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CERTIFICATE

This is to certify that the work embodied in this thesis entitled **"Design, Synthesis and Pharmacological Evaluation of Small Organic Molecules for Therapeutic Agents"** has been carried out by **Mr. Subal Kumar Dinda** under my supervision. He has fulfilled all the requirements of the Jawaharlal Nehru University (JNU), New Delhi, India for the award of the degree of Doctor of Philosophy. He has completed the prescribed attendance at the research centre in accordance with the rules. The work included in this thesis is original, carried out by the candidate himself, unless stated otherwise and has not been submitted in part or full, for any other degree or diploma of any other University/Institute.

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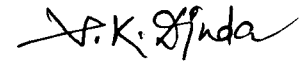

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Contents

List of Abbreviations

$[\alpha]_D^{25}$	specific rotation at 25 °C and wavelength of sodium D line
AD	Sharpless asymmtric dihydroxylation
AE	Sharpless asymmtric epoxidation
aq	aqueous
anhyd	anhydrous
$AlCl_3$	aluminium trichloride
ATPγS	adenosine-5'-O-(3-thiotriphosphate)
cAMP	cyclic 3', 5'-adenosinemonophosphate
Bn	benzyl
Boc	*tert*-butyloxy carbonyl
9-BBN	9-Borabicyclo[3.3.1]nonane
br	broad (in NMR)
c	concentration in g/ml
°C	degree Celcius
calcd.	calculated
$CDCl_3$	deuterated chloroform
^1H-^1H COSY	proton proton correlated spectroscopy
d	doublet (in NMR)
DCM	dichloromethane
dd	double doublet (in NMR)
DIBAL-H	diisobutyl aluminum hydride
DIPT	diisopropyl tartrate
DET	diethyl tartrate
DEAD	diethyl azodicarboxylate
DIPEA	*N,N*-diisopropyl ethyl amine
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
equiv	equivalent
ee	enantiomeric excess
g	gram (s)
h	hour (s)
HMBC	heteronuclear multiple bond correlation
HSQC	heteronuclear single quantum coherence
Hz	hertz
IC_{50}	inhibitory concentration for 50% inhibition
IR	infrared
J	coupling constant (in NMR)
LAH	lithium aluminum hydride
LHMDS	lithium hexamethyldisilazide

m	multiplet (in NMR)
MDR	multi drug-resistant
mg	milligram (s)
mL	milliliter (s)
mmole	millimole (s)
m/z	mass to charge ratio
M^+	molecular ion peak
MeI	iodomethane
MHz	megahertz
mp	melting point
MS	mass spectroscopy
2-MeSADP	2-methylthio-adenosine-5'-diphosphate
N	normality
NMR	nuclear magnetic resonance
Py	pyridine
PDC	pyridinium dichromate
PMB	4-methoxybenzyl
PPh_3	triphenylphosphine
PNB	4-nitrobenzoyl
PCC	pyridinium chlorochromate
Pd/C	palladium on charcoal
ppm	parts per million (in NMR)
q	quartet (in NMR)
R_f	retardation factor
rt	room temperature
s	singlet (in NMR)
t	triplet (in NMR)
TBHP	*tert*-butylhydroperoxide
TFA	trifluoroacetic acid
$Ti(O^iPr)_4$	titanium (IV) tetraisopropoxide
tert	tertiary
THF	tetrahydrofuran
TMS	tetramethyl silane
TBS	*tert*-butyldimethylsilyl
TBDMS	*tert*-butyldimethylsilyl
TLC	thin layer chromatography
TMSCl	trimethylsilyl chloride
Ts	*p*-toluenesulfonyl
UV	ultraviolet
μM	micromolar
WHO	world health organization

Preface

The role played by modern organic synthesis in the pharmaceutical industry sustains to be one of the main drivers in the drug discovery process. The problem of the limited availability of natural products in bio-evaluation has been tackled in many cases by modern methods of organic synthesis with which small molecules including natural products can be prepared in sufficient quantities. Small molecules include new drugs and drug candidates and reagents and these have proven to be invaluable tools for investigating biological systems. Synthetic small molecules make up a major portion of the modern screening palette. Advances in the field of synthetic organic chemistry have led to the development of many stereoselective methodologies for proficient assemblage of small organic molecules. Much endeavor has been applied on the development of asymmetric variants of well-established reactions, and upon finding of a new reaction that generates new stereogenic centers, development of stereoselective versions using chiral auxiliaries, reagents, or catalysts. The use of small molecules by 'Chemical Genetics' approach provide fast, conditional, dose-dependent, and often reversible control of biological functions. Thus, dynamic processes such as the cell cycle and development can be dissected in details by adding or removing the small molecule at appropriate times. Moreover, in contrast to Classical Genetic approach (gene knockouts and RNA knockdowns), selective small molecule probes can be used to study the individual functions of multifunctional proteins and can distinguish between different conformational and post-translational modification states of their targets. Thus, the use of small molecules to activate or inactivate proteins by direct interactions has come out as a powerful tool for the study of complex biological systems. Small molecules can also be used to illuminate new potential therapeutic targets and provide a direct means of validating these targets in model systems. Thus, the identification of new, highly specific small molecule probes remains a significant current challenge in chemical biology and drug discovery.

The thesis entitled **"Design, Synthesis and Pharmacological Evaluation of Small Organic Molecules for Therapeutic Agents"** is arranged under **seven** chapters:

Chapter 1: Utilization of enantiopure 2,3-epoxyalcohols and *syn*-2,3-dihydroxy esters in the synthesis of natural products and natural product-like molecules

Chapter 1 includes an overview of selected literature reports covering synthesis of natural products (NPs) and natural product-like molecules (NPLMs) involving 1,2-aminoalcohol, 2,3-dihydrobenzofuran & 1-benzopyran moieties employing the Sharpless asymmetric dihydroxylation and epoxidation as the key chirality inducing steps.

Chapter 2: Synthesis and Pharmacological Evaluation of a series of Aryl aryl methyl thio arenes (AAMTAs) as Antimalarial Therapeutics

Chapter 2 deals with our study with Aryl aryl methyl thio arenes (AAMTAs) showing antimalarial activity *in vivo* in animal model. Interestingly, AASMPs exhibit acceptable selectivity against the malaria parasite and show antimalarial activity *in vivo* against the MDR rodent malaria parasite *P. yoelii.*

Chapter 3: Stereoselective Synthesis of Functionalized 2,3-Dihydrobenzofurans and 1-Benzopyrans by Phenoxide ion-Mediated Carbocyclization

This chapter is divided into two sections: **Sections 3A** and **Section 3B**.

Sections 3A deals with efficient asymmetric synthesis of 2-isopropenyl-2,3-dihydrobenzofurans and 4-(2,3-dihydrobenzofuran-2-yl)-2-methylbut-3-en-2-ols. Key steps include Sharpless asymmetric epoxidation reaction on suitable allyl alcohols and construction of the 2,3-dihydrobenzofuran nucleus by phenolate ion-mediated intramolecular 5-*exo-tet* epoxide ring opening reactions. The simplicity of the reaction sequence, as well as the commercial accessibility of large array of starting 2-hydroxyaromatic aldehydes, makes this process a convenient method for the preparation of "natural-product-like" 2-substituted 2,3-dihydrobenzofuran frameworks. In addition, the scope the reaction sequence is much broader, and synthesis of various substituted aromatic and heteroaromatic nuclei can be envisioned from the starting aldehydes.

Section 3B deals with efficient asymmetric synthetic methods of enantiomerically pure 2-hydroxymethyl chromans and 4-chroman-2-yl-2-methyl-but-3-en-2-ols. Key steps include Sharpless asymmetric epoxidation reaction on suitable allyl alcohol and construction of the benzopyran nucleus by phenoxide ion mediated intramolecular 6-*exo-tet* epoxide ring opening. The ease of the reaction sequence, as well as the rapid accessibility of the starting 2-allylphenols, makes this process a practical method for the preparation of optically active 2-hydroxymethyl-chromans.

Chapter 4: Asymmetric Total Syntheses of Spisulosine, Its Diastereo- and Regioisomers

Chapter 4 illustrates the first protecting group-free syntheses of spisulosine, an anticancer marine natural product and its diastereo- and regioisomers employing Sharpless asymmetric epoxidation reaction as the source of chirality. The other merits of this synthesis are high-yielding reaction steps, high enantioselectivity and various possibilities available for structural modification and thus it might be considered as a general synthetic strategy to enantiomerically pure 2-amino-3-alkanols.

Chapter 5: Stereoselective Synthesis of Functionalized 1-Benzoxepines

Chapter 5 deals with an asymmetric synthesis of 2,3-disubstituted 1-benzoxepines by an easy and high yielding reaction sequence. Key steps include Sharpless asymmetric dihydroxylation reaction on suitable α,β-unsaturated esters and construction of the 1-benzoxepine nuclei by phenoxide ion-directed intramolecular 7-*endo-tet* carbocyclization of *syn*-2,3-dihydroxy ester-derived cyclic sulphates. Presence of methoxy group *ortho* to the phenolic –OH functionality on the phenyl ring rendered the cyclization reaction completely regioselective producing 1-benzoxepine derivatives only. In the absence of a methoxy group on the phenyl ring, the reaction furnished both 1-benzoxepine and 1-benzopyran derivatives with the former being the major one.

Chapter 6. An Enantioselective Approach towards Synthesis of a potent $C_{17,20}$-Lyase Inhibitor

Chapter 6 deals with our preliminary studies for enatioselective synthesis of a potent $C_{17,20}$-lyase inhibitor and its other analogues. Notable features of this approach include the use of Sharpless asymmetric dihydroxylation to synthesize the enantiomerically pure 1,2-diol and and thus both enatiomers could be obtained by varying the ligands. The other merits of this synthesis are high-yielding reaction steps, high enantioselectivity and various possibilities available for structural modification and thus it might be considered as a general synthetic strategy to enantiomerically pure tertiary alcohols bearing the two aromatic rings.

Chapter 7. Design and Synthesis of Small Organic Molecules for P2X ion and P2Y G Protein-Coupled Receptors (GPCRs)

Chapter 7 deals with our preliminary studies for the quest to improve the bioavailability of Suramin related molecules, a polysulfonated naphthylurea, that served as a highly successful chemical lead for the development of potent and selective P2X antagonists. We have successfully replaced the sulfonate group of suramin analogs with esters and acids. Furthermore, the position of *bis*-urea has also been replaced by different spacers as shown in the chaper 7. It is also suggested that the size of the molecule might play a vital role in approaching the P2 receptors. In this quest, the bulk of the suramin has been reduced to small organic molecules having requisite functional groups with different spacers like amides (-CONH-), saturated amines, acids and esters.

Chapter 1:

Utilization of enantiopure 2,3-epoxyalcohols and *syn*-2,3-dihydroxy esters in the synthesis of natural products and natural product-like molecules

1.1 Introduction

Though molecular chirality might seem to be simply of academic importance, it has in reality, a great impact in our everyday life. The innate chirality of living systems dictates surprising specificity in the recognition of chiral molecules. Almost all living organisms have single enantiomer molecular components. [1] Hence the bioactivity of the two enantiomers or diastereomers of a food ingredient or drug can be entirely dissimilar.[2] The smells of lemon and orange vary in being the left- and right-handed versions of the same molecule, Limonene (Figure 1.1). In the same way, caraway and spearmint seeds smell pretty different. The natural α-amino acids, which are the building blocks of proteins, are found only in the left-handed L-configuration (L–amino acids) whereas sugars are found in the right-handed D-configuration. Our enzymes and nucleic acids are chiefly composed of D–sugars and L–amino acids. The varied chemical processes in living cells depend on, and sustain, these asymmetries, since they are constrained by enzyme catalysis. The enzymes are simply protein molecules providing an active site into which reactants (drug) can fit. Especially, after the most infamous *Thalidomide* drug tradegy[3], and also with the black history of chiral drugs like *Seldane*, *Albuterol*, *Ketamine*, the importance of chirality in pharmaceuticals have become very intense. Consequently, issues related to chirality have gradually encompassed pharmaceutical chemistry research. It resulted in the FDA (Food and Drug Administration, USA) regulations governing chiral active pharmaceutical ingredients (APIs) (with >30% market share)[4] and hence the rapidly increasing demand from the pharmaceutical industry for enantiomerically pure compounds. And this has triggered extensive development in asymmetric synthesis[5].

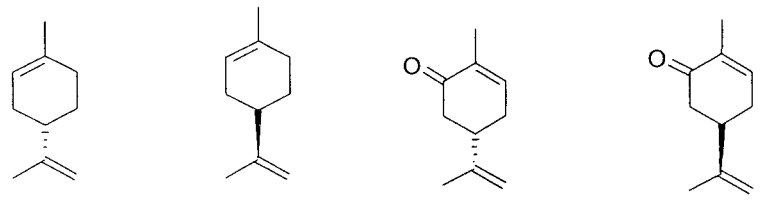


Figure 1.1 *S*-(-)-limonene (Smell of lemons) *R*-(+)-limonene (Smell of oranges) *S*-(+)-Carvone (smell of Spearmint) *R*-(-)-Carvone (smell of Caraway)

1.2 Different Approaches for Accessing Chiral Molecules

Three chief ways for synthesis of enantiomerically pure compounds, namely, resolution, chiral pool and asymmetric synthesis, are schematically represented in Figure 1.2[2].

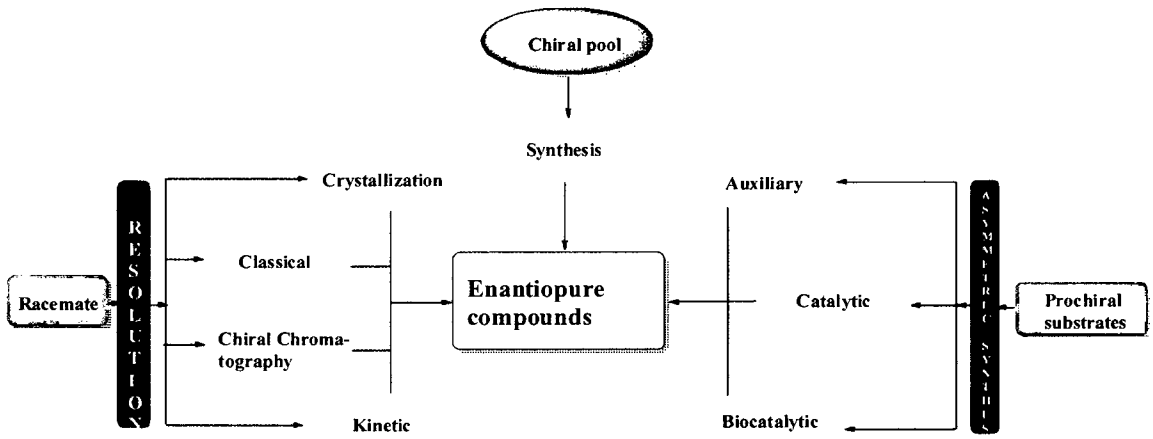


Figure 1.2 Approaches for synthesis of enantio pure compounds

1.2.1 Resolution of Racemates

Resolution still provides the main technique for obtaining pure enantiomers industrially.[6] In classical resolution, a stoichiometric amount of a chiral resolving agent is associated to the substrate to furnish a pair of diastereisomers, which are then separated through a chemical transformation, the substrate being released from the resolving agent.[7] Kinetic resolution[8] is based on the principle that in the presence of an optically active catalyst, reagent or biocatalyst, one of the enantiomers of a racemic mixture is more rapidly transformed or metabolized to the product than the other. In the direct crystallization,[9] the two enantiomers are allowed to crystallize at the same time in different vessels by adding seeds of the opposite enantiomers to a racemic supersaturated solution and the racemic filtrate is recycled after concentration, provided the target molecule exists as crystalline conglomerates (racemic mixture) rather than racemic compounds. But only a few chiral compounds exist in crystalline conglomerate form. Because of long separation times, the large volume of solvent used and relatively high cost of the chiral chromatographic supports, chiral chromatography[10] often is carried out only on analytical or preparative scale.

1.2.2 The Chiral Pool Approach

In this approach, inexpensive naturally occurring compounds such as α-amino acids, carbohydrates, terpenes, alkaloids etc. are used as chiral building blocks to obtain the target molecules with retention of chirality through successive synthetic manipulations. It is especially attractive for target molecules having the similar chirality to the chiral compounds used. This strategy suffers from some limitations such as only limited number of starting materials are available, sometimes quantity of the desired molecule is very small and its isolation from the source demands for high degree of labour and cost factors.

1.2.3 Asymmetric Synthesis

Asymmetric synthesis or stereoselective synthesis is the preferential formation in a chemical reaction of one enantiomer or diastereoisomer over the other as a result of the influence of a chiral feature present in the substrate, reagent, catalyst or environment.

Asymmetric synthesis can be substrate-controlled, auxillary-controlled, reagent-controlled or catalyst-controlled. The creation of a new chiral centre is directed by the presence of a stereogenic unit already present within the chiral substrate (substrate-controlled) while in the auxiliary-control a chiral auxiliary is attached to an achiral substrate so as to direct the enantioselectivity, and after transformation, the chiral auxiliary is removed and often recycled. An achiral substrate is directly transformed to a chiral product utilizing an enantiopure chiral reagent (reagent-control). Nevertheless, these three chiral transformations need at least one equivalent of an enantiopure compound that is not very pleasing from economical as well as environmental viewpoints. Hence, over the last forty years the most significant advancement in asymmetric synthesis has been the development and application of chiral catalysts to induce the stereoselectivity.

Over the last few decades a number of potent asymmetric reactions have appeared as an outcome of the growing need to develop efficient and practical syntheses of bio-active compounds. Catalytic asymmetric reactions afford an especially practical entry into the chiral world due to their economical use of asymmetry inducing agents.[11] In 2001 the Nobel Prize in Chemistry was awarded to

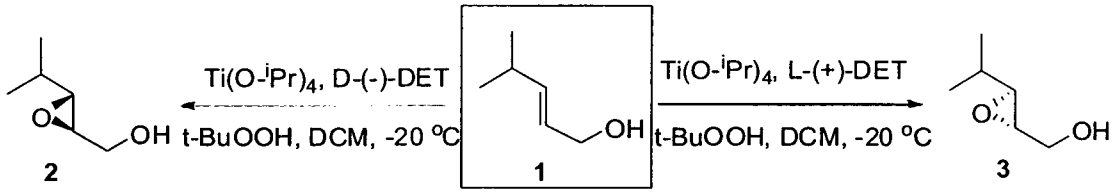
Prof. William S. Knowles, **Prof. Ryoji Noyori**, and **Prof. K. Barry Sharpless** for their contributions in the development of catalytic asymmetric synthesis.[12]

1.3 Sharpless Asymmetric Epoxidation (AE) and Dihydroxylation (AD)

Two most important chirality inducing reactions developed by the Sharpless group are the asymmetric epoxidation (AE) of allylic alcohols and the osmium-catalyzed asymmetric dihydrxylation (AD) of olefins.

1.3.1 Sharpless Asymmetric Epoxidation (AE)

Since its discovery in 1980, Sharpless Asymmetric Epoxidation (AE) was by far the best asymmetric reaction known to date. It is one of the central transformations used for the enantioselective chemical reaction to furnish 2,3-epoxyalcohols from primary and secondary allylic alcohols.[13] When a prochiral E-allylic alcohol is treated with a 10- membered fluxional complex (two titanium atoms bridged by two tartrate ligands) formed by equimolar reaction of dialkyl tartrate and titanium tetraisopropoxide at -20 °C in the presence of 4Å molecular sieves (4Å MS) in dry DCM followed by treatment with allylic alcohol and t-butyl hydroperoxide (TBHP), it leads to the formation of 10 membered dissymmetric complex which delivers the epoxidation stereoselectively. The absolute configuration of the epoxide is predictable with the CH_2OH group of the allylic alcohol **1** written on the lower right in the rectangular plane as shown in Scheme 1.1. D-(-)-DIPT (diisopropyl tartrate) or DET (diethyl tartrate) deliver the epoxidation from the β-face of the allylic alcohol furnishing compound **2** while L-(+)-DET delivers the epoxidation from the α-face of the allylic alcohol furnishing compound **3**. Easy availability of reagents involved, and high enantiomeric (or diastereomeric) excess obtained in the reaction made the AE to find widespread application in the introduction of chirality in the complex target molecules. The easy and precise prediction of stereochemical outcome irrespective of the substitution pattern on the allylc alcohol further emphasized the reaction application. Since chiral epoxides can be easily converted into dialcohols, aminoalcohols or ethers etc, this reaction has found wide application in asymmetric organic transformation, especially in the synthesis of complex chiral molecules, that has led to several reviews.[14]



Scheme 1.1

1.3.2 Sharpless Asymmetric Dihydroxylation (AD)

The catalytic asymmetric dihydroxylation (AD) of olefins allows access to a wide variety of vicinal diols of high enantiomeric purity.[15] The highly enantiopure 1,2- diol compounds obtained are versatile and convenient building blocks in the synthesis of bioactive compounds. This osmium tetroxide catalyzed dihydroxylation has been developed into an extremely efficient and selective process for wide variety of substituted olefins. Chiral amine ligands provide the required rate enhancement and asymmetric induction, by coordinating to the osmium atom. The most popular ligands are based on the naturally occurring cinchona alkaloids dihydroquinidine (DHQD) or dihydroquinine (DHQ). In particular, the ligands $(DHQ)_2PHAL$ **7** and $(DHQD)_2PHAL$ **8** (Figure 1.4), in which two of the alkaloids are connected to a 1,4-phathalazine (PHAL) ring, have found widespread use. The dihydroxylation reaction can be carried out with osmium tetroxide as a catalyst, typically added in the lower oxidation state as the solid $[K_2OsO_4.2H_2O]$, and the favoured co-oxidant of choice is potassium ferricyanide $[K_3Fe(CN)_6]$. The additive methanesulfonamide $CH_3SO_2NH_2$ often enhances the rate of the reaction. The reagent combination with the ligand $(DHQ)_2PHAL$ **7** is referred to as AD-mix-α and with $(DHQD)_2PHAL$ **8** as to AD-mix-β. These two ligands provide the opposite enantioselectivity and therefore either enantiomer of the vicinal diol can be accessed readily. The ligands **7** and **8** are not enantiomeric, but do provoke each mirror image environment around the metal, which co-ordinates to the quinuclidine nitrogen atom (the chiral center adjacent to this nitrogen atom and that bearing the oxygen atom are enantiomeric in these two ligands). The alkene must approach in a preferred orientation and this has been depicted by mnemonic in Figure 1.3. With the largest olefin substituent in the lower left corner as drawn, the DHQ based ligand system promotes dihydroxylation from the lower (α), while the top (β) face reacts with the DHQD-based ligand system. A typical example of AD is shown in Scheme 1.2[33].

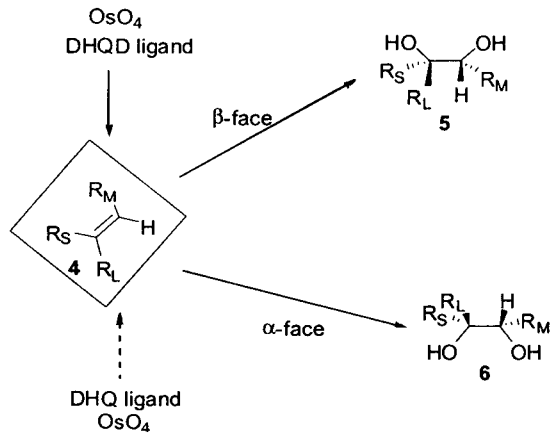
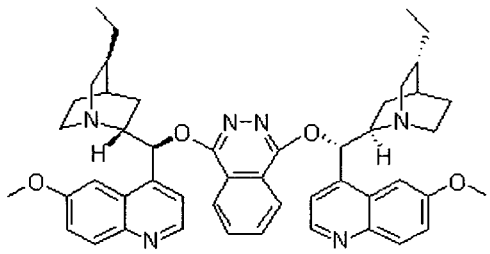
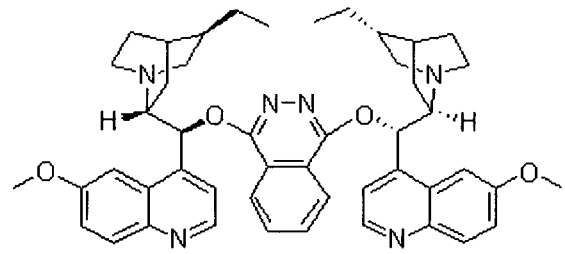


Figure 1.3

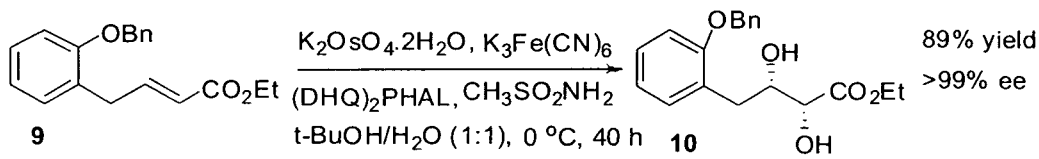


Hydroquinine 1,4-phthalazinediyl diether
$(DHQ)_2PHAL$ **7**



Hydroquinidine 1,4-phthalazinediyl diether
$(DHQD)_2PHAL$ **8**

Figure 1.4



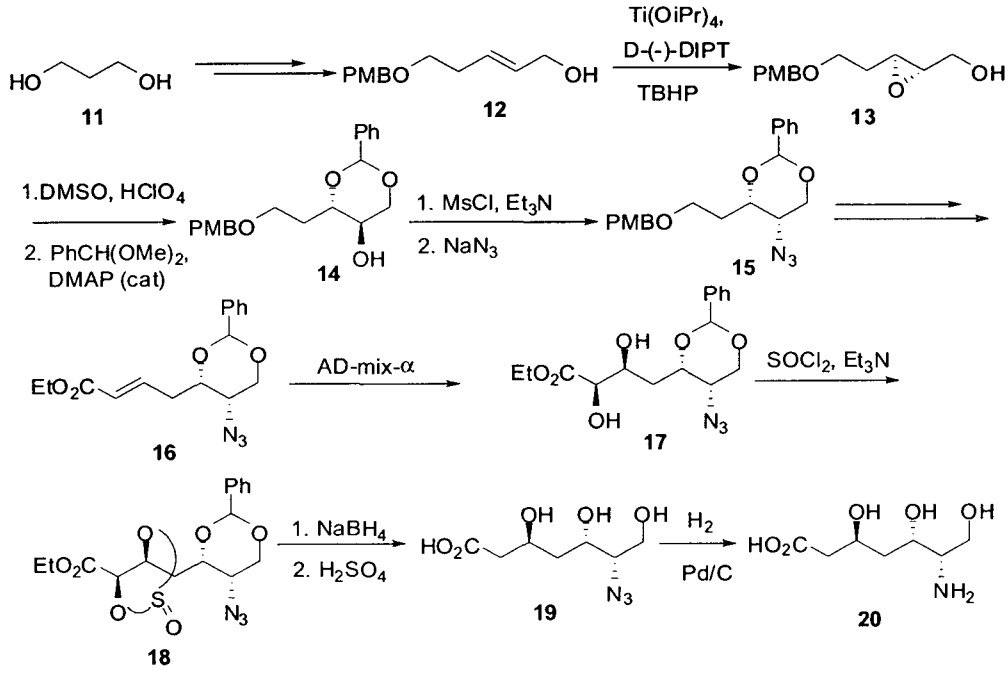
Scheme 1.2

In the following sections, we have depicted some selected literature reports covering synthesis of natural products (NPs) and natural product-like molecules (NPLMs) containing 1,2-aminoalcohol, 2,3-dihydrobenzofuran, 1-benzopyran moieties, and some cyclic natural products employing the Sharpless asymmetric dihydroxylation and epoxidation as the key chirality inducing steps.

1.4 Synthesis of NPs and NPLMs containing 1,2-Aminoalcohol Functionality

1.4.1 Enantioselective synthesis of (-)-galantinic acid

An efficient enantioselective synthesis of (-)-galantinic acid **20**, a non-proteogenic amino acid was achieved by Kumar *et al* using Sharpless asymmetric epoxidation (of compound 12), dihydroxylation (of 16) and the regioselective nucleophilic opening of a cyclic sulfite **18** as the key steps (Scheme 1.3)[16].

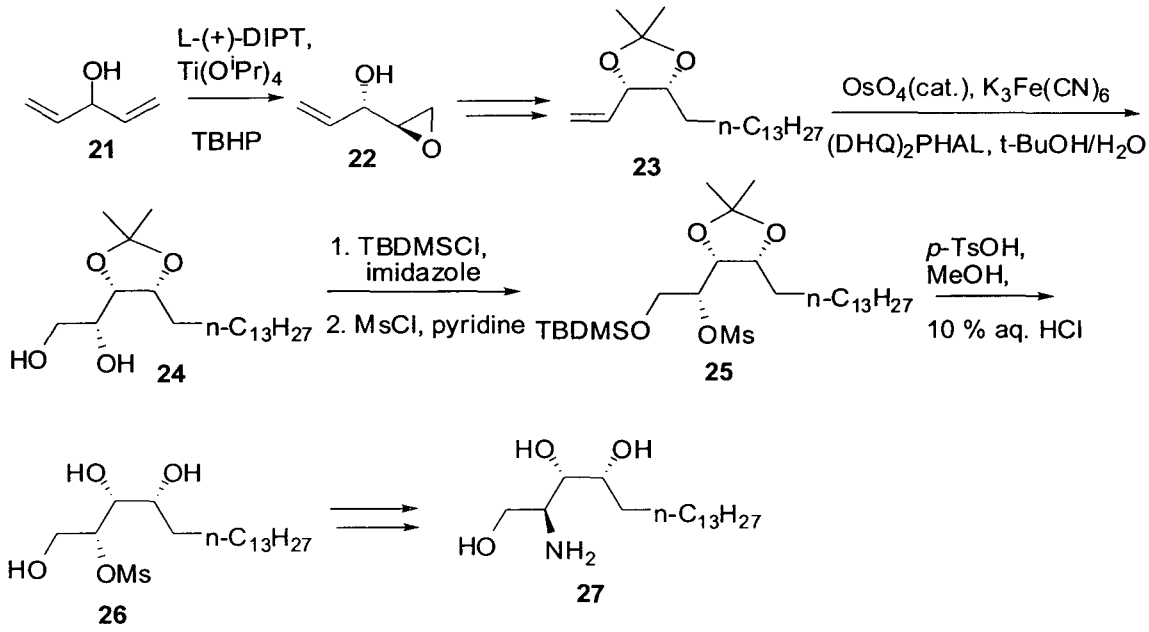


Scheme 1.3

1.4.2 Synthesis of D-ribo-Crs-Phytosphingosine

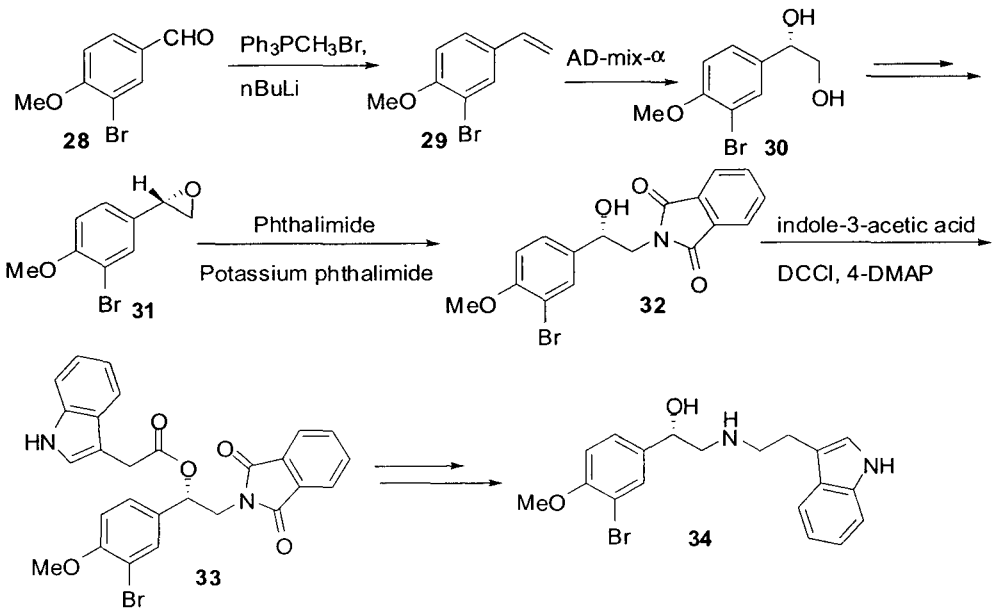
A facile synthesis of D-ribo-Crs-Phytosphingosine **27** *via* Sharpless AE and AD was accomplished by Guo-qiang Lin *et al.*[17] The oxirane **22** was readily obtained from divinylcarbinol **21** (Scheme 1.4) using AE with high de (98%) and ee (97%). Subsequent regioselective ring opening with n-tridecyl magnesium bromide in the presence of copper (I) iodide followed by removal of silyl group furnished a diol, which upon treatment with dimethoxy propane easily provided the isopropylidene derivative **23**. Dihydroxylation of compound **23** with Sharpless conditions gave a mixture of diastereoisomers. The desired product **24** was favoured when the $(DHQ)_2PHAL$ was used as the chiral ligand. Then protection of the primary alcohol with TBDMSCl, and mesylation of the secondary alcohol followed by removal of the ketyl group and sillyl group provides the triol **26**, which upon treatment with sodium

azide followed by LAH reduction (Schmidt's protocol)[18] afforded the target molecule **27**.



Scheme 1.4

1.4.3 First Total Synthesis of the Marine Natural Product (*S*)-(+)-Chelonin B



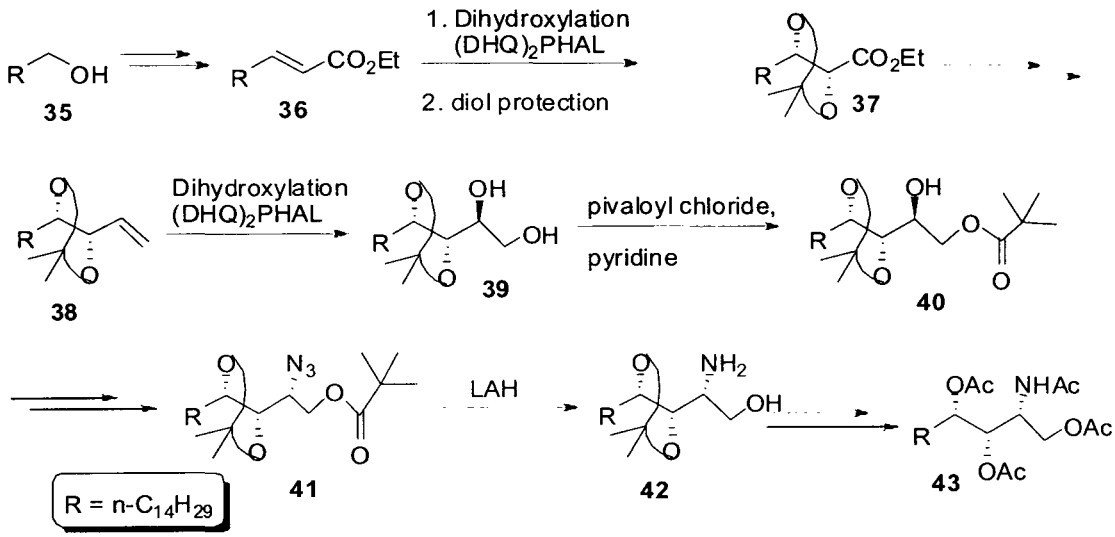
Scheme 1. 5

Another example of applications of the Sharpless AD is the first total synthesis of the marine natural product (*S*)-(+)-chelonin B **34** (Scheme 1.5).[19] Transformation of the

(*S*)-diol **30** into the corresponding enantiomerically enriched (*S*)-isomer of oxirane **31** was accomplished according to the method of Sharpless *et al.*[20] Compound **31** upon catalytic epoxide ring-opening followed by sequential deprotection–rearrangement of a phthalimido indole acetate provided the target molecule **34**.

1.4.4 First Synthesis of L-*xylo*-(2*R*,3*S*,4*S*)-C18-phytosphingosine

Pradeep Kumar and co-workers accomplished the first diastereoselective synthesis of L-*xylo*-(2*R*,3*S*,4*S*)-C18-phytosphingosine **43** by utilizing double stereodifferentiation[21] in asymmetric dihydroxylation (Scheme 1.6).[22]

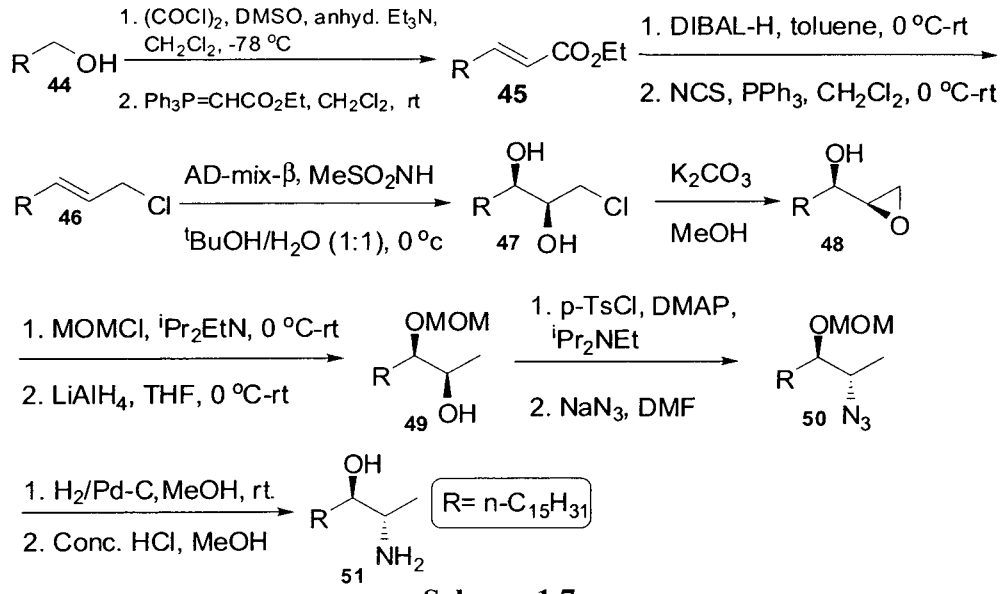


Scheme 1.6

Through stepwise synthetic manipulations such as the dihydroxy protection as acetonide followed by LAH reduction of ester group, Swern oxidation of the primary alcohol and Wittig olefination, the first dihydroxylation product **37** was converted to the enantiomerically enriched terminal olefin **38**. Compound **38** was then subjected to the second dihydroxylation using $(DHQD)_2PHAL$ ligand while compound **39** was obtained with a diastereomeric ratio of 83:17. The primary hydroxyl group was protected as pivaloate and the secondary hydroxyl group converted to the azide functionality. Subsequent treatment with LAH furnished the aminoalcohol **42** through pivaloate deprotection and azide reduction simultaneously. Acetonide deprotection followed by acetylation provided the final compound **43**.

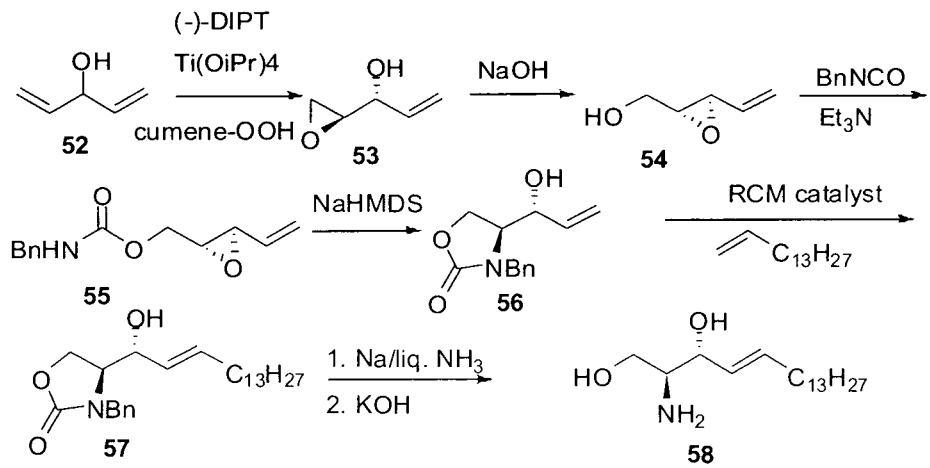
1.4.5 Synthesis of Spisulosine

Panda *et al.* utilized the diol **47** easily derived from Sharpless asymmetric hydroxylation of allyl chloride **46** for synthesis of spisulosine **51**, an anticancer marine natural product (Scheme 1.7).[23] Treatment of diol **46** with powdered NaOH in THF at 0 °C afforded the epoxide **48.** The hydroxyl group of **48** was protected as methoxymethyl ether with MOMCl in CH_2Cl_2 in the presence of *N,N*-diisopropylethylamine, and subsequent LAH mediated regioselective reductive ring opening at 0 °C provided **49**. Tosylation of the hydroxyl group of compound **49** follwed by S_N^2 substitution of the tosyl with azide group furnished compound **50**, which on azide reduction followed by MOM deprotection afforded the final compound **51**.



Scheme 1.7

1.4.6 Synthesis of D-*erythro*-sphingosine

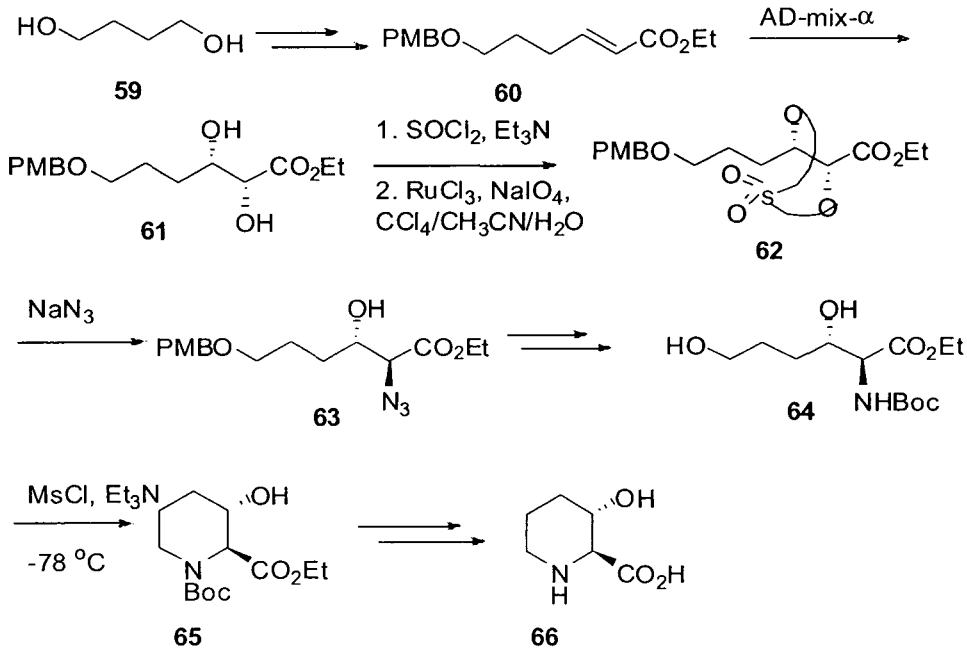


Scheme 1.8

The Somfai group employed Sharpless AE in the synthesis of D-*erythro*-sphingosine **58**. The key steps were regioselective opening (Pyne rearrangement) of the AE product vinylepoxide **53** and an *E*-selective cross-metathesis (of **56**) (Scheme 1.8)[24].

1.4.7 Synthesis of (2S,3S)-3-hydroxypipecolic acid

Pradeep Kumar and co-workers accomplished enantioselective synthesis of (2*S*,3*S*)-3-hydroxypipecolic acid **66** using Sharpless AD and the regioselective nucleophilic opening of a cyclic sulfate **62** as the key steps as depicted in Scheme 1.9.[25] The enantiopure diol **61** was converted to cyclic sulfate **62** which underwent the nucleophilic ring opening with NaN_3 regiospecifically at the α-carbon atom to furnish the azido alcohol **63**. The amino diol **64** obtained by deprotection of the PMB group followed by reduction of the azido group under hydrogenation in the presence of Boc_2O was transformed into the cyclized product on treatment with mesyl chloride and triethylamine at -78 °C to **65**. Subsequent ester hydrolysis with LiOH followed by Boc deprotection with TFA provided the final product **66**.



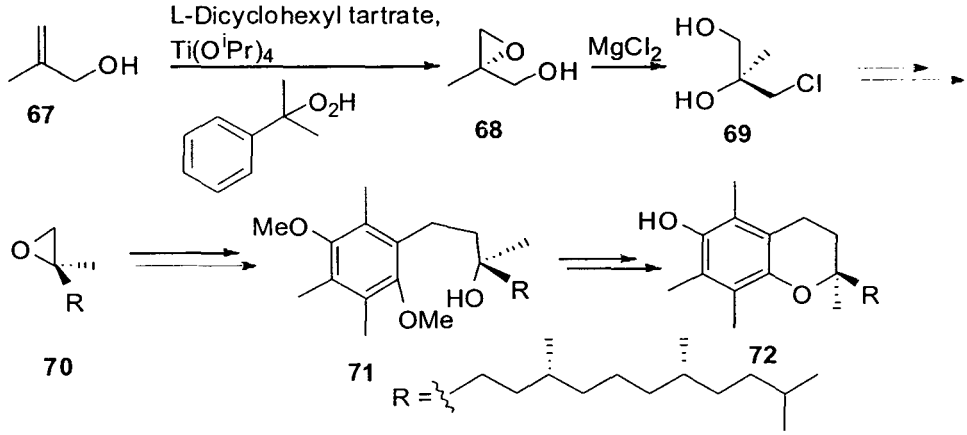
Scheme 1.9

1.5 Synthesis of NPs and NPLMs containing 2,3-Dihydrobenzofurans, 1-Benzopyrans and 1-Benzoxepine moieties

1.5.1 1-Benzopyrans

1.5.1.1 Total Synthesis of α-tocopherol

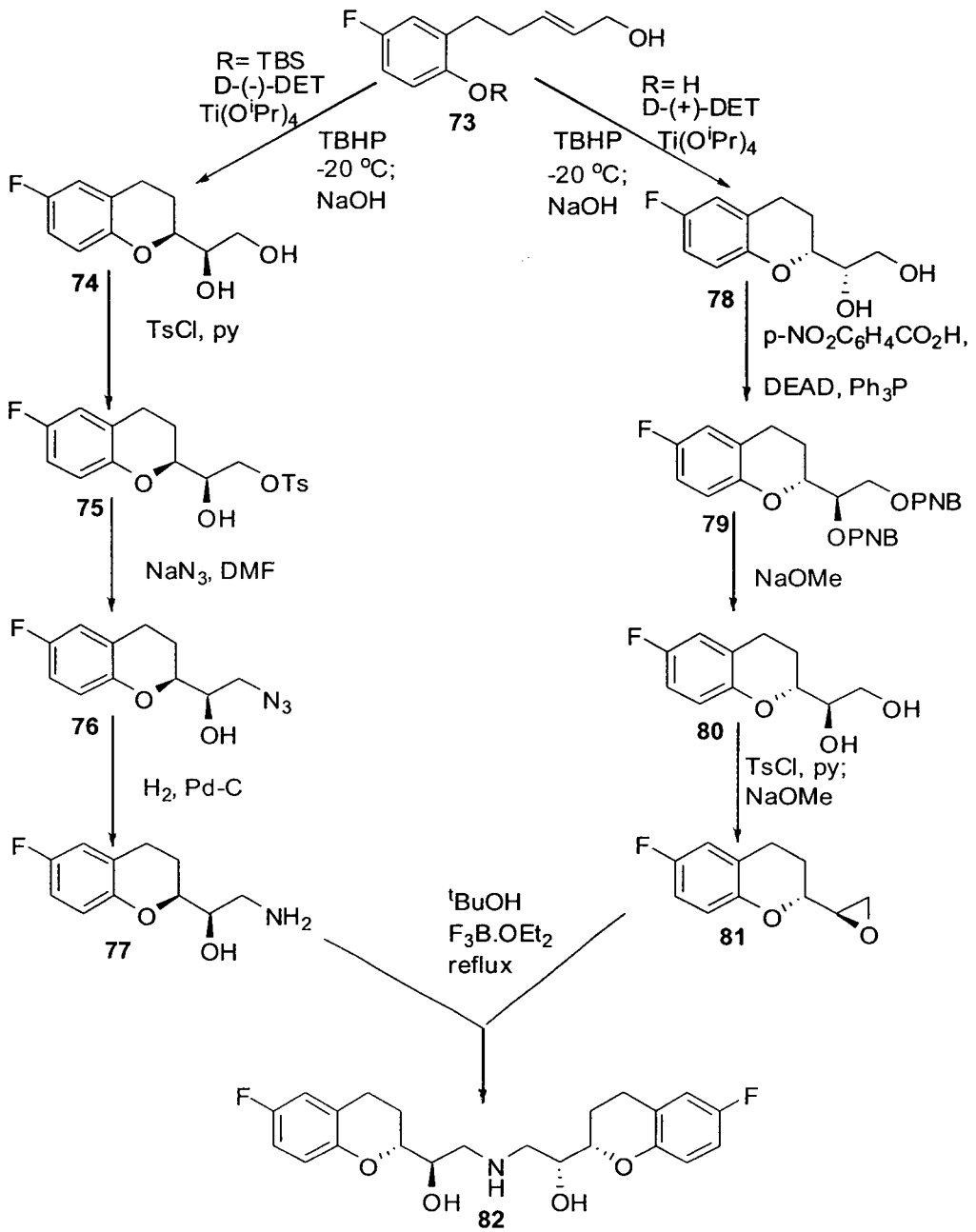
The Barner group achieved the total synthesis of α-tocopherol **72** by utilizing the Sharpless asymmetric epoxidation for chiral induction.[26] The chiral tertiary alcohol **69** was converted to epoxide **70**, of which the chiral center was eventually incorporated in the chroman unit of α-tocopherol **72** (Scheme 1.10).



Scheme 1.10

1.5.1.2 Total Synthesis of Nebivolol

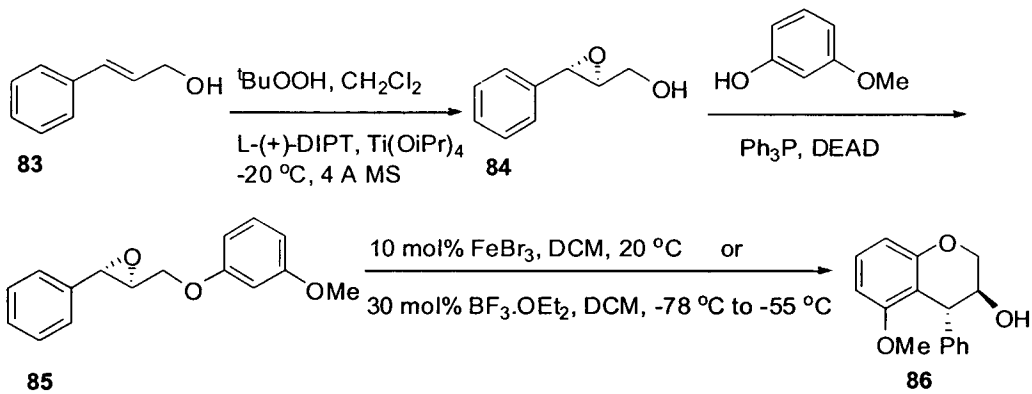
The total synthesis of Nebivolol **82** (Scheme 1.11), a hypertensive agent, was reported by Chandrasekhar et al.[27] In this synthesis, one-pot Sharpless AE followed by intramolecular epoxide opening with internal phenoxide anion to generate the chiral chromane was the key step. Two chroman intermediates **74** and **78**, their chirality being controlled by the epoxidation catalyst were obtained this way. Chromane **74** on treatment with tosylchloride followed by NaN_3 furnished the azido alcohol **76.** Reduction of the azide to its corresponding amine **77** accomplished the synthesis of the left fragment of **82**. Under Mitsunobu conditions (p-$NO_2C_6H_4CO_2H$, Ph_3P and DEAD) followed by deprotection of di-PNB ester **79**, chromane **78** furnished diol **80** with inversion at C2. Next, monotosylation followed by treatment with base (NaOMe) provided epoxide **81**. The total synthesis of **82** was completed by assembling the two fragments involving nucleophilic opening of epoxide **81** with hydroxy amine **77**.



Scheme 1.11

1.5.1.3 Lewis acid-catalyzed ring closure of Glycidyl esters

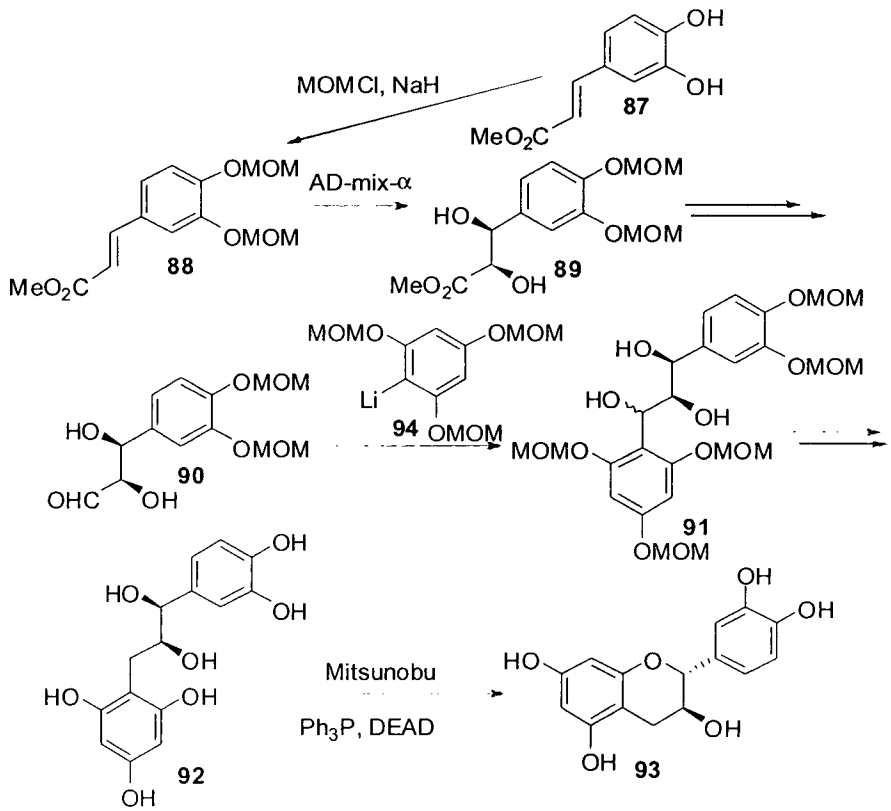
Aryl glycidyl ether **85**, readily available in enantiomerically pure form by Sharpless AE were shown to undergo a Lewis acid-catalyzed (using $BF_3.OEt_2$ at -55 °C, or $FeBr_3$ at 20 °C) stereospecific ring closure leading to enantiopure 3-chromanol **86** (Scheme 1.12).[28]



Scheme 1.12

1.5.1.4 Enantioselective Synthesis of (2R,3S)-(+)-catechin

Park and co-workers utilized Sharpless AD product **89** to achieve enantioselective synthesis of natural product, (2*R*,3*S*)-(+)-catechin **93** (Scheme 1.13).[29] The dihydroxyaldehyde compound **90** obtained from enantiopure diol **89** by successive synthetic manipulation was treated with lithiated compound **94** to furnish **91**.

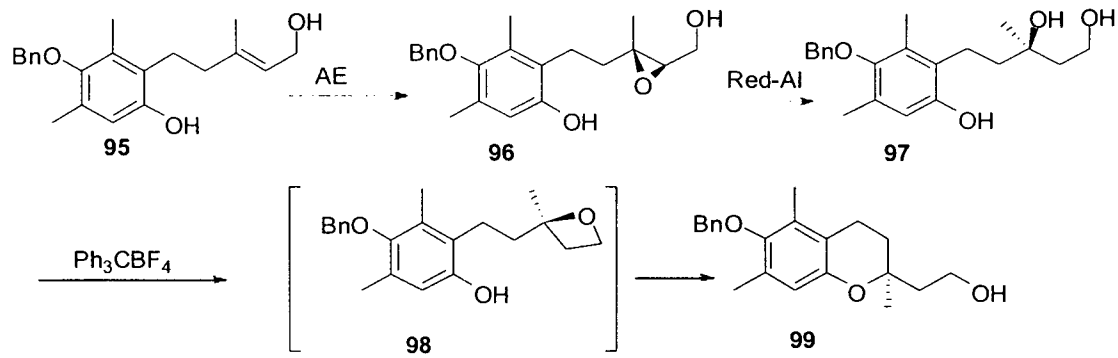


Scheme 1.13

Subsequent by selective deoxygenation *via* Barton–McCombie reaction [30] followed by MOM deprotection provided **92** which on Mitsunobu reaction gave **93**. Later, the Chan group applied the same strategy to access to various catechin analogues for studying their cytotoxic properties.[31]

1.5.1.5 Contribution from Achiwa group

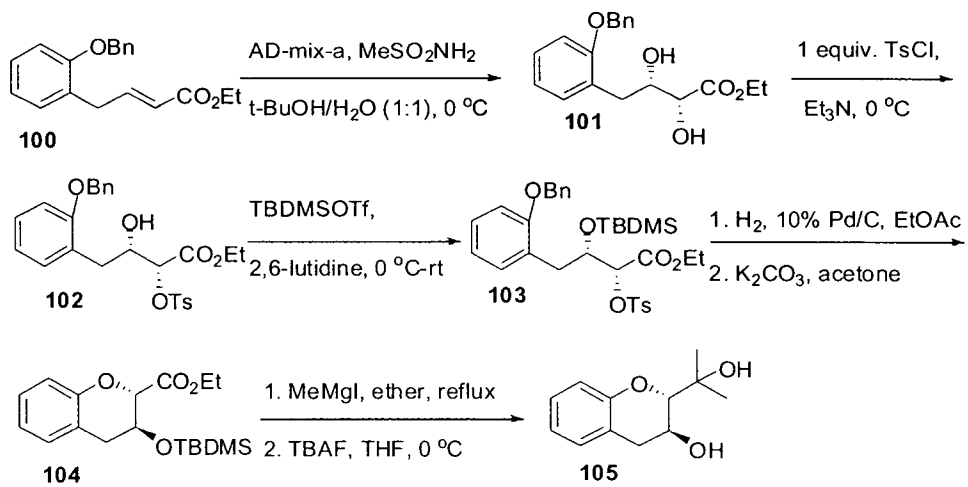
Another example of the application of Sharpless AE is the synthesis of chiral chroman **99** by Achiwa group. Epoxide **96** had undergone regioselective ring opening to give 1,3-diol **97** and further to chiral chroman **99** following an acid-promoted cyclization (Scheme 1.14)[32] through the oxetane intermediate **98**.



Scheme 1.14

1.5.1.6 Contribution from Panda's group

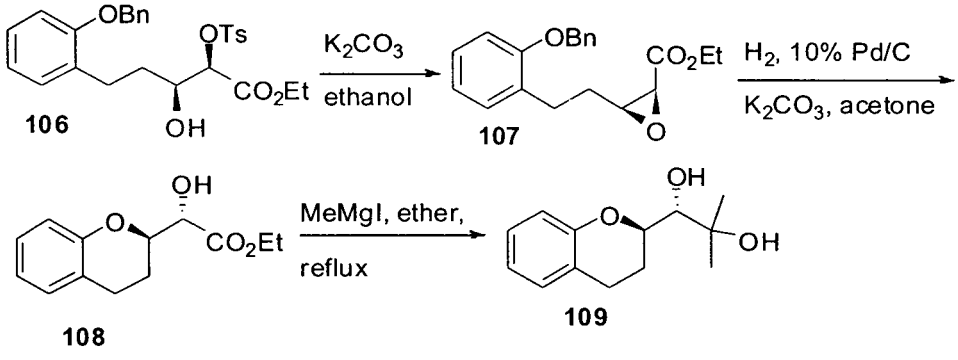
Panda et al. developed enentioselective routes towards a number of NPLMs containing substituted benzo-fused 1-oxaheterocycle moieties employing Sharpless asymmetric dihydroxylation as a source of chirality.[33]



Scheme 1.15

The *trans*-cinnamate ester **100** was subjected to Sharpless asymmetric dihydroxylation to furnish enantiopure diol **101**, which upon regioselective α-tosylation and TBDMS-protection of β-hydroxy group provided compound **103** (Scheme 1.15). The later compound underwent phenoxide ion mediated S_N2 cyclization to furnish chiral chroman **104** and further to **105**.

Again, α-tosyloxy-β-hydroxy ester **106** on treatment with K_2CO_3 to *syn*-glycidic ester **107**, which underwent phenoxide ion mediated carbocyclization to furnish "natural-product-like" dihydrobenzofuran skeleton furnished enantiopure chroman **108** and eventually **109** (Scheme 1.16).

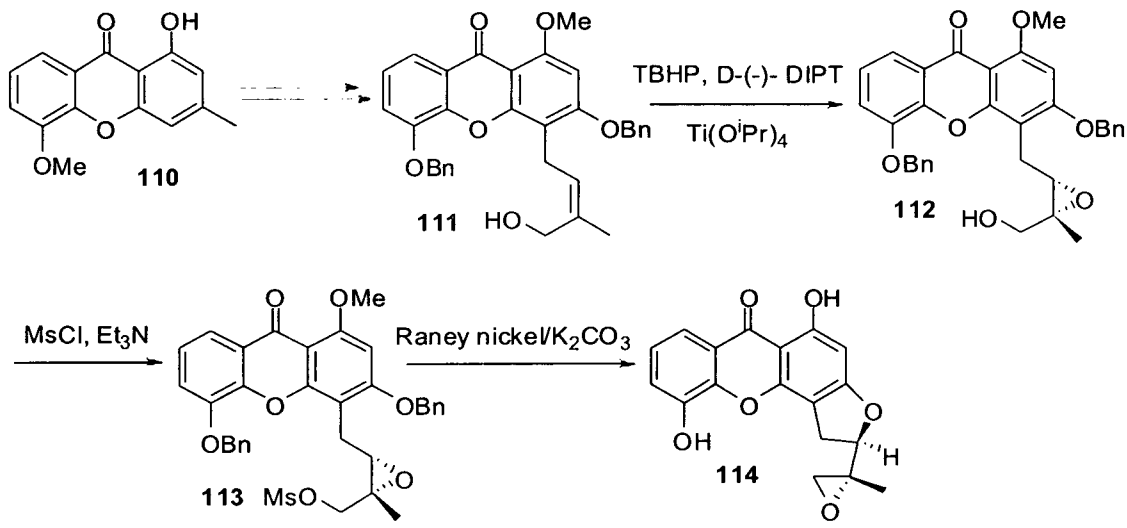


Scheme 1.16

1.5.2 2,3-Dihydrobenzofurans

1.5.2.1 Total synthesis of psorospermin

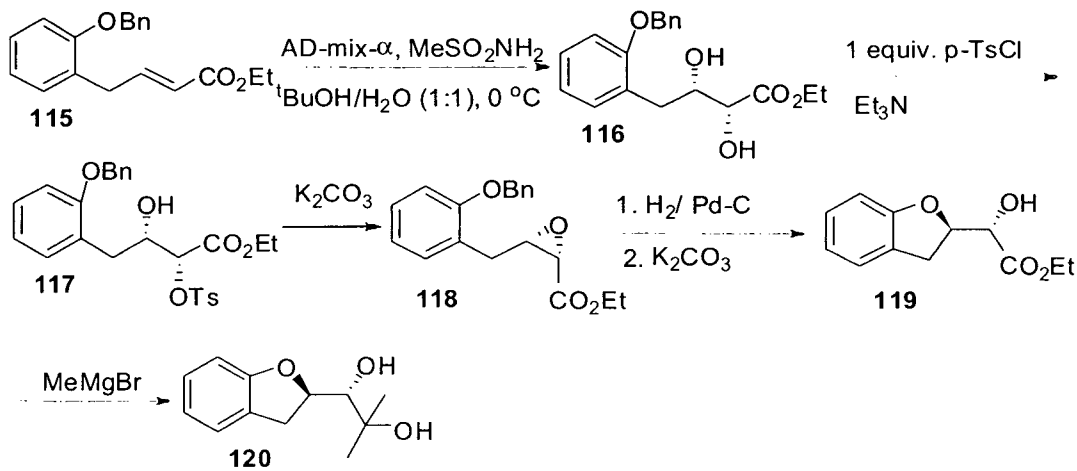
The total synthesis of psorospermin **114**, a novel fused tetracyclic xanthone natural products containing two chiral centers and a reactive epoxide, showing novel antineoplastic properties was accomplished by the Schwaebe group employing Sharpless asymmetric epoxidation on Z-allylic alcohol **111** (Scheme 1.17).[34] Phenoxide ion mediated ring opening of the epoxide **113** followed an intramolecular S_N2 type pathway. Here phenoxide ion mediated carbocyclization led to the simultaneous formation of 1-benzofuran and epoxide ring through a zipper-type cyclization.[35]



Scheme 1.17

1.5.2.2 Contribution from Panda's laboratory

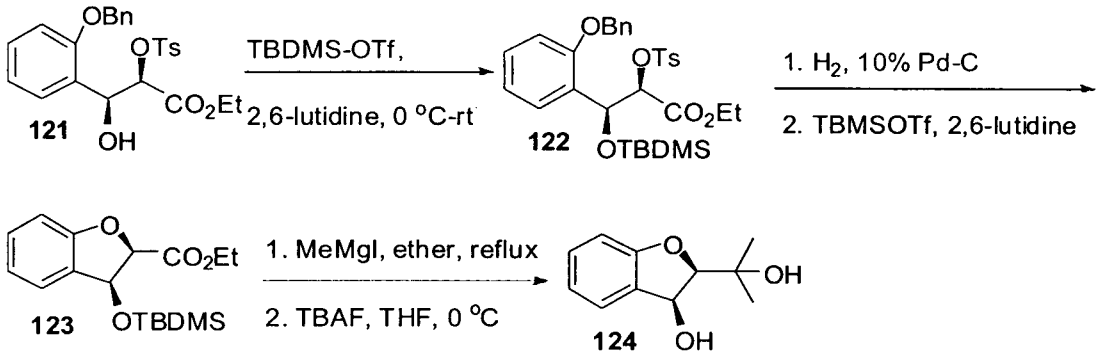
Panda *et al.* developed simple but efficient routes to access a number of various dihydrobenzofurans.[33] Dihydroxylation product **116** was converted to β-hydroxy-α-tosyloxy ester **117** by regioselective monotosylation. Compound **117** on treatment with K_2CO_3 afforded *syn*-glycidic ester **118**, which underwent phenoxide ion mediated carbocyclization to furnish "natural-product-like" dihydrobenzofuran skeleton **119** (Scheme 1.18).



Scheme 1.18

Alternatively, the hydroxyl group of β-hydroxy-α-tosyloxy ester **121** was protected with TBDMSOTf to afford **122**. Upon phenoxide ion mediated intramolecular S_N2

reaction on the tosyloxy group, 2,3-dihydrobenzofuran derivative **123** was obtained (Scheme 1.19).

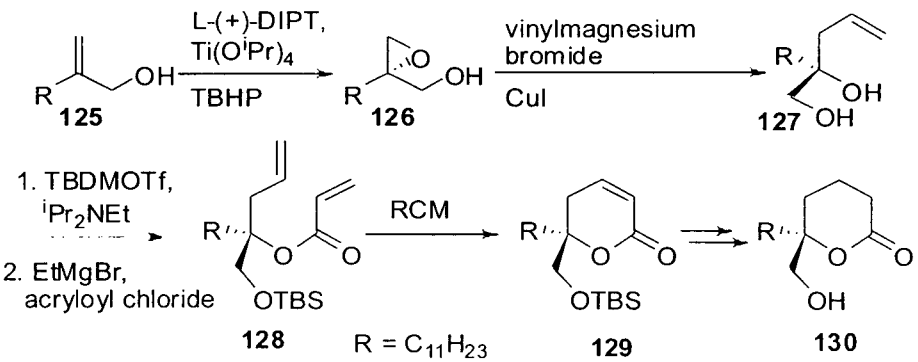


Scheme 1.19

1.6 Synthesis of cyclic natural products

1.6.1 Synthesis of δ-lactonic marine natural product, (+)-tanikolide

The Honda group synthesized the δ-lactonic marine natural product, (+)-tanikolide **130** (Scheme 1.20) by introduction of a chiral quaternary carbon center by the Sharpless AE.[36] Regioselective epoxy-ring opening of chiral epoxide **126** with a vinyl group followed by a ring-closing metathesis (RCM) of diene **128** furnished the required δ-lactone moiety **129**. Treatment of compound **129** with *p*-toluenesulfonic acid for TBDMS deprotection followed by catalytic hydrogenation over 5% palladium on carbon under hydrogen provided the final compound **130**.

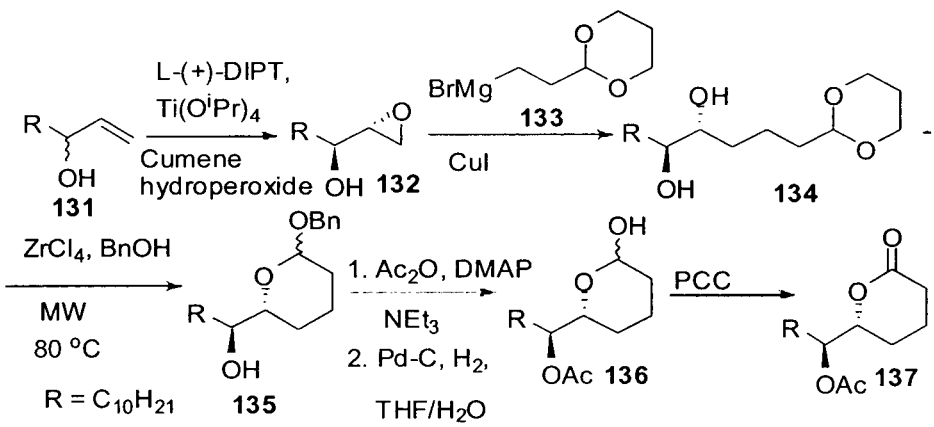


Scheme 1.20

1.6.2 Synthesis of Both Enantiomers of 6-acetoxy-5-hexadecanolide

The stereoselective synthesis of (–)-(5*R*,6*S*)-*erythro*-6-acetoxy-5-hexadecanolide **137** (Scheme 1.21), the major component of a mosquito ovipositor

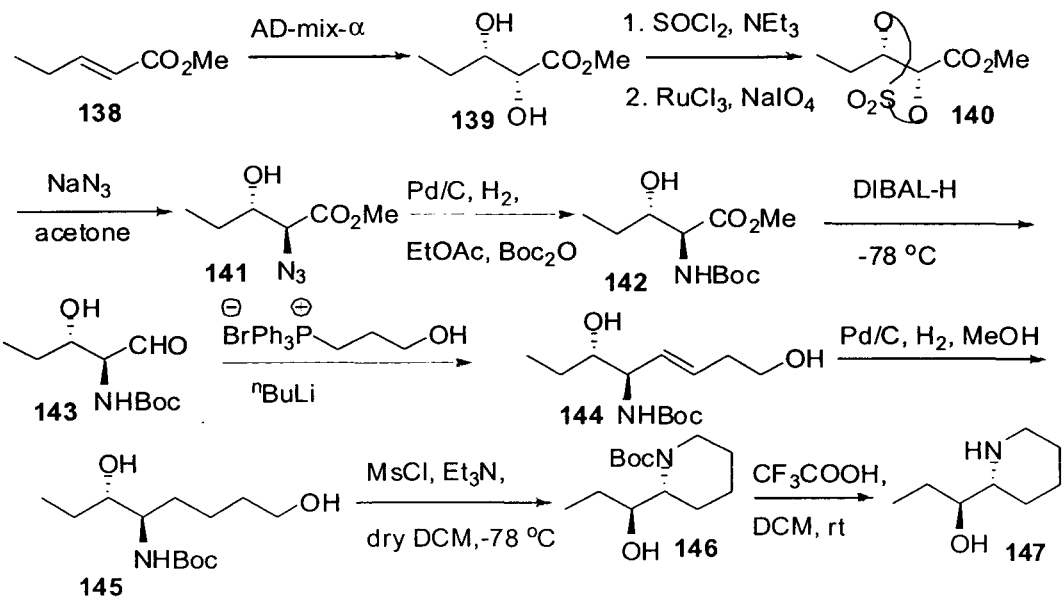
attractant pheromone, and its enantiomer was completed using Sharpless AE and $ZrCl_4$-catalyzed cyclic acetal formation as the key steps.[37]The chiral epoxide **132** derived from **131** by AE using $Ti(O^iPr)_4$, L-(+)-diisopropyl tartrate and cumene hydroperoxide as the oxidant, was treated with the Grignard reagent **133** in the presence of 20 mol % CuI to furnish **134**. When compound **134** was treated with $ZrCl_4$, deprotection of the 1,3-dioxane and its subsequent cyclisation under microwave irradiation afforded the benzyl-substituted tetrahydropyrane **135**. Acetylation of the hydroxyl group followed by debenzylation and oxidation of the resulting lactol **136** using PCC afforded the final product **137**.



Scheme 1.21

1.6.3 Enantioselective Synthesis of (-)-α-conhydrine

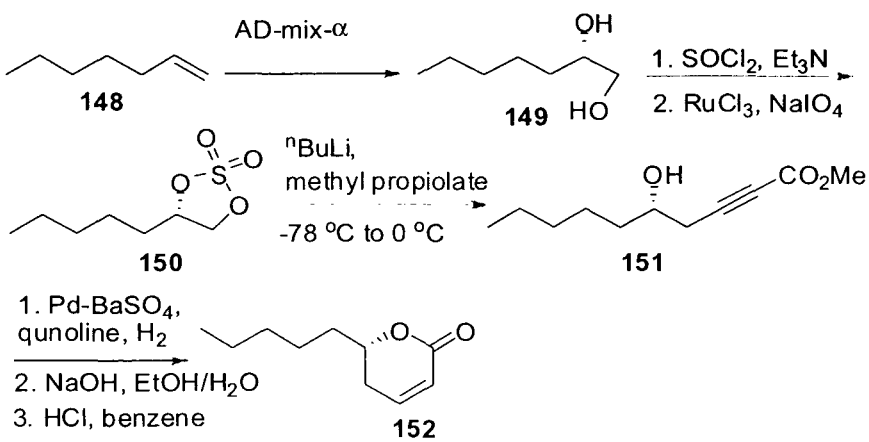
Synthesis of (-)-α-conhydrine **147** (Scheme 1.22) was achieved by Pradeep Kumar and co-workers by applying Sharpless AD.[38] Enantiopure diol **139** was treated with thionyl chloride and triethyl amine to give the cyclic sulfite, which was oxidized using $RuCl_3/NaIO_4$ to afford cyclic sulfate **140**. Regioselective ring opening of cyclic sulfate **140** with NaN_3 followed by reduction of azide and protection of the amine with Boc_2O afforded **142**, which upon reduction with DIBAL-H provided the aldehyde **143**. Next, three carbon chain elongation *via* Wittig olefination and subsequent cyclization furnished the target molecule **147**.



Scheme 1.22

1.6.4 Asymmetric Synthesis of (*S*)-Massiolactone

Asymmetric synthesis of (*S*)-Massiolactone **25** (Scheme 1.23) was accomplished by the Kumar group using Sharpless AD as the source of chirality for the first time.[39]



Scheme 1.23

The diol **149** was converted into cyclic sulfate **150**, which upon regiospecific nucleophilic opening with methyl propiolate at -78 °C provided the alcohol **151**. Next, sequential partial hydrogenation, saponification and lactonization afforded the final molecule **152**.

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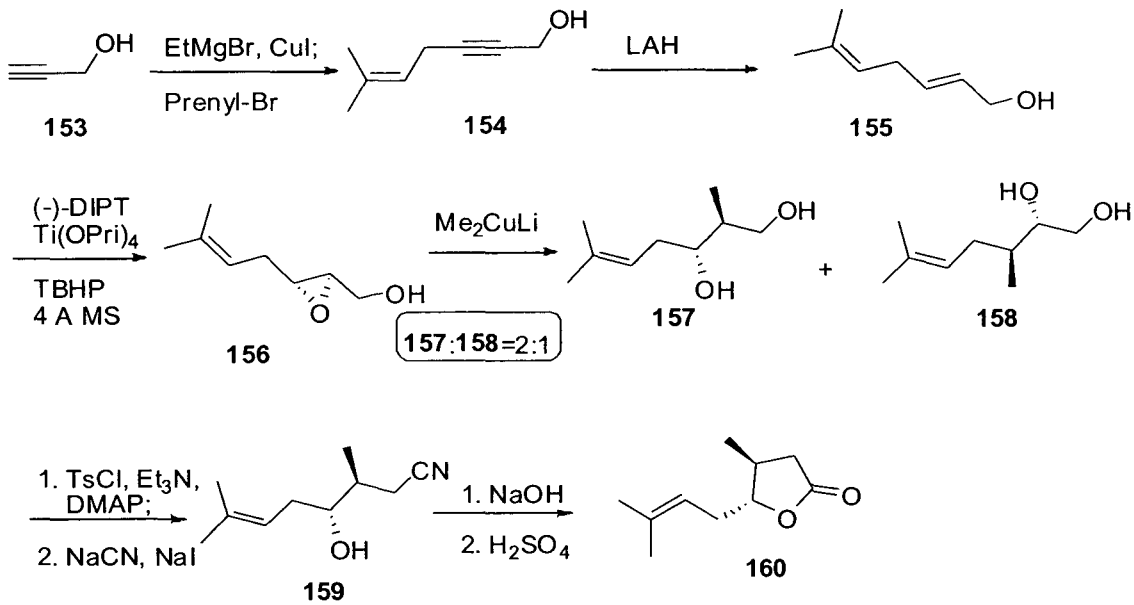
1.6.5 Asymmetric Synthesis of (+)-eldanolide

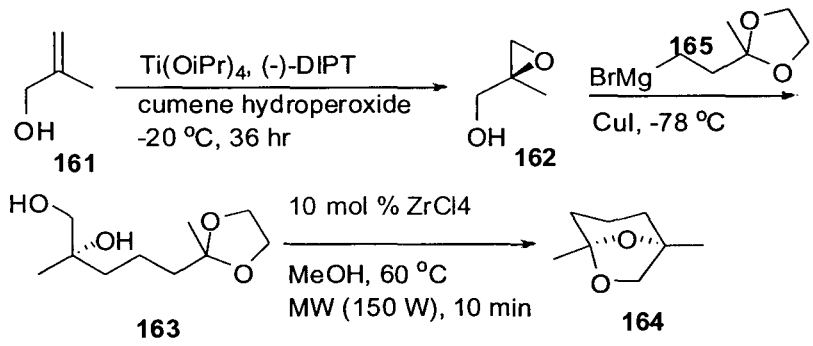
The monoterpenoid pheromone (+)-eldanolide **160** (Scheme 1.24) was synthesized by Zhai et al[40], the key step being the formation of the 1,3-diol **157** by regio- and stereoselective ring opening of 2,3-epoxy alcohol **156**, derived from Sharpless AE of the allylic alcohol **155**. One carbon elongation through selective tosylation of diol **157** at the primary hydroxyl followed by S_N2 displacement of the tosyl group by cyanide furnished nitrile **159**. Next, saponification of the nitrile followed by lactonization afforded the final molecule **160**.



Scheme 1.24

1.6.6 Asymmetric Synthesis of (-)-frontalin

The natural product, (-)-frontalin **164** was synthesized applying Sharpless AE and $ZrCl_4$-catalyzed intramolecular acetalization as the key steps[41]. The epoxide **162** was synthesized employing Sharpless asymmetric epoxidation using 10 mol % of $Ti(O^iPr)_4$ and 15 mol % of D(-)-DIPT as the source of chirality and cumene hydroperoxide as oxidant at -20 °C for 36 h. The ring opening of chiral epoxide **162** was accomplished at -78 °C using a catalytic amount of CuI (10 mol %) with 3 equiv of the Grignard reagent **165** (Scheme 1.25).

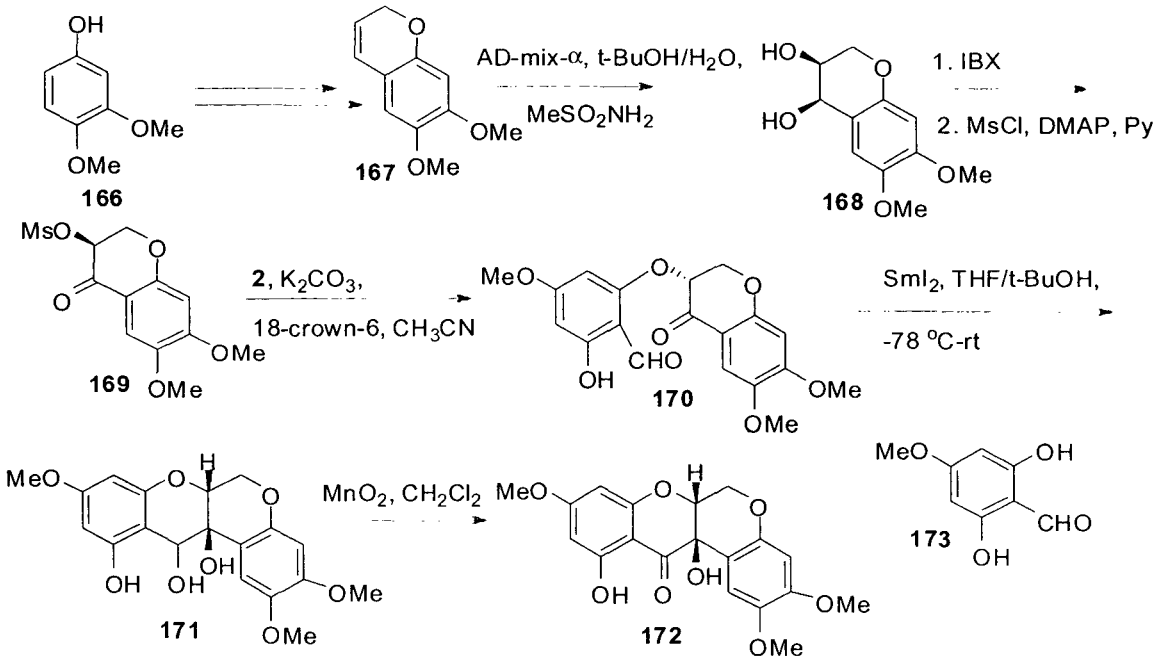


Scheme 1.25

The diol **163** was then treated with $ZrCl_4$ (10 mol %) in methanol under microwave irradiation (150 W) for 10 min to afford (-)-frontalin **164**.

