Synthesis of Optically Pure Pharmaceuticals Employing Aziridines/Epoxides as Chiral Synthons and Development of Novel Biologically Active Compounds based on Benzopyran-4-ones

Thesis Submitted to AcSIR

For the Award of the Degree of

DOCTOR OF PHILOSOPHY

In

CHEMICAL SCIENCES

By

Viswanadh Nalla

(Registration Number**:** 10CC13J26008)

Under the guidance of

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April 2018

Dedicated to

My Beloved Family,

My Guide and Friends

For their endless love, support and encouragement

सीएसआईआर - राष्ट्रीय रासायनिक प्रयोगशाला
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THESIS CERTIFICATE

This is to certify that the work incorporated in this Ph.D. thesis entitled **"Synthesis of optically pure pharmaceuticals employing aziridines/epoxides as chiral synthons and development of novel biologically active compounds based on benzopyran-4-ones"** submitted by **Mr. Viswanadh Nalla** to Academy of Scientific and Innovative Research (AcSIR) in fulfillment of the requirements for the award of the Degree of **Doctor of Philosophy**, embodies original research work under my supervision. I further certify that this work has not been submitted to any other University or Institution in part or full for the award of any degree or diploma. Research material obtained from other sources has been duly acknowledged in the thesis. Any text, illustration, table etc., used in the thesis from other sources, have been duly cited and acknowledged.

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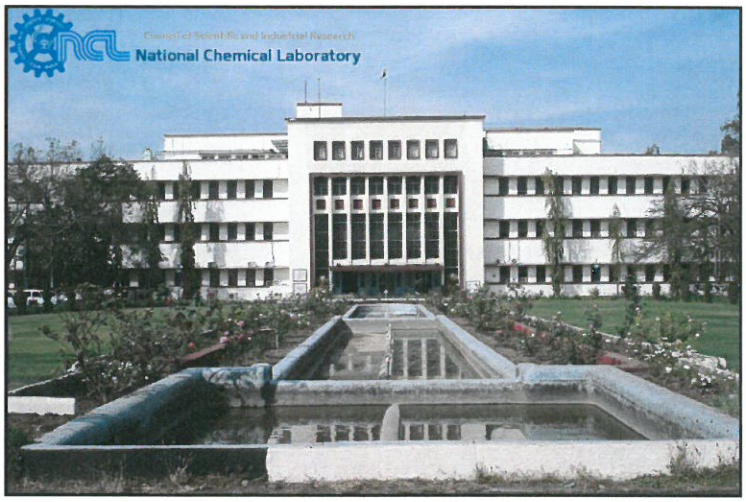

Declaration by the Candidate

I hereby declare that the original research work embodied in this thesis entitled, **"Synthesis of optically pure pharmaceuticals employing aziridines/epoxides as chiral synthons and development of novel biologically active compounds based on benzopyran-4-ones''** submitted to Academy of Scientific and Innovative Research for the award of degree of Doctor of Philosophy (Ph.D.) is the outcome of experimental investigations carried out by me under the supervision of **Dr. M. Muthukrishnan**, Principal Scientist, Organic Chemistry Division, CSIR-National Chemical Laboratory, Pune. I affirm that the work incorporated is original and has not been submitted to any other academy, university or institute for the award of any degree or diploma.

2nd April 2018
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Acknowledgement

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My family is always a source of inspiration and great moral support for me in perceiving my education, I used to thank the god of almighty for providing me such a beautiful family. I take this opportunity to my sense of gratitude to my parents Rukumini (mother), Ramarao (father), my lovely brothers Suresh Kumar, Venkatesh and my grandparents Savitramma, Laxmanrao, Thavitamma, Venkanna, Kalavathi, Prakashrao for their tons of love, sacrifice, blessings, unconditional support, and encouragement.

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Viswanadh Nalla

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Abbreviations

Ac	Acetyl
AcCl	Acetyl chloride
AcOH	Acetic acid
Ac_2O	Acetic anhydride
ACN	Acetonitrile
ADME	Absorption, distribution, metabolism, and excretion
ATP	Adenosine triphosphate
$AlCl_3$	Aluminium chloride
Aq.	Aqueous
NH_4Cl	Ammonium chloride
NH_4OH	Ammonium hydroxide
Å	Angstrom
atm	Atmosphere
Br	Bromo
Br_2	Bromine
Bn	Benzyl
PhH	Benzene
BH_3	Boron hydride
BuOH	Butanol
n-Bu_4NBr	tetra-*n*-Butylammonium bromide
TBAI	tetra-*n*-Butylammonium iodide
Boc	*tert*-Butoxy carbonyl
t-Bu	*tertiary*-Butyl
DBAD	Di-*tert*-butyl azodicarboxylate
TBHP	*tert*-Butyl hydroperoxide
C	Carbon
CBr_4	Carbon tetrabromide
Cbz	Carboxybenzyl
Cat.	Catalytic
cm^{-1}	1/centimetre
DCM (CH_2Cl_2)	Dichloromethane

Abbreviations

$CHCl_3$	Chloroform
$CDCl_3$	Deuterated chloroform
CuI	Copper iodide
CuOTf	Copper triflate
$CuSO_4$	Copper sulfate
Conc.	Concentrated
J	Coupling constant (in NMR)
°C	Degree Celsius
DEA	Diethylamine
DEAD	Diethylazocarboxylate
DET	Diethylltartarate
DIAD	Diisopropylazocarboxylate
DIPT	Diisopropyl tartrate
DMAP	*N*, *N*'-dimethylaminopyridine
DMF	*N*, *N*'-dimethylformamide
DMSO	Dimethylsulphoxide
DMSO-*d*6	Deutriated dimethylsulphoxide
ee	Enantiomeric Excess
EtOH	Ethanol
Et	Ethyl
ESI	Electrospray ionization
EtOAc	Ethyl acetate
Et_2O	Diethyl ether
equiv.	Equivalent
ν	Frequency
g	Gram (s)
h	Hour (s)
HRMS	High resolution mass spectrometry
HPLC	High-pressure liquid chromatography
HCl	Hydrochloric acid
H_2	Hydrogen

Hz	Hertz
h	Hour (s)
In vitro	Outside a living organism
IC_{50}	Half-maximal inhibitory concentration
IR	Infrared
In vivo	Inside a living organism
I	Iodo
Kcal	Kilocalorie (s)
lit.	Literature
$LiAlH_4$ (LAH)	Lithium aluminium hydride
LiBr	Lithium bromide
m/z	Mass to charge ratio
MP	Melting point
Me	Methyl
MeOH	Methanol
MHz	Megahertz
M.P	Melting point
MsCl	Methanesulfonyl chloride
min	Minute(s)
µg	Microgram
µM	Micromolar
mg	Milligram (s)
mL	Milliliter (s)
mmol	Millimole (s)
MIC	Minimum inhibitory concentration
M	Molarity
Mtb	*Mycobacterium tuberculosis*
MS	Molecular sieves
N	Normality
nM	Nanomolar (s)
NMR	Nuclear magnetic resonance

ppm	Parts per million
Pd	Palladium
$Pd(OH)_2$	Palladium hydroxide
Pr	Propyl
i-Pr	iso-Propyl
Ph	Phenyl
psi	Pounds per square inch
K_2CO_3	Potassium carbonate
KOH	Potassium hydroxide
t-BuOK	Potassium tertiary butoxide
PDB	Protein Data Bank
Py	Pyridine
RMSD	Root-mean-square deviation
rt	Room temperature
RM	Reaction mixture
RuO_2	Ruthenium oxide
Na	Sodium
$NaNH_2$	Sodium amide
NaN_3	Sodium azide
$NaBH_4$	Sodium borohydride
Na_2CO_3	Sodium carbonate
$NaIO_4$	Sodium periodate
NaOPh	Sodium phenoxide
NaH	Sodium hydride
NaOH	Sodium hydroxide
Na_2SO_4	Sodium sulfate
Red-Al	Sodium bis(methoxyethoxy)aluminum hydride
tert	Tertiary
$Ti(OEt)_4$	Titanium(IV) ethoxide
$Ti(O^iPr)_4$	Titanium(IV) isopropoxide
TEA	Triethyl amine

PBu_3	Tributylphosphine
$HSiCl_3$	Trichlorosilane
TFA	Trifluoroacetic acid
$(CF_3CO)_2O$	Trifluoroacetic anhydride
CF_3SO_3H	Trifluoromethanesulfonic acid
PPh_3	Triphenylphosphine
THF	Tetrahydrofuran
TLC	Thin layer chromatography
$SOCl_2$	Thionylchloride
p-TsCl	*para*-Toluenesulfonyl chloride
UV	ultraviolet
H_2O	water

Abbreviations used for NMR spectral information

br	broad	s	singlet	dd	doublet of doublets
d	doublet	t	triplet	ddd	doublet of doublet of doublets
m	multiplet	q	quartet	quint	quintet
sept	septet				

Abbreviations used for amino acids

Ala	Alanine	Asn	Asparagine	Asp	Aspartic acid
Gln	Glutamine	Gly	Glycine	Glu	Glutamic acid
Leu	Leucine	Lys	Lysine	Met	Methionine
Phe	Phenylalanine	Thr	Threonine	Trp	Tryptophan
Tyr	Tyrosine	Ser	Serine	Val	Valine

- All reagents, starting materials, and solvents were obtained from commercial suppliers and used as such without further purification.
- Solvents were distilled and dried using standard protocols. Reactions were carried out in anhydrous solvents under argon atmosphere in oven-dried glassware.
- Petroleum ether refers to the fraction collected in the boiling range 60-80 °C. Organic layers after every extraction were dried over anhydrous sodium sulfate.
- Air sensitive reagents and solutions were transferred *via* syringe or cannula and were introduced to the apparatus *via* rubber septa.
- All reactions are monitored by thin layer chromatography (TLC) with 0.25 mm pre-coated E-Merck silica gel plates (60F-254). Visualization was accomplished with either UV light, Iodine adsorbed on silica gel or by immersion in an ethanolic solution of phosphomolybdic acid (PMA), *p*-anisaldehyde or $KMnO_4$ followed by heating with a heat gun for ~15 sec.
- All evaporations were carried out under reduced pressure on Buchi rotary evaporator below 50 °C unless otherwise specified.
- Column chromatography was performed on silica gel (100-200 or 230-400 mesh size).
- Deuterated solvents for NMR spectroscopic analyses were used as received. 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 ^{1}H NMR and 100.03 MHz for ^{13}C NMR) and DRX 500 (500.13 MHz ^{1}H NMR and 125.03 MHz for ^{13}C NMR) spectrometers.
- Chemical shifts (δ) reported are referred to internal reference tetramethylsilane (TMS). Chemical shifts have been expressed in ppm units relative to TMS, using the residual solvent peak as a reference standard. Coupling constants were measured in Hertz.
- All the melting points are uncorrected and were recorded using a scientific melting point apparatus (Buchi B-540).
- 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) were recorded on a Thermo Scientific Q-Exactive, Accela 1250 pump and also EI Mass spectra were recorded on Finnigan MAT-1020 spectrometer at 70 *eV* using a direct inlet system.
- Infrared (IR) spectra were recorded on an FT-IR spectrometer as thin films in chloroform using NaCl plates and absorptions were expressed in cm^{-1}.
- Optical rotations were recorded on a P-2000 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.
- Chemical nomenclature (IUPAC) and structures were generated using Chem Bio Draw Ultra 13.0 software.

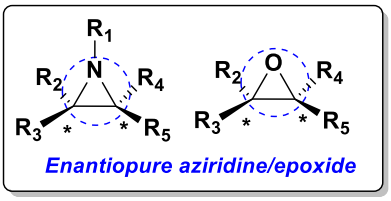
Synopsis

AcSIR	**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	Viswanadh Nalla
AcSIR Enrolment No. & Date	Ph. D in Chemical Sciences (10CC13J26008); January 2013
Title of the Thesis	Synthesis of Optically Pure Pharmaceuticals Employing Aziridines/Epoxides as Chiral Synthons and Development of Novel Biologically Active Compounds based on Benzopyran-4-ones
Research Supervisor	Dr. M. Muthukrishnan

The proposed thesis is divided into three chapters. The first chapter gives a brief introduction to epoxides and aziridines, their significance in organic synthesis. In addition, facile synthesis of (*R*)-mexiletine, (*R*)-phenoxybenzamine hydrochloride and (*S*)-metolachlor *via* aziridine ring opening is also described in this chapter. Asymmetric synthesis of (*R*)-benzylmorpholine, *R* and *S* enantiomers of bepridil and anti-obesity drug lorcaserin is described in the second chapter. The third chapter deals with the design, synthesis, biological and molecular modeling studies of two series of chromone embedded triazoles and chromone/aza-chromone fused α-aminophosphonates. The details are given below.

Chapter 1: Chiral aziridine ring opening: Facile syntheses of (*R*)-mexiletine, (*R*)-phenoxybenzamine hydrochloride and (*S*)-metolachlor

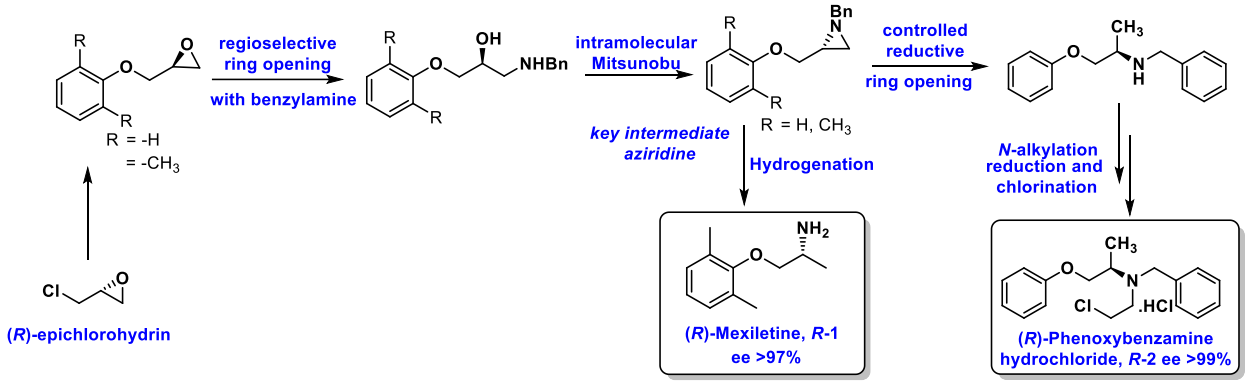
***Section I*:** Introduction to epoxides and aziridines in organic synthesis



Epoxides and aziridines are valuable synthetic intermediates in organic synthesis. Due to its high ring strain and reactivity, they can be ring opened with wide variety of nucleophiles and that 1,2-difunctional ring-opened products represent common motifs in many interesting organic molecules. A brief account of the significant organic transformations utilizing epoxides and aziridines is presented in this section.

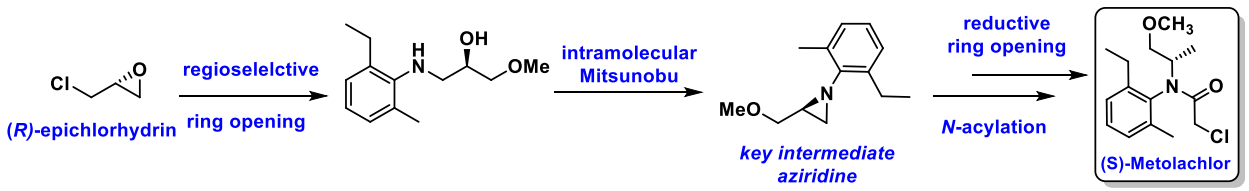
***Section II*:** Facile synthesis of (*R*)-mexiletine and (*R*)-phenoxybenzamine hydrochloride *via* chiral aziridine ring opening

Mexiletine is an important β-amino aryl ether class of anti-arrhythmic drug and phenoxybenzamine hydrochloride (Dibenzyline®) is the β-chloroethylamine class of drug belongs to α-blocker series, widely used in the treatment of hypertension. In this section, concise and efficient synthetic routes developed for the active enantiomers of these two important drugs, employing chiral aziridine as a key intermediate have been described. Simple procedures, readily available starting materials and high enantioselectivity are some of the salient features of this approach.[1,5]



***Section III*:** Efficient synthesis of optically active (*S*)-metolachlor *via* reductive ring opening of aziridine

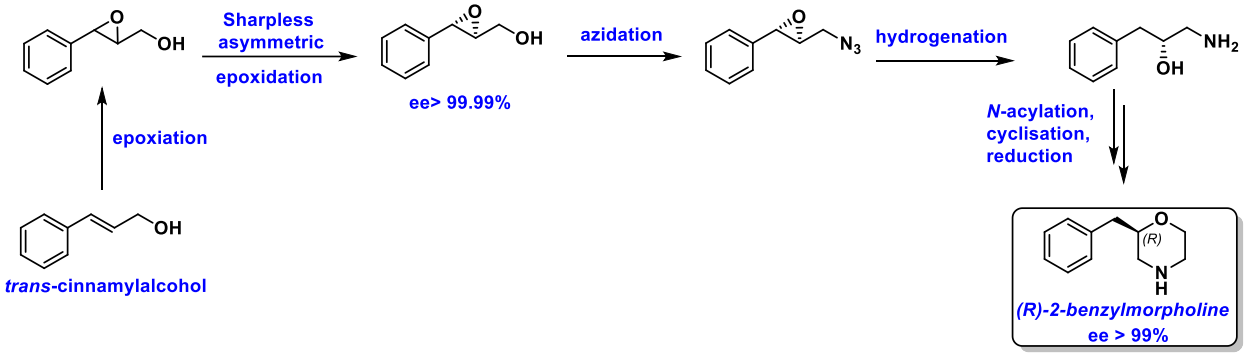
Metolachlor belongs to the class of chloroacetamide herbicide. The high herbicidal activity resides in (*S*)-enantiomer of metolachlor. Some of the drawbacks associated with the previous synthetic routes are low enantioselectivity, protection-deprotection steps and expensive reagents etc. In this section, we describe an efficient synthesis of (*S*)-metolachlor in five steps via reductive ring opening of aziridine.



***Chapter 2*: Asymmetric syntheses of (*R*)-2-benzylmorpholine, both enantiomers of calcium channel blocker bepridil and anti-obesity drug lorcaserin**

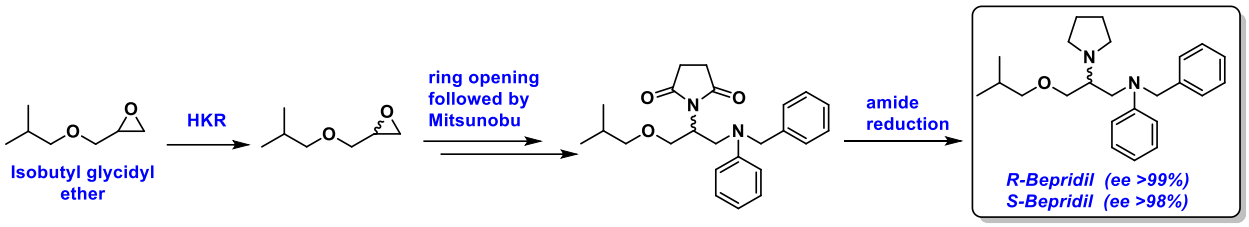
***Section I*:** An enantioselective synthesis of appetite suppressant (*R*)-2-benzylmorpholine employing Sharpless asymmetric epoxidation strategy

C-substituted morpholine analogues, in particular, the non-racemic ones are important structural scaffolds present in many pharmaceutically important compounds (Reboxetine, Viloxazine etc). In that series, (*R*)-2-benzylmorpholine is a classical example, known to be a potent appetite suppressant. Despite their wide utility, synthetic routes to these valuable compounds especially the non-racemic ones are very limited. In this section, we describe an alternate synthesis of (*R*)-2-benzylmorpholine starting from readily available *trans*-cinnamyl alcohol employing Sharpless asymmetric epoxidation strategy.[2]



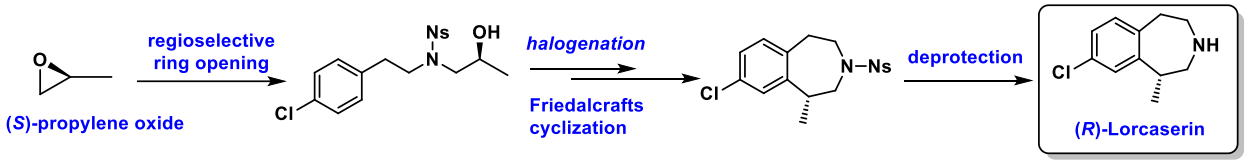
***Section II*:** A new and efficient enantioselective synthesis of both enantiomers of calcium channel blocker bepridil

Bepridil (Trade name: Vascor®) is a long-acting calcium-blocking agent with significant antianginal activity. Pharmacological studies reveal that (*R*)-isomer of bepridil is more active than (*S*)-enantiomer. However, there are no reports on the enantioselective preparation of bepridil enantiomers are available. Recent studies indicate the potential use of bepridil in many new therapeutic indications including anti-ebola virus activity. In this section, the development of a new enantioselective synthetic route to bepridil enantiomers using Jacobsen's hydrolytic kinetic resolution strategy has been described.[3]



***Section III*:** An alternate synthesis of anti-obesity drug lorcaserin

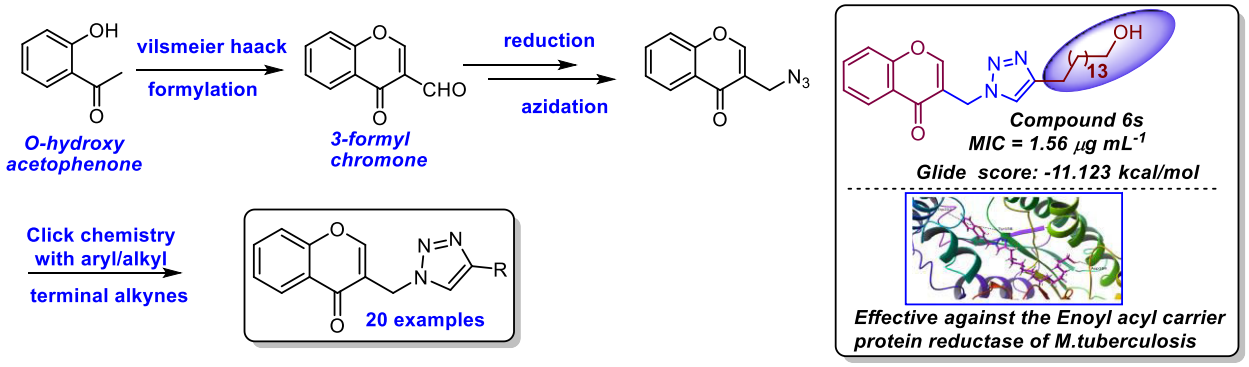
Lorcaserin is a novel anti-obesity drug approved by the FDA in 2012 for the treatment of obesity. Several methods have been reported for the synthesis of lorcaserin and some of the drawbacks associated these methods are complicated workups, expensive and unstable reagents etc. This section illustrates the development of an alternate strategy for the synthesis of lorcaserin from commercially available (*S*)-propylene oxide.[5]



***Chapter 3*: Development of novel biologically active compounds based on benzopyran-4-one motif**

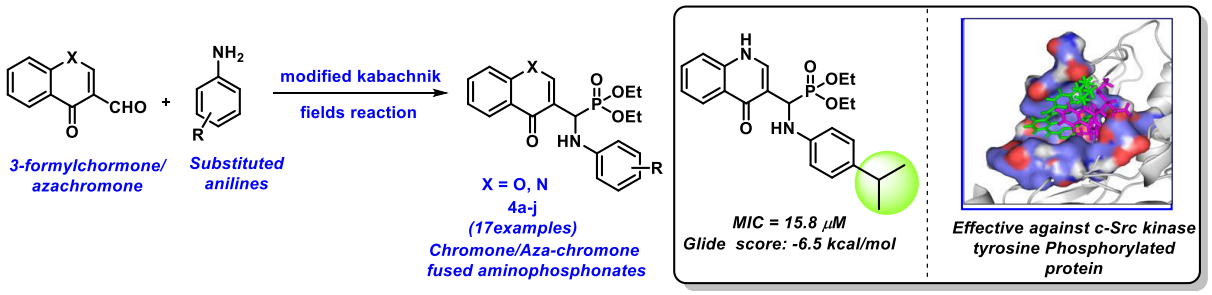
***Section I*:** Synthesis, biological evaluation and molecular modeling studies of novel triazole-chromone conjugates as potent anti-TB agents

Tuberculosis (TB) remains one of the leading contagious diseases caused by bacterial pathogen *Mycobacterium tuberculosis* responsible for high mortality worldwide. Therefore, the discovery and development of effective anti-TB drugs are urgently needed. In this section, synthesis of novel chromone embedded 1,4 disubstituted [1,2,3]-triazole derivatives, followed by their *in vitro* biological evaluation against *Mycobacterium tuberculosis* H37Rv is described. We identified one potent compound with MIC value 1.56 µg mL^{-1}. In addition, molecular docking and chemoinformatics tools were applied to identify the probable molecular target as well drug likeliness of the synthesized compounds.



***Section II*:** Synthesis, biological evaluation and molecular modeling studies of novel chromone/aza-chromone fused α-aminophosphonates as c-Src kinase inhibitors

This section deals with the synthesis of new series of chromone/azachromone fused α-amino phosphonate conjugates and evaluated their c-Src kinase inhibitory activity. Few compounds exhibit moderate inhibition. Further, molecular docking analyses were performed to study the interactions with kinase Src tyrosine protein.



Noteworthy Findings:

- Accomplished a new synthesis of (*R*)-mexiletine, (*R*)-phenoxybenzamine hydrochloride and (*R*)-metolachlor employing aziridine as a key intermediate.
- Developed a simple and efficient synthetic route to (*R*)-benzylmorpholine, (*R*) & (*S*)-bepridil and lorcaserin utilizing epoxide as a valuable chiral synthon.
- Designed and synthesized chromone embedded 1,2,3-triazole conjugates & chromone/aza-chromone based α-aminophosphonates, studied their biological potential against MTB and c-Src kinase protein respectively.

References:

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3. Mujahid, M.; Subramanian, J.; **Viswanadh, N**.; Sasikumar, M.; Kunte, S. S.; Muthukrishnan, M. *New J. Chem.***2017**, *41*, 824.
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CHAPTER 1

Chiral aziridine ring opening: Facile syntheses of (R)-mexiletine, (R)-phenoxybenzamine hydrochloride and (S)-metolachlor

1.1. SECTION 1

Introduction to enantiopure epoxides and aziridines in organic synthesis

1.1.1. Introduction to chirality

Chirality is a fundamental and structural property of three-dimensional structure of an object. The term chirality means "handedness", the two enantiomers or optical isomers of a chiral compound cannot be superimposed which are mirror images to each other.[1] In an achiral environment, a pair of enantiomers of a chiral drug exhibit same physical and chemical properties except that they differ in their interaction with plane polarized light. The mirror image of a pair of enantiomers that rotates the plane polarized light in opposite directions with equal magnitude. Optical activity refers to the ability of a chiral molecule to rotate the plane of polarized light and it can be measured by an instrument known as a polarimeter.[2] If an optically active compound rotates the plane of polarized light to the right (clockwise direction) are said to be dextrorotatory (d) or (+) enantiomer and the same substance that rotates the plane of polarized light to the left (anticlockwise direction) are said to be levorotatory (l) or (-) enantiomer. The D/L (dexter/laevus) notation (Fischer-Rosanoff convention)[3] corresponds to the relative configuration of the molecule, compared with the relative configurations of glyceraldehyde enantiomers as the standard compound mainly applied for assigning the α-amino acids and carbohydrates. The Cahn-Ingold-Prelog (CIP) convention[4] describes the R/S (rectus/sinister) nomenclature to assign the absolute configurations of each chiral center.

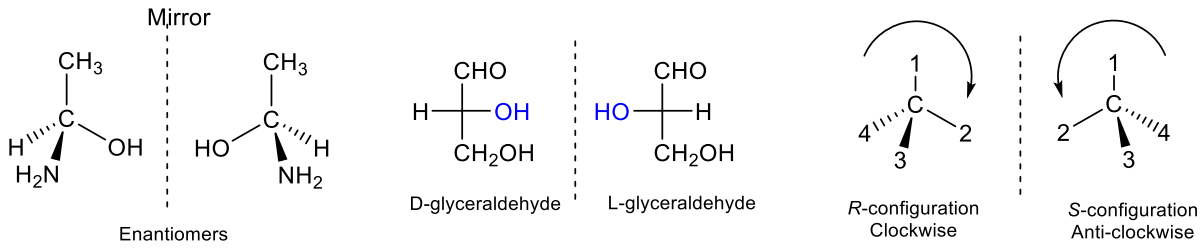


Figure 1. Chiral molecules and its nomenclature

1.1.1.1. Chirality in living systems

Chirality is an important phenomenon and plays a vital role in living systems. Living organisms are composed of chiral biological molecules such as amino acids, sugars, proteins, enzymes, lipids and nucleic acids. In nature, these biomolecules exist in only one of the two possible enantiomeric forms e.g., amino acids in proteins are configurated only in L-form while sugars in DNA and RNA are configurated in D-form.[5] In a chiral environment (i.e. chiral living systems), one enantiomer of a chiral drug may have different pharmacokinetics, pharmacodynamics and toxicity responses over the other enantiomer.[6] Thus, one enantiomeric form of a chiral drug may have desired therapeutic activity (eutomer), while the other isomer (distomer) may be inactive or less active or adverse or toxic effect, or may give rise to an entirely different pharmacological response.[7]

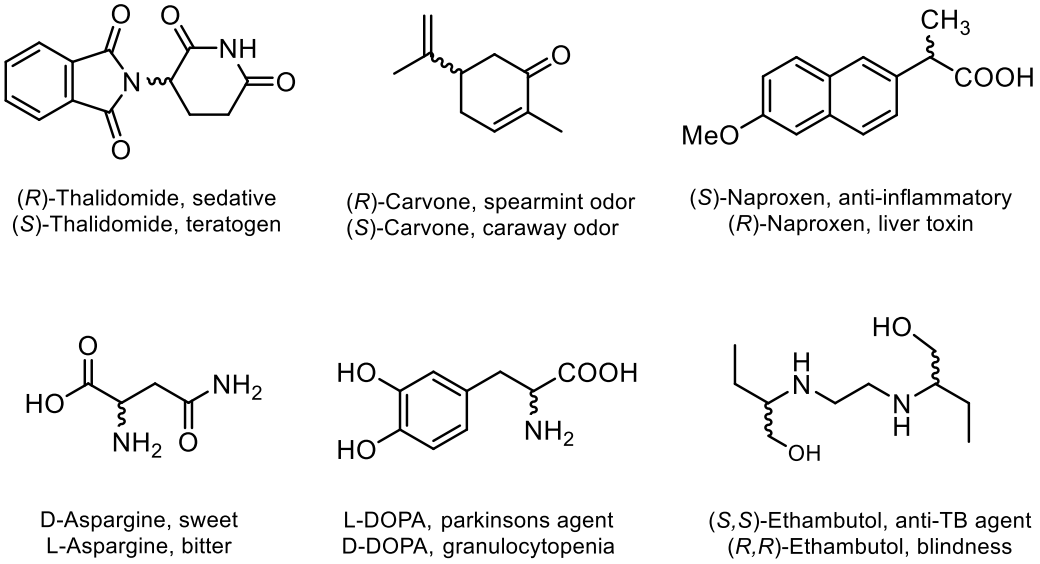


Figure 2. Chiral drugs with different biological activity

According to the receptor theory, enantiomer of the chiral drugs specifically binds to the biologically active sites such as proteins (enzymes, receptors), nucleic acids (DNA, RNA) and biomembranes (phospholipids, glycolipids, sterols). The pharmacological activity of drugs mainly depends on their interaction between the enantiomers of a drug molecule and a biologically active site. All these receptor binding sites composed of homochiral biomolecules having three-dimensional complex structures preferably interacts with only one of the enantiomeric form of a chiral drug.[8] In 1957, a drug namely thalidomide has been introduced into the market as a racemate for treating morning sickness

and nausea in pregnant women. Unfortunately, due to the remarkable side-effects of this drug, more than 10,000 babies were born with missing or abnormal arms, hands, legs, or feet. The reason is, only (*R*)-enantiomer of thalidomide possessed the desired therapeutic activity, whereas (*S*)-thalidomide caused severe birth defects such as a mutation in the fetus.[9] Due to the differential, the biological and pharmacological behavior of two enantiomers and the adverse side effects associated with the non-functional enantiomer in the racemic drug, the stereochemistry in chiral drugs is a critically important issue for the pharmaceutical industries as well as the regulatory authorities. The regulatory authorities United States Food and Drug Administration (US FDA) in 1992 and European Union agency (EU) in 1994 have officially announced a policy statement concerning the development of stereoisomeric drugs, which favor the development of single-enantiomers over the racemates.[10]

1.1.1.2. Chirality in pharmaceuticals

In 1990's, most of the chiral synthetic drugs introduced into the market were racemates, only 12% of the chiral drugs were single enantiomers.[11] After the enforcement of new FDA's marketing guidelines on chiral drugs, there was a sharp decrease in the market of racemic drugs.[12] In 2004, out of 16 newly approved drugs, 13 synthetic drugs were marketed as a single enantiomer and rest of the 3 drugs were racemates. In 2007, 70% of the FDA approved drugs of the newly launched synthetic drugs were single enantiomers. Currently, a single-enantiomer product is a major area of concern in the modern pharmaceutical research.[13] Many drugs that have been approved as racemates are being re-evaluated and re-marketed as single-enantiomers. Therefore, the production of a single-enantiomer of a chiral drug has become an intense research area.

1.1.1.3. Methods for the preparation of enantiopure compounds[14]

The classical methods for the synthesis of enantiomerically pure compounds are mainly categorized into three groups:

1) Resolution of racemates
2) Chiral pool approach
3) Asymmetric synthesis

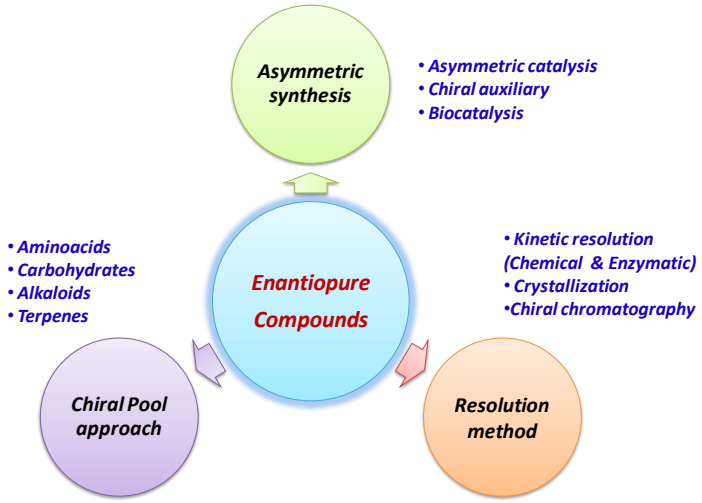
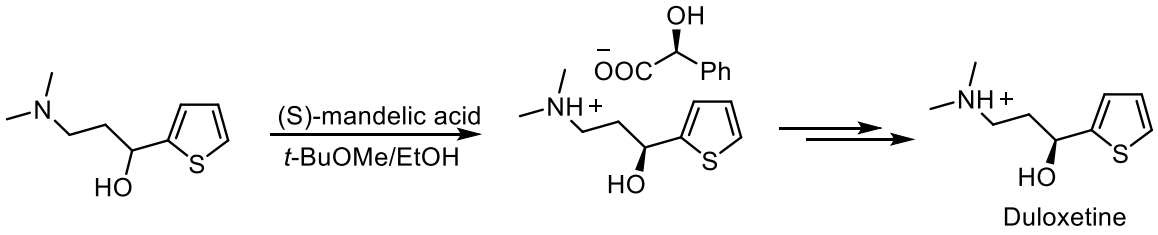


Figure 3. Various methods for the synthesis of enantiopure compounds.

1) Resolution of racemates: For the preparation of enantiopure compounds, one of the oldest and widely used protocols is the resolution of racemates in which resolution is performed at the end of a racemic reaction sequence with the help of a chiral compound. As in most of the cases, only one enantiomer is useful, half of the synthetic product is often discarded. Further, this method requires an equimolar amount of an enantiomerically pure compound which cannot be reused or recycled. But, still, this method is widely used in industries. For example, synthesis of antidepressant drug duloxetine was carried out *via* resolution method using (*S*)-mandelic acid (**Scheme 1**).[15]



Scheme 1. Synthesis of duloxetine *via* resolution method

2) Chiral pool approach: The term "chiral pool" refers to the many naturally occurring chiral building blocks with a high degree of enantiomeric purity. Chiral pool approach is one of the most economical and scalable methods to synthesize the enantiomerically pure intermediate compounds from the inexpensive chiral precursors derived from natural sources. Commonly used naturally occurring enantiopure starting compounds includes *α*-

amino acids, carbohydrates, alkaloids, α-hydroxy acids, terpenes etc.,[16] In this approach, these enantiopure starting compounds are incorporated into the molecule or manipulated using an achiral reagent retaining its chirality to provide the desired target molecule during the course of successive synthetic sequences. The most significant considerations for the chiral pool approach are: (i) the cost and availability of the enantiopure starting materials; (ii) Whether accessible to both enantiomers; (iii) Whether racemization will occur while manipulation or introduction of the chiral center during the synthetic steps.

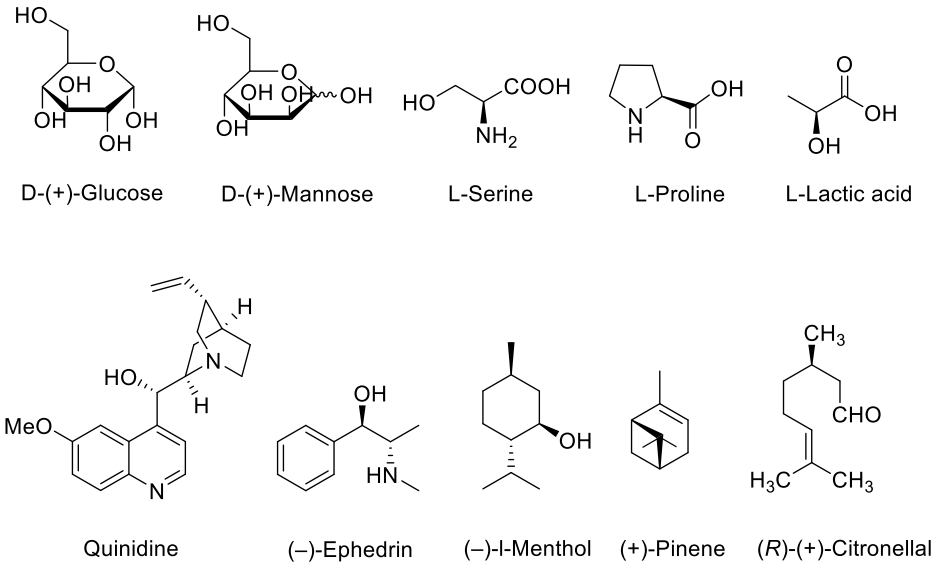


Figure 4. Examples of naturally occurring chiral building blocks

3) Asymmetric synthesis: Asymmetric synthesis (enantioselective synthesis or stereoselective synthesis) is the synthesis of enantiopure compounds from the prochiral or achiral substrates in the presence of a chiral entity (chiral catalyst, chiral auxiliary, chiral solvent). It can be catalyzed by a chemocatalyst or a biocatalyst. It has emerged as a powerful and widely employed method for the effective preparation of enantioenriched biologically active compounds. In an asymmetric reaction, the prochiral substrate combines with chiral entity results in the formation of two diastereomeric transition states. These asymmetric reactions can be categorized into four methods, according to how the chiral induction is exerted.[17]

a) First generation asymmetric synthesis (substrate-controlled diastereoselectivity).

b) Second generation asymmetric synthesis (auxiliary-controlled diastereoselectivity).

c) Third generation asymmetric synthesis (reagent-controlled diastereoselectivity).
d) Fourth generation asymmetric synthesis (catalyst-controlled diastereoselectivity).

a) Substrate-controlled asymmetric synthesis: In these reactions, the new chiral center is formed by the reaction of the chiral substrate with the achiral reagent at a diastereotopic center controlled intramolecularly by an adjacent chiral center which already exists in the chiral substrate.

b) Auxiliary-controlled asymmetric synthesis: The formation of the new stereogenic center by reaction of the prochiral substrate with the chiral auxiliary *via* diastereoselective reaction. The asymmetric induction is controlled by the chiral auxiliary in the substrate intramolecularly. This method is similar to the first generation method but the only difference is the extra two steps in the synthesis, is the attachment and removal of the chiral auxiliary in the later stage. The requirements of the chiral auxiliary are: easily available in the pure enantio-enriched form, can be easily attached to the functional group in the prochiral substrate, induce excellent stereocontrol, can be easily removable of chiral auxiliary and preferably recycled.

c) Reagent-controlled asymmetric synthesis: The direct formation of enantiopure products from the prochiral substrate in the presence of the chiral reagent. The newly formed chiral center is achieved by the reagent control and stereoselectivity is controlled by the structure and chirality of the chiral reagent used. In contrast to the above two methods, the chiral induction is not the part of the substrate and the stereocontrol is achieved intermolecularly in this method.

d) Catalyst-controlled asymmetric synthesis: The above three methods require enantiomerically pure compounds in stoichiometric quantities which lowers the efficiency of the above methods. To overcome this drawback, the most important development in asymmetric synthesis in the last few decades was the introduction of chiral catalysts to produce an enantiomerically enriched product from the prochiral substrate. In this method, the prochiral substrate is transformed into an enantiomerically pure compound in the presence of catalytic amounts of chiral catalyst in a single step.

Asymmetric catalysis: A chiral catalyst promotes selective conversion of one enantiomer or formation of one enantiomer over the other enantiomer. In this method, catalytic amounts of chiral catalysts are required to provide large quantities of enantiomerically enriched product. The chiral catalyst may be biocatalyst (enzymes) or chemocatalyst (small organic molecules) or metal catalyst (metal complexes). The major breakthrough in asymmetric catalysis from the pioneering works of three eminent scientists William S. Knowles, R. Noyori and K Barry Sharpless shared the Nobel Prize in Chemistry based on the development of asymmetric catalyzed hydrogenation and oxidation reactions in the production of single enantiomer drugs or chemicals in 2001. Enantioselective catalyzed reactions such as asymmetric hydrogenation, asymmetric epoxidation, asymmetric dihydroxylation, asymmetric aldol reaction, asymmetric Diels-Alder reactions and asymmetric ring opening reactions have become very important tools in the production of enantiomerically pure pharmaceuticals and chemicals.[18] These diverse asymmetric catalytic reactions provide access to wide variety of products with new chiral C-C, C-H, C-N, C-O and C-S bond forming reactions.

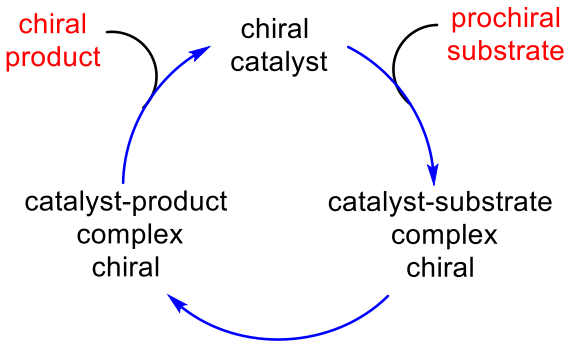


Figure 5. Schematic representation of enantioselective synthesis using a chiral catalyst

1.1.2. Epoxides in organic synthesis

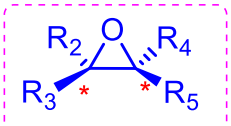


Fig 6. Epoxide

Epoxide or oxirane is a three-membered strained oxygen-containing heterocyclic compound (**Fig. 6**).[19] Due to its high Baeyer ring strain (27 kcal/mol) and induced a partial positive charge on carbon atoms due to the electronegative oxygen atom, these serve as reactive electrophiles. These are highly reactive and it can easily undergo nucleophilic ring opening reactions with a wide range of nucleophiles (carbon, nitrogen, hydrogen, oxygen, halogen, and sulfur) to access

many 1,2-disubstituted or defunctionalized ring-opened products in highly regio- and stereoselective manner.[20] In particular, enantiopure epoxides are versatile synthetic intermediates in pharmaceutical and natural product synthesis.[21] In addition, these strained epoxide moieties are found in a number of biologically significant natural products.

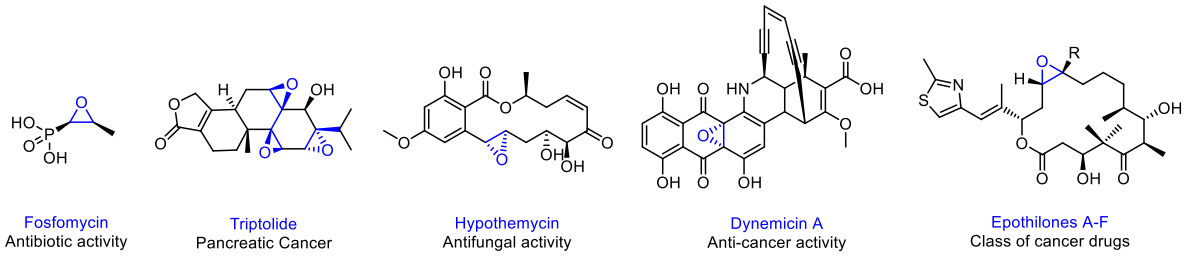
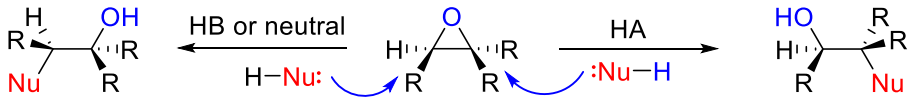


Figure 6. Examples of few natural products possessing epoxide ring

1.1.2.1. Reactivity of epoxides

Epoxides participate in nucleophilic ring opening reactions under acidic, basic or neutral conditions. Under these conditions, non-symmetrical epoxides provide different products. In general, the nucleophile approaches from the rear side of the epoxide carbon resulting in the inversion of configuration at the electrophilic center. In acid-catalyzed nucleophilic ring opening reactions, the nucleophile attacks predominantly at the sterically more hindered carbon due to the formation of stable protonated transition state supported by the borderline S_N2 mechanism. In case of basic or neutral medium, the attack of nucleophile predominantly at the sterically less substituted carbon through S_N2 type mechanism (**Scheme 2**).



Scheme 2. Ring opening of epoxides under acid and base-catalyzed reaction conditions.

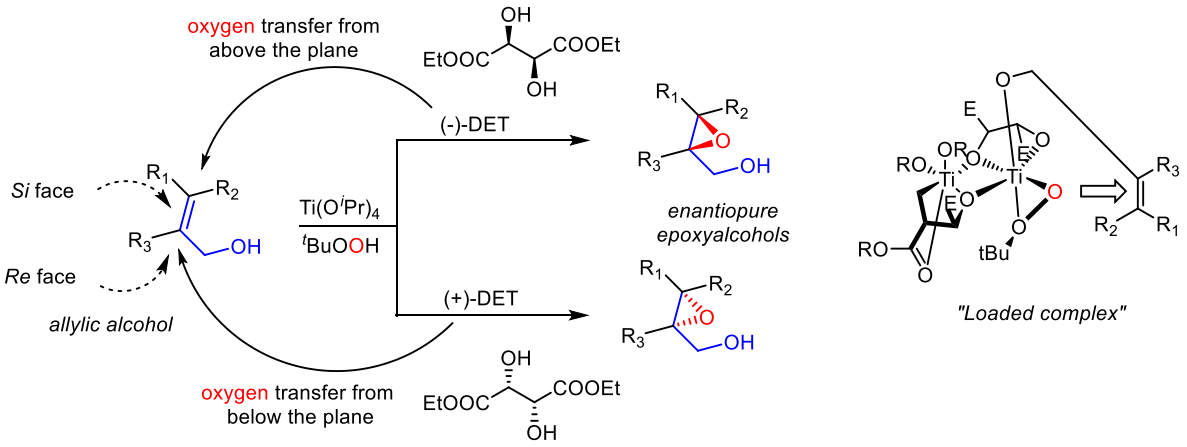
Importantly, enantiopure epoxides are powerful synthons for the synthesis of many natural products as well as pharmaceutically important compounds. Due to their immense utility in organic synthesis, the preparation of enantiopure epoxides has been of great interest to organic chemists.

1.1.2.2. Important synthetic methods to enantiopure epoxides

In the last three decades, several enantioselective strategies have been developed for the preparation of enantiopure epoxides, however, only a few of them are of significant value and lead to the desired enantiomers in high yields with excellent enantiomeric excess. Currently, direct asymmetric epoxidation of alkenes and kinetic resolution of racemic epoxides are the most widely employed chemical methods for the preparation of enantiopure epoxides.

a) Sharpless Asymmetric Epoxidation

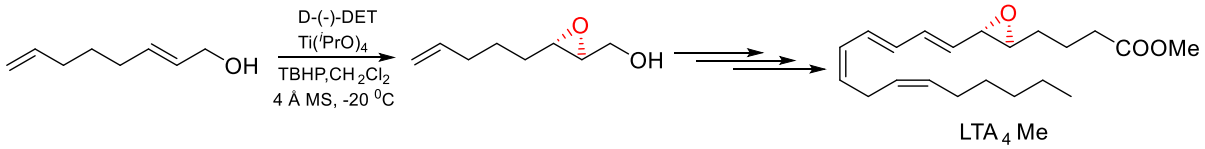
In 1980, Sharpless and co-workers reported the enantioselective epoxidation of alkenes popularly known as the Sharpless-Katsuki asymmetric epoxidation reaction.[22] In this asymmetric transformation, the allylic alcohols are transformed into their corresponding enantiopure epoxyalcohols using titanium isopropoxide [$Ti^{(IV)}(O^iPr)_4$] as the catalyst, *tert*-butyl hydroperoxide (TBHP) as the terminal oxidant and chiral tartrate as the chiral ligand (**Scheme 3**). In this reaction, the epoxy alcohols are obtained in high yields with excellent enantiomeric excesses. These reactions are performed at low temperature (-20^{0}C) and at inert atmosphere using anhydrous CH_2Cl_2 as a common solvent.



Scheme 3. Sharpless asymmetric epoxidation of allylic alcohols

Mechanistically, the titanium complex and desired tartrate ligand formed the titanium-tartrate complex. After addition of TBHP oxidant, the titanium-tartrate complex exchanges the ligands by displacement of one of the tartrate carbonyl group as well as an isopropoxide ligand. Finally, the diacetal oxygen atom from TBHP oxidant coordinates to

the titanium-tartrate complex activates the peroxide and facilitates the intramolecular transfer of peroxide. The hydroxyl group of allylic alcohol coordinates to the axial site of the titanium complex, therefore, the olefin bond is activated and facilitates for intramolecular oxygen transfer leads to complete stereoselectivity control. Sharpless developed the empirical rule to predict the absolute configuration of the resulting epoxyalcohol. The mechanism of the oxygen atom deliver depends upon the tartrate ligand used. When (*S, S*)-(-)-tartrate ligand is used as a chiral ligand, the oxygen atom is transferred from "above" the plane of allylic alcohol where (*R, R*)-(+)-tartrate ligand is used the oxygen atom is transferred from "below" the plane of allylic alcohol. This catalytic asymmetric reaction is an effective robust method and it can be performed with highly functionalized allylic alcohols. Sharpless asymmetric epoxidation reaction serves as a powerful tool in the key steps for the total synthesis of many natural products and biologically active molecules.[23] For example, Spur *et al.* have utilized the Sharpless asymmetric epoxidation to generate key stereochemistry and functionality in the total synthesis of leukotrienes LTA_4 methyl ester (**Scheme 4**).[24]

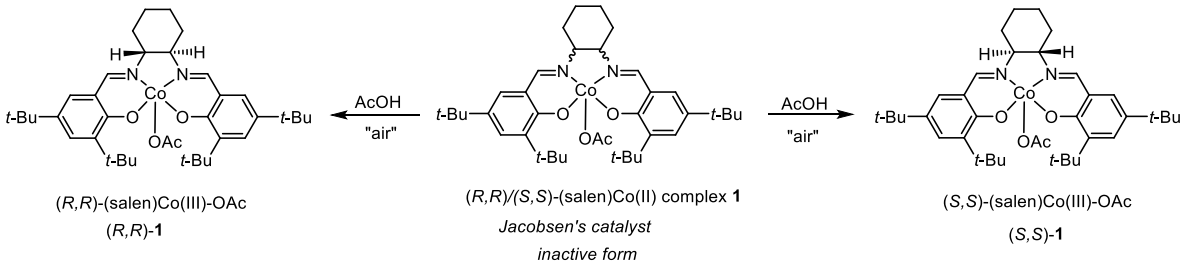


Scheme 4. Total synthesis of leukotriene LTA_4 methyl ester

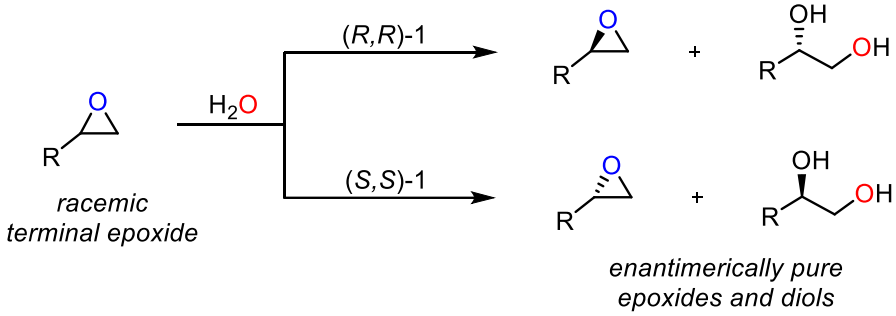
b) Hydrolytic Kinetic Resolution (HKR)

Kinetic resolution involves resolution of a racemic mixture into its enantiomers based on the differential rates of reaction of the enantiomers of a racemate towards a chiral catalyst or reagent. Hydrolytic kinetic resolution (HKR) is an efficient protocol to resolve the variety of terminal racemic epoxides using metal-salen complexes as a chiral catalyst. In 1997, Jacobsen group[25] developed an effective method for the preparation of enantio-enriched terminal epoxides along with enantiopure vicinal diols from its corresponding racemic terminal epoxides using Co(III)-(Salen)-OAc {(*R, R*)-**1** & (*S, S*)-**1**} catalyst and water as the nucleophile. The commercially available inactive Co(II)-(Salen)complex catalyst converted into the corresponding Co(III)-(salen)-OAc precatalyst by aerobic oxidation with acetic acid at room temperature (**Scheme 5**). In this method, the selective hydrolysis of one of the enantiomer of the racemic epoxide leads to the enantiomeric 1,2-

diol, while the opposite enantiomer remains as unhydrolyzed epoxide in pure enantiomeric form (**Scheme 6**).



Scheme 5. Aerobic oxidation of Jacobsen's catalyst provides Co(III)-Salen precatalyst



Scheme 6. Co-catalyzed hydrolytic kinetic resolution of racemic terminal epoxide

The salient features of HKR strategy includes: Commercial availability of both cobalt salen catalysts {(*R*,*R*)/(*S*,*S*)-Co(II)-(salen)complex **1**}, readily available racemic epoxides, low catalytic loading (0.2 to 2 mol%), safe and inexpensive reagent (0.55 equiv H_2O), mild reaction conditions (RT), both products obtained in high enantiopurity (≥98% ee), easy isolation of the products due to large difference in the boiling point and polarity and applicable to large scale production. Since its discovery, HKR has been successfully utilized for the synthesis of complex biologically active natural products and pharmaceuticals.[26]

1.1.2.3. Nucleophilic ring opening reactions of epoxides

Epoxides have been extensively used as versatile synthetic precursors that serve as "reactive electrophiles" for nucleophilic ring-opening reactions. Despite the importance of reactivity of strained ring, epoxides undergo synthetically useful transformations with a wide range of nucleophiles to provide a variety of oxygen-containing 1,2-functionalized

compounds (**Figure 6**).[27] The regioselectivity of the ring opening reactions depends on several factors such as reaction conditions, nature of nucleophile and the nature of the substituent in the epoxide.

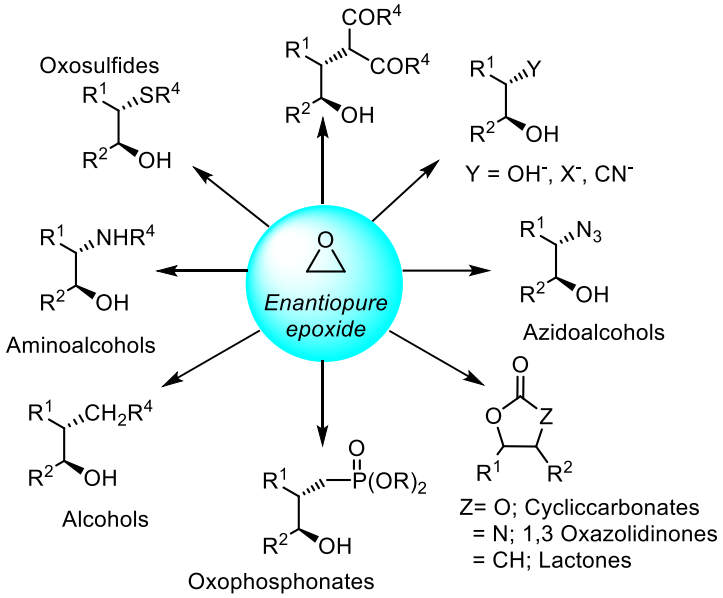


Figure 6. Nucleophilic ring opening reactions of epoxides

The ring opening reactions and rearrangements of epoxides have provided efficient ways to generate molecular complexity during the synthesis of many biologically active molecules (**Figure 7**).

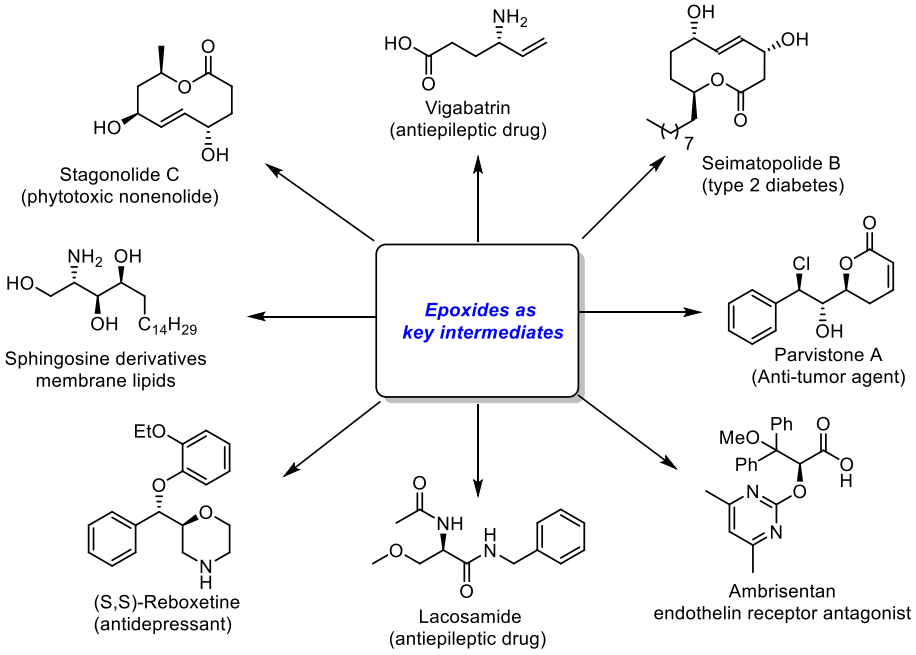


Figure 7. Synthesis of various bioactive molecules *via* an epoxide intermediate

1.1.3. Aziridines in organic synthesis

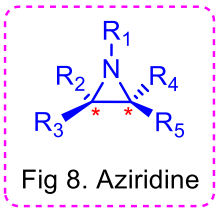


Fig 8. Aziridine

Like epoxides, aziridines[28] are also valuable three-membered nitrogen-containing heterocyclic compounds. Due to its high ring strain (26.7 kcal/mol) and reactivity, these are highly prone to undergo regio- and stereoselective ring opening reactions[29] with a wide variety of carbon and heteroatom nucleophiles, there as to allow access towards various functionalized nitrogen-containing compounds like amino alcohols, amino acids etc.,[29] In particular, enantiopure aziridines (**Fig. 8**) are used as important chiral building blocks, chiral auxiliaries and chiral ligands in asymmetric synthesis.[30] Growing synthetic accessibility of chiral aziridines has propelled their use in ring opening reactions in organic synthesis.[31] In addition, the biological activity of aziridines lies in their property as powerful alkylating agents. The aziridine functionality is also present in many naturally occurring molecules such as azinomycins, mitomycins, FR-900482, ficellomycin, miraziridine, maduropeptin, and azicemicins are of significant interest (**Figure 9**).[32]

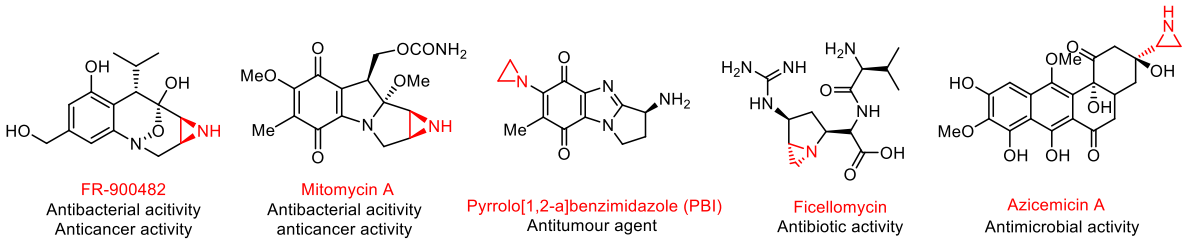


Figure 9. Representative natural products possessing aziridine ring moiety

1.1.3.1. Reactivity of aziridines

Aziridines are reactive and versatile substrate because of certain inherent features within their structure. These include high ring strain, a reactive π bond, a lone pair of electrons on the nitrogen, and the ability to undergo ring cleavage reactions. The more S-character of the lone pair on nitrogen results in the lower basicity than the acyclic aliphatic amines which reduce the ability of π-character. To release the strain of the ring these are participating in the ring opening reactions. The pyramidal inversion of the nitrogen atom in aziridine (8-12 kcal/mol) is higher than the open chain amines. In aziridine, due to increase in ring strain makes to prevent the inversion of nitrogen (**Figure 10**).[33]

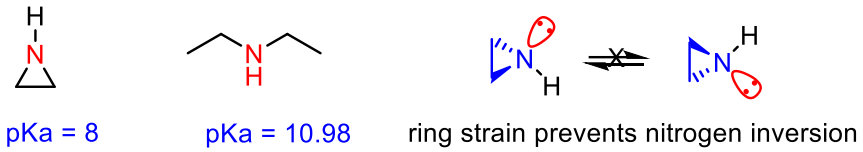


Figure 10. Basicity and pyramidal inversion of the nitrogen atom of aziridine

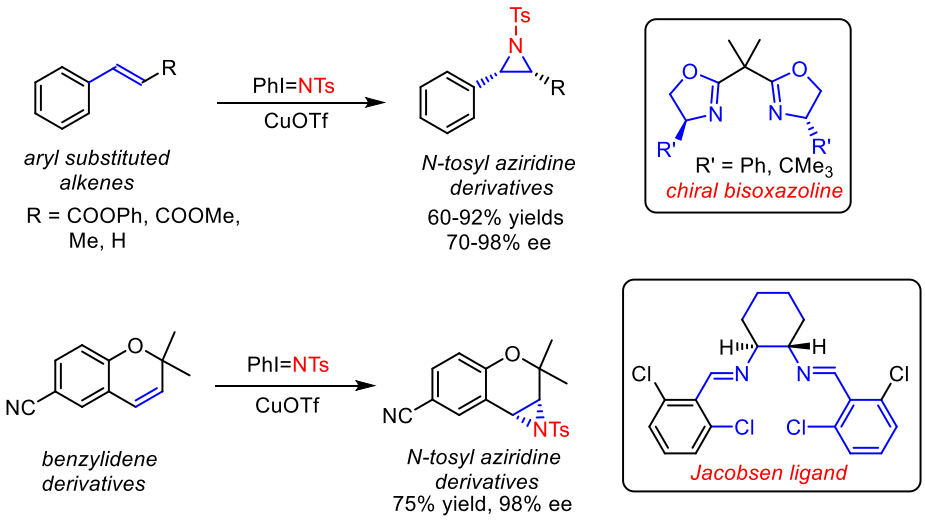
Due to the lower electronegativity of nitrogen compared to oxygen, ring-opening reactions of aziridines are less facile than the opening of the corresponding epoxides. The main and characteristic property of aziridine is their high reactivity towards a variety of nucleophilic reagents either by employing acidic catalysis or by electron withdrawing group on nitrogen atom to activate the aziridine ring.[34] Depending upon the nature of the substituent on nitrogen atom, aziridines are classified as "activated" and "non-activated" aziridines. Activated aziridines have an electron-withdrawing substituent on nitrogen during nucleophilic ring-opening capable of stabilizing the negative charge that develops on the nitrogen atom. Non-activated aziridines contain a basic nitrogen atom or an *N*-alkylated or *N*-arylated substituent and nucleophilic ring-opening reactions usually take place under acid catalysis (Lewis or Bronsted acids).[35]

1.1.3.2. Synthetic methods to enantiopure aziridines

Over the past few decades, numerous synthetic routes have been developed for the preparation of aziridines. However, most of the traditional methods are the insertion of nitrogen source to olefins, transfer of a suitable carbon source to imines, and intramolecular cyclization of vicinal amino derivatives. The asymmetric strategy is the important tool in the synthesis of enantiopure aziridines which are useful precursors in the synthesis of various nitrogen-containing biologically important molecules *via* ring opening transformations.[36] Chiral aziridines can be prepared from other chiral pool starting materials such as amino acids, carbohydrates, and hydroxy acids. In general, methods such as asymmetric aziridination of olefins or imines in the presence of chiral catalysts/chiral auxiliaries, or asymmetric synthesis of aziridines starting from enantiopure epoxides, amino alcohols and diols has been widely employed.[37] Only selected methods are described below.

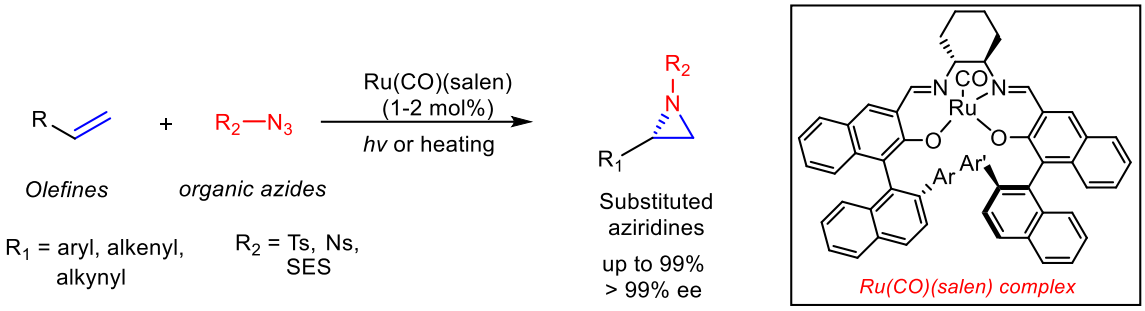
a) Metal-catalyzed asymmetric aziridination

The insertion of nitrene to olefin is the most popular methods to synthesize aziridines due to easy availability of olefins starting materials. In the recent years, several enantioselective methodologies have been developed towards the synthesis of many natural products and bioactive molecules.[38] Jacobsen[39] and Evans[40] independently reported the catalytic asymmetric aziridination of olefins for direct conversion of alkenes to an aziridine. In both the methods, the addition of nitrene source (PhI=NTs; *N*-(p-toluenesulfonyl)-iminophenyliodinane) to the aryl-substituted alkenes are catalyzed by copper (I) triflate. Evans employed the enantioselective aziridination of aryl substituted alkenes utilizing chiral 4-4'-disubstituted bis-oxazolinones as a chiral ligand. Jacobsen has also been successfully employed the asymmetric aziridination of benzylidene derivatives using Salen complex as a chiral catalyst. In these protocols, the *N*-tosyl-aziridines were obtained in moderate to excellent yields with enantioselectivity up to 97% ee (**Scheme 7**).



Scheme 7. Catalytic asymmetric aziridination of olefins using a copper catalyst

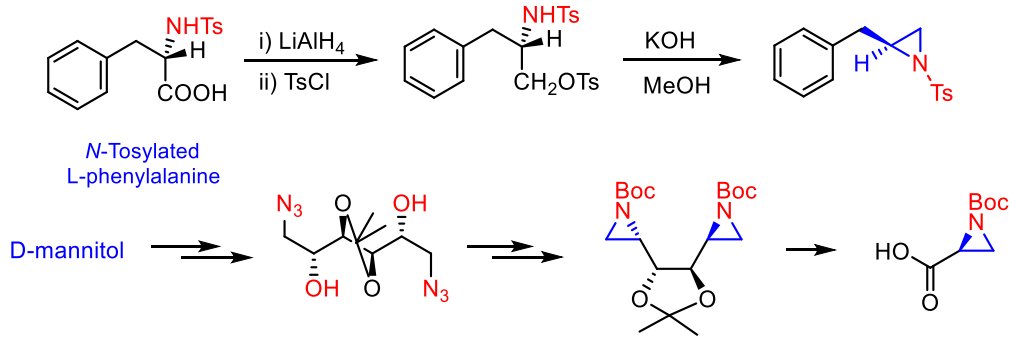
Katsuki and co-workers[41] have been reported the catalytic asymmetric aziridination of olefins using organic azide as a nitrene source and chiral Ru-Salen(CO) complexes as a chiral catalyst. In this reaction, nitrene was generated either by induction of UV irradiation or heating. Under these conditions, numbers of various olefins are transformed to corresponding N-substituted aziridines with high enantiopurities (>99% ee) excellent yields (up to 99%) (**Scheme 8**).



Scheme 8. Asymmetric aziridination of olefins using chiral Ru-Salen (CO) complexes

b) From chiral pool starting material[42]

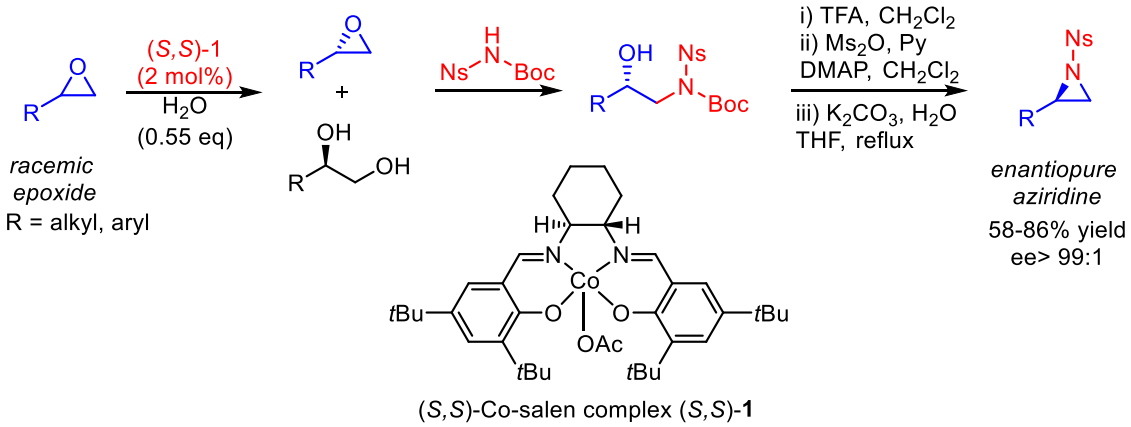
Naturally occurring amino acids and carbohydrates are used as chiral starting materials for the preparation of enantiopure aziridines (**Scheme 9**). Using this strategy, the synthesis of enantiopure aziridines are limited due to the restricted availability of suitable chiral starting materials and it requires multistep synthesis.



Scheme 9. Enantiopure aziridines from amino acids and carbohydrates

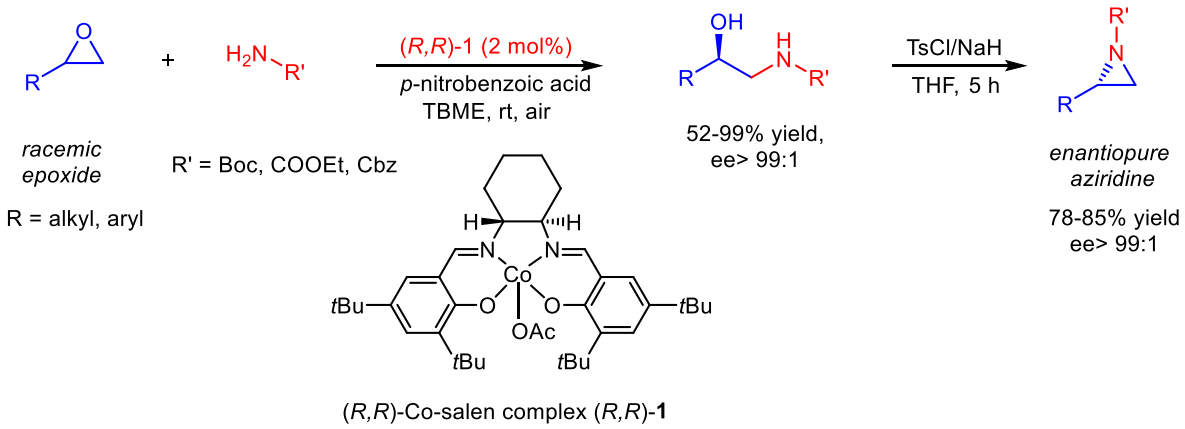
c) Asymmetric hydrolytic and aminolytic kinetic resolution (HKR & AKR)

A large number of highly enantiopure aliphatic and aromatic terminal aziridines are synthesized from the wide range of inexpensive racemic terminal epoxides *via* hydrolytic kinetic resolution and aminolytic kinetic resolution by using water or a protected amine as a nucleophile using Jacobsen's chiral Co^{III}-Salen complex as a catalyst. Jacobsen *et.al.* have developed the hydrolytic kinetic resolution strategy using carbamates as a nucleophile and the chiral Co^{III}-Salen complex as the catalyst to produce *N*-protected amino alcohol derivatives followed by intramolecular ring closure reaction afforded the enantiopure aziridine in high enantiopurity (**Scheme 10**).[43]



Scheme 10. Hydrolytic kinetic resolution of terminal epoxides to enantiopure aziridines

In 2004, Bartoli and co-workers has utilized Jacobsen's Co^{III}-Salen-catalyzed asymmetric aminolytic kinetic resolution (AKR) of racemic terminal epoxides with *N*-protected amines results in enantiopure *N*-protected 1,2-amino alcohols followed by intramolecular ring closure of 1,2 amino alcohol derivative to produce *N*-protected aziridine derivatives in moderate to good yields with high enantiopurity (>99% ee) (**Scheme 11**).[44]



Scheme 11. Aminolytic kinetic resolution of terminal epoxides to enantiopure aziridines

1.1.3.3. Nucleophilic ring opening reactions of aziridines

Nucleophilic ring opening reactions are the most characteristic reactions of aziridines. Like epoxides, aziridines are facile to stereo- and regioselective nucleophilic ring opening reactions to release ring strain, provides various nitrogen-containing 1,2-difunctional enantiopure products (**Figure 11**).[45]

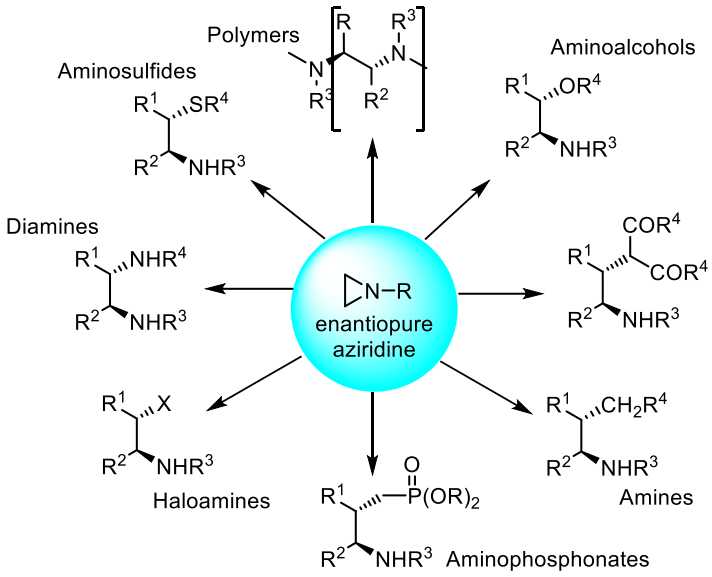


Figure 11. Nucleophilic ring opening reactions of aziridine

Various methods have been reported for the nucleophilic ring opening reactions of aziridines with a wide range of heteroatom nucleophiles such as carbon-centered, nitrogen-centered, halogen centered, sulfur centered nucleophiles and with organometallic reagents and reducing agents. These are also capable of participating in cycloaddition reactions, polymerization and rearrangement reactions. The regioselectivity of nucleophilic ring opening reactions of aziridine depends upon the reaction conditions and the nature of substituent on the nitrogen atom of an aziridine.[46]

Aziridines serves as versatile intermediates which undergo a variety of synthetic transformations. Over the years, many research groups have developed various methodologies to generate highly valuable enantiopure aziridines and utilized them successfully for the synthesis of many pharmaceutically important drugs and biologically active natural products (**Figure 12**).[47]

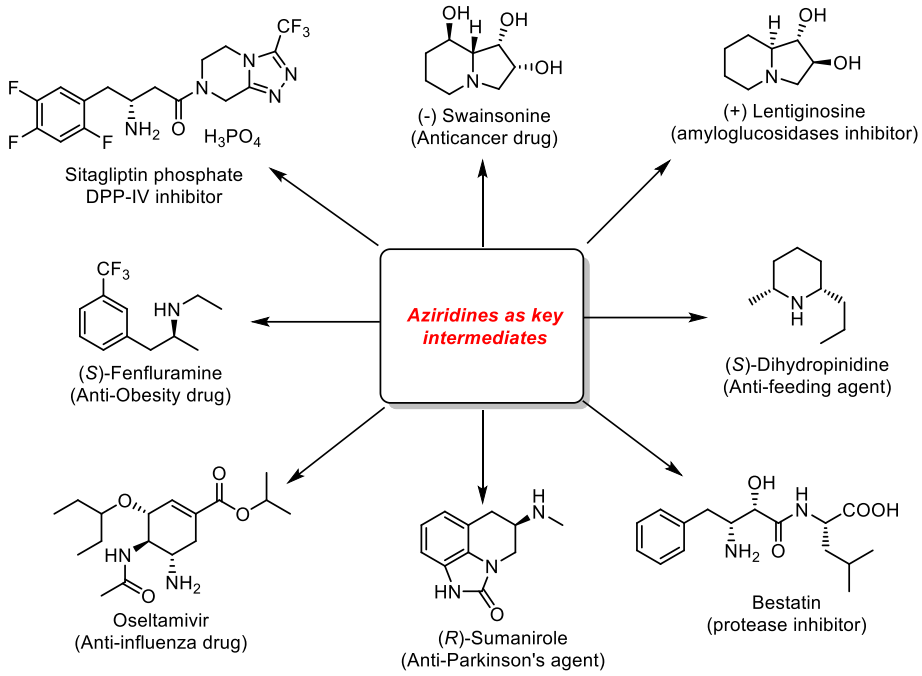


Figure 12. Synthesis of various bioactive molecules *via* aziridine intermediate

Thus, chiral epoxides and aziridines are an important starting point for the preparation of a plethora of biologically active natural products as well as pharmaceutically important compounds.

1.1.4. References

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1.2. SECTION 2

Facile synthesis of (*R*)-mexiletine and (*R*)-phenoxybenzamine hydrochloride *via* chiral aziridine ring opening

1.2.1. Introduction

Synthesis of compounds in their enantio-enriched form became very important in the marketplace, especially in the pharmaceutical sector.[1] This is mainly because; the enantiomers of chiral drugs often exhibit significantly different pharmacological, toxicological, pharmacodynamic and pharmacokinetic properties.[2] Hence the development of newer methods aiming the synthesis of active enantiomer or both isomers (for careful evaluation of individual enantiomers) of chiral drugs is a main focus of research in many academic and industrial laboratories.[3] In this context, optically pure amines are prevalent and versatile building blocks in biologically active compounds such as pharmaceutical drugs, several natural products, and agrochemical active ingredients (**Figure 1**). These are also incorporated as chiral ligands, resolving agents and chiral auxiliaries or chiral bases in many asymmetric transformations and also serves as valuable precursors in the synthesis of many biologically active compounds. Hence, the development of novel and efficient synthetic strategies to access this kind of enantiopure compounds are in high demand.

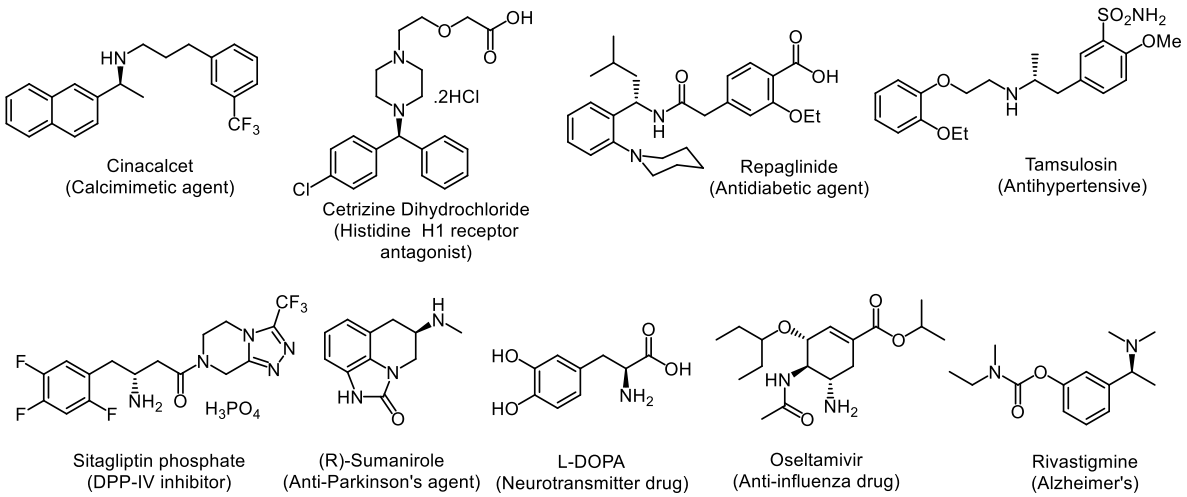
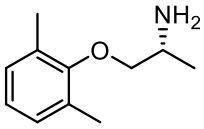
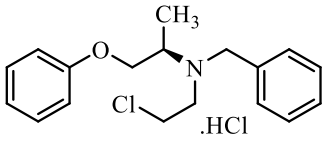


Figure 1. Examples of pharmaceutically significant chiral amines

Numerous methods have been established to obtain enantiopure compounds employing resolution methods, chiral pool approaches, and asymmetric synthesis.[4] In the last two decades, asymmetric synthesis has proven attractive and efficient methods to access optically pure compounds.[5] In this context, optically pure epoxides and aziridines are considered as an important chiral non-racemic precursor that can be readily transformed into valuable enantiomerically enriched building blocks such as amines, amino alcohols and amino acids etc.,[6] Aziridines, in particular, enantiopure aziridines are versatile chiral building blocks or intermediates in organic synthesis for the preparation of various nitrogen-containing natural products as well as biologically significant compounds. Due to its high ring strain (26.7 kcal/mol), and versatile reactivity, they can easily undergo regio- and stereoselective ring opening under mild conditions to provide useful amino derivatives.[7] This inherent ability of aziridines has been well studied in the case of natural product synthesis, however its potential utility in the preparation of chiral drug molecules are scarce. In this section, the development of new and efficient synthetic routes to (*R*)-mexiletine *R*-**1** and (*R*)-phenoxybenzamine hydrochloride *R*-**2** using chiral aziridine as a key intermediate has been described (**Figure 2**).



R-Mexiletine *R*-**1**
antiarrhythmic



R-Phenoxybenzamine. HCl *R*-**2**
antihypertensive

Figure 2. Structure of (*R*)-mexiletine *R*-**1** and (*R*)-phenoxybenzamine hydrochloride *R*-**2**

(*R*)-Mexiletine

Mexiletine, an orally active anti-arrhythmic drug belongs to Type IB class of antiarrhythmic agents that includes tocainide, lidocaine, and phenytoin (**Figure 3**).[8] It acts as non-selective voltage-gated sodium channel (VGSC) antagonist used in the treatment of ventricular arrhythmia, allodynia and myotonic syndromes, etc,. Racemic form of mexiletine is available in the market with the trade name Mexitil®. However, *in vivo* and *in vitro* pharmacological studies shows that (*R*)-isomer of mexiletine *R*-**1** is more potent than the (*S*)-isomer in experimental arrhythmias and in binding studies on cardiac sodium

channels.[9]

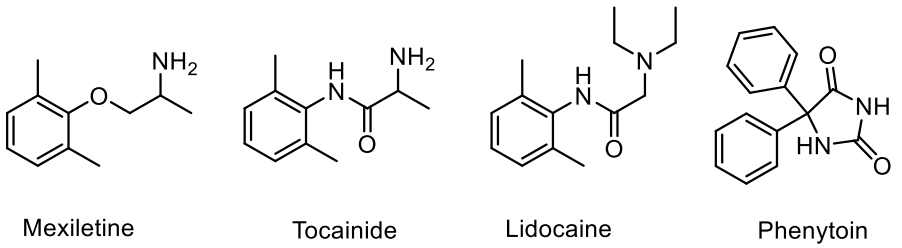


Figure 3. Type IB antiarrhythmic agents

Further, mexiletine undergoes rapid and extensive *in vitro* metabolism in human liver mediated by cytochrome P450 to the number of metabolites *via* by aromatic hydroxylation to *p*-hydroxy mexiletine (PHM) and *m*-hydroxy mexiletine (MHM), aliphatic hydroxylation to hydroxymethyl mexiletine (HMM) and *N*-oxidation to *N*-hydroxy-mexiletine (NHM) and are shown in **Figure 4**.[10]

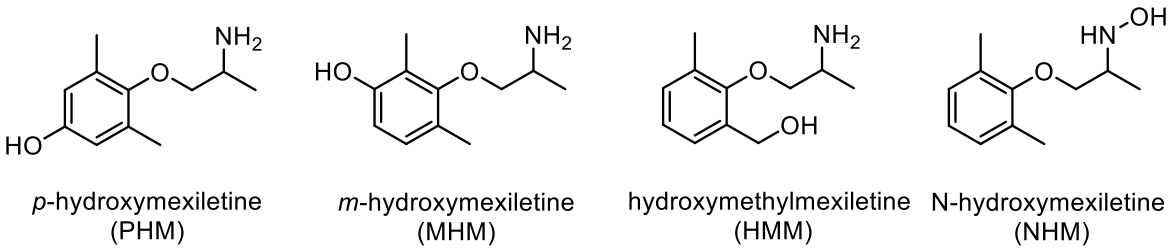


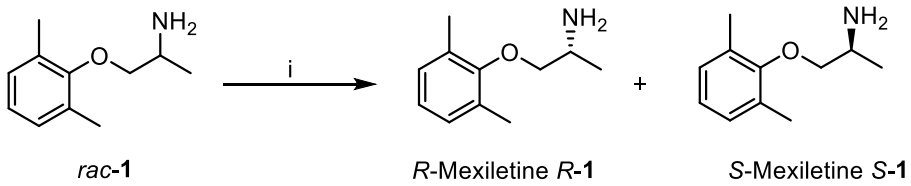
Figure 4. Mexiletine metabolites in human liver

1.2.2. Review of Literature

Various methods for the synthesis of optically pure mexiletine have been reported in the literature. Detail reports of significant syntheses of (*R*)-mexiletine *R*-**1** are described below.

Turgeon approach (1991)[11]

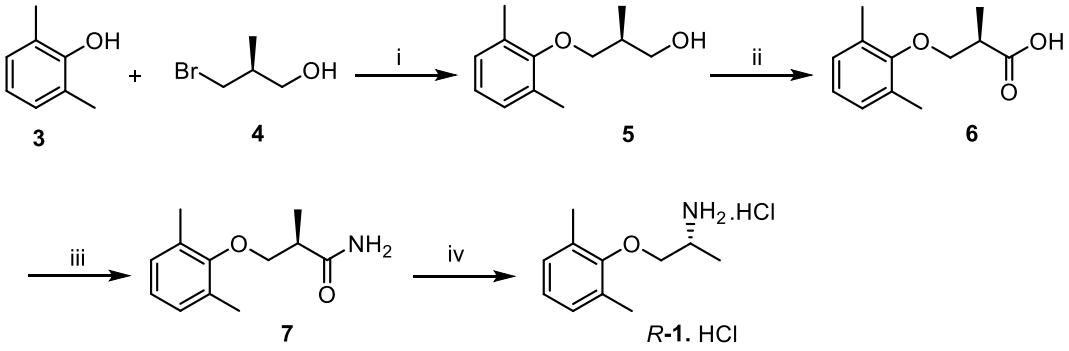
Turgeon *et al.* employed chemical resolution method to prepare the mexiletine enantiomers *R*-**1** and *S*-**1** from the *rac*-mexiletine *rac*-**1** using a chiral resolving agent (+)-Di-*p*-toluoyl-D-tartaric acid (**Scheme 1**).



Scheme 1. *Reagents and conditions*: (i) (+)-Di-*p*-toluoyl-D-tartaric acid, MeOH.

Franchini's approach (1994)[12]

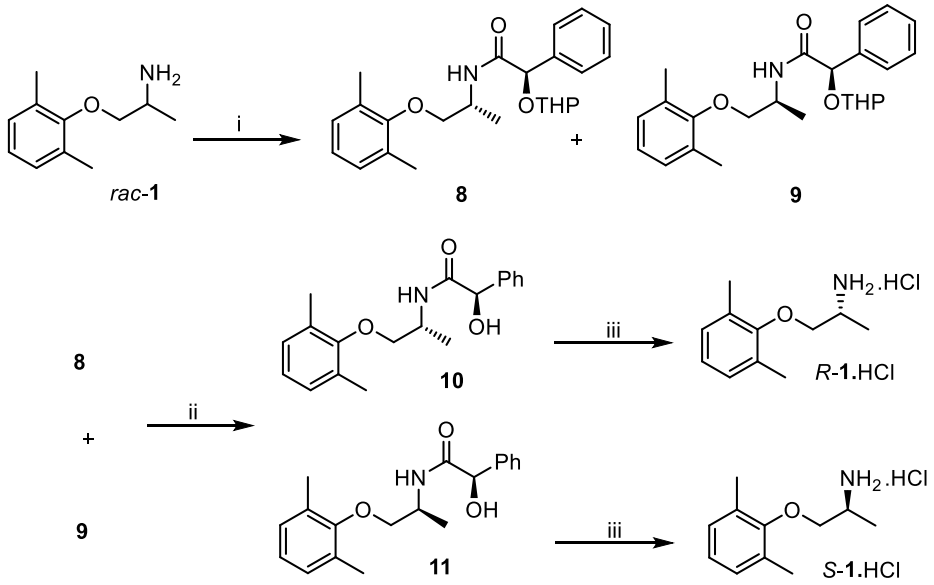
Franchini *et al.* have reported the synthesis of (*R*)-mexiletine *R*-**1** with chiral starting material (+)-(*S*)-3-bromo-2-methyl-1-propanol **4**. Phenol **3** on *O*-alkylation with bromo-alcohol **4** under basic condition gave hydroxy ether **5** (**Scheme 2**). The alcohol **5** on oxidation in the presence of catalytic amount of ruthenium dioxide and sodium periodate as a co-oxidant to its corresponding acid **6**. Subsequently, carboxylic acid **6** was converted to amide **7** followed by Hofmann degradation condition afforded *R*-**1**.HCl



Scheme 2. *Reagents and conditions:* (i) 10% NaOH, reflux to rt, 3 h, 40%; (ii) cat. RuO_2, EtOAc/10% aq. $NaIO_4$ (1:1), rt, 2 days, 75%; (iii) (a) $SOCl_2$, reflux, 1 h, (b) conc. NH_4OH, 0 °C to rt, 12 h, 60%; (iv) (a) Br_2/NaOH, reflux, 4 min, (b) 0.5 N HCl, rt, 40%.

Martin's approach (1999)[13]

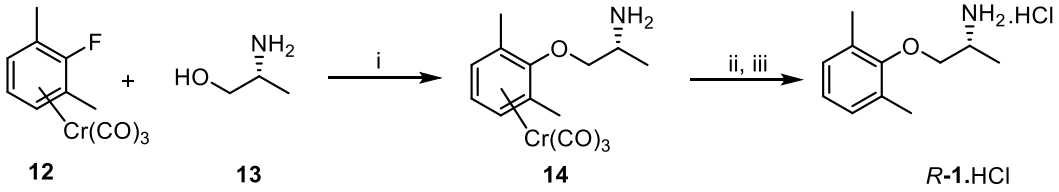
Martin and co-workers prepared both enantiomers of mexiletine *R*-**1** and *S*-**1** using chiral derivatizing agent tetrahydropyranyl protected (*R*)-mandelic acid (THPMA) from the *rac*-mexiletine **1** (**Scheme 3**). The *rac*-mexiletine *rac*-**1** was subjected to chemical resolution method with tetrahydropyranyl-protected (*R*)-mandelic acid *(THPMA)* provided diastereomeric mixture of the acylated products **8** and **9** followed by deprotection of tetrahydropyranyl (THP) in the presence of acidic medium gave the diastereomeric mixture amides **10** and **11**. After separation by column chromatography, hydrolysis of **10** and **11** gave enantiopure mexiletine hydrochloride *R*-**1**.HCl and *S*-**1**.HCl respectively.



Scheme 3. *Reagents and conditions:* (i) dicyclohexylcarbodiimide (DCC), THPMA, EtOAc, 0 °C to rt, 2 h, 97%; (ii) conc. HCl, MeOH/H_2O (4:1), 12 h, 85%; (iii) (a) 4 M H_2SO_4, H_2O/Dioxane, 80 °C, 72 h, 88%, (b) 20% HCl in methanol.

Loughhead's approach (1999)[14]

In this approach, Loughhead and co-workers described the stereospecific synthesis of both enantiomers of mexiletine *R*-**1** and *S*-**1** starting from commercially available homo chiral (*R*)-2-amino-1-propanol **13**. Aromatic nucleophilic substitution of compound **13** with 1,3-dimethyl-2-fluorobenzene tricarbonyl chromium **12** in toluene gave chromium complex **14**. Oxidative decomposition of chromium complex **14** in the presence of iodine in THF afforded (*R*)-mexiletine in 32% yield and 99%ee (**Scheme 4**).

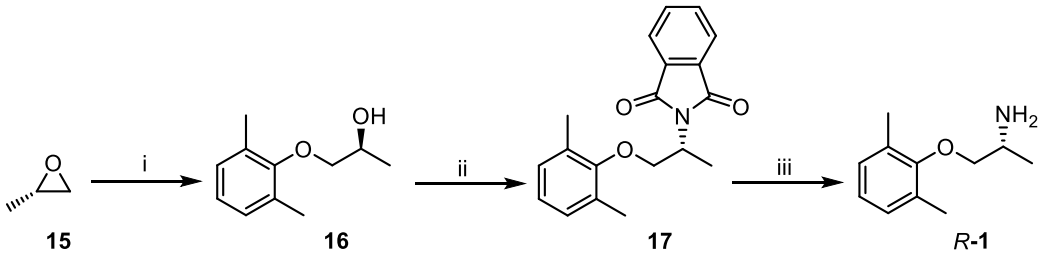


Scheme 4. *Reagents and conditions:* (i) NaH, THF, 2 h, rt; (ii) I_2, rt, 2 h, 64%; (iii) 1 M HCl, Et_2O, 32%.

Lentini's approach (2000)[15]

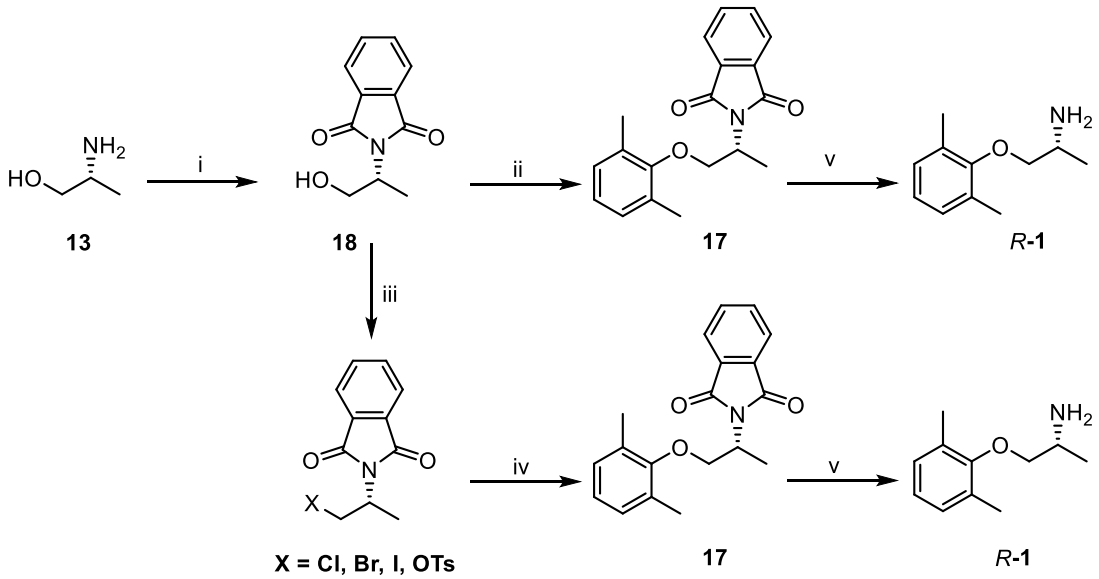
Lentini and co-workers developed a synthetic route to both enantiomers of mexiletine from the commercially available enantiomerically pure propylene oxide **15**

(**Scheme 5**). Regioselective ring opening of epoxide **15** with 2,6-dimethyl phenol **3** afforded the (*S*)-1-(2,6-dimethylphenyloxy)-2-propanol **16**. Secondary alcohol **16** was subjected to Mitsunobu reaction with phthalimide gave phthalimido derivative **17**, which on hydrazinolysis afforded (*R*)-mexiletine *R*-**1** in 66% yield and 96%ee.



Scheme 5. *Reagents and conditions:* (i) 2,6-dimethyl phenol **3**, NaOH, ACN, 12 h, rt, 53%; (ii) phthalimide, Ph_3P, DEAD, THF, 12 h, rt, 68%; (iii) hydrazine hydrate ($N_2H_4.H_2O$), AcOH, MeOH, reflux, 6 h, 66%.

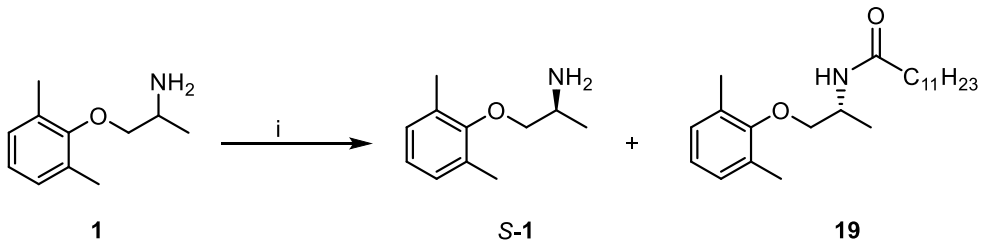
The same research group[16] have reported an alternative synthetic route starting from homochiral (*R*)-(-)-2-amino-1-propanol **13** that is depicted in **Scheme 6**.



Scheme 6. *Reagents and conditions:* (i) phthalic anhydride, Et_3N, toluene, reflux, 3 h, 78%; (ii) 2,6-dimethyl phenol **3**, PPh_3, DEAD (or) DIAD, THF, rt, 24 h, 65%,; (iii) $SOCl_2$, Py, THF, 60 °C, 6 h (when X = Cl); HBr (g) or HI (g), -5 °C to rt, 18 h, 80 °C, 2h (when X = Br or I); *p*-TsCl, pyridine, rt, 18 h (when X = OTs); (iv) 2,6-dimethyl phenol **3**, DMF, Na_2CO_3, 130 °C, 24 h; (v) N_2H_4, AcOH, EtOH, reflux, 2 h.

Gastaldi's approach (2007)[17]

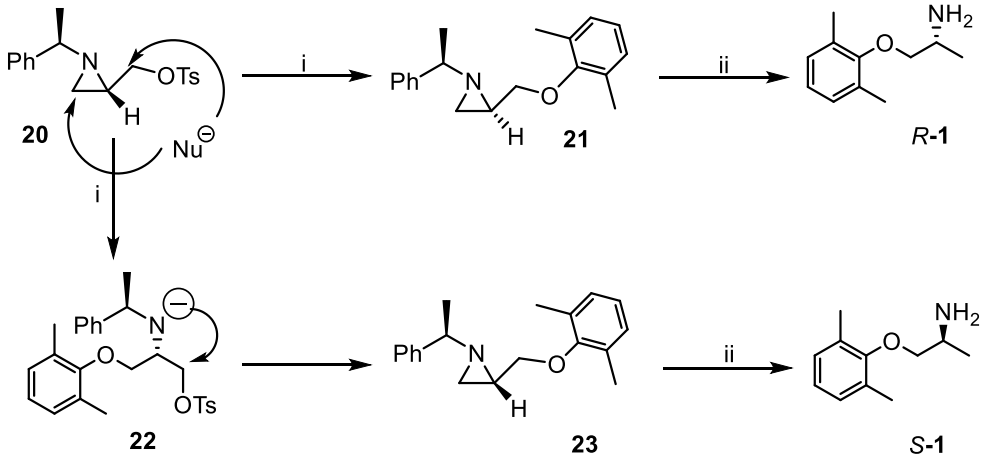
Gastaldi and co-workers reported the enzymatic resolution of *rac*-mexiletine **1** using *Candida antartica* lipase-B (CALB, Novozym 435) and lauric acid as an acyl donor. The acetylation is more selective towards the (*R*)-enantiomer under these reaction conditions (**Scheme 7**).



Scheme 7. *Reagents and conditions:* (i) lauric acid, CAL-B, heptane, 80 °C, 7 h, **19** (39%, 98%ee), *S*-**1** (27%, 99%ee).

Han & Lee's approach (2008)[18]

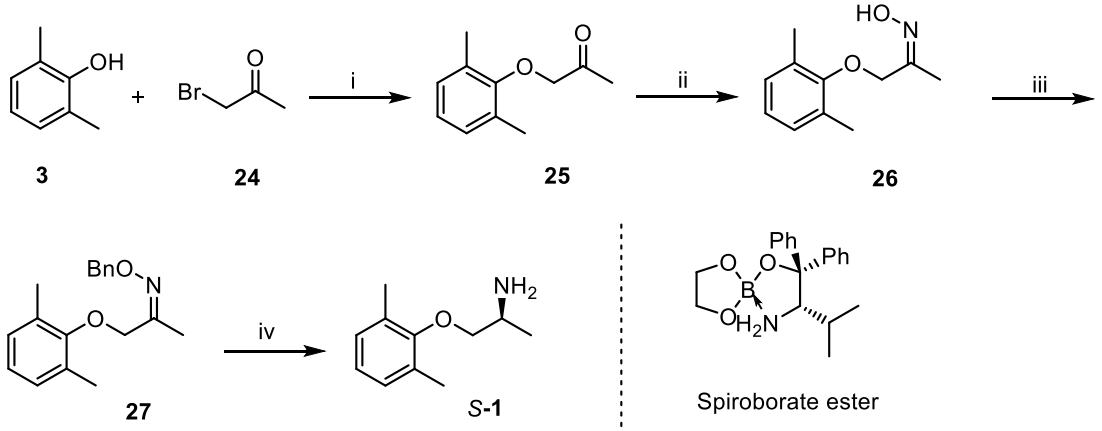
In this approach, Han and co-workers synthesized both isomers of mexiletine *via* nucleophilic substitution of 2-(sulfonyloxymethyl)aziridine **20** with 2,6-dimethylphenol **3** as shown in the **Scheme 8**. The synthesis started from (*2R*)-1-[1'(*R*)-α-methylbenzyl](p-toluenesulfonyloxymethyl)aziridine **20** *via* two diastereomers 2*R*- and 2*S*-(2,6-dimethyl-phenoxy)methyl aziridines **21** and **23** followed by hydrogenolysis afforded both *R*-**1** and *S*-**1** in 84% and 87% yields respectively.



Scheme 8. *Reagents and conditions*: (i) 2,6-dimethyl phenol **3**, acetone/DMF (1:1), K_2CO_3, reflux, 4 h, 76% (**21:23** = 84:16); (ii) H_2 (1 atm), $Pd(OH)_2$, MeOH, rt, 84% (*R*-**1**), 97% (*S*-**1**).

Ortiz-Marciales approach (2008)[19]

In this approach, Ortiz-Marciales *et al.* employed asymmetric reduction strategy for the preparation of (*S*)-enantiomer of mexiletine ***S*-1** (**Scheme 9**).

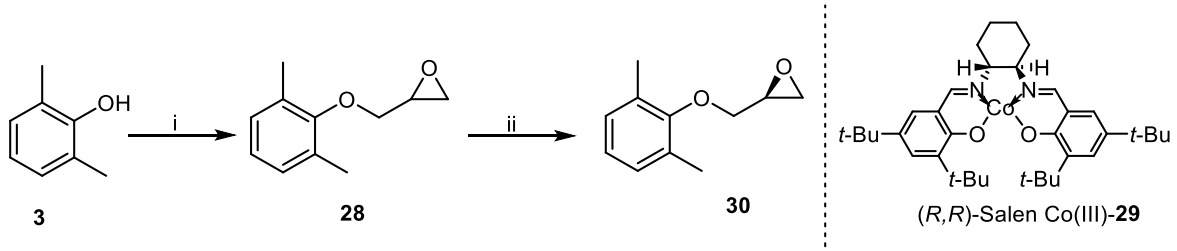


Scheme 9. *Reagents and conditions:* (i) K_2CO_3, DMF, rt, 24 h, 84%; (ii) $NH_2OH.HCl$, pyridine, EtOH, 0 °C, 12 h, 57%; (iii) BnBr, NaH, DMF, -30 °C to rt, 12 h, 84%; (iv) 10 mol% Spiroborate ester, BH_3.THF, 1,4-dioxane, 0 °C, 48 h, 84%, 94%ee

Treatment of 2,6-Xylenol **3** with 1-bromoacetone **24** under basic condition afforded desired ketone **25** in 84% yield. Ketone **25** was converted into oxime in the presence of $NH_2OH{\cdot}HCl$ and pyridine followed by *O*-benzylation with benzyl bromide gave (*Z*)-benzyloxime ether **27**. Finally, reduction of oxime ether **27** using 10% spiroborate ester developed by Marciales gave *S*-**1**.

Muthukrishnan'sapproach (2009)[21]

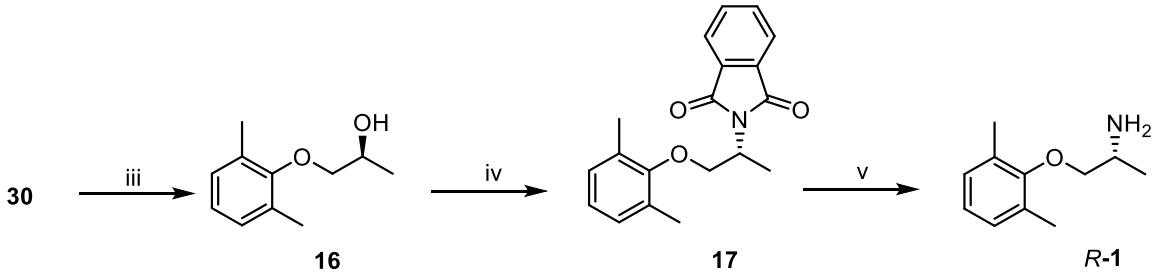
Muthukrishnan and co-workers employed Jacobsen's hydrolytic kinetic resolution strategy to synthesize (*R*)-mexiletine (**Scheme 10**).



Scheme 10. *Reagents and conditions:* (i) epichlorohydrin, K_2CO_3, dry acetone, reflux, 24 h,

80%; (ii) (*R*,*R*)-Salen Co (III)-**29** (0.5 mol %), H_2O (0.55 equiv.), 0 °C to rt, 30 h.

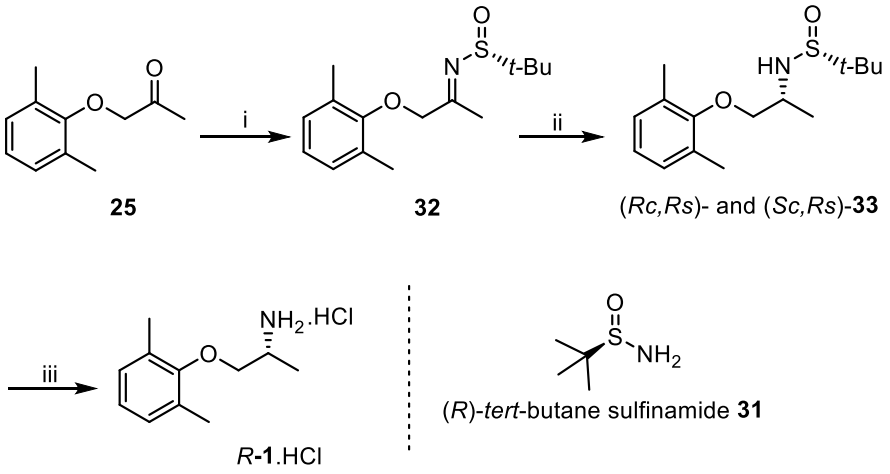
The epichlorohydrin on treatment with 2,6-Xylenol **3** under reflux condition afforded the 2,6 dimethylglycidyl ether **28**. The *rac*-glycidyl ether **28** was subjected to Jacobsen's hydrolytic kinetic resolution employing Jacobsen's catalyst (*R, R*)-salen Co(III)-**29** and water afforded enantiomerically pure epoxide **30** in 43% yield along with enantiopure diol in 47% yield. Reductive ring opening of enantiomerically pure epoxide **30** with LAH, followed by Mitsunobu reaction with phthalimide and phthalimido ether **17** formed was treated with hydrazine hydrate in ethanol afforded (*R*)-mexiletine ***R*-1** in 86% yield and >99% ee (**Scheme 11**).



Scheme 11: *Reagents and conditions:* (iii) LAH, dry THF, 0 °C, 30 min, 92%; (iv) phthalimide, Ph_3P, DIAD, dry THF, rt, 2 h, 83%; (v) $N_2H_4.H_2O$, EtOH, reflux, 3 h; 86%.

Ryan's approach (2015)[20]

Ryan *et al.* utilized chiral auxiliary approach for the synthesis of both isomers *R*-**1**.HCl and *S*-**1**.HCl.



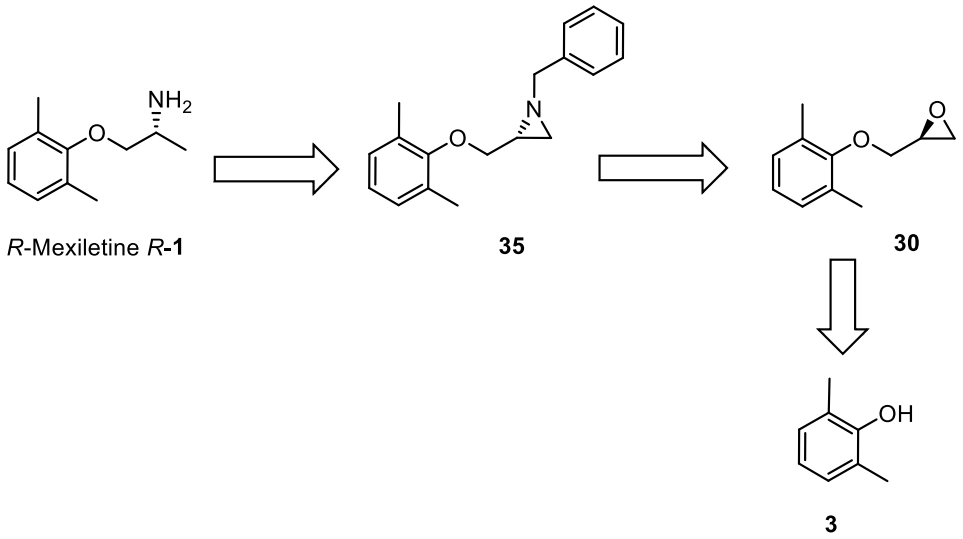
Scheme 12. *Reagents and conditions:* (i) (*R*)-*tert*-butanesulfinamide **31**, $Ti(OEt)_4$, 90 °C, MW, 1 h; (ii) $NaBH_4$, $CuSO_4$, 20 °C, THF, 5h; (iii) 4 M HCl, 1,4-dioxane, 3 h.

As depicted in **Scheme 12**, the 3-(2,6-dimethylphenyloxy)-2-propanone **25** on condensation with (*R*)-*tert*-butanesulfinamide **31** in neat $Ti(OEt)_4$ under microwave condition gave (*R*)-*tert*-butanesulfinyl imine **32**. Further, reduction of imine intermediate **32** gave the two diastereomeric *N*-tertbutanesulfinyl chiral amines (*Rc, Rs*)- and (*Sc, Rs*)-**33** in dr = 4.0:1.0 ratio. Finally, the removal of the chiral auxiliary from the major product (*Rc, Rs*)-**33** with 4 N HCl afforded (*R*)-**1**.HCl in high enantiopurity.

1.2.3. Present work

Objective

The medicinal properties of (*R*)-mexiletine have attracted a great deal of interest among various chemists. Approaches that have been used so far to prepare enantiopure mexiletine enantiomers involve a chiral pool, chemo/enzymatic resolution strategy or using stereoselective protocols. In this section, the development of simple and efficient approach towards the preparation of *R*-mexiletine *R*-**1** *via* the reductive ring opening reaction of the enantiopure aziridine as a key step has been described. A retrosynthetic analysis of *R*-**1** is outlined in **Scheme 13**.



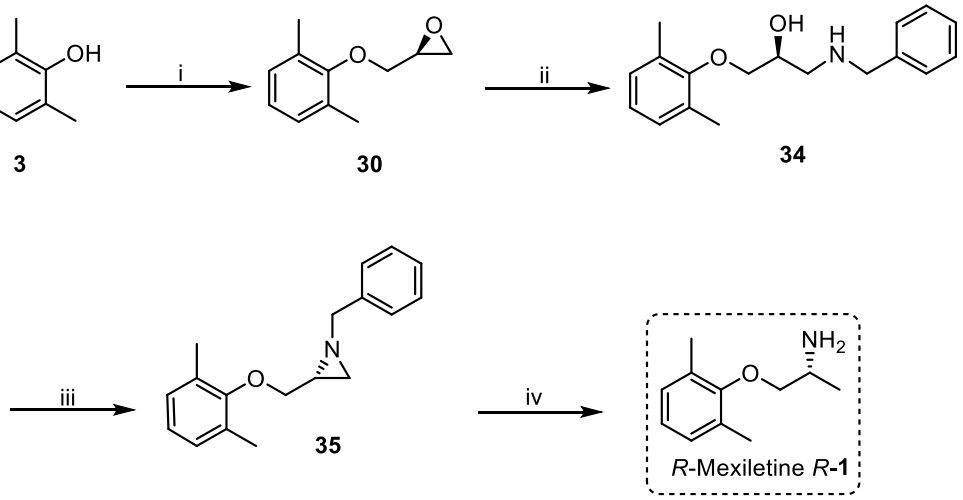
Scheme 13. Retrosynthetic analysis of (*R*)-mexiletine

As shown in **Scheme 13**, it has been envisaged that chiral aziridine **35** could serve as a key intermediate for the synthesis of (*R*)-mexiletine *R*-**1**. The intermediate **35** can be converted to the target molecule *via* simple reductive ring opening reaction. The chiral aziridine **35** in turn, could be prepared from regioselective ring opening of enantiopure

epoxide **30** followed by intramolecular ring closure employing Mitsunobu reaction. The epoxide **30** could be easily obtained from the commercially available starting material (*R*)-epichlorohydrin and 2,6-dimethylphenol **3**.

1.2.4. Results and Discussion

As illustrated in **Scheme 14**, synthesis of (*R*)-mexiletine *R*-**1** began with the commercially available 2,6-dimethyl phenol **3** which on *O*-alkylation with (*R*)-epichlorohydrin in anhydrous acetone in the presence of potassium carbonate at reflux for 20 h gave enantiopure 2,6-dimethyl phenyl glycidyl ether **30** in 73 % yield. In the ^{1}H NMR spectrum of glycidyl ether **30,** the methylene protons of epoxide resonate at δ 3.78 and 4.07 ppm as a doublet of doublet, while in the ^{13}C NMR the methylene carbon resonate at δ 72.9 ppm. Next, the regioselective ring opening of enantiopure epoxide **30** with benzylamine in the presence of catalytic amount of lithium bromide under the neat condition for 6 h furnished amino alcohol derivative **34** in 76% yield.[22] In the ^{1}H NMR spectrum, the methylene protons of amino alcohol **34** resonate as a multiplet at δ 3.81-3.90 ppm. On the other hand, the methylene carbons displayed resonance signals at δ 53.9 and 51.3 ppm in ^{13}C NMR supported the formation of amino alcohol **34**. The IR spectrum showed the characteristic -OH absorption at 3593 cm^{-1}. Subsequently, the amino alcohol **34** was subjected to intramolecular ring closure employing Mitsunobu reaction in the presence of



Scheme 14. *Reagents and conditions*: (i) (*R*)-epichlorohydrin, K_2CO_3, dry acetone, reflux, 20 h, 73%; (ii) benzylamine, cat. LiBr (neat), 6 h, 76%; (iii) PPh_3, DIAD, dry toluene, 0 °C to reflux, 12 h, 84%; (iii) H_2 (50 psi), 10% Pd/C, MeOH, rt, 6 h, 73%.

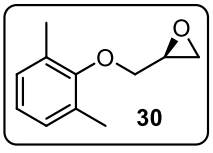
triphenylphosphine, DIAD using toluene as a solvent at reflux condition for 12 h gave key intermediate aziridine **35** in 84 % yield. In the ^{1}H NMR spectrum of **35,** the shielded methylene protons of aziridine unit resonated as a doublet at δ 1.58 and δ 1.83 ppm. The formation of aziridine **35** was further confirmed by HRMS spectrum at *m/z* 268.1696 corresponding to molecular formula $C_{18}H_{22}ON$ $[M+H]^+$ with calculated value *m/z* 268.1696. Finally, the aziridine intermediate **35** was subjected to reductive ring opening under H_2 pressure (50 psi) in the presence of 10 % Pd/C in methanol for 6 h gave the (*R*)-mexiletine (*R*-**1**) in 73 % yield with an overall yield of 34%, $[\alpha]^{25}_D$ -2.4 (*c* 5.0, $CHCl_3$) {lit.[15] $[\alpha]^{25}_D$ -2.7 (*c* 4.7, $CHCl_3$)}. In the ^{1}H NMR spectrum of *R*-**1**, the disappearance of benzyl protons and appearance of characteristic signals of methyl protons resonate as a doublet at δ 1.17 ppm and -NH_2 as a broad singlet at δ 1.67 ppm confirmed the formation of the target molecule (*R*)-mexiletine *R*-**1**. The physical and spectroscopic data of all synthesized molecules were confirmed by means of IR, ^{1}H NMR, ^{13}C NMR and HRMS analysis.

1.2.5. Conclusion

In conclusion, a concise and efficient route for the synthesis of active enantiomer of antiarrhythmic drug (*R*)-mexiletine *R*-**1** has been realized using reductive ring opening of chiral aziridine intermediate as a key step. Simple procedures, the ready availability of the starting materials and good overall yields are some of the salient features of this approach. Further, this strategy can be exploited for the preparation of other optically active mexiletine analogs.

1.2.6. Experimental Section

1) (*S*)-2-((2,6-dimethylphenoxy)methyl)oxirane (30)



A solution (*R*)-epichlorohydrin (2.6 mL, 0.0327 mol) was added slowly to a stirred solution of 2,6-dimethylphenol **3** (2 g, 0.0163 mol) and K_2CO_3 (6.3 g, 0.0458 mol) in anhydrous acetone (25 mL), and the resulting mixture was heated at reflux for 20 h. When the reaction was complete (TLC), the reaction mixture was filtered, washed with acetone and concentrated the solvent. The crude residue was dissolved in ethyl acetate (3 x 20 mL) and

extracted with 1 M NaOH (3 x 10 mL) and the collected organic layers were washed with brine (2 x 10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography [silica-gel, petroleum ether/acetone (96:4)] gave **30** as a yellow oil.

