-**26**, (*S*)-**29**, (*R*)-**30**, (*S*)-**35** and (*R*)-**38** showed selective antiplasmodial activities against 3D7 strain only and showed IC_{50} values of 5.79, 3.85, 4.72, 6.77, 6.97, 5.95 and 7.42 µM, respectively with cytotoxicity (CC_{50}) values of 28.43, 31.69, 63.88, 140.67, 49.36, 31.79, >200 µM, respectively.



21 33

It was observed that analogs **4**, **6**, **7** and **11** showed high cytotoxicities with CC_{50} values of 5.87, 5.08, 6.44 and 14.04 µM, respectively (**Table 4**). Analog **6** exhibited more cytotoxicity than the standard drug, podophyllotoxin and analogs **4** and **7** showed comparable cytotoxicities to podophyllotoxin.



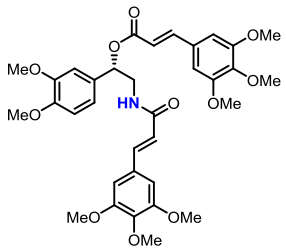
4 6 7 11

2.1.5.1.2. *In vitro* antiplasmodial activity of analogs of syncarpamide with different acid chains (39-52)

Analogs **39**-**52** were synthesized by varying the two side chains of syncarpamide. Analogs (*S*)-**39** and (*R*)-**40** contain benzoyl groups in the side chains instead of cinnamoyl groups and

were found to exhibit selective activity against 3D7 strain with IC_{50} values of 6.67, 3.66 µM and CC_{50} values of 38.94, and 103.76 µM, respectively (**Table 5**).

Analogs (*S*)-**41** and (*R*)-**42** were synthesized using 3,4,5-trimethoxy cinnamic acid. When the side chains with cinnamoyl groups in syncarpamide were replaced with 3,4,5-trimethoxy cinnamoyl groups, an increase in antiplasmodial activities was observed against both the strains of *P. falciparum*. Compound (*S*)-**41** showed IC_{50} values of 3.16 and 1.78 µM against 3D7 and K1 strains of *P. falciparum* with CC_{50} value of 87.98 µM against Vero cell line. Compound (*R*)-**42** showed IC_{50} values of 2.28 and 2.07 µM against 3D7 and K1 strains with CC_{50} value of 88.94 µM. The better activity shown by the analogs (*S*)-**41** and (*R*)-**42** could be attributed to the increase in electron-density in the side chain of both the ester as well as amide groups when compared to parent compound syncarpamide.



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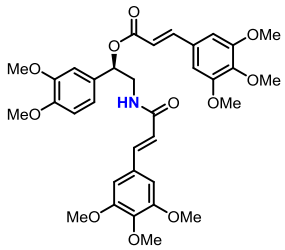
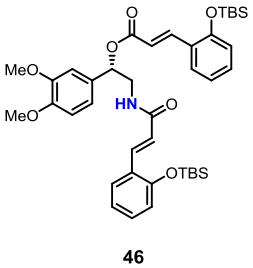
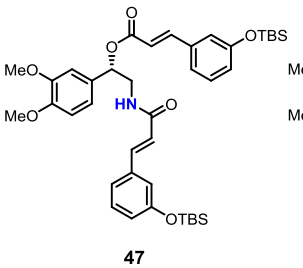


42

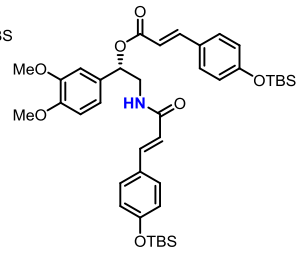
Compounds (*S*)-**43** and its enantiomer, (*R*)-**44** with dihydro cinnamoyl groups in both side chains were also exhibiting selective antiplasmodial activity against 3D7 strain with IC_{50} values of 2.86, 5.86 µM with CC_{50} values of 39.34, and 73.19 µM, respectively. Compounds (*S*)-**46** and (*S*)-**48** exhibited IC_{50} values of 8.49 and 7.92 µM against 3D7 and 4.56 and 3.37 µM against K1, respectively. (*S*)-**46** and (*S*)-**48** showed CC_{50} values of 58.91 and 40.79 µM, respectively. The activities observed in (*S*)-**46** and (*S*)-**48** may be due to the possibility of resonance in the aromatic ring of side chains with OTBS groups present in *o* and *p* positions, respectively. However, compound (*S*)-**47** lacked the corresponding activity due to the presence of OTBS group at the *m*-position on the aromatic ring of the side chains.



46



47



48

Table 5. *In vitro* antiplasmodial activity of analogs of syncarpamide with different acid chains (**39**-**52**).

Analogs	IC_{50} in µM (mean ± sd) against		CC_{50} in µM (mean ± sd)	SI (CC_{50}/IC_{50}) against
	3D7 *P. falciparum*	K1 *P. falciparum*	Vero cell line	3D7 *P. falciparum*
39	6.67 ± 1.2	>10.0	38.94 ± 5.56	5.83
40	3.66 ± 0.5	>10.0	103.76 ± 10.22	28.35
41	3.16 ± 0.2	1.78 ± 0.22	87.98 ± 7.14	27.84
42	2.28 ± 0.39	2.07 ± 0.34	88.94 ± 5.75	39.01
43	2.86 ± 0.53	>10.0	39.34 ± 3.55	13.76
44	5.86 ± 0.8	>10.0	73.19 ± 6.21	12.49
45	>10.0	>10.0	51.84 ± 3.4	<5.18
46	8.49 ± 0.8	4.56 ± 0.81	58.91 ± 5.5	6.94
47	>10.0	7.52 ± 0.63	52.62 ± 2.58	<5.26
48	7.92 ± 0.75	3.37 ± 0.4	40.79 ± 3.56	5.15
49	>10.0	>10.0	36.9 ± 2.1	<3.69
50	>10.0	>10.0	44.29 ± 4.35	<4.43
51	>10.0	>10.0	42.43 ± 3.45	<4.24
52	>10.0	>10.0	29.89 ± 2.88	<2.98
CQ	0.005 ± 0.0	0.25 ± 0.020	75 ± 15	15000
Podophyllotoxin	nd	nd	5.74 ± 0.65	-

sd values for >10.0 were ‘0’; nd = not done; sd = standard deviation; SI = selectivity index.

The remaining compounds (*S*)-**45**, (*S*)-**49**, (*S*)-**50**, (*S*)-**51** and (*S*)-**52** did not show any *in vitro* antiplasmodial activities against any of the strains due to the complete lack of aromaticity in the side chains of (*S*)-**45** and partial lack of aromaticity in the side chains of (*S*)-**51** and (*S*)-**52**. Effect of partial resonance on the antiplasmodial activity could be observed in the compounds (*S*)-**49** and (*S*)-**50** as these two compounds possessed cinnamoyl group in the amide and ester side chains, respectively (**Table 5**).

2.1.5.1.3. *In vitro* antiplasmodial activity of other analogs of syncarpamide (53-58)

(*S*)-**53** and (*R*)-**54** are analogs of syncarpamide having naphthalene ring in place of the benzene ring. Only (*R*)-**54** showed antiplasmodial activity with an IC_{50} value of 2.15 µM against K1 strain and CC_{50} value of 180.94 µM (**Table-6**).

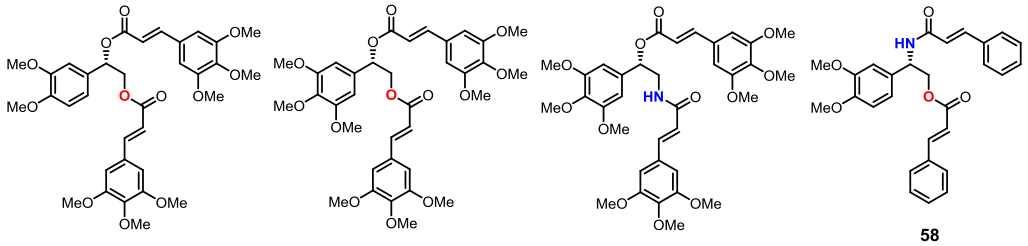
The two oxy-analogs (*S*)-**55** and (*S*)-**56** contain trimethoxy benzoyl groups in both the side chains and showed good activity against both the strains. Compounds (*S*)-**55** and (*S*)-**56** are highly electron-rich due to the presence of methoxyl groups on the aromatic rings of side chains and also on the aromatic ring of the core structure. Compound (*S*)-**55** showed the IC_{50} value of 2.34 against 3D7 and 2.62 µM against K1 having CC_{50} value of 55.70 µM whereas compound (*S*)-**56** exhibited an IC_{50} value of 6.29 against 3D7 and 2.23 µM against K1 and CC_{50} value of 47.38 µM.

Table 6. *In vitro* antiplasmodial activity of other analogs of syncarpamide (**53-58**).

Analogs	IC_{50} in µM (mean ± sd) against		CC_{50} in µM (mean ± sd)	SI (CC_{50}/IC_{50}) against
	3D7 *P. falciparum*	K1 *P. falciparum*	Vero cell line	3D7 *P. falciparum*
53	>10.0	>10.0	>200	~20
54	>10.0	2.15 ± 0.22	180.94 ± 21.33	<18.09
55	2.34 ± 0.4	2.62 ± 0.42	55.70 ± 4.8	23.80
56	6.29 ± 1.3	2.23 ± 0.23	47.38 ± 2.31	7.53
57	1.89 ± 0.26	1.93 ± 0.2	42.68 ± 4.4	22.58
58	>10.0	>10.0	55.59 ± 5.36	<5.56
CQ	0.005 ± 0.0	0.25 ± 0.020	75 ± 15	15000
Podophyllotoxin	nd	nd	5.74 ± 0.65	-

Similarly, compound (*S*)-**57** showed good antiplasmodial activity with an IC_{50} value of 1.89 and 1.93 µM against 3D7 and K1 strains of *P. falciparum*, respectively and CC_{50} value of 42.68 µM. Compound (*S*)-**57** is highly electron-rich due to the presence of three methoxy groups each on the core structure (aromatic ring) and also on the aromatic rings of the two side chains. Interestingly, compound (*S*)-**58** which is a regioisomer of syncarpamide **1** was found to be inactive against both the strains (3D7 and K1) of *P. falciparum* (**Table 6**). This

explains the role of oxygen at C-7 and nitrogen at C-8 positions in the syncarpamide **1** on antiplasmodial activity.



55 **56** **57** **58**

2.1.5.2. *In vivo* antimalarial assay of selected compounds (1, 41, 42, 55 and 57)

Five compounds, syncarpamide **1**, (*S*)-**41**, (*R*)-**42**, (*S*)-**55** and (*S*)-**57** which showed high *in vitro* antiplasmodial activities against both the strains (3D7 and K1) of *P. falciparum* were selected for their *in vivo* antimalarial assay against chloroquine-resistant *P. yoelii* (N-67) strain of *Plasmodium*.[24] The results are depicted in **Table 7**.

Table 7. *In vivo* antimalarial activity of selected compounds (**1**, **41**, **42**, **55** and **57**).

Compound	% Parasitaemia on day 4 in 5 individual mice				
	Mice-1	Mice-2	Mice-3	Mice-4	Mice-5
1	09.50	13.76	11.93	11.72	13.20
41	11.78	11.93	11.12	11.26	12.13
42	14.07	12.50	15.10	12.16	11.46
55	06.06	08.31	16.23	13.92	11.13
57	10.10	10.16	13.50	07.50	11.92
Untreated	11.40	13.60	16.16	09.80	10.06
Arteether	0.0	0.0	0.0	0.0	0.0

Unfortunately, none of the five molecules, **1**, (*S*)-**41**, (*R*)-**42**, (*S*)-**55** and (*S*)-**57** showed any promising *in vivo* antimalarial activity against *P. yoelii* (N-67) strain. There was no significant difference in percent parasite survival on day 4 in respect to untreated mice, however in mice treated with arteether no parasite survived on day 4.

2.1.6. CONCLUSION

In conclusion, the first asymmetric synthesis of the antiplasmodial compound, syncarpamide **1** and its enantiomer, (*R*)-**2** has been achieved using Sharpless asymmetric dihydroxylation of 3,4-dimethoxy styrene. A library of 56 analogs (**3**-**58**) of syncarpamide has been synthesized by taking different substituted aromatic aldehydes, by changing the stereocenter at the C-7 carbon and also by varying different ester and amide side chains. Regioisomer (*S*)-**58** of syncarpamide was synthesized using Sharpless amino hydroxylation of 3,4-dimethoxy styrene but was found to be inactive. Analogs (*S*)-**41**, (*R*)-**42**, (*S*)-**55**, and (*S*)-**57** having 3,4,5-trimethoxy cinnamoyl groups as side chains showed better antiplasmodial activity against both the strains (3D7 and K1) than the natural product, syncarpamide **1**. In case of enantiomers (*S*)-**41** and (*R*)-**42**, the stereochemistry at C-7 was not a major contributing factor towards antiplasmodial activities since both were found to possess better antiplasmodial activity against both the strains (3D7 and K1) than the natural product, syncarpamide **1**. The electron density and possibility of resonance in both ester and amide side chains increases the *in vitro* antiplasmodial activity. However, four synthesized compounds (**4**, **6**, **7** and **11**) showed high cytotoxicities with CC_{50} values of 5.87, 5.08, 6.44 and 14.04 µM, respectively. Further, five compounds, syncarpamide **1**, (*S*)-**41**, (*R*)-**42**, (*S*)-**55** and (*S*)-**57** were assayed *in vivo* assay against chloroquine-resistant *P. yoelii* (N-67) strain of *Plasmodium*. However, none of the five molecules exhibited any promising *in vivo* antimalarial activity against *P. yoelii* (N-67) strain. The high order of *in vitro* antiplasmodial activities exhibited by compounds (*S*)-**41**, (*R*)-**42**, (*S*)-**55**, (*S*)-**56** and (*S*)-**57** qualifies these compounds as candidates for further development as new antimalarial agents.

2.1.7. EXPERIMENTAL SECTION

General procedure for the synthesis of substituted styrenes

To a stirred suspension of methyltriphenylphosphonium bromide in THF, base (inorganic base like NaH, $NaNH_2$ or organic base like *n*-BuLi) was added under argon at 0 ºC and stirred for 30 min. After the mixture was cooled again to 0 ºC, aromatic aldehyde in THF was added dropwise and stirred for 12 h. The mixture was concentrated in *vacuo* and residue was dissolved in water, and the aqueous layer was extracted with DCM (3X35 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated and purified by

silica gel (100-200 mesh) column chromatography in petroleum ether: ethyl acetate (95:5) to afford pure styrene (85-99% yield).

General procedure for dihydroxylation of styrenes

A round-bottomed flask, equipped with a magnetic stirrer, was charged with 5 mL of *tert*-butyl alcohol, 5 mL of water, and 1.4 g of AD-mix-α or AD-mix-β. Stirring at rt produced two clear phases; the lower aqueous phase appears bright yellow. The mixture was cooled to 0 ºC after that some of the dissolved salts precipitated. One mmol of olefin was added at once, and the heterogeneous slurry was stirred vigorously at 0 ºC for 6-24 h (progress was monitored by TLC). While the mixture was stirred at 0 ºC, anhydrous sodium sulfite (1.5 g) was added, and the mixture was allowed to warm to rt and further stirred for 30-60 min. EtOAc (10 mL) was added to the reaction mixture, and after separation of the layers, the aqueous phase was further extracted with EtOAc (3X5 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated to afford the diol and the ligand. This crude reaction mixture was purified by silica gel column chromatography (100-200 mesh) eluting with EtOAc/petroleum ether (1:1) to afford the pure 1,2-diol in 80-98% yield.

General procedure for monotosylation of 1,2-diol

To a stirred solution of diol (1.0 equiv.) in DCM (10 mL) was added pyridine (10.0 equiv.) at 0 ºC followed by *p*-toluenesulfonyl chloride (2.0 equiv.) and the reaction mixture was stirred for 3-4 h at 0 ºC. After completion of the reaction (TLC), the reaction mixture was treated with 10% HCl at 0 ºC and extracted with EtOAc (3X20 mL). The combined organic layers were pooled and washed with water (2X20 mL) and brine (1X20 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The concentrate was purified by silica gel chromatography (100-200 mesh) using EtOAc/petroleum ether (2:3) to furnish the desired monotosylated product (75-90% yield).

General procedure for preparation of 1,2-azido alcohol

A solution of monotosylate (1.0 equiv.), tetrabutylammonium iodide (0.05 equiv.) and sodium azide (3.0 equiv.) in DMF was heated at 80-90 ºC for 3-4 h. After completion of the reaction (TLC), the reaction mixture was cooled to 0 ºC, and water (5 mL) was added. The aqueous layer was extracted with ethyl acetate (3X35 mL). The combined organic layers were washed with brine (2X20 mL), dried over anhydrous Na_2SO_4, filtered, and

concentrated. The concentrate was purified by silica gel column chromatography (100-200 mesh) using EtOAc/petroleum ether (3:7) to afford the desired product in 80-95% yield.

General procedure for the preparation of 1,2-amino alcohol

1,2-Azido alcohol was dissolved in anhydrous MeOH, and 10% palladium on carbon (10% w/w) was added. The reaction mixture was stirred under a hydrogen atmosphere until completion as determined by TLC. The reaction mixture was filtered through Celite (pre-washed with MeOH), and the solvent was removed *in vacuo*, which gave the crude compound that was used in the next step without further purification.

General procedure for coupling with DCC/DMAP

To a stirred solution of 1,2-diol/1,2-amino alcohol (1 mmol) in dry DCM (5 mL), corresponding acid (2.5 mmol), DCC (2.5 mmol) and DMAP (4.0 mmol) were added and stirred at rt for 12 h. After completion of the reaction (TLC), the reaction mixture was filtered to remove insoluble DCU, and the filtrate was washed with water (10 mL), brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated to furnish a crude residue. The crude residue was purified by silica gel column chromatography (100-200 mesh) using EtOAc/petroleum ether (1:1) to afford the final compound in 75-80% yield.

In vitro* antiplasmodial activity assay against chloroquine-sensitive (3D7) and resistant (K1) strains of *P. falciparum

Cultures of chloroquine-sensitive (3D7) and resistant (K1) strains of *P. falciparum* were maintained in medium RPNI supplemented with 25 mM HEPES, 0.2% D-glucose, 0.21% sodium bicarbonate and 0.5% ALBUMAX-II[21] The stock (5 mg/mL or 10 mM) solutions of compounds were prepared in DMSO and required dilutions were made in culture medium. For evaluation of 50% inhibitory concentration (IC_{50}) of the compounds, Malaria SYBR Green I-based fluorescence (MSF) assay was carried out.[22] Two-fold serial dilutions of test compounds were made in 96 well plates and incubated with 1.0% parasitized cell suspension containing 0.8% parasitaemia (Asynchronous culture with more than 80% ring stages). The plates were incubated at 37 °C in a CO_2 incubator in an atmosphere of 5% CO_2 and air mixture. 72 hours later 100 µL of lysis buffer containing 2x concentration of SYBR Green-I (Invitrogen) was added to each well and incubated for one hour at 37 °C. The plates were examined at 485±20 nm of excitation and 530±20 nm of emission for relative fluorescence units (RFUs) per well using the fluorescence plate reader (FLX800, BIOTEK). IC_{50} values

were obtained by Logit regression analysis of dose-response curves.[22] Chloroquine was used as the standard reference drug.

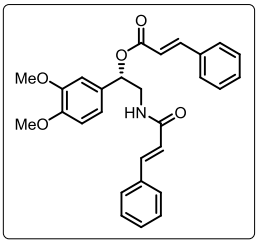
Cytotoxicity of the samples was carried out using Vero cell line (C1008; Monkey kidney fibroblast).[23] The cells were incubated with test sample dilutions for 72h and MTT was used as a reagent for detection of cytotoxicity. Podophyllotoxin (Sigma) was used as the standard reference drug. 50% cytotoxic concentration (CC_{50}) was determined using non-linear regression analysis of dose-response curves.

***In vivo* assay against chloroquine-resistant *P. yoelii* (N-67)**

Five molecules syncarpamide **1**, (*S*)-**41**, (*R*)-**42**, (*S*)-**55** and (*S*)-**57** were evaluated *in vivo* against chloroquine-resistant *P. yoelii* (N-67)-Swiss mice model.[24] The molecules were tested at 100 mg/kg dose through intraperitoneal route. The mice received infective inoculum containing $1x10^5$ parasites on day 0 and then received four doses of the test compound on day 0-3 once daily. Five infected mice were used for each molecule, and five infected mice were kept as untreated control. Arteether (5 mg/kg, x4, intramuscular) was used as positive control. Thin blood smear from the tail blood of each mouse was prepared on day 4. The blood smears were fixed in methanol, stained with Giemsa's stain and examined under the light microscope (100 X oil immersion). At least 10-15 fields per smear were scanned to obtain percent parasitaemia in treated as well as untreated mice. If significant suppression in percent parasitaemia is observed on day 4, the monitoring continues up to day 28.

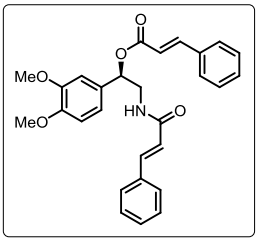
Compounds characterization data for final molecules 1-58

(*S*)-2-Cinnamamido-1-(3,4-dimethoxyphenyl)ethyl cinnamate (1):



Colorless solid; **m.p.:** 132-135 °C; R_f = 0.34 (EtOAc-petroleum ether, 2:3); $[\alpha]_D^{26}$ +41.5 (*c* 1, $CHCl_3$); **IR ($CHCl_3$):** 3364, 3301, 3068, 3017, 2932, 2846, 1710, 1664, 1627, 1516, 1455, 1336, 1258, 1217, 1163, 1030, 984, 861, 758, 672 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.72 (d, *J* = 16.1 Hz, 1H), 7.63 (d, *J* = 15.7 Hz, 1H), 7.55-7.43 (m, 4H), 7.42-7.29 (m, 6H), 7.03-6.85 (m, 3H), 6.49 (d, *J* = 16.1 Hz, 1H), 6.39 (d, *J* = 15.7 Hz, 1H), 6.07 (brs, 1H), 6.01 (dd, *J* = 7.7, 5.0 Hz, 1H), 3.89 (s, 3H), 3.86 (s, 3H), 3.92-3.83 (m, 2H, overlapped); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 166.5, 165.9, 149.2, 149.1, 145.8, 141.5, 134.7, 134.1, 130.5, 130.2, 129.7, 128.9, 128.7, 128.1, 127.8, 120.3,119.0, 117.6, 111.2, 109.8, 74.7, 55.9, 55.9, 44.6; **ESI-LCMS:** *m/z* 479.95 $(M+Na)^+$; **HRMS**: *m*/z for $C_{28}H_{27}NO_5Na$ $(M+Na)^+$: calcd 480.1781, found 480.1772; **HPLC**: *ee* 99.2% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 30% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R =17.0 min].

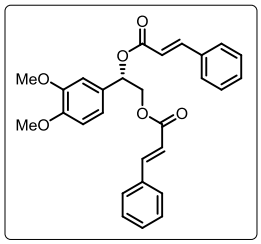
(*R*)-2-Cinnamamido-1-(3,4-dimethoxyphenyl)ethyl cinnamate (2):



Colorless solid; **m.p.:** 123-125 °C; R_f = 0.34 (EtOAc-petroleum ether, 2:3); $[\alpha]_D^{23}$ -42.0 (*c* 1, $CHCl_3$); **IR ($CHCl_3$):** 3364, 3301, 3068, 3017, 2932, 2846, 1710, 1664, 1627, 1516, 1455, 1336, 1258, 1217, 1163, 1030, 984, 861, 758, 672 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.80-7.57 (m, 2H, two doublets overlapped), 7.57-7.44 (m, 4H), 7.43-7.29 (m, 6H), 7.06-6.82 (m, 3H), 6.50 (d, *J* = 15.9 Hz, 1H), 6.40 (d, *J* = 15.7 Hz, 1H), 6.15-5.94 (m, 2H), 3.90 (s, 3H), 3.87 (s, 3H), 3.97-3.80 (m, 2H, overlapped); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.5, 165.9, 149.2, 149.1, 145.8, 141.5, 134.7, 134.1, 130.5, 130.2, 129.7, 128.9, 128.7, 128.1,

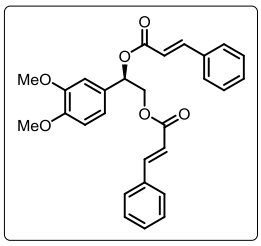
127.8, 120.3,119.0, 117.6, 111.2, 109.8, 74.7, 55.9, 55.9, 44.6; **ESI-LCMS:** *m/z* 480.25 $(M+Na)^+$; **HRMS:** *m*/z for $C_{28}H_{27}NO_5Na$ $(M+Na)^+$: calcd 480.1781, found 480.1770; **HPLC:** *ee* 96.2% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 30% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R =21.0 min].

(*S*)-1-(3,4-Dimethoxyphenyl)ethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (3)



Colorless viscous liquid; R_f = 0.48 (EtOAc-petroleum ether, 3:7); $[\alpha]_D^{24}$ +29.4 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3021, 2961, 2938, 1713, 1637, 1579, 1519, 1451, 1422, 1330, 1310, 1256, 1216, 1161, 1071, 1028, 863, 756, 711, 684 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.82-7.65 (m, 2H, two doublets overlapped), 7.59-7.44 (m, 4H), 7.44-7.31 (m, 6H), 7.09-6.84 (m, 3H), 6.59-6.39 (m, 2H, two doublets overlapped), 6.19 (dd, *J* = 8.2, 4.0 Hz, 1H), 4.67-4.42 (m, 2H), 3.91 (s, 3H), 3.88 (s, 3H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.6, 166.1, 149.3, 149.1, 145.5, 145.5, 134.2, 130.4, 129.1, 128.9, 128.1, 119.4, 117.7, 117.5, 73.4, 66.1, 56.0, 55.9; **ESI-LCMS:** *m/z* 481.02 $(M+Na)^+$; **HRMS:** *m/z* for $C_{28}H_{26}O_6Na$ $(M+Na)^+$: calcd 481.1622, found 481.1626; **HPLC:** *ee* 97.4% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 30% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R =14.0 min].

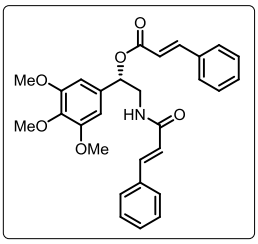
(*R*)-1-(3,4-Dimethoxyphenyl)ethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (4)



Colorless viscous liquid; R_f = 0.48 (EtOAc-petroleum ether, 3:7); $[\alpha]_D^{25}$ -26.9 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3364, 3020, 1712, 1664, 1637, 1518, 1464, 1450, 1421, 1388, 1329, 1310, 1216, 1161, 1027, 988, 928, 864, 771, 668 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.82-7.64 (m, 2H, two doublets overlapped), 7.60-7.44 (m, 4H), 7.44-7.31 (m, 6H), 7.09-6.83 (m, 3H), 6.59-6.37 (m, 2H, two doublets overlapped), 6.18 (dd, *J* = 8.2, 3.9 Hz, 1H), 4.67-4.42

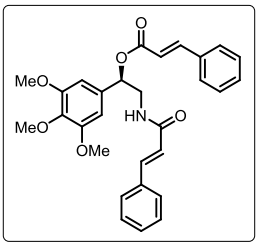
(m, 2H), 3.91 (s, 3H), 3.88 (s, 3H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.6, 166.1, 149.3, 149.1, 145.6, 145.6, 134.3, 134.3, 130.5, 128.9, 128.2, 119.4, 117.8, 117.5, 111.2, 73.5, 66.2, 56.0, 56.0; **ESI-LCMS:** *m/z* 481.02 $(M+Na)^+$; **HRMS:** *m/z* for $C_{28}H_{26}O_6Na$ $(M+Na)^+$: calcd 481.1622, found 481.1626; **HPLC:** *ee* 97.9% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 30% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R =22.5 min].

(*S*)-2-Cinnamamido-1-(3,4,5-trimethoxyphenyl)ethyl cinnamate (5)



Colorless solid; **m.p.:** 126-128 °C; R_f = 0.37 (EtOAc-petroleum ether, 2:3); **$[\alpha]_D^{26}$** +39 (*c* 1, $CHCl_3$); **IR ($CHCl_3$):** 3361, 3165, 3018, 1711, 1664, 1633, 1594, 1509, 1463, 1422, 1330, 1216, 1164, 1130, 770, 668 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.75 (d, *J* = 16.0 Hz, 1H), 7.65 (d, *J* = 15.5 Hz, 1H), 7.57-7.44 (m, 4H), 7.44-7.30 (m, 6H), 6.65 (s, 2H), 6.52 (d, *J* = 16.0 Hz, 1H), 6.40 (d, *J* = 15.5 Hz, 1H), 6.10-5.92 (m, 2H), 3.95-3.78 (m, 11H) or {3.87 (s, 6H), 3.84 (s, 3H), 3.90-3.78 (m, 2H, overlapped)}; **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.6, 166.0, 153.4, 149.2, 149.1, 145.8, 141.5, 134.7, 134.1, 130.5, 130.2, 129.7, 128.9, 128.8, 128.2, 127.8, 120.3, 119.0, 117.5, 111.1,109.8, 103.5, 74.7, 60.8, 55.9, 55.9, 44.6; **ESI-LCMS:** *m/z* 510.26$(M+Na)^+$; **HRMS:** *m/z* for $C_{29}H_{29}NO_6Na$ $(M+Na)^+$: calcd 510.1887, found 510.1876; **HPLC:** *ee* 100% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 10.0 min].

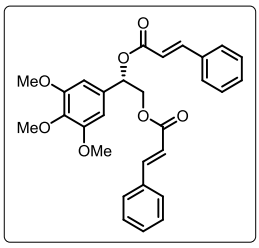
(*R*)-2-Cinnamamido-1-(3,4,5-trimethoxyphenyl)ethyl cinnamate (6)



Colorless solid; **m.p.:** 126-127 °C; R_f = 0.37 (EtOAc-petroleum ether, 2:3); **$[\alpha]_D^{26}$** -39 (*c* 1, $CHCl_3$); **IR ($CHCl_3$):** 3361, 3165, 3018, 1711, 1664, 1633, 1594, 1509, 1463, 1422, 1330, 1216, 1164, 1130, 770, 668 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.75 (d, *J* = 16.0 Hz, 1H),

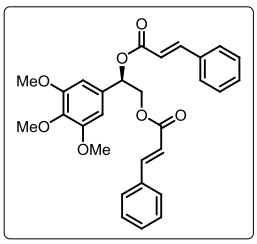
7.65 (d, *J* = 15.5 Hz, 1H), 7.57-7.44 (m, 4H), 7.44-7.30 (m, 6H), 6.65 (s, 2H), 6.52 (d, *J* = 16.0 Hz, 1H), 6.40 (d, *J* = 15.5 Hz, 1H), 6.10-5.92 (m, 2H), 3.95-3.78 (m, 11H) or {3.87 (s, 6H), 3.84 (s, 3H), 3.90-3.78 (m, 2H, overlapped)}; **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.6, 166.0, 153.4, 149.2, 149.1, 145.8, 141.5, 134.7, 134.1, 130.5, 130.2, 129.7, 128.9, 128.8, 128.2, 127.8, 120.3, 119.0, 117.5, 111.1,109.8, 103.5, 74.7, 60.8, 55.9, 55.9, 44.6; **ESI-LCMS:** *m*/*z* 510.08 $(M+Na)^+$; **HRMS:** *m*/z for $C_{29}H_{29}NO_6Na$ $(M+Na)^+$: calcd 510.1887, found 510.1873; **HPLC:** *ee* 98.3% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 21.6 min].

(*S*)-1-(3,4,5-Trimethoxyphenyl)ethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (7)



Colorless viscous liquid; R_f = 0.45 (EtOAc-petroleum ether, 3:7); **$[\alpha]_D^{28}$** +23 (c 1, $CHCl_3$); **IR ($CHCl_3$):** 3019, 2956, 2921, 1713, 1636, 1593, 1503, 1458, 1377, 1311, 1213, 1158, 1129, 982, 751, 669 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.79-7.67 (m, 2H, two doublets overlapped), 7.57-7.48 (m, 4H), 7.44-7.34 (m, 6H), 6.68 (s, 2H), 6.55 (d, *J* = 15.9 Hz, 1 H), 6.46 (d, *J* = 15.9 Hz, 1 H), 6.16 (dd, *J* = 8.3, 3.4 Hz, 1H), 4.62-4.46 (m, 2H), 3.89 (s, 6H), 3.84 (s, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.6, 160.0, 153.4, 145.8, 145.6, 138.1, 134.2, 132.2, 130.5, 130.5, 128.9, 128.1, 117.5, 117.4, 103.8, 73.7, 66.2, 60.8, 56.2; **ESI-LCMS:** *m*/*z* 511.04 $(M+Na)^+$; **HRMS:** *m*/z for $C_{29}H_{28}O_7Na$ $(M+Na)^+$: calcd 511.1727, found 511.1736; **HPLC:** *ee* 98.6% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 30% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R =12.7 min].

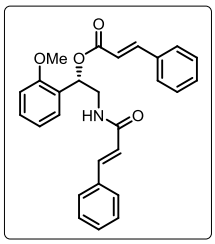
(*R*)-1-(3,4,5-Trimethoxyphenyl)ethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (8)



Colorless viscous liquid; R_f = 0.45 (EtOAc-petroleum ether, 3:7); **$[\alpha]_D^{25}$** -22.4 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3360, 3164, 3020, 2935, 2848, 1713, 1635, 1603, 1509, 1458, 1419,

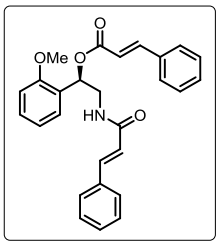
1319, 1216, 1164, 866, 762, 669cm^{-1}; **^{1}H NMR (500 MHz, CDCl$_3$)** δ 7.78-7.68 (m, 2H, two doublets overlapped), 7.57-7.48 (m, 4H), 7.42-7.35 (m, 6H), 6.68 (s, 2H), 6.54 (d, *J* = 15.9 Hz, 1H), 6.45 (d, *J* = 15.9 Hz, 1H), 6.16 (dd, *J* = 8.6, 3.4 Hz, 1H), 4.57 (dd, *J* = 11.9, 8.6 Hz, 1H), 4.49 (dd, *J* = 11.9, 3.4 Hz, 1H), 3.89 (s, 6H), 3.84 (s, 3H); **^{13}C NMR (125 MHz, CDCl$_3$)** δ 166.6, 166.0, 153.4, 145.8, 145.6, 138.2, 134.2, 132.2, 130.5, 130.5, 128.9, 128.2, 128.1, 117.5, 117.4, 103.8, 73.7, 66.2, 60.8, 56.2; **ESI-LCMS:** *m*/*z* 511.06 (M+Na)$^+$; **HRMS:** *m*/z for $C_{29}H_{28}O_7Na$ (M+Na)$^+$: calcd 511.1727, found 511.1733; **HPLC:** *ee* 97.9% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 30% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 28.0 min].

(*S*)-2-Cinnamamido-1-(2-methoxyphenyl)ethyl cinnamate (9)



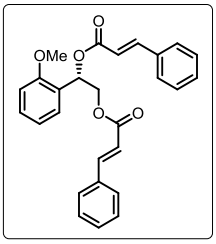
Colorless solid; **m.p.:** 112-114 °C; ***R*$_f$** = 0.45 (EtOAc-petroleum ether, 2:3); **[α]$_D^{27}$** +31.5 (*c* 0.5, $CHCl_3$); **IR (CHCl$_3$):** 3369, 3016, 2925, 2856, 2404, 1710, 1666, 1629, 1526, 1455, 1323, 1215, 1170, 1040, 982, 859, 764, 673 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.74 (d, *J* = 16.1 Hz, 1H), 7.59 (d, *J* = 15.7 Hz, 1H), 7.55-7.26 (m, 12H), 7.02-6.88 (m, 2H), 6.55 (d, *J* = 16.1 Hz, 1H), 6.47 (dd, *J* = 7.8, 3.4 Hz, 1H), 6.40 (d, *J* = 15.7, 1H), 6.15 (brs, 1H), 3.88 (s, 3H), 3.97-3.77 (m, 2H, overlapped); **^{13}C NMR (100 MHz, CDCl$_3$)** δ 166.5, 165.9, 156.2, 145.7, 141.0, 134.8, 134.2, 130.4, 129.6, 129.4, 128.9, 128.7, 128.2, 127.8, 126.4, 126.1, 120.7, 120.6, 117.7, 110.7, 69.9, 55.5, 43.9; **ESI-LCMS:** *m*/*z* 450.13(M+Na)$^+$; **HRMS:** *m*/z for $C_{27}H_{25}NO_4Na$ (M+Na)$^+$: calcd 450.1676, found 450.1668; **HPLC:** *ee* 94.4% [Chiralpak-IB (0.46 mm ϕ X 250 mmL), 20% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R =10.0 min].

(*R*)-2-Cinnamamido-1-(2-methoxyphenyl)ethyl cinnamate (10)



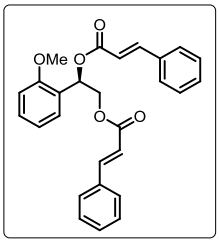
Colorless solid; **m.p.:** 112-114 °C; $\boldsymbol{R_f}$ = 0.45 (EtOAc-petroleum ether, 2:3); $\boldsymbol{[\alpha]_D^{27}}$ -28.7 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3369, 3016, 2925, 2856, 2404, 1710, 1666, 1629, 1526, 1455, 1323, 1215, 1170, 1040, 982, 859, 764, 673 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 16.1 Hz, 1H), 7.59 (d, *J* = 15.7 Hz, 1H), 7.55-7.25 (m, 12H), 7.03-6.87 (m, 2H), 6.52 (d, *J* = 16.1 Hz, 1H), 6.45 (dd, *J* = 8.1, 3.7 Hz, 1H), 6.38 (d, *J* = 15.7, 1H), 6.17 (brs, 1H), 3.88 (s, 3H), 3.98-3.77 (m, 2H, overlapped); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.5, 165.9, 156.2, 145.7, 141.0, 134.8, 134.2, 130.4, 129.6, 129.4, 128.9, 128.7, 128.2, 127.8, 126.4, 126.1, 120.7, 120.6, 117.7, 110.7, 69.9, 55.5, 43.9; **ESI-LCMS:** *m*/*z* 450.12 $(M+Na)^+$; **HRMS:** *m*/z for $C_{27}H_{25}NO_4Na$ $(M+Na)^+$: calcd 450.1676, found 450.1673; **HPLC:** *ee* 96.9% [Chiralpak-IB (0.46 mm ϕ X 250 mmL), 20% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R =7.9 min].

(*S*)-1-(2-Methoxyphenyl)ethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (11)



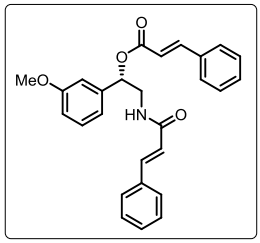
Colorless viscous liquid; $\boldsymbol{R_f}$ = 0.44 (EtOAc-petroleum ether, 1:4); $\boldsymbol{[\alpha]_D^{28}}$ +0.8 (*c* 1, $CHCl_3$); **IR ($CHCl_3$):** 2956, 2919, 1713, 1635, 1600, 1493, 1455, 1355, 1308, 1278, 1245, 1157, 1048, 1024, 981, 863, 758, 709, 683 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.83-7.61 (m, 2H, two doublets overlapped), 7.60-7.29 (m, 12H), 7.04-6.87 (m, 2H), 6.67-6.38 (m, 3H), 4.58-4.47 (m, 2H), 3.89 (s, 3H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.6, 165.9, 156.3, 145.4, 145.2, 134.4, 130.3, 130.3, 129.4, 128.8, 128.1, 128.1, 126.9, 125.0, 120.6, 118.0, 117.8, 110.6, 68.9, 65.4, 55.5; **ESI-LCMS:** *m*/*z* 451.04 $(M+Na)^+$; **HRMS:** *m*/*z* for $C_{27}H_{24}O_5Na$ $(M+Na)^+$: calcd 451.1516, found 451.1507; **HPLC:** *ee* 97.2% [Chiralpak-IB (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 6.4 min].

(*R*)-1-(2-Methoxyphenyl)ethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (12)



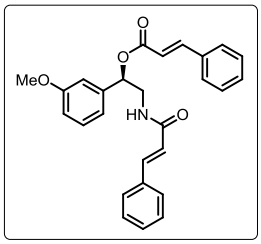
Colorless viscous liquid; $\boldsymbol{R_f}$ = 0.44 (EtOAc-petroleum ether, 1:4); $\boldsymbol{[\alpha]_D^{28}}$ -0.8 (*c* 1, $CHCl_3$); **IR ($CHCl_3$):** 3019, 2360, 2341, 1713, 1663, 1579, 1495, 1439, 1329, 1310, 1283, 1248, 1216, 1162, 1050, 980, 770, 711, 684 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.83-7.62 (m, 2H, two doublets overlapped), 7.60-7.29 (m, 12H), 7.05-6.87 (m, 2H), 6.66-6.38 (m, 3H), 4.57-4.48 (m, 2H), 3.89 (s, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.7, 166.0, 156.3, 145.4, 145.2, 134.4, 130.4, 130.4, 129.5, 128.9, 128.9, 128.2, 128.2, 127.0, 125.0, 120.7, 118.0, 117.8, 110.6, 69.0, 65.4, 55.6; **ESI-LCMS:** *m*/*z* 451.10 $(M+Na)^+$; **HRMS:** *m*/z for $C_{27}H_{24}O_5Na$ $(M+Na)^+$: calcd 451.1516, found 451.1514; **HPLC:** *ee* 96.7% [Chiralpak-IB (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 9.5 min].

(*S*)-2-Cinnamamido-1-(3-methoxyphenyl)ethyl cinnamate (13)



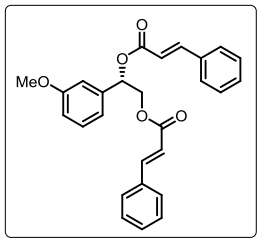
Colorless solid; **m.p.:** 139-141 °C; $\boldsymbol{R_f}$ = 0.39 (EtOAc-petroleum ether, 2:3); $\boldsymbol{[\alpha]_D^{23}}$ +42.5 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3361, 3164, 3019, 2400, 1710, 1664, 1634, 1514, 1450, 1411, 1389, 1330, 1311, 1215, 1165, 1129, 1043, 978, 769, 668 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.73 (d, *J* = 15.9 Hz, 1H), 7.63 (d, *J* = 15.6 Hz, 1H), 7.55-7.42 (m, 4H), 7.41-7.26 (m, 7H), 7.03 (d, *J* = 7.6 Hz, 1H), 6.99 (s, 1H), 6.88 (d, *J* = 7.9 Hz, 1H), 6.50 (d, *J* = 16.2 Hz, 1H), 6.39 (d, *J* = 15.6 Hz, 1H), 6.14 (brs, 1H), 6.03 (dd, *J* = 7.9, 3.4 Hz, 1H), 3.80 (s, 3H), 3.94-3.78 (m, 2H, overlapped); **^{13}C NMR (125 MHz, $CDCl_3$)** δ 166.4, 166.0, 159.8, 145.9, 141.5, 139.3, 134.7, 134.1, 130.5, 129.8, 129.7, 128.9, 128.8, 128.2, 127.8, 120.3,118.6, 117.5, 113.9, 112.1, 74.7, 55.3, 44.8; **ESI-LCMS:** *m*/*z* 450.02 $(M+Na)^+$; **HRMS:** *m*/z for $C_{27}H_{25}NO_4Na$ $(M+Na)^+$: calcd 450.1676, found 450.1673; **HPLC:** *ee* 96.9% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R =13.7 min].

(*R*)-2-Cinnamamido-1-(3-methoxyphenyl)ethyl cinnamate (14)



Colorless solid; **m.p.:** 138-140 °C; R_f = 0.39 (EtOAc-petroleum ether, 2:3); **$[\alpha]_D^{23}$** -47.8 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3361, 3164, 3019, 2400, 1710, 1664, 1634, 1514, 1450, 1411, 1389, 1330, 1311, 1215, 1165, 1129, 1043, 978, 769, 668 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.74 (d, *J* = 16.0 Hz, 1H), 7.64 (d, *J* = 15.7 Hz, 1 H), 7.55-7.43 (m, 4H), 7.42-7.28 (m, 7H), 7.05-6.95 (m, 2H), 6.86 (ddd, *J* = 8.2, 2.7, 0.9 Hz, 1H), 6.52 (d, *J* = 16.0 Hz, 1 H), 6.39 (d, *J* = 15.7 Hz, 1 H), 6.13-5.98 (m, 2H), 3.81 (s, 3H), 3.99-3.73 (m, 2H, overlapped); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.3, 165.9,159.8, 145.9, 141.5, 139.4, 134.7, 134.2, 130.5, 129.8, 129.7, 128.9, 128.8, 128.2, 127.8, 120.3,118.7, 117.5, 113.9, 112.2, 74.7, 55.3, 44.8; **ESI-LCMS:** *m*/*z* 450.04 $(M+Na)^+$; **HRMS:** *m*/z for $C_{27}H_{25}NO_4Na$ $(M+Na)^+$: calcd 450.1676, found 450.1671; **HPLC:** *ee* 96.6% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R =17.8 min].

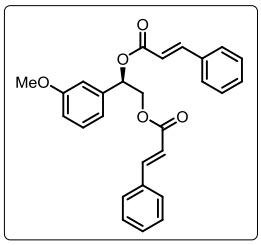
(*S*)-1-(3-Methoxyphenyl)ethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (15)



Colorless solid; **m.p.:** 67-69 °C; R_f = 0.45 (EtOAc-petroleum ether, 1:4); **$[\alpha]_D^{23}$** +22.7 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3365, 3169, 3021, 2931, 1713, 1633, 1451, 1316, 1268, 1216, 1165, 1042, 986, 865, 765, 668 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.83-7.64 (m, 2H, two doublets overlapped), 7.58-7.45 (m, 4H), 7.44-7.26 (m, 7H), 7.09-6.98 (m, 2H), 6.88 (ddd, *J* = 8.2, 2.5, 0.9 Hz, 1H), 6.59-6.38 (m, 2H, two doublets overlapped), 6.22 (dd, *J* = 7.2, 4.7 Hz, 1H), 4.63-4.46 (m, 2H), 3.82 (s, 3H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.5, 165.9, 159.8, 145.6, 145.5, 138.2, 134.3, 130.4, 129.8, 128.9, 128.2, 119.0, 117.7, 117.5, 114.0, 112.5, 73.5, 66.2, 55.3; **ESI-LCMS:** *m*/*z* 451.00$(M+Na)^+$; **HRMS:** *m*/z for $C_{27}H_{24}O_5Na$ $(M+Na)^+$: calcd 451.1516, found 451.1513; **HPLC:** *ee* >99.9% [Chiralpak-IB (0.46 mm ϕ

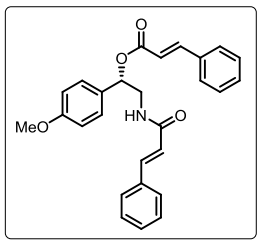
X 250 mmL), 30% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R =17.0 min].

(*R*)-1-(3-Methoxyphenyl)ethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (16)



Colorless solid; **m.p.:** 67-69 °C; R_f = 0.45 (EtOAc-petroleum ether, 1:4); **[α]$_D^{23}$** -25.6 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3365, 3169, 3021, 2931, 1713, 1633, 1451, 1316, 1268, 1216, 1165, 1042, 986, 865, 765, 668 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.81-7.63 (m, 2H, two doublets overlapped), 7.58-7.46 (m, 4H), 7.43-7.26 (m, 7H), 7.09-6.97 (m, 2H), 6.88 (ddd, *J* = 8.2, 2.5, 0.9 Hz, 1H), 6.59-6.38 (m, 2H, two doublets overlapped), 6.21 (dd, *J* = 7.2, 4.6 Hz, 1H), 4.63-4.47 (m, 2H), 3.82 (s, 3H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.5, 165.9, 159.8, 145.6, 145.5, 138.2, 134.3, 130.4, 129.8, 128.9, 128.2, 119.0, 117.7, 117.5, 114.0, 112.5, 73.5, 66.2, 55.3; **ESI-LCMS:** *m*/*z* 451.01 $(M+Na)^+$; **HRMS:** *m*/z for $C_{27}H_{24}O_5Na$ $(M+Na)^+$: calcd 451.1516, found 451.1513; **HPLC:** *ee* 98.4% [Chiralpak-IB (0.46 mm ϕ X 250 mmL), 30% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 45.4 min].

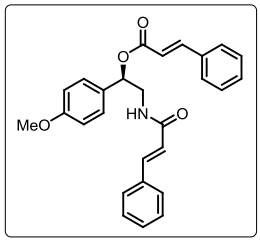
(*S*)-2-Cinnamamido-1-(4-methoxyphenyl)ethyl cinnamate (17)



Colorless solid; **m.p.:** 137-139 °C; R_f = 0.38 (EtOAc-petroleum ether, 2:3); **[α]$_D^{26}$** +51.5 (*c* 0.6, $CHCl_3$); **IR ($CHCl_3$):** 3361, 3165, 3019, 1688, 1620, 1525, 1411, 1390, 1216, 1043, 771, 669 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.77-7.57 (m, 2H, two doublets overlapped), 7.54-7.42 (m, 4H), 7.41-7.28 (m, 8H), 6.91 (d, 2H, *p*-disubstituted), 6.54-6.32 (m, 2H, two doublets overlapped), 6.16-6.06 (m, 1H), 6.01 (dd, *J* = 7.3, 5.6 Hz, 1H), 3.92-3.81 (m, 2H), 3.79 (s, 3H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.5, 165.9, 159.7, 145.7, 141.4, 134.7, 134.2, 130.4, 129.8, 129.7, 128.9, 128.7, 128.1, 128.0, 127.8, 120.4, 117.5, 114.1, 74.7, 55.3, 44.6;

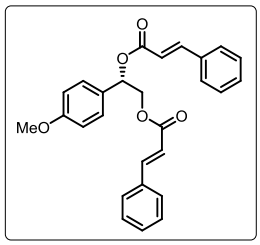
ESI-LCMS: *m/z* 449.96 $(M+Na)^+$; **HRMS:** *m/z* for $C_{27}H_{25}NO_4Na$ $(M+Na)^+$: calcd 450.1676, found 450.1673; **HPLC:** *ee* 96.8% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R =15.0 min].

(*R*)-2-Cinnamamido-1-(4-methoxyphenyl)ethyl cinnamate (18)



Colorless solid; **m.p.:** 138-140 °C; $\boldsymbol{R_f}$ = 0.38 (EtOAc-petroleum ether, 2:3); $\boldsymbol{[\alpha]_D^{26}}$ -59.5 (*c* 0.58, $CHCl_3$); **IR ($CHCl_3$):** 3361, 3165, 3019, 1688, 1620, 1525, 1411, 1390, 1216, 1043, 771, 669 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.78-7.57 (m, 2H, two doublets overlapped), 7.54-7.42 (m, 4H), 7.42-7.28 (m, 8H), 6.91 (d, 2H, *p*-disubstituted), 6.55-6.32 (m, 2H, two doublets overlapped), 6.13 (t, *J* = 5.5 Hz, 1H), 6.01 (dd, *J* = 7.6, 5.3 Hz, 1H), 3.93-3.81 (m, 2H), 3.79 (s, 3H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.5, 165.9, 159.7, 145.7, 141.4, 134.7, 134.2, 130.4, 129.8, 129.7, 128.9, 128.7, 128.1, 128.0, 127.8, 120.4, 117.5, 114.1, 74.7, 55.3, 44.6; **ESI-LCMS:** *m/z* 450.31 $(M+Na)^+$; **HRMS:** *m/z* for $C_{27}H_{25}NO_4Na$ $(M+Na)^+$: calcd 450.1676, found 450.1671; **HPLC:** *ee* 99.4% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 26.0 min].

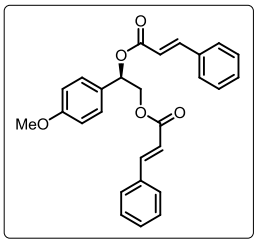
(*S*)-1-(4-Methoxyphenyl)ethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (19)



Colorless solid; **m.p.:** 93-96 °C; $\boldsymbol{R_f}$ = 0.43 (EtOAc-petroleum ether, 1:4); $\boldsymbol{[\alpha]_D^{26}}$ +32.5 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3022, 2964, 2358, 1713, 1633, 1513, 1452, 1251, 1215, 1167, 1036, 986, 930, 896, 832, 762, 672, 607 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.76-7.66 (m, 2H, two doublets overlapped), 7.56-7.47 (m, 4H), 7.43-7.33 (m, 8H), 6.93 (d, 2H, *p*-disubstituted), 6.52 (d, *J* = 16.2 Hz, 1 H), 6.46 (d, *J* = 15.9 Hz, 1 H), 6.19 (dd, *J* = 8.6, 3.7 Hz, 1H), 4.57 (dd, *J* = 11.9, 8.5 Hz, 1H), 4.48 (dd, *J* = 11.9, 3.7 Hz, 1H), 3.80 (s, 3H); **^{13}C**

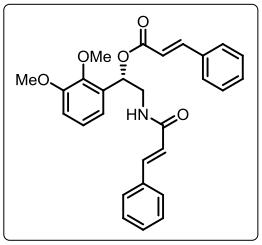
NMR (125 MHz, $CDCl_3$) δ 166.6, 166.1,159.8, 145.5, 134.3, 130.4, 128.9, 128.2, 117.8, 117.5, 114.1, 73.2, 66.1, 55.3; **ESI-LCMS:** *m/z* 451.07 $(M+Na)^+$; **HRMS:** *m/z* for $C_{27}H_{24}O_5Na$ $(M+Na)^+$: calcd 451.1516, found 451.1514; **HPLC:** *ee* 98.3% [Chiralpak-IB (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R =17.7 min].

(*R*)-1-(4-Methoxyphenyl)ethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (20)



Colorless solid; **m.p.:** 76-79 °C; R_f = 0.43 (EtOAc-petroleum ether, 1:4); $[\alpha]_D^{26}$ -31.3 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3022, 2964, 2358, 1713, 1633, 1513, 1452, 1251, 1215, 1167, 1036, 986, 930, 896, 832, 762, 672, 607 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.77-7.65 (m, 2H, two doublets overlapped), 7.57-7.46 (m, 4H), 7.44-7.33 (m, 8H), 6.94 (d, 2H, *p*-disubstituted), 6.52 (d, *J* = 16.1 Hz, 1H), 6.46 (d, *J* = 16.1 Hz, 1H), 6.19 (dd, *J* = 8.6, 3.9 Hz, 1H), 4.59 (dd, *J* = 11.7, 8.3 Hz, 1H), 4.49 (dd, *J* = 12.0, 3.9 Hz, 1H), 3.80 (s, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.6, 166.0, 159.8, 145.4, 134.3, 134.2, 130.4, 128.9, 128.7, 128.2, 128.1, 117.8, 117.5, 114.1, 73.2, 66.1, 55.3; **ESI-LCMS:** *m/z*451.09 $(M+Na)^+$; **HRMS:** *m/z* for $C_{27}H_{24}O_5Na$ $(M+Na)^+$: calcd 451.1516, found 451.1514; **HPLC:** *ee* 98.7% [Chiralpak-IB (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R =31.5 min].

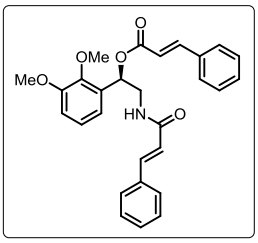
(*S*)-2-Cinnamamido-1-(2,3-dimethoxyphenyl)ethyl cinnamate (21)



Colorless solid; **m.p.:** 105-108 °C; R_f = 0.38 (EtOAc-petroleum ether, 2:3); $[\alpha]_D^{26}$ -7.9 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3366, 3170, 3020, 2358, 1706, 1666, 1627, 1520, 1419, 1216, 1168, 1040, 927, 763, 668 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.73 (d, *J* = 15.9 Hz, 1H), 7.61 (d, *J* = 15.7 Hz, 1H), 7.56-7.43 (m, 4H), 7.42-7.29 (m, 6H), 7.15-6.99 (m, 2H), 6.90

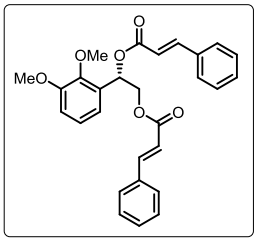
(dd, *J* = 7.6, 2.2, 1H), 6.51 (d, *J* = 16.0 Hz, 1H), 6.44-6.32 (m, 2H), 6.14 (t, *J* = 5.4 Hz, 1H), 3.98 (s, 3H), 3.87 (s, 3H), 3.90-3.84 (m, 2H, overlapped); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.5, 166.0, 152.6, 146.2, 145.8, 141.3, 134.8, 134.2, 131.9, 130.5, 129.7, 128.9, 128.8, 128.2, 127.9, 124.6, 120.5, 118.4, 117.6, 112.4, 69.9, 60.9, 55.8, 44.3; **ESI-LCMS:** *m*/*z* 480.22 $(M+Na)^+$; **HRMS:** *m*/*z* for $C_{28}H_{27}NO_5Na$ $(M+Na)^+$: calcd 480.1781, found 480.1777; **HPLC:** *ee* 96.5% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 15% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 32.8 min].

(*R*)-2-Cinnamamido-1-(2,3-dimethoxyphenyl)ethyl cinnamate (22)



Colorless solid; **m.p.:** 105-107 °C; R_f = 0.38 (EtOAc-petroleum ether, 2:3); **$[\alpha]_D^{26}$** +8.0 (*c* 0.58, $CHCl_3$); **IR ($CHCl_3$):** 3366, 3170, 3020, 2358, 1706, 1666, 1627, 1520, 1419, 1216, 1168, 1040, 927, 763, 668 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.75 (d, *J* = 16.0 Hz, 1H), 7.62 (d, *J* = 15.7 Hz, 1H), 7.56-7.45 (m, 4H), 7.43-7.30 (m, 6H), 7.15-6.99 (m, 2H), 6.90 (dd, *J* = 7.6, 2.2 Hz, 1H), 6.50 (d, *J* = 16.0 Hz, 1H), 6.45-6.32 (m, 2H), 6.16 (t, *J* = 4.9 Hz, 1H), 3.99 (s, 3H), 3.88 (s, 3H), 3.95-3.81 (m, 2H, overlapped); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.5, 166.0, 152.6, 146.2, 145.8, 141.3, 134.8, 134.2, 131.9, 130.5, 129.7, 128.9, 128.8, 128.2, 127.9, 124.6, 120.5, 118.4, 117.6, 112.4, 69.9, 60.9, 55.8, 44.3; **ESI-LCMS:** *m*/*z* 480.23 $(M+Na)^+$; **HRMS:** *m*/z for $C_{28}H_{27}NO_5Na$ $(M+Na)^+$: calcd 480.1781, found 480.1780; **HPLC:** *ee* 93.8% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 15% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 29.1 min].

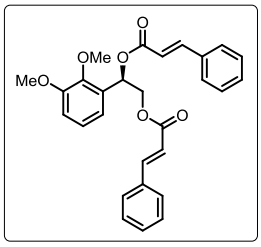
(*S*)-1-(2,3-Dimethoxyphenyl)ethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (23)



Colorless viscous liquid; R_f = 0.4 (EtOAc-petroleum ether, 1:4); **$[\alpha]_D^{23}$** -17.0 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3021, 2943, 2843, 2358, 1713, 1637, 1482, 1448, 1298, 1272, 1215, 1168,

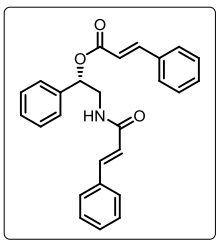
1084, 1042, 993, 929, 868, 760, 672, 611 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.78-7.65 (m, 2H, two doublets overlapped), 7.52 (m, 4H), 7.42-7.32 (m, 6H), 7.12-7.01 (m, 2H), 6.92 (d, *J* = 7.6 Hz, 1H), 6.60-6.49 (m, 2H), 6.47 (d, *J* = 16.2 Hz, 1H), 4.61 (dd, *J* = 11.9, 8.6 Hz, 1H), 4.46 (dd, *J* = 11.6, 3.1 Hz, 1H), 4.01 (s, 3H), 3.89 (s, 3H); **^{13}C NMR (125 MHz, $CDCl_3$)** δ 166.6, 166.0, 152.6, 146.4, 145.4, 145.3, 134.3, 134.3, 130.5, 130.4, 130.3, 128.9, 128.8, 128.1, 124.2, 118.8, 117.8, 117.7, 112.6, 69.1, 65.6, 60.8, 55.8; **ESI-LCMS:** *m/z* 481.04 $(M+Na)^+$; **HRMS:** *m/z* for $C_{28}H_{26}O_6Na$ $(M+Na)^+$: calcd 481.1622, found 481.1616; **HPLC:** *ee* 97.3% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 7.4 min].

(*R*)-1-(2,3-Dimethoxyphenyl)ethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (24)



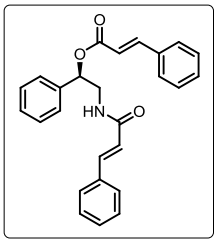
Colorless viscous liquid; R_f = 0.4 (EtOAc-petroleum ether, 1:4); $[\alpha]_D^{23}$ +18.0 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3021, 2943, 2843, 2358, 1713, 1637, 1482, 1448, 1298, 1272, 1215, 1168, 1084, 1042, 993, 929, 868, 760, 672, 611 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.80-7.67 (m, 2H, two doublets overlapped), 7.58-7.48 (m, 4H), 7.44-7.33 (m, 6H), 7.13-7.02 (m, 2H), 6.92 (d, *J* = 7.6 Hz, 1H), 6.61-6.51 (m, 2H), 6.47 (d, *J* = 16.2 Hz, 1H), 4.61 (dd, *J* = 11.9, 8.2 Hz, 1H), 4.48 (dd, *J* = 11.9, 3.1 Hz, 1H), 4.01 (s, 3H), 3.89 (s, 3H); **^{13}C NMR (125 MHz, $CDCl_3$)** δ 166.6, 166.0, 152.6, 146.4, 145.5, 145.3, 134.3, 134.3, 130.5, 130.4, 130.3, 128.9, 128.8, 128.2, 124.2, 118.8, 117.8, 117.7, 112.5, 69.1, 65.6, 60.8, 55.8; **ESI-LCMS:** *m/z* 481.09 $(M+Na)^+$; **HRMS:** *m/z* for $C_{28}H_{26}O_6Na$ $(M+Na)^+$: calcd 481.1622, found 481.1619; **HPLC:** *ee* 98.2% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 8.5 min].

(*S*)-2-Cinnamamido-1-phenylethyl cinnamate (25)



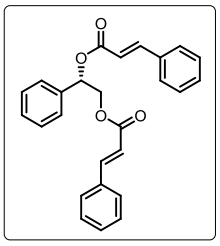
Colorless solid; **m.p.:** 181-183 °C; R_f = 0.52 (EtOAc-petroleum ether, 2:3); **[α]$_D^{28}$** +59.8 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3292, 3022, 2927, 2864, 2404, 2358, 1711, 1667, 1630, 1516, 1419, 1327, 1216, 1167, 1095, 1030, 929, 764, 670 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.26-7.82 (m, 17H), 6.30 - 6.60 (m, 2H), 6.06 (m, 2H), 3.69 - 4.06 (m, 2H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.5, 166.1, 146.0, 141.6, 137.9, 134.8, 134.2, 130.6, 129.8, 129.0, 128.9, 128.6, 128.3, 127.9, 126.6, 120.4, 117.6, 74.9, 44.9; **ESI-LCMS:** *m/z* 420.11 $(M+Na)^+$; **HRMS:** *m/z* for $C_{26}H_{23}NO_3Na$ $(M+Na)^+$: calcd 420.1570, found 420.1566; **HPLC:** *ee* 97.1% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 12.8 min].

(*R*)-2-Cinnamamido-1-phenylethyl cinnamate (26)



Colorless solid; **m.p.:** 183-185°C; R_f = 0.52 (EtOAc-petroleum ether, 2:3); **[α]$_D^{28}$** -57.3 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3292, 3022, 2927, 2864, 2404, 2358, 1711, 1667, 1630, 1516, 1419, 1327, 1216, 1167, 1095, 1030, 929, 764, 670 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.81-7.26 (m, 17H), 6.59-6.30 (m, 2H), 6.17-5.99 (m, 2H), 4.07-3.71 (m, 2H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.5, 166.1, 146.0, 141.6, 137.9, 134.8, 134.3, 130.6, 129.8, 129.0, 128.9, 128.6, 128.3, 127.9, 126.6, 120.4, 117.6, 74.9, 44.9; **ESI-LCMS:** *m/z* 420.15 $(M+Na)^+$; **HRMS:** *m/z* for $C_{26}H_{23}NO_3Na$ $(M+Na)^+$: calcd 420.1570, found 420.1562; **HPLC:** *ee* 94.8% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 20.4 min].

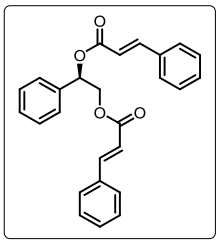
(*S*)-1-Phenylethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (27)



Colorless solid; **m.p.:** 131-133 °C; R_f = 0.26 (EtOAc-petroleum ether, 1:9); **[α]$_D^{26}$** +42.7 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3022, 1714, 1638, 1436, 1320, 1216, 1166, 767, 671 cm^{-1}; **^{1}H**

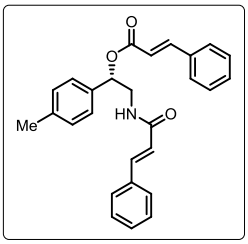
NMR (200 MHz, $CDCl_3$) δ 7.84-7.65 (m, 2H, two doublets overlapped), 7.60-7.28 (m, 15H), 6.60-6.37 (m, 2H, two doublets overlapped), 6.24 (dd, *J* = 7.5, 4.6 Hz, 1H), 4.65-4.44 (m, 2H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.6, 166.0, 145.6, 145.5, 136.7, 134.2, 130.4, 128.9, 128.7, 128.2, 126.8, 117.7, 117.5, 73.6, 66.2; **ESI-LCMS:** *m*/*z* 421.24 $(M+Na)^+$; **HRMS:** *m*/z for $C_{26}H_{22}O_4Na$ $(M+Na)^+$: calcd 421.1410, found 421.1411; **HPLC:** *ee* 96.5% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 8.8 min].

(*R*)-1-Phenylethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (28)



Colorless solid; **m.p.:** 132-134 °C; R_f = 0.26 (EtOAc-petroleum ether, 1:9); **$[\alpha]_D^{26}$** -42.0 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3022, 1714, 1638, 1436, 1320, 1216, 1166, 767, 671 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.84-7.65 (m, 2H, two doublets overlapped), 7.60-7.28 (m, 15H), 6.61-6.37 (m, 2H, two doublets overlapped), 6.24 (dd, *J* = 7.5, 4.4 Hz, 1H), 4.66-4.45 (m, 2H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.6, 166.0, 145.6, 145.5, 136.7, 134.2, 130.4, 128.9, 128.7, 128.2, 126.8, 117.7, 117.5, 73.6, 66.2; **ESI-LCMS:** *m*/*z* 421.17 $(M+Na)^+$; **HRMS:** *m*/z for $C_{26}H_{22}O_4Na$ $(M+Na)^+$: calcd 421.1410, found 421.1404; **HPLC:** *ee* 92.6% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 16.6 min].

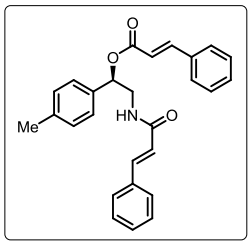
(*S*)-2-Cinnamamido-1-(p-tolyl)ethyl cinnamate (29)



Colorless solid; **m.p.:** 139-140 °C; R_f = 0.5 (EtOAc-petroleum ether, 2:3); **$[\alpha]_D^{28}$** +55.4 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3441, 3021, 2926, 2403, 1711, 1669, 1630, 1515, 1438, 1326, 1216, 1168, 1036, 928, 858, 768, 671 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.79-7.56 (m, 2H, two doublets overlapped), 7.55-7.13 (m, 14H), 6.51 (d, *J* = 15.9 Hz, 1H), 6.39 (d, *J* =

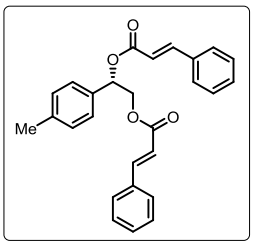
15.5 Hz, 1H), 6.16-5.96 (m, 2H), 4.00-3.73 (m, 2H), 2.35 (s, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.6, 166.1, 145.8, 141.5, 138.5, 134.9, 134.8, 134.3, 130.6, 129.8, 129.5, 129.0, 128.9, 128.3, 127.9, 126.6, 120.5, 117.7, 74.9, 44.8, 21.3; **ESI-LCMS:** *m/z* 434.07 $(M+Na)^+$; **HRMS:** *m*/z for $C_{27}H_{25}NO_3Na$ $(M+Na)^+$: calcd 434.1727, found 434.1725; **HPLC:** *ee* 95.2% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 12.2 min].

(*R*)-2-Cinnamamido-1-(p-tolyl)ethyl cinnamate (30)



Colorless solid; **m.p.:** 139-141°C; R_f = 0.5 (EtOAc-petroleum ether, 2:3); **$[\alpha]_D^{28}$** -59.6 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3441, 3021, 2926, 2403, 1711, 1669, 1630, 1515, 1438, 1326, 1216, 1168, 1036, 928, 858, 768, 671 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.79-7.56 (m, 2H, two doublets overlapped), 7.55-7.13 (m, 14H), 6.51 (d, *J* = 15.9 Hz, 1H), 6.39 (d, *J* = 15.5 Hz, 1H), 6.16-5.96 (m, 2H), 4.00-3.73 (m, 2H), 2.34 (s, 3H); **^{13}C NMR (100 MHz,$CDCl_3$)** δ 166.6, 166.0, 145.8, 141.6, 138.5, 134.9, 134.8, 134.3, 130.6, 129.8, 129.5, 129.0, 128.9, 128.3, 127.9, 126.6, 120.4, 117.7, 74.9, 44.8, 21.3; **ESI-LCMS:** *m/z* 434.07 $(M+Na)^+$; **HRMS:** *m*/z for $C_{27}H_{25}NO_3Na$ $(M+Na)^+$: calcd 434.1727, found 434.1721; **HPLC:** *ee* 95.3% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 20.4 min].

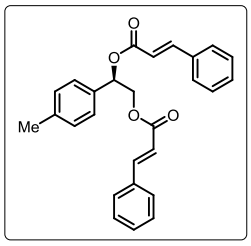
(*S*)-1-(*p*-Tolyl)ethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (31)



Colorless solid; **m.p.:** 103-105 °C; R_f = 0.26 (EtOAc-petroleum ether, 1:9); **$[\alpha]_D^{27}$** +40.1 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3361, 3164, 3022, 1712, 1634, 1523, 1445, 1401, 1316, 1268, 1215, 1165, 1036, 762, 670 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.81-7.66 (m, 2H, two doublets overlapped), 7.59-7.48 (m, 4H), 7.45-7.33 (m, 8H), 7.22 (d, 2H, *p*-disubstituted),

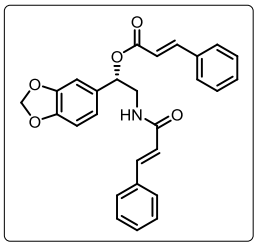
6.54 (d, 1H, *J* = 16.0), 6.46 (d, 1H, *J* = 16.0), 6.23 (dd, *J* = 8.2, 3.7 Hz, 1H), 4.61-4.45 (m, 2H), 2.37 (s, 3H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.6, 166.0, 145.5, 138.5, 134.3, 133.7, 130.4, 129.4, 128.9, 128.2, 126.8, 117.8, 117.5, 73.5, 66.2, 21.2; **ESI-LCMS:** *m/z* 435.16 $(M+Na)^+$; **HRMS:** *m/z* for $C_{27}H_{24}O_4Na$ $(M+Na)^+$: calcd 435.1567, found 435.1559; **HPLC:** *ee* 98.3% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 30% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 10.1 min].

(*R*)-1-(*p*-Tolyl)ethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (32)



Colorless solid; **m.p.:** 103-105 °C; R_f = 0.26 (EtOAc-petroleum ether, 1:9); **$[\alpha]_D^{27}$** -42.3 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3361, 3164, 3022, 1712, 1634, 1523, 1445, 1401, 1316, 1268, 1215, 1165, 1036, 762, 670 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.77-7.66 (m, 2H, two doublets overlapped), 7.57-7.47 (m, 4H), 7.42-7.32 (m, 8H), 7.22 (d, 2H, *p*-disubstituted), 6.54 (d, 1H, *J* = 16.0), 6.46 (d, 1H, *J* = 16.0), 6.23 (dd, *J* = 8.2, 3.7 Hz, 1H), 4.63-4.46 (m, 2H), 2.37 (s, 3H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.6, 166.0, 145.5, 138.5, 134.3, 133.7, 130.4, 129.4, 128.9, 128.2, 126.8, 117.8, 117.5, 73.5, 66.2, 21.2; **ESI-LCMS:** *m/z* 435.16 $(M+Na)^+$; **HRMS:** *m/z* for $C_{27}H_{24}O_4Na$ $(M+Na)^+$: calcd 435.1567, found 435.1563; **HPLC:** *ee* 97.6% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 30% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 23.3 min].

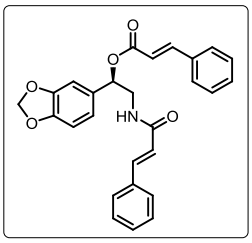
(*S*)-1-(Benzo[*d*][1,3]dioxol-5-yl)-2-cinnamamidoethyl cinnamate (33)



Colorless solid; **m.p.:** 186-190 °C; R_f = 0.23 (EtOAc-petroleum ether, 3:7); **$[\alpha]_D^{26}$** +42.4 (*c* 0.53, $CHCl_3$); **IR ($CHCl_3$):** 3361, 3164, 3020, 2932, 2358, 1710, 1666, 1628, 1506, 1444, 1327, 1215, 1167, 1040, 984, 932, 862, 762, 670 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.72 (d, 1H, *J* = 16.0 Hz), 7.64 (d, 1H, *J* = 15.6 Hz), 7.56-7.44 (m, 4H), 7.43-7.30 (m, 6H), 6.95-

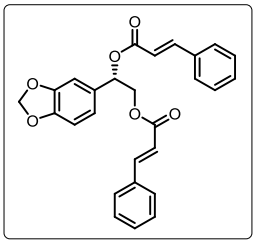
6.89 (m, 2H), 6.80 (d, *J* = 8.2 Hz, 1H), 6.49 (d, 1H, *J* = 16.0), 6.40 (d, *J* = 15.6 Hz, 1H), 6.11 (t, *J* = 5.5 Hz, 1H), 6.00-5.92 (m, 3H), 3.93-3.75 (m, 2H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.4, 166.0, 147.9, 147.7, 145.9, 141.6, 134.6, 134.1, 131.6, 130.6, 129.7, 128.9, 128.8, 128.2, 127.8, 120.4, 120.2, 117.5, 108.4, 106.9, 101.2, 74.7, 44.7; **ESI-LCMS:** *m*/*z* 464.16 $(M+Na)^+$; **HRMS:** *m*/*z* for $C_{27}H_{23}NO_5Na$ $(M+Na)^+$: calcd 464.1468, found 464.1465; **HPLC:** *ee* 94.1% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 50% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 14.7 min].

(*R*)-1-(Benzo[*d*][1,3]dioxol-5-yl)-2-cinnamamidoethyl cinnamate (34)



Colorless solid; **m.p.:** 200-202 °C; R_f = 0.23 (EtOAc-petroleum ether, 3:7); **$[\alpha]_D^{26}$** -38.3 (*c* 0.57, $CHCl_3$); **IR ($CHCl_3$):** 3361, 3164, 3020, 2932, 2358, 1710, 1666, 1628, 1506, 1444, 1327, 1215, 1167, 1040, 984, 932, 862, 762, 670 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.71 (d, 1H, *J* = 16.0 Hz), 7.63 (d, 1H, *J* = 15.6 Hz), 7.55-7.43 (m, 4H), 7.41-7.30 (m, 6H), 6.95-6.88 (m, 2H), 6.79 (d, *J* = 7.8 Hz, 1H), 6.48 (d, *J* = 16.0 Hz, 1H), 6.39 (d, *J* = 15.6 Hz, 1H), 6.10 (t, *J* = 5.5 Hz, 1H), 5.99-5.92 (m, 3H), 3.92-3.74 (m, 2H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.4, 166.0, 147.9, 147.7, 145.9, 141.6, 134.6, 134.1, 131.6, 130.6, 129.7, 128.9, 128.8, 128.2, 127.8, 120.4, 120.2, 117.5, 108.4, 106.9, 101.2, 74.7, 44.7; **ESI-LCMS:** *m*/*z* 464.10 $(M+Na)^+$; **HRMS:** *m*/z for $C_{27}H_{23}NO_5Na$ $(M+Na)^+$: calcd 464.1468, found 464.1467; **HPLC:** *ee* 93.4% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 50% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 29.5 min].

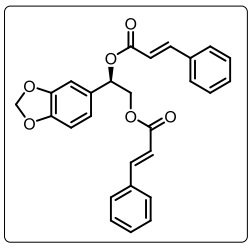
(*S*)-1-(Benzo[*d*][1,3]dioxol-5-yl)ethane-1,2-diyl(2*E*,2'*E*)-bis(3-phenylacrylate) (35)



Colorless solid; **m.p.:** 106-107 °C; R_f = 0.45 (EtOAc-petroleum ether, 1:4); **$[\alpha]_D^{26}$** +29.1 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3022, 1714, 1637, 1496, 1446, 1317, 1249, 1215, 1166, 1040,

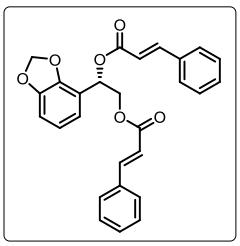
985, 934, 865, 761, 673, 620 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.73 (m, 2H, two doublets overlapped), 7.58-7.45 (m, 4H), 7.43-7.31 (m, 6H), 7.02-6.77 (m, 3H), 6.60-6.38 (m, 2H, two doublets overlapped), 6.15 (dd, *J* = 7.7, 4.4 Hz, 1H), 5.98 (s, 2H), 4.63-4.41 (m, 2H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.5, 166.0, 147.9, 147.8, 145.6, 145.5, 134.2, 130.4, 128.9, 128.2, 120.6, 117.6, 117.4, 108.4, 107.3, 101.2, 73.4, 66.2; **ESI-LCMS:** *m/z* 465.14 $(M+Na)^+$; **HRMS:** *m/z* for $C_{27}H_{22}O_6Na$ $(M+Na)^+$: calcd 465.1309, found 465.1307; **HPLC:** *ee* 97.5% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 14.8 min].

(*R*)-1-(Benzo[*d*][1,3]dioxol-5-yl)ethane-1,2-diyl(2*E*,2'*E*)-bis(3-phenylacrylate) (36)



Colorless solid; **m.p.:** 108-110 °C; R_f = 0.45 (EtOAc-petroleum ether, 1:4); **$[\alpha]_D^{26}$** -29.8 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3022, 1714, 1637, 1496, 1446, 1317, 1249, 1215, 1166, 1040, 985, 934, 865, 761, 673, 620 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.79-7.63 (m, 2H, two doublets overlapped), 7.60-7.46 (m, 4H), 7.45-7.31 (m, 6H), 7.00-6.88 (m, 2H), 6.82 (d, *J* = 7.8 Hz, 1H), 6.51 (d, *J* = 15.9 Hz, 1H), 6.44 (d, *J* = 16.1 Hz, 1H), 6.13 (dd, *J* = 8.1, 3.7 Hz, 1H), 5.96 (s, 2H), 4.59-4.41 (m, 2H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.5, 166.0, 147.9, 147.8, 145.6, 145.5, 134.2, 130.4, 128.9, 128.2, 120.6, 117.6, 117.4, 108.4, 107.3, 101.2, 73.4, 66.2; **ESI-LCMS:** *m/z* 465.26 $(M+Na)^+$; **HRMS:** *m/z* for $C_{27}H_{22}O_6Na$ $(M+Na)^+$: calcd 465.1309, found 465.1306; **HPLC:** *ee* 97.9% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 26.7 min].

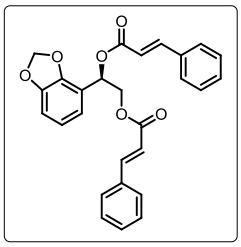
(*S*)-1-(Benzo[*d*][1,3]dioxol-4-yl)ethane-1,2-diyl(2*E*,2'*E*)-bis(3-phenylacrylate) (37)



Colorless viscous liquid; R_f = 0.48 (DCM-petroleum ether, 7:3); **$[\alpha]_D^{28}$** +12.9 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3287, 3022, 2923, 2860, 2358, 1716, 1637, 1459, 1317, 1215, 1165,

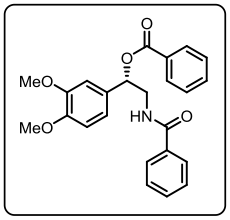
1040, 987, 934, 855, 761, 672 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.83-7.62 (m, 2H, two doublets overlapped), 7.60-7.45 (m, 4H), 7.44-7.29 (m, 6H), 6.99-6.76 (m, 3H), 6.61-6.30 (m, 3H), 6.02 (m, 2H), 4.75-4.49 (m, 2H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.6, 166.0, 147.7, 145.9, 145.6, 145.1, 134.4, 130.6, 130.5, 129.0, 129.0, 128.3, 128.3, 122.0, 120.1, 118.3, 117.6, 117.6, 108.9, 101.4, 69.3, 64.8; **HRMS:** *m/z* for $C_{27}H_{22}O_6Na$ $(M+Na)^+$: calcd: 465.1309, found 465.1305; **HPLC:** *ee* 96.7% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 10.3 min].

(*R*)-1-(Benzo[*d*][1,3]dioxol-4-yl)ethane-1,2-diyl(2*E*,2'*E*)-bis(3-phenylacrylate) (38)



Colorless viscous liquid; R_f = 0.48 (DCM-petroleum ether, 7:3); **$[\alpha]_D^{28}$** -12.8 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3287, 3022, 2923, 2860, 2358, 1716, 1637, 1459, 1317, 1215, 1165, 1040, 987, 934, 855, 761, 672 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.83-7.62 (m, 2H, two doublets overlapped), 7.60-7.45 (m, 4H), 7.44-7.29 (m, 6H), 6.99-6.76 (m, 3H), 6.61-6.30 (m, 3H), 6.02 (m, 2H), 4.75-4.49 (m, 2H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.6, 166.0, 147.7, 145.9, 145.6, 145.1, 134.4, 130.6, 130.5, 129.0, 128.3, 128.3, 122.0, 120.1, 118.3, 117.6, 117.6, 108.9, 101.4, 69.3, 64.8; **HRMS:** *m/z* for $C_{27}H_{22}O_6Na$ $(M+Na)^+$: calcd 465.1309, found 465.1309; **HPLC:** *ee* 94.3% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 14.0 min].