1.6.7 Total synthesis of 6-deoxyclitoriacetal

A total synthesis of the rotenoid, 6-deoxyclitoriacetal **172**, a cytotoxic natural product, was successfully achieved by Khorphueng et al.[42]



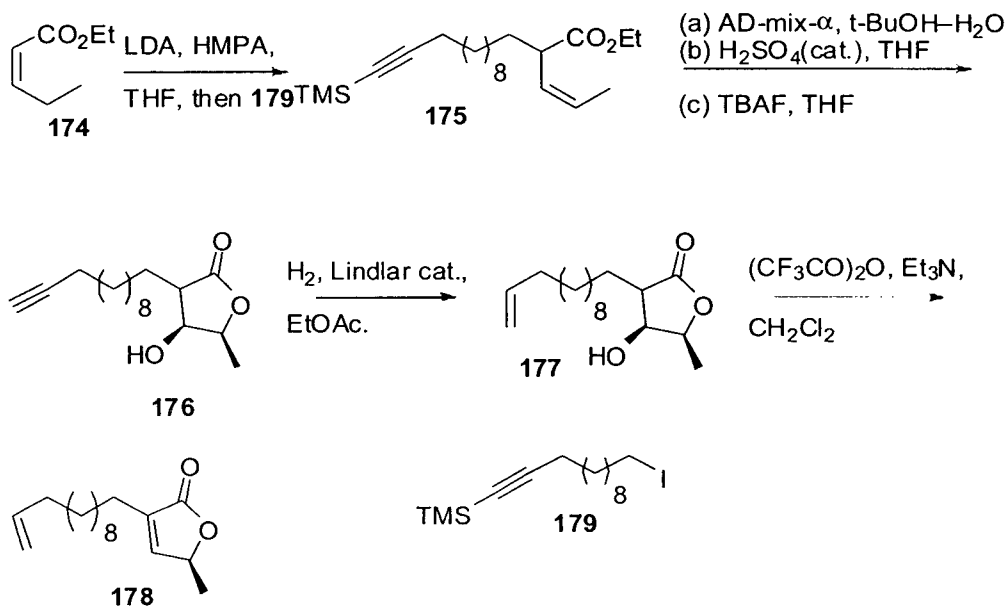
Scheme 1.26

The vicinal diol **168** upon regioselective IBX oxidation followed by mesyl protection furnished **169** which coupled with compound **173** on treatment with K_2CO_3 in the presence of 18-crown-6 to provide **170** (Scheme 1.26). Subsequent stereoselective intramolecular keto-aldehyde pinacol coupling[43] using samarium diiodide in THF/t-

BuOH at -78 °C provided **171** which on MnO_2 oxidation furnished the target compound **172**.

1.6.8 Enantioselective synthesis of butenolide natural products

Based on Sharpless asymmetric dihydroxylation, the Yao group developed a novel synthetic strategy (Scheme 1.27) to synthesize simple natural products with butenolide segments enantioselectively[44]. Asymmetric dihydroxylation of compound **175** followed by acid-catalyzed cyclization of the crude AD product and then desilylation proceeded smoothly to afford a mixture of diastereomers **176**. Recrystallization of **176** from a cold DCM/hexane solution was accomplished to increase ee. Compound **178**, a butenolide natural product exhibiting mosquito larvicidal activity, can be obtained by partial reduction of **176** with Lindlar catalyst followed by dehydration of **177**.

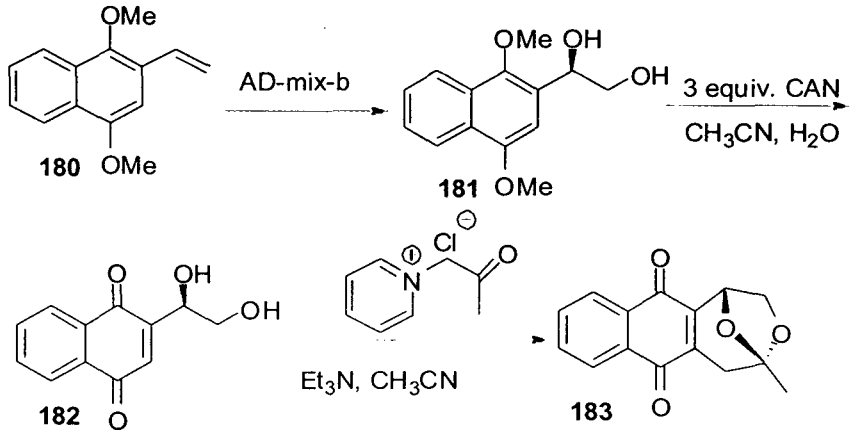


Scheme 1.27

1.6.9 First enantioselective synthesis of isagarin

Kimpe *et al* achieved first enantioselective synthesis of both enantiomers of isagarin, a new type of tetracyclic naturally occurring 1,4- naphthoquinone[45]. The enantiomerically pure diol **52** obtained from AD of 1,4-dimethoxy-2-vinyl-naphthalene **2** was converted to the corresponding 1,4-naphthoquinone **53** on

treatment with CAN via oxidative demethylation (Scheme 1.32). Next, compound **53** was treated with a acetylmethyl pyridinium ylid **55** to furnish the final compound 1*R*,4*S*-isagarin **54.**

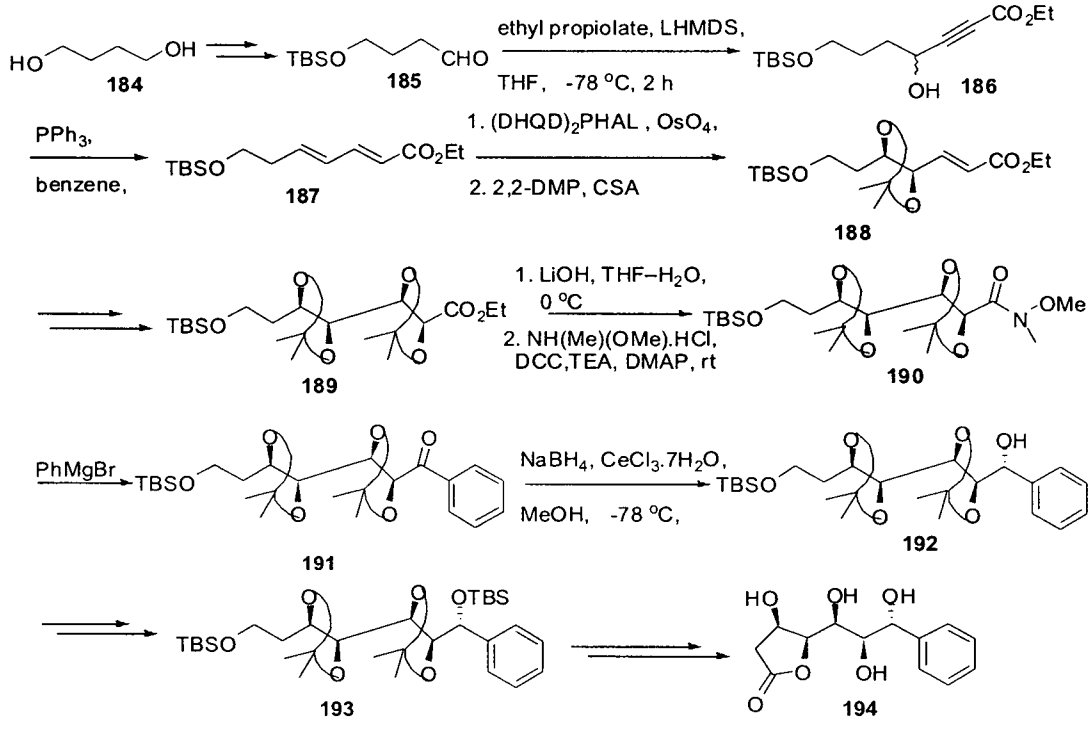


Scheme 1.28

1.6.10 Total synthesis of (+)-cardiobutanolide

Total synthesis of (+)-cardiobutanolide **194**, a polyhydroxylated natural product, was achieved by Chandrasekhar et al.[46] The lithiated ethyl propiolate was added to aldehyde **185** at -78 °C to furnish γ-hydroxy ethyl propiolate derivative **186** (Scheme 1.29). Applying the Lu and Guo protocol[47] the hydroxy ethyl propiolate **186** was converted to the (*E,E*) diene ester **187**. When exposed to $(DHQD)_2PHAL/OsO_4/NMO\text{-}H_2O$ in PEG[48] followed by acetonide formation **187** provided compound **188**. Following second Sharpless AD and acetonide formation, diisopropylidine derivative **189** was obtained with good diastereoselectivity (8:2). Weinreb amide **190** was exposed to PhMgBr to yield aryl ketone **191**. The diastereoselective reduction of prochiral ketone **191** generated the desired alcohol **192** with appreciable diastereoselectivity (3:1). The silylation of the benzylic hydroxyl group followed by the selective deprotection of the primary silyl ether furnished the alcohol **193**. The oxidation of primary alcohol **193** to carboxylic acid followed by esterification furnished globally protected aryl pentol acid. The deprotection under aq TFA in the presence of a drop of conc. HCl allowed simultaneous removal of

isopropylidine groups and silyl ether and also lactonization to provide (+)-cardiobutanolide **194**.

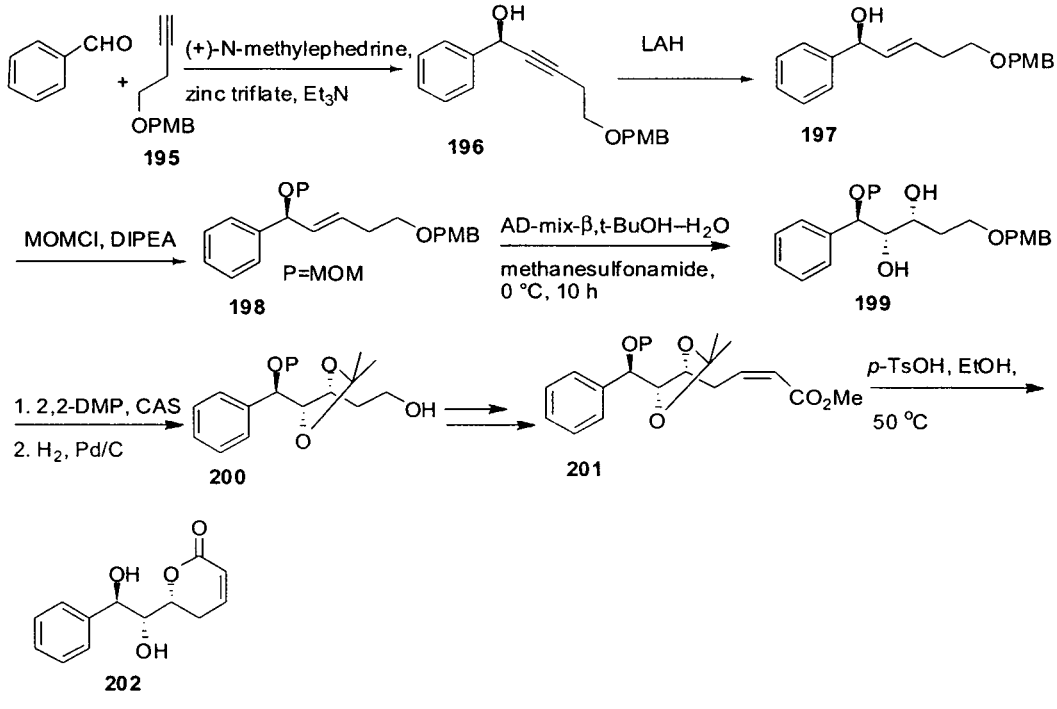


Scheme 1.29

1.6.11 Total Synthesis of (+)-Goniodiol

Gowravaram Sabitha and co-workers reported[49] total synthesis of (+)-goniodiol **202** (Scheme 1.30) using Carreira alkynylation and Sharpless AD for introducing chirality. Benzaldehyde underwent Carreira alkynylation with compound **195** in the presence of (+)-*N*-methylephedrine, zinc triflate, and triethylamine[50] to furnish **196** with high enantioselectivity. Compound **196** upon LAH reduction furnished the *E*-allylic alcohol **197**. The secondary hydroxy group of compound **197** was protected as its MOM ether **198** by treating with MOMCl in the presence of DIPEA. Compound **198** was then treated with AD-mix-β in *t*-BuOH–H_2O (1:1) to afford diol **199** as a single isomer. Subsequent acetonide protection of hydroxyl groups followed by the deprotection of PMB group with H_2 over Pd/C furnished **200**. Oxidation of alcohol **200** to its corresponding aldehyde and subsequent chain elongation[51] with Still–Gennari reagent furnished the *Z*-isomer **201** in along with traces of *E*-isomer. Compound **201** on treating with catalytic amount of PTSA in

EtOH at 50 °C afforded the target molecule **202** by tandem deprotection and in situ cyclization process.



Scheme 1.30

1.7. Conclusion

Over the last three decades, Sharpless asymmetric epoxidation and dihydroxylation, two very powerful catalytic oxidation reactions of olefins, have been and being utilized most widely as potent tools for inducing chirality in synthetic organic chemistry. And hence these are reviewed many times. With the optimization of the ligands and amount of primary oxidants, these two catalytic oxidation reactions provide chiral 2,3-epoxyalcohols and *syn*-1,2-diols in high yields and enantioselectivities. Their developments have provided academic research with many important tools, thereby contributing to more rapid advances in research – not only in chemistry but also in materials science, biology and medicine. These two reactions have been and being widely applied in the modern synthetic organic chemistry giving access to new molecules needed to investigate hitherto unexplained and undiscovered phenomena in the molecular world.

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Chapter 2:

Synthesis and Pharmacological Evaluation of a series of Aryl aryl methyl thio arenes (AAMTAs) as Antimalarial Therapeutics

2.1 Introduction

Malaria is a global health emergency. It has had a greater impact on world history than any other infectious disease. More than 300 to 500 million individuals worldwide are infected with *Plasmodium* species, and 1.5 to 2.7 million people a year, most of whom are children, die from the infection.[1] Malaria is endemic in over 90 countries in which 2400 million people live; this represents 40% of the world's population. Approximately 90% of malaria deaths occur in Africa. This disease becomes marked clinically when any one of the human protozoa parasites namely *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae* comes into the intraerythrocytic cycle. *P. falciparum* by itself is responsible for about 80% of infections and 90% of deaths.[2] The management of malaria relies solely on chemotherapeutics and chemoprophylaxis due to limitations associated with vaccine development and vector control. Despite continuing efforts in vaccine development, malaria prevention is difficult, and no drug is universally effective. The global spread of multiple drug resistant *P. falciparum*[3] has sharply limited the choice of chemoprophylactic drugs and has worsened the chronic shortages of affordable, effective treatment drugs[4]. Hence, intense interest has been aimed at the quest for novel antimalarial therapeutic agents.[5]

2.2 Basis of the Present Work

Trisubstituted methanes (TRSMs) with or without sulfur spacers have been reported to exhibit various biological activities as anti-breast cancer[6], antitubercular[7], antiimplantation[8], antiproliferative[9], etc. Aryl aryl methyl thio arenes (AAMTAs) belong to the class of TRSMs with sulfur spacer. Among TRSMs with sulfur spacers, the antimalarial activity of several arylacridinyl sulfones has been reported[10]. Initial antimalarial activity of [(Aryl)-arylsulfanyl-methyl]-pyridines against chloroquine resistant *P. yoelii in vivo* have been reported as well [11]. These preliminary findings encouraged us to synthesize and evaluate a new series of AAMTAs with sulfur spacer for antimalarial efficacy. Here in this work, we have screened a number of Aryl aryl methyl thio arenes based on their heme binding affinity and subsequently tested for antimalarial activity *in vitro* and *in vivo* using multidrug resistant strain (MDR strain *P. yoelli*). These compounds offer antimalarial activity by promoting the development of oxidative stress through the inhibition of heme polymerization and generating reactive oxygen species.

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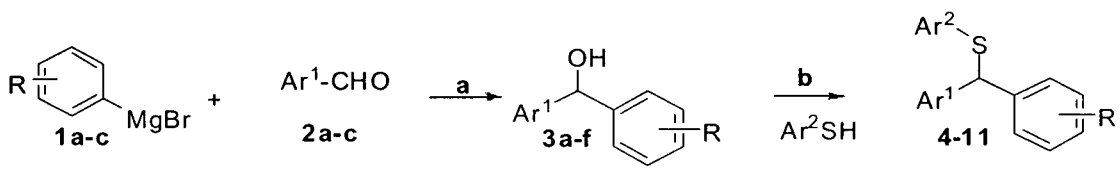
2.3 Results and Discussion

2.3.1 Chemistry

Synthesis of Aryl aryl methyl thio arenes (AAMTAs) has been accomplished from a series of carbinols on which *S*-alkylation of different aryl or heteroaryl thiols has been performed. Carbinols were synthesized by Grignard reaction of arylmagnesium bromide with a series of arylcarbaldehydes **2a-c** (Scheme 2.1). Thus, *S*-alkylation of aryl or heteroaryl thiols utilizing carbinols **3a-f** as the alkylating agents (Scheme 2.1) was obtained in the presence of a catalytic amount of conc. H_2SO_4 in dry benzene under reflux condition whereas *S*-alkylation reactions on carbinols **3a-f** were achieved in the presence of anhydrous $AlCl_3$ (1.1 equiv.) in dry benzene at room temperature (Scheme 2.1). However, in case of *S*-alkylation of 2-mercaptobenzothiazole on carbinol **3a,** reaction was performed at reflux condition since 2-mercaptobenzoxazole is insoluble in benzene at room temperature.



1a, R = *m*-OCH_3
1b, R = *p*-OCH_3
1c, R = *o*-OCH_3

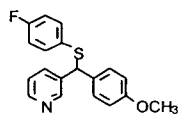
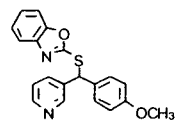
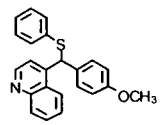
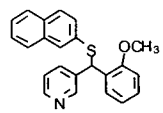
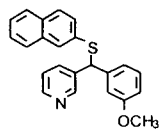
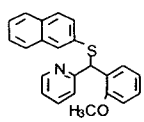
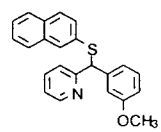
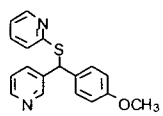
2a, Ar^1= 2-Pyridyl
2b, Ar^1= 3-Pyridyl
2c, Ar^1 = 4-Quinolinyl

3a, R = *p*-OCH_3, Ar^1= 3-Pyridyl
3b, R = *p*-OCH_3, Ar^1 = 4-Quinolyl
3c, R = *o*-OCH_3, Ar^1= 3-Pyridyl
3d, R = *o*-OCH_3, Ar^1= 2-Pyridyl
3e, R = *m*-OCH_3, Ar^1= 3-Pyridyl
3f, R = *m*-OCH_3, Ar^1= 2-Pyridyl

4, R= *p*-OCH_3, Ar^1= 3-Pyridyl, Ar^2=4-flouro-phenyl
5, R= *p*-OCH_3, Ar^1= 3-Pyridyl, Ar^2= 2-benzooxazolyl
6, R= *p*-OCH_3, Ar^1= 4-quinolyl, Ar^2= phenyl
7, R= *o*-OCH_3, Ar^1= 3-Pyridyl, Ar^2= Naphthalenyl
8, R= *m*-OCH_3, Ar^1= 3-Pyridyl, Ar^2= Naphthalenyl
19 R= *o*-OCH_3, Ar^1= 2-Pyridyl, Ar^2=Naphthalenyl
10, R= *m*-OCH_3, Ar^1= 2-Pyridyl, Ar^2=Naphthalenyl
11, R= *p*-OCH_3, Ar^1= 3-Pyridyl, Ar^2=2-Pyridyl

Scheme 2.1 Synthesis of Aryl aryl methyl thio arenes (AAMTAs) **(4-11)**; *Reagents and conditions*: (a) dry THF, rt. (b) conc. H_2SO_4, dry benzene, reflux, 0.5 h or anhyd. $AlCl_3$, dry benzene, rt or reflux, 0.5 h.

Table 2.1 Synthesized Aryl aryl methyl thio arenes (AAMTAs) **4-11.**

Compound no.	Structure	*CSLogP ± SD[a]	*CSpK$_a$ ± SD[b] (mol/l)	K$_D$(μM) M±SE	IC$_{50}$ (μM) Hypoxanthine uptake M±SE	IC$_{50}$ (μM) Hemozoin formation *P. yoelii* MDR M±SE
4		3.45± 1.01	4.96±0.8	12.3 ±1.2	4.2 ±0.4	70 ±5.4
5		3.41± 1.03	4.24±1.1	6.25 ±0.8	1.45±0.08	5±.024
6		5.20± 1.21	4.11±1.5	8.25 ±0.9	> 4	5±.03
7		5.07± 1.11	4.92±1.4	7.67± 1.5	3.4±0.29	10 ±0.9
8		5.1± 1.31	4.72±1.4	4.76 ±0.6	1 ±.003	5.9 ±0.7
9		5.15± 1.04	4.44±1.3	7.22 ±0.3	3 ±0.2	12.5
10		5.25± 1.19	4.37±1.4	5.11±0.5	>4	>100
11		3.09±0.9	4.67±0.9	4.65 ±0.8	1.5±0.07	5 ±0.4

[a] *CSLogP = Chem Silico LogP;* [b] *CSpK$_a$= Chem Silico pK$_a$;* CSLogP and CSpK$_a$ of the AAMTAs was calculated using property prediction software CSPredict developed by ChemSilico LLC which is a registered trademark of ChemSilico LLC, Tewksbury, MA 01876, USA (www.chemsilico.com)[12].

2.3.2 Biology

2.3.2.1 AAMTAs interact with heme and inhibit hemozoin (β-hematin) formation

Malaria parasite possesses efficient mechanism of digestion of hemoglobin (Hb) and subsequently the detoxification of resultant heme (ferriprotoporphyrin IX (FP) to protect itself from heme-induced oxidative stress. The major heme detoxification system involved mainly hemozoin formation[13] inside the food vacuole of parasite. In general, compound, which interacts with heme, inhibits hemozoin formation, and inhibition of hemozoin formation is the most validated rationale to develop antimalarial therapeutics[14]. In order to find new antimalarial compounds, different AAMTAs compound were synthesized to evaluate their heme interacting property and effect on hemozoin formation. Interaction of AAMTAs with heme was followed at different concentrations. Addition of AAMTAs perturbed the heme spectrum (Fig 2.1), which is indicative of an interaction between the AAMTAs and the heme moieties.

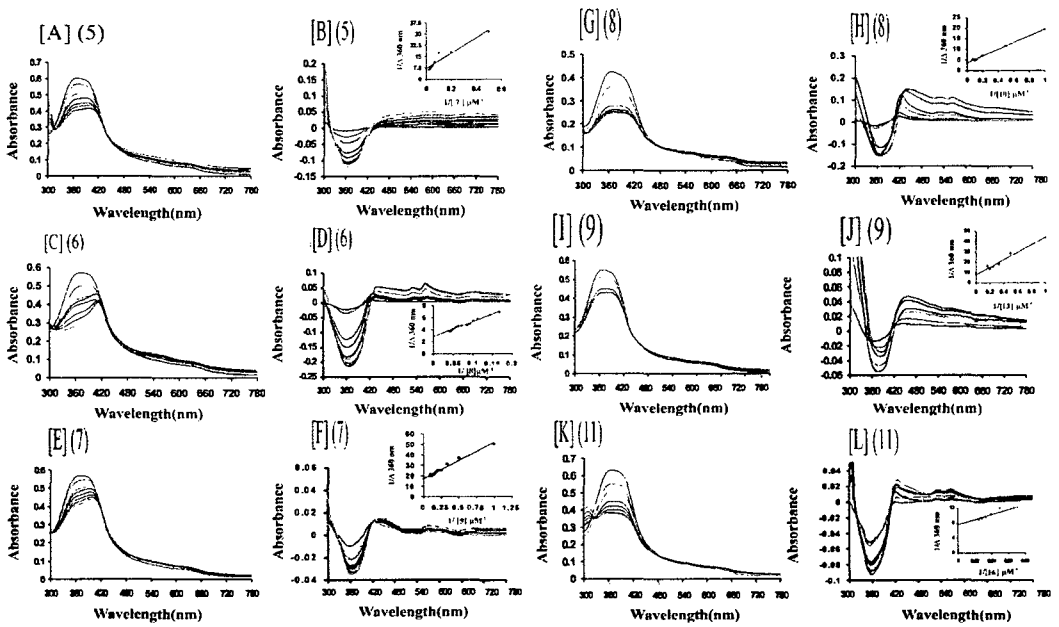


Figure 2.1. Interaction of AAMTAs with heme; Optical and differential optical soret spectroscopy for AAMTAs - hemin interaction at different concentrations of AAMTAs (1-20 μM); Compound names are indicated in bracket. Inset shows the plot of 1/Δ360 nm vs 1/[AAMTAs] to calculate K_D.

This is followed by titration with increasing amounts of AAMTAs into the heme solution produced spectra with well-defined isosbestic point in the Soret range with reduction in the heme Soret molar absorptivity, and a shift of the Soret band to longer wavelengths. Interaction of AAMTAs with heme was determined at pH 5.2, approximating to the pH of the parasite food vacuole to mimic the cellular mileu inside parasite. Since heme have optical absorption at 362 nm , it represented a broad peak , indicating that dimers of the μ-oxo type or β-hematin type predominate under our *in vitro* conditions[15]. The binding of AAMTAs to heme were also studied by optical difference spectroscopy to measure and calculate binding affinity of these compounds (Fig 2.1). The apparent K_D values for the binding of AAMTAs to heme, calculated from the plot of $1/\Delta A_{362}$ against 1/ [AAMTAs] were shown in the insets (Fig 2.1). Out of various AAMTAs, compounds **5, 8** and **11** showed the highest affinity towards heme (Table 2.1). Antimalrial drugs such as chloroquine and amodiaquine (type-1 blood schizontocides) act by forming complexes with heme (the hydroxo or aqua complex of ferriprotoporphyrin IX (Fe (III) PPIX). Such interaction with heme leads to the inhibition of hemozoin formation and subsequently causes parasite death (antimalarial). Therefore, we checked whether interaction of AAMTAs with heme can cause any inhibition of hemozoin formation and offer antimalrial activity. Results clearly indicated that AAMTAs inhibited hemozoin formation in a concentration dependent manner with an IC_{50} value in the μM range (Table 2.1). Compound **5, 8** and **11** showed much higher activity in inhibiting hemozoin formation (IC_{50} ~ 5 and >5μM). However, some of AAMTAs showed less activity when compared with the others. Association with heme of any test compound is not fully sufficient to inhibit hemozoin formation, there is much concerned about the lipophilicity and *p*Ka of the test compound[16].

2.3.2.2 Inhibition of *P. falciparum* growth by AAMTAs

Inhibition of hemozoin formation is a most validated antimalarial drug target; hence we were interested to test the *in vitro* antimalarial activity of AAMTAs. Interestingly, AAMTAs inhibited the growth and development of malaria parasite effectively as evident from the inhibition of ^{3H}hypoxantine uptake. But out of various AAMTAs, compounds **5, 7, 8, 9** and **11** showed very potent antimalarial activity (IC_{50} = > 2μM) (Table 2.1). The dose used in *in vitro* experiment should not be correlated with the effective dose under *in vivo* conditions. Pharmacokinetic evaluations and analysis

during pre-clinical studies show that under *in vivo* conditions effectiveness of any drugs is based on absorption, distribution, metabolism, and excretion. These influence the drug levels and kinetics of drug exposure to the target region and hence influence the performance and pharmacological activity of the compound as a drug. Existing literature suggested that AAMTAs offer antiproliferation and anticancer properties along with promising antimalarial activity and are shown to inhibit hemozoin formation[17]. However, compound showing antiproliferative activity in tumor cells follows some different mode of action. Therefore, we cannot exclude that antimalarial action of these molecules followed directly due to hemozoin formation or it may be targeted multiple sites of malaria parasite like tumor cells.

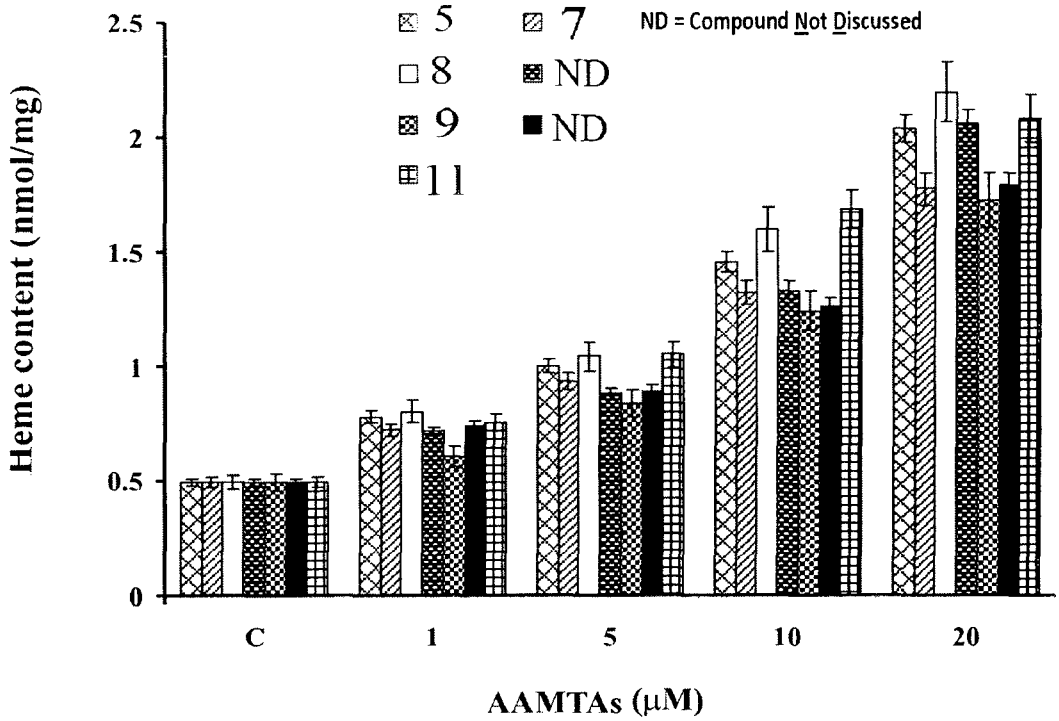


Figure 2.2

2.3.2.3 AAMTAs promotes oxidative stress in *P. falciparum*

The inhibition of hemozoin formation as a result of heme interaction causes death of the parasite due to the accumulation of toxic free heme and subsequent development of the oxidative stress[18]. It is well known that free heme is very toxic possessing detergent-like properties, interfering with membrane integrity and has the ability to undergo redox reactions causing the generation of reactive oxygen species (ROS)[14]. In view of above finding, we were interested to check whether AAMTAs can develop oxidative stress in *P. faciparum* by favouring the accumulation of free heme. The

selected AAMTAs (**5, 7, 8, 9** and **11**), which showed better anti-plasmodial activity were tested for this purpose. Results clearly indicated that the inhibition of hemozoin formation by AAMTAs allowed the accumulation of heme in the parasite (Fig 2.2).

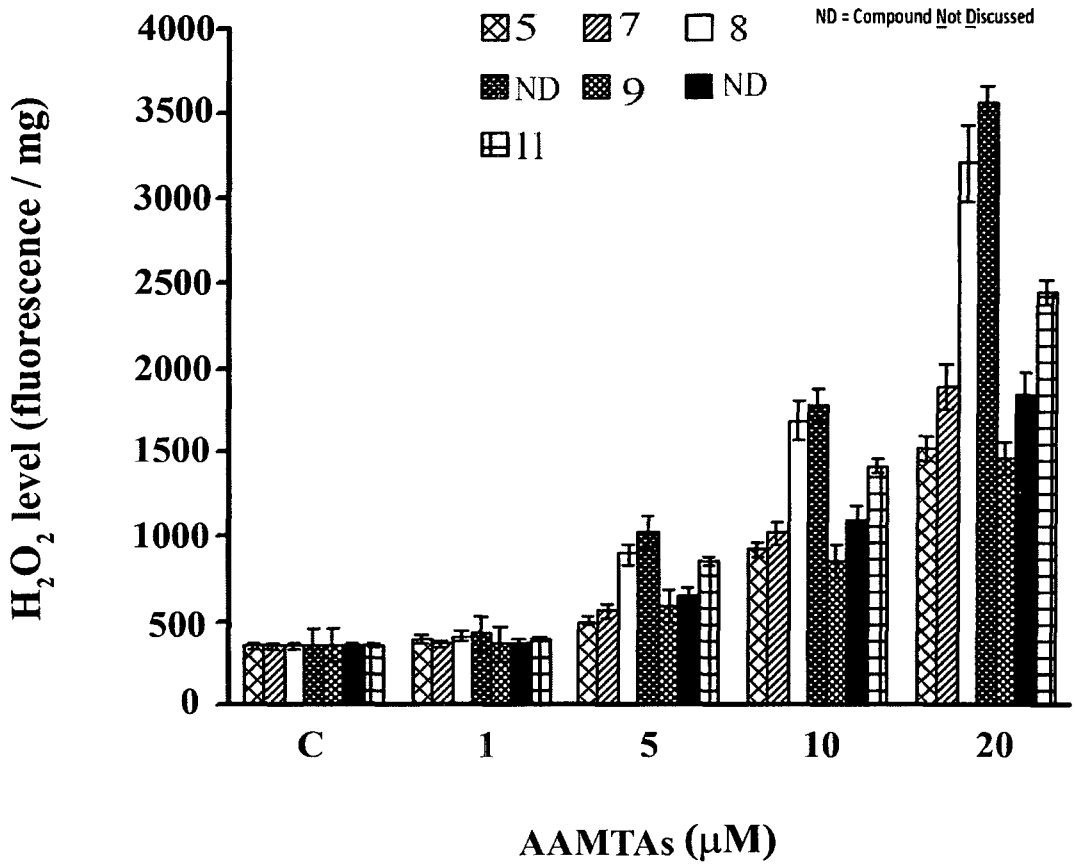


Figure 2.3

Out of various AAMTAs tested, compound **5, 8** and **11** were shown to be highly active. Intra-parasitic H_2O_2 was measured in control and AAMTAs treated parasite by measuring the fluorescent dichlorofluorescein (DCF) oxidation product formed from non-fluorescent probe dichlorofluorescein diacetate after interation with ROS. Interestingly, AAMTAs treatment causes significant induction of the generation of intra-parasitic H_2O_2 (Fig 2.3). The capabilities of iron of heme to accept and donate electrons contribute towards its potential toxicity. A possible route for degradation of the heam is by reacting with H_2O_2 under conditions designed to resemble those found in the food vacuole, i.e., at pH 5.2. However, the estimation of concentrations of heme and H_2O_2 in the food vacuole is difficult. The peroxidative degradation of heme is thought to involve a reaction with H_2O_2 to form a ferryl intermediate[19]. Transport of electrons within the Fe(IV) intermediate of heme causes widening of the porphyrin

ring and release of iron, which can interact with H_2O_2 and formed hydroxyl radical ($^{\bullet}OH$)[20].

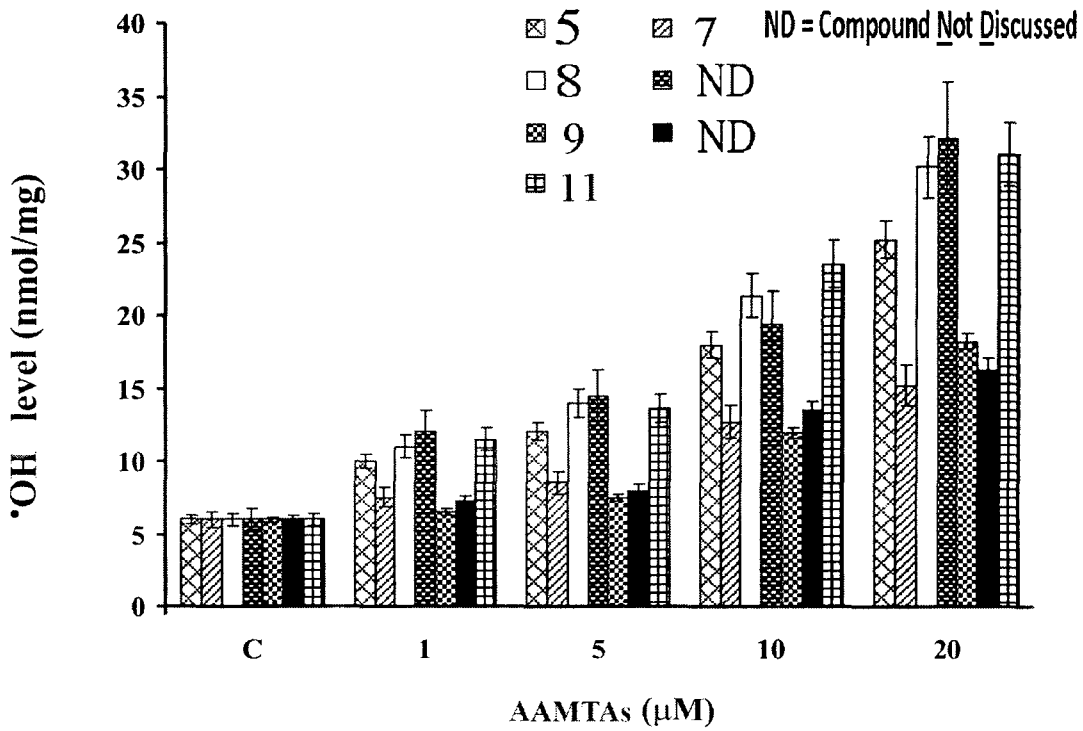


Figure 2.4

In view of above, we checked the formation of $^{\bullet}OH$ inside parasite after treating parasite in culture with different concentrations of AAMTAs. AAMTAs lead to the generation of highly reactive $^{\bullet}OH$ in a concentration dependent manner (Fig 2.4). The inhibition of heme detoxification to hemozoin by AAMTAs resulted in the accumulation of vast amount of reactive oxygen species (ROS) in *P. falciparum*. An excess of ROS will cause oxidative damage to critical biomolecules such as lipids and proteins leading to the formation of the lipid peroxidation product and protein carbonylation respectively. We have checked that incubation of parasite culture with AAMTAs leads to the formation of the lipid peroxidation product and protein carbonylation. Among AAMTAs compound **5, 7, 8, 9** and **11** caused significant formation of lipid peroxide and protein carbonyl (Fig 2.5 and 2.6). GSH plays a pivotal role in the antioxidant defense through the maintenance of the redox state of protein-SH moieties, reduces the noxious hydrogen and lipid peroxides and the extrusion of heme[21]. GSH uptake from erythrocyte cytosol through the process of

pinocytosis and inside the food vacuole (parasite) binds with free heme and reduces its toxicity[22]. Apart from its role in heme detoxification, GSH acts as a cofactor for a variety of vital proteins including glutathione-dependent peroxidases, glutathione S-transferases (GSTs), glutaredoxins and glyoxalases of the parasite (http://plasmodb.org)[23]. It is also directly involved in antioxidant reactions – for instance, the termination of radical-based chain reactions where single electrons are transferred from thiyl radicals or disulphide radicals[24], thus depletion of GSH would result in a less efficient detoxification of free FP IX and inactivation of many enzymes, development of oxidative stress and consequently the death of parasite. To examine whether AAMTAs *via* stimulating the accumulation of intra-parasitic H_2O_2 reduced the cellular GSH level, selected AAMTAs were incubated with *P. falciparum* in culture and GSH level was measured. AAMTAs significantly decreased the GSH level concentration dependently (Fig. 2.7).

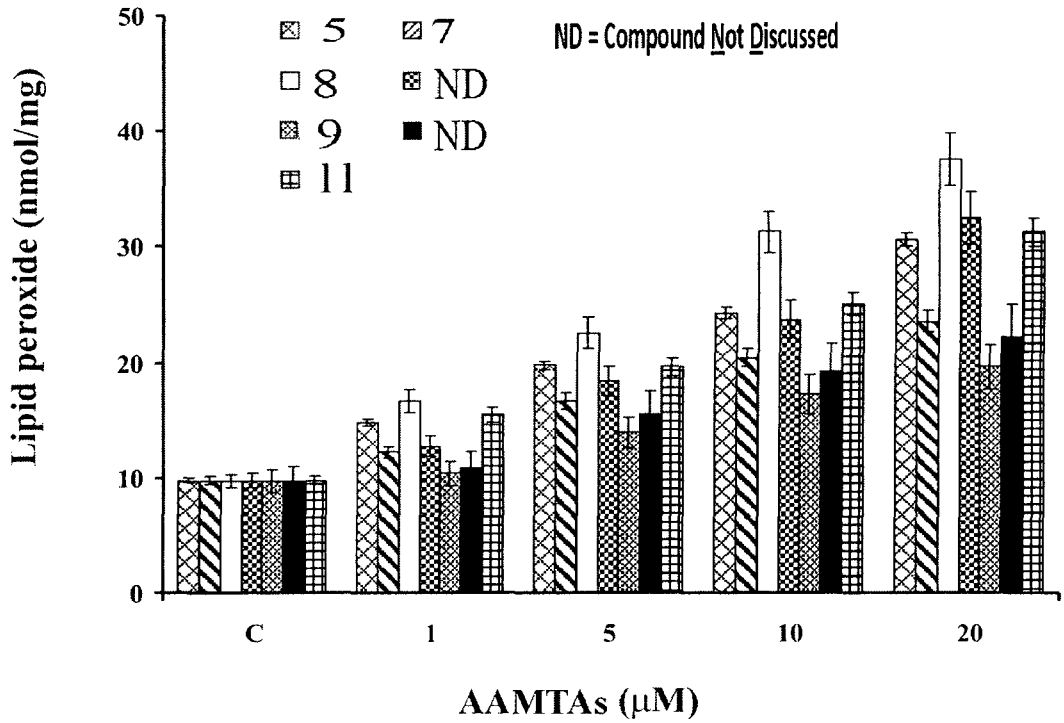


Figure 2.5

In order to assess whether the oxidative stress induced by AAMTAs is actually responsible for the inhibition of parasite growth and development or any other factor is also involved. Therefore, we have checked the restoration of parasite growth and

development after antioxidant therapy (effect of different antioxidant or •OH scavengers; such as mannitol and spin traps like PBN on AAMTAs induced *P. rfalciparum* death). Results clearly indicated that •OH scavengers significantly protected *P. falciparum* from AAMTAs induced growth inhibition. Thus from the above data it is evident that the development of oxidative stress is the antimalarial mode of action of AAMTAs.

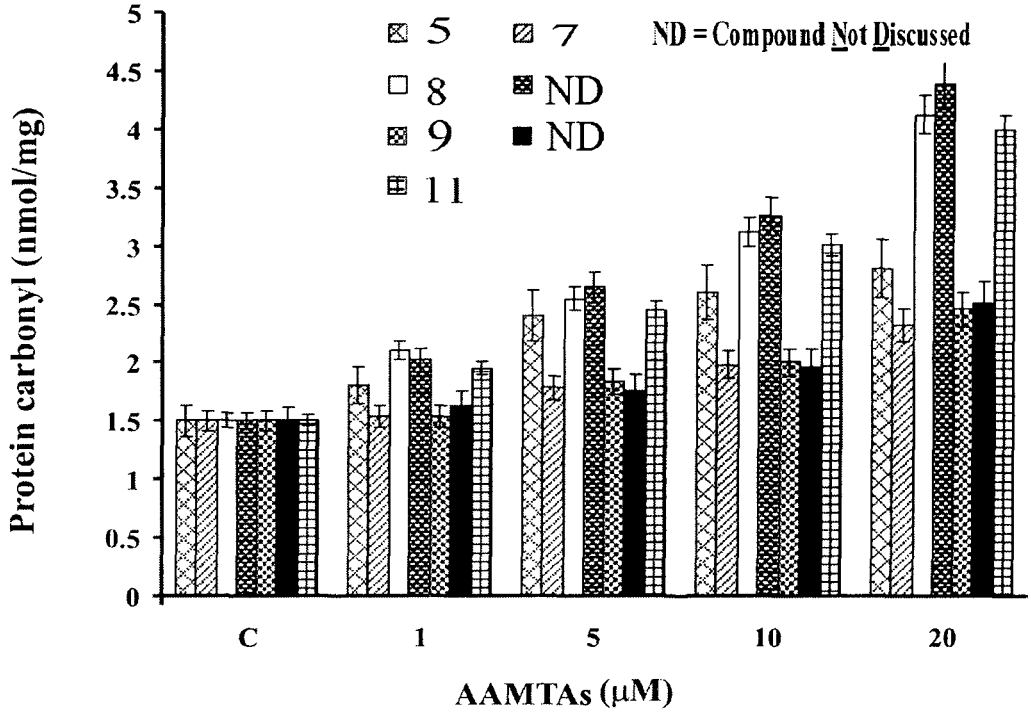


Figure 2.6

2.3.2.4 *In vivo* antimalarial activity against multidrug resistant malaria parasite *P. yoelii* (MDR)

In vitro antimalarial activity of AAMTAs encouraged us to evaluate the effect of efficient AAMTAs against multidrug resistant rodent malaria parasite Plasmodium yoelii (MDR) under *in vivo* conditions. Since here in this study we have found that AAMTAs showed potent antimalarial activity against chloroquine (CQ) sensitive strain of *P. falciparum*, but the whole world is concerned towards the identification of new antimalarial drugs against multi drug resistant parasite. Moreover, there is ample evidence in the literature that compounds, which are active *in vitro* even at lower concentration, did not show activity when tested *in vivo*. To overcome all these issues,

we have used MDR strain of *Plasmodium* (*P. yoelli*) to test the antimalarial activity of AAMTAs under *in vivo* condition using mice model (balb/C).

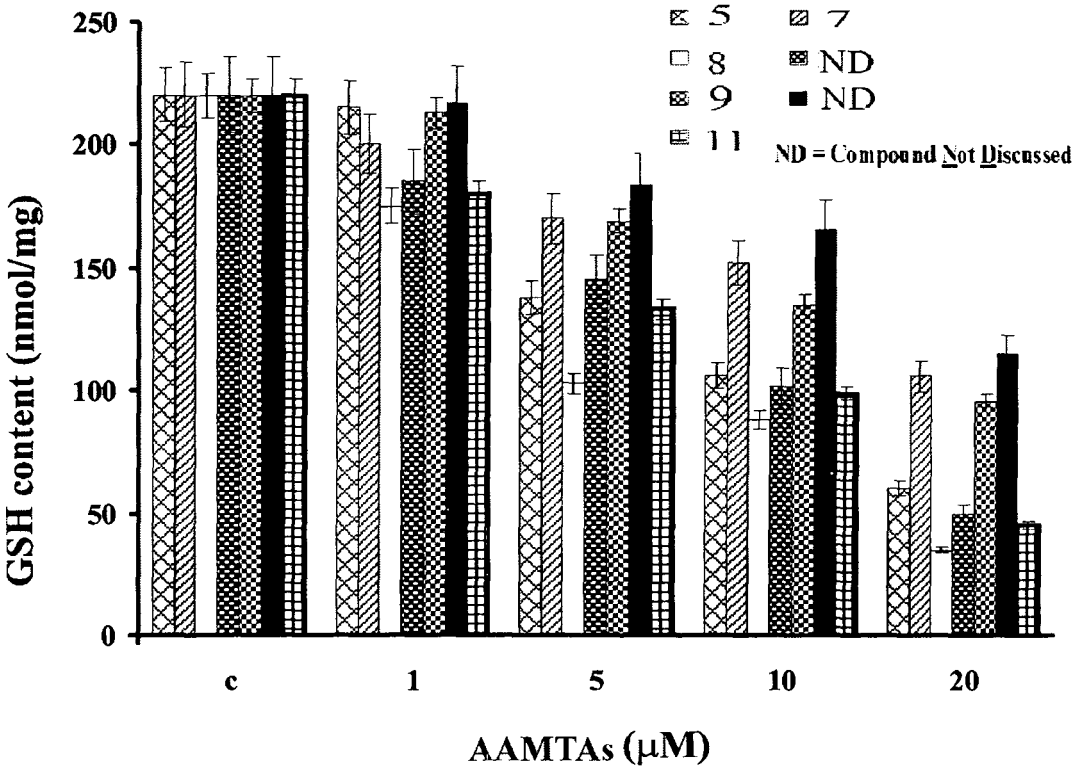
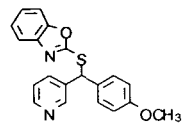
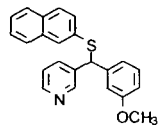
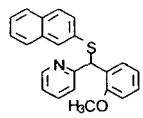
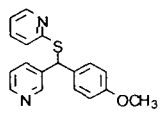


Figure 2.7

We have selected few in vitro screened AAMTAs, which showed highest activity. These issues comprises several aspects involves rapid metabolic degradation, selective uptake as well as ability of the drug to accumulate to pharmacologically relevant concentrations at the site of drug action. But, in vitro assay of antimalarial activity is beneficial to screen large number of drugs to select effective one. AAMTAs showed good antimalarial activity in vivo and out of various AAMTAs, the most active during in vivo study were compounds **5** and **11**, which suppressed the mean parasitemia (day 8) by 50%, 68% and 79% (Compound **5**) and 55%, 72% and 80% (compound **11**) at dose levels of 5 mg/kg, 10 mg/kg and 25 mg/kg, respectively (Table 2.2). While compounds **8** and **9** are comparatively less effective and suppressed the (day 8) mean parasitemia by 23%, 41% and 50% (compound **8**), 33%, 53% and 58% (compound **9**) at dose levels of 5 mg/kg, 10 mg/kg and 25 mg/kg, respectively. Thus, results indicated that compounds **5** and **11** were highly effective but compounds **8** and **9** were found to be comparatively less effective. However,

compound **8** showed best activity during different experiments like heme binding, hemozoin formation and in vitro parasite growth but not to be effective during *in vivo* testing.

Table 2.2

AAMTAs	Structure	Dose (mg/kg/day) for 4 days	***Mean % suppression of parasitemia (Day 8) M±SE
5		5	50±6
		10	68±8
		25	79±9
8		5	23±3
		10	41±5
		25	85±8
9		5	33±4
		10	53±5
		25	58±5
11		5	55±7
		10	72±6
		25	80±8
Vehicle		0	0

Percentage suppression was calculated as [(C-T)/C] x 100, where C is the parasitemia in the control group and T is the parasitemia in the treated group.

2.3.2.5 *In vitro* cytotoxicity assay

To study the toxicity of AAMTAs on nucleated rapidly proliferating cells, we used leukemia cell line U 937. Cells were incubated with 1mM- 10 mM concentration of AAMTAs, followed by MTT reduction assay. It was found that AAMTAs had no

significant toxicity on rapidly proliferating leukemia cells (Table 2.3) as evident from selectivity index, which were greater than 142.

Table 2.3.

AAMTAs	IC_{50} (μM) Hypoxanthine uptake	U 937 IC_{50} (μM)	IC_{50} (μM) [U 937/ Hypoxanthine uptake] S_I[a]
5	1.4	200	142.85
11	1.5	380	253.33

[a]Selectivity index (S_I) = cytotoxicity IC_{50}/antiplasmodial IC_{50}.

2.4 Conclusion

In conclusion, AAMTAs showed antimalarial activity *in vivo*. Drug resistance of malaria parasite is a major public health problem which hinders the control of malaria. AAMTAs show prominent antimalarial activity against multi drug resistance strain of *Plamodium yoelli* under in vivo condition. Results of *in vivo* screening of active AAMTAs compounds show that these compounds suppress mean % parasitemia (in AAMTAs treated group) more than 50% even at a dose of 25 mg/kg body weight in experimental Balb/c mice model. Since AAMTAs show interaction with heme and inhibits hemozoin formation, (which is basically a physical process rather than genetic one) development of resistance against this compound will not be easy and quick. AAMTAs show interaction with heme effectively even at very low concentration and causes free heme accumulation which ultimate leads to parasite death (as monitored by ^3H hypoxanthine incorporation assay using *P. falciparum* culture) via generation of oxidative stress. Selective active AAMTAs compounds do not show *in vitro* cytotoxicity on nucleated proliferating leukemia cell line U 937 and the selectivity index (S_I) value was in a range of 142- 253, when analyzed by MTT reduction assay.

2.5 Experimental Section

2.5.1 Chemistry

2.5.1.1 General remarks

All dry reactions were carried out under argon or nitrogen in oven-dried glassware using standard gas-light syringes, cannulas and septa. All reagents and solvents were dried prior to use according to standard methods. Commercial reagents were used without further purification unless otherwise stated. Reactions were monitored on silica gel TLC plates (coated with TLC grade silica gel, obtained from Merck). Detecting agents used (for TLC) were iodine vapors and/or spraying with an aqueous solution of vanillin in 10% sulfuric acid followed by heating at 150 °C. Column chromatography was performed over silica gel (60-120 mesh) procured from Qualigens (India) using freshly distilled solvents. Mass spectra were recorded as electron spray ionization (ESI-MS) or Fast atom bombardment spectra (FAB-MS) on a JEOL SX 102 spectrometer using Argon/xenon as the FAB gas. Elemental analyses were done on Varian EL-III C H N analyzer. Melting points were determined on COMPLAB melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR RXI spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker DPX-200 (operating at 200 MHz for ^{1}H and 50 MHz for ^{13}C) or DPX-300 (operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C) spectrometer using $CDCl_3$. Tetramethylsilane (0.00 ppm) served as an internal standard in ^{1}H NMR and $CDCl_3$ (77.0 ppm) in ^{13}C NMR. All spectra were recorded at 25 °C. Coupling constants (*J* values) are given in hertz (Hz). Chemical shifts are expressed in parts per million.

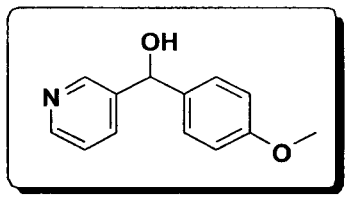
2.5.1.2 General procedure for preparation of carbinols (3a-f).

To a suspension of Mg (1.5g, 61.70 mmol) in dry THF (40 mL) was added dropwise a solution of 4-bromoanisole (6.52 mL, 52.08 mmol) in dry THF (40 mL). After stirring the mixture for 30 min. a solution of aryl and heteroaryl carbaldehyde (45 mmol) in dry THF (30 mL) was added dropwise and the resulting solution was allowed to stir for an additional 30 min. After quenching by adding a saturated solution of NH_4Cl (20 mL), the reaction mixture was extracted with ethyl acetate (100 mL), washed with water (100 mL), brine (2x50mL) and then dried over Na_2SO_4. The organic layer was

removed under reduced pressure. The crude product was purified by silica gel column chromatography.

(4-Methoxy-phenyl)-pyridin-3-yl-methanol (3a):

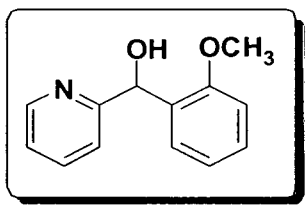
R_f: 0.28 (ethyl acetate). Isolated as pale yellow solid (yield 68%, mp 105–106 °C) by



elution with 45% ethyl acetate in hexane on silica gel. IR (KBr): 3216, 2362, 1608, 1511, 1427, 1248, 1175, 1030, 812, 712, 573 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.42 (d, 1H, *J*= 1.68), 8.29 (dd, 1H, J_1= 1.32, J_2= 4.76), 7.67 (d, 1H, *J*= 7.86), 7.26-7.16 (m, 3H), 6.84 (d, 2H, *J*= 8.62), 5.74 (s, 1H), 4.24 (s, br, 1H), 3.76 (s, 3H). ^{13}C NMR ($CDCl_3$+CCl_4, 50 MHz): δ 159.50, 148.22, 148.18, 140.74, 136.18, 134.84, 128.73, 128.30, 123.82, 114.37, 73.60, 55.65. MS (FAB): *m/z* 216 $[M+H]^+$, 198 $[M-OH]^+$. Anal. Calcd. for $C_{13}H_{13}NO_2$: C, 72.54; H, 6.09; N, 6.51. Found: C, 72.59; H, 6.22; N, 6.47.

(2-methoxyphenyl)(pyridin-2-yl)methanol (3d):

R_f: 0.30 (20% ethyl acetate in hexane). Isolated as yellowish liquid (yield 71%) by



elution with 6% ethyl acetate in hexane on silica gel. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.47-8.44 (m, 1H), 7.55 (d, 1H, *J* = 1.5 Hz), 7.24-7.10 (m, 3H), 6.96-6.94 (m, 2H), 6.80-6.75 (m, 1H), 5.71 (s, 1H), 3.71 (s, 3H). MS (ESI): *m/z* 215.4 $[M]^+$. Anal. Cacld. for $C_{13}H_{13}NO_2$: C, 72.54; H, 6.09; N, 6.51. Found: C, 72.69; H, 6.00; N, 6.62.

2.5.1.3 General Procedure for Preparation of Aryl aryl methyl thio arenes (4-11)

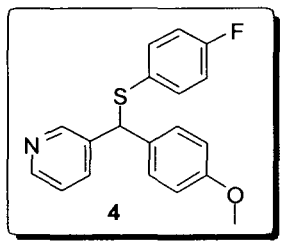
Method a: To a solution of carbinol **3a-f** (2.50 mmol) and electron-rich arene or arylthiol (3.75 mmol) in dry benzene (25 mL), a catalytic amount of conc. H_2SO_4 was added and the mixture was refluxed for half an hour. After adding water, the reaction mixture was extracted with ethyl acetate (25 mL), washed by brine (25 mL), and dried over Na_2SO_4. The combined organic layer was removed under reduced pressure. The crude product was purified by silica gel column chromatography to furnish Aryl aryl methyl thio arenes (**4-11**).

Method b: To a solution of carbinol **3a-f** (2.50 mmol) and electron-rich arene or arylthiol (3.75 mmol) in dry benzene, anhydrous $AlCl_3$ (2.52 mmol) was added and the mixture was stirred at room temperature for half an hour. After adding ice-cooled

water, the reaction mixture was extracted with ethyl acetate (25 mL), washed by brine (25 mL), and dried over Na_2SO_4. The combined organic layer was removed under reduced pressure. The crude product was purified by silica gel column chromatography to furnish Aryl aryl methyl thio arenes (**4-11**).

3-[(4-Fluoro-phenylsulfanyl)-(4-methoxy-phenyl)-methyl]-pyridine 4:

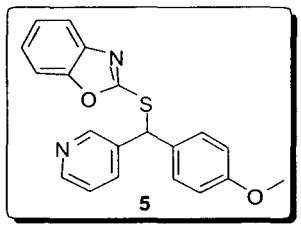
R_f: 0.48 (50% ethyl acetate in hexane). Isolated as pale yellow solid (yield 63%, mp



87-89 ^{0}C) by elution with 15% ethyl acetate in hexane from silica gel. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.57 (s, 2H), 8.41 (s, 1H), 7.68 (d, 1H, J = 7.8), 7.25-7.13 (m, 5H), 6.86- 6.77 (m, 3H), 5.66 (s, 1H), 3.74 (s, 3H). MS (ESI): *m/z* 326 (M^++1). Anal. Calcd for $C_{19}H_{16}FNOS$: C, 70.13; H, 4.96, N, 4.30. Found: C, 70.23; H, 5.19; N, 4.30.

2-[(4-Methoxy-phenyl)-pyridin-3-yl-methylsulfanyl]-benzooxazole 5:

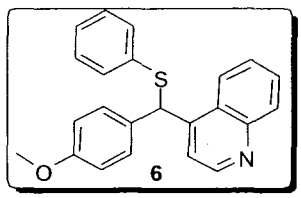
R_f: 0.38 (50% ethyl acetate in hexane). Isolated as pale yellow solid (yield 61%, mp



83-85 ^{0}C) by elution with 15% ethyl acetate in hexane from silica gel. ^{1}H NMR (200 MHz, $CDCl_3$) : δ 8.76 (d, 1H, J = 1.7), 8.48 (s, 1H), 7.83 (d, 2H, J = 7.9), 7.69-7.65 (m, 1H), 7.40-7.21 (m, 5H), 6.88-6.83 (m, 2H), 6.34 (s, 1H), 3.75 (s, 3H). MS (ESI): *m/z* 349 (M^++1). Anal. Calcd for $C_{20}H_{16}N_2O_2S$: C, 68.94; H, 4.63; N, 8.04. Found: C, 69.02; H, 4.42; N, 8.19.

[(4-Methoxy-phenyl)-phenylsulfanyl-methyl]-quinoline 6:

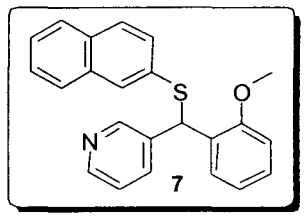
R_f: 0.44 (50% ethyl acetate in hexane). Isolated as pale yellow solid (yield 64%, mp



89-92 ^{0}C) by elution with 15% ethyl acetate in hexane from silica gel. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.90 (d, 1H, J = 4.54), 8.16-8.03 (m, 2H), 7.80 (d, 1H, J = 4.52), 7.67 (m, 1H), 7.55 (m, 1H), 7.32-7.12 (m, 7H), 6.81 (d, 2H, J = 8.6), 6.24 (s, 1H), 3.74 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 159.5, 150.6, 148.8, 146.5, 136.2, 131.4, 130.6, 130.2, 130.1, 129.6, 129.4, 127.9, 127.3, 127.2, 126.8, 123.8, 121.3, 114.6, 114.4, 55.6, 52.8. MS (ESI): *m/z* 358 (M^++1). Anal. Calcd for $C_{25}H_{43}N_3O_4S$: C, 62.34; H, 9.00; N, 8.72. Found: C, 62.52; H, 9.21; N, 8.46

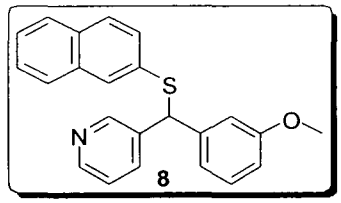
3-[(2-Methoxy-phenyl)-(naphthalen-2-ylsulfanyl)-methyl]-pyridine 7: R_f: 0.48 (50% ethyl acetate in hexane). Isolated as pale yellow solid (yield 64%, mp 102-104

^{0}C) by elution with 15% ethyl acetate in hexane from silica gel. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.65 (d, 1H, *J* = 1.94), 8.42 (m, 1H), 7.75-7.61 (m, 6H), 7.43-7.38 (m, 3H), 7.25-7.19 (m, 2H), 6.87 (m, 2H), 6.13 (s, 1H), 3.79 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 156.7, 150.4, 148.5, 137.4, 136.2, 134.0, 133.5, 132.4, 129.4, 129.3, 128.7, 128.5, 128.0, 127.7, 126.8, 126.3, 123.6, 121.3, 111.2, 56.0, 48.0. MS (ESI): *m/z* 358 (M^++1), 199.3 (M^+-$C_{10}H_7S$). Anal. Calcd for $C_{23}H_{19}NOS$: C, 77.28; H, 5.36; N, 3.92. Found: C, 77.43; H, 5.48; N, 4.05.



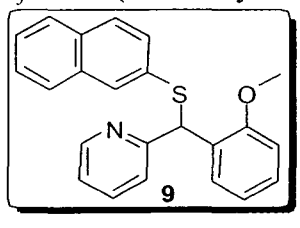
3-[(3-Methoxy-phenyl)-(naphthalen-2-ylsulfanyl)-methyl]-pyridine 8:

R_f: 0.48 (50% ethyl acetate in hexane). Isolated as pale yellow solid (yield 64%, mp 102-104 ^{0}C) by elution with 15% ethyl acetate in hexane from silica gel. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.63 (d, 1H, *J* = 2.2), 8.43 (d, 1H, *J* = 3.4), 7.74-7.61 (m, 5H), 7.43-7.36 (m, 5H), 7.32-7.24 (m, 1H), 6.86-6.82 (m, 2H), 5.62 (s, 1H), 3.76 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 159.4, 150.2, 148.9, 137.4, 136.1, 133.9, 132.9, 132.6, 132.2, 130.3, 129.8, 128.8, 128.0, 127.7, 126.9, 126.5, 123.8, 114.6, 55.6, 54.8. MS (ESI): *m/z* 358 (M^++1), 198 (M^+-$C_{10}H_7S$). Anal. Calcd for $C_{23}H_{19}NOS$: C, 77.28; H, 5.36; N, 3.92. Found: C, 77.19; H, 5.51; N, 4.06.



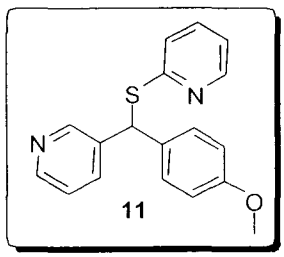
2-[(2-Methoxy-phenyl)-(naphthalen-2-ylsulfanyl)-methyl]-pyridine 9:

R_f: 0.38 (60% ethyl acetate in hexane). Isolated as pale yellow solid (yield 60%) by elution with 15% ethyl acetate in hexane from silica gel. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.51-8.49 (m, 1H), 7.56-7.51 (m, 1H), 7.32-7.19 (m, 4H), 6.94-6.85 (m, 2H), 6.20 (s, 1H), 3.82 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 161.7, 157.0, 148.1, 137.0, 132.1, 129.1, 128.2, 122.5, 121.6, 121.3, 111.1, 69.6, 55.9, . MS(ESI): *m/z* (M^++1), 230 (M^+-$C_{10}H_7S$). Anal. Calcd for $C_{23}H_{19}NOS$: C, 77.28; H, 5.36; N, 3.92. Found: C, 77.48; H, 5.17; N, 4.07.



2-((4-methoxyphenyl)(pyridin-3-yl)methylthio)pyridine 11:

R_f: 0.52 (ethyl acetate in hexane). Isolated as colourless solid (60%, mp 139-140 °C) by elution with 45% ethyl acetate in hexane from silica gel. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.67



(s, 1H), 8.50 (d, 1H, J = 4.2), 8.36 (d, 1H, J = 4.4), 7.86 (d, 2H, J = 7.8), 7.08-7.06 (m, 1H), 6.88 (d, 2H, J = 8.52), 6.16 (s, 1H), 2.30 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 160.39, 153.85, 149.87, 139.03, 138.29, 137.10, 134.61, 132.12, 130.01, 129.68, 128.76, 126.80, 125.09, 124.14, 123.54, 114.28, 96.54, 77.76, 55.56, 30.11, 21.55. MS (ESI): *m/z* 308 $[M]^+$. Anal. Calcd for $C_{18}H_{16}N_2OS$: C, 70.10; H, 5.23; N, 9.08. Found: C, 70.21; H, 5.34; N, 9.27.

2.5.2 Biology

2.5.2.1 Materials used

Hemin, RPMI-1640, saponin, SDS, chloroquine, glutathione (GSH), dichlorofluorescein diacetate, thiobarbituric acid (TBA), trichloroacetic acid (TCA), dichlorofluorescein diacetate, fetal calf serum (FCS); DMSO; 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), Penicillin, streptomycin and tetraethoxypropane were purchased from Sigma (St. Louis, MO, USA). Albumax II was procured from Life Technologies, USA, Giemsa Stain was purchased from Qualigens Fine Chemicals, India. ^{3}H hypoxanthine was purchased from Amersham Biosciences, USA. All other chemicals were of analytical grade purity.

2.5.2.2 Parasite Culture

P. falciparum (clone NF54) was grown as described earlier[25]. Parasite culture was maintained at a hematocrit level of 5% in complete RPMI medium (CRPMI; RPMI 1640 medium, supplemented with 25 mM HEPES, 50 μg ml^{-1} gentamycin, 370 μM hypoxanthine and 0.5% (w/v) AlbuMaxII) using tissue-culture flasks (25 cm^2 and 75 cm^2) with loose screw caps. Old medium was changed with fresh medium once in 24 h and the culture was routinely monitored through Giemsa-staining of thin smears.

2.5.2.3 Preparation of parasite lysate

Parasite was isolated as described previously[26]. In brief, erythrocytes with ~10% parasitemia (*P. falciparum*) or 50% parasitemia (*P.yoelii*) were centrifuged at 800 x g for 5 mins, washed twice and resuspended in cold phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 5.3 mM Na_2HPO_4 and 1.8 mM KH_2PO_4). An equal volume of 0.5% saponin in PBS (final concentration 0.25%) was added to the erythrocyte suspension and kept on ice for 15-20 mins. Lysate was centrifuged at 1300 x g for 5 mins to get parasite pellet, and pellet was washed with PBS thrice and either used

immediately or kept at –80°C. The isolated parasite was lysed in PBS by mild sonication (30 sec pulse, bath type sonicator) at 4°C and the whole lysate was then stored at –20°C for future use. Protein content of the parasite lysate was estimated by Lowry method[27].

2.5.2.4 Assay of Hemozoin (β-Hematin) Formation

In vitro hemozoin (β-hematin) formation was assayed as described earlier[28]. In brief, the assay mixture contained final volume of 1 ml (100 mM sodium acetate buffer pH 5.2), 100 μM hemin, parasite lysate (20 μl), and different concentrations of AAMTAs compounds. The reaction was initiated by the addition of hemin and further incubated for 12 h at 37^0 C. The reaction was terminated by centrifugation at 15,000x g for 10 min at room temperature. The pellet was washed thrice with 100 mM Tris buffer pH 7.8 containing 2.5% SDS and finally with 100 mM bicarbonate buffer pH 9.2. The insoluble pellet (hemozoin) was solubilized in 50 μl of 2 N NaOH and diluted further to 1 ml with 2.5% SDS. The absorbance of the solution was recorded at 400 nm and an extinction coefficient of $91 mM^{-1}cm^{-1}$ was used to quantitate the heme converted to hemozoin[28a].

To see the effect of AAMTAs on hemozoin formation in *P. falciparum,* the amount of hemozoin formed in presence or absence of AAMTAs was measured as described earlie[29]. In brief, D-sorbitol (5%) synchronized *P. falciparum* culture (5% parasitemia was treated with different concentrations of AAMTAs compounds and incubated further for 48 hours. The *P. falciparum* culture was then harvested and parasite was isolated from infected RBC by saponin (0.5%, 10 minutes) treatment. Parasite was washed three times with PBS and parasite lysate was prepared after mild sonication. Parasite lysate was washed three times with 2% SDS and the resulting pellet was suspended in a solution of 10 mM Tris-HCl (pH 8.0), 0.5% SDS, and 1 mM $CaCl_2$ containing 2 μg /ml of proteinase K and was then incubated at 37^0C overnight. The pellet was then washed three times in 2% SDS and incubated in 6 M urea for 3 h at room temperature on a shaker. After incubation, it was centrifuged (4000 x g for 10 minutes) at room temperature and again washed three times with 2% SDS. Finally, the hemozoin pellet was dissolved in 20 mM NaOH containing 2% SDS and the OD of solution was measured at 400 nm to quantitate hemozoin.

2.5.2.5 Heme interaction studies

Heme interaction of synthesized AAMTAs compounds were analysed by optical and differential optical spectroscopy as described earlier[11]. In brief, reaction assembled in a total volume of 1 ml containing heme (1 μM) in 100 mM acetate buffer, pH 5.2 in a Perkin Elmer Lamda 15 UV/VIS spectrophotometer at 25 ± 1 ^{0}C with quartz cells of 1 cm light- path. Different concentrations of AAMTAs compounds (1-20 μM) were added successively. Soret spectrum without AAMTAs compounds and after addition of AAMTAs compounds was recorded immediately. Interaction of AAMTAs compounds with native heme was also measured by optical difference spectroscopy as described earlier[30]. For measurement of difference spectra of heme- AAMTAs compounds versus the heme, both the reference and sample cuvettes were filled with 1 ml of heme solution (1 μM) to provide the baseline trace. This was followed by addition of different concentration of AAMTAs compounds to the sample cuvette with concomitant addition of the same volume of DMSO to the reference cuvette. The contents were mixed well before the spectrum was recorded. The equilibrium dissociation constant (K_D) for complex formation was calculated from the following expression as described by Schejter et. Al[26].

$$1/\triangle A = (K_D/\triangle A_\alpha)1/S + 1/\triangle A_\alpha$$

Where K_D is the dissociation constant of the heme – AAMTAs compound complex, S is the concentration of AAMTAs , $\triangle A$ is the observed absorption changes at a particular wavelength and $\triangle A_\alpha$ is the absorption changes at saturating concentration of the ligand (AAMTAs compounds).

2.5.2.6 *In vitro* antimalarial activity

Inhibition of *P. falciparum* growth was studied by following (^{3}H) hypoxanthine uptake as described earlier[31]. *P. falciparum* (NF-54 strain) was cultured *in vitro* as described earlier[25]. D-sorbitol synchronized parasites culture was used to achieve uniform ring stages as described previously[32]. To check the antimalarial activity of AAMTAs compounds, the ring synchronized *P. falciparum* (parasitemia 0.5% - 1%) was cultured in multiwells (200μl/well) plate in presence or absence of different concentrations of AAMTAs. Chloroquine was used as a positive control. After 48 hours (^{3}H) hypoxanthine (0.7μCi/well) was added in each well and further incubated

for 48 hours to monitor parasite viability by measuring incorporation of (^{3}H) hypoxanthine in parasite nucleic acids. After that culture was harvested and washed thrice in phosphate buffered saline (PBS). Parasite pellets were dissolved in 100 µl 3N NaOH by keeping at $37^{0}C$ for 6 hours. After incubation, it was dissolved/ added in (10 ml/vial) scintillation fluid (PPO, 4 g; POPOP, 200mg; naphthalene, 60 g; ethylene glycol, 20ml; methanol 100 ml in one litre of 1, 4 dioxane). After 12 hours of incubation, (^{3}H) hypoxanthine uptake was measured using β- scintillation counter.

2.5.2.7 Free heme quantitation in *P. falciparum*

The heme content in control and AAMTAs compounds-treated *P. falciprum* was measured as described earlier[11]. In brief, *P. falciparum* culture was incubated in the presence or absence of various concentrations of AAMTAs compounds for 48 h. The culture was then centrifuged to pellet the cells, and the cell pellet was washed in PBS. Concentrated formic acid (1 ml) was then added to solubilize pellets and then heme concentration of the formic acid solution was determined in a Shimadzu UV/VIS1700 spectrophotometer at 398 nm (extinction coefficient = $1.56 \times 10^{5}\ M^{-1}\ cm^{-1}$). The heme content was expressed as nmol/mg of cell protein.

2.5.2.8 Measurement of reactive oxygen species

P. falciparum (4% parasitemia) was cultured in the presence or absence of different concentrations of AAMTAs compounds for a period of 48 hours as described[11]. The culture was then further incubated for a period of 30 min in complete RPMI medium containing 10 µM 2', 7'-dichlorofluorescein diacetate. The culture was then washed thrice with phosphate-buffered saline, and the parasite was isolated from control and treated groups as described. Isolated parasites were lysed by mild sonication (5-sec pulse, bath type sonicator) at $4^{0}C$. Intra-paraitic H_2O_2 leve lwas measured in control or AAMTAs compounds treated parasite by measuring the fluorescent dichlorofluorescein (DCF) oxidation product of non-fluorescent probe dichlorofluorescein diacetate by H_2O_2[33]. Fluorescent intensities were recorded from the lysate in a Hitachi F-7000 fluorescence spectrophotometer in a 5-mm path length quartz cell in a total volume of 1 ml at wavelength 502 and 523 nm for excitation and

emission, respectively. H_2O_2 was measured as relative fluorescence and expressed as fluorescence intensity /mg of parasite lysate.

•OH radicals generated as a consequence of oxidative stress in the *P. falciparum* after AAMTAs compounds treatment at different concentrations was measured as described earlier [34] using dimethyl sulfoxide (DMSO) as •OH scavenger. In brief, *P. falciparum* culture (200 µl) (2-4% parasitemia, ring + early trophozoites stage) was grown in multi-well plate in presence or absence of different concentrations of AAMTAs compounds containing 20 µl of 25% DMSO for 48 hours. DMSO (20 µl) was added in each time along with the specific concentrations of AAMTAs compounds when the medium was changed (once in 24 hours). Parasite alone (without DMSO and AAMTAs compounds) was used as negative control. After 48 hours, the culture was centrifuged at 800 xg for 5 min washed and resuspended in cold PBS. The parasite was isolated as described above and the isolated parasite was lysed in triple distilled water and processed for the extraction of methanesulfinic acid formed by the reaction of •OH with DMSO. Methanesulfinic acid formed was allowed to react it with Fast Blue BB salt and the intensity of the resulting yellow chromophore was measured at 425 nm using benzene-sulfinic acid as standard.

2.5.2.9 Assessment of oxidative stress in *P. falciparum*

To analyze the oxidative stress induced by AAMTAs, we have checked the formation of lipid peroxidation, protein carbonyl formation as well as total GSH content. *P. falciparum* culture (4% parasitemia) was incubated with different concentration of AAMTAs compounds for 48 hours. After incubation, parasite was isolated and mixed with PBS (500 µl) to prepare parasite lysate as described above and the lipid peroxidation product from these lysates was measured as described earlier[35]. In brief, parasite lysate (500 µl) was treated with 1ml TCA-TBA mixture in 1N HCl and incubated 15 minutes at 100^0 C. After incubation, it was cooled and centrifuged (4000 rpm for 10 minutes). Then supernatant was collected and OD was measured at 535nm using Shimadzu UV-VIS spectrophotometer. Formation of lipid peroxide in parasite membrane was expressed as nmol/ mg protein. Tetraethoxypropane was used as a standard. In brief, parasite lysate (500 µl) was treated with 10% trichloroacetic acid for protein precipitation and allowed to react

with 0.5 ml of 10 mM 2,4-dinitrophenylhydrazine for 1 h. After precipitation with 10% trichloroacetic acid, the protein was washed three times with a mixture of ethanol: ethylacetate (1:1), dissolved in 0.6 ml of a solution containing 6 M guanidine-HCl in 20 mM potassium phosphate adjusted to pH 2.3 with trifluoroacetic acid. The solution was centrifuged, and the supernatant was used for measurement of carbonyl content by measuring the OD at 362 nm.

2.5.2.10 Measurement of reduced glutathione (GSH)

P. falciparum (4% parasitemia) was cultured in the presence or absence of different concentrations of AAMTAs. GSH content of control and AAMTAs treated parasite culture was measured by using a fluorometric method using the commercially available glutathione assay kit (BioVision, 980 Linda Vista Avenue Mountain View, California, USA). To perform the experiments, essentially same protocol were followed as described in kit with slight modification. In the assay, kit provided probe o-phthalaldehyde (OPA) which reacts with GSH but not with GSSG, generating fluorescence to specifically quantify GSH. GSH was used as a standard. After 48 h of treatment, the culture was washed twice with PBS, and subsequently parasite was isolated. Isolated parasite was sonicated in 200 µl of 20 mM ice-cold using sonicator (by using a 9 s on/10 s off cycle for a period of 60 s) and centrifuged at 10,000 × *g* for 20 min to get clear lysate. After that lysate (200 µl) was mixed with an equal volume of 10% trichloroacetic acid, and protein precipitate was removed by centrifugation. After that 10 µl of OPA was mixed with supernatant and incubated at room temperature for 40 min according to the manufacturer's instruction. Samples were then read on a fluorescence plate reader equipped with Ex/Em = 340/420 nm (HITACHI F7000, Nishi-Shimbashi 1-chome, Minato-ku, Tokyo, Japan). Each experiment was carried out in triplicate and the mean was used in calculations.

2.5.2.11 *In vivo* antimalarial activity

The *in vivo* antimalarial efficacy of AAMTAs was evaluated as described earlier[11]. In brief, rodent malarial model BALB/c mice was infected by MDR (chloroquine, mefloquine, and halofantrine) strain Plasmodium yoelii and subsequently treated intrapertonialy at three dose levels (5 mg/kg, 10 mg/kg, 25 mg/kg body weight) of AAMTAs compounds (**5, 8, 9** and **11**). Briefly, for each dose level a group of six mice

(25 ±5 g) were inoculated intraperitoneally (i.p.) with 1 x10^5 parasitized RBCs on day 0 and AAMTAs compounds derivatives was administered on days 2, 3 , 4 and 5 of post-infection via the i.p. route. The treatment was continued at each dose level from day 2 to 5 *via* the intraperitoneal route. The aqueous suspension (emulsion) of drugs was prepared in ground-nut oil so as to obtain the required drug dose per animal in 0.2 ml volume. The efficacy of active AAMTAs were assessed by continues monitoring the effects of the drugs on % parasitemia and survival. Levels of parasitemia from individual mice were evident in Giemsa-stained thin blood smears every day. The mean value resulted for each group of mice was used to calculate the percentage of suppression in parasitemia with respect to the vehicle control group. Arteemal (α-β-Arteether) treated mice were used as a positive control at a dose level of 50 mg/kg/day body weight.

2.5.2.12 In vitro cytotoxicity

Cytotoxicity of AAMTAs compounds was evaluated by monitoring (MTT) reduction assay using U 937 leaukemia cell line. The cytotoxic effect of different concentrations of AAMTAs on U 937 leaukemia cell line was evaluated. In brief, U937 cells were obtained from culture medium by centrifugation at 500g for 5 minutes. The cell pellets were resuspended and loaded in a haemocytometer for cell count before plating of the cells. U 937 cells were seeded in 96-well flat-bottomed tissue culture microplate at a concentration of 10^5 cells per well treated with different concentrations of AAMTAs (1μM- 10 mM), DMSO (negative control) and sodium azide (1 mM) and incubated for 24 hrs at 37 °C in a humidified atmosphere containing 5% CO_2 respectively. At the end of incubation, 10μl of WST-1 reagent was added to each well and incubated for 3 h. After incubation, the plates were thoroughly shaken on ELISA shaker for 30 seconds. The colour absorbance of each well was recorded at 450nm using an ELISA reader (Beckman Coulter, CA, USA) with a reference serving as blank. The IC_{50} value of each fraction was calculated for U937 cells. All experiment were performed in triplicate and presented as mean ± standard error of mean of three different experiments.

2.6 References

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2.7 Spectra of selected compounds

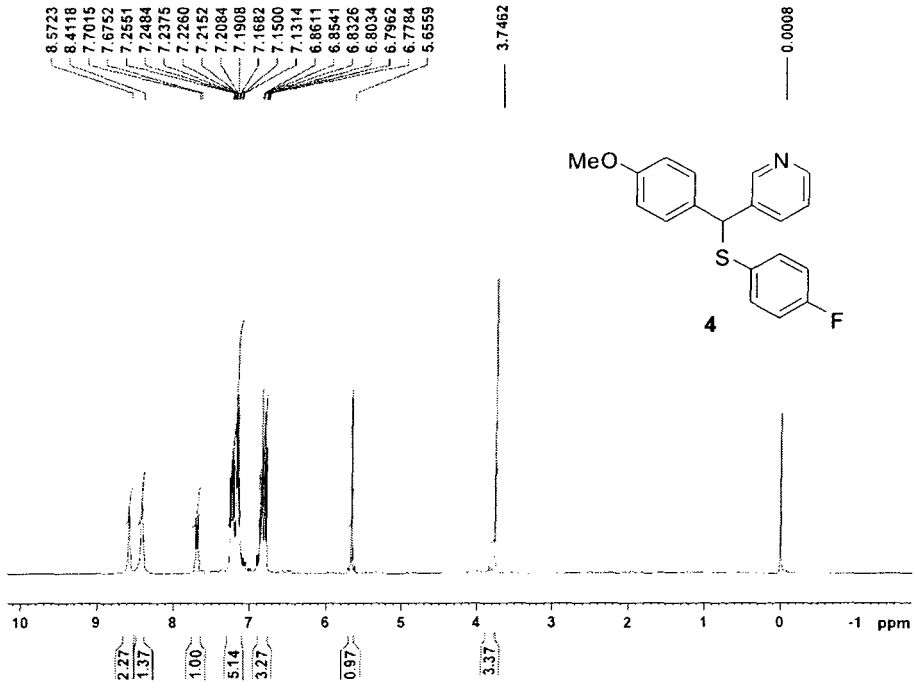


Figure 2.8 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of **4**.

