SYNTHETIC STUDIES TOWARD EUPOMATILONE-6, 4-*O*-β-D-GALACTOSYL MALTOSE AND AMPHIDINOL-3

BY

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A THESIS

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BY

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MAY 2008

DEDICATED

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C E R T I F I C A T E

The research work presented in thesis entitled "**Synthetic studies toward Eupomatilone-6, 4-*O*-β-D-galactosyl maltose- LG-2 and amphidinol-3**" has been carried out under my supervision and is a bonafide work of **Mr. Bhargava Karumudi**. This work is original and has not been submitted for any other degree or diploma of this or any other University.

(Dr. M. K. Gurjar)
Research Guide

D E C L A R A T I O N

The research work embodied in this thesis has been carried out at National Chemical Laboratory, Pune under the supervision of **Dr. M. K. Gurjar**, Division of Organic Chemistry, National Chemical Laboratory, Pune – 411 008. This work is original and has not been submitted part or full, for any degree or diploma of this or any other University.

(Bhargava Karumudi)
Candidate

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ABBREVIATIONS

Ac	-	Acetyl
AcOH	-	Acetic acid
Ac_2O	-	Acetic anhydride
AIBN	-	2,2'-Azobisisobutyronitrile
Bn	-	Benzyl
BnBr	-	Benzyl bromide
BSA	-	*N*,*O*-Bis(trimythlsilyl)acetamide
BH_3·DMS	-	Boron dimethylsulfide complex
BF_3·Et_2O	-	Boron trifluoride diethyl etherate
Boc	-	*tert*-Butoxy carbonyl
$(Boc)_2O$	-	Di-*tert*-butyl dicarbonate
CAN	-	Ceric ammonium nitrate
DMP	-	Dess–Martin periodinane
DCC	-	Dicyclohexylcarbodiimide
DIBAL-H	-	Diisobutylaluminium hydride
DIPEA	-	Diisopropyl ethylamine
DMM	-	Dimethoxymethane (Methylal)
DMA	-	*N*,*N'*-Dimethylacetamide
DMF	-	*N*,*N'*-Dimethylformamide
DMAP	-	*N*,*N'*-Dimethylaminopyridine
DMSO	-	Dimethyl sulfoxide
EtOH	-	Ethanol
Et	-	Ethyl
EtOAc	-	Ethyl acetate
EOE	-	Ethyloxyethyl
HMDS	-	Hexamethyldisilazane
HMPA	-	Hexamethylphosphoramide
Im	-	Imidazole
IBX	-	Iodoxybenzoic acid
LDA	-	Lithium diisopropylamide

MeOH	-	Methanol
MsCl	-	Methanesulfonyl chloride
Me	-	Methyl
MeI	-	Methyl iodide
m-CPBA	-	*met*a-Chloroperbenzoic acid
MTPA	-	2-Methoxy-2-phenyl-2-(trfluoromethyl)acetyl
NaOMe	-	Sodium methoxide
NIS	-	N-Iodosuccinimide
Pd/C	-	Palladium on Carbon
$Pd(OH)_2/C$	-	Palladium hydroxide on Carbon
Ph	-	Phenyl
py	-	Pyridine
PDC	-	Pyridiniumdichromate
p-TSA	-	*par*a-Toluenesulfonic acid
TBAI	-	Tetra-*n*-butylammonium iodide
TBAF	-	Tetra-*n*-butylammonium fluoride
TBDMS	-	*tert*-Butyldimethyl silyl
THF	-	Tetrahydrofuran
TMS	-	Trimethylsilyl
$Yb(OTf)_3$	-	Ytterbium trifluromethanesulfonate

GENERAL REMARKS

❋ ^{1}H NMR spectra were recorded on AC-200 MHz, MSL-300 MHz, and DRX-500 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts have been expressed in ppm units downfield from TMS.

❋ ^{13}C NMR spectra were recorded on AC-50 MHz, MSL-75 MHz, and DRX-125 MHz spectrometer

❋ EI Mass spectra were recorded on Finngan MAT-1020 spectrometer at 70 *eV* using a direct inlet system.

❋ Infrared spectra were scanned on Shimadzu IR 470 and Perkin-Elmer 683 or 1310 spectrometers with sodium chloride optics and are measured in cm^{-1}.

❋ Optical rotations were measured with a JASCO DIP 370 digital polarimeter.

❋ Melting points were recorded on Buchi 535 melting point apparatus and are uncorrected.

❋ All reactions are monitored by Thin Layer chromatography (TLC) carried out on 0.25 mm E-Merck silica gel plates (60F-254) with UV light, I_2 and anisaldehyde in ethanol as development reagents.

❋ All solvents and reagents were purified and dried by according to procedures given in Vogel's Text Book of Practical Organic Chemistry. All reactions were carried out under Nitrogen or Argon atmosphere with dry, freshly distilled solvents under anhydrous conditions unless otherwise specified. Yields refer to chromatographically and spectroscopically homogeneous materials unless otherwise stated.

❋ All evaporations were carried out under reduced pressure on Buchi rotary evaporator below 40 °C.

❋ Silica gel (60–120) used for column chromatography was purchased from ACME Chemical Company, Bombay, India.

CONTENTS

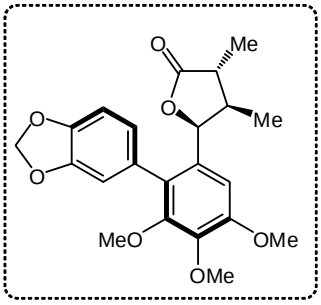
ABSTRACT

Abstract

The thesis entitled "**Synthetic studies towards Eupomatilone-6, 4-O-β-D-galactosyl maltose- LG-2 and amphidinol-3**" consists of three chapters. First chapter describes total synthesis of eupomatilone-6. Second chapter deals with the synthetic studies towards the 4-O-β-D-galactosyl maltose LG-2. The final chapter III deals with synthetic studies towards C (43) – C (52) fragment of amphidinol-3, using chiral pool strategy.

CHAPTER 1:

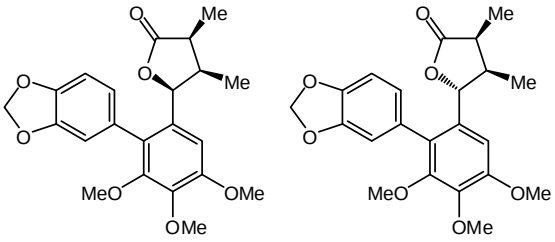
Total Synthesis of Eupomatilone-6.



(+)-Eupomatilone-6

In 1991, Taylor *et.*a*l.* isolated a series of novel lignans eupomatilones 1–7 from the shrubs *Eupomatia bennettii* F.Muell. and their relative configurations were elucidated by the extensive NMR studies. Eupomatilones are characterized by the biaryl system with a substituted γ-lactone ring attached to one of the aryl rings. Although they exist as a mixture of atropisomers, efforts to separate them by HPLC were unsuccessful. Apart from a recent synthesis of (±)-5-*epi*-eupomatilone-6 (**2**), no report has yet appeared on the total synthesis of any of these natural products until our exploration in this direction (figure 1).

Figure 1.



Eupomatilone-6 (**1**) Proposed

5-epi-eupomatilone-6 (**2**) McIntosh

The first total synthesis of the putative structure of Eupomatilone-6 (**1**) was reported from our group consists Suzuki coupling, Sharpless asymmetric dihydroxylation and intramolecular Horner-Wadsworth-Emmons reactions as key transformations. As the physical data of synthetic compound (specific rotation and ^{1}H & ^{13} NMR spectral data) is not in agreement with the reported data, we have analyzed the spectral data of available eupomatilones along with the synthetic eupomatilones and came to a conclusion that the assigned relative stereochemistry of eupomatilone-6 needs revision. Based upon these NMR analyses we have also provided two alternative formulations (figure 2) for eupomatilone-6 (**3** and **4**).

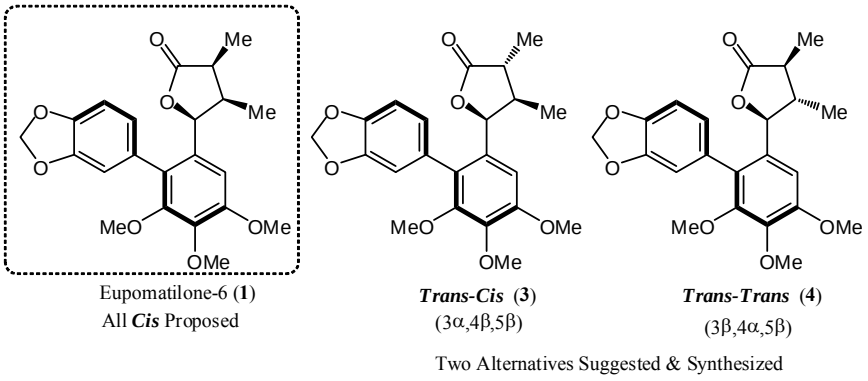
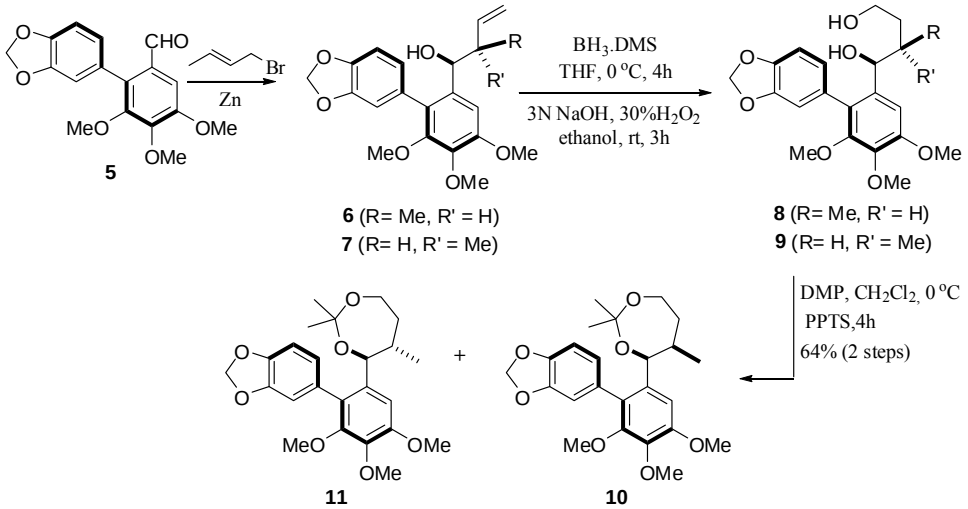


Figure 2: Putative Structure of Eupomatilone-6 (**1**) Proposed Alternatives (**3** & **4**)

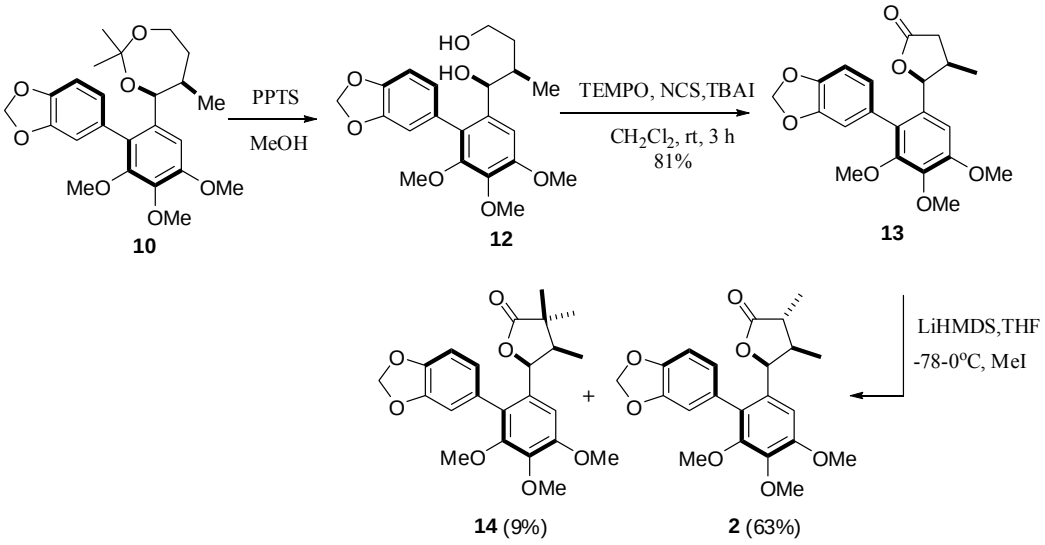
We initiated our synthesis by crotylation of the known aldehyde **5** with crotyl bromide in the presence of zinc and ammonium chloride gave an inseparable 1:1 mixture of *syn*- and *anti*-homoallylic alcohols **6** and **7** (scheme 1).

Scheme 1.



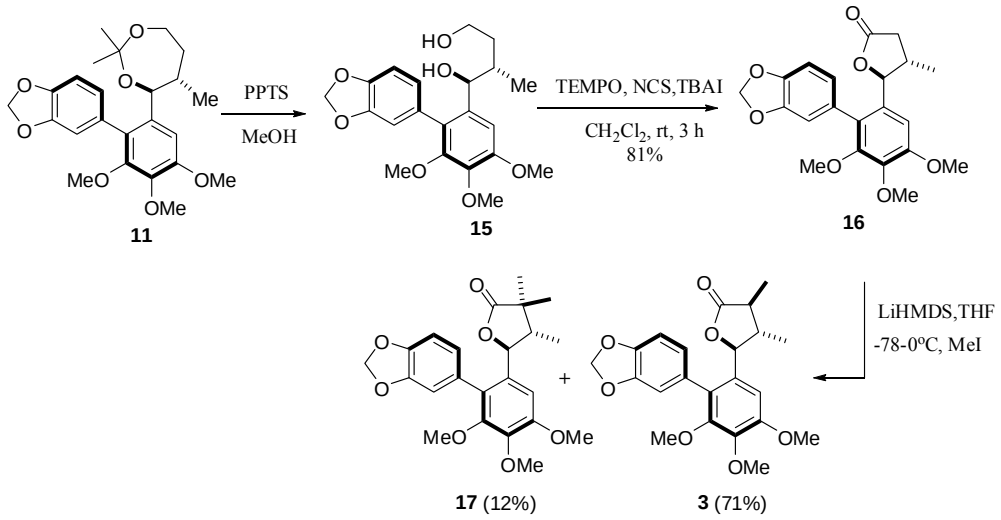
Since the efforts to separate the mixture by column chromatography failed, we decided to proceed further with the mixture. However, subsequent hydroboration-oxidation of 6/7 by using $Me_2S{:}BH_3$ and H_2O_2 followed by treatment with DMP and PPTS gave the acetonide derivatives 10 and 11 which were separated by silica gel chromatography. The *Syn* acetonide 10 was deprotected by ppts in methanol gave the corresponding diol 12. The diol 12 was oxidised with NCS-TEMPO to obtain the corresponding γ-butyrolactones 13 in good yield (scheme 2). After screening several reagents, we observed that the diastereoselectivity of methylation at C-3 was highest with LiHMDS-MeI. The dialkylated product 14 was also isolated as a minor product. The spectral data of 2 was in complete agreement with the data published for the naturally occurring eupomatilone-6.

Scheme 2.



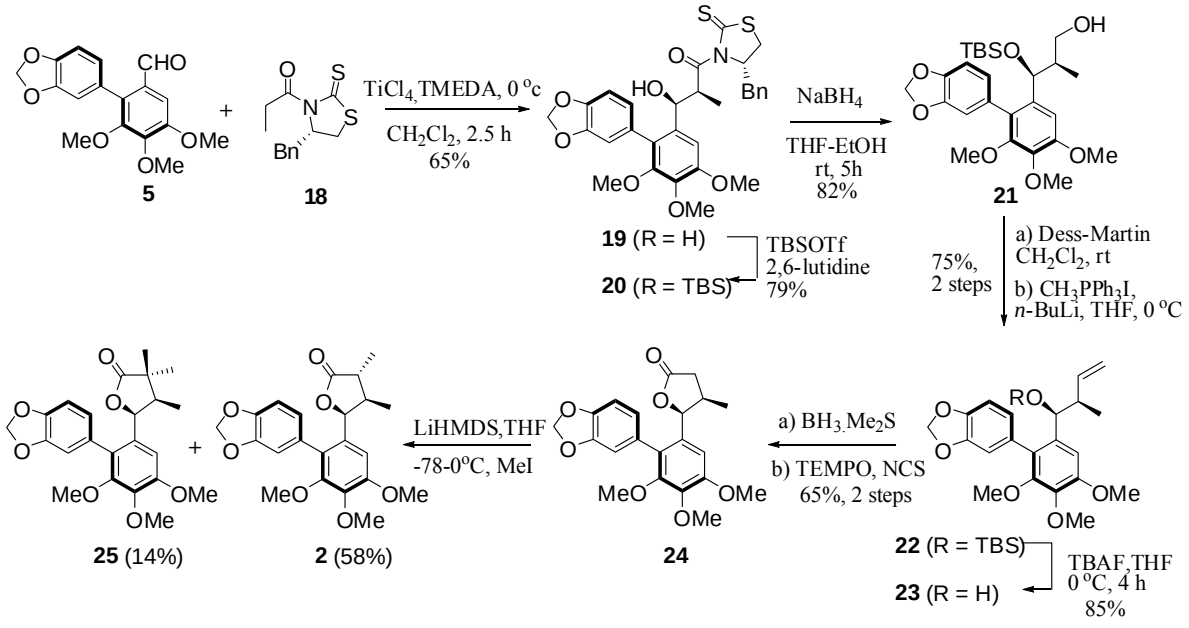
Similarly, compound **11** was transformed by adopting the same strategy, into diastereomeric eupomatilone **3** (scheme 3). The ^{1}H NMR spectrum of **3** was substantially different from the natural eupomaltinone-6. After successfully establishing the relative stereochemistry of racemic eupomatilone-6, we felt the need to design, based on our new findings, the asymmetric synthetic protocol which would establish the absolute configuration of eupomatilone-6. It is pertinent to mention that eupomatilone-6 inherently exists in nature, as a mixture of atropisomers whose separation is still being precluded.

Scheme3.



Our asymmetric synthetic plan is as depicted in (scheme 4). Our asymmetric synthesis began with aldol condensation of *N*-propionyl-thiazolidinethione **18** with aldehyde **5**. After having an easy access for the key aldol adduct **19**, the free hydroxyl group was protected as its TBS-ether **20**; thiazolidinethione auxiliary was removed by treating the TBS ether with $NaBH_4$ to afforded primary alcohol **21**.

Scheme 4.



The alcohol **21** was subjected to DessMartin periodinane oxidation to aldehyde, which was used with out purification and consequent Wittig reaction with PPh_3=CH_2 gave

the olefin derivative **22**. The crotyl compound **23** was afforded by removeing TBS ether from compound **22**. Compound **23** was subjected to successive hydroboration and oxidation to give the diol, which was subjected to selective oxidation and lactonization as depicted earlier afforded lactone derivative **24**. The alkylation of **24** was executed by using essentially the same reaction (LiHMDS-MeI) as reported above to give (3*R*,4*R*,5*S*)-eupomatilone-6 (2).

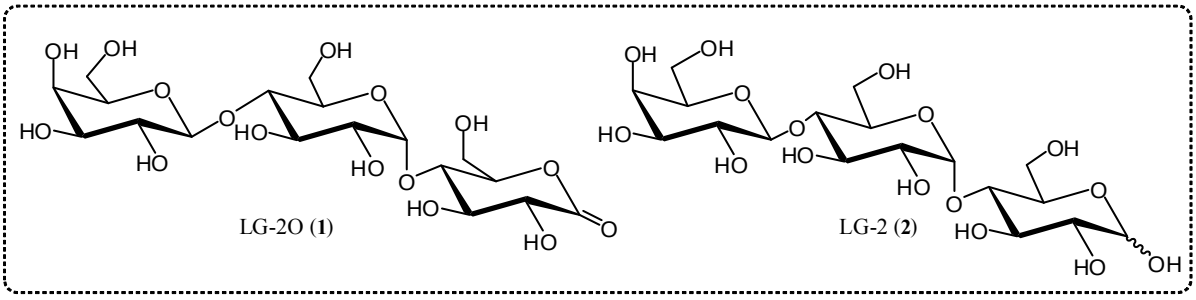
In continuation I have recently completed the synthesis of the proposed diasteromers of eupomatilone-6 and confirmed the revised structure of eupomatilone-6 as **2**. The center of our synthesis is the stereoselective methylation at C-3 of a 4,5-disubstituted γ-butyrolactone to address the requisite 3,4,5-*trans*-*cis* and *trans*-*trans*- relative configuration. We extended this synthetic exercise further by completing the first synthesis of optically active (3*R*,4*R*,5*S*)-eupomatilone-6 and established the absolute configuration of naturally occurring eupomatilone-6 as (3*S*,4*S*,5*R*) (**2**).

CHAPTER 2:

Synthetic studies towards 4-O-ß-D-galactosyl maltose LG-2.

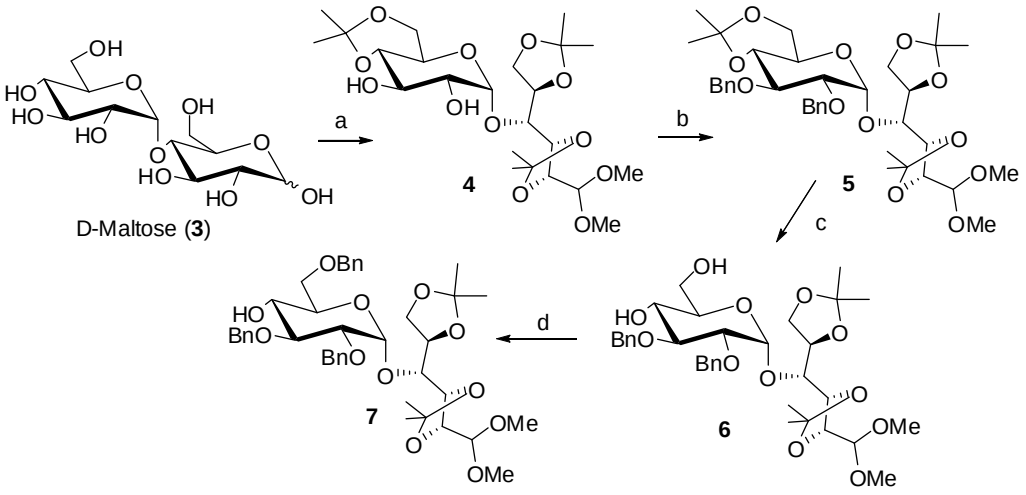
α-Amylases are a group of enzymes widely distributed in microorganisms, plants and animal secretions, which catalyze the hydrolysis of the α-(1→4) glycosidic linkages in starch and various oligosaccharides. Inhibition of α -amylases reduces the post-prandial glucose peaks, which is of particular importance in patients with diabetes. LG2O and LG3O, which are competitive inhibitors for mammalian alpha-amylases, exhibited Ki values of the order of 2.8 – 18 μM. LG-2O was synthesized enzymatically, but there is no chemical synthesis. The interesting glycosidase inhibition activity of LG-2O and lack of any synthesis led us to investigate its synthesis. We planed to make the lactone at the late stages, from the corresponding lactol LG-2 (figure 1).

Figure 1.



The key step in our reaction is the pentenyl orthoester activated glycosylation between a galatosyl donor **8** and maltose unit as an acceptor **7**. The synthesis of the glycosyl acceptor started with intermediate maltose disaccharide **4** which can be made from maltose **3** in a single transformation in good yield. Following a sequence of benzylation, selective acetonide deprotection and selection 1°-OH protection as its benzyl ether provided the glycosyl acceptor **7** (scheme 1).

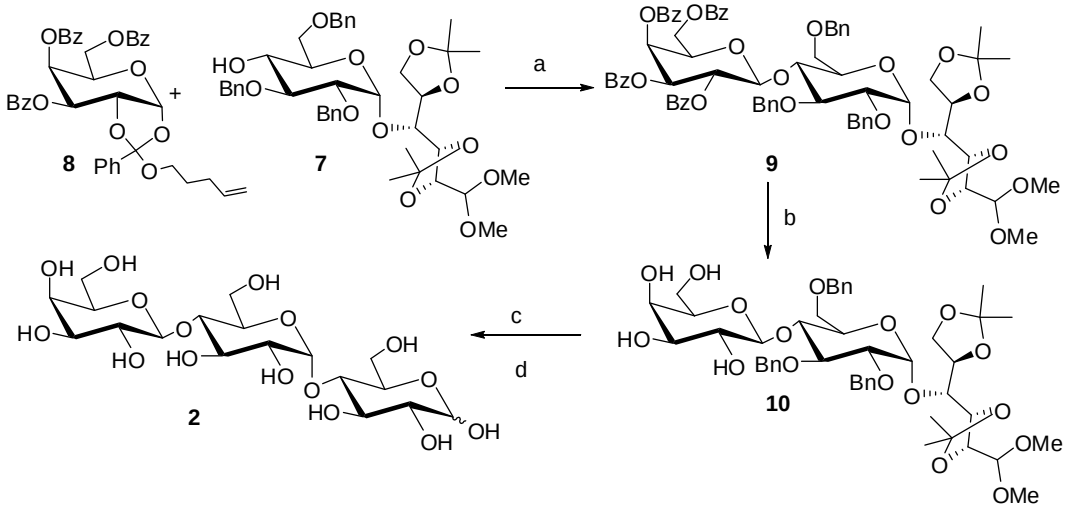
Scheme 1.



Reagents and conditions: a) 1, 4-Dioxane, 2, 2-Dimethoxy propane, PTSA, 80 ^{o}C; b) NaH, BnBr, DMF; c) 0.6% H_2SO_4, MeOH; d) Bu_2SnO, BnBr; $PhCH_3$

The glycosyl acceptor **8** was made according to the reported procedures. Using NIS and $Yb(OTf)_3$ as activators, the key glycosylation was carried out in 62% yield to get the compound **9** (scheme 2). The debenzyolation followed debenzylation with concommitant hydrolysis using Pd/C in presence of TFA provided LG-2 (**2**). The conversion of lactol to lactone was known.

Scheme 2.



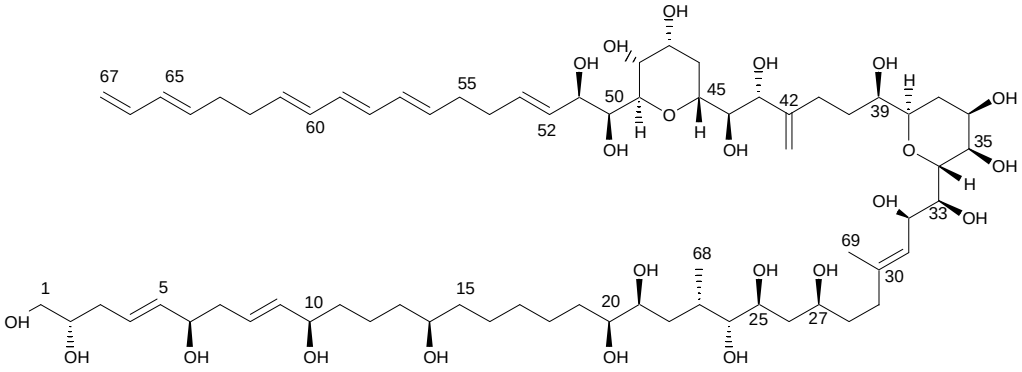
Reagents and conditions: a) NIS, $Yb(OTf)_3$, DCM; b) NaOMe, MeOH; c) Pd/C, Dioxane; d) TFA, H_2O.

CHAPTER 3:

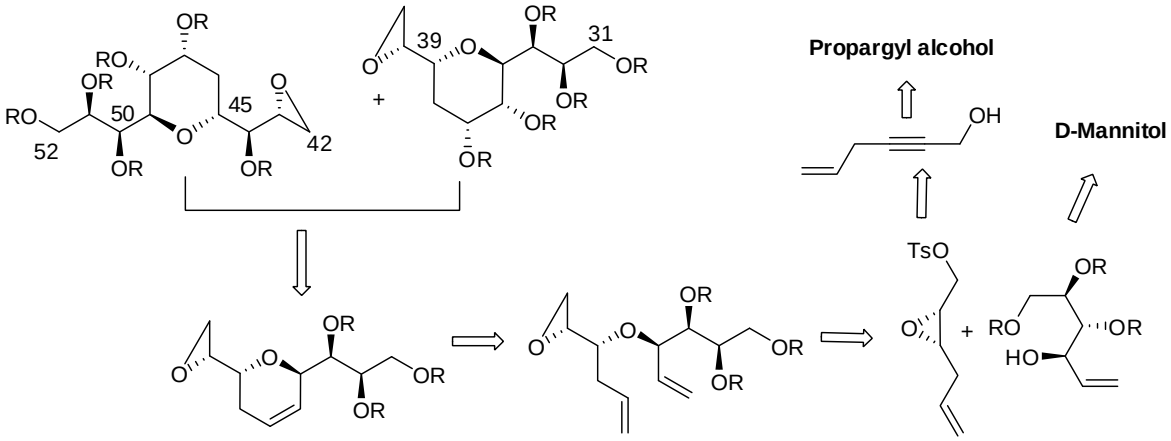
Synthetic studies towards Amphidinol- 3.

The amphidinols belongs to the class of natural products isolated from the marine dinoflagelates *Amphidinium* sp. that display antifungal, hemolytic, cytoxic, and ichthyotoxic activities. Dinoflagellates, a type of primitive unimolecular alage, are a rich source of structurally and biologically intriguing natural products; eg., okadaic acid, brevetoxins, ciguatoxins and maitotoxin. Among these polyether-cyclic compounds, amphidinols are unique dinoflagellate metaholites since they are primerly made up of linear polyhydroxy structures. Of the 13-polyketide metabolites in this family, amphidinol-3 is one of the most biologically active, with antifungal activity against Aspergillus niger and hemolytic activity on human erythrocytes. Amphidinol-3 effects cholesterol dependent membrane disruption, leading to speculation that its mode of action may, in part, be due to disruption of cell membranes (figure 1).

Figure 1: Amphidinol 3 (**1**).

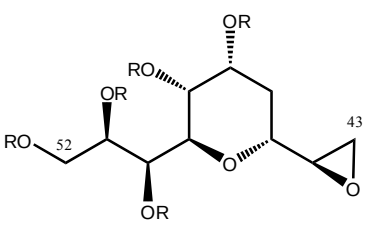


Retro-synthesis:

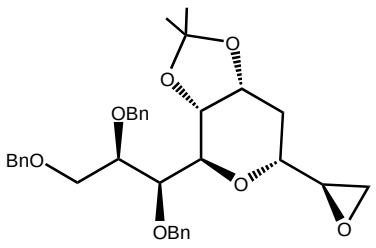


The long chain polyhydroxy compounds may be one of the most challenging targets. The novel architecture and the bioactive properties of the amphidinol 3 led us to investigate its synthesis. It contains a C (52) – C (67) skipped polyene chain, a series of 1,5-diols within the C (2) – C (15) region, two highly substituted tetrahydropyran units, and a total of 25 stereocenters on a contiguous 67-carbon backbone. Herein we describe the synthesis of C (41) – C (52) fragment 1 of amphidinol 3 (figure 2).

Figure 2:



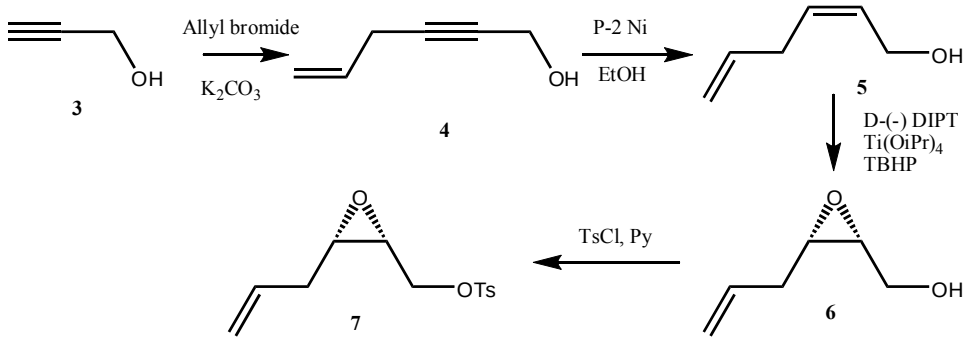
*C 43-C 52 Fragment **1***



*Protected C 43-C 52 Fragment 1 (**2**)*

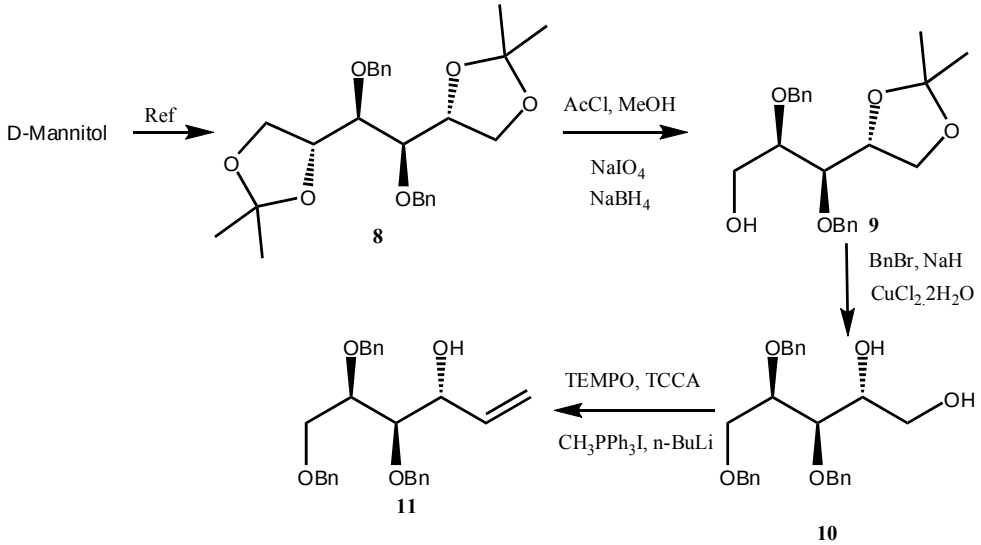
We initiated our synthesis known propargyl alcohol (**3**) which is alkylated with allyl bromide followed by cis hydrogenation using P-2 Ni gave hexa-2,5-dienol **5**. Sharpless epoxidation of the allylic alcohol under standard conditions (D (-) DIPT, TBHP, $Ti(O^iPr)_4$) yielding required epoxide **6**, whose free hydroxyl was protected as it's tosyl ether gave coupling fragment **7** as shown in scheme 1.

Scheme 1.



Other fragment was initiated with known diisopropylidine mannitol which was benzylated using BnBr, NaH in DMF gave compound **8** .Selective removal of one of the terminal isopropylidine group followed by oxidative cleavage with periodate and borohydride reduction gave compound **9** (scheme 2).

Scheme 2:



The hydroxyl group is protected as its benzyl ether, followed by subsequent removal of terminal acetonide group by mild acidic hydrolysis with $CuCl.2H_2O$ in CH_3CN to give diol **10**. Selective oxidation of the primary hydroxyl group with TEMPO and TCAA followed by Wittig olifination gave the compound **11**.

After having both the coupling fragments our next task was nucleophilic epoxide opening under mild acidic conditions. Nucleophilic opening of the epoxide **7** with vinyl alcohol **11** under standard conditions ($BF_3.Et_2O$, 4 A molecular sieves). However the desired coupling reaction did not take place under these conditions and the olefin **11** was recovered (scheme 3). A careful survey of other conditions for the crucial coupling was conducted (Table 1). Unfortunately, it was found that all the attempts to couple olefin **11** and epoxy tosylate **7** by intermolecular nuclephilic epoxide opening procedures under various reaction conditions turned out to be failures (scheme 3).

Scheme 3:

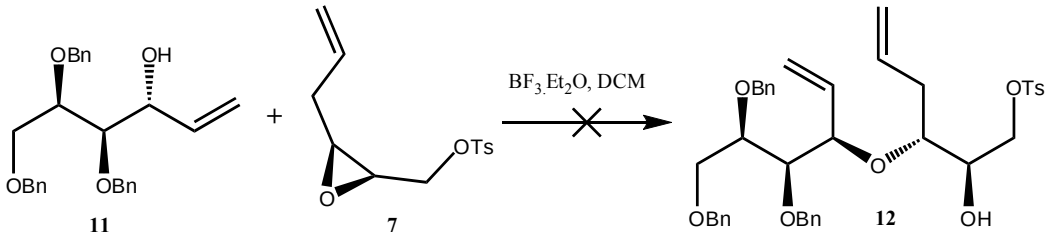
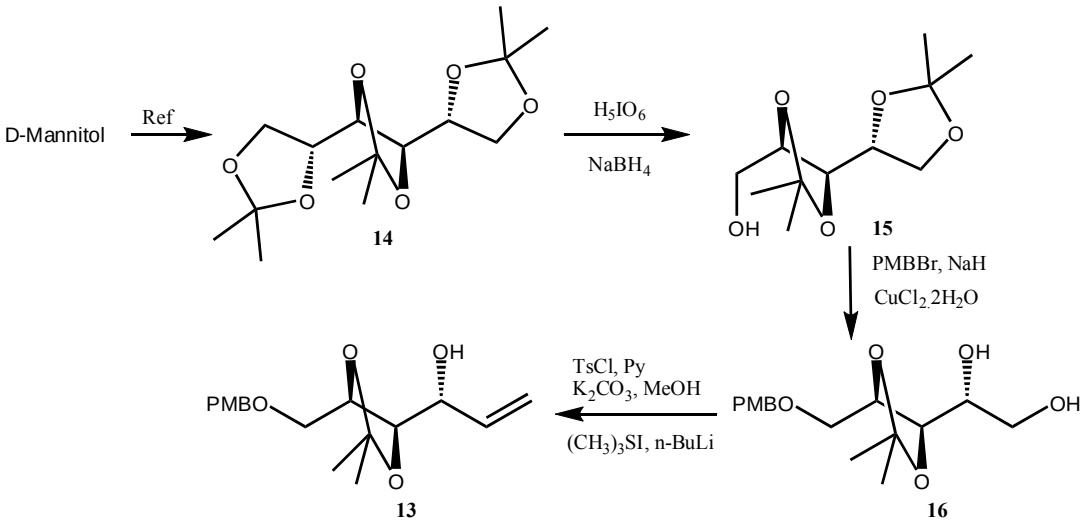


Table 1: *Conditions applied and results obtained in epoxide opening*

Entry	Reaction conditions	Results obtained
1	$BF_3.Et_2O$, DCM, -20 ^oC , 12h	No reaction, olefin **5** recovered.
2	$BF_3.Et_2O$, DCM, -20 ^oC to rt, 12h	No reaction, olefin **5** recovered.
3	$BF_3.Et_2O$ (1 eq), DCM, -20 ^oC to rt, 12h	Complex mixture.
4	$Cu(OTf)_2$, DCM, -20 ^oC, 12h	Both the starting materials recovered.
5	$Cu(OTf)_2$, DCM, -20 ^oC to rt, 48h	No reaction.

Having met with failures to open the epoxide using acid catalysts, we redesigned our retrosynthetic strategy and it was decided to attempt the epoxide opening with some what less bulkier group instead of benzyl ethers. It was assumed that the bulkier benzyl groups could not allow the incoming electrophile which met with failure. Thus the benzyl ethers in olefin moiety **11** were replaced by isopropylidene. The modified coupling partner **13** was synthesized as follows (scheme 4).

Scheme 4:



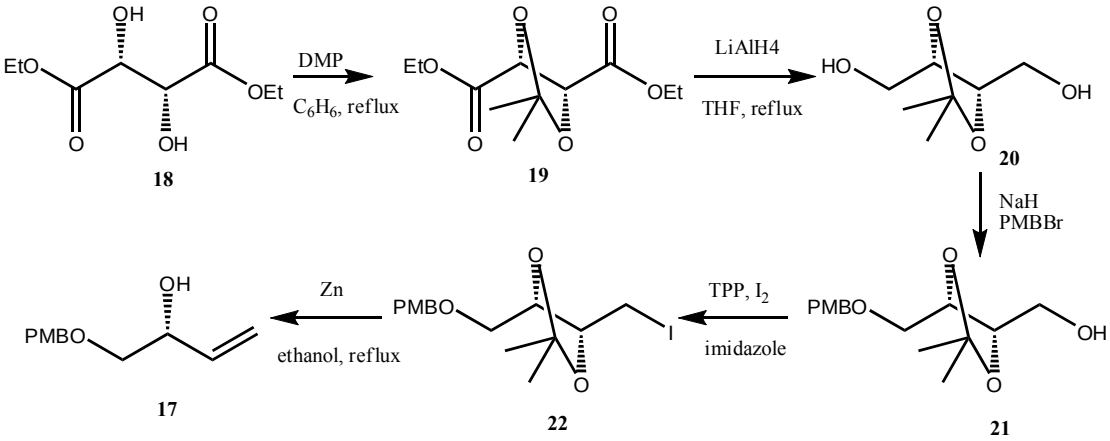
The olefin **13** commenced from D mannitol using known protocol. Mannitol triacetonide was selectively cleaved with periodic acid followed by borohydrate reduction gave compound **15**. Protection of the free hydroxyl as PMB ether followed by acetonide deprotection furnished diol **16**. Mono tosylation of diol followed by epoxide formation and subsequent nucleophilic opening with trimethylsulphonium iodide gave olefin **13**.

With the required fragments **13** and **7** in hand, the crucial epoxide opening was investigated. For the selective opening of epoxide, various conditions and reagents were applied. But unfortunately all the conditions failed to give the desired product. In most of the cases compound **13** was recovered.

Having met with failures to open the epoxide using acid catalysts, we decided to open the epoxide with simple homo allylic alcohol. For this olefin **17** was selected as coupling fragment which can easily prepared from diethyl tartarate by reported protocol. The required olefin was achieved by series of reactions, acetonation of L-diethyl tartrate, reduction of

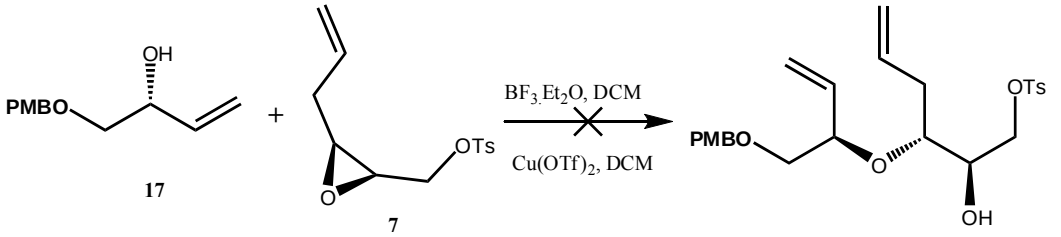
diester, mono PMB protection, iodination of free hydroxyl group followed by zinc mediated elimination furnished compound **17** (scheme 5**)**.

Scheme 5:



After having both the coupling partners in hand our next task was crucial epoxide opening. Again both the fragments were subjected to various reaction conditions as mentioned above to get the desired product were met with failure (scheme 6).

Scheme 6:



In fact, the amphidinol 3 natural products present several challenges to the synthetic organic chemist some of them were solved. Finally, in contrast, that different solutions were devised for some of the problems demonstrates that natural products often stimulate the development of and provide a proving ground for new strategies and methods of organic synthesis.

In summary, we have successfully accomplished the syntheses of crucial coupling segments with suitable protections for the projected fragment synthesis of Amphidinol 3. Our strategy presents a very different approach from the previous syntheses of tetrahydrofuran moiety. Studies toward the C (43)-C (52) fragment of Amphidinol 3 are under progress in our laboratory.

CHAPTER I

Total Synthesis of Eupomatilone-6

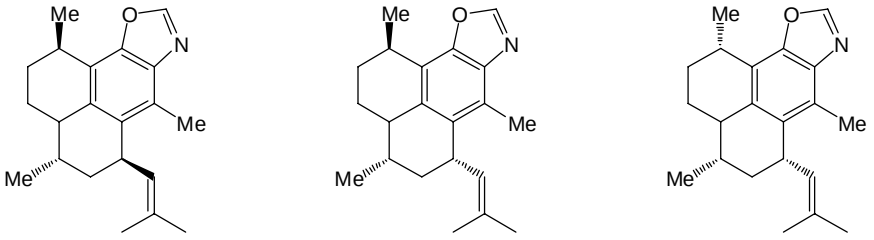
Introduction

Introduction

Structural Misassignments and Ambiguities in Natural Product Elucidation:

Synthesis has played a major role in natural product chemistry. Until the evolution of spectroscopic methods, independent synthesis of a compound was thought to give the ultimate proof for the proposed structure. The proponents of X-ray crystallography and spectroscopy have often claimed that with the advent of these powerful techniques, the need for synthesis abolished. Even a quick look at the recent literature shows, however, that no matter how sophisticate are the instrumental methods used, there is still the ambiguity of experimental error and even today structures elucidated through instrumental means are revised as a result of synthesis. This is increasingly true as the level of complexity of compounds increases. For e.g., spectral data of pseudopteroxazole (proposed structure **1**) did not match either its synthetic version or its analogue (**2**). Corey[1] et al suggested that the natural product might be having structure **3** (figure 1).

Figure 1:



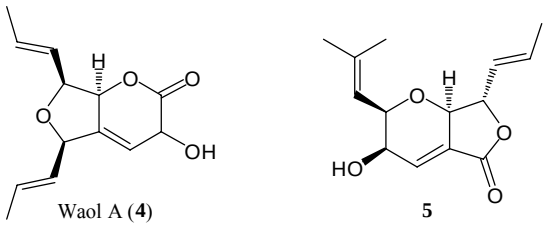
1 Proposed structure

2

3 Probable structure

Similarly, Snider[2] *et al* has found that the assigned structure of Waol A **4** to be revised as **5** (figure 2).

Figure 2:

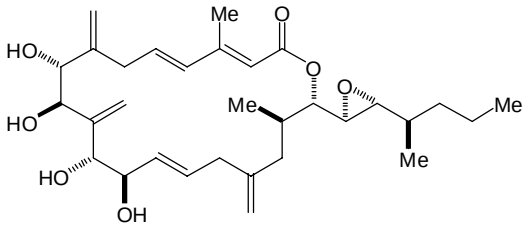


Waol A (**4**) Praposed structure

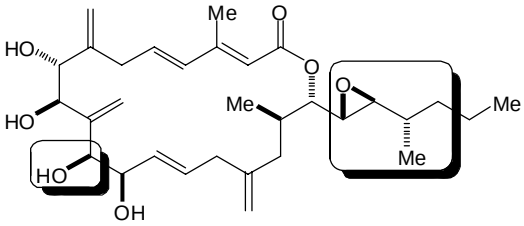
5 revised structure

Some recent natural products that were misassigned and their revised structures where the structure was conformed by chemical synthesis[3] (figure 3).

Figure 3:

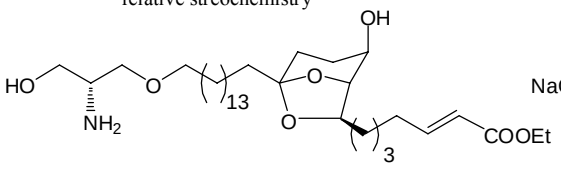


Reported structure[4]
relative streochemistry

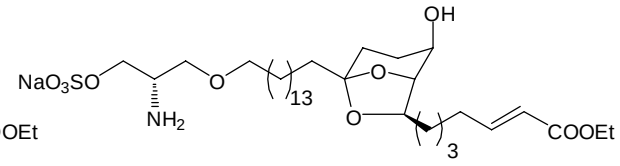


Amphidinolide A

Corected structure[5]

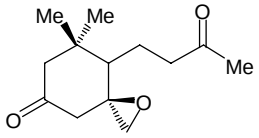


Praposed[6]



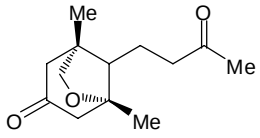
(+) Didemniserinolipid B

Revised[7]



Praposed[8]

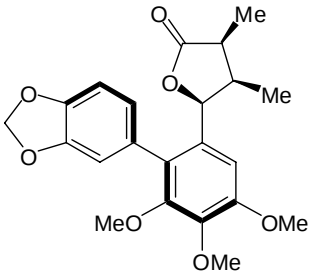
Annuionone A



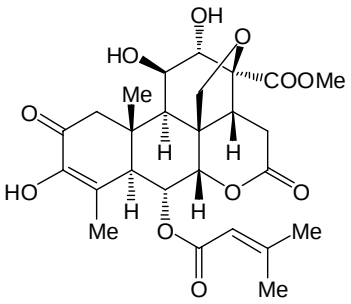
Revised[9]

Some natural products whose proposed structures have been disproved by synthesis, but are awaiting a revised proposal at the start of the project (figure 4).

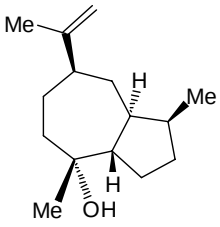
Figure 4:



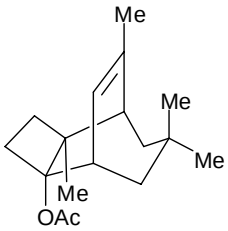
Eupomatilone-6
Isolation: Taylor[10]
Synthesis: Gurjar[11] *et al*



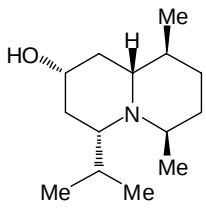
Bruceoside A aglycon
Isolation: Okanor[12] *et al*
Synthesis: VanderRoest[13] *et al*



Pogostol
Isolation: Wateman[14] *et al*
Synthesis: Booker-Milburn[15] *et al*



Paesslerin A
Isolation: Palermo[16] *et al*
Synthesis: Ihara[17] *et al*

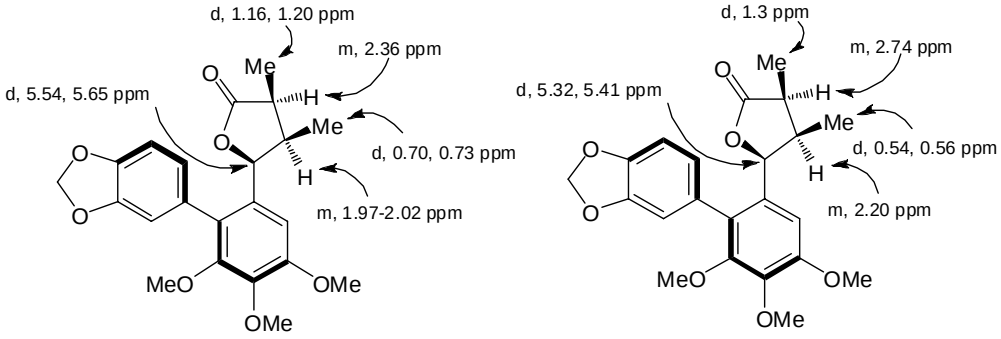


Plumerinine
Isolation: Malik[18] *et al*
Synthesis: Comins[19] *et al*

Mistaken Identity-Case Study: Eupomatilone-6:

Synthesis of eupomatilone-6 had been reported from our group[11]. As the physical data of our synthetic eupomatilone-6 with the proposed stereochemistry[10] (specific rotation and ^{1}H & ^{13}C NMR spectral data) is not in agreement, we have analyzed the spectral data of available eupomatilones along with the synthetic eupomatilones (figure 5).

Figure 5:



All *Cis* (**11**)
Praposed Eupomatilone-6

All *Cis* (**11**)
Synthesised

NMR Signals for both of the Attropoisomers.

Reported optical rotation = - 25.6 (c = 0.5)

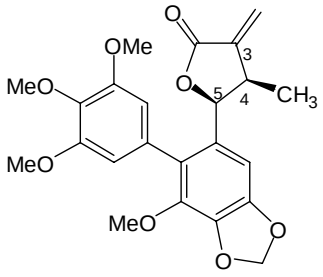
Optical Rotation = - 30.0 (c = 0.5, $CHCl_3$)

We came to a conclusion that the assigned relative stereochemistry of eupomatilone-6 needs revision.

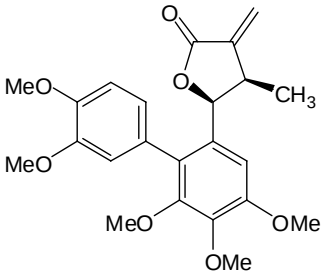
Eupomatilones:

The Australian shrub *Eupomatia Bennettii* F. Muell is a source[10] of rich variety of lignans like eupomatenoids, eupodienones, eupobennettin, bennettinone and eupomatilones. The Eupomatilones are a family of seven degraded lignans. Their relative stereochemistry has been assigned to be as in figure 6 (**1-7**) based on nOe studies. The eupomatilones are unusual among the lignan family in that the C_{α}-phenyl linkage in one of the phenylpropanoid unit has been cleaved. All members of the family possess C_4-C_5 cis stereochemistry in the butyrolactone ring. They also possess a biaryl system with a γ-lactone ring attached to one of the aryl rings. Eupomatilones **4**, **6** and **7** are having the methyl groups on the butyrolactone ring *cis* to each other. Eupomatilones **5**, **6** and **7**-exhibited atropisomerism.

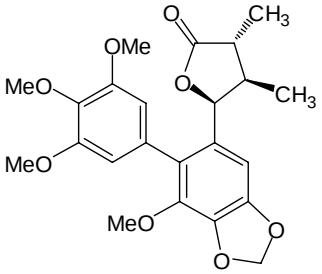
Figure 6:



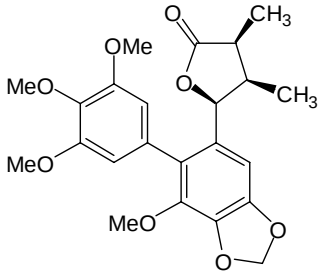
Eupomatilone-1(**6**)



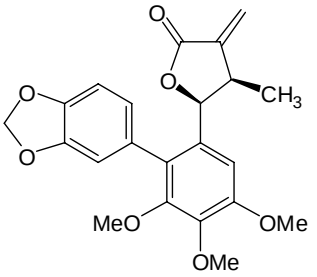
Eupomatilone-2 (**7**)



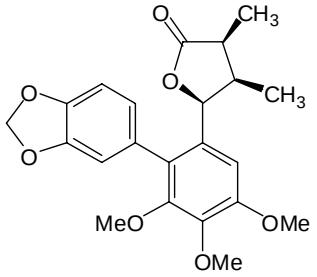
Eupomatilone-3 (**8**)



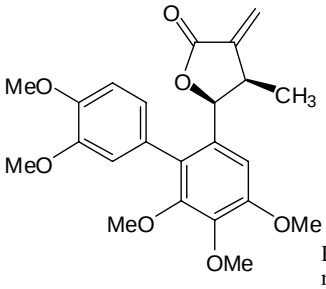
Eupomatilone-4 (**9**)



Eupomatilone-5 (**10,** 1:1 mixture of atropisomers)



Eupomatilone-6 (**11,** 1:1 mixture of atropisomers)



Eupomatilone-7 (**12,** 1:1 mixture of atropisomers)

The biaryl is the central building block in a vary large number of natural products of differing structures, biological activity and biosynthetic origin, including for example polyketides, terpenes, lignans, coumarins, flavonoids, tannins, peptides and alkaloids.[20-21] Because of their interesting properties, not only as pharmacologically active natural products, but also as chiral reagents[22] and crown ethers[23] as chiral host molecules for inclusion compounds,[24] as the basis of chiral phases for chromatography,[25] as inflexible spacers between two halves of a molecule[26-27] or also as the basis of chiral liquid crystals,[28] natural and unnatural biaryls constitute attractive synthetic goals.

In many of the natural or synthetic biaryls, bulky ortho substituents next to the biaryl axis lead to hindrance of free rotation around the biaryl axis and thus existence of stable atropisomerism. Some times only three ortho substituents are present while in some

extreme cases only two ortho substituents may suffice. The stereochemistry around the biaryl axis is as important as any other chiral center in showing the biological acitivity and pharmacological properties. In nature, most of the sterically hindered biaryls are available in both atropisomeric states although in differing quantities.

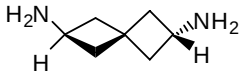
Atropisomerism:

Compounds that do not contain asymmetric atoms can nevertheless be chiral. Some examples[29] of these are shown in figure 7.

Figure 7:

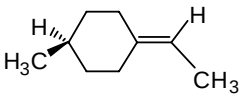
an allene

13



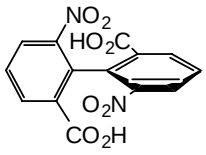
a spirane

14



compound with exocyclic double bond

15

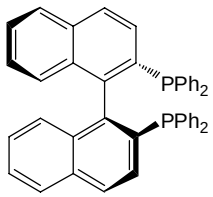


a biaryl

16

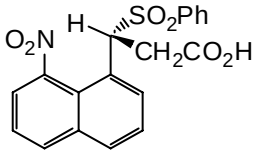
Whereas compounds **13**, **14** and **15** are thermally stable enantiomers, the biaryl system **16** contains chirality due to restricted rotation about a single bond. Stereoisomers resulting from hindered rotation about single bonds where the steric strain barrier to rotation is high enough to allow for the isolation of the conformers. Isomers that can be separated only because of restricted rotation about single bond and have half-life of racemisation of greater than 1000 seconds at RT are called atropisomers.[30]

Among the best known ligands for homogeneous catalysts used in asymmetric synthesis are the atropoisomeric ligands R (+) – BINAP (**17**) and S (-) – BINAP (**18**), which are used for hydrogenation.[31]



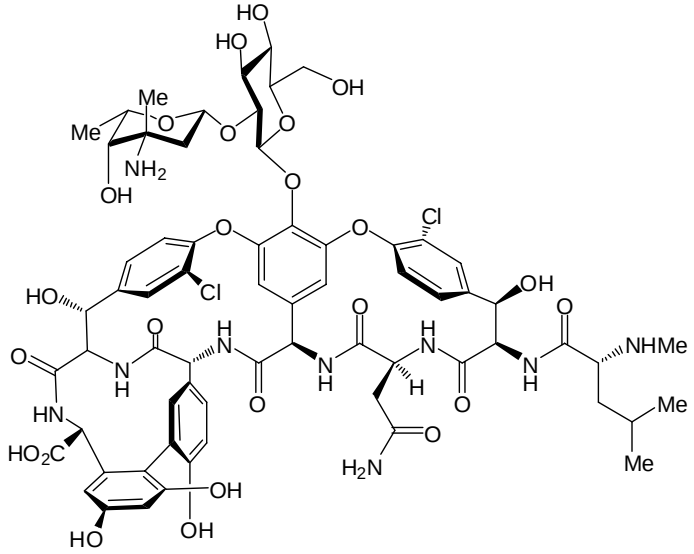
(R)-(+)-BINAP (**17**)

Biaryl systems are not the only ones with sufficient restriction about a single bond for atropisomerism to occur. One of the earliest examples of non-biaryl atropisomerism was the *peri*-substituted compound **19** isolated by Mills and Elliot in 1928.[32]

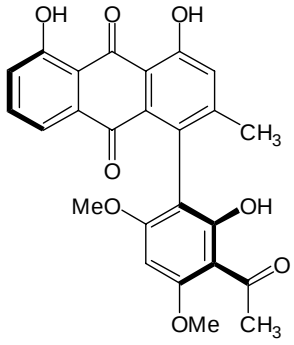


19

Examples of naturally occurring atropisomers include vancomycin (**20**) and knipholone (**21**) which is found in the roots of *Kniphofia Foliosa* of the family *Asphodelaceae*.[33]



Vancomycin (**20**)



Knipholone (**21**)

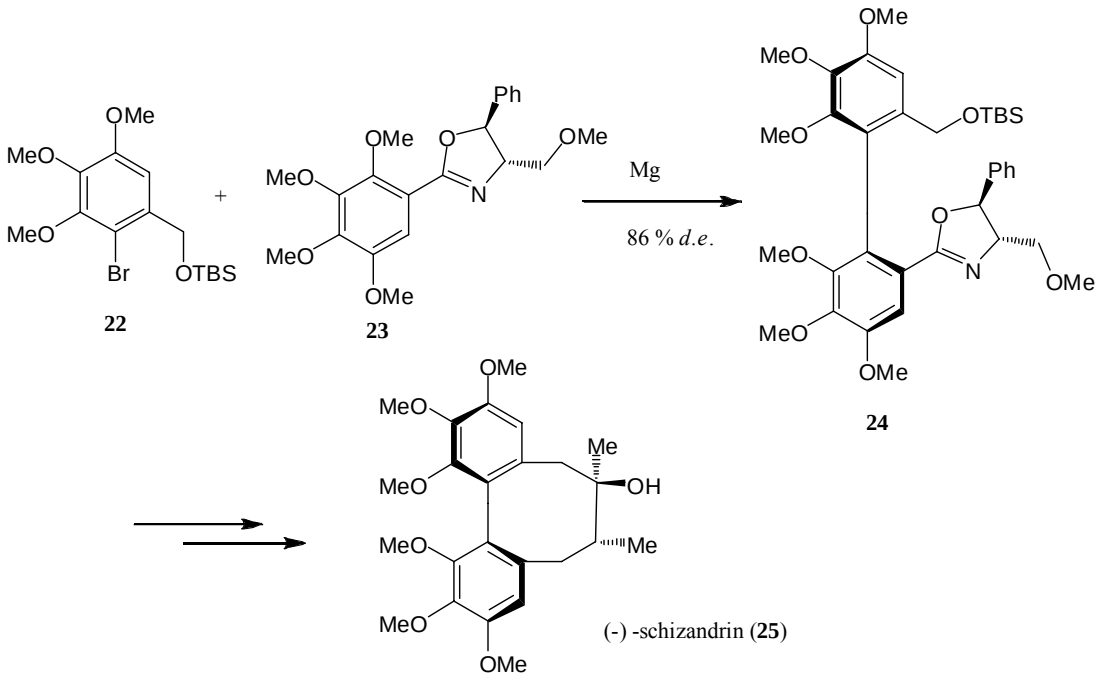
Separation of atropisomers is possibly by chiral resolution methods such as selective crystallization. In an atropo-enantioselective or atropselective synthesis one atropisomer is formed at the expense of the other. Several efficient methods are available for the synthesis of atropisomerically pure biaryl compounds. The commonly used methods will be discussed briefly here.

Atrop-selective biaryl synthesis *Meyer's oxazoline approach*

Meyers[34] elaborated a method based on an aromatic nucleophilic substitution using a chiral oxazoline auxiliary. The oxazoline moiety readily promotes displacement of ortho-methoxy and fluoro group by strong nucleophiles, generally Grignard reagents. The method

works well for biaryls bearing three ortho substituents. Lignan (-)-schizandrin[35] (**25**) was prepared by atrop-diastereoselective crossed biaryl coupling using grignard generated from **22** and chiral oxazoline **23** (scheme 1).

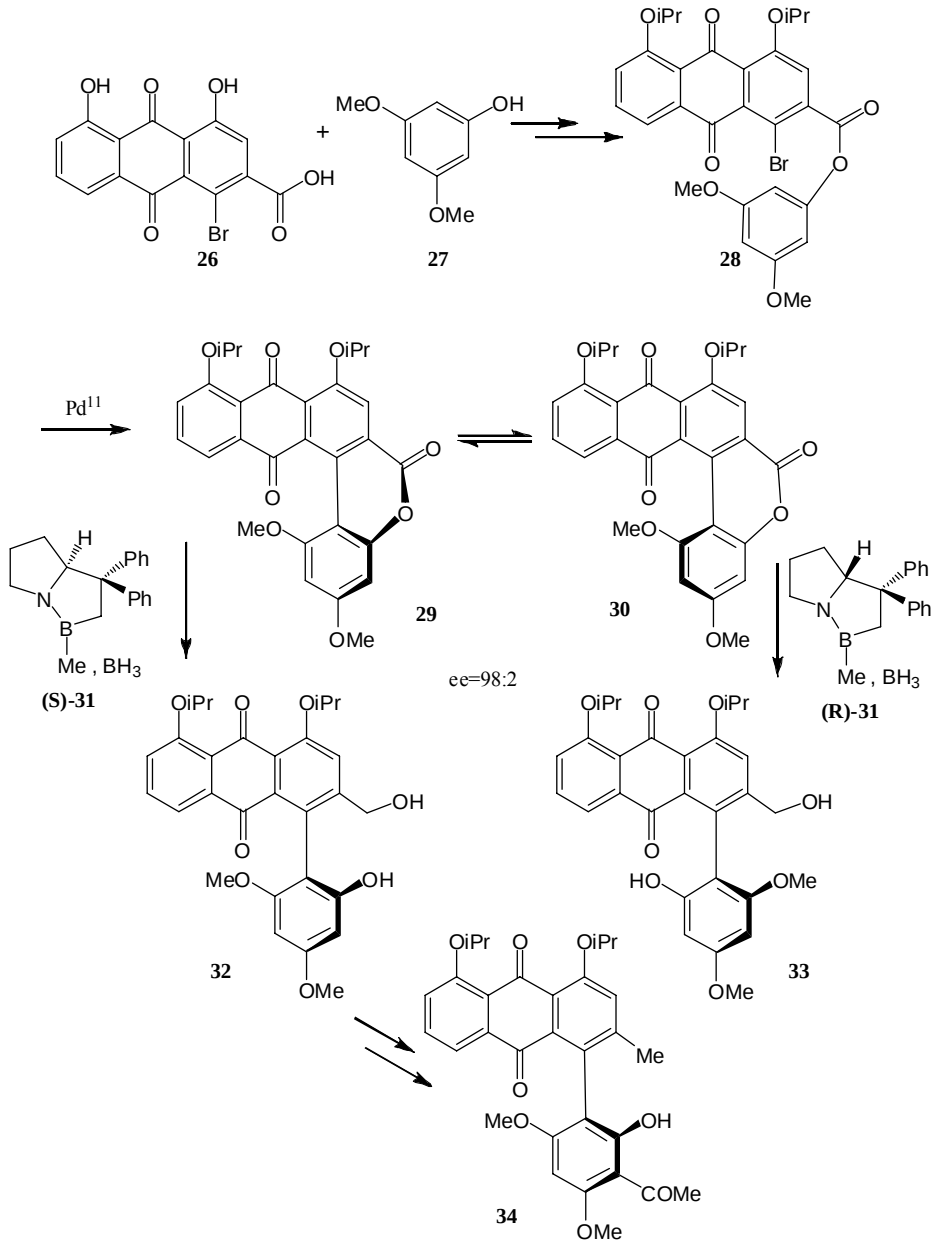
Scheme 1:



Bringmann's *lactone approach*

Bringmann prepared a wide range of natural biaryl compounds such as (+)-knipholone (**34**) *via* the "lactone method"[36] consisting of the atropselective ring cleavage of configurationally unstable lactone bridged biaryls. This method offers the advantage of controlling the axial chirality using catalytic chiral reagents. In their synthesis[37] of (+)-knipholone (scheme 2), carboxylic acid **26** and 3,5-dimethoxyphenol **27** were coupled to form the ester bridge. Pd-catalyzed intramolecular coupling produced the key intermediate lactone **29**. The most significant function of the ester bridge is that it dramatically lowers the rotational isomerisation barrier at the axis so that in contrast to the corresponding open biaryls, six membered biaryl lactones of type **29** are still configurationally unstable and exists as racemic mixtures of rapidly interconverting atropo-enantiomerns **29** and **30**. Out of this racemic mixture, one form can be cleaved with high stereoselectivity by a number of chiral nucleophiles. By use of the other nucleophilic enantiomer, the other isomeric product can be obtained. Thus cleavage of **29** with (*S*)-**31** gave **32** while (*R*)-**31** gave the enantiomer **33**.

Scheme 2:

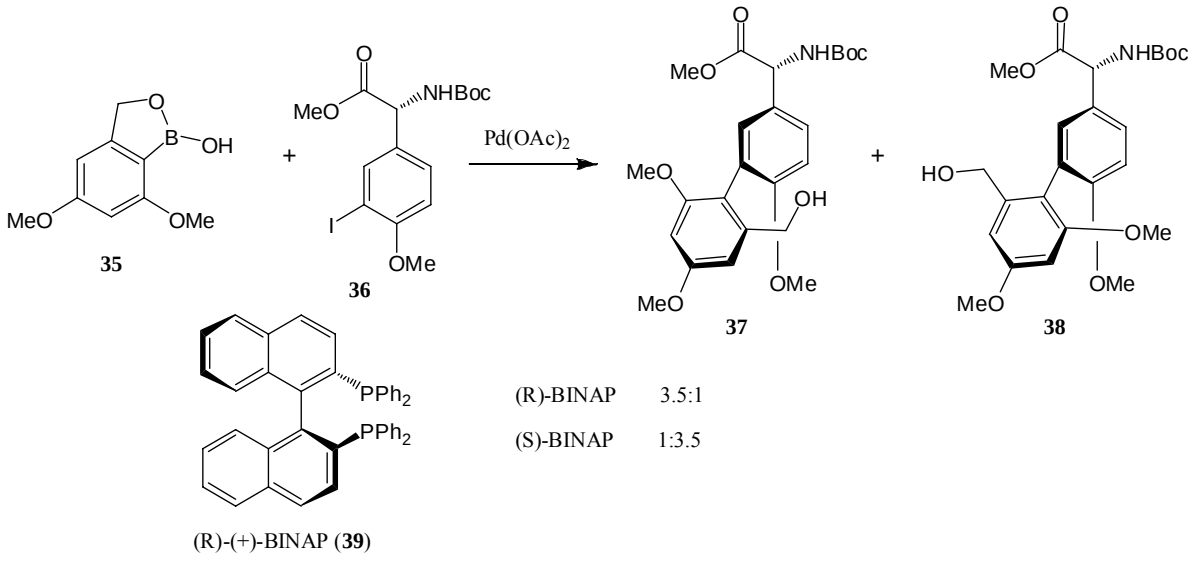


Using chiral catalysts:

The use of aryl reactants incorporating a chiral auxiliary as a control element for inducing atropisomer selectivity can however, be limited by their commercial availability as well as by limitation to a particular biaryl substitution pattern. Stereoselective aryl couplings have also been reported using aromatic systems that themselves do not incorporate chiral control elements through the use of chiral catalysts or reagents.

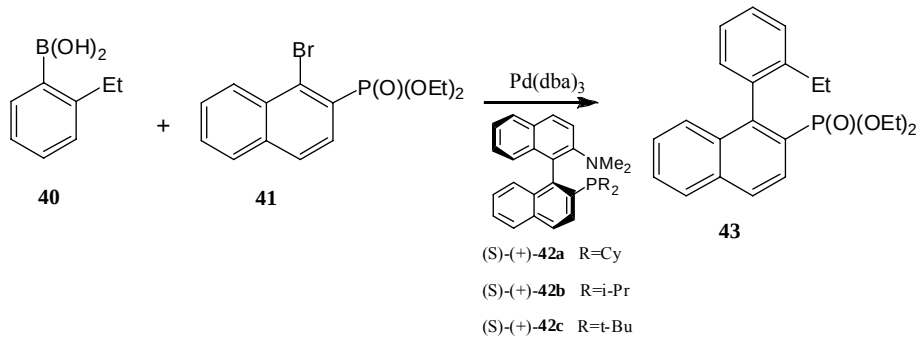
Atropo-enantioselective Suzuki coupling reactions are now carried out, mainly using binaphthyl and ferrocenyl ligands. Nicolaou *et al* reported an atropo-diastereoselective Suzuki cross coupling reaction using chiral ligands for the synthesis of biaryl **37** (scheme 3) in their total synthesis of vancomycin.[38] A variety of chiral and achiral ligands were screened and they found that while achiral ligands lead to no selectivity, among chiral ligands, only BINAP ligands gave good selectivity. Atropselectivity was reversed upon switching the chirality of the ligand. When the reaction was conducted in toluene (at 90 ^{o}C), atropisomerisation was observed. However in THF (at 60 ^{o}C), no isomerisation was detected.

Scheme 3:



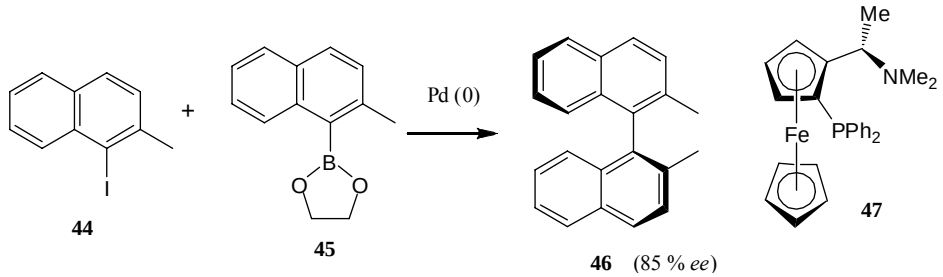
Ligands **42a-c** ware used by Buchwald[39] to obtain *ee* values upto 92% in their synthesis of the biaryl (+)-**43** (scheme 4).

Scheme 4:



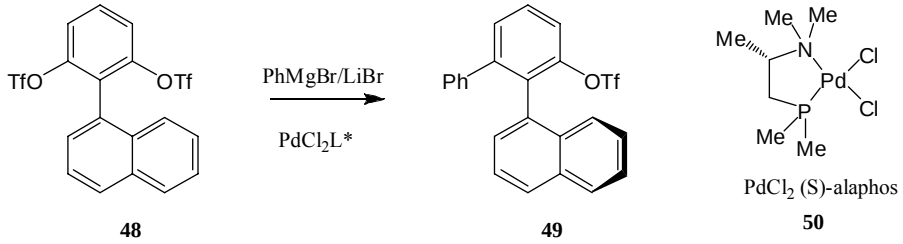
Cammidge reported[40] an atropselective Suzuki coupling with the ferrocenyl ligand **47** with *ee* upto 85 % (scheme 5).

Scheme 5:



Asymmetric cross coupling of achiral biaryl ditriflates like **48** with aryl grignard reagents in presence of LiBr and palladium complex $PdCl_2$[(S)-alaphos] (**50**) gave axially chiral monoarylated products **49** with high ee (scheme 6).[41]

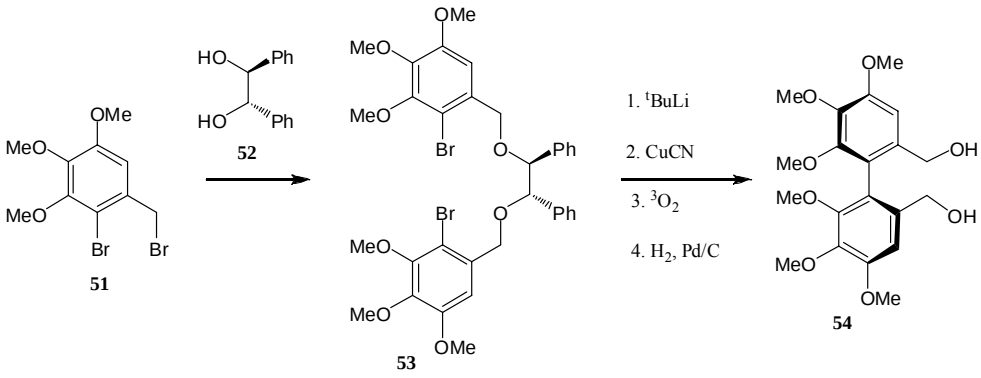
Scheme 6:



Other methods:

Cyanocuprate mediated intramolecular biaryl coupling has been reported by Lipshutsz *et al* (scheme 7)[42] The key to controlling the directionality of these couplings lies in the proper choice of the tether, which must: i) join the individual aryl subunits efficiently; ii) allow for cuprate generation and exert 100% stereocontrol in the reductive

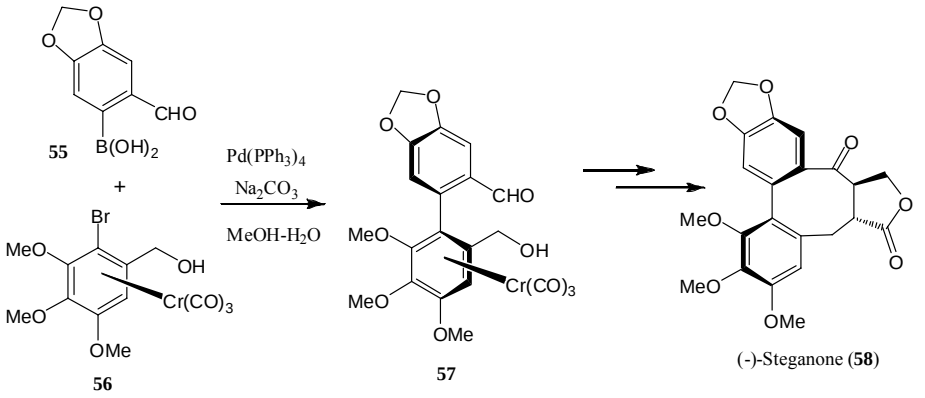
Scheme 7:



elimination step; and iii) undergo facile and high yield removal. The tethered product **53** was prepared from **51** using tether *S*, *S*-stilbene diol (**52**). Converting **53** to the diarylcuprate, oxidation with molecular oxygen followed by hydrogenation afforded optically pure **54**.

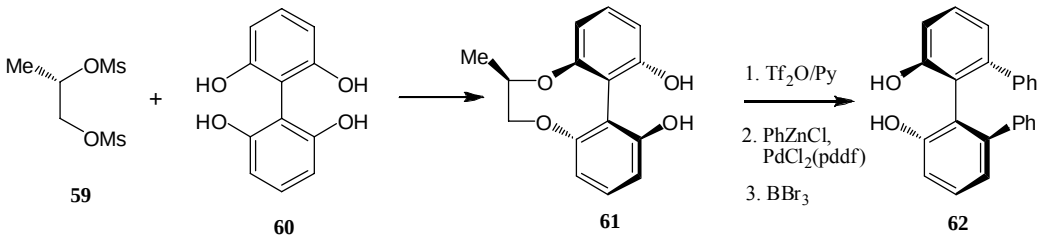
Natural product (-)-steganone (**58**) has been synthesized using an atropselective Suzuki coupling between the achiral boronic acid **55** and the chiral arene chromium complex **56**, to yield the desired atropisomer **57** (scheme 8).[43]

Scheme 8:



6,6′-disubstituted biphenyldiols like **62** have been synthesized (scheme 9) by asymmetrically desymetrizing the achiral biaryl **60** using (*S*)-*bis*-mesylate **59** giving biaryl **61** as a single enantiomer.[44] The free hydroxyls are then converted into trifaltes and replaced by alkyl or aryl group using Negishi reaction with a organozinc reagent. Lewis acid promoted cleavage of the *bis*-ether bond then gives 6,6′-disubstituted biphenyl diols enantioselectively.

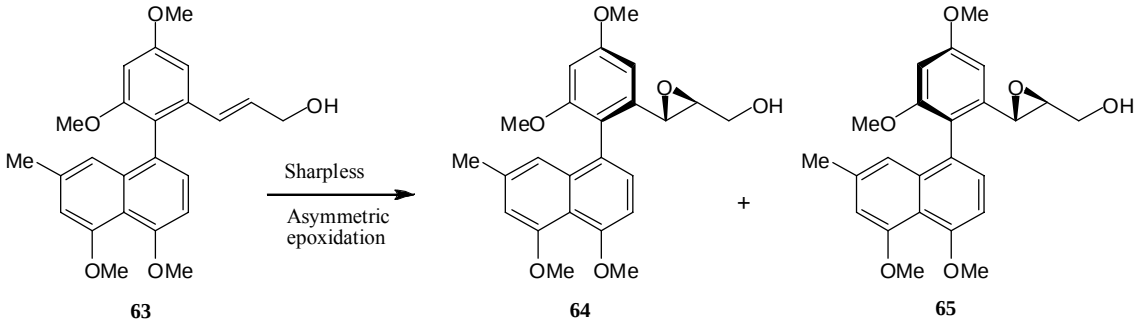
Scheme 9:



Rama Rao *et al* achieved the resolution of biaryls *via* Sharpless asymmetric epoxidation (scheme 10).[45] Racemic biaryl allylic alcohols (**63**) when subjected to

Sharpless asymmetric epoxidation gave rise to mixture of atropisomers **64** and **65**, which were easily separated by chromatography.

Scheme 10:



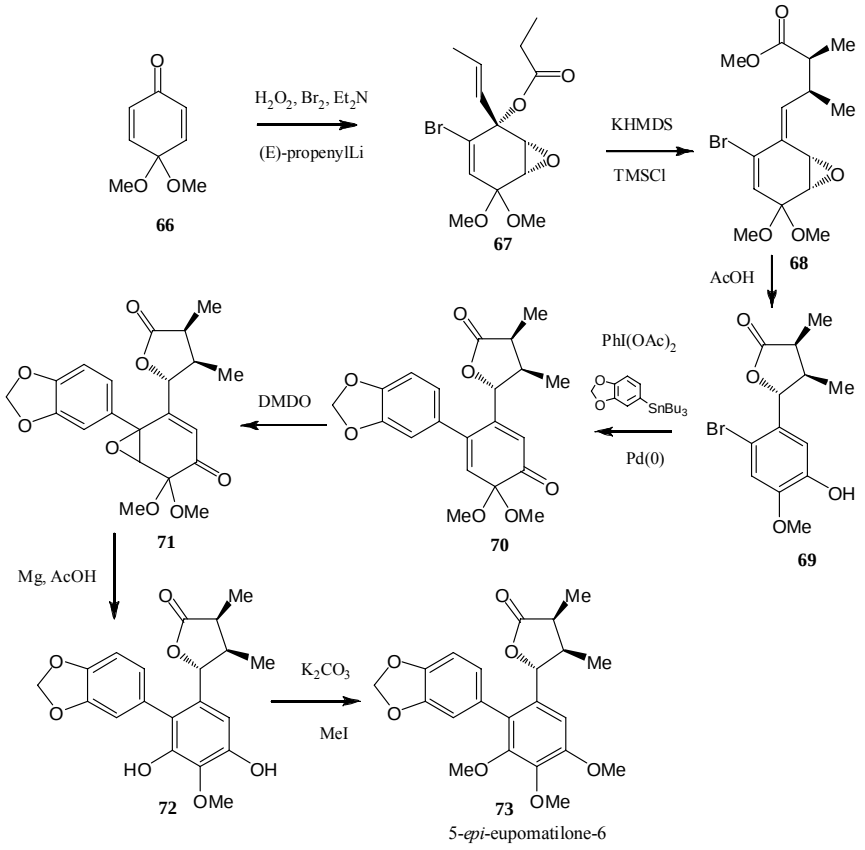
63 **64** **65**

Past Work

McIntosh's Approach:

McIntosh[46] *et al* reported the synthesis of 5-*epi*-eupomatilone-6 (**73**) *via* Ireland-Claisen rearrangement as the key step (scheme 11). The synthesis involved conversion of

Scheme 11:



66 **67** **68** **69** **70** **71** **72** **73**

5-*epi*-eupomatilone-6

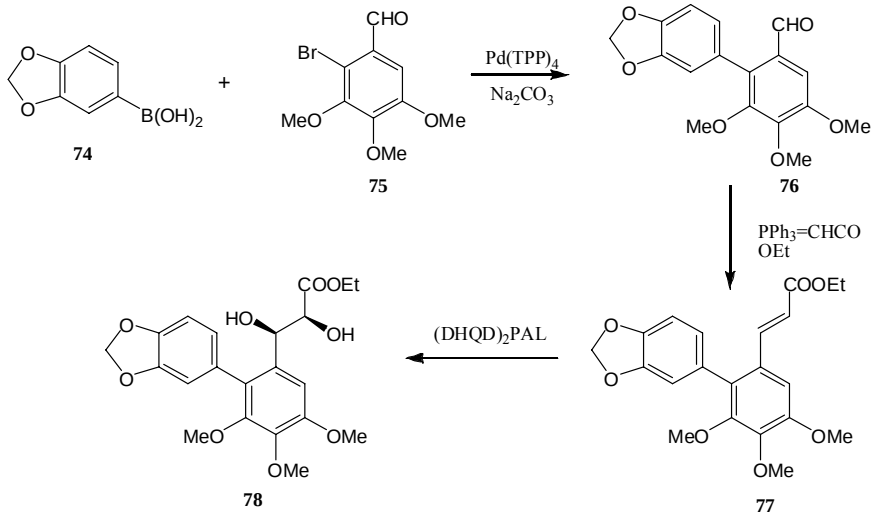
the *p*-quinone monoketal **66** to the bromo epoxide followed by the addition of (*E*)-propenyl lithium and *in situ* esterficiation to afford **67** as a single stereoisomer. **67** was converted to **68** by Ireland-Claisen rearrangement. SN2 lactonization and *insitu* aromatization by heating the acid **68** in AcOH afforded **69**. Oxidation of phenol **69** using $PhI(OAc)_2$ and Stille coupling with piperonyl tributylstannane gave **70**. Regioselective epoxidation of **70** using DMDO and reductive ring opening of the epoxide produced biaryl **72** which existed as non-separable 1:1 mixture of atropisomers. Finally, 5-*epi*-eupomatilone-6 (**73**) was obtained by methylation of the phenolic hydroxyl group.

Apart from a recent synthesis of (±)-5-*epi*-eupomatilone-6 (**73**), no report has yet appeared on the total synthesis of any of these natural products until our exploration in this direction. As part of our continuing interest in biaryl-containing natural products coupled with the structural peculiarity of eupomatilones, the first total synthesis of the putative structure of Eupomatilone-6 (**11**) was reported from our group.[11]

Our Approach:

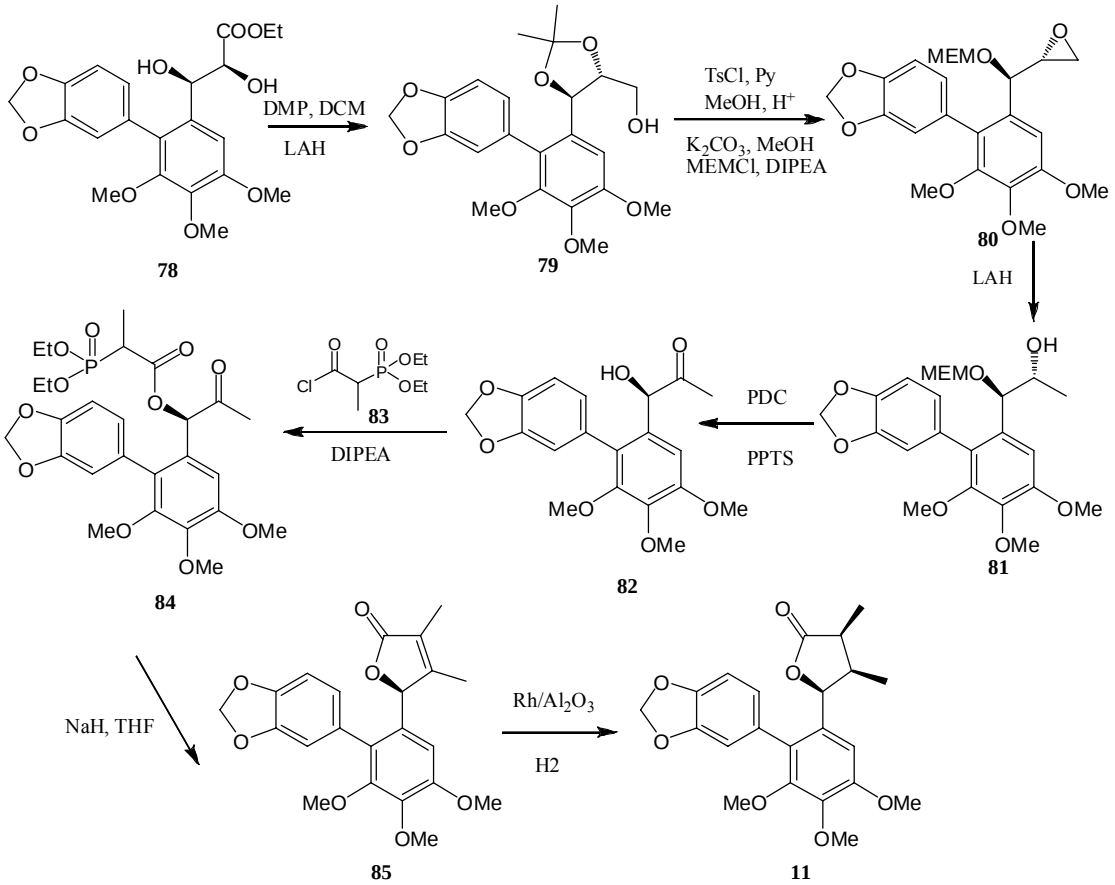
Our lab reported the synthesis of eupomatilone-6 (**11**), based on an intramolecular Horner-Wadsworth-Emmons reaction and found out that the final product did not match spectroscopically with with assigned structure of natural eupomatilone-6. Our synthesis starts with the Suzuki coupling reaction between **74** and **75** in the presence of Pd (0) to afford the biaryl aldehyde derivative **76**. Two carbon wittig homologation of **76** followed by asymmetric dihydroxylation gave the dihydroxy derivative **78** (scheme 12).

Scheme 12:



Protection of **78** as an isopropylidene derivative followed by reduction of the ester group with $LiAlH_4$ gave the alcohol **79**. Tosylation of primary hydroxyl group, deprotection of isopropylidene group, cyclization to the epoxide derivative, and protection of free hydroxyl group as the MEM ether, **79** was converted into **80**. Reductive ring opening of the epoxide ring with $LiAlH_4$ formed **81**. Oxidation of **81** with PDC gave the keto derivative, which was treated with PPTS in *t*BuOH to cleave the MEM group and afford the R-hydroxyketone derivative **82**. Compound **82** was reacted with the acid chloride **83** in the presence of DIPEA-CH_2Cl_2 to afford the phosphonate **84** (scheme 13). The intramolecular Horner-Wadsworth-Emmons reaction of **84** was carried out by using NaH in DMF. The resulting unsaturated-γ-lactone **85** was hydrogenated in the presence of Rh/Al_2O_3 in EtOAc at 60 psi to give **11.**

Scheme 13:



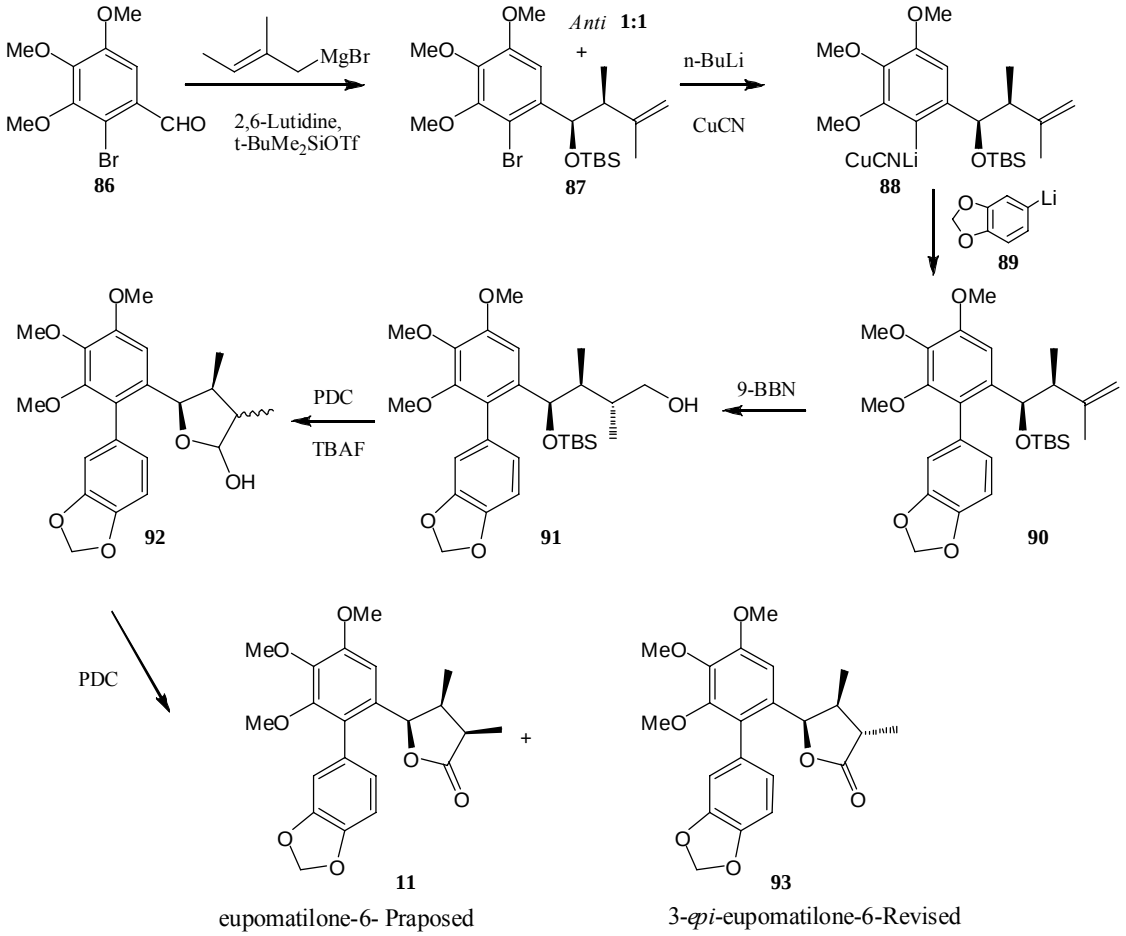
Interestingly, the ^1H NMR spectrum of synthetic **11** did not match that of either the natural product[10] or the 5-*epi* eupomatilone-6[46] (**73**) isomer, we have analyzed the spectral

data and came to a conclusion that the assigned relative stereochemistry of eupomatilone-6 needs revision. Eupomatilone-6's stereochemical assignments were still debated at the start of this project. We proposed two alternative formulations for eupomatilone-6. When our work is in progress Coleman *et al* reported the synthesis of eupomatilone-6 (**11**) and corrected the structure of eupomatilone-6.

Coleman's Approach:

Coleman[47] *et al* reported the structure of eupomatilone-6 as **93** (scheme 14). The synthetic strategy was based on a Lipshutz oxidative biarylcuprate cross coupling of **88** with **89** and a subsequent diastereocontrolled hydroboration/oxidation sequence on the alkene of **90** as a key steps. The synthesis involved the Cuprate coupling of the corresponding aryllithium reagents **88** and **89** afforded biphenyl **90**.

Scheme 14:



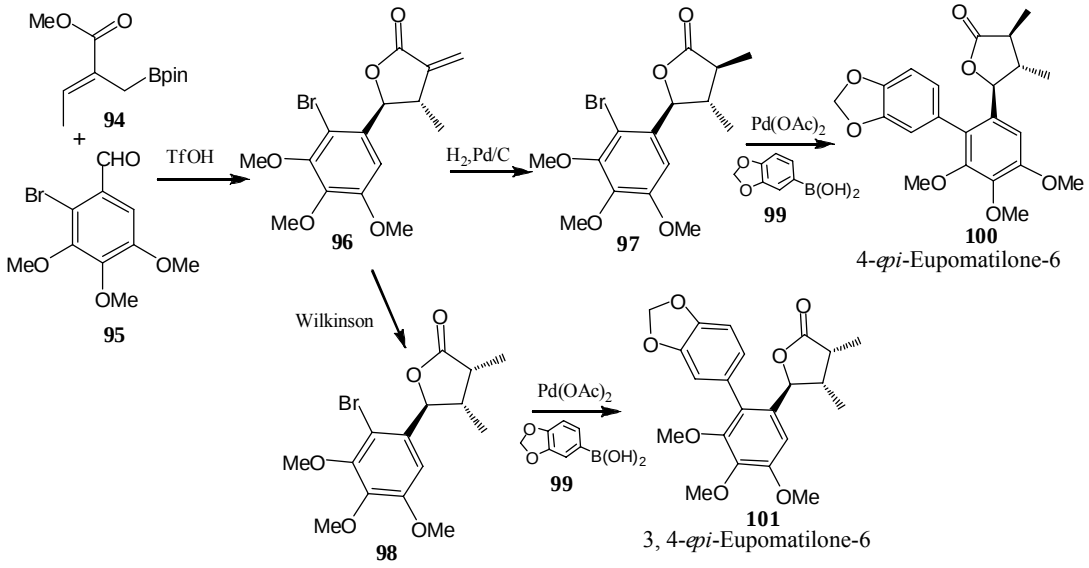
Hydroboration/oxidation of the alkene of **90** afforded the corresponding primary alcohol **91**. Oxidation of alcohol **91** with pyridinium dichromate afforded the corresponding aldehyde, which cyclized to lactol **92,** after fluoride-mediated deprotection of the silyl ether. Lactol to lactone oxidation afforded a 1:3 mixture of **11** and its epimer **93**, which could be separated.

We established the relative stereochemistry of the natural eupomatilone-6 and proposed the absolute configuration of naturally occurring eupomatilone-6. After our work Hall *et al* reported the all four diastereomers of eupomatilone-6.