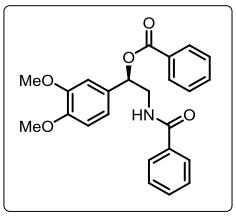
(*S*)-2-Benzamido-1-(3,4-dimethoxyphenyl)ethyl benzoate (39)



Colorless solid; **m.p.:** 129-131 °C; R_f = 0.33 (EtOAc-petroleum ether, 1:1); **$[\alpha]_D^{28}$** +13.1 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3021, 2928, 1717, 1660, 1603, 1520, 1457, 1376, 1320, 1264, 1218, 1152, 1107, 1029, 927, 855, 763, 666 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.10-8.05 (m, 2H), 7.74-7.68 (m, 2H), 7.60-7.53 (m, 1H), 7.50-7.36 (m, 5H), 7.06 (dd, *J* = 8.2, 1.8 Hz,

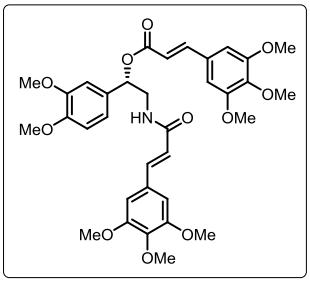
1H), 7.01 (d, *J* = 2.3 Hz, 1H), 6.88 (d, *J* = 8.2 Hz, 1H), 6.63 (t, *J* = 5.5 Hz, 1H), 6.18 (dd, *J* = 8.2, 4.6 Hz, 1H), 4.08-3.92 (m, 2H), 3.88 (s, 3H), 3.87 (s, 3H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 167.6, 166.3, 149.3, 149.2, 134.3, 133.3, 131.6, 130.2, 129.9, 129.8, 128.6, 128.5, 126.9, 119.0, 111.3, 109.8, 77.7, 77.1, 76.5, 75.2, 56.0, 55.9, 45.2; **ESI-LCMS:** *m*/z 428.12 $(M+Na)^+$; **HRMS:** *m*/z for $C_{24}H_{23}NO_5Na$ $(M+Na)^+$: calcd 428.1468, found 428.1462; **HPLC:** *ee* 91.4% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 6.3 min].

(*R*)-2-Benzamido-1-(3,4-dimethoxyphenyl)ethyl benzoate (40)



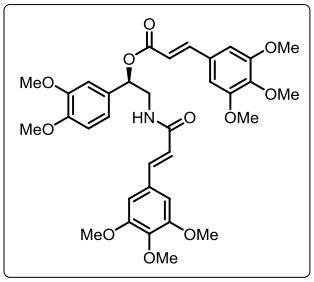
Colorless solid; **m.p.:** 128-130 °C; R_f = 0.33 (EtOAc-petroleum ether, 1:1); **$[\alpha]_D^{28}$** -13.0 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3021, 2928, 1717, 1660, 1603, 1520, 1457, 1376, 1320, 1264, 1218, 1152, 1107, 1029, 927, 855, 763, 666 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.11-8.05 (m, 2H), 7.74-7.68 (m, 2H), 7.60-7.53 (m, 1H), 7.50-7.36 (m, 5H), 7.06 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.01 (d, *J* = 2.3 Hz, 1H), 6.88 (d, *J* = 8.2 Hz, 1H), 6.63 (t, *J* = 5.0 Hz, 1H), 6.18 (dd, *J* = 8.2, 4.6 Hz, 1H), 4.07-3.93 (m, 2H), 3.88 (s, 3H), 3.87 (s, 3H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 167.7, 166.3, 149.2, 149.2, 134.3, 133.4, 131.6, 130.2, 129.9, 129.8, 128.6, 128.5, 126.9, 119.0, 111.2, 109.7, 77.7, 77.1, 76.5, 75.2, 56.0, 55.9, 45.2; **ESI-LCMS:** *m*/z 428.12 $(M+Na)^+$; **HRMS:** *m*/z for $C_{24}H_{23}NO_5Na$ $(M+Na)^+$: calcd 428.1468, found 428.1468; **HPLC:** *ee* 93.8% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 13.6 min].

(*S*)-1-(3,4-Dimethoxyphenyl)-2-((*E*)-3-(3,4,5-trimethoxyphenyl)acrylamido)ethyl(*E*)-3-(3,4,5-trimethoxyphenyl)acrylate (41)



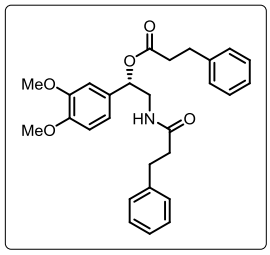
Colorless solid; **m.p.:** 138-140 °C; R_f = 0.42 (EtOAc-petroleum ether, 4:1); **[α]$_D^{27}$** +45.4 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3021, 2932, 2848, 1710, 1669, 1628, 1587, 1511, 1461, 1423, 1326, 1266, 1217, 1138, 1032, 928, 766, 669 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.71-7.48 (m, 2H, two doublets overlapped), 7.06-6.83 (m, 3H), 6.74 (s, 2H), 6.70 (s, 2H), 6.47-6.24 (m, 2H, two doublets overlapped), 6.11-5.93 (m, 2H), 3.95-3.82 (m, 26H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.6, 166.0, 153.5, 153.5, 149.3, 149.2, 145.8, 141.6, 140.4, 139.7, 130.3, 130.2, 129.7, 119.6, 119.2, 116.8, 111.3, 110.0, 105.4, 105.1, 105.1, 77.5, 77.3, 77.1, 77.0, 76.8, 74.8, 61.0, 56.2, 56.2, 56.1, 56.0, 44.7; **HRMS:** *m/z* for $C_{34}H_{39}NO_{11}Na$ $(M+Na)^+$: calcd 660.2415, found 660.2419; **HPLC:** *ee* 97.0% [Chiralpak-IB (0.46 mm ϕ X 250 mmL), 20% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 8.0 min].

(*R*)-1-(3,4-Dimethoxyphenyl)-2-((*E*)-3-(3,4,5-trimethoxyphenyl)acrylamido)ethyl(*E*)-3-(3,4,5-trimethoxyphenyl)acrylate (42)



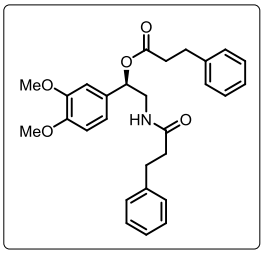
Colorless solid; **m.p.:** 138-140 °C; R_f = 0.42 (EtOAc-petroleum ether, 4:1); **[α]$_D^{27}$** -43.6 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3021, 2932, 2848, 1710, 1669, 1628, 1587, 1511, 1461, 1423, 1326, 1266, 1217, 1138, 1032, 928, 766, 669 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.75-7.45 (m, 2H, two doublets overlapped), 7.08-6.83 (m, 3H), 6.82-6.65 (m, 4H), 6.50-6.21 (m, 2H, two doublets overlapped), 6.02 (m, 2H), 4.07-3.72 (m, 26H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.6, 166.0, 153.5, 153.5, 149.3, 149.2, 145.9, 141.6, 140.4, 139.7, 130.3, 130.2, 129.7, 119.6, 119.2, 116.8, 111.3, 110.0, 105.4, 105.1, 77.6, 77.6, 77.5, 77.3, 77.1, 76.8, 74.8, 61.0, 61.0, 56.2, 56.2, 56.1, 56.0, 44.7; **HRMS:** *m*/z for $C_{34}H_{39}NO_{11}Na$ $(M+Na)^+$: calcd 660.2415, found 660.2407; **HPLC:** *ee* 96.2% [Chiralpak-IB (0.46 mm ϕ X 250 mmL), 20% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 11.6 min].

(*S*)-1-(3,4-Dimethoxyphenyl)-2-(3-phenylpropanamido)ethyl 3-phenylpropanoate (43)



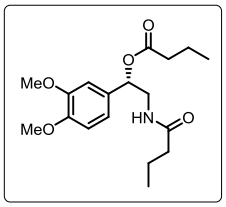
Colorless solid; **m.p.:** 82-84 °C; $\boldsymbol{R_f}$ = 0.45 (EtOAc-petroleum ether, 1:1); $\boldsymbol{[\alpha]_D^{28}}$ +31.1 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3313, 3069, 3017, 2926, 2853, 1733, 1658, 1601, 1514, 1455, 1372, 1255, 1230, 1151, 1029, 856, 813, 758, 701, 665 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.37-7.07 (m, 10H), 6.87-6.72 (m, 3H), 5.73 (dd, *J* = 8.3, 4.2 Hz, 1H), 5.37 (m, 1H), 3.85 (s, 6H, two OMe singlets), 3.73-3.36 (m, 2H), 3.04-2.80 (m, 4H), 2.75-2.56 (m, 2H), 2.46-2.25 (m, 2H); **^{13}C NMR (100 MHz,$CDCl_3$)** δ 172.2, 172.2, 149.2, 149.1, 140.9, 140.4, 130.2, 128.6, 128.5, 128.4, 128.3, 126.5, 126.4, 126.3, 119.0, 111.2, 109.7, 74.6, 56.0, 44.3, 38.3, 35.8, 31.5, 30.9; **HRMS:** *m/z* for $C_{28}H_{31}NO_5Na$ $(M+Na)^+$: calcd 484.2094, found 484.2092; **HPLC:** *ee* 92.3% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 5.8 min].

(*R*)-1-(3,4-Dimethoxyphenyl)-2-(3-phenylpropanamido)ethyl 3-phenylpropanoate (44)



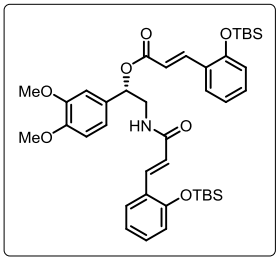
Colorless solid; **m.p.:** 77-78 °C; $\boldsymbol{R_f}$ = 0.45 (EtOAc-petroleum ether, 1:1); $\boldsymbol{[\alpha]_D^{28}}$ -30.2 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3313, 3069, 3017, 2926, 2853, 1733, 1658, 1601, 1514, 1455, 1372, 1255, 1230, 1151, 1029, 856, 813, 758, 701, 665 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.35-7.10 (m, 10H), 6.85-6.74 (m, 3H), 5.73 (dd, *J* = 8.3, 4.3 Hz, 1H), 5.43-5.30 (m, 1H), 3.85 (s, 6H, two OMe singlets), 3.72-3.37 (m, 2H), 3.00-2.83 (m, 4H), 2.73-2.60 (m, 2H), 2.44-2.29 (m, 2H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 172.2, 172.2, 149.2, 149.1, 140.9, 140.4, 130.2, 128.6, 128.4, 128.3, 126.5, 126.3, 119.0, 111.2, 109.7, 74.6, 56.0, 44.3, 38.3, 35.8, 31.5, 30.9; **HRMS:** *m/z* for $C_{28}H_{31}NO_5Na$ $(M+Na)^+$: calcd 484.2094, found 484.2088; **HPLC:** *ee* 90.6% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 6.7 min].

(*S*)-2-Butyramido-1-(3,4-dimethoxyphenyl)ethyl butyrate (45)



Colorless viscous liquid; R_f = 0.47 (EtOAc-petroleum ether, 3:2); $[\alpha]_D^{26}$ +56.2 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3449, 3019, 2962, 2404, 1732, 1668, 1600, 1516, 1459, 1372, 1256, 1218, 1172, 1090, 1030, 929, 855, 761, 668 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 6.97-6.78 (m, 3H), 5.88-5.67 (m, 2H), 3.88 (s, 3H, OMe singlet), 3.87 (s, 3H, OMe singlet), 3.74-3.55 (m, 2H), 2.41-2.26 (m, 2H), 2.21-2.06 (m, 2H), 1.75-1.5 (m, 4H), 1.00-0.82 (m, 6H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 173.1, 173.0, 149.0, 130.4, 119.0, 111.1, 109.7, 74.2, 55.9, 44.2, 38.6, 36.3, 19.1, 18.4, 13.7, 13.7; **ESI-LCMS:** *m*/*z* 359.98 $(M+Na)^+$; **HRMS:** *m*/z for $C_{18}H_{27}NO_5Na$ $(M+Na)^+$: calcd 360.1781, found 360.1774; **HPLC:** Chiralpak-IA (0.46 mm ϕ X 250 mmL), 25% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 4.8 min.

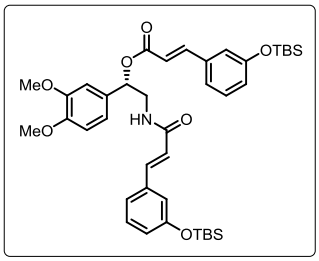
(*S*)-2-((*E*)-3-(2-((*tert*-Butyldimethylsilyl)oxy)phenyl)acrylamido)-1-(3,4-dimethoxyphenyl) ethyl(*E*)-3-(2-((*tert*-butyldimethylsilyl)oxy)phenyl)acrylate (46)



Light yellow liquid; R_f = 0.61 (EtOAc-petroleum ether, 2:3); $[\alpha]_D^{26}$ +7.8 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3437, 3021, 2930, 2858, 2402, 1715, 1666, 1628, 1516, 1470, 1260, 1216, 1157, 1031, 922, 841, 762, 669 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.13 (d, *J* = 16.1 Hz, 1H), 7.96 (d, *J* = 15.9 Hz, 1H), 7.56 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.49 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.25-7.18 (m, 2H), 7.02-6.78 (m, 7H), 6.46 (d, *J* = 16.1 Hz, 1H), 6.36 (d, *J* = 15.7 Hz, 1H), 6.02-5.94 (m, 1H), 5.85 (t, *J* = 4.8 Hz, 1H), 3.95-3.70 (m, 8H), 1.10-0.96 (m, 18H), 0.28-0.12 (m, 12H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.5, 166.1, 154.7, 154.6, 149.1, 149.1, 140.9, 137.0, 131.6, 130.7, 130.4, 130.3, 127.6, 127.5, 126.3, 126.2, 125.7, 121.6, 121.4, 120.2, 120.1, 120.1, 119.9, 119.0, 117.2, 111.1, 109.8, 74.6, 56.1, 55.9, 49.2, 44.6, 34.0,

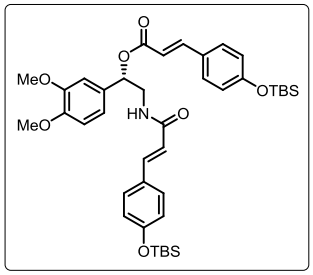
25.8, 25.8, 24.9, 18.3, -4.2, -4.2; **HRMS:** *m/z* for $C_{28}H_{27}NO_5Na$ $(M+Na)^+$: calcd 740.3409, found 740.3381; **HPLC:** Chiralpak-IB (0.46 mm ϕ X 250 mmL), 20% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 7.9 min.

(*S*)-2-((*E*)-3-(3-((*tert*-Butyldimethylsilyl)oxy)phenyl)acrylamido)-1-(3,4-dimethoxyphenyl) ethyl(*E*)-3-(3-((*tert*-butyldimethylsilyl)oxy)phenyl)acrylate (47)



Viscous liquid; R_f = 0.28 (EtOAc-petroleum ether, 3:7); $[\alpha]_D^{27}$ +25.4 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3438, 3020, 2938, 2859, 2403, 1713, 1632, 1590, 1516, 1475, 1257, 1218, 1162, 1024, 850, 761, 670 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.66 (d, *J* = 15.9 Hz, 1H), 7.56 (d, *J* = 15.6 Hz, 1H), 7.29-7.17 (m, 2H), 7.15-7.07 (m, 2H), 7.03-6.93 (m, 4H), 6.91-6.79 (m, 3H), 6.45 (d, *J* = 15.9 Hz, 1H), 6.33 (d, *J* = 15.6 Hz, 1H), 5.99 (brs, 2H), 3.95-3.83 (m, 8H), 0.99 (s, 18H), 0.20 (s, 12H); **^{13}C NMR (125 MHz, $CDCl_3$)** δ 166.5, 165.9, 156.1, 156.0, 149.2, 149.2, 145.7, 141.5, 136.1, 135.6, 130.2, 129.9, 129.7, 122.4, 121.5, 121.0, 120.4, 119.5, 119.3, 119.1, 117.7, 111.2, 109.9, 74.7, 56.0, 55.9, 44.6, 34.0, 25.7, 18.2, -4.4; **HRMS:** *m/z* for $C_{28}H_{27}NO_5Na$ $(M+Na)^+$: calcd 740.3409, found 740.3384; **HPLC:** Chiralpak-IB (0.46 mm ϕ X 250 mmL), 20% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 4.2 min.

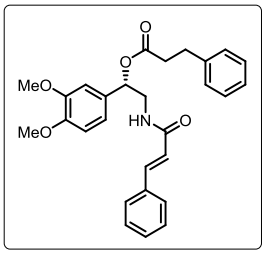
(*S*)-2-((*E*)-3-(4-((*tert*-Butyldimethylsilyl)oxy)phenyl)acrylamido)-1-(3,4-dimethoxyphenyl) ethyl(*E*)-3-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)acrylate (48)



Colorless solid; **m.p.:** 63-65 °C; R_f = 0.45 (EtOAc-petroleum ether, 2:3); $[\alpha]_D^{27}$ +38.1 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3328, 3019, 2940, 2858, 2405, 1707, 1663, 1602, 1514, 1462, 1424, 1266, 1217, 1160, 1101, 1029, 911, 840, 760, 670 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$)**

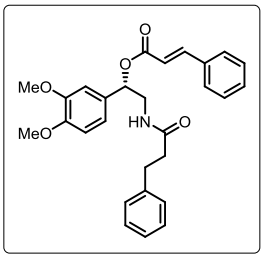
δ 7.67 (d, J = 15.9 Hz, 1H), 7.56 (d, J = 15.3 Hz, 1H), 7.41 (m, 2H), 7.37 (m, 2H), 6.99 (dd, J = 1.5, 8.2 Hz, 1H), 6.96-6.94 (m, 1H), 6.89-6.78 (m, 5H), 6.35 (d, J = 15.9 Hz, 1H), 6.23 (d, J = 15.6 Hz, 1H), 5.98 (dd, J = 8.2, 4.6 Hz, 1H), 5.94 (t, J = 5.5 Hz, 1H), 3.93-3.83 (m, 8H), 0.98 (m, 18H), 0.21 (m, 12H); **^{13}C NMR (125 MHz, $CDCl_3$)** δ 166.8, 166.3, 158.1, 157.4, 149.1, 145.5, 141.2, 130.4, 129.8, 129.4, 128.0, 127.5, 120.5, 120.4, 119.0, 118.1, 115.2, 111.2, 109.9, 74.5, 56.0, 55.9, 44.7, 34.0, 25.6, 24.9, 18.2, -4.4; **HRMS:** *m*/*z* for $C_{28}H_{27}NO_5Na$ $(M+Na)^+$: calcd 740.3409, found 740.3384; **HPLC:** Chiralpak-IB (0.46 mm ϕ X 250 mmL), 20% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 8.8 min.

(*S*)-2-Cinnamamido-1-(3,4-dimethoxyphenyl)ethyl 3-phenylpropanoate (49)



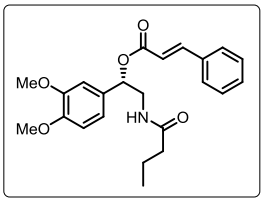
Colorless solid; **m.p.:** 101-103 °C; R_f = 0.34 (EtOAc-petroleum ether, 1:1); **$[\alpha]_D^{29}$** -13.9 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3444, 3021, 2925, 2858, 2358, 1712, 1670, 1515, 1457, 1371, 1318, 1216, 1163, 1030, 928, 857, 763, 670 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.70 (d, J = 16.0 Hz, 1H), 7.57-7.46 (m, 2H), 7.45-7.34 (m, 3H), 7.33-7.09 (m, 5H), 6.98-6.77 (m, 3H), 6.45 (d, J = 15.9 Hz, 1H), 5.87 (dd, J = 7.7, 5.2 Hz, 1H), 5.72 (t, J = 5.3 Hz, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.77-3.64 (m, 2H), 3.01-2.88 (m, 2H), 2.55-2.41 (m, 2H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 172.3, 166.4, 149.2, 149.1, 145.8, 140.7, 134.2, 130.6, 130.2, 129.0, 128.6, 128.3, 128.2, 126.3, 119.0, 117.6, 111.1, 109.7, 74.7, 62.6, 56.0, 55.9, 44.4, 38.3, 31.5; **HRMS:** *m*/*z* for $C_{28}H_{29}NO_5Na$ $(M+Na)^+$: calcd 482.1938, found 482.1932; **HPLC:** Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 7.7 min.

(*S*)-1-(3,4-Dimethoxyphenyl)-2-(3-phenylpropanamido)ethyl cinnamate (50)



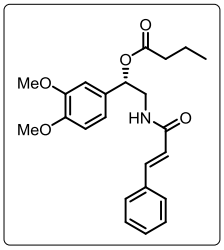
Colorless solid; **m.p.:** 112-114 °C; R_f = 0.45 (EtOAc-petroleum ether, 1:1); **$[\alpha]_D^{26}$** +27.2 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3434, 3020, 2926, 2855, 2403, 1733, 1668, 1625, 1514, 1457, 1334, 1217, 1152, 1030, 859, 760, 669 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.61 (d, *J* = 15.7 Hz, 1H), 7.54-7.44 (m, 2H), 7.42-7.33 (m, 3H), 7.30-7.14 (m, 5H), 6.94-6.78 (m, 3H), 6.24 (d, *J* = 15.7 Hz, 1H), 5.86 (dd, *J* = 8.3, 4.7 Hz, 1H), 5.64 (t, *J* = 5.4 Hz, 1H), 3.87 (s, 3H), 3.87 (s, 3H), 3.82-3.57 (m, 2H), 3.04-2.89 (m, 2H), 2.79-2.64 (m, 2H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 172.3, 165.9, 149.2, 149.1, 141.6, 140.3, 134.7, 130.1, 129.8, 128.9, 128.6, 128.3, 127.9, 126.4, 120.2, 119.0, 111.1, 109.8, 74.6, 56.0, 44.5, 35.8, 30.8; **HRMS:** *m*/*z* for $C_{28}H_{29}NO_5Na$ $(M+Na)^+$: calcd 482.1938, found 482.1934; **HPLC:** Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 8.1 min.

(*S*)-2-Butyramido-1-(3,4-dimethoxyphenyl)ethyl cinnamate (51)



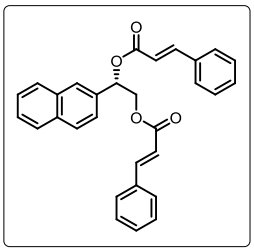
Colorless solid; **m.p.:** 93-94 °C; R_f = 0.45 (EtOAc-petroleum ether, 1:1); **$[\alpha]_D^{26}$** +31.5 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3442, 3020, 2927, 2403, 1732, 1668, 1626, 1515, 1458, 1334, 1255, 1216, 1173, 1031, 929, 858, 762, 669 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.62 (d, *J* = 15.6 Hz, 1H), 7.49 (m, 2H), 7.40-7.33 (m, 3H), 6.96-6.83 (m, 3H), 6.36 (d, *J* = 15.6 Hz, 1H), 5.93-5.86 (m, 2H), 3.89 (s, 3H), 3.87 (s, 3H), 3.85-3.72 (m, 2H), 2.35 (dt, *J* = 7.6, 2.1 Hz, 2H), 1.69-1.63 (m, 2H), 0.92 (t, *J* = 7.3 Hz, 3H); **^{13}C NMR (125 MHz, $CDCl_3$)** δ 173.2, 165.9, 149.2, 149.1, 141.6, 134.7, 130.3, 129.8, 128.8, 127.8, 120.2, 119.0, 111.2, 109.8, 77.3, 77.0, 76.8, 74.2, 56.0, 55.9, 44.6, 36.4, 18.4, 13.7; **HRMS:** *m*/*z* for $C_{23}H_{27}NO_5Na$ $(M+Na)^+$: calcd 420.1781, found 420.1774; **HPLC:** Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 254 nm, t_R = 6.3 min.

(*S*)-2-Cinnamamido-1-(3,4-dimethoxyphenyl)ethyl butyrate (52)



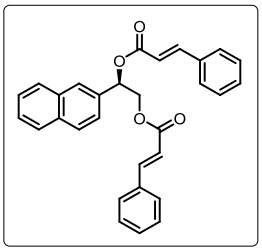
Colorless liquid; R_f = 0.28 (EtOAc-petroleum ether, 1:1); **[α]$_D^{23}$** -2.9 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3446, 3388, 3018, 2966, 1712, 1605, 1516, 1457, 1317, 1257, 1217, 1165, 927, 861, 761, 670 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.72 (d, *J* = 16.1 Hz, 1H), 7.53 (m, 2H), 7.44-7.36 (m, 3H), 6.99-6.95 (dd, *J* = 1.7, 8.1 Hz, 1H), 6.93 (d, *J* = 1.7 Hz, 1H), 6.86 (d, *J* = 8.1 Hz, 1H), 6.49 (d, *J* = 15.9 Hz, 1H), 5.94 (t, *J* = 6.5 Hz, 1H), 5.85-5.74 (m, 1H), 3.95-3.80 (m, 8H), 2.18-2.11 (m, 2H), 1.63 (m, 2H), 0.94-0.90 (m, 3H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 173.1, 166.5, 149.2, 149.1, 145.8, 134.2, 130.6, 130.2, 129.0, 128.2, 119.0, 117.6, 111.1, 109.8, 74.7, 55.9, 44.3, 38.7, 19.1, 13.7; **HRMS:** *m*/*z* for $C_{28}H_{27}NO_5Na$ $(M+Na)^+$: calcd 420.1781, found 420.1762; **HPLC:** Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 14.8 min.

(*S*)-1-(Naphthalen-2-yl)ethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (53)



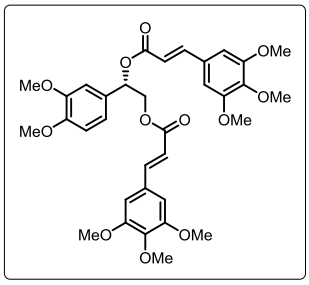
Colorless solid; **m.p.:** 120-121 °C; R_f = 0.48 (EtOAc-petroleum ether, 1:4); **[α]$_D^{28}$** -19.5 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3290, 3022, 2926, 2862, 2404, 2358, 1714, 1637, 1524, 1422, 1318, 1216, 1166, 1030, 929, 763, 671 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 8.05-7.30 (m, 19H), 6.68-6.32 (m, 3H), 4.78-4.51 (m, 2H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.7, 166.2, 145.8, 145.7, 134.4, 134.2, 133.5, 133.3, 130.6, 130.5, 129.0, 129.0, 128.7, 128.3, 128.2, 127.8, 126.5, 126.3, 124.3, 117.8, 117.6, 73.8, 66.3; **ESI-LCMS:** *m*/*z* 471.19 $(M+Na)^+$; **HRMS:** *m*/z for $C_{30}H_{24}O_4Na$ $(M+Na)^+$: calcd 471.1567, found 471.1566; **HPLC:** *ee* 100% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 12.5 min].

(*R*)-1-(Naphthalen-2-yl)ethane-1,2-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (54)



Colorless solid; **m.p.:** 123-124 °C; R_f = 0.48 (EtOAc-petroleum ether, 1:4); $[\alpha]_D^{28}$ +23.9 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3290, 3022, 2926, 2862, 2404, 2358, 1714, 1637, 1524, 1422, 1318, 1216, 1166, 1030, 929, 763, 671 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.96-7.30 (m, 19H), 6.65-6.34 (m, 3H), 4.76-4.53 (m, 2H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 166.7, 166.2, 145.8, 145.7, 134.4, 134.2, 133.5, 133.3, 130.6, 130.5, 129.0, 129.0, 128.7, 128.3, 128.2, 127.8, 126.5, 126.3, 124.3, 117.8, 117.6, 73.8, 66.3; **ESI-LCMS:** *m*/*z* 471.16 $(M+Na)^+$; **HRMS:** *m*/z for $C_{30}H_{24}O_4Na$ $(M+Na)^+$: calcd 471.1567, found 471.1570; **HPLC:** *ee* 98.4% [Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 22.8 min].

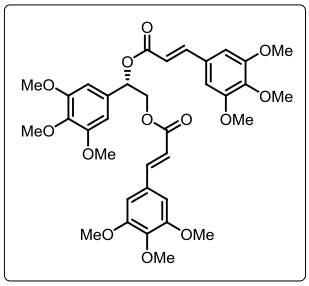
(*S*)-1-(3,4-Dimethoxyphenyl)ethane-1,2-diyl(2*E*,2'*E*)-bis(3-(3,4,5-trimethoxyphenyl) acrylate) (55)



Colorless viscous liquid; R_f = 0.43 (EtOAc-petroleum ether, 1:1); $[\alpha]_D^{27}$ +33.4 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3686, 3021, 2937, 2845, 2403, 1712, 1635, 1585, 1510, 1460, 1423, 1319, 1218, 1139, 1032, 928, 766, 670 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.67-7.59 (m, 2H, two doublets overlapped), 7.04 (dd, *J* = 8.2, 2.3 Hz, 1H), 6.97 (d, *J* = 1.8 Hz, 1H), 6.89 (d, *J* = 8.2 Hz, 1H), 6.75 (s, 2H), 6.74 (s, 2H), 6.43 (d, *J* = 15.6 Hz, 1H), 6.35 (d, *J* = 15.6 Hz, 1H), 6.19 (dd, *J* = 8.7, 3.2 Hz, 1H), 4.62 (dd, *J* = 12.4, 8.9 Hz, 1H), 4.47 (dd, *J* = 11.9, 3.7 Hz, 1H), 3.94-3.84 (m, 26H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.6, 166.1, 153.5, 145.5, 140.4, 129.7, 129.1, 119.4, 117.0, 116.7, 111.3, 110.2, 105.4, 73.5, 66.1, 61.0, 56.2, 56.0, 56.0; **HRMS:** *m*/*z* for $C_{34}H_{38}O_{12}Na$ $(M+Na)^+$: calcd 661.2255, found 661.2252; **HPLC:**

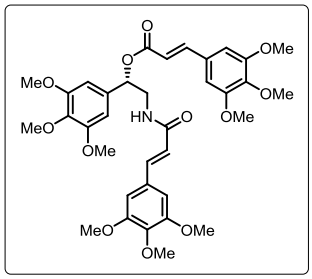
Chiralpak-IB (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 15.1 min.

(*S*)-1-(3,4,5-Trimethoxyphenyl)ethane-1,2-diyl(2*E*,2'*E*)-bis(3-(3,4,5-trimethoxyphenyl) acrylate) (56)



Colorless solid; **m.p.:** 61-62 °C; R_f = 0.43 (EtOAc-petroleum ether, 1:1); **$[\alpha]_D^{29}$** +29.2 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3290, 3021, 2932, 2404, 2358, 1712, 1634, 1589, 1508, 1460, 1421, 1327, 1217, 1135, 1016, 929, 764, 670 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.69-7.58 (m, 2H, two doublets overlapped), 6.76 (s, 2H), 6.74 (s, 2H), 6.68 (s, 2H), 6.44 (d, *J* = 15.6 Hz, 1H), 6.36 (d, *J* = 16.0 Hz, 1H), 6.16 (dd, *J* = 9.2, 3.7 Hz, 1H), 4.61 (dd, *J* = 11.9, 8.7 Hz, 1H), 4.46 (dd, *J* = 11.5, 3.2 Hz, 1H), 3.92-3.82 (m, 29H, 9-OMe and two diastereotopic protons); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.6, 153.5, 145.8, 140.5, 138.4, 132.1, 129.7, 116.8, 116.6, 105.5, 104.1, 73.8, 61.0, 60.8, 56.2; **HRMS:** *m/z* for $C_{35}H_{40}O_{13}Na$ $(M+Na)^+$: calcd 691.2361, found 691.2354; **HPLC:** Chiralpak-IB (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 13.7 min.

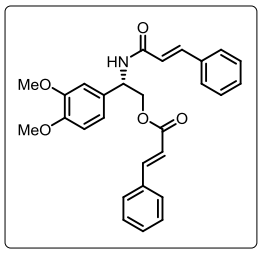
(*S*)-1-(3,4,5-Trimethoxyphenyl)-2-((*E*)-3-(3,4,5-trimethoxyphenyl)acrylamido)ethyl(*E*)-3-(3,4,5-trimethoxyphenyl)acrylate (57)



Colorless solid; **m.p.:** 67-69 °C; R_f = 0.28 (EtOAc-petroleum ether, 7:3); **$[\alpha]_D^{27}$** +46.6 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3686, 3441, 3021, 2937, 2403, 1711, 1629, 1589, 1509, 1462, 1424, 1326, 1217, 1136, 1005, 927, 767, 670 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.71-

7.49 (m, 2H, two doublets overlapped), 6.75 (s, 2H), 6.71 (s, 2H), 6.66 (s, 2H), 6.48-6.24 (m, 2H, two doublets overlapped), 6.08-5.92 (m, 2H, NH and methine protons), 3.96-3.79 (m, 29H, 9-OMe and two diastereotopic protons); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 166.5, 166.0, 153.5, 153.5, 153.4, 146.0, 141.7, 139.7, 138.1, 133.3, 130.2, 129.6, 119.5, 116.6, 105.4, 105.0, 103.7, 74.9, 61.0, 60.8, 56.3, 56.2, 44.7; **ESI-LCMS:** *m/z* 690.25 $(M+Na)^+$; **HRMS:** *m*/z for $C_{35}H_{41}NO_{12}Na$ $(M+Na)^+$: calcd 690.2521, found 690.2516; **HPLC:** Chiralpak-IA (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 10.3 min.

(*S*)-2-Cinnamamido-2-(3,4-dimethoxyphenyl)ethyl cinnamate (58)



Colorless solid; **m.p.:** 117-120 °C; R_f = 0.47 (EtOAc-petroleum ether, 1:1); **$[\alpha]_D^{29}$** +45.9 (*c* 0.5, $CHCl_3$); **IR ($CHCl_3$):** 3366, 3021, 2926, 2858, 2405, 2358, 1708, 1630, 1511, 1457, 1321, 1216, 1168, 1029, 928, 762, 670 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.76-7.57 (m, 2H, two doublets overlapped), 7.55-7.42 (m, 4H), 7.41-7.28 (m, 6H), 7.00-6.79 (m, 3H), 6.58 (d, *J* = 7.8 Hz, 1H), 6.53-6.36 (m, 2H, two doublets overlapped), 5.45 (m, 1H), 4.66 (dd, *J* = 11.6, 7.7 Hz, 1H), 4.43 (dd, *J* = 11.5, 4.7 Hz, 1H), 3.87 (s, 3H), 3.84 (s, 3H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ 167.4, 165.6, 149.2, 148.8, 146.0, 141.7, 134.7, 134.1, 131.0, 130.6, 129.8, 128.9, 128.8, 128.2, 127.9, 120.3, 118.9, 117.2, 111.4, 110.3, 66.2, 55.9, 52.9; **HRMS:** *m/z* for $C_{28}H_{27}NO_5Na$ $(M+Na)^+$: calcd 480.1781, found 480.1776; **HPLC:** Chiralpak-IB (0.46 mm ϕ X 250 mmL), 40% EtOH in hexane, flow rate 1.0 mL min^{-1}, UV detection at 220 nm, t_R = 10.8 min.

2.1.8. REFERENCES

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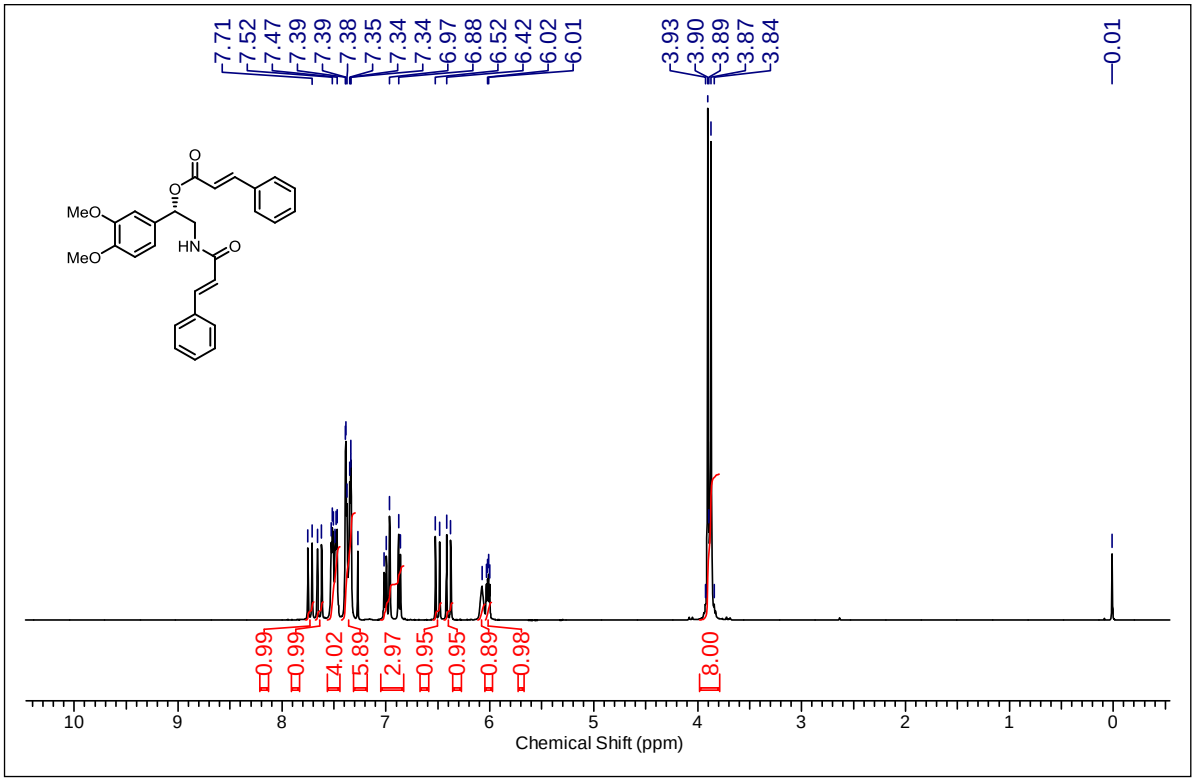
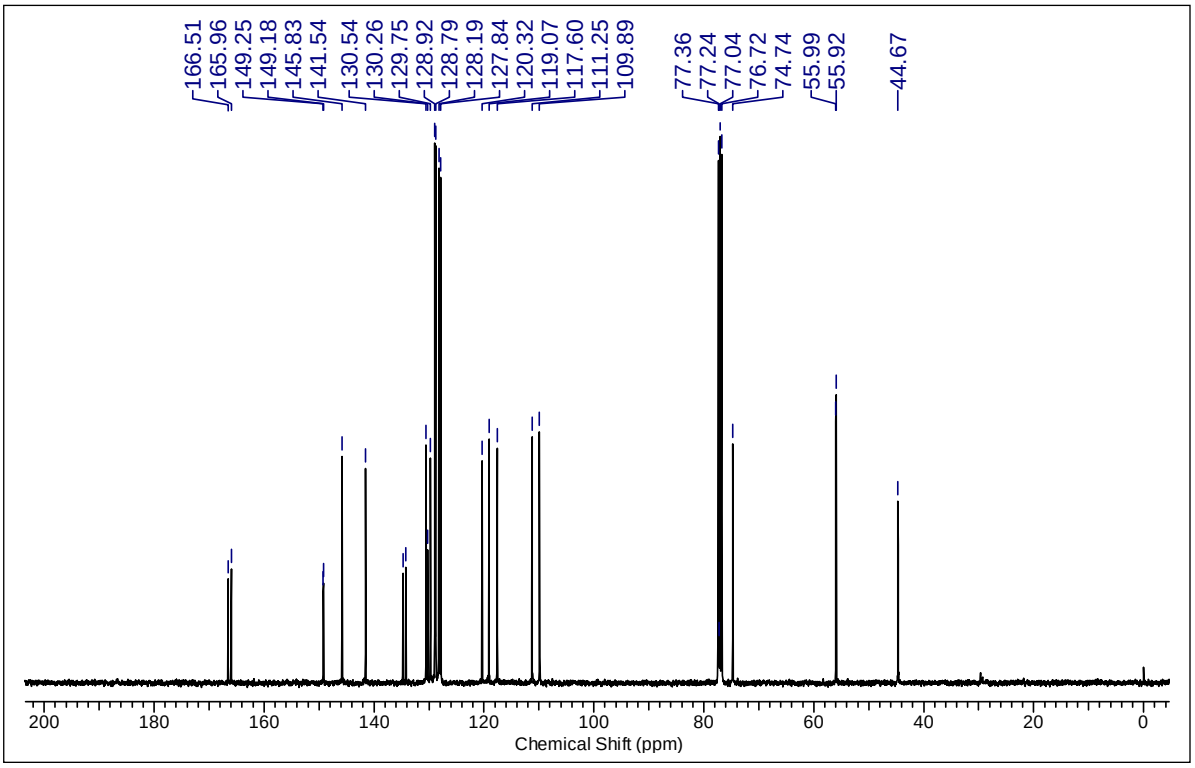
2.1.9. SPECTRA OF SELECTED FINAL COMPOUNDS

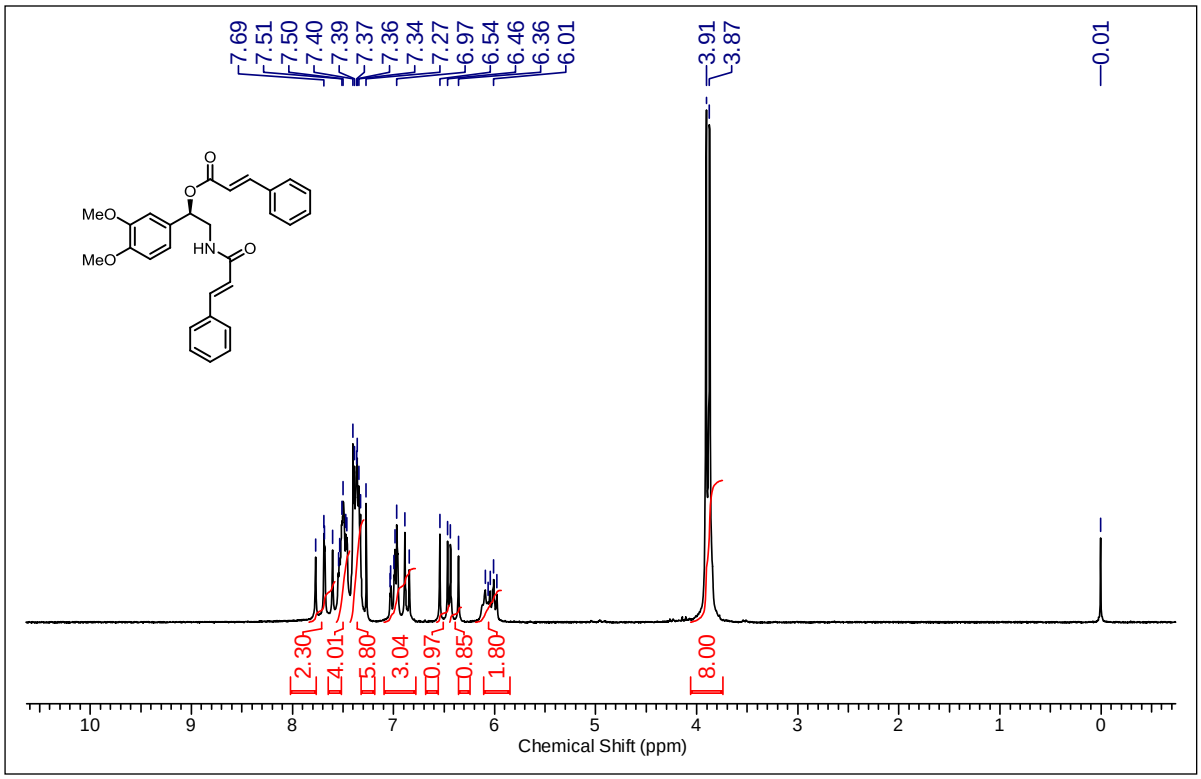
^{1}H-NMR spectrum of 1 (400 MHz, $CDCl_3$)



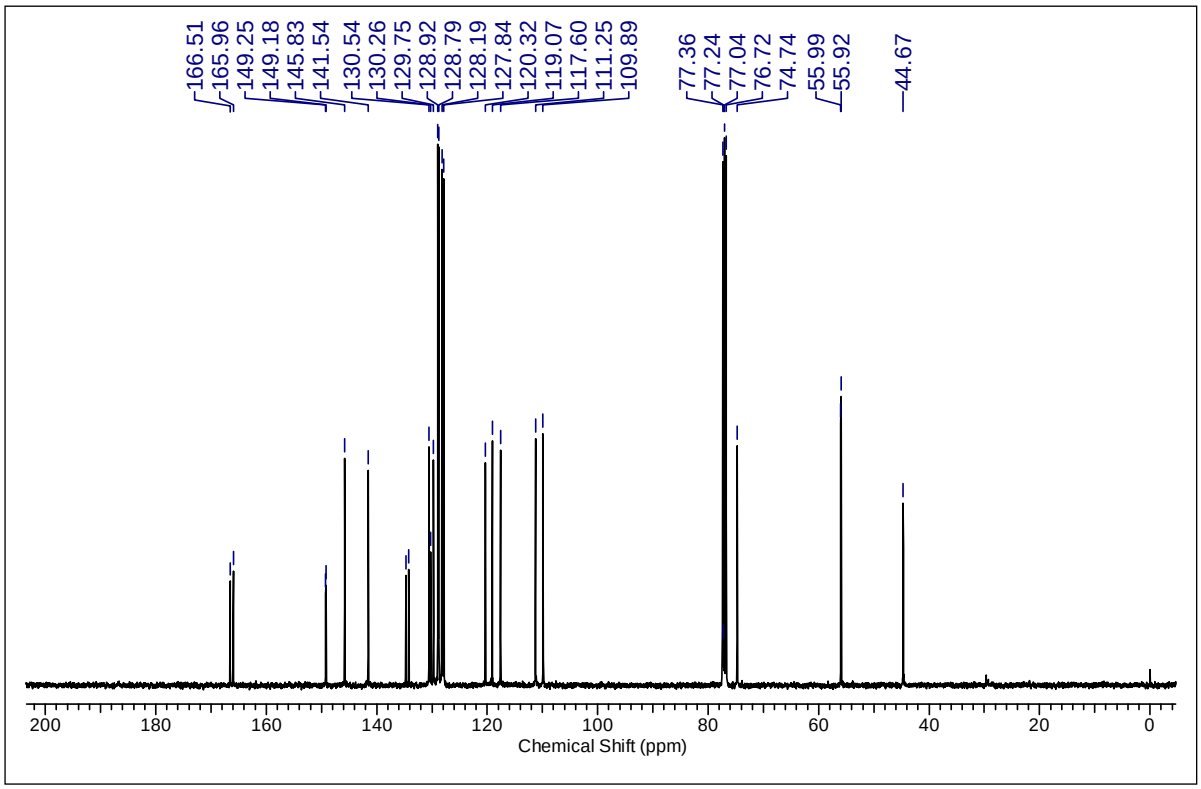
^{13}C-NMR spectrum of 1 (100 MHz, $CDCl_3$)



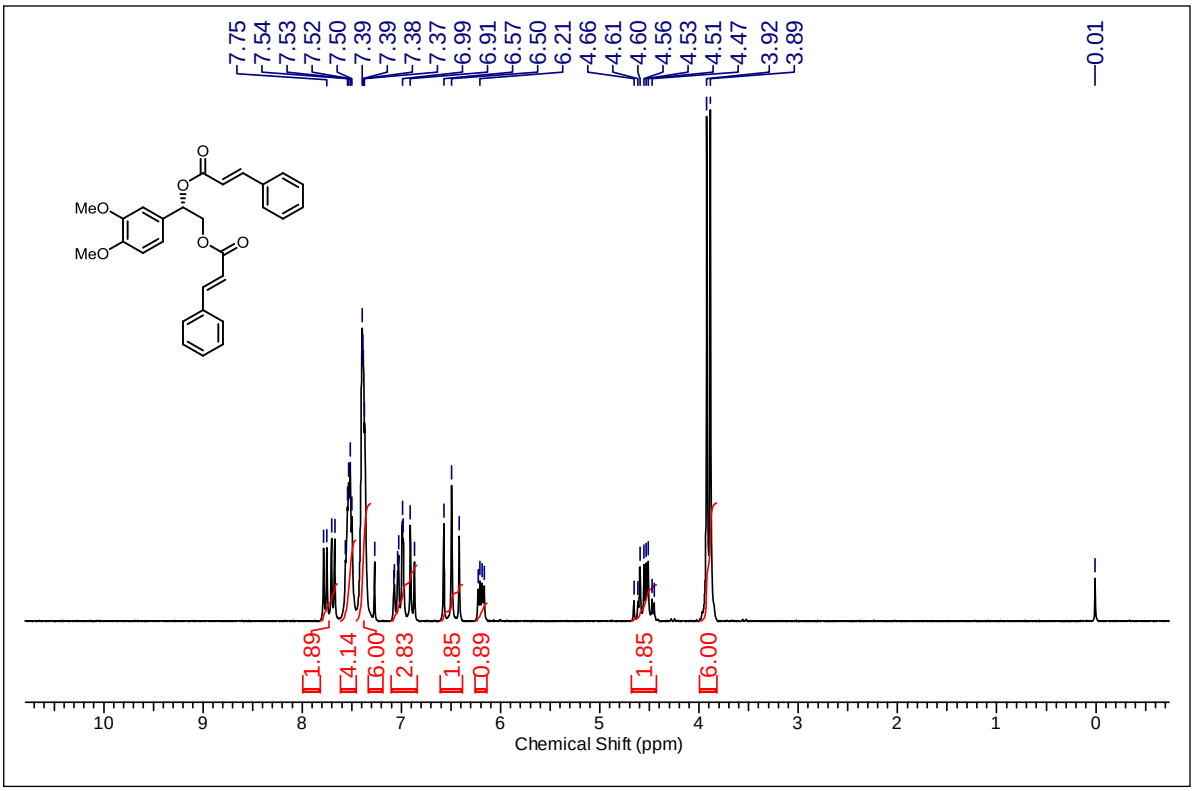
^{1}H-NMR spectrum of 2 (200 MHz, $CDCl_3$)



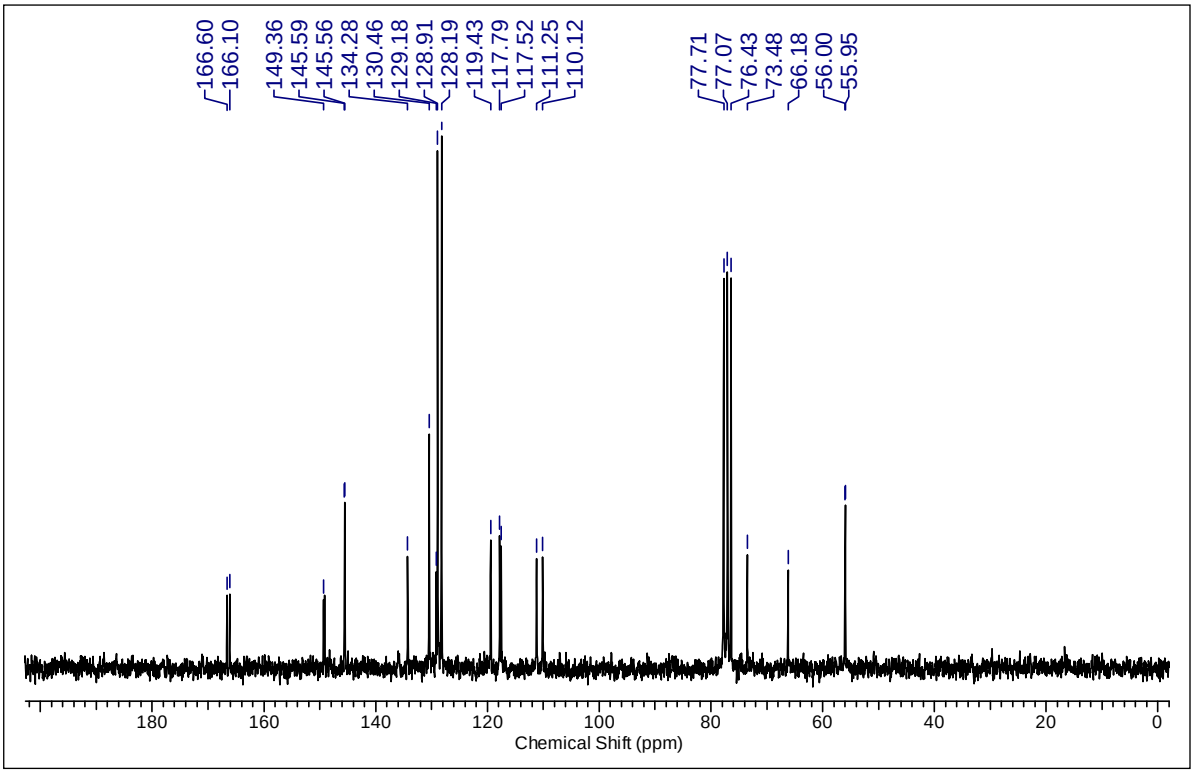
^{13}C-NMR spectrum of 2 (100 MHz, $CDCl_3$)



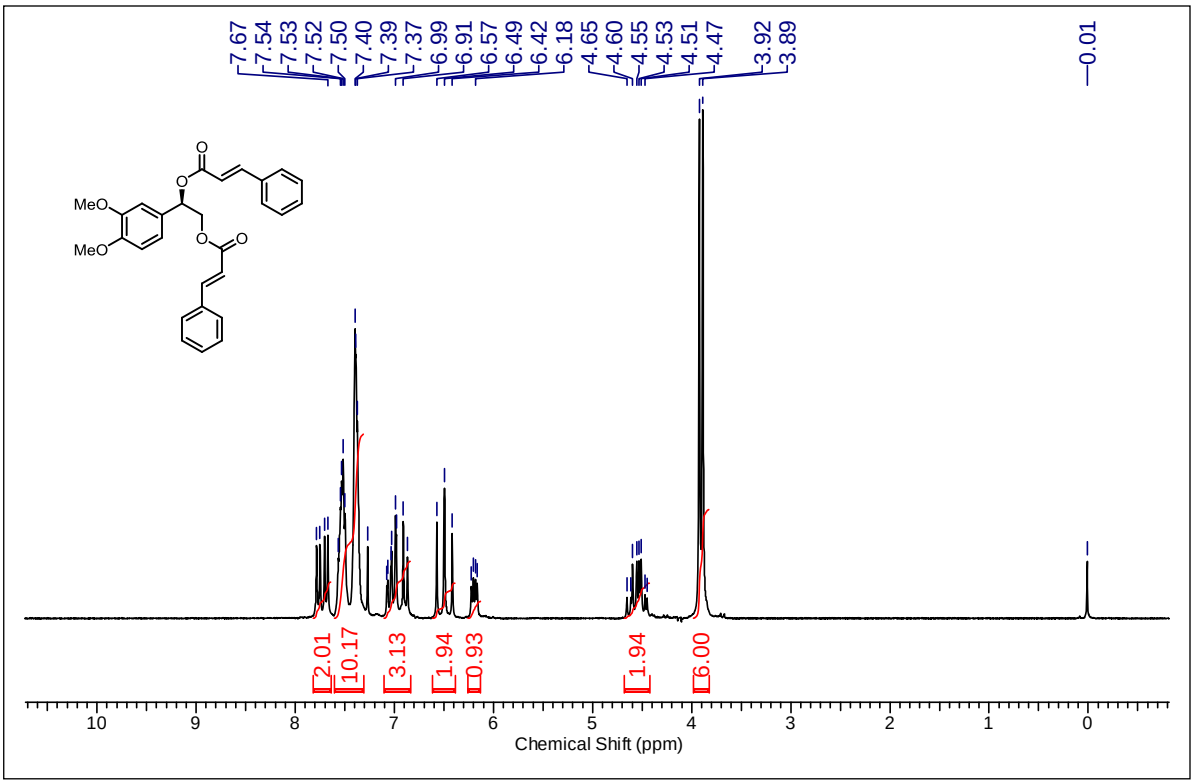
^{1}H-NMR spectrum of 3 (200 MHz, $CDCl_3$)



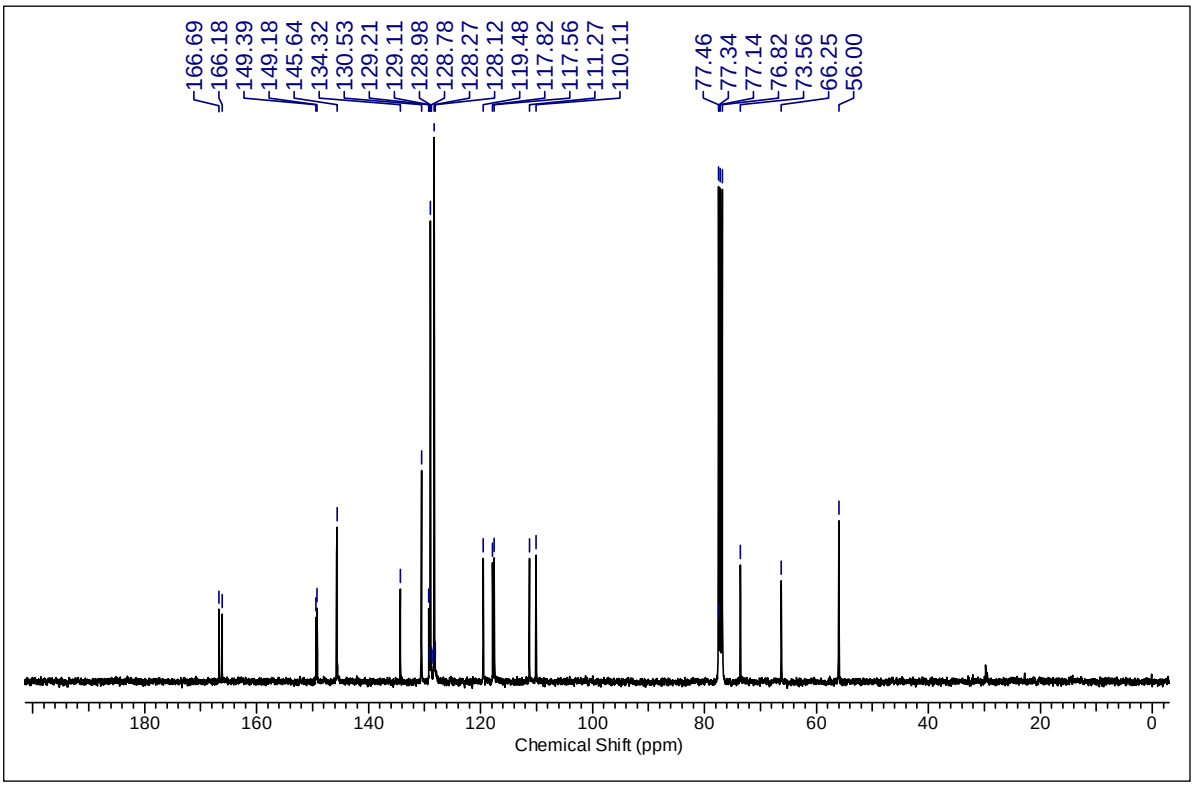
^{13}C-NMR spectrum of 3 (50 MHz, $CDCl_3$)



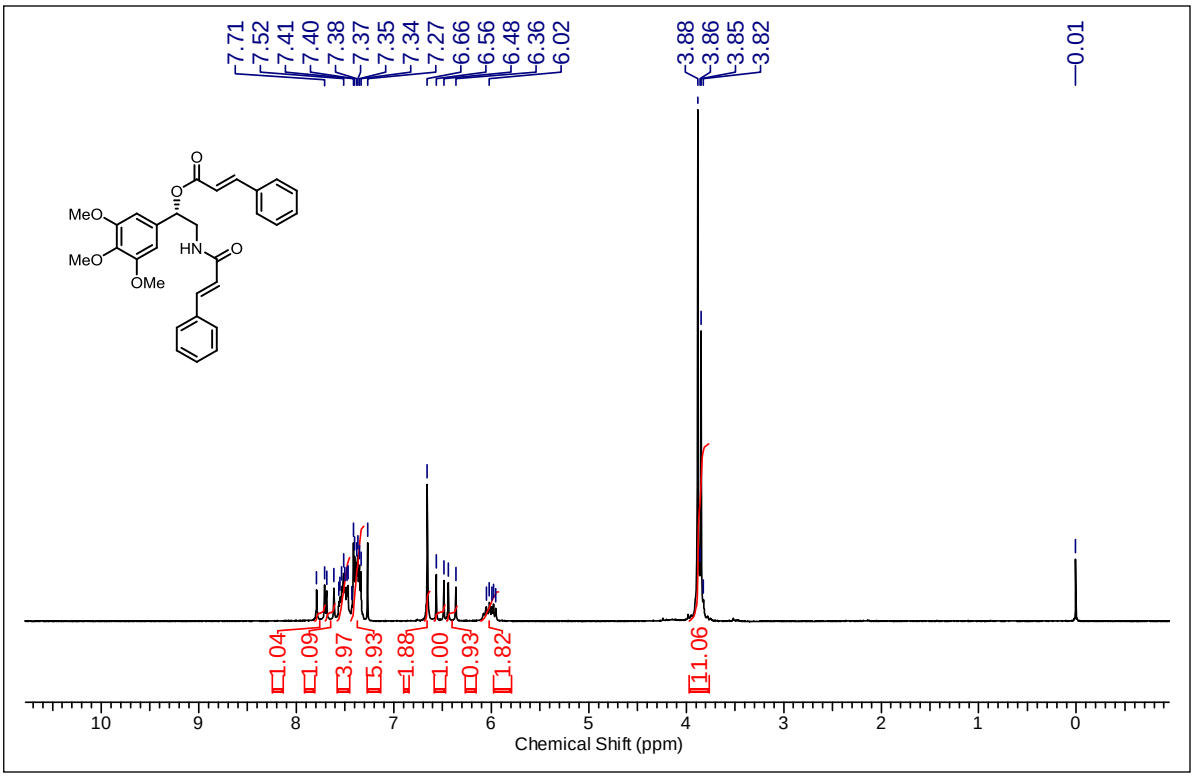
^{1}H-NMR spectrum of 4 (200 MHz, $CDCl_3$)



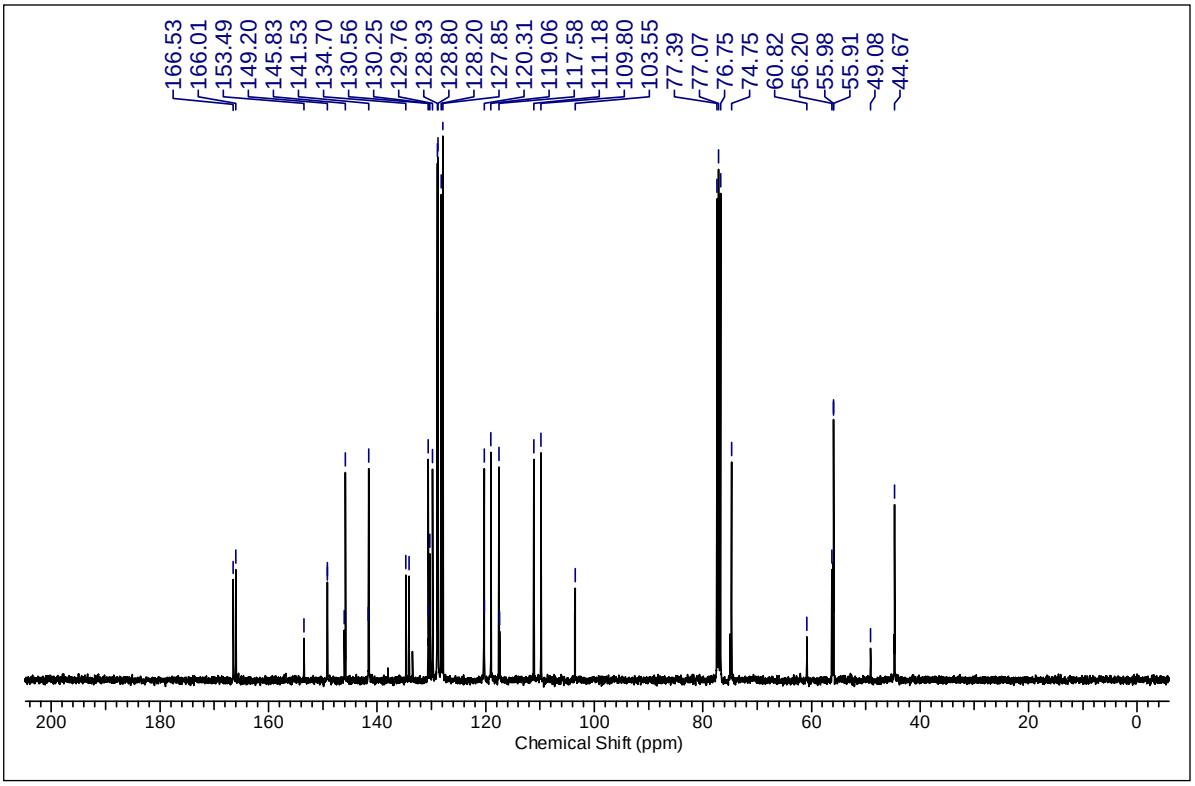
^{13}C-NMR spectrum of 4 (50 MHz, $CDCl_3$)



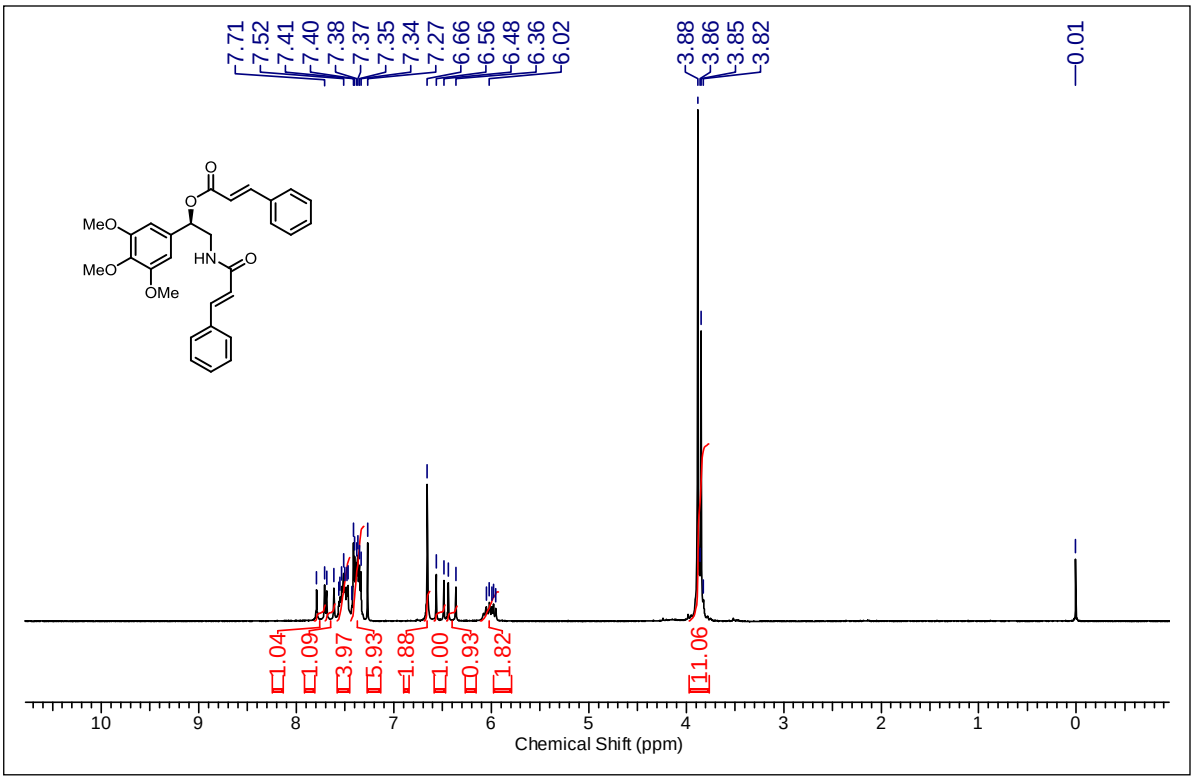
^{1}H-NMR spectrum of 5 (200 MHz, $CDCl_3$)



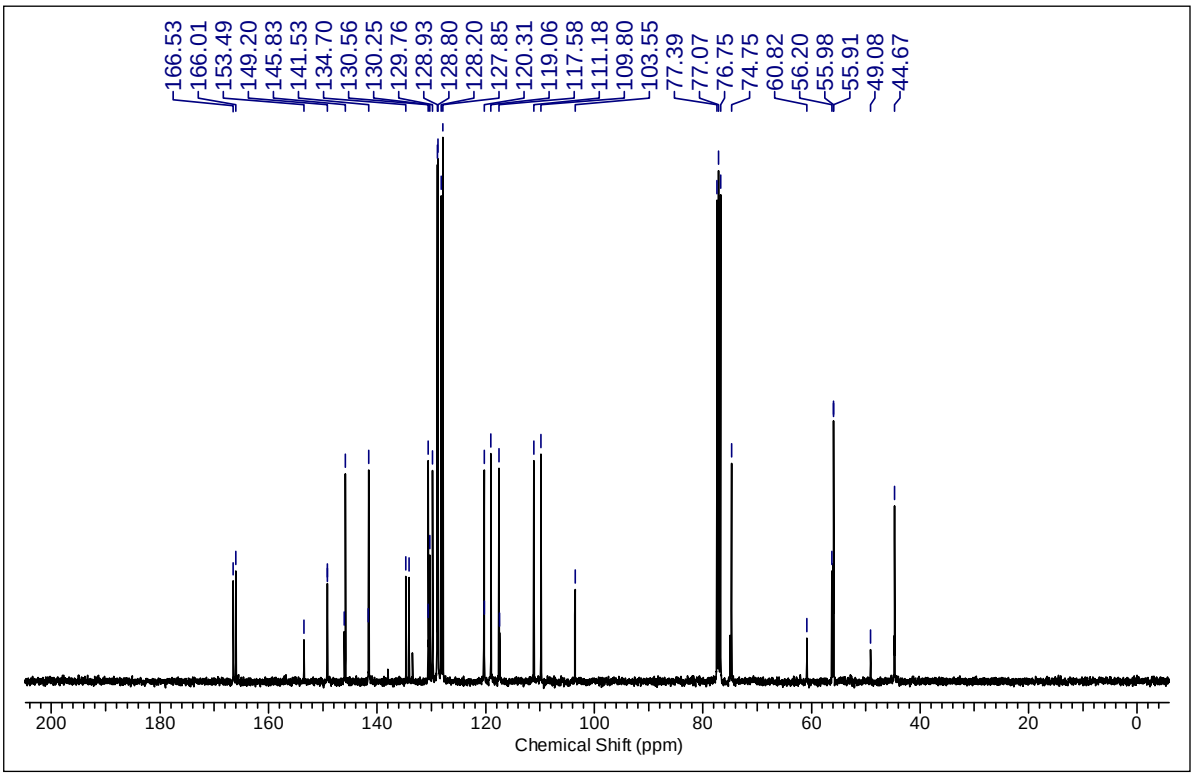
^{13}C-NMR spectrum of 5 (100 MHz, $CDCl_3$)



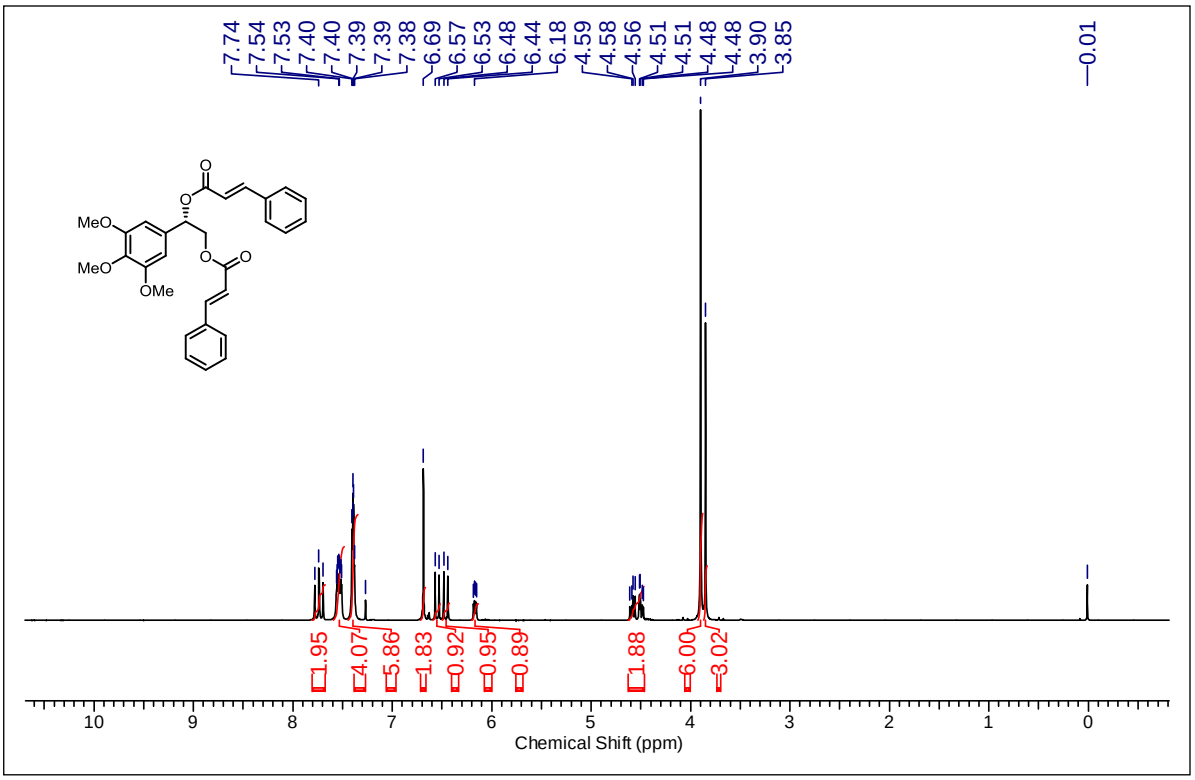
^{1}H-NMR spectrum of 6 (200 MHz, $CDCl_3$)



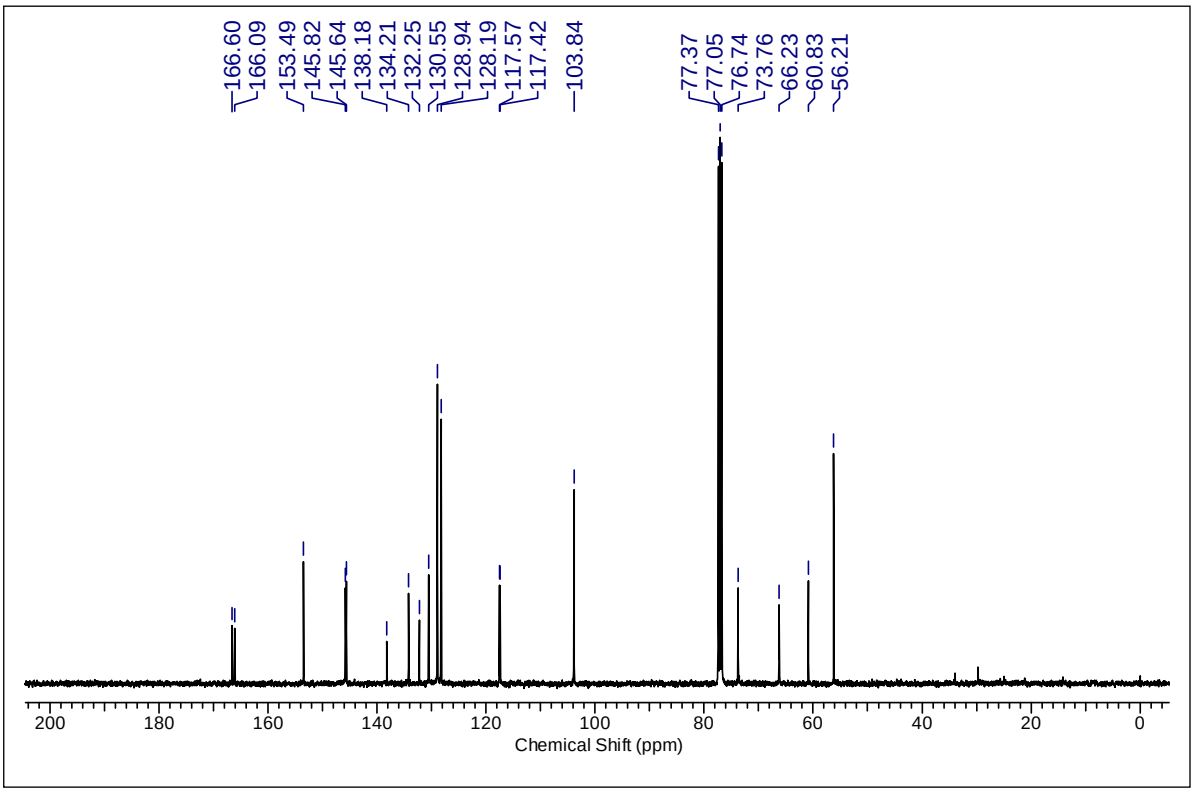
^{13}C-NMR spectrum of 6 (100 MHz, $CDCl_3$)



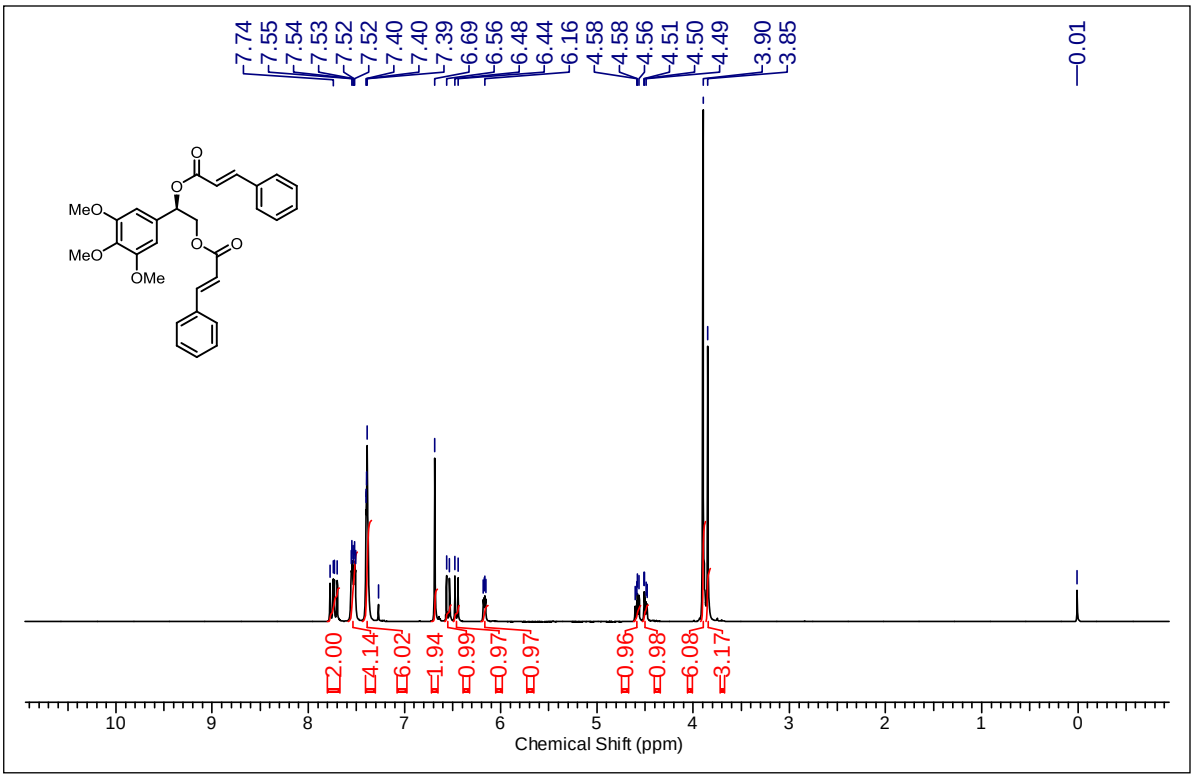
^{1}H-NMR spectrum of 7 (400 MHz, $CDCl_3$)



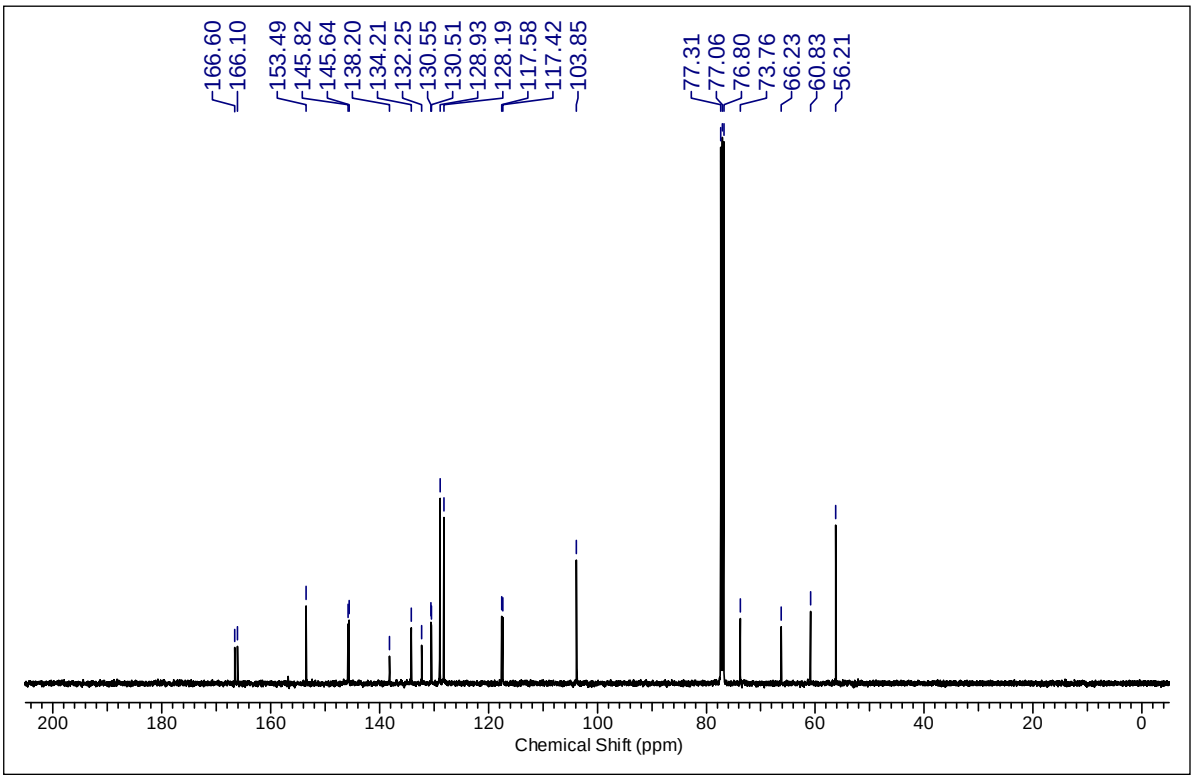
^{13}C-NMR spectrum of 7 (100 MHz, $CDCl_3$)