Yield: 2.11 g, 73%;

Molecular Formula: $C_{11}H_{14}O_2$;

Specific rotation: $[\alpha]^{25}_D$ = +2.5 (*c* 2.0,$CHCl_3$) {lit.[21] $[\alpha]^{25}_D$ = +2.5 (*c* 2.0, $CHCl_3$)};

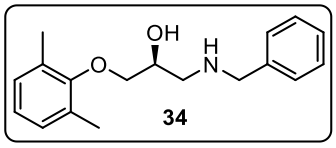
IR ($CHCl_3$, cm^{-1}): ν_{max} 3436, 3020, 1600, 1497, 1215, 1045, 929, 669;

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.34 (s, 6 H), 2.73-2.76 (m, 1 H), 2.90-2.91 (m, 1 H), 3.37-3.41 (m, 1 H), 3.78 (dd, *J* = 11.0, 6.1 Hz, 1 H), 4.07 (dd, *J* = 11.0, 2.3 Hz, 1 H), 6.95-6.98 (m, 1 H), 7.04 (d, *J* = 7.3 Hz, 2 H);

^{13}C NMR (125 MHz, $CDCl_3$): δ 155.4 (C), 130.7 (C, 2 carbons), 128.7 (CH, 2 carbons), 123.9 (CH), 72.9 (CH_2), 50.4 (CH), 44.4 (CH_2), 16.1 (CH_3, 2 carbons);

HRMS (ESI): *m/z* calculated for $C_{11}H_{15}O_2$ $[M+H]^+$ 179.1067, found 179.1068.

2) (*S*)-1-(benzylamino)-3-(2,6-dimethylphenoxy)propan-2-ol (34)



34

A round bottomed flask charged with(*S*)-2-((2,6-dimethylphenoxy)methyl)oxirane **30** (1.9 g, 0.0106 mol), LiBr (cat.) and benzylamine (0.93 mL, 0.0085 mol) was stirred at room temperature for 6 h. When the reaction was complete (TLC), H_2O (10 mL) was added and the mixture was extracted with (methyl *tert*-butyl ether) MTBE (3 x 20 mL). The organic layers were combined, washed with brine (2 x 10 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography [silica-gel, EtOAc/petroleum ether (18:82)] gave **34** as a colorless solid.

Yield: 2.31g, 76%;

MP: 61-62 °C;

Molecular Formula: $C_{18}H_{23}NO_2$;

Specific rotation: $[\alpha]^{25}_D$ = +2.7 (*c* 2.0, $CHCl_3$);

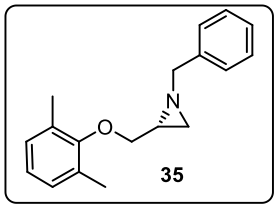
IR ($CHCl_3$, cm^{-1}): ν_{max} 3593, 3445, 3322, 3059, 3016, 2945, 2962, 1666, 1590, 1495, 1481, 1433, 1206, 1127, 1049, 719, 676;

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.29 (s, 6 H), 2.86 (dd, *J* = 11.7, 7.8 Hz, 1 H), 2.93 (dd, *J* = 12.2, 3.6 Hz, 1 H), 3.81-3.90 (m, 4 H), 4.08-4.13 (m, 1 H), 6.93-6.96 (m, 1 H), 7.01 (d, *J* = 7.3 Hz, 2 H), 7.27-7.30 (m, 1 H), 7.35 (d, *J* = 4.3 Hz, 4 H);

^{13}C NMR (125 MHz, $CDCl_3$): δ 155.3 (C), 140.0 (C), 130.7 (C, 2 carbons), 128.9 (CH, 2 carbons), 128.4 (CH, 2 carbons), 128.1 (CH, 2 carbon), 127.1 (CH), 123.9 (CH), 74.3 (CH_2), 69.2 (CH), 53.9 (CH_2), 51.3 (CH_2), 16.2 (CH_3, 2 carbons);

HRMS (ESI): *m/z* calculated for $C_{18}H_{24}O_2N$ $[M+H]^+$ 286.1802, found 286.1803.

3) (*R*)-1-benzyl-2-((2,6-dimethylphenoxy)methyl)aziridine (35)



To a stirred solution of **34** (2 g, 0.0070 mol) in dry toluene (3 mL), was added PPh_3 (3.67 g, 0.0140 mol) at 0 °C, and the solution was stirred for 15 min. A solution of DIAD (2.1 mL, 0.0105 mol) in dry toluene (2 mL) was added dropwise over 20 min at 0 °C and the mixture was then stirred for 30 min. After 30 min the reaction mixture allowed to room temperature and reflux for 12 h. When the reaction was complete (TLC), the reaction mixture was concentrated under reduced pressure and the crude residue was purified by column chromatography [silca-gel, EtOAc/petroleum ether (4:96)] gave **35** as a colorless oil.

Yield: 1.57 mg, 84%;

Molecular Formula: $C_{18}H_{21}NO$;

Specific rotation: $[\alpha]^{25}_D$ = +40.5 (*c* 1.0, $CHCl_3$);

Chiral HPLC: ee >97% [The ee of **35** was determined by chiral HPLC analysis; Chiralcel OD-H (250 X 4.6 mm) column; eluent: n-hexane/isopropanol (99.5:0.5); flow rate: 1 mL/min; detector 220 nm; (*R*)-isomer t_R= 12.59 min.; (*S*)-isomer t_R= 13.8 min.];

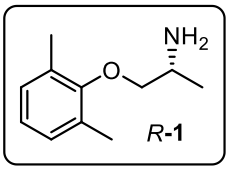
IR ($CHCl_3$, cm^{-1}): ν_{max} 3017, 2925, 1595, 1474, 1262, 1020, 915, 764;

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.58 (d, *J* = 6.4 Hz, 1 H), 1.83 (d, *J* = 2.7 Hz, 1 H), 2.03-2.07 (m, 1 H), 2.30 (s, 6 H), 3.49 (d, *J* = 13.4 Hz, 1 H), 3.59 (d, *J* = 13.4 Hz, 1 H), 3.78-3.84 (m, 2 H), 6.93 (t, *J* = 7.3, 1 H), 7.01 (d, *J* = 7.3 Hz, 2 H), 7.27-7.30 (m, 1 H), 7.34-7.37 (m, 2 H), 7.41 (d, *J* = 7.6, 2 H);

^{13}C NMR (125 MHz, $CDCl_3$): δ 155.7 (C), 139.0 (C), 130.9 (C, 2 carbons), 128.7 (CH, 2 carbons), 128.3 (CH, 2 carbons), 128.0 (CH, 2 carbons), 127.0 (CH), 123.7 (CH), 74.5 (CH_2), 64.3 (CH_2), 38.6 (CH), 31.7 (CH_2), 16.3 (CH_3, 2 carbons);

HRMS (ESI): *m/z* calculated for $C_{18}H_{22}ON$ $[M+H]^+$ 268.1696, found 268.1696.

4) (*R*)-1-(2,6-dimethylphenoxy)propan-2-amine (*R*-1)



To the stirred solution of **35** (0.250 g) in methanol (10 mL) was added palladium on activated carbon (0.060 g, 10 wt %) and the reaction mixture was stirred under hydrogen atmosphere (50 psi) for 6 h at room temperature. When the reaction was completed (TLC), the catalyst was filtered over the bed of Celite (EtOAc eluent) and the solvent was evaporated under reduced pressure. The crude product was purified by chromatography [basic alumina, methanol/EtOAc (5:95)] afforded *R*-**1** as a colorless oil.

Yield: 0.122 g, 73%;

Molecular Formula: $C_{11}H_{17}NO$;

Specific rotation: $[\alpha]^{25}_D$ = -2.4 (*c* 5.0, $CHCl_3$) {lit.[15] $[\alpha]^{25}_D$ = -2.7 (*c* 4.7, $CHCl_3$)};

Chiral HPLC: ee >97% [The ee of *R*-**1a** (as *N*-acetyl derivative) was determined by chiral HPLC analysis of the corresponding *N*-acetyl derivative; Chiralcel OD-H (250 X 4.6 mm) column; eluent: *n*-hexane/isopropanol (90:10); flow rate: 0.6 mL/min; detector 220 nm; (*R*)-isomer t_R= 13.43 min.; (*S*)-isomer t_R = 20.58 min.];

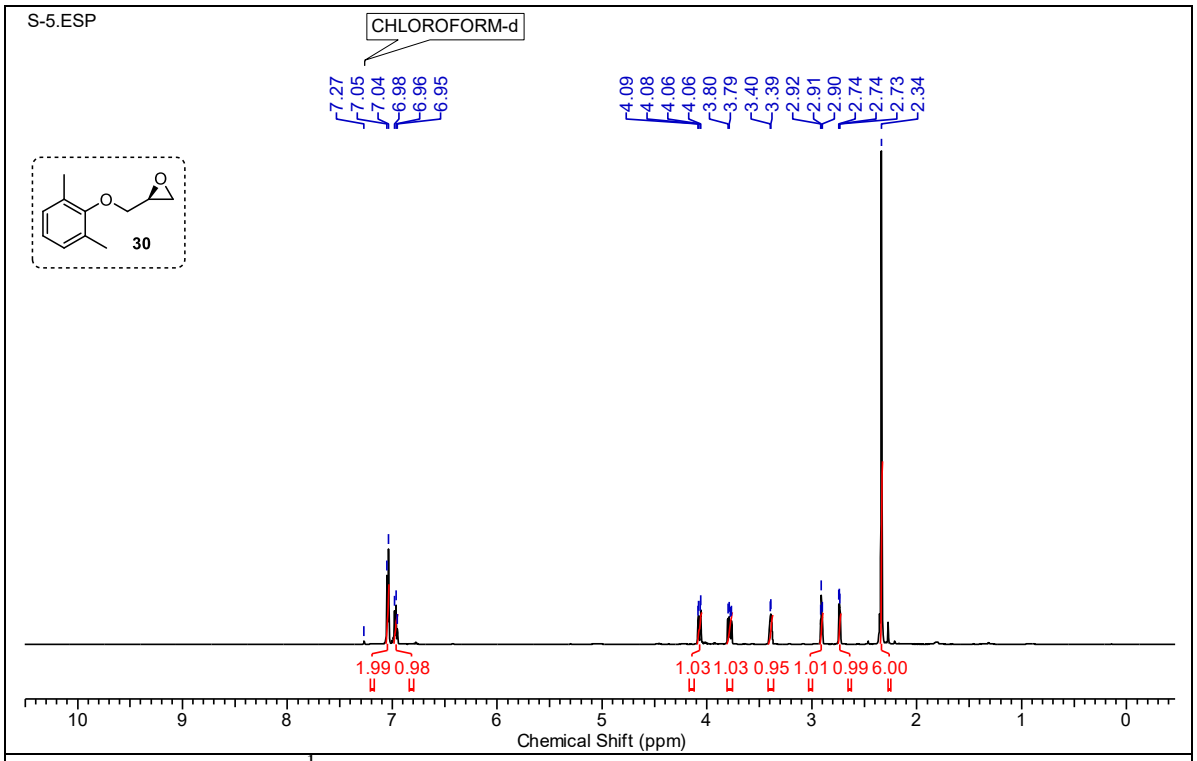
IR (Neat, cm^{-1}): ν_{max} 3019, 2400, 1667, 1476, 1263, 1215, 1092, 1028, 928, 853;

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.17 (d, *J* = 6.4 Hz, 3 H), 1.67 (bs, 2 H), 2.30 (s, 6 H), 3.35-3.41 (m, 1 H), 3.53-3.56 (m, 1 H), 3.64-3.67 (m, 1 H), 6.91-6.94 (m, 1 H), 7.01 (apparent d, *J* = 7.6 Hz, 2 H);

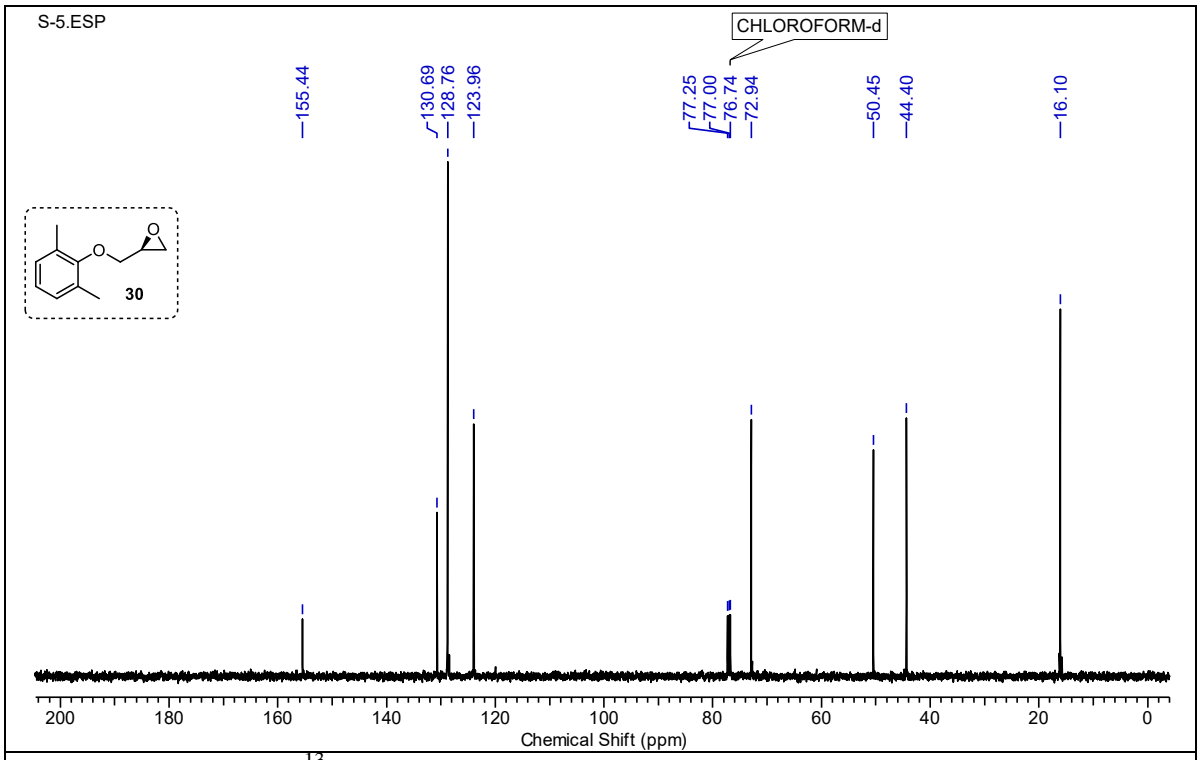
^{13}C NMR (125 MHz, $CDCl_3$): δ 155.5 (C), 130.8 (C, 2 carbons), 128.8 (CH, 2 carbons), 123.8 (CH), 78.3 (CH_2), 47.3 (CH), 19.8 (CH_3), 16.3 (CH_3, 2 carbons);

HRMS (ESI): *m/z* calculated for $C_{11}H_{18}ON$ $[M+H]^+$ 180.1383, found 180.1383.

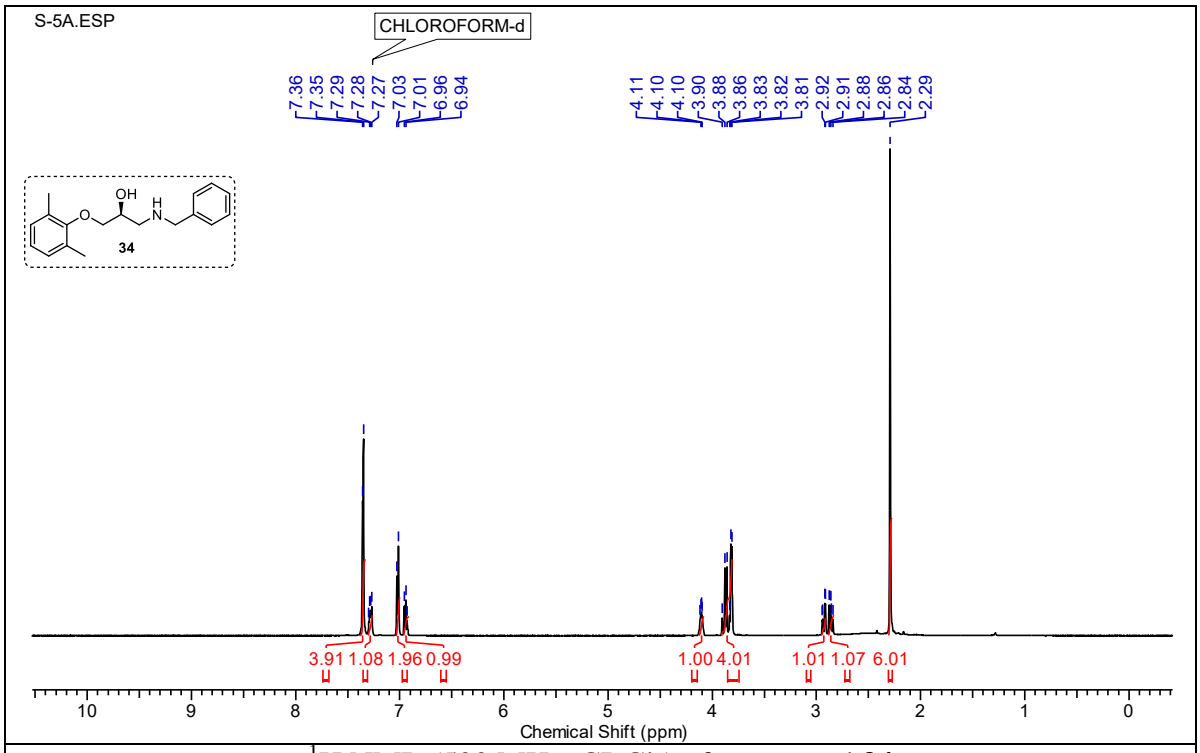
1.2.7. Spectra



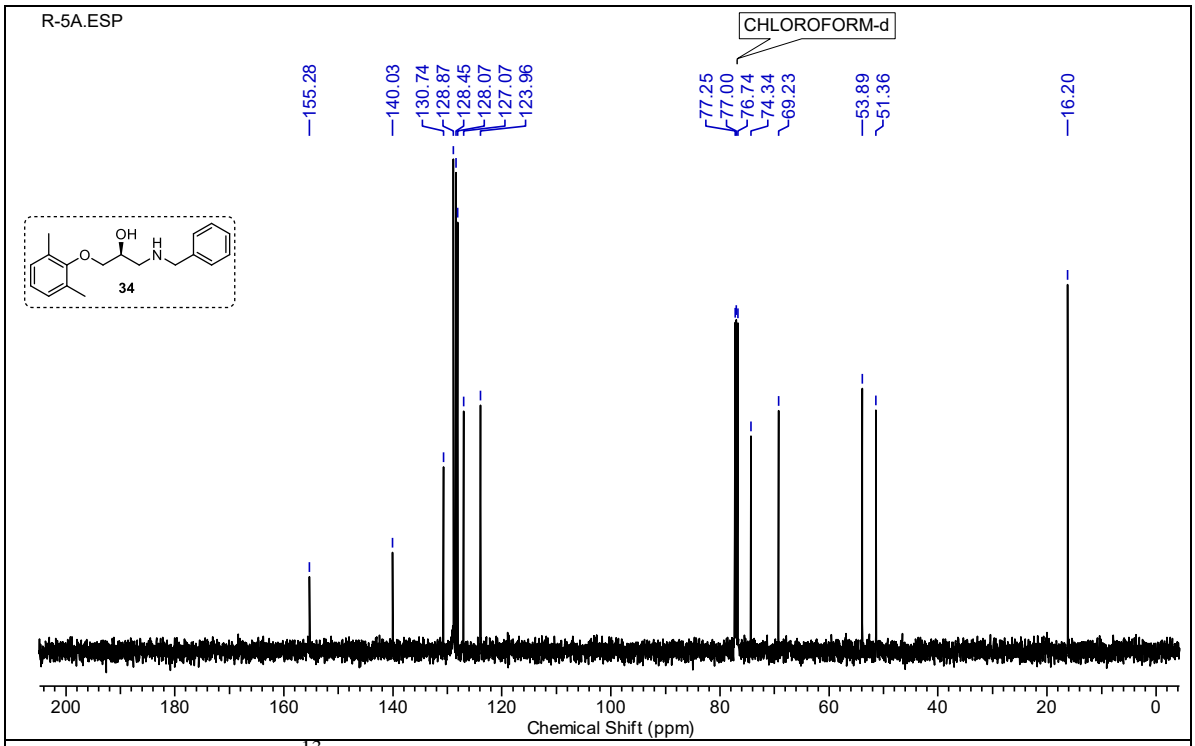
^{1}H NMR (500 MHz, $CDCl_3$) of compound **30**



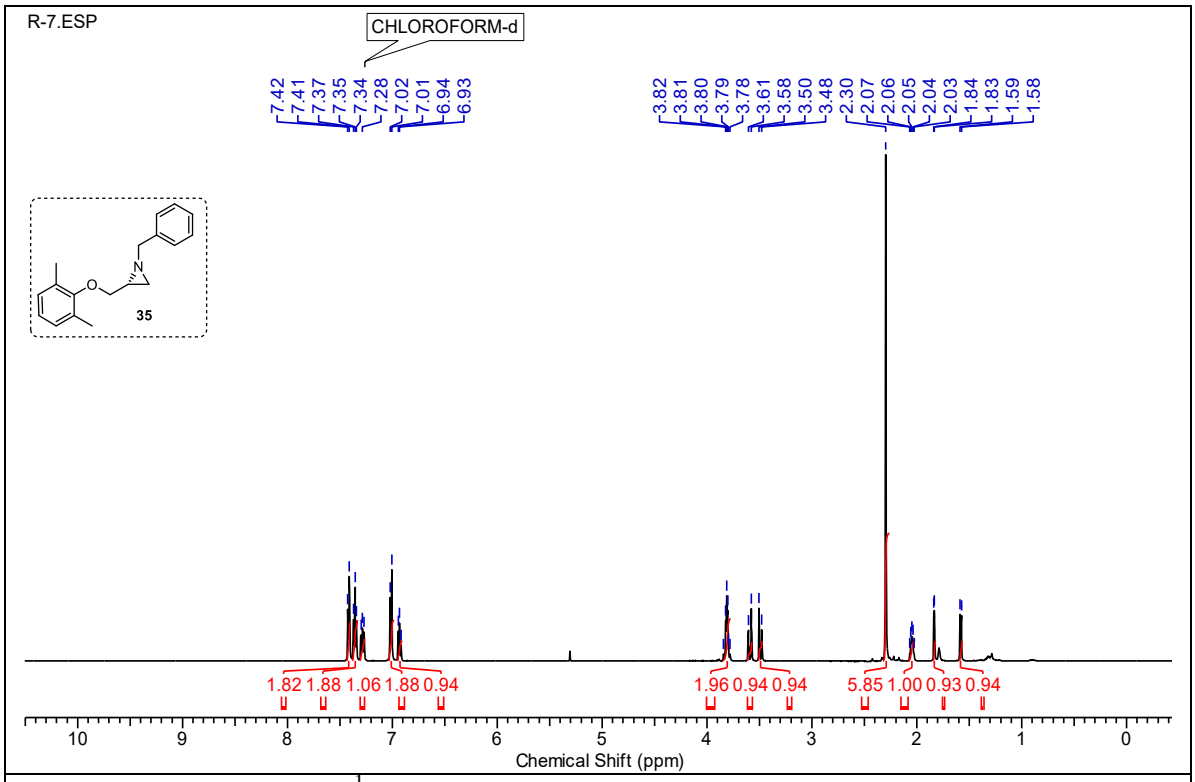
^{13}C NMR (125 MHz, $CDCl_3$) of compound **30**



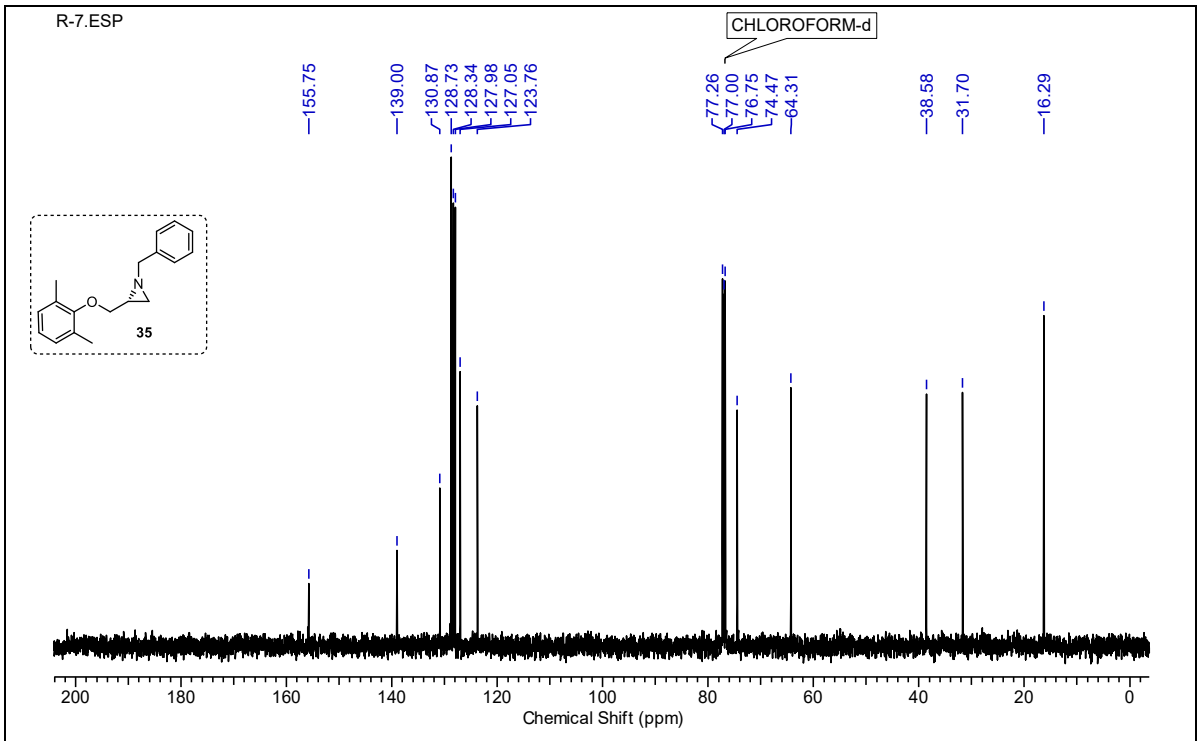
^{1}H NMR (500 MHz, $CDCl_3$) of compound **34**



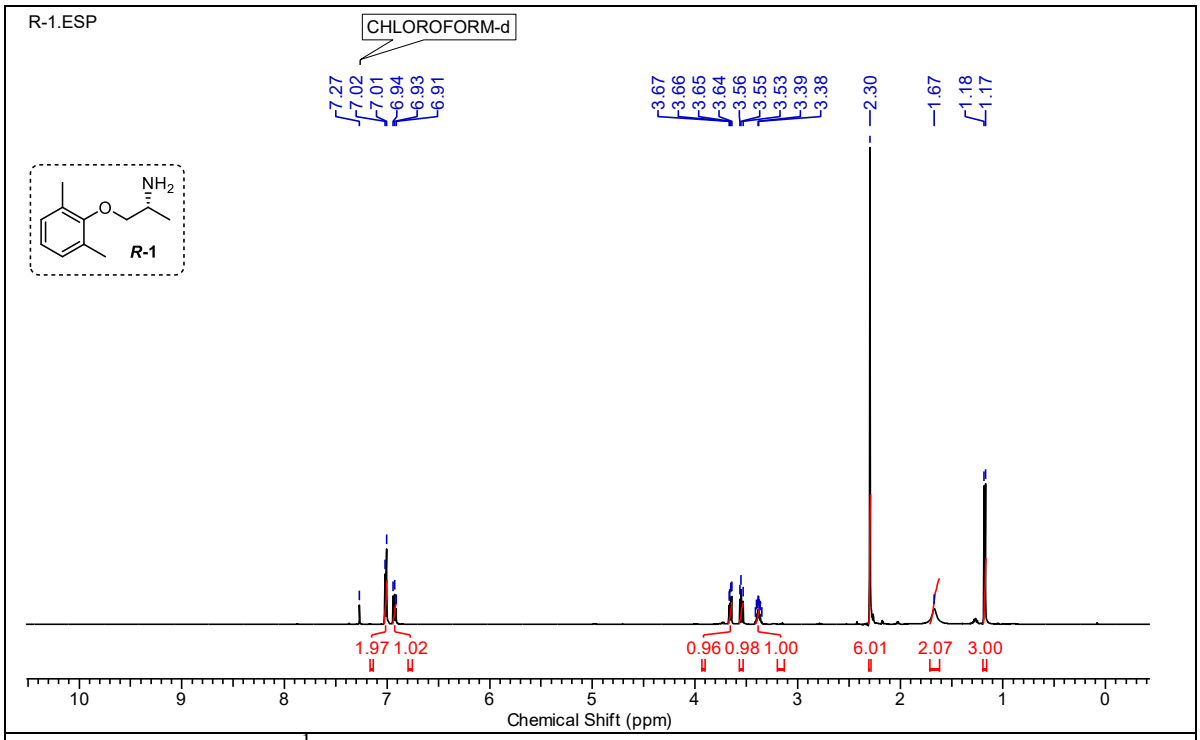
^{13}C NMR (125 MHz, $CDCl_3$) of compound **34**



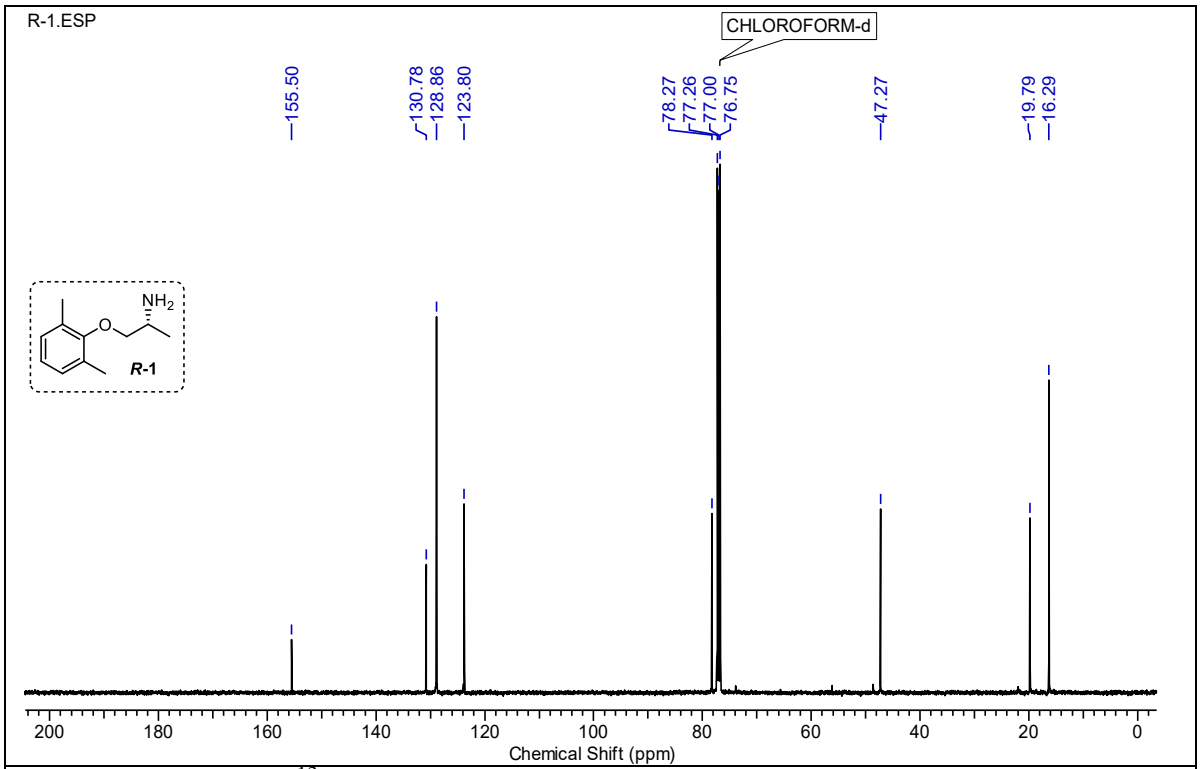
^{1}H NMR (500 MHz, $CDCl_3$) of compound **35**



^{13}C NMR (125 MHz, $CDCl_3$) of compound **35**



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



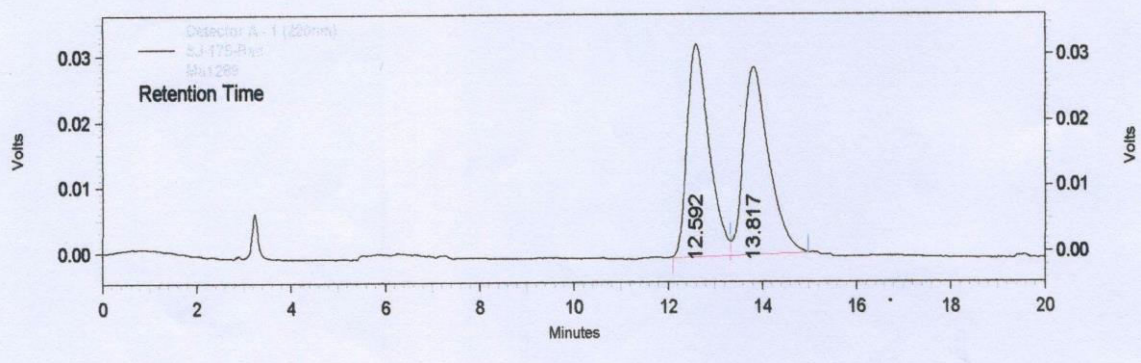
^{13}C NMR (125 MHz, $CDCl_3$) of compound *R*-**1**

1.2.8. Chiral HPLC analysis data

Chiral HPLC analysis of Compound 35

Conditions: Chiralcel OD-H (250 X 4.6 mm) column; eluent: n-hexane/isopropanol (99.5:0.5); flow rate: 1 mL/min; detector 220 nm.

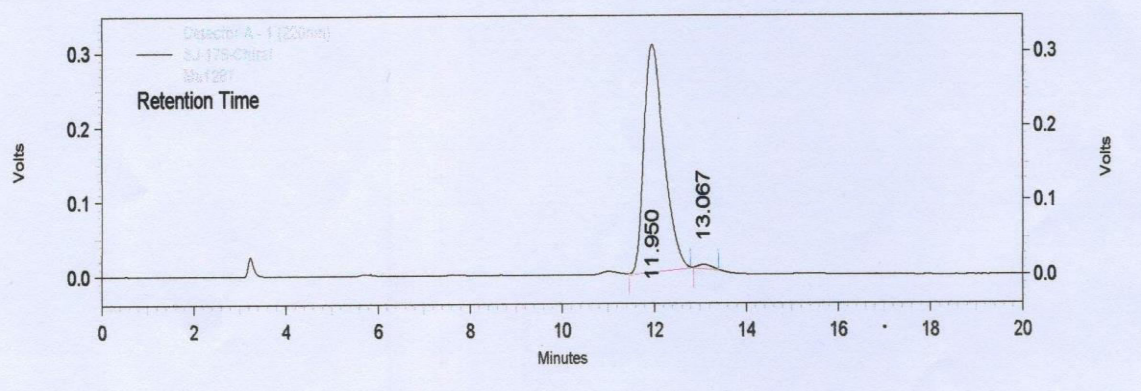
Racemic



Racemic Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	12.592	522199	49.331
2	13.817	536370	50.669
Totals		1058569	100.000

Chiral



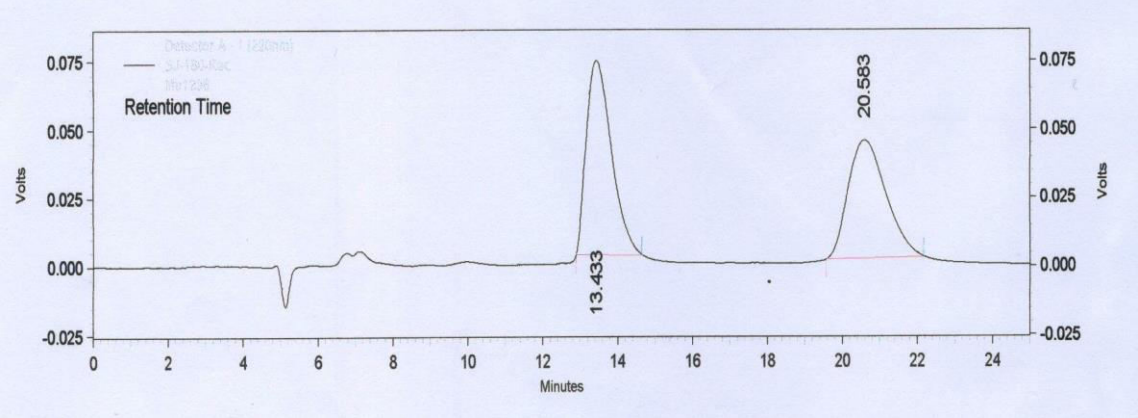
Chiral Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	11.950	4571621	98.990
2	13.067	46644	1.010
Totals		4618265	100.000

Chiral HPLC analysis of Compound *R*-1a (as *N*-acetyl derivative)

Conditions: Chiralcel OD-H (250 X 4.6 mm) column; eluent: n-hexane/isopropanol (90:10); flow rate: 0.6 mL/min; detector 220 nm.

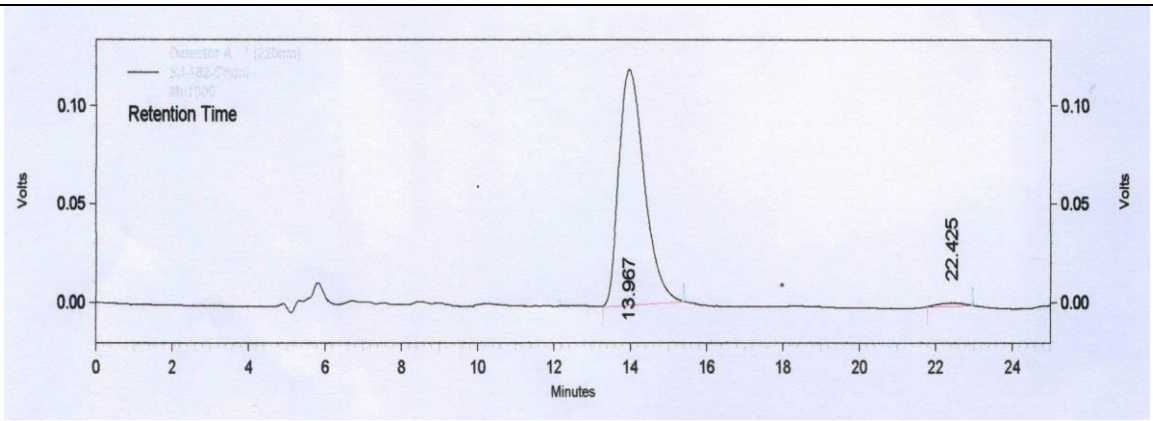
Racemic



Racemic Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	13.433	3310909	52.371
2	20.583	3011159	47.629
Totals		6322068	100.000

Chiral:



Chiral Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	13.967	5525451	98.792
2	22.425	67542	1.208
Totals		5592993	100.000

(*R*)-Phenoxybenzamine hydrochloride

Phenoxybenzamine hydrochloride (*R*-**2**, PB; trade name Dibenzyline®) is an important β-chloroethylamine class of drug belongs to α-blocking agents (**Figure 5**).[23] It is an irreversible, long-acting, non-competitive α-adrenoreceptor antagonist widely used in the treatment of hypertension associated with phaeochromocytoma.[24] It has also found application in treating benign prostatic hyperplasia (BPH) and hypoplastic left heart syndrome etc,.[25] Pharmacological studies reveal that (*R*)-enantiomer of phenoxybenzamine hydrochloride (*R*-**2**) is 14.5 times more potent than its (*S*)-enanatiomer.[26]

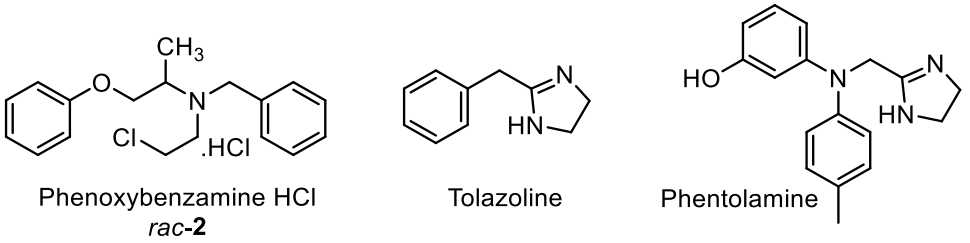


Figure 5. Representative non-selective α-adrenergic receptor blockers

Therapeutic actions of phenoxybenzamine

The chloroethylamine group in phenoxybenzamine binds to α-adrenergic receptors covalently causing irreversible blockade. The drug action of phenoxybenzamine is slower but the duration of action is long-lasting (3-4 days of single dose) due to its irreversible antagonism. It promotes the relaxation of vascular smooth muscle and leads to dilation of blood vessels results in a lowering of blood pressure (**Figure 6**). In addition to this, phenoxybenzamine inhibits the release of norepinephrine from the adrenergic nerve endings and prevents the neuronal uptake at higher concentrations (10^{-5} g/mL) results may enhance in transmitter release.[27]

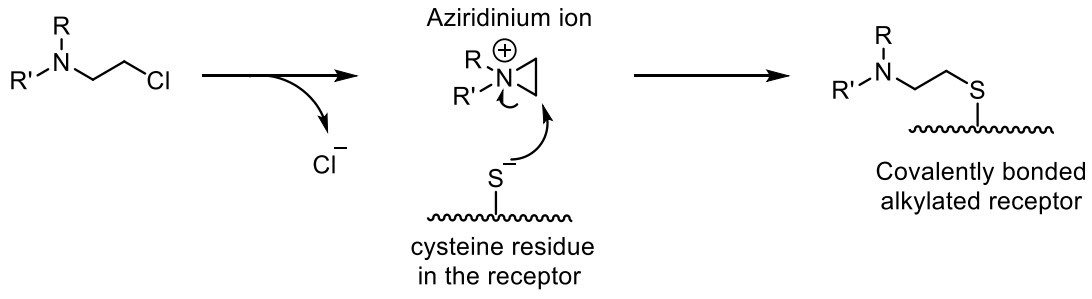


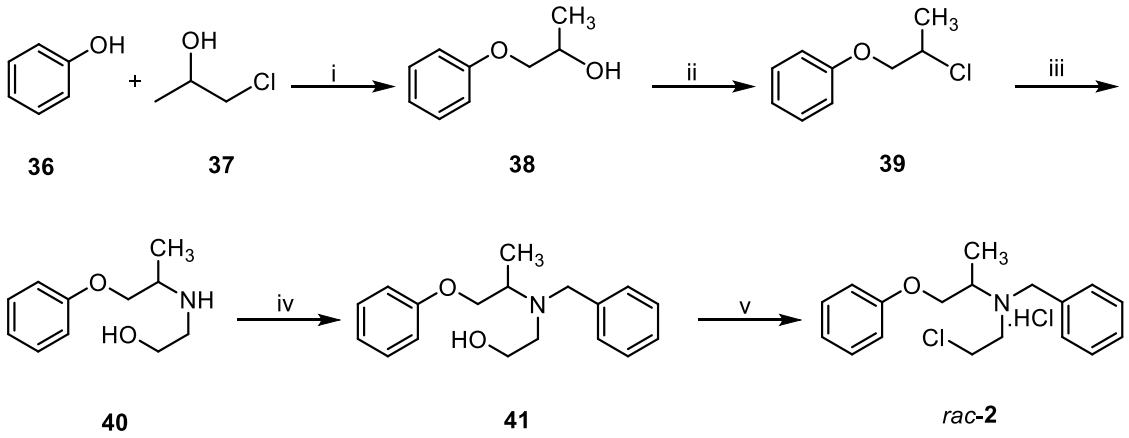
Figure 6. Mechanism of action of PB

1.2.9. Review of Literature

A few reports have appeared in the literature for the synthesis of phenoxybenzamine hydrochloride enantiomers. A detailed report of these syntheses is described below.

Kerwin approach (1951)[28]

Kerwin *et al.* reported the synthesis of racemic phenoxybenzamine hydrochloride *rac*-**2** starting from phenol **36** (**Scheme 15**). The reaction of phenol **36** with propylene



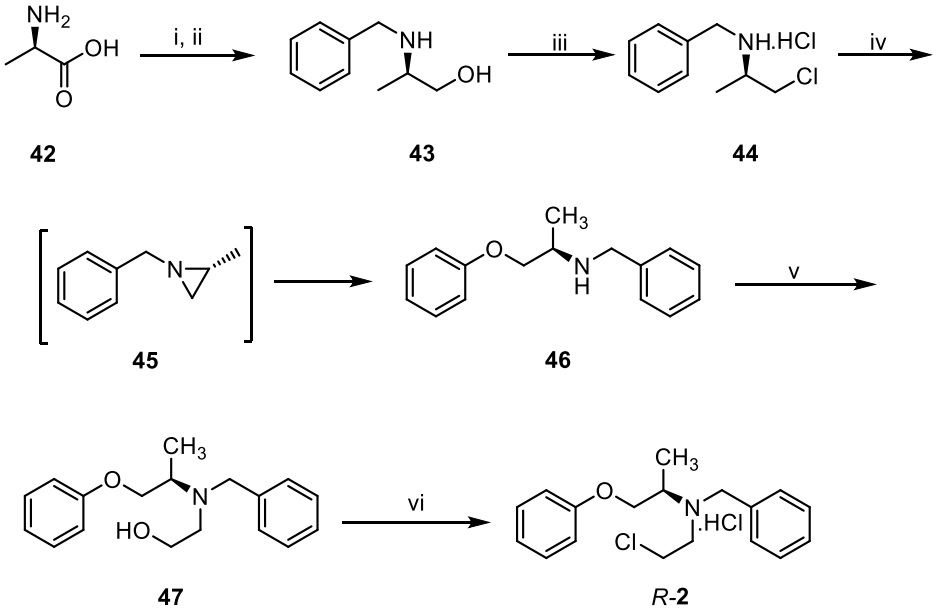
Scheme 15. *Reagents and conditions:* (i) NaOH, H_2O, EtOH, reflux, 5 h; (ii) $SOCl_2$, benzene, reflux; (iii) ethanolamine, reflux; (iv) benzylchloride, K_2CO_3, EtOH, reflux, 9 h; (iv) $SOCl_2$, HCl, $CHCl_3$, reflux.

chlorohydrin **37** in the presence of a base gave secondary alcohol **38**. Alcohol **38** was converted to chloro derivative **39** followed by treatment with ethanolamine at 160 °C under neat condition afforded amino alcohol **40**. *N*-benzylation followed by chlorination of compound **41** using thionyl chloride gave *rac*-phenoxybenzamine hydrochloride (*rac*-**2**).

Portoghese approach (1971)[26]

Portoghese and co-workers accomplished the synthesis of both enantiomers of phenoxybenzamine hydrochloride *R*-**2** and *S*-**2** employing chiral pool approach (**Scheme 16**). Commercially available unnatural amino acid (*D*)-alanine **42** was *N*-benzoylated followed by reduction with LAH provided (*R*)-*N*-benzylalanine **43**. Replacement of hydroxy group by chloride by treating aminoalcohol **43** with thionyl chloride afforded chloro derivative **44**. Compound **44** on treatment with sodium phenoxide for prolonged

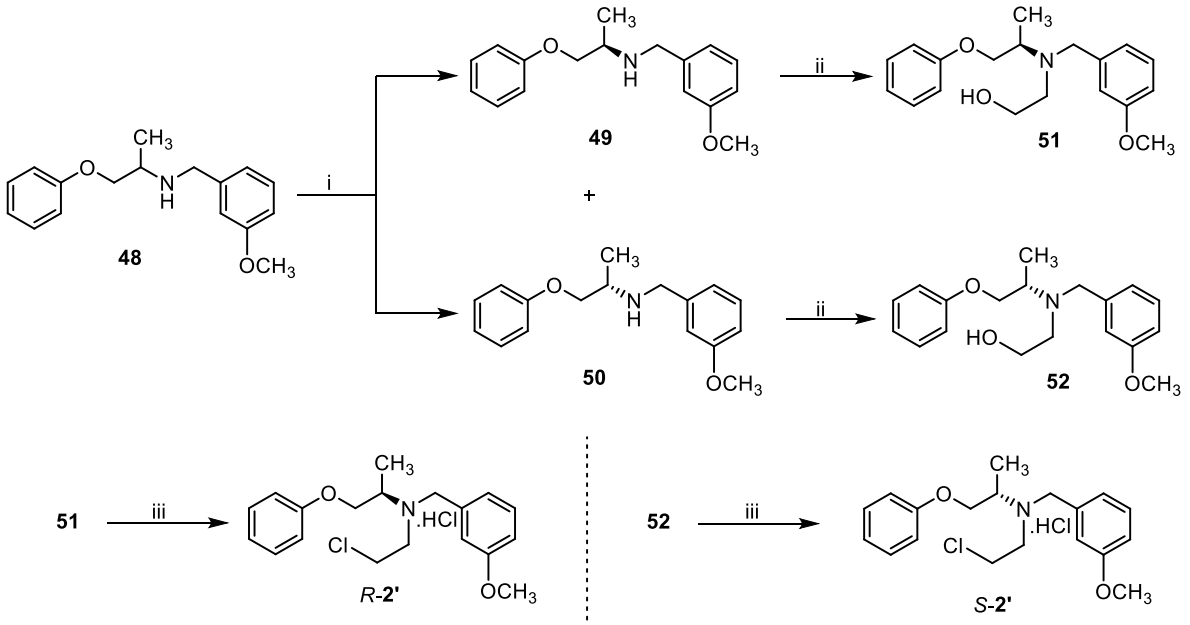
reaction period (120 h) gave secondary amine **46**. Finally, the target molecule *R*-**2**.HCl was obtained by treating amine derivative **46** with ethylene oxide followed by treating amino alcohol derivative **47** with thionyl chloride in refluxing chloroform. Using the same strategy, *S*-enantiomer has also been prepared.



Scheme 16. *Reagents and conditions:* (i) benzoylchloride, aq.$NaHCO_3$; (ii) $LiAlH_4$, THF, reflux; (iii) $SOCl_2$, PhH, reflux, 80%; (iv) NaOPh, EtOH, reflux, 120 h, 43%; (v) Ethylene oxide, H_2O, sealed tube, 120 °C, 6 h, 84%; (vi) $SOCl_2$, $CHCl_3$, reflux, 55%.

Giardina approach (1997)[29]

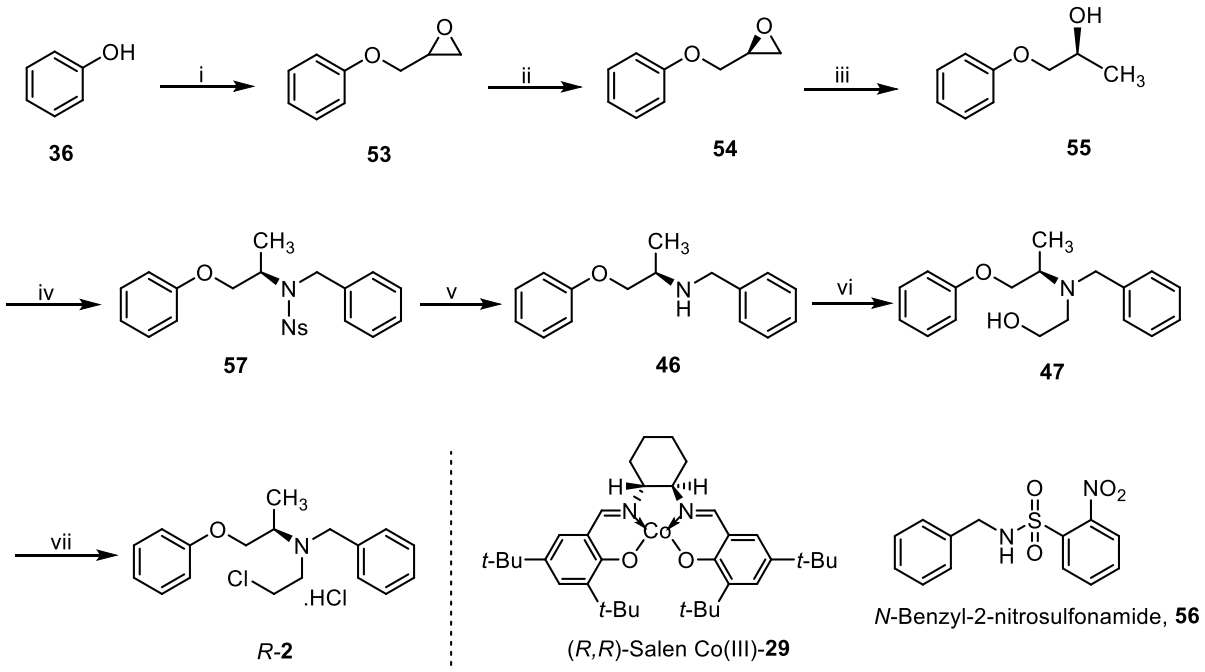
Giardina and co-workers reported the chemical resolution strategy for the synthesis of PB analogue enantiomers *R*-**2'** and *S*-**2'** (**Scheme 17**). As shown in **Scheme 17**, chemical resolution of *rac-N*-(3-methoxybenzyl)-1-phenoxypropan-2-amine intermediate **48** with D-(+)- or L-(-)-*O,O'*-dibenzoyl tartaric acids afforded the respective enantiomers **49** and **50** respectively. Further treatment of secondary amine derivatives **49** and **50** with 2-bromoethanol gave amino alcohol derivatives **51** and **52** respectively. Finally, chlorination of amino alcohols **51** and **52** on treatment with thionyl chloride in chloroform, saturated with HCl (g) provided *R*-**2'** and *S*-**2'** respectively.



Scheme 17. *Reagents and conditions:* (i) D-(+)- or L-(-)-*O,O'*-dibenzoyl tartaric acid, MeOH, rt; (ii) 2-bromoethanol, K_2CO_3, EtOH; (iii) $SOCl_2$, HCl (g), $CHCl_3$.

Muthukrishnan's approach (2010)[30]

Muthukrishnan and co-workers described the enantioselective synthesis of (*R*)-phenoxybenzamine hydrochloride *via* Jacobsen's hydrolytic kinetic resolution and Fukuyama-Mitsunobu strategy as a key reaction steps (**Scheme 18**). *O*-alkylation of phenol **36** with *rac*-epichlorohydrin under basic condition afforded the *rac*-phenyl glycidyl ether **53** in 90% yield. The *rac*-glycidyl ether **53** was subjected to Jacobsen's hydrolytic kinetic resolution conditions gave the enantiopure (*S*)-glycidyl ether **54** along with its diol. The reductive ring opening of epoxide **54** with LAH provided β-hydroxy ether **55**. Subsequently, the β-hydroxy ether **55** was subjected to Fukuyama-Mitsunobu protocol with *N*-benzyl-2-nitrosulfonamide **56**, followed by denosylation afforded the secondary amine derivative **46**. *N*-alkylation followed by chlorination of amino alcohol **47** on treatment with thionyl chloride afforded (*R*)-phenoxybenzamine hydrochloride *R*-**2** in 52% yield and 99% ee enantiopurity.



Scheme 18. *Reagents and conditions:* (i) epichlorohydrin, K_2CO_3, dry acetone, reflux, 8 h, 90%; (ii) (*R*,*R*)-Salen Co (III)-**29** (0.5 mol %), H_2O (0.55 equiv.), 0 °C to rt, 30 h; (iii) $LiAlH_4$, dry THF, 0 °C, 30 min, 93%; (iv) *N*-benzyl-2-nitro-benzenesulfonamide **56**, Ph_3P, DIAD, dry THF, rt, 2 h, 81%; (v) thiophenol, K_2CO_3, dry acetonitrile, rt, 2 h, 87%; (vi) bromoethanol, K_2CO_3, ethanol, 110 °C, sealed tube, 72 h, 82%; (vii) $SOCl_2$, HCl (g), dry PhH, 0 °C to reflux, 8 h, 52%.

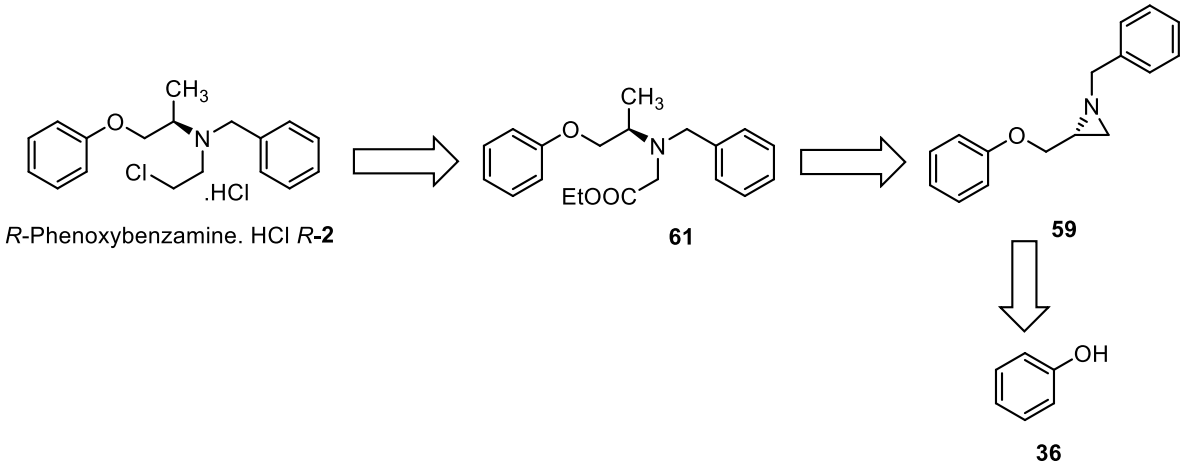
1.2.10. Present work

Objective

The syntheses documented in the literature for the preparation of PB *R*-**2** mainly utilizes the chiral pool or chemical resolution strategies. Further, reported methods suffer from drawbacks such as expensive starting materials, catalysts, harsh reaction conditions and multistep synthesis etc,. In this section, we explored the chemistry of aziridine to develop an improved and efficient synthesis of (*R*)-phenoxybenzamine HCl *R*-**2** *via* reductive ring opening reaction of the enantiopure aziridine as a key step.

A retrosynthetic analysis of (*R*)-phenoxybenzamine HCl *R*-**2** is outlined in **Scheme 19**. The aziridine derivative **59** was visualized as a key intermediate for the synthesis of (*R*)-phenoxybenzamine HCl *R*-**2**. The key intermediate **59** can be extended to the ester derivative **61** *via* reductive ring opening and *N*-alkylation reactions. Simple reduction and chlorination of ester amine **61** can lead to the target molecule *R*-**2**. Further, the chiral

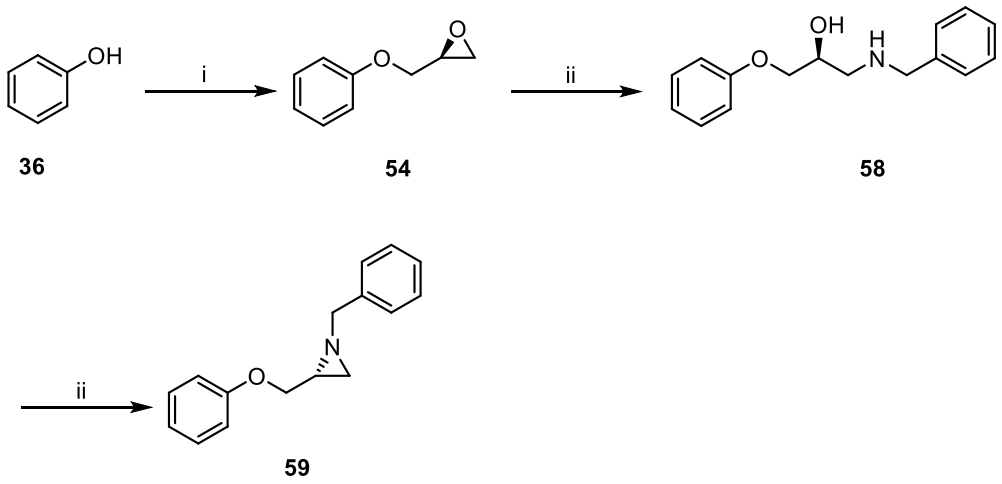
aziridine **59**, in turn, could be derived from commercially available phenol **36** *via O*-alkylation followed by intramolecular ring closure sequences.



Scheme 19. Retrosynthetic analysis of (*R*)-phenoxybenzamine HCl

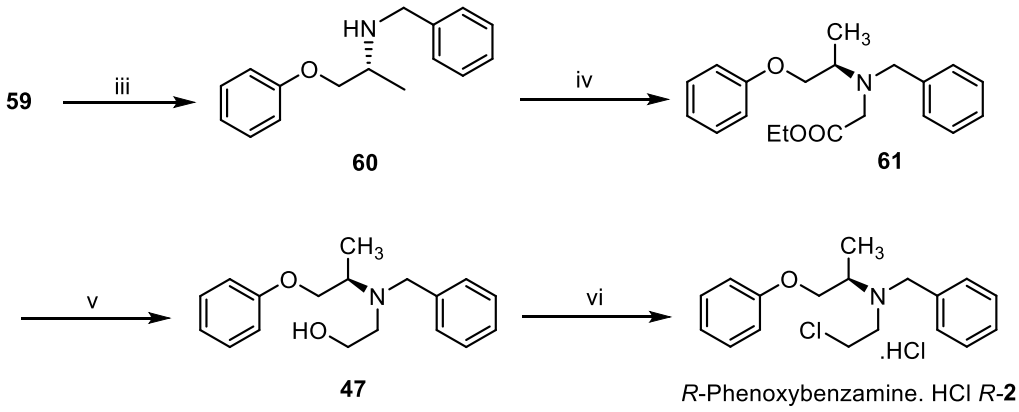
1.2.11. Results and Discussion

Synthetic strategy followed for the synthesis of (*R*)-phenoxybenzamine HCl ***R*-2** is outlined in **Scheme 20**. At first, phenol **36** was *O*-alkylated with (*R*)-epichlorohydrin in anhydrous acetone in the presence of potassium carbonate at reflux condition for 16 h gave epoxide **54** in 82 % yield. In the ^{1}H NMR spectrum of **54**, the methylene protons of ether resonate as a doublet of doublet at δ 3.95 and 4.22 ppm, while in the ^{13}C NMR spectrum, methylene carbon of ether was discernible at δ 68.6 ppm indicates the formation of product **54**. Our next objective was to convert the enantiopure epoxide **54** into the key aziridine intermediate **59** by employing the same two step sequences (ring opening followed by Mitsunobu protocols) used for the preparation of (*R*)-mexiletine *R*-**1**). The epoxide **54** was opened regioselectively with benzylamine in the presence of catalytic amount of lithium bromide under a neat condition at room temperature for 9 h gave amino alcohol derivative **58** in 83% yield. In the ^{1}H NMR spectrum, the signals corresponding to methine and benzylic protons in **58** resonated at δ 4.09-4.15 (m) and 3.98 (d) while in the ^{13}C NMR spectrum, the benzylic carbon displayed the resonance signal at δ 51.2 ppm ascertain the formation of product **58**. In the IR spectrum of amino alcohol **58**, the absorption bands corresponding to -OH and -NH group at 3593 cm^{-1} and 3322 cm^{-1} respectively. Subsequently, the amino alcohol derivative **58** was subjected to Mitsunobu reaction with triphenylphosphine, DIAD in toluene at reflux condition for 16 h afforded the key



Scheme 20. *Reagents and conditions*: (i) (*R*)-epichlorohydrin, K_2CO_3, dry acetone, reflux, 16 h, 82%; (ii) (a) benzylamine, cat. LiBr (neat), 9 h, 83%; (b) PPh_3, DIAD, dry toluene, 0 °C to reflux, 16 h, 78%;

intermediate aziridine **59** in 78 % yield. In the ^{1}H NMR spectrum, appearance of signals at δ 1.59 (d), 1.87 (d) and 1.98-2.04 (m) corresponds to the shielded protons of aziridine unit confirms the formation of **59**. On the other hand, the methine and methylene carbons of aziridine unit in the ^{13}C NMR spectrum of **59** displayed resonance signals at δ 37.9 and δ 32.0 ppm respectively. Next, our aim was to convert aziridine derivative **59** to secondary amine derivative **60** without a deprotecting *N*-benzyl group (**Scheme 21**). We tried several reaction conditions to achieve conversion. Gratifyingly, reductive ring opening of **59** in the presence of 10 % Pd/C under H_2 pressure (50 psi) in MeOH for 45 min afforded the



Scheme 21. *Reagents and conditions:* (iii) H_2 (50 psi), 10% Pd/C, MeOH, rt, 45 min, 72%; (iv) $BrCH_2COOEt$, K_2CO_3, dry DMF, 80 °C, 12 h, 66%; (v) $LiAlH_4$, dry THF, 0 °C to rt, 3h, 72%; (vi) $SOCl_2$, HCl (g), dry PhH, reflux, 8 h, 56%.

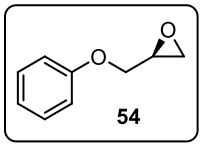
secondary amine derivative **60** in 72% yield. In the ^{1}H NMR spectrum, the signals correspond to methyl protons of ring-opened product **60** resonate as a doublet at δ 1.23 ppm and in the ^{13}C NMR spectrum, the signal correspond to methyl carbon resonated at δ 17.1 ppm. It was further confirmed by HRMS, which displayed a peak at *m/z* 242.1532 corresponding to molecular formula $C_{16}H_{20}ON$ $[M+H]^{+}$, with calculated value *m/z* 242.1539. To incorporate the chloroethyl moiety on nitrogen, firstly the secondary amine **60** was *N*-alkylated with ethyl bromoacetate in the presence of potassium carbonate as a base to obtain a tertiary amine product **61** in 66% yield. In the ^{13}C NMR spectrum of **61**, the characteristic peak of ester carbonyl carbon resonated at δ 172.5 ppm and in the IR spectrum of **61**, the absorption band of ester carbonyl group displayed at 1724 cm^{-1} confirmed the formation of product **61**. Reduction of aminoester **61** using LAH in anhydrous THF for 3 h gave amino alcohol **47** in 72% yield. In the ^{13}C NMR spectrum of **47**, the disappearance of ester carbonyl carbon signal at δ 172.5 ppm and appearance of the absorption band of the primary hydroxyl group in the IR spectrum of **47** at 3323 cm^{-1} indicates the formation of amino alcohol derivative **47**. Finally, the hydroxyl group of **47** was replaced by a chloride employing thionyl chloride in dry benzene under HCl (g) condition for 8 h furnished (*R*)-phenoxybenzamine HCl *R*-**2** in 56% with an overall yield of 10.5%, $[\alpha]^{22}_{D}$ +18.2 (*c* 4.0, EtOH) {lit.[30] $[\alpha]^{22}_{D}$ + 18.0 (*c* 4.0, EtOH)}. The physical and spectroscopic data were in full agreement with the literature.[30]

1.2.12. Conclusion

In conclusion, a practical and efficient route for the synthesis of active enantiomer of antihypertensive agent (*R*)-phenoxybenzamine HCl *R*-**2** has been described *via* controlled reductive ring opening of chiral aziridine intermediate as a key step. The merits of the present approach being high enantioselectivity, simple procedures and ready availability of the starting materials. The synthetic strategy described herein has significant potential for the synthesis of a variety of other biologically important nitrogen-containing compounds.

1.2.13. Experimental procedure

1) (*S*)-2-(phenoxymethyl)oxirane (54)



To a stirred solution of phenol **36** (2 g, 0.0212 mol) in anhydrous acetone (20 mL) was added potassium carbonate (5.87 g, 0.0425 mol), (*R*)-epichlorohydrin (2.5 mL, 0.0318 mol) under an inert atmosphere and refluxed for 16 h. When the reaction was complete (TLC), the reaction mixture was filtered, washed with acetone and concentrated the solvent. The crude residue was dissolved in ethyl acetate (3 x 20 mL) and extracted with 1 M NaOH (3 x 10 mL) and the collected organic layers were washed with brine (2 x 10 mL), dried over $NaSO_4$ and concentrated under reduced pressure. The crude residue was purified by column chromatography [silica-gel, petroleum ether/acetone (98:2)] afforded **54** as pale yellow colored oil.

Yield: 2.61 g, 82%;

Molecular Formula: $C_9H_{10}O_2$;

Specific rotation: $[\alpha]^{25}_D$ = + 5.1 (*c* 7.5, $CHCl_3$) {lit.[31] $[\alpha]^{23}_D$ = + 5.2 (*c* 7.5, $CHCl_3$)};

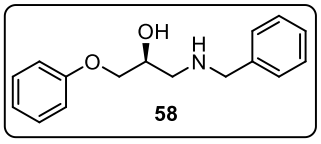
IR ($CHCl_3$, cm^{-1}): ν_{max} 3436, 3020, 1600, 1497, 1215, 1045, 929, 669;

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.75 (dd, *J* = 4.9, 2.6 Hz, 1 H), 2.88-2.92 (m, 1 H), 3.32-3.40 (m, 1 H), 3.95 (dd, *J* = 11.0, 5.7 Hz, 1 H), 4.22 (dd, *J* = 11.1, 3.2 Hz, 1 H), 6.91-7.03 (m, 3 H), 7.26-7.36 (m, 2 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 158.5 (C), 129.5 (CH, 2 carbons), 121.2 (CH), 114.6 (CH, 2 carbons), 68.7 (CH_2), 50.1 (CH), 44.7 (CH_2);

HRMS (ESI): *m/z* calculated for $C_{18}H_{21}O_4$ $[M+H]^+$ 301.1434, found 301.1405.

2) (*S*)-1-(benzylamino)-3-phenoxypropan-2-ol (58)



58

To (*S*)-2-(phenoxymethyl)oxirane **54** (2.5 g, 0.0166 mol) and LiBr (cat.) was added dropwise benzylamine (3.64 mL, 0.0332 mol) and the reaction mixture was stirred at room temperature for 9 h. After completion of the reaction (TLC), H_2O (5 mL) was added and the mixture was extracted with diethyl ether (2 x 10 mL). The organic layers were combined, washed with brine (2 x 5 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified over column chromatography [silica-gel, EtOAc/petroleum ether (30:70)] afforded **58** as a colorless solid.

Yield: 3.55 g, 83 %;

MP: 73-74 °C;

Molecular Formula: $C_{16}H_{19}NO_2$;

Specific rotation: $[\alpha]^{25}_D$ = -6.3 (*c* 1.05, $CHCl_3$);

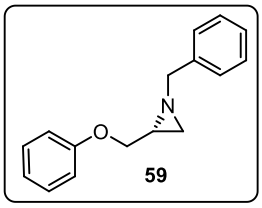
IR ($CHCl_3$, cm^{-1}): ν_{max} 3593, 3445, 3322, 3059, 3016, 2945, 2962, 1666, 1590, 1495, 1481, 1433, 1206, 1127, 1049, 719, 676;

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.46 (bs, 2 H), 2.80-2.85 (m, 1 H), 2.91-2.95 (m, 1 H), 3.84-3.91 (m, 2 H), 3.98 (d, *J* = 5.0 Hz, 2 H), 4.09-4.15 (m, 1 H), 6.90-6.99 (m, 3 H), 7.26-7.38 (m, 7 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 158.6 (C), 139.8 (C), 129.4 (CH, 2 carbons), 128.4 (CH, 2 carbons), 128.1 (CH, 2 carbons), 127.1 (CH), 121.0 (CH), 114.5 (CH, 2 carbons), 70.3 (CH_2), 68.4 (CH), 53.7 (CH_2), 51.2 (CH_2);

HRMS (ESI): *m/z* calculated for $C_{16}H_{20}O_2N$ $[M+H]^+$ 258.1489, found 258.1490.

3) (*R*)-1-benzyl-2-(phenoxymethyl)aziridine (59)



59

A solution of DIAD (3.44 mL, 0.0174 mol) was added slowly to a stirred solution of amino alcohol **54** (3 g, 0.0116 mol), triphenylphosphine (6.12 g, 0.0233 mol), in anhydrous Toluene (10 mL) at 0 °C under inert atmosphere and the resulting solution was stirred for 30 min. The reaction mixture was then refluxed for 16 h. After completion of the reaction (TLC), H_2O (10 mL) was added and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were washed with brine (2 x 5 mL), dried over $NaSO_4$ and concentrated under reduced pressure. The crude residue was purified by column chromatography [silica-gel, EtOAc/petroleum ether (18:82)] gave **59** as a colorless liquid.

Yield: 2.25 g, 78%.

Molecular Formula: $C_{16}H_{17}NO$;

Specific rotation: $[\alpha]^{25}_D$ = + 5.9 (*c* 1.0, $CHCl_3$);

Chiral HPLC: ee >98% [The ee of **59** was determined by chiral HPLC analysis; Chiralcel OD-H (250 X 4.6 mm) column; eluent: n-hexane/isopropanol (98:2); flow rate: 1 mL/min; detector 220 nm; (*R*)-isomer t_R= 16.0 min.; (*S*)-isomer t_R= 11.64 min.];

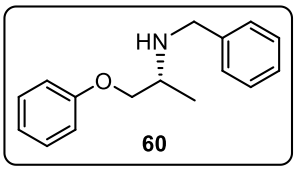
IR ($CHCl_3$, cm^{-1}): ν_{max} 3324, 3060, 2983, 2829, 2073, 1735, 1707, 1599, 1586, 1497, 1692, 1350, 1240, 1033, 733, 694;

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.59 (d, *J* = 6.4 Hz, 1 H), 1.87 (d, *J* = 2.7 Hz, 1 H), 1.98-2.04 (m, 1 H), 3.47-3.58 (m, 2 H), 3.97 (d, *J* = 5.5 Hz, 2 H), 6.89-6.96 (m, 3 H), 7.25-7.30 (m, 3 H), 7.33-7.40 (m, 4 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 158.6 (C), 138.8 (C), 129.4 (CH, 2 carbons), 128.3 (CH, 2 carbons), 128.0 (CH, 2 carbons), 127.1 (CH), 120.7 (CH), 114.5 (CH, 2 carbons), 70.0 (CH_2), 64.2 (CH_2), 37.9 (CH), 32.0 (CH_2);

HRMS (ESI): *m/z* calculated for $C_{16}H_{18}ON$ $[M+H]^+$ 240.1383, found 240.1378.

4) (*R*)-*N*-benzyl-1-phenoxypropan-2-amine (60)



60

To a stirred solution of aziridine **59** (2 g, 0.0083 mol) in methanol (20 mL) was added 10% Pd/activated carbon (0.2 g) and the mixture was vigorously stirred under hydrogen pressure (50 psi) at room temperature for 45 min. After completion of the reaction (TLC), the catalyst was filtered through a bed of Celite and washed with methanol (10 mL). The filtrate was evaporated under reduced pressure and purified by column chromatography [silica-gel, EtOAc/petroleum ether (14:86)] gave **60** as a colorless oil.

Yield: 1.45 g, 72%;

Molecular Formula: $C_{16}H_{19}NO$;

Specific rotation: $[\alpha]^{22}_D$ = +10.7 (*c* 4.0, EtOH) {lit.[24] $[\alpha]^{22}_D$ = +10.2 (*c* 4.0, EtOH)};

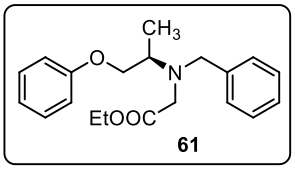
IR ($CHCl_3$, cm^{-1}): ν_{max} 3433, 3327, 3059, 3032, 2962, 2962, 1724, 1597, 1494, 1458, 1481, 1244, 1074, 1039, 725, 696;

^{1}H NMR (400 MHz $CDCl_3$): δ 1.23 (d, *J* = 8.0 Hz, 3 H), 2.70 (bs, 1 H), 3.16-3.23 (m, 1 H), 3.83-3.98 (m, 4 H), 6.91-6.98 (m, 3 H), 7.25-7.39 (m, 7 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 158.7 (C), 140.0 (C), 129.4 (CH, 2 carbons), 128.4 (CH, 2 carbons), 128.1 (CH, 2 carbons), 127.0 (CH), 120.8 (CH), 114.5 (CH, 2 carbons), 71.7 (CH_2), 51.7 (CH), 51.1 (CH_2), 17.1 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{16}H_{20}ON$ $[M+H]^+$ 242.1539, found 242.1532.

5) (*R*)-ethyl-2-(benzyl(1-phenoxypropan-2-yl)amino)acetate (61)



61

A solution of ethyl-2-bromoacetate (2.22 mL, 0.0199 mol) in anhydrous DMF (3 mL) was added slowly to a stirred solution of amine **60** (1.2 g, 0.0049 mol), K_2CO_3 (0.330 g, 2.39 mmol) in anhydrous DMF (2 mL) was added dropwise and heated to reflux at 80 °C for 12 h. When the reaction was complete (TLC), H_2O (3 x 10 mL) was added and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were washed with brine (2 x 5 mL), dried over $NaSO_4$ and concentrated under reduced pressure. The crude residue was purified by column chromatography [silica-gel, EtOAc/petroleum ether (10:90)] afforded **61** as a colorless liquid.

Yield: 1.07 g, 66%;

Molecular Formula: $C_{20}H_{25}NO_3$;

Specific rotation: $[\alpha]^{25}_D$ = +33.4 (*c* 0.92, $CHCl_3$);

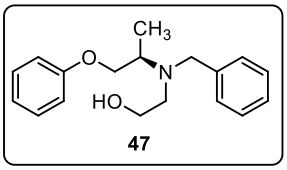
IR ($CHCl_3$, cm^{-1}): ν_{max} 3194, 3080, 2962, 2905, 2253, 1724, 1646, 1554, 1470, 1393, 1264, 1191, 1160, 1003, 911, 720, 650;

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.21-1.24 (m, 3 H), 1.25-1.27 (m, 3 H), 3.31-3.37 (m, 1 H), 3.40-3.54 (m, 2 H), 3.89-3.97 (m, 3 H), 4.05-4.13 (m, 3 H), 6.86-6.96 (m, 3 H), 7.24-7.33 (m, 5 H), 7.41-7.43 (m, 2 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 172.5 (CO), 158.8 (C), 139.8 (C), 129.4 (CH, 2 carbons), 128.6 (CH, 2 carbons), 128.2 (CH, 2 carbons), 127.0 (CH), 120.6 (CH), 114.5 (CH, 2 carbons), 70.5 (CH_2), 60.3 (CH), 55.3 (CH_2), 54.7 (CH_2), 52.1 (CH_2), 14.2 (CH_3), 14.1 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{20}H_{26}O_3N$ $[M+H]^+$ 328.1907, found 328.1902.

6) (*R*)-2-(benzyl(1-phenoxypropan-2-yl)amino)ethan-1-ol (47)



47

A solution of ester **61** (0.900 g, 0.0027 mol) in anhydrous THF (3 mL) was added drop wise

to a suspension of $LiAlH_4$ (0.208 g, 0.0054 mol) in anhydrous THF (15 mL) at 0 °C and the mixture was stirred at room temperature for 2 h. The reaction mixture was cooled to 0 °C and cold water (1 ml) was added drop wise followed by the addition of 3 M KOH solution (1 ml). After being stirred at room temperature for 30 min, the mixture was filtered through the bed of Celite and concentrated under reduced pressure. The crude residue was purified by column chromatography [silica-gel, EtOAc/petroleum ether (5:95)] afforded **47** as a colorless oil.

Yield: 0.562 g, 72%;

Molecular Formula: $C_{18}H_{23}NO_2$;

Specific rotation: $[\alpha]^{25}_D$ = + 15.2 (*c* 4.0, EtOH) {lit.[26] $[\alpha]^{25}_D$ = + 15.0 (*c* 4.0, EtOH)};

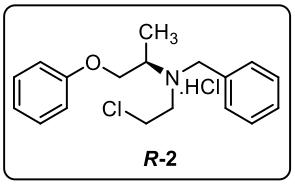
IR ($CHCl_3$, cm^{-1}): ν_{max} 3323, 3061, 3061, 3028, 2972, 2928, 2872, 1799, 1599, 1494, 1379, 1244, 1170, 1059, 1039;

^{1}H NMR (400 MHz , $CDCl_3$): δ 1.16 (d, *J* = 6.7 Hz, 3 H), 2.72-2.77 (m, 1 H), 2.84-2.90 (m, 1 H), 3.31-3.39 (m, 1 H), 3.47-3.52 (m, 1 H), 3.55-3.61 (m, 1 H), 3.70 (d, *J* = 13.7 Hz, 1 H), 3.87-3.90 (m, 2 H), 4.00-4.04 (m, 1 H), 6.92 (d, J = 7.6 Hz, 2 H), 6.96-7.00 (m, 2 H), 7.25-7.38 (m, 7 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 158.5 (C), 139.7 (C), 129.4 (CH, 2 carbons), 128.6 (CH, 2 carbons), 128.3 (CH, 2 carbons), 127.0 (CH), 120.8 (CH), 114.4 (CH, 2 carbons), 69.5 (CH_2), 58.7 (CH_2), 54.6 (CH_2), 53.3 (CH), 51.0 (CH_2), 11.7 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{18}H_{24}O_2N$ $[M+H]^+$ 286.1802, found 286.1794.

7) (*R*)-*N*-benzyl-*N*-(2-chloroethyl)-1-phenoxypropan-2-amine hydrochloride (*R*-2)



***R*-2**

To a pre-cooled (0 °C) solution of alcohol **47** (0.3 g, 0.0011 mol) in anhydrous benzene (20 mL), HCl gas was bubbled slowly for 15 min. Then $SOCl_2$ (0.1 mL, 0.0014 mol) in anhydrous benzene (5 mL) was added dropwise and the resulting mixture was refluxed for 8 h. The excess thionyl chloride was removed under reduced pressure. The residue was basified with a $NaHCO_3$ solution and extracted with diethyl ether (2 x 10 mL). Removal of the solvent afforded the crude product which was purified by flash chromatography [silica-

gel, EtOAc/petroleum ether (5:95)] afforded (*R*)-phenoxybenzamine hydrochloride *R*-**2** as a colorless solid.

Yield: 0.28 g, 56%;

MP: 125-26 °C (lit.[26] mp: 123-24 °C);

Molecular Formula: $C_{18}H_{22}ClNO$;

Specific rotation: $[\alpha]^{22}_{D}$ = +18.2 (*c* 4.0, EtOH) {lit.[30] $[\alpha]^{22}_{D}$ = + 18.0 (*c* 4.0, EtOH)};

Chiral HPLC: ee >99% after recrystallization in MeOH [The ee of *R*-**2** was determined by chiral HPLC analysis; Chiralcel OJ-H (250 X 4.6 mm) column; eluent: petroleum ether/isopropanol (90:10); flow rate: 1 mL/min; detector 254 nm; (*R*)-isomer t_R = 14.42 min.; (*S*)-isomer t_R = 9.10 min.];

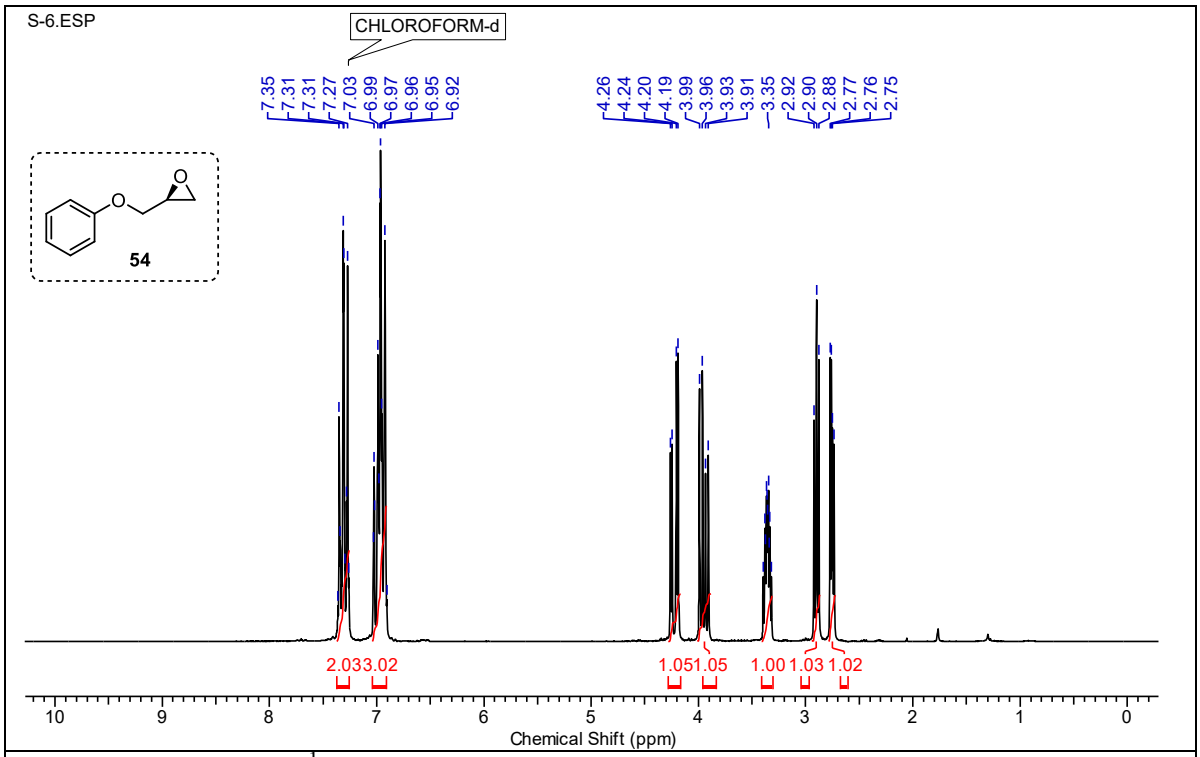
IR ($CHCl_3$, cm^{-1}): ν_{max} 3063, 2958, 2928, 2884, 1726, 1595, 1492, 1458, 1284, 1246, 1126, 1074, 1035, 750, 696;

^{1}H NMR (400 MHz, $CDCl_3$): δ1.20 (d, *J* = 6.8 Hz, 3 H), 2.91-3.05 (m, 2 H), 3.22-3.30 (m, 1 H), 3.39 (apparent t, *J* = 7.3 Hz, 2 H), 3.76-3.89 (m, 3 H), 4.02-4.06 (m, 1 H), 6.90 (d, *J* = 8.1 Hz, 2 H), 6.94-6.97 (m, 1 H), 7.25-7.39 (m, 7 H);

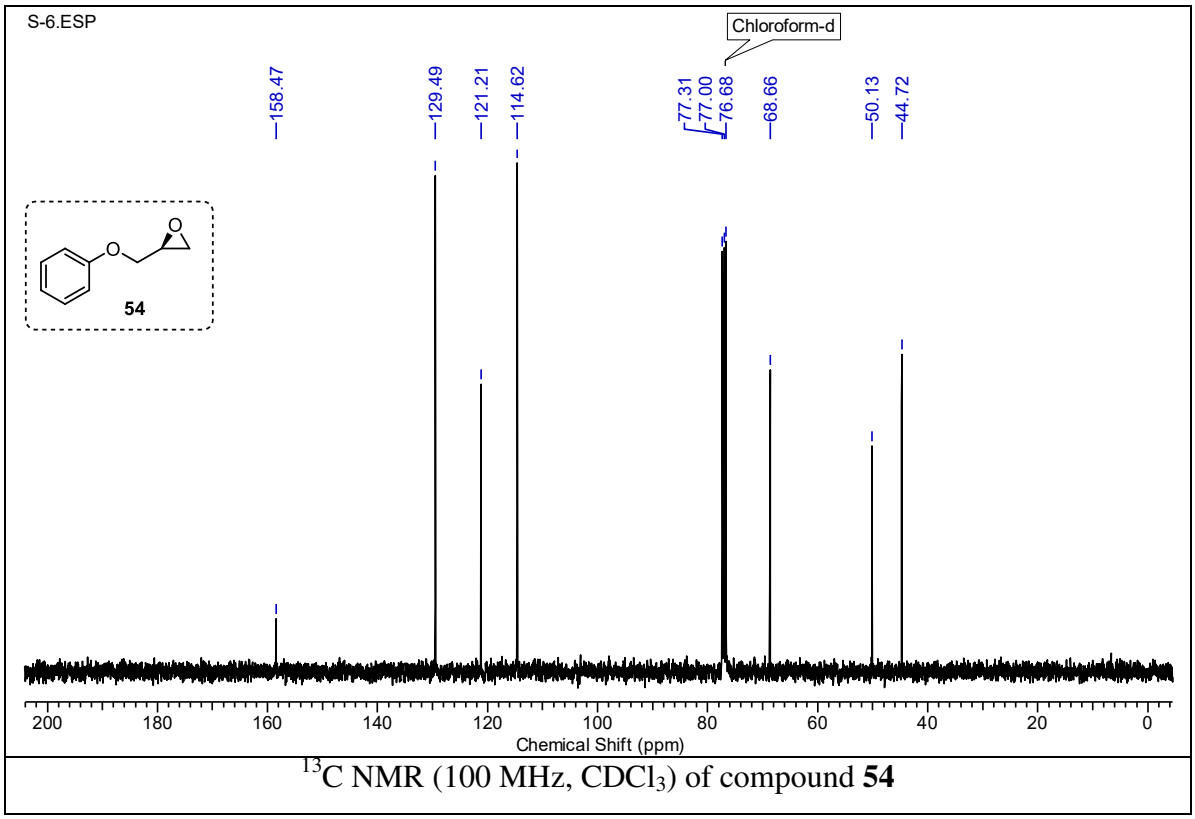
^{13}C NMR (100 MHz, $CDCl_3$): δ 158.8 (C), 140.3 (C), 129.4 (CH, 2 carbons), 128.4 (CH, 2 carbons), 128.3 (CH, 2 carbons), 127.0 (CH), 120.7 (CH), 114.4 (CH, 2 carbons), 70.3 (CH_2), 55.8 (CH_2), 54.8 (CH), 53.0 (CH_2), 43.0 (CH_2), 13.3 (CH_3);

MS (*m/z*): 322 $[M+NH_4]^+$, 268 $[M-Cl]^+$;

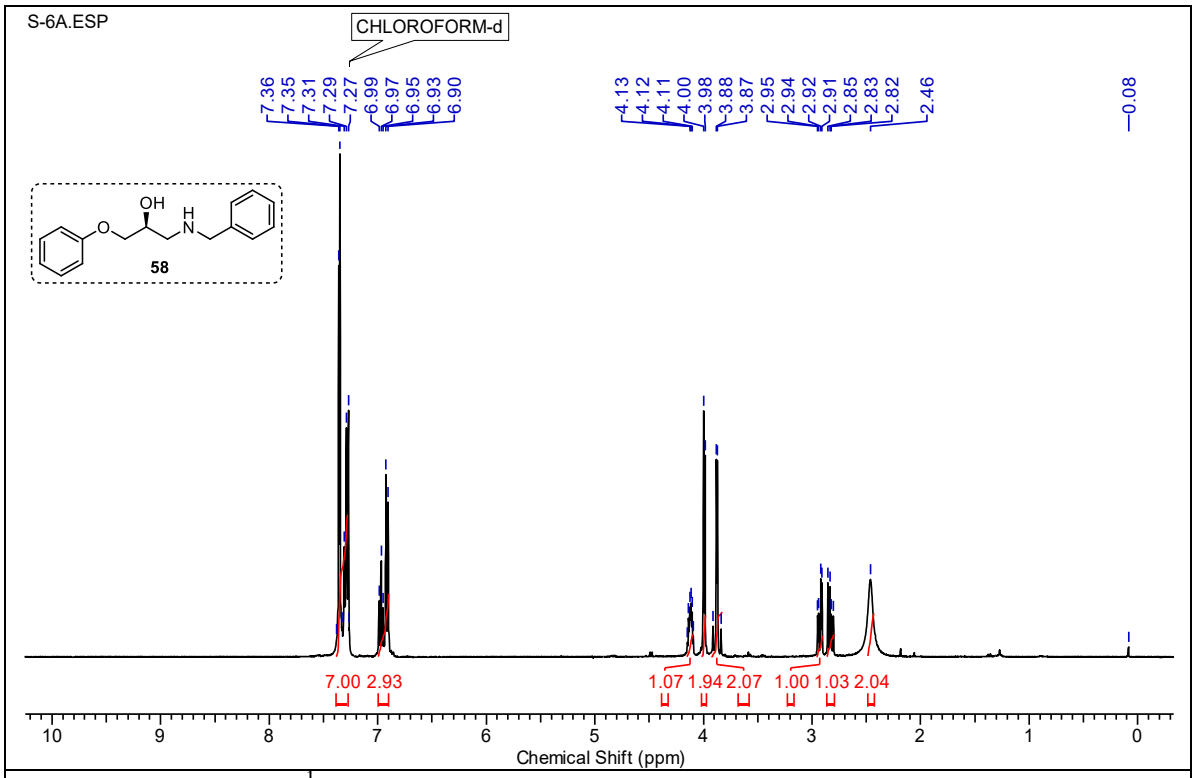
1.2.14. Spectra



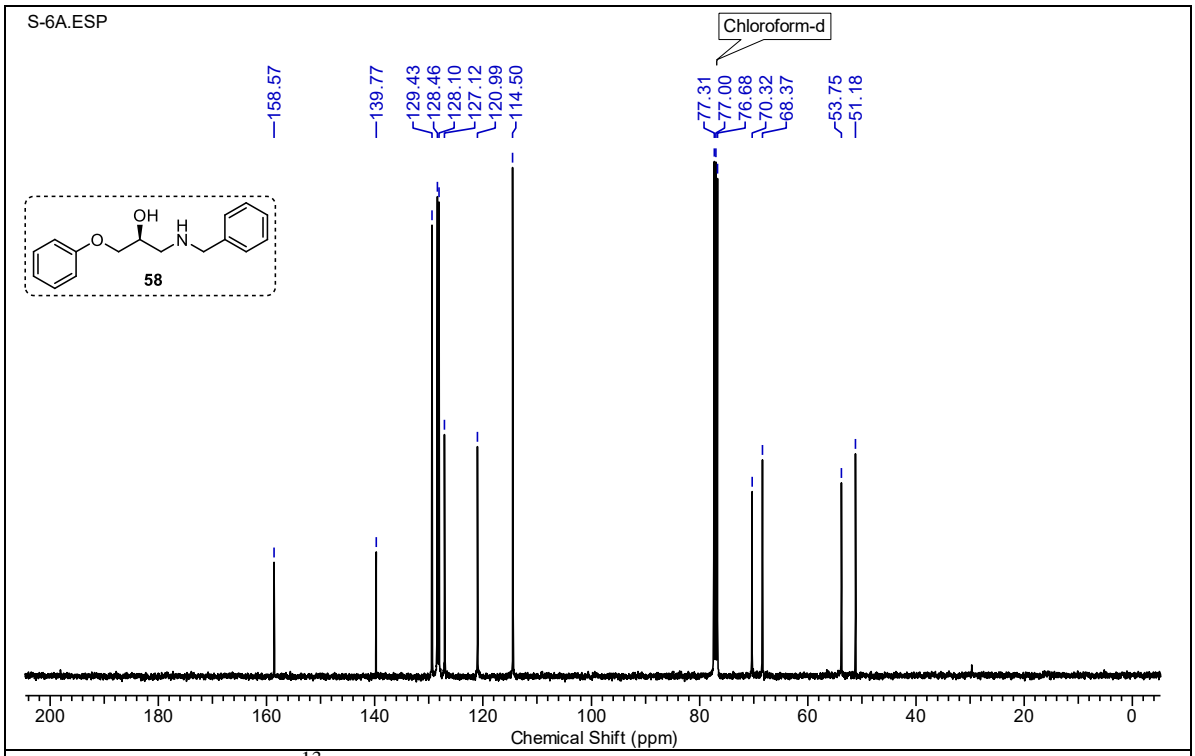
^{1}H NMR (200 MHz, $CDCl_3$) of compound **54**



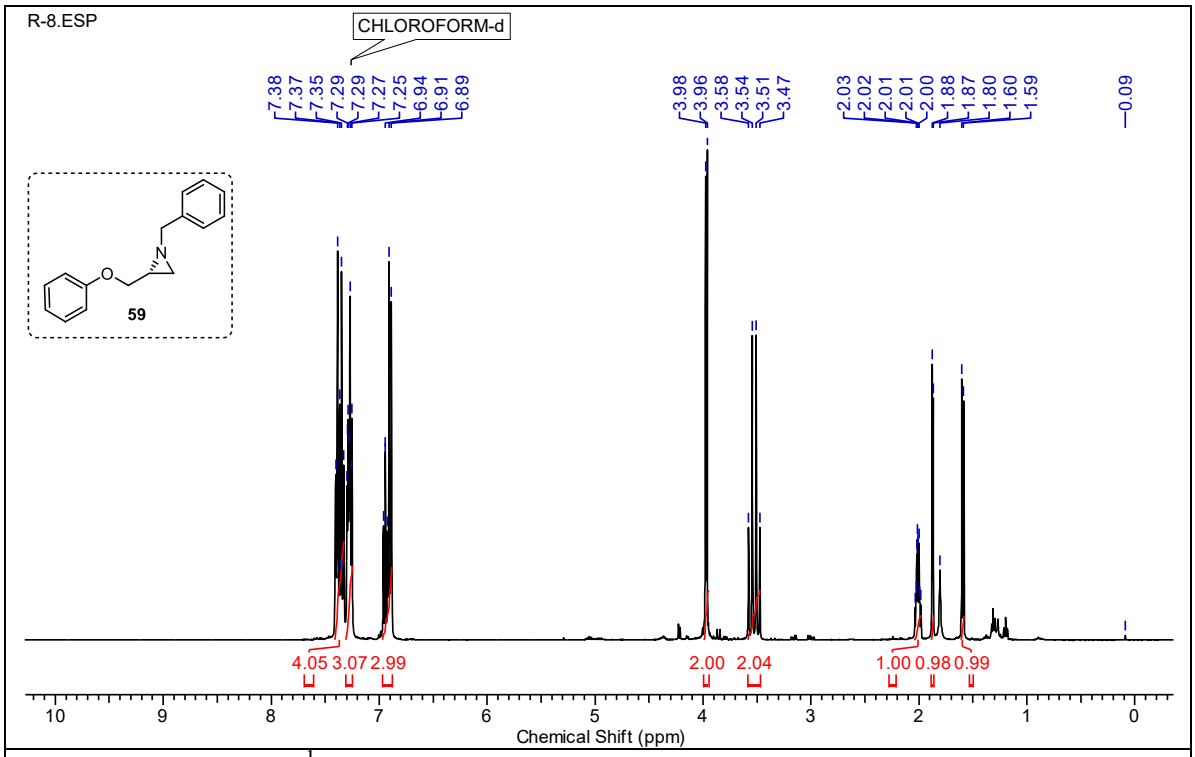
^{13}C NMR (100 MHz, $CDCl_3$) of compound **54**



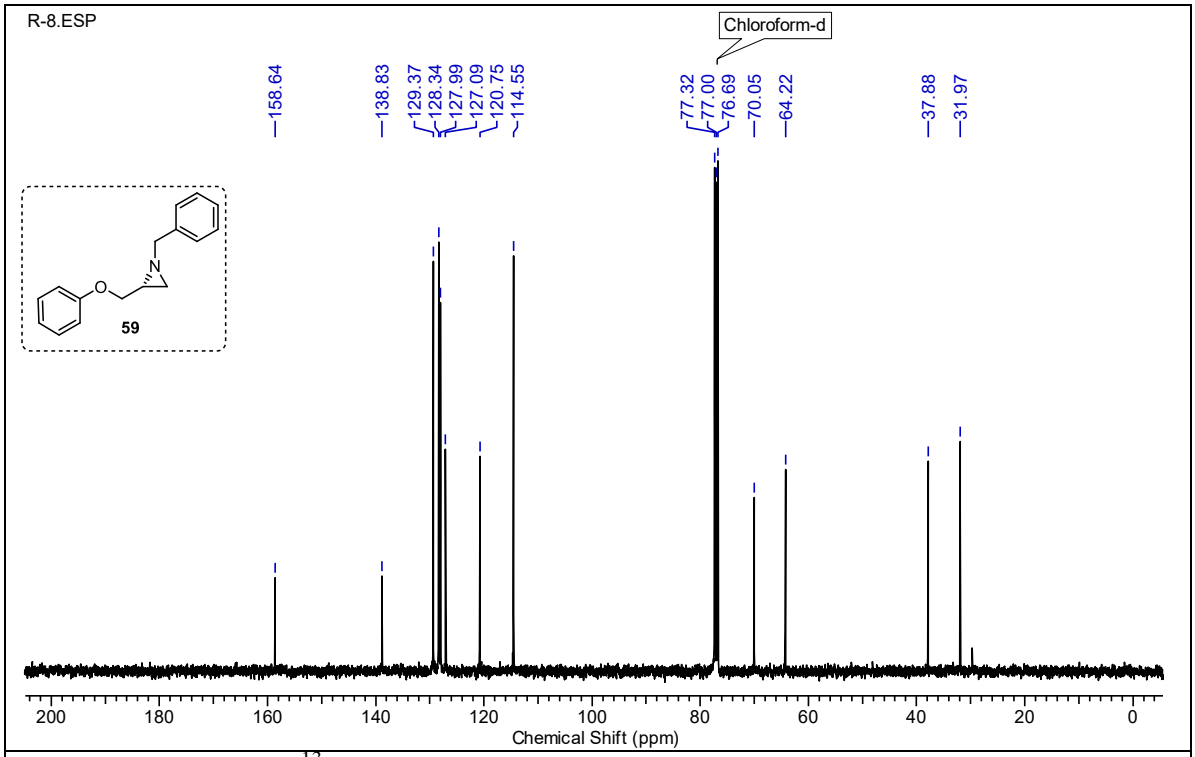
^{1}H NMR (400 MHz, $CDCl_3$) of compound **58**



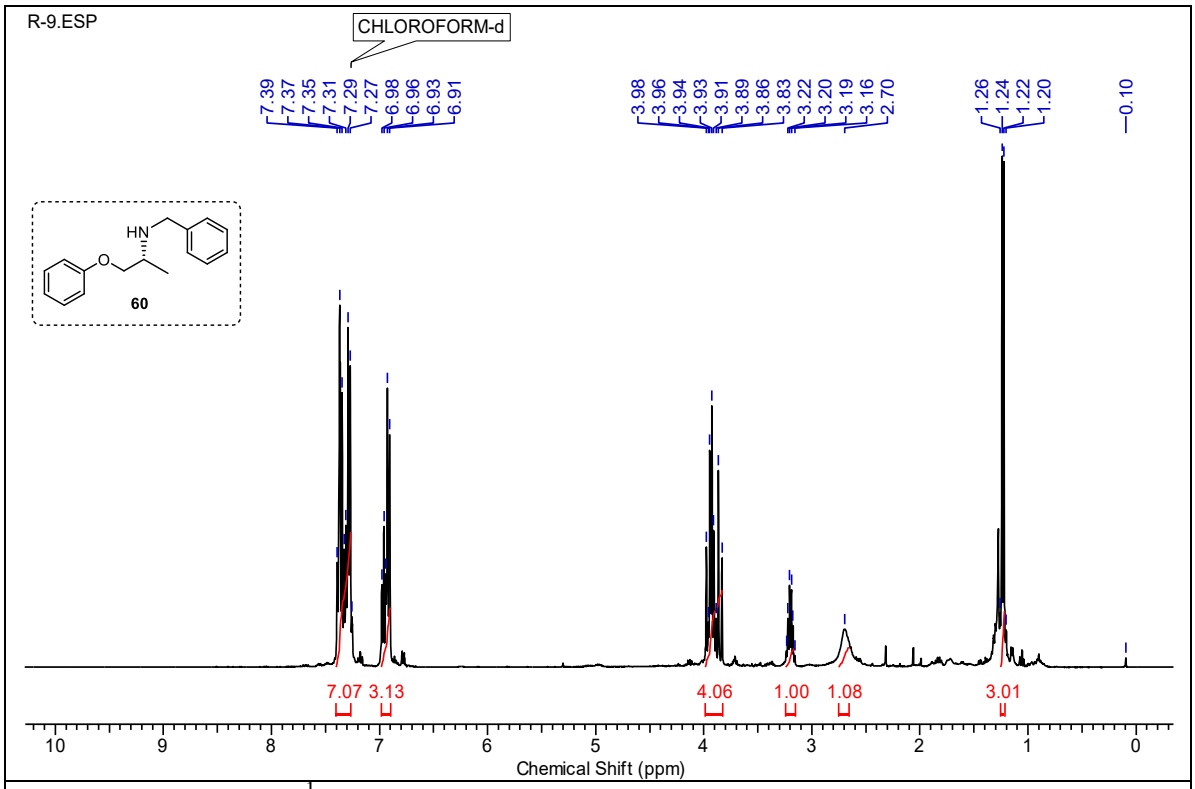
^{13}C NMR (100 MHz, $CDCl_3$) of compound **58**



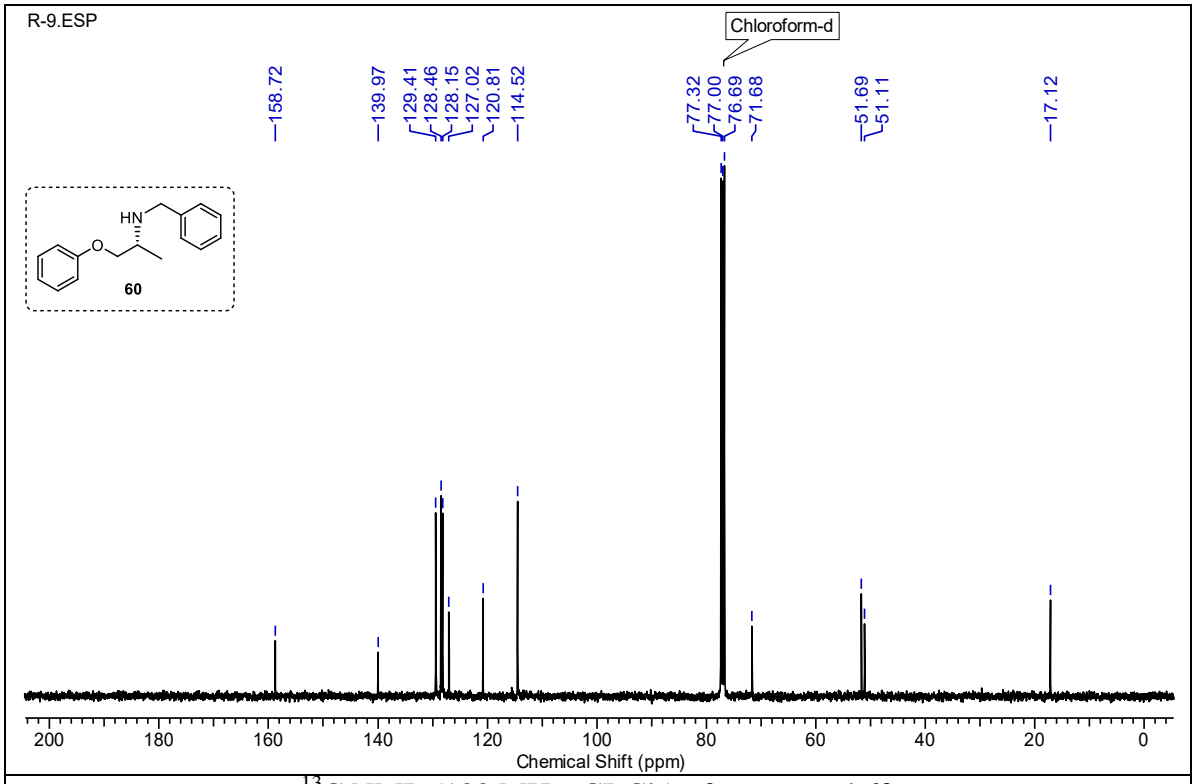
^{1}H NMR (400 MHz, $CDCl_3$) of compound **59**



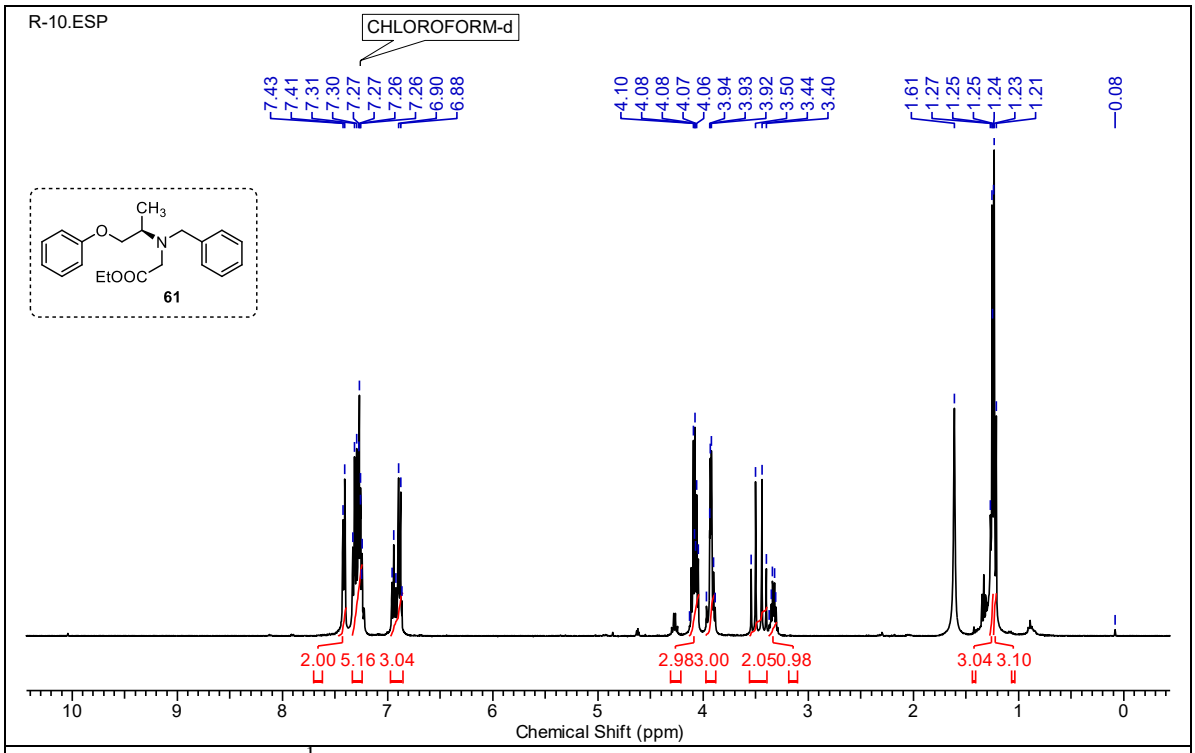
^{13}C NMR (100 MHz, $CDCl_3$) of compound **59**



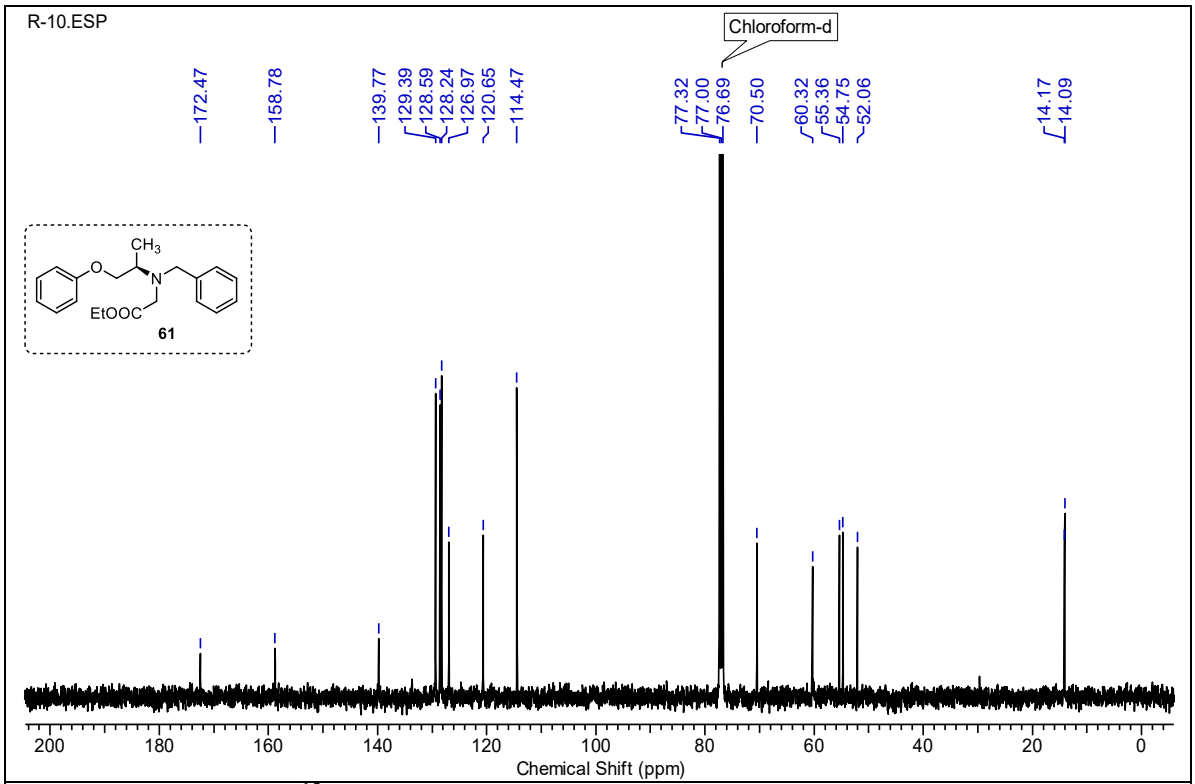
^{1}H NMR (400 MHz, $CDCl_3$) of compound **60**



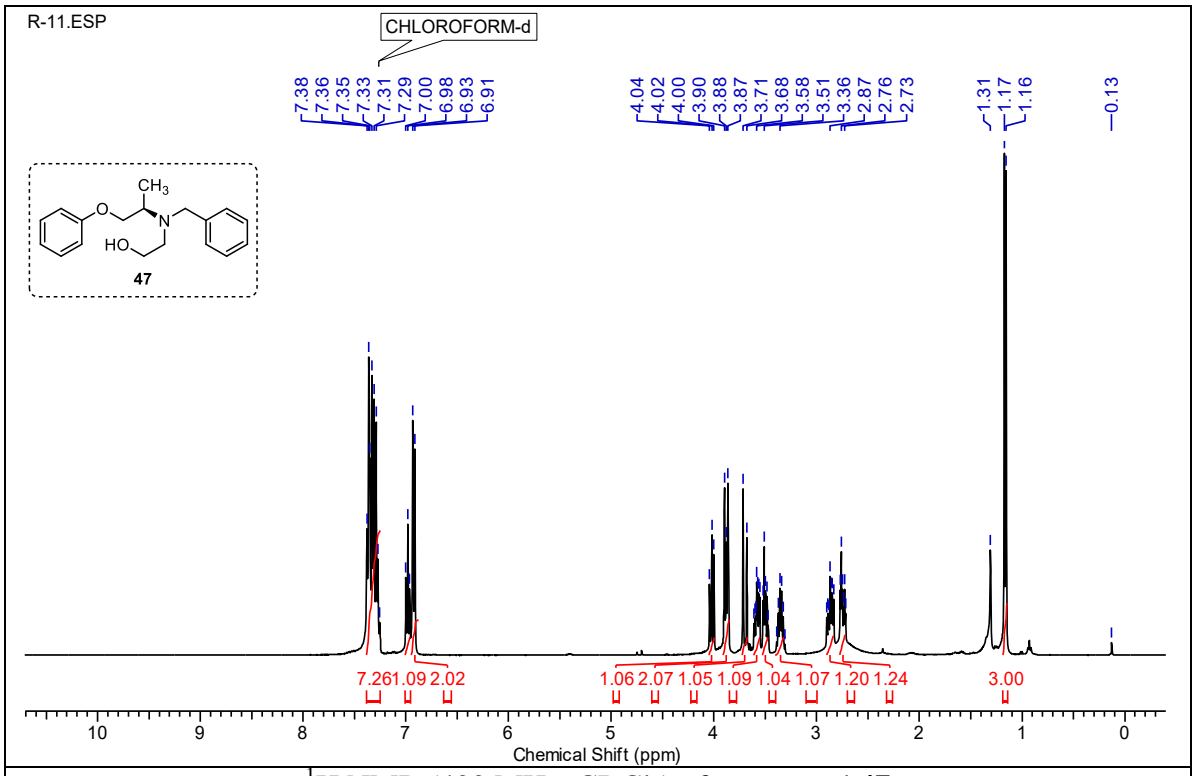
^{13}C NMR (100 MHz, $CDCl_3$) of compound **60**



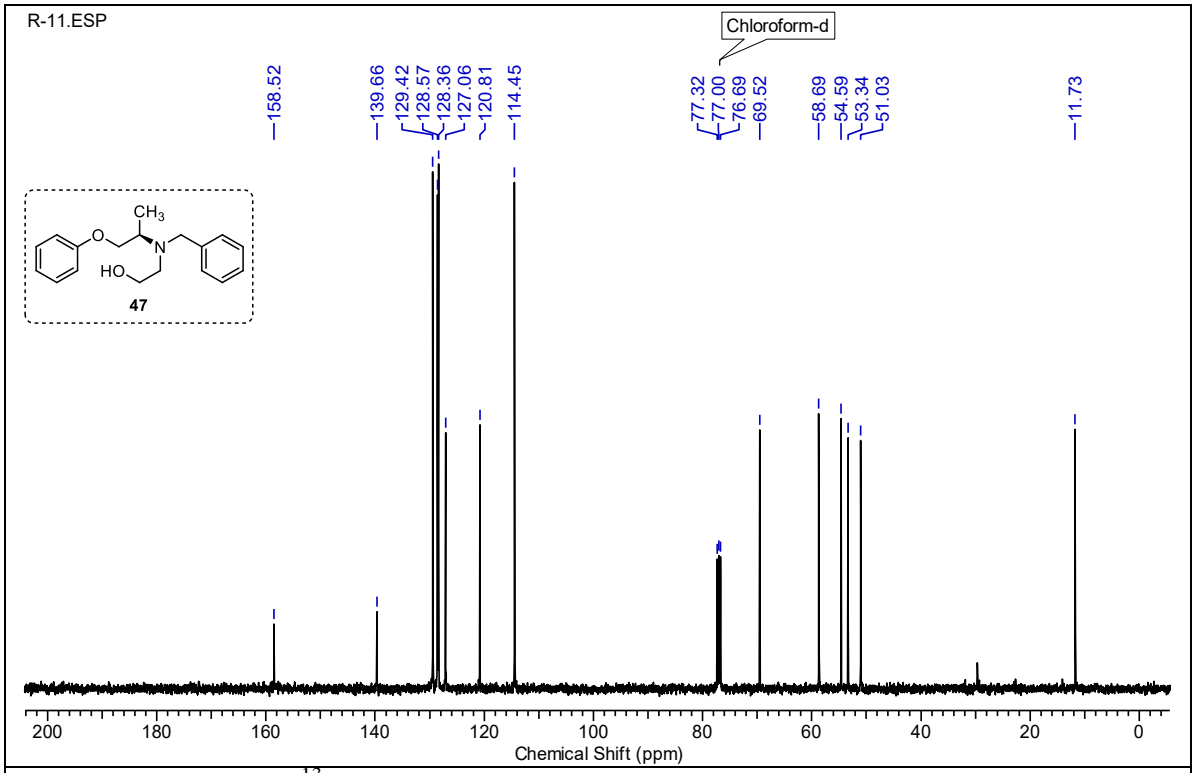
^{1}H NMR (400 MHz, $CDCl_3$) of compound **61**



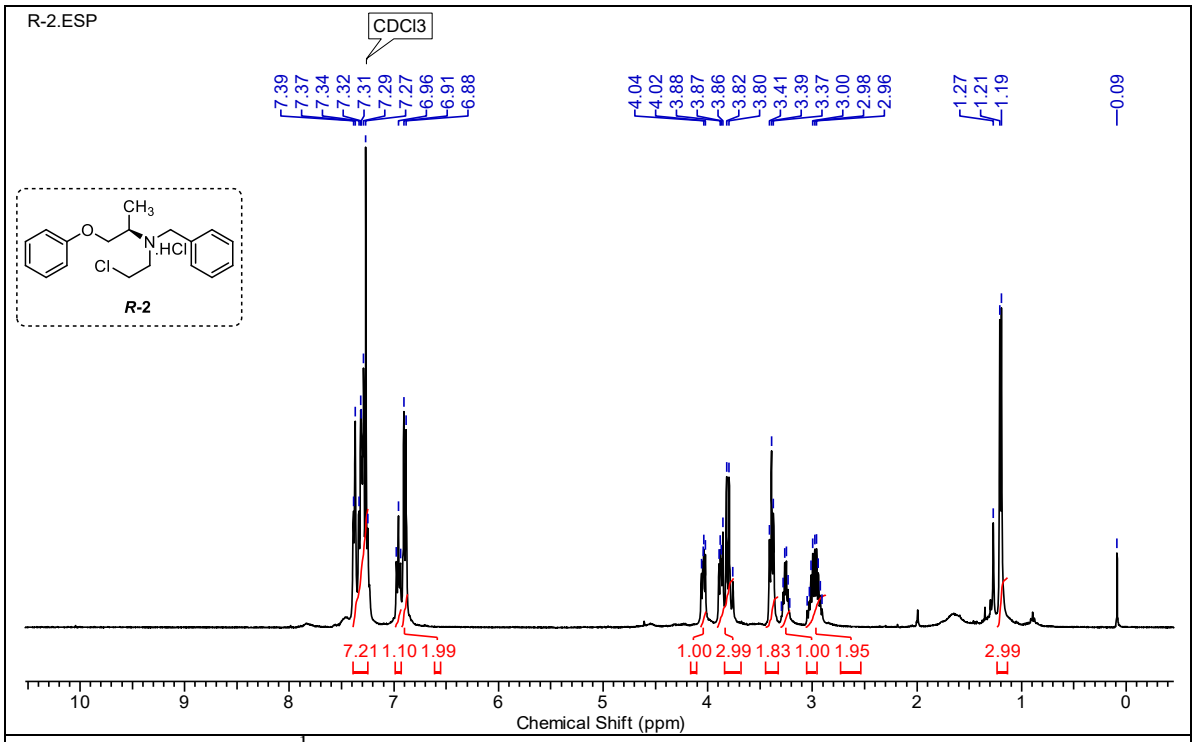
^{13}C NMR (100 MHz, $CDCl_3$) of compound **61**



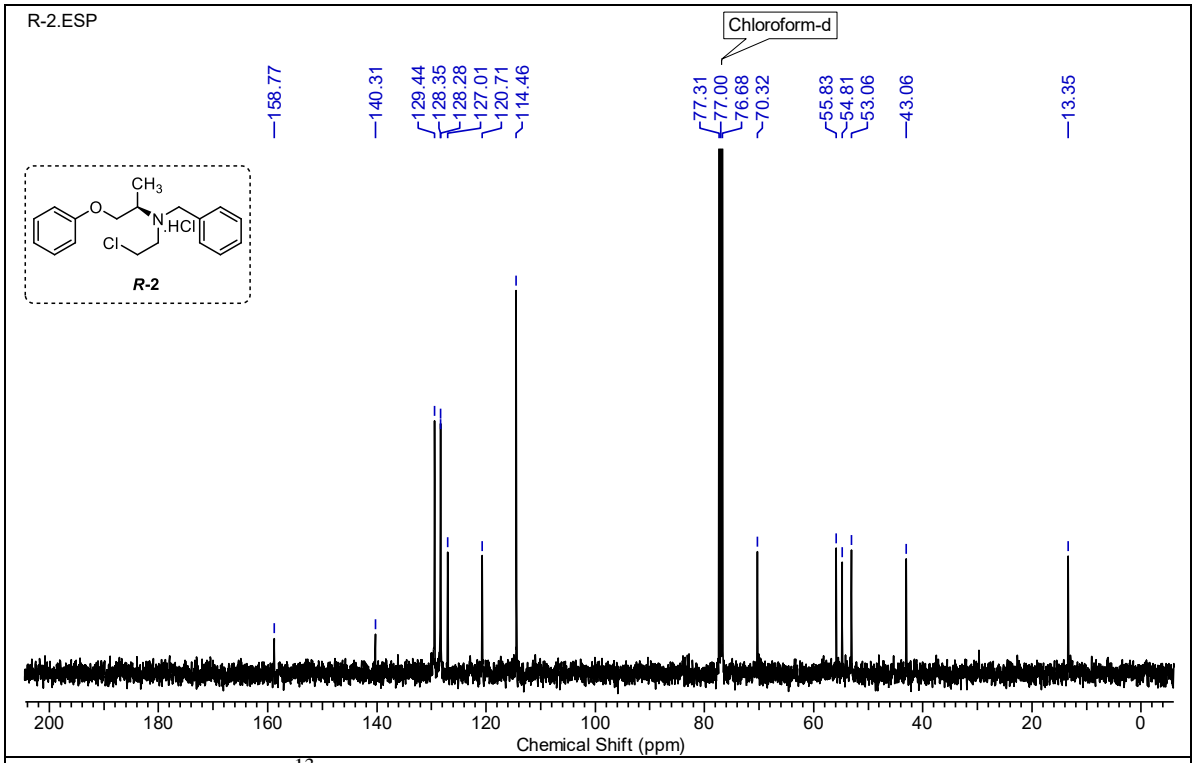
^{1}H NMR (400 MHz, $CDCl_3$) of compound **47**



^{13}C NMR (100 MHz, $CDCl_3$) of compound **47**



^{1}H NMR (400 MHz, $CDCl_3$) of compound *R*-**2**



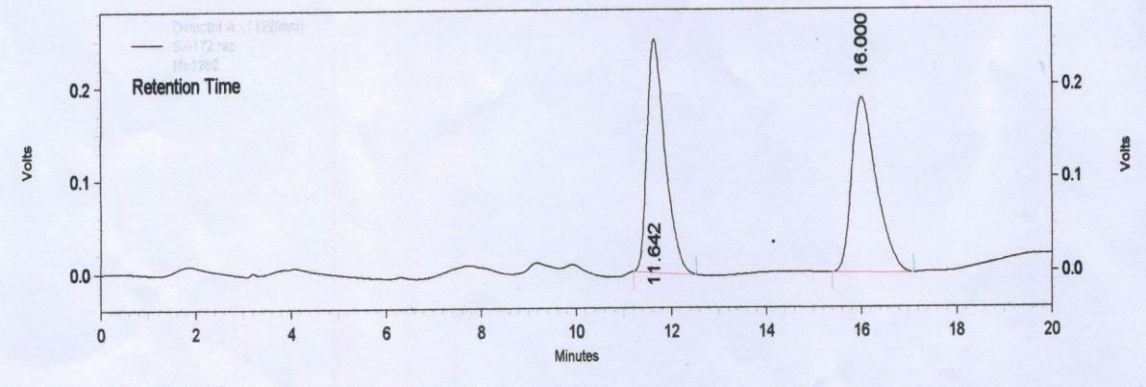
^{13}C NMR (100 MHz, $CDCl_3$) of compound *R*-**2**

1.2.15. Chiral HPLC analysis data

Chiral HPLC analysis of Compound 59

Conditions: Chiralcel OD-H (250 X 4.6 mm) column; eluent: n-hexane/isopropanol (98:2); flow rate: 1 mL/min; detector 220 nm.