Hall's Approach:

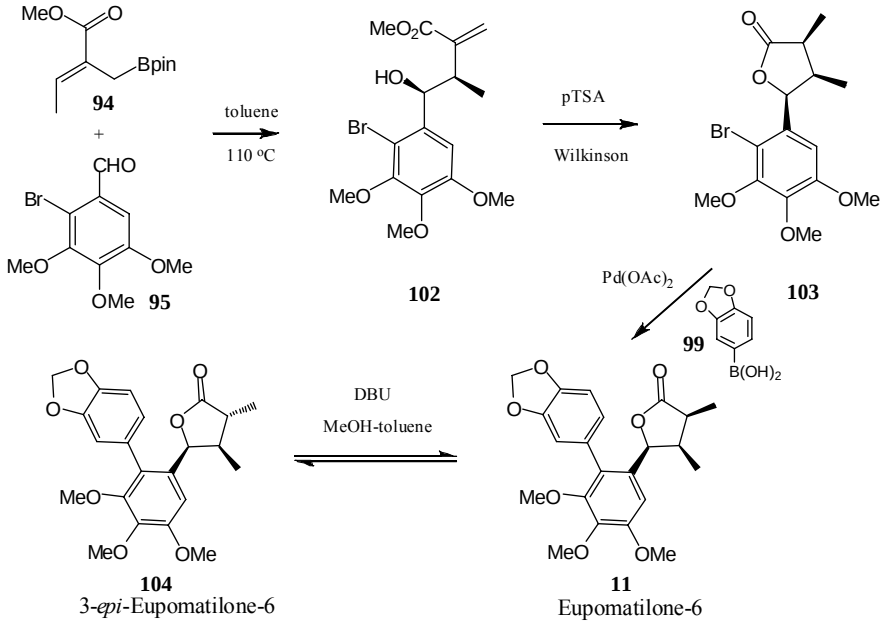
Hall[48] *et al* reported the synthesis of all four diastereomers of eupomatilone-6 (**11)**. The key step was triflic acid catalyzed addition of deactivated allylboronates to aldehydes (scheme 15). The allylation of aldehyde **95** with 2-alkoxycarbonyl allylboronate **94** leads to *R*-*exo*-methylene *γ*- lactones reminiscent of the lactone **96** unit of the eupomatilones. Heterogeneous hydrogenation led to **97** as the major diastereome; on the other hand, homogeneous hydrogenation conditions using Wilkinson's catalyst provided **98** as the major diastereomer. Both **97** and **98** were coupled with commercial boronic acid **99** under Buchwald's conditions to give 4-*epi*-Eupomatilone-6 **(100**) and 3, 4-*epi*-Eupomatilone-6 **(101)**.

Scheme 15:



To access the two other diastereomers (scheme 16), a thermal allylboration between **94** and **95**, proceeded without isomerization to give *syn*-hydroxy ester **102**. The ester was lactonized under mildly acidic conditions. The resulting lactone was hydrogenated to **103**, and then subjected to the Suzuki coupling with **99** to give Eupomatilone-6 (**11).** The isomerization of **11** with DBU in methanol, gave an equilibrium ratio of ca. 1:1 of the two separable epimers of 3-*epi*- Eupomatilone-6 (**104**).

Scheme 16:



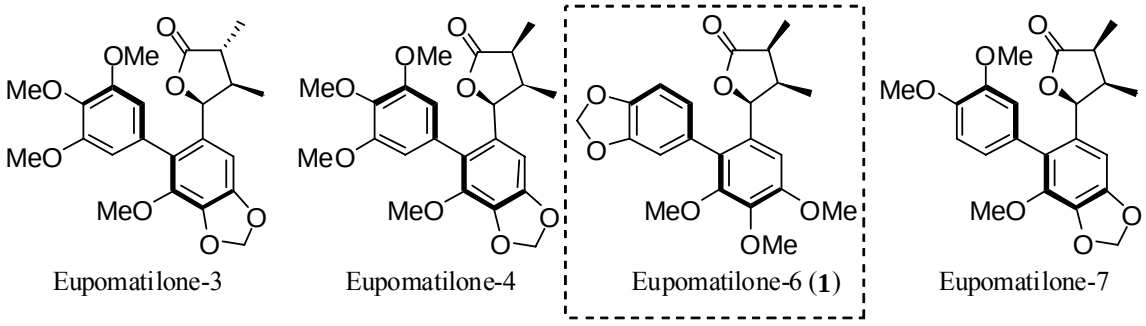
Present Work

Present Work

Lignans are a widely distributed class of dimeric phenyl propanoid derivatives, many of which have strong antimicrobial, antiviral or antifeedant activity and thus they possess a variety of pharmacological actions in humans, though the most interesting of these are subtle and not easily studied. At the ecological level, there is evidence that lignans play a role in plant-fungus, plant-plant and plant-insect interactions. At the molecular level, on the other hand, some are known to bind to the tubulin of microtubules to interrupt nucleotide transport and DNA synthesis and to be specific inhibitors of certain enzymes.

The Australian shrubs *Eupomatia bennettii* F.Muell is the source of a variety of lignans such as eupodienones, eupobennettin, bennettinone and seven closely related substances termed eupomatilones. Extensive spectroscopic studies have established that eupomatilones are degraded lignans, each having a biphenyl system with a γ-lactone ring attached to one of the aryl rings and some of them exhibit atropisomerism. Under ambient conditions, eupomatilone-6 exists as an equilibrating mixture of biaryl atropisomers (1:1 mixture). Eupomatilone-6 (**1**) is particular among the seven eupomatilones in that all the three substituents on the lactone moiety are *syn* to each other. The relative stereochemistry of eupomatilone-6 is depicted in figure 1.

FIGURE 1. Representative natural eupomatilones.[10]

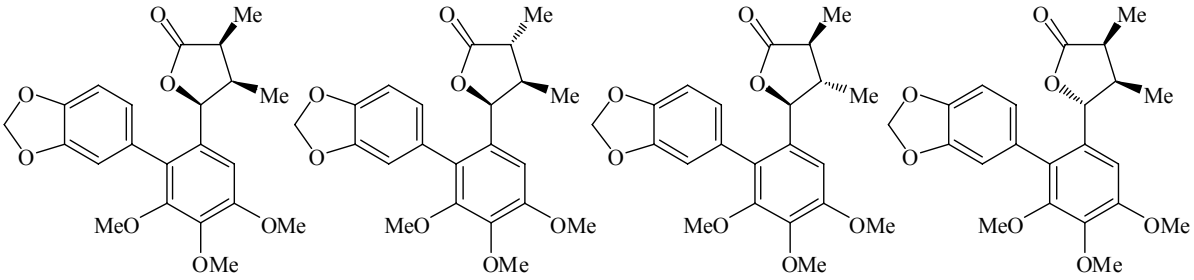


Synthesis has played a major role in natural product chemistry. Until the evolution of spectroscopic methods, independent synthesis of a compound was thought to give the ultimate proof for the proposed structure. The proponents of X-ray crystallography and

spectroscopy have often claimed that with the advent of these powerful techniques, the need for synthesis abolished. Even a quick look at the recent literature shows, however, that no matter how sophisticate are the instrumental methods used, there is still the ambiguity of experimental error and even today structures elucidated through instrumental means are revised as a result of synthesis. This is increasingly true as the level of complexity of compounds increases.

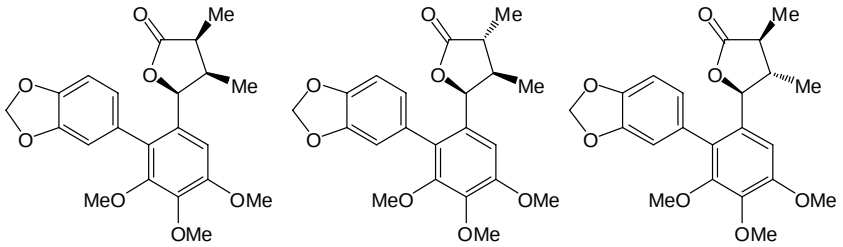
The structural diversity compared to other eupomatilones prompted us to attempt the synthesis of eupomatilone-6 (**1**). First synthesis of eupomatilone-6 had been reported from our group.[11] As the physical data of our synthetic eupomatilone-6 with the proposed stereochemistry (specific rotation and ^{1}H & ^{13}C NMR spectral data) is not in agreement with the reported data, the spectral data of available eupomatilones analyzed along with the synthetic eupomatilones and came to a conclusion that the assigned relative stereochemistry of eupomatilone-6 needs revision. Having three contiguous stereogenic centers in the lactone moiety, there exist four possible diastereomers, namely all-*cis* (**1**), 3-*epi*-**1** (*trans-cis*) (**2**), 4-*epi*-**1** (*cis-trans*) (**3**), and 3,4-*epi*-**1** (all-*trans*) (**4**) as shown in figure 2. Due to difficult configuration analysis of five-membered rings, eupomatilone-6's stereochemical assignments were still debated at the start of this project.

Figure-2: All possible diastereomers of eupomatilone-6.



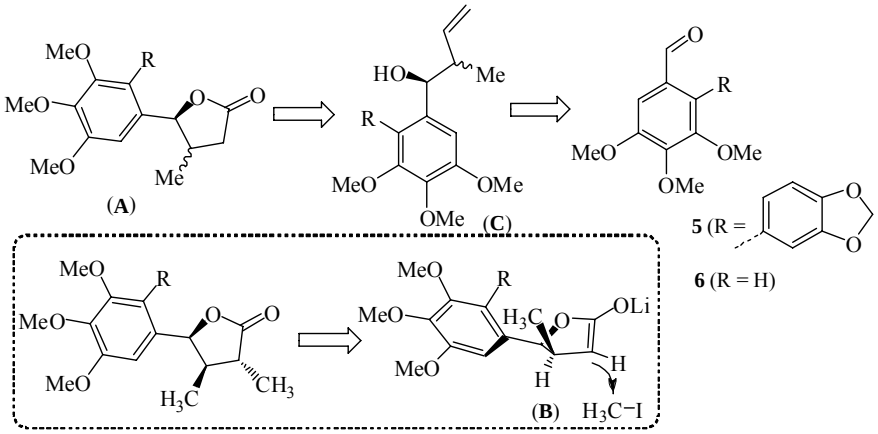
Based upon these NMR analyses of synthetic eupomatilones **1** and **4**, two alternative formulations for eupomatilone-6 (**2** and **3**) were recognized. We proposed the revised structure as either $3\alpha,4\beta,5\beta$-configuration (**2**) or $3\beta,4\alpha,5\beta$-configuration (**3**). The relative stereochemistry of eupomatilone-6 is depicted in figure 3.

FIGURE 3. Alternative structures suggested for the eupomatilone-6.



The important stereochemical feature of the two identified alternative structures for eupomatilone-6 is the 3,4-*trans* stereochemistry of the butyrolactone unit. Keeping this in mind we devised a retrosynthetic strategy envisaging (figure 4) a stereoselective *anti*-methylation at C-3 of the 4,5-disubstituted γ-butyrolactones (**B**) to furnish 3,4-*trans*-4,5-*cis* and 3,4-*trans*-4,5-*trans*-diastereomeric products.[49,50] The intended synthesis of both *cis*- and *trans*-4,5-disubstited- γ-butyrolactones[51] (**A**) respectively from the *syn*- and *anti*-homoallylic alcohols (**C**) was a straightforward proposition. The crotylation of the biaryl aldehyde **5** under Barbier type reaction[52] conditions would give rise to both the requisite *syn*- and *anti*-homoallylic alcohols. Prior to continue with original biaryl aldehyde **5**, we intended to use the simple 3,4,5-trimethoxybenzaldehyde **6** as a model system to execute the proposed synthetic transformations and correlate the spectral features and relative stereochemistry of the products on the way. This was mainly because of the expected complex NMR spectra with products derived from **5** as it exist as a mixture of atropisomers in solution which can be observed on NMR time scale.

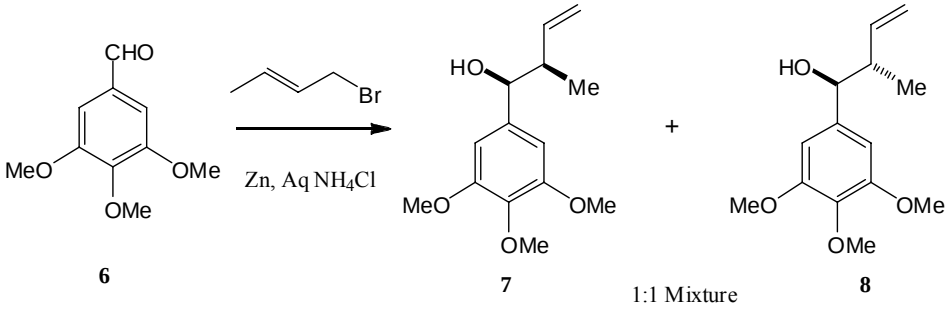
FIGURE 4. *Retrosynthetic strategy and intended C3-anti methylation*



Model study:

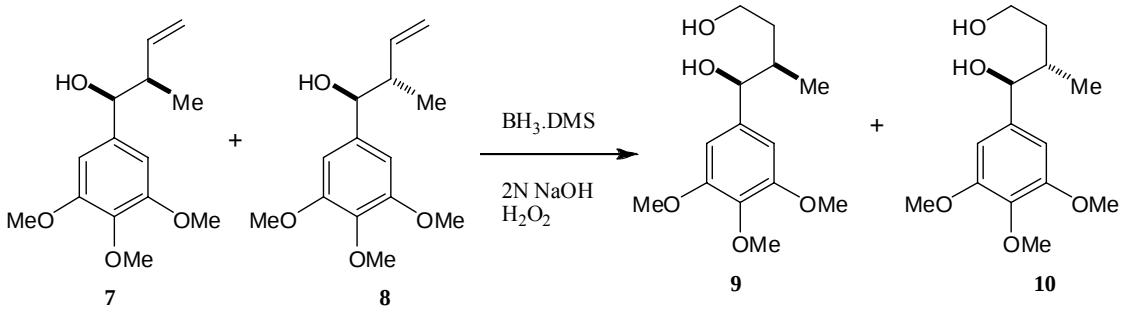
The model study began with the Barbier reaction of trimethoxy benzaldehyde **6** with crotyl bromide in the presence of activated zinc and aqueous NH_4Cl in THF to get an inseparable 1:1 mixture of *syn* (**7)** and *anti* (**8)** homoallylic alcohols (scheme 1). In the ^1H NMR spectrum the benzylic-H of *syn*- and *anti*- isomers was well separated and resonates at δ 4.54 (J = 5.4 Hz) and δ 4.28 (J = 7.9 Hz) ppm respectively for **7** and **8**.

Scheme 1:



Since our attempts to separate the homoallylic alcohols **7/8** by column chromatography failed, we decided to proceed further with the mixture. Hydroboration, and oxidation of the olefin (scheme 2) afforded an inseparable mixture of *syn* (**9**) and *anti* (**10**) diols, whose structure was conformed by the absence of the olefinic protons in the ^1H NMR as well as in ^{13}C NMR spectrum.

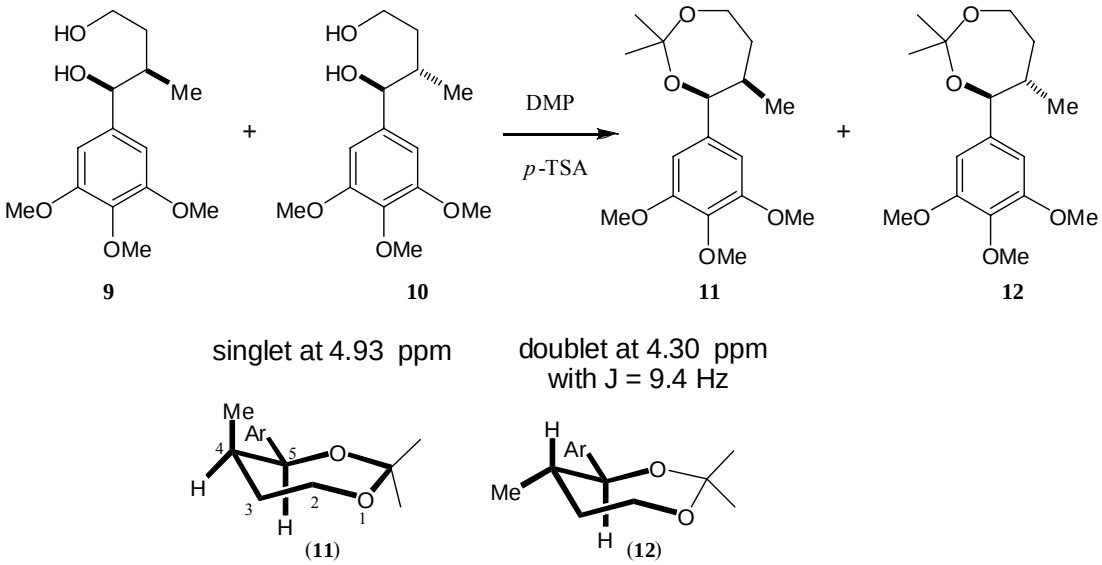
Scheme 2:



At this stage, in order to separate the mixture as well as to characterize their stereochemistry, the diols mixture **9**/**10** was subjected for acetonide protection employing

2,2 dimethoxy propane, catalytic *p*-TSA in DCM. Gratifyingly, the *syn* (**11**) and *anti* (**12**) (Scheme 3) acetonides were easily separable by flash column chromatography. The ^{1}H NMR spectral analysis, particularly the characteristic chemical shifts and coupling constants of benzylic proton at C-5, were useful in assigning the relative stereochemistries as depicted in structures **11** and **12**. For example, **11** revealed signal of benzylic proton as singlet at 4.90; whereas, it appeared as doublet at 4.26 ppm with J = 9.4 Hz in case of compound **12**.

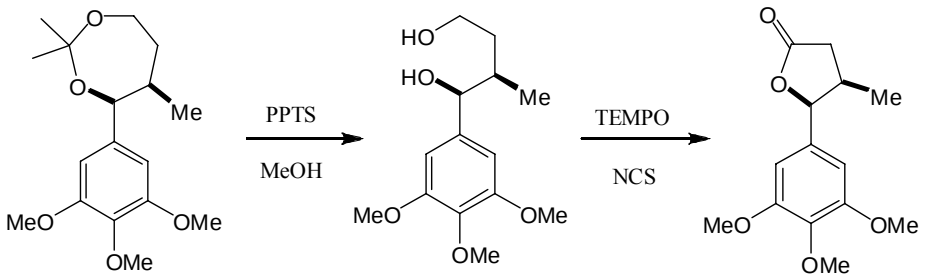
Scheme 3:



After establishing the relative stereochemistries of the acetonides **11** and **12**, our next task was to construct the key lactone moieties (**A**). Thus the isopropylidene group of the *syn* acetonide **11** was deprotected with PPTS in MeOH to get the pure diol **9**. The crude product was oxidized to get the lactone **13** in the presence of TEMPO, NCS in DCM (scheme 4).[53] It is known that with this reagents system primary hydroxyl group will be selectively oxidized to acid and subsequent under go lactonization. In the ^{1}H NMR spectrum of compound **13**, benzylic-H signal was resonated much down field (δ 5.52 ppm) as a doublet indicating the formation of lactone, and all other protons resonated at expected positions. The assigned structure for compound **13** was further confirmed by its ^{13}C NMR

where the carbonyl group resonates at δ 172.2 ppm, and a characteristic lactone carbonyl stretching signal at 1775 cm^{-1} in the IR spectrum.

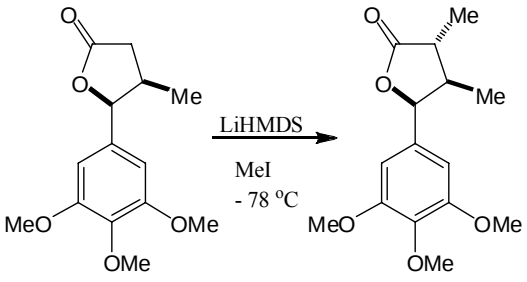
Scheme 4:



11 **9** **13**

After having the 4,5-*syn*-lactone **13** in our hands, we next investigated the proposed stereoselective 3,4-*anti*-methylation of the lactone **13**. After screening several reagents, we observed that the diastereoselectivity of methylation at C-3 was highest with LiHMDS-MeI and afforded **14** in excellent yields. The structure of compound **14** was determined by ^{1}H NMR spectrum where the newly formed methyl group resonates at δ 0.78 ppm as doublet and all other signals were in well agreement with the assigned structure. The coupling constant of the C-3 and C-4 protons clearly indicates that they are *trans* to each other. The methylation exclusively occurred *anti* to the existing methyl. The structure was further confirmed by ^{13}C NMR spectrum.

Scheme 5:

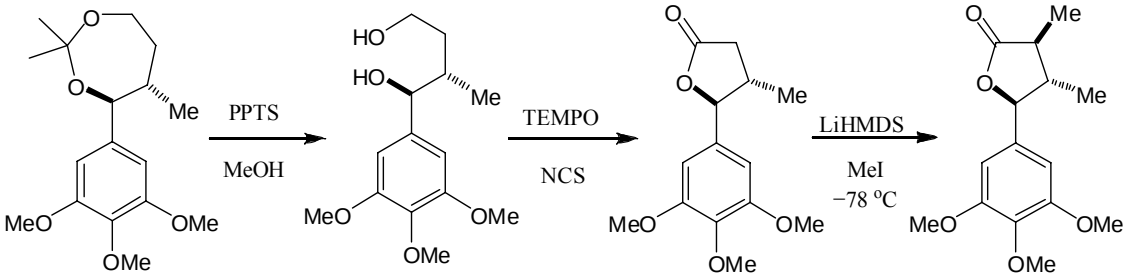


13 **14**

In similar lines, the acetonide **12** was subjected to the same set of reaction conditions to get the lactone **15**, which upon methylation gave the *trans*, *trans* lactone **16**

(scheme 6). The ^{1}H NMR and ^{13}C NMR were in complete agreement with the assigned structures (the benzylic proton for the lactone at δ 4.83 ppm as doublet and the methyl group at δ 0.97 ppm as doublet for the compound **16**), the coupling constant further indicates both the methyl groups are *trans* to each other.

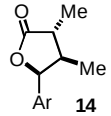
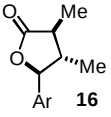
Scheme 6:



12 **10** **15** **16**

After having the spectral data of both *trans*, *cis* (**14**) and *trans*, *trans* (**16**) lactones, we compared the spectral data of these model lactones with the data of eupomatilone- 6 (Table 1). To our surprise, the data of **14** has maximum overlap with the natural eupomatilone 6 indicating the possible stereochemistry of eupomatilone- 6 as **2** (Figure 3).

Table 1:

	H - 3	H - 4	H - 5	Me - 3	Me - 4
Eupomatilone 6 **(1)**	2.36, 2.37 multiplet $J_{3,Me}$=7.0 Hz $J_{3,4}$=5.3 Hz	2.02 multiplet $J_{4,Me}$=$J_{3,4}$=7.0Hz $J_{4,5}$= 5.3 Hz 1.97 multiplet	5.54 d $J_{4,5}$=7.0 Hz 5.65 d	1.20 d $J_{3,Me}$=7.0 Hz 1.19 d	0.70 d $J_{4,Me}$=7.0 Hz 0.73 d
 **14** *Trans,Cis*	2.25-2.33 multiplet	1.97-2.03 multiplet	5.42 d $J_{4,5}$=7.2 Hz	1.10 s	0.74 d $J_{4,Me}$=6.8 Hz
 **16** *Trans,Trans*	2.40 2.48 multipltet	2.28 2.36 multiplet	4.79 d $J_{4,5}$=10.2 Hz	1.26 d $J_{3,Me}$=7.0 Hz	0.98 d $J_{4,Me}$=6.6 Hz

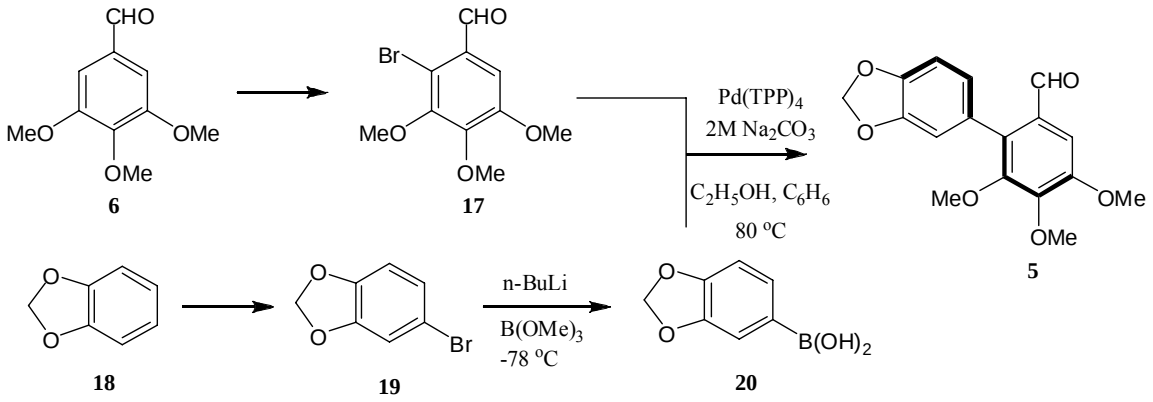
After establishing the feasibility of proposed strategy by completing the synthesis of model lactones with complete stereochemical control for the C-3 methyl introduction, we

next proceeded further for the synthesis of two possible diastereomers of eupomatilone 6 employing the aldehyde **5**.

Synthesis of Racemic Eupomatilone-6 (2 and 3).

The synthetic endeavor began with the construction of biaryl aldehyde **5** according to the procedures established in this lab. Thus bromination of 3,4,5-trimethoxybenzaldehyde (**6)** followed by Suzuki coupling of resulting bromoaldehyde **17** with easily available boronic acid **20** afforded the key biaryl aldehyde **5** as mixture of atropisomers (scheme 7).

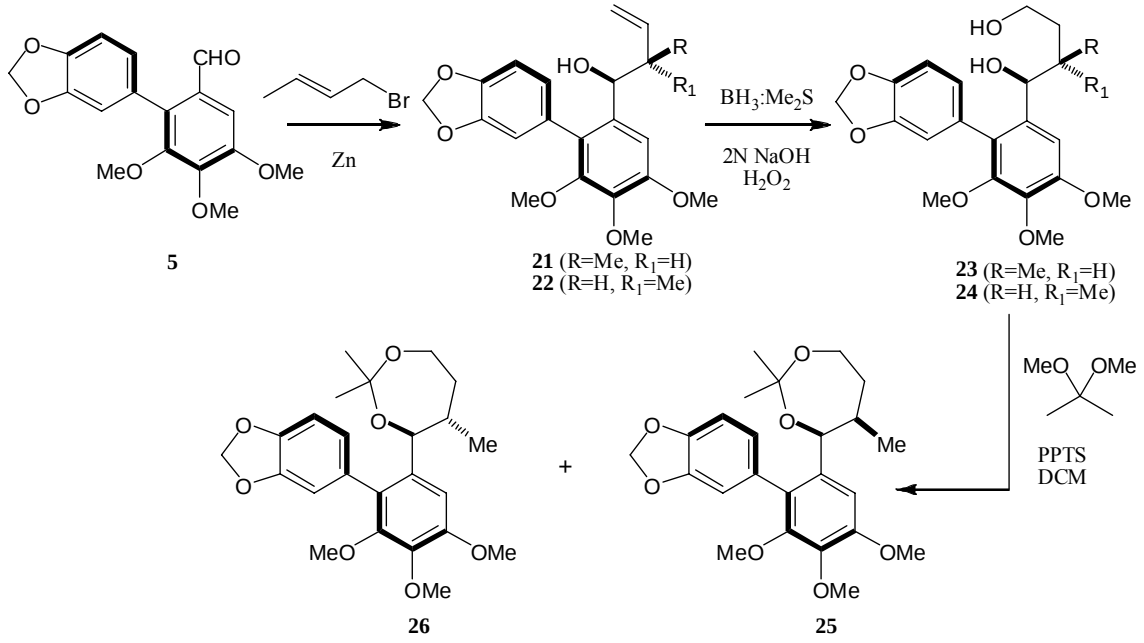
Scheme 7:



In the ^{1}H NMR spectrum of **5**, the methylene protons of dioxole ring resonated as a singlet at δ 6.0 while the aldehyde proton appeared at δ 9.67. All the other resonances were in agreement with the assigned structure. Having **5** in hand, the next task was crotylation. Thus, treatment of **5** with crotyl bromide in the presence of zinc and ammonium chloride gave an inseparable 1:1 mixture of *syn*- and *anti*-homoallylic alcohols **21** and **22** (scheme 8). The ^{1}H NMR spectrum is well agreement with the assigned structure. For example the methyl groups resonate at 0.70, 0.72 ppm and 0.94, 0.96 ppm as doublets (of both atropisomers, *J*= 6.8 Hz). The benzylic proton was observed at 4.24, 4.26 ppm as doublets (*J* = 8.5 Hz) and at 4.49, 4.53 ppm as doublets (*J* = 6.2 Hz) of both atropisomers. Since the efforts to separate the mixture by column chromatography failed, as established earlier we proceeded further with the mixture, to make acetonide derivatives thus, hydroboration-

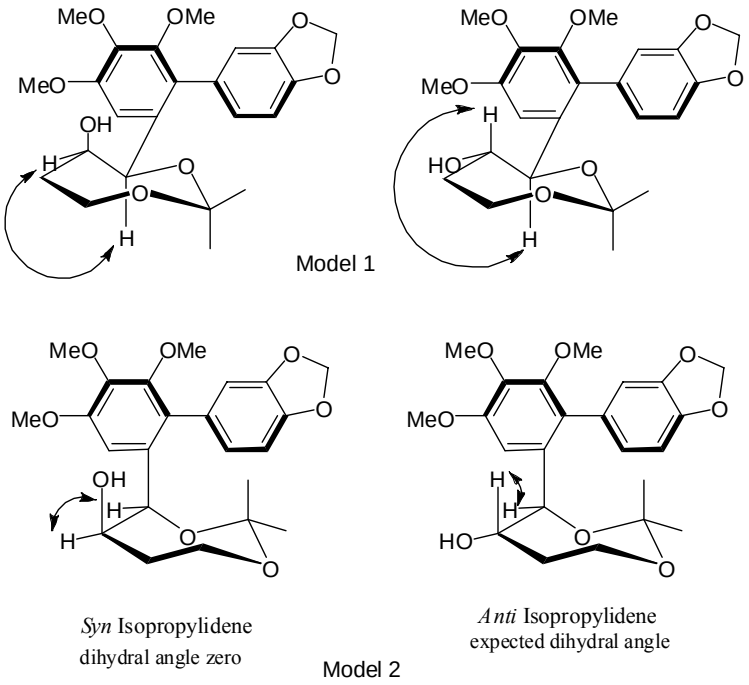
oxidation of **21**/**22** by using Me_2S:BH_3 and H_2O_2 afforded diols **23** and **24** as inseparable mixture. The crude ^{1}H NMR spectra of the mixture **23** and **24** clearly showed the disappearance of olefinic protons as well as the new methylene groups in the DEPT spectrum. As established in model studies, we protected the diols mixture as their acetonides by employing 2,2-dimethoxy propane and PPTS in dichloromethane and the resulting acetonides **25** and **26** are separable by silica gel chromatography and characterized with the help of NMR spectroscopy.

Scheme 8:



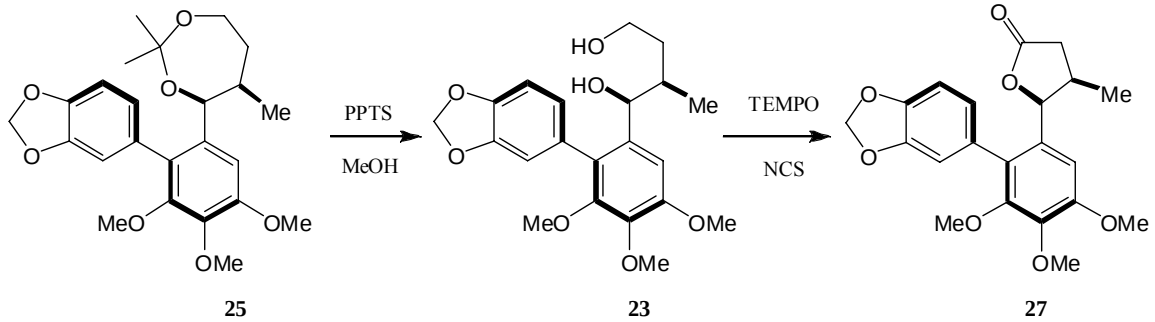
The ^{1}H NMR spectral analysis, particularly the characteristic chemicals shifts and coupling constants of benzylic proton at C-5, were useful in assigning the relative stereochemistries as depicted in structures **25** and **26**. For example, **25** revealed signals of benzylic proton of the equilibrating atropisomers as singlets at 4.77 and 4.87 ppm; whereas, it appeared as doublets at 4.33 and 4.35 ppm with J = 9.4 Hz in case of compound **26** (figure 5).

Figure 5: possible conformers for the isopropylidene derivatives.



Having both the ketals **25** and **26** in hand, next stage in our synthetic plan was to convert them to the proposed lactones (**A**) via selective oxidation of the primary alcohol (scheme 9) and subsequent lactone ring formation. Thus, the hydrolysis *syn*-acetonide **25** by PPTS in MeOH and subsequent selective oxidation followed by ring closer of resulting diol **23** with NCS-TEMPO[53,47] gave the corresponding 4,5-*syn*-γ-butyrolactone **27** in good yield.

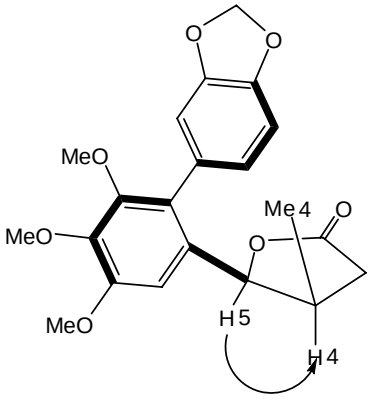
Scheme 9:



In the ^{1}H NMR spectrum of compound **27**, the methylene adjacent to carbonyl group resonates at δ 2.20-2.30 ppm as multiplet. The benzylic proton was observed at δ

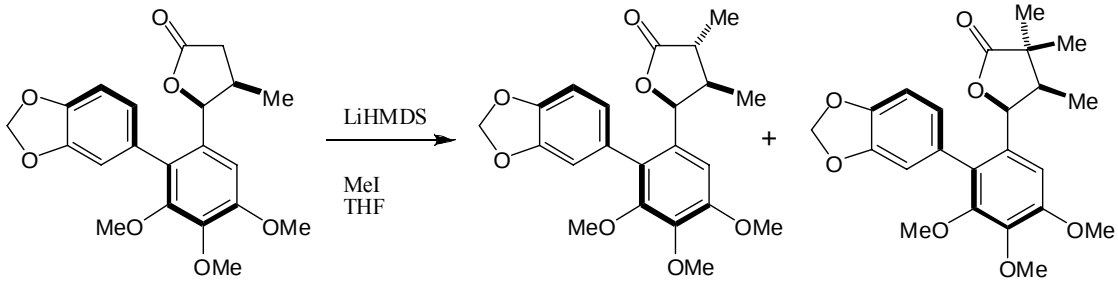
5.42 and 5.51 ppm as doublets (of both atropoisomers, *J*= 5.6 Hz). Further the ^{13}C NMR clearly showed the carbonyl group at δ 176.1 ppm. Only one methylene group as compared to diol in the DEPT spectra also supports the proposed structure. IR spectrum showed absorption at 1776 cm^{-1}, characteristic of lactone. The assigned *syn* configuration to **27** was substantiated by NOE experiments, where a strong NOE was found between H-4 and H-5 (figure 6). After confirming the configuration of the lactone **27**, we proceeded to accomplish the synthesis of *trans*, *cis*-diastereomer **2** of eupomatilone-6.

Figure 6: nOe study on **27**.



As it was established in the model studies, the intended stereoselective methylation of lactone **27** (scheme 10) was attempted with LiHMDS in THF at - 78 °C and quenching with methyl iodide gave compound **2,** along with some dimethylated compound **28**.

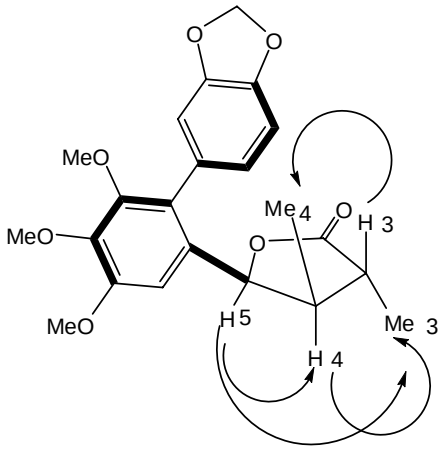
Scheme 10:



27 **2** **28**

The spectral data of **2** was in complete agreement with the data published[10,47] for the naturally occurring eupomatilone-6. The structure of **2** was extensively characterized by its ^{1}H NMR, ^{13}C NMR spectra, elemental analysis and nOe experiments. In the ^{1}H NMR spectrum the newly formed methyl group resonates at δ 1.19, 1.20 ppm as doublets (*J*= 7.4 HZ) of both the atropisomers. The benzylic proton resonates at δ 5.53, 5.64 ppm as doublets (*J*= 6.9 HZ). Disappearance of the methylene group in the DEPT spectra clearly indicates the assigned structure. The predominate diastereoselectivity was supported by nOe studies. The *α*-configuration at C-3 was supported by NOESY spectrum, where a strong nOe was found between H-3 and Me-4, as well as H-4 and Me-3 indicating an *anti*-relationship between the two-methyl groups thus confirming its assigned structure (figure 7).

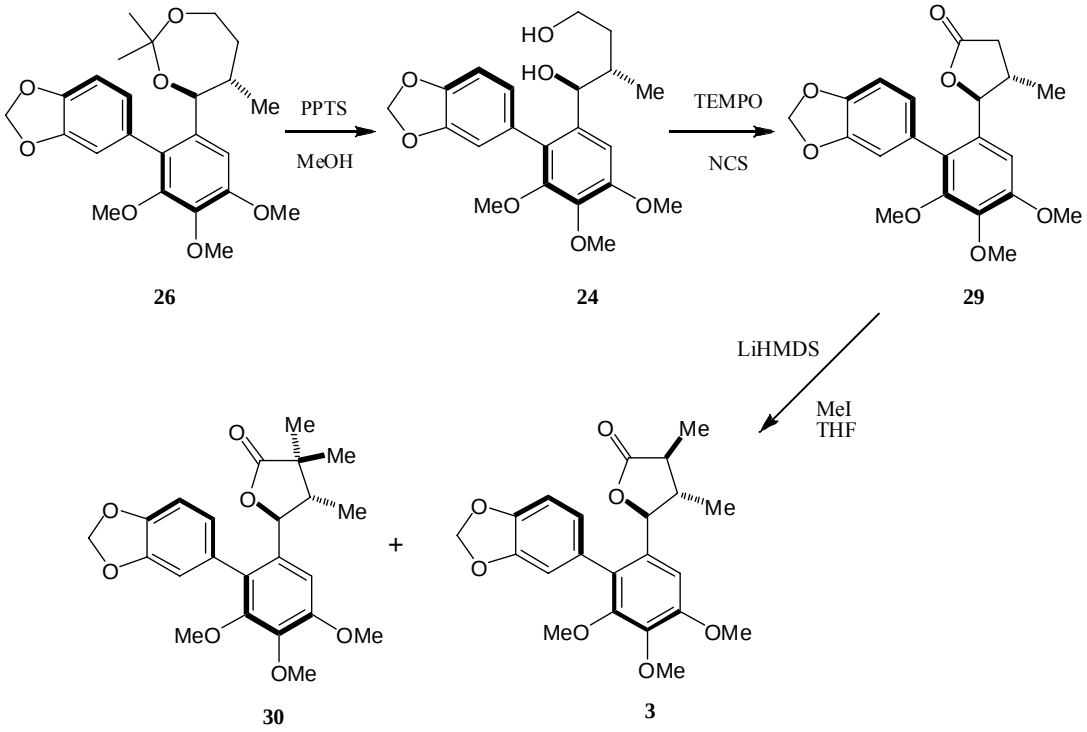
Figure 7: Important through space interactions observed in the NOSEY spectrum of compound **2** (eupomatilone-6).



The dimethylated product **28** was confirmed by the presence of additional methyl group adjacent to carbonyl in the ^{1}H NMR as well as ^{13}C NMR spectra. The singlets at δ 1.11, 1.12, 1.13, 1.14 ppm (of both the atropisomers) in the ^{1}H NMR confirmed the structure.

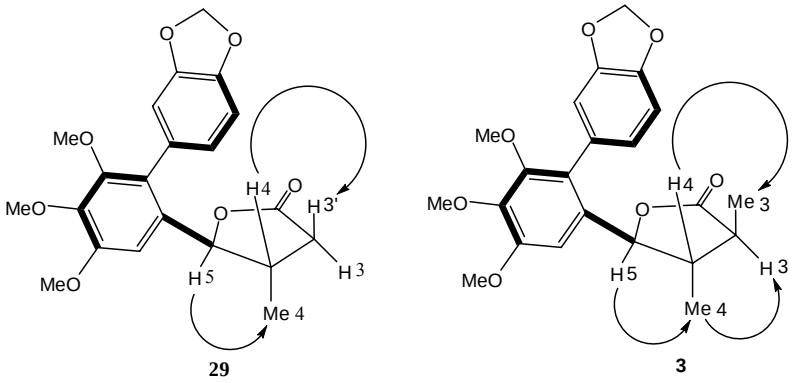
After the successful synthesis of the possible diastereomer **2**, which turned out to be eupomatilone-6, we proceeded further for remaining *trans*,*trans*-diasteromer **3**, (Scheme 11) to prove the structure of eupomatilone-6 with out any ambiguity.

Scheme 11:



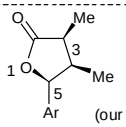
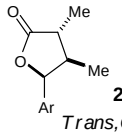
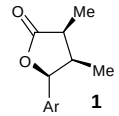
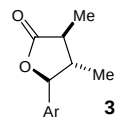
Compound **26** was subjected to acid hydrolysis in the presence of PPTS and methanol, to procure diol **24** whose ^{1}H NMR spectrum (absence of isopropylidene group) is in full agreement with the assigned structure. Both the benzylic protons resonate at δ 4.87, 4.92 ppm as doublets (J = 8.54 Hz). The diol **24** was subjected to oxidation followed by lactone formation afforded compound **29**. In the ^{1}H NMR spectra benzylic proton resonates at δ 4.27, 4.29 ppm as doublets (J = 7.6 Hz). Lactone further confirmed by ^{13}C NMR where the carbonyl group resonates at δ 176.2, 176.3 ppm of both atropisomers. IR spectrum also supports the assigned structure. The relative stereochemistry of the methyl and oxygen groups further confirmed by nOe study (figure 8).

Figure 8: nOe study on **29** and **3**.



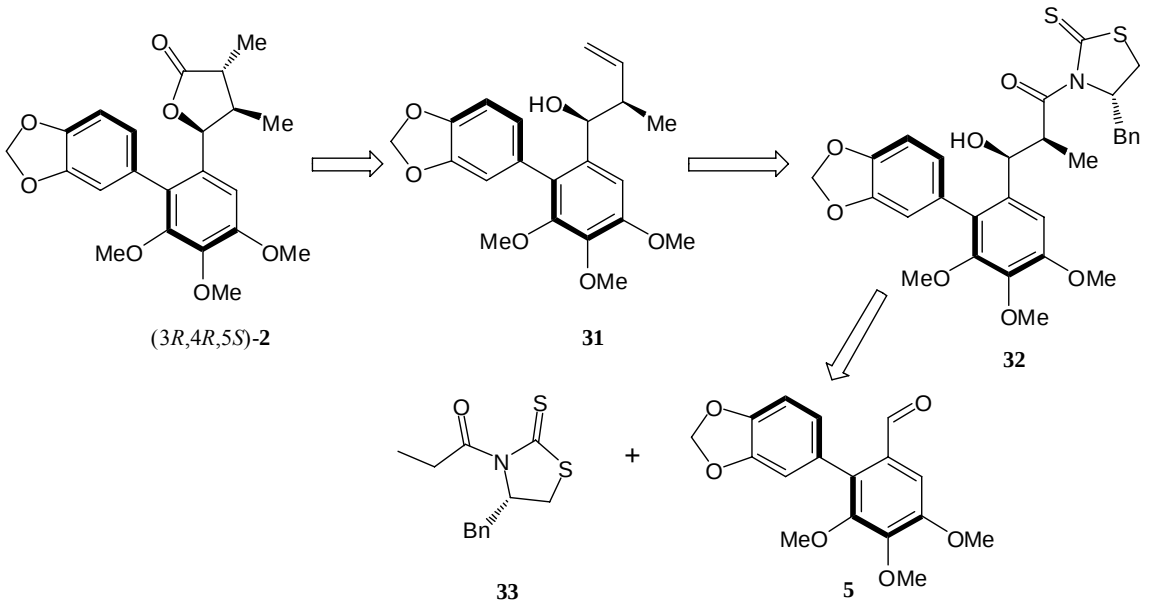
Compound **29** was methylated according to established conditions to afford compound **3** along with small amount of dimethylated compound **30**. The spectral data of compound **3** was entirely different (table 2) from the natural eupomatilone-6 thus establishing the assigned relative stereochemistry (as in compound **2**) beyond the doubt. In the ^{1}H NMR spectrum the newly formed methyl group resonates at δ 1.23, 1.26 ppm as singlets of both the attropoisomers. The benzylic protons resonates at δ 4.78, 4.80 ppm as doublets (J= 9.46 HZ). Further dimethylated product **30** was confirmed by the presence of additional methyl group adjacent to carbonyl in the ^{1}H NMR as singlets at δ 0.96, 0.97 and 1.22 ppm (of both the attropoisomers). NOE clearly showed that both of the methyl groups are *anti* to each other (figure 8).

Table 2:

	H - 3	H - 4	H - 5	Me - 3	Me - 4
 (our lab) *Cis,Cis*	2.74 quintet $J_{3,Me}$=$J_{3,4}$ =7.2 Hz	2.20 multiplet	5.32, 5.41 doublet $J_{4,5}$=5.0 Hz	1.13 doublet $J_{3,Me}$=7.2 Hz	0.54, 0.56 doublet $J_{4,Me}$=7.2 Hz
 **2** *Trans,Cis*	2.37 sextet J=7.2 Hz	1.93-2.04 multiplet	5.53,5.64 doublet $J_{4,5}$=6.9 Hz	1.19, 1.20 doublet $J_{3,Me}$=7.4 Hz	0.70, 0.73 doublet $J_{4,Me}$=7.1 Hz
 **1** Eupomatilone 6	2.36, 2.37 multiplet $J_{3,Me}$=7.0 Hz $J_{3,4}$=5.3 Hz	2.02, 1.97 multiplet $J_{4,Me}$=$J_{3,4}$=7.0Hz $J_{4,5}$= 5.2 Hz	5.54,5.65 doublet $J_{4,5}$=7.0 Hz	1.19, 1.20 doublet $J_{3,Me}$=7.0 Hz	0.70, 0.73 doublet $J_{4,Me}$=7.0 Hz
 **3** *Trans,Trans*	2.08-2.24 multiplet	1.93-2.06 multiplet	4.78, 4.80 doublet $J_{4,5}$=9.46 Hz	1.23, 1.26 singlet	0.87, 0.89 doublet $J_{4,Me}$=6.0 Hz

After successfully establishing the relative stereochemistry of racemic eupomatilone-6, we felt the need to design, based on our new findings, the asymmetric synthetic protocol which would establish the absolute configuration of eupomatilone-6. It is pertinent to mention that eupomatilone-6 inherently exists in nature, as a mixture of atropisomers. Our asymmetric synthetic plan is based on the retro synthetic analysis as depicted in scheme 12.

Scheme 12:



The key intermediate of our asymmetric synthesis is the construction of the homoallyllic alcohol **31** with the requisite stereochemistry by aldol protocol consisting the chiral thiazolidinethione **33**.

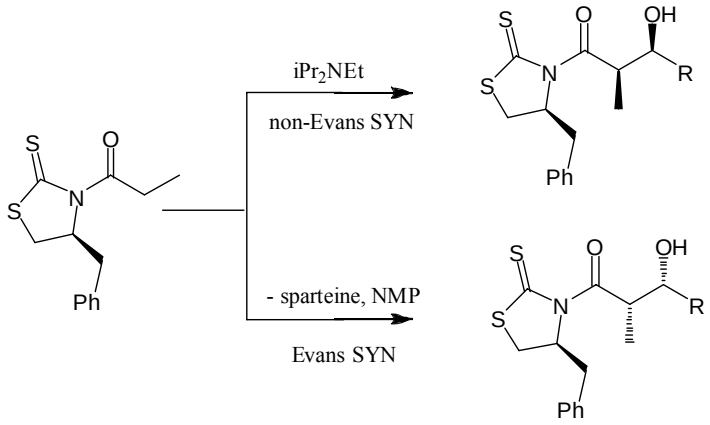
<u>A short account of Thiazolidinethione Methodology.</u>

The aldol reaction is arguably one of the most significant carbon-carbon bond forming reactions available to synthetic organic chemists.[54] *This is a reliable reaction, which can be applied to a broad range of substrates for the construction of new carbon-carbon bonds in a regio-, diastereo-, and enantioselective manner. Indeed, with the invention of the Evans chiral oxazolidinone* [55] *the aldol reaction became one of the first reactions for which a truly reliable and highly selective asymmetric variant was known. This advance,*

along with the discovery of the Sharpless asymmetric epoxidation,[56] ushered in a new era of asymmetric synthesis. Since the initial report by Evans, many other reports of chiral metal enolate based asymmetric and catalytic asymmetric aldol reactions have appeared, but the Evans method remains the benchmark system for the synthesis of syn-propionate aldol constructs.

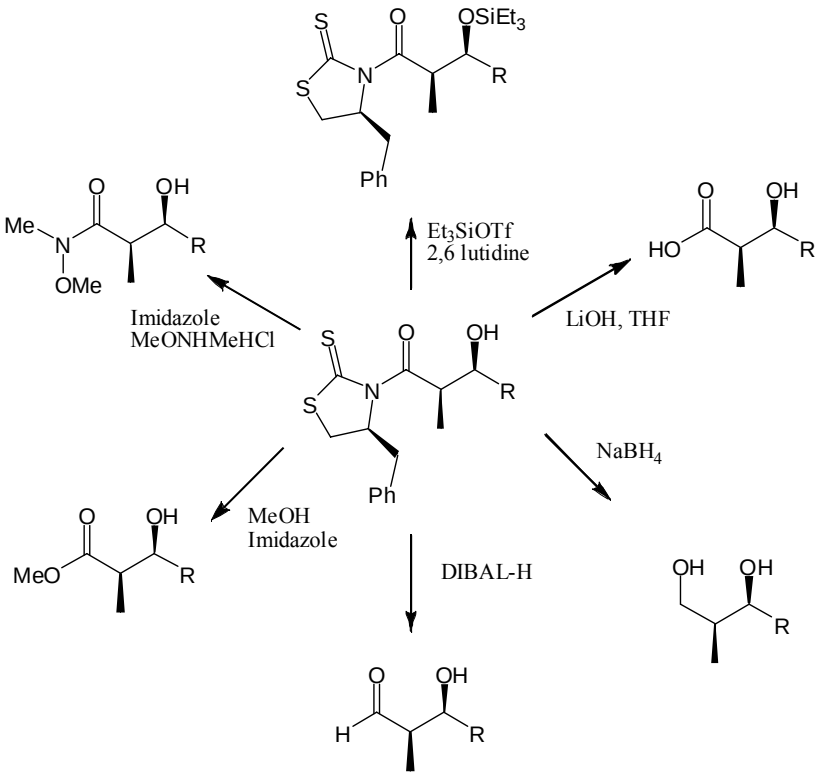
Crimmins et al, developed a highly efficient chiral auxiliary-based propionate aldol methodology.[57] Based on acylated thiazolidinethiones, the technology contains several distinct advantages over other propionate aldol approaches. Unlike Evans' chiral oxazolidinone boron-mediated aldol approach, the reagents employed are relatively inexpensive. Furthermore, both Evans syn and non-Evans syn aldol products can be accessed from the same antipode of the auxiliary simply by changing the amount and type of base employed. Thus, the inexpensive natural L-amino acids can be employed to obtain either desired stereochemical outcome of the aldol reaction (figure 9).

Figure 9:



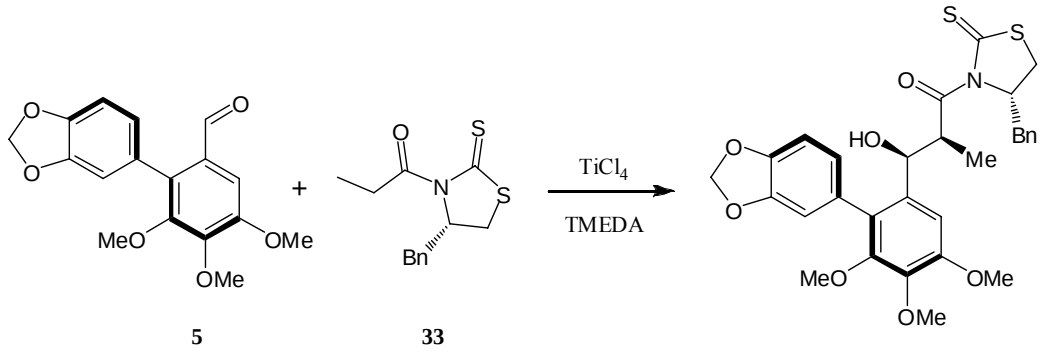
One of the desirable qualities of the thiazolidinethione auxiliary is the ease in which it can be converted into a variety of products. The highly enhanced leaving group ability of the thiazolidinethione allows for the use of milder conditions in the known transformations of oxazolidinones as well as additional reaction pathways. Finally, the most important of these additional transformations is the ability to reductively cleave the auxiliary directly to the aldehyde in a single step with DIBAL, a reaction which is not possible with oxazolidinones and oxazolidinethiones (figure 10).

Figure 10:



Our asymmetric synthesis began with aldol condensation of *N*-propionyl-thiazolidinethione with aldehyde **5**. Gratingly, *N*-propionyl-thiazolidinethione **33** when reacted with $TiCl_4$ to generate the titanium enolate followed by addition of the biarylaldehyde **5** gave aldol adduct **32** (scheme 12) exclusively. The crude product was purified by flash chromatography, homogeneous by TLC and NMR. Diastereomeric ratios were determined by ^1H NMR (>98:2 indicates that the minor isomer could not be detected by NMR). The benzylic proton resonates at δ 5.53 and 5.64 ppm as doublets (for both the atropisomers) and the rest of the spectrum was in complete conformity with the assigned structure. IR spectrum supports the characteristic amide and thiocarbonyl stretching. ^{13}C NMR and elemental analysis further confirmed the structure.

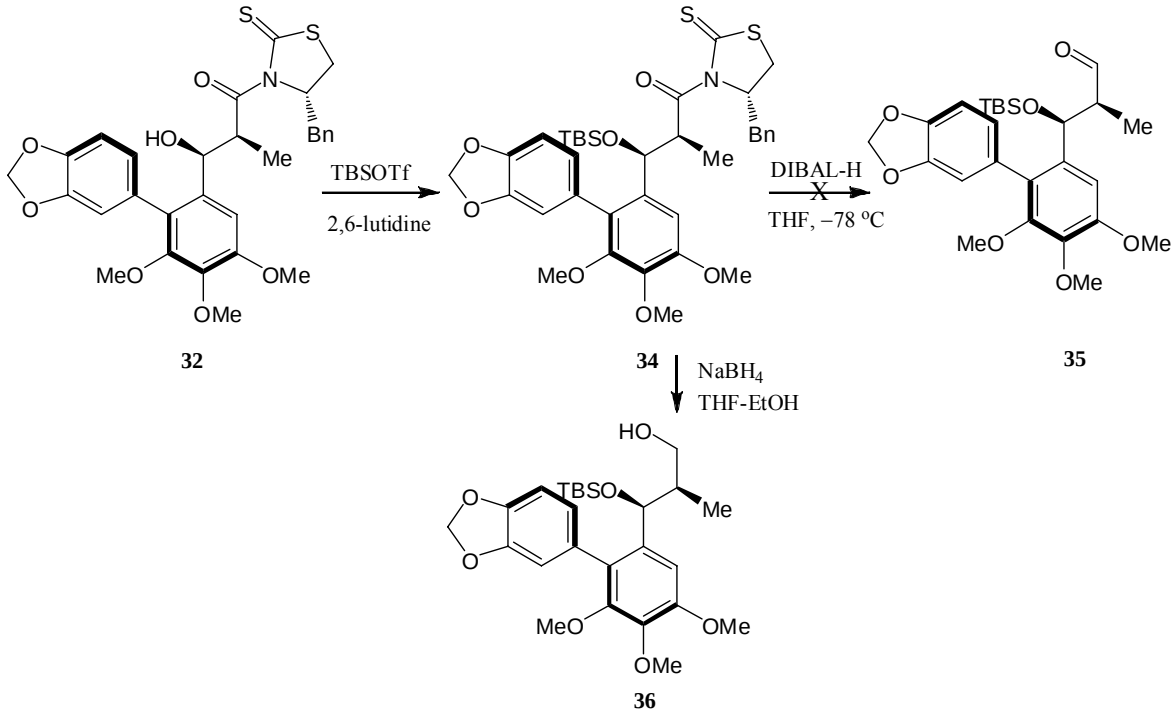
Scheme 12:



5 **33** **32**

After having an easy access for the key aldol adduct **32**, the free hydroxyl group was protected as its TBS-ether, by treating with TBSOTf and 2,6 lutidine in DCM (scheme 13) to afford **34**. The structure of the compound **34** was confirmed by its ^{1}H and ^{13}C NMR analysis, where the presence of typical silyl ether signals (at δ –0.26, -0.23, -0.07, -0.05, 0.84, 0.86 ppm as singlet in ^{1}H NMR and at δ –4.8, -4.7, -4.6, -4.5, 13.4, 13.6 ppm in ^{13}C NMR) were seen confirming the presence of both attropoisomers as well as the silyl ether.

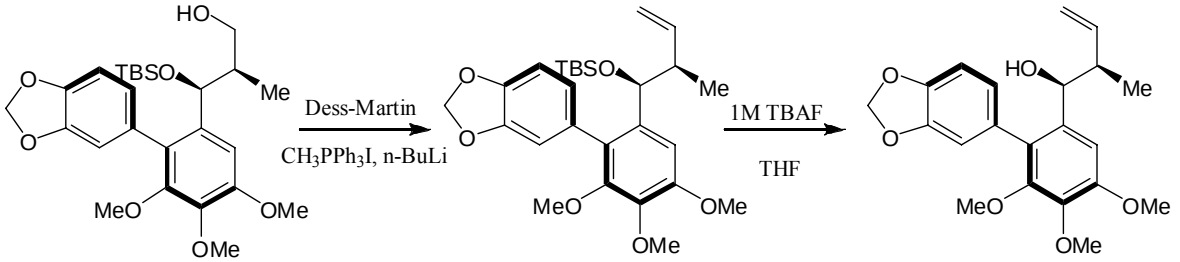
Scheme 13:



32 **34** **35** **36**

Our initial attempts for the reductive removal of the thiazolidinethione auxiliary from **34** to the corresponding aldehyde **35** with DIBAL-H were futile. To over come, a two-step strategy to construct the aldehyde **35** was adopted. Thus, thiazolidinethione auxiliary was removed by treating the TBS ether **34** with $NaBH_4$ in THF-EtOH[58] to afforded primary alcohol **36** (scheme 13). The absence of the characteristic thiazolidinethione moiety signals in ^1H, ^{13}C NMR and IR spectrum clearly suggests the assigned structure. The alcohol **36** was subjected to Dess̃Martin periodinane oxidation to get the aldehyde **35**, which was used with out purification and consequent Wittig reaction with $PPh_3{=}CH_2$ gave the olefin derivative **37** (scheme 14). In the ^1H NMR spectrum the, olefinic proton resonates at δ 5.96 ppm and the rest of the spectrum was in complete conformity with the assigned structure. The key *syn* crotyl alcohol **31** was obtained from olefin **37** by the TBS group removal employing 1M solution of Bu_4NF in THF. In the ^1H, ^{13}C NMR spectrum clearly showed the absence of typical silyl group signals and the rest of the protons were resonated at their expected values.

Scheme 14:

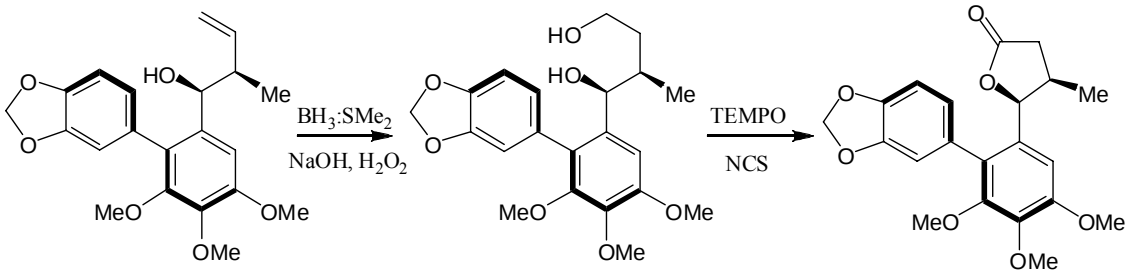


36 **37** **31**

After having key alcohol **31** in hand, we next focused our attention on hydroboration and lactonization of **31** as established with the respective racemic alcohol earlier. Thus, compound **31** was subjected to successive hydroboration and oxidation to give the diol **38** (scheme 15) whose ^1H and ^{13}C NMR was in complete agreement with the data of racemic diol **23**. The diol **38** was subjected to selective oxidation and lactonization as established earlier (TEMPO, NCS) to prepare the lactone derivative **39**. The chiral HPLC analysis (*Chiracel-OD* column) of **39** established the enantiomeric excess about 97%. All

other spectral data were in well agreement with the data of corresponding racemic compound **27**.

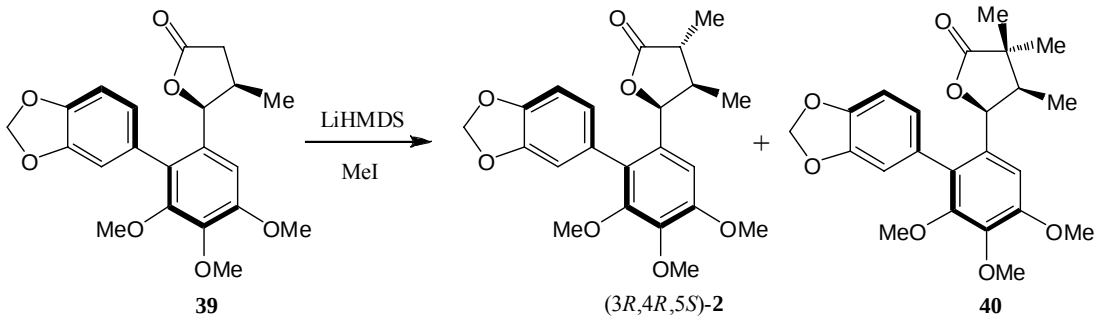
Scheme 15:



31 **38** **39**

Our final investigation for the establishment of absolute configuration of eupomatilone-6 only required alkylation of lactone **39**. Thus, the alkylation of **39** was executed by using essentially the same reaction (LiHMDS-MeI) as reported above to give (3*R*,4*R*,5*S*)-eupomatilone-6 (**2**) (scheme 16) whose spectroscopic data was in full agreement with reported spectra. The chiral HPLC showed that enantiomeric excess was >99%. The optical rotation observed $\{[\alpha]_D^{25} = +24.7$ ($c = 0.5$, $CHCl_3$)$\}$ for synthetic product was in agreement with reported valve $\{[\alpha]_D^{25} = -25.6$ ($c = 0.5$)$\}$[10] but with opposite sign to the natural eupomatilone-6. The minor quantity of dialkylated product **40** was also isolated and characterized by ^1H and ^{13}C NMR spectrum.

Scheme 16:



39 (3*R*,4*R*,5*S*)-**2** **40**

In summary (figure 11), a concise, four-step synthesis of eupomatilone-6 (**2**) and its diastereomer **3** has been devised which established the relative stereochemistry of the natural eupomatilone-6. Further studies on optically active (3*R*,4*R*,5*S*)-**2** proposed the

absolute configuration of naturally occurring eupomatilone-6 as (3*S*,4*S*,5*R*). As summarized in table 3, our streochemical assignments were all correlated with the previously reported NMR spectral data, and further confirmed Coleman's recent structural revision of **1** putting an end to the ambiguity in the original stereochemical assignments.

Figure 11: Racemic and Stereodivergent Synthesis of Eupomatilone-6.

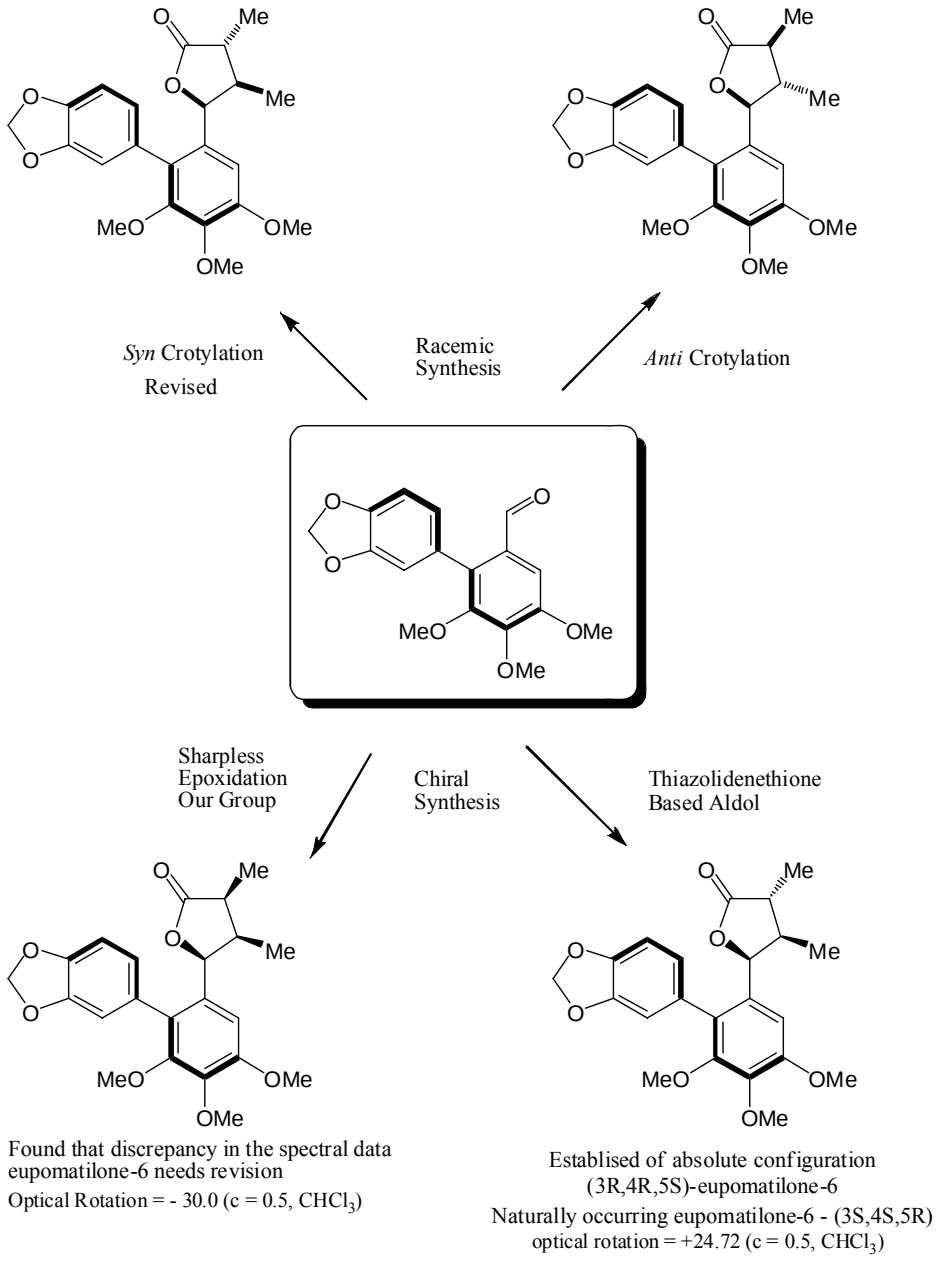
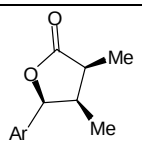
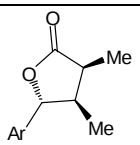
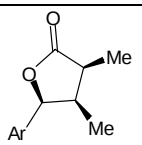
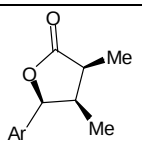
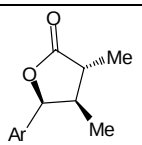
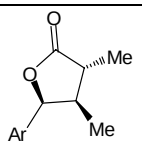
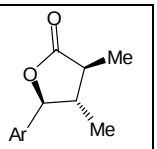
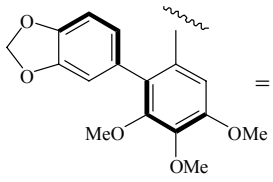


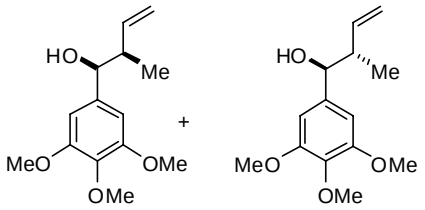
Table 3: Observations of the ^{1}H NMR signals for the corresponding lacrone.

							
	Original (Taylor)	McIntosh	Our Lab (Gurjar)	Coleman	Revised (Coleman)	This Work (Revised)	This Work
H-3	δ 2.36, 2.37, m, *J*=7.0, 5.3	δ 2.75, m	δ 2.74, quintet *J*=7.2	δ 2.74, app sextet, *J*=7.0	δ 2.37, sextet, *J*=7.2	δ 2.37, sextet, *J*=7.2	δ 2.24-2.08, m
H-4	δ 2.02, 1.97, m, *J*=7.0, 5.2	δ 2.36, m	δ 2.20, m	δ 2.20-2.10, m	δ 2.05-1.96, m	δ 2.04-1.93, m	δ 2.06-1.93, m
H-5	δ 5.54, 5.65, d, *J*=7.0	δ 5.00, 5.10, d, *J*=4.4	δ 5.32, 5.41, d, *J*=5.0	δ 5.32, 5.40, d, *J*=4.9	δ 5.54, 5.65, d, *J*=6.9	δ 5.53, 5.64, d, *J*=6.9	δ 4.78, 4.80, d, *J*=9.46
Me-3	δ 1.20, 1.19, d, *J*=7.0	δ 1.06, 1.07, d, *J*=7.5	δ 1.13, d, *J*=7.2	δ 1.12, d, *J*=7.3	δ 1.19, 1.20, d, *J*=7.4	δ 1.19, 1.20, d, *J*=7.4	δ 1.23, 1.26, s
Me-4	δ 0.70, 0.73, d, *J*=7.0	δ 0.65, 0.67, d, *J*=6.8	δ 0.54, 0.56, d, *J*=7.2	δ 0.54, 0.56, d, *J*=7.3	δ 0.70, 0.73, d, *J*=7.1	δ 0.70, 0.73, d, *J*=7.1	δ 0.87, 0.89, d, *J*=6.0

 = Ar

Experimental Section

2-methyl-1-(3,4,5-trimethoxyphenyl)but-3-en-1-ol (7 &8).



7 **8**

To a solution of the aldehyde **6** (5.0 g, 25.5 mmol), crotyl bromide (5.24 ml, 51 mmol), aqueous saturated NH_4Cl (15 ml) in THF (25 ml) at 0 ^oC was added Zn dust (5.0 g, 76 mmol) over a period of 30 min. After an hour, the reaction mixture was filtered over Celite and the layers separated. The aqueous phase was extracted with ethyl acetate. The combined organic layer was dried over sodium sulphate, filtered and concentrated. The crude residue was purified by silica gel column chromatography with light petroleum: ethyl acetate (9:1) as eluent to afford a 1:1 mixture of *syn*- and *anti*-alcohols (**7** & **8**) (6.2 g, 96%).

Mol. Formula : $C_{14}H_{20}O_4$

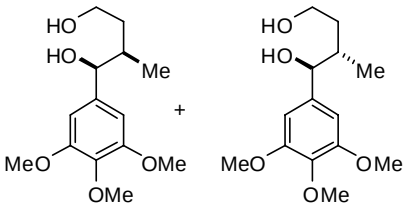
IR ($CHCl_3$) : 3015, 1587, 1461, 1409, 1226, 1128, 1037, 938, 752, 661 cm^{-1}.

^{1}H NMR (200 MHz, $CHCl_3$) : δ 0.89 (d, *J* = 6.8 Hz, 3H), 1.03 (d, *J* = 6.8 Hz, 3H), 1.81 (br s, 2 x OH), 2.01 (br s, 1 x OH), 2.22 (br s, 1 x OH), 2.40–2.62 (m, 2H), 3.84 (s, 6H), 3.86 (s, 6H), 3.87 (s, 6H), 4.28 (d, *J* = 8.0 Hz, 1H), 4.55 (d, *J* = 5.5 Hz, 1H), 5.03–5.28 (m, 4H), 5.71–5.91 (m, 2H), 6.54 (s, 2H), 6.57 (s, 2H).

^{13}C NMR (50 MHz, $CHCl_3$) : 13.71 (q), 15.69 (q), 43.94 (d), 44.90 (d), 55.14 (q), 59.88 (q), 76.69 (d), 77.23 (d), 102.81 (d), 102.94 (d), 113.95 (t), 114.94 (t), 135.88 (s), 136.10 (s), 138.17 (s), 138.45 (s), 139.99 (d), 140.10 (s), 151.93 (s), 152.05 (s) ppm.

Elemental Analysis : Calcd: C, 66.65; H, 7.99%.
Found: C, 66.58; H, 7.95%.

2-methyl-1-(3,4,5-trimethoxyphenyl)butane-1,4-diol (9 & 10).



9 **10**

To a solution of olefines **7**/**8** (6 g, 23.78 mmol) in anhydrous THF (30 ml) at 0 °C BH_3.DMS (3.39 ml, 35.7 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. It was cooled to 0 ^oC and treated with aqueous NaOH (39 ml, 3N, 119 mmol), 30% H_2O_2 (3.64 ml, 119 mmol) and EtOH (3 ml). The reaction mixture allowed to warm to room temperature. After 3 h, the volatiles were removed under reduced pressure and the residue diluted with ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulphate, filtered, and concentrated, crude diols **9**/**10** (5.5 g, 86%) was purified by column chromatography.

Mol. Formula : $C_{14}H_{22}O_5$

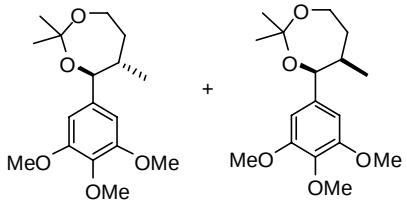
IR ($CHCl_3$) : 3014, 1592, 1475, 1409, 1211, 1123, 1046, 931, 751, 668 cm^{-1}.

^{1}H NMR (200 MHz, $CHCl_3$) : δ 0.76 (d, *J* = 6.8 Hz, 3H), 0.86 (d, *J* = 6.8 Hz, 3H), 1.40–1.96 (m, 6H), 3.53–3.74 (m, 4H), 3.81 (s, 6H), 3.83 (s, 12H), 4.23 (d, *J* = 7.7 Hz, 1H), 4.54 (d, *J* = 4.4 Hz, 1H), 6.52 (s, 2H), 6.53 (s, 2H).

^{13}C NMR (50 MHz, $CHCl_3$) : 13.51 (q), 17.03 (q), 35.62 (t), 35.81 (t), 37.35 (d), 38.19 (d), 55.66 (q), 59.79 (t), 60.14 (t), 60.44 (q), 76.86 (d), 78.85 (d), 102.98 (d), 103.24 (d), 136.10 (s), 136.40 (s), 139.09 (s), 139.49 (s), 152.37 (s), 152.51 (s) ppm.

Elemental Analysis : Calcd: C, 62.20; H, 8.20%.
Found: C, 62.17; H, 8.23%.

2,2,5-trimethyl-4-(3,4,5-trimethoxyphenyl)-1,3-dioxepane11 and 12.



12 **11**

To a solution of the above diols **9**/**10** (5 g, 18.5 mmol) in CH_2Cl_2 (20 ml) was added 2,2-dimethoxypropane (34 ml, 277.5 mmol) and PPTS (cat) and the contents were stirred for 4 h at room temperature. The reaction mixture was quenched with Et_3N (0.3 ml) and concentrated to give a crude product which was purified on silica gel by using light petroleum:ethyl acetate (19:1) to give **11** (2.2 g, 38.3%) and **12** (2.1 g, 36.6%).

Spectral data of *syn* acetonide 11:

Mol. Formula : $C_{17}H_{26}O_5$

IR ($CHCl_3$) : 3015, 1591, 1484, 1412, 1326, 1219, 1128, 1047, 971, 755, 663 cm^{-1}.

^{1}H NMR (200 MHz, $CHCl_3$) : δ 0.77 (d, *J* = 7.0 Hz, 3H), 1.34 (s, 3H), 1.44 (s, 3H), 1.90–2.18 (m, 3H), 3.60 (dt, *J* = 12.5, 3.2 Hz, 1H), 3.83 (d, *J* = 1.9 Hz, 1H), 3.84 (s, 3H), 3.87 (s, 6H), 4.92 (s, 1H), 6.55 (s, 2H).

^{13}C NMR (50 MHz, $CHCl_3$) : 11.2 (q), 24.4 (q), 24.8 (q), 36.2 (d), 36.4 (t), 55.5 (q), 57.7 (t), 60.1 (q), 73.3 (d), 100.0 (s), 102.4 (d), 136.6 (s), 137.6 (s), 152.5 (s) ppm.

Maldi top : 333.18 $[M+Na]^+$

Elemental Analysis : Calcd: C, 65.78; H, 8.44%.
Found: C, 65.72; H, 8.39%.

Spectral data of *anti* acetonide 12:

Mol. Formula : $C_{17}H_{26}O_5$

IR ($CHCl_3$) : 3015, 1591, 1484, 1412, 1326, 1219, 1128, 1047, 971, 755, 663 cm^{-1}.

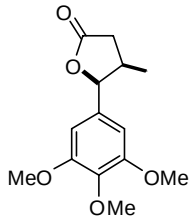
^{1}H NMR (200 MHz, $CHCl_3$) **:** δ 0.58 (d, *J* = 6.6 Hz, 3H), 1.37 (s, 3H), 1.38 (s, 3H), 1.72–1.76 (m, 1H), 1.81–1.93 (m, 1H), 3.64 (dt, *J* = 12.2, 3.2 Hz, 1H), 3.81 (s, 1H), 3.84 (s, 3H), 3.88 (s, 6H), 4.28 (d, *J* = 9.4 Hz , 1H), 6.49 (s, 2H).

^{13}C NMR (50 MHz, $CHCl_3$) **:** 18.1 (q), 24.7 (q), 25.1 (q), 39.02 (t), 39.5 (d), 55.8 (q), 60.5 (q), 60.7 (t), 79.3 (d), 100.4 (s), 103.7 (d), 136.8 (s), 137.9 (s), 152.8 (s) ppm.

Maldi top **:** 333.18 $[M+Na]^+$

Elemental Analysis **:** Calcd: C, 65.78; H, 8.44%.
Found: C, 65.72; H, 8.39%.

4-methyl-5-(3,4,5-trimethoxyphenyl)dihydrofuran-2(3H)-one 13.



13

Compound **11** (2 g, 6.44 mmol) and PPTS (cat) in MeOH (10 ml) were stirred at room temperature for 5 h. Quenched with Et_3N (0.1 ml), concentrated to obtain **9** (1.6 g, 92%). The crude material was used without purification.

A biphasic mixture of diol **9** (1.6 g, 5.92 mmol), TEMPO (90 mg, 0.59 mmol), TBAI (220 mg, 0.59 mmol) and N-chlorosuccinimide (2.37 g, 17.76 mmol), aqueous $NaHCO_3$ (0.5M, 10 ml) and aqueous K_2CO_3 (0.05M, 10 ml) and CH_2Cl_2 (15 ml) was vigorously stirred for 3 h at rt. The organic layer was separated, and the aqueous phase extracted with CH_2Cl_2. The organic layer was washed with brine, dried, and evaporated. The residue was purified by silica gel column chromatography by eluting with light petroleum: ethyl acetate (4:1) to give **13** (1.2 g, 81%).

Mol. Formula **:** $C_{14}H_{18}O_5$

IR ($CHCl_3$) **:** 3018, 1776, 1592, 1486, 1321, 1219, 1072, 1038, 921,

752, 662 cm^{-1}.

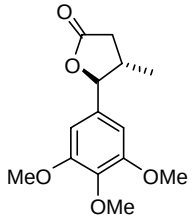
^{1}H NMR (200 MHz, $CHCl_3$) **:** δ 0.74 (d, *J* = 7.0 Hz, 3H), 2.46–2.55 (m, 1H), 2.81–2.91 (m, 2H), 3.84 (s, 3H), 3.87 (s, 6H), 5.53 (d, *J* = 5.6 Hz, 1H), 6.44 (s, 2H).

^{13}C NMR (50 MHz, $CHCl_3$) **:** 18.1 (q), 24.7 (q), 25.1 (q), 39.02 (t), 39.5 (d), 55.8 (q), 60.5 (q), 60.7 (t), 79.3 (d), 100.4 (s), 103.7 (d), 136.8 (s), 137.9 (s), 152.8 (s) ppm.

Maldi top **:** 289.12 $[M+Na]^+$

Elemental Analysis **:** Calcd: C, 63.15; H, 6.81%.
Found: C, 63.12; H, 6.78%.

4-methyl-5-(3,4,5-trimethoxyphenyl)dihydrofuran-2(3H)-one 15.



15

Compound **12** (2 g, 5.1 mmol) was subjected to essentially the same reaction conditions to produce **15** (1.2 g, 81 %).

Mol. Formula **:** $C_{14}H_{18}O_5$

IR ($CHCl_3$) **:** 3018, 1776, 1592, 1486, 1321, 1219, 1072, 1038, 921, 752, 662 cm^{-1}.

^{1}H NMR (200 MHz, $CHCl_3$) **:** δ 1.17 (d, *J* = 6.5 Hz, 3H), 2.27–2.36 (m, 1H), 2.42–2.51 (m, 1H), 2.67–2.80 (m, 1H), 3.82 (s, 3H), 3.84 (s, 6H), 4.84 (d, *J* = 8.5 Hz, 1H), 6.51 (s, 2H).

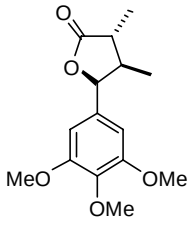
^{13}C NMR (50 MHz, $CHCl_3$) **:** 16.6 (q), 37.2 (t), 39.8 (d), 56.2 (q), 60.8 (q), 88.2 (d), 102.9 (d), 128.3 (d), 133.4 (s), 153.5 (s), 175.8 (s) ppm.

Maldi top **:** 289.12 $[M+Na]^+$

Elemental Analysis **:** Calcd: C, 63.15; H, 6.81%.

Found: C, 63.12; H, 6.78%.

3,4-dimethyl-5-(3,4,5-trimethoxyphenyl)dihydrofuran-2(3H)-one 14.



14

To a solution of **13** (1 g, 3.76 mmol) in anhydrous THF (5 ml) at –78 °C was added LiHMDS (1M solution in THF, 4.13 ml). After 1 h, MeI (0.26 ml, 4.5 mmol) was added and stirring continued for additional 1 h at –78 °C. The reaction mixture was quenched with saturated ammonium chloride, extracted with ethyl acetate, dried over sodium sulphate and concentrated. The crude residue was purified by silica gel chromatography by eluting with light petroleum: ethyl acetate (9:1) to give **14** (0.7 g, 66.5%).

Mol. Formula : $C_{15}H_{20}O_5$

IR ($CHCl_3$) : 3422, 2928, 1779, 1598, 1452, 1409, 1316, 1236, 1192, 1121, 1036, 932, 759, 664 cm^{-1}.

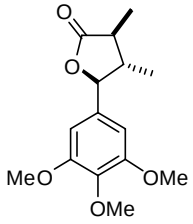
^{1}H NMR (200 MHz, $CHCl_3$) : δ 0.74 (d, *J* = 6.8 Hz, 3H), 1.10 (s, 3H), 1.97-2.03 (m, 1H), 2.25-2.33 (m, 1H), 3.77 (s, 3H), 3.78 (s, 6H), 5.42 (d, *J* = 7.2 Hz, 1H), 6.29 (s, 2H).

^{13}C NMR (50 MHz, $CHCl_3$) : 13.6 (q), 14.5 (q), 43.1 (d), 49.6 (d), 56.1 (q), 60.5 (q), 84.4 (d), 103.5 (d), 133.1 (s), 138.5 (s), 181.1 (s) ppm.

Maldi top : 303.13 $[M+Na]^+$

Elemental Analysis : Calcd: C, 64.27; H, 7.19%.
Found: C, 64.25; H, 7.12%.

3,4-dimethyl-5-(3,4,5-trimethoxyphenyl)dihydrofuran-2(3H)-one 16.



16

Compound **15** (1 g, 3.76 mmol) was subjected to essentially the same reaction conditions to produce **16** (0.75 g, 71.2 %).

Mol. Formula : $C_{15}H_{20}O_5$

IR ($CHCl_3$) : 3422, 2928, 1779, 1598, 1452, 1409, 1316, 1236, 1192, 1121, 1036, 932, 759, 664 cm^{-1}.

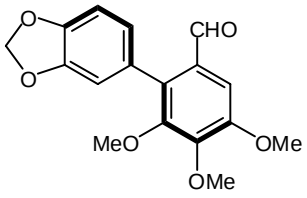
^{1}H NMR (200 MHz, $CHCl_3$) : δ 0.98 (d, *J* = 6.6 Hz, 3H), δ 1.26 (d, *J* = 7.0 Hz, 3H), 2.28–2.36 (m, 1H), 2.40–2.48 (m, 1H), 3.81 (s, 6H), 3.84 (s, 3H), 4.79 (d, *J* = 10.2 Hz, 1H), 6.50 (s, 2H).

^{13}C NMR (50 MHz, $CHCl_3$) : 18.1 (q), 23.1 (q), 40.1 (d), 42.2 (d), 56.1 (q), 60.5 (q), 82.1 (d), 103.1 (d), 128.1 (s), 131.7 (s), 138.4 (s), 153.4 (s), 179.1 (s) ppm.

Maldi top : 303.13 $[M+Na]^+$

Elemental Analysis : Calcd: C, 64.27; H, 7.19%.
Found: C, 64.25; H, 7.12%.

2-Benzo[1,3]dioxol-5-yl-3,4,5-trimethoxy benzaldehyde (5).



Preparation of Pd (0)

A mixture of $PdCl_2$ (17.72 g, 0.1 mol). PPh_3 (131 g, 0.5 mol) and 1.2 lit. of DMSO is heated to 140 ^oC till a clear solution is obtained. The oil bath is removed and the solution is stirred for 15 min. Hydrazine hydrate is then added rapidly (20 .0 g, 0.4 mol). The dark solution is immediately cooled with water bath, crystallization begins at 125 ^oC. At this

point, the mixture is allowed to cool without external cooling. The crystals are filtered under nitrogen, washed successively with ethanol (2x50 mL) and ether (2x50 mL), dried, stored under nitrogen and protected from light.

A mixture of $Pd(PPh_3)_4$ (150 mg, 0.13 mmol), benzene (9 mL), bromo aldehyde **17** (1.2 g, 4.3 mmol), 2 M aq. solution of Na_2CO_3 (4.3 mL, 8.6 mmol), EtOH (2 mL) and boronic acid **20** (800 mg, 4.8 mmol) was degassed and refluxed under argon. After 24 h, both the layers were separated and the organic layer was washed with water, brine, dried and evaporated. The crude residue on purification by column chromatography (llight petroleum:EtOAc 1:19 gave biaryl aldehyde **5** (1.1 g, 79 %).

Mol. Formula : $C_{17}H_{16}O_6$

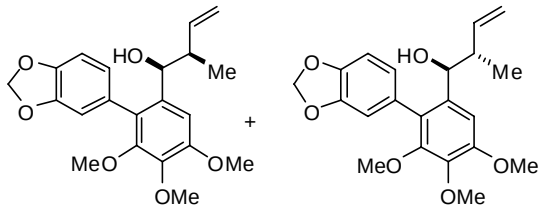
IR ($CHCl_3$) : 3012, 1595, 1671, 1046, 933, 752, 661 cm^{-1}.

^{1}H NMR (200 MHz, $CHCl_3$) : δ 3.65 (s, 3 H), 3.95 (s, 3 H), 3.98 (s, 3 H), 6.02 (s, 2 H), 6.62-6.66 (m, 1 H), 6.85 (m, 2 H), 7.26 (s, 1 H), 9.67 (s, 1 H).

^{13}C NMR (50 MHz, $CHCl_3$) : δ 55.7, 60.7, 100.9, 105.0, 107.5, 111.0, 124.5, 126.1, 129.7, 133.5, 147.2, 150.9, 152.7, 190.4. ppm.

Elemental Analysis : Calcd: C, 64.56; H, 5.06%.
Found: C, 64.73; H, 5.27%.

1-(2-(benzo[d][1,3]dioxol-5-yl)-3,4,5-trimethoxyphenyl)-2-methylbut-3-en-1-ol 21 &22.



21 **22**

To a solution of the aldehyde **5** (10.0 g, 31.6 mmol), crotyl bromide (8.5 g, 63.6 mmol), aqueous saturated NH_4Cl (25 ml) in THF (50 ml) at 0 ^oC was added Zn dust (6.19 g, 94.7 mmol) over a period of 30 min. After an hour, the reaction mixture was filtered over Celite and the layers separated. The aqueous phase was extracted with ethyl acetate. The combined organic layer was dried over sodium sulphate, filtered and concentrated. The crude residue was purified by silica gel column chromatography with light petroleum: ethyl

acetate (9:1) as eluent to afford a 1:1 mixture of *syn*- and *anti*-alcohols (**21** & **22**) (10.95 g, 93%).

Mol. Formula : $C_{21}H_{24}O_6$

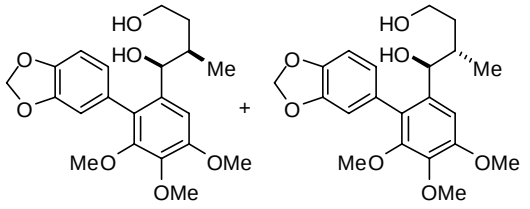
IR ($CHCl_3$) : 3019, 1597, 1481, 1403, 1216, 1126, 1041, 938, 758, 669 cm^{-1}.

^{1}H NMR (200 MHz, $CHCl_3$) : δ 0.70 (d, *J* = 6.8 Hz, 3H), 0.72 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H), 1.81 (br s, 2 x OH), 2.06 (br s, 2 x OH), 2.35–2.46 (m, 4H), 3.60 (s, 6 H), 3.61 (s, 6 H), 3.88 (s, 12H), 3.90 (s, 6H), 3.92 (s, 6H), 4.24(d, *J* = 8.5 Hz, 1H), 4.26 (d, *J* = 8.5 Hz, 1H), 4.49 (d, *J* = 6.2 Hz, 1H), 4.53 (d, *J* = 6.2 Hz, 1H), 4.81–4.95 (m, 4H), 5.09–5.20 (m, 4H), 5.47–5.76 (m, 4H), 5.99–6.01 (m, 8H), 6.58–6.74 (m, 8H), 6.81–6.89 (m, 8H).

^{13}C NMR (50 MHz, $CHCl_3$) : 13.3 (q), 13.5 (q), 16.6 (q), 21.0 (q), 43.4 (d), 45.6 (d), 45.7 (d), 55.5 (q), 60.3 (q), 60.4 (q), 60.5 (q), 72.8 (d), 72.9 (d), 100.6 (t), 104.8 (d), 105.3 (d), 107.4 (d), 107.5 (d), 110.0 (d), 110.2 (d), 111.1 (d), 111.2 (d), 114.2 (t), 114.3 (t), 116.0 (t), 122.5 (d), 122.8 (d), 123.9 (d), 124.9 (d), 127.3 (s), 127.8 (s), 128.2 (s), 128.3 (s), 128.6 (s), 129.4 (s), 129.4 (s), 129.5 (s), 136.3 (s), 136.4 (s), 136.9 (s), 136.9 (s), 137.3 (s), 140.5 (d), 140.6 (d), 140.7 (d), 141.0 (s), 146.1 (s), 146.9 (s), 150.6 (s), 150.6 (s), 152.3 (s), 152.6 (s) ppm.

Elemental Analysis : Calcd: C, 67.73; H, 6.50%.
Found: C, 67.58; H, 6.66%.

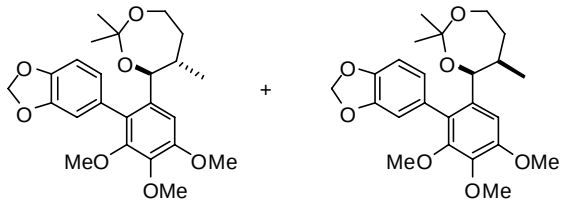
1-(2-(benzo[d][1,3]dioxol-5-yl)-3,4,5-trimethoxyphenyl)-2-methylbutane-1,4-diol 23 & 24.



23 **24**

To a solution of olefines **21**/**22** (10 g, 26.8 mmol) in anhydrous THF (50 ml) at 0 °C BH_3.DMS (2.4 g, 40.8 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. It was cooled to 0 ^oC and treated with aqueous NaOH (45 ml, 3N, 135 mmol), 30% H_2O_2 (15.5 ml, 135 mmol) and EtOH (3 ml). The reaction mixture allowed to warm to room temperature. After 3 h, the volatiles were removed under reduced pressure and the residue diluted with ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulphate, filtered, and concentrated, gave crude diols **23**/**24** (8.98 g, 86%).

5-(2,3,4-trimethoxy-2, 2,5-trimethyl-1, 3-dioxepan-4-yl) phenyl) benzo [1,3] dioxide 25 and 26.



26 **25**

To a solution of the above crude diols **23**/**24** (8.98 g, 23 mmol) in CH_2Cl_2 (40 ml) was added 2,2-dimethoxypropane (42 ml, 346.5 mmol) and PPTS (0.115 g, 0.46 mmol) and the contents were stirred for 4 h at room temperature. The reaction mixture was quenched with Et_3N (0.3 ml) and concentrated to give a crude product which was purified on silica gel by using light petroleum:ethyl acetate (19:1) to give **25** (3.68 g, 37%) and **26** (3.68 g, 37%).

Spectral data of *syn* acetonide 25:

Mol. Formula : $C_{24}H_{30}O_7$

IR ($CHCl_3$) : 3019, 1597, 1480, 1402, 1322, 1216, 1126, 1043, 973, 757, 668 cm^{-1}.

^{1}H NMR (200 MHz, $CHCl_3$) : δ 0.77 (d, *J* = 7.1 Hz, 3H), 0.83 (d, *J* = 7.1 Hz, 3H), 1.23 (s, 6H), 1.26–1.42 (m, 4H), 1.36 (s, 6H), 1.65–1.77 (m, 2H), 3.40 (m, 1H), 3.46 (m, 1H), 3.62 (s, 6H), 3.76–3.88 (m, 2H), 3.90 (s, 6H), 3.92 (s, 6H), 4.78 (s, 1H), 4.88 (s, 1H), 6.00–6.03 (br.s, 4H), 6.57–6.67 (m, 4H), 6.83–6.92 (m, 4H).

^{13}C NMR (50 MHz, $CHCl_3$) : 11.5 (q), 24.9 (q), 25.1 (q), 35.1 (d), 35.3 (d), 36.7 (t), 36.8 (t), 55.9 (q), 58.1 (t), 60.7 (q), 60.8 (q), 60.9 (q), 71.0 (d), 71.1 (d), 100.3 (t), 100.9 (s), 106.7 (d), 107.9 (d), 108.2 (d), 109.7 (d), 111.1 (d), 122.3 (d), 123.8 (d), 126.2 (s), 126.3 (s), 130.0 (s), 130.1 (s), 136.9 (s), 140.6 (s), 146.4 (s), 146.5 (s), 147.4 (s), 147.5 (s), 150.9 (s), 151.9 (s) ppm.

Maldi top : 431.24 $[M+H]^+$, 453.22 $[M+Na]^+$

Elemental Analysis : Calcd: C, 66.96; H, 7.02%.
Found: C, 66.90; H, 7.26%.

Spectral data of *anti* acetonide 26:

Mol. Formula : $C_{24}H_{30}O_7$

IR ($CHCl_3$) : 3019, 1597, 1480, 1402, 1322, 1216, 1126, 1043, 973, 757, 668 cm^{-1}.

^{1}H NMR (200 MHz, $CHCl_3$) : δ 0.57 (d, *J* = 6.8 Hz, 6H), 1.13 (s, 3H), 1.15 (s, 3H), 1.31 (s, 6H), 1.60–1.88 (m, 6H), 3.53 (dt, *J* = 12.1, 3.0 Hz, 2H), 3.61 (s, 3H), 3.62 (s, 3H), 3.56 (br.t, *J* = 12.1 Hz,2H), 3.90 (s, 6H), 3.94 (s, 6H), 4.33 (d, *J* = 9.4 Hz, 1H), 4.36 (d, *J* = 9.4 Hz, 1H), 5.99–6.02 (m, 4H), 6.00

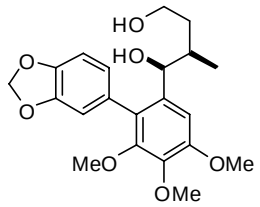
(br.s, 2H), 6.02 (d, *J* = 1.4 Hz, 1H), 6.04 (d, *J* = 1.4 Hz, 1H), 6.58 (dd, *J* = 7.9, 1.6 Hz, 1H), 6.62–6.68(m, 3H), 6.77(s, 2H), 6.82 (d, *J* = 7.9 Hz, 1H), 6.84 (d, *J* = 7.9 Hz, 1H).