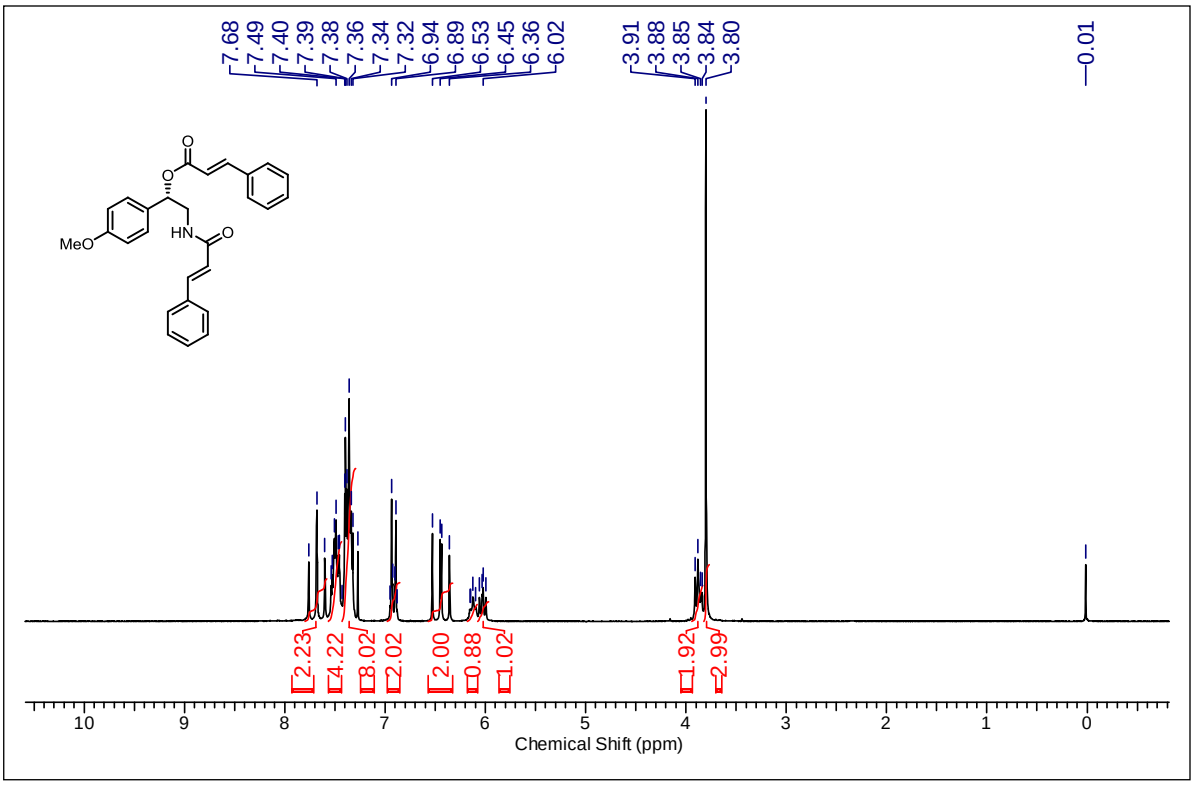
^{1}H-NMR spectrum of 8 (500 MHz, $CDCl_3$)



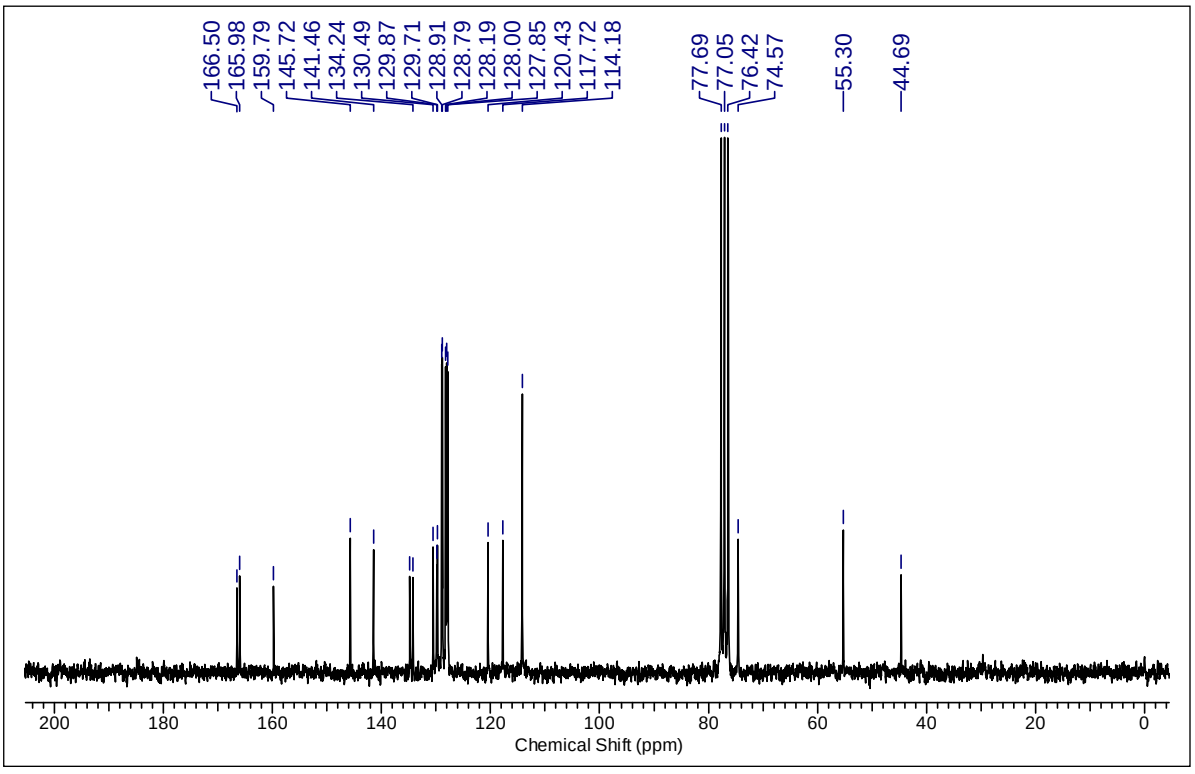
^{13}C-NMR spectrum of 8 (125 MHz, $CDCl_3$)



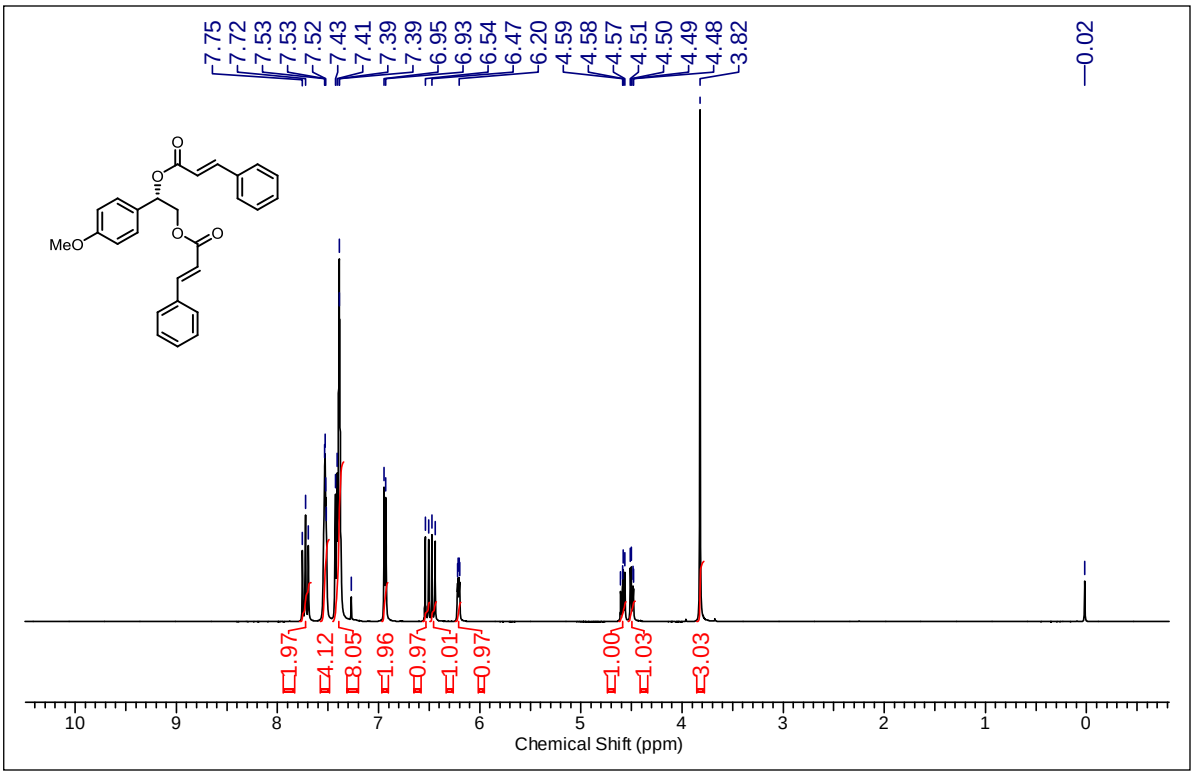
^{1}H-NMR spectrum of 17 (200 MHz, $CDCl_3$)



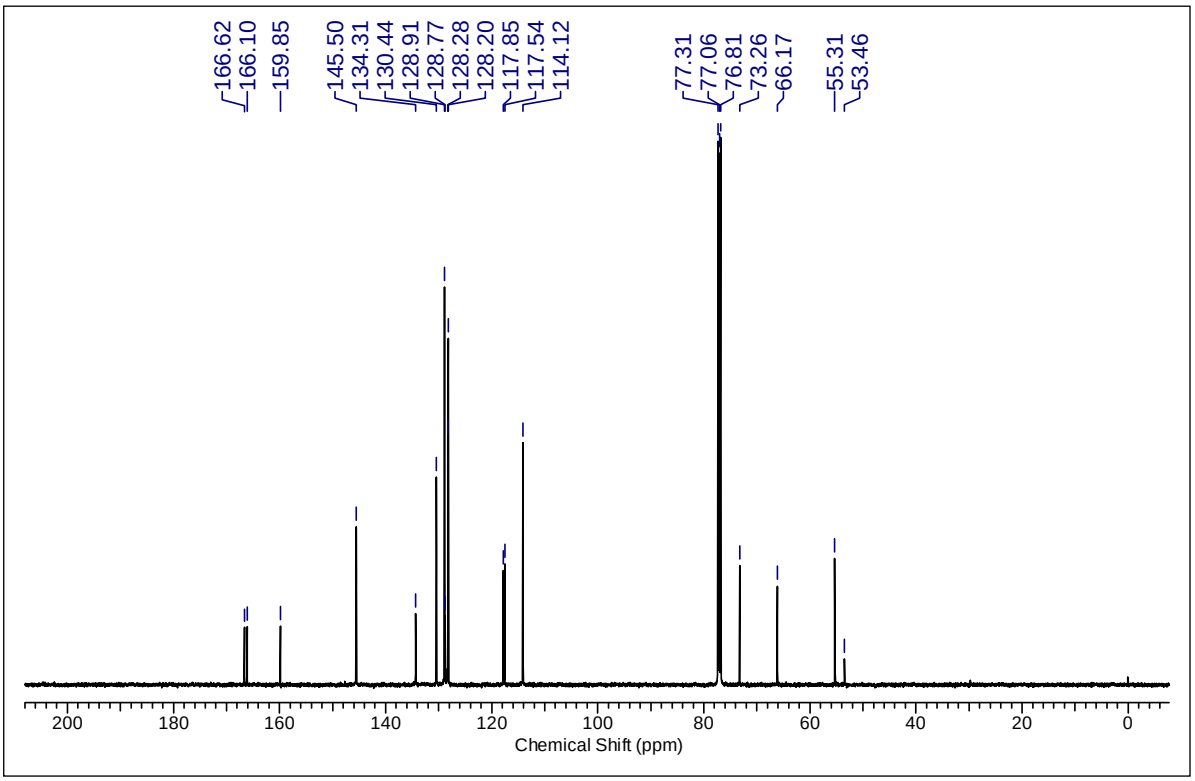
^{13}C-NMR spectrum of 17 (50 MHz, $CDCl_3$)



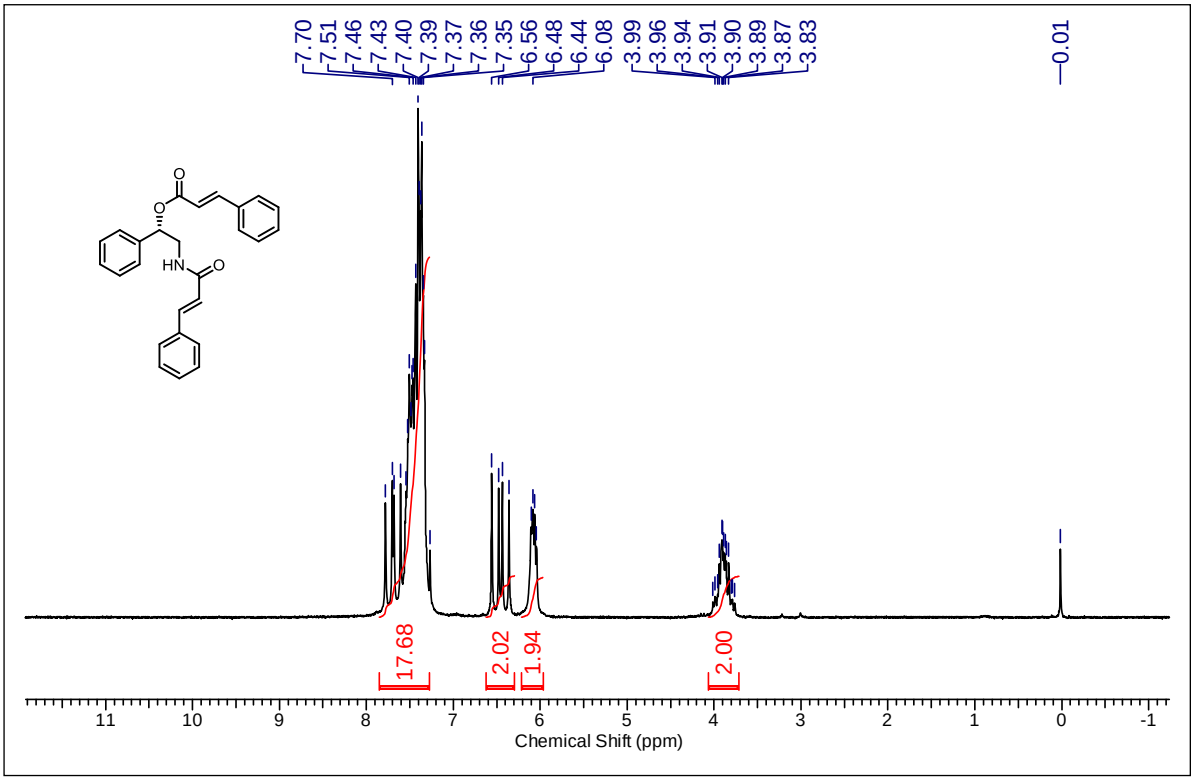
^{1}H-NMR spectrum of 19 (500 MHz, $CDCl_3$)



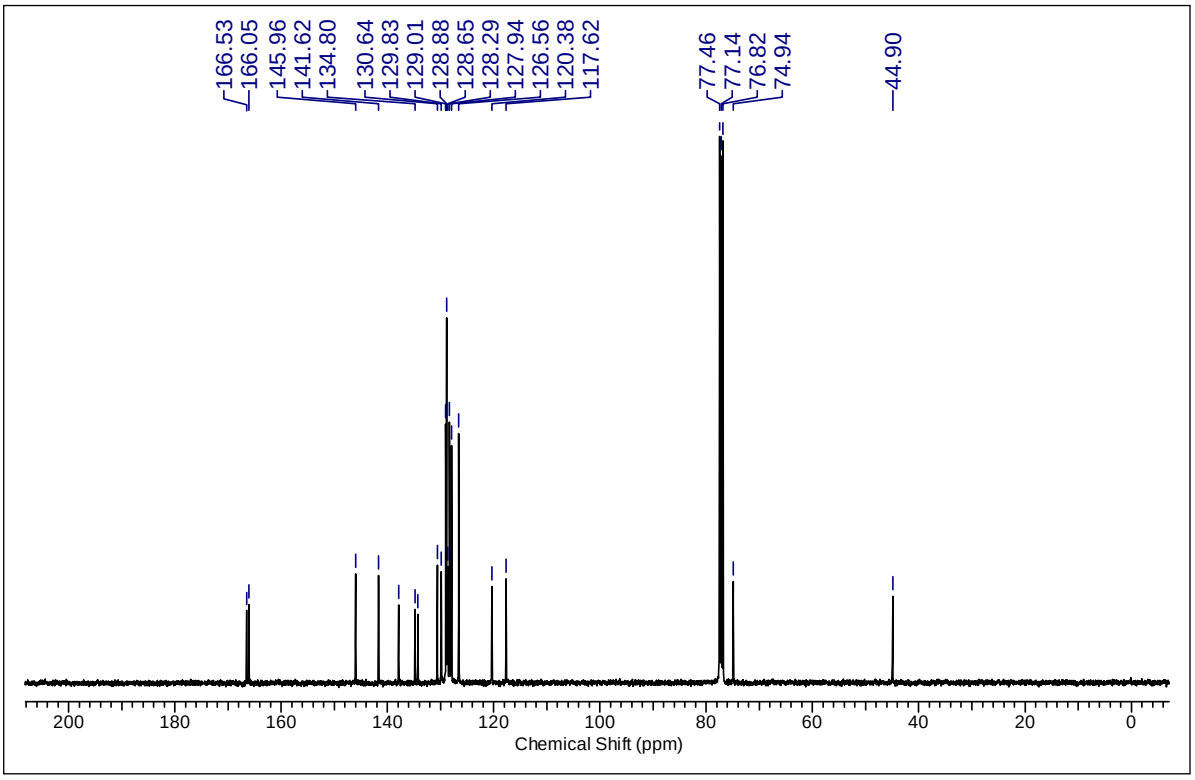
^{13}C-NMR spectrum of 19 (125 MHz, $CDCl_3$)



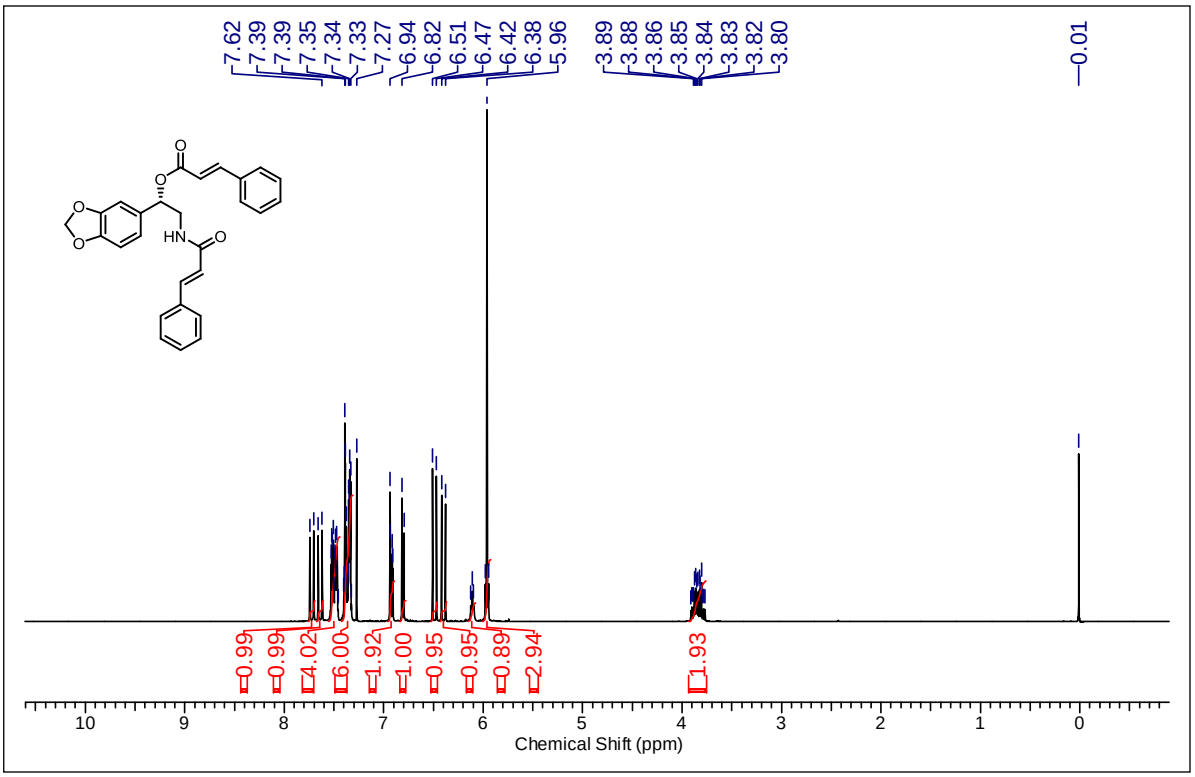
^{1}H-NMR spectrum of 25 (200 MHz, $CDCl_3$)



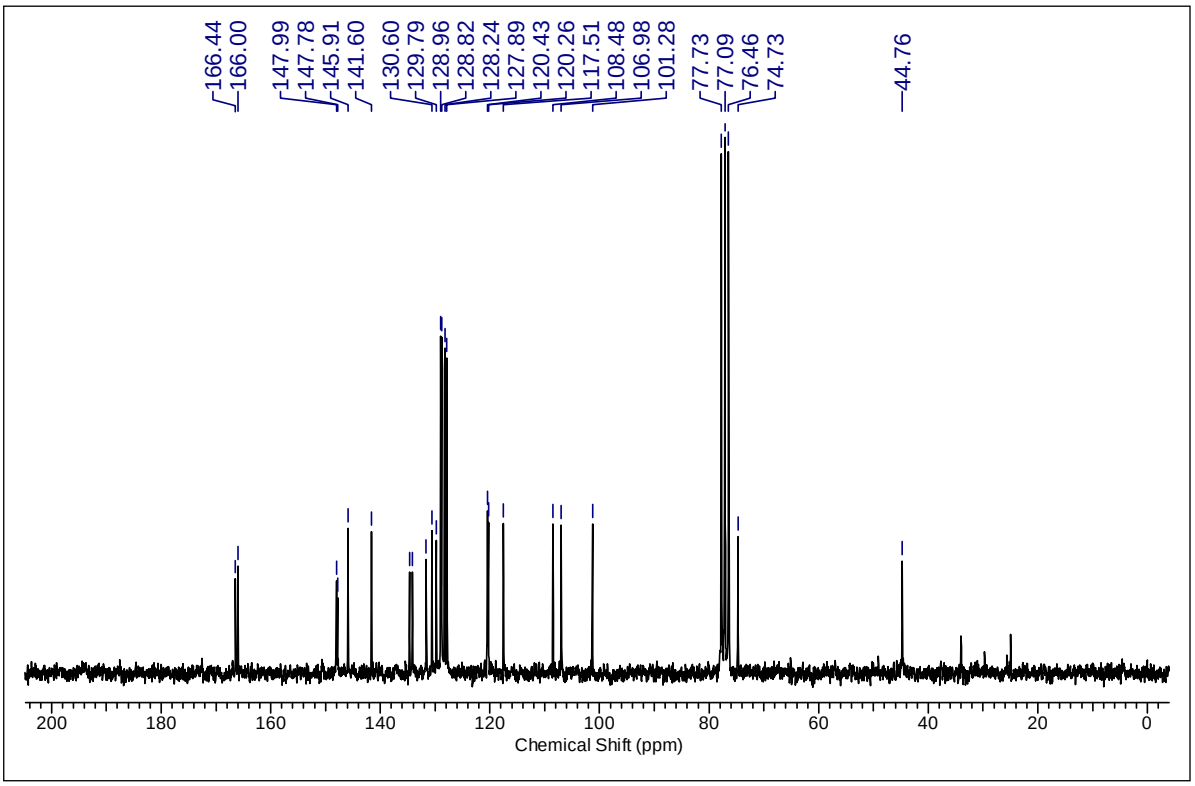
^{13}C-NMR spectrum of 25 (100 MHz, $CDCl_3$)



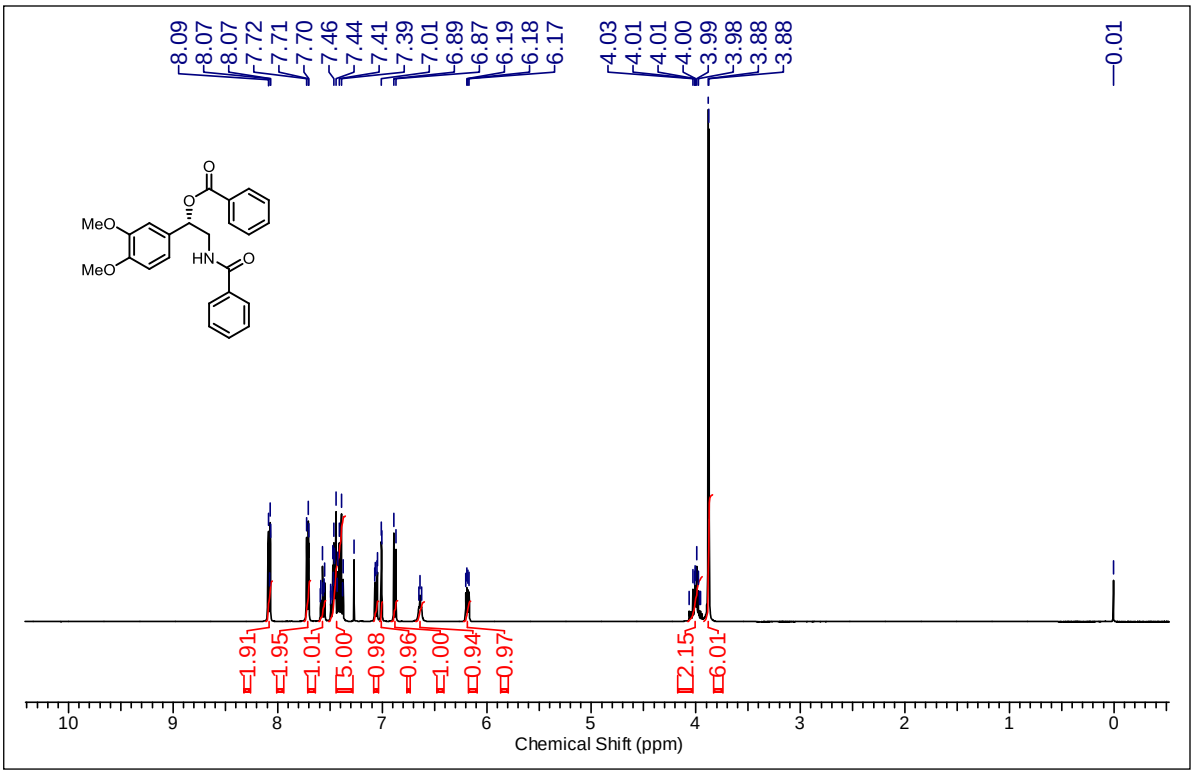
^{1}H-NMR spectrum of 33 (400 MHz, $CDCl_3$)



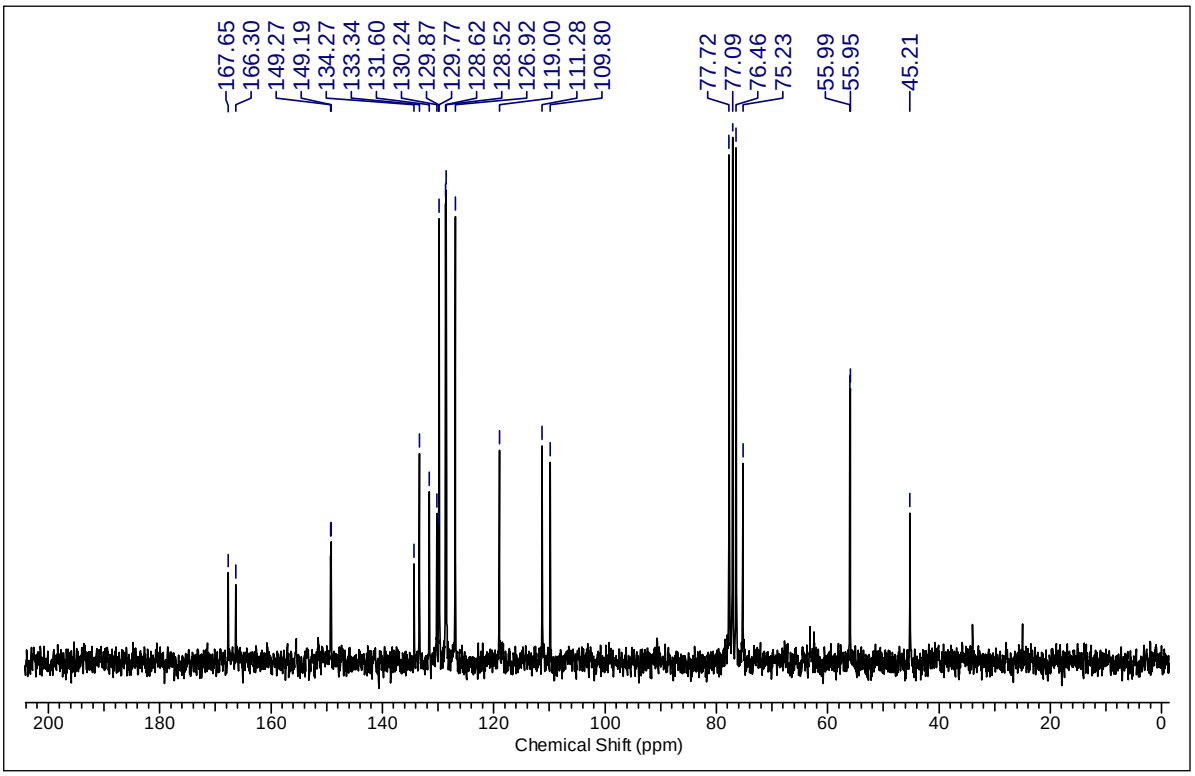
^{13}C-NMR spectrum of 33 (50 MHz, $CDCl_3$)



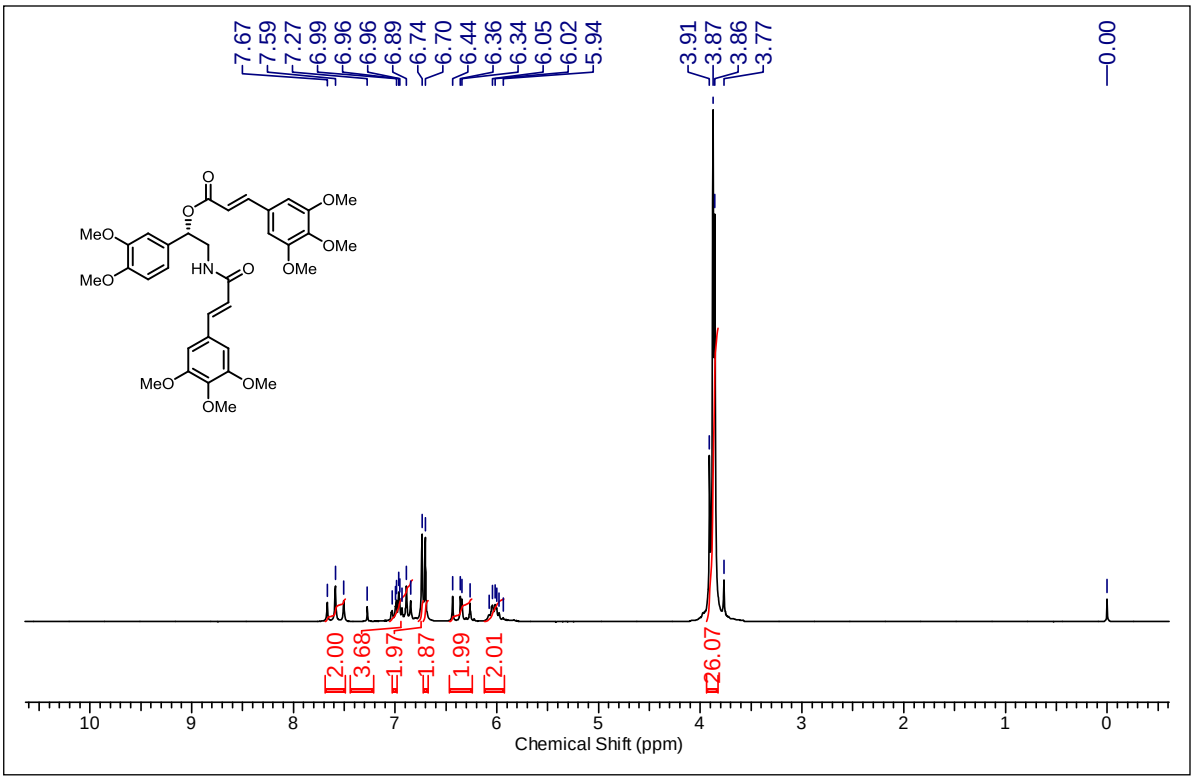
^{1}H-NMR spectrum of 39 (400 MHz, $CDCl_3$)



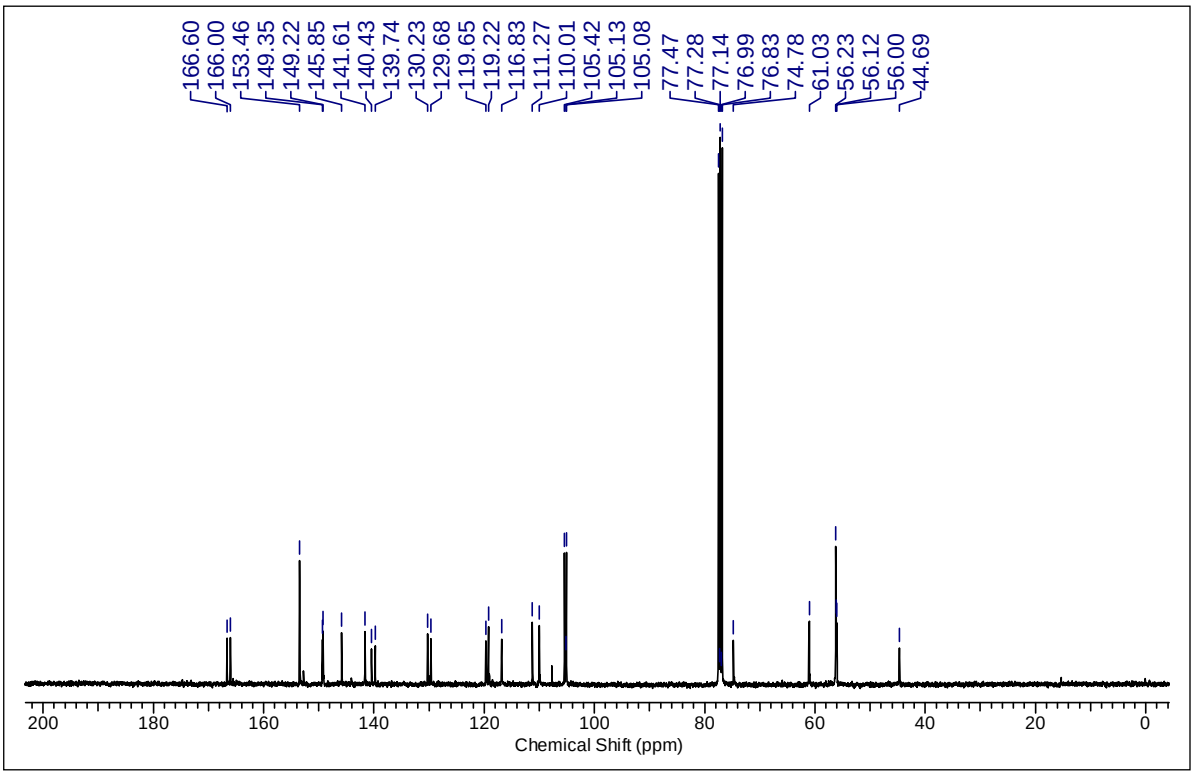
^{13}C-NMR spectrum of 39 (50 MHz, $CDCl_3$)



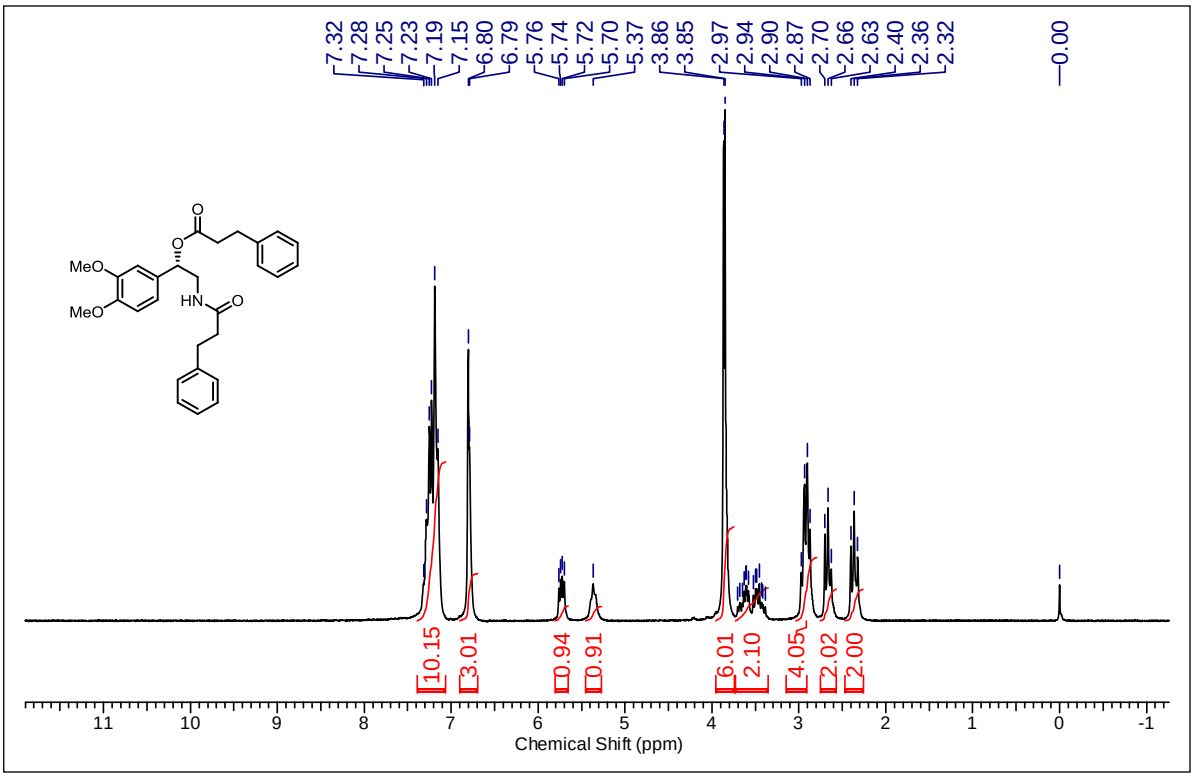
^{1}H-NMR spectrum of 41 (200 MHz, $CDCl_3$)



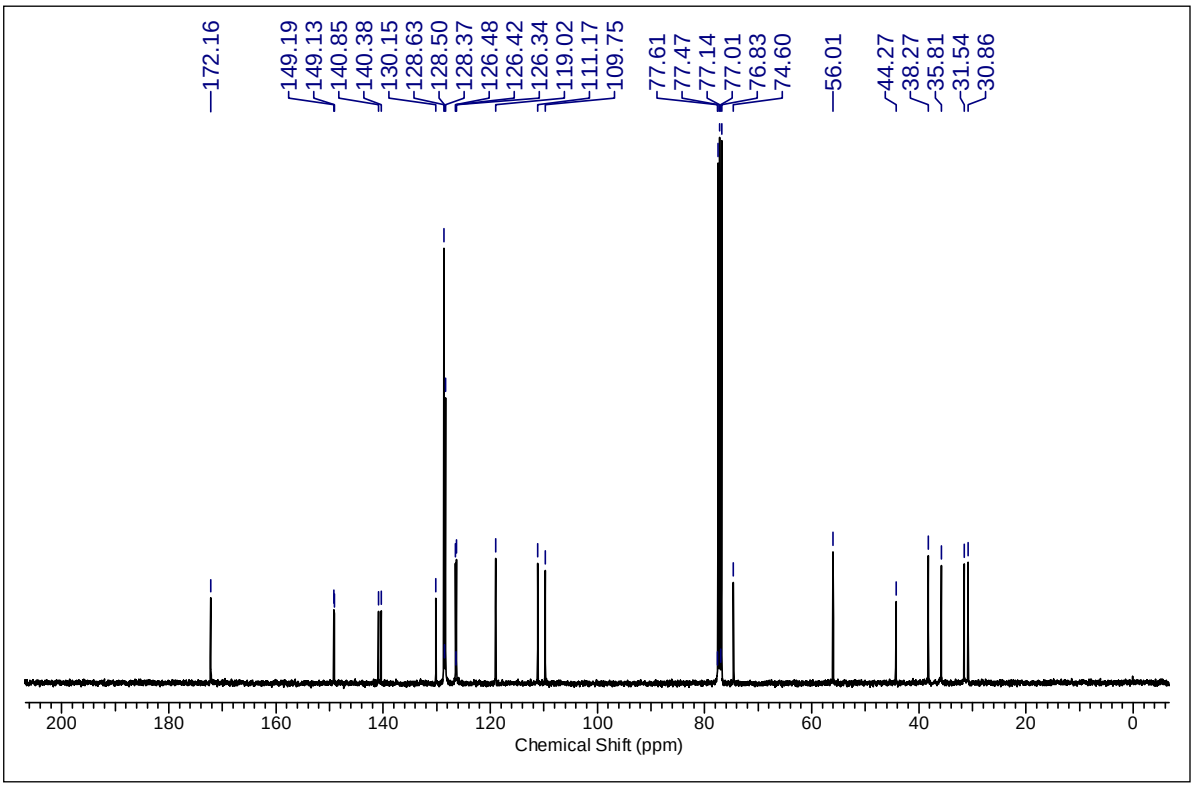
^{13}C-NMR spectrum of 41 (100 MHz, $CDCl_3$)



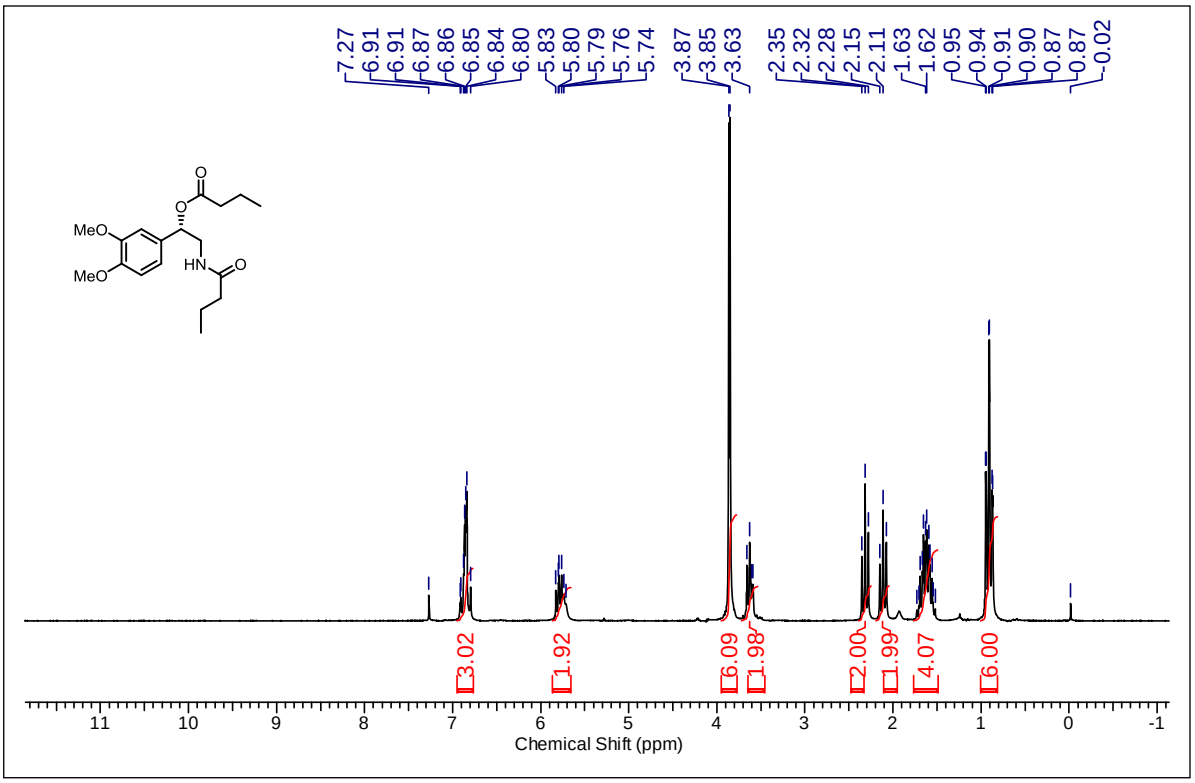
^{1}H-NMR spectrum of 43 (200 MHz, $CDCl_3$)



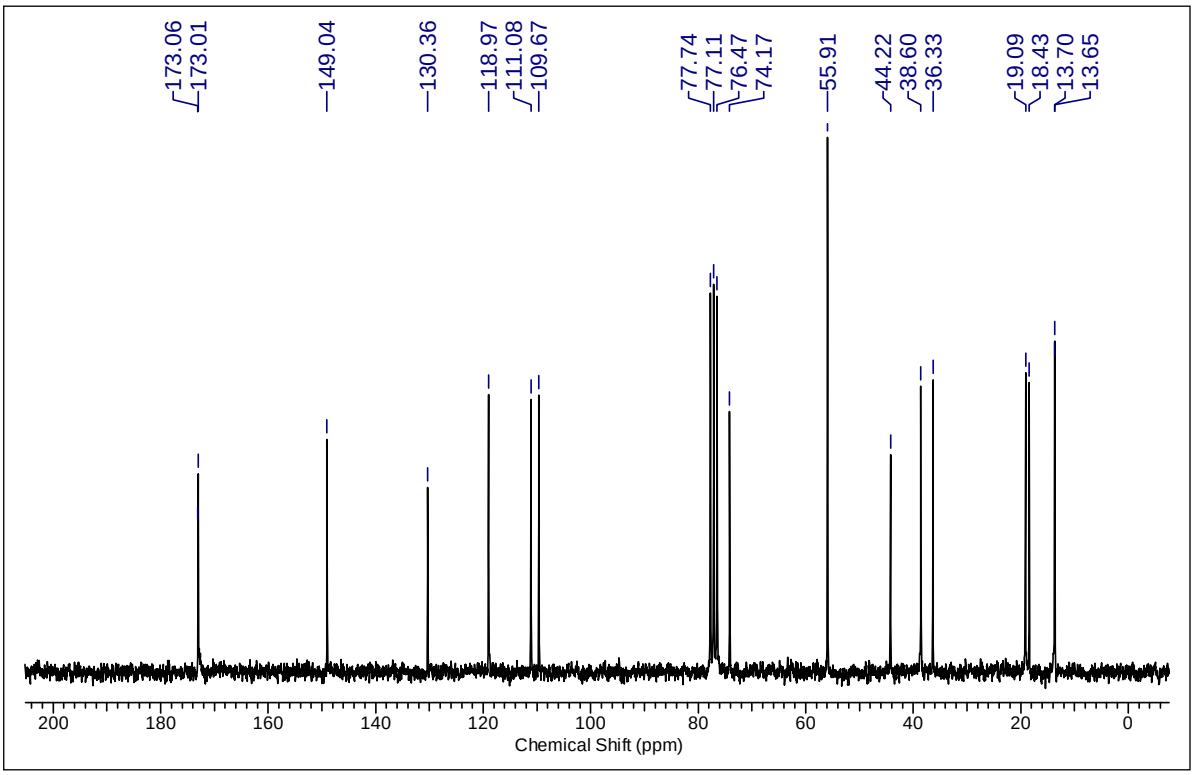
^{13}C-NMR spectrum of 43 (100 MHz, $CDCl_3$)



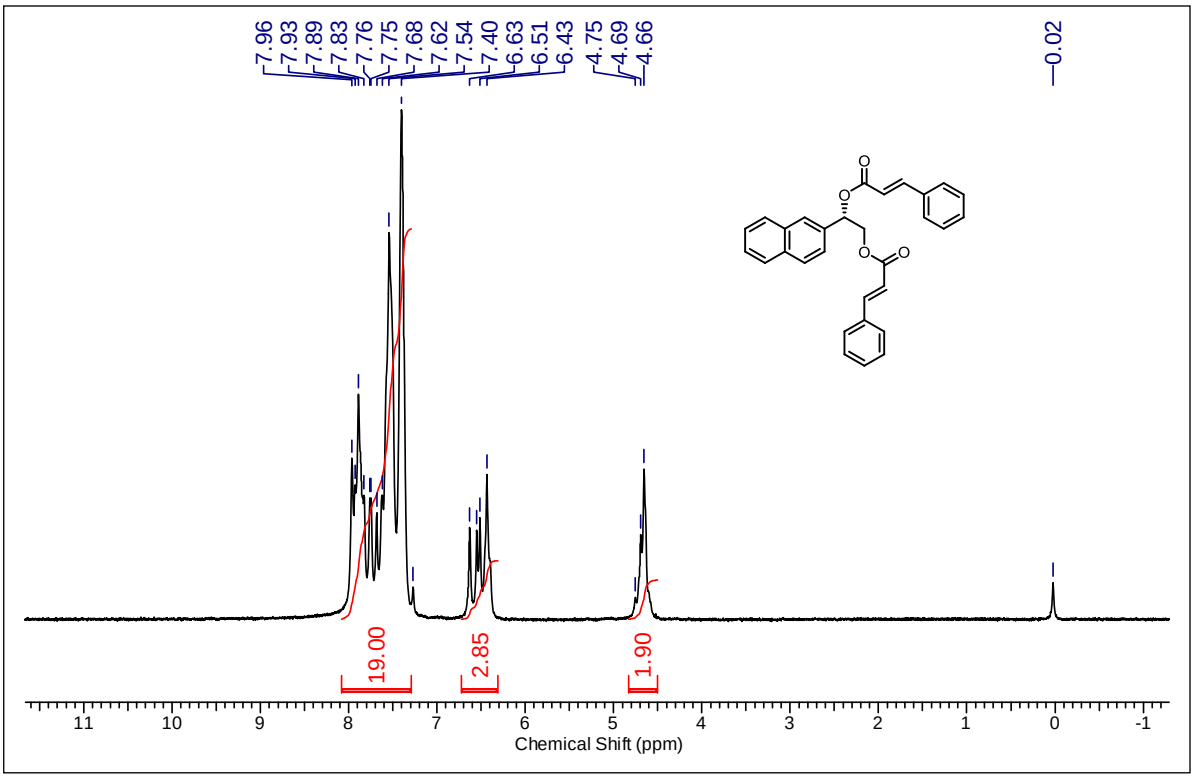
^{1}H-NMR spectrum of 45 (200 MHz, $CDCl_3$)



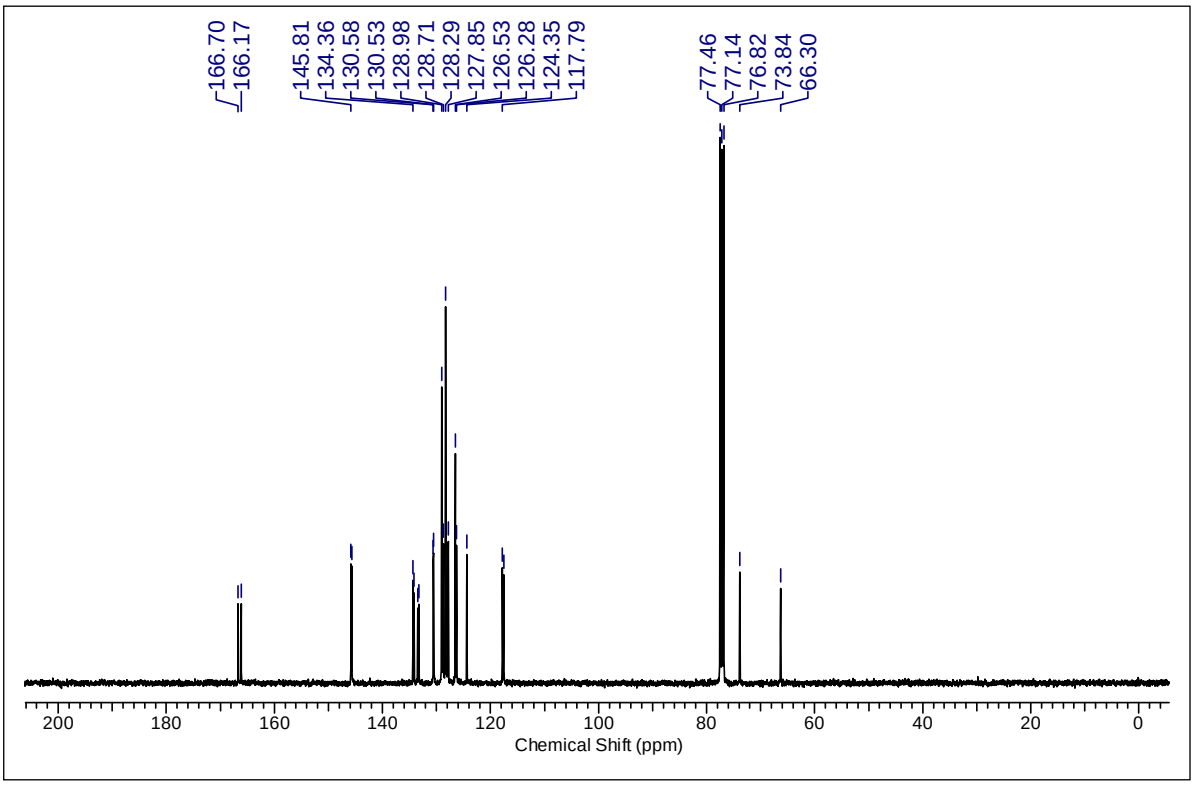
^{13}C-NMR spectrum of 45 (50 MHz, $CDCl_3$)



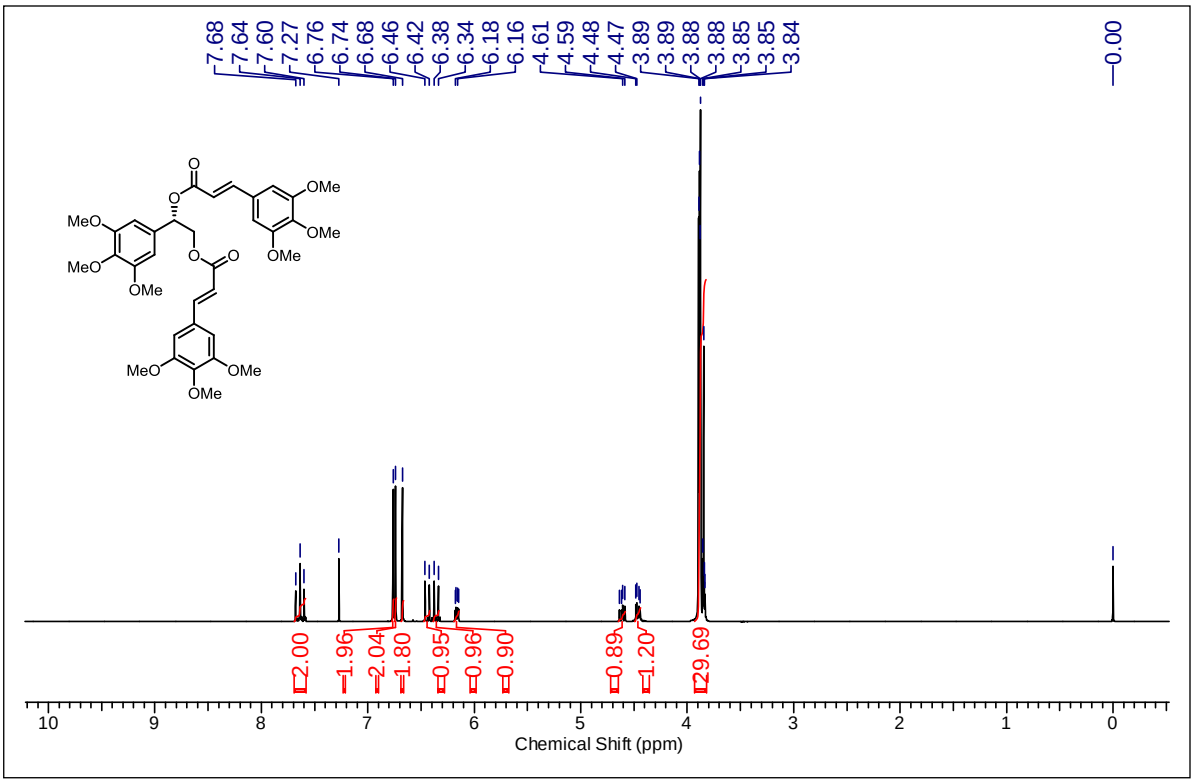
^{1}H-NMR spectrum of 53 (200 MHz, $CDCl_3$)



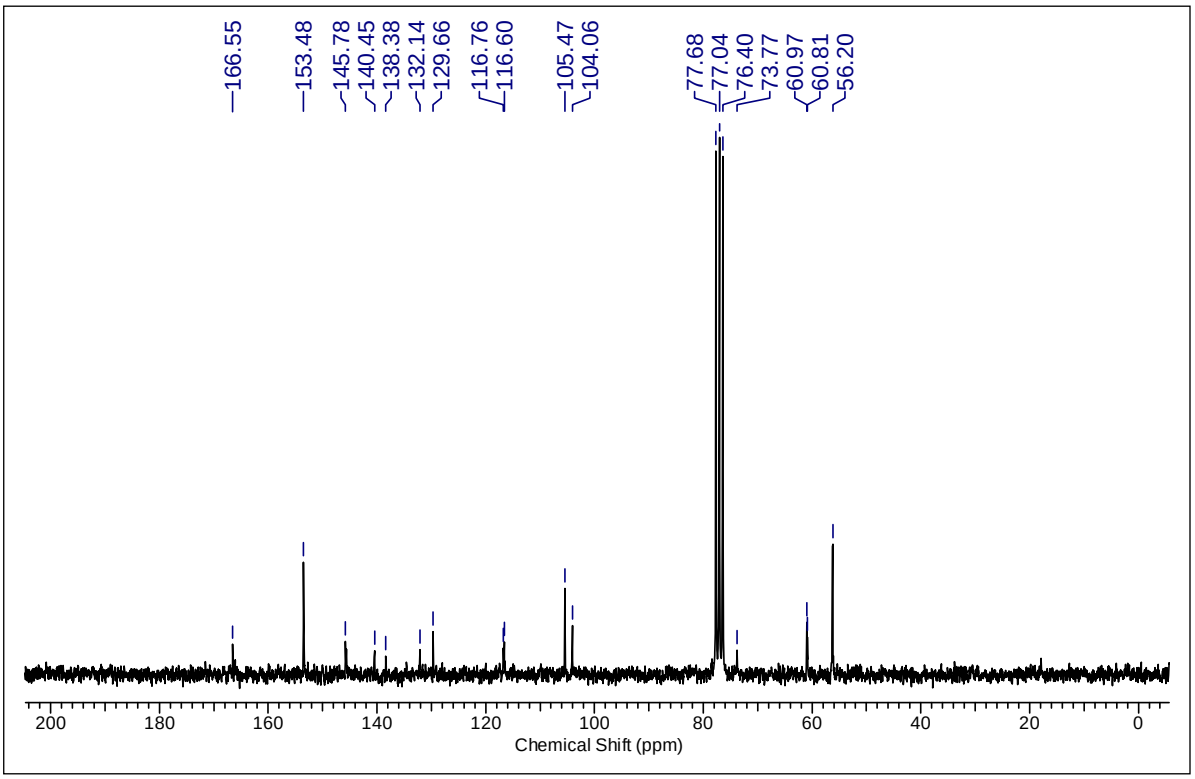
^{13}C-NMR spectrum of 53 (100 MHz, $CDCl_3$)



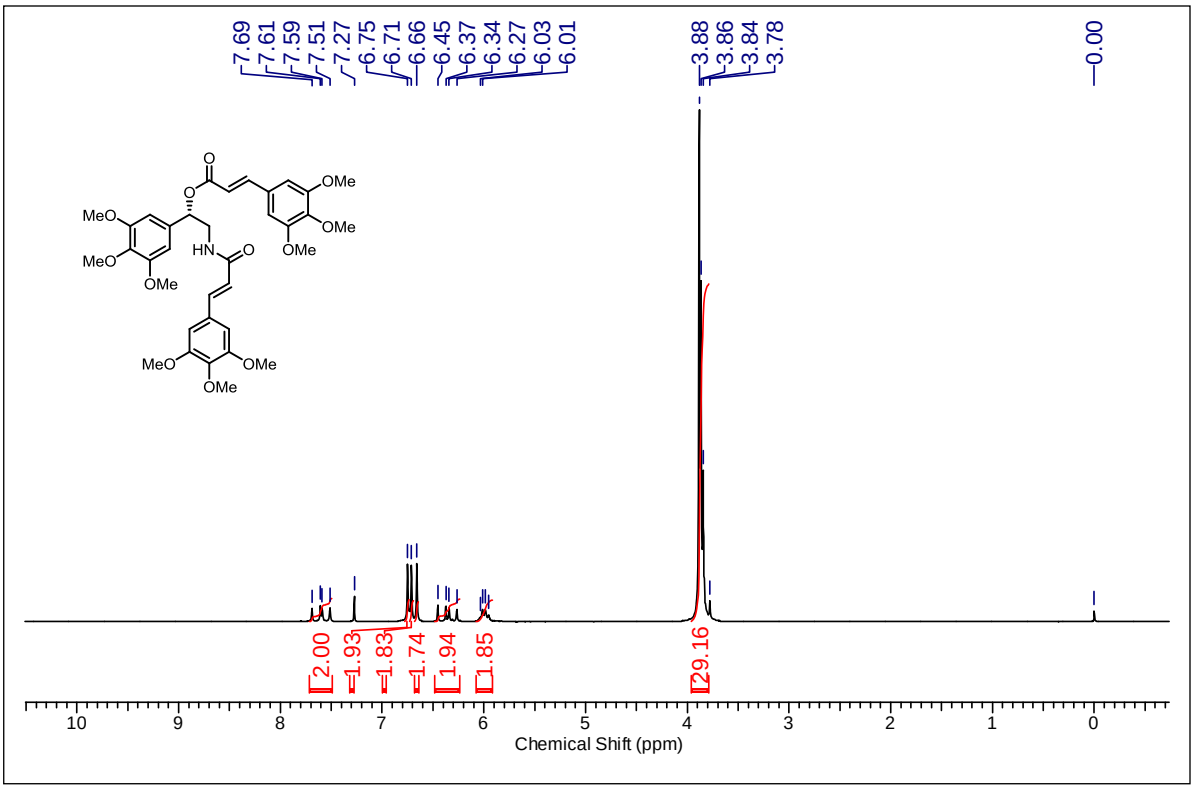
^{1}H-NMR spectrum of 56 (400 MHz, $CDCl_3$)



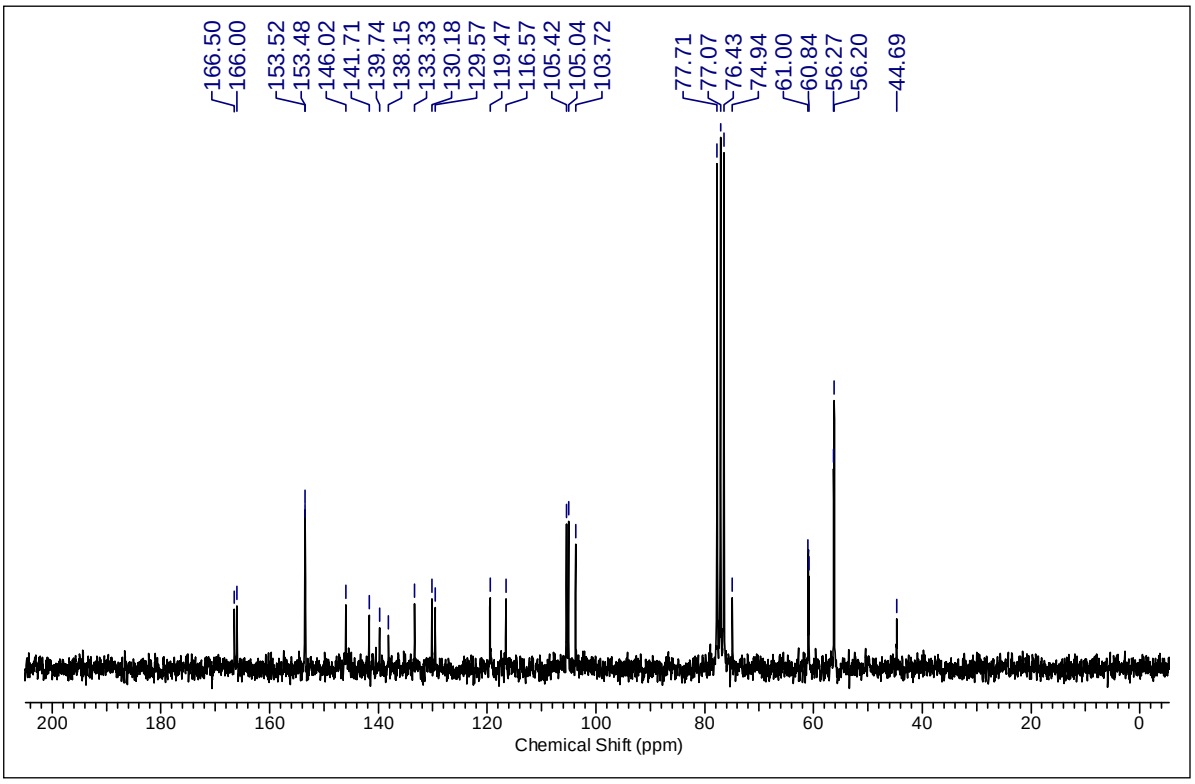
^{13}C-NMR spectrum of 56 (50 MHz, $CDCl_3$)



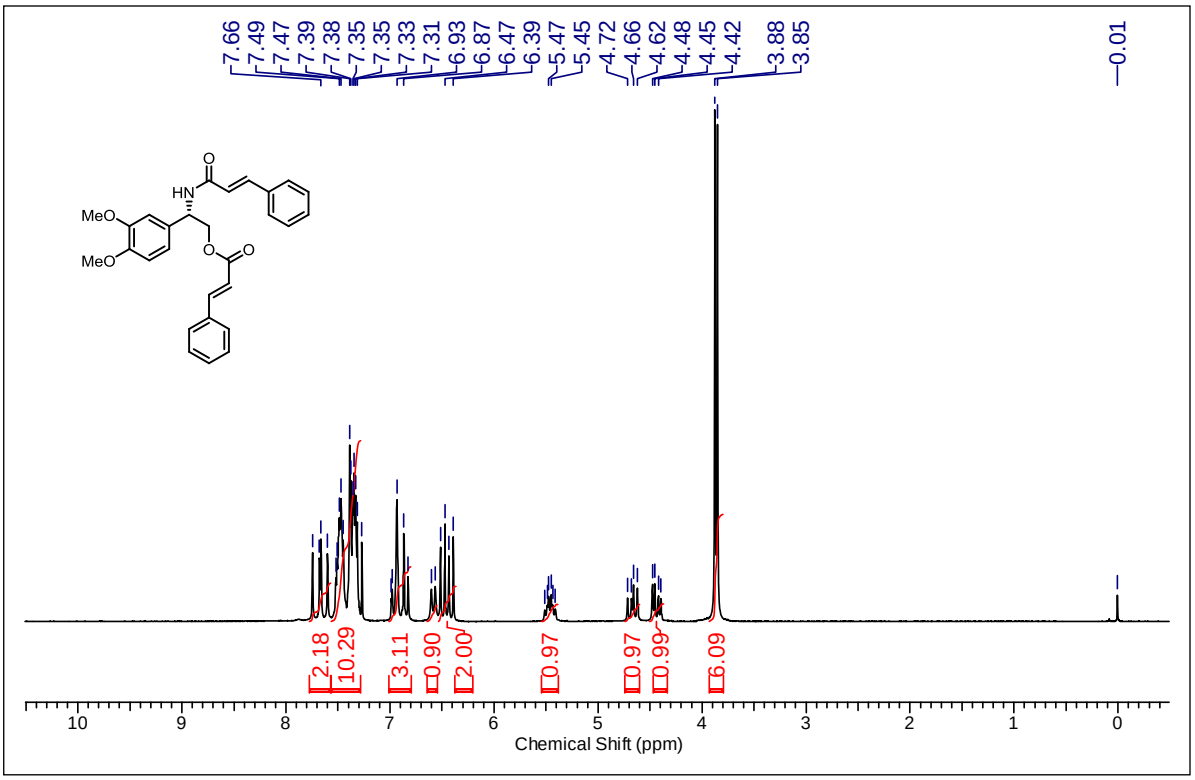
^{1}H-NMR spectrum of 57 (200 MHz, $CDCl_3$)



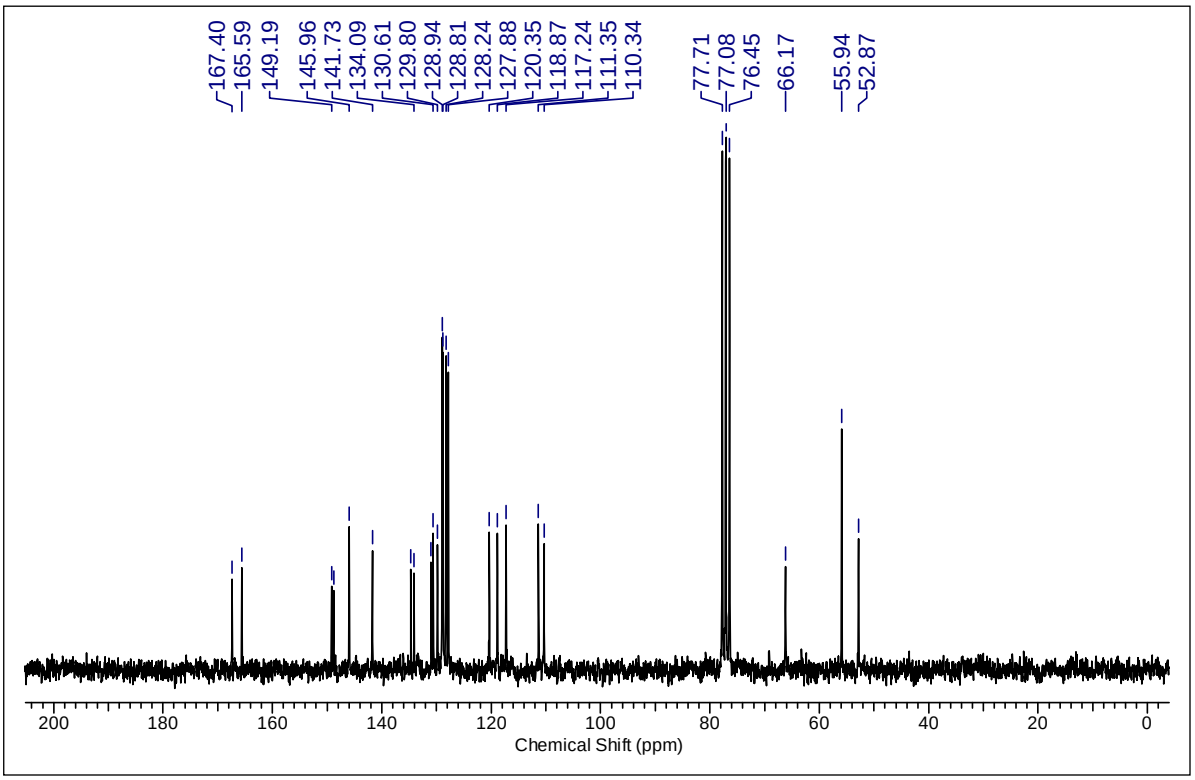
^{13}C-NMR spectrum of 57 (50 MHz, $CDCl_3$)



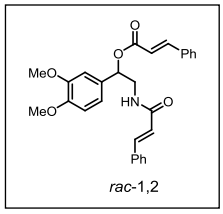
^{1}H-NMR spectrum of 58 (200 MHz, $CDCl_3$)



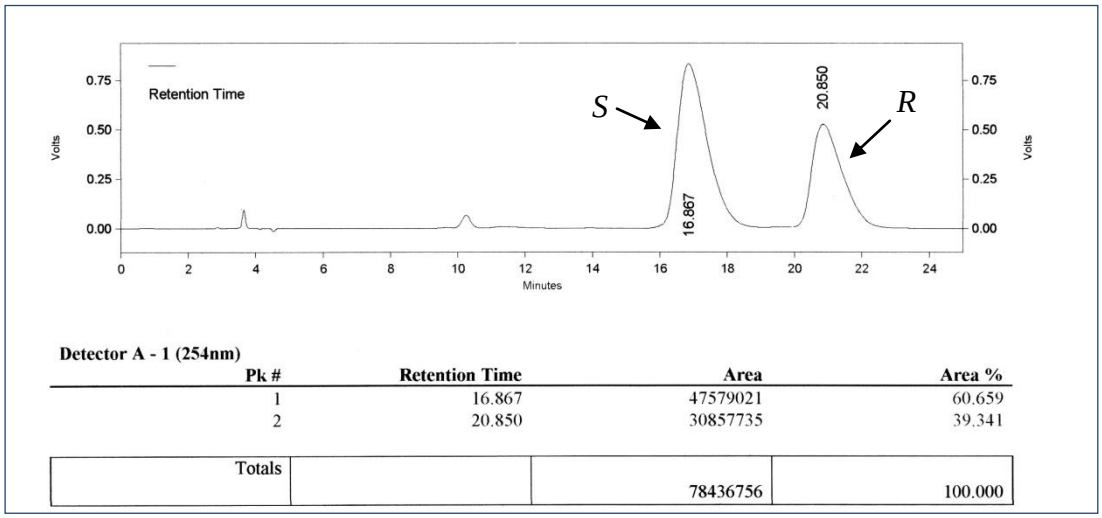
^{13}C-NMR spectrum of 58 (50 MHz, $CDCl_3$)



2.1.10. HPLC Chromatograms of enantiomers 1, 2 and their racemic mixture Chiralpak-IA (250X4.6 cm), EtOH:Hexane (30:70), flow 1 mL/min, det.: UV 254 nm

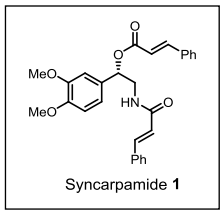


rac-1,2

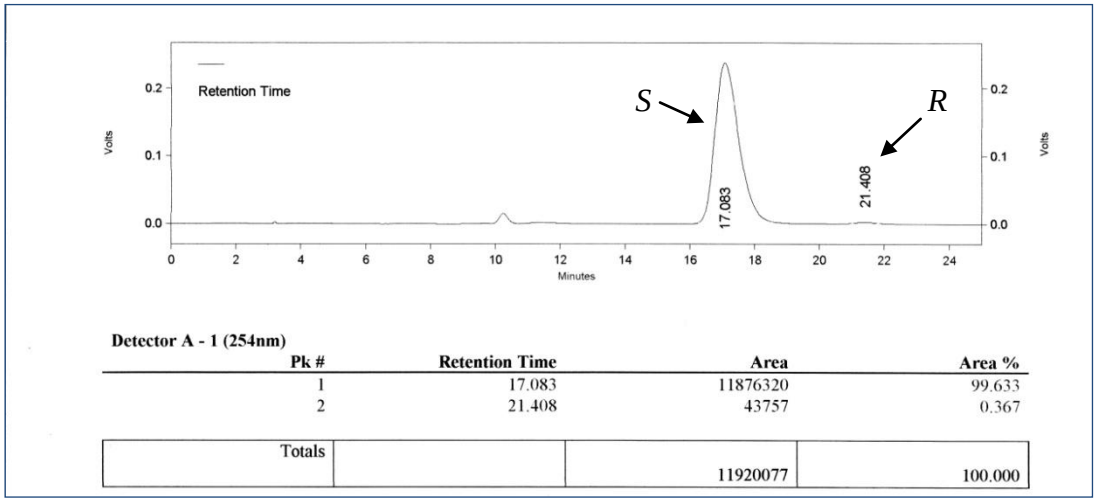


Detector A - 1 (254nm)

Pk #	Retention Time	Area	Area %
1	16.867	47579021	60.659
2	20.850	30857735	39.341
Totals		78436756	100.000

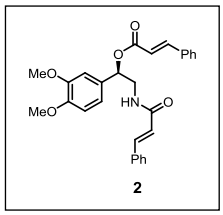


Syncarpamide **1**

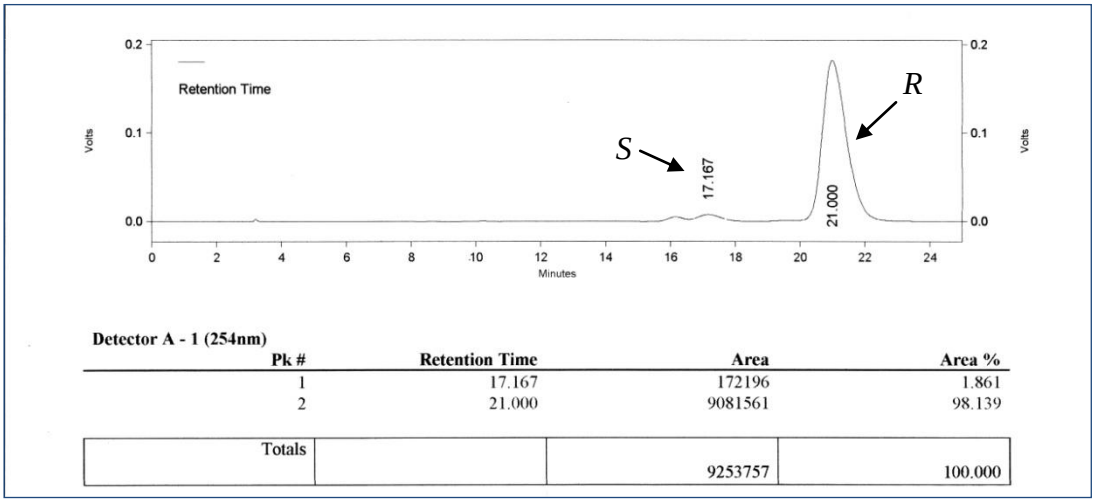


Detector A - 1 (254nm)

Pk #	Retention Time	Area	Area %
1	17.083	11876320	99.633
2	21.408	43757	0.367
Totals		11920077	100.000



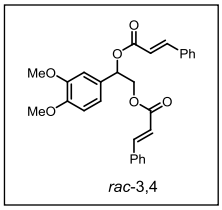
2



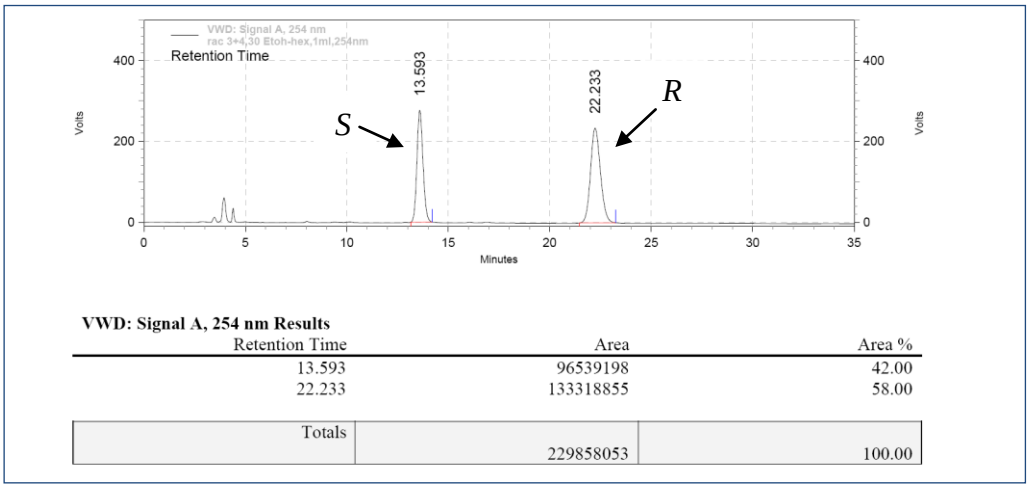
Detector A - 1 (254nm)

Pk #	Retention Time	Area	Area %
1	17.167	172196	1.861
2	21.000	9081561	98.139
Totals		9253757	100.000

HPLC Chromatograms of enantiomers 3, 4 and their racemic mixture
Chiralpak-IA (250X4.6 cm), EtOH:Hexane (30:70), flow 1 mL/min, det.: UV 254 nm

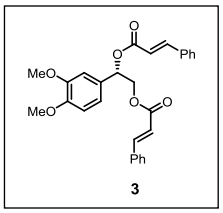


rac-3,4

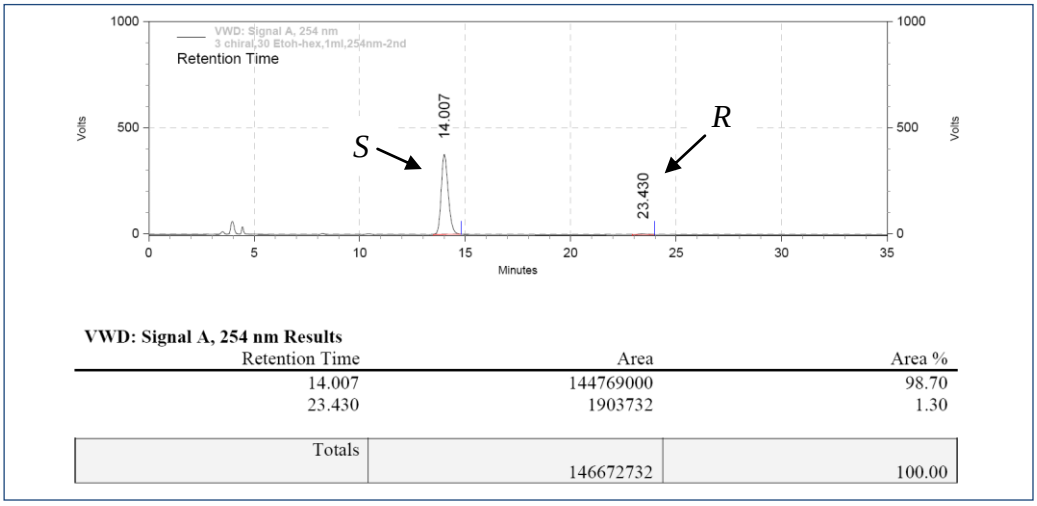


VWD: Signal A, 254 nm Results

Retention Time	Area	Area %
13.593	96539198	42.00
22.233	133318855	58.00
Totals	229858053	100.00

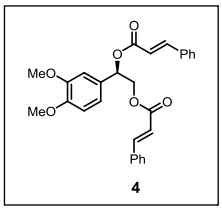


3



VWD: Signal A, 254 nm Results

Retention Time	Area	Area %
14.007	144769000	98.70
23.430	1903732	1.30
Totals	146672732	100.00



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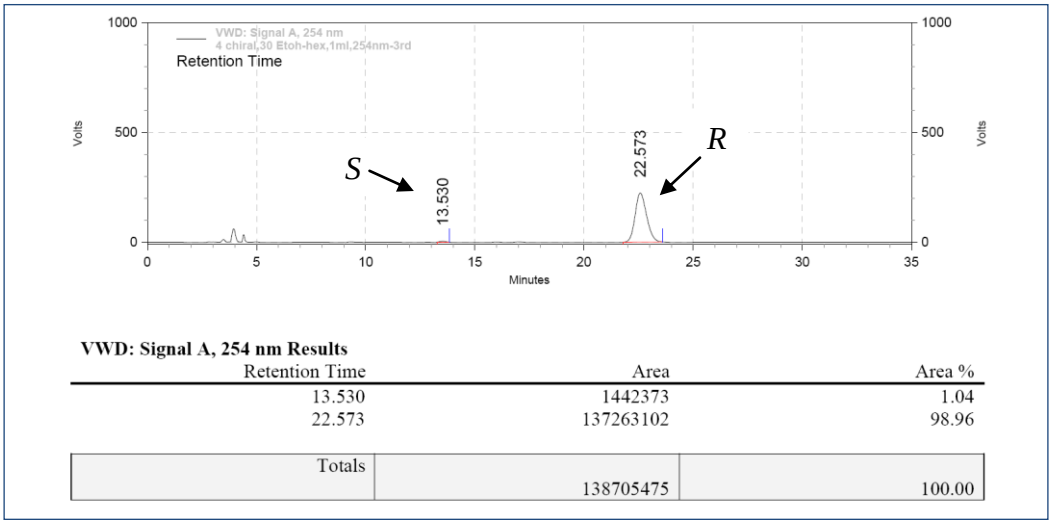
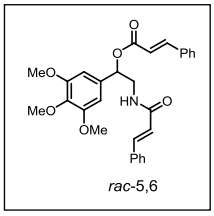
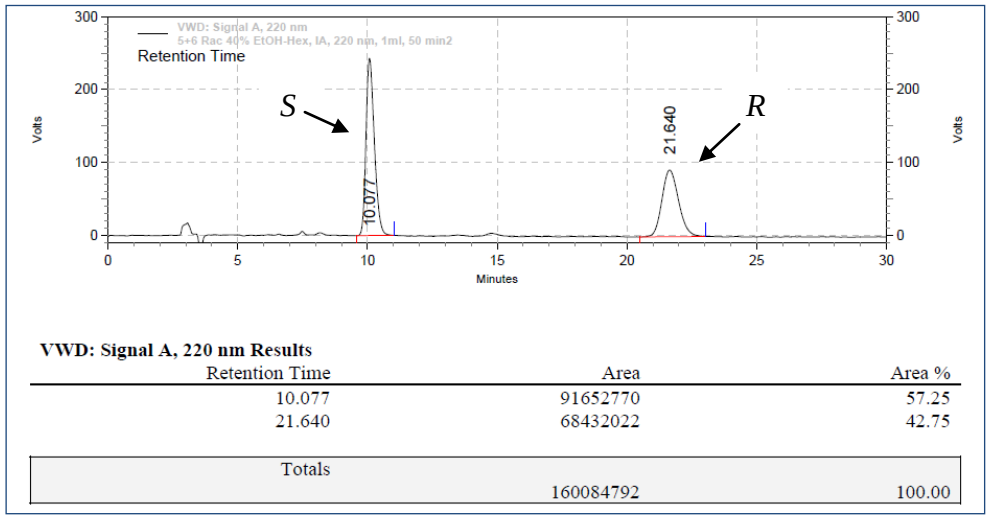