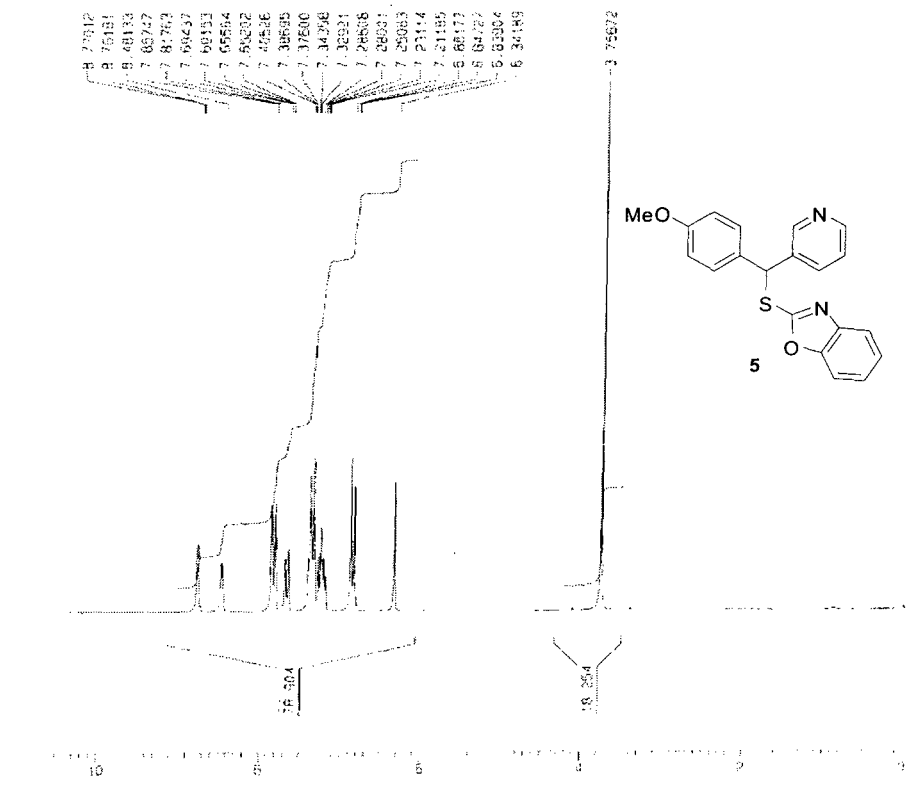


Figure 2.9 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of **5**.

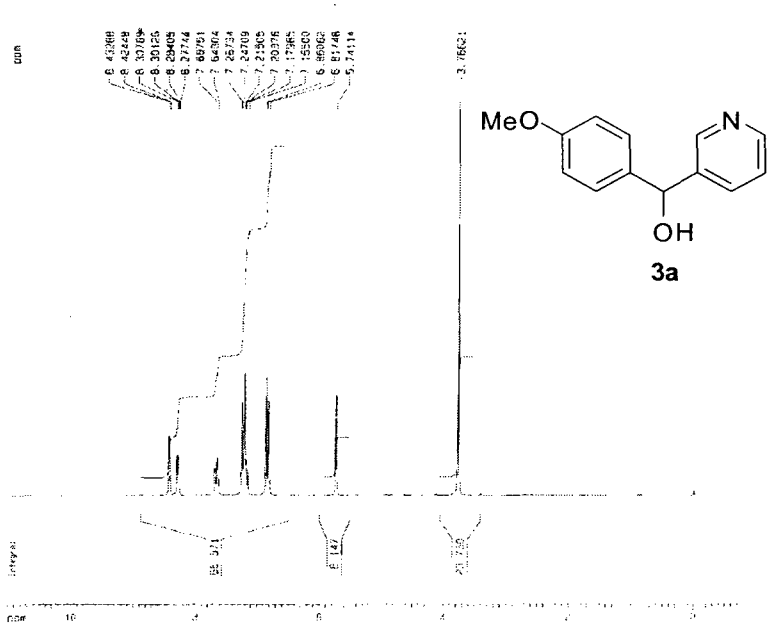


Figure 2.10 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of **3a**.

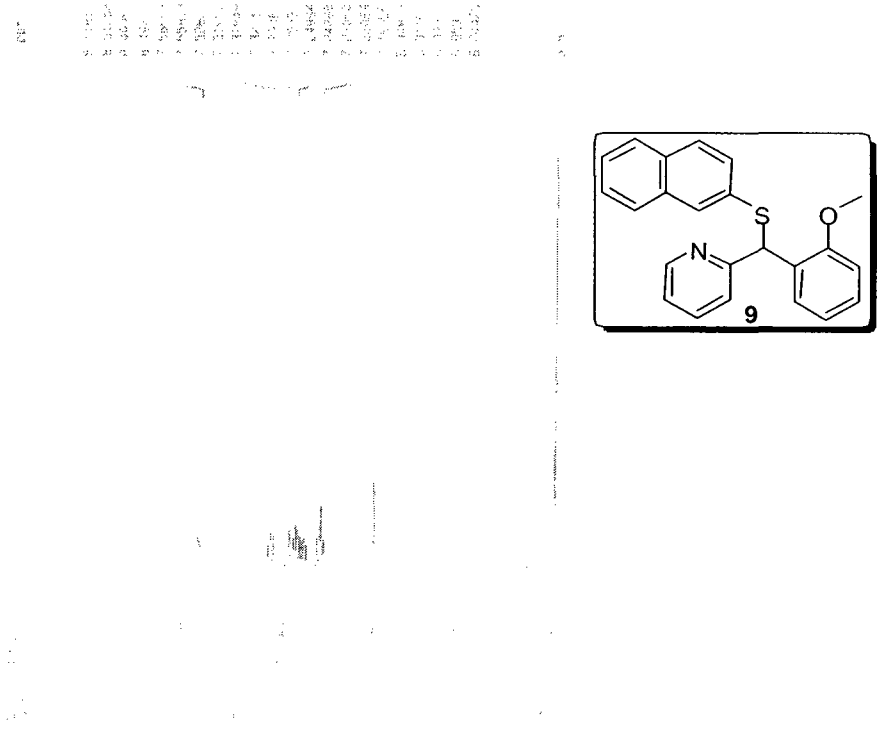


Figure 2.11 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of **9**.

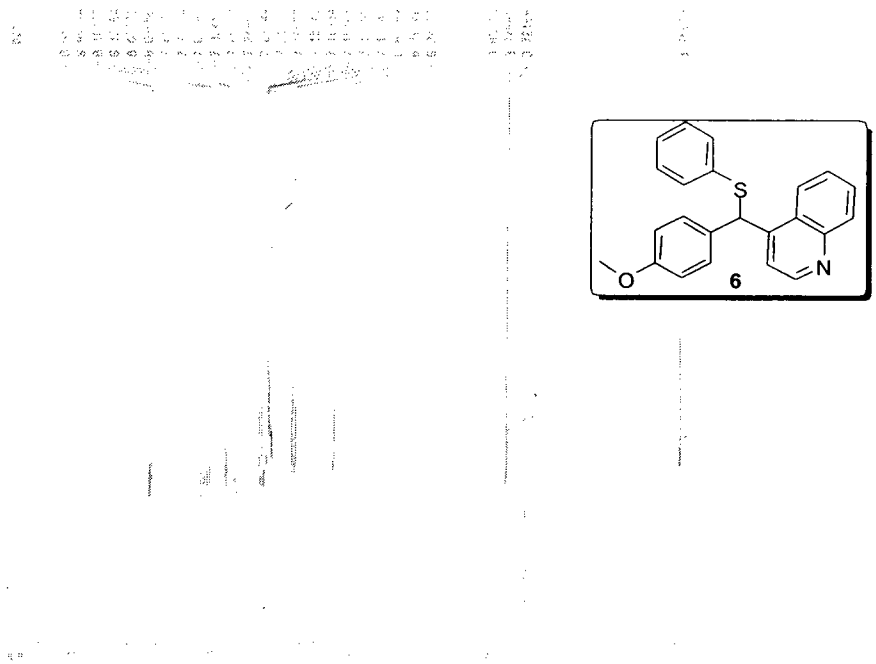


Figure 2.12 ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of **6.**

Chapter 3:
Stereoselective Synthesis of Functionalized 2,3-Dihydrobenzofurans and 1-Benzopyrans by Phenoxide ion-Mediated Carbocyclization

Section 3A:
Stereoselective Synthesis of 2-Substituted 2,3-Dihydrobenzofurans

3.1.1 Introduction

Over the last some decades the outcome of molecular chirality on biological activity has created extreme interest in asymmetric synthesis.[1] Much endeavor has been applied on the development of asymmetric variants of well-established reactions, and upon finding of a new reaction that generates new stereogenic centers focus often immediately shifts to the development of stereoselective versions using chiral auxiliaries, reagents, or catalysts. 2,3-Dihydrobenzofuran derivatives are common in numerous natural products and unnatural molecules.[2] The wide ranging biological properties of these heterocycles have attracted the considerable attention of organic and medicinal chemists.[3] The 2-isopropenyl-2,3-dihydrobenzofuran framework is an integral part of numerous bioactive natural products[4] that contains an asymmetric carbon at the 2-position such as tremetone **1**, 6-hydroxytremetone **2**, fomannoxin **3**, and xyloallenolide B **4** (Figure 3.1.1). In addition to these structurally simple molecules, more complex compounds such as rotenone **5**, 3',4'-deoxypsorospermin **6**, and xyloketal J **7** also contain the same embedded unit.

Tremetone **1** is the major toxic component in white snakeroot (Ageratina altissima) extracts that can cause trembles in livestock and milk sickness in humans.[4a] (2*R*)-6-hydroxytremetone **2** is a potent germination inhibitor of onion, lettuce, and tomato seeds. A phytotoxic secondary metabolite fomannoxin **3** shows growth inhibiting effects on callus and suspension cultures of conifer cells.[4b] Rotenone **5** was isolated from the plant *Derris eliptica* found in Southeast Asia or from the plant *Lonchocarpus utilis* or *L. urucu* native to South America. It is an insecticidal principle and also used as a fish poison. Xyloallenolide B **4** and xyloketal J **7** are secondary metabolites isolated from the mangrove endophytic fungus *Xylaria* sp. (#2508), but when tested for antibacterial activity they showed no effect against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Sarcina lutea* at 50 μg/mL.[5] 3',4'-deoxypsorospermin **6** is one of cytotoxic dihydrofuranoxanthones isolated from bioassay-directed chemical studies of the ethanolic extract of the root bark of the tropical African plant *Psorospermum febrifugum* Spach. (Guttiferae) exhibiting significant *in vitro* cytotoxic (against 9PS cells in culture) and *in vivo* antitumor activity in the P388 mouse leukemia assay.[6] The heliannuols G and H, **8a,b,** heliannane sesquiterpenes isolated from the fresh leaf aqueous extracts of Helianthus annuus L. SH-222 and YPP,[7] were found to have phytotoxic allelopathic activity.

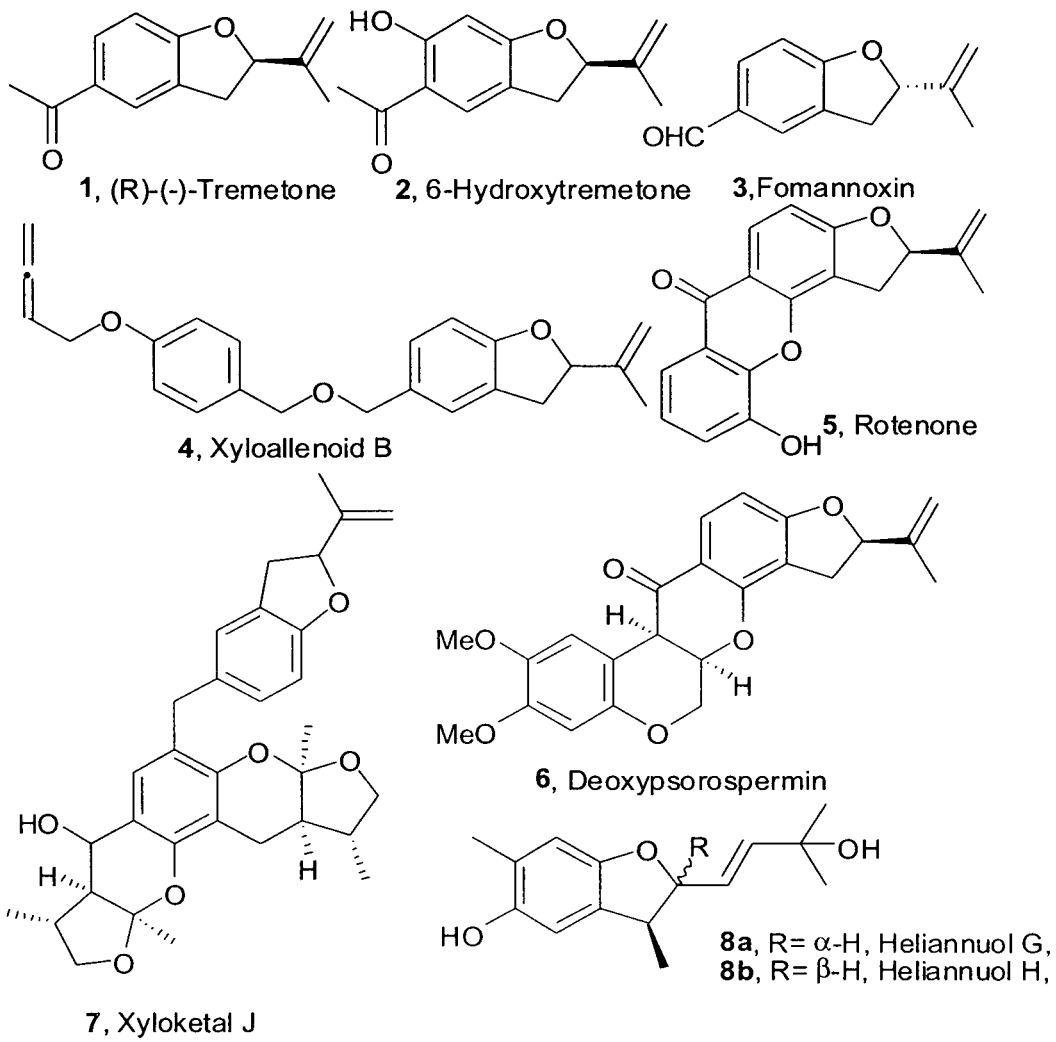


Figure 3.1.1. Representative examples of natural products containing the 2-substituted 2,3-dihydrobenzofuran moiety.

3.1.2 Basis of the Present Work

The history of the synthesis of this class of compounds dates back to 1963 when the first asymmetric synthesis of **1** was achieved from the corresponding chiral 2,3-dihydrobenzofuran-2-carboxylic acid, both enantiomers of which were obtained by a chiral resolution procedure.[4a] Recently, Yamaguchi and co-workers achieved kinetic resolution of the enantiomers of several racemic 2-isopropenyl-2,3-dihydrobenzofurans utilizing Sharpless asymmetric dihydroxylation.[8] However, the above two methods of synthesizing chiral 2-isopropenyl-2,3-dihydrobenzofurans suffered from various drawbacks such as very low yield of the resolution step and low enantiomeric excess (ee) of the desired isomer. As an alternative, several attempted asymmetric cyclizations have been reported in the literature, but these have all proven

unsuccessful in providing high enantiomeric excesses of 2-isopropenyl-2,3-dihydrobenzofuran.[9] Very recently, Koning and co-workers achieved an efficient enantioselective synthesis of the 2-isopropenyl-2,3-dihydrobenzofuran skeleton of tremetone and hydroxytremetone from (*E*)-4-(2-hydroxyphenyl)-2-methyl-2-butenyl methyl carbonate and (*E*)-4-(2,6- dihydroxyphenyl)-2-methyl-2-butenyl methyl carbonate, respectively.[10] The key step was a catalytic palladium-mediated reaction in the presence of the chiral Trost ligand.

Taking into account of all these facts and our interest in the synthesis and biological evaluation of enantiomerically pure medium ring benzo-annulated heterocycles,[11] we herein describe our results that illustrate efficient enantioselective synthetic routes to our designed molecules containg 2-isopropenyl-2,3-dihydrobenzofurans (**9a,b**) and 4-(2,3-dihydrobenzofuran-2-yl)-2-methylbut-3-en-2-ols (**10a,b**) [Figure 3.1.2] through phenolate ion-mediated intramolecular 5-*exo-tet* ring opening of Sharpless asymmetric epoxidation-derived enantiomerically enriched epoxy alcohols.

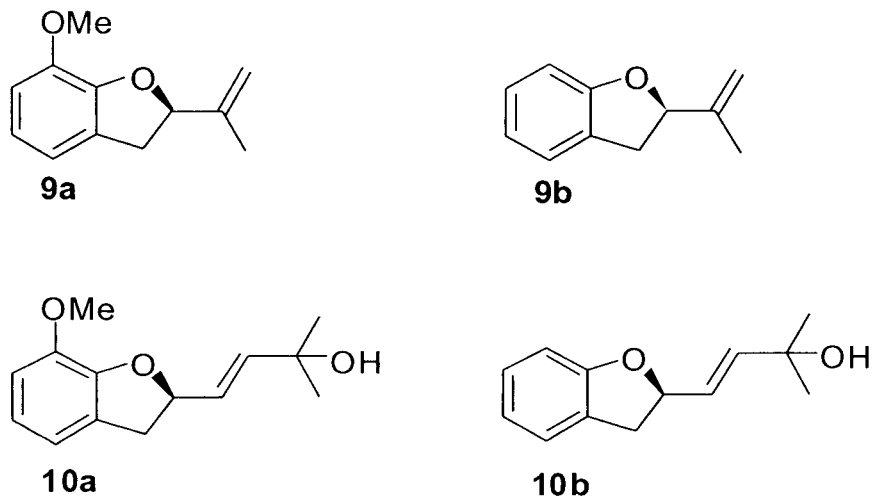
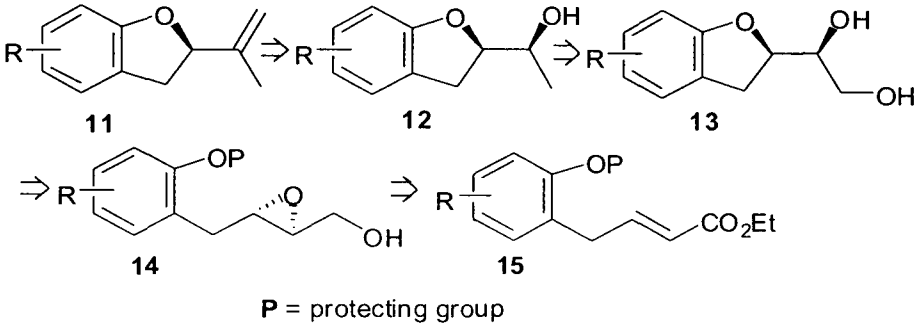


Figure 3.1.2. Our "designed" molecules containing the 2-substituted 2,3-dihydrobenzofuran moiety.

3.1.3 Results and Discussion

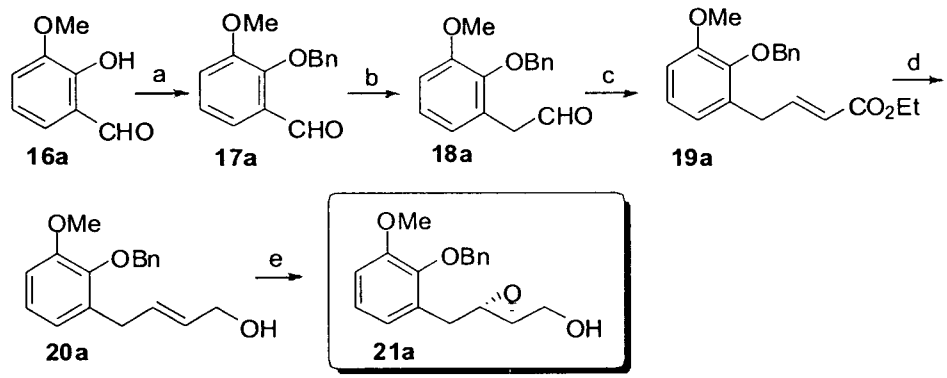
Since its discovery, the Sharpless asymmetric epoxidation of allyl alcohols has become a highly efficient methodology for the synthesis of a wide range of enantiomerically pure natural and unnatural molecules.[12] We planned to apply the methodology as the source of chirality for a general enantioselective synthesis of 2-isopropenyl-2,3-dihydrobenzofurans. Our envisioned retrosynthetic analysis for a

general enantioselective synthetic strategy to 2-isopropenyl-2,3-dihydrobenzofuran **11** is depicted in Scheme 3.1.1. The double bond of the target molecule **11** was anticipated to be fashioned by a Wittig olefination reaction of 2-acetyl-2,3-dihydrobenzofuran obtained from the oxidation of alcohol **12.** Compound **12** was expected to be accessible from dihydroxy compound **13.** Epoxide **14** was thought to be the key intermediate to provide diol **13** *via* a phenolate ion-mediated intramolecular 5-*exo*-*tet* epoxide ring opening reaction.[11c]



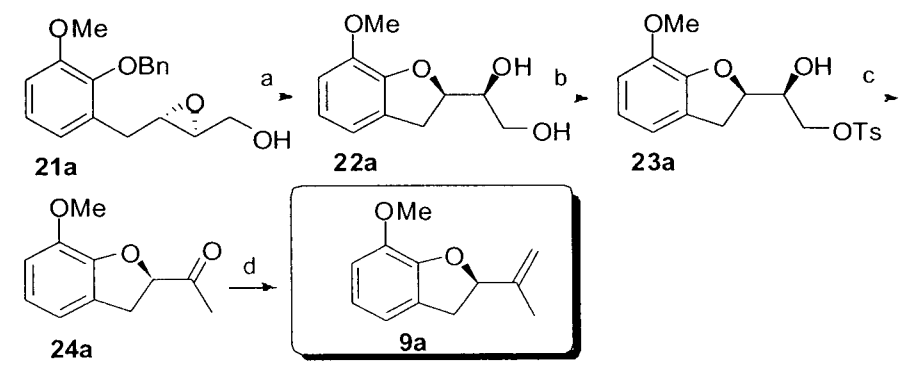
Scheme 3.1.1. Retrosynthetic analysis of 2-isopropenyl-2,3-dihydrobenzofurans.

Our model synthesis to obtain enantiopure 2-isopropenyl-2,3-dihydrobenzofurans commenced with the benzylation of commercially available 2-hydroxy-3-methoxybenzaldehyde **16a** with (bromomethyl)benzene and anhydrous K_2CO_3 in dry acetone under reflux condition furnishing 2-benzyloxybenzaldehydes **17a** in good yields (Scheme 3.1.2). Next, Wittig olefination of **17a** with methoxymethyl-triphenylphosphonium chloride and LHMDS in dry THF at 0 °C followed by hydrolysis of the resulting olefines with 1.5 (N) HCl in THF under reflux condition furnished corresponding one-carbon homologated aldehydes **18a**. Another Wittig olefination of aldehydes **18a** with [(ethoxycarbonyl)methylene]-triphenylphosphorane in dry CH_2Cl_2 at room temparature furnished the corresponding *trans*-unsaturated esters **19a** exclusively. Next, DIBAL-H reduction of **19a** in dry toluene at 0 °C gave *trans*-allylic alcohols **20a** which were used for the subsequent SAE reaction.[12] Thus, **20a** were treated with titanium tetraisopropoxide and *tert*-butyl hydroperoxide in the presence of L-(+)-DIPT under asymmetric epoxidation conditions to get chiral epoxides **21a** in high yields. The enantiomeric excess (ee) values of **21a** were determined to be 96%.



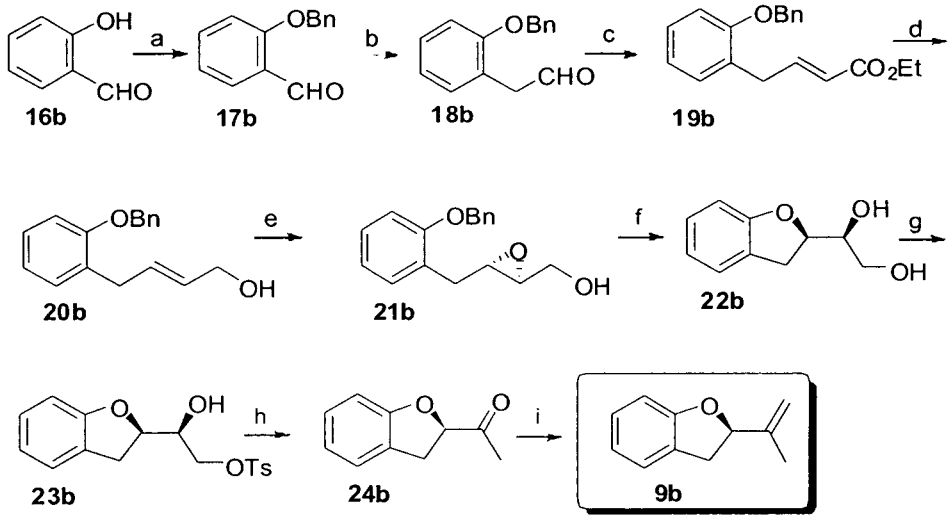
Scheme 3.1. 2. *Reagents and conditions:* (a) BnBr, anhyd. K_2CO_3, dry acetone, reflux, 4 h, 85%; (b) (i) $CH_3OCH_2Ph_3P^+Cl^-$, LHMDS, dry THF, 0 °C-rt, 3 h. (ii) 1.5 (N) HCl, THF, reflux, 3 h, 70%; based on two steps. (c) $Ph_3P{=}CHCO_2Et$, dry CH_2Cl_2, rt, overnight, 81%. (d) DIBAL-H, dry toluene, 0 °C-rt, 2 h, 95%. (e) L-(+)-DIPT, $Ti(O^iPr)_4$, TBHP, CH_2Cl_2, −25 °C, 4 Å MS, 18 h, 85%.

With **21a** in our hand, attention was then turned to their elaboration into the corresponding 2-isopropenyl-2,3-dihydrobenzofuran derivatives. Thus debenzylation of **21a** by means of 10% Pd-C catalyzed hydrogenolysis followed by treatment of the resulting phenolic derivatives with 10% NaOH solution saturated with NaCl furnished the corresponding dihydroxy compound **22a** *via* 5-*exo*-*tet* intramolecular epoxide ring opening. Next, selective tosylation of the primary hydroxyl group of **22a** gave monotosyloxy products **23a**. LAH mediated reductive removal of the tosyloxy functionality of **23a** followed by PDC oxidation of the resulting alcohols yielded 2-acetyl-2,3-dihydrobenzofuran derivatives **24a**. Finally, Wittig olefination of **24a** with methyl-triphenylphosphonium iodide and potassium *tert*-butanolate in dry THF gave 2-isopropenyl-2,3-dihydrobenzofuran derivative **9a** (Scheme 3.1.3). ^1H NMR spectrum of **9a** showed protons of the methylene group at δ 5.10 (s, 1H) and 4.91 (s, 1H). Similarly, ^{13}C NMR spectrum of **9a** showed one signal at δ 111.3 due to the presence of methylene group.The ee value of **9a** was determined to be 94% by chiral HPLC analysis.



Scheme 3.1.3. *Reagents and conditions:* (a) (i) 10% Pd-C, ethyl acetate, H_2, rt, 2 h. (ii) 10% NaOH solution saturated with NaCl, 0 °C, 3 h, 80%; based on two steps. (b) TsCl, anhyd. Et_3N, dry CH_2Cl_2, 0 °C,overnight, 75 %. (c) (i) $LiAlH_4$, dry THF, 0 °C-rt, 3 h. (ii) PDC, dry CH_2Cl_2, 0 °C-rt, 24 h, 68%; based on the last two steps. (e) $CH_3Ph_3P^+I^-$, *t*-BuOK, dry THF, 0 °C-rt, 12 h, 78%.

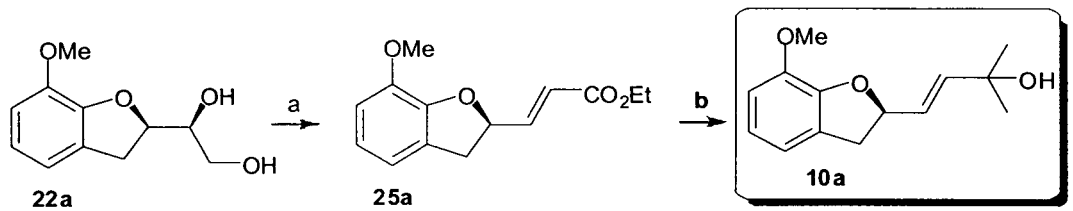
After the enantioselective synthesis of 2-isopropenyl-2,3-dihydrobenzofuran derivative **9a**, our next attention was to synthesize structurally related analogue of **9a** with no substituents in the benzene ring. Thus, commercially available 2-hydroxybenzaldehyde **16b** was converted into **9b** using the same reaction sequence as that described for the synthesis of **9a** (Scheme 3.1.4).



Scheme 3.1.4. *Reagents and conditions:* (a) BnBr, anhyd. K_2CO_3, dry acetone, reflux, 4 h, 85%. (b) (i) $CH_3OCH_2Ph_3P^+Cl^-$, LHMDS, dry THF, 0 °C-rt, 3 h. (ii) 1.5 (N) HCl, THF, reflux, 3 h, 71%; based on two steps. (c) $Ph_3P{=}CHCO_2Et$, dry CH_2Cl_2, rt, overnight, 75%. (d) DIBAL-H, dry toluene, 0 °C-rt, 2 h, 94%. (e) L-(+)-DIPT, $Ti(O^iPr)_4$, TBHP, CH_2Cl_2, −25 °C, 4 Å MS, 18 h, 88%.(f) (i) 10% Pd-C, ethyl acetate, H_2, 2 h. (ii) 10% NaOH solution saturated with NaCl, 0 °C, 3 h, 78%; based on two steps. (g) TsCl, anhyd. Et_3N, dry CH_2Cl_2, 0 °C, overnight, 77%. (h) (i) $LiAlH_4$, dry THF, 0 °C-rt, 3 h. (ii) PDC, dry CH_2Cl_2, 0 °C-rt, 24 h, 66%; over two steps. (i) $CH_3Ph_3P^+I^-$, *t*-BuOK, dry THF, 0 °C-rt, 12 h, 75%.

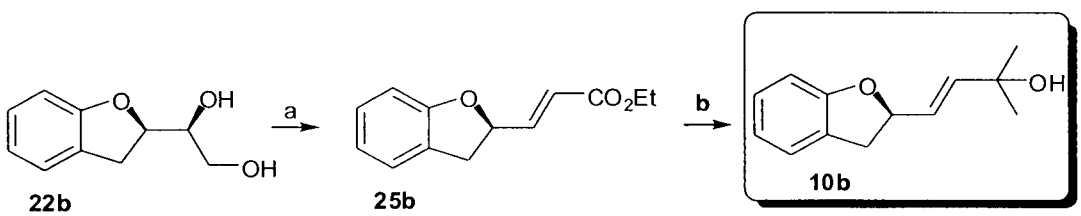
After the enantioselective synthesis of 2-isopropenyl-2,3-dihydrobenzofurans, our next attention was to synthesize 4-(2,3-dihydrobenzofuran-2-yl)-2-methylbut-3-en-2-ols. This type of structural motif is the core structure of the revised structure of helliannuol G **8a** and helliannuol H **8b** (Figure 3.1.1).[7] Towards that objective, diols **22a** were cleaved with $NaIO_4$ in MeOH-H_2O furnishing the corresponding 2-formyl-2,3-dihydrobenzofurans which on treatment with [(ethoxycarbonyl)methylene]-triphenylphosphorane in dry CH_2Cl_2 at room temparature furnished the corresponding *trans*-unasaturated esters **25a** (Scheme

3.1.5). Next, treatment of the esters **25a** with an excess of methylmagnesium iodide furnished the tertiary alcohols **10a** in very high yields. ^{1}H NMR spectrum of **10a** showed protons of the two methyl groups at δ 1.26 (s, 6H). Similarly, ^{13}C NMR spectrum of **10a** showed one signal at δ 29.6 due to the presence of two methyl groups.



Scheme 3.1.5. Reagents and conditions: (a) (i) $NaIO_4$, MeOH-H_2O, 0 °C, 2 h. (ii) $Ph_3P{=}CHCO_2Et$, dry CH_2Cl_2, rt, overnight, 85%; based on two steps. (c) MeMgI, dry ether, 0 °C-reflux, 4 h, 94%.

Similarly, diols **22b** was converted into **10b** (the corresponding analogue of **10a**) (Scheme 3.1.6) using the same reaction sequence as that described for the synthesis of **10a** (Scheme 3.1.5).



Scheme 3.1.6. Reagents and conditions: (a) (i) $NaIO_4$, MeOH-H_2O, 0 °C, 2 h. (ii) $Ph_3P{=}CHCO_2Et$, dry CH_2Cl_2, rt, overnight, 87%; based on two steps. (c) MeMgI, dry ether, 0 °C-reflux, 4 h, 93%.

3.1.4 Conclusion

In conclusion, an efficient asymmetric synthesis of 2-isopropenyl-2,3-dihydrobenzofurans and 4-(2,3-dihydrobenzofuran-2-yl)-2-methylbut-3-en-2-ols has been developed. Key steps include Sharpless asymmetric epoxidation reaction on suitable allyl alcohols and construction of the 2,3-dihydrobenzofuran nucleus by phenolate ion-mediated intramolecular 5-*exo*-*tet* epoxide ring opening reactions. The simplicity of the reaction sequence, as well as the commercial accessibility of large array of starting 2-hydroxyaromatic aldehydes, makes this process a convenient method for the preparation of "natural-product-like" 2-substituted 2,3-dihydrobenzofuran frameworks. In addition, the scope the reaction sequence is much broader, and synthesis of various substituted aromatic and heteroaromatic nuclei can be envisioned from the starting aldehydes.

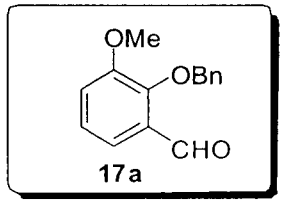
3.1.5 Experimental Section

3.1.5.1 General Remarks

All dry reactions were carried out under nitrogen in oven-dried glassware using standard gas-light syringes and septa. All reagents and solvents were dried prior to use according to the standard methods. Commercial reagents were used without further purification unless otherwise stated. Reactions were monitored on silica gel TLC plates (coated with TLC grade silica gel, obtained from Merck). Detecting agents used (for TLC) were iodine vapors and/or spraying with an aqueous solution of vanillin in 10% sulfuric acid followed by heating at 150 °C. Column chromatography was performed over silica gel (60-120 mesh, 100-200 mesh, 230-400 mesh) procured from Qualigens (India) using freshly distilled solvents. Mass spectra were recorded as electron spray ionization (ESI-MS) or fast atom bombardment spectra (FAB-MS) on a JEOL SX 102 spectrometer using argon/xenon as the FAB gas. Elemental analyses were done on Varian EL-III C H N analyzer. Melting points were determined on COMPLAB melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR RXI spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker DPX-200 (operating at 200 MHz for ^{1}H and 50 MHz for ^{13}C) or DPX-300 (operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C) spectrometer using $CDCl_3$ or DMSO-d_6 as solvent. Tetramethylsilane (0.00 ppm) served as an internal standard in ^{1}H NMR and $CDCl_3$ (77.0 ppm) in ^{13}C NMR. All spectra were recorded at 25°C. Coupling constants (*J* values) are given in hertz (Hz). Chemical shifts are expressed in parts per million. The enantiomeric excess was determined by Lichro CART Chiradex column (250x4 mm, 5 μm) using water and methanol as eluent at 25 °C. Optical rotations were measured at the sodium D-line at ambient temperature, with a Perkin Elmer 141 polarimeter.

3.1.5.2 Synthesis of Compounds

2-Benzyloxy-3-methoxybenzaldehyde (17a). To a solution of commercially available 2-hydroxy-3- methoxybenzaldehyde (6.00 g, 39.43 mmol) in dry acetone (100 mL) was added anhyd. K_2CO_3 (8.17 g, 59.14 mmol) and (bromomethyl)benzene (5.63 mL, 47.31 mmol) and the resulting mixture was then refluxed for 4 h. The mixture was then filtered through celite and the filter cake was washed with



17a

acetone (100 mL). The filtrate was concentrated and the resulting residue was redissolved in ethyl acetate (100 mL), washed with water (2x100 mL) and brine (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and then concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography (6% ethyl acetate in *n*-hexane) afforded **17a** (8.12 g, 85%) as a colorless solid. M.p. 59-60°C.

IR (KBr): 2929, 2363, 1687, 1586, 1479, 1371, 1257, 1070, 755 cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$): δ 10.22 (s, 1H), 7.40-7.32 (m, 6H), 7.16-7.12 (m, 2H), 5.17 (s, 2H), 3.93 (s, 3H).

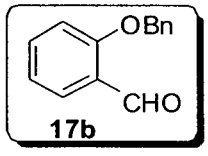
^{13}C NMR (50 MHz, $CDCl_3$): δ 190.5, 153.3, 151.3, 136.6, 130.6, 129.0, 128.9, 128.8, 124.5, 119.3, 118.3, 76.6, 56.3.

MS (ESI): *m/z* 242 $[M]^+$, 91 $[C_6H_5CH_2]^+$.

Anal. Calcd for $C_{15}H_{14}O_3$: C, 74.36; H, 5.82. Found: C, 74.43; H, 5.95.

The above physical and spectroscopic data were as consistent with as literature data.[13]

2-Benzyloxybenzaldehyde (17b): Using commercially available 2-hydroxybenzaldehyde (10 mL, 95.64 mmol), the title compound was prepared in the same manner as that described for **17a**. Purification of the crude product by silica gel column chromatography (4% ethyl acetate in hexane) afforded **17b** (17.25 g, 85%) as a colorless oil. R_f: 0.52 (20% ethyl acetate in hexane).



17b

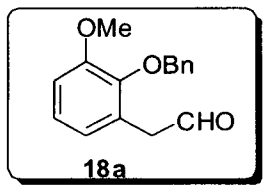
IR (Neat): 2874, 1680, 1597, 1237, 991, 755 cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$): δ 10.55 (s, 1H), 7.85 (dd, 1H, J_1 = 2.0, J_2 = 7.9), 7.52-7.36 (m, 6H), 7.06-7.02 (m, 2H), 5.17 (s, 2H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 189.5, 160.9, 135.9, 135.8, 128.6, 128.2, 128.1, 127.1, 124.9, 120.8, 112.9, 70.2.

MS (ESI): *m/z* 212 $[M]^+$, 91 $[C_6H_5CH_2]^+$. Anal. Calcd for $C_{14}H_{12}O_2$: C, 79.22; H, 5.70. Found: C, 79.37; H, 5.88. The above spectroscopic data are in consistence with the literature data.[11c]

2-[2-(benzyloxy)-3-methoxyphenyl]acetaldehyde (18a). To a stirring suspension of methoxymethyl-triphenylphosphonium chloride (16.97 g, 49.52 mmol) in dry THF (60 mL) was added a solution of LHMDS (1 M in THF, 50 mL, 50 mmol) dropwise under nitrogen atmosphere at 0 °C. The resulting red solution was stirred at this temperature for 45 min, at which point compound **17a** (6.00 g, 24.76 mmol) in THF (30 mL) was added dropwise over 15 min. The reaction mixture was allowed to stirr at room temperature for 2 h, at which point saturated aq. NH_4Cl (50 mL) was added. The resulting mixture was extracted with diethyl ether (3×50 mL) and the combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to give yellow oil. The resulting oil was loaded on a small pad of silica gel and eluted with diethyl ether to remove the baseline impurities. The crude product thus obtained was used for the next step without further purification.



18a

A solution of the above crude product in THF (60 mL) and 1.5 *N* HCl (25 mL) was refluxed for 3 h. The reaction mixture was then diluted with water (50 mL) and extracted with ether (2×100 mL). The combined organic layer was washed with aqueous $NaHCO_3$ solution and brine, and dried over $MgSO_4$. Evaporation of the solvent and purification of the residue over a silica gel column using 6% ethyl acetate in *n*-hexane as eluent furnished aldehyde **18a** as a colorless oil (4.76 g, 70%).

IR (Neat): 3019, 2360, 1722, 1588, 1476, 1270, 1215, 1082, 759 cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$): δ 9.51 (t, 1H, J = 2.0), 7.40-7.27 (m, 5H), 7.06-6.98 (m, 1H), 6.87 (dd, 1H, J_1 = 1.6, J_2 = 8.2), 6.69 (dd, 1H, J_1 = 1.4, J_2 = 8.2), 5.00 (s, 2H), 3.85 (s, 3H), 3.52 (d, 2H, J = 2.0).

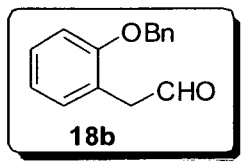
^{13}C NMR (50 MHz, $CDCl_3$): δ 199.4, 152.7, 145.9, 137.2, 128.2, 128.1, 127.8, 126.6, 124.1, 122.6, 111.8, 74.3, 55.5, 45.0.

MS (ESI): *m/z* 256 $[M]^+$.

Anal. Calcd for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29. Found: C, 74.85; H, 6.42.

(2-Benzyloxyphenyl)acetaldehyde (18b): Using 1.5 g (5.71 mmol) of **17b**, the title compound was prepared in the same manner as that described for **18a**. Purification of the residue by silica gel column chromatography (4% ethyl acetate in hexane)

afforded **18b** (2.25 g, 71%) as a colorless gum. R_f: 0.55 (20% ethyl acetate in hexane).



18b

IR (Neat): 3035, 2950, 2360, 1720, 1597, 1500, 1230, 758 cm^{-1}.

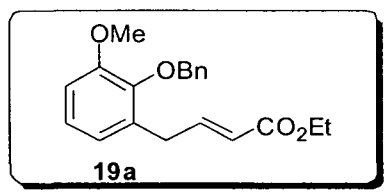
^1H NMR (300 MHz, $CDCl_3$): δ 9.81 (t, 1H, J = 1.9), 7.50-7.35 (m, 6H), 7.25-7.27 (m, 1H), 7.09-7.05 (m, 2H), 5.14 (s, 2H), 3.79 (d, 2H, J = 1.9).

^{13}C NMR (75 MHz, $CDCl_3$): δ 199.5, 156.4, 136.5, 131.1, 128.6, 128.3, 127.6, 126.9, 121.3, 120.8, 111.5, 69.6, 45.2.

MS (FAB): *m/z* 226 $[M]^{+\cdot}$.

Anal. Calcd for $C_{15}H_{14}O_2$: C, 79.62; H, 6.24. Found: C, 79.73; H, 6.33. The above physical and spectroscopic data were as consistent with as literature data.[11c]

(*E*)-ethyl 4-[2-(benzyloxy)-3-methoxyphenyl]but-2-enoate (19a). To a solution of **18a** (4.00 g, 15.6 mmol) in dry CH_2Cl_2 (40 mL) was added [(ethoxycarbonyl)methylene]-triphenylphosphorane (7.06 g, 20.28 mmol) at room temperature. The reaction mixture was stirred at room temperature for overnight. Solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (4% ethyl acetate in *n*-hexane) to afford **19a** (4.10 g, 81%) as a colorless oil.



19a

IR (KBr): 3019, 2361, 1709, 1475, 1271, 1216, 1082, 756, 669 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ 7.43-7.27 (m, 5H), 7.04-6.95 (m, 2H), 6.83 (dd, 1H, J_1 = 1.2, J_2 = 8.1), 6.71 (dd, 1H, J_1 = 1.2, J_2 = 7.6), 5.70 (td, 1H, J_1 = 1.5, J_2 = 15.6), 4.99 (s, 2H), 4.13 (q, 2H, J = 7.1), 3.91 (s, 3H), 3.43 (dd, 2H, J_1 = 1.4, J_2 = 6.6), 1.23 (t, 2H, J = 7.1).

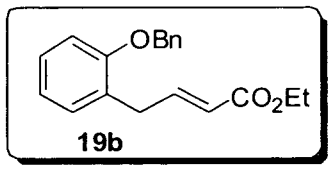
^{13}C NMR (75 MHz, $CDCl_3$): δ 166.4, 152.8, 147.2, 145.7, 137.6, 131.8, 128.3, 128.0, 127.8, 124.0, 122.0, 111.0, 74.6, 60.0, 55.6, 32.5, 14.1.

MS (FAB): *m/z* 327 $[M+1]^+$, 281 $[M\text{-}OEt]^+$.

Anal. Calcd for $C_{20}H_{22}O_4$: C, 73.60; H, 6.79. Found: C, 73.67; H, 6.92.

(2*E*)-4-(2-Benzyloxyphenyl)but-2-enoic Acid Ethyl Ester (19b): Starting from 2.0 g (8.83 mmol) of **18b**, the title compound was prepared in the same manner as that described for **19a**. Purification of the crude product by silica gel column

chromatography (6% ethyl acetate in hexane) afforded **19b** (1.96 g, 75%) as a colorless gum. R_f: 0.58 (20% ethyl acetate in hexane).



19b

IR (Neat): 2368, 1720, 1502, 1253, 1045, 750 cm^{-1}.

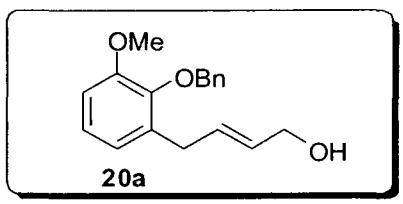
^1H NMR (300 MHz, $CDCl_3$): δ 7.40-7.26 (m, 6H), 7.20-7.07 (m, 2H), 6.92-6.87 (m, 2H), 5.77 (d, 1H, J = 15.5), 5.03 (s, 2H), 4.14 (q, 2H, J = 7.1), 3.55-3.53 (m, 2H), 1.24 (t, 3H, J = 7.1).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.51, 156.32, 147.10, 136.98, 130.20, 128.42, 127.91, 127.75, 127.14, 126.54, 121.88, 120.83, 111.72, 69.86, 59.98, 32.97, 14.16.

MS (ESI): *m/z* 319 $[M+Na]^+$.

Anal. Calcd for $C_{19}H_{20}O_3$: C, 77.00; H, 6.80. Found: C, 77.09; H, 6.65. The above physical and spectroscopic data were as consistent with as literature data.[11c]

***(E)*-4-[2-(benzyloxy)-3-methoxyphenyl]but-2-en-1-ol (20a).** To an ice-cooled stirred solution of **19a** (3.50 g, 10.72 mmol) in dry toluene (30 mL) was added DIBAL-H (1 M in toluene, 27 mL, 27 mmol)) dropwise under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 2 h, cooled to 0 °C, carefully quenched with methanol (5 mL) and a saturated aq. sodium potassium tartarate solution (25 mL). The resulting mixture was vigorously stirred for 45 min. at rt and then extracted with ethyl acetate (2x50 mL), washed with water (50 mL) and brine (100 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and then concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography (20% ethyl acetate in *n*-hexane) afforded **20a** (2.90 g, 95%) as a colorless gum.



20a

IR (KBr): 3421, 3017, 2361, 1638, 1474, 1272, 1216, 1081, 759 cm^{-1}.

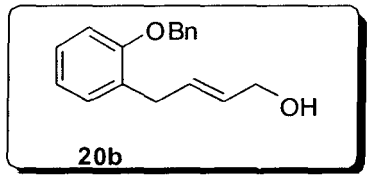
^1H NMR (300 MHz, $CDCl_3$): 7.42-7.28 (m, 5H), 7.01-6.93 (m, 1H), 6.81-6.68 (m, 2H), 5.74-5.65 (m, 1H), 5.59-5.50 (m, 1H), 4.97 (s, 2H), 3.99 (d, 2H, J = 5.6), 3.86 (s, 3H), 3.29 (d, 2H, J = 6.3).

^{13}C NMR (75 MHz, $CDCl_3$+CCl_4): δ 152.9, 145.9, 138.1, 134.2, 131.2, 130.2, 128.5, 128.3, 128.0, 127.8, 123.9, 121.2, 110.8, 74.6, 63.5, 55.8, 32.8.

MS (ESI): *m/z* 302 $[M+NH_4]^+$, 267 $[M-OH]^+$.

Anal. Calcd for $C_{18}H_{20}O_3$: C, 76.03; H, 7.09. Found: C, 76.15; H, 7.21.

(*E*)-4-[2-(benzyloxy)phenyl]but-2-en-1-ol (20b). Using 3.50 g (11.8 mmol) of **19b**, the title compound was prepared in the same manner as that described for **20a**.



20b

Purification of the crude product by silica gel column chromatography (18% ethyl acetate in *n*-hexane) afforded **20b** (2.82 g, 94%) as a colorless gum.

IR (Neat): 3385, 3032, 2921, 2863, 1597, 1493, 1452, 1240, 1010, 974, 750, 697 cm^{-1}.

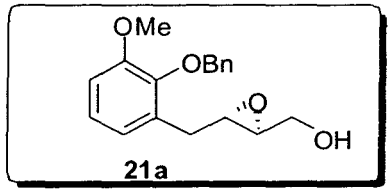
^1H NMR (200 MHz, $CDCl_3$): δ 7.44-7.33 (m, 5H), 7.17-7.13 (m, 2H), 6.94-6.87 (m, 2H), 5.84-5.80 (m, 1H), 5.72-5.67 (m, 1H) ,5.07 (s, 2H), 4.07 (d, 2H, J = 5.6), 3.43 (d, 2H, J = 6.6).

^{13}C NMR (75 MHz, $CDCl_3$): δ 156.3, 137.2, 131.1, 129.94, 129.91, 128.8, 128.4, 127.7, 127.4, 127.1, 120.8, 111.7, 69.8, 63.6, 33.0.

MS (ESI): *m/z* 237 $[M\text{-}OH]^+$, 272 $[M+NH_4]^+$.

Anal. Calcd for $C_{17}H_{18}O_2$: C, 80.28; H, 7.13. Found: C, 80.21; H, 7.26.

[(2*S*,3*S*)-3-(2-(benzyloxy)-3-methoxybenzyl]oxiran-2-yl)methanol (21a). To a cooled (−25 °C) suspension of activated and powdered 4 Å MS (2.00 g) in dry CH_2Cl_2 (30 mL) were added L-(+)-DIPT (1.80 mL, 10.55 mmol) and $Ti(O^iPr)_4$ (2.87 mL, 9.67 mmol). The resulting mixture was then stirred for 20 min at the same temperature and then TBHP (5.6 M in n-decane, 4.7 mL, 26.37 mmol) was added dropwise. After 20 min, a solution of **20a** (2.50 g, 8.79 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise over 15 min. The resulting mixture was kept at -25 °C freeze for 18 h. The reaction mixture was allowed to warm to 0 °C and poured into a freshly prepared and cooled (0 °C) solution of ferrous sulfate and tartaric acid (2.50 g and 1.00 g, respectively) in deionised water (20 mL). The two-phase mixture was stirred for 30 min, aqueous phase separated and extracted with CH_2Cl_2. The combined organic phases were treated with a pre-cooled (0 °C) solution of 30% NaOH (20 mL) in saturated brine. The two-phase mixture was then stirred for 1 h at room temperature and the aqueous layer separated. It was extracted with CH_2Cl_2 (2x30 mL), washed with brine (30 mL), and the combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude product by silica gel column



21a

chromatography (30% ethyl acetate in n-hexane) afforded **21a** (2.24 g, 85%) as a colorless oil. $[\alpha]^{25}_{D}$: -12.37 (*c* 2.6, $CHCl_3$).

IR (Neat): 3020, 2360, 1730, 1474, 1216, 760, 670 cm^{-1}.

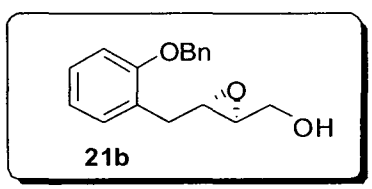
^1H NMR (300 MHz, $CDCl_3$): δ 7.46-7.32 (m, 5H), 7.04-6.99 (m, 1H), 6.87-6.82 (m, 2H), 5.10-4.9 (m, 2H), 3.90 (s, 3H), 3.77 (d,1H, J = 12.5), 3.50 (d, 1H, J = 11.4), 3.07- 3.03 (m, 1H), 2.92-2.76 (m, 3H), 2.17 (s, 1H).

^{13}C NMR (75 MHz, $CDCl_3$+CCl_4): δ 152.7, 146.0, 137.8, 131.2, 128.3, 127.9, 124.0, 122.4, 111.2, 74.6, 61.6, 58.5, 55.7, 55.5, 32.2.

MS (ESI): *m/z* 301 $[M+1]^+$, 318 $[M+NH_4]^+$.

Anal. Calcd for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71. Found: C, 72.17; H, 6.68.

[(2*S*,3*S*)-3-(2-(benzyloxy)benzyl)oxiran-2-yl]methanol (21b). Using 2.50 g (9.83 mmol) of **20b**, the title compound was prepared in the same manner as that described for **21a**. Purification of the crude product by silica gel column chromatography (30% ethyl acetate in *n*-hexane) afforded **21b** (2.34 g, 88%) as a colorless oil. $[\alpha]^{25}_{D}$: -1.7 (*c* 2.2, $CHCl_3$).



21b

IR (Neat): 3434, 3019, 2926, 1495, 1216, 1020, 760, 669 cm^{-1}.

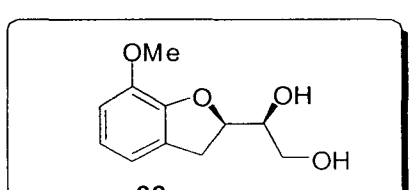
^1H NMR (200 MHz, $CDCl_3$): δ 7.43-7.30 (m, 5H), 7.24-7.17 (m, 2H), 6.94-6.87 (m, 2H), 5.05 (s, 2H), 3.85-3.74 (m, 1H), 3.59-3.48 (m, 1H), 3.22-3.17 (m, 1H), 2.99-2.89 (m, 3 H), 2.15 (s, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 156.7, 137.1, 130.7, 128.67, 128.62, 128.1, 128.0, 127.4, 127.3, 125.7, 120.9, 111.7, 70.0, 61.6, 58.7, 55.2, 32.6.

MS (ESI): *m/z* 271 $[M+1]^+$, 288 $[M+NH_4]^+$.

Anal. Calcd for $C_{17}H_{18}O_3$: C, 75.53; H, 6.71. Found: C, 75.47; H, 6.88.

(*R*)-1-[(*S*)-7-methoxy-2,3-dihydrobenzofuran-2-yl]ethane-1,2-diol (22a). To a stirred solution of **21a** (2.00 g, 6.65 mmol) in ethyl acetate (30 mL) was added 10% Pd-C (250 mg). After stirring for 2 h at room temperature under pressure of a hydrogen balloon, the reaction mixture was filtered through a pad of Celite® and the filtrate was concentrated under



22a

reduced pressure to get the corresponding debenzylated product as a colorless solid which was used for the next step without further purification.

To an ice-cooled stirred solution of the above debenzylated product in CH_2Cl_2 (20 mL), was added a pre-cooled (0 °C) solution of 30% NaOH (20 mL) in saturated brine. The two-phase mixture was then stirred for 3 h at room temperature and the aqueous layer separated. It was extracted with CH_2Cl_2 (2x30 mL), washed with brine (30 mL), and the combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography (35% ethyl acetate in *n*-hexane) afforded **22a** (1.12 g, 80%) as a colorless crystalline solid. M.p.: 102-103 0°C. $[\alpha]^{25}_D$: -7.4 (*c* 1.07, MeOH).

IR (KBr): 3408, 3020, 2360, 1710,1215, 761, 670 cm^{-1}.

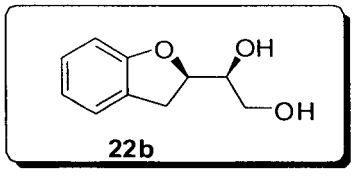
^1H NMR (300 MHz, $CDCl_3$): δ 6.84-6.80 (m, 2H), 6.75-6.70 (m, 1H), 4.84-4.76 (m, 1H), 4.04-3.99 (m, 1H), 3.84 (s, 3H), 3.79-3.68 (m, 2H), 3.33-3.12 (m, 2H), 3.00 (s, 1H), 2.63 (s, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 147.2, 144.2, 127.8, 121.3, 117.2, 110.8, 83.6, 72.7, 63.1, 55.8, 31.2.

MS (ESI): *m/z* 211 $[M+1]^+$, 228 $[M+NH_4]^+$.

Anal. Calcd for $C_{11}H_{14}O_4$: C, 62.85; H, 6.71. Found: C, 62.95; H, 6.55.

***(R)*-1-[(*S*)-2,3-dihydrobenzofuran-2-yl]ethane-1,2-diol (22b).** Using 2.00 g (7.39 mmol) of **21b**, the title compound was prepared in the same manner as that described for **22a**. Purification of the crude product by silica gel column chromatography (35% ethyl acetate in *n*-hexane) afforded **22b** (1.04 g, 78%) as a colorless crystalline solid. M.p.: 55-56 0°C. $[\alpha]^{25}_D$ -6.68 (*c* 0.49, MeOH).



22b

IR (KBr): 3430, 3021, 2924, 1216, 1020, 760, 669 cm^{-1}.

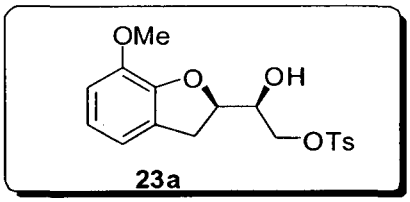
^1H NMR (200 MHz, $CDCl_3$): δ 7.26-7.07 (m, 2H), 6.89-6.75 (m, 2H), 4.83-4.72 (m, 1H), 3.99-3.92 (m, 1H), 3.88-3.79 (m, 2H), 3.25 (d, 2H, J = 8.6), 2.38 (s, br, 1H), 2.04 (s, br, 1H).

^{13}C NMR (75 MHz, $CDCl_3$+CCl_4): δ 159.1, 128.0, 126.4, 125.1, 120.8, 109.3, 82.7, 73.0, 63.3, 31.3.

MS (ESI): *m/z* 181 $[M+1]^+$, 198 $[M+NH_4]^+$.

Anal. Calcd for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.79; H, 6.51.

***(R)*-2-hydroxy-2-[(*S*)-7-methoxy-2,3-dihydrobenzofuran-2-yl]ethyl 4-methylbenzenesulfonate (23a).** To a solution of diol **22a** (1.00 g, 4.75 mmol) in dry CH_2Cl_2 (15 mL) at 0 °C was added dry triethyl amine (1.16 mL, 8.31 mmol) followed by tosyl chloride (0.91 g, 4.75 mmol) and kept in the refrigerator for overnight. The reaction mixture was diluted with H_2O, and extracted with CH_2Cl_2 (2x30 mL). The combined organic layers were washed with water (25 mL), brine (25 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and purification of the crude product by silica gel column chromatography (20% ethyl acetate in *n*-hexane) afforded **23a** (1.30 g, 75%) as a colorless solid. M.p.: 108-110 °C. $[\alpha]^{25}_D$: -1.9 (*c* 2.58, MeOH).



23a

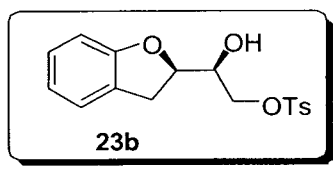
IR (KBr): 3493, 2363, 1593, 1490, 1192, 1088, 809 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ 7.79 (d, 2H, J = 8.4), 7.34 (d, 2H, J = 8.1), 6.84-6.70 (m, 3H), 4.86-4.69 (m, 1H), 4.30-3.95 (m, 3H), 3.81 (s, 3H), 3.30-3.14 (m, 2H), 2.71-2.65 (m, 1H), 2.44 (s, 3H).

MS (ESI): *m/z* 382 $[M+NH_4]^+$, 387 $[M+Na]^+$.

Anal. Calcd for $C_{18}H_{20}O_6S$: C, 59.33; H, 5.53. Found: C, 59.42; H, 5.71.

***(R)*-2-[(*S*)-2,3-dihydrobenzofuran-2-yl]-2-hydroxyethyl4-methylbenzenesulfonate (23b).** Using 1.00 g (5.55 mmol) of **22b**, the title compound was prepared in the same manner as that described for **23a**. Purification of the crude product by silica gel column chromatography (20% ethyl acetate in *n*-hexane) afforded **23b** (1.43 g, 77%) as a colorless semi-solid. $[\alpha]^{25}_D$: -7.66 (*c* 0.95, EtOH).



23b

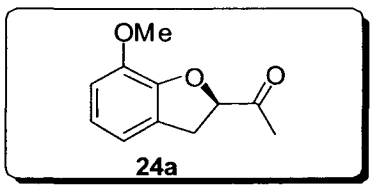
IR (KBr): 3429, 2921, 1635, 1474, 1357, 1175, 757 cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$): δ 7.81 (d, 2H, J = 8.3), 7.35 (d, 2H, J = 8.1), 7.17-7.05 (m, 2H), 6.88-6.80 (m, 1H), 6.70 (d, 2H, J = 7.9), 4.70-4.62 (m, 1H), 4.33-4.26 (m, 1H), 4.19-4.11 (m, 1H), 3.97-3.95 (m, 1H), 3.22 (d, 2H, J = 8.2), 2.45 (s, 3H).

MS (ESI): *m/z* 352 $[M+NH_4]^+$, 357 $[M+Na]^+$.

Anal. Calcd for $C_{17}H_{18}O_5S$: C, 61.06; H, 5.43. Found: C, 61.23; H, 5.28.

(*R*)-1-(7-methoxy-2,3-dihydrobenzofuran-2-yl)ethanone (24a). To an ice-cooled suspension of $LiAlH_4$ (0.26 g, 6.85 mmol) in dry THF (10 mL) was added a solution of **23a** (1.00 g, 2.74 mmol) in dry THF (15 mL) dropwise and the resulting mixture was stirred for 3h at rt. It was quenched by dropwise addition of ethyl acetate (5 mL) at 0°C and water (20 mL). The mixture was extracted with ethyl acetate (2x30 mL), washed with brine (25 mL), The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and then concentrated under reduced pressure to obtain a colorless gum which was used for the next step without further purification.



24a

To an ice-cooled stirring solution of the above crude product in dry CH_2Cl_2 (30 mL), was added PDC (2.78 g, 7.38 mmol). The mixture was then stirred for 24 h at room temperature. After evaporating dichloromethane the reaction mixture was diluted with diethyl ether (50 mL) and filtered through a small pad of silica gel. Concentration of the filtrate and purification of the residue by silica gel column chromatography (10% ethyl acetate in *n*-hexane) afforded **24a** (0.37 g, 68%) as a colorless semi-solid. $[\alpha]^{25}_D$: +4.7 (*c* 1.0, $CHCl_3$).

IR (KBr): 3021, 2360, 1719, 1493, 1289, 1215, 1086, 760, 670 cm^{-1}.

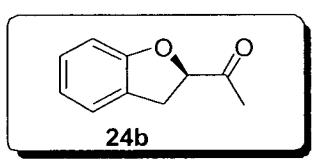
^1H NMR (200 MHz, $CDCl_3$): δ 6.91-6.75 (m, 3H), 5.13-5.04 (m, 1H), 3.90 (s, 3H), 3.57-3.26 (m, 2H), 2.33 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$+CCl_4): δ 208.5, 147.1, 144.5, 126.3, 121.9, 116.9, 111.5, 86.1, 56.0, 33.1, 26.0.

MS (ESI): *m/z* 210 $[M+NH_4]^+$.

Anal. Calcd for $C_{11}H_{12}O_3$: C, 68.74; H, 6.29. Found: C, 68.85; H, 6.37.

(*S*)-1-(2,3-dihydrobenzofuran-2-yl)ethanone (24b). Using 1.00 g (2.99 mmol) of **23b**, the title compound was prepared in the same manner as that described for **24a**. Purification of the crude product by silica gel column chromatography (10% ethyl acetate in *n*-hexane) afforded **24b** (0.35 g, 66%) as a light yellow gum. $[\alpha]^{25}_D$: +3.7 (*c* 3.2, $CHCl_3$).



24b

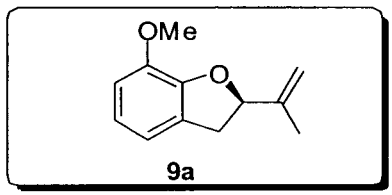
IR (KBr): 3021, 2927, 2361, 1729, 1217, 1045, 761, 670 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.20-7.12 (m, 2H), 6.93-6.86 (m, 2H), 5.08-4.99 (m, 1H), 3.48-3.31 (m, 2H), 2.29 (s, 3H).

MS (ESI): *m/z* 180 $[M+NH_4]^+$.

Anal. Calcd for $C_{10}H_{10}O_2$: C, 74.06; H, 6.21. Found: C, 74.22; H, 6.31.

(*R*)-7-methoxy-2-(prop-1-en-2-yl)-2,3-dihydrobenzofuran (9a). To a suspension of methyltriphenylphosphonium iodide (1.88 g, 4.65 mmol) in dry THF (15 mL) under a nitrogen atmosphere at 0 °C was added *t*-BuOK (0.52 g, 4.65 mmol). The reaction mixture was stirred for 15 min at 0 °C and was then warmed to rt while stirring was continued for 45 min. The solution was then recooled to 0 °C, and a solution of ketone **20a** (0.30 g, 1.55 mmol) in dry THF (10 mL) was added dropwise. The reaction mixture was then warmed to rt and stirred for an additional 12 h. The reaction was quenched with saturated aq. NH_4Cl solution (10 mL) and extracted with Et_2O (3×10 mL). The combined organic extracts were washed with brine (10 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (4% ethyl acetate in *n*-hexane) to give alkene **9a** (0.23 g, 78%) as a colorless oil. $[\alpha]^{25}_D$: +12.9 (c 0.79, EtOH).



9a

IR (Neat): 3020, 2928, 2361, 1490, 1287, 1216, 1087, 761, 669 cm^{-1}.

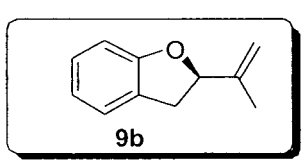
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.83-6.72 (m, 3H), 5.29-5.19 (m, 1H), 5.10 (s, 1H), 4.91 (s, 1H), 3.87 (s, 3H), 3.37-3.29 (m, 1H), 3.12-3.03 (m, 1H), 1.78 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 148.1, 144.3, 143.6, 127.8, 120.8, 117.0, 112.4, 111.3, 86.5, 56.0, 35.1, 17.1.

MS (ESI): *m/z* 190 $[M]^+$, 208 $[M+NH_4]^+$.

Anal. Calcd for $C_{12}H_{14}O_2$: C, 75.76; H, 7.42. Found: C, 75.83; H, 7.31.

(*R*)-2-(prop-1-en-2-yl)-2,3-dihydrobenzofuran (9b). Using 0.30 g (1.85 mmol) of **24b**, the title compound was prepared in the same manner as that described for **9a**. Purification of the crude product by silica gel column chromatography (4% ethyl acetate in *n*-hexane) afforded **9b** (0.22 g, 75%) as a colorless oil.[14] $[\alpha]^{25}_D$: +10.6 (*c* 1.0, EtOH), (literature[15] $[\alpha]^{25}_D$: +10.9, EtOH).



9b

IR (KBr): 3020, 2927, 2360, 1216, 1045, 761, 670 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ 7.16-7.08 (m, 2H), 6.85-6.78 (m, 2H), 5.16 (1H, t, J = 8.9), 5.09 (1H, s), 4.91 (1H, s), 3.37-3.29 (m, 2H), 3.08-3.00 (m, 2H), 1.77 (s, 3H).

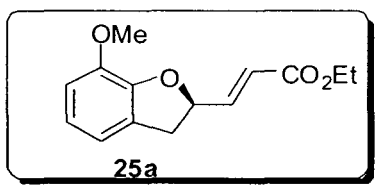
^{13}C NMR (75 MHz, $CDCl_3$): δ 159.7, 144.0, 128.0, 126.5, 124.7, 120.3, 111.9, 109.2, 85.6, 34.7, 17.2.

MS (ESI): *m/z* 160 $[M]^+$, 178 $[M+NH_4]^+$.

Anal. Calcd for $C_{10}H_{10}O_2$: C, 74.06; H, 6.21. Found: C, 74.22; H, 6.31.

The above physical and spectroscopic data were as consistent with as literature data.[10]

(*R*,*E*)-ethyl 3-(7-methoxy-2,3-dihydrobenzofuran-2-yl)acrylate (25a). To a stirred solution of diol **22a** (100 mg, 0.47 mmol) in methanol (5 mL) at 0°C was added a solution of $NaIO_4$ (152 mg, 0.71 mmol) in water (2 mL). After stirring the reaction mixture at 0°C for 2 h, methanol was evaporated in vacuo at low temperature. The aqueous solution was extracted with CH_2Cl_2 (2x10 mL). The combined organic layers were washed with water (10 mL), brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield the crude aldehyde as a colorless gum which was used for the next step without further purification.



25a

To a solution of the above crude aldehyde in dry CH_2Cl_2 (5mL) was added [(ethoxycarbonyl)methylene]-triphenylphosphorane (235 mg, 0.67 mmol) at room temperature. The reaction mixture was stirred at room temperature for overnight. Solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (4% ethyl acetate in *n*-hexane) to afford **25a** (99 mg, 85%) as a colorless gum. $[\alpha]^{25}_D$: +18.08 (*c* 0.62, MeOH).

IR (Neat): 3020, 2935, 2361, 1715, 1492, 1273, 1216, 1087, 759 cm^{-1}.

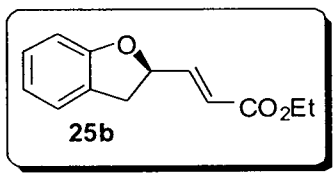
^1H NMR (300 MHz, $CDCl_3$): δ 6.94 (dd, 1H, J_1 = 5.2, J_2 = 15.6), 6.75-6.66 (m, 3H), 6.06 (dd, 1H, J_1 = 1.4, J_2 = 15.6), 5.36-5.28 (m, 1H), 4.12 (q, J = 7.1), 3.81 (s, 3H), 3.44-3.36 (m, 1H), 3.03-2.95 (m, 1H), 1.22 (t, J = 7.1).

^{13}C NMR (75 MHz, $CDCl_3$): δ 165.7, 147.3, 145.3, 144.5, 126.5, 121.5, 121.4, 117.0, 111.7, 81.0, 60.3, 55.8, 35.9, 14.1.

MS (ESI): *m/z* 266 $[M+NH_4]^+$.

Anal. Calcd for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50. Found: C, 67.85; H, 6.63.

(*R*,*E*)-ethyl 3-(2,3-dihydrobenzofuran-2-yl)acrylate (25b). Using 100 mg (0.55 mmol) of **22b**, the title compound was prepared in the same manner as that described for **25a**. Purification of the crude product by silica gel column chromatography (4% ethyl acetate in *n*-hexane) afforded **25b** (104 mg, 87%) as a colorless gum. $[\alpha]^{25}_D$: +18.08 (*c* 1.46, MeOH).



25b

IR (Neat): 3021, 2929, 2361, 1726, 1475, 1216, 1087, 761 cm^{-1}.

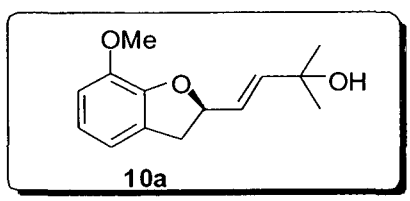
^1H NMR (300 MHz, $CDCl_3$): δ 7.15-7.10 (m, 2H), 7.01 (dd, 1H, J_1 = 5.0, J_2 = 15.6), 6.88-6.80 (m, 2H), 6.12 (dd, 1H, J_1 = 0.8, J_2 = 15.6), 5.38-5.31 (m, 1H), 4.20 (q, J = 7.1), 3.51-3.43 (m, 1H), 3.08-3.00 (m, 1H), 1.31 (t, J = 7.1).

^{13}C NMR (75 MHz, $CDCl_3$): δ 165.7, 159.1, 145.7, 128.3, 125.2, 124.8, 121.4, 120.8, 109.6, 80.4, 60.4, 35.6, 14.2.

MS (ESI): *m/z* 236 $[M+NH_4]^+$.

Anal. Calcd for $C_{13}H_{14}O_3$: C, 71.54; H, 6.47. Found: C, 71.68; H, 6.37.

(*R*,*E*)-4-(7-methoxy-2,3-dihydrobenzofuran-2-yl)-2-methylbut-3-en-2-ol (10a). To a freshly prepared, magnetically stirred, ice-cold suspension of methylmagnesium iodide [prepared from iodomethane (0.28 mL, 3.5 mmol) and magnesium (0.085 g, 3.5 mmol) in 10 mL of dry diethyl ether] was added a solution of ester **25a** (75 mg, 0.30 mmol) in dry THF (5 mL). The reaction mixture was refluxed for 4 h, cooled, and quenched with aqueous NH_4Cl solution (10 mL). The mixture was extracted with ethyl acetate (2x25 mL), washed with water (25 mL) and brine (25 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography (12% ethyl acetate in *n*-hexane) afforded **10a** (0.07 mg, 94%) as a colorless gum. $[\alpha]^{25}_D$: -2.08 (*c* 0.75, MeOH).



10a

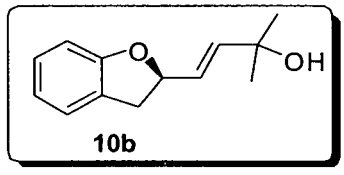
IR (Neat): 3021, 2361, 1593, 1216, 763 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ 6.77-6.66 (m, 3H), 5.93-5.75 (m, 2H), 5.21-5.13 (m, 1H), 3.79 (s, 3H), 3.33-3.25 (m, 1H), 2.99-2.9 (m, 1H), 1.26 (s, 6H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 147.6, 144.4, 141.2, 127.8, 125.8, 121.0, 117.0, 111.0, 83.9, 70.5, 55.8, 36.6, 29.6. MS (ESI): *m/z* 217 $[M\text{-}OH]^+$, 233 $[M\text{-}1]^+$.

Anal. Calcd for $C_{14}H_{18}O_3$: C, 71.77; H, 7.74. Found C, 71.90; H, 7.67.

***(R,E)*-4-(2,3-dihydrobenzofuran-2-yl)-2-methylbut-3-en-2-ol (10b).** Using 75 mg (0.34 mmol) of **25b**, the title compound was prepared in the same manner as that described for **10a**. Purification of the crude product by silica gel column chromatography (12% ethyl acetate in *n*-hexane) afforded **10b** (65 mg, 93%) as a light yellow gum. $[\alpha]^{25}_D$: -7.72 (*c* 0.6, MeOH).



10b

IR (Neat): 3430, 3019, 2361, 1729, 1478, 1219, 761 cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.15-7.08 (m, 2H), 6.86-6.76 (m, 2H), 6.01-5.83 (m, 2H), 5.23-5.15 (m, 1H), 3.41-3.33 (m, 1H), 3.04-2.96 (m, 1H).

^{13}C NMR (75 MHz, $CDCl_3$+CCl_4): δ 159.3, 140.9, 128.1, 126.4, 126.2, 124.8, 120.5, 109.4, 82.9, 70.5, 36.3, 29.8.

MS (ESI): *m/z* 287 $[M\text{-}OH]^+$, 203 $[M\text{-}1]^+$.

Anal. Calcd for $C_{13}H_{16}O_2$: C, 76.44; H, 7.90. Found: C, 76.63; H, 7.99.