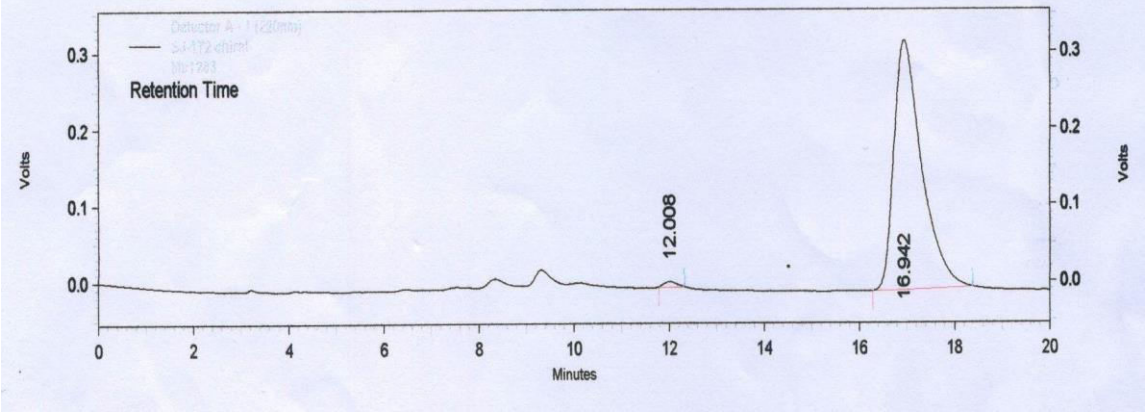
Racemic



Racemic Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	11.642	6592932	49.430
2	16.000	6745102	50.570
Totals		13338034	100.000

Chiral



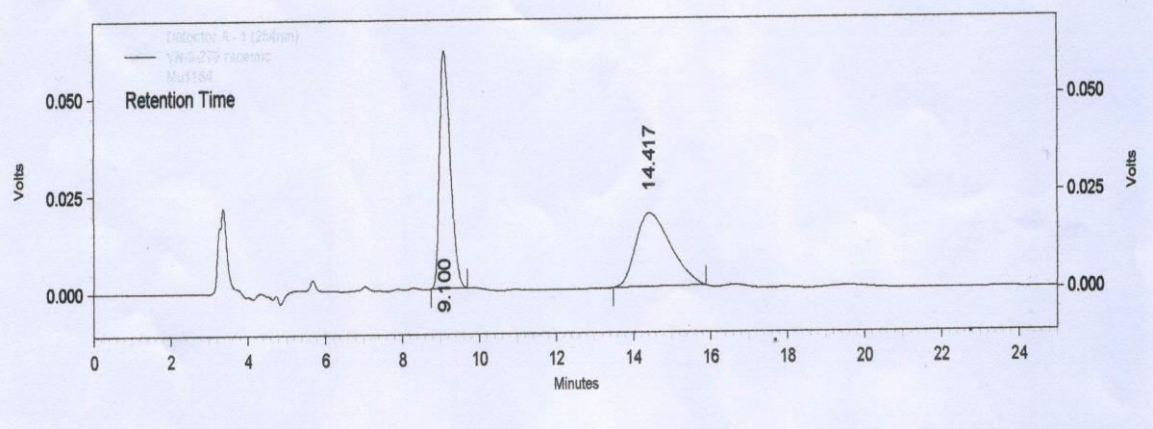
Chiral Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	12.008	112636	0.850
2	16.942	13137370	99.150
Totals		13250006	100.000

Chiral HPLC analysis of Compound *R*-2

Conditions: Chiralcel OJ-H (250 X 4.6 mm) column; eluent: pet. ether/isopropanol (90:10); flow rate: 1 mL/min; detector 254 nm.

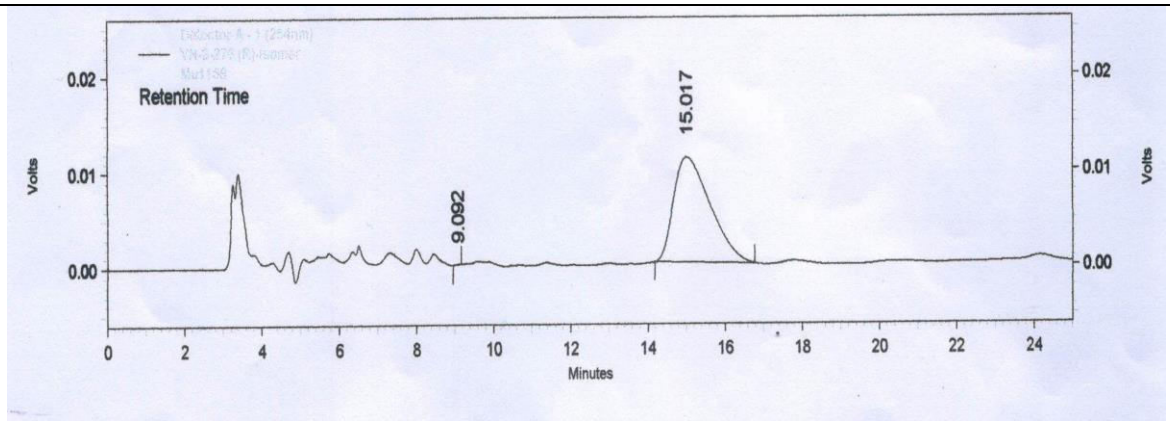
Racemic



Racemic Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	9.100	1177740	49.876
2	14.417	1183585	50.124
Totals		2361325	100.000

Chiral



Chiral Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	9.092	534	0.037
2	15.017	1457586	99.963
Totals		1458120	100.000

1.2.16. References

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1.3. SECTION 3

Efficient synthesis of optically active (*S*)-metolachlor *via* reductive ring opening of aziridine

1.3.1. Introduction

Chloroacetanilides are selective, systemic pre-emergence and early post-emergence herbicides used for effective control of most annual grass and broadleaf weeds using in corn, soybeans, peanuts, rice, sorghum, maize, cotton and in various agronomically several crops.[1] It acts as growth inhibitors by suppressing the biosynthesis of several important plant constituents like proteins, lipids, fatty acids, flavonoids and isoprenoids.[2] These are *N, N*-disubstituted aniline derivatives and usually differ by their alkyl substituents on the nitrogen atom. Alachlor, acetochlor, butachlor, metolachlor, and propachlor are most commonly and extensively used herbicides (Fig.1) and these are rapidly biodegradable and extensively metabolized in soil, plant and mammals system.[3] The mode of action of these herbicides is *via* inhibition of very long chain fatty acids and elongation in plants due to interference with the function of specific enzymes. Alachlor was a first commercialized herbicide in this chemical group and metolachlor is widely used and most abundantly applied herbicides.[4]

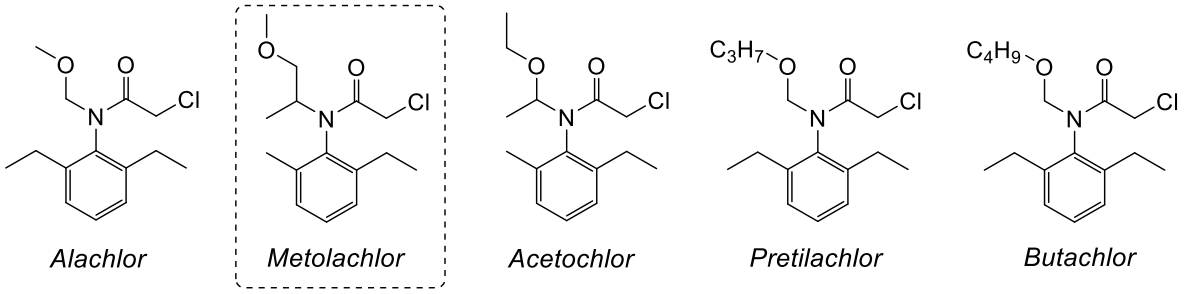


Figure 1. Chloroacetanilides based herbicide chemical family

Firstly, in 1972 metolachlor was described as an *N*-chloroacetylated, *N*-alkoxyalkylated ortho disubstituted aniline and marketed under the trade name DUAL®. It is widely used for selective weed control in maize and protecting a variety of other important

crops over 20 years in more than 70 crops across the world.[5] It comprises four stereoisomers, two of which are inactive and other two are active towards the herbicidal activity. The stereoisomers arise due to the presence of two chiral elements from the combination of a chiral centre in the aliphatic side chain and a chiral axis (atropisomerism, due to restricted rotation around the C-N) between aromatic ring and the nitrogen atom.[6] Previously, metolachlor was applied as a racemate, but later it was found that about 95% of the herbicidal activity of metolachlor exists in the two 1-*S* diastereomers which means that (*S*)-enantiomers exhibits high herbicidal activity than the (*R*)-enantiomers (**Figure 2**).[7] The herbicidal activity mainly governed by the stereogenic carbon atom in the alkyl moiety. In 1996, the active ingredient was commercialized by Novartis (formerly Ciba-Geigy). Since 1997, the racemate was replaced by enantiopure (*S*)-metolachlor which is an active ingredient of DUAL MAGNUM® to reduce the ca 40% of environment load.[8] The estimated production of this chiral grass herbicide is more than 30,000 t/year in world-wide.

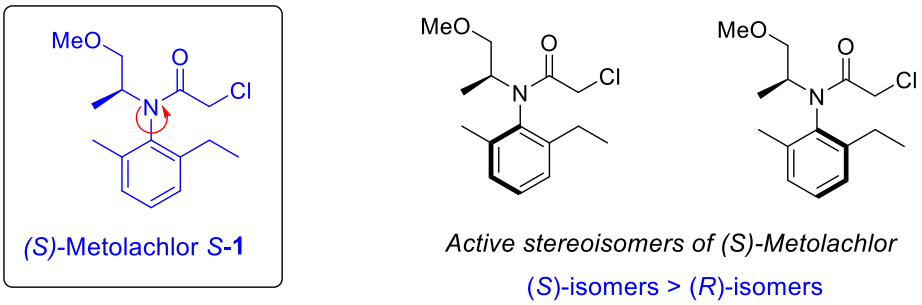


Figure 2. Active isomers of metolachlor

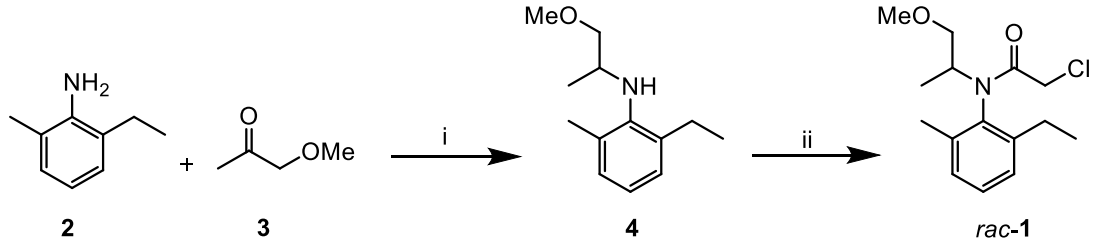
1.3.2. Review of Literature

Several approaches have been reported in the literature for the synthesis of racemic as well as optically active (*S*)-metolachlor. Most of these methods rely upon asymmetric hydrogenation as a key step.[9] A detailed report of these syntheses is described below.

Blaser's approach (1976)[10]

Blaser and co-workers reported the industrial process of *rac*-metolachlor *rac*-**1**. Thus as shown in **Scheme 1**, the reductive alkylation of 2-ethyl-6-methylaniline **2** (MEA) with aqueous methoxyacetone **3** in the presence of Pt/C and catalytic amounts of sulfuric acid at 50 °C and 5 bar to give methyl ester derivative **4** followed by *N*-acetylation with

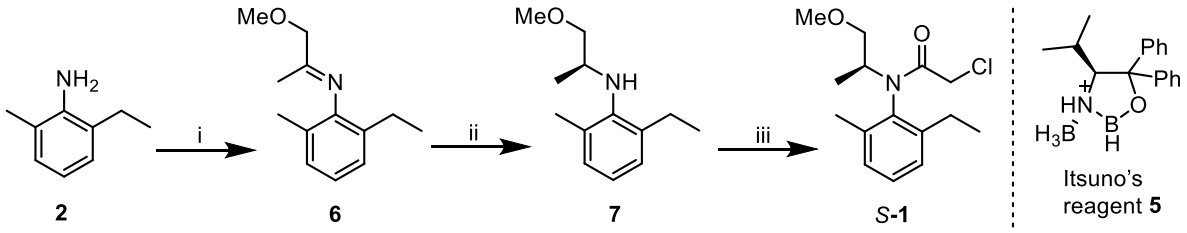
chloroacetylchloride afforded the *rac*-metolachlor *rac*-**1**.



Scheme 1. *Reagents and conditions*: (i) Pt/C, cat. H_2SO_4, 50 °C, 5 bar; (ii) $ClCOCH_2Cl$

Cho's approach (1992)[11]

Cho and co-workers utilized the asymmetric hydrogenation of *N*-phenyl ketimine derivative **6** using the chiral boron hydride **5** as a chiral catalyst for the synthesis of (*S*)-metolachlor *S*-**1** (**Scheme 2**). Thus, *N*-phenyl ketimine derivative **6** was prepared by treating 2-ethyl-6-methyl aniline **2** with methoxyacetone in the presence of catalytic amount of *p*-toluene sulfonic acid. Further, the imine derivative **6** was subjected to asymmetric hydrogenation with 1 M Itsuno's reagent **5** (chiral hydride), afforded the corresponding amine derivative **7** in 87% yield. Finally, *N*-chloroacetylation of amine precursor **7** afforded the optically active metolachlor *S*-**1** in 62% ee.

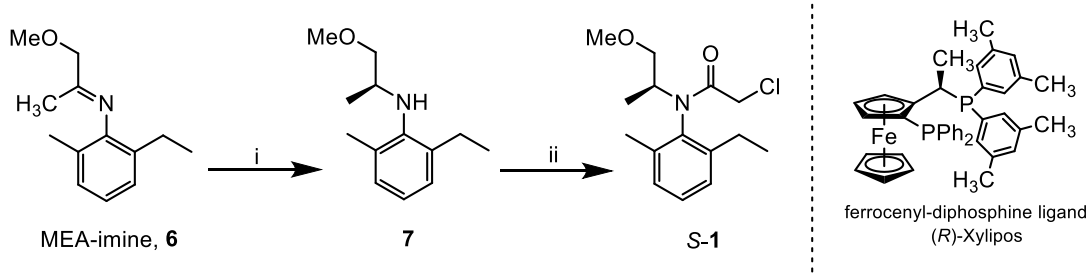


Scheme 2. *Reagents and conditions*: (i) methoxyacetone, cat. *p*-TsOH, benzene; (ii) 1 M Itsuno's reagent **5**, THF, 30 °C, 2 days, 87%, (iii) $ClCOCH_2Cl$, Na_2CO_3, benzene.

Blaser's approach (1999)[12]

In 1999, Blaser and co-workers again reported the asymmetric synthesis of (*S*)-metolachlor *via* enantioselective hydrogenation strategy using Iridium catalyst (**Scheme 3**). Thus, imine derivative **6** was subjected to enantioselective C=N hydrogenation in the presence of Iridium/(*R*)-Xylipos catalyst in the presence of iodine and acetic acid under H_2 atmosphere gave *(S)-N*-alkylated aniline **7** in 80% ee. Subsequently, *N*-acetylation of aniline

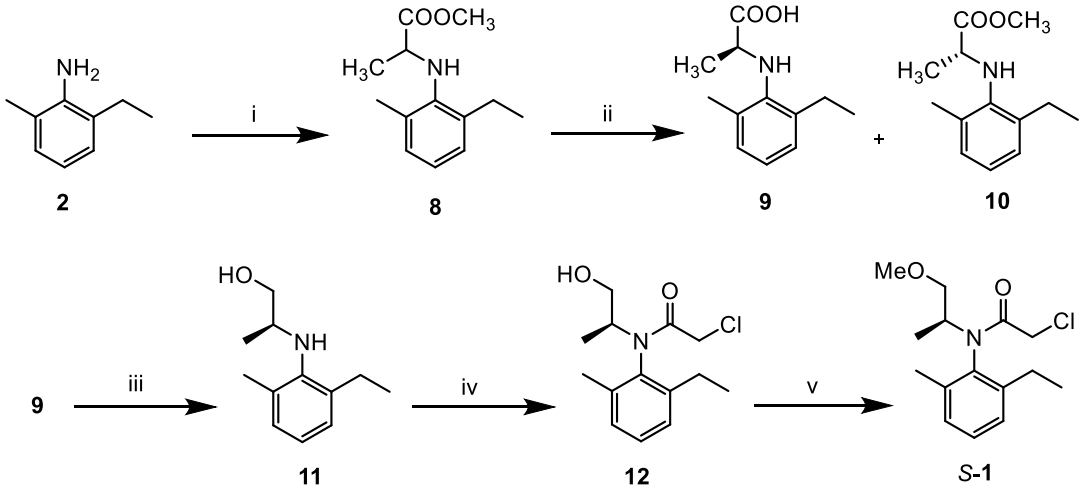
derivative **7** under basic condition afforded (*S*)-metolachlor *S*-**1**.



Scheme 3. *Reagents and conditions*: (i) $[Ir(COD)Cl]_2$/(*R*)-Xylipos, H_2 (80 bar), I_2, AcOH, 50 °C, 14 h; (ii) $ClCOCH_2Cl$, Na_2CO_3.

Zheng's approach (2006)[13]

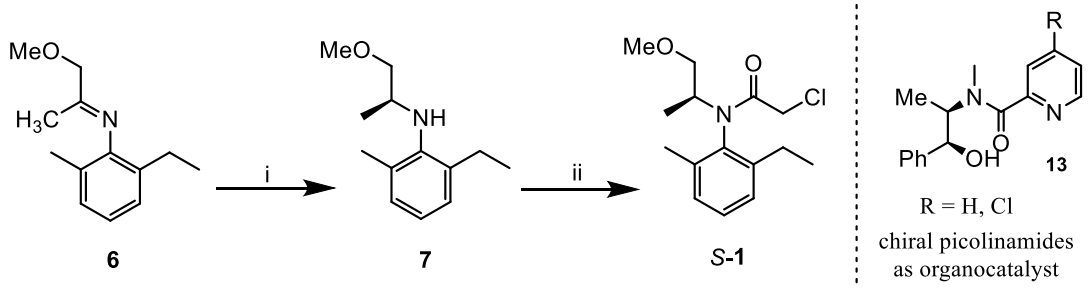
Zheng and co-workers employed chemoenzymatic approach towards the synthesis of (*S*)-metolachlor (**Scheme 4**). Treatment of 2-ethyl-6-methyl aniline **2** with methyl-2-bromopropionate in $NaHCO_3$ gave *N*-(2-ethyl-6-methylphenyl)alanine methyl ester **8**. Next, biocatalytic hydrolysis of racemic methyl ester **8** using CAL-B in aqueous buffer afforded **9** and **10**. The desired product **9** was reduced to its alcohol derivative **11** in 92% yield. Treatment of compound **11** with chloroacetylchloride under basic condition followed by etherification of compound **12** yielded the *S*-**1** with the optical purity 91% ee**.**



Scheme 4. *Reagents and conditions*: (i) methyl-2-bromopropionate, $NaHCO_3$, 125 °C, 18 h, 67%; (ii) CAL-B, phosphate buffer (P^H 8), 15% v/v Et_2O; (iii) $NaBH_4$, THF, Conc. H_2SO_4, rt, 24 h, 92%; (iv) $ClCOCH_2Cl$, Na_2CO_3, benzene, 15-20 °C, 3 h, 95%; (v) 2,2-dimethoxy-propane, *p*-TsOH, MeOH, reflux, 36 h, 66%.

Stefania's approach (2009)[14]

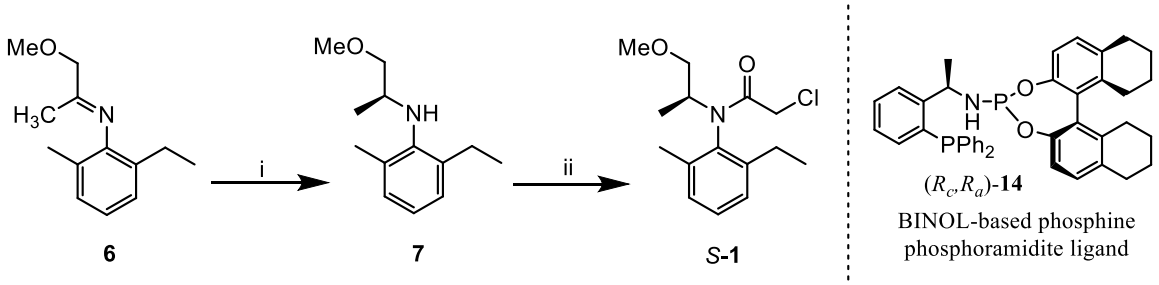
Stefania and co-workers reported the enantioselective preparation of (*S*)-metolachlor *via* Lewis base promoted the asymmetric reduction of methoxyacetone imine **6** using 10 mol% *N*-picolinoylamide of (*1S,2R*)-ephedrine **13**. As usual, the aniline derivative **7** was chloroacetylated to give (*S*)-metolachlor *S*-**1** in 67-70% ee (**Scheme 5**).



Scheme 5. *Reagents and conditions*: (i) **13** (10 mol%), $HSiCl_3$, $CHCl_3$, 0 °C or -20 °C, 15 h, 67-84%; (ii) $ClCOCH_2Cl$.

Hou's approach (2012)[15]

Hou *et al.* utilized the Iridium-catalyzed asymmetric hydrogenation using BINOL-based phosphine phosphoramidite ligand for the synthesis of (*S*)-metolachlor (**Scheme 6**). Thus, imine **6** was hydrogenated to its corresponding amine **7** in the presence of Iridium catalyst and BINOL derived phosphine phosphoramidite ligand under hydrogen atmosphere (80 bar) in DCM solvent followed by *N*-acetylation yielded (*S*)-metolachlor in 80%ee**.**

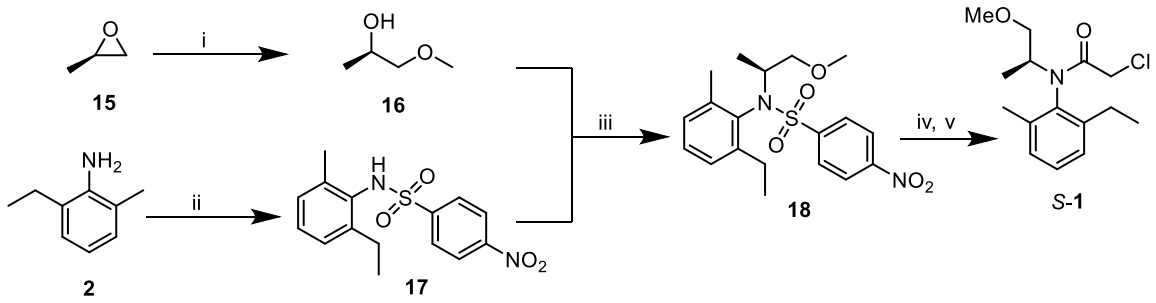


Scheme 6. *Reagents and conditions*: (i) $[Ir(COD)Cl]_2$ (0.0005%), (*Rc,Ra*)-**14** (0.0011%), Bu_4NI, H_2 (80 bar), DCM, 100 °C, 18 h; (ii) $ClCOCH_2Cl$, 96%.

Wang approach (2017)[16]

Very recently, Wang *et al.* accomplished the enantioselective preparation of (*S*)-metolachlor *S*-**1** from the commercially available (*R*)-propylene oxide **15** (**Scheme 7**). Thus,

regioselective ring opening of (*R*)-propylene oxide **15** with methanol under basic condition afforded compound **16**, which was further subjected to Mitsunobu reaction condition with 4-nitro-*N*-phenylbenzenesulfonamide **17** afforded compound **18** with inversion of configuration. Finally, deprotection followed by chloroacetylation of amine derivative **18** gave (*S*)-metolachlor *S*-**1** in 99% ee.

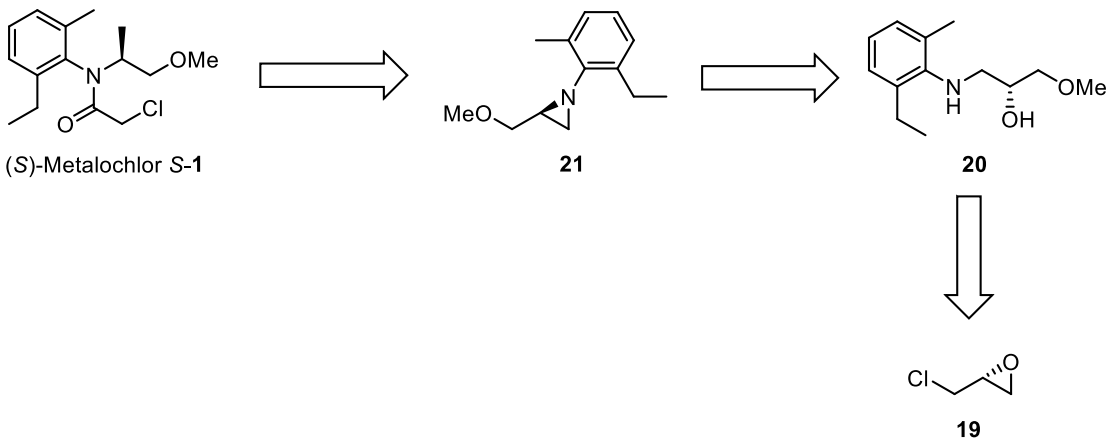


Scheme 7. *Reagents and conditions*: (i) NaOH, MeOH, reflux, 5 h, 79%; (ii) 4-nitrobenzene sulfonyl chloride, pyridine, DCM, rt, 2.5 h, 95%; (iii) DIAD, PPh_3, toluene, 50 °C, 2 h, 99%; (iv) 2-mercaptonic acid, DBU, CH_3CN, rt, 85%; (v) $ClCOCH_2Cl$, KOH, 80%.

1.3.3. Present work

Objective

The reports available for the synthesis of (*S*)-metolachlor, which involves asymmetric processes mainly hydrogenation of imine or enamide, enzymatic resolution, and chiral pool approaches. Some of these methods suffer from certain drawbacks such as low enantioselectivity, less overall yield, protection-deprotection steps, expensive reagents and catalysts, drastic reaction conditions etc. Very recently, Wang and co-workers demonstrated a new route for the synthesis of (*S*)-metolachlor.[16] Although this method seems to be impressive, it involves nosylation-denosylation steps which limit the superiority of this method. So, still, there is a scope for developing a new method that can overcome these drawbacks. In this section, we explored an alternative route for the synthesis of (*S*)-metolachlor starting from commercially available 2-ethyl-6-methyl aniline **2** and **(***R***)**-epichlorohydrin **19**.

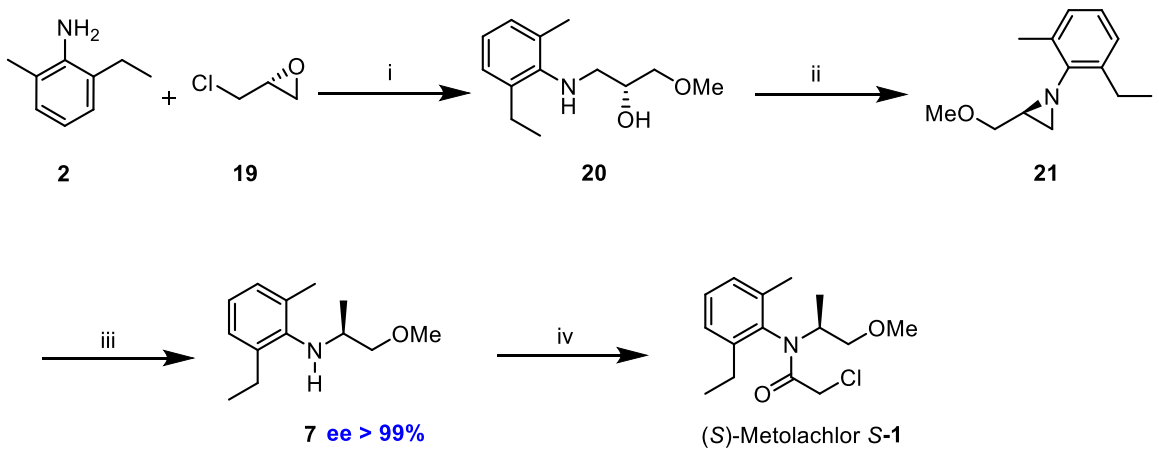


Scheme 8. Retrosynthetic analysis of (*S*)-metolachlor *S*-**1**

Retrosynthetically, it is envisioned that enantiomerically pure epichlorohydrin **19** can be used a chiral starting material for the synthesis of (*S*)-metolachlor. The aziridine **21** was visualized as a key intermediate for the synthesis of *S*-**1**, which in turn could be obtained from the epoxide **19** by regioselective ring opening followed by intramolecular Mitsunobu reaction. This aziridine **21** intermediate can be transformed to the final product *S*-**1** *via* reductive ring opening followed by acetylation protocols (**Scheme 8**).

1.3.4. Results and Discussion

Synthetic strategy followed for the synthesis of (*S*)-metolachlor *S*-**1** is outlined in **Scheme 9**. At first, 2-ethyl-6-methyl aniline **2** was treated with (*R*)-epichlorohydrin **19** in refluxing methanol for 6 h. Subsequently, the crude reaction mixture was treated with oven dried freshly crushed KOH at 0 °C, followed by stirring at room temperature for 8 h afforded the amino alcohol **20** in 96% yield. In the ^{1}H NMR spectrum of **20**, the signals resonated as a singlet at δ 3.42 ppm due to -O**CH$_3$** and multiplet in the range of δ 3.96-4.00 ppm due -**CH**OH proton, while in the ^{13}C NMR spectrum, signals corresponding to methoxy and methine carbon resonated at δ 69.6 and 59.2 ppm respectively. The IR spectrum of **20**, the absorption band of hydroxyl group displayed at 3421 cm^{-1}. Next, the secondary alcohol **20** was subjected to intramolecular ring closure reaction employing Mitsunobu condition in the presence of PPh_3 and DIAD using anhydrous toluene as a solvent gave key intermediate aziridine **21** in 86% yield without any loss in enantiopurity.



Scheme 9. *Reagents and conditions*: (i) (a) dry methanol, reflux, 6 h; (b) KOH, rt, 8 h, 96% (2steps); (ii) PPh_3, DIAD, dry toluene, 0 °C to reflux, 3 h, 86%; (iii) H_2 (1 atm), 10% Pd/C, MeOH, 1 h, rt, 78%; (iv) $ClCH_2COCl$, Na_2CO_3, toluene, 0 °C-rt, 1 h, 79%.

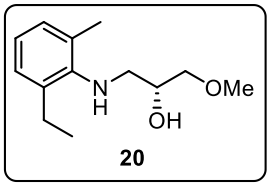
The key aziridine **21** on reductive ring opening under H_2 pressure in the presence of catalytic amount of 10% Pd/C and using methanol as a solvent furnished the required metolachlor precursor **7** in high enantiopurity (ee>99%). In the ^{13}C NMR spectrum of **7**, signals corresponding to CH_2, CH and CH_3 carbons of ring-opened product resonate at δ 76.2, 52.9 and 18.5 ppm respectively indicates the formation of secondary amine **7**. Finally, *N*-chloroacetylation of secondary amine derivative **7** was carried out with chloroacetyl-chloride under basic condition in toluene afforded (*S*)-metolachlor *S*-**1** with an overall yield of 50.8%, $[\alpha]^{25}_D$ -5.7 (*c* 2.80, n-hexane) {lit.[5] $[\alpha]^{25}_D$ -8.2 (*c* 2.1, n-hexane}. The structure of *S*-**1** was confirmed by its IR, ^1H NMR, ^{13}C NMR, and mass analysis.

1.3.5. Conclusion

In conclusion, developed an efficient new route for the synthesis of enantiopure (*S*)-metolachlor, an active ingredient of DUAL MAGNUM® *S*-**1** *via* reductive ring opening of aziridine. The attractive feature of the present protocol includes ready availability of the starting materials, simple chemical transformations, high enantiopurity and good overall yield. This simple protocol may find application in the large-scale synthesis of the (*S*)-metolachlor in high enantiopurity.

1.3.6. Experimental Section

1) (*R*)-1-((2-ethyl-6-methylphenyl)amino)-3-methoxypropan-2-ol (20)



20

To a stirred solution of epichlorohydrin **19** (2 g, 21.6 mmol) in methanol (15 mL) was added 2-ethyl-6-methyl aniline **2** (3.2 g, 23.7 mmol) and the resulting mixture was refluxed for 6 h. After completion of the reaction (monitored by TLC), crushed KOH (3.0 g, 54.0 mmol) was added portion wise at a temperature <25 °C. After completing the addition, the reaction mixture was stirred vigorously for 8 h at room temperature. After completion of the reaction (monitored by TLC), excess methanol was evaporated under reduced pressure. The crude mixture was then poured into water (20 mL) and extracted with EtOAc (2 x 15 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography using EtOAc/petroleum ether (10:90) gave alcohol product **20** as a pale brown oil.

Yield: 4.6 g, 96%;

Molecular Formula: $C_{13}H_{21}NO_2$;

Specific rotation: $[\alpha]^{21}_D$ = +4.93 (*c* 2.09, $CHCl_3$);

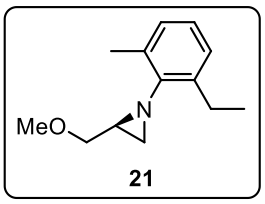
IR ($CHCl_3$, cm^{-1}): ν_{max} 3421, 3009, 2966, 1593, 1466, 1377, 1216, 1129, 968, 667;

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.26 (t, *J* = 7.6 Hz, 3 H), 2.33 (s, 3 H), 2.66-2.71 (m, 2 H), 2.98 (dd, *J* = 12.5, 7.0 Hz, 1 H), 3.10 (dd, *J* = 12.5, 4.0 Hz, 1 H), 3.42 (s, 3 H), 3.47 (dd, *J* = 9.4, 6.4 Hz, 1 H), 3.51 (dd, *J* = 9.7, 3.6 Hz, 1 H), 3.96-4.00 (m, 1 H), 6.91 (apparent t, *J* = 7.2 Hz, 1 H), 7.02 (d, *J* = 7.3 Hz, 1 H), 7.04 (d, *J* = 7.6 Hz, 1 H);

^{13}C NMR (50 Hz, $CDCl_3$): δ 145.0 (C), 136.2 (C), 130.6 (C), 128.8 (CH), 126.7 (CH), 122.6 (CH), 75.3 (CH_2), 69.6 (CH), 59.2 (CH_3), 51.5 (CH_2), 24.2 (CH_2), 18.5 (CH_3), 14.8 (CH_3);

MS: *m/z* 224 $[M+1]^+$, 246 $[M+Na]^+$.

2) (*S*)-1-(2-ethyl-6-methylphenyl)-2-(methoxymethyl)aziridine (21)



21

A solution of DIAD (3.0 mL, 15.4 mmol) in dry toluene (5 mL) was added dropwise to a solution of amino alcohol **20** (2.3 g, 10.3 mmol) and triphenylphosphine (4.0 g, 15.4 mmol) in a dry toluene (25 mL) under N_2 atmosphere at 0 °C. The reaction mixture was refluxed for 3 h. After completion of reaction (monitored by TLC), the solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using EtOAc/petroleum ether (5:95) afforded aziridine product **21** as a yellow oil.

Yield: 1.8 g, 86 %;

Molecular Formula: $C_{13}H_{19}NO$;

Specific rotation: $[\alpha]^{21}_{D}$ = -120.5 (*c* 1.0, $CHCl_3$);

Chiral HPLC: ee >99% [Chiral HPLC analysis: Chiralcel OD-H (250 x 4.6 mm) column; eluent: n-hexane/isopropanol = 99.75:0.25; flow rate: 0.5mL/min; detector: 220 nm];

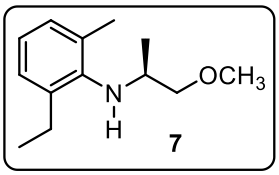
IR ($CHCl_3$, cm^{-1}): ν_{max} 3419, 2967, 2875, 1915, 1745, 1592, 1460, 1378, 1355, 1276, 1217, 1188, 1108, 965, 929, 900, 666;

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.28 (t, *J* = 7.6 Hz, 3 H), 2.04 (d, *J* = 6.3 Hz, 1 H), 2.39 (s, 3 H), 2.41-2.50 (m, 2 H), 2.80 (q, *J* = 7.5 Hz, 2 H), 3.46 (s, 3 H), 3.49-3.54 (m, 1 H), 3.93 (dd, *J* = 10.4, 4.3 Hz, 1 H), 6.88 (apparent t, *J* = 7.3 Hz, 1 H), 6.95-7.04 (m, 2 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 149.9 (C), 134.9 (C), 129.1 (C), 128.8 (CH), 126.9 (CH), 122.0 (CH), 74.0 (CH_2), 59.1 (CH_3), 39.4 (CH), 34.9 (CH_2), 24.3 (CH_2), 19.3 (CH_3), 14.3 (CH_3);

MS: *m/z* 206 $[M+1]^+$, 228 $[M+Na]^+$.

3) (*S*)-2-ethyl-*N*-(1-methoxypropan-2-yl)-6-methylaniline (7)



7

To a solution of aziridine **21** (1.0 g, 4.87 mmol) in methanol (10 mL) was added palladium on activated carbon (0.065 g, 10-20 wt %) and the reaction mixture was stirred under

hydrogen atmosphere (balloon) for 1 h. After completion of the reaction (monitored by TLC) the catalyst was filtered over the Celite bed (EtOAc eluent) and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography using EtOAc/petroleum ether (2:98) afforded amine product **7** as a pale yellow oil.

Yield: 0.79 g, 78 %;

Molecular Formula: $C_{13}H_{21}NO$;

Specific rotation: $[\alpha]^{21}_{D} = +11.68$ (*c* 2.0, $CHCl_3$);

Chiral HPLC: ee >99% [Chiral HPLC analysis: Chiralcel OD-H (250 x 4.6 mm) column; eluent: n-hexane/isopropanol = 99.75:0.25; flow rate: 0.5mL/min; detector: 220 nm];

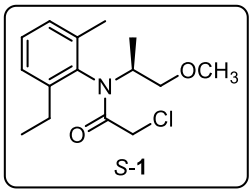
IR ($CHCl_3$, cm^{-1}): ν_{max} 3409, 3019, 2969, 2877, 2401, 1593, 1465, 1385, 1215, 1103, 928, 669;

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.19-1.21 (m, 3 H), 1.26 (t, *J* = 7.5 Hz, 3 H), 2.31 (s, 3 H), 2.67 (q, *J* = 7.6 Hz, 2 H), 3.32-3.37 (m, 3 H), 3.39 (s, 3 H), 6.88 (apparent t, *J* = 7.4 Hz, 1 H), 7.00-7.06 (m, 2 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 144.2 (CH), 135.5 (CH), 129.8 (CH), 128.7 (CH), 126.5 (CH), 121.7 (CH), 76.2 (CH_2), 58.9 (CH_3), 52.9 (CH), 24.2 (CH_2), 18.9 (CH_3), 18.5 (CH_3), 14.5 (CH_3);

MS: *m/z* 208 $[M+1]^+$, 230 $[M+Na]^+$.

4) (*S*)-2-Chloro-*N*-(2-ethyl-6-methyl-phenyl)-N-(2-methoxy-1-methyl-ethyl)-acetamide ((*S*)-metolachlor *S*-1)



S-1

To a stirred solution of **7** (0.1 g, 0.48 mmol) and sodium carbonate (0.102 g, 0.96 mmol) in toluene (3 mL) was added chloroacetyl chloride (0.065 g, 0.57 mmol, 46 µL) at 0 °C. The resulting mixture was stirred for 1 h at room temperature. After completion of the reaction (monitored by TLC), toluene was removed under reduced pressure and the residue was diluted with water (3 mL) and extracted with EtOAc (3 x 5 mL). The phases were separated, and the organic phase was washed with brine (2 x 5 mL), dried (Na_2SO_4) and filtered. The

solvent was removed under reduced pressure and the crude residue was purified by silica gel column chromatography using EtOAc/petroleum ether (10:90) gave *S*-**1** as a colorless oil.

Yield: 0.108 g; 79%;

Molecular Formula: $C_{15}H_{22}ClNO_2$;

Specific rotation: $[\alpha]^{25}_D$ = -5.7 (*c* 2.80, n-hexane) {lit.[13] $[\alpha]^{25}_D$ = -8.2 (*c* 2.1, n-hexane};

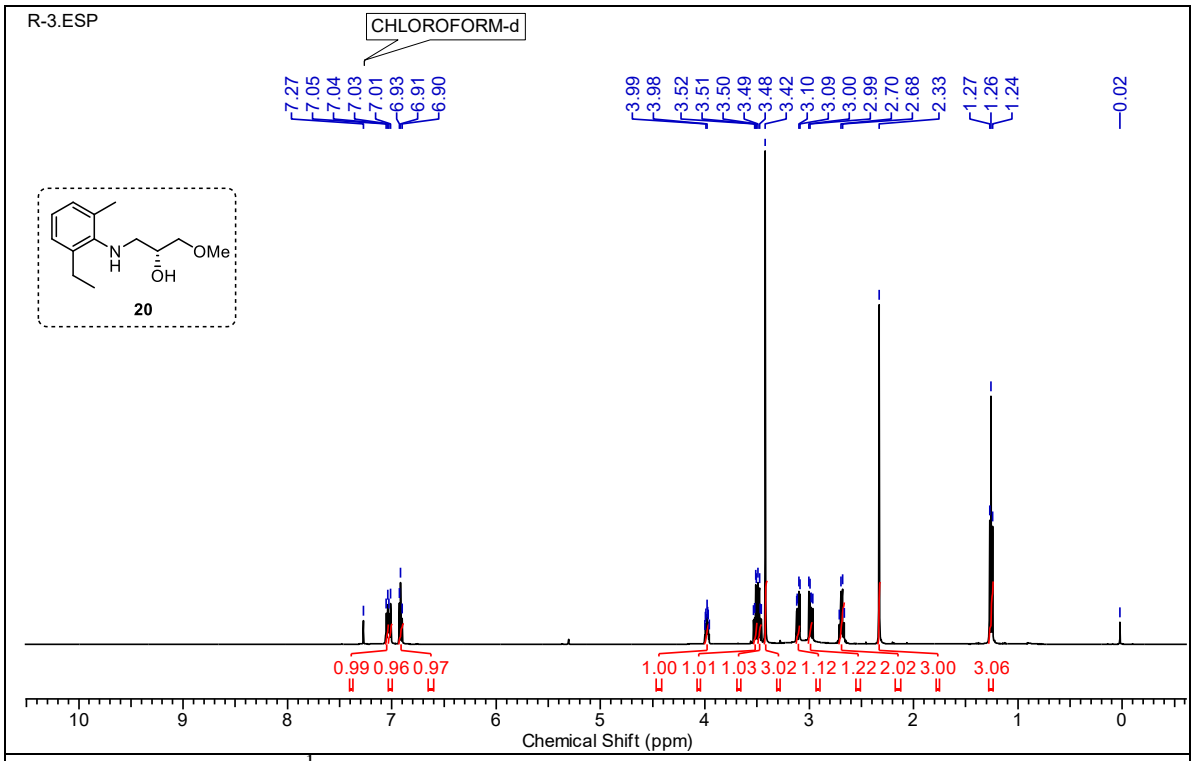
IR ($CHCl_3$, cm^{-1}): ν_{max} 3464, 3019, 1664, 1462, 1215, 1112, 765, 669;

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.13-1.18 (m, 1 H), 1.25 (t, *J* = 7.5 Hz, 3 H), 2.23 (s, 3 H, major+minor), 2.48-2.69 (m, 2 H), 3.28 (s, 3 H, major+minor), 3.45-3.54 (m, 1 H), 3.61 (s, 2 H, major+minor), 3.66-3.78 (m, 1 H), 4.15-4.28 (m, 1 H), 7.11-7.31 (m, 3 H);

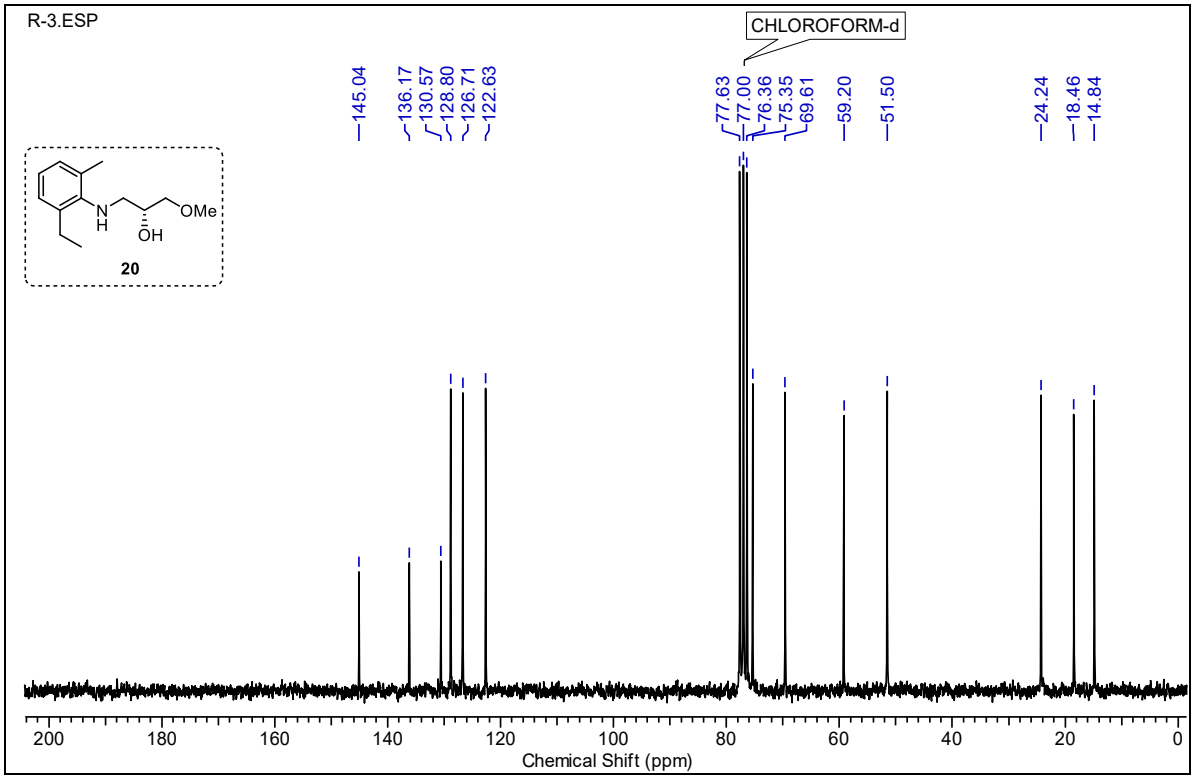
^{13}C NMR (50 MHz, $CDCl_3$): δ 166.8 (CO), 142.5 (C, minor), 142.4 (C, major), 137.1 (C), 136.9 (C, major), 136.8 (C, minor), 128.9 (CH, 2 carbons), 126.9 (CH, major), 126.79 (CH, minor), 74.54 (CH_2), 58.5 (CH_3), 55.3 (CH, minor), 55.2 (CH, major), 42.9 (CH_2, major), 42.8 (CH_2, minor), 23.8 (CH_2, minor), 23.6 (CH_2, major), 18.9 (CH_3), 15.5 (CH_3, minor), 15.3 (CH_3, major), 14.2 (CH_3, major), 13.9 (CH_3, minor);

MS: *m/z* 284 $[M+1]^+$, 306 $[M+Na]^+$.

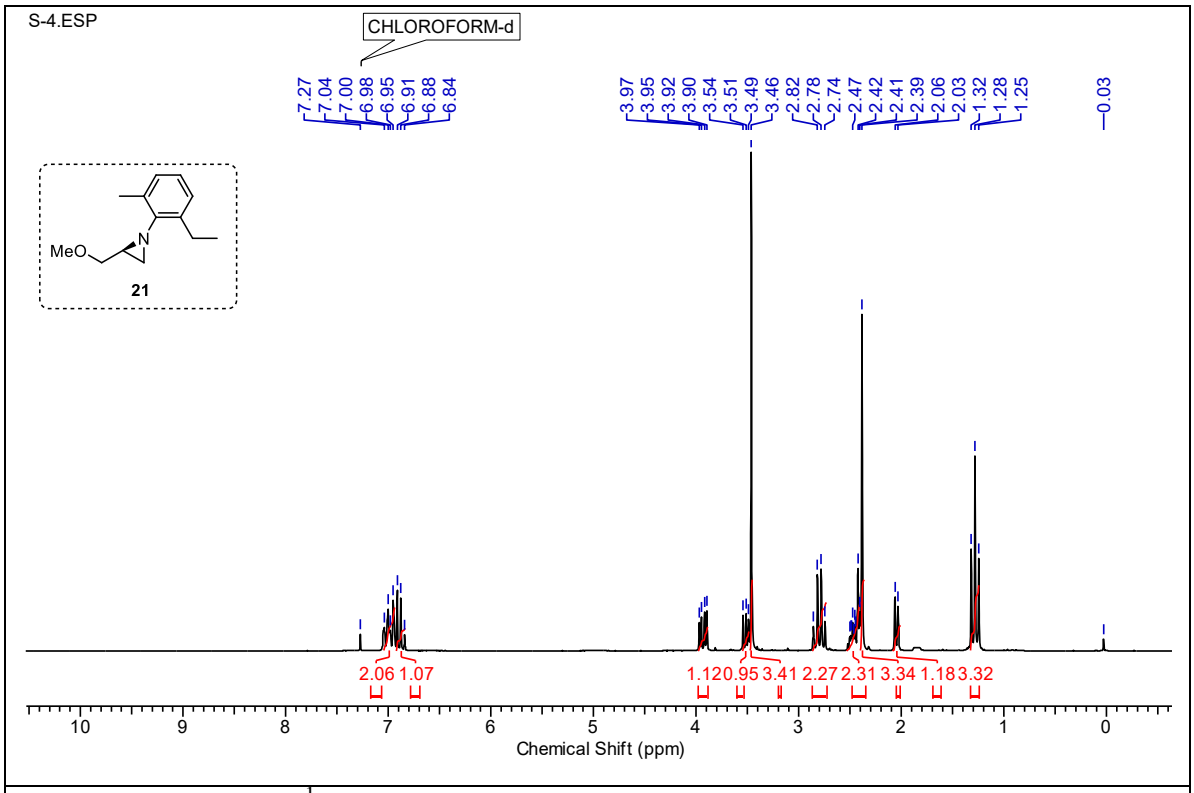
1.3.7. Spectra



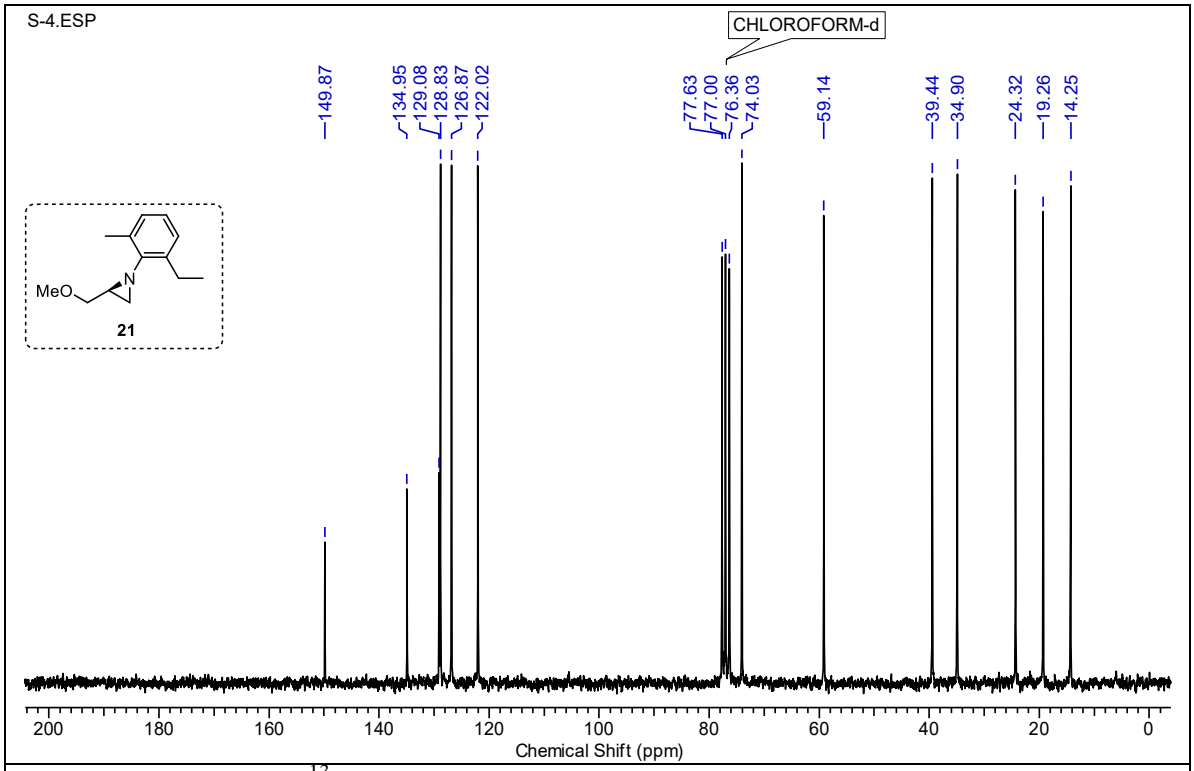
^{1}H NMR (500 MHz, $CDCl_3$) of compound **20**



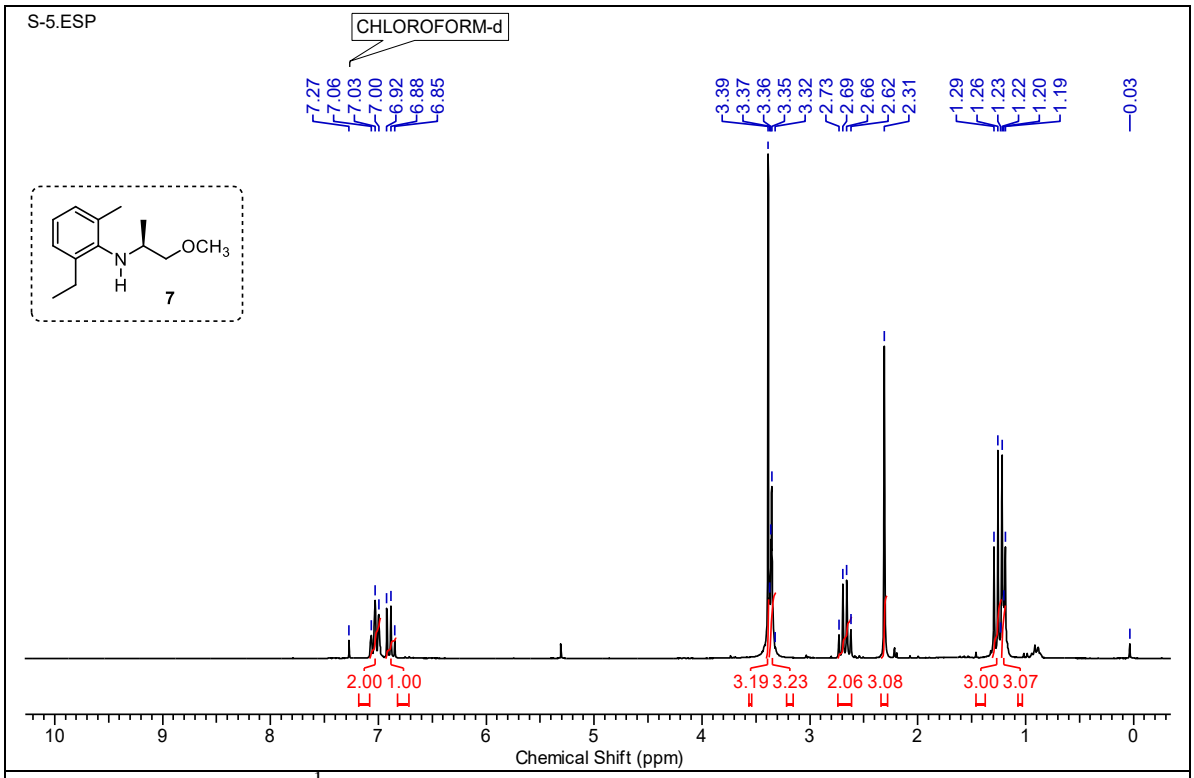
^{13}C NMR (50 MHz, $CDCl_3$) of compound **20**



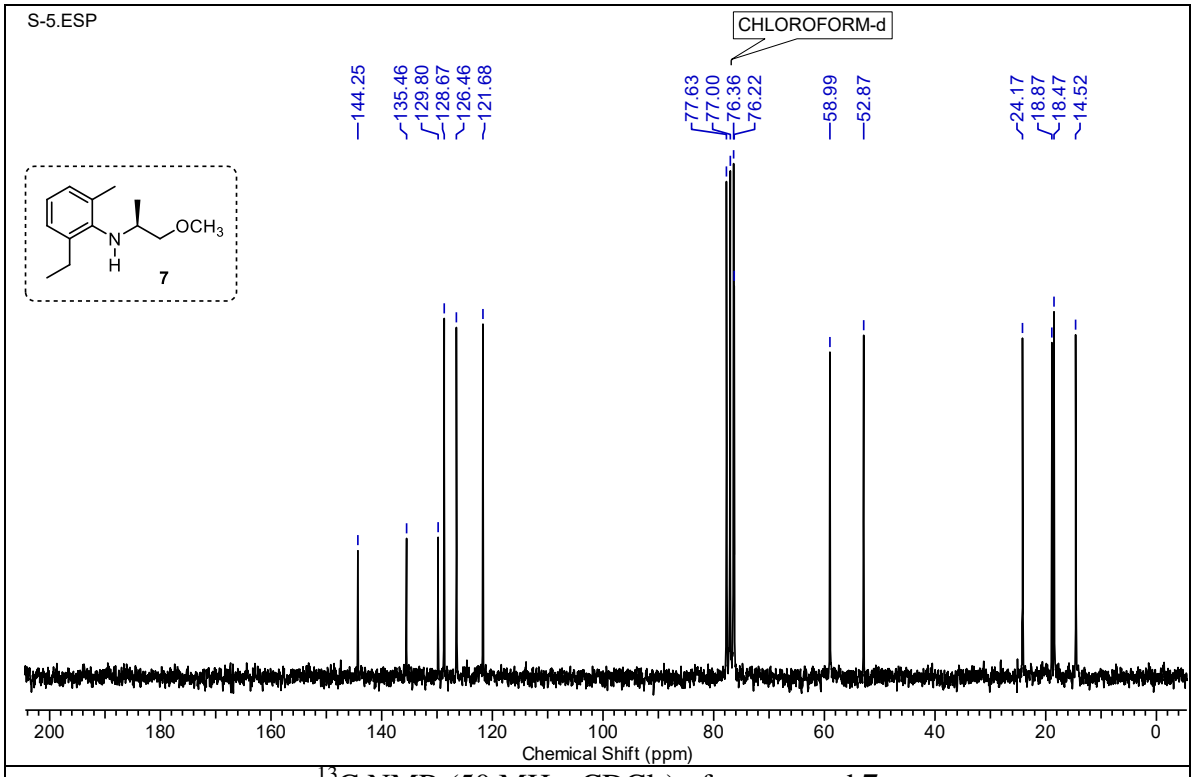
^{1}H NMR (200 MHz, $CDCl_3$) of compound **21**



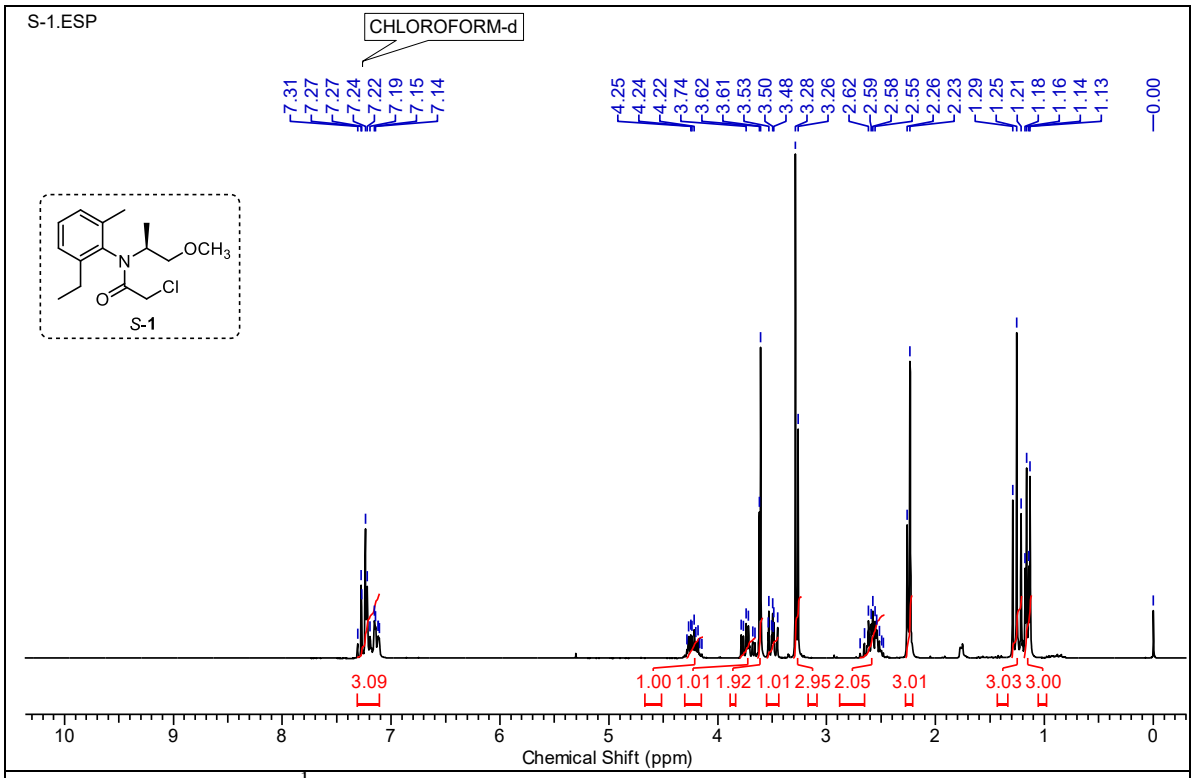
^{13}C NMR (50 MHz, $CDCl_3$) of compound **21**



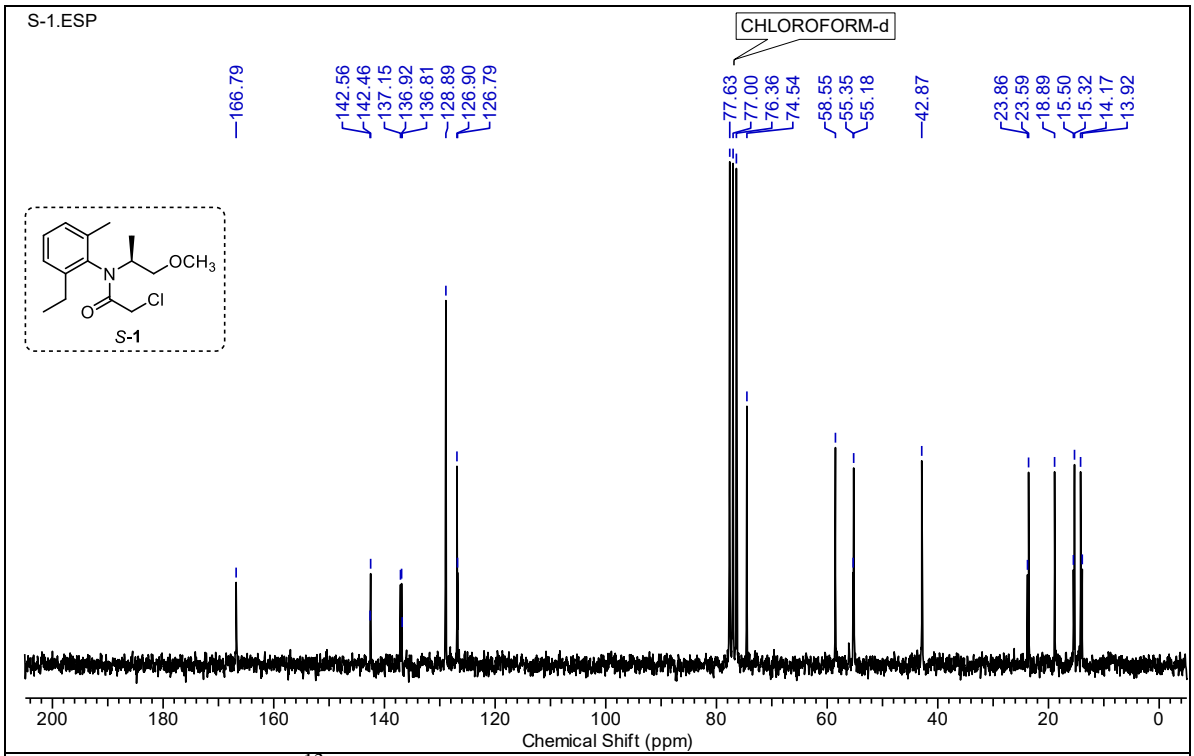
^{1}H NMR (200 MHz, $CDCl_3$) of compound **7**



^{13}C NMR (50 MHz, $CDCl_3$) of compound **7**



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



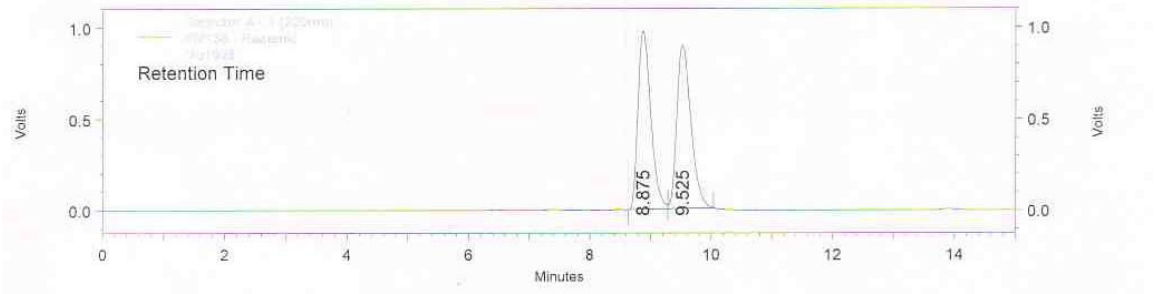
^{13}C NMR (50 MHz, $CDCl_3$) of compound *S*-**1**

1.3.8. Chiral HPLC analysis data

Chiral HPLC analysis of Compound 7

Conditions: Chiralcel OD-H (250 X 4.6 mm) column; eluent: n-Hexane/isopropanol (99.75:0.25); flow rate: 0.5mL/min; detector 220 nm.

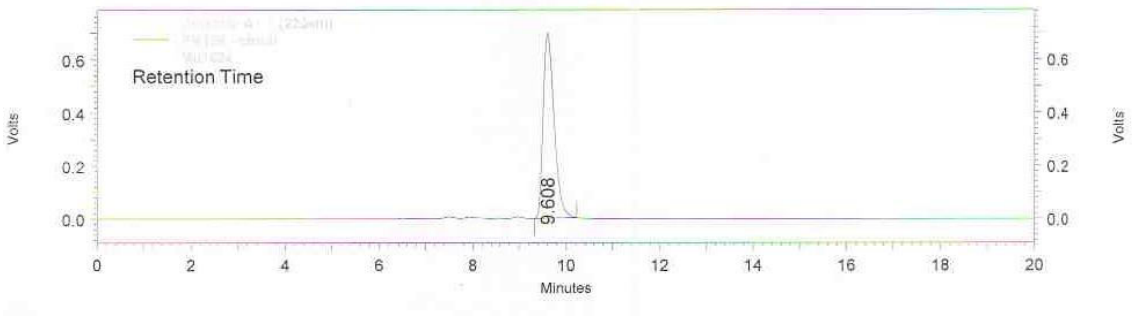
Racemic



Racemic Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	8.875	14342294	49.725
2	9.525	14500801	50.275
Totals			

Chiral



Chiral Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	9.608	11219758	100.000
Totals		11219758	100.000

1.3.9. References

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CHAPTER 2

Asymmetric syntheses of (R)-2-benzyl-morpholine, both enantiomers of calcium channel blocker bepridil and anti-obesity drug lorcaserin

2.1. SECTION 1

An enantioselective synthesis of appetite suppressant (*R*)-2-benzylmorpholine

2.1.1. Introduction

Morpholines are widely used in organic synthesis, mainly as a simple base or as an *N*-alkylating agent.[1] However, synthesis of C-functionalized morpholine derivatives is quite less explored area.[2] C-Functionalized morpholines are found in various natural products as well as in drugs.[3] They are potential therapeutic agents for a wide variety of medical disorders such as depression (Reboxetine, Viloxesine),[4] anorectics (Phenmetrazine, Phendimetrazine),[5] chemotherapy-induced nausea & vomiting (Aprepitant)[6] etc,. The synthesis of morpholine moiety bearing a reactive functional at C2-position is more challenging. Despite their wide utility, synthetic routes to these valuable compounds especially the non-racemic ones are very limited.

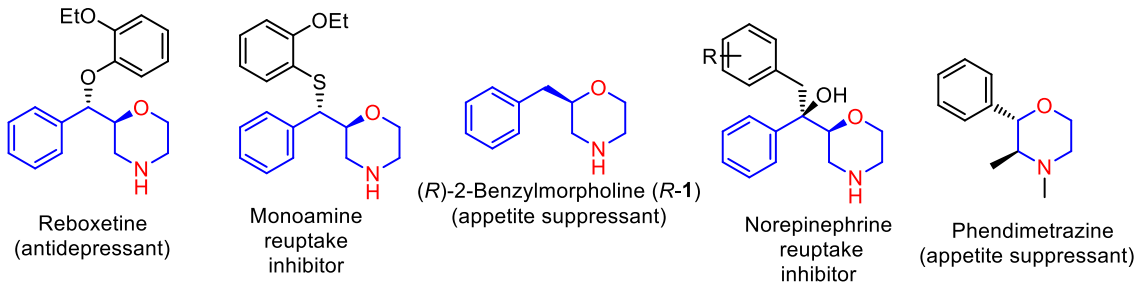


Figure 1. Representative pharmaceutically significant chiral C2-substituted morpholine analogues

In general, synthetic routes to construct the enantiopure morpholine moiety rely upon naturally occurring amino acids or optically pure amino alcohols or other chiral precursors chiral pool approaches.[7] In recent years, enantioselective oxidation reactions such as Sharpless epoxidation, aminohydroxylation, dihydroxylation and related reactions have been extensively studied to access enantiopure morpholine analogues.[8] (*R*)-2-Benzylmorpholine *R*-**1** is a classical example of chiral 2-morpholine analogues, known to be a potent appetite suppressant and widely studied for its pharmacological properties.[9]

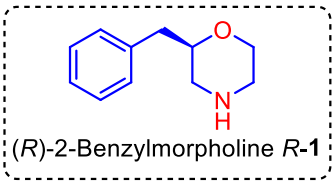


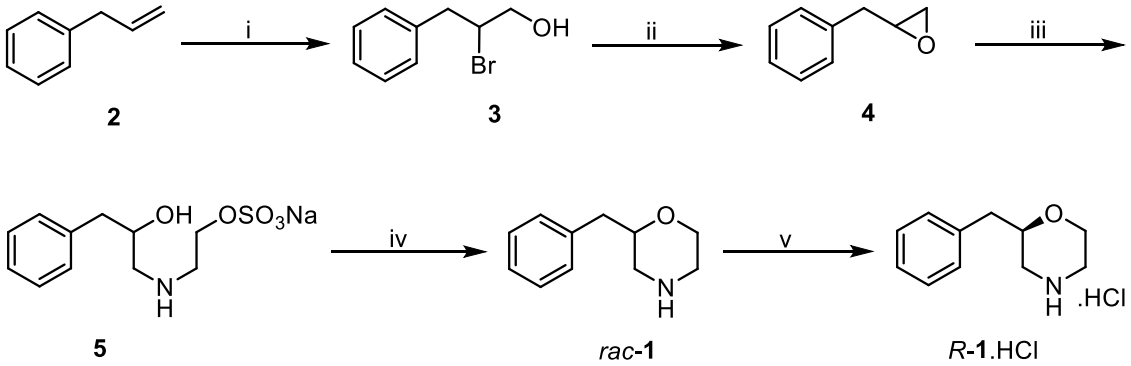
Figure 2. (*R*)-2-Benzylmorpholine *R*-**1**

2.1.2. Review of Literature

A few approaches have appeared on asymmetric synthesis of (*R*)-benzylmorpholine. These involve the use of chemical/enzymatic resolution, proline-catalyzed α-aminoxylation strategy etc,. Some significant synthesis of (*R*)-2-benzylmorpholine *R*-**1** is described below.

Brown's approach (1990)[10]

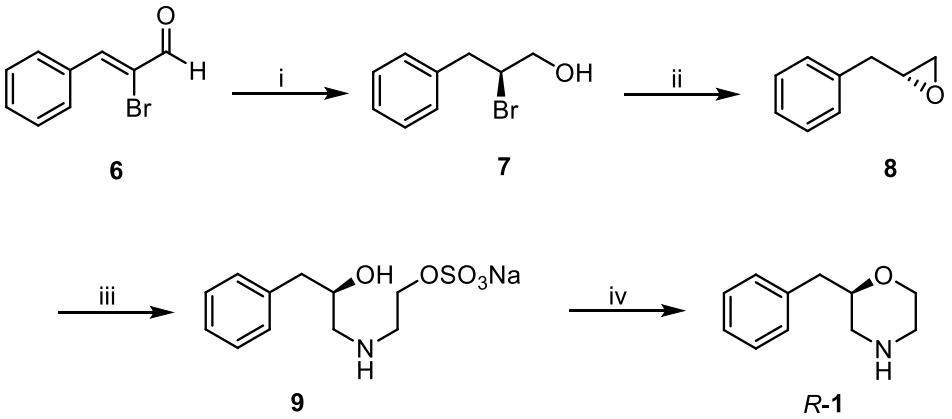
Brown *et.al.* described the chemical resolution method to synthesize both enantiomers of *R*-**1**.HCl and *S*-**1**.HCl (**Scheme 1**). As shown in **Scheme 1**, at first benzyloxirane **4** was prepared from allylbenzene **2** in two steps. Regioselective ring opening of epoxide **4** with ethanolamine-*O*-sulphate in basic medium gave sulfate ester **5**. Subsequent ring closure of compound **5** with sodium hydroxide in toluene at 65 °C for 8 h afforded *rac*-benzylmorpholine *rac*-**1** in 38% yield. Finally, *rac*-benzylmorpholine upon chemical resolution employing (+)- and (-)-dibenzoyltartaric acids as resolving agents in 2-propanol under reflux condition for 18 h gave the (*R*)-benzylmorpholine hydrochloride *R*-**1**.HCl and (*S*)-benzylmorpholine hydrochloride *S*-**1**.HCl in 18% and 26% yields respectively.



Scheme 1. *Reagents and conditions:* (i) *N*-bromosuccinimide, H_2O, rt, 48 h; (ii) NaOH, H_2O, 65 °C, 45 min, 67%; (iii) ethanolamine-*O*-sulphate, 16 M NaOH, MeOH, 40 °C, 2 h; (iv) NaOH, toluene, 65 °C, 8 h, 38%; (v) (+)-dibenzoyltartaric acid, reflux, 18h, 18%

Arrigo's approach (1998)[11]

Arrigo and co-workers have prepared (*R*)-2-benzylmorpholine *R*-**1** employing chemo-enzymatic approach (**Scheme 2**). Accordingly, (Z)-α-bromo cinnamaldehyde **6** upon reduction in the presence of Baker's yeast, XAD1180 resin at 25 °C for 48 h afforded the (*S*)-bromoalcohol **7** in 91% yield. The intramolecular ring closure reaction of bromo alcohol **7** in aqueous basic condition gave (*R*)-benzyloxirane **8** in 73% yield. The chiral epoxide **8** was treated with ethanolamine-*O*-sulphate in the presence of sodium hydroxide in methanol at 40 °C for 2 h gave sulfate ester **9**. Base-catalyzed ring closure of compound **9** with NaOH in toluene at 65 °C for 7 h afforded (*R*)-2-benzylmorpholine *R*-**1** in 66% yield.

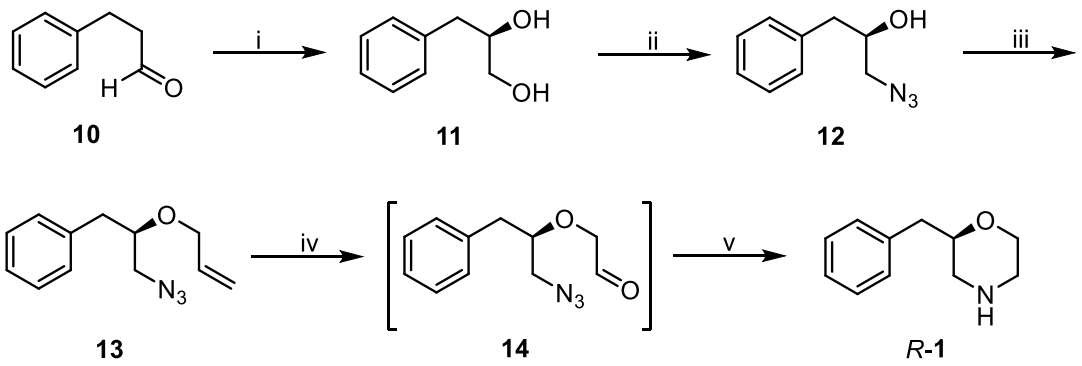


Scheme 2. *Reagents and conditions:* (i) Baker's yeast, XAD1180 resin, 25 °C, 48 h, 91%; (ii) NaOH, H_2O, 65 °C, 45 min, 73%; (iii) ethanolamine-*O*-sulphate, 16 M NaOH, MeOH, 40 °C, 2 h; (iv) NaOH, toluene, 65 °C, 7 h, 66%.

Waghmode's approach (2010)[12]

Waghmode and co-workers developed an enantioselective synthesis of (*R*)-2-benzyl-morpholine *R*-**1** employing L-proline catalyzed asymmetric α-aminoxylation and palladium-catalyzed intramolecular reductive amination as a key steps (**Scheme 3**). Readily available 3-phenylpropanaldehyde **10** was subjected to L-proline-catalyzed asymmetric α-amin-oxylation protocol afforded the diol **11** in 69% yield. Selective tosylation of diol **11** followed by azidation gave azido derivative **12** in 88% yield. Azido alcohol **12** on treatment with allyl bromide gave azidoallyl ether **13** in 98% yield. The azido ether **13** on dihydroxylation with potassium osmate in NMO at room temperature for 10 h followed by oxidative cleavage of an olefin with sodium metaperiodate gave azido aldehyde **14**. Finally, reductive amination of azido aldehyde **14** in the presence of 10% Pd/C afforded target

molecule *R*-**1** in 57% yield and >95% ee.



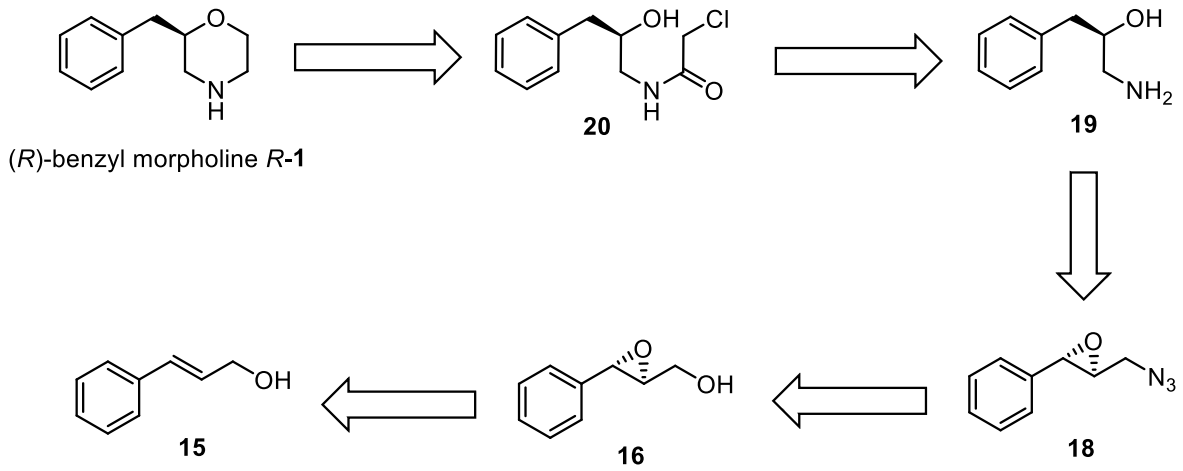
Scheme 3. *Reagents and conditions*: (i) (a) PhNO, L-proline (25 mol %), CH_3CN, -20 °C, 24 h then MeOH, $NaBH_4$, 30 min, (b) H_2 (1 atm), 10% Pd/C, MeOH, 10 h, 69% (2 steps); (ii) (a) dibutyltin oxide (2 mol %), *p*-TsCl, Et_3N, CH_2Cl_2, 0 °C to rt, 1 h; (b) NaN_3, DMF, 70 °C, 10 h, 88% (2 steps); (iii) allyl bromide, NaH, DMF, 0 °C, 1.5 h, 98%; (iv) (a) $K_2OsO_4.H_2O$ (2 mol %), NMO, acetone:H_2O (8:2), rt, 10 h, (b) $NaIO_4$, acetone:H_2O (9:1), 0 °C, rt, 4 h; (v) H_2 (1 atm), 10 % Pd/C, MeOH, 12 h, 57% (3 steps).

2.1.3. Present work

Objective

Literature search revealed that although there are few reports available on the synthesis of (*R*)-2-benzylmorpholine *R*-**1**, most of them suffer from drawbacks such as the use of expensive reagents, low overall yields, low enantiopurity, etc,. Hence the development of new and alternative route that can overcome these drawbacks is highly desirable. Thus, the objective of the present work is to develop a new alternative route for the asymmetric synthesis of (*R*)-2-benzylmorpholine *R*-**1** with high enantiopurity.

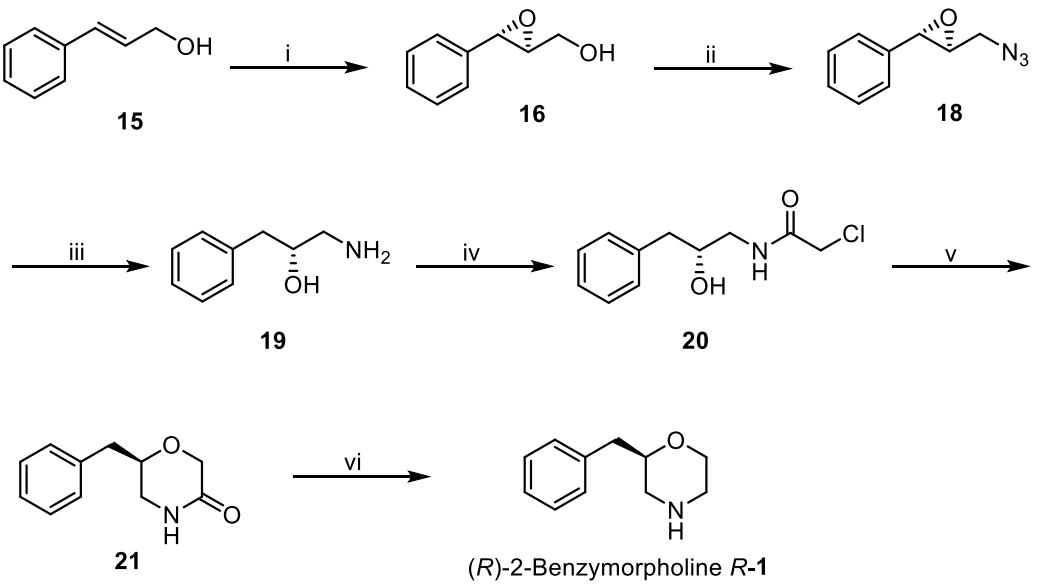
The retrosynthetic analysis of *R*-**1** is presented in **Scheme 4**. As shown in **Scheme 4**, we envisaged that the amino alcohol **19** would serve as a key intermediate for the synthesis of (*R*)-2-benzylmorpholine *R*-**1** which can be transformed to the final product *via N*-acetylation, cyclization followed by amide reduction. Now the key intermediate **19** could be obtained from the chiral epoxy alcohol **16** *via* tosylation, azidation followed by hydrogenation. Further, this chiral epoxide **16** can be obtained from commercially available *trans*-cinnamyl alcohol **15** using Sharpless asymmetric epoxidation (SAE) strategy.



Scheme 4. Retrosynthetic analysis of (*R*)-benzylmorpholine *R*-**1**

2.1.4. Results and Discussion

Synthetic strategy followed for the synthesis of (*R*)-2-benzylmorpholine *R*-**1** is outlined in **Scheme 5**. As illustrated in **Scheme 5**, synthesis commenced with the readily available *trans*-cinnamyl alcohol **15** which was subjected to Sharpless asymmetric epoxidation conditions ((+)-DIPT, $Ti(OiPr)_4$, TBHP, CH_2Cl_2, -20 °C) gave enantiomerically pure epoxy alcohol **16** in 86% yield (ee >99%). In the ^{1}H NMR spectrum of epoxyalcohol **16**, the disappearance of olefinic unit and signals of methine protons of epoxide unit resonate as a multiplet at δ 3.18-3.23 ppm and a doublet at 3.89 ppm confirms the formation of compound **16**. On the other hand, in the ^{13}C NMR spectrum of **16**, the methine carbons of epoxide moiety resonated at δ 51.63 and 56.28 ppm. Subsequently, the epoxy alcohol **16** was converted into the corresponding azido epoxide **18** by carrying out *O*-tosylation at a low temperature (-20 °C) followed by azidation using sodium azide in dry DMF. In the IR spectrum of **18**, the absorption band at 2101 cm^{-1} indicating the presence of azide functionality. Next, the azido epoxide **18** was subjected to palladium carbon-catalyzed concomitant hydrogenolysis of epoxide and hydrogenation of azide moiety in a single step afforded amino alcohol **19** in 75% yield. In the ^{1}H NMR spectrum of **19**, the signal corresponds to methine hydrogen resonates as a multiplet at δ 3.68-3.80 ppm, while in the ^{13}C spectrum, resonance signals of methine carbon appeared at δ 73.0 ppm. The formation of **19** was further confirmed by the IR spectrum, the absorption band of amine functionality displayed at 3570 cm^{-1}. It is worth noting that although the regioselective ring opening of azido epoxide **18** with various nucleophiles have been studied, the concomitant



Scheme 5. *Reagents and conditions:* (i) TBHP, L-(+)-DIPT, $Ti(O^iPr)_4$, 4 Å MS, dry CH_2C1_2, -20 °C to 0 °C, 4 h, 86%; (ii) (a) *p*-TsCl, TEA, DMAP, dry CH_2Cl_2, -20 °C, 8 h, (b) NaN_3, dry DMF, 0 °C to rt, 12 h, 72% (2 steps); (iii) H_2 (50 psi), 10% Pd/C, MeOH, 6 h, RT, 75%; (iv) chloroacetychloride, Na_2CO_3, toluene-H_2O, 5 °C, 2 h, 87%; (v) *t*-BuOK, dry isopropanaol, 0 °C to rt, 3 h, 69%; (vi) Red-Al, dry THF, 0 °C to rt, 12 h, 85%.

hydrogenolysis of epoxide and hydrogenation of azide in a single step to produce enantiopure *β*-amino alcohols have not been examined. Considering the significance of enantiopure *β*-amino alcohols as important structural elements present in many natural products as well as pharmaceuticals, this reaction represents the valuable alternative to prepare enantiopure *β*-amino alcohol, utilizing SAE strategy. Further, the amino alcohol **19** on *N*-acylation with chloroacetyl chloride under basic condition provided compound **20** in 87% yield. In the ^{1}H NMR spectrum of **20**, the signals correspond to methylene protons of acetyl group resonated at δ 4.07 ppm, while in the ^{13}C NMR spectrum, the characteristic carbonyl carbon of acetyl group resonated at δ 166.70 ppm. Further, the formation of product **20** was supported by the IR spectrum, absorption band was observed at 1741 cm^{-1} corresponds to the carbonyl of the amide moiety. Subsequent cyclization induced by *t*-BuOK in isopropanol gave the morpholinamide derivative **21** in 69% yield. In the ^{1}H NMR spectrum of **21**, the signals corresponding to methylene protons of morpholinone moiety resonated as a multiplet at δ 3.16-3.37 ppm (-CH**CH_2**NHC=O-) and 4.09-4.33 ppm (-O**CH_2**C=ONH-). Further, in the ^{13}C NMR spectrum, the deshielded methylene carbon (-NH(C=O)**CH_2**) was discernible at δ 67.59 ppm confirms the formation of amide product **21**.

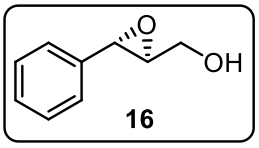
Finally, the reduction of amide bond using Red-Al in THF completed the synthesis of (*R*)-2-benzylmorpholine *R*-**1**, an overall 24% yield with enantiopurity >99% ee, $[\alpha]_{25}^{D}$ = +1.31 (c 5, $CHCl_3$); {lit.[12] $[\alpha]_{25}^{D}$ = +1.28 (c 5, $CHCl_3$)}. The structure of (*R*)-2-benzylmorpholine *R*-**1** was confirmed by means of IR, ^{1}H NMR, ^{13}C NMR and HRMS analysis.

2.1.5. Conclusion

In conclusion, the development of new and alternate synthesis of (*R*)-2-benzyl-morpholine *R*-**1**, an appetite suppressant agent employing Sharpless asymmetric epoxidation as a key step has been described here. Simple procedures, inexpensive starting materials, and high enantiopurity are some of the salient features of this approach. Further, this simple approach offers flexibility in making diverse lead like C-2 chiral morpholine scaffolds for application in a range of therapeutic areas.

2.1.6. Experimental Section

1) ((2*S*,3*S*)-3-phenyloxiran-2-yl)methanol (16)



16

To a stirred solution of 1 g of activated 4 Å molecular sieves (activated at 180 °C overnight in the oven) in CH_2Cl_2 (100 mL), $Ti(OiPr)_4$ (0.44 mL, 1.49 mmol) was added under an inert atmosphere. The reaction mixture was cooled to -20 °C and L-(+)-diisopropyl tartrate (0.37 mL, 1.78 mmol) added and stirred for 15 min after that 5-6 M solution of TBHP in undecane (6 mL, 29.8 mmol) was added at the same temperature. The resulting mixture was allowed to stir at -20 °C for 1 h and then a solution of (*E*)-3-phenyl-2-propenol **15** (2 g, 14.9 mmol) in CH_2Cl_2 (10 mL) was added dropwise over 30 min. After 3 h at -20 °C, the reaction was quenched with 10% aqueous solution of NaOH and saturated with NaCl (2 mL) at -20 °C. After diethyl ether (30 mL) was added, the cold bath was allowed to warm to 10 °C, stirring was maintained at 10 °C, while $MgSO_4$ (1.5 g) and Celite (400 mg) were added. After another 15 min of stirring, the mixture was allowed to settle, and the clear solution was filtered through a pad of Celite and washed with diethyl ether. Azeotropic removal of TBHP with toluene at a reduced pressure and high vacuum crude product as yellow oil. Recrystallization from petroleum ether-diethyl ether gave epoxy alcohol **16** as

white crystals.

Yield: 1.92 g, 86%;

MP: 52-53 °C (lit.[13] mp: 51.5-53 °C);

Molecular Formula: $C_9H_{10}O_2$;

Specific rotation: $[\alpha]^{25}_D$ = -54.3 (*c* 2.4, $CHCl_3$); {lit.[13] $[\alpha]^{25}_D$ = -49.6 (*c* 2.4, $CHCl_3$)};

Chiral HPLC: ee > 99% [The ee of **16** was determined by chiral HPLC analysis; Chiralcel OD-H (250 x 4.6 mm) column; eluent: isopropanol/n-hexane (5:95); flow rate: 1.0 mL/min; detector: 220 nm [(*S*)-isomer t_R = 17.275 (major); (*R*)-isomer t_R = 19.317 (minor)].

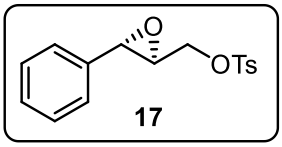
IR ($CHCl_3$, cm^{-1}): ν_{max} 3444, 3019, 2401, 1673, 1462, 1216, 1068, 929, 863, 769;

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.80 (bs, 1 H), 3.18-3.23 (m, 1 H), 3.75 (dd, *J* = 12.8, 4.0 Hz, 1 H), 3.89 (d, *J* = 2.0 Hz, 1 H), 4.0 (dd, *J* = 12.8, 2.3 Hz, 1 H), 7.22-7.37 (m, 5 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 136.5 (C), 128.4 (CH, 2 carbons), 128.2 (CH), 125.6 (CH, 2 carbons), 62.5 (CH), 61.2 (CH_2), 55.6 (CH);

HRMS (ESI): *m/z* calculated for $C_9H_{10}O_2$ $[M+Na]^+$ 173.0572, found 173.0572.

2) ((2*S*,3*S*)-3-phenyloxiran-2-yl)methyl 4-methylbenzenesulfonate (17)



17

To a stirred solution of epoxyalcohol **16** (1.8 g, 11.9 mmol) in anhydrous CH_2Cl_2 (10 mL) were added DMAP (0.15 g, 1.19 mmol), anhydrous triethylamine (3.34 mL, 23.9 mmol), at -20 °C and stir it for 30 min. After that the solution of *p*-toluenesulfonyl chloride (2.97 g, 15.6 mmol) in anhydrous CH_2Cl_2 (5 mL) was added dropwise about 15 min at -20 °C and resulting mixture was stirred at -20 °C for 7 h. After completion of reaction (monitored by TLC), water (2 x 10 mL) was added and then the organic phase was separated. The aqueous layer was extracted with dichloromethane (2 x 15 mL). The combined organic extracts were washed with brine (2 x 10 mL), dried over Na_2SO_4 and the solvent was evaporated under reduced pressure gave tosylated product **17** as a colorless solid which was used for further step without purification.

Yield: 3.45 g, 95%;

MP: 64-66 °C (lit.[14] mp: 68-69 °C); ;

Molecular Formula: $C_{16}H_{16}O_4S$;

Specific rotation: $[\alpha]^{25}_{D}$ = -44.5 (*c* 2.5, $CHCl_3$); {lit.[14] $[\alpha]^{25}_{D}$ = -45.0 (*c* 2.5, $CHCl_3$)};

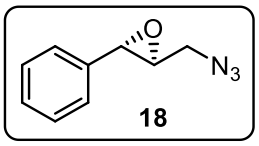
IR ($CHCl_3$, cm^{-1}): ν_{max} 3375, 3024, 2404, 1649, 1600, 1450, 1366, 1217, 1037, 978, 851, 762, 669;

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.46 (s, 3 H), 3.23-3.26 (m, 1 H), 3.76 (apparent d, *J* = 1.7 Hz, 1 H), 4.14 (dd, *J* = 11.5, 5.6 Hz, 1 H), 4.34 (dd, *J* = 11.5, 3.7 Hz, 1 H), 7.21-7.23 (m, 2 H), 7.32-7.37 (m, 5H), 7.83 (d, *J* = 8.1 Hz, 2 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 145.1 (C), 135.5 (C), 132.6 (C), 129.9 (CH, 2 carbons), 128.6 (CH, 2 carbons), 128.5 (CH), 128.0 (CH, 2 carbons), 125.6 (CH, 2 carbons), 69.4 (CH_2), 58.5 (CH), 56.4 (CH), 21.6 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{16}H_{16}O_4S$ $[M+H]^+$ 305.0842, found 305.0836; $C_{16}H_{16}O_4S$ $[M+Na]^+$ 327.0662, found 327.0655.

3) (2*S*,3*S*)-2-(azidomethyl)-3-phenyloxirane (18)



18

To a stirred solution of tosylated compound **17** (3 g, 9.8 mmol) in anhydrous DMF (15 mL) was added sodium azide (0.64 g, 9.8 mmol) at 0 °C and the resulting mixture was stirred for 5 h at 0 °C and allowed to room temperature for further 10 h. After completion of reaction (monitored by TLC), EtOAc (20 mL) and ice cold water (3 x 10 mL) were added and then the organic phase was separated. The aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic extracts were washed with brine (2 x 10 mL), dried over Na_2SO_4 and the solvent was evaporated under reduced pressure gave azide product **18**.

Yield: 1.31 g, 76%;

Molecular Formula: $C_9H_9N_3O$;

Specific rotation: $[\alpha]^{25}_{D}$ = -59.6 (*c* 1.86, $CHCl_3$);

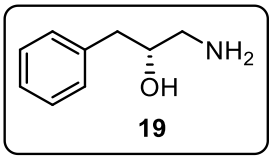
IR ($CHCl_3$, cm^{-1}): ν_{max} 3346, 3017, 2927, 2863, 2101, 1670, 1450, 1357, 1266, 1220, 1098, 1027, 977, 877, 761, 665;

^{1}H NMR (200 MHz, $CDCl_3$): δ 3.21 (ddd, *J* = 5.2, 3.4, 2.0 Hz, 1 H), 3.42 (dd, *J* = 13.6, 5.1 Hz, 1 H), 3.63 (dd, *J* = 13.6, 3.3 Hz, 1 H), 3.83 (d, *J* = 2.0 Hz, 1 H), 7.22-7.37 (m, 5 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 136.0 (C), 128.5 (CH, 2 carbons), 128.4 (CH), 125.6 (CH, 2 carbons), 60.2 (CH), 56.3 (CH), 51.6 (CH_2);

HRMS (ESI): *m/z* calculated for C_9H_9O $[M-N_3]^+$ 133.0648, found 133.0647.

4) (*R*)-1-amino-3-phenylpropan-2-ol (19)



19

To a stirred solution of azide **18** (1.5 g, 8.46 mmol) in methanol (10 mL) was added 10% palladium on activated carbon (0.15 g) and the reaction mixture was stirred under hydrogen atmosphere (50 psi) for 5 h. After completion of the reaction (monitored by TLC), the catalyst was filtered through the bed of Celite (EtOAc eluent) and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography [basic alumina, EtOAc/petroleum ether (10:90)] afforded amino alcohol **19** as pale yellow oil.

Yield: 0.97 g, 75 %;

Molecular Formula: $C_9H_{13}NO$;

Specific rotation: $[\alpha]^{25}_D$ = +1.84 (*c* 1.02, $CHCl_3$);

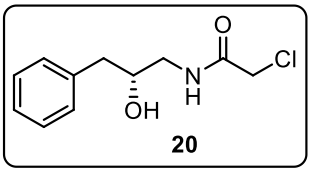
IR ($CHCl_3$, cm^{-1}): ν_{max} 3668, 3570, 3367, 3019, 2929, 1651, 1577, 1492, 1457, 1330, 1217, 1084, 1035, 927, 764, 668;

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.02 (bs, 3 H), 2.52-2.63 (m, 1 H), 2.71-2.85 (m, 2 H), 3.68-3.80 (m, 1 H), 7.19-7.35 (m, 5 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 138.3 (C), 129.3 (CH, 2 carbons), 128.4 (CH, 2 carbons), 126.3 (CH), 73.0 (CH), 46.8 (CH_2), 41.4 (CH_2);

HRMS (ESI): *m/z* calculated for $C_9H_{13}NO$ $[M+H]^+$ 152.1070, found 152.1070; $C_9H_{13}NO$ $[M+Na]^+$ 174.0889, found 174.0889.

5) (*R*)-2-chloro-*N*-(2-hydroxy-3-phenylpropyl)acetamide (20)



20

To a stirred solution of amino alcohol **19** (0.7 g, 4.6 mmol) in toluene (5 mL) was added sodium carbonate (0.932 g, 8.8 mmol) in water (1 mL) at 0 °C. After 15 min, to the above resulting mixture chloroacetyl chloride (0.590 g, 5.2 mmol, 416 µL) in toluene (5 mL) was

added over 30 min. The resulting mixture was stirred for 1 h at 5 °C. After completion of the reaction (monitored by TLC), the residue was diluted with water (5 mL) and extracted with toluene (3 x 5 mL). The phases were separated, and the organic phase was washed with brine (2 x 5 mL), dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure and the crude residue was purified by column chromatography [silica gel, EtOAc/petroleum ether (25:75)] gave acetylated product **20** as a colorless oil.

Yield: 0.915 g, 87%;

Molecular Formula: $C_{11}H_{14}ClNO_2$;

Specific rotation: $[\alpha]^{25}_D$ = -6.3 (*c* 1.4, $CHCl_3$);

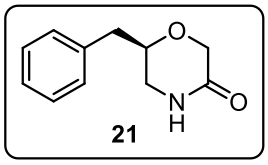
IR ($CHCl_3$, cm^{-1}): ν_{max} 3423, 3022, 2928, 2403, 1741, 1594, 1532, 1423, 1216, 1030, 927, 765, 672;

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.33 (bs, 1 H), 2.74 (dd, *J* = 13.6, 8.2 Hz, 1 H), 2.85 (dd, *J* = 13.6, 5.0 Hz, 1 H), 3.22-3.28 (m, 1 H), 3.60-3.66 (m, 1 H), 3.97-4.01 (m, 1 H), 4.07 (s, 2 H), 6.99 (bs, 1 H), 7.21-7.23 (m, *J* = 7.5 Hz, 1 H), 7.26-7.36 (m, 4 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7 (CO), 137.1 (C), 129.3 (CH, 2 carbons), 128.8 (CH, 2 carbons), 126.9 (CH), 71.6 (CH), 45.0 (CH_2), 42.6 (CH_2), 41.5(CH_2);

HRMS (ESI): *m/z* calculated for $C_{11}H_{14}O_2NCl$ $[M+H]^+$ 228.0786, found 228.0785; $C_{11}H_{14}O_2NCl$ $[M+Na]^+$ 250.0605, found 250.0605.

6) (*R*)-2-benzylmorpholin-3-one (21)



21

Potassium tertiary butoxide (0.98 g, 8.78 mmol) in dry isopropanol (10 mL) was added dropwise to a stirred solution of acetylated compound **20** (0.80 g, 3.51 mmol) in dry isopropanol (5 mL) at 0 °C under N_2. The resulting mixture was stirred for 2 h at room temperature. After completion of the reaction (monitored by TLC), the reaction was quenched with 1 N HCl solution (10 mL) extracted with ethyl acetate (2 x 20 mL). The combined organic layers were washed with brine (2 x 5 mL) dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by column chromatography [basic alumina, methanol/CH_2Cl_2 (4:96)] afforded amide product **21** as a colorless solid.

Yield: 0.465 g, 69%;

MP: 106-108 °C;

Molecular Formula: $C_{11}H_{13}NO_2$;

Specific rotation: $[\alpha]^{25}_D$ = -30.7 (*c* 1.81, $CHCl_3$);

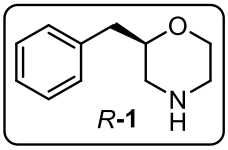
IR ($CHCl_3$, cm^{-1}): ν_{max} 3406, 3019, 2400, 1681, 1496, 1455, 1427, 1341, 1215, 1114, 1027, 928, 669;

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.76 (dd, *J* = 13.9, 6.4 Hz, 1 H), 2.99 (dd, *J* = 13.9, 6.8 Hz, 1 H), 3.16-3.37 (m, 2 H), 3.84-3.97 (m, 1 H), 4.2 (q, *J* = 16.9, 14.5 Hz, 2 H), 6.95 (bs, 1 H), 7.18-7.36 (m, 5 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 169.1 (CO), 136.7 (C), 129.1 (CH, 2 carbons), 128.6 (CH, 2 carbons), 126.8 (CH), 73.8 (CH), 67.6 (CH_2), 45.9 (CH_2), 39.1 (CH_2);

HRMS (ESI): *m/z* calculated for $C_{11}H_{13}O_2N$ $[M+H]^+$ 192.1019, found 192.1019; $C_{11}H_{13}O_2N$ $[M+Na]^+$ 214.0838, found 214.0838.

7) (*R*)-2-benzylmorpholine (*R*-1)



R-**1**

To a stirred solution of amide derivative, **21** (0.2 g, 1.04 mmol) in THF (10 mL) was added slowly a solution of Red-Al (65% w/w in toluene, 845 µL, 4.18 mmol) at 0 °C. After being stirred at room temperature for 12 h, the reaction mixture was cooled to 0 °C and quenched with dropwise addition of water (1 mL) followed by the addition of an aqueous 4 N KOH solution (2 mL). The resulting crude residue was filtered over Celite bed, dried over Na_2SO_4 and concentrated in vacuo. Purification of the crude residue by flash chromatography [silica-gel, methanol/CH_2Cl_2 (3:97)] afforded (*R*)-2-benzylmorpholine *R*-**1** as a colorless oil.

Yield: 0.157 g, 85%;

Molecular Formula: $C_{11}H_{15}NO$;

Specific rotation: $[\alpha]^{25}_D$ = +1.31 (*c* 5.0, $CHCl_3$); {lit.[12] $[\alpha]^{25}_D$ = +1.28 (*c* 5.0, $CHCl_3$)};

Chiral HPLC: ee > 99.9% [The ee of *R*-**1** was determined by chiral HPLC analysis; Chiralcel OD-H (250 x 4.6 mm) column; eluent: EtOH/n-hexane/TFA (10:90:0.1); flow rate: 1.0 mL/min; detector: 254 nm [(*R*)-isomer t_R = 6.625 (major); (*S*)-isomer t_R = 8.075 (minor)];

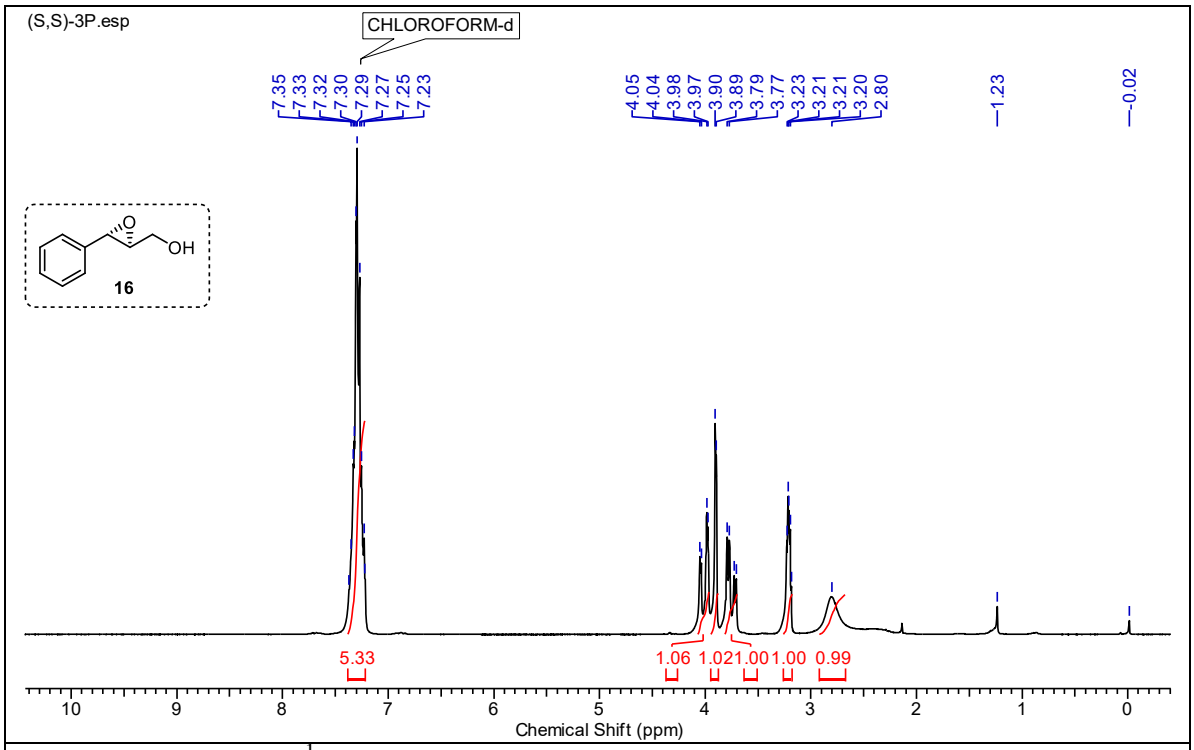
IR ($CHCl_3$, cm^{-1}): ν_{max} 3020, 2400, 1652, 1403, 1215, 1099, 929, 669;

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.40 (bs, 1 H), 2.51-2.68 (m, 2 H), 2.75-2.93 (m, 4 H), 3.51-3.71 (m, 2 H), 3.83-3.89 (m, 1 H), 7.17-7.32 (m, 5 H);

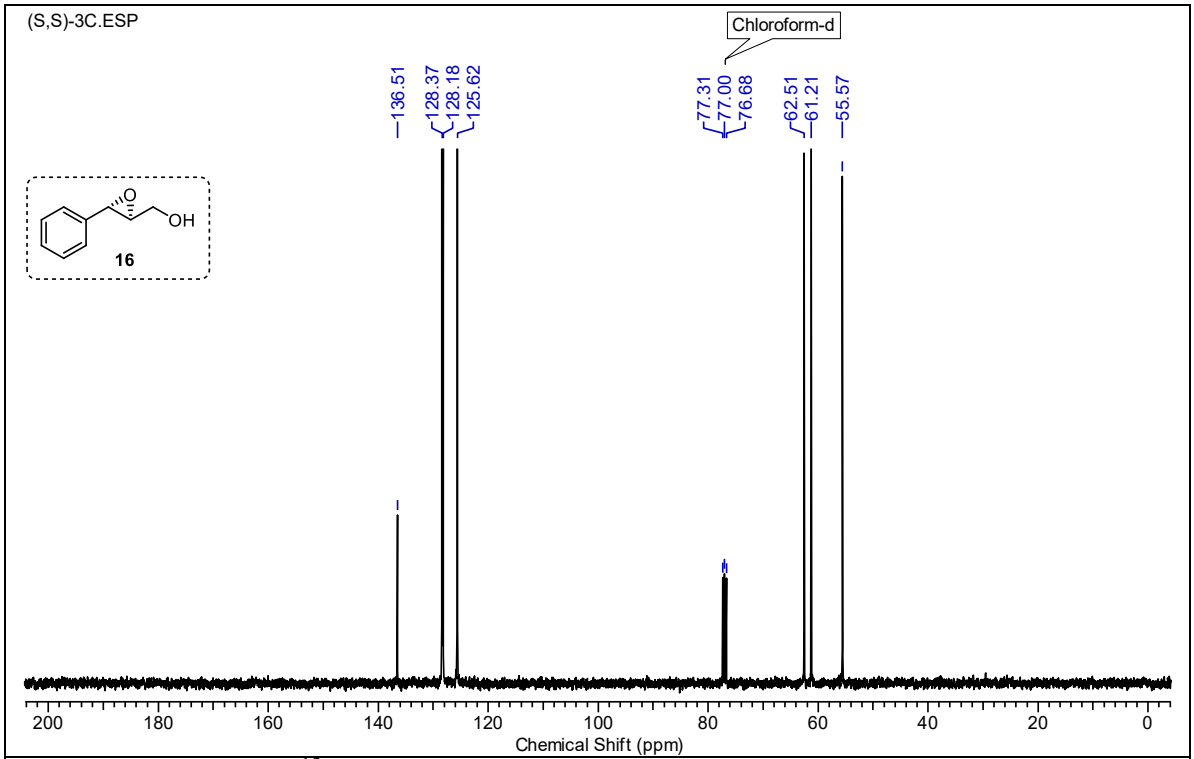
^{13}C NMR (125 MHz, $CDCl_3$): δ 137.6 (C), 129.2 (CH, 2 carbons), 128.3 (CH, 2 carbons), 126.4 (CH), 77.1 (C), 67.6 (CH_2), 50.3 (CH_2), 45.3 (CH_2), 40.2 (CH_2);

HRMS (ESI): *m/z* calculated for $C_{11}H_{15}ON$ $[M+H]^+$ 178.1226, found 178.1226.