VWD: Signal A, 254 nm Results

Retention Time	Area	Area %
13.530	1442373	1.04
22.573	137263102	98.96
Totals	138705475	100.00

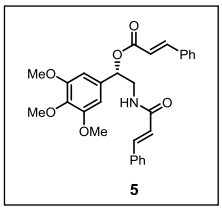
HPLC Chromatograms of enantiomers 5, 6 and their racemic mixture
Chiralpak-IA (250X4.6 cm), EtOH:Hexane (40:60), flow 1 mL/min, det.: UV 220 nm

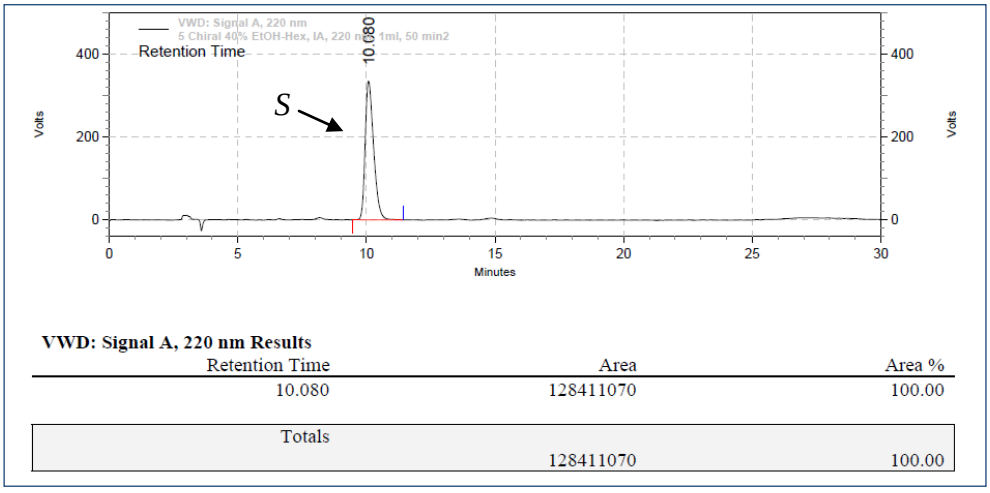




VWD: Signal A, 220 nm Results

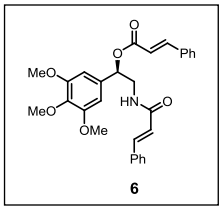
Retention Time	Area	Area %
10.077	91652770	57.25
21.640	68432022	42.75
Totals	160084792	100.00

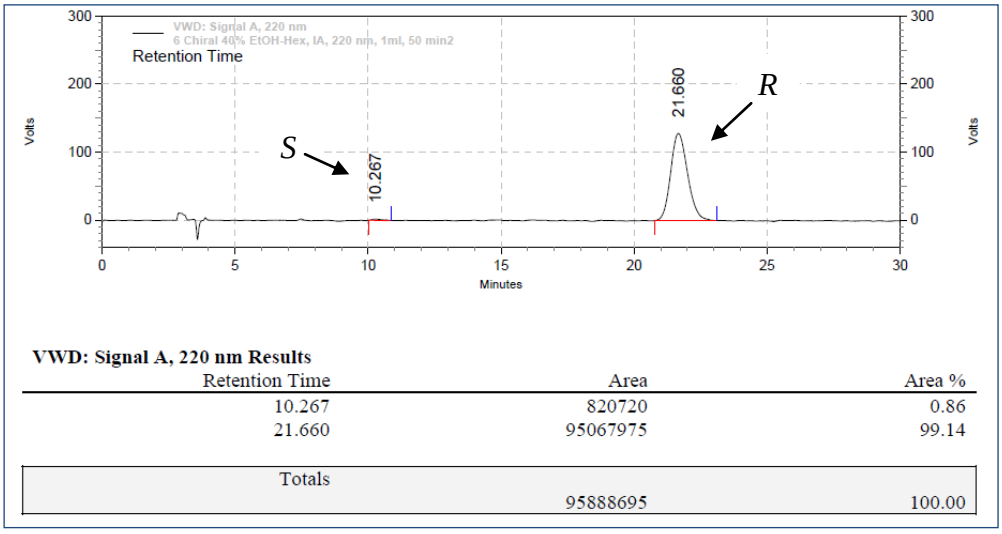




VWD: Signal A, 220 nm Results

Retention Time	Area	Area %
10.080	128411070	100.00
Totals	128411070	100.00

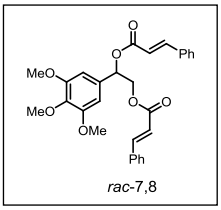




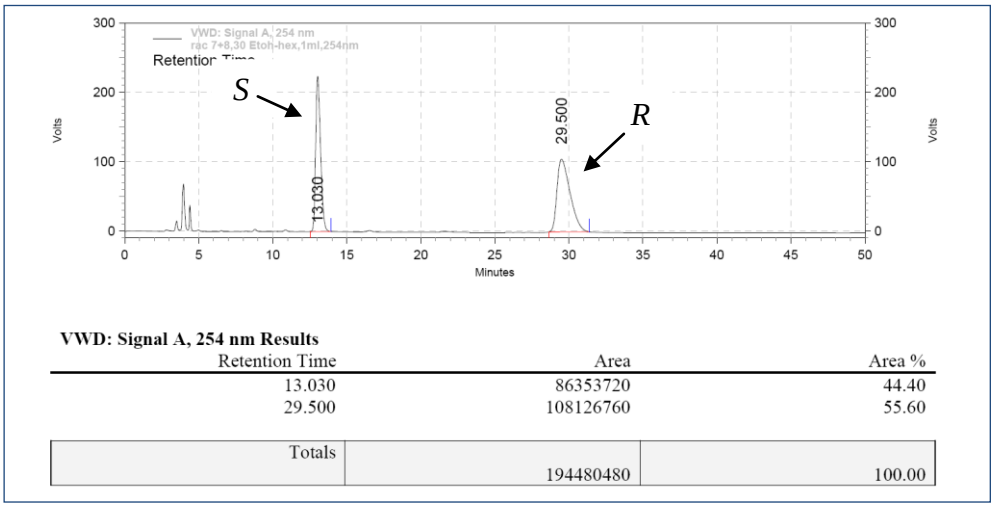
VWD: Signal A, 220 nm Results

Retention Time	Area	Area %
10.267	820720	0.86
21.660	95067975	99.14
Totals	95888695	100.00

HPLC Chromatograms of enantiomers 7, 8 and their racemic mixture
Chiralpak-IA (250X4.6 cm), EtOH:Hexane (30:70), flow 1 mL/min, det.: UV 254 nm

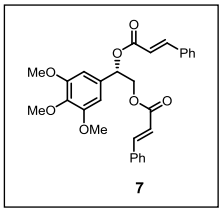


rac-7,8

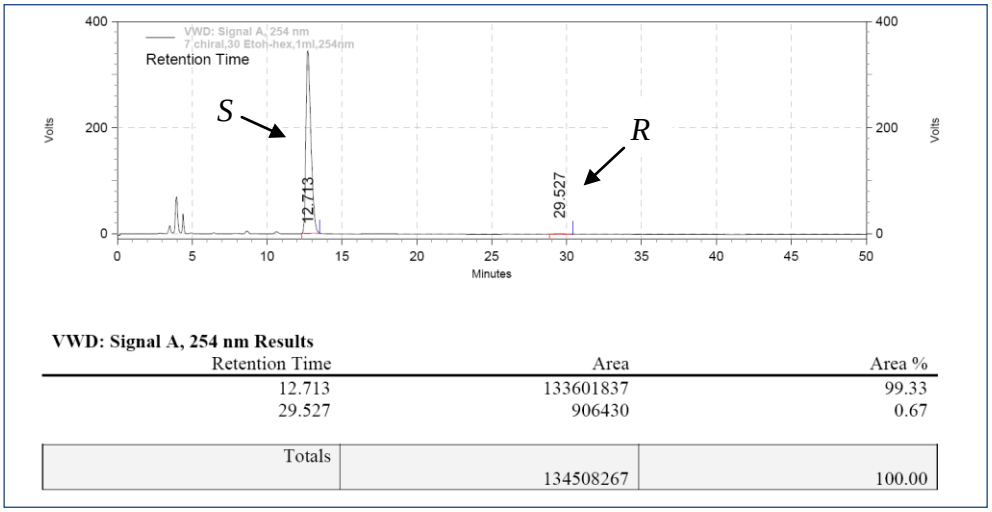


VWD: Signal A, 254 nm Results

Retention Time	Area	Area %
13.030	86353720	44.40
29.500	108126760	55.60
Totals	194480480	100.00

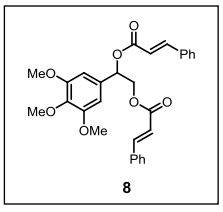


7

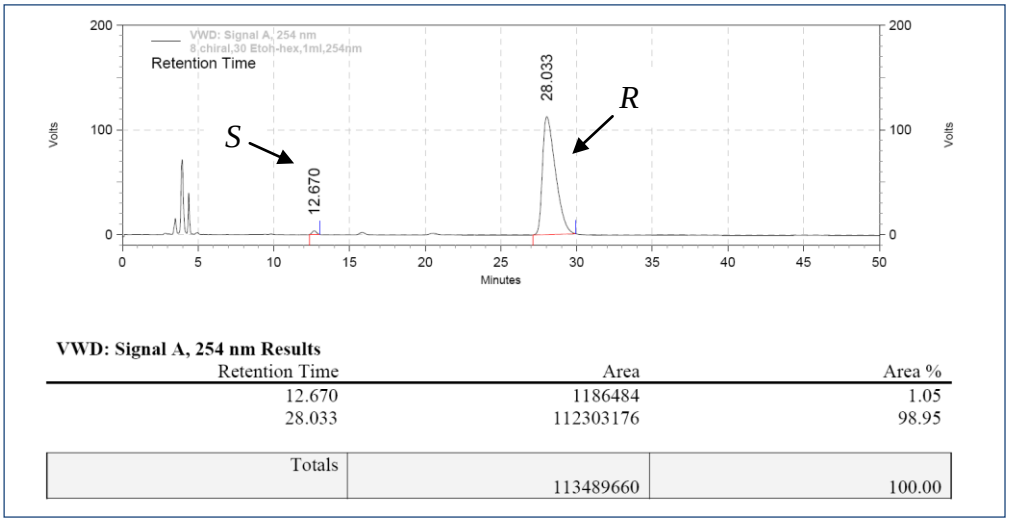


VWD: Signal A, 254 nm Results

Retention Time	Area	Area %
12.713	133601837	99.33
29.527	906430	0.67
Totals	134508267	100.00



8



VWD: Signal A, 254 nm Results

Retention Time	Area	Area %
12.670	1186484	1.05
28.033	112303176	98.95
Totals	113489660	100.00

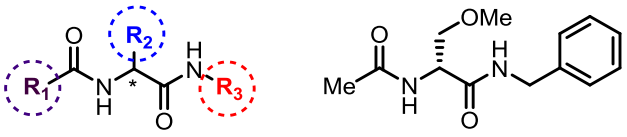
2.2. SECTION B

Synthesis of functionalized amino acids using chiral pool approach: synthesis of antiepileptic drug (*R*)-lacosamide

2.2.1. INTRODUCTION

Epilepsy is a neurological disorder that disturbs the normal activity of brain cells. It describes the types of recurrent seizures produced by paroxysmal excessive neuronal discharges in the brain.[1] It is estimated that epilepsy affects approximately 50 million people worldwide and over 10 million people in India alone.[2] Unfortunately, many medications such as valproic acid, carbamazepine, phenobarbital, phenytoin, etc. are ineffective for approximately one-third of patients with epilepsy.[3] Many continue to have seizures, while others experience side effects such as drowsiness, dizziness, nausea, liver damage, etc.[4]

Functionalized amino acids (FAAs) of formula **A** were identified as a novel class of anticonvulsant agents (also commonly known as antiepileptic drugs or as antiseizure drugs). Anticonvulsants are a diverse group of pharmacological agents used in the treatment of epileptic seizures.[5] The lead compound among the functionalized amino acids, (*R*)-lacosamide (Vimpat) **1** was identified as a best antiepileptic drug (AED) and has been suggested for the treatment of partial-onset seizures in patients with epilepsy and as add-on treatment in brain tumor patients.[6] Currently, (*R*)-lacosamide is marketed in U.S. and Europe and according to UCB pharma, its worldwide expected sale in 2015-2020 is set to be €1.2 billion.



FAAs (**A**) (*R*)-Lacosamide **1**

Figure 1. Structures of FAAs (A) and (*R*)-lacosamide **1**.

The exact mode of action of this drug in humans has not yet been fully elucidated but it is believed that it increases the slow inactivation of the voltage-gated sodium channels thus

inhibiting repetitive neuronal firing.[7] Additionally, (*R*)-lacosamide **1** is also under clinical trials for the treatment of neuropathic pains.[8] Few examples of functionalized amino acids (FAAs) are shown in **Figure 2**.

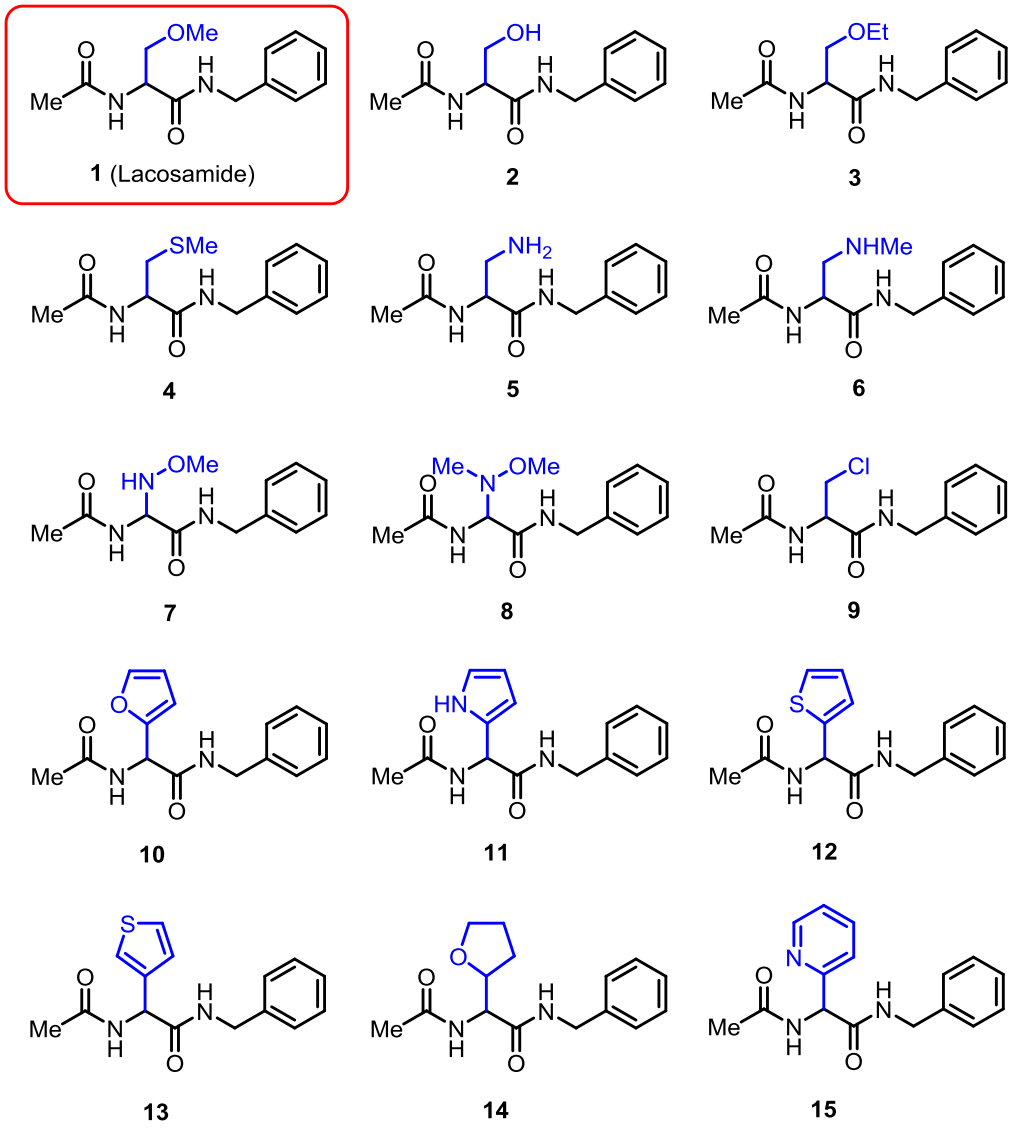
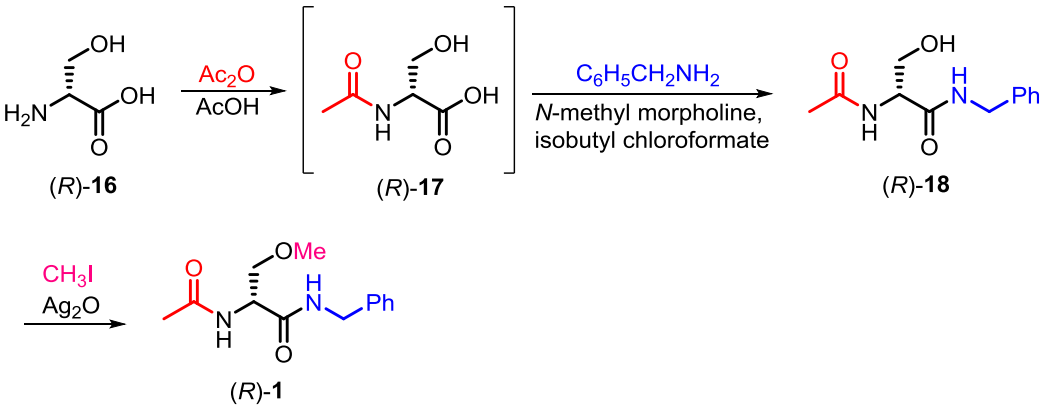


Figure 2. Selected examples of functionalized amino acids (FAAs) **1-15**.

2.2.2. REVIEW OF LITERATURE

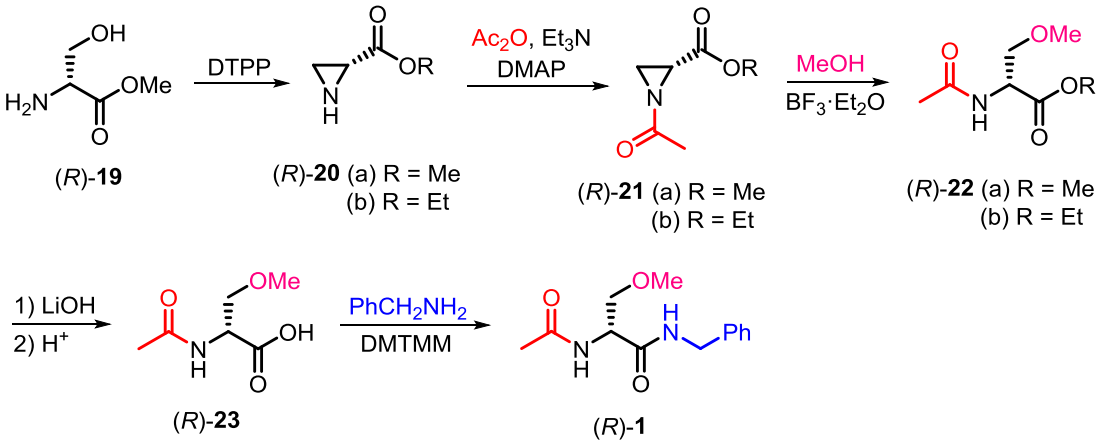
Because of the medical importance of (*R*)-lacosamide **1**, its synthesis has attracted the attention of researchers around the world and several chiral pool and asymmetric syntheses were reported in literature.[9b-15] (*R*)-Lacosamide **1** could be considered as a functionalized derivative of amino acid D-serine (*R*)-**16**. Initial strategies for the synthesis of this drug used non-natural amino acid, D-serine as a starting material. The conversion of D-serine into **1**

involves *N*-acylation, and *O*-methylation steps with protecting groups introduction and manipulation used in different order.[9] *O*-Methylation was the problematic step because of possible racemization of amino acid during the step.[9b,g–j,l] To overcome this possible racemization, Kuhn's *O*-methylation protocol[9a] involving MeI and Ag_2O was used in the three-step synthesis of (*R*)-lacosamide **1** starting from D-serine as a starting material (**Scheme 1**).[9b] The drawbacks of this method are high cost and nonregenerability of silver catalyst and longer reaction time (3-5 days) required for the methylation step.



Scheme 1. Three step synthesis of (*R*)-lacosamide **1**.

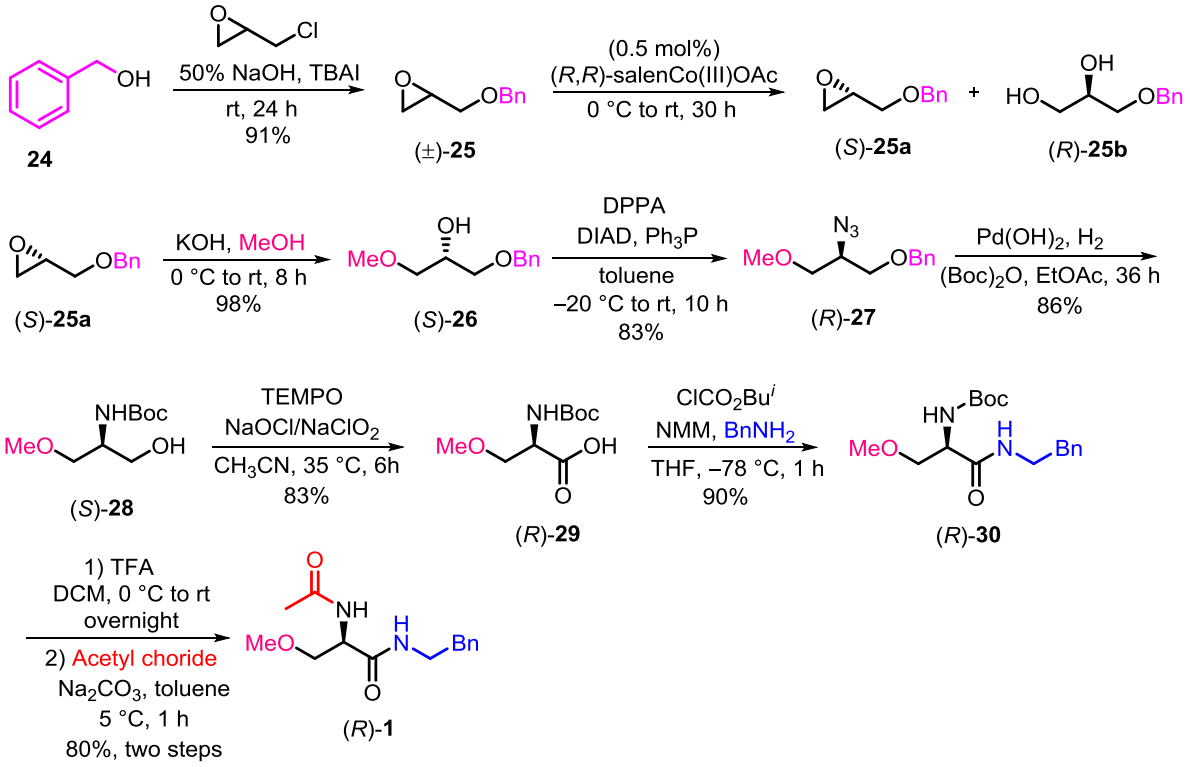
In another approach, D-serine methyl ester (*R*)-**19** was transformed into the corresponding aziridine using diethoxytriphenylphosphorane (DTPP), which upon ring opening with MeOH using $BF_3{\cdot}Et_2O$ as a catalyst followed by hydrolysis of the ester to acid and then amide coupling with benzyl amine furnished (*R*)-lacosamide **1** (**Scheme 2**).[9c]



Scheme 2. Synthesis of (*R*)-lacosamide **1** through aziridine intermediate.

Muthukrishnan *et al.* (2011)[10]

Jacobsen's hydrolytic kinetic resolution (HKR) strategy was utilized for the synthesis of (*R*)-lacosamide **1**. The *rac*-benzyl glycidyl ether (±)-**25** was subjected to Jacobsen's hydrolytic kinetic resolution conditions with water using the catalyst (*R*,*R*)-Salen Co(III)OAc (0.5 mol %) to furnish the required enantiomerically pure epoxide (*S*)-**25a** in 47% yield and >99% *ee* along with its diol (*R*)-**25b** in 43% yield. Further, functional group transformations provided (*R*)-lacosamide **1** in 8 steps with 18% overall yield from *rac*-epichlorohydrin as starting material (**Scheme 3**).



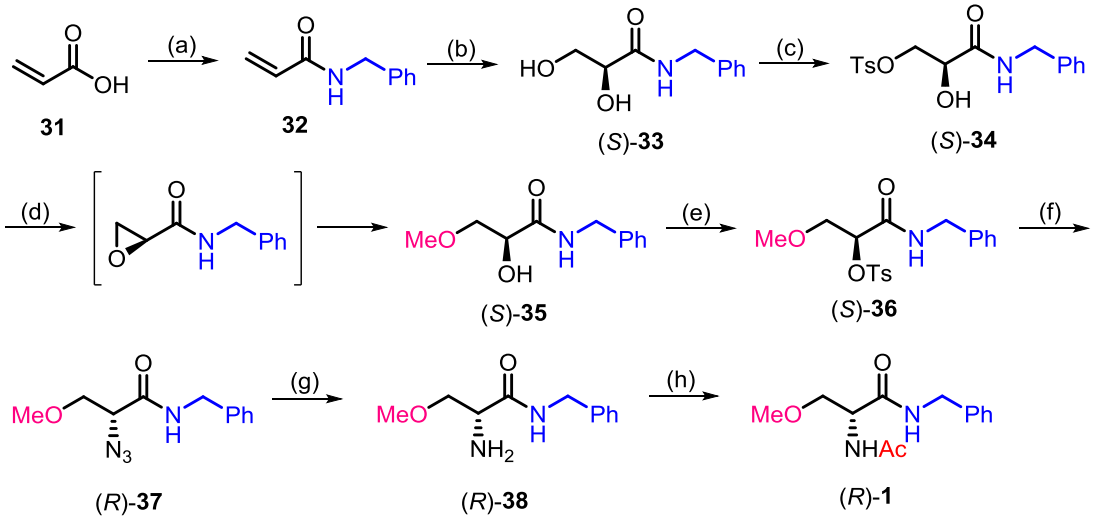
Scheme 3. Synthesis of (*R*)-lacosamide **1** using Jacobsen's HKR strategy.

The drawbacks of this method are loss of yield in Jacobsen's hydrolytic kinetic resolution step and use of expensive Jacobsen's catalyst hence, this synthesis of (*R*)-lacosamide **1** is not commercially viable.

Narsaiah *et al.* (2013) [11]

Sharpless asymmetric dihydroxylation was used to synthesize (*R*)-lacosamide **1**. The asymmetric synthesis commenced from protection of acrylic acid **31** with benzyl amine.

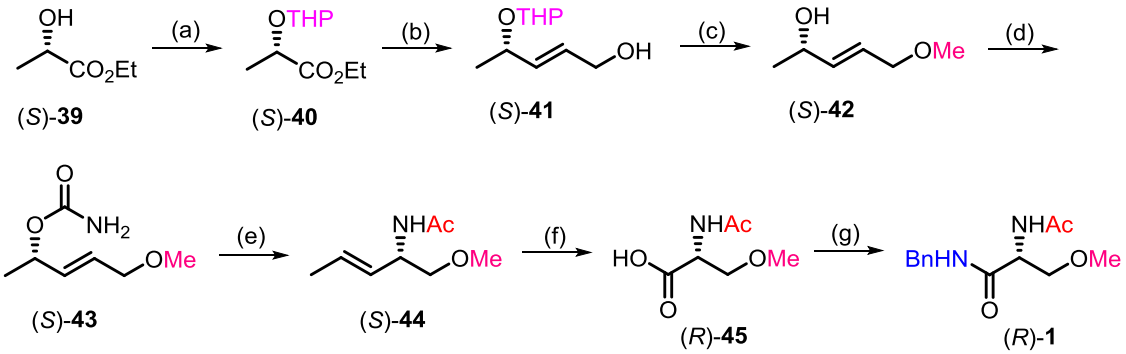
Sharpless asymmetric dihydroxylation of *N*-benzylacrylamide **32** with AD-mix-β furnished diol (*S*)-**33** which on further functional group transformations provided (*R*)-lacosamide **1** in 8 steps with 29% overall yield from acrylic acid as starting material (**Scheme 4**).



Scheme 4. Asymmetric synthesis of (*R*)-lacosamide **1** using asymmetric dihydroxylation. *Reagents and conditions:* (a) HOBt, EDCI, Et_3N, CH_2Cl_2, 0 °C to rt, 8 h, 70%; (b) AD-mix-β, $MsNH_2$, *t*-BuOH-H_2O (1:1), 0 °C, 80%; (c) Bu_2SnO, TsCl (1 equiv), Et_3N, CH_2Cl_2, 0 °C, 2 h, 95%; (d) K_2CO_3, MeOH, 0 °C, 8 h, 90%; (e) TsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C, 92%; (f) NaN_3, DMF, 70 °C, 6 h, 87%; (g) Ph_3P, THF-H_2O (9:1), 50 °C, 12 h, 85%; (h) Ac_2O, DMAP, CH_2Cl_2, 0 °C, 1 h, 90%.

Stecko *et al.* (2014)[12]

Stecko *et al.* synthesized (*R*)-lacosamide **1** from ethyl L-lactate (*S*)-**39** employing stereospecific allyl cyanate to isocyanate rearrangement as a key step. The target molecule was synthesized in 7 steps with 22% overall yield using ethyl L-lactate as starting molecule.

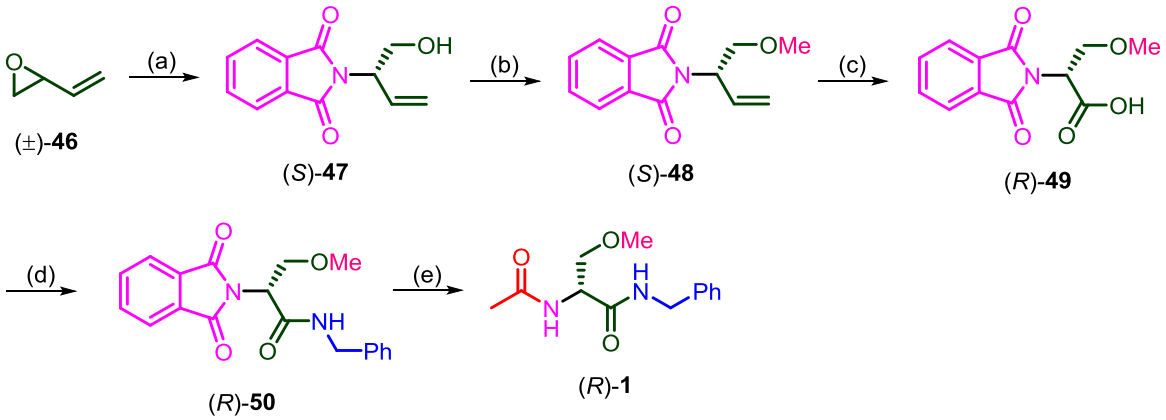


Scheme 5. Synthesis of (*R*)-lacosamide **1** using allyl cyanate to isocyanate rearrangement. *Reagents and conditions:* (a) DHP, PPTS, CH_2Cl_2, rt, 95%; (b) (i) DIBAL-H, CH_2Cl_2, −78 °C; (ii) NaH, $(EtO)_2P(O)CH_2COOEt$, THF, 0 °C; (iii) DIBAL-H, CH_2Cl_2, −78 °C, (75%, 3 steps); (c) (i) NaH, MeI, THF, rt; (ii) AcCl (5 mol %), MeOH, rt, (78%, 2 steps: *O*-methylation and deprotection); (d) (i) TCA-NCO, CH_2Cl_2, 0 °C, 1 h; (ii) aq. K_2CO_3, MeOH, rt, 2 h, (82%, 2 steps); (e) (i) TFAA, Et_3N, THF, 0 °C, 30 min; (ii) MeMgBr, THF, −10 °C to rt, (74%, 2 steps); (f) $RuCl_3 \cdot H_2O$ (3 mol %), $NaIO_4$, acetone/water (5:1), 79%; (g) IBCF, NMM, $BnNH_2$, THF, −20 °C to rt, 82%.

The drawbacks of this method are a large number of steps involved in the synthesis and use of pyrophoric reagents such as Grignard reagents, DIBAL-H, other expensive reagents such as $RuCl_3 \cdot H_2O$, trichloroacetyl isocyanate (TCA-NCO) and trifluoroacetic anhydride (TFAA).

Pandey *et al.* (2015)[13]

Trost's Dynamic Kinetic Asymmetric Transformation (DYKAT) was employed as a key step to synthesize (*R*)-lacosamide **1**. Enantiopure phthaloyl alcohol derivative (*S*)-**47** was prepared from the racemic butadiene monoepoxide (±)-**46** by the application Trost's DYKAT strategy using (*R*,*R*)-DACH ligand (**Figure 3**). Compound (*S*)-**47** on further functional group transformations furnished (*R*)-lacosamide **1** in 5 steps with 52% overall yield starting from butadiene monoepoxide (±)-**46** as starting material.



Scheme 6. Synthesis of (*R*)-lacosamide **1** using Trost's Dynamic Kinetic Asymmetric Transformation (DYKAT). *Reagents and conditions*: (a) Phthalimide, Na_2CO_3, 1.2 mol % (*R*,*R*)-DACH, 0.4 mol % $[\eta^3\text{-}C_3H_5PdCl]_2$, dry CH_2Cl_2, rt, 14 h, 98%; (b) MeI, NaH, DMF, 0 °C to rt, 3 h, 86%; (c) (i) OsO_4, $NaIO_4$, 2,6-lutidine, dioxane:water 3:1 v/v, 0 °C to rt, 2 h; (ii) Oxone, DMF, rt, 12 h (78% over two steps); (d) $C_6H_5CH_2NH_2$, *N*-methyl morpholine, isobutyl chloroformate, THF, −78 °C to rt, 1 h, 88%; (e) (i) $NH_2NH_2.H_2O$, isopropyl

alcohol, 0 °C to rt, 2 h; (ii) CH_3COCl, Na_2CO_3, dry toluene, 0 to 5 °C, 1 h, (91% over two steps).

The main draw of this method is the use of expensive Trost's (*R*,*R*)-DACH ligand which renders this synthesis to be not commercially viable considering that (*R*)-**1** is currently being used as a drug.

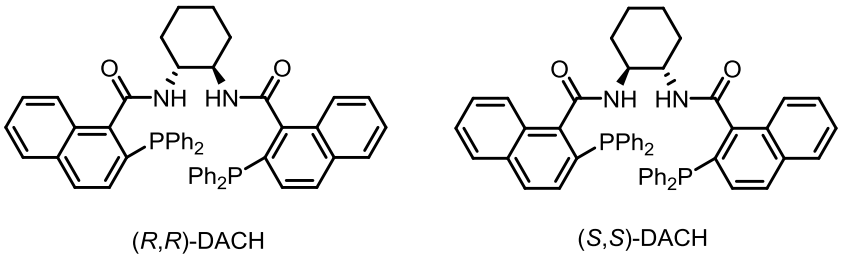


Figure 3. Structures of (*R*,*R*) and (*S*,*S*)-DACH ligands.

Miscellaneous methods for synthesis of (*R*)-lacosamide

Some other synthetic methods of (*R*)-lacosamide **1** were also reported using Ugi multicomponent reaction and chiral Rhodium complexes etc.[14]

2.2.3. PRESENT WORK

Objective

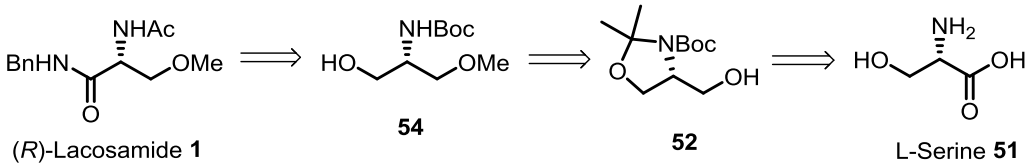
As a part of our research programme in the synthesis of biologically active molecules using chiral building blocks, we considered developing an industrially feasible chiral pool synthesis of (*R*)-lacosamide **1** from simple and cheap starting materials without the need of any expensive chiral ligands, hydrolytic kinetic resolution and harsh reaction conditions. We devised the chiral pool strategy for the synthesis of (*R*)-lacosamide **1** from natural amino acid L-serine[15] whereas the previous syntheses relied on using expensive chiral ligands in Trost's dynamic kinetic asymmetric transformation (DYKAT), Jacobsen's hydrolytic kinetic resolution (HKR), use of non-natural amino acid, D-serine, chiral Rhodium complexes and other industrially non-feasible methods.[9b-14]

2.2.4. RESULTS AND DISCUSSION

2.2.4.1. Retrosynthetic analysis of (*R*)-lacosamide 1

Our synthetic strategy for the synthesis of (*R*)-lacosamide **1** is outlined in **Scheme 7**. We envisioned that the target molecule could be synthesized from a key starting material *N*-Boc-*N*,*O*-isopropylidene-L-serinol **52** which could be readily synthesized in gram-scale from

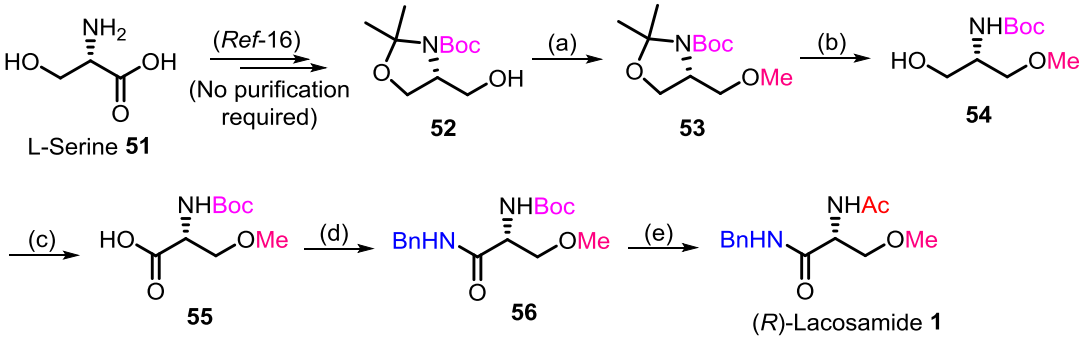
natural amino acid L-serine **51** in four steps without the need of any purification of the intermediates.[16] Further protection, deprotection and functional group transformations of **52** would lead to the desired target molecule, (*R*)-lacosamide **1**.



Scheme 7. Retrosynthetic analysis of (*R*)-lacosamide **1**.

2.2.4.2. Synthesis of (*R*)-lacosamide 1

As shown in **Scheme 8**, the synthesis of (*R*)-lacosamide **1** started from natural amino acid L-serine **51** which was converted to protected primary alcohol (*N*-Boc-*N*,*O*-isopropylidene-L-serinol) **52**. *N*-Boc-*N*,*O*-isopropylidene-L-serinol **52** was synthesized in gram-scale from L-serine in 4 steps and 81% overall yield without the need of purification of any intermediates.[16] The protected primary alcohol **52** was methylated with MeI and NaH to furnish **53** in 88% yield.[17] It is pertinent to mention here that no racemization was observed during the methylation step whereas in Kuhn *et. al.*[9a] has used neutral conditions for *O*-methylation[9a] using MeI and Ag_2O to avoid racemization (see **Scheme 1**) in the three-step synthesis of (*R*)-lacosamide **1** starting from D-Serine as a starting material. The flexibility of using basic conditions and several industrially preferable methylating agents (such as MeI, dimethyl sulfate, dimethyl carbonate, etc.) for methylation step are the added advantages to the present synthesis.



Scheme 8. Synthesis of (*R*)-lacosamide **1**. *Reagents and conditions:* (a) NaH, MeI, THF, rt, 30 min, 88%; (b) PTSA, MeOH, rt, 5 h, 86%; (c) TEMPO, NaOCl, $NaClO_2$, CH_3CN, rt, 3 h, 99%; (d) $C_6H_5CH_2NH_2$, *N*-methyl morpholine, isobutyl chloroformate, THF, −78 °C to rt, 1

h, 90%; (e) (i) TFA, DCM, rt, overnight; (ii) Ac_2O, DMAP, DCM, rt, 4h, (80% over 2 steps).

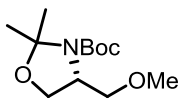
Deprotection of acetonide group of **53** was carried out in acidic conditions using *p*-toluene sulfonic acid in methanol to furnish compound **54** in 86% yield. Oxidation of primary alcohol in **54** with TEMPO resulted in the clean formation of acid **55** (99% yield) which was used in the next step as such without any further purification. Acid **55** was coupled with benzyl amine using the well-established mixed anhydride method involving *N*-methyl morpholine and isobutyl chloroformate to furnish amide **56** (90%). The final Boc deprotection of **56** with TFA followed by acetylation of the crude amine with acetic anhydride and DMAP followed by flash chromatography (SiO_2) resulted in the formation of final product (*R*)-lacosamide **1** in 80% yield over two steps. Chiral HPLC of **1** proved that no racemization had taken place during the synthesis (100% *ee*).

2.2.5. CONCLUSION

In summary, (*R*)-lacosamide **1** was synthesized using the chiral pool strategy with 100% *ee* without any racemization during the intermediate steps. Further, **1** was synthesized in 5 steps with an overall yield of 54% starting from *N*-Boc-*N*,*O*-isopropylidene-L-serinol **52** which can be readily synthesized in gram-scale from natural amino acid L-serine. The advantages of this present synthesis over the other existing methods are the use of natural amino acid L-serine as a starting material, short and high yielding steps without the need of kinetic resolution or expensive chiral ligands thereby making the synthesis of the drug molecule more easier for the industrial synthesis. Further, several *O*-substituted, *N*-substituted and amide substituted diverse analogs could be synthesized by using the chiral pool strategy with 100% *ee* for further development of new potent anti-epileptic agents.

2.2.6. EXPERIMENTAL SECTION

^{t}Butyl (*R*)-4-(methoxymethyl)-2,2-dimethyloxazolidine-3-carboxylate (53)

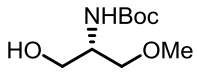


53

To a dry THF solution (30 mL) of alcohol 5**2** (3 g, 12.97 mmol) was added NaH (0.985 g of 60% dispersion in mineral oil, 24.64 mmol) and then MeI was added dropwise (1.6 mL,

25.94 mmol), and the mixture was stirred at room temperature for 30 min. After the reaction was complete (TLC), it was cooled on an ice bath and quenched by slowly adding cold water (30 mL). The mixture was extracted with dichloromethane (3 X 40 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After concentration of the combined organic layers, the residual oil was subjected to flash chromatography (CombiFlash R_f 200*i*, Teledyne Isco) using RediSep® silica gel column (12 g) eluting with petroleum ether-EtOAc (9:1, isocratic) to furnish methyl ether **53** as a colourless liquid (2.8 g, 88%); $\mathbf{R_f}$ = 0.35 (10% EtOAc in petroleum ether); $\mathbf{[\alpha]_D^{25}}$ -46.6 (*c* 1.03, $CHCl_3$) [Lit. $[\alpha]_D^{25}$ -42.4 (*c* 1.37, $CHCl_3$)[17]]; **IR** ($CHCl_3$) υ_{max}: 3370, 3017, 2934, 1688, 1464, 1387, 1252, 1217, 1168, 1095, 1030, 851, 766, 668 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 4.04-3.88 (m, 3H), 3.50 (m, 1H), 3.37 (s, 3H), 3.34-3.26 (m, 1H), 1.61-1.44 (m, 15H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ: 152.2, 151.7, 93.7, 93.3, 80.3, 79.8, 72.1, 71.3, 65.6, 65.3, 59.0, 58.9, 56.3, 56.1, 28.4, 28.4, 27.5, 26.7, 24.3, 23.0; **HRMS** (ESI): *m*/*z* calcd for $C_{12}H_{23}NO_4Na$ $[M + Na]^+$268.1519; found: 268.1506.

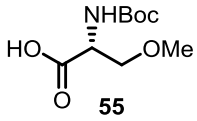
^{t}Butyl (*S*)-(1-hydroxy-3-methoxypropan-2-yl)carbamate (54)



54

To a solution of **53** (1.5 g, 6.11 mmol) in MeOH (27 mL) was added *p*-toluene sulfonic acid monohydrate (0.210 g, 1.22 mmol) and the reaction mixture was stirred at room temperature for 5 h. After the completion of the reaction, the reaction mixture was treated with saturated aqueous sodium bicarbonate solution (30 mL) and then extracted with dichloromethane (3 X 30 mL). The combined organic extracts were concentrated and the residue was purified by flash chromatography (CombiFlash R_f 200*i*, Teledyne Isco) using RediSep®silica gel column (12 g) eluting with petroleum ether-EtOAc (1:1, isocratic) to furnish alcohol **54** as a colourless liquid (1.08 g, 86%); $\mathbf{R_f}$ = 0.35 (50% EtOAc in petroleum ether); $\mathbf{[\alpha]_D^{25}}$ +4.8 (*c* 1.04, $CHCl_3$) [Lit. $[\alpha]_D^{25}$ +3.8 (*c* 0.95, $CHCl_3$)[10]]; **IR** ($CHCl_3$) υ_{max}: 3440, 3015, 2934, 1699, 1506, 1376, 1318, 1221, 1169, 1095, 1056, 978, 926, 761, 668 cm^{-1}; **^{1}H NMR** (200 MHz, $CDCl_3$) δ: 5.22 (brs, 1H), 3.86-3.44 (m, 5H), 3.37 (s, 3H), 2.98 (brs, 1H), 1.45 (s, 9H); **^{13}C NMR** (50 MHz, $CDCl_3$) δ: 156.1, 79.7,73.0, 63.7, 59.2, 51.5, 28.4; **HRMS** (ESI): *m*/*z* calcd for $C_9H_{19}NO_4Na$ $[M + Na]^+$ 228.1206; found: 228.1196.

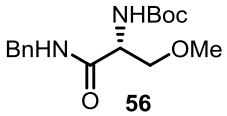
***N*-(*t*Butoxycarbonyl)-*O*-methyl-D-serine (55)**



55

To a solution of compound **54** (0.920 g, 4.48 mmol) in CH_3CN (4.6 mL) and saturated solution of KH_2PO_4 (4.6 mL) were added $NaClO_2$ (0.810 g, 8.96 mmol), TEMPO (0.190 g, 1.21 mmol) and NaOCl (0.19 mL). After stirring for 3 h, the mixture was poured into water (9 mL) and extracted with dichloromethane (3 X 30 mL). The combined organic extracts were washed with water, brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the product **55** was used in the next step without further purification (0.985 g, 99%); light orange liquid; $\mathbf{R_f}$ = 0.16 (60% EtOAc in petroleum ether); $\mathbf{[\alpha]_D^{26}}$ -13.4 (*c* 1.03, $CHCl_3$) [Lit. $[\alpha]_D^{26}$ -19.2 (*c* 1.4, $CHCl_3$)[10]]; **IR ($CHCl_3$)** υ_{max}: 3441, 3018, 2985, 2935, 1713, 1505, 1405, 1376, 1218, 1166, 1118, 1063, 925, 850, 762, 669 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ: 5.43 (brs, 1H), 4.42 (brs, 1H), 3.85 (d, *J* = 8.5 Hz, 1H), 3.64 (d, *J* = 7.3 Hz, 1H), 3.37 (s, 3H), 1.45 (s, 9H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ: 174.8, 155.8, 80.3, 72.5, 59.4, 28.4; **HRMS** (ESI): *m/z* calcd for $C_9H_{17}NO_5Na$ $[M + Na]^+$ 242.0999; found: 242.0987.

^{t}Butyl (*R*)-(1-(benzylamino)-3-methoxy-1-oxopropan-2-yl) carbamate (56)

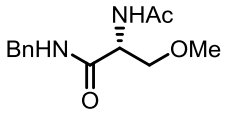


56

To a solution of acid **55** (0.985 g, 4.49 mmol) in dry THF (10 ml) was added *N*-methyl morpholine (0.6 mL, 5.39 mmol) at −78 °C under an argon atmosphere. After 5 min, isobutyl chloroformate (0.7 mL, 5.39 mmol) was added and stirred for another 5 min. To this reaction mixture benzyl amine (0.6 mL, 5.39 mmol) was added at −78 °C and the reaction mixture was allowed to come up to room temperature and stirred for another 1 h. After completion of the reaction (TLC), the reaction mixture was filtered through a pad of celite, and washed with ethyl acetate (20 mL). The solvent was removed under reduced pressure and the crude reaction mixture was subjected to flash chromatography (CombiFlash R_f 200*i*, Teledyne Isco) using RediSep® silica gel column (12 g) eluting with petroleum ether-EtOAc (3:2, isocratic) to yield **56** as a white solid (1.24 g, 90%); $\mathbf{R_f}$ = 0.35 (40% EtOAc in petroleum ether); mp 63-64 °C [Lit. 63-64 °C[10]]; $\mathbf{[\alpha]_D^{26}}$ -24.3 (*c* 1.04, $CHCl_3$) [Lit. $[\alpha]_D^{26}$ -20.5 (*c* 0.9, $CHCl_3$)[10]]; **IR ($CHCl_3$)** υ_{max}: 3428, 3345, 3017, 2933, 2405, 1708, 1671,

1493, 1365, 1220, 1167, 1116, 1075, 1025, 922, 860, 762, 668 cm^{-1}; **^{1}H NMR (200 MHz, $CDCl_3$)** δ: 7.40-7.19 (m, 5H), 6.78 (brs, 1H), 5.55-5.33 (m, 1H), 4.48 (d, *J* = 4.3 Hz, 2H), 4.27 (brs, 1H), 3.84 (dd, *J* = 3.9, 9.2 Hz, 1H), 3.50 (dd, *J* = 6.2, 9.1 Hz, 1H), 3.36 (s, 3H), 1.43 (s, 9H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ: 170.3, 155.5, 138.0, 128.7, 127.5, 80.4, 72.1, 59.1, 43.5, 28.3; **HRMS** (ESI): *m*/*z* calcd for $C_{16}H_{24}N_2O_4Na$ $[M + Na]^+$ 331.1628; found: 331.1612.

(*R*)-2-Acetamido-*N*-benzyl-3-methoxypropanamide (Lacosamide) (1)



(*R*)-Lacosamide **1**

To a solution of compound **56** (0.320 g, 1.03 mmol) in dichloromethane (4 mL) was added trifluoroacetic acid (1.6 mL) and the reaction mixture was stirred at room temperature overnight. After the completion of reaction (TLC), the solvent was evaporated under reduced pressure. The crude reaction mixture was dissolved in anhydrous CH_2Cl_2 (7.5 mL) and slowly acetic anhydride (1.0 mL, 10.57 mmol) dissolved in anhydrous CH_2Cl_2 (1.5 mL) and a catalytic amount of DMAP (27 mg, 0.103 mmol) was added. The resulting reaction mixture was stirred at room temperature for further 4 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (CombiFlash R_f 200*i*, Teledyne Isco) using RediSep® silica gel column (12 g) eluting with $CHCl_3$-MeOH (95:5, isocratic) to afford pure **1** as a white solid (0.207 g, 80% for 2 steps); **R_f** = 0.4 (10% MeOH in CH_2Cl_2); mp 140-141 °C [Lit. 140-141 °C,[11] 142-143 °C,[12] 143-144 °C[13]]; **$[\alpha]_D^{25}$** +15.9 (*c* 1.01, MeOH) [Lit. +16.2 (*c* 1, MeOH),[11] +16.1 (*c* 1.2, MeOH),[12] +16.1 (*c* 1, MeOH)[13]]; **IR ($CHCl_3$)** υ_{max}: 3671, 3421, 3304, 3015, 2933, 2404, 1657, 1515, 1456, 1376, 1218, 1115, 927, 762, 668 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 7.36-7.21 (m, 5H), 7.02 (brs, 1H), 6.66 (d, *J* = 6.1 Hz, 1H), 4.60 (dt, *J* = 4.4, 7.0 Hz, 1H), 4.51-4.37 (m, 2H), 3.77 (ddd, *J* = 1.7, 4.2, 9.2 Hz, 1H), 3.46 (dd, *J* = 7.5, 8.7 Hz, 1H), 3.36 (s, 3H), 1.99 (s, 3H); **^{13}C NMR (100 MHz , $CDCl_3$)** δ: 170.5, 170.0, 137.9, 128.7, 127.4, 71.9, 59.1, 52.5, 43.5, 23.1; **HRMS** (ESI): *m*/*z* calcd for $C_{13}H_{18}N_2O_3Na$ $[M + Na]^+$ 273.1210; found: 273.1195; **HPLC**: *ee* 100% [The *ee* of (*R*)-lacosamide (**1**) was determined by chiral HPLC analysis: Chiralcel OD-H (0.46 cm X 25 cm), *iso*-propyl alcohol-^{n}hexane-trifluoroacetic acid (30:70:0.1), flow rate 0.5 mL/min; UV detection at 220 nm; t_R = 10.5 min].

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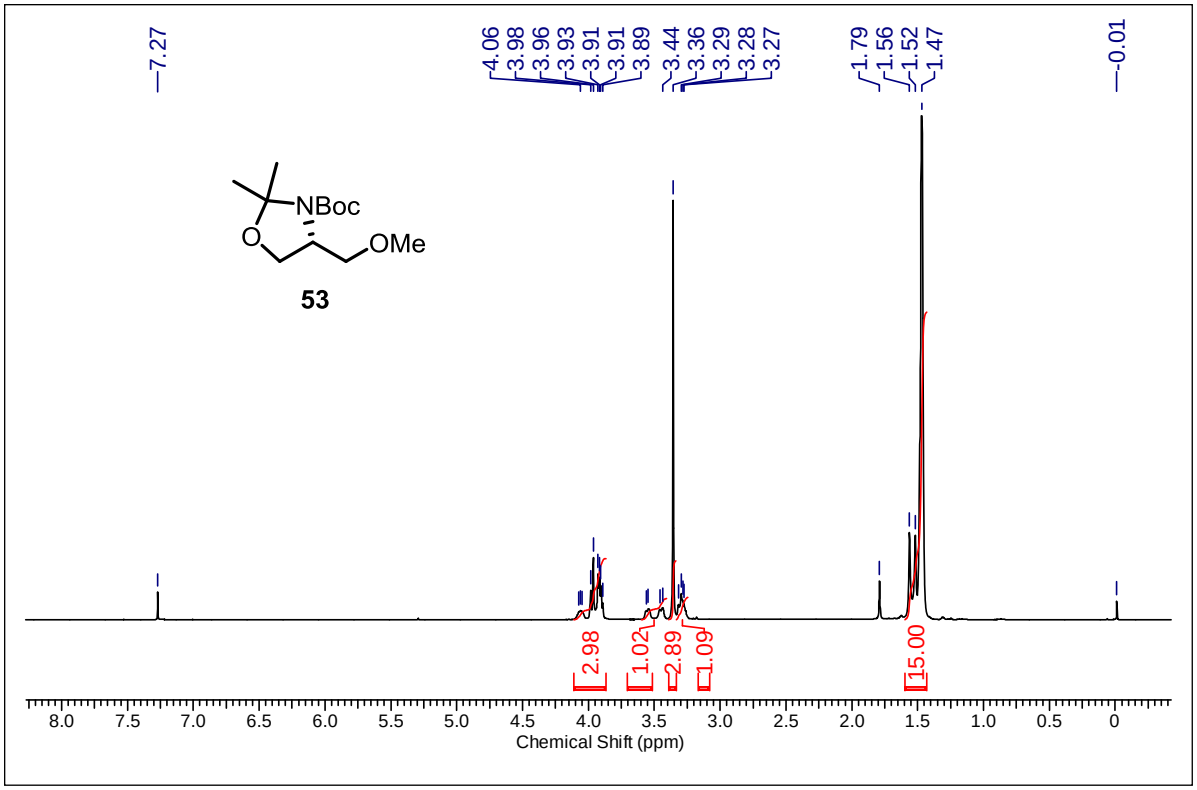
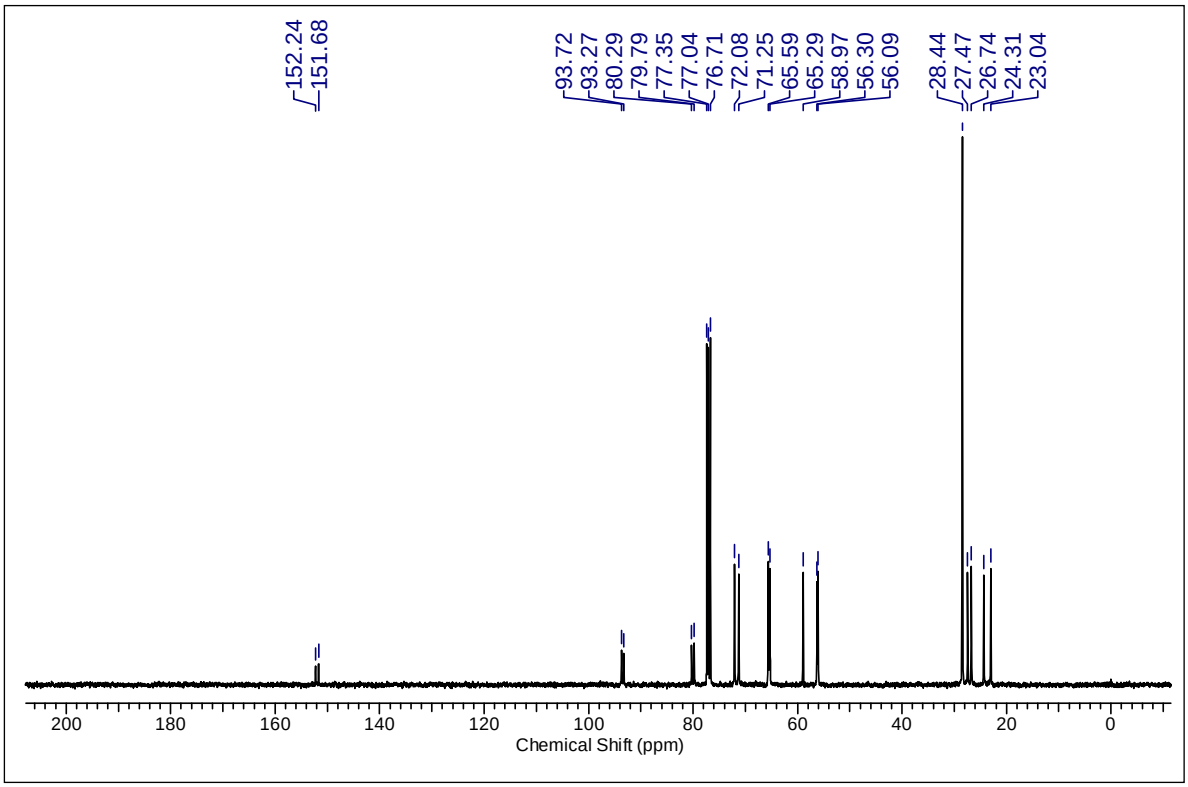
2.2.8. SPECTRA

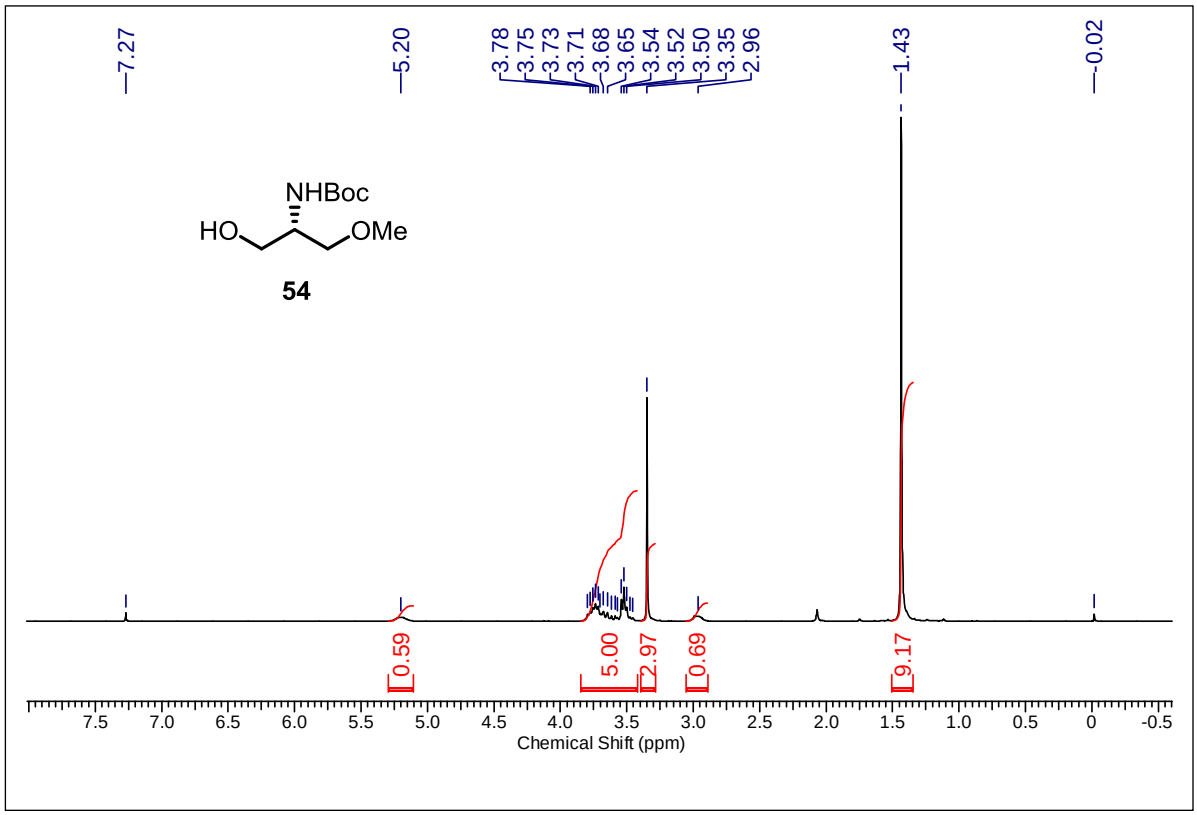
^{1}H-NMR spectrum of compound 53 (400 MHz, $CDCl_3$)



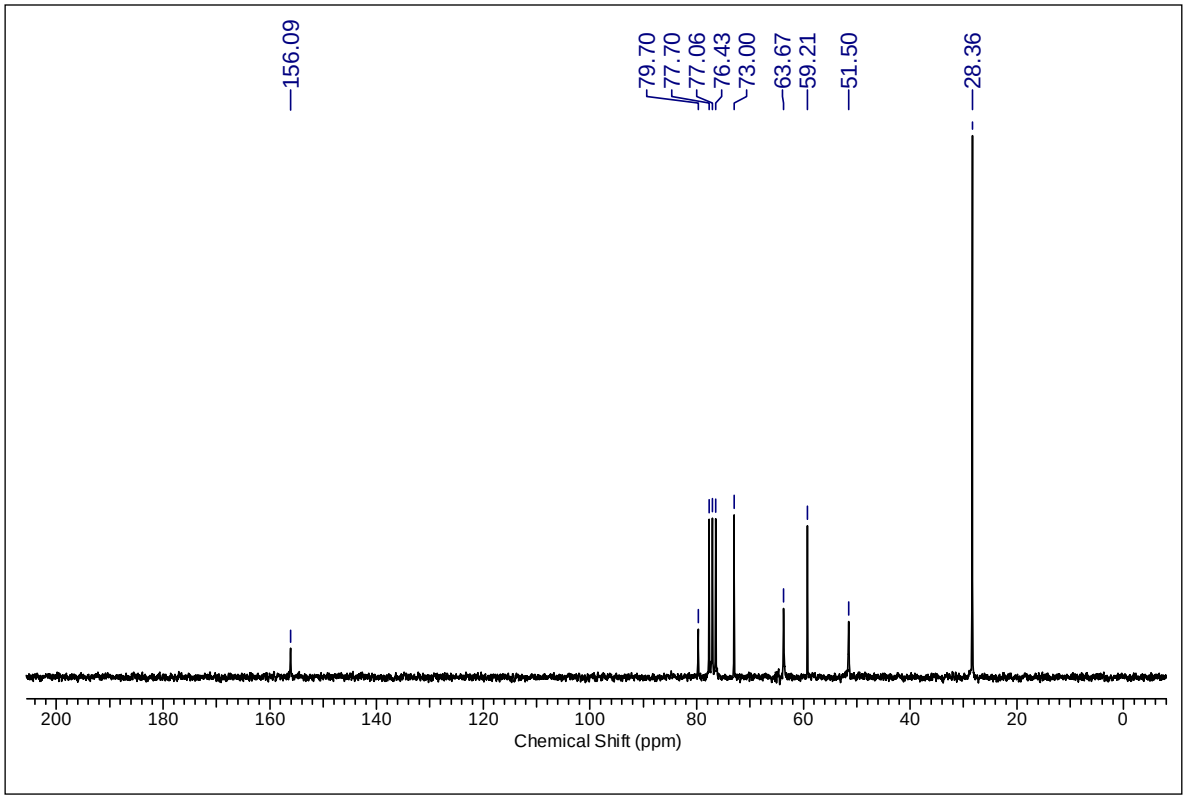
^{13}C-NMR spectrum of compound 53 (100 MHz, $CDCl_3$)



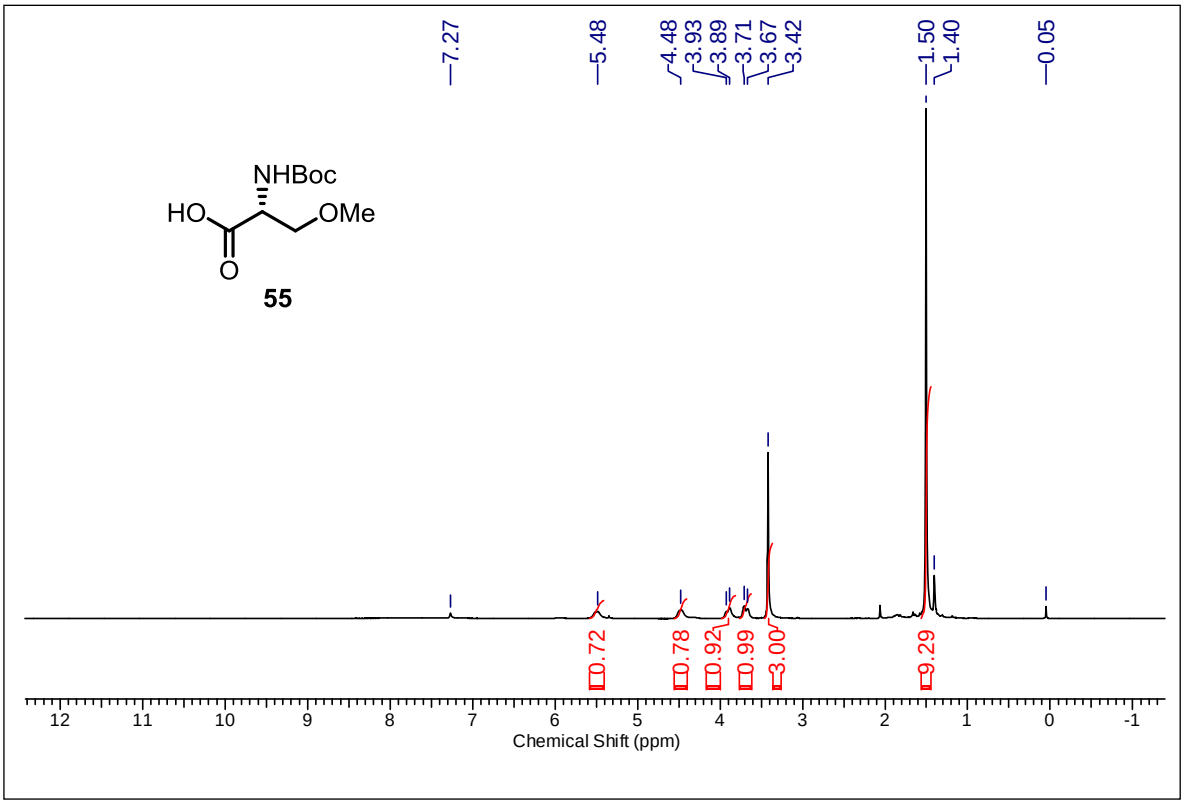
^{1}H-NMR spectrum of compound 54 (200 MHz, $CDCl_3$)



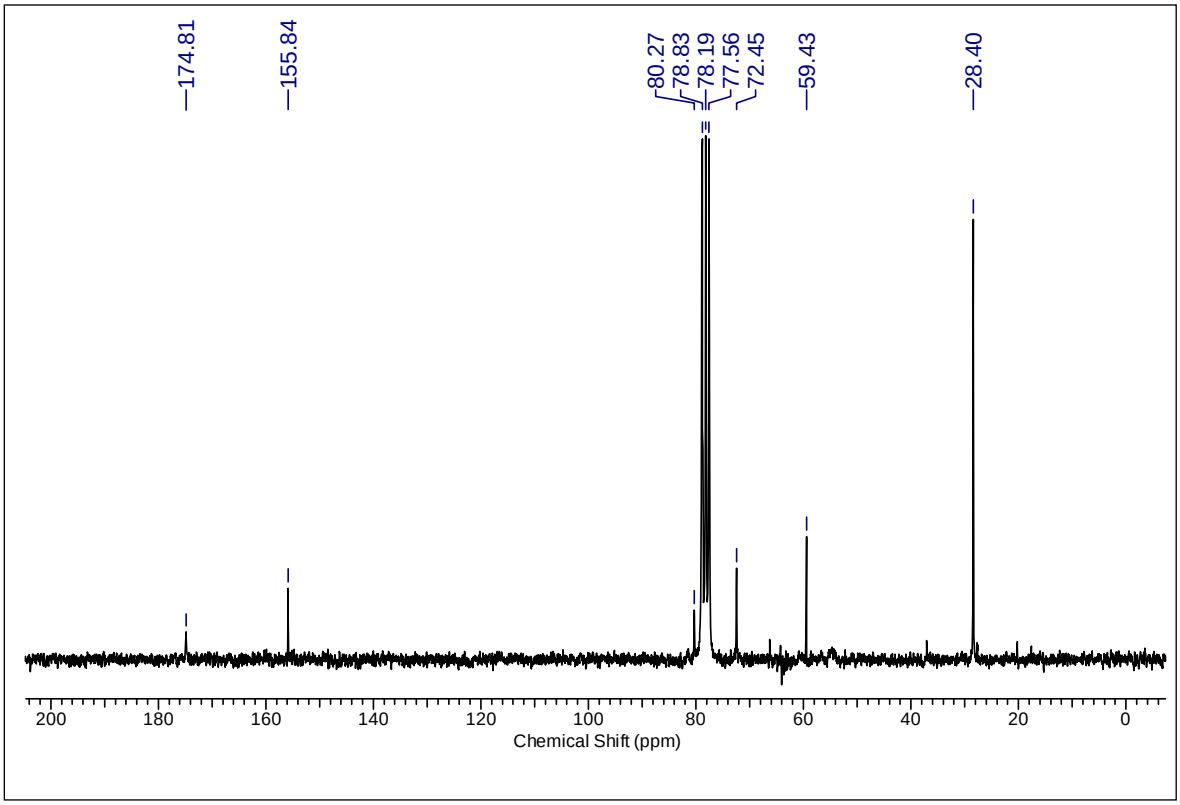
^{13}C-NMR spectrum of compound 54 (50 MHz, $CDCl_3$)



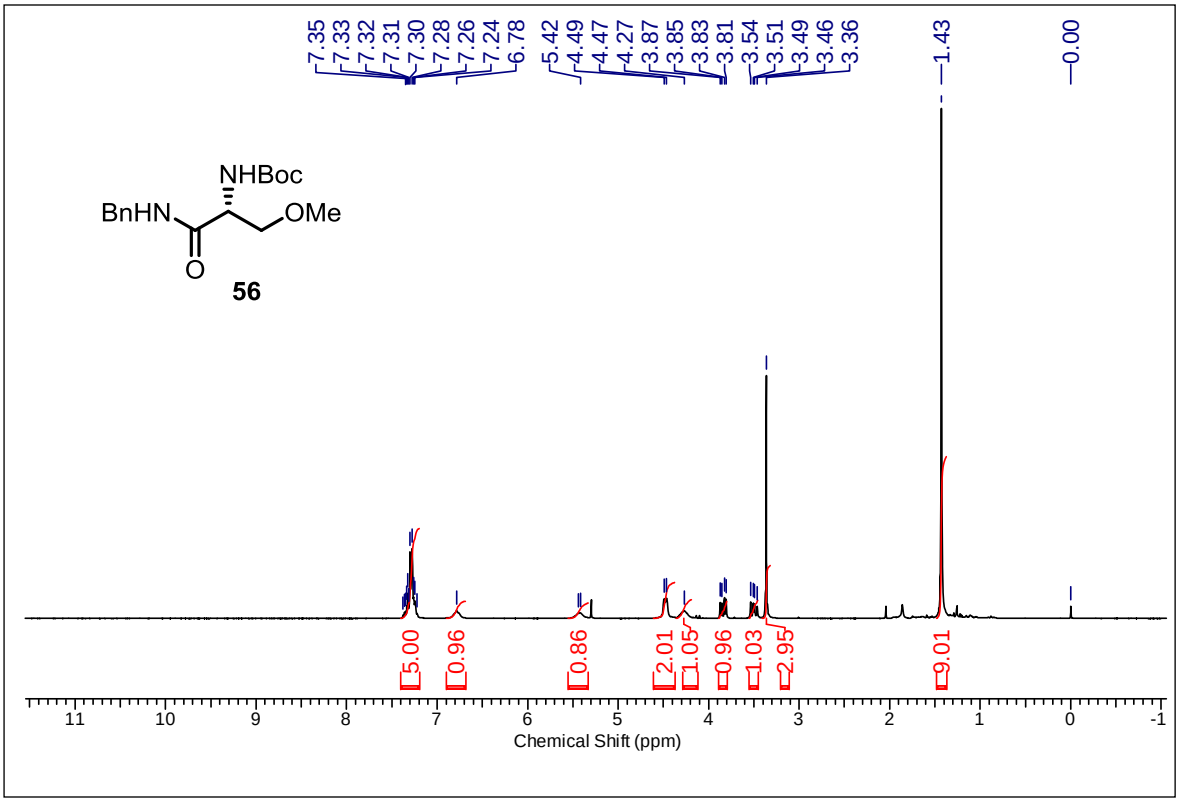
^{1}H-NMR spectrum of compound 55 (200 MHz, $CDCl_3$)



^{13}C-NMR spectrum of compound 55 (50 MHz, $CDCl_3$)



^{1}H-NMR spectrum of compound 56 (200 MHz, $CDCl_3$)



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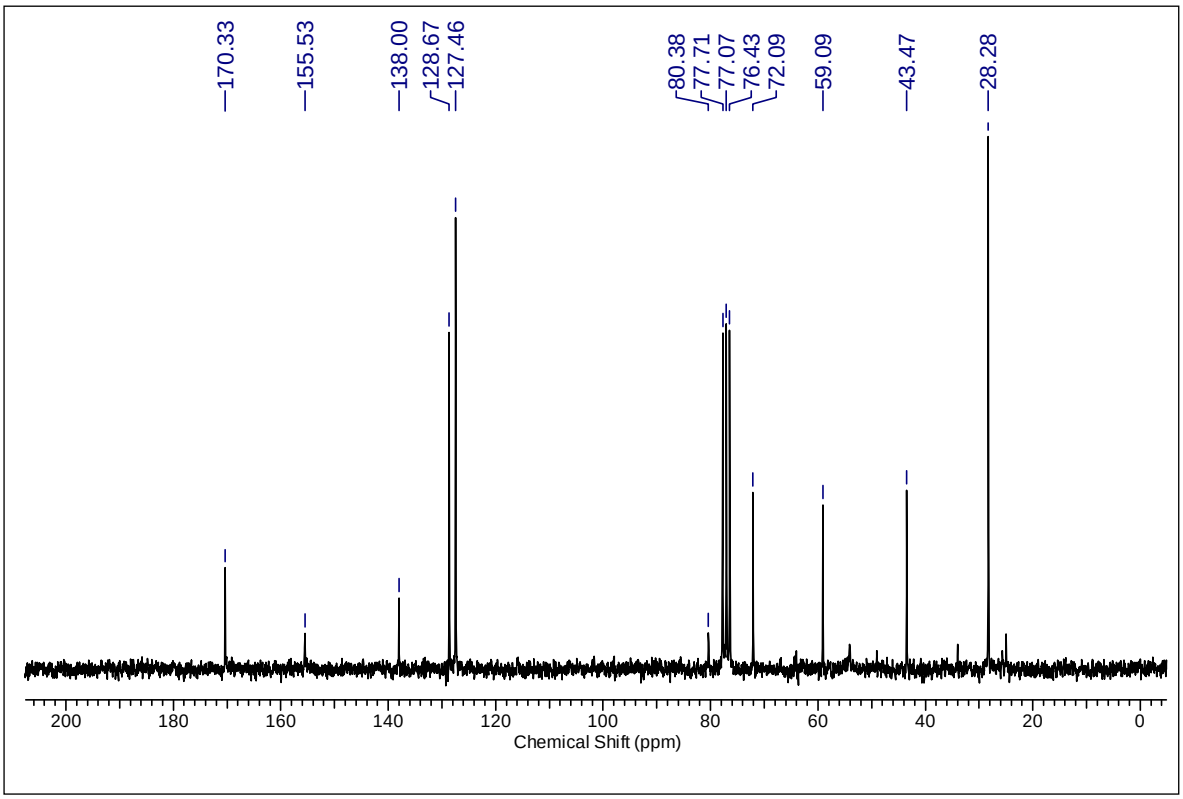
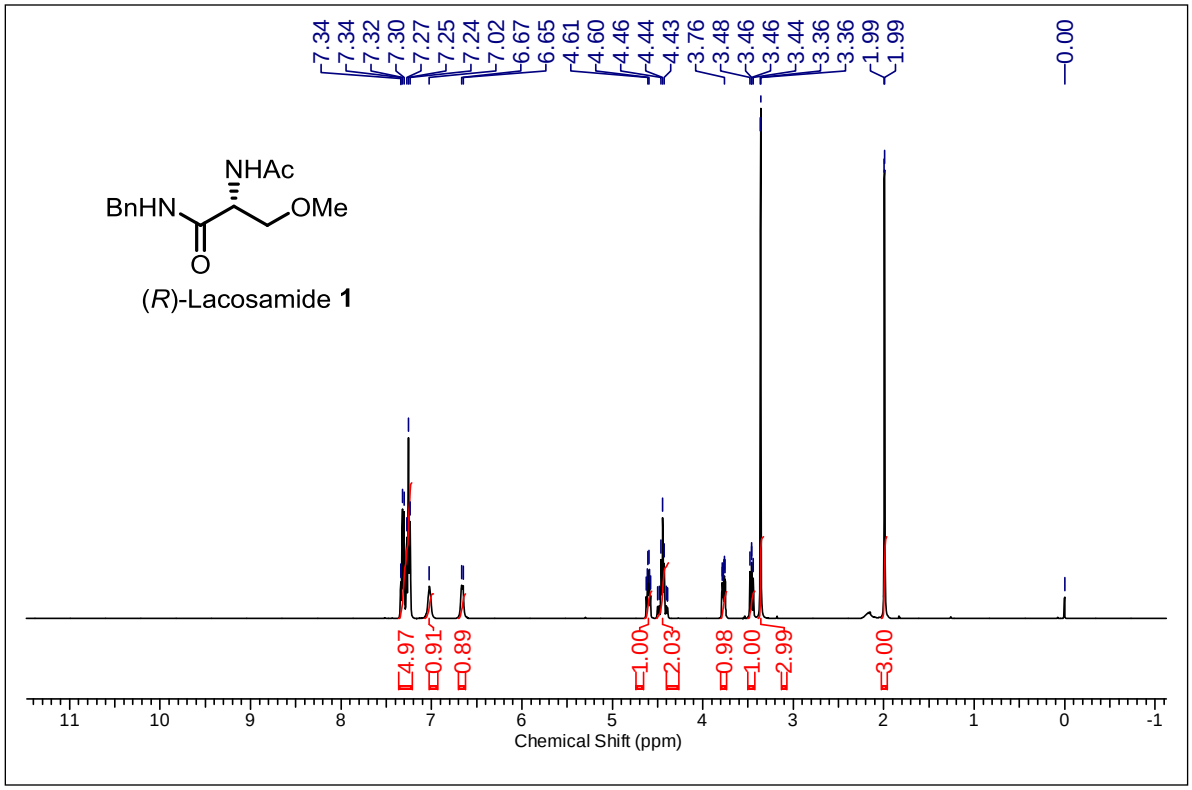
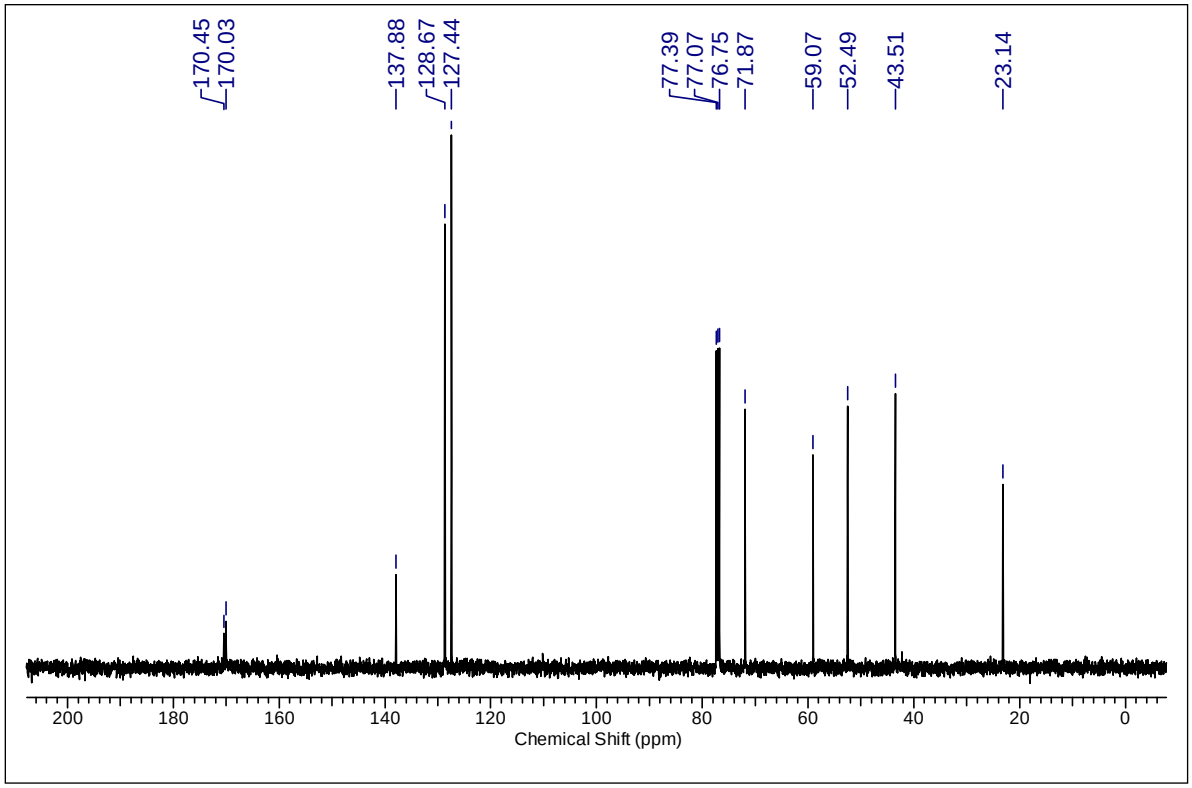


^{1}H-NMR spectrum of (*R*)-lacosamide 1 (400 MHz, $CDCl_3$)



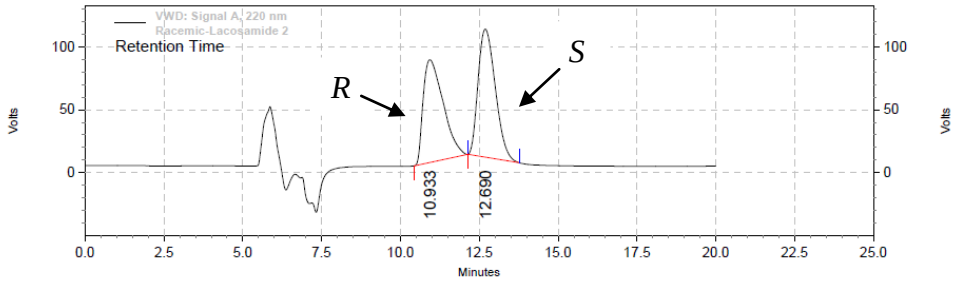
^{13}C-NMR spectrum of (*R*)-lacosamide 1 (100 MHz, $CDCl_3$)



2.2.9. Chiral HPLC analysis data

HPLC Analysis of *rac*-lacosamide

Chiralcel OD-H, PE:IPA:TFA (70:30:0.1), flow 0.5 mL/min, det.: UV 220 nm



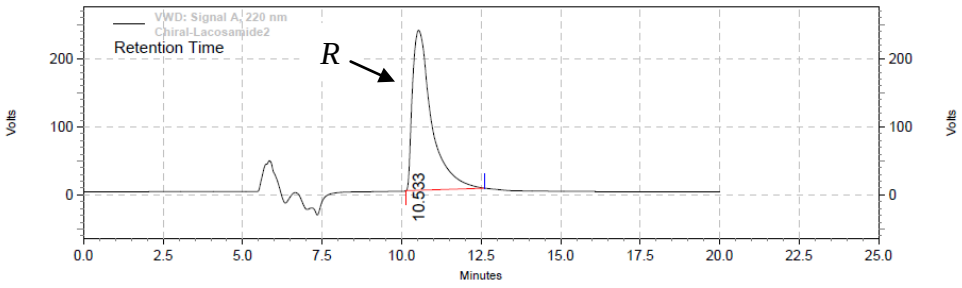
VWD: Signal A, 220 nm Results

Retention Time	Area	Area %
10.933	60362527	48.13
12.690	65048227	51.87
Totals	125410754	100.00

Column : Chiral OD-H
Eluent System : 70:30:0.1 (PE:IPA:TFA)
Flow rate: 0.5 mL/min
Injection vol..: 20ul
Wavelength: 220 nm
Sample Conc.: 1 mg/ ml

HPLC Analysis of (*R*)-lacosamide 1

Chiralcel OD-H, PE:IPA:TFA (70:30:0.1), flow 0.5 mL/min, det.: UV 220 nm



VWD: Signal A, 220 nm Results

Retention Time	Area	Area %
10.533	163938209	100.00
Totals	163938209	100.00

Column : Chiral OD-H
Eluent System : 70:30:0.1 (PE:IPA:THF)
Flow rate: 0.5mL/min
Injection vol..: 20ul
Wavelength: 220 nm
Sample Conc.: 1 mg/ ml

Chapter 3

Studies Directed Towards Bioactives from *Vernonia arborea*

Synthesis and anticancer studies of Michael adducts and Heck arylation products of sesquiterpene lactones, zaluzanin D and zaluzanin C from *Vernonia arborea*

3.1. INTRODUCTION

The generation of diverse chemical libraries using natural products as scaffolds is considered one of the effective methods for drug discovery.[1] Sesquiterpene lactones are sesquiterpenoids (3 isoprene units) and contain a lactone ring. Sesquiterpenes bearing α,β-unsaturated γ-lactones are ubiquitous in nature and have been reported to be isolated from various genera of the family Asteraceae but also occur sporadically in other angiosperm families, Apiaceae, Magnoliaceae and even in some liverworts. They exhibit diverse biological activities such as anti-microbial, anti-inflammatory, antiulcer, antiviral, anticancer and antimalarial activities.[2] Sesquiterpene lactones can be divided into several main classes such as germacranolides, heliangolides, guaianolides, pseudoguaianolides, hypocretenolides, eudesmanolides depending on ring framework as shown in **Figure 1**.