3.1.6 References

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3.1.7 Spectra of Selected Compounds

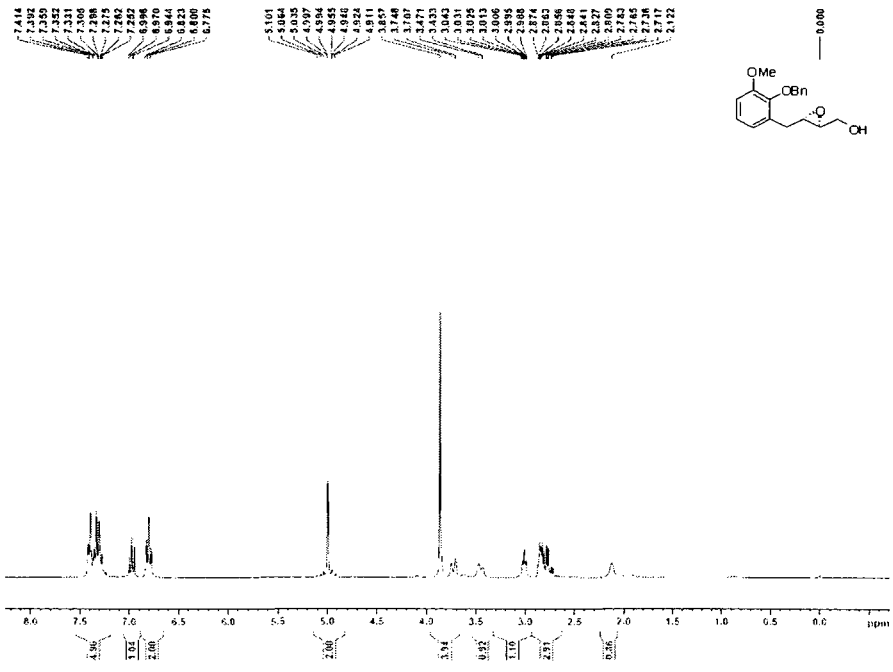


Figure 3.1.3: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **21a.**

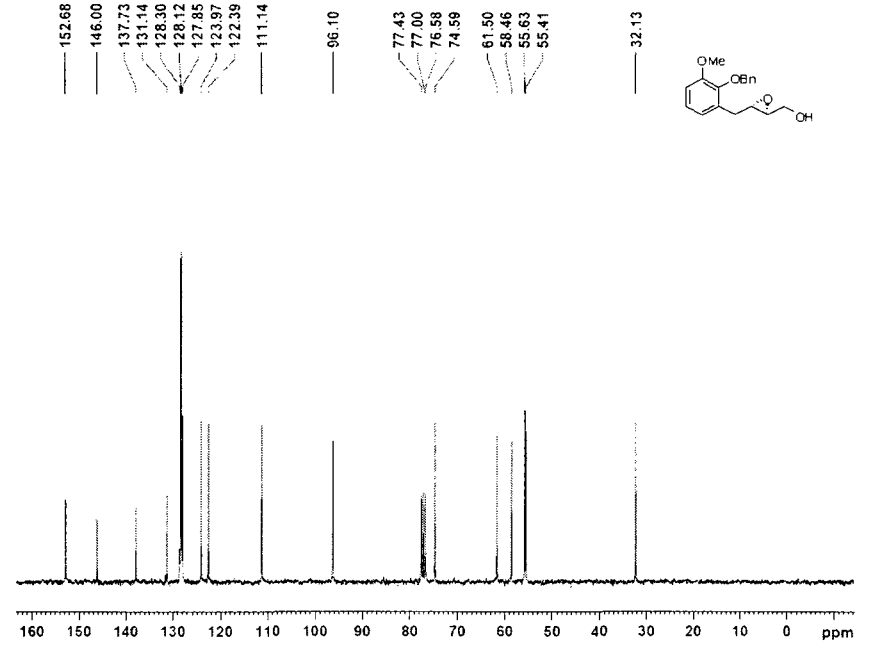


Figure 3.1.4: ^{13}C NMR ($CDCl_3$+CCl_4, 75 MHz) spectrum of **21a.**

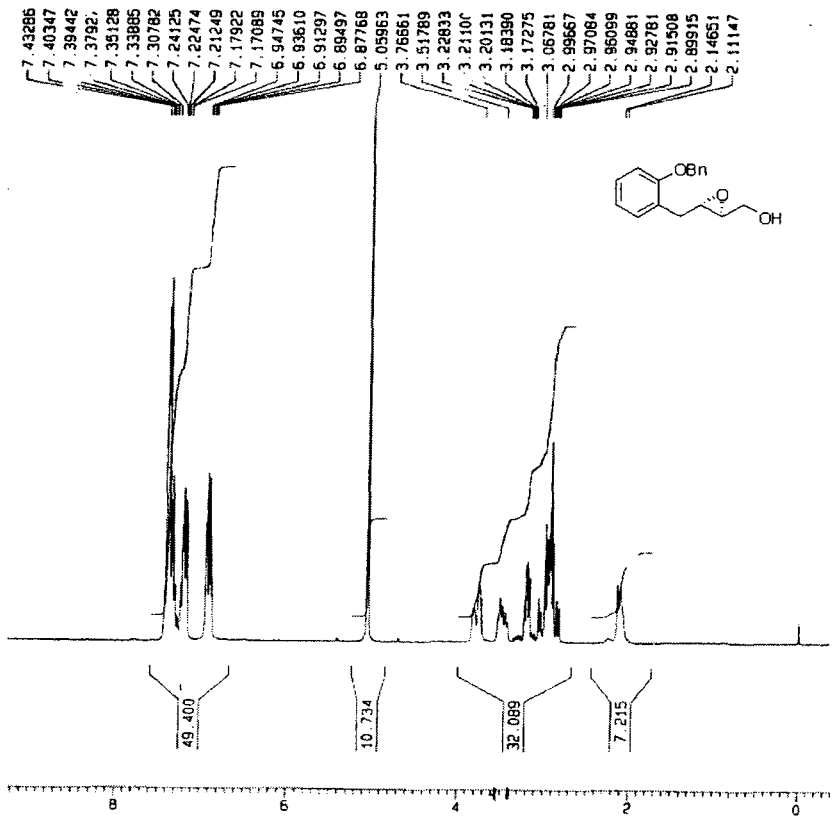


Figure 3.1.5: ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of **21b.**

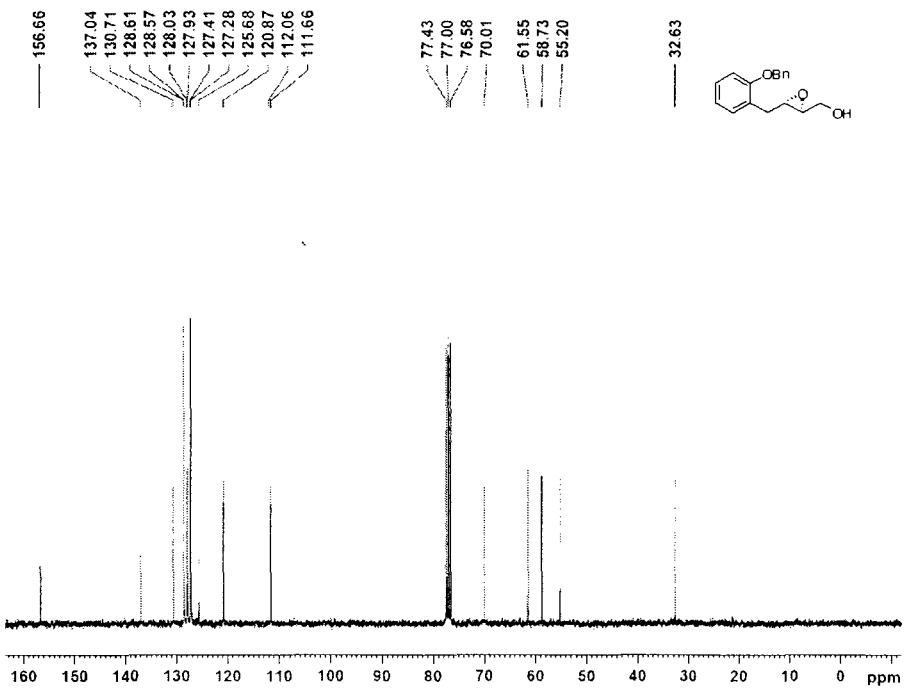


Figure 3.1.6: ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **21b.**

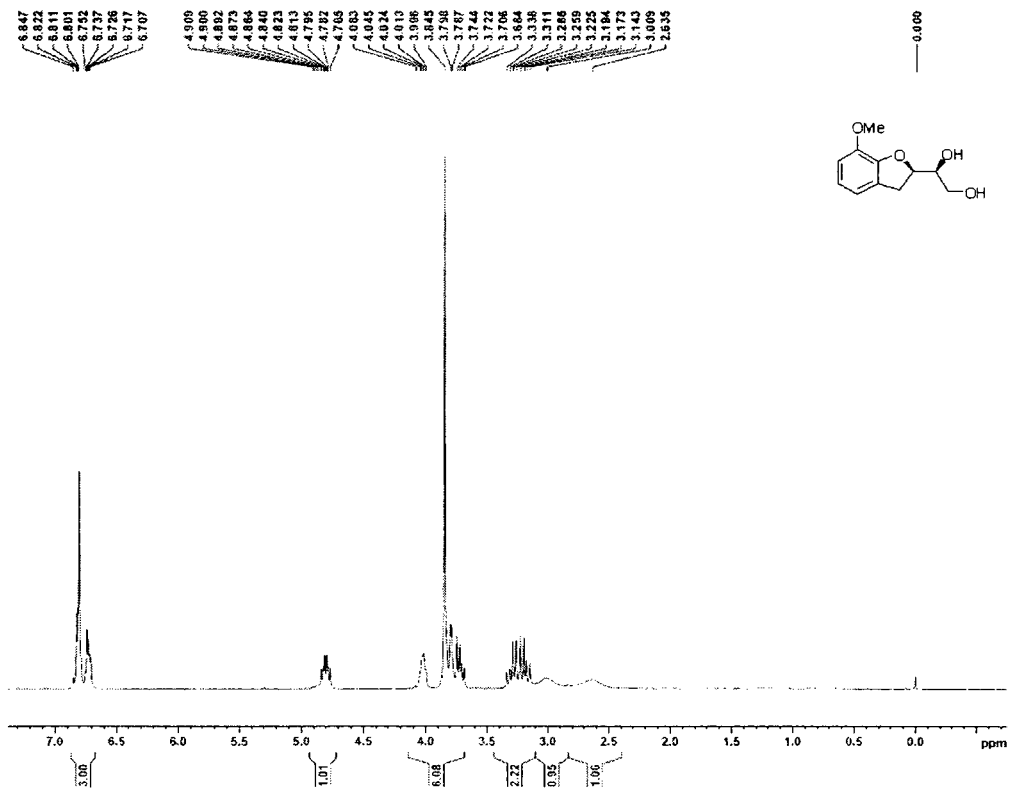


Figure 3.1.7: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **22a.**

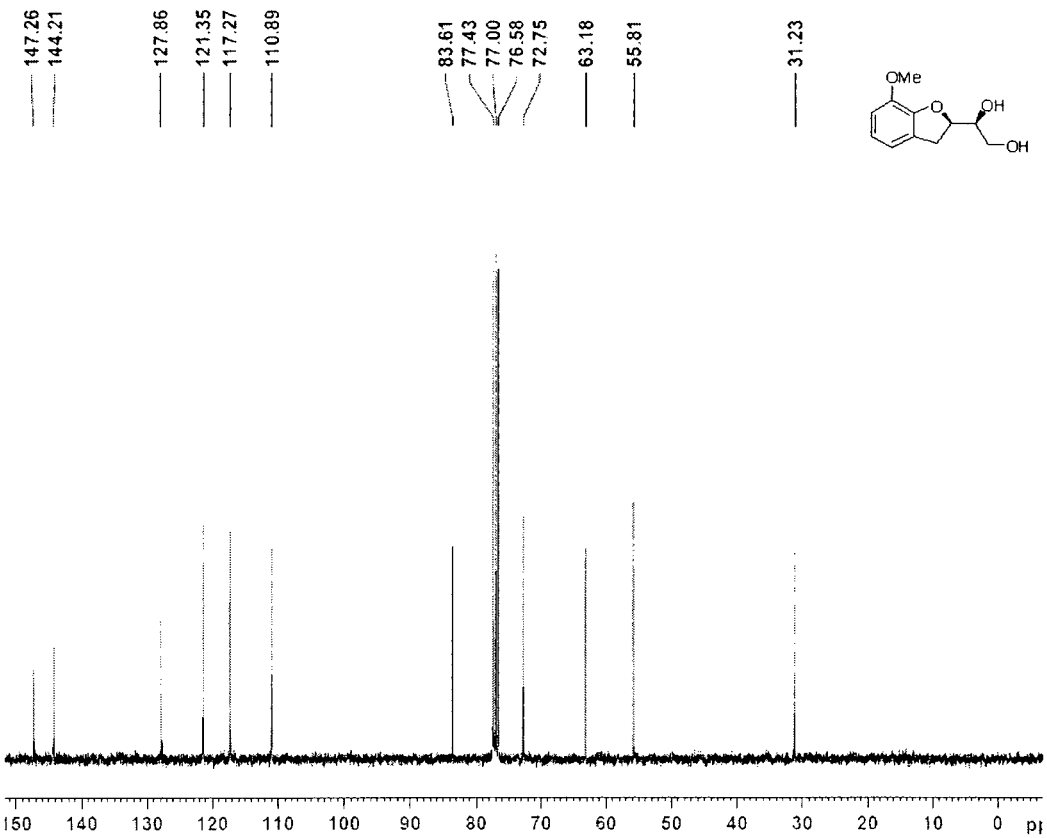


Figure 3.1.8: ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **22a.**

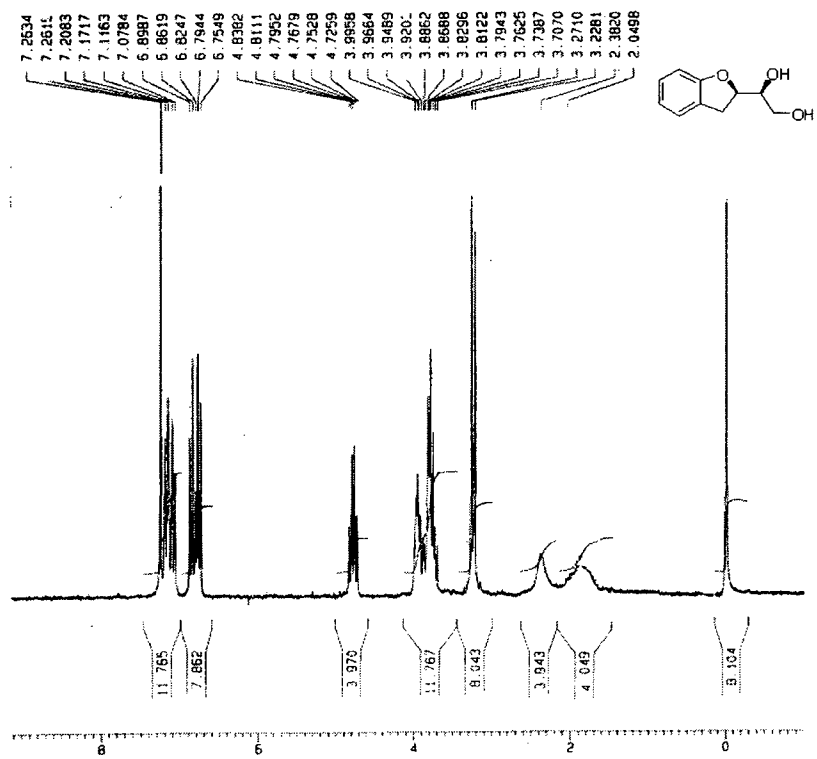


Figure 3.1.9: ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of **22b.**

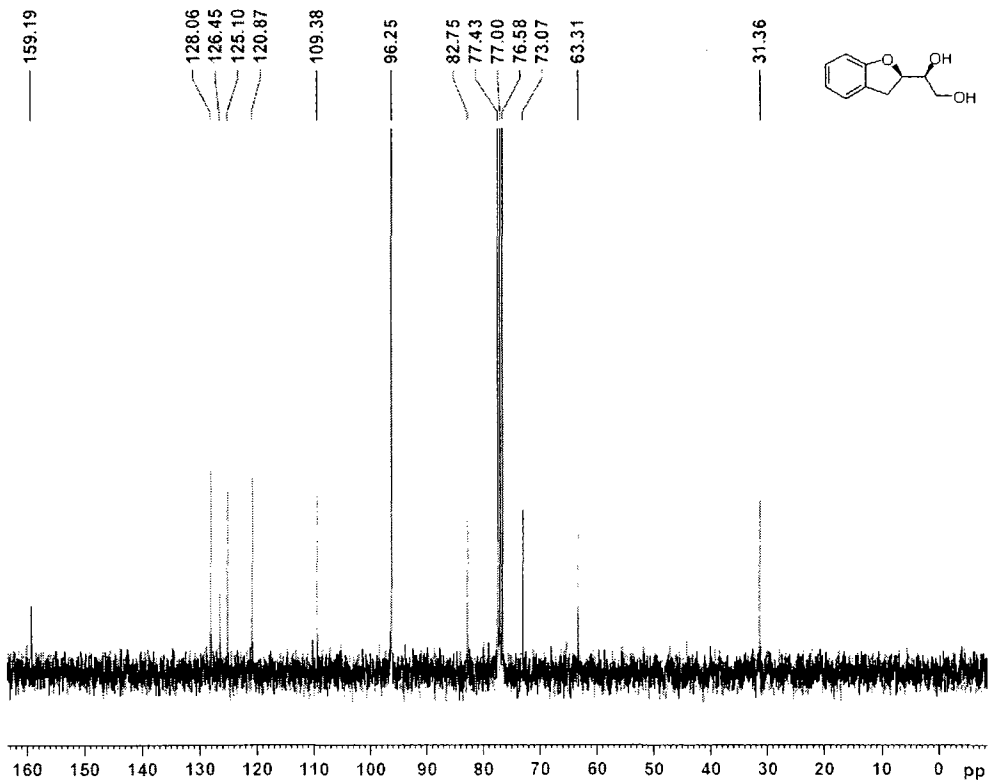


Figure 3.1.10: ^{13}C NMR ($CDCl_3$+CCl_4, 75 MHz) spectrum of **22b.**

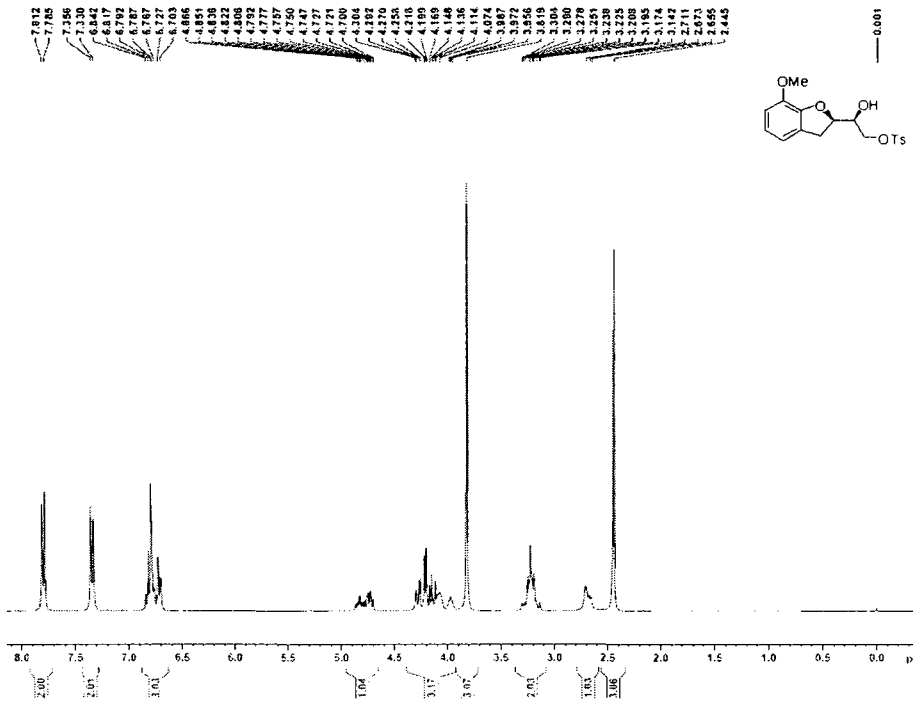


Figure 3.1.11: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **23a.**

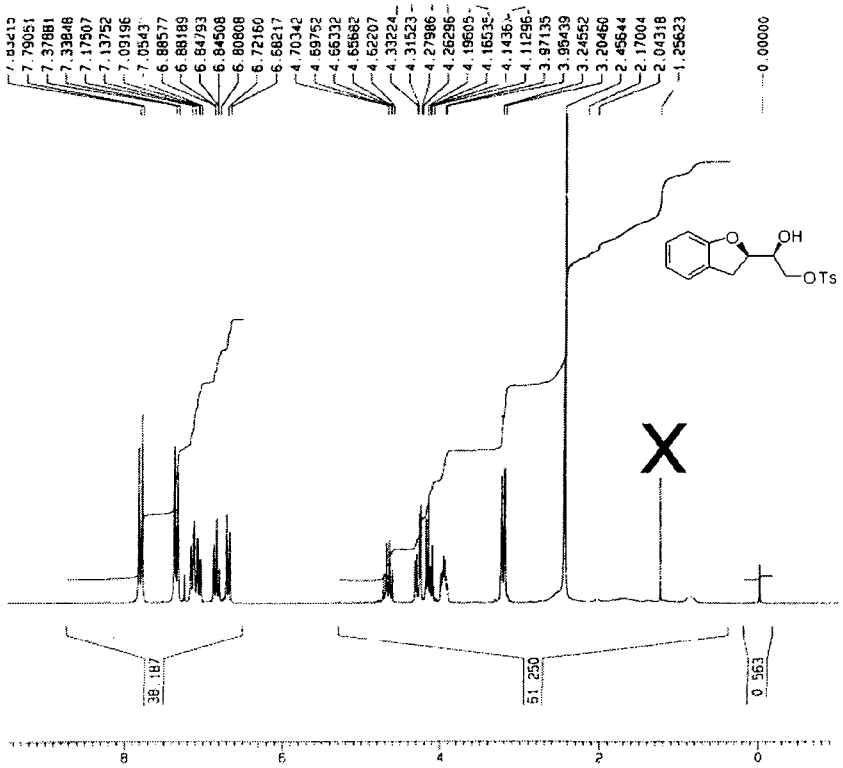


Figure 3.1.12: ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of **23b.**
['X' peak doesn't belong to compound **23b**]

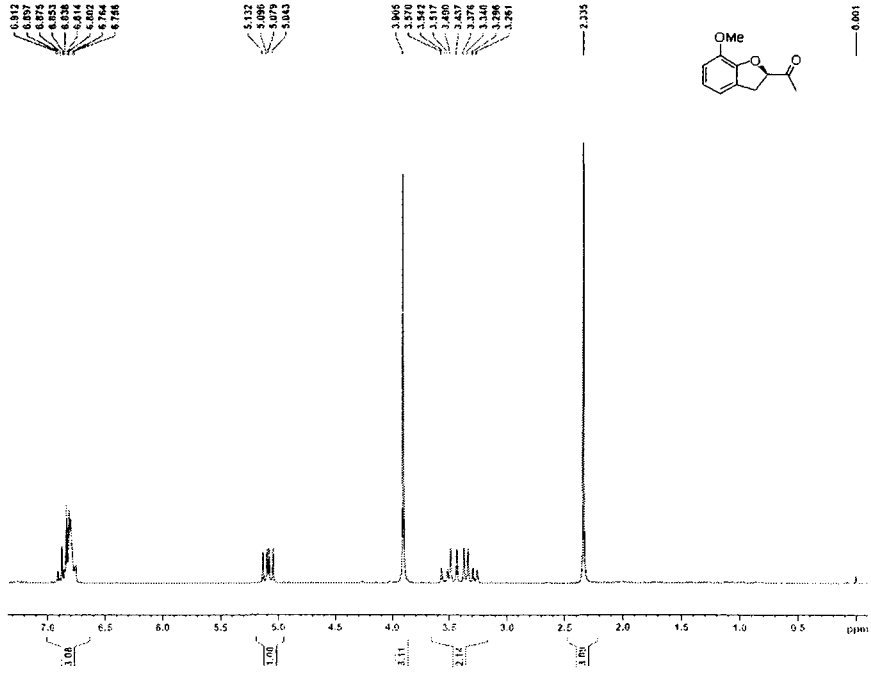


Figure 3.1.13: ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of **24a.**

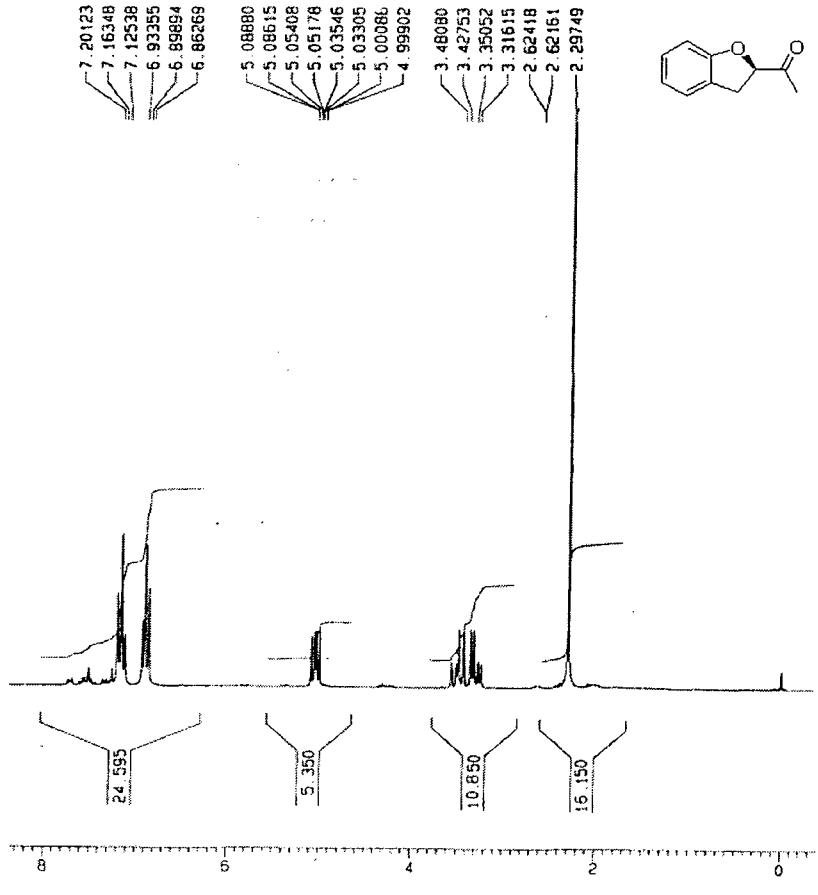


Figure 3.1.14: ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of **24b.**

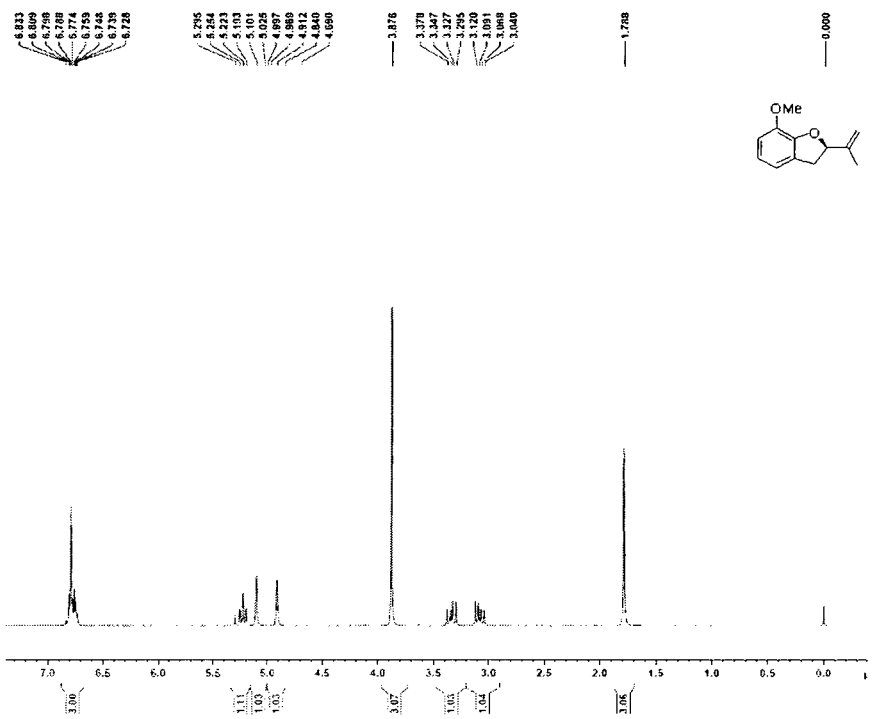


Figure 3.1.15: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **9a.**

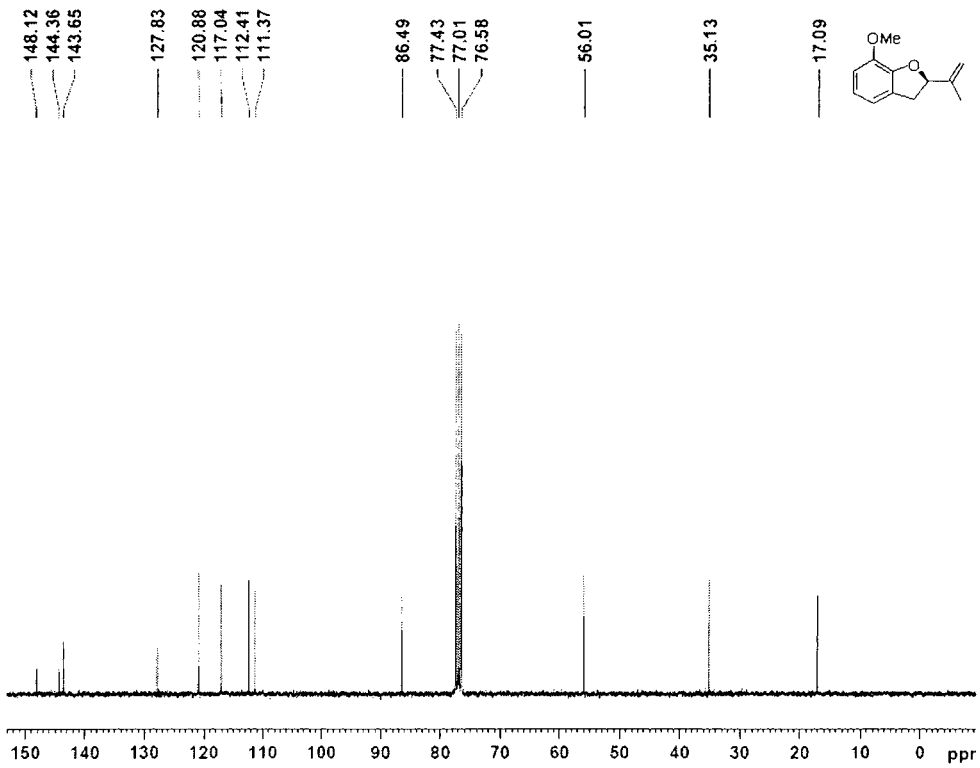


Figure 3.1.16: ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **9a.**

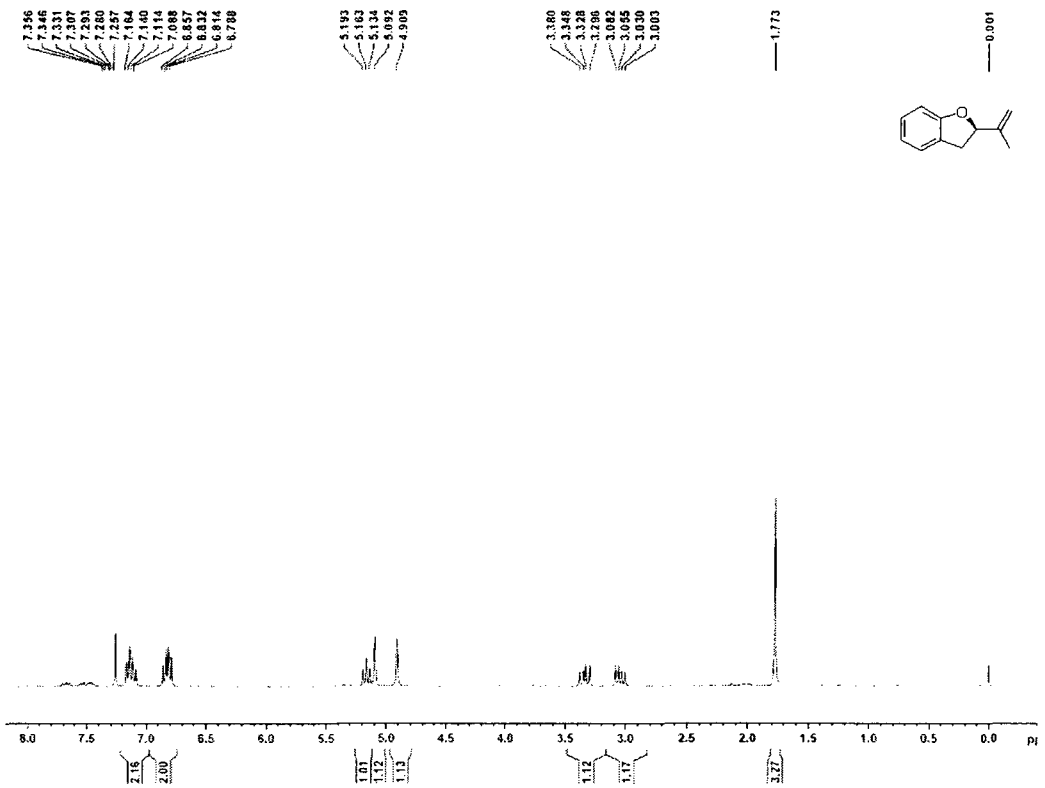


Figure 3.1.17: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **9b.**

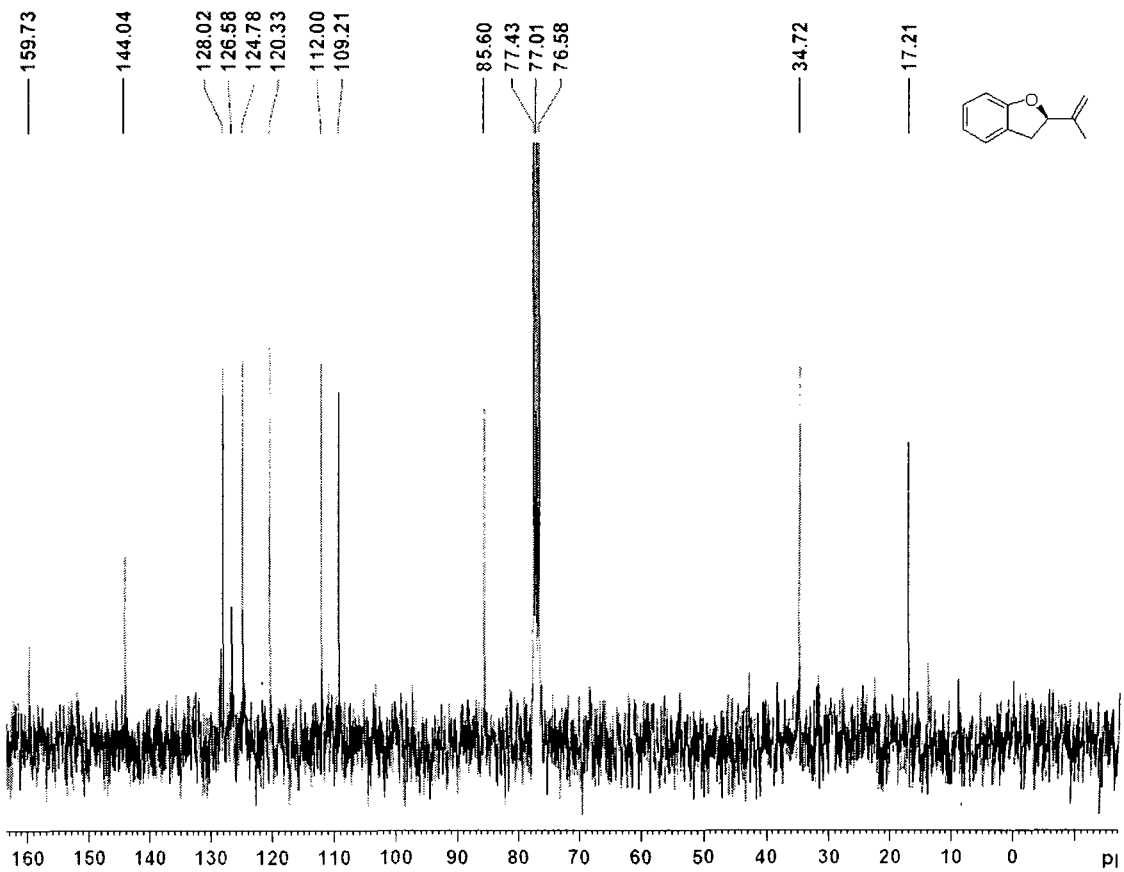


Figure 3.1.18: ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **9b.**

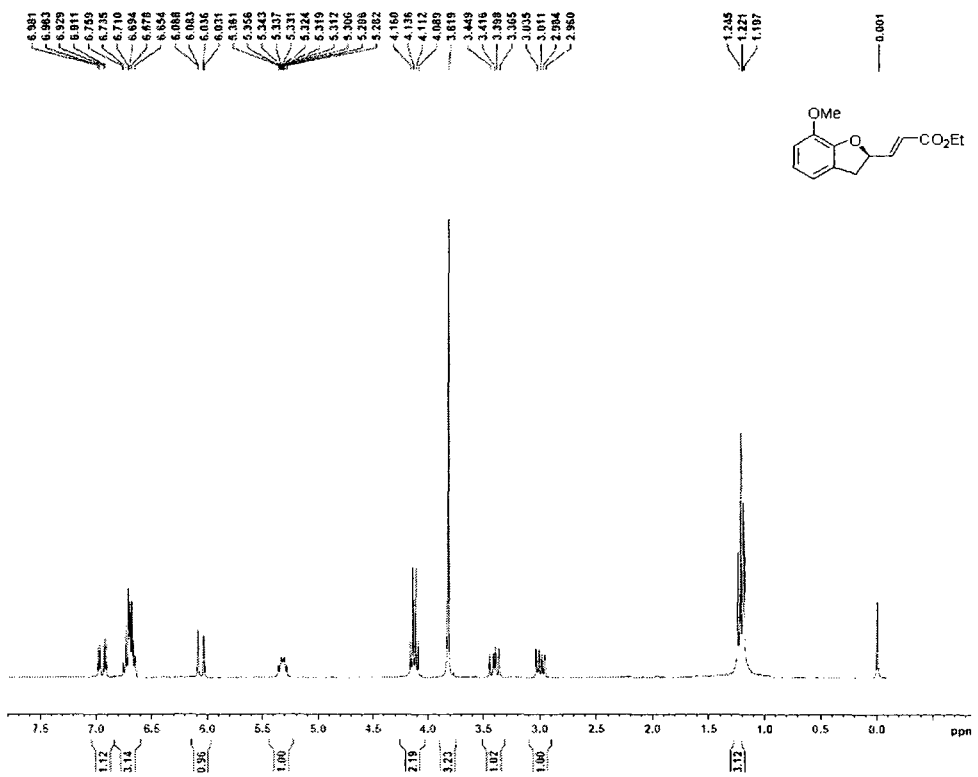


Figure 3.1.19: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **25a.**

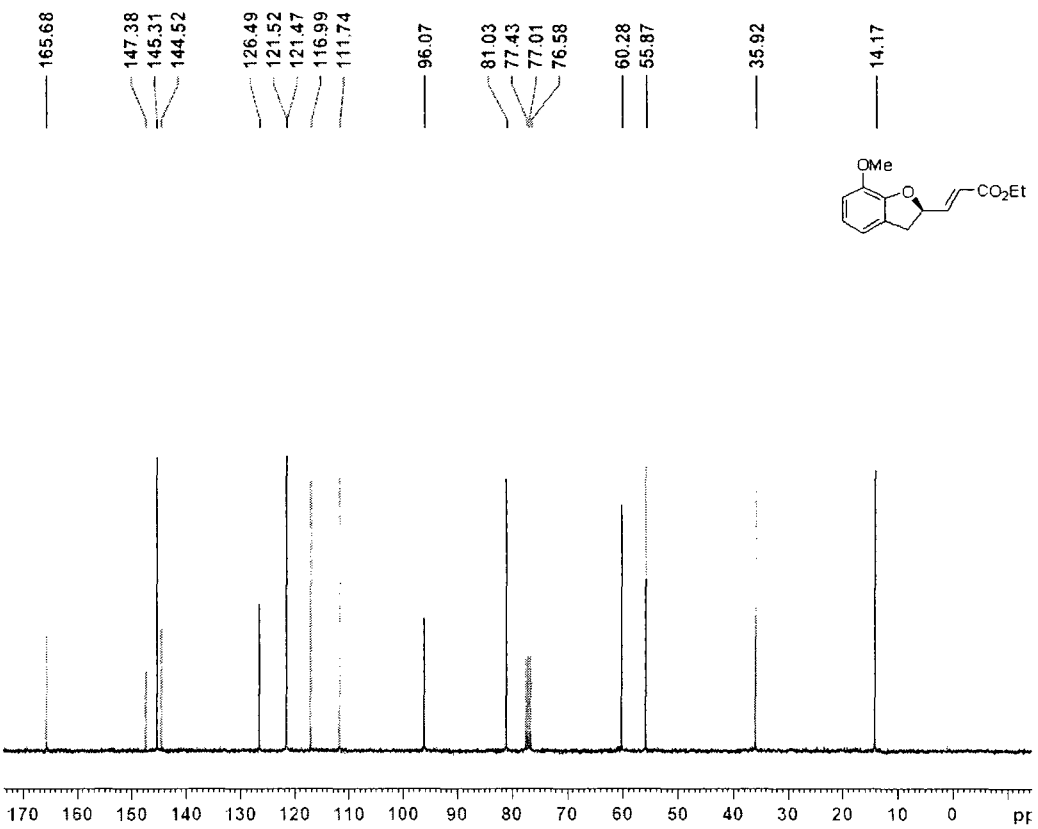


Figure 3.1.20: ^{13}C NMR ($CDCl_3$+CCl_4, 75 MHz) spectrum of **25a.**

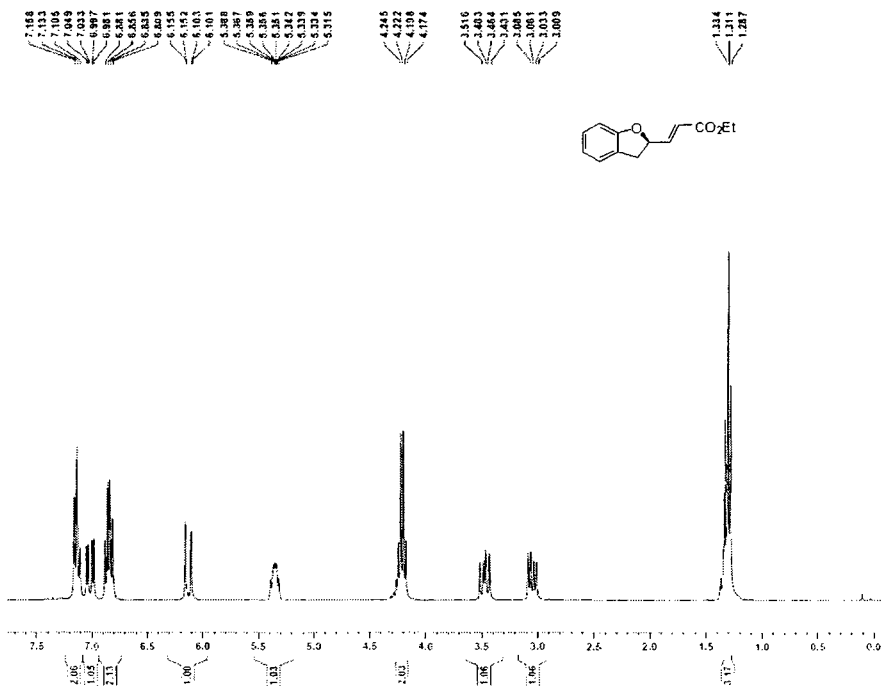


Figure 3.1.21: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **25b.**

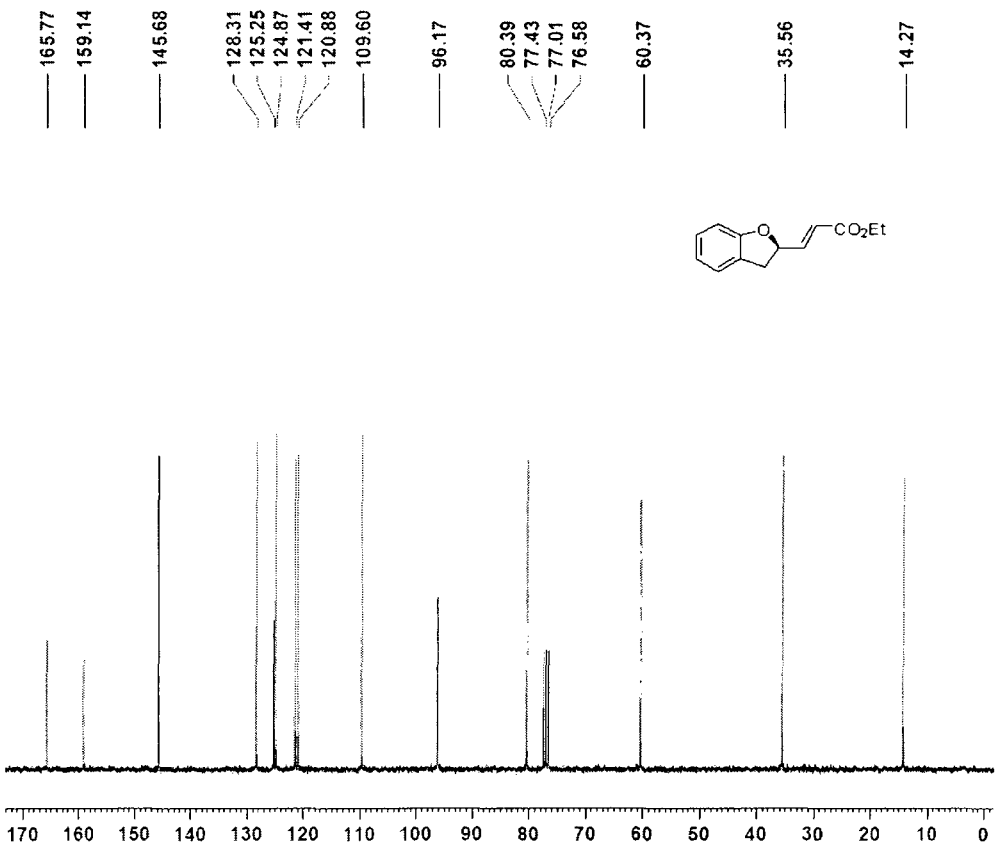


Figure 3.1.22: ^{13}C NMR ($CDCl_3$+CCl_4, 75 MHz) spectrum of **25b.**

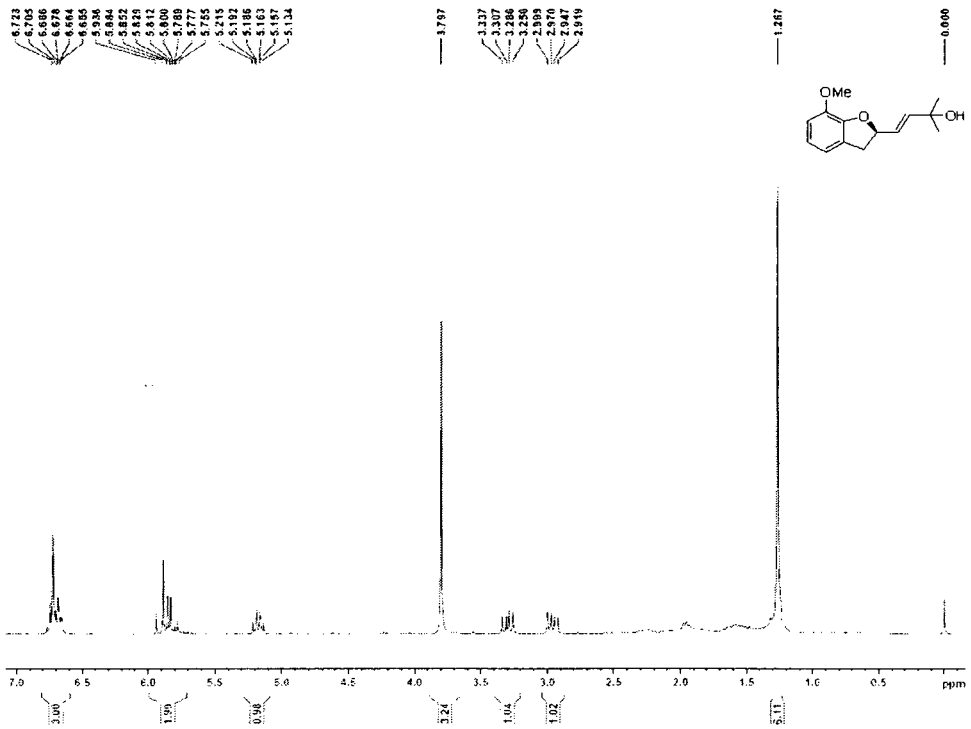


Figure 3.1.23: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **10a.**

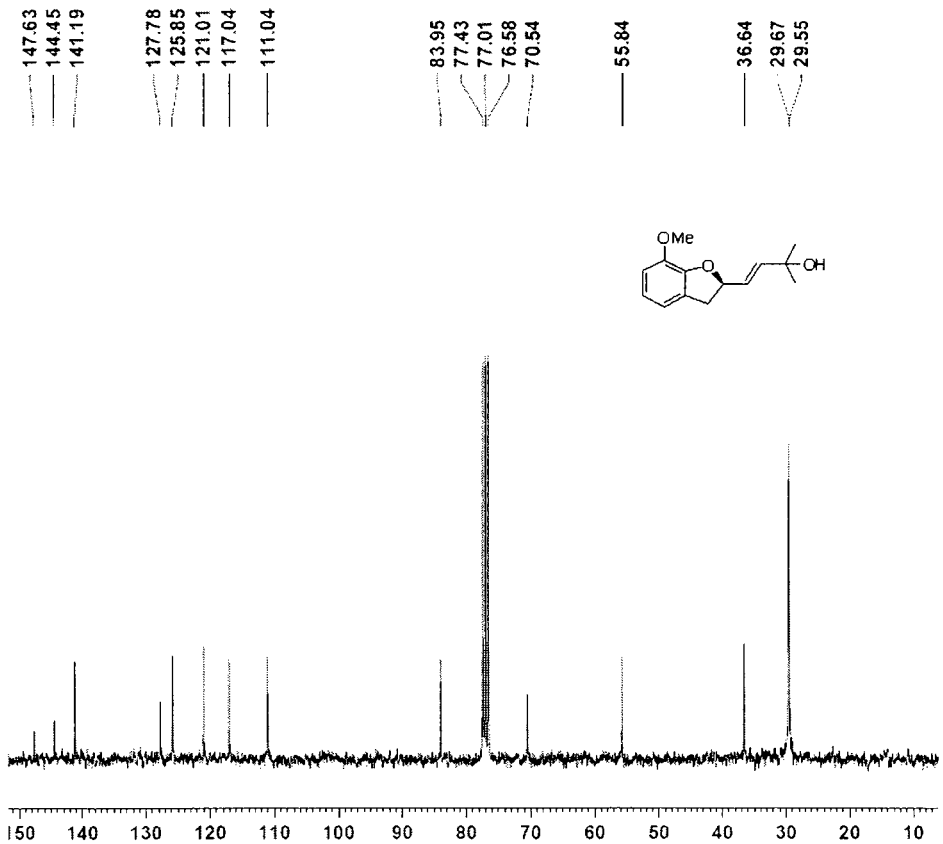


Figure 3.1.24: ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **10a.**

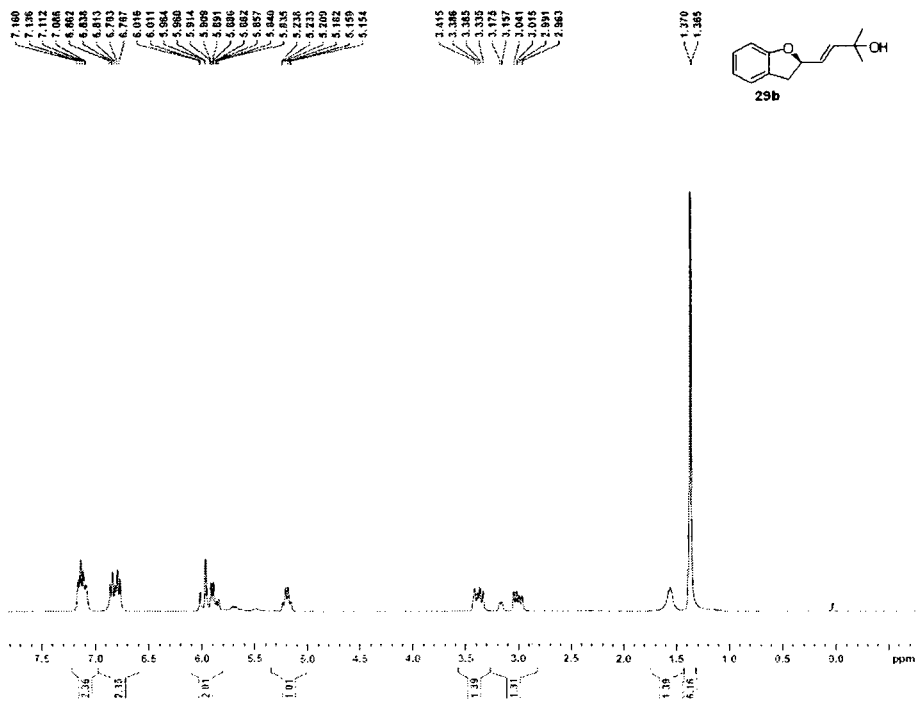


Figure 3.1.26: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **10b.**

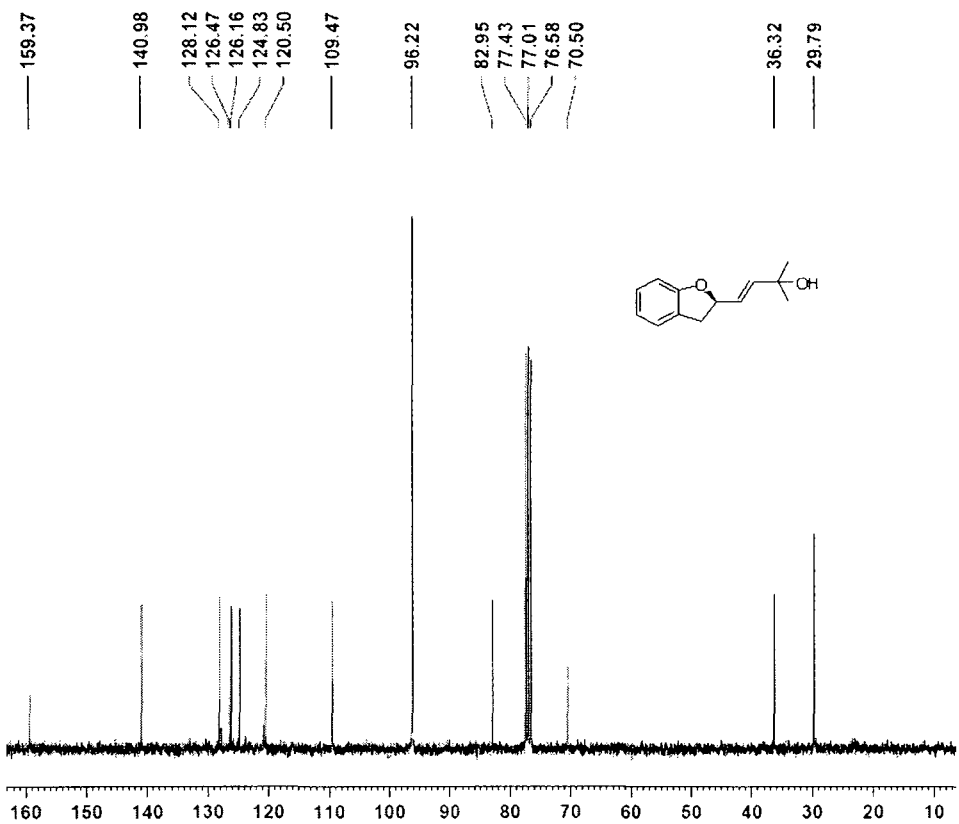


Figure 3.1.27: ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **10b.**

Section 3B:
Stereoselective Synthesis of
2-Substituted 1-Benzopyrans

3.2.1 Introduction

Over the past several years, the asymmetric synthesis of the key intermediates leading to a target based bioactive natural or unnatural molecule or diversity oriented pharmacologically active compounds have received a great deal of attention to the organic and medicinal chemists.[1] 2-Substituted 1-benzopyran derivatives are ubiquitous in natural products as well as in large number of unnatural molecules.[2] Owing to their wide ranging bioactivity profile, they continue to be a source of inspiration to chemists.[3] Enantiomerically pure 2-hydroxymethyl chromans are important synthetic intermediates for the synthesis of numerous pharmacologically active 2-substituted chroman derivatives, Figure 3.2.1.[4-9] For example, 2-hydroxymethyl chromans have been used for the synthesis of 2-azaheterocyclylmethyl chromans that are useful for controlling diseases of the central nervous system.[4] These compounds have also been used for the synthesis of various 2-(aminomethyl)chroman derivatives which have shown inhibition of iron-dependent lipid peroxidation, a pathophysiological process involved in many disease states.[5] Some newer generations of dopaminergic agents also contain 2-(aminomethyl) chromans.[6] The 2-(aminomethyl)chroman derivative, repinotan **3** (Figure 3.2.2), a high affinity 5-HT_{1A} receptor agonist[7] developed by Bayer is used for the treatment of ischemic stroke and traumatic brain injury.[8] Another 2-(aminomethyl)chroman derivative, Sarizotan **4** is currently in phase III clinical trials for patients with Parkinson disease suffering from treatment induced dyskinesia.[9] It is worth mentioning that biological activities of 2-substituted chromans are dependent on the absolute configuration of the stereogenic centre as illustrated in the development of repinotan **3** and sarizotan **4** as single enantiomers.

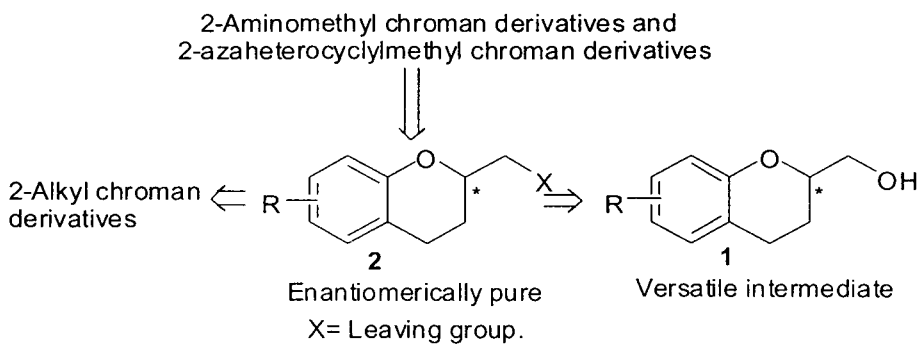
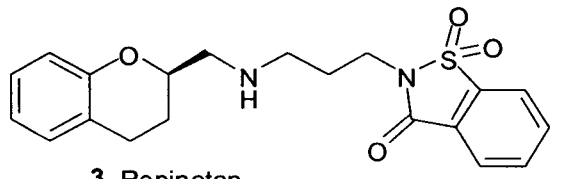
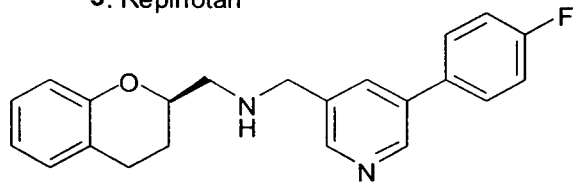


Figure 3.2.1. Synthetic utilities of 2-hydroxymethyl chromans

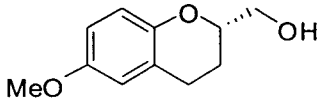


3. Repinotan

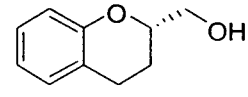


4. Sarizotan

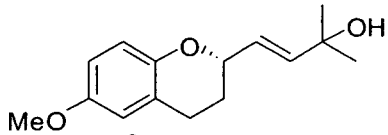
Figure 3.2.2. Some pharmacologically active 2-substituted chroman derivatives



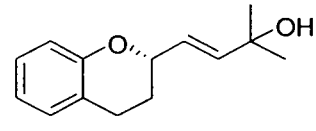
5a



5b



6a



6b

Figure 3.2.3. Our "designed" molecules containing the 2-substituted 1-benzopyran moiety

3.2.2 Basis of the Present Work

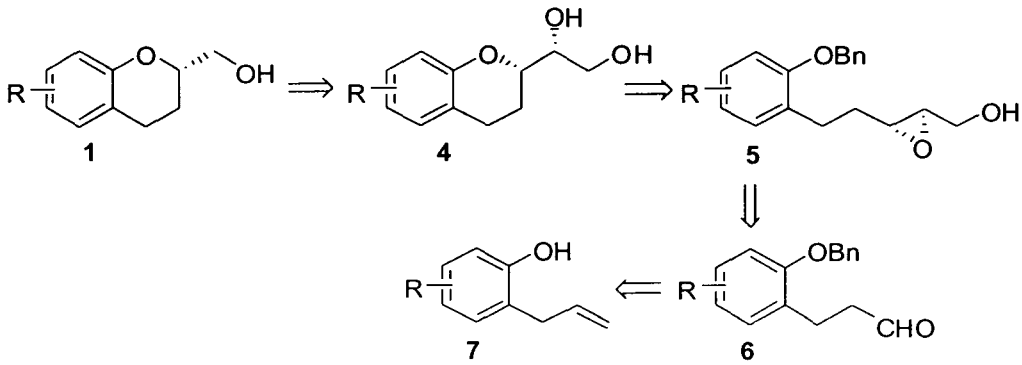
A literature survey shows that enantiomerically pure 2-hydroxymethyl chromans can be synthesized from optically pure chroman-2-carboxylic acids,[10] which, in turn can be obtained *via* chemical[11] as well as enzymatic[12] resolution of the racemate for which facile syntheses[13] have been reported. However, we could find only two reported enantioselective routes to **1**. The first one involved the synthesis of 2-hydroxymethyl chromans from enantiomerically enriched 2-vinylchroman (*ee* upto 53%), which was synthesized from palladium-catalyzed cyclization of hydroxy allylic carbonate in the presence of various chiral ligands.[14] The second one described in an US patent involved Sharpless asymmetric dihydroxylation reaction of 4-aryl-1-butene derivatives furnishing 2-hydroxymethyl chromans of low ee.[15]

Taking into account of all these facts and our interest in the asymmetric synthesis of heterocycles,[16] we herein describe our results that illustrate efficient enantioselective synthetic routes to our designed molecules 2-hydroxymethyl

chromans, and 4-chroman-2-yl-2-methyl-but-3-en-2-ols [Figure 3.2.3] through phenolate ion-mediated intramolecular 6-*exo-tet* ring opening of Sharpless asymmetric epoxidation-derived enantiomerically enriched epoxy alcohols.

3.2.3 Results and Discussion

Our retrosynthetic approach is shown in Scheme 3.2.1.

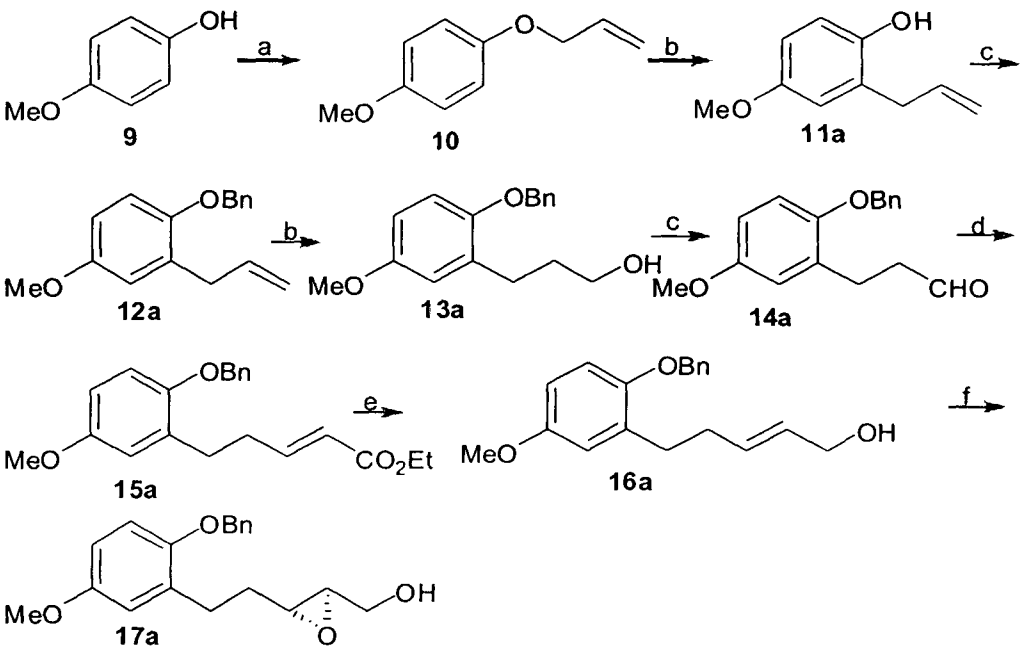


Scheme 3.2.1. Retrosynthetic analysis of 2-hydroxymethyl chromans

We envisioned that enantiomerically pure 2-hydroxymethyl chroman **1** can be synthesized from the dihydroxy compound **5** which in turn can be made from epoxide **6** *via* phenoxide ion mediated intramolecular 6-*exo-tet* epoxide ring opening. The epoxide **6** can be synthesized by Sharpless asymmetric epoxidation of the corresponding allyl alcohol, which in turn can be synthesized from 2-allylphenol **8**.

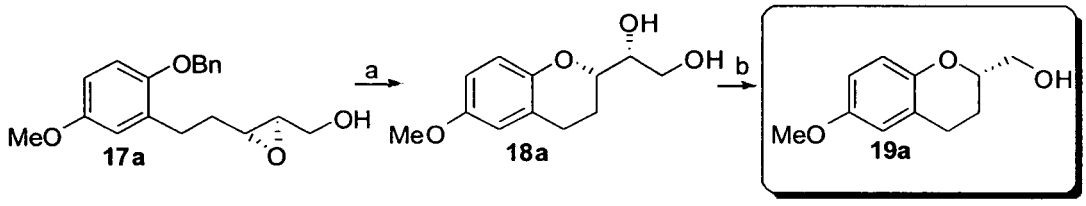
Our approach commenced with the allylation of commercially available 4-methoxyphenol **9** with allyl bromide and anhydrous K_2CO_3 in dry acetone under reflux condition followed by Claisen rearrangement of the resulting allyl ether **10** producing 5-methoxy-2-allylphenol[17] **11**, Scheme 3.2.2. Next, benzylation of **11** with (bromomethyl)benzene and anhydrous K_2CO_3 in dry acetone under reflux condition to gave the corresponding benzylated products **12a** in excellent yields. Next, hydroboration of **12a** with 9-BBN followed by oxidation with $NaOH/H_2O_2$ furnished the primary alcohols **13a** in high yields. PCC oxidation of **13a** followed by Wittig olefination of the resulting aldehydes **14a** furnished the corresponding *trans*-unasaturated esters **15a**. DIBAL-H reduction of **15a** gave *trans*-allyl alcohols **16a**. Next, **16a** were treated with titanium tetraisopropoxide and *tert*-butyl hydroperoxide in the presence of D-(-)-DIPT under asymmetric epoxidation conditions to get chiral

epoxides **17a** in good yields. The enantiomeric excess (*ee*) values of **17a** were determined to be 98% by Mosher esters technique.



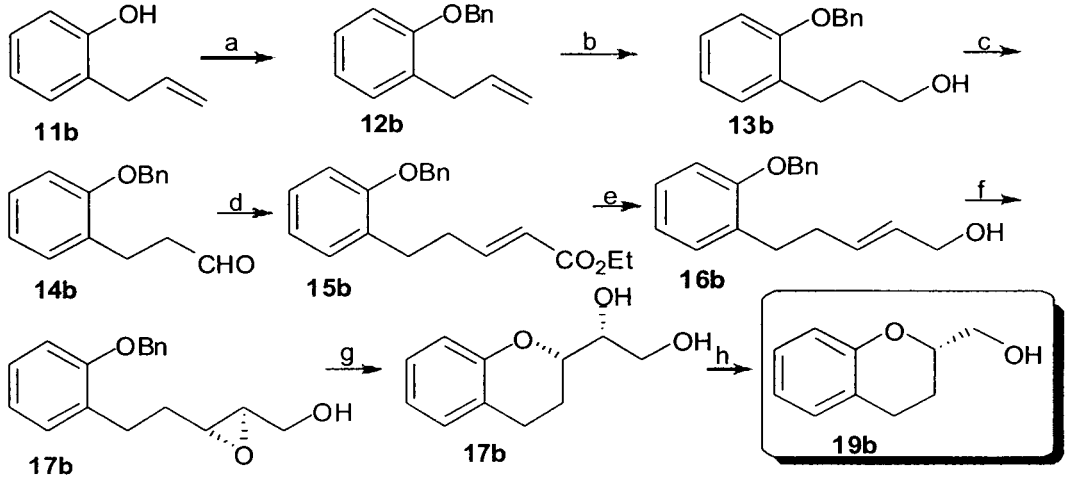
Scheme 3.2.2. Reagents and conditions: (a) Allyl bromide, anhyd. K_2CO_3, dry acetone, reflux, 3h, 98%. (b) 180 °C, 6h, 80%. (a) BnBr, anhyd. K_2CO_3, dry acetone, reflux, 4 h, 95% (b) (i) 9-BBN, dry THF, rt, 4 h. (ii) 30% H_2O_2, NaOH, reflux, 2 h, 94%. (c) PCC, dry CH_2Cl_2, 0 °C–rt, 5 h, 76%. (d) $Ph_3P{=}CHCO_2Et$, dry CH_2Cl_2, rt, overnight, 80%. (e) DIBAL-H, dry toluene, 0 °C–rt, 4 h, 96%. (f) D(-)-DIPT, $Ti(O^iPr)_4$, TBHP, CH_2Cl_2, −25 °C, 18 h, 88%.

With **17a** in our hand, attention was then turned to their elaboration into the corresponding 2-hydroxymethyl chroman derivatives. Thus debenzylation of **17a** by 10% Pd-C catalyzed hydrogenolysis followed by treatment of the resulting phenolic derivatives with 10% NaOH solution saturated with NaCl furnished the corresponding dihydroxy compound **18a** (Scheme 3.2.3) as a solid crystalline compound *via* 5-*exo-tet* intramolecular epoxide ring opening[18]. $NaIO_4$ mediated cleavage reaction of **37a** furnished the corresponding 2-formyl benzopyrans which were then reduced to their respective 2-hydroxymethyl chromans **19a**. The enantiomeric excess (*ee*) values of **19a** were determined to be >99% by chiral HPLC analysis [Chiral Cel OD, petroleum ether/*i*-PrOH (98:2) 1 mL/min, 254 mm].



Scheme 3.2.3. *Reagents and conditions:* (a) (i) 10% Pd-C, ethyl acetate, H_2, 2 h. (ii) 10% NaOH solution saturated with NaCl, 0 °C, 3 h, 83%; based on two steps. (b) (i) $NaIO_4$, MeOH-H_2O, 0 °C, 2 h. (ii) $NaBH_4$, MeOH, 0 °C to rt, 0.5 h, 87%; based on two steps.

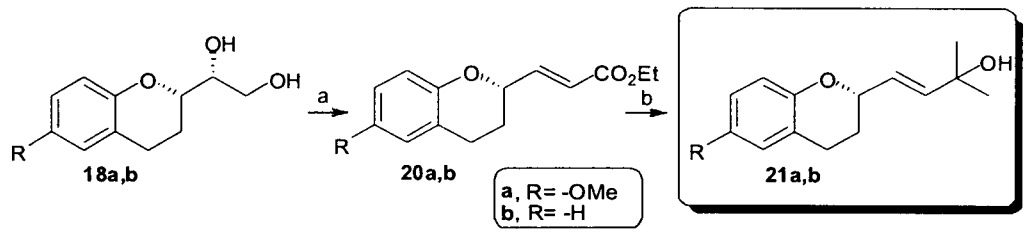
After the enantioselective synthesis of (*S*)-(6-methoxychroman-2-yl)methanol **19a**, our next attention was to synthesize structurally related analogue of **19a** with no substituent in the benzene ring. Thus, commercially available 2-allylphenol **11b** was converted into **19b** (Scheme 3.2.4) using the same reaction sequence as that described for the synthesis of **19a** (Scheme 3.2.2 & Scheme 3.2.3).



Scheme 3.2.4. Reagents and conditions: (a) BnBr, anhyd. K_2CO_3, dry acetone, reflux, 4 h, 96% (b) (i) 9-BBN, dry THF, rt, 4 h. (ii) 30% H_2O_2, NaOH, reflux, 2 h, 96%. (c) PCC, dry CH_2Cl_2, 0 °C–rt, 5 h, 72%. (d) Ph_3P=$CHCO_2Et$, dry CH_2Cl_2, rt, overnight, 77%. (e) DIBAL-H, dry toluene, 0 °C–rt, 4 h, 95%. (f) D(-)-DIPT, Ti(O^iPr)$_4$, TBHP, CH_2Cl_2, −25 °C, 18 h, 90%. (g) (i) 10% Pd-C, ethyl acetate, H_2, 2 h. (ii) 10% NaOH solution saturated with NaCl, 0 °C, 3 h, 84%; based on two steps. (h) (i) $NaIO_4$, MeOH-H_2O, 0 °C, 2 h. (ii) $NaBH_4$, MeOH, 0 °C to rt, 0.5 h, 89%; over two steps.

Next, we were interested to utilize diols **18a,b** for the synthesis of 1-benzopyran analogues of 2-(2,3-dihydrobenzofuran-2-yl)-2-methylbut-3-en-2-ol **10a** (**Chapter 3, Section 3A, Scheme 3.1.5**). Towards that direction, **18a,b** were

converted into **21a,b** (Scheme 3.2.5) using the same reaction sequence as that described for the synthesis of **10a** from **22a** (Scheme 3.1.5).



Scheme 3.2.5. Reagents and conditions: (a) (i) $NaIO_4$, MeOH-H_2O, 0 °C, 2 h. (ii) $Ph_3P{=}CHCO_2Et$, dry CH_2Cl_2, rt, overnight, **20a** (88%), **20b** (90%); based on two steps. (c) MeMgI, dry ether, 0 °C-reflux, 4 h, **21a** (93%), **21b** (95%).

3.2.4 Conclusion

In conclusion, an efficient asymmetric synthetic method of enantiomerically pure 2-hydroxymethyl chromans, and 4-chroman-2-yl-2-methyl-but-3-en-2-ols has been developed. Key steps include Sharpless asymmetric epoxidation reaction on suitable allyl alcohol and construction of the benzopyran nucleus by phenoxide ion mediated intramolecular 6-*exo-tet* epoxide ring opening. The ease of the reaction sequence, as well as the rapid accessibility of the starting 2-allylphenols, makes this process a practical method for the preparation of optically active 2-hydroxymethyl-chromans. In addition, the scope the reaction sequence is much broader, and synthesis of various substituted aromatic and heteroaromatic nuclei can be envisioned from the starting allyl arene derivatives.

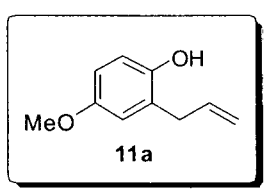
3.2.5 Experimental Section

3.2.5.1 General Remarks

As described in **Section 3.1.5.1 (Chapter 3, Section 3A).**

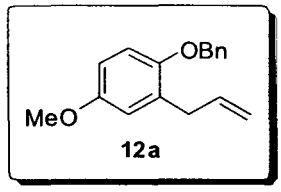
3.2.5.2 Synthesis of Compounds

2-Allyl-4-methoxyphenol (11a). Compound **11a** was synthesized according to published procedure[17] with some modifications. To a solution of 4-methoxyphenol (5.00 g, 40.28 mmol) in dry acetone (30 mL) were added allyl bromide (4.10 mL, 48.33 mmol), anhydrous K_2CO_3 (5.60 g, 40.35 mmol) and potassium iodide (0.20 g) and the mixture was refluxed for 3 hr with continuous stirring. The yield of



the product was 6.5 g (98%). The allyl ether (6.00 g) was rearranged by refluxing with 6.00 g of DMA at 180 °C for 6 h. Compound **11a** was obtained as colorless oil (4.80 g, 80%). The spectroscopic data of **11a** was consistent with literature data.[17]

3-Allyl-4-benzyloxyanisole (12a). To a solution of **11a** (4.00 g, 24.36 mmol) in dry acetone (50 mL) was added anhydrous K_2CO_3 (5.0 g, 36.17 mmol) and (bromomethyl)benzene (2.9 mL, 24.36 mmol) and refluxed for 4 h. The mixture was then filtered through celite and the filter cake was well washed with acetone (100 mL). The filtrate was concentrated and the resulting residue was redissolved in ethyl acetate (100 mL), washed with water (2x50 mL) and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and then concentrated under vacuo. Purification of the crude product by silica gel column chromatography (2% ethyl acetate in *n*-hexane) afforded **12a** (5.88 g, 95%) as a colorless oil.



12a

IR (Neat): 2924, 2362, 1639, 1495, 1558, 1216, 1043, 731, 693 cm^{-1}.

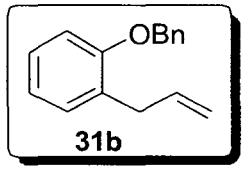
^1H NMR (200 MHz, $CDCl_3$): δ 7.40-7.29 (m, 5H), 6.84-6.66 (m, 3H), 6.01-5.92 (m, 1H), 5.09 (m, 2H), 5.00 (s, 2H), 3.73 (s, 3H), 3.42 (d, 2H, J = 6.5).

^{13}C NMR (75 MHz, $CDCl_3$): δ 154.2, 151.0, 138,0, 137.2, 130.8, 128.9, 128.1, 127.6, 116.5, 116.2, 113.5, 111.7, 78.1, 71.2, 56.0, 35.0.

MS (ESI): *m/z* 254 $[M]^+$, 91 $[C_6H_5CH_2]^+$.

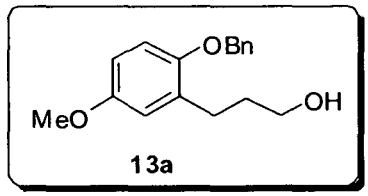
Anal. Calcd for $C_{17}H_{18}O_2$: C, 80.28; H, 7.13. Found: C, 80.35; H, 7.32.

2-Benzyloxyallylbenzene (12b): Starting from 4.0 g (17.67 mmol) of **11b**, the title compound was prepared in the same manner as that described for **12a**. Purification of the crude product by silica gel column chromatography (2% ethyl acetate in hexane) afforded **12b** (3.50 g, 96%) as a colorless oil. R_f: 0.46 (10% ethyl acetate in hexane). IR (Neat): 3069, 2908, 2370, 1597, 1494, 1453, 1241, 1022, 749 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.44-7.28 (m, 5H), 7.20-7.14 (m, 2H), 6.93-6.89 (m, 2H), 6.08-5.95 (m, 1H), 5.08 (s, 2H), 5.05-5.02 (m, 2H), 3.45 (d, 2H, J = 6.6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.4, 137.4, 137.0, 129.9, 129.0, 128.5, 127.7, 127.3, 127.1, 120.8, 115.4, 111.7, 69.9, 34.4. MS (ESI): *m/z* 225 $[M+1]^+$, 91 $[C_6H_5CH_2]^+$. Anal. Calcd for $C_{16}H_{16}O$: C, 85.68; H, 7.19. Found: C, 85.76; H, 7.30. The above physical and spectroscopic data were as consistent with as literature data.[16c]



31b

3-[2-(benzyloxy)-5-methoxyphenyl]propan-1-ol (13a). To a stirred solution of **12a** (4.00 g, 15.72 mmol) in anhydrous THF (30 mL) was added a 0.5 M THF solution of 9-BBN (47 mL) dropwise under a nitrogen atmosphere at 0 °C and the mixture was stirred at room temperature for 4 h. H_2O (5 mL) was added followed by 3 N NaOH solution (40 mL) and 30% aqueous hydrogen peroxide solution (30 mL). The reaction mixture was stirred for 2 h at 60 °C. The mixture was extracted with ethyl acetate (2x50 mL), washed with water (150 mL) and brine (150 mL). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography (8% ethyl acetate in *n*-hexane) afforded alcohol **13a** (4.02 g, 94%) as a colorless gum. R_f: 0.32 (20% ethyl acetate in n-hexane).



13a

IR (Neat): 3431, 2929, 2361, 1497, 1214, 1042, 756, 698 cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$): δ 7.44-7.34 (m, 5H), 7.85 (d, 1H, J = 8.7), 6.76-6.66 (m, 2H), 5.02 (s, 2H), 3.75 (s, 3H), 3.57 (t, 2H, J = 6.3), 2.74 (t, 2H, J = 7.2), 1.88-1.70 (m, 2H), 1.77 (s, br, 1H).

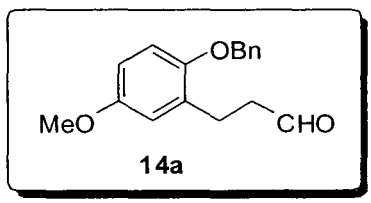
^{13}C NMR (75 MHz, $CDCl_3$): δ 154.3, 151.3, 137.7, 132.3, 129.0, 128.3, 127.7, 116.8, 113.5, 111.6, 71.5, 62.1, 56.0, 33.4, 26.7.

MS (ESI): *m/z* 272 $[M]^+$, 255 $[M\text{-}OH]^+$, 91 $[C_6H_5CH_2]^+$.

Anal. Calcd for $C_{17}H_{20}O_3$: C, 74.97; H, 7.40. Found: C, C, 74.88; H, 7.58.

Compound **13b**, **14b** and **15b** were synthesized by known method. [16c]

3-[2-(benzyloxy)-5-methoxyphenyl]propanal (14a). To a stirred solution of **13a** (3.00 g, 11.01 mmol) in dry CH_2Cl_2 (50 mL) was added 4 Å molecular sieve (3.85 g) and PCC (3.20 g, 14.84 mmol) at 0 °C and the mixture was stirred at room temperature for 5 h. After evaporating CH_2Cl_2 the reaction mixture was diluted with ether (50 mL) and filtered through a small pad of silica gel. Concentration of the filtrate and purification of the residue by silica gel column chromatography (4% ethyl acetate in *n*-hexane) afforded **14a** (2.26 g, 76%) as a colorless gum.



14a

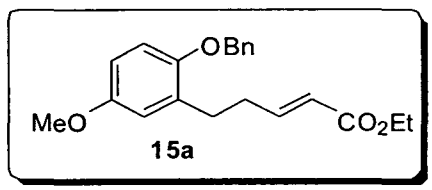
^1H NMR (200 MHz, $CDCl_3$): δ 9.69 (t, 1H, J = 1.5), 7.36-7.29 (m, 5H), 6.81-6.64 (m, 3H), 4.96 (s, 2H), 3.68 (s, 3H), 2.93 (t, 2H, J = 7.3), 2.70-2.63 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 202.5, 154.2, 151.1, 137.8, 130.8, 129.0, 128.3, 127.6, 117.4, 113.3, 112.3, 71.0, 56.0, 44.3, 24.0.

MS (FAB): *m/z* 270 $[M]^{+\cdot}$.

Anal. Calcd for $C_{17}H_{18}O_3$: C, 75.53; H, 6.71. Found: C, 75.63; H, 6.88.

(*E*)-ethyl 5-[2-(benzyloxy)-5-methoxyphenyl]pent-2-enoate (15a). To a solution of **14a** (2.00 g, 7.39 mmol) in dry CH_2Cl_2 (30 mL) was added [(ethoxycarbonyl]methylene)triphenylphosphorane (3.21 g, 9.21 mmol) at room temperature. The reaction mixture was stirred at room temperature for overnight. Solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (4% ethyl acetate in *n*-hexane) to afford **15a** (2.01 g, 80%) as a colorless gum.



15a

IR (KBr): 2926, 2359, 1708, 1649, 1496, 1211, 1035, 736 cm^{-1}.

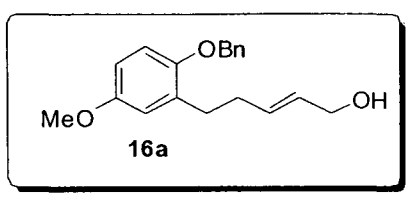
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37-7.22 (m, 5H), 7.04-6.94 (m, 1H), 6.77-6.60 (m, 3H), 5.79 (d, 1H, *J* = 15.6), 4.93 (s, 2H), 4.14-4.07 (m, 2H), 3.65 (s, 3H), 2.77-2.72 (m, 2H), 2.48-2.41 (m, 2H), 1.20 (t, 3H, *J* = 7.1).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1, 153.4, 150.3, 148.1, 137.2, 130.5, 128.1, 127.3, 126.7, 121.2, 115.9, 112.4, 110.9, 70.1, 59.6, 55.0, 32.1, 28.8, 13.9.

MS (FAB): *m/z* 340 $[M]^+$.

Anal. Calcd for $C_{21}H_{24}O_4$: C, 74.09; H, 7.11. Found: C, 74.21; H, 7.03.

(*E*)-5[2-(benzyloxy)-5-methoxyphenyl]pent-2-en-1-ol (16a). To an ice-cooled stirred solution of **15a** (2.00 g, 5.87 mmol) in dry toluene (16 mL) was added DIBAL-H (1 M in toluene, 14.8 mL, 14.8 mmol)) dropwise under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 2 h, cooled to 0 °C, carefully quenched with methanol (3 mL) and a saturated aq. sodium potassium tartarate solution (15 mL). The resulting mixture was vigorously stirred for 45 min. at rt and then extracted with ethyl acetate (2x30 mL), washed with water (30 mL) and brine (50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and then concentrated under reduced



16a

pressure. Purification of the crude product by silica gel column chromatography (20% ethyl acetate in n-hexane) afforded **16a** (1.68 g, 96%) as a colorless semi-solid.

IR (Neat): 3417, 2930, 2361, 1499, 1217, 1043, 754 cm^{-1}.

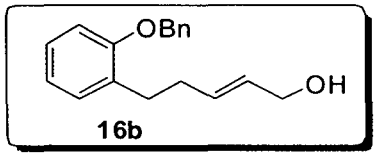
^1H NMR (300 MHz, $CDCl_3$): δ δ 7.42-7.27 (m, 5H), 6.81 (d, 1H, J = 8.8), 6.73 (d, 1H, J = 3.0), 6.66 (dd, 1H, J_1 = 3.1, J_2 = 8.7), 5.78-5.55 (m, 2H), 5.01 (s, 2H), 4.04 (d, 2H, J = 5.5), 3.74 (s, 3H), 2.75-2.70 (m, 2H), 2.35 (m, 2H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 153.6, 150.8, 137.6, 132.6, 131.8, 129.3, 128.4, 127.6, 127.0, 116.3, 112.8, 110.9, 70.6, 63.6, 55.5, 32.4, 30.2.

MS (ESI): *m/z* 316.1 $[M+NH_4]^+$.

Anal. Calcd for $C_{19}H_{22}O_3$: C, 76.48; H, 7.43. Found: C, 76.35; H, 7.68.

(*E*)-5-[2-(benzyloxy)phenyl]pent-2-en-1-ol (16b). Starting from 2.00 g (6.44 mmol) of **15b**, the title compound was prepared in the same manner as that described for **16a**.



16b

Purification of the crude product by silica gel column chromatography (20% ethyl acetate in *n*-hexane) afforded **16b** (1.64 g, 95%) as a colorless gum.

IR (Neat): 3417, 2930, 2361, 1499, 1217, 1043, 754, 697 cm^{-1}.

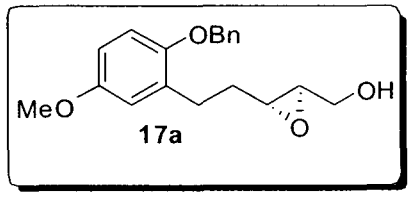
^1H NMR (200 MHz, $CDCl_3$): δ 7.42-7.35 (m, 5H), 7.29-7.13 (m, 2H), 6.91(d, 2H, J = 6.0), 5.75-5.58 (m, 2H), 5.08 (s, 2H), 4.05 (d, 2H, J = 5.0), 2.77 (t, 2H, J =7.2), 2.43-2.42 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 157.0, 137.9, 133.3, 130.9, 129.7, 128.9, 128.2, 127.5, 127.4, 121.1, 112.0, 70.2, 64.1, 32.8, 30.6.

MS (ESI): *m/z* 268 $[M]^+$.

Anal. Calcd for $C_{18}H_{20}O_2$: C, 80.56; H, 7.51. Found: C, 80.59; H, 7.45.

{(2*R*,3*R*)-3-[2-(benzyloxy)-5-methoxyphenethyl]oxiran-2-yl}methanol (17a). To a cooled (−25 °C) suspension of activated and powdered 4 Å MS (1.50 g) in dry



17a

CH_2Cl_2 (20 mL) were added L-(+)-DIPT (1.05 mL, 6.03 mmol) and $Ti(O^iPr)_4$ (1.65 mL, 5.55 mmol). The resulting mixture was then stirred for 20 min at the same temperature and then TBHP (5.6 M in n-decane, 2.9 mL, 15.06 mmol) was added dropwise. After 20 min, a solution of **16a** (1.50 g, 5.02 mmol) in dry CH_2Cl_2 (12 mL) was added dropwise over 15 min. The

resulting mixture was kept at -25 °C refrigerator for 18 h. The reaction mixture was allowed to warm to 0 °C and poured into a freshly prepared and cooled (0 °C) solution of ferrous sulfate and tartaric acid (1.50 g and 0.60 g, respectively) in deionised water (12 mL). The two-phase mixture was stirred for 30 min, aqueous phase separated and extracted with CH_2Cl_2. The combined organic phases were treated with a pre-cooled (0 °C) solution of 30% NaOH (15 mL) in saturated brine. The two-phase mixture was then stirred for 1 h at room temperature and the aqueous layer separated. It was extracted with CH_2Cl_2 (2x20 mL), washed with brine (20 mL), and the combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography (30% ethyl acetate in *n*-hexane) afforded **17a** (1.39 g, 88%) as a colorless gum. $[\alpha]^{25}_D$: +15.2 (*c* 1.9, $CHCl_3$).

IR (Neat): 3425, 2926, 1621, 1500, 1219, 1042, 745, 700 cm^{-1}.

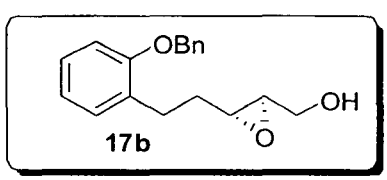
^1H NMR (300 MHz, $CDCl_3$): δ 7.43-7.28 (m, 5H), 6.83 (d, 1H, J = 8.8), 6.75 (d, 1H, J = 3.0), 6.68 (dd, 1H, J_1 = 3.1, J_2 = 8.8), 5.01 (s, 2H), 3.79-3.78 (m, 1H), 3.75 (s, 3H), 3.55-3.48 (m, 1H), 2.98-2.94 (m, 1H), 2.87-2.70 (m, 3H), 1.99-1.72 (m, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 153.0, 148.4, 139.7, 126.2, 121.9, 117.2, 113.8, 113.2, 75.3, 70.3, 55.3, 29.6, 29.5, 27.8, 24.5.

MS (ESI): *m/z* 315 $[M+1]^+$, 332 $[M+NH_4]^+$.

Anal. Calcd for $C_{19}H_{22}O_4$: C, 72.59; H, 7.05. Found: C, 72.71; H, 7.11.