^{13}C NMR (50 MHz, $CHCl_3$) **:** 18.2 (q), 25.2 (q), 25.7 (q), 38.9 (t), 40.9 (d), 41.0 (d), 55.8 (q), 60.5 (q), 60.7 (t), 60.8 (q), 74.6 (d), 74.7 (d), 100.7 (t), 100.8 (d), 105.7 (d), 107.5 (d), 107.6 (d), 110.9 (d), 111.4 (d), 123.6 (d), 124.2 (d), 127.8 (s), 129.9 (s), 136.7 (s), 136.8 (s), 141.1 (s), 146.5 (s), 147.0 (s), 150.7 (s), 153.0 (s) ppm.

Maldi top **:** 431.24 $[M+H]^+$, 453.22 $[M+Na]^+$

Elemental Analysis **:** Calcd: C, 66.96; H, 7.02%.
Found: C, 67.10; H, 7.15%.

1-(2-(benzo[d][1,3]dioxol-5-yl)-3,4,5-trimethoxyphenyl)-2-methylbutane-1,4-diol 23.



23

Compound **25** (3 g, 7 mmol) and PPTS (35 mg, 0.14 mmol) in MeOH (15 ml) were stirred at room temperature for 5 h. Quenched with Et_3N (0.1 ml), concentrated and the residue was purified by silica gel chromatography by eluting with light petroleum: ethyl acetate (3:1) to obtain **23** (2.48 g, 91%).

Mol. Formula **:** $C_{21}H_{26}O_7$

IR ($CHCl_3$) **:** 3620, 3019, 1638, 1522, 1423, 1215, 1045, 928, 669 cm^{-1}.

^{1}H NMR (200 MHz, $CHCl_3$) **:** δ 0.84 (d, *J* = 6.8 Hz, 3H), 0.86 (d, *J* = 6.8 Hz, 3H), 1.16–1.33 (m, 2H), 1.38–1.54 (m, 2H), 1.69–1.84 (m, 2H), 2.50 (br, 4H, 4 x OH), 3.34–3.53 (m, 4H), 3.61 (s,

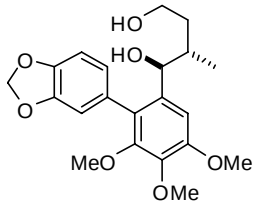
6H), 3.88 (s, 6H), 3.90 (s, 6H), 4.53 (d, *J* = 4.8 Hz, 1H), 4.59 (d, *J* = 4.8 Hz, 1H), 5.99 – 6.00 (s, 4H), 6.59–6.70 (m, 4H), 6.82–6.87 (m, 2H), 6.92 (s, 2H).

^{13}C NMR ($CHCl_3$) : 13.3 (q), 36.1 (2d), 36.4 (2t), 55.9 (1), 60.2 (t), 60.7 (q), 60.9 (q), 72.8 (d), 72.9 (d), 100.9 (t), 105.5 (d), 107.9 (d), 110.2 (d), 111.3 (d), 122.8 (d), 124.1 (d), 127.3 (s), 129.7 (s), 129.9 (s), 137.6 (s), 141.0 (s), 146.4 (s), 147.3 (s), 151.0 (s), 152.5 (s) ppm.

Maldi top : 413.16 $[M+Na]^+$

Elemental Analysis : Calcd: C, 64.60; H, 6.71%.
Found: C, 64.79; H, 6.96%.

(1-(2-(benzo[d][1,3]dioxol-5-yl)-3,4,5-trimethoxyphenyl)-2-methylbutane-1,4-diol 24.



24

Compound **26** was transformed into **24** as reported above.

Mol. Formula : $C_{21}H_{26}O_7$

IR ($CHCl_3$) : 3620, 3019, 1638, 1522, 1423, 1215, 1045, 928, 669 cm^{-1}.

^{1}H NMR ($CHCl_3$) : δ 0.61 (d, *J* = 7.0 Hz, 3H), 0.62 (d, *J* = 7.0 Hz, 3H), 1.43–1.58 (m, 2H), 1.64–1.80 (m, 2H), 1.83–1.97 (m, 2H), 2.95 (s, 4xOH), 3.54–3.76 (m, 4H), 3.60 (s, 3H), 3.61(s, 3H), 3.89 (s, 6H), 3.90 (s, 6H), 4.27 (d, *J* = 8.5 Hz, 1H), 4.29(d, *J* = 8.5 Hz, 1H), 5.99–6.03 (m, 4H), 6.58–6.71 (m, 4H), 6.81–6.87 (m, 2H), 6.86 (s, 2H).

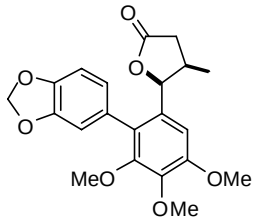
^{13}C NMR ($CHCl_3$) : 17.5 (q), 36.4 (t), 38.4 (d), 55.8 (d), 60.7 (t), 60.7 (q), 60.8 (q), 60.9 (q), 74.5 (d), 74.6 (d), 100.8 (t), 104.9 (d),

107.7 (d), 107.8 (d), 110.3 (d), 111.2 (d), 122.9 (d), 124.1 (d), 128.2 (s), 129.7 (s), 129.8 (s), 137.8 (s), 137.8 (s), 141.0 (s), 146.3 (s), 147.1 (s), 150.7 (s), 150.8 (s), 152.9 (s) ppm.

Maldi top : 413.16 $[M+Na]^+$

Elemental Analysis : Calcd: C, 64.60; H, 6.71%.
Found: C, 64.81; H, 6.65%.

5-(2-(benzo[d][1,3]dioxol-5-yl)-3,4,5-trimethoxyphenyl)-4-methyldihydrofuran-2(3H)-one 27.



27

A biphasic mixture of diol **23** (2 g, 5.1 mmol), TEMPO (80 mg, 0.51 mmol), TBAI (190 mg, 0.51 mmol) and N-chlorosuccinimide (2.05 g, 15.4 mmol), aqueous $NaHCO_3$ (0.5M, 10 ml) and aqueous K_2CO_3 (0.05M, 10 ml) and CH_2Cl_2 (20 ml) was vigorously stirred for 3 h at rt. The organic layer was separated, and the aqueous phase extracted with CH_2Cl_2. The organic layer was washed with brine, dried, and evaporated. The residue was purified by silica gel column chromatography by eluting with light petroleum: ethyl acetate (4:1) to give **27** (1.6 g, 81%).

Mol. Formula : $C_{21}H_{22}O_7$

IR ($CHCl_3$) : 3020, 1776, 1599, 1483, 1324, 1215, 1080, 1042, 929, 757, 669 cm^{-1}.

^{1}H NMR (200 MHz, $CHCl_3$) : δ 0.68 (d, *J* = 7.3 Hz, 3H), 0.70 (d, *J* = 7.3 Hz, 3H), 2.20–2.30 (m, 4H), 2.69 (dd, *J* = 7.8,6.3 Hz, 1H), 2.72 (dd, *J* = 7.8,6.3 Hz, 1H), 3.65 (s, 3H), 3.66 (s, 3H), 3.91 (s, 12H), 5.42 (d, *J* = 5.6 Hz, 1H), 5.51 (d, *J* = 5.6 Hz,

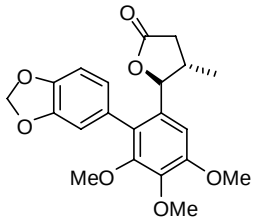
1H), 6.02–6.03 (m, 2H), 6.04–6.05(m, 2H), 6.58 (dd, *J* = 7.9,1.6 Hz, 1H), 6.62 (d, *J* = 1.6 Hz, 1H), 6.70 (dd, *J* = 7.9,1.6 Hz, 1H), 6.73 (d, *J* = 1.6 Hz, 1H), 6.81(s, 1H), 6.82(s, 1H), 6.85 (d, *J* = 7.9 Hz, 1H), 6.88 (d, *J* = 7.9 Hz, 1H).

^{13}C NMR ($CHCl_3$) **:** 15.2 (q), 15.3 (q), 33.6 (d), 33.8 (d), 37.7 (t), 56.0 (q), 60.6 (q), 60.9 (q), 81.9 (d), 101.0 (t), 104.8 (d), 108.0 (d), 108.2 (d), 109.5 (d), 110.5 (d), 122.3 (d), 123.3 (d), 126.3 (s), 126.4 (s), 128.9 (s), 130.0 (s), 141.6 (s), 146.8 (s), 146.9 (s), 147.5 (s), 151.4 (s), 152.7 (s), 176.1 (s) ppm.

Maldi top **:** 387.16 $[M+H]^+$, 409.13 $[M+Na]^+$

Elemental Analysis **:** Calcd: C, 65.28; H, 5.74%.
Found: C, 65.21; H, 5.53%.

5-(2-(benzo[d][1,3]dioxol-5-yl)-3,4,5-trimethoxyphenyl)-4-methyldihydrofuran-2(3H)-one 29.



29

Compound **24** (2 g, 5.1 mmol) was subjected to essentially the same reaction conditions to produce **29** (1.68 g, 85 %).

Mol. Formula **:** $C_{21}H_{22}O_7$

IR ($CHCl_3$) **:** 3020, 1776, 1599, 1483, 1324, 1215, 1080, 1042, 929, 757, 669 cm^{-1}.

^{1}H NMR ($CHCl_3$) **:** δ 0.81 (d, *J* = 6.6 Hz, 3H), 0.83 (d, *J* = 6.6 Hz, 3H), 2.06 (dd, *J* = 9.0,1.0 Hz, 1H), 2.14(dd, *J* = 9.0,1.0 Hz, 1H), 2.36–2.53 (m, 2H), 2.63 (dd, *J* = 7.9,2.3 Hz, 1H), 2.71

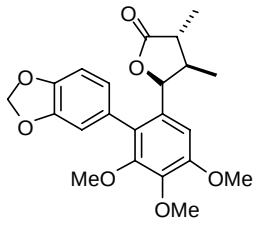
(dd, *J* = 7.9,2.3 Hz, 1H), 3.57 (s, 3H), 3.58 (s, 3H), 3.84 (s, 12H), 4.87 (d, *J* = 7.6 Hz, 1H), 4.92 (d, *J* = 7.6 Hz, 1H), 5.94–5.95 (m, 4H), 6.59 (dd, *J* = 7.9,1.7 Hz, 1H), 6.62–6.68 (m, 5H), 6.79 (s, 1H), 6.83 (s, 1H).

^{13}C NMR (50 MHz, $CHCl_3$) **:** 16.8 (q), 36.8 (t), 39.2 (d), 56.1 (q), 60.8 (q), 60.9 (q), 61.0 (q), 84.6 (d), 84.7 (d), 101.1 (t), 101.1 (d), 104.4 (d), 107.9 (d), 108.2 (d), 110.3 (d), 111.2 (d), 123.1 (d), 124.1 (d), 128.8 (s), 128.8 (s), 131.7 (s), 131.8 (s), 142.3 (s), 146.8 (s), 146.9 (s), 147.4 (s), 147.5 (s), 151.4 (s), 151.4 (s), 153.2 (s), 176.2 (s), 176.3 (s) ppm.

Maldi top **:** 387.16 $[M+H]^+$, 409.13 $[M+Na]^+$

Elemental Analysis **:** Calcd: C, 65.28; H, 5.74%.
Found: C, 65.07; H, 5.61%.

Total synthesis of (±)-eupomatilone-6 and its 3-methylated derivative 2.



2 (63%)

To a solution of **27** (0.50 g, 1.3 mmol) in anhydrous THF (5 ml) at –78 °C was added LiHMDS (1M solution in THF, 1.55 ml). After 1 h, MeI (0.09 ml, 1.45 mmol) was added and stirring continued for additional 1 h at –78 °C. The reaction mixture was quenched with saturated ammonium chloride, extracted with ethyl acetate, dried over sodium sulphate and concentrated. The crude residue was purified by silica gel chromatography by eluting with light petroleum: ethyl acetate (9:1) to give **28** (0.05 g, 9 %) and **2** (0.33 g, 63%).

Spectral data of (±)-eupomatilone-6 (2)

Mol. Formula **:** $C_{22}H_{24}O_7$

IR ($CHCl_3$) **:** 3429, 2932, 1773, 1596, 1457, 1406, 1326, 1226, 1197,

1127, 1040, 937, 754, 667 cm^{-1}.

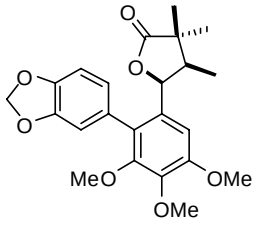
^{1}H NMR (200 MHz, $CHCl_3$) **:** δ 0.70 (d, *J* = 7.1 Hz, 3H), 0.73 (d, *J* = 7.1 Hz, 3H), 1.19 (d, *J* = 7.4 Hz, 3H), 1.20 (d, *J* = 7.4 Hz, 3H), 1.93–2.04 (m, 2H), 2.37 (sextet, J = 7.2 Hz, 2H), 3.63 (s, 3H), 3.64 (s, 3H), 3.89 (s, 6H), 3.90 (s, 6H), 5.53 (d, *J* = 6.9 Hz, 1H), 5.64 (d, *J* = 6.9 Hz, 1H), 6.01–6.03 (m, 4H), 6.59 (dd, *J* = *J* = 7.9, 1.6 Hz, 1H), 6.64 (d, *J* = 1.5 Hz, 1H), 6.66 (2s, 2H), 6.70 (dd, *J* = 1.6, *J* = 7.9 Hz, 1H), 6.72 (d, *J* = 1.5 Hz, 1H), 6.86 (d, *J* = 7.9 Hz, 1H), 6.88 (d, *J* = 7.9 Hz, 1H).

^{13}C NMR (50 MHz, $CHCl_3$) **:** 14.8 (q), 14.9 (q), 15.3 (q), 15.5 (q), 41.2 (d), 41.5 (d), 42.5 (d), 42.6 (d), 56.1 (q), 60.8 (q), 61.0 (q), 61.1 (q), 79.8 (d), 79.8 (d), 101.1 (t), 101.2 (t), 104.6 (d), 104.7 (d), 108.1 (d), 108.3 (d), 109.9 (d), 110.9 (d), 122.6 (d), 123.7 (d), 127.0 (s), 127.1 (s), 128.9 (s), 129.0 (s), 130.3 (s), 141.7 (s), 146.8 (s), 146.9 (s), 147.6 (s), 147.6 (s), 151.5 (s), 152.8 (s), 179.7 (s) ppm.

Maldi top **:** 401.57 $[M+H]^+$, 423.57 $[M+Na]^+$

Elemental Analysis **:** Calcd: C, 65.99; H, 6.04%.
Found: C, 65.85; H, 5.91%.

Spectral data of 3-methyl eupomatilone-6 (28).



28

Mol. Formula **:** $C_{23}H_{26}O_7$

^{1}H NMR (200 MHz, $CHCl_3$) **:** δ 0.54 (d, *J* = 7.5 Hz, 3H), 0.59 (d, *J* = 7.5 Hz, 3H), 1.11 (s, 3H), 1.12 (s, 3H), 1.13 (s, 3H), 1.14 (s, 3H),

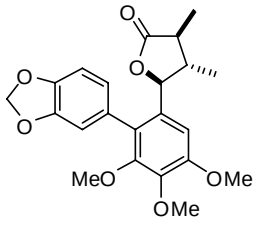
1.73–1.79 (m, 1H), 1.81–1.86 (m, 1H) 3.65 (s, 3H), 3.66 (s, 3H), 3.90 (s, 6H), 3.91 (s, 6H), 5.51 (d, *J* = 5.8 Hz, 1H), 5.65 (d, *J* = 5.8 Hz, 1H), 6.02–6.06 (m, 4H), 6.56 (d, *J* = 8.2 Hz, 1H), 6.62 (s, 1H), 6.71–6.75 (m, 2H), 6.80 (s, 1H), 6.82 (s, 1H), 6.87 (d, *J* = 8.2 Hz, 1H), 6.89 (d, *J* = 8.2 Hz, 1H).

^{13}C NMR ($CHCl_3$) **:** 12.0 (q), 12.1 (q), 20.0 (q), 24.7 (q), 24.8 (q), 44.1 (d), 44.4 (d), 44.9 (d), 56.2 (q), 60.9 (q), 61.1 (q), 61.2 (q), 78.9 (d), 79.0 (d), 101.2 (t), 104.8 (d), 104.9 (d), 108.2 (d), 108.4 (d), 109.7 (d), 110.7 (d), 122.3 (d), 123.4 (d), 129.1 (s), 130.6 (s), 141.5 (s), 146.9 (s), 147.7 (s), 151.5 (s), 152.9 (s), 181.3 (s) ppm.

Maldi top **:** 415.98 $[M+H]^+$, 438.02 $[M+Na]^+$

Elemental Analysis **:** Calcd: C, 66.65; H, 6.32%.
Found: C,66.90; H, 6.41%.

Total synthesis of (±)-4-*epi*-eupomatilone-6 (3) and its 3-methylated derivative 30.



3

Compound **29** (0.5 g, 1.29 mmol) was subjected to essentially the same reaction conditions to produce **30** (0.068 g, 12 %) and **3** (0.37 g, 71 %).

Spectral data of compound 3.

Mol. Formula **:** $C_{22}H_{24}O_7$

IR ($CHCl_3$) **:** 3429, 2932, 1772, 1596, 1457, 1406, 1326, 1226, 1197, 1127, 1040, 937, 754 cm^{-1}.

^{1}H NMR ($CHCl_3$) **:** δ 0.87 (d, 3 H, *J* = 6.00 Hz), 0.89 (d, 3 H, *J* = 6.00 Hz), δ 1.23 (s, 3 H), 1.26 (s, 3 H), 1.92–2.24 (m, 4 H), 3.62(s, 3

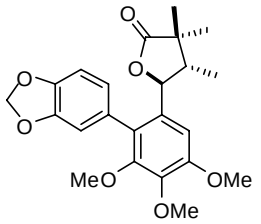
H), 3.63(s, 3 H), 3.91 (s, 12 H), 4.78 (d, 1 H, *J* = 9.46 Hz), 4.80 (d, 1 H, *J* = 9.46 Hz), 6.01–6.03 (m, 4 H), 6.61–6.73 (m, 6 H), 6.83–6.87 (m, 2 H).

^{13}C NMR ($CHCl_3$) **:** 12.9 (q), 14.3 (q), 43.3 (d), 47.6 (d), 56.1 (d), 60.8 (d), 60.9 (d), 61.0 (d), 82.5 (d), 82.6 (d), 101.1 (t), 101.1 (t), 105.1 (d), 105.1 (d), 107.8 (d), 108.1 (d), 110.5 (d), 111.4 (d), 123.3 (d), 124.3 (d), 128.8 (s), 128.9 (s), 129.9 (s), 130.6 (s), 130.8 (s), 142.6 (s), 146.8 (s), 146.9 (s), 147.3 (s), 147.5 (s), 151.2 (s), 151.2 (s), 153.3 (s), 178.4 (s), 178.5 (s) ppm.

Maldi top **:** 401.57 $[M+H]^+$, 423.57 $[M+Na]^+$

Elemental Analysis **:** Calcd: C, 65.99; H, 6.04%.
Found: C, 66.15; H, 6.07%.

Spectral data of 3-methyl 4-*epi*-eupomatilone-6 (30).



30

Mol. Formula **:** $C_{22}H_{24}O_7$

^{1}H NMR ($CHCl_3$) **:** δ 0.73 (d, *J* = 7.1 Hz, 3H), 0.74 (d, *J* = 7.1 Hz, 3H), 0.96(s, 3H), 0.97 (s, 3H), 1.22 (s, 6H), 2.10-2.16(m, 2H), 3.62 (s, 3H), 3.63 (s, 3H), 3.91 (s, 6H), 3.92 (s, 6H), 4.85 (d, *J* = 10.5 Hz, 1H), 4.90 (d, *J* = 10.5 Hz, 1H), 6.02–6.04 (m, 2H), 6.05–6.06 (m, 2H), 6.63 (dd, *J* = 7.9, 1.6 Hz, 1H), 6.69–6.74 (m, 5H), 6.86 (d, *J* = 7.9 Hz, 1H), 6.87 (d, *J* = 7.9 Hz, 1H).

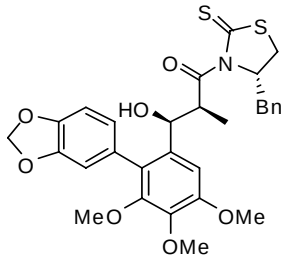
^{13}C NMR ($CHCl_3$) **:** 9.7 (q), 18.2 (q), 23.2 (q), 43.2 (s), 49.6 (d), 56.1 (q), 60.7 (q), 60.9 (q), 61.0 (q), 80.8 (d), 80.8 (d), 101.0 (t),

104.9 (d), 105.0 (d), 107.7 (d), 108.0 (d), 110.4 (d), 111.4 (d), 123.2 (d), 124.3 (d), 125.2 (d), 128.1 (d), 128.7 (s), 128.8 (s), 128.9 (d), 129.7 (s), 129.7 (s), 130.9 (s), 130.9 (s), 142.4 (s), 146.7 (s), 146.8 (s), 147.3 (s), 147.4 (s), 151.1 (s), 151.2 (s), 153.2 (s), 181.5 (s), 181.6 (s) ppm.

Maldi top : 401.57 $[M+H]^+$, 423.57 $[M+Na]^+$

Elemental Analysis : Calcd: C, 66.65; H, 6.32%.
Found: C, 66.79; H, 6.42%.

(2S,3S)-3-(2-(benzo[d][1,3]dioxol-5-yl)-3,4,5-trimethoxyphenyl)-1-((S)-4-benzyl-2-thioxothiazolidin-3-yl)-3-hydroxy-2-methylpropan-1-one (**32**).



32

To a solution of *N*-propionyl-thiazolidinethione (**33**, 9.2 g, 34.7 mmol) in dry CH_2Cl_2 (90 ml) at 0 ^oC was added $TiCl_4$ (6.3 g, 33.4 mmol) and TMEDA (8.9 g, 87 mmol) and stirred for 20 min. To this dark red enolate solution was added biaryl aldehyde **5** (10.0 g, 31.6 mmol) in dry CH_2Cl_2 (30 ml) and stirring continued for additional 2 h at 0 ^oC. The reaction was quenched with aqueous NH_4Cl (60 ml) and layers separated. The aqueous layer was extracted with ethyl acetate. The combined organic layer was dried over sodium sulphate, filtered and concentrated. Purification of the residue by column chromatography on silica gel with light petroleum: ethyl acetate (4:1) as eluent gave **32** (12 g, 65%).

Mol. Formula : $C_{30}H_{31}NO_7S_2$

$[\alpha]_D^{25}$: +133 (c = 1.0, $CHCl_3$).

IR ($CHCl_3$) : 3400, 3019, 1672, 1597, 1456, 1341, 1215, 1041, 938, 759, 668 cm^{-1}.

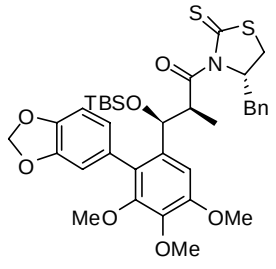
^1H NMR (200 MHz, $CHCl_3$) : δ 1.23 (d, J = 7.0 Hz, 3H), 1.27 (d, J = 7.0 Hz, 3H), 2.79

(d, *J* = 11.4 Hz, 1H), 2.80 (d, *J* = 11.4 Hz, 1H), 2.87–3.19 (m, 8H), 3.56 (s, 3H), 3.58 (s, 3H), 3.86 (s, 6H), 3.94 (s, 6H), 4.31–4.39 (m, 2H), 4.72–4.89 (m, 4H), 5.94 (d, *J* = 1.3 Hz, 1H), 5.96 (d, *J* = 1.3 Hz, 1H), 6.02 (d, *J* = 1.5 Hz, 1H), 6.08 (d, *J* = 1.5 Hz, 1H), 6.59–6.66 (m, 4H), 6.78 (d, *J* = 7.7 Hz, 1H), 6.99 (d, *J* = 7.8 Hz, 1H), 6.98 (s, 1H), 6.99 (s, 1H), 7.19–7.39 (m, 10H).

^{13}C NMR ($CHCl_3$) **:** 11.3 (q), 11.5 (q), 32.4 (t), 32.5 (t), 36.2 (t), 44.4 (d), 55.8 (q), 60.5 (q), 60.7 (q), 60.8 (q), 68.7 (d), 68.8 (d), 71.5 (d), 71.7 (d), 95.9 (d), 100.7 (t), 101.0 (t), 105.2 (d), 105.3 (d), 107.4 (d), 108.2 (d), 110.0 (d), 111.6 (d), 122.4 (d), 124.1 (d), 127.0 (d), 127.2 (s), 128.7 (d), 128.8 (s), 129.1 (d), 134.7 (s), 134.8 (s), 136.1 (s), 141.3 (s), 141.3 (s), 146.4 (s), 146.5 (s), 147.0 (s), 147.3 (s), 151.0 (s), 151.1 (s), 152.5 (s), 152.5 (s), 178.2 (s), 178.3 (s), 200.2 (s), 200.5 (s) ppm.

Elemental Analysis **:** Calcd: C, 61.94; H, 5.37; N, 2.41; S, 11.02 %.
Found: C, 62.02; H, 5.54; N, 2.40; S, 11.29%.

(2S,3S)-3-(2-(benzo[d][1,3]dioxol-5-yl)-3,4,5-trimethoxyphenyl)-1-((S)-4-benzyl-2-thioxothiazolidin-3-yl)-3-(tert-butyldimethylsilyloxy)-2-methylpropan-1-one 34.



34

A solution of **32** (8 g, 13.7 mmol), 2,6-lutidine (14.7 g, 137 mmol) and *t*-butyldimethylsilyl trifluoromethanesulphonate (7.245 g, 27.5 mmol) in CH_2Cl_2 (40 ml) was stirred for 5 h at 0 ^oC and then quenched with saturated aqueous $NaHCO_3$. The aqueous

phase was extracted with CH_2Cl_2 and the combined organic layer washed sequentially with saturated aqueous $NaHCO_3$, H_2O, brine and dried over sodium sulfate. The solvent was removed and residue purified by silica gel chromatography by eluting with light petroleum: ethyl acetate (19:1) to afford **34** (7.6 g, 79%).

Mol. Formula : $C_{36}H_{45}NO_7S_2Si$

$[\alpha]_D^{25}$: +106.8 (*c* = 1, $CHCl_3$).

IR ($CHCl_3$) : 3019, 1697, 1596, 1482, 1302, 1216, 1040, 938, 836, 758, 668 cm^{-1}.

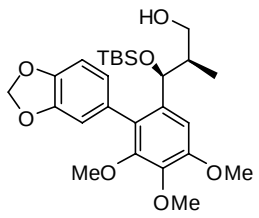
^{1}H NMR (200 MHz, $CHCl_3$) : δ –0.26 (s, 3H), –0.23 (s, 3H), –0.07 (s, 3H), –0.05(s, 3H), 0.84 (s, 9H), 0.86(s, 9H), 1.30 (d, *J* = 6.4 Hz, 3H), 1.32 (d, *J* = 6.4 Hz, 3H), 2.65–2.78 (m, 4H), 2.86 (dd, *J* = 11.0, 2.0 Hz, 1H), 2.95 (dd, *J* = 11.0, 2.0 Hz, 1H), 3.22 (dd, *J* = 13.3, 3.4 Hz, 2H), 3.49 (s, 3H), 3.50 (s, 3H), 3.84 (s, 6H), 3.94 (s, 6H), 4.46–4.53 (m, 2H), 4.61–4.72 (m, 2H), 4.73 (d, *J* = 7.2 Hz, 1H), 4.76 (d, *J* = 7.2 Hz, 1H), 6.01 (d, *J* = 1.4 Hz, 1H), 6.03 (d, *J* = 1.4 Hz, 1H), 6.07 (d, *J* = 1.4 Hz, 1H), 6.13 (d, *J* = 1.4 Hz, 1H), 6.60–6.65 (m, 2H), 6.75–6.82 (m, 2H), 6.83 (d, *J* = 8.1 Hz, 1H), 6.93 (s, 2H), 6.99 (d, *J* = 8.1 Hz, 1H), 7.17–7.35 (m, 10H).

^{13}C NMR (50 MHz, $CHCl_3$) : –4.8 (q), –4.7 (q), –4.6 (q), –4.5 (q), 13.4 (q), 13.6 (q), 18.3 (s), 25.8 (q), 32.4 (t), 36.5 (t), 47.4 (d), 47.6 (d), 55.8 (q), 60.7 (q), 60.9 (q), 69.6 (d), 69.6 (d), 73.0 (d), 73.2 (d), 101.0 (t), 101.1 (t), 105.8 (d), 107.4 (d), 108.1 (d), 110.9 (d), 112.0 (d), 123.5 (d), 124.5 (d), 126.9 (d), 127.2 (d), 128.5 (d), 128.7 (d), 128.9 (d), 129.3 (d), 136.6 (s), 136.7 (s), 141.4 (s), 146.6 (s), 146.7 (s), 147.1 (s), 147.4 (s), 151.0 (s), 152.5 (s), 176.5 (s), 176.7 (s), 200.7 (s), 200.8 (s) ppm.

Elemental Analysis : Calcd: C, 62.13; H, 6.52; N, 2.01; S, 9.21%.

Found: C, 61.96; H, 6.43; N, 2.00; S, 9.01 %.

(2R,3S)-3-(2-(benzo[d][1,3]dioxol-5-yl)-3,4,5-trimethoxyphenyl)-3-(tert-butyldimethylsilyloxy)-2-methylpropan-1-ol 36.



36

Compound **34** (5 g, 7.2 mmol) and $NaBH_4$ (540 mg, 14.3 mmol) in THF (25 ml) and EtOH (5 ml) were stirred at room temperature for 5 h. Excess borohydride was quenched at 0 °C with dil. HCl and concentrated. The residue was partioned between water-ether, organic layer separated and washed with 1 M NaOH, H_2O, brine, dried over sodium sulphate, and concentrated. The crude was purified on silica gel by using light petroleum: ethyl acetate (4:1) as an eluent to give **36** (2.91 g, 82%).

Mol. Formula : $C_{26}H_{38}O_7Si$

$[\alpha]_D^{25}$: –8.5 (*c* = 1.05, $CHCl_3$).

IR ($CHCl_3$) : 3389, 2930, 1598, 1481, 1402, 1384, 1322, 1232, 1125, 1040, 939, 872, 838, 757 cm^{-1}.

^{1}H NMR (200 MHz, $CHCl_3$) : δ –0.15 (s, 3H), –0.15(s, 3H), 0.01 (s, 6H), 0.75 (d, *J* = 6.9 Hz, 3H), 0.78 (d, *J* = 6.9 Hz, 3H), 0.92 (s, 18H), 1.53–1.68 (br m, 2H), 3.33 (s, 2H), 3.36 (s, 2H), 3.62 (s, 3H), 3.63 (s, 3H), 3.89 (s, 6H), 3.90 (s, 6H), 4.80 (d, *J* = 2.7 Hz, 1H), 4.89 (d, *J* = 2.7 Hz, 1H), 6.01–6.04 (m, 4H), 6.60 (dd, *J* = 7.8, 1.7 Hz, 1H), 6.63–6.67 (m, 3H), 6.85 (d, *J* = 7.8 Hz, 1H), 6.87 (d, *J* = 8.1 Hz, 1H), 6.93 (s, 2H).

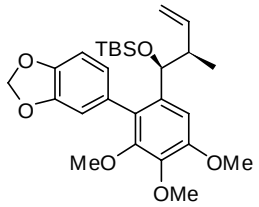
^{13}C NMR (50 MHz, $CHCl_3$) : –5.2 (q), –4.5 (q), 9.6 (q), 18.0 (s), 25.8 (q), 42.4 (d), 42.6 (d), 55.6 (q), 60.7 (q), 60.8 (q), 60.9 (q), 65.8 (t), 70.7 (d), 100.9 (t), 106.3 (d), 107.8 (d), 107.9 (d), 109.9

(d), 111.4 (d), 122.5 (d), 124.0 (d), 126.0 (s), 129.6 (s), 129.7 (s), 137.7 (s), 137.8 (s), 140.6 (s), 146.4 (s), 146.5 (s), 147.3 (s), 147.4 (s), 150.9 (s), 152.0 (s) ppm.

Maldi top : 513.57 $[M+Na]^+$

Elemental Analysis : Calcd: C, 63.64; H, 7.81%.
Found: C, 63.81; H, 7.76%.

((1S,2R)-1-(2-(benzo[d][1,3]dioxol-5-yl)-3,4,5-trimethoxyphenyl)-2-methylbut-3-enyloxy)(tert-butyl)dimethylsilane 37.



19

A suspension of alcohol **36** (2.0 g, 4.1 mmol) and Dess Martin periodinane (2.08 g, 4.9 mmol) in CH_2Cl_2 (10 ml) was stirred at 0 ^oC for 1.5 h, filtered through Celite and concentrated to procure the aldehyde (1.8 g, 90%) which was used immediately for the next reaction.

To a suspension of methyl-triphenylphosphonium iodide (7.4 g, 18.3 mmol) in anhydrous THF (30 ml) at 0°C was added *n*-BuLi (23 ml of 1.6 M in hexane). The mixture was stirred for 1 h at room temperature and then cannulated into the above prepared aldehyde solution in THF (10 ml) maintained at 0 ^oC. The reaction mixture was allowed to attain to room temperature and further stirred for 12 h. The reaction was quenched with saturated aqueous NH_4Cl, extracted with ethyl acetate, dried (Na_2SO_4) and concentrated. Purification of the residue on silica gel by using light petroleum: ethyl acetate (19:1) to give **37** (1.49 g, 75%).

Mol. Formula : $C_{27}H_{38}O_6Si$

$[\alpha]_D^{25}$: –7.98 (*c* = 1, $CHCl_3$).

IR ($CHCl_3$) : 3379, 2932, 2856, 1597, 1481, 1401, 1322, 1195, 1157, 1083, 1040, 938, 837, 775 cm^{-1}.

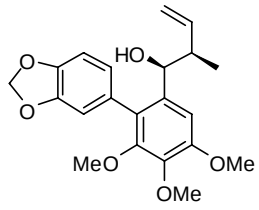
^1H NMR (200 MHz, $CHCl_3$) : δ –0.19 (s, 3H), –0.18 (s, 3H), –0.06 (s, 6H), 0.88–0.91

(m, 6H), 0.89 (s, 18H), 2.11–2.25 (m, 2H), 3.63 (s, 6H), 3.89 (s, 6H), 3.90 (s, 6H), 4.52 (d, *J* = 3.6 Hz, 1H), 4.57 (d, *J* = 3.6 Hz, 1H), 4.67 (ddd, *J* = 17.2, 1.8, 1.3 Hz, 2H), 4.82 (br.ddd, *J* = 10.2, 1.7, 0.9 Hz, 2H), 5.58 (ddd, *J* = 17.2, 10.2, 7.3 Hz, 1H), 5.60 (ddd, *J* = 17.2, 10.2, 7.3 Hz, 1H), 6.01–6.04 (m, 4H), 6.60–6.69 (m, 4H), 6.84–6.95 (m, 4H).

^{13}C NMR (50 MHz, $CHCl_3$) **:** –4.9 (q), –4.6 (q), 12.3 (q), 12.4 (q), 18.2 (s), 25.8 (q), 44.7 (d), 44.8 (d), 55.7 (q), 60.7 (q), 60.9 (q), 61.0 (q), 74.3 (d), 100.9 (t), 106.2 (d), 106.3 (d), 107.8 (d), 107.9 (d), 109.9 (d), 111.8 (d), 113.6 (t), 115.3 (d), 120.0 (d), 122.5 (d), 124.4 (d), 126.4 (s), 129.4 (d), 129.7 (s), 129.9 (s), 138.2 (s), 140.6 (s), 142.5 (s), 142.6 (s), 146.4 (s), 146.5 (s), 147.3 (s), 150.8 (s), 152.0 (s) ppm.

Elemental Analysis **:** Calcd: C, 66.63; H, 7.87%.
Found: C, 66.87; H, 7.78%.

(1S,2R)-1-(2-(benzo[d][1,3]dioxol-5-yl)-3,4,5-trimethoxyphenyl)-2-methylbut-3-en-1-ol 31.



31

Compound **37** (1 g, 2.1 mmol) and *n*-Bu_4NF (4.1 ml, 1 M in THF) in anhydrous THF (5 ml) were stirred for 4 h at room temperature. Diluted with water and extracted with ether. The organic layer was washed with brine, dried over anhydrous sodium sulphate, filtered and concentrated. The crude was purified on silica gel by eluting with light petroleum: ethyl acetate (6:1) to afford **31** (0.65 g, 85 %).

Mol. Formula **:** $C_{21}H_{24}O_6$

$[\alpha]_D^{25}$ **:** –11.9 (*c* = 1, $CHCl_3$).

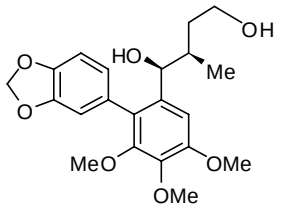
IR ($CHCl_3$) : 3019, 1594, 1481, 1216, 1125, 1041, 849, 758, 669 cm^{-1}.

^{1}H NMR (200 MHz, $CHCl_3$) : δ 0.93 (d, *J* = 6.7 Hz, 3H), 0.94 (d, *J* = 6.7 Hz, 3H), 2.26 (br s, 2H), 2.32–2.43 (m, 2H), 3.57 (s, 3H), 3.58 (s, 3H), 3.86 (s, 6H), 3.88 (s, 6H), 4.45 (d, *J* = 6.3 Hz, 1H), 4.49 (d, *J* = 6.3 Hz, 1H), 4.77–4.93 (m, 4H), 5.53 (ddd, *J* = 17.2, 10.3, 6.8 Hz, 1H), 5.56 (ddd, *J* = 17.2, 10.3, 6.8 Hz, 1H), 5.94–5.99 (br m, 4H), 6.58 (dd, *J* = 7.7, 1.6 Hz, 1H), 6.63–6.66 (m, 3H), 6.79 (br.d, *J* = 7.8 Hz, 1H), 6.81 (d, *J* = 7.8 Hz, 1H), 6.88 (s, 2H).

^{13}C NMR (50 MHz, $CHCl_3$) : 14.3 (q), 14.3 (q), 42.4 (d), 43.5 (d), 55.8 (q), 60.6 (q), 60.8 (q), 60.9 (d), 73.1 (d), 73.2 (d), 100.8 (t), 105.1 (d), 105.5 (d), 107.8 (d), 110.0 (d), 110.1 (d), 110.2 (d), 111.4 (d), 114.7 (t), 114.8 (t), 117.5 (d), 117.6 (d), 122.5 (d), 122.7 (d), 124.2 (s), 127.7 (s), 129.4 (s), 129.6 (s), 135.8 (s), 135.9 (s), 136.5 (s), 136.6 (s), 141.2 (s), 146.5 (s), 147.2 (s), 150.9 (s), 152.5 (s), 152.6 (s) ppm.

Elemental Analysis : Calcd: C, 67.73; H, 6.50%.
Found: C, 67.58; H, 6.79%.

(1S,2R)-1-(2-(benzo[d][1,3]dioxol-5-yl)-3,4,5-trimethoxyphenyl)-2-methylbutane-1,4-diol 38.



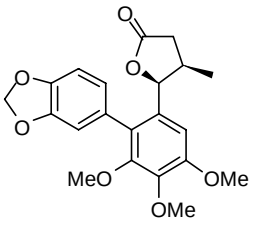
38

To a solution of compound **31** (0.5 g, 1.34 mmol) in anhydrous THF (3 ml) at 0 °C BH_3.DMS (0.15 ml, 1.608 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. It was cooled to 0 ^oC and 3 N aqueous solution of NaOH (0.268 g(ml), 6.7 mmol), aqueous 30 % H_2O_2 (0.76 ml, 6.7 mmol), and EtOH (0.15 ml) were added. The reaction mixture allowed to warm to room temperature. After 3 h, the

volatiles were removed under reduced pressure and the residue diluted with ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulphate, filtered, and concentrated. The crude residue was purified by silica gel chromatography by eluting with light petroleum: ethyl acetate (3:1) to give **38** (0.43 g, 82%).

$[\alpha]_D^{25}$ **:** + 7.51 (*c* = 1, $CHCl_3$).

(4R,5S)-5-(2-(benzo[d][1,3]dioxol-5-yl)-3,4,5-trimethoxyphenyl)-4-methyldihydrofuran-2(3H)-one 39.

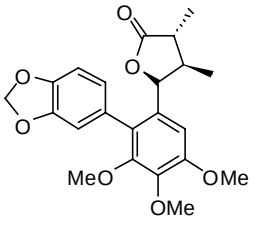


39

A suspension of **38** (0.3 g, 0.77 mmol), TEMPO (0.012 g, 0.077 mmol), *n*-Bu_4NI (0.029g, 0.077 mmol) and N-chlorosuccinimide (0.3 g, 2.2 mmol) in CH_2Cl_2 (3 ml), 0.5M aqueous $NaHCO_3$ (1.5 ml) and 0.05M aqueous K_2CO_3 (1.5 ml) were vigorously stirred for 3 h. The organic layer was separated, and the aqueous phase extracted with CH_2Cl_2. The organic layer was washed with brine, dried, and evaporated. The residue was purified by silica gel column chromatography by eluting with light petroleum: ethyl acetate (4:1) to give **39** (0.24 g, 80%).

$[\alpha]_D^{25}$ **:** –26.39 (*c* = 1, $CHCl_3$).

Synthesis of (+)-eupomatilone-6 (2).



2

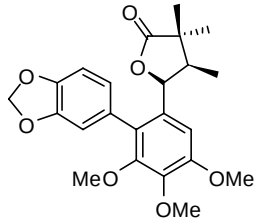
To solution of **39** (0.10 g, 0.258 mmol) in anhydrous THF (1 ml) at –78 ^{0}C, 1M LiHMDS solution in THF (0.3 ml) was added. After 1 h, MeI (0.02 ml, 0.32 mmol) was added and stirring continued for additional 1 h. The reaction mixture was quenched with

saturated ammonium chloride, extracted with ethyl acetate, dried over sodium sulphate and concentrated. The crude residue was purified by silica gel chromatography by eluting with light petroleum: ethyl acetate (9:1) to give **40** (0.015 g, 14 %) and **2** (0.06 g, 58 %).

2:

$[\alpha]_D^{25}$ **:** +24.72 (*c* = 0.5, $CHCl_3$).

3-methyl (+)-eupomatilone-6 derivative 40.

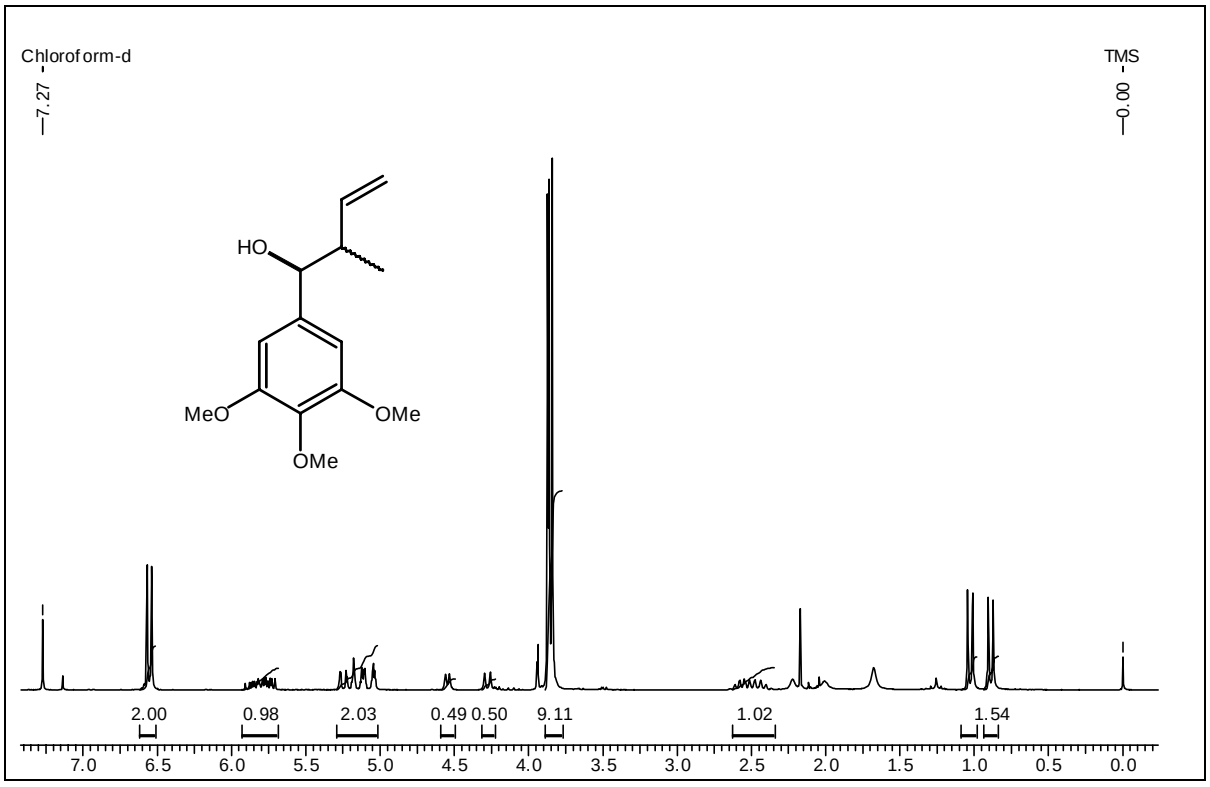


40

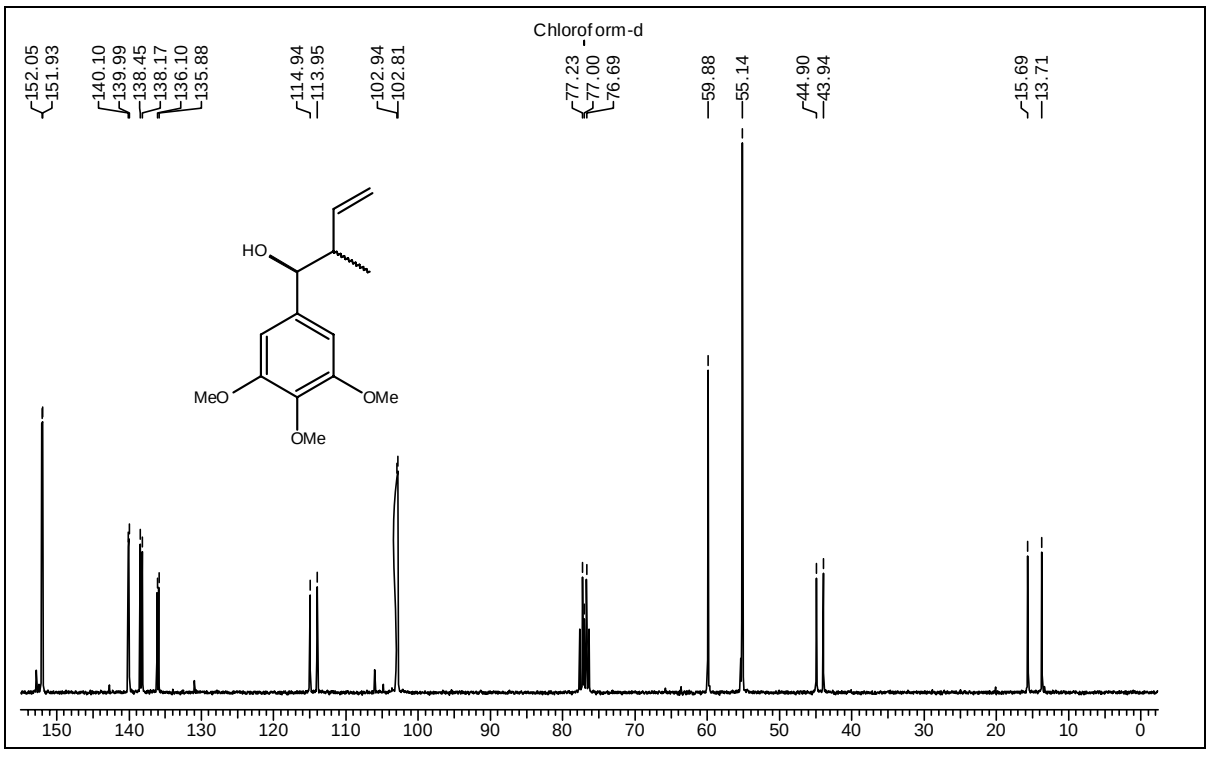
40:

$[\alpha]_D^{25}$ **:** –40.8 (*c* = 0.5, $CHCl_3$).

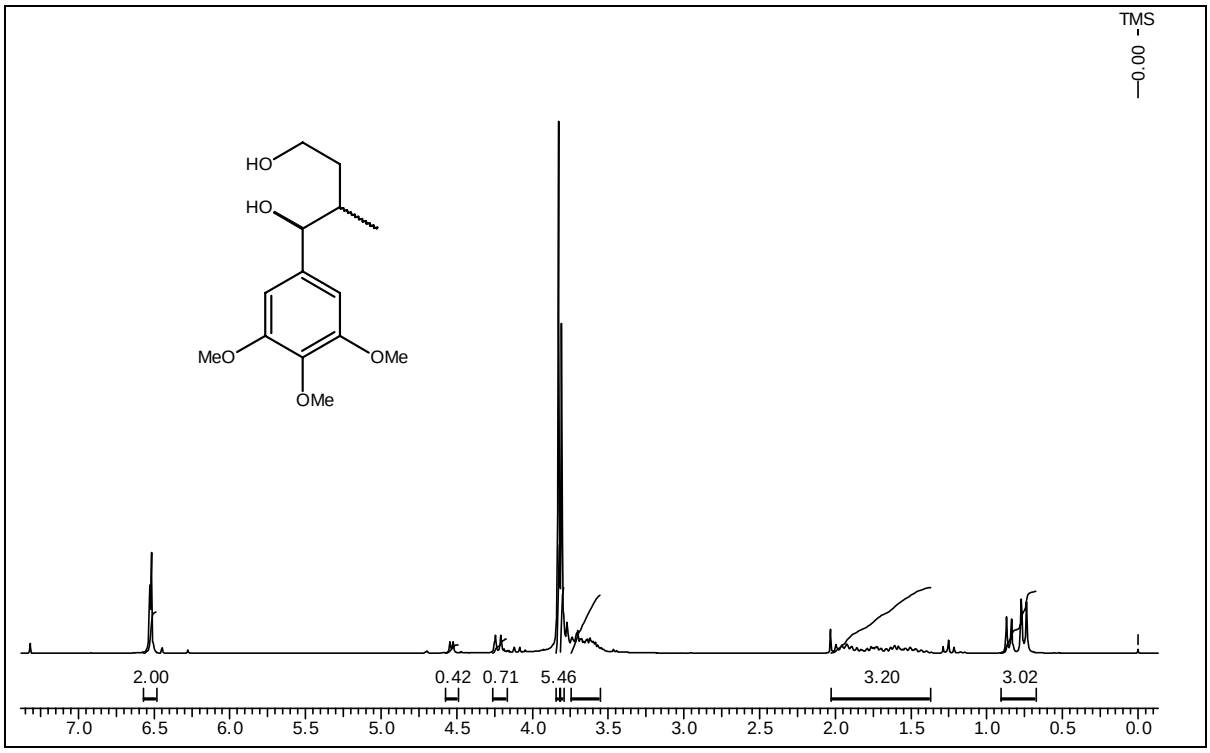
Spectra



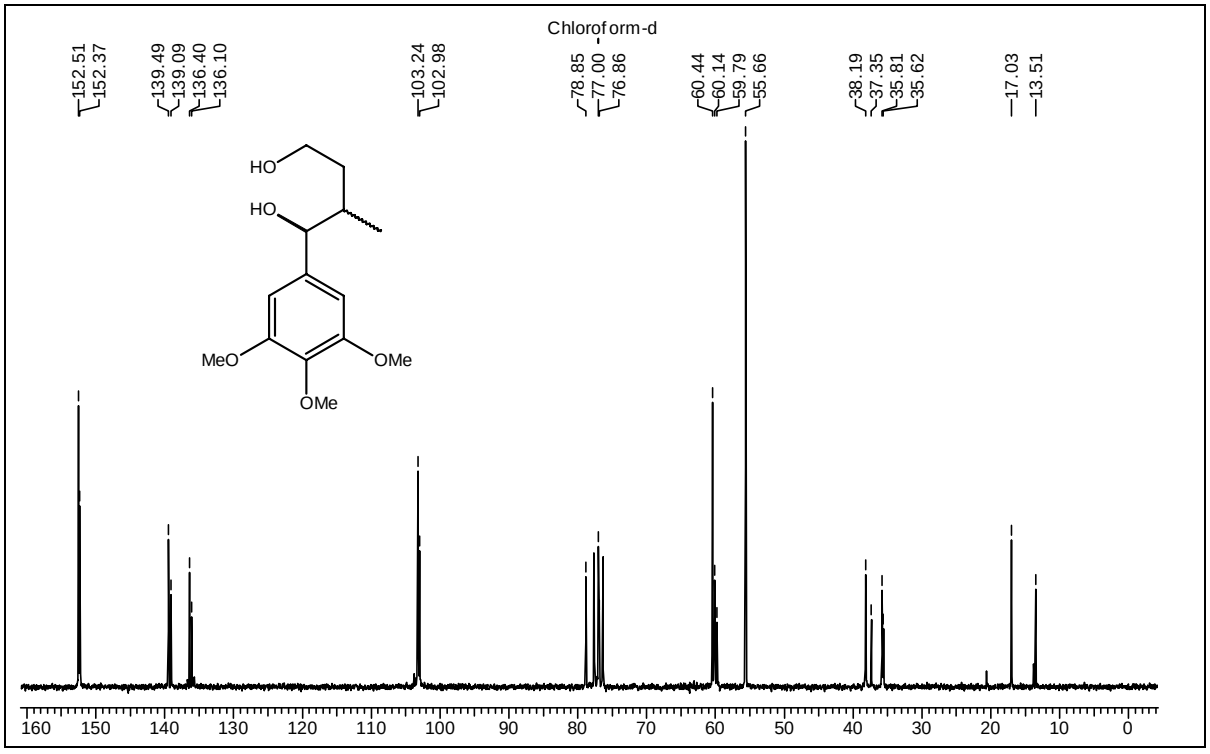
^{1}H NMR spectrum of compound 7/8 in $CDCl_3$



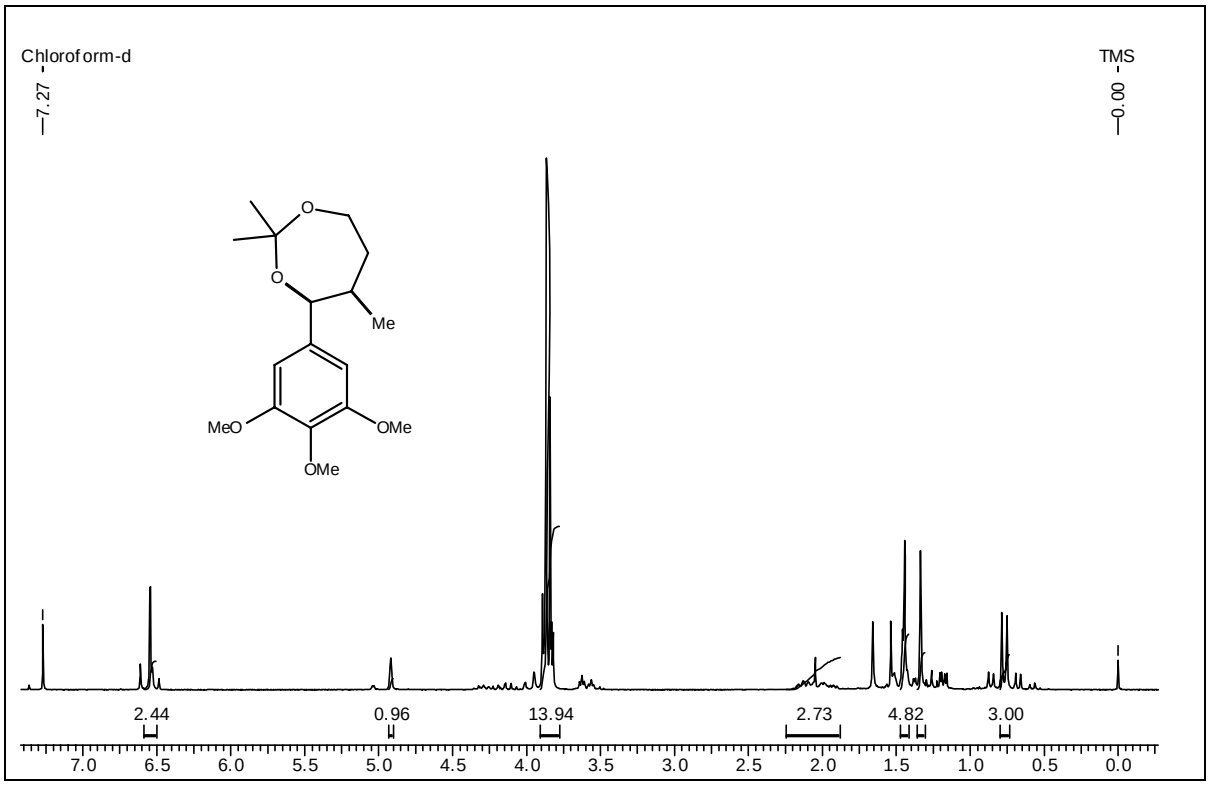
^{13}C NMR spectrum of compound 7/8 in $CDCl_3$



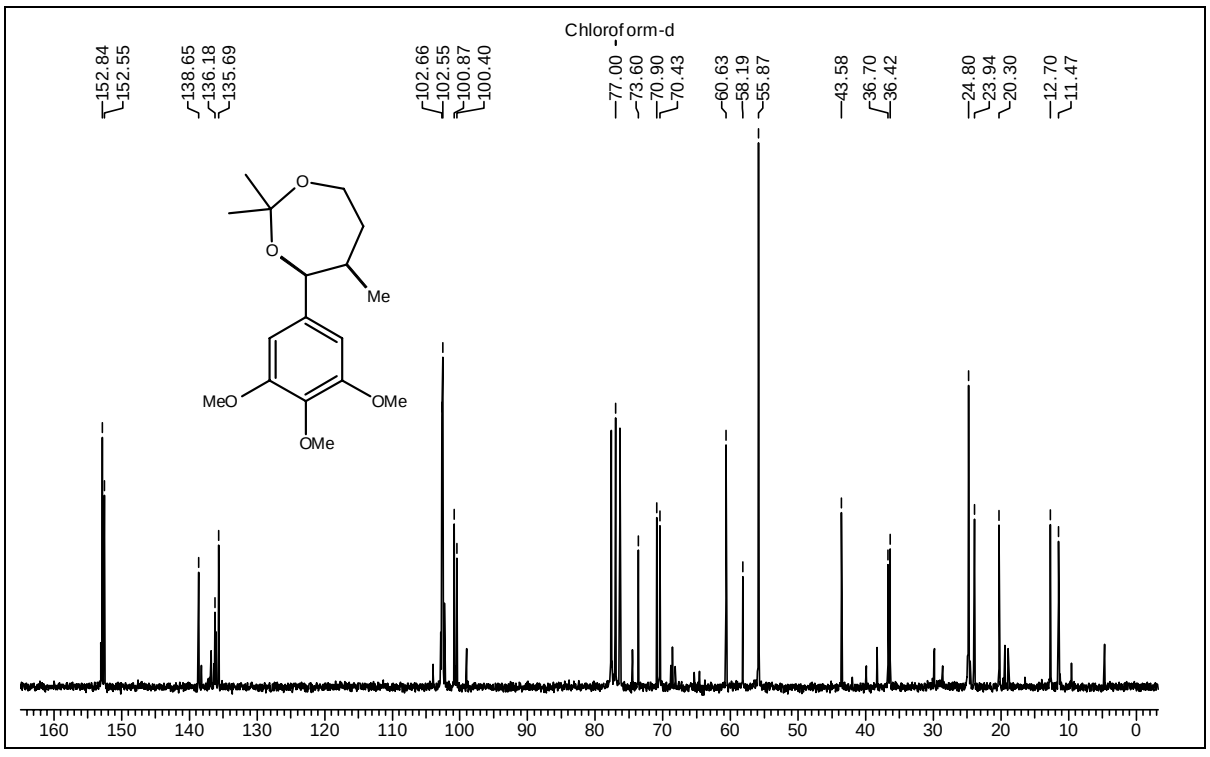
^{1}H NMR spectrum of compound 9/10 in $CDCl_3$



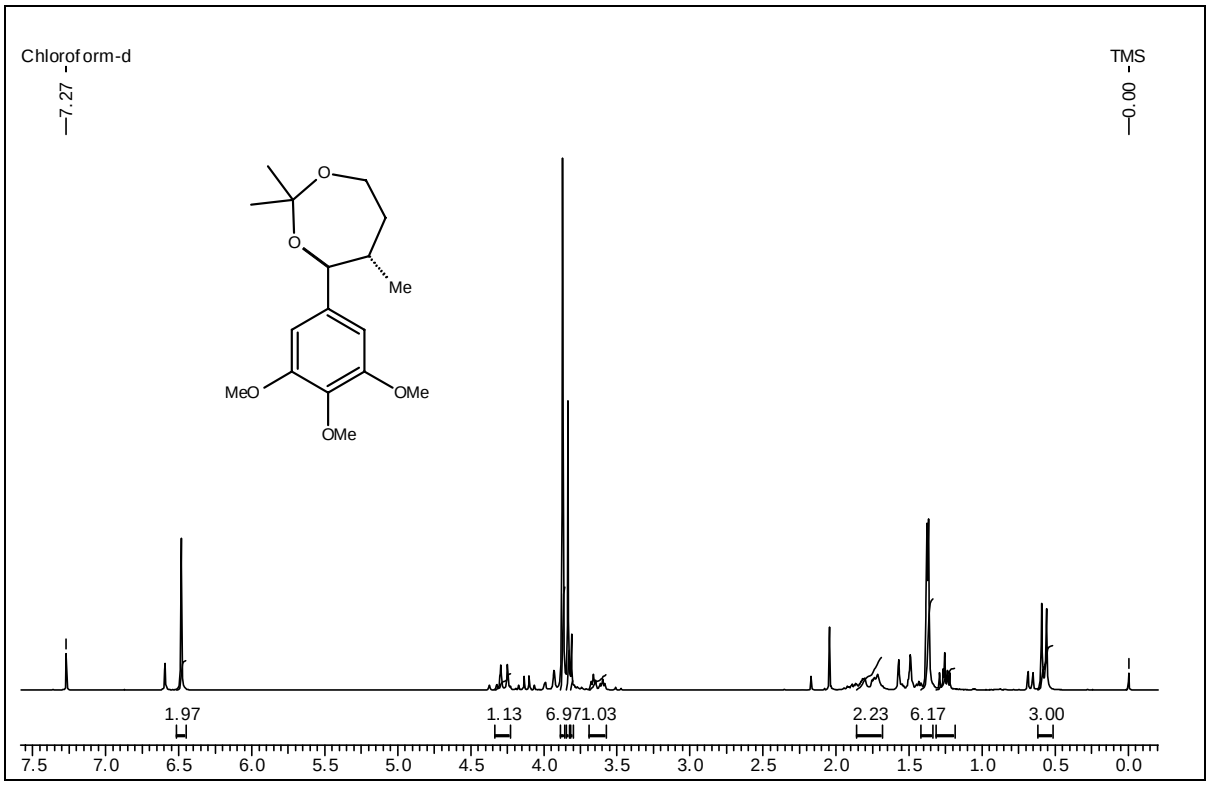
^{13}C NMR spectrum of compound 9/10 in $CDCl_3$



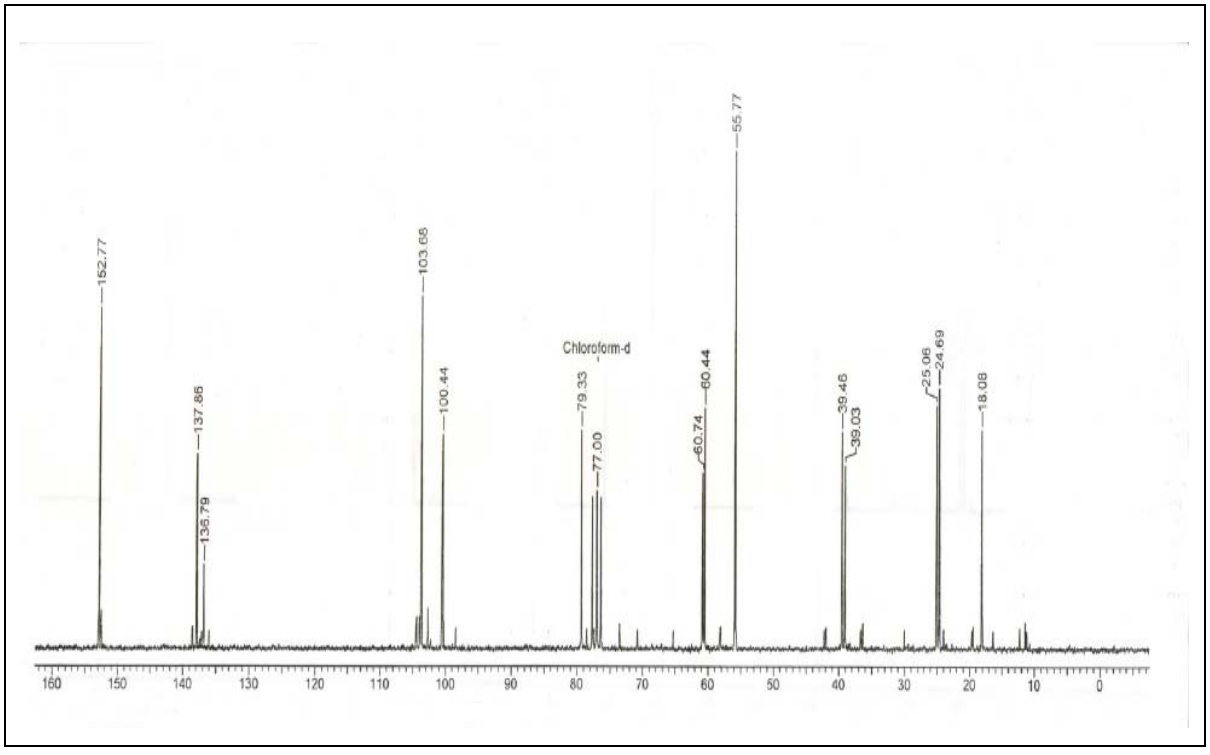
^{1}H NMR spectrum of compound 11 in $CDCl_3$



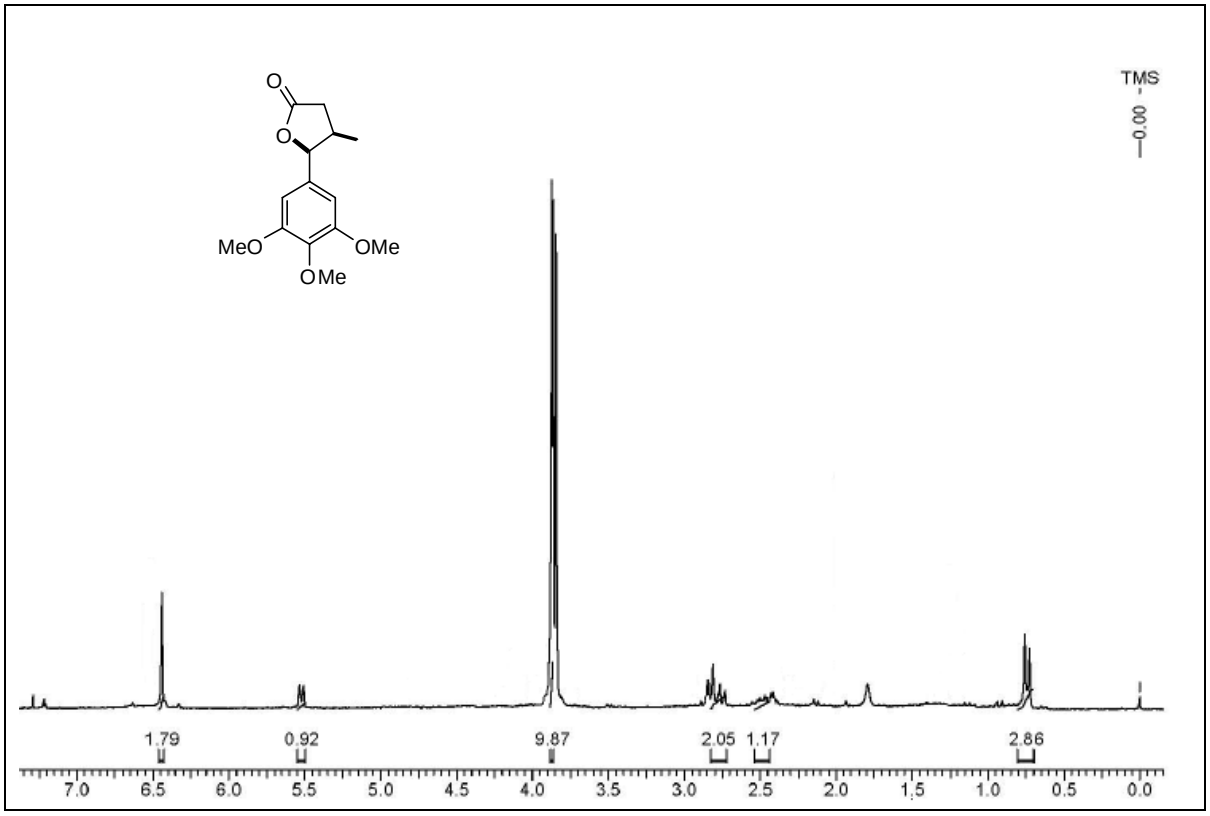
^{13}C NMR spectrum of compound 11 in $CDCl_3$



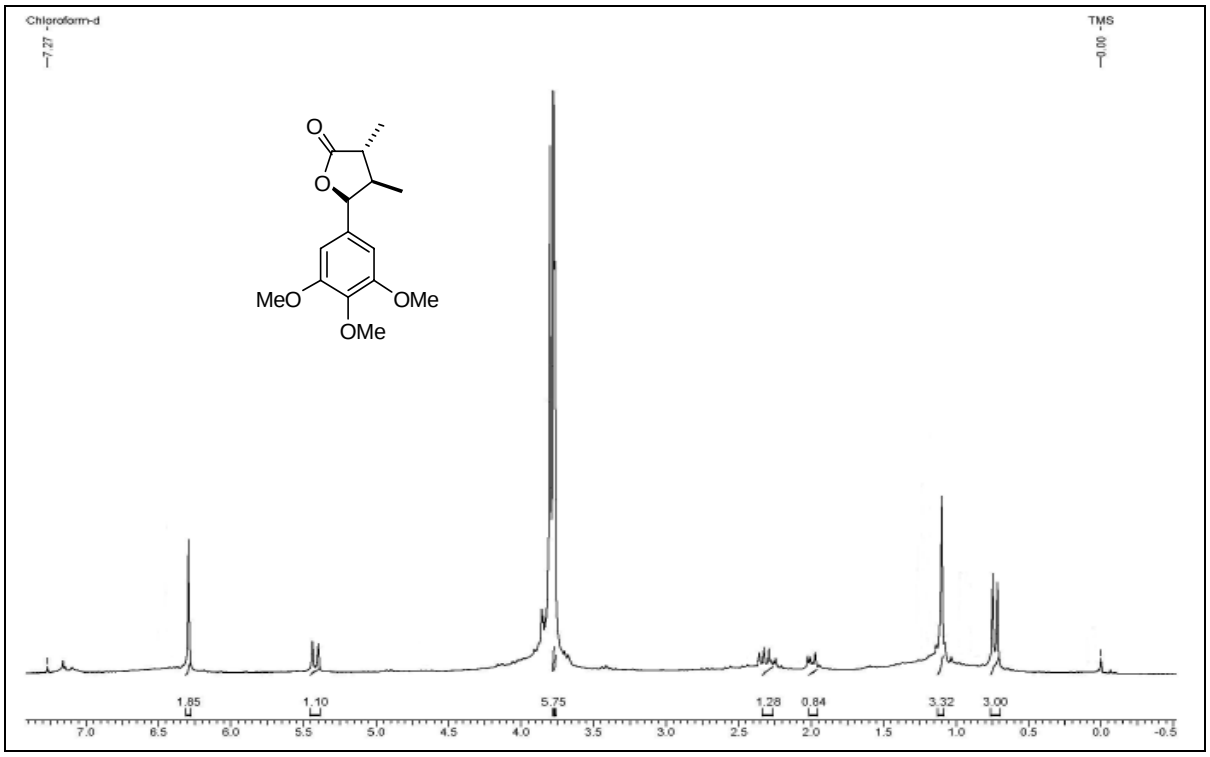
^{1}H NMR spectrum of compound 12 in $CDCl_3$



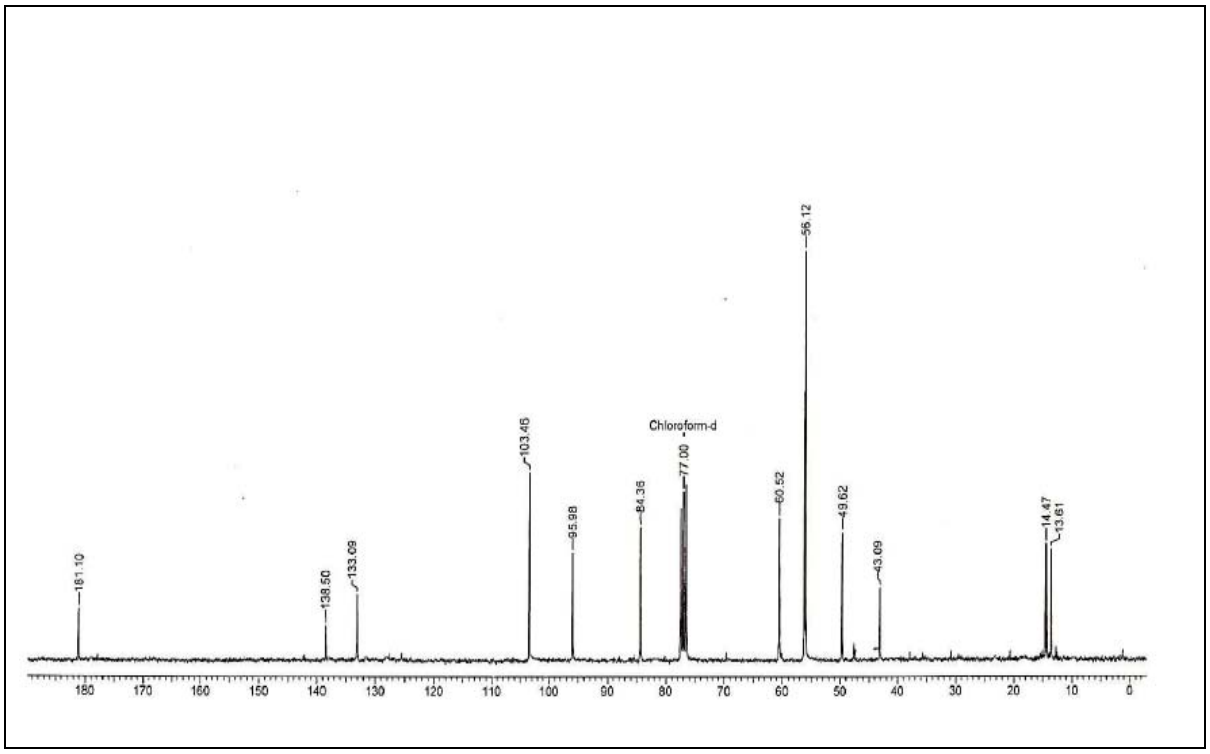
^{13}C NMR spectrum of compound 12 in $CDCl_3$



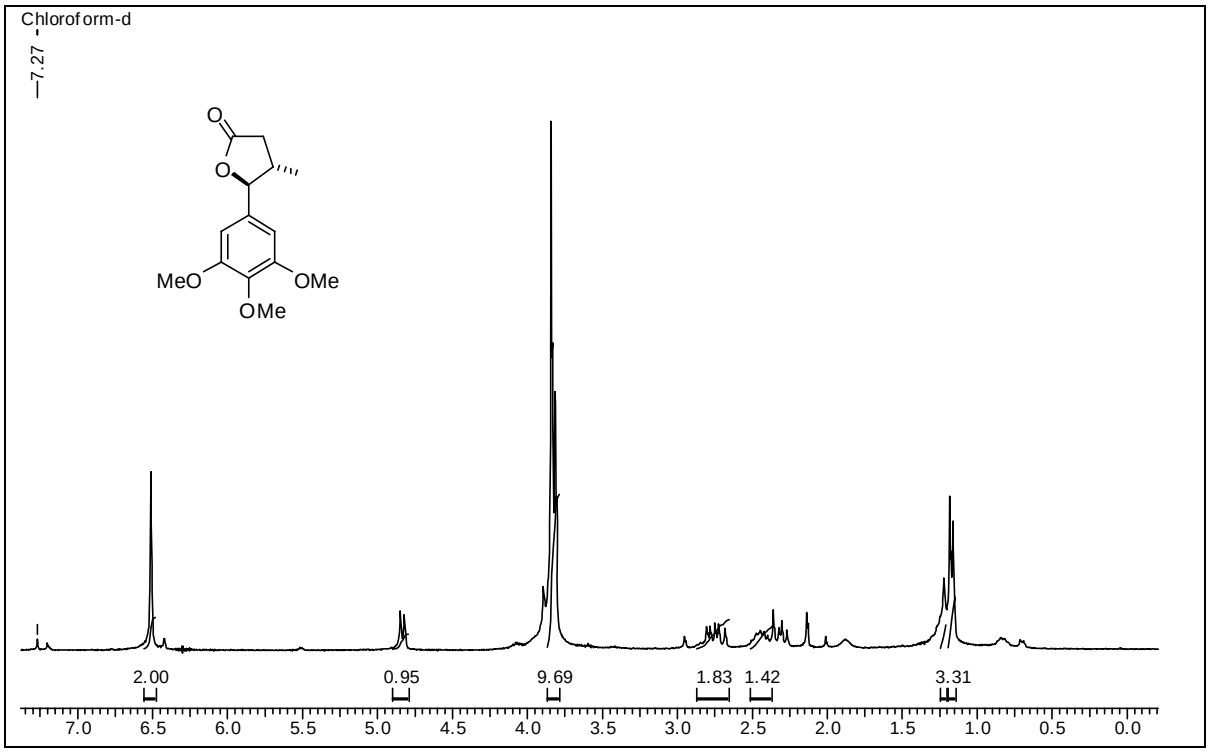
^{1}H NMR spectrum of compound 13 in $CDCl_3$



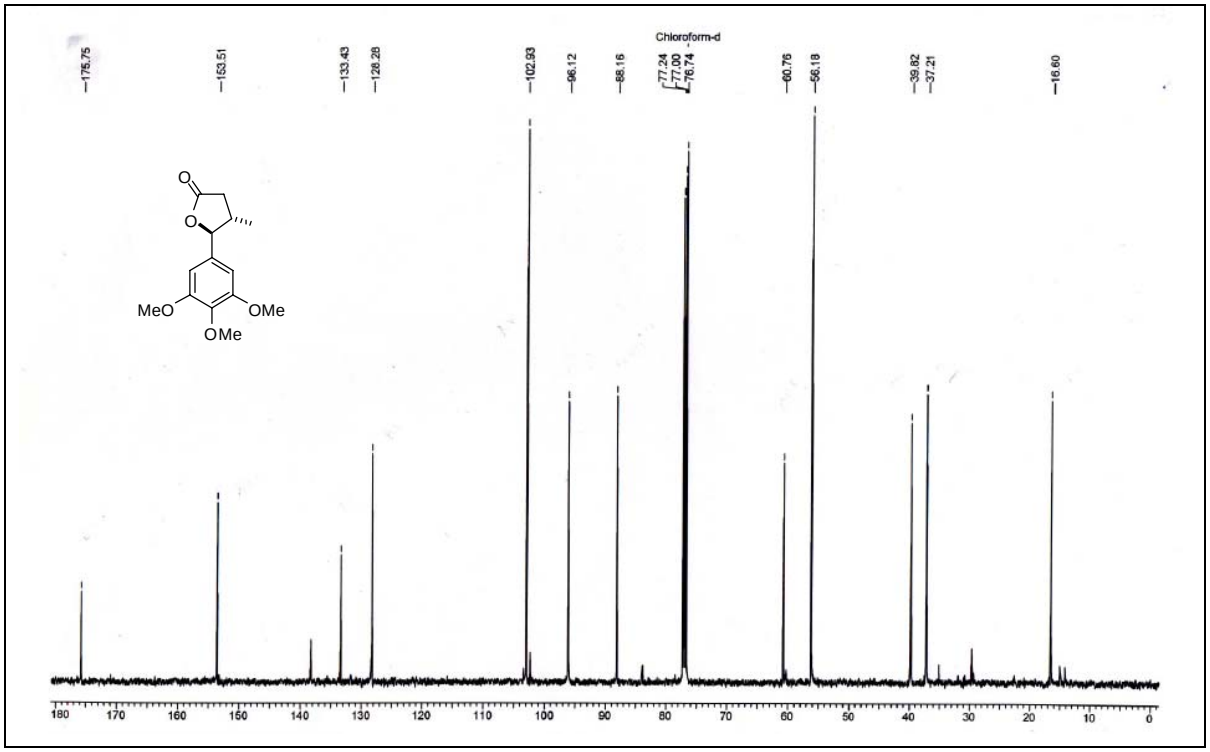
^{1}H NMR spectrum of compound 14 in $CDCl_3$



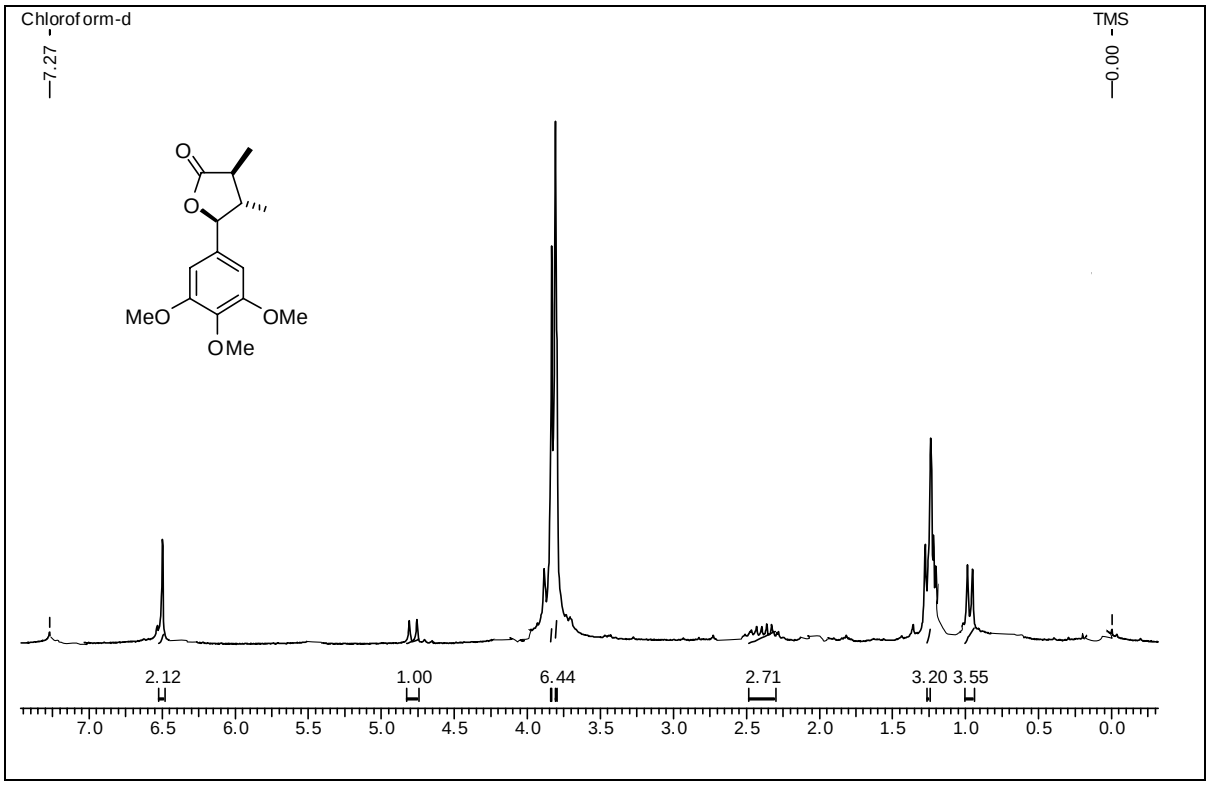
^{13}C NMR spectrum of compound 14 in $CDCl_3$



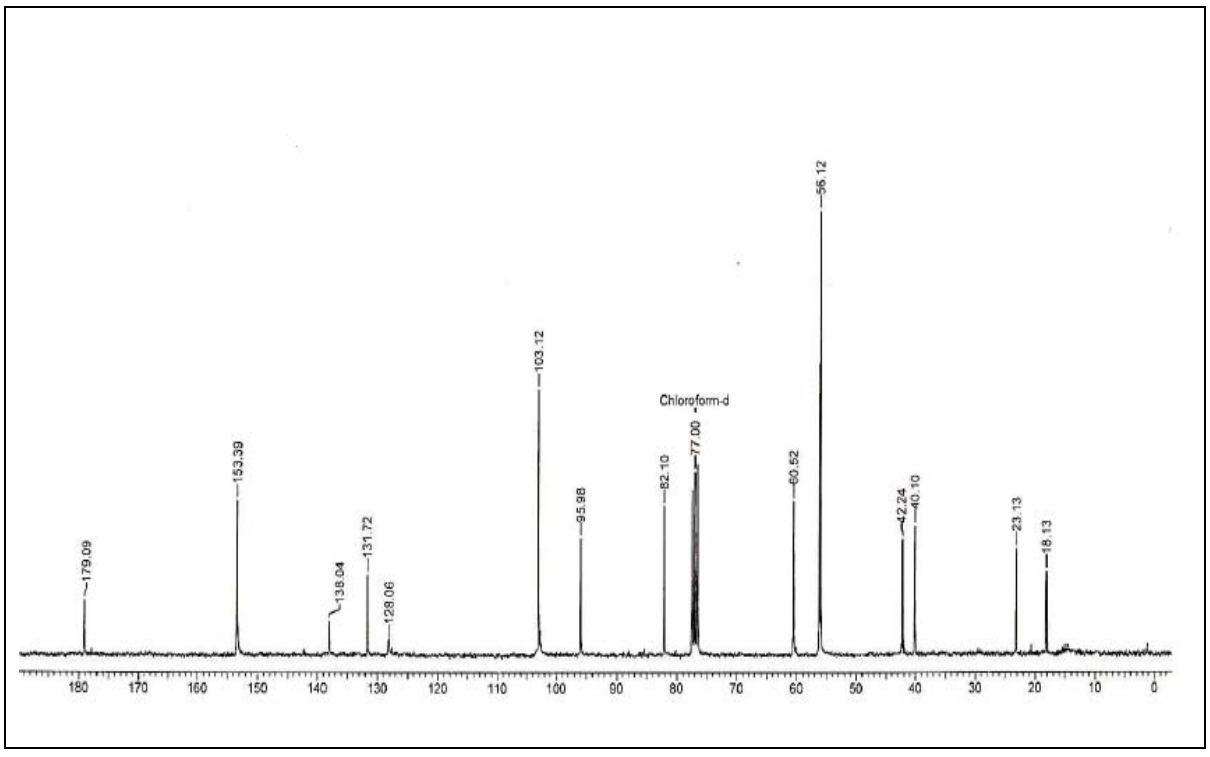
^{1}H NMR spectrum of compound 15 in $CDCl_3$



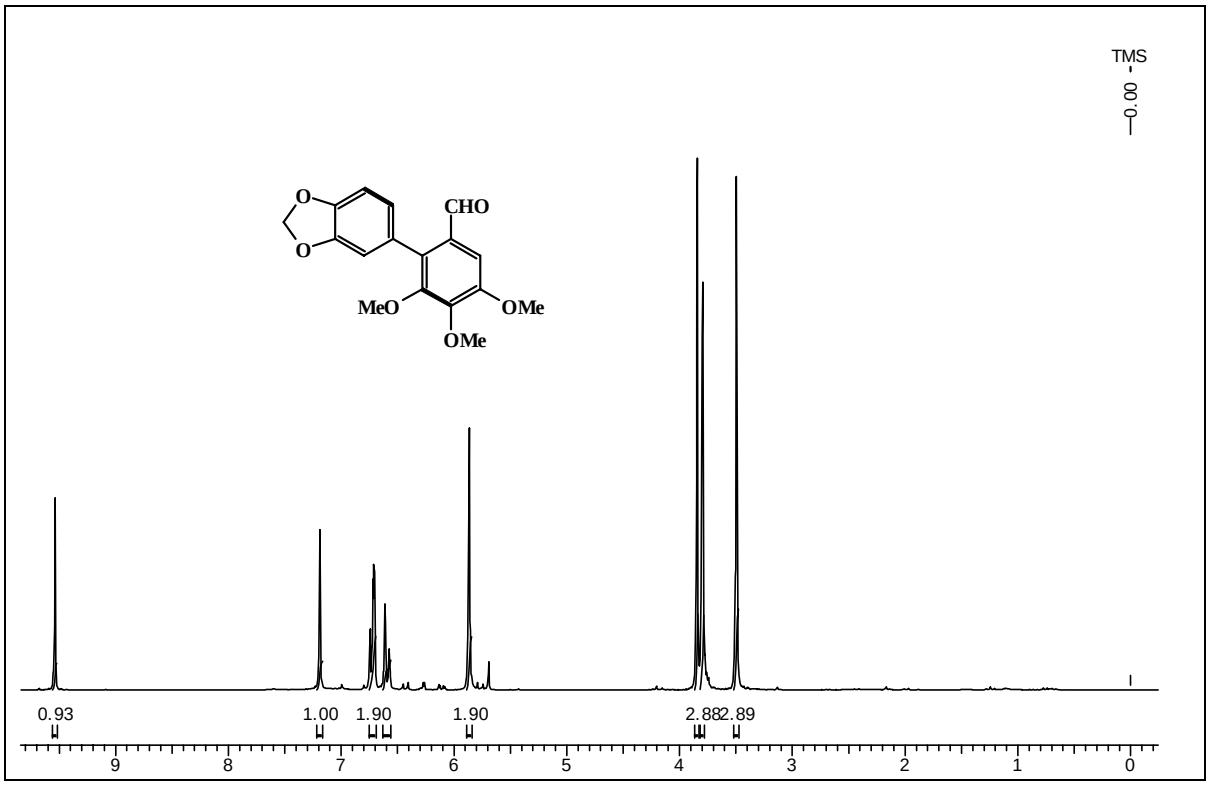
^{13}C NMR spectrum of compound 15 in $CDCl_3$



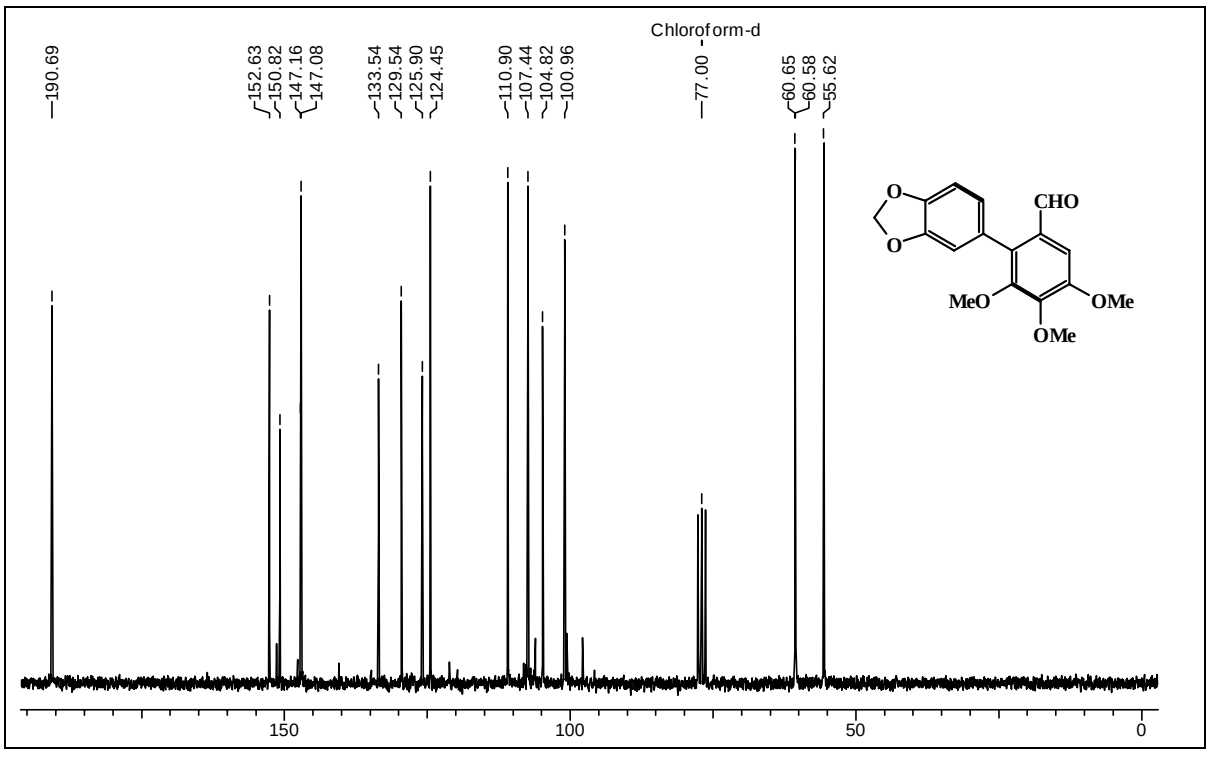
^{1}H NMR spectrum of compound 16 in $CDCl_3$



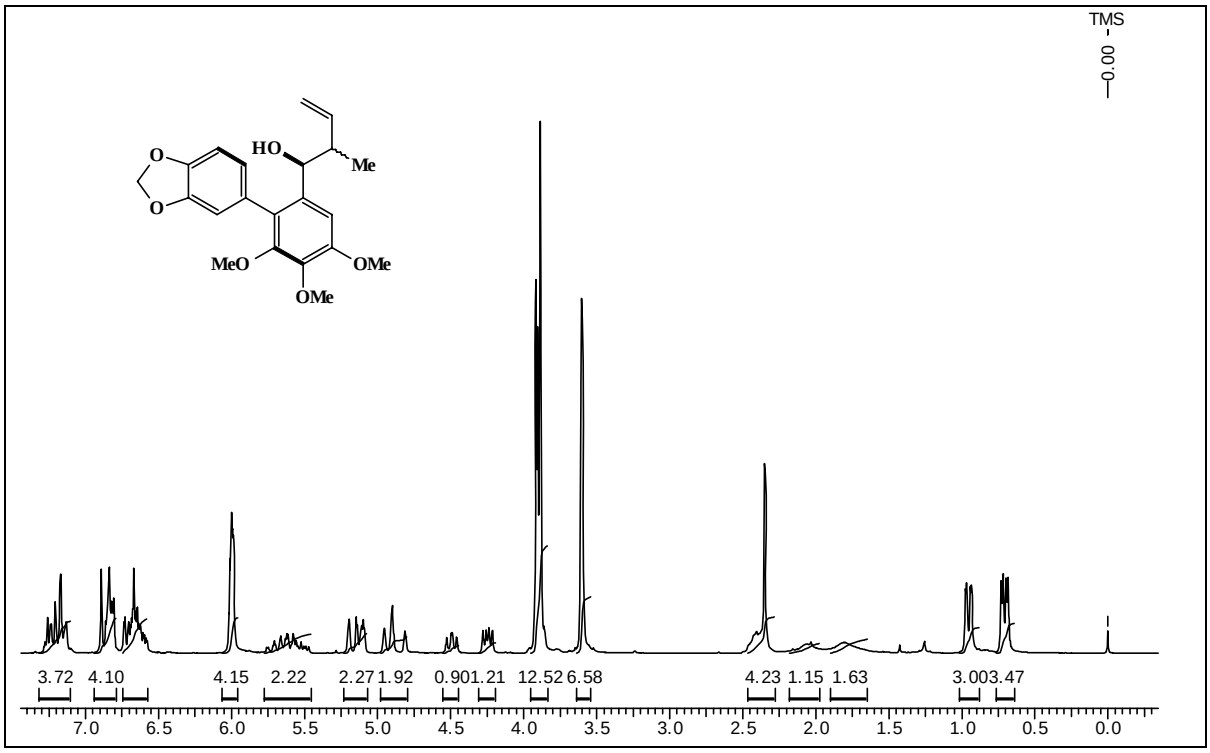
^{13}C NMR spectrum of compound 16 in $CDCl_3$