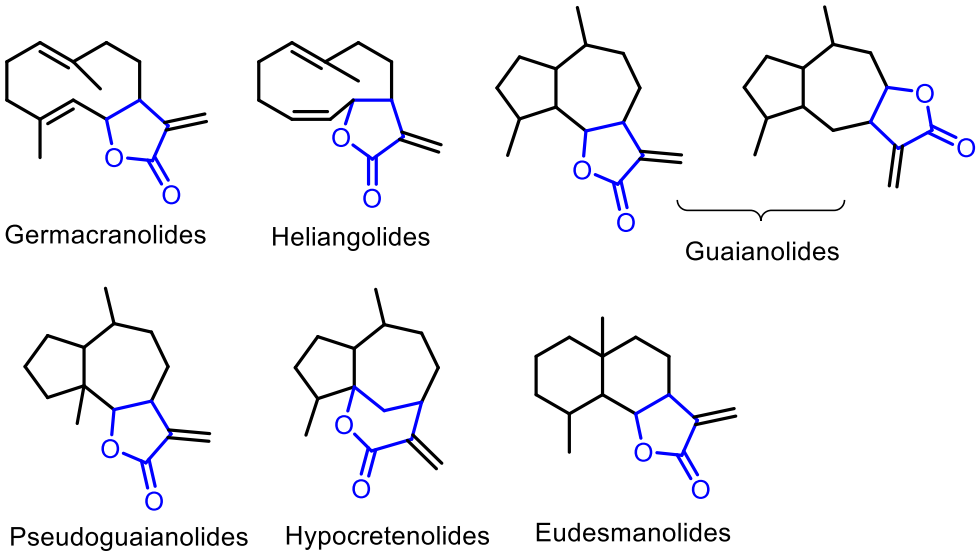


Figure 1. Some main classes of sesquiterpene lactones.

The genus *Vernonia* (family Asteraceae) consists of approximately 1000 species of herbs and shrubs.[3] Several research groups have isolated structurally diverse sesquiterpene lactones from various species of genus *Vernonia* possessing various biological properties.[4,5]

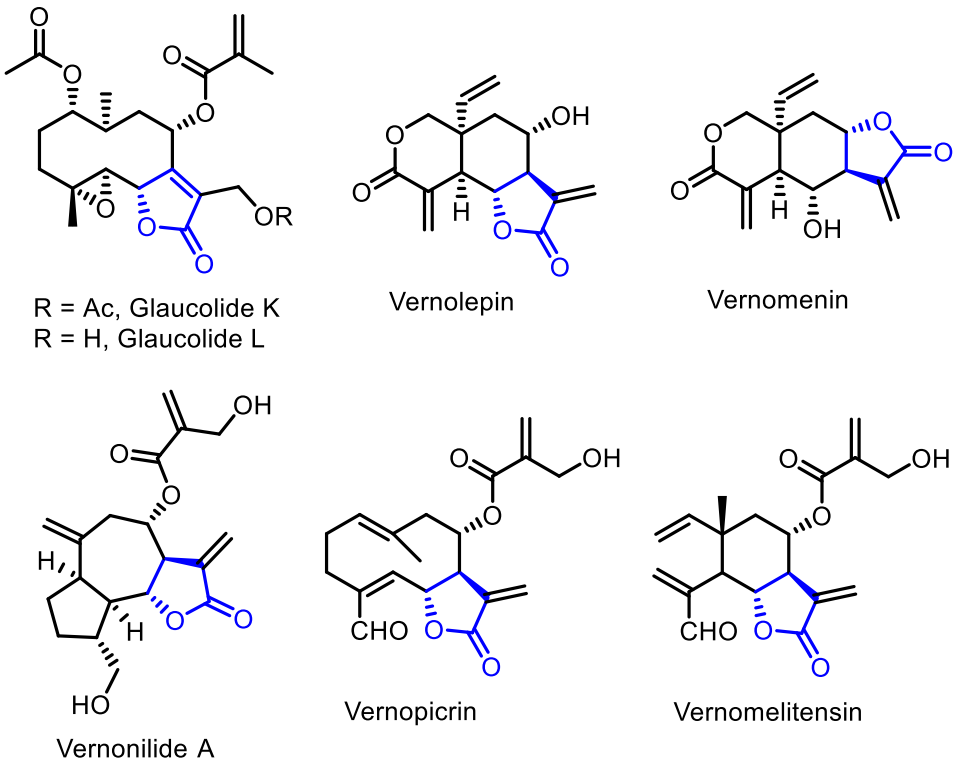


Figure 2. Selected examples of sesquiterpene lactones isolated from genus *Vernonia*.

3.2. REVIEW OF LITERATURE

Sesquiterpene lactones react with nucleophilic sulfhydryl groups present in enzymes, proteins, and glutathione.[6] The use of sesquiterpene lactones as therapeutic agents is limited due to their poor water solubility. To overcome this, amino-adducts of sesquiterpene lactones have been prepared by adding different amines to the *α*-methylene-*γ*-lactone substructure to enhance the water solubility of the parent molecules and to retain their biological activity.[7,8] Regeneration of parent α,β-unsaturated γ-lactone occurs by the *retro*-Michael reaction, potentially through bioactivation at the site of action (**Figure 3**). This prodrug approach has transformed several sesquiterpene lactones such as alantolactone, ambrosin, arglabin, costunolide, helenalin, parthenolide and ivangustin, into successful clinical candidates.[8]

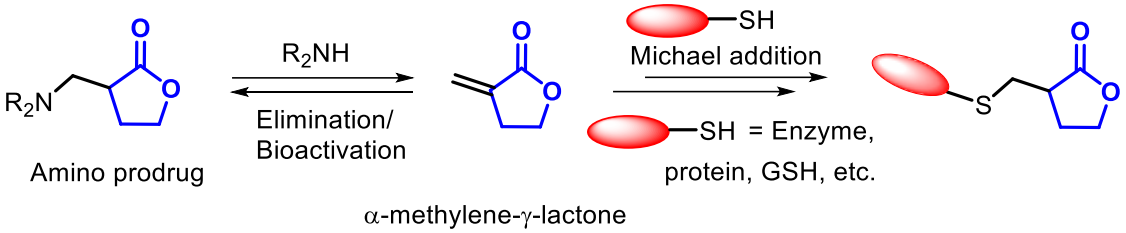


Figure 3. Conversion of α-methylamino-γ-butyrolactones to α-methylene-γ-butyrolactones and trapping with biological thiols.

3.3. PRESENT WORK

Objective

Continuing our interest in naturally occurring sesquiterpene lactones[9] and other bioactive secondary metabolites, we wished to take up the chemical examination of *V. arborea* leaves for the isolation of bioactive secondary metabolites, zaluzanin C and zaluzanin D. In the present study, several structurally diverse Michael adducts of zaluzanin D have been synthesized with the formation of one or two new C-N bonds. Analogs of zaluzanin D with a new C-C bond formation have also been synthesized by using Pd catalyzed Heck coupling reaction. The *in vitro* anticancer activities of all the analogs were tested against human breast cancer cell lines MCF7 and MDA-MB-231.

3.4. RESULTS AND DISCUSSION

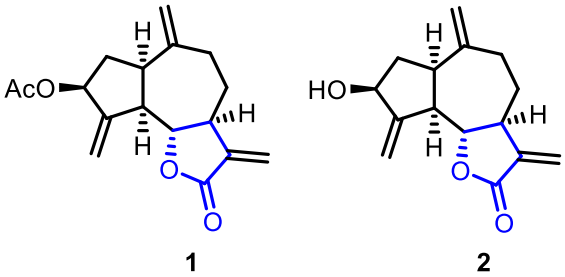
3.4.1. Plant material

The aerial parts of *V. arborea* were collected from the Kolli Hills (Perumakkai Shola), Tamilnadu, India during March 2013. The plant was identified by Prof. Dr. N. Parthasarathy, Department of Ecology and Environmental Sciences, Pondicherry University, India, where a voucher specimen (No. 5318) is being maintained.

Extraction and Isolation

Air-dried and grounded leaves (2.8 kg) of *V. arborea* were extracted with petroleum ether (5 x 5.0 L) at room temperature for five days. After completion of the extraction, the solvent was evaporated under reduced pressure to afford the petroleum ether extract (105 g). The remaining plant material was further extracted with MeOH (5 x 5.0 L) at room temperature for five days. After completion of the extraction, the solvent was evaporated under reduced pressure to afford the MeOH extract (224.15 g).

A portion of the petroleum ether extract (5.3 g) was flash chromatographed on CombiFlash Companion, Isco Teledyne Inc., USA using RediSep® column (SiO_2, 2x12 g, stacked together) and isocratic elution was done with ethyl acetate-petroleum ether (4:96) to furnish the pure compound (66.3 mg, 0.04%) which was identified as zaluzanin D **1** on the basis of its spectral data, comparison of spectral data with reported data[10] and co-TLC with an authentic sample. Further flash chromatography with ethyl acetate-petroleum ether (6:94) furnished a pure compound, which was identified as zaluzanin C **2** (16.3 mg, 0.0098%) by its spectral data and comparison of spectral data with reported data.[10]



1 **2**

Figure 4. Structures of zaluzanin **1** and zaluzanin C **2** isolated from *V. arborea*.

Further flash chromatography with ethyl acetate-petroleum ether (6:94) furnished zaluzanin C **2** (**Figure 4**). Finally, we proved the structure of zaluzanin D **1** by its single crystal X-ray analysis (**Figure 5**).[11a]

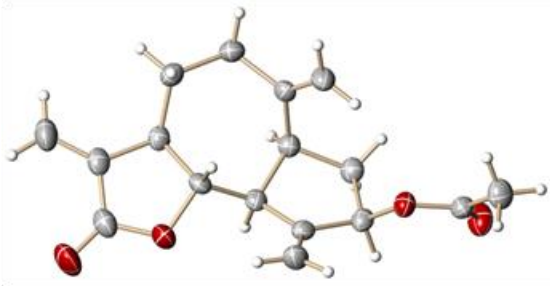


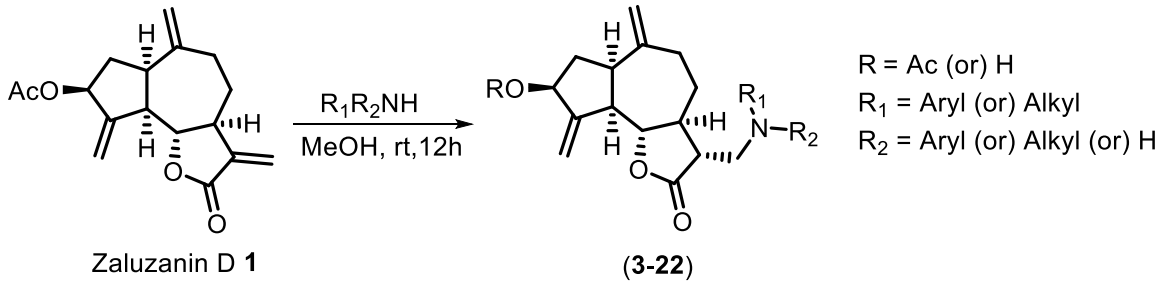
Figure 5. ORTEP diagram of zaluzanin D **1**.

3.4.2. Chemistry

3.4.2.1. Synthesis of Michael adducts of zaluzanin D using different chiral/achiral amines

Since Michael addition of primary or secondary amines to α,β-unsaturated lactones resulted in the compounds possessing higher anticancer activities compared to the original

compound, we wished to synthesize a library of amino adducts of zaluzanin D with one or more new C-N bonds. The amino derivatives of zaluzanin D **1** were synthesized *via* Michael addition of different amines to the α,β-unsaturated γ-lactone functionality present in zaluzanin D. As shown in **Scheme 1**, a methanolic solution of chiral/achiral amine and zaluzanin D underwent Michael addition at room temperature to furnish various Michael adducts of zaluzanin D, **3-22** (**Table 1**).[11b] Michael addition of chiral amine (*S*)-(-)-α-methylbenzylamine to zaluzanin D furnished a mixture of two products, compound **3** and its corresponding deacetylated product **4** (entry 1, **Table 1**) which were isolated by flash chromatography. The formation of deacetylated product **4** may be due to the *in situ* formation of zaluzanin C which in turn formed due to the basicity of amine used in the Michael addition.



Scheme 1. Synthesis of Michael adducts of zaluzanin D **1**.

Addition of the amine to zaluzanin D was found to be diastereoselective, and the stereochemistry of C-11 in compound **3** was assigned[12] as α by its NOESY experiment. In the NOESY spectra of compound **3**, correlations were observed between 6β-H and 11β-H. Also, 5α-H showed a correlation with 7α-H (**Figure 6**).

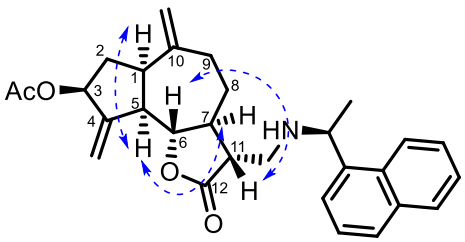
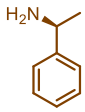
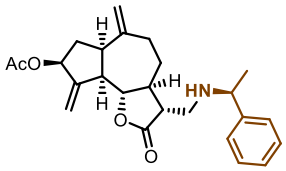
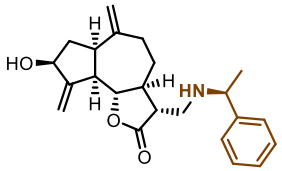
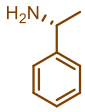
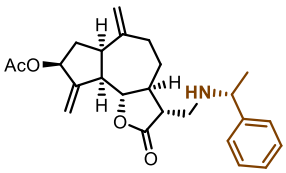
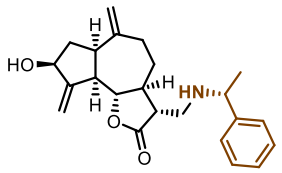
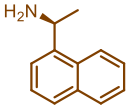
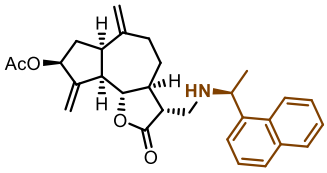
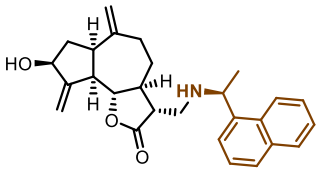


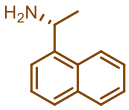
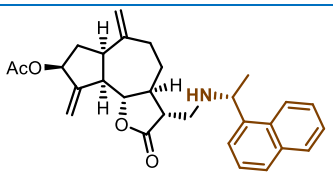
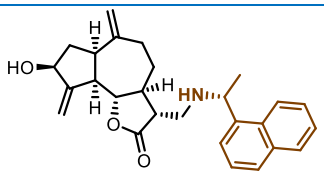
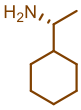
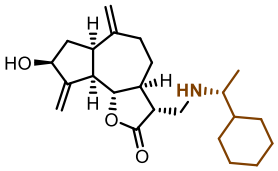
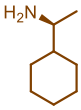
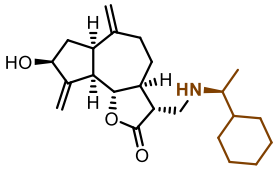
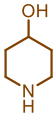
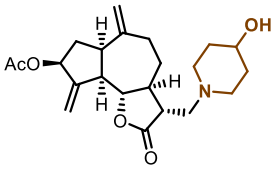
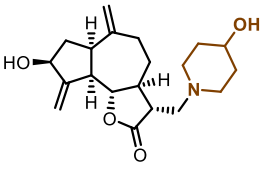
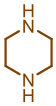
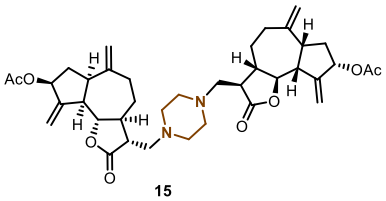
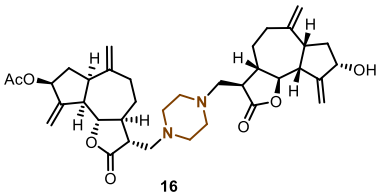
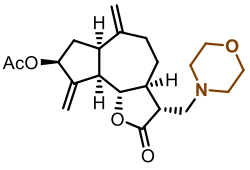
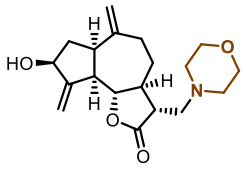
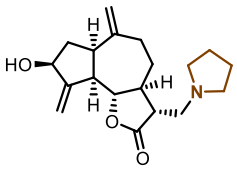
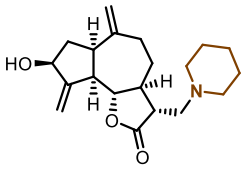
Figure 6. NOE correlations of compound **3**.

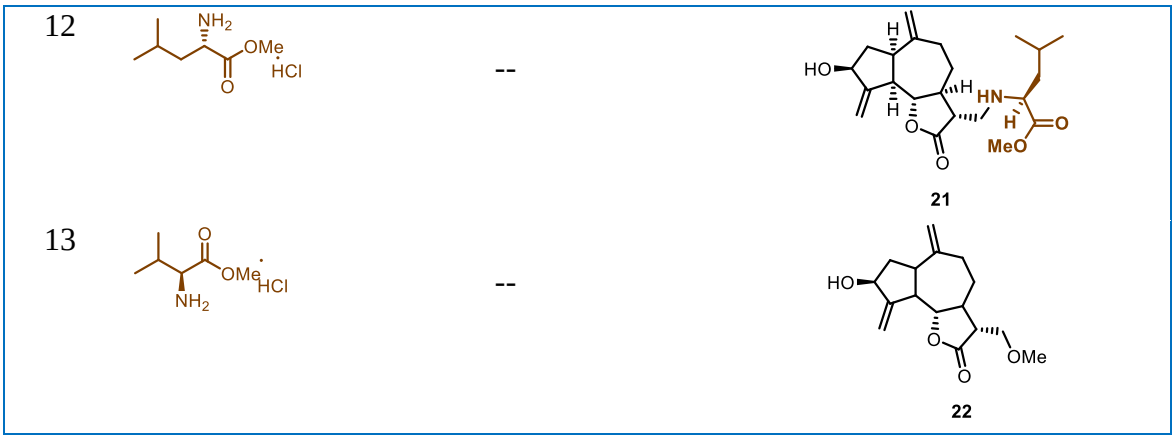
Similarly, the reaction of zaluzanin D **1** with (*R*)-(+)-α-methylbenzylamine resulted in the formation of compounds **5** and **6**. The reaction of zaluzanin D **1** with (*S*)- and (*R*)-1-(1-

naphthyl) ethylamine also furnished of compounds **7**, **8** and **9**, **10** respectively (entries 3 and 4, **Table 1**). However, reaction with (*R*)- and (*S*)-1-cyclohexylethylamine furnished the deacetylated product only (compounds **11** and **12**, entries 5 and 6, **Table 1**). Next, we carried out the reaction of zaluzanin D **1** with various six and five-membered cyclic amines, which furnished corresponding C-N derivatives of zaluzanin D **1** (entries 7-11, **Table 1**). 4-Hydroxypiperidine and morpholine on reaction with zaluzanin D **1** yielded acetylated products (**13** and **17**) and deacetylated products (**14** and **18**), respectively (entries 7 and 9, respectively, **Table 1**). An interesting result was obtained in case of piperazine (entry 9, **Table 1**), which furnished acetylated dimer **15** and mono deacetylated dimer **16** with two new C-N bonds on both sides of piperazine. However, pyrrolidine and piperidine resulted in the formation of deacetylated products only (**19** and **20**, respectively) (entries 10 and 11, **Table 1**). Further, we tried to form a C-N bond of zaluzanin D **1** with amino acid methyl ester hydrochlorides (entries 12 and 13, **Table 1**). In this case, K_2CO_3 was used as a base for *in situ* generation of the free amine, which on reaction with zaluzanin D **1** furnished the deacetylated product **21**. However, in case of valine methyl ester hydrochloride, excess use of K_2CO_3 resulted in the C-O bond formed product **22**.

Table 1. Michael adducts of zaluzanin D synthesized using various amines.

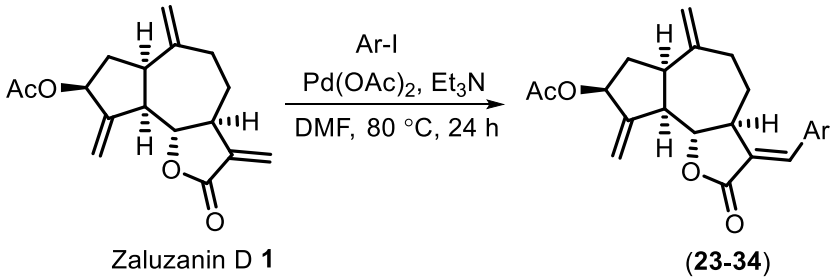
Entry	Amine	Product (s)	
1		 **3**	 **4**
2		 **5**	 **6**
3		 **7**	 **8**

4		 9	 10
5		--	 11
6		--	 12
7		 13	 14
8		 15	 16
9		 17	 18
10		--	 19
11		--	 20



3.4.2.2. Synthesis of Heck arylated analogs of zaluzanin D using different aryl iodides (23-34)

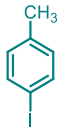
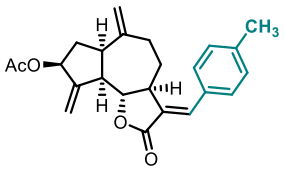
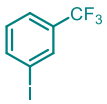
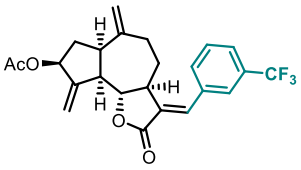
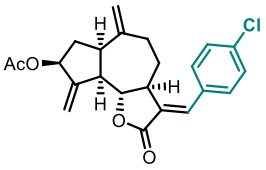
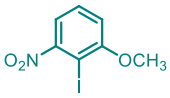
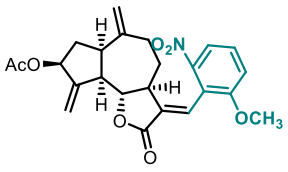
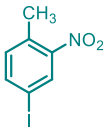
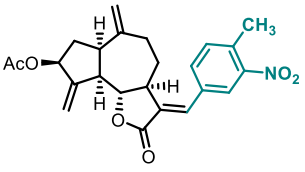
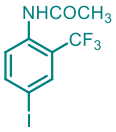
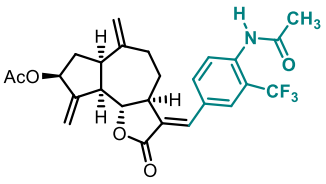
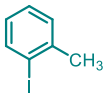
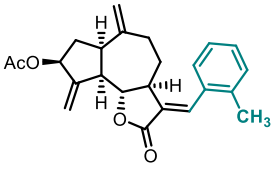
Although several structural modifications such as Michael addition, reduction, cyclopropanation of the double bond, oxidation of hydroxyl group of sesquiterpene lactones have been made in literature, transition metal catalyzed cross coupling reactions of sesquiterpene lactones were less explored.[13] We presumed that Heck arylation of α,β-unsaturated γ-lactone core of zaluzanin D would provide additional information on structure-activity data of zaluzanin D. As shown in **Scheme 2**, Heck arylation analogs (**23-34**) of zaluzanin D were synthesized[11b] using the standard Heck coupling conditions (5 mol% $Pd(OAc)_2$, Et_3N in DMF at 80 °C) and readily available aryl iodides.

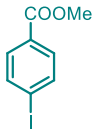
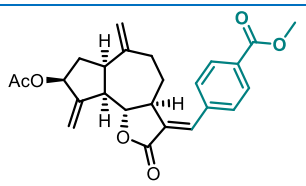
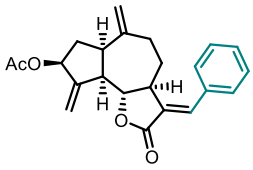
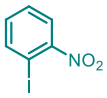
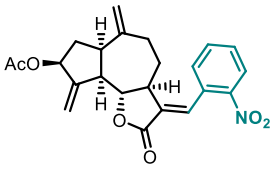
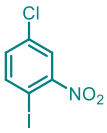
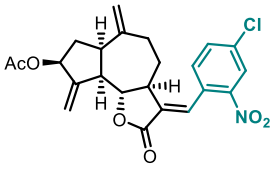
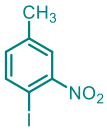
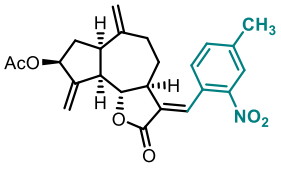


Scheme 2. Synthesis of Heck analogs of zaluzanin D **1**.

It should be mentioned here that arylation preferred to take place at the exo methylene of α,β-unsaturated γ-lactone substructure over the other two isolated exo methylene groups present in zaluzanin D and resulted in the exclusive formation of *E*-olefin[14] containing products only (**Table 2**).

Table 2. Heck analogs of zaluzanin D synthesized using various aryl iodides.

Entry	Substrate	Aryl iodide	Product
1	zaluzanin D		 **23**
2	zaluzanin D		 **24**
3	zaluzanin D		 **25**
4	zaluzanin D		 **26**
5	zaluzanin D		 **27**
6	zaluzanin D		 **28**
7	zaluzanin D		 **29**

8	zaluzanin D		 **30**
9	zaluzanin D		 **31**
10	zaluzanin D		 **32**
11	zaluzanin D		 **33**
12	zaluzanin D		 **34**

3.4.3. Biology

3.4.3.1. *In vitro* anticancer activities of Michael adducts of zaluzanin D (3-22)

Since zaluzanin D **1** had exhibited anticancer activity against human breast cancer cell line (**Table 3**), we screened all the synthesized compounds for their antiproliferative activity against breast cancer cell line MCF7 and selected compounds **1**, **9**, **10** and **14** against MDA-MB-231. Cells were treated with different concentrations (0–100 μM) of the synthesized compounds for 48 h and then MTT assay was performed as described in the experimental section. Among these compounds **1**, **9**, **10** and **14** showed activity (IC_{50}<50 μM) against MCF7 and MDA-MB-231 (**Figure 5** and **Table 3**). Among these four compounds, (*R*)-1-(1-naphthyl) ethylamine adducts of zaluzanin D and C (*i.e.*, **9** and **10**) were found to show most potent antiproliferative effect (IC_{50} 30 μM and 18.8 μM, respectively) against MCF7 cells as compared to other synthesized compounds. Interestingly, compound **9** was found to possess

more potent antiproliferative activity in highly metastatic MDA-MB-231 cell line (IC_{50} 13.33 µM) as compared to MCF7 (IC_{50} 30 µM) suggesting that **9** could be more effective in inducing cell death in higher grades of breast cancer. However, it also has potent cytotoxic effect on the normal breast epithelial MCF10A cells suggesting that compound **9** could not be useful for therapeutic purpose (**Figure 5c** and **Table 3**). Interestingly, compound **10** has the minimal cytotoxic effect against the MCF10A suggesting that it could be a better candidate compound for further designing the small molecule to chemotherapy (**Figure 5c** and **Table 3**). The *in vitro* anticancer activities of all the synthesized Michael adducts are depicted in **Table 3**.

Table 3. *In vitro* anticancer activities of Michael adducts of zaluzanin D (**3-22**).

Compound	IC_{50} in µM (mean ± sd) against		
	MCF7	MDA-MB-231	MCF10A
1 (zaluzanin D)	53.7 ± 4.11	34.17 ± 4.48	29.6 ± 0.84
2 (zaluzanin C)	>100	Not tested	Not tested
3	71.2 ± 0.9	Not tested	Not tested
4	>100	Not tested	Not tested
5	>100	Not tested	Not tested
6	>100	Not tested	Not tested
7	>100	Not tested	Not tested
8	75.8 ± 3	Not tested	Not tested
9	30 ± 0.7	13.33 ± 1.53	42 ± 4.1
10	18.83 ± 1.7	23 ± 1	>100
11	>100	Not tested	Not tested
12	>100	Not tested	Not tested
13	56.1 ± 0.9	Not tested	Not tested
14	40 ± 2.16	25 ± 2.3	>50
15	>100	Not tested	Not tested
16	>100	Not tested	Not tested
17	>100	Not tested	Not tested

18	>100	Not tested	Not tested
19	>100	Not tested	Not tested
20	>100	Not tested	Not tested
21	>100	Not tested	Not tested
22	>100	Not tested	Not tested

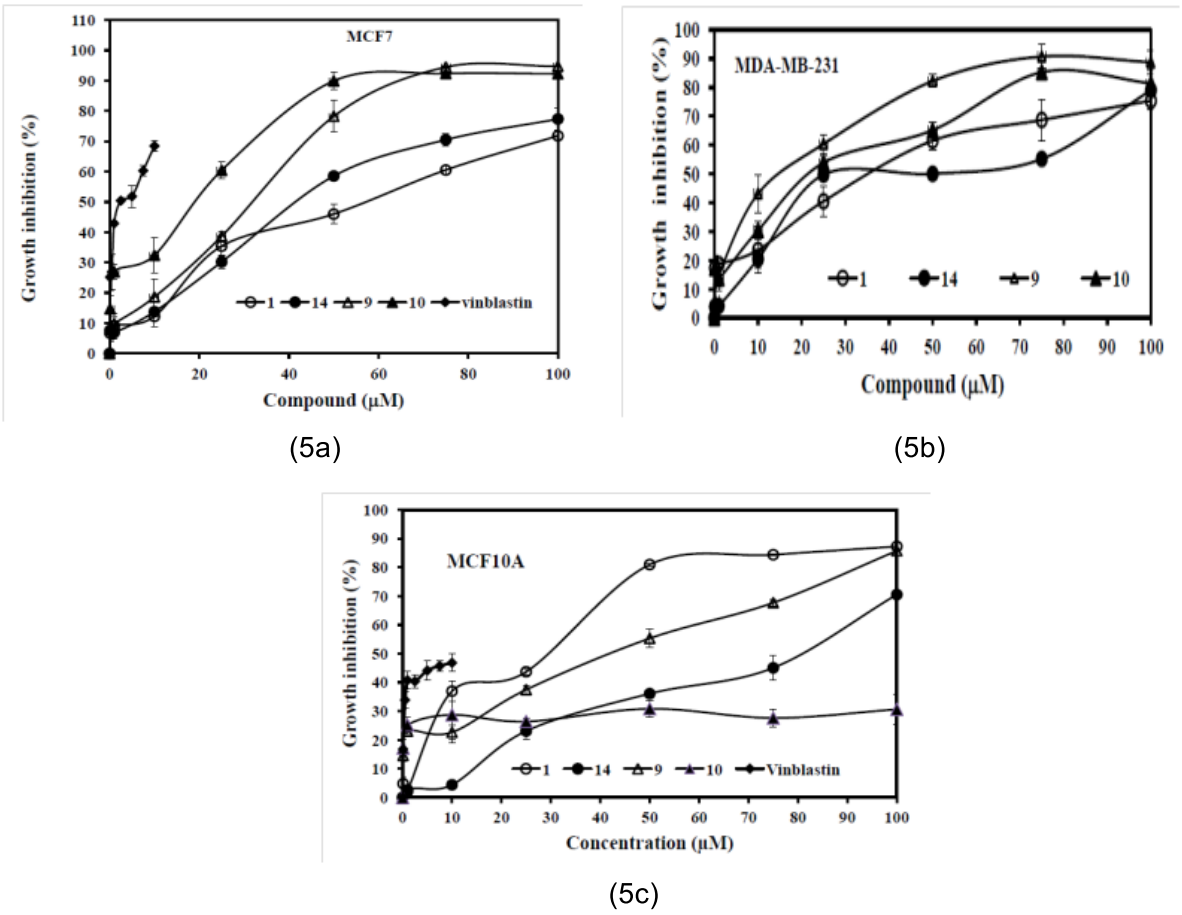


Figure 5. Effect of selected compounds on growth of breast cancer cell lines. Breast cancer cell line (a) MCF7 (b) MDA-MB-231 and (c) MCF10A were treated with different concentrations of compounds for 48 h, and then MTT assay was performed as described in experimental section. MTT assay was repeated three times and mean values were plotted with standard deviation.

To understand the inhibition of proliferation of cancer cells by these compounds, we have performed the FACS as described in the experimental section. Results showed that treatment of MCF7 cells with **9** and **10** significantly increased the apoptotic populations while

compound **1** and **14** induced apoptosis to a lesser extent indicating that compounds **9** and **10** execute their antiproliferative activity through induction of apoptosis (**Figure 6a**). To validate the apoptotic induction by these two compounds (**9** and **10**), we then checked fragmentation profile of genomic DNA following their treatment.[15] Results demonstrated that the presence of substantial fragmented DNA ladder following treatment of MCF7 cells with these compounds (**Figure 6b**). Similar results were also observed *in vivo* upon staining the DNA with Hoechst (**Figure 6c**). Results taken together revealed that compounds **9** and **10** are the potent inducer of apoptosis and thereby inhibit the cancer cells proliferation. We then checked the apoptotic pathway and found that **9** and **10** induced apoptosis through the intrinsic pathway of apoptosis through cleavage of caspase 9 (**Figure 6d**).[16-17] Collectively, these results suggest that **9** and **10** may function as anticancer agents through induction of intrinsic apoptosis.

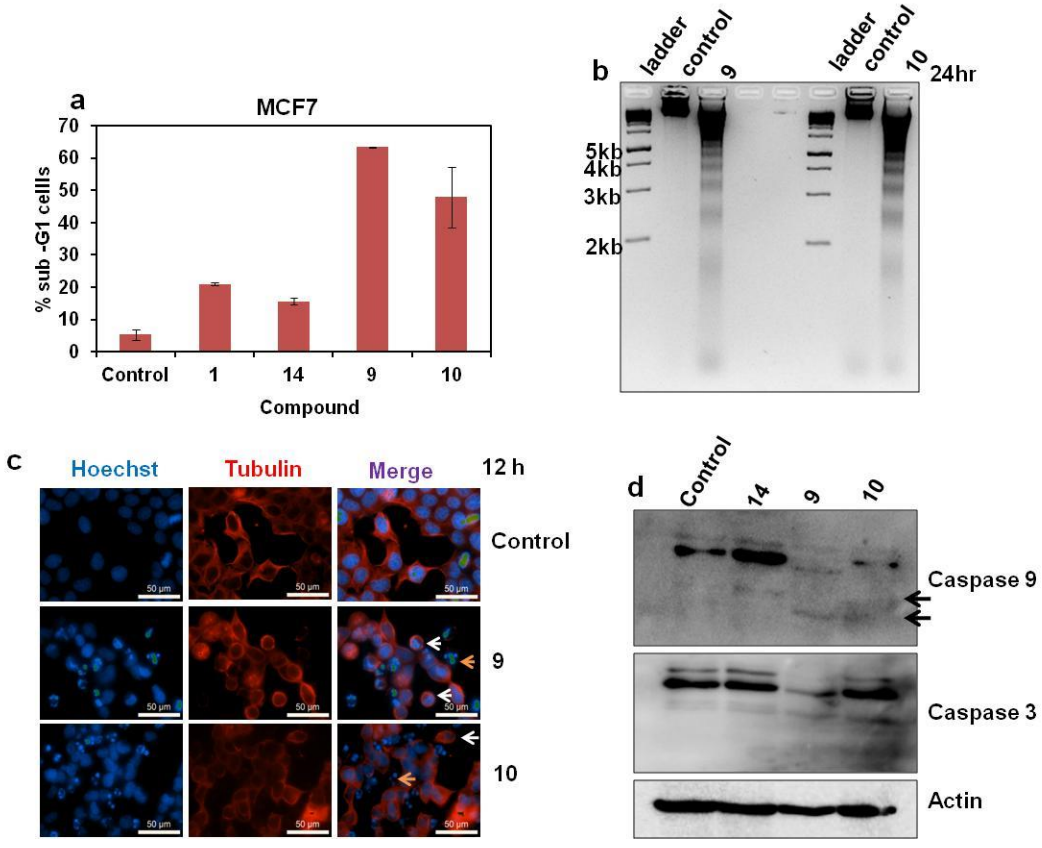


Figure 6. Compound **9** and **10** promote apoptosis in cancer cell line. (a) MCF7 cells were treated with 30 μM of **9,** and 20 μM of **10** for 24 h and cells were then collected for FACS analysis as mentioned in material and methods. The experiment was repeated three times

and mean of the sub-G1 population was plotted. (b) Compound **9** and **10** promoted DNA fragmentation of MCF7 cells. MCF7 cells were either vehicle-treated (DMSO) or 30 μM of **9** or 20 μM of **10** for 24 h. Cells were then collected, and fragmented DNA was isolated as described in the experimental section. (c) Immunofluorescence data depicts the fragmentation of genomic DNA following treatment of Compound **9** and **10** clearly show fragmented DNA (white arrows) and stressed tubulin morphology (orange arrows). Red: tubulin, blur: Hoechst. (d) Compound **9** and **10** induce apoptosis through intrinsic pathway. MCF7 cells were treated with either vehicle-treated (DMSO) or 30 μM of **9** or 20 μM of **10** for 24 h. Cells were then collected, lysed, and whole cell protein extracts were immunoblotted for indicated proteins.

3.4.3.2. *In vitro* anticancer activities of Heck analogs of zaluzanin D (23-34)

All the synthesized Heck analogs **23-34** were assayed for their antiproliferative activity against breast cancer cell line MCF7 as described in experimental section. Among all the synthesized Heck analogs, three analogs **29**, **31** and **32** exhibited good antiproliferative activity with IC_{50} values of 74 μM, 37 μM and 36.5 μM, respectively. The *in vitro* anticancer activities of all the synthesized Heck analogs are shown in **Table 4**.

Table 4. *In vitro* anticancer activities of Heck analogs of zaluzanin D (**23-34**).

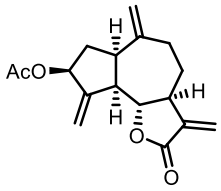
Compound	IC_{50} in μM against MCF7
23	>100
24	>100
25	>100
26	>100
27	>100
28	>100
29	74
30	>100
31	37
32	36.5
33	>100
34	>100

3.5. CONCLUSION

In summary, we have isolated two guaianolide class of sesquiterpene lactones, zaluzanin D **1** and zaluzanin C **2** from the leaves of *V. arborea*. A library of several diverse Michael adducts (**3-22**) and Heck arylation analogs (**23-34**) of zaluzanin D **1** have been synthesized by reacting with various amines and aryl iodides, respectively. All the new functionalized molecules were assayed for their *in vitro* anticancer activities against human breast cancer cell lines MCF7 and MDA-MB-231 and selected compounds were checked in MCF10A. Isolated zaluzanin D **1** exhibited IC_{50} values of 53.7 and 34.17 μM against MCF7 and MDA-MB-231 cell lines whereas zaluzanin C **2** was inactive to both the cell lines. Four Michael analogs (**9**, **10**, **13** and **14**) were found to possess potent anti-cancer activity as compared to other synthesized compounds. Out of these four compounds, compound **9** and **10** were found to exhibit potent antiproliferative effect against MCF7 cells. Compound **9** exhibited IC_{50} values of 30 μM and 13.33 μM, whereas compound **10** exhibited IC_{50} values of 18.83 μM and 23 μM against MCF7 and MDA-MB-231 cell lines, respectively. However, compound **10** has minimal cytotoxic effect against the normal breast MCF10A cells indicating that compound **10** could be a potential compound for the development of superior anticancer therapeutic compound. Further, amongst all the synthesized Heck analogs **23-34**, two analogs **31** and **32** exhibited good antiproliferative activity with IC_{50} values of 37 and 36.5 μM, respectively.

3.6. EXPERIMENTAL SECTION

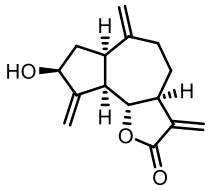
Zaluzanin D (1):



Colorless solid; **m.p.:** 105-106 °C [reported[10] 103-104 °C]; $\boldsymbol{R_f}$ = 0.35 (DCM); $\boldsymbol{[\alpha]_D^{25}}$ +23.5 (*c* 1, $CHCl_3$) [reported[10] +21.43 (c 0.28, $CHCl_3$)]; **^{1}H NMR (200 MHz, $CDCl_3$)** δ_H 6.22 (d, *J* = 3.5 Hz, 1H), 5.63-5.45 (m, 3H), 5.30 (t, *J* = 2.0 Hz, 1H), 4.96 (s, 2H), 4.07 (t, *J* = 9.2 Hz, 1H), 3.04-2.78 (m, 3H), 2.58-2.35 (m, 2H), 2.34-2.15 (m, 2H), 2.11 (s, 3H), 1.90-1.72 (m, 1H), 1.58-1.45 (m, 1H); **^{13}C NMR (50 MHz, $CDCl_3$)** δ_C 170.8, 170.0, 148.1, 147.8, 139.6,

120.4, 114.4, 113.6, 83.8, 74.7, 50.3, 45.3, 44.6, 36.5, 34.6, 30.6, 21.3; **LC-MS (ESI):** *m/z* at 311.06 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{17}H_{21}O_4$ $[M+H]^+$ 289.1434, found 289.1433.

Zaluzanin C (2):



Brown viscous liquid; $\boldsymbol{R_f}$ = 0.30 (MeOH-DCM, 1:19); $\boldsymbol{[\alpha]_D^{26}}$ +45.4 (*c* 1, $CHCl_3$) [reported[10] +50 (c 0.1, $CHCl_3$)]; **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H 6.22 (d, *J* = 3.7 Hz, 1H), 5.50 (d, *J* = 3.1 Hz, 1H), 5.46 (s, 1H), 5.33 (s, 1H), 5.01 (s, 1H), 4.95 (s, 1H), 4.58 (t, *J* = 7.3 Hz, 1H), 4.11 (t, *J* = 9.2 Hz, 1H), 2.96-2.88 (m, 1H), 2.87-2.81 (m, 1H), 2.54-2.46 (m, 1H), 2.37-2.21 (m, 3H), 2.22-2.12 (m, 1H), 1.82-1.73 (m, 1H), 1.52-1.41 (m, 1H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 170.1, 153.0, 148.0, 139.7, 120.3, 114.4, 111.3, 83.9, 73.6, 49.9, 45.6, 44.2, 39.0, 34.2, 30.6; **HRMS (ESI)** calcd for $C_{15}H_{19}O_3$ $[M+H]^+$ 247.1329, found 247.1329.

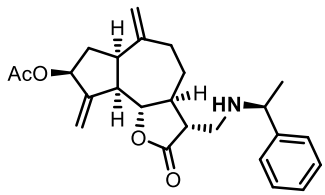
General procedure for the synthesis of amino derivatives of zaluzanin D 1 (3-22):

Zaluzanin D **1** (1 equiv.) was dissolved in dry MeOH (5 mL) and then amine (1.5 equiv.) was added to it and stirred at rt for overnight under argon. After completion of the reaction (TLC), the reaction mixture was evaporated to dryness and the residue was purified using CombiFlash *R*f 200*i* with UV/VIS and ELSD, Isco Teledyne Inc. using RediSep® column (12 g, SiO_2) and eluted with EtOAc-petroleum ether (0→70%, gradient) to furnish the pure amino derivatives **3-22**.

General procedure for the synthesis of Heck arylated analogs of zaluzanin D 1 (23-34)

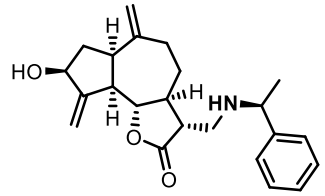
To a mixture of aryl halide (3 equiv.) and palladium (II) acetate (5 mol%) in DMF was added zaluzanin D (1 equiv.) and stirred at room temperature for 10 min and then triethylamine (7 equiv.) was added to the reaction mixture and heated at 80 °C under air for 24 h. After completion of the reaction (TLC), the reaction mixture was allowed to cool to room temperature, water (2 mL) was added, and the resultant mixture was extracted with EtOAc (5 mL X 3). The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure and then purified using CombiFlash *R*f 200*i* with UV/VIS and ELSD, Isco Teledyne Inc. using RediSep® column (12 g, SiO_2) and eluted with EtOAc-petroleum ether (0→70%, gradient) which furnished the pure aryl derivatives of zaluzanin D **(23-34)**.

(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-6,9-dimethylene-2-oxo-3-((((*S*)-1-phenylethyl)amino)methyl) dodecahydroazuleno[4,5-*b*]furan-8-yl-acetate (3):



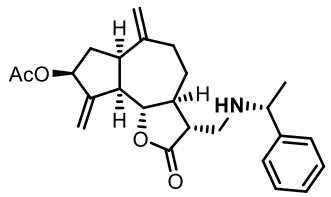
Dark brown viscous liquid (62%); R_f = 0.30 (EtOAc-petroleum ether, 2:3); $[\alpha]_D^{25}$ +25.3 (*c* 1, $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H 7.36-7.32 (m, 1H), 7.32-7.28 (m, 3H), 7.27-7.22 (m, 1H), 5.57-5.51 (m, 1H), 5.39 (t, *J* = 2.1 Hz, 1H), 5.26 (t, *J* = 2.1 Hz, 1H), 4.90 (s, 2H), 4.01 (t, *J* = 9.6 Hz, 1 H), 3.96 (s, 1H), 3.77 (q, *J* = 6.4 Hz, 1H), 2.93-2.85 (m, 2H), 2.84-2.77 (m, 1H), 2.49-2.36 (m, 4H), 2.30-2.20 (m, 1H), 2.10 (s, 3H), 2.04 (s, 1H), 1.97-1.88 (m, 1H), 1.86-1.74 (m, 2H), 1.36 (d, *J* = 6.9 Hz, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 177.8, 170.8, 148.6, 148.5, 144.9, 128.6, 127.2, 126.8, 113.6, 113.2, 84.3, 74.8, 58.4, 50.0, 47.3, 45.0, 44.5, 44.1, 36.4, 36.1, 32.1, 29.8, 24.5, 21.3; **LCMS (ESI):** *m/z* 432.05 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{25}H_{32}O_4N$ $[M+H]^+$ 410.2326, found 410.2328.

(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-8-hydroxy-6,9-dimethylene-3-((((*S*)-1-phenylethyl)amino) methyl)decahydroazuleno[4,5-*b*]furan-2(3*H*)-one (4):



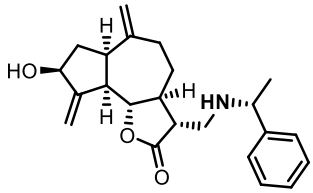
Dark brown viscous liquid (32%); R_f = 0.30 (EtOAc-petroleum ether, 7:3); $[\alpha]_D^{26}$ +21.1 (*c* 1, $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H 7.36-7.35 (m, 2H), 7.34 (s, 1H), 7.32-7.30 (m, 2H), 5.36 (t, *J* = 1.8 Hz, 1H), 5.29 (t, *J* = 2.3 Hz, 1H), 4.54 (tt, *J* = 7.3, 1.8 Hz, 1H), 4.18 (q, *J* = 6.8 Hz, 1H), 4.04 (t, *J* = 9.6 Hz, 1H), 3.77 (q, *J* = 6.6 Hz, 1H), 2.93-2.75 (m, 3H), 2.48-2.35 (m, 3H), 2.34-2.27 (m, 1H), 2.26-2.16 (m, 1H), 1.96-1.89 (m, 1H), 1.89-1.85 (m, 4H), 1.84-1.77 (m, 1H), 1.78-1.68 (m, 1H), 1.36 (d, *J* = 6.4 Hz, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 177.9, 153.4, 148.8, 144.9, 128.8, 128.6, 127.8, 126.8, 126.2, 113.6, 110.9, 84.4, 73.5, 58.4, 51.1, 47.2, 45.4, 44.5, 43.7, 38.9, 35.9, 29.8, 24.5, 23.3; **LCMS (ESI):** *m/z* 390.05 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{23}H_{30}O_3N$ $[M+H]^+$ 368.2220, found 368.2220.

(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-6,9-dimethylene-2-oxo-3-((((*R*)-1-phenyl ethyl)amino)methyl) dodecahydroazuleno[4,5-*b*]furan-8-yl-acetate (5):



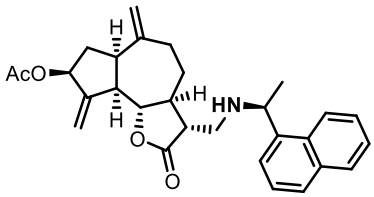
Brown viscous liquid (66%); $\boldsymbol{R_f}$ = 0.30 (EtOAc-petroleum ether, 2:3); $\boldsymbol{[\alpha]_D^{26}}$ +34.4 (*c* 1, $CHCl_3$); **^{1}H NMR (200 MHz, $CDCl_3$)** δ_H 7.37-7.22 (m, 5H), 5.59-548 (m, 1H), 5.40 (t, *J* = 2.0 Hz, 1H), 5.26 (t, *J* = 2.0 Hz, 1H), 4.90 (s, 2 H), 4.00 (t, *J* = 9.2 Hz, 1H), 3.75 (q, *J* = 6.6 Hz, 1H), 2.96-2.75 (m, 2H), 2.70 (s, 1H), 2.67 (s, 1H), 2.55-2.28 (m, 3H), 2.26-2.14 (m, 1H), 2.10 (s, 3H), 2.05-1.96 (m, 3H), 1.79 (td, *J* = 14.2, 6.4 Hz, 1H), 1.56 (d, *J* = 6.3 Hz, 1H), 1.35 (d, *J* = 6.6 Hz, 3H); **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 177.6, 170.8, 148.5, 148.3, 144.3, 128.6, 127.2, 126.7, 113.6, 113.2, 84.2, 74.7, 58.7, 49.9, 47.2, 45.9, 45.7, 44.0, 36.3, 36.1, 32.4, 29.7, 23.9, 21.3; **LCMS (ESI):** *m/z* 432.05 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{25}H_{32}O_4N$ $[M+H]^+$ 410.2326, found 410.2329.

(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-8-hydroxy-6,9-dimethylene-3-((((*R*)-1-phenylethyl)amino) methyl)decahydroazuleno[4,5-*b*]furan-2(3*H*)-one (6):



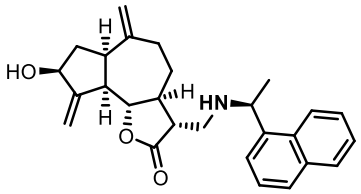
Brown viscous liquid (28%); $\boldsymbol{R_f}$ = 0.30 (EtOAc-petroleum ether, 7:3); $\boldsymbol{[\alpha]_D^{26}}$ +33.1 (*c* 1, $CHCl_3$); **^{1}H NMR (200 MHz, $CDCl_3$)** δ_H 7.36-7.26 (m, 5H), 5.37 (t, *J* = 1.9 Hz, 1H), 5.29 (t, *J* = 1.8 Hz, 1H), 4.96 (s, 1H), 4.91 (s, 1H), 4.59-4.48 (m, 1H), 4.03 (t, *J* = 9.4 Hz, 1H), 3.75 (q, *J* = 6.6 Hz, 1H), 2.93-2.75 (m, 2H), 2.69 (s, 1H), 2.66 (s, 1H), 2.51-2.27 (m, 3H), 2.00-1.96 (m, 6H), 1.80-1.66 (m, 1H), 1.62-1.47 (m, 1H), 1.35 (d, *J* = 6.7 Hz, 3H); **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 177.8, 153.3, 148.7, 144.6, 128.6, 127.2, 126.7, 113.7, 110.9, 84.3, 73.5, 58.7, 49.6, 47.3, 46.3, 45.8, 43.6, 38.8, 35.9, 32.4, 29.7, 24.0; **LCMS (ESI):** *m/z* 390.07 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{23}H_{30}O_3N$ $[M+H]^+$ 368.2220, found 368.2208.

(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-6,9-dimethylene-3-((((*S*)-1-(naphthalen-1-yl)ethyl)amino) methyl)-2-oxododecahydroazuleno[4,5-*b*]furan-8-yl acetate (7):



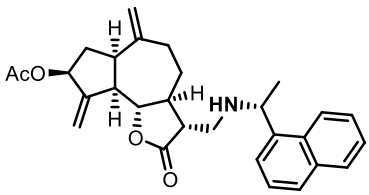
Dark brown viscous liquid (52%); $\boldsymbol{R_f}$ = 0.60 (EtOAc-petroleum ether, 1:1); $\boldsymbol{[\alpha]_D^{25}}$ +20.4 (*c* 1, $CHCl_3$); **^{1}H NMR (200 MHz, $CDCl_3$)** δ_H 8.29 (d, *J* = 7.6 Hz, 1H), 7.94-7.83 (m, 1H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.62 (d, *J* = 7.0 Hz, 1H), 7.55-7.42 (m, 3H), 5.54 (t, *J* = 7.0 Hz, 1H), 5.40 (s, 1H), 5.26 (s, 1H), 4.86 (s, 2H), 4.72-4.52 (m, 1H), 3.98 (t, *J* = 8.2 Hz, 1H), 3.03 (d, *J* = 11.2 Hz, 2H), 2.84-2.72 (m, 2H), 2.60-2.33 (m, 4H), 2.10 (s, 3H), 1.85-1.66 (m, 3H), 1.53 (d, *J* = 6.4 Hz, 3H), 1.18-1.04 (m, 2H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 177.8, 170.9, 148.8, 148.5, 143.2, 140.5, 134.0, 130.8, 129.1, 127.4, 126.1, 125.7, 125.5, 123.0, 121.5, 113.4, 113.0, 84.3, 74.8, 53.9, 49.9, 48.0, 46.6, 45.5, 45.0, 43.9, 36.4, 32.5, 24.8, 23.3, 21.4; **LCMS (ESI):** *m/z* 482.09 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{29}H_{34}O_4N$ $[M+H]^+$ 460.2482, found 460.2487.

(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-8-hydroxy-6,9-dimethylene-3-((((*S*)-1-(naphthalene-1-yl) ethyl)amino)methyl)decahydroazuleno[4,5-*b*] furan-2(3*H*)-one (8):



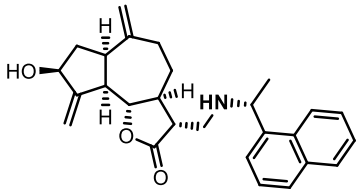
Dark brown viscous liquid (27%); $\boldsymbol{R_f}$ = 0.30 (EtOAc-petroleum ether, 1:1); $\boldsymbol{[\alpha]_D^{25}}$ +35.1 (*c* 1, $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H 8.22 (d, *J* = 8.2 Hz, 1H), 7.90-7.84 (m, 1H), 7.75 (d, *J* = 8.2 Hz, 1H), 7.64 (d, *J* = 6.9 Hz, 1H), 7.54-7.45 (m, 3H), 5.35 (s, 1H), 5.28 (s, 1H), 4.92 (s, 1H), 4.86 (s, 1H), 4.65 (q, *J* = 6.4 Hz, 1H), 4.52 (t, *J* = 7.8 Hz, 1H), 4.05 - 3.95 (m, 1H), 2.85 (dd, *J* = 12.4, 4.1, Hz, 1H), 2.77-2.65 (m, 3H), 2.43-2.22 (m, 4H), 2.21-2.13 (m, 1H), 2.11-2.01 (m, 1H), 1.91-1.82 (m, 1H), 1.83-1.65 (m, 3H), 1.52 (d, *J* = 6.4 Hz, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 177.9, 153.4, 148.9, 140.4, 134.1, 131.5, 129.0, 127.4, 125.8, 125.7, 125.4, 123.4, 123.0, 113.5, 110.8, 84.3, 73.6, 53.9, 49.6, 47.9, 46.0, 45.2, 43.5, 38.8, 36.1, 32.5, 23.3; **LCMS (ESI):** *m/z* 440.14 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{27}H_{32}O_3N$ $[M+H]^+$ 418.2377, found 418.2373.

(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-6,9-dimethylene-3-((((*R*)-1-(naphthalen-1-yl)ethyl)amino) methyl)-2-oxododecahydroazuleno[4,5-*b*]furan-8-yl acetate (9):



Pale yellow viscous liquid (57%); R_f = 0.70 (EtOAc-petroleum ether, 7:3); $[\alpha]_D^{25}$ +30.9 (*c* 1, $CHCl_3$); **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 8.29 (d, *J* = 7.9 Hz, 1H), 7.87 (d, *J* = 7.6 Hz, 1 H), 7.75 (d, *J* = 7.9 Hz, 1H), 7.61 (d, *J* = 7.0 Hz, 1H), 7.55-7.40 (m, 3H), 5.54 (t, *J* = 7.0 Hz, 1H), 5.40 (s, 1H), 5.27 (s, 1H), 4.88-4.85 (m, 2H), 4.60 (q, *J* = 6.3 Hz, 1H), 3.98 (t, *J* = 8.7 Hz, 1H), 3.02 (dd, *J* = 12.2, 2.8 Hz, 1H), 2.87-2.69 (m, 2H), 2.52 (dd, *J* = 12.2, 5.5 Hz, 1H), 2.48-2.40 (m, 1H), 2.40-2.25 (m, 3H), 2.10 (s, 3H), 1.84-1.62 (m, 3H), 1.52 (d, *J* = 6.7 Hz, 3H), 1.19-1.07 (m, 2H); **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 177.7, 170.8, 148.6, 148.4, 143.1, 140.3, 133.9, 130.7, 129.0, 127.2, 126.0, 125.6, 125.4, 122.9, 121.4, 113.3, 112.9, 84.1, 74.7, 53.8, 49.8, 47.9, 46.4, 45.4, 44.9, 43.8, 36.3, 32.3, 24.7, 23.1, 21.2; **LCMS (ESI):** *m/z* 482.05 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{29}H_{34}O_4N$ $[M+H]^+$ 460.2482, found 460.2487.

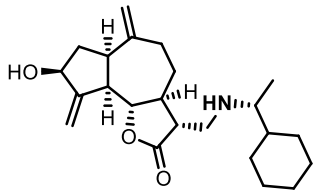
(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-8-hydroxy-6,9-dimethylene-3-((((*R*)-1-(naphthalen-1-yl)ethyl) amino)methyl)decahydroazuleno[4,5-*b*] furan-2(3H)-one (10):



Pale yellow viscous liquid (34%); R_f = 0.40 (EtOAc-petroleum ether, 7:3); $[\alpha]_D^{25}$ +24.6 (*c* 1, $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H 8.24 (d, *J* = 8.2 Hz, 1H), 7.89-7.85 (m, 1H), 7.75 (d, *J* = 8.2 Hz, 1H), 7.64 (d, *J* = 6.9 Hz, 1H), 7.53-7.46 (m, 3H), 5.35 (s, 1H), 5.28 (s, 1H), 4.92 (s, 1H), 4.86 (s, 1H), 4.66 (q, *J* = 6.4 Hz, 1H), 4.53 (t, *J* = 7.8 Hz, 1H), 4.04-3.97 (m, 1H), 2.87 (dd, *J* = 12.4, 4.1 Hz, 1H), 2.76-2.67 (m, 3H), 2.43-2.26 (m, 4H), 2.20-2.14 (m, 1H), 2.06 (dq, *J* = 11.9, 3.7 Hz, 1H), 1.90-1.82 (m, 1H), 1.83-1.66 (m, 3H), 1.53 (d, *J* = 6.4 Hz, 3H), 1.30-1.16 (m, 2H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 177.8, 153.3, 148.7, 140.3, 134.0, 131.4, 128.9, 127.3, 125.7, 125.6, 125.3, 123.2, 122.9, 113.4, 110.6, 84.2, 76.7, 73.5,

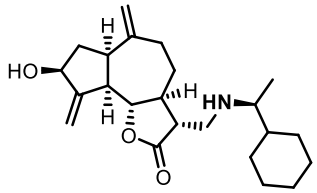
53.8, 49.5, 47.8, 45.9, 45.0, 43.4, 38.7, 36.0, 32.3, 23.2; **LCMS (ESI):** *m/z* 440.02 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{27}H_{32}O_3N$ $[M+H]^+$ 418.2377, found 418.2379.

(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-3-((((*R*)-1-cyclohexylethyl)amino)methyl)-8-hydroxy-6,9-dimethylenedecahydroazuleno[4,5-*b*]furan-2(3*H*)-one (11):



Brown viscous liquid (88%); R_f = 0.40 (EtOAc); $[\alpha]_D^{24}$ +17.5 (*c* 1, $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H 5.34 (s, 1H), 5.31 (s, 1H), 4.99-4.89 (m, 2H), 4.54 (t, *J* = 7.3 Hz, 1H), 4.23-4.08 (m, 1H), 3.42 (dd, *J* = 11.2, 3.4 Hz, 1H), 2.97-2.73 (m, 4H), 2.55-2.45 (m, 1H), 2.36-2.27 (m, 1H), 2.23-2.07 (m, 2H), 2.04-2.01 (m, 5H), 1.84-1.65 (m, 6H), 1.65-1.51 (m, 2H), 1.49-1.34 (m, 2H), 1.20 (d, *J* = 6.9 Hz, 3H), 1.10-1.01 (m, 2H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 178.3, 176.1, 153.0, 148.2, 114.0, 111.1, 85.1, 73.4, 59.7, 49.4, 46.5, 45.0, 44.5, 43.6, 40.5, 38.7, 35.3, 31.5, 29.7, 29.5, 29.3, 27.2, 26.2, 26.1, 25.9, 21.7, 14.0; **LCMS (ESI):** *m/z* 396.15 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{23}H_{36}O_3N$ $[M+H]^+$ 374.2690, found 374.2685.

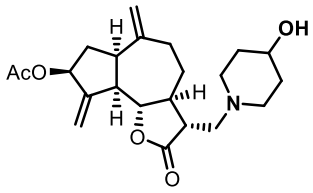
(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-3-((((*S*)-1-cyclohexylethyl)amino)methyl)-8-hydroxy-6,9-dimethylenedecahydroazuleno[4,5-*b*]furan-2(3*H*)-one (12):



Dark brown viscous liquid (86%); R_f = 0.30 (EtOAc-petroleum ether, 7:3); $[\alpha]_D^{26}$ +25.9 (*c* 1, $CHCl_3$); **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 5.38-5.26 (m, 2H), 4.9-4.91 (m, 2H), 4.55 (t, *J* = 7.6 Hz, 1H), 4.15 (t, *J* = 9.5 Hz, 1H), 3.19-3.15 (m, 1H), 2.97-2.74 (m, 4H), 2.54-2.47 (m, 1H), 2.38-2.28 (m, 1H), 2.23-2.10 (m, 2H), 2.05-1.96 (m, 5H), 1.80-1.65 (m, 6H), 1.59-1.52 (m, 1H), 1.47-1.36 (m, 2H), 1.30-1.27 (m, 1H), 1.14 (d, *J* = 6.5 Hz, 3H), 1.09-1.00 (m, 2H); **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 177.8, 176.0, 153.1, 148.4, 113.9, 111.0, 84.9, 77.3, 77.0, 76.8, 73.5, 58.0, 49.4, 46.3, 45.0, 43.6, 43.3, 40.9, 38.7, 35.6, 31.9, 29.7, 27.3, 26.3, 26.2,

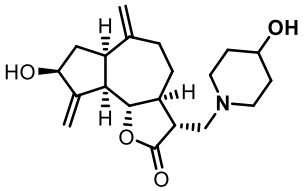
26.0, 21.7, 14.3; **LCMS (ESI):** *m/z* 396.13 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{23}H_{36}O_3N$ $[M+H]^+$ 374.2690, found 374.2693.

(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-3-((4-hydroxypiperidin-1-yl)methyl)-6,9-dimethylene-2-oxo dodecahydroazuleno[4,5-*b*]furan-8-yl acetate (13):



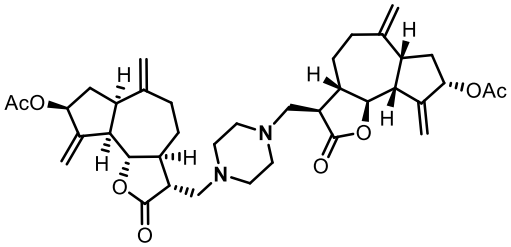
Pale yellow viscous liquid (73%); R_f = 0.50 (MeOH-DCM, 1:9); $[\alpha]_D^{25}$ +63.2 (*c* 1, $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H 5.54 (t, *J* = 7.3 Hz, 1H), 5.40 (m, 1H), 5.27 (m, 1H), 4.93-4.89 (m, 2H), 4.01 (t, *J* = 9.4 Hz, 1H), 3.74-3.65 (m, 1H), 2.93 (q, *J* = 7.9 Hz, 1H), 2.84-2.74 (m, 3H), 2.74-2.66 (m, 1H), 2.64-2.56 (m, 1H), 2.52-2.31 (m, 4H), 2.31-2.15 (m, 3H), 2.11 (s, 3H), 2.09-2.02 (m, 1H), 1.93-1.76 (m, 4H), 1.64-1.48 (m, 2H), 1.42-1.32 (m, 1H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 177.5, 170.9, 148.8, 148.4, 113.5, 113.3, 83.9, 74.8, 67.9, 57.6, 52.2, 50.9, 50.2, 47.9, 45.3, 44.2, 36.4, 36.3, 34.6, 34.3, 32.8, 21.4; **LCMS (ESI):** *m/z* 412.07 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{22}H_{32}O_5N$ $[M+H]^+$ 390.2275, found 390.2272.

(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-8-hydroxy-3-((4-hydroxypiperidin-1-yl)methyl)-6,9-dimethylenedecahydroazuleno[4,5-*b*]furan-2(3*H*)-one (14):



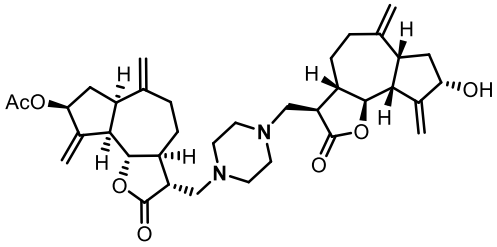
Pale yellow viscous liquid (20%); R_f = 0.40 (MeOH-DCM, 1:9); $[\alpha]_D^{25}$ +61.8 (*c* 1, $CHCl_3$); **^{1}H NMR (400 MHz, CD_3OD)** δ_H 5.28-5.23 (m, 2H), 5.01-4.88 (m, 1H), 4.56-4.45 (m, 1H), 4.17-4.06 (m, 1H), 3.66-3.53 (m, 1H), 3.20-3.10 (m, 1H), 3.00-2.88 (m, 2H), 2.86-2.79 (m, 2H), 2.77-2.70 (m, 2H), 2.63-2.53 (m, 2H), 2.52-2.46 (m, 1H), 2.31-2.20 (m, 4H), 2.17-2.03 (m, 2H), 1.96-1.78 (m, 3H), 1.73-1.66 (m, 1H), 1.58-1.49 (m, 2H), 1.45-1.37 (m, 1H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ δ_C 178.7, 153.3, 149.4, 112.0, 108.2, 84.2, 72.4, 66.9, 56.9, 51.8, 50.8, 48.9, 44.8, 42.9, 38.0, 35.5, 33.6, 33.5, 32.2; **LCMS (ESI):** *m/z* 370.12 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{20}H_{30}O_4N$ $[M+H]^+$ 348.2169, found 348.2156.

(3*R*,3a*S*,3'*R*,3a'*S*,6a*R*,6a'*R*,8*S*,8'*S*,9a*R*,9b*S*,9a'*R*,9b'*S*)-(piperazine-1,4diylbis(methylene))bis(6,9-dimethylene-2-oxododeca-hydro-azuleno[4,5-*b*]furan-3,8-diyl) diacetate (15):



Pale yellow viscous liquid (26%); R_f = 0.40 (EtOAc-petroleum ether, 7:3); $[\alpha]_D^{26}$ +67.7 (*c* 1, $CHCl_3$); **^{1}H NMR (200 MHz, $CDCl_3$)** δ_H 5.61-5.49 (m, 2H), 5.72-5.39 (m, 2H), 5.29-5.26 (m, 2H), 4.95-4.90 (m, 4H), 4.01 (t, *J* = 9.5 Hz, 2H), 2.96-2.74 (m, 6H), 2.67-2.58 (m, 2H), 2.56-2.38 (m, 14H), 2.33-2.27 (m, 2H), 2.26-2.16 (m, 2H), 2.11 (s, 6H), 2.04-1.96 (m, 2H), 1.88-1.74 (m, 2H), 1.46-1.38 (m, 2H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 177.4, 170.9, 148.8, 148.4, 113.5, 113.4, 83.9, 74.8, 57.6, 57.6, 53.5, 53.5, 50.1, 47.9, 47.9, 45.2, 44.2, 36.4, 36.3, 32.8, 21.4; **LCMS (ESI):** *m/z* 685.39 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{38}H_{51}O_8N_2$ $[M+H]^+$ 663.3640, found 663.3637.

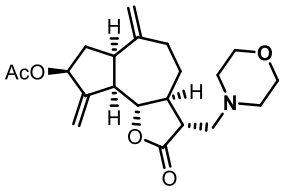
(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-3-((4-(((3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-8-hydroxy-6,9-dimethylene-2-oxododecahydroazuleno[4,5-*b*]furan-3-yl)methyl)piperazin-1-yl)methyl)6,9-dimethyl-ene-2-oxododeca-hydroazuleno[4,5-*b*]furan-8-yl acetate (16):



Pale yellow viscous liquid (15%); R_f = 0.30 (EtOAc-petroleum ether, 7:3); $[\alpha]_D^{26}$ +69.0 (*c* 1, $CHCl_3$); ^{1}H **NMR (200 MHz, $CDCl_3$)** δ_H 5.58-5.52 (m, 1H), 5.42-5.37 (m, 2H), 5.32-5.26 (m, 2H), 4.97 (s, 1H), 4.94-4.91 (m, 2H), 4.56 (t, *J* = 7.6 Hz, 1H), 4.08-3.96 (m, 2H), 3.48-3.40 (m, 3H), 2.97-2.85 (m, 2H), 2.83-2.76 (m, 3H), 2.64-2.56 (m, 2H), 2.54-2.45 (m, 6H), 2.44-2.35 (m, 4H), 2.35-2.30 (m, 2H), 2.27-2.14 (m, 2H), 2.11 (s, 3H), 2.09-2.01 (m, 2H), 1.84-1.71 (m, 2H), 1.39-1.33 (m, 6H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 177.4, 177.3, 170.8, 153.4, 148.9, 148.8, 148.4, 113.5, 113.5, 113.3, 111.0, 83.9, 83.8, 74.8, 73.6, 58.6, 57.6, 57.5, 53.5, 50.1, 49.8, 48.2, 47.8, 45.2, 44.1, 43.8, 38.9, 36.4, 36.2, 36.0, 32.8, 21.3, 8.3;

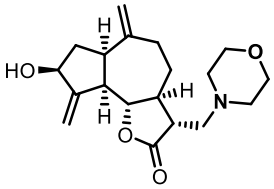
LCMS (ESI): *m/z* 643.38 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{36}H_{49}O_7N_2$ $[M+H]^+$ 621.3534, found 621.3508.

(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-6,9-dimethylene-3-(morpholinomethyl)-2-oxododecahydro azuleno[4,5-*b*]furan-8-yl acetate (17):



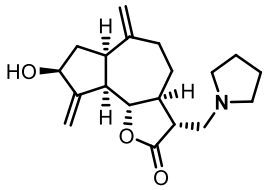
Brown viscous liquid (84%); $\boldsymbol{R_f}$ = 0.50 (EtOAc-petroleum ether, 7:3); $\boldsymbol{[\alpha]_D^{26}}$ +69.7 (*c* 1, $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H 5.59-5.52 (m, 1H), 5.41 (m, 1H), 5.28 (m, 1H), 4.94-4.90 (m, 2H), 4.02 (t, *J* = 9.8 Hz, 1H), 3.73-3.64 (m, 4H), 2.97-2.89 (m, 1H), 2.84-2.76 (m, 2H), 2.65-2.58 (m, 1H), 2.53-2.40 (m, 7H), 2.39-2.32 (m, 1H), 2.23 (dq, *J* = 11.5, 3.4 Hz 1H), 2.11 (s, 3 H), 2.08-2.03 (m, 1 H), 1.81 (quint, *J* = 6.9 Hz, 1H), 1.40-1.29 (m, 1H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 177.1, 170.8, 148.6, 148.3, 113.5, 113.4, 83.7, 74.7, 66.9, 58.0, 54.0, 50.1, 47.8, 44.9, 44.1, 36.3, 36.1, 32.7, 21.3; **LCMS (ESI):** *m/z* 398.07 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{21}H_{30}O_5N$ $[M+H]^+$ 376.2118, found 376.2115.

(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-8-hydroxy-6,9-dimethylene-3-(morpholinomethyl)decahydro azuleno[4,5-*b*]furan-2(3*H*)-one (18):



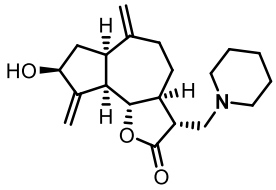
Brown viscous liquid (14%); $\boldsymbol{R_f}$ = 0.40 (EtOAc-petroleum ether, 7:3); $\boldsymbol{[\alpha]_D^{26}}$ +66.1 (*c* 1, $CHCl_3$); **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 5.39 (s, 1H), 5.31 (s, 1H), 4.97 (s, 1H), 4.93 (s, 1H), 4.56 (t, *J* = 7.3 Hz, 1H), 4.05 (t, *J* = 9.5 Hz, 1H), 3.71-3.65 (m, 4H), 3.45 (q, *J* = 7.0 Hz, 1H), 2.88 (q, *J* = 8.2 Hz, 1H), 2.83 - 2.76 (m, 2H), 2.64-2.57 (m, 1H), 2.53-2.41 (m, 5H), 2.37-2.32 (m, 2H), 2.24-2.18 (m, 1H), 2.08-2.02 (m, 1H), 1.81-1.71 (m, 1H), 1.38-1.32 (m, 2H); **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C ; 177.3, 153.3, 148.8, 113.5, 111.1, 83.8, 73.6, 66.9, 58.0, 54.1, 49.7, 48.2, 45.0, 43.7, 38.8, 35.9, 32.8; **LCMS (ESI):** *m/z* 356.06 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{19}H_{28}O_4N$ $[M+H]^+$ 334.2013, found 334.2013.

(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-8-hydroxy-6,9-dimethylene-3-(pyrrolidin-1-ylmethyl)deca hydroazuleno[4,5-*b*]furan-2(3*H*)-one (19):



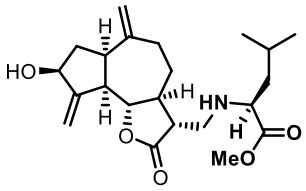
Brown viscous liquid (78%); $\boldsymbol{R_f}$ = 0.20 (EtOAc); $\boldsymbol{[\alpha]_D^{26}}$ +42.0 (*c* 1, $CHCl_3$); **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 5.39-5.26 (m, 2H), 5.00-4.88 (m, 2H), 4.14-3.95 (m, 1H), 3.55-3.36 (m, 1H), 2.93-2.78 (m, 4H), 2.76-2.68 (m, 1H), 2.66-2.59 (m, 2H), 2.54-2.44 (m, 2H), 2.38-2.21 (m, 3H), 2.09-1.99 (m, 2H), 1.99-1.92 (m, 1H), 1.91-1.84 (m, 1H), 1.82-1.77 (m, 2H), 1.76-1.70 (m, 1H), 1.52 (d, *J* = 6.8 Hz, 1H), 1.42-1.30 (m, 1H); **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 177.3, 153.3, 148.9, 113.4, 110.8, 83.9, 73.5, 54.5, 54.4, 49.6, 47.7, 46.3, 43.5, 38.8, 36.2, 32.6, 23.5; **LCMS (ESI):** *m/z* 318.18 $(M+H)^+$; **HRMS (ESI)** calcd for $C_{19}H_{28}O_3N$ $[M+H]^+$ 318.2064, found 318.2060.

(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-8-hydroxy-6,9-dimethylene-3-(piperidin-1-ylmethyl)deca hydroazuleno[4,5-*b*]furan-2(3*H*)-one (20):



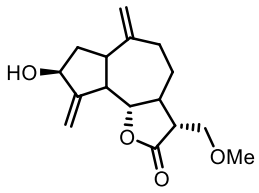
Dark brown viscous liquid (66%); $\boldsymbol{R_f}$ = 0.30 (EtOAc); $\boldsymbol{[\alpha]_D^{26}}$ +46.1 (*c* 1, $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H 5.37 (d, *J* = 1.8 Hz, 1H), 5.30 (d, *J* = 1.8 Hz, 1H), 4.96 (s, 1H), 4.93 (s, 1H), 4.55 (dt, *J* = 7.3, 1.4, Hz, 1H), 4.06 (dt, *J* = 9.6, 1.8 Hz, 1H), 2.92-2.75 (m, 3H), 2.70-2.63 (m, 1H), 2.61-2.53 (m, 3H), 2.52-2.46 (m, 3H), 2.38-2.29 (m, 2H), 2.22-2.10 (m, 1H), 2.08-1.98 (m, 2H), 1.79-1.71 (m, 1H), 1.65-1.57 (m, 4H), 1.50-1.42 (m, 2H), 1.38-1.32 (m, 1H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 177.6, 153.4, 149.0, 113.5, 110.9, 84.0, 73.6, 57.7, 54.6, 49.7, 48.3, 44.7, 43.6, 38.8, 36.1, 32.5, 29.8, 25.3, 23.8; **LCMS (ESI):** *m/z* 354.14 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{20}H_{30}O_3N$ $[M+H]^+$ 332.2220, found 332.2219.