{(2*R*,3*R*)-3-[2-(benzyloxy)phenethyl]oxiran-2-yl}methanol (17b). Starting from 1.50 g (5.59 mmol) of **16b**, the title compound was prepared in the same manner as that described for **17a**. Purification of the crude product by silica gel column chromatography (30% ethyl acetate in *n*-hexane) afforded **17b** (1.43 g, 90%) as a colorless gum. $[\alpha]^{25}_D$: +24.7 (*c* 1.54, $CHCl_3$).



17b

IR (Neat): 3434, 2927, 1598, 1495, 1240, 1023, 752 cm^{-1}.

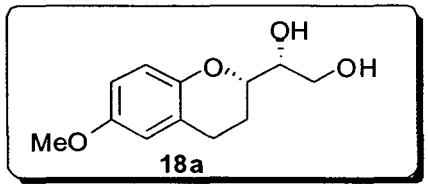
^1H NMR (300 MHz, $CDCl_3$): δ 7.44-7.29 (m, 5H), 7.20-7.15 (m, 2H), 6.92-6.87 (m, 2H), 5.07 (s, 2H), 3.79-3.75 (m, 1H), 3.55-3.47 (m, 1H), 2.98-2.74 (m, 4H), 2.00-1.78 (m, 2H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 156.5, 137.2, 130.0, 129.8, 128.5, 127.8, 127.4, 127.1, 120.8, 111.7, 69.9, 61.7, 58.6, 55.7, 31.7, 26.9.

MS (ESI): *m/z* 285 $[M+1]^+$, 302 $[M+NH_4]^+$.

Anal. Calcd for $C_{18}H_{20}O_3$: C, 76.03; H, 7.09. Found: C, 76.19; H, 7.16.

(*R*)-1-[(*S*)-6-methoxychroman-2-yl]ethane-1,2-diol (18a). To a stirred solution of **17a** (1.25 g, 3.97 mmol) in ethyl acetate (20 mL) was added 10% Pd-C (150 mg).



18a

After stirring for 2 h at room temperature under pressure of a hydrogen balloon, the reaction mixture was filtered through a pad of Celite® and the filtrate was concentrated under reduced pressure to get the corresponding debenzylated product as a colorless solid which was used for the next step without further purification.

To an ice-cooled stirred solution of the above debenzylated product in CH_2Cl_2 (15 mL), was added a pre-cooled (0 °C) solution of 30% NaOH (15 mL) in saturated brine. The two-phase mixture was then stirred for 3 h at room temperature and the aqueous layer separated. It was extracted with CH_2Cl_2 (2x25 mL), washed with brine (20 mL), and the combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography (40% ethyl acetate in *n*-hexane) afforded **18a** (0.74 g, 83%) as a colorless solid. M.p.: 87-88 °C. $[\alpha]^{25}_D$: +16.56 (*c* 0.56, MeOH).

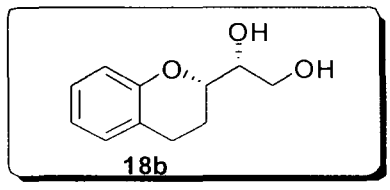
IR (KBr): 3397, 2924, 2360, 1496, 1219, 1041, 761 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.72 (d, 1H, J = 8.8), 6.65 (dd, 1H, J_1= 2.7, J_2 = 8.8), 6.59 (d, 1H, J = 2.5), 4.01-3.96 (m, 1H), 3.88-3.84 (m, 3H), 3.74 (s, 3H), 2.90-2.71 (m, 3H), 2.34-2.29 (m, 1H), 2.14-2.07 (m, 1H), 1.91-1.77 (m, 2H).

MS (ESI): *m/z* 285 $[M+1]^+$, 302 $[M+NH_4]^+$.

Anal. Calcd for $C_{12}H_{16}O_4$: C, 64.27; H, 7.19. Found: C, 64.19; H, 7.32.

(*R*)-1-[(*S*)-chroman-2-yl]ethane-1,2-diol (18b). Starting from 1.25 g (4.39 mmol) of



18b

17b, the title compound was prepared in the same manner as that described for **18a**. Purification of the crude product by silica gel column chromatography (40% ethyl acetate in *n*-hexane) afforded **18b** (0.71 g,

84%) as a colorless solid. M.p.: 63-64 °C. $[\alpha]^{25}_D$: +26.28 (*c* 0.76, MeOH).

IR (KBr): 3385, 3020, 2361, 1586, 1216, 761 cm^{-1}.

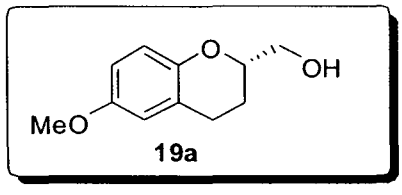
^1H NMR (300 MHz, $CDCl_3$): δ 7.12-7.05 (m, 2H), 6.89-6.79 (m, 2H), 4.08-4.03 (m, 1H), 3.92-3.84 (m, 3H), 2.88-2.81 (m, 3H), 2.55 (s, br, 2H), 2.18-2.12 (m, 1H), 1.92-1.85 (m, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 152.9, 128.3, 126.0, 120.8, 119.2, 115.3, 75.1, 72.0, 62.1, 23.0, 22.0.

MS (ESI): *m/z* 285 $[M+1]^+$, 302 $[M+NH_4]^+$.

Anal. Calcd for $C_{11}H_{14}O_3$: C, 68.02; H, 7.27. Found: C, 68.16; H, 7.29.

(*S*)-(6-methoxychroman-2-yl)methanol (19a). To a stirred solution of diol **18a** (250 mg, 1.11 mmol) in methanol (8 mL) at 0°C was added a solution of $NaIO_4$ (380 mg, 1.77 mmol) in water (4 mL). After stirring the reaction mixture at 0°C for 2 h, methanol was evaporated in vacuo at low temperature. The aqueous solution was extracted with CH_2Cl_2 (2x10 mL). The combined organic layers were washed with water (10 mL), brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to yield the crude aldehyde as a colorless gum which was used for the next step without further purification.



19a

To an ice-cooled solution of the above crude aldehyde in methanol (6 mL) was added $NaBH_4$ (50 mg, 1.31 mmol) and the reaction mixture was stirred for 30 min at rt. Methanol was removed under reduced pressure and the residue was dissolved in ethyl acetate (15 mL) and water (15 mL). The organic layer was separated and washed with brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography (20% ethyl acetate in *n*-hexane) afforded **19a** (18 mg, 87%) as a colorless gum. $[\alpha]_D^{20}$: +104.8 (*c* 1.5, $CHCl_3$).

IR (Neat): 3397, 2924, 2360, 1496, 1219, 1041, 761 cm^{-1}.

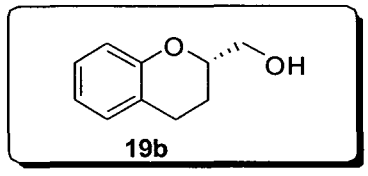
^1H NMR($CDCl_3$, 300 MHz) δ 6.76 (d, 1H, J = 8.8), 6.65 (dd, 1H, J_1= 2.9, J_2 = 8.8), 6.60 (d, 1H, J = 2.8), 4.10–4.02 (m, 1H), 3.87-3.66 (m, 5H), 2.94-2.70 (m, 2H), 2.10 (br, s, 1H), 1.97–1.76 (m, 2H).

^{13}C NMR (75 MHz): δ 153.4, 148.4, 122.4, 117.2, 114.0, 113.3, 76.2, 65.6, 55.7, 24.7, 23.7.

MS (ESI): *m/z* 194 $[M]^+$.

Anal. Calcd for $C_{11}H_{14}O_3$: C, 68.02; H, 7.27. Found: C, 68.11; H, 7.35.

(*S*)-chroman-2-ylmethanol (19b). Starting from 250 mg (1.28 mmol) of **18b**, the title compound was prepared in the same manner as that described for **19a**. Purification of the crude product by silica gel column chromatography (20% ethyl acetate in *n*-hexane) afforded **19b** (188 mg, 89%) as a colorless gum. $[\alpha]_D^{20}$: +110.6 (*c* 1.55, MeOH), (literature[31] $[\alpha]^{25}_D$: +113.4, *c* 1.1, MeOH).



19b

IR (Neat): 3422, 2933, 1651, 1430, 1054, 706 cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ 7.14–7.07 (m, 2H), 6.90–6.84 (m, 2H), 4.18–4.11 (m, 1H), 3.89-3.76 (m, 2H), 2.92-2.81 (m, 2H), 2.02 (br, s, 1H), 1.98–1.86 (m, 2H).

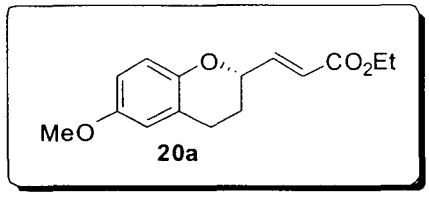
^{13}C NMR (75 MHz): δ 153.2, 128.3, 126.0, 120.6, 119.2, 115.4, 75.1, 64.3, 23.2, 22.4.

MS (ESI): *m/z* 164 $[M]^+$.

Anal. calcd for $C_{10}H_{12}O_2$: C, 73.15; H, 7.37. Found: C, 73.27; H, 7.48.

The above spectral data agreed with literature data.[19]

(*S*,*E*)-ethyl 3-(6-methoxychroman-2-yl)acrylate (20a). To a stirred solution of diol **22a** (100 mg, 0.44 mmol) in methanol (5 mL) at 0°C was added a solution of $NaIO_4$ (144 mg, 0.66 mmol) in water (2 mL). After stirring the reaction mixture at 0°C for 2 h, methanol was evaporated in vacuo at low temperature. The aqueous solution was extracted with CH_2Cl_2 (2x10 mL). The combined organic layers were washed with water (10 mL), brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield the crude aldehyde as a colorless gum which was used for the next step without further purification.



20a

To a solution of the above crude aldehyde in dry CH_2Cl_2 (5mL) was added [(ethoxycarbonyl)methylene]-triphenylphosphorane (235 mg, 0.67 mmol) at room temperature. The reaction mixture was stirred at room temperature for overnight. Solvent was removed under reduced pressure and the residue was purified by silica

gel column chromatography (4% ethyl acetate in *n*-hexane) afforded **20a** (0.10 g, 88%) as a colorless gum. $[\alpha]^{25}_{D}$: +5.4 (*c* 1.21, MeOH).

IR (Neat): 3020, 2400, 1708, 1496, 1426, 1216, 761, 699 cm^{-1}.

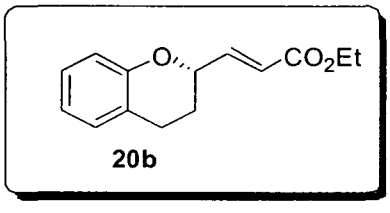
^1H NMR (300 MHz, $CDCl_3$): δ 6.98 (dd, 1H, J_1= 4.1, J_2 = 15.7), 6.78 (d, 1H, J = 8.9), 6.67 (dd, 1H, J_1= 2.8, J_2 = 8.9), 6.56 (d, 1H, J = 2.7), 6.14 (dd, 1H, J_1= 1.7, J_2 = 15.7), 4.69-4.64 (m, 1H), 4.21 (q, 2H, J = 7.1), 3.74 (s, 3H), 2.91-2.69 (m, 2H), 2.16-2.07 (m, 1H), 1.91-1.78 (m, 1H), 1.31 (q, 3H, J = 7.1).

^{13}C NMR (75 MHz, $CDCl_3$+CCl_4): δ 166.0, 153.5, 147.9, 146.1, 121.7, 121.4, 117.3, 113.8, 113.5, 73.7, 60.3, 55.4, 27.0, 24.3, 14.2.

MS (ESI): *m/z* 280 $[M+NH_4]^+$.

Anal. Calcd for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92. Found: C, 68.83; H, 6.88.

(*S*,*E*)-ethyl 3-(chroman-2-yl)acrylate (20b). Starting from 0.10 g (0.51 mmol) of **19b**, the title compound was prepared in the same manner as that described for **20a**.



20b

Purification of the crude product by silica gel column chromatography (4% ethyl acetate in *n*-hexane) afforded **20b** (0.11 g, 90%) as a colorless gum. $[\alpha]^{25}_{D}$: -17.8 (*c* 1.46, MeOH).

IR (Neat): 3020, 2361, 1713, 1480, 1217, 763, 699 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ 7.12-6.96 (m, 3H), 6.86-6.82 (m, 2H), 6.15 (dd, 1H, J_1= 1.8, J_2 = 15.7), 4.75-4.69 (m, 1H), 4.20 (q, 2H, J = 7.2), 2.90-2.70 (m, 2H), 2.17-2.08 (m, 1H), 1.90-1.78 (m, 1H), 1.29 (q, 3H, J = 7.1).

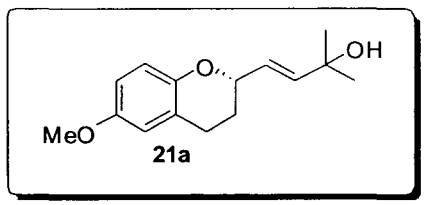
^{13}C NMR (75 MHz, $CDCl_3$): δ 166.2, 153.8, 146.0, 129.4, 127.4, 121.4, 120.5, 116.7, 73.8, 60.4, 26.9, 23.9, 14.1.

MS (ESI): *m/z* 250 $[M+NH_4]^+$.

Anal. Calcd for $C_{14}H_{16}O_3$: C, 72.39; H, 6.94. Found: C, 72.53; H, 6.85.

(*S*,*E*)-4-(6-methoxychroman-2-yl)-2-methylbut-3-en-2-ol (21a). To a freshly prepared, magnetically stirred, ice-cold suspension of methylmagnesium iodide [prepared from iodomethane (0.26 mL, 3.3 mmol) and magnesium (0.080 g, 3.3 mmol) in 10 mL of dry diethyl ether] was added a solution of ester **21a** (75 mg, 0.28 mmol) in dry THF (5 mL). The reaction mixture was refluxed for 4 h, cooled, and

quenched with aqueous NH_4Cl solution (10 mL). The mixture was extracted with ethyl acetate (2x25 mL), washed with water (25 mL) and brine (25 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography (18% ethyl acetate in *n*-hexane) afforded **21a** (66 mg, 93%) as a colorless gum. $[\alpha]^{25}_D$: +13.91 (*c* 1.75, MeOH).



21a

IR (Neat): 3413, 3019, 2361, 1713, 1496, 1216, 758, 699 cm^{-1}.

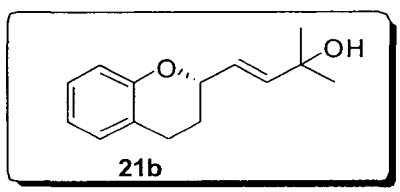
^1H NMR (300 MHz, $CDCl_3$): δ 6.75 (d, 1H, J = 8.9), 6.64 (dd, 1H, J_1= 2.8, J_2 = 8.9), 6.55 (d, 1H, J = 2.8), 5.94 (dd, 1H, J_1= 0.9, J_2 = 15.7), 5.77 (dd, 1H, J_1= 5.8, J_2 = 15.7), 4.46-4.41 (m, 1H), 3.72 (s, 3H), 2.88-2.64 (m, 2H), 2.29 (s, br, 1H), 2.04-1.95 (m, 1H), 1.85-1.72 (m, 1H), 1.34 (s, 6H).

^{13}C NMR (75 MHz, $CDCl_3$+CCl_4): δ 153.0, 148.4, 139.7, 126.2, 121.9, 117.2, 113.8, 113.2, 75.3, 70.3, 55.3, 29.6, 29.5, 27.8, 24.5.

MS (ESI): *m/z* 231 $[M\text{-}OH]^+$, 247 $[M\text{-}1]^+$.

Anal. Calcd for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.43; H, 8.18.

(*S*,*E*)-4-(chroman-2-yl)-2-methylbut-3-en-2-ol (21b). Starting from 75 mg (0.32 mmol) of **20b**, the title compound was prepared in the same manner as that described for **21a**. Purification of the crude product by silica gel column chromatography (18% ethyl acetate in n-hexane) afforded **21b** (67 mg, 95%) as a colorless gum. $[\alpha]^{25}_D$: +7.5 (*c* 0.4, $CHCl_3$).



21b

IR (Neat): 3410, 3019, 2361, 1713, 1586, 1216, 760 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ 7.10-7.01 (m, 2H), 6.84-6.82 (m, 2H), 5.97 (d, 1H, J = 15.8), 5.81 (dd, 1H, J_1= 5.8, J_2 = 15.7), 4.55-4.49 (m, 1H), 2.91-2.70 (m, 2H), 2.08-1.99 (m, 1H), 1.89-1.76 (m, 1H), 1.67 (s, br, 1H), 1.34 (s, 6H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 154.4, 139.9, 129.4, 127.2, 126.2, 121.7, 120.1, 116.7, 75.6, 70.55, 29.3, 29.6, 27.9, 24.3.

MS (ESI): *m/z* 201 $[M\text{-}OH]^+$, 217 $[M\text{-}1]^+$.

Anal. Calcd for $C_{14}H_{18}O_2$: C, 77.03; H, 8.31. Found: C, 77.19; H, 8.47.

3.2.6 References

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3.2.7 Spectra of Selected Compounds

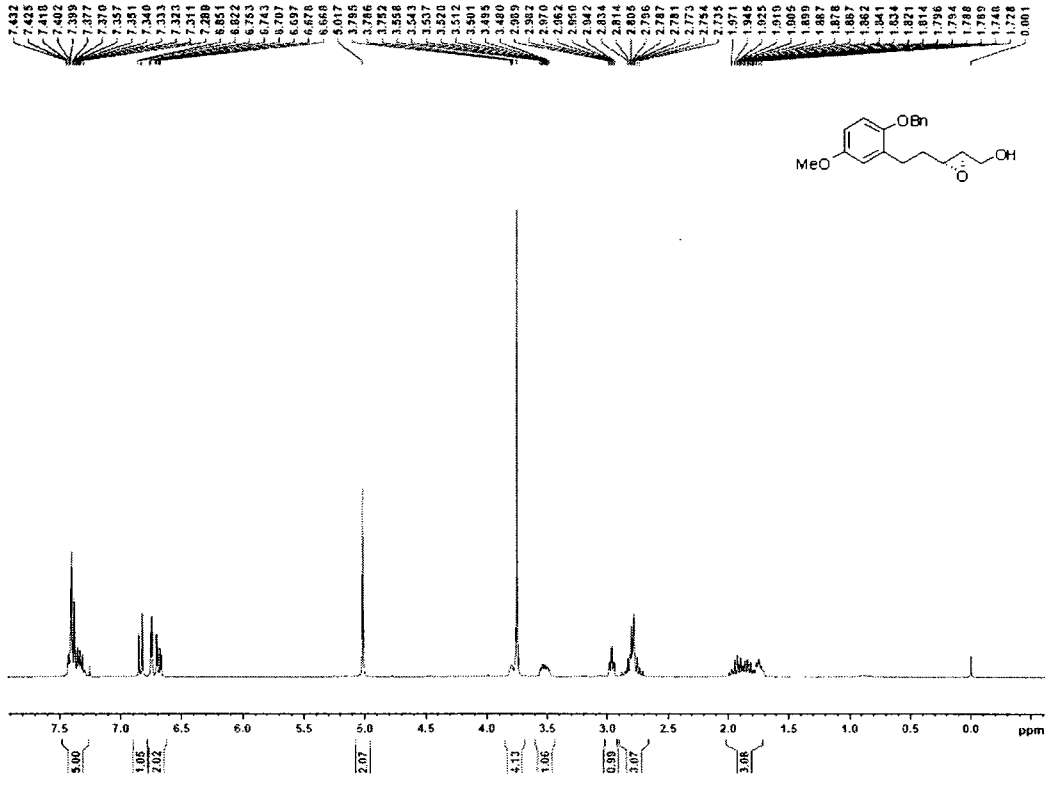


Figure 3.3.4: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **17a.**

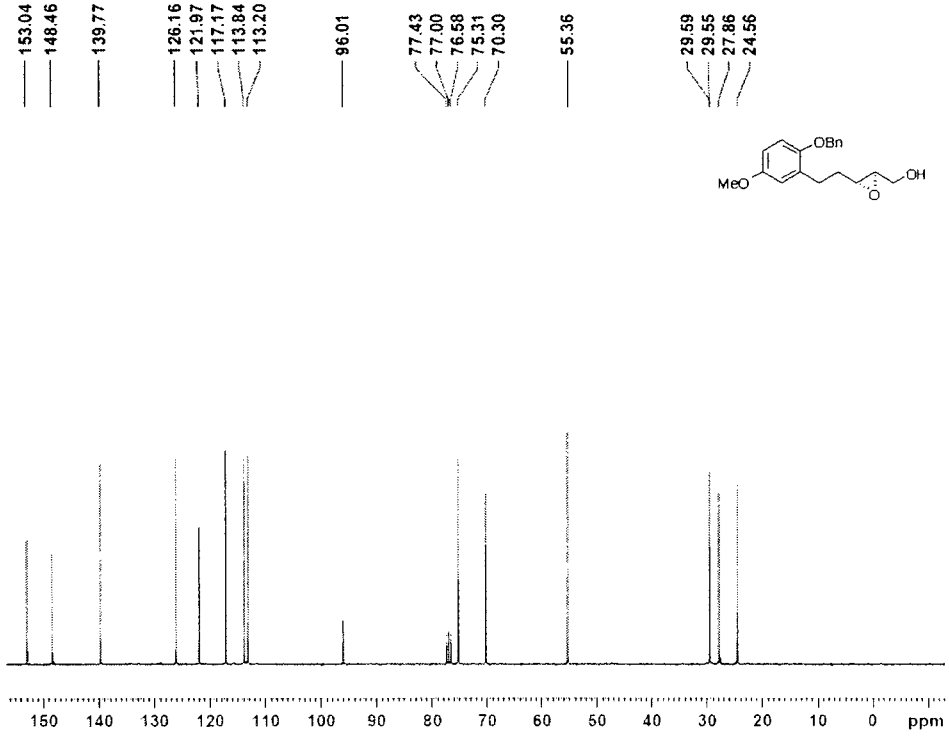


Figure 3.2.5: ^{13}C NMR ($CDCl_3$+CCl_4, 75 MHz) spectrum of **17a.**

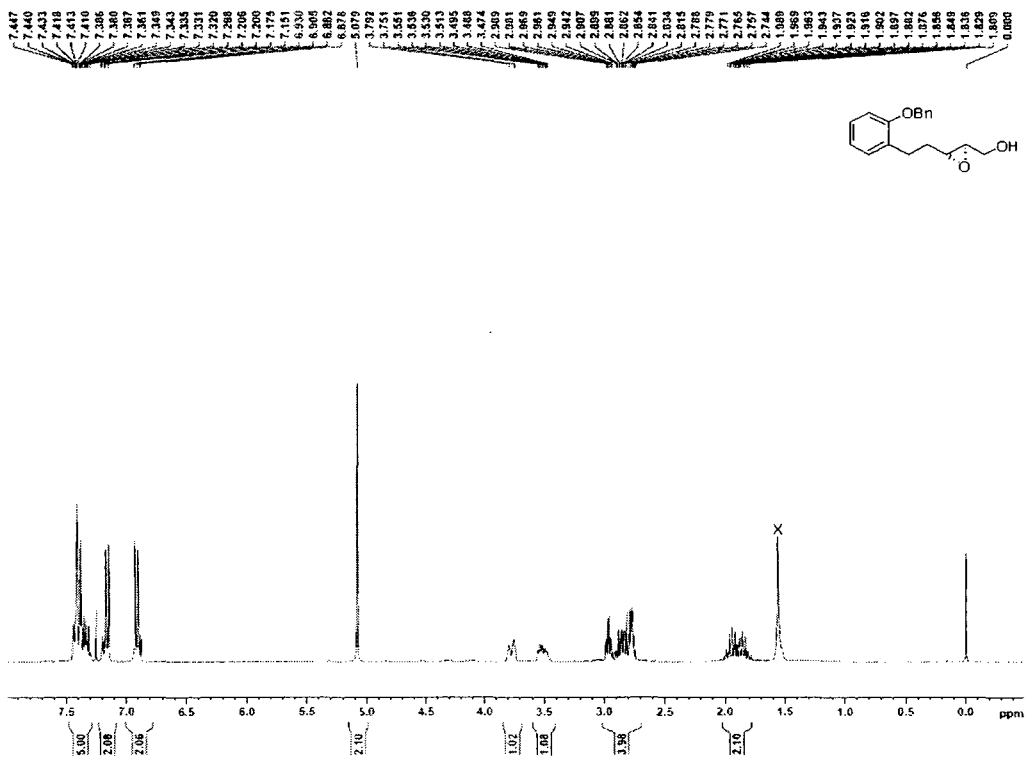


Figure 3.3.6: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **17b.**

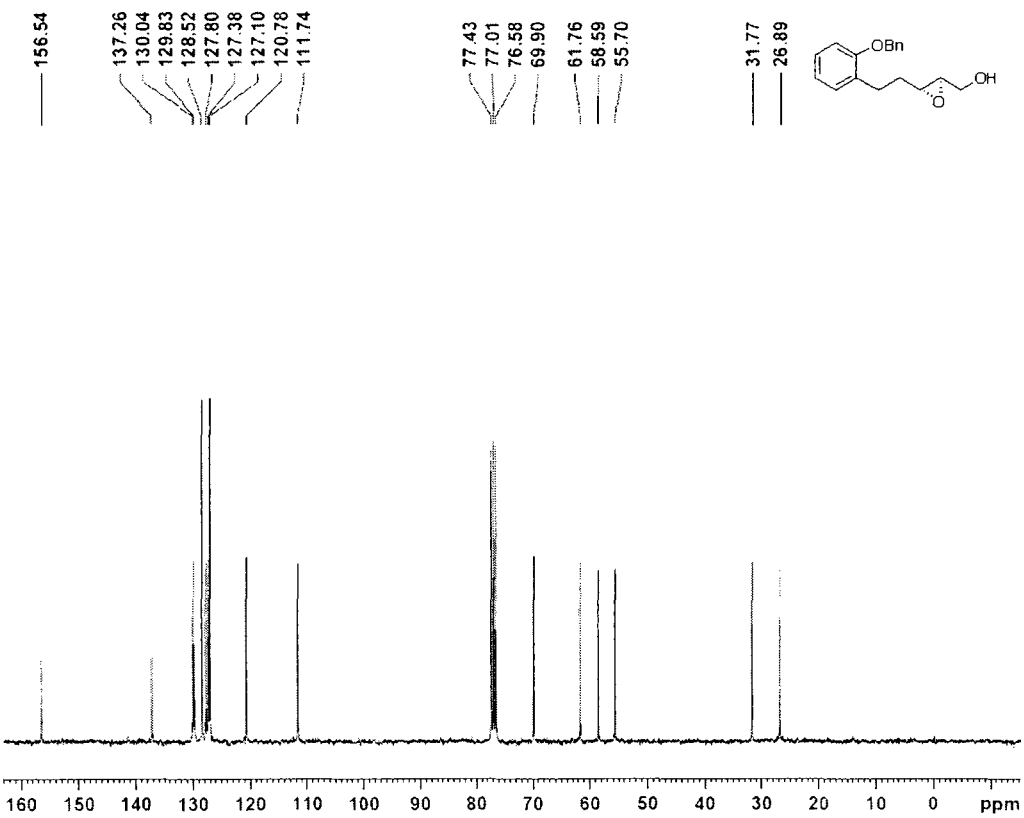


Figure 3.2.7: ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **17b.**

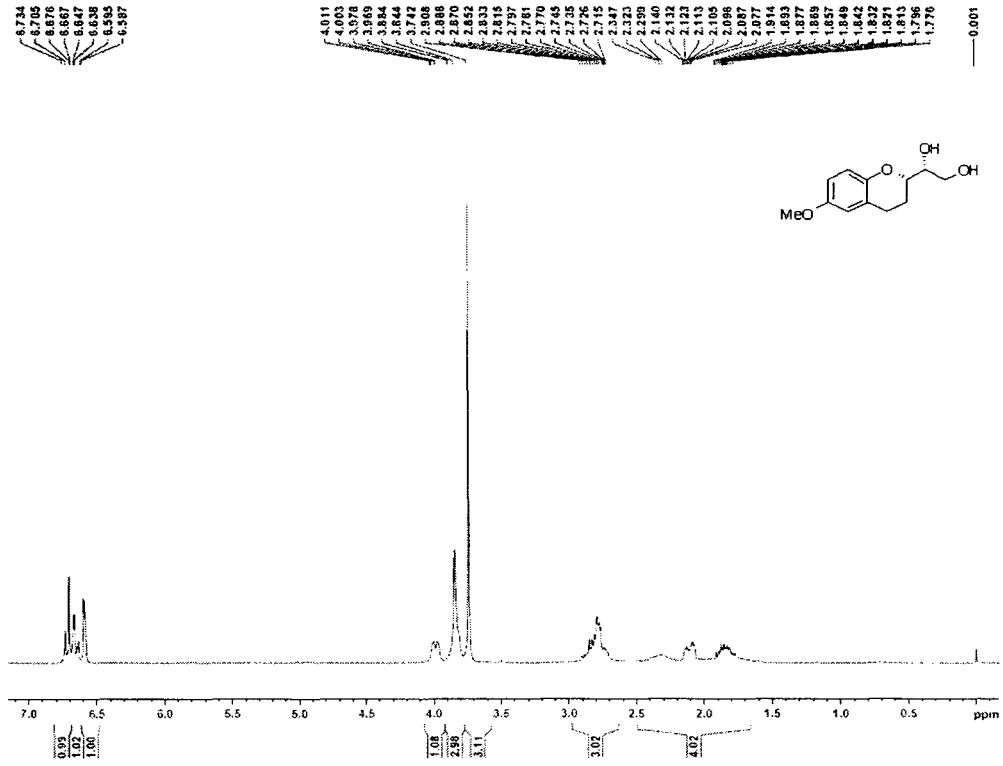


Figure 3.2.8: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **18a.**

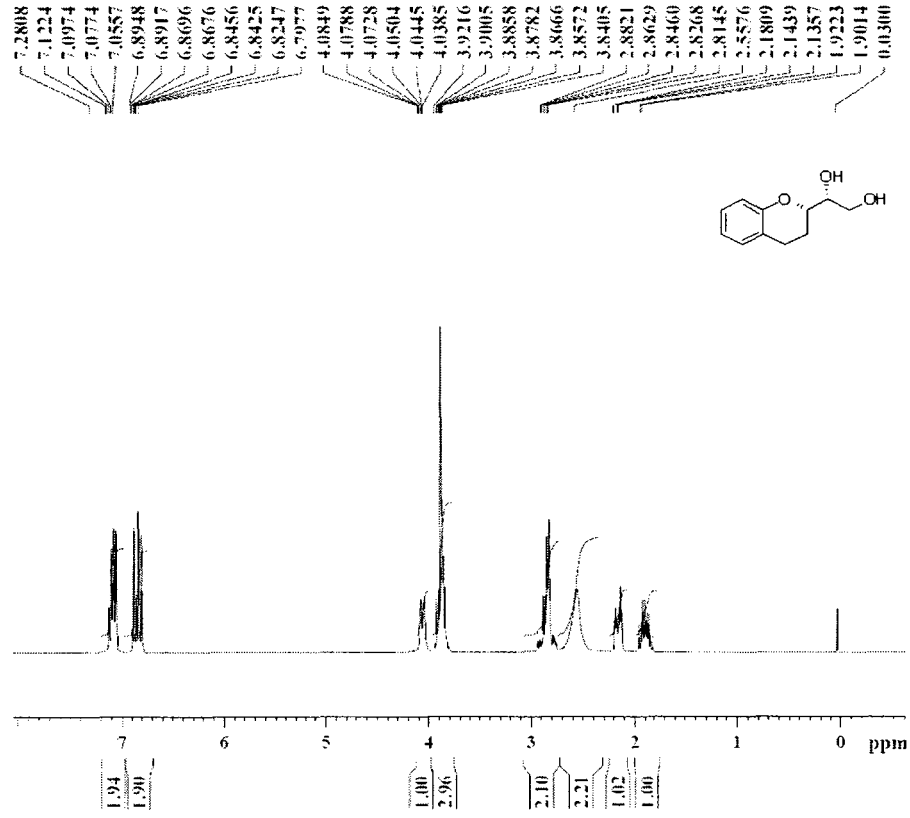


Figure 3.2.9: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **18b.**

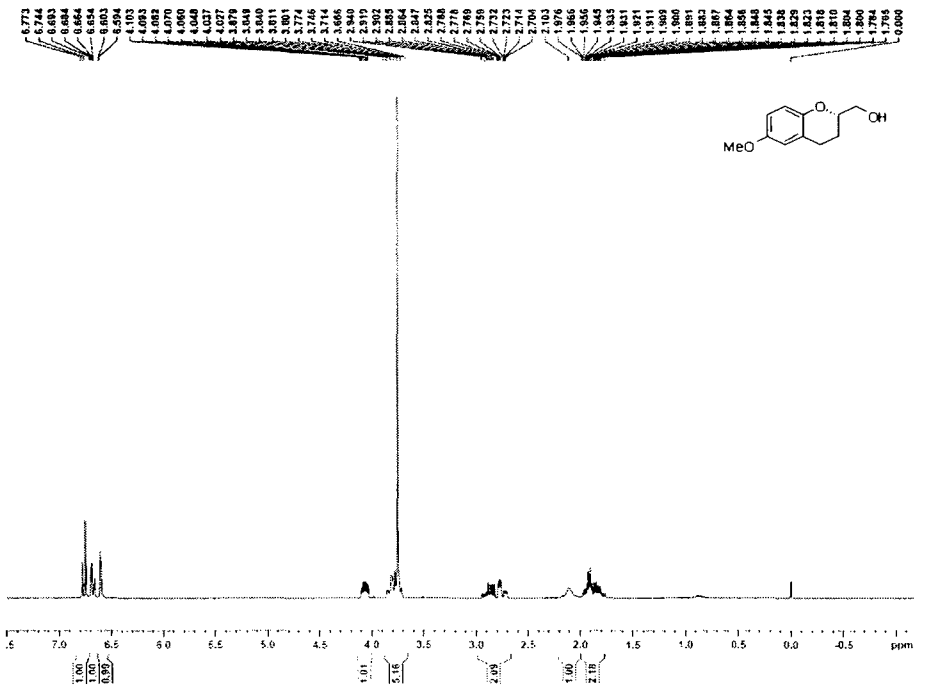


Figure 3.2.10: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **19a.**

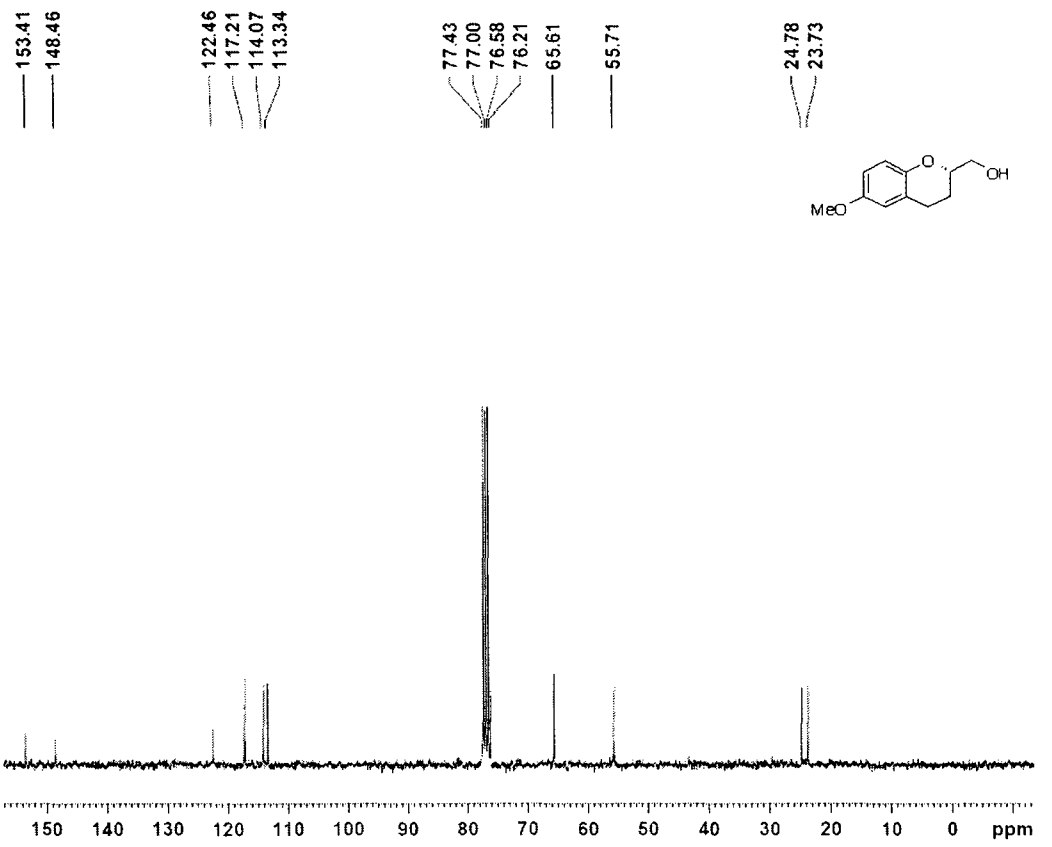


Figure 3.2.11: ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **19a.**

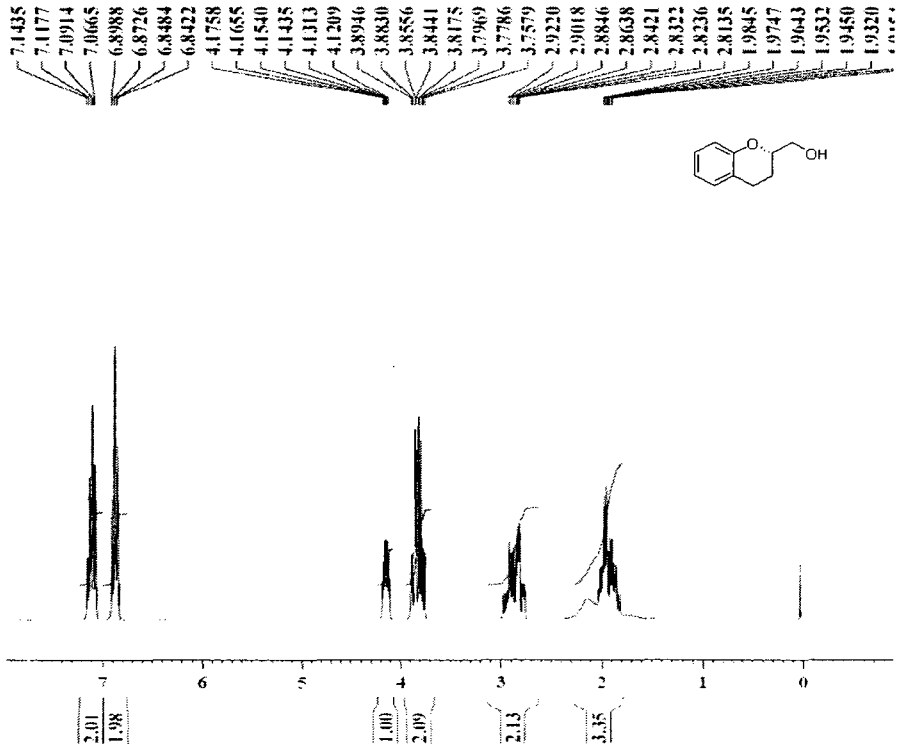


Figure 3.2.12: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **19b.**

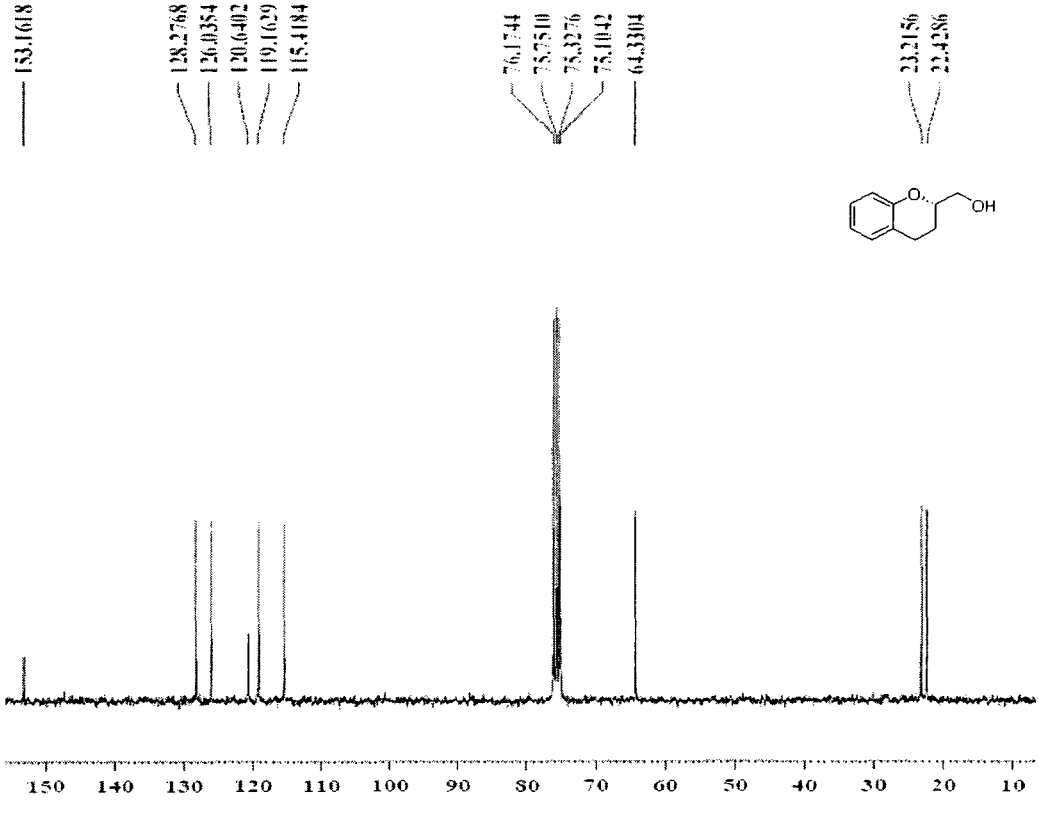


Figure 3.2.13: ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **19b.**

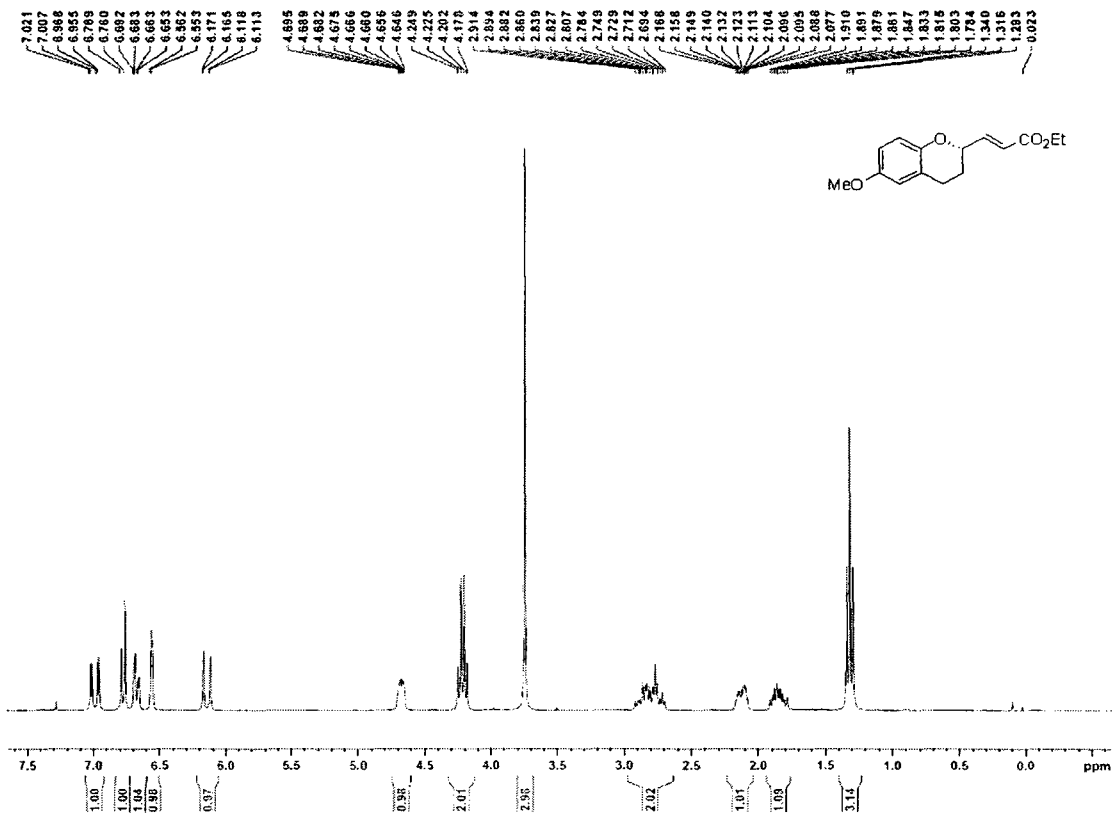


Figure 3.2.14: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **20a.**

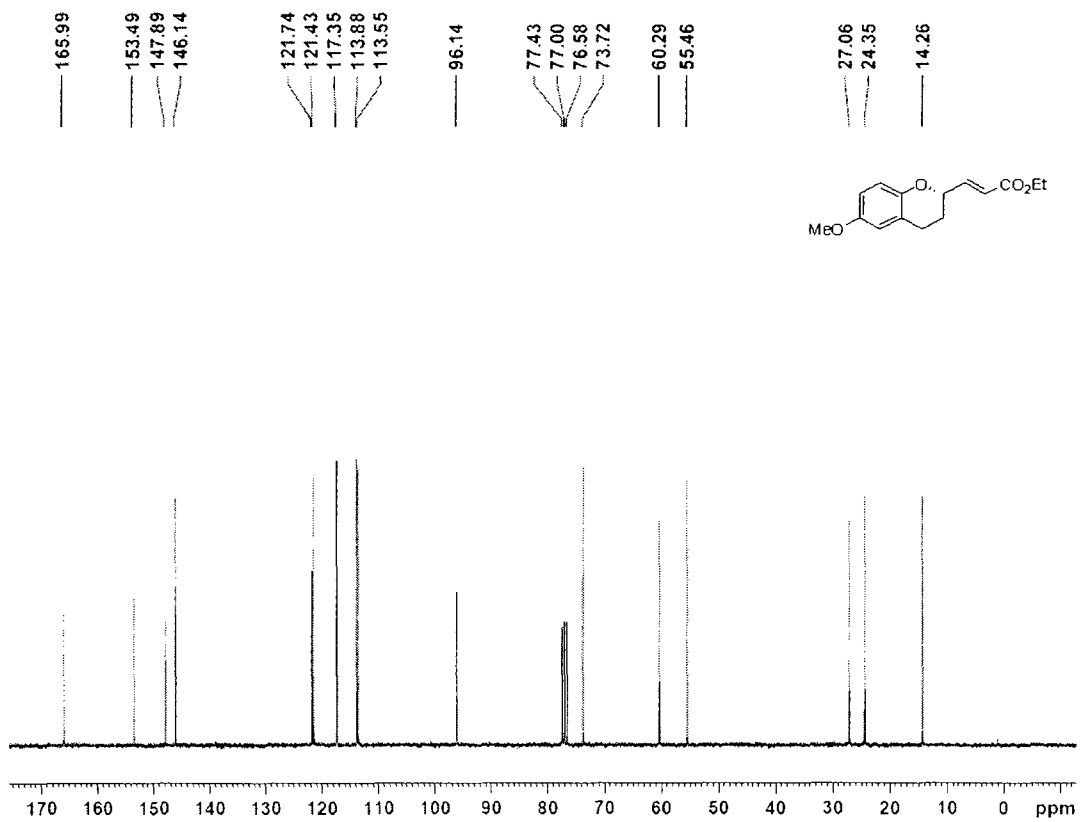


Figure 3.2.15: ^{13}C NMR ($CDCl_3$+CCl_4, 75 MHz) spectrum of **20a.**

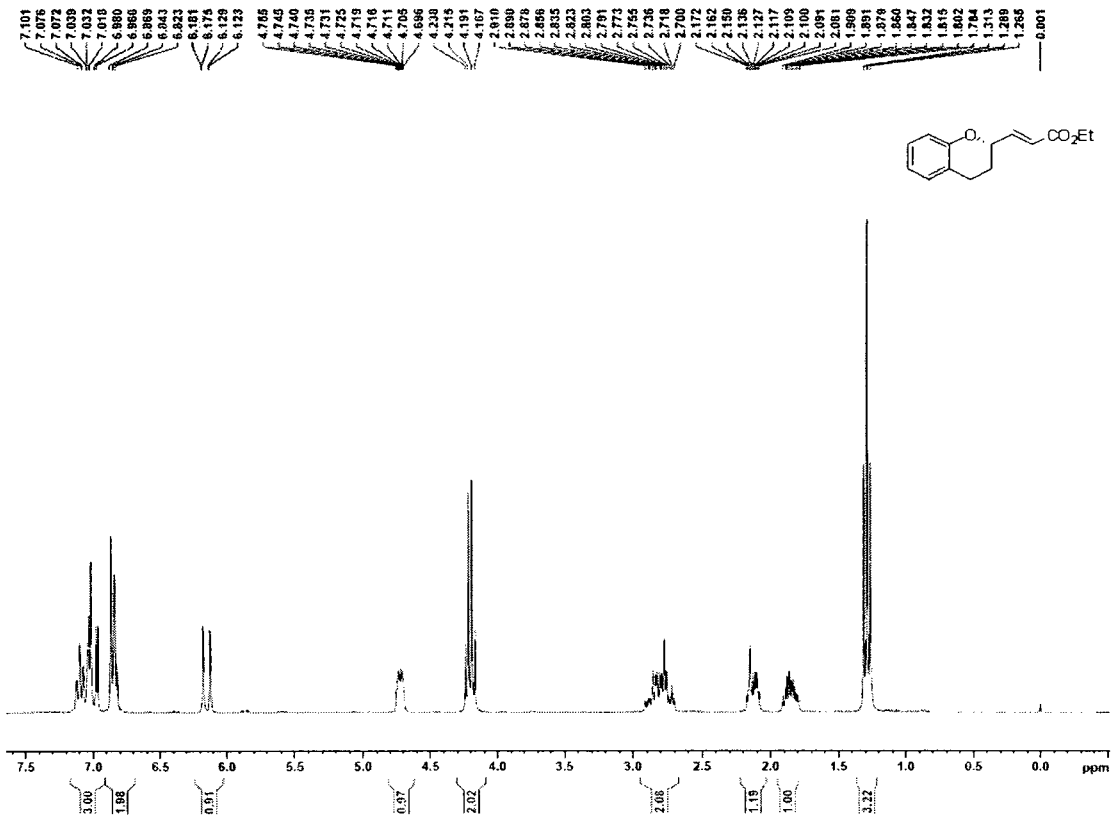


Figure 3.2.16: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **20b.**

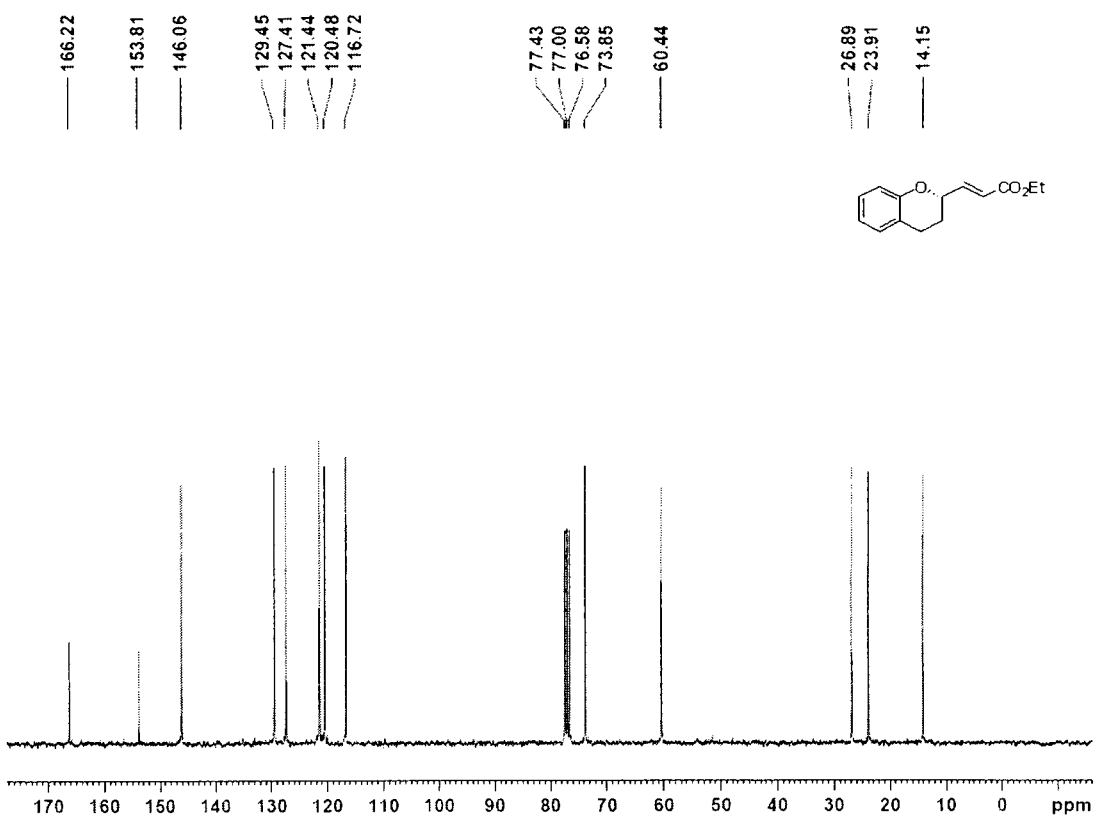


Figure 3.2.17: ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **20b.**

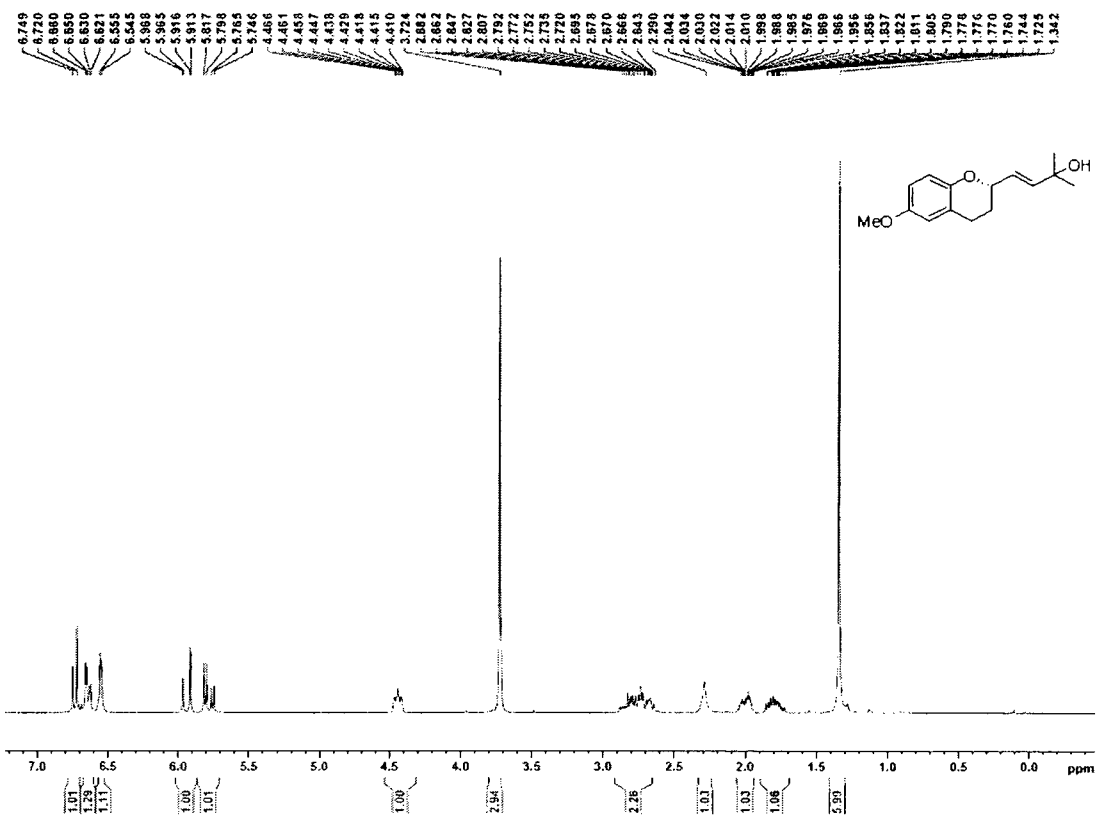


Figure 3.2.18 ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **21a.**

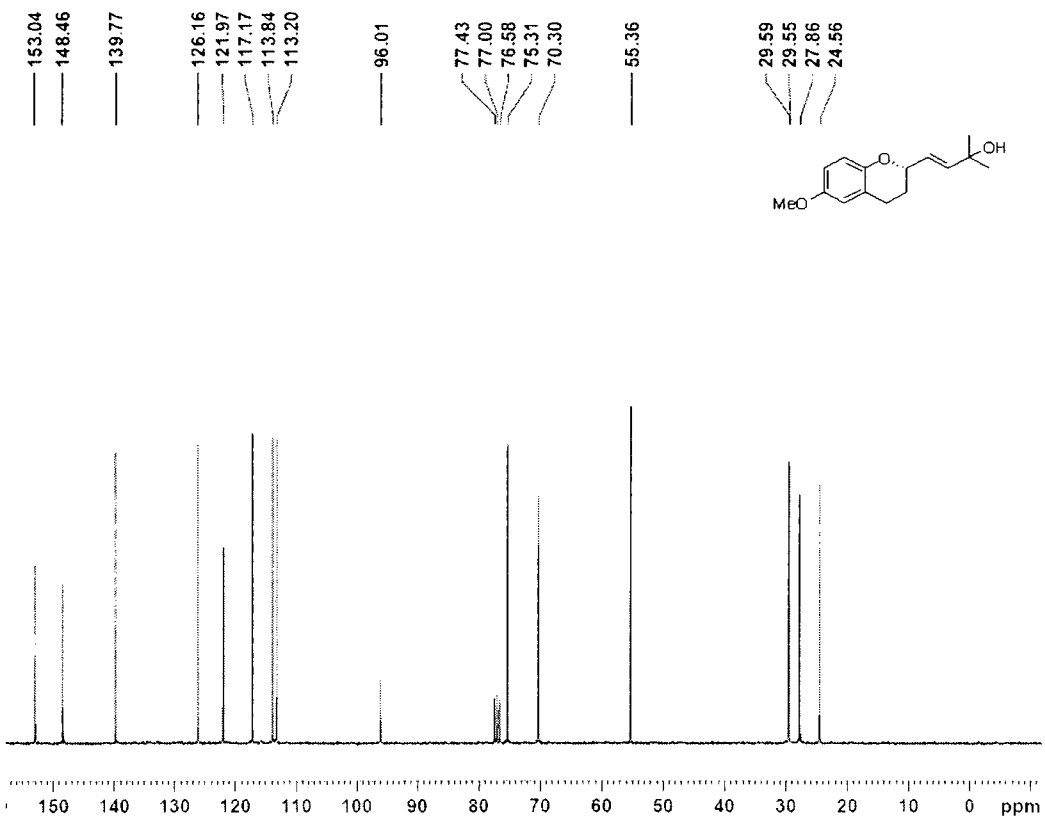


Figure 3.2.19: ^{13}C NMR ($CDCl_3$+CCl_4, 75 MHz) spectrum of **21a.**

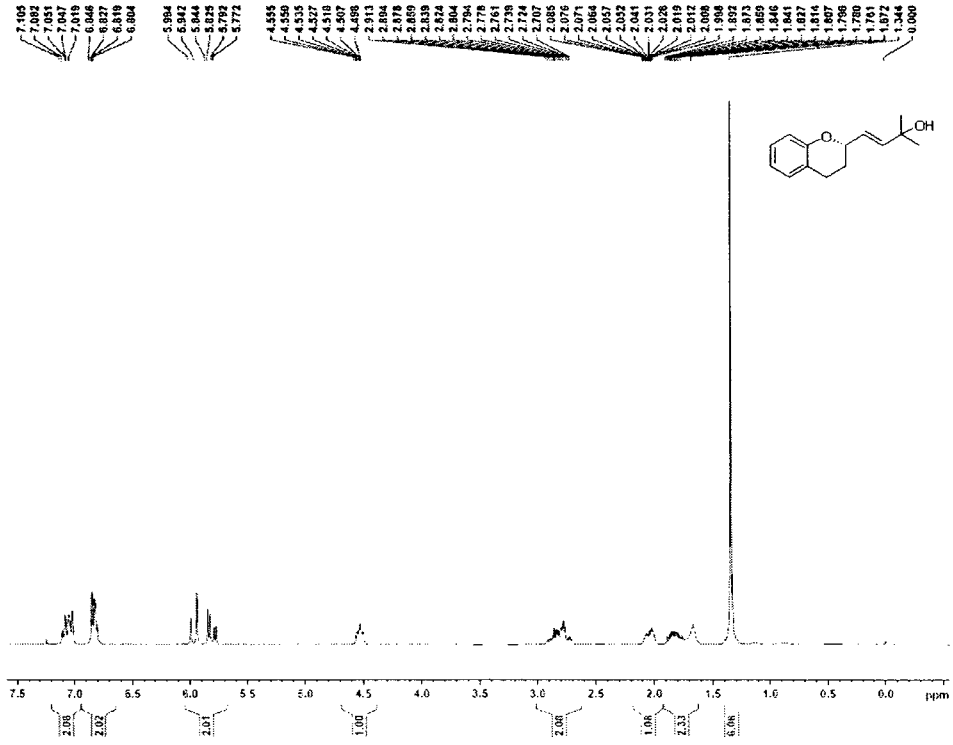


Figure 3.2.20: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **21b.**

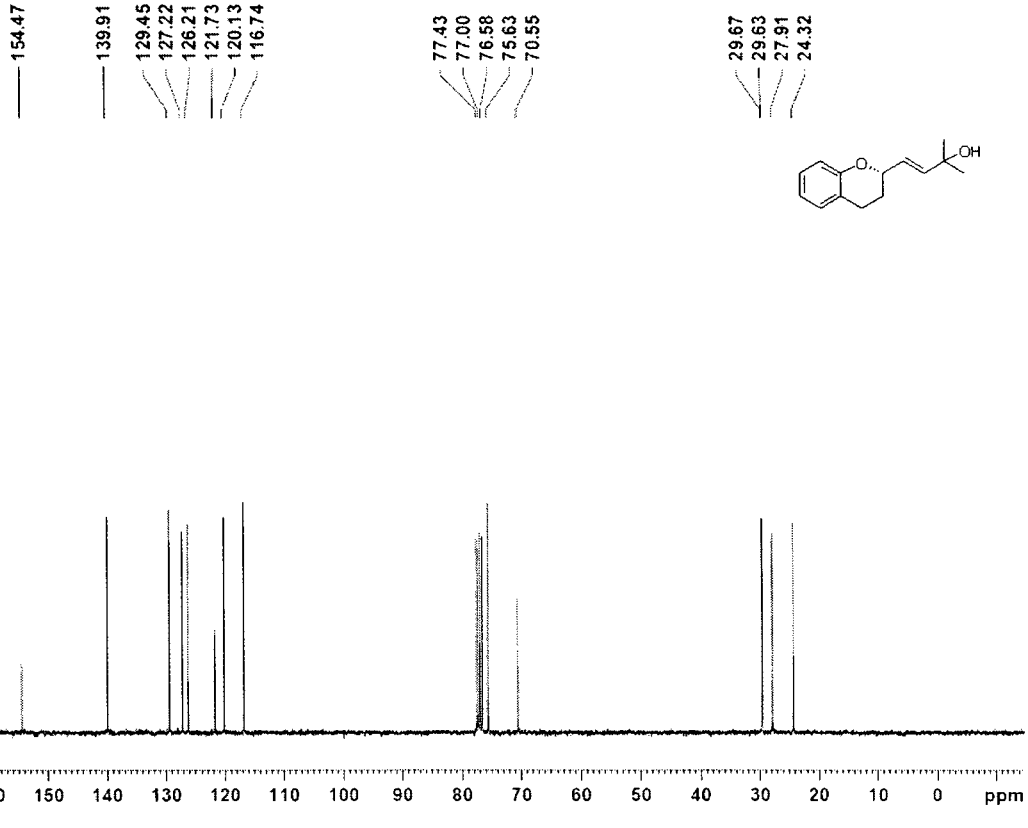


Figure 3.2.21: ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **21b.**

Chapter 4:
Asymmetric Total Syntheses of Spisulosine, Its Diastereo- and Regioisomers

4.1 Introduction

Marine natural products and their analogues, which have been shown to display huge structural diversity normally associated with important biological activity, sustain to be a source of potential pharmaceutical leads and therapeutic agents as well as inspiration for developing new synthetic strategies for their construction[1]. 2-Amino-3-alkanols are commonly found in tunicates and some sponges. The structures of these molecules are generally related to the widely distributed amphiphilic sphingosine, the central structural element of sphingolipids; their carbon-chain length varies from C_{12} to C_{30}, and polyunsaturated variants have been also reported. Ascidians from *Pseudodistoma* and *Clavelina* genera have been prolific in the production of 2-amino-3-hydroxyhydrocarbons. Examples are crucigasterins 277, 275, and 225,[2] obscuraminols A–F,[3] clavaminols A–F,[4] and (2*S*,3*R*)-2-aminododecan-3-ol.[5] Spisulosine **1** (ES-285) (Figure 4.1) is a marine-derived[6] sphingoid-type base containing a long saturated C_{18} alkyl chain with an erythro-1,2-amino alcohol, and the antiproliferative activity[7] of its hydrochloride has included it in clinical trials as a potential antitumoral agent.

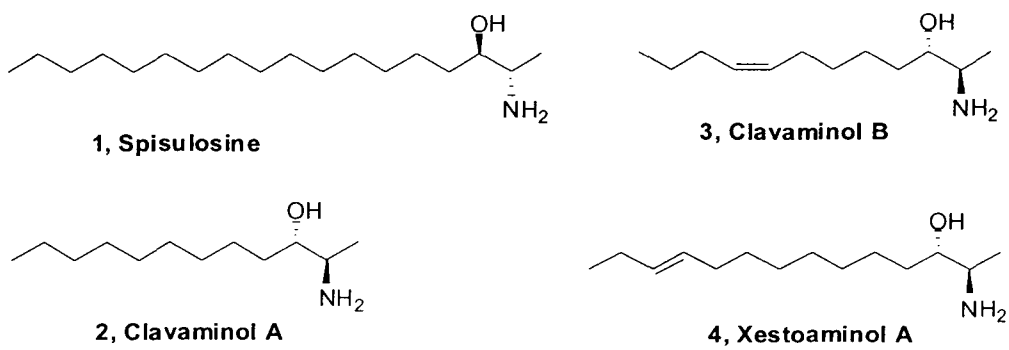


Figure 4.1. Spisulosine **1** and some other natural long chain 2-amino-3-alkanols

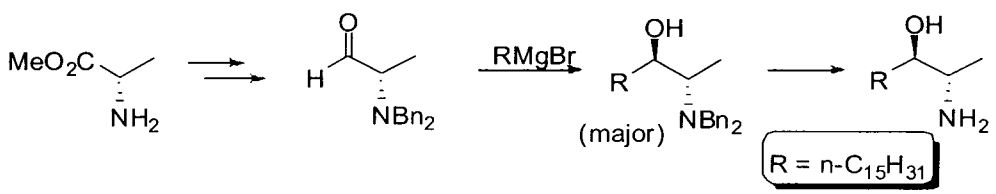
This compound caused a loss of actin stress fibers[7]. It has demonstrated *in vitro* antiproliferative activity against a variety of human tumor cell lines, such as colon, gastric, pancreas, pharynx and renal tumors, but a particular selectivity for hepatomas and slowly growing tumors was noted.[8c] During *in vivo* studies in mice significant tumor growth inhibition was observed by ES-285 of human renal tumors, melanoma and prostate tumors.[8c] Cultured cells showed altered morphology on treatment with Spisulosine (ES-285). Studies confirmed that Spisulosine works on the cell's

microfilaments but not on the microtubule network.[7,8] It inhibits the growth of the prostate tumor cell lines PC-3 and LNCaP and could have a role as prostate anti-tumoral agent[9]. It exhibited in vitro activity against HT-29 (0.05 l μg/mL; P-388 (0.01 μg/mL) and MEL-28 (0.05 μg/mL) tumor cell lines.[6]

4.2 Basis of the Present Work

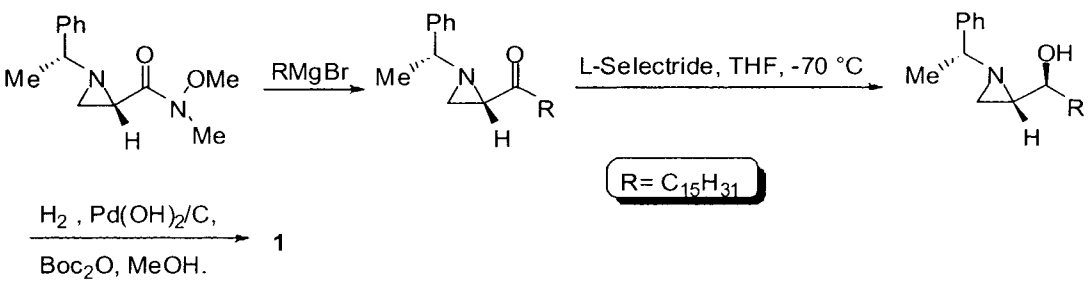
The structural simplicity associated with the antiproliferative activity of **1** has stimulated several elegant and efficient approaches for its total synthesis.[10,11] However, a synthetic protocol to obtain this valuable compound starting from a cheap and readily available precursor would be interesting. The chemistry of **1** and the corresponding *syn*-diastereomer dates back to 1957 when Croatian researchers synthesized these two molecules for the first time in the determination of absolute configurations of lipid bases with two or more asymmetric carbon atoms.[10a] In this context it is important to mention that in the above synthesis, **1** was known as a purely synthetic molecule as its isolation as a natural product became known afterwards.[7]

In recent time, efficient synthesis of spisulosine **1** and related compounds were achieved by a diastereoselective addition reaction of appropriate long alkyl chain Grignard reagents on L-alanine derived *N*-protected amino aldehyde derivative (Scheme 4.1).[11]



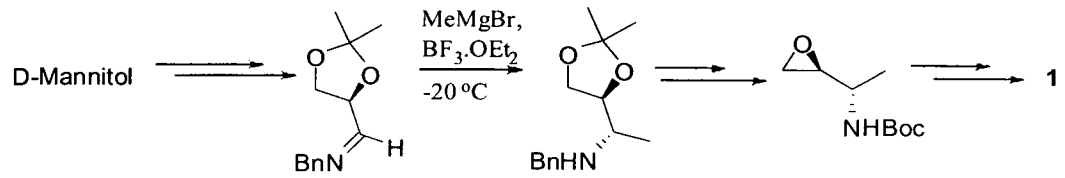
Scheme 4.1. Synthesis of spisulosine and related molecules from L-alanine

Lee and co-workers reported asymmetric synthesis of *N*-Boc-spisulosine from an enantiomerically pure 2-acylaziridine derivative (Scheme 4.2).[10b]



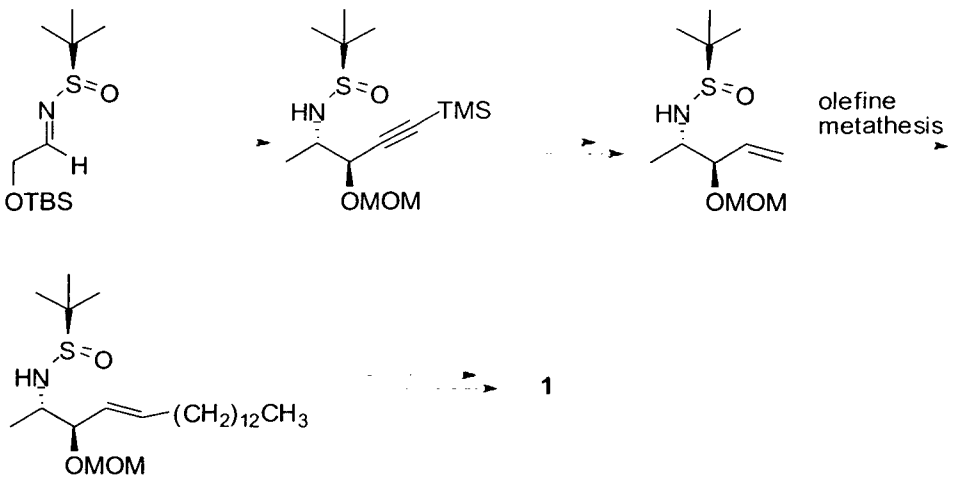
Scheme 4.2. Synthesis of *N*-Boc-spisulosine by the Lee group

Very recently, Galvez and co-workers reported asymmetric synthesis of the hydrochloride salt of spisulosine from D-mannitol (Scheme 4.3).[10c]



Scheme 4.3. Synthesis of spisulosine starting from the inexpensive precursor D-mannitol

Another recent report of synthesis of **1** involved the stereoselective addition of a racemic 3-alkoxy allenylzinc to enantiopure *N*-tert-butylsulfinyl imines and a cross-metathesis reaction as the key steps (Scheme 4.4).[10d]

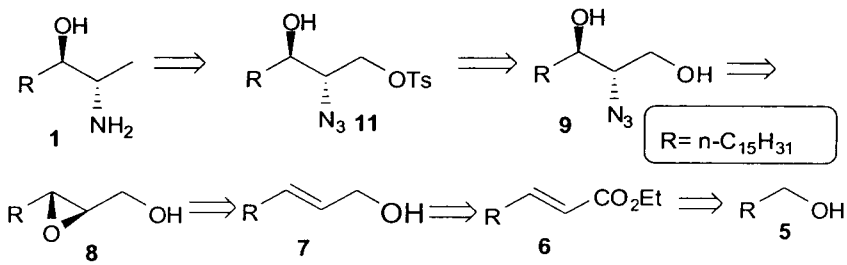


Scheme 4.4. Synthesis of spisulosine starting from *N*-tert-butylsulfinyl imines

In this **Chapter**, we herein report the first protecting group-free syntheses of spisulosine **1** and its diastereo- and regioisomers employing Sharpless asymmetric epoxidation reaction as the source of chirality.

4.3 Results and Discussion

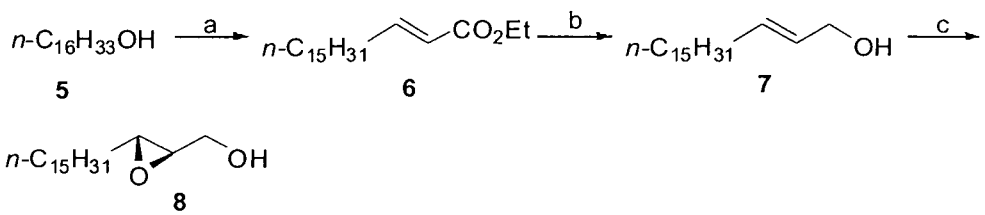
Total synthesis of natural or synthetic biologically important molecules employing a protecting-group-free strategy has been an important challenge in organic chemistry.[12] Our envisioned retrosynthetic analysis for the protecting group-free enantioselective preparation of spisulosine is depicted in Scheme 4.5.



Scheme 4.5. Retrosynthetic analysis of spisulosine **1**

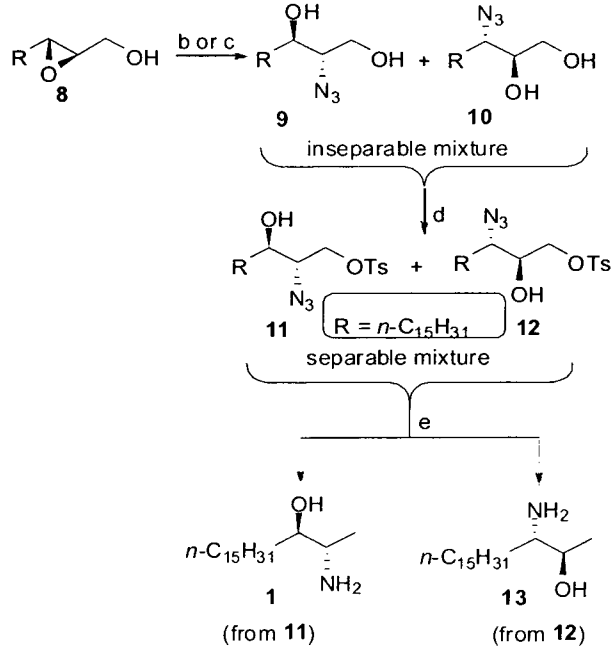
The target molecule **1** was anticipated to be prepared by one-pot reduction of azido tosylate **11** which, in turn, could be derived from azido diol **9**. The compound **9** could be obtained from epoxy alcohol **8** by the trialkyl borate mediated C-2 selective azidolysis under Miyashita's condition.[13] Although Miyashita's pioneering C-2 selective nucleophilic substitution reactions including the azidolysis have been utilized for natural product synthesis,[14] it has never been employed in protecting group-free synthesis of a target molecule. Epoxy alcohol **8** could be derived from allylic alcohol **7** which, in turn, could be derived from alcohol **5** via α,β-unsaturated ester **6**.

We started by Swern oxidation of commercial palmityl alcohol **5** (Scheme 4.6) to provide 1-hexadecanal which was used directly in the subsequent Wittig reaction without further purification to avoid decomposition. Thus, reaction of the above crude aldehyde with $Ph_3P{=}CHCO_2Et$ in dry CH_2Cl_2 furnished the desired *trans*-α,β-unsaturated ester **6** in high yield (80% from **5**). Next, DIBAL-H reduction of **6** in dry toluene furnished the corresponding *trans*-α,β-unsaturated alcohol **7** in excellent yield. Sharpless asymmetric epoxidation[15] of **7** with D-(-)-diethyl tartrate was then performed to get epoxy alcohol **8** in 85% yield, which showed >98% ee by ^1H NMR analysis of the corresponding Mosher ester derivative.



Scheme 4.6. *Reagents and conditions*: (a) (i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -78 to 0 °C, 2 h. (ii) $Ph_3P{=}CHCO_2Et$, dry CH_2Cl_2, 8 h, 80% (for two steps). (b) DIBAL-H (1 M in toluene), dry toluene, 0 °C-rt, 1 h, 95%.

Next, C-2 selective azidolysis of enantiomerically pure epoxide **8** by sodium azide in dry DMF in the presence of trimethylborate furnished mixture of azidodiols **9** (major) and **10** (minor) (Scheme 4.7). Unfortunately, these two regioisomers did not show any separation on TLC, and an attempt to purify by column chromatography was also unsuccessful. Miyashita and co-workers isolated the regioselective C-2 substituted products by $NaIO_4$-mediated oxidative cleavage of the corresponding C-3 substituted minor product (thus, removing the minor azidodiol as one-carbon degraded less polar aldehydes). However, we were also interested in synthesizing the regioisomer of **1,** in which case compound **10** was needed as an intermediate. Thus, utilization of the $NaIO_4$-mediated oxidative cleavage reaction in a mixture of **9** and **10** was not a desired option for us. We were delighted to see that the corresponding tosylates **11** and **12**, which were obtained by tosylation of a mixture of **9** and **10** with 1.1 equivalent of tosyl chloride in dry CH_2Cl_2 in the presence of triethylamine at 0 °C, were completely separable by silica gel column chromatography.



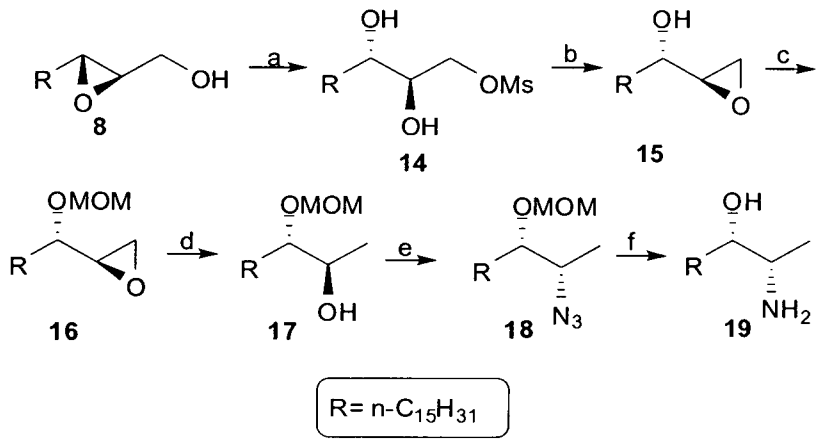
Scheme 4.7. *Reagents and conditions*: (a) D-(-)-DET, Ti(O*i*-Pr)$_4$, TBHP, CH_2Cl_2, –25 °C, 24 h, 85%.(b) NaN_3, $(MeO)_3B$, DMF, 50 °C, 5 h. (c) NaN_3, NH_4Cl, MeOH/H_2O(8:1), 80 °C, 18 h. (d) TsCl (1.1 equiv.), CH_2Cl_2, anhyd. Et_3N, 0 °C, 6 h, 65% (for **11**, using reaction condition "b"), 67% (for **12**, using reaction condition "c"); for combined two steps. (e) $LiAlH_4$, THF, 0 °C-rt, 5 h, 70% (**1**), 62% (**13**).

Amounts of isolated **11** and **12** suggested that in the azidolysis reaction compounds **9** and **10** were formed in 85:15 ratio. Subsequently, compound **11** was treated with $LiAlH_4$ in THF to get spisulosine **1** in 70% yield. Although the primary alcohol functionality of azidodiol was selectively converted to its tosylate, this was not protection of functional group, rather functional group interconversion, because subsequent treatment with LAH resulted in reduction of azide along with simultaneous conversion of $-CH_2OTs$ into $-CH_3$.Thus, this synthetic route of spisulosine did not require any protecting group.

Since the above synthetic route allowed to access compound **12** only in minor amount, it was necessary to search for an alternative route for getting sufficient amount of **12** which could allow the synthesis of the regioisomer of **1**. Towards that objective, epoxide **13** was subjected to the standard azidolysis ring opening reaction[16] carried out with NaN_3/NH_4Cl in an 8:1 MeOH and H_2O solution to get **10** (major) and **9** (minor amount), Scheme 5. Next, tosylate **12** was isolated in pure form in the similar way as that is described for **11**. Treatment of compound **12** with $LiAlH_4$ in THF furnished aminoalcohol **13** in 62% yield.

Next, we turned our attention towards the synthesis of a diastereoisomer **19** of spisulosine with the (2*S*,3*S*) configuration. Towards that objective, the hydroxyl group of epoxide **8** was mesylated and the resulting epoxy mesylate was then treated with perchloric acid to afford dihydroxy mesylate **14** (Scheme 4.8).[17] Treatment of **14** with anhyd. K_2CO_3 in dry methanol gave epoxy alcohol **15** in 89% yield. With the enantiomerically pure epoxy alcohol **15** in hand, our next target was to convert it into **19**. Towards that objective, hydroxyl group of **15** was protected as methoxymethyl ether with MOM-Cl in CH_2Cl_2 in the presence of *N*,*N*-diisopropylethylamine to give compound **16** in 98% yield. Next, regioselective reductive ring opening of the epoxide ring of **16** with $LiAlH_4$ in THF at 0 ^{0}C provided **17** in 95% yield. Subsequent tosylation of alcohol **17** with tosyl chloride in the presence of triethylamine (1.5 equiv.) and a catalytic amount of DMAP furnished the corresponding tosylate which was used for the next step without further purification. Thus, the above crude tosylate on refluxing with NaN_3 in dry DMF gave the azide **18** in good yield. Finally, reduction of the azido group of **18** by hydrogen in the presence of 10% Pd-C in

methanol followed by deprotection of MOM group of the resulting amine derivative with conc. HCl furnished the diastereomer **19**.