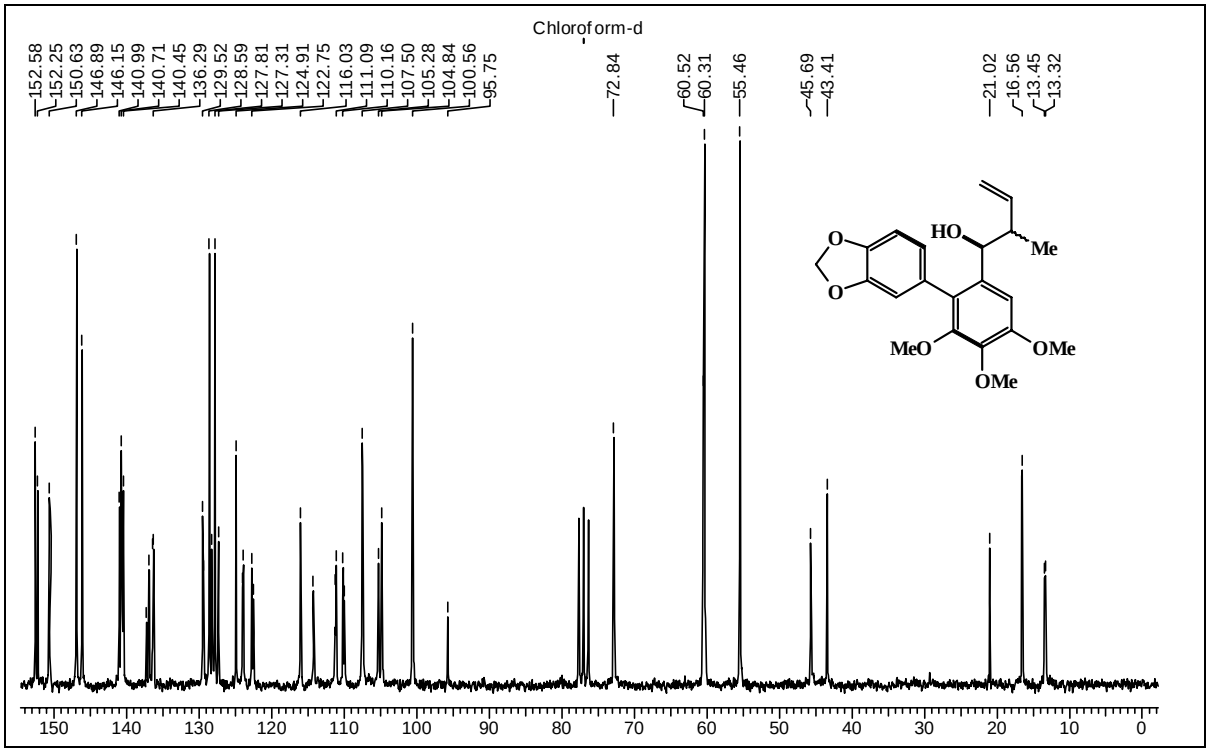
^{1}H NMR spectrum of compound 5 in $CDCl_3$



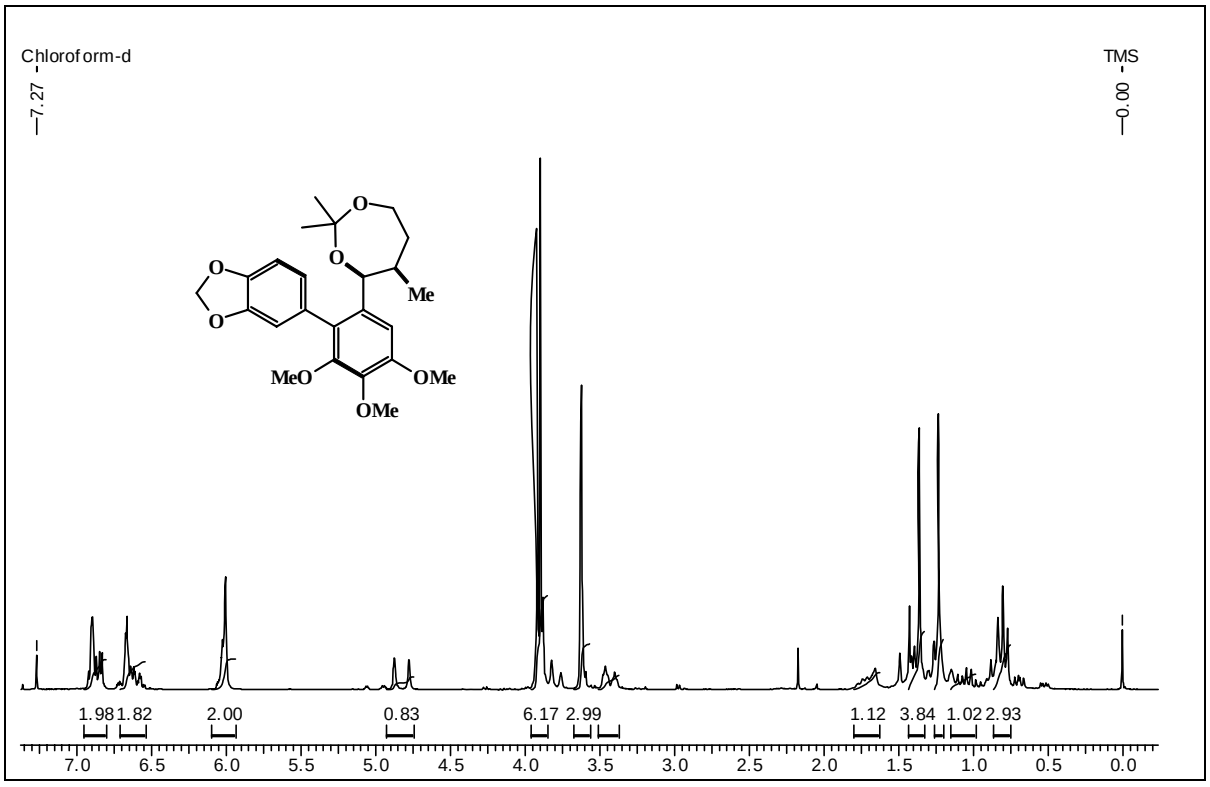
^{13}C NMR spectrum of compound 5 in $CDCl_3$



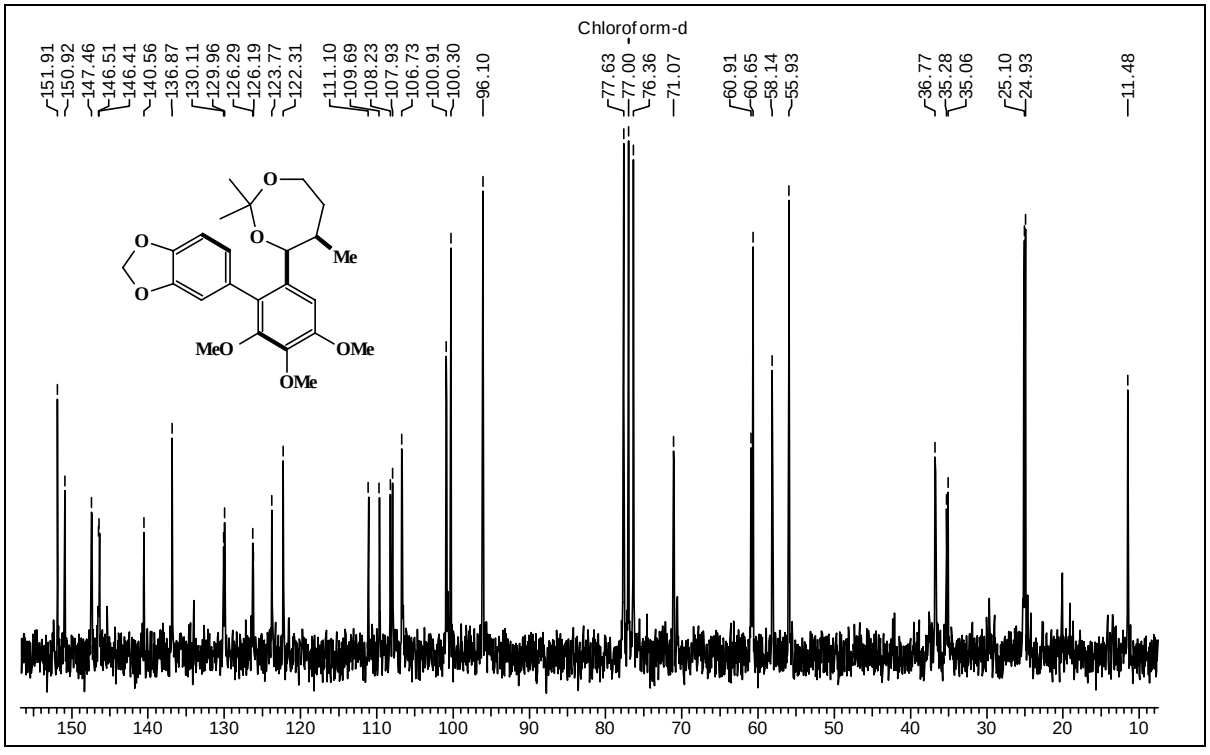
^{1}H NMR spectrum of compound 21/22 in $CDCl_3$



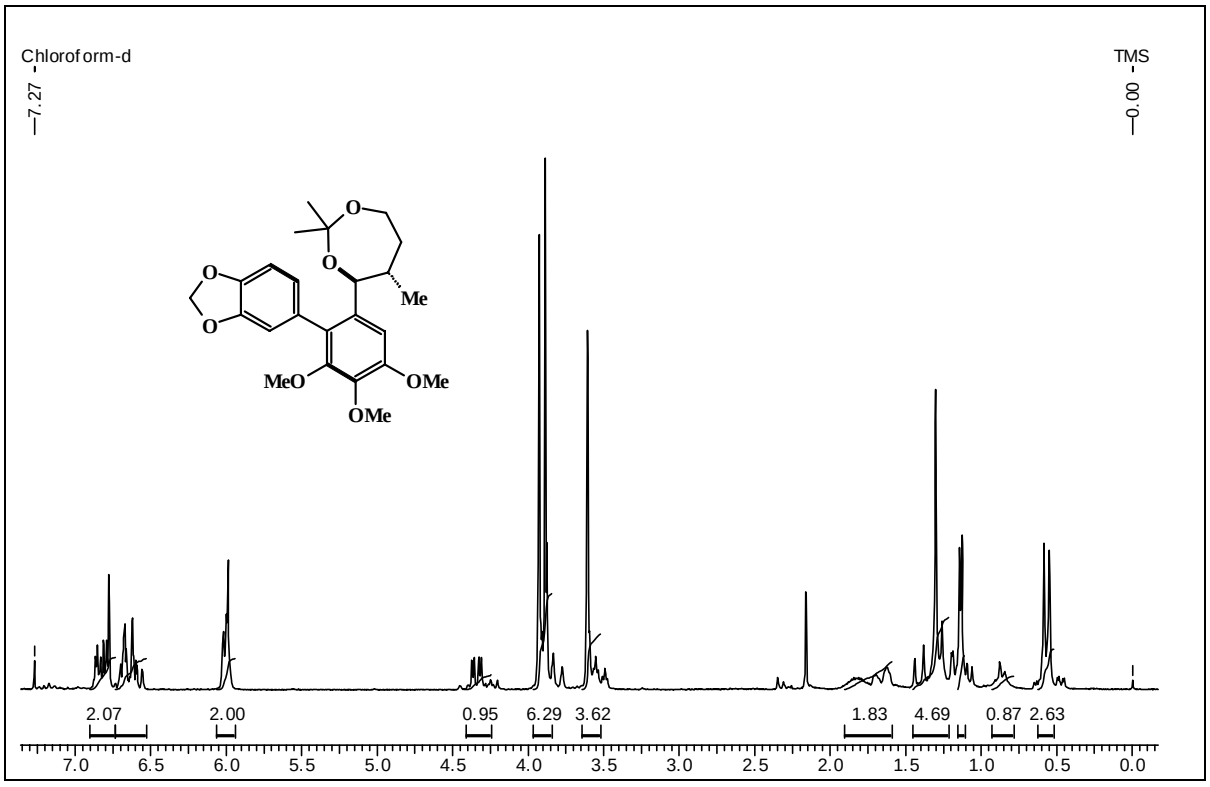
^{13}C NMR spectrum of compound 21/22 in $CDCl_3$



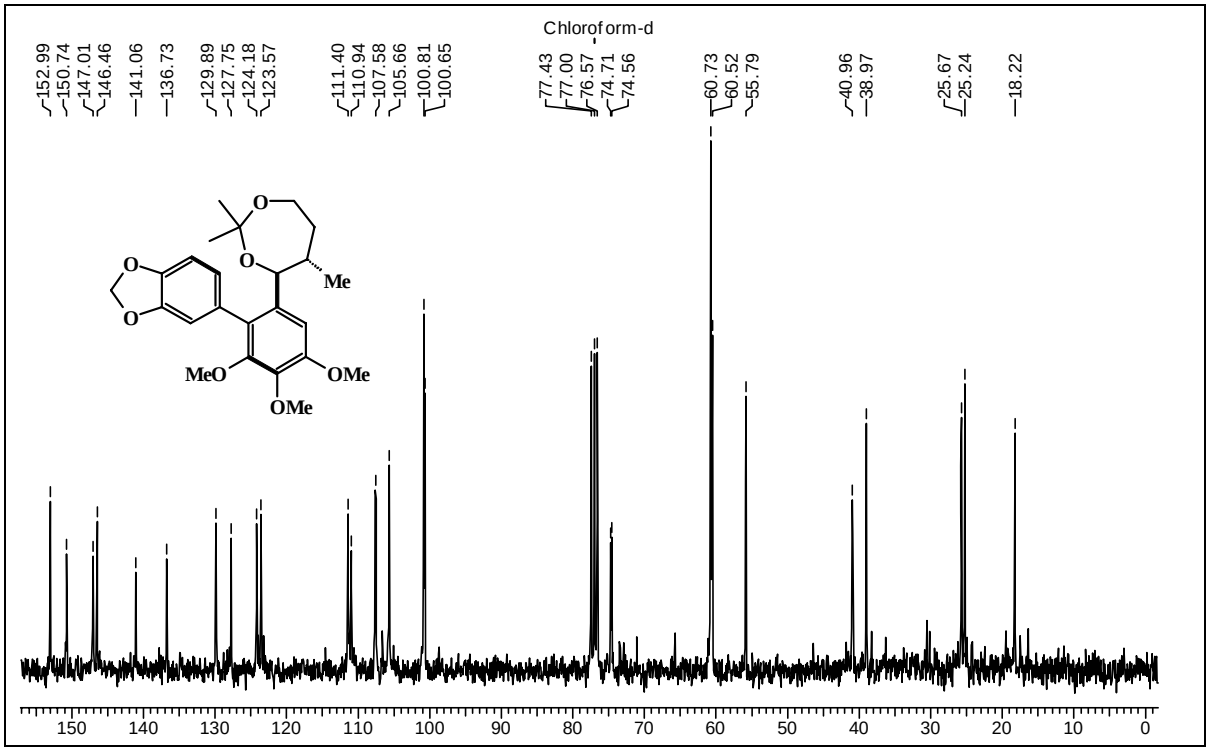
^{1}H NMR spectrum of compound 25 in $CDCl_3$



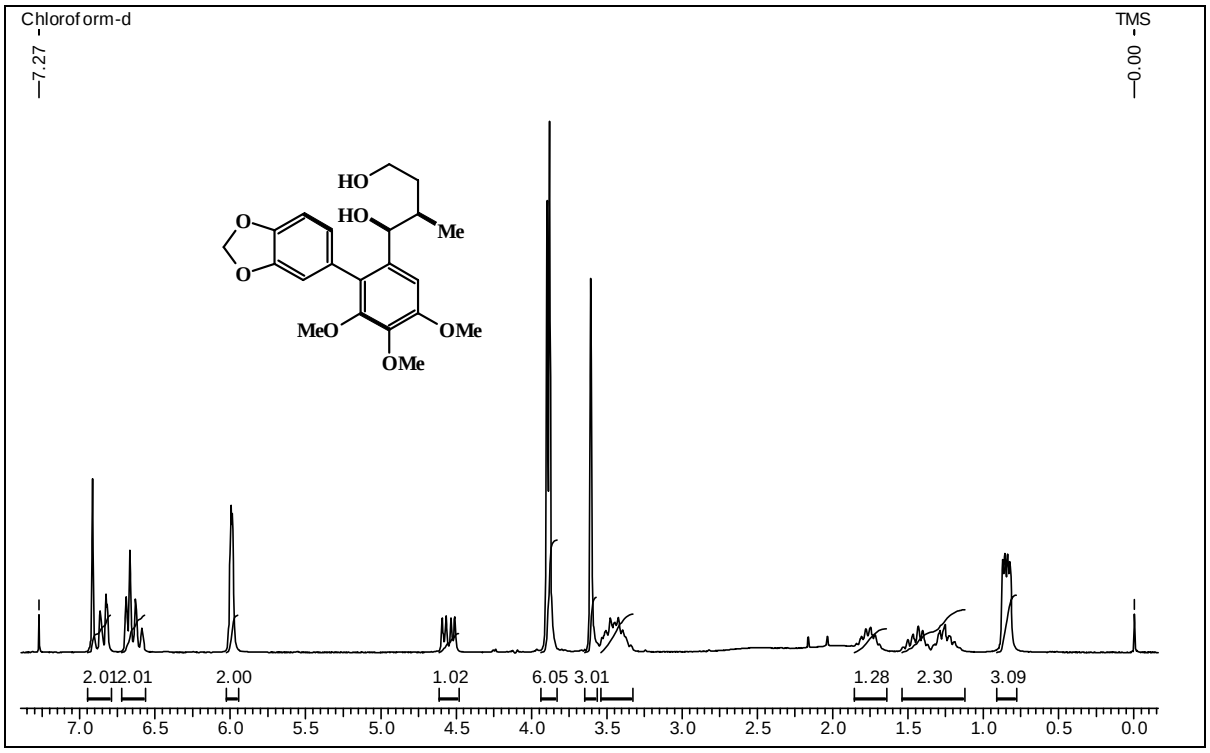
^{13}C NMR spectrum of compound 25 in $CDCl_3$



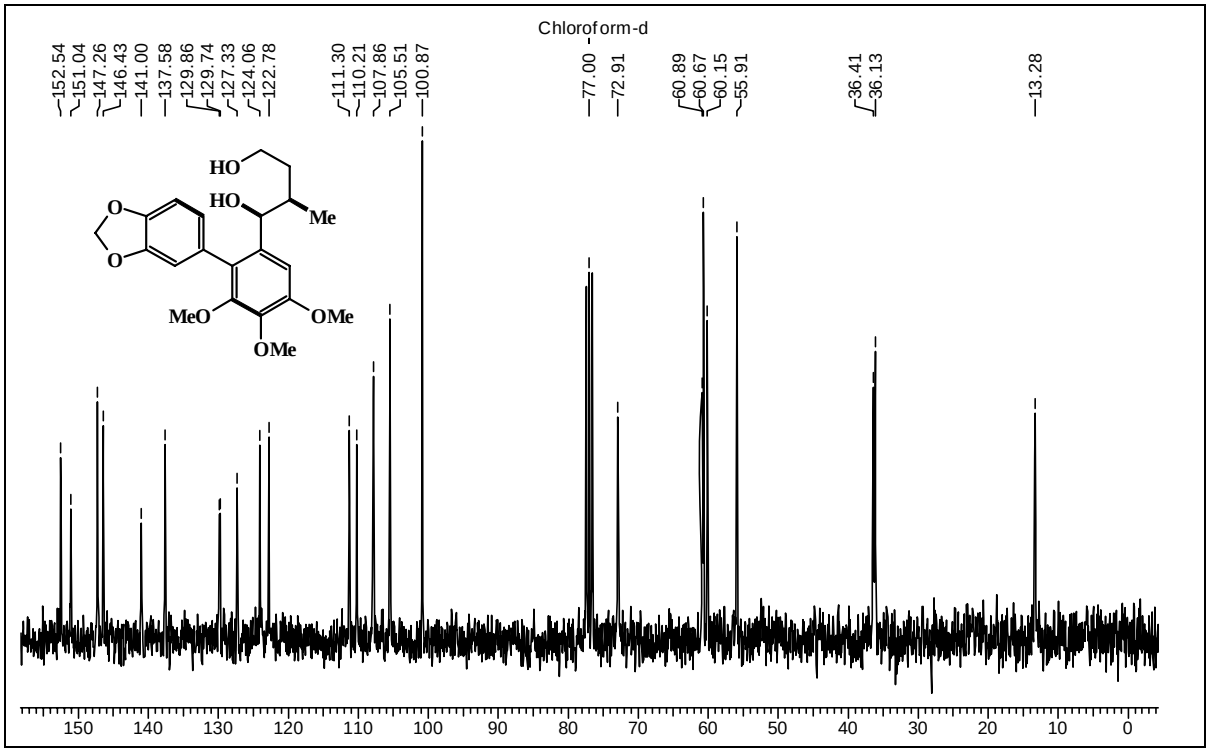
^{1}H NMR spectrum of compound 26 in $CDCl_3$



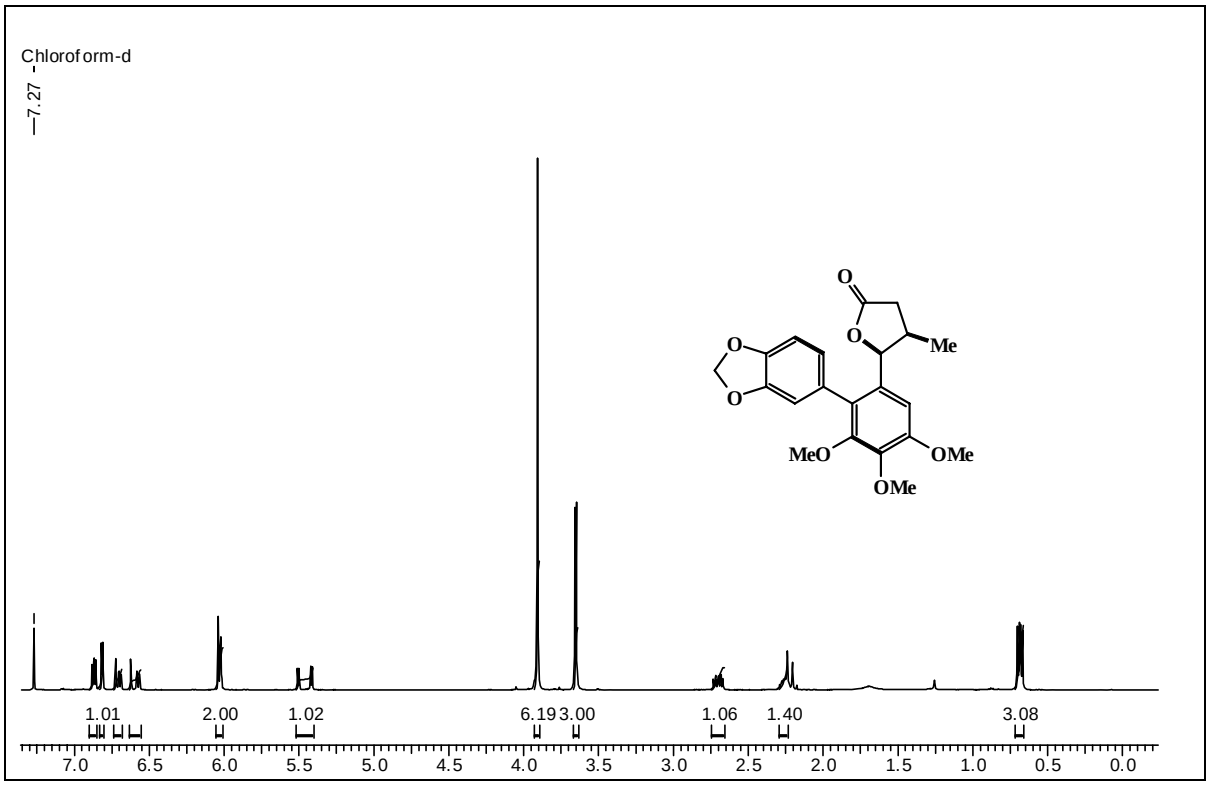
^{13}C NMR spectrum of compound 26 in $CDCl_3$



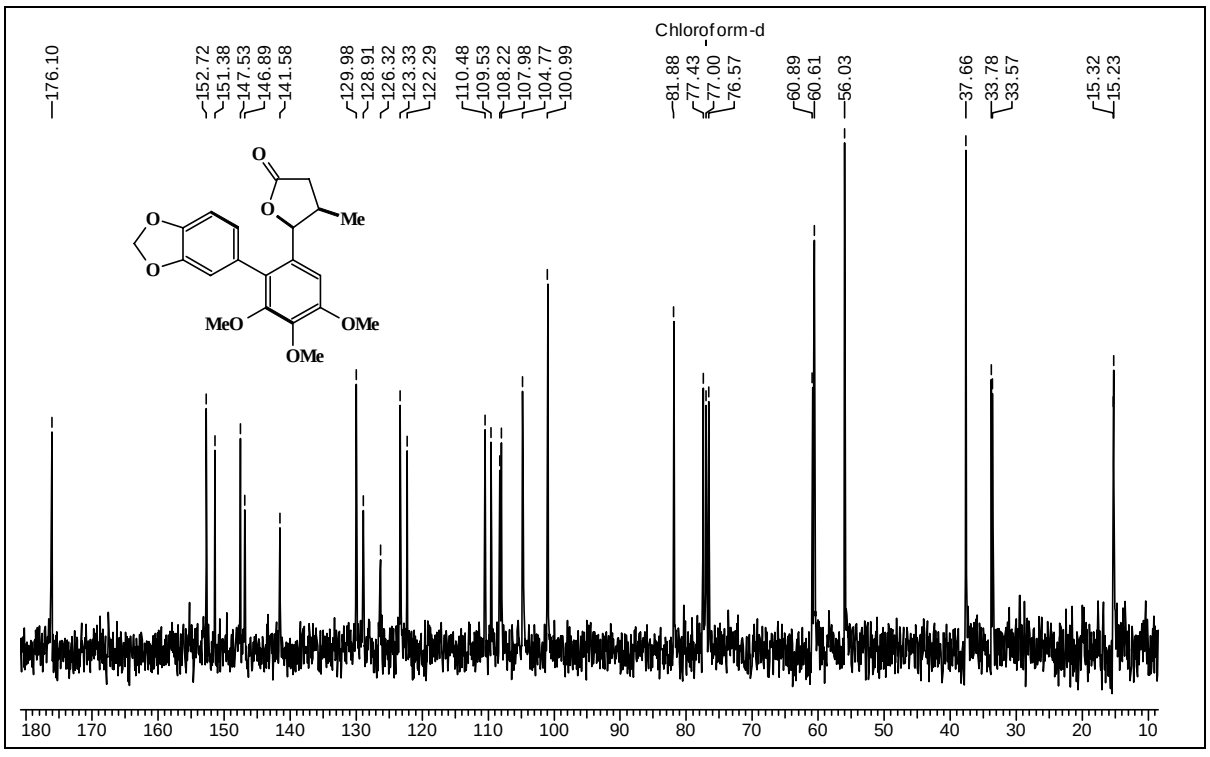
^{1}H NMR spectrum of compound (+/-) 23 in $CDCl_3$



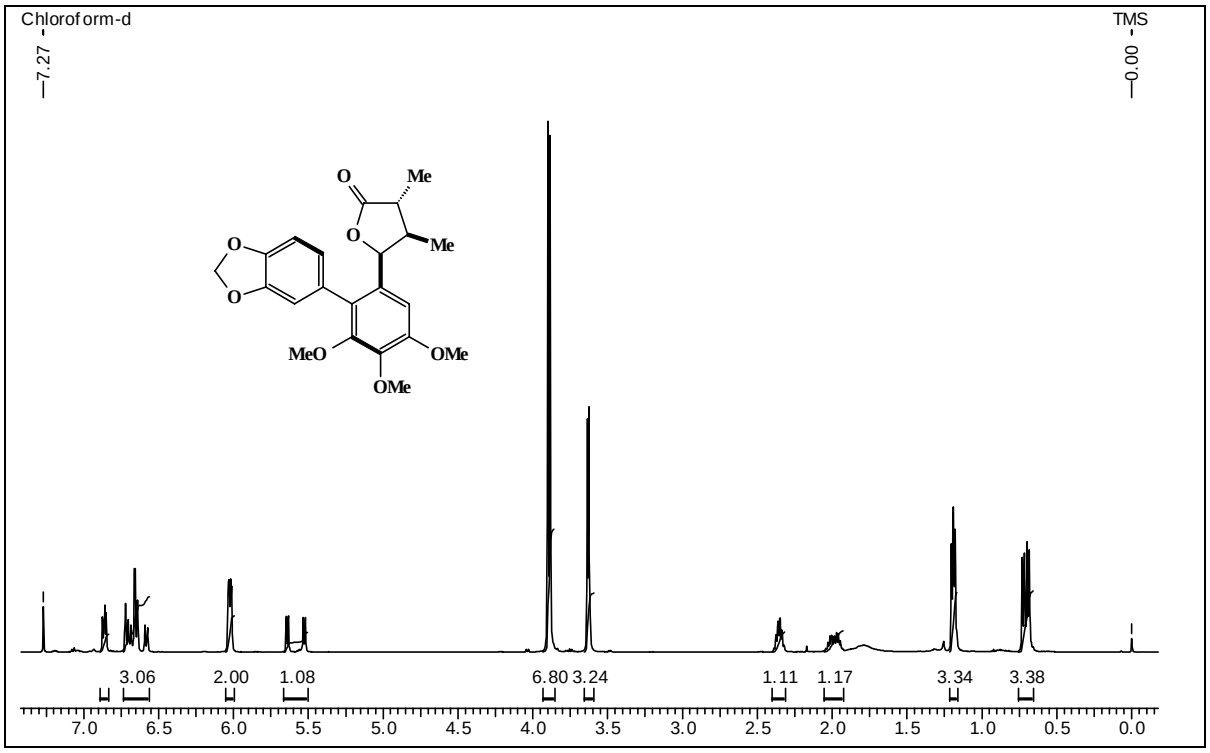
^{13}C NMR spectrum of compound (+/-) 23 in $CDCl_3$



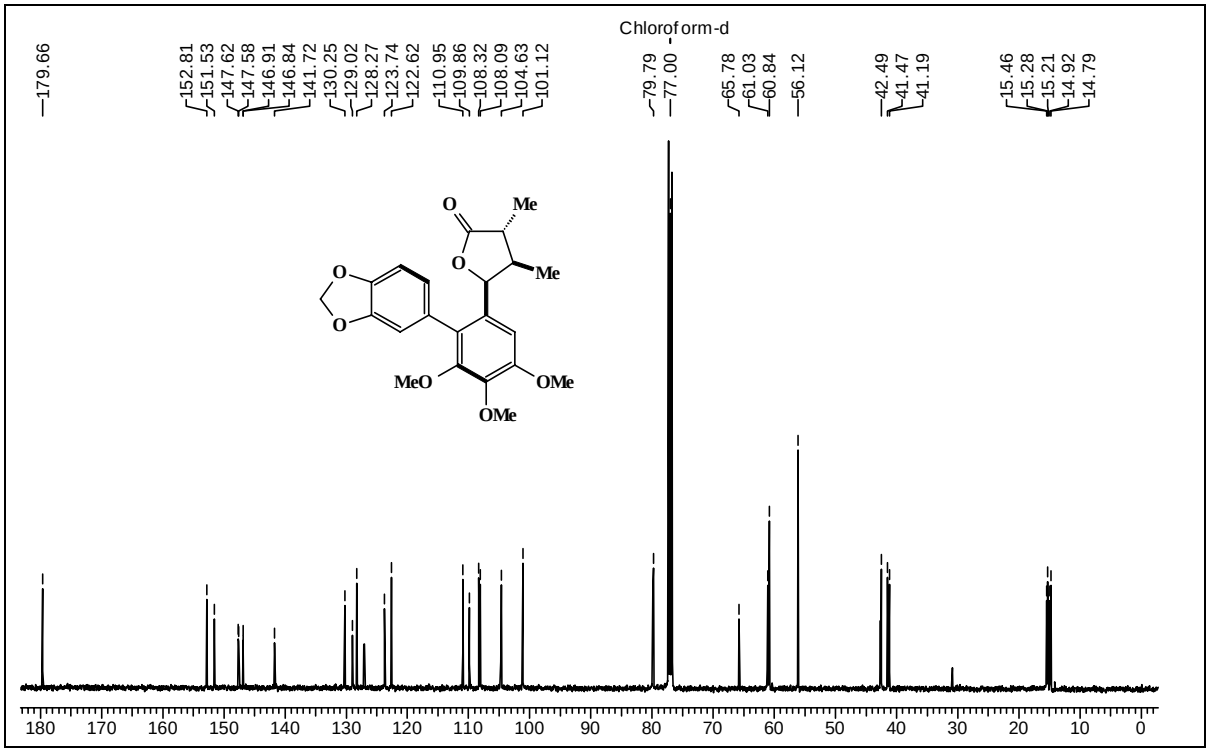
^{1}H NMR spectrum of compound (+/-) 27 in $CDCl_3$



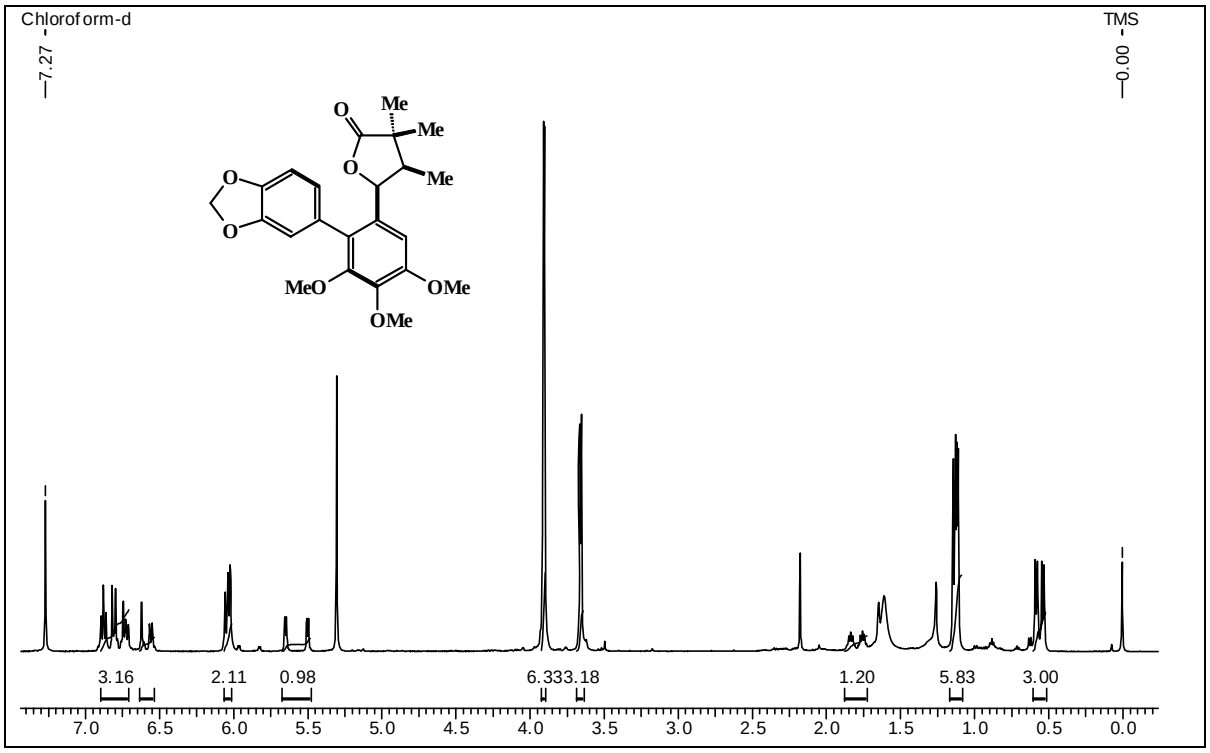
^{13}C NMR spectrum of compound (+/-) 27 in $CDCl_3$



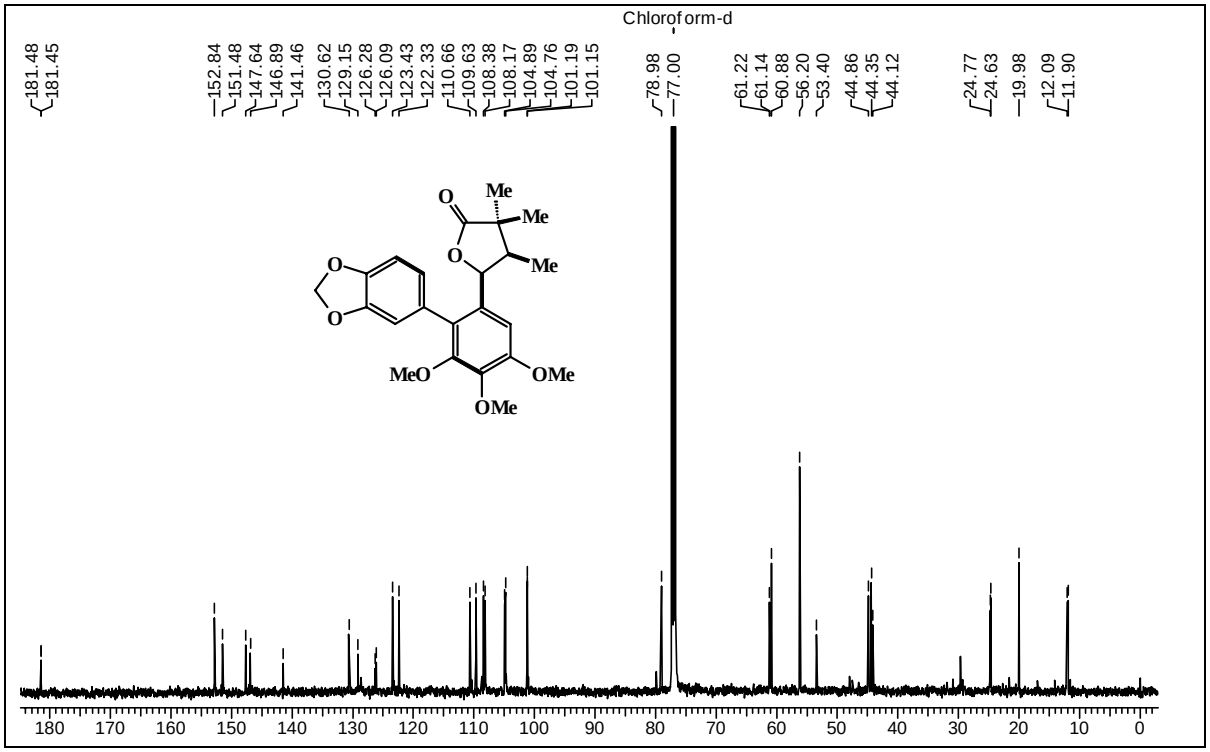
^{1}H NMR spectrum of compound (+/-) 2 in $CDCl_3$



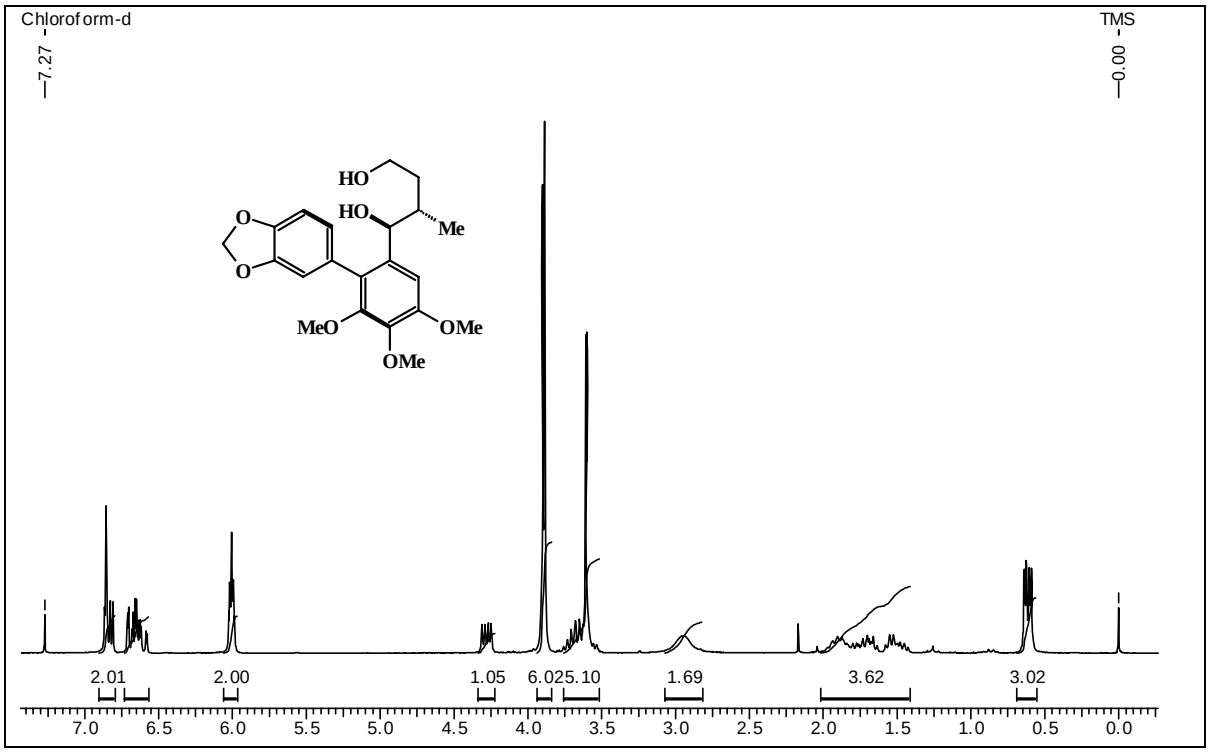
^{13}C NMR spectrum of compound (+/-) 2 in $CDCl_3$



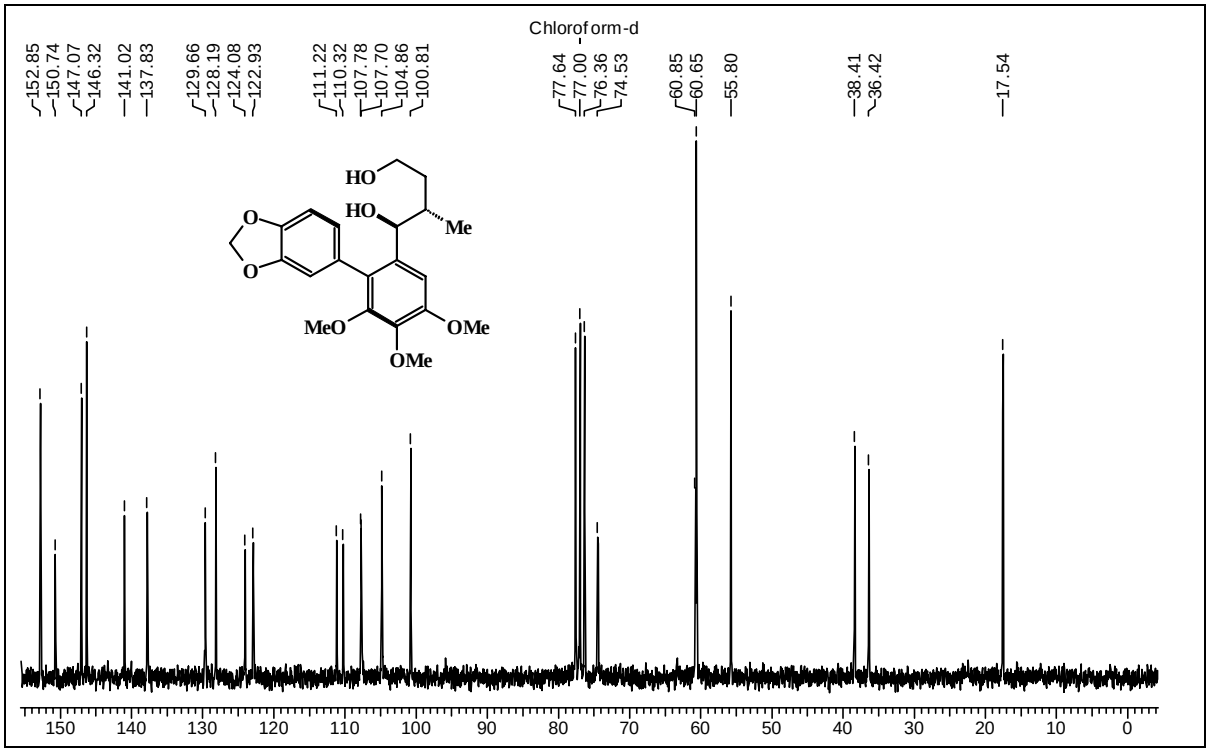
^{1}H NMR spectrum of compound (+/-) 28 in $CDCl_3$



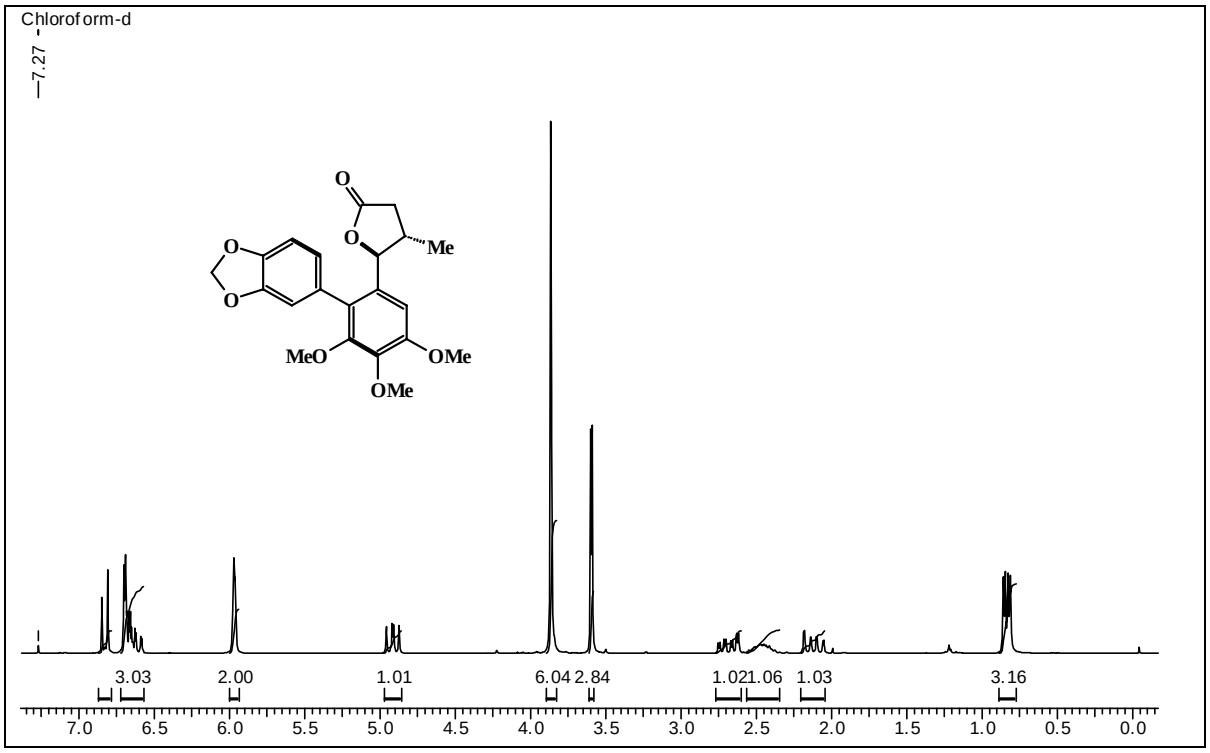
^{13}C NMR spectrum of compound (+/-) 28 in $CDCl_3$



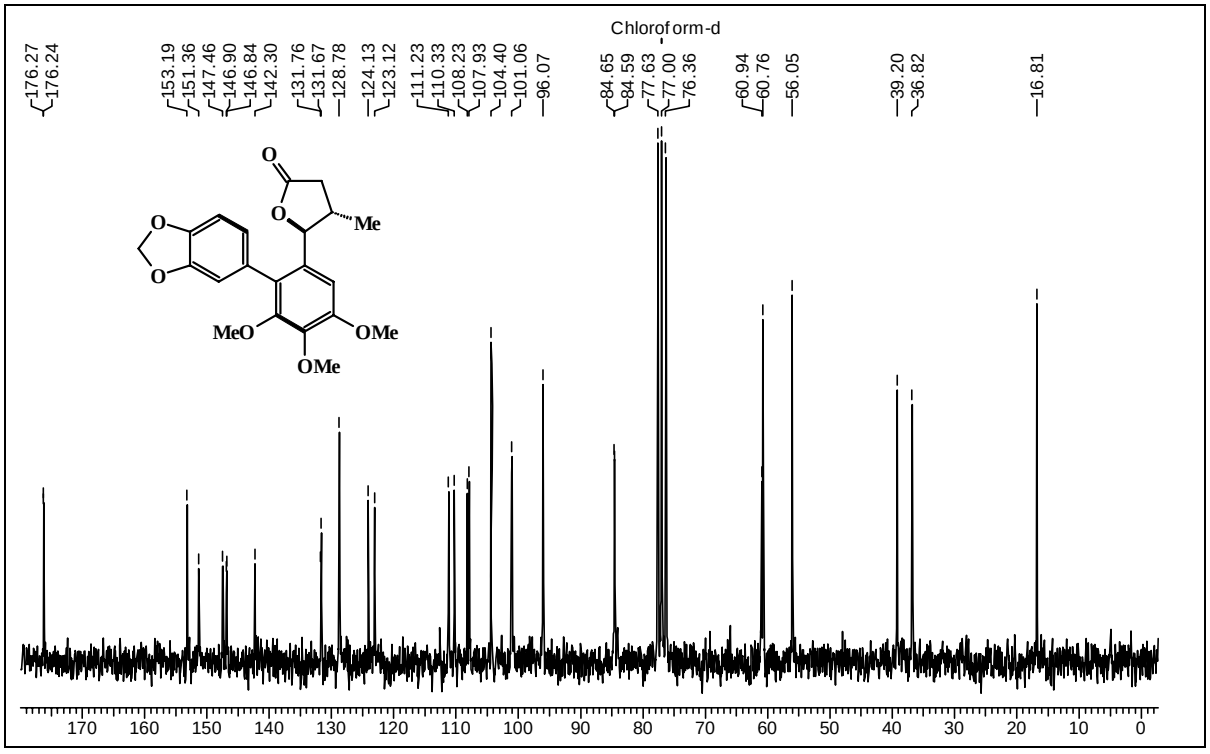
^{1}H NMR spectrum of compound 24 in $CDCl_3$



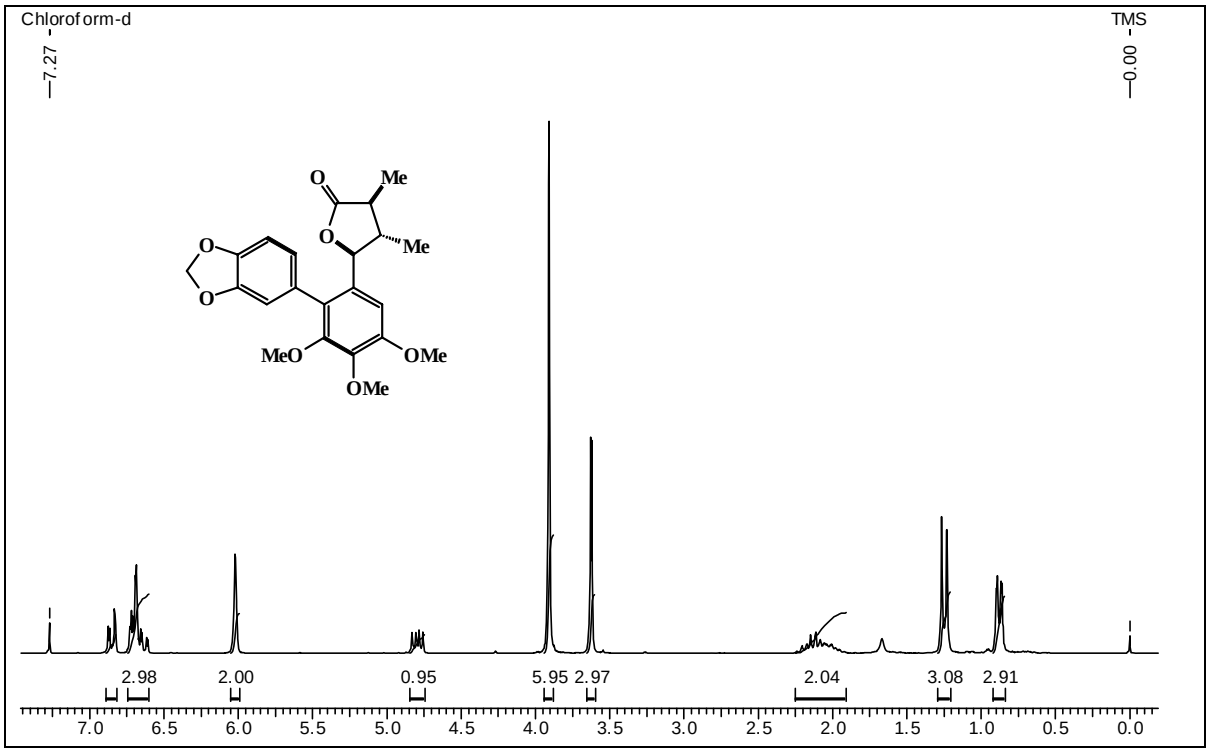
^{13}C NMR spectrum of compound 24 in $CDCl_3$



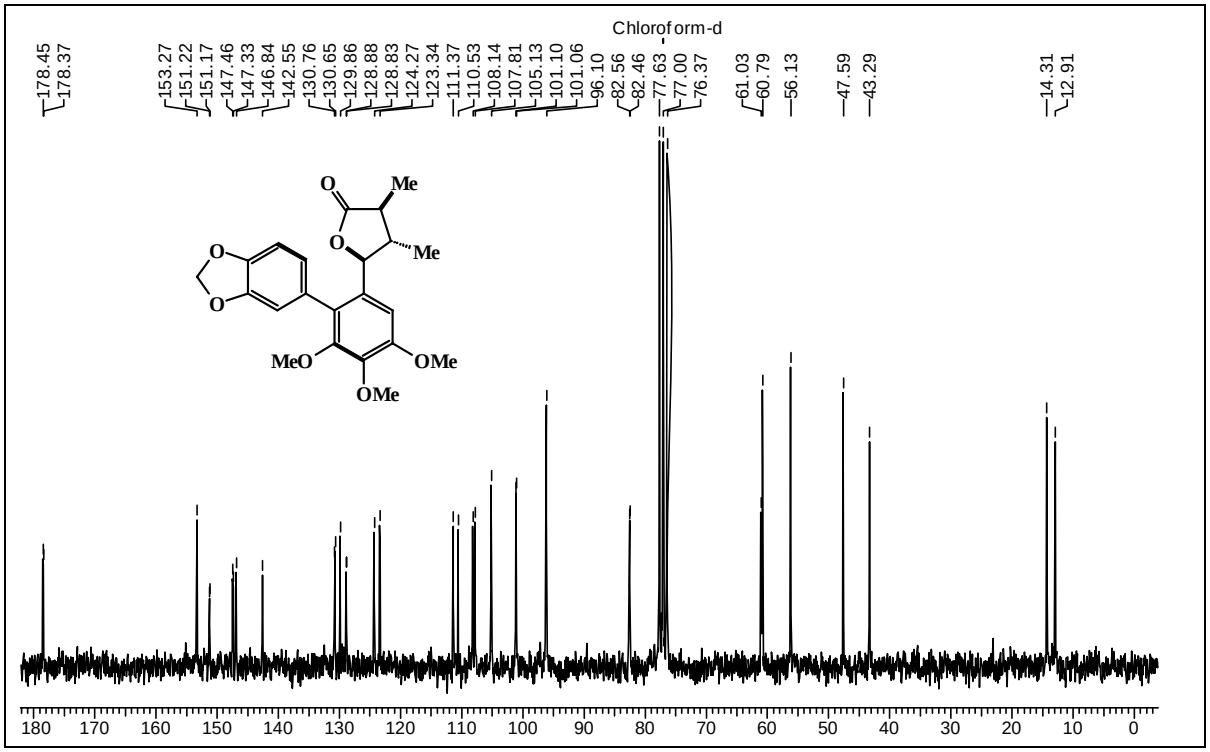
^{1}H NMR spectrum of compound 29 in $CDCl_3$



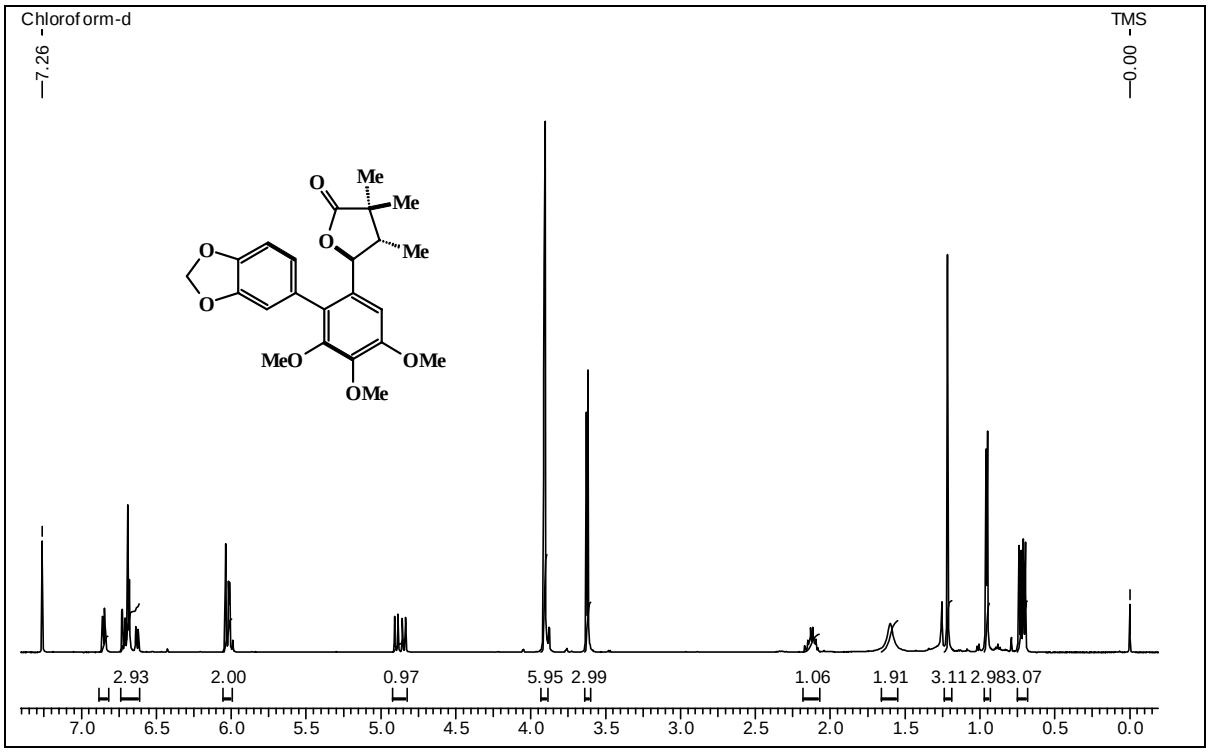
^{13}C NMR spectrum of compound 29 in $CDCl_3$



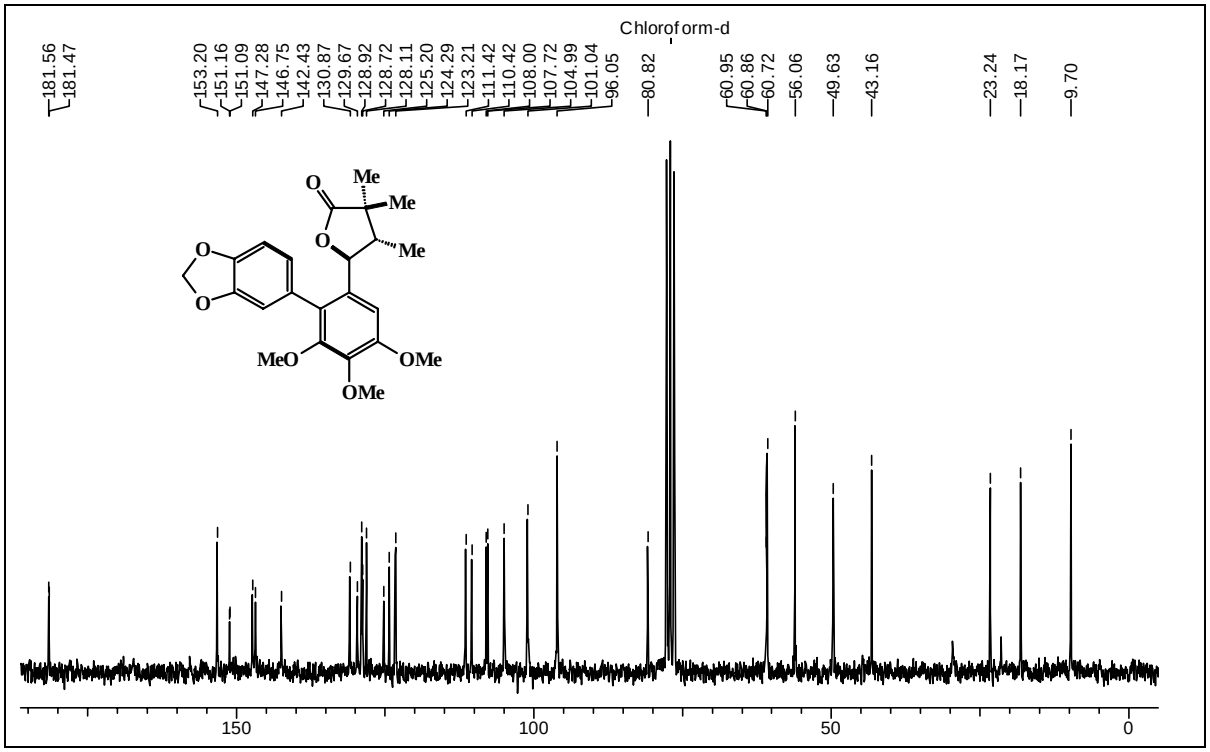
^{1}H NMR spectrum of compound 3 in $CDCl_3$



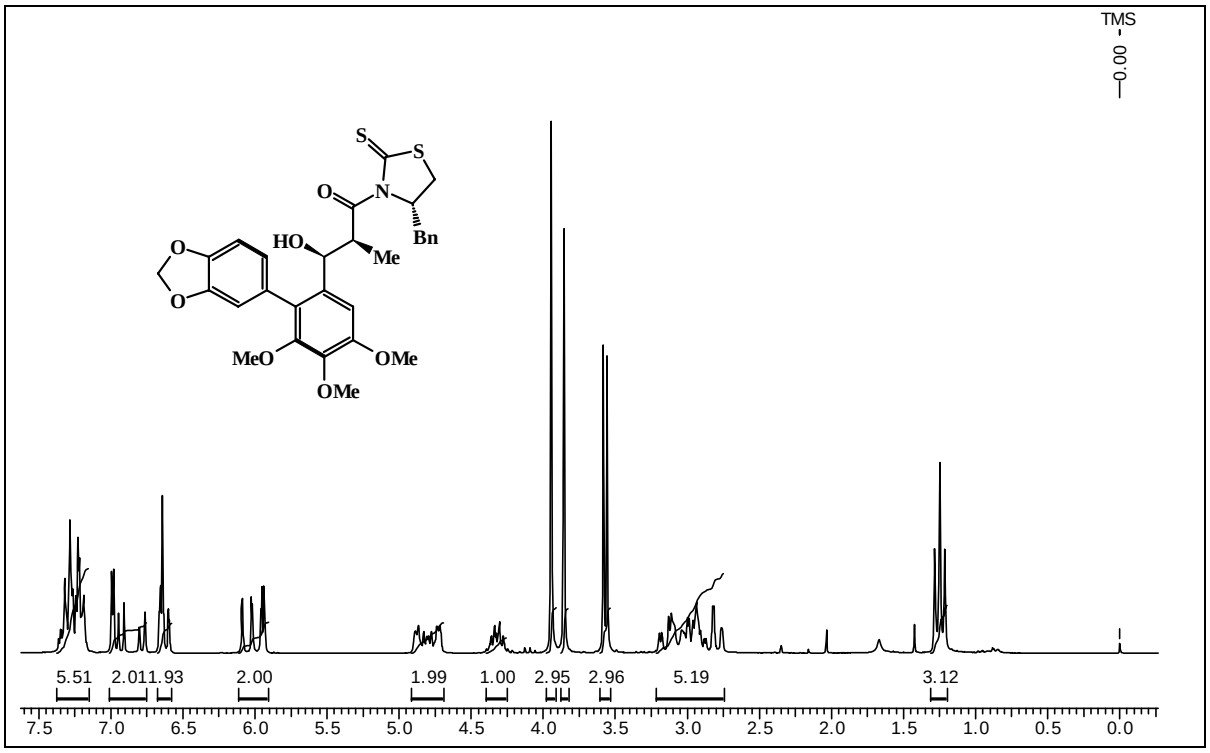
^{13}C NMR spectrum of compound 3 in $CDCl_3$



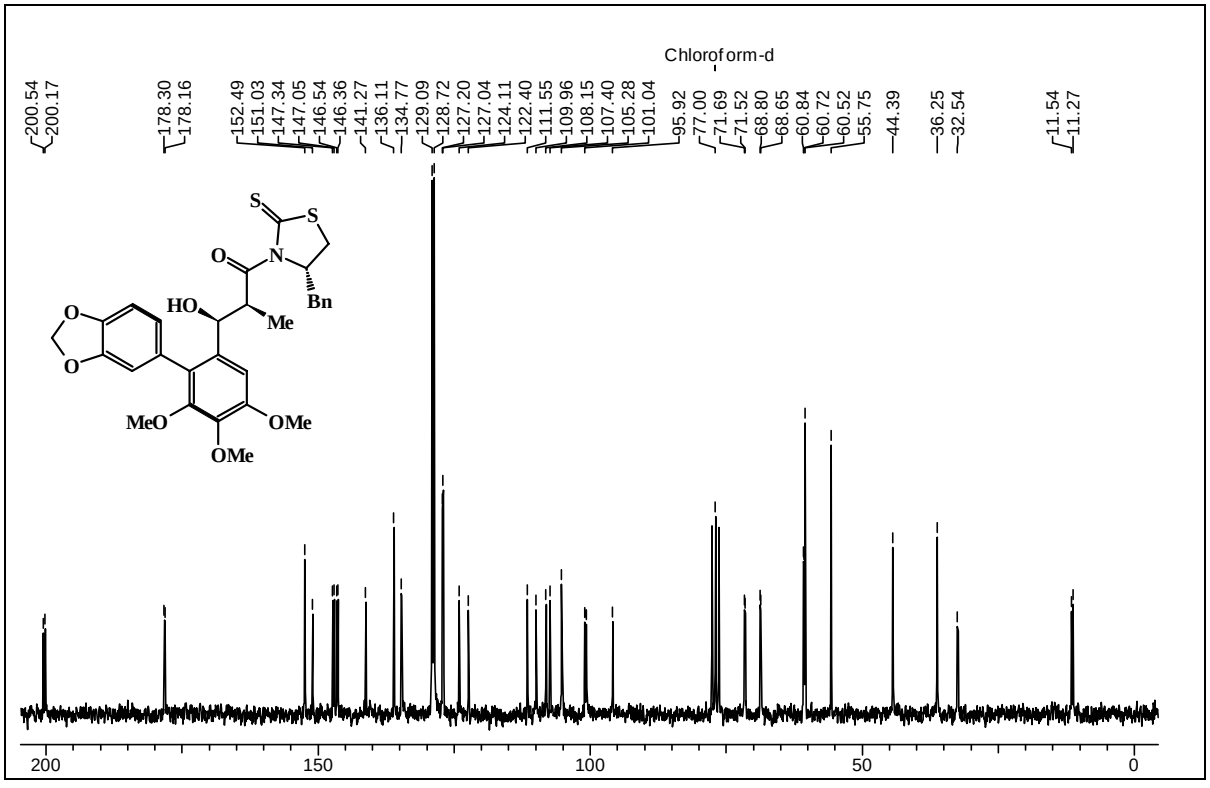
^{1}H NMR spectrum of compound 30 in $CDCl_3$



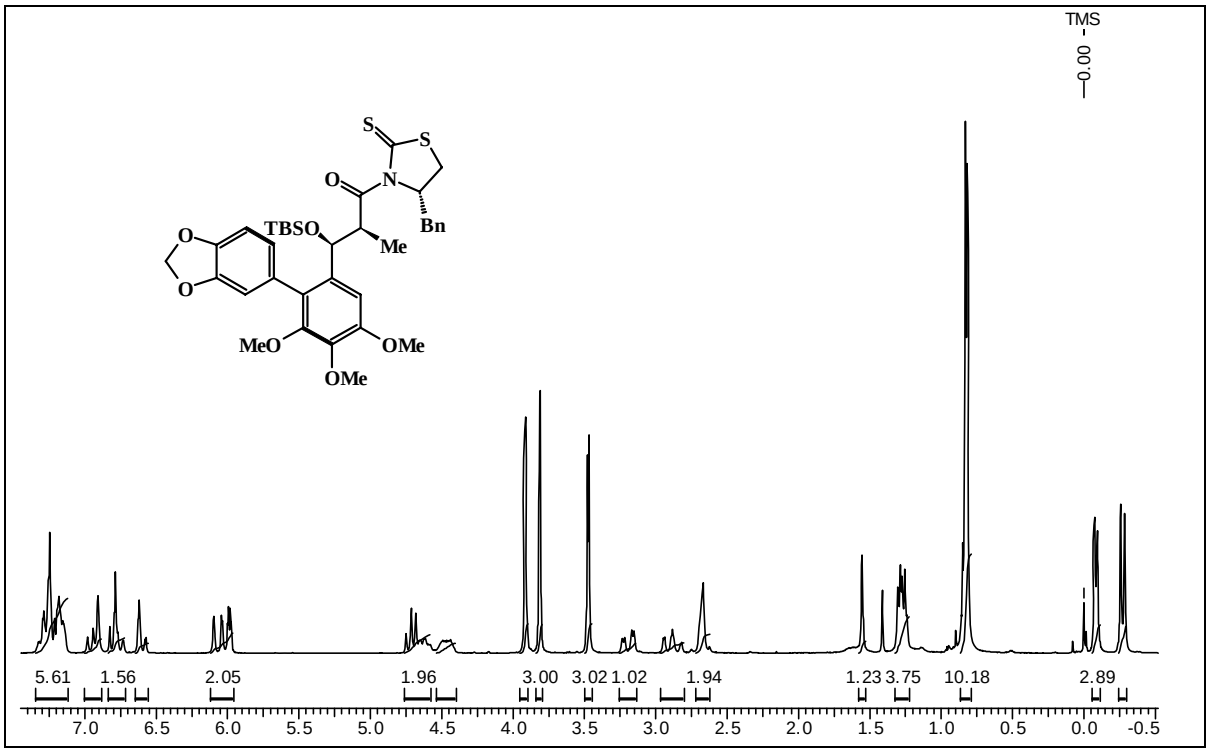
^{13}C NMR spectrum of compound 30 in $CDCl_3$



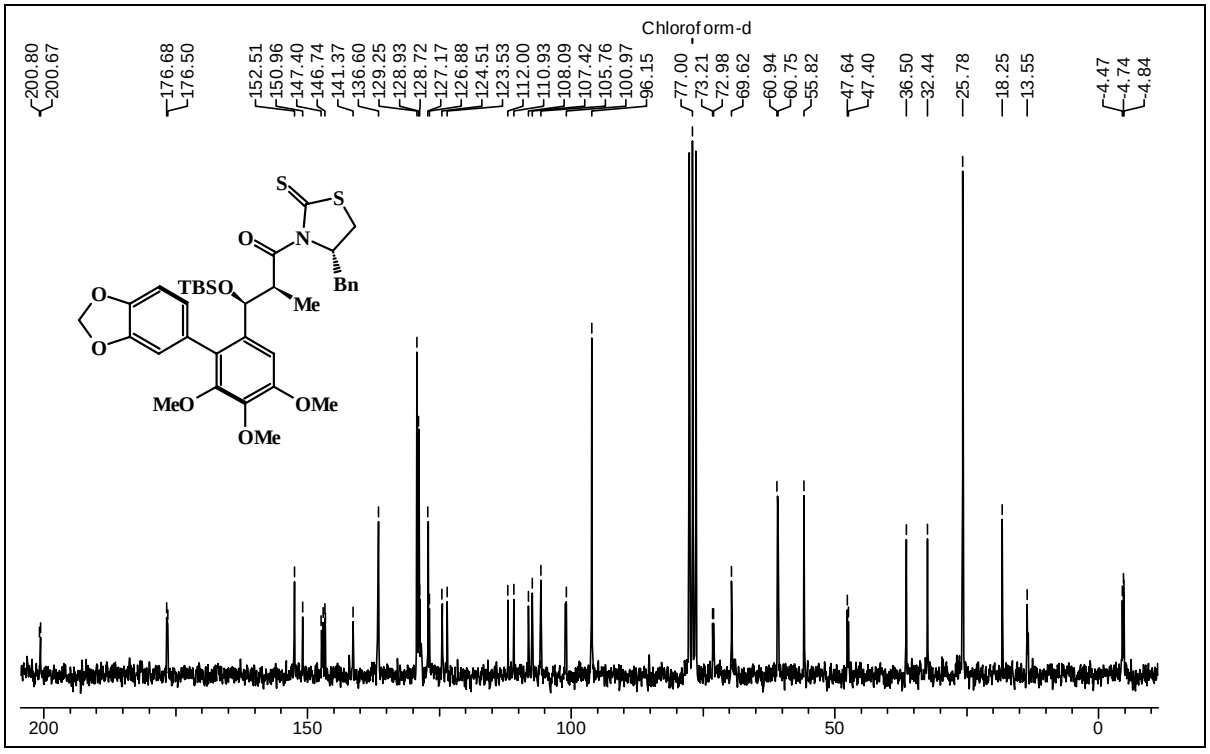
^{1}H NMR spectrum of compound 32 in $CDCl_3$



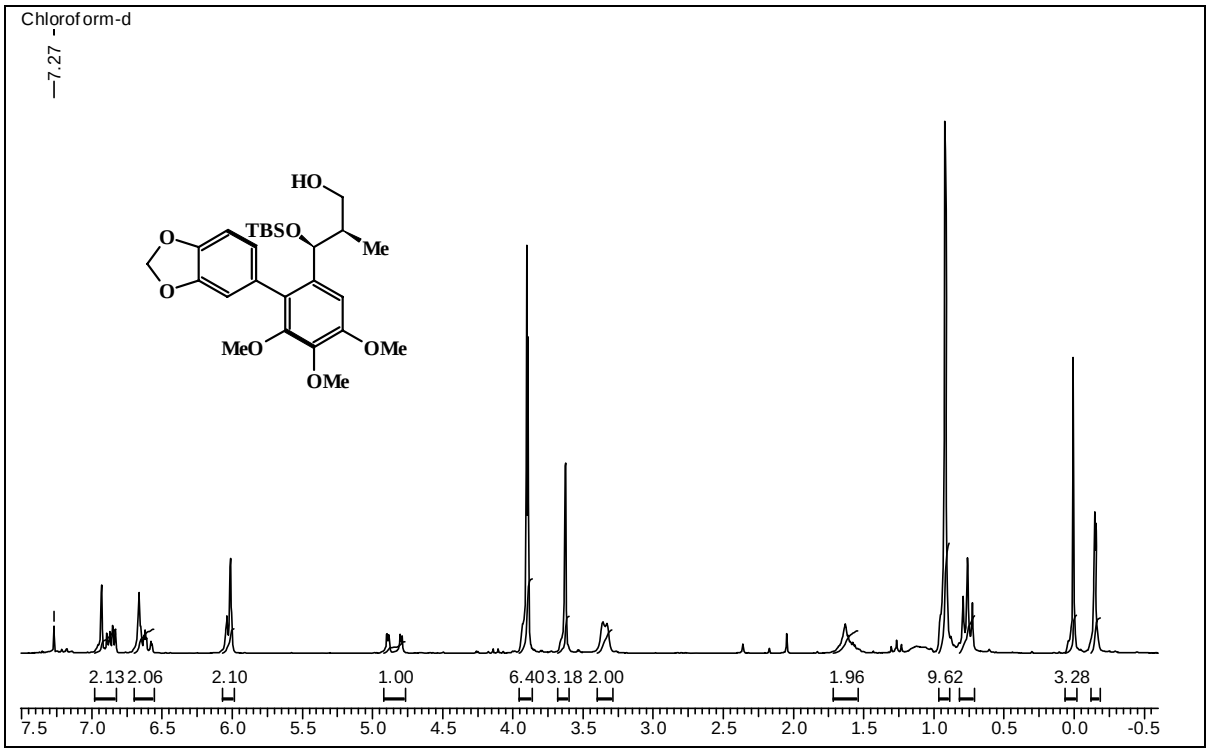
^{13}C NMR spectrum of compound 32 in $CDCl_3$



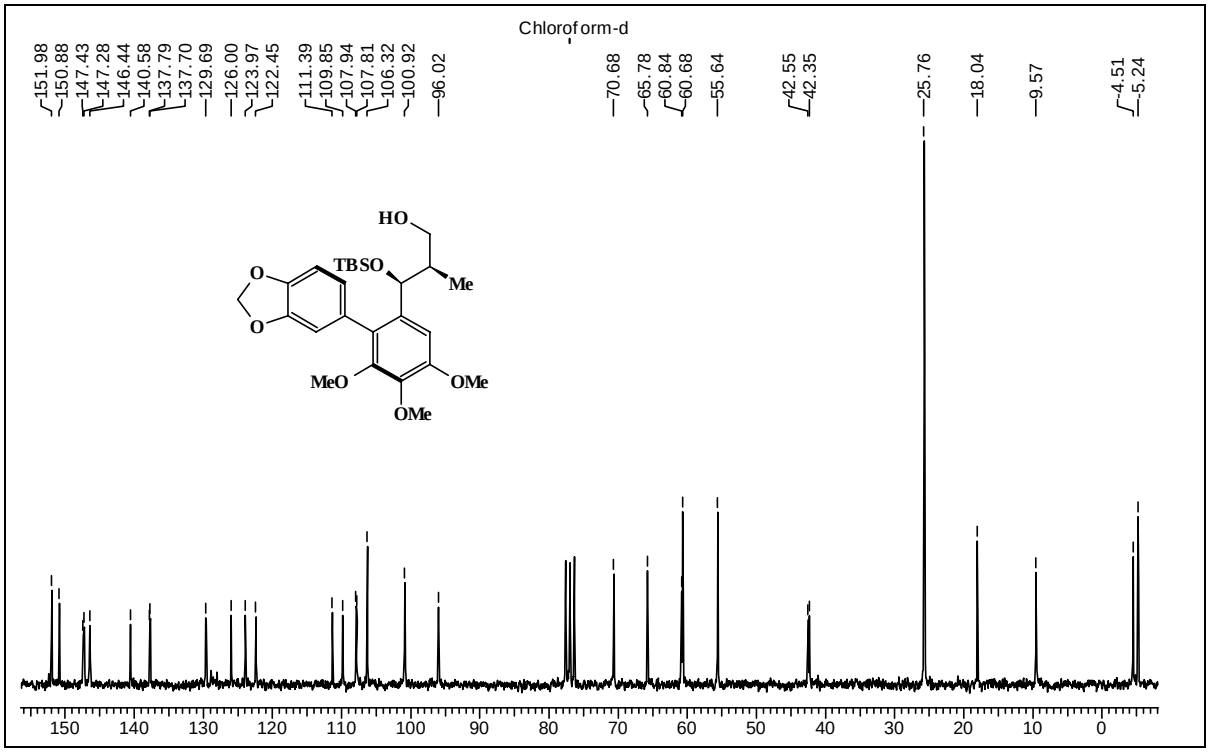
^{1}H NMR spectrum of compound 34 in $CDCl_3$



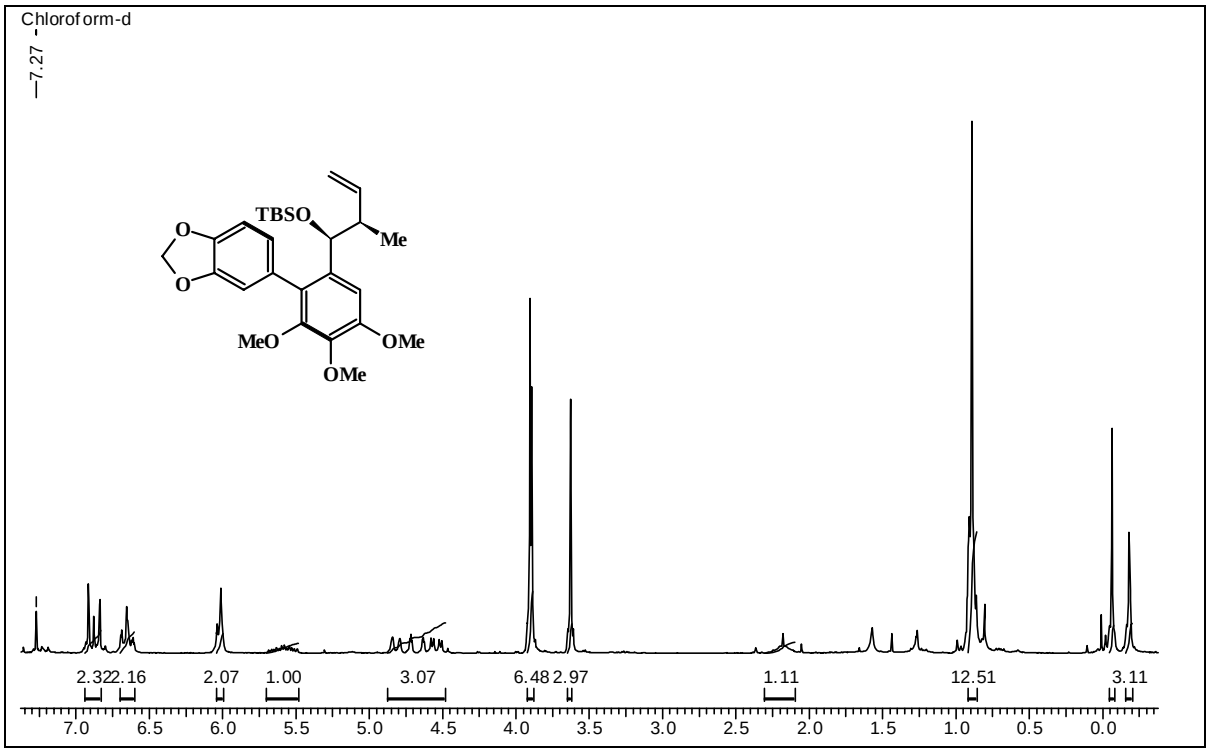
^{13}C NMR spectrum of compound 34 in $CDCl_3$



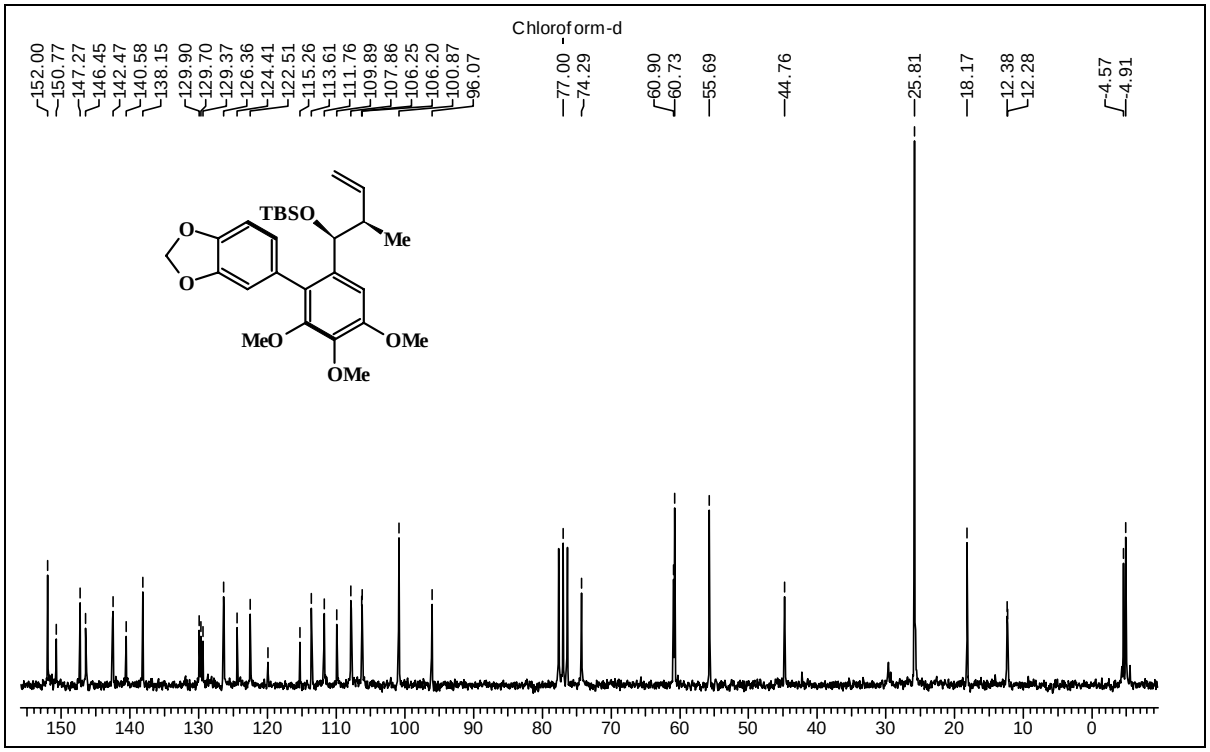
^{1}H NMR spectrum of compound 36 in $CDCl_3$



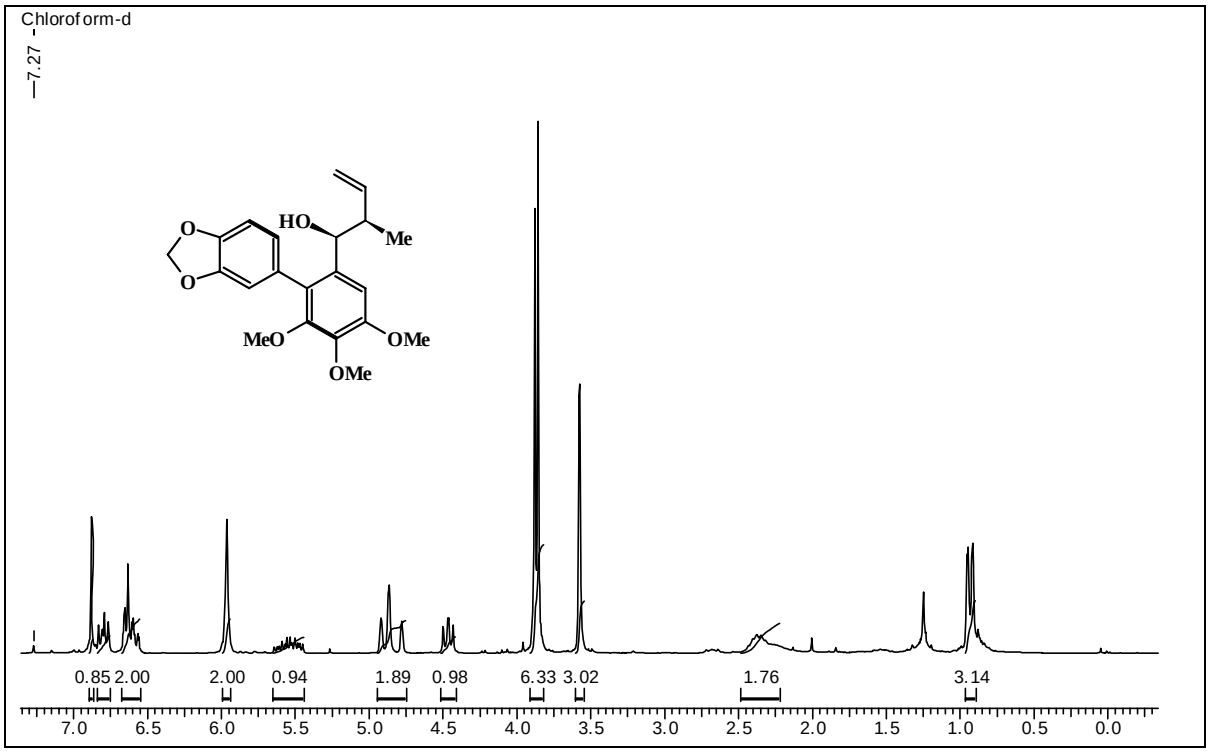
^{13}C NMR spectrum of compound 36 in $CDCl_3$



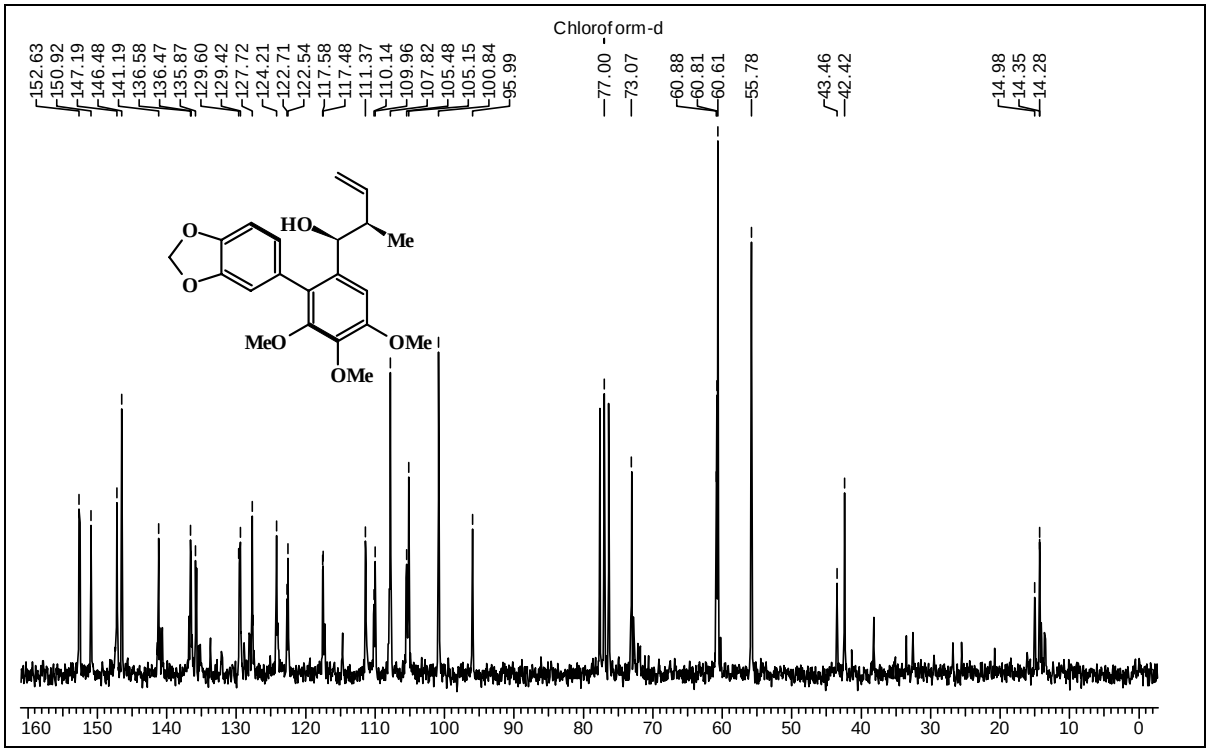
^{1}H NMR spectrum of compound 37 in $CDCl_3$



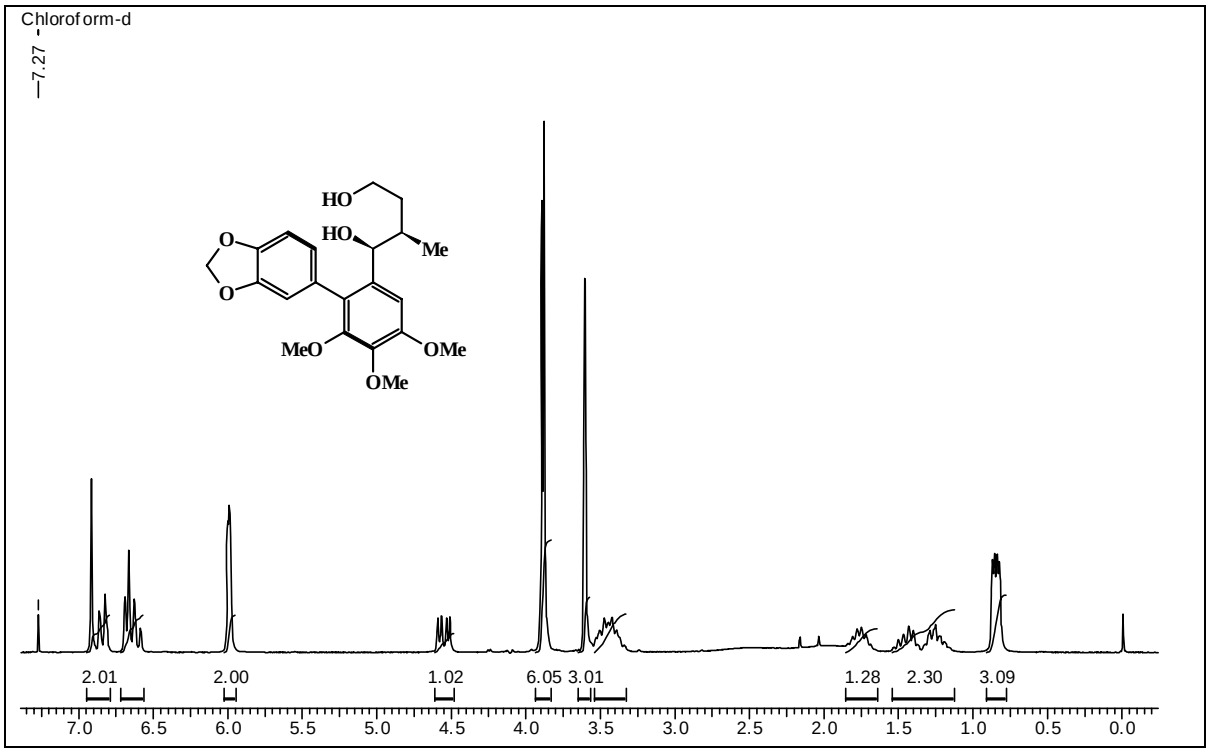
^{13}C NMR spectrum of compound 37 in $CDCl_3$



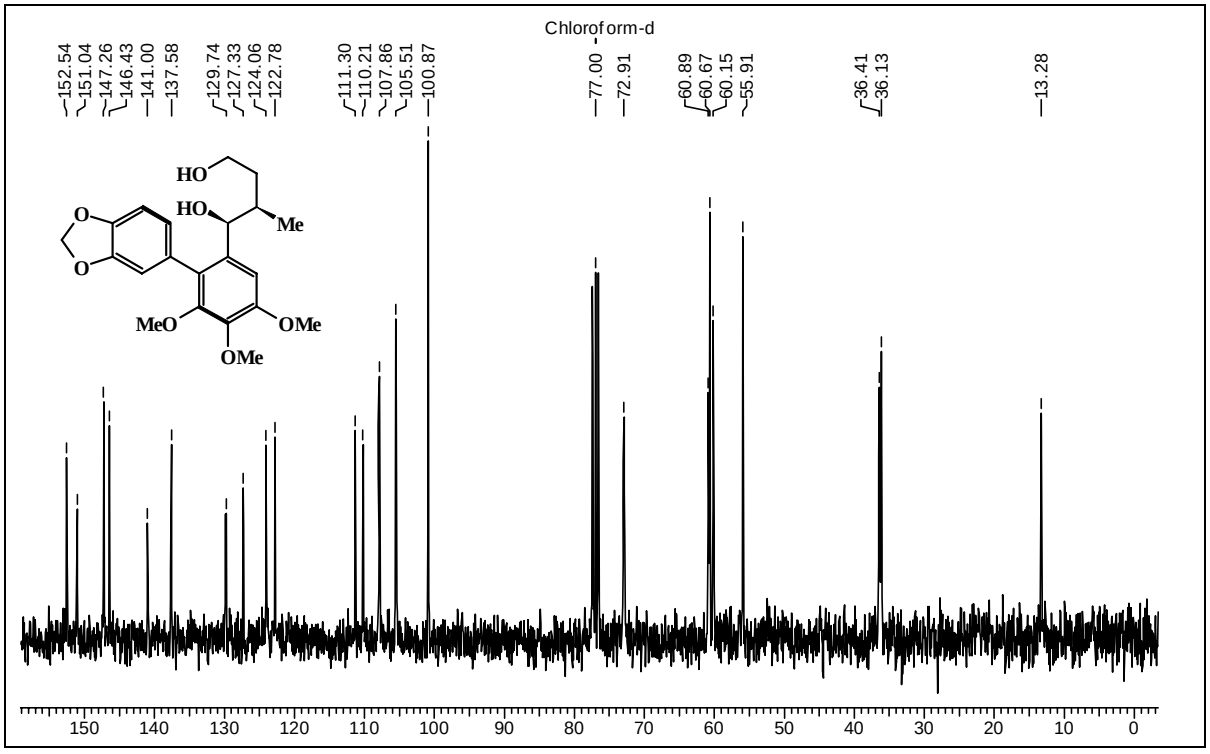
^{1}H NMR spectrum of compound (-) 31 in $CDCl_3$