Methyl(((3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-8-hydroxy-6,9-dimethylene-2-oxododecahydro azuleno[4,5-*b*]furan-3-yl)methyl)-*L*-leucinate (21):



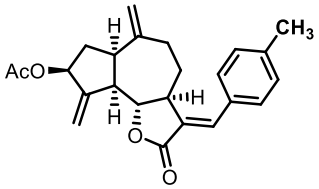
Yellow viscous liquid (82%); $\boldsymbol{R_f}$ = 0.30 (EtOAc-petroleum ether, 1:1); $\boldsymbol{[\alpha]_D^{25}}$ +29.8 (*c* 1, $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H 5.37 (s, 1H), 5.30 (s, 1H), 4.97 (s, 1H), 4.93 (s, 1H), 4.55 (t, *J* = 7.8 Hz, 1H), 4.05 (t, *J* = 9.6 Hz, 1H), 3.72 (s, 3H), 3.30 (t, *J* = 7.3 Hz, 1H), 2.91-2.84 (m, 2H), 2.84-2.77 (m, 1H), 2.72 (dd, *J* = 11.9, 4.6 Hz, 1H), 2.50 (td, *J* = 13.3, 4.6 Hz, 1H), 2.44-2.26 (m, 3H), 2.26-2.11 (m, 2H), 2.06-1.98 (m, 1H), 1.79-1.67 (m, 2H), 1.43-1.28 (m, 2H), 1.53-1.43 (m, 2H), 0.94-0.88 (m, 6H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 177.4, 176.1, 153.4, 148.8, 113.7, 111.1, 84.1, 73.6, 60.7, 51.8, 49.7, 48.0, 46.1, 46.0, 43.7, 42.6, 38.9, 35.9, 32.5, 25.0, 22.8, 22.3; **LCMS (ESI):** *m/z* 414.27 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{22}H_{34}O_5N$ $[M+H]^+$ 392.2431, found 392.2434.

(3*R*,3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-8-hydroxy-3-(methoxymethyl)-6,9-dimethylenedecahydro azuleno[4,5-*b*]furan-2(3*H*)-one (22):



Yellow viscous liquid (87%); $\boldsymbol{R_f}$ = 0.40 (EtOAc-petroleum ether, 1:1); $\boldsymbol{[\alpha]_D^{26}}$ +62.4 (*c* 1, $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H 5.38 (m, 1H), 5.30 (m, 1H), 4.97 (m, 1H), 4.93 (m, 1H), 4.55 (t, *J* = 7.3 Hz, 1H), 4.05 (t, *J* = 8.7 Hz, 1H), 3.73-3.68 (m, 1H), 3.63 (dd, *J* = 9.6, 1.8 Hz, 1H), 3.37 (s, 3H), 2.93-2.80 (m, 2H), 2.51 (td, *J* = 12.8, 4.6 Hz, 1H), 2.42-2.29 (m, 3H), 2.24-2.16 (m, 1H), 2.09-2.01 (m, 1H), 1.80-1.71 (m, 1H), 1.42-1.28 (m, 2H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 176.1, 153.4, 148.9, 113.7, 110.9, 84.1, 73.6, 68.9, 59.4, 49.5, 48.1, 45.1, 43.6, 38.9, 36.2, 32.6; **LCMS (ESI):** *m/z* 300.99 $(M+Na)^+$; **HRMS (ESI)** calcd for $C_{16}H_{22}O_4Na$ $[M+Na]^+$ 301.1410, found 301.1398.

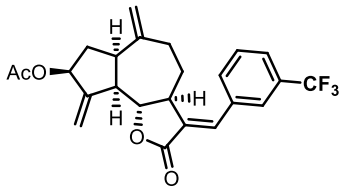
(3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-3-((*E*)-4-methylbenzylidene)-6,9-dimethylene-2-oxododecahydro azuleno[4,5-*b*]furan-8-yl acetate (23):



Brown viscous liquid (48%); $\boldsymbol{R_f}$ = 0.45 (EtOAc-petroleum ether, 1:4); **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 7.63 (d, *J* = 2.7 Hz, 1H), 7.30 (d, *J* = 7.9 Hz, 2H), 7.24 (d, *J* = 7.9 Hz, 2H), 5.61 (t, *J* = 7.2 Hz, 1H), 5.56 (s, 1H), 5.33 (s, 1H), 5.01 (s, 1H), 4.94 (s, 1H), 4.21 (dd, *J* = 9.9,

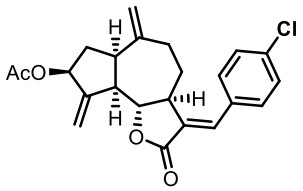
8.1 Hz, 1H), 3.41 (td, *J* = 7.4, 3.8 Hz, 1H), 3.03 (q, *J* = 8.2 Hz, 1H), 2.95-2.83 (m, 1H), 2.49-2.44 (m, 1H), 2.42 (s, 3H), 2.37-2.29 (m, 2H), 2.21-2.15 (m, 1H), 2.14 (s, 3H), 1.89-1.81 (m, 1H); **^{13}C NMR (125 MHz, CDCl$_3$)** δ_C 171.8, 170.9, 148.0, 147.5, 139.9, 138.1, 131.0, 129.7, 129.3, 128.4, 114.7, 113.8, 83.7, 74.7, 50.6, 45.2, 43.5, 36.7, 33.3, 28.4, 21.5, 21.3; **HRMS (ESI)** calcd for $C_{24}H_{26}O_4Na$ $[M+Na]^+$ 401.1723, found 401.1720.

(3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-6,9-dimethylene-2-oxo-3-((*E*)-3-(trifluoromethyl)benzylidene) dodecahydroazuleno[4,5-*b*]furan-8-yl acetate (24):



Brown viscous liquid (53%); ***R*$_f$** = 0.42 (EtOAc-petroleum ether, 1:4); **^{1}H NMR (400 MHz, CDCl$_3$)** δ_H 7.67-7.61 (m, 3H), 7.57-7.54 (m, 2H), 5.63-5.56 (m, 1H), 5.56-5.53 (m, 1H), 5.35-5.31 (m, 1H), 5.01 (s, 1H), 4.92 (s, 1H), 4.23 (dd, *J* = 10.4, 7.7 Hz, 1H), 3.42 (td, *J* = 7.5, 3.8 Hz, 1H), 3.03 (q, *J* = 8.3 Hz, 1H), 2.91-2.82 (m, 1H), 2.43 (dt, *J* = 14.2, 8.0 Hz, 1H), 2.34-2.21 (m, 3H), 2.19-2.13 (m, 1H), 2.11 (s, 3H), 1.87-1.78 (m, 1H); **^{13}C NMR (100 MHz, CDCl$_3$)** δ_C 170.9, 170.8, 147.9, 146.9, 135.9, 134.7, 132.8, 131.7, 129.2, 128.6, 125.9, 125.9, 122.4, 115.1, 114.2, 83.6, 74.6, 50.6, 45.3, 43.3, 36.7, 32.3, 28.1, 21.3; **HRMS (ESI)** calcd for $C_{24}H_{23}O_4F_3Na$ $[M+Na]^+$ 455.1441, found 455.1436.

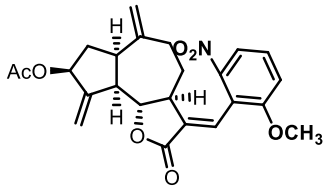
(3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-3-((*E*)-4-chlorobenzylidene)-6,9-dimethylene-2-oxododecahydro azuleno[4,5-*b*]furan-8-yl acetate (25):



Brown viscous liquid (64%); ***R*$_f$** = 0.42 (EtOAc-petroleum ether, 1:4); **^{1}H NMR (400 MHz, CDCl$_3$)** δ_H 7.60-7.54 (m, 1H), 7.39 (d, *J* = 8.1 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 2H), 5.59 (t, *J* = 7.1 Hz, 1H), 5.53 (s, 1H), 5.32 (s, 1H), 4.99 (s, 1H), 4.92 (s, 1H), 4.24-4.17 (m, 1H), 3.39-3.30 (m, 1H), 3.01 (q, *J* = 8.1 Hz, 1H), 2.85 (t, *J* = 9.3 Hz, 1H), 2.48-2.38 (m, 1H), 2.36-2.26 (m, 2H), 2.20-2.14 (m, 1H), 2.11 (s, 3H), 1.87-1.77 (m, 1H); **^{13}C NMR (100 MHz, CDCl$_3$)** δ_C 171.3, 170.8, 147.9, 147.2, 136.5, 135.4, 132.4, 130.8, 130.3, 128.9, 115.0, 114.1, 83.5,

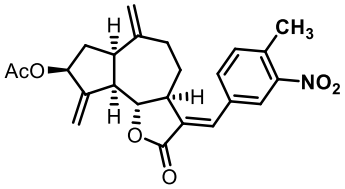
74.6, 50.6, 45.2, 43.4, 36.7, 32.9, 28.3, 21.3; **HRMS (ESI)** calcd for $C_{23}H_{23}O_4ClNa$ $[M+Na]^+$ 421.1177, found 421.1171.

(3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-3-((*E*)-2-methoxy-6-nitrobenzylidene)-6,9-dimethylene-2-oxodo decahydroazuleno[4,5-*b*]furan-8-yl acetate (26):



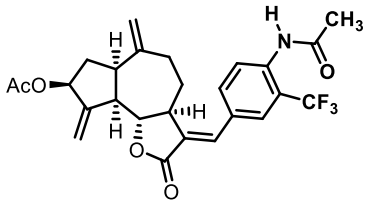
Brown viscous liquid (42%); R_f = 0.37 (EtOAc-petroleum ether, 3:7); **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H 7.67 (d, *J* = 7.8 Hz, 1H), 7.42 (t, *J* = 8.3 Hz, 1H), 7.15 (d, *J* = 8.1 Hz, 1H), 6.86 (brs, 1H), 5.56 (t, *J* = 7.3 Hz, 1H), 5.45 (s, 1H), 5.27 (s, 1H), 4.98 (s, 2H), 4.10 (t, *J* = 9.5 Hz, 1H), 3.83 (s, 3H), 3.11-3.01 (m, 1H), 2.97 (q, *J* = 8.1 Hz, 1H), 2.90-2.82 (m, 1H), 2.57-2.50 (m, 1H), 2.48-2.36 (m, 2H), 2.28-2.19 (m, 1H), 2.11 (s, 3H), 1.86-1.78 (m, 1H), 1.64-1.53 (m, 1H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 170.9, 170.9, 148.6, 148.0, 147.8, 132.4, 129.3, 122.7, 119.7, 116.4, 115.4, 114.3, 113.7, 83.1, 74.7, 56.5, 50.3, 46.4, 44.7, 36.5, 34.3, 30.6, 21.3; **HRMS (ESI)** calcd for $C_{24}H_{25}O_7NNa$ $[M+Na]^+$ 462.1523, found 462.1521.

(3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-3-((*E*)-4-methyl-3-nitrobenzylidene)-6,9-dimethylene-2-oxodo decahydroazuleno[4,5-*b*]furan-8-yl acetate (27):



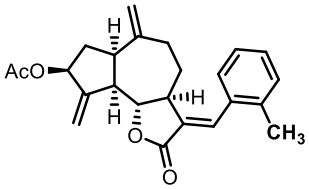
Light brown semi solid (54%); R_f = 0.56 (EtOAc-petroleum ether, 3:7); **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H 8.00 (s, 1H), 7.57 (d, *J* = 3.4 Hz, 1H), 7.53-7.48 (m, 1H), 7.41 (d, *J* = 7.8 Hz, 1H), 5.63-5.55 (m, 1H), 5.55-5.51 (m, 1H), 5.32 (t, *J* = 1.7 Hz, 1H), 5.02 (s, 1H), 4.96 (s, 1H), 4.23 (dd, *J* = 10.4, 7.7 Hz, 1H), 3.47-3.39 (m, 1H), 3.10-2.94 (m, 1H), 2.93-2.80 (m, 1H), 2.65 (s, 3H), 2.49-2.38 (m, 1H), 2.38-2.26 (m, 2H), 2.26-2.15 (m, 1H), 2.11 (s, 3H), 1.87-1.78 (m, 1H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 170.8, 170.8, 149.1, 147.9, 146.9, 134.7, 133.9, 133.2, 132.6, 131.9, 126.6, 125.1, 115.3, 114.1, 83.6, 74.6, 50.6, 45.2, 43.3, 36.6, 32.5, 28.2, 21.2, 20.5; **HRMS (ESI)** calcd for $C_{24}H_{25}O_6NNa$ $[M+Na]^+$ 446.1574, found 446.1569.

(3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-3-((*E*)-4-acetamido-3-(trifluoromethyl)benzylidene)-6,9-dimethylene-2-oxododecahydroazuleno[4,5-*b*]furan-8-yl acetate (28):



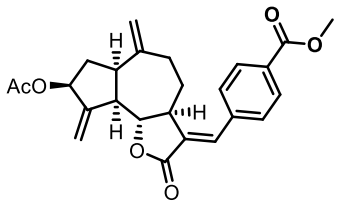
Brown viscous liquid (68%); R_f = 0.15 (EtOAc-petroleum ether, 3:7); **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 8.37 (d, *J* = 7.9 Hz, 1H), 7.62 (s, 1H), 7.59-7.53 (m, 3H), 5.62-5.56 (m, 1H), 5.54 (s, 1H), 5.34-5.31 (m, 1H), 5.02 (s, 1H), 4.94 (s, 1H), 4.23 (dd, *J* = 10.4, 7.6 Hz, 1H), 3.40 (dt, *J* = 7.6, 3.7 Hz, 1H), 3.03 (q, *J* = 8.2 Hz, 1H), 2.90-2.82 (m, 1H), 2.43 (td, *J* = 14.0, 8.1 Hz, 1H), 2.35-2.29 (m, 2H), 2.26 (s, 3H), 2.24-2.15 (m, 2H), 2.12 (s, 3H), 1.87-1.79 (m, 1H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 171.1, 170.8, 168.5, 147.9, 147.0, 136.0, 135.4, 133.9, 130.7, 127.4, 123.8, 115.2, 114.1, 83.6, 74.6, 50.6, 45.3, 43.2, 36.7, 32.4, 28.1, 24.9, 21.3; HRMS (ESI) calcd for $C_{26}H_{26}O_5NF_3Na$ $[M+Na]^+$ 512.1655, found 512.1650.

(3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-3-((*E*)-2-methylbenzylidene)-6,9-dimethylene-2-oxododecahydroazuleno[4,5-*b*]furan-8-yl acetate (29):



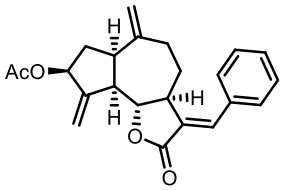
Brown viscous liquid (56%); R_f = 0.45 (EtOAc-petroleum ether, 1:4); **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 7.73 (d, *J* = 3.4 Hz, 1H), 7.27-7.16 (m, 4H), 5.62-5.55 (m, 1H), 5.53 (t, *J* = 2.0 Hz, 1H), 5.32 (t, *J* = 1.8 Hz, 1H), 4.94 (s, 1H), 4.88-4.82 (m, 1H), 4.17 (dd, *J* = 10.4, 8.2 Hz, 1H), 3.33-3.23 (m, 1H), 2.97 (q, *J* = 8.0 Hz, 1H), 2.89-2.81 (m, 1H), 2.44-2.38 (m, 1H), 2.31 (s, 3H), 2.27-2.15 (m, 2H), 2.11 (s, 3H), 2.06-2.00 (m, 2H), 1.84-1.76 (m, 1H); **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 171.4, 170.9, 148.0, 147.4, 137.5, 136.9, 133.4, 130.6, 130.3, 129.3, 127.9, 125.7, 114.6, 114.1, 83.4, 74.7, 50.7, 45.1, 43.9, 36.6, 33.0, 28.7, 21.3, 19.9; **HRMS (ESI)** calcd for $C_{24}H_{26}O_4Na$ $[M+Na]^+$ 401.1723, found 401.1718.

Methyl-4-((*E*)-((3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-8-acetoxy-6,9-dimethylene-2-oxodecahydroazuleno[4,5-*b*]furan-3(2*H*)-ylidene)methyl)benzoate (30):



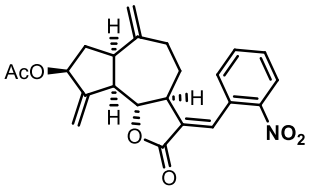
Brown viscous liquid (67%); $\boldsymbol{R_f}$ = 0.24 (EtOAc-petroleum ether, 1:4); **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H 8.07-8.02 (m, 1H), 7.58-7.52 (m, 1H), 7.48-7.43 (m, 1H), 7.36-7.23 (m, 2H), 5.60-5.51 (m, 2H), 5.45 (d, *J* = 11.2 Hz, 1H), 4.95-4.89 (m, 2H), 4.17 (dd, *J* = 10.3, 8.3 Hz, 1H), 3.90 (s, 3H), 3.24-3.17 (m, 1H), 2.98-2.90 (m, 1H), 2.86-2.78 (m, 1H), 2.59-2.51 (m, 1H), 2.47-2.32 (m, 2H), 2.21-2.12 (m, 1H), 2.10 (s, 3H), 2.01-1.93 (m, 1H), 1.79-1.72 (m, 1H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 171.0, 170.8, 166.6, 147.9, 147.3, 138.3, 136.4, 132.2, 130.9, 129.3, 128.9, 128.8, 128.4, 114.5, 114.3, 83.0, 74.7, 52.4, 50.8, 45.2, 43.7, 36.6, 32.6, 29.0, 21.3; **HRMS (ESI)** calcd for $C_{25}H_{26}O_6Na$ $[M+Na]^+$ 445.1622, found 445.1616.

(3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-3-((*E*)-benzylidene)-6,9-dimethylene-2-oxododecahydroazuleno [4,5-*b*]furan-8-yl acetate (31):



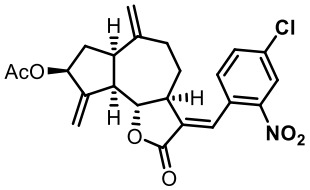
Light brown viscous liquid (55%); $\boldsymbol{R_f}$ = 0.57 (EtOAc-petroleum ether, 1:4); **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H 7.41-7.36 (m, 6H), 5.63-5.56 (m, 1H), 5.54 (t, *J* = 2.0 Hz, 1H), 5.32 (t, *J* = 2.0 Hz, 1H), 4.98 (s, 1H), 4.92 (s, 1H), 4.20 (dd, *J* = 10.3, 7.8 Hz, 1H), 3.43-3.35 (m, 1H), 3.01 (q, *J* = 8.1 Hz, 1H), 2.92-2.80 (m, 1H), 2.48-2.36 (m, 2H), 2.33-2.27 (m, 1H), 2.19-2.13 (m, 1H), 2.11 (s, 3H), 1.96-1.89 (m, 1H), 1.87-1.78 (m, 1H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 171.6, 170.9, 148.0, 147.4, 138.0, 133.9, 129.6, 129.4, 128.6, 128.4, 114.8, 113.9, 83.6, 74.7, 50.6, 45.2, 43.5, 36.7, 33.1, 28.4, 21.3; **HRMS (ESI)** calcd for $C_{23}H_{24}O_4Na$ $[M+Na]^+$ 387.1567, found 387.1563.

(3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-6,9-dimethylene-3-((*E*)-2-nitrobenzylidene)-2-oxododecahydro azuleno[4,5-*b*]furan-8-yl acetate (32):



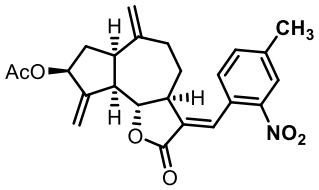
Brown viscous liquid (47%); $\boldsymbol{R_f}$ = 0.20 (EtOAc-petroleum ether, 1:4); **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H 8.23-8.13 (m, 1H), 7.87 (d, *J* = 3.7 Hz, 1H), 7.73-7.65 (m, 1H), 7.63-7.55 (m, 1H), 7.42 (d, *J* = 7.3 Hz, 1H), 5.59-5.51 (m, 2H), 5.36-5.29 (m, 1H), 4.93 (s, 1H), 4.81 (s, 1H), 4.20 (dd, *J* = 10.3, 8.3 Hz, 1H), 3.24-3.12 (m, 1H), 2.94 (q, *J* = 8.4 Hz, 1H), 2.87-2.77 (m, 1H), 2.43-2.34 (m, 1H), 2.23-2.14 (m, 1H), 2.11 (s, 3H), 2.01-1.95 (m, 1H), 1.83-1.73 (m, 1H), 1.66-1.53 (m, 2H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 170.9, 170.3, 147.7, 147.5, 146.9, 134.2, 133.6, 132.5, 130.8, 130.1, 129.9, 125.1, 114.9, 114.6, 82.9, 74.6, 50.7, 45.2, 43.7, 36.6, 32.2, 29.0, 21.3; **HRMS (ESI)** calcd for $C_{23}H_{23}O_6NNa$ $[M+Na]^+$ 432.1418, found 432.1413.

(3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-3-((*E*)-4-chloro-2-nitrobenzylidene)-6,9-dimethylene-2-oxodo decahydroazuleno[4,5-*b*]furan-8-yl acetate (33):



Brown viscous liquid (52%); $\boldsymbol{R_f}$ = 0.26 (EtOAc-petroleum ether, 1:4); **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 8.18 (d, *J* = 2.1 Hz, 1H), 7.78 (d, *J* = 3.7 Hz, 1H), 7.66 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.37 (d, *J* = 8.2 Hz, 1H), 5.60-5.55 (m, 1H), 5.52 (t, *J* = 1.8 Hz, 1H), 5.33 (t, *J* = 1.8 Hz, 1H), 4.96-4.93 (m, 1H), 4.84 (s, 1H), 4.21 (dd, *J* = 10.4, 8.2 Hz, 1H), 3.19-3.12 (m, 1H), 2.98-2.92 (m, 1H), 2.84-2.77 (m, 1H), 2.42-2.35 (m, 1H), 2.23-2.17 (m, 1H), 2.11 (s, 3H), 2.04-1.98 (m, 1H), 1.91-1.82 (m, 1H), 1.82-1.76 (m, 1H), 1.67-1.60 (m, 1H); **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 170.8, 170.0, 147.9, 147.6, 146.7, 135.8, 133.7, 133.3, 132.8, 131.2, 129.1, 125.4, 115.1, 114.7, 82.8, 74.6, 50.8, 45.2, 43.7, 36.6, 32.0, 29.1, 21.3; **HRMS (ESI)** calcd for $C_{23}H_{22}O_6NClNa$ $[M+Na]^+$ 466.1028, found 466.1027.

(3a*S*,6a*R*,8*S*,9a*R*,9b*S*)-3-((*E*)-4-methyl-2-nitrobenzylidene)-6,9-dimethylene-2-oxodo decahydroazuleno[4,5-*b*]furan-8-yl acetate (34):



Brown viscous liquid (67%); $\boldsymbol{R_f}$ = 0.26 (EtOAc-petroleum ether, 1:4); **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H 8.02-7.94 (m, 1H), 7.82 (d, *J* = 3.7 Hz, 1H), 7.48 (d, *J* = 7.3 Hz, 1H), 7.30 (d, *J* = 7.8 Hz, 1H), 5.62-5.50 (m, 2H), 5.36-5.27 (m, 1H), 5.03-4.90 (m, 2H), 4.82 (s, 1H), 4.25-4.10 (m, 1H), 3.21-3.14 (m, 1H), 2.99-2.90 (m, 1H), 2.88-2.75 (m, 1H), 2.51 (s, 3H), 2.42-2.36 (m, 1H), 2.22-2.16 (m, 1H), 2.11 (s, 3H), 2.03-1.95 (m, 1H), 1.87-1.63 (m, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C 170.9, 170.4, 147.8, 147.4, 147.0, 140.8, 134.3, 132.0, 129.9, 127.8, 125.4, 124.9, 114.8, 114.5, 82.9, 74.6, 50.7, 45.2, 43.8, 36.6, 32.3, 29.0, 21.3, 21.2; **HRMS (ESI)** calcd for $C_{24}H_{25}O_6NNa$ $[M+Na]^+$ 446.1574, found 446.1571.

Biology

Anticancer studies

Compounds were dissolved in DMSO (Sigma) to prepare 50 mM concentrations stock solutions. All the further dilutions were also made in DMSO. During the treatment, the final concentration of DMSO was maintained at <0.02%.

Antibodies

Anti-Caspase 9 and Anti-Caspase 3 antibodies were purchased from Cell Signaling and Anti-αTubulin antibody was procured from Sigma, Goat anti-rabbit HRP conjugated secondary antibody was purchased from Bio-Rad, and Goat anti-mouse HRP conjugated secondary antibody was purchased from Cell Signaling.

Cell culture

Breast cancer cell line MCF7 was grown in DMEM (GIBCO), MBA-MB-231 in RPMI (GIBCO) with 10 % FBS, 100 U/mL penicillin and 100 µg/mL streptomycin and MCF10A in DMEM/F12 (Gibco) containing, horse Serum (10% final), EGF (20ng/ml), Hydrocortisone (0.5 mg/ml), Cholera Toxin (100 ng/ml), Insulin (10 µg/ml) and Penicillin/Streptomycin mix (1ml/100ml) at 37 °C in a humid, 5% CO_2 regulated incubator.

Growth inhibition by cytotoxicity assay

The cytotoxic effect of the compounds was determined using MTT (3-(4, 5 dimethylthiazol-2-yl)-2-5 diphenyltetrazolium bromide) assay. Cells were seeded (4 X 10^3 per well) in 96

well plates. After 24 hours of seeding, cells were exposed with varying concentrations (0-100 µM) of respective compounds for 48 hours in triplicates. Then, MTT solution (20 µL of 5 mg/mL stock for each well of 96 well plate) was added and further incubated for 3.5 hours in humid 5% CO_2 incubator. Media containing MTT solution was then replaced by MTT solvent (*iso*-propanol, HCl and Triton X-100), incubated for 15 min at room temperature with gentle shaking for complete dissolution of Formazan. Absorbance was measured at 570 nm using a Thermo Scientific Multiskan G0 Elisa plate reader. All experiments were carried out at least in triplicate, and the percentage of viable cells was calculated as the mean with respect to the controls.

Western Blot analysis

The cells were harvested, washed with 1XPBS and were lysed in lysis buffer (50 mM Tris pH 7.4, 5 mM EDTA, 250 mM NaCl, 10 mM sodium fluoride, 0.5 mM sodium orthovanadate and 0.5% Triton X100) with 100 µL lysis buffer per 35 mm cell culture plate. The lysate was incubated on ice for 20 minutes followed by centrifugation at 16000xg for 20 minutes at 4 °C. The supernatant was collected, and the protein content was estimated by Bradford method using bovine serum albumin as a standard. The protein samples were prepared in 1X Lamelli Buffer and boiled for 5 minutes. The protein samples were then resolved by SDS-PAGE and transferred onto polyvinylidene fluoride membrane (Merck Millipore, Billerica, MA, USA). Skimmed milk (3%) in 0.05% Tween (TBST) was used for blocking the membrane for 1 h. The membrane was then washed and incubated with the respective primary antibodies at 4 °C for overnight. The membrane was then washed thrice with TBST and incubated with respective HRP conjugated secondary antibody. Protein bands were detected using the Super Signal West Pico substrate (Thermo Scientific).

Cell cycle analysis by fluorescence-activated cell shortening (FACS)

Cells were seeded one-day prior the treatment of compounds. Next day cells were incubated with and without selected compounds for 24 h and were then collected for FACS analysis**.** Propidium iodide staining was performed for the total DNA content of the cells. Briefly, the cells were washed with 1X PBS, trypsinized and then spin down at 3000xg for 2 minutes at 4 °C. The cell pellet obtained was fixed and permeabilized using 900 µL of 95% chilled ethanol, which was added dropwise along with continuous vortexing. The cells were then stored overnight at 4 °C. The fixed cells were then pelleted at 3000xg for 2-3 minutes. The

supernatant was discarded, and the pellet was washed twice with 1X PBS. The pellet was dissolved and stained with 1 mL staining solution (900 µL 1XPBS, 2 mM $MgCl_2$, 50 µL Propidium Iodide stock solution (5 mg/mL of 1XPBS) 50 µL RNase stock solution (1 mg/mL) and incubated at 37 °C for 20 minutes. Cells were then passed through cell strainers and proceed for FACS accusation on BD FACS Calibur. The data were then analyzed using Cell Quest pro software.

DNA fragmentation assay

DNA fragmentation assay was done after treating MCF7 cells with respective compounds as per the protocol described previously.[15] The fragmentation ladder of the DNA was observed on a 2% agarose gel.

Immunostaining

The cells were grown on a cover slip as a monolayer overnight. The cells were then exposed with respective compounds at IC_{50} concentration and incubated for 12 hours. The cells on coverslips were fixed in 3.7% formaldehyde for 20 minutes in the dark at room temperature. The cover slips were washed 2–3 times with 1XPBS. The cells were then permeabilized by 0.5% Tween-20 at room temperature for 30 minutes and then washed with PBS for 4 times. The permeabilized cells were blocked with 3% BSA followed by staining with α-tubulin (Sigma) in 3% BSA solution for 1 h at room temperature. The cells were washed with 1XPBS 5 times and then stained with ALEXA Flour 594 conjugated secondary antibody for 1 h and then washed with 1XPBS for 5 times. Finally, DNA was stained with Hoechst and coverslips were mounted on slides in mounting media (8 mg/mL DABCO in 80% glycerol and 20% PBS).

3.7. REFERENCES

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11. (a) Crystallographic data (excluding structure factors): CCDC-1525170 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif; (b) Valkute, T. R.; Aratikatla, E. K.; Gupta, N. A.; Ganga S.; Santra, M. K.; Bhattacharya, A. K. *RSC Adv.* **2018**, 8, 38289.

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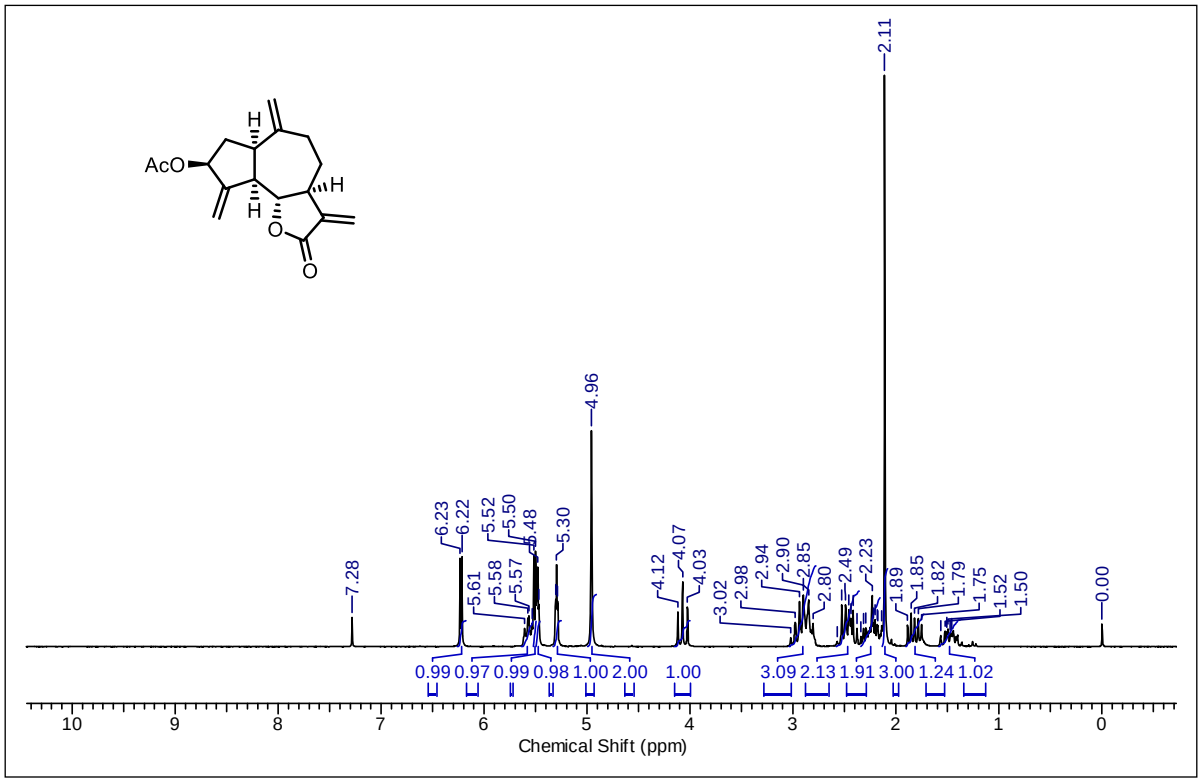
3.8. SPECTRA

^{1}H NMR of Compound 1 (200 MHz, $CDCl_3$)



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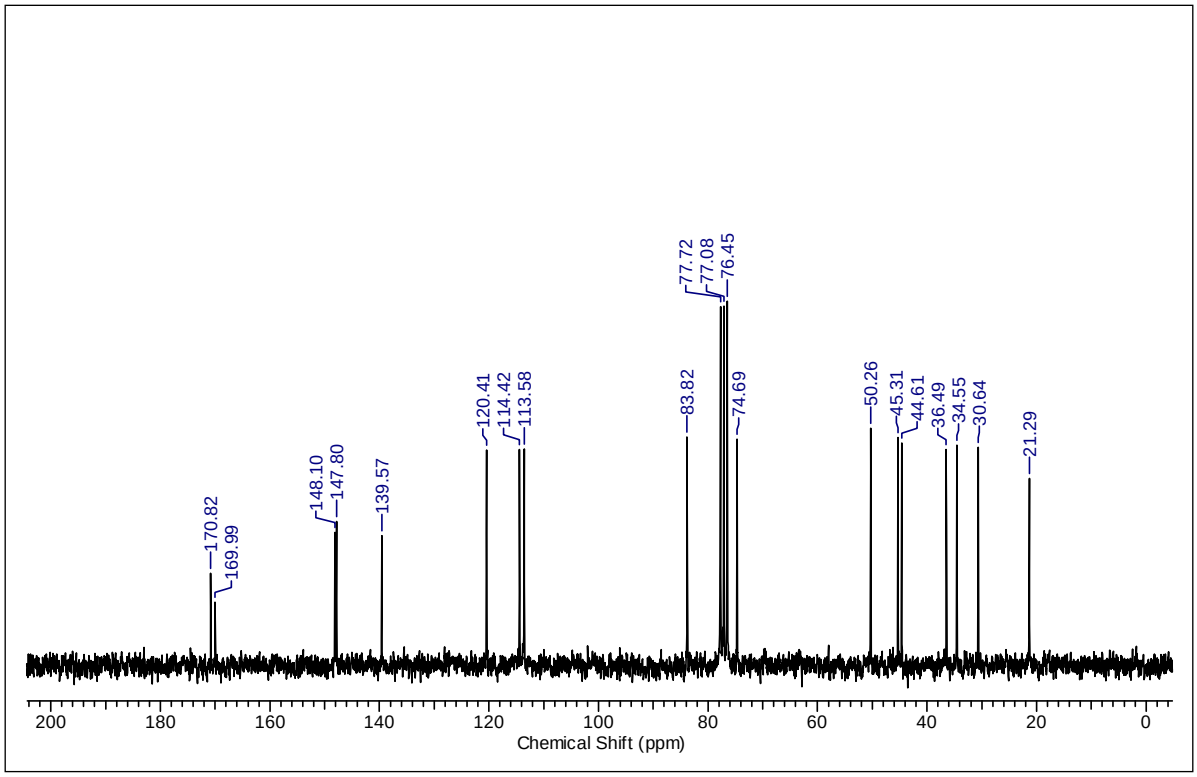

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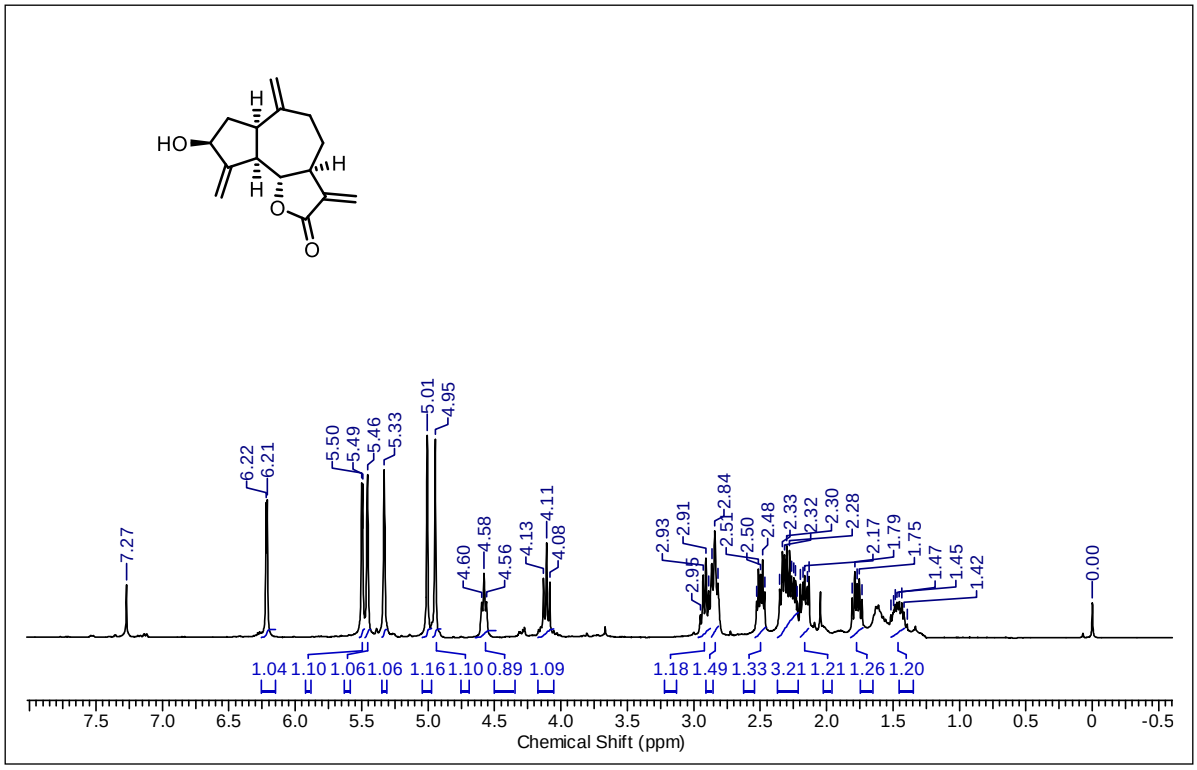

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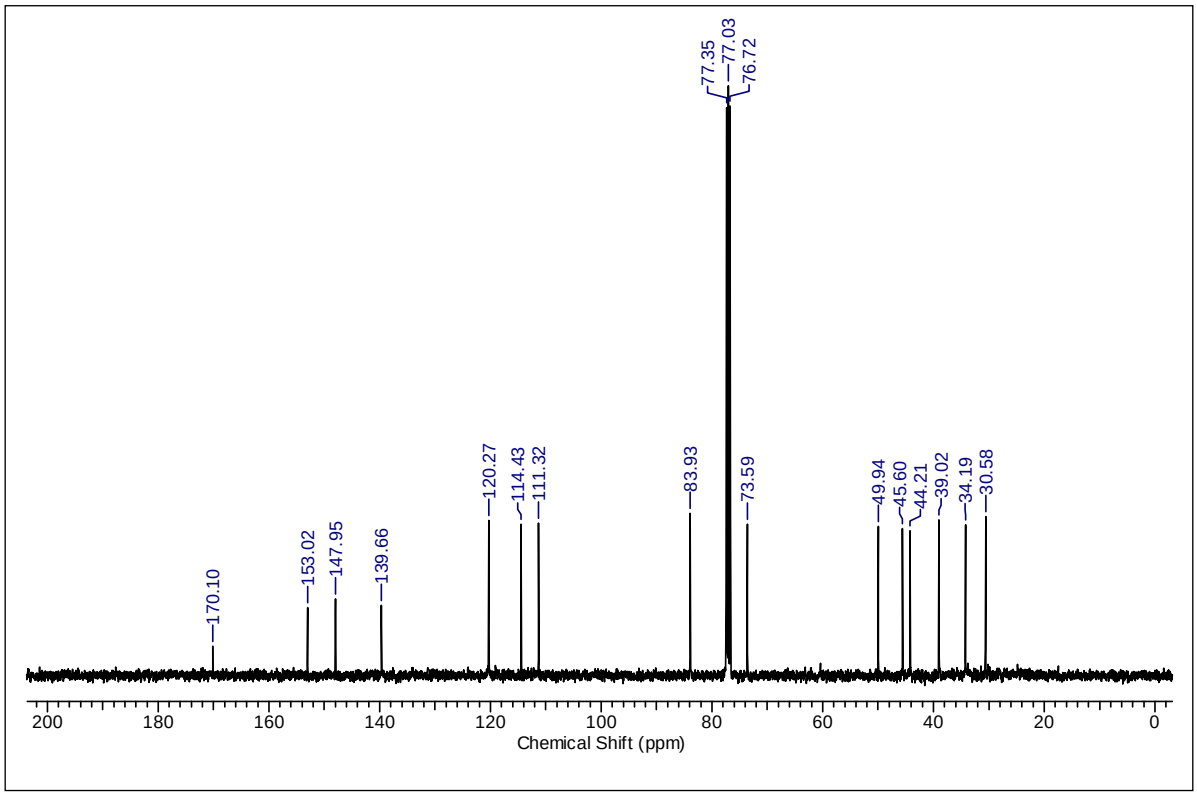

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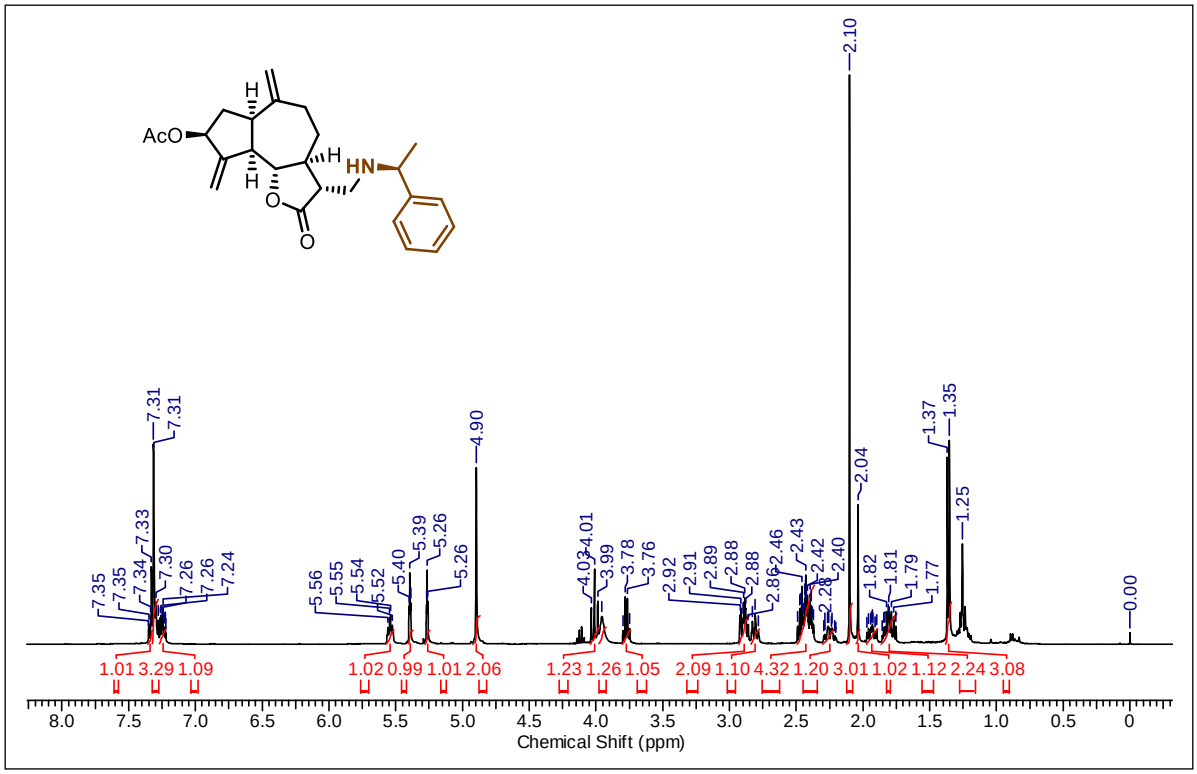

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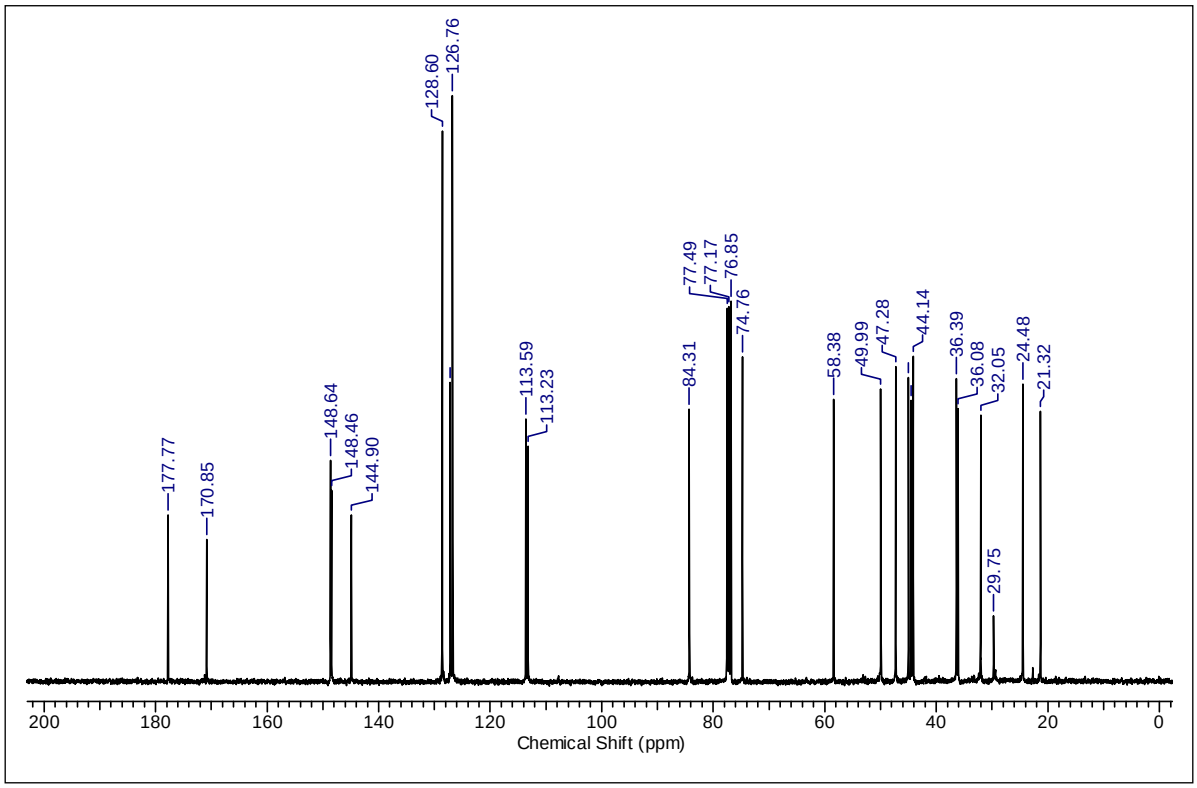

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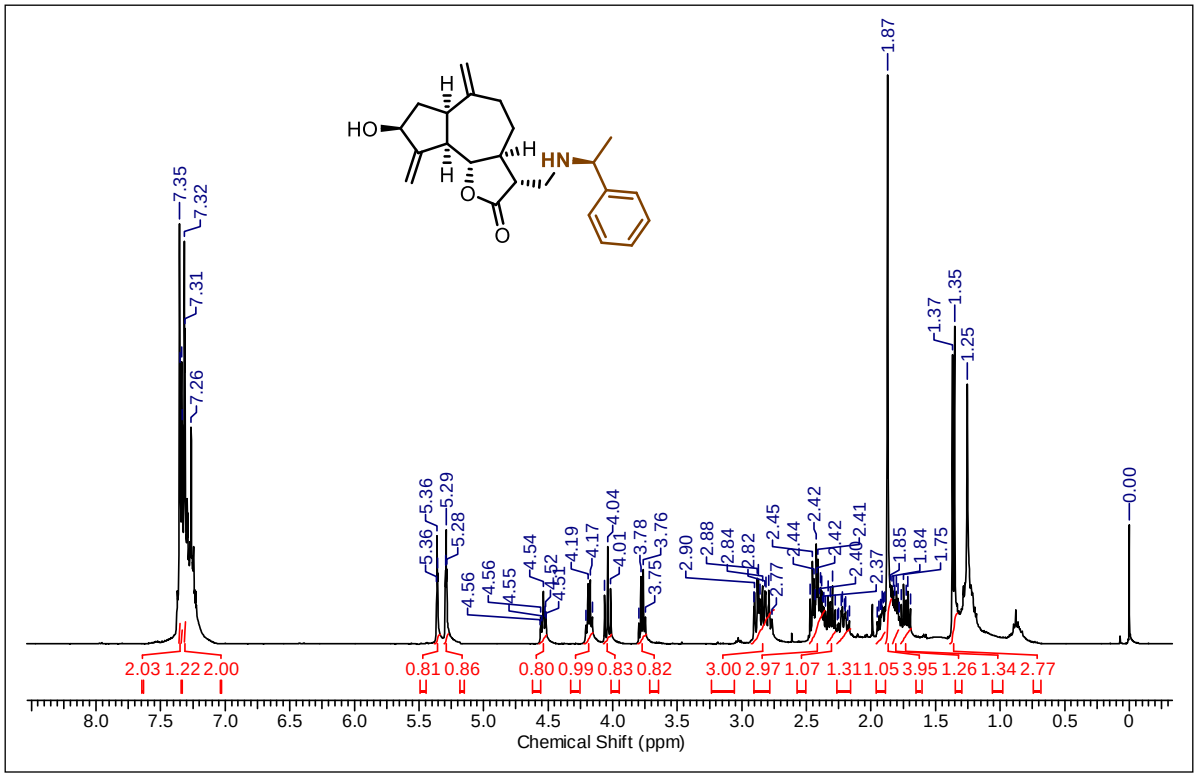

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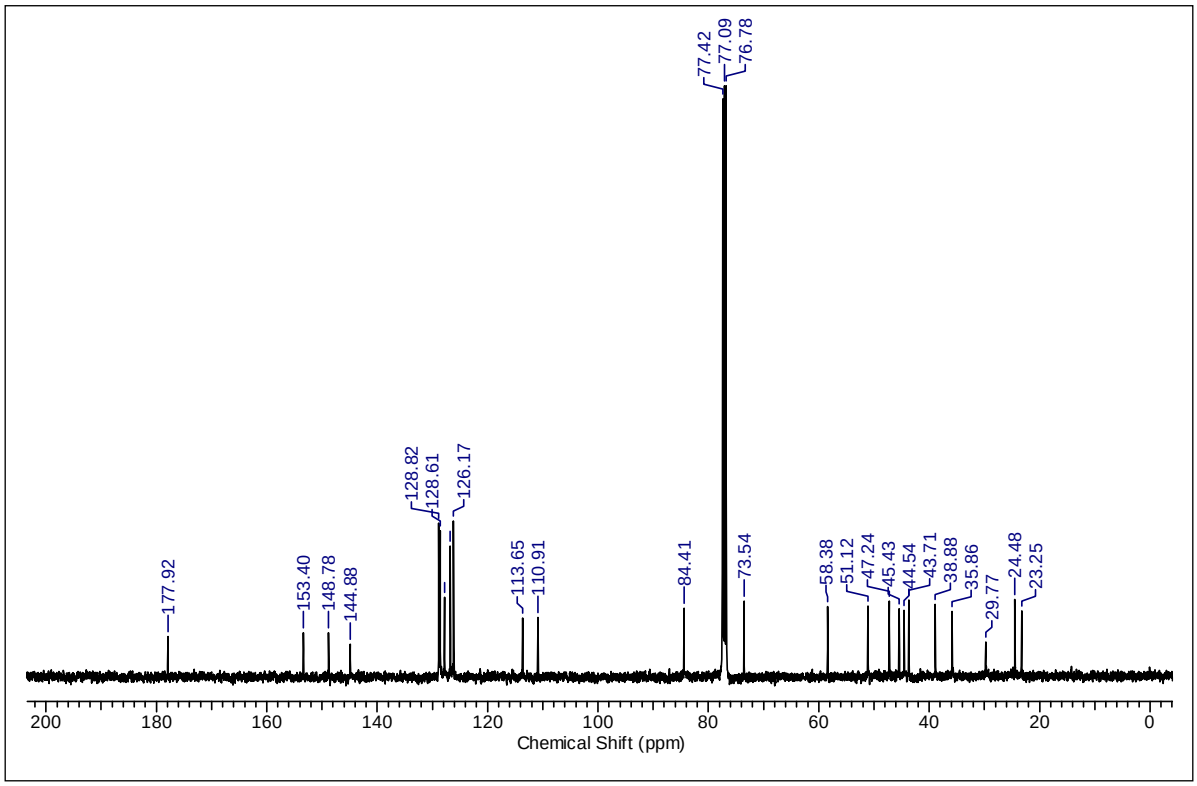

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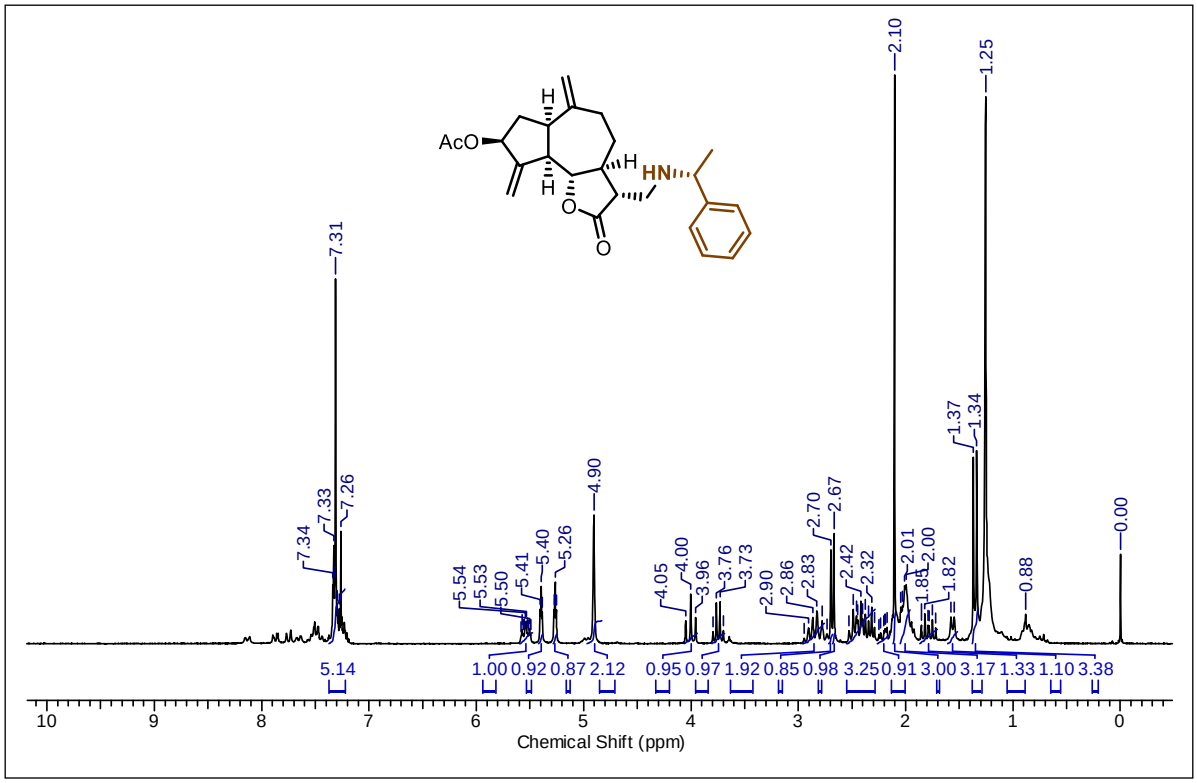

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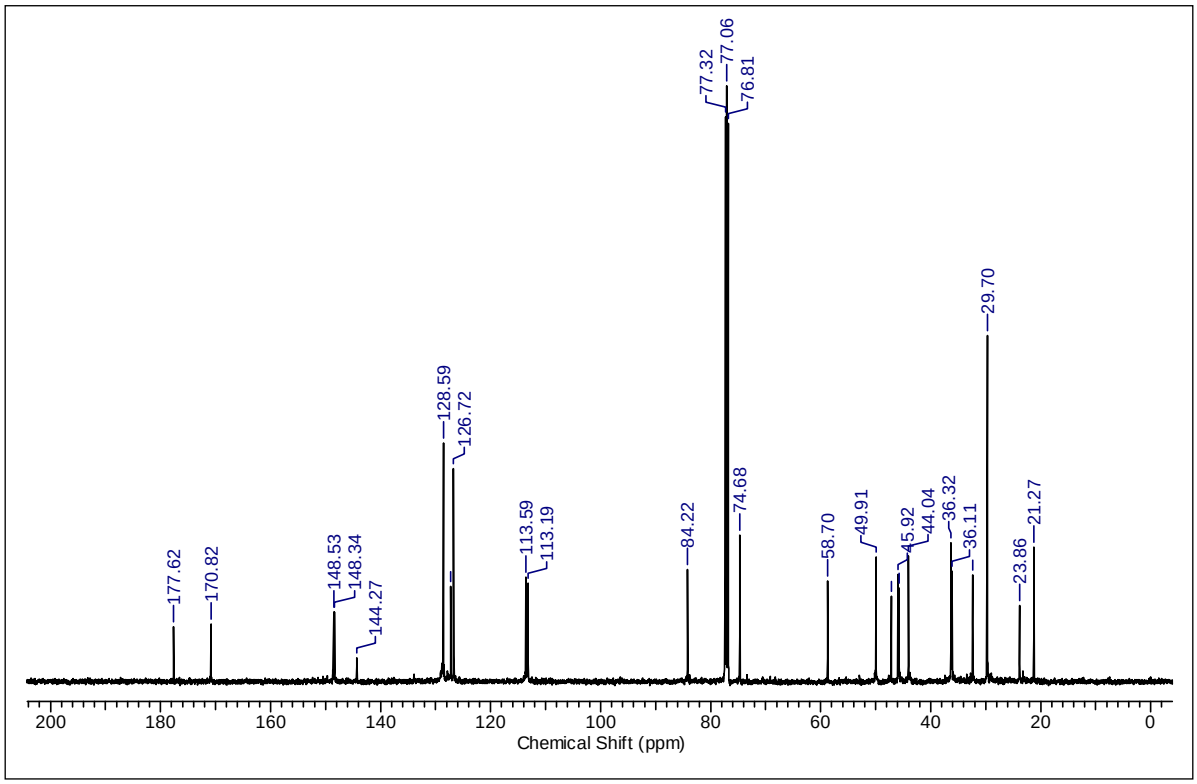

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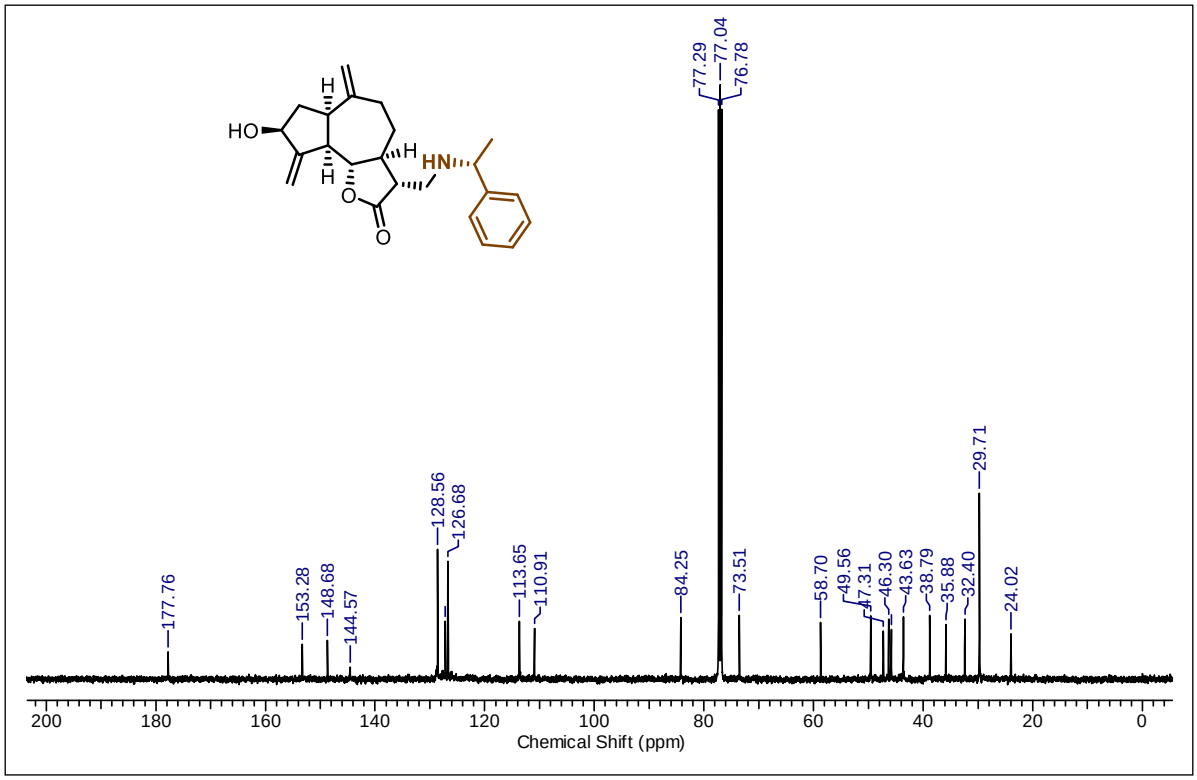

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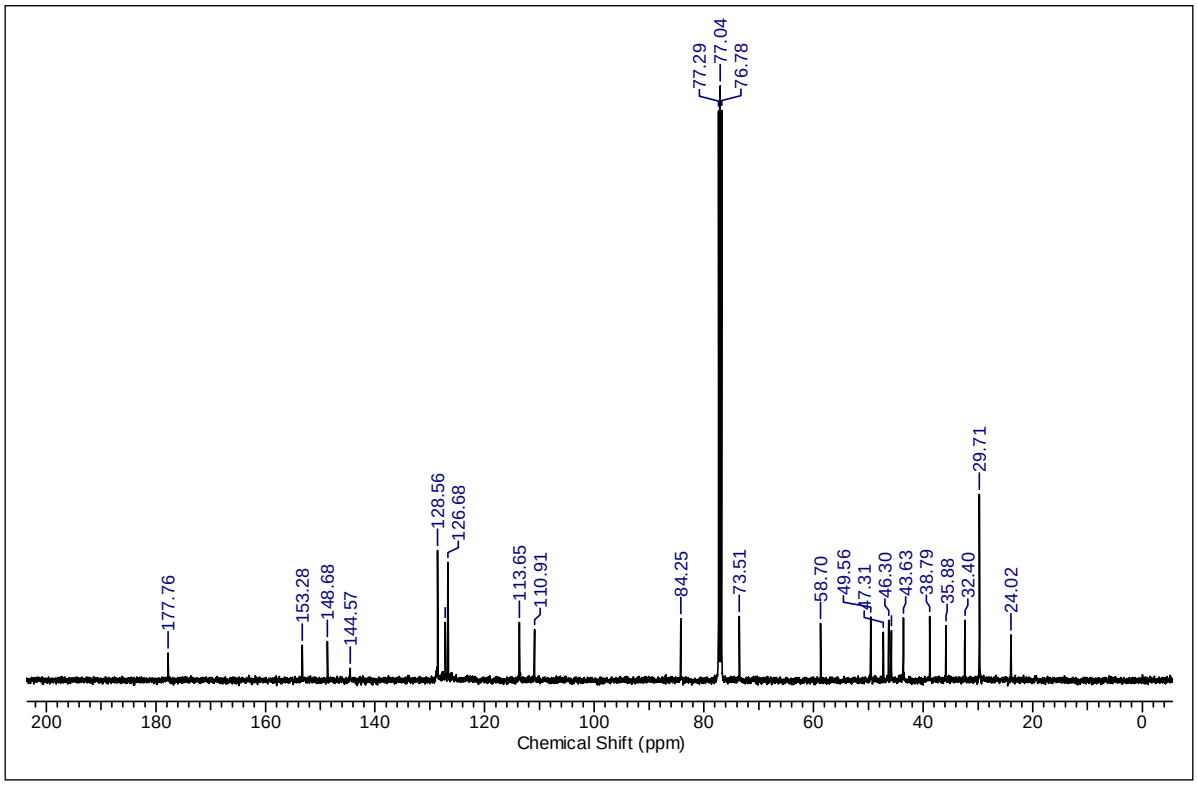

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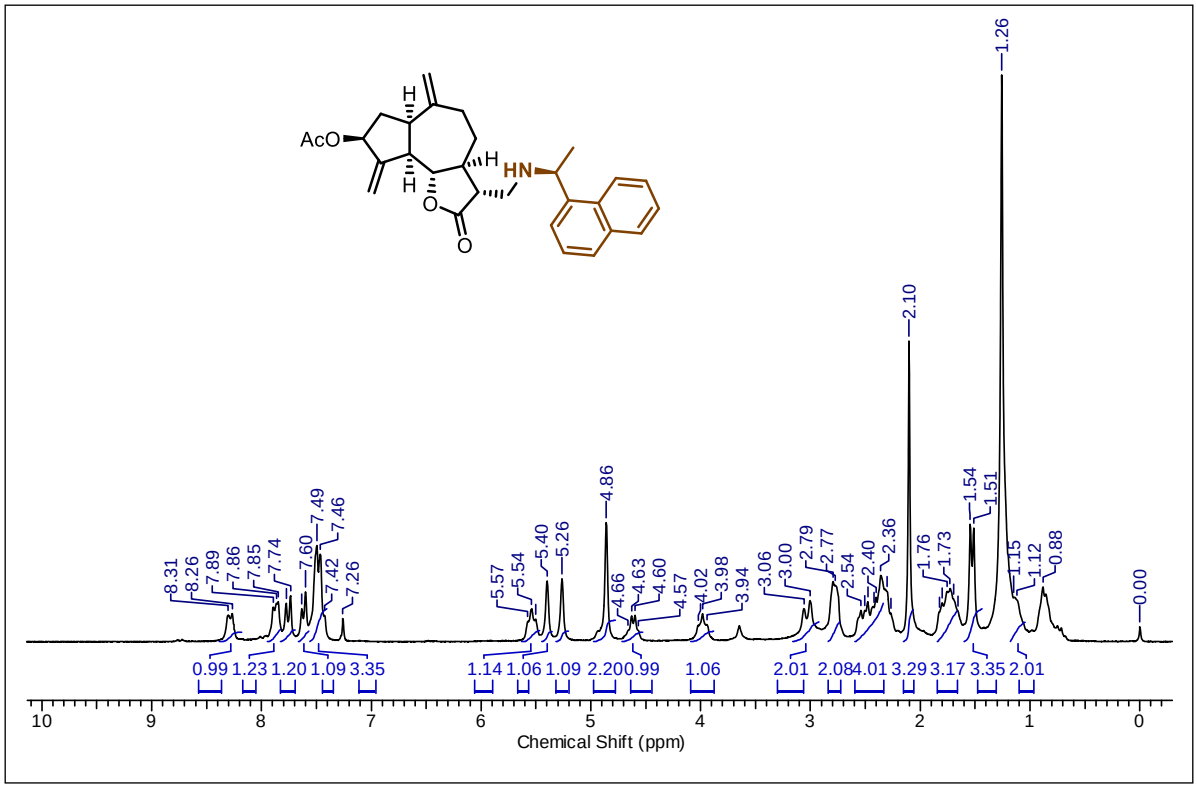

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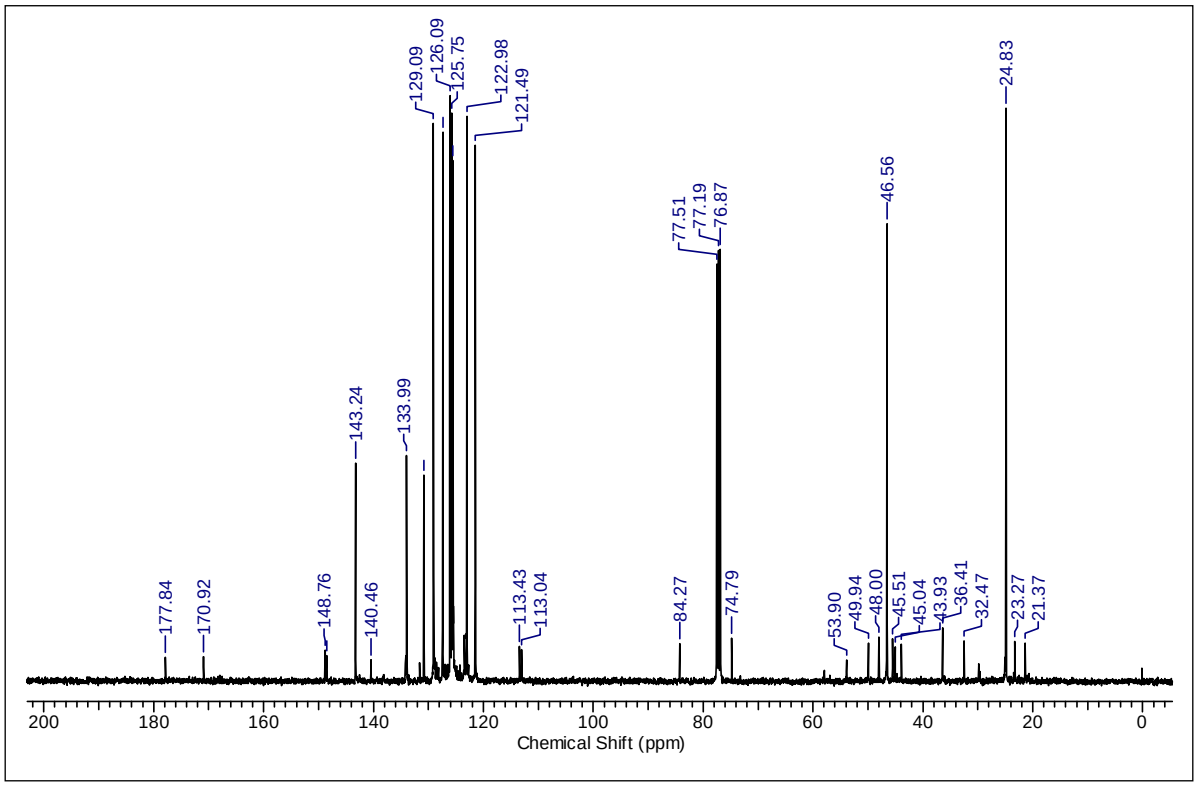

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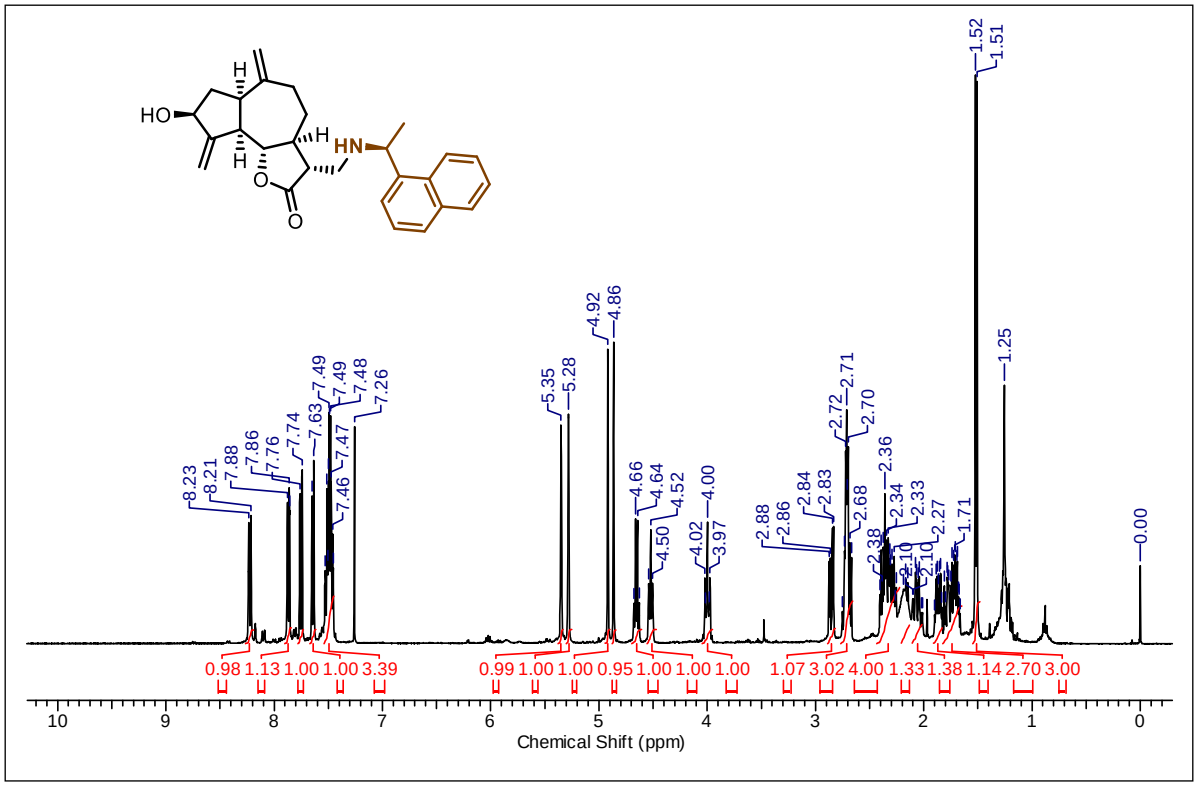

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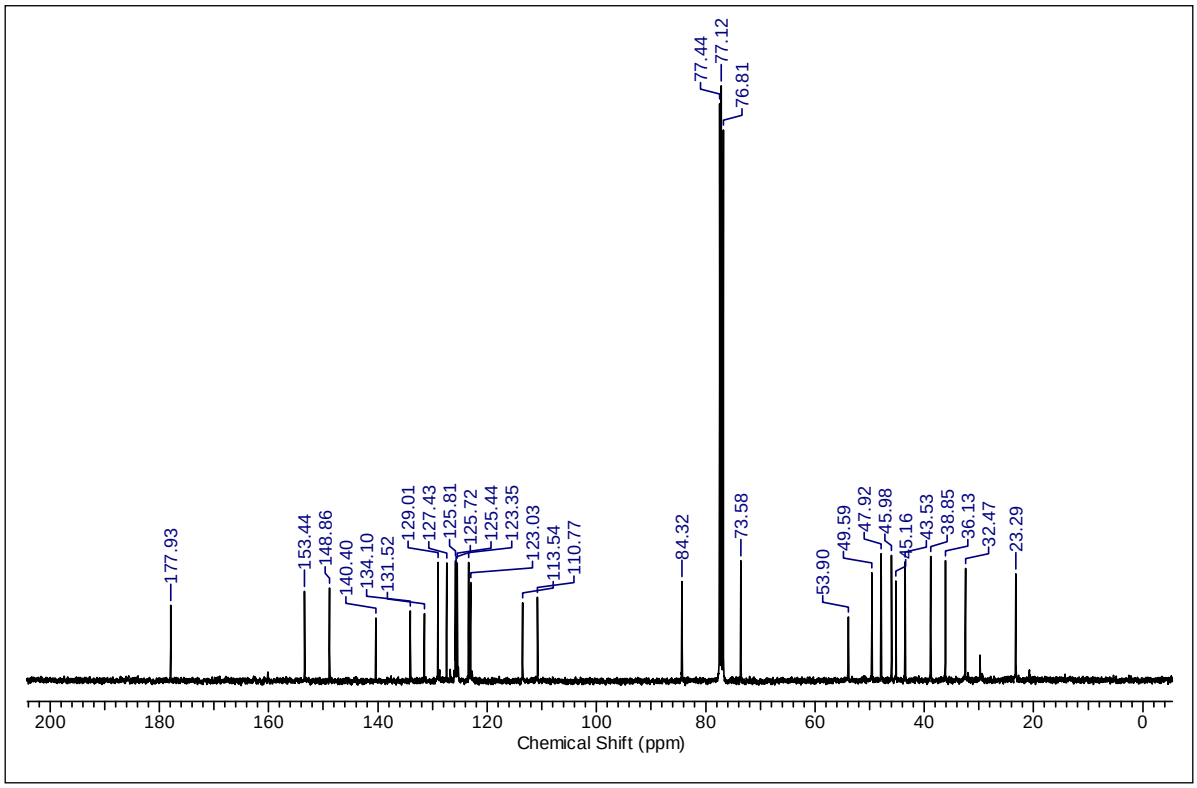

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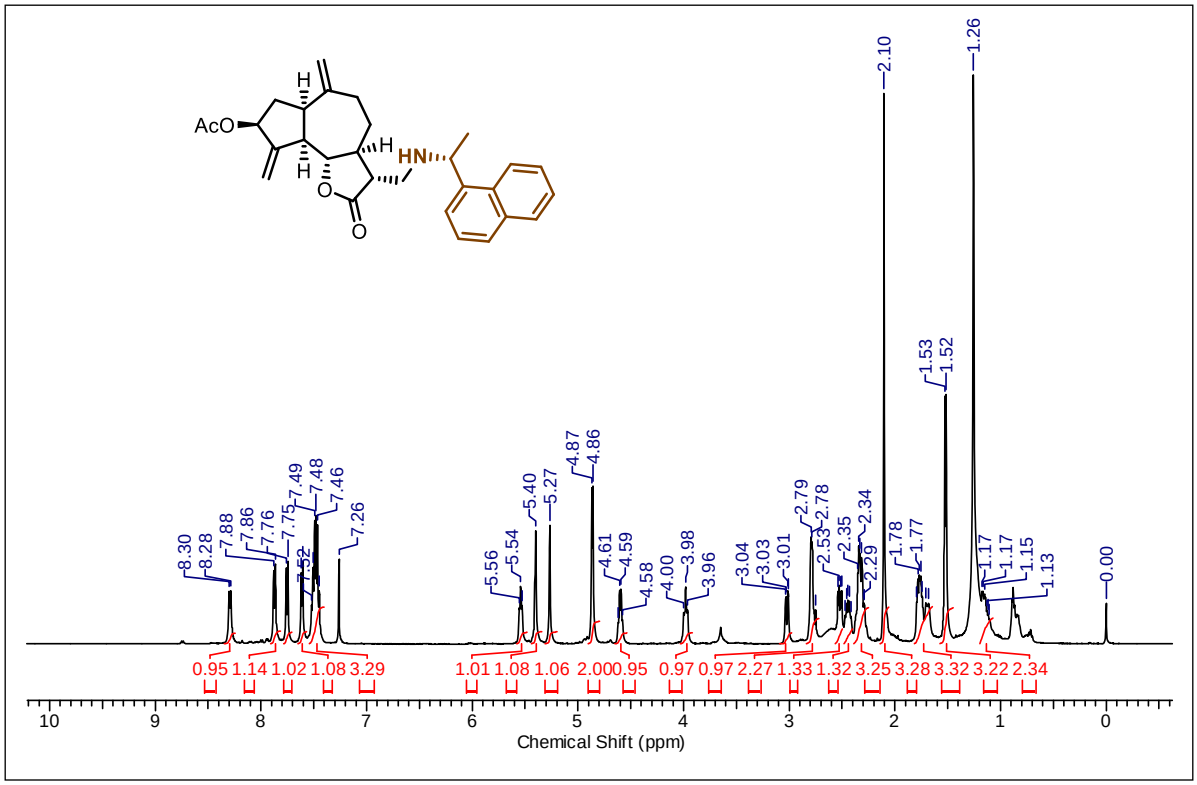

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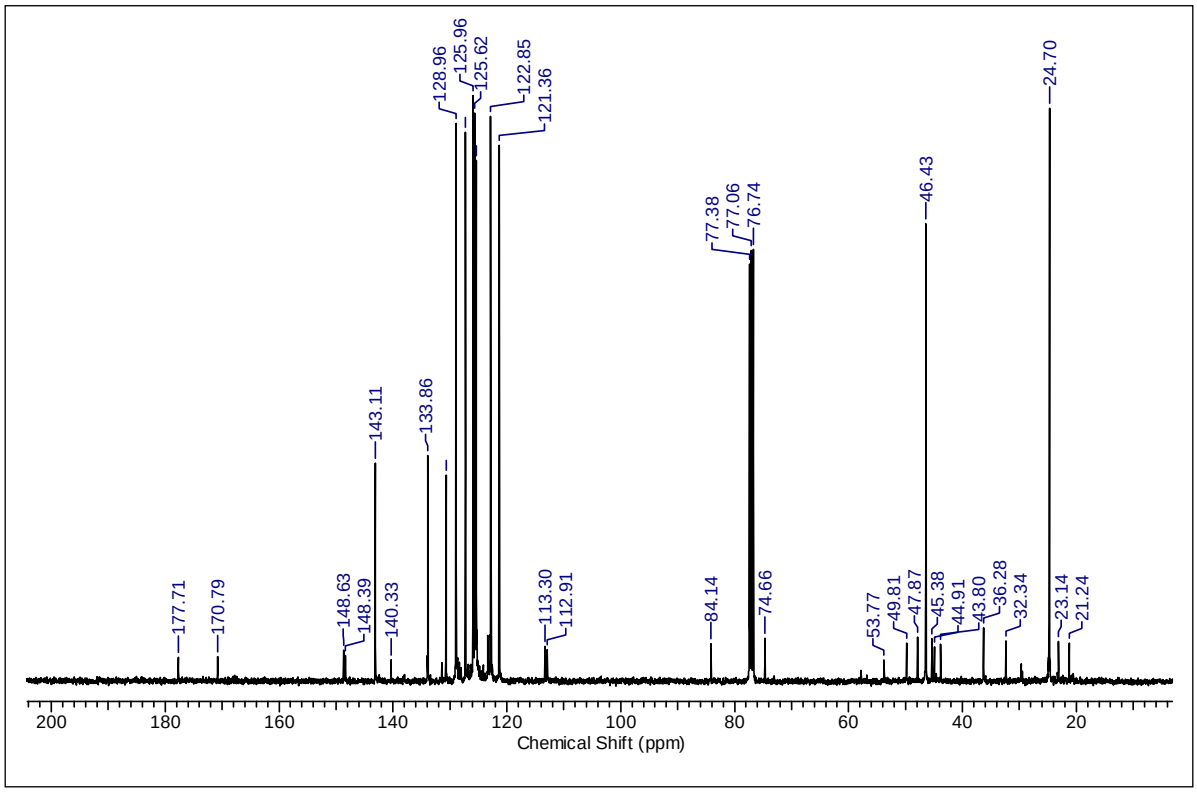