Scheme 4.8. *Reagents and conditions*: (a) (i) MsCl, Et_3N, CH_2Cl_2, 0 °C; (ii) $HClO_4$, DMSO, 60 °C, 87% (for two steps). (b) K_2CO_3, MeOH, rt, 89%. (c) MOMCl, *i*-DIPEA, CH_2Cl_2, 0 °C-rt, 12 h, 96%. (d) $LiAlH_4$, THF, 0 °C-rt, 5 h, 94%. (e) (i) TsCl, CH_2Cl_2, anhyd. Et_3N, DMAP (cat.), 0 °C-rt, 48 h. (ii) NaN_3, DMF, 90 °C, 5 h, 71% (over two steps). (f) (i) H_2/Pd-C, methanol, 6 h, rt. (ii) Conc. HCl, methanol, 75% (over two steps).

4.4 Conclusion

In conclusion, starting from commercially available palmityl alcohol, new asymmetric total syntheses of spisulosine **1** and its regio- and diastereomers have been developed. Notable features of this approach include the use of Sharpless asymmetric epoxidation reactions to synthesize the enantiomerically pure epoxy alcohol and regioselective epoxide azidolysis for the first protecting group free synthesis of **1**. The other merits of this synthesis are high-yielding reaction steps, high enantioselectivity and various possibilities available for structural modification and thus it might be considered as a general synthetic strategy to enantiomerically pure 2-amino-3-alkanols.

4.5 Experimental Section

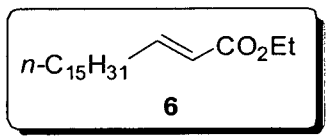
4.5.1 General Remarks

As that described in **Section 3A** of **Chapter 3**.

4.5.2 Synthesis of Compounds

Ethyl (2*E*)-octadec-2-enoate (6): To a stirring solution of oxalyl chloride (4.53 g, 35.69 mmol) in dry CH_2Cl_2 (50 mL) at -78 °C was added dropwise dry DMSO (5.6

mL, 71.35 mmol) in CH_2Cl_2 (20 mL). After 30 min, alcohol **5** (5.76 g, 23.76 mmol) in



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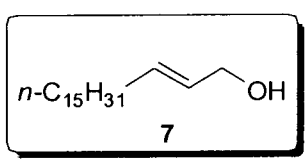
CH_2Cl_2 (20 mL) was added over 10 min giving copious white precipitate. After stirring for 1 h at -78 °C the reaction mixture was brought to -60 °C and anhyd. Et_3N (13.26 mL, 95.12 mmol) was added slowly and stirred for 30 min allowing the reaction mixture to warm to room temperature. The reaction mixture was then diluted with water (75 mL) and CH_2Cl_2. The organic layer was separated and washed with water and brine, dried (Na_2SO_4) and passed through short pad of celite. The filtrate was concentrated to give the aldehyde as a pale yellow oil, which was used as such for the next step without purification.

To a stirring solution of the above crude aldehyde in dry CH_2Cl_2 was added (ethoxycarbonylmethylene)triphenylphosphorane (9.25 g, 26.54 mmol) and the reaction mixture was stirred for 8 h at rt. It was then concentrated and purification of the crude product by silica gel column chromatography (2% ethyl acetate in *n*-hexane) afforded **6** (5.90 g, 80%) as a colorless liquid. R_f: 0.54 (5% ethyl acetate in n-hexane). IR (Neat): 1716, 1654 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.01-6.91 (m, 1H, H_3), 5.80 (d, 1H, J= 15.4, H_2), 4.17 (q, 2H, J = 7.2, CH_2), 2.18 (br q, 2H, J =6.9, H_4), 1.47-1.26 (m, 29H), 0.87 (t, 3H, J= 6.5, CH_3). MS (ESI): *m/z* 311 $[M]^+$. Anal. Calcd for $C_{20}H_{38}O_2$: C, 77.36; H, 12.33. Found: C, 77.51; H, 12.47. The above spectroscopic data are in consistence with the literature data.[18]

(2*E*)-Octadec-2-en-1-ol (7): To a stirring solution of 3.62 g (11.66 mmol) of **6** in 40 mL of dry toluene was added dropwise 35 mL (35.0 mmol) of DIBAL-H (1 M



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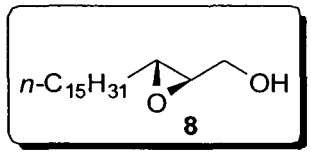
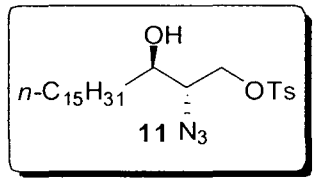
in toluene) at 0 °C. The resulting mixture was allowed to warm to room temperature over 1 h, then quenched by adding water (50 mL). The resulting gel was dissolved by dropwise addition of 6N HCl. The organic layer was separated, and the aqueous phase was extracted with ethyl acetate (2x50 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ solution (75 mL), then dried over dried over anhydrous Na_2SO_4, filtered, and then concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography (8% ethyl acetate in *n*-hexane) afforded **7** (2.97 g, 95%) as a colorless solid. M.p.: 46-48 °C. R_f: 0.54 (20% ethyl acetate in n-hexane). IR (Neat): 3420, 2926, 2854, 1637, 1463, 1216, 972, 766 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 5.75-5.55 (m,

2H, $H_{2,3}$), 4.08 (d, 2H, J = 4.9, H_1), 2.04 (br q, 2H, J =6.3, H_4), 1.48 -1.25 (m, 27H), 0.86 (t, 3H, J = 6.5, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 133.5, 128.8, 63.8, 32.2, 31.9, 29.7, 29.6, 29.4, 29.3, 29.1, 22.7, 14.1. MS (ESI): *m/z* 268 $[M]^+$. Anal. Calcd for $C_{18}H_{36}O$: C, 80.53; H, 13.52. Found: C, 80.42; H, 13.66. The above spectroscopic data are in consistence with the literature data.[19]

{(*2R,3R*)-3-pentadecyloxiran-2-yl}methanol (8): Freshly distilled $Ti(OPr^i)_4$ (3.9 mL, 13.1 mmol) was added to CH_2Cl_2 and the resulting solution was cooled to -25 °C. D-(-)-DET (3.0 mL, 17.4 mmol) was then added. The resulting mixture was then stirred for 20 min, and then a solution of **7** (2.92 g, 10.9 mmol) in CH_2Cl_2 (20 mL) was added. 20 min later TBHP (5.6 M in *n*-decane, 5.8 mL, 32.7 mmol) was added and then the reaction mixture was stored in the -20 °C refrigerator for 24h. The reaction was then quenched by addition of Me_2S (3.2 mL, 43.6 mmol). The resulting mixture was stirred for 30 min at -20 °C and then saturated aqueous Na_2SO_4 (15 mL) was added. This suspension was allowed to warm to room temperature, then filtered through a pad of celite, washed with diethyl ether and purification of the crude product by silica gel column chromatography (6% ethyl acetate in *n*-hexane) afforded **8** (2.64 g, 85%) as a colorless solid. M.p.: 67-68 °C. R_f: 0.53 (5% ethyl acetate in n-hexane). $[\alpha]_D^{25}$: +22.7 (*c* 0.93, MeOH). IR (Neat): 3428, 2923, 1216, 762 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 3.95-3.89 (m, 1H, H_{1a}), 3.66-3.58 (m, 1H, H_{1b}), 2.98-2.91 (m, 2H, $H_{2,3}$), 1.61 -1.14 (m, 28H), 0.86 (t, 3H, J = 6.5, CH_3).^{13}C NMR (50 MHz, $CDCl_3$): δ 61.7, 58.4, 56.0, 31.9, 31.5, 29.7, 29.5, 29.4, 25.9, 22.7, 14.1. MS (ESI): *m/z* 284 $[M]^+$. Anal. Calcd for $C_{18}H_{36}O_2$: C, 76.00; H, 12.76. Found: C, 75.96; H, 12.84. The above spectroscopic data are in consistence with the literature data.[19]



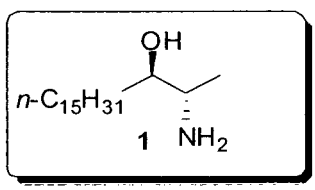
(*2S,3R*)-2-azido-3-hydroxyoctadecyl-4-methyl benzenesulfonate (11): A solution of the epoxide **8** (1.3 g, 4.57 mmol), $(MeO)_3B$ (1.05 mL, 9.14 mmol), and NaN_3 (0.6 g, 9.14 mmol) in DMF was stirred at 50 °C for 3 h. After cooling to 0 °C , a saturated aqueous solution of $NaHCO_3$ was added, and the mixture was stirred for 30 min. The mixture was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layer was successively



washed with water, a saturated aqueous solution of $NaHCO_3$, brine, and dried over Na_2SO_4. Concentration under reduced pressure followed by column chromagraphy affored 4.34 g (95 %) of the azido diol as a 84:16 mixture of regio isomers.

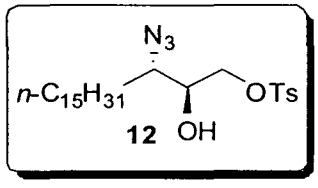
To an ice-cooled solution of the above regioisomeric azido diol in dry CH_2Cl_2 (10 mL) was added anhyd Et_3N (1.16 mL, 8.31 mmol) followed by TsCl (0.91 g, 4.75 mmol) and the mixture was kept overnight at 0 °C. The mixture was diluted with H_2O (25 mL) and extracted with CH_2Cl_2 (2x30 mL). The combined organic layers were washed with H_2O (25 mL), brine (25 mL), and dried (Na_2SO_4). The solvent was removed under reduced pressure and purification of the crude product by silica gel flash column chromatography (20% EtOAc in *n*-hexane) afforded **11** (1.43 g, 65%) as a colorless gum. R_f: 0.48 (30% ethyl acetate in *n*-hexane). $[\alpha]_D^{25}$: -3.22 (*c* 2.58, MeOH). IR (Neat): 3431, 3021, 2928, 2364, 1636, 1216, 1102, 765, 669 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.82 (d, 2H, J = 8.3, ArH), 7.37 (d, 2H, J = 8.4, ArH), 4.34 (dd, 1H, J_1 = 3.17, J_2 =3.13), 3.64-3.51 (m, 2H), 2.69 (d, 1H, J = 5.18), 2.46 (s, 3H, $ArCH_3$), 1.48-1.23 (m, 28H), 0.88 (t, 3H, J = 6.4, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.2, 132.5, 130.0, 128.0, 71.0, 69.0, 64.6, 33.2, 31.9, 29.6, 29.5, 29.4, 29.3, 25.4, 22.7, 21.7, 14.1. MS (ESI): *m/z* 482 $[M]^+$. Anal. Calcd for $C_{25}H_{43}N_3O_4S$: C, 62.34; H, 9.00; N, 8.72. Found: C, 62.52; H, 9.21; N, 8.46.

(*2S,3R*)-2-aminooctadecan-3-ol (1): To a stirring suspension of $LiAlH_4$ (76 mg, 2.0 mmol) in dry THF (8 mL) was dropwise added a solution of tosylate **11** (0.12 g, 0.25 mmol) in dry THF (3 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 8 h. The reaction was quenched by the addition of water (5 mL). The mixture was extracted with ethyl acetate (3x10 mL), washed with water (10 mL), brine (10 mL), and dried over Na_2SO_4. It was then filtered and concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography (50% methanol in $CHCl_3$) afforded **1** (44 mg, 70%) as a white solid. M.p.: 67-68 °C. R_f: 0.48 (20% methanol in chloroform). $[\alpha]_D^{25}$: +21.4 (*c* 0.5, $CHCl_3$). IR (KBr): 3408 (OH), 3345-3200 (NH), 2922, 2854, 1652, 1468, 1257, 1190, 1070, 1044, 744, 621 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ 3.68 (m, 1H,



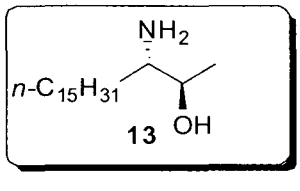
CH-3), 3.34-3.22 (m, 1H, CH-2), 1.49 -1.27 (m, 31H), 1.20 (d, 3H, J = 6.7, CH_3-1), 0.89 (t, 3H, J = 6.2, CH_3-18). ^{13}C NMR (75 MHz, $CDCl_3$): δ 74.9, 50.5, 32.6, 32.1, 30.1, 29.9, 29.8, 26.4, 22.9, 17.1, 14.3. MS (ESI): *m/z* 287 $[M+1]^+$. Anal. Calcd for $C_{18}H_{39}NO$: C, 75.72; H, 13.77; N, 4.91. Found: C, 75.79; H, 13.88; N, 5.07. The physical and spectral data were in agreement with the literature data.[11]

(*2S,3S*)-3-azido-2-hydroxyoctadecyl-4-methylbenzenesulfonate (12): A solution of the epoxy alcohol **8** (1.2 g, 4.21 mmol) in an 8:1 MeOH/H_2O mixture (18.0 mL) was



treated with NaN_3 (1.26 g, 19.37 mmol) and NH_4Cl (0.45 g, 8.40 mmol) and the resulting reaction mixture was stirred at 80 °C for 18h. Dilution with ether and evaporation of the solution afforded a crude reaction product. This crude mixture containg regiomeric azidodiols was treated in the same way as that described for **11**. The solvent was removed under reduced pressure and purification of the crude product by silica gel flash column chromatography (20% EtOAc in *n*-hexane) afforded **12** (1.42 g, 67%) as a colorless gum. R_f: 0.48 (30% ethyl acetate in *n*-hexane). $[\alpha]_D^{25}$: -4.53 (*c* 1.74, MeOH). IR (Neat): 3428, 2927, 2366, 1635, 1218, 769 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.82 (d, 2H, J = 8.3, ArH), 7.37 (d, 2H, J = 8.4, ArH), 4.34 (dd, 1H, J_1 = 3.2, J_2 =3.1), 3.64-3.51 (m, 2H), 2.69 (d, 1H, J = 5.18), 2.46 (s, 3H, $ArCH_3$), 1.48-1.23 (m, 28H), 0.88 (t, 3H, J = 6.4, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.2, 132.5, 130.0, 128.0, 71.0, 69.0, 64.7, 33.2, 31.9, 29.6, 29.5, 29.4, 29.3, 25.4, 22.7, 21.7, 14.1. MS (ESI): *m/z* 482 $[M]^+$. Anal. Calcd for $C_{25}H_{43}N_3O_4S$: C, 62.34; H, 9.00; N, 8.72. Found: C, 62.52; H, 9.21; N, 8.46.

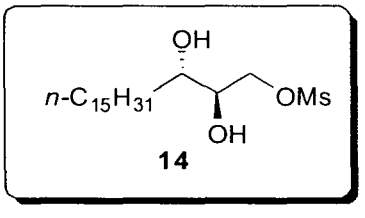
(*2R, 3S*)-3-aminooctadecan-2-ol (13): To a stirring suspension of $LiAlH_4$ (76 mg, 2.0 mmol) in dry THF (8 mL) was dropwise added a solution of tosylate **12** (0.12 g, 0.25



mmol) in dry THF (3 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 8 h. The reaction was quenched by the addition of water (5 mL). The mixture was extracted with ethyl acetate (3x10 mL), washed with water (10 mL), brine (10 mL), and dried over Na_2SO_4. It was then filtered and concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography (50% methanol in $CHCl_3$) afforded **13** (44 mg,

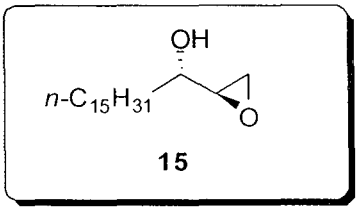
62%) as a white solid. M.p.: 61-63 °C. R_f: 0.48 (20% methanol in chloroform). $[\alpha]_D^{25}$: +7.3 (*c* 1.7, MeOH). IR (KBr): 3408, 3022, 2925, 2855, 1628, 1464, 1257, 1216, 1041, 762, 670 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ 3.78-3.75 (m, 1H, CH-2), 2.81 (m, 1H, CH-3), 1.45 -1.26 (m, 28H), 1.10 (d, 3H, J = 6.4, CH_3-1), 0.88 (t, 3H, J = 6.2, CH_3-18). ^{13}C NMR (75 MHz, $CDCl_3$): δ 70.2, 56.3, 33.3, 32.3, 30.2-29.7 (10xCH_2), 26.5, 23.0, 17.2, 14.2. MS (ESI): *m/z* 287 $[M+1]^+$. Anal. Calcd for $C_{18}H_{39}NO$: C, 75.72; H, 13.77; N, 4.91. Found: C, 75.63; H, 13.91; N, 5.02.

(*2R,3S*)-2,3-dihydroxyoctadecyl methanesulfonate (14). A solution of **8** (1.82 g, 6.39 mmol) in CH_2Cl_2 (40 mL) was treated with MsCl (0.75 mL, 9.6 mmol) and Et_3N (1.8 mL, 12.8 mmol) at 0 °C. Upon being stirred for 15 min, the reaction mixture was diluted with diethyl ether, washed with saturated NH_4Cl, water and concentrated. The resulting mixture was dissolved in 60% DMSO (40 mL) solution, being treated with 70% $HClO_4$(0.2 mL). After being stirred for 6h at 50°C, the mixture was diluted with EtOAc, washed with saturated $NaHCO_3$ and water, dried over Na_2SO_4, and concentrated. Purification by silica gel column chromatagraphy (*n*-hexane : EtOAc = 7:3~ 1:1) gave **14** (2.12g, 87%) as a colorless solid. M.p.: 91-93 °C. R_f: 0.45 (30% ethyl acetate in *n*-hexane). $[\alpha]_D^{25}$: +5.1 (*c* 1.07, MeOH). IR (KBr): 3387, 3322, 2920, 2362, 1339, 1173 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 5.38-5.32 (m, 1H), 4.45-4.29 (m, 2H), 3.83-3.73 (m, 2H), 3.08 (s, 3H), 2.78 (d, 1H, J = 6.7), 1.85-1.13 (m, 28H), 0.88 (t, 3H, J = 6.6). ^{13}C NMR (50 MHz, $CDCl_3$): δ 72.5, 72.2, 71.0, 37.5, 32.7, 31.9, 31.8, 29.8, 29.6, 29.5, 29.4, 29.3, 29.2, 27.2, 25.7, 22.6, 14.1. MS (ESI): *m/z* 380 $[M]^+$, 403 $[M+Na]^+$. Anal. Calcd for $C_{19}H_{40}O_5S$: C, 59.96; H, 10.59. Found: C, 59.74; H, 9.87.



14

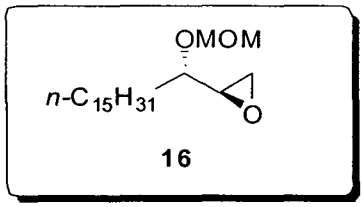
4.1.15.(*2R,3S*)-1,2-Epoxyoctadecan-3-ol (15). A solution of compound **14** (o.80 g, 2.1 mmol) in dry MeOH (30 mL) was treated with K_2CO_3 (0.44 g, 3.15 mmol) and the mixture was stirred for 45 min at rt. After completion of the reaction, the mixture was concentrated and extracted with diethyl ether, washed with water and brine. The organic layer was dried



15

over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography(10% ethyl acetate in *n*-hexane) gave **15** (0.53 g, 89%) as a colorless solid. M.p.: 69-72°C. R_f: 0.48 (20% ethyl acetate in *n*-hexane). $[\alpha]_D^{25}$: +0.23 (*c* 1.54, MeOH). IR (KBr): 3328, 2963, 2838, 1446, 963, 852, 718 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 3.39-3.27 (m, 1H), 2.93-2.87 (m, 1H), 2.77-2.72 (m, 1H), 2.65-2.61 (m, 1H), 2.51-2.49 (m, 1H), 1.59–1.19 (m, 28H), 0.81 (t, 3H, J = 6.5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 71.6, 55.4, F45.0, 34.0, 31.6, 29.6, 29.5, 29.3, 25.1, 22.6, 14.0. MS (ESI): *m/z* 285 $[M]^+$. Anal. Calcd for $C_{18}H_{36}O_2$: C, 76.00; H, 12.76. Found: C, 76.09; H, 12.83.

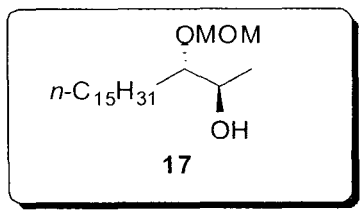
4.1.16.(*2R,3S*)-1,2-Epoxy-3-methoxymethoxyocta- decane (16). To a stirring solution of alcohol **14** (0.51 g, 1.79 mmol) and *i*-Pr_2NEt (0.60 mL, 3.58 mmol) in



16

CH_2Cl_2 (10 mL) was added MOMCl (0.24 mL, 2.68 mmol) at 0 °C. The mixture was stirred for 12 h at rt. The reaction was quenched with saturated aqueous NH_4Cl (10 mL) and the mixture was extracted with CH_2Cl_2 (2x20 mL). The organic layer was washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10% ethyl acetate in hexane) to afford **16** (0.564 g, 96%) as a colorless oil. R_f: 0.5 (35% ethyl acetate in *n*-hexane). $[\alpha]_D^{25}$: +18.4 (*c* 1.2, MeOH). IR (Neat): 2917, 2843, 1408, 1369, 1249, 1220, 1141, 1043, 724 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 4.88 (d, 1H, J = 6.6, OCH_aH_bO), 4.66 (d, 1H, J = 6.7, OCH_aH_bO), 3.40 (s, 3H, OCH_3), 3.29-3.22 (m, 1H, H_3), 3.00-2.96 (m, 1H, H_2), 2.78 (t, 1H, J = 4.6, CH_aH_b-1), 2.54-2.52 (m, 1H, CH_aH_b-1), 1.65–1.25 (m, 28H), 0.88 (t, 3H, J = 6.9). ^{13}C NMR (75 MHz, $CDCl_3$): δ 95.4, 78.0, 55.5, 54.7, 43.8, 32.3, 31.9, 29.67, 29.63, 29.62, 29.56, 29.51, 29.34, 25.4, 22.6, 14.1. MS (ESI): *m/z* 329 $[M]^+$. Anal. Calcd for $C_{20}H_{40}O_3$: C, 73.12; H, 12.27. Found: C, 73.28; H, 12.46.

4.1.17.(*2R,3S*)-3-Methoxymethoxyoctadecan-2-ol (17). To a stirring suspension of $LiAlH_4$ (77 mg, 2.06 mmol) in dry THF (12 mL) was dropwise added a solution of

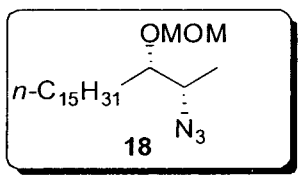


17

epoxide **16** (0.54 g, 1.64 mmol) in dry THF (10 mL) at 0 °C. The reaction mixture was allowed to warm to

room temperature, and stirred for 5 h. The reaction was quenched by the addition of water (8 mL). The mixture was extracted with ethyl acetate (3x15 mL), washed with water (15 mL), brine (15 mL), and dried over Na_2SO_4. It was then filtered and concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography (15% ethyl acetate in *n*-hexane) afforded **17** (509 mg, 94%) as a colorless liquid. R_f: 0.41 (35% ethyl acetate in *n*-hexane). $[\alpha]_D^{25}$: +17.3 (*c* 1.3, MeOH). IR (Neat): 3443, 2913, 2839, 1461, 1244, 1204, 1140, 1028, 913, 712 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 4.74-4.68 (m, 2H), 3.73-3.65 (m, 1H), 3.42 (s, 3H), 3.29-3.23 (m, 1H), 3.04 (s, br, 1H), 1.59–1.25 (m, 28H), 1.15 (d, 3H, J = 6.3), 0.88 (t, 3H, J = 6.3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 97.3, 85.4, 69.2, 55.7, 31.9, 31.1, 29.8, 29.6, 29.3, 25.0, 22.6, 19.0, 14.0. MS (ESI): *m/z* 331 $[M]^+$. Anal. Calcd for $C_{20}H_{42}O_3$: C, 72.67; H, 12.81. Found: C, 72.81; H, 12.94.

(2*S*,3*S*)-2-Azido-3-methoxymethoxyoctadecane (18): To an ice-cooled solution of alcohol **17** (410 mg, 1.24 mmol), anhyd. triethyl amine (0.29 mL, 1.96 mmol) and DMAP (10 mg) in dry CH_2Cl_2 (10 mL) was added TsCl (200 mg, 1.05 mmol). After being stirred for 1 h in an ice bath and then 48 h at rt, the mixture was diluted with CH_2Cl_2 (30 mL) and water (20 mL). The organic layer was separated, washed with brine (15 mL), and dried over Na_2SO_4. It was then filtered and the filtrate was concentrated under reduced pressure to get the crude tosylate which was used for the next step without further purification.

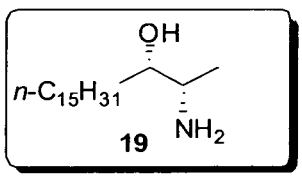


18

To a solution of the above crude tosylate in dry DMF (10 mL) was added NaN_3 (0.34 g, 5.25 mmol). After being stirred for 5 h at 90 °C, the mixture was diluted with ethyl acetate (20 mL) and water (10 mL). The organic layer was separated, washed with brine (15 mL), and dried over Na_2SO_4. It was then filtered and the filtrate was concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography (4% ethyl acetate in *n*-hexane) afforded **18** (313 mg, 71%) as a colorless liquid. R_f: 0.58 (10% ethyl acetate in *n*-hexane). $[\alpha]_D^{25}$: +11.3 (*c* 1.7, MeOH). ^1H NMR (300 MHz, $CDCl_3$): δ 4.73-4.64 (m, 2H), 3.62-3.51 (m, 2H), 3.39 (s, 3H), 1.61–1.19 (m, 31H), 0.86 (t, 3H, J = 6.9). ^{13}C NMR (75 MHz, $CDCl_3$): δ 96.4, 80.3, 59.6, 55.6, 31.8, 30.4, 29.62, 29.60, 29.52, 29.48, 29.3, 25.4, 22.6, 14.2, 14.0. MS (ESI): *m/z* 356 $[M]^+$, 342 [M-

$N_2]^+$. Anal. Calcd for $C_{20}H_{41}N_3O_2$: C, 67.56; H, 11.62; N, 11.82. Found: C, 67.71; H, 11.83; N, 12.02.

(*2S,3S*)-2-aminooctadecan-3-ol (19): To a stirred solution of **18** (95 mg, 0.27 mmol) in methanol (10 mL) was added 10% Pd-C (20 mg). After stirring for 4 h at room temperature under pressure of a hydrogen balloon, the reaction mixture was filtered through a pad of Celite® and the filtrate was concentrated under reduced pressure to get the corresponding amine derivative as a colorless semi-solid which was used for the next step without further purification.



19

To a solution of the above crude amine in methanol (5 mL) was added two drops of conc. HCl. After being stirred for 12 h at rt, the mixture was concentrated under reduced pressure. The residue was redissolved in $CHCl_3$ (10 mL) and the resulting solution was treated with saturated aq. $NaHCO_3$ solution (10 mL).The organic layer was separated, washed with brine (15 mL), and dried over Na_2SO_4. It was then filtered and the filtrate was concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography (50% methanol in $CHCl_3$) afforded **19** (58 mg, 75%) as a white solid. M.p.: 73-75 °C. R_f: 0.48 (20% methanol in chloroform). $[\alpha]_D^{25}$: +3.4 (*c* 1.8, MeOH). IR (KBr): 3408, 3345-3200, 2922, 2854, 1652, 1468, 1257, 1190, 1070, 1044, 744, 621 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ 3.68 (m, 1H, CH-3), 3.26-3.22 (m, 1H, CH-2), 1.49 -1.27 (m, 31H), 1.20 (d, 3H, J = 6.7, CH_3-1), 0.89 (t, 3H, J = 6.2, CH_3-18). ^{13}C NMR (75 MHz, $CDCl_3$): δ 75.0, 50.6, 32.7, 32.2, 30.1, 29.5, 26.3, 22.9, 17.1, 14.2. MS (ESI): *m/z* 287 $[M+1]^+$. Anal. Calcd for $C_{18}H_{39}NO$: C, 75.72; H, 13.77; N, 4.91. Found: C, 75.77; H, 13.92; N, 5.11.

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4.7 Spectra of selected molecules

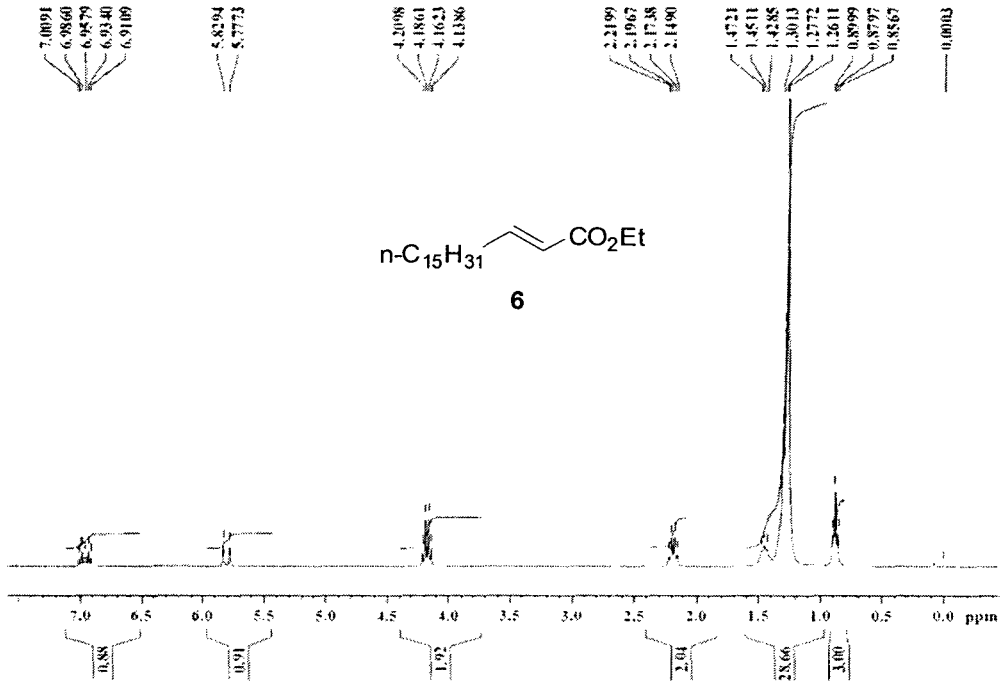


Figure 4.2 ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **6**.

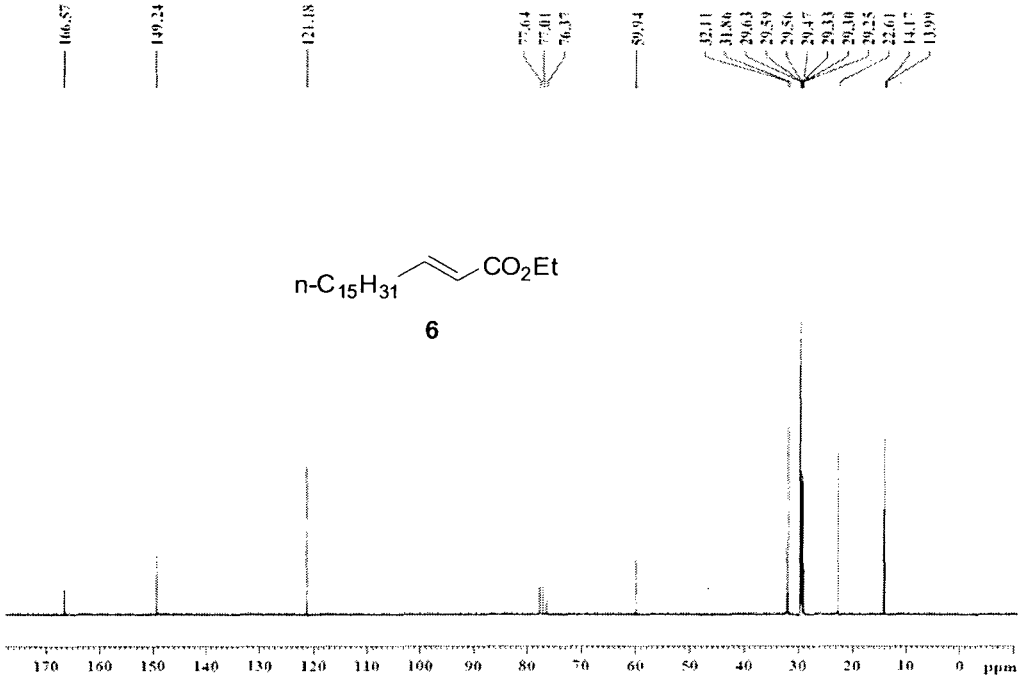


Figure 4.3 ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **6**.

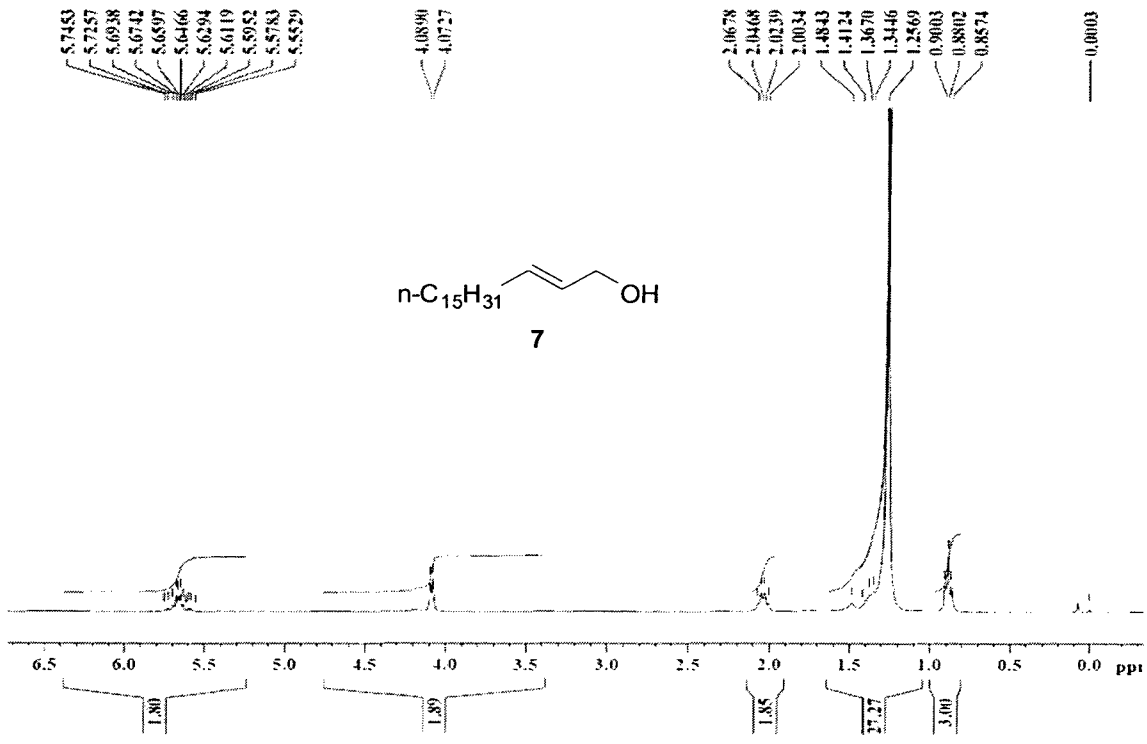


Figure 4.4 ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **7**.

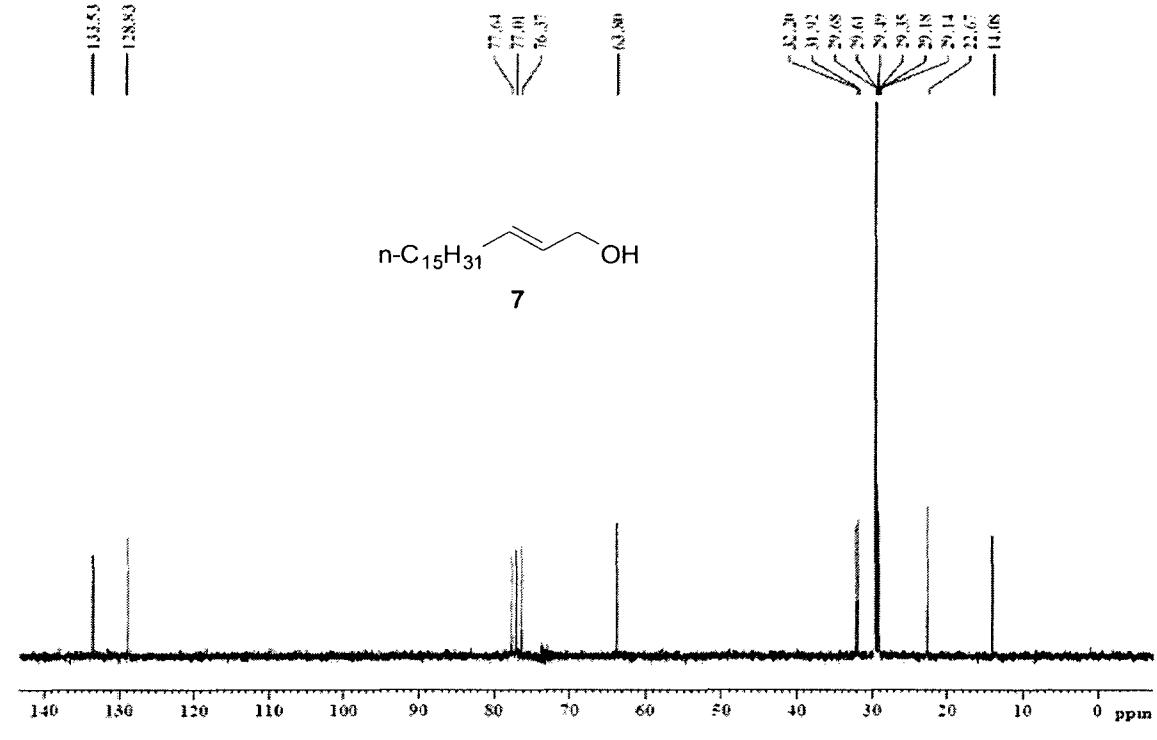


Figure 4.5 ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **7**.

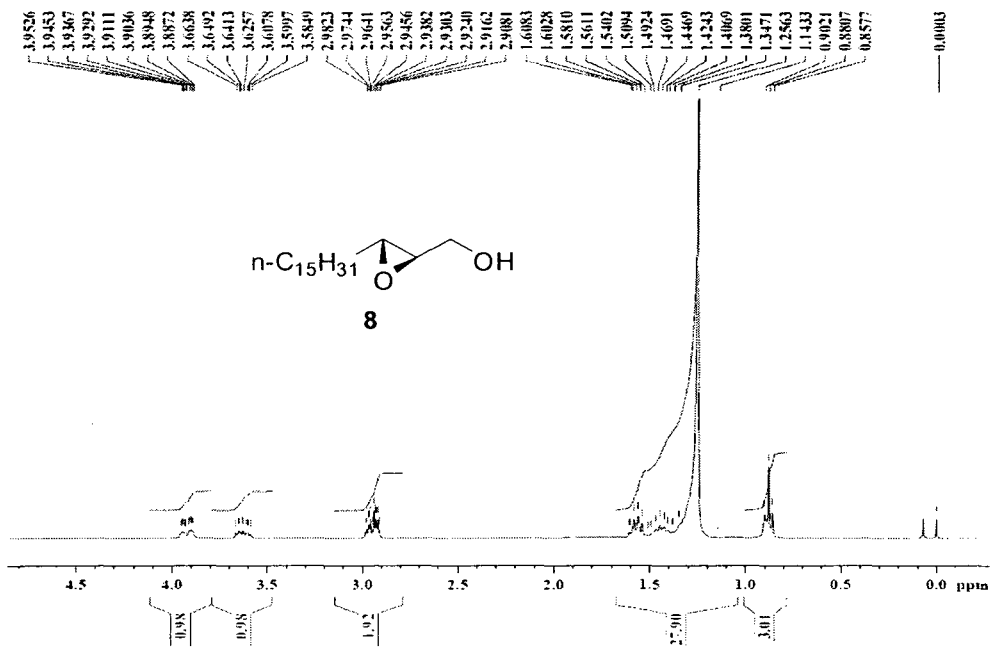


Figure 4.6 ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **8**.

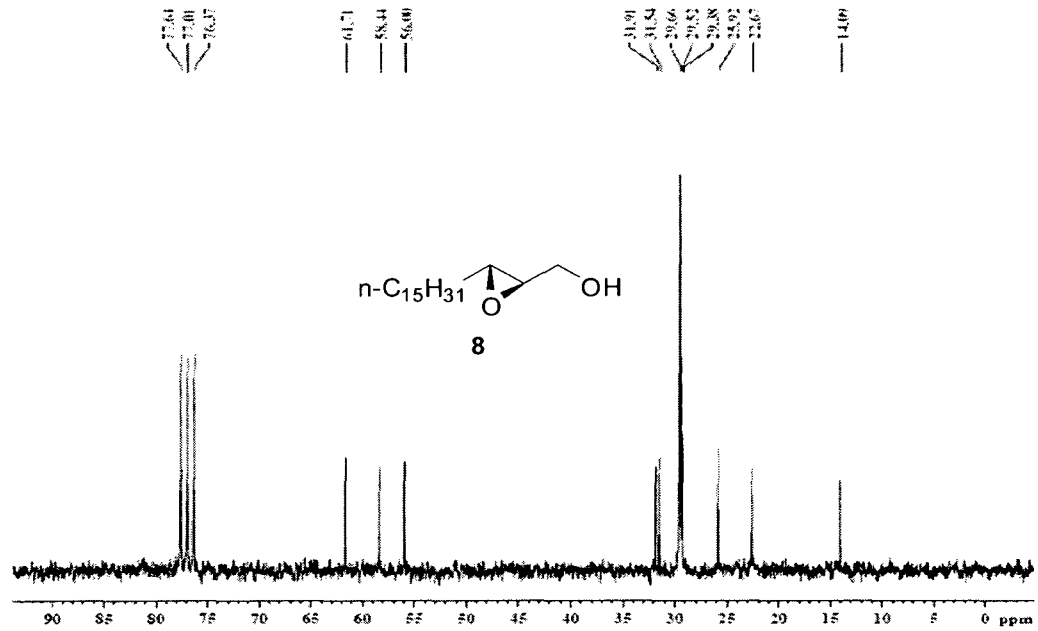


Figure 4.7 ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **8**.

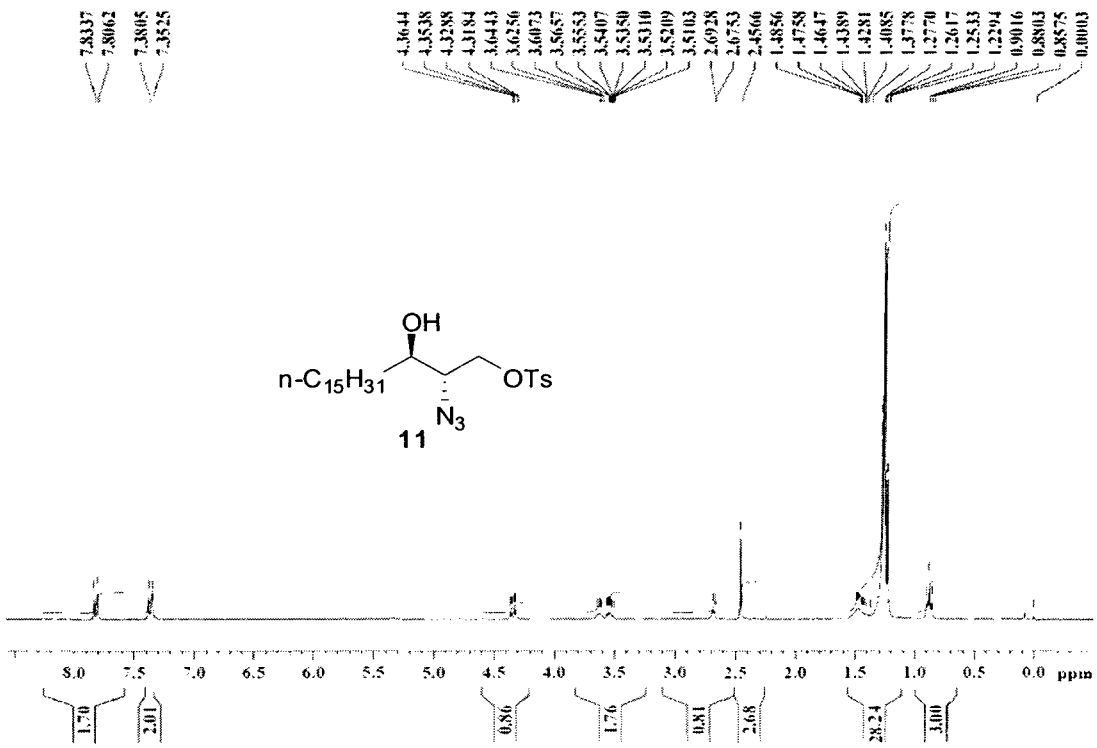


Figure 4.8 ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **11**.

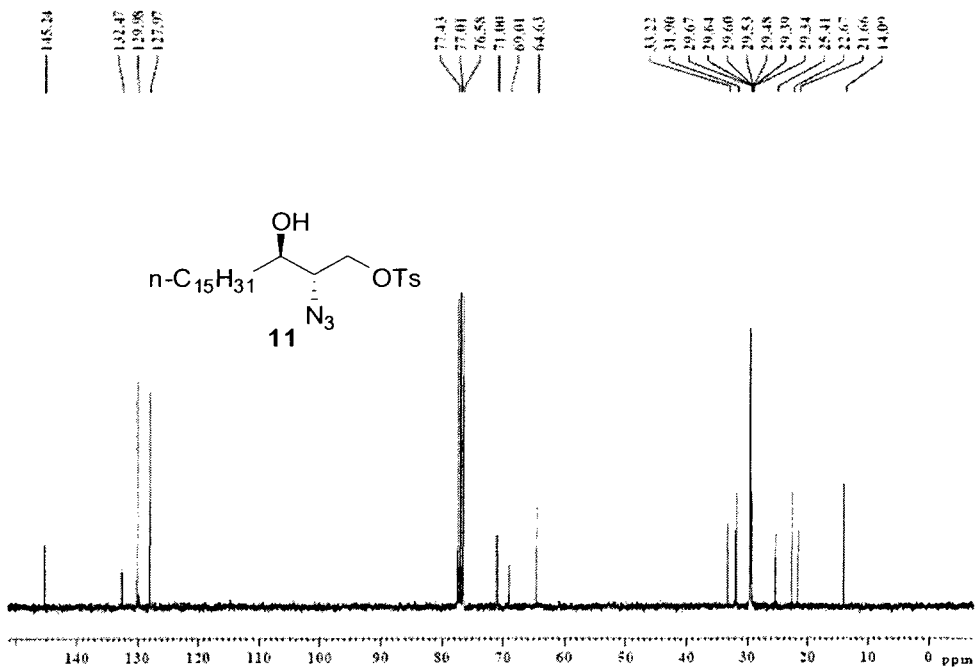


Figure 4.9 ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **11**.

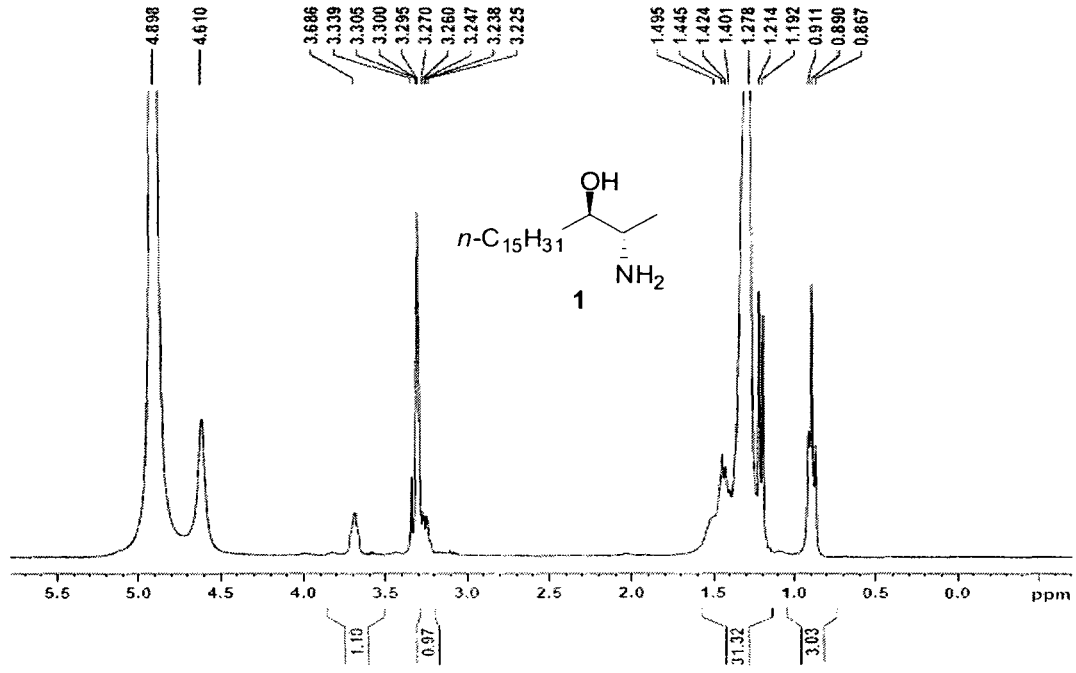


Figure 4.10 ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **1**.

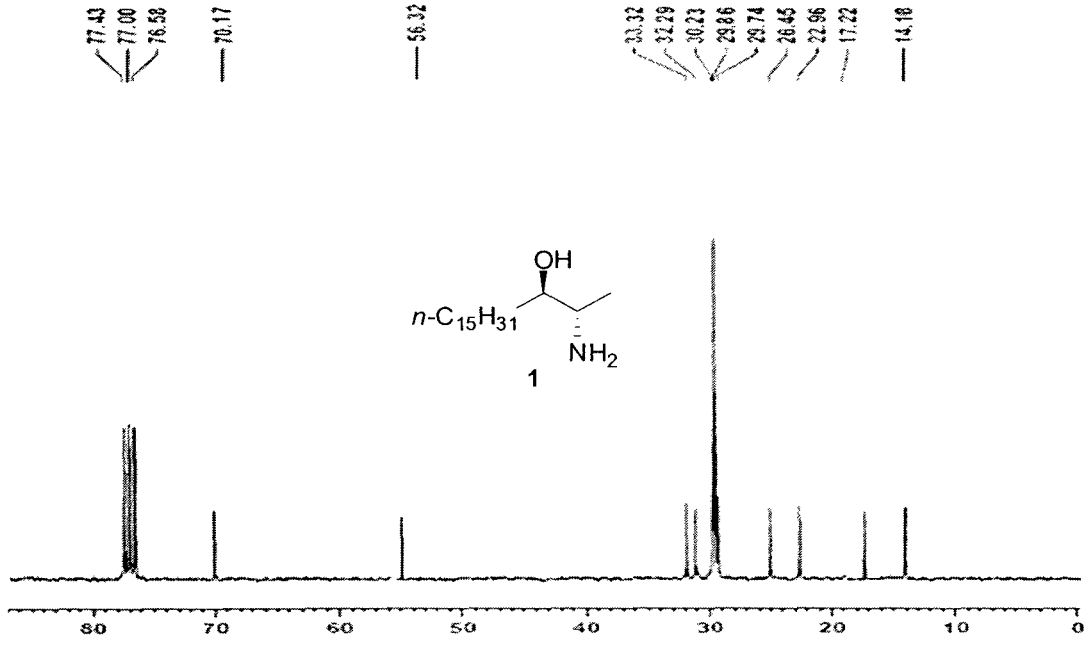


Figure 4.11 ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **1**.

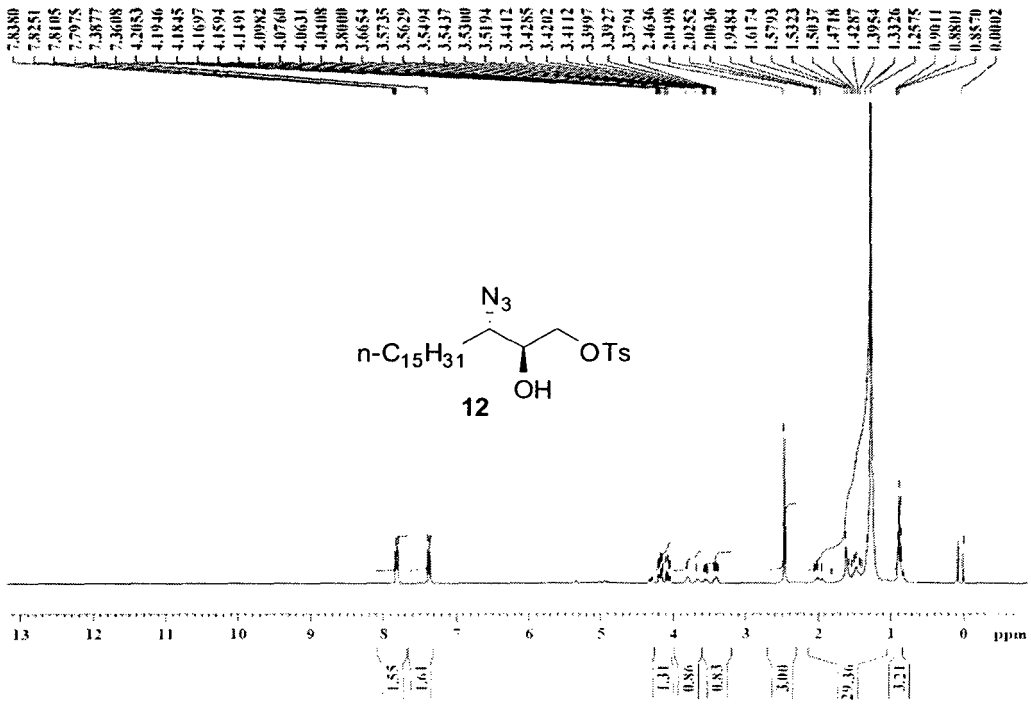


Figure 4.12 ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **12**.

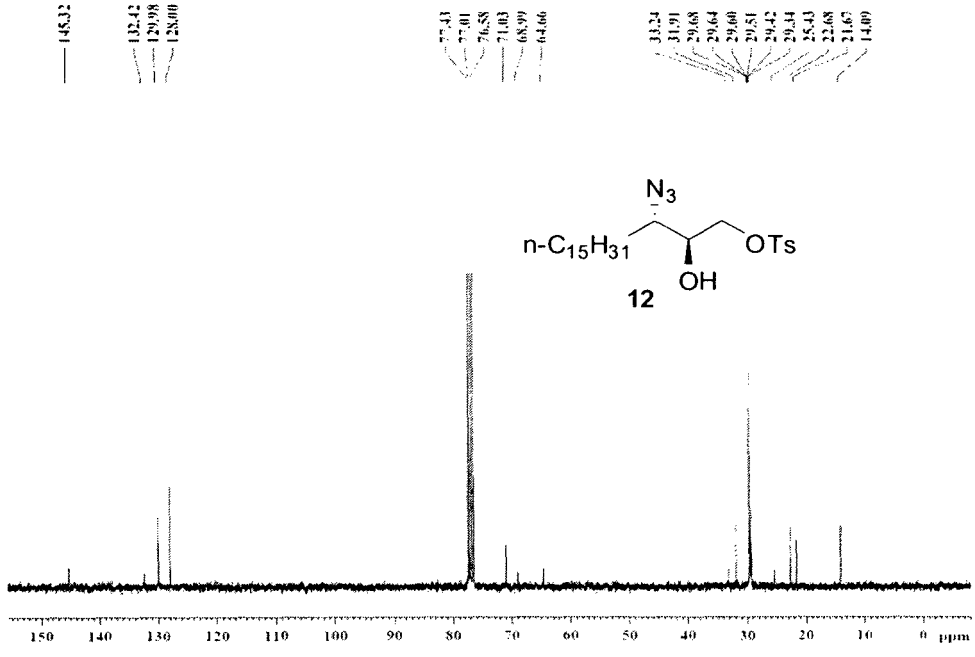


Figure 4.13 ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **12**.

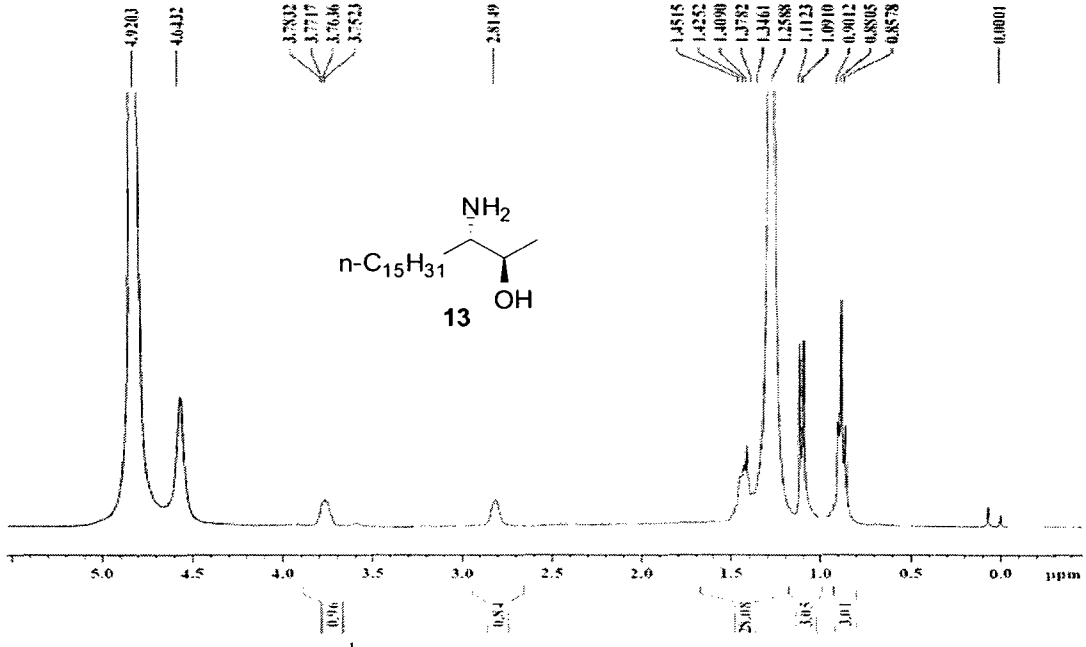


Figure 4.14 ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **13**.

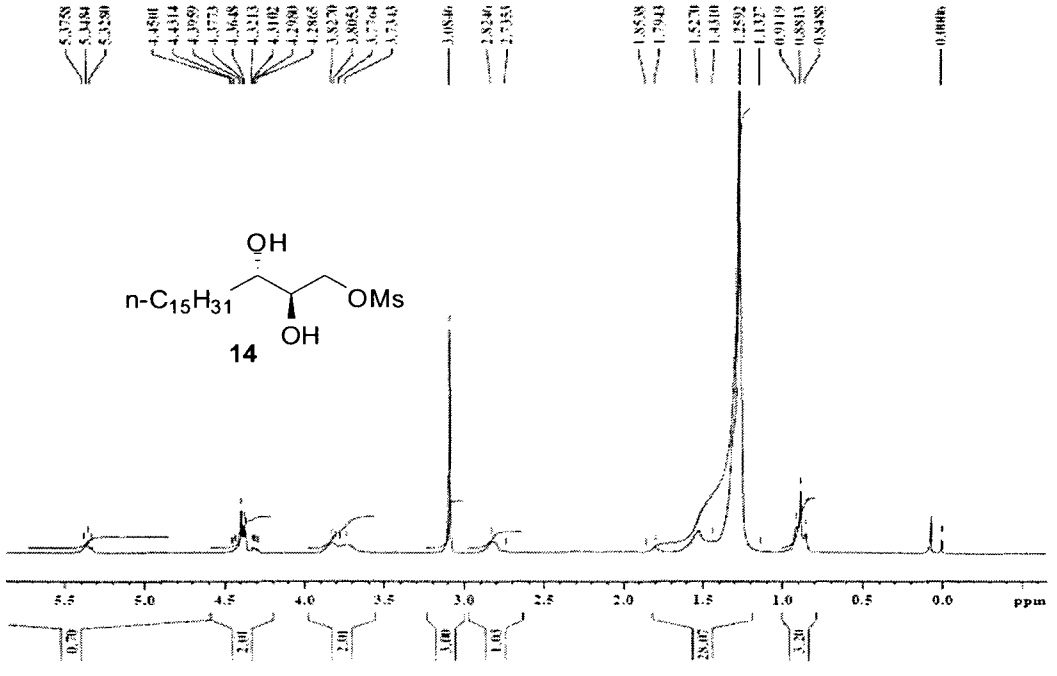


Figure 4.15 ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **14**.

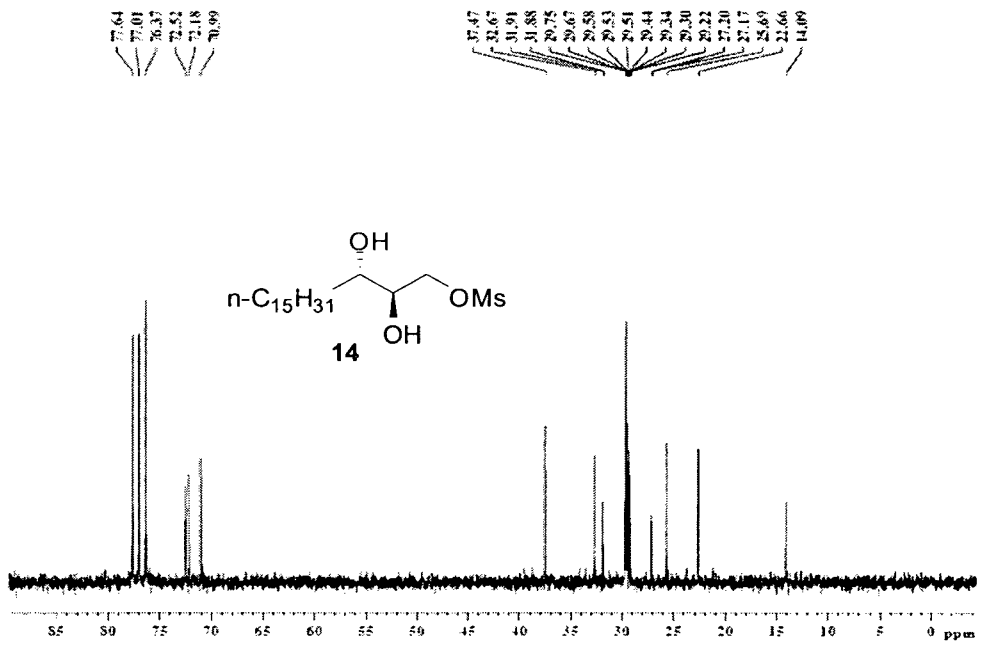


Figure 4.16 ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **14**.

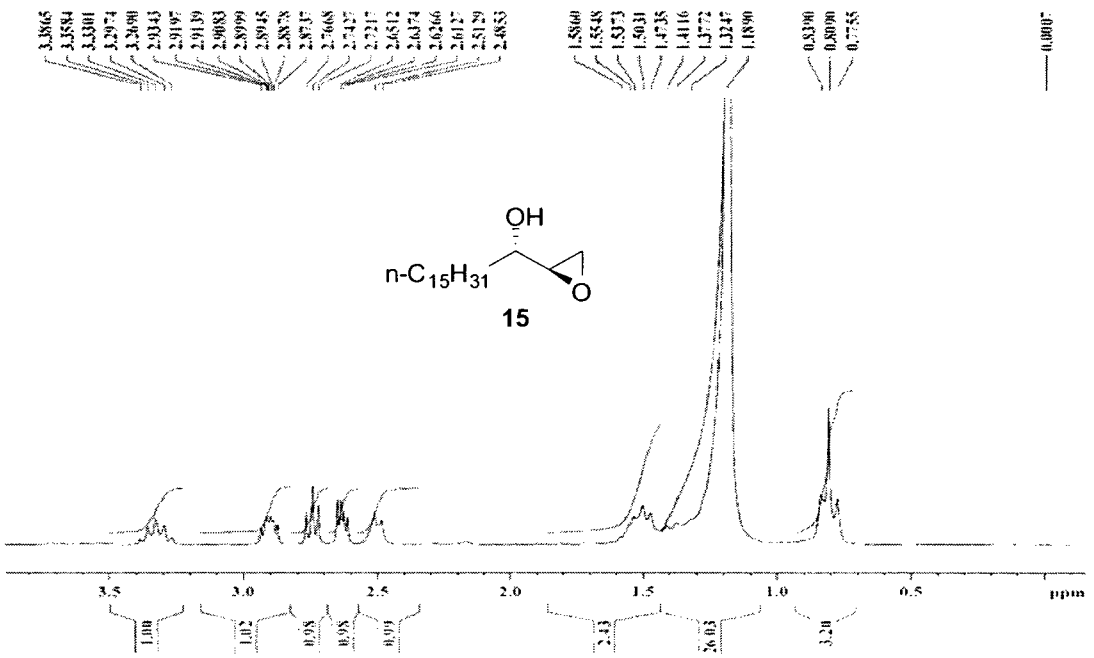


Figure 4.17 ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **15**.

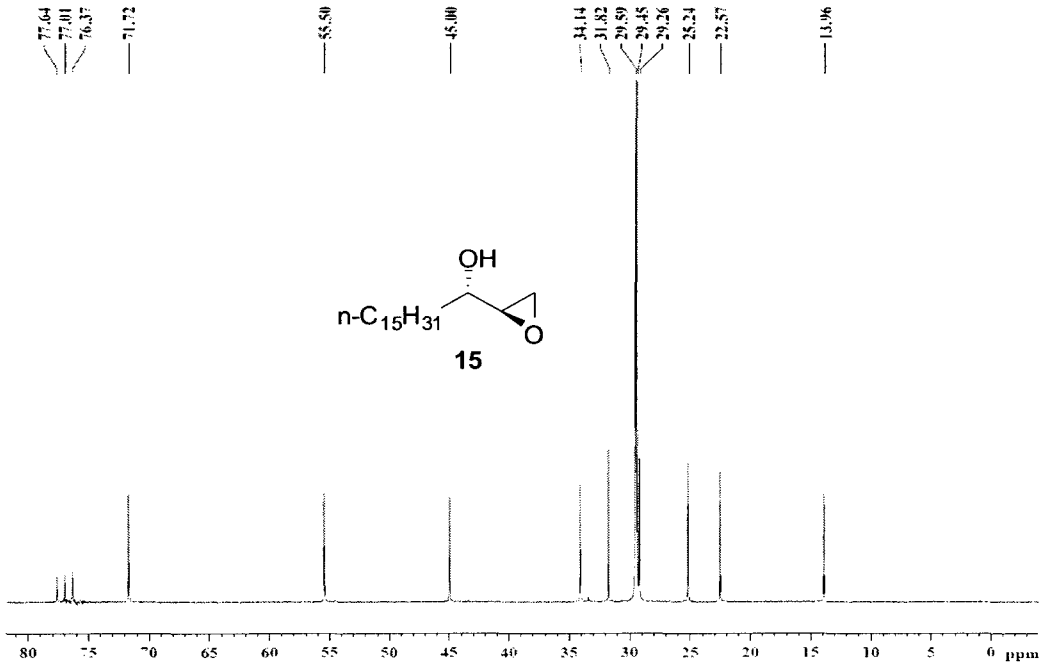


Figure 4.18 ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **15**.

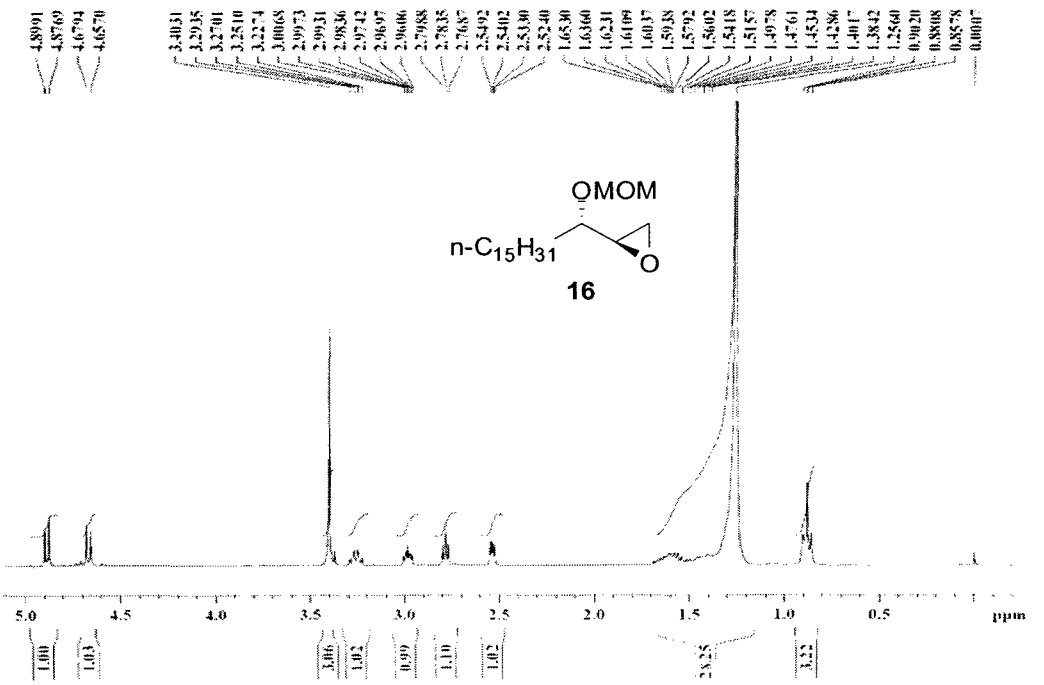


Figure 4.19 ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **16**

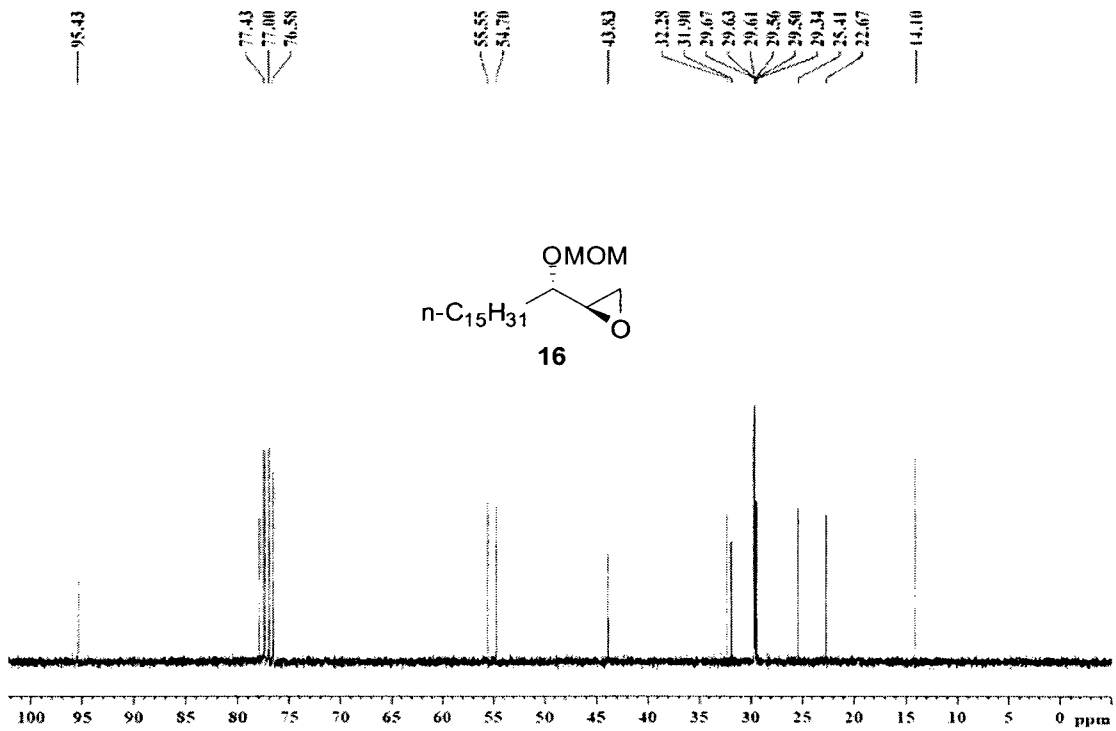


Figure 4.20 ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **16**.

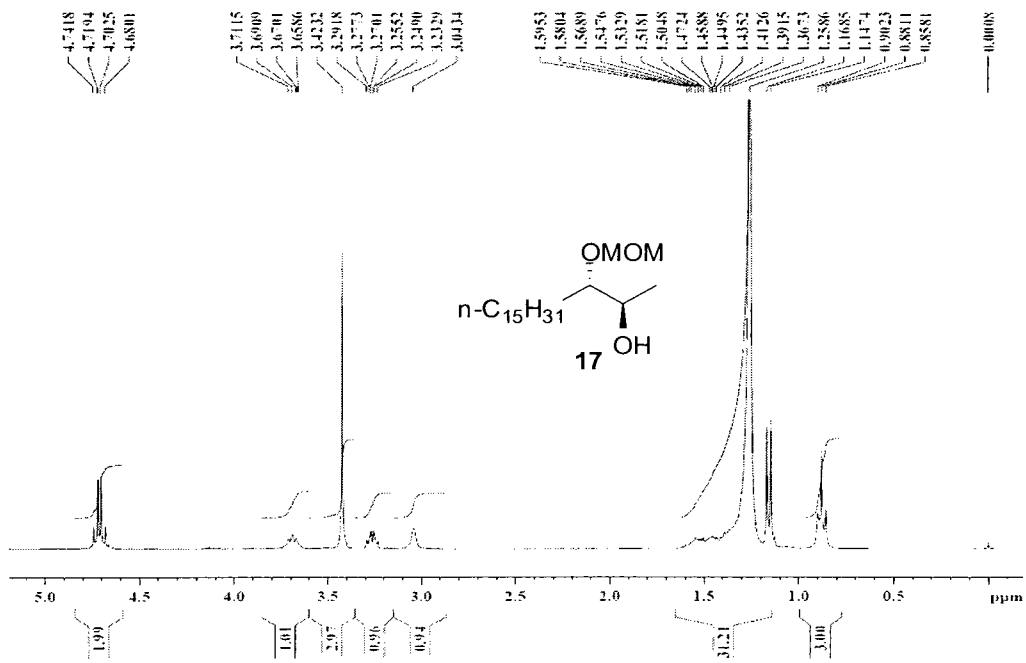


Figure 4.21 ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **17**.

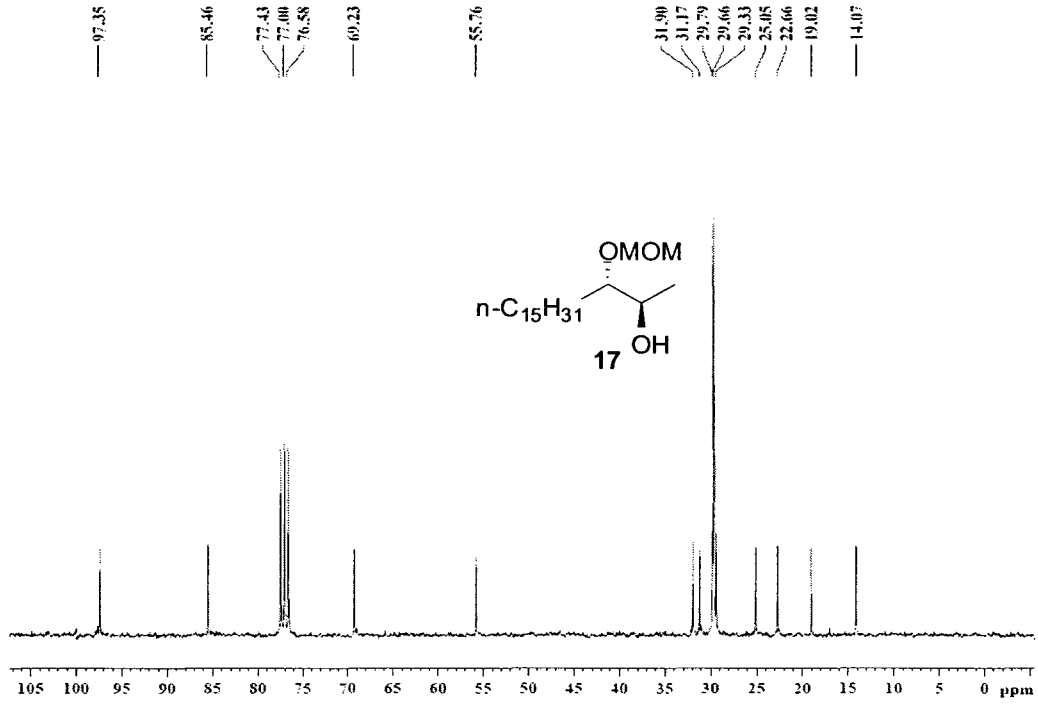


Figure 4.22 ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **17**.

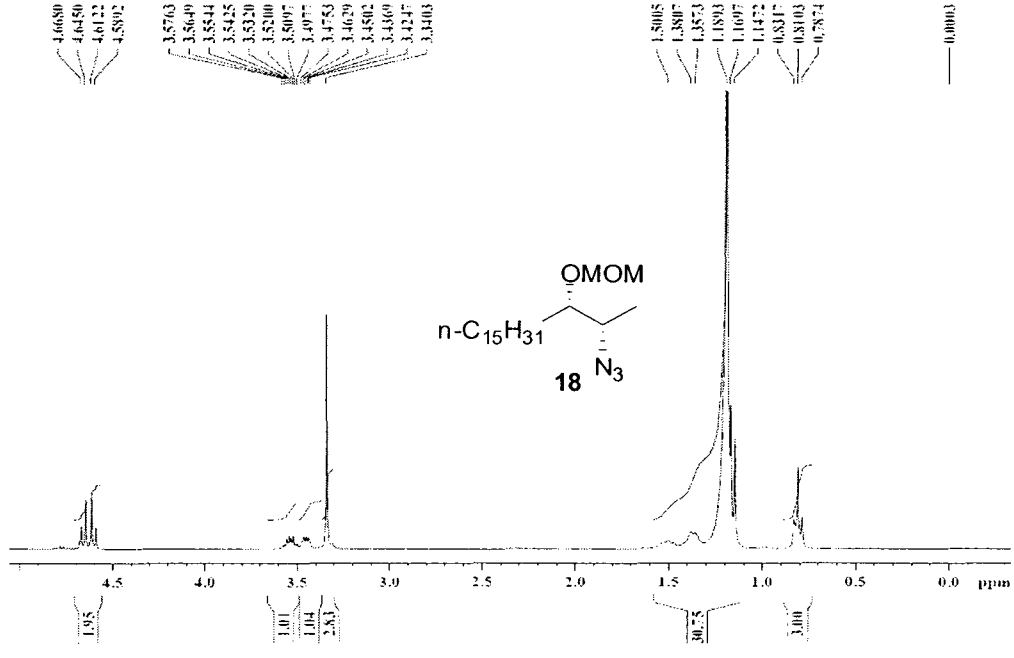


Figure 4.23 ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **18**.

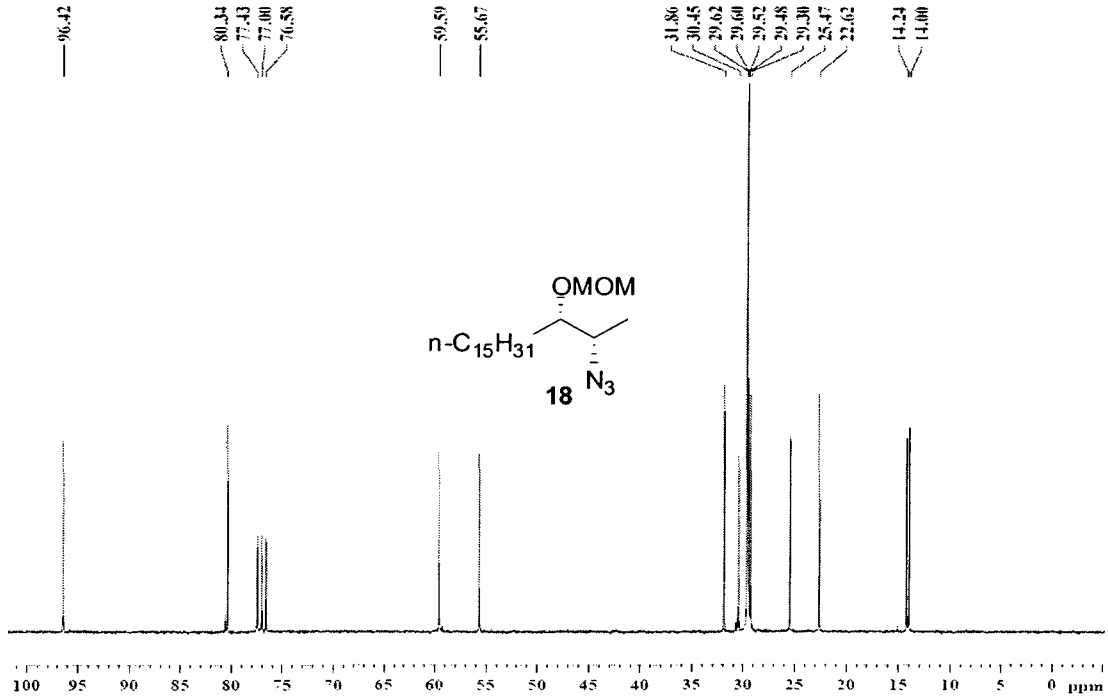


Figure 4.24 ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **18**.

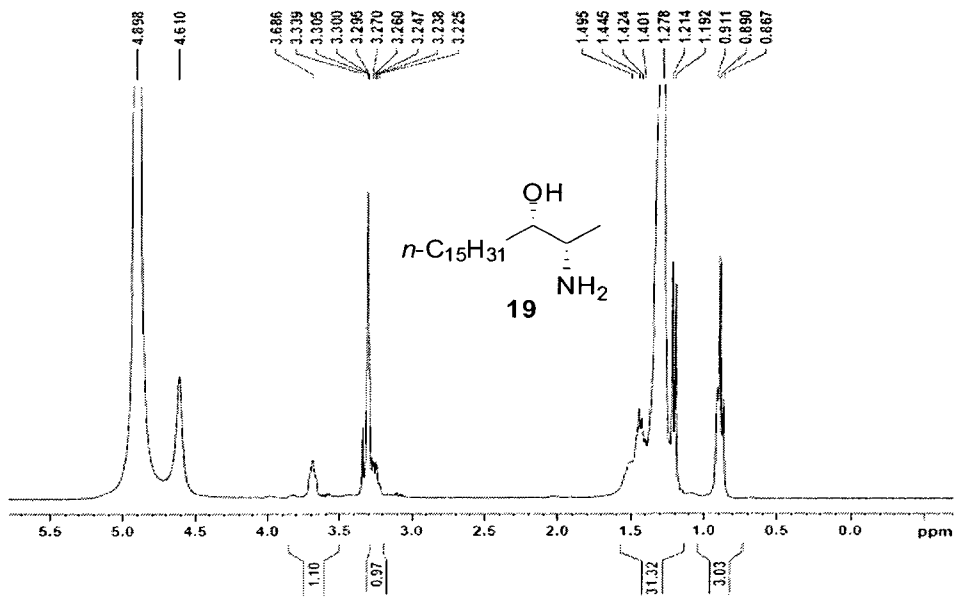


Figure 4.25 ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **19**.