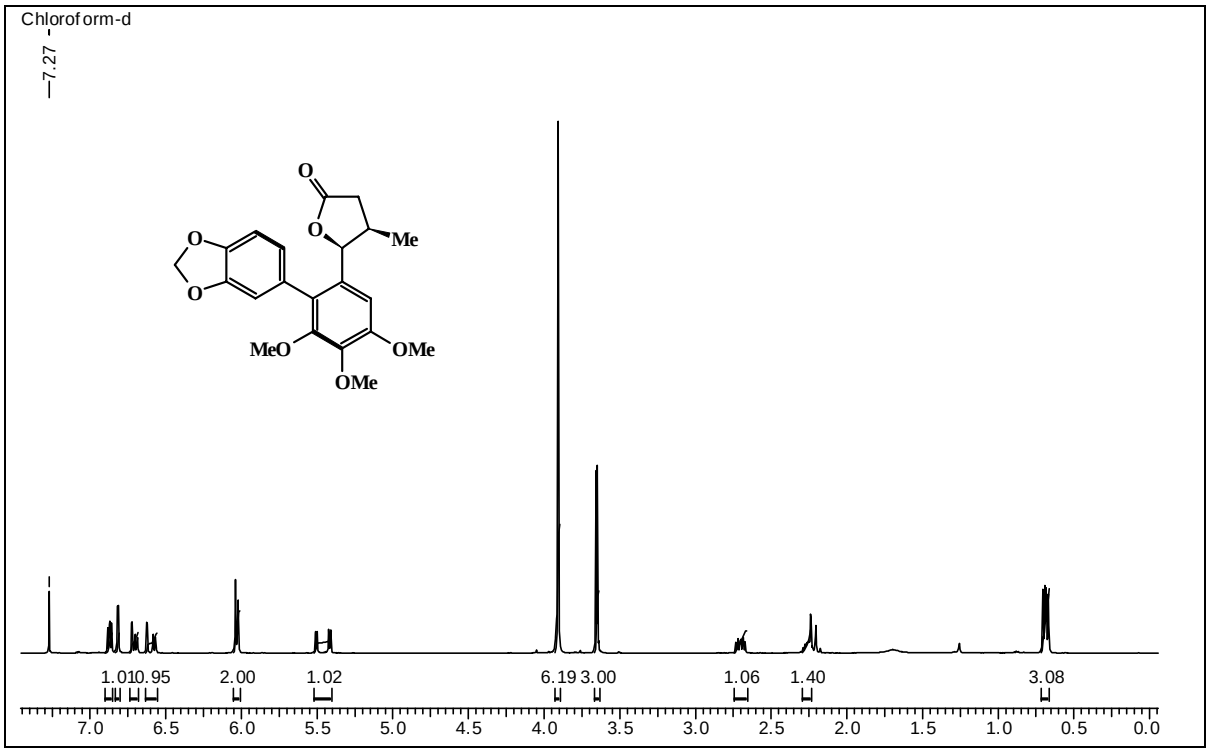
^{13}C NMR spectrum of compound (-) 31 in $CDCl_3$



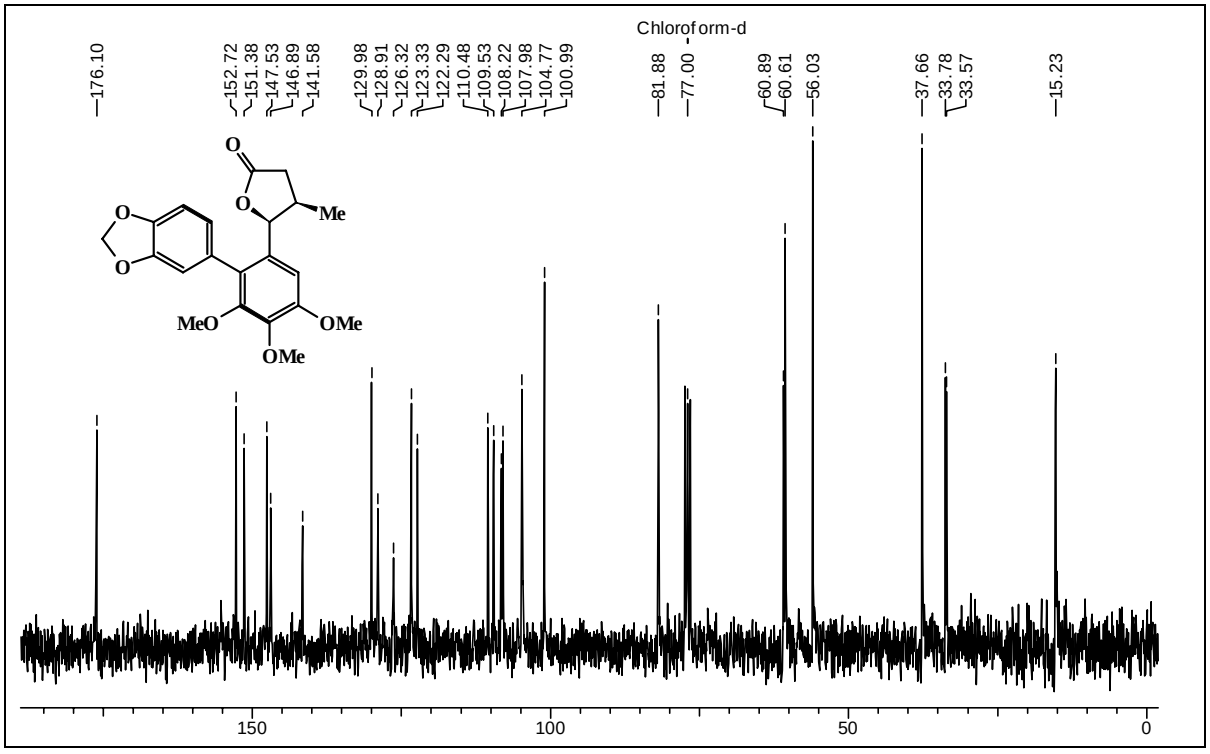
^{1}H NMR spectrum of compound (+) 38 in $CDCl_3$



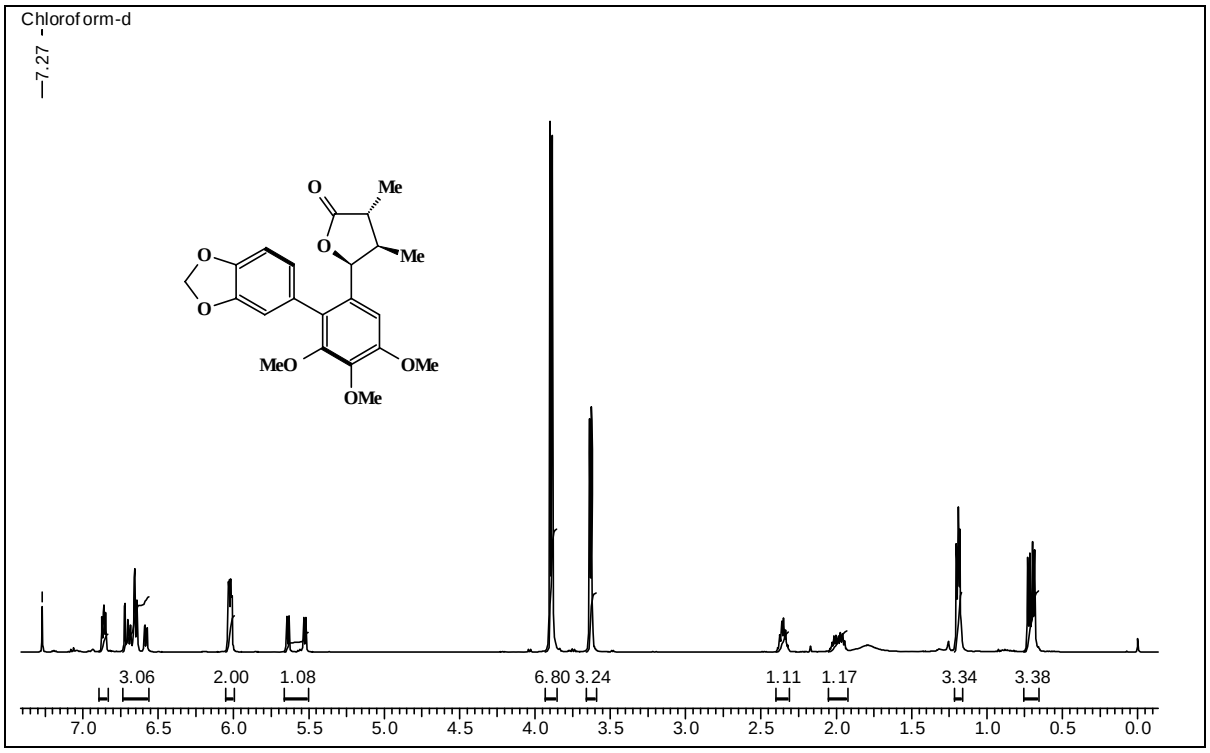
^{13}C NMR spectrum of compound (+) 38 in $CDCl_3$



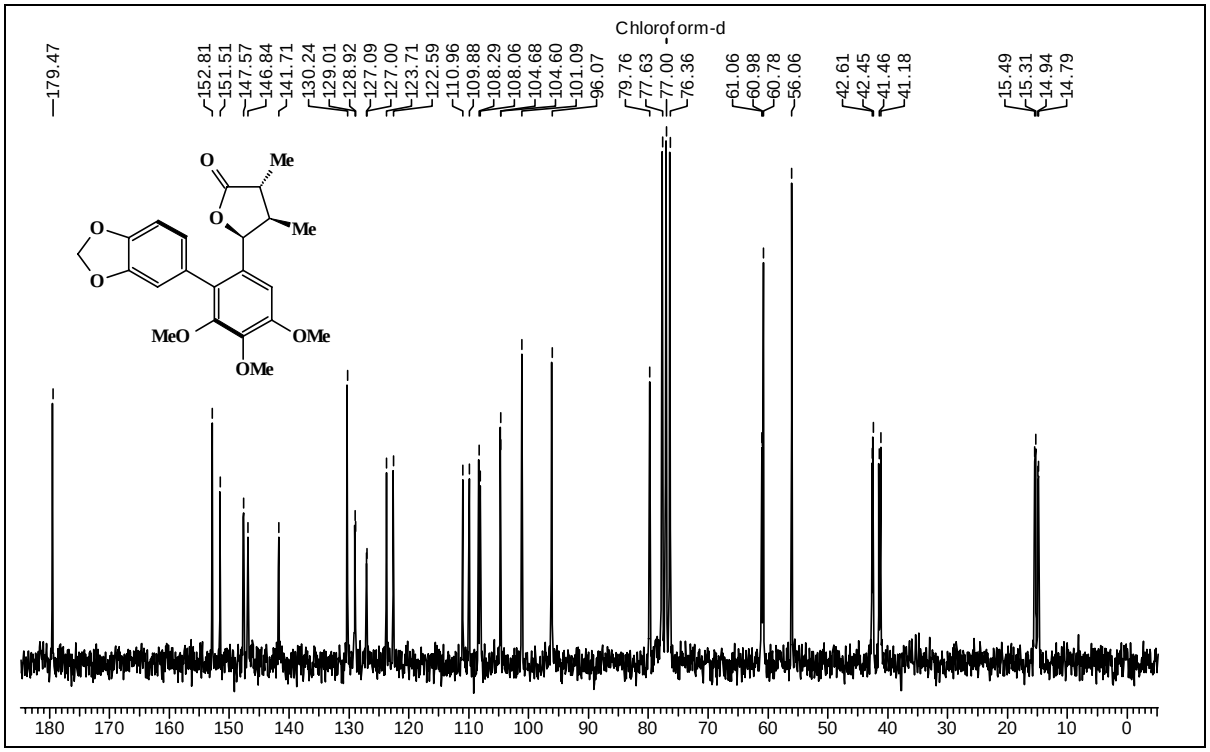
^{1}H NMR spectrum of compound (-) 39 in $CDCl_3$



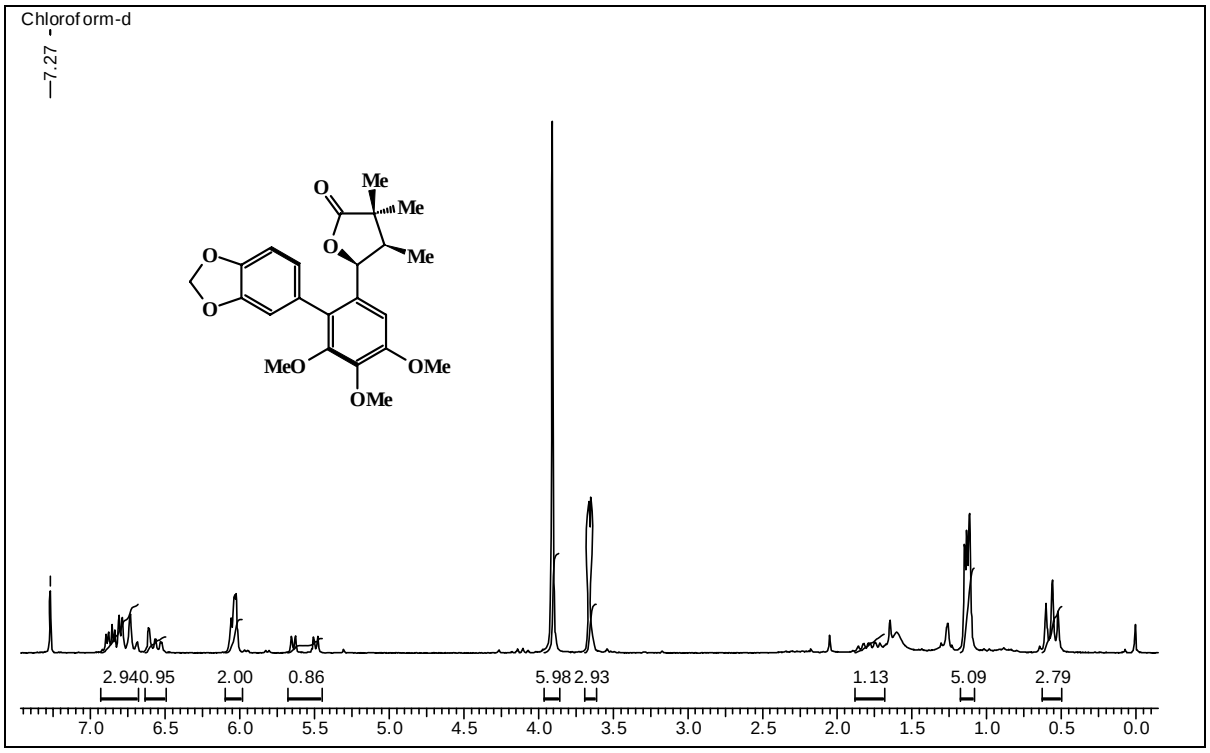
^{13}C NMR spectrum of compound (-) 39 in $CDCl_3$



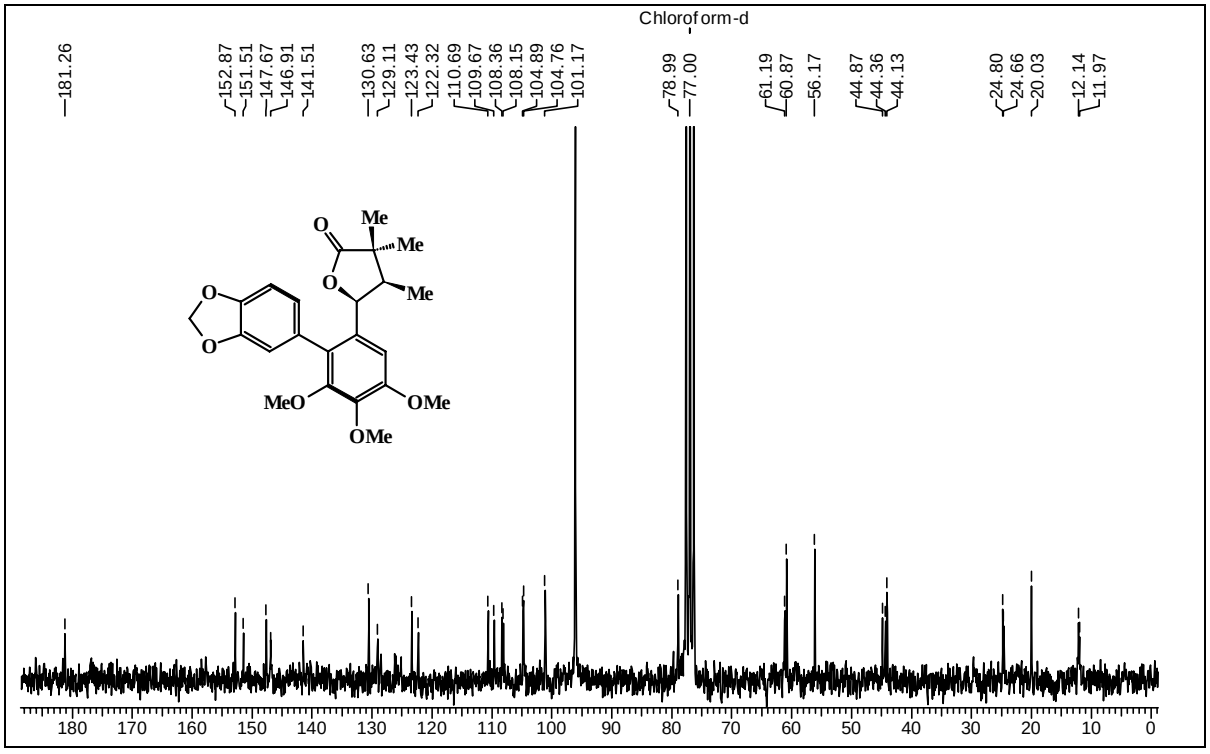
^{1}H NMR spectrum of compound (+) 2 in $CDCl_3$



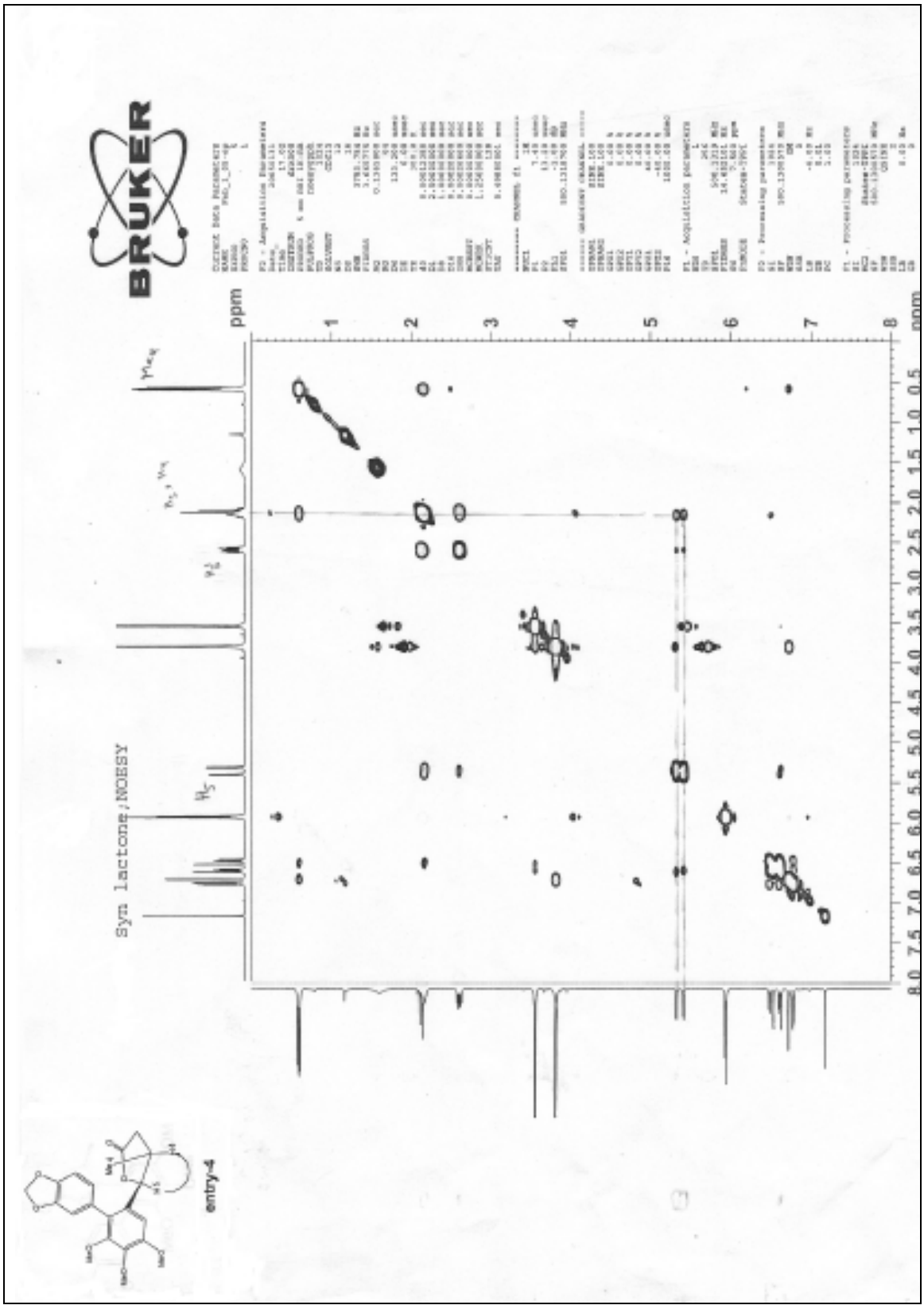
^{13}C NMR spectrum of compound (+) 2 in $CDCl_3$



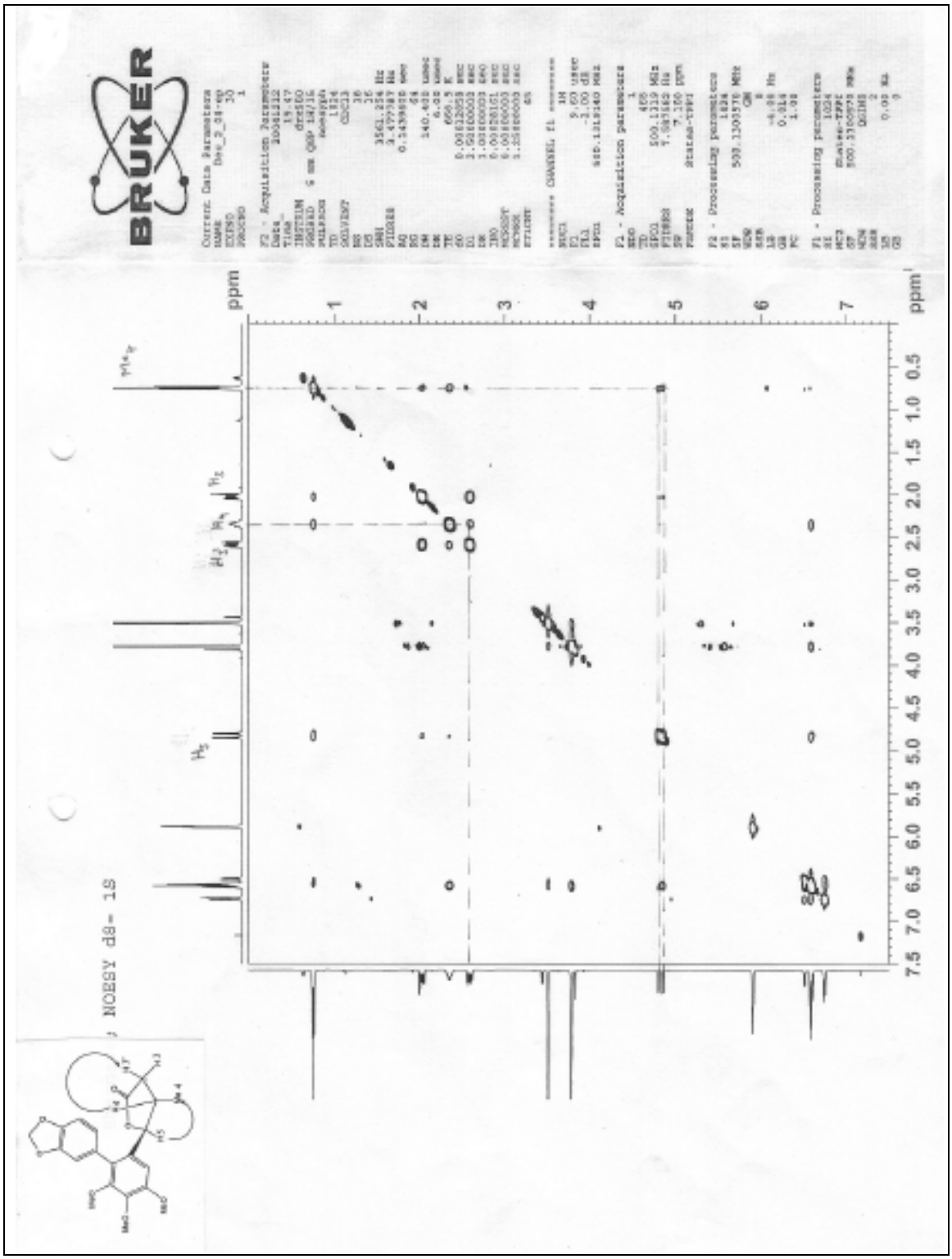
^{1}H NMR spectrum of compound (-) 40 in $CDCl_3$



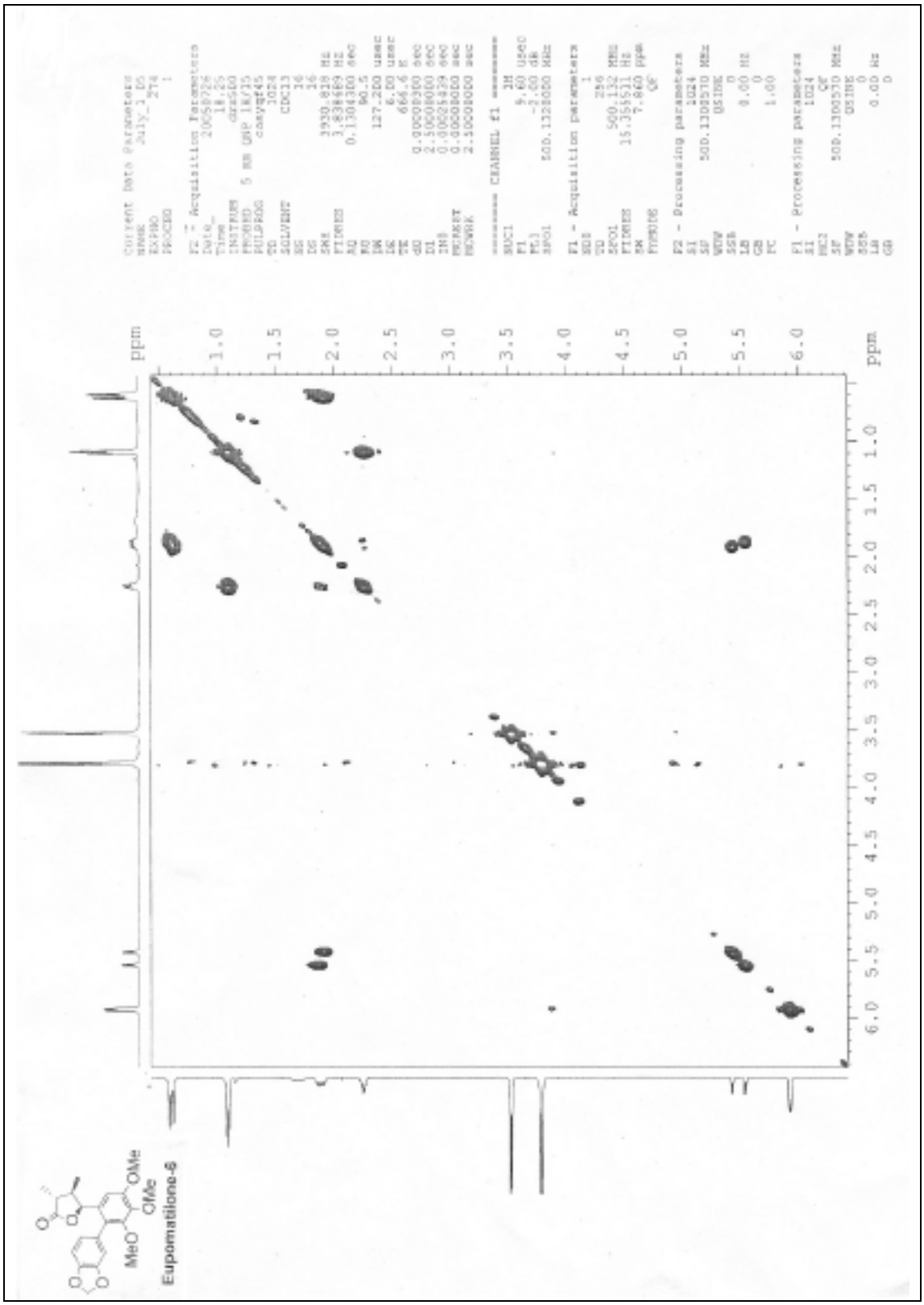
^{13}C NMR spectrum of compound (-) 40 in $CDCl_3$



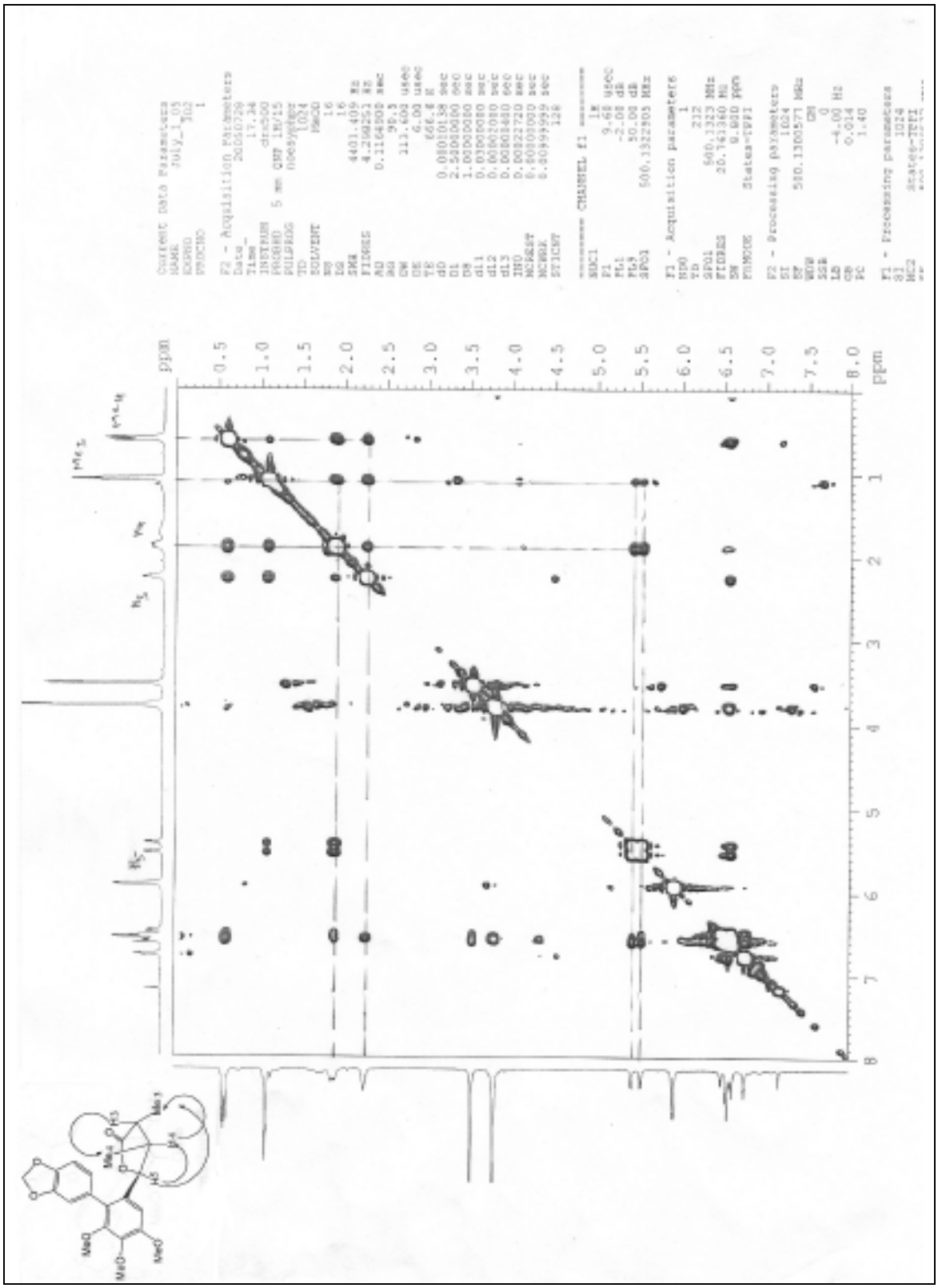
NOESY spectrum of compound 27 in $CDCl_3$



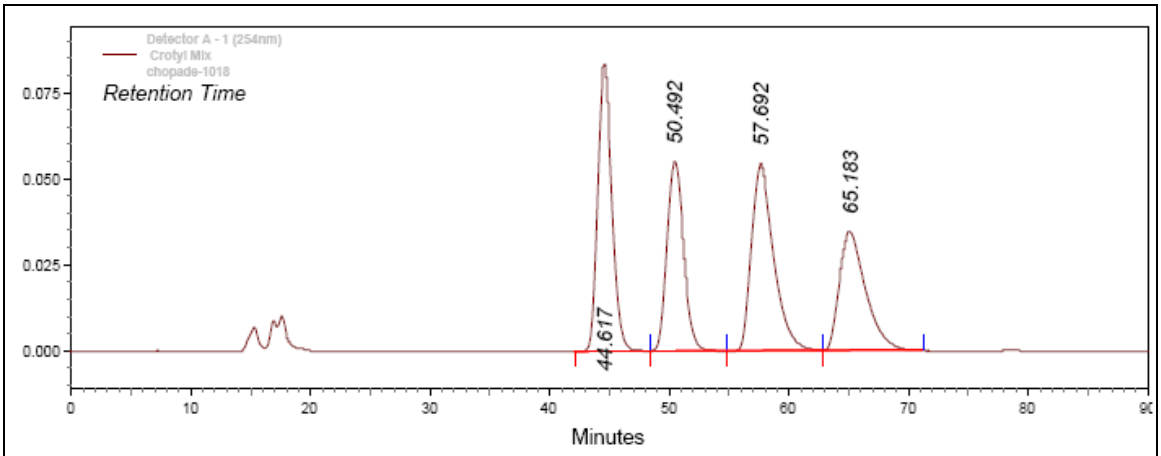
NOESY spectrum of compound 29 in $CDCl_3$



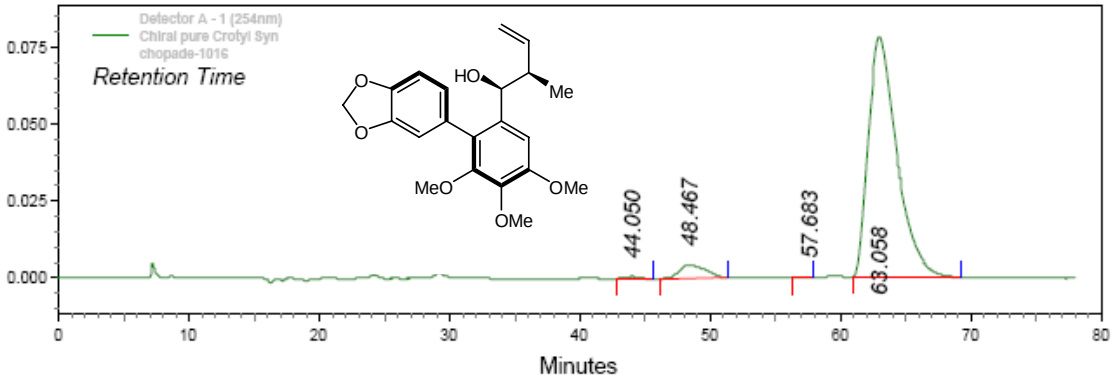
COSY spectrum of compound 2 in $CDCl_3$



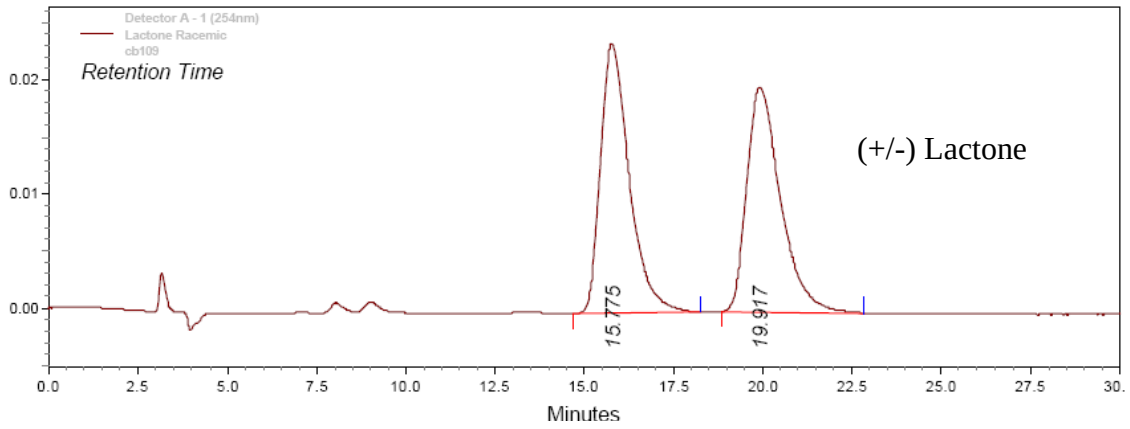
NOESY spectrum of compound 2 in $CDCl_3$



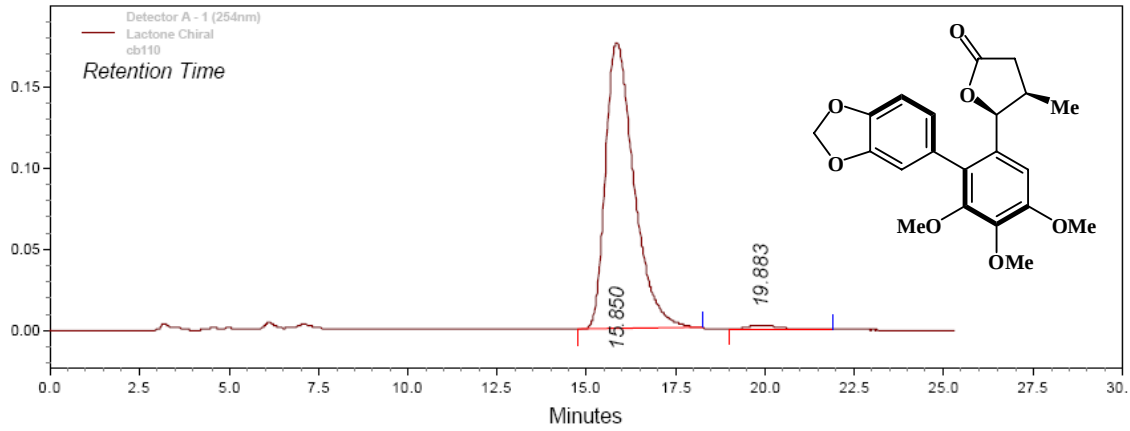
HPLC of Compound **21/22** (Racemic)



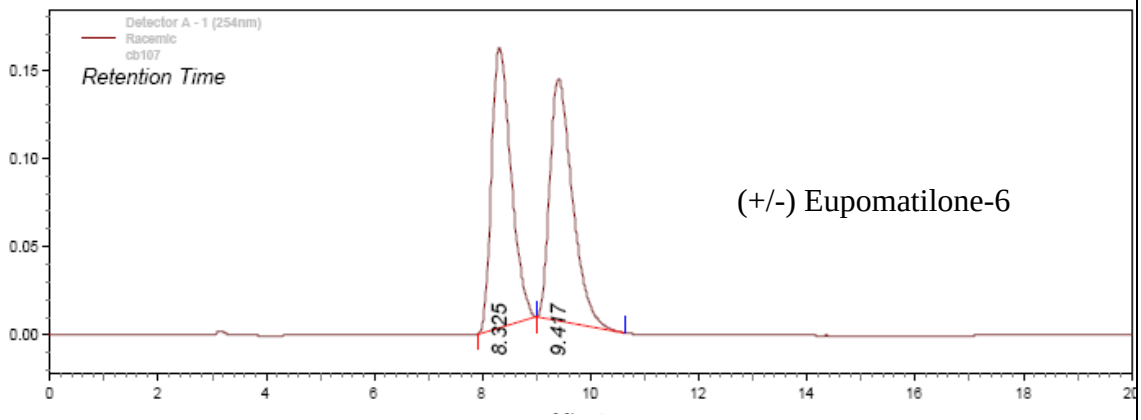
HPLC of Compound **31** (Chiral)



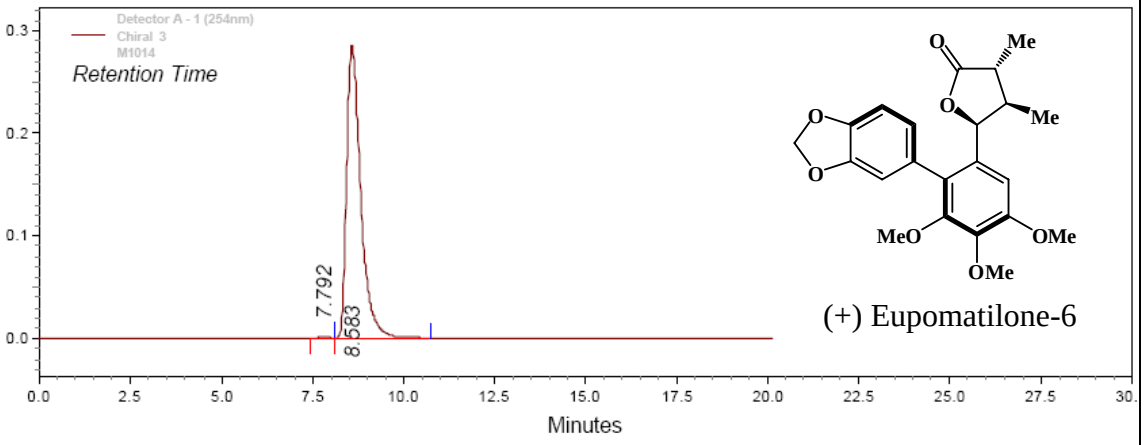
HPLC of Compound **27** (Racemic)



HPLC of Compound **39** (Chiral)



HPLC of Compound **2** (Racemic)



HPLC of Compound **2** (Chiral)

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CHAPTER II

First Formal Synthesis of 4-*O*-β-D-Galactosyl Maltobionolactone: A Substrate Analogue Inhibitors for Mammalian α-Amylases

Introduction

Introduction

A metabolic disorder is a medical disorder which affects the production of energy within individual human (or animal) cells. Most *metabolic disorders* are genetic, though a few are "acquired" as a result of diet, toxins, infections, etc. Genetic metabolic disorders are also known as inborn errors of metabolism. In general, the genetic *metabolic disorders* are caused by genetic defects that result in missing or improperly constructed enzymes necessary for some step in the metabolic process of the cell.

Metabolism[1] is the complete set of chemical reactions that occur in living cells. These processes are the basis of life, allowing cells to grow and reproduce, maintain their structures, and respond to their environments. Metabolism is usually divided into two categories. Catabolism yields energy, an example being the breakdown of food in cellular respiration. Anabolism, on the other hand, uses this energy to construct components of cells such as proteins and nucleic acids. The chemical reactions of metabolism are organized into metabolic pathways, in which one chemical is transformed into another by a sequence of enzymes. Enzymes are crucial to metabolism because they allow cells to drive desirable but thermodynamically unfavorable reactions by coupling them to favorable ones. Enzymes also allow the regulation of metabolic pathways in response to changes in the cell's environment or signals from other cells. The metabolism of an organism determines which substances it will find nutritious and which it will find poisonous. For example, some prokaryotes use hydrogen sulfide as a nutrient, yet this gas is poisonous to animals. The speed of metabolism, the metabolic rate, also influences how much food an organism will require.

Most of the structures that make up animals, plants and microbes are made from three basic classes of molecule: amino acids, carbohydrates and lipids (often called fats). As these molecules are vital for life, metabolism focuses on making these molecules, in the construction of cells and tissues, or breaking them down and using them as a source of energy, in the digestion and use of food. Many important biochemicals can be joined together to make polymers such as DNA and proteins. These macromolecules are essential parts of all living organisms.

The largest classes of *metabolic disorders* are:

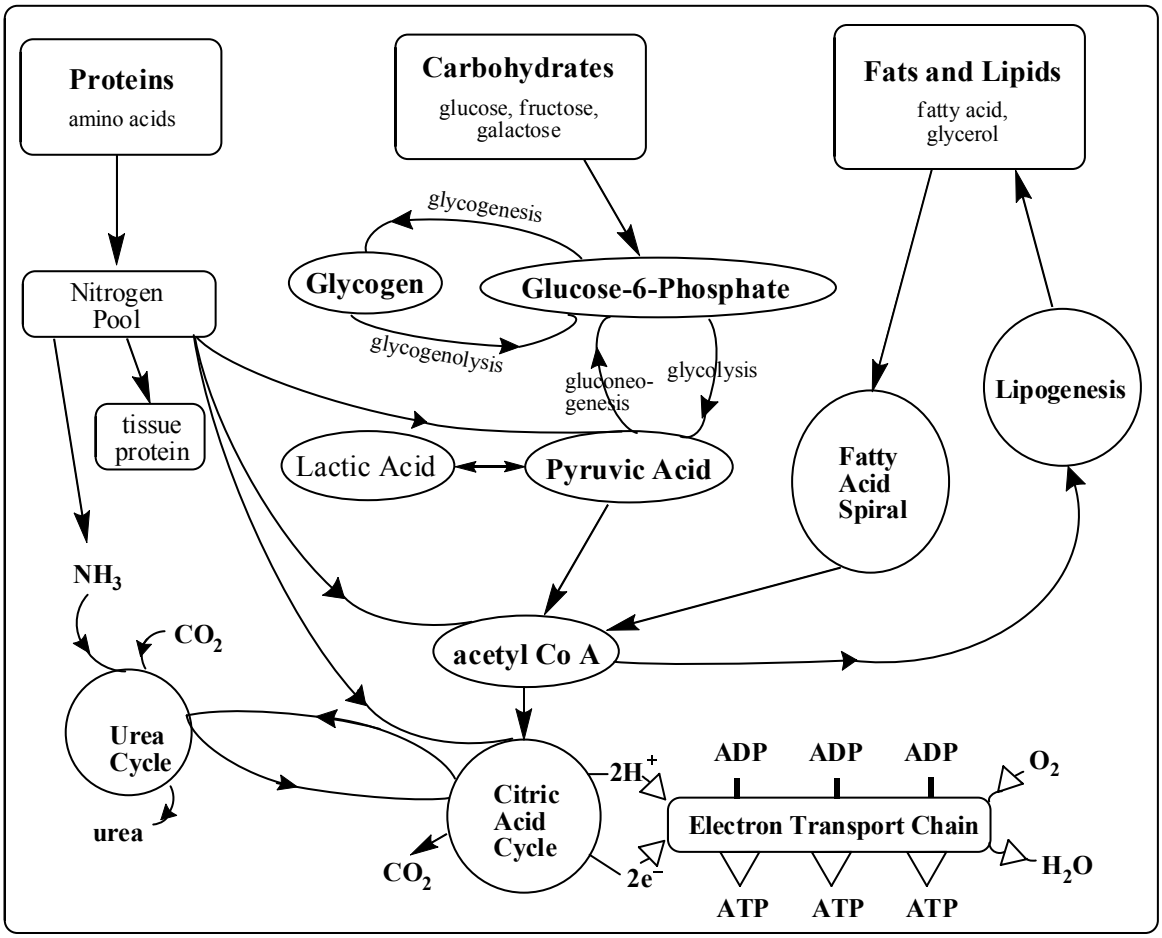
- Disorders of carbohydrate metabolism
- Disorders of amino acid metabolism
- Disorders of organic acid metabolism (organic acidurias)
- Disorders of fatty acid oxidation and mitochondrial metabolism
- Disorders of porphyrin metabolism
- Disorders of purine or pyrimidine metabolism
- Disorders of steroid metabolism
- Disorders of mitochondrial function
- Disorders of peroxisomal function

Carbohydrate Metabolism

Carbohydrates are a superior short-term energy reserve for organisms, because they are much simpler to metabolize than fats or proteins. In animals, all dietary carbohydrates are delivered to cells in the form of glucose. Carbohydrates are typically stored as long polymers of glucose molecules with Glycosidic bonds for structural support (e.g. chitin, cellulose) or energy storage (e.g. glycogen, starch). However, the strong affinity of carbohydrates for water makes storage of large quantities of carbohydrates inefficient due to the large molecular weight of the solvated water-carbohydrate complex. In some organisms, excess carbohydrates are catabolised to form Acetyl-CoA, where they enter the fatty acid synthesis pathway. Fatty acids, triglycerides, and other lipids are commonly used for long-term energy storage. The hydrophobic character of lipids makes them a much more compact form of energy storage than hydrophilic carbohydrates.

The most important carbohydrate is glucose, a simple sugar (monosaccharide) that is metabolized by nearly all known organisms. Glucose and other carbohydrates are part of a wide variety of metabolic pathways across species: plants synthesize carbohydrates from atmospheric gases by photosynthesis, which can then be consumed by other organisms and used as fuel for cellular respiration. Oxidation of one gram of carbohydrate yields approximately 4 kcal of energy. Energy obtained from carbohydrate metabolism is usually stored in the form of ATP. Organisms capable of aerobic respiration metabolize glucose and oxygen to release energy with carbon dioxide and water as byproducts.

Metabolic pathways



- Carbon fixation, whereby CO_2 is reduced to carbohydrate.
- Glycolysis - the breakdown of the glucose molecule in order to obtain ATP and Pyruvate.
- Pyruvate from glycolysis enters the Krebs cycle in aerobic organisms.
- The Pentose phosphate pathway, which acts in the conversion of hexoses into pentoses and in NADPH regeneration.
- Glycogenesis - the conversion of excess glucose into glycogen in order to prevent excessive osmotic pressure buildup inside the cell
- Glycogenolysis - the breakdown of glycogen into glucose, in order to provide a steady level of glucose supply for glucose-dependent tissues.
- Gluconeogenesis - *de novo* synthesis of glucose molecules from simple organic compounds.

Various enzymes are involved in the breaking down and synthesis of glucosidic linkages in starch, which can be grouped into four general classes:

Breakdown of starch:

- Hydrolysis of α-1,4-glucosidic linkages, e.g. α-amylase
- Hydrolysis of α-1,6-glucosidic linkages, e.g. pullulanase

Synthesis of starch:

- Transglycosylation to form α-1,4-glucosidic linkages: cyclodextrin glucanotransferase
- Transglycosylation to form α-1,6-glucosidic linkages: branching enzyme

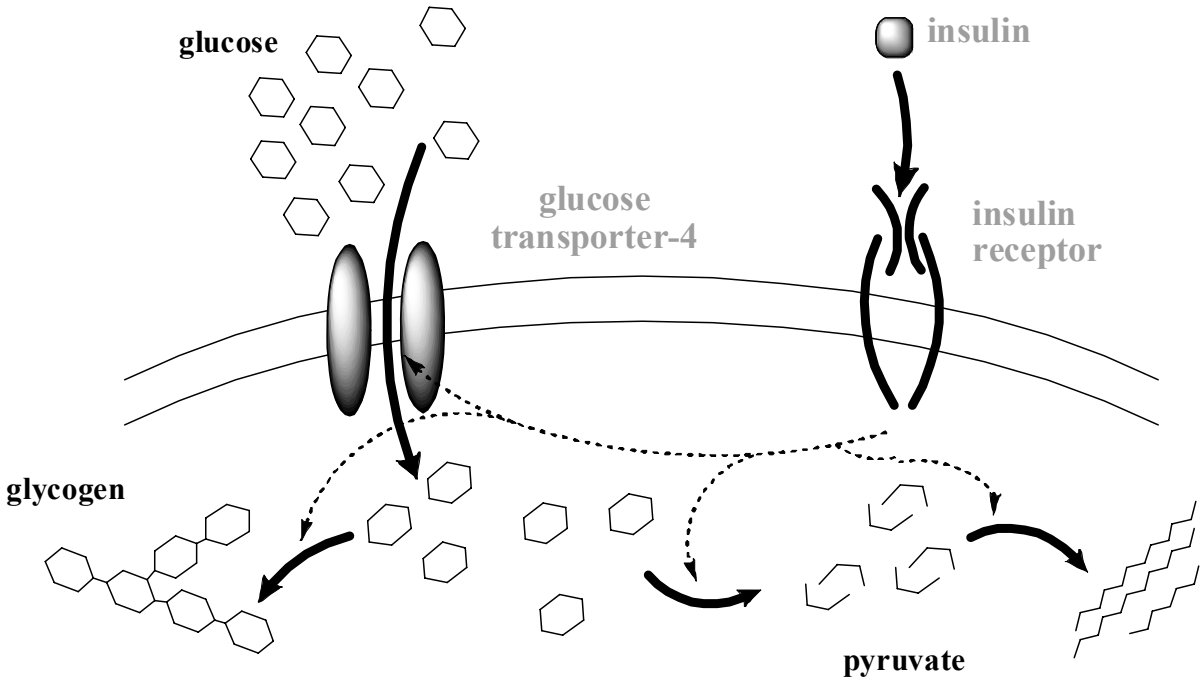
Glucose metabolism

The major site of daily glucose consumption (75%) is the brain via aerobic pathways. Most of the remainder of is utilized by erythrocytes, skeletal muscle, and heart muscle. The body obtains glucose either directly from the diet or from amino acids and lactate via gluconeogenesis. Glucose obtained from these two primary sources either remains soluble in the body fluids or is stored in a polymeric form, glycogen. Glycogen[2] is considered the principal storage form of glucose and is found mainly in liver and muscle, with kidney and intestines adding minor storage sites. With up to 10% of its weight as glycogen, the liver has the highest specific content of any body tissue. Muscle has a much lower amount of glycogen per unit mass of tissue, but since the total mass of muscle is so much greater than that of liver, total glycogen stored in muscle is about twice that of liver. Stores of glycogen in the liver are considered the main buffer of blood glucose levels.

Because insulin[3] is the principal hormone that regulates uptake of glucose into most cells from the blood (primarily muscle and fat cells, but not central nervous system cells), deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus. Much of the carbohydrate in food is converted within a few hours to the monosaccharide glucose, the principal carbohydrate found in blood. Some carbohydrates are not converted. Notable examples include fruit sugar (fructose) that is usable as cellular fuel, but it is not converted to glucose and does not participate in the insulin/glucose metabolic regulatory mechanism; additionally, the carbohydrate cellulose (though it is actually many glucose molecules in long chains) is not converted to glucose, as humans and many animals have no digestive pathway capable of handling cellulose. Insulin is released into the blood by beta cells (*β*-cells) in the pancreas in response to rising levels of blood

glucose (e.g., after a meal). Insulin enables most body cells (about 2/3 is the usual estimate, including muscle cells and adipose tissue) to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage. Insulin is also the principal control signal for conversion of glucose (the basic sugar used for fuel) to glycogen for internal storage in liver and muscle cells. Reduced glucose levels result both in the reduced release of insulin from the beta cells and in the reverse conversion of glycogen to glucose when glucose levels fall, although only glucose thus recovered by the liver re-enters the bloodstream as muscle cells lack the necessary export mechanism.

Effect of insulin on glucose uptake and metabolism.



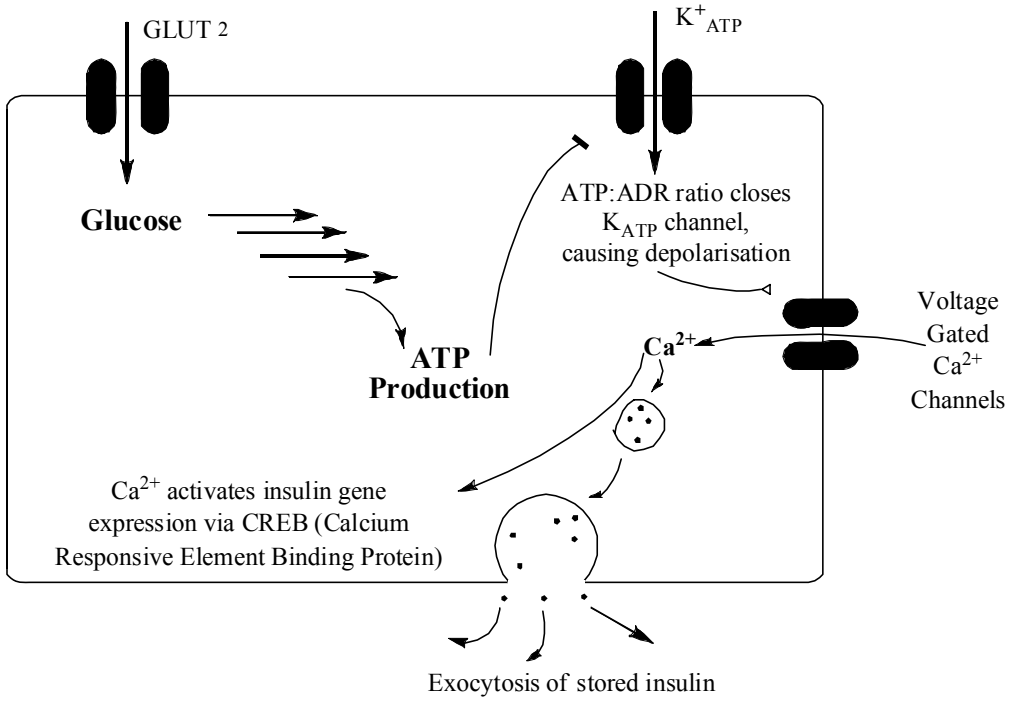
Insulin production is more or less constant within the beta cells, irrespective of blood glucose levels. It is stored within vacuoles pending release, via exocytosis, which is triggered by increased blood glucose levels. Insulin resistance means that body cells do not respond appropriately when insulin is present.

Higher insulin levels increase many anabolic ("building up") processes such as cell growth and duplication, protein synthesis, and fat storage. Insulin is the principal signal in converting many of the bidirectional processes of metabolism from a catabolic to an

anabolic direction, and vice versa. In particular, it is the trigger for entering or leaving ketosis (the fat burning metabolic phase).

If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or resistance), or if the insulin itself is defective, glucose will not be handled properly by body cells (about ⅔ require it) or stored appropriately in the liver and muscles. The net effect is persistent high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as diabetes, acidosis.

Mechanism of insulin release in normal pancreatic beta cells



Human diseases of carbohydrate metabolism

- Diabetes mellitus
- Lactose intolerance
- Fructose intolerance
- Galactosemia
- Glycogen storage disease

Diabetes mellitus

Diabetes mellitus, often simply diabetes, is a metabolic disorder characterized by hyperglycemia (high blood sugar). The characteristic symptoms are polyuria (excessive

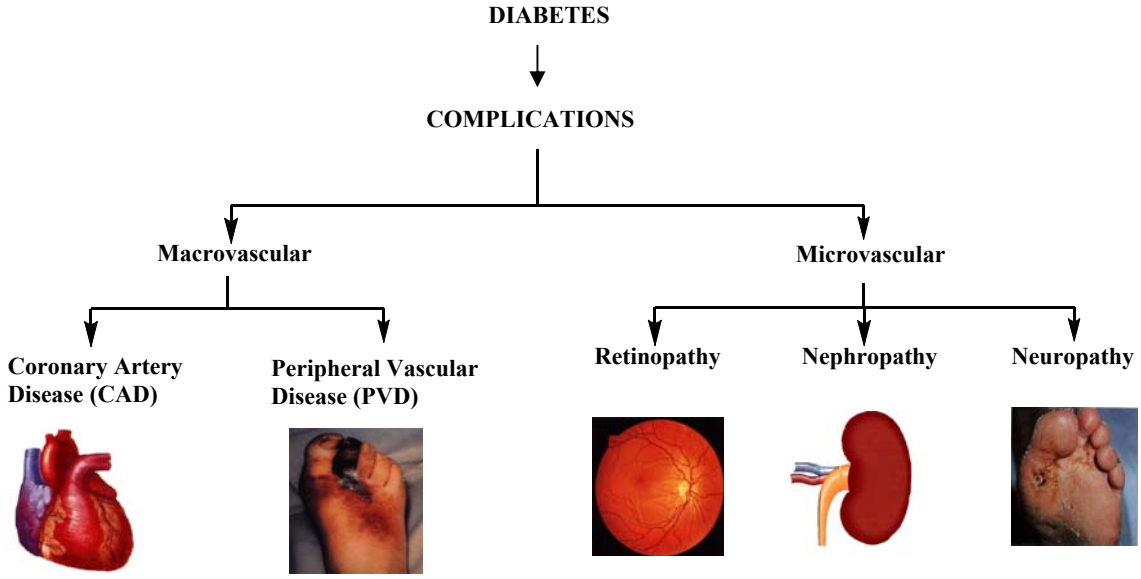
urine production), polydipsia (thirst and increased fluid intake) and blurred vision; these symptoms may be absent if the blood sugar is only mildly elevated. The ancient Indians tested for diabetes by observing whether ants were attracted to a person's urine, and called the ailment "sweet urine disease" (Madhumeha). The Korean, Chinese, and Japanese words for diabetes are based on the same ideographs which mean "sugar urine disease".

The World Health Organization recognizes[1] three main forms of diabetes mellitus: *type 1*, *type 2*, and *gestational diabetes* (occurring during pregnancy), which have similar signs, symptoms, and consequences, but different causes and population distributions. Ultimately, all forms are due to the beta cells of the pancreas being unable to produce sufficient insulin to prevent hyperglycemia.[3] Type 1 is usually due to autoimmune destruction of the pancreatic beta cells which produce insulin. Type 2 is characterized by tissue-wide insulin resistance, but impairment of beta cell function is necessary for its development. Gestational diabetes is similar to type 2 diabetes, in that it involves insulin resistance; the hormones of pregnancy cause insulin resistance in those women genetically predisposed to developing this condition.

Types 1 and 2 are incurable chronic conditions, but have been treatable since insulin became medically available in 1921, and today are usually managed with a combination of dietary treatment, tablets (in type 2) and, frequently, insulin supplementation. Gestational diabetes typically resolves with delivery. Diabetes can cause many complications. Acute complications (hypoglycemia, ketoacidosis or nonketotic hyperosmolar coma) may occur if the disease is not adequately controlled. Serious long-term complications include cardiovascular disease (doubled risk), chronic renal failure (diabetic nephropathy is the main cause of dialysis in developed world adults), retinal damage (which can lead to blindness and is the most significant cause of adult blindness in the non-elderly in the developed world), nerve damage (of several kinds), and microvascular damage, which may cause erectile dysfunction (impotence) and poor healing.

Poor healing of wounds, particularly of the feet, can lead to gangrene which can require amputation, the leading cause of non-traumatic amputation in adults in the developed world. Adequate treatment of diabetes, as well as increased emphasis on blood pressure control and lifestyle factors (such as not smoking and keeping a healthy body weight), may improve the risk profile of most aforementioned complications.

Prevalence of Diabetes Complications



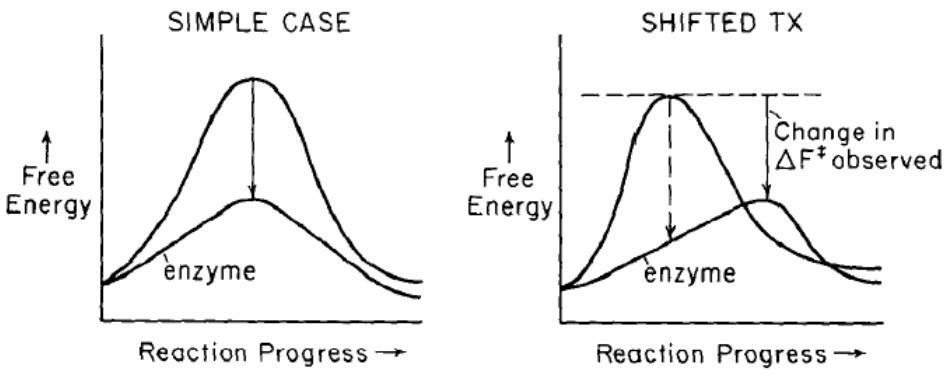
The most important drug now used in Type 2 diabetes is the Biguanide metformin which works primarily by reducing liver release of blood glucose from glycogen stores as well as some increase in uptake of glucose by the body's tissues. Both historically and currently commonly used are the Sulfonylurea group, of which several members (including glibenclamide and gliclazide) are widely used; these increase glucose stimulated insulin secretion by the pancreas.

Postprandial hyperglycemia plays an important role in the development of type 2 diabetes mellitus and complications associated with the disease such as micro- and macro-vascular disease.[4] However, therapeutic approaches to the treatment of postprandial hyperglycemia have been ineffective except for insulin therapy, creating a challenge for researchers involved in the development of new agents to control postprandial hyperglycemia. One strategy used to stabilize postprandial blood glucose has been the utilization of specific enzyme inhibitors which act against gut digestive enzymes by interrupting rapid nutrient absorption. The powerful synthetic α-glucosidase inhibitors, such as acarbose and voglibose, act directly to reduce the sharp increases in glucose concentration that occur immediately following ingestion of a meal.[5] These inhibitors also produce a reduction in insulinaemic responses and have a beneficial effect on insulin resistance. However, the powerful inhibitory action causes side effects like flatulence,

diarrhea and abdominal cramping, all of which are associated with incomplete carbohydrate absorption.[6] In addition, there are reports of an increased incidence of renal tumors and serious hepatic injury and acute hepatitis.[7]

The other promising approach to control postprandial hyperglycaemia[8] is based on the inhibitory action of α-amylase derived from saliva and the pancreas. By contrast to the a-glucosidase inhibitors, starch malabsorption induced by an α-amylase inhibitor might not be associated with as large an osmotic load, as the colonic flora are capable of metabolizing large quantities of starch without producing diarrhea.[9] In early studies using a proteinaceous α-amylase inhibitor, investigators failed to control postprandial blood glucose concentration because such inhibitors had a very little *anti*-amylase activity.[10] However, recent studies have demonstrated that inhibition of carbohydrate digestion by a wheat α-amylase inhibitor reduced postprandial plasma insulin and glucose response to dietary carbohydrate in rats, dogs and humans.[11]

Transition State Inhibitors



Acarbose is an anti-diabetic drug used to treat type 2 diabetes mellitus and, in some countries, prediabetes. It is an inhibitor of *α-glucosidase*, an enteric enzyme that releases glucose from larger carbohydrates.

Glucose is a major source of energy in our body, but unfortunately, free glucose is relatively rare in our typical diet. Instead, glucose is locked up in many larger forms, including lactose and sucrose, where two small sugars are connected together and long chains of glucose like starches and glycogen. One of the major jobs of digestion is to break these chains into their individual glucose units, which are then delivered by the blood to hungry cells throughout your body.

Starches are glucose polymers in which glucopyranose units are bonded by α-linkages. It is made up of a mixture of Amylose and Amylopectin. Amylose consists of a linear chain of several hundred glucose molecules and Amylopectin is a branched molecule made of several thousand glucose units. Starches are insoluble in water. They can be digested by hydrolysis, catalyzed by enzymes called amylases, which can break the α-linkages (glycosidic bonds). Humans and other animals have amylases, so they can digest starches. Potato, rice, wheat, and maize are major sources of starch in the human diet.

The structural components of plants are formed primarily from cellulose. Wood is largely cellulose and lignin, while paper and cotton are nearly pure cellulose. Cellulose is a polymer made with repeated glucose units bonded together by β-linkages. Humans and many other animals lack an enzyme to break the β-linkages, so they do not digest cellulose. Certain animals can digest cellulose, because bacteria possessing the enzyme are present in their gut. The classic example is the termite.

Glycosidase inhibitors[12-15]

Glycosidase inhibitors are molecules that bind to Glycosidase (enzymes) and decrease their activity. The binding of an inhibitor can stop a substrate from entering the enzyme's active site and/or hinder the enzyme from catalyzing its reaction (Figure 3). Since blocking an enzyme's activity can kill a pathogen or correct a metabolic imbalance, many drugs are enzyme inhibitors. They are also used as herbicides and pesticides.

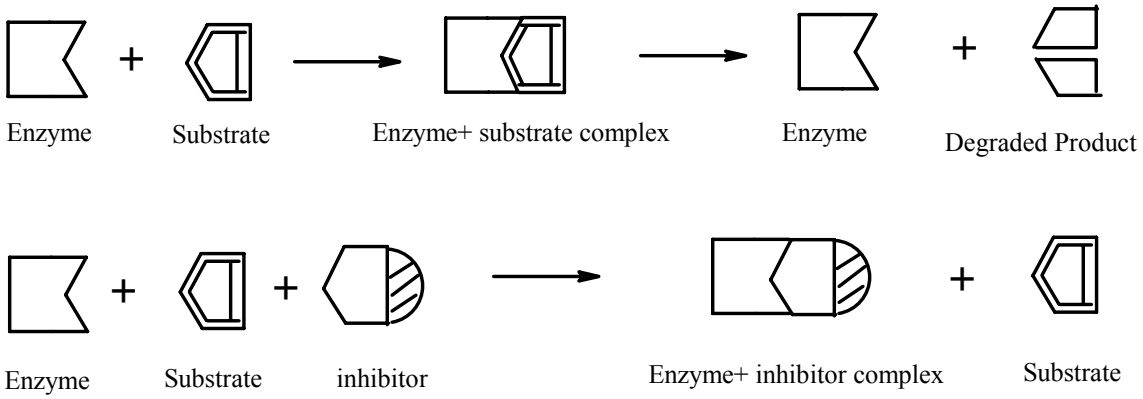


***Figure* 3:** *Inhibitor mimicking the enzyme*

A range of inhibitors of glycoside hydrolases have been developed. These include sugar shaped nitrogen heterocycles such as deoxynojirimycin, isofagomine, and various unsaturated species such as PUGNAc. Several drugs in clinical use are inibitors of

glycoside hydrolases including acarbose, Relenza (zanamivir), miglitol and Tamiflu (oseltamivir).

Glycoside hydrolases

Glycoside hydrolases (also called glycosidases) catalyze the hydrolysis of the glycosidic linkage to generate two smaller sugars. They are extremely common enzymes with roles in nature including degradation of biomass such as cellulose and hemicellulose, in anti-bacterial defense strategies (eg lysozyme), in pathogenesis mechanisms (eg viral neuraminidases) and in normal cellular function (eg trimming mannosidases involved in N-linked glycoprotein biosynthesis). Together with glycosyltransferases, glycosidases form the major catalytic machinery for the synthesis and breakage of glycosidic bonds.

Glycoside hydrolases are found in essentially all domains of life. In bacteria and prokaryotes, they are found both as intracellular and extracellular enzymes largely involved in nutrient acquisition. One of the important occurences of glycoside hydrolases in bacteria is the enzyme beta-galactosidase, which is involved in regulation of expression in *E. coli* through the [[*che* operon]]. In higher organisms glycoside hydrolases are found within the endoplasmic reticulum where they are involved in processing of N-linked glycoproteins, and in the lysozome as enzymes involved in the degradation of carbohydrate structures. Deficiency in specific lysozomal glycoside hydrolases can lead to a range of lysosomal storage disorders that result in developmental problems or death. Glycoside hydrolases are found in the intestinal tract and in saliva where they degrade complex carbohydrates such as lactose, starch, sucrose and trehalose. In the gut they are found as glycosylphosphatidyl anchored enzymes on endothelial cells. The enzyme lactase is required for degradation of the milk sugar lactose and is present at high levels in infants, but in most populations will decrease after weaning or during infancy, potentially leading to lactose intolerance in adulthood. The enzyme O-GlcNAcase is involved in removal of N-acetylglucoamine groups from serie residues in the nucleus of the cell. The glycoside hydrolases are involved in the biosynthesis and degradation of glycogen in the body.

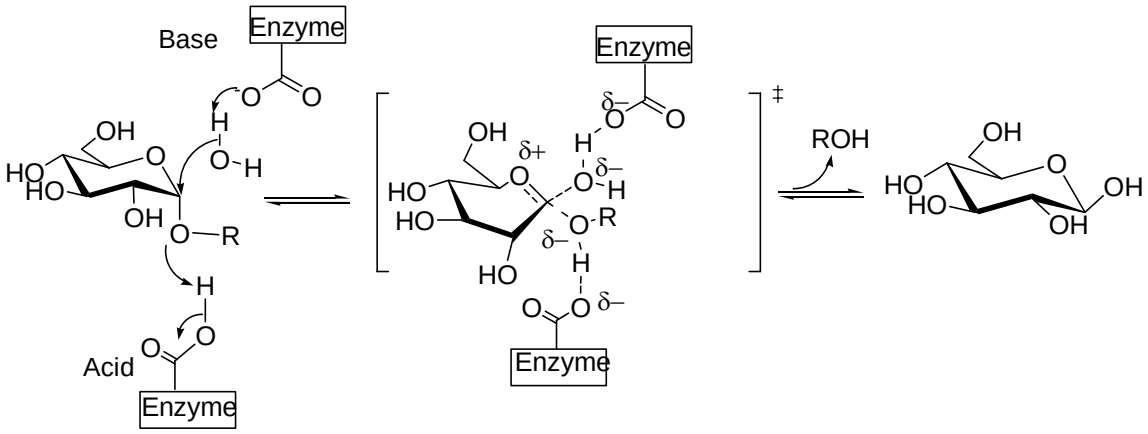
Glycoside hydrolases are classified into EC 3.2.1 as enzymes catalyzing the hydrolysis of O- or S-glycosides. Glycoside hydrolases can also be classified according to the stereochemical outcome of the hydrolysis reaction: thus they can be classified as either *retaining* or *inverting* enzymes.[16] Glycoside hydrolases can also be classified as exo or endo

acting, dependent upon whether they act at the (usually non-reducing) end or in the middle, respectively, of an oligo/polysaccharide chain. Glycoside hydrolases may also be classified by sequence based methods.

Reaction Mechanism of Glycosidases[17]

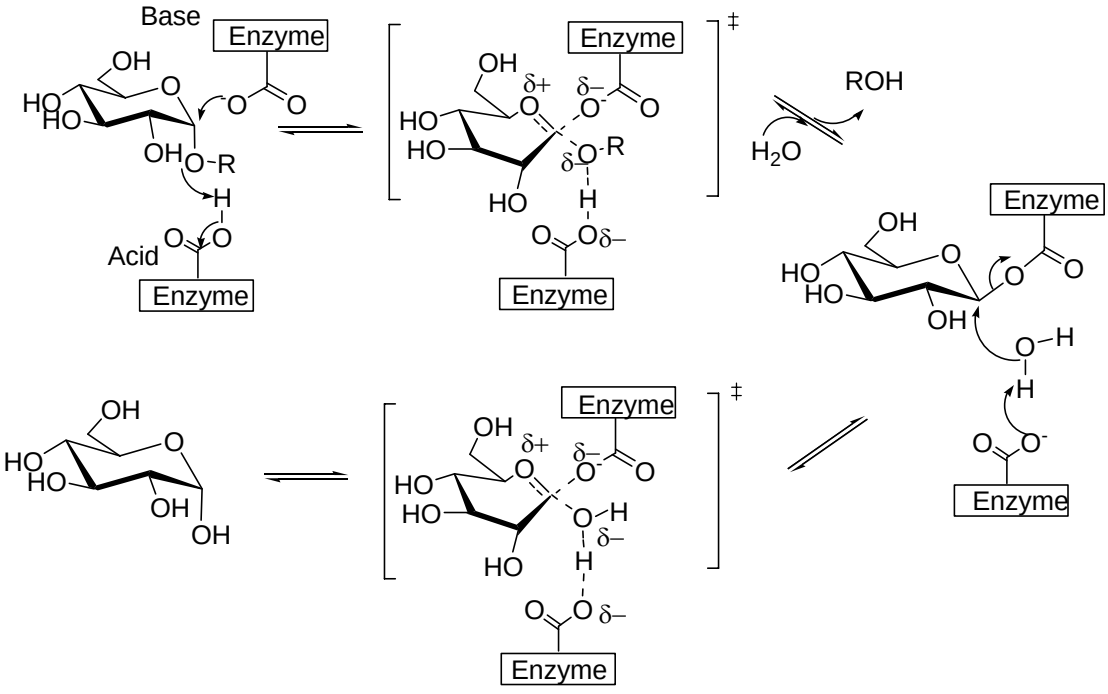
Inverting Glycosidases

Inverting enzymes utilize two enzymic residues, typically carboxylate residues, that act as acid and base respectively, as shown below for a *beta*-glucosidase.



Retaining Glycosidases

Retaining glycosidases operate through a two-step mechanism, with each step resulting in inversion, for a net retention of stereochemistry.



Retaining glycosidases operate through a two-step mechanism, with each step resulting in inversion, for a net retention of stereochemistry. Again, two residues are involved, which are usually enzyme-borne carboxylates. One acts as a nucleophile and the other as an acid/base. In the first step the nucleophile attacks the anomeric centre, resulting in the formation of a glycosyl enzyme intermediate, with acidic assistance provided by the acidic carboxylate. In the second step the now deprotoned acidic carboxylate acts as a base and assists a nucleophilic water to hydrolyze the glycosyl enzyme intermediate, giving the hydrolyzed product.[18]

An alternative mechanism for hydrolysis with retention of stereochemistry can occur that proceeds through a nucleophilic residue that is bound to the substrate, rather than being attached to the enzyme. Such mechanisms are common for certain N-acetylhexosaminidases, which have an acetamido group capable of neighboring group participation to form an intermediate oxazoline or oxazolinium ion. Again, the mechanism proceeds in two steps through individual inversions to lead to a net retention of configuration.

Amylase

Amylase is the name given to glycoside hydrolase enzymes that break down starch into glucose molecules. Although the amylases are designated by different Greek letters, they all act on *α*-1,4-glycosidic bonds. Under the original name of *diastase*, amylase was the first enzyme to be found and isolated (by Anselme Payen in 1833). *α*-Amylase is the major form of amylase found in humans and other mammals.

Classification:

α-Amylase

The *α*-amylases are calcium metalloenzymes, completely unable to function in the absence of calcium. By acting at random locations along the starch chain, *α*-amylase breaks down long-chain carbohydrates, ultimately yielding maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin. Because it can act anywhere on the substrate, *α*-amylase tends to be faster acting than *β*-amylase. In animals, it is a major digestive enzyme. In human physiology, both the salivary and pancreatic amylases are *α*-amylases. They are discussed in much more detail at *α*-amylase. *α*-Amylase is the major form of amylase found in humans and other mammals.

β-Amylase

Another form of amylase, *β*-amylase is also synthesized by bacteria, fungi and plants. Working from the non-reducing end, *β*-amylase catalyzes the hydrolysis of the second *α*-1,4 glycosidic bond, cleaving off two glucose units (maltose) at a time. During the ripening of fruit, *β*-amylase breaks starch into sugar, resulting in the sweet flavor of ripe fruit. Both are present in seeds; *β*-amylase is present prior to germination whereas *α*-amylase and proteases appear once germination has begun. Cereal grain amylase is key to the production of malt. Many microbes also produce amylase to degrade extracellular starches. Animal tissues do not contain *β*-amylase, although it may be present in microrganisms contained within the digestive tract.

γ-Amylase

In addition to cleaving the last *α*-(1-4)glycosidic linkages at the nonreducing end of amylose and amylopectin, yielding glucose, γ-amylase will cleave *α*-(1-6) glycosidic linkages.

Amylase in human physiology:

Although found in many tissues, amylase is most prominent in pancreatic juice and urine which each have their own isoform of human *α*-amylase. They behave differently on isoelectric focusing, and can also be separated in testing by using specific monoclonal antibodies. In humans, all amylase isoforms link to chromosome 1p21.

Salivary amylase (ptyalin)

Amylase is found in saliva and breaks starch down into maltose and dextrin. This form is also called ptyalin. Ptyalin will break large, insoluble starch molecules into soluble starches (amylodextrin, erythrodextrin, achrodextrin) producing successively smaller starches and ultimately maltose. Ptyalin acts on linear *α*-(1-4) glycosidic linkages, but compound hydrolysis requires an enzyme which acts on branched products. Salivary amylase is inactivated in the stomach by gastric acid.

Pancreatic amylase

Pancreatic *α*-amylase randomly cleaves the *α*-(1-4)glycosidic linkages of amylose to yield dextrin, maltose or maltotriose. It adopts a double displacement mechanism with retention of anomeric configuration.

Attacking Starch

α-Amylase begins the process of starch digestion. It takes starch chains and breaks them into smaller pieces with two or three glucose units. Two similar types of amylase are made in our body, one is secreted in saliva, where it starts to break down starch grains as you chew, and the other is secreted by the pancreas, where it finishes its job. Then, these little pieces are broken into individual glucose units by a collection of enzymes that are tethered to the walls of the intestine.

α-Amylase Inhibitors

Microorganisms, higher plants, and animals produce a large number of different protein inhibitors of *α*-amylases in order to regulate the activity of these enzymes. These inhibitors can be grouped into six classes based on their tertiary structures: lectin-like, knottin-like, cereal-type, Kunitz-like, γ-purothionin-like and thaumatin-like inhibitors. Some of these inhibitors act by directly blocking the active centre of the enzyme at various local sites.

In animals, *α*-amylase inhibitors reduce the glucose peaks that can occur after a meal, slowing the speed with which *α*-amylase can convert starch to simple sugars until the body can deal with it. This is of particular importance in people with diabetes, where low insulin levels prevent extra cellular glucose from being cleared quickly from the blood. Therefore, diabetics tend to have low *α*-amylase levels in order to keep glucose levels under control, except after taking insulin, which causes a rise in pancreatic *α*-amylase.

Plants also use *α*-amylase inhibitors as a defense strategy. These inhibitors impede the digestive action of *α*-amylases and proteinases in the insect gut, thereby acting as insect anti-feed ants. As a result, *α*-amylase inhibitors have potential in various fields, including crop protection and the treatment of diabetes.

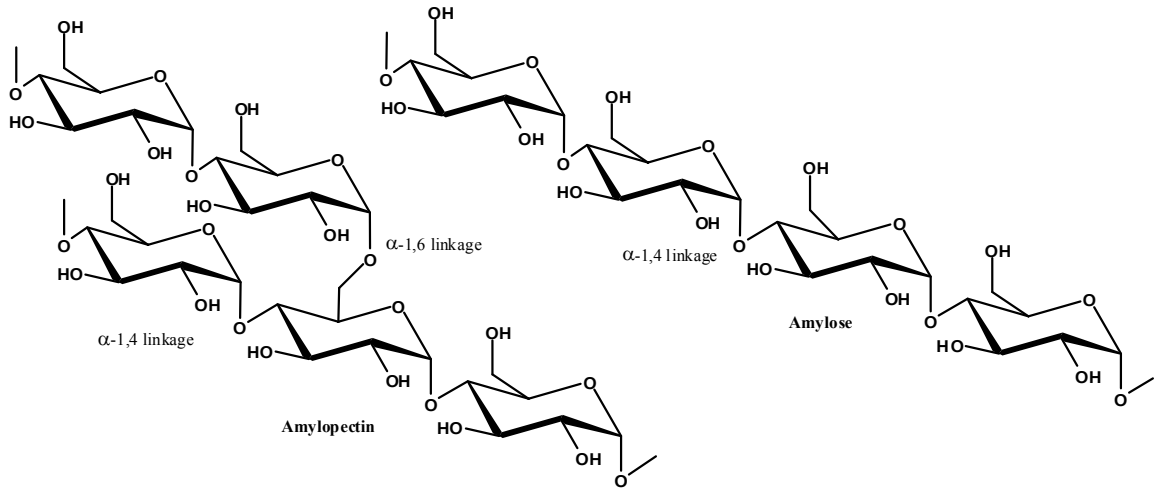
The enzymatic mechanism of *α*-amylases is very complex and not yet completely elucidated. Neither is inhibition by the widely occurring natural *α*-amylase inhibitors. Up to now, all structural studies on the interaction of *α*-amylases with substrate analogs, carbohydrate inhibitors and proteinaceous inhibitors have been performed with the pig pancreatic enzyme (PPA). Although the amino acid sequences of mammalian and insect *α*-amylases are very similar, several inhibitors of insect *α*-amylases have been reported to inhibit PPA and other mammalian *α*-amylases only to a very limited extent or not at all .

Carbohydrates usually form the bulk of the human diet, their breakdown products providing much of the energy required to sustain life. Carbohydrates consist of simple sugars (monosaccharides and disaccharides), as well as more complex compounds composed of multiple sugar units (polysaccharides), such as starch and cellulose. The largest source of starch in our diets comes from corn (maize), as well as from wheat, potatoes and rice. The starch we eat is generally a mixture of amylose, which is composed of α-1,4-linked glucose polymers (link between C-1 and C-4 atoms), and amylopectin, which is composed of α-1,4-linked glucose polymers branched by α-1,6-linkages.

To provide energy, these complex carbohydrates first need to be broken down into their sugar components, which ultimately enter the tricarboxylic acid cycle to yield ATP and carbon dioxide using water and oxygen. Digestion of starch begins in the mouth, where salivary α-amylase provides partial digestion, breaking down the polysaccharides into shorter oligomers. Once these reach the stomach, they are digested further by pancreatic α-amylase, which produces even smaller oligosaccharides that can be broken down by various α glucosidases to monosaccharide such as glucose that are readily absorbed into the bloodstream.

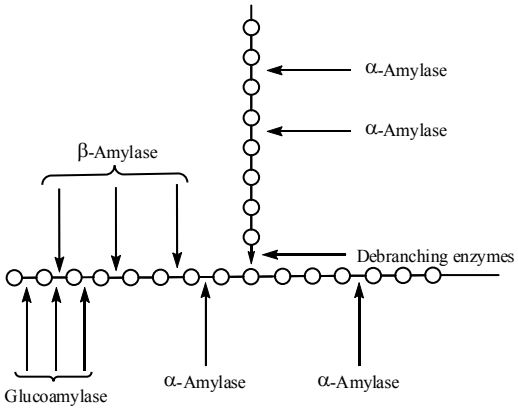
α-Glucosidases are among the most important carbohydrate-splitting enzymes. They catalyze the hydrolysis of α-glucosidic linkages. Their substrates are depending on their specificity oligo and polysaccharides. Substrate analogue inhibitors for mammalian α-amylases and other mammalian intestinal carbohydrate-splitting enzymes studied during the last few years have aroused medical interest in the treatment of metabolic diseases such as diabetes. The ability to control carbohydrate digestion is useful in the treatment of diabetes mellitus and obesity. Natural enzyme inhibitors, especially inhibitors of hydrolases are found in many plants and animals as well as in bacteria and fungi.[19]

The natural substrate of the amylases is starch, the main storage form of carbohydrate in most of the higher plants.[20-22] Starch is composed of two polysaccharides, amylose and amylopectin. Amylose is a linear α-(1-4) -glucan consisting of helically arranged chains of polysaccharides. Amylopectin, on the other hand, is a branched polysaccharide. The molecules have a treelike structure[23] consisting of two types of glucan residues. α-(1-4) and that are connected by α-(1-6)-linkages.



α-Amylases attack the substrate molecule for the example of amylopectin from inside acting as "endoenzymes", and split off maltose residues released in the α-configuration. Amylose is thus degraded mainly to maltose; in addition, slight amounts of maltotriose and glucose may result. When amylopectin is degraded, so called α-dextrins[21] are also formed which still contain the original inter-chain α-(1-6)-glucosidic linkages not cleaved by α-amylases. β-Amylases are found preferentially in plants but are not produced by the mammalian organism. They act as "exoenzymes", splitting successive maltose residues (which due to inversion are released in the β-configuration) from amylase and amylopectin from the non-reducing end. The degradation of amylose is sometimes complete only after pretreatment with a so-called debranching enzyme due to the presence of a very small number of branch points. The β-amylolytic degradation of amylopectin, on the other hand, remains incomplete even under these conditions, since the outermost branching sites can be neither split nor bypassed.

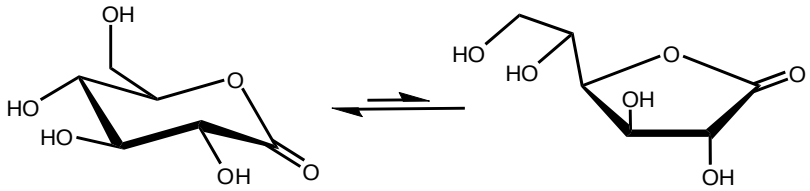
Sites of action of various starch-degrading enzymes.



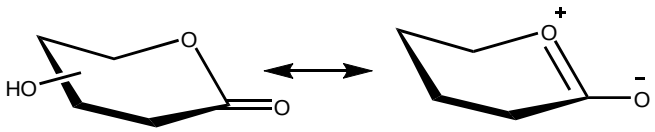
Glucoamylases also known as exo-α-(1-4)-glucosidases, amyloglucosidases or γ-amylases are found preferentially in microorganisms but also occur as enzyme complexes in the intestinal wall of mammals. They successively split off glucose residues from the non-reducing end. The "debranching enzymes" which split α-(1-6)-glucosidic linkages in the native amylopectin molecule, occur preferentially in microorganisms and plants. Corresponding enzymes of the mammalian organisms are not active in the intestinal tract. Here the intestinal oligo- and disaccharidases act on the α-(1-6)-glucosidic linkages of degradation products of amylopectin.

Aldonolactones as α-amylase inhibitors:

The strong inhibition of glycosidases by aldonolactones was first mentioned[24] in 1940. The aldonic acids themselves are non-inhibitory, and that 1,5-lactones are better inhibitors than their 1,4-isomers.[25] Also, the 1,4-lactones are not stable and easily convert to their 1,5-isomers.



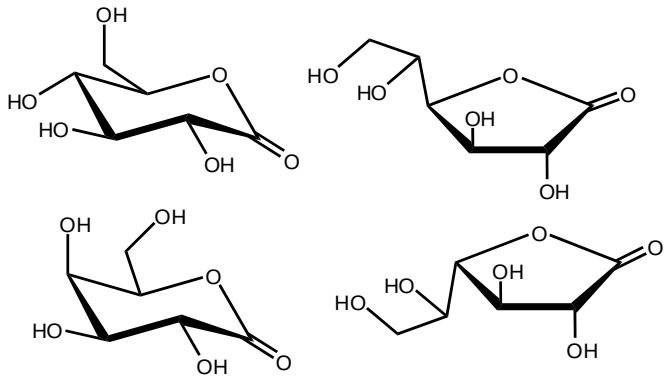
Various models have been proposed for the reaction mechanisms of glycosidases in the transition-state. The oxocar-bonium ion model has been applied to interpret the reaction mechanisms of many glycosidases, including lysozyme, glucoamylase, and glucosidase. In these mechanisms, when a glycosidic bond is cleaved, a glycosyl oxocar-bonium ion intermediate is formed for catalysis. Evidence of the role of the transition-state intermediates came from inhibition studies involving lactones as transition-state analogues, because of their structural similarity. Based on this hypothesis, the inhibition studies on glycosidases with aldonolactones, which were derived from the corresponding sugars have been studied.



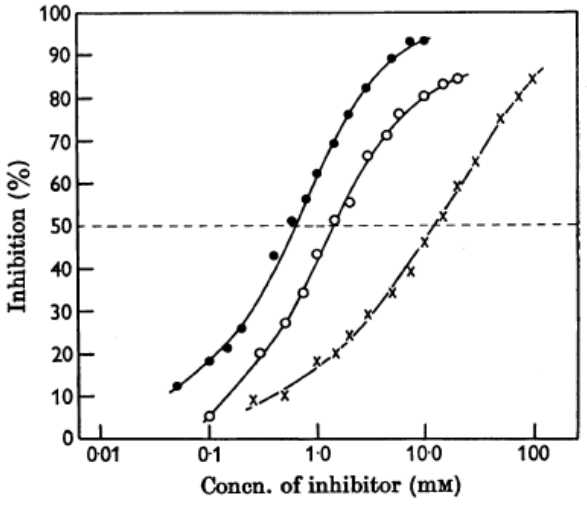
The fact that the aldonolactones might exert their powerful inhibition by virtue of their structural similarity with a glycosyl oxocarbonium ion intermediate or a related transition state.[26] Both the lactone and the oxocarbonium ion have a trigonal, planar configuration at C-l and adopt a half-chair conformation which is in marked contrast with the tetrahedral C-l configuration and $^{1}C_{4}$ conformation of aldopyranoside substrates and aldoses. In addition to these geometrical factors, there have to be considered electrostatic interactions arising from the large dipole moment of the lactone which would enhance lactone binding if there is a negatively charged group in close proximity to C-l of the bound inhibitor. There is a general agreement that the aldono-1,5-lactones are better inhibitors for glycosidases than are the 1,4-isomers[27] which are probably no better inhibitors than aldoses or polyols of comparable structure. It was found that the a-specific enzymes were inhibited by D-gluconolactone at least 100-fold less potently than the *β*-specific ones. The latter are inhibited by aldono-l, 5-lactones several hundred to many thousand-fold better than by the corresponding aldoses. It has been demonstrated that the inhibition is competitive; that is, the inhibitor competes with the substrate for the free enzyme.

In spite of the difference in ring-size between inhibitor and pyranosyl substrate, *β*-glucuronidase is powerfully inhibited by saccharo 1,4-lactone.[28] In the somewhat feebler inhibition of *β*-glucosidase by gluconolactone, the 1,4- and 1,5-lactones are equally effective. Study of the powerful inhibition of *β*-N-acetylglucosaminidase by 2-acetamido-2-deoxygluconolactone and 2-acetamido- 2-deoxygalactonolactone revealed changes in the composition of the inhibitor solutions that were attributed to interconversion of the 1,4- and 1,5-lactones. It was concluded that the two forms of 2-acetamido-2-deoxygluconolactone were equally inhibitory, whereas 2-acetamido-2-deoxygalactono-1,5-lactone was considerably more powerful than its 1,4- isomer.

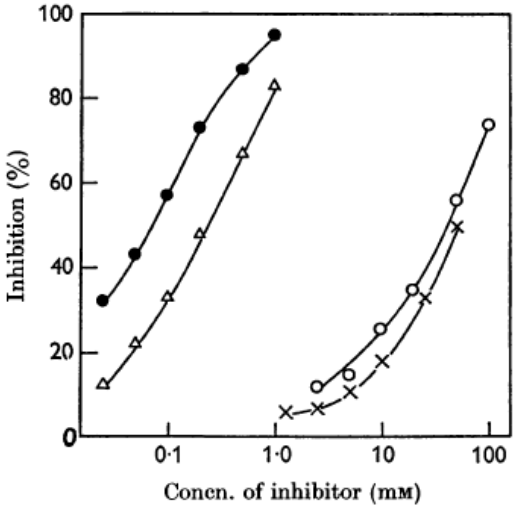
It was decided to investigate the possibility that this difference between the 1,4- and 1,5-lactone[28] was a general phenomenon applying to the galactose series, in contrast with the glucose series, and having its origin in the configuration of the sugar at C-4. Because the hydroxyl groups at C-4 and C-5 are trans in galactose, the relative position of the side chain is reversed on changing from a 1,5 to a 1,4-ring, there is a complete reversal of rotation when galactose or fucose (6-deoxygalactose) is converted into the 1,4-lactone.



It has been shown that the inhibitory power of D-galactonolactone and of D-fuconolactone (6-deoxy-D-galactonolactone) towards the appropriate glycosidases is dependent upon the proportion of the uncharacterized (1-5)-lactone formed in the aqueous solution of the crystalline (1-4)-lactone.[29] This was explained by the reversal of the configuration of the ring (and of the optical rotation), compared with the pyranosyl substrate, that occurs in the galactose series when one passes from the (1-5)-lactone to the (1-4)-lactone. This argument does not apply to the glucose or the mannose series. Nevertheless, the inhibitory power of crystalline D-mannono-(1-4)-lactone towards mammalian α-D-mannosidase is greatly enhanced when partial conversion into the (1-5)-lactone is effected in solution. The same difference in inhibitory power was encountered when the two lactones were tested against limpet α- and β-mannosidase.