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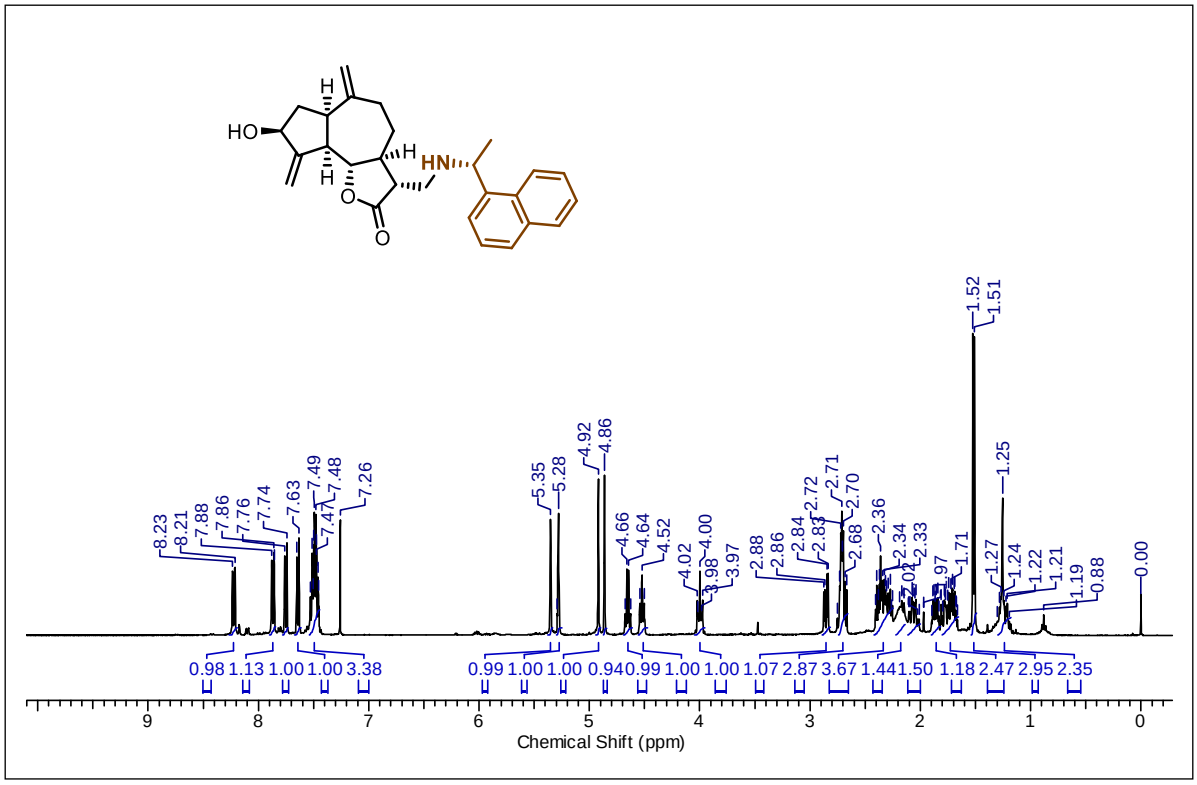

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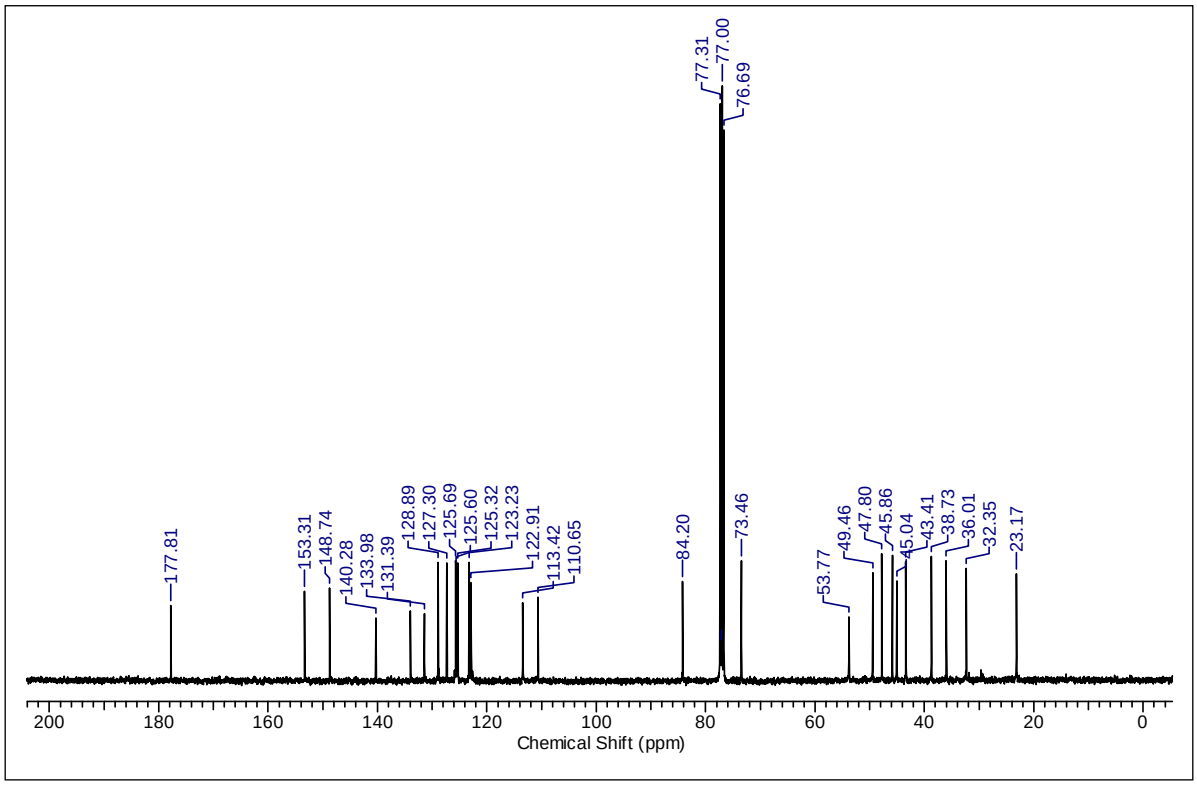

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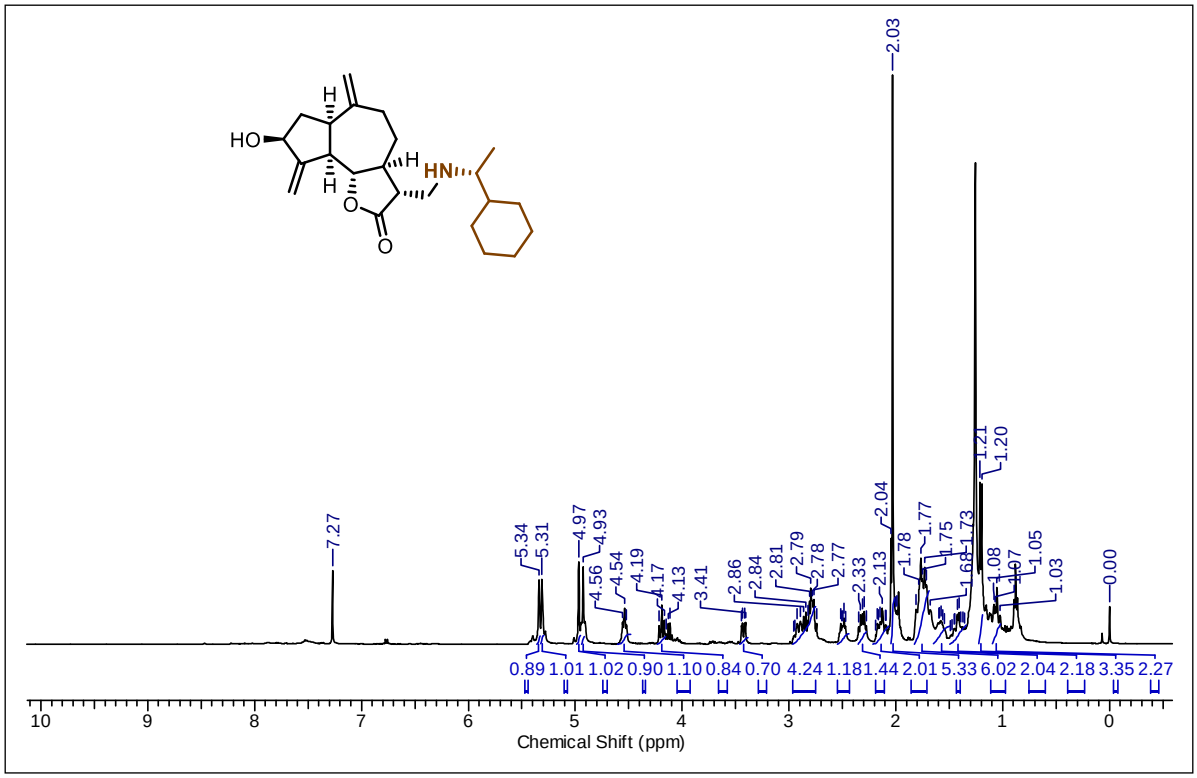

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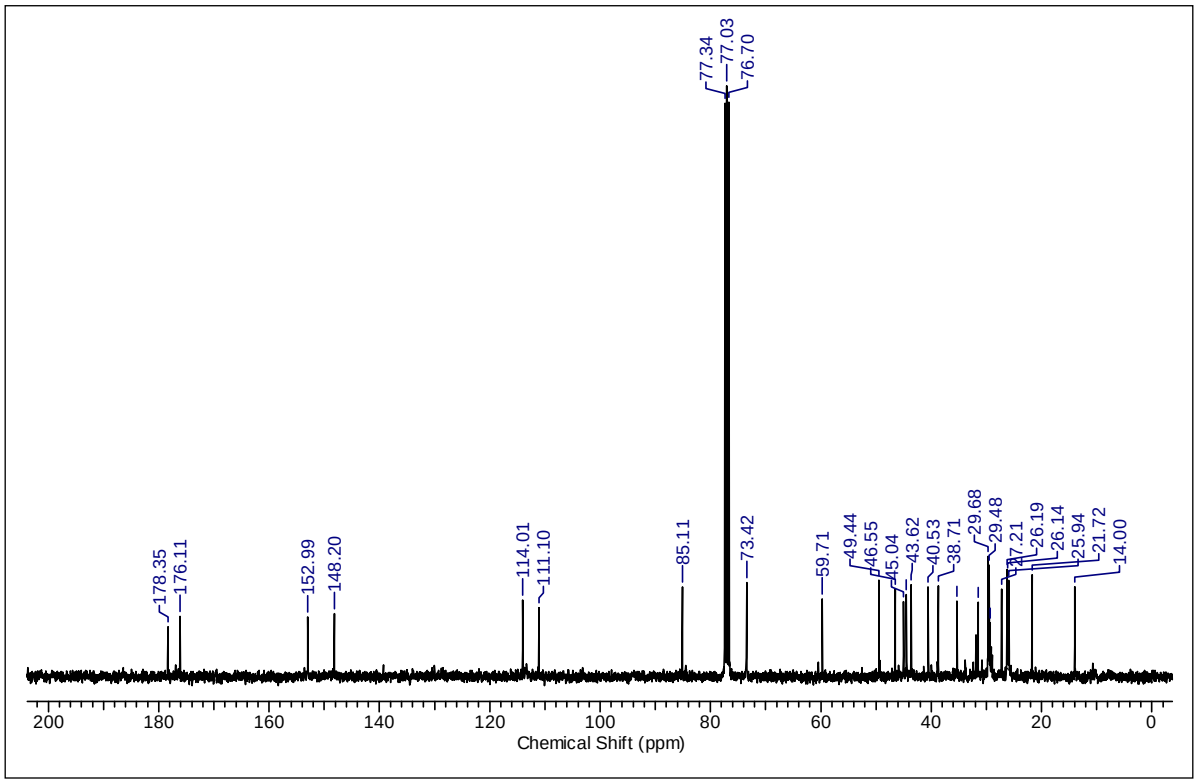

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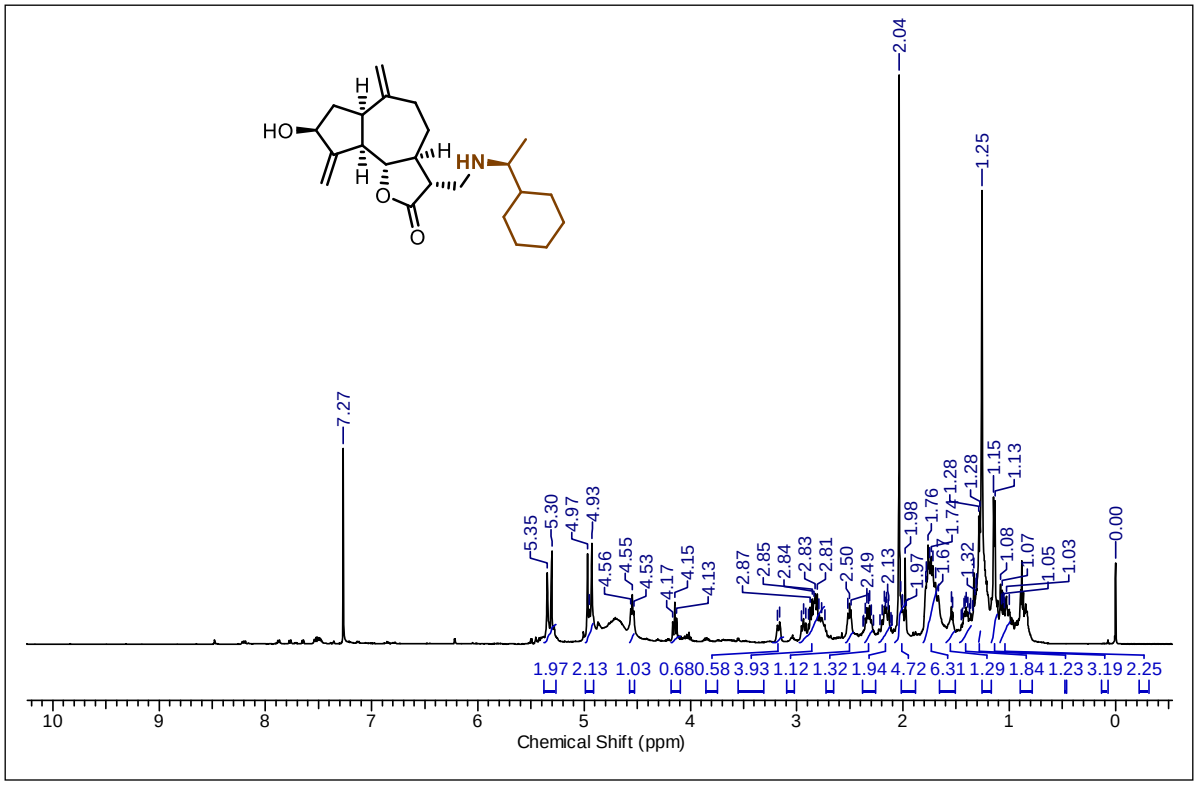

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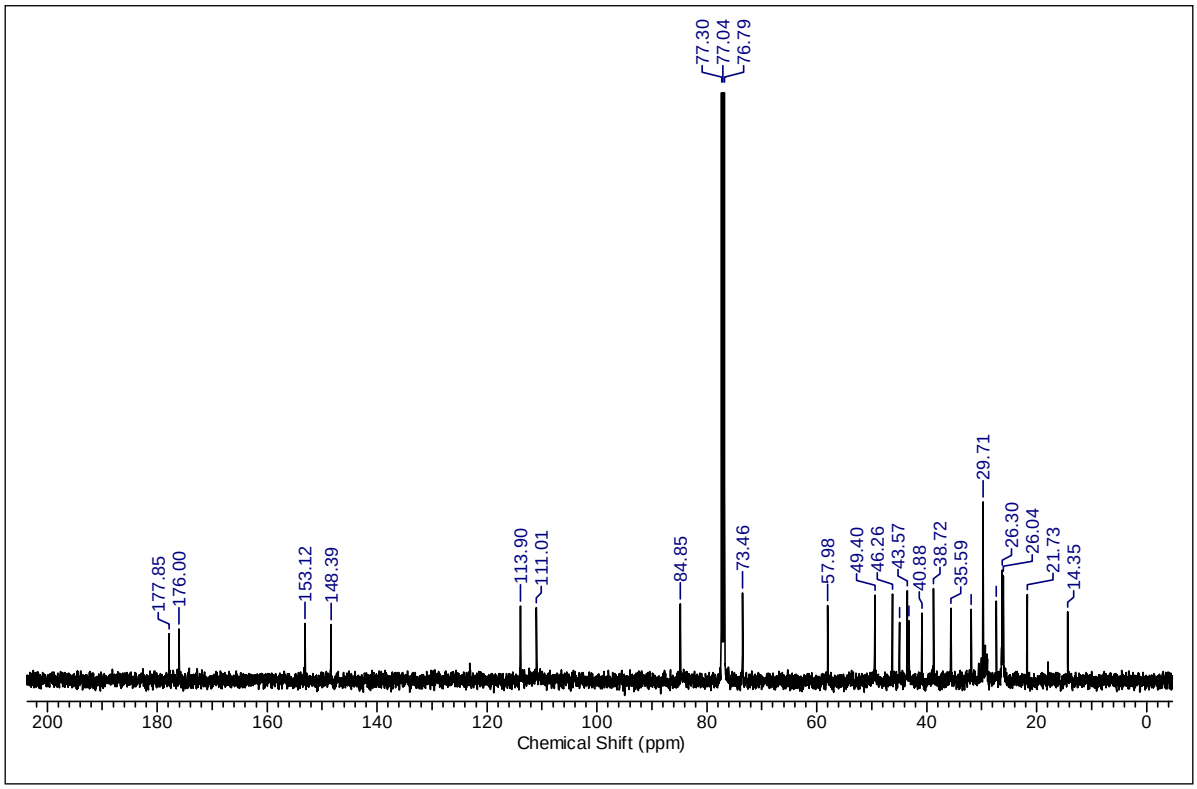
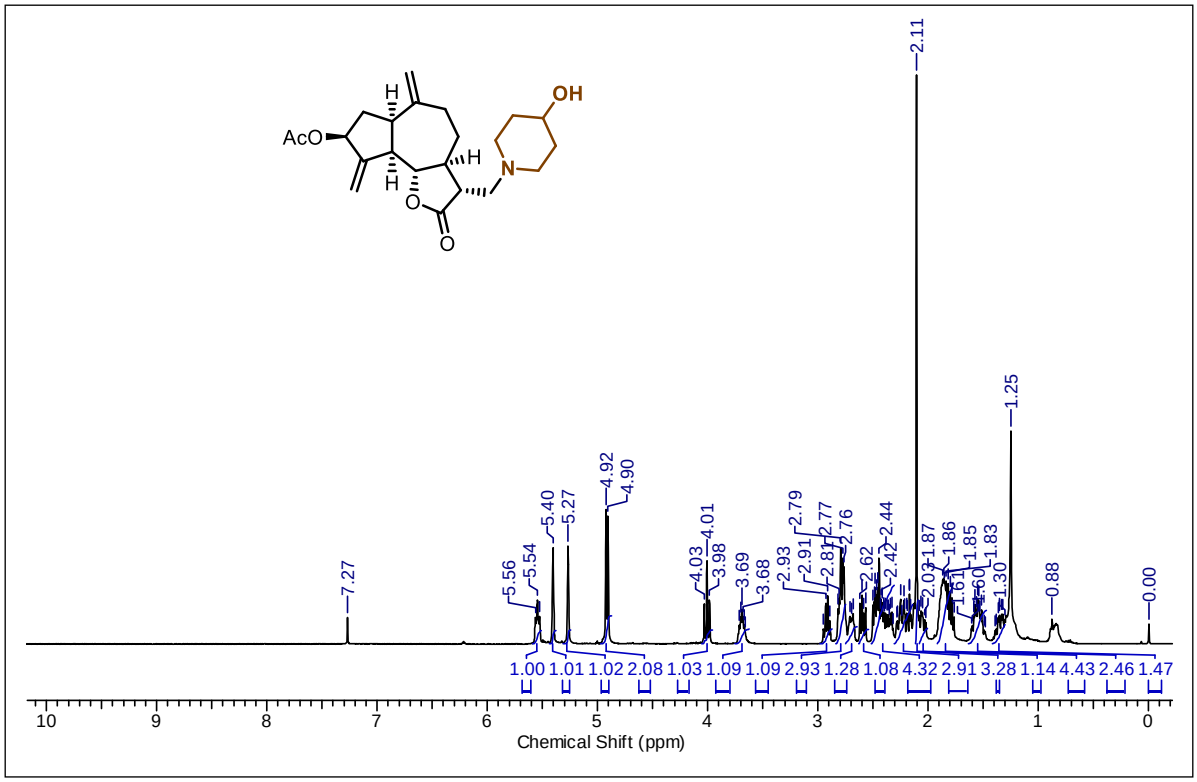
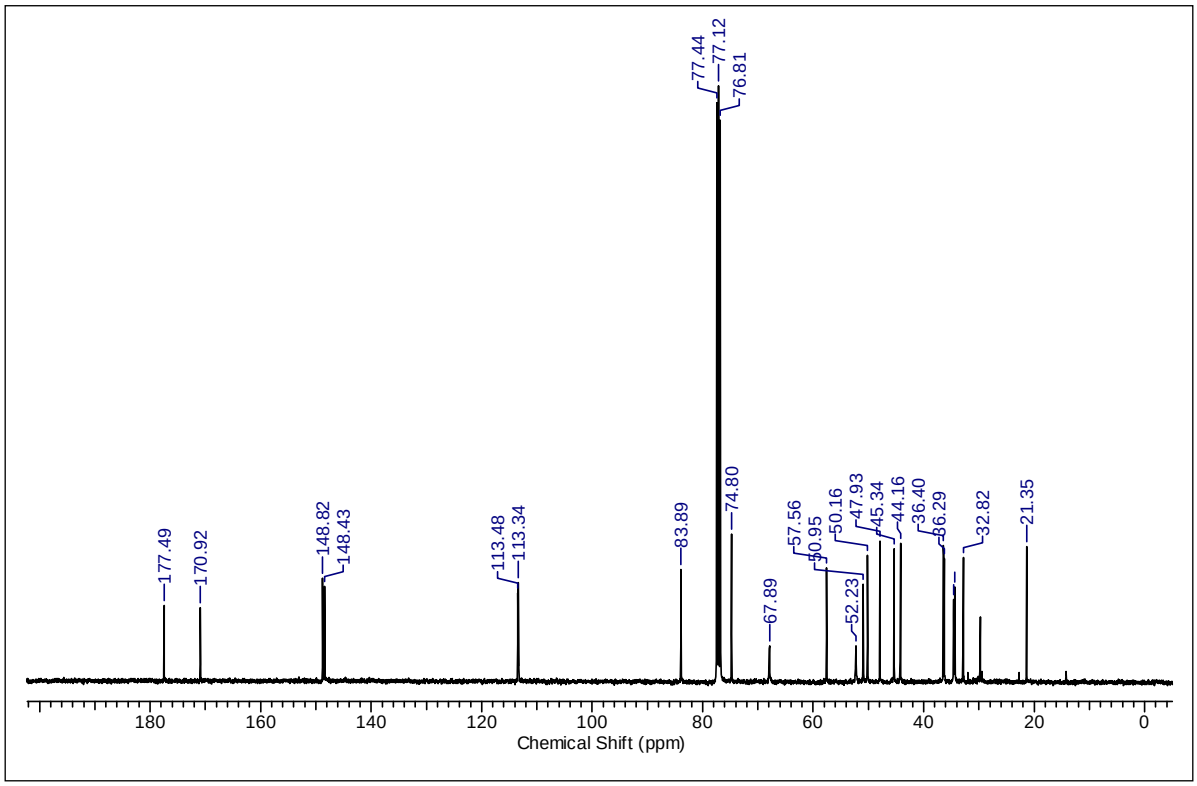


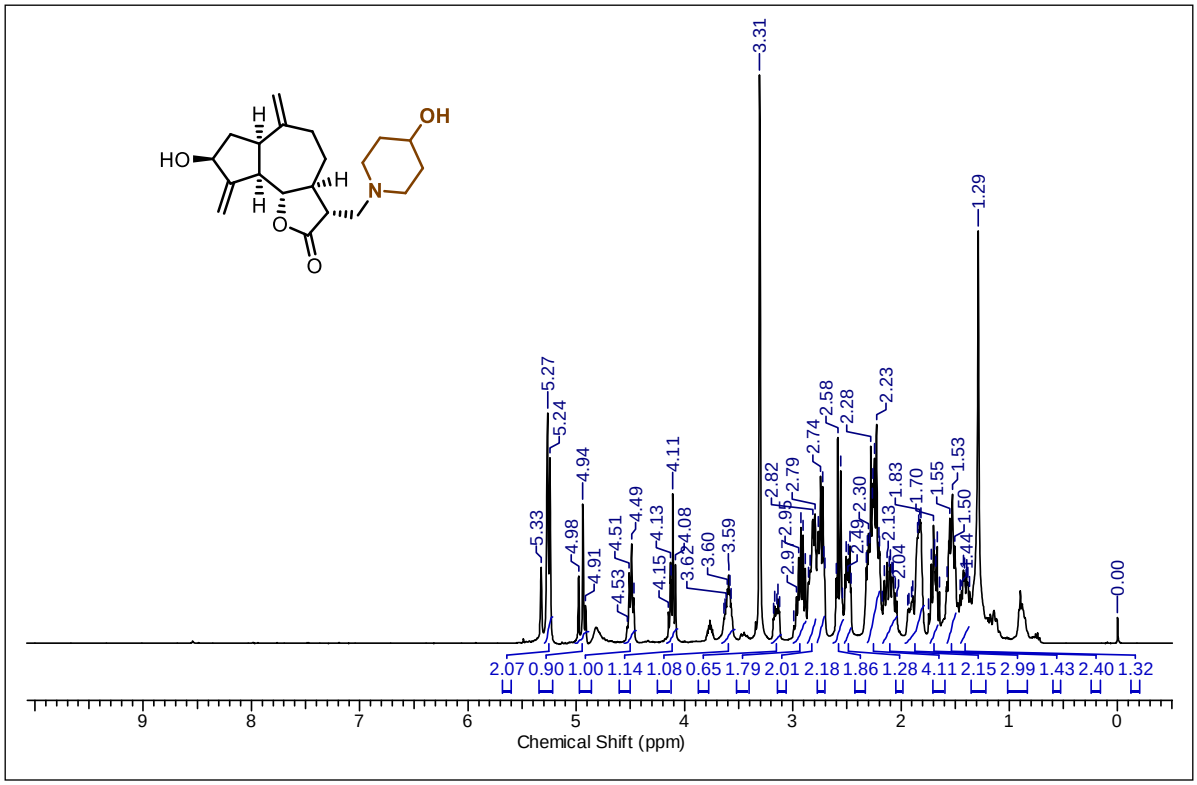
^{1}H NMR of Compound 13 (400 MHz, $CDCl_3$)



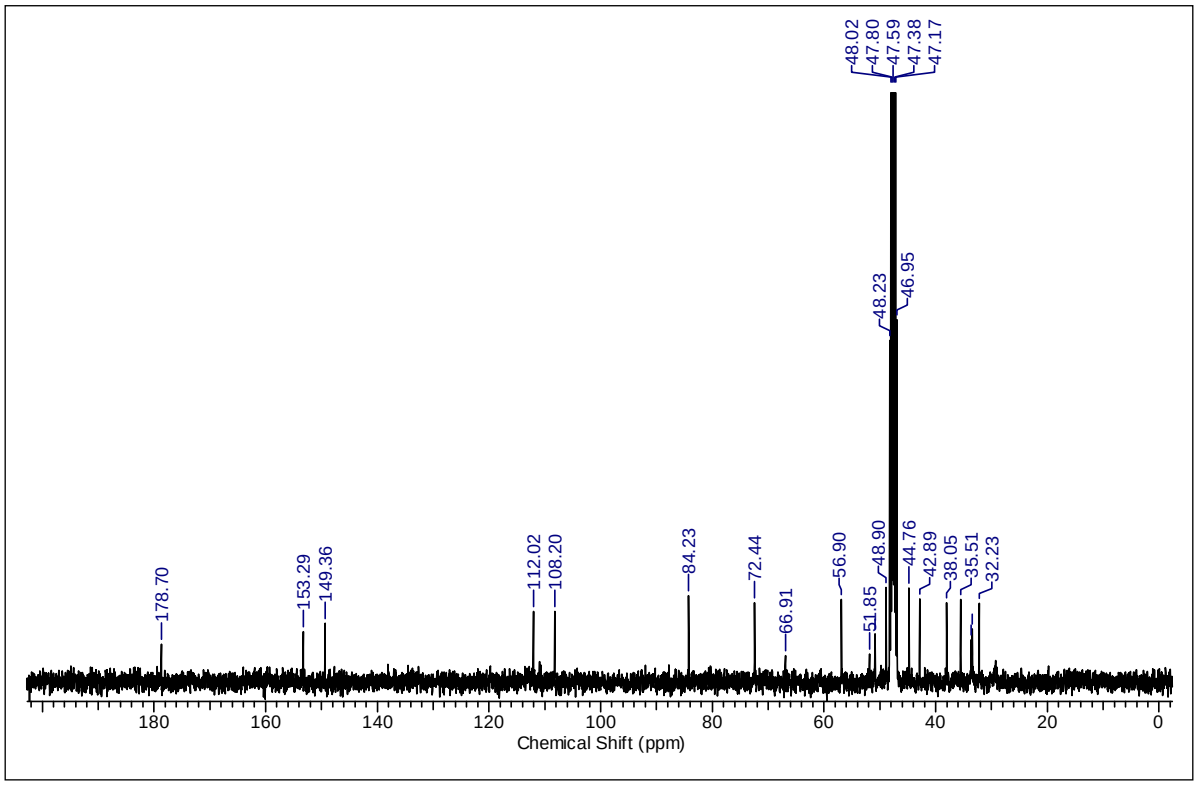
^{13}C NMR of Compound 13 (100 MHz, $CDCl_3$)



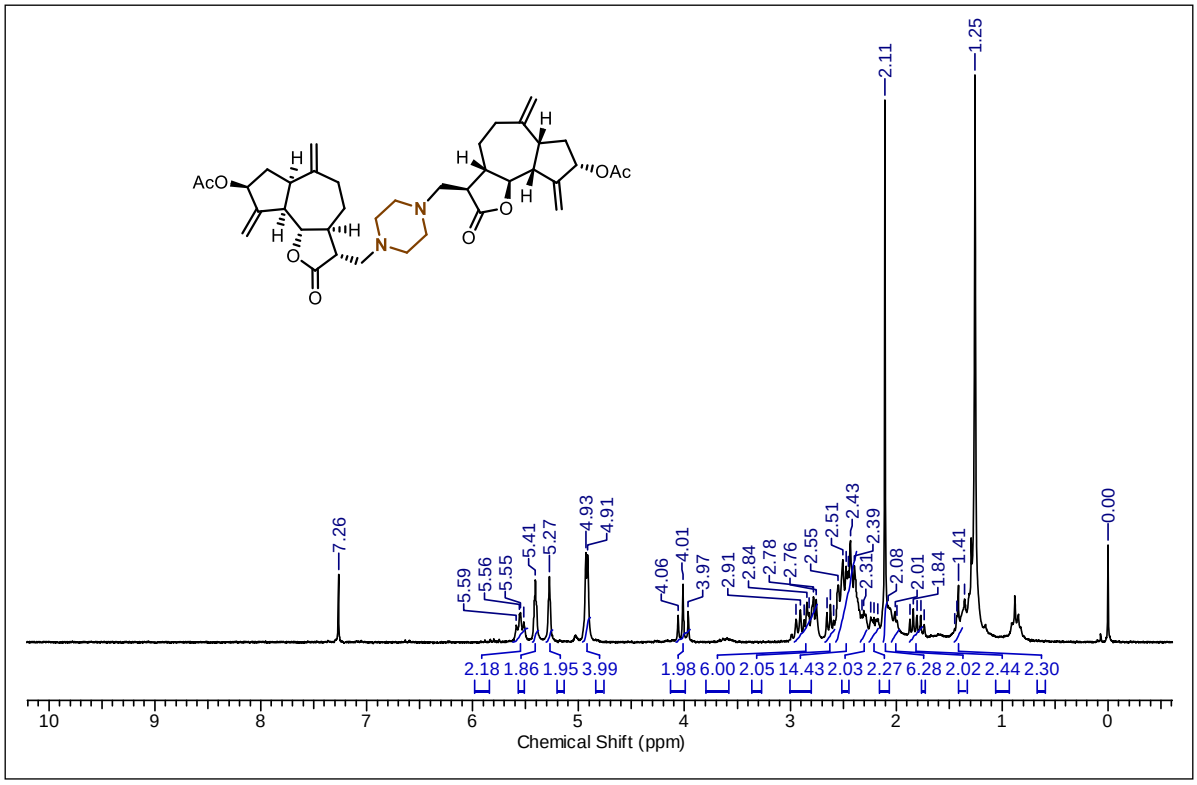
^{1}H NMR of Compound 14 (400 MHz, $CDCl_3$)



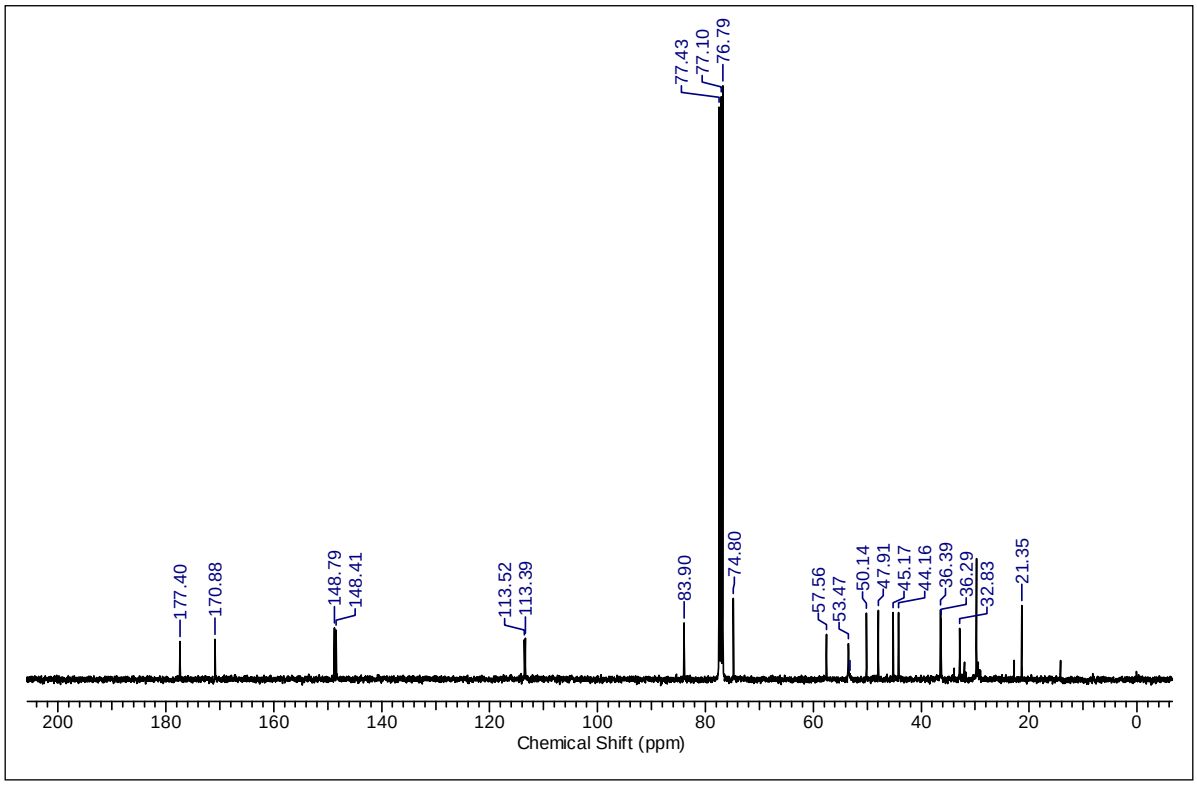
^{13}C NMR of Compound 14 (100 MHz, $CDCl_3$)



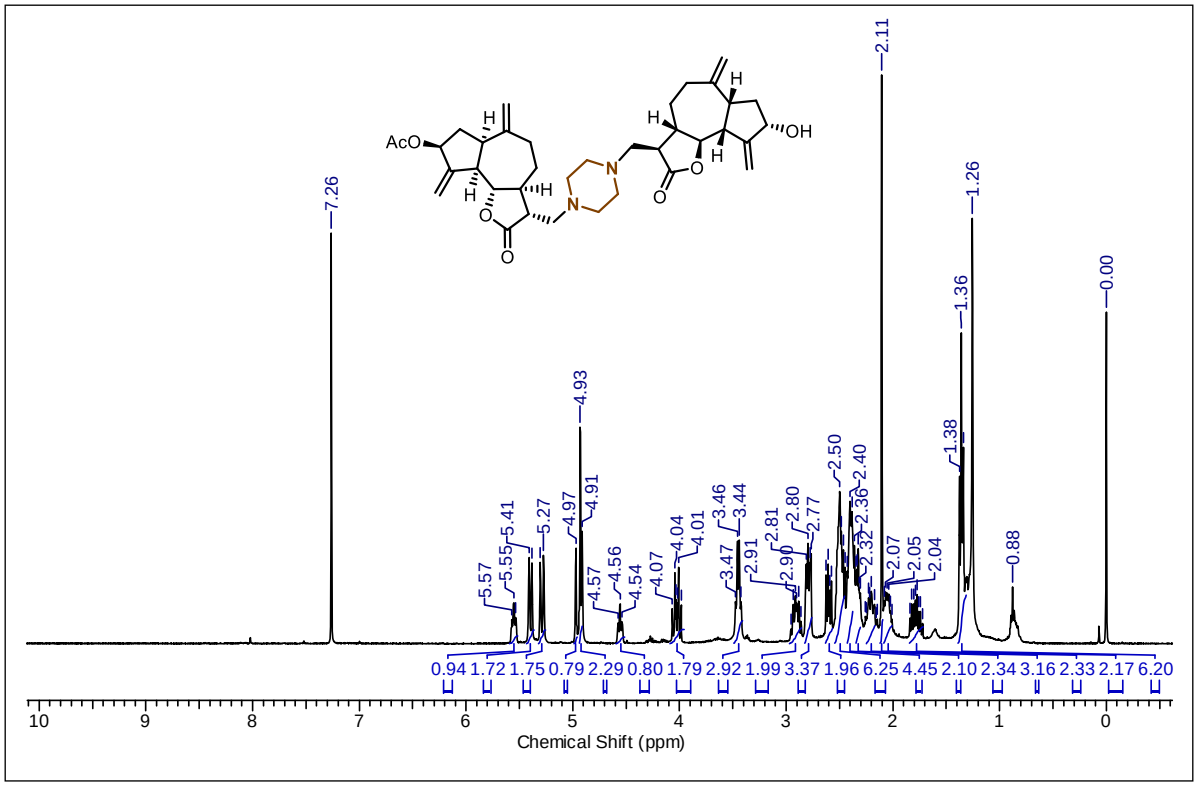
^{1}H NMR of Compound 15 (200 MHz, $CDCl_3$)



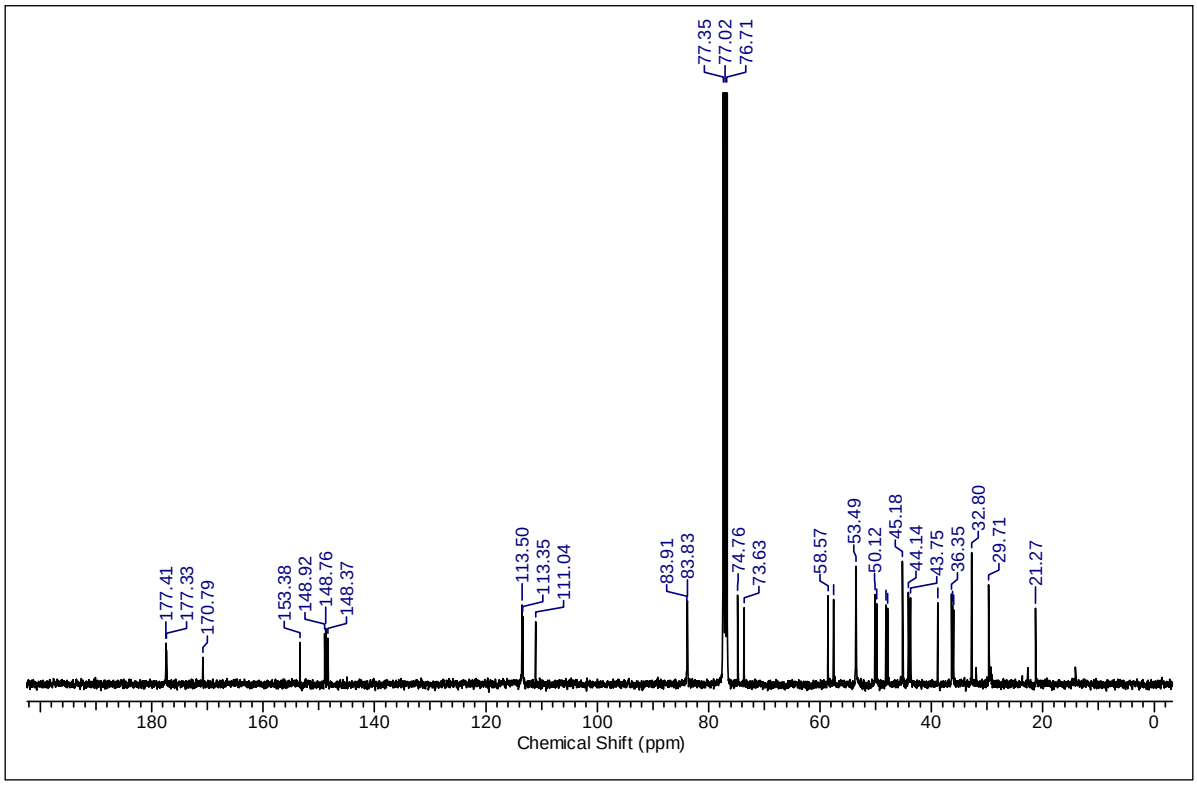
^{13}C NMR of Compound 15 (100 MHz, $CDCl_3$)



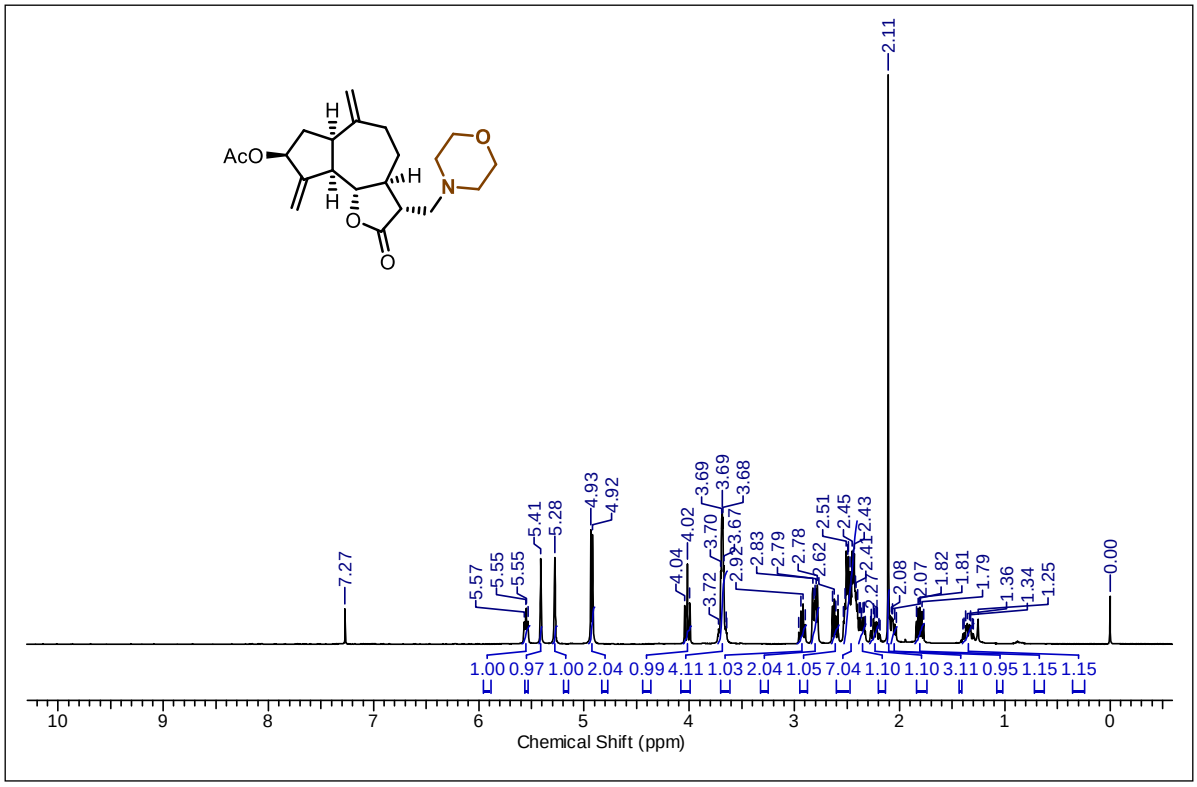
^{1}H NMR of Compound 16 (200 MHz, $CDCl_3$)



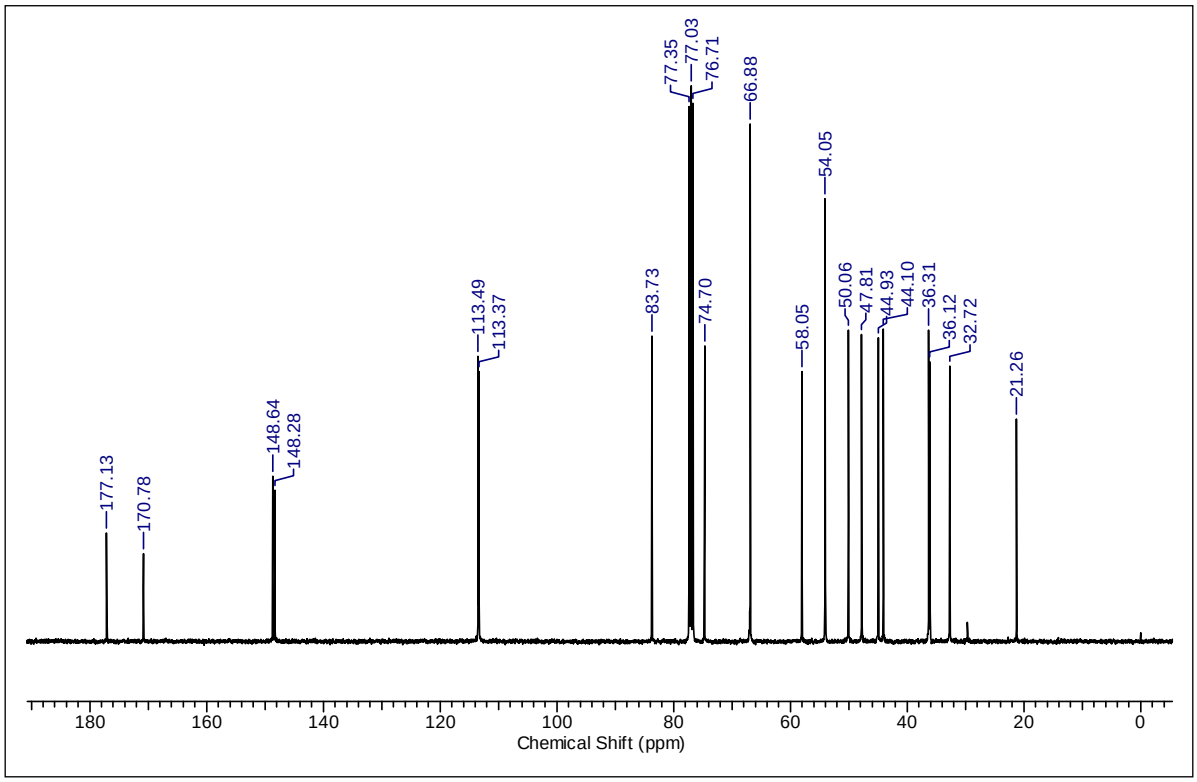
^{13}C NMR of Compound 16 (100 MHz, $CDCl_3$)



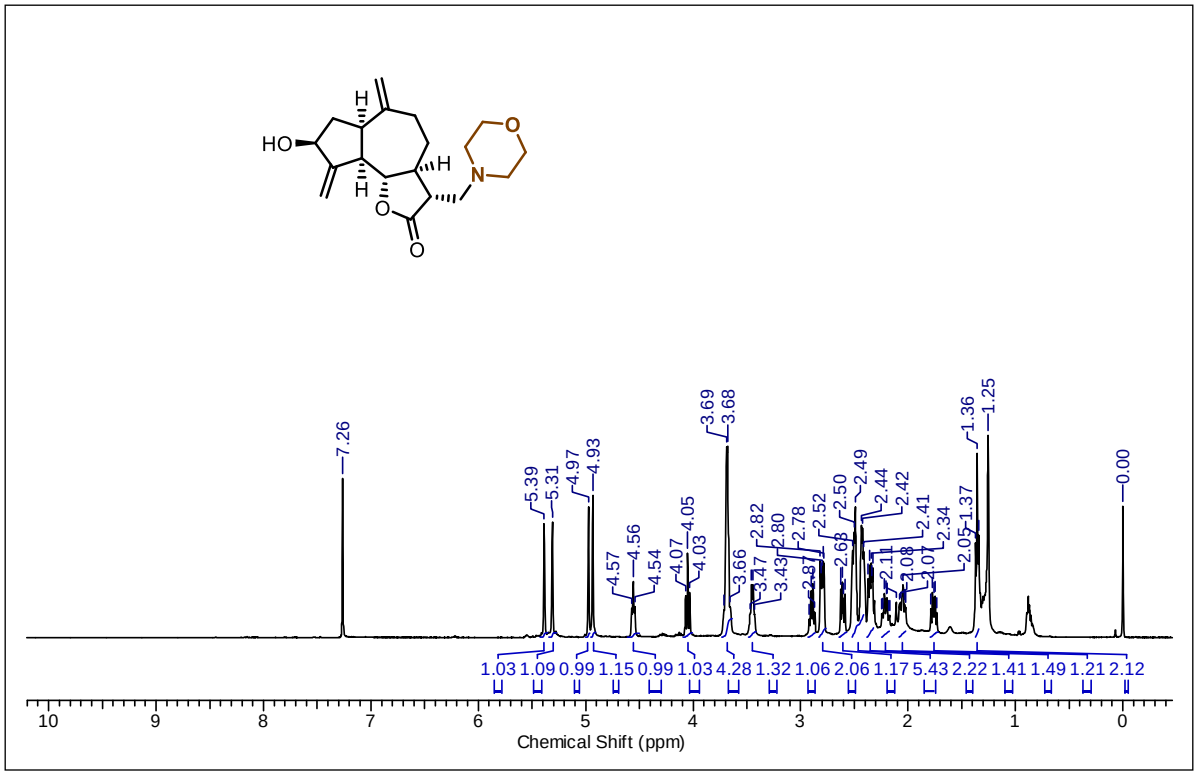
^{1}H NMR of Compound 17 (400 MHz, $CDCl_3$)



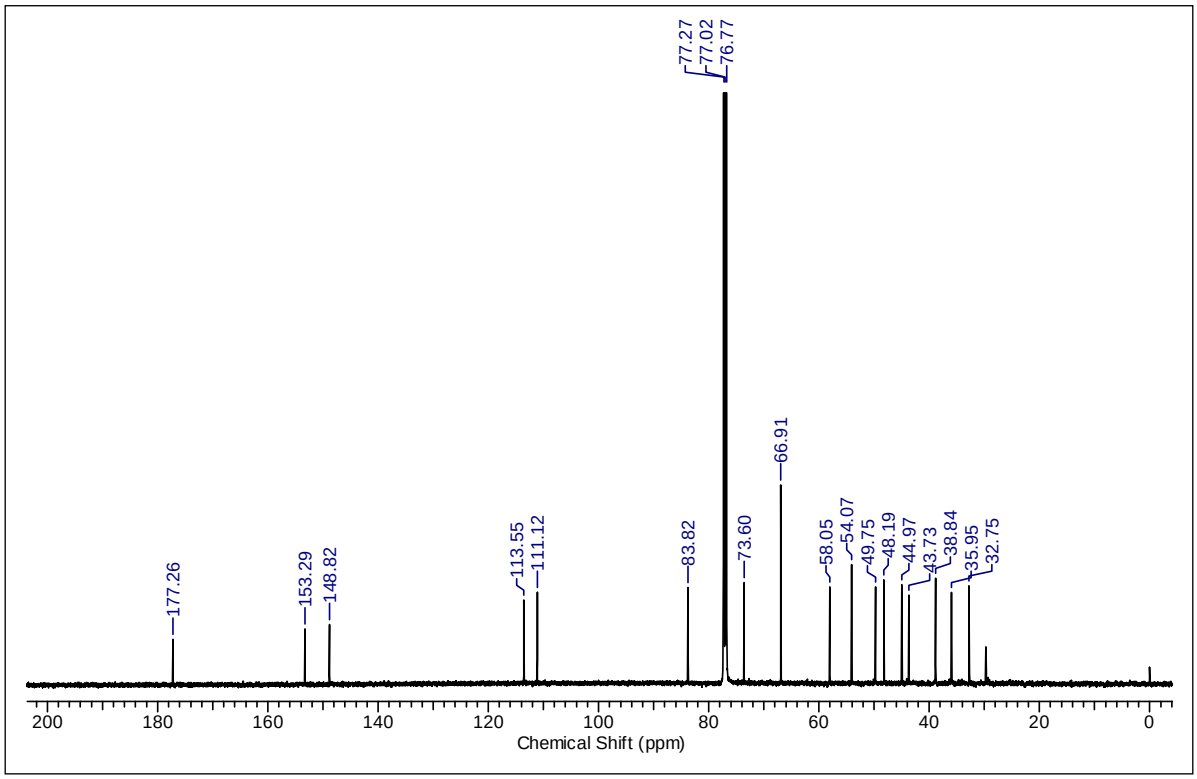
^{13}C NMR of Compound 17 (100 MHz, $CDCl_3$)



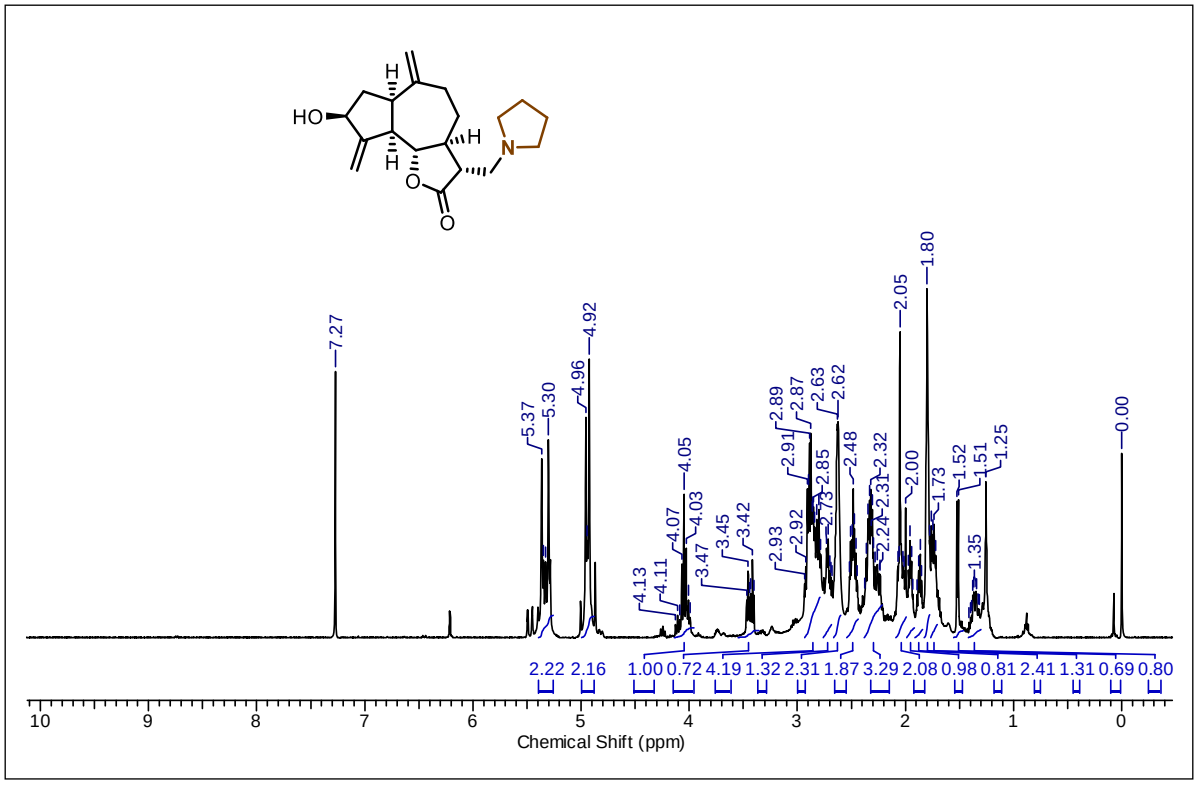
^{1}H NMR of Compound 18 (500 MHz, $CDCl_3$)



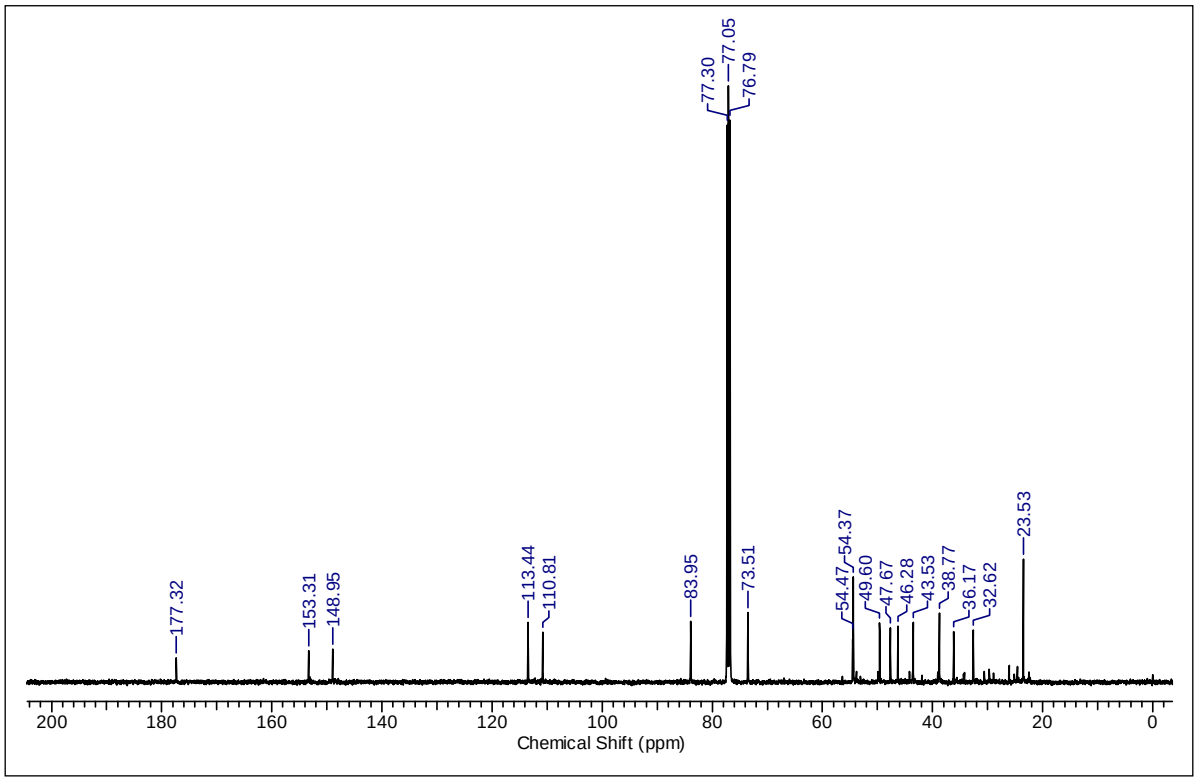
^{13}C NMR of Compound 18 (125 MHz, $CDCl_3$)



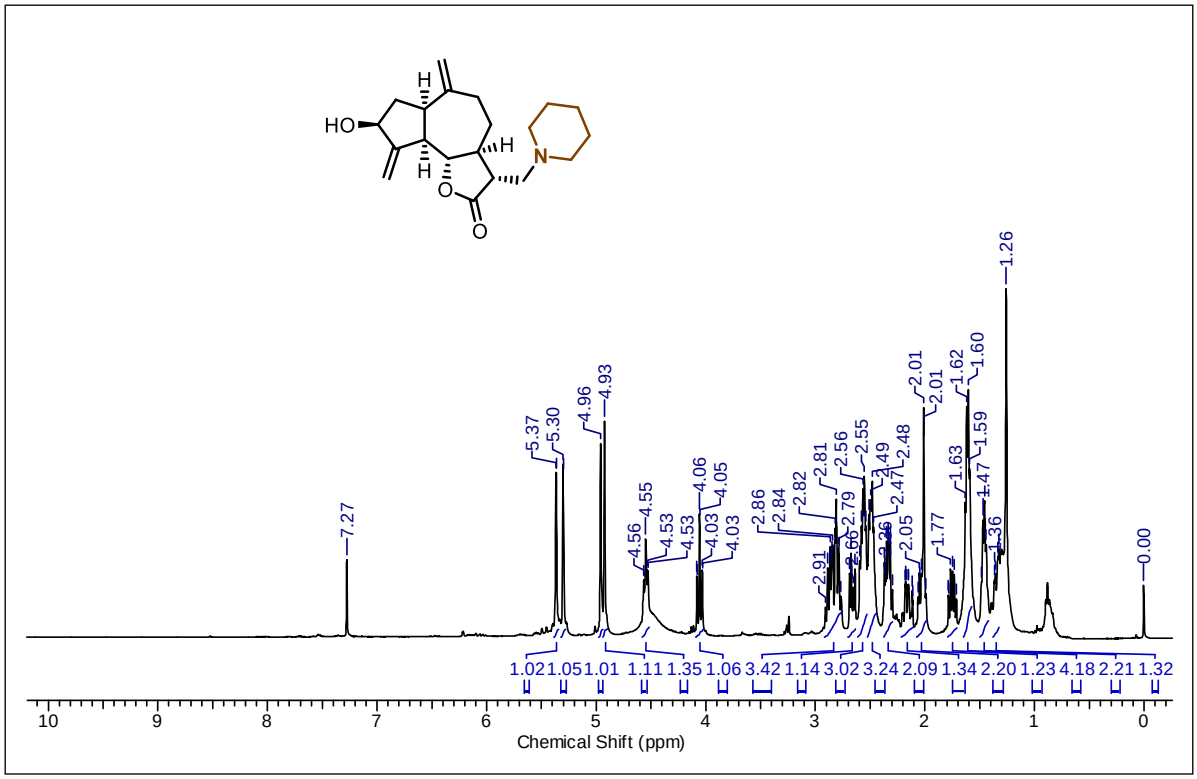
^{1}H NMR of Compound 19 (500 MHz, $CDCl_3$)



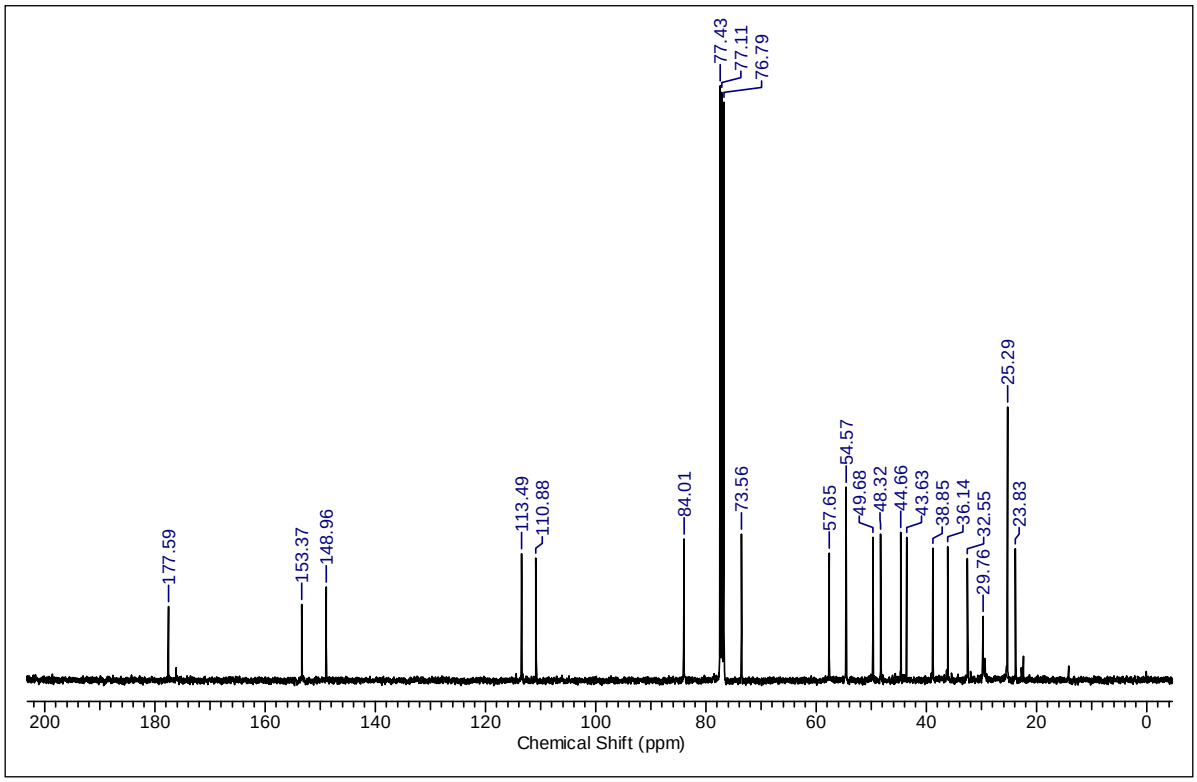
^{13}C NMR of Compound 19 (125 MHz, $CDCl_3$)



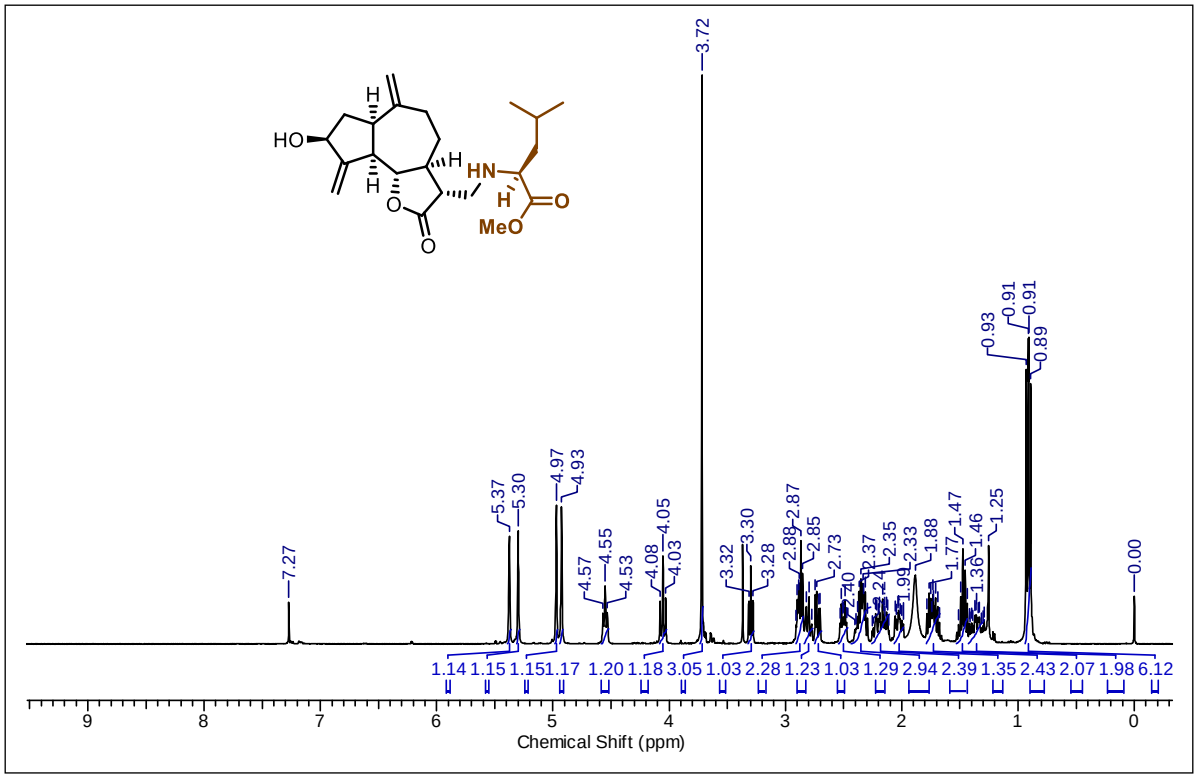
^{1}H NMR of Compound 20 (400 MHz, $CDCl_3$)



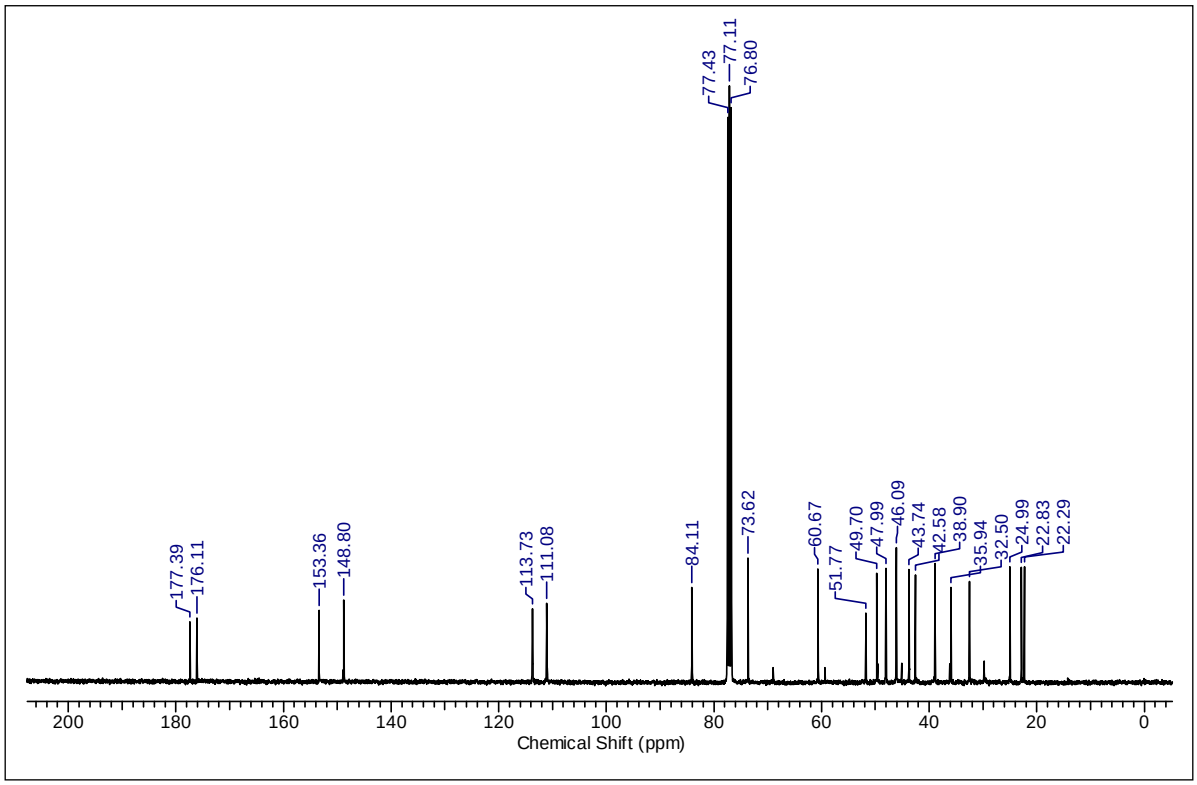
^{13}C NMR of Compound 20 (100 MHz, $CDCl_3$)



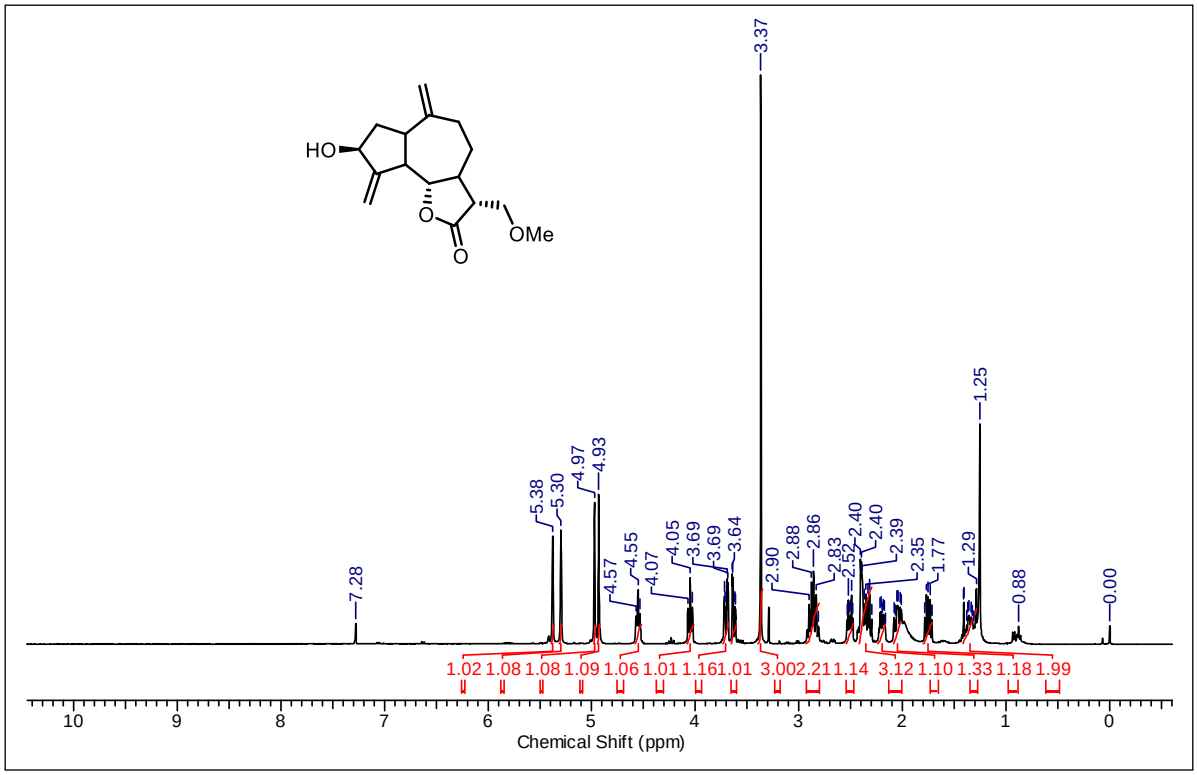
^{1}H NMR of Compound 21 (400 MHz, $CDCl_3$)



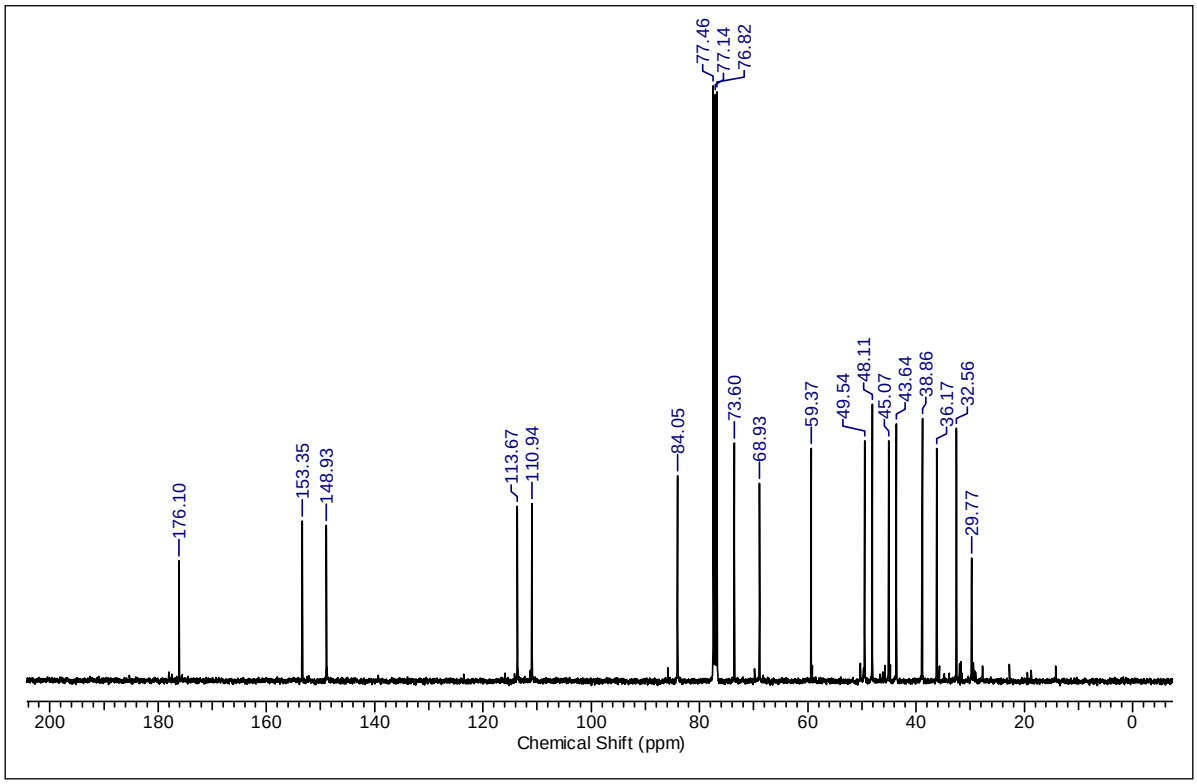
^{13}C NMR of Compound 21 (100 MHz, $CDCl_3$)



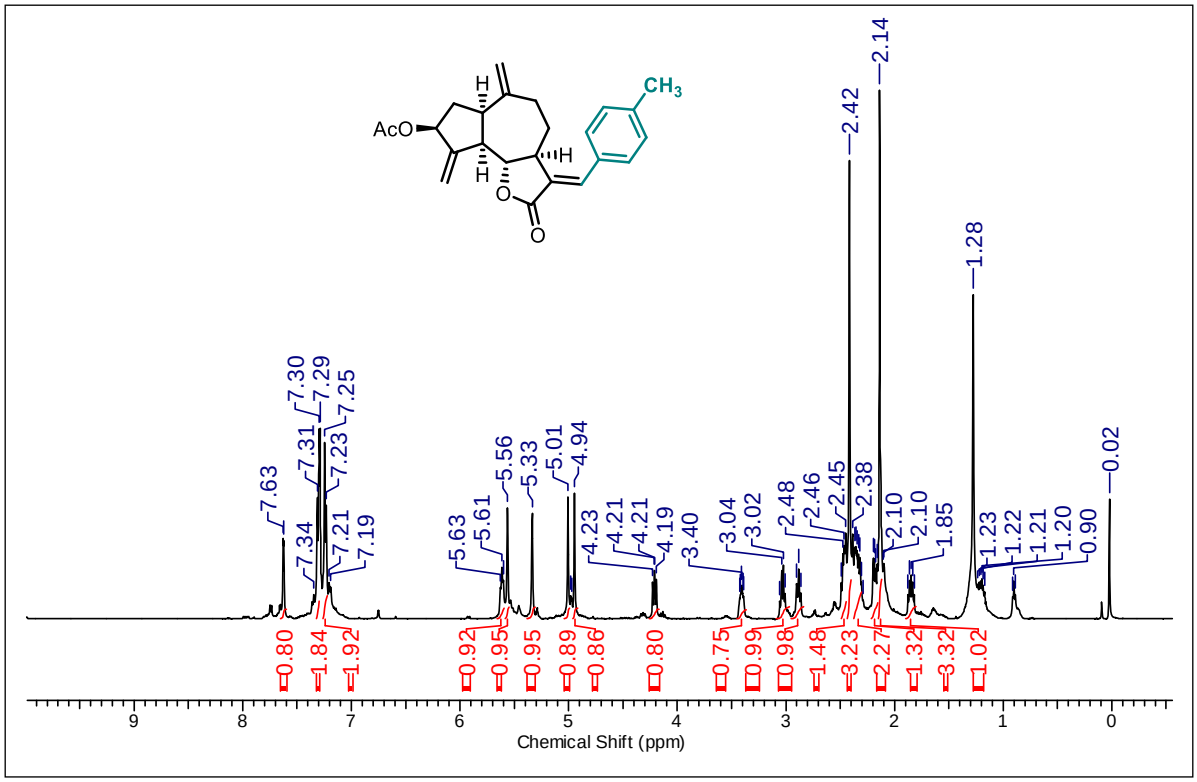
^{1}H NMR of Compound 22 (400 MHz, $CDCl_3$)



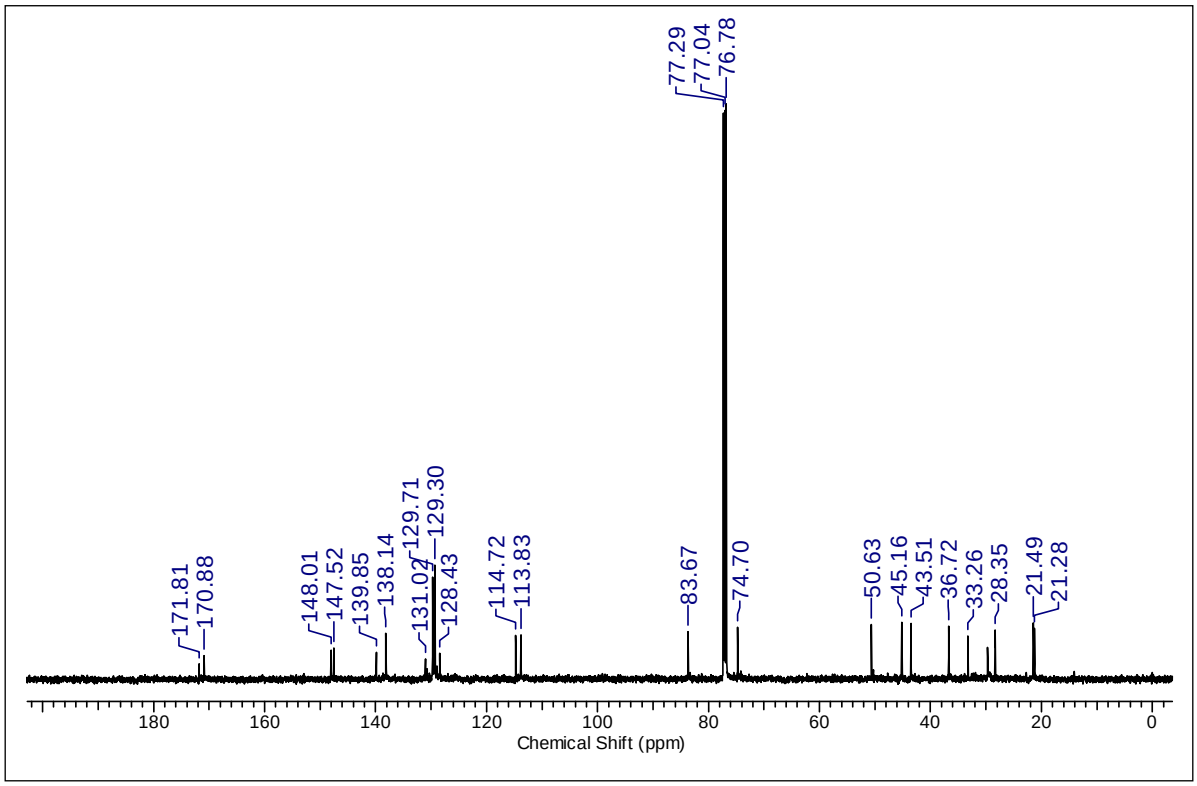
^{13}C NMR of Compound 22 (100 MHz, $CDCl_3$)