Chapter 5:
Stereoselective Synthesis of Functionalized 1-Benzoxepines

5.1 Introduction

Partially or fully hydrogenated 1-benzoxepines (Figure 5.1) are important benzo-fused medium ring oxa-heterocycle motifs found in numerous pharmaceutically interesting and potentially bioactive natural compounds[1] and synthetic molecules[2]. There are simple molecules like radulanin A **2**, heliannuol D **3**, and pterulone **6**, while edulisone A **5** represents structurally complex one. Hence, the development of new synthetic methods to access this important class of compounds has received substantial amount of attention from modern organic chemists.[3] To construct functionalized 1-benzoxepines, there are two types of synthetic strategies: (i) manipulation of a pre-existing oxygen-containing cyclic core and (ii) assembling from acyclic precursors. Although some methods are available for their construction, the establishment of new synthetic protocols, especially chiral variants, starting from readily available substrates remains highly desirable.

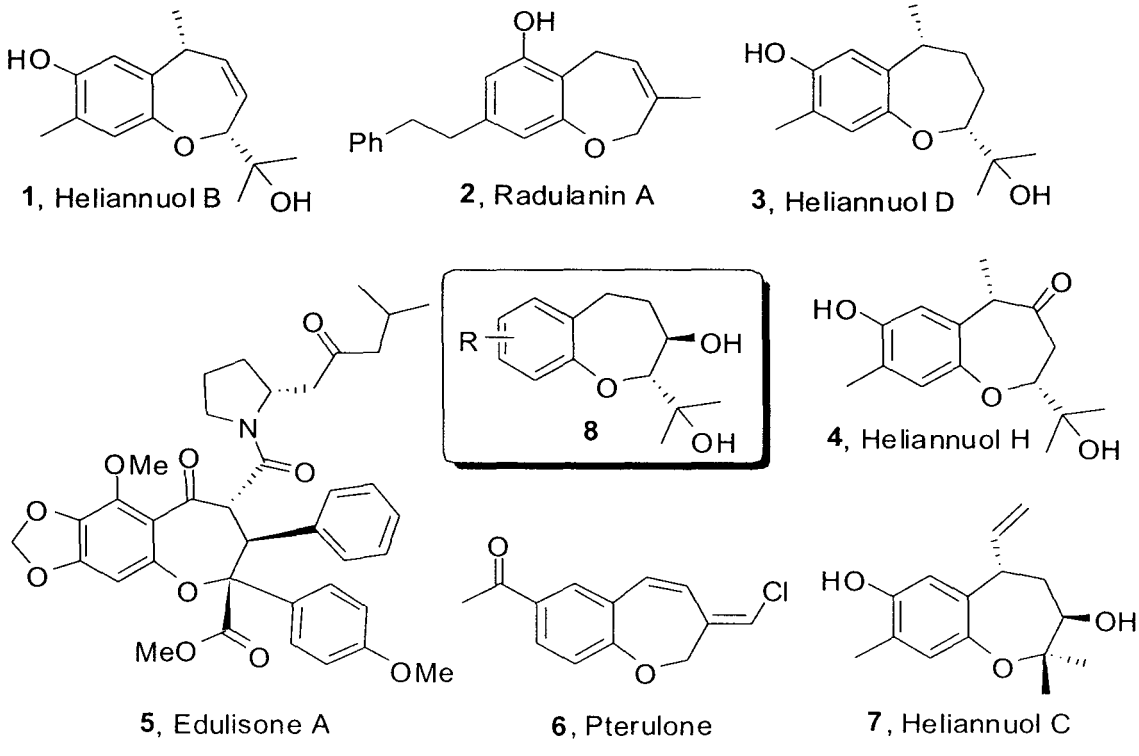
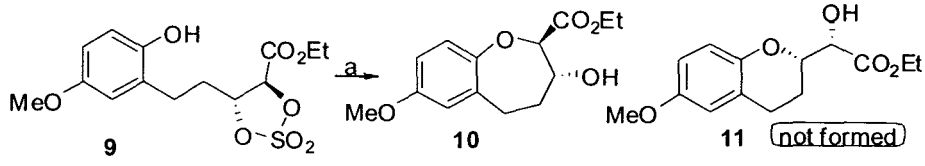


Figure 5.1. Some natural products **1-7** and our molecule **8** containing 1-benzoxepine moiety

5.2 Basis of the Present Work

In our laboratory, it was found that phenoxide ion mediated intramolecular 7-*endo-tet* S_N2 ring opening of *syn*-2,3-dihydroxy ester-derived cyclic sulfate derivative **9** leads to construction of 1-benzoxepine derivative **10** over 1-benzopyran ring **11** (Scheme 5.1).[4]



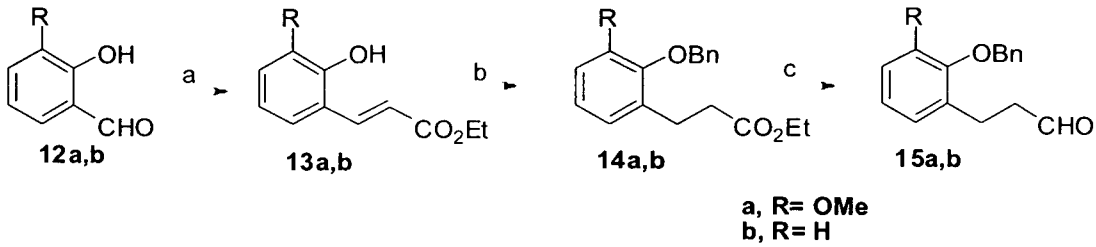
Scheme 5.1. *Reagents and conditions*: (a) (i) anhyd. K_2CO_3, dry acetone, rt, 8h. (ii) 20% H_2SO_4, THF, rt, overnight, 70% (over two steps).

In that study only one example of 1-benzoxepine derivative having methoxy group at *para* to the phenolic–OH functionality on the phenyl ring was synthesized. Later, we carried out studies to observe how the key cyclization reaction would be affected by the absence and presence of methoxy substituent at other positions of the phenyl ring. Herein we disclose this study which reveals that the presence of methoxy group *para*- and *ortho* to the phenolic–OH functionality on the phenyl ring renders the cyclization reaction completely regioselective producing 1-benzoxepine derivatives only, while in the absence of a methoxy group on the phenyl ring, the reaction furnishes both 1-benzoxepine and 1-benzopyran derivatives with the former being the major one.

5.3 Results and Discussion

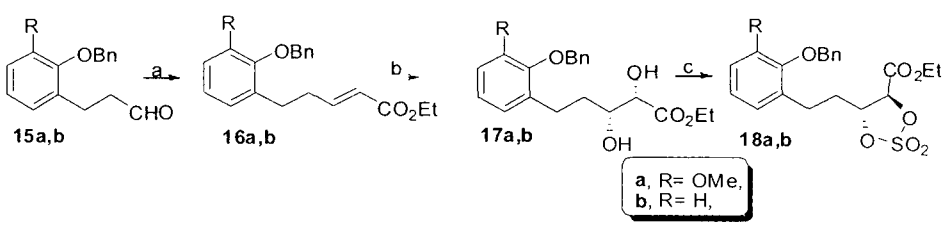
In our study, commercially available 2-hydroxy-3-methoxybenzaldehyde **12a** and 2-hydroxybenzaldehyde **12b** were selected as starting materials. Compounds **12a,b** were converted into the corresponding phenolic–OH protected and two-carbon homologated aldehydes **15a,b** by essentially following the steps as depicted in Scheme 5.2. Thus, Wittig olefination of **12a,b** with (carbethoxymethylene)triphenylphosphorane in dry CH_2Cl_2 at room temparature furnished the corresponding *trans* cinnamate esters **13a,b** in very high yields. Next, hydrogenation of **13a,b** in the presence of 10% Pd-C followed by benzylation of the resulting hydroxy esters with benzyl bromide and anhydrous K_2CO_3 in dry acetone

under reflux condition yielded **14a,b** in high yields. DIBAL-H reduction of **14a,b** in dry toluene at -78°C furnished the corresponding aldehydes **15a,b** in very high yield.



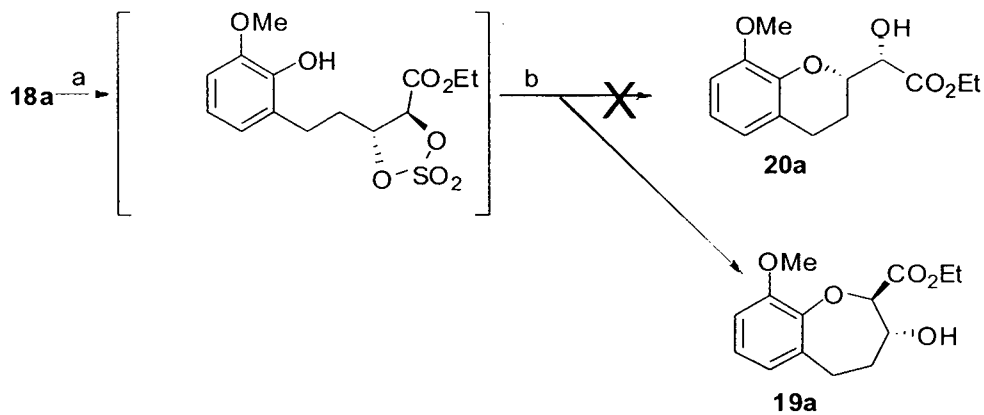
Scheme 5.2. *Reagents and conditions*: (a) $Ph_3P{=}CHCO_2Et$, CH_2Cl_2, rt, 1h, **13a** (95%) and **13b** (95%). (b) (i) H_2, 10%Pd-C, EtOAc, 8 h, (ii) BnBr, anhyd. K_2CO_3, dry acetone, reflux, 4h, **14a** (87%) and **14b** (91%); for combined two steps (c) DIBAL-H, dry toluene, -78°C, 1h, **15a** (94%) and **15b** (95%).

With the ready availability of aldehydes **15a,b** attention was turned to their elaboration into cyclic sulfate derivatives (Scheme 5.3). Towards that objective, 15a,b were treated with (carbethoxymethylene)triphenylphosphorane in dry CH_2Cl_2 at room temperature to obtain the corresponding *trans* unsaturated esters 16a,b. Subjection of 16a,b to Sharpless asymmetric dihydroxylation[5] with AD mix β in ^tBuOH–H_2O (1:1) at 0 °C for 24 h furnished enantiopure dihydroxyl derivatives **17a,b** in good yields and high enantiomeric excess (90%, ee >99%; determined by chral HPLC analysis). Treatment of diol **17a,b** with thionyl chloride and triethylamine in CH_2Cl_2 gave the respective cyclic sulfites which were further oxidized using $NaIO_4$ and a catalytic amount of ruthenium trichloride to furnish the corresponding cyclic sulfates **18a,b** in good yields. ^1H NMR spectrum of **18a** showed two signals at δ 4.85-4.79 (m, 1H), 4.65 (d, 1H, J = 7.0) corresponding to the two protons α-H & β-H with respect to ester functionality; these two protons appeared upfield while the diols (compound **17a**) were not protected. Again, MS (FAB) showed a peak at *m/z* 436 corresponding to the molecular ion of compound **18a**.



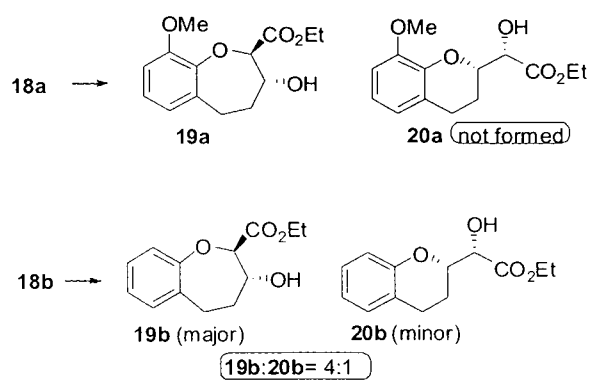
Scheme 5.3. *Reagents and conditions*: (a) $Ph_3P{=}CHCO_2Et$, dry DCM, rt, overnight, 82% (**16a**) and 77% (**16b**). (b) AD mix β, $MeSO_2NH_2$, *t*-BuOH/H_2O (1:1), 0 °C, 24 h, 94% (**17a**) and 88% (**17b**). (c) (i)$SOCl_2$, Et_3N, CH_2Cl_2, 0 °C, 20 min (ii) $RuCl_3$, $NaIO_4$, MeCN–H_2O; 1:9, 0 °C, 10 h, 87% (**18a**) and 85% (**18b**) (over last two steps).

To perform phenoxide ion mediated intramolecular cyclic sulfate ring opening reaction (Scheme 5.4), **18a** was subjected to hydrogenolysis under H_2/10% Pd-C condition to yield the corresponding phenolic derivative. Without purification the phenol was reacted with anhydrous K_2CO_3 in dry acetone followed by hydrolysis with 20% H_2SO_4 in THF. After extensive NMR studies (^1H, ^{13}C, COSY, HMBC, HSQC), we found the final cyclic product as 1-benzoxepine skeleton **19a** rather than 1-benzopyran ring system **20a**, the latter being the expected product.



Scheme 5.4. *Reagents and conditions:* (a) H_2, 10%Pd-C, EtOAc, 8 h, 89%. (b) (i) anhyd. K_2CO_3, dry acetone, rt, 8h. (ii) 20% H_2SO_4, THF, rt, overnight, 70% (for two steps).

It is important to mention that in the cyclization reaction of the cyclic sulfate, **18a** did not provide corresponding products **20a** with 1-benzopyran ring system. However, cyclic sulfate **18b** furnished major cyclic product **19b** containing a 1-benzoxepine skeleton (60%) and minor product **20b** (15%) with a benzopyran ring system.



^{1}H NMR Spectrum of **19a** showed the presence of two multiplets at δ 2.34-2.25 and 1.65-1.52 attributable to the protons H-4_a and H-4_b and another multiplet at δ 2.89-2.65 due to two H-5 protons. H-3 appeared as a multiplet at δ 4.18-4.11 whereas the proton H-2 showed a doublet at δ 3.85 (J = 9.2). The presence of the –OH group was indicated by a broad singlet at δ 3.11. The above attribution of various protons was done by incisive analysis of COSY spectrum of **19a**. Based on the coupling observed in the HSQC spectrum of **19a**, ring carbons appearing at δ 83.9, 71.9, 33.4 and 28.0 were attributed to C-2, C-3, C-4 and C-5, respectively. Similarly, the signals at δ 61.6, 55.4, and 14.0 were assigned to the $-O\underline{C}H_2CH_3$, $-O\underline{C}H_3$ and $-OCH_2\underline{C}H_3$ respectively. Finally, six signals at δ 156.0, 151.9, 135.6, 121.8, 115.0, and 112.1 were assigned to the aromatic carbons C-7, C-10, C-11, C-8, C-6 and C-9. In the HMBC spectrum of **19a** (Figure 5.2.), H-2 [δ 3.85 (J = 9.2)] showed coupling with $\underline{C}$=O (δ 170.7), C-3 (δ 71.9), C-4 (δ 33.4) and C-10 (δ 151.9). The long range coupling of H-2 with C-10 confirmed formation of 1-benzoxepine ring over 1-benzopyran ring as this coupling would not be possible in case of **20a**. Again, had the cyclic product been **20a**, the proton [δ 4.18-4.11 (m, 1H)] would have shown long range coupling with the aromatic carbon atom [C-9 (δ 151.9) and carbonyl carbon (δ 170.7) in structure **20a**) which is completely absent in the HMBC spectrum. Thus, the 1-benzoxepine structure **19a** was confirmed. The structures of other isolated cyclic products **19b** and **20b** were similarly assigned.

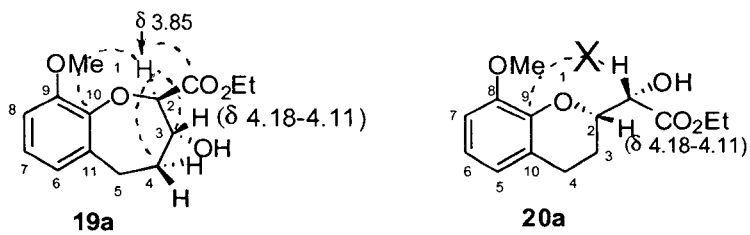
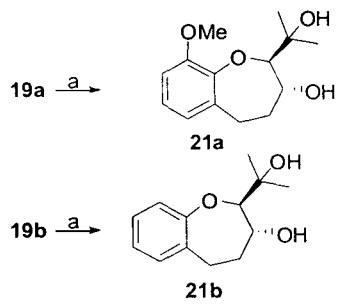


Figure 5.2. *Coupling of H-2 in the HMBC spectrum of* **19a**.

It is a well known fact that due to the presence of ester group, the α-carbon atom of an α,β-dihydroxy ester-derived cyclic sulfate possesses higher reactivity than the β-carbon and hence nucleophilic attack occurs at α-carbon almost exclusively.[6] Thus, formation of 1-benzoxepine derivatives **19a** and **19b** (*via* 7-*endo-tet* cyclization) might be explained on the basis of high reactivity at α-carbon. At the same time, 1-benzopyran derivatives **20a** and **20b** (formed *via* 6-*exo-tet* cyclization) might be

considered as entropically favorable. Since the presence of methoxy group *ortho*- and *para*- to phenolic–OH functionality on a phenyl ring increases the nucleophilicity of the phenoxide ion, cyclic sulfates **18a** gave only 1-benzoxepine derivative **19a**. In the absence of methoxy group nucleophilicity of a phenoxide ion gets reduced. Thus, cyclic sulfate **18b** furnished 1-benzoxepine derivative **19b** along with minor amount of 1-benzopyran derivative **20b**.

Finally, treatment of **19a** and **19b** with an excess of methylmagnesium iodide furnished the corresponding tertiary alcohols **21a-b** in high yields, Scheme 5.5.



Scheme 5.5. *Reagents and conditions*: (a) MeMgI, dry THF, 0 °C-reflux, 3h, (88%), **21a** (90%) and **21b** (94%).

5.4 Conclusion

In summary, we have described an asymmetric synthesis of 2,3-disubstituted 1-benzoxepines by an easy and high yielding reaction sequence. Key steps include Sharpless asymmetric dihydroxylation reaction on suitably substituted α,β-unsaturated esters and construction of the 1-benzoxepine nuclei by phenoxide ion-directed intramolecular 7-*endo-tet* carbocyclization of *syn*-2,3-dihydroxy ester-derived cyclic sulphates. Presence of methoxy group *ortho* to the phenolic–OH functionality on the phenyl ring rendered the cyclization reaction completely regioselective producing 1-benzoxepine derivatives only. In the absence of a methoxy group on the phenyl ring, the reaction furnished both 1-benzoxepine and 1-benzopyran derivatives with the former being the major one. To the best of our knowledge this is the first report where α,β-dihydroxy ester cyclic sulfates are utilized in the stereoselective synthesis of benzo-annulated heterocycles.

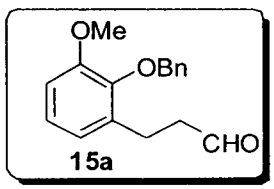
5.5 Experimental Section

5.5.1 General Remarks

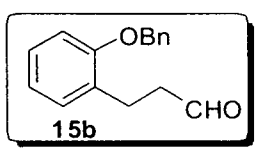
As described in **Section 3.1.5.1 (Chapter 3, Section 3A).**

5.5.2 Synthesis of compounds

3-(2-(benzyloxy)-2-methoxyphenyl)propanal (15a): To a stirring solution of **14a** (3.0 g, 9.54 mmol) in dry toluene (50 mL) was added DIBAL-H (1 M in toluene, 12 mL, 12 mmol)) dropwise under nitrogen atmosphere at -78°C. The reaction mixture was stirred at the same temperature for 1 h and quenched with dry methanol (5 mL). The reaction was allowed to come at 0°C and a saturated aq. sodium potassium tartarate solution (25 mL) was added. The resulting mixture was vigorously stirred for 45 min. at rt and then extracted with ethyl acetate (2x50 mL), washed with water (50 mL) and brine (100 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and then concentrated under reduced pressure. Concentration of the filtrate and purification of the residue by silica gel column chromatography (4% ethyl acetate in hexane) afforded **15a** (2.35 g, 94%) as a colorless gum. IR (Neat): 3020, 2361, 1722, 1610, 1506, 1216, 761 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 9.59 (s, 1H), 7.42-7.37 (m, 2H), 7.33-7.22 (m, 3H), 6.94-6.89 (m, 1H), 6.76-6.67 (m, 2H), 4.98 (s, 2H), 3.80 (s, 3H), 2.80 (t, 2H, J = 7.5), 2.70-2.53 (t, 2H, J = 7.6). MS (FAB): *m/z* 270 $[M]^+$. Anal. Calcd for $C_{17}H_{18}O_3$: C, 75.53; H, 6.71. Found: C, 75.49; H, 6.87.

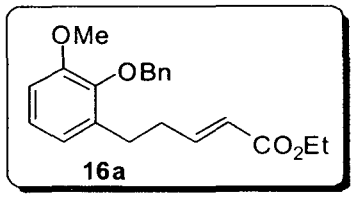


3-(2-(benzyloxy)phenyl)propanal (15b): Starting from 3.0 g (10.55 mmol) of **14b,** compound **15b** was prepared in the same manner as that described for the synthesis of **15a**. Purification of the crude product by silica gel column chromatography (4% ethyl acetate in hexane) afforded **15b** (2.40 g, 95%) as a colorless gum. The physical and spectroscopic data of **15b** were in full agreement with our previously reported data of the same compound.[7]



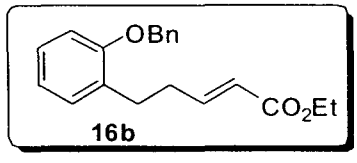
(*E*)-ethyl 5-(2-(benzyloxy)-3-methoxyphenyl)pent-2-enoate (16a): To a solution of **15a** (2.0 g, 7.39 mmol) in dichloromethane (30 mL) was added (carbethoxymethylene)triphenylphosphorane (3.21 g, 9.21 mmol) at room

temperature. The reaction mixture was stirred at room temperature for overnight. Solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (4% ethyl acetate in hexane) to afford **16a** (2.06 g, 82%) as a colorless gum. IR (KBr): 2929, 2355, 1709, 1655, 1499, 1212, 1035, 738 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.35-7.33 (m, 2H), 7.29-7.20 (m, 3H), 6.91-6.80 (m, 2H), 6.71 (dd, 1H, J_1 = 1.2, J_2 = 8.2), 6.64 (dd, 1H, J_1 = 1.2, J_2 = 7.6), 5.67 (dd, 1H, J_1 = 1.3, J_2 = 14.3), 4.91 (s, 2H), 4.06 (q, 2H, J = 7.1), 3.75 (s, 3H), 2.62-2.57 (m, 2H), 2.34-2.27 (m, 2H), 1.16 (t, 3H, J = 7.1). MS (FAB): *m/z* 340 $[M]^+$. Anal. Calcd for $C_{21}H_{24}O_4$: C, 74.09; H, 7.11. Found: C, 74.15; H, 7.24.



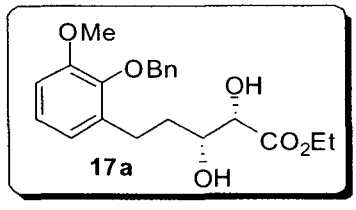
16a

(*E*)-ethyl 5-(2-(benzyloxy)phenyl)pent-2-enoate (16b): Starting from 2.0 g (8.32 mmol) of **15b,** compound **16b** was prepared in the same manner as that described for the synthesis of **16a**. Purification of the crude product by silica gel column chromatography (4% ethyl acetate in hexane) afforded **16b** (2.0 g, 77%) as a colorless gum. The physical and spectroscopic data of **16b** were in full agreement with our previously reported data of the same compound.[7]



16b

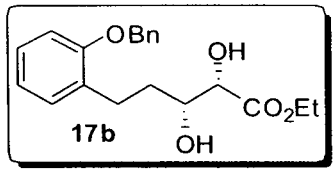
(2*S*,3*R*)-ethyl 5-(2-(benzyloxy)-3-methoxyphenyl)-2,3-dihydroxypentanoate (17a): To a stirred solution of *tert*-butyl alcohol (17 mL) and water (22 mL) were added AD mix β (6.2 g) and methanesulfonamide (0.42 g, 4.4 mmol) at room temperature. The mixture was vigorously stirred at room temperature until both phases were clear and then cooled to 0°C. A solution of cinnamate ester **16a** (1.5 g, 4.4 mmol) in *tert*-butyl alcohol (5 mL) was added 0°C. The reaction was stirred at the same temperature for 28 h. The reaction was quenched at 0°C by the addition of sodium sulfite (6.5 g), warmed to room temperature, and further stirred for 1 h. The reaction mixture was then extracted with ethyl acetate (3x50 mL). The combined organic layer was washed with aq. 2 N KOH solution (50 mL), water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and then concentrated under vacuo. Purification of the crude product by silica gel column chromatography (25% ethyl acetate in hexane)



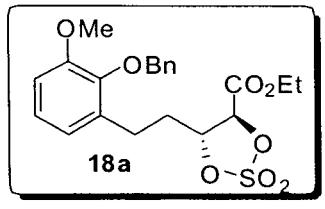
17a

furnished **17a** as a colorless gum (1.5 g, 94%). $[\alpha]^{25}_{D}$ +12.2 (*c* 1.23, MeOH). The enantiomeric excess was estimated to be >99% by chiral HPLC analysis (instrument: HP1100, column: LichroCART Chiradex column 250 mm × 4 mm, 5 μm), flow rate: 0.5 mL/min, detection: UV 254 nm, eluent: methanol/H_2O). IR (KBr): 3442, 2929, 2361, 1735, 1635, 1501, 1217, 758 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$+CCl_4): δ 7.45-7.24 (m, 5H), 6.99-6.91 (m, 1H), 6.74 (d, 2H, *J* = 8.3), 5.04-4.92 (m, 2H), 4.19 (q, 2H, *J* = 7.1), 3.92 (s, 1H), 3.85 (s, 3H), 3.72 (s, br, 1H), 3.17 (s, br, 1H), 2.75-2.54 (m, 3H), 2.44 (d, 1H, *J* = 2.5), 1.94-1.62 (m, 2H), 1.24 (t, 3H, *J* = 7.1). MS (FAB): *m/z* 374 $[M]^+$. Anal. Calcd for $C_{21}H_{26}O_6$: C, 67.36; H, 7.00. Found: C, 67.29; H, 7.19.

(2*S*,3*R*)-ethyl 5-(2-(benzyloxy)phenyl)-2,3-dihydroxypentanoate (17b): Starting from 1.5 g (4.83 mmol) of **16b**, compound **17b** was prepared in the same manner as that described for the synthesis of **17a**. Purification of the crude product by silica gel column chromatography (25% ethyl acetate in hexane) furnished **17b** as a colorless gum (1.46 g, 88%). $[\alpha]^{25}_{D}$ +19.2 (*c* 3.55, $CHCl_3$). The enantiomeric excess (>99%) was determined in the same manner as that described for **17a**. The spectroscopic data of **17b** were in full agreement with our previously reported data of the same compound.[7]

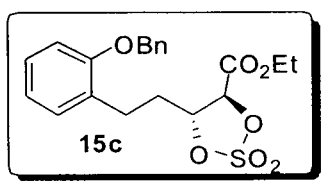


(2*S*,3*R*)-5-(2-(2-Benzyloxy-3-methoxyphenyl)-ethyl)-2,2-dioxo-2l6-[1,3,2]dioxathiolane-4-carboxylic acid ethyl ester (18a): To an ice cooled stirring solution of diol **17a** (1.0 g, 2.67 mmol) in dry CH_2Cl_2 (15 mL) and anhydrous Et_3N (1.48 mL, 10.62), was dropwise added thionyl chloride (0.3 mL, 4.12 mmol). The reaction mixture was stirred for 20 min and then quenched by adding water (10 mL). The phases were separated and aqueous phase extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were dried over Na_2SO_4 and concentrated. The residue was dissolved in CH_3CN (10 mL) and CCl_4 (10 mL) and resulting solution was cooled with an ice-water bath. Next, $RuCl_3 \cdot H_2O$ (5 mg, 0.024 mmol) and $NaIO_4$ (1.23 g, 5.66 mmol) were added followed by water (10 mL). The resulting orange mixture was stirred at room temperature for 10 h. The mixture was then diluted with ether (50 mL), and the two phases separated. The organic layer was washed with water (30 mL), saturated aq $NaHCO_3$ (20 mL), brine, dried over



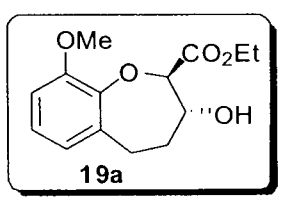
Na_2SO_4, and concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography (15% ethyl acetate in hexane) furnished **18a** as a colorless gum (1.1 g, 87%). $[\alpha]^{25}_D$ +44.9 (*c* 1.56, MeOH). IR (KBr): 3020, 2931, 2361, 1767, 1477, 1214, 1040, 761 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$+CCl_4): δ 7.41-7.30 (m, 5H), 7.00-6.95 (m, 1H), 6.83 (dd, 1H, J_1 = 1.4, J_2 = 8.2), 6.72 (dd, 1H, J_1 = 1.3, J_2 = 7.6), 5.06-4.97 (m, 2H), 4.85-4.79 (m, 1H), 4.65 (d, 1H, J = 7.0), 4.25 (q, 2H, J = 7.1), 3.89 (s, 3H), 2.78-2.57 (m, 2H), 2.18-2.10 (m, 2H), 1.27 (t, 3H, J = 7.1). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.7, 152.7, 146.1, 137.7, 133.3, 128.5, 128.3, 128.1, 124.2, 122.0, 111.3, 83.2, 79.4, 74.8, 63.0, 55.7, 33.6, 26.1, 14.0. MS (FAB): *m/z* 436 $[M]^+$. Anal. Calcd for $C_{21}H_{24}O_8S$: C, 57.79; H, 5.54. Found: C, C, 57.83; H, 5.49.

(2*S*,3*R*)-5-(2-(2-Benzyloxyphenyl)-ethyl)-2,2-dioxo-2l6-[1,3,2]dioxathiolane-4-carboxylic acid ethyl ester (18b): Starting from 1.0 g (2.90 mmol) of **17b,** compound **18b** was prepared in the same manner as that described for the synthesis of



18a. Purification of the crude product by silica gel column chromatography (15% ethyl acetate in hexane) furnished **18b** as a colorless solid (0.99 g, 85%). $[\alpha]^{25}_D$ +58.8 (*c* 2.37, MeOH). IR (KBr): 2936, 2362, 1773, 1497, 1398, 1205, 1055, 760 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.42-7.28 (m, 5H), 7.23-7.12 (m, 2H), 6.93-6.87 (m, 2H), 5.05 (s, 2H), 4.94-4.87 (m, 1H), 4.78 (d, 1H, J = 7.1), 4.21 (q, 2H, J = 7.1), 2.96-2.74 (m, 2H), 2.37-2.17 (m, 2H), 1.22 (t, 3H, J = 7.1). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.5, 156.3, 136.8, 130.0, 128.5, 128.0, 127.8, 127.6, 127.0, 120.8, 111.6, 83.5, 79.5, 69.7, 63.0, 32.8, 25.9, 13.7. MS (FAB): *m/z* 406 $[M]^+$. Anal. Calcd for $C_{20}H_{22}O_7S$: C, 59.10; H, 5.46. Found: C, 59.22; H, 5.56.

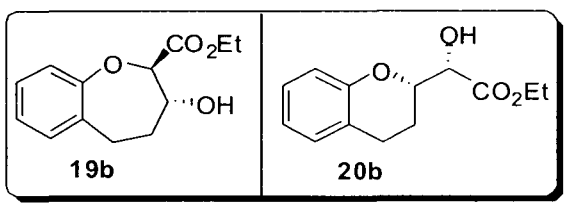
(2*R*,3*R*)-ethyl 3-hydroxy-3-methoxy-2,3,4,5-tetrahydrobenzo[b]oxepine-2-carboxylate (19a): To a stirred solution of **18a** (0.9 g, 2.06 mmol) in ethyl acetate (20 mL) was added 10% Pd-C (50 mg). After stirring for 3 h at room temperature under pressure of a hydrogen balloon, the reaction mixture was filtered through a pad of Celite® and the filtrate was



concentrated under reduced pressure to get the corresponding debenzylated product (0.64 g) as a colorless semi-solid which was used for the next step without further purification.

To a stirring solution of the above debenzylated product in dry acetone (20 mL), was added anhyd. K_2CO_3 (0.4 g, 2.89 mmol) and the mixture was stirred for 5 h at room temperature. After removing acetone of the reaction mixture under reduced pressure, the residue was stirred with 20% aq H_2SO_4 (20 mL) and THF (10 mL) for 16 h. The resultant solution was then neutralized with aq. saturated $NaHCO_3$ solution and extracted with ethyl acetate. The combined organic phases were washed with water, brine dried Na_2SO_4, and concentrated. Purification of the crude product by silica gel column chromatography (20% ethyl acetate in hexane) furnished **19a** as a colorless semi-solid (0.394 g, 72%). $[\alpha]^{25}_D$ -22.1 (*c* 3.44, MeOH). IR (KBr): 3434, 2923, 2358, 1648, 1511, 1218, 768 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 6.95-6.90 (m, 1H), 6.76-6.68 (m, 2H), 4.33 (q, 2H, *J* = 7.1), 4.21-4.13 (m, 1H), 3.84 (d, 1H, *J* = 10.3), 3.82 (s, 3H), 3.08 (s, br, 1H), 2.92-2.69 (m, 2H), 2.33-2.22 (m, 1H), 1.64-1.52 (m, 1H), 1.38 (t, 3H, *J* = 7.1). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6,151.9, 147.6, 136.3, 124.5, 121.7, 111.4, 83.6, 72.0, 61.5, 56.2, 33.4, 27.8, 14.2. MS (FAB): *m/z* 266 $[M]^+$. Anal. Calcd for $C_{14}H_{18}O_5$: C, 63.15; H, 6.81. Found: C, 63.22; H, 6.93.

((2*R*,3*R*)-ethyl 3-hydroxy-2,3,4,5-tetrahydrobenzo[b]oxepine-2-carboxylate (19b) and (S)-ethyl 2-((S)-chroman-2-yl)-2-hydroxyacetate (20b): 0.9 g (2.21 mmol) of **18b** was subjected to the same cyclization reaction as that described for the synthesis of **19a**. Purification of the crude product by silica gel column chromatography (20% ethyl acetate in hexane) furnished **19b** (0.304 g, 60%) and **20b** (76 mg, 15%).

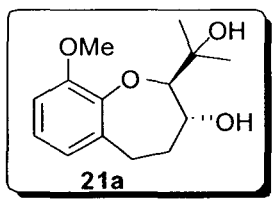


Physical and spectral data of 19b: Colorless gum. $[\alpha]^{25}_D$ +18.2 (*c* 2.62, MeOH). IR (KBr): 3439, 2925, 2359, 1648, 1515, 1216, 768 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$+CCl_4): δ 7.16-7.10 (m, 2H), 7.06-6.97 (m, 2H), 4.32 (q, 2H, *J* = 7.1), 4.22-4.15 (m, 1H), 3.92 (d, 1H, *J* = 9.3), 2.98 (s, br, 1H), 2.91-2.75 (m, 2H), 2.35-2.26 (m,

1H), 1.68-1.56 (m, 1H), 1.37 (t, 3H, J = 7.1). ^{13}C NMR (75 MHz, $CDCl_3$+CCl_4): δ 170.8, 158.3, 134.2, 130.1, 127.7, 124.5, 121.3, 83.5, 71.8, 61.6, 33.2, 27.8, 14.2. MS (FAB): *m/z* 236 $[M]^+$. Anal. Calcd for $C_{13}H_{16}O_4$: C, 66.09; H, 6.83. Found: C, 66.21; H, 6.72.

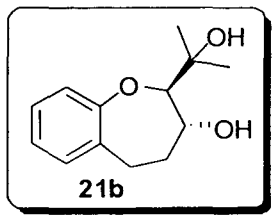
Physical and spectral data of 20b: Colorless gum. $[\alpha]^{25}_D$ +64.1 (*c* 0.78, MeOH). IR (KBr): 3445, 2926, 2362, 1650, 1511, 1218, 761 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$+CCl_4): δ 7.07-6.99 (m, 2H), 6.83-6.77 (m, 2H), 4.41-4.38 (m, 1H), 4.34-4.21 (m, 2H), 2.99 (d, 1H, J = 6.6), 2.93-2.74 (m, 2H), 2.13-1.99 (m, 1H), 1.95-1.87 (m, 1H), 1.31 (t, 3H, J = 7.1). ^{13}C NMR (75 MHz, $CDCl_3$+CCl_4): δ 172.0, 154.5, 129.4, 127.4, 121.6, 120.6, 116.6, 77.5, 72.9, 61.9, 24.6, 22.5, 14.3. MS (FAB): *m/z* 236 $[M]^+$. Anal. Calcd for $C_{13}H_{16}O_4$: C, 66.09; H, 6.83. Found: C, 66.17; H, 6.94.

(2*R*,3*R*)-2-(2-hydroxypropan-2-yl)-3-methoxy-2,3,4,5-tetrahydrobenzo[b]oxepin-3-ol (21a): To a freshly prepared, magnetically stirred, ice-cold suspension of methylmagnesium iodide [prepared from methyl iodide (1.2 mL, 14.0 mmol) and magnesium (0.34 g, 14.0 mmol) in 10 mL of dry ether] was added a solution of ester **19a** (0.25 g, 0.93 mmol) in dry THF (10 mL). The reaction mixture was refluxed for 4 h, cooled, and quenched with aqueous NH_4Cl solution (10 mL). The mixture was extracted with ethyl acetate (2x25 mL), washed with water and brine. The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography (30% ethyl acetate in hexane) afforded **21a** as a colorless solid (0.215 g, 90%). $[\alpha]^{25}_D$ -46.4 (*c* 3.38, MeOH). IR (KBr): IR (KBr): 3415, 3022, 2933, 2363, 1499, 1216, 762 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$+CCl_4): δ 6.93-6.85 (m, 1H), 6.72-6.65 (m, 2H), 4.07-3.96 (m, 1H), 3.80 (s, 3H), 3.75 (s, br, 1H,), 3.66 (s, br, 1H), 3.19 (d, 1H, J = 9.3), 2.92-2.68 (m, 2H), 2.32-2.18 (m, 1H), 1.70-1.52 (m, 1H), 1.42 (s, 3H), 1.37 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$+CCl_4): δ 151.3, 147.8, 135.7, 123.7, 121.7, 110.1, 91.5, 73.4, 72.8, 55.6, 35.5, 29.7, 28.2, 26.1, 25.5. MS (FAB): *m/z* 252 $[M]^+$, 235 $[M\text{-}OH]^+$. Anal. Calcd for $C_{14}H_{20}O_4$: C, 66.65; H, 7.99. Found: C, 66.82; H, 8.15.



21a

(2*R*,3*R*)-2-(2-hydroxypropan-2-yl)-2,3,4,5-tetrahydrobenzo[b]oxepin-3-ol (21b):



21b

Starting from 0.25 g (1.06 mmol) of **19b,** compound **21b** was prepared in the same manner as that described for the synthesis of **21a**. Purification of the crude product by silica gel column chromatography (30% ethyl acetate in hexane) furnished **21b** as a colorless solid (0.221 g, 94%). $[\alpha]^{25}_{D}$ -10.8 (*c* 1.34, MeOH). IR (KBr): 3421, 3025, 2930, 2363, 1498, 1216, 765 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.10-7.05 (m, 2H), 6.96-6.87 (m, 2H), 4.55-4.07 (m, 3H), 3.21 (d, 1H, J = 9.3), 2.83-2.73 (m, 2H), 2.24-2.15 (m, 1H), 1.67-1.55 (m, 1H), 1.44 (s, 3H), 1.36 (s, 3H). MS (FAB): *m/z* 222 $[M]^+$, 205 $[M\text{-}OH]^+$. Anal. Calcd for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 70.29; H, 8.31.

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7. Das, S. K.; Panda, G. *Tetrahedron*, **2008**, *64*, 4162.

5.7 Spectra of selected compounds

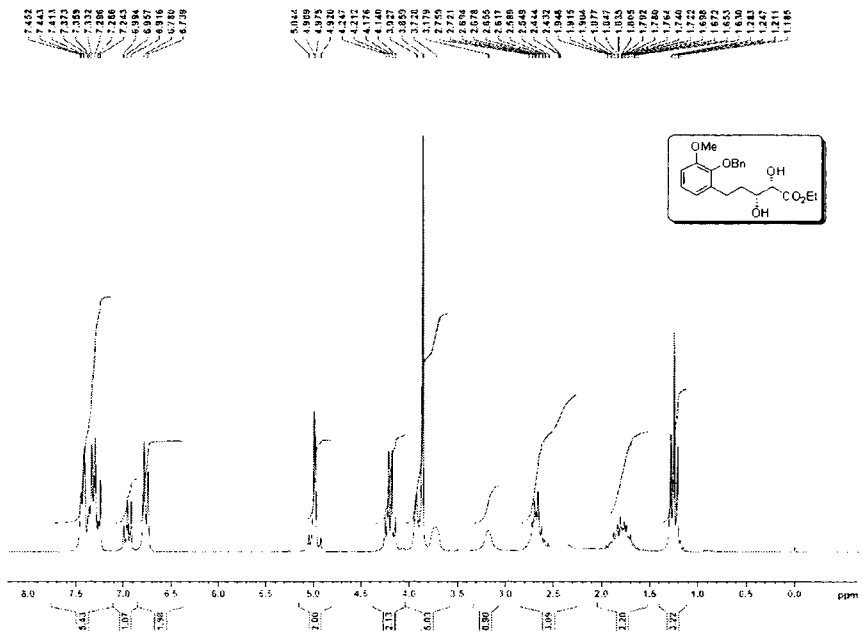


Figure 5.3: ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of **17a**.

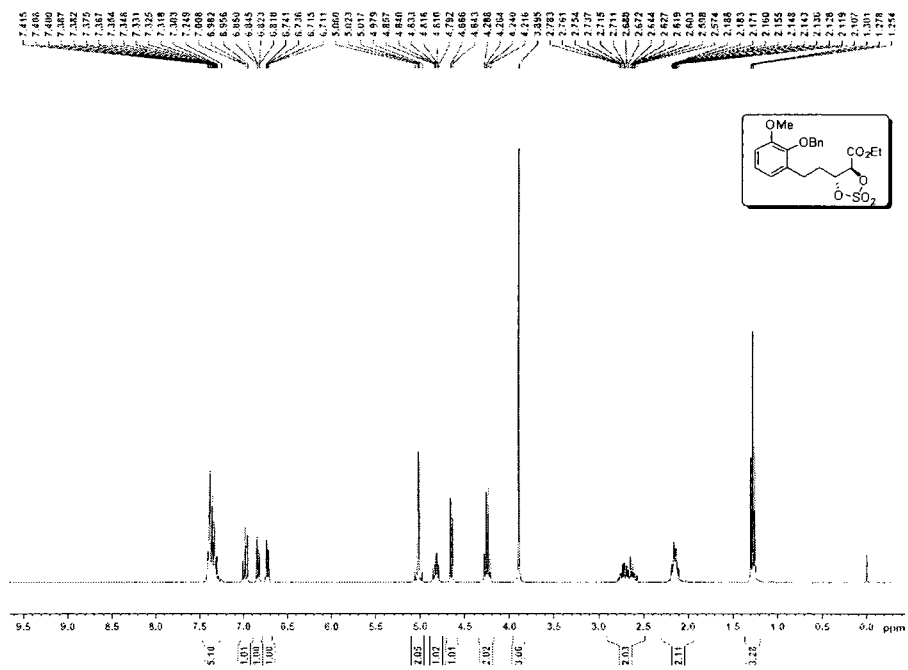


Figure 5.4: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **18a**.

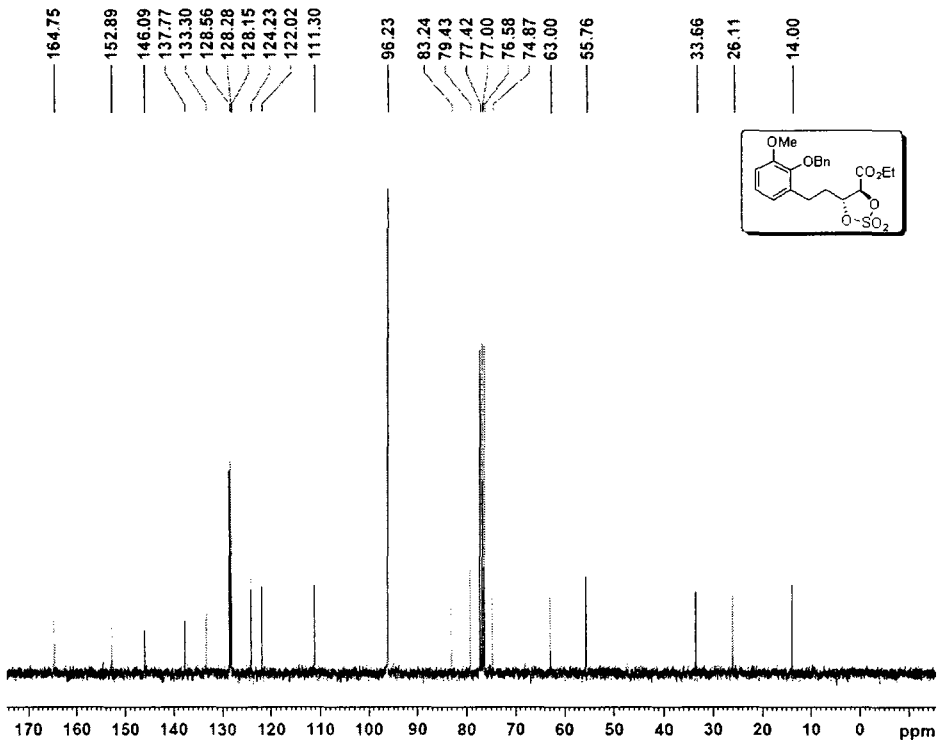


Figure 5.5: ^{13}C NMR ($CDCl_3$+CCl_4, 75 MHz) spectrum of **18a**.

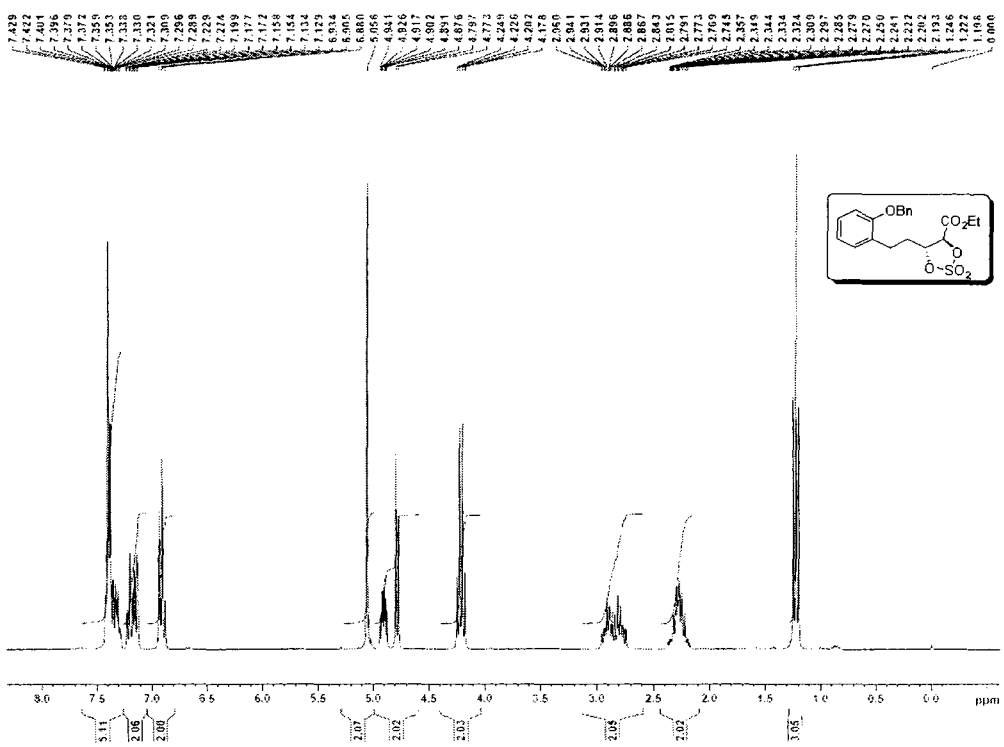


Figure 5.6: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **18b**.

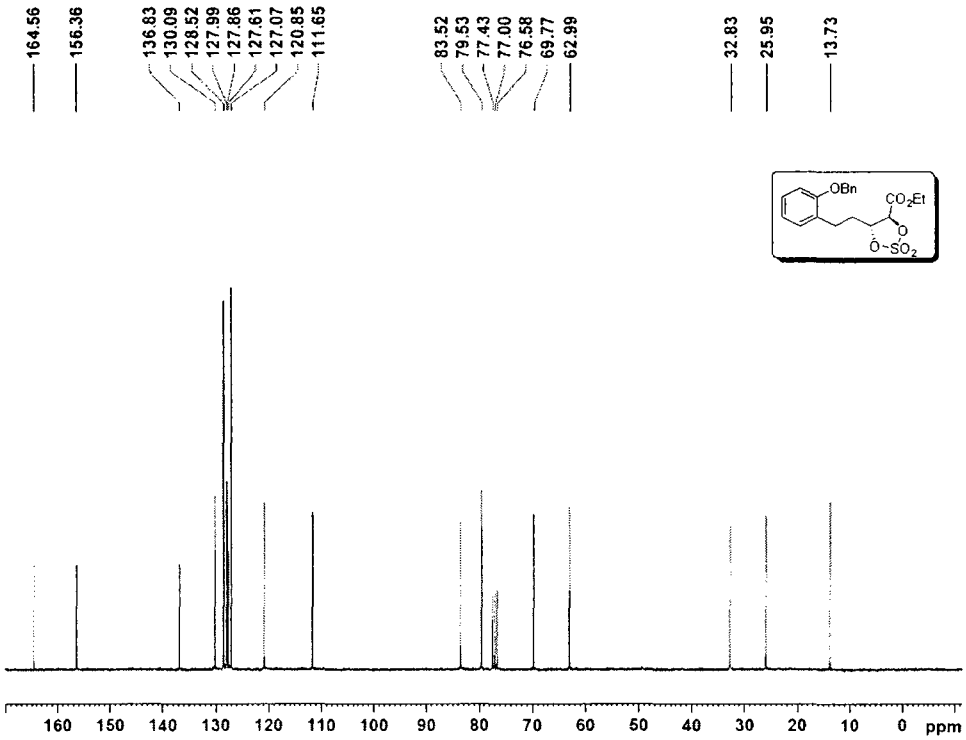


Figure 5.7: ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **18b.**

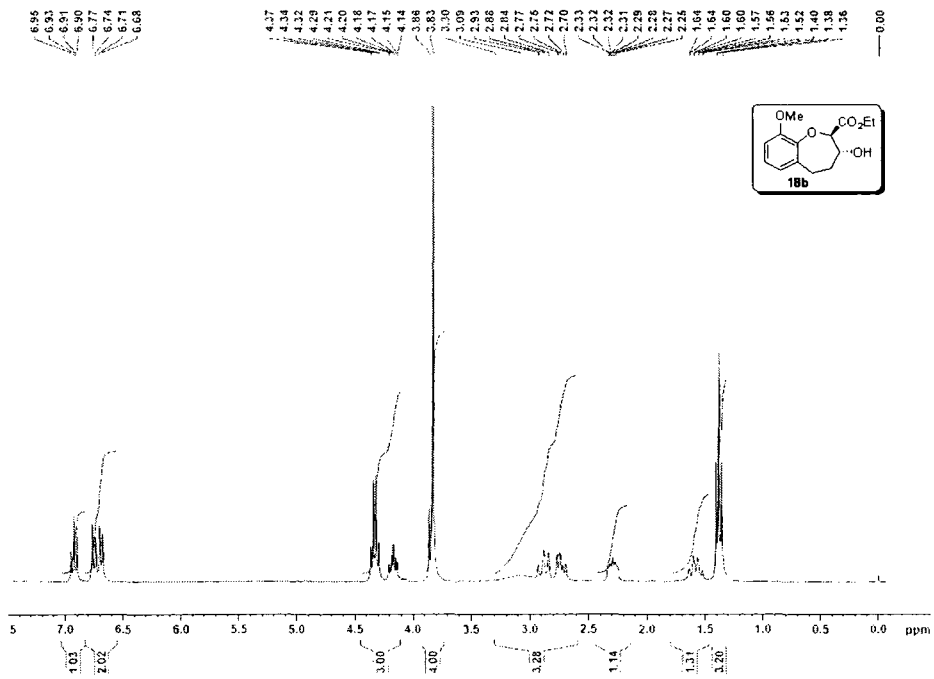


Figure 5.8: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **19a.**

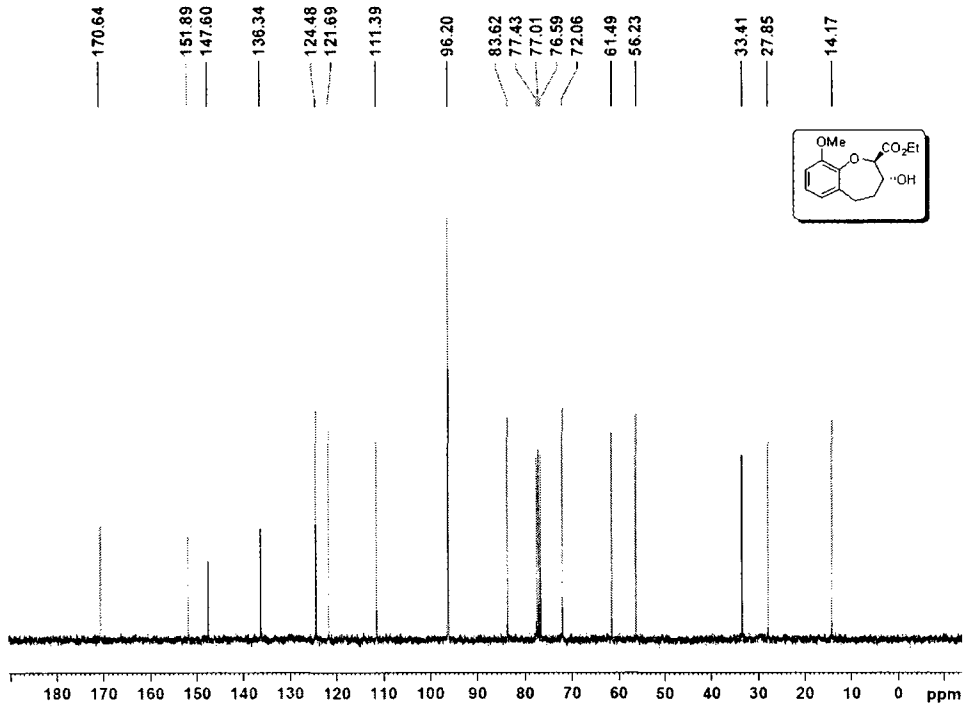


Figure 5.9: ^{13}C NMR ($CDCl_3$+CCl_4, 75 MHz) spectrum of **19a**.

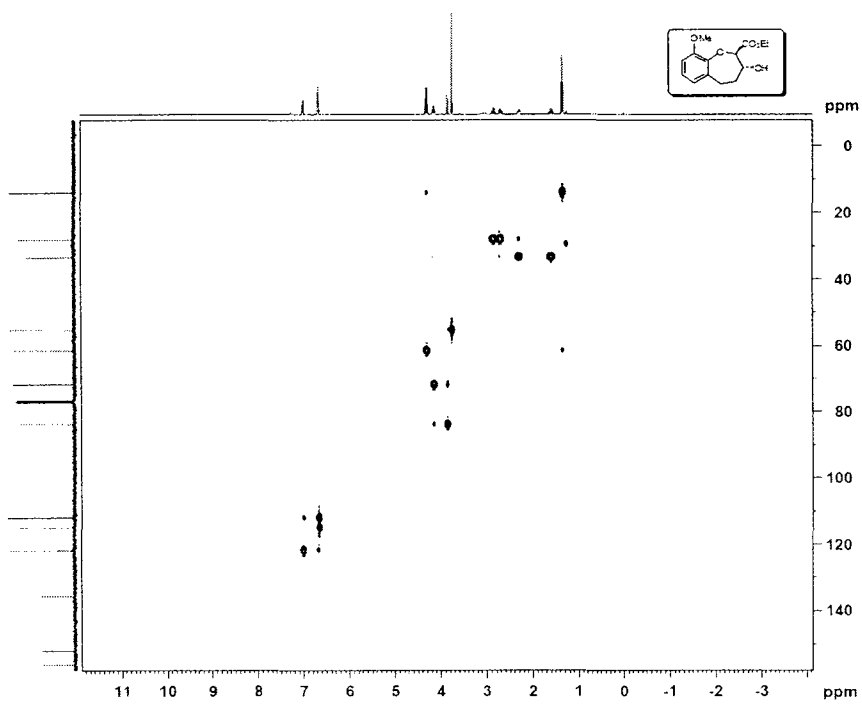


Figure 5.10: HSQC spectrum of **19a.**

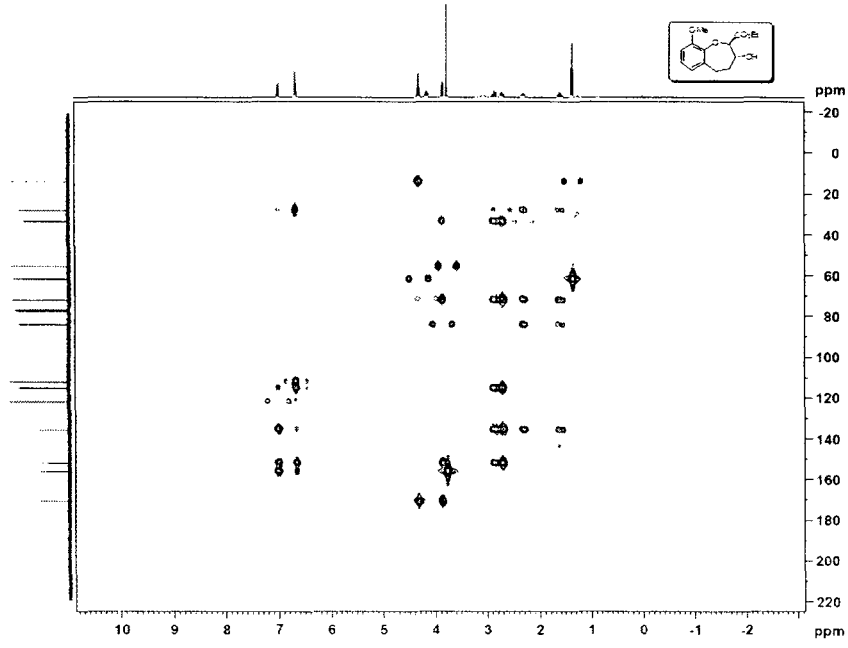


Figure 5.11: HMBC spectrum of **19a**.

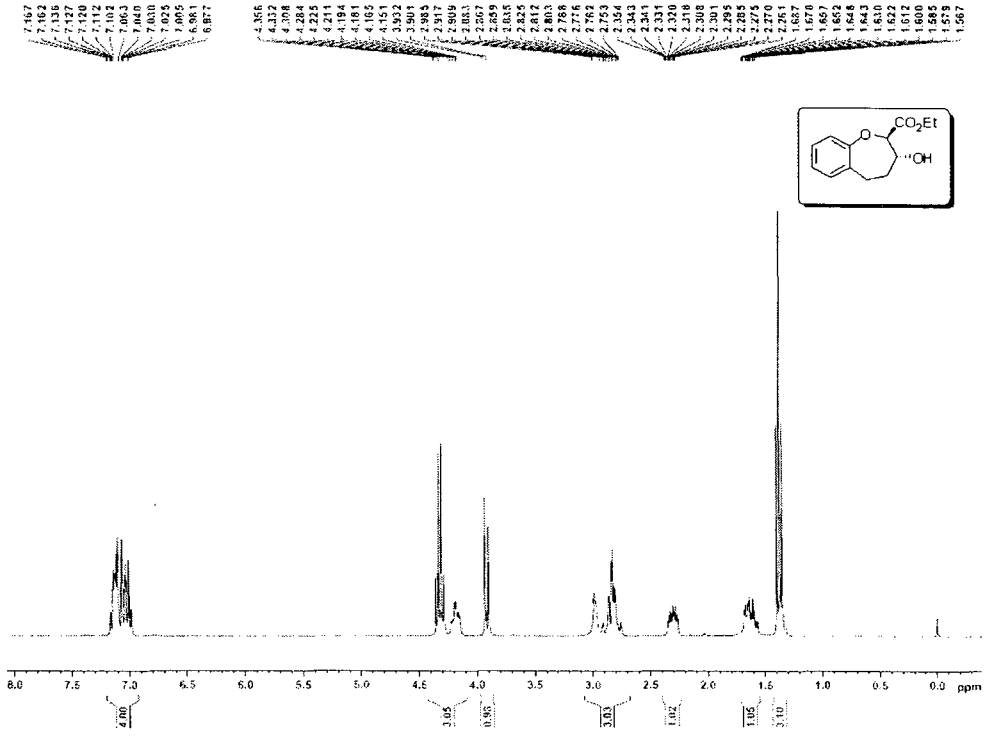


Figure 5.12: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **19b**.

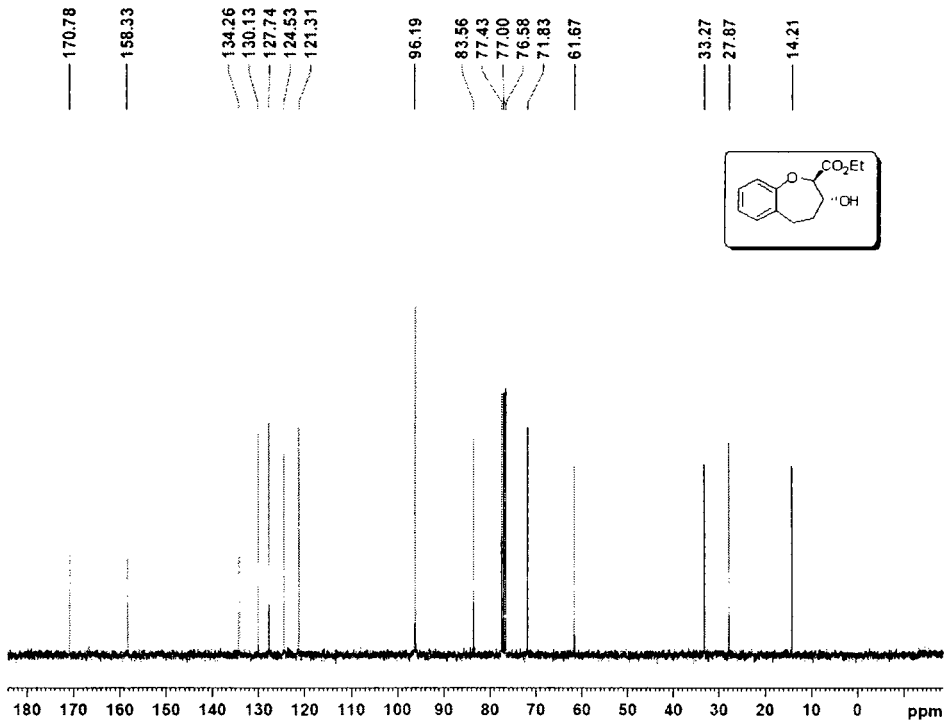


Figure 5.13: ^{13}C NMR ($CDCl_3$+CCl_4, 75 MHz) spectrum of **19b**.

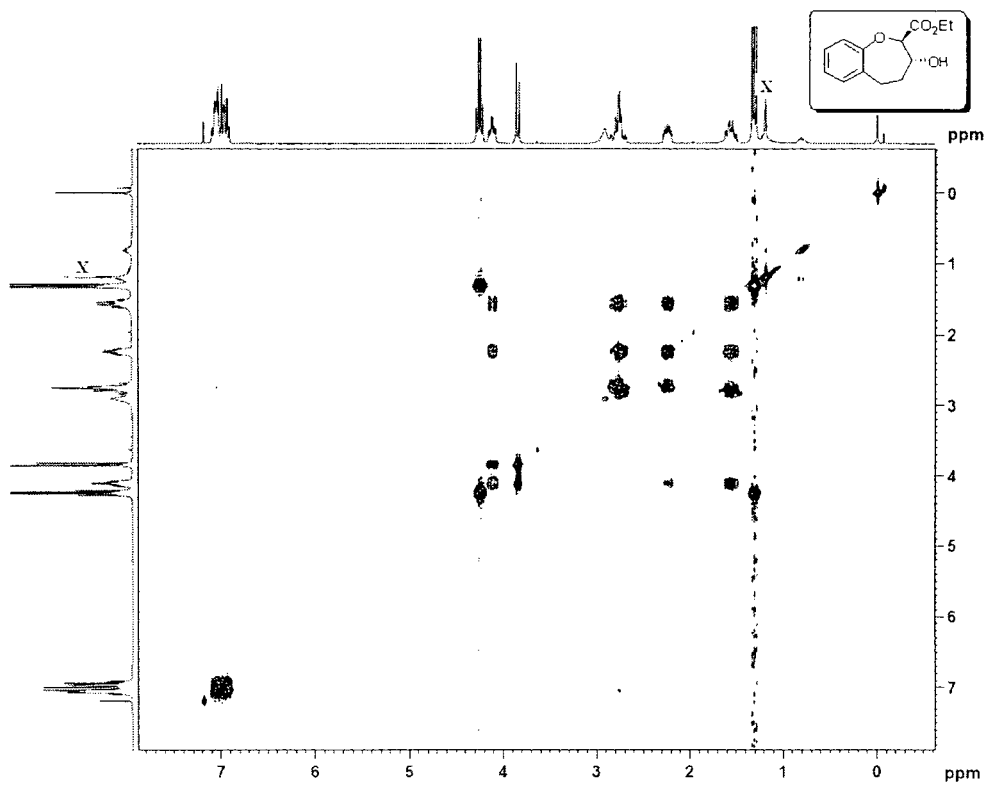


Figure 5.14: COSY spectrum of **19b**.

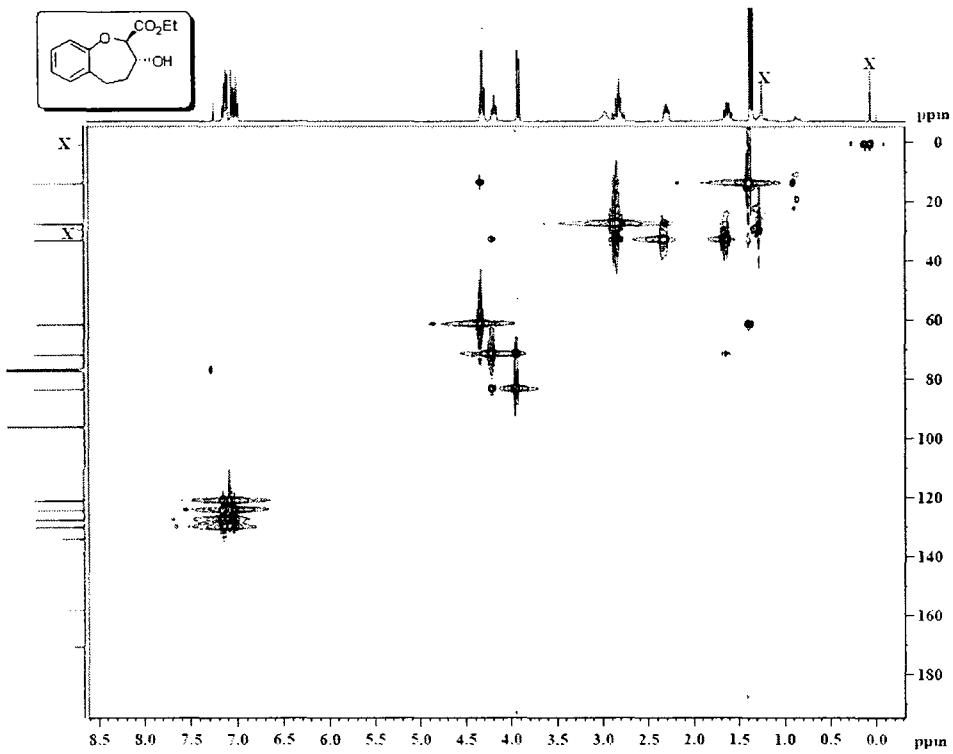


Figure 5.15: HSQC spectrum of **19b**.

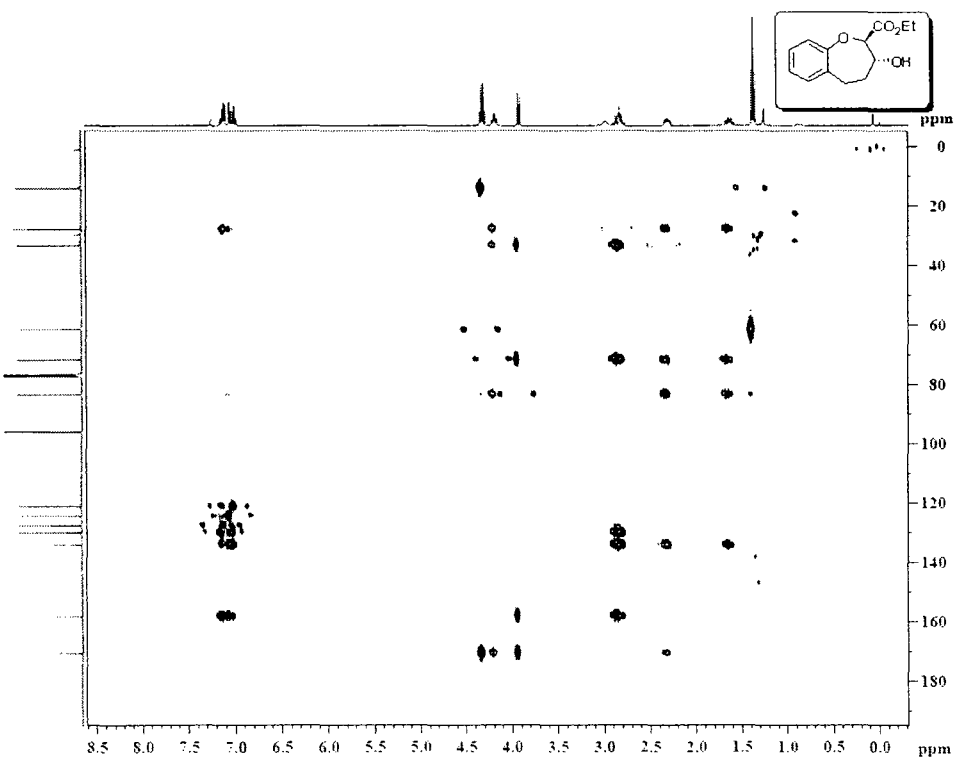


Figure 5.16: HMBC spectrum of **19b**.

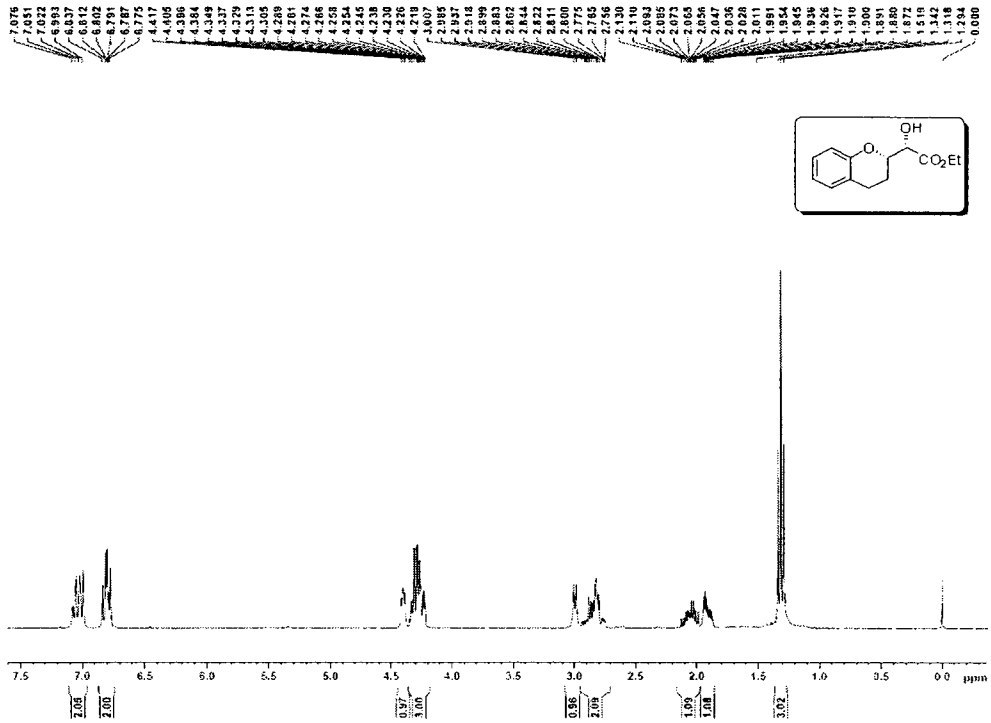


Figure 5.17: ^{1}H NMR ($CDCl_3$+CCl_4, 300 MHz) spectrum of **20b**.

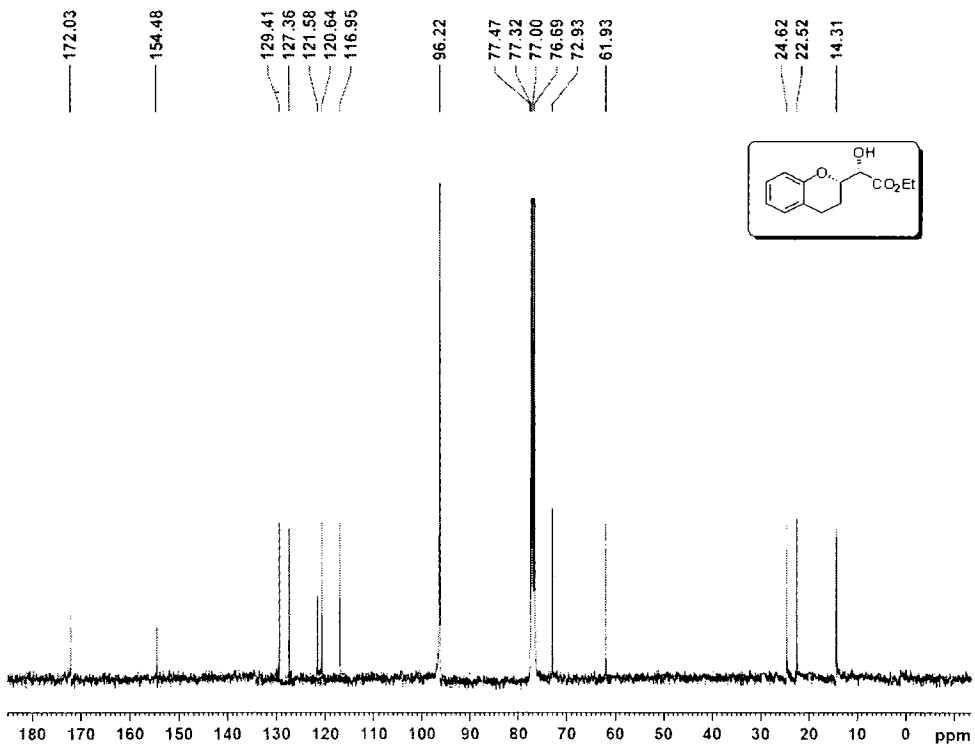


Figure 5.18: ^{13}C NMR ($CDCl_3$+CCl_4, 75 MHz) spectrum of **20b**.

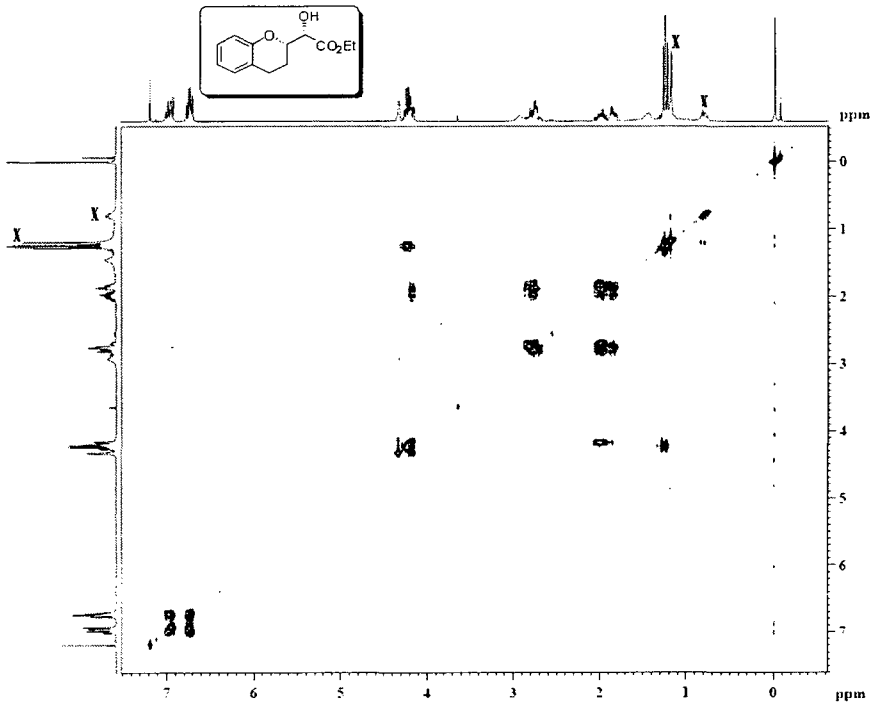


Figure 5.19: COSY spectrum ($CDCl_3$+CCl_4) of **20b**

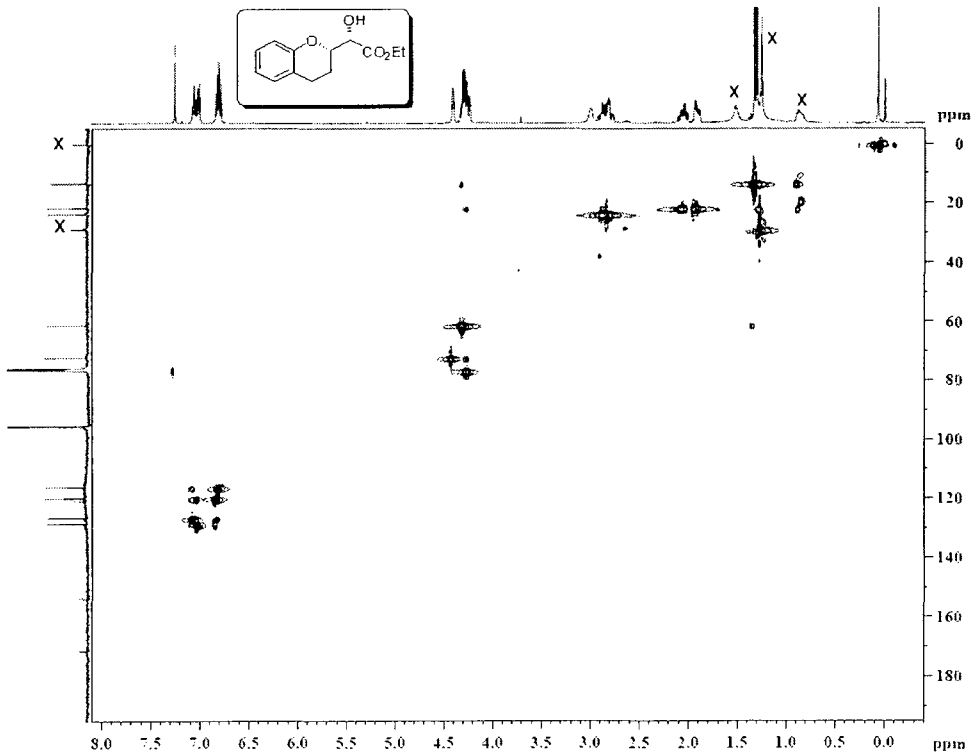


Figure 5.20: HSQC spectrum ($CDCl_3$+CCl_4) of **20b**.

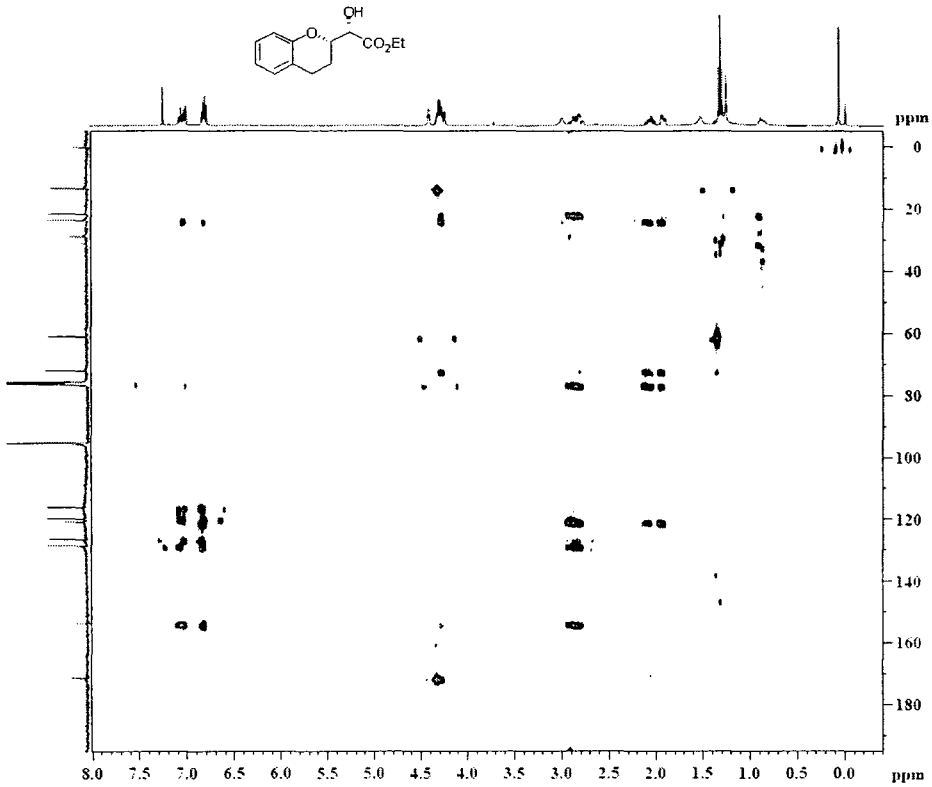


Figure 5.21: HMBC spectrum ($CDCl_3$+CCl_4) of **20b**.

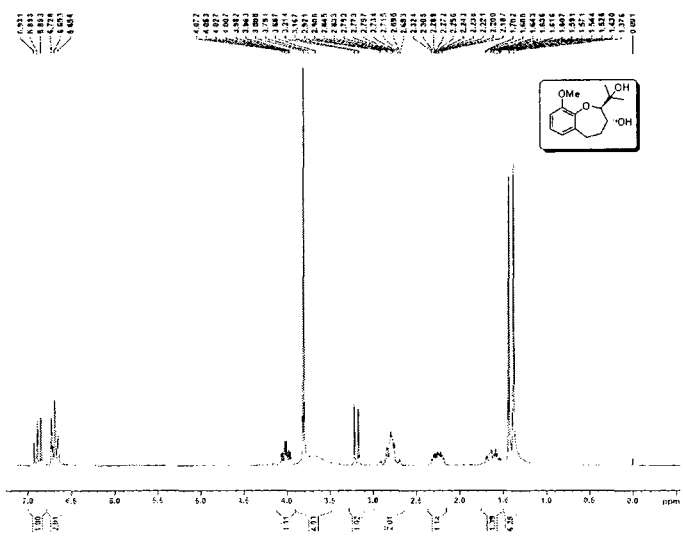


Figure 5.22: ^{1}H NMR ($CDCl_3$+CCl_4, 300 MHz) spectrum of **21a**.