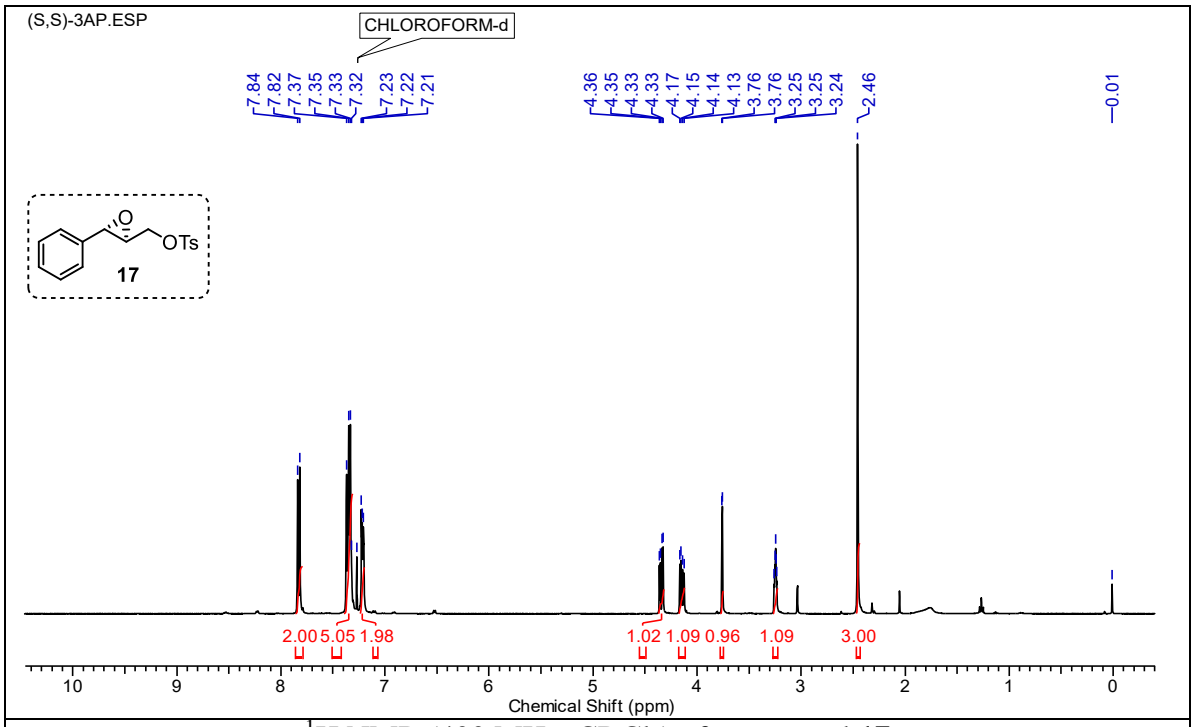
2.1.7. Spectra



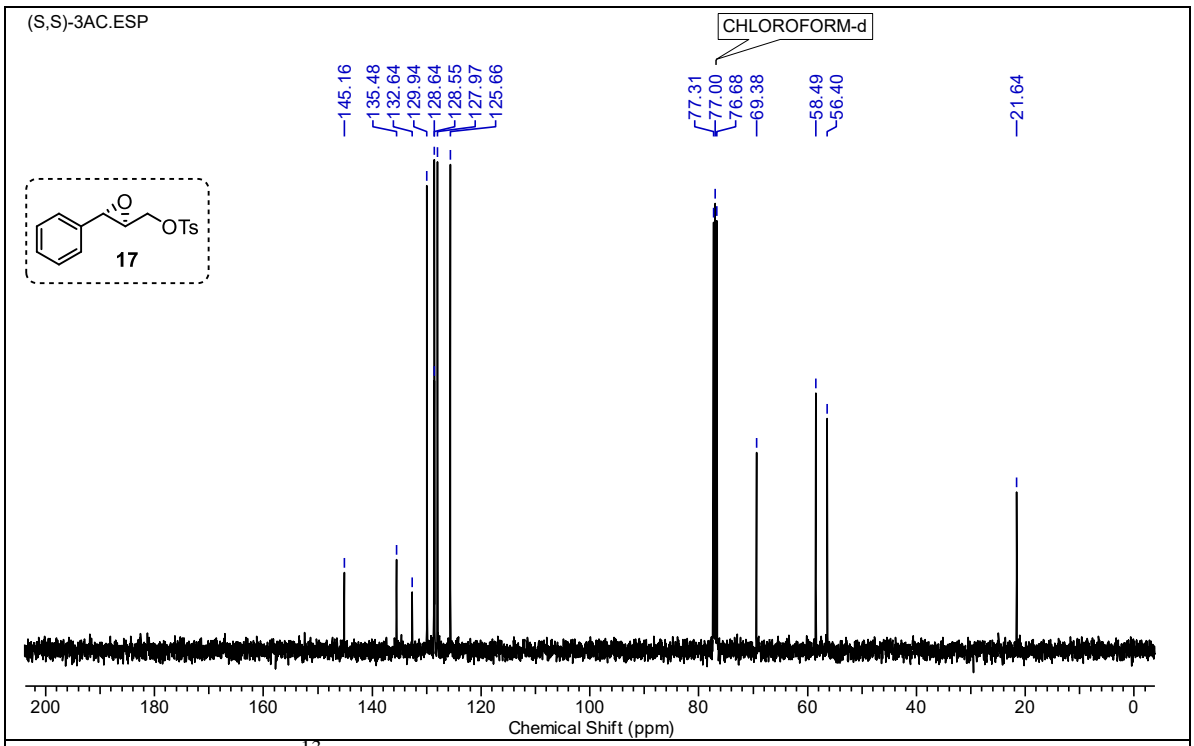
^{1}H NMR (200 MHz, $CDCl_3$) of compound **16**



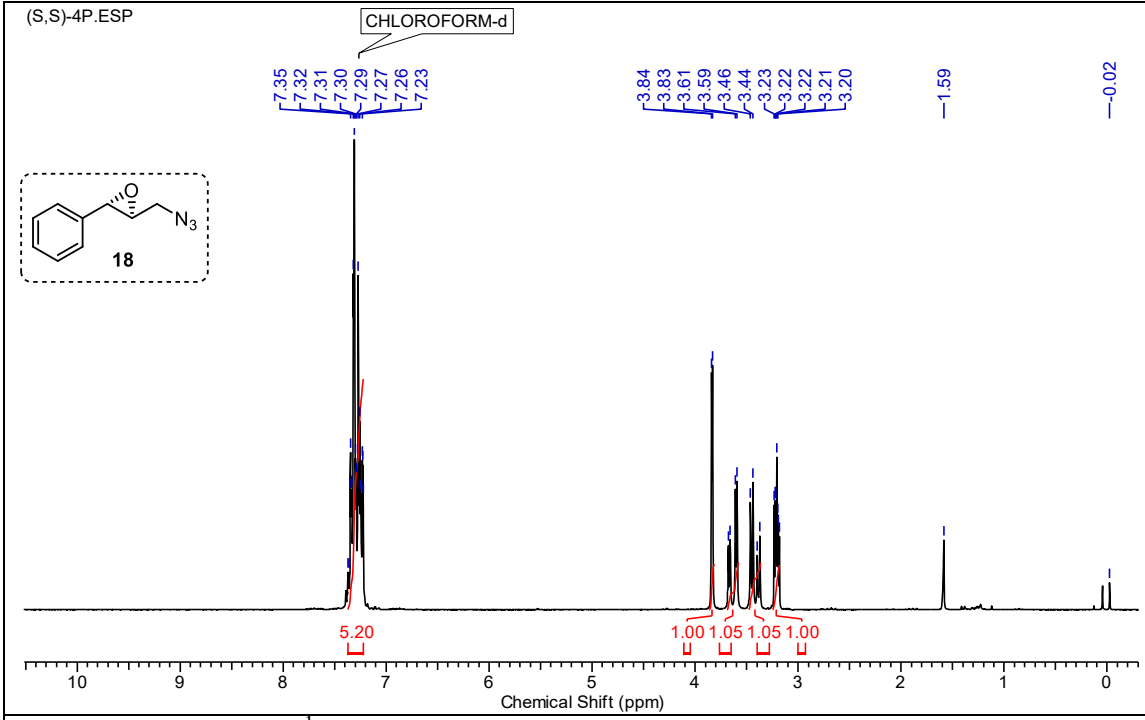
^{13}C NMR (100 MHz, $CDCl_3$) of compound **16**



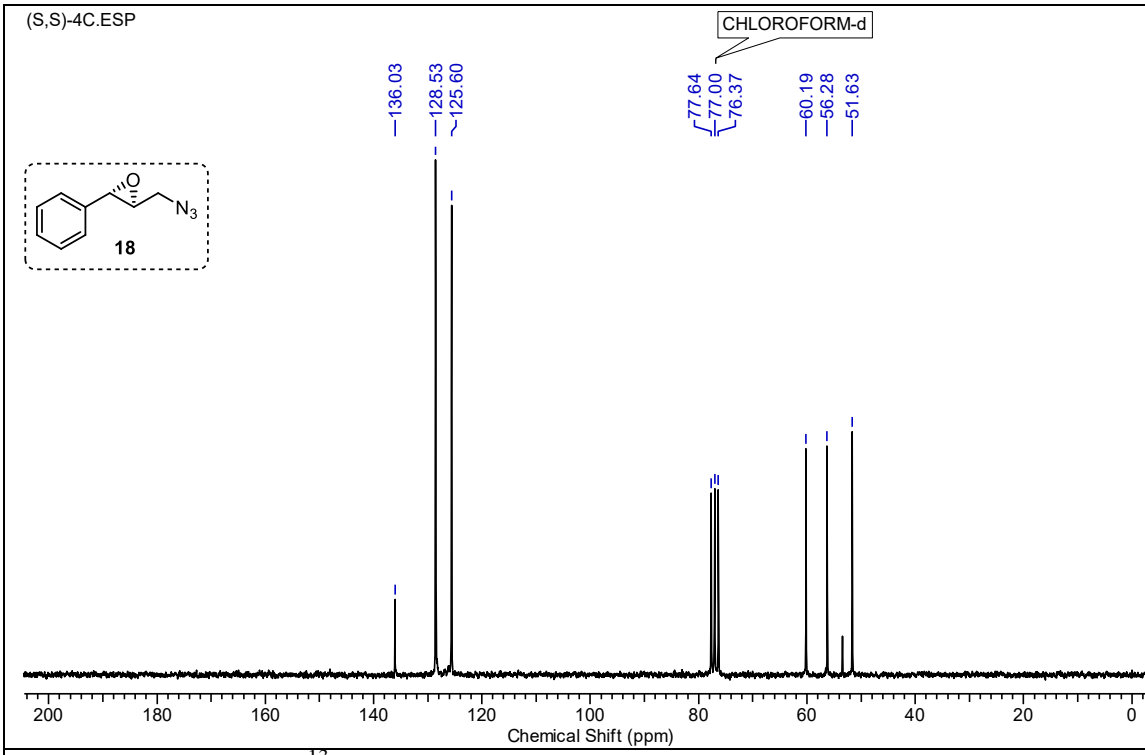
^{1}H NMR (400 MHz, $CDCl_3$) of compound **17**



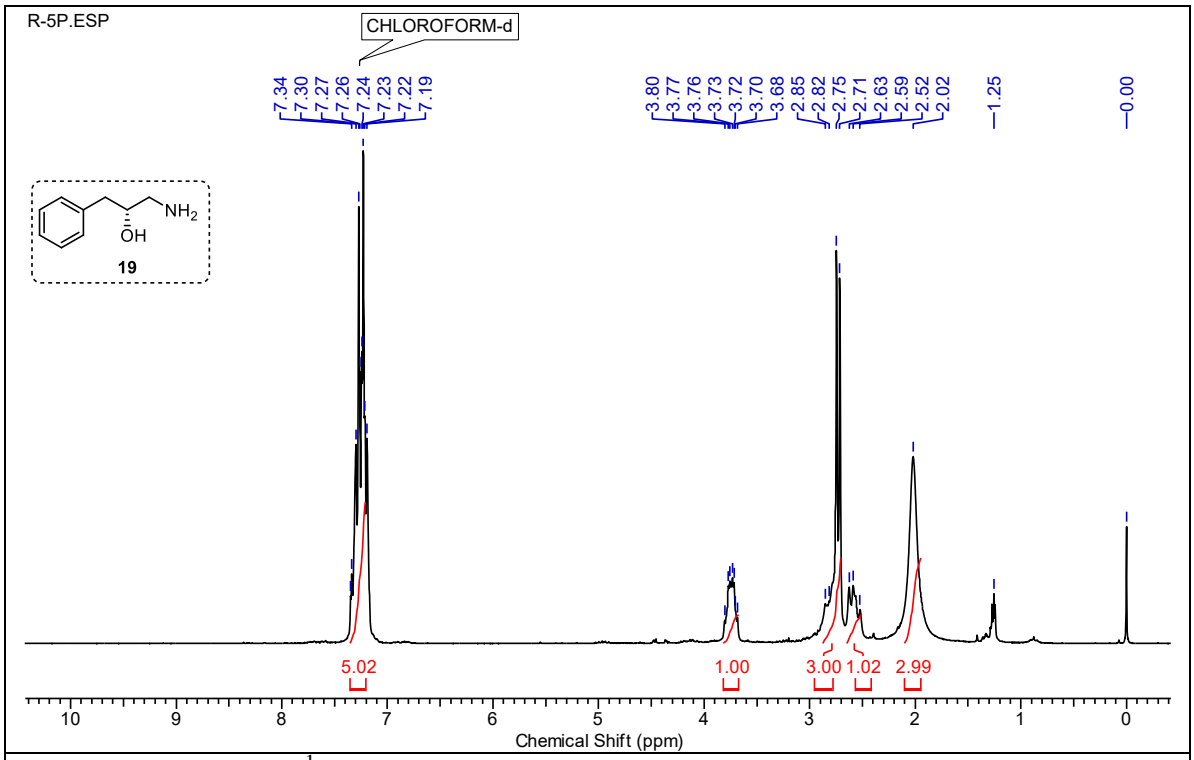
^{13}C NMR (100 MHz, $CDCl_3$) of compound **17**



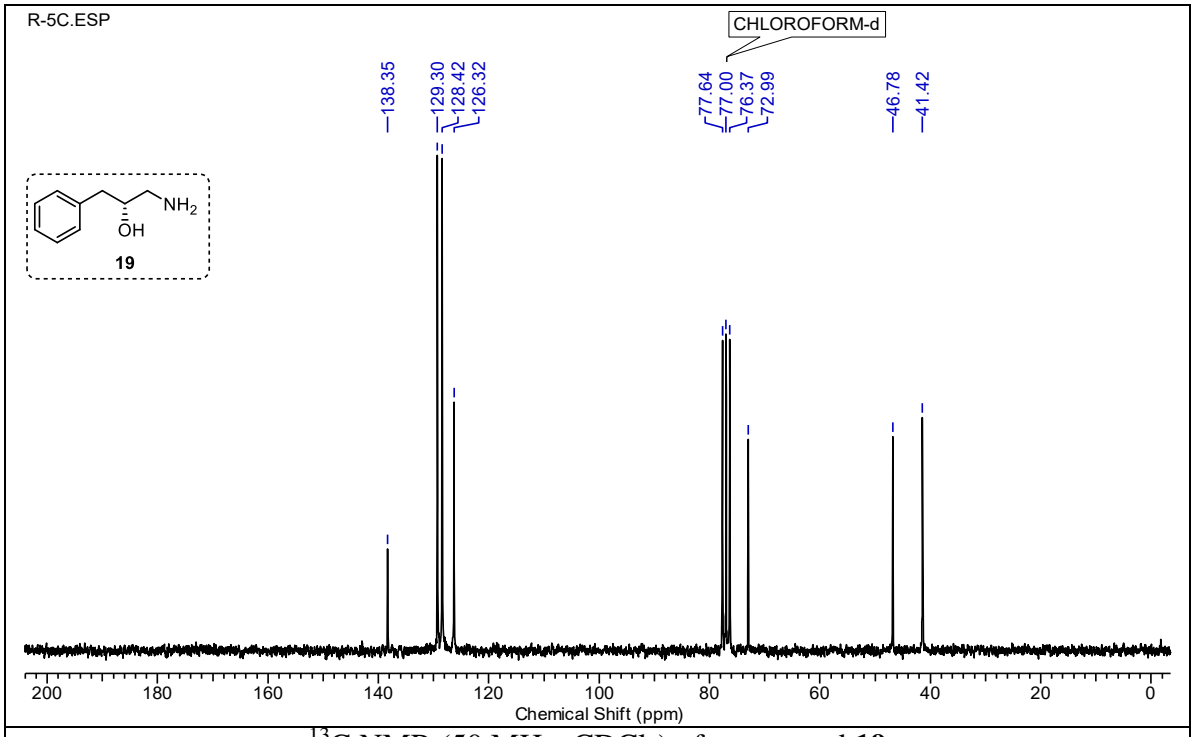
^{1}H NMR (200 MHz, $CDCl_3$) of compound **18**



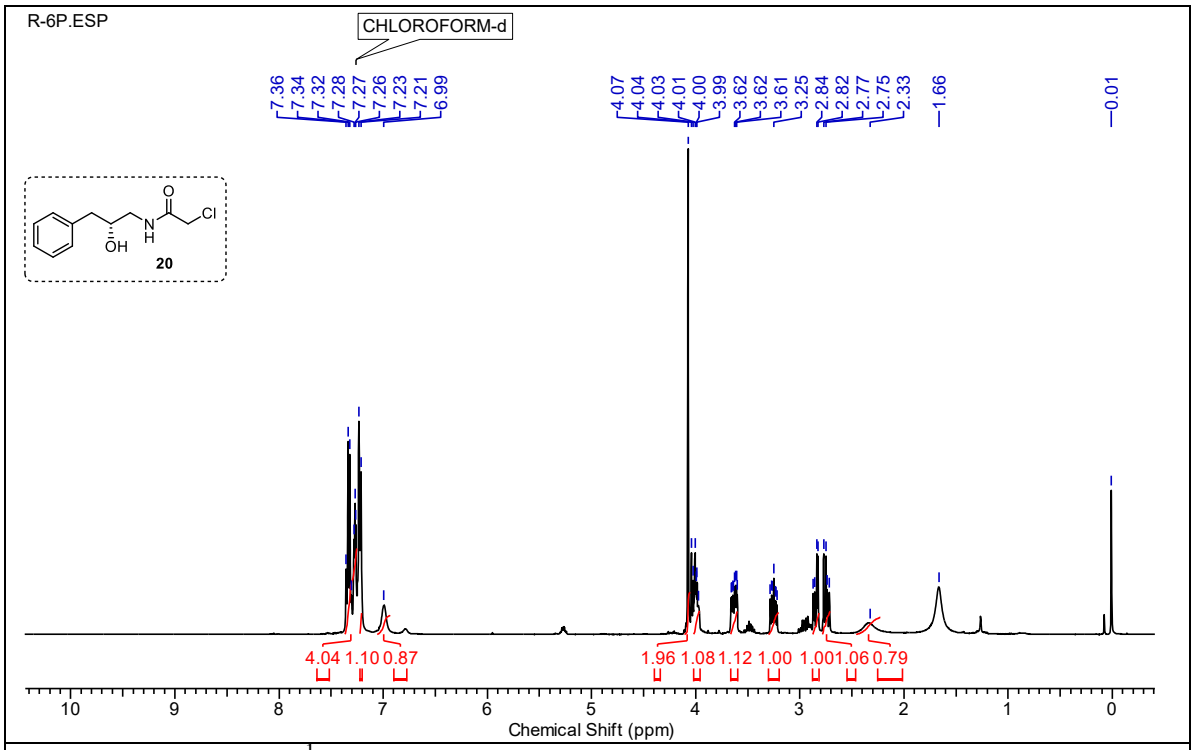
^{13}C NMR (50 MHz, $CDCl_3$) of compound **18**



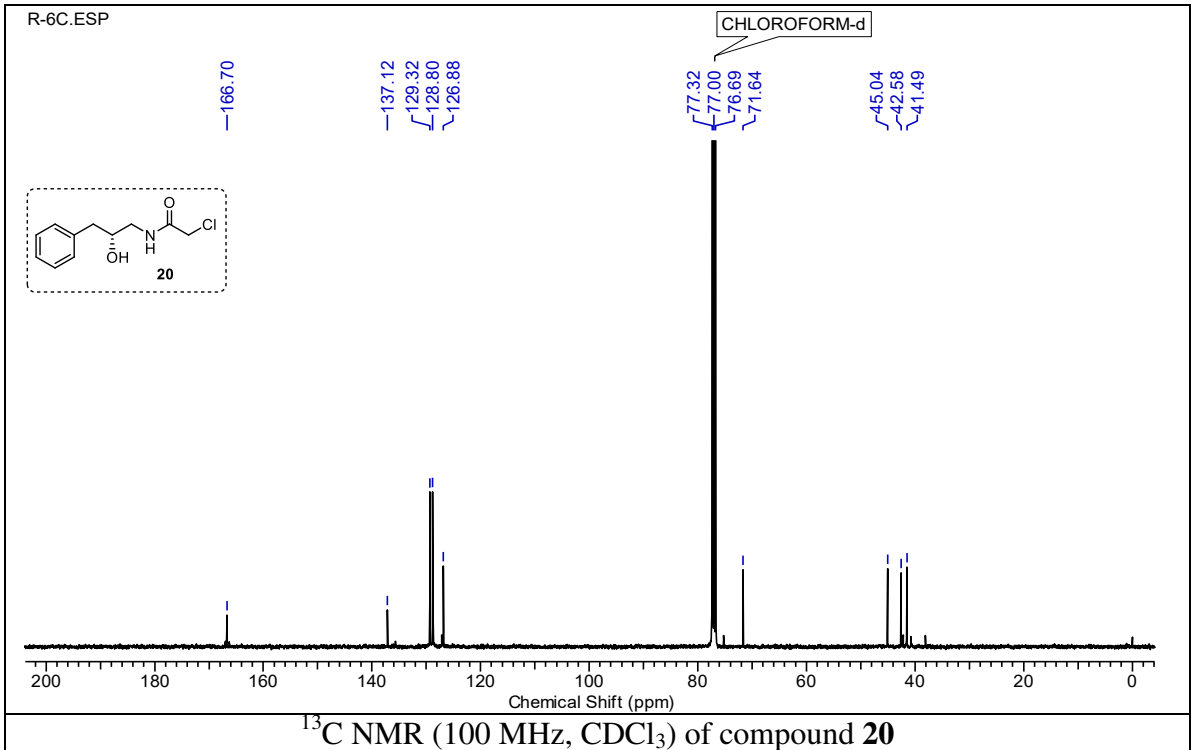
^{1}H NMR (200 MHz, $CDCl_3$) of compound **19**



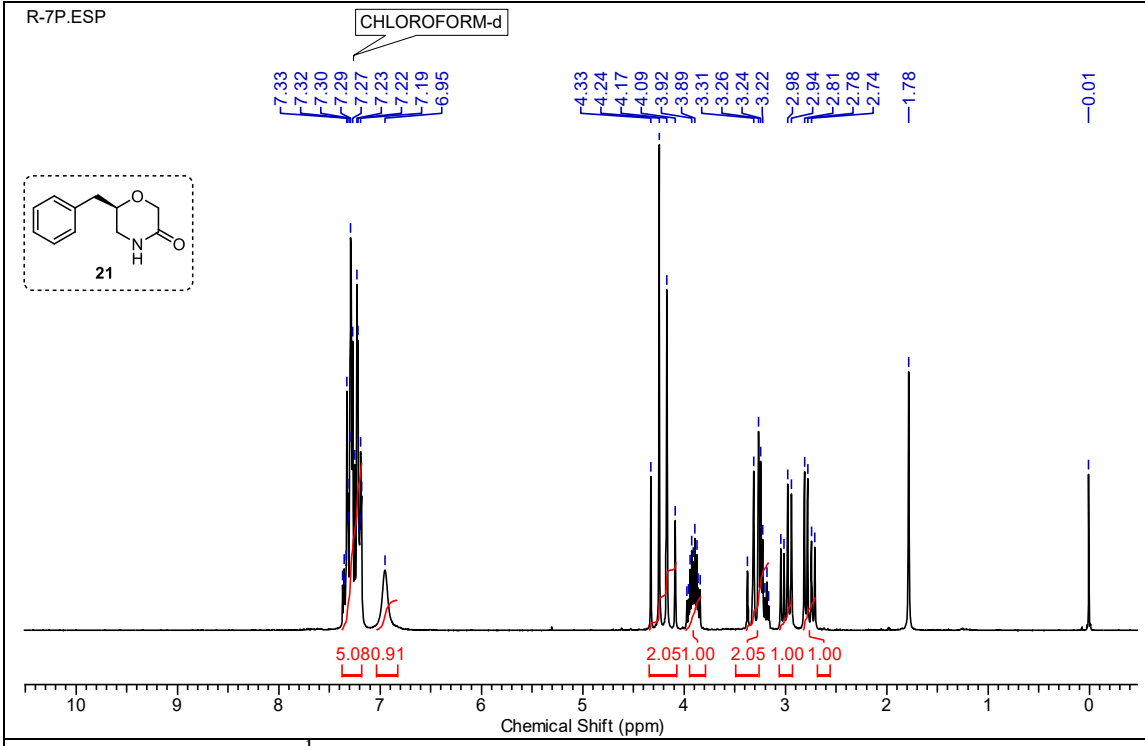
^{13}C NMR (50 MHz, $CDCl_3$) of compound **19**



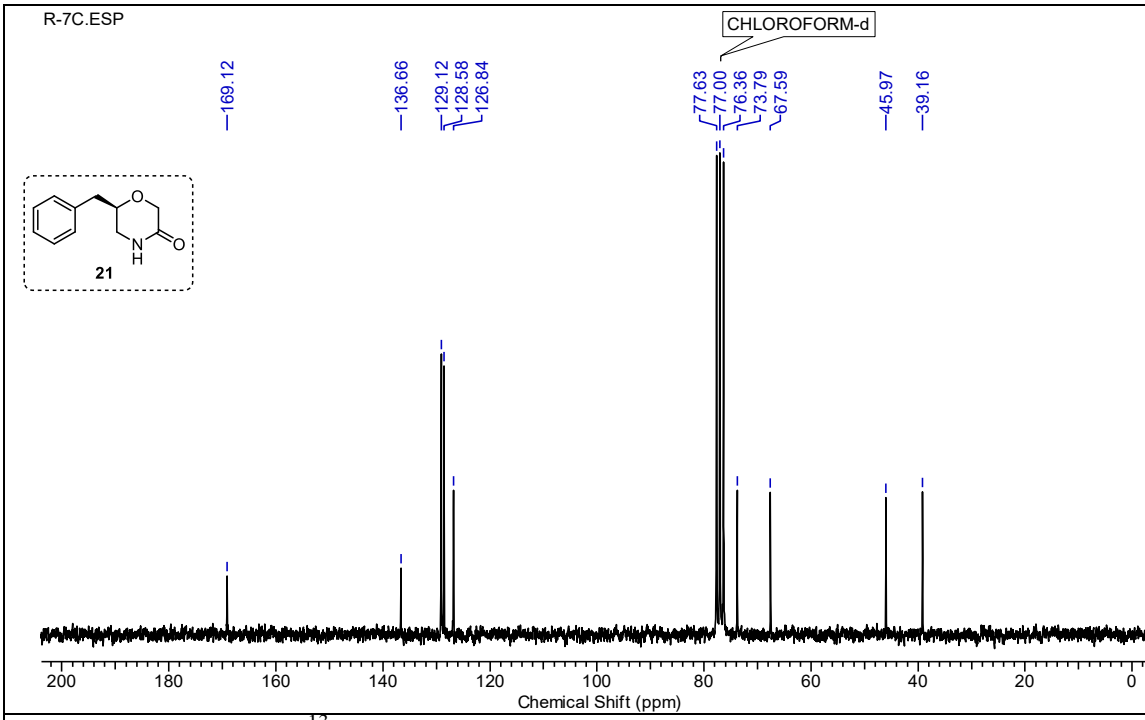
^{1}H NMR (400 MHz, $CDCl_3$) of compound **20**



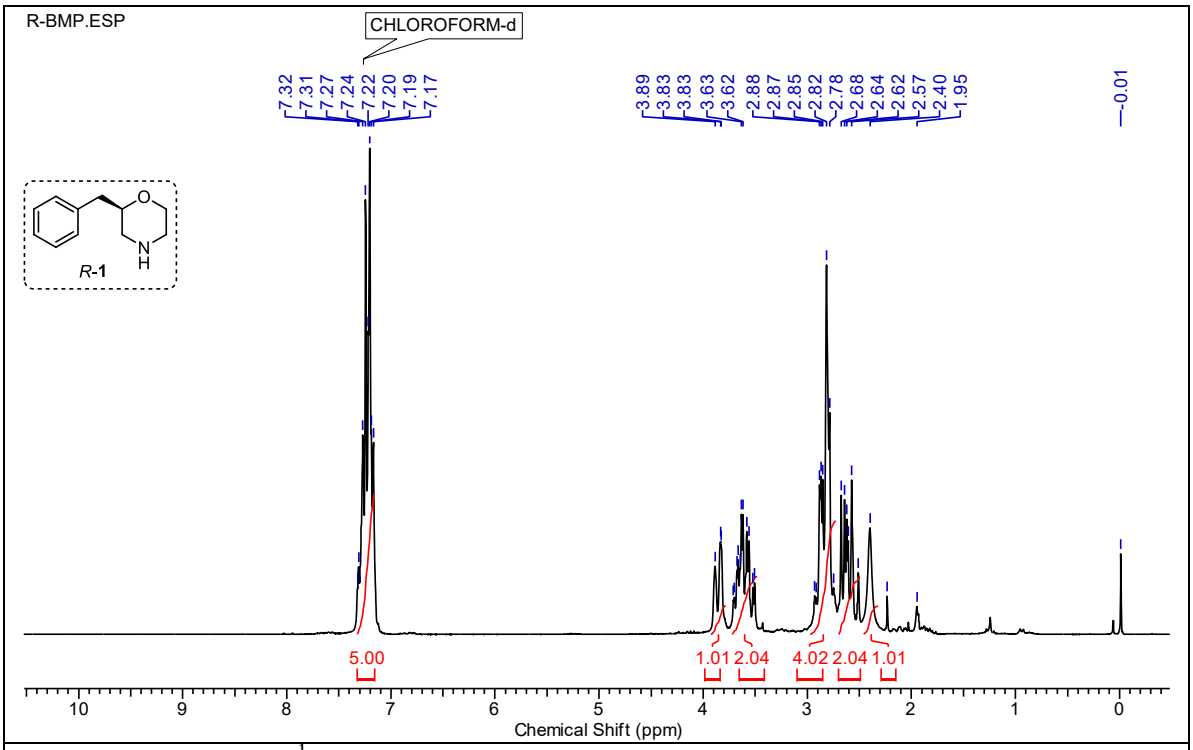
^{13}C NMR (100 MHz, $CDCl_3$) of compound **20**



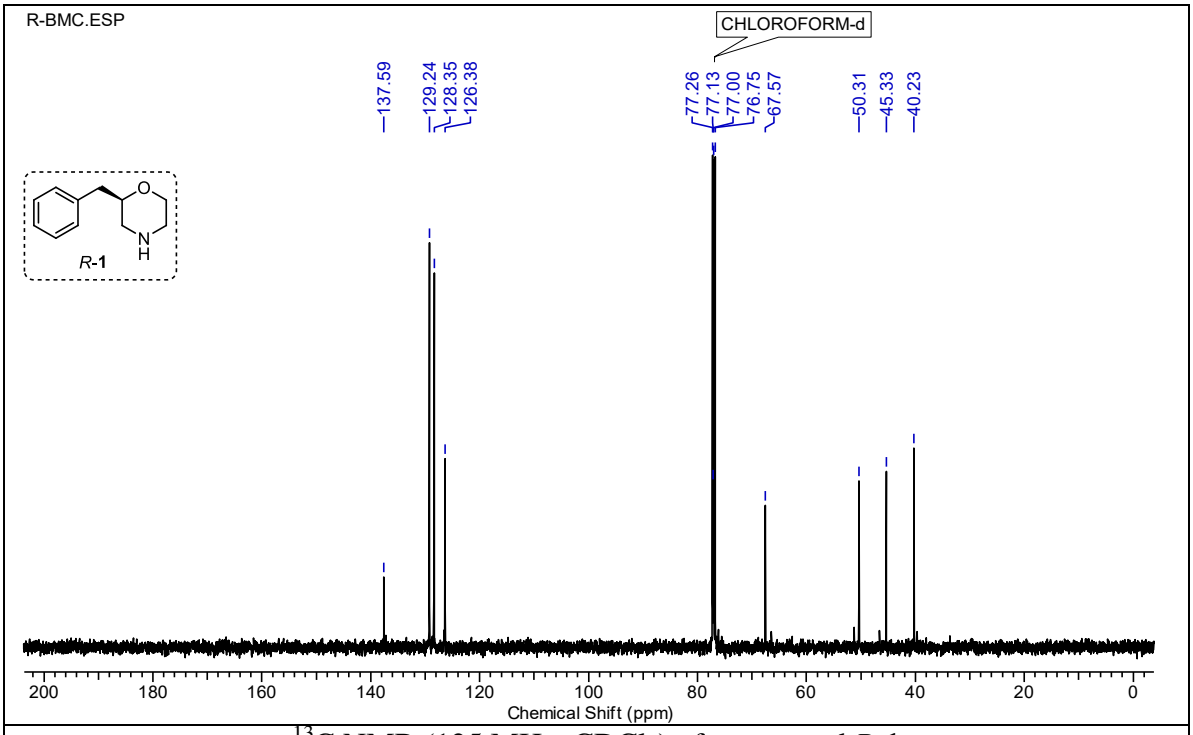
^{1}H NMR (200 MHz, $CDCl_3$) of compound **21**



^{13}C NMR (50 MHz, $CDCl_3$) of compound **21**



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



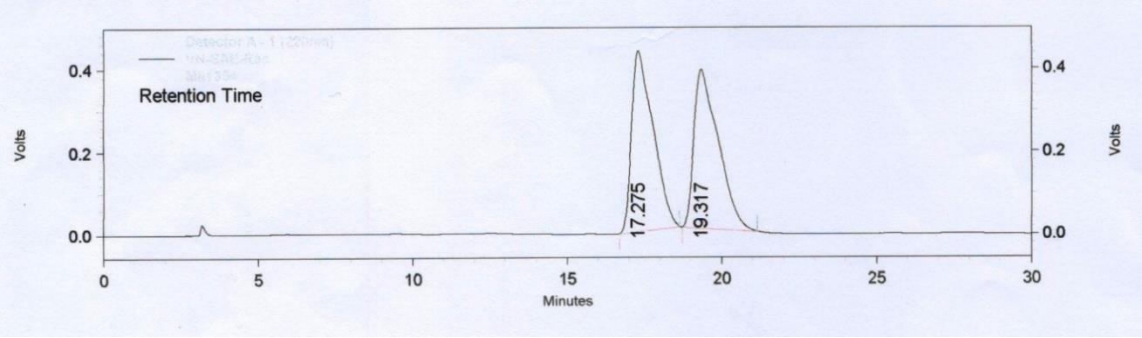
^{13}C NMR (125 MHz, $CDCl_3$) of compound *R*-**1**

2.1.8. Chiral HPLC analysis data

Chiral HPLC analysis of Compound 16

Conditions: Chiralcel OD-H (250 X 4.6 mm) column; eluent: n-Hexane/isopropanol (95:05); flow rate: 1 mL/min; detector 254 nm.

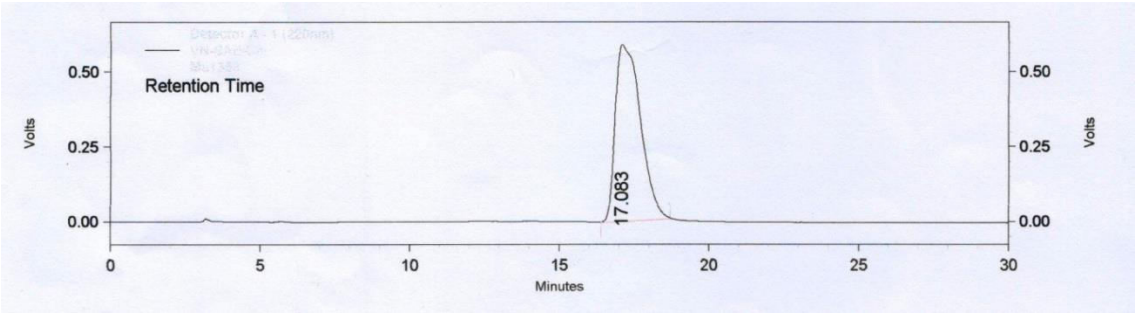
Racemic



Racemic Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	17.275	10554460	50.062
2	19.317	10528178	49.938
Totals		21082638	100.000

Chiral



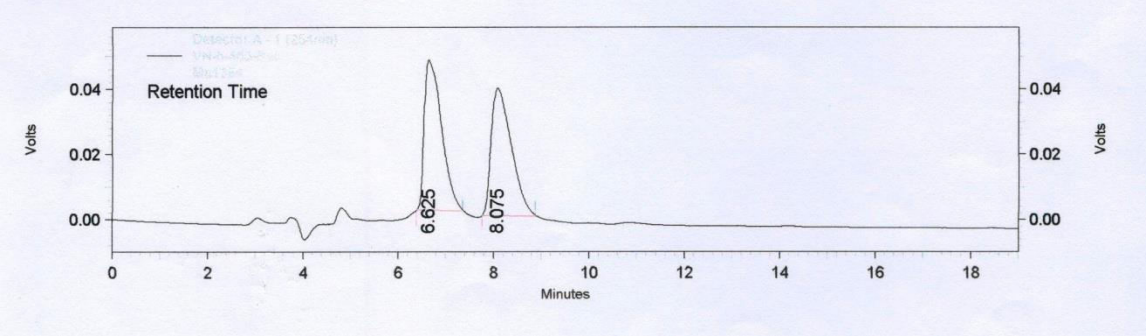
Chiral Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	17.083	17052835	100
Totals		17052835	100

Chiral HPLC analysis of Compound *R*-1

Conditions: Chiralcel OD-H (250 X 4.6 mm) column; eluent: n-Hexane/ethanol/TFA (90:10:0.1); flow rate: 1 mL/min; detector 254 nm.

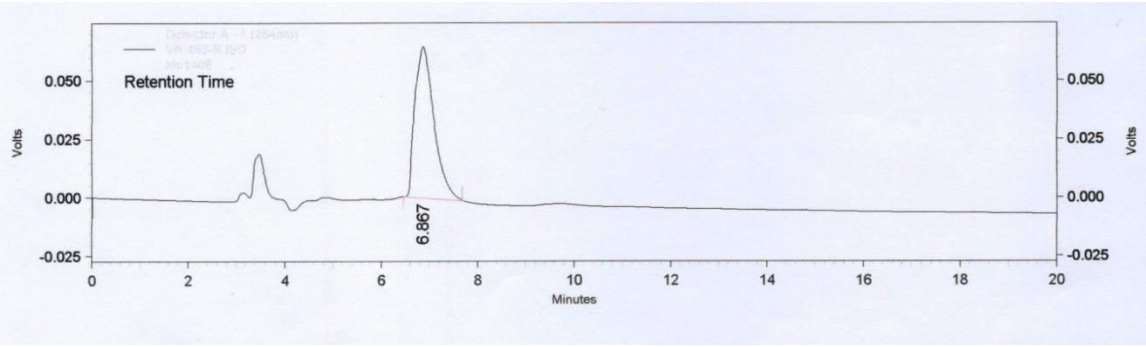
Racemic



Racemic Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	6.625	579798	50.450
2	8.075	569460	49.550
Totals		1149258	100.000

Chiral



Chiral Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	6.867	1777358	100
Totals		1777358	100

2.1.9. References

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2.2. SECTION 2

A new and efficient enantioselective synthesis of both enantiomers of calcium channel blocker bepridil

2.2.1. Introduction

Calcium channel blockers (CCBs) or calcium antagonists are a class of drugs developed as potent vasodilators and identified by the German physiologist Albrecht Fleckenstein in mid-1960's.[1] Calcium channel blockers are widely used in treating various cardiovascular diseases such as hypertension, angina pectoris, cardiac arrhythmia (abnormal heart rhythms), Raynaud's phenomenon and migraine. It inhibits the influx of calcium (Ca^{2+}) ions into cell membrane by blockade of calcium ion channel.[2] Based on the chemical structure and functional distinctions, L-type calcium channels are categorized into three classes namely (**Figure 1**),[3]

a) 1,4-Dihydropyridines (DHPs) (e.g. Amlodipine, felodipine, isradipine, nicardipine)
b) Phenylalkylamines (PAAs) (e.g. Verapamil, bepridil)
c) Benzothiazepines (BTZs) (e.g. Diltiazem)

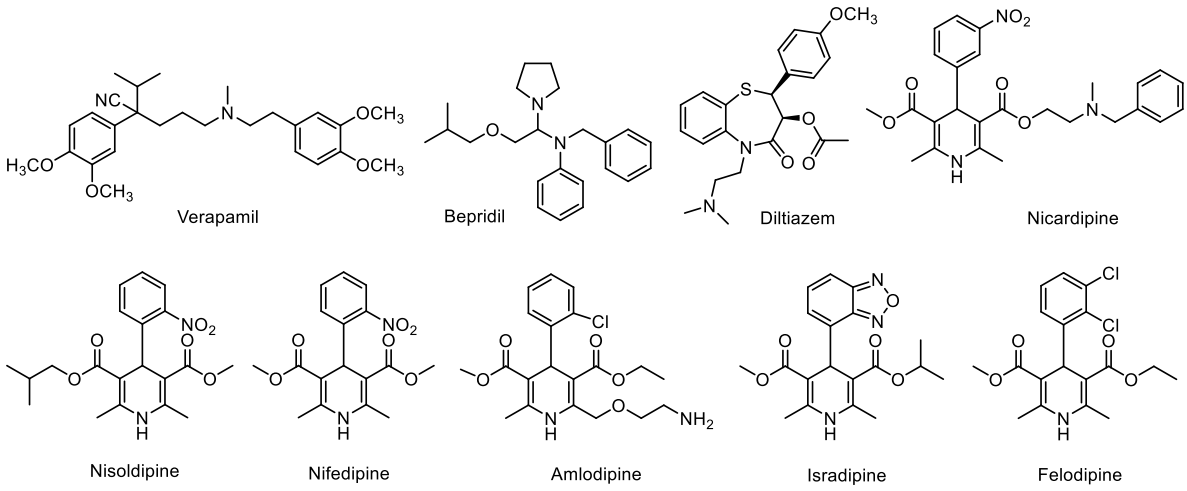


Figure 1. Representative examples of calcium channel blockers (CCBs)

Majority of CCBs are 1,4-Dihydropyridines. The pharmacostudies of these classes of drugs shows that dihydropyridines have greater selectivity for vasodilator activity than benzothiazepines and phenylalkylamines. One newer class, the diarylaminopropanolethers e.g. bepridil operates through a slightly different mechanism associated with T (transient)-type channels.[4]

The basic mechanism of action of calcium channel blocker is a blockade of voltage-gated calcium channels (VGCCs). When calcium enters into the vascular and cardiac muscles it causes smooth muscle contraction. Many CCBs (antihypertensive/antianginal), that block the passage of calcium ions into heart and blood vessel muscle cells promotes the relaxation of smooth muscles around the blood vessels results in widening of blood vessels (vasodilation), reduces the flow of blood supply to the heart muscle results in reduction of systolic and diastolic blood pressure (arterial pressure), and helping to prevent hypertension, chest pain as well as abnormal heart rhythms. Most of the CCBs are high lipophilic in nature and well absorbed orally (>90%) and extensively metabolized in the liver *via* first-pass hepatic metabolism.[5]

Bepridil

Bepridil (Trade name: Vascor®) is a non-selective, long-acting calcium channel blocker with significant antianginal and anti-ischemic activities (**Figure 2**).[6] It has antihypertensive and selective anti-arrhythmia activities and acts as a calmodulin antagonist.[7] It is generally administered as a racemate. However, as expected, pharmacological studies reveal that there are significant differences in activity amongst bepridil enantiomers and (*R*)-enantiomer of bepridil is more active than (*S*)-enantiomer.[8]

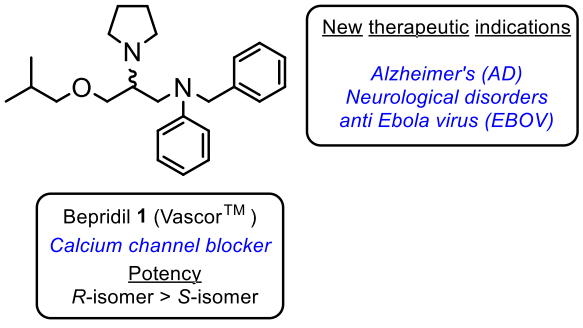


Figure 2. Bepridil

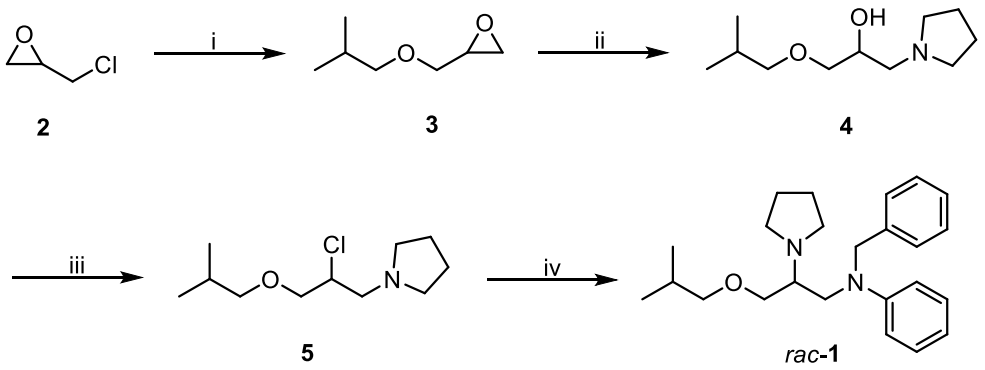
Importantly, repurposing or repositioning of approved drugs has significant benefits over traditional drug development for finding new indications for rare and neglected diseases to minimize costs, duration and risks.[9] In recent years, drug repositioning has accounted for approximately 30% of the newly approved drugs by the FDA. In this context, bepridil has been recognized as a potential candidate in new therapeutic areas such as alzheimer's, antiviral, atrial fibrillation and in certain neurological disorders. Recently, Olinger and co-workers identified bepridil as a potential lead molecule against Ebola virus disease (EBOV).[10] They screened about 2600 bioactive small molecules, including FDA approved drugs and molecular probes against Ebola virus. In the preliminary *in vitro* screening, they identified 171 scaffolds as novel inhibitors of Ebola virus disease (EBOV) infection. Among these, they selected thirty promising drugs for further study (*in vivo* studies in mice model). The *in vivo* studies revealed that two drugs such as bepridil (a calcium channel blocker) and sertraline (a selective serotonin reuptake inhibitor) are a promising candidate to treat Ebola virus infection. These findings are a really significant discovery as there is no effective treatment currently available for Ebola infection. According to this research, bepridil being an approved drug, repurposing of this may rapidly move to human testing and have a potential to become a frontline against Ebola virus infection.

2.2.2. Review of Literature

Very few reports are available in the literature for the synthesis of racemic as well as optically active (*R*)-bepridil *R*-**1**. Detail reports of these syntheses of *R*-**1** are described below.

Mauvernay's approach (1976)[11]

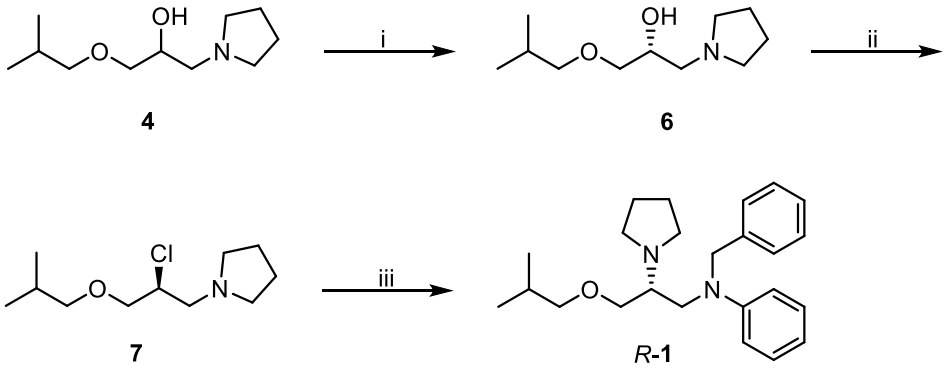
Mauvernay *et al.* described the synthesis of *rac*-bepridil *rac*-**1** starting from epichlorohydrin **2** (**Scheme 1**). Epichlorohydrin **2** on treatment with isobutanol in NaOH afforded isobutyl glycidyl ether **3**. Subsequently, the regioselective opening of epoxide **3** with pyrrolidine provided amino alcohol derivative **4**, followed by treatment with thionyl chloride in chloroform gave corresponding chloro derivative **5**. Finally, the chloro derivative **5** was treated with *N*-benzyl aniline and sodium amide in xylene afforded the *rac*-bepridil *rac*-**1**.



Scheme 1. *Reagents and conditions*: (i) isobutanol, NaOH; (ii) pyrrolidine; (iii) $SOCl_2$, $CHCl_3$, 45 °C; (iv) *N*-benzylaniline, $NaNH_2$, xylene, 135 °C, 6 h.

Winslow's approach (1985)[12]

Winslow *et al.* reported the chemical resolution strategy for the preparation of (*R*)-isomer of bepridil *R*-**1** (**Scheme 2**). *α*-[(2-methylpropoxy)-methyl]-1-pyrrolidine-ethanol **4** was resolved with D-(+)dibenzoyl tartaric acid monohydrate in anhydrous ethanol gave corresponding salt, which was further treated with NaOH afforded enantiopure secondary alcohol **6** in 27% yield. Alcohol **6**, on treatment with thionyl chloride in anhydrous toluene at 75 °C afforded the corresponding chloro derivative **7**, which on treatment with *N*-benzylaniline afforded (*R*)-bepridil *R*-**1**.

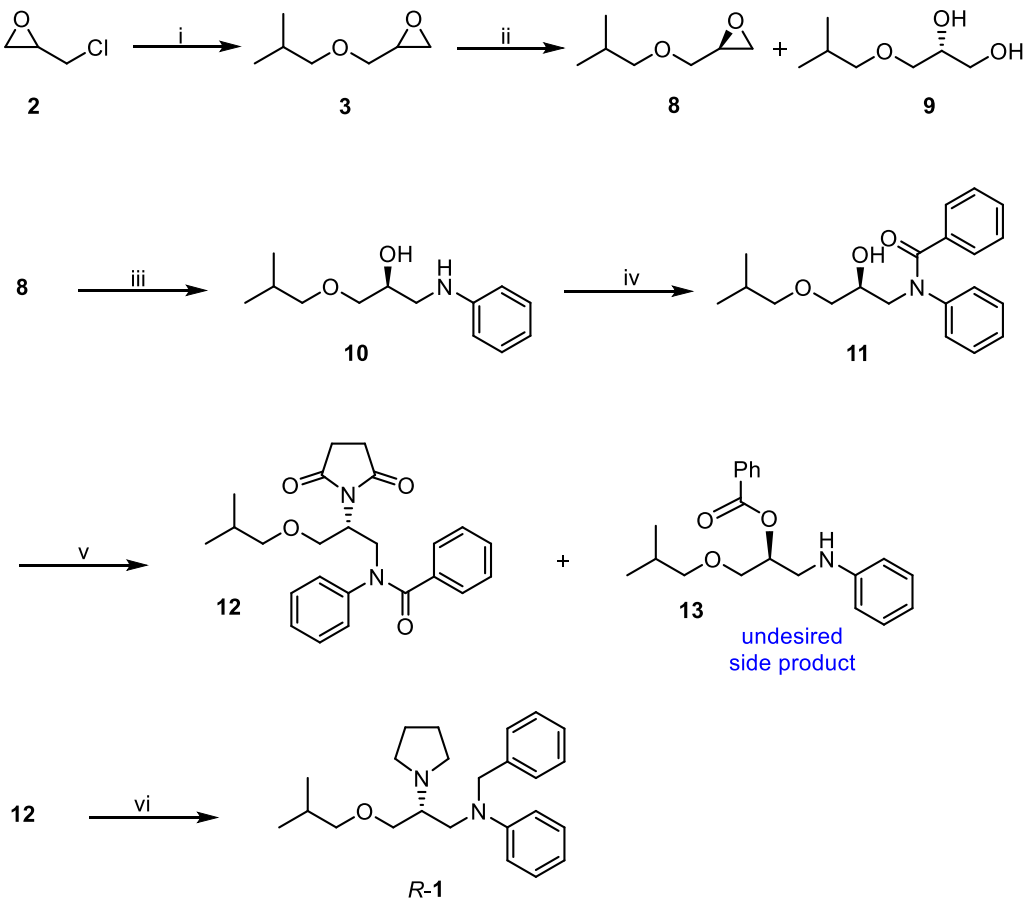


Scheme 2. *Reagents and conditions*: (i) D-(+) dibenzoyl tartaric acid monohydrate, EtOH, NaOH, 27%; (ii) $SOCl_2$, toluene, 75 °C, 2 h, 75%; (iii) *N*-benzylaniline, toluene, 80 °C, 74%.

2.2.3. Present work

Objective

In view of the significance of bepridil in many new therapeutic indications, it seemed timely to develop a new and effective enantioselective synthetic route to bepridil enantiomers. Importantly, there is no asymmetric synthetic route to this molecule is reported yet. Previously, we developed a new enantioselective synthesis of (*R*)-bepridil from the easily available starting materials by taking advantage of Jacobsen's hydrolytic kinetic resolution strategy.[13] In this approach, the required epoxide **3** was obtained by treating epichlorohydrin **2** with isobutanol under basic condition (**Scheme 3**). Epoxide **3** was subjected to Jacobsen's hydrolytic kinetic resolution condition with (*R, R)*-Salen Co (III)-OAc afforded the enantiopure epoxide **8** in 40% along with diol **9** in 42% yield.

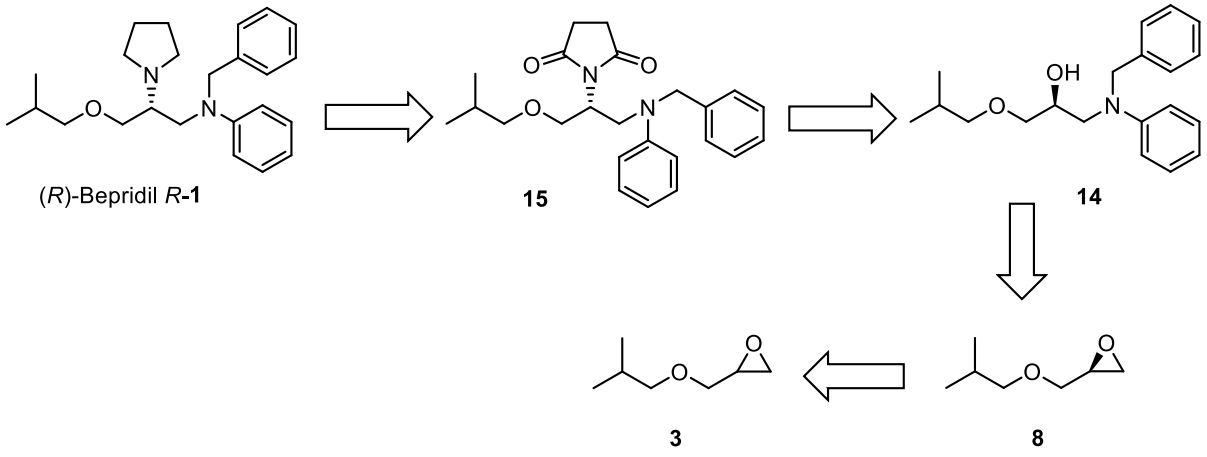


Scheme 3. *Reagents and conditions:* (i) isobutanol, cat. TBAI, aq. KOH (50% w/w), rt, 12 h, 62%; (ii) 0.5 mol % (*R,R*)-Salen Co(III)-OAc, H_2O, 0 °C-rt, 24 h; (iii) aniline, cat. LiBr, MeOH, 12 h, 90%; (iv) C_6H_5COCl, Et_3N, DCM, 0 °C, 1 h, 85%; (v) succinimide, DIAD, Ph_3P, THF, 0 °C-rt, 12 h, 40%; (vi) Borane-DMS, THF, reflux, 12 h, 70%.

Subsequently, the regioselective ring opening of epoxide **8** with aniline followed by *N*-benzoylation of amino alcohol **10** with benzoyl chloride gave amide derivative **11** in 85% yield. Amide **11** was subjected to Mitsunobu reaction with succinimide in PPh_3 and DIAD gave succinimide derivative **12** in 40% yield along with undesired benzoyl migrated product **13** in 25% yield. Finally, the reduction of amide bonds in compound **12** with borane-DMS afforded (*R*)-bepridil *R*-**1**. Although *R*-enantiomer of bepridil *R*-**1** has been successfully synthesized using this strategy mentioned above, this method suffered from two limitations such as (1) *overall yield is not satisfactory (only 9 %)* (2) *problematic Mitsunobu step that gives undesired side product in 25% yield*. Hence, in this section, the development of modified approach towards the synthesis of both enantiomers of bepridil *R*-**1** & *S*-**1** has been described.

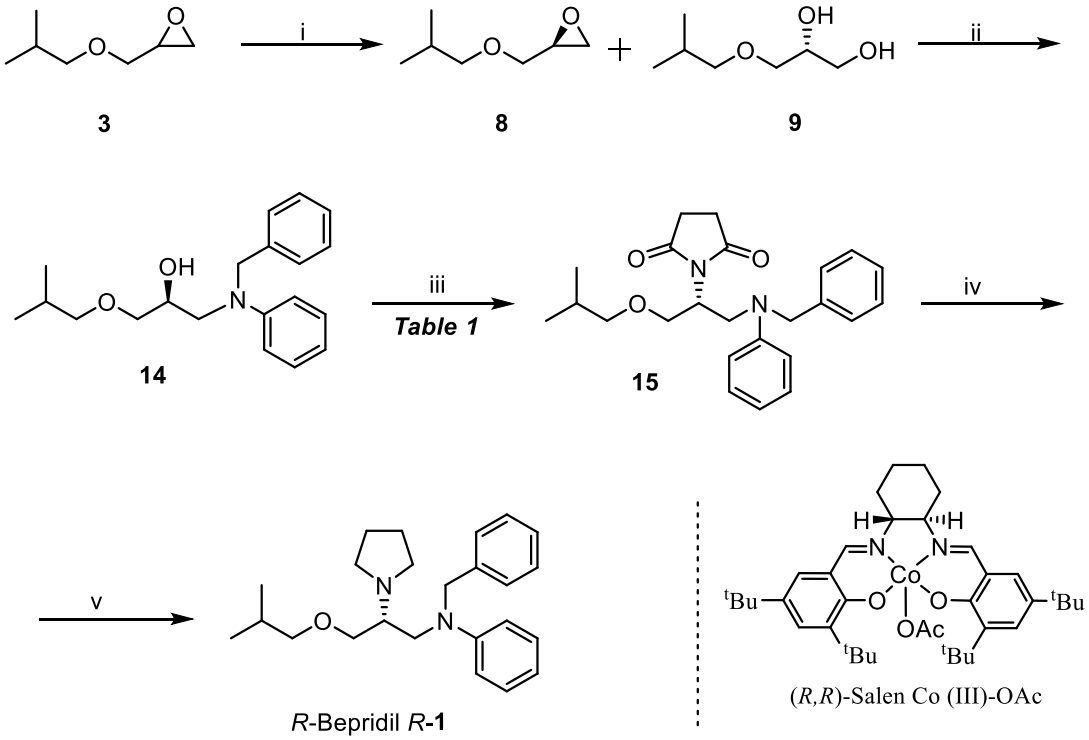
2.2.4 Results and Discussion

Highly active enantiomer (*R*)-bepridil *R*-**1** has been selected for initial investigation and the retrosynthetic analysis is outlined in **Scheme 4**. It has been envisioned that the amino alcohol **14** could be visualized as a key intermediate for the synthesis, which can be transformed to the amide precursor **15** using Mitsunobu protocol. Further, compound **15** can be transformed to the target molecule *R*-**1** *via* simple amide reduction. The key intermediate **14**, in turn, can be obtained from the regioselective ring opening of **8** with *N*-benzylaniline. The chiral epoxide **8** could be easily prepared from its racemic epoxide **3** with high enantiopurity employing hydrolytic kinetic resolution (HKR) strategy.



Scheme 4. Retrosynthetic analysis of (*R*)-bepridil *R*-**1**

A modified synthetic sequence for the synthesis of (*R*)-bepridil *R*-**1** is shown in **Scheme 5**. The synthesis commenced with the readily available starting material 2-(isobutoxymethyl)oxirane **3**, which was subjected to Jacobsen's hydrolytic kinetic resolution with 0.55 equivalents of water in the presence of a Jacobsen cobalt catalyst [(*R,R*)-Salen Co(III)-OAc (0.5 mol %)] at ambient temperature for 24 h gave the enantiomerically pure epoxide **8** in 40 % yield $\{[\alpha]^{22}_{D} = +3.4$ (*c* 1.99, $CHCl_3)\}$ followed by (*R*)-3-isobutoxypropane-1,2-diol **9** in 42% yield. The desired epoxide **8** (75 °C, 8 mbar) was easily isolated from the more polar diol **9** (150 °C, 8 mbar) by vacuum distillation. Importantly, the undesired diol **9** can be easily converted to the desired epoxide **8** as per the reported procedure.[14]

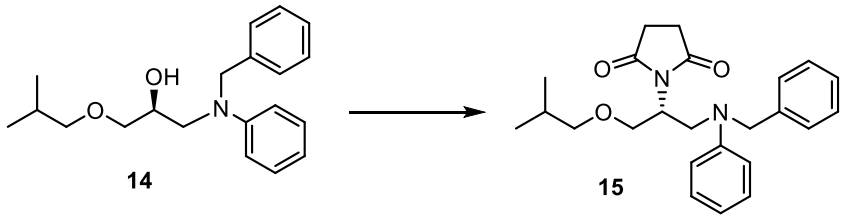


Scheme 5. *Reagents and conditions*: (i) 0.5 mol % (*R,R*) Salen Co (III)-OAc, H_2O, 0 °C-rt, 24 h; (ii) *N*-benzylaniline, MeOH, reflux, 12 h, 73 %; (iii) succinimide, PPh_3, DIAD, THF, reflux, 12 h, 88% (Table 1); (iv) Red-Al, toluene, rt, 20 h, 86%.

As the HKR on epoxide **8** is unknown, we wanted to ensure its enantiopurity before proceeding further. However, our attempts to separate the *rac* epoxide **2** under various HPLC conditions were unsuccessful. Hence, it has been decided to check its enantiopurity at the next step. Accordingly, the epoxide **8** on regioselective ring opening with readily available *N*-benzyl aniline in refluxing methanol for 12 h afforded the corresponding amino

alcohol **14** in 73% yield. In the ^{1}H NMR spectrum of compound **14**, signals corresponding to methine proton and hydroxyl proton resonated at δ 4.15-4.23 ppm and δ 2.57 ppm and in the ^{13}C NMR, the signals of methine carbon attached to the hydroxy group was discernible at δ 68.6 ppm confirms the formation of ring-opened product **14**. Further, the IR spectrum of **14** displayed absorption band of the hydroxyl group at 3394 cm^{-1}. Our next aim was to replace the hydroxyl group by a succinimide moiety without loss of enantioselectivity. A variety of Mitsunobu conditions were examined to achieve a good yield of the Mitsunobu product with high enantiopurity (**Table 1**). It has been found that Mitsunobu reaction in the presence of PPh_3 (4 equiv.), DIAD (3 equiv.), succinimide (4 equiv.) using THF as a solvent under reflux conditions (Table 1, entry **8**) is optimal to obtain relatively higher yields (88%) and high enantioselectivity (ee >99%). In the ^{1}H NMR spectrum of compound

Table: 1. Attempted Mitsunobu reaction conditions



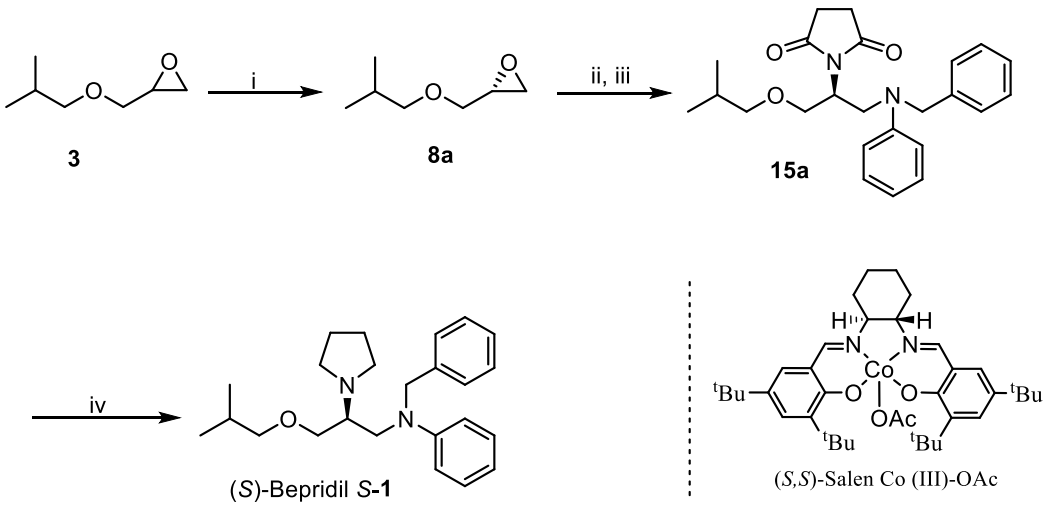
Entry	Reaction conditions	Yield[c] (%) **15**	ee (%)[d] *R*-**1**
1[a]	PPh_3, DIAD, toluene, rt, 24 h	N.R	--
2[a]	PPh_3, DIAD, toluene, 60 °C, 24 h	<5	--
3[a]	PPh_3, DIAD, toluene, reflux, 12 h	92	89
4[a]	PPh_3, DEAD, toluene, 60 °C, 12 h	90	95
5[a]	PPh_3, DEAD, ether, reflux, 12 h	20	91
6[a]	PPh_3, DIAD, ether, reflux, 12 h	15	91
7[a]	PPh_3, DIAD, THF, rt, 24 h	N.R	--
8[b]	**PPh_3, DIAD, THF, reflux, 12 h**	**88**	**99**
9[a]	PBu_3, DEAD, EtOAc, reflux, 12 h	N.R	--
10[a]	PPh_3, DBAD, ether, reflux, 12 h	N.R	--

a) PPh_3 (2 equiv.), DIAD/DEAD/DBAD (1.5 equiv.) used b) PPh_3 (4 equiv.), DIAD (3 equiv.), succinimide (4 equiv.) used c) isolated yields. d) Determined by chiral HPLC analysis of final molecule *R*-**1**

15, the signals of methylene protons ranging from δ 2.19-2.25 (m, 4 H) while in the ^{13}C NMR spectrum, the characteristic signals of two carbonyl carbons resonated at δ 177.7 ppm correspond to succinimide moiety in compound **15**. In the IR spectrum of **15**, the absorption band of carbonyl group displayed at 1660 cm^{-1}. Finally, the amide reduction of succinimido derivative **15** using Red-Al in anhydrous toluene at room temperature for 20 h furnished the target molecule *R*-bepridil *R*-**1** {$[\alpha]^{22}_D$ = -5.2 (*c* 1.23, MeOH)} with an overall yield of 18%. The enantiomeric purity of *R*-**1** was found to be 99%.

(*S*)-Bepridil *S*-1

Similarly, *S*-enantiomer of bepridil *S*-**1** was also synthesized successfully employing the same procedure (**Scheme 6**). Here, (*S, S*) Salen Co (III)-OAc was used as a catalyst to get the required (*R*)-epoxide as a starting material. Using the same sequences depicted in **Scheme 6**, (*S*)-bepridil *S*-**1** was obtained with an overall yield of 17%; $[\alpha]^{22}_D$ = +5.15 (*c* 1.23, MeOH). The enantiomeric excess of *S*-**1** was determined by chiral HPLC analysis and found to be 98% ee.



Scheme 6. *Reagents and conditions* (i) 0.5 mol % (*S,S*) Salen Co (III)-OAc, H_2O, 0 °C-rt, 24 h; (ii) *N*-benzylaniline, MeOH, reflux, 12 h, 70 %; (iii) PPh_3, DIAD, THF, reflux, 12 h, 89%; (iv) Red-Al, toluene, rt, 20 h, 84%.

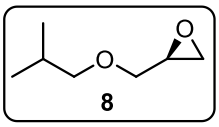
2.2.5. Conclusion

In conclusion, motivated by the lack of efficient methods for the preparation of bepridil enantiomers, we have developed a short and efficient method for the enantioselective preparation of both the enantiomers of bepridil for the first time in an overall yield about

18% and ee >98%. Simple procedures, high enantioselectivities, and the ready availability of the starting materials are some of the salient features of this approach. Further, the strategy could be exploited for the preparation of chiral bepridil analogues that are required for ongoing drug research focused on finding new therapeutic applications of bepridil.

2.2.6. Experimental Section

1) (*S*)-2-(isobutoxymethyl)oxirane (8)



8

A mixture of epoxide **3** (5 g, 38.4 mmol) and (*R, R*)-Salen Co(III)-OAc complex (0.055 g, 0.0812 mmol) was vigorously stirred for 15 min, then cooled to 0 °C. H_2O (0.38 mL, 21.12 mmol) was added from a microsyringe over 15 min and the resulting mixture was stirred at room temperature for 12 h. Additional of (*R, R*)-Salen Co(III)-OAc complex (0.055 g, 0.0812 mmol) was then added, and stirring was continued for additional 12 h. The resulting mixture was diluted with ethyl acetate (20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The epoxide **8** from residual liquid was isolated by vacuum distillation (75 °C, 8 mbar) as a colorless oil, followed by the diol **9** (150 °C, 8 mbar) as light yellow oil.

Epoxide 8

Yield: 2.0 g, 40%;

Molecular Formula: $C_7H_{14}O_2$;

Specific rotation: $[\alpha]^{22}_D = +3.45$ (*c* 1.99, $CHCl_3$);

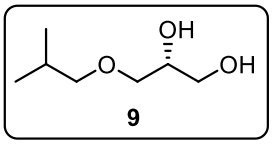
IR ($CHCl_3$, cm^{-1}): ν_{max} 2960, 2873, 1522, 1474, 1421, 1097;

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (d, *J* = 6.7 Hz, 6 H), 1.86 (sp, *J* = 6.7 Hz, 6 H), 2.59 (dd, *J* = 5.0, 2.8 Hz, 1 H), 2.76-2.80 (m, 1 H), 3.09-3.17 (m, 1 H), 3.20-3.28 (m, 2 H), 3.22-3.41 (m, 1 H), 3.69 (dd, *J* = 11.6, 3.0 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 78.3 (CH_2), 71.4 (CH_2), 50.8 (CH), 44.1 (CH_2), 28.4 (CH), 19.2 (CH_3, 2 carbons);

HRMS (ESI): *m/z* calculated for $C_7H_{14}O_2$ $[M+H]^+$ 131.1067, found 131.1067.

Diol 9



Yield: 2.4 g, 42%;

Molecular Formula: $C_7H_{16}O_3$;

Specific rotation: $[\alpha]^{22}_D$ = -2.02 (*c* 2.1, $CHCl_3$);

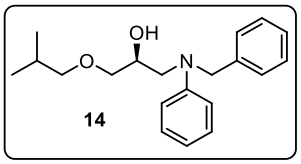
IR ($CHCl_3$, cm^{-1}): ν_{max} 3751, 3574, 2961, 2875, 1524, 1477, 1400, 1031;

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.93 (d, *J* = 6.7 Hz, 6 H), 1.78-1.98 (m, 1 H), 2.60-2.64 (dd, *J* = 5.0, 2.6 Hz, 1 H), 2.78-2.82 (dd, *J* = 5.0, 4.1 Hz, 1 H), 3.15-3.25 (m, 2 H), 3.48-3.51 (m, 2 H), 3.68-3.75 (m, 2 H), 3.82-3.92 (m, 1 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 78.4 (CH_2), 72.5 (CH_2), 70.6 (CH), 64.2 (CH_2), 28.3 (CH), 19.2 (CH_3, 2 carbons);

MS: m/z 171 $[M+Na]^+$.

2) (*S*)-1-(*N*-benzyl-*N*-phenylamino)-3-isobutoxypropan-2-ol (14)



To stirred solution of **8** (0.2 g, 1.53 mmol) in dry MeOH (4 mL) was added *N*-benzyl aniline (0.281 g, 1.53 mmol) and the resulting mixture was refluxed for 12 h under N_2 atmosphere. After completion of the reaction (indicated by TLC), the solvent was evaporated under reduced pressure. Water (15 mL) was added and the mixture was extracted with EtOAc (3 x 10 mL). The organic layers were combined, washed with brine (2 x 10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude residue by column chromatography [silica gel, EtOAc/petroleum ether (8:92)] gave **14** as a colorless oil.

Yield: 0.35 g, 73%;

Molecular Formula: $C_{20}H_{27}NO_2$;

Specific rotation: $[\alpha]^{22}_D$ = +4.8 (*c* 1, $CHCl_3$);

Chiral HPLC: ee >99% [The ee of **14** was determined by chiral HPLC analysis; Chiralcel OD-H (250 x 4.6 mm) column; eluent: *n*-hexane/ethanol/trifluoroacetic acid (85:15:0.1);

flow rate 0.5 mL/min; detector: 254 nm; *(S)*-isomer t_R = 9.49 min];

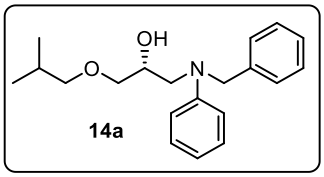
IR ($CHCl_3$, cm^{-1}): ν_{max} 3687, 3394, 3022, 2403, 1600, 1216, 1116, 929;

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.99 (dd, *J* = 6.6, 3.2 Hz, 6 H), 1.90-2.00 (m, 1 H), 2.57 (bs, 1 H), 3.29 (t, *J* = 6.4 Hz, 2 H), 3.49 (dd, *J* = 9.8, 5.4 Hz, 1 H), 3.56-3.60 (m, 2 H), 3.66 (dd, *J* = 15.1, 5.9 Hz, 1 H), 4.15-4.23 (m, 1 H), 4.71 (d, *J* = 10.3 Hz, 2 H), 6.77 (t, *J* = 7.1 Hz, 1 H), 6.84 (d, *J* = 7.8 Hz, 2 H), 7.23-7.30 (m, 5 H), 7.34-7.38 (m, 2 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 148.5 (C), 138.4 (C), 129.1 (CH, 2 carbons), 128.5 (CH, 2 carbons), 126.7 (CH), 126.5 (CH, 2 carbons), 116.7 (CH), 112.6 (CH, 2 carbons), 78.3 (CH_2), 72.4 (CH_2), 68.6 (CH), 54.9 (CH_2), 54.1 (CH_2), 28.3 (CH), 19.31 (CH_3), 19.3 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{20}H_{27}NO_2$ $[M+H]^+$ 314.2115, found 314.2112.

3) (*R*)-1-(*N*-benzyl-*N*-phenylamino)-3-isobutoxypropan-2-ol (14a)



14a

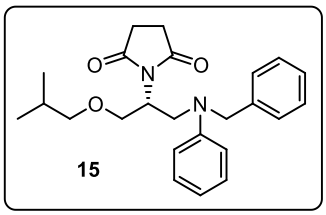
The enantiomeric amino alcohol prepared in the same way as **14** by the reaction of **8a** (0.2 g, 1.5 mmol) and *N*-benzyl aniline (0.281 g, 1.53 mmol) in dry MeOH to obtain **14a** as a colorless oil. The spectroscopic data corresponds with those of **14**.

Yield: 0.33 g, 70%;

Specific rotation: $[\alpha]^{22}_D$ = -4.4 (*c* 1, $CHCl_3$);

Chiral HPLC: ee >99% [The ee of **14a** was determined by chiral HPLC analysis; Chiralcel OD-H (250 x 4.6 mm) column; eluent: n-hexane/ethanol/TFA (85:15:0.1); flow rate 0.5 mL/min; detector: 254 nm; (*R*)-isomer t_R = 11.27 min].

4) ((*R*)-1-(1-(benzyl(phenyl)amino)-3-isobutoxypropan-2-yl)pyrrolidine-2,5-dione (15)



15

A solution of DIAD (0.37 mL, 1.9 mmol) in dry THF (5 mL) was added dropwise to a solution of **14** (0.2 g, 0.64 mmol), succinimide (0.25 g, 2.55 mmol) and triphenylphosphine (0.67 g, 2.55 mmol) in dry THF (10 mL) at 0 °C under N_2 atmosphere. Subsequently, the

reaction mixture was refluxed for 12 h. After completion of the reaction (monitored by TLC), the reaction mixture was cooled to room temperature, the solvent was removed under reduced pressure and the residue was purified by column chromatography [silica gel, EtOAc/petroleum ether (12:88)] to afford **15** as a colorless oil.

Yield: 0.22 g, 88%;

Molecular Formula: $C_{24}H_{30}N_2O_3$;

Specific rotation: $[\alpha]^{22}_D$ = +46.4 (*c* 1.4, $CHCl_3$);

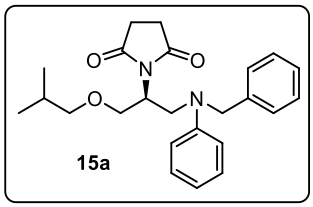
IR ($CHCl_3$, cm^{-1}): ν_{max} 3397, 3023, 2403, 1708, 1660, 1515, 1217, 1116;

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (dd, *J* = 6.8, 2.2 Hz, 6 H), 1.82 (sp, *J* = 6.6 Hz, 1 H), 2.19-2.25 (m, 4 H), 3.14 (dd, *J* = 8.9, 6.6 Hz, 1 H), 3.22 (dd, *J* = 8.9, 6.6 Hz, 1 H), 3.65-3.71 (m, 2 H), 3.78 (dd, *J* = 9.5, 8.1 Hz, 1 H), 4.21 (dd, *J* = 15.2, 10.1 Hz, 1 H), 4.39 (d, *J* = 16.6 Hz, 1 H), 4.68 (d, *J* = 16.6 Hz, 1 H), 4.72-4.78 (m, 1 H), 6.73 (t, *J* = 7.3 Hz, 1 H), 6.79 (d, *J* = 8.3 Hz, 1 H), 7.19-7.26 (m, 5 H), 7.30-7.34 (m, 2 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 177.7 (CO, 2 carbons), 148.5 (C), 138.5 (C), 129.1 (CH, 2 carbons), 128.4 (CH, 2 carbons), 126.9 (CH, 2 carbons), 126.8 (CH), 117.3 (CH), 113.5 (CH, 2 carbons), 77.9 (CH_2), 67.8 (CH_2), 54.4 (CH_2), 49.6 (CH), 49.1 (CH_2), 28.3 (CH), 27.8 (CH_2, 2 carbons), 19.1 (CH_3, 2 carbons);

HRMS (ESI): *m/z* calculated for $C_{24}H_{30}N_2O_3$ $[M+H]^+$ 395.2329, found 395.2327.

5) (*S*)-1-(1-(benzyl(phenyl)amino)-3-isobutoxypropan-2-yl)pyrrolidine-2,5-dione (15a)



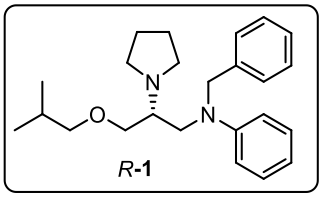
15a

The enantiomeric amide derivative prepared in the same way as **15** by reaction of DIAD (0.2 mL, 1.1 mmol), succinimide (0.14 g, 1.4 mmol), triphenyl phosphine (0.37 g, 1.4 mmol) and **14a** (0.11 g, 0.35 mmol) in dry THF to obtain **15a** as a colorless oil. The spectroscopic data corresponds with those of **15**.

Yield: 0.12 g, 89%;

Specific rotation: $[\alpha]^{22}_D$ = -42.8 (*c* 1.4, $CHCl_3$).

6) (*R*)-*N*-benzyl-*N*-(3-isobutoxy-2-(pyrrolidin-1-yl)propyl)aniline (*R*-1)



To a stirred solution of **15** (0.1 g, 0.24 mmol) in toluene (2 mL) was added, at 0 °C, a solution of Red-Al (70 % in toluene, 0.34 mL, 1.2 mmol). The resulting mixture was stirred at room temperature for 20 h. The reaction mixture, diluted with ethyl acetate (6 mL), was washed with 1 M aq. NaOH. The aqueous phase was extracted with ethyl acetate (2 x 10 mL), and the combined organic layers, after being washed with water, were dried over sodium sulfate, filtered, and evaporated in vacuo. The crude product was purified by column chromatography [silica gel, EtOAc/petroleum ether (20:80)] afforded (*R*)-**1** as a pale yellow oil.

Yield: 0.075 g, 86%;

Molecular Formula: $C_{24}H_{34}N_2O$;

Specific rotation: $[\alpha]^{22}_D$ = -5.2 (*c* 1.23, MeOH);

Chiral HPLC: ee >99% [The ee of *R*-**1** was determined by chiral HPLC analysis; Conditions: Chiralcel OD-H (250 X 4.6 mm) column; eluent: ethanol/n-hexane/DEA (2:98:0.1); flow rate: 1 mL/min; detector 254 nm; (*R*)-isomer t_R = 3.90 min];

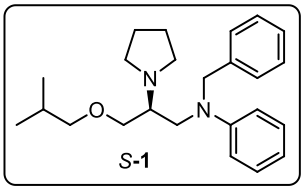
IR ($CHCl_3$, cm^{-1}): ν_{max} 3061, 3026, 2957, 2799, 1943, 1806, 1735, 1598, 1505, 1452, 1354, 1294, 1224, 1112, 987, 873, 746, 694;

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (dd, *J* = 9.1, 6.7 Hz, 3 H), 1.77-1.83 (m, 4 H), 1.94 (sp, *J* = 6.6 Hz, 1 H), 2.67-2.88 (m, 5 H), 3.15-3.22 (m, 2 H), 3.55-3.62 (m, 2 H), 3.66-3.81 (m, 2 H), 4.61-4.73 (m, 2 H), 6.69 (t, *J* = 7.2 Hz, 1 H), 6.80 (d, *J* = 8.3 Hz, 2 H), 7.17-7.25 (m, 5 H), 7.26-7.33 (m, 2 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 148.5 (C), 138.7 (C), 129.1 (CH, 2 carbons), 128.4 (CH, 2 carbons), 128.2 (CH), 126.5 (CH, 2 carbons), 116.1 (CH), 112.4 (CH, 2 carbons), 78.3 (CH_2), 69.0 (CH_2), 60.9 (CH), 54.6 (CH_2), 51.1 (CH_2, 3 carbons), 28.3 (CH), 23.3 (CH_2, 2 carbons), 19.6 (CH_3), 19.5 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{24}H_{34}N_2O$ $[M+H]^+$ 367.2744, found 367.2740.

7) (*S*)-*N*-benzyl-*N*-(3-isobutoxy-2-(pyrrolidin-1-yl)propyl)aniline (*S*-1)



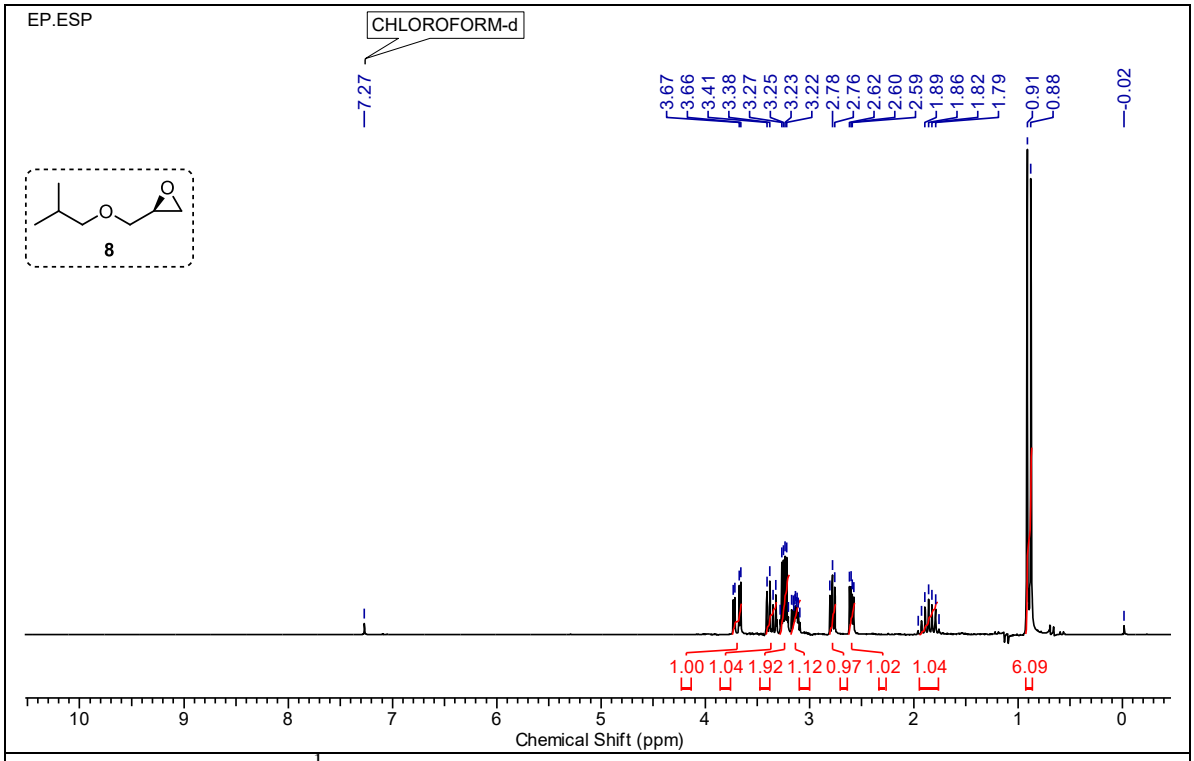
(*S*)-bepridil prepared in the same way as *R*-**1** by reaction of Red-Al (70 % in toluene, 0.34 mL, 1.2 mmol) and **15a** (0.1 g, 0.24 mmol) in toluene to obtain *S*-**1** as a pale yellow oil.

Yield: 0.074 g, 84%;

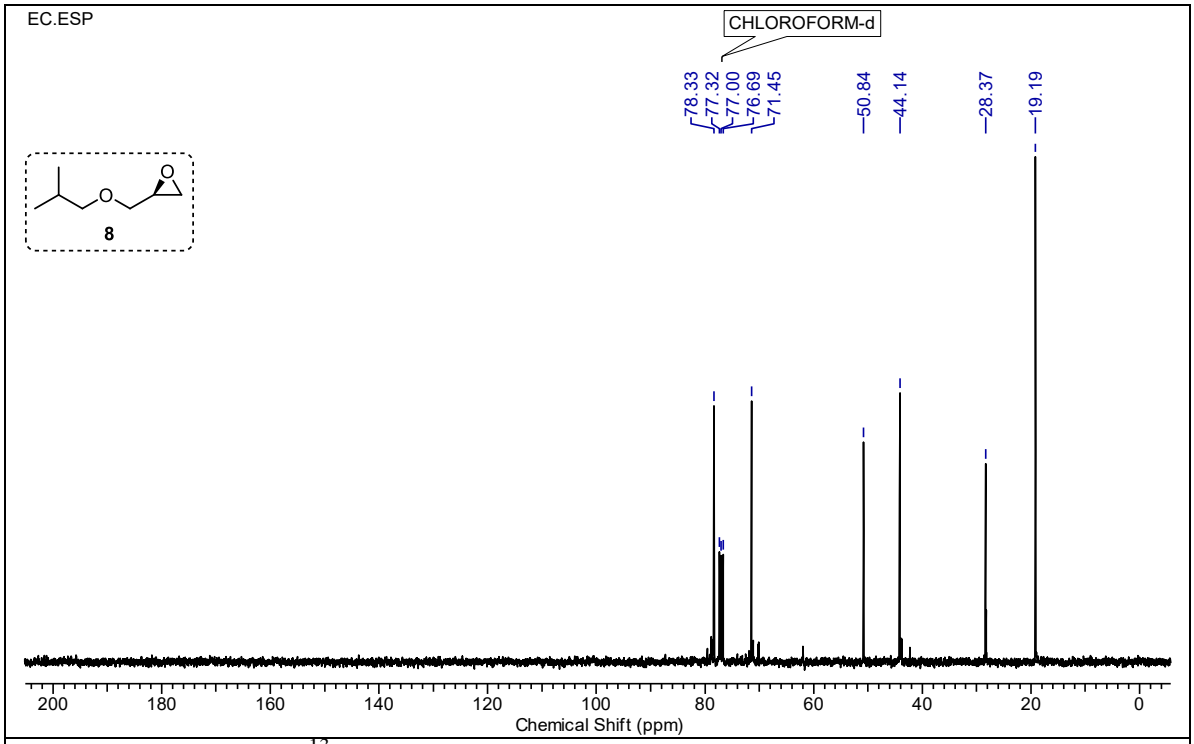
Specific rotation: $[\alpha]^{22}_{D}$ = +5.15 (*c* 1.23, MeOH);

Chiral HPLC: ee >98% [The ee of *S*-**1** was determined by chiral HPLC analysis; Conditions: Chiralcel OD-H (250 X 4.6 mm) column; eluent: ethanol/n-Hexane/DEA (2:98:0.1); flow rate: 1 mL/min; detector 254 nm; (*S*)-isomer t_R = 4.32 min]; The spectroscopic data corresponds with those of *S*-**1**.

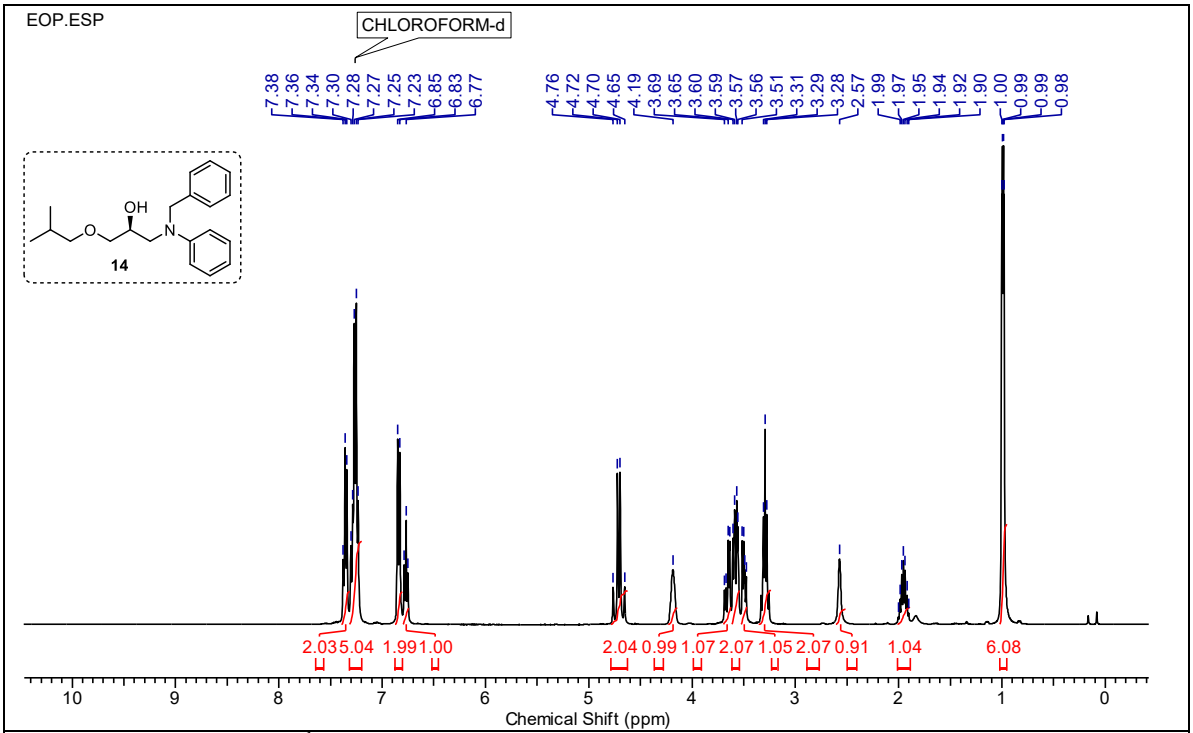
2.2.7. Spectra



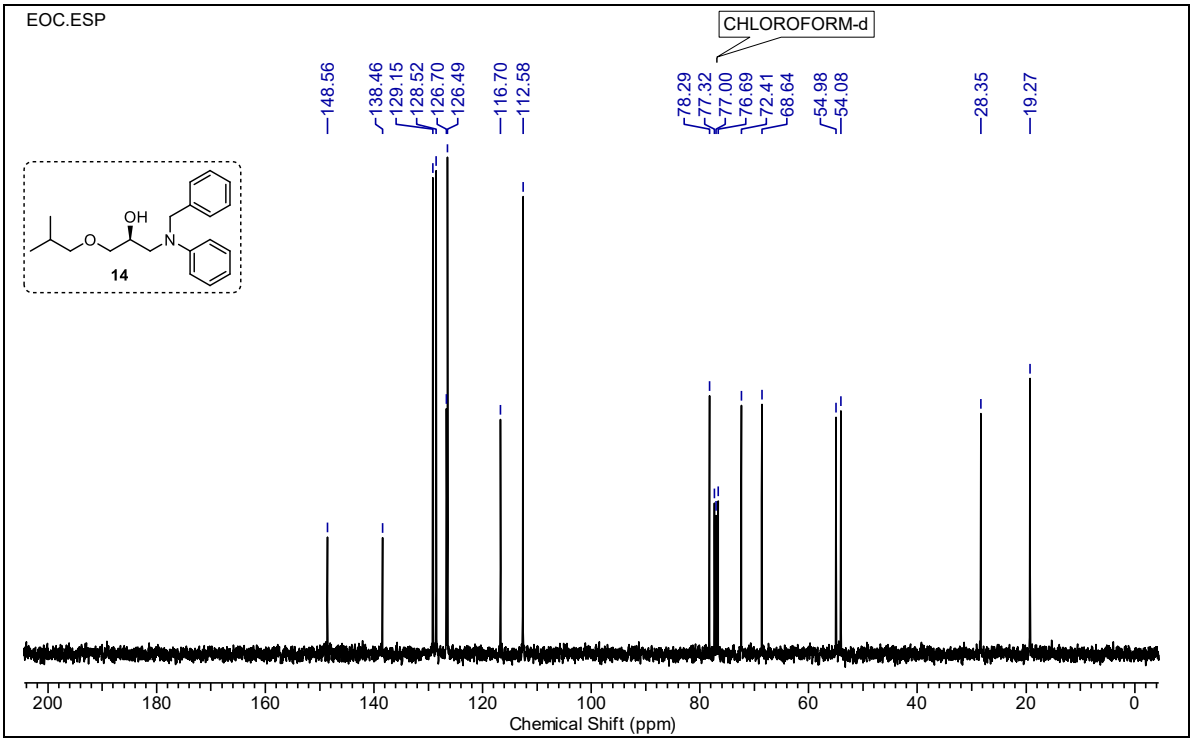
^{1}H NMR (200 MHz, $CDCl_3$) of compound **8**



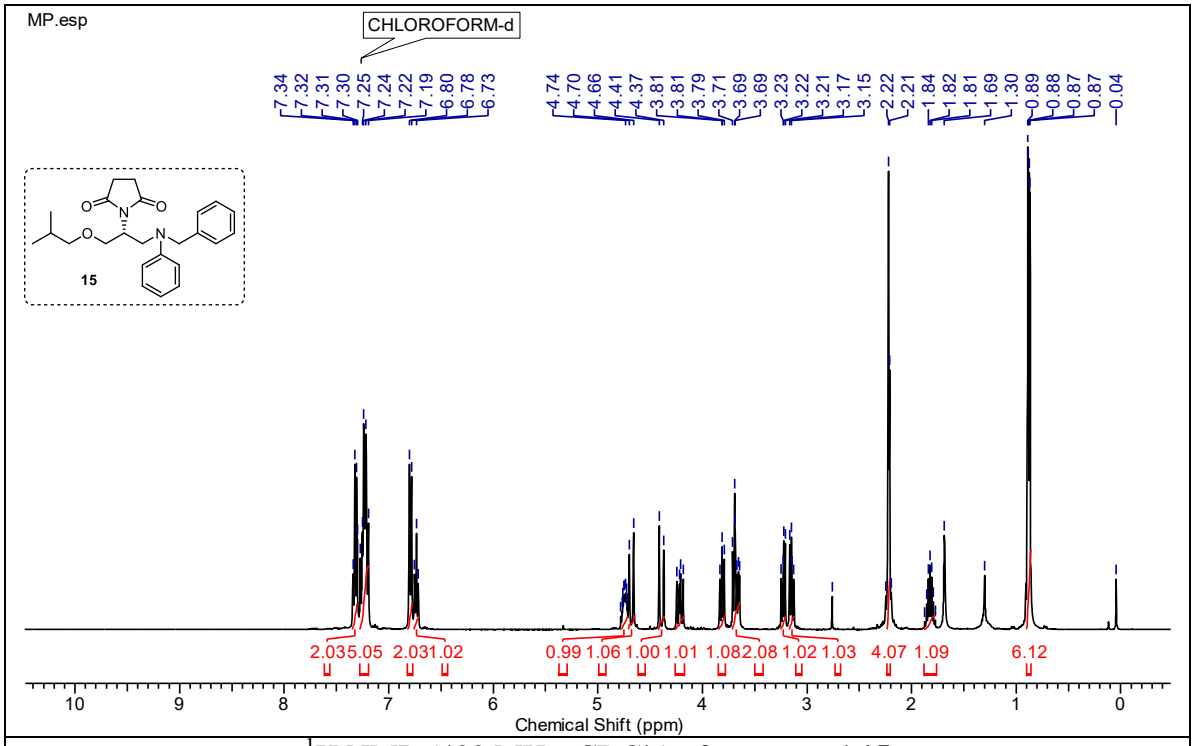
^{13}C NMR (100 MHz, $CDCl_3$) of compound **8**



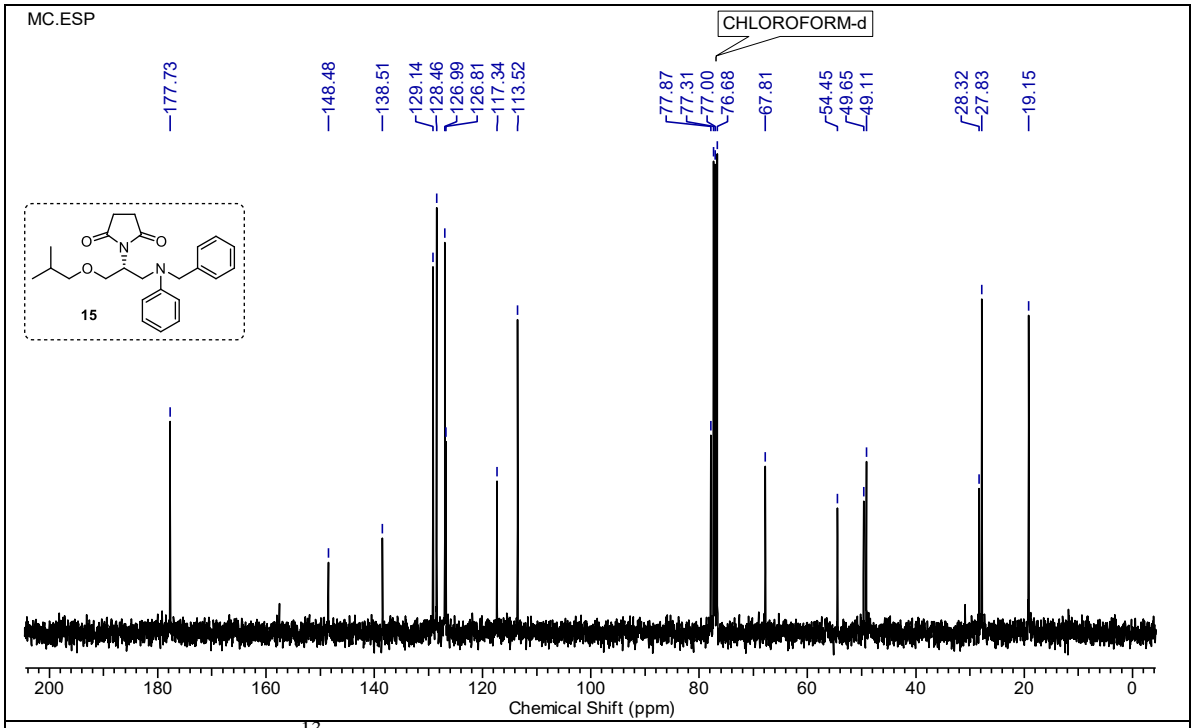
^{1}H NMR (400 MHz, $CDCl_3$) of compound **14**



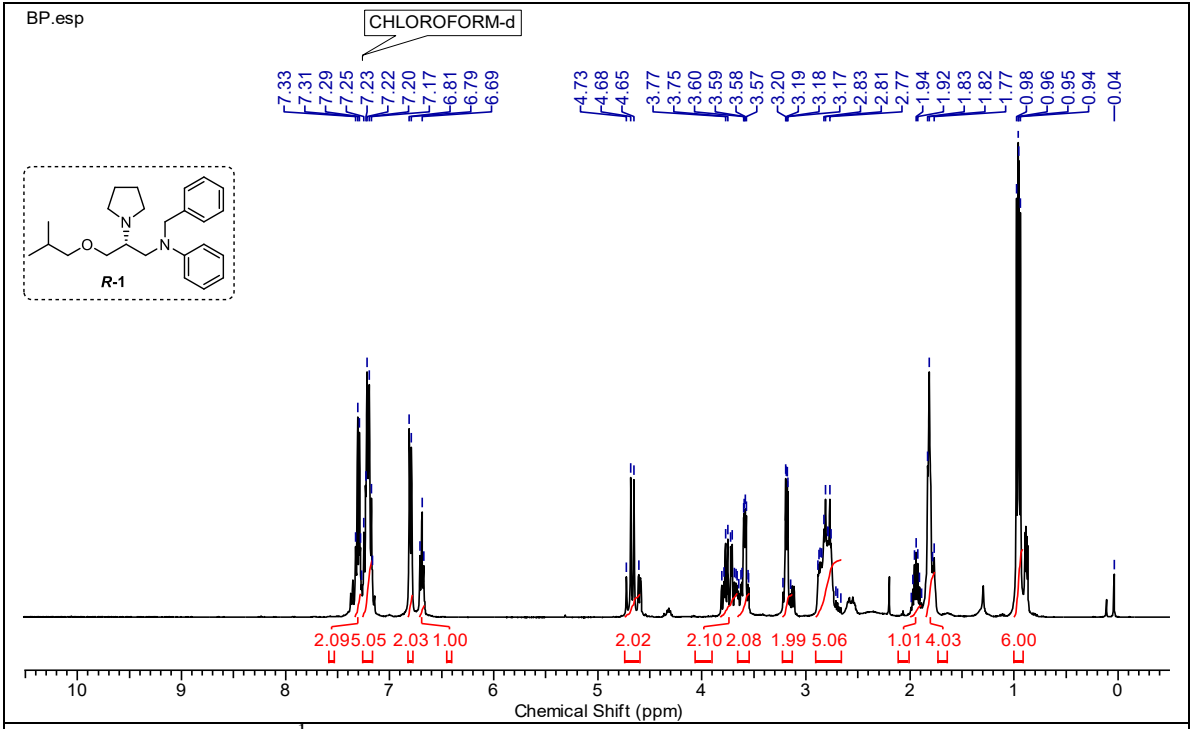
^{13}C NMR (100 MHz, $CDCl_3$) of compound **14**



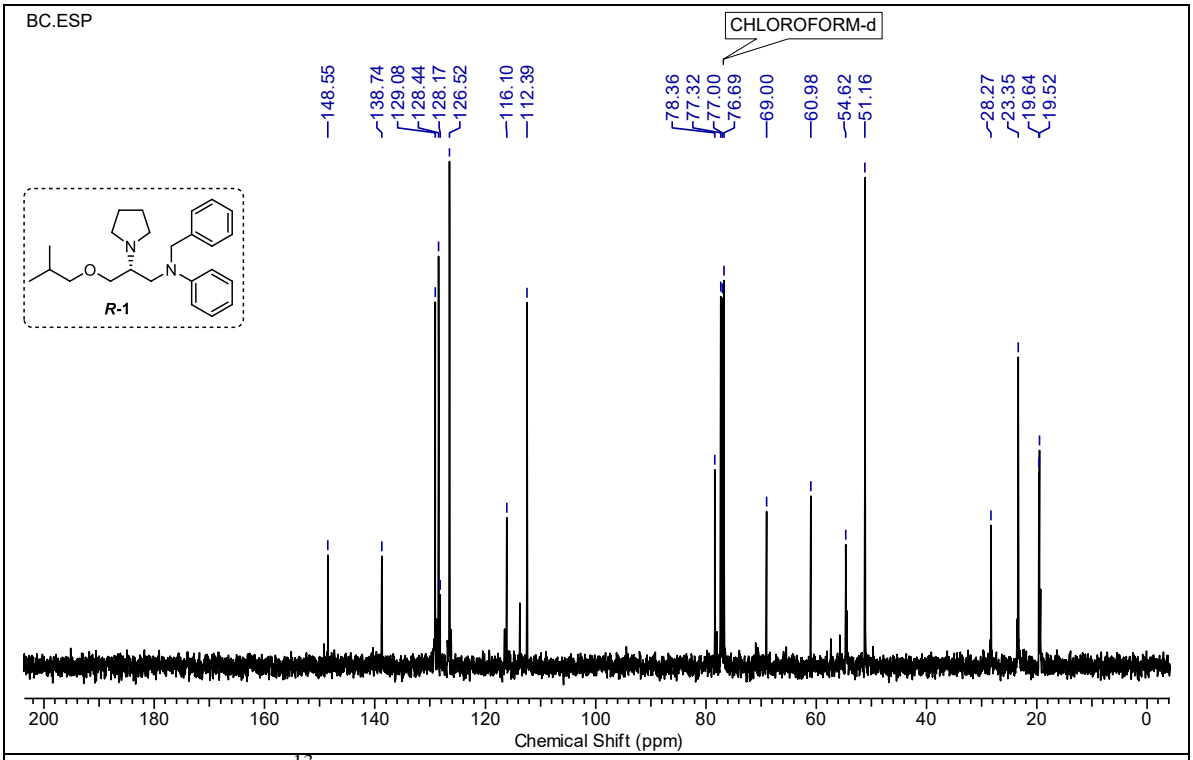
^{1}H NMR (400 MHz, $CDCl_3$) of compound **15**



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



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



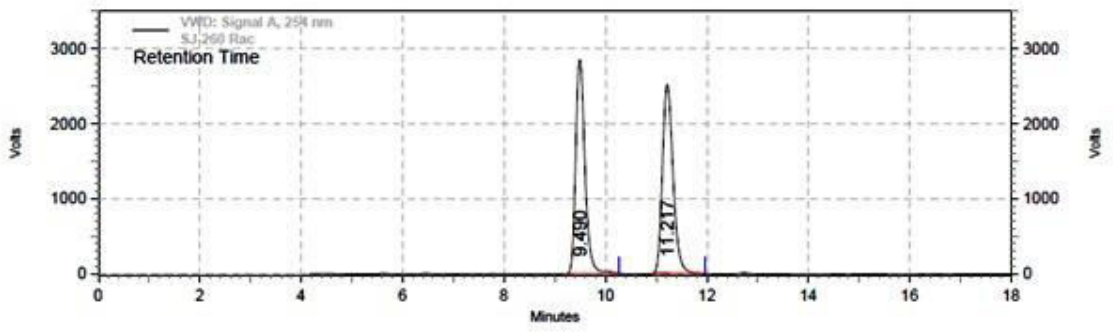
^{13}C NMR (100 MHz, $CDCl_3$) of compound ***R*-1**

2.2.8. Chiral HPLC analysis data

Chiral HPLC analysis of compound 14

Conditions: Chiralcel OD-H (250 X 4.6 mm) column; eluent: n-hexane/ethanol/trifluoro acetic acid (85:15:0.1); flow rate: 0.5 mL/min; detector: 254 nm

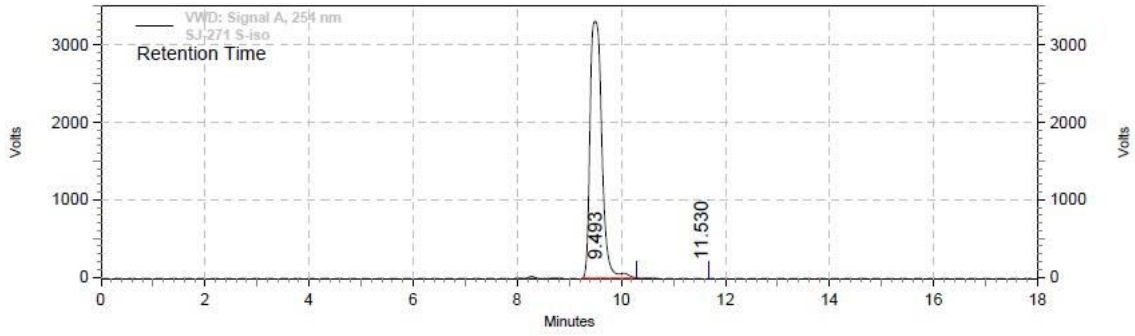
Racemic



Racemic Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	9.490	608243143	49.70
2	11.217	615288876	50.30
Totals		1223332019	100.000

Chiral

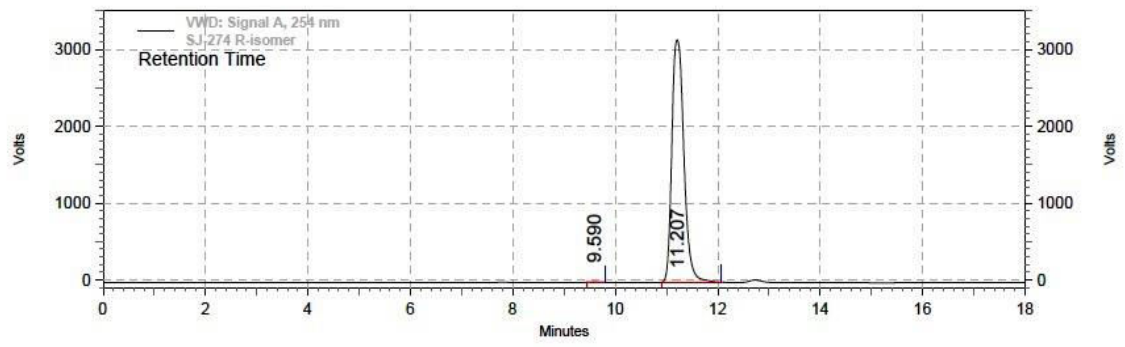


Chiral Sample Chromatograph (S-isomer)

Pk #	Retention Time (mins)	Area	Area %
1	9.493	878103139	99.94
2	11.530	545776	0.06
Totals		878648915	100.000

Chiral HPLC analysis of compound 14a

Chiral



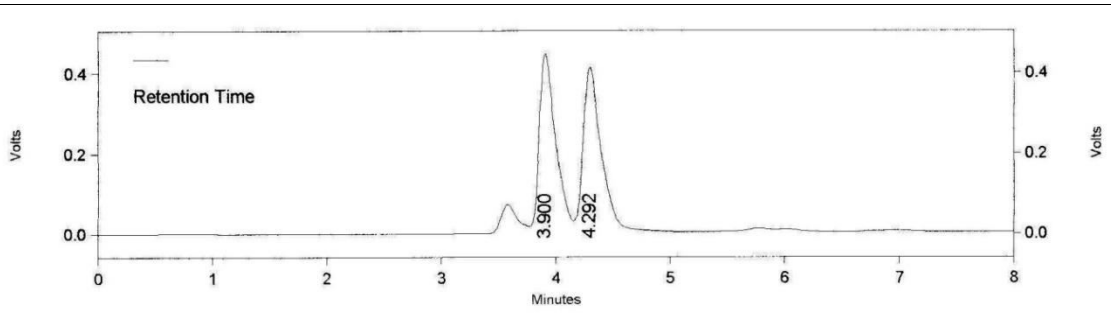
Chiral Sample Chromatograph (R-isomer)

Pk #	Retention Time (mins)	Area	Area %
1	9.590	1189183	0.14
2	11.207	864570961	99.86
Totals		865760144	100.00

Chiral HPLC analysis of compound *R*-1

Conditions: Chiralcel OD-H (250 X 4.6 mm) column; eluent: Ethanol/n-Hexane/DEA (2:98:0.1); flow rate: 1 mL/min; detector 254 nm.

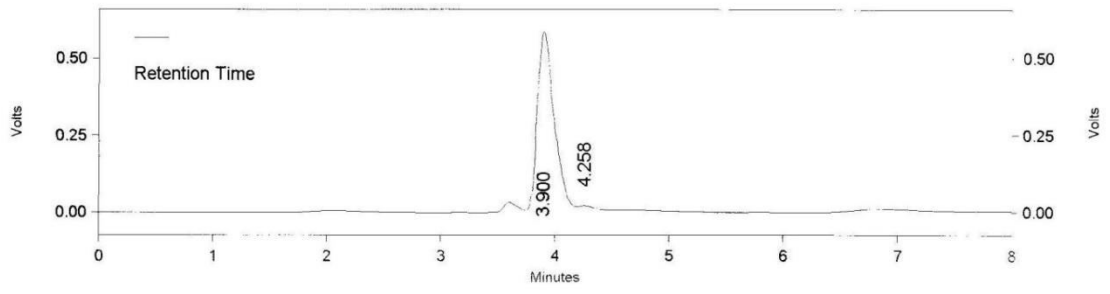
Racemic



Racemic Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	3.900	2045706	50.114
2	4.292	2036406	49.886
Totals		4082112	100.000

Chiral

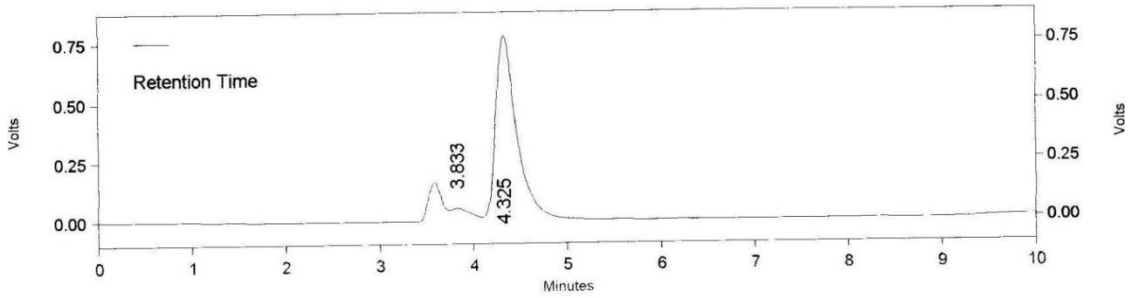


Chiral Sample Chromatograph (R-isomer)

Pk #	Retention Time (mins)	Area	Area %
1	3.900	2848992	99.558
2	4.258	12653	0.442
Totals		2861645	100.000

Chiral HPLC analysis of compound *S*-1

Chiral



Chiral Sample Chromatograph (S-isomer)

Pk #	Retention Time (mins)	Area	Area %
1	3.833	58926	1.036
2	4.325	5630039	98.964
Totals		5688965	100.000

2.2.9. References

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2.3. SECTION 3

An alternate synthesis of anti-obesity drug lorcaserin

2.3.1. Introduction

Obesity or overweight is considered as one of the most serious chronic and stigmatized diseases and become a significant public health concern globally.[1] According to the World Health Organization, obesity has been described as 'global epidemic' and the rate of prevalence of obesity in adults and children has increased more than doubled across the world over the past three decades.[2] According to the new predictions, eight in 10 men and seven in 10 women will be obese or overweight by 2020. Obesity significantly enhances the risk factor for other disorders such as type 2 diabetes mellitus, hypertension, cardiovascular diseases, stroke, and certain other serious cancer diseases.[3] The degree of obesity was measured in terms of body mass index (BMI). It is measured by dividing the body mass by height and expressed in kg per square meter units. Generally in adults, overweight defined as BMI of 25.0 to 29.9 kg/m^2, obesity as BMI of 30.0 kg/m^2 or more in adults.[4]

Obesity-medications

In the late 1950's, central nervous acting agents, such as amphetamines and their derivatives were introduced as primary appetite-suppressant drugs used for the treatment of obesity.[5] Appetite-suppressants phentermine (1959), diethylpropion (1959), benzphetamine (1960) and phendimetrazine (1982) are approved for short-term obesity.[6] The FDA approved appetite suppressants fenfluramine (1973) and dexfenfluramine (1996), stimulates serotonin release in the brain and remarkably reduces body weight when used alone or in combination therapy with phentermine. However, in 1997 both fenfluramine and dexfenfluramine were discontinued due to the adverse cardiovascular risk, significant side effects, and safety concern.[7] During the same period, three drugs were approved namely sibutramine (Meridia® and Reductil®), rimonabant (Acomplia®) and orlistat (Xenical® and Alli®) in the European market.[8] Later, rimonabant (2008) and sibutramine (2010) were withdrawn from the market due to adverse side effects. During the past six years, the newly approved anti-obesity drugs are lorcaserin, phentermine/topiramate, naltrexone /bupropion

and liraglutide (**Figure 1**).[9]

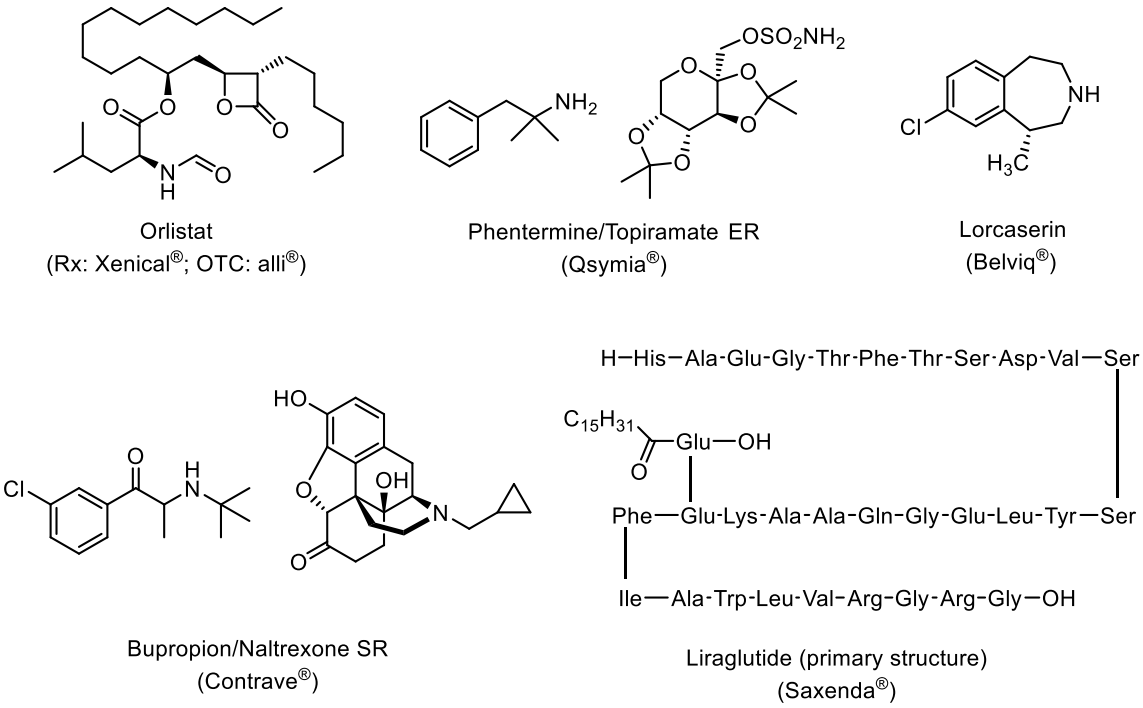


Figure 1. Currently available weight loss drugs in the market

Lorcaserin

Lorcaserin hydrochloride (formerly known as APD356, Lorqess) chemically (*R*)-8-chloro-1-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride, is a novel, potent and selective 5-hydroxytryptamine (5-HT_{2C}) receptor agonist used in long-term treatment for weight loss developed by Arena pharmaceuticals marketed under the trade name Belviq® (**Figure 2**).[10] It was approved by FDA in June 2012.[11] It helps to promote weight loss in obese and overweighted people associated with diabetes type 2, high blood pressure and dyslipidemia.

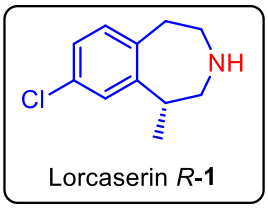


Figure 2. Lorcaserin *R*-**1**

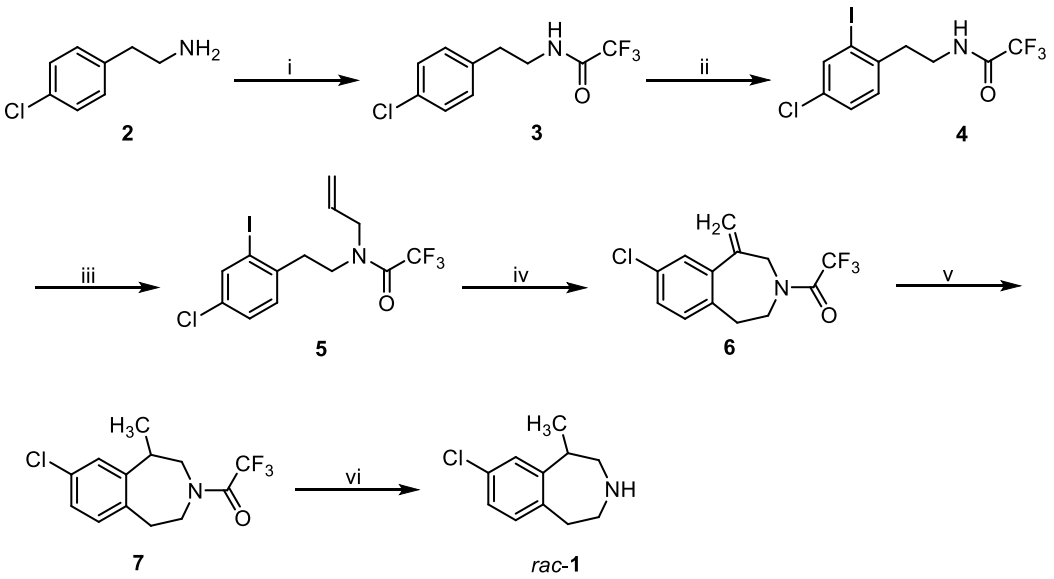
The mechanism of action of lorcaserin is believed to suppress appetite and promotes feelings of satiety by selectively stimulates the serotonin 2C receptors present in the hypothalamus.[12] The *in vitro* and *in vivo* studies showed that the affinity of the drug towards the 5-HT_{2C} receptors about 104-fold selectivity compared with the 5-HT_{2B} receptors and 18-fold than that of the 5-HT_{2A} receptors.[13]

2.3.2. Review of Literature

The synthetic methods available for the preparation of lorcaserin *R*-**1** are reviewed. These involve chemical resolution methods, chiral pool approaches, and enantioselective protocols. Some of the important reports of these syntheses are described below.