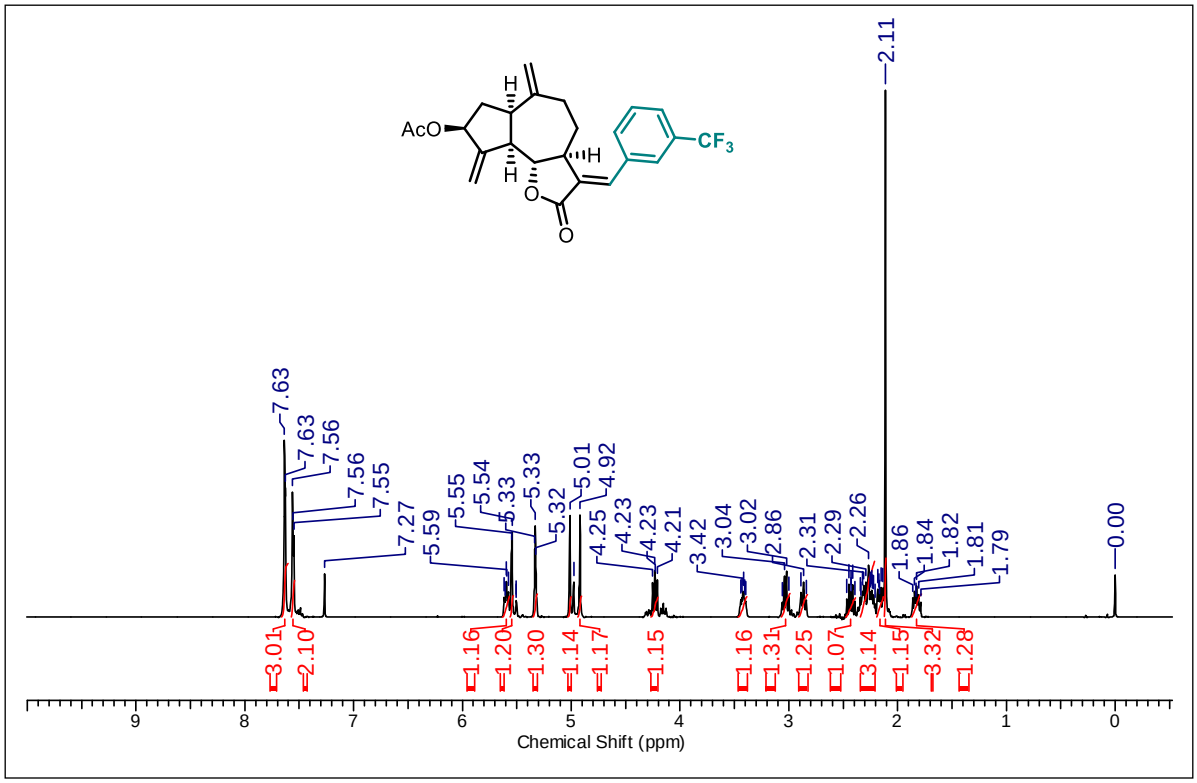
^{1}H NMR of Compound 23 (500 MHz, $CDCl_3$)



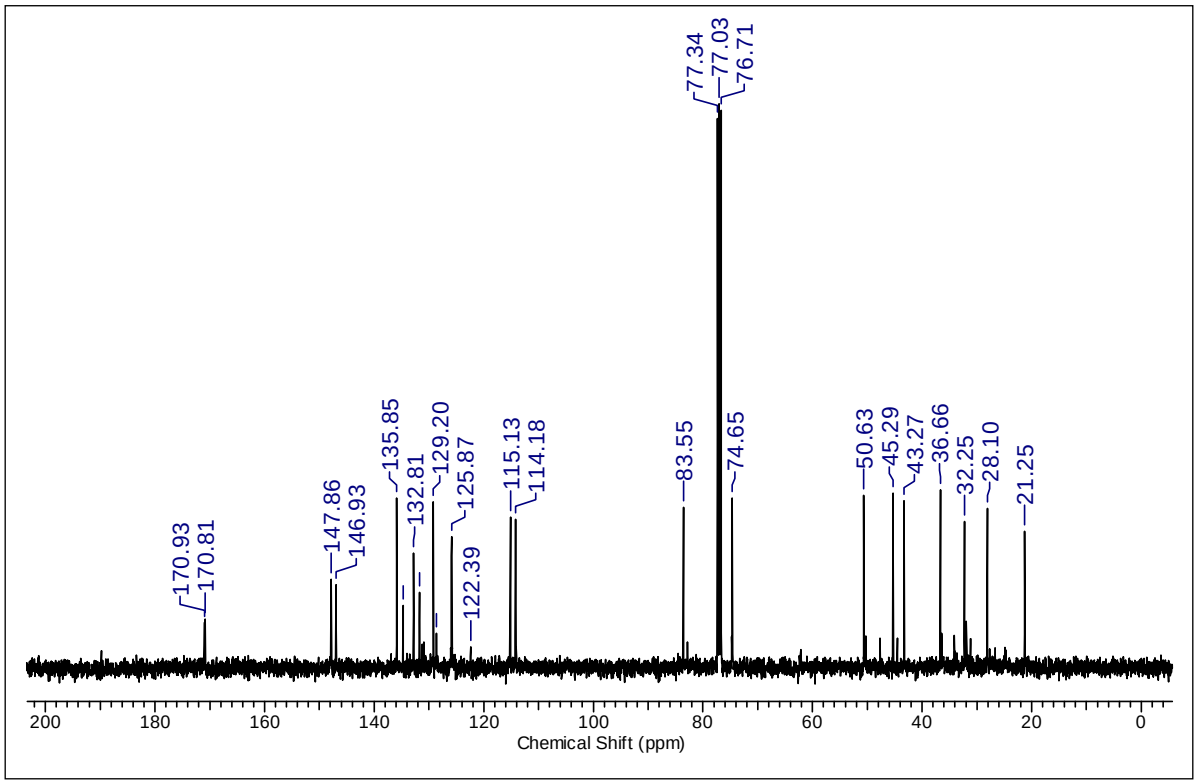
^{13}C NMR of Compound 23 (125 MHz, $CDCl_3$)



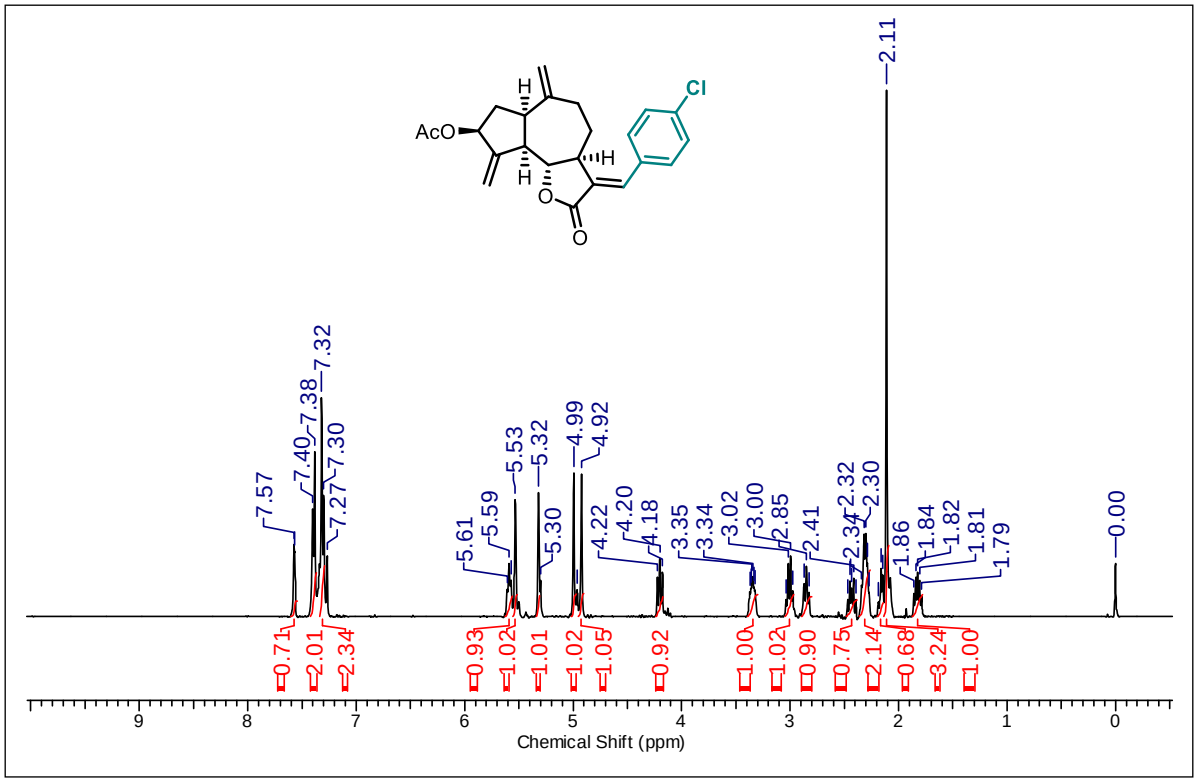
^{1}H NMR of Compound 24 (400 MHz, $CDCl_3$)



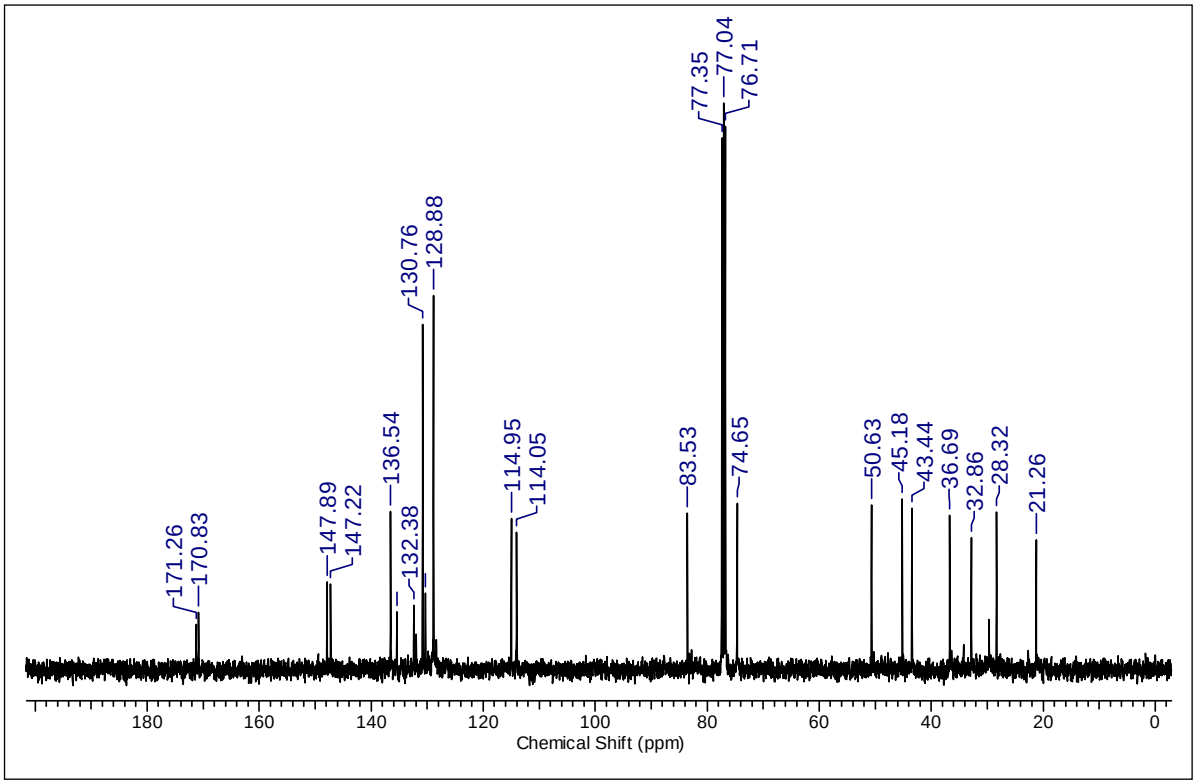
^{13}C NMR of Compound 24 (100 MHz, $CDCl_3$)



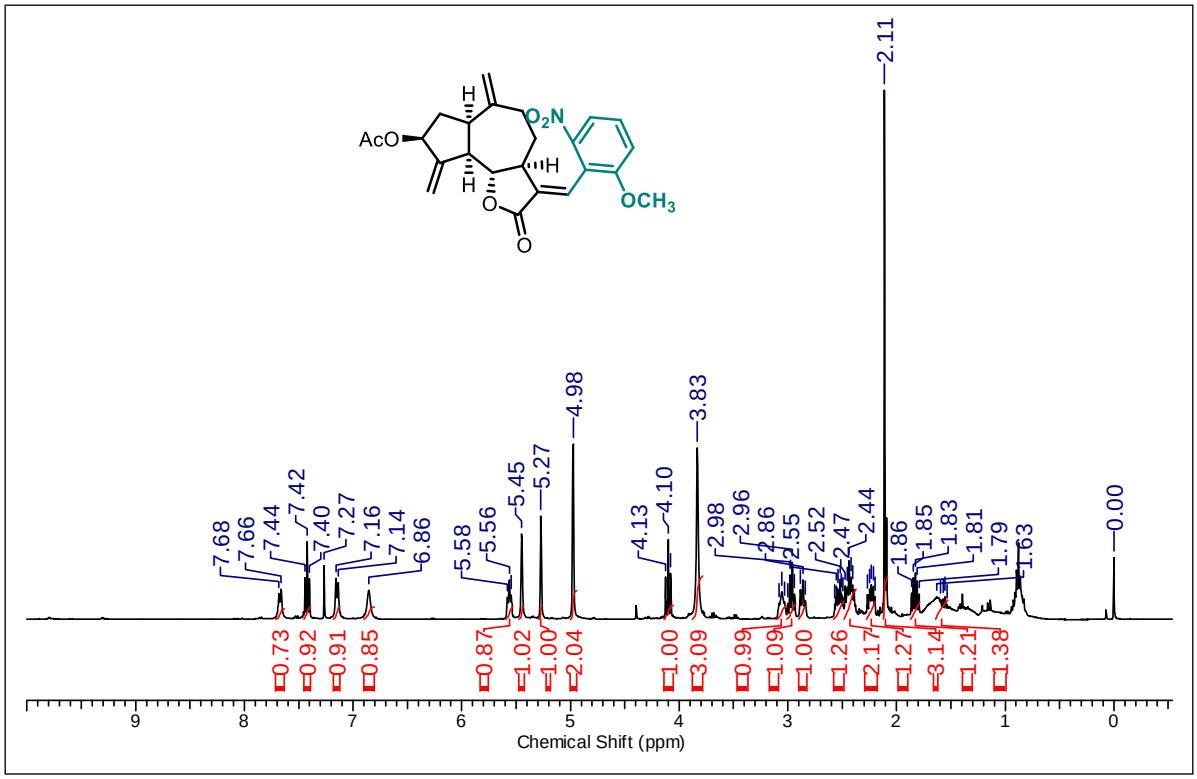
^{1}H NMR of Compound 25 (400 MHz, $CDCl_3$)



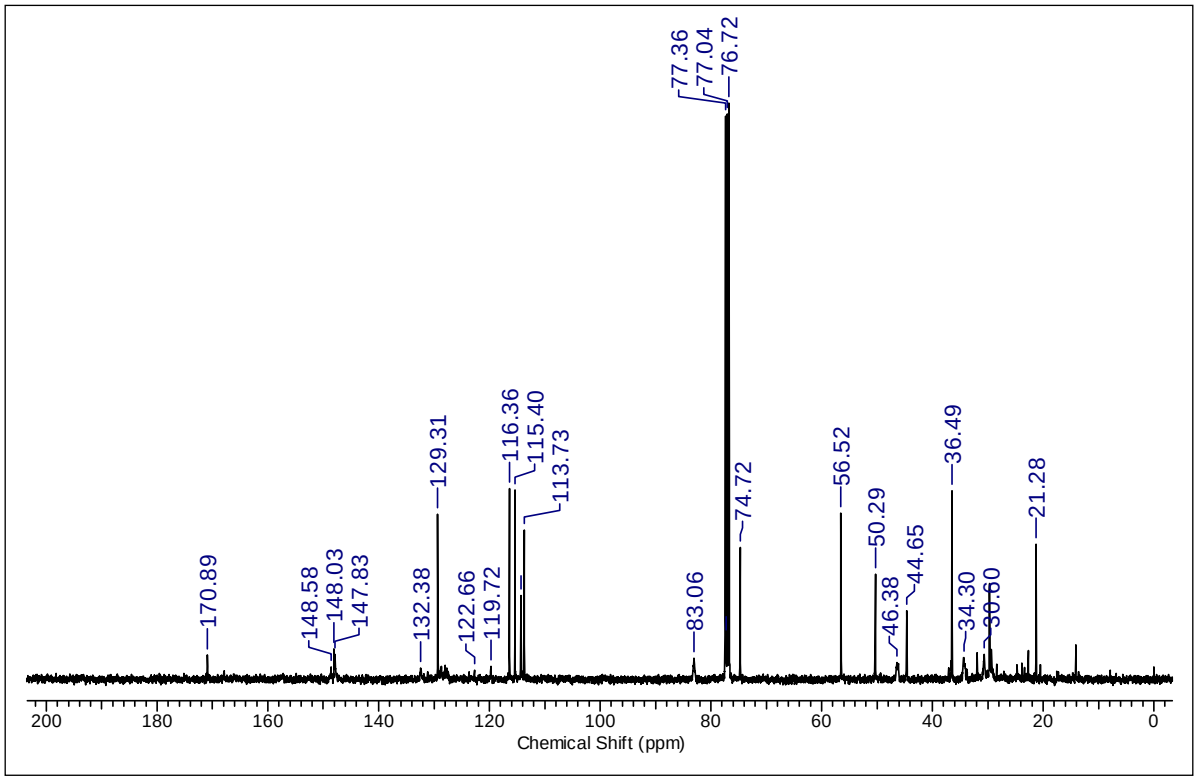
^{13}C NMR of Compound 25 (100 MHz, $CDCl_3$)



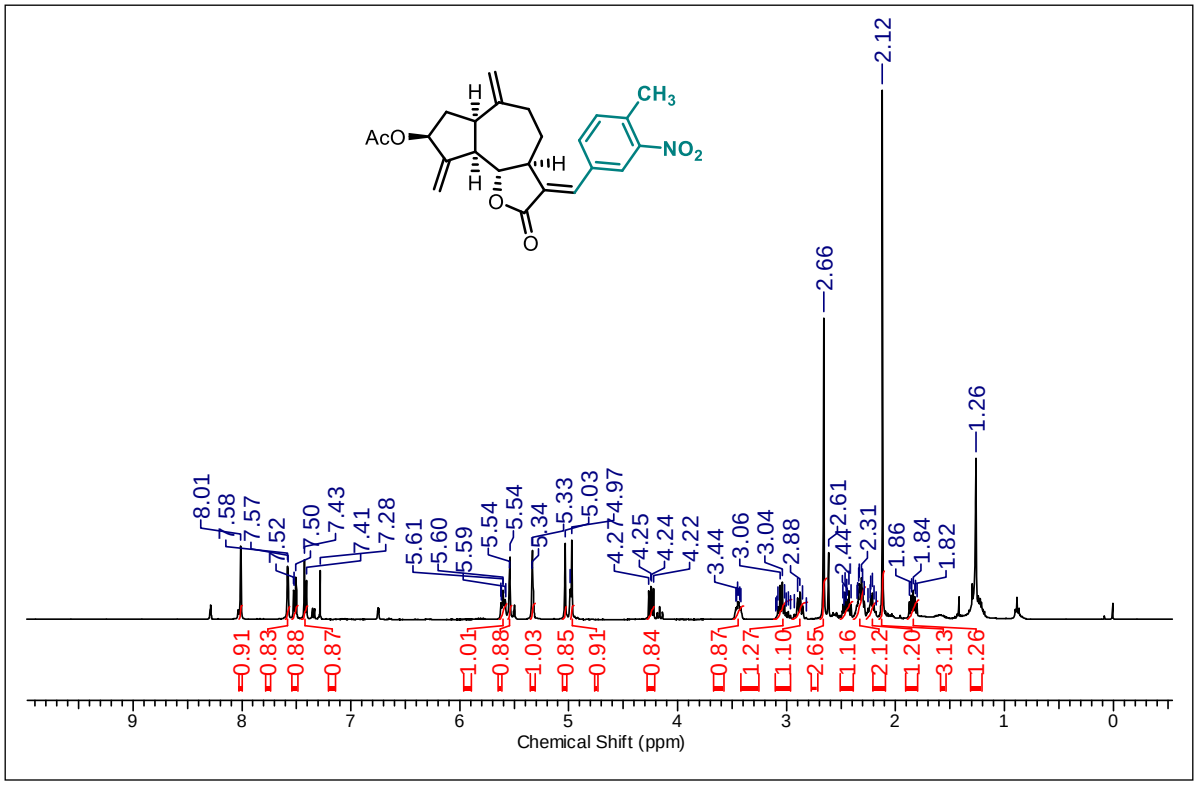
^{1}H NMR of Compound 26 (400 MHz, $CDCl_3$)



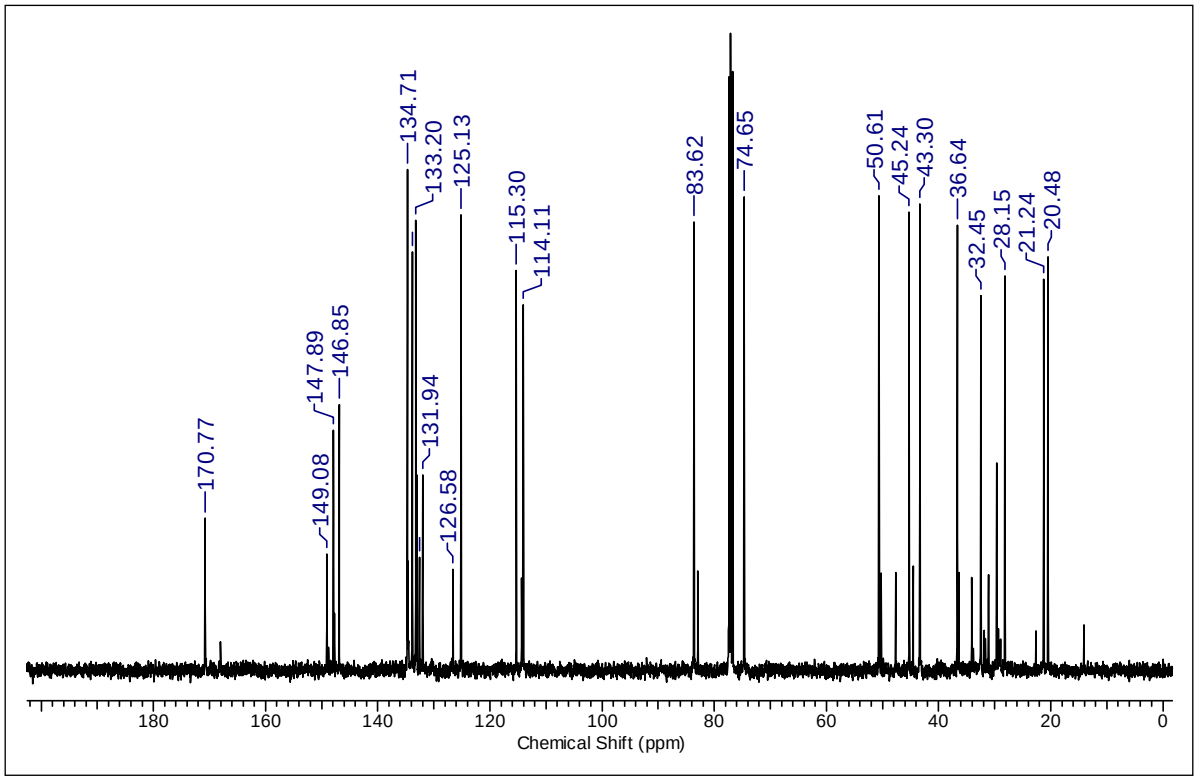
^{13}C NMR of Compound 26 (100 MHz, $CDCl_3$)



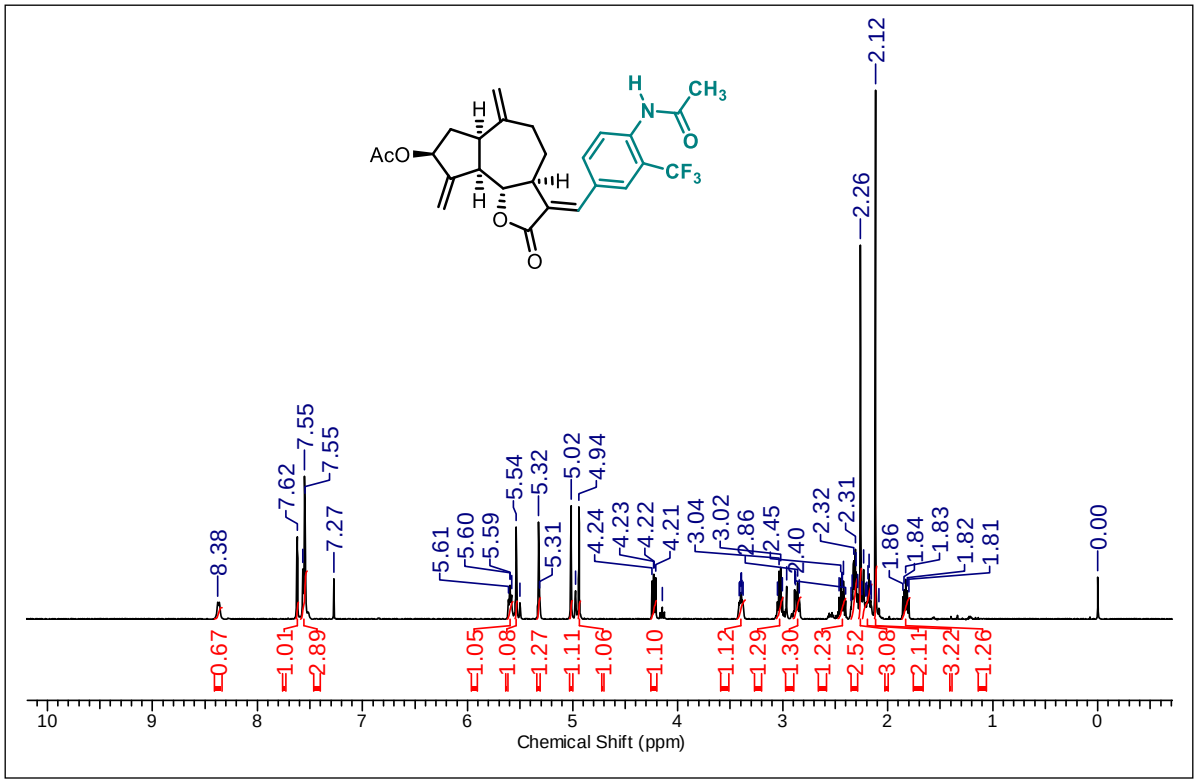
^{1}H NMR of Compound 27 (400 MHz, $CDCl_3$)



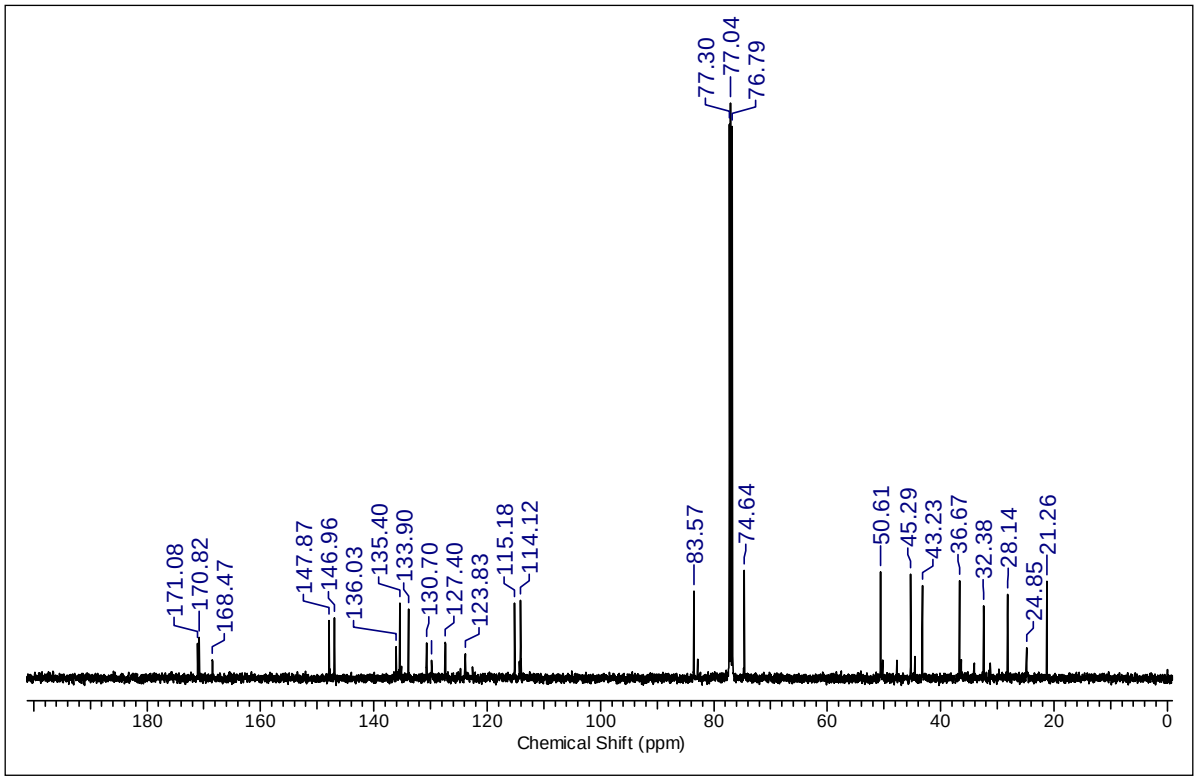
^{13}C NMR of Compound 27 (100 MHz, $CDCl_3$)



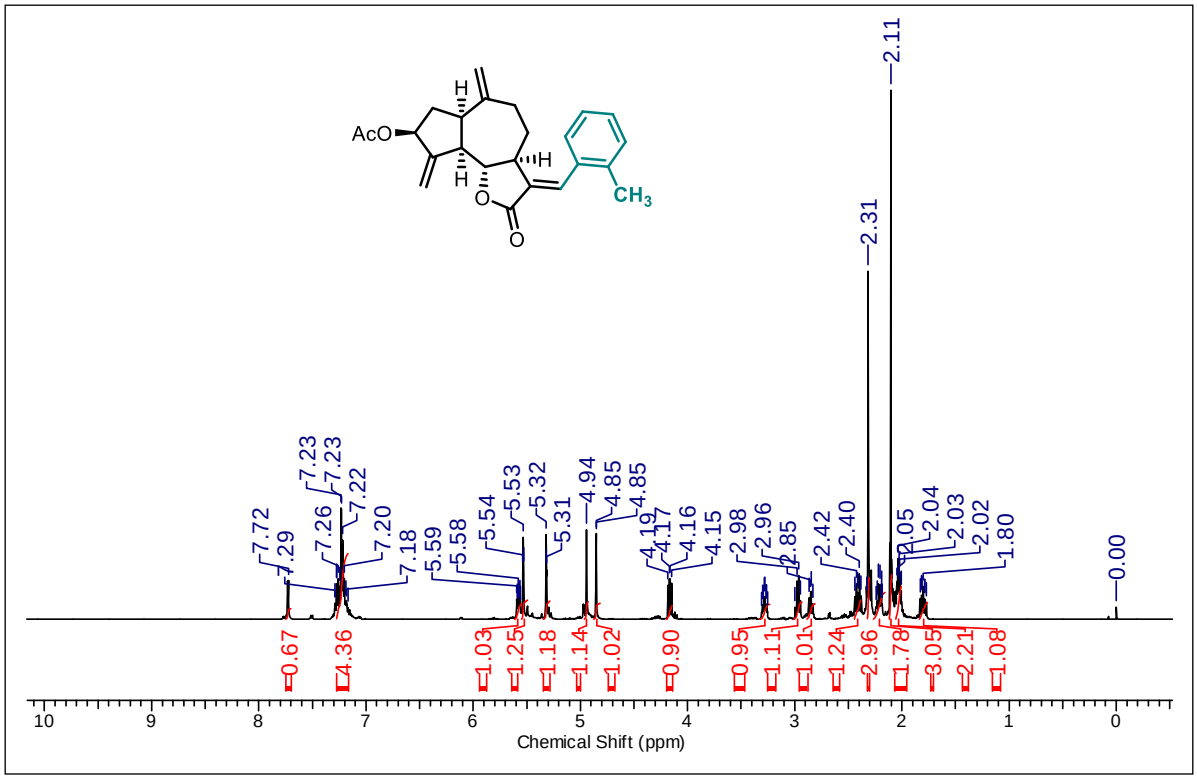
^{1}H NMR of Compound 28 (500 MHz, $CDCl_3$)



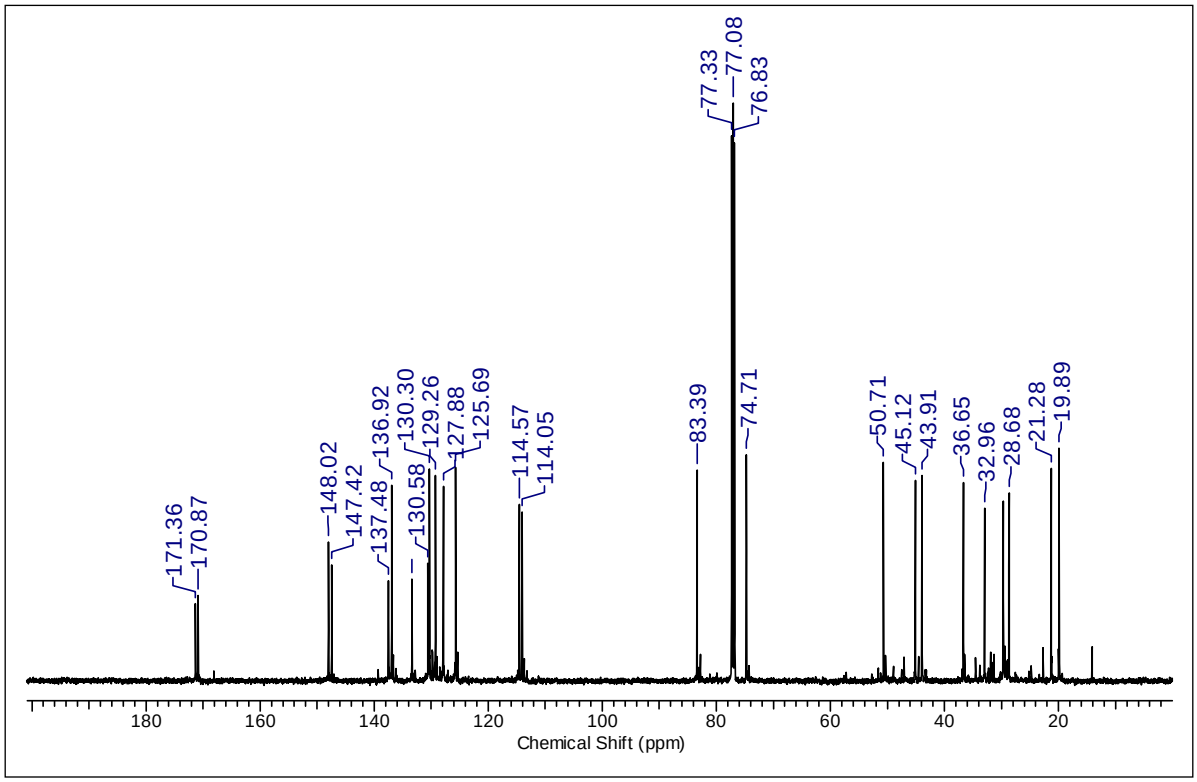
^{13}C NMR of Compound 28 (125 MHz, $CDCl_3$)



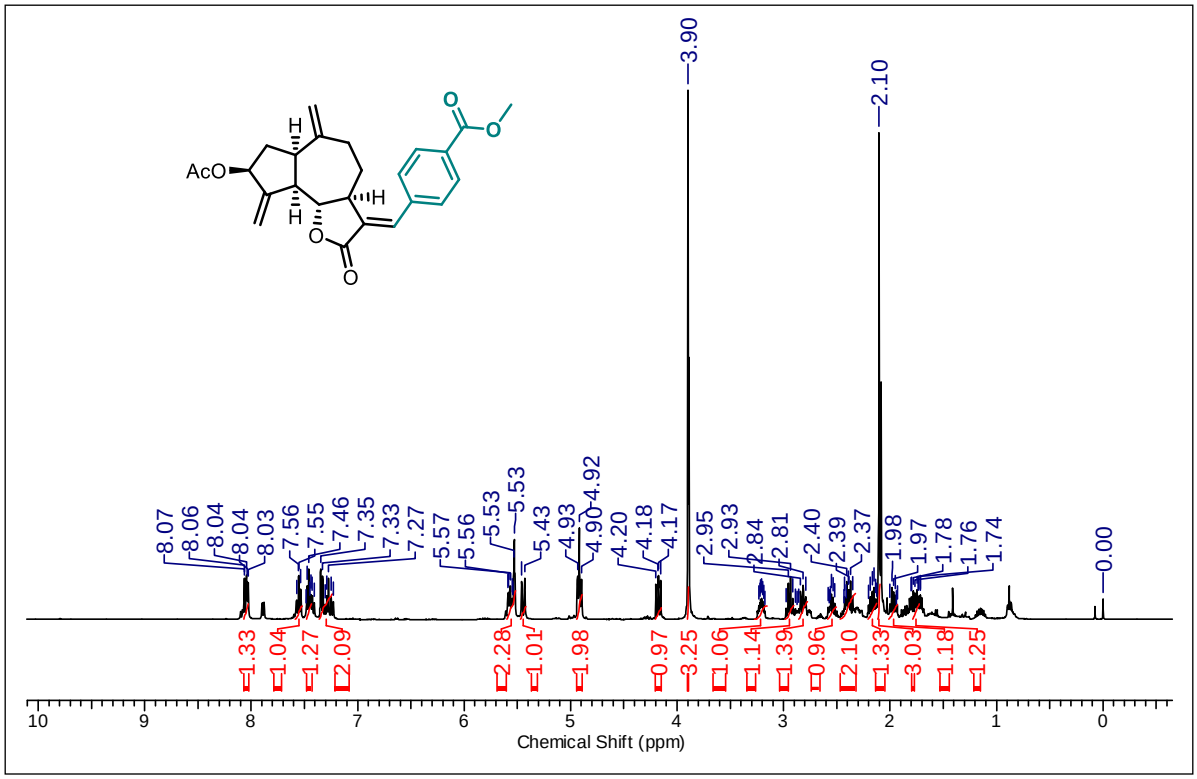
^{1}H NMR of Compound 29 (500 MHz, $CDCl_3$)



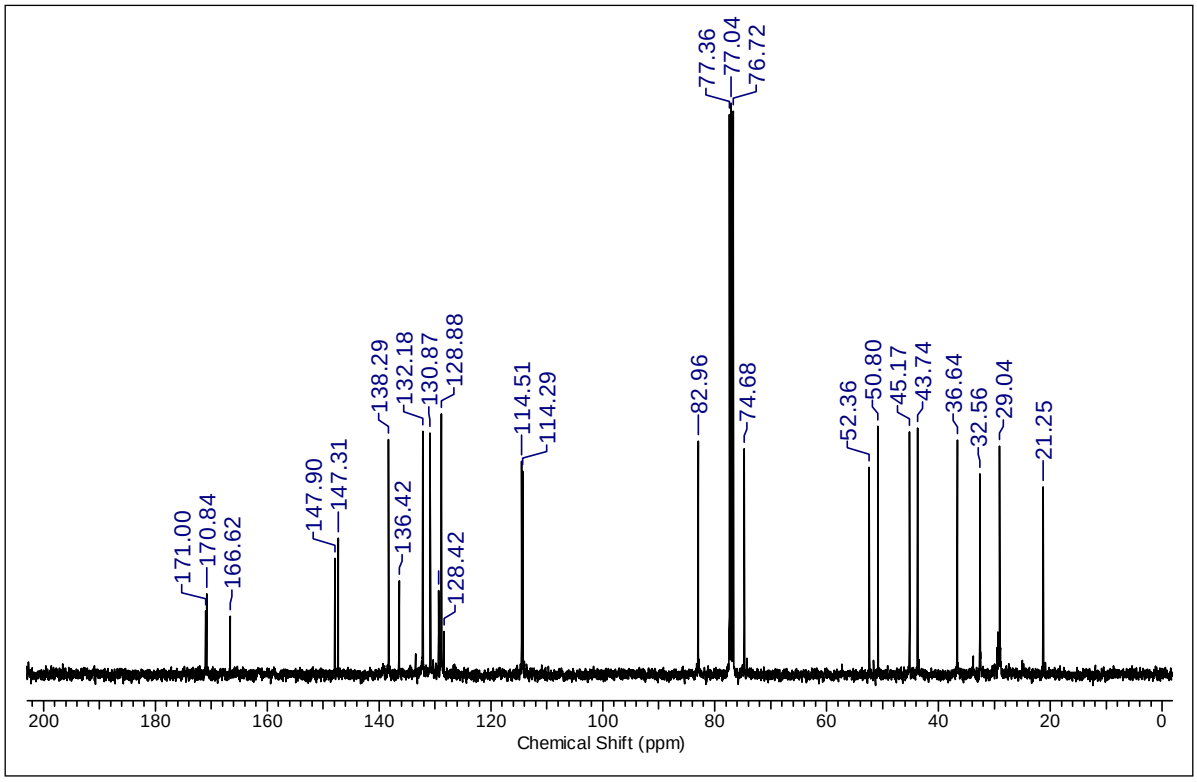
^{13}C NMR of Compound 29 (125 MHz, $CDCl_3$)



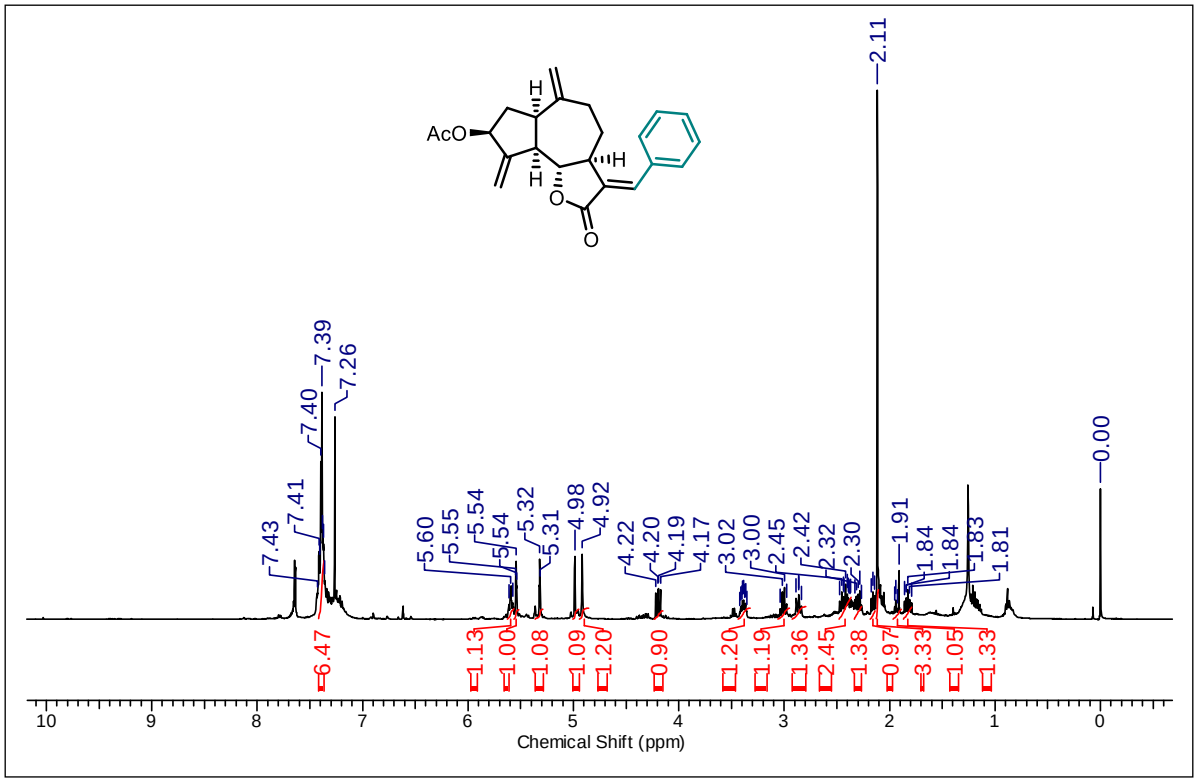
^{1}H NMR of Compound 30 (400 MHz, $CDCl_3$)



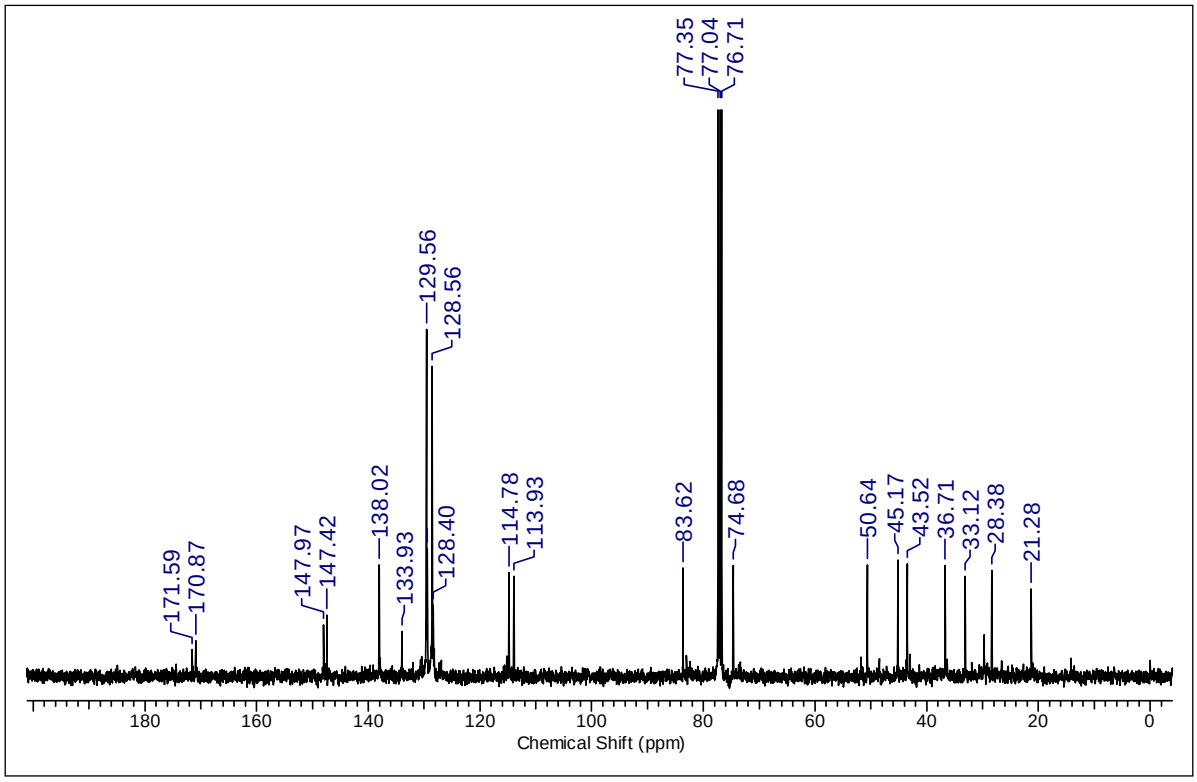
^{13}C NMR of Compound 30 (100 MHz, $CDCl_3$)



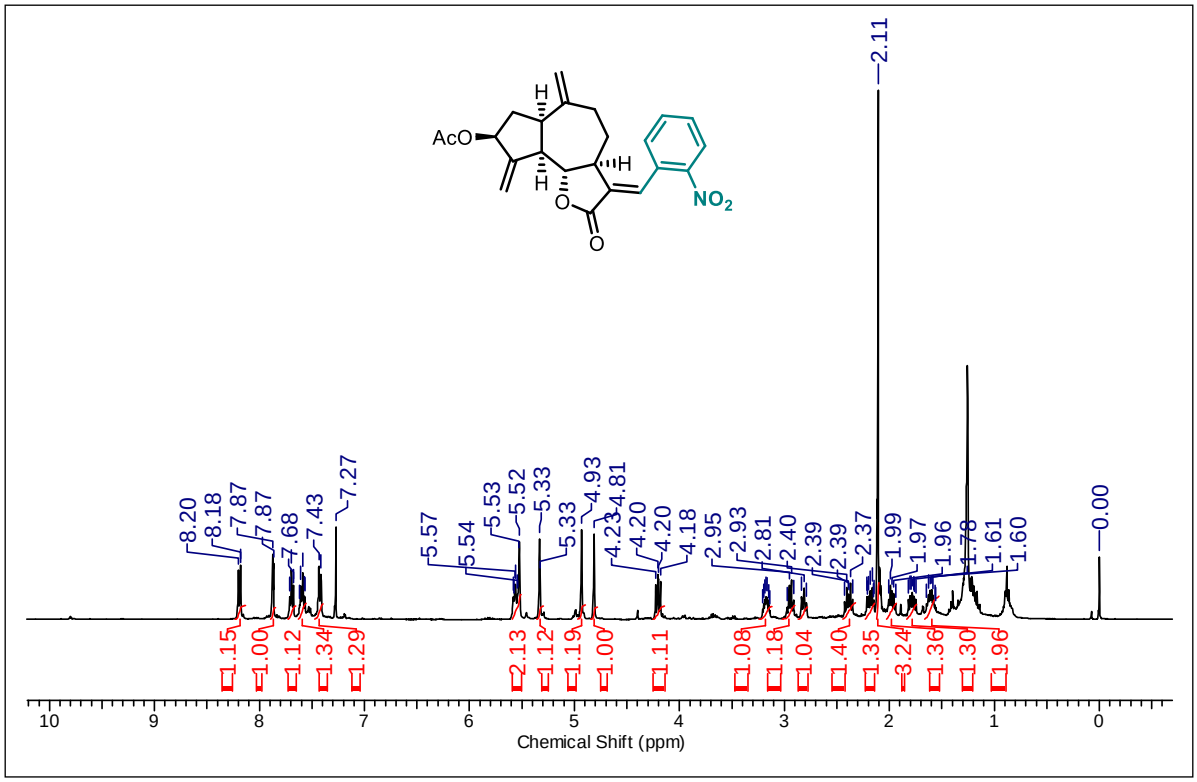
^{1}H NMR of Compound 31 (400 MHz, $CDCl_3$)



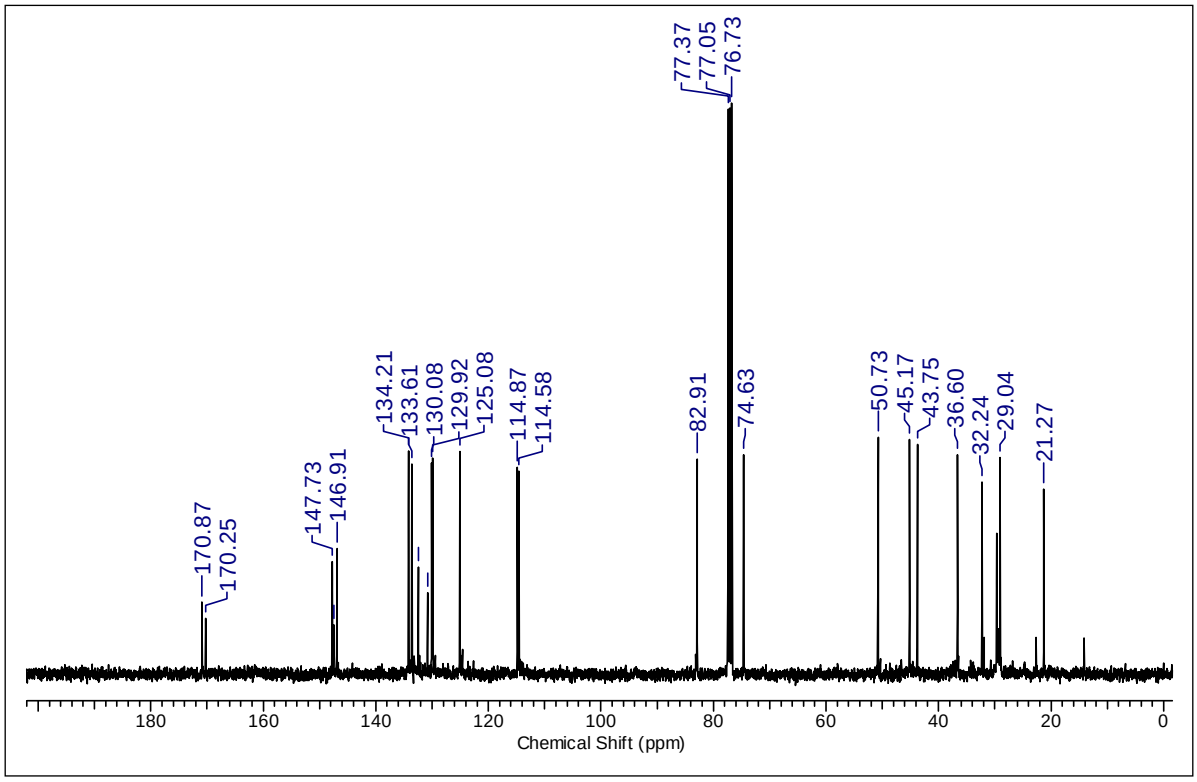
^{13}C NMR of Compound 31 (100 MHz, $CDCl_3$)



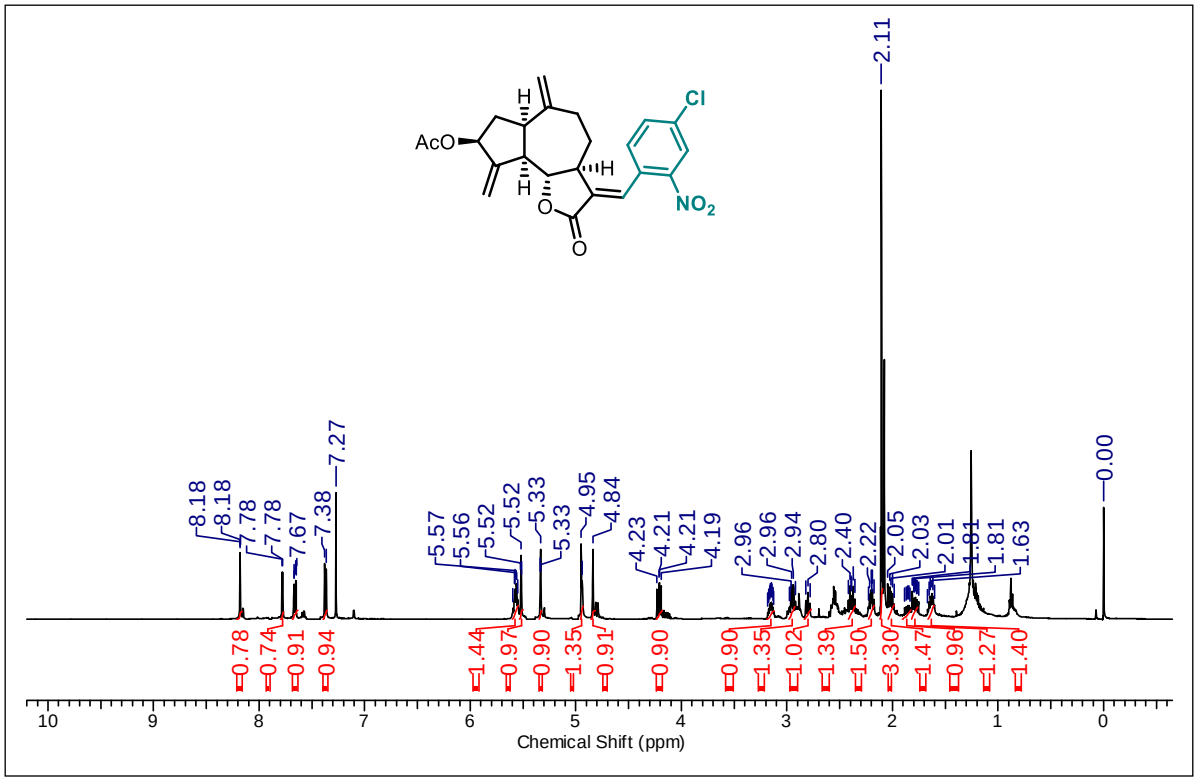
^{1}H NMR of Compound 32 (400 MHz, $CDCl_3$)



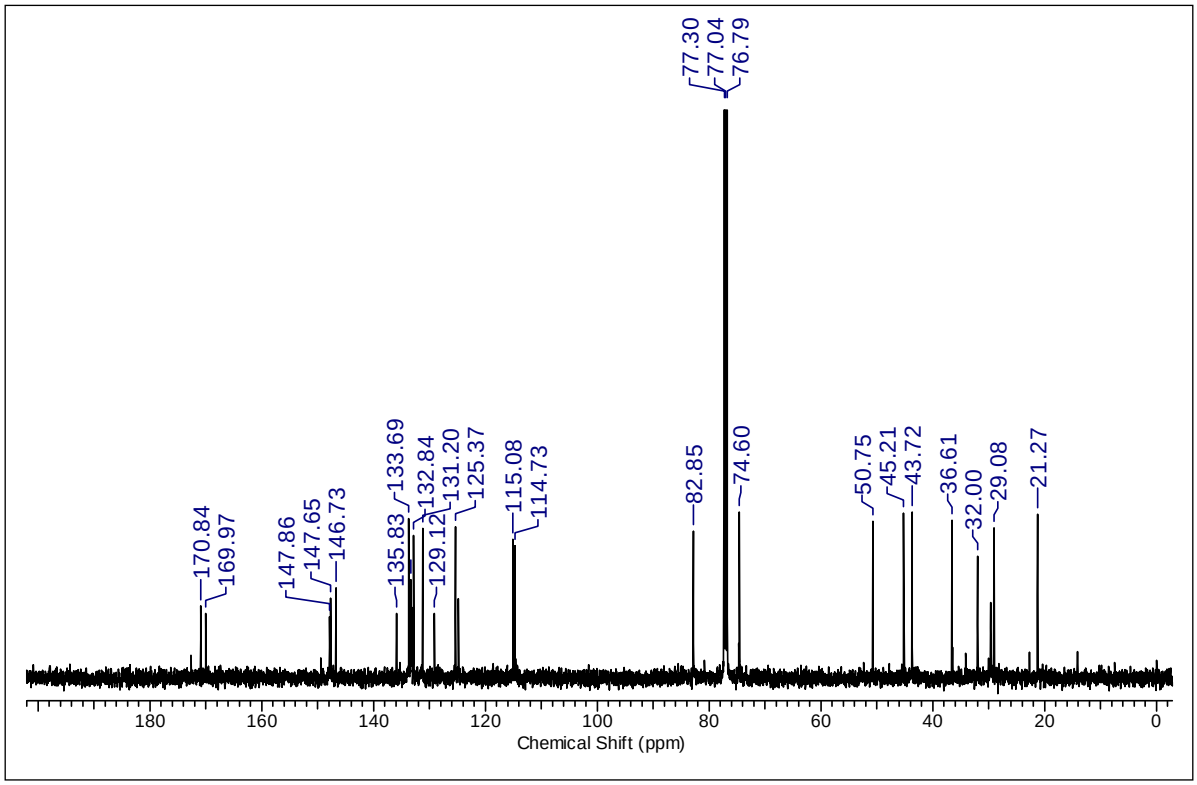
^{13}C NMR of Compound 32 (100 MHz, $CDCl_3$)



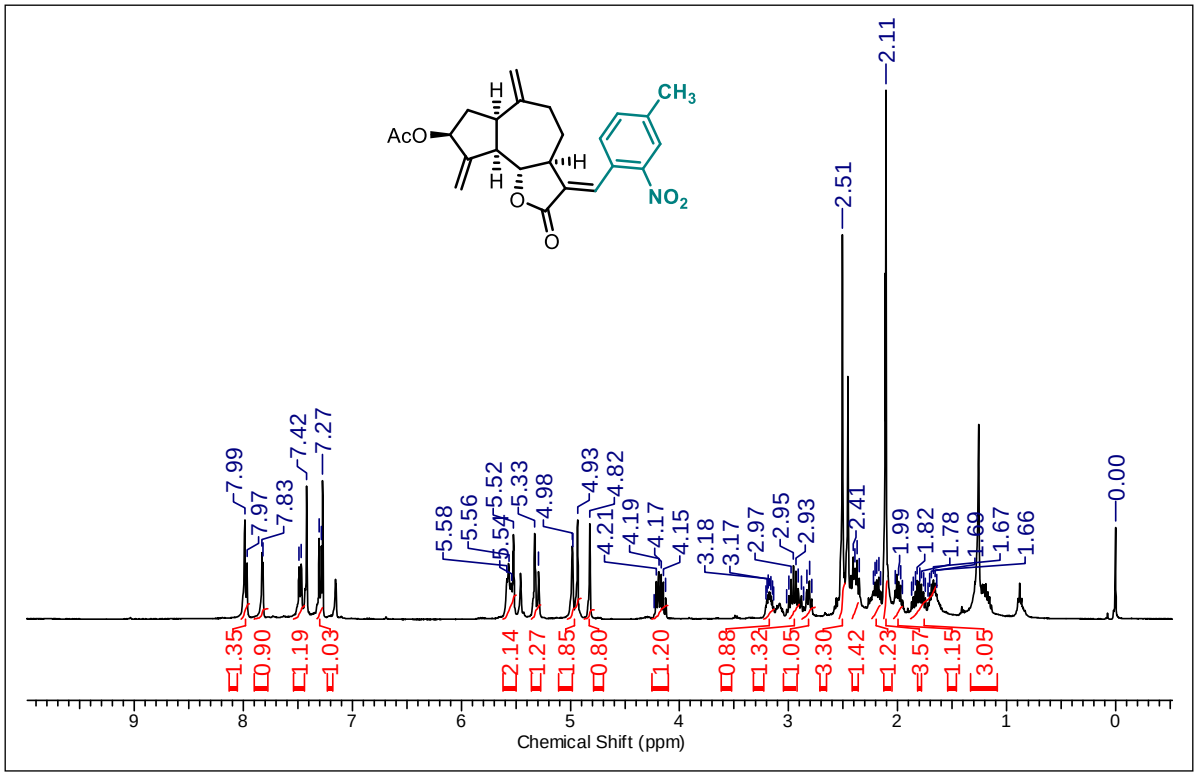
^{1}H NMR of Compound 33 (500 MHz, $CDCl_3$)



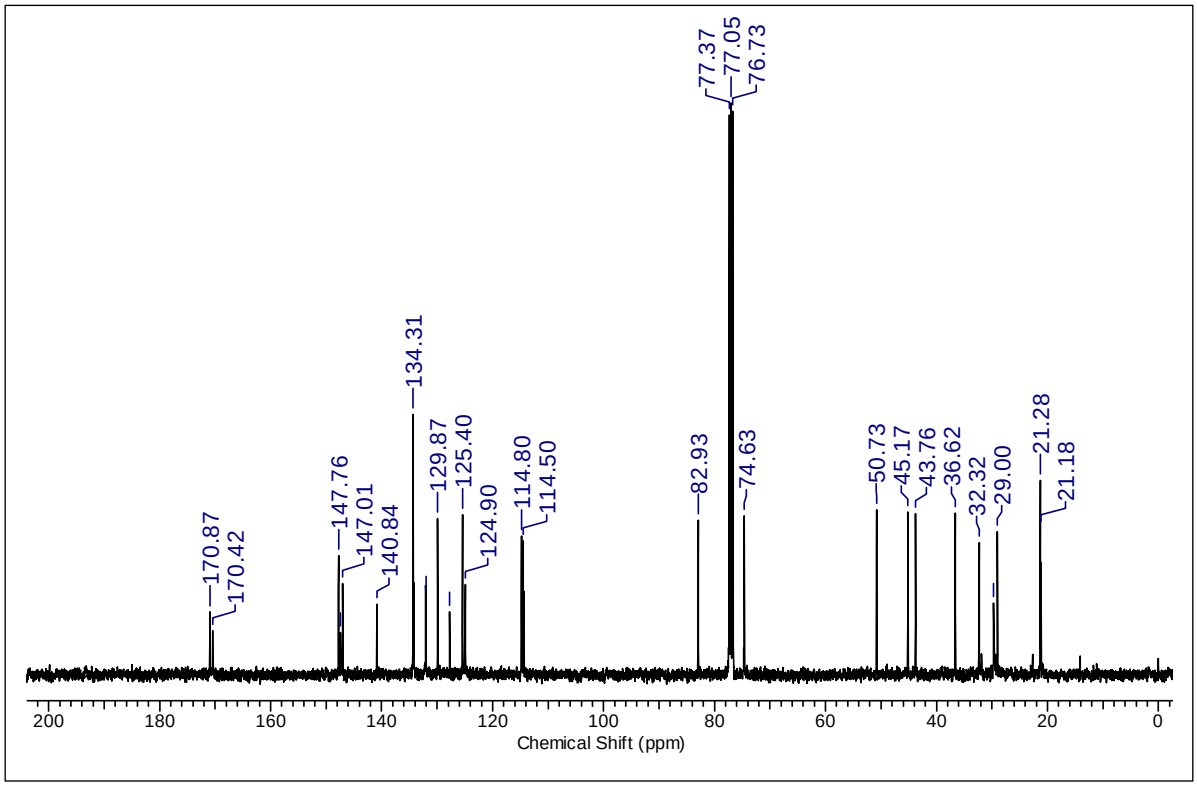
^{13}C NMR of Compound 33 (125 MHz, $CDCl_3$)



^{1}H NMR of Compound 34 (400 MHz, $CDCl_3$)



^{13}C NMR of Compound 34 (100 MHz, $CDCl_3$)



Bio - Data

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Education

- **Ph. D. in Organic Chemistry (2012-present)**
  Academy of Scientific & Innovative Research, CSIR-National Chemical Laboratory, Pune, India.

  Research supervisor: **Dr. Asish K. Bhattacharya**, Senior Scientist, CSIR-NCL, Pune.

  Thesis title: **"Design and Synthesis of Artemisinin-Dipeptidyl Vinyl Phosphonate Hybrid Molecules as Novel Antimalarial Agents; Synthesis of Biologically Active Molecules and Studies Directed Towards Bioactives from *Vernonia arborea*"**

- **Master of Science (M.Sc) in Organic Chemistry (2008-2010)**
  First division with 7.95 CGPA, Andhra University Campus, Visakhapatnam, A.P, India.

- **Bachelor of Science (B.Sc.) (2005-2008)**
  Mathematics, Physics and Chemistry 2008, First Division with 81%, Andhra University, Visakhapatnam, A.P, India.

Awards/Fellowships Received

- Awarded Senior Research Fellowship (**SRF**) from the Council for Scientific and Industrial Research (CSIR), New Delhi, India (2014-2017).
- Awarded Junior Research Fellowship (**JRF**) from the Council for Scientific and Industrial Research (CSIR), New Delhi, India (2012-2014).
- Awarded National Eligibility Test (**NET**) for Ph. D. and Lectureship, June-2010 from UGC-CSIR, New Delhi, India.
- Awarded National Eligibility Test (**NET**) for Ph. D. and Lectureship, December-2009 from UGC-CSIR, New Delhi, India.

- Best poster award (**1st prize**) presented by Royal Society of Chemistry (**RSC**) at **XI J-NOST** conference for research scholars held during December 14-17, 2015 in NISER, Bhubaneswar.

Industrial Research Experience

- Worked in R&D as a **Research Chemist** in **Laxai Avanti Life Sciences,** Hyderabad, Telangana, India from May-2010 to November-2011.
- Worked in R&D as a Project Intern in **Dr. Reddy's Laboratories**, IPDO, Bachupally, Hyderabad, Telangana, India from May-2009 to July-2009.

Research Interests

- Design and synthesis of novel chemical entities for medicinal and pharmaceutical chemistry.
- Total synthesis of biologically active natural products.

Conferences and Symposia

- Presented poster on "Total synthesis of antiepileptic drug (*R*)-lacosamide from L-serine" at **XI J-NOST** conference for research scholars held during December 14-17, 2015 in NISER, Bhubaneswar and received best poster award (**1st prize**) presented by Royal Society of Chemistry (**RSC**) for the poster.
- Oral presentation on "Synthesis of syncarpamide, its analogues and antimalarial activity" at **X J-NOST** conference for research held during December 4-6, 2014 in IIT Madras, Chennai.
- Participated in **17th CRSI National Symposium in Chemistry and 9th CRSI-RSC Symposium** held at CSIR- National Chemical Laboratory Pune, India, February 2015.
- Participated in a satellite meeting on **"Frontiers in Chemistry & Biology of Oligosaccharides 2014 (FCBO)"** held at Indian Institute of Science Education and Research, Pune.
- Presented poster on "Synthesis of antiepileptic drug (*R*)-lacosamide from L-serine" during **National Science day 2016** at CSIR-National Chemical Laboratory Pune, India, February 2016.

Publications and patents

(1) **Eswar K. Aratikatla**, and Asish K. Bhattacharya. Chiral Pool Approach for the Synthesis of Functionalized Amino Acids: Synthesis of Antiepileptic Drug (*R*)-Lacosamide. ***Tetrahedron Lett.* 2015, *56*, 5802**.

(2) **Eswar K. Aratikatla**, Tushar R. Valkute, Sunil K. Puri, Kumkum Srivastava, and Asish K. Bhattacharya. Norepinephrine alkaloids as antiplasmodial agents: Synthesis of syncarpamide and insight into the structure-activity relationships of its analogues as antiplasmodial agents. ***Eur. J. Med. Chem.* 2017, *138*, 1089**.

(3) Tushar R. Valkute, **Eswar K. Aratikatla**, and Asish K. Bhattacharya. Efficient synthesis of functionalized olefins by Wittig reaction using Amberlite resin as a mild base. ***Synth. Commun.* 2017, *47*, 581**.

(4) Tushar R. Valkute, **Eswar K. Aratikatla**, Neha A. Gupta, S. Ganga, Manas K. Santra, and Asish K. Bhattacharya. Synthesis and anticancer studies of Michael adducts and Heck arylation products of sesquiterpene lactones, zaluzanin D and zaluzanin C from *Vernonia arborea*. ***RSC Adv.* 2018, 8, 38289**.

(5) **Eswar K. Aratikatla**, and Asish K. Bhattacharya. Enantioselective Synthesis of γ-Aryl-γ-Amino Vinyl Phosphonate and Sulfonates via Wittig Olefination of Trityl Protected α-Amino Aldehyde (***Manuscript submitted***).

(6) **Eswar K. Aratikatla**, Asif Mohammed, Pawan Malhotra, and Asish K. Bhattacharya. Design and Synthesis of Artemisinin-Dipeptidyl Vinyl Phosphonate Hybrid Molecules as Novel Antimalarial Agents targeting falcipain-2 enzyme (***Manuscript under preparation***).

(7) Asish K. Bhattacharya, **Eswar K. Aratikatla**, Kumkum Srivastava and Ashan Manhas, Anti-malarial compounds and process for preparation thereof. **WO 2016/063301 A2**.

(8) **Eswar K. Aratikatla**, Asif Mohammed, Pawan Malhotra, and Asish K. Bhattacharya. Novel antimalarial compounds targeting dual pathways of hemoglobin degradation and hemozoin development in the parasite, and their use for therapy of drug-resistant malaria. Provisional patent filed (application no. **201811014401**).

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Chiral pool approach for the synthesis of functionalized amino acids: synthesis of antiepileptic drug (*R*)-lacosamide

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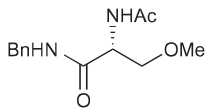
ABSTRACT

An efficient total synthesis of (*R*)-lacosamide **1** has been achieved from *N*-Boc-*N*,*O*-isopropylidene-L-serinol **2** which could easily be obtained from natural L-serine. Our synthesis of **1** starting from **2** using chiral pool strategy resulted in 54% overall yield.

Introduction

Epilepsy is a neurological disorder due to which patients suffering from this disease witness recurrent spontaneous seizures. It is believed that this disease affects approximately 50 million people worldwide and India alone accounts for approximately 10 million cases.[1] (*R*)-lacosamide **1** (Vimpat, Fig. 1) is currently being used clinically in the U.S. and Europe to treat people suffering from this disease. Although, the exact mode of action of this drug in humans is not yet deciphered however, it is widely believed that it increases the slow inactivation of the voltage-gated sodium channels thus inhibiting repetitive neuronal firing.[2]

Till date several synthesis of **1** have been reported using racemic epichlorohydrin[3] or acrylic acid[4] or ethyl L-lactate[5] or racemic butadiene monoepoxide[6] or D,L-serine[7] or D-serine,[8] respectively as starting materials. The kinetic resolution of the key intermediate using Hydrolytic Kinetic Resolution (HKR)[3] leads to the loss of yield during the resolution step whereas Trost's dynamic kinetic asymmetric transformation (DYKAT)[6] requires ligands, which are expensive. In some of the synthesis racemic lacosamide has been resolved to (*R*)-enantiomer either using crystallization[7a] with chiral carboxylic acid or chiral chromatography.[7b] The synthesis of **1** utilizing D-serine as a starting material involves *O*-methylation which undergoes racemization.[8c,g–j,l] In order to overcome this drawback, Kohn et al.[8c] used neutral but rather expensive Kuhn's *O*-methylation protocol,[8a] which requires Ag_2O.



(*R*)-Lacosamide (**1**)

Figure 1. Structure of antiepileptic drug, Lacosamide **1**.

Results and discussions

We thought of an efficient and straightforward synthesis of **1**, which could be achieved on an industrial scale at a much cheaper cost. We envisaged the synthesis of **1** could be achieved as delineated in Scheme 1. The key starting material, *N*-Boc-*N*,*O*-isopropylidene-L-serinol **2** could easily be synthesized[9] in gram scale from natural L-serine in four steps without the need of any purification of the intermediates. *N*-Boc-protected alcohol **4** would lead us to the desired product **1** after protection/deprotection and functional group manipulations. Also, our synthesis would enable the generation of various *O*-substituted congeners of (*R*)-lacosamide **1** for further development of new potent anti-epileptic agents.

We started the synthesis of (*R*)-lacosamide **1** from readily available *N*-Boc-*N*,*O*-isopropylidene-L-serinol **2**.[9] Methylation of the

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Research paper

Norepinephrine alkaloids as antiplasmodial agents: Synthesis of syncarpamide and insight into the structure-activity relationships of its analogues as antiplasmodial agents

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ABSTRACT

Syncarpamide **1**, a norepinephrine alkaloid isolated from the leaves of *Zanthoxylum syncarpum* (Rutaceae) exhibited promising antiplasmodial activities against *Plasmodium falciparum* with reported IC_{50} values of 2.04 μM (D6 clone), 3.06 μM (W2 clone) and observed by us 3.90 μM (3D7 clone) and 2.56 μM (K1 clone). In continuation of our work on naturally occurring antimalarial compounds, synthesis of syncarpamide **1** and its enantiomer, (*R*)-**2** using Sharpless asymmetric dihydroxylation as a key step has been accomplished. In order to study structure-activity-relationship (SAR) in detail, a library of 55 compounds (**3–57**), which are analogues/homologues of syncarpamide **1** were synthesized by varying the substituents on the aromatic ring, by changing the stereocentre at the C-7 and/or by varying the acid groups in the ester and/or amide side chain based on the natural product lead molecule and further assayed *in vitro* against 3D7 and K1 strains of *P. falciparum* to evaluate their antiplasmodial activities. In order to study the effect of position of functional groups on antiplasmodial activity profile, a regioisomer (*S*)-**58** of syncarpamide **1** was synthesized however, it turned out to be inactive against both the strains. Two compounds, (*S*)-**41** and its enantiomer, (*R*)-**42** having 3,4,5-trimethoxy cinnamoyl groups as side chains showed better antiplasmodial activity with IC_{50} values of 3.16, 2.28 μM (3D7) and 1.78, 2.07 μM (K1), respectively than the natural product, syncarpamide **1**. Three compounds (*S*)-**13**, (*S*)-**17**, (*S*)-**21** exhibited antiplasmodial activities with IC_{50} values of 6.39, 6.82, 6.41 μM against 3D7 strain, 4.27, 7.26, 2.71 μM against K1 strain and with CC_{50} values of 147.72, 153.0, >200 μM respectively. The *in vitro* antiplasmodial activity data of synthesized library suggests that the electron density and possibility of resonance in both the ester and amide side chains increases the antiplasmodial activity as compared to the parent natural product **1**. The natural product syncarpamide **1** and four analogues/homologues out of the synthesized library of 55, (*S*)-**41**, (*R*)-**42**, (*S*)-**55** and (*S*)-**57** were assayed *in vivo* assay against chloroquine-resistant *P. yoelii* (N-67) strain of *Plasmodium*. However, none of the five molecules, **1**, (*S*)-**41**, (*R*)-**42**, (*S*)-**55** and (*S*)-**57** exhibited any promising *in vivo* antimalarial activity against *P. yoelii* (N-67) strain. Compounds **4**, **6**, **7** and **11** showed high cytotoxicities with CC_{50} values of 5.87, 5.08, 6.44 and 14.04 μM, respectively. Compound **6** was found to be the most cytotoxic as compared to the standard drug, podophyllotoxin whereas compounds **4** and **7** showed comparable cytotoxicities to podophyllotoxin.

1. Introduction

The protozoan parasites of the genus *Plasmodium* cause malaria, which affects more than 50% of the world's population living in tropical and subtropical regions of America, Asia, and Africa. The WHO reported that more than 212 million people were diagnosed

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Efficient synthesis of functionalized olefins by Wittig reaction using Amberlite resin as a mild base

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ABSTRACT

A convenient procedure for the synthesis of olefins by the reaction of stabilized, semistabilized, and nonstabilized phosphorous ylides with various aldehydes or ketone using Amberlite resin as a mild base is described. Our developed method offers facile and racemization-free synthesis of α,β-unsaturated amino esters and chiral allylic amine. The developed methodology offers mild reaction conditions, high efficiency, and facile isolation of the final products, a practical alternative to known procedures.

GRAPHICAL ABSTRACT

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Aldehyde; alkene; Amberlite resin; phosphonium ylides; Wittig reaction

Introduction

Carbon–carbon bond formation reactions play a major role in the total synthesis of bioactive molecules. Several C–C bond-forming reactions are known in the literature, however, Wittig olefination remains one of the most favored when an alkene group is to be introduced with specificity of bond placement. In the classical Wittig method[1–3] forms a phosphorous ylide is formed upon treatment with appropriate base, which then reacts with a ketone or aldehyde to furnish the corresponding alkene. However, the Wittig method has a few drawbacks[4] such as substrate sensitivity to base, racemization of the adjacent stereogenic center, and self-condensation of the carbonyl to name a few. Several new modifications of Wittig reaction conditions using various bases such as tertiary amines,[5] lithium 1,1,1,3,3,3-hexafluoro-isopropoxide,[6] LiCl/DBU,[7] $KOSiMe_3$,[8] LiOH,[9] or KH[10] have been reported. Recently, Ag_2CO_3 as a mild base for ylide formation for the Wittig reaction has been reported.[11] Using K_2CO_3 as a mild base, Wittig

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Color versions of one or more of the figures in this article can be found online on at www.tandfonline.com/lsyc.
Supplemental data (full experimental detail with spectral data of all the synthesized compounds, copies of ^1H, ^{13}C NMR, and DEPT spectra, HRMS of all the compounds, HPLC chromatogram of chiral compounds) can be accessed on the publisher's website.

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Synthesis and anticancer studies of Michael adducts and Heck arylation products of sesquiterpene lactones, zaluzanin D and zaluzanin C from *Vernonia arborea*†

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Sesquiterpene lactones containing α-methylene-γ-lactones, zaluzanin D **1** and zaluzanin C **2** were isolated from the leaves of *Vernonia arborea*. Several diverse Michael adducts (**3–22**) and Heck arylation analogs (**23–34**) of **1** have been synthesized by reacting with various amines and aryl iodides, respectively and were assayed for their *in vitro* anticancer activities against human breast cancer cell lines MCF7 and MDA-MB-231. Among all the synthesized analogs, Michael adducts **9** and **10** showed better anticancer activities as compared to **1**. However, among these compounds, only **10** has minimal cytotoxic effect on normal breast epithelial MCF10A cells. Our detailed mechanistic studies reveal that compounds **9** and **10** execute their antiproliferative activity through induction of apoptosis and thereby inhibit the cancer cells proliferation and compound **10** could be a lead compound for designing potential anti-cancer compound.

Introduction

The generation of diverse chemical libraries using natural products as scaffolds is considered as one of the effective methods for drug discovery.[1] Sesquiterpenes bearing α,β-unsaturated γ-lactones are ubiquitous in nature and have been reported to be isolated from various genera of the family Asteraceae but also occur sporadically in other angiosperm families, Apiaceae, Magnoliaceae and even in some liverworts. They exhibit diverse biological activities such as anti-microbial, anti-inflammatory, antiulcer, antiviral, anticancer and antimalarial activities.[2] The genus *Vernonia* (family Asteraceae) consists of approximately 1000 species of herbs and shrubs.[3] Several research groups have isolated structurally diverse sesquiterpene lactones from various species of genus *Vernonia* possessing various biological properties (Fig. 1).[4,5] Sesquiterpene lactones react with nucleophilic sulfhydryl groups present in enzymes, proteins, and glutathione.[6] The use of sesquiterpene lactones as therapeutic agents is limited due to their poor water solubility. To overcome this, amino-adducts of sesquiterpene lactones have been prepared by adding different amines to the α-methylene-γ-lactone substructure to enhance the water solubility of the parent molecules and to retain their biological activity.[7,8] Regeneration of parent α,β-unsaturated γ-lactone occurs by the retro-Michael reaction, potentially through bioactivation at the site of action. This prodrug approach has transformed several sesquiterpene lactones such as alantolactone, ambrosin, arglabin, costunolide, helenalin, parthenolide and ivangustin, into successful clinical candidates (Fig. 1).[8]

Continuing our interest in naturally occurring sesquiterpene lactones[9] and other bioactive secondary metabolites, we wished to take up the chemical examination of *V. arborea* leaves for the isolation of bioactive secondary metabolites, zaluzanin C and zaluzanin D. In the present study, several structurally diverse Michael adducts of zaluzanin D have been synthesized with the formation of one or two new C–N bonds. Analogs of zaluzanin D with a new C–C bond formation have also been synthesized by using Pd catalyzed Heck coupling reaction. The *in vitro* anticancer activities of all the analogs were tested against human breast cancer cell lines MCF7 and MDA-MB-231.

Results and discussion

A portion of the petroleum ether extract (5.3 g) was flash chromatographed on CombiFlash Companion, Isco Teledyne Inc., USA using RediSep® column (SiO_2, 2 × 12 g) and elution was carried out isocratically with ethyl acetate–petroleum ether (4 : 96) to furnish a colorless solid. It was identified as zaluzanin

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† Electronic supplementary information (ESI) available:Additional information includes spectral copies. CCDC 1525170. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c8ra06238b

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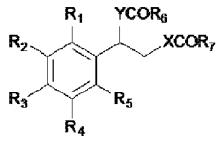
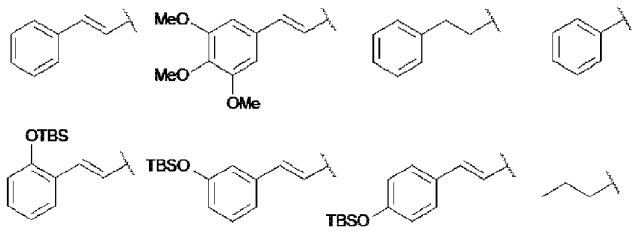
[Continued on next page]

(54) Title: ANTI-MALARIAL COMPOUNDS AND PROCESS FOR PREPARATION THEREOF



Formula (I)

(57) Abstract: The present invention discloses anti-malarial compound of formula (I) Formula (I) wherein, X is selected from O or NH; R_1, R_2, R_3, R_4 and R_5 is selected from H or OMe or CH_3, $-CH_2-O-CH_2-$ or -CH=CH-CH=CH-; Y is selected from O or NH and R_6, R_7 is selected from the following compounds: or pharmaceutically acceptable salts thereof, process for preparation and a pharmaceutical composition containing the same.



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Erratum

Erratum

Erratum