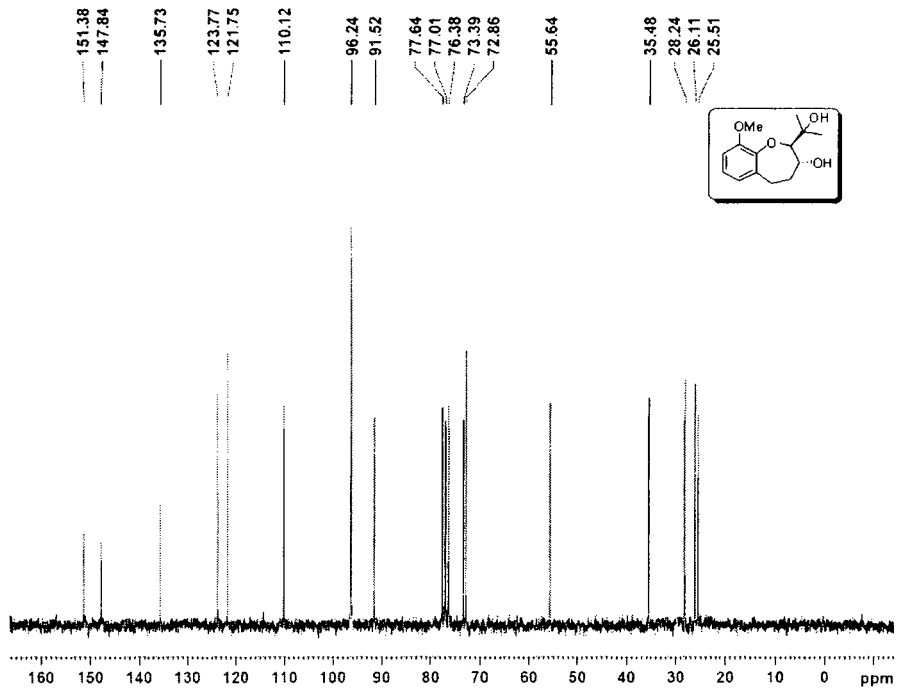


Figure 5.23: ^{1}H NMR ($CDCl_3$+CCl_4, 300 MHz) spectrum of **21a**.

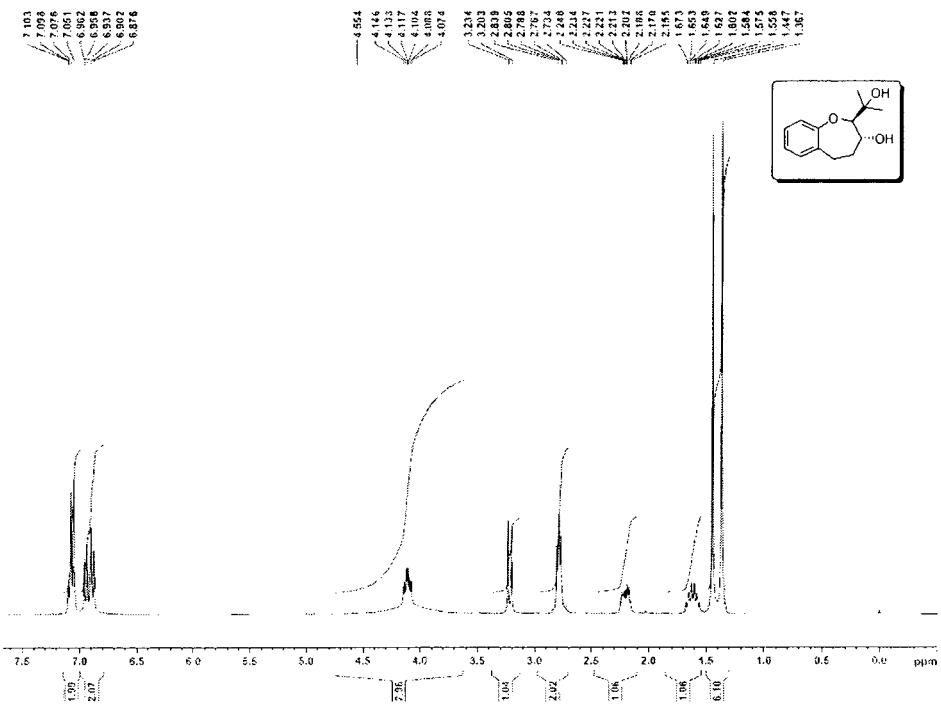


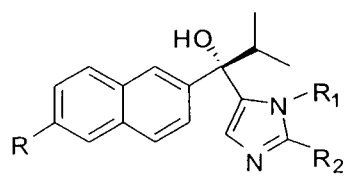
Figure 5.24: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **21b**.

Chapter 6:
An Enantioselective Approach towards Synthesis of a potent $C_{17,20}$-Lyase Inhibitor

6.1 Introduction

Nowadays prostate cancer is the most common cancer for males in the US and Europe. Cancer in nearly 80% of prostate cancer patients develops androgen-dependently and responds to first-line endocrine therapy. Presently, the standard treatment includes orchidectomy and its medical equivalent, the administration of gonadotropin-releasing hormone (GnRH) analogues, which eradicate the production of testosterone in the testes. Nevertheless, these treatments do not inhibit adrenal androgen production and hence are frequently combined with an androgen receptor antagonist to block the action of residual adrenal androgens.[1,2] The combination strategy is named combined androgen blockade (CAB), although to date none of the androgen antagonists attain effective therapeutic results due to suboptimal pharmacokinetic properties or substantial efficacy-limiting adverse effects. Moreover, long term treatment with androgen antagonists cause the selection of androgen receptor mutations in prostate cancers, and antagonists may become agonists.[3–6]

$C_{17,20}$-Lyase, which is a key enzyme involved in androgen biosynthesis, is thought to be a promising target for the treatment of androgen-dependent prostate cancer.[7] (1*S*)-1 (6,7-dimethoxy-2-naphthyl)-1-(1*H*-imidazol-4-yl)-2-methylpropan-1-ol **1** is one of novel $C_{17,20}$-lyase inhibitors under clinical trials.[8,9] It had a potent $C_{17,20}$-lyase inhibition with IC_{50} values of 21 and 28 nM toward rat and human enzymes, respectively, and markedly reduced the serum testosterone concentration in animal models and decreased the weights of androgen-dependent organs such as prostate and seminal vesicles in rats.[9a] Further studies revealed that **1** had a relatively potent inhibitory activity (IC_{50} =140 nM) on rat steroid 11β-hydroxylase, which is responsible for the production of mineralocorticoids.

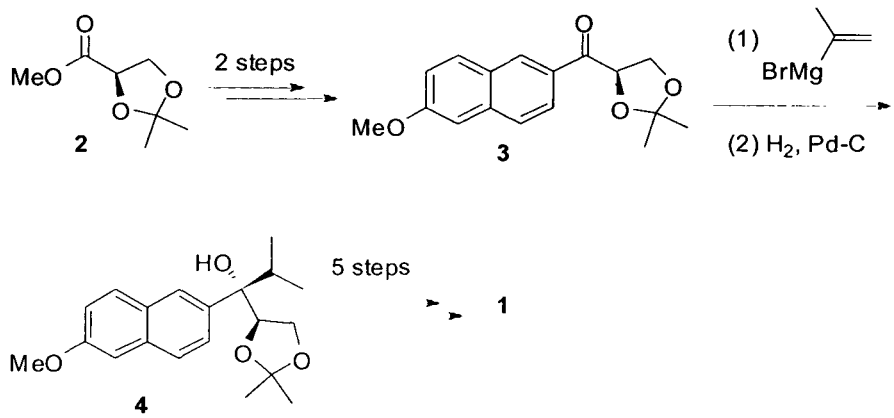


1, R_1, R_2=H; R=OMe

Figure 6.1

6.2 Basis of the Present Work

In spite of its potential bioactivity profile, compound **1** has only one stereocontrolled synthesis[9b] known so far. This chiral pool approach started with the optically active ester **2**, which is commercially available or can be readily prepared[10] from D-mannitol. In this approach, a diastereoselective Grignard reaction was the key step as illustrated in Scheme 6.1.



Scheme 6.1. Chiral Pool Approach towards synthesis of **1**

Kawakami and co-workers reported[11] large-scale racemic synthesis of **1**. Therefore, a short and efficient enantioselective synthetic route to **1** would be highly desirable. As part of our research programme aimed at developing efficient enantioselective routes to access bioactive molecules,[12] the Sharpless asymmetric dihydroxylation[13] was envisaged as a powerful tool to chiral dihydroxy compounds offering considerable opportunities for synthetic manipulations. In this chapter, we report our preliminary studies to develop a short and elegant approach towards synthesis of **1** and its other related analogues applying Sharpless asymmetric dihydroxylation for introduction of chirality.

6.3 Results and Discussion

The van Leusen imidazole synthesis[14], a unique multicomponent reaction, allows to access novel imidazoles (Figure 6.2) from aldehydes using substituted TosMIC reagents in the presence of suitable amines.

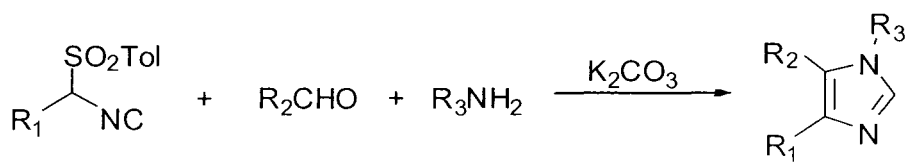
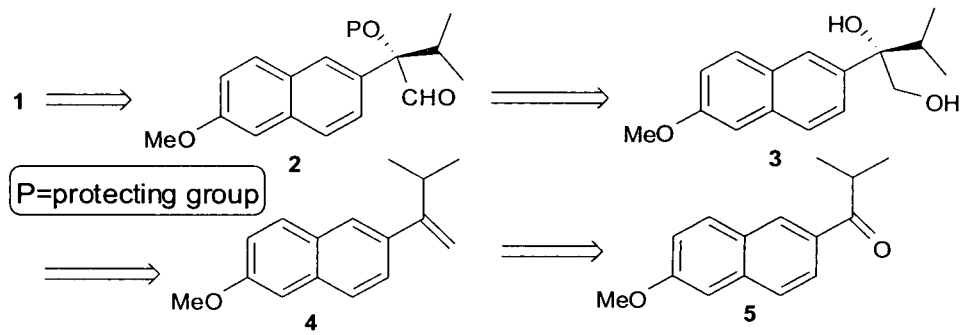


Figure 6.2. van Leusen three-component reaction to imidazoles

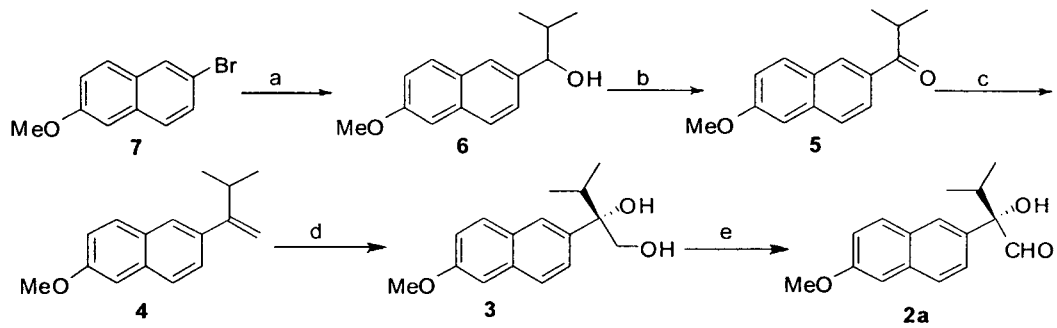
We were interested to apply this protocol for introducing the imidazole moiety into the taget molecule **1**. Our synthetic strategy for the synthesis of **1** was envisioned through the retrosynthetic analysis depicted in Scheme 6.2.



Scheme 6.2. Retrosynthetic analysis of **1**

The target molecule **1** could be anticipated to be prepared from aldehyde **2**, which, in turn, could be derived from enantiopure diol **3** *via* selective oxidation of the primary alcohol. The compound **3** could be readily obtained from vinyl aryl **4** by Sharpless asymmetric dihydroxylation. The compound **4** could be derived from the ketone **5** *via* Wittig olefination.

We started by litiation of commercially available 2-bromo-6-methoxy naphthalene **7** as illustrated in Scheme 6.3. Thus, **7** was treated with n-BuLi at -78 °C followed by addition of isobutyraldehyde to furnish the benzyl alcohol **6**. Next, compound **6** upon Swern oxidation provided ketone **5**. Next, compound **5** was treated with methylene triphenylphosphorane (generated *in situ*) to give the Wittig olefination product **4** in 78% yield. Sharpless AD of olefin **4** with AD-mix-α [i.e., with osmium tetroxide and potassium ferricyanide as cooxidant in the presence of $(DHQ)_2PHAL$ as the chiral ligand][13,15] in ^{t}BuOH–H_2O (1:1) at 0 °C for 28 h provided the crude product which on recrystallisation twice from EtOAc/pet. ether furnished the pure diol **3** in 72% yield with 92% ee. The subsequent selective oxidation of the primary alcohol in **3** was achieved under standard Swern oxidation conditions to furnish the corresponding α-hydroxyaldehyde **2a** in 81% yield. ^{1}H NMR spectrum of **2a** showed one signal at δ 9.68 (s, 1H) indicating the presence of aldehyde proton. Similarly, ^{13}C NMR spectrum of **2a** showed one signal at δ 200.8 due to the presence of aldehyde functionality.



Scheme 6.3. *Reagents and conditions*: (a) n-BuLi, THF, -78 °C, 20 min, then isobutyraldehyde, 2 h, 88%. (b) $(COCl)_2$, DMSO, -78 °C, Et_3N, CH_2Cl_2, 2 h, 80% . (c) $Ph_3P^+CH_3I^-$, *t*-BuOK, THF, 0 °C - rt, 10 h, 78%. (d) AD-mix-α, t-BuOH/H_2O (1:1), 0 °C, 24 h, 72%.(e) $(COCl)_2$, DMSO, -78 °C, Et_3N, CH_2Cl_2, 2 h, 81% .

With the enantiopure α-hydroxy aldehyde **2a** in our hand, we turned our attention towards its conversion into **1** and its other related analogues to evaluate their bioefficacy. Moreover, compound **2a,** a chiral tertiary α-hydroxyaldehyde, is a very versatile building block in synthetic chemistry, especially for the synthesis of many natural products.[16] At present, studies are in progress in these directions, and will be disclosed in due course.

6.4 Conclusion

In conclusion, new enatioselective synthesis of a potent $C_{17,20}$-lyase inhibitor and its other analogues have been initiated. Notable features of this approach includes the use of Sharpless asymmetric dihydroxylation to synthesize the enantiomerically pure 1,2-diol and and thus both enatiomers could be obtained by varying the ligands. The other merits of this synthesis are high-yielding reaction steps, high enantioselectivity and various possibilities available for structural modification and thus it might be considered as a general synthetic strategy to enantiomerically pure tertiary alcohols bearing the two aromatic rings.

6.5 Experimental Section

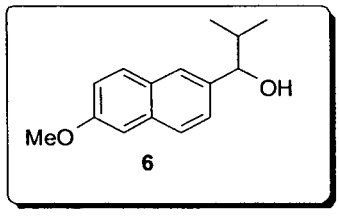
6.5.1 General Remarks

As that described in **Section 3A** of **Chapter 3**.

6.5.2 Synthesis of Compounds

1-(6-methoxynaphthalen-2-yl)-2-methylpropan-1-ol (6):

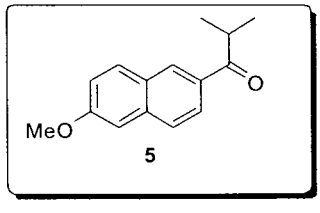
To a stirred solution of 2-bromo-6-methoxy naphthalene **7** (2.0 g, 8.44 mmol) in anhydrous THF (25 mL) at -78 °C and under N_2, *n*-BuLi (1.6 M in hexane, 5.3 mL, 8.44 mmol) was added. The resulting yellow solution was stirred at -78 °C for 20 minutes after which isobutyraldehyde (0.70 mL, 7.66 mmol) in THF (2 mL) were added at the same temperature and stirred at room temperature for 1 h. After quenching with water, THF was removed in vacuo. The mixture was extracted with ethyl acetate (3x20 ml), washed with brine and dried over Na_2SO_4. The concentrated extract was subjected to column chromatography on silica gel and elution with 10% ethyl acetate in hexane furnished alcohol **6** (1.71 g, 88%) as colourless oil. R_f: 0.58 (20% ethyl acetate in hexane). IR (Neat): 2963, 2364, 1608, 1468, 1265, 1218, 1167, 1031, 765 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.69-7.63 (m, 3H), 7.38 (d, 1H, *J*=8.3), 7.12 (d, 2H, *J*=10.4), 4.43 (d, 1H, *J*=6.9), 3.89 (s, 3H), 5.17 (s, 2H), 2.12 (s, 1H), 2.08-1.97 (m, 1H), 1.02 (d, 3H, *J*=6.6), 0.79 (d, 3H, *J*=6.7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.6, 138.9, 134.0, 129.4, 128.6, 126.7, 125.3, 125.2, 118.9, 105.7, 80.1, 55.2, 35.2, 19.1, 18.3. MS (ESI): *m/z* 230 $[M]^+$, 213 $[M\text{-}OH]^+$. Anal. Calcd for $C_{15}H_{18}O_2$: C, 78.23; H, 7.88. Found: C, 78.39; H, 7.74.



6

1-(6-methoxynaphthalen-2-yl)-2-methylpropan-1-one (5):

To a stirring solution of oxalyl chloride (1.65 g, 13.02 mmol) in dry CH_2Cl_2 (20 mL) at -78 °C was added dropwise dry DMSO (1.38 mL, 19.53 mmol) in CH_2Cl_2 (5 mL). After 30 min, alcohol **6** (1.5 g, 6.51 mmol) in CH_2Cl_2 (15 mL) was added over 10 min giving copious white precipitate. After stirring for 1 h at -78 °C the reaction mixture was brought to -60 °C and anhyd. Et_3N (4.55 mL, 32.55 mmol) was added slowly and stirred for 30 min allowing the reaction mixture to warm to room temperature. The reaction mixture was then diluted with water (25 mL)

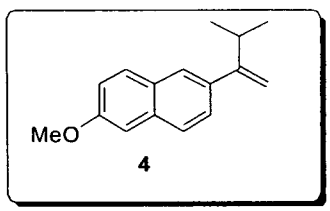


5

and CH_2Cl_2. The organic layer was separated and washed with water and brine, dried (Na_2SO_4) and passed through short pad of celite. The filtrate was concentrated to give ketone **5** as a pale yellow oil (1.19 g, 80%). R_f: 0.35 (10% ethyl acetate in hexane). IR (Neat): 3449, 3020, 2361, 1625, 1480, 1215, 1030, 761 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.40 (m, 1H), 7.03-6.93 (m, 2H), 5.42 (s, 1H), 57.21-7.14 (m, 2H), 3.92 (s,3H), 3.76-3.62 (m, 1H), 1.28 (s, 3H), 1.25 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 204.1, 159.6, 137.1, 131.5, 131.0, 129.5, 127.9, 127.1, 125.0, 119.5, 105.7, 55.3, 35.1, 19.3. MS (ESI): *m/z* 228 $[M]^+$. Anal. Calcd for $C_{15}H_{16}O_2$: C, 78.92; H, 7.06. Found: C, 78.78; H, 7.23.

2-methoxy-6-(3-methylbut-1-en-2-yl)naphthalene (4):

To a suspension of methyltriphenylphosphonium iodide (5.34 g, 13.2 mmol) in dry THF (40 mL) under a nitrogen atmosphere at 0 °C was added *t*-BuOK (1.50 g, 13.2 mmol). The reaction mixture was stirred for 15 min at 0 °C and was then warmed to rt while stirring was continued for 45 min. The solution was then recooled to 0 °C, and a solution of ketone **5** (1.00 g, 4.4 mmol) in dry THF (10 mL) was added dropwise. The reaction mixture was then warmed to rt and stirred for an additional 12 h. The reaction was quenched with saturated aq. NH_4Cl solution (10 mL) and extracted with Et_2O (3×30 mL). The combined organic extracts were washed with brine (20 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (2% ethyl acetate in *n*-hexane) to give alkene **4** (0.78 g, 78%) as a colorless solid. Mp: 84-89 °C. R_f: 0.80 (10% ethyl acetate in hexane). IR (KBr): 3449, 2964, 2362, 1630, 1483, 1215, 1163, 1033, 854, 761 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.72-7.66 (m, 3H), 7.08 (d, 2H, *J*=8.2), 7.15-7.11 (m, 2H), 5.25 (s, 1H), 5.09 (s, 1H), 3.90 (s, 3H), 3.02-2.89 (m, 1H), 1.15 (s, 3H), 1.13 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.6, 155.6, 137.9, 133.8, 129.6, 128.8, 126.5, 125.8, 124.8, 118.8, 109.8, 105.6, 55.3, 32.2, 29.7, 22.2. MS (ESI): *m/z* 226 $[M]^+$. Anal. Calcd for $C_{16}H_{18}O$: C, 84.91; H, 8.02. Found: C, 85.03; H, 8.26.

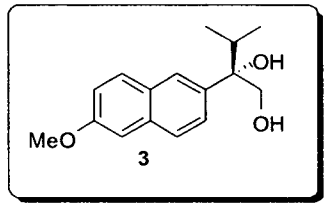


4

(S)-2-(6-methoxynaphthalen-2-yl)-3-methylbutane-1,2-diol (3):

To a stirred solution of *tert*-butyl alcohol (15 mL) and water (15 mL) were added AD mix α (3.71 g) at room temperature. The mixture was vigorously stirred at room

temperature until both phases were clear and then cooled to 0°C. A solution of olefin

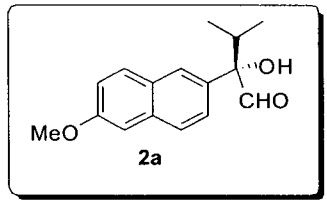


3

4 (0.60 g, 2.65 mmol) in *tert*-butyl alcohol (5 mL) was added 0°C. The reaction was stirred at the same temperature for 28 h. The reaction was quenched at 0°C by the addition of sodium sulfite (4.0 g), warmed to room temperature, and further stirred for 1 h. The reaction mixture was then extracted with ethyl acetate (3x15 mL). The combined organic layer was washed with aq. 2 N KOH solution (20 mL), water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and then concentrated under vacuo. Purification of the crude product by silica gel column chromatography (25% ethyl acetate in hexane) furnished the crude diol **3** as a colorless solid. $[\alpha]^{25}_D$ +12.2 (*c* 1.23, MeOH). It was recrystallised twice from EtOAc/petroleum ether to furnish the pure diol **3** (1.5 g, 72%). The enantiomeric excess was estimated to be 92%. R_f: 0.6 (30% ethyl acetate in petroleum ether). IR (KBr): 3450, 3018, 2968, 2362, 1606, 1481, 1266, 1216, 1167, 1033, 854, 763 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 4.73-4.64 (m, 2H), 3.62-3.51 (m, 2H), 3.39 (s, 3H), 1.61–1.19 (m, 31H), 0.86 (t, 3H, J = 6.9). ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.8, 138.1, 133.5, 129.6, 128.6, 126.8, 125.2, 124.6, 119.0, 105.4, 68.4, 55.3, 35.1, 17.5, 16.8. MS (ESI): *m/z* 260 $[M]^+$, 243 $[M\text{-}OH]^+$. Anal. Calcd for $C_{16}H_{20}O_3$: C, 73.82; H, 7.74. Found: C, 73.94; H, 7.63.

(S)-2-hydroxy-2-(6-methoxynaphthalen-2-yl)-3-methylbutanal (2a):

To a stirring solution of oxalyl chloride (0.50 g, 3.85 mmol) in dry CH_2Cl_2 (10



2a

mL) at -78 °C was added dropwise dry DMSO (0.42 mL, 5.77 mmol) in CH_2Cl_2 (10 mL). After 30 min, diol **3** (0.50 g, 1.92 mmol) in CH_2Cl_2 (10 mL) was added over 10 min giving copious white precipitate. After stirring for 1 h at -78 °C the reaction mixture was brought to -60 °C and anhyd. Et_3N (1.40 mL, 9.65 mmol) was added slowly and stirred for 30 min allowing the reaction mixture to warm to room temperature. The reaction mixture was then diluted with water (25 mL) and CH_2Cl_2. The organic layer was separated and washed with water and brine, dried (Na_2SO_4) and passed through short pad of celite. The filtrate was concentrated to provide the aldehyde **2a** as a colorless solid

(o.40 g, 81%). R_f: 0.68 (15% ethyl acetate in hexane). $[\alpha]_D^{25}$: +17.2 (*c* 1.5, $CHCl_3$). The enantiomeric excess was estimated to be >95% by chiral HPLC analysis (instrument: HP1100, column: LichroCART Chiradex column 250x4 mm, 5 μm), flow rate: 0.5 mL/min, detection: UV 254 nm (eluent: methanol/ H_2O). IR (KBr): 3435, 3018, 2971, 2366, 1723, 1601, 1479, 1385, 1266, 1194, 1035, 985, 800, 669 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 9.68 (s, 1H), 7.9 (m, 1H), 7.74 (d, 2H, *J*=4.2), 7.54 (d, 1H, *J*=7.9), 7.17-7.11 (m, 2H), 3.88 (s, 1H), 2.66 (s, 1H) 0.96 (d, 2H, *J*=5.0), 0.81 (d, 2H, *J*=5.2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 96.4, 80.3, 59.6, 55.6, 31.8, 30.4, 29.62, 29.60, 29.52, 29.48, 29.3, 25.4, 22.6, 14.2, 14.0. MS (ESI): *m/z* 258 $[M+H]^+$, 241 $[M-OH]^+$. Anal. Calcd for $C_{16}H_{18}O_3$: C, 74.39; H, 7.02. Found: C, 74.68; H, 7.28.

6.6 References

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S. K. *Eur. J. Org. Chem.* **2010**, 5100. (d) Panda, G.; Dinda, S. K.; Das, S. K. *Tetrahedron* **2010**, *66*, 9304.

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6.7 Spectra of Selected molecules

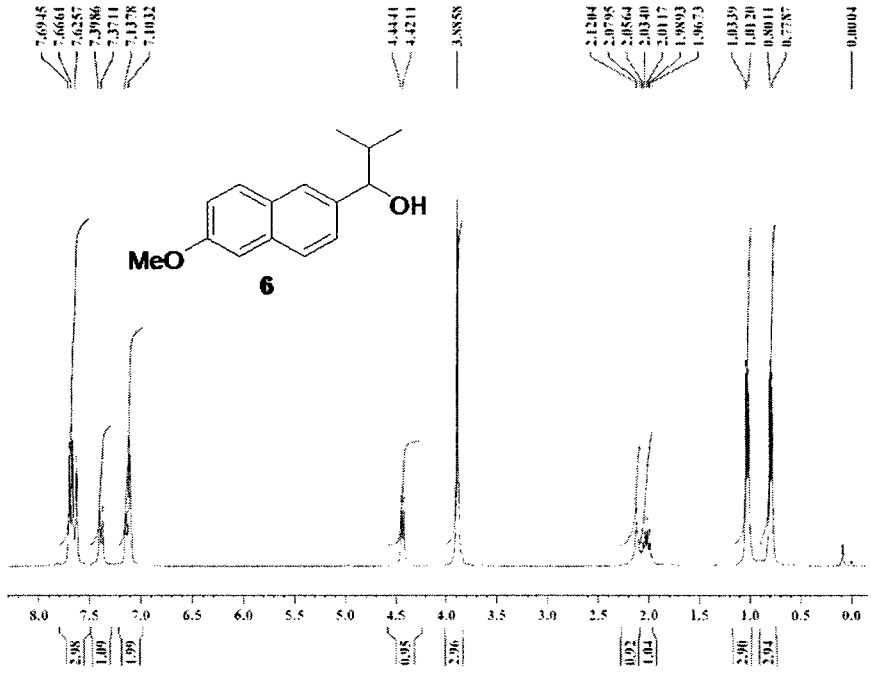


Figure 6.3: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **6**.

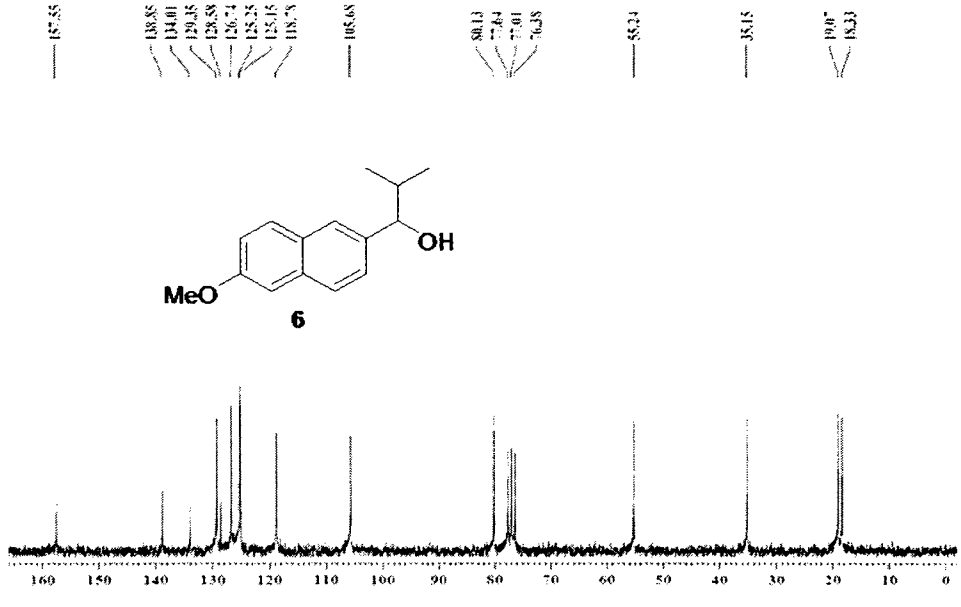


Figure 6.4: ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **6**.

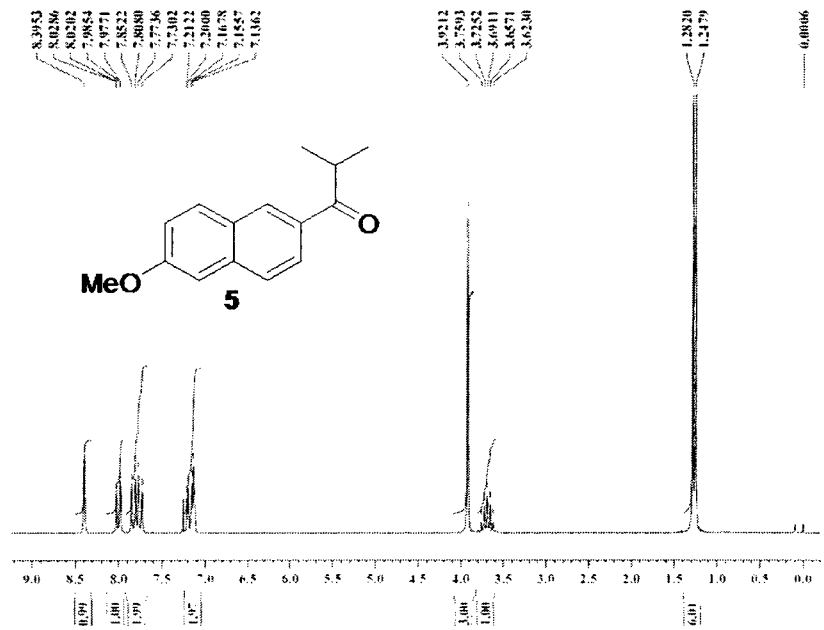


Figure 6.5: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **5**.

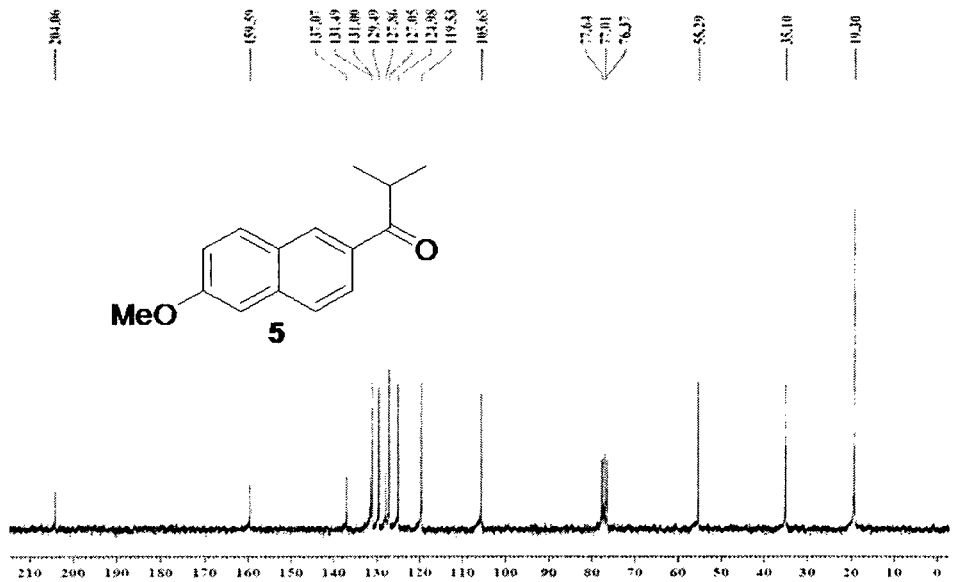


Figure 6.6: ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **5**.

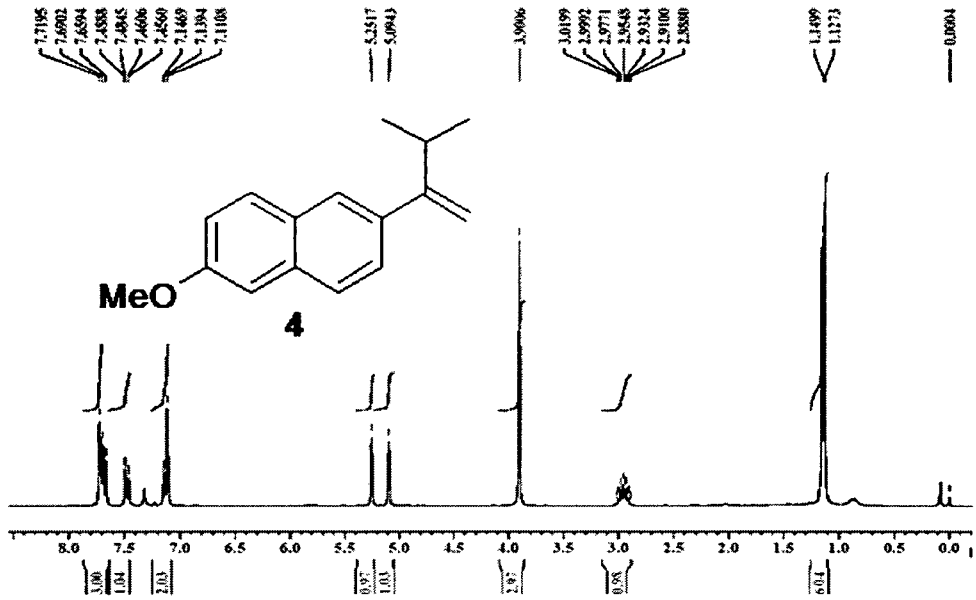


Figure 6.7: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **4**.

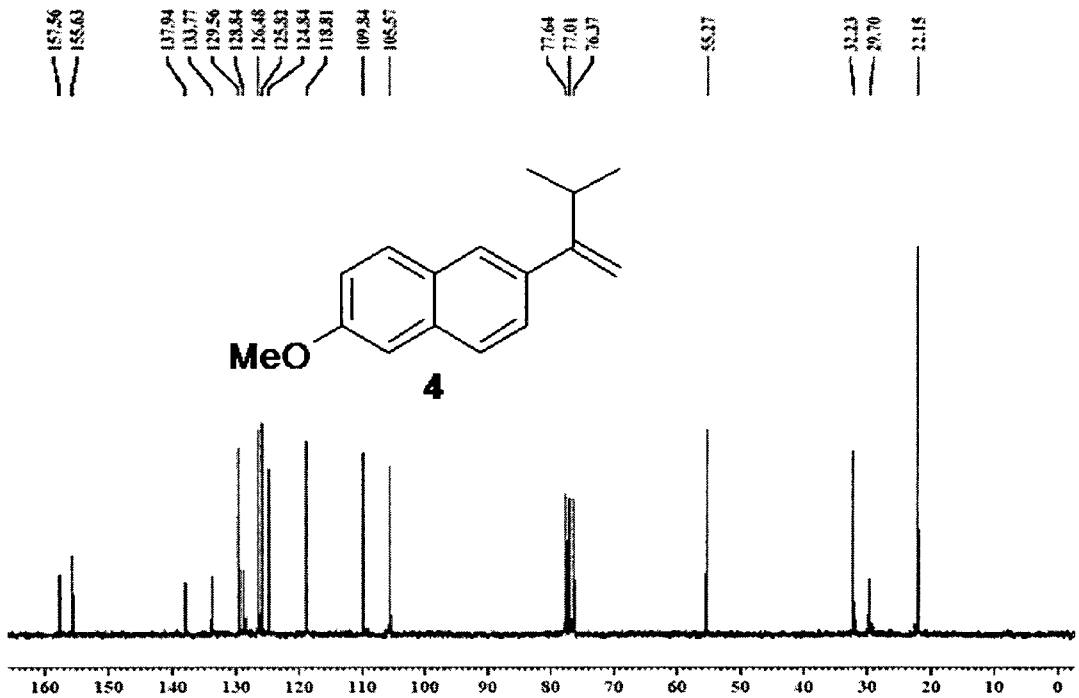


Figure 6.8: ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **4**.

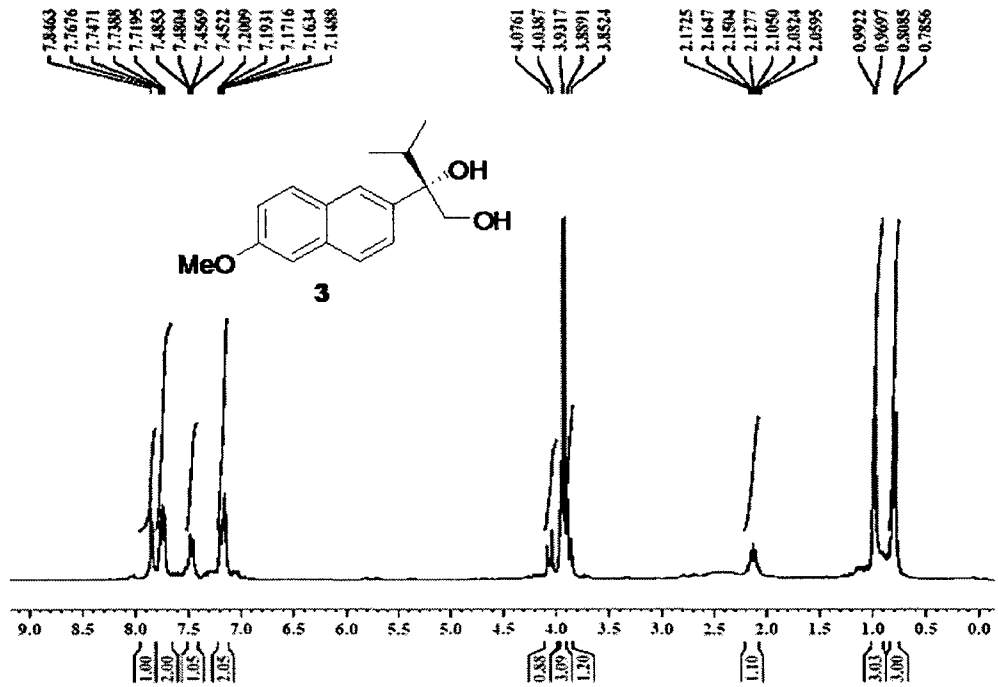


Figure 6.9: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **5**.

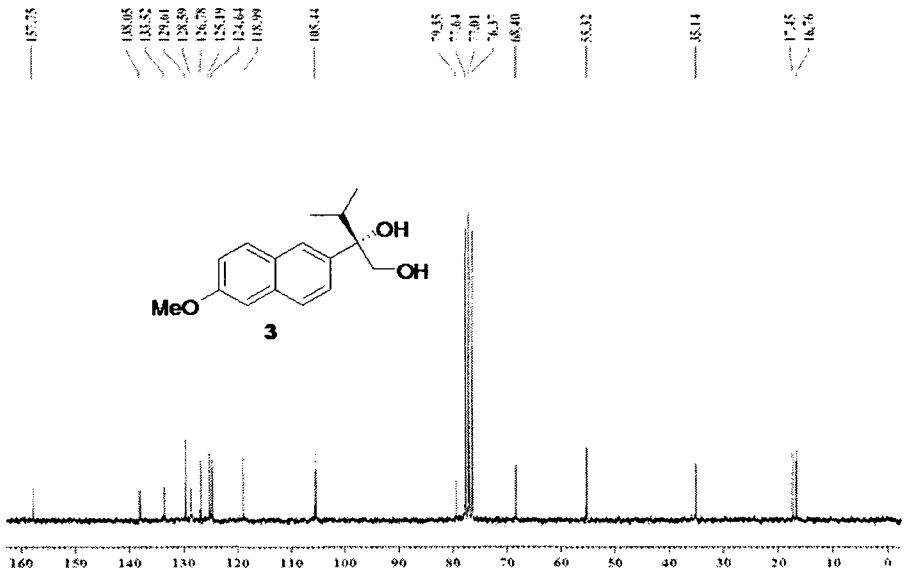


Figure 6.10: ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **5**.

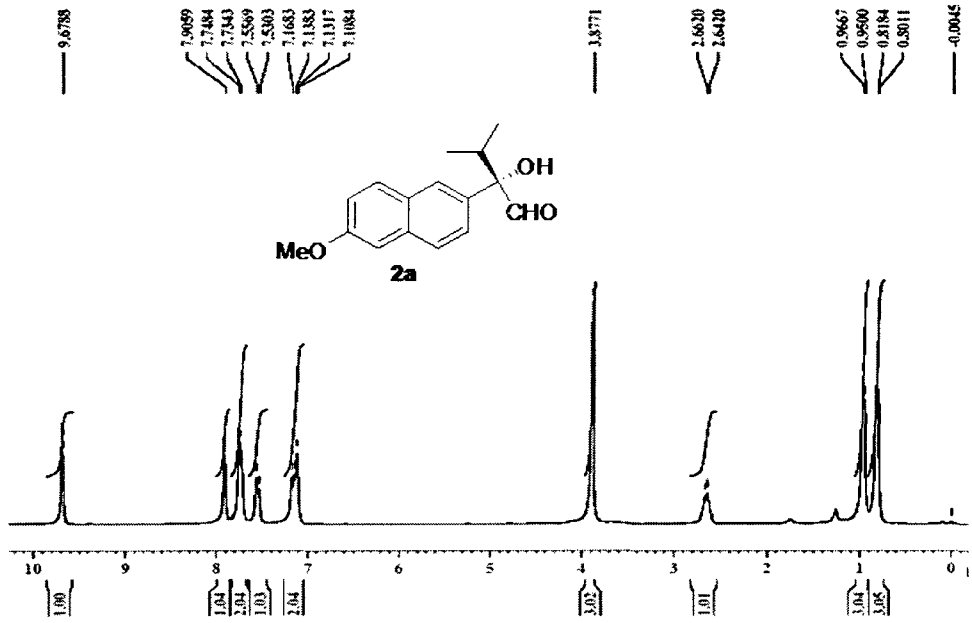


Figure 6.11: ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **2a**.

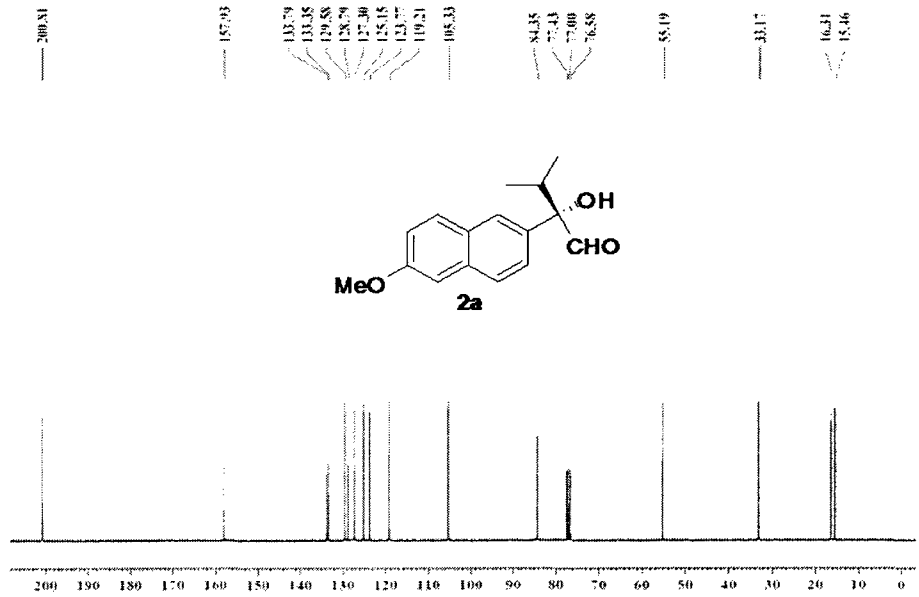


Figure 6.12: ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **2a**.

Chapter 7:
Design and Synthesis of Small Organic Molecules for P2X ion and P2Y G Protein-Coupled Receptors (GPCRs)

7.1 Introduction

P2 receptors exist in most tissues.[1-4] These plasma membrane nucleotide P2-receptors subdivided in ligand gated ion channels (P2X) and G-protein coupled receptors (P2Y) are interesting targets not only in experimental pharmacology but also in drug research.[1-7] To date, seven mammalian P2X-receptors termed $P2X_1$ to $P2X_7$ and eight P2Y-receptors termed $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ have been identified by molecular cloning.[8-10] Ionotropic P2X and metabotropic P2Y receptors exhibit a wide expression profile and are involved in a variety of physiological functions.[11,12] However, exploration of the physiological function of P2 receptors with pharmacological and genetic approaches has been hampered by a lack of subtype selective ligands. In this context, Suramin **1**, a polysulfonated naphthylurea, served as a highly successful chemical lead for the development of potent and selective P2X antagonists.[13-15]

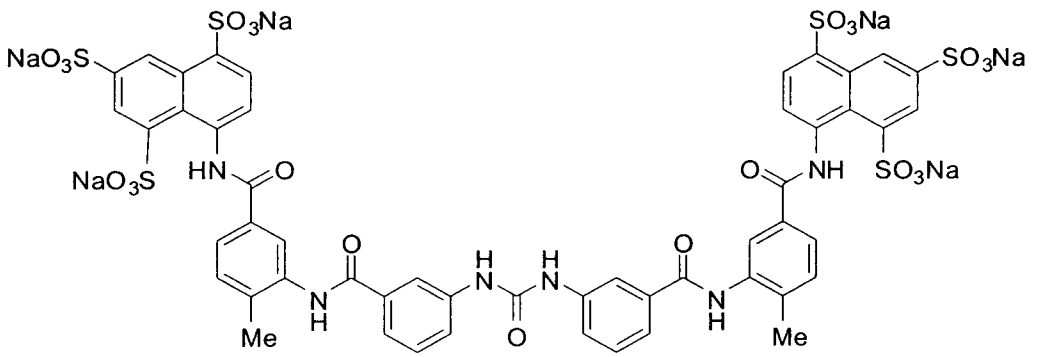


Figure 7.1 Structure of suramin **1**.

The current goal of our research is to identify and apply potent, selective, and stable $P2Y_{11}$ receptor ligands to define the physiological role(s) of this receptor. The another objective is to synthesize suramin like molecules having enhanced activity. The $P2Y_{11}$ receptor is specifically activated by ATP and more potently by some related ATP analogues.[16]

7.2 Basis of the Present Work

The major limitation associated with the suramin-derived compounds[17] is their poor bioavailablity due to the presence of polyanionic charge on the molecules. In order to improve the bioavailability properties, we planned to replace the sulfonate group with esters and acids. Furthermore, the position of *bis*-urea was to be replaced by different spacers as shown in the targeted molecules (Figure 7.2). It is also

suggested that the size of the molecule would play a vital role in approaching the P2 receptors. In this quest, the bulk of the suramin has been reduced to small organic molecules having requisite functional groups like sulfonates or esters or acids with different spacers (Figure 7.2). To minic some of the already existing ligands, efforts were made for the synthesis of new structural prototypes where amide (-CONH-) functional group would be replaced by saturated amine (-CH_2NH-) group in some of the bonding cases. To improve the bioavailability and remove toxicity due to the sulfonate group, the designed prototypes having methyl or ethyl ester functionality or acids were planned.

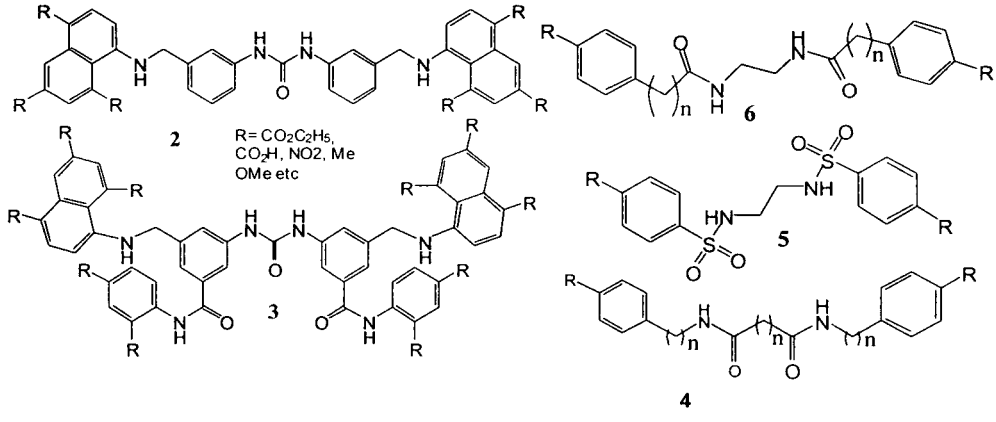
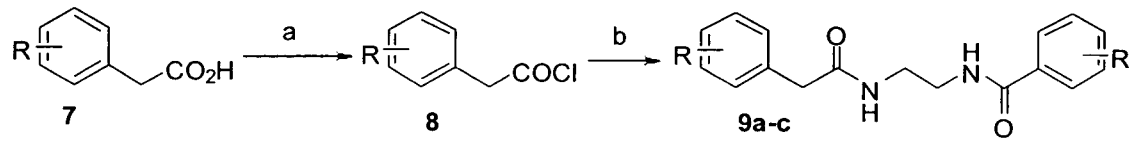


Figure 7.2 Designed prototype ligands for P2 receptors

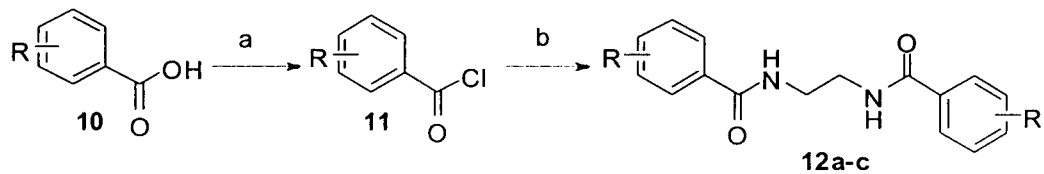
7.3 Results and Discussion

7.3.1 Chemistry

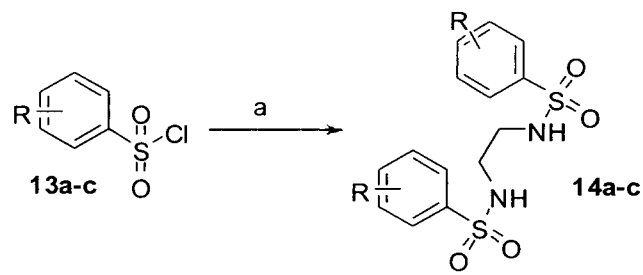
The amide, benzamide or sulfonamide functionalities were introduced by reacting acyl chloride (Scheme 7.1), benzoyl chloride (Scheme 7.2) or sulfonyl chloride (Scheme 7.3), respectively, with amines. In most cases, the amine used was ethylenediamine (EDA). Thionyl chloride $SOCl_2$ is commonly used to generate acyl chlorides from their corresponding carboxylic acids in the presence of triethyl amine at 0 °C. The various benzoyl chlorides were obtained directly by treating substituted aromatic acids with thionyl chloride at 80 °C. Subsequent coupling with ethylenediamine was performed at -20 °C. However, sulfonamides are obtained well at room temperature.



Scheme 7.1. *Reagents and conditions*: (a) $SOCl_2$, Et_3N, CH_2Cl_2, 0 °C, 20 min (b) EDA, dry DCM, Et_3N, -20 °C, 12 h, 74%, over two steps.



Scheme 7.2. *Reagents and conditions*: (a) $SOCl_2$, dry benzene, reflux, 3 h. (e) EDA, dry DCM, Et_3N, -20 °C, 12 h, 72%, over two steps.

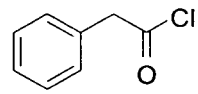
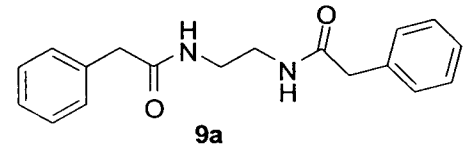
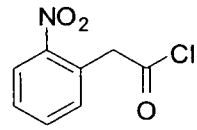
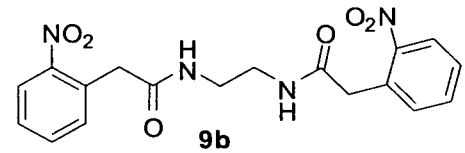
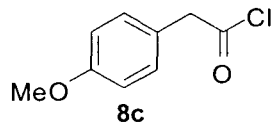
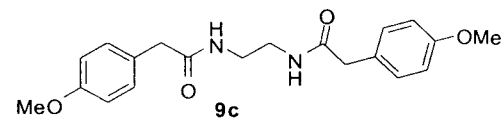
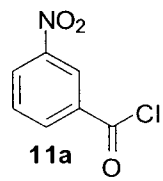
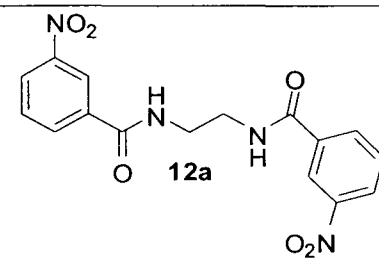
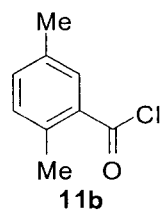
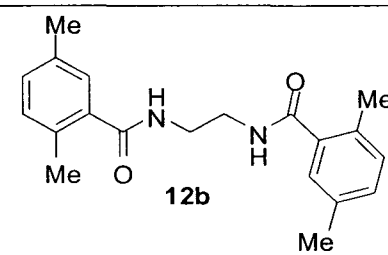
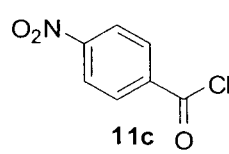
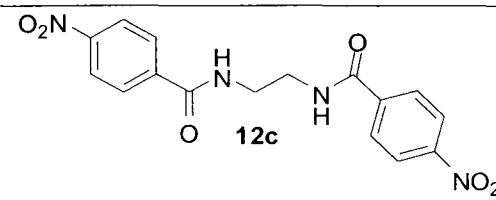


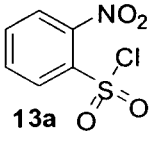
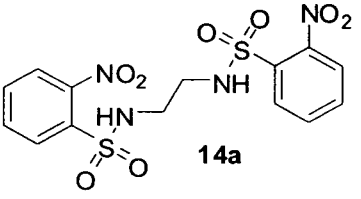
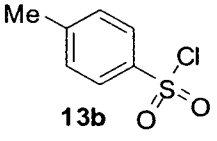
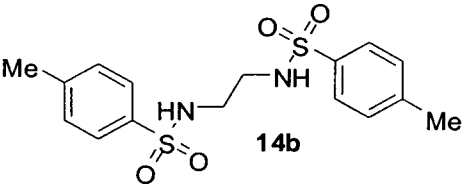
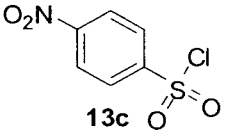
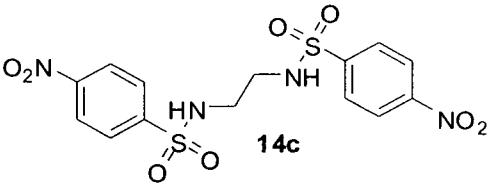
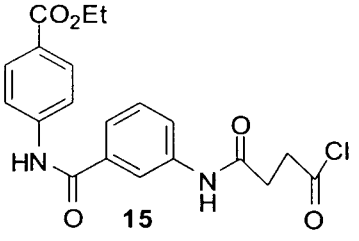
Scheme 7.3. *Reagents and conditions*: (a) EDA, dry DCM, Et_3N, rt, 1 h, 80%.

We synthesized some of the designed molecules as shown in Table 7.1 for their preliminary pharmacological screening at P2Y receptors.

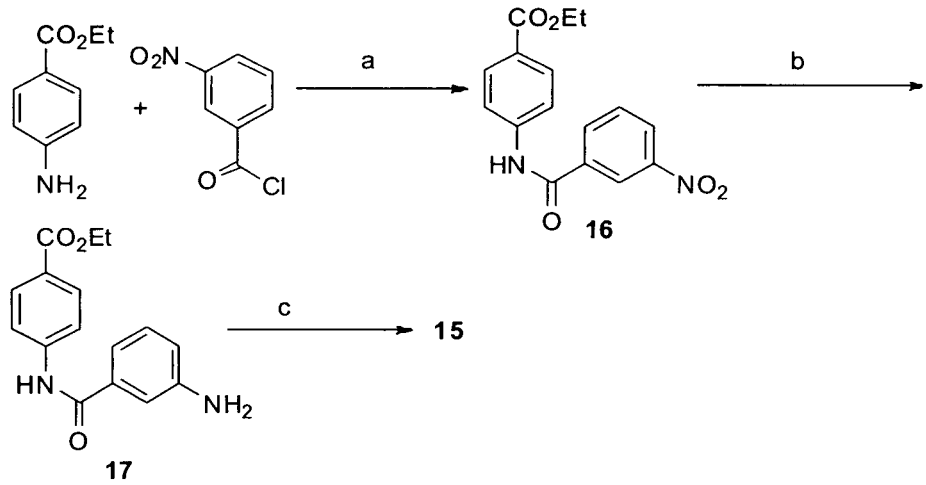
Table 7.1 Synthesized diamide 9a-c, and 12a-c, disulfonamide 14a-c

Amine	Acyl or aryl or sulfonyl chloride	Diamide or disulfonamide

Ethylene diamine (EDA)	 **8a**	 **9a**
EDA	 **8b**	 **9b**
EDA	 **8c**	 **9c**
EDA	 **11a**	 **12a**
EDA	 **11b**	 **12b**
EDA	 **11c**	 **12c**

EDA	 **13a**	 **14a**
EDA	 **13b**	 **14b**
EDA	 **13c**	 **14c**
		 **15**

Compound **15** was prepared following the steps as shown in Scheme 7.4. Ethyl 4-aminobenzoate reacted with 3-nitrobenzoyl chloride in the presence of pyridine to furnish amide **16**. The nitro group in compound **16** was reduced to amine following standard hydrogenation conditions. Next, compound **17** reacted with succinyl dichloride in the presence of triethyl amine to provide compound **15**.



Scheme 7.4. *Reagents and conditions*: (a) py, 0 °C, 3 h, 68%. (b) H_2, 10% Pd-C, EtOAc, rt, 1 h, 85%. (c) succinyl dichloride, dry DCM, Et_3N, 0 °C-rt, 4 h, 67%.

7.3.2 Pharmacology

All the synthesized compounds were tested for interaction with $P2Y_{11}$ receptors recombinantly expressed in 1321N1 astrocytoma cells[16] and at human embryonic kidney (HEK293) cells endogenously expressing $P2Y_1$ and $P2Y_2$ receptors as has been shown by Schachter *et al.* and Yu *et al.*[18,19] The expression of $P2Y_1$ and $P2Y_2$ receptors in our HEK293 cells was confirmed by RT-PCR using $P2Y_1$ and $P2Y_2$ specific primer pairs and yielded cDNA fragments with an expected size of 247 bp for P2Y2 and 389 bp for $P2Y_1$ as shown by agarose gel electrophoresis. Interaction of test compounds with P2Y receptors was analyzed by using a fluorescence calcium assay described by Kassack et al.[20] However, none of the synthesized compounds showed any agonist or antagonist activity at $P2Y_1$, $P2Y_2$, or $P2Y_{11}$ receptors up to a concentration of 100 μM (data not shown).

7.4 Conclusion

This study presents an initial description of our designed molecules and their preliminary bioevaluation. Further studies are ongoing in our lab and will be reported in due course.

7.5 Experimental Section

7.5.1 Chemistry

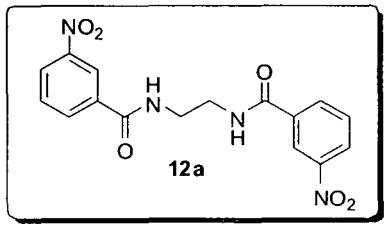
7.5.1.1 General remarks

As described in **Section 2.5.1.1 (Chapter 2).**

5.1.2 Procedure for preparation of

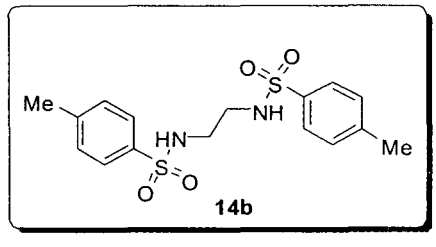
***N,N'*-(ethane-1,2-diyl)bis(3-nitrobenzamide) 12a:**

To a stirring solution of benzoyl chloride **11a** (0.50 g, 2.69 mmol) in dry CH_2Cl_2 (10 mL) at -20 °C was added dropwise a mixture of dry EDA (0.060 mL, 0.90 mmol) and dry Et_3N (0.40 mL, 2.69 mmol) in dry CH_2Cl_2 (5 mL). After stirring for 12 h at -20 °C the reaction mixture was brought to rt and the reaction mixture was filtered off, and the residue was washed with DCM and MeOH, dried to give diamide **12a** as a colorless solid (1.19 g, 80%). IR (KBr): 3303, 3078, 2369, 1640, 1525, 1350, 1098, 722 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 9.02 (s, 2H, NH), 8.67-8.66 (m, 2H, ArH), 8.39-8.35 (m, 2H, ArH), 8.31-8.28 (m, 2H, ArH), 7.80-7.75 (m, 2H, ArH), 3.51 (t, 4H, J = 2.6 Hz, CH_2). MS (ESI): *m/z* 359 $[M+H]^+$. Anal. Calcd for $C_{16}H_{14}N_4O_6$: C, 53.63; H, 3.94; N, 15.64. Found: C, 53.74; H, 4.03; N, 15.74.



12a

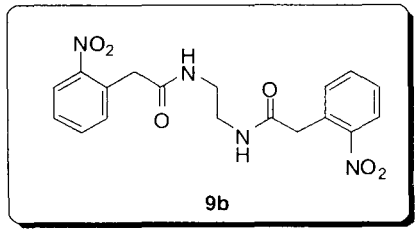
N,N'-(ethane-1,2-diyl)bis(4-methylbenzenesulfonamide) 14b: IR (KBr): 3452, 3290, 2364, 1597, 1410, 1334, 1157, 1062, 669 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.71 (d, 4H, J = 8.2, ArH), 7.32-7.26 (m, 4H, ArH), 5.03 (s, 2H, NH), 3.06 (t, 4H, J = 2.8 Hz, CH_2), 2.43 (s, 6H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 129.9, 127.1, 43.0, 21.5. MS (ESI): *m/z* 369 $[M+H]^+$, 387 $[M+NH_4]^+$.



14b

Anal. Calcd for $C_{16}H_{20}N_2O_4S_2$: C, 52.15; H, 5.47; N, 7.60. Found: C, 52.34; H, 5.64; N, 7.73.

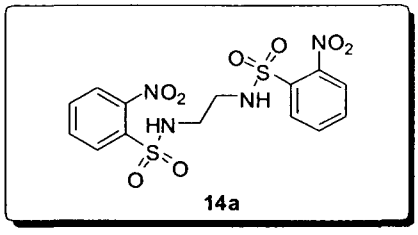
N,N'-(ethane-1,2-diyl)bis(2-(2-nitrophenyl)acetamide) 9b: ^1H NMR (300 MHz, DMSO-d_6): δ 8.09 (s, 2H, NH), 7.29-7.23 (m, 8H, ArH), 3.39 (s, 4H, benzylicH), 3.11 (t, 4H, J = 2.6 Hz, CH_2), 2.43 (s, 6H, CH_3). MS (ESI): *m/z* 387



9b

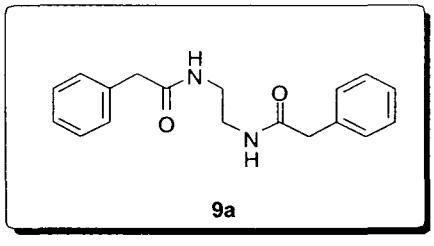
$[M+H]^+$, 409 $[M+Na]^+$. Anal. Calcd for $C_{18}H_{18}N_4O_6$: C, 55.96; H, 4.70; N, 14.50. Found: C, 55.79; H, 4.81; N, 14.67.

N,N'-(ethane-1,2-diyl)bis(2-nitrobenzenesulfonamide) 14a: ^1H NMR (300 MHz, $CDCl_3$): δ 8.13-8.10 (m, 2H, ArH), 7.90-7.87 (m, 2H, ArH), 7.77-7.74 (m, 2H, ArH), 5.66 (s, 2H, NH), 3.32 (t, 4H, J = 2.6 Hz, $C\underline{H}_2$). MS (ESI): *m/z* 431 $[M+H]^+$, 448 $[M+NH_4]^+$. Anal. Calcd for $C_{14}H_{14}N_4O_8S_2$: C, 39.07; H, 3.28; N, 13.02. Found: C, 39.56; H, 3.36; N, 13.23.



14a

N,N'-(ethane-1,2-diyl)bis(2-phenylacetamide) 9a: ^1H NMR (300 MHz, DMSO-d_6): δ 8.09 (s, 2H, NH), 7.31-7.19 (m, 10H, ArH), 3.39 (s, 4H, benzylicH), 3.11 (t, 4H, J = 2.6 Hz, $C\underline{H}_2$), 2.43 (s, 6H, CH_3). MS (ESI): *m/z* 297 $[M+H]^+$, 319 $[M+Na]^+$. Anal. Calcd for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80; N, 9.45. Found: C, 73.04; H, 6.87; N, 9.68.



9a

7.5.2 Pharmacology

7.5.2.1 Cell Culture and Measurements of Intracellular Calcium

All methods have been previously described in detail.[20,21] HEK293 cells were grown in Dulbecco's modified Eagle Medium Nutrient Mixture F-12 Ham (DMEM/F12 1:1 Mixture) (Sigma-Aldrich) containing 100 U/mL penicillin G, 100 μg/mL streptomycin, 10% fetal bovine serum, and 5 mM L-glutamine (Sigma-Aldrich). 1321N1- $P2Y_{11}$ astrocytoma cells stably transfected with a plasmid containing the human $P2Y_{11}$ coding sequence (AF030335)27 were cultured in Dulbecco's modified Eagle Medium (DMEM) with glutamax-I, sodium pyruvate, glucose (4500 mg/L), and pyridoxine (Gibco) supplemented with 100 U/mL penicillin G, 100 μg/mL streptomycin, 10% fetal bovine serum, and 200 μg/ mL G418 (Sigma-Aldrich). Cells were incubated at 37 °C in 5% CO_2.

Ca^{2+} fluorescence was measured as previously described using a fluorescence microplate reader with a pipettor system (NOVOstar; BMG LabTech, Offenburg, Germany).[20] Harvested cells (0.05% trypsin/0.02% EDTA, Sigma Aldrich) were rinsed with the appropriate culture medium. After centrifugation, the pelleted cells were resuspended in fresh medium and kept at 37 °C under 5% CO_2 for 20 min. After washing two times with Krebs-HEPES buffer, cells were loaded with Oregon Green 488 BAPTA-1/AM (3 μM; Molecular Probes, Eugene, OR) for 60 min at 25 °C in the same buffer containing 1% Pluronic F-127 (Sigma-Aldrich). After rinsing three times with Krebs-HEPES buffer, the cell suspension was diluted and evenly plated into 96-well plates (Greiner, Frickenhausen, Germany) at a density of 50-100 000 cells/well. Concentration-response curves of agonists were obtained by injection of increasing concentrations of 2-MeSADP (native $P2Y_1$ receptors in HEK293 cells) or UTP (native $P2Y_2$ receptors in HEK293 cells) or ATPγS(1321N1-$P2Y_{11}$ cells). Excitation wavelength was 485 nm (bandwidth 12 nm), and fluorescence intensity was monitored at 520 nm (bandwidth 35 nm) for 30 s at 0.4 s intervals. Concentration-inhibition curves of antagonists were obtained by preincubating the cells with test compounds for 30 min at 37 °C and subsequent injection of agonist (31.6 nM 2-MeSADP, 3.16 μM UTP, or 1 μM ATPγS, respectively).

7.5.2.2 Data Analysis of Intracellular Calcium Measurements

Effects of single doses of antagonists (100 μM) were expressed as a percentage of the agonist control responses. Antagonist IC_{50} values (pIC_{50} = -log IC_{50}) represent the concentration needed to inhibit by 50% the effect elicited by single doses of agonists. Apparent functional Ki values (pK_i = -log K_i) were calculated according to the equation of Cheng and Prusoff:[22]

$$K_i = IC_{50}/(1 + L/EC_{50})$$

where IC_{50} is the inhibitory concentration 50% of the antagonist, EC_{50} is the effective concentration 50% of the used agonist, and L is the molar concentration of the used agonist. IC_{50} values for antagonists and EC_{50} values for agonists were derived from -log concentration - effect (inhibition) curves fitted to the pooled data by logistic, nonlinear regression analysis (Prism 4.00, GraphPad Software, San Diego, CA).

7.6 References

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7.7 Spectra of selected compounds

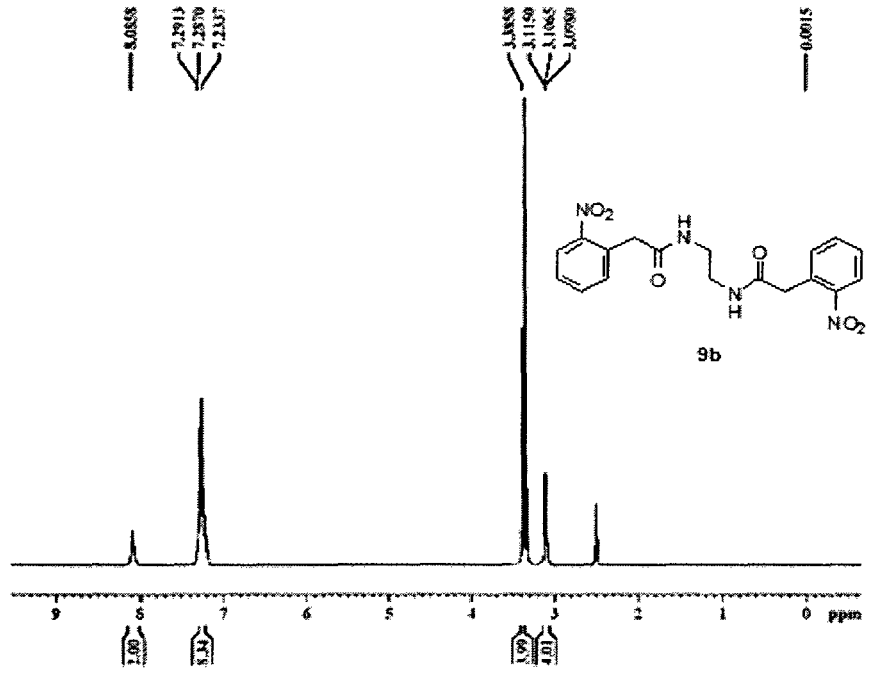


Figure 7.5 ^{1}H NMR (DMSO-d_6, 300 MHz) spectrum of **9b**

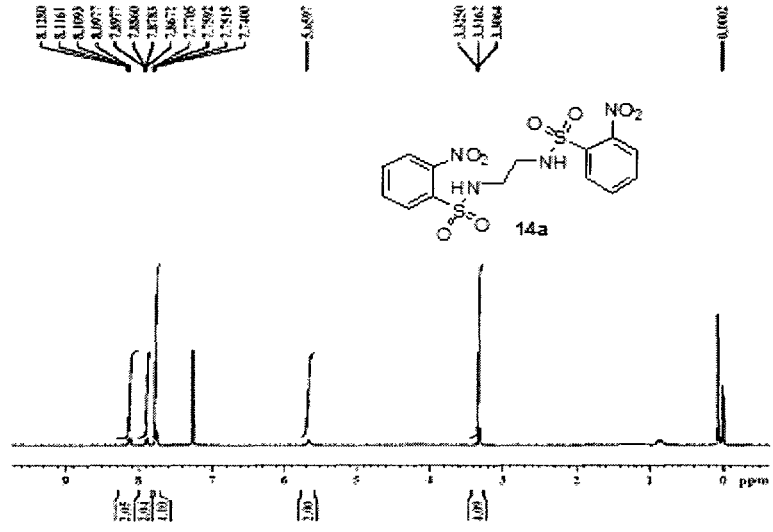


Figure 7.6 ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **14a**

Figure 7.7 ^{1}H NMR (DMSO-d_6, 300 MHz) spectrum of **9a**

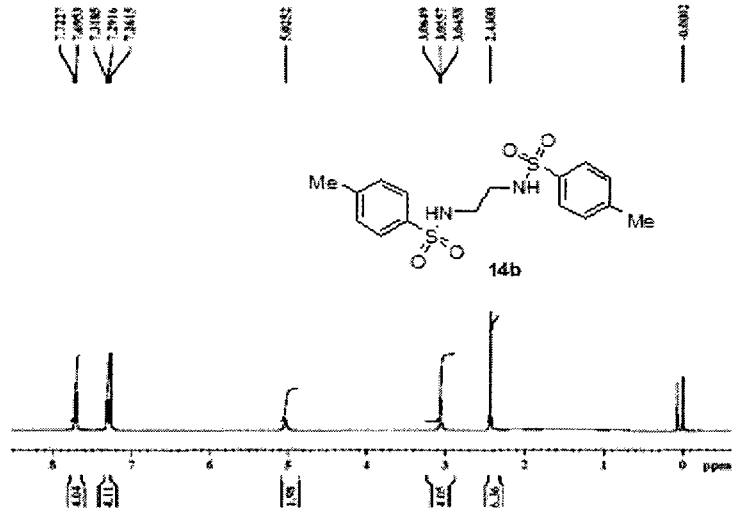


Figure 7.8 ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of **14b**

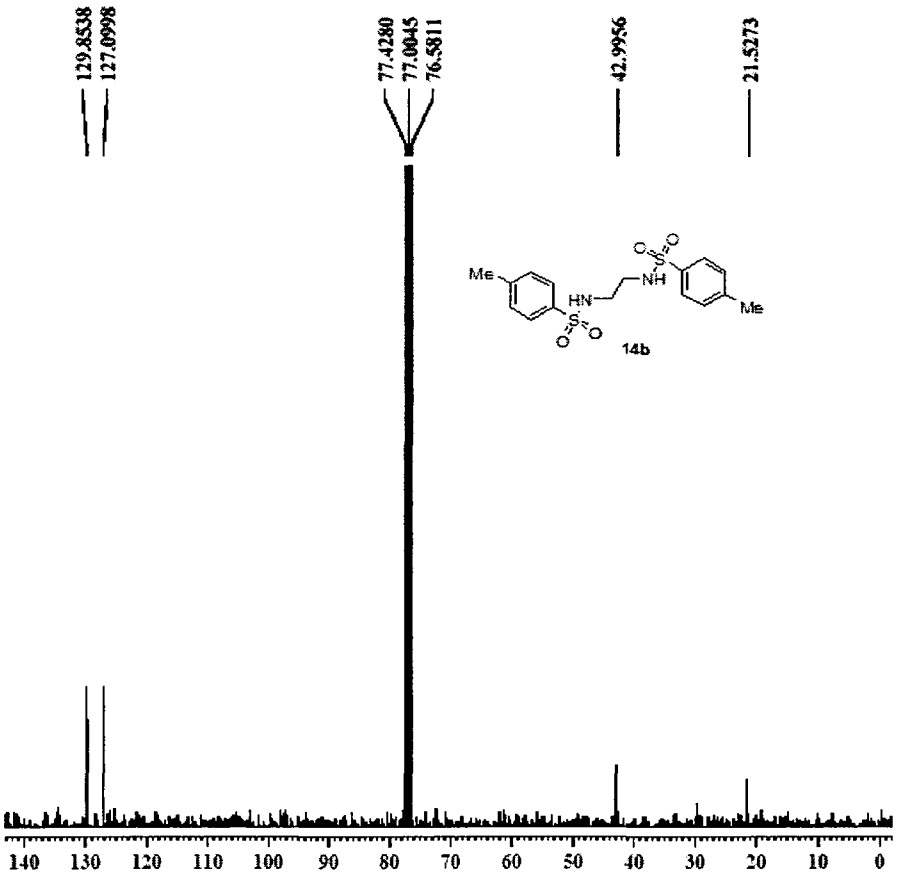


Figure 7.9 ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of **14b**.

About the Author

Subal Kumar Dinda was born in the village of Raghunathchak in the district of Purba Medinipur, West Bengal, India on August 8, 1982 to mother Mrs. Bharati Dinda and father Mr. Ranajit Kumar Dinda. He received his B.Sc. degree in 2004 in Chemistry (Hons.) from Vidyasagar University. In 2006, he obtained his M.Sc. degree in Chemistry at the same university. He has been working as research fellow (CSIR) towards his Ph.D. in the Medicinal and Process Chemistry Division at CSIR-Central Drug Research Institute, Lucknow under the guidance of Dr. Gautam Panda since November 24, 2006 and has been registered as a Ph.D. student from Jawaharlal Nehru University. His research interests focus on the design and stereoselective synthesis of novel bioactive molecules utilizing alternative synthetic methodologies.

Fellowships:

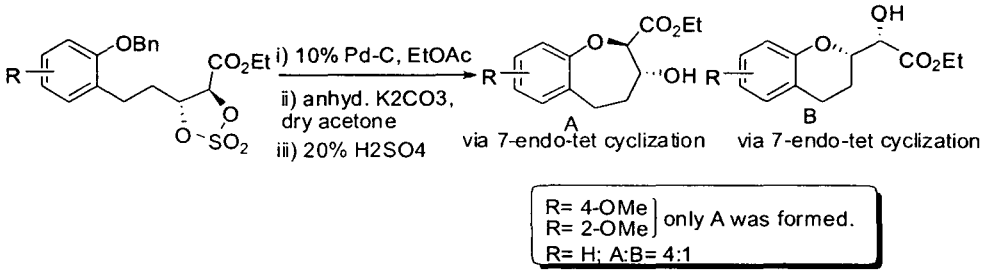
CSIR Junior Research Fellowship (24.11.2006-23.11.2008).

CSIR Senior Research Fellowship (24.11.2008-till date).

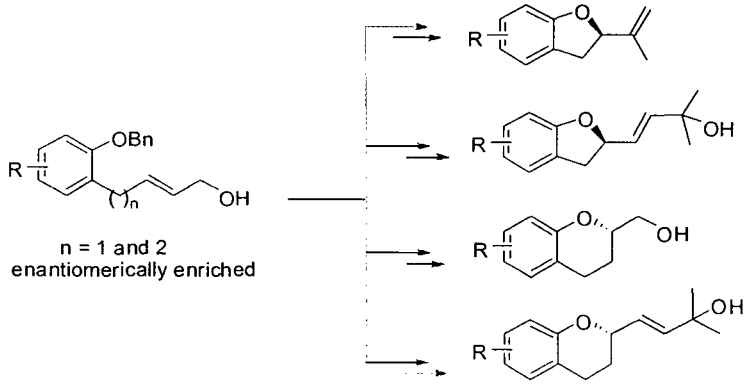
List of Publications

Papers in SCI Journals:

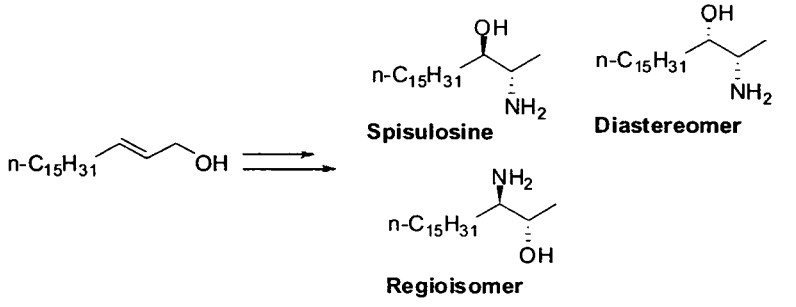
Enantioselective Synthesis of Funtionalized 1-Benzoxepines by Phenoxide Ion Mediated *7-endo-tet* Carbocyclization of Cyclic sulfates: Sajal Kumar Das, Subal Kumar Dinda and Gautam Panda. *Eur. J. Org. Chem.* **2009**, 204-207.



Application of Phenolate Ion Mediated Intramolecular Epoxide Ring Opening in the Enantioselective Synthesis of Funtionalized 2,3-Dihydrobenzofurans and 1-Benzopyrans: Subal Kumar Dinda, Sajal Kumar Das and Gautam Panda. *Synthesis* **2009**, 1886-1896.

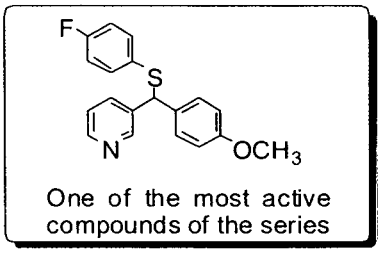


Asymmetric total syntheses of spisulosine and its diastereo- and regioisomers: Subal Kumar Dinda, Sajal Kumar Das and Gautam Panda. *Tetrahedron* **2010**, *66*, 9303-9309.



Patent Filed:

Aryl aryl methyl thio arenes (AAMTAs) as Antimalarial Agents and a process for the preparation thereof :Gautam Panda, Priyanka Singh, Sanjit Kumar Das, Subal Kumar Dinda, Manish Goyal, Uday Bandyopadhyay; (0364DEL2011) dt 14-Feb-2011.



Conference attended:

14th ISCB International Conference, Central Drug Research Institute, Lucknow, 15-18 January, **2010**. Presented a poster entitled "Stereoselective Synthesis of Functionalized 2,3-dihydrobenzofurans, 1-benzopyrans and 1-benzoxepines by Phenoxide ion-mediated Carbocyclization".