Smith's approach (2003)[14]

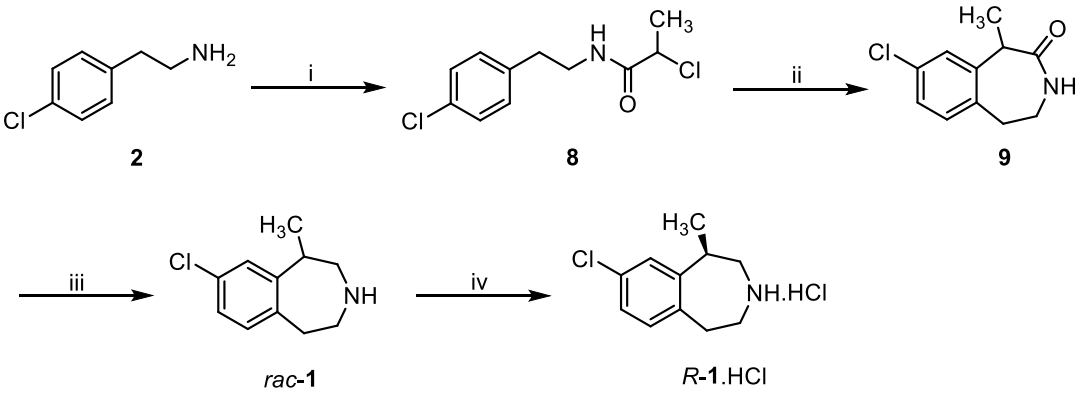
Smith and co-workers reported the first synthetic process for the preparation of *rac*-1orcaserin *rac*-**1**. Thus, commercially available 2-(4-chlorophenyl)ethanamine **2** on treatment with trifluoroacetic anhydride afforded the trifluoroacetamide **3**, which was further iodination at aromatic ring gave iodinated compound **4**. The amide intermediate **4** on *N*-allylation followed by palladium catalyzed intramolecular Heck reaction gave exo-methylene derivative **6**. Finally, hydrogenation followed by deprotection afforded the *rac*-lorcaserin *rac*-**1** (**Scheme 1**).



Scheme 1. *Reagents and conditions:* (i) $(CF_3CO)_2O$, pyridine, CH_2Cl_2; (ii) Iodochloride, MeOH or bis(pyridine)iodonium tetrafluoroborate, CF_3SO_3H, CH_2Cl_2; (iii) allylbromide, NaOH, K_2CO_3, *n*-Bu_4NBr, toluene-H_2O; (iv) PPh_3, $Pd(OAc)_2$, *n*-Bu_4NBr, CH_3COOK,

DMF; (v) 10% Pd/C, H_2, ethanol; (vi) NaOH, MeOH-H_2O

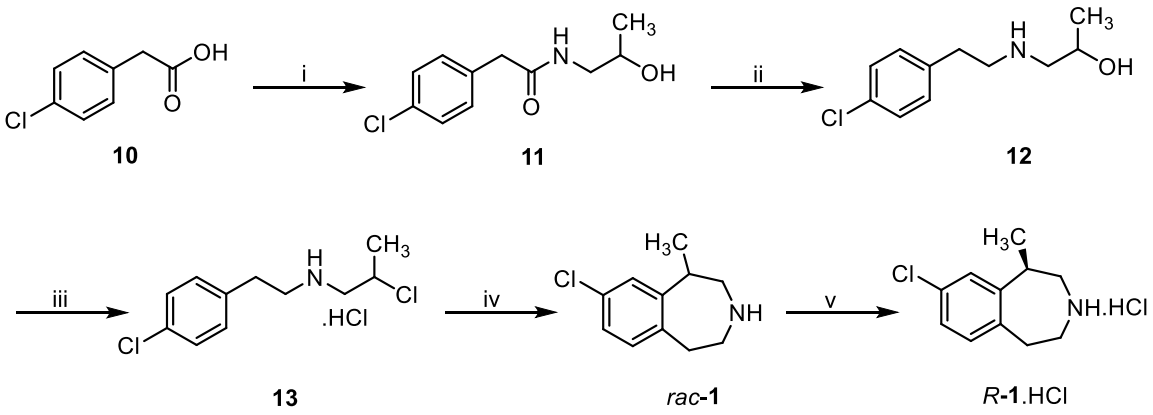
In 2005, the same research group have reported the chemical resolution method for the synthesis of optically pure lorcaserin hydrochloride *R*-**1**.HCl employing intramolecular Friedel-Craft's alkylation as a key step (**Scheme 2**). Thus, acylation of 2-(4-chlorophenyl)-ethanamine **2** with 2-chloropropionyl chloride gave the amide intermediate **8**, which was further cyclized in the presence of anhydrous aluminium chloride at 150-200 °C gave the cyclized amide precursor **9**. The reduction of amide **9** in the presence of BH_3-ether afforded the *rac*-lorcaserin *rac*-**1**. Finally, chemical resolution of racemate compound *rac*-**1** with L-(+)-tartaric acid followed by treatment with sodium hydroxide gave *R*-**1**.HCl.



Scheme 2. *Reagents and conditions:* (i) 2-chloropropionyl chloride, Et_3N, CH_3CN; (ii) $AlCl_3$, 150-200 °C; (iii) BH_3, ether; (iv) a) L-(+)-tartaric acid, b) NaOH, c) 1 M HCl in ether.

Fritch's approach (2008)[15]

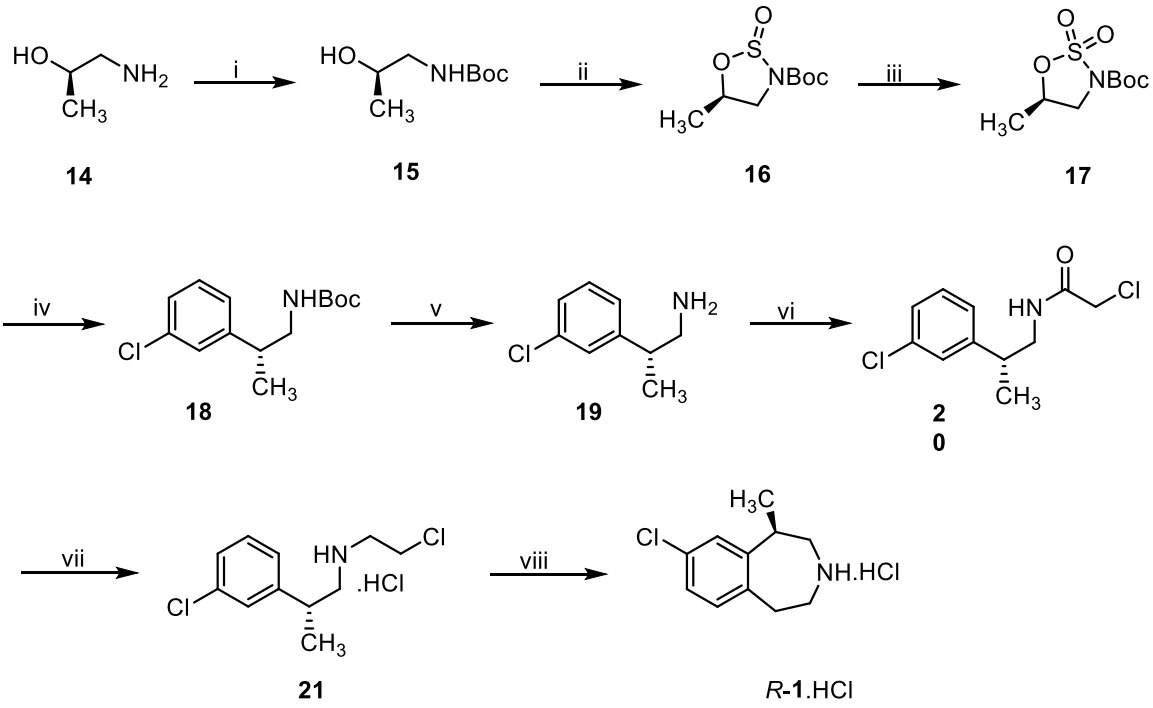
In 2008, Fritch and co-workers also reported chemical resolution method for the synthesis of lorcaserin hydrochloride ***R*-1**.HCl starting from 2-(4-chlorophenyl)acetic acid **10** (**Scheme 3**). Accordingly, the coupling of compound **10** with 1-amino2-propanol in the presence of coupling agent 3,4,5-fluorobenzeneboronic acid afforded the amide derivative **11**. The amide derivative **11** was transformed to its chloro derivative **13** by amide reduction followed by treatment with thionyl chloride. Further, chloro derivative **13** was subjected to intramolecular Friedel-Craft's alkylation with anhydrous aluminium chloride gave *rac*-lorcaserin *rac*-**1**. Finally, chemical resolution of *rac*-lorcaserin *rac*-**1** using L-(+)-tartaric acid as a chiral resolving agent afforded the optically pure *R*-**1**.HCl.



Scheme 3. *Reagents and conditions:* (i) 1-aminopropan-2-ol, 3,4,5-fluorobenzene boronic acid; (ii) BH_3/THF; (iii) $SOCl_2$, DMA; (iv) $AlCl_3$, 1,2-dichlorobenzene; (v) a) L-(+)-tartaric acid, b) NaOH, HCl-saturated EtOAc.

Ivana's approach (2014)[16]

Ivana and co-workers described the stereoselective synthesis of optically pure *R*-**1**.HCl starting from homochiral (*R*)-1-aminopropan-2-ol **14** (**Scheme 4**). At first,

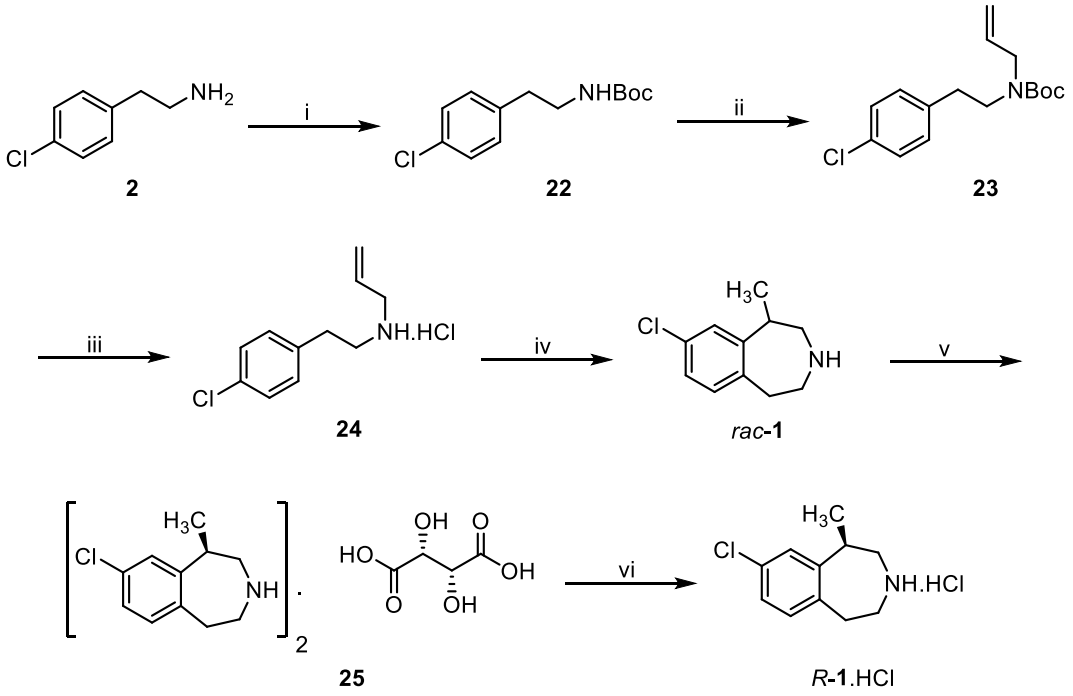


Scheme 4. *Reagents and conditions:* (i) Boc_2O, Et_3N, MeOH, 60 °C, 30 min, 97%; (ii) a) $SOCl_2$, imidazole, CH_2Cl_2, 20 °C, 3 h, 90%; (iii) aq. $NaIO_4$, cat. $RuO_2.H_2O$, 0 °C to rt, 4 h, 80%; (iv) 1-chloro-3-iodo-benzene, *i*PrMgCl, Et_2O, cat. CuI, -10 °C to 0 °C, 12 h, 86%; (v) 6 M HCl, THF, 45 °C, 3 h, 78%; (vi) chloroacetylchloride, Na_2CO_3, CH_2Cl_2, 10 °C, 3 h, 95%; (vii) BH_3/THF, HCl, Et_2O, 25 °C, 15 h, 76%; (viii) $AlCl_3$, 150 °C, 12 h.

Boc-protection of homochiral (*R*)-1-aminopropan-2-ol **14**, followed by treatment with thionyl chloride and further oxidation with $NaIO_4$/cat. RuO_2 afforded oxathiazolidine derivative **17**. Subsequently, the ring opening of oxathiazolidine derivative **17** with 1-chloro-3-iodo-benzene in *i*-PrMgCl gave amine derivative **18** in 86% yield. The deprotection of amine **18** followed by *N*-acetylation provided the acetamide derivative **20**. Reduction of amide functionality of compound **20** followed by intramolecular Friedel-Craft's alkylation in the presence of aluminium chloride at 150 °C furnished *R*-**1**.HCl.

Yugen's approach (2015)[17]

Yugen and co-workers also described chemical resolution method for the preparation of lorcaserin hydrochloride *R*-**1**.HCl, very similar to Smith's method (**Scheme 5**). Thus, Boc protection followed by *N*-allylation of 2-(4-chlorophenyl)ethanamine **2** afforded the key intermediate *N*-(4-chlorophenethyl)prop-2-en-1-amine **23**. Deprotection followed by intramolecular Friedel-Craft's alkylation gave *rac*-lorcaserin *rac*-**1** in 92%. Finally, *rac*-lorcaserin *rac*-**1** was resolved with L-(+)-tartaric acid gave *R*-**1**.HCl.

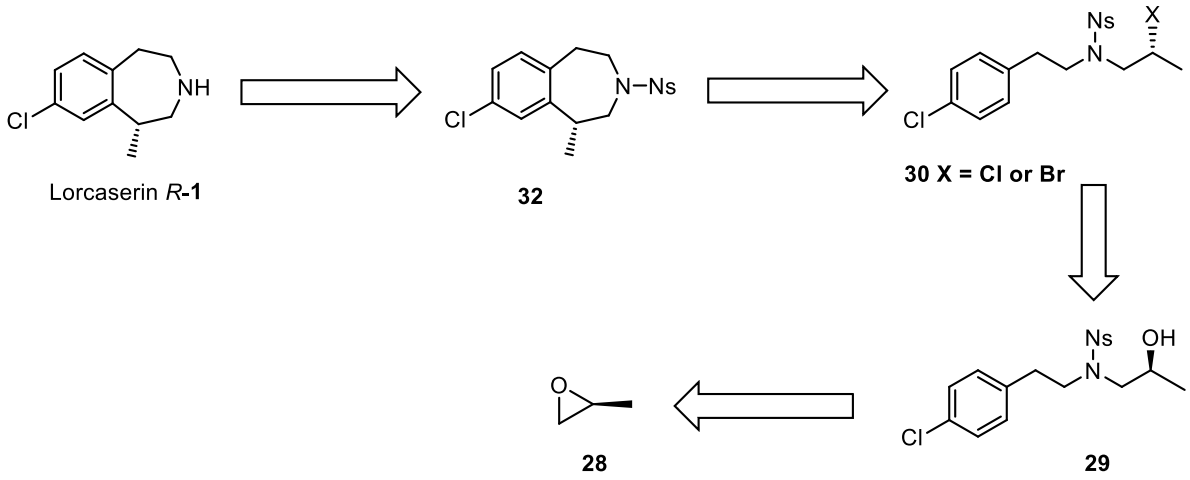


Scheme 5. *Reagents and conditions:* (i) Boc_2O, CH_2Cl_2, cat. DMAP, 0 °C to rt, 2 h, 95%; (ii) allylbromide, K_2CO_3, toluene, KOH, TBAI, 80 °C, 5 h, 96%; (iii) HCl, EtOAc, P^H 2, rt, 89%; (iv) $AlCl_3$, 1,2-dichlorobenzene, 110 °C, 4 h, 92%; (v) L-(+)-tartaric acid, H_2O, 50 °C, acetone, 10 °C, 33% (vi) a) 20% K_2CO_3, P^H 8-9, cyclohexane, b) HCl-saturated EtOAc, EtOH, P^H 2, 5 h, rt, 91%.

2.3.3. Present work

Objective

As discussed above lorcaserin has attracted a great deal of attention due to its unique weight-lowering action. As most of the reported methods utilize chemical resolution strategy, still there is an avenue for developing an asymmetric synthetic route to this valuable molecule. In this section, the development of the facile synthesis of lorcaserin *R*-**1** starting from readily available starting materials has been discussed. The retrosynthetic analysis of *R*-**1** is outlined in **Scheme 6**.



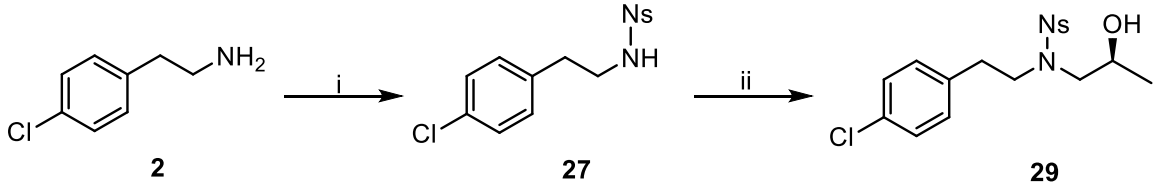
Scheme 6. Retrosynthetic analysis of lorcaserin *R*-**1**

Retrosynthetic analysis of lorcaserin *R*-**1** reveals that protected chiral amino alcohol derivative **29** could serve as a key intermediate for the synthesis. The protected amino alcohol derivative **29** could be achieved by the regioselective ring opening of (*S*)-propylene oxide **28** with an appropriate amine derivative **27**. The key intermediate **29** can be transformed into the target molecule *R*-**1** by halogenation followed by intramolecular Friedel-Craft's alkylation and *N*-deprotection sequences.

2.3.4. Results and Discussion

Synthetic strategy followed for the synthesis of lorcaserin *R*-**1** is outlined in **Scheme 7**. Accordingly, synthesis commenced with the readily available starting material 2-(4-chlorophenyl)ethanamine **2**, which on protection using 2-nitrobenzenesulfonylchloride

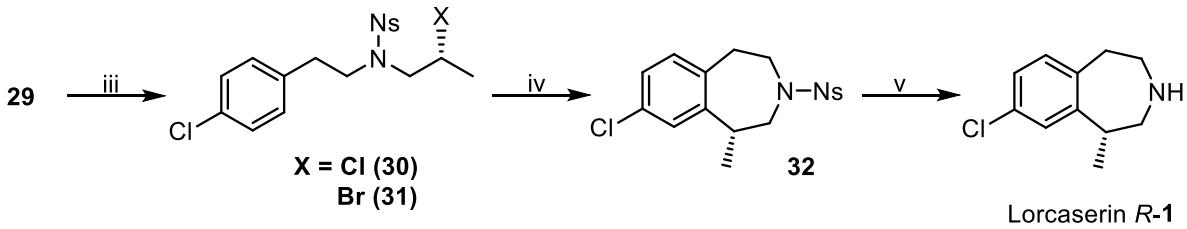
26 in dry CH_2Cl_2 and triethylamine at 0 °C to room temperature for 12 h gave nosyl protected secondary amine derivative **27** in 96% yield. In the ^{1}H NMR spectrum of **27**, signals corresponding to the nosyl group resonated at δ 7.69-7.75 (m), 7.83 (d), and δ 8.04 (d). Subsequently, the regioselective ring opening of (*S*)-propylene oxide **28** with nosylamine **27** and a catalytic amount of lithium bromide in anhydrous CH_2Cl_2 under reflux condition for 12 h afforded the key intermediate protected amino alcohol derivative **29**. In the ^{1}H NMR spectrum of **29**, the resonance signals corresponding to methyl and methine protons resonated at δ 1.20 (d), 3.99-4.06 (m) and in the ^{13}C NMR spectrum, signals corresponds to methine and methyl carbon resonated at δ 20.8 ppm and 66.0 ppm respectively.



2 **27** **29**

Scheme 7. *Reagents and conditions:* (i) 2-nitrobenzene sulfonylchloride **26**, Et_3N, DCM, 0 °C-rt, 12 h, 96%; (ii) (*S*)-propylene oxide **28**, cat. LiBr, DCM, reflux, 12 h, 89%.

Next, we turned our attention to convert the secondary -OH into a halo compound then try for cyclization. Accordingly, amino alcohol **29** was converted into its corresponding halo derivatives **30** and **31** by treating with thionyl chloride for chloro compound **30** and with CBr_4/PPh_3 (Appel reaction) for bromo compound **31** (**Scheme 8**). Subsequently, both the halo derivatives **30** and **31** were subjected to intramolecular Friedel-Crafts's alkylation with anhydrous aluminium chloride in chlorobenzene at room temperature for 12 h afforded the cyclized product **32**. It has been observed that chloro compound **30** produced cyclized



X = Cl (30)
Br (31)

32

Lorcaserin *R*-**1**

Scheme 8. *Reagents and conditions*: (iii) $SOCl_2$, pyridine, 50 °C, 12 h, 79% (**30**); CBr_4, PPh_3, DCM, 50 °C, 12 h, 98% (**31**); (iv) $AlCl_3$, chlorobenzene, rt, 12 h, (from **30**, 45%, 74% ee), (from **31**, 33%, 76% ee); (v) thiophenol, K_2CO_3, DMF, 2 h, rt, 71%.

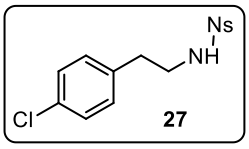
product **32** in 45% yield with 74% enantiopurity. Similarly, bromo compound **31** gave compound **32** with 33% yield and 76% enantiopurity. Finally, compound **32** was denosylated by treating with thiophenol and potassium carbonate in anhydrous DMF at room temperature for 2 h afforded lorcaserin *R*-**1**. The structures of all the synthesized compounds were confirmed by its ^{1}H NMR, ^{13}C NMR, and HRMS analysis.

2.3.5. Conclusion

In conclusion, developed a new and alternative synthesis of anti-obesity agent lorcaserin, starting from readily available starting materials. Although expected enantiopurity couldn't achieved in this method, efforts are in progress to achieve high enantiopurity by modifying reaction parameters.

2.3.6. Experimental Procedure

1) *N*-(4-chlorophenethyl)-2-nitrobenzenesulfonamide (27)



27

To a solution of 4-chlorophenethylamine **2** (1 g, 6.5 mmol) in dry dichloromethane (10 mL) and triethylamine (1.2 mL, 8.4 mmol) was added 2-nitrobenzenesulfonylchloride (1.2 gm, 5.2 mmol) at 0 °C and the resulting mixture was stirred at room temperature for 12 h. Upon completion of the reaction (TLC), the reaction mixture was diluted with water (25 mL) and extracted with DCM (3 x 15 mL). The combined organic layer was washed with brine (2 x 10 mL), dried over anhydrous Na_2SO_4. The filtrate was concentrated under reduced pressure to give the crude product, which was purified by column chromatography packed with silica gel using EtOAc/petroleum ether (15:85) afforded nosylated product **27** as a pale yellow solid.

Yield: 2.1 gm, 96 % yield;

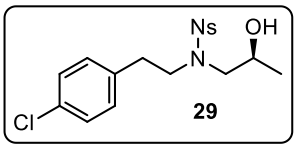
Molecular Formula: $C_{14}H_{13}ClN_2O_4S$;

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.82 (t, *J* = 6.8 Hz, 2 H), 3.40 (q, *J* = 6.4 Hz, 2 H), 5.35 (apparent t, *J* = 5.3 Hz, 1 H), 7.03 (d, *J* = 8.1 Hz, 2 H), 7.17 (d, *J* = 8.3 Hz, 2 H), 7.69-7.75 (m, 2 H), 7.83 (apparent d, *J* = 8.1 Hz, 1 H), 8.04 (apparent d, *J* = 8.3 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 147.7 (C), 135.9 (C), 133.8 (C), 133.4 (CH), 132.8 (CH), 132.7 (C), 130.7 (CH), 130.0 (CH, 2 carbons), 128.7 (CH, 2 carbons), 125.4 (CH), 44.9 (CH_2), 35.4 (CH_2);

HRMS (ESI): *m/z* calculated for $C_{14}H_{13}O_4N_2ClS$ $[M+Na]^+$ 363.0177, found 363.0170.

2) (*S*)-*N*-(4-chlorophenethyl)-*N*-(2-hydroxypropyl)-2-nitrobenzenesulfonamide (29)



To a solution of (*S*)-propylene oxide **28** (4.1 mL, 51.61 mmol) in dry dichloromethane (25 mL) were added catalytic amount of lithium bromide (5 mol%) and a solution of nosylated compound **27** (2.0 gm, 5.88 mmol) in anhydrous CH_2Cl_2 (15 mL) at 0 °C under argon atmosphere. The resulting reaction mixture was refluxed (under cold water circulation) for 12 h. Upon completion of the reaction (TLC), the reaction mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (3 x 25 mL). The combined organic layer was washed with brine (2 x 10 mL), dried over anhydrous Na_2SO_4. The filtrate was concentrated under reduced pressure to give the crude product, which was purified by column chromatography packed with silica gel using EtOAc/petroleum ether (30:70) furnished alcohol product **29** as a pale yellow semi-solid.

Yield: 2.1 g, 89 %;

Molecular Formula: $C_{17}H_{19}ClN_2O_5S$;

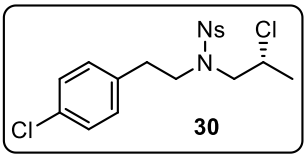
Specific rotation: $[\alpha]^{25}_D$ = + 2.233 (*c* 1.2, $CHCl_3$);

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20 (d, *J* = 6.0 Hz, 3 H), 2.83-2.95 (m, 2 H), 3.29 (d, *J* = 15.1, 8.7 Hz, 1 H), 3.36 (d, *J* = 15.1, 3.6 Hz, 1 H), 3.51-3.59 (m, 1 H), 3.62-3.69 (m, 1 H), 3.99-4.06 (m, 1 H), 7.09 (d, *J* = 8.2 Hz, 2 H), 7.20 (d, *J* = 8.2 Hz, 2 H), 7.61 -7.73 (m, 3 H), 7.98 (dd, *J* = 7.8, 1.4 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 147.9 (C), 136.4 (C), 133.6 (CH), 133.1 (C), 132.4 (C), 131.7 (CH), 130.9 (CH), 130.1 (CH, 2 carbons), 128.7 (CH, 2 carbons), 124.2 (CH), 66.0 (CH), 54.8 (CH_2), 50.1 (CH_2), 34.2 (CH_2), 20.8 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{17}H_{19}O_5N_2ClS$ $[M+Na]^+$ 421.0595, found 421.0589.

3) (*R*)-*N*-(4-chlorophenethyl)-*N*-(2-chloropropyl)-2-nitrobenzenesulfonamide (30)



To the stirred solution of alcohol **29** (1.2 g, 3.01 mmol) in dry pyridine (10 mL) was added freshly distilled thionyl chloride (0.7 mL, 6.03 mmol) drop-wise at room temperature. After completion of the addition, the reaction mixture was stirred at 50 °C for 12 h. After completion of the reaction (TLC), the reaction mixture was cooled to room temperature and diluted with water (20 mL) which was extracted with ethyl acetate (3 x 25 mL). The collected organic phase was washed with diluted HCl (2 x 20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product, which was purified by column chromatography packed with silica gel using EtOAc/petroleum ether (15:85) gave chloro product **30** as pale yellow semi-solid.

Yield: 0.97 g, 79%;

Molecular Formula: $C_{17}H_{18}Cl_2N_2O_4S$;

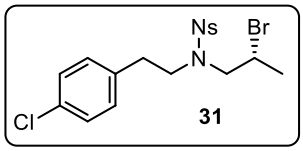
Specific rotation: $[\alpha]^{25}_D$ = -11.184 (*c* 1.8, $CHCl_3$);

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.54 (d, *J* = 6.4 Hz, 3 H), 2.79-2.91 (m, 2 H), 3.51-3.59 (m, 2 H), 3.63-3.74 (m, 2 H), 4.17-4.25 (m, 1 H), 7.05 (d, *J* = 8.2 Hz, 2 H), 7.16 (d, *J* = 8.2 Hz, 2 H), 7.61-7.74 (m, 3 H), 7.95 (apparent d, *J* = 8.2 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 147.8 (C), 136.1 (C), 133.7 (CH), 132.9 (C), 132.4 (C), 131.8 (CH), 130.7 (CH), 130.0 (CH, 2 carbons), 128.6 (CH, 2 carbons), 124.3 (CH), 55.3 (CH), 54.9 (CH_2), 49.9 (CH_2), 33.8 (CH_2), 22.4 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{17}H_{18}O_4N_2Cl_2S$ $[M+Na]^+$ 439.0257, found 439.0250.

4) (*R*)-*N*-(2-bromopropyl)-*N*-(4-chlorophenethyl)-2-nitrobenzenesulfonamide (31)



To a stirred solution of alcohol **29** (0.5 g, 1.24 mmol) and in dry CH_2Cl_2 (3 mL) was added recrystallized PPh_3 (1.0 g, 3.72 mmol) at room temperature under inert atmosphere. After stirred for 5 minutes, to the above reaction mixture recrystallized CBr_4 (0.84 g, 2.50 mmol) was added. The resulting mixture was refluxed for 8h. After completion of the reaction

(TLC), the reaction mixture was quenched by addition of water (10 mL), and then neutralized with saturated aqueous $NaHCO_3$ (10 mL). The reaction mixture was filtered through a pad of Celite and washed with ethyl acetate. The resulting filtrate was extracted with ethyl acetate (3 x 10 mL) and water (3 x 5 mL). The collected organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by column chromatography using EtOAc/petroleum ether (15:85) afforded the bromo product **31** as a colorless oil.

Yield: 0.56 g, 98%;

Molecular Formula: $C_{17}H_{18}BrClN_2O_4S$;

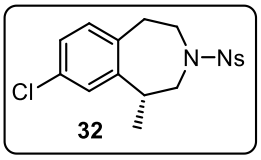
Specific rotation: $[\alpha]^{25}_D$ = -27.132 (*c* 2.3, $CHCl_3$);

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.72 (d, *J* = 6.4 Hz, 3 H), 2.79-2.91 (m, 2 H), 3.51-3.59 (m, 1 H), 3.63-3.71 (m, 2 H), 3.76-3.82 (m, 1 H), 4.23 (q, *J* = 6.9 Hz, 1 H), 7.06 (apparent d, *J* = 8.2 Hz, 2 H), 7.19 (apparent d, *J* = 8.2 Hz, 2 H), 7.63-7.75 (m, 3 H), 7.98 (dd, *J* = 7.8, 1.4 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 147.9 (C), 136.0 (C), 133.7 (CH), 133.0 (C), 132.6 (C), 131.8 (CH), 130.9 (CH), 130.1 (CH, 2 carbons), 128.7 (CH, 2 carbons), 124.4 (CH), 55.4 (CH_2), 49.8 (CH_2), 46.0 (CH), 33.8 (CH_2), 23.3 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{17}H_{18}O_4N_2BrClS$ $[M+Na]^+$ 484.9731, found 484.9719.

5) (*R*)-8-chloro-1-methyl-3-((2-nitrophenyl)sulfonyl)-2,3,4,5-tetrahydro-1*H*-benzo[*d*]-azepine (32)



32

To a stirred solution of **30** (0.5 g, 1.20 mmol) or **31** (0.5 g, 1.08 mmol) in anhydrous chlorobenzene (6 mL) was added anhydrous $AlCl_3$ (0.83 g, 6.0 mmol), under an inert atmosphere. The resulting reaction mixture was stirred at room temperature for 12 h. After completion of reaction (TLC), the reaction mixture was quenched by slow addition of water (15 mL), and then neutralized with saturated aqueous $NaHCO_3$. The reaction mixture was filtered through a pad of Celite and washed with ethyl acetate. The resulting filtrate was partitioned between ethyl acetate (3 x 15 mL) and water (3 x 10 mL). The combined organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by

column chromatography using EtOAc/petroleum ether (15:85) gave cyclized product **32** as a colorless liquid.

Yield: (0.21 g, 45% from **30**); (0.14 g, 33% from **31**);

Molecular Formula: $C_{17}H_{17}ClN_2O_4S$;

Specific rotation: $[\alpha]^{25}_{D}$ = -9.120 (*c* 0.3, $CHCl_3$);

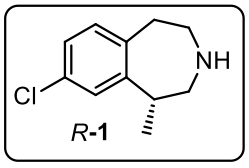
Chiral HPLC: ee > 74% (from **30**) [The ee of **32** was determined by chiral HPLC analysis; Kromasil OJ-H (250 x 4.6 mm) column; eluent: n-hexane/isopropanol (70:30); flow rate: 1.0 mL/min; detector: 222 nm [(*S*)-isomer t_R = 39.667 (minor); (*R*)-isomer t_R = 44.967 (major)]; ee > 76% (from **31**) [The ee of **32** was determined by chiral HPLC analysis; Kromasil OJ-H (250 x 4.6 mm) column; eluent: n-hexane/isopropanol (70:30); flow rate: 1.0 mL/min; detector: 222 nm [(*S*)-isomer t_R = 40.442 (minor); (*R*)-isomer t_R = 45.667 (major)];

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39 (d, *J* = 7.1 Hz, 3 H), 2.95-3.01 (m, 1 H), 3.11-3.19 (m, 2 H), 3.36-3.55 (m, 4 H), 7.01 (d, *J* = 8.1 Hz, 1 H), 7.08-7.13 (m, 2 H), 7.59-7.71 (m, 3 H), 7.95-7.98 (m, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 147.9 (C), 145.8 (C), 137.5 (C), 133.4 (CH), 133.0 (C), 132.5 (C), 131.6 (CH), 131.5 (CH), 130.8 (CH), 127.5 (CH), 126.5 (CH), 124.1 (CH), 53.4 (CH_2), 47.7 (CH_2), 40.5 (CH), 36.3 (CH_2), 17.3 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{17}H_{17}O_4N_2ClS$ $[M+Na]^+$ 403.0490, found 403.0484.

6) (*R*)-8-chloro-1-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine (*R*-1)



R-**1**

To a stirred solution of **32** (0.15 g, 0.39 mmol) and K_2CO_3 (0.16 g, 1.18 mmol) in dry DMF (5 mL) was added drop-wise thiophenol (0.05 mL, 0.47 mmol) at room temperature. The resulting mixture was stirred at room temperature for 2 h. After completion of reaction (TLC), the reaction mixture was diluted with water (8 mL) and washed with cold aqueous $NaHCO_3$ (5 mL). The reaction mixture was extracted with ethyl acetate (3 x 10 mL). The collected organic layer was dried over anhydrous Na_2SO_4, distilled off under reduced pressure and the crude residue was then purified over column chromatography (methanol/EtOAc 20:80) afforded target product *R*-**1**.

Yield: 0.055 g, 71%;

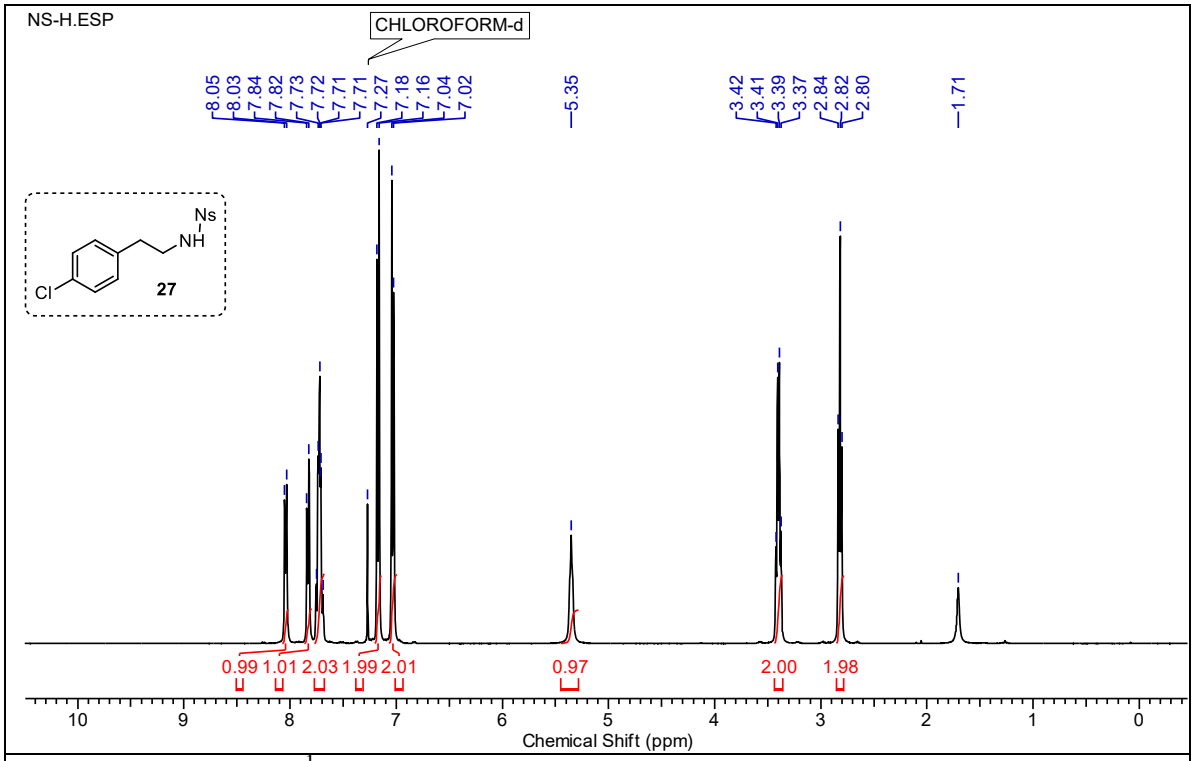
Molecular Formula: $C_{11}H_{14}ClN$;

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.32 (d, *J* = 7.3 Hz, 3 H), 2.00 (bs, 1 H), 2.61-2.74 (m, 1 H), 2.85-3.10 (m, 6 H), 7.00 (d, *J* = 7.9 Hz, 1 H), 7.08 (d, *J* = 7.9 Hz, 1 H), 7.13 (s, 1 H);

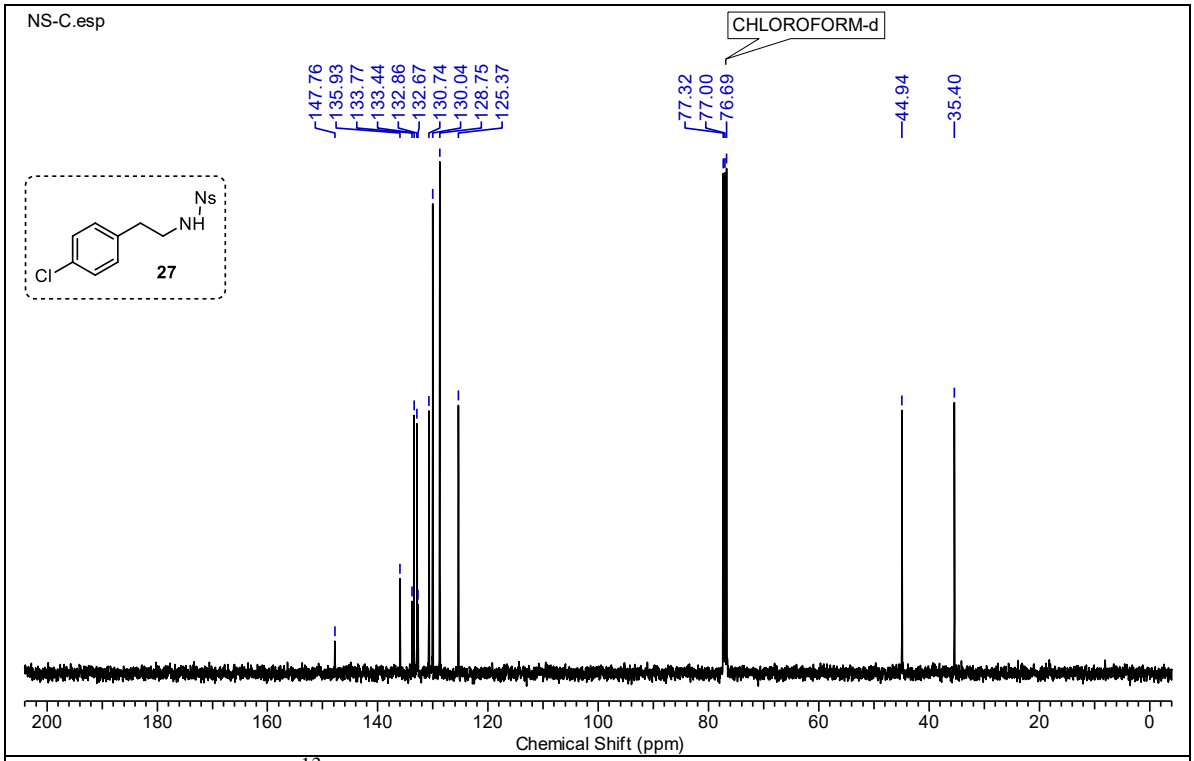
^{13}C NMR (100 MHz, $CDCl_3$): δ 147.4 (C), 139.7 (C), 131.8 (C), 130.9 (CH), 126.6 (CH), 125.7 (CH), 54.6 (CH_2), 47.8 (CH_2), 41.6 (CH), 39.0 (CH_2), 17.5 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{11}H_{14}NCl$ $[M+H]^+$ 196.0888, found 196.0888.

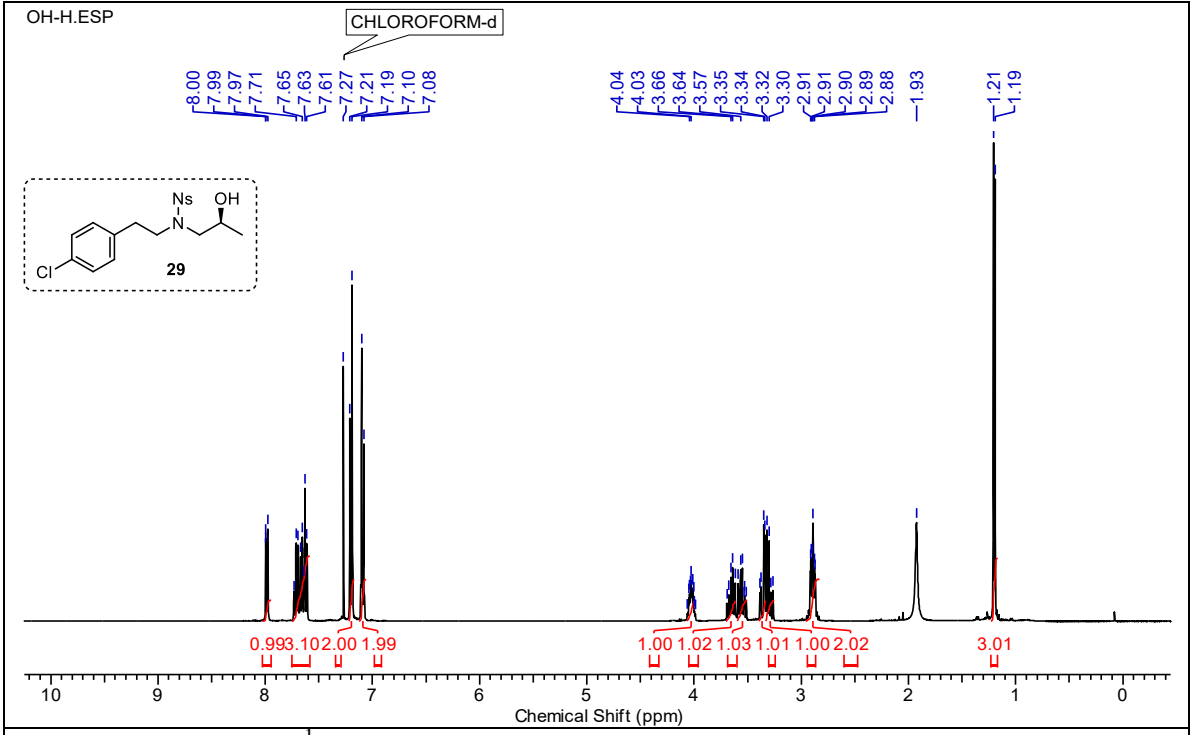
2.3.7. Spectra



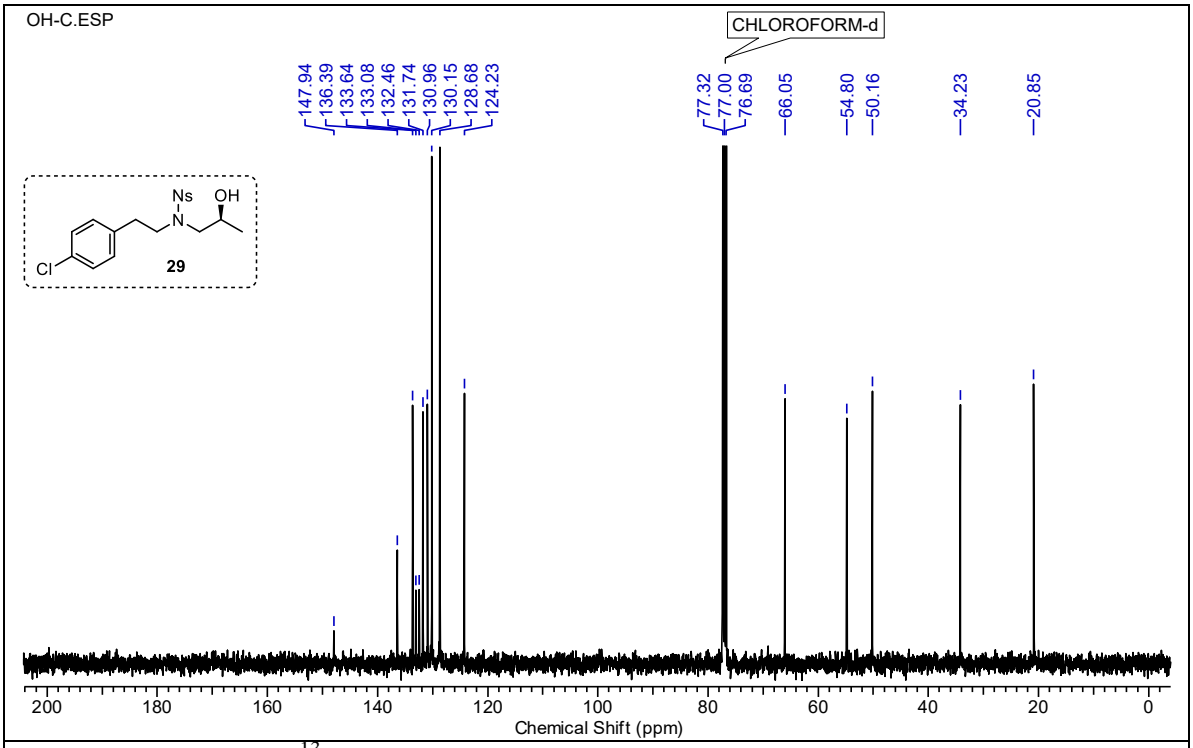
^{1}H NMR (400 MHz, $CDCl_3$) of compound **27**



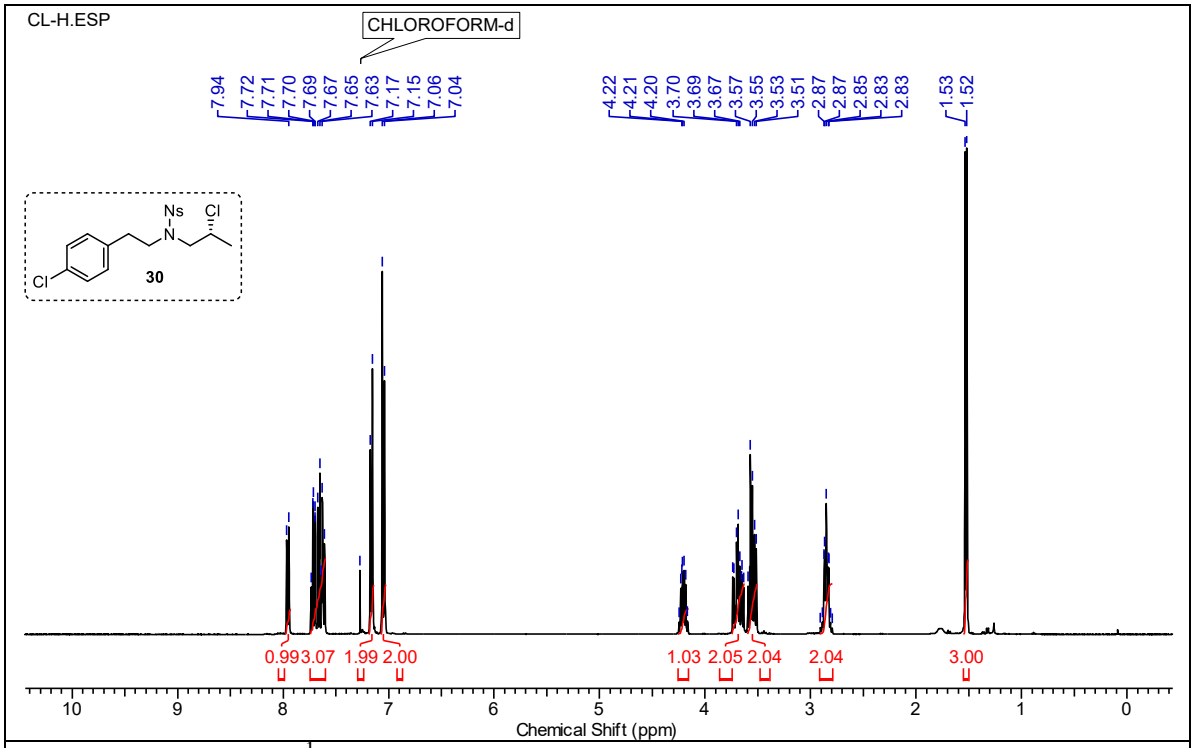
^{13}C NMR (100 MHz, $CDCl_3$) of compound **27**



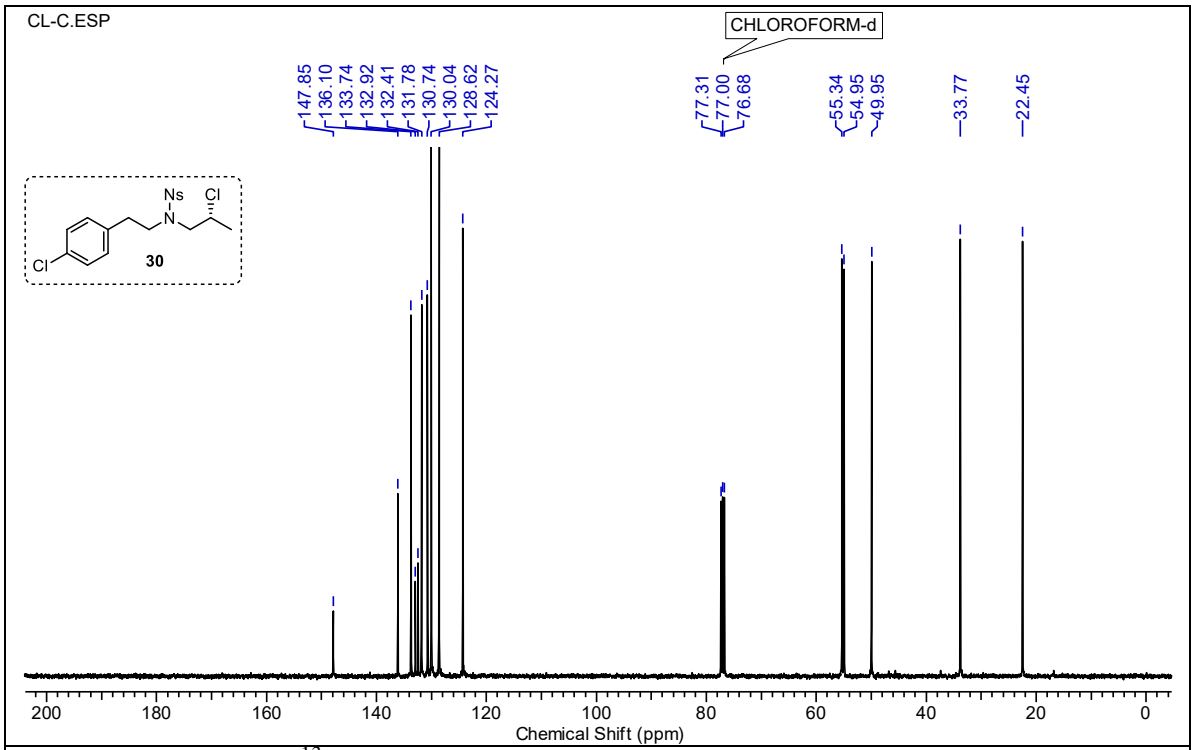
^{1}H NMR (400 MHz, $CDCl_3$) of compound **29**



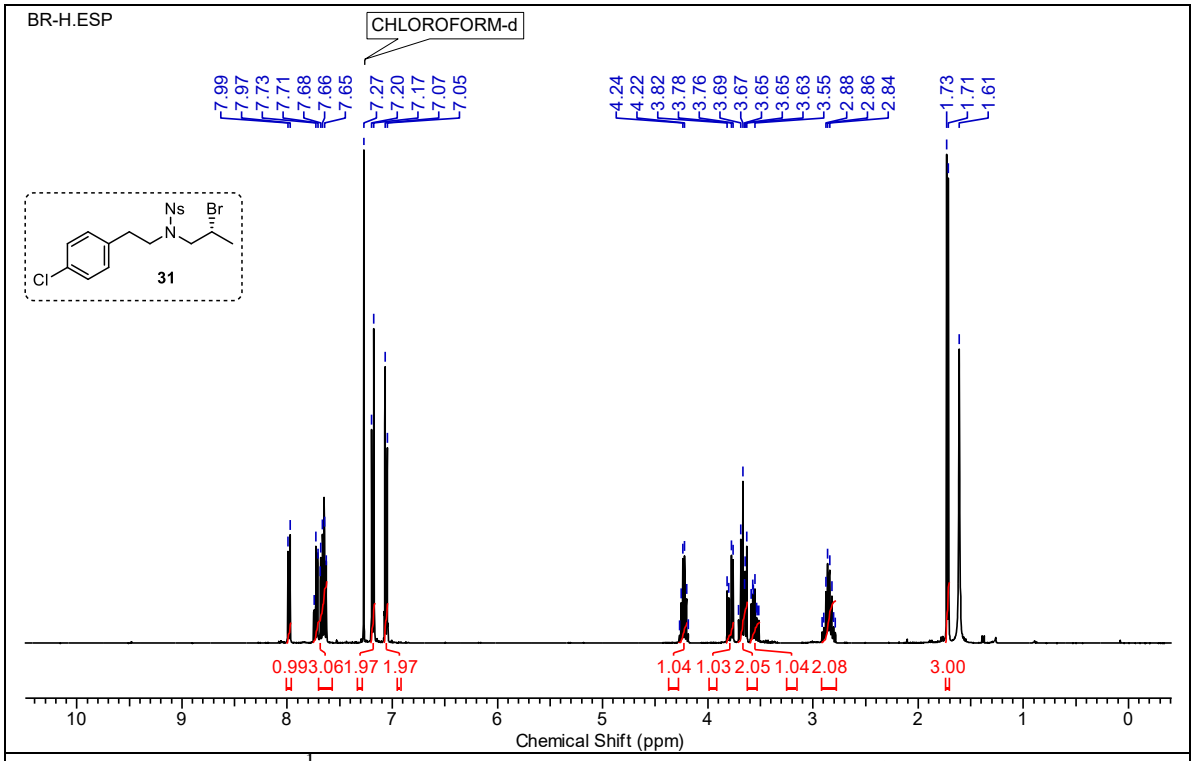
^{13}C NMR (100 MHz, $CDCl_3$) of compound **29**



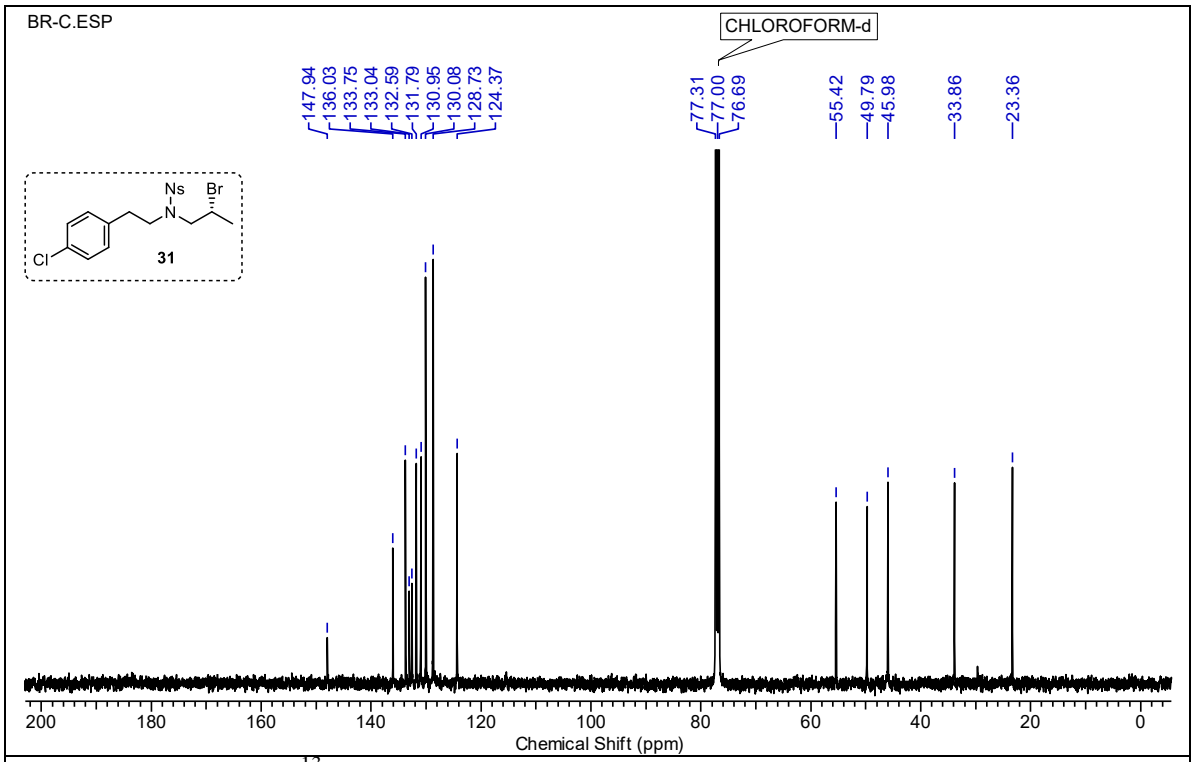
^{1}H NMR (400 MHz, $CDCl_3$) of compound **30**



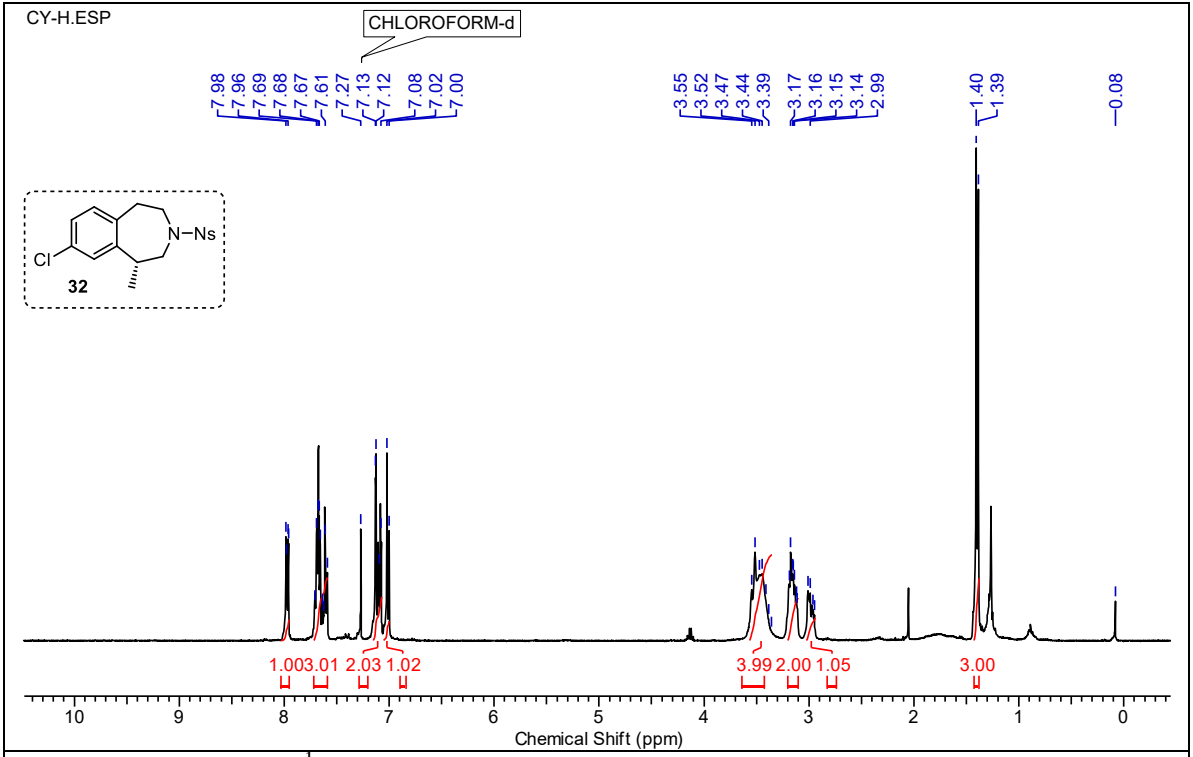
^{13}C NMR (100 MHz, $CDCl_3$) of compound **30**



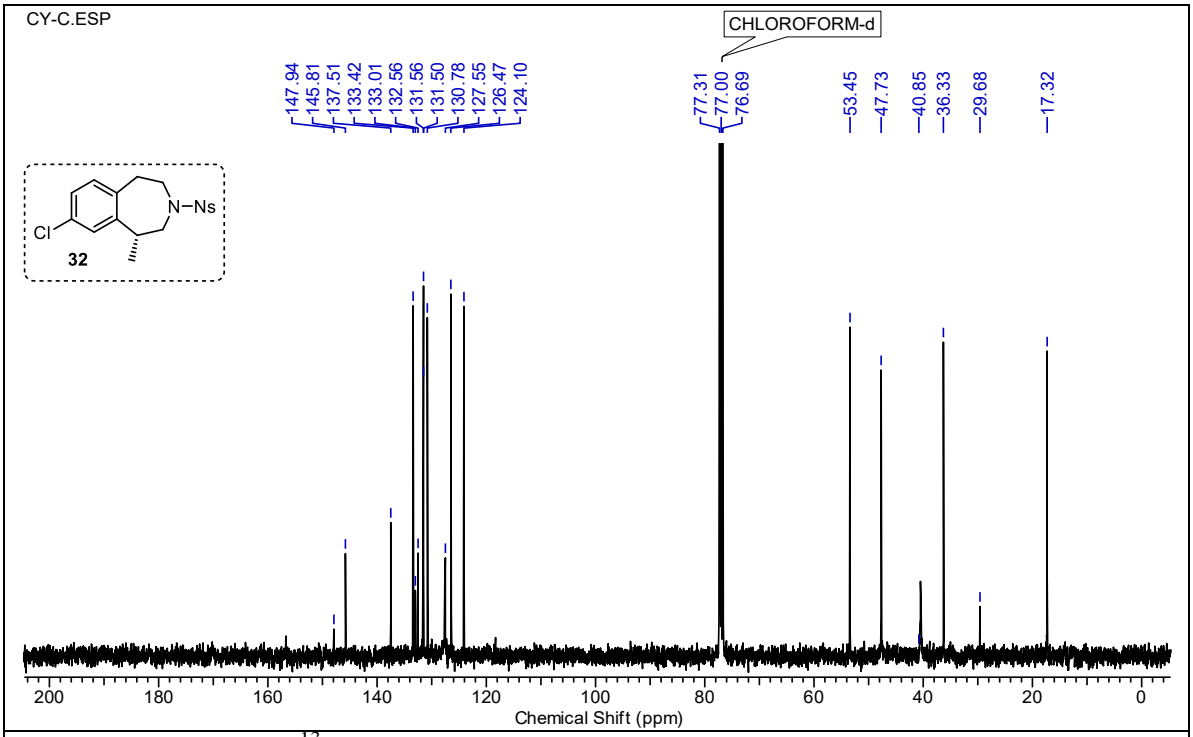
^{1}H NMR (400 MHz, $CDCl_3$) of compound **31**



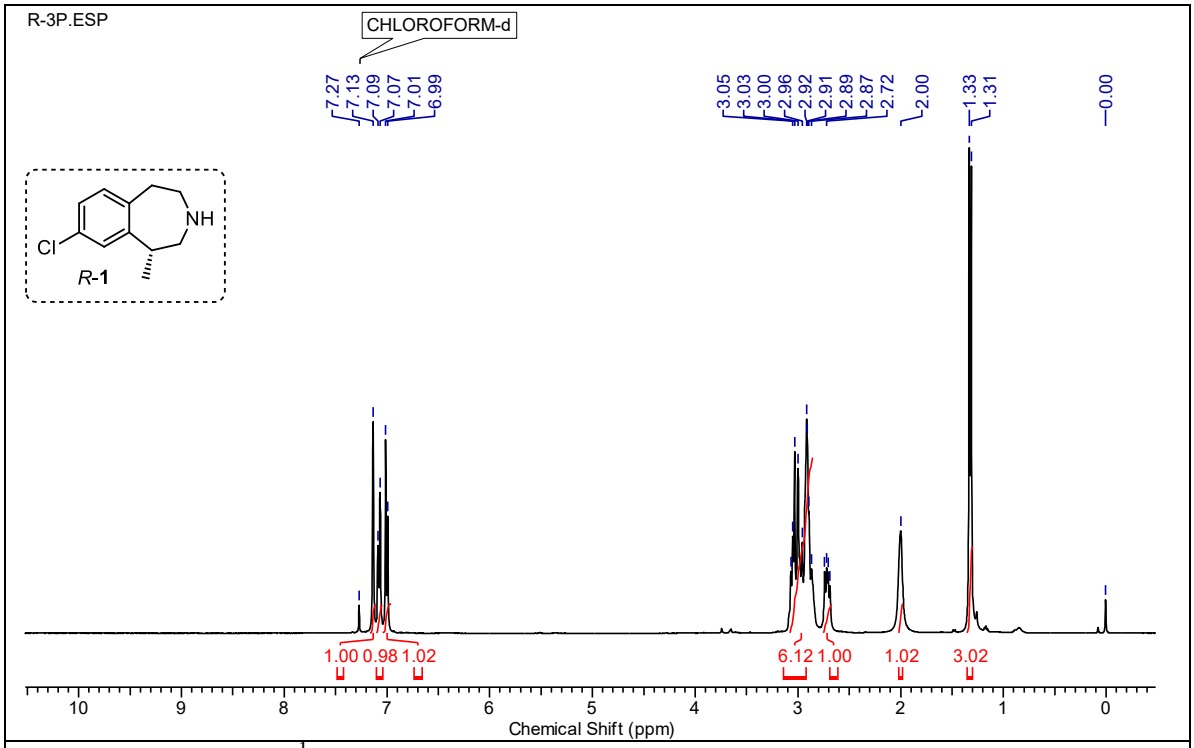
^{13}C NMR (100 MHz, $CDCl_3$) of compound **31**



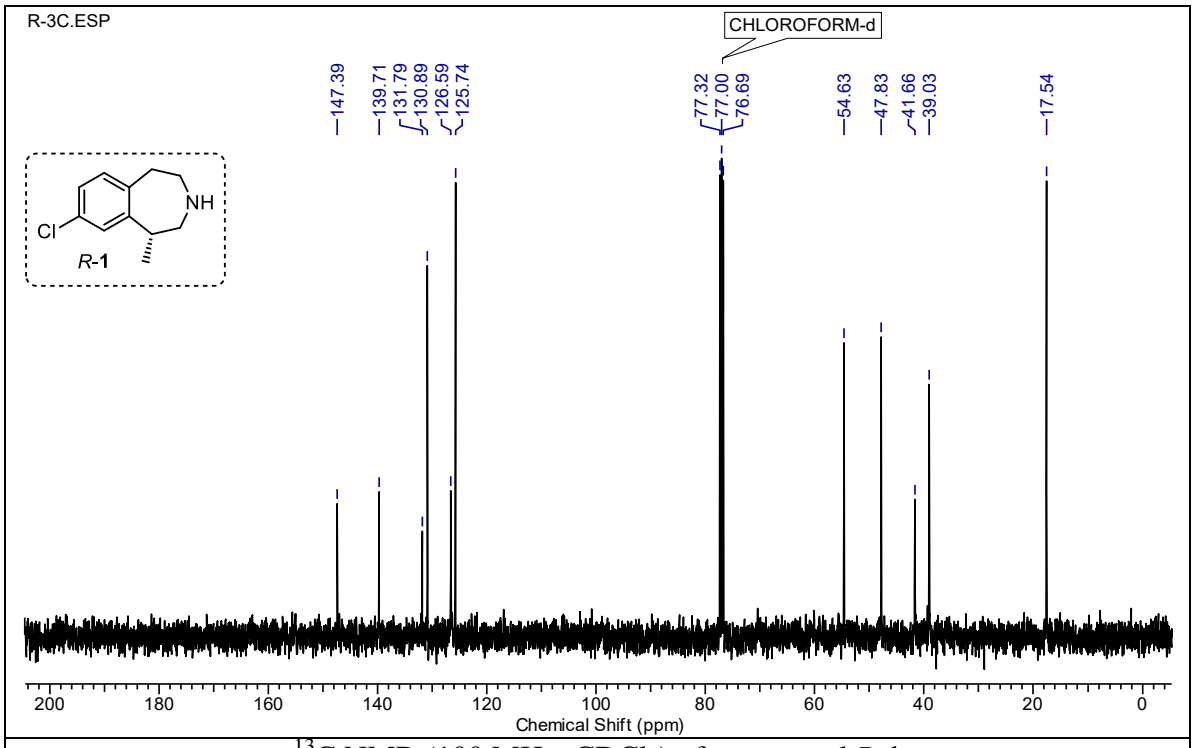
^{1}H NMR (400 MHz, $CDCl_3$) of compound **32**



^{13}C NMR (100 MHz, $CDCl_3$) of compound **32**



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



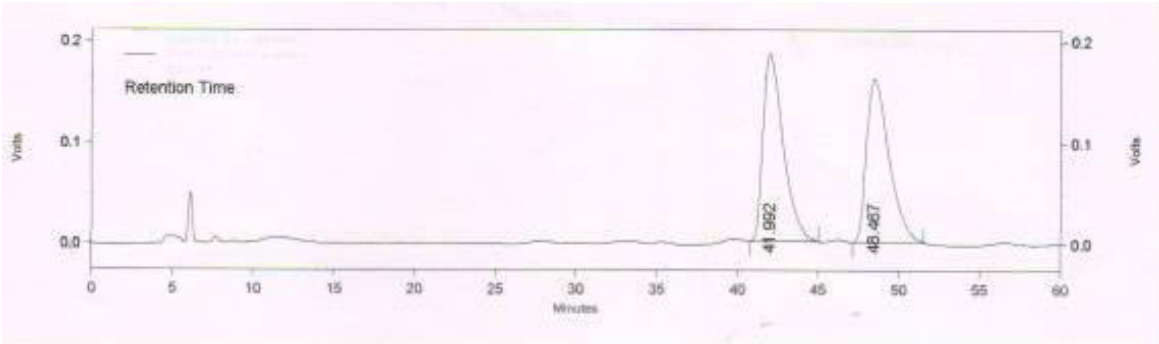
^{13}C NMR (100 MHz, $CDCl_3$) of compound *R*-**1**

2.3.8. Chiral HPLC analysis data

Chiral HPLC analysis of Compound 32

Conditions: Chiralcel OJ-H (250 X 4.6 mm) column; eluent: n-hexane/isopropanol (70:30); flow rate: 1 mL/min; detector 220 nm.

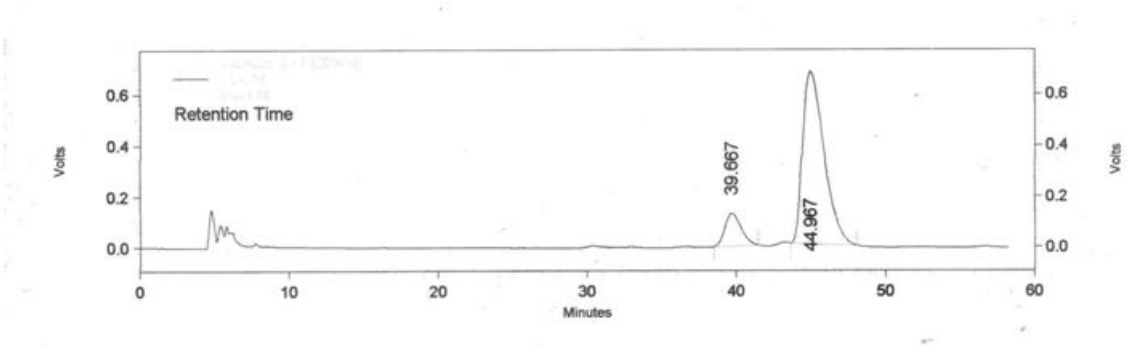
Racemic



Racemic Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	41.992	16286721	50.813
2	48.467	15765679	49.187
Totals		32052400	100.000

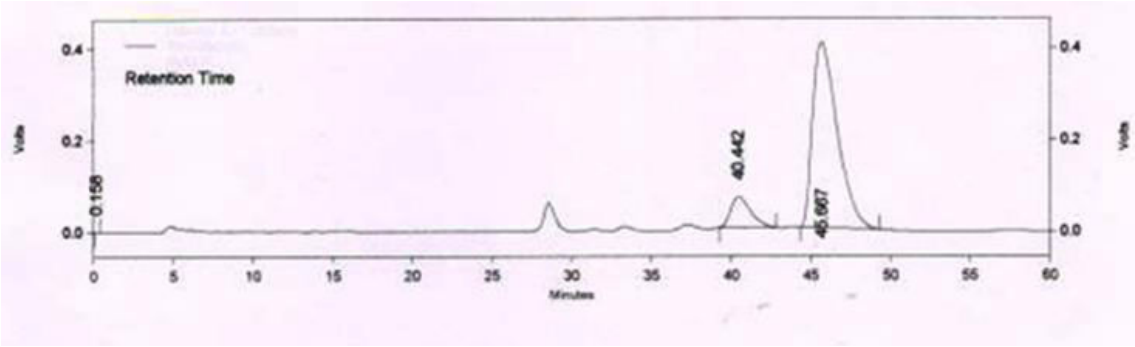
Chiral Sample Chromatograph (from compound 30)



Chiral Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	39.667	9697588	12.639
2	44.967	67029277	87.361
Totals		76726865	100.000

Chiral Sample Chromatograph (from compound 31)



Chiral Sample Chromatograph

Pk #	Retention Time (mins)	Area	Area %
1	40.442	5761455	11.674
2	45.667	43589498	88.325
Totals		49351144	100.000

2.3.9. References

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CHAPTER 3

Development of novel biologically active compounds based on benzopyran-4-one motif

3.1. SECTION 1

Synthesis, biological evaluation and molecular modeling studies of novel triazole-chromone conjugates as potent anti-TB agents

3.1.1. Introduction

Tuberculosis is a major infectious disease caused by pathogenic bacterial species *Mycobacterium tuberculosis* (*Mtb*) and it is a leading global threat to public health.[1] In 2017 alone, according to World health organization (WHO) report 10.4 million new cases and 1.7 million deaths, of which developing countries showed 95% of the share.[2] Globally, 45% of the total estimated TB cases from South Asian countries, in which the leading number of TB cases from India followed by Indonesia, China, Bangladesh, Philippines, and Pakistan. A recent study reveals that a large number of people with estimated TB cases in India is two to three times higher than previous estimates.[3] Furthermore, the emergence of a drug-resistant microorganism, especially multidrug-resistant (MDR-TB) one along with lethal combination of TB and HIV infection and extensively drug-resistant TB (XDR-TB) strains makes this disease even more challenging.[4] MDR-TB strains of *M. tuberculosis* that resists to two key potent anti-TB drugs rifampicin and isoniazid, with or without resistance to other first and second line TB drugs. XDR-TB is defined as MDR-TB with additional resistance to any fluoroquinolone and any one of the three-second line injectable agents.[5] The rate of mortality is more in XDR-TB than MDR-TB due to the limited number of effective treatments. In the past few decades, only a few drugs have been approved by the FDA to treat TB.[6] Therefore, the discovery and development of novel anti-TB agents with new chemotypes, acting on novel drug targets is an important task for infectious diseases research program.[7]

Nature has always been proved to be an important source of new drugs and they are recognized as evolutionarily selected and biologically pre-validated starting point for any successful drug discovery.[8] Additionally, natural products have been extensively utilized to elucidate complex cellular mechanisms, including signal transduction and cell cycle regulation leading to the identification of important targets for therapeutic intervention.[9] As a result of recent advances in biology, there is now an increased demand for new natural

product-like small molecules.[10] Despite the increased need for new natural products, their isolation and structure elucidation still remains a highly laborious and time-consuming process.[11] Therefore, synthesis of natural products inspired compound collections and their biological evaluation is a highly promising strategy for the identification of unique biologically relevant compound classes.

In this context, Chromones (4*H*-chromen-4-one, 4*H*-1-benzopyran-4-one) are ubiquitous structures, widely distributed naturally occurring compounds of plant origin.[12] They are oxygen-containing benzo-annulated heterocycles with oxa-pyran ring and it is the core backbone of flavonoid (2-aryl substituted chromones) family, such as flavones, isoflavones, and flavonols (**Figure 1**).[13] Due to their abundance in nature and their low mammalian toxicity, naturally occurring and synthetic chromone analogues exhibit a vast range of pharmacological properties like antioxidant, anti-inflammatory, antiviral, anticancer, anti-HIV, immunostimulators and anti-bacterial.[14] Some pharmaceutically important compounds possessing chromone framework are presented in **Figure 2**.

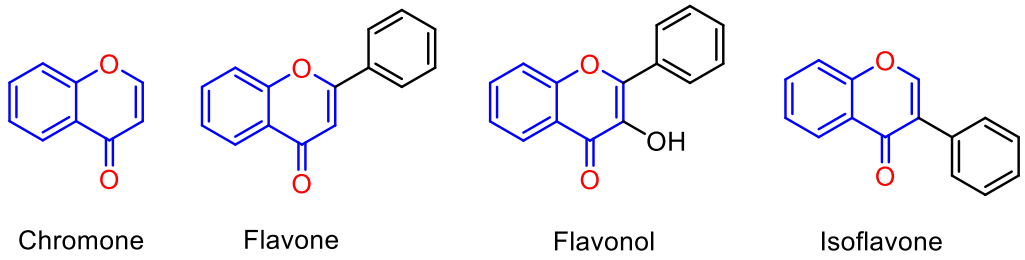


Figure 1. Chromone and flavonoid scaffolds.

Interestingly, many natural and natural product inspired chromone analogs exhibit significant antimycobacterial activity (**Figure 3**).[15] Recently, several research groups successfully demonstrated their mode of action by identifying their molecular targets.[16] A good safety profile, the possibility of oral administration,[17] and easy synthesis are the major factors contributing to the growing interest in exploring the pharmacological activities of chromones.

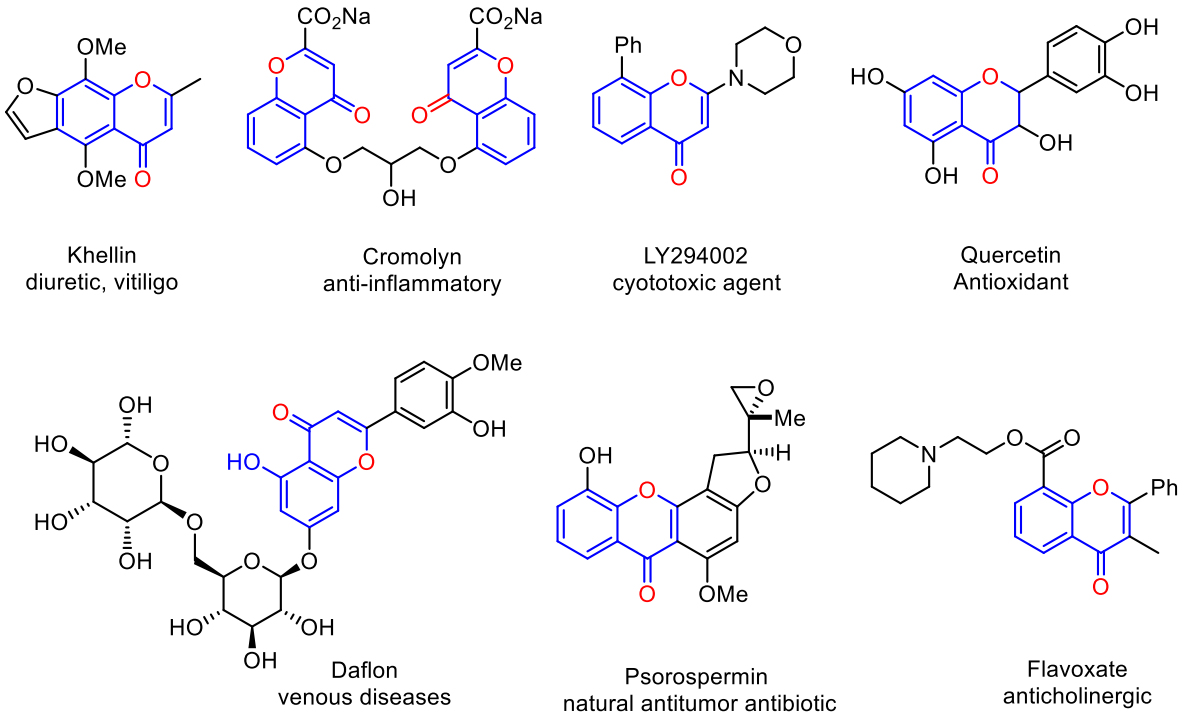


Figure 2. Chromone based compounds used as clinical agents

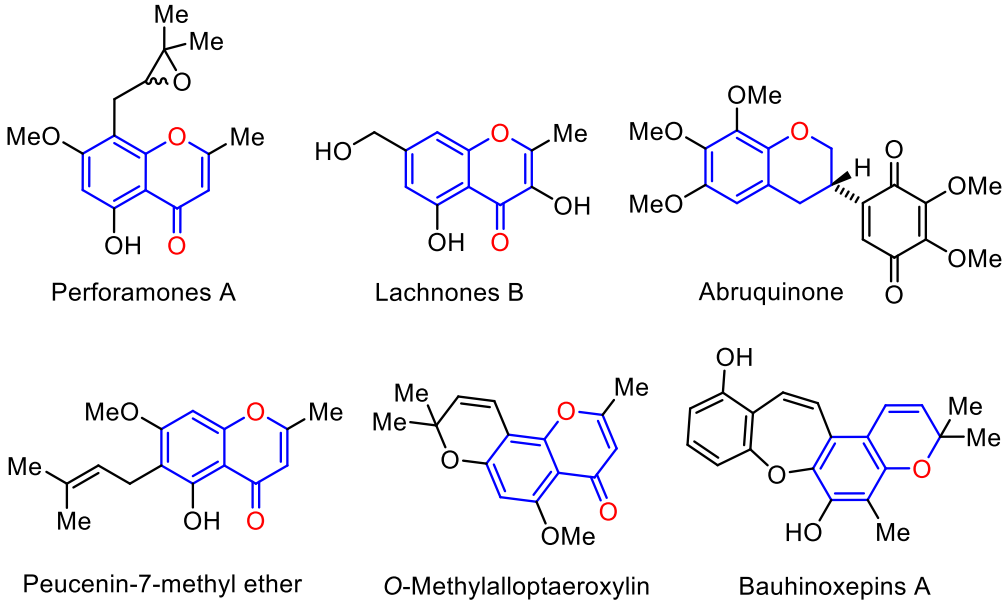


Figure 3. Some naturally occurring chromone scaffolds possessing antimycobacterial activity

3.1.2. Present work

Objective

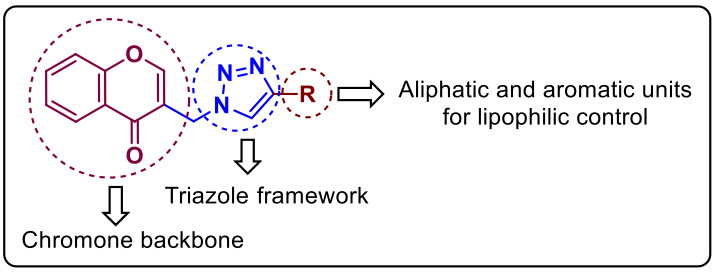


Figure 4. Design strategy for chromone embedded [1,2,3]-triazole as an antitubercular agent

In view of our continuing interest in the chemistry of privileged chromone motif, in particular, the design and synthesis of novel natural products like small molecules based on the chromone motif for various biological applications, in this section, design and synthesis of a series of novel chromone embedded 1,4 disubstituted [1,2,3]-triazole analogues and their biological evaluation against *M. Tuberculosis* H37Rv has been described (**Figure 4**). The interest in incorporation of [1,2,3]-triazole moiety stems from the advent of click chemistry protocol which has been used in various applications including drug discovery process.[18] In addition, triazole embedded heterocyclic frameworks exhibit a plethora of biological activities, especially anti-mycobacterial activity (**Figure 5**).[19]

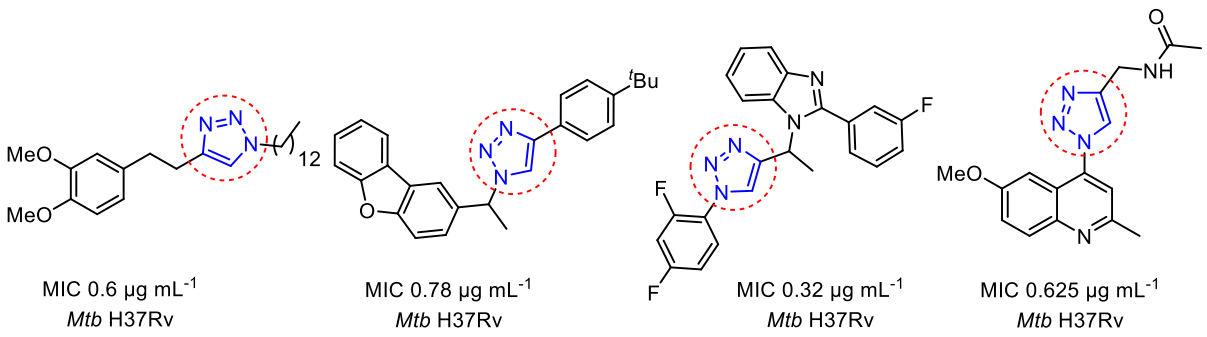
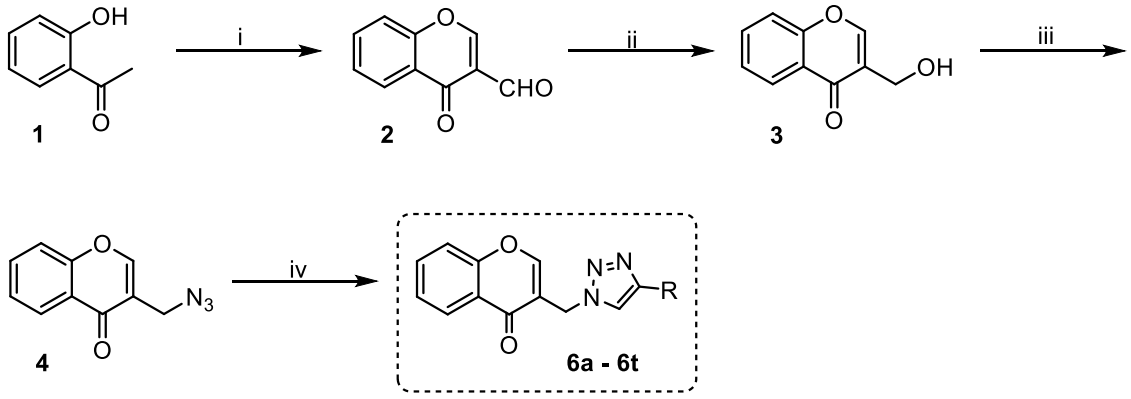


Figure 5. Representative [1,2,3]-triazole analogues possessing anti-mycobacterial activity

3.1.3. Results and Discussion

Chemistry

A four-step synthetic strategy was followed for the preparation of novel chromone embedded [1,2,3]-triazoles **6a-t** is outlined in **Scheme 1**. At first, 3-formyl chromone **2** was synthesized by formylation of *O*-hydroxy acetophenone **1** using Vilsmeier-Haack reagent ($POCl_3$ in DMF) at 55 °C for 5 h in 75% yield. In the ^1H NMR of **2**, the signals appeared for newly formed -CHO was discernible at δ 10.4 ppm while in the ^{13}C NMR, the resonance signals for characteristic carbonyl carbon was observed at δ 188.6 ppm confirmed the formation of aldehyde derivative **2**.



Scheme 1. *Reagents and conditions*: (i) DMF/$POCl_3$, 55 °C, 5 h, 75%; (ii) basic Al_2O_3, ^{i}PrOH, 75 °C, 4 h, 82 %; (iii) a) MsCl, NEt_3, CH_2Cl_2, 0 °C, 1 h, 71%; b) NaN_3, DMF, 50 °C, 5 h, 93%; (iv) alkyl/aryl terminal alkynes **5a-t**, sodium ascorbate, $CuSO_4.5H_2O$, *t*-BuOH/H_2O (1:1, v/v), 60 °C, 1-3 h.

Entry	R	Yield of 6 (%)	Entry	R	Yield of 6 (%)
6a	C_6H_5	90	**6k**	Napthyl-	93
6b	(4-Me) C_6H_4	82	**6l**	(C_5H_4N)	60
6c	(4-^{n}Et)C_6H_4	82	**6m**	$^nC_4H_9$	93
6d	(4-^{n}Pr) C_6H_4	84	**6n**	$^nC_6H_{13}$	82
6e	(4-$^nC_5H_{11}$) C_6H_4	88	**6o**	Cyclopentyl-	86
6f	(4-^{t}Bu) C_6H_4	96	**6p**	Cyclohexyl-	80
6g	(4-OMe)C_6H_5	60	**6q**	(Cyclohexyl)CH_2-	88
6h	(4-$^nC_5H_{11}O$)C_6H_5	88	**6r**	(9-fluorenol)	58
6i	(3-CH_3)C_6H_4	85	**6s**	Tridecan-1-ol	84
6j	(2-CH_3, 4-OCH_3) C_6H_3	60	**6t**	-H	38

Further, reduction of 3-formylchromone **2** with solid supported basic alumina in isopropanol at 75 °C for 4 h afforded 3-hydroxymethyl chromone **3** in 82%. In the ^{1}H NMR spectrum of compound **3**, the signals discernible as a singlet at δ 2.12 ppm and 4.60 ppm corresponding to hydroxyl proton and methylene protons respectively indicate the formation of compound **3**. In the IR spectra, the absorption band for hydroxyl group displayed at 3423 cm^{-1} further confirmed the formation of alcohol derivative **3**. Subsequently, mesylation of chromone alcohol derivative **3** followed by azidation with sodium azide in DMF at 50 °C for 5 h afforded the key chromone embedded azide intermediate **4** in 93% yield. The formation of azide derivative **4** was confirmed by its IR spectra, the absorption band displayed for azide group at 2107 cm^{-1}. Finally, the [1,2,3]-triazole core was incorporated through copper catalyzed 1,3 dipolar cycloaddition of 2-azidomethylchromone **4** with commercially available different alkyl/aryl terminal alkynes **5a-t** in the presence of sodium ascorbate in *t*-BuOH/H_2O (1:1, v/v) solvent mixture resulted in the formation of chromone embedded triazole compounds **6a-t** respectively in good to excellent yields (**Scheme 1**).

The structures of all the newly synthesized compounds **6a-t** were completely characterized by the ^{1}H NMR, ^{13}C NMR, and HRMS analysis. In the ^{1}H NMR spectrum of compound **6a** (representative example), a signal corresponds to the CH_2 protons that bridges the chromone with triazole moiety was observed at δ 5.48 ppm (as a singlet) and the corresponding ^{13}C resonance signal was delineated at δ 45.5 ppm and the chromone carbonyl was discernible at δ 176.7 ppm. In addition, the appearance of a sharp singlet for 1 proton observed at δ 8.22 ppm in the PMR, suggested the presence of 1,2,3 triazole CH. The appearance of a sharp singlet (1H) observed at δ 8.15 ppm in the PMR, suggested the presence olefinic C-H of chromone moiety. The HRMS (ESI) for **6a** shows the m/z at 304.1086 for $C_{18}H_{13}O_2N_3$ $[M+H]^+$.

Anti-mycobacterial evaluation

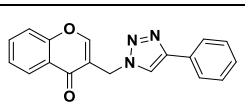
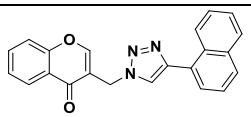
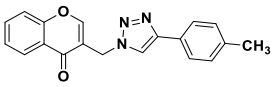
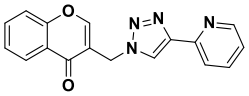
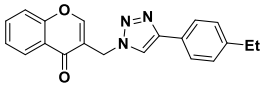
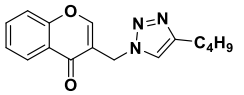
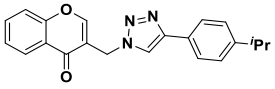
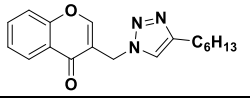
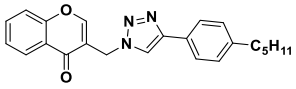
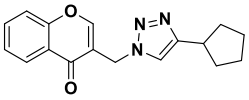
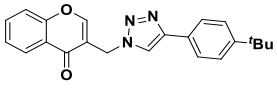
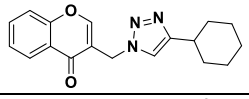
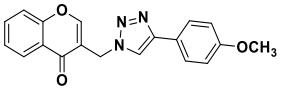
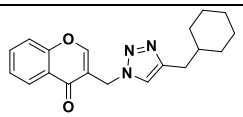
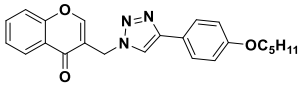
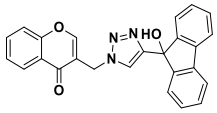
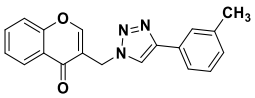
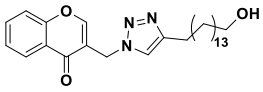
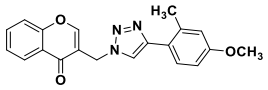
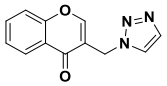
All the newly synthesized chromone embedded [1,2,3]-triazoles derivatives **6a-t** were screened for their *in vitro* anti-tubercular activity against *Mycobacterium tuberculosis* H37Rv (ATCC27294) using Microplate Alamar Blue Assay (MABA) method. The minimum inhibitory concentration (MIC; μg mL^{-1}) was determined for each compound. The MIC is defined as the lowest concentration at which complete inhibition was observed of

bacterial growth. Rifampicin and ethambutol were used as reference compounds. The MIC values of the synthesized compounds along with the standard drugs for comparison are reported in **Table 1**.

Among the 20 chromone embedded [1,2,3]-triazole derivatives tested, seven compounds (**6f**-**6h**, **6m**, **6o**, **6p** and **6s**) were found to be active with MIC values in the range of 1.56 to 12.5 μg mL^{-1}. The compound **6s** was found to be highly active among all the compounds tested with a MIC value of 1.56 μg mL^{-1}, which is 4.8 times more active than the standard drug ethambutol (MIC 7.64 μg mL^{-1}). Based on the results of anti-mycobacterial assay, the preliminary SAR of the chromone embedded triazole analogues reveals that the compounds bearing phenyl group (**6a**) as well as substituted phenyl such as 4-methyl, 4-ethyl, 4-propyl, and 4-pentyl (**6b**, **6c**, **6d**, and **6e**) do not favour better activity, with the exception of **6f** possessing 4-*t*-butyl group (MIC, 3.125 μg mL^{-1}). It was also observed that alkoxy substitution at 4-position of the phenyl ring (**6g** and **6h)** enhances the activity against *Mtb*. However, the addition of another methyl group at 2-position of **6g** leads to complete loss of activity, **6i** (MIC, 50 μg mL^{-1}). Further, replacement of phenyl group with naphthyl (**6k**) and pyridyl (**6l**) does not appear to enhance the activity (MIC, greater than 50 μg mL^{-1}). Interestingly, modification on the triazole core by changing R group from aromatic to aliphatic group (cyclic or acyclic) enhances the activity against *Mtb*. For example, the compound **6o** possessing cyclopentyl substituent at R position and compound **6m**, possessing *n*-butyl substituent exhibits better activity with MIC 3.125 and 6.25 μg mL^{-1} respectively, with an exception of **6n** possessing *n*-hexyl group (MIC, greater than 50 μg mL^{-1}). Importantly, the most active compound in the series, 6s possess long aliphatic chain terminated with hydrophilic -OH as a capping group (MIC, 1.56 μg mL^{-1}).

All chromone embedded [1,2,3]-triazole analogues were also tested for *in vitro* cytotoxicity against RAW 264.7 cells at 50 μg concentration using (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. All the analogues showed less than 50% inhibition, percentage inhibitions of cells are represented in **Table 1**. The most promising anti-tubercular analogous **6f**, **6g**, **6h**, **6m**, **6o** and **6s** exhibited 20.12%, 28.40%, 18.68%, 36.82%, 18.42% and 24.68% growth inhibition, respectively, at 50 μg mL^{-1}. The results indicated that potent analogues **6f**, **6h**, **6o,** and **6s** are comparatively less toxic and are suitable for further studies.

Table 1. *In vitro* anti-tubercular activity of chromone embedded [1,2,3]-triazoles against *Mycobacterium tuberculosis* H37Rv

Sr. No.	Compound	MIC (μg mL^{-1})[a]	Cytoto-xicity[b]	Sr. No.	Compound	MIC (μg mL^{-1})[a]	Cytoto-xicity[b]
6a		25	12.86	**6k**		50	26.12
6b		25	26.82	**6l**		50	30.60
6c		25	16.12	**6m**		6.25	36.82
6d		50	20.60	**6n**		50	23.74
6e		25	20.12	**6o**		3.125	18.42
6f		3.125	20.12	**6p**		12.5	20.12
6g		6.25	28.40	**6q**		25	28.62
6h		3.125	18.68	**6r**		50	30.60
6i		25	26.82	**6s**		1.56	24.68
6j		50	30.34	**6t**		50	30.12

Note: [a] Rifampicin (MIC 0.24 μg mL^{-1}); Ethambutol (MIC 7.64 μg mL^{-1}); [b] Cytotoxicity at 50 μg (RAW 264.7 cells) % inhibition.

Computational studies

Mycobacterium tuberculosis inhibitors perform an inhibitory action *via* different mechanistic pathways in the cell. We selected six validated protein targets from each class based on their role and importance in the mechanistic pathways (**Table 2**).[20] The biological significance of the selected proteins is discussed in detail herein. Thymidylate kinase (PDB ID: 1G3U) plays role in the catalysis of the transfer of the phosphoryl moiety from the

phosphoryl donor, ATP to TMP which is key intermediate for the DNA blocking builds.[21] Lumazine synthase (PDB ID: 1W19) catalyzes certain steps in riboflavin biosynthesis.[22] Enoyl-acyl carrier protein (PDB ID: 1ZID) is essential for fatty acid synthase system (FAS-II) pathway in mycobacterial cells.[23] Whereas pantothenate synthase (PDB ID: 3IUB) catalyzes the condensation of pantonate with β-alanine to form pantothenate, a precursor coenzyme A biosynthesis.[24] MTB phosphotyrosine B [MtbPtpB] (PDB ID: 2OZ5) blocks the signal-regulated kinase and p-38 mediated by IL-6 thereby promoting mycobacterial survival in the host.[25] Dihydrofolate reductase (PDB ID: 1DG5) helps in regulating the amount of tetrahydrofolate in the cell. Tetrahydrofolate derivatives are key components in purine and thymidylate synthesis which is important for cell proliferation and cell growth.[26]

Table 2. List of tuberculosis targets and mechanistic pathway class

PDB ID	Name of targets	Class
1G3U	Thymidylate kinase	DNA synthesis
1W19	6,7-dimethyl-8-ribityllumazine synthase	Cofactor biosynthesis
1ZID	Enoyl-acyl carrier protein	Mycolic acid biosynthesis
2OZ5	MTB phosphotyrosine phosphatase B	Arrest of phagosome maturation
3IUB	Pantothenate synthetase	β-Alanine metabolism
1DG5	Dihydrofolate reductase	Folate metabolism

Methodology

Preparation of ligands

The two-dimensional structures (.mol) of four compounds i.e **6f**, **6h**, **6o** and **6s** were drawn and the structure was analyzed by using Marvin view. The compounds were converted to three-dimensional structure (.pdb) using LigPrep tool.[27] LigPrep is a Schrödinger suite tool which is used to generate three-dimensional structures from two-dimensional structures, search tautomers, isomers for compounds and carry out energy minimization by applying the OPLS 2005 force field.

Preparation of macromolecule

The protein targets retrieved from RCSB Protein Data Bank are proteins associated with metabolic functioning and proliferation of *M. tuberculosis*. Enzymes thymidylate kinase (PDB code 1G3U), MTB phosphotyrosine phosphatase B [MtbPtpB] (PDB code 2OZ5) proteins, Enoyl-acyl carrier protein (PDB code 1ZID), Dihydrofolate reductase (PDB code 1DG5), Pantothenate synthetase (PDB code 3IUB) and 6,7-dimethyl-8-

ribityllumazine synthase (PDB code 1W19) served as docking receptors. The proteins were fixed for errors in atomic representations and optimized using Protein Preparation Wizard Maestro v10.3 (Maestro, version 10.3: Schrodinger, LLC, New York, NY, USA). The bond orders were assigned to residues, hydrogen atoms were added at p^H 7.0. Minimization was carried out using OPLS 2005 force field with an RMSD cut-off value of 0.3Å.

Molecular docking

The molecular docking was performed and analyzed *via* the Glide v 6.8 docking tool.[28] The receptor grid was centered based on the active site of the protein using receptor grid generation tool. Ligands prepared using LigPrep were flexibly docked in grid box using Monte Carlo based simulation algorithm. An extra precision (XP) method was employed that generated binding poses based on energy. The favourably docked molecules were ranked according to the Glide Score (**Table 3** and **4**).

Table 3. Molecular docking analysis of 6 protein targets with selected compounds. The binding energy was calculated for GLIDE in kcal/mol

PDB target	GLIDE score Binding energy (kcal/mol)			
	Compound **6f**	Compound **6h**	Compound **6o**	Compound **6s**
1G3U	-6.551	-6.782	-5.617	-5.912
1W19	-6.852	-4.880	-4.165	-5.600
1ZID	**-7.826**	**-9.189**	**-7.316**	**-11.123**
2OZ5	-6.899	-7.344	-5.572	-7.967
3IUB	-6.600	-6.602	-5.104	-5.291
1DG5	-4.521	-4.793	-4.475	-6.233

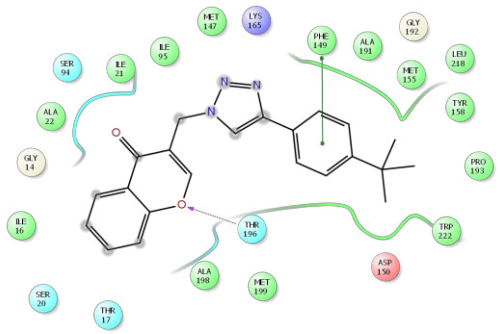
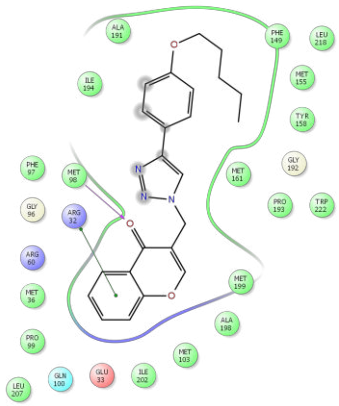
Table 4. Molecular docking analysis of selected compounds

Protein Target	Compound name	Amino acids involved in intermolecular interactions	Binding Energy (kcal/mol)
1ZID	Compound **6f**	Thr196 Phe149	-7.826
	Compound **6h**	Met98 Arg32	-9.189
	Compound 6s	Asp64 Trp222 Tyr158	**-11.123**
	Compound **6o**	Thr196	-7.316

Molecular docking analysis

Automated docking was used to assess the binding modes and conformation of the ligand molecules. Among the 20 chromone embedded [1,2,3]-triazoles, compounds **6f**, **6h**, **6o,** and **6s** were considered as they showed significant activities (**Table 1**). 1ZID, enoyl-acyl carrier protein showed better binding score with four chromone-based triazoles when compared with the rest of the proteins (**Table 2**). Compound **6s** gave a better score when compared with other compounds for the target proteins, with a binding score ranging from -7.3 to -11.123 kcal/mol. Enoyl-acyl carrier protein reductase is involved in mycolic acid biosynthesis, the inhibition of which leads to the lysis of *Mtb*. The key intermolecular protein-ligand interactions are depicted in **Figure 6**. **Figure 6** represents the intermolecular amino acid interaction with the compounds **6f**, **6h, 6s,** and **6o**. Compound **6s** showed highest binding energy value of -11.123 kcal/mol. Asp64, Trp222, and Tyr158 amino acids interacted with compound **6s** showing high ligand exposure. Trp222 and Tyr158 had π-π interaction with the compound **6s.** Compound **6f** and **6o** similarly showed π-π interaction with Phe149 and Thr196, respectively. Thus above results suggest that π-π interaction improves the docking scores.

Compound **6s** is bound to the active site amino acid residues in the pocket region as shown in **Figure 7** (**A** and **B**). The pocket region of 1ZID is present in a loop region flanked by alpha-helix chains seen in **Figure 7 (A)**. The location and orientation of the triazole group are complementary to the surrounding InhA side chains, which create a specific binding pocket. These observations indicate that compound **6s** may have an important role in anchoring within the active site of the receptor.

a) Compound **6f**	**b)** Compound **6h**
	

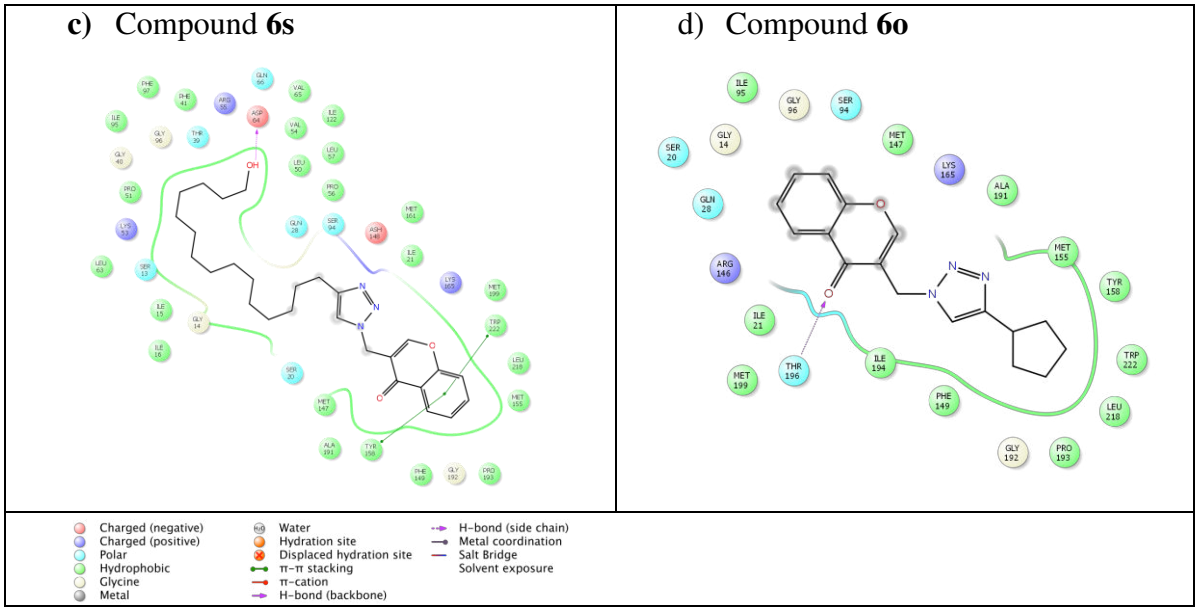


Figure 6. Amino acids involved in intermolecular interactions

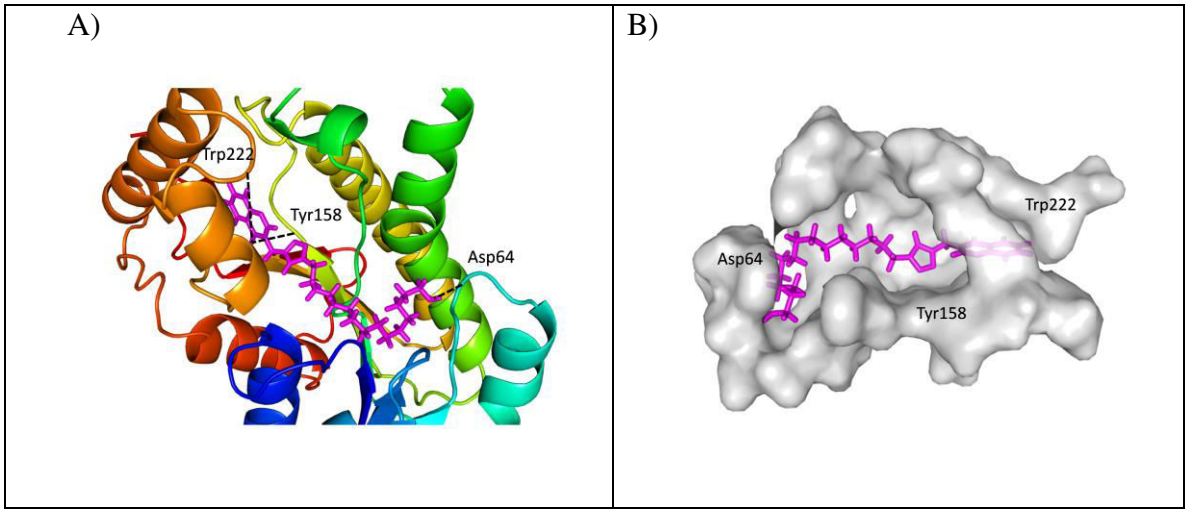


Figure 7. A) Compound **6s** (in magenta) in enoyl-acyl carrier protein (PDB ID:1ZID). B) Alignment of compound **6s** in the binding pocket.

Chemoinformatics Analysis

Six active compounds were analyzed for their drug-like properties (**Table 5**). Lipinski rule of five was predicted using Screening Assistant 2 tool.[29] All these compounds including compound **6s** displayed good drug-like properties. The drug-like and lead-like property analysis for most compounds generated a score of 0.25 which gave support to the positive results obtained in Rule of 5. ADME properties were predicted using PreADMET software[30] in order to check their potential as anti-tubercular compounds.

Table 5. Chemoinformatics analysis

Properties	Compounds					
	6f	**6g**	**6h**	**6m**	**6o**	**6s**
Lipinski Rule[a]						
Molecular weight	359.429	333.347	389.455	283.331	295.342	453.627
HB accept	3	4	4	3	3	4
HB donor	0	0	0	0	0	1
LogP	3.335	1.792	3.631	1.533	1.409	5.188
Chemical properties						
Weiner path[a]	2075	1661	2737	1015	1117	4787
Ring count[a]	4	4	8	5	3	17
PDL/PLL[a]	0.25	0.25	0.25	0.25	0.25	0.25
ADME properties						
BBB (-3.0 – 1.2)[b]	2.88269	1.161	0.23527	0.5112	0.36551	1.14616
CaCo2 (nms) (<25, poor, >500, best) [b]	34.0619	23.231	32.9739	25.026	16.0711	39.8231
HIA (50-100%) [b]	97.41	97.16	97.72	98.34	98.27	96.48
Rotatable bonds (0-15)[a]	4	4	8	5	3	17
TPSA (7.0 – 200.0)[b]	57.01	66.24	66.24	57.01	57.01	77.24
Toxicity properties[c]						
DSSTox Carcinogenic potency Mutagenecity	Neg. (C:0.161)	Neg. (C:0.202)	Neg. (C:0.112)	Neg. (C:0.110)	Neg. (C:0.189)	Neg. (C:0.115)
DSSTox Carcinogenic potency Mouse	Neg. (C: 0.083)	Neg. (C: 0.093)	Neg. (C: 0.099)	Neg. (C: 0.107)	Neg. (C: 0.141)	Neg. (C: 0.208)

[a]Computed using Screening Assistant 2 program. PDL (Progressive drug-like), PLL (Progressive lead like).
[b] PreADMET software. [c]LAZAR wherein Neg = Negative and C = Confidence value

The blood-brain barrier (BBB) model values for compound **6s** was 1.14616 which clearly lay in range suggesting the compound can penetrate the BBB on theoretical grounds. Most compounds displayed CaCo2 cell permeability values above 25 nms,[31] topological polar surface area (TPSA) above 7.0 and the human intestinal absorption (HIA) quantities in the 50-100 % range, indicating that they may be further developed in an oral dosage form.[32] Lazy structure-activity relationships (LAZAR) software[33] predicted all the compounds as noncarcinogenic and nonmutagenic, and the probability greater than 0.025 suggesting the

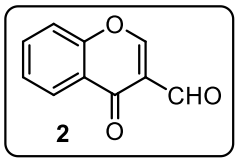
predictions to be reliable. The predicted favourable ADME features for compound **6s** further indicates that it is a promising anti-tubercular lead candidate.

3.1.4. Conclusion

In conclusion, a series of novel chromone embedded [1,2,3]-triazole derivatives were synthesized *via* an easy and convenient synthetic protocol starting from 2-hydroxy acetophenone. The new 20 analogues **6a-t** accomplished in four-step synthetic sequences using click chemistry as a key step and were fully characterized by their NMR and mass spectral data. The *in vitro* anti-mycobacterial evaluation study of all the compounds revealed that seven compounds found to be active against *M.tuberculosis* H37Rv. The compound **6s** is the most potent compound *in vitro* with a MIC value of 1.56 µg mL^{-1}. Cross-docking studies revealed compound **6s** to be more effective against the enoyl-acyl carrier protein reductase of *Mtb*. Molecular docking and chemoinformatics studies proved that compound **6s** displayed drug-like properties against the enoyl-acyl carrier protein reductase. Docking results also indicated that Asp64, Trp222 and Tyr158 amino acids in the binding pocket as potential ligand binding hot-spot residues.

3.1.5. Experimental Section

1) 4-oxo-4*H*-chromene-3-carbaldehyde (2)



2

To a stirred solution of dry DMF (40 mL), $POCl_3$ (20.6 mL, 220.34 mmol) was added dropwise at 5 °C. The mixture was stirred for 15 min and then the solution of 2-hydroxy acetophenone (10 g, 73.44 mmol) in DMF (20 mL) was added dropwise at 5 °C. The reaction mixture was stirred at the same temperature for 30 min, then heated and stirred at 55 °C for another 4 h. The mixture was cooled to room temperature, poured into ice-water (approx. 400 mL) and stirred for 1.5 h. The precipitate was filtered off, washed with ethanol afforded **2.**

Yield: 75% (colorless solid);

MP: 152-154 °C (lit.[34] mp: 152-154 °C);

Molecular Formula: $C_{10}H_6O_3$;

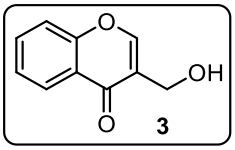
IR ($CHCl_3$, cm^{-1}): ν_{max} 3370, 3023, 2921, 2403, 1659, 1612, 1569, 1464, 1423, 1310, 1217, 1027, 930, 766, 671;

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50-7.56 (m, 2 H), 7.74-7.79 (m, 1 H), 8.31 (dd, *J* = 7.9, 1.6 Hz, 1 H), 8.56 (s, 1 H), 10.40 (s, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 188.6 (CO), 175.9 (CO), 160.6 (CH), 156.2 (C), 134.8 (CH), 126.6 (CH), 126.2 (CH), 125.3 (C), 120.3 (C), 118.6 (CH);

HRMS (ESI): *m/z* calculated for $C_{10}H_6O_3$ $[M+H]^+$ 175.0390, found 175.0392.

2) 3-(hydroxymethyl)-4*H*-chromen-4-one (3)



To a stirred solution of 3-formyl chromone **2** (2 g, 5% of alumina weight) in 100 ml of 2-propanol, about 40 g of basic alumina was added. The resulting solution was stirred at 75 °C for 4 hours. The reaction mixture was filtered through Celite bed and the solvent was removed under reduced pressure and the residue was purified by column chromatography over silica gel using EtOAc/petroleum ether (3:7) afforded compound **3** as a viscous liquid.

Yield: 82%;

Molecular Formula: $C_{10}H_8O_3$;

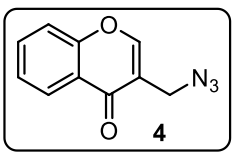
IR ($CHCl_3$, cm^{-1}): ν_{max} 3423, 3019, 2925, 2403, 1643, 1469, 1406, 1347, 1217, 1155, 1023, 971, 918, 852, 763, 670;

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.12 (bs, 1 H), 4.60 (s, 2 H), 7.40-7.51 (m, 2 H), 7.67-7.75 (m, 1 H), 7.96 (s, 1 H), 8.24 (dd, *J* = 7.9, 1.7 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 178.4 (CO), 156.6 (C), 152.8 (CH), 133.9 (CH), 125.6 (CH), 125.23 (CH), 123.8 (C), 123.3 (C), 118.2 (CH), 58.5 (CH_2);

HRMS (ESI): *m/z* calculated for $C_{10}H_8O_3$ $[M+H]^+$ 177.0546, found 177.0546.

3) 3-(azidomethyl)-4*H*-chromen-4-one (4)



To a stirred solution of **3** (2.5 g, 14.2mmol) and Et_3N (5.14 mL, 36.92 mmol), methane sulfonyl chloride (1.49 mL, 18.46 mmol) in CH_2Cl_2 (30 mL) was added dropwise at 0 °C. The resulting reaction mixture was stirred at 0 °C for 1 h. After completion of the reaction (monitored by TLC), the reaction mixture was diluted with water (approx. 20 mL) and extracted with CH_2Cl_2 (3 x 10mL). The combined organic layers were washed with water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude mesylated product **3a** (2.56 g, 71%) was further used for next step without any purification.

To a solution of crude mesylate **3a** (2.5 g, 9.84 mol) in anhydrous DMF (20 mL), sodium azide (1.6 g, 24.6 mmol) was added batchwise at room temperature. The resulting solution was heated to 50 °C for 5 h. After completion of reaction (monitored by TLC) reaction mixture was poured into ice-cold water (20 mL) and extracted with ethyl acetate (3 x 10 mL). The combined ethyl acetate layers were washed with brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by flash chromatography gave azide **4**.

Yield: 93% (colorless solid);

MP: 50-52 °C;

Molecular Formula: $C_{10}H_7N_3O_2$;

IR ($CHCl_3$, cm^{-1}): ν_{max} 3369, 3018, 2922, 2855, 2107, 1648, 1416, 1407, 1349, 1268, 1217, 1106, 1028, 842, 759, 668;

^{1}H NMR (200 MHz, $CDCl_3$): δ 4.33 (s, 2 H), 7.41-7.51 (m, 2 H), 7.67-7.76 (m, 1 H), 7.97 (s, 1 H), 8.26 (dd, *J* = 7.9, 1.7 Hz, 1 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 176.9 (CO), 156.5 (C), 153.8 (CH), 134.0 (CH), 125.9 (CH), 125.5 (CH), 123.7 (C), 119.7 (C), 118.2 (CH), 46.4 (CH_2);

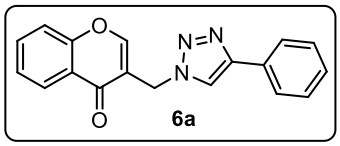
HRMS (ESI): *m/z* calculated for $C_{10}H_7O_2N_3$ $[M+Na]^+$ 224.0430, found 224.0432.

General procedure for the synthesis of Chromones embedded 1,2,3-triazole derivatives (6a-t)

To a stirred solution of azide **4** (1 equiv.) and aliphatic/aromatic alkynes, **5a-5t** (1.3 equiv) in *t*-butanol (3 mL) was added sequentially copper sulfate pentahydrate (20 mol %), sodium ascorbate (20 mol %) and distilled water (3 mL). The resulting reaction mixture was stirred for 1-3 h at 60 °C. After completion of the reaction (monitored by TLC), the reaction mixture was diluted with EtOAc (1 x 10 mL) and then washed with water (2 x 5 mL), the organic layer was separated, washed with brine solution (2 x 5 mL), dried over anhydrous

sodium sulfate and concentrated in vacuo. The crude residue thus obtained was purified over silica gel column chromatography eluted with EtOAc/petroleum ether (1:1) to furnish corresponding chromone embedded [1,2,3]-triazole derivatives **6a-6t**.

1) 3-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6a)



6a

Yield: 90% (pale yellow solid);

MP: 154-155 °C;

Molecular Formula: $C_{18}H_{13}N_3O_2$;

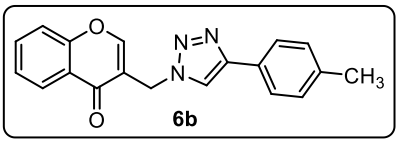
IR ($CHCl_3$, cm^{-1}): ν_{max} 3685, 3357, 3022, 2923, 2402, 1649, 1523, 1469, 1423, 1353, 1216, 1030, 927, 765, 671;

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.48 (s, 2 H), 7.30-7.34 (m, 1 H), 7.39-7.51 (m, 4 H), 7.70-7.74 (m, 1 H), 7.83 (d, *J* = 7.3 Hz, 2 H), 8.15 (s, 1 H), 8.22 (s, 1 H), 8.24 (dd, *J* = 8.0, 1.6 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7 (CO), 156.5 (C), 155.8 (CH), 134.4 (CH, 2 carbons), 130.1 (C), 128.8 (CH, 2 carbons), 128.3 (CH), 125.8 (CH, 3 carbons), 123.8 (C), 121.3 (C), 119.1 (C), 118.4 (CH), 45.5 (CH_2);

HRMS (ESI): *m/z* calculated for $C_{18}H_{13}O_2N_3$ $[M+H]^+$ 304.1081, found 304.1086.