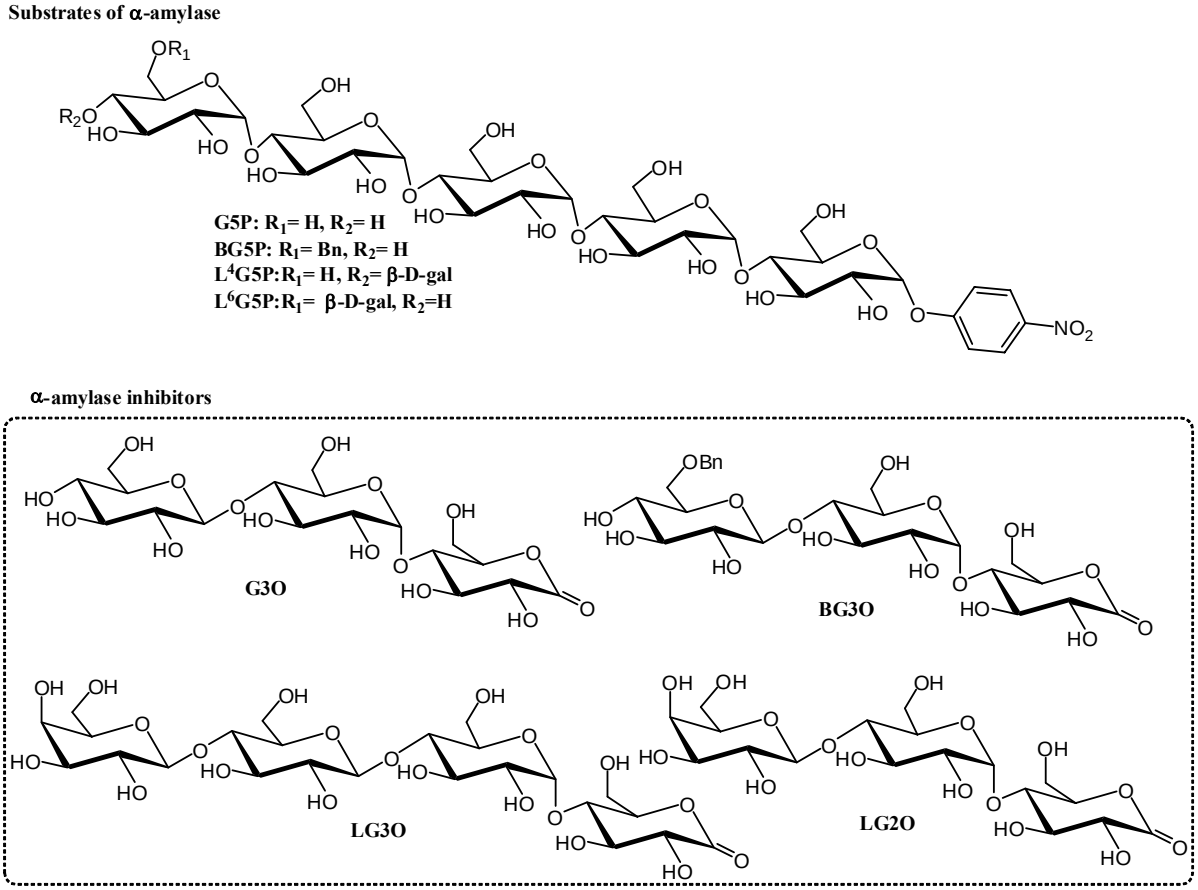
Inhibition of β-galactosidase from rat epididymis by varying concentrations of galactono-1,4-lactone (x), sodium galactonate (0) and the most inhibitory solution derived from the galactonate ion (•)



Inhibition of mammalian rc-mannosidase by varying the concentration of mannono-(1-5)-lactone (0), mannono-(1-4) lactone (•), sodium mannonate (x) and the 'most inhibitory solution' derived from the mannonate ion (Δ)

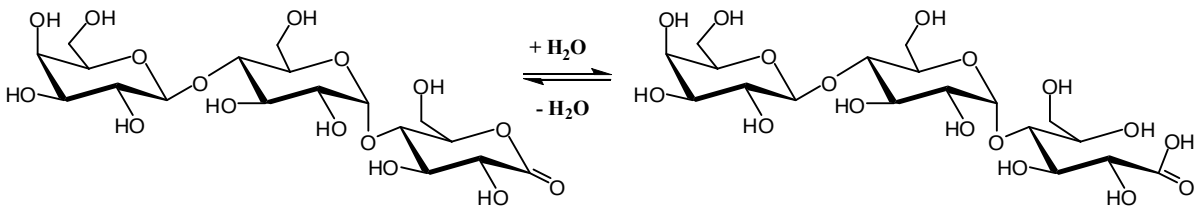
Maltooligosaccharides as α-amylases inhibitors:

Maltooligosaccharidonolactone and its analogue are exhibit α-amylase inhibition[30] activity. Both end-modified maltooligosaccharide derivatives, which would be expected to exhibit tolerance as to digestion with the digestive enzymes, α-glucosidase and glucoamylase, were designed as substrate analogue inhibitors for mammalian α-amylases. Systematic trends in inhibition studies on the structural modification of substrates would be helpful in revealing with the requirements for binding and catalytic specificity.



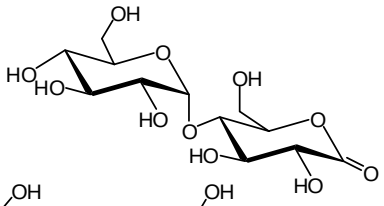
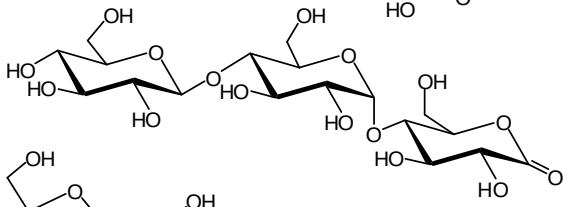
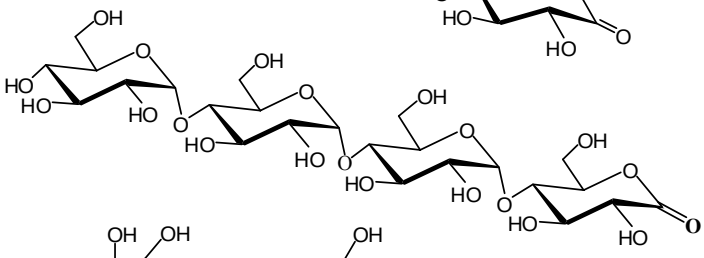
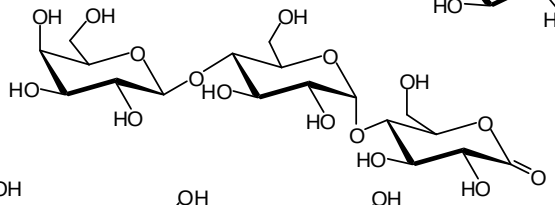
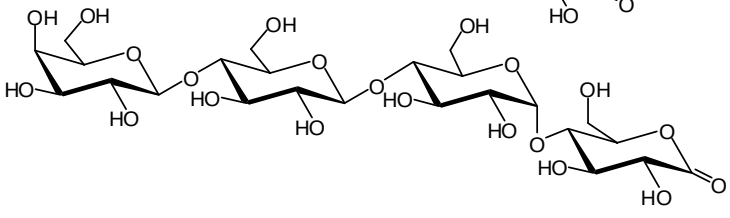
LG2O exhibits *anti*-amylase activity and is a transition state inhibitor.[31] Lg2O possess possesses a lactone ring at the non-reducing end, and this compound exhibits *anti*-α-amylase activity at modest concentrations. Interestingly, LG2O has two important characteristics: 1) LG2O does not possess *anti*-α-glucosidase activity (No sharp decrease in glucose levels); 2) LG2O gradually loses its *anti*-amylase activity in solution because of the cleavage to the lactone ring. These findings raised the possibility that LG2O might be

suitable as a mild *anti*-amylase agent for diabetic patients without producing the serious side effects.



The mode of inhibition of LG2O and LG3O for HSA, HPA and PPA were shown to be competitive.[30c] The modes of inhibition of maltobiono-lactone (G2O), maltotrionolactone (G3O), and maltotetra-onolactone (G4O), as control samples, were also of the competitive types. The two compounds were shown to be strong competitive inhibitors with K_i values of 2.8-18.0 μM, as shown in table 1.

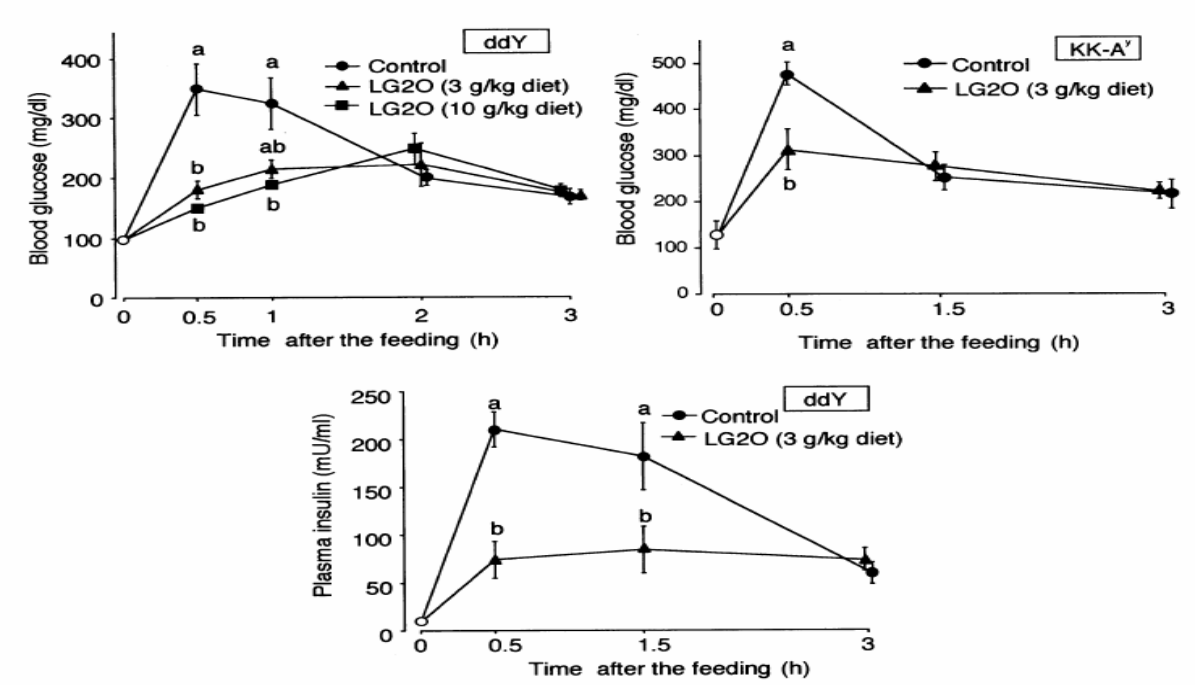
Table 1:

	HSA	HPA	PPA
	45.4	NT	135.9
	5.0	16.7	0.9
	2.6	9.5	0.9
	4.9	18.0	2.8
	3.4	11.4	2.9

Despite the introduction of a galactosyl group at the terminal position, the K_i values of LG2O and LG3O for HSA and HPA were close to those of G3O and G4O, as control samples, respectively. In this process, LG2O and LG3O were not hydrolyzed by these amylases. Furthermore, maltotriose and maltotetraose did not show any inhibitory activity against these α-amylases. It is evident that a lactone form with a certain glucose-chain length is essential for enzyme inhibition. Accordingly, the actual *Ki* values of LG2O and LG3O should be as high as they appear, because they exist in the active lactone and inactive aldonic acid forms.[30c, 32]

Administration of a new α-amylase inhibitor LG2O, as a food-drug mixture to ddY mice, resulted in significant improvements in both blood glucose and insulin response following ingestion of the drug containing diet. A significant reduction in postprandial blood glucose was also observed in KK-Ay mice manifesting symptoms of type 2 diabetes.[31]

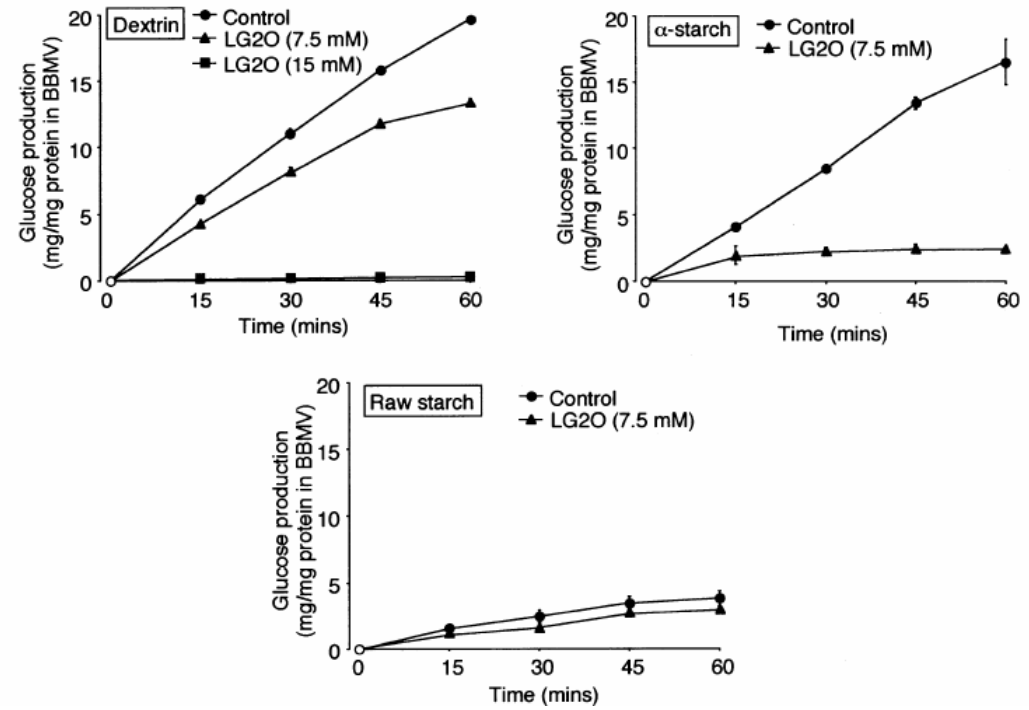
Changes in blood glucose and plasma insulin concentrations in ddY and KK-Ay mice given starch Rich diet containing various concentrations LC20.



LG2O produced a 1.5–3.5 fold increase in gut content and reducible sugar remaining in the small intestine. This fact suggests that LG2O slows down the delivery of

carbohydrates into the body. The in vitro glucose production to reproduce the inhibitory action of LG2O mesurments showed that reduced glucose production derived from substrates, and the rank order of inhibitory potency by LG2O at 7.5 mM was as follows: α-starch > raw starch > dextrin.

In vitro hydrolysis of dextrin, a-starch and raw starch. The reaction mixture included pancreatic juice (α-amylase) and brush border membrane vesicles (α-glucosidase) prepared from ddY mice.[31]



A previous study has reported that the mode of inhibition of LG2O for saliva and pancreatic amylase was of a competitive type.[30c] Although there is no clear explanation for the substrate specificity observed here, the difference in water solubility and affinity to α-amylase might affect the inhibitory potency of LG2O. LG2O controls postprandial hyperglycemia by inhibiting carbohydrate digestion in the gut. Interestingly, the amount of stomach contents did not differ between the control and LG2O mice. In addition, a long feeding duration for LG2O had no influence on food intake. LG2O had no influence on satiety and gastric emptying in spite of delayednutrient delivery in the small intestine.

LG2O gradually loses *anti*-amylase activity in the process of digestion, LG2O markedly inhibited α-amylase activity in the stomach. Since most α-amylase existing in the stomach is produced from saliva, LG2O might inhibit α-amylase derived from saliva.

However, LG2O had little or no influence on *α*-amylase activity in the small intestine. Although we have not been able to show exactly why LG2O lost *anti*-amylase activity, it must be related to the chemical characteristics of LG2O. This compound takes only a lactone form in the solid phase, but in the aqueous phase the lactone form gradually transforms into its corresponding aldonic acid form. Although the lactone form exhibits an *anti*-amylase effect, the aldonic acid form loses its activity. In addition, incubation at moderate temperature promotes transformation of LG2O from lactone to its aldonic acid form.[30c]This evidence suggests that LG2O can easily lose its *anti*-amylase activity in the gastrointestinal lumen.

The loss of *anti*-amylase activity is desirable from the view point of clinical application. It is reported that *α*-glucosidase inhibitors cause various gastrointestinal symptoms and hepatic disorders. This is because inhibition of *α*-glucosidase causes an accumulation of an excessive number of short-chain carbohydrate polymers and the *α*-glucosidase inhibitor itself in the distal gut, which produces these symptoms. The presence of undigested and unabsorbed hyperosmolar solution also results in osmotic fluid shifts and intestinal hurry which might reduce the contact time between the mucosa and all nutrients existing in intestine. *α*-Amylase inhibitors slow hydrolysis of macromolecules but not glucoside; as a result, osmotically active digestive products do not accumulate in the intestinal lumen . Until now, various proteinaceous *α*-amylase inhibitors have been isolated from plants and microbes. However, the low specific activity and lack of enough inhibitors to perform long-term experiment have hindered further investigation and use of *α*-amylase inhibitors in clinical practice. Since LG2O is produced from lactose and maltotriose by enzymatic and chemical reaction, the difficulty of mass-production can be largely overcome. Administration of LG2O inhibits carbohydrate digestion in the gut, and produces significant improvements in both blood glucose and insulin response following ingestion of a LG2O containing diet, and provides support for its therapeutic potential in treating diabetes mellitus and obesity.[31]

Introduction to *O*-glycosylation methods

In nature carbohydrates are present as *C*-glycosides, *O*-glycosides, and *N*-glycosides. Of all the three *O*-glycosides are the most important class of compounds. *O*-glycosides are formed from the condensation of the anomeric hydroxyl group of sugar with

the hydroxyl group of another molecule. The latter can be a simple alcohol, a hydroxylated amino acid, another sugar or a more complex molecule.

The importance of cell surface carbohydrates in biological processes ranging from antibody-antigen interaction to cell-cell recognition and development has led to a great deal of activity at the carbohydrate frontier. An integral part of this has involved the development of new chemical methods of oligosaccharide synthesis requiring fewer manipulations and/or resulting in higher yields, increased stereoselectivity and selective activation. Although many advances have been made in the synthesis of oligosaccharides, each oligosaccharide synthesis remains an independent problem, whose resolution requires considerable systematic research and a good deal of know how. There are no universal reaction conditions for oligosaccharide synthesis. In an oligosaccharide synthesis, two poly functional sugar units must be coupled. Regioselectivity in such coupling reactions is generally achieved when the glycosylating agent (glycosyl donor) possesses selectively protected hydroxyl groups and an activating group at the anomeric carbon atom and when the sugar component with free hydroxyl group (glycosyl acceptor) possesses protecting groups at all other hydroxyl functions. Thus, complicated protecting strategies and suitable procedures for activation at the anomeric carbon atom are required.

Stereoselective formation of *O*-glycosidic bond is one of the most important problems in carbohydrate chemistry.[33] Recently, much effort has been devoted to the stereoselective synthesis of glycopyranosides and several efficient methods have been developed by the appropriate combination of sugar donors and activators (figure 3).[34]

Despite significant advances in the synthesis of complex oligosaccharides, construction of *O*-furanosidic linkages remains a challenging task.[35] To address at the stereoselectivities, it is advantageous to clarify glycosylation methods as 1,2-*cis* and 1,2-*trans*- furanosides instead of α- and β- glycosides as the latter would be confusing.

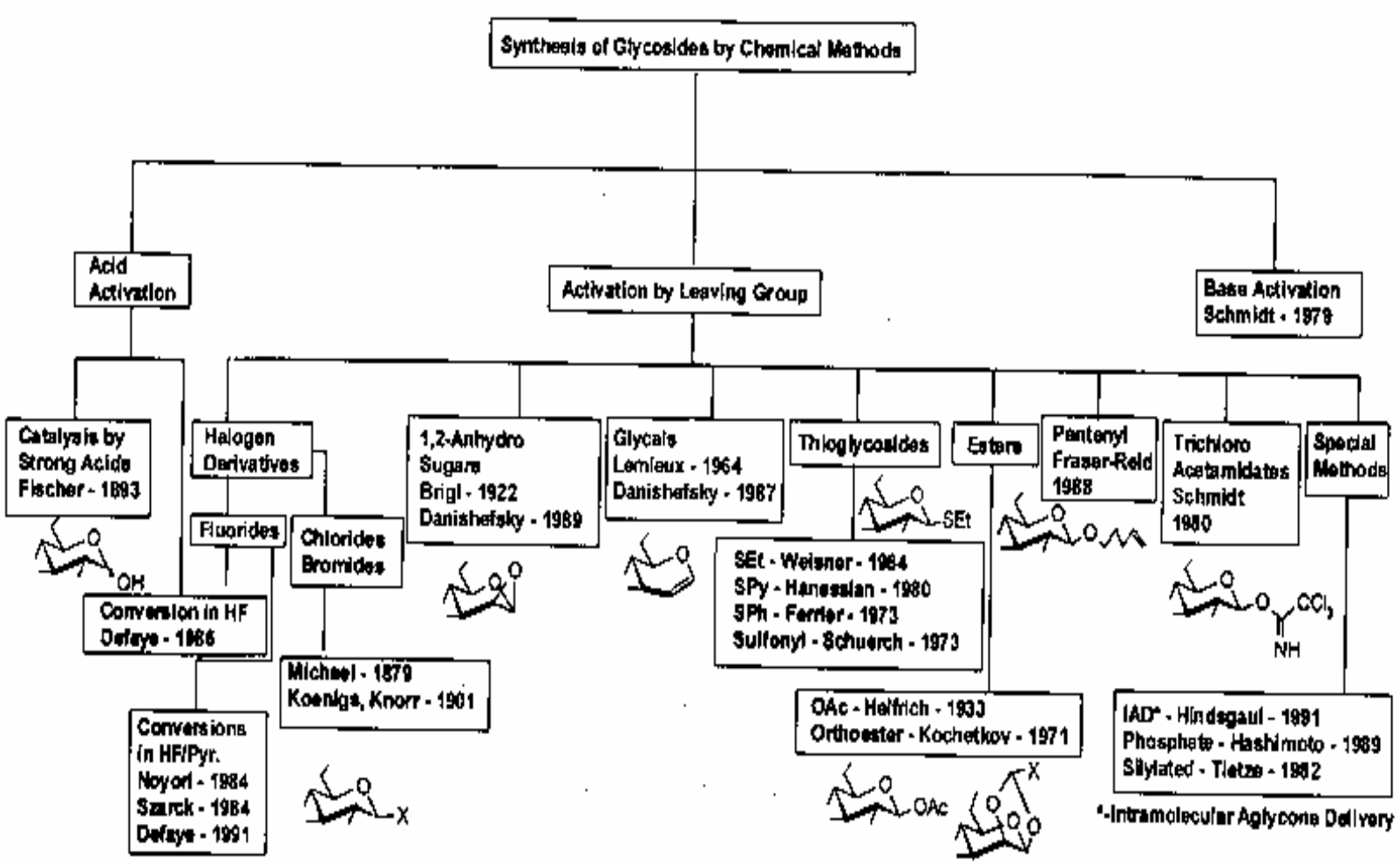


Figure 3: Development of Pyranoside Glycosylation Methods

Present Work

α-Amylases are a group of enzymes widely distributed in microorganisms, plants and animal secretions, which catalyze the hydrolysis of the α-(1→4) glycosidic linkages in starch and various oligosaccharides.[30c] In mammalians inhibition of α-amylases reduces the post-prandial glucose levels, which is of particular importance in patients with diabetes. LG-2O and LG-3O, which are competitive inhibitors for mammalian α-amylases, exhibited K_i values of the order of 2.8 – 18 μM. LG-2O was synthesized by oxidation of LG-2, which in turn is an enzymatic product. The interesting glycosidase inhibition activity of LG-2O and lack of any chemical synthesis for its precursor lactol LG-2 has prompted us to investigate its synthesis.

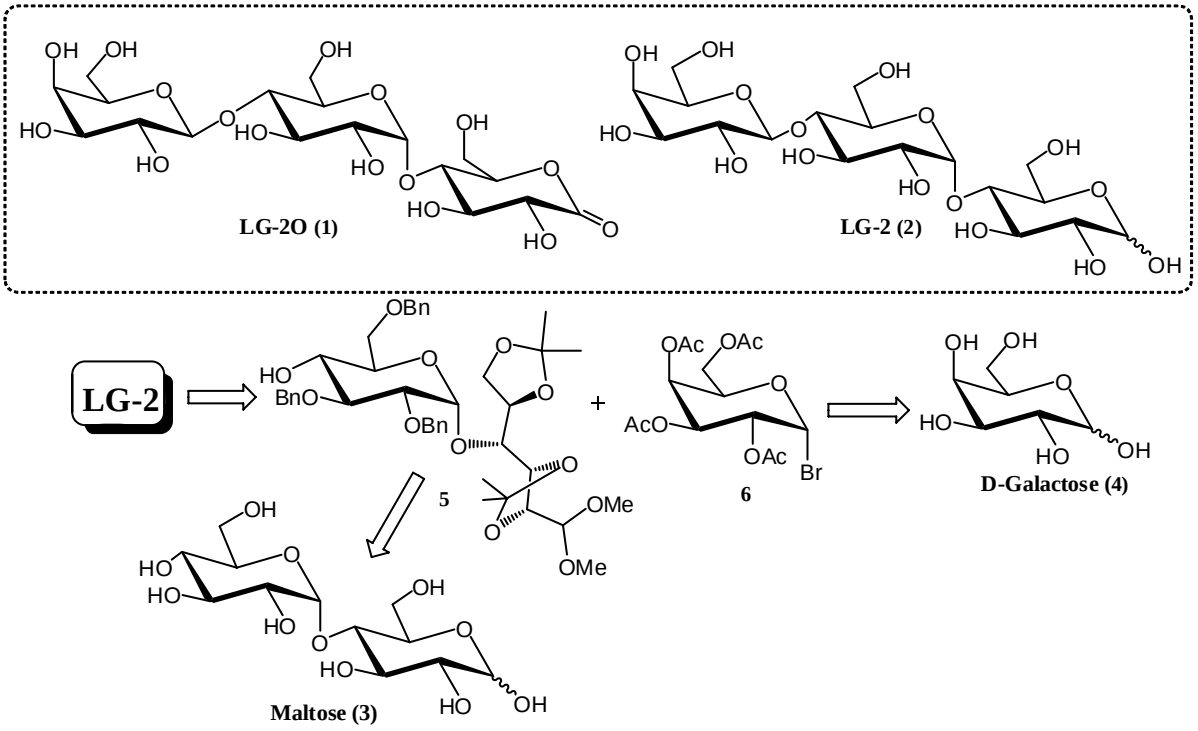


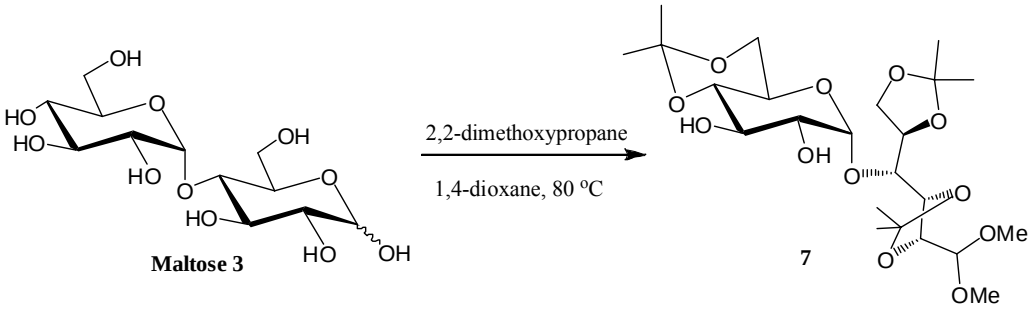
Figure 1. *Potential α-Amylase inhibitor LG-2O (**1**) and its enzymatic source LG-2 (**2**) and intended synthetic strategy*

Considering ease of availability, D-maltose (**3**) was recognized as the potential glycosyl acceptor and being addition of D-Galactose (**4**) at C4[1]-OH of the non-reducing glucose end as depicted in figure 1, the two key building units were the differentially

protected maltose unit **5** (glycosyl acceptor) and tetraacetylgalactosyl bromide **6** (glycosyl donor).

Our synthetic endeavor started with the acetonation of maltose (**3**).[36] The investigation of various conditions for the reaction of 2,2-dimethoxypropane has been conducted on several disaccharides. The nature of the acetals thus obtained depends largely on the temperature, the solvent, and the amount of acid catalyst that are chosen for the procedure. Thus, maltose **3** was treated with 2,2-dimethoxypropane in 1,4-dioxane at 80 °C to afford the tri acetonide **7** (Scheme 1) in which the $C4^1$-O/$C6^1$-O of the non-reducing glucose unit, all the free hydroxyl groups of the reducing glucose were protected selectively as acetonides and C1-aldehyde as its dimethyl acetal leaving only the $C2^1$-OH and $C3^1$-OH of non-reducing glucose unit free.

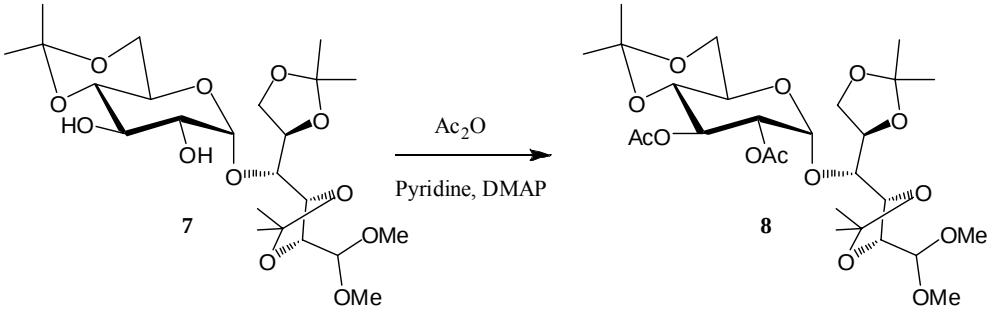
Scheme 1:



Characteristic singlets were seen at δ 1.35, 1.43, 1.46 and 1.51 ppm due to isopropylidene groups, two singlets corresponding to two methoxy groups of the acetal at δ 3.44 and 3.46 ppm, a doublet at δ 5.08 ppm (J= 3.9 Hz) for α-anomer and doublet at δ 4.42 ppm (J= 5.9 Hz) to acetal C-H. In the ^{13}C NMR spectrum, anomeric carbon resonated at δ 101.4 ppm and acetal carbon at δ 105.8 ppm. The 1,3-dioxalane (5 membered) acetonide carbons appeared as singlets at δ 110.2 and 109.1 ppm (27.5, 27.3, 26.6 and 25.2 for the methyl carbons) whereas 1,3 dioxane (6 membered) acetonide at δ 99.9 ppm (29.3 and 19.4 for the methyl carbons). Rest of the spectrum was in complete agreement with that of reported data.

Further, the compound was confirmed by acetylating the two free hydroxyl groups to obtain acetate **8** by acetic anhydride in pyridine (scheme 2). The characteristic signals at δ 2.30 and 2.36 ppm as singlets in ^{1}H NMR as well as at δ 176.2 and 176.8 ppm for the corresponding carbonyls in ^{13}C NMR supported the assigned structure.[36b]

Scheme 2:



ASSIGNMENT OF RING SIZE IN ISOPROPYLIDENE ACETALS BY ^{13}C N.M.R.[37]

Polyols capable of reacting with more than one equivalent of acetone can form 5,6 or 7-membered rings. Structure determination of these acetals using partial acidic hydrolysis may be difficult because of the possibility of rearrangements. Substantial differences in the ^{13}C chemical shifts of the acetal carbon in the acetals were useful in assigning the ring size.

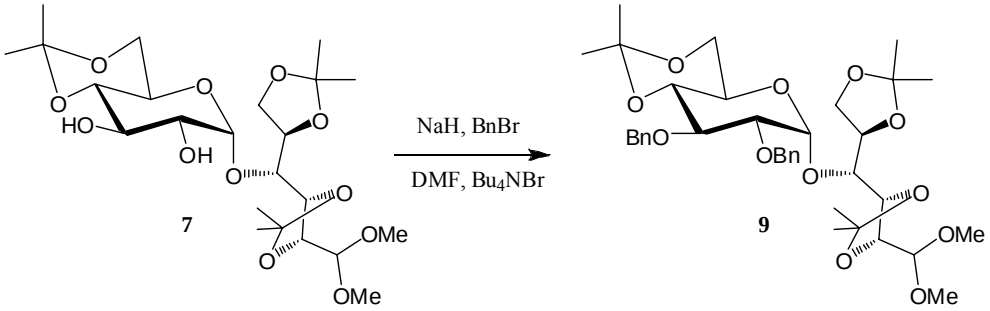
In a 6-membered ring the ^{13}C chemical shifts of the methyl groups are separated by about 10 p.p.m. This large difference presumably reflects the difference in environment of equatorial and axial methyl groups in a chair conformation and the higher field signal is due to the axial group, which is more crowded. For 5 membered acetals the methyl signals are only slightly separated, probably due to the conformational mobility of the system and to the fact that the methyl groups are pseudoequatorial and pseudoaxial. In the 7-membered acetal the isopropylidene methyl signals were coincident. This is a monocyclic system with considerable conformational freedom not present in bicyclic systems.

^{13}C Chemical Shift Correlations for Isopropylidene Acetals.

Ring Size	*Acetal C*	*Me*	*Δδ Me*
5	*108.5-111.4 (mono cyclic or fused to pyranose ring) 111.4-115.7 (fused to furanose ring)*	*23.7-27.8*	*0.0-2.4*
6	*97.1-99.5*	*18.2-19.3 and 28.6-29.2*	*9.8-10.9*
7	*100.7-100.9*	----	-----

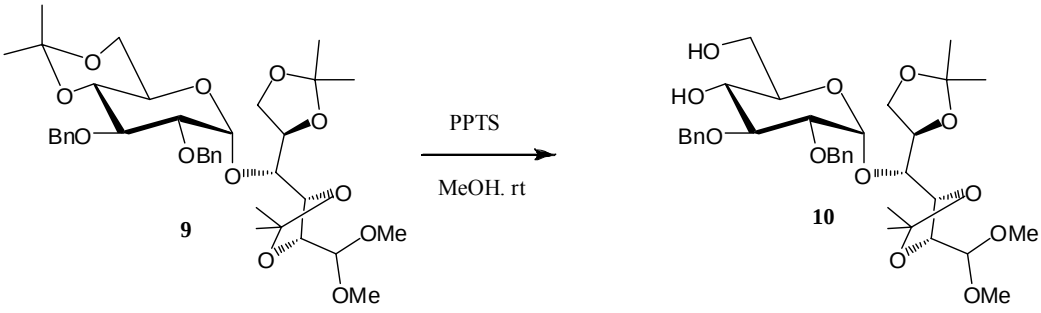
Having confirmed the structure of compound **7,** we proceeded further for the synthesis of key glycosyl acceptor **5**. Thus, treatment of compound **7** with NaH, BnBr in DMF at room temperature for 6 h to afford dibenzyl maltose derivative **9** (scheme 3). In the ^{1}H NMR spectrum, aromatic protons were seen at δ 7.24 to 7.36 ppm as multiplet. In the ^{13}C NMR spectrum of compound **9**, the anomeric carbons were identified at δ 98.5 for *α*-anomer and δ 104.6 ppm for the acetal with two benzylic CH_2's resonating at δ 72.8 and 73.6 ppm. The structure was further supported by elemental analysis.

Scheme 3:



After a careful screening of various conditions for the acetonide deprotection, we could accomplish the selective removal of 6-membered ring isopropylidene group (1,3-dioxane) in preference to the other isopropylidene groups (1,3-dioxalane) by employing PPTS in MeOH and carrying the reaction at 0 °C for 8 h and procured the diol **10** in excellent yield.[36]

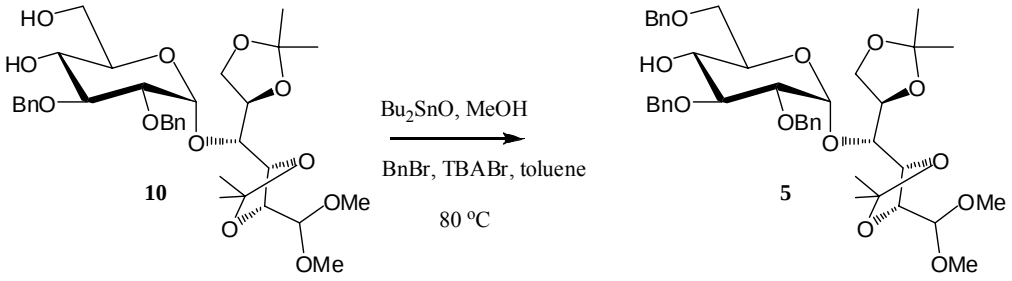
Scheme 4:



The disappearance of characteristic isopropylidene methyls at δ 19.2 and 29.2 ppm as well as acetal carbon at δ 98.0 ppm in the ^{13}C NMR spectrum of compound **10** was well in agreement with the reported literature values. The structure of compound **10** was further confirmed by its elemental analysis.

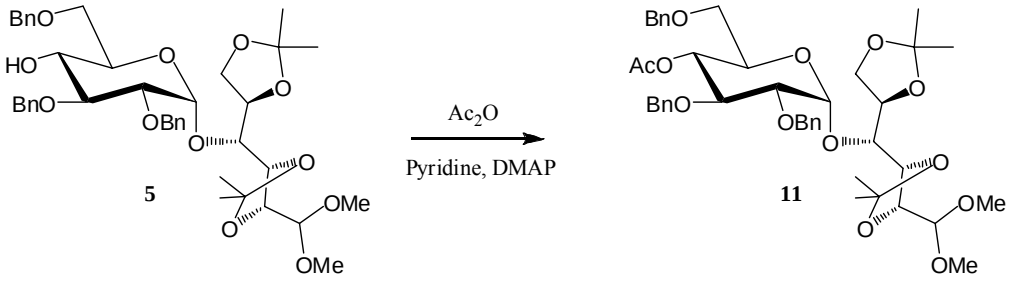
After having compound **10** in hand, our next task was selective benzylation of 1°-OH, and we opted for dibutyltin[38] protocol that has been employed in carbohydrate chemistry very frequently. Thus, compound **10**, dibutyltinoxide were heated to reflux in methanol and concentrated. The resulting stannylidene acetal was treated with benzyl bromide and tetra butylammonium bromide in toluene to afford mono benzyl compound **5** (scheme 5). In the ^{1}H NMR spectrum of **5**, aromatic protons resonate at δ 7.22-7.36 ppm as multiplet for 15 H, as well as in the ^{13}C NMR the three benzylic CH_2's resonate at δ 73.28, 73.66 and 75.18 ppm thus confirming the selective monobenzylation.

Scheme 5:



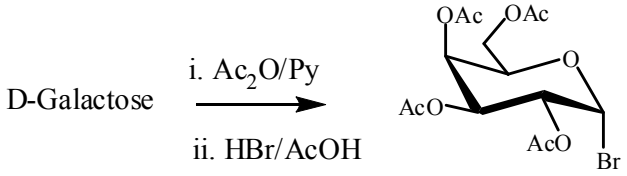
In order to confirm the regiochemistry of benzylation, compound **5** was converted to corresponding acetate **11**, (scheme 6) by treating it with acetic anhydride in pyridine at rt. The ^{1}H NMR spectrum showed characteristic signal at δ 1.78 ppm as singlet and δ 5.07 as a triplet (*J*=9.8 Hz). In the ^{13}C NMR signals at δ 20.74 and 169.45 ppm due to corresponding methyl and carbonyl groups were noted.

Scheme 6:



After successful completion of aglycon (glycosyl acceptor) **5**, we focused our attention to make the glycosyl donor identified in the retrosynthesis. The galactosyl donor was made by adopting the reported protocol. D-galactose (**4**) was converted into tetraacetyl galactosyl bromide **6** (scheme 7) by treating galactose with acetic anhydride in pyridine followed by HBr in AcOH.[39]

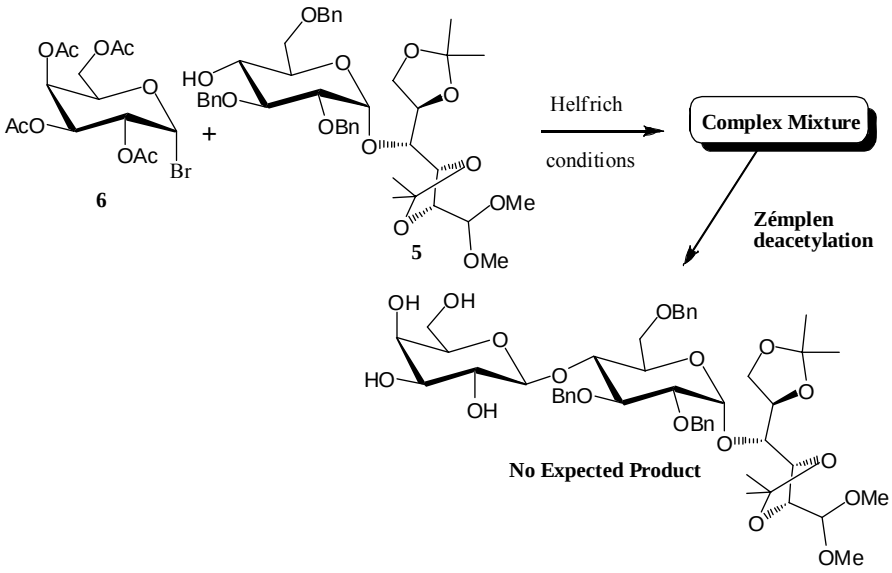
Scheme 7:



6

After having both of the coupling partners (acceptor and donor) in hand, our next task was crucial glycosidation under mild acidic conditions. Our initial efforts for the glycosylation were started with classical *Koenigs Knorr*[40] type using modified Helfrich[41] conditions. Thus the acceptor **5** was treated with tetraacetylgalactosyl bromide **6** in the presence of $Hg(CN)_2$, $HgCl_2$ in acetonitrile which was not successful. We always ended with complex mixture, which was subsequently deacylated using Zémplen deacetylation[42] (NaOMe in MeOH) to identify a mixture of compounds (scheme 8).

Scheme 8:

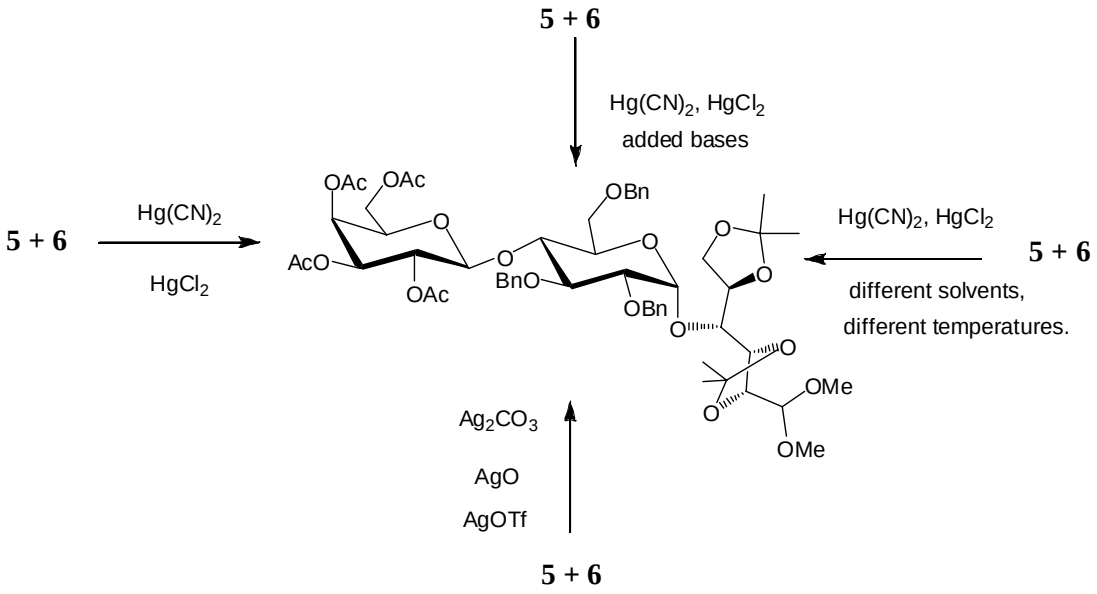


The ^1H NMR showed complex spectrum in which some of the characteristic acetonide signals were missing. This was probably due to the byproduct HBr that is liberated during the course of reaction which could deprotect the isopropylidene group, thus opening the different acceptors within the compound and leading to complex mixture.

Our attempts to neutralize the acid liberated by employing additives such as 2,6-lutidine, collidine and triethylamine were met with failure. In some cases the reaction could not proceed, starting material was recovered. All efforts for glycosidation by varying

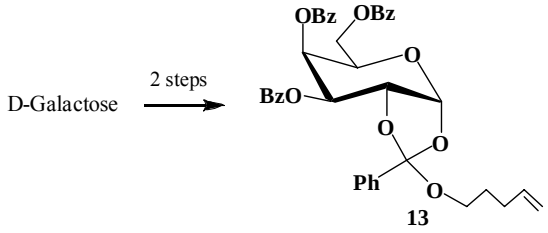
reaction conditions such as solvent, temperature, activator (HgO, Ag_2CO_3, AgOTf) were unsuccessful (scheme 9).

Scheme 9:



As the synthesis of trisaccharide turned out to be a Herculean task *via* classical *Koenigs Knorr*, Helferich methods and other metal carbonates, triflate mediated glycosidation methods also failed.

Having met with failures using various glycosidation conditions we turned our attention on pentenyl glycosyl donors, which was pioneered by Fraser-Reid *et al*.[43] The desired tribenzoyl galactosyl pentenyl orthoester **13** was prepared from D-galactose.[44]



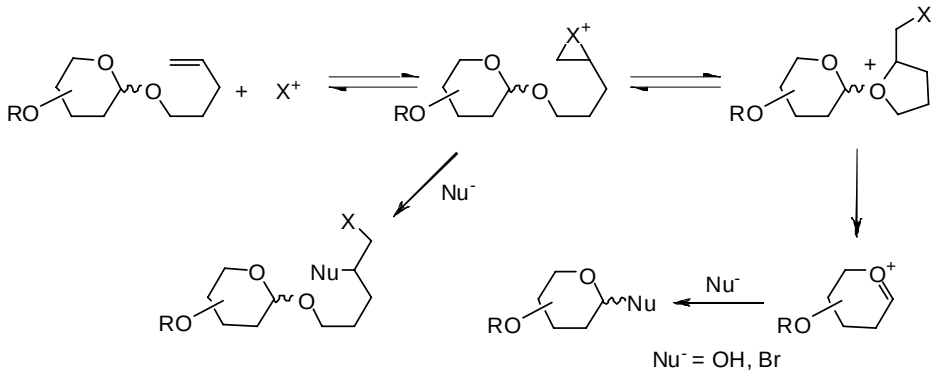
Before we discuss the approach it is pertinent to mention the concepts of *n*-pentenyl mediated *O*-glycosylation reaction and further developments of *n*-pentenyl orthoesters as glycosyl donors.

Salient Features of Fraser-Reid's Glycosylation Method

The serendipitous discovery of n-pentenyl leaving group strategy by Fraser-Reid's group[43] opened a new era in the art of oligosaccharide synthesis. n-Pentenyl glycosides (NPGs) are readily

obtained by standard glycosidations including Fischer's direct method and although they are stable to a wide range of reagents, they are easily activated by treatment with a halonium ion (figure 2). The effect of some of the commonly used protecting groups upon glycoside reactivity has been probed with these substrates, and the "armed/disarmed" strategy for oligosaccharide assembly emanated directly from these investigations. Thus esters disarm electronically, while benzylidene and isopropylidene groups disarm by torsional strain.

Figure 2:



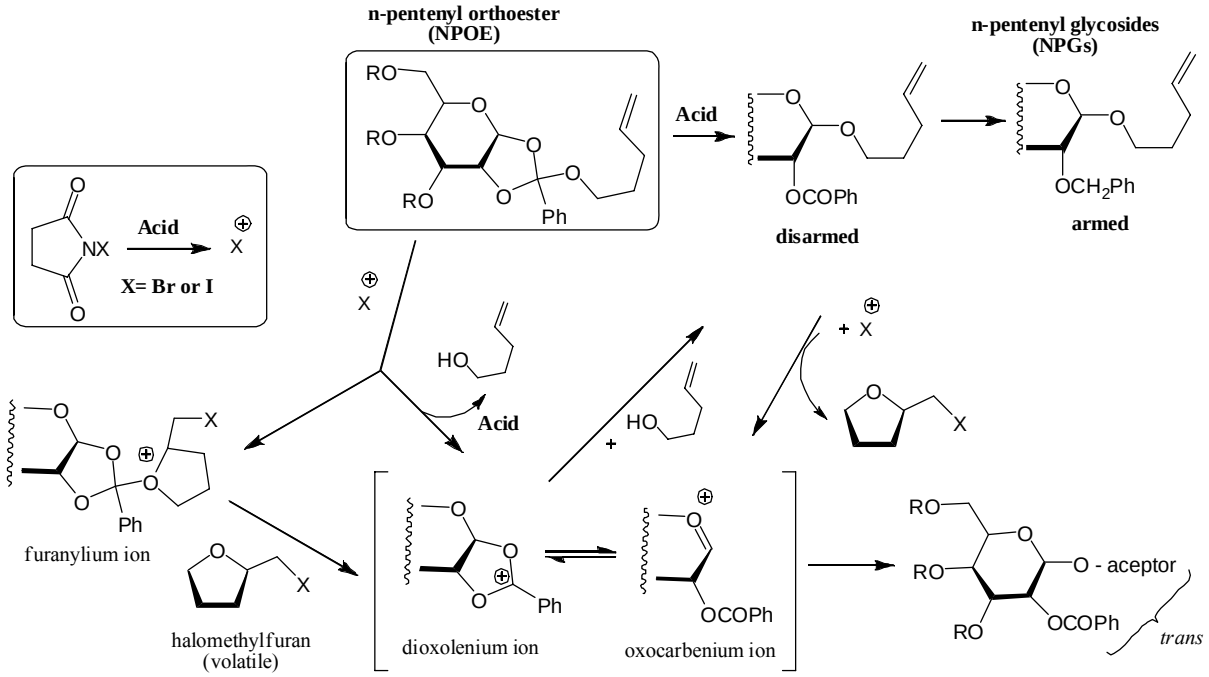
n-Pentenyl mediated Glycosylation method

Most commonly used activators for NPGs are NBS, IDCP (Iodonium dicollidine per chlorate). However, IDCP is not commercially available, a circumstance which compromised its attractiveness. Latter on alternative promoters were sought that would, among other things not require laboratory preparation. A non-nucleophilic counter anion was essential, and trifluoro methanesulfonate (triflate) was preferred and it was found that NIS reacted with TfOH to generate a ready source of iodonium ion solved the problem, as both NIS and TfOH are commercially available. A general drawback of this protocol is the use of strong acid and thus care should be taken while planning and executing the glycosylation reaction.

Pentenyl Orthoester

Glycosyl orthoesters have been used extensively to prepare trans-1,2-glycosides by acid catalyzed rearrangement. The advent of n-pentenyl chemistry[45] added a dimension to the capability of glycosyl orthoesters, that Kotchetkov, the major exponent of glycosyl orthoester chemistry, had found wanting.[46]

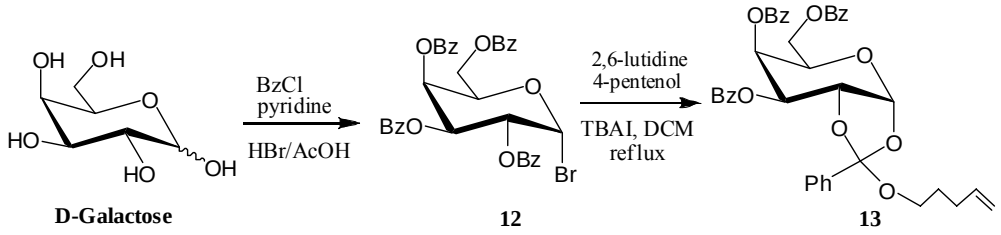
Figure 3:



*Thus the n-pentenyloxy moiety (a) could not only be transferred to the anomeric center by the normal rearrangement, but (b) could be extricated via the furanylium ion, into the halomethyl furan so that an in situ acceptor would not face competition in providing a new glycoside. Of course the latter, could alternatively have been obtained from the disarmed donor via the oxocarbenium ion, but the low reactivity sometimes causes iodo-alkoxylation of the double bond of disarmed one (*Figure 3). *Such products are never obtained with NPOEs.*

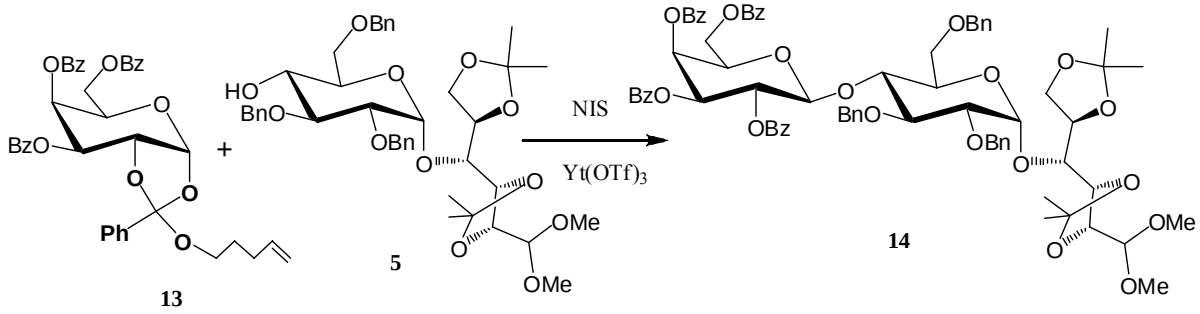
Thus treatment of galactose **4** with benzoyl chloride in pyridine followed by HBr in acetic acid gave crude galactosyl bromide **12,** which upon treatment with 2,6-lutidine, 4-pentenol in refluxing DCM afforded the orthoester **13** (scheme 10). In the ^{1}H NMR spectrum of orthoester **13**, anomeric proton resonated at δ 6.20 ppm as doublet (*J*= 5.1 Hz) and the characteristic olefinic protons at δ 5.68-5.83 ppm as multiplet. In the ^{13}C NMR spectrum benzoate carbonyl resonates at δ 165.0, 165.1 and 165.8 ppm. Further, the signals of the orthoester carbon at δ 120.1 ppm and the olefinic methylene at δ 114.8 ppm confirmed the assigned structure. Rest of the spectrum is in full agreement with the reported data. The structure was further supported by mass as well as elemental analysis. The LC-MS analysis confirmed mass of **13** m/z at 687.65 (M^{+} +Na).

Scheme 10:



To our pleasant delight the effective glycosidation of **5** and **13** to form the trisaccharide was achieved using NIS, $Yt(OTf)_3$ (cat.) and $4A^o$ molecular sieves in CH_2Cl_2 at room temperature gave the trisaccharide **14** (scheme 11) (contaminated with minor quantity of aglycone **5**), which was purified by flash column chromatography.

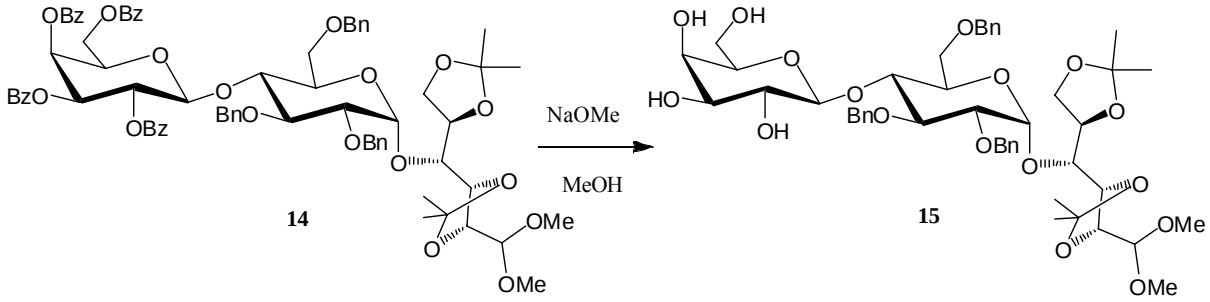
Scheme 11:



The high-resolution ^{1}H NMR spectrum of **14** showed three anomeric protons at δ 5.83 (J = 3.5 Hz), 4.71 (J = 5.6 Hz) and 4.68 (J = 7.8 Hz) ppm as distinguishable doublets. As we have already fixed stereochemistry at anomeric positions in the disaccharide as 1,2-*cis* linkages, the remaining doublet indicates the newly formed galactopyranosyl linkage as 1,2-*trans* (β-linkage) configuration. This was further confirmed by the ^{13}C NMR and DEPT spectral studies wherein three anomeric carbons were identified at δ 98.79 (α-linkage), 100.16 (β-linkage) and 105.45 ppm (acetal). In addition, LC MS (Na) [(M^++23) at 1339.88] and elemental analysis (Calcd. for $C_{75}H_{80}O_{21}$: C, 68.38, H, 6.12. Found: C, 68.35, H, 6.07) supported the structure of **14**.

After successful establishment of 1,2 *trans* glycosidic bond we proceeded to debenzoylation of **14** under Zémplen conditions to provided the pure tri-*O*-benzylated derivative **15** (scheme 12).

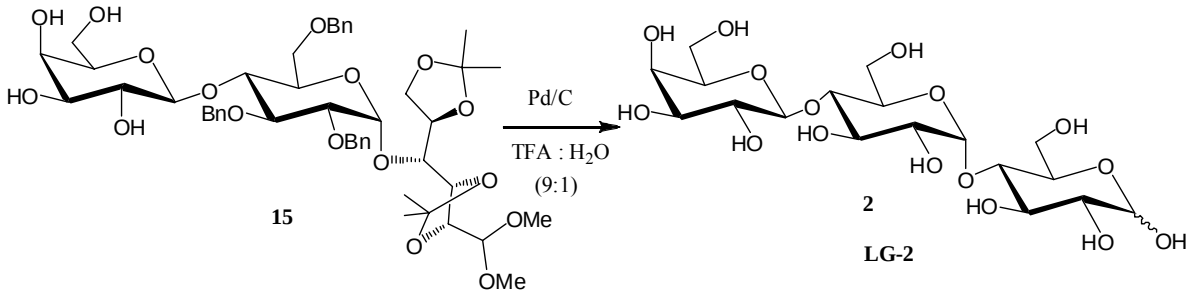
Scheme 12:



The high-resolution ^{1}H NMR spectrum of **15** showed three anomeric protons at δ 4.31, 4.34 and 5.11 ppm as distinguishable doublets. This was further confirmed by the ^{13}C NMR and DEPT spectral studies wherein three anomeric carbons were identified at δ 105.65, 102.74 and 99.41 ppm. In addition, LC MS (Na) [(M^{+}+23) at 923.93] and elemental analysis (Calcd. for $C_{47}H_{64}O_{17}$: C, 62.65, H, 7.16. Found: C, 62.63, H, 7.17) supported the structure of **15**.

Finally compound **15** was subjected to exhaustive hydrogenolysis over 10% Pd/C in methanol at normal temperature and pressure (ntp) to provide the debenzylated compound as a complex mixture whose ^{1}H NMR spectrum showed disappearance of characteristic aromatic signals but at the same time some of the acetonide signals were not seen. Attempts to synthesize pure debenzylated ($Pd(OH)_2/C$, Pd/C and K_2CO_3) compound were not successful. Unable to debenzylate selectively, we proceeded with the complex reaction mixture further and treated with TFA: H_2O (9:1) at room temperature[47] which yielded the target trisaccharide **2** (**LG-2**) (scheme 13).

Scheme 13:



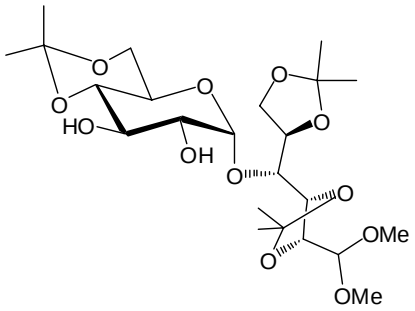
The high-resolution ^{1}H, ^{13}C NMR and DEPT spectral data established the structure of **2**. For example, in the ^{1}H NMR spectrum of **2**, resonances typical of anomeric proton (1,2-*trans*) was identified at δ 4.47 (*J* = 7.5 Hz), for (1,2-*cis*) was identified at 5.43 (*J* = 3.9 Hz) and 5.24 (*J* = 3.6 Hz), 4.66 ppm (*J* = 7.9 Hz) for the lactol anomers as doublet, while rest of the spectrum was in complete agreement with the assigned structure. The chemical shifts of anomeric carbons were observed at δ 105.78, 102.27 102.18 and 98.70 ppm in its ^{13}C NMR spectrum. Further, LC MS (Na) analysis of **2** showed the mass m/z at: 527.17 accounting for M^{+}+23 while elemental analysis (Calcd. for $C_{18}H_{32}O_{16}$: C, 42.86, H, 6.39. Found: C, 42.82, H, 6.35) was satisfactory. Further the specific rotation of synthetic trisaccharide {$[\alpha]_D^{25}$ = +101.6, *c* = 1, H_2O} closely matches with the reported trisaccharide[30c] {$[\alpha]_D^{25}$ = +103.1-+107.7, *c* = 1, H_2O} unambiguously establishing its structure. Compound **2** constitutes the oligosaccharide segment of LG-2O, which is a potent substrate for α-amylase inhibitor.

Conclusion

Since the conversion of lactol to lactone was reported in literature, for the first time the formal synthesis of LG-2O was successfully achieved. The advanced intermediate **14**, a potential substrate for the effective synthesis of various biologically active analogues of nojirimycin, deoxy nojirimycin that can serve as potential mammalian α-amylases, an ongoing project in our lab.

Experimental Section

4,6-*O*-isopropylidene-α-glucopyranosyl-(1→4)2,3:5,6-di-*O*-isopropylidene-aldehydo-glucose dimethyl acetal (7).



Maltose monohydrate was dehydrated azeotropically with toluene, followed by heating at 45-60 ^{o}C under diminished pressure. To a solution of anhydrous maltose (11.5 g, 36 mmol) in 1,4-dioxane (100 ml) were added 2,2-dimethoxypropane (40 ml, 0.33 mol) and *p*-toluenesulfonic acid (100 mg), and the mixture was stirred for 20 h at 80 ^{o}C. Concentrated ammonia was added dropwise until the solution was neutral (PH paper), and the solvents were evaporated. The residue was chromatographed on silica by using light petroleum ether: ethyl acetate (1:1) to afford compound **7** (9.0 g, 51%) as thick oil.

Mol. Formula : $C_{23}H_{40}O_{12}$

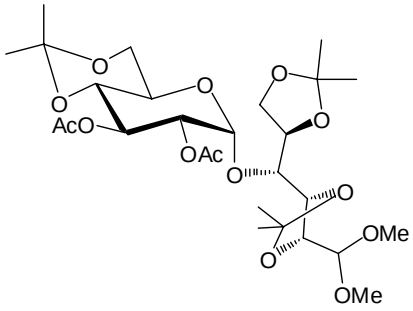
$[\alpha]_D^{25}$: 74.1 (*c* = 2.1, $CHCl_3$)

IR ($CHCl_3$) : 3459, 2992, 1631, 1457, 1382, 1215, 1067, 1025, 945, 849, 755, 667 cm^{-1}.

^{1}H NMR (500 MHz, $CHCl_3$) : δ 1.35 (s, 3H), 1.43 (s, 6H), 1.46 (s, 6H), 1.51 (s, 3H), 2.76 (br s, 1H), 3.17 (d, *J* = 9.6 Hz, 1H), 3.44 (s, 3H), 3.46 (s, 3H), 3.53 (t, *J* = 9.3 Hz, 1H), 3.58 (dd, *J* = 9.3, 4.0 Hz, 1H), 3.71-3.81 (m, 3H), 3.88 (dd, *J* = 9.3, 4.0 Hz, 2H), 3.97 (dd, *J* = 8.0, 6.5 Hz, 1H), 4.04 (dd, *J* = 8.0,

		6.5 Hz, 1H), 4.15 (dd, *J* = 6.5, 4.0 Hz, 1H), 4.22-4.26 (m, 2H), 4.41 (d, *J* = 6.0 Hz, 1H), 5.07 (d, *J* = 4.0 Hz, 1H).
^{13}C NMR (125 MHz, $CHCl_3$)	**:**	19.09 (q), 24.97 (q), 26.34 (q), 27.10 (q), 27.26 (q), 29.04 (q), 53.69 (d), 56.58 (d), 62.12 (t), 63.92 (d), 65.84 (t), 72.10 (d), 73.34 (d), 73.57 (d), 75.21 (d), 75.99 (d), 78.30 (d), 80.16 (d), 99.67 (s), 100.96 (d), 104.81 (d), 108.92 (s), 110.17 (s) ppm.
Maldi top	**:**	531.25 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 54.32; H, 7.93%. Found: C, 54.36; H, 7.92%.

2,3-Di-*O*-acetyl-4,6-*O*-isopropylidene-α-glucopyranosyl-(1→4)-2,3:5,6-di-*O*-isopropylidene-aldehydo-glucose dimethyl acetal (8).



Compound **7** (0.1 g, 0.17 mmol) was treated overnight with a mixture of acetic anhydride and pyridine (2 mL, 1:1). After evaporation in vacuo, the residue was subjected to column chromatography (light petroleum ether: ethyl acetate, 4:1) to yield compound **8** (0.11 g, 95%) as colorless oil.

Mol. Formula	**:**	$C_{27}H_{44}O_{14}$
$[\alpha]_D^{25}$	**:**	68.2 (*c* = 0.11, $CHCl_3$)
IR ($CHCl_3$)	**:**	3459, 2992, 1631, 1457, 1382, 1215, 1067, 1025, 945, 849, 755, 667 cm^{-1}.

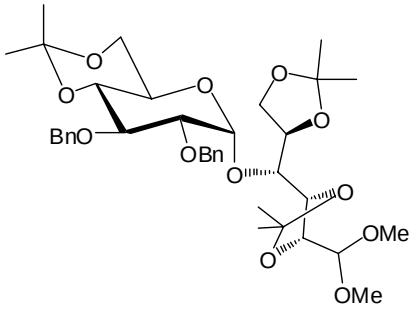
^{1}H NMR (200 MHz, $CHCl_3$) **:** δ 1.35 (s, 3H), 1.37 (s, 3H), 1.41(s, 3H), 1.46 (s, 9H), 2.04 (s, 3H), 2.08 (s, 3H), 3.43 (s, 3H), 3.44 (s, 3H), 3.71 (d, *J* = 5.9 Hz, 1H), 3.76 (d, *J* = 6.6, 1H), 3.82-4.32 (m, 9H), 4.94 (dd, *J* = 10, 3.9 Hz, 1H), 5.18 (d, *J* = 3.9 Hz, 1H), 5.37 (dd, *J* = 10.0, 9.0 Hz, 1H).

^{13}C NMR (50 MHz, $CHCl_3$) **:** 18.78 (q), 20.51 (q), 20.63 (q), 24.86 (q), 26.11 (q), 26.54 (q), 27.03 (q), 28.77 (q), 54.33 (d), 56.69 (d), 61.91 (t), 63.59 (d), 65.56 (t), 69.12 (d), 71.27 (d), 71.81 (d), 75.41 (d), 75.46 (d), 77.00 (d), 78.63 (d), 78.69 (d), 97.99 (d), 99.50 (s), 105.78 (d), 108.37 (s), 109.92 (s), 169.65 (s), 170.23 (s), ppm.

LC MS **:** 615.2 $[M+Na]^+$

Elemental Analysis **:** Calcd: C, 54.72; H, 7.48%.
Found: C, 54.68; H, 7.45%.

2,3-Di-*O*-benzyl-4,6-*O*-isopropylidene-α-glucopyranosyl-(1→4)-2,3:5,6-di-*O*-isopropylidene-aldehydo-glucose dimethyl acetal (9).

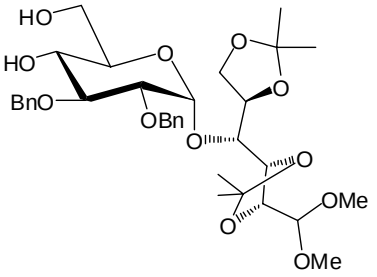


A solution of **8** (8 g, 15.73 mmol) in DMF (40 ml) was added to a suspension of sodium hydride (60 %) (2.41 g, 62.9 mmol) in DMF (20 ml) at 0 ^oC. After 30 min benzyl bromide (5.6 ml, 47.2 mmol) and tetrabutylammonium bromide (1 g) were added and the mixture was stirred for 12 h at rt. After cooling to 0 ^oC methanol was added, and the solvents were removed. The residue was dissolved in dichloromethane, washed with water,

brine and dried over Na_2SO_4. The syrup was chromatographed on silica using light petroleum ether: ethyl acetate (4:1) as an eluent to give compound **9** (10.2 g, 94%) as syrup.

Mol. Formula	**:**	$C_{37}H_{52}O_{12}$
$[\alpha]_D^{25}$	**:**	48.56 (*c* = 2.2, $CHCl_3$)
IR ($CHCl_3$)	**:**	3492, 3064, 2988, 1605, 1496, 1380, 1252, 1159, 1075, 906, 855, 788, 698 cm^{-1}.
^{1}H NMR (500 MHz, $CHCl_3$)	**:**	δ 1.34 (s, 3H), 1.37 (s, 3H), 1.41 (s, 6H), 1.46 (s, 6H), 3.33 (s, 3H), 3.42 (s, 3H), 3.48 (dd, *J* = 9.7, 3.3 Hz, 1H), 3.63 (t, *J* = 9.5 Hz, 1H), 3.67 (t, *J* = 10.5 Hz, 1H), 3.84 (dd, *J* = 10.5, 5.1 Hz, 1H), 3.88-3.93 (m, 3H), 4.02 (dd, *J* = 6.1, 3.3 Hz, 2H), 4.08 (dd, *J* = 7.6, 2.4 Hz, 1H), 4.25 (q, *J* = 6.1 Hz, 1H), 4.30 (d, *J* = 6.1 Hz, 1H), 4.58 (dd, *J* = 7.6, 6.1 Hz, 1H), 4.75 (2d, *J* = 11.2 Hz, 2H), 4.78 (d, *J* = 11.2 Hz, 1H), 4.83 (d, *J* = 11.2 Hz, 1H), 5.03 (d, *J* = 3.3 Hz, 1H), 7.24-7.36 (m, 10H).
^{13}C NMR (125 MHz, $CHCl_3$)	**:**	18.02 (q), 23.88 (q), 25.18 (q), 25.43 (q), 26.16 (q), 28.04 (q), 52.43 (d), 55.46 (d), 61.31 (t), 62.59 (d), 64.90 (t), 72.85 (t), 73.60 (d), 73.64 (t), 73.90 (d), 75.03 (d), 75.66 (d), 75.92 (d), 77.81 (d), 78.14 (d), 98.02 (s), 98.54 (d), 104.60 (d), 107.21 (s), 108.50 (s), 126.25 (d), 126.42 (d), 126.73 (d), 127.02 (d), 127.06 (d), 137.11 (s), 137.76 (s) ppm.
Maldi top	**:**	711.8 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 64.53; H, 7.55%. Found: C, 64.35; H, 7.75%.

2,3-Di-*O*-benzyl-α-glucopyranosyl-(1→4)-2,3:5,6-di-*O*-isopropylidene-aldehydo-glucose dimethyl acetal (10).



A solution of **9** (5 g, 7.26 mmol) and pyridine *p*-toluenesulphonate (100 mg) in methanol (50 ml) was stirred for 8 h. A few drops of triethylamine was added to neutralize the reaction mixture. Solvent was removed under reduced pressure, and the residue was chromatographed on silica by using light petroleum ether: ethyl acetate (1:1) as an eluent to afford compound **10** (3.2 g, 68%) as a syrup.

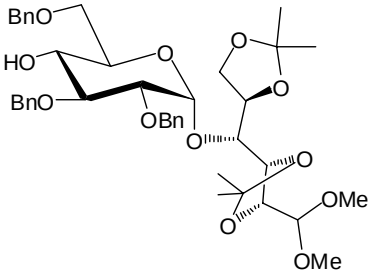
Mol. Formula	**:**	$C_{34}H_{48}O_{12}$
$[\alpha]_D^{25}$	**:**	23.6 (*c* = 2.2, $CHCl_3$)
IR ($CHCl_3$)	**:**	3445, 3063, 2986, 1604, 1496, 1380, 1251, 1154, 1065, 917, 862, 740, 699 cm^{-1}.
^{1}H NMR (500 MHz, $CHCl_3$)	**:**	δ 1.32 (s, 3H), 1.41(s, 3H), 1.44 (s, 3H), 1.47 (s, 3H), 2.44 (br s, 1H), 3.08 (br s, 1H), 3.29 (s, 3H), 3.38 (dd, *J* = 9.7, 3.3 Hz, 1H), 3.41 (s, 3H), 3.48 (dd, *J* = 9.7, 3.3 Hz, 1H), 3.63 (dd, *J* = 11.2, 6.1 Hz, 1H), 3.78 (t, *J* = 9.5 Hz, 1H), 3.80-3.86 (m, 2H), 3.98 (t, *J* = 7.6 Hz, 2H), 4.07 (t, *J* = 8.2 Hz, 2H), 4.34 (d, *J* = 6.1 Hz, 2H), 4.67 (dd, *J* = 11.2, 7.6 Hz, 3H), 4.75 (d, *J* = 11.2 Hz, 1H), 4.94 (d, *J* = 11.2 Hz, 1H), 5.16 (d, *J* = 3.3 Hz, 1H), 7.26-7.38 (m, 10H).
^{13}C NMR (125 MHz, $CHCl_3$)	**:**	24.01 (q), 25.55 (q), 26.75 (q), 27.30 (q), 52.87 (d), 56.44 (d), 62.68 (t), 64.96 (t), 70.38 (d), 72.99 (d), 73.05

(t), 74.86 (d), 75.15 (t), 78.37 (d), 79.06 (d), 80.28 (d), 80.80 (d), 98.28 (d), 105.40 (d), 108.20 (s), 110.18 (s), 127.67 (d), 127.83 (d), 127.90 (d), 128.06 (d), 128.31 (d), 128.57 (d), 138.04 (s), 138.40 (s) ppm.

Maldi top : 671.3 $[M+Na]^+$

Elemental Analysis : Calcd: C, 62.95; H, 7.46%.
Found: C, 62.9; H, 7.42%.

2,3,6-Tri-*O*-benzyl-α-glucopyranosyl-(1→4)-2,3:5,6-di-*O*-isopropylidene-aldehydo-glucose dimethyl acetal (5).



A suspension of diol **10** (3 g, 4.6 mmol) and Bis-dibutyltin oxide (2.3 g, 9.25 mmol) in anhyd MeOH (30 ml) was heated under reflux for 3 h. The reaction mixture was concentrated and dried in vacuo for several hours. After addition of dry toluene (40 ml), tetrabutylammonium iodide (1.7 g, 4.62 mmol) and benzyl bromide (0.82 ml, 6.93 mmol) were added, and the mixture was stirred for 12 h at 80 °C. The solvent was evaporated; methanol was added to solidify the tin impurities. The solid was filtered, washed with methanol, concentrated and the crude product was purified by column chromatography [light petroleum ether: ethyl acetate (4:1)] to afford **5** (2.8 g, 82.4 %) as an oil.

Mol. Formula : $C_{41}H_{54}O_{12}$

$[\alpha]_D^{25}$: 26.9 (c = 1.9, $CHCl_3$)

IR ($CHCl_3$) : 3479, 3063, 2986, 1878, 1605, 1496, 1380, 1251, 1150,

1063, 908, 861, 788, 698 cm^{-1}.

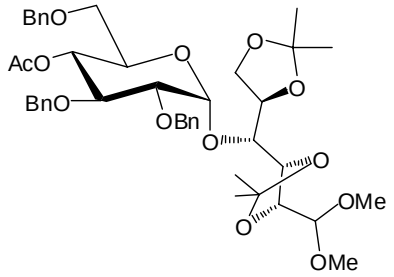
^{1}H NMR (500 MHz, $CHCl_3$) **:** δ 1.30 (s, 3H), 1.38 (s, 3H), 1.42 (s, 3H), 1.44 (s, 3H), 2.36 (br s, 1H), 3.32 (s, 3H), 3.43 (s, 3H), 3.48 (dd, *J* = 9.7, 3.3 Hz, 1H), 3.59 (d, *J* = 9.7 Hz, 1H), 3.62 (d, *J* = 3.3 Hz, 1H), 3.68 (dd, *J* = 10.3, 4.2 Hz, 1H), 3.79-3.82 (m, 2H), 3.90 (dt, *J* = 9.7, 3.7 Hz, 1H), 4.01 (dd, *J* = 6.1, 2.1 Hz, 2H), 4.14 (dd, *J* = 7.6, 2.4 Hz, 1H), 4.20 (q, *J* = 6.1 Hz, 1H), 4.29 (d, *J* = 6.1 Hz, 1H), 4.50 (d, *J* = 12.1 Hz, 1H), 4.57 (d, *J* = 12.1 Hz, 1H), 4.61 (dd, *J* = 7.6, 6.1 Hz, 1H), 4.69 (dd, *J* = 12.1, 6.1 Hz, 2H), 4.75 (d, *J* = 12.1 Hz, 1H), 4.91 (d, *J* = 11.6 Hz, 1H), 5.09 (d, *J* = 3.3 Hz, 1H), 7.22-7.36 (m, 15H).

^{13}C NMR (125 MHz, $CHCl_3$) **:** 25.15 (q), 26.54 (q), 26.94 (q), 27.43 (q), 53.65 (d), 56.52 (d), 66.62 (t), 69.66 (t), 70.73 (d), 71.12 (d), 73.28 (t), 73.66 (t), 75.18 (t), 76.19 (d), 78.85 (d), 79.23 (d), 79.95 (d), 81.00 (d), 99.01 (d), 105.86 (d), 108.43 (s), 109.79 (s), 127.60 (d), 127.64 (d), 127.73 (d), 127.87 (d), 127.99 (d), 128.28 (d), 128.33 (d), 128.48 (d), 137.99 (s), 138.16 (s), 138.74 (s) ppm.

Maldi top **:** 761.3 $[M+Na]^+$

Elemental Analysis **:** Calcd: C, 66.6; H, 7.3%.
Found: C, 65.78; H, 6.91%.

4-*O*-Acetyl-2,3,6-tri-*O*-benzyl-α-glucopyranosyl-(1→4)-2,3:5,6-di-*O*-isopropylidene-aldehydo-glucose dimethyl acetal (11).



Compound **10** (100 mg, 0.14 mmol) was treated overnight with a mixture of acetic anhydride and pyridine (2 mL, 1:1). After evaporation in vacuo, the residue was subjected to column chromatography (light petroleum ether /ethylacetate, 4:1) to yield **11** (96 mg, 91%) as colorless oil.

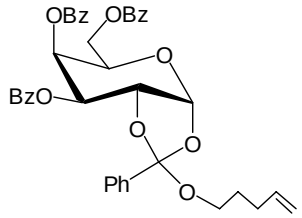
Mol. Formula : $C_{43}H_{56}O_{13}$

^{1}H NMR (200 MHz, $CHCl_3$) : δ 1.30 (s, 3H), 1.39 (s, 3H), 1.44 (s, 3H), 1.47 (s, 3H), 1.78 (s, 3H), 3.33 (s, 3H), 3.39 (d, *J*= 6.4 Hz, 2H), 3.44 (s, 3H), 3.55 (dd, *J* = 9.7, 3.3 Hz, 1H), 3.82 (dd, *J* = 6.0, 2.5 Hz, 1H), 3.95 (t, *J* = 9.7 Hz, 1H), 4.01 (d, *J* = 6.0 Hz, 2H), 4.15 (dd, *J* = 7.8, 2.5 Hz, 1H), 4.21 (d, *J* = 6.0 Hz, 1H), 4.31 (d, *J* = 6.0 Hz, 1H), 4.45 (d, *J* = 8.2 Hz, 2H), 4.55-4.66 (m, 3H), 4.72 (s, 2H), 4.76 (d, *J* = 11.6, 1H), 5.07 (t, *J* = 9.8 Hz, 1H), 5.08 (d, *J* = 3.3 Hz, 1H), 7.23-7.36 (m, 15H).

^{13}C NMR (50 MHz, $CHCl_3$) : 20.74 (d), 25.11 (d), 26.49 (d), 26.92 (d), 27.40 (d), 53.59 (d), 56.52 (d), 66.60 (t), 68.85 (t), 69.33 (d), 70.51 (d), 73.53 (t), 73.58 (d), 75.03 (t), 76.05 (d), 78.70 (d), 78.87 (d), 79.22 (d), 79.89 (d), 98.95 (d), 105.77 (d), 108.44 (s), 109.74 (s), 127.52 (d), 127.68 (d), 127.90 (d), 128.06 (d), 128.22 (d), 128.28 (d), 137.75 (s), 137.97 (s), 138.45 (s), 169.45 (s) ppm.

LC MS	**:**	803.3 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 66.14; H, 7.23%.
		Found: C, 66.1; H, 7.25%.

3,4,6-Tri-*O*-benzoyl-α-galactopyranosyl pentenyl orthoester (13).



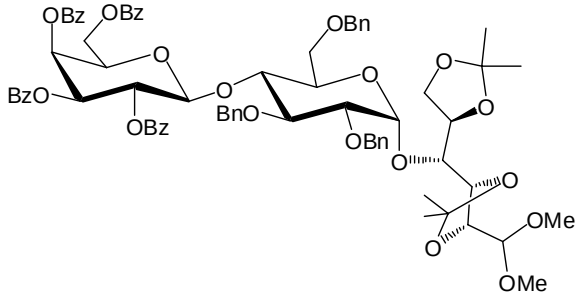
D-Galactose (2 g, 11.1 mmol) and DMAP (0.01 g, 0.08 mmol) were dissolved in pyridine (20 ml) and benzoyl chloride (10 ml, 86.2 mmol) was slowly added with cooling and stirring. The resulting reaction mixture was stirred for 24 h at room temperature and then evaporated to dryness. The solid residue was dissolved in CH_2Cl_2, and water was carefully added with cooling and vigorous stirring to decompose the excess of benzoyl chloride. The product was extracted with CH_2Cl_2, and dried to provide the crude pentabenzoate, in approximately 85% yield. The crude material (8.5 g, 12.1 mmol) was dissolved in CH_2Cl_2 (100 ml) and acetic anhydride (3.0 ml, 31.8 mmol) was added to ensure anhydrous conditions. The reaction mixture was cooled to 0 ^oC, 30% HBr/AcOH solution (50 ml) was slowly added and the reaction mixture was stirred at 0 ^oC for 1 h. The temperature of the reaction mixture was then raised up to, ~10 ^oC, the flask was sealed and stored in the refrigerator (+5 ^oC) overnight. The reaction mixture was then diluted with cold CH_2Cl_2 and cold water was added. The organic layer was washed with cold water, cold saturated $NaHCO_3$ and dried. After evaporation of solvent the crude glycosyl bromide 2,3,4,6-tetra-O-benzoyl-α-D-galactopyranosyl bromide, **12** (Rf 0.59 in Hex/EtOAc 2:1) was

obtained in approximate yield 98% (7.86 g). The crude glycosyl bromide **12** (7.3g, 11.1 mmol) was dissolved in dry CH_2Cl_2 (100 ml), and 2,6-lutidine (2.0 ml, 17.2 mmol), 4-pentenyl alcohol (1.4 ml, 14.1 mmol), tetra-n-butylammonium iodide (0.2 g, 540 mmol) were added. The resulting mixture was refluxed under argon for 24 h, when TLC (Rf 0.59 in Hex/EtOAc 3:1) showed complete disappearance of **12**. After cooling to room temperature, water and diethyl ether were added, and the organic layer was washed with water, brine and dried. After evaporation the residue was purified by silica gel using hexane/ethyl acetate (from 9:1 to 4:1) to give compound **13** (4.7 g, 63.7 %) as a viscous liquid.

Mol. Formula	**:**	$C_{39}H_{36}O_{10}$
$[\alpha]_D^{25}$	**:**	64.4 (*c* = 2, $CHCl_3$)
IR ($CHCl_3$)	**:**	3443, 3065, 2944, 1818, 1725, 1602, 1584, 1451, 1315, 1270, 1177, 1069, 920, 803, 757, 709 cm^{-1}.
^{1}H NMR (200 MHz, $CHCl_3$)	**:**	1.64-1.71 (m, 2H), 2.03-2.17 (m, 2H), 3.40 (t, *J*= 6.5 Hz, 2H), 4.38 (dd, *J* = 9.6, 4.1 Hz, 1H), 4.49-4.64 (m, 2H), 4.77 (dd, *J* = 6.0, 5.1 Hz, 1H), 4.90-5.04 (m, 2H), 5.54 (dd, *J* = 6.0, 4.1 Hz, 1H), 5.68-5.83 (m, 2H), 6.20 (d, *J* = 5.1 Hz, 1H), 7.33-7.98 (m, 20H).
^{13}C NMR (50 MHz, $CHCl_3$)	**:**	28.52 (t), 30.08 (t), 62.25 (t), 62.98 (t), 66.41 (d), 68.74 (d), 70.03 (d), 73.26 (d), 98.07 (d), 114.83 (t), 120.13 (s), 125.92 (d), 128.21 (d), 128.24 (d), 128.33 (d), 128.40 (d), 128.79 (s), 128.99 (s), 129.28 (s), 129.41 (d), 129.61 (d), 129.69 (d), 133.04 (d), 133.26 (d), 133.43 (d), 136.54 (s), 137.81 (d), 165.08 (s), 165.11 (s), 165.83 (s) ppm.
LC MS	**:**	687.65 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 70.47; H, 5.46%.

Found: C, 70.42; H, 5.44%.

2,3,4,6-Tetra-*O*-benzoyl-β-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-glucopyranosyl-(1→4)-2,3:5,6-di-*O*-isopropylidene-aldehydo-glucose dimethyl acetal (14).



The acceptor **5** (1 g, 1.35 mmol) and NPOE **13** (1.8 g, 2.71mmol) were dissolved together in a small quantity of toluene, azeotroped to dryness and kept overnight under vacuum. The mixture was dissolved in dry CH_2Cl_2 (15 mL) cooled to 0 ^oC under an argon atmosphere, NIS (0.91 g, 4.05 mmol) was added, and after stirring for 5 min, $Yt(OTf)_3$ (0.25 g, 0.405 mmol) was added. The reaction was monitored by TLC, and when complete, the reaction was quenched with 10% aqueous sodium thiosulfate and saturated sodium bicarbonate solutions, extracted with CH_2Cl_2, and purified by flash column chromatography using light petroleum ether /ethyl acetate (9:1) gave pure compound **14** (900 mg, 51 %) as a thick syrup.

Mol. Formula : $C_{75}H_{80}O_{21}$

$[\alpha]_D^{25}$: +34.1 (*c* = 1, $CHCl_3$)

IR ($CHCl_3$) : 3443, 3019, 2944, 1732, 1603, 1541, 1316, 1268, 1215, 1094, 713, 669 cm^{-1}.

^{1}H NMR (400 MHz, $CHCl_3$) : δ 1.07 (s, 3H), 1.16 (s, 3H), 1.37 (s, 3H), 1.39 (s, 3H), 3.30 (s, 3H), 3.40 (s, 3H), 3.43 (d, *J* = 3.5 Hz, 1H), 3.49

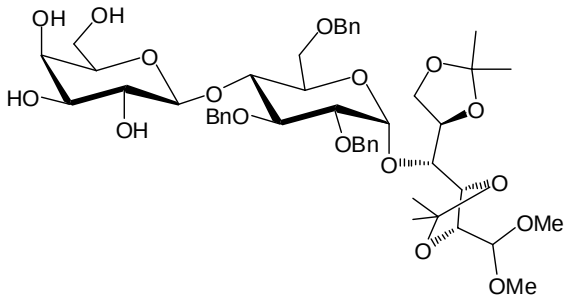
(dd, *J* = 9.7, 3.3 Hz, 1H), 3.72 (dd, *J* = 10.6, 2.1 Hz, 1H), 3.77 (d, *J* = 2.1 Hz, 1H), 3.81-3.86 (m, 3H), 3.90 (d, *J* = 2.1 Hz, 1H), 3.94 (d, *J* = 2.1 Hz, 1H), 4.01-4.08 (m, 3H), 4.17 (dd, *J* = 11.2, 7.3 Hz, 1H), 4.29-4.34 (m, 2H), 4.38 (dd, *J* = 11.2, 6.1 Hz, 1H), 4.52 (dd, *J* = 7.6, 6.1 Hz, 1H), 4.68 (t, *J* = 8.0 Hz, 2H), 4.71 (d, *J* = 5.6 Hz, 1H), 4.81 (d, *J* = 4.7 Hz, 1H), 4.84 (d, *J* = 3.7 Hz, 1H), 5.13 (d, *J* = 3.7 Hz, 1H), 5.16 (d, *J* = 11.2 Hz, 1H), 5.24 (dd, *J* = 10.4, 3.5 Hz, 1H), 5.66 (dd, *J* = 10.4, 8.0 Hz, 1H), 5.83 (d, *J* = 3.5 Hz, 1H), 7.14-7.63 (m, 25H), 7.75 (dd, *J* = 8.3, 1.3 Hz, 2H), 7.80 (dd, *J* = 8.3, 1.3 Hz, 2H), 7.91 (dd, *J* = 8.3, 1.3 Hz, 2H), 8.03 (dd, *J* = 8.3, 1.3 Hz, 2H), 8.11 (dd, *J* = 8.3, 1.3 Hz, 2H).

^{13}C NMR (100 MHz, $CHCl_3$) **:** 24.97 (q), 26.10 (q), 26.62 (q), 27.29 (q), 53.30 (d), 56.46 (d), 61.44 (t), 65.90 (t), 67.31 (t), 67.81 (d), 70.09 (d), 70.86 (d), 71.82 (d), 73.64 (t), 73.70 (t), 74.76 (d), 75.07 (t), 75.99 (d), 76.48 (d), 77.95 (d), 78.76 (d), 79.51 (d), 98.78 (d), 100.16 (d), 105.45 (d), 108.12 (s), 109.76 (s), 127.07 (d), 127.22 (d), 127.47 (d), 127.96 (d), 128.04 (d), 128.15 (d), 128.19 (d), 128.41 (d), 128.49 (d), 128.53(d), 128.77 (d), 128.82 (d), 128.99 (s), 129.05 (s), 129.38 (s), 129.48 (s), 129.59 (d), 129.66 (d), 129.72 (d), 129.76 (d), 130.07 (d), 133.13 (d), 133.27 (d), 133.36 (d), 133.52 (d), 137.72 (s), 138.28 (s), 139.25 (s), 164.69 (s), 165.39 (s), 165.85 (s), 170.74 (s) ppm.

LC MS **:** 1339.87 $[M+Na]^+$

Elemental Analysis **:** Calcd: C, 68.38; H, 6.12%.
Found: C, 68.35; H, 5.99%.

β-Galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-glucopyranosyl-(1→4)-2,3:5,6-di-*O*-isopropylidene-aldehydo-glucose dimethyl acetal (15).



Compound **14** (0.5 g, 0.38 mmol) was treated with small amount of sodium (cat) in dry methanol. After 4 h (TLC monitoring), the solution was neutralized by addition of acidic resin (Amberlite IR 120, H1-form). The resin was washed with methanol. The combined organic layers were concentrated to dryness, which was purified by column chromatography using light petroleum ether /ethyl acetate (2:3) to give compound **15** (310 mg, 91%) as thick mass.

Mol. Formula : $C_{47}H_{64}O_{17}$

$[\alpha]_D^{25}$: 58.1 (*c* = 1, $CHCl_3$)

IR ($CHCl_3$) : 3065, 3012, 2935, 1716, 1603, 1496, 1455, 1372, 1218, 1046, 918, 864, 771, 667 cm^{-1}.

^{1}H NMR (400 MHz, $CHCl_3$) : δ 1.33 (s, 3H), 1.41 (s, 3H), 1.42 (s, 3H), 1.44 (s, 3H), 3.31 (t, *J* = 1.6 Hz, 1H), 3.33 (s, 3H), 3.44 (s, 3H), 3.55 (dd, *J* = 9.5, 7.8 Hz, 2H), 3.60 (d, *J* = 4.8 Hz, 2H), 3.66 (dd, *J* = 11.0, 1.3 Hz, 2H), 3.83 (d, J= 3.3 Hz, 2H), 3.86 (dd, *J* = 5.5, 2.7 Hz, 2H), 3.94-4.05 (m, 6H), 4.13(dd, *J* = 7.8, 2.7 Hz, 1H), 4.25 (d, *J* = 5.5 Hz, 1H), 4.31 (d, *J* = 6.1 Hz, 1H), 4.34 (d, *J* = 7.8 Hz, 1H), 4.48 (d, *J* = 12.0 Hz, 1H), 4.59 (dd, *J* = 6.1, 7.8 Hz, 2H), 4.65 (d, *J* = 5.2 Hz, 1H), 4.68 (d, *J* = 5.5 Hz, 1H), 4.71 (d, *J* = 6.1 Hz, 1H), 4.74 (d, *J* = 12.0 Hz, 1H), 4.84 (d, *J* = 11.2 Hz,

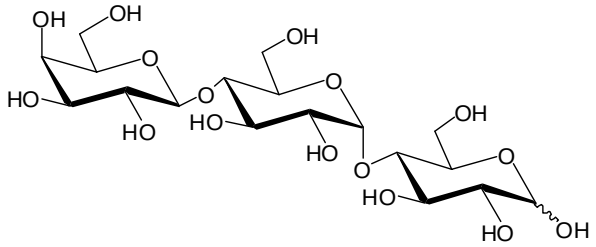
1H), 4.89 (d, J = 11.2 Hz, 1H), 5.11 (d, J = 3.5 Hz, 1H), 7.28-7.37 (m, 15H).

^{13}C NMR (50 MHz, $CHCl_3$) **:** 24.83 (q), 26.38 (q), 26.73 (q), 27.24 (q), 53.58 (d), 56.60 (d), 62.08 (t), 66.33 (t), 68.30 (t), 69.15 (d), 70.59 (d), 72.08 (d), 73.51 (t), 74.14 (d), 74.75 (d), 75.07 (t), 76.03 (d), 77.20 (d), 78.65 (d), 79.08 (d), 79.47 (d), 80.13 (d), 99.08 (d), 102.73 (d), 105.66 (d), 108.46 (s), 109.69 (s), 127.44 (d), 127.57 (d), 127.76 (d), 127.95 (d), 128.16 (d), 128.20 (d), 128.38 (d), 128.93 (d), 129.68 (d), 137.57 (s), 138.01 (s), 138.78 (s) ppm.

LC MS **:** 923.9 $[M+Na]^+$

Elemental Analysis **:** Calcd: C, 62.65; H, 7.16%.
Found: C, 62.64; H, 7.13%.

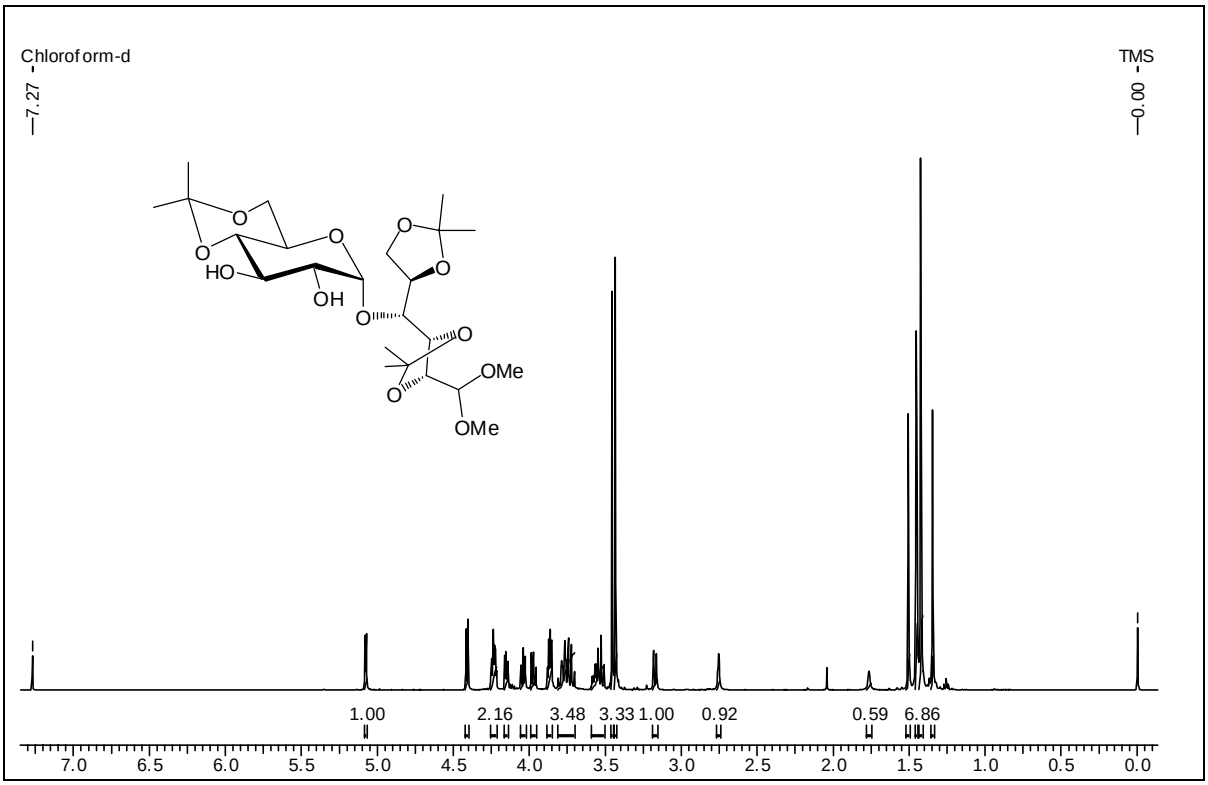
LG-2: 4^1-*O*-β-galactosyl-maltose (2).