2) 3-((4-(*p*-tolyl)-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6b)



6b

Yield: 82% (colorless solid);

MP: 170-172 °C;

Molecular Formula: $C_{19}H_{15}N_3O_2$;

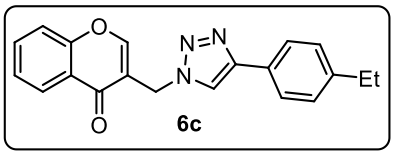
IR ($CHCl_3$, cm^{-1}): ν_{max} 3687, 3189, 3022, 2403, 2356, 1645, 1523, 1469, 1422, 1216, 1037, 927, 770, 672;

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.37 (s, 3 H), 5.47 (s, 2 H), 7.20 (s, 1 H), 7.24 (s, 1 H), 7.42-7.51 (m, 2 H), 7.68-7.77 (m, 3 H), 8.08 (s, 1 H), 8.20 (s, 1 H), 8.24 (dd, *J* = 7.9, 1.6 Hz, 1 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 176.7 (CO), 156.5 (C), 155.6 (CH), 148.0 (C), 137.9 (C), 134.3 (C), 129.4 (CH, 2 carbons), 127.6 (C), 125.8 (CH), 125.8 (CH), 125.6 (CH, 2 carbons), 123.8 (C), 120.6 (CH), 119.3 (C), 118.4 (CH), 45.2 (CH_2), 21.2 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{19}H_{15}O_2N_3$ $[M+H]^+$ 318.1237, found 318.1240.

3) 3-((4-(4-ethylphenyl)-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6c)



6c

Yield: 82% (pale yellow solid);

MP: 149-150 °C;

Molecular Formula: $C_{20}H_{17}N_3O_2$;

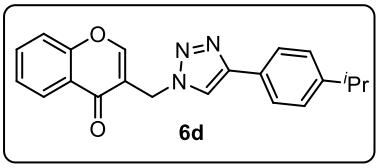
IR ($CHCl_3$, cm^{-1}): ν_{max} 3687, 3394, 3022, 2403, 1648, 1529, 1424, 1217, 1030, 927, 769, 672;

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.25 (t, *J* = 7.6 Hz, 3 H), 2.67 (q, *J* = 15.3, 7.6 Hz, 2 H), 5.47 (s, 2 H), 7.22 (s, 1 H), 7.26 (s, 1 H), 7.42-7.51 (m, 2 H), 7.68-7.76 (m, 3 H), 8.09 (s, 1 H), 8.20 (s, 1 H), 8.20 (s, 1 H), 8.24 (dd, *J* = 7.9, 1.6 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7 (CO), 156.5 (C), 155.6 (CH), 148.1 (C), 144.3 (C), 134.3 (CH), 128.2 (CH, 2 carbons), 127.9 (C), 125.8 (CH), 125.8 (CH), 125.7 (CH, 2 carbons), 123.8 (C), 120.7 (CH), 119.3 (C), 118.4 (CH), 45.2 (CH_2), 28.6 (CH_2), 15.5 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{20}H_{17}O_2N_3$ $[M+H]^+$ 332.1394, found 332.1401.

4) 3-((4-(4-propylphenyl)-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6d)



6d

Yield: 84% (colorless solid);

MP: 135-136 °C;

Molecular Formula: $C_{21}H_{19}N_3O_2$;

IR ($CHCl_3$, cm^{-1}): ν_{max} 3188, 3019, 2596, 2406, 1631, 1433, 1218, 1041, 768, 671;

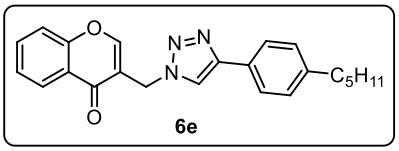
^{1}H NMR (200 MHz, $CDCl_3$): δ 0.94 (t, *J* = 7.3 Hz, 3 H), 1.56-1.75 (m, 4 H), 2.60 (t, *J* = 7.6

Hz, 2 H), 5.47 (s, 2 H), 7.20 (s, 1 H), 7.24 (s, 1 H), 7.42-7.51 (m, 2 H), 7.67-7.76 (m, 3 H), 8.08 (s, 1 H), 8.19 (s, 1 H), 8.23 (dd, *J* = 7.9, 1.6 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7 (CO), 156.5 (C), 155.6 (CH), 148.1 (C), 142.7 (C), 134.3 (CH), 128.8 (CH, 2 carbons), 127.9 (C), 125.8 (CH), 125.7 (CH), 125.6 (CH, 2 carbons), 123.8 (C), 120.7 (CH), 119.3 (C), 118.4 (CH), 45.2 (CH_2), 37.8 (CH_2), 24.4 (CH_2), 13.8 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{21}H_{19}O_2N_3$ $[M+H]^+$ 346.1550, found 346.1558.

5) 3-((4-(4-pentylphenyl)-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6e)



6e

Yield: 88% (colorless solid);

MP: 147-148 °C;

Molecular Formula: $C_{23}H_{23}N_3O_2$;

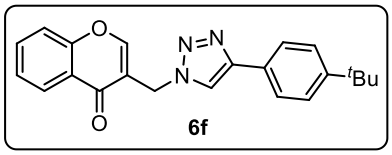
IR ($CHCl_3$, cm^{-1}): ν_{max} 3370, 3022, 2926, 2403, 1648, 1524, 1466, 1421, 1353, 1216, 1029, 927, 763, 670;

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (t, *J* = 6.7 Hz, 3 H), 1.29-1.36 (m, 4 H), 1.63-1.70 (m, 2 H), 2.58-2.66 (m, 2 H), 5.48 (s, 2 H), 7.20 (s, 1 H), 7.24 (s, 1 H), 7.42-7.51 (m, 2 H), 7.68-7.76 (m, 3 H), 8.08 (s, 1 H), 8.19 (s, 1 H), 8.25 (dd, *J* = 7.9, 1.6 Hz, 1H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7 (CO), 156.5 (C), 155.6 (CH), 148.1 (C), 143.0 (C), 134.3 (CH), 128.8 (CH, 2 carbons), 127.8 (C), 125.8 (CH), 125.7 (CH), 125.6 (CH, 2 carbons), 123.8 (C), 120.7 (CH), 119.3 (C), 118.4 (CH), 45.2 (CH_2), 35.6 (CH_2), 31.4 (CH_2), 31.0 (CH_2), 22.5 (CH_2), 14.0 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{23}H_{23}O_2N_3$ $[M+H]^+$ 374.1863, found 374.1868.

6) 3-((4-(4-(*tert*-butyl)phenyl)-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6f)



6f

Yield: 96% (colorless solid);

MP: 218-219 °C;

Molecular Formula: $C_{22}H_{21}N_3O_2$;

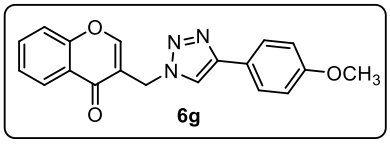
IR ($CHCl_3$, cm^{-1}): ν_{max} 3390, 3021, 2963, 2404, 1648, 1464, 1218, 1032, 927, 769, 673;

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.34 (s, 9 H), 5.48 (s, 2 H), 7.41-7.51 (m, 4 H), 7.68-7.78 (m, 3 H), 8.10 (s, 1 H), 8.20 (s, 1 H), 8.24 (dd, *J* = 7.9, 1.6 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.6 (CO), 156.5 (C), 155.6 (CH), 151.1 (C), 147.9 (C), 134.3 (CH), 127.7 (C), 125.8 (CH), 125.7 (CH), 125.6 (CH, 2 carbons), 125.4 (CH, 2 carbons), 123.8 (C), 120.7 (CH), 119.3 (C), 118.3 (CH), 45.2 (CH_2), 34.6 (C), 31.2 (CH_3, 3 carbons);

HRMS (ESI): *m/z* calculated for $C_{22}H_{21}O_2N_3$ $[M+H]^+$ 360.1707, found 360.1712.

7) 3-((4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6g)



Yield: 60% (colorless solid);

MP: 170-171 °C;

Molecular Formula: $C_{19}H_{15}N_3O_3$;

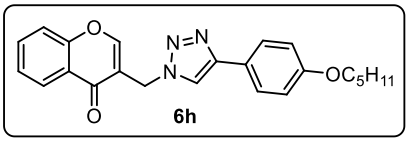
IR ($CHCl_3$, cm^{-1}): ν_{max} 3687, 3022, 2403, 2356, 1648, 1511, 1467, 1424, 1351, 1217, 1030, 927, 770, 672;

^{1}H NMR (200 MHz, $CDCl_3$): δ 3.84 (s, 3 H), 5.47 (s, 2 H), 6.92 (s, 1 H), 6.97 (s, 1 H), 7.42-7.51 (m, 2 H), 7.68-7.77 (m, 3 H), 8.04 (s, 1 H), 8.20 (s, 1 H), 8.22-8.27 (m, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.8 (CO), 159.5 (C), 156.5 (C), 155.7 (CH), 147.9 (C), 134.4 (CH), 127.0 (CH, 3 carbons), 125.8 (CH), 123.8 (C), 123.2 (C), 120.2 (CH), 119.3 (C), 118.3 (CH), 114.1 (CH, 2 carbons), 55.3 (CH_3), 45.2 (CH_2);

HRMS (ESI): *m/z* calculated for $C_{19}H_{15}O_3N_3$ $[M+H]^+$ 334.1186, found 334.1192.

8) 3-((4-(4-(pentyloxy)phenyl)-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6h)



Yield: 85% (colorless solid);

MP: 154-155 °C;

Molecular Formula: $C_{23}H_{23}N_3O_3$;

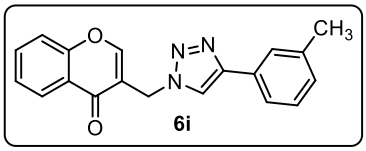
IR ($CHCl_3$, cm^{-1}): ν_{max} 3686, 3189, 3021, 2953, 2403, 1647, 1466, 1418, 1352, 1310, 1217, 1039, 926, 768, 671;

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.94 (t, *J* = 6.9 Hz, 3 H), 1.38-1.50 (m, 4 H), 1.77-1.86 (m, 2 H), 3.98 (t, *J* = 6.6 Hz, 2 H), 5.47 (s, 2 H), 6.91 (s, 1 H), 6.95 (s, 1 H), 7.42-7.51 (m, 2 H), 7.68-7.76 (m, 3 H), 8.03 (s, 1 H), 8.19 (s, 1 H), 8.24 (dd, *J* = 7.9, 1.6 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7 (CO), 159.1 (C), 156.5 (C), 155.6 (CH), 147.9 (C), 134.3 (CH), 127.0 (CH, 2 carbons), 125.8 (CH), 125.7 (CH), 123.8 (C), 123.0 (C), 120.1 (CH), 119.4 (C), 118.4 (CH), 114.7 (CH, 2 carbons), 68.0 (CH_2), 45.2 (CH_2), 28.9 (CH_2), 28.1 (CH_2), 22.5 (CH_2), 14.0 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{23}H_{23}O_3N_3$ $[M+H]^+$ 390.1812, found 390.1821.

9) 3-((4-(*m*-tolyl)-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6i)



6i

Yield: 85% (pale yellow solid);

MP: 124-125 °C;

Molecular Formula: $C_{19}H_{15}N_3O_2$;

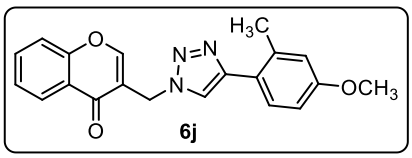
IR ($CHCl_3$, cm^{-1}): ν_{max} 3685, 3190, 3021, 2403, 1646, 1523, 1468, 1418, 1352, 1217, 1043, 926, 767, 671;

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.37 (s, 3 H), 5.45 (s, 2 H), 7.09-7.13 (m, 1 H), 7.23-7.31 (m, 1 H), 7.39-7.49 (m, 2 H), 7.57-7.74 (m, 3 H), 8.09 (s, 1 H), 8.18-8.24 (m, 2 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7 (CO), 156.4 (C), 155.6 (CH), 148.0 (C), 138.4 (C), 134.3 (CH), 130.3 (C), 128.8 (CH), 128.6 (CH), 126.3 (CH), 125.7 (CH, 2 carbons), 123.7 (C), 122.8 (CH), 120.9 (CH), 119.2 (C), 118.3 (CH), 45.2 (CH_2), 21.3 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{19}H_{15}O_2N_3$ $[M+H]^+$ 318.1237, found 318.1245.

10)3-((4-(4-methoxy-2-methylphenyl)-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6j)



6j

Yield: 60% (pale yellow solid);

MP: 172-173 °C;

Molecular Formula: $C_{20}H_{17}N_3O_3$;

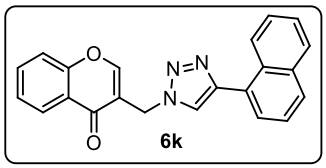
IR ($CHCl_3$, cm^{-1}): ν_{max} 3686, 3392, 3022, 2403, 1648, 1473, 1425, 1217, 1033, 927, 769, 672;

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.45 (s, 3 H), 3.82 (s, 3 H), 5.49 (s, 2 H), 6.79-6.83 (m, 2 H), 7.42-7.52 (m, 3 H), 7.66-7.77 (m, 2 H), 7.99 (s, 1 H), 8.21-8.25 (m, 2 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7 (CO), 159.3 (C), 156.5 (C), 155.6 (CH), 147.1 (C), 137.1 (C), 134.3 (CH), 130.1 (CH), 125.8 (CH), 125.7 (CH), 123.8 (C), 122.6 (CH), 119.4 (C, 2 carbons), 118.3 (CH), 116.1 (CH), 111.3 (CH), 55.2 (OCH_3), 45.1 (CH_2), 21.5 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{20}H_{17}O_3N_3$ $[M+H]^+$ 348.1343, found 348.1353.

11) 3-((4-(naphthalen-1-yl)-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6k)



Yield: 93% (brick red solid);

MP: 154-155 °C;

Molecular Formula: $C_{22}H_{15}N_3O_2$;

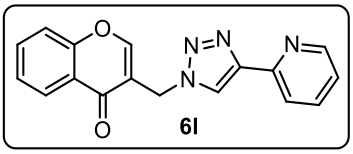
IR ($CHCl_3$, cm^{-1}): ν_{max} 3687, 3189, 3022, 2403, 2355, 1643, 1523, 1472, 1424, 1216, 1038, 928, 770, 672;

^{1}H NMR (200 MHz, $CDCl_3$): δ 5.56 (s, 2 H), 7.42-7.56 (m, 6 H), 7.68-7.77 (m, 2 H), 7.86-7.91 (m, 2 H), 8.22-8.26 (m, 2 H), 8.28 (s, 1 H), 8.36-8.41 (m, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7 (CO), 156.5 (C), 155.7 (CH), 147.0 (C), 134.3 (CH), 133.8 (C), 130.9 (C), 128.8 (CH), 128.3 (CH), 127.9 (C), 127.2 (CH), 126.6 (CH), 125.9 (CH), 125.8 (CH), 125.7 (CH), 125.4 (CH), 125.3 (CH), 123.9 (CH), 123.8 (C), 119.2 (C), 118.3 (CH), 45.3 (CH_2);

HRMS (ESI): *m/z* calculated for $C_{22}H_{15}O_2N_3$ $[M+H]^+$ 354.1237, found 354.1246.

12) 3-((4-(pyridin-2-yl)-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6l)



Yield: 60% (greenish solid);

MP: 175-176 °C;

Molecular Formula: $C_{17}H_{12}N_4O_2$;

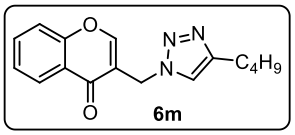
IR ($CHCl_3$, cm^{-1}): ν_{max} 3686, 3189, 3022, 2403, 2355, 1648, 1523, 1469, 1420, 1352, 1217, 1040, 927, 770, 672;

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.52 (s, 2 H), 7.32 (s, 1 H), 7.43-7.50 (m, 2 H), 7.69-7.73 (m, 1 H), 7.84-7.93 (m, 1 H), 8.16 (s, 1 H), 8.23 (dd, *J* = 8.0, 1.6 Hz, 2 H), 8.60 (s, 1H), 8.71 (s, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.4 (CO), 156.5 (C), 155.4 (CH), 150.1 (C), 149.2 (C), 148.4 (C), 136.9 (CH), 134.3 (CH), 125.9 (CH), 125.8 (CH), 123.8 (CH), 123.3 (CH), 122.8 (CH), 120.3 (C), 119.1 (CH), 118.2 (CH), 45.4 (CH_2);

HRMS (ESI): *m/z* calculated for $C_{17}H_{12}O_2N_4$ $[M+H]^+$ 305.1033, found 305.1038.

13) 3-((4-butyl-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6m)



Yield: 93% (colorless solid);

MP: 87-88 °C;

Molecular Formula: $C_{16}H_{17}N_3O_2$;

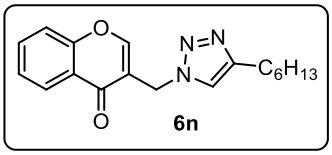
IR ($CHCl_3$, cm^{-1}): ν_{max} 3686, 2412, 3022, 2963, 2403, 1648, 1529, 1468, 1424, 1350, 1217, 1032, 927, 769, 672;

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.92 (t, *J* = 7.1 Hz, 3 H), 1.26-1.45 (m, 2 H), 1.57-1.74 (m, 2 H), 2.75 (t, *J* = 7.2 Hz, 2 H), 5.44 (s, 2 H), 7.42-7.52 (m, 2 H), 7.69-7.77 (m, 2 H), 8.17-8.26 (m, 2 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7 (CO), 156.5 (C), 155.5 (CH), 148.7 (C), 134.3 (CH), 125.8 (CH), 125.7 (CH), 123.8 (C), 121.9 (CH), 119.5 (C), 118.3 (CH), 44.9 (CH_2), 31.5 (CH_2), 25.3 (CH_2), 22.3 (CH_2), 13.8 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{16}H_{17}O_2N_3$ $[M+H]^+$ 284.1394, found 284.1396.

14) 3-((4-hexyl-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6n)



Yield: 82% (colorless solid);

MP: 84-85 °C;

Molecular Formula: $C_{18}H_{21}N_3O_2$;

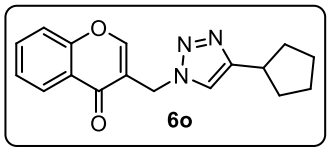
IR ($CHCl_3$, cm^{-1}): ν_{max} 3414, 3022, 2404, 1647, 1433, 1218, 1030, 928, 769, 673;

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, *J* = 6.9 Hz, 3 H), 1.26-1.37 (m, 6 H), 1.61-1.68 (m, 2 H), 2.67-2.71 (m, 2 H), 5.40 (s, 2 H), 7.43-7.49 (m, 2 H), 7.64 (s, 1 H), 7.69-7.73 (m, 1 H), 8.15 (s, 1 H), 8.22 (dd, *J* = 8.0, 1.6 Hz, 1 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 176.7 (CO), 156.5 (C), 155.6 (CH), 148.4 (C), 134.3 (CH), 125.8 (CH), 125.7 (CH), 123.8 (C), 122.2 (CH), 119.3 (C), 118.3 (CH), 45.2 (CH_2), 31.5 (CH_2), 29.3 (CH_2), 28.9 (CH_2), 25.5 (CH_2), 22.5 (CH_2), 14.0 (CH_3);

HRMS (ESI): *m/z* calculated for $C_{18}H_{21}O_2N_3$ $[M+H]^+$ 312.1707, found 312.1711.

15) 3-((4-cyclopentyl-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6o)



Yield: 88% (pale yellow solid);

MP: 157-158 °C;

Molecular Formula: $C_{17}H_{17}N_3O_2$;

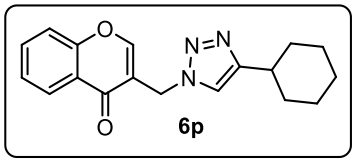
IR ($CHCl_3$, cm^{-1}): ν_{max} 3190, 3010, 1630, 1450, 1220, 1040, 770, 670;

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.64-1.81 (m, 7 H), 2.03-2.13 (m, 2 H), 3.09-3.22 (m, 1 H), 5.39 (s, 2 H), 7.41-7.50 (m, 2 H), 7.58 (s, 1 H), 7.67-7.76 (m, 1 H), 8.14 (s, 1 H), 8.23 (dd, *J* = 7.9, 1.7 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7 (CO), 156.5 (C), 155.6 (CH), 152.9 (C), 134.3 (CH), 125.8 (CH), 125.7 (CH), 123.8 (C), 120.9 (CH), 119.4 (C), 118.3 (CH), 44.9 (CH_2), 36.7 (CH), 33.1 (CH_2, 2 carbons), 25.1 (CH_2, 2 carbons);

HRMS (ESI): *m/z* calculated for $C_{17}H_{17}O_2N_3$ $[M+H]^+$ 296.1394, found 296.1398.

16) 3-((4-cyclohexyl-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6p)



Yield: 80% (pale yellow solid);

MP: 145-146 °C;

Molecular Formula: $C_{18}H_{19}N_3O_2$;

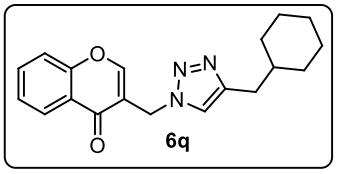
IR ($CHCl_3$, cm^{-1}): ν_{max} 3188, 3020, 1632, 1433, 1218, 1042, 768, 670;

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.28-1.49 (m, 5 H), 1.70-1.85 (m, 3 H), 1.96-2.07 (m, 2 H), 2.64-2.80 (m, 1 H), 5.38 (s, 2 H), 7.41-7.50 (m, 2 H), 7.56 (s, 1 H), 7.67-7.75 (m, 1 H), 8.12 (s, 1 H), 8.23 (dd, *J* = 7.9, 1.6 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7 (CO), 156.5 (C), 155.6 (CH), 153.9 (C), 134.2 (CH), 125.8 (CH), 125.7 (CH), 123.8 (C), 120.6 (CH), 119.4 (C), 118.3 (CH), 44.9 (CH_2), 35.3 (CH), 32.9 (CH_2, 2 carbons), 26.1 (CH_2, 2 carbons), 25.9 (CH_2);

HRMS (ESI): *m/z* calculated for $C_{18}H_{19}O_2N_3$ $[M+H]^+$ 310.1550, found 310.1557.

17) 3-((4-(cyclohexylmethyl)-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6q)



Yield: 88% (colorless solid);

MP: 133-134 °C;

Molecular Formula: $C_{19}H_{21}N_3O_2$;

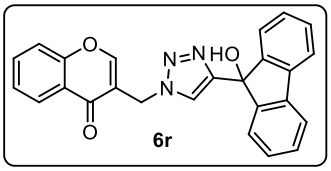
IR ($CHCl_3$, cm^{-1}): ν_{max} 3686, 3189, 3021, 2928, 2853, 2403, 1647, 1524, 1465, 1417, 1350, 1216, 1043, 926, 768, 671;

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89-1.03 (m, 2 H), 1.14-1.28 (m, 3 H), 1.60-1.72 (m, 6 H), 2.56 (d, *J* = 6.7 Hz, 2 H), 5.39 (s, 2 H), 7.41-7.51 (m, 2 H), 7.59 (s, 1 H) 7.67-7.76 (m, 1 H), 8.13 (s, 1 H), 8.23 (dd, *J* = 8.0, 1.6 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7 (CO), 156.5 (C), 155.5 (CH), 147.2 (C), 134.3 (CH), 125.8 (CH), 125.7 (CH), 123.8 (C), 122.5 (CH), 119.5 (C), 118.3 (CH), 44.9 (CH_2), 38.0 (CH), 33.4 (CH_2), 33.0 (CH_2, 2 carbons), 26.4 (CH_2), 26.1 (CH_2, 2 carbons);

HRMS (ESI): *m/z* calculated for $C_{19}H_{21}O_2N_3$ $[M+H]^+$ 324.1707, found 324.1710.

18) 3-((4-(9-hydroxy-9*H*-fluoren-9-yl)-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6r)



Yield: 58% (pale yellow solid);

MP: 239-240 °C;

Molecular Formula: $C_{25}H_{17}N_3O_3$;

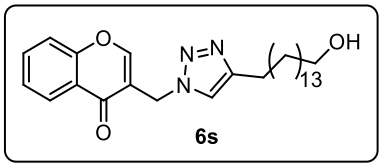
IR ($CHCl_3$, cm^{-1}): ν_{max} 3687, 3188, 3022, 2403, 1645, 1522, 1467, 1421, 1216, 1043, 926, 769, 671;

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.74 (bs, 1 H), 5.35 (s, 2 H), 7.30 (s, 1 H), 7.33-7.53 (m, 6 H), 7.61-7.74 (m, 5 H), 8.13 (s, 1 H), 8.14-8.19 (m, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.5 (CO), 156.5 (C), 155.7 (CH), 147.6 (C, 2 carbons), 139.6 (C, 2 carbons), 134.3 (CH), 129.7 (C), 129.5 (CH, 2 carbons), 128.5 (C), 128.3 (CH, 2 carbons), 125.9 (CH), 125.7 (CH), 124.9 (CH), 123.7 (C), 120.3 (CH, 2 carbons), 120.2 (CH, 2 carbons), 119.0 (C), 118.3 (CH), 45.0 (CH_2).

HRMS (ESI): *m/z* calculated for $C_{25}H_{17}O_3N_3$ $[M+H]^+$ 408.1343, found 408.1349.

19) 3-((4-(15-hydroxypentadecyl)-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6s)



Yield: 84% (pale yellow solid);

MP: 123-124 °C;

Molecular Formula: $C_{27}H_{39}N_3O_3$;

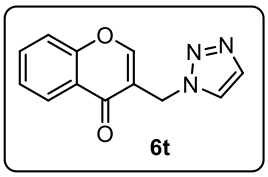
IR ($CHCl_3$, cm^{-1}): ν_{max} 3688, 3391, 3023, 2930, 2403, 2354, 1648, 1524, 1427, 1216, 1026, 928, 768, 671;

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.25-1.37 (m, 22 H), 1.57-1.72 (m, 4 H), 2.68 (t, *J* = 6.57 Hz, 2 H), 3.65 (t, *J* = 6.6 Hz, 2 H), 5.39 (s, 2 H), 7.41-7.51 (m, 2 H), 7.61 (s, 1 H), 7.68-7.76 (m, 1 H), 8.13 (s, 1 H), 8.23 (dd, *J* = 7.9, 1.6 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7 (CO), 156.5 (C), 155.5 (CH), 148.7 (C), 134.3 (CH), 125.8 (CH), 125.7 (CH), 123.8 (C), 121.9 (CH), 119.5 (C), 118.4 (CH), 63.0 (CH_2), 44.9 (CH_2), 32.8 (CH_2), 29.6 (CH_2, 7 carbons), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 25.7 (CH_2), 25.6 (CH_2);

HRMS (ESI): *m/z* calculated for $C_{27}H_{39}O_3N_3$ $[M+H]^+$ 454.3064, found 454.3074.

20) 2-((1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-chromen-4-one (6t)



6t

Yield: 38% (pale yellow solid);

MP: 117-118 °C;

Molecular Formula: $C_{12}H_9N_3O_2$;

IR ($CHCl_3$, cm^{-1}): ν_{max} 3687, 3412, 3022, 2403, 2356, 1649, 1523, 1470, 1421, 1216, 1069, 1025, 927, 770, 672;

^{1}H NMR (200 MHz, $CDCl_3$): δ 5.47 (s, 2 H), 7.42-7.51 (m, 2 H), 7.69-7.76 (m, 2 H), 7.92 (s, 1 H), 8.16 (s, 1 H), 8.23 (dd, *J* = 7.9, 1.6 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7 (CO), 156.5 (C), 155.5 (CH), 134.3 (CH), 133.9 (CH), 125.8 (CH, 2 carbons), 124.8 (CH), 123.8 (C), 119.3 (C), 118.4 (CH), 45.0 (CH_2);

HRMS (ESI): *m/z* calculated for $C_{12}H_9O_2N_3$ $[M+H]^+$ 228.0768, found 228.0771.

***In-vitro* MTB MABA assay**

Briefly, the inoculum was prepared from fresh LJ medium re-suspended in 7H9-S medium (7H9 broth, 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase [OADC]), adjusted to a McFarland tube No. 1, and diluted 1:20; 100 µl was used as inoculum. Each drug stock solution was thawed and diluted in 7H9-S at four-fold the final highest concentration tested. Serial two-fold dilutions of each drug were prepared directly in a sterile 96-well microtiter plate using 100 µl 7H9-S. A growth control containing no antibiotic and a sterile control was also prepared on each plate. Sterile water was added to

all perimeter wells to avoid evaporation during the incubation. The plate was covered, sealed in plastic bags and incubated at 37 ^{o}C in normal atmosphere. After 7 days incubation, 30 ml of Alamar blue solution was added to each well, and the plate was re-incubated overnight. A change in color from blue (oxidized state) to pink (reduced) indicated the growth of bacteria, and the MIC was defined as the lowest concentration of drug that prevented this change in color.

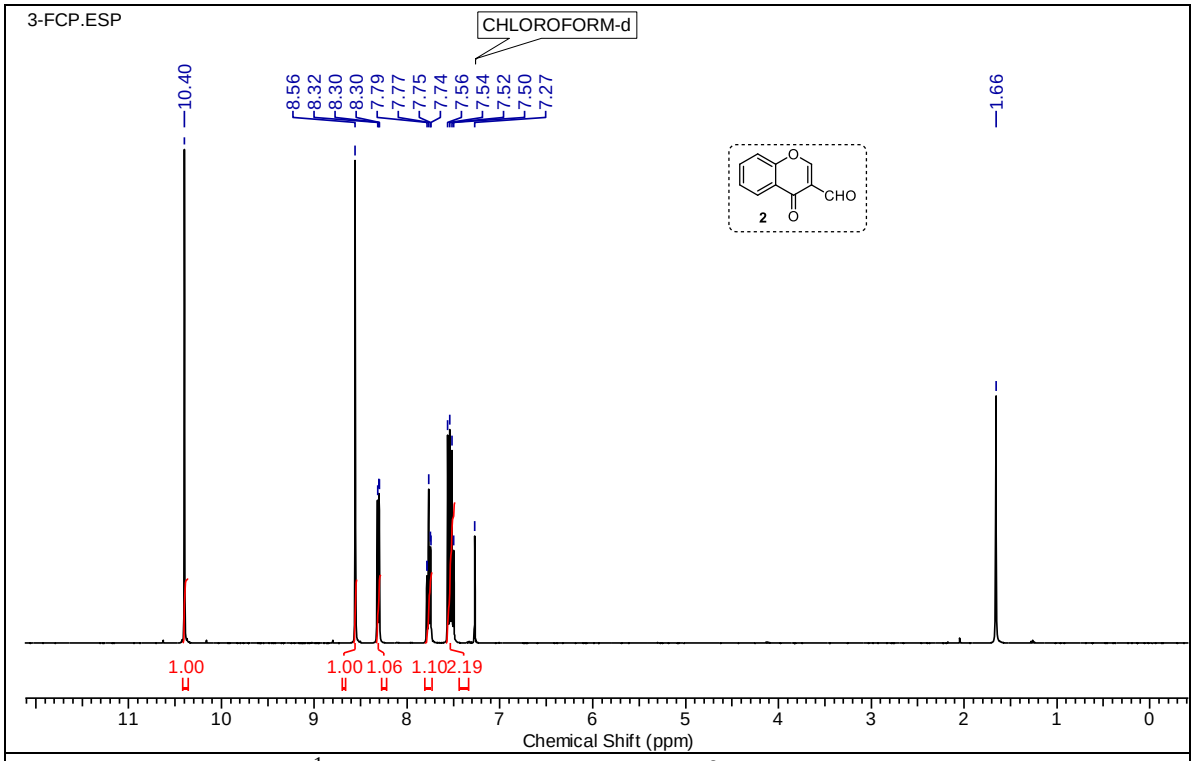
***In-vitro* cytotoxicity screening**

Some compounds were further examined for toxicity in a RAW 264.7 cell line at the concentration of 50 µM.

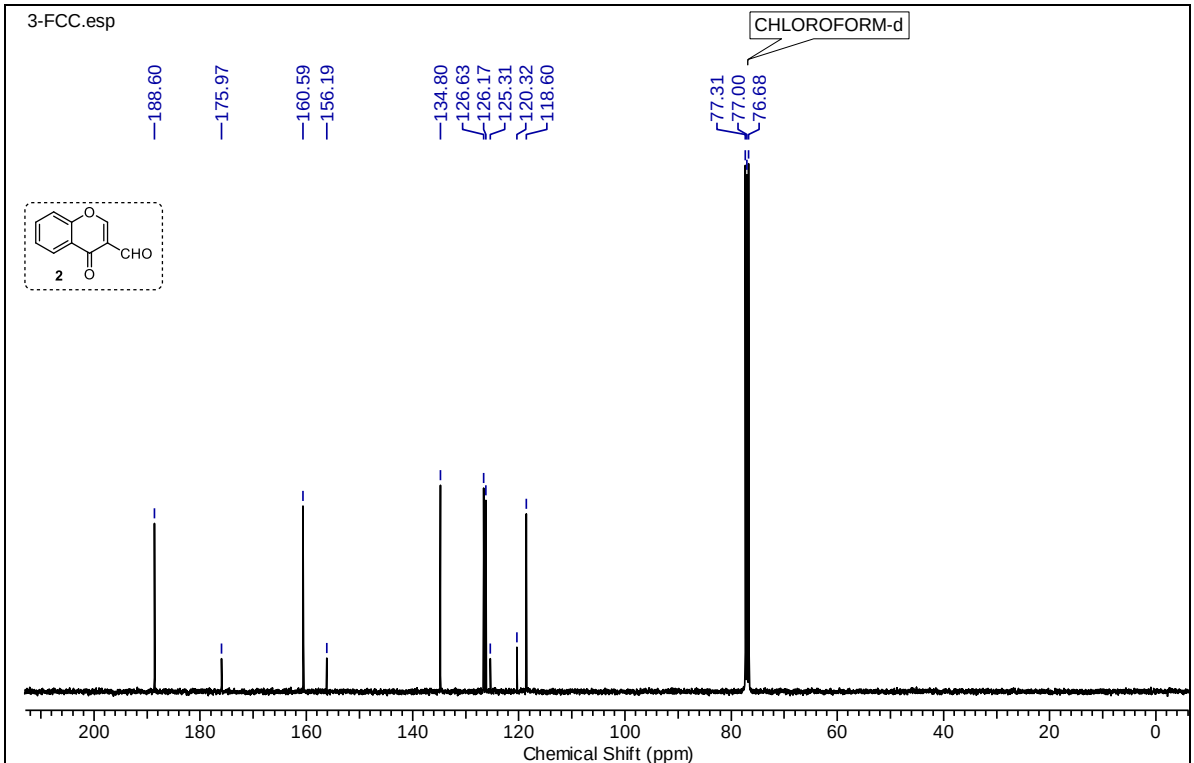
	MIC in µM
Rifampicin	0.24
Ethambutol	7.64

After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay.

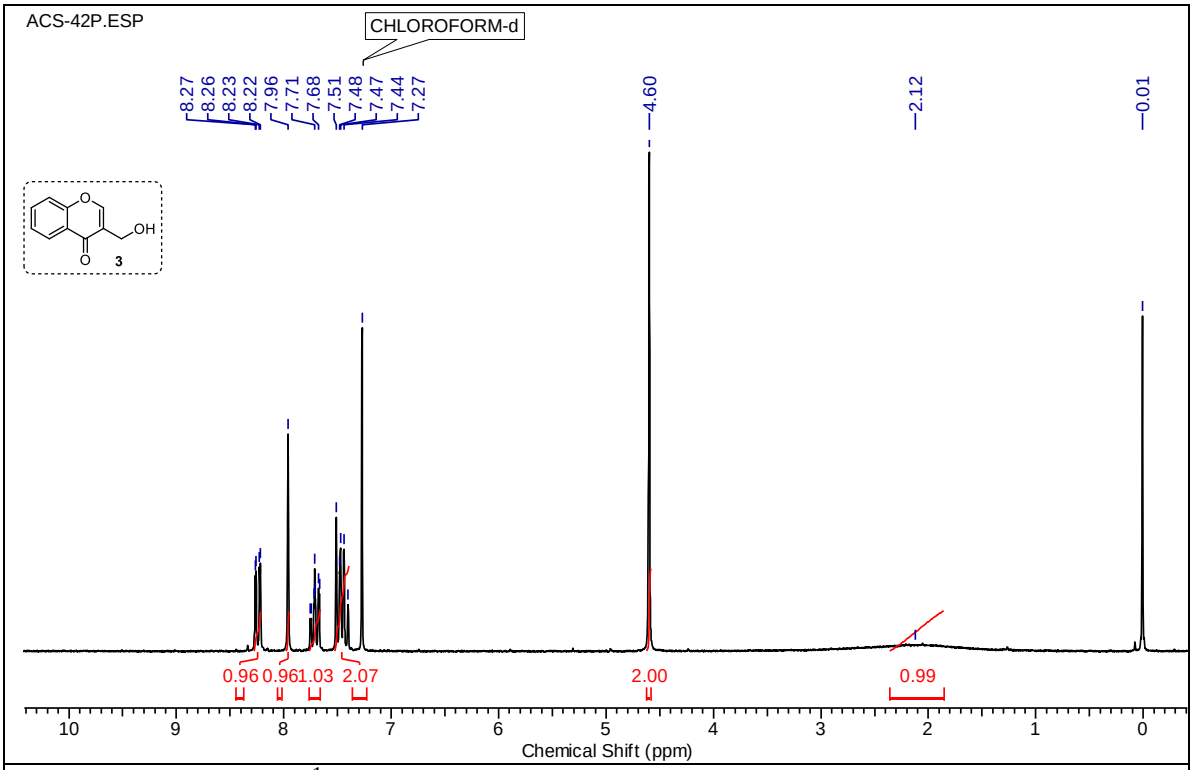
3.1.6. Spectra



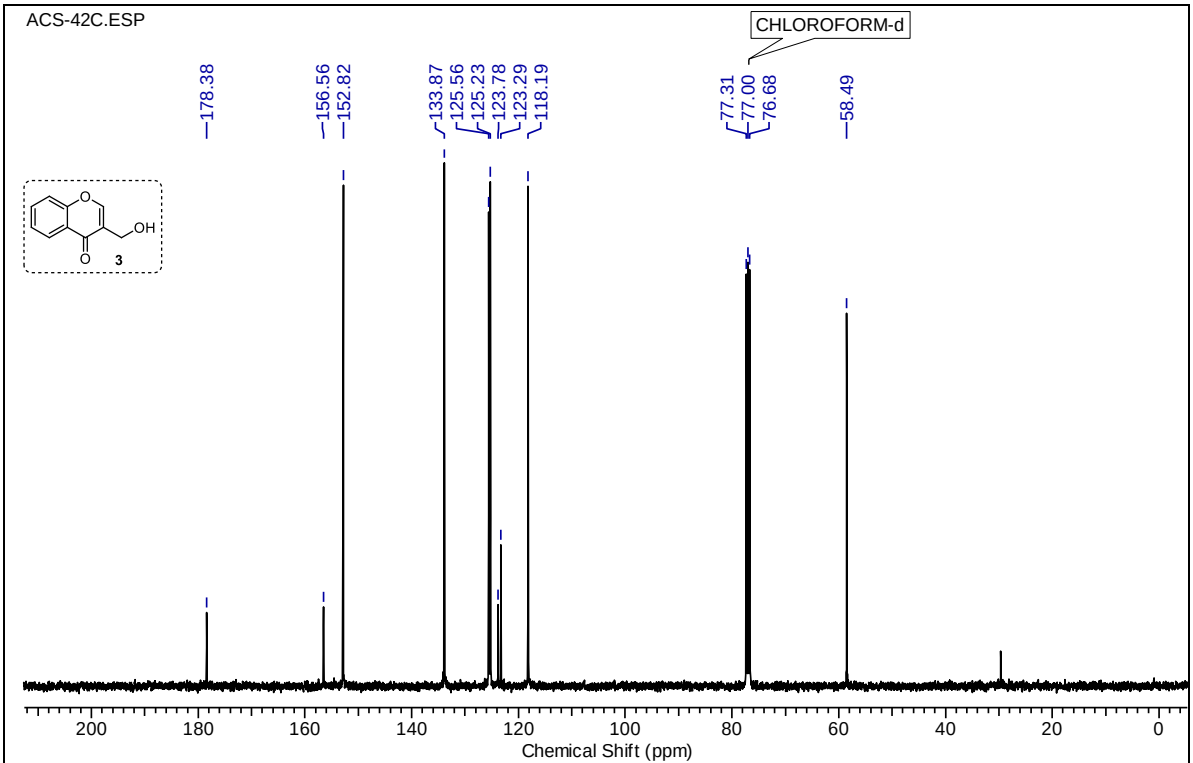
^{1}H NMR (400 MHz, $CDCl_3$) of compound **2**



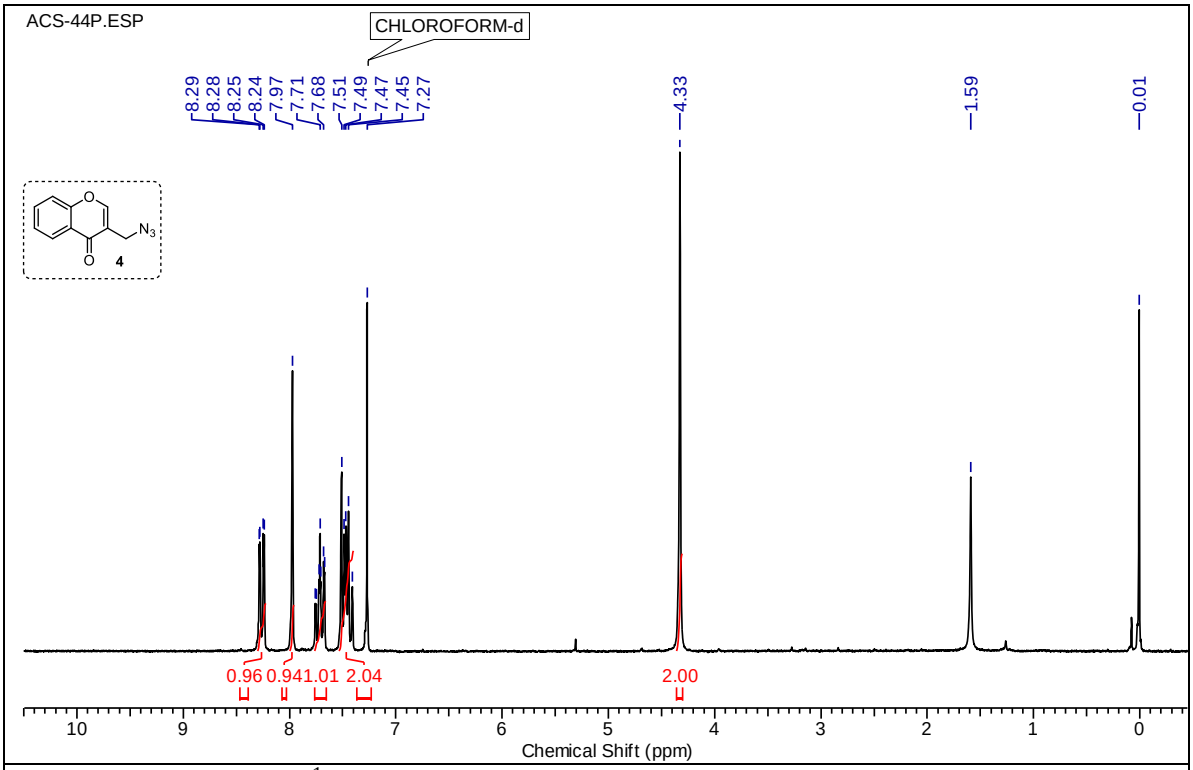
^{13}C NMR (100 MHz, $CDCl_3$) of compound **2**



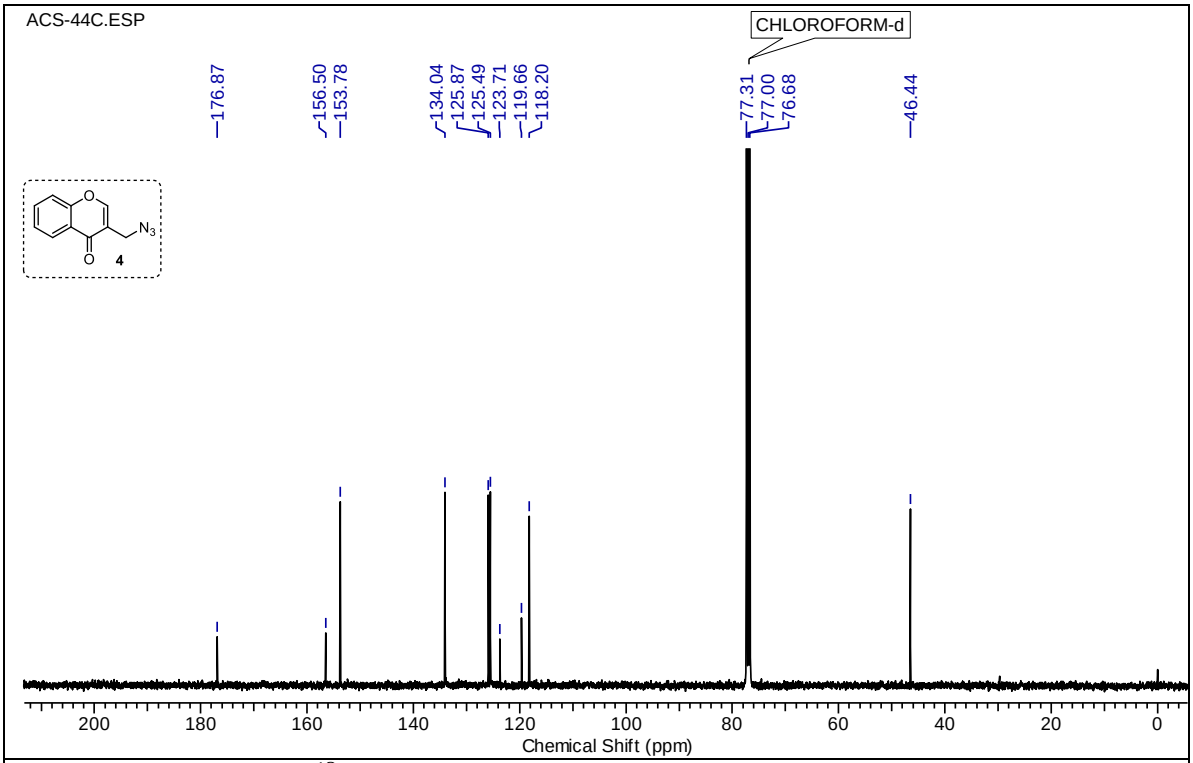
^{1}H NMR (200 MHz, $CDCl_3$) of compound **3**



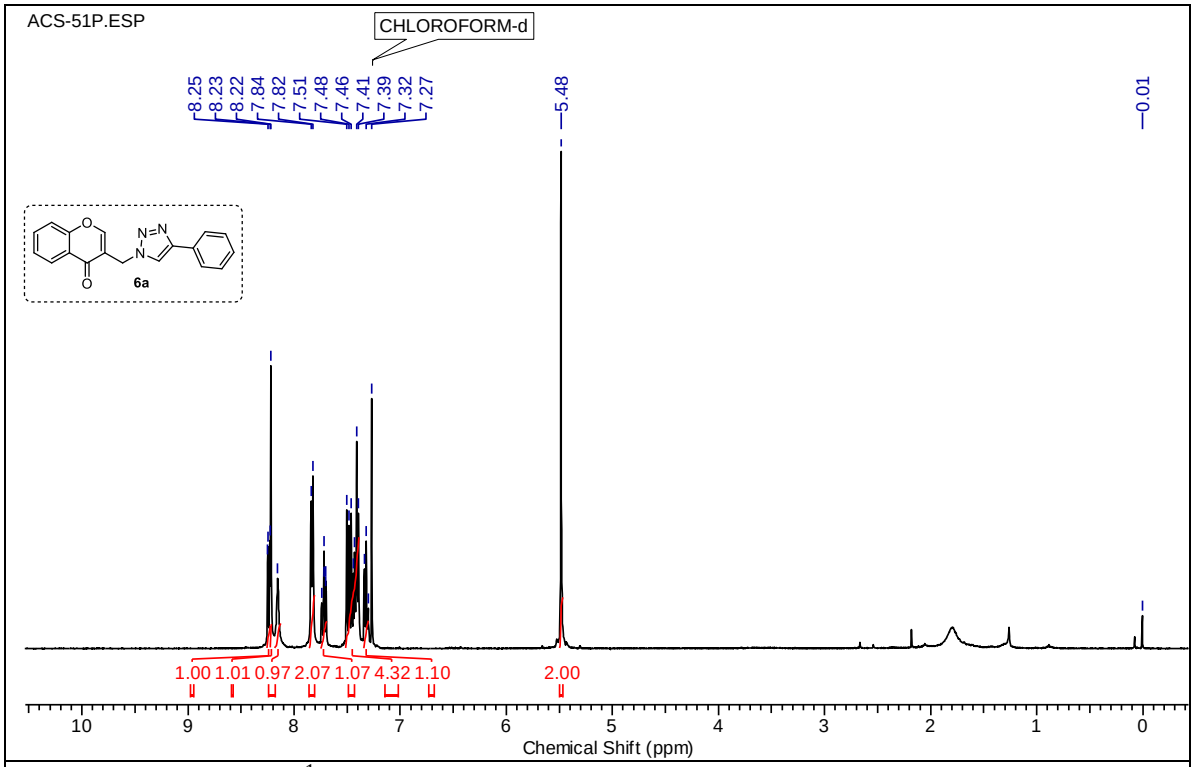
^{13}C NMR (100 MHz, $CDCl_3$) of compound **3**



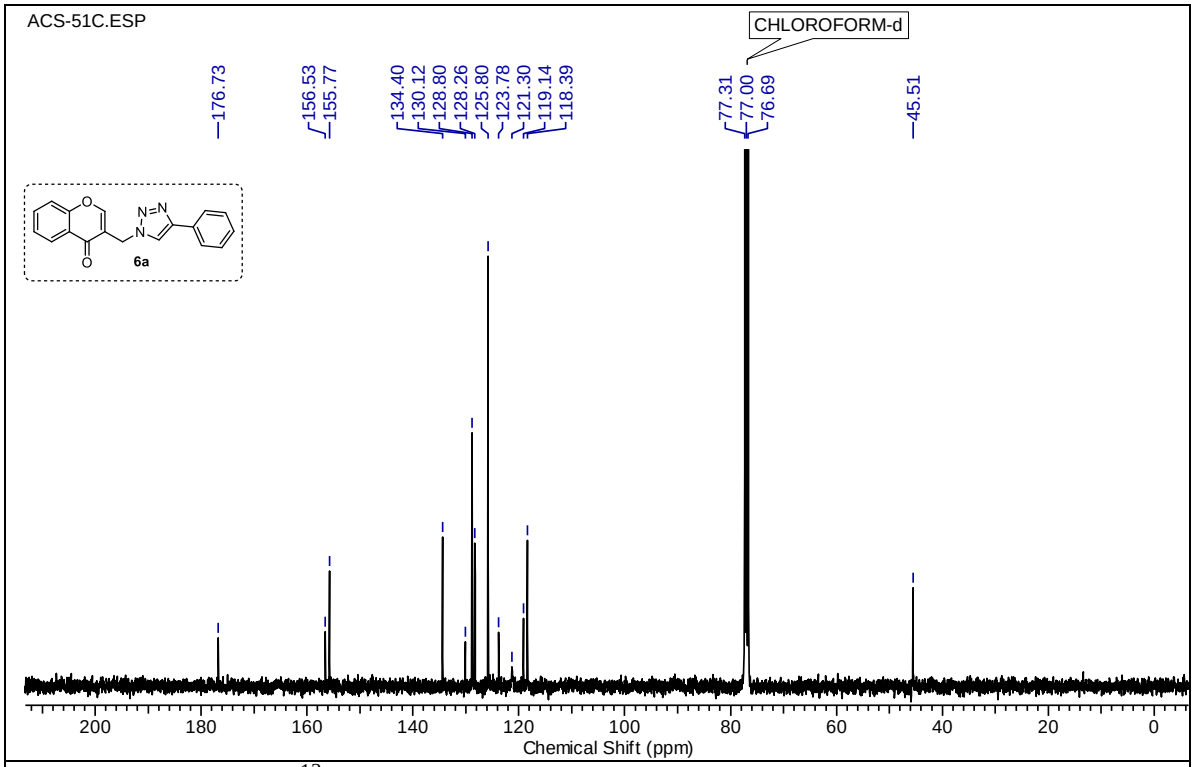
^{1}H NMR (200 MHz, $CDCl_3$) of compound **4**



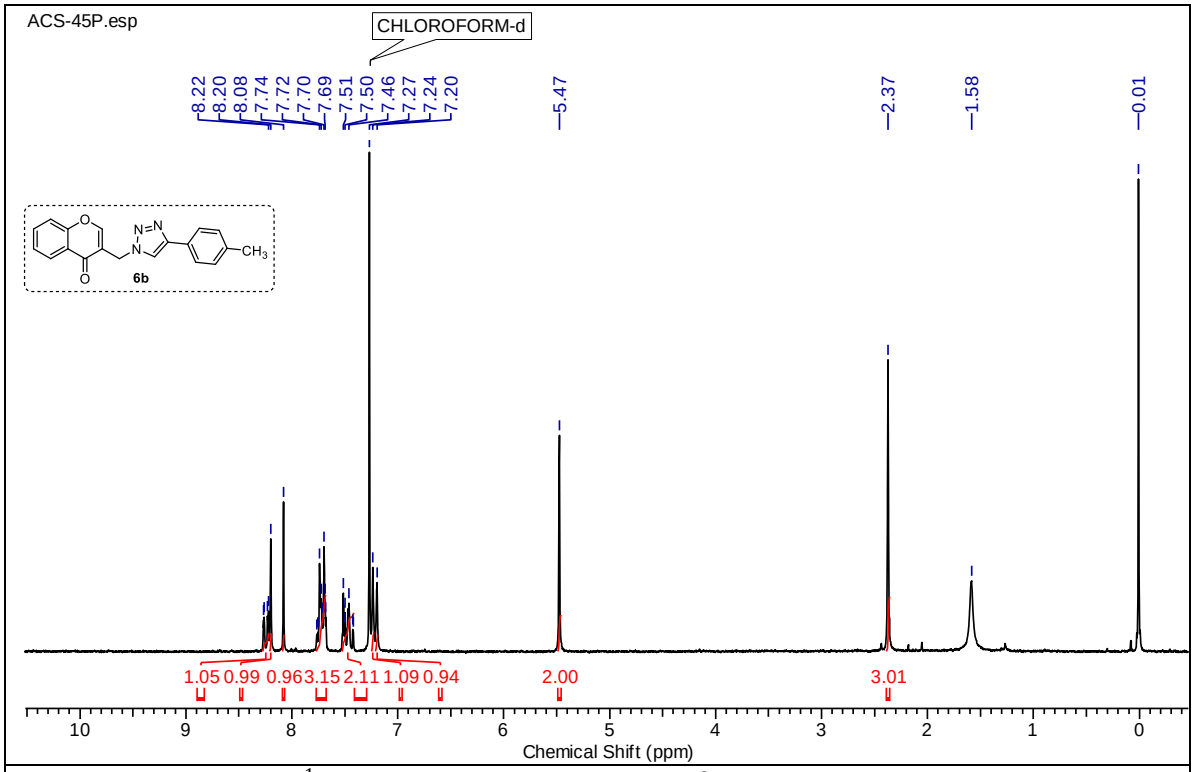
^{13}C NMR (50 MHz, $CDCl_3$) of compound **4**



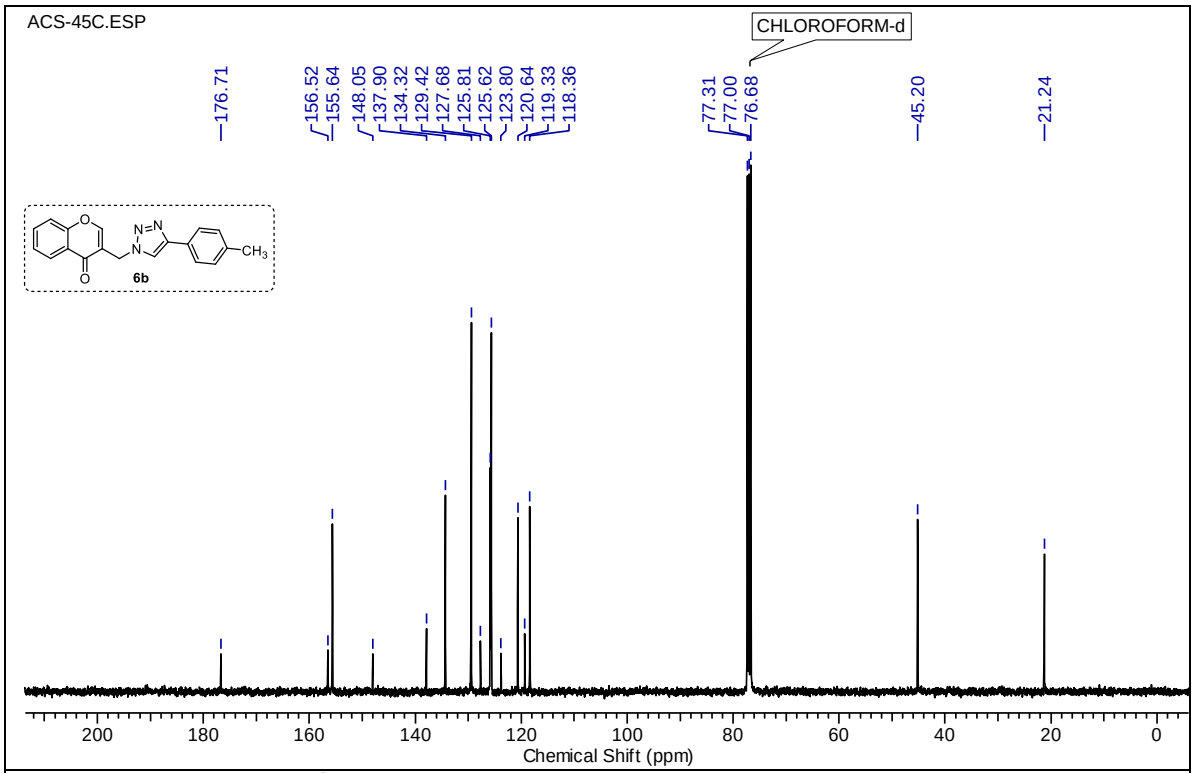
^{1}H NMR (400 MHz, $CDCl_3$) of compound **6a**



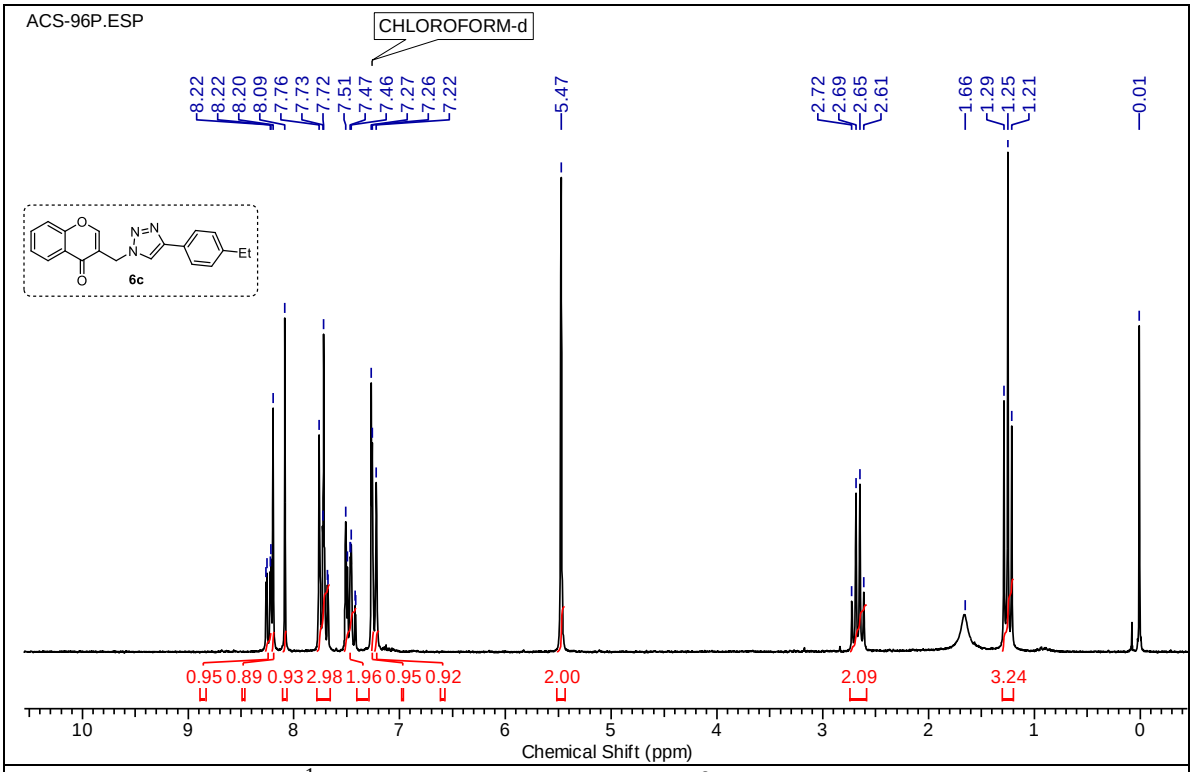
^{13}C NMR (100 MHz, $CDCl_3$) of compound **6a**



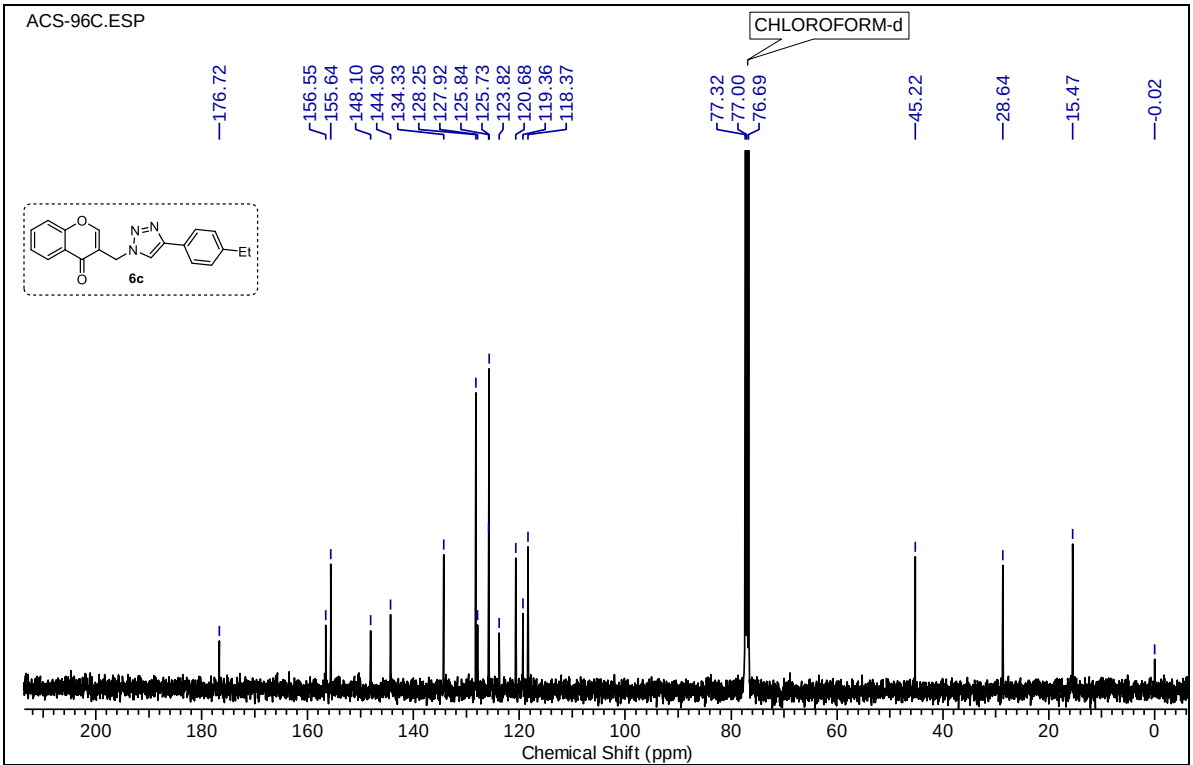
^{1}H NMR (200 MHz, $CDCl_3$) of compound **6b**



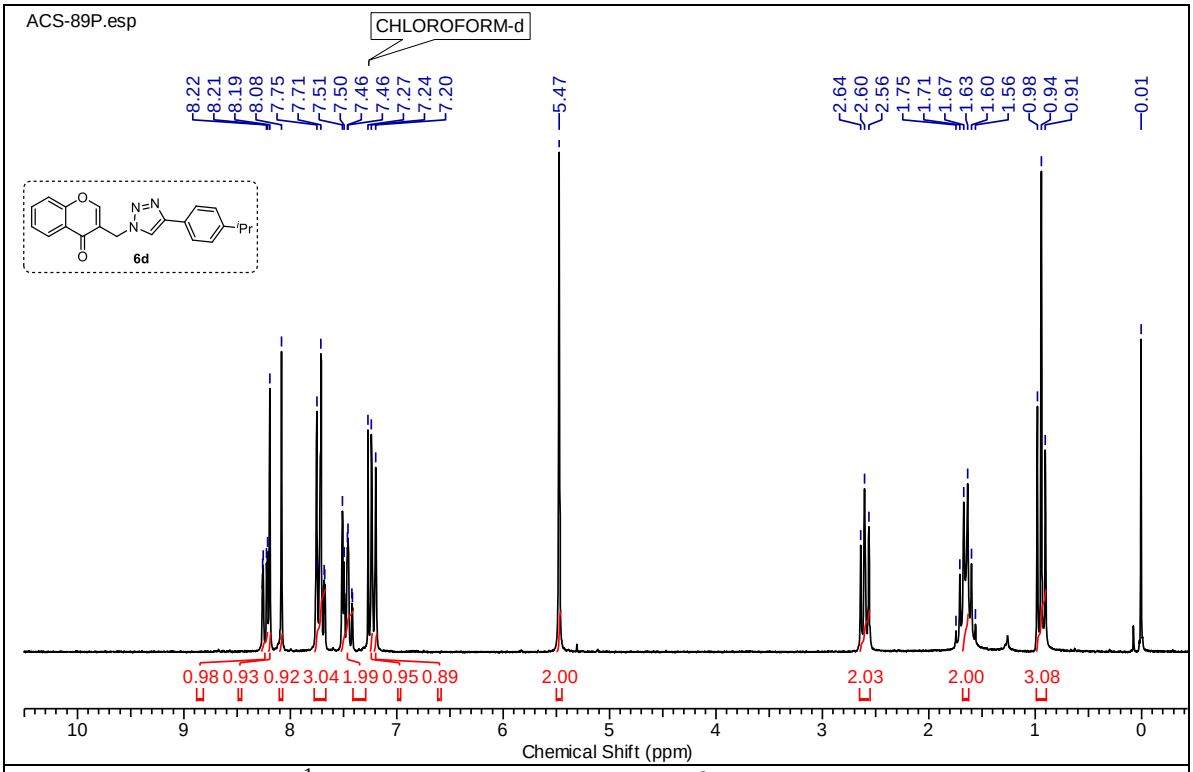
^{13}C NMR (50 MHz, $CDCl_3$) of compound **6b**



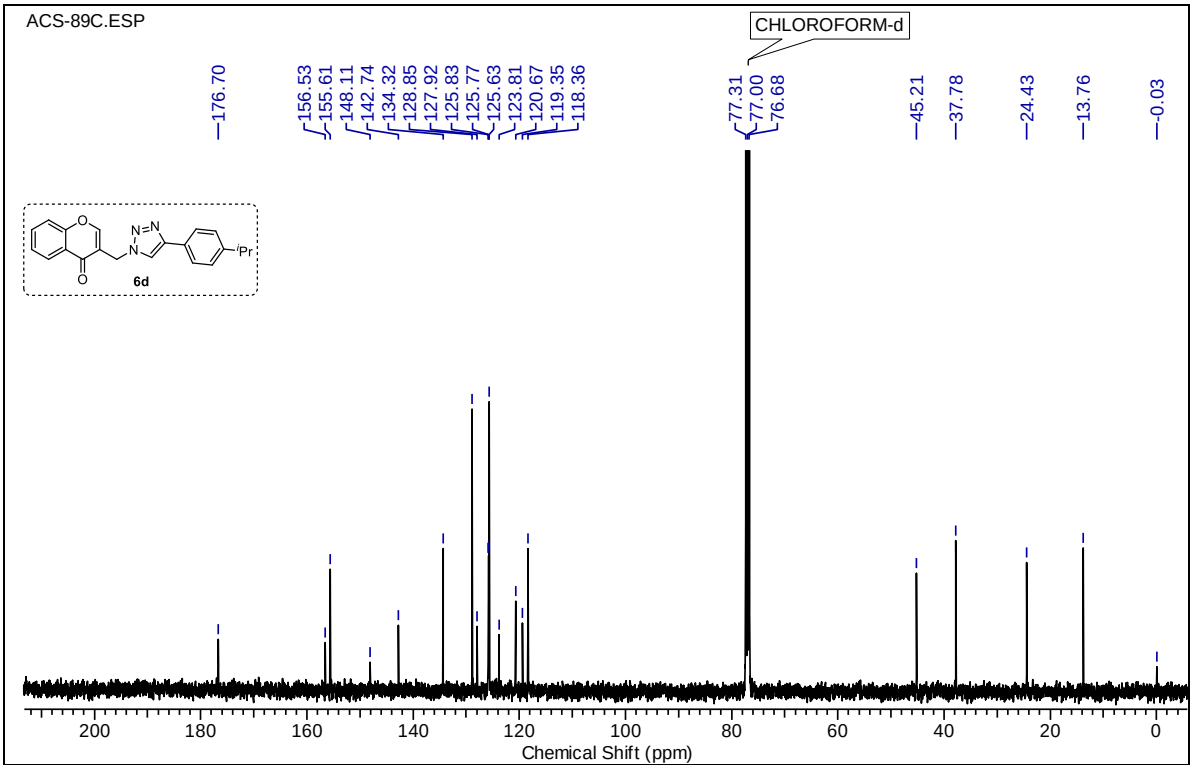
^{1}H NMR (200 MHz, $CDCl_3$) of compound **6c**



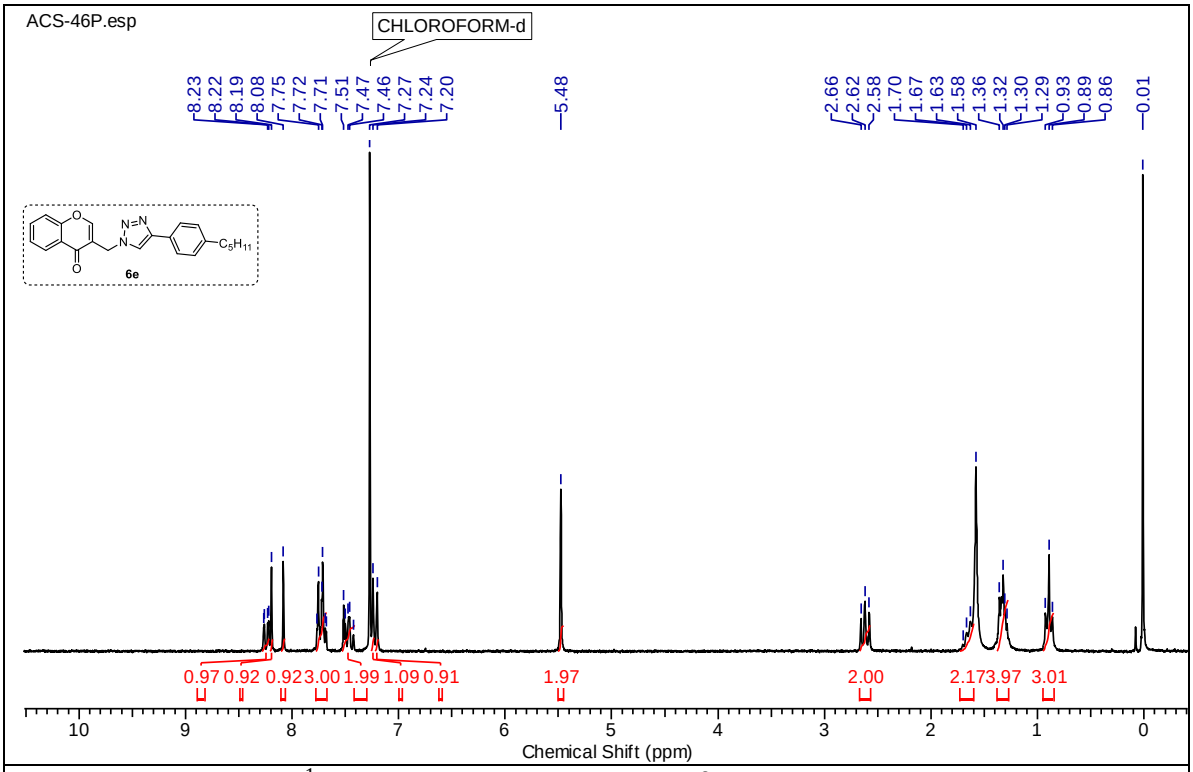
^{13}C NMR (100 MHz, $CDCl_3$) of compound **6c**



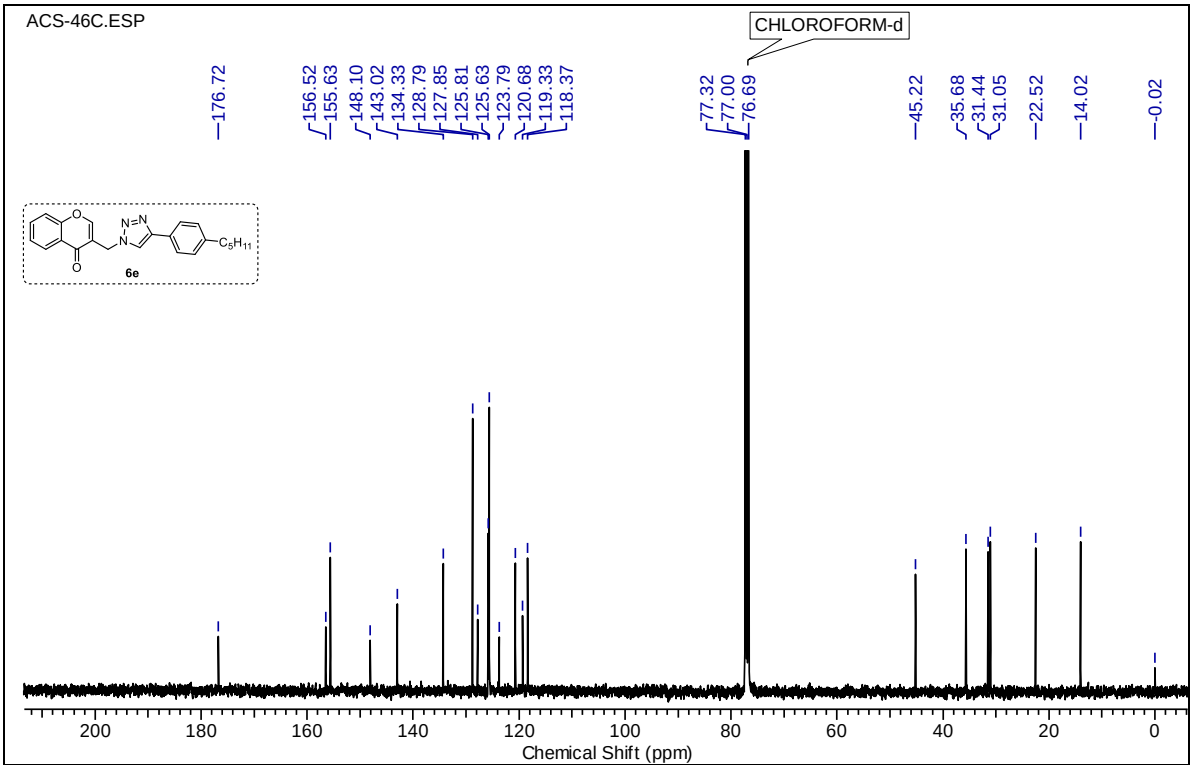
^{1}H NMR (200 MHz, $CDCl_3$) of compound **6d**



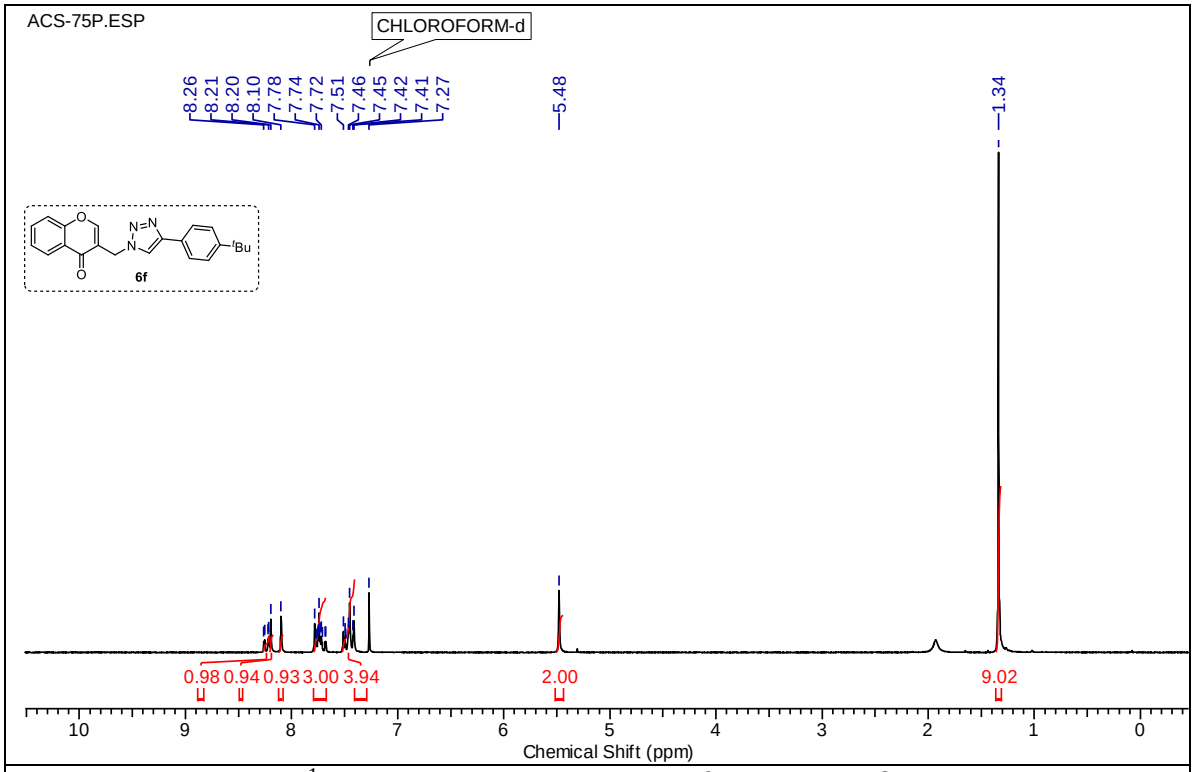
^{13}C NMR (100 MHz, $CDCl_3$) of compound **6d**



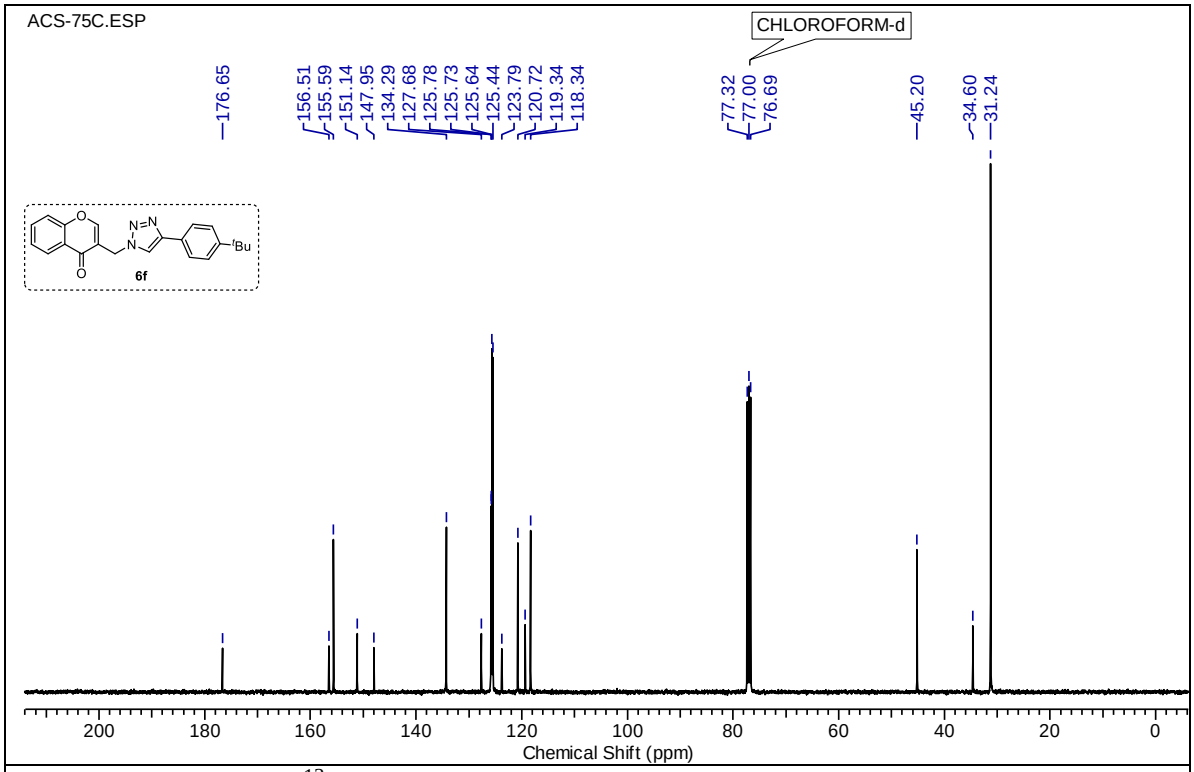
^{1}H NMR (200 MHz, $CDCl_3$) of compound **6e**



^{13}C NMR (100 MHz, $CDCl_3$) of compound **6e**



^{1}H NMR (200 MHz, $CDCl_3$) of compound **6f**



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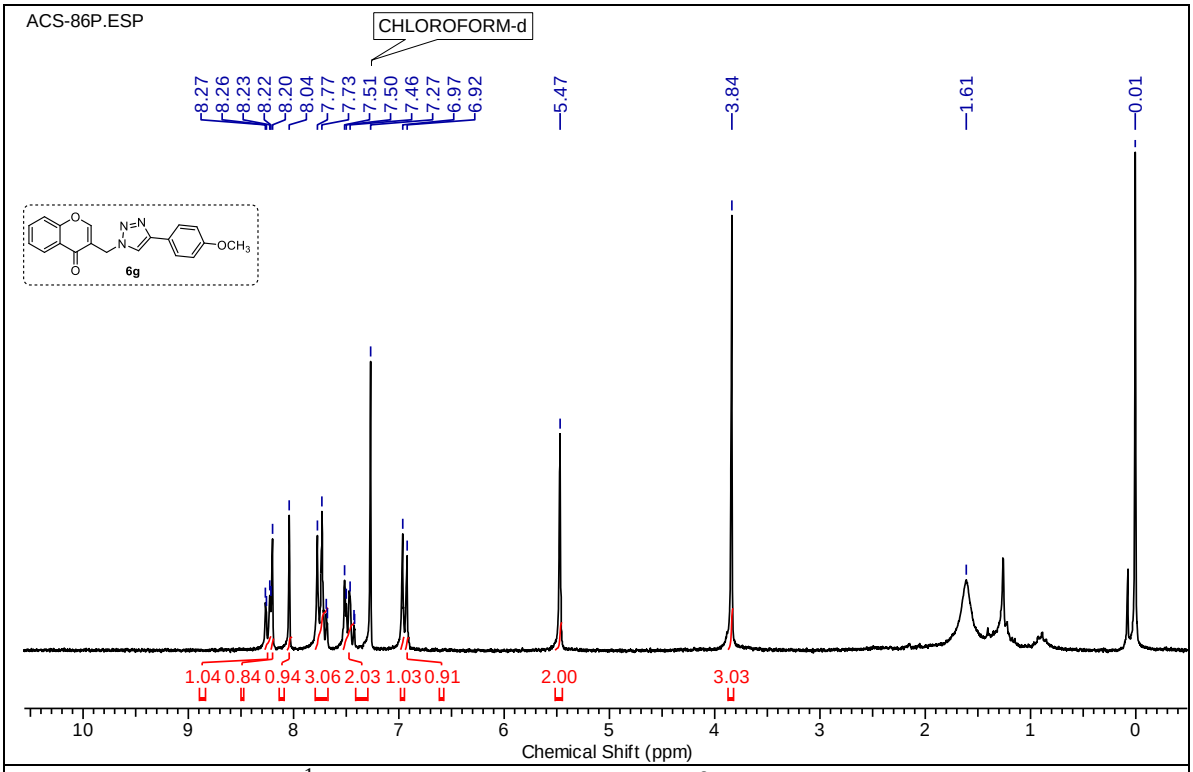
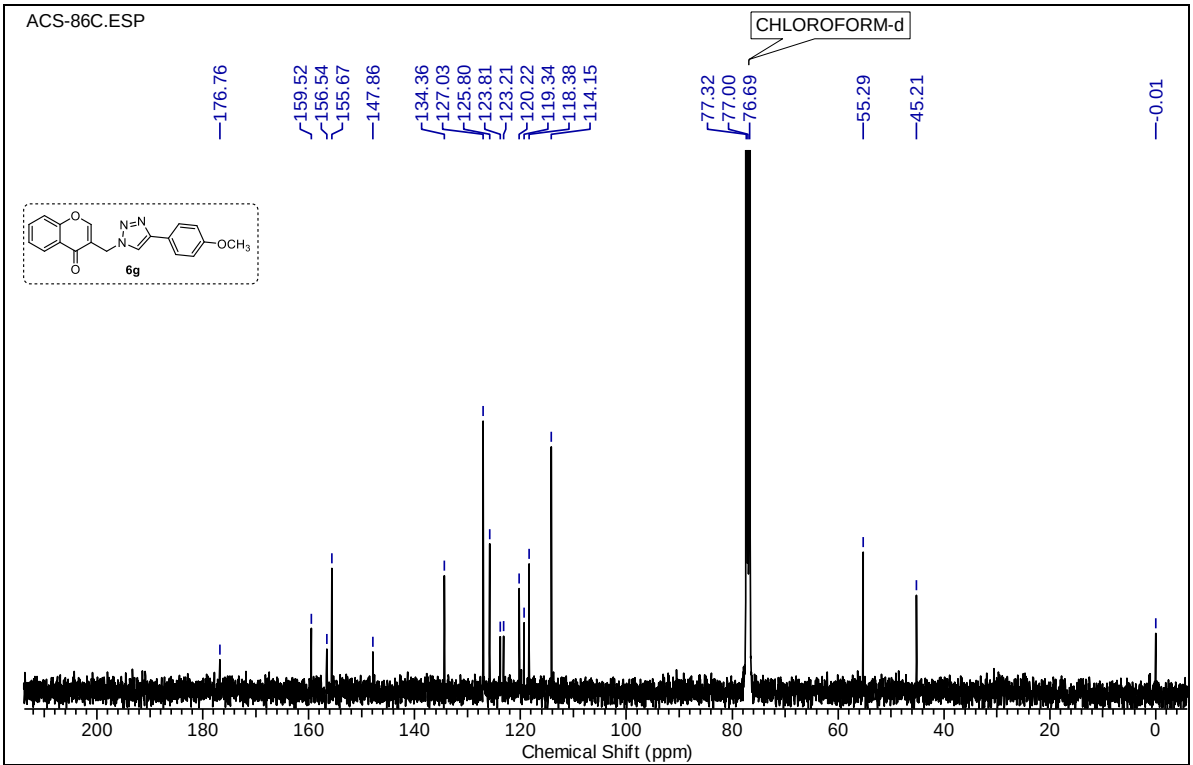
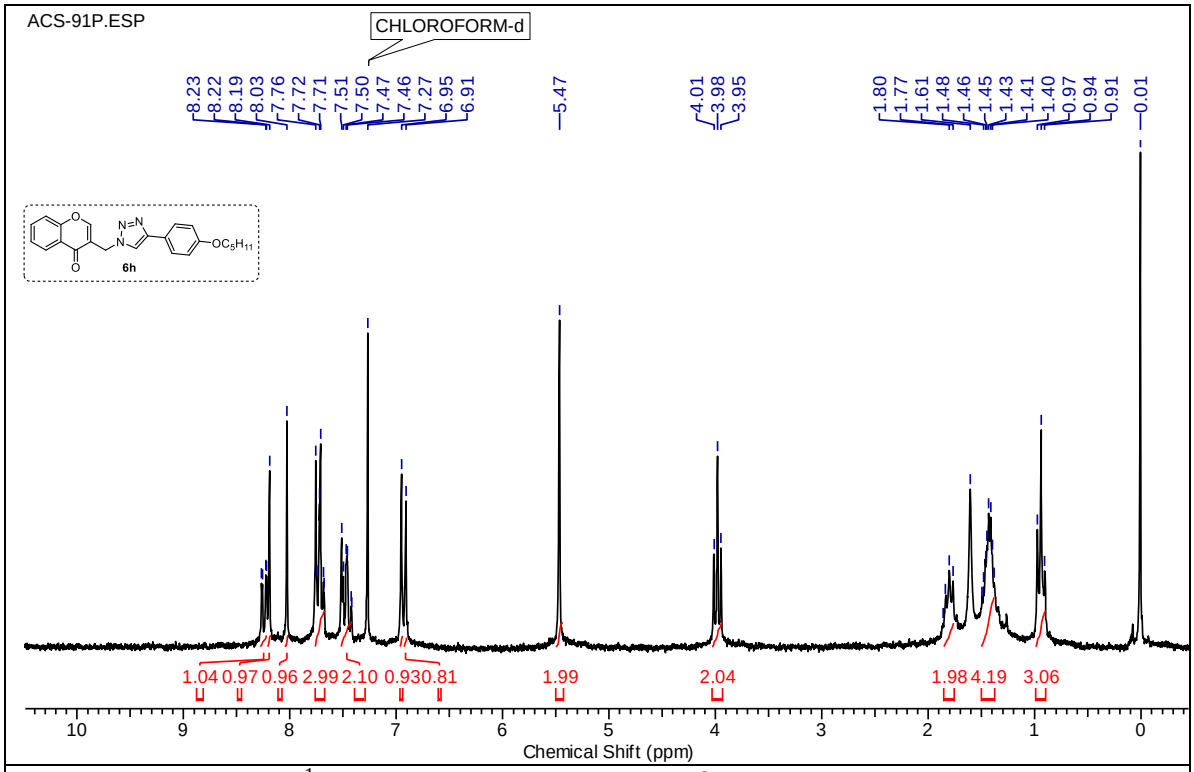


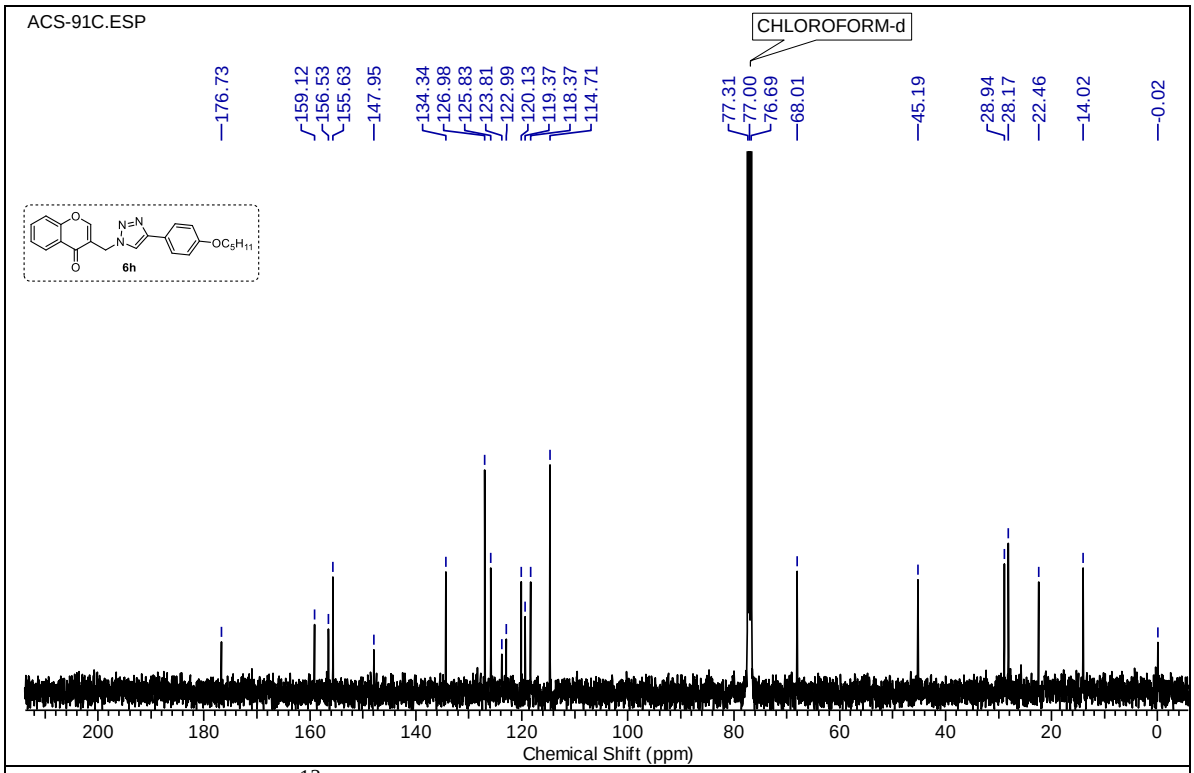
^{1}H NMR (200 MHz, $CDCl_3$) of compound **6g**



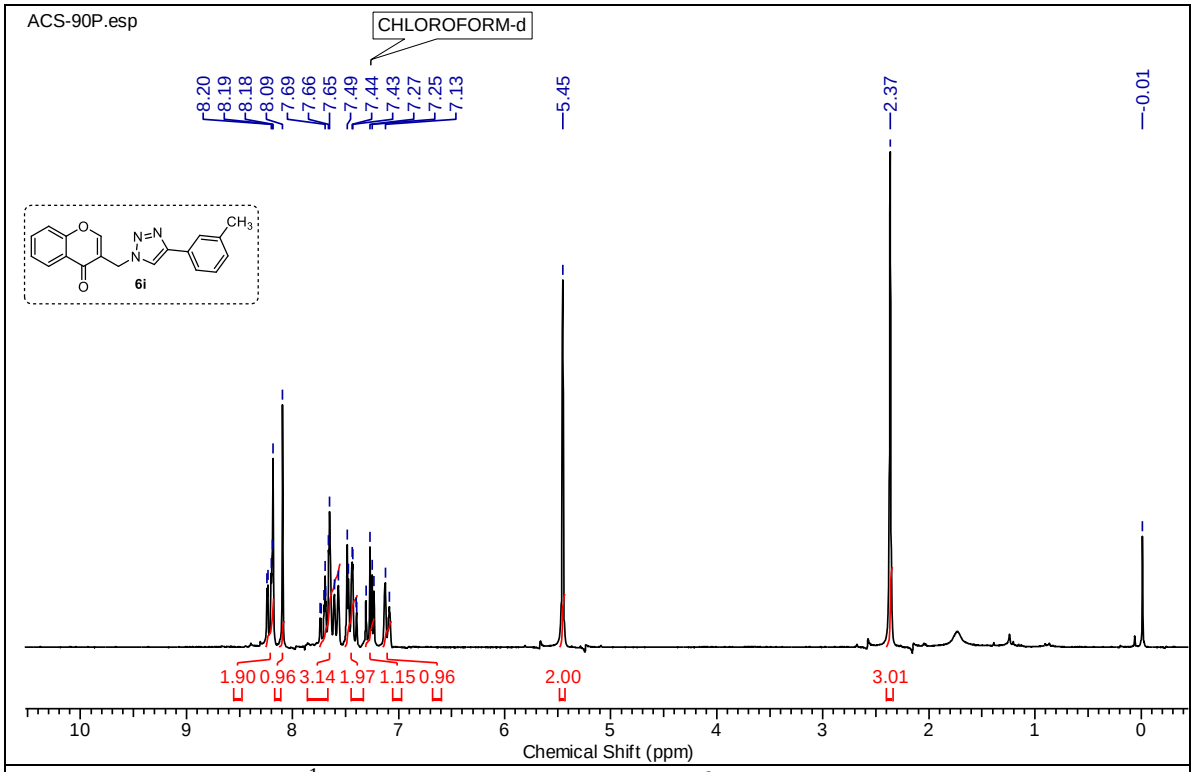
^{13}C NMR (100 MHz, $CDCl_3$) of compound **6g**



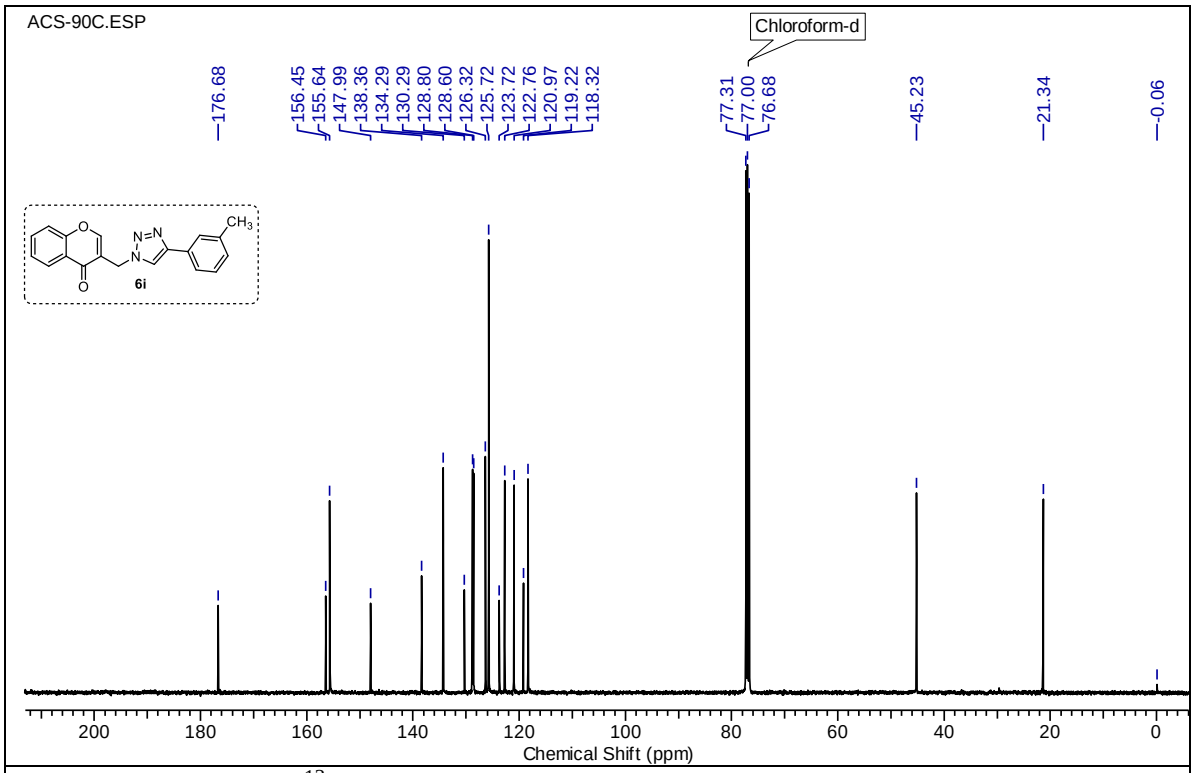
^{1}H NMR (200 MHz, $CDCl_3$) of compound **6h**



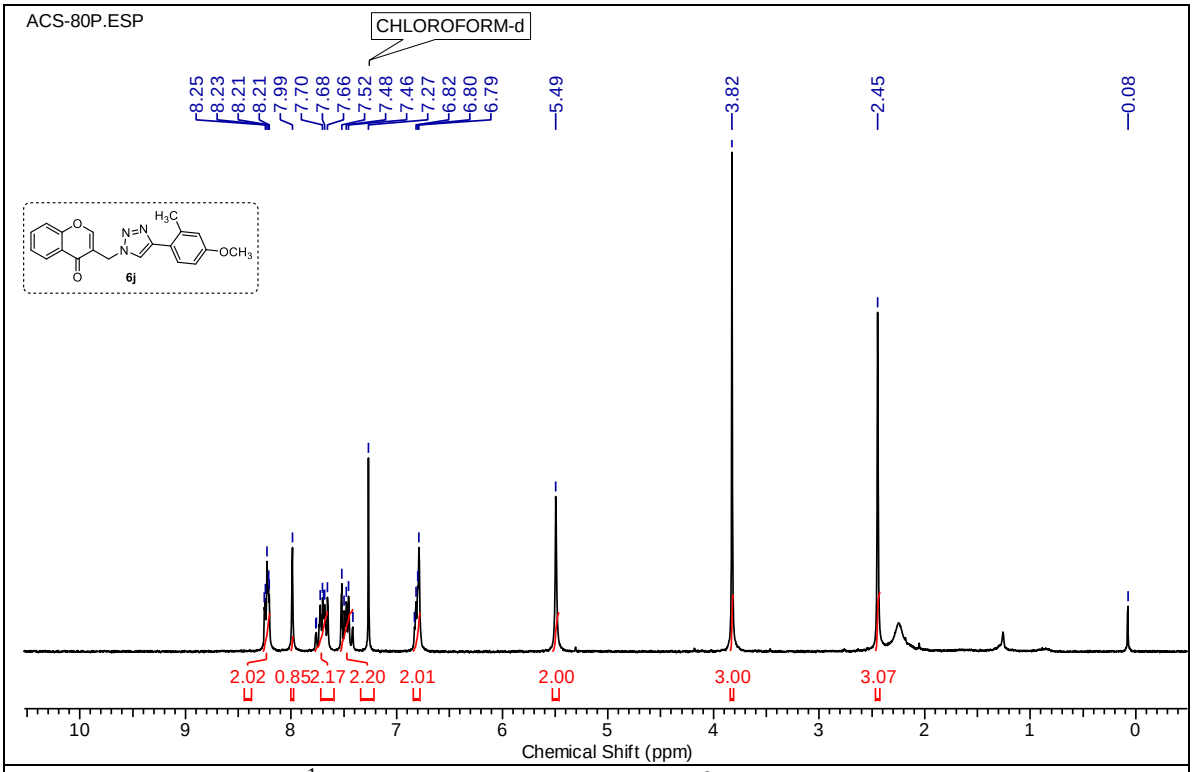
^{13}C NMR (100 MHz, $CDCl_3$) of compound **6h**



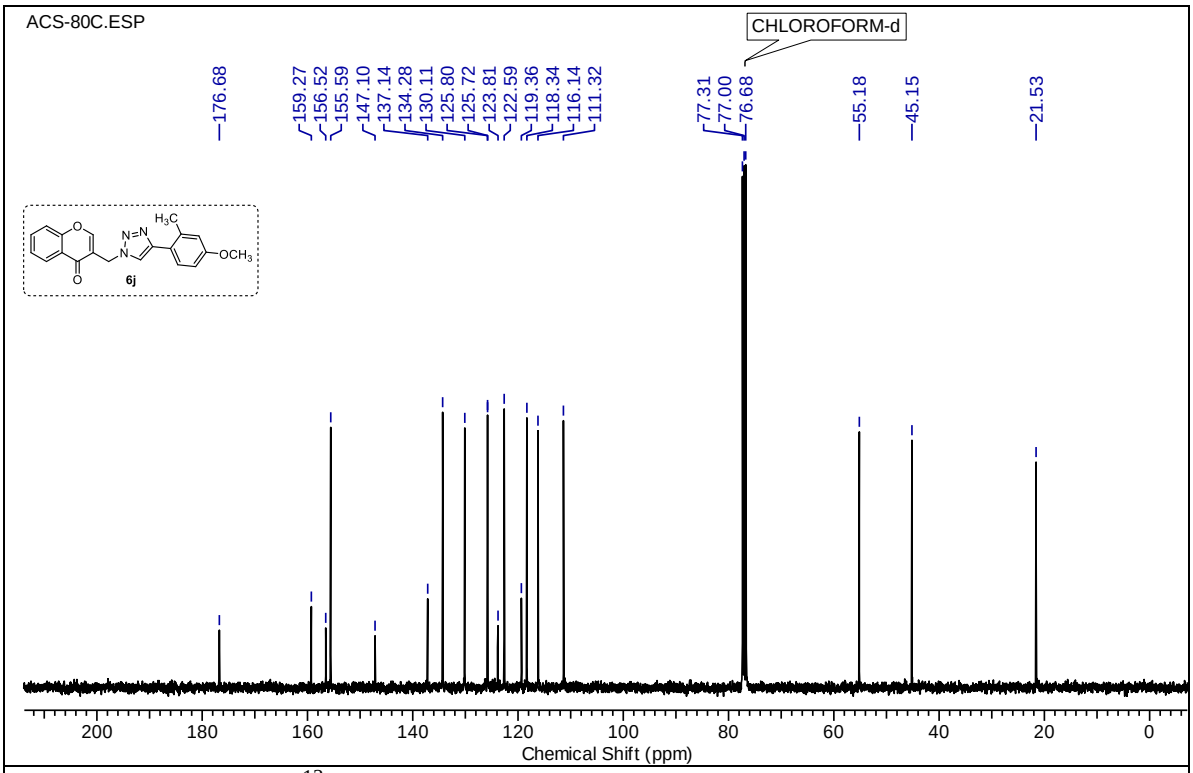
^{1}H NMR (200 MHz, $CDCl_3$) of compound **6i**



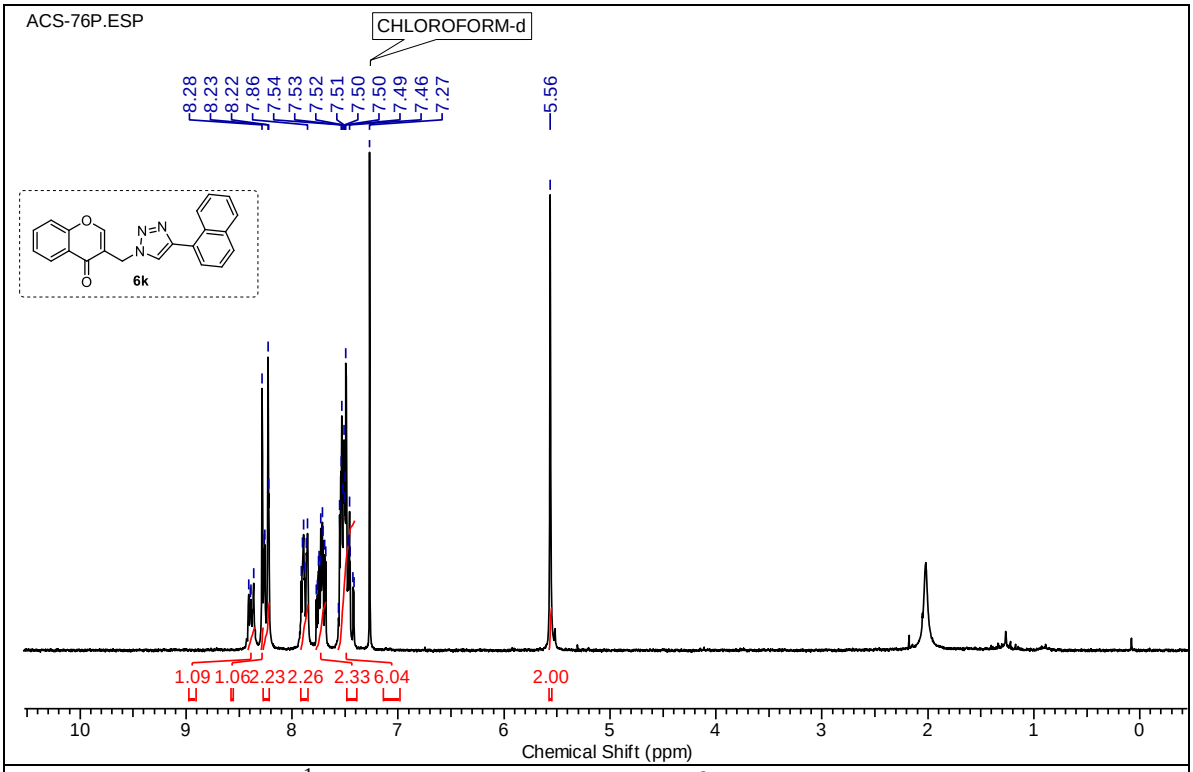
^{13}C NMR (100 MHz, $CDCl_3$) of compound **6i**



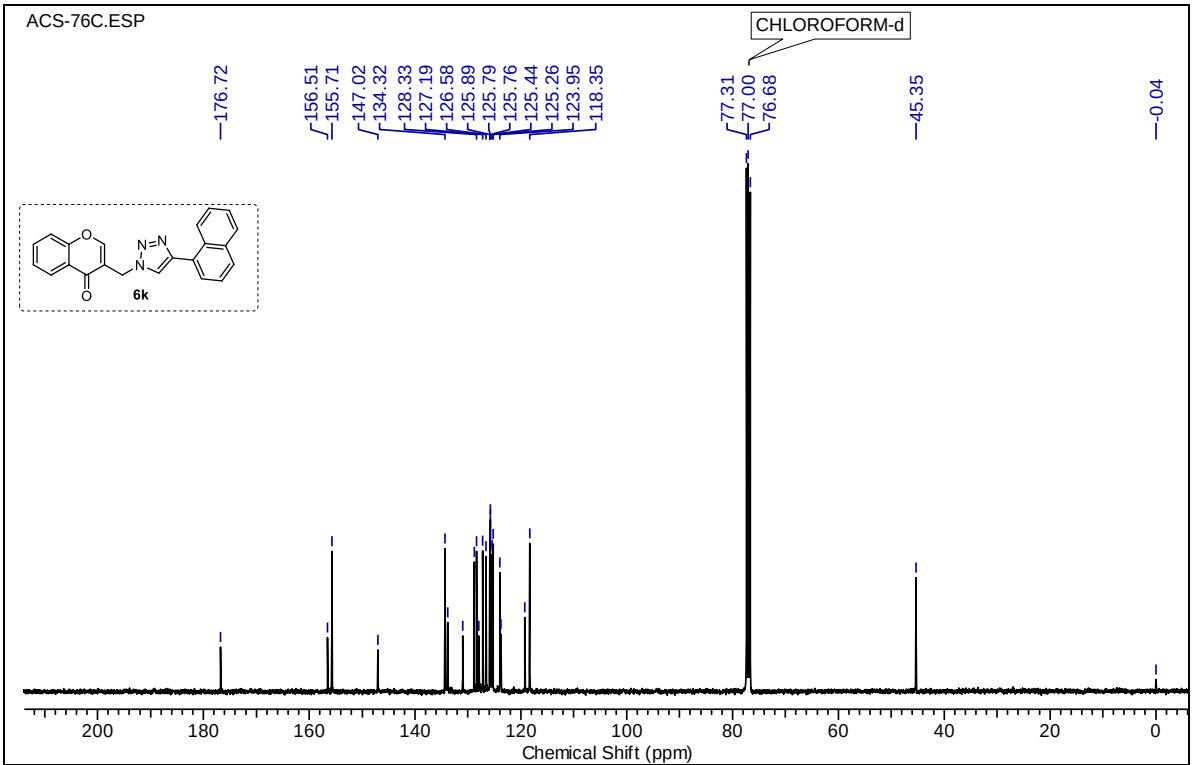
^{1}H NMR (200 MHz, $CDCl_3$) of compound **6j**



^{13}C NMR (100 MHz, $CDCl_3$) of compound **6j**



^{1}H NMR (200 MHz, $CDCl_3$) of compound **6k**



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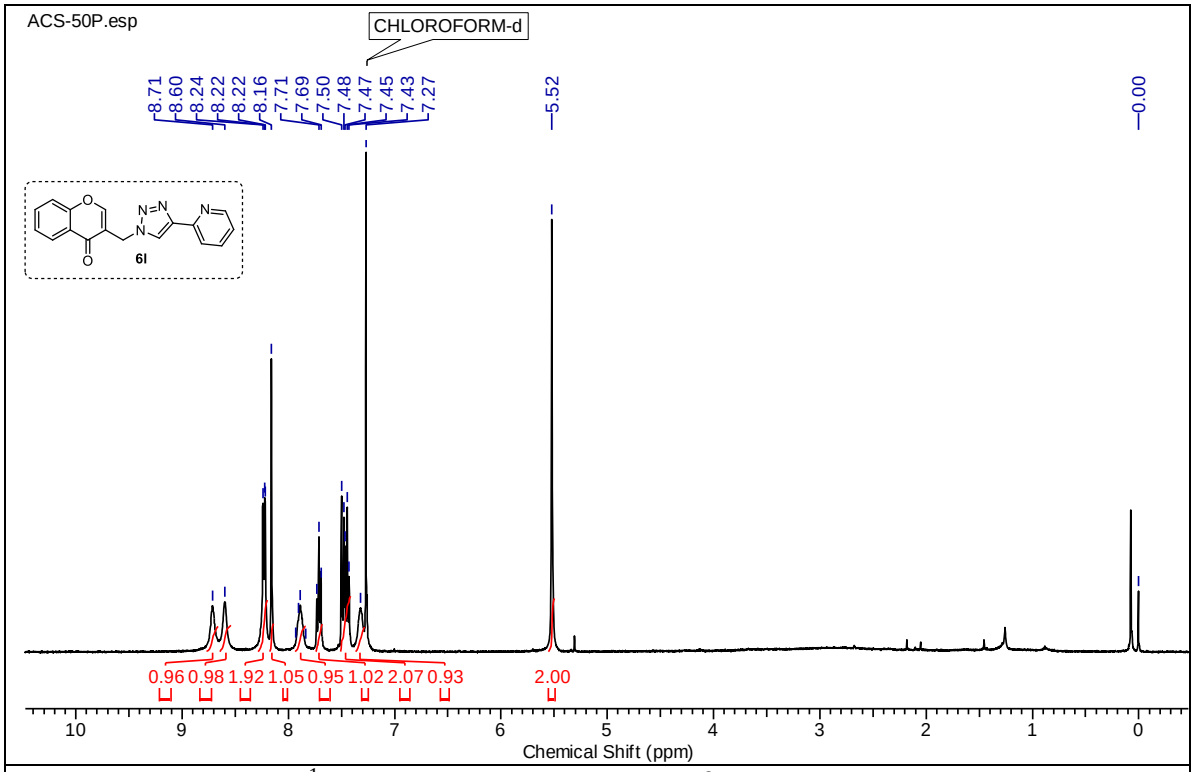
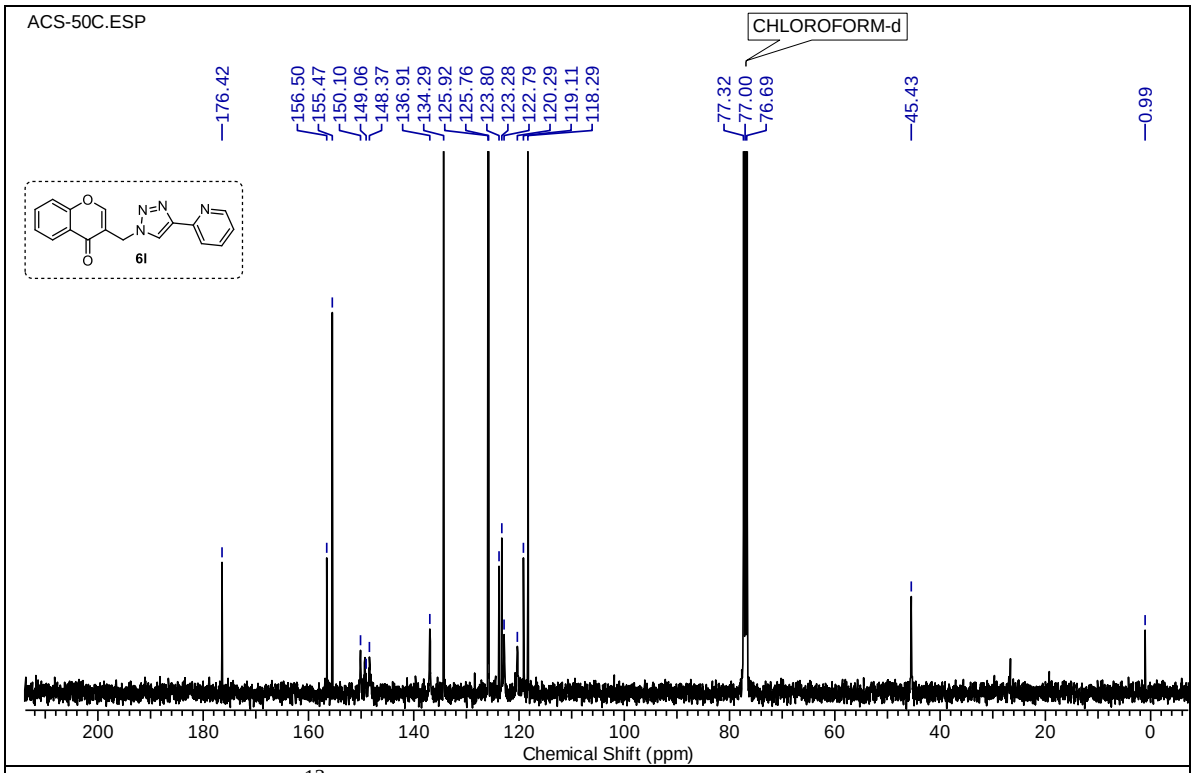
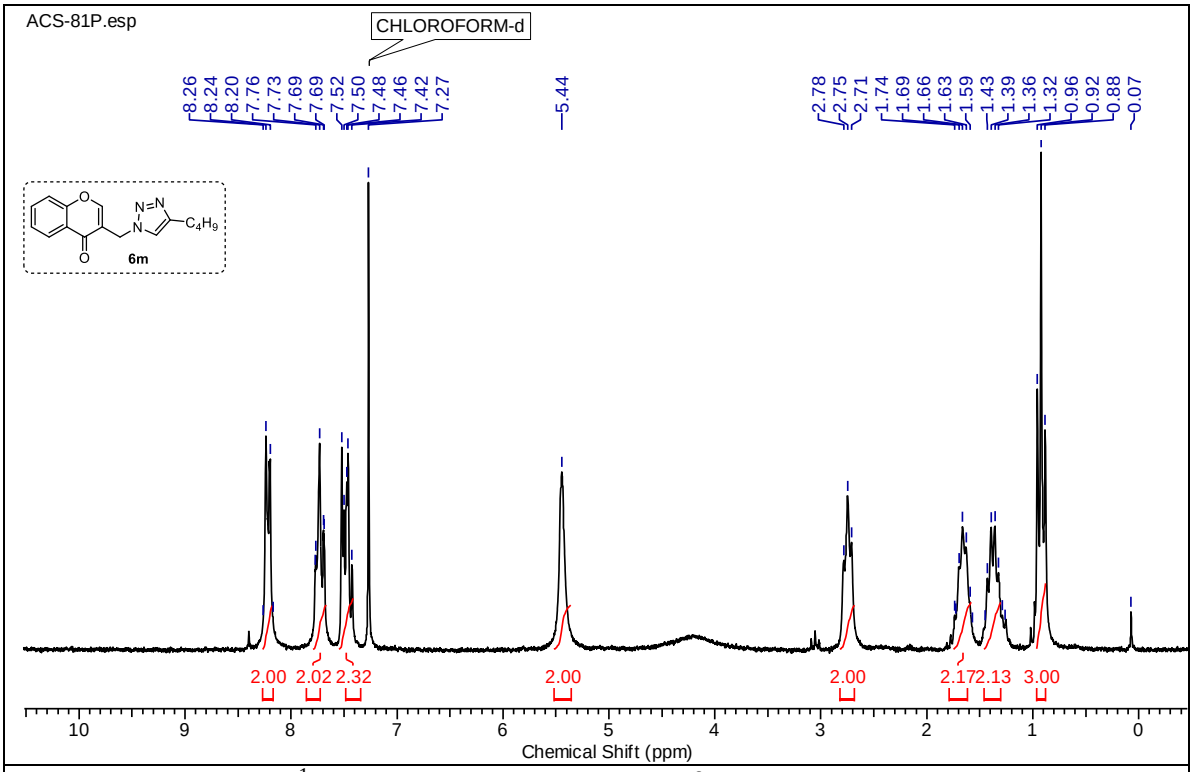