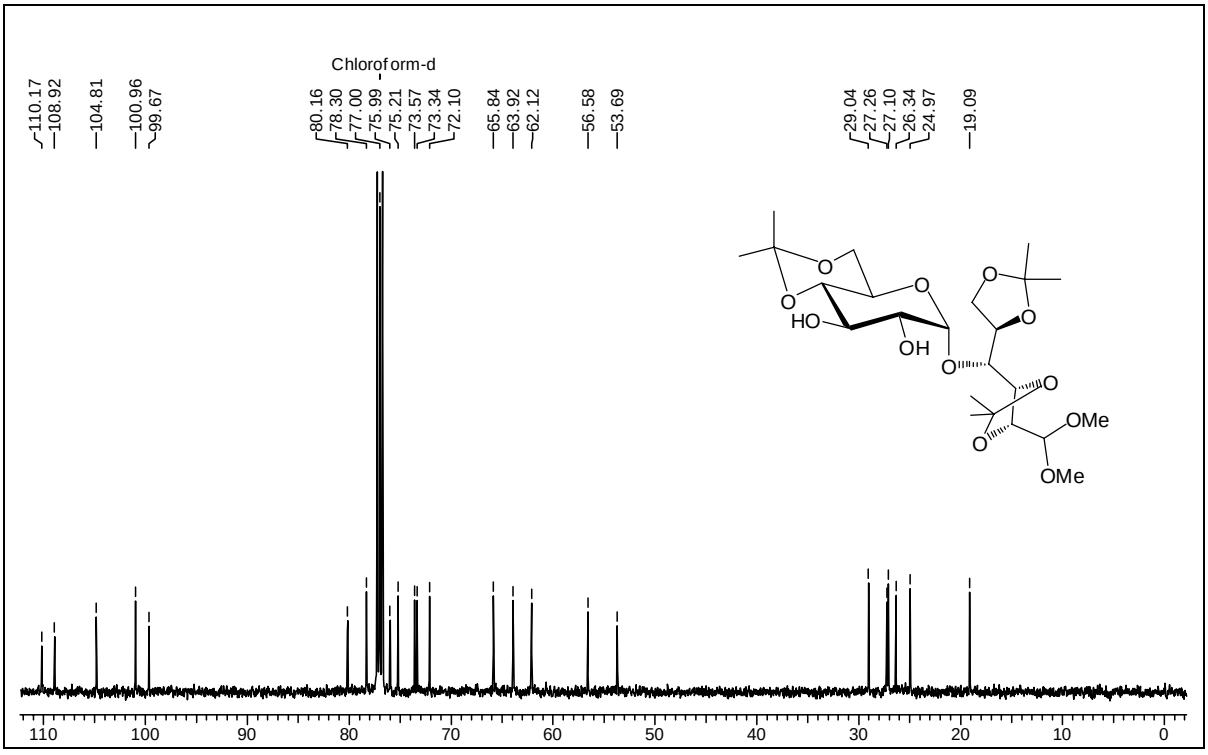
The compound (0.1 g, 0.11 mmol) **15** in MeOH (1 ml) was added 10% Pd/C (20 mg) under H_2 at normal temperature and pressure. After 10 h the reaction mixture was filtered through celite pad and concentrated. The crude product was used without further purification. Trisaccharide was stirred in THF (1 ml) and a 9:1 mixture of TFA: H_2O (1 ml) at 0 ^oC for 10 h, solvents were evaporated under diminished pressure. The crude material was purified by column chromatography by using $CHCl_3$/MeOH (4:1) gave solid compound **2** (16 mg, 29 %).

Mol. Formula	**:**	$C_{18}H_{32}O_{16}$
$[\alpha]_D^{25}$	**:**	101.6 (*c* = 1, H_2O)
IR ($CHCl_3$)	**:**	3065, 3012, 2935, 1716, 1603, 1496, 1455, 1372, 1218, 1046, 918, 864, 771, 667 cm^{-1}.
^{1}H NMR (400 MHz, D_2O)	**:**	δ 3.29 (t, *J* = 8.7 Hz, 1H), 3.53-4.01 (m, 17H), 4.47 (d, *J* = 7.7 Hz, 1H), 4.67 (d, *J* = 7.9 Hz, 1H), 5.24 (d, *J* = 3.7 Hz, 1H), 5.42 (d, *J*= 3.9 Hz, 1H).
^{13}C NMR (100 MHz, D_2O)	**:**	62.77 (t), 63.46 (t), 63.59 (t), 63.98 (t), 71.50 (d), 72.82 (d), 73.90 (d), 74.23 (d), 74.32 (d), 74.42 (d), 75.48 (d), 76.12 (d), 76.92 (d), 77.42 (d), 78.27 (d), 79.09 (d), 79.74 (d), 79.97 (d), 81.03 (d), 94.82 (d), 98.69 (s), 102.24 (s), 102.32 (s), 105.78 (s) ppm.
LC MS	**:**	527.17 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 42.86, H, 6.39 %. Found: C, 42.82; H, 6.35%.

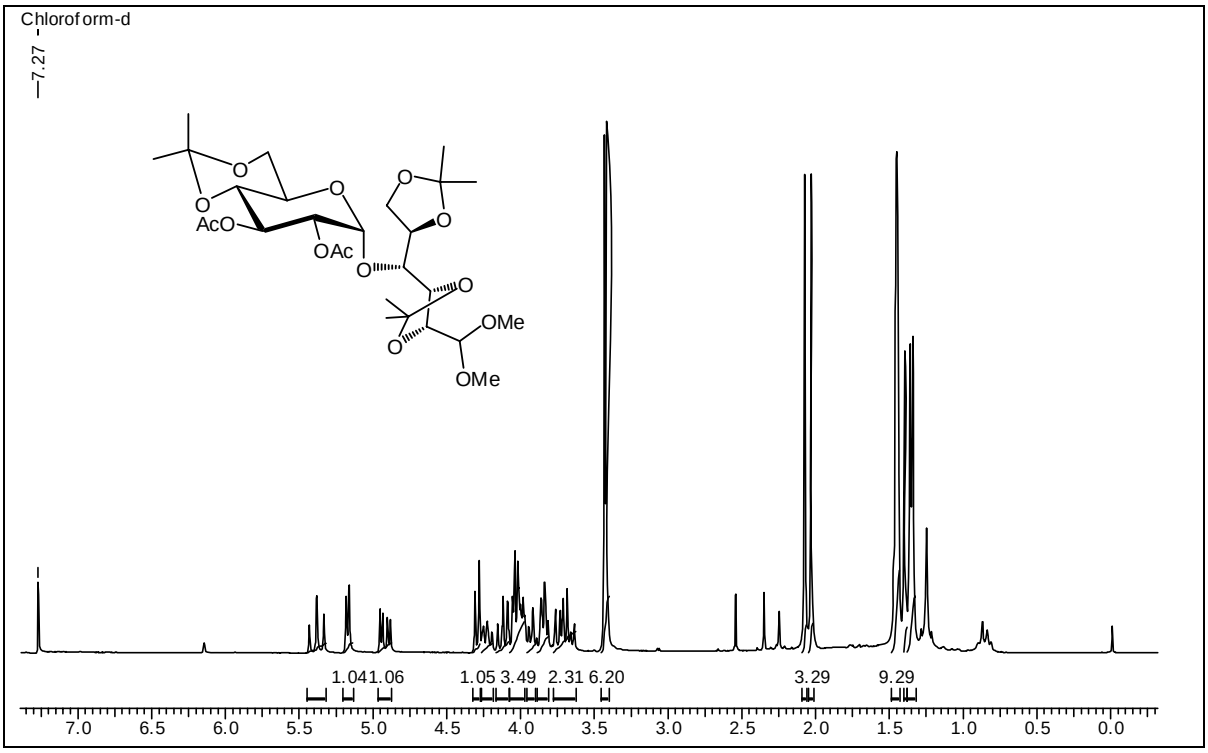
Spectra



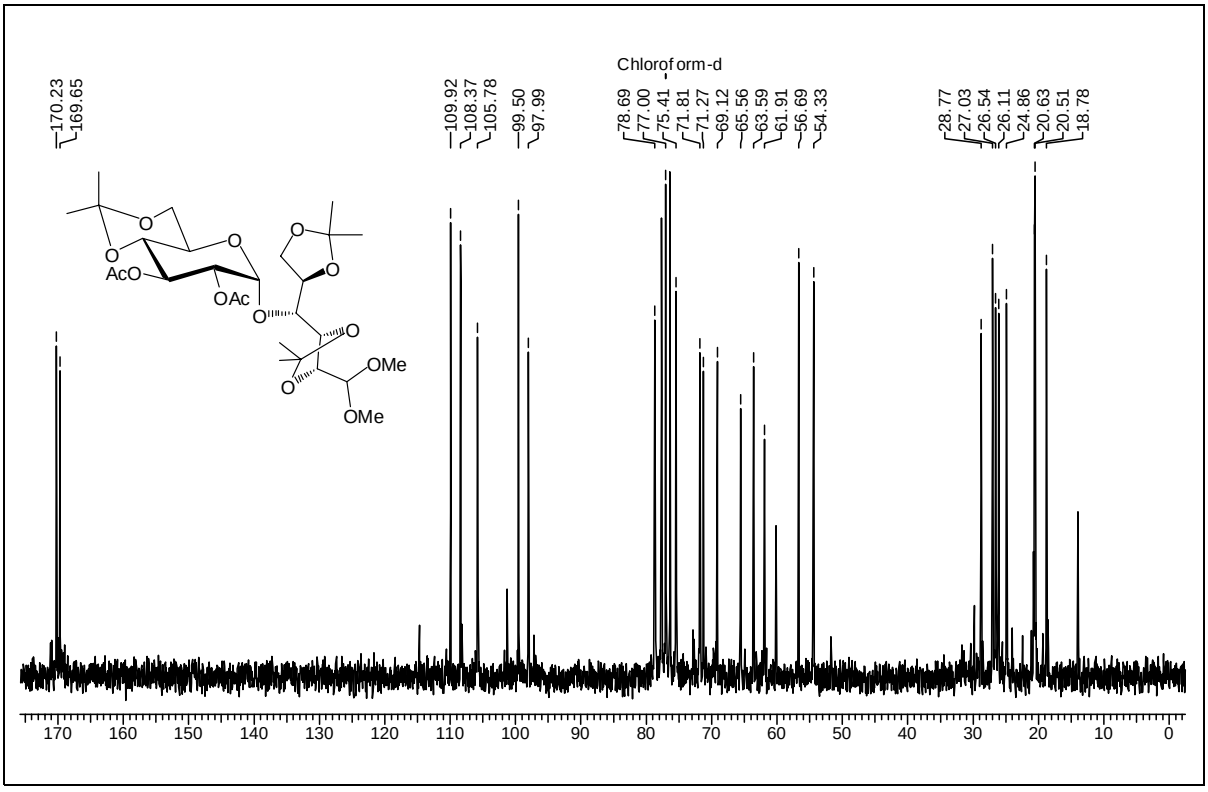
^{1}H NMR spectrum of compound 7 in $CDCl_3$



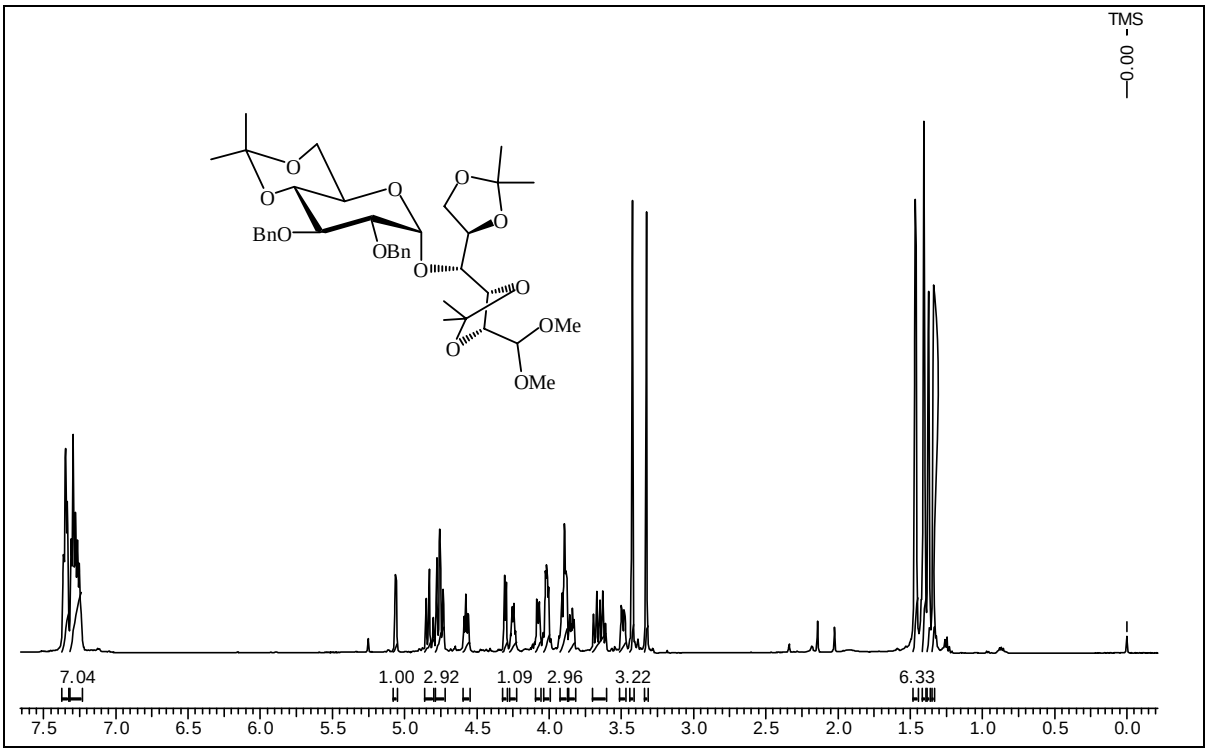
^{13}C NMR spectrum of compound 7 in $CDCl_3$



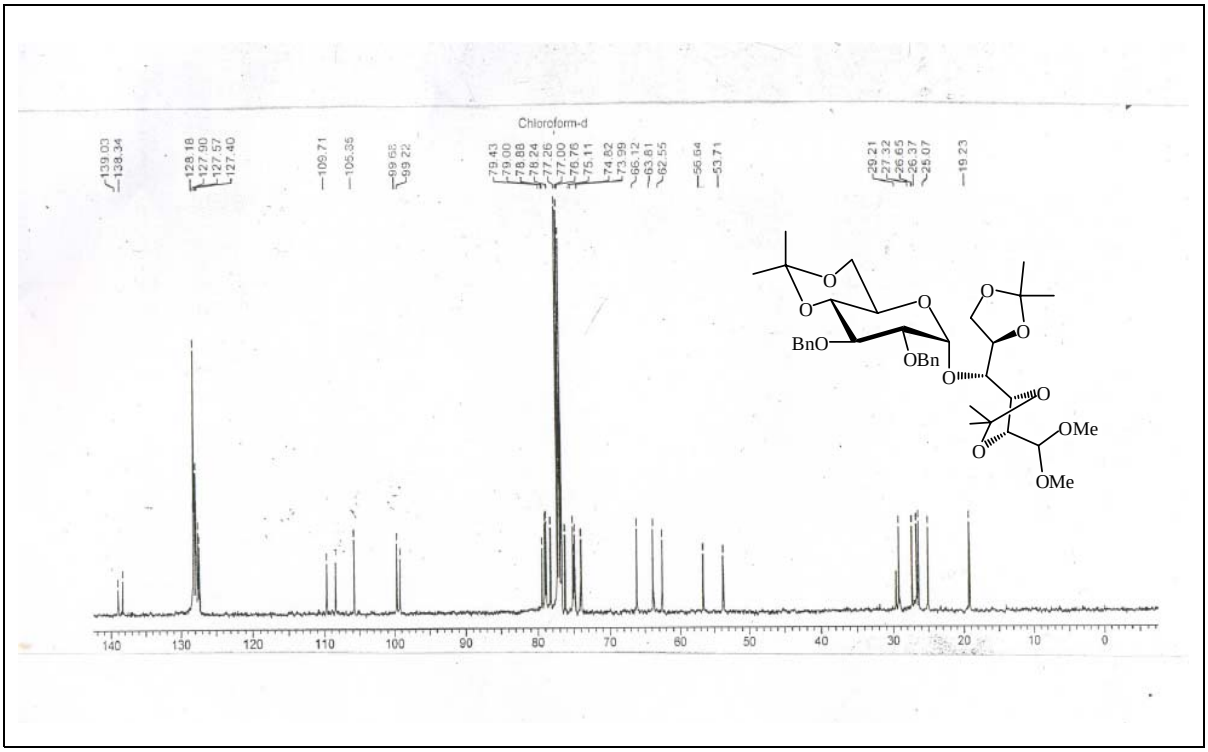
^{1}H NMR spectrum of compound 8 in $CDCl_3$



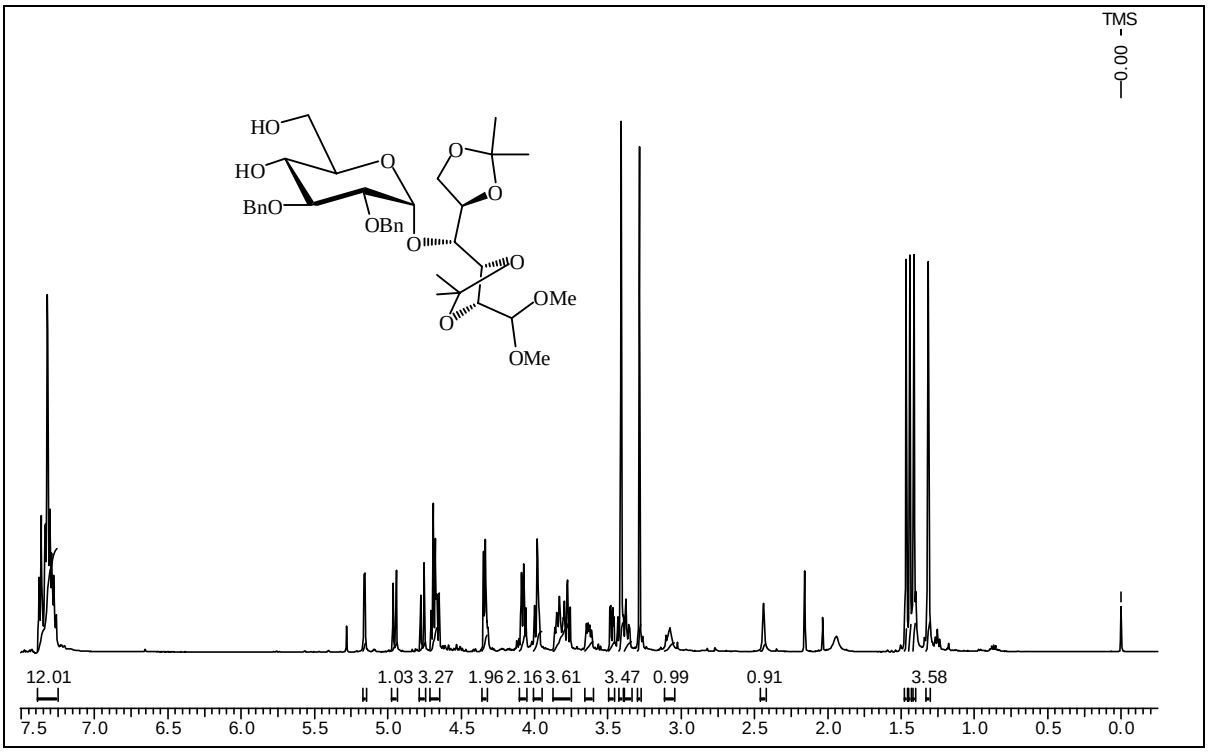
^{13}C NMR spectrum of compound 8 in $CDCl_3$



^{1}H NMR spectrum of compound 9 in $CDCl_3$



^{13}C NMR spectrum of compound 9 in $CDCl_3$



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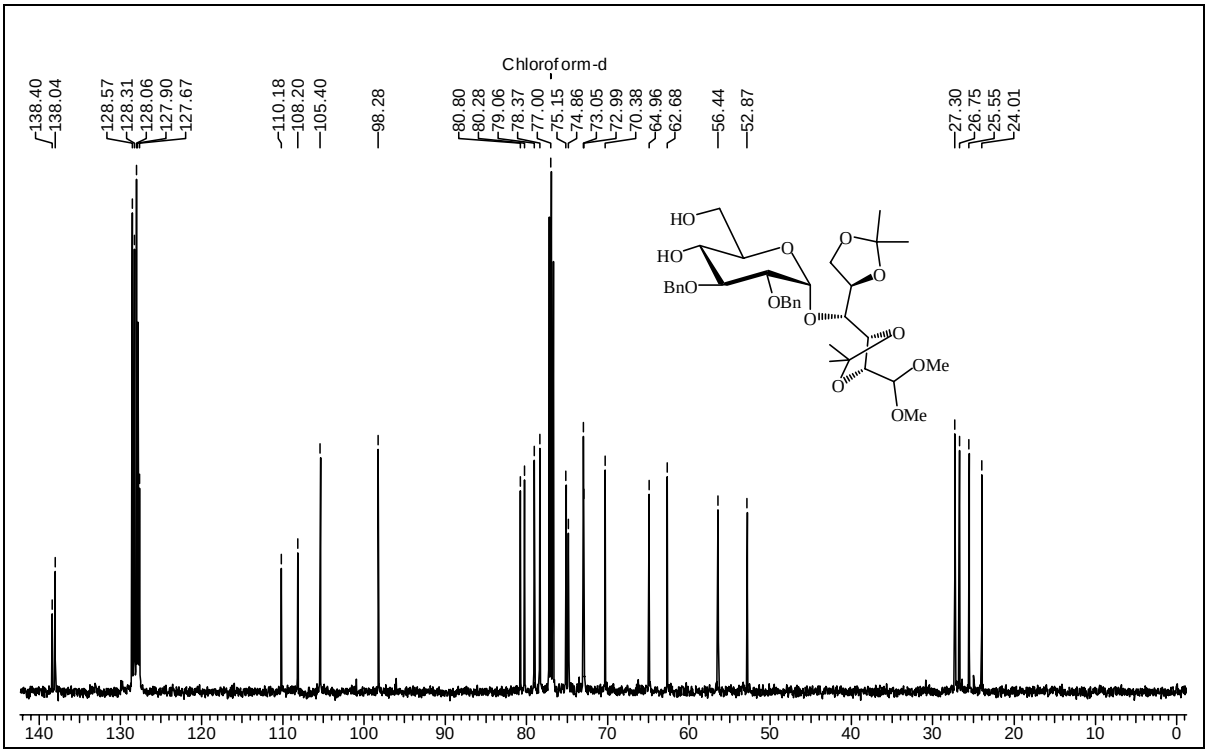

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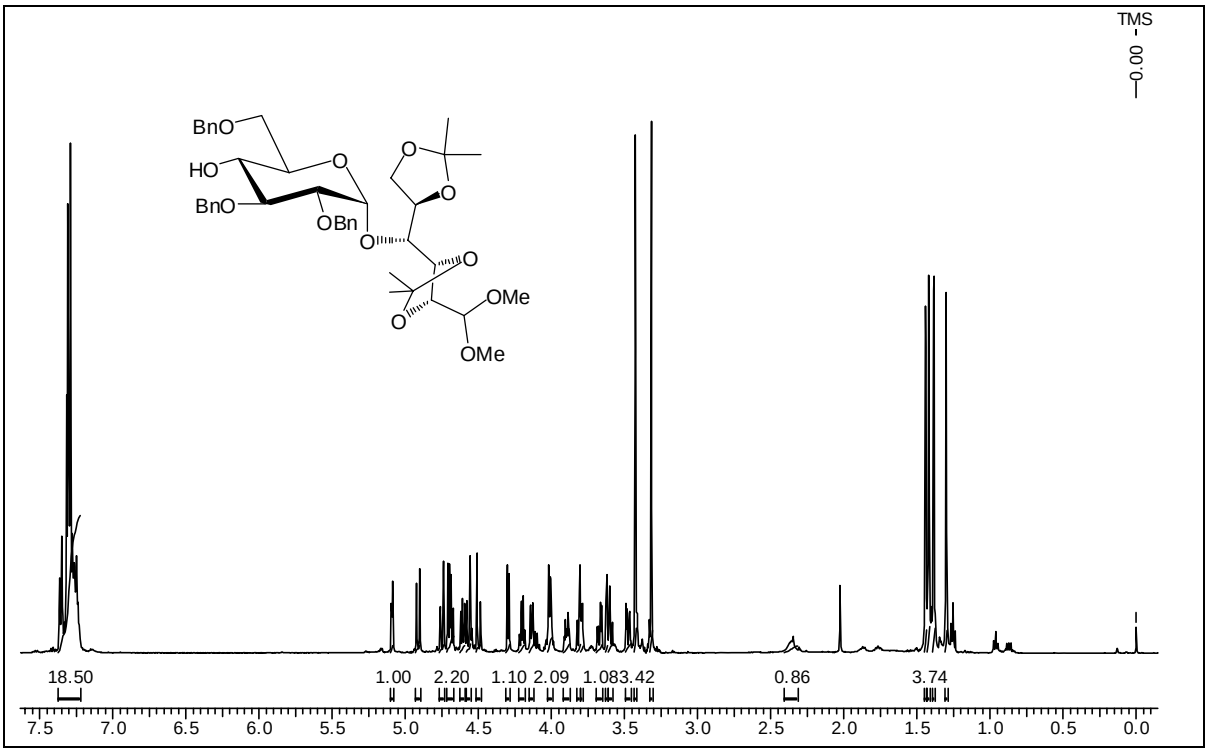
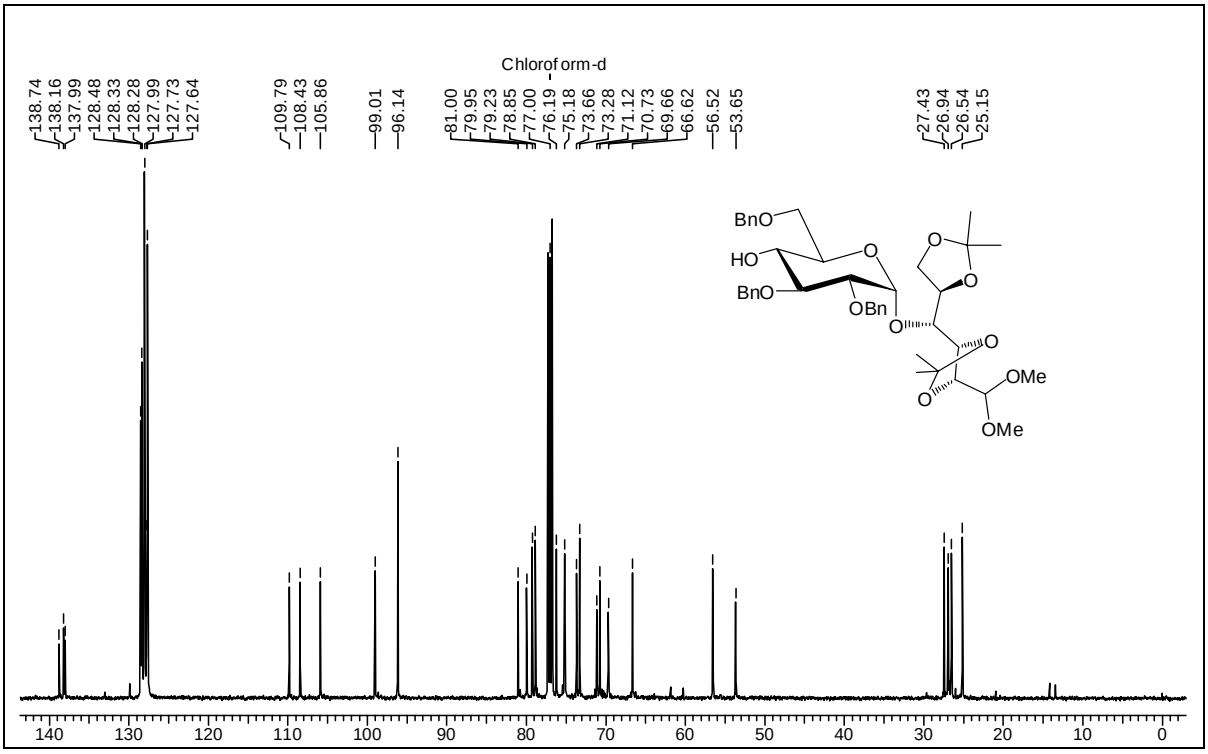


^{1}H NMR spectrum of compound 5 in $CDCl_3$



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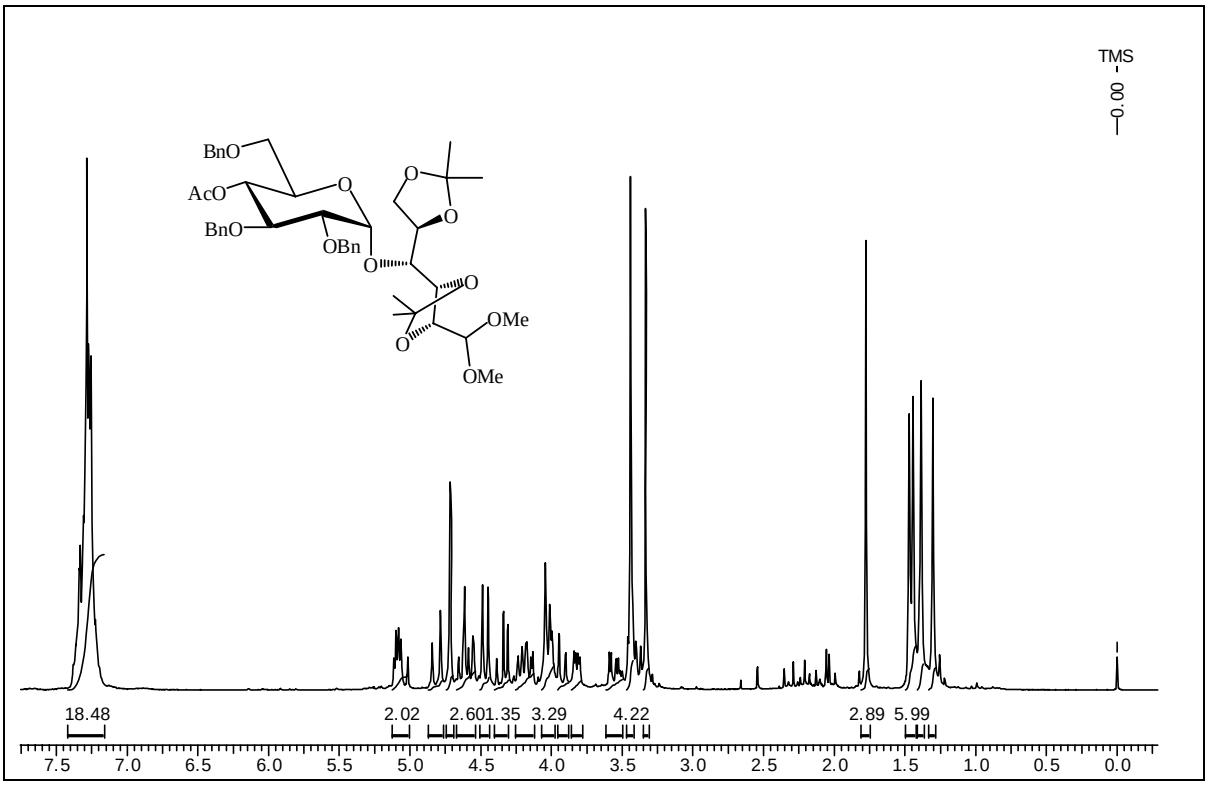
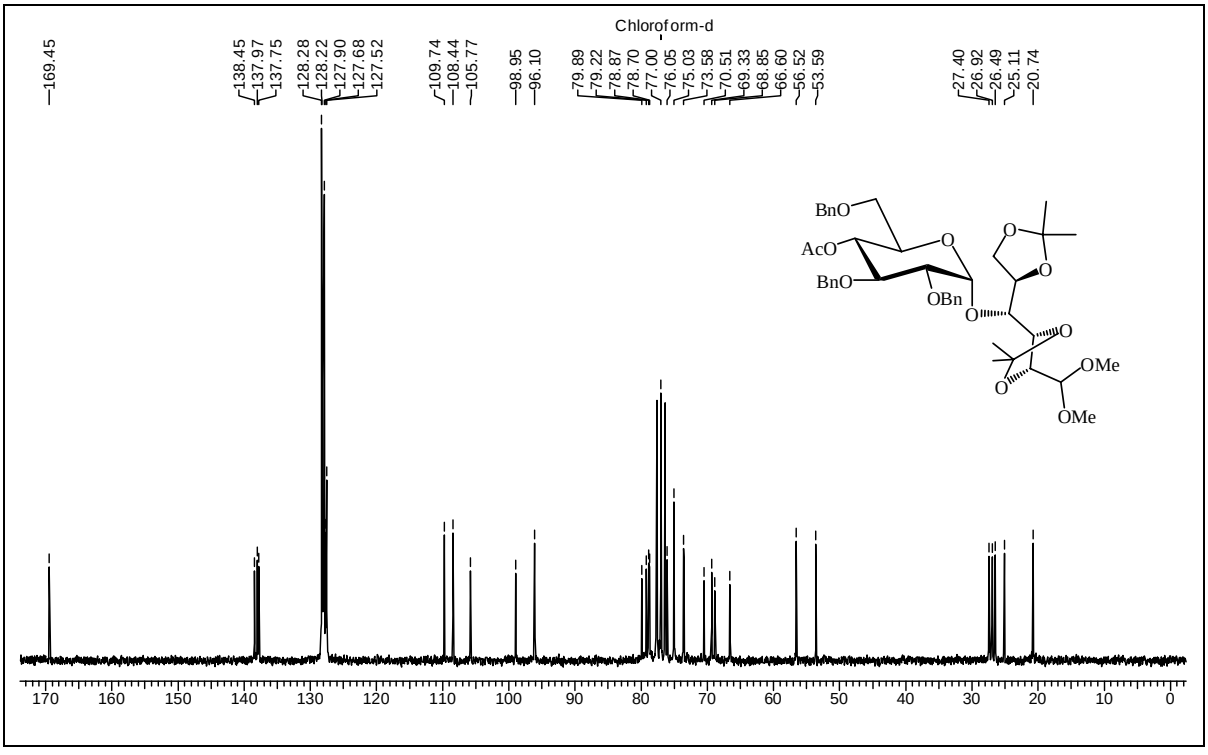
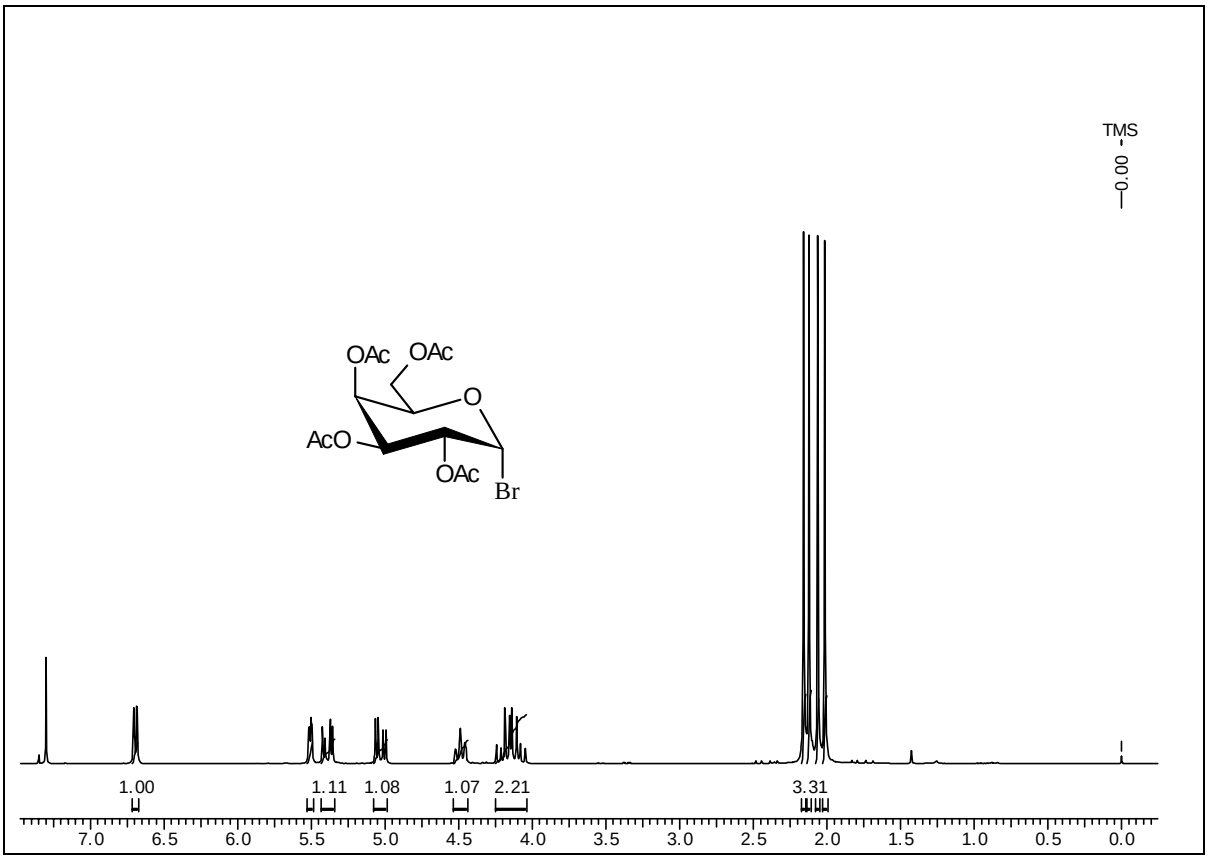


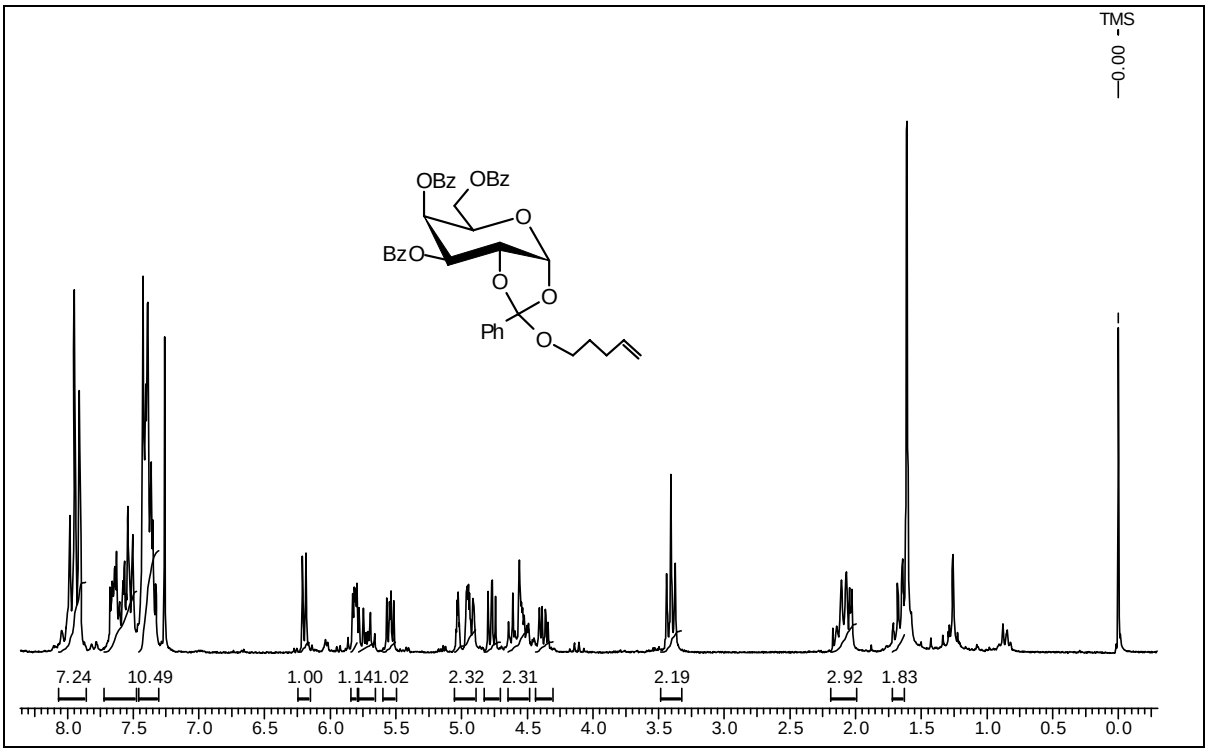
^{1}H NMR spectrum of compound 11 in $CDCl_3$



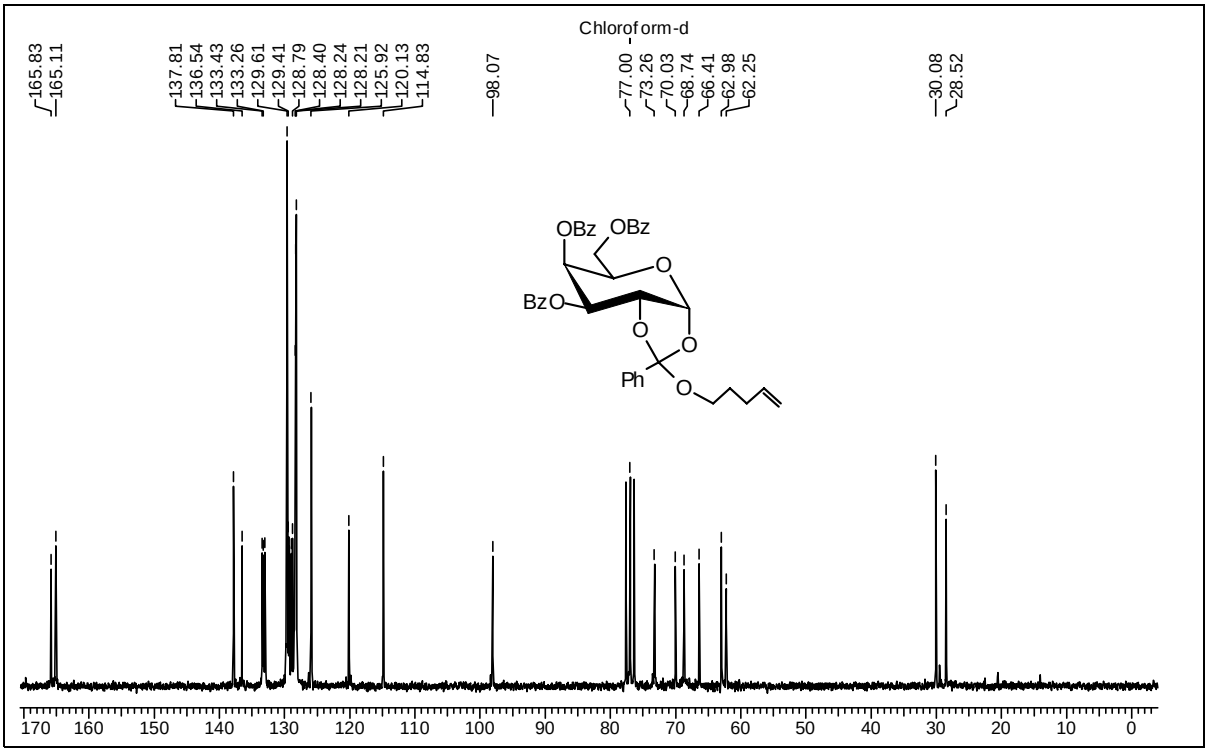
^{13}C NMR spectrum of compound 11 in $CDCl_3$



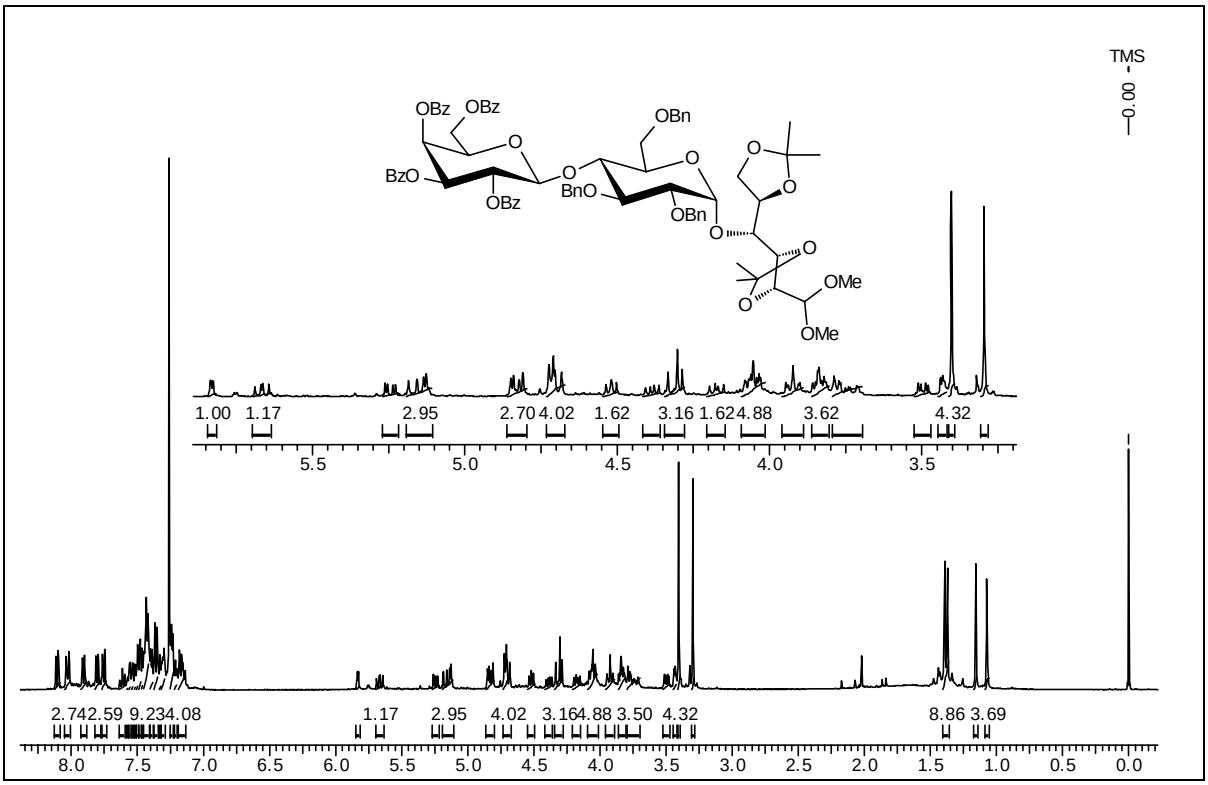
^{1}H NMR spectrum of compound 6 in $CDCl_3$



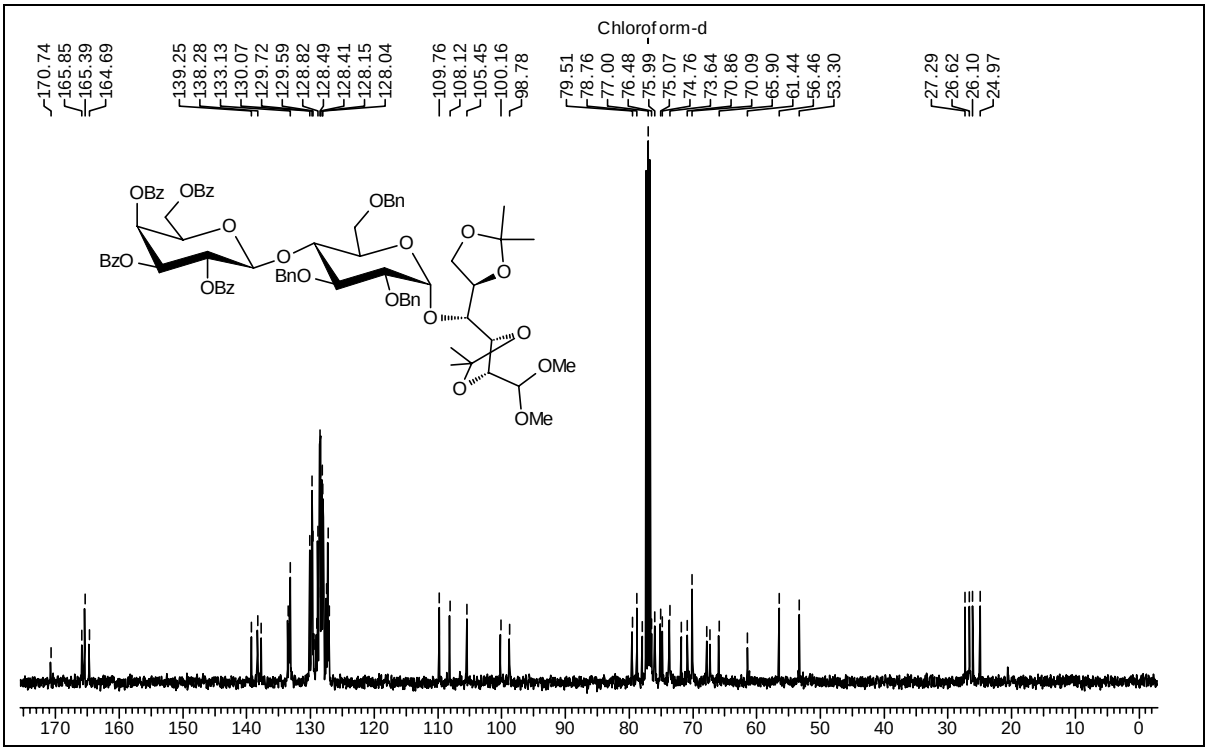
^{1}H NMR spectrum of compound 13 in $CDCl_3$



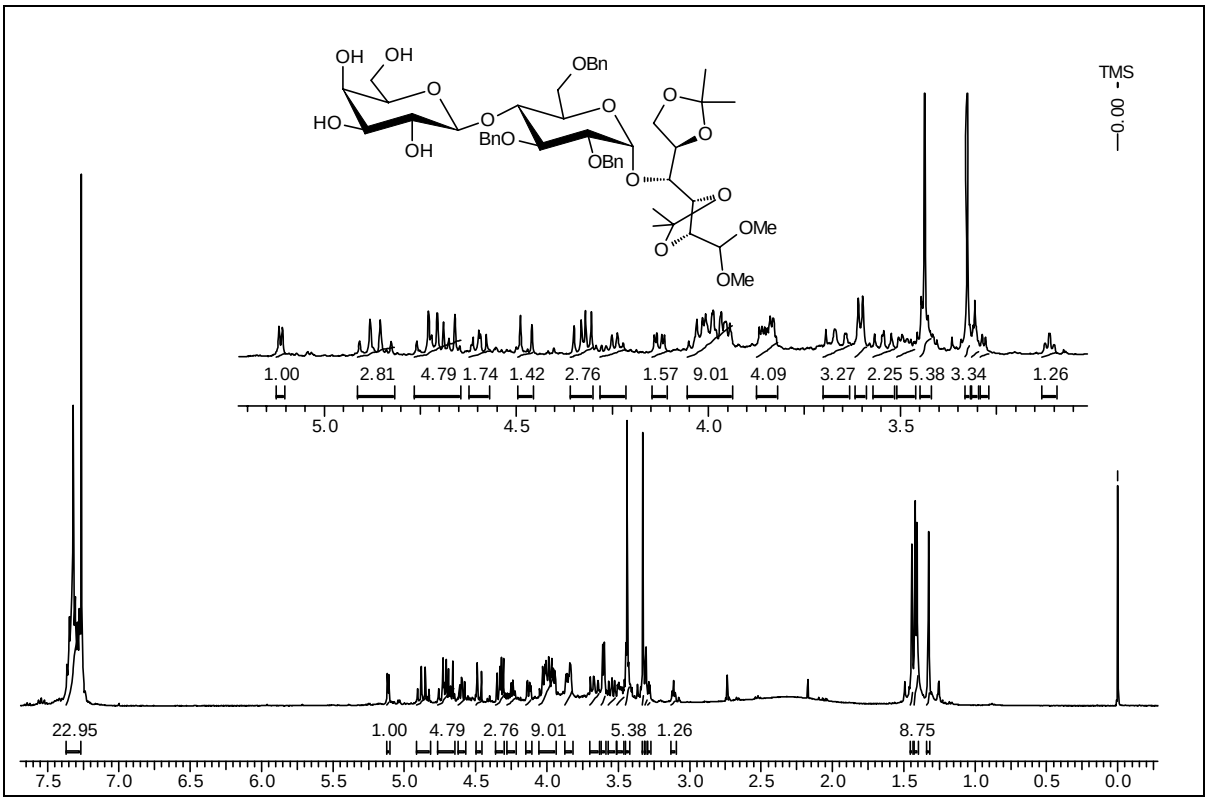
^{13}C NMR spectrum of compound 13 in $CDCl_3$



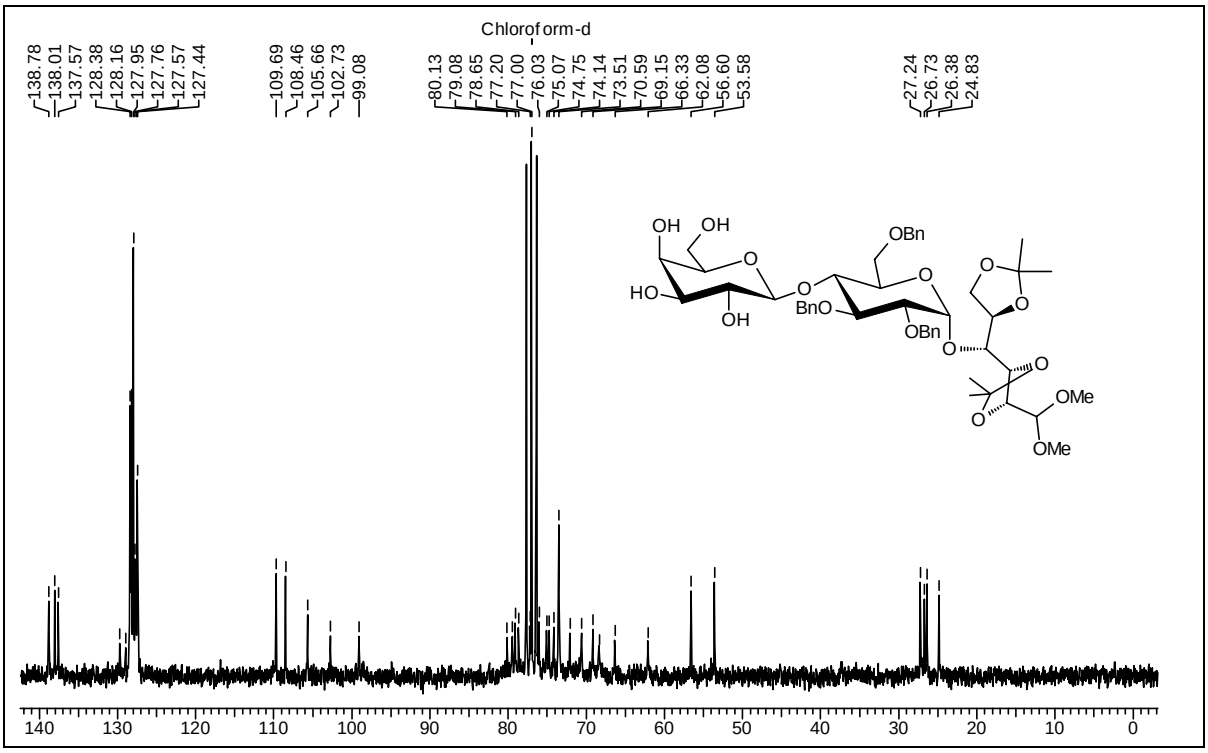
^{1}H NMR spectrum of compound 14 in $CDCl_3$



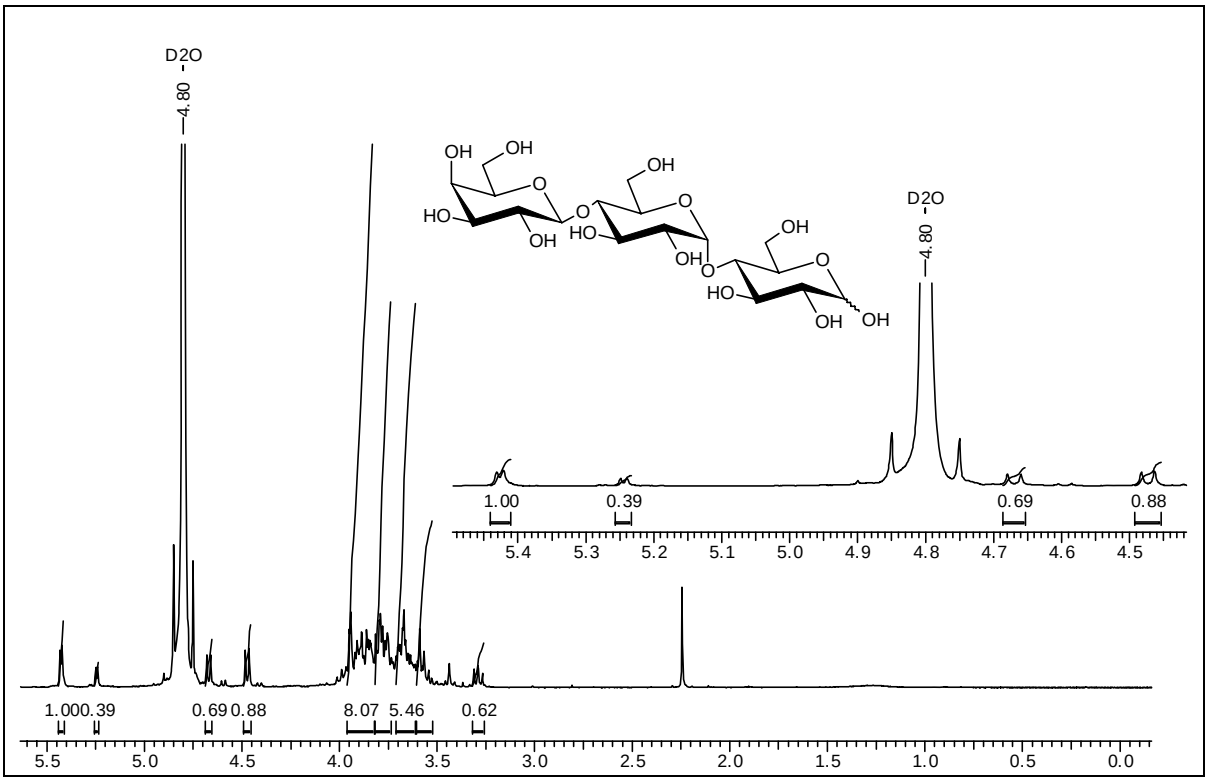
^{13}C NMR spectrum of compound 14 in $CDCl_3$



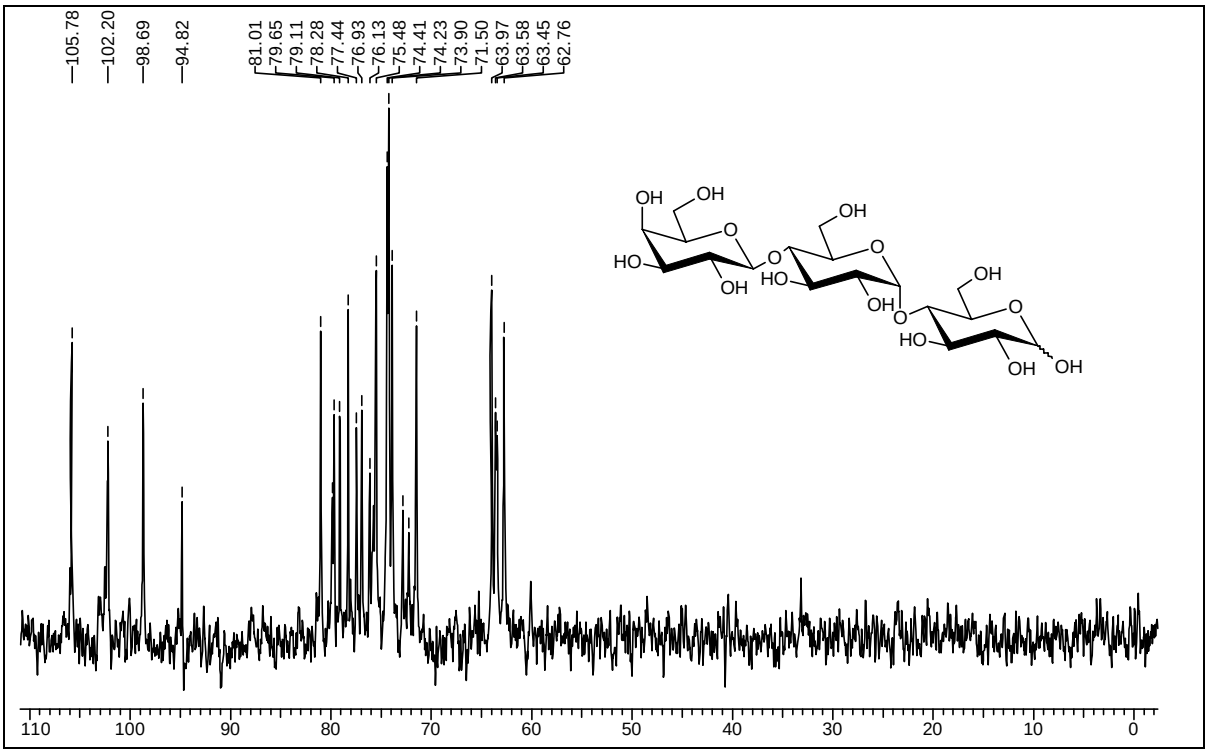
^{1}H NMR spectrum of compound 15 in $CDCl_3$



^{13}C NMR spectrum of compound 15 in $CDCl_3$



^{1}H NMR spectrum of compound 2 in D_2O



^{13}C NMR spectrum of compound 2 in D_2O

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CHAPTER III

Synthetic Studies Toward C_{43} – C_{52} Fragment of Amphidinol-3

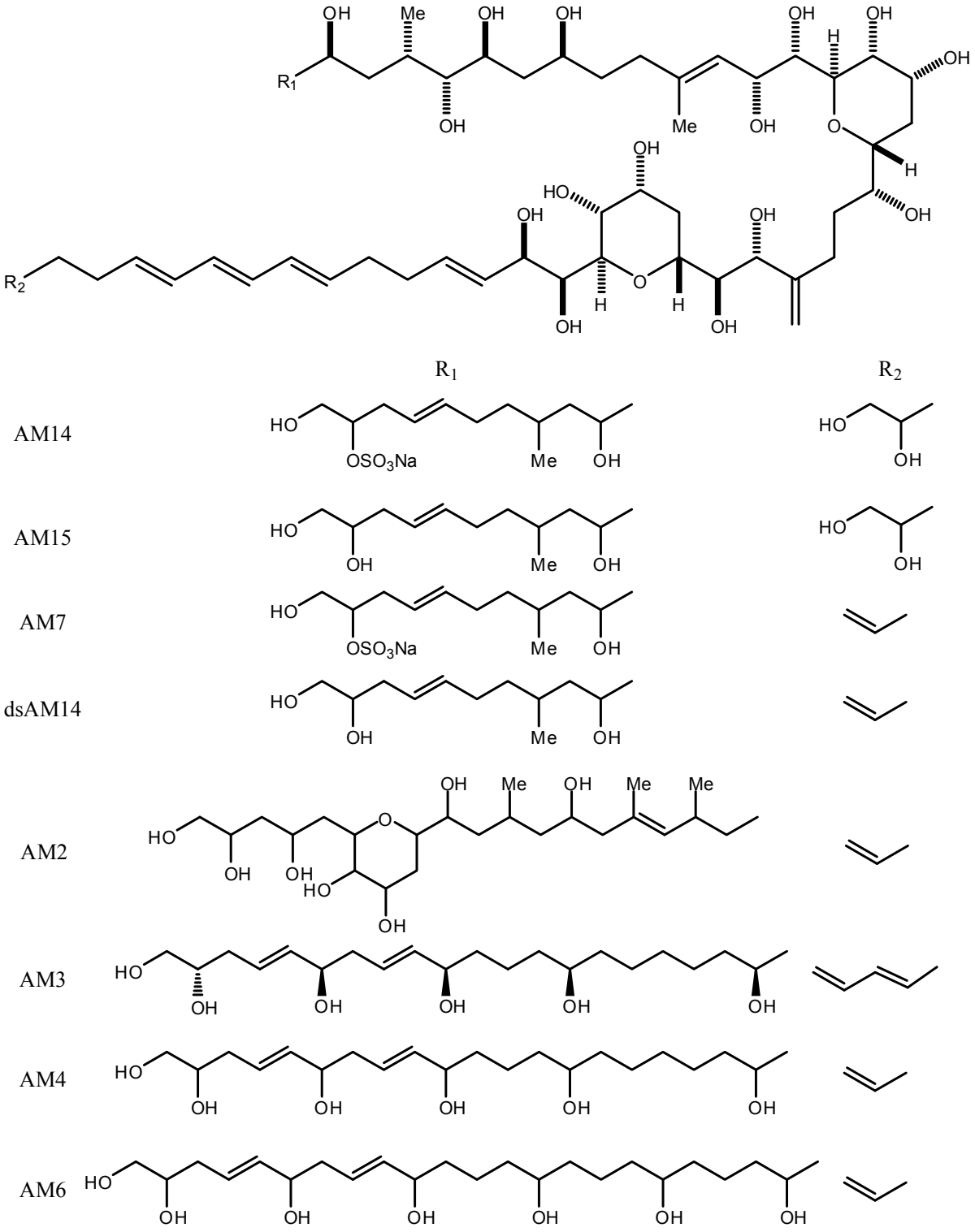
Introduction

Introduction

Dinoflagellates are known to be one of the most promising sources of diverse natural products with intriguing biological/pharmacological activities,[1,2] such as amphidinolides,[3] ciguatoxins, maitotoxin, okadaic acid, and saxitoxins. Amphidinols (AMs),[4–9] a class of polyhydroxy- polyene compounds isolated from the dinoflagellate Amphidinium species, mainly consist of a linear polyene-polyhydroxyl structure and showed potent antifungal activity, which significantly exceeds that of commercial drugs such as amphotericin B. Among known antifungal agents, AMs are unique in possessing neither nitrogenous polycycles as seen for synthetic drugs, nor macrocyclic structures commonly found in polyenemacrolide antibiotics. Therefore, a hitherto unknown mechanism of action should be involved in their biological action. Thus, AMs may provide an intriguing model to gain a better understanding of the mechanism of antifungal activities, which eventually helps to develop better drugs for treatment of AIDS-related diseases and those upon transplantation.

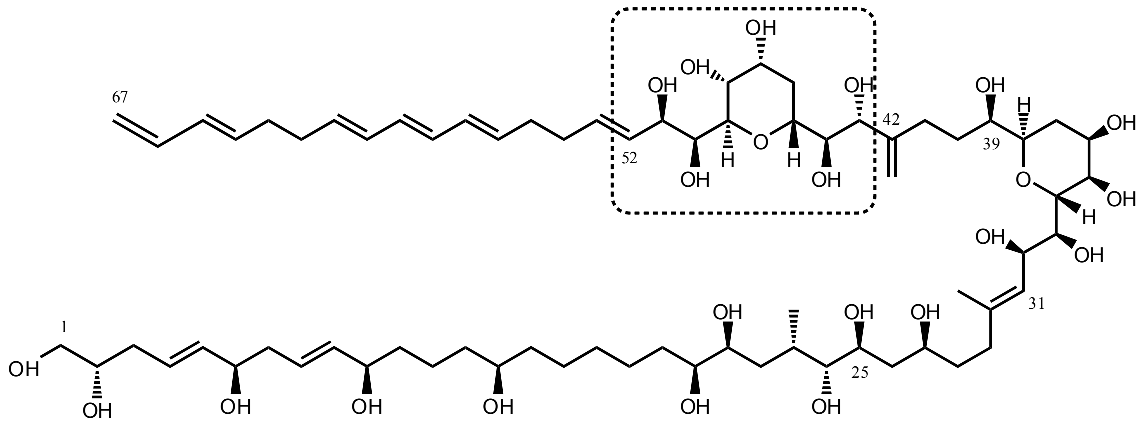
Amphidinols are unique dinoflagellate metabolites since they are primarily made up of linear polyhydroxy structures, exhibiting high hemolytic and antifungal properties. The first member of this family was isolated by Yasumoto in 1991[2] and new derivatives are continuously being identified.[3] The relative and absolute stereochemistry of amphidinol 3 (**1**) has recently been elucidated through an elegant combination of spectroscopic analysis and degradation studies.[4] Of the 13-polyketide metabolites in this family, amphidinol-3 (figure 1) is one of the most biologically active, with antifungal activity against Aspergillusniger and hemolytic activity on human erythrocytes. Amphidinol-3 effects cholesterol dependent membrane disruption, leading to speculation that its mode of action may, in part, be due to disruption of cell membranes.

Figure 1. Structures of some known amphidinols.



Absolute Configuration of Amphidinol 3:

J-based configuration analysis,[10] which has been proven to be a powerful tool for the stereochemical determination of acyclic structures.[10b] In this method, 1,2-diastereomeric relationships between chiral centers are determined by choosing a correct staggered rotamer among six possibilities arising from *erythro* and *threo* configurations using spin-coupling constants ($^{3}J_{H,H}$ and $^{2,3}J_{C,H}$,).[10a]



Amphidinol-3 (**1**)

The stereochemical assignment of **1** was accomplished as follows; (a) the *J*-based method[10] was used for acyclic parts with 1,2- and 1,3-chiral centers, C20-C27, C32-C34, C38-C39, C44-C45, and C50-C51; (b) NOE analysis combined with *J* analysis was used for two ether cycles and their linkage C39-C44; (c) the modified Mosher method[11] and chromatographic/NMR comparison were used for degradation products to determine the absolute stereochemistry at C2, C6, C10, C14, C23, and C39.

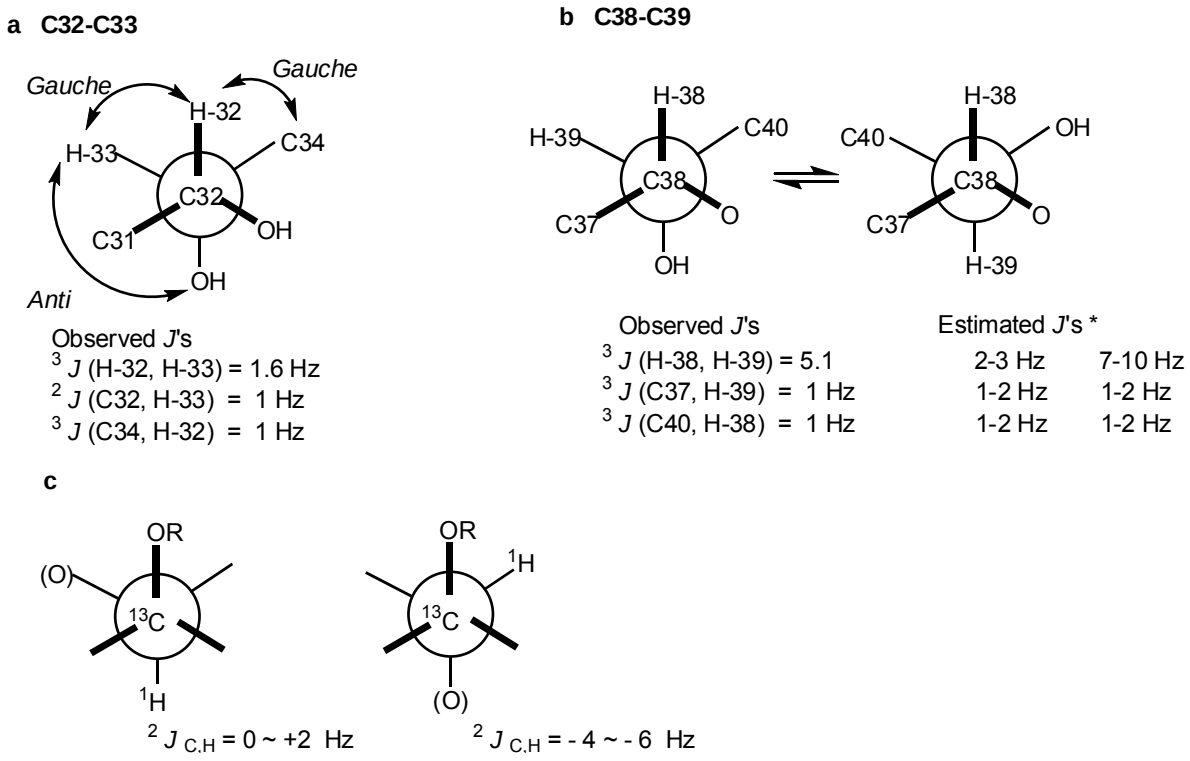
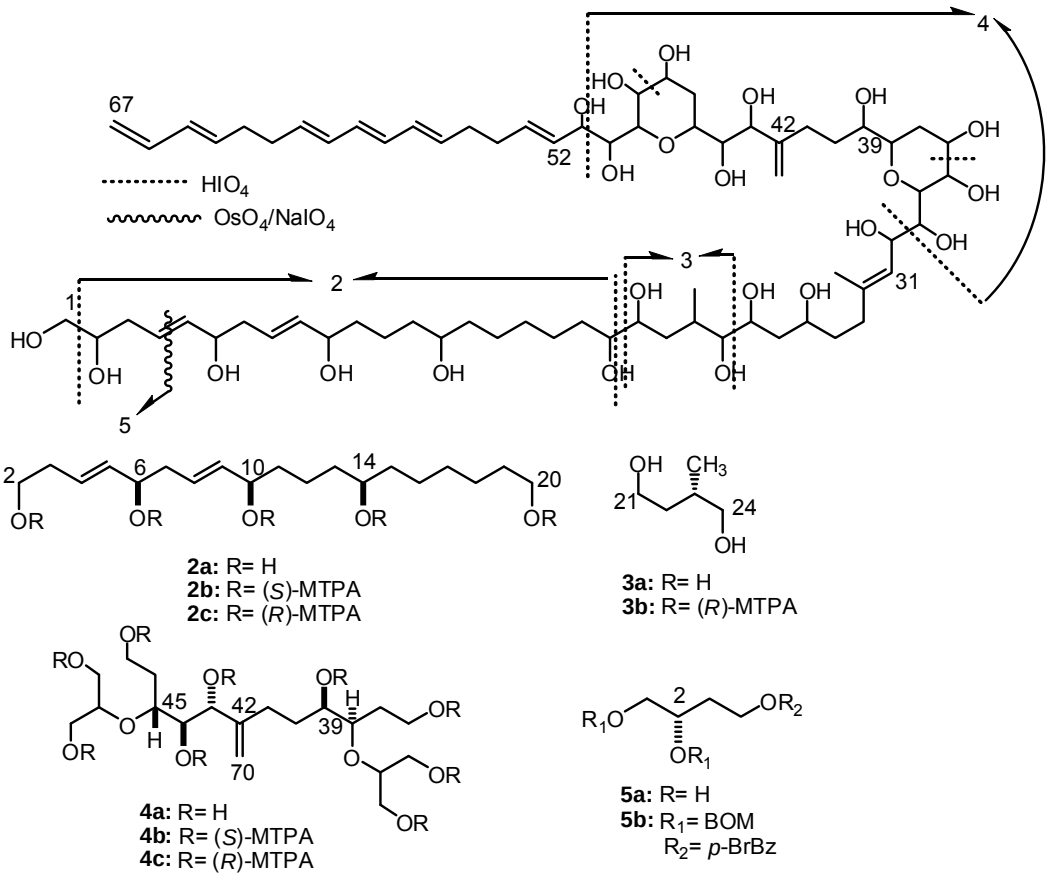


Figure 1. Rotamers and coupling constants for C32-C33 (a) and C38-C39 (b). **J* values for each rotamer estimated from stereochemically known compounds.[10a] (c) $^{2}J_{C,H}$ values depend on the dihedral angle between an oxygen atom on a relevant carbon and a proton on a neighboring carbon; in 1,2-dioxygenated systems such as C32-C33 (a), the anti O/H orientation gives $^{2}J_{C,H}$ of 0 to +2 Hz while the gauche orientation gives $^{2}J_{C,H}$ of -4 to -6 Hz.[12]

The absolute configurations of C20-C27 and C32-C51 and those at C2, C6, C10, and C14 were investigated using degradation products; treatment of **1** with $HIO_4/NaBH_4$, followed by esterification with (*R*)- and (*S*)-MTPA (2-methoxy-2-trifluoromethyl-2-phenylacetic acid) and separation by HPLC, furnished MTPA esters of fragments corresponding to C2-C20 (**2b**,**c**), C21-C24 (**3b**) and C33-C50 (**4b**,**c**). The absolute stereochemistries of C6, C10, C14, and C39[13] were elucidated by the modified Mosher method using **2b**/**2c** and **4b**/**4c**.[11] The configuration of **3a** was determined to be 23*S* by comparison of the NMR data of the bis-(*R*)-MTPA esters **3b** with (*S*)- and (*R*)-MTPA esters of authentic (*R*)-methyl-1,4-butanediol.[14] The configuration of C2 was determined using the C1-C4 fragment obtained from the *O*-benzyloxy-methyl derivative of **1** by treatment with $OsO_4/NaIO_4$.[15] The resulting 1,2-dibenzyloxylmethoxy-butyl *p*-bromobenzoate (**5b**) was chromatographed on a chiral resolution column[16] and determined to be an (*S*)-enantiomer.

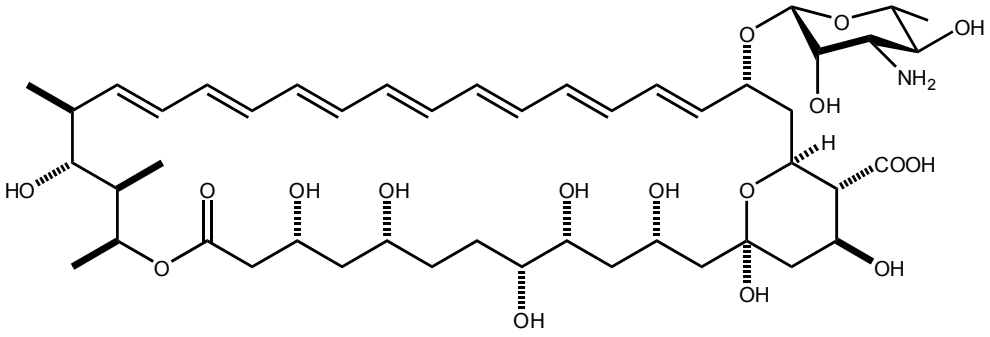
Considering all of these partial configurations led to the complete structure of amphidinol 3 with the absolute stereochemistry of 2*S*, 6*R*, 10*R*, 14*R*, 20*S*, 21*S*, 23*S*, 24*R*, 25*S*, 27*S*, 32*R*, 33*S*, 34*R*, 35*R*, 36*R*, 38*R*, 39*R*, 43*R*, 44*R*, 45*R*, 47*R*, 48*R*, 49*R*, 50*S*, and 51*R*.



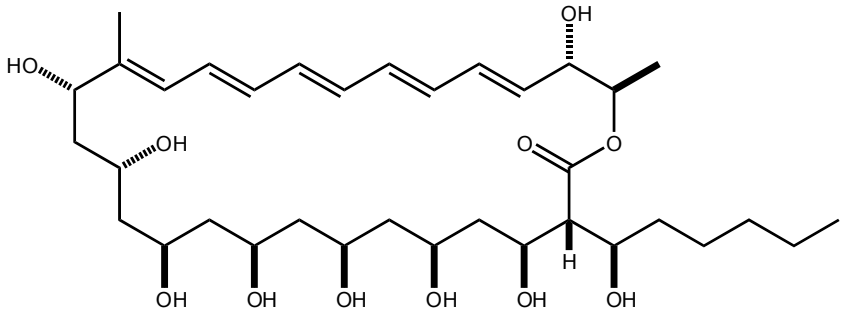
Bioactivity of amphidinols:

Amphidinols (AMs) show potent membrane-permeabilizing effects in terms of antifungal and hemolytic activities due to their special structural features.[17-22] Their structures are best characterized by a long carbon chain encompassing multiple hydroxyl groups and polyolefins, and the lopsided distribution of these hydrophilic and hydrophobic moieties may be reminiscent of polyene macrolides such as amphotericin B (AmB, 2). Besides the antifungal activity, AMs possess potent toxicity against diatoms,[23] implying the allelochemical roles against other epiphytic microbes in marine ecosystems. Their powerful membrane-disrupting activity is supposed to originate from a channel-like assemblage.[23]

Efflux assays of a fluorescent dye using small unilamellar vesicles (SUV)[23,24] have demonstrated the enhancement of the activity by cholesterol and ergosterol, which further infers the resemblance between AMs and AmB in their membrane activities.



Amphotericine B (**2**)



Filipin (**3**)

AmB (**2**) and filipin (**3**) are polyene antibiotics, both of which are known to interact with sterols in biomembranes. However, they permeabilize the membrane by different mechanisms;[25–27] AmB exerts its action by forming ion channels (channel-type) while filipin induces the damage-type of membrane disruption.[26] In the 1970s, the well known barrel-stave model was proposed for an ion channel of AmB, in which about eight pairs of AmB/sterol comprise a single ion channel.[28,29] Its selective toxicity against microorganisms could be attributed to a higher affinity for ergosterol, a principal fungal sterol, over cholesterol comprising mammalian plasma membranes.[30,31] Recent investigations using covalent conjugate analogues have demonstrated that the direct bimolecular interactions of AmB/AmB and AmB/lipid play an important role in channel formation in phospholipids membranes.[32–35] In the present study, we examined the membrane permeabilizing actions of AM3, the most potent amphidinol homologue,[24,36] by using hemolytic tests, Na+ efflux

assays, and ultraviolet–visible (UV) spectra; and compared its mode of membrane action with those of AmB, filipin and synthetic detergent Triton X-100.

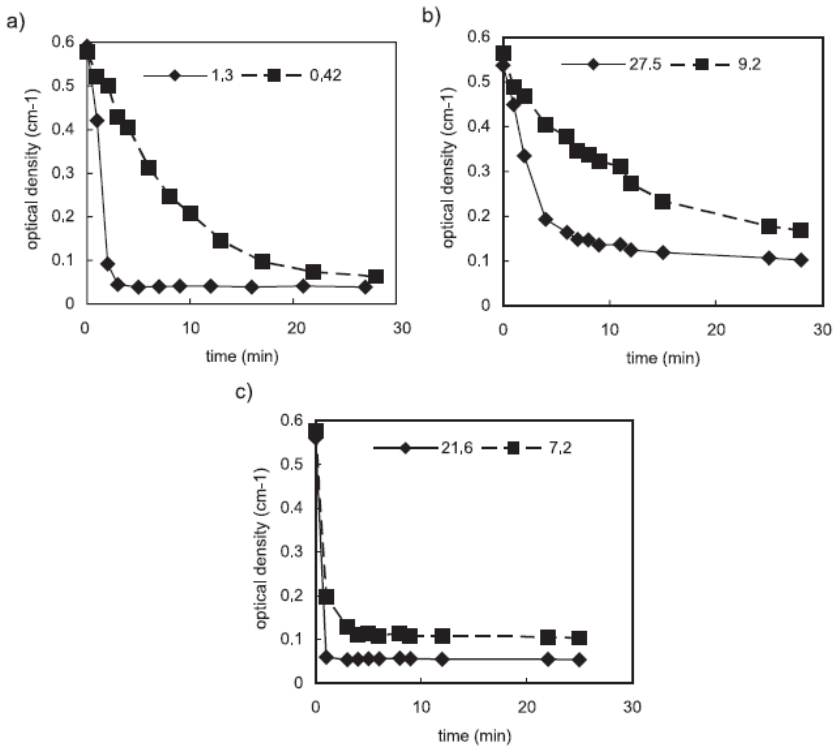


Fig. 2. *Time courses of hemolysis elicited by AM3 (a), AmB (b) and filipin III (c) Hemolytic activities of AM3, AmB and filipin III in two concentrations were monitored by the absorbance of erythrocyte suspension at 650 nm as described in Section 2, and plotted against incubation time. The numbers in figures show the concentrations of the agent in micromoles.*

In comparison with AM 7 and AM 11 to AM 13, AM 3 showed the most potent antifungal and hemolytic activities (Table 1.1). This implies the importance of molecular structures in bioactivities. The shorter polyol moiety in AM 7 than that in AM 3 was suggested to be responsible for the lower potent bioactivities of AM 7 than those of AM 3; therefore, the polyhydroxyl chain could be important for channel size and conductance.[22] Satake et al. showed that AM 11 to AM 13 possess lower antifungal and hemolytic activities than AM 3 does because of sulfate ester groups attached in their C1 positions. This shows that the terminal hydroxyl group plays a critical role in the bioactivities of the AMs.[19]

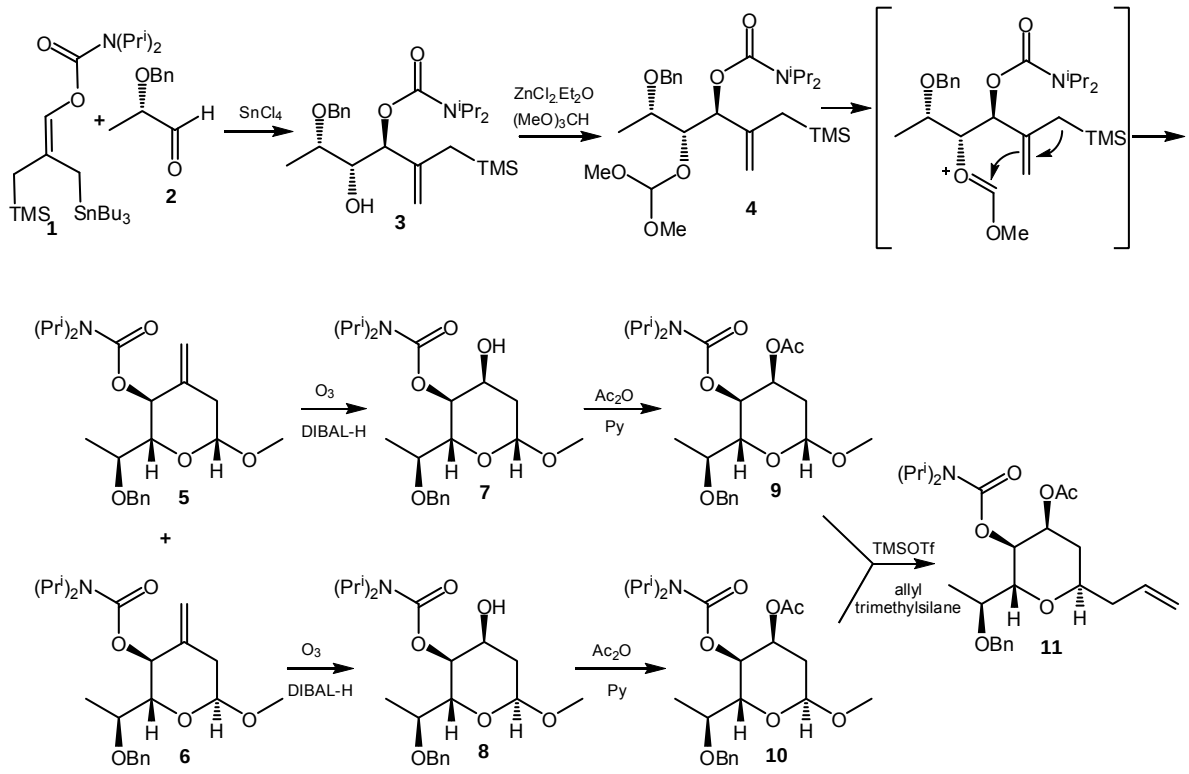
Activity	AM2	AM3	AM4	AM7	AM9	AM10	AM11	AM12	AM13	AmB	Filipin III
Antifungal (MEC, μg/disk; A. niger)	44.3	9.0	58.2	10.0	32.9	154.0	256.6	>100	132.0	6.3	1.4
Hemolysis (EC_{50}, μM; human erythrocytes)	1.2	0.25	0.21	3.0	0.18	6.5	28.9	3.0	2.0	2.0	2.0

Figure 3: *Biological Activity of Amphidinols, AmB and Fililin III.*

Marko's Approach:

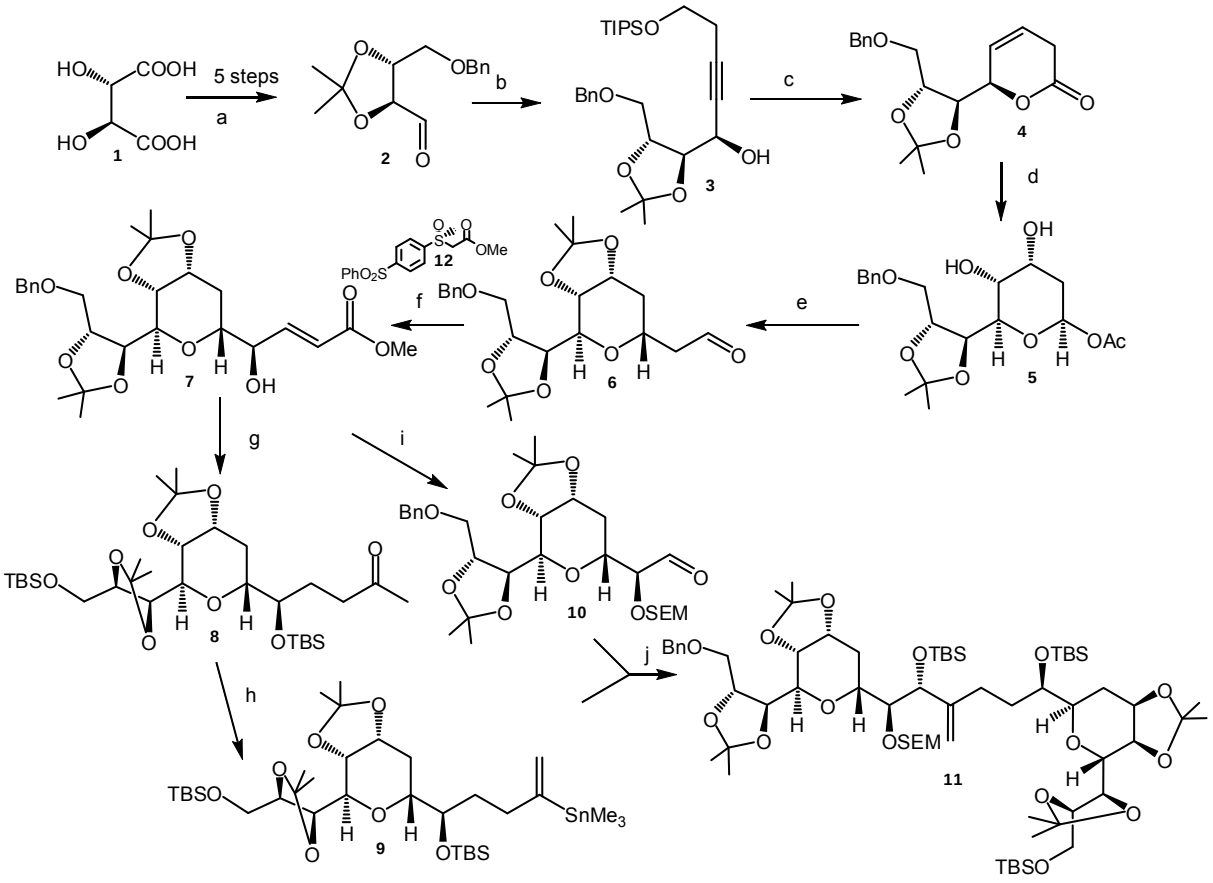
Marko[37] *et al*. showed the synthesis of the 2,6-*anti*-configured tetrahydropyrans, possessing all the functions enantiomerically related to the pyran systems of amphidinol-3, via an *anti*-allylation and an intramolecular Sakurai cyclization[38] as key steps shown in scheme 5. The synthesis of the similar core of amphidinol-3 commenced from enantiomerically pure *syn–anti* configured triol **3** could be synthesized efficiently by the $SnCl_4$-mediated allylation of chiral α-benzyloxyaldehydes **2** with the uniquely functionalised allylstannane **1**. The unprotected compound **3** was submitted to this condensation reaction, in the presence of an orthoester and zinc dichloride etherate, allylsilane **3** generated the diastereoisomeric cyclic acetals **5** and **6** (*syn*:*anti* = 3:1). The exocyclic double bond of acetals **5** and **6** was cleaved by ozonolysis, the ketone derived was reduced with DIBAL-H to afford axial alcohol **7** and **8**. The hydroxy function of **7** and **8** was next converted into an acetate group and the resulting acetals **9** and **10** were submitted to the allylation protocol, by treatment of a mixture of **9** and **10** with allyltrimethylsilane, in the presence of TMSOTf, gave exclusively **11**. This final product possesses all the functions and the correct stereochemical relationship present in tetrahydropyran rings of the Amphidinol-3.

Scheme 5:



Rychnovsky's approach:

Rychnovsky[39] synthesis commenced from aldehyde **2**, a known chiral synthon conveniently obtained by standard refunctionalization of D-(-)-tartaric acid (Scheme 2). Aldehyde **2** was coupled with a titanium acetylide leading to alcohol **3** with high diastereoselectivity (95:5 dr). Partial reduction of the triple bond under P-2 nickel reduction conditions and alcohol desilylation afforded diol, whose primary- alcohol was selectively oxidized with TEMPO, followed by subsequent lactol oxidation efficiently provided lactone **4**. Reductive acetylation of lactone **4** diastereoselectively produced acetoxy ether, which was then subjected to dihydroxylation conditions to provided **5**. TMSOTf-mediated allylation of the oxocarbenium ion derived from acetoxy ether 5 proceeded in excellent yield and furnished the desired 2,6-*trans*-THP ring as a 10:1 mixture of diastereomers. Protection of the diol as the acetonide and subsequent ozonolysis provided aldehyde **6**. [2,3] sulfoxide/sulfenate rearrangement furnished allylic alcohol **7**. Both halves of the core section were initiated from common intermediate **7**. The alcohol **7** was first protected as the TBS ether.