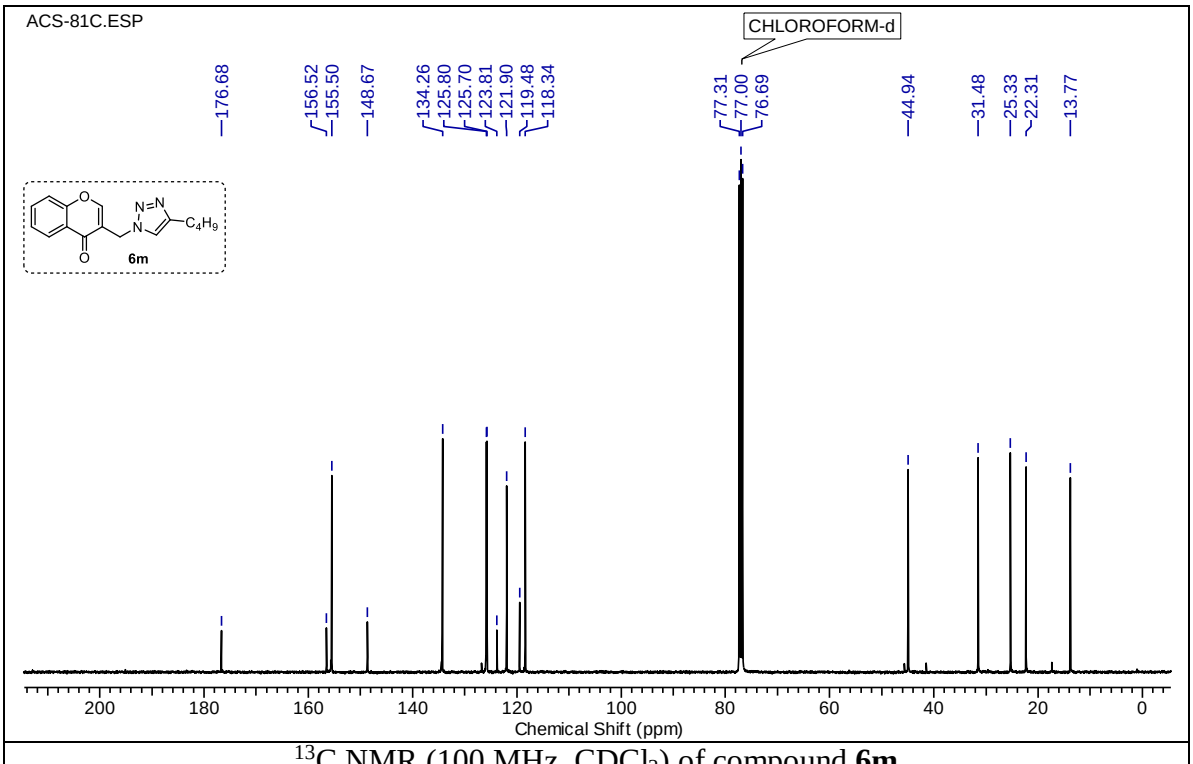
^{1}H NMR (400 MHz, $CDCl_3$) of compound **6l**



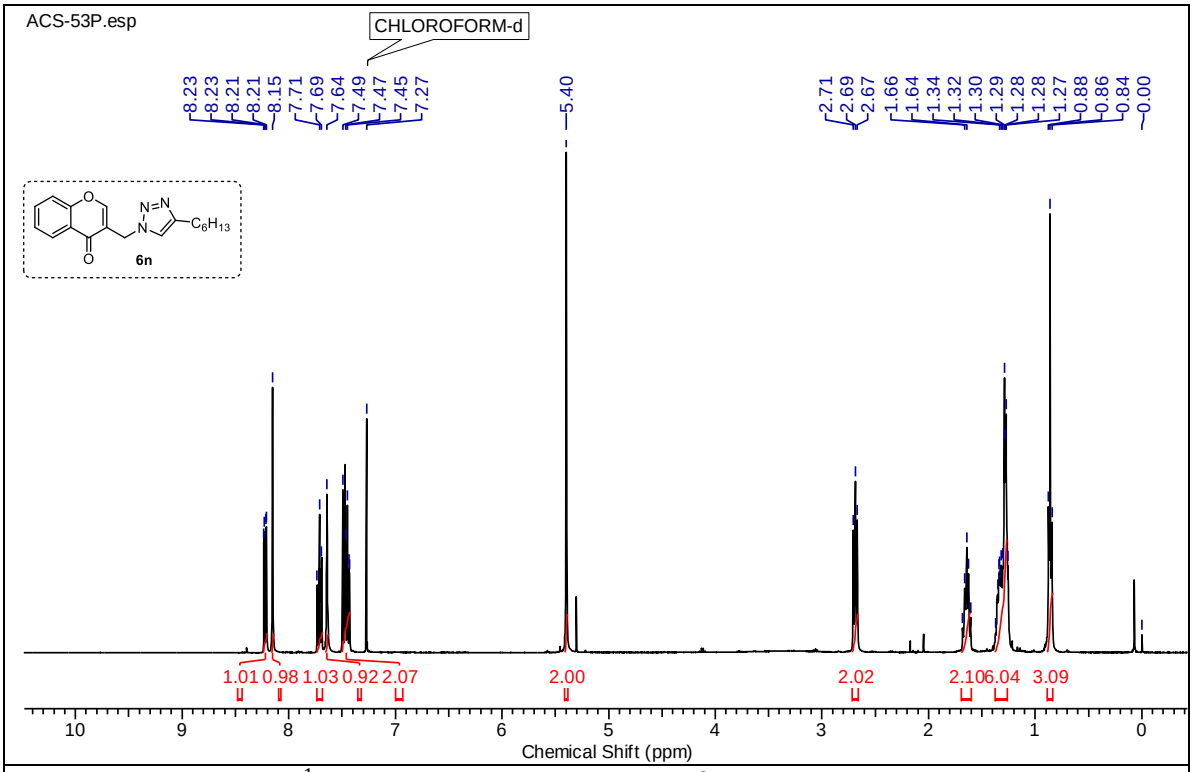
^{13}C NMR (100 MHz, $CDCl_3$) of compound **6l**



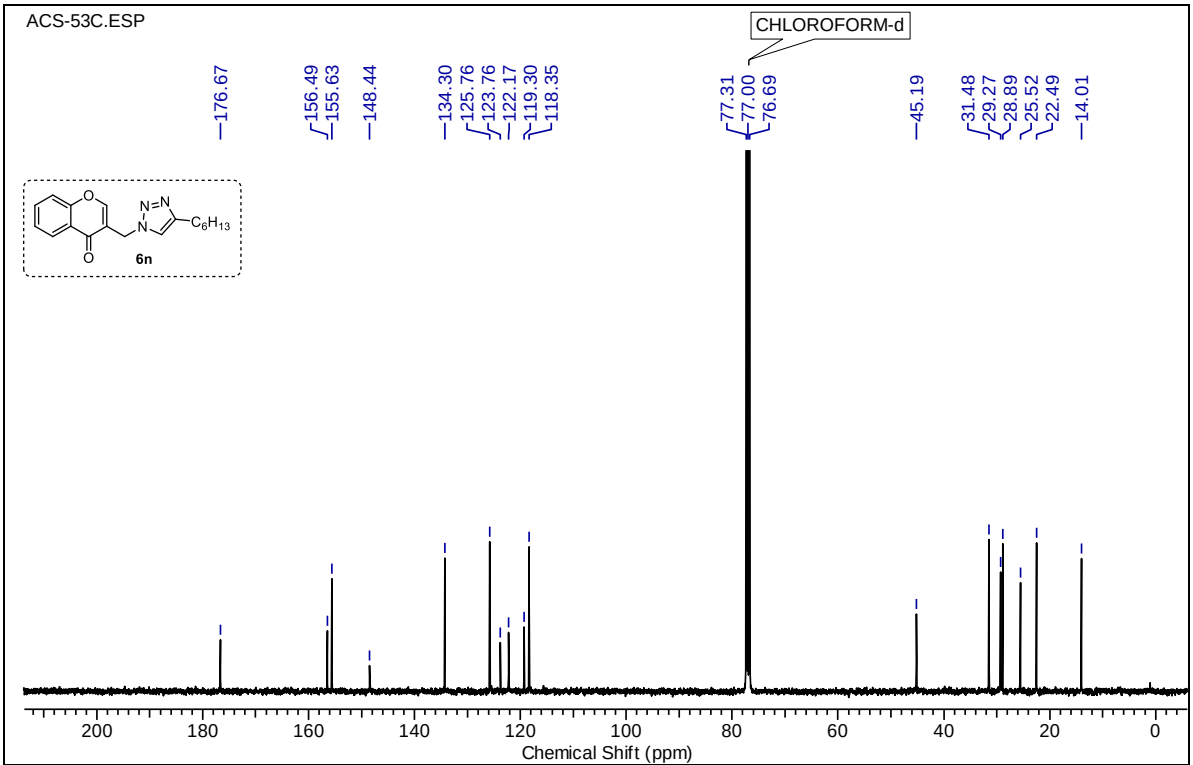
^{1}H NMR (200 MHz, $CDCl_3$) of compound **6m**



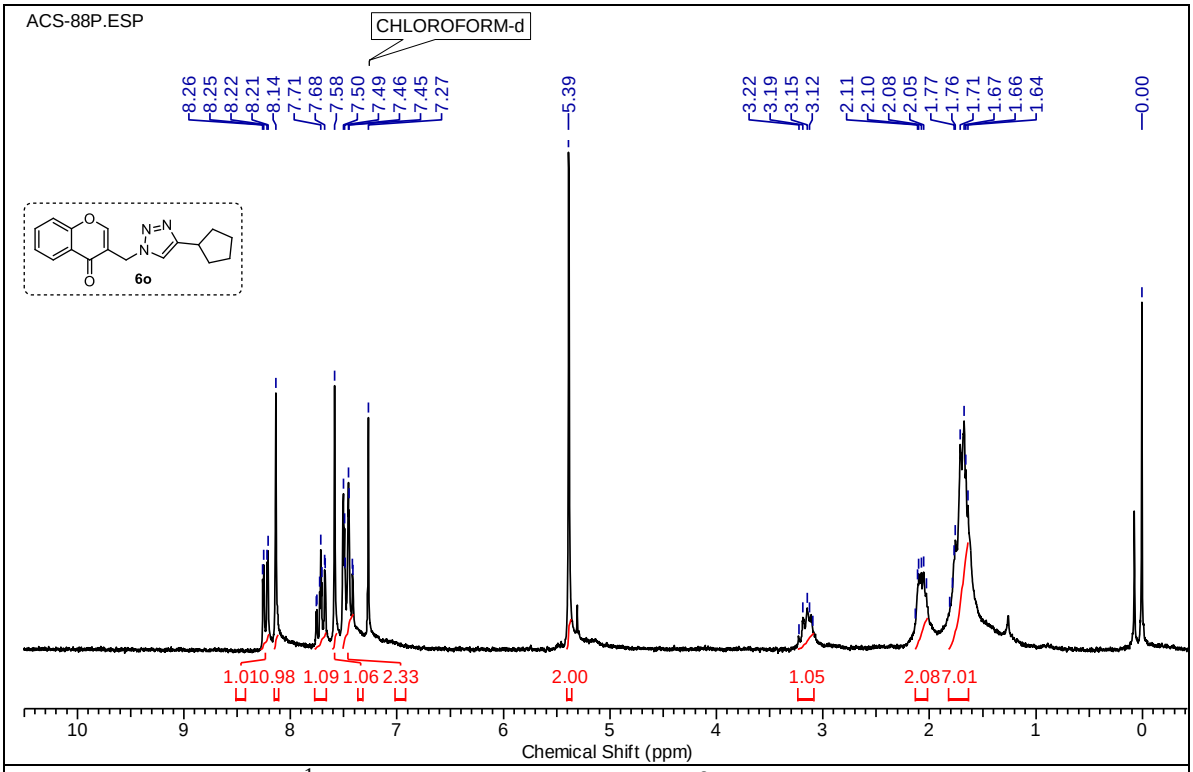
^{13}C NMR (100 MHz, $CDCl_3$) of compound **6m**



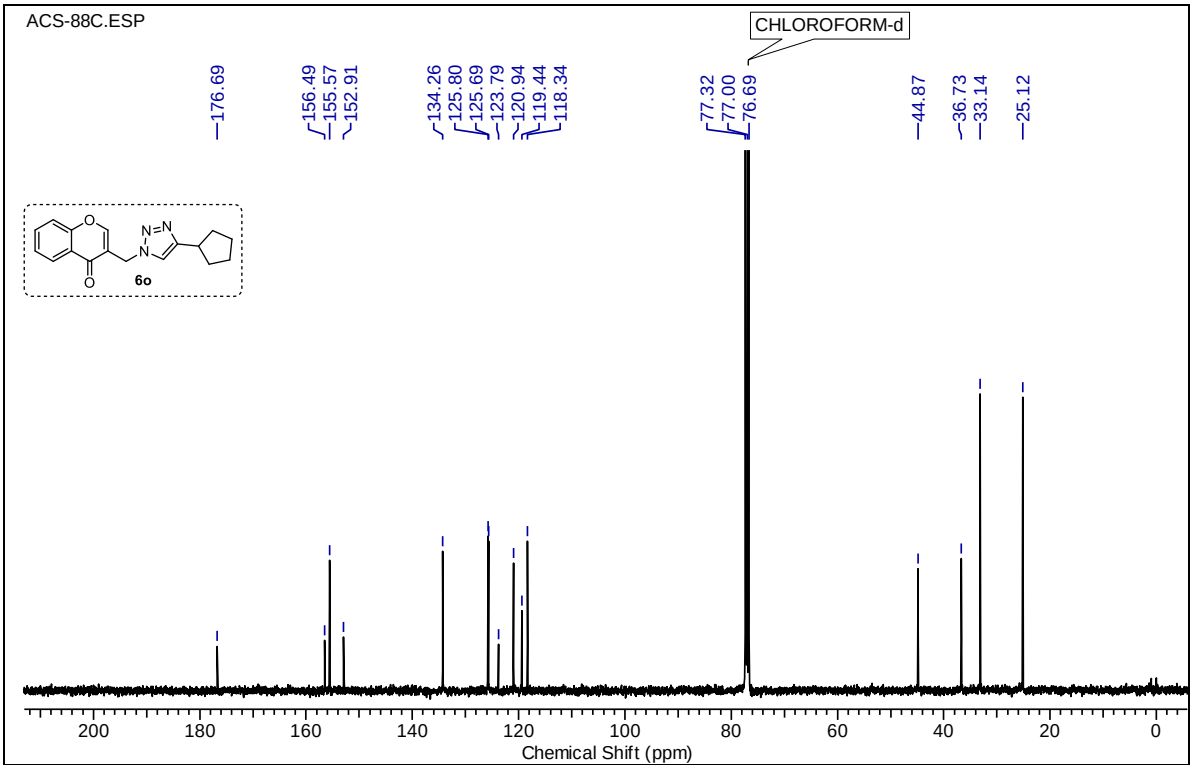
^{1}H NMR (400 MHz, $CDCl_3$) of compound **6n**



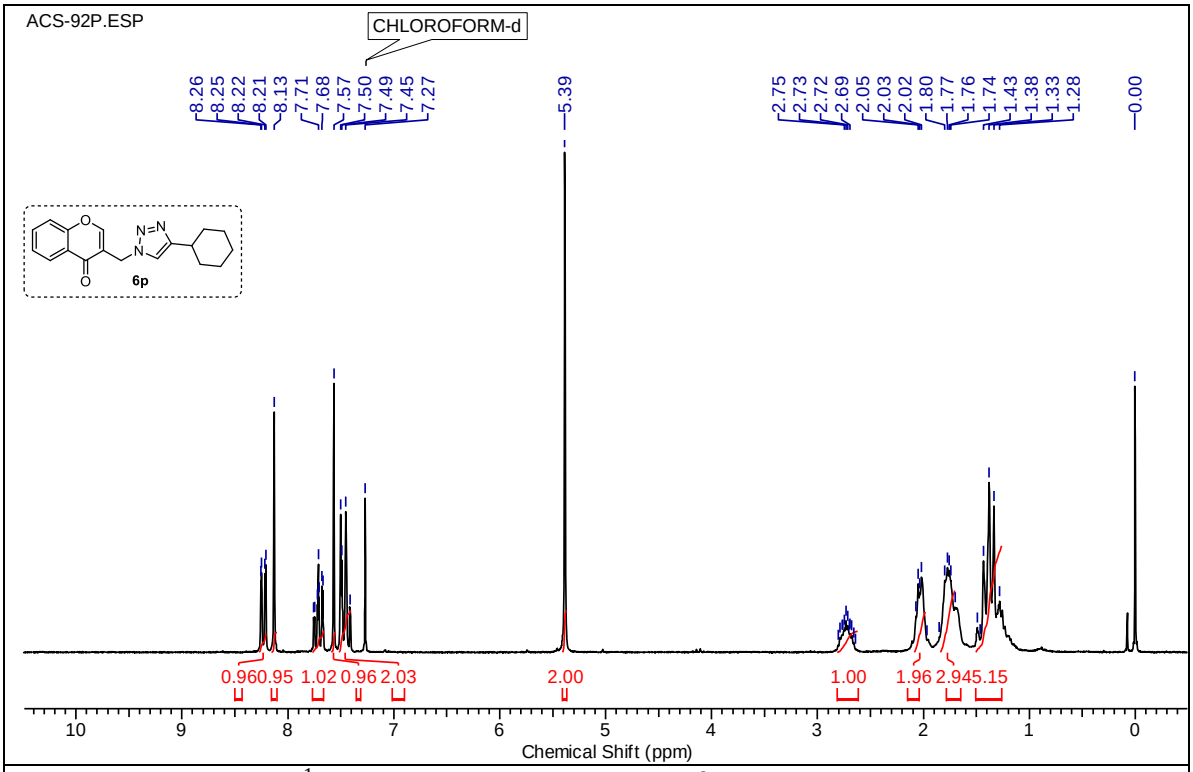
^{13}C NMR (50 MHz, $CDCl_3$) of compound **6n**



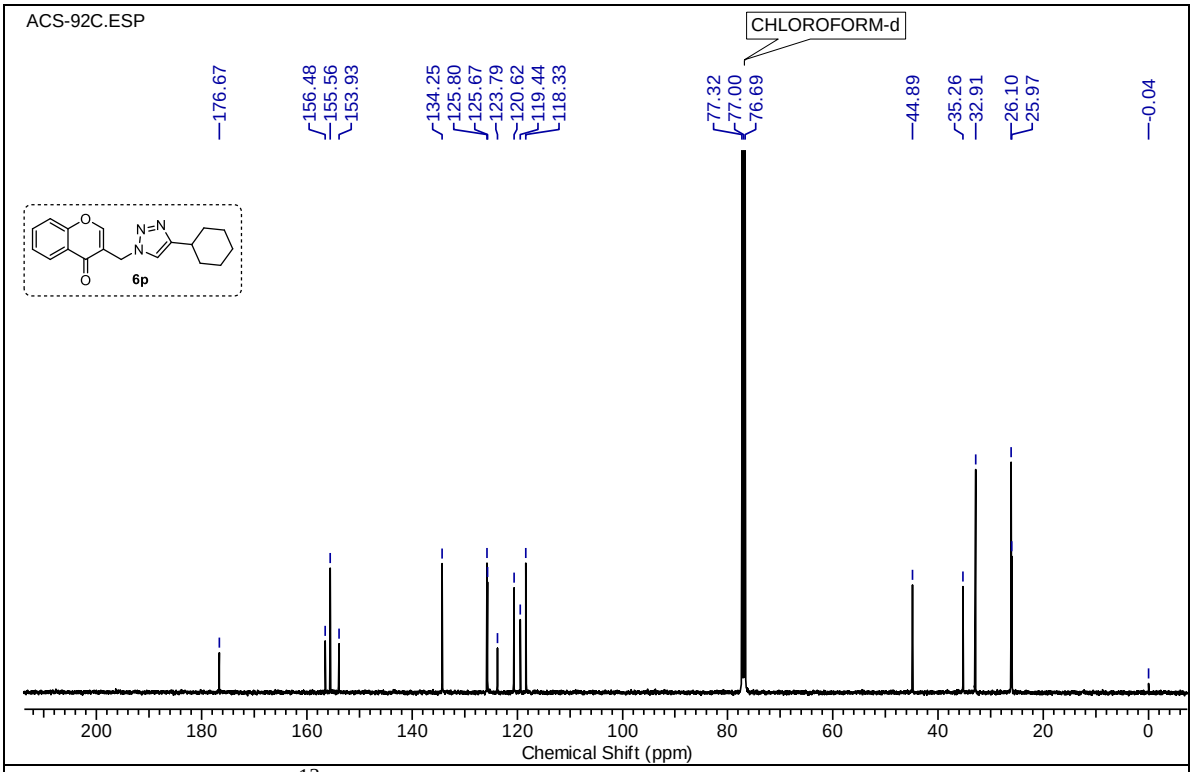
^{1}H NMR (200 MHz, $CDCl_3$) of compound **6o**



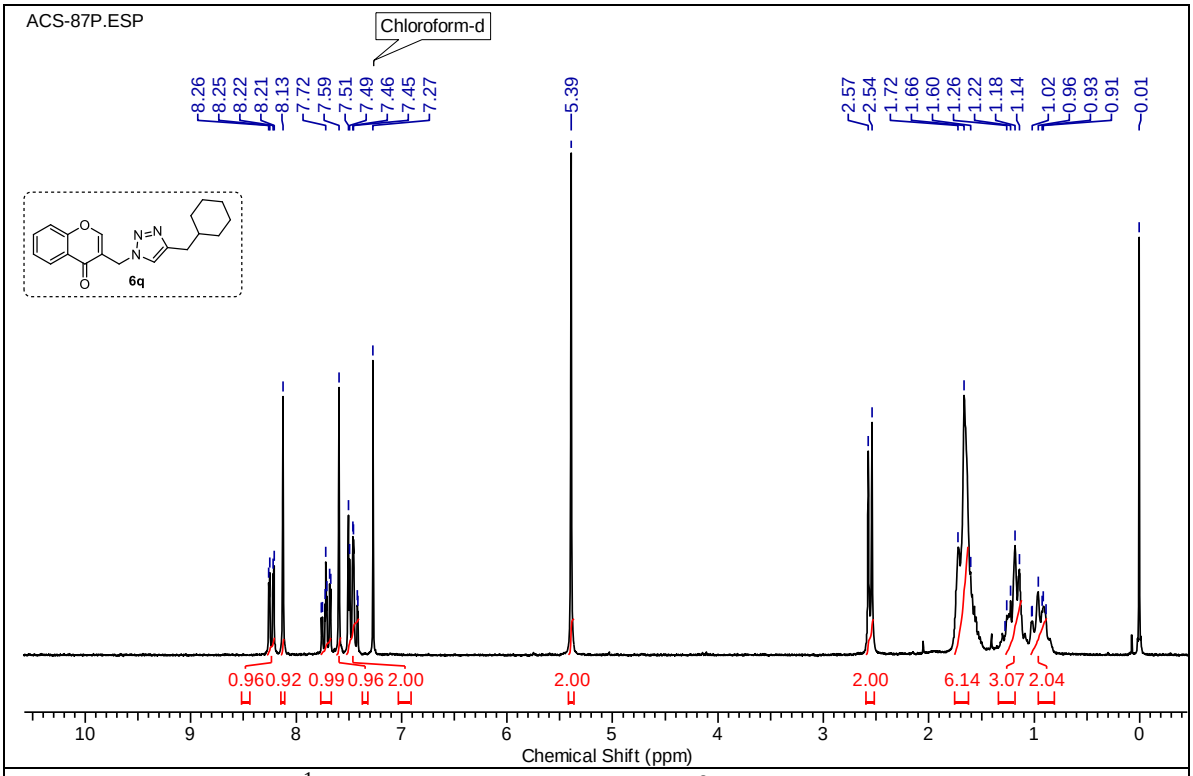
^{13}C NMR (100 MHz, $CDCl_3$) of compound **6o**



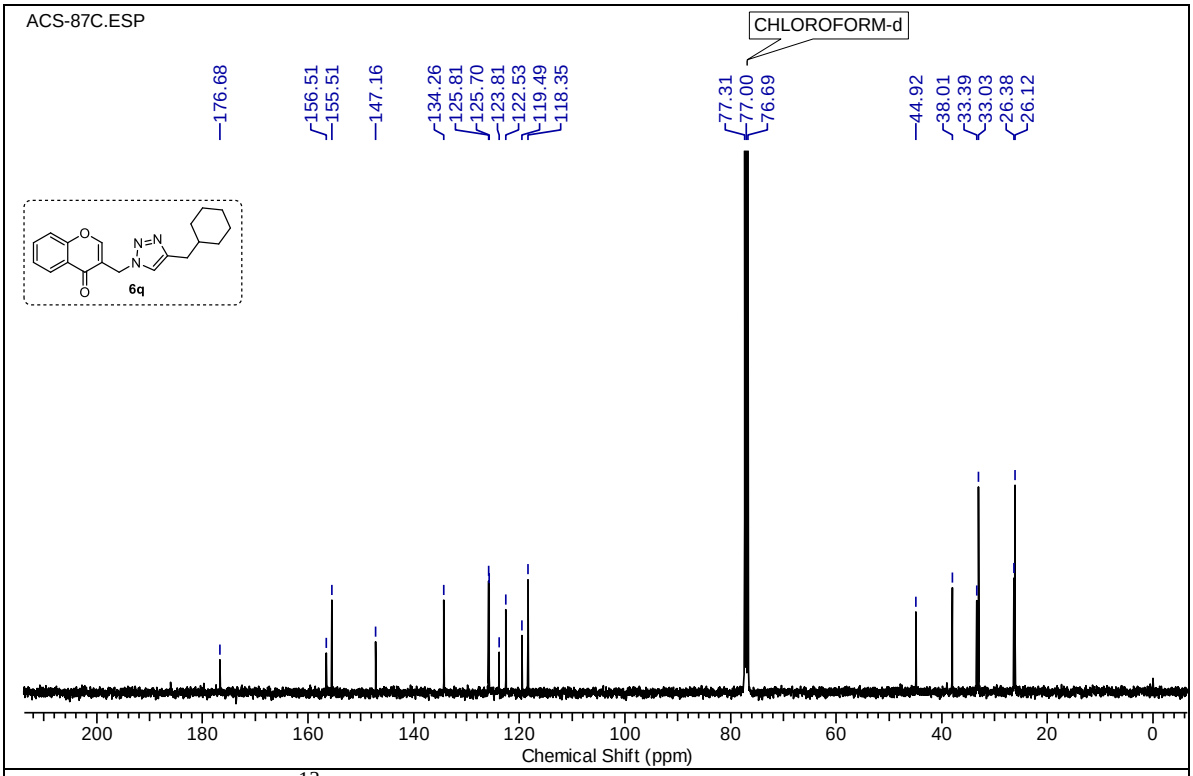
^{1}H NMR (200 MHz, $CDCl_3$) of compound **6p**



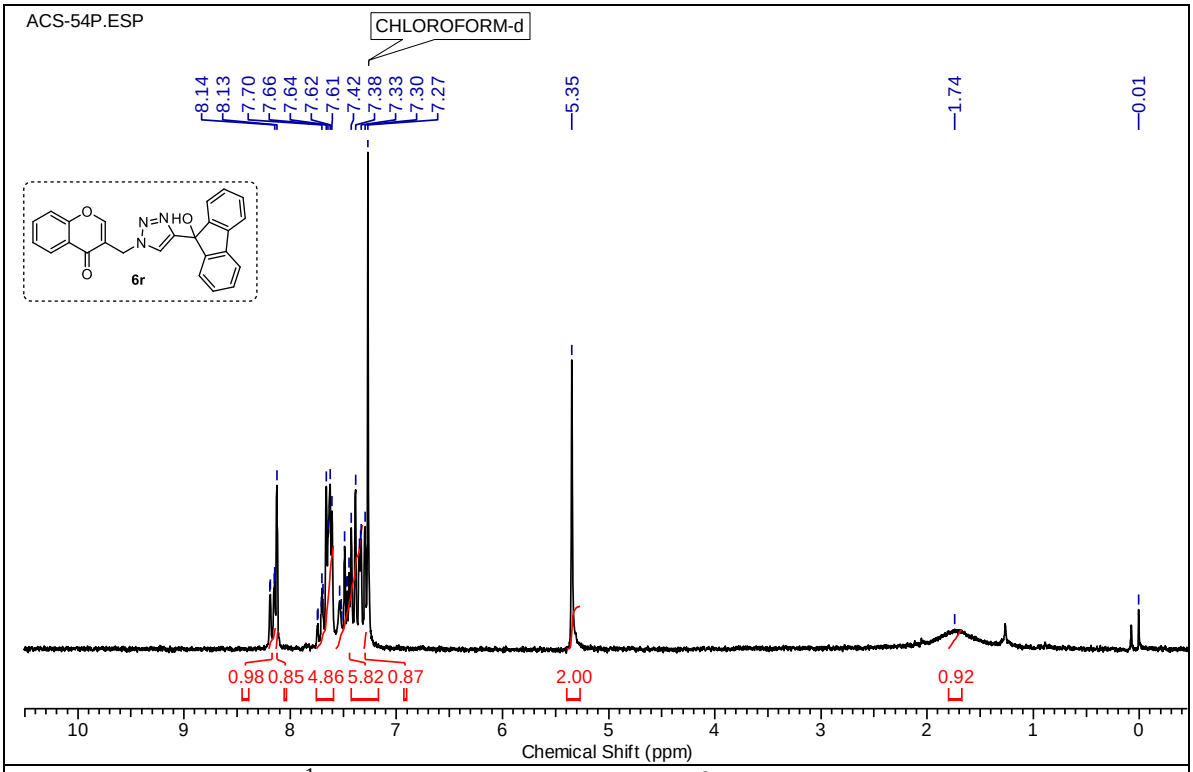
^{13}C NMR (100 MHz, $CDCl_3$) of compound **6p**



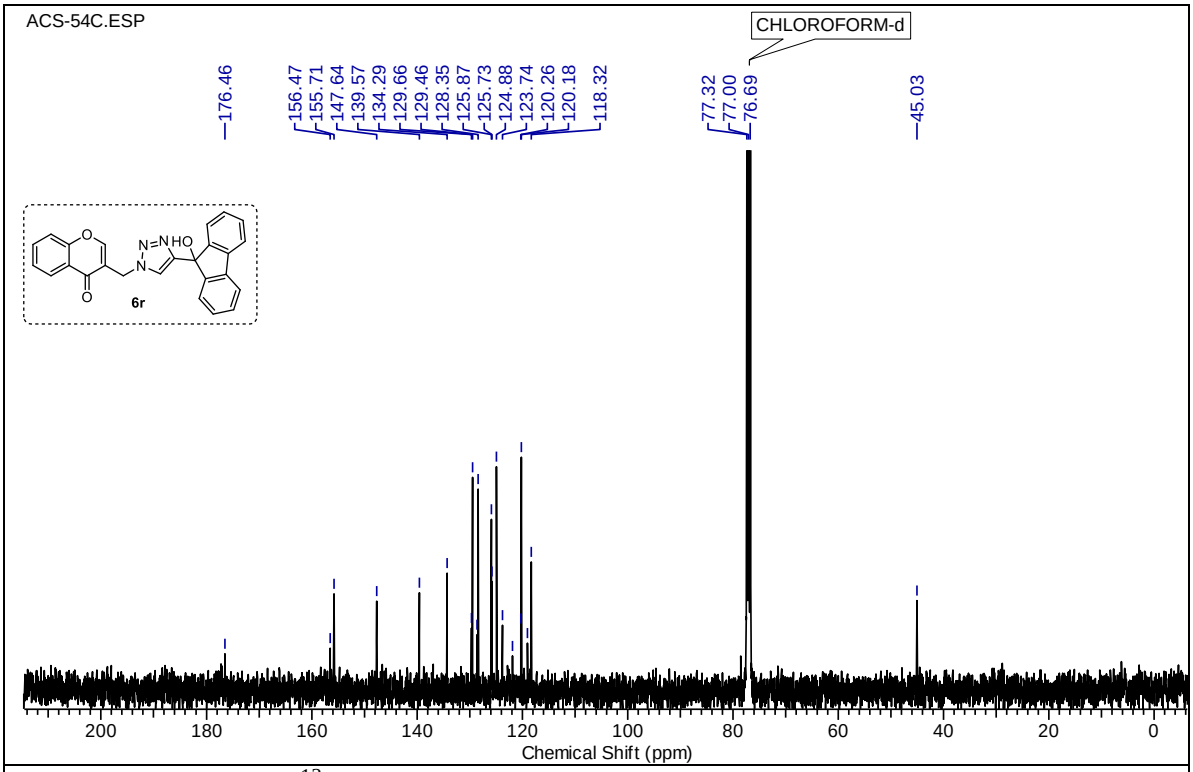
^{1}H NMR (200 MHz, $CDCl_3$) of compound **6q**



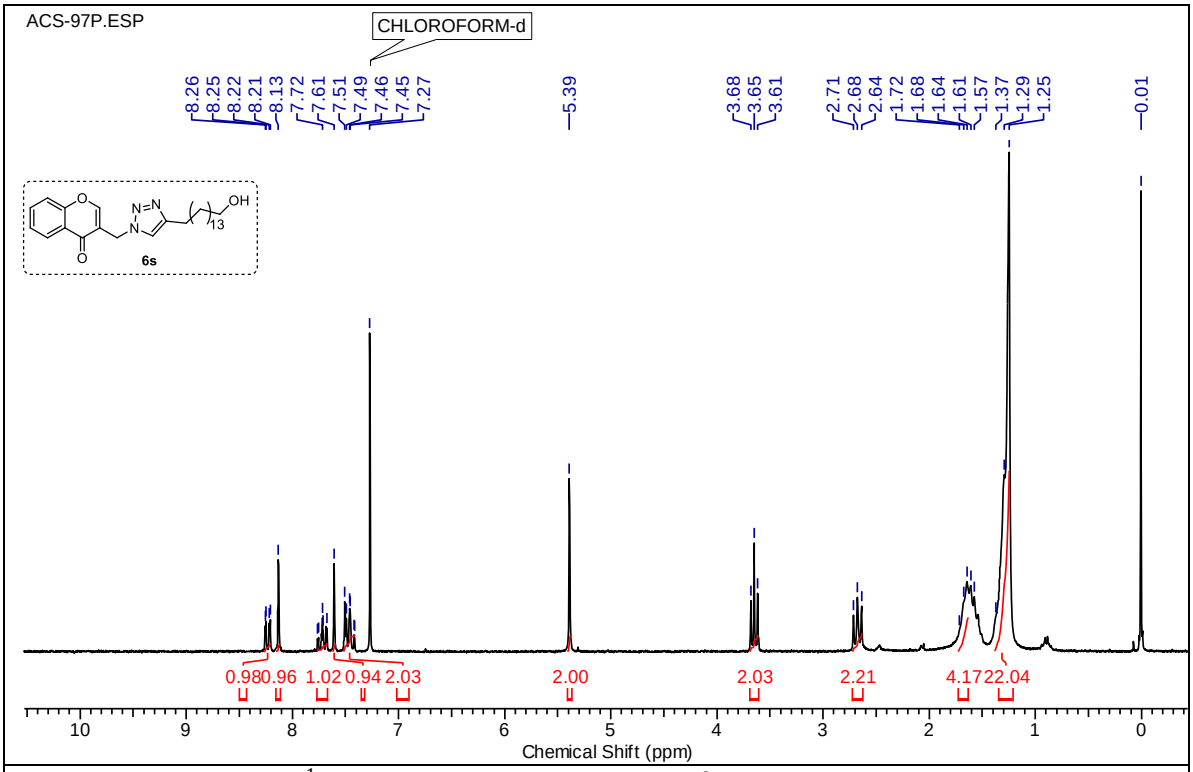
^{13}C NMR (100 MHz, $CDCl_3$) of compound **6q**



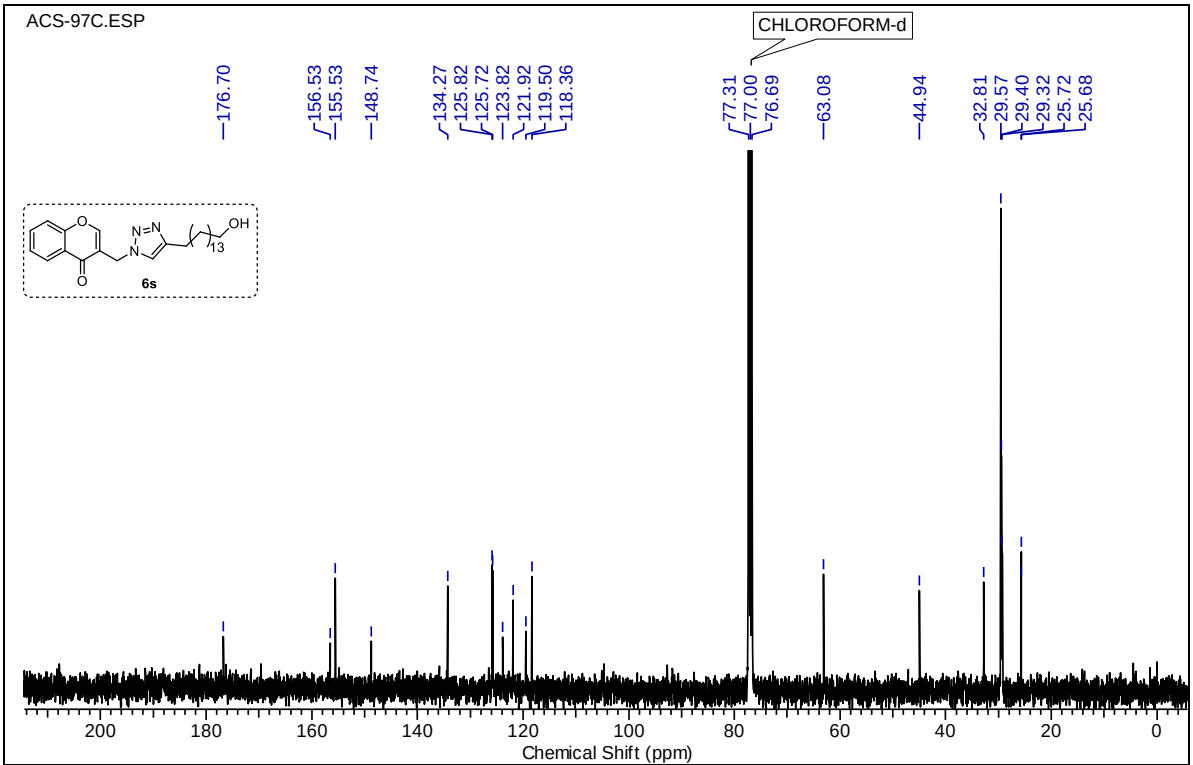
^{1}H NMR (200 MHz, $CDCl_3$) of compound **6r**



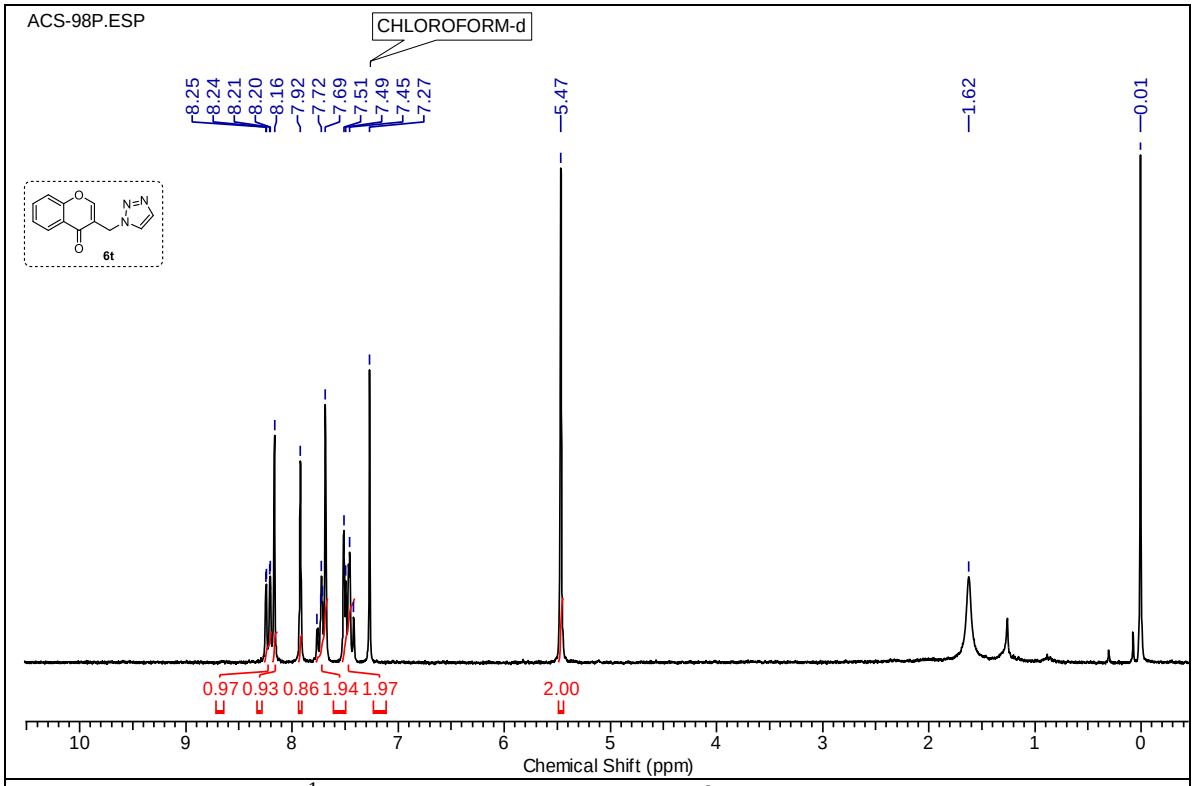
^{13}C NMR (100 MHz, $CDCl_3$) of compound **6r**



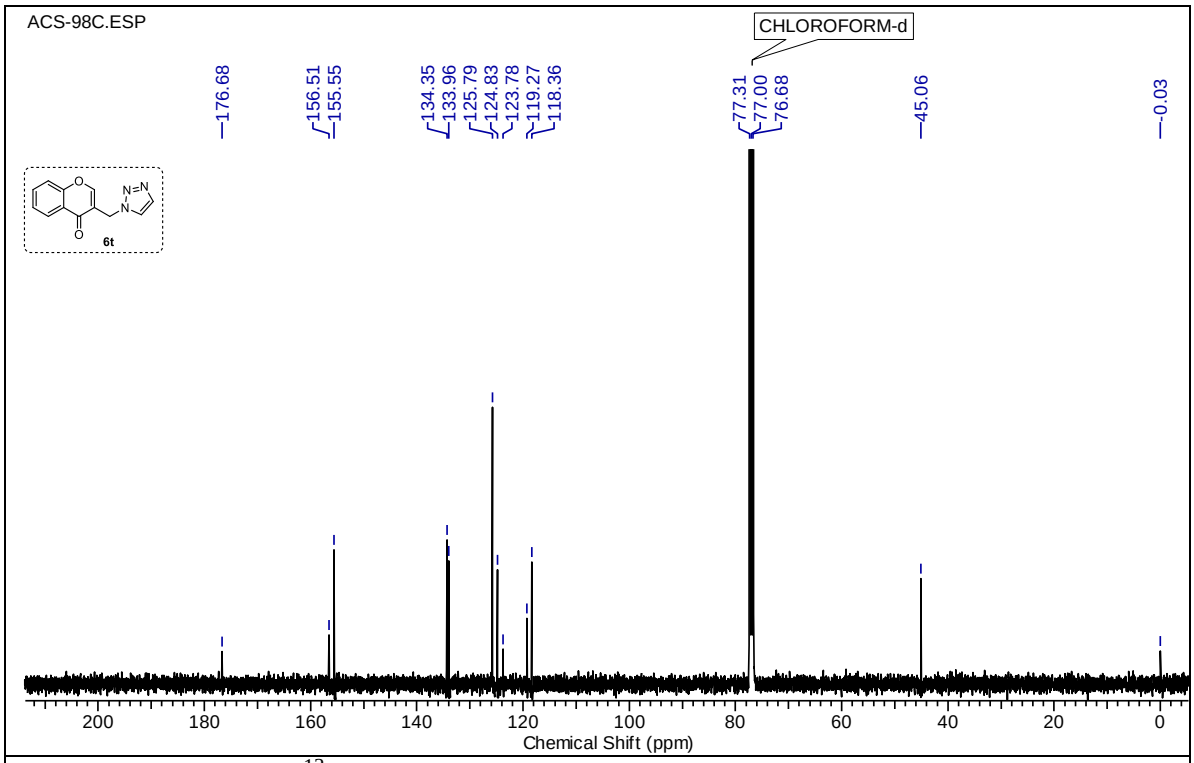
^{1}H NMR (200 MHz, $CDCl_3$) of compound **6s**



^{13}C NMR (100 MHz, $CDCl_3$) of compound **6s**



^{1}H NMR (200 MHz, $CDCl_3$) of compound **6t**



^{13}C NMR (100 MHz, $CDCl_3$) of compound **6t**

3.1.7. References

1. (a) Zumla, A.; Raviglione, M.; Hafner, R.; von Reyn, F. *N. Engl. J. Med.* **2013**, *368*, 745; (b) Russell, D. G.; Barry, C. E., III; Flynn, J. L. *Science* **2010**, *328*, 852.
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4. (a) Corbett, E. L.; Watt, C. J.; Walker, N.; Maher, D.; Williams, B. G.; Raviglione, M. C.; Dye, C. *Arch Intern Med* **2003**, *163*, 1009; (b) Nikalje, A. G.; Mudassar, P. *Asian J. Biol. Sci.* **2011**, *4*, 101.
5. Caminero, J. A.; Sotgiu, G.; Zumla, A.; Migliori, G. B. *Lancet Infect. Dis.* **2010**, *10*, 621.
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3.2. SECTION 2

Synthesis, biological evaluation and molecular modeling studies of novel chromone/aza-chromone fused α-aminophosphonates as c-Src kinase inhibitors

3.2.1. Introduction

Protein tyrosine kinases (PTKs) are a large family of cytoplasmic enzymes that catalyze the phosphorylation of proteins to change their protein activity. Protein phosphorylation is an enzymatic process which involves the transport of the γ-phosphoryl group from ATP to hydroxyl group of the serine, threonine, or tyrosine residue in many proteins.[1] In this process, adenosine triphosphate (ATP) or guanosine triphosphate (GTP) serves as the phosphoryl donor, while serine and threonine and tyrosine residues are the phosphoryl receptors. The Src family kinases (SFKs) are non-receptor tyrosine kinases comprises of nine different PTKs in mammalian cells including c-Src, c-Yes, Fyn, Lck, Lyn, Hck, Frk, Blk and c-Fgr.[2] Based on expression pattern in the body, these members are further subclassified into three groups. The first group includes Src, Yes, Fgr, and Fyn, are expressed in wide range of tissues; the second group includes Lyn, Hck, Lck, and Blk, are primarily expressed in hematopoietic cells; in the third group, Frk-related kinases are mainly expressed in epithelial-derived cells.[3] The organization of Src family kinases consists of five distinct regions: a 14-carbon N-terminal myristoylated segment attached to an SH4 domain, unique domain (non-conserved region) followed by modular SH3 domain, SH2 domain, SH2-kinase regulatory linker region and tyrosine kinase domain (the SH1 domain), and a carboxyl (C)-terminal regulatory segment (**Figure 1**).[4]

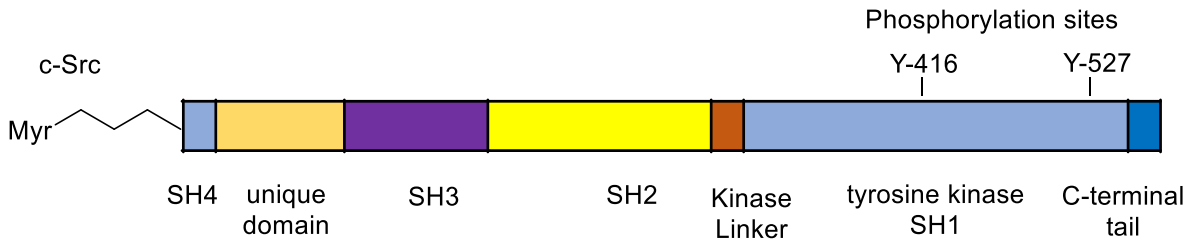


Figure 1. Structural domain of Src family kinases

Tyr416 or Y416 and Tyr527 or Y527 are the two regulatory phosphorylation sites at the activation loop of the kinase domain (SH1) promotes the activity of the enzyme and at C-terminal tail to inactivate the enzyme respectively.[5] SFKs play important roles in the regulation of a wide array of normal cellular signal transduction pathways, such as cell division, growth factor signaling differentiation, survival, adhesion, migration and invasion.[6] Src tyrosine kinase overexpression or mutations are associated with malignant transformation in many human cancer diseases, such as colon, lung, breast, prostate, ovary and pancreas.[7] Boustinib (SKI-6606), dasatinib (BMS-354825), saracatinib (AZD-0530) and ponatinib are some of the Src/multikinase inhibitors that have been approved by the FDA for the treatment of chronic myelogenous leukemia and few others KX2-391, INNO-406, XL-999 and XL-228 are in clinical trial for a variety of solid tumors (**Figure 2**).[8] In addition, it has been recognized that Src kinases could be important in diseases related to multiple organ systems. For example, Src inhibitor saracatinib is being investigated in six non-oncological indications. Hence, there has been rising interest in the development of Src kinase inhibitors in recent years, both for their use as research probes, delineating the specific functions of these kinases, and as potential anticancer/related therapeutics.[9]

Natural products are well recognized as biologically prevalidated starting points for drug discovery.[10] Many strategies are being adapted to exploit advantageously the features of natural products in the design of novel small molecules for various biological applications. One such a successful approach is based on the concept of "molecular hybridization approach" as it combines the structural features of different classes of compounds to provide compounds with improved or unprecedented biological activities.[11]

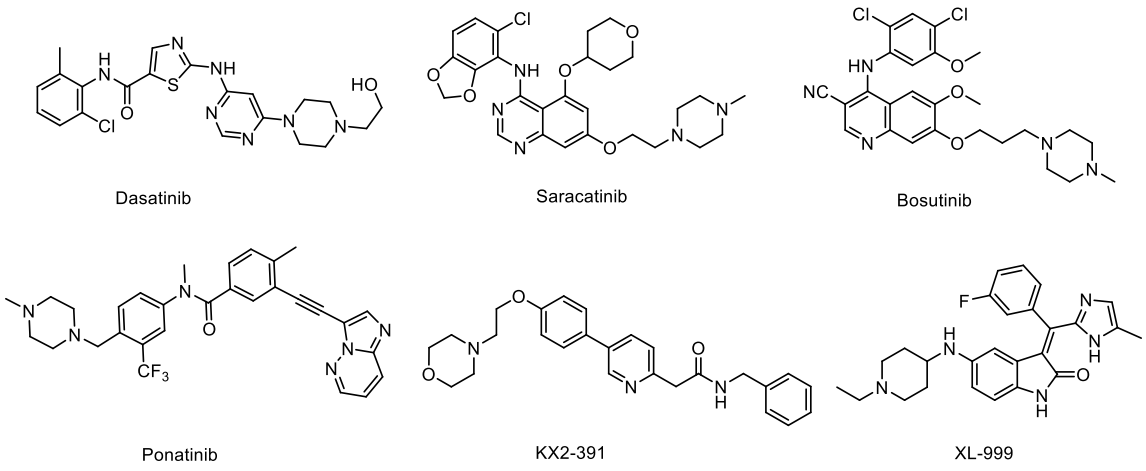


Figure 2. Representative examples of Src kinase inhibitors (marketed drugs/clinical trials)

α-aminophosphonates and α-aminophosphonic acids constitute an important group of naturally occurring compounds that are considered as structural analogues of the natural α-amino acids.[12] They have a broad range of medicinal applications and employed as antibiotics, enzyme inhibitors, herbicides, HIV protease, peptide mimics, plant growth regulators etc,.[13] Further, they are well known for their biological activities such as anticancer, antiviral, antifungal and anti-leishmanial.[14] In many instances, it has been shown that these α-aminophosphonates has been incorporated into the organic heterocycles/natural products and newly generated hybridized molecules often exhibit improved or unprecedented biological properties.

3.2.2. Present work

Objective

In the previous section, synthesis of various chromone-triazole conjugates were prepared and evaluated their anti-TB potential. Further, chromone and coumarin derivatives are known for the inhibition of Src kinase protein as well.[15] Intrigued by these reports, in this section we describe the synthesis of new chromone/azachromone fused α-amino phosphonates and study their c-Src kinase inhibitory activity. Further, we studied the molecular docking and chemoinformatics analysis to understand the drug-likeliness and effectiveness of these molecules against phosphorylated/unphosphorylated form of Src kinase.

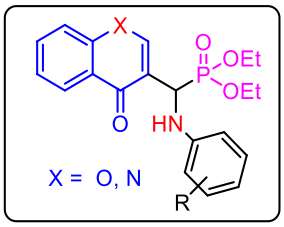


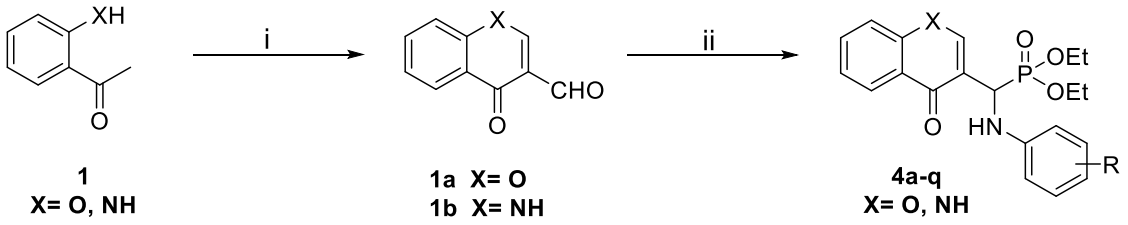
Figure 3. Design of chromone/azachromone fused α-aminophosphonate conjugates

3.2.3 Results and Discussion

Chemistry

Firstly, the precursor 3-chromone/azachromone carboxaldehyde **1a,b** was synthesized from commercially available 2-hydroxyacetophenone/2-aminoacetophenone using a Vilsmeier condition as per well known reported procedure.[16] Synthesis of the

targeted chromone/azachromone fused α-aminophosphonate derivatives (**4a-q**) were prepared through a three-component reaction involving aldehydes, amines and phosphites employing Kabachnik-Fields reaction condition. Usually, this reaction is performed in the presence of suitable Lewis and Bronsted acid catalysts, alkali metal alkoxides, solid catalysts, rare earth element or metal triflates and in ionic liquids.[17] Furthermore, few methods are catalyzed under solvent-free conditions for the synthesis of α-aminophosphonates.[18] However, many of these reported methods associated with disadvantages such as harsh reaction conditions, moisture sensitive, toxic or hazardous reagents, expensive catalysts, low yields, prolonged reaction times, and tedious workup procedures. Quite, incidentally, we discovered that silica chloride can act as a better catalyst for the Kabachnik-Fields reaction. Accordingly, 3-chromone carboxaldehyde/3-aza-chromone carboxaldehyde **1a,b** on condensation with different aryl amines (**2**) and diethyl phosphite (**3**) in the presence of silica chloride in ethanol at 60 °C afforded chromone/azachromone fused α-aminophosphonate conjugates **4a-q** (**Scheme 1**).

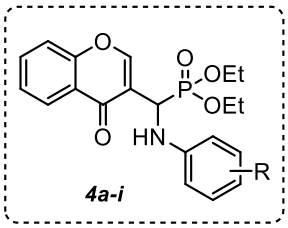


1
X= O, NH

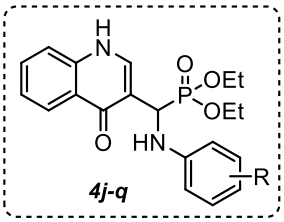
1a X= O
1b X= NH

4a-q
X= O, NH

Scheme 1. *Reagents and conditions*: (i) $POCl_3$, DMF, H_2O, 55 °C, 7 h, **1a** (75%) **1b** (85%); (ii) aromatic substituted amines **2**, diethylphosphite **3**, silica chloride, EtOH, 60 °C, 8-12 h .



4a-i



4j-q

Entry	R	Time; Yield
4a	C_6H_5-	(8h, 85%)
4b	(*p*-OMe)$C_6H_4CH_2$-	(12h, 60%)
4c	(*o*-OMe)C_6H_5-	(10h, 82%)
4d	(*m*-OH)C_6H_5-	(12h, 35%)
4e	(*m*-F)C_6H_5-	(12h, 70%)
4f	(*o*-^{i}Pr)C_6H_5-	(10h, 80%)
4g	(*o*-Cl)C_6H_5-	(10h, 78%)
4h	(*p*-OMe)C_6H_5-	(8h, 80%)
4i	(*m*-Cl)C_6H_5-	(12h, 76%)

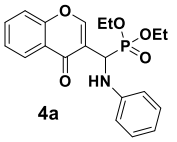
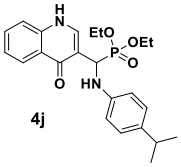
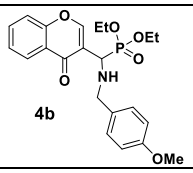
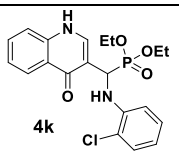
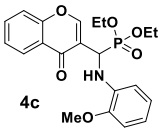
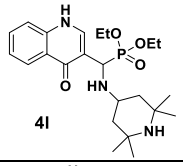
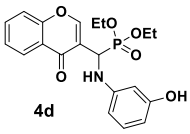
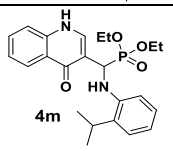
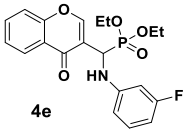
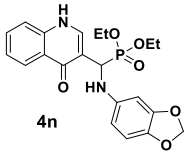
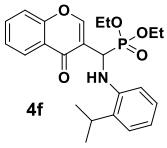
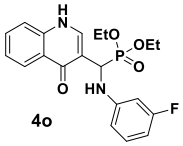
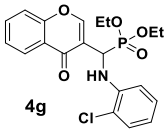
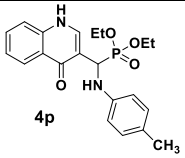
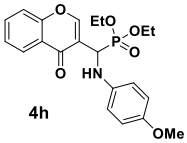
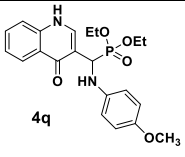
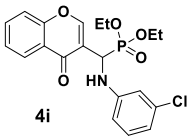
Entry	R	Time; Yield
4j	(*p*-^{i}Pr)C_6H_5-	(8h, 88%)
4k	(*m*-Cl)C_6H_5-	(12h, 76%)
4l	2,2,6,6-tetramethylpiperidine	(12h, 62%)
4m	(*o*-^{i}Pr)C_6H_5-	(10h, 80%)
4n	benzo[d][1,3]dioxole	(10h, 75%)
4o	(*m*-F)C_6H_5-	(12h, 72%)
4p	(*p*-Me)C_6H_5-	(8h, 82%)
4q	(*p*-OMe)C_6H_5-	(8h, 85%)

Most of the compounds were obtained in good yields (60-85%) with a reaction time of 8-12 hours. All the new compounds **4a-q** were fully characterized by ^{1}H NMR and ^{13}C NMR spectroscopic analysis. For example, in the ^{1}H NMR spectrum of **4a**, the signals of the two methyleneoxy (-OC**H**$_2$CH$_3$) protons attached with phosphorus was observed at δ 4.0-4.40 ppm as multiplets and two methyl (-OCH$_2$C**H**$_3$) protons were discernible at δ 1.19 ppm and δ 1.34 ppm as triplets. The chemical shifts correspond to two methyl protons (-OCH$_2$C**H**$_3$) were different due to the low rate of environmental exchange caused by the slow rotation of the P-C bond. Due to the coupling with the phosphorous atom, the signals of methylene proton (P(O)C**H**) appeared at δ 5.37 ppm as a doublet with coupling constant $^{2}J_{PH}$ = 24.0 Hz. In the ^{13}C NMR spectrum of **4a**, the signals appeared as doublets at δ 176.3, 155.1, 63.8, 63.4, 45.1, 16.4, and δ 16.3 ppm corresponding to carbonyl (CO), C-2 chromone carbon, -O**C**H$_2$CH$_3$, -**C**HP(O) and -OCH$_2$**C**H$_3$ with coupling constants 3.8 Hz, 5.4 Hz, 7.0 Hz and 5.4 Hz respectively. Further, the formation of **4a** was confirmed by its IR spectra, the absorption band corresponds to carbonyl and -NH displayed at 1643 cm^{-1} and 3409 cm^{-1}. The structures of all the compounds **4a-q** were further confirmed by HRMS analysis.

c-Src kinase inhibitory activity

An array of all the synthesized compounds (**4a-q)** was evaluated for their c-Src kinase inhibitory activity. The results of c-Src kinase inhibitory potency **4a-q** are depicted in **Table 1**. The IC$_{50}$ value (μM) was determined for each compound. Protein kinase inhibitor, Staurosporine, and an Src kinase inhibitor, PP2 were employed as the positive controls. Out of the seventeen compounds screened, three compounds (**4c, 4j, 4o**) showed moderate c-Src kinase inhibition in the range of IC$_{50}$ values between 15.8-63.6 μM. In particular, 4-isopropyl derivative (**4j**), was identified as the most potent compound with IC$_{50}$=15.8 μM in the series. Structure-activity relationship studies suggest that the presence electron donating group 2-methoxy group in compound **4c** exhibits modest activity with IC$_{50}$ value 40.6 μM and presence of methoxy group present at C4-position of aniline ring (**4b**, **4h**, **4q**) didn't show the inhibitory activity. Introduction of electronegative fluorine group at C3-position in amine aromatic ring of aza-chromone derivative **4o** showed significant activity with IC$_{50}$ value 63.6 μM, whereas in chromone derivative **4e** showed the poor inhibitory activity. The replacement with chlorine group at C2-position in amine aromatic ring (**4i**) exhibited the weaker activity with IC$_{50}$ value 85.0 μM, whereas in

Table 1. *In vitro* c-Src kinase inhibitory activity of chromone/aza-chromone fused α-aminophosphonates

Entry	Compound	IC_{50} (μM)[a]	Entry	Compound	IC_{50} (μM)[a]
1.	 4a	>150	10.	 4j	15.8
2.	 4b	>150	11.	 4k	>150
3.	 4c	40.6	12.	 4l	98.1
4.	 4d	>150	13.	 4m	>150
5.	 4e	>150	14.	 4n	>150
6.	 4f	>150	15.	 4o	63.6
7.	 4g	>150	16.	 4p	>150
8.	 4h	>150	17.	 4q	>150
9.	 4i	85.0		Staurosporine PP2	0.6 0.5

[a] The concentration of the compound that inhibited enzyme activity by 50%

compounds **4g**, **4k** didn't show any significant c-Src kinase inhibitory activity. Furthermore, the 2,2,6,6-tetramethylpiperidine (**4l**) derivative showed weak inhibitory activity with IC_{50} value 98.1 µM. The unsubstituted aromatic amine ring of compound **4a** and the rest of the substituted derivatives (**4d**, **4f**, **4m-n**, **4p**) with IC_{50} values more than 150 µM showed the poor activity against c-Src kinase.

Computational studies

Methodology

Preparation of macromolecule

The protein targets retrieved from RCSB Protein Data Bank were unphosphorylated proto-oncogenic tyrosine protein kinase Src (PDB code 1Y57) and phosphorylated tyrosine protein kinase Src (PDB code 2H8H) which served as docking receptors. The proteins were fixed for errors in atomic representations and optimized using Protein Preparation Wizard Maestro v10.3.[19] The bond orders were assigned to residues, hydrogen atoms were added at pH 7.0. Minimization was carried out using OPLS 2005 force field with an RMSD cut-off value of 0.3Å.

Preparation of ligands

The 2D structures of all compounds i.e. **4c, 4i, 4j**, staurosporine, and PP2 were drawn and analyzed by Marvin view. The compounds were converted to 3D structure (.pdb) using LigPrep tool.[20] LigPrep is a Schrödinger suite tool which is used to generate 3D structures from 2D structures, search tautomers, isomers for compounds and carry out energy minimization by applying the OPLS 2005 force field.

Molecular docking

The molecular docking was performed and analyzed via the Glide v6.8 docking tool.[21] The receptor grid was centered based on the active site of the protein using receptor grid generation tool. Ligands prepared using LigPrep were flexibly docked in grid box using Monte Carlo based simulation algorithm. An extra precision (XP) method was employed that generated binding poses based on energy. The favourably docked molecules were ranked according to the Glide Score.

Molecular docking analysis

With an objective to explore the binding potential of the synthesized and tested molecules for c-Src tyrosine kinase, we performed docking studies as shown in **Table 2**. Among the 17 structures, compounds **4c**, **4i**, and **4j** were mainly investigated further for docking simulations since they exhibit higher inhibitory activity as ascertained from their MIC data (**Table 1**).

Table 2. Molecular docking analysis of phosphorylated and unphosphorylated c-Src kinase tyrosine protein with selected compounds. The binding energies were calculated using Glide v6.8 docking tool: No docking score was obtained.

		Unphosphorylated Protein: 1Y57		Phosphorylated Protein: 2H8H	
Sr. No	Entry	Aminoacids involved in intermolecular interactions	GLIDE Score (kcal/mol)	Amino acids involved in intermolecular interactions	GLIDE Score (kcal/mol)
1	4c	Lys295 Asp404	-4.5	Lys295 Ala390	-5.0
2	4i	Asp404	-4.4	Lys295	-4.6
3	4j	Lys295 Ala390	**-5.4**	Asn391 Lys295	**-6.5**
4	4p	-	-	Ala390 Ser345	-6.10
5	4m	-	-	Lys295 Ala390	-4.18
6	Staurosporine	Met341	-8.3	-	-
7	PP2	Met341	-7.6	-	-

Human c-Src tyrosine kinase is a multi-domain protein consisting of 535 amino acids possessing SH3, SH2 domains followed by a short N-terminal and C-terminal regulation segment.[22] In the unphosphorylated structure, the SH2 and SH3 domains lie at a right angle to each other, with the only SH3 domain in contact with the N-terminal lobe. The SH3 domain is bound between SH2 and kinase domains in this inactive conformation.[23] The C-terminal tail folds back to N-terminal, and the active site is exposed as it is not blocked by the activation loop.[24] Upon phosphorylation of Tyr527 in C-terminal or Tyr 416 in the activation loop, the SH2 and SH3 domains lie parallel to the N and C lobes forming an open and closed cleft that determines the access to the catalytic site.[25] Phosphorylation of Tyr 527 down-regulates the kinase activity and phosphorylation of Tyr 416 is deemed necessary for exhibiting full kinase activity. The binding site for c-Src protein is an ATP

pocket present in the N-lobe region.[26] The residues lining the binding site of the protein were extracted from the X-ray data (**Table 3**). **Figure 3** displays the four subunits of the unphosphorylated c-Src Tyrosine kinase protein as well as the native ligand imatinib derivative.

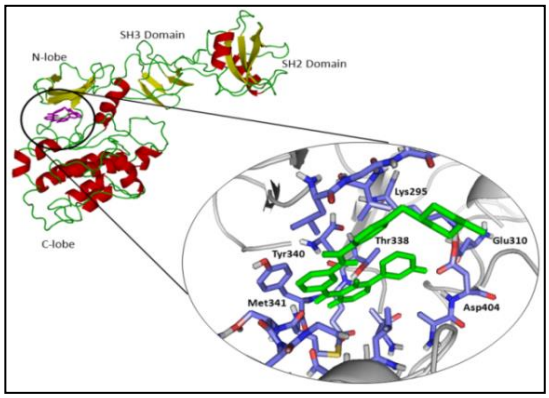


Figure 3. A) Structure of c-Src kinase protein (PDB ID: 1Y57) composed of the SH2 domain, SH3 domain, N-lobe, and C-lobe. B) Native ligand imatinib derivative (green) bound to the active site residues (blue).

Table 3. Amino acids residues present in the Src kinase pocket region

Sr no.	Pocket region	Amino acid residues in the binding region
1	Glycine-rich region and P-loop region	Leu273, Gly274, Gln275, Val281
2	Hinge region	Thr338, Gly339, Tyr340, Met341
3	Activation loop	Leu393
4	C terminal region	Trp260, Glu310,
5	DGF Motif	Asn404, Phe405, Gly406
6	β3 of the N-terminal lobe	Ala293

All the synthesized compounds were docked into the ATP binding site which is located at a cleft between the N and C-terminal lobes, flanked by the hinge region, P-loop, helix α C and the activation loop. The docked orientations of the **4j** compound with respect to the native ligands imatinib derivative in unphosphorylated protein (1Y57) and anilino quinazoline derivative with phosphorylated protein (2H8H) in the pocket site are depicted in **Figure 4** and **5** respectively. Compound **4j** and **4c** were bound to the Lys295 residue present in the C-terminal region of the pocket that acts as an important site for catalysis, binding of a compound to the terminal inhibits further kinase activity. Docking against the unphosphorylated protein (1Y57) showed that compound **4j** performed better with -5.4 kcal/mol binding energy as compared to **4c** and **4i** which displayed -4.5 and -4.4 kcal/mol binding energy respectively and formed only a single hydrogen acceptor bond with Asp 404

(**Table 2**). It is to be noted here that the compound **4j** displayed a similar good docking score of -6.5 kcal/mol with the phosphorylated protein (2H8H) as well. It formed two key interactions with both the proteins. The interaction with Lys 295 is common in both the receptors, however in the unphosphorylated protein (1Y57) it formed a bonded interaction with Ala390 and in the phosphorylated protein, it formed a bond with the Asn 391 residue (**Figure 6**).

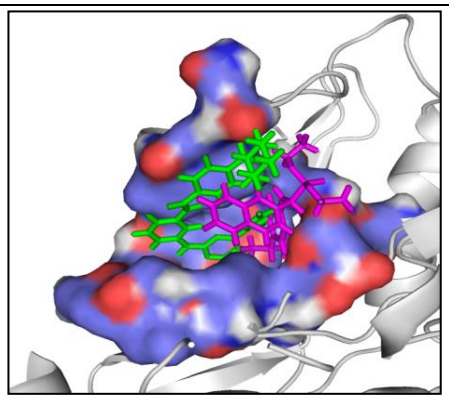


Fig (4). Surface view of Compound 4j (pink) and native ligand (green) in the binding pocket of receptor 1Y57 (Unphosphorylated protein).

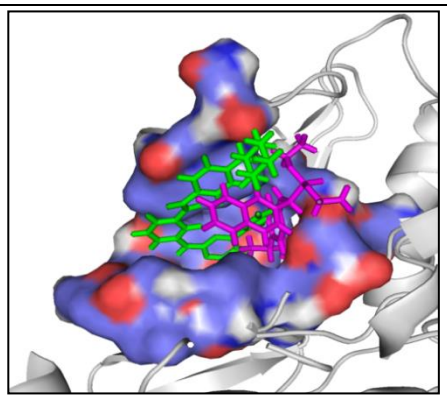
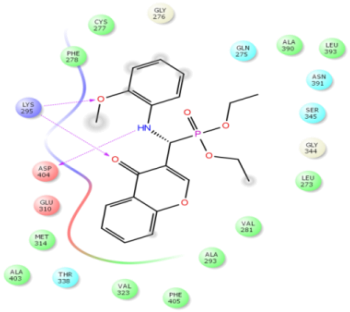
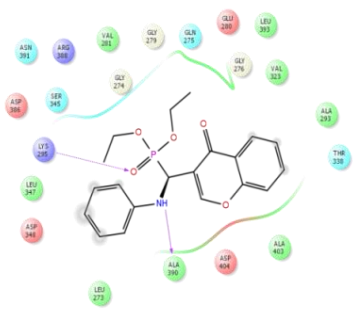


Fig (5). Surface view of Compound 4j (pink) and native ligand (green) in the binding pocket of receptor 2H8H (Phosphorylated protein)

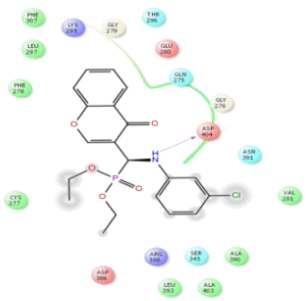
A) Compound 4c (1Y57 protein)



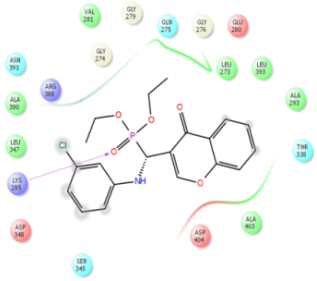
A) Compound 4c (2H8H protein)



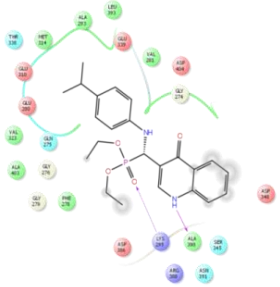
B) Compound 4i (1Y57 protein)



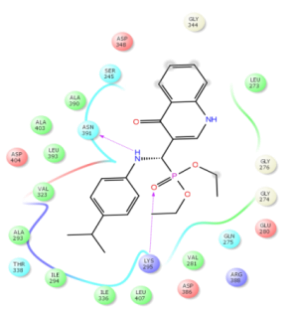
B) Compound 4i (2H8H protein)



C) Compound 4j (1Y57 protein)



C) Compound 4j (2H8H protein)



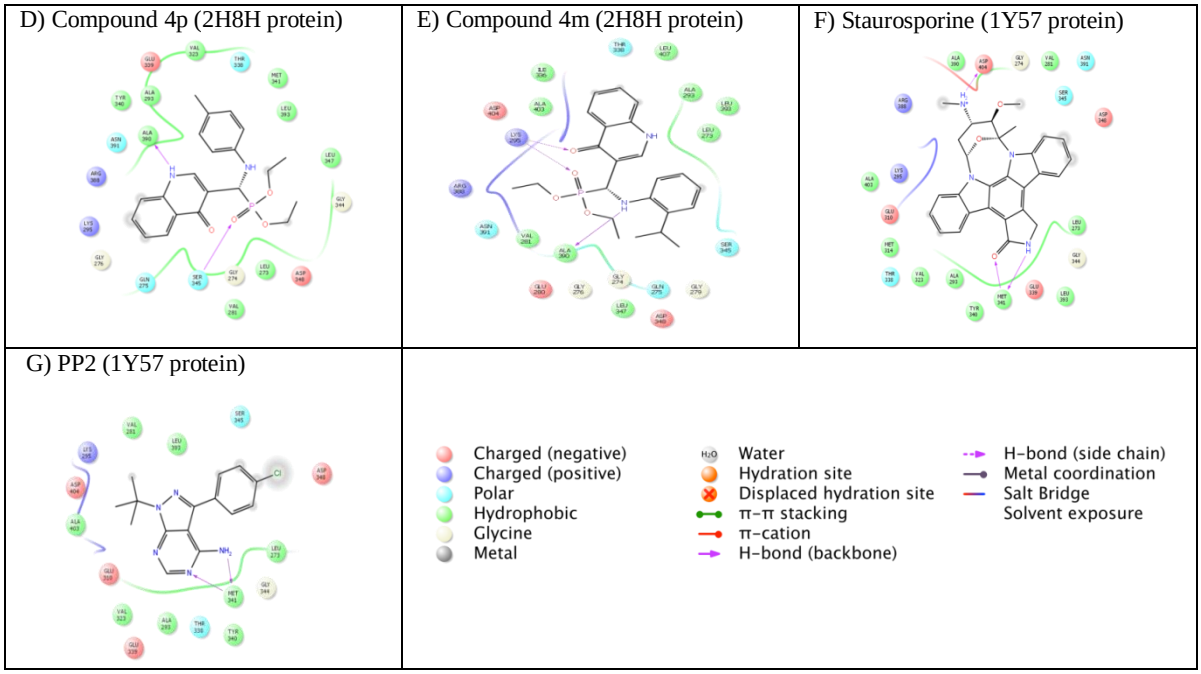
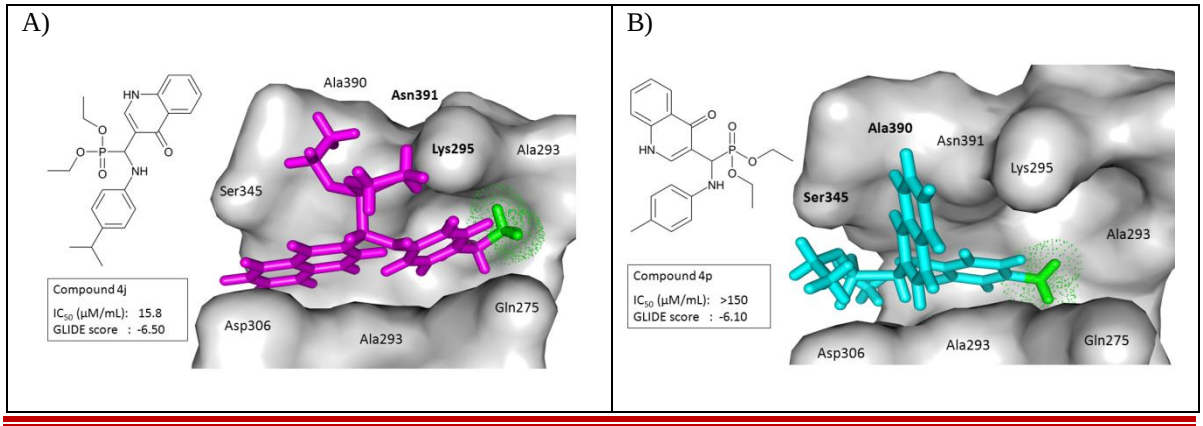


Fig (6). Amino acids involved in key intermolecular interactions for A) Compound 4c B) Compound 4i C) Compound 4j D) Compound 4p E) Compound 4m F) Staurosporine and G) PP2 for unphosphorylated protein (1Y57) and phosphorylated protein (2H8H).

As the compounds **4p** and **4j** had no significant difference in the docking scores there was a need to explore a plausible explanation of higher *in vitro* bioactivity exhibited by the compound **4j**. A comparative conformation analysis of the docked poses of **4j**, **4p**, and **4m** in the 2H8H pocket region led to a few interesting observations (**Figure 7**). It is to be noted that the isopropyl aniline and methyl groups of compound **4j** and **4p** respectively fit in the pocket regions, whereas the isopropyl aniline group in the compound **4m** seems to protrude out of the pocket region thereby leading to a lower binding efficiency. In the docked conformation of **4p**, the methyl group does not seem to fit well into the active site cavity.



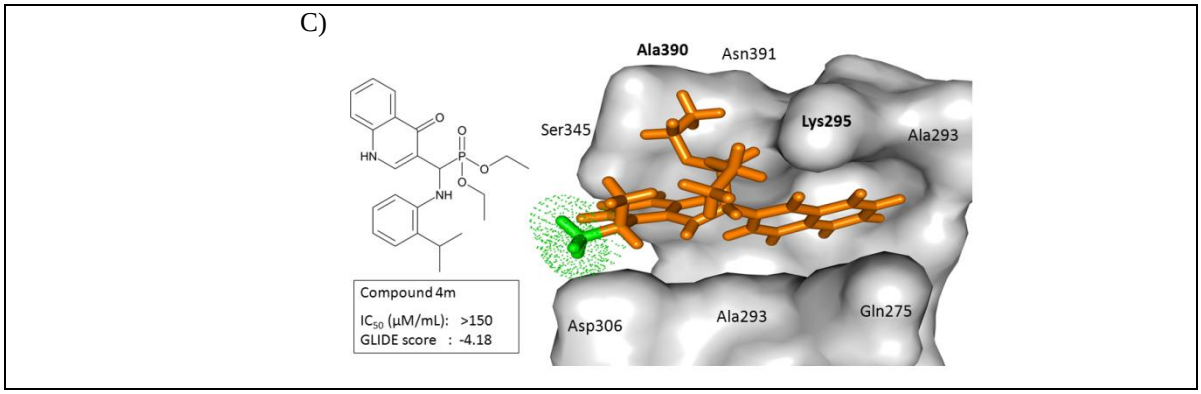


Fig (7). Figure showing the orientation of the compounds A) 4j B) 4p and C) 4m in the 2H8H pocket region. The green dots represent the functional group position of the compounds in the docked complex.

These observations indicate that the *para*-orientation of the isopropyl group in the aniline ring in compound **4j** may have an important role in anchoring it within the active site of the receptor.

Chemoinformatics Analysis

A druggability check was performed for all the 17 synthesized compounds (**Table 4**). Lipinski rule of 5 predictions was performed using the Screening Assistant 2 tool.[27] Most of the compounds displayed no violation of the standard Rule of 5 indicating they possess good drug-like properties. A drug-like and lead-like property analysis generated a score of 0.372 and 0.125 respectively. The notion that these compounds could be further developed as anti-cancer compounds were further assisted by ADME properties predicted using the PreADMET software.[28] The compounds possessed the desirable range of BBB model suggesting good pharmacological profile. It is well established that for a compound to be accepted in an oral dosage form, CaCo2 cell permeability selected compound **4k** prioritized in this study fulfilled the above 25 nms and the Human Intestinal Absorption (HIA) quantities should lie in the 50-100% range. The selected compound **4j** prioritized in this study fulfilled the above criteria indicating that it may be further developed in an oral dosage form. TPSA (Topological polar surface area) results indicated satisfactory values for all the 17 compounds.[29] Thus most synthesized compounds predicted favourable ADME predictions. LAZAR (Lazy structure-activity relationships) software detects carcinogenic properties based on the similarities in functional group with carcinogenic compounds present in the Lazar database.[30] Based on this software, all the compounds predicted non-carcinogenic properties wherein the confidence value greater than 0.025 suggested the

model to give highly reliable predictions.

Table 4. Chemoinformatics analysis

Properties	**Compounds**								
	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**
Lipinski Rule[a]									
Molecular weight	387.372	431.425	417.398	403.371	405.362	429.453	421.817	417.398	421.817
HB accept	4	6	5	5	4	4	4	5	4
HB donor	1	1	1	2	1	1	1	1	1
LogP	9.1135	9.0915	9.7864	8.2982	9.1296	11.2118	9.6439	9.7864	9.6439
Chemical properties									
Weiner path[b]	1704	2424	2046	1882	1882	2233	1861	2130	1882
Ring count[a]	3	3	3	3	3	3	3	3	3
PDL[a]	0.375	0.25	0.375	0.25	0.375	0.375	0.375	0.375	0.375
PLL[a]	0.125	0.25	0.125	0.125	0.125	0.25	0.125	0.125	0.125
ADME properties									
BBB (-3.0 – 1.2)[c]	1.27	0.65	1.12	0.71	1.21	0.90	1.29	1.07	1.19
CaCo2 (nms) (<25, poor, >500, best)[c]	21.70	21.71	21.70	21.57	21.70	21.71	21.48	21.70	21.51
HIA (50-100%) [c]	96.916	97.519	97.30	94.45	96.91	96.73	96.61	98.87	95.02
Rotatable bonds (0 - 15)[a]	10	13	12	11	10	13	10	12	10
TPSA (7.0 – 200.0)[b]	73.86	83.09	83.09	94.09	73.86	73.86	73.86	83.09	73.86
Toxicity properties[d]									
DSSTox Carcinogenic potency Mouse	Neg. (C: 0.066)	Neg. (C: 0.003)	Neg. (C: 0.080)	Neg. (C: 0.080)	Neg. (C: 0.066)	-	Neg. (C: 0.066)	Neg. (C: 0.080)	Neg. (C: 0.006)
Properties	**Compounds**								
	4j	**4k**	**4l**	**4m**	**4n**	**4o**	**4p**	**4q**	
Lipinski Rule[a]									
Molecular weight	**428.469**	420.833	449.532	428.469	430.397	404.378	400.415	416.414	
HB accept	**4**	4	6	4	6	4	4	5	
HB donor	**1**	2	3	2	2	2	2	3	
LogP	**9.56**	9.5932	11.0418	11.1611	9.2098	9.0789	9.7475	8.6768	
Chemical properties									
Weiner path[b]	**2359**	1861	2436	2233	2287	1882	1903	2130	
Ring count[a]	**3**	3	3	3	4	3	3	3	
PDL[a]	**0.375**	0.375	0.375	0.375	0.375	0.25	0.375	0.25	
PLL[a]	**0.125**	0.125	0.25	0.25	0.125	0.125	0.125	0.125	
ADME properties									
BBB (-3.0 –	**1.35**	1.06	0.40	1.49	0.43	0.73	0.93	0.41	

1.2)[c]									
CaCo2 (nms) (<25, poor, >500, best)[c]	**21.69**	21.19	21.65	21.70	21.62	21.67	21.68	21.48	
HIA (50-100%) [c]	**94.51**	98.07	91.24	94.51	96.80	97.27	94.28	97.89	
Rotatable bonds (0 - 15)[a]	**9**	10	14	13	10	10	11	12	
TPSA (7.0 – 200.0)[b]		76.66	76.66	88.69	76.66	95.12	76.66	76.66	
Toxicity properties[d]									
DSSTox Carcinogenic potency Mouse		Neg. (C: 0.081)	-	Pos. (C: 0.009)	-	Neg. (C: 0.071)	-	Neg. (C: 0.081)	

[a] Computed using Screening Assistant 2 program. PDL (Progressive drug-like), PLL(Progressive lead like).
[b] Calculated using MOE(CCG) Chemoinformatics suite. [c]PreADMET software. [d]LAZAR wherein Neg = Negative, Pos = Positive and C = Confidence value

3.2.4. Conclusion

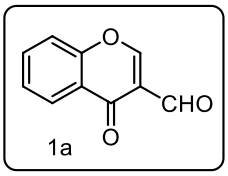
In conclusion, a series of chromone/azachromone fused α-aminophosphonate conjugates have been synthesized using silica chloride as a new catalyst. The structures of compounds were confirmed by ^{1}H NMR, ^{13}C NMR, IR, and HRMS analysis. All the synthesized compounds were evaluated for their c-Src kinase inhibitory activity and one of the compounds **4j** was found to be effective with an IC_{50} value of 15.8 µM. Docking studies revealed that compound **4j** to be more effective against the phosphorylated form of Src kinase.

3.2.5. Experimental Section

Preparation of 3-chromone carboxaldehyde/3-azachromone carboxaldehyde (1a, b)

To a stirred solution of dry DMF (40 mL), phosphorous oxychloride (3 equiv.) was added dropwise at 5 °C. The mixture was stirred for 15 min and then the solution of 2-hydroxyacetophenone or 2-aminoacetophenone **1** (1 equiv) in DMF (20 mL) was added dropwise at 5 °C. The reaction mixture was stirred at same temperature for 30 min and then heated and stirred at 55 °C for 4 h. The mixture was cooled to room temperature, poured into ice-water (approx. 400 mL) and stirred for 1.5 h. The precipitate was filtered off, washed ethanol yields **1a** (75%, colorless solid) or **1b** (85%, pale yellow solid).

1) 4-oxo-4*H*-chromene-3-carbaldehyde (1a)



Yield: 75% (colorless solid);

MP: 152-154 °C (lit.[31] mp: 152-154 °C);

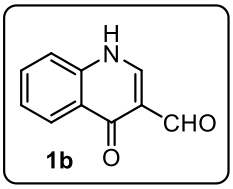
Molecular Formula: $C_{10}H_6O_3$;

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50-7.56 (m, 2 H), 7.74-7.79 (m, 1 H), 8.31 (dd, *J* = 7.8, 1.6 Hz, 1 H), 8.56 (s, 1 H), 10.40 (s, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 188.6 (CO), 176.0 (CO), 160.6 (CH), 156.2 (C), 134.8 (CH), 126.6 (CH), 126.2 (CH), 125.3 (C), 120.3 (C), 118.6 (CH);

HRMS (ESI): *m/z* calculated for $C_{10}H_6O_3$ $[M+H]^+$ 175.0390, found 175.0388.

2) 4-oxo-1,4-dihydroquinoline-3-carbaldehyde (1b)



Yield: 85% (pale yellow solid);

MP: >270 °C (lit.[32] mp: > 278 °C);

Molecular Formula: $C_{10}H_7NO_2$;

^{1}H NMR (400 MHz, DMSO-d_6): ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 (t, *J* = 7.3 Hz, 1 H), 7.66 (d, *J* = 7.8, 1 H), 7.55 (t, *J* = 7.3 Hz, 1 H), 8.20 (d, *J* = 7.8, 1 H), 8.47 (d, *J* = 6.8 Hz, 1 H), 10.19 (s, 1 H), 12.69 (bs, 1 H);

^{13}C NMR (100 MHz, DMSO-d_6): δ 188.7 (CO), 176.2 (CO), 143.2 (CH), 139.4 (C), 133.1 (CH), 127.7 (C), 125.4 (C), 125.3 (CH), 119.4 (CH), 116.3 (C);

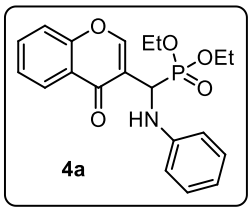
HRMS (ESI): *m/z* calculated for $C_{10}H_7O_2N$ [M+H]+ 174.0550, found 174.0549.

General method for the synthesis of chromone/aza-chromone fused α-aminophosphonates (4a-4q)

To a mixture of 3-chromone/aza-chromone carboxaldehyde (1.0 mmol), substituted amines **2** (1 mmol) and diethyl phosphate **3** (1.0 mmol) in ethanol (3 mL) was added silica chloride

(0.1 mol%). The resulting reaction mixture was stirred for 8-12 h at 60 °C. After completion of the reaction, the catalyst was filtered and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography [silica gel, EtOAc/petroleum ether (30:70)] afforded pure product **4a-4q**. Spectroscopic data of all compounds are given below.

1) Diethyl((4-oxo-4*H*-chromen-3-yl)(phenylamino)methyl)phosphonate (4a)



Yield: 85% (red brownish solid);

MP: 134-136 °C;

Molecular Formula: $C_{20}H_{22}NO_5P$;

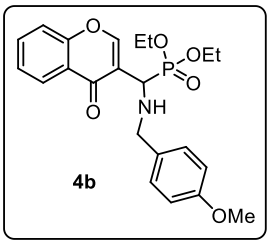
IR ($CHCl_3$, cm^{-1}): ν_{max} 3684, 3409, 2927, 2400, 1643, 1604, 1509, 1467, 1349, 1027, 974, 928, 849;

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.19 (t, *J* = 7.1 Hz, 3 H), 1.34 (t, *J* = 7.1 Hz, 3 H), 4.02-4.15 (m, 2 H), 4.22-4.29 (m, 2 H), 4.77 (bs, 1 H), 5.37 (d, J_{PH} = 24.0 Hz, 1 H, NCH), 6.67 (d, *J* = 7.8 Hz, 2 H), 6.73 (t, *J* = 7.3 Hz, 1 H), 7.14 (t, *J* = 7.8 Hz, 2 H), 7.42-7.45 (m, 2 H), 7.68 (t, *J* = 8.3 Hz, 1 H), 8.15 (d, *J* = 3.0 Hz, 1 H), 8.27 (d, *J* = 7.8 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.3 (d, *J* = 3.8 Hz, CO), 156.2 (C), 155.1 (d, *J* = 5.4 Hz, CH), 145.2 (C), 133.8 (CH), 129.4 (CH, 2 carbons), 125.9 (C), 125.4 (CH), 123.4 (C), 120.2 (C), 118.8 (CH), 118.3 (CH), 113.6 (CH, 2 carbons), 63.8 (d, J_{PC} = 7.0 Hz, -O$\underline{CH_2}CH_3$), 63.4 (d, J_{PC} = 6.9 Hz, -O$\underline{CH_2}CH_3$), 45.1 (d, J_{PC} = 156.4 Hz, -CHP), 16.4 (d, J_{PC} = 5.4 Hz, -OCH$_2\underline{CH_3}$), 16.3 (d, J_{PC} = 5.4 Hz, -OCH$_2\underline{CH_3}$);

HRMS (ESI): *m/z* calculated for $C_{20}H_{22}NO_5P$ $[M+Na]^+$ 410.1128, found 410.1121.

2) Diethy(((4-methoxybenzyl)amino)(4-oxo-4*H*-chromen-3-yl)methyl)phosphonate (4b)



Yield: 60% (pale yellow solid);

MP: 152-154 °C;

Molecular Formula: $C_{22}H_{26}NO_6P$;

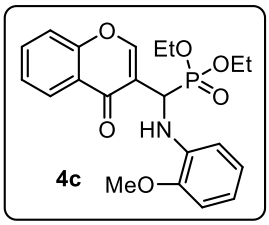
IR ($CHCl_3$, cm^{-1}): ν_{max} 3662, 3350, 2933, 2400, 1643, 1603, 1573, 1512, 1465, 1401, 1315, 1128, 1051, 1029, 974, 668;

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22 (t, *J* = 7.1 Hz, 3 H), 1.32 (t, *J* = 7.1 Hz, 3 H), 3.76 (s, 3H), 3.79 (s, 2 H), 4.04-4.10 (m, 2 H), 4.16-4.27 (m, 2 H), 4.41 (t, *J* = 5.9 Hz, 1 H), 4.57 (d, J_{PH} = 20.0 Hz, 1 H, NCH), 6.82 (d, *J* = 8.8 Hz, 2 H), 6.87-6.92 (m, 1 H), 7.19-7.21 (m, 1 H), 7.41-7.49 (m, 2 H), 8.20-8.24 (m, 2 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.4 (d, *J* = 4.6 Hz, CO), 158.7 (C), 156.1 (C), 155.8 (d, *J* = 6.9 Hz, CH), 155.8 (d, *J* = 5.4 Hz, CH), 133.6 (CH), 131.3 (C), 129.5 (CH, 2 carbons), 125.9 (CH), 125.3 (CH), 123.6 (C), 120.1 (C), 113.7 (CH, 2 carbons), 63.3 (d, J_{PC} = 6.9 Hz, -O$CH_2$$CH_3$), 62.7 (d, J_{PC} = 6.9 Hz, -O$CH_2$$CH_3$), 55.2 ($CH_3$), 51.4 (d, J_{PC} = 15.4 Hz, CH_2), 49.2 (d, J_{PC} = 157.2 Hz, -CHP), 16.4 (d, J_{PC} = 6.2 Hz, -$OCH_2$$CH_3$), 16.3 (d, J_{PC} = 6.2 Hz, -$OCH_2$$CH_3$);

HRMS (ESI): *m/z* calculated for $C_{22}H_{26}NO_6P$ $[M+H]^+$ 432.1571, found 432.1566; $C_{22}H_{26}NO_6P$ $[M+Na]^+$ 454.1390, found 454.1383.

3)Diethyl(((2-methoxyphenyl)amino)(4-oxo-4*H*-chromen-3-yl)methyl)phosphonate (4c)



Yield: 82% (pale yellow solid);

MP: 130-131 °C;

Molecular Formula: $C_{21}H_{24}NO_6P$;

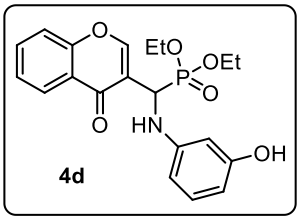
IR ($CHCl_3$, cm^{-1}): ν_{max} 3683, 3358, 2916, 2400, 1643, 1601, 1514, 1466, 1425, 1027, 928, 624;

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20 (t, *J* = 7.1 Hz, 3 H), 1.33 (t, *J* = 7.1 Hz, 3 H), 3.87 (s, 3 H), 4.03-4.16 (m, 2 H), 4.22-4.29 (m, 2 H), 5.21 (bs, 1 H), 5.40 (d, J_{PH} = 23.5 Hz, 1 H, NCH), 6.59 (d, *J* = 7.3 Hz, 1 H), 6.67-6.70 (m, 1 H), 6.75-6.79 (m, 2 H), 7.40-7.44 (m, 2H), 7.66 (t, *J* = 7.0 Hz, 1 H), 8.13 (d, *J* = 3.4 Hz, 1 H), 8.26 (d, *J* = 7.8 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.2 (d, J_{PC} = 3.8 Hz, CO), 156.2 (C), 155.2 (d, J = 5.4 Hz, CH), 147.2 (C), 135.3 (d, J_{PC} = 13.1 Hz, C), 133.7 (CH), 125.88 (CH), 125.3 (CH), 123.35 (C), 121.2 (CH), 120.4 (C), 118.2 (CH), 118.0 (CH), 110.9 (CH), 109.5 (CH), 63.7 (d, J_{PC} = 6.9 Hz, -O$\underline{CH_2}CH_3$), 63.3 (d, J_{PC} = 6.9 Hz, -O$\underline{CH_2}CH_3$), 55.4 (CH_3), 45.1 (d, J_{PC} = 155.7 Hz, -CHP), 16.3 (d, J_{PC} = 6.2 Hz, -OCH$_2\underline{CH_3}$), 16.2 (d, J_{PC} = 6.2 Hz, -OCH$_2\underline{CH_3}$);

HRMS (ESI): *m/z* calculated for $C_{21}H_{24}NO_6P$ $[M+H]^+$ 418.1414, found 418.1409; $C_{21}H_{24}NO_6P$ $[M+Na]^+$ 440.1233, found 440.1227.

4) Diethyl(((3-hydroxyphenyl)amino)(4-oxo-4*H*-chromen-3-yl)methyl)phosphonate (4d)



4d

Yield: 35% (colorless solid);

MP: 130-131 °C;

Molecular Formula: $C_{20}H_{22}NO_6P$;

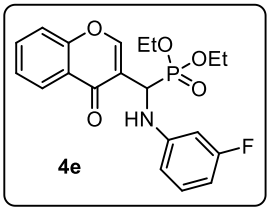
IR ($CHCl_3$, cm^{-1}): ν_{max} 3671, 3291, 2929, 2400, 1644, 1612, 1575, 1523, 1467, 1424, 1350, 1317, 1167, 1146, 1027, 973, 928;

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20 (t, J = 7.1 Hz, 3 H), 1.33 (t, J = 7.1 Hz, 3 H), 4.02-4.17 (m, 2 H), 4.22-4.28 (m, 2 H), 5.24-5.37 (m, 2 H), 6.53 (d, J = 7.8 Hz, 1 H), 6.32-6.44 (m, 1 H), 6.32-6.44 (m, 1 H), 7.02-7.06 (m, 1 H), 7.36-7.47 (m, 2 H), 7.63-7.71 (m, 1 H), 8.17-8.22 (m, 1 H), 8.26 (d, J = 3.3 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.1 (d, J_{PC} = 3.0 Hz, CO), 165.6 (C), 163.0 (C), 156.2 (C), 155.2 (d, J_{PC} = 5.2 Hz, CH), 147.2 (t, J_{PC} = 11.2 Hz, C), 133.6 (CH), 130.3 (CH), 126.0 (CH), 125.8 (CH), 124.0 (CH), 120.4 (C), 118.6 (CH), 109.5 (CH), 105.3 (CH), 63.8 (d, J_{PC} = 7.1 Hz, -O$\underline{CH_2}CH_3$), 63.4 (d, J_{PC} = 7.1 Hz, -O$\underline{CH_2}CH_3$), 45.0 (d, J_{PC} = 156.6 Hz, -CHP), 16.3 (d, J_{PC} = 5.6 Hz, -OCH$_2\underline{CH_3}$), 16.2 (d, J_{PC} = 5.6 Hz, -OCH$_2\underline{CH_3}$);

HRMS (ESI): *m/z* calculated for $C_{20}H_{22}NO_6P$ $[M+H]^+$ 404.1258, found 404.1256.

5) Diethyl(((3-fluorophenyl)amino)(4-oxo-4*H*-chromen-3-yl)methyl)phosphonate (4e)



Yield: 70% (pale yellow solid);

MP: 129-130 °C;

Molecular Formula: $C_{20}H_{21}FNO_5P$;

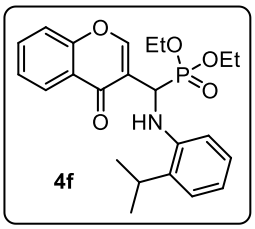
IR ($CHCl_3$, cm^{-1}): ν_{max} 3682, 3409, 1914, 2400, 1643, 1616, 1513, 1467, 1424, 1316, 1052, 1031, 928;

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.19 (t, *J* = 7.1 Hz, 3H), 1.33 (t, *J* = 7.1 Hz, 3H), 4.02-4.16 (m, 2H), 4.22-4.29 (m, 2H), 5.32-5.38 (m, 2H), 6.35-6.46 (m, 3H), 7.01-7.07 (m, 1H), 7.40-7.44 (m, 2H), 7.63-7.68 (m, 1H), 8.23-8.27 (m, 2H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.1 (d, J_{PC} = 3.1 Hz, CO), 165.0 (C), 162.6 (C), 156.1 (C), 155.2 (d, J_{PC} = 5.4 Hz, CH), 147.6 (t, J_{PC} = 11.6 Hz, C), 133.8 (CH), 130.3 (d, J_{PC} = 9.2 Hz, CH), 125.8 (CH), 125.4 (CH), 123.3 (C), 120.0 (C), 118.2 (CH), 109.0 (CH), 105.1 (d, J_{PC} = 21.6 Hz, CH), 100.7 (d, J_{PC} = 25.4 Hz, CH), 63.8 (d, J_{PC} = 7.1 Hz, -O$\underline{CH_2}CH_3$), 63.4 (d, J_{PC} = 6.9 Hz, -O$\underline{CH_2}CH_3$), 45.0 (d, J_{PC} = 156.4 Hz, -CHP), 16.3 (d, J_{PC} = 5.4 Hz, -$OCH_2\underline{CH_3}$), 16.2 (d, J_{PC} = 5.4 Hz, -$OCH_2\underline{CH_3}$);

HRMS (ESI): *m/z* calculated for $C_{20}H_{21}FNO_5P$ $[M+H]^+$ 406.1214, found 406.1208; $C_{20}H_{21}FNO_5P$ $[M+Na]^+$ 428.1034, found 428.1026.

6) Diethyl(((2-isopropylphenyl)amino)(4-oxo-4*H*-chromen-3-yl)methyl)phosphonate (4f)



Yield: 80% (pale yellow solid);

MP: 92-93 °C;

Molecular Formula: $C_{23}H_{28}NO_5P$;

IR ($CHCl_3$, cm^{-1}): ν_{max} 3683, 3380, 2926, 2400, 1643, 1524, 1467, 1424, 1052, 1031, 928;

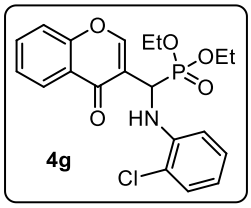
^{1}H NMR (400 MHz, $CDCl_3$): δ 1.21 (t, *J* = 6.8 Hz, 3 H), 1.31-1.36 (m, 9 H), 3.03 (q, *J* = 6.8 Hz, 2 H), 4.04-4.14 (m, 2 H), 4.22-4.29 (m, 2 H), 4.83 (bs, 1 H), 5.37 (d, J_{PH} = 23.5 Hz,

1 H, NCH), 6.56 (d, *J* = 7.8 Hz, 1 H), 6.76 (t, *J* = 7.3 Hz, 1 H), 7.02 (t, *J* = 7.3 Hz, 1 H), 7.16 (d, *J* = 7.3 Hz, 1 H), 7.42-7.45 (m, 1 H), 7.66-7.70 (m, 1 H), 8.10 (d, *J* = 3.4 Hz, 1 H), 8.28 (d, *J* = 7.8 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.3 (d, J_{PC} = 3.1 Hz, CO), 156.2 (C), 154.9 (d, J_{PC} = 6.2 Hz, CH), 142.1 (d, J_{PC} = 12.3 Hz, CH), 133.8 (CH), 133.0 (C), 126.9 (CH), 125.9 (CH), 125.4 (CH), 125.2 (CH), 123.4 (C), 120.2 (C), 118.7 (CH), 118.2 (CH), 111.3 (CH), 63.6 (d, J_{PC} = 6.9 Hz, -O$\underline{CH_2}$$CH_3$), 63.5 (d, J_{PC} = 6.9 Hz, -O$\underline{CH_2}$$CH_3$), 45.3 (d, J_{PC} = 155.0 Hz, -CHP), 27.4 (CH), 22.3 (CH_3, 2 carbons), 16.4 (d, J_{PC} = 5.4 Hz, -OCH$_2$$\underline{CH_3}$), 16.3 (d, J_{PC} = 5.4 Hz, -OCH$_2$$\underline{CH_3}$);

HRMS (ESI): *m/z* calculated for $C_{23}H_{28}NO_5P$ $[M+H]^+$ 430.1778, found 430.1771.

7) Diethyl(((2-chlorophenyl)amino)(4-oxo-4*H*-chromen-3-yl)methyl)phosphonate (4g)



4g

Yield: 78% (pale yellow solid);

MP: 82-83 °C;

Molecular Formula: $C_{20}H_{21}ClNO_5P$;

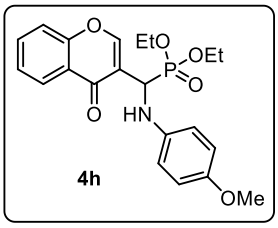
IR ($CHCl_3$, cm^{-1}): ν_{max} 3667, 3322, 2934, 1643, 1612, 1596, 1574, 1466, 1420, 1349, 1315, 1167, 1095, 1050, 1030, 975, 929;

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (t, *J* = 6.8 Hz, 3 H), 1.36 (t, *J* = 7.0 Hz, 3 H), 4.08-4.19 (m, 2 H), 4.22-4.31 (m, 2 H), 5.33 (bs, 1 H), 5.42 (d, J_{PH} = 23.5 Hz, 1 H, NCH), 6.68 (d, *J* = 6.8 Hz, 2 H), 7.08 (t, *J* = 7.6 Hz, 1 H), 7.28 (d, *J* = 7.8 Hz, 1 H), 7.45-7.49 (m, 2 H), 7.71 (t, *J* = 7.6 Hz, 1 H), 8.15-8.17 (m, 1 H), 8.29 (d, *J* = 7.8 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.1 (d, J_{PC} = 3.1 Hz, CO), 156.2 (C), 155.1 (d, J_{PC} = 5.4 Hz, CH), 141.6 (d, *J* = 12.3 Hz, CH), 133.9 (CH), 129.2 (CH), 128.0 (CH), 125.9 (CH), 125.5 (CH), 123.3(C), 120.0 (C), 119.1 (C), 118.8 (CH), 118.3 (CH), 112.3 (CH), 63.9 (d, J_{PC} = 6.9 Hz, -O$\underline{CH_2}$$CH_3$), 63.5 (d, J_{PC} = 6.9 Hz, -O$\underline{CH_2}$$CH_3$), 44.8 (d, J_{PC} = 155.7 Hz, -CHP), 16.4 (d, J_{PC} = 6.2 Hz, -OCH$_2$$\underline{CH_3}$), 16.3 (d, J_{PC} = 6.2 Hz, -OCH$_2$$\underline{CH_3}$);

HRMS (ESI): *m/z* calculated for $C_{20}H_{21}ClNO_5P$ $[M+H]^+$ 422.0919, found 422.0912; $C_{20}H_{21}ClNO_5P$ $[M+Na]^+$ 444.0738, found 444.0731.

8) Diethyl(((4-methoxyphenyl)amino)(4-oxo-4*H*-chromen-3-yl)methyl)phosphonate (4h)



Yield: 80% (red brownish solid);

MP: 116-117 °C;

Molecular Formula: $C_{21}H_{24}NO_6P$;

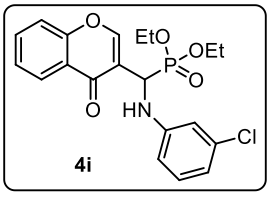
IR ($CHCl_3$, cm^{-1}): ν_{max} 3683, 3380, 1925, 2400, 1643, 1513, 1466, 1424, 1026, 928, 624;

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20 (t, *J* = 6.8 Hz, 3 H), 1.34 (t, *J* = 6.8 Hz, 3 H), 3.69 (s, 3 H), 4.04-4.15 (m, 2 H), 4.22-4.29 (m, 2 H), 4.50 (bs, 1 H), 5.29 (d, J_{PH} = 24.5 Hz, 1 H, NCH), 6.62 (d, *J* = 8.8 Hz, 2 H), 6.73 (d, *J* = 8.3 Hz, 2 H), 7.42-7.45 (m, 2 H), 7.68 (t, *J* = 7.8 Hz, 1 H), 8.13-8.15 (m, 1 H), 8.26 (d, *J* = 7.8 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.3 (d, J_{PC} = 3.8 Hz, CO), 156.2 (C), 155.1 (d, J_{PC} = 5.4 Hz, CH), 152.9 (C), 139.4 (d, J_{PC} =14.6 Hz, C), 133.8 (CH), 125.9 (CH), 125.4 (CH), 123.4 (C), 120.2 (C), 118.2 (CH), 114.9 (CH, 2 carbons), 114.8 (CH, 2 carbons), 63.7 (d, J_{PC} = 6.9 Hz, -O$\underline{CH_2}CH_3$), 63.3 (d, J_{PC} = 6.9 Hz, -O$\underline{CH_2}CH_3$), 55.6 (CH), 46.0 (d, J_{PC} = 156.7 Hz, -CHP), 16.4 (d, J_{PC} = 5.4 Hz, -$OCH_2\underline{CH_3}$), 16.3 (d, J_{PC} = 5.4 Hz, -$OCH_2\underline{CH_3}$);

HRMS (ESI): *m/z* calculated for $C_{21}H_{24}NO_6P$ $[M+H]^+$ 418.1414, found 418.1407; $C_{21}H_{24}NO_6P$ $[M+Na]^+$ 440.1233, found 440.1225.

9) Diethyl(((3-chlorophenyl)amino)(4-oxo-4*H*-chromen-3-yl)methyl)phosphonate (4i)



Yield: 76% (pale yellow solid);

MP: 141-142 °C;

Molecular Formula: $C_{20}H_{21}ClNO_5P$;

IR ($CHCl_3$, cm^{-1}): ν_{max} 3679, 3384, 2969, 2400, 1644, 1467, 1424, 1054, 1032, 1016, 928;

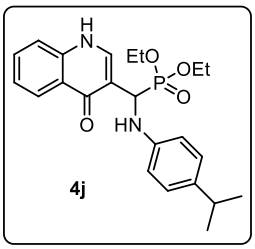
^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20 (t, *J* = 6.8 Hz, 3 H), 1.33 (t, *J* = 6.8 Hz, 3 H), 4.02-

4.16 (m, 2 H), 4.22-4.29 (m, 2 H), 5.26-5.38 (m, 2 H), 6.55 (d, *J* = 7.8 Hz, 1 H, NCH), 6.66 (d, *J* = 7.8 Hz, 1 H), 6.72 (s, 1 H), 7.02 (t, *J* = 7.8 Hz, 1 H), 7.41-7.44 (m, 2 H), 7.66 (t, *J* = 8.3Hz, 1 H), 8.21 (d, *J* = 3.1 Hz, 1 H), 8.26 (d, *J* = 7.8 Hz, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.1 (d, J_{PC} = 3.8 Hz, CO), 156.2 (C), 155.2 (d, J_{PC} = 5.4 Hz, CH), 147.0 (d, J_{PC} = 13.1 Hz, C), 134.9 (C), 133.8 (CH), 130.3 (CH), 125.9 (CH), 125.4 (CH), 123.3 (C), 119.9 (C), 118.5 (CH), 118.2 (CH), 113.8 (CH), 111.3 (CH), 63.8 (d, J_{PC} = 6.9 Hz, -O$\underline{CH_2}CH_3$), 63.4 (d, J_{PC} = 6.9 Hz, -O$\underline{CH_2}CH_3$), 45.0 (d, J_{PC} = 157.2 Hz, -CHP), 16.4 (d, J_{PC} = 5.4 Hz, -OCH$_2\underline{CH_3}$), 16.2 (d, J_{PC} = 5.4 Hz, -OCH$_2\underline{CH_3}$);

HRMS (ESI): *m/z* calculated for $C_{20}H_{21}ClNO_5P$ $[M+H]^+$ 422.0919, found 422.0912; $C_{20}H_{21}ClNO_5P$ $[M+Na]^+$ 444.0738, found 444.0732.

10) Diethyl(((4-isopropylphenyl)amino)(4-oxo-1,4-dihydroquinolin-3-yl)methyl)phosphonate (4j)



Yield: 88% (pale yellow solid);

MP: 197-198 °C;

Molecular Formula: $C_{23}H_{29}N_2O_4P$;

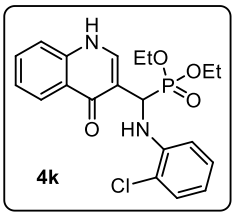
IR ($CHCl_3$, cm^{-1}): ν_{max} 3684, 3321, 2934, 2401, 1598, 1517, 1477, 1425, 1029, 928, 851;

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09-1.13 (m, 9 H), 1.38 (t, *J* = 7.1 Hz, 3 H), 2.72 (spt, *J* = 6.8 Hz, 1 H), 3.95-4.03 (m, 1 H), 4.07-4.16 (m, 1 H), 4.27-4.34 (m, 2 H), 5.56 (d, J_{PH} = 23.7 Hz, 1 H, NCH), 6.55 (d, *J* = 8.3 Hz, 2 H), 6.95 (d, *J* = 8.3 Hz, 2 H), 7.25-7.29 (m, 1 H), 7.41 (d, *J* = 8.3 Hz, 1 H), 7.49 (t, *J* = 7.3 Hz, 1 H), 7.85-7.90 (m, 1 H), 8.33 (d, *J* = 7.8 Hz, 1 H), 11.47 (bs, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.4 (d, J_{PC} = 3.8 Hz, CO), 143.6 (d, J_{PC} = 14.6 Hz, C), 139.4 (C), 139.2 (C), 137.6 (d, J_{PC} = 4.6 Hz, CH), 131.7 (CH), 127.1 (CH, 2 carbons), 125.8 (CH), 125.0 (C), 123.8 (CH), 118.1 (CH), 115.4 (C), 113.7 (CH, 2 carbons), 64.1 (d, J_{PC} = 6.9 Hz, -O$\underline{CH_2}CH_3$), 63.7 (d, J_{PC} = 6.9 Hz, -O$\underline{CH_2}CH_3$), 46.4 (d, J_{PC} = 155.7 Hz, -CHP), 33.1 (CH), 24.1 (CH_3, 2 carbons), 16.4 (d, J_{PC} = 5.4 Hz, -OCH$_2\underline{CH_3}$), 16.2 (d, J_{PC} = 5.4 Hz, -OCH$_2\underline{CH_3}$);

HRMS (ESI): *m/z* calculated for $C_{23}H_{29}N_2O_4P$ $[M+H]^+$ 429.1938, found 429.1935; $C_{23}H_{29}N_2O_4P$ $[M+Na]^+$ 451.1757, found 451.1753.

11) Diethyl(((2-chlorophenyl)amino)(4-oxo-1,4-dihydroquinolin-3-yl)methyl)phosphonate (4k)



Yield: 76% (orange red liquid);

Molecular Formula: $C_{20}H_{22}ClN_2O_4P$;

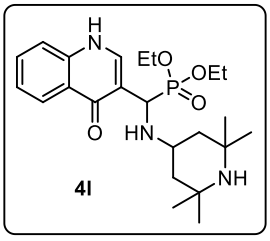
IR ($CHCl_3$, cm^{-1}): ν_{max} 3672, 3344, 2932, 2400, 1645, 1611, 1513, 1467, 1441, 1401, 1349, 1176, 1104, 1031, 970, 929;

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.14 (t, *J* = 7.1 Hz, 3 H), 1.39 (t, *J* = 6.8 Hz, 3 H), 4.03-4.21 (m, 2 H), 4.24-4.39 (m, 2 H), 5.32 (bs, 1 H), 5.67 (d, J_{PH} = 23.0 Hz, 1 H, NCH), 6.56-6.65 (m, 1 H), 6.69-6.73 (m, 1 H), 6.96-7.05 (m, 1 H), 7.17 (dd, *J* = 7.8, 1.3 Hz, 1 H), 7.24-7.32 (m, 1 H), 7.48-7.54 (m, 2 H), 7.91-7.99 (m, 1 H), 8.32 (d, *J* = 8.1 Hz, 1 H) 11.78 (bs, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.3 (d, J_{PC} = 4.0 Hz, CO), 141.6 (d, J_{PC} = 13.9 Hz, C), 139.4 (CH), 137.5 (d, J_{PC} = 4.4 Hz, CH), 131.8 (CH), 128.9 (CH), 128.0 (CH), 125.7 (CH), 124.9 (C), 124.0 (CH), 119.6 (C), 118.7 (CH), 118.3 (CH), 114.8 (d, *J* = 1.8 Hz, C), 112.7 (CH), 64.3 (d, J_{PC} = 7.0 Hz, -O$\underline{CH_2}CH_3$), 63.9 (d, J_{PC} = 7.3 Hz, -O$\underline{CH_2}CH_3$), 46.7 (d, J_{PC} = 155.5 Hz, -CHP), 16.4 (d, J_{PC} = 5.5 Hz, -$OCH_2\underline{CH_3}$), 16.2 (d, J_{PC} = 5.8 Hz, -$OCH_2\underline{CH_3}$);

HRMS (ESI): *m/z* calculated for $C_{20}H_{22}ClN_2O_4P$ $[M+H]^+$ 421.1078, found 421.1075; $C_{20}H_{22}ClN_2O_4P$ $[M+Na]^+$ 443.0898, found 443.0895.

12) Diethyl((4-oxo-1,4-dihydroquinolin-3-yl)((2,2,6,6-tetramethylpiperidin-4-yl)amino)methyl)phosphonate (4l)



Yield: 62% (pale yellow solid);

Molecular Formula: $C_{23}H_{36}N_3O_4P$;

MP: 242-244 °C;

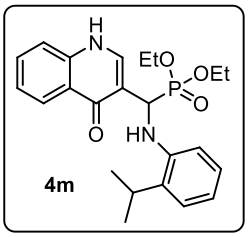
IR ($CHCl_3$, cm^{-1}): ν_{max} 3858, 3307, 2944, 2832, 2522, 2227, 2045, 1654, 1450, 1115, 1038,971, 822, 756, 667;

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.14-1.30 (m, 17 H), 1.44 (t, *J* = 7.1 Hz, 3 H), 1.65 (d, *J* = 12.2 Hz, 1 H), 2.04 (d, *J* = 12.2 Hz, 1 H), 2.60-2.68 (m, 2 H), 2.94-3.00 (m, 1 H), 4.00-4.16 (m, 2 H), 4.28-4.41 (m, 2 H), 5.0 (d, J_{PH} = 19.1 Hz, 1 H, NCH), 7.25-7.29 (m, 1 H), 7.46-7.54 (m, 2 H), 8.05 (s, 1 H), 8.28 (d, *J* = 8.3 Hz, 1 H), 11.98 (s, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.2 (CO), 139.7 (C), 139.5 (C), 138.6 (C), 138.5 (CH), 131.5 (CH), 125.9 (CH), 123.7 (CH), 118.4 (CH), 63.6 (d, J_{PC} = 6.9 Hz, -O$CH_2$$CH_3$), 63.5 (d, J_{PC} = 6.9 Hz, -O$CH_2$$CH_3$), 53.0 (C, 2 carbons), 47.2 (CH), 45.1 (CH), 44.1 (CH), 33.3 (CH), 27.5 (CH_3, 2 carbons), 27.3 (CH_3, 2 carbons), 16.6 (d, J_{PC} = 5.4 Hz, -$OCH_2$$CH_3$), 16.3 (d, J_{PC} = 6.8 Hz, -$OCH_2$$CH_3$);

HRMS (ESI): *m/z* calculated for $C_{23}H_{36}N_3O_4P$ $[M+H]^+$ 450.2516, found 450.2514.

13) Diethyl(((2-isopropylphenyl)amino)(4-oxo-1,4-dihydroquinolin-3-yl)methyl)phosphonate (4m)



Yield: 80% (colorless solid);

MP: 186-187 °C;

Molecular Formula: $C_{23}H_{29}N_2O_4P$;

IR ($CHCl_3$, cm^{-1}): ν_{max} 3683, 3385, 2927, 2400, 1629, 1575, 1524, 1476, 1424, 1031, 928, 849, 626;

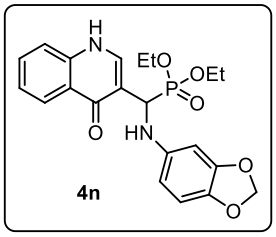
^{1}H NMR (400 MHz, $CDCl_3$): δ 1.06 (d, *J* = 6.8 Hz, 3 H), 1.14 (t, *J* = 7.1 Hz, 3 H), 1.22 (d, *J* = 6.4 Hz, 3 H), 1.41 (t, *J* = 6.8 Hz, 3 H), 2.83 (spt, *J* = 6.8 Hz, 1 H), 3.96-4.06 (m, 1 H), 4.10-4.20 (m, 1 H), 4.29-4.37 (m, 2 H), 4.85 (bs, 1 H), 5.60 (d, J_{PH} = 23.0 Hz, 1 H, NCH), 6.64 (d, *J* = 7.8 Hz, 1 H), 6.71 (t, *J* = 7.3 Hz, 1 H), 6.99 (t, *J* = 7.6 Hz, 1 H), 7.08 (d, *J* = 7.3 Hz, 1 H), 7.31 (t, *J* = 7.3 Hz, 1 H), 7.44 (d, *J* = 8.3 Hz, 1 H), 7.54 (d, *J* = 7.3 Hz, 1 H), 7.78-

7.80 (m, 1 H), 8.36 (d, *J* = 8.3 Hz, 1 H), 11.31 (bs, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.4 (d, J_{PC} = 3.8 Hz, CO), 142.3 (d, J_{PC} = 13.1 Hz, CH), 139.5 (C), 137.1 (d, J_{PC} = 3.8 Hz, CH), 132.7 (C), 131.7 (CH), 127.0 (CH), 126.0 (CH), 125.1 (C), 124.9 (CH), 123.9 (CH), 118.6 (C), 118.0 (CH), 115.4 (C), 111.8 (CH), 64.2 (d, J_{PC} = 7.0 Hz, -O$\underline{CH_2}CH_3$), 63.7 (d, J_{PC} = 7.7 Hz, -O$\underline{CH_2}CH_3$), 46.3 (d, J_{PC} = 155.7 Hz, -CHP), 27.4 (CH), 22.2 (CH_3), 22.0 (CH_3), 16.5 (d, J_{PC} = 5.4 Hz, -OCH$_2\underline{CH_3}$), 16.2 (d, J_{PC} = 5.4 Hz, -OCH$_2\underline{CH_3}$);

HRMS (ESI): *m/z* calculated for $C_{23}H_{29}N_2O_4P$ $[M+H]^+$ 429.1938, found 429.1933; $C_{23}H_{29}N_2O_4P$ $[M+Na]^+$ 451.1757, found 451.1750.

14) Diethyl((benzo[d][1,3]dioxol-5-ylamino)(4-oxo-1,4-dihydrquinoin-3-yl)methyl)phosphonate (4n)



Yield: 75% (red brownish solid);

MP: 193-194 °C;

Molecular Formula: $C_{21}H_{23}N_2O_6P$;

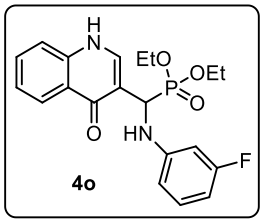
IR ($CHCl_3$, cm^{-1}): ν_{max} 3681, 3386, 2923, 2400, 1628, 1525, 1476, 1424, 1054, 1032, 1017, 928, 850, 625;

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.16 (t, *J* = 7.1 Hz, 3 H), 1.40 (t, *J* = 7.1 Hz, 3 H), 4.03-4.17 (m, 2 H), 4.25-4.35 (m, 2 H), 5.40 (d, J_{PH} = 22.5 Hz, 1 H, N$\underline{CH}$), 5.77 (s, 2 H), 6.06 (d, *J* = 8.3 Hz, 1 H), 6.25 (s, 1 H), 6.54 (d, *J* = 8.3 Hz, 1 H), 7.24-7.28 (m, 1 H), 7.39-7.41 (m, 1 H), 7.46-7.50 (m, 1 H), 7.87 (s, 1 H), 8.29 (d, *J* = 7.8 Hz, 1 H), 11.41 (bs, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.4 (d, J_{PC} = 3.8 Hz, CO), 148.3 (C), 141.3 (d, *J* = 15.4 Hz, C), 140.5 (CH), 139.4 (C), 137.6 (d, *J* = 4.6 Hz, CH), 131.7 (C), 125.8 (CH), 125.0 (C), 123.9 (CH), 118.1 (CH), 115.2 (C), 108.5 (CH), 105.6 (CH), 100.6 (CH_2), 97.0 (CH), 64.1 (d, J_{PC} = 7.7 Hz, -O$\underline{CH_2}CH_3$), 63.9 (d, J_{PC} = 6.7 Hz, -O$\underline{CH_2}CH_3$), 47.6 (d, J_{PC} = 154.9 Hz, -CHP), 16.5 (d, J_{PC} = 6.2 Hz, -OCH$_2\underline{CH_3}$), 16.2 (d, J_{PC} = 6.2 Hz, -OCH$_2\underline{CH_3}$);

HRMS (ESI): *m/z* calculated for $C_{21}H_{23}N_2O_6P$ $[M+H]^+$ 431.1366, found 431.1364; $C_{21}H_{23}N_2O_6P$ $[M+Na]^+$ 453.1186, found 453.1182.

15) Diethyl(((3-fluorophenyl)amino)(4-oxo-1,4-dihydroquinolin-3-yl)methyl)phosphonate (4o)



Yield: 72% (colorless solid);

MP: 152-154 °C;

Molecular Formula: $C_{20}H_{22}FN_2O_4P$;

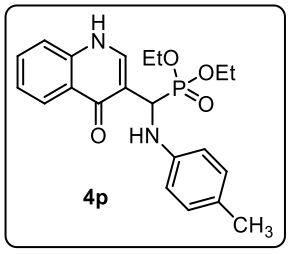
IR ($CHCl_3$, cm^{-1}): ν_{max} 3671, 3424, 2954, 2400, 1641, 1624, 1527, 1475, 1424, 1316, 1048, 1024, 928, 756, 667;

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.14 (t, J_{PH} = 7.1 Hz, 3 H), 1.33 (t, J_{PH} = 7.1 Hz, 3 H), 4.03-4.17 (m, 2 H), 4.23-4.38 (m, 2 H), 5.66 (d, J_{PH} = 23.0 Hz, 1 H, NCH), 6.32-6.47 (m, 3 H), 6.96-7.05 (m, 1 H), 7.39-7.46 (m, 2 H), 7.61-7.66 (m, 1 H), 8.23-8.27 (m, 2 H), 11.35 (bs, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.1 (d, J_{PC} = 3.1 Hz, CO), 164.9 (C), 162.5 (C), 156.2 (C), 155.1 (d, J_{PC} = 5.3 Hz, CH), 147.5 (C), 133.8 (CH), 130.3 (d, J_{PC} = 9.2 Hz, CH), 125.7 (CH), 125.3 (CH), 123.3 (C), 118.3 (CH), 109.1 (CH), 105.1 (d, J_{PC} = 21.7 Hz, CH), 100.6 (d, J_{PC} = 25.2 Hz, CH), 64.1 (d, J_{PC} = 7.1 Hz, -O$\underline{CH_2}$$CH_3$), 63.9 (d, J_{PC} = 6.9 Hz, -O$\underline{CH_2}$$CH_3$), 45.2 (d, J_{PC} = 156.0 Hz, -CHP), 16.4 (d, J_{PC} = 5.6 Hz, -$OCH_2\underline{CH_3}$), 16.2 (d, J_{PC} = 5.6 Hz, -$OCH_2\underline{CH_3}$);

HRMS (ESI): *m/z* calculated for $C_{20}H_{22}FN_2O_4P$ $[M+H]^+$ 405.1374, found 405.1367.

16) Diethyl((4-oxo-1,4-dihydroquinolin-3-yl)(p-tolylamino)methyl)phosphonate (4p)



Yield: 82% (pale yellow solid);

MP: 201-202 °C;

Molecular Formula: $C_{21}H_{25}N_2O_4P$;

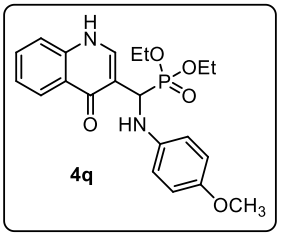
IR ($CHCl_3$, cm^{-1}): ν_{max} 3683, 3278, 2934, 2400, 1629, 1518, 1476, 1424, 1029, 928, 850, 625;

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.13 (d, *J* = 6.8 Hz, 3 H), 1.40 (t, *J* = 7.1 Hz, 3 H), 2.15 (s, 3 H), 3.95-4.04 (m, 1 H), 4.07-4.17 (m, 1 H), 4.29-4.32 (m, 2 H), 4.63 (bs, 1 H), 5.54 (d, J_{PH} = 22.5 Hz, 1 H, NCH), 6.52 (d, *J* = 7.8 Hz, 2 H), 6.89 (t, *J* = 7.8 Hz, 2 H), 7.25-7.29 (m, 1 H), 7.40 (d, *J* = 7.8 Hz, 1 H), 7.47-7.51 (m, 1 H), 7.84 (s, 1 H), 8.32 (d, *J* = 7.8 Hz, 1 H), 11.36 (bs, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.4 (d, J_{PC} = 3.8 Hz, CO), 143.3 (d, J_{PC} = 14.6 Hz, CH), 139.4 (C), 137.5 (CH), 131.7 (CH), 129.7 (CH, 2 carbons), 127.9 (C), 125.9 (CH), 125.1 (C), 123.8 (CH), 118.1 (CH), 115.3 (C), 113.9 (CH, 2 carbons), 64.1 (d, J_{PC} = 7.0 Hz, -O$CH_2$$CH_3$), 63.8 (d, J_{PC} = 7.0 Hz, -O$CH_2$$CH_3$), 46.5 (d, J_{PC} = 155.7 Hz, -CHP), 20.3 (CH_3), 16.5 (d, J_{PC} = 6.2 Hz, -OCH$_2$$CH_3$), 16.2 (d, J_{PC} = 6.2 Hz, -OCH$_2$$CH_3$);

HRMS (ESI): *m/z* calculated for $C_{21}H_{25}N_2O_4P$ $[M+H]^+$ 401.1625, found 401.1619; $C_{21}H_{25}N_2O_4P$ $[M+Na]^+$ 423.1444, found 423.1437.

17) Diethyl(((4-methoxyphenyl)amino)(4-oxo-1,4-dihydroquinolin-3-l)methyl)phosphonate (4q)



4q

Yield: 85% (dark red semisolid);

Molecular Formula: $C_{21}H_{25}N_2O_5P$;

IR ($CHCl_3$, cm^{-1}): ν_{max} 3662, 3230, 2835, 2401, 1621, 1565, 1476, 1442, 1392, 971, 822, 756, 667;

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.13 (d, *J* = 7.1 Hz, 3 H), 1.39 (t, *J* = 7.1 Hz, 3 H), 3.65 (s, 3 H), 3.95-4.04 (m, 1 H), 4.07-4.15 (m, 1 H), 4.28-4.38 (m, 2 H), 5.51 (d, J_{PH} = 22.5 Hz, 1 H, NCH), 6.58 (d, *J* = 8.8 Hz, 2 H), 6.67 (t, *J* = 8.8 Hz, 2 H), 7.25-7.29 (m, 1 H), 7.42-7.50 (m, 2 H), 7.92 (s, 1 H), 8.32 (d, *J* = 7.8 Hz, 1 H), 11.63 (bs, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.4 (d, J_{PC} = 3.8 Hz, CO), 152.8 (C), 139.7 (d, *J* = 14.6 Hz, C), 139.4 (C), 137.7 (d, J_{PC} = 4.6 Hz, CH), 131.6 (CH), 125.8 (CH), 125.0 (C), 123.8 (CH), 118.1 (CH), 115.3 (C), 115.1 (CH, 2 carbons), 114.7 (CH, 2 carbons), 64.0 (d, J_{PC} =

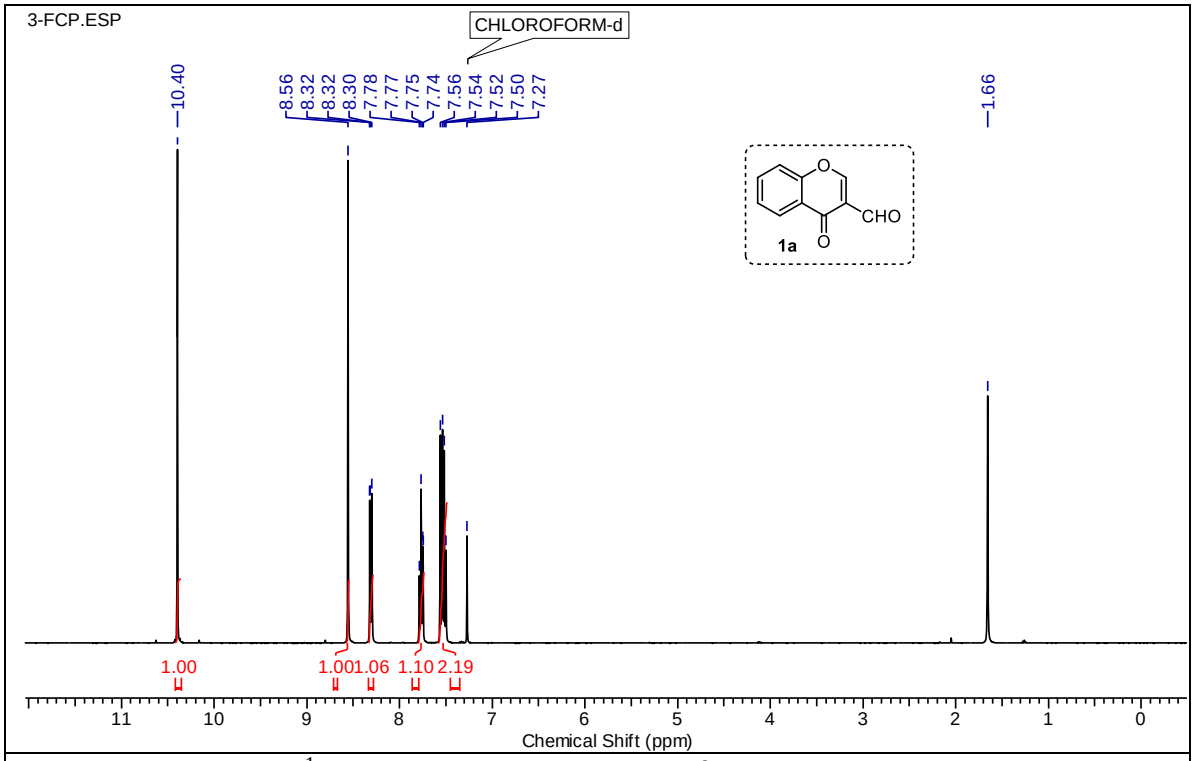
7.0 Hz, -O<u>CH$_2$</u>CH$_3$), 63.7 (d, J_{PC} = 7.0 Hz, -O<u>CH$_2$</u>CH$_3$), 55.6 (CH$_3$), 47.1 (d, J_{PC} = 155.7 Hz, -CHP), 16.4 (d, J_{PC} = 6.2 Hz, -OCH$_2$<u>CH$_3$</u>), 16.2 (d, J_{PC} = 6.2 Hz, -OCH$_2$<u>CH$_3$</u>);

HRMS (ESI): *m/z* calculated for $C_{21}H_{25}N_2O_5P$ $[M+H]^+$ 417.1574, found 417.1570.

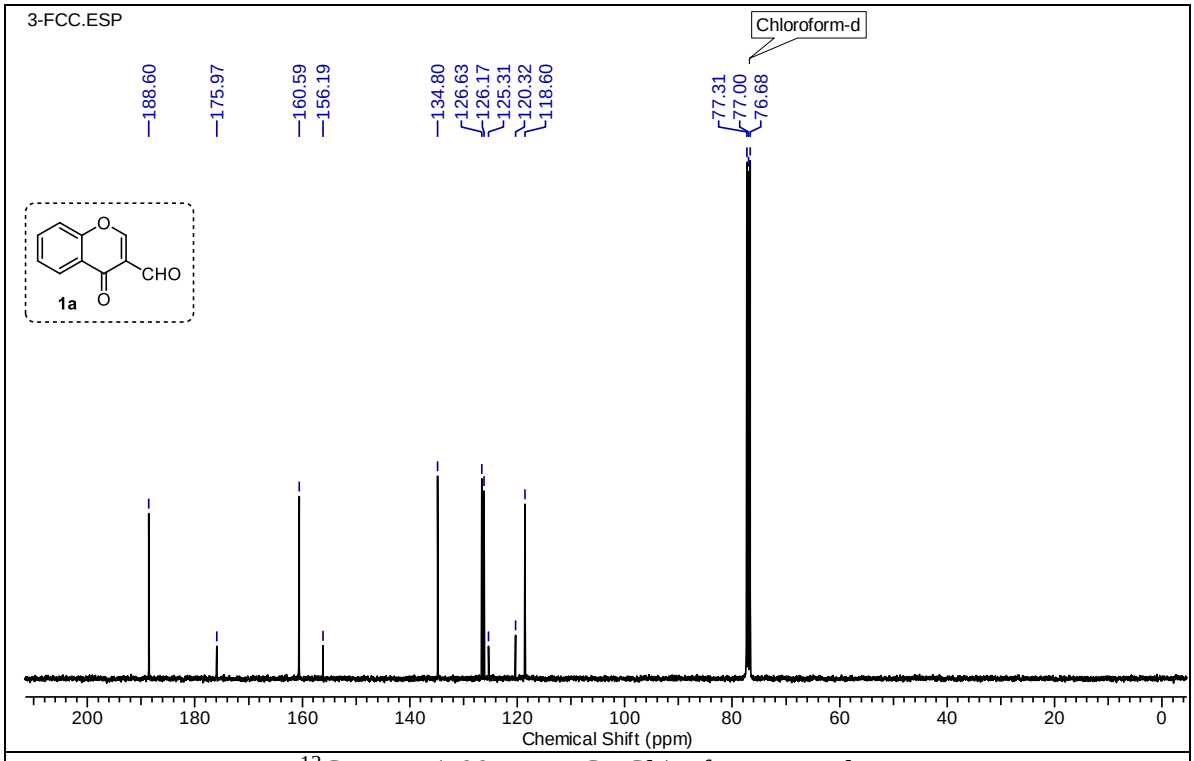
c-Src kinase assay

The effect of synthesized compounds on the activity of c-Src kinase was assessed by Transcreener® ADP^2 FI Assay, from Bell Brook Labs, Madison, WI, (catalog no. 3013-1K) according to manufacturer's protocol. 384-well Low volume Black nonbinding surface round bottom microplate was purchased from Corning (#3676). In summary, the kinase reaction was started in 384-well low volume black microplate with the incubation of the 2.5 μL of the reaction cocktail (0.7 nM of His$_6$-Src kinase domain in kinase buffer) with 2.5 μL of prediluted compounds (dissolved in 10% DMSO, 4X target concentration) for 10 min at room temperature using microplate shaker. The reaction cocktail was made using the kinase buffer HEPES (200 mM, pH 7.5), $MgCl_2$ (16 mM), EGTA (8 mM), DMSO (4%), Brij-35 (0.04%), and 2-mercaptoethanol (43 mM). Kinase reaction was started by adding 5 μL of ATP/substrate (40 μM/600μM) cocktail and incubated for 30 min at room temperature on microplate shaker. Src optimal peptide (AEEEIYGEFEAKKKK) was used as the substrate for the kinase reaction. Kinase reaction was stopped by adding 10 μL of the 1X ADP Detection Mixture to the enzyme reaction mixture and mixed using a plate shaker. The mixture was incubated at room temperature for 1 h, and the fluorescence intensity was measured. The 1X ADP Detection Mixture was prepared by adding ADP^2 Antibody-IRDyeR QC-1 (10 μg/mL) and ADP Alexa594 Tracer (8 nM) to Stop & Detect Buffer B(1X). Fluorescence Intensity measurements were performed using fluorescence intensity optical module using the excitation of 580 nm and emission of 630 nm with bandwidths of 10 nm by Optima, BMG Labtech microplate reader. IC_{50} of the compounds were calculated using ORIGIN 6.0 (origin lab) software. IC_{50} is the concentration of the compound that inhibited enzyme activity by 50%. All the experiments were carried out in triplicate.

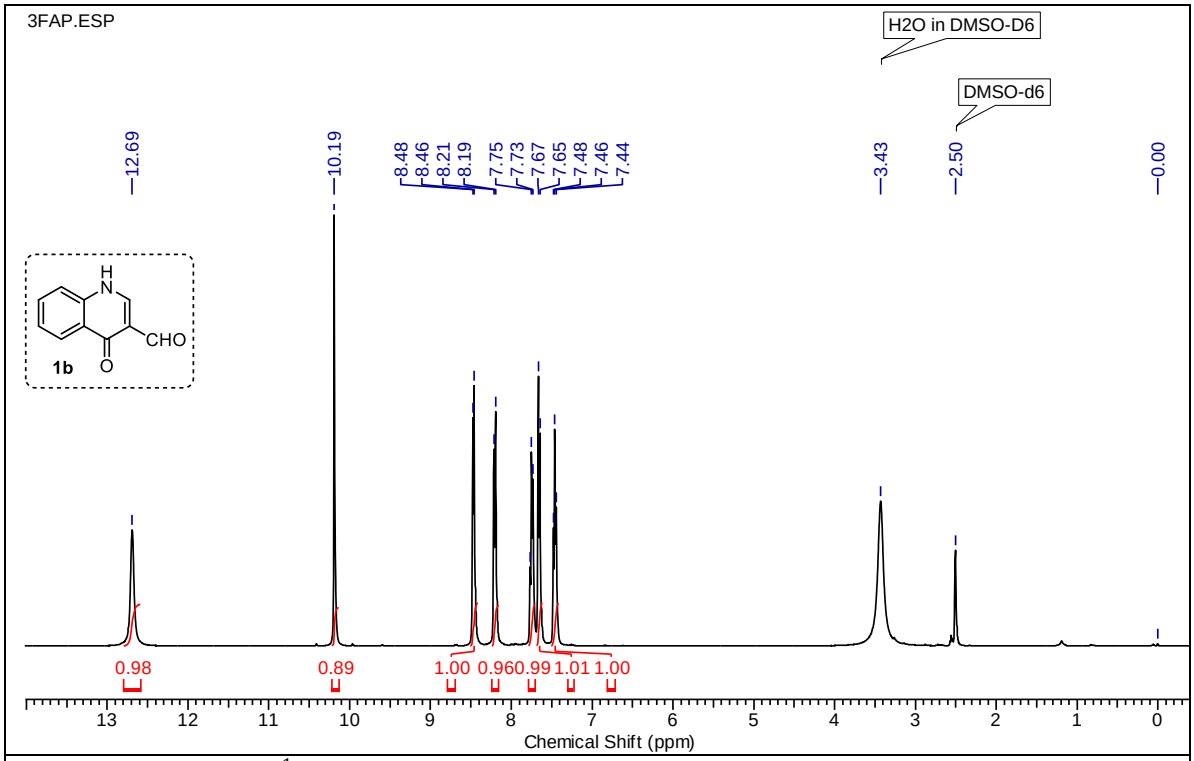
3.2.6. Spectra



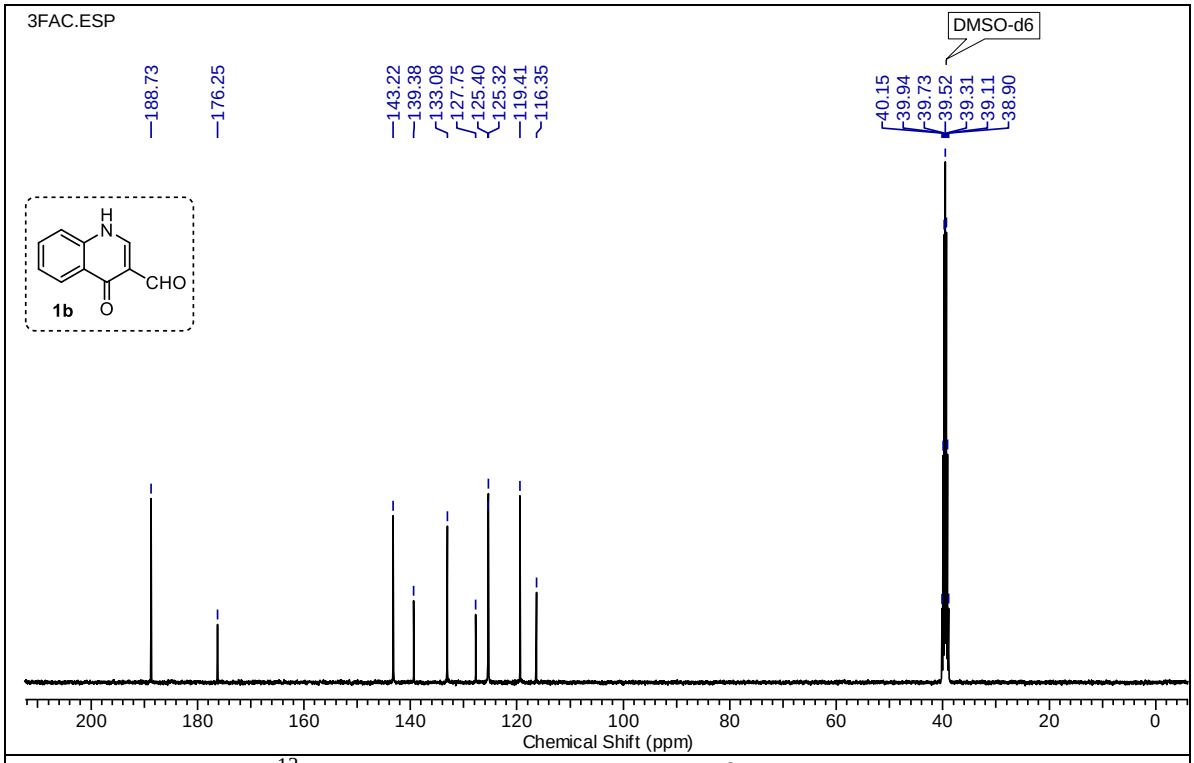
^{1}H NMR (400 MHz, $CDCl_3$) of compound **1a**



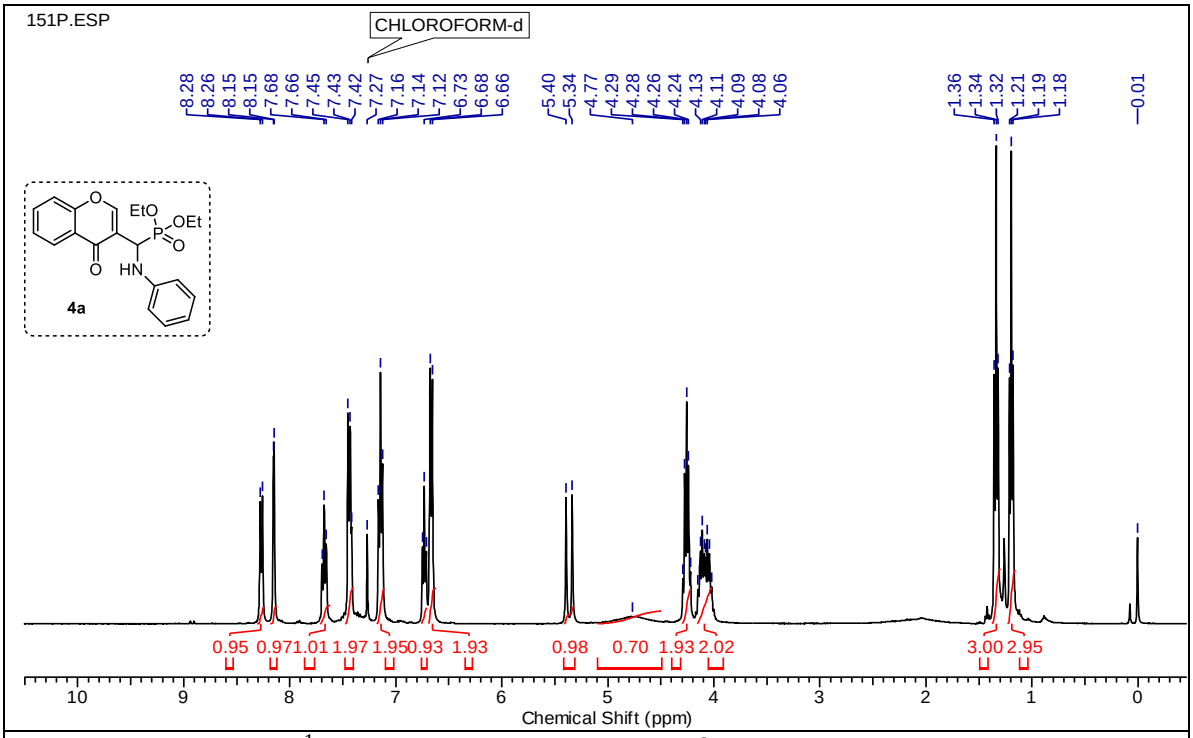
^{13}C NMR (100 MHz, $CDCl_3$) of compound **1a**



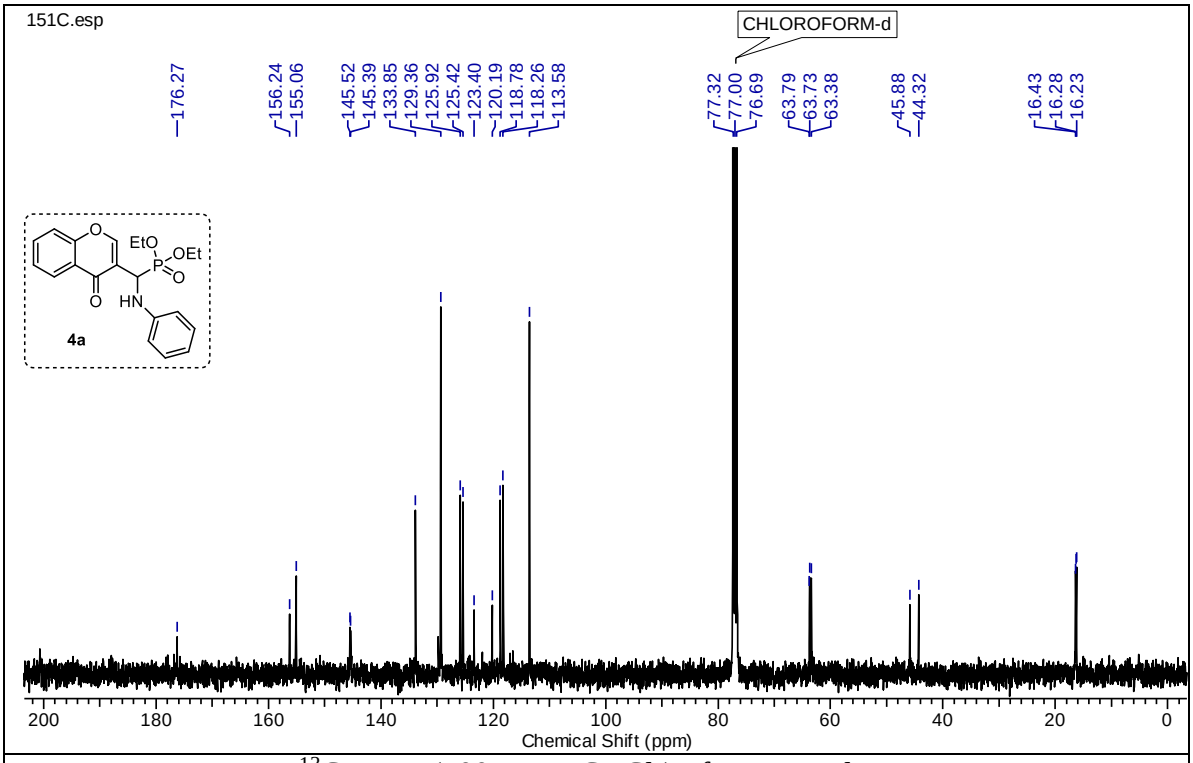
^{1}H NMR (400 MHz, DMSO-d_6) of compound **1b**



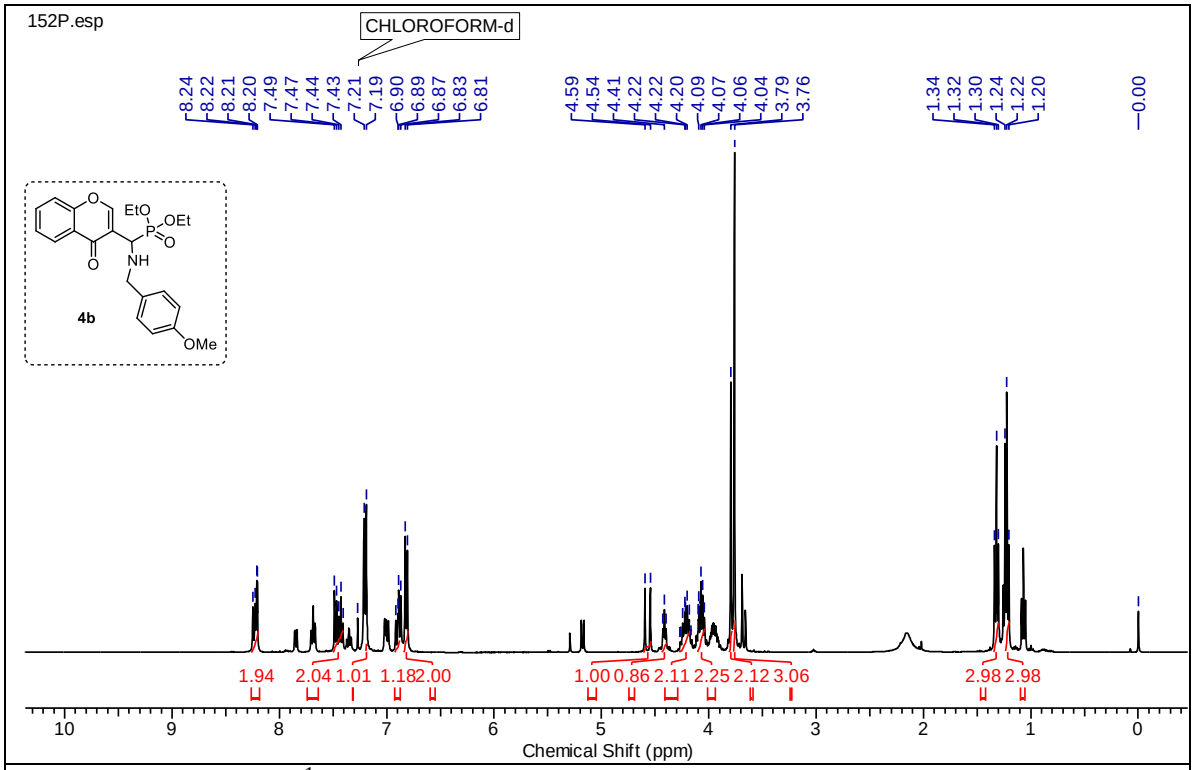
^{13}C NMR (100 MHz, DMSO-d_6) of compound **1b**



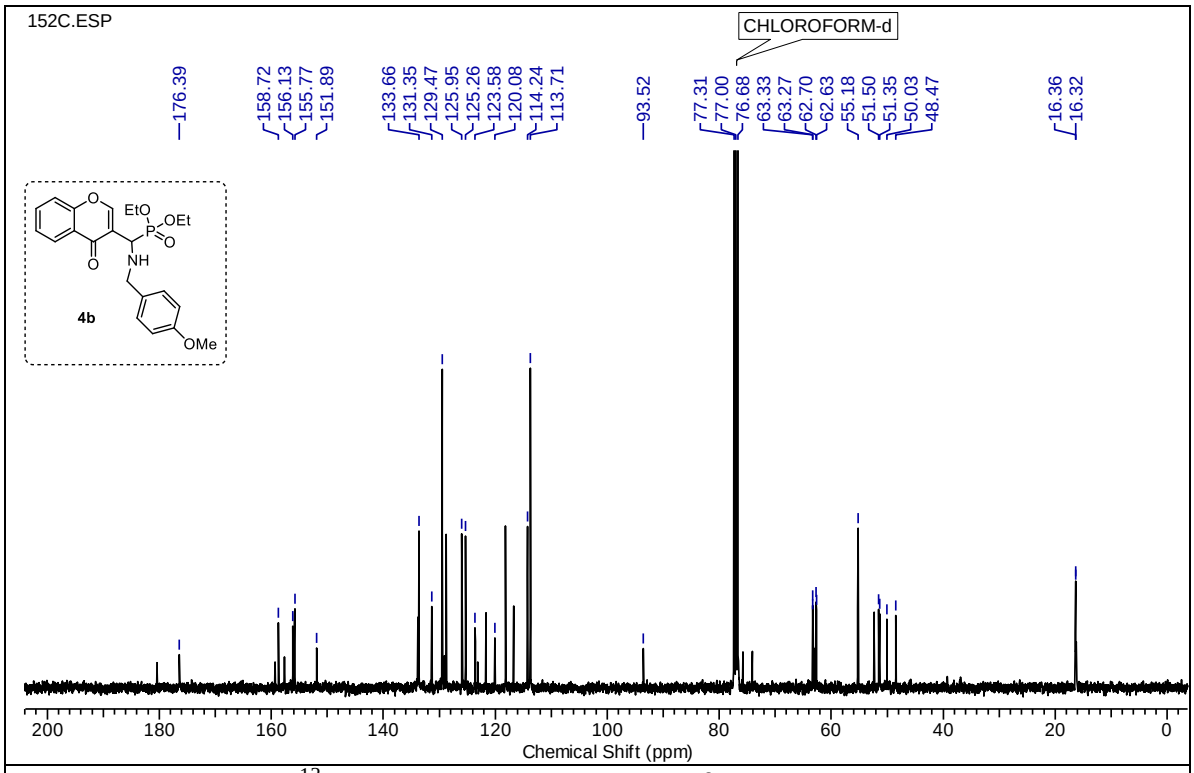
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4a**



^{13}C NMR (100 MHz, $CDCl_3$) of compound **4a**



^{1}H NMR (400 MHz, $CDCl_3$) of compound **4b**



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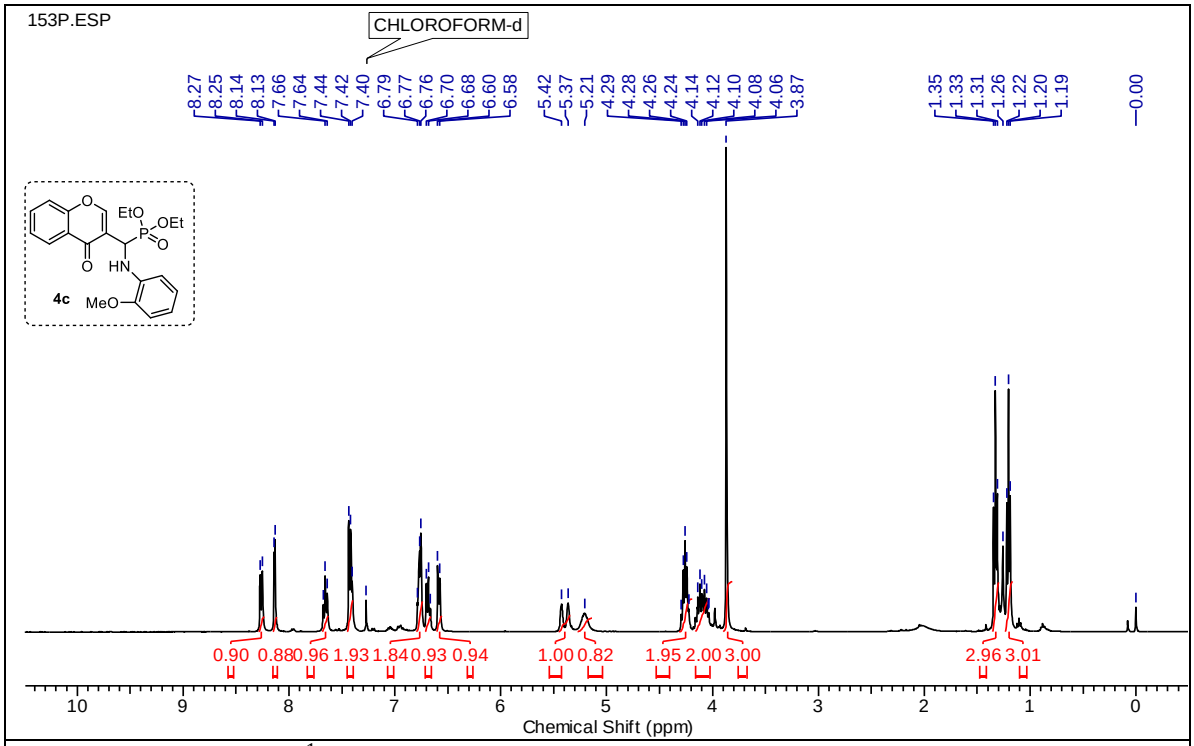
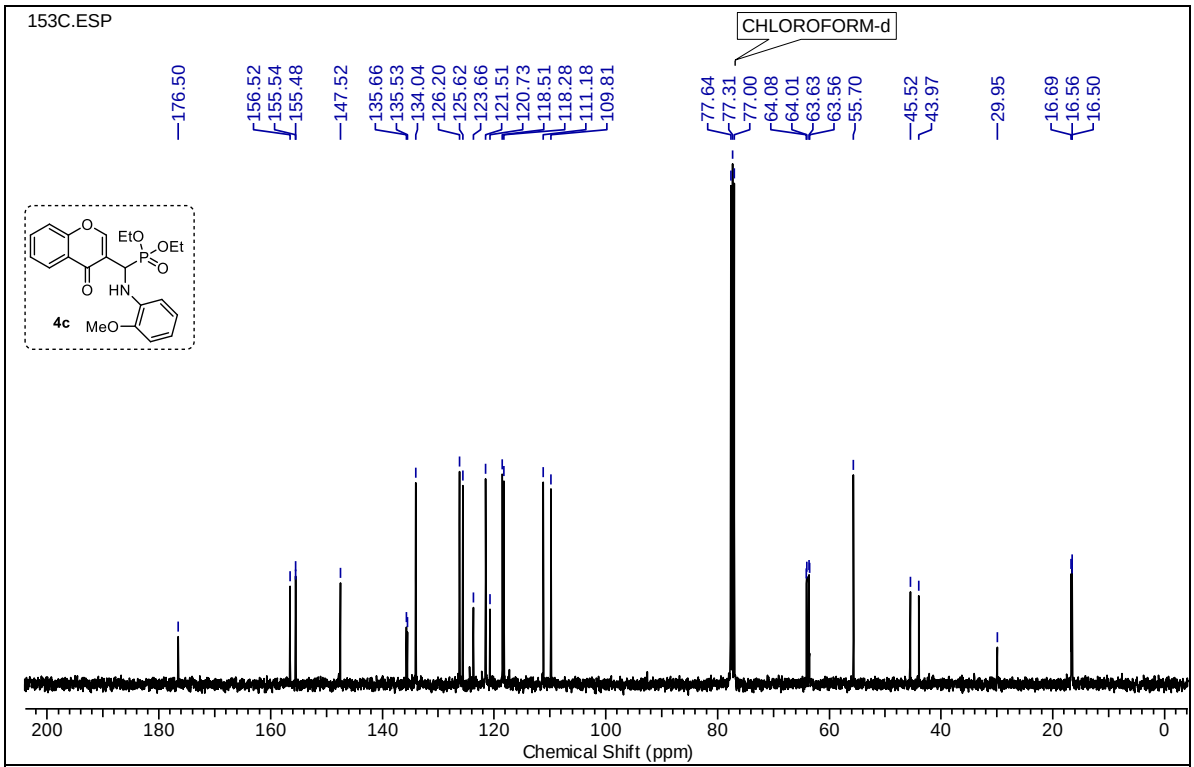


^{1}H NMR (400 MHz, $CDCl_3$) of compound **4c**



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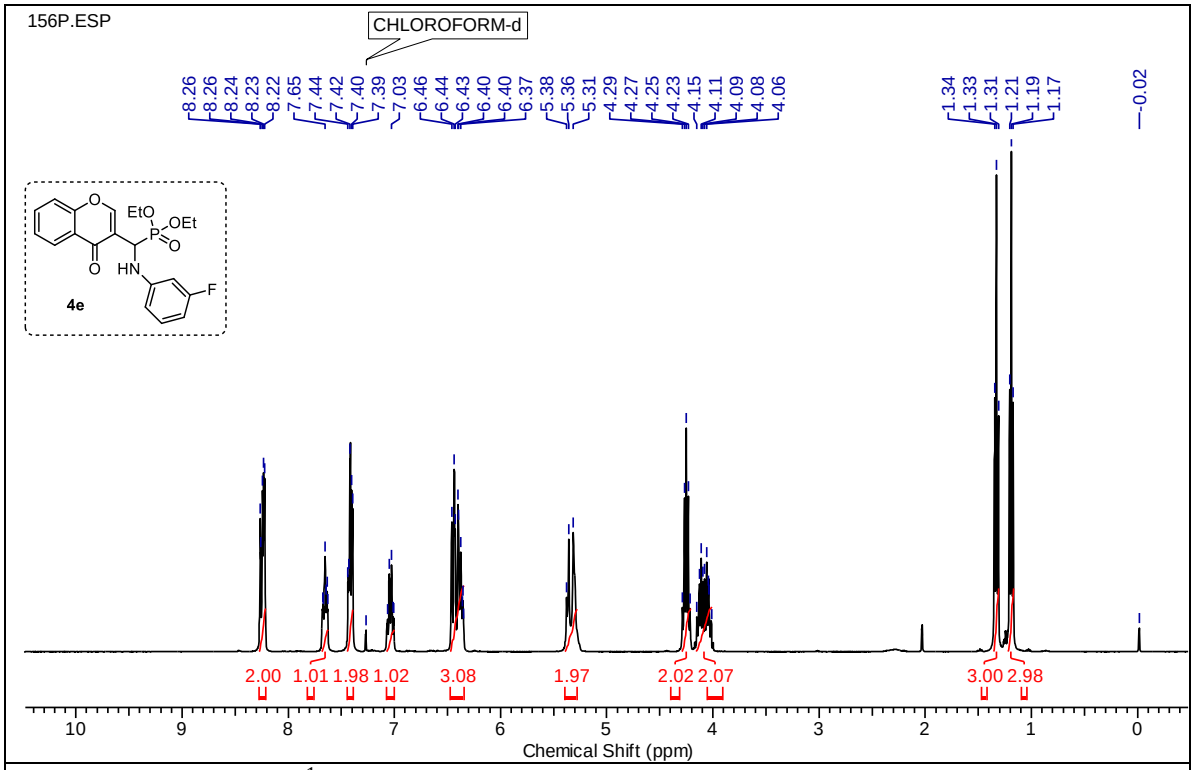
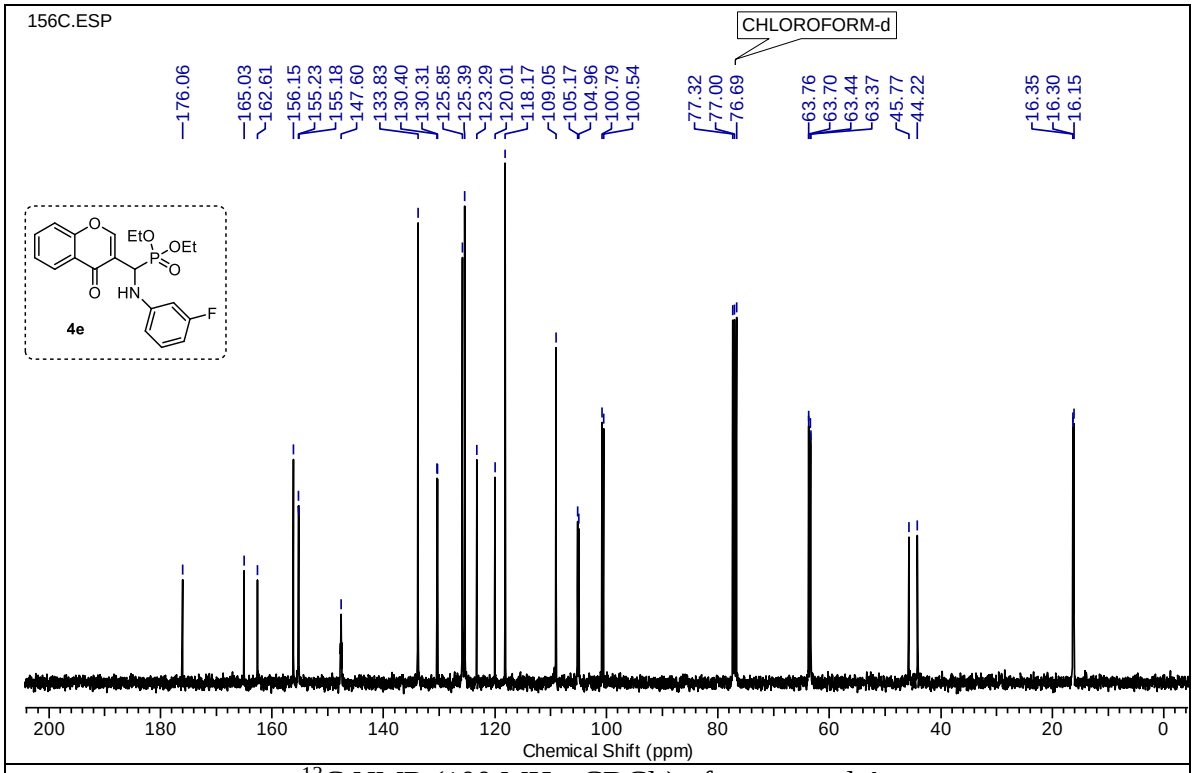


^{1}H NMR (400 MHz, $CDCl_3$) of compound **4e**



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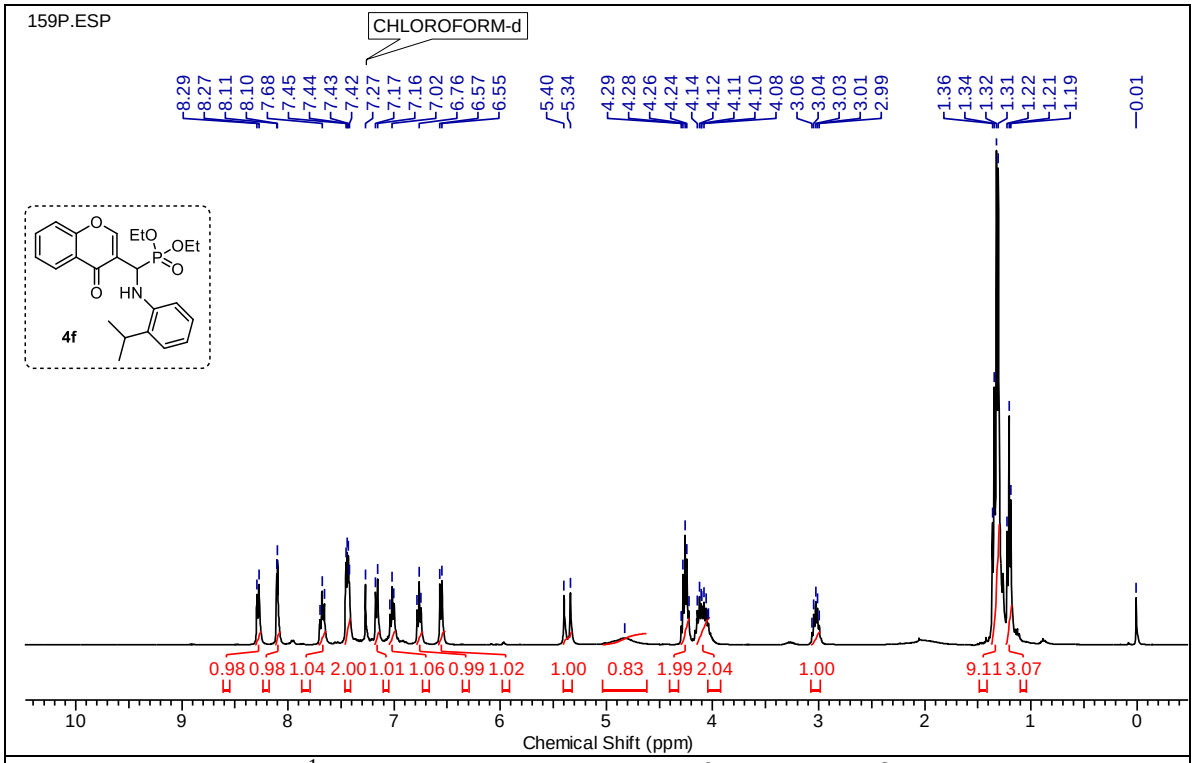

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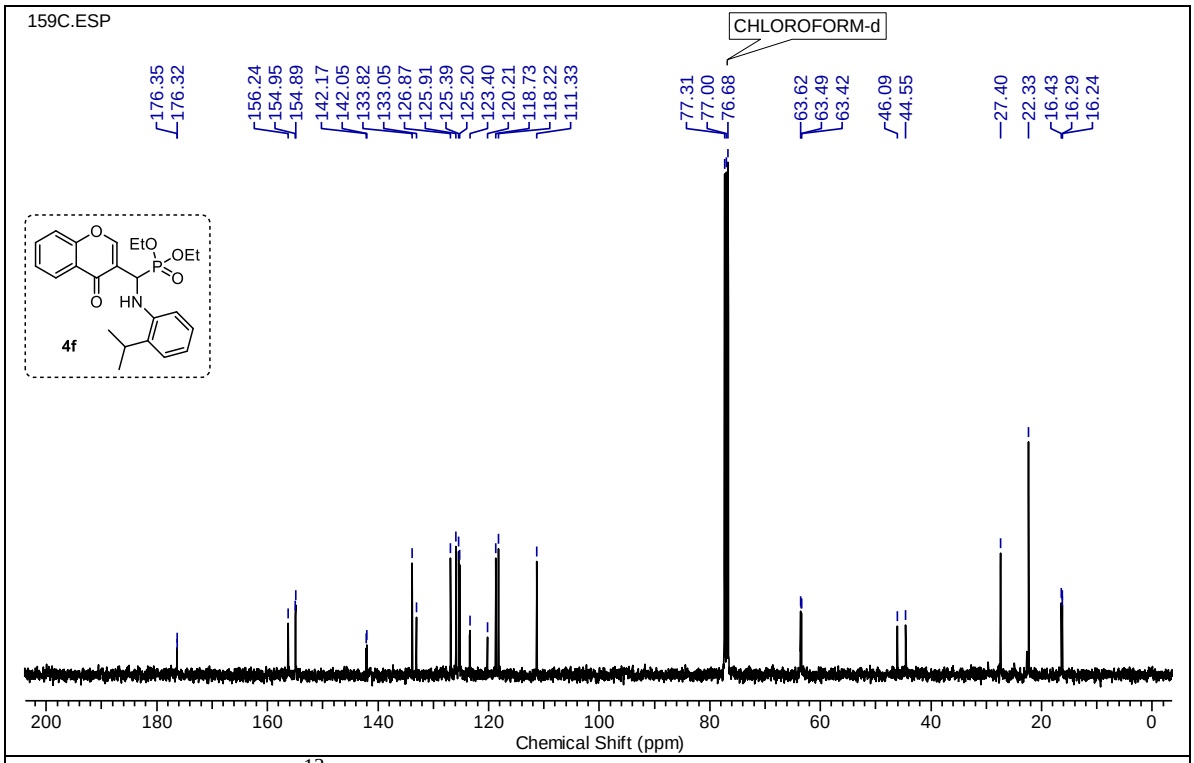
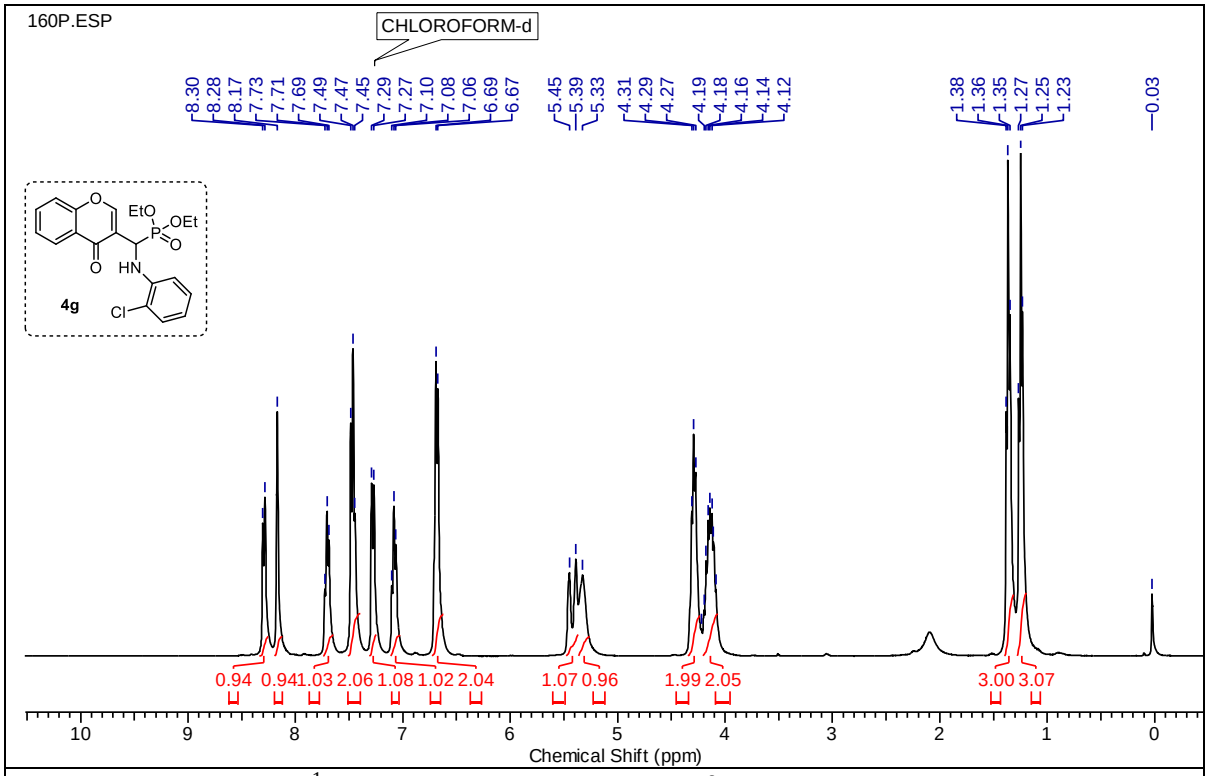
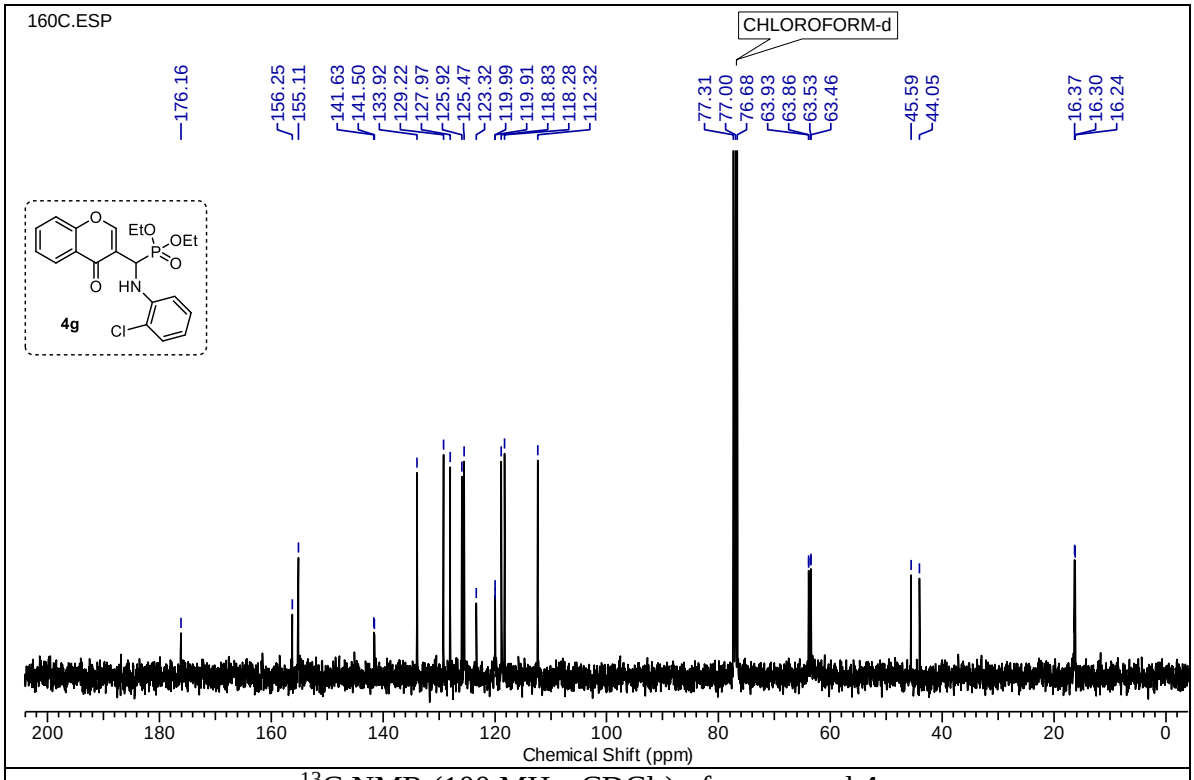


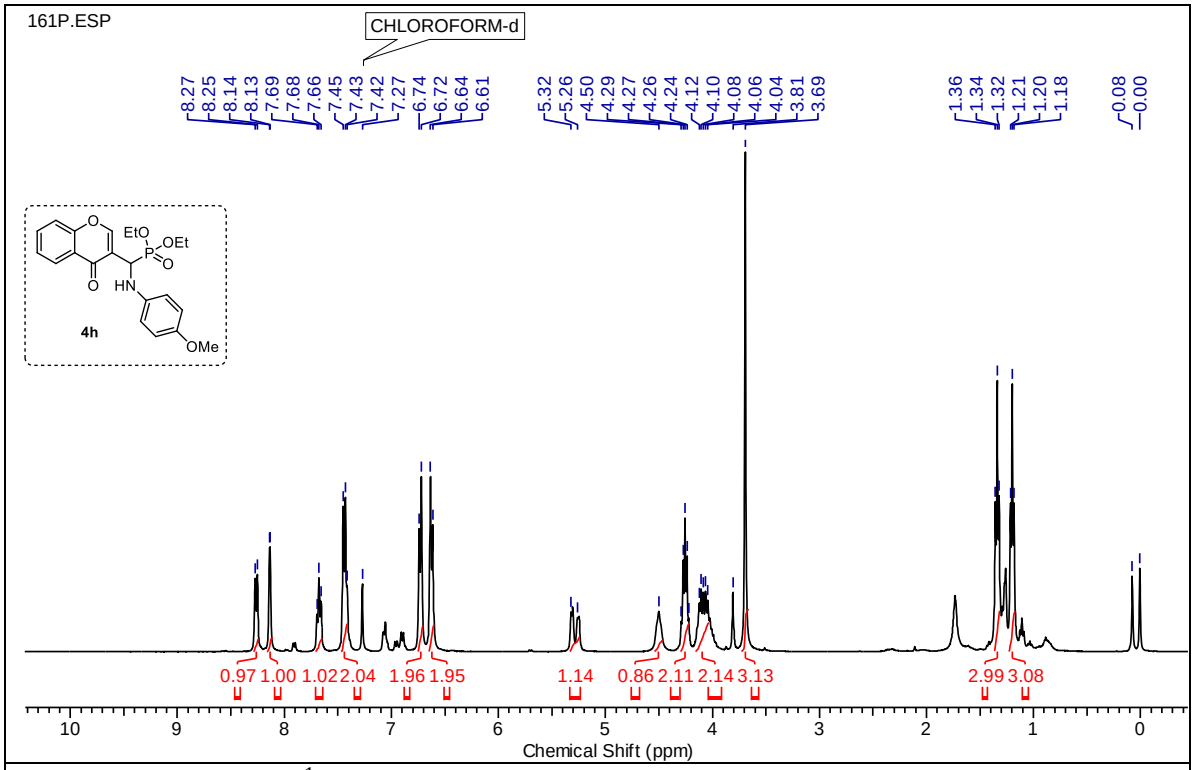
^{13}C NMR (100 MHz, $CDCl_3$) of compound **4f**



^{1}H NMR (400 MHz, $CDCl_3$) of compound **4g**



^{13}C NMR (100 MHz, $CDCl_3$) of compound **4g**



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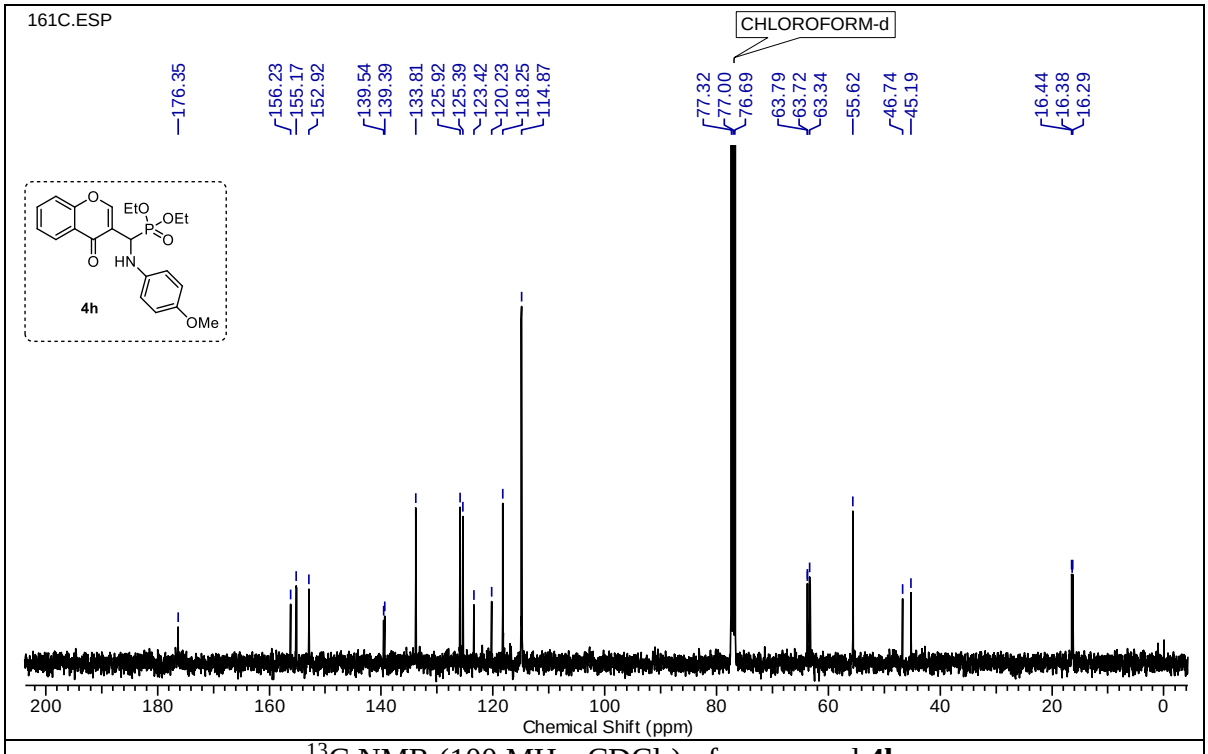

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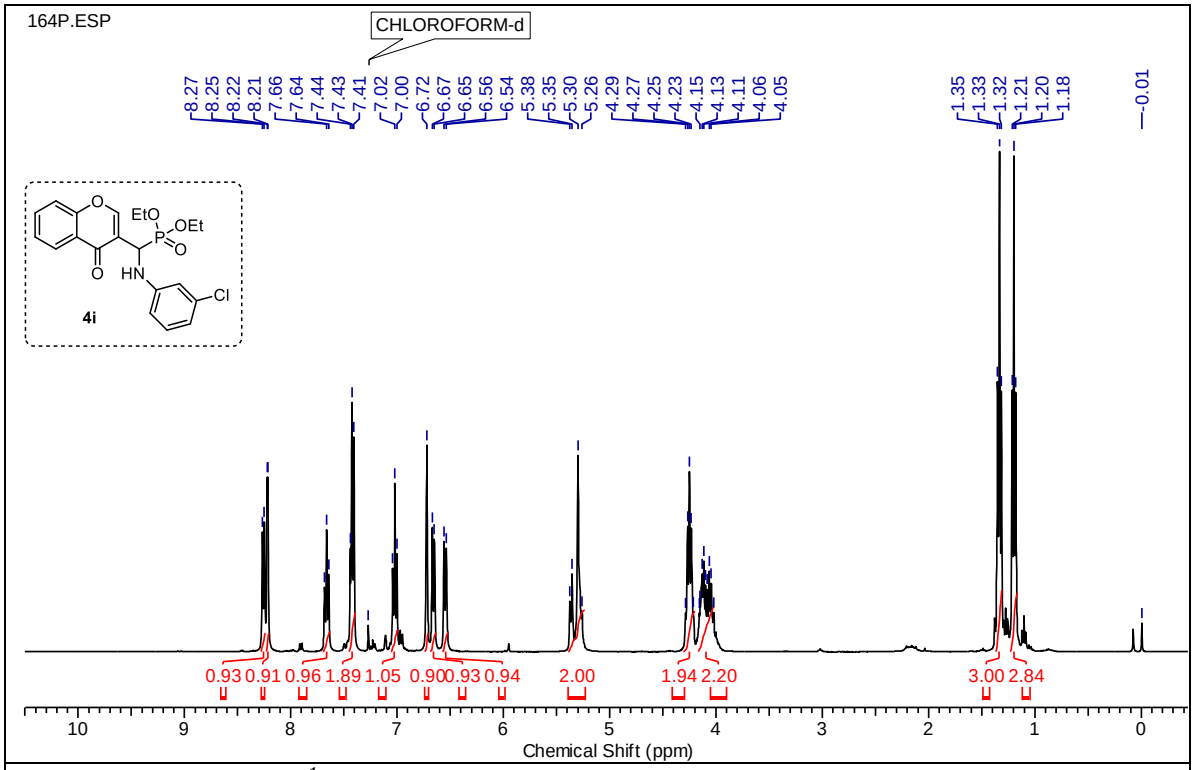
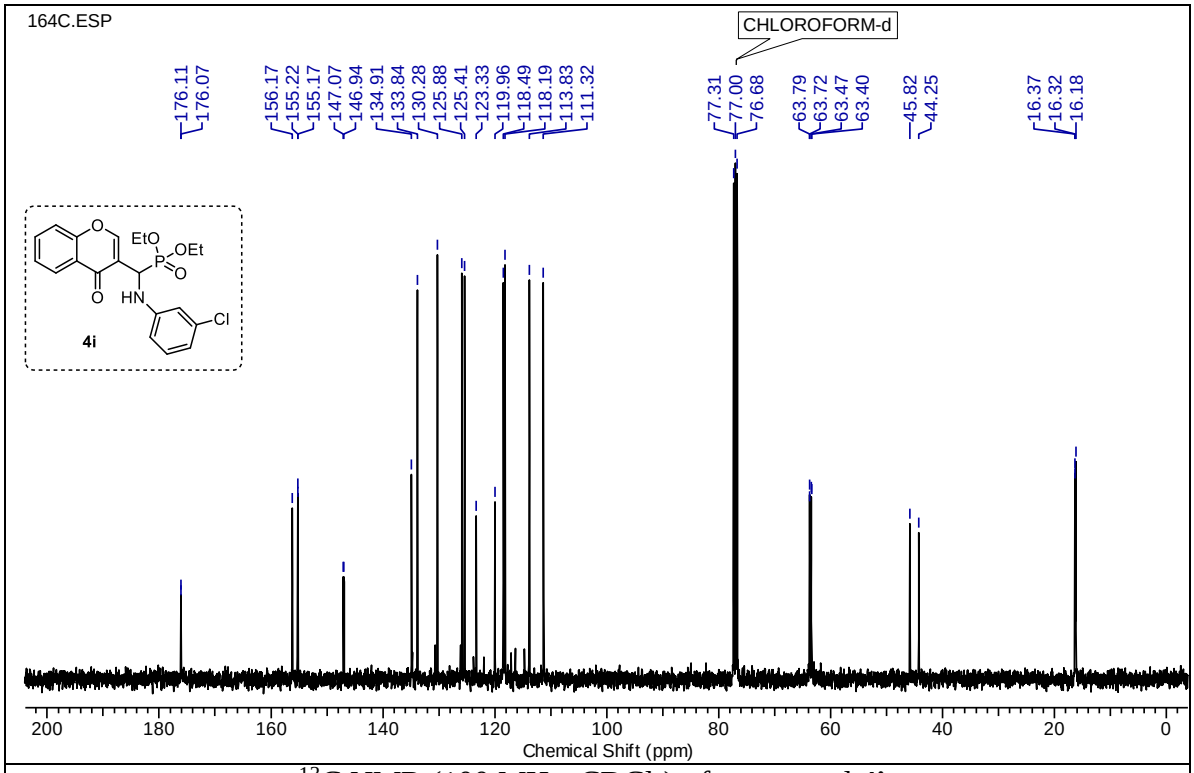


^{1}H NMR (400 MHz, $CDCl_3$) of compound **4i**



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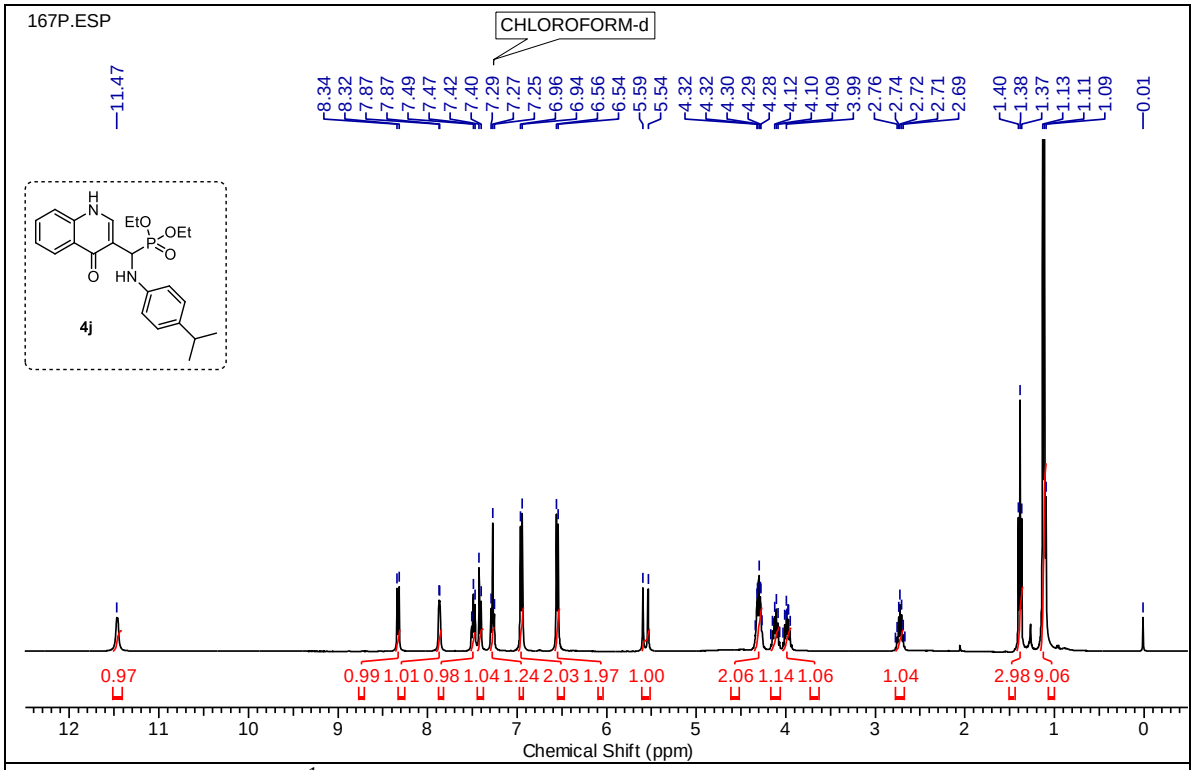

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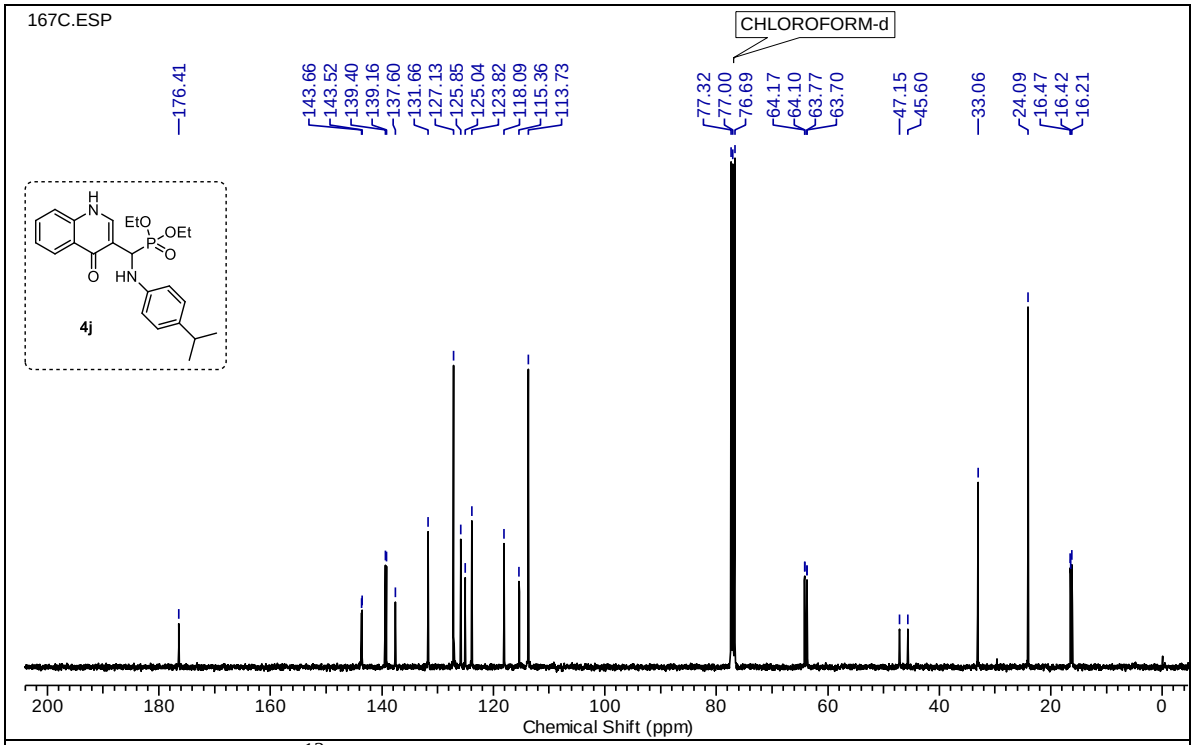

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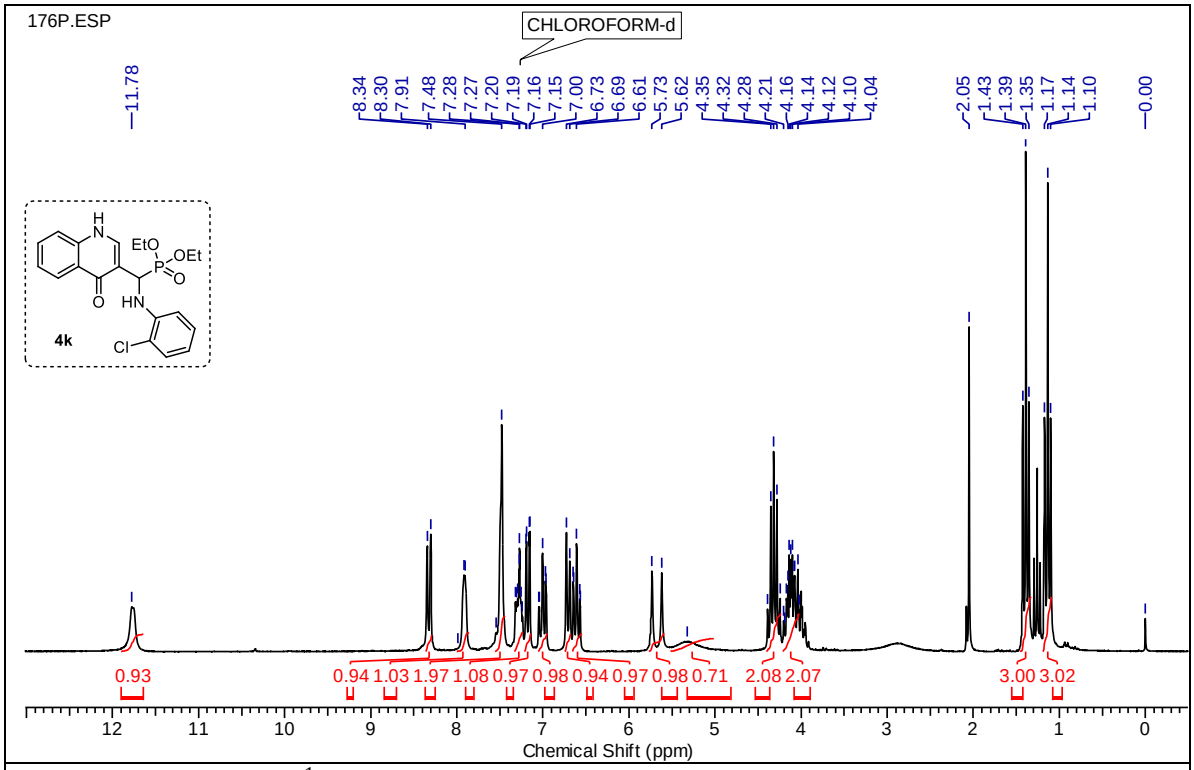

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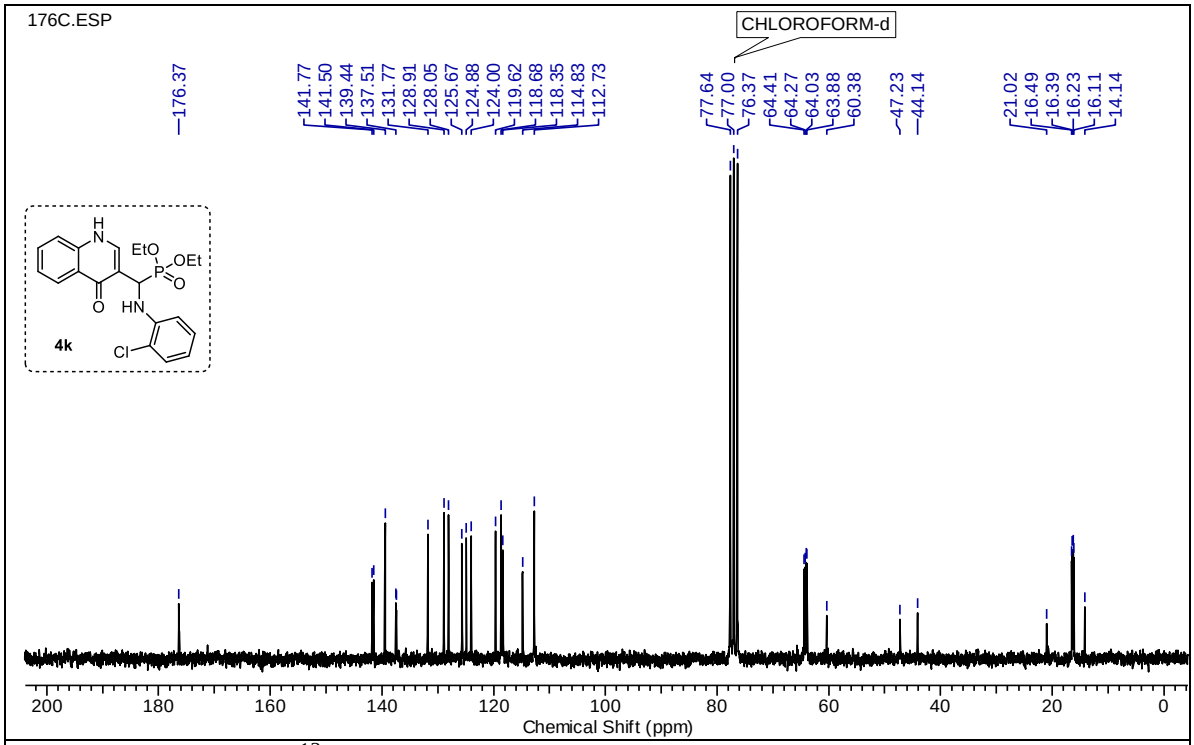

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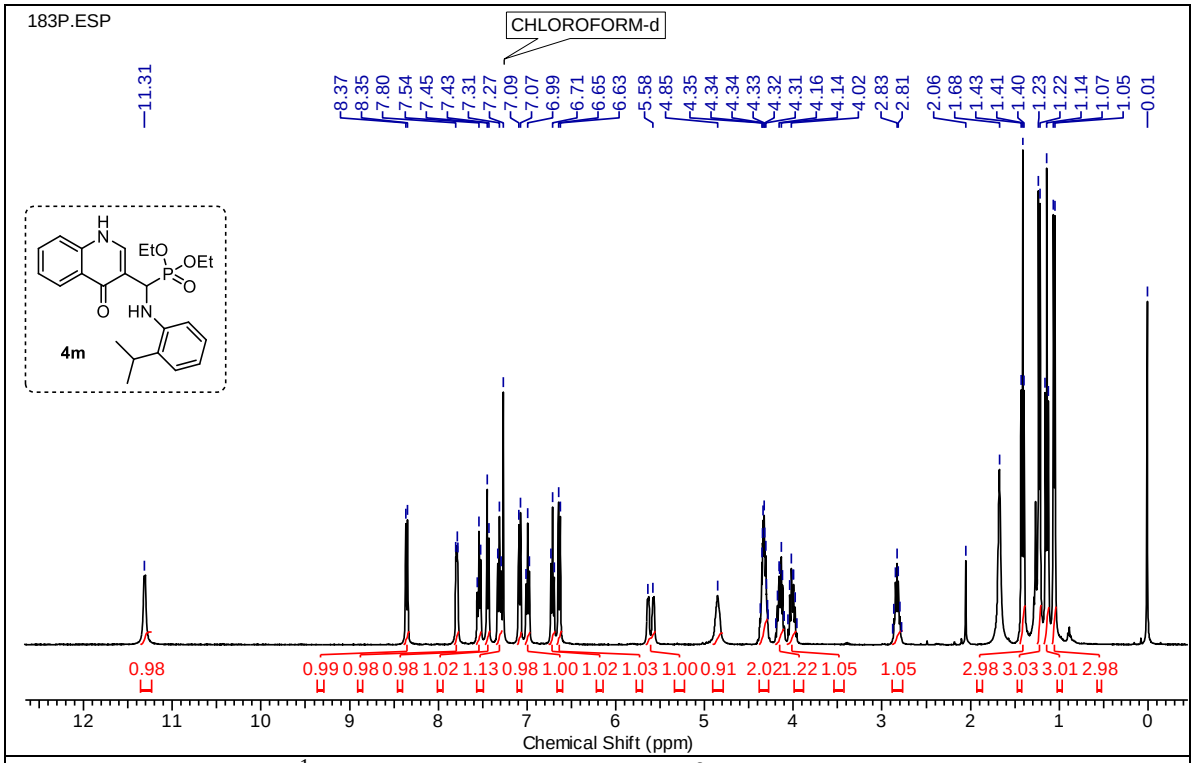
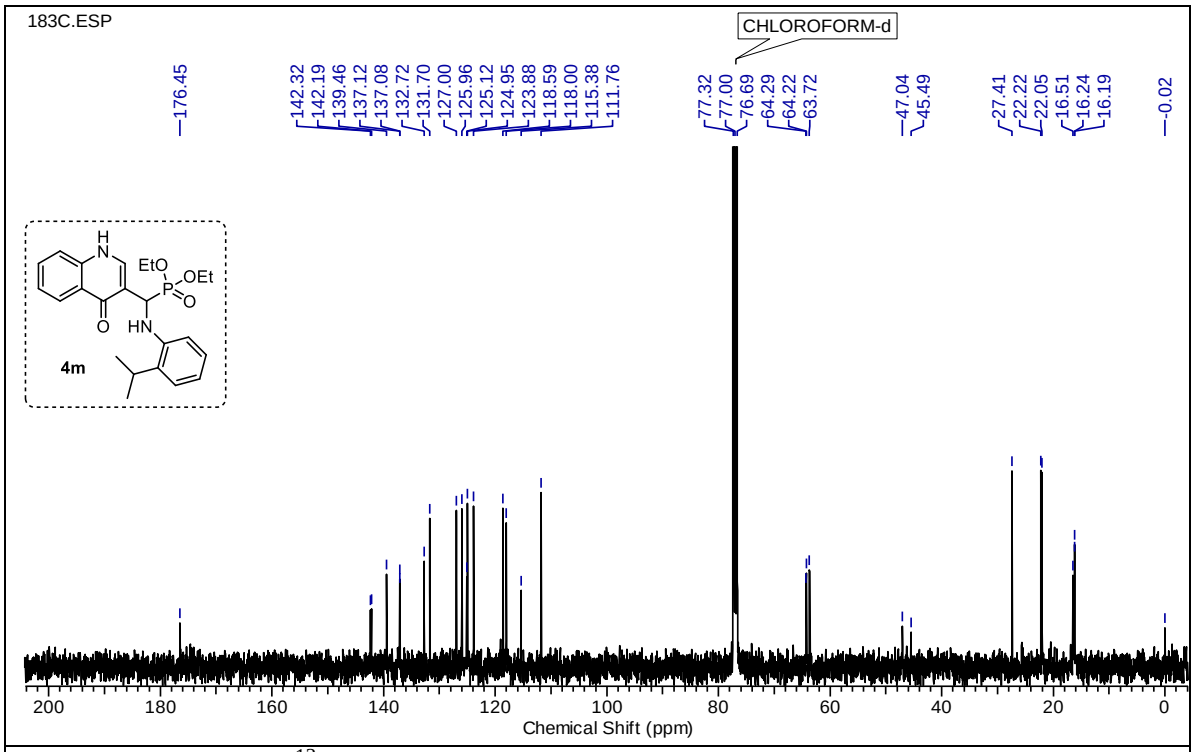
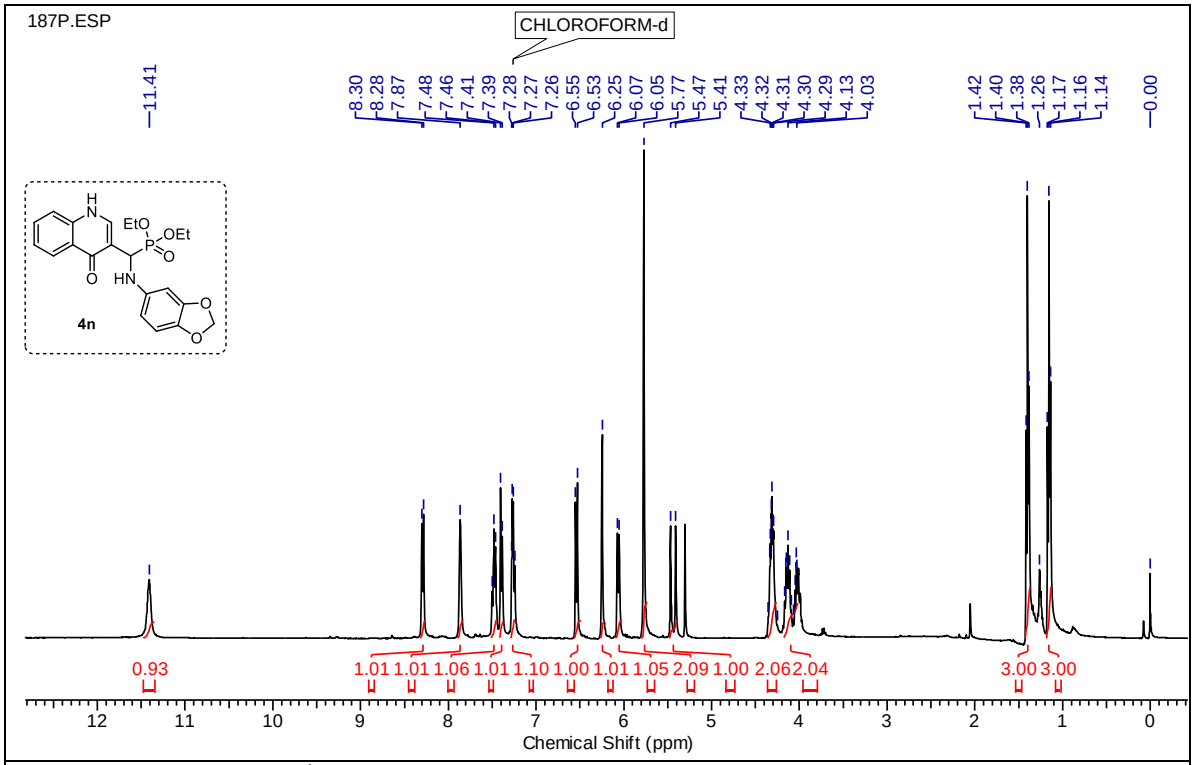


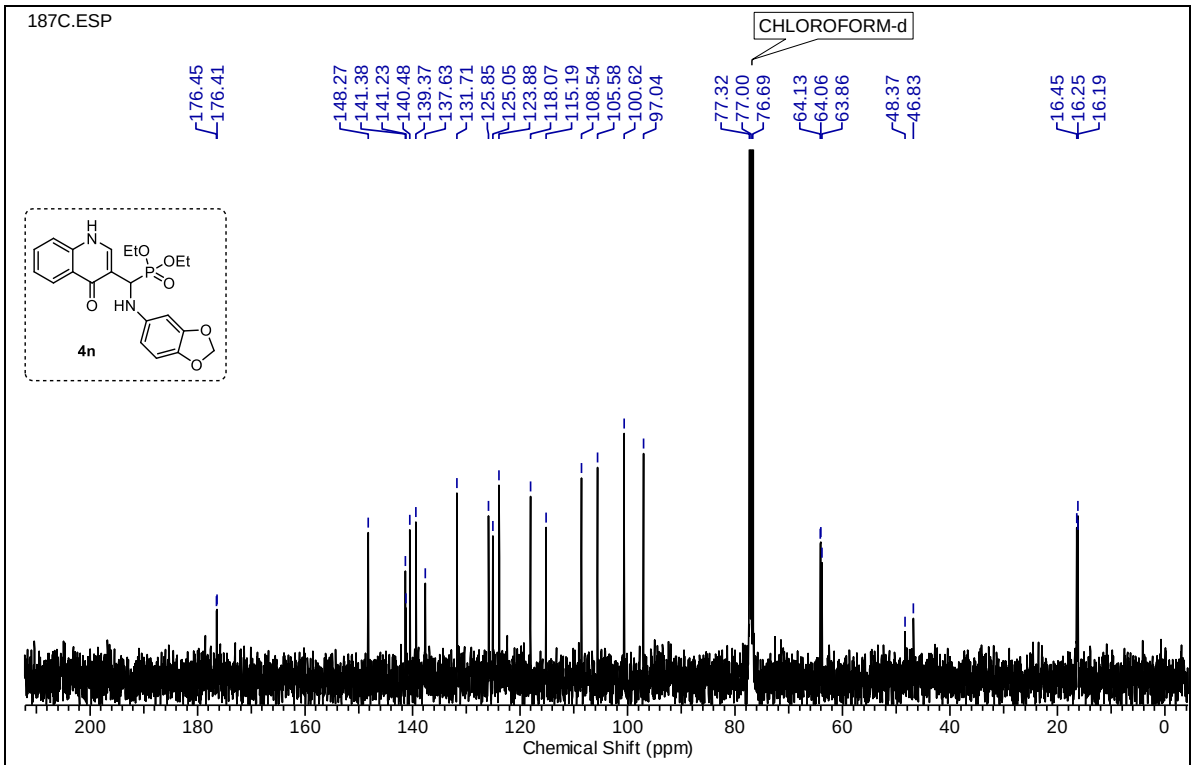
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4m**



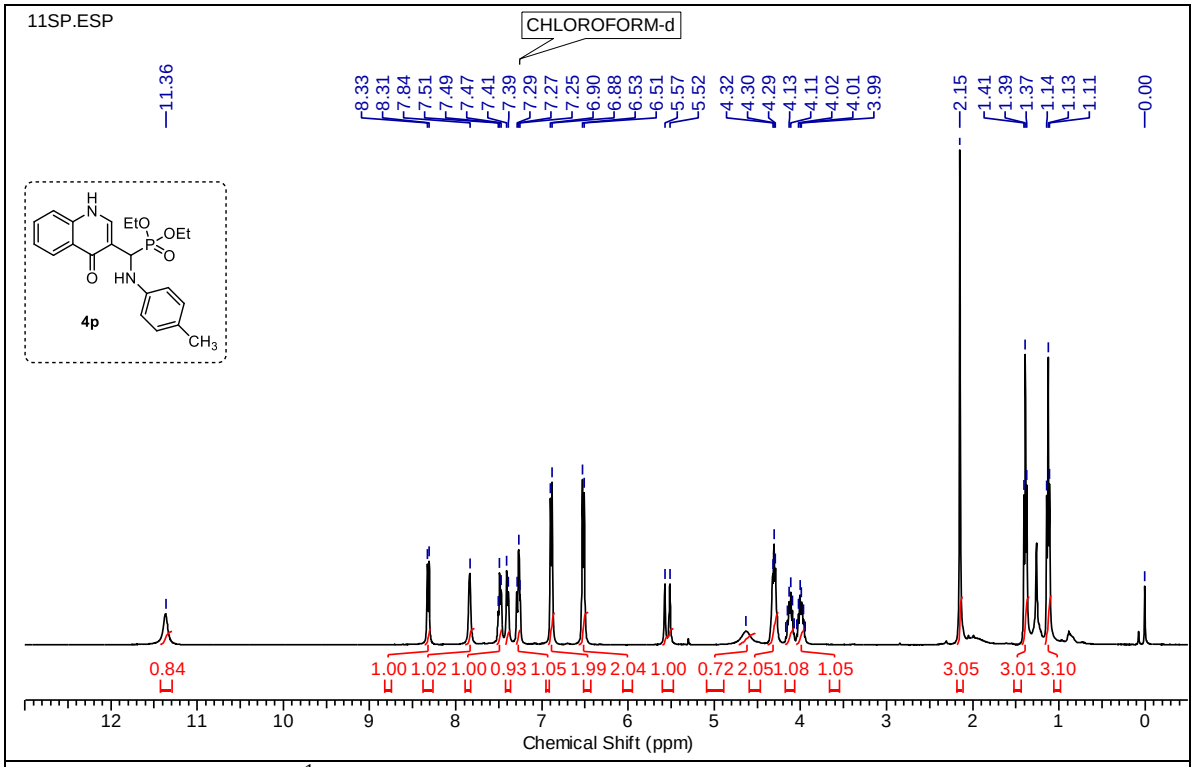
^{13}C NMR (100 MHz, $CDCl_3$) of compound **4m**



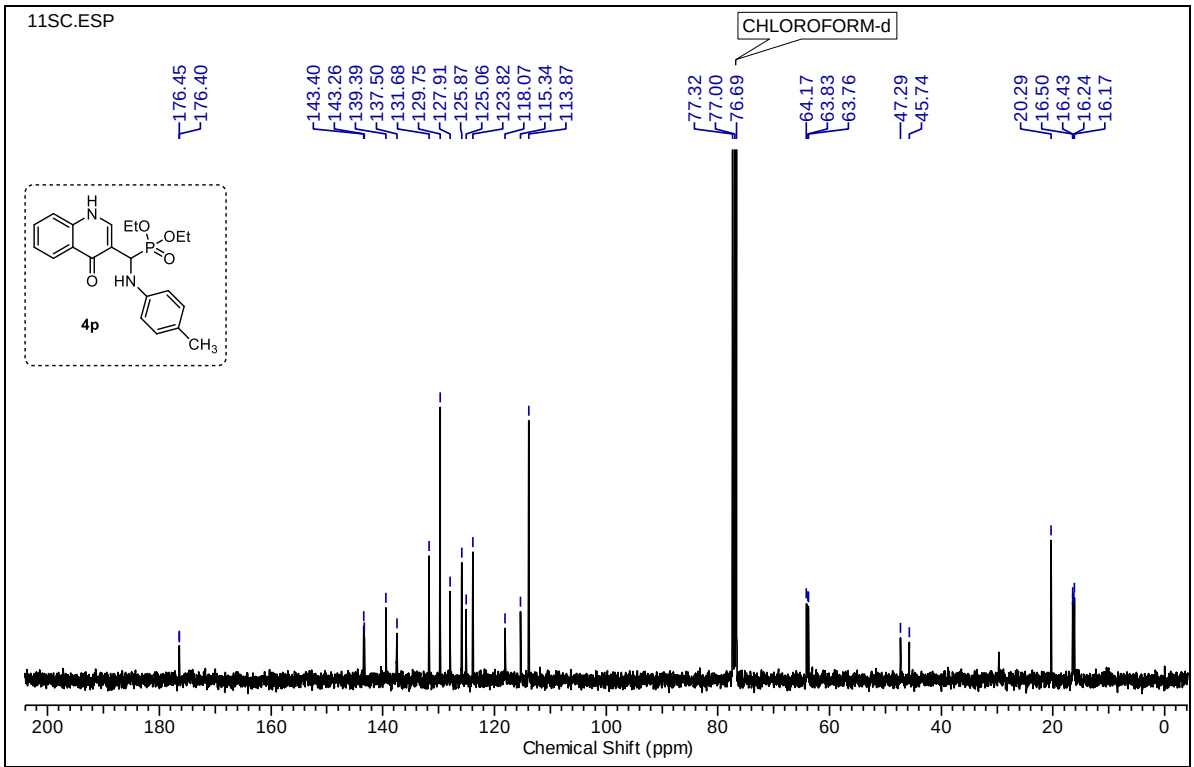
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4n**



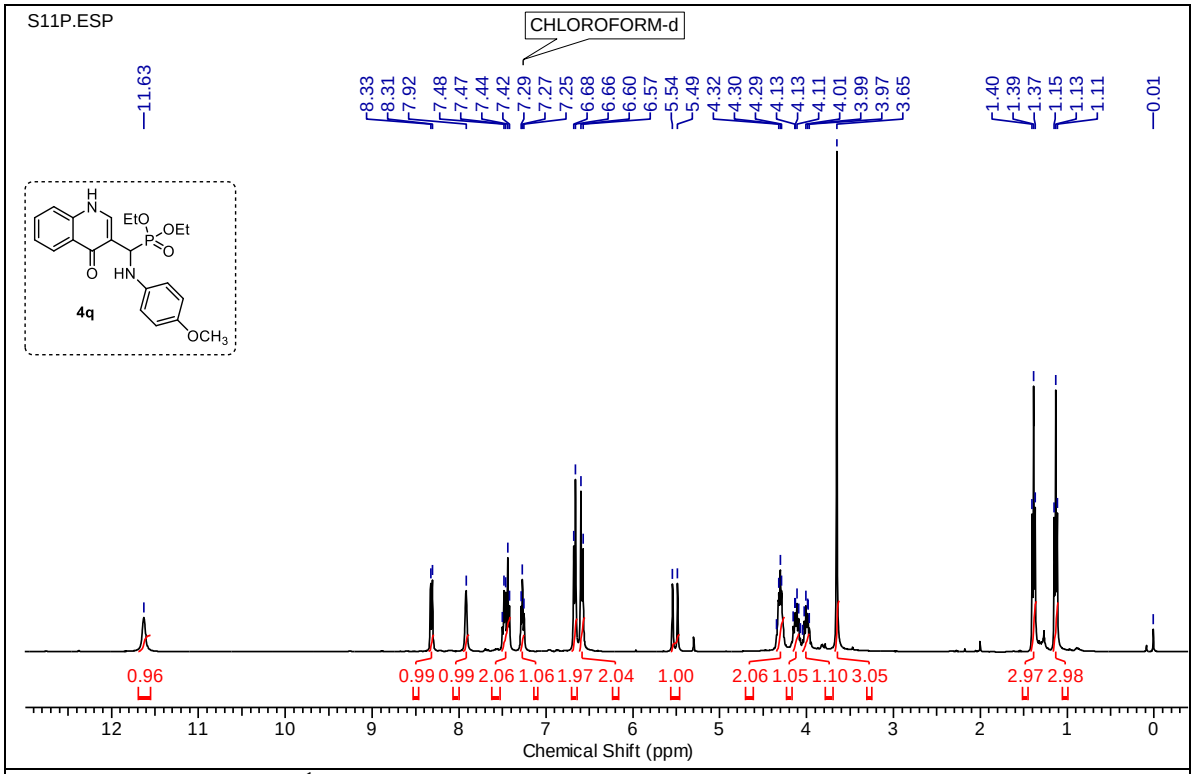
^{13}C NMR (100 MHz, $CDCl_3$) of compound **4n**



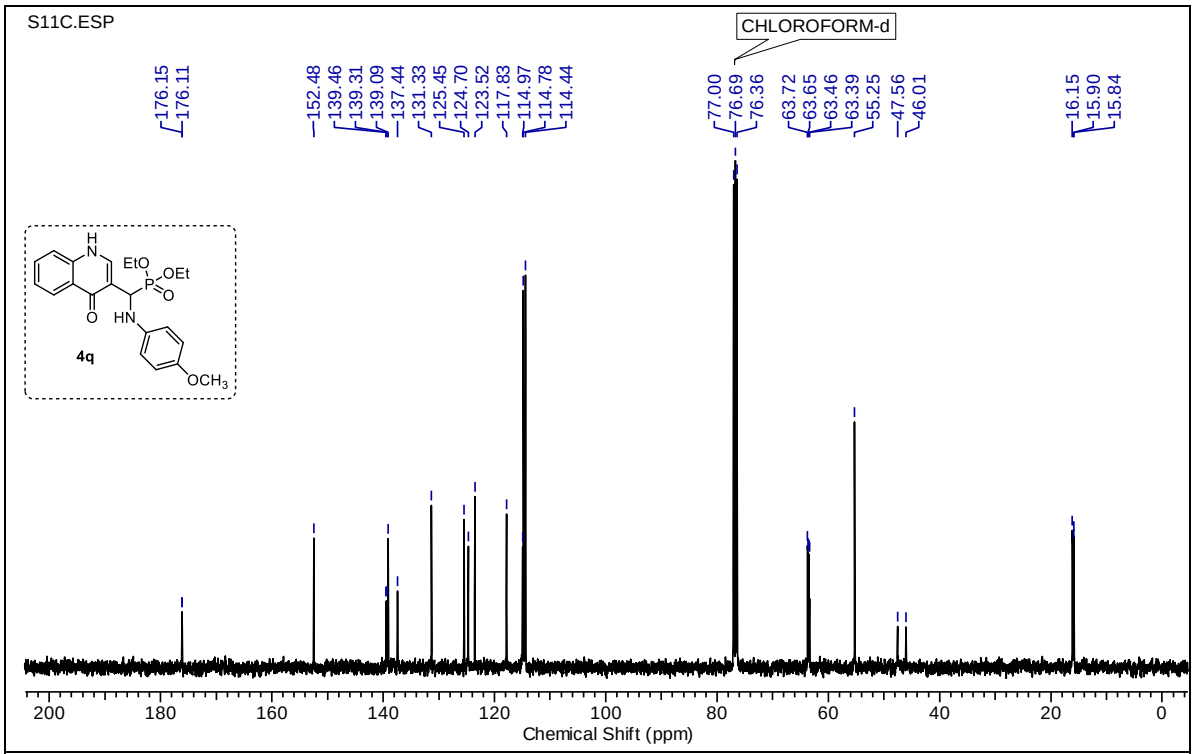
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4p**



^{13}C NMR (100 MHz, $CDCl_3$) of compound **4p**



^{1}H NMR (400 MHz, $CDCl_3$) of compound **4q**



^{13}C NMR (100 MHz, $CDCl_3$) of compound **4q**

3.2.7. References

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[In: RSC Drug Discovery Ser., 2012; 25]; RSC, 2012.

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Publications and patents

(1) Chiral aziridine ring opening: facile synthesis of (*R*)-mexiletine and (*R*)-phenoxybenzamine hydrochloride. **Viswanadh, N.**; Velayudham, R.; Jambu, S.; Sasikumar, M.; Muthukrishnan, M*. *Tetrahedron Lett.* **2015**, *56*, 5269.

(2) An alternate synthesis of appetite suppressant (*R*)-2-benzylmorpholine employing Sharpless asymmetric epoxidation strategy. **Viswanadh, N.**; Mujumdar, P.; Sasikumar, M.; Kunte, S. S.; Muthukrishnan, M*. *Tetrahedron Lett.* **2016**, *57*, 861.

(3) A new and efficient enantioselective synthesis of both enantiomers of the calcium channel blocker bepridil. Mujahid, M.; Subramanian, J.; **Viswanadh, N.**; Sasikumar, M.; Kunte, S. S.; Muthukrishnan, M*. *New J. Chem.* **2017**, *41*, 824.

(4) Transition metal free regio-selective C-H hydroxylation of chromanones towards the synthesis of hydroxyl-chromanones using $PhI(OAc)_2$ as the oxidant. **Viswanadh, N.**; Ganesh S. Ghotekar.; Mahesh, B. Thoke.; Velayudham, R.; Aslam C. Shaikh.; Karthikeyan, M.; Muthukrishnan, M*. *Chem. Commun.,* **2018**, *54*, 2252.

(5) Identification of potent chromone embedded [1,2,3]-triazoles as novel antitubercular agents. **Viswanadh, N**.; Aslam, S.; Sanket, B.; Vyas, R.; Karthikeyan, M.; Yogeeswari, P.; Sriram, D.; Muthukrishnan, M*. *Royal Soc. Open Sci.* **2018** (*In press*).

(6) Synthesis, biological evaluation and molecular modeling studies of novel chromone/aza-chromone fused α-aminophosphonates as Src kinase inhibitors. Sanket B.; **Viswanadh, N.**; Mujahid, M.; Amir N. S.; Rakesh K. T.; Keykavous P.; Vyas, R.; Karthikeyan, M.; Muthukrishnan, M*. (*Manuscript communicated*).

(7) New method for the synthesis of (*R*)-phenoxybenzamine hydrochloride employing aziridine ring opening as a key step. **Viswanadh Nalla.**; Velayudham, R.; Karthikeyan, M.; Muthukrishnan, M. Indian patent *Appln No. 1844/DEL/2014*.

(8) A process for the preparation of 8-chloro-1-methyl-2,3,4,5-tetrahydro-1-*H*-3-benzo[D]azepine and its enantiomers. Muthukrishnan, M.; Velayudham, R.; **Viswanadh Nalla.**; PCT, *WO 2015/170346 A1*.

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Chiral aziridine ring opening: facile synthesis of (*R*)-mexiletine and (*R*)-phenoxybenzamine hydrochloride

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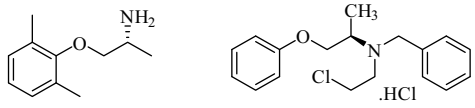
ABSTRACT

A simple and efficient synthesis of chiral drugs (*R*)-mexiletine **1**, an anti-arrhythmic drug and (*R*)-phenoxybenzamine hydrochloride **2**, an anti-hypertensive drug has been described via controlled reductive ring opening of chiral aziridine as a key step. The target compounds **1** and **2** were obtained in overall yields of 34% and 10.5%, respectively.

Synthesis of compounds in their enantio-enriched form became very important in the market place, especially in the pharmaceutical sector. This is mainly because; the enantiomers of chiral drugs often exhibit significantly different pharmacological, toxicological, pharmacodynamic and pharmacokinetic properties. Hence the development of newer methods aiming the synthesis of active enantiomer or both enantiomers (for careful evaluation of individual enantiomers) of chiral drugs is a main focus of research in many academic and industrial laboratories.[1]

Mexiletine is an important β-amino aryl ether class of drug used in the treatment of arrhythmia, allodynia and myotonic syndromes, etc. and its racemic form of mexiletine is available in the market with the trade name Mexitil®.[2] However, the (*R*)-isomer of mexiletine (**1**) (Fig. 1) is more potent than the (*S*)-isomer in experimental arrhythmias and in binding studies on cardiac sodium channels.[3] Similarly, phenoxybenzamine hydrochloride (**2**, PB; Commercial name Dibenzyline®) is an important β-chloroethylamine class of drug in the α-blocker series, widely used in the treatment of hypertension.[4] It has also found application in treating benign prostatic hyperplasia (BPH) hypoplastic left heart syndrome, etc. However, the (*R*)-isomer of phenoxybenzamine hydrochloride (**2**) is 14.5 times more potent than its (*S*)-isomer.[5] Approaches that have been used so far to prepare enantiopure mexiletine and PB includes chiral pool, chemo/enzymatic resolution strategy or using stereoselective protocols.[6,7]



R-Mexiletine **1** — *antiarrhythmic*

R-Phenoxybenzamine. HCl **2** — *antihypertensive*

Figure 1. Structure of (*R*)-mexiletine and (*R*)-phenoxybenzamine hydrochloride.

As part of our ongoing programme on developing a new and improved process for the preparation of various pharmaceutically important compounds for industrial applications,[8,9] sometime ago we reported the preparation of **1** and **2** employing hydrolytic kinetic resolution strategy.[9a,b] Importantly, the potential utility of compounds **1** and **2** in new therapeutic areas inspired us to develop a robust method for their synthesis preferably from a common precursor there as to make a diverse range of chiral analogues of **1**[10] and **2** in a simple manner to test their biological activities. We herein report a simple and efficient approach towards the preparation of **1** and **2** via the reductive ring opening reaction of the enantiopure aziridine as a key step.

A retrosynthetic analysis of (*R*)-mexiletine (**1**) and (*R*)-phenoxybenzamine. HCl (**2**) is outlined in Scheme 1. As shown in Scheme 1, we envisaged that chiral aziridines ***R*-7** & ***R*-8** would be an ideal key intermediate for the synthesis of both (*R*)-mexiletine (**1**) and (*R*)-phenoxybenzamine HCl (**2**), respectively. These intermediates ***R*-7** and ***R*-8** can be converted to the target molecules via reductive ring opening followed by simple synthetic sequences. The chiral aziridines ***R*-7** and ***R*-8** in turn, can be prepared from commercially available phenols via O-alkylation, regioselective ring opening followed by intramolecular ring closure sequences.

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An alternate synthesis of appetite suppressant (*R*)-2-benzylmorpholine employing Sharpless asymmetric epoxidation strategy

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ABSTRACT

An alternate synthesis of (*R*)-2-benzylmorpholine **1**, an appetite suppressant agent has been accomplished starting from readily available *trans*-cinnamyl alcohol employing Sharpless asymmetric epoxidation strategy as a key step, with an overall yield of 24%.

C-Substituted morpholine analogues, in particular the non-racemic ones are important structural scaffolds present in many pharmaceutically important compounds (Fig. 1).[1] They are potential therapeutic agents for a wide variety of medical disorders such as depression (Reboxetine, Viloxazine),[2] anorectic (Phenmetrazine, Phendimetrazine),[3] chemotherapy induced nausea and vomiting (Aprepitant),[4] etc. In that series, (*R*)-2-benzyl morpholine is a classical example of chiral 2-morpholine analogues, known to be a potent appetite suppressant and widely studied for its pharmacological properties.[5] Further, its potential utility in treating diabetes mellitus and certain CNS disorders are also under investigation.[6] Despite their wide utility, synthetic routes to these valuable compounds especially the non-racemic ones are very limited.[7] So far, methods described in the literature to afford (*R*)-2-benzyl morpholine involve optical resolution,[5] chemoenzymatic route[8] or enantioselective method employing proline catalyzed α-aminooxylation strategy.[9]

As a part of our ongoing programme on developing a new and improved process for the preparation of various pharmaceutically important compounds for industrial applications,[10,11] we herein report a simple and efficient approach towards the preparation of (*R*)-2-benzylmorpholine employing Sharpless asymmetric epoxidation (SAE)[12] strategy.

A retrosynthetic analysis of **1** is outlined in Scheme 1. As shown in Scheme 1, the amino alcohol **5** can be visualized as a key intermediate for the synthesis of (*R*)-2-benzylmorpholine (**1**) which can be elaborated to the amide derivative **6** by simple N-acylation. Further, compound **6** might be transformed to the target molecule **1** via cyclization followed by amide reduction.

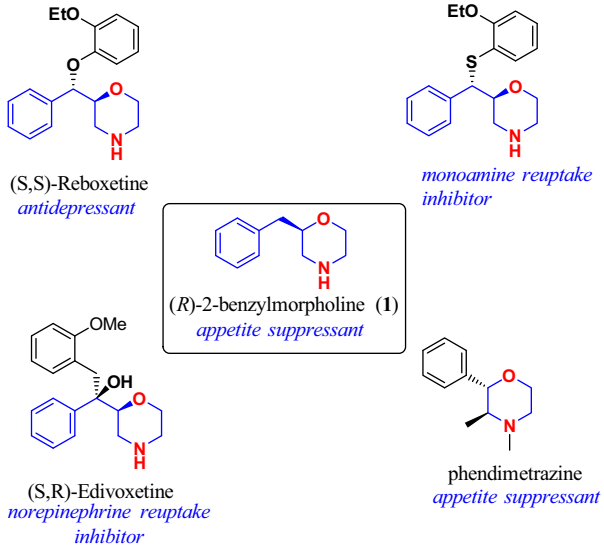

Figure 1. Few pharmaceutically important compounds possessing chiral C-2 substituted morpholine structure.

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A new and efficient enantioselective synthesis of both enantiomers of the calcium channel blocker bepridil†

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A concise and efficient enantioselective synthesis of both enantiomers of bepridil, a calcium channel blocker, is reported. Jacobsen's hydrolytic kinetic resolution method was utilized to resolve racemic 2-(isobutoxymethyl)oxirane. The incorporation of the succinimide moiety by the Mitsunobu reaction, which was investigated in detail, occurred without any loss of enantioselectivity. Using this strategy, both enantiomers of the target molecule were obtained in good yield and with high enantiopurity (ee > 99%).

Introduction

Calcium channel blockers are an important group of drugs and they have prevalent use in treating hypertension, heart failure, cardiac arrhythmias, *etc.*[1] Bepridil (trade name: Vascor®) is a long acting calcium-blocking agent with significant antianginal activity (Fig. 1). It has antihypertensive and selective anti-arrhythmia activities and acts as a calmodulin antagonist.[2] Although it contains one stereogenic centre, it is generally administered as a racemate. However, as expected, pharmacological studies reveal that there are significant variations in the activity amongst bepridil enantiomers and the (*R*)-enantiomer of bepridil is more active than the (*S*)-enantiomer in certain cases.[3]

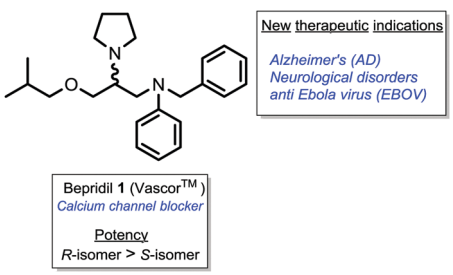

Fig. 1 Bepridil.

Importantly, many racemic drugs, which previously received FDA approval, are being re-evaluated to determine the potential benefits of the pure enantiomers.[4] In spite of the fact that the enantiomers of bepridil have been separated from the racemic mixture by the capillary electrophoresis method, there are no reports available on the enantioselective preparation of bepridil enantiomers.[5] Furthermore, in recent years, it has been recognized that bepridil could be important in new therapeutic areas such as Alzheimer's disease,[6a] viral infections,[6b] and atrial fibrillation[6c] and in certain neurological disorders.[6d] Very recently, bepridil has been identified as a potential lead molecule against Ebola virus disease (EBOV) by inhibiting a later stage of viral entry. Presumably, with bepridil being an approved drug, its repurposing may rapidly move to human testing and it has potential to become a frontline against Ebola virus infection.[7]

By understanding the significance of bepridil in many new therapeutic indications, it seemed timely to develop a new and effective enantioselective synthetic route to bepridil enantiomers. These bepridil enantiomers and their analogues would be extremely useful in the early phase of the drug discovery program to ascertain their pharmacological, toxicological, pharmacodynamic and pharmacokinetic characteristics.

Epoxides constitute one of the most widely used functional groups in organic transformations and serve as important building blocks in the industrial syntheses of a wide variety of organic materials.[8] Over the past few years, investigations in our laboratory have demonstrated the potential utility of these epoxides for the synthesis of many pharmaceutically important compounds.[9,10] Herein we report a new and simple enantioselective synthetic strategy to access both enantiomers of bepridil starting from commercially available epoxide.

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Research

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Identification of potent chromone embedded [1,2,3]-triazoles as novel antitubercular agents

Viswanadh Nalla[1,2], Aslam Shaikh[1,2], Sanket Bapat[4], Renu Vyas[4], M. Karthikeyan[1,2], P. Yogeeswari[3], D. Sriram[3] and M. Muthukrishnan[1,2]

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A series of 20 novel chromone embedded [1,2,3]-triazoles derivatives were synthesized via an easy and convenient synthetic procedure starting from 2-hydroxy acetophenone. The *in vitro* anti-mycobacterial evaluation studies carried out in this work reveal that seven compounds exhibits significant inhibition against *Mycobacterium tuberculosis* H37Rv strain with MIC in the range of 1.56–12.5 µg ml^{-1}. Noticeably, compound **6s** was the most potent compound *in vitro* with a MIC value of 1.56 µg ml^{-1}. Molecular docking and chemoinformatics studies revealed that compound **6s** displayed drug-like properties against the enoyl-acyl carrier protein reductase of *M. tuberculosis* further establishing its potential as a potent inhibitor.

1. Introduction

Tuberculosis is a major infectious disease caused by *Mycobacterium tuberculosis* (Mtb) and it is estimated that there are 10.4 million new cases and 1.4 million deaths in 2015 alone, of which developing countries showed a major share [1]. Recent study reveals that the numbers of TB cases in India are two to three times higher than previously estimated suggesting that global number of TB cases might be largely underestimated [2]. Furthermore, the emergence of a drug resistant microorganism responsible for TB, especially multidrug-resistant one along with lethal combination of TB and HIV infection makes this

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(57) Abstract: The present invention discloses a process for synthesis of Lorcaserin and its analogues using epoxide/chiral epoxide.

Erratum

Erratum

Erratum