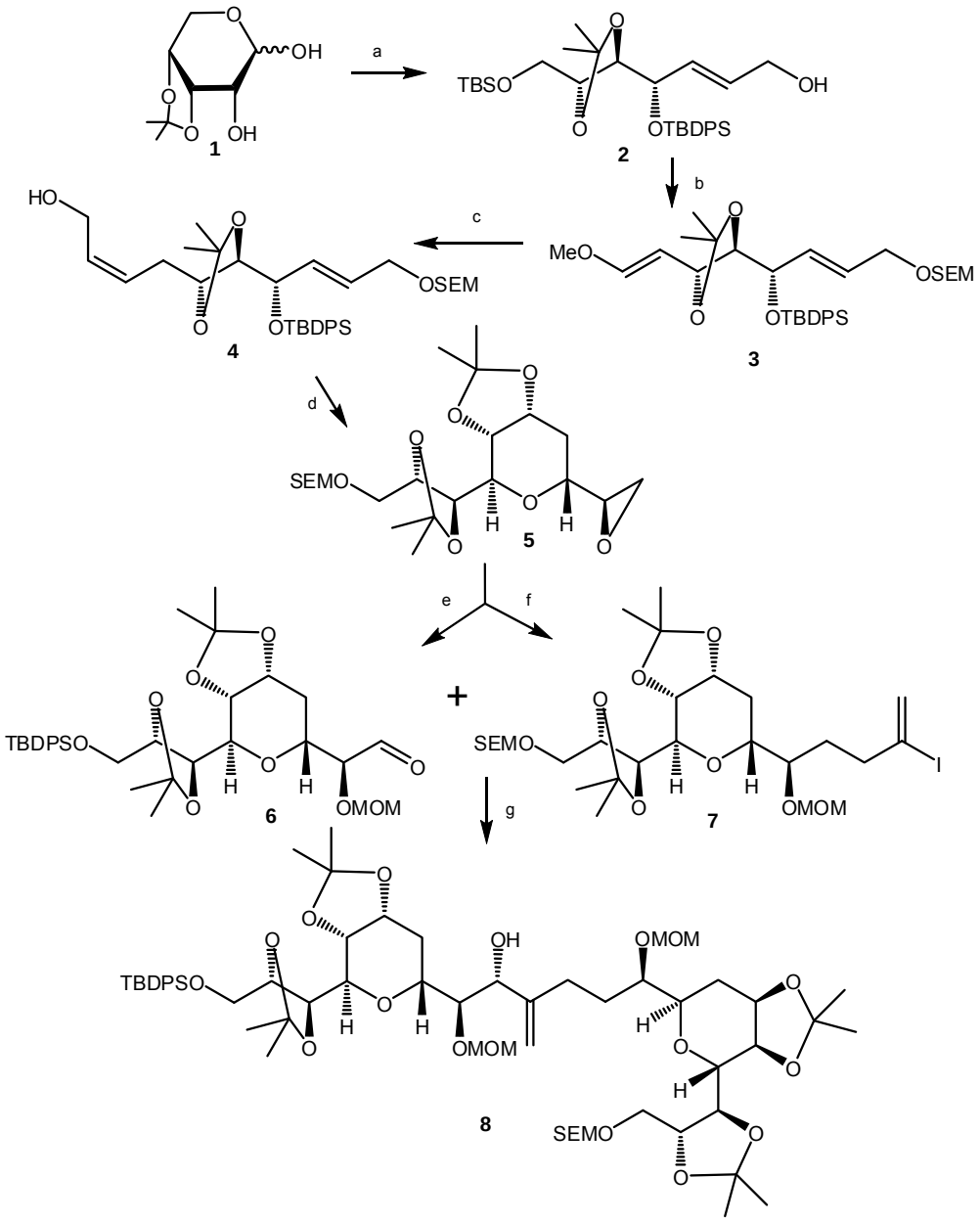
Reagents and conditions: a) ref 12; b) i) alkyne, n-BuLi, THF -78 °C ii) $TiCl_4/Ti(O^iPr)_4$, then **2**; c) i. H_2. $Ni(OAc)_2$ $4H_2O$. $NaBH_4$, $NH_2(CH_2)_2NH_2$, EtOH, rt; ii. TBAF, THF, rt; iii. TEMPO, TBAI, NCS, DCM/H_2O rt, d) i. DIBAL, DCM, -78 °C; ii. Py, DMAP, Ac_2O, DCM, -78 °C to rt iii. OsO_4, NMO; e) i. allyltrimethylsilane, DTBMP, TMSOTf, CH_2Cl_2, -78 °C; ii. K_2CO_3, MeOH; iii. 2,2-DMP, TsOH, C_6H_6; iv. O_3, sudan III, CH_2Cl_2; v. PPh_3, -78°C, f) 12, piperidine, CH_3CN, -58 °C; g) i. TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, rt; ii. H_2 (90 psi), Pd/C, THF; iii. TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, rt; iv. Me(OMe)NH·HCl, nBuLi, THF, -78→ 0 °C; v. MeMgBr, THF, 0 °C; h) i. LDA, THF, -78 °C; ii. N-(5-chloro-2-pyridyl)triflimide, -70 °C; iii. $Me_3SnSnMe_3$, LiCl, $[Pd(PPh_3)_4]$, THF, 80 °C in a sealed tube; i) i. SEMCl, $^iPr_2NEt$, CH_2Cl_2, 40 °C; ii. OsO_4, NMO, acetone/H_2O (8:1); iii. $NaIO_4$, THF/H_2O; j) i. 9, nBuLi, THF, -78 °C, then 10; ii. Dess–Martin periodinane, pyridine, CH_2Cl_2 ; iii. $Zn(BH_4)_2$, Et_2O, -35 °C; iv. TBSOTf, 2,6-lutidine, CH_2Cl_2.

A strategic protecting-group exchange was affected by hydrogenolysis of the benzyl group, which was accompanied by reduction of the enoate alkene, followed by silylation of the primary alcohol. Methyl ketone **8** was obtained from ester via the intermediate Weinreb amide. Kinetic deprotonation with LDA followed by trapping with Comins reagent

furnished the enol triflate, which was then transformed into vinyl stannane **9** by a Stille cross-coupling with hexamethylditin. Intermediate **7** was next converted into the requisite aldehyde **10** in which the free alcohol was protected as SEM ether, Oxidative cleavage of the alkene by using the Johnson–Lemieux protocol afforded aldehyde coupling partner **10**. Union of the two THP fragments **9** and **10** was accomplished by treatment of stannane **9** with nBuLi to afford the nucleophilic vinyl lithium species and subsequent addition to aldehyde **10**. This reaction furnished the C31–C52 bisTHP core of AM3 as a 1:1 mixture of diastereomers. Oxidation of the allylic alcohol mixture with Dess–Martin periodinane followed by chelation-controlled reduction of the resultant ketone with $Zn(BH_4)_2$ afforded the desired *R*- alcohol as the sole product. Protection of the alcohol as the TBS ether afforded the complete, fully protected core fragment **11**.

Paquette's approach:

Paquette[40] synthesis started with ready available 3,4-*O*-isopropylidene-β-D-ribopyranose **1**. Homologation of **1** by means of Wittig chain extension, followed by protection of the resulting primary hydroxyl as its TBS ether, and masking the secondary hydroxyl group with the more bulky TBDPS group. Unsaturated ester was reduced to to allylic alcohol by DIBAL-H, fallowed by borohydrate reduction provided **2**. The free hydroxyl group protected as its SEM ether, followed by selective deprotection of the primary silly ether by means of *N*-bromosuccinimide in aqueous DMSO. Alcohol subjected to periodinane oxidation, and the aldehyde was treated with methoxymethylenetriphenylphosphorane gave **3**. Mercuration–reductive demercuration of **3** under basic conditions to provide the homologated aldehyde, which is treated with the Still–Gennari trifluoroethylphosphono ester followed by subsequent reduction with DIBAL-H provided **4**. Sharpless asymmetric epoxidation of 4 followed by selective removal of the TBDPS group provided THF ring. Monotosylation of the mixture in the presence of catalytic amounts of dibutyltin oxide, followed by oxerane formation, which was then subjected to dihydroxylation with AD-mix-β followed by acetonide formation in the presence of 2,2-dimethoxypropane provided **5**. Both halves of the core section were initiated from common intermediate **5**.

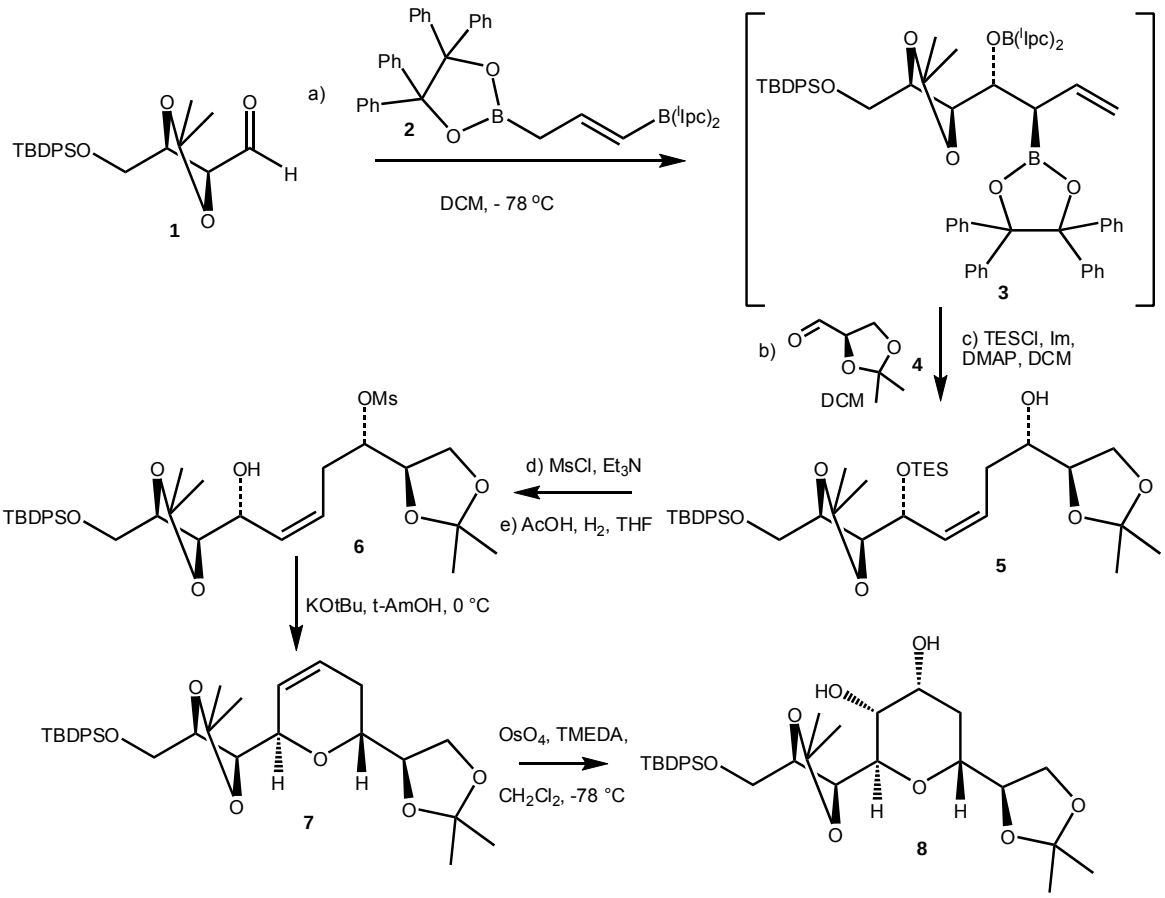


Reagents and conditions: a) i.$Ph_3P{=}CHCO_2{}^tBu$, DMF/dioxane (1:1), 75 °C; ii. TBSCl, Et_3N, DMAP; iii. TBDPSCl, imidazole, DMAP, DMF; iv. DIBAL-H, CH_2Cl_2; v. $NaBH_4$, THF/MeOH; b) i. SEMCl, $^iPr_2Net$, Bu_4NI; ii. NBS, DMSO, H_2O, r.t.; iii. DMP, py, CH_2Cl_2; iv. $Ph_3P{=}CHOMe$; c) i. $Hg(OAc)_2$; $NaBH_4$, THF/H_2O, 0 °C; ii. $(CF_3CH_2O)_2P(O)CH_2CO_2Me$, 18-Cr-6, KHMDS; iii. DIBAL-H, CH_2Cl_2; d) i. TBAF, THF; ii. TsCl, Et_3N, Bu_2SnO; iii. K_2CO_3, MeOH; iv. AD-mix-β; v. 2,2-dimethoxypropane, PPTS; e) i. $Me_3S^+I^-$, n-BuLi; ii. MOMCl, DIPEA, DCM; iii. TBAF, 55 °C; iv. TBDPSCl, imidazole, DMAP, DCM; v. OsO_4, $NaIO_4$, THF/buffer; f) i. propargyl megnesiumbromide, $HgCl_2$; ii. MOMCl, DIPEA, $CHCl_3$; iii. $Bu_2SnSi(CH_3)_2Ph$, $Pd(PPh_3)_4$; iv. TBAF, DMSO, 80 °C; v. I_2, DCM; g) i. $CrCl_2$, $NiCl_2$, DMF, rt; ii. DMP, Py, DCM; iii. *R*-Me-CBS, BH_3.DMS, DCM.

The oxirane ring in **5** was cleaved efficiently using trimethylsulfonium iodide (TMSI) and *n*-butyl lithium giving rise smoothly to the terminal allylic alcohol which was treated with MOMCl and replacement of the primary SEM group by TBDPS. Carboxaldehyde functionality in **6** was subsequently liberated by application of the Johnson-Lemieux oxidation. Reaction of **5** with the Grignard reagent derived from propargyl bromide and ensuing MOM protection of the resulting secondary carbinol. The protected alkyne was then treated with neat dimethylphenylsilyl tributylstannane in the presence of $Pd(Ph_3)_4$ and the exposure to TBAF in DMSO provided tin derivative, which was subjected to tin-iodide exchange to deliver **7**. The union of **7** with aldehyde **6** by Nozaki-Hiyama-Kishi reaction followed by oxidation to the conjugated ketone was accommodated by the Dess-Martin periodinane (DMP) reagent, followed by enantioselective *R*-Me-CBS reduction, as driven by borane-dimethyl sulfide to provided **8**.

Roush's approach:

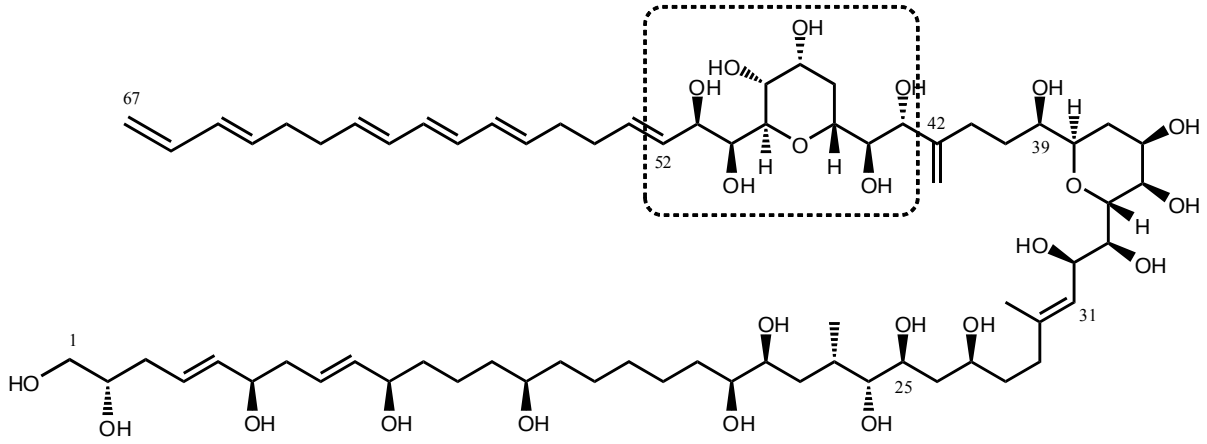
Roush[41] synthesis commenced from known aldehyde **1**. In situ generated *γ*-boryl substituted allylborane **2** was treated with aldehyde **1** at -78 °C. After the first allylboration was allowed to proceed to completion at -78 °C, D-glyceraldehyde acetonide **4** was added and the reaction mixture was allowed to warm to ambient temperature overnight. This sequence provided the *syn*-1,5-diol **5** after silylation of the allylic alcohol as TES ether. The homoallylic hydroxyl group was then functionalized as a mesylate and the allylic TES group removed under acidic conditions to give cyclization precursor **6**. Hydroxy mesylate **6** was cyclized to dihydropyran **7** by treating with KO-*t*-Bu. Dihydroxylation of **7** with OsO_4 and TMEDA in CH_2Cl_2 at -78 °C provided tetrahydropyran **8**.


a)
DCM, - 78 °C
b)
DCM
c) TESCl, Im,
DMAP, DCM
d) MsCl, Et3N
e) AcOH, H2, THF
KOtBu, t-AmOH, 0 °C
OsO4, TMEDA,
CH2Cl2, -78 °C
TBDPSO
1
2
3
4
5
6
7
8

Present Work

The amphidinols belongs to the class of natural products isolated from the marine dinoflagelates[4-9] *Amphidinium* sp. that display antifungal, hemolytic, cytoxic, and ichthyotoxic activities. Dinoflagellates, a type of primitive unimolecular alage, are a rich source of structurally and biologically intriguing natural products; eg., okadaic acid, brevetoxins, ciguatoxins and maitotoxin. Among these polyether-cyclic compounds, amphidinols are unique dinoflagellate metaholites since they are primerly made up of linear polyhydroxy structures. Of the 13-polyketide metabolites in this family, amphidinol-3 (Fig 1) is one of the most biologically active, with antifungal activity against Aspergillus niger and hemolytic activity on human erythrocytes. Amphidinol-3 effects cholesterol dependent membrane disruption, leading to speculation that its mode of action may, in part, be due to disruption of cell membranes.

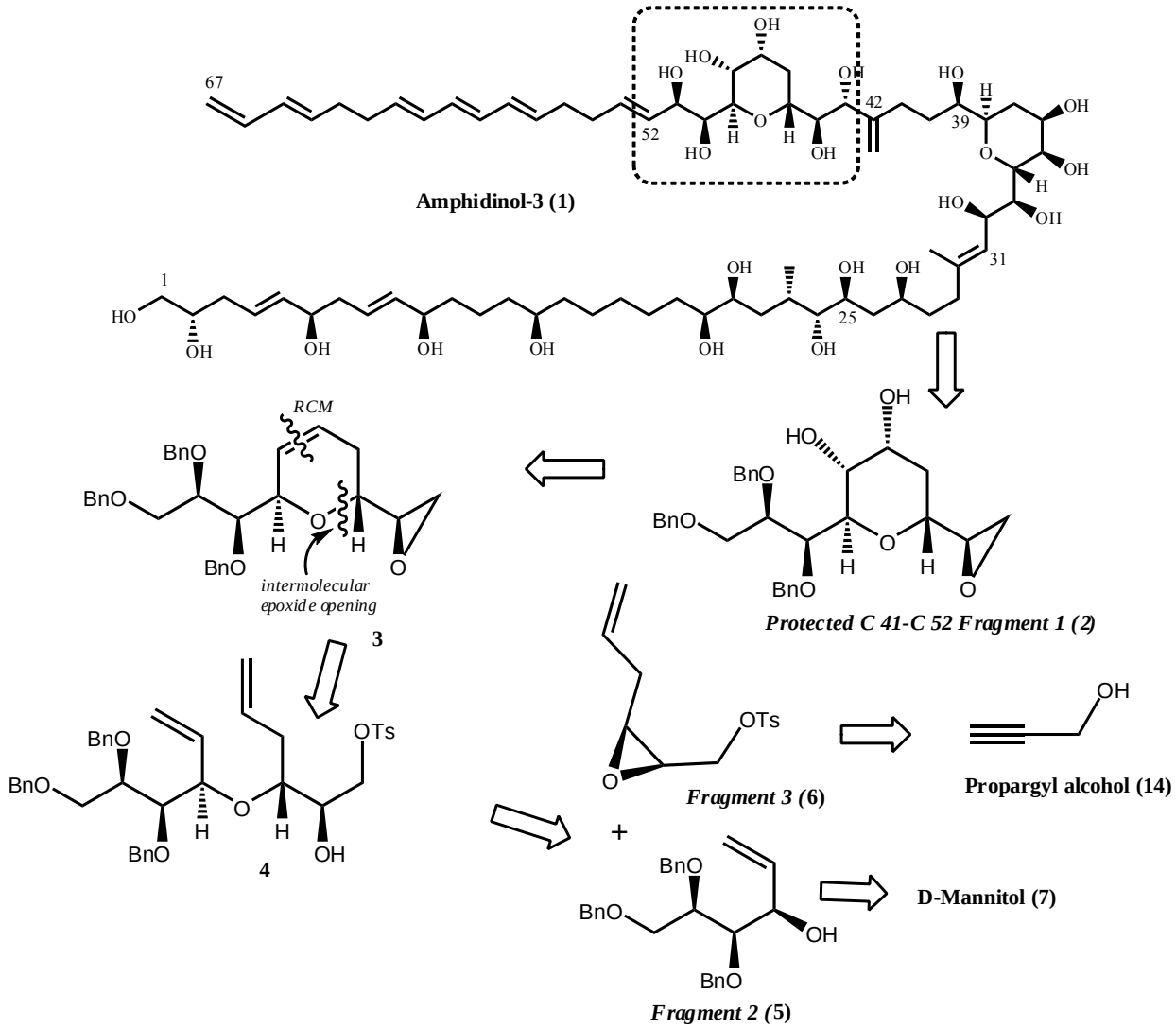
Figure 1: Amphidinol 3 (**1**).



Amphidinol-3 (**1**) contains a C (52) – C (67) skipped polyene chain, a series of 1,5-diols within the C (2) – C (15) region, two highly substituted tetrahydropyran units, and a total of 25 stereocenters on a contiguous 67-carbon backbone. The long chain polyhydroxy compounds may be one of the most challenging targets. The novel architecture and the bioactive properties of the amphidinol 3 led us to investigate its synthesis. On careful observation the two highly substituted tetrahydropyran rings (C (32)-C (39) and C (44)-C

(51)) were identical and they can be made from the same common intermediate. Herein we describe the synthesis of protected C (43) – C (52) fragment 1 (**2**) of amphidinol 3 (scheme 1).

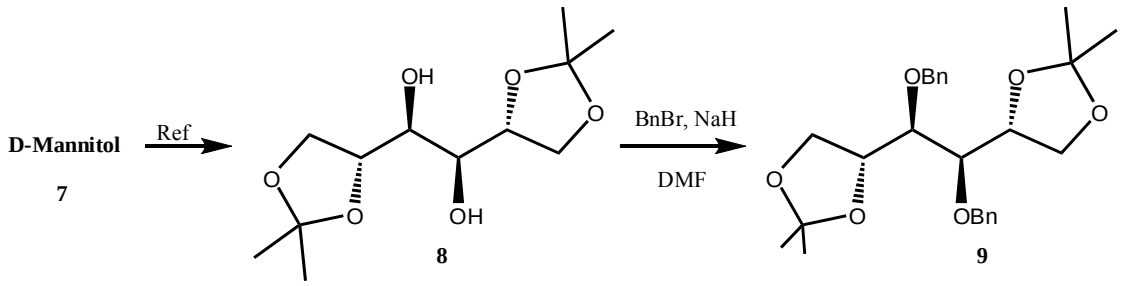
Scheme 1: retrosynthetic analysis of amphidinol 3.



The starting material was identified as D-mannitol and propargyl alcohol from which the key intermediates **5** and **6** were made. The synthesis of building block **5** was initiated by known protocol, i.e. acetonation D-mannitol. Thus D-mannitol **7** was converted to mannitol diacetonide (**8**) by known procedure.[42] Compound **8** was benzylated[43] using BnBr, NaH in

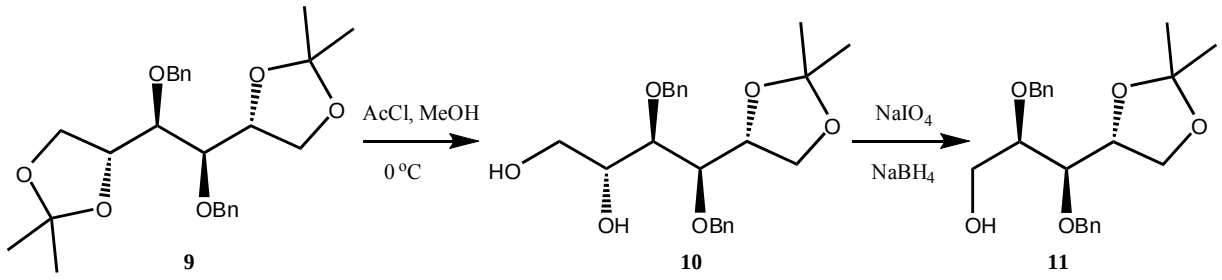
DMF gave compound **9** (scheme 2). In the ^{1}H NMR spectra of compound **9**, benzylic methylene protons resonate at δ 4.7 as singlet for 4H as well as the aromatic region at δ 7.28-7.34 as multiplet for 10H. Rest of the spectra was well agreement with the reported data suggesting the assigned structure for the compound **9**.

Scheme 2:



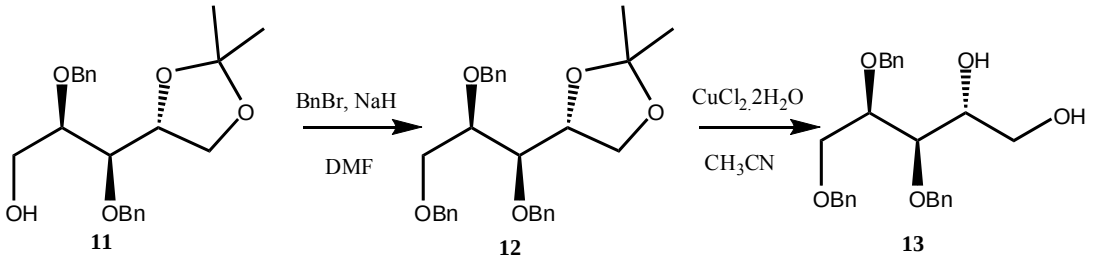
Selective removal[43] of one of the isopropylidene group was achieved by mild acidic hydrolysis under standered conditions. Thus compound **9** was treated with AcCl in MeOH at 0 ^{o}C for 5 min ended up with mono acetonide compound **10**. The compound was characterized by ^{1}H NMR spectra, in which one of the characteristic signals due to isopropylidene moiety was missing. All other protons were resonates at their respected values, confirming the assigned structure for compound **10**. Oxidative cleavage of diol **10** with sodium metaperiodate followed by $NaBH_4$ reduction in methanol gave the compound **11** (scheme 3). In the ^{1}H NMR spectra of compound **11** methylene protons attached to primary hydroxyl group resonates at δ 4.26 as multiplet. ^{13}C NMR spectra and elemental analysis further supported the compound **11**.

Scheme 3:



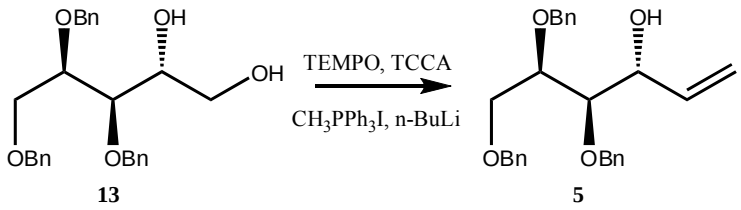
The primary alcohol was protected as benzyl ether by standard (BnBr, NaH in DMF) conditions yielded tribenzyl compound **12**. In the ^{1}H NMR spectra of compound **12,** the new benzylic methylene resonates at δ 4.46 as singlet as well as in DEPT the methylene resonates at δ 73.9 ppm. Elemental analysis further confirmed the structure **12**. The isopropylidene group in compound **12** was removed by using $CuCl_2.2H_2O$ in acetonitrile[44] at 0 ^oC gave diol **13** (scheme 4). The characteristic isopropylidene group signals were absent in the ^{1}H NMR spectra, all other protons were resonates at their respected values. Compound **13** further supported by ^{13} C NMR spectra and elemental analysis.

Scheme 4:



The key building block **5** was achieved by selective oxidation primary alcohol (compound **13**) using TEMPO, Trichloroisocyanuric acid[45] (TCCA) in DCM to aldehyde followed by one carbon wittig homologation with $PPh_3{=}CH_2$ in THF at 0 ^oC (scheme 5). In the ^{1}H NMR spectrum the characteristic olefinic protons resonate at δ 5.81 and the methylene protons appeared at δ 5.15 as multiplets. Rest of the spectrum was in complete conformity with the assigned structure **5**.

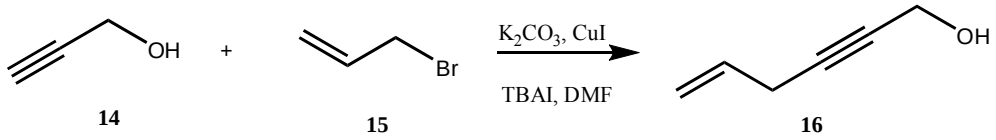
Scheme 5:



After having the compound **5** in hand our next task was synthesis of building block **6**. Our synthetic endeavor begins with propargyl alcohol **14** by known protocal.[46] Thus

propargyl alcohol (**14**) was treated with allyl bromide in the presence of K2CO3, CuI and TBAI in DMF to give compound **16** in good yield (scheme 6). In the ^{1}H NMR spectrum of compound **16** olefinic signal ware resonates at δ 5.09 and 5.73 ppm as multliplet, allylic methylene protons were seen at δ 2.98 ppm as multiplet. In the ^{13}C NMR spectrum olefinic carbons resonates at δ 115.9 and 132.1 ppm. Rest of the spectrum was in full agreement with assigned structure **16**.

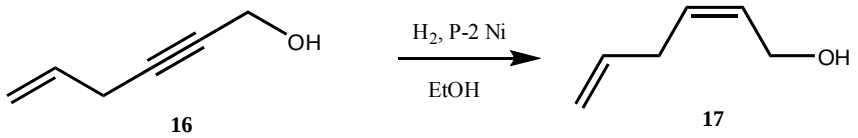
Scheme 6:



14 **15** **16**

Cis hydrogenation of the alkyne was carried out using P-2 Nickel.[47] Thus compound **16** was treated with P-2 Ni (prepared from $Ni(OAc)_4$ and $NaBH_4$ in ethanol) poisoned with ethylenediamine under H_2 atmosphere gave compound **17** (scheme 7). In the 1H NMR spectrum of compound **17** characteristic signals due to internal olefine were seen at δ 5.48-5.60, 5.63-5.68 and 5.75-5.83 ppm as multiplets.Where as terminal olefinic protons were at δ 4.98 and 5.03 ppm as dq. ^{13}C NMR and elemental analysis further confirmed the assigned structure **17**.

Scheme 7:



16 **17**

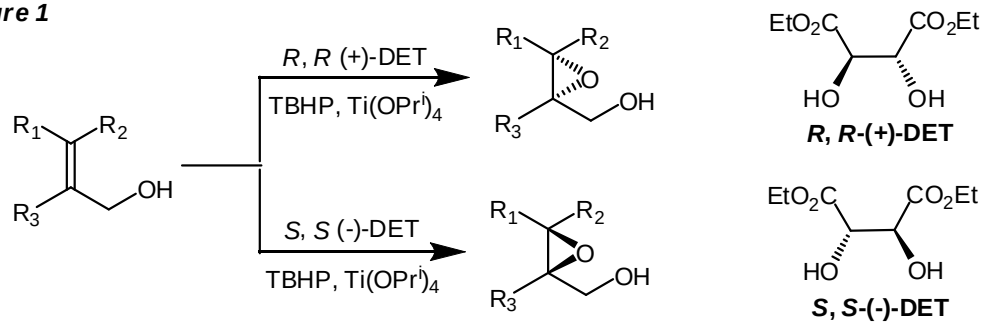
After having the compound **17** in hand our next task was sharples epoxidation of chiral secondary allylic alcohol.

A short account of Sharpless asymmetric epoxidation (SAE) .

Sharpless asymmetric epoxidation (SAE) is one of the most popular reactions used for the enantioselective epoxidation of achiral allylic alcohols in organic synthesis. When a

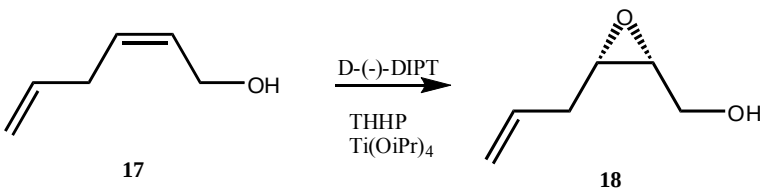
prochiral Z- or E-allylic alcohol is treated with dialkyl tartarate (generally Et or ^{i}Pr), titanium tetraisopropoxide and ter-butylhydroperoxide, produces the corresponding chiral epoxyalcohol with high ee. Easy availability of reagents involved, and high enantiomeric (or diastereomeric) excess obtained in the reaction made the Sharpless asymmetric epoxidation to find wide spread application in the introduction of chirality in the complex target molecules. The easy and accurate prediction of stereochemical outcome irrespective of substitution on the allylic alcohol further asserted the reaction application (Figure 2).

Figure 1



Our next task was epoxidation of allylic alcohol. Sharpless asymmetric epoxidation[46b,48] of the *Z*-allylic alcohol **17** using diisopropyl-D-tartarate, titanium tetraisopropoxide and *t*-butylhydroperoxide in the presence of 4Å molecular sieves powder in CH_2Cl_2 at –20 °C provided the corresponding epoxy alcohol **18** with excellent diastereoselectivity based on spectroscopic analysis (scheme 8). The absolute configuration of **18** was established based on the empirical rules published by Sharpless.[27] Formation of a single diastereomer **18** was adjudged by the analysis of its ^{1}H and ^{13}C spectral data. The ^{1}H NMR spectrum of **18** displayed the epoxy proton at δ 3.13 (ddd, *J* = 4.4, 6.5, 10.8 Hz, 1H) and 3.20 (ddd, *J* = 4.1, 6.8, 8.5 Hz, 1H). All the other protons resonated at their expected chemical shifts. Further ^{13}C NMR spectrum of **18** showed signals due to the epoxy carbons at δ 55.6 and 56.6 ppm.

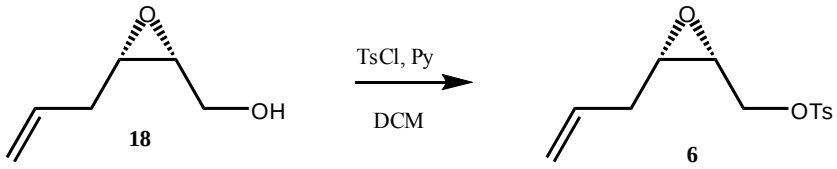
Scheme 8:



17 **18**

The key intermediate **6** was achieved by protecting free hydroxyl group of **18** as Tosyl with TsCl, Pyridine in DCM (scheme 9). The ^{1}H NMR spectrum of **6** displayed the typical tosyl methyl protons as singlets at δ 2.44 ppm and aromatic protons at δ 7.36 (d, *J* = 8.23 Hz, 2H), 7.80 (d, *J* = 8.23 Hz, 2H). All the other protons resonated at their expected chemical shifts.

Scheme 9:



After having both the key intermediates in hand our next task was nucleophilic epoxide opening under mild acidic conditions as depicted in retrosynthesis.[49] Thus, epoxide **6** was treatment with vinyl alcohol **5** and $BF_3.Et_2O$ in DCM. However the desired coupling reaction did not take place under these conditions and the olefin **5** was recovered (scheme 10). A careful survey of other conditions for the crucial coupling was conducted (Table 1). Unfortunately, it was found that all the attempts to couple olefin **5** and epoxy tosylate **6** by intermolecular nuclephilic epoxide opening procedures under various reaction conditions turned out to be failures.

Scheme 10:

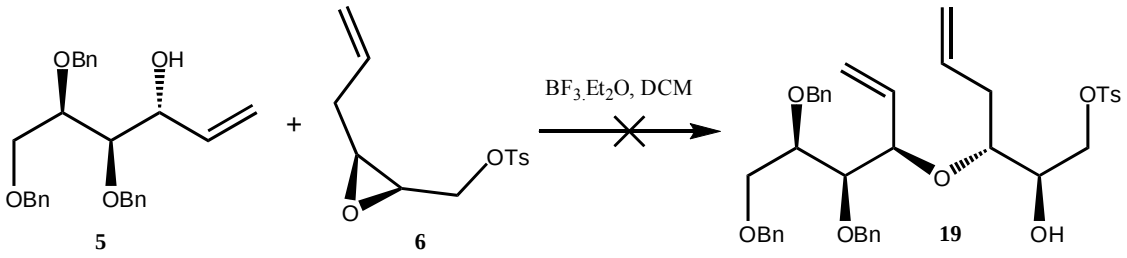
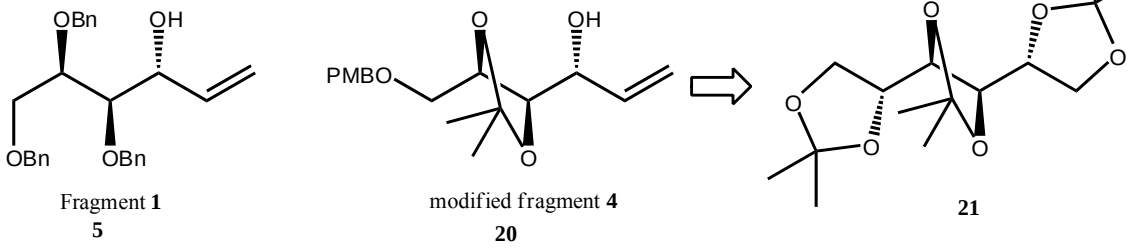


Table 1: *Conditions applied and results obtained in epoxide opening*

Entry	Reaction conditions	Results obtained
1	$BF_3.Et_2O$, DCM, -20 ^oC , 12h	No reaction, olefin **5** recovered.
2	$BF_3.Et_2O$, DCM, -20 ^oC to rt, 12h	No reaction, olefin **5** recovered.
3	$BF_3.Et_2O$ (1 eq), DCM, -20 ^oC to rt, 12h	Complex mixture.
4	$Cu(OTf)_2$, DCM, -20 ^oC, 12h	Both the starting materials recovered.
5	$Cu(OTf)_2$, DCM, -20 ^{o}Cto rt, 48h	No reaction.

Having met with failures to open the epoxide using acid catalysts, we redesigned our retrosynthetic strategy and it was decided to attempt the epoxide opening with some what less bulkier group instead of benzyl ethers (Figure 2). It was assumed that the bulkier benzyl groups could not allow the incoming electrophile which met with failure. Thus the benzyl ethers in olefin moiety **5** were replaced by isopropylidene. The modified coupling partner **20** was synthesized as follows.

Figure 2: alternative strategy.



Fragment **1**
5

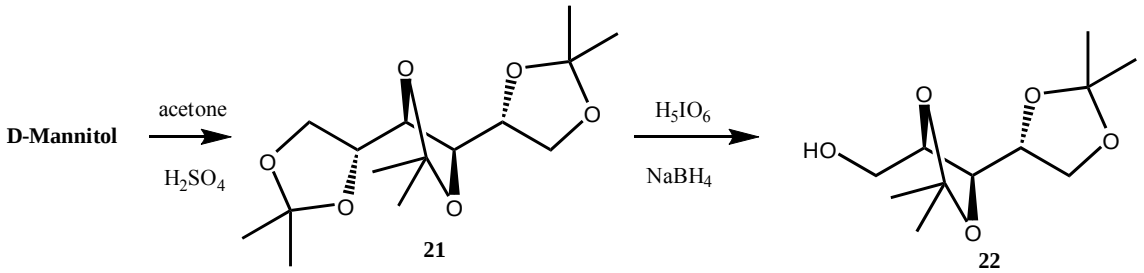
modified fragment **4**
20

21

The key fragment commenced from D-Mannitol triacetonide by reported protocol.[50] Thus D-Mannitol was treated with acetone, H_2SO_4 to give the triacetonide moiety **21** (scheme 11). The spectral and analytical data were in full agreement with the reported values.

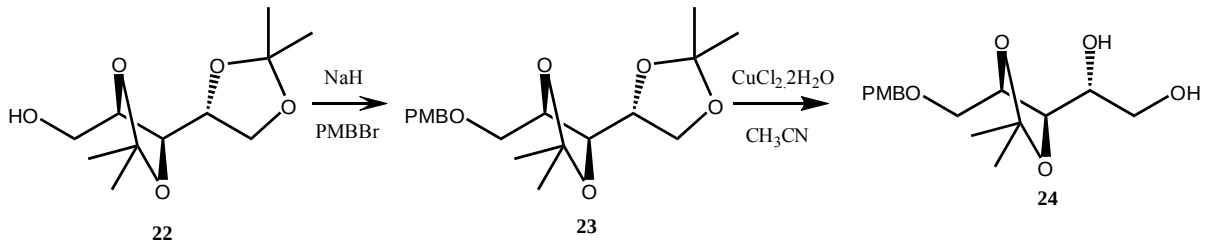
One of the teriminal acetonide group of compound **21** was selectively cleaved by periodic acid in diethyl ether to give aldehyde, which was subsequently reduced with $NaBH_4$ to give the alcohol **22**. In the ^1H NMR spectra the characteristic signals due to one acetonide were missing. Rest of the spectra was in full agreement with the reported values.

Scheme 11:



The primary hydroxyl group was protected as PMB ether by treating compound **22** with NaH and PMBBr in DMF yielded compound **23** (scheme 12). In the ^1H NMR spectra signals at δ 3.79 and δ 4.52 ppm as singlet were observed. Aromatic signals were resonates at δ 6.86 (d, J = 7.67 Hz, 2H) and 7.26 (d, J = 7.67 Hz, 2H). Rest of the spectra was in full agreement with the assigned structure. Compound **23** was treated with $CuCl_2.2H_2O$ in CH_3CN gave diol **24**. In the ^1H and ^{13}C NMR spectra the characteristic signals due to terminal acetonide were missing. Rest of the spectra was in full agreement with the assigned structure.

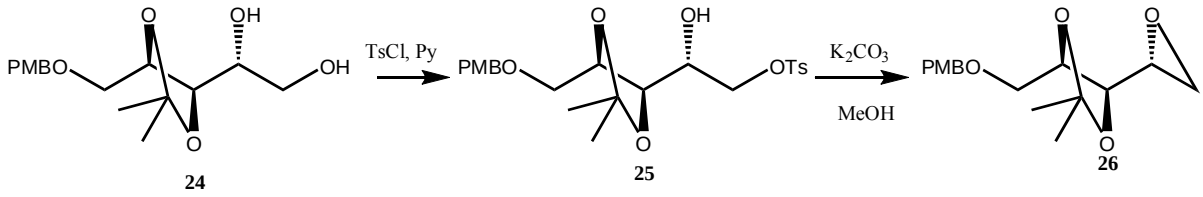
Scheme 12:



The primary hydroxyl group of diol 24 was protected as tosyl by TsCl in pyridine to give mono tosylate **25** (scheme 13). The ^1H NMR spectrum of **6** displayed the typical Tosyl methyl protons as singlets at δ 2.44 as singlet. All the other protons resonated at their expected chemical shifts. The mono tosyl compound **24** was converted to epoxide **26** by treating it with K_2CO_3 in methanol. The ^1H NMR spectrum of **26** displayed the epoxy proton at δ 2.69 (dd, 1H, J = 2.6, 5.1 Hz) and 2.82 (dd, 1H, J = 4.0, 5.0 Hz). All the other protons

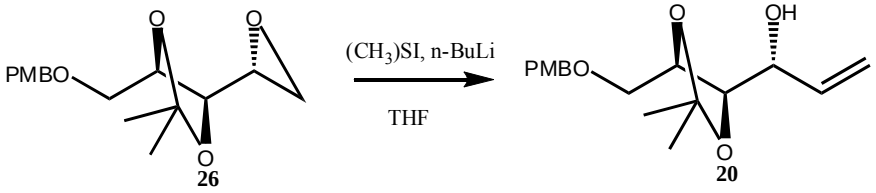
resonated at their expected chemical shifts. Further ^{13}C NMR spectrum of **26** showed signals due to the epoxy carbons at δ 51.7 and 55.2 ppm.

Scheme 13:



Our next task was nucleophilic opening of epoxide to get olefin.[51] Thus compound **26** treated with trimethylsulphonium iodide and n-butyl lithium in THF give olefin fragment **20** (scheme 14). In the ^1H NMR spectrum the characteristic olefinic protons resonates at δ 5.17, ppm as multplet. The methylene protons appeared at δ 5.78 as multplet and the rest of the spectrum was in complete conformity with the assigned structure **20**.

Scheme 14:



With the required fragments compounds **20** and **6** in hand, the crucial epoxide opening was investigated. For the selective opening of epoxide, various conditions and reagents were applied and the results are summarized in table 2. But unfortunately all the conditions failed to give the desired product **27** (Scheme 15). In most of the cases compound **20** was recovered.

Scheme 15:

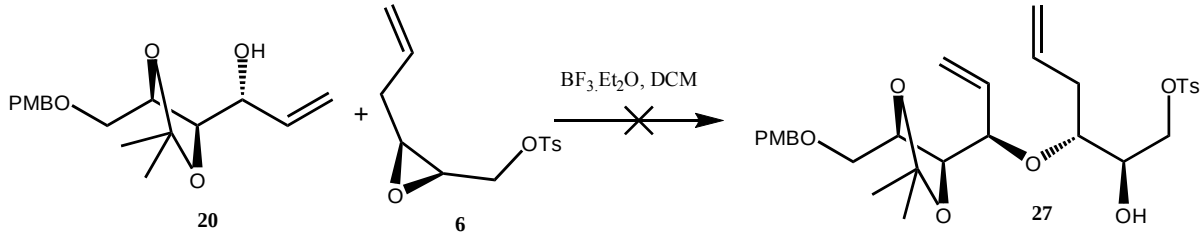
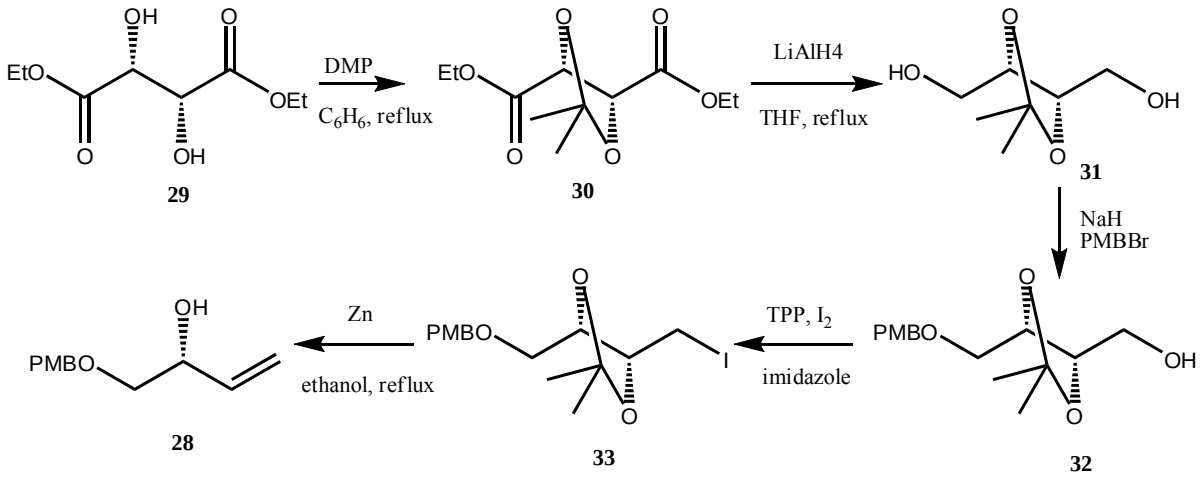


Table 2: *Conditions applied and results obtained in epoxide opening*

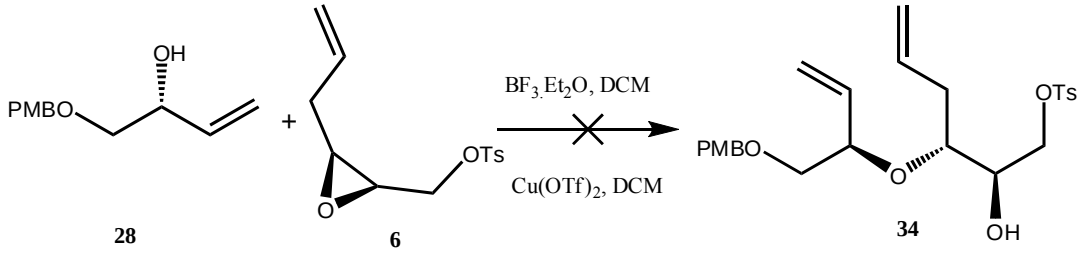
Entry	Reaction conditions	Results obtained
1	$BF_3.Et_2O$ (cat), DCM, -20 ^oC , 12h	No reaction, olefin **20** recovered.
2	$BF_3.Et_2O$ (cat), DCM, -20 ^oC to rt, 12h	No reaction, olefin **20** recovered.
3	$BF_3.Et_2O$ (1 eq), DCM, -20 ^oC to rt, 12h	Complex mixture.
4	$Cu(OTf)_2$, DCM, -20 ^oC, 12h	Both the starting materials recovered.
5	$Cu(OTf)_2$, DCM, -20 ^{o}Cto rt, 48h	No reaction.

Having met with failures to open the epoxide using acid catalysts, we decided to open the epoxide with simple homo allylic alcohol. For this olefin **28** was selected as coupling fragment 5 which can easily prepared from diethyl tartarate by reported protocol.[52] Thus, L-diethyl tartrate was converted to acetonide moiety, which was subsequently reduceded with $LiAlH_4$ furnished the diol **31** (scheme 16). Mono protection of the diol **31** was acheieved by using NaH and PMBBr in THF. The free hydroxyl group in compound **32** was converted to iodo **33** by TPP, I_2 and imidazole in refluxing toluene.[53] In the ^1H NMR spectrum of compound **33** the characteristic acetonide protons resonates at δ 1.41, 1.46 ppm. The methylene protons adjacent to iodo appeared at δ 3.26 and 3.34 as doublet of doublet and the rest of the spectrum was in complete conformity with the assigned structure **33**. The coupling fragment (**28**) was achieved by eliminating the iodo using Zn in refluxing ethanol. In the ^1H NMR spectrum iodo methylene protons were absent. The characteristic olefinic protons resonate at δ 5.15, 5.74 ppm as multiplets, and the rest of the spectrum was in complete conformity with the assigned structure **28**.

Scheme 16:



After having both the coupling partners in hand our next task was crucial epoxide opening. Again both the fragments were subjected to various reaction conditions as mentioned above to get the desired product **34** (scheme 17) were met with failure.

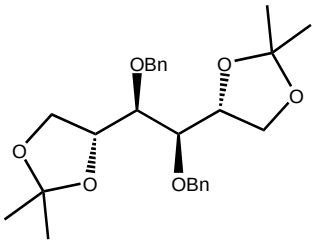


In fact, the amphidinol 3 natural products present several challenges to the synthetic organic chemist some of them were solved. Finally, in contrast, that different solutions were devised for some of the problems demonstrates that natural products often stimulate the development of and provide a proving ground for new strategies and methods of organic synthesis.

In summary, we have successfully accomplished the syntheses of crucial coupling segments with suitable protections for the projected fragment synthesis of Amphidinol 3. Our strategy presents a very different approach from the previous syntheses of tetrahydrofuran moiety. Studies toward the C (43)-C (52) fragment of Amphidinol 3 are under progress in our laboratory.

Experimental Section

(1R,2R)-1,2-bis(benzyloxy)-1,2-bis((R)-2,2-dimethyl-1,3-dioxolan-4-yl)ethane (9).

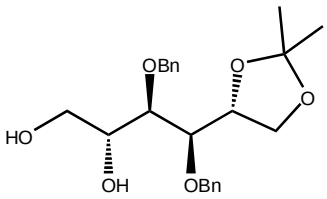


A solution of **8** (10 g, 38.1 mmol) in DMF (40 ml) was added to a suspension of sodium hydride (60 %) (3.51 g, 152.0 mmol) in DMF (20 ml) at 0 ^{o}C. Aafter 30 min benzyl bromide (9.98 ml, 84.0 mmol) and tetrabutylammonium bromide (1 g) were added and the mixture was stirred for 12 h at rt. Reaction mixture was quenched with methanol at 0 ^{o}C, water was added and extracted with EtOAc. Combined organic layers were dried over Na_2SO_4. The syrup was chromatographed on silica using lightpetrolium ether: ethyl acetate (9:1) as an eluent to give compound **9** (15.9 g, 94 %) as syrup.

Mol. Formula	**:**	$C_{26}H_{34}O_6$
$[\alpha]_D^{25}$	**:**	36 (*c* = 1, $CHCl_3$)
IR ($CHCl_3$)	**:**	2952, 2864, 1612, 1485, 1445, 1239, 1221, 1151, 1078, 918 cm^{-1}.
^{1}H NMR (200 MHz, $CHCl_3$)	**:**	δ 1.33 (s, 6 H), 1.41 (s, 6 H), 3.79 (d, *J* = 6.08 Hz, 2 H), 3.85 (dd, *J* = 8.4, 6.5 Hz, 2 H), 4.01 (dd, *J* = 8.4, 6.2 Hz, 2H), 4.24 (q, *J* = 12.4, 6.2 Hz, 2 H), 4.70 (s, 4 H), 7.28-7.34 (m, 10H).
^{13}C NMR (50 MHz, $CHCl_3$)	**:**	δ 25.12 (q), 26.56 (q), 66.65 (t), 74.46 (t), 75.71 (d), 79.81 (d), 108.41 (d), 127.62 (d), 127.90 (d), 128.21 (d), 138.10 (s) ppm.
LC MS	**:**	465.24 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 70.56; H, 7.74%.

Found: C, 70.13; H, 7.8%.

(2R,3R,4R)-3,4-bis(benzyloxy)-4-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)butane-1,2-diol (10).



To a stirred solution of compound **9** (10.0 g, 22.6 mmol) in dry MeOH (50 mL) was added AcCl (8.03 ml, 113.0 mmol) at icebath temperature. The reaction mixture was stirred at icebath temperature for 5 min. After completion of the reaction (monitored by TLC), CH_2Cl_2 was added (50 mL), the reaction mixture was neutralized with 10% $NaHCO_3$ (10 mL) and washed with H_2O (10 mL). Finally, the organic layer was dried (Na_2SO_4) and concentrated. The crude residue, which was purified on silica gel column chromatography by using light petroleum: ethyl acetate (3:2) as an eluent to give **10** (7.6 g, 84 %).

Mol. Formula : $C_{23}H_{30}O_6$

$[\alpha]_D^{25}$: 101.6 (*c* = 1, $CHCl_3$)

IR ($CHCl_3$) : 2956, 2867, 1622, 1475, 1485, 1219, 1251, 1121, 1073, 912 cm^{-1}.

^{1}H NMR (200 MHz, $CHCl_3$) : δ 1.35 (s, 3 H), 1.44 (s, 3 H), 3.55-3.76 (m, 3 H), 3.82 (t, *J* = 4.75, 1 H), 3.96 (dd, *J* = 8.21, 7.26 Hz, 1H), 4.05 (dd, *J* = 8.21, 6.29 Hz, 1H), 4.23 (dd, *J* = 5.0, 0.76 Hz, 1H), 4.26 (dd, *J* = 13.4, 5.0 Hz, 1H), 4.63 (s, 2 H), 4.73 (d, *J* = 1.53, 2H), 7.32-7.36 (m, 10H).

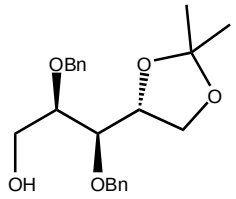
^{13}C NMR (50 MHz, $CHCl_3$) : δ 24.93 (q), 26.29 (q), 61.20 (t), 65.90 (t), 72.61 (t), 74.49 (t), 76.14 (d), 78.96 (d), 79.79 (d), 108.35 (s), 127.61 (d), 127.65 (d), 127.79 (d), 127.92 (d), 128.21

(d), 137.87 (s) ppm

LC MS : 425.48 $[M+Na]^+$

Elemental Analysis : Calcd: C, 68.84; H, 7.51%.
Found: C, 68.74; H, 7.53%.

(2R,3S)-2,3-bis(benzyloxy)-3-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)propan-1-ol (11).



To a solution of **10** (8 g, 19.88 mmol), 5% aq. $NaHCO_3$ (10 mL) in DCM was added $NaIO_4$ (6.38 g, 29.8 mmol) portion wise over period of 20 min at room temperature. After 1 h, solids were filtered through celite, washed with DCM, dried over sodium sulphate and concentrated. The crude aldehyde was used with out purification.

Crude aldehyde was treated with $NaBH_4$ (0.9 g, 23.85 mmol) in MeOH (30 ml) at room temperature for 3 h. Excess borohydride was quenched at 0 °C with dil. HCl and concentrated. The residue was partioned between water-ether, organic layer separated and washed with 1 M NaOH, H_2O, brine, dried over sodium sulphate, and concentrated. The crude was purified on silica gel by using light petroleum: ethyl acetate (4:1) as an eluent to give **11** (5.8 g, 78%).

Mol. Formula : $C_{22}H_{28}O_5$

IR ($CHCl_3$) : 2965, 2862, 1625, 1475, 1465, 1214, 1191, 1123, 1053, 914 cm^{-1}.

^{1}H NMR (200 MHz, $CHCl_3$) : δ 1.35 (s, 3 H), 1.44 (s, 3 H), 2.50-2.17 (m, 1H), 3.60 (dd, *J* = 9.5, 4.8 Hz, 1H), 3.71 (dt, *J* = 9.73, 5.0 Hz 2 H), 3.82 (t, *J* = 4.75, 1 H), 3.97 (dd, *J* = 8.21, 7.26 Hz, 1H),

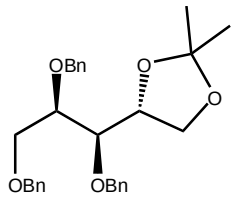
4.06 (dd, *J* = 8.21, 6.29 Hz, 1H), 4.23 (dd, *J* = 5.0, 0.76 Hz, 1H), 4.26 (dd, *J* = 13.4, 5.0 Hz, 1H), 4.63 (s, 2 H), 4.74 (d, *J* = 1.53, 2H), 7.29-7.36 (m, 10H).

^{13}C NMR ($CHCl_3$) : δ 24.98 (q), 26.33 (q), 61.27 (t), 65.96 (t), 72.65 (t), 74.55 (t), 76.20 (d), 79.04 (d), 79.84 (d), 108.42 (d), 127.67 (d), 127.71 (d), 127.84 (d), 127.98 (d), 128.27 (d), 137.91 (s) ppm.

LC MS : 395.45 $[M+Na]^+$

Elemental Analysis : Calcd: C, 70.94; H, 7.58%.
Found: C, 70.8; H, 7.49%.

(R)-2,2-dimethyl-4-((1S,2R)-1,2,3-tris(benzyloxy)propyl)-1,3-dioxolane (12).



A solution of **11** (5 g, 13.42 mmol) in DMF (20 ml) was added to a suspension of sodium hydride (60 %) (0.617 g, 26.8 mmol) in DMF (20 ml) at 0 ^oC. Aafter 30 min benzyl bromide (1.916 ml, 16.11 mmol) and tetrabutylammonium bromide (0.5 g) were added and the mixture was stirred for 12 h at rt. Reaction mixture was quenched with methanol at 0 ^oC, water was added and extracted with EtOAc. Combined organic layers were dried over Na_2SO_4. The syrup was chromatographed on silica using lightpetrolium ether: ethyl acetate (9:1) as an eluent to give compound **12** (5.8 g, 93%) as syrup.

Mol. Formula : $C_{22}H_{28}O_5$

IR ($CHCl_3$) : 2925, 2845, 1678, 1464, 1419, 1225, 1181, 1129, 1056, 925 cm^{-1}.

^{1}H NMR ($CHCl_3$) : δ 1.32 (s, 3 H), 1.41 (s, 3 H), 2.50-2.17 (m, 1H), 3.58 (d, *J* = 1.8 Hz, 1H), 3.61 (d, *J* = 0.76 Hz, 1H), 3.75 (dd, *J* =

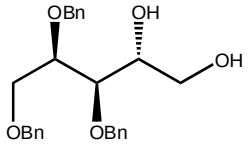
5.7, 3.3 Hz, 1H), 3.86 (dd, *J* = 4.5, 3.3 Hz, 1 H), 3.92 (d, *J* = 3.0 Hz, 1H), 3.95 (d, *J* = 1.8 Hz, 1H), 4.23 (dd, *J* = 5.0, 0.9 Hz, 1H), 4.25 (dd, *J* = 13.4, 4.52 Hz, 1H), 4.46 (s, 2 H), 4.61 (dd, *J* = 11.6, 7.0 Hz, 2H), 4.70 (dd, *J* = 11.43, 8.5 Hz, 2H), 7.25-7.33 (m, 15H).

^{13}C NMR (50 MHz, $CHCl_3$) **:** δ 24.41 (q), 25.78 (q), 65.22 (d), 68.67 (t), 72.43 (t), 72.54 (t), 73.90 (t), 75.78 (d), 77.36 (d), 107.40 (d), 126.88 (d), 127.30 (d), 127.33 (d), 127.49 (d), 127.59 (d), 137.25 (s), 137.51 (s), 137.62 (s) ppm.

LC MS **:** 485.58 $[M+Na]^+$

Elemental Analysis **:** Calcd: C, 75.30; H, 7.41%.
Found: C, 75.22; H, 7.46%.

(2R,3R,4R)-3,4,5-tris(benzyloxy)pentane-1,2-diol (13).



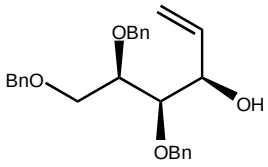
To a solution of the benzyl ether **12** (5 g, 10.81 mmol) in MeCN (40 mL) was added $CuCl_2.2H_2O$ (1.74 g, 12.97 mmol) at 0 °C. After 40 min, the reaction was quenched by addition of saturated $NaHCO_3$ at the same temperature. The reaction mixture was filtered through Celite and washed with EtOAc, the organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, concentrated. Purification of the residue on silica gel by using light petroleum: ethyl acetate (3:2) to give **13** (3.5 g, 77%).

Mol. Formula **:** $C_{26}H_{30}O_5$

IR ($CHCl_3$) **:** 2816, 2745, 1679, 1452, 1405, 1215, 1173, 1103, 1023, 956 cm^{-1}.

^{1}H NMR (200 MHz, $CHCl_3$)	**:**	δ 3.30 (m, 1H), 3.66 (dd, *J* = 7.55, 3.8 Hz, 2H), 3.74-3.84 (m, 3H), 3.92 (dt, *J* = 5.43, 4.15 Hz, 1H), 4.52 (d, *J* = 1.8 Hz, 2H), 4.58 (s, 2 H), 4.60 (d, *J* = 11.8 Hz, 1H), 4.76 (d, *J* = 11.8 Hz, 1H), 7.25-7.33 (m, 15H).
^{13}C NMR (50 MHz, $CHCl_3$)	**:**	δ 63.04 (t), 68.99 (t), 70.97 (d), 72.48 (t), 72.94 (t), 73.12 (t), 75.97 (d), 77.25 (d), 127.19 (d), 127.23 (d), 127.41 (d), 127.43 (d), 127.63 (d), 127.92 (d), 127.96 (d), 137.35 (s) ppm.
LC MS	**:**	445.51 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 73.91; H, 7.16%. Found: C, 73.7; H, 7.12%.

(3R,4R,5R)-4,5,6-tris(benzyloxy)hex-1-en-3-ol (5).



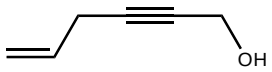
Trichloroisocyanuric acid (1.815 g, 7.81 mmol) was added to a solution of the diol **13** (3.0 g, 7.10 mmol) in CH_2Cl_2 (20 mL), and the solution was stirred and maintained at 0 °C, followed by addition of TEMPO (0.011 g, 0.071 mmol). After the addition, the mixture was warmed to room temperature and stirred for 15 min and then filtered on Celite, and the organic phase was washed with 15 mL of a saturated solution of Na_2CO_3, followed by 1 N HCl and brine. The organic layer was dried (Na_2SO_4), and the solvent was evaporated. The crude aldehyde was used without purification.

To a suspension of methyl-triphenylphosphonium iodide (9.63 g, 21.3 mmol) in anhydrous THF (40 ml) at 0°C was added *n*-BuLi 1.6 M (6.65 ml, 10.65 mmol). The mixture was stirred for 1 h at room temperature and then cannulated into the above prepared aldehyde solution in THF (10 ml) maintained at 0 ^oC. The reaction mixture was

allowed to attain he room temperature and further stirred for 12 h. The reaction was quenched with saturated aqueous NH_4Cl, extracted with ethyl acetate, dried (Na_2SO_4) and concentrated. Purification of the residue on silica gel by using light petroleum: ethyl acetate (19:1) to give **5** (1.8 g, 60.6%).

Mol. Formula	**:**	$C_{27}H_{30}O_4$
IR ($CHCl_3$)	**:**	3048, 3035, 2895, 1716, 1615, 1392, 1415, 1384, 1216, 1042, 918, 854, 776, 664 cm^{-1}.
^{1}H NMR (200 MHz, $CHCl_3$)	**:**	δ 3.16 (d, 1H, *J*= 5.8 Hz), 3.54 (dd, *J* = 3.7, 5.8 Hz, 1H), 3.70 (d, *J* = 5.2 Hz, 1H), 3.87-3.94 (m, 1H), 4.33-4.75 (m, 8H), 5.15-5.38 (m, 2H), 5.81-5.98 (m, 1H), 7.30-7.37 (m, 15H).
^{13}C NMR (50 MHz, $CHCl_3$)	**:**	δ 64.6 (t), 69.4 (t), 71.8 (d), 72.7 (t), 72.8 (t), 73.1 (t), 78.1 (d), 79.8 (d), 115.8 (t), 126.6 (d), 127.1(d), 127.4 (d), 127.4 (d), 127.5 (d), 127.8 (d), 127.9 (d), 128.1 (d), 128.1 (d), 133.2 (d), 133.6 (d), 137.5 (d), 137.6 (s), 137.7 (s), 137.7 (s), 140.8 (s) ppm.
LC MS	**:**	441.52 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 77.48; H, 7.22%. Found: C, 77.43; H, 7.2%.

hex-5-en-2-yn-1-ol (16).

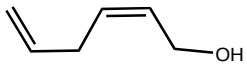


To a well stirred solution of propargyl alcohol (10g, 0.13mol) in DMF (50ml) was added Na_2CO_3 (18.8g, 0.178mol) followed by tetra n-butyl ammonium iodide (3.8g, 0.011mol) and cuprous iodide (1.13g, 0.005mol) at r.t under N_2. To this was added the allyl bromide (15.8g, 0.13mol) at same temperature over a period of 20 min. The contents were stirred overnight at r.t. Ether was added to reaction mixture. After filtration through a bed of

celite the organic phase was washed with water, brine and evaporated. The residue on purification by column chromatography using light petroleum: ethyl acetate (8:2) furnished the compound **16** (9.37g, 82%) as a yellow oil.

Mol. Formula	**:**	C_6H_8O
IR ($CHCl_3$)	**:**	3426, 3079, 2923, 2234, 1653 cm^{-1}.
^{1}H NMR (200 MHz, $CHCl_3$)	**:**	δ 2.18 (br s, 1H), 2.98-3.04 (m, 2H), 4.29 (t, *J* = 2.2 Hz, 2H), 5.09-5.37 (m, 2H), 5.73-5.91 (m, 1H).
^{13}C NMR (50 MHz, $CHCl_3$)	**:**	δ 22.8 (t), 50.6 (t), 80.6 (s), 82.2 (s), 115.9 (t), 132.1 (d) ppm.
LC MS	**:**	119.06 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 74.97; H, 8.39%. Found: C, 74.91; H, 8.32%.

(Z)-hexa-2,5-dien-1-ol (17).

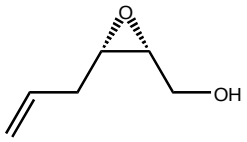


Sodium borohydride (5.90 g, 156.04 mmol) was added to a suspension of nickel acetate terahydrate (28.48 g, 114.43 mmol) in ethanol (200 mL). The green suspension instantly turned black with gas evolution. After 30 min ethylenediamine (27.82 mL, 416.12 mmol) was added drop wise. After 15 min compound **16** (10.0 g, 104.03 mmol) in 50 mL of ethanol was added. The black reaction mixture was stirred vigorously under an atmosphere of hydrogen at rt. After hydrogen uptake ceased **(5** h), the reaction mixture was filtered through a pad of Celite and the ethanol filtrate was concentrated *in vacuo.* The crude diene was dissolved in ether (40 mL), and the ether layer was washed with saturated

aqueous NaCl, dried over $NaS0_4$, concentrated, and chromatographed on silicagel using light petroleum: ethyl acetate (8:2) to give **17** (8.9 g, 87% **)** as a pale yellow oil.

Mol. Formula	**:**	$C_6H_{10}O$
IR ($CHCl_3$)	**:**	3422, 2985, 2937, 2858, 1659 cm^{-1}.
^{1}H NMR (200 MHz, $CHCl_3$)	**:**	δ 2.80-2.83 (m, 2H), 4.15-4.17 (m, 2H), 4.98 (dq, *J* = 1.5, 3.1 Hz, 1H), 5.03 (dq, *J* = 1.5, 3.1 Hz, 1H), 5.48-5.60 (m, 1H), 5.63-5.68 (m, 1H), 5.75-5.83 (m, 1H).
^{13}C NMR (50 MHz, $CHCl_3$)	**:**	δ 31.4 (t), 58.7 (t), 116.9 (t), 127.8 (d), 130.2 (d), 135.7 (d) ppm.
LC MS	**:**	121.07 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 73.43; H, 10.27%;.
		Found: C, 73.42; H, 10.19%.

((2R,3S)-3-allyloxiran-2-yl)methanol (18).

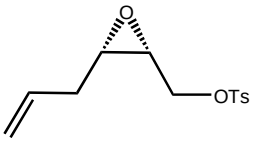


Anhydrous powdered $4A^o$ molecular sieves (4 g)and anhydrous CH_2Cl_2 (100 mL) were placed in a 250 mL round-bottomed flask under Ar. After cooling the flask to – 20 ^oC, the following reagents were added sequentially through an addition funnel with stirring: D-(-)-diisopropyl tartrate (4.77 g, 20.38 mmol) in CH_2Cl_2 (10 mL), $Ti(OiPr)_4$ (2.90 g, 10.19 mmol), and 6.25 m tBuOOH in toluene (20.38 mL, 122.27 mmol). After stirring the reaction mixture for 1 h at to – 20 ^oC, a solution of (Z)-2,5-dihexen-1-ol **17** (10.0 g, 101.89 mmol, previously stored for 24 h over 4 a molecular sieves) in CH_2Cl_2 (50 mL) was added dropwise. After stirring for 4 h at – 20 ^oC, the reaction was quenched by addition of 10% aq. NaOH solution (10 mL) and diethyl ether (100 mL). The mixture was then allowed to

warm to 10 ^{o}C before anhydrous $MgSO_4$ and celite were added. After stirring at rt for 15 min, the mixure was filtered through a short pad of Celite, the solvents were evaporated, and the excess tBuOOH was removed by azeotropic distillation with toluene. The crude product was then purified by column chromatography on silica gel (petroleum ether/ethyl acetate 5:1) to give compound **18** (9.8 g, 84.2%) as an oil.

Mol. Formula	**:**	$C_6H_{10}O_2$
IR ($CHCl_3$)	**:**	3435, 2974, 2919, 2826, 1659 cm^{-1}.
^{1}H NMR (200 MHz, $CHCl_3$)	**:**	δ 2.23-2.30 (m, 1H), 2.39-2.46 (m, 1H), 2.71 (br s, 1H), 3.13 (ddd, *J* = 4.4, 6.5, 10.8 Hz, 1H), 3.20 (ddd, *J* = 4.1, 6.8, 8.5 Hz, 1H), 3.70 (dd, *J* = 6.8, 12.3 Hz, 1H), 3.87 (d, *J* = 12.3 Hz, 1H), 5.12-5.20 (m, 2H), 5.86 (dt, *J*= 6.5 Hz, 1H).
^{13}C NMR (50 MHz, $CHCl_3$)	**:**	δ 32.2 (t), 55.6 (d), 56.6 (d), 60.3 (t), 117.1 (t), 133.1 (d) ppm.
LC MS	**:**	137.07 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 63.14; H, 8.83%.
		Found: C, 63.21; H, 8.79%.

((2R,3S)-3-allyloxiran-2-yl)methyl 4-methylbenzenesulfonate (6).



To a solution of alcohol **18** (10.0 g, 87.61 mmol) and pyridine (14.17 mL, 175.22 mmol) in CH_2Cl_2 (100 mL) at 0 ^{o}C was added p-toluenesulfonyl chloride (18.37 g, 96.37 mmol). The solution was then stirred at room temperature for 12 h, whereupon H_2O (100 mL) was added. The layers were separated, and the aqueous layer was extracted with

CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The resulting oil was purified by flash chromatography over silica gel eluting with petroleum ether/ethyl acetate (9:1) to provide **6** (20.2 g 86%) as a clear, colorless oil.

Mol. Formula	**:**	$C_{13}H_{16}O_4S$
IR ($CHCl_3$)	**:**	3077, 2971, 2951, 1658, 1589, 1452, 1357, 1185 cm^{-1}.
^{1}H NMR (200 MHz, $CHCl_3$)	**:**	δ 2.12-2.19 (m, 1H), 2.23-2.30 (m, 1H), 2.44 (s, 3H), 3.07 (ddd, *J* = 4.4, 6.5, 10.8 Hz, 1H), 3.19 (ddd, *J* = 4.1, 6.8, 8.5 Hz, 1H), 4.09 (dd, *J* = 6.5, 11.3 Hz, 1H), 3.87 (d, *J* = 4.8, 11.3 Hz, 1H), 5.08-5.15 (m, 2H), 5.78 (dt, *J*= 6.5 Hz, 1H), 7.36 (d, *J* = 8.23 Hz, 2H), 7.80 (d, *J* = 8.23 Hz, 2H).
^{13}C NMR (50 MHz, $CHCl_3$)	**:**	δ21.4 (q), 31.9 (t), 52.7 (d), 55.1 (d), 67.8 (t), 117.5 (t), 127.7 (d), 129.7 (d), 132.4 (s), 145.1(s) ppm.
LC MS	**:**	291.08 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 58.19; H, 6.01%.
		Found: C, 58.23; H, 6.12%.

Genaral procedure for epoxide opening:

Using $BF_3.Et_2O$:

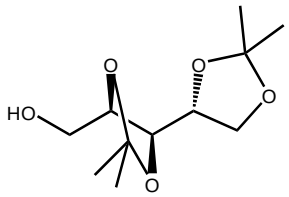
To a solution of epoxy tosylate (1.0 eq) and alcohol (1.5 eq) in CH_2Cl_2 (10 mL/ mmol) under exclusion of moisture under N_2 atmosphere at – 20 ^oC was added 5 mole % $BF_3 \cdot Et_2O$. After stirring at same temperature for 12 h, the solvent was removed under reduced pressure.

Using $Cu(OTf)_2$:

To a solution of epoxy tosylate (1.0 eq) and alcohol (1.5 eq) in CH_2Cl_2 (10 mL/ mmol) under exclusion of moisture under N_2 atmosphere at – 20 ^oC was added cat

$Cu(OTf)_2$ (0.01 eq). After stirring at same temperature for 12 h, the solids were filtered through celite and the solvent removed under reduced pressure.

((4S,4'R,5R)-2,2,2',2'-tetramethyl-4,4'-bi(1,3-dioxolan)-5-yl)methanol (22).



To a solution of 1,2:3,4:5,6-tri-O-isopropylidene-D-mannitol **21** (12.5 g, 41.4 mmol) in dry ether (180 mL) was added periodic acid (12.26g, 53.80 mmol) portionwise at 0 ^oC under a nitrogen atmosphere. After stirring for 6h at room temperature, the reaction mixture was filtered through a pad of Celite, and the filtrate concentrated under reduced pressure to give crude arabinose derivative, which was used in the next step without any purification.

To the above arabinose derivative in dry MeOH (40 mL) was added $NaBH_4$ (1.81 g, 47.86mmol) portionwise at 0 ^oC under a nitrogen atmosphere. After being stirred for 3 h at room temperature, saturated aq NH_4Cl was added to the reaction mixture, MeOH concentrated under reduced pressure and residue extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified by column chromatography (ethyl acetate/ hexane) (2:8) to afford **22** as a syrup.

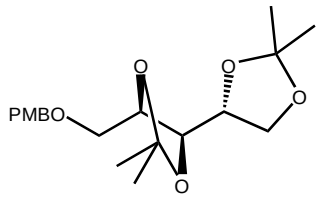
Mol. Formula : $C_{11}H_{20}O_5$

$[\alpha]_D^{25}$: - 1.1 (*c* = 1, $CHCl_3$)

IR ($CHCl_3$) : 3488, 2987, 2935, 2878, 1429, 1352, 1218, 1146, 918, 864, cm^{-1}.

^{1}H NMR (200 MHz, $CHCl_3$)	**:**	δ 1.35 (s, 3H), 1.39 (s, 3H), 1.40 (s, 3H), 1.43 (s, 3H), 2.40 (br s, 1H), 3.67–3.81 (m, 3H), 3.95–4.11 (m, 3H), 4.18 (dd, 1H, *J* = 5.4, 7.6Hz).
^{13}C NMR (50 MHz, $CHCl_3$)	**:**	δ 25.0 (q), 26.5 (q), 26.7 (q), 26.8 (q), 62.5 (t), 67.6 (t), 76.8 (d), 78.2 (d), 80.8 (d), 109.2 (s), 109.6 (s) ppm.
LC MS	**:**	255.15 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 56.88; H, 8.68%. Found: C, 56.85; H, 8.55%.

(4S,4'R,5R)-5-((4-methoxybenzyloxy)methyl)-2,2,2',2'-tetramethyl-4,4'-bi(1,3-dioxolane) (23).

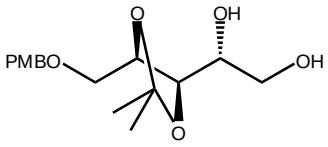


To a stirred suspension of NaH (3.44 g, 60%) in dry DMF (50 mL) was added a solution of **22** (10.0 g, 43.05 mmol) in dry DMF (50 mL) dropwise at 0 ^oC under a nitrogen atmosphere. After stirring at room temperature for 30 min, PMB bromide (9.56 g, 51.66 mmol) was added and the reaction mixture stirred overnight, quenching with saturated aq NH_4Cl and extracted with ether. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified by column chromatography (ethyl acetate/hexane) (1:9) to afford pure **23** (14.8 g, 97.5%) as a pale yellow oil.

Mol. Formula	**:**	$C_{19}H_{28}O_6$
$[\alpha]_D^{25}$	**:**	8.02 (*c* = 1, $CHCl_3$)

IR ($CHCl_3$)	**:**	2935, 2879, 1633, 1496, 1455, 1242, 1218, 1146, 1087, 918 cm^{-1}.
^{1}H NMR (200 MHz, $CHCl_3$)	**:**	δ 1.31 (s, 3H), 1.33 (s, 3H), 1.38 (s, 3H), 1.40 (s, 3H), 3.53 (dd, 1H, *J* = 6.0, 10.6 Hz), 3.67–3.76 (m, 2H), 3.79 (s, 3H), 3.88–3.95 (m, 1H), 3.97–4.04 (m, 1H), 4.07-4.15 (m, 2H), 3.94–4.02 (m, 1H), 4.52 (s, 2H), 6.86 (d, *J* = 7.67 Hz, 2H), 7.26 (d, *J* = 7.67 Hz, 2H).
^{13}C NMR (50 MHz, $CHCl_3$)	**:**	25.0 (q), 26.4 (d), 26.8 (q), 54.8 (d), 67.3 (t), 70.0 (t), 72.8 (t), 76.9 (d), 77.4 (d), 79.3 (d), 109.2 (s), 109.4(s), 113.4 (d), 129.0 (s), 129.9 (s), 158.9 (s) ppm.
LC MS	**:**	375.19 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 64.75; H, 8.01%. Found: C, 64.72; H, 7.92%.

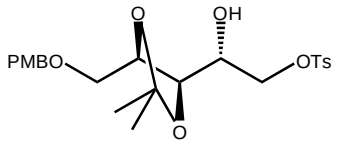
(R)-1-((4R,5R)-5-((4-methoxybenzyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)ethane-1,2-diol (24).



To a solution of the PMB ether **23** (5 g, 14.19 mmol) in MeCN (40 mL) was added $CuCl_2.2H_2O$ (2.9 g, 17.03 mmol) at 0 °C. After 40 min, the reaction was quenched by addition of saturated $NaHCO_3$ at the same temperature. The reaction mixture was filtered through Celite and washed with EtOAc, the organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, concentrated. Purification of the residue on silica gel by using light petroleum: ethyl acetate (3:2) to give **24** (3.5 g, 77%).

Mol. Formula	**:**	$C_{16}H_{24}O_6$
$[\alpha]_D^{25}$	**:**	-16.44 (*c* = 1, $CHCl_3$)
IR ($CHCl_3$)	**:**	3427, 2985, 2926, 2357, 1638, 1453, 1375, 1216, 1083, 860 cm^{-1}.
^{1}H NMR (200 MHz, $CHCl_3$)	**:**	δ 1.37 (s, 6H), 3.52 (dd, 1H, *J* = 7.5, 9.3 Hz), 3.63–3.69 (m, 2H), 3.70–3.77 (m, 3H), 3.80 (s, 3H), 4.02 (dt, 1H, *J* = 4.5, 7.5 Hz), 4.52 (s, 2H), 6.87 (d, *J* = 7.67 Hz, 2H), 7.25 (d, *J* = 7.67 Hz, 2H).
^{13}C NMR (50 MHz, $CHCl_3$)	**:**	δ 26.7(q), 54.9(d), 64.0(t), 70.3 (t), 72.7 (d), 73.1 (t), 78.4 (d), 78.6 (d), 95.9(s), 109.1 (s), 113.6 (d), 129.3 (d), 159.1 (s) ppm.
LC MS	**:**	335.16 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 61.52; H, 7.74%. Found: C, 61.58; H, 7.66%.

(R)-2-hydroxy-2-((4R,5R)-5-((4-methoxybenzyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl 4-methylbenzenesulfonate (25).

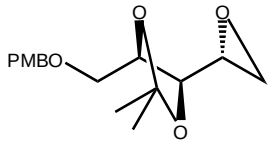


To the diol **24** (5.0 g, 16.01 mmol) in a mixture of dry DCM (40 mL) and dry pyridine (2 mL, 24.01 mmol) was added tosyl chloride (3.36 g, 17.61 mmol) in portions at 0 °C. The reaction was allowed to proceed at rt for 12 h. The reaction mixture was poured into a mixture of ice-cold ether and 6 N HCl. The ether layer was separated, and the aqueous layer was extracted with ether. The combined organic layers were washed with water and brine. Finally, it was dried over anhydrous Na_2SO_4 and concentrated in vacuo.

Purification of the residue on silica gel by using light petroleum: ethyl acetate (7:1) to give **25** (6.5 g, 87%).

Mol. Formula	**:**	$C_{23}H_{30}O_8S$
$[\alpha]_D^{25}$	**:**	7.08 (*c* = 1, $CHCl_3$)
IR ($CHCl_3$)	**:**	3427, 2985, 2926, 2357, 1638, 1453, 1375, 1216, 1083, 860 cm^{-1}.
^{1}H NMR (200 MHz, $CHCl_3$)	**:**	δ 1.31 (s, 6H), 2.44 (s, 3H), 3.50 (dd, 2H, *J* = 6.6, 9.4 Hz), 3.62–3.70 (m, 2H), 3.78 (dd, 1H, *J* = 2.4, 5.6 Hz), 3.80 (s, 3H), 3.97-4.13 (m, 2H), 4.26 (dd, 1H, *J* = 2.3, 10.3 Hz), 4.50 (s, 2H), 6.87 (d, *J* = 7.67 Hz, 2H), 7.20-7.34 (m, 5H), 7.79 (d, *J* = 7.67 Hz, 2H).
^{13}C NMR (50 MHz, $CHCl_3$)	**:**	δ 21.4 (q), 26.6 (q), 26.7(q), 55.0(d), 70.0 (t), 70.9 (d), 71.6 (t), 73.1 (t), 77.9(d), 78.7(d), 95.9 (s), 109.5 (s), 113.7 (d), 127.8 (d), 129.1(d), 129.4 (d), 129.6 (d), 132.7 (s), 144.5 (s), 149.2 (s), 159.2 (s) ppm.
LC MS	**:**	489.17 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 59.21; H, 6.48%. Found: C, 59.19; H, 6.45%.

(4R,5R)-4-((4-methoxybenzyloxy)methyl)-2,2-dimethyl-5-((R)-oxiran-2-yl)-1,3-dioxolane (26).

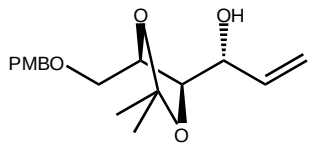


To a stirred solution of monotosylate derivative **25** (5.0 g, 10.72 mmol) in dry MeOH (50 mL) was added K_2CO_3 (2.96 g, 21.43 mmol) under a nitrogen atmosphere. After being stirred for 30 min at room temperature, filtered through a bed of celite and the MeOH

was evaporated under reduced pressure keeping the temperature below 30 ^{o}C. The residue was extracted with $CHCl_3$. The organic extract was washed with water, brine, dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified by column chromatography (ethyl acetate/ hexane) (1:11) to give **26** (2.5 g, 79%) as a syrup.

Mol. Formula	**:**	$C_{16}H_{22}O_5$
IR ($CHCl_3$)	**:**	2978, 2954, 2825, 1675, 1464, 1384, 1227, 1055, 975, 858 cm^{-1}.
^{1}H NMR (200 MHz, $CHCl_3$)	**:**	δ 1.43 (s, 6H), 2.69 (dd, 1H, *J* = 2.6, 5.1 Hz), 2.82 (dd, 1H, *J* = 4.0, 5.0 Hz), 3.05–3.11 (m, 1H), 3.58 (d, 1H, *J*= 2.0 Hz), 3.61 (d, 1H, *J*= 1.0 Hz), 3.65 (dd, 1H, *J* = 5.28, 7.9 Hz), 3.81 (s, 3H), 4.10-4.20 (m, 1H), 4.53 (s, 2H), 6.88 (d, *J* = 7.67 Hz, 2H), 7.27 (d, *J* = 7.67 Hz, 2H).
^{13}C NMR (50 MHz, $CHCl_3$)	**:**	δ 26.6 (q), 26.9 (q), 45.0 (t), 51.7 (d), 55.2 (d), 70.1 (t), 73.2 (t), 78.0 (d), 78.1 (d), 110.1 (s), 113.7 (d), 129.3 (d), 129.9 (s), 159.2 (s) ppm.
LC MS	**:**	317.15 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 65.29; H, 7.53%.
		Found: C, 65.25; H, 7.61%.

(R)-1-((4R,5R)-5-((4-methoxybenzyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)prop-2-en-1-ol (20).

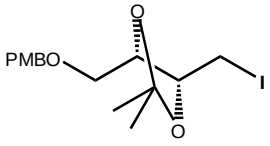


A solution of trimethylsulfonium iodide (13.87 g, 67.95 mmol) in THF (100 mL) was cooled to -15 ^{o}C. *n*-BuLi (1.6 M in hexane, 42.47 mL, 67.95 mmol) was added

dropwise and the resulting solution was stirred for 1 h at -15 ^{o}C. A solution of the previous epoxide **26** (5.0 g, 16.99 mmol) in THF (25 mL) was added and a cloudy suspension was formed. The reaction was allowed to slowly warm to 25 ^{o}C over 1 h and stirred for another 1 h. The reaction mixture was cooled in an ice/water bath and quenched with water. The layers were separated and the aqueous layer was extracted twice with 1:1 EtOAc/hexanes. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography using EtOAc/hexanes (1:9) gave allylic alcohol **20 (**4.8 g, 91.6%) of as a yellow oil.

Mol. Formula	**:**	$C_{17}H_{24}O_5$
IR ($CHCl_3$)	**:**	3433, 2924, 2825, 1464, 1384, 1135, 1075, 975, 858 cm^{-1}.
^{1}H NMR (200 MHz, $CHCl_3$)	**:**	δ 1.41 (s, 6H), 3.04 (d, 1H, *J*= 2.6 Hz), 3.59 (dd, 2H, *J* = 1.8, 5.1 Hz), 3.75–3.82 (m, 1H), 3.80 (s, 3H), 4.07-4.25 (m, 2H), 4.52 (s, 2H), 5.17-5.42 (m, 2H), 5.78-5.95 (m, 1H), 6.88 (d, *J* = 7.67 Hz, 2H), 7.27 (d, *J* = 7.67 Hz, 2H).
^{13}C NMR (50 MHz, $CHCl_3$)	**:**	δ 26.8 (q), 55.1 (d), 70.2 (t), 72.2 (d), 73.0 (t), 80.8 (d), 109.2(s), 113.6 (d), 116.5 (t), 129.3 (d), 129.5 (s), 136.2 (d), 159.1 (s) ppm.
LC MS	**:**	331.16 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 66.21; H, 7.84%. Found: C, 66.32; H, 7.78%.

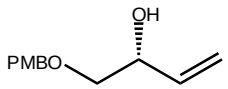
(4R,5S)-4-(iodomethyl)-5-((4-methoxybenzyloxy)methyl)-2,2-dimethyl-1,3-dioxolane (33).



The primary alcohol **32** (10.0 g, 35.42 mmol) is dissolved in toluene (6-10 mL/mmol of alcohol) and the solution is heated to reflux. The heating is stopped and as soon as boiling has ceased, triphenylphosphine (11.15 g, 42.50 mmol), imidazole (7.23 g, 106.26 mmol) and iodine (11.69 g, 46.05 mmol are added (caution: vigorous formation of HI). After refluxing for 20 minutes, the solution is cooled to rt. The organic phase is washed with a saturated solution of $Na_2S_2O_3$ until the brown colour disappears. After separation of the organic phase the aqueous phase is extracted with ether. The organic phases are combined, dried over $NaSO_4$, filtered and concentrated. Purification by flash chromatography using EtOAc/hexanes (1:9) gave compound **33** (13.2 g, 95%) as a yellow syrup.

Mol. Formula	**:**	$C_{15}H_{21}IO_4$
IR ($CHCl_3$)	**:**	3433, 2924, 2825, 1464, 1384, 1135, 1075, 975, 858 cm^{-1}.
^{1}H NMR (200 MHz, $CHCl_3$)	**:**	δ 1.41 (s, 3H), 1.46 (s, 3H), 3.26 (dd, 1H, *J* =5.4,10.5 Hz), 3.34 (dd, 1H, *J* = 5.3,10.6 Hz), 3.62 (dd, 2H, *J* = 1.2, 5.1 Hz), 3.81 (s, 3H), 3.82–3.99 (m, 2H), 4.52 (s, 2H), 6.89 (d, 2H, *J* = 8.6 Hz), 7.26 (d, 2H, *J* = 7.6 Hz).
^{13}C NMR (50 MHz, $CHCl_3$)	**:**	δ 6.3 (t), 27.1 (q), 27.2 (q), 55.1 (d), 70.0 (t), 73.0 (t), 77.5 (d), 79.9 (d), 109.5 (s), 113.7 (d), 129.1 (d), 129.7(s), 159.1 (s) ppm.
LC MS	**:**	415.05 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 45.93; H, 5.40%. Found: C, 45.91; H, 5.38%.

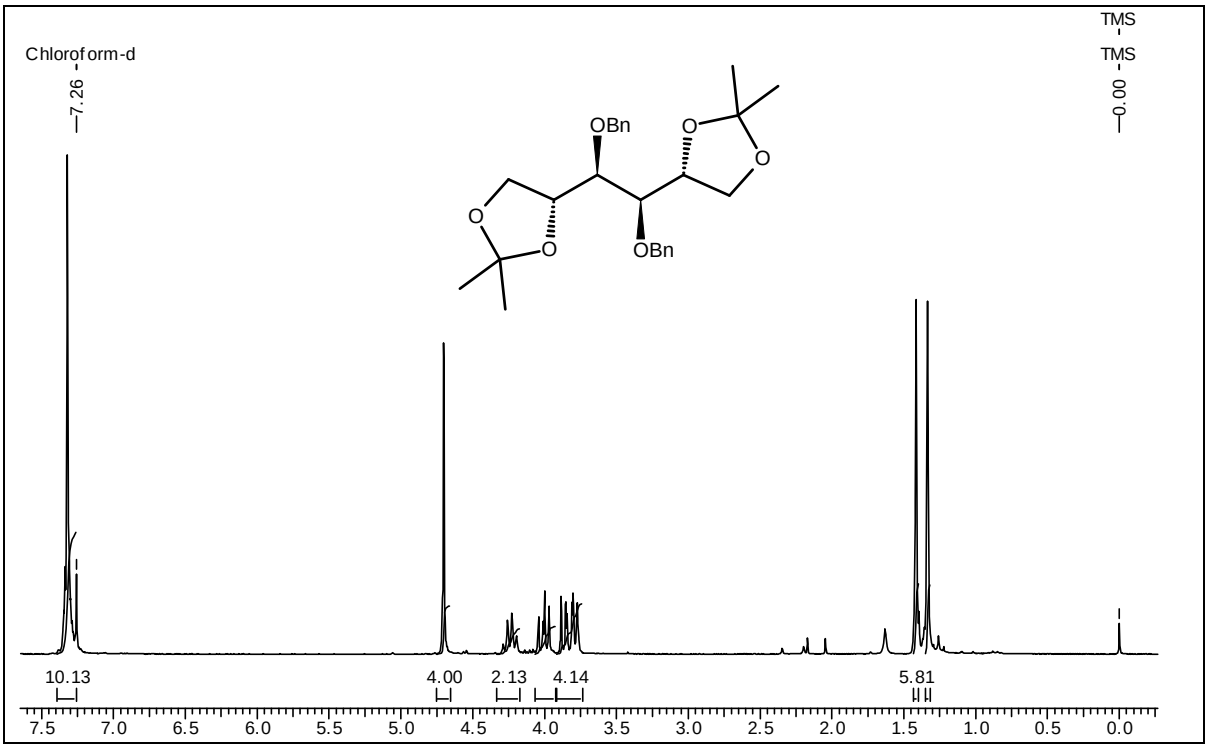
(R)-1-(4-methoxybenzyloxy)but-3-en-2-ol (28).



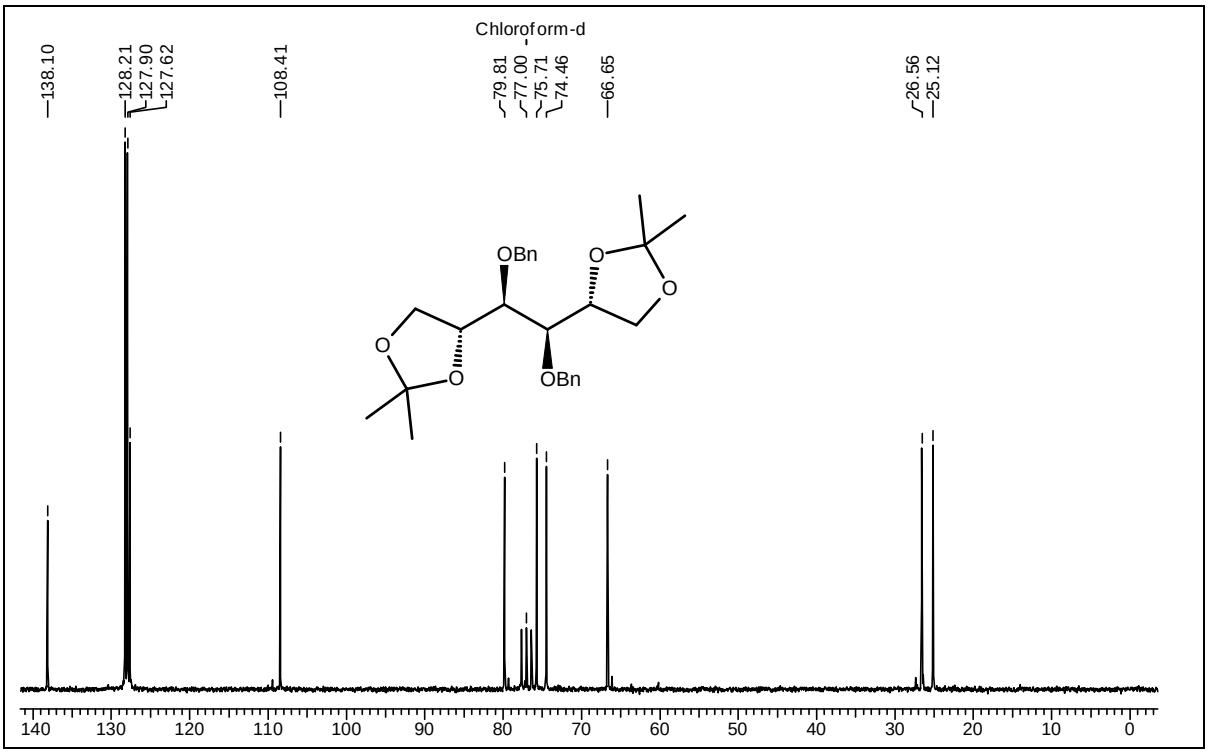
To a stirred solution of iodide **33** (5.0 g, 12.75 mmol) in absolute EtOH (50 mL) was added zinc powder (8.34 mg, 127.5 mmol). The solution was heated to reflux and upon completion of the reaction, as judged by TLC analysis, the mixture was allowed to cool to rt. The mixture was diluted with Et_2O (200 mL) and filtered through Celite. The filtrate was washed with sat. aqueous $Na_2S_2O_3$. The Et_2O solution was separated, dried over $NaSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel (4:1 hexanes/EtOAc) to give compound **28** (2.5 g, 94%).

Mol. Formula	**:**	$C_{12}H_{16}O_3$
$[\alpha]_D^{25}$	**:**	3.4 (*c* = 1, $CHCl_3$)
IR ($CHCl_3$)	**:**	3362, 2955, 1424, 1254, 1155, 1025, 925, 898 cm^{-1}.
^{1}H NMR (200 MHz, $CHCl_3$)	**:**	δ 2.62 (br s, 1H), 3.33 (dd, 1H, *J* = 8.0,9.6 Hz), 3.41 (dd, 1H, *J* = 3.5,9.6 Hz), 3.80 (s, 3H), 4.32 (br s, 1H), 4.50 (s, 2H), 5.15-5.39 (m, 2H), 5.74-5.91 (m, 1H), 6.89 (d, 2H, *J* = 8.6 Hz), 7.26 (d, 2H, *J* = 8.6 Hz).
^{13}C NMR (50 MHz, $CHCl_3$)	**:**	δ 55.0 (d), 71.2 (d), 72.8 (t), 73.6 (t), 113.6 (d), 116.0 (t), 129.2 (d), 129.8 (d), 136.7 (d), 159.1 (s) ppm.
LC MS	**:**	231.11 $[M+Na]^+$
Elemental Analysis	**:**	Calcd: C, 69.21; H, 7.74%. Found: C, 69.26; H, 7.69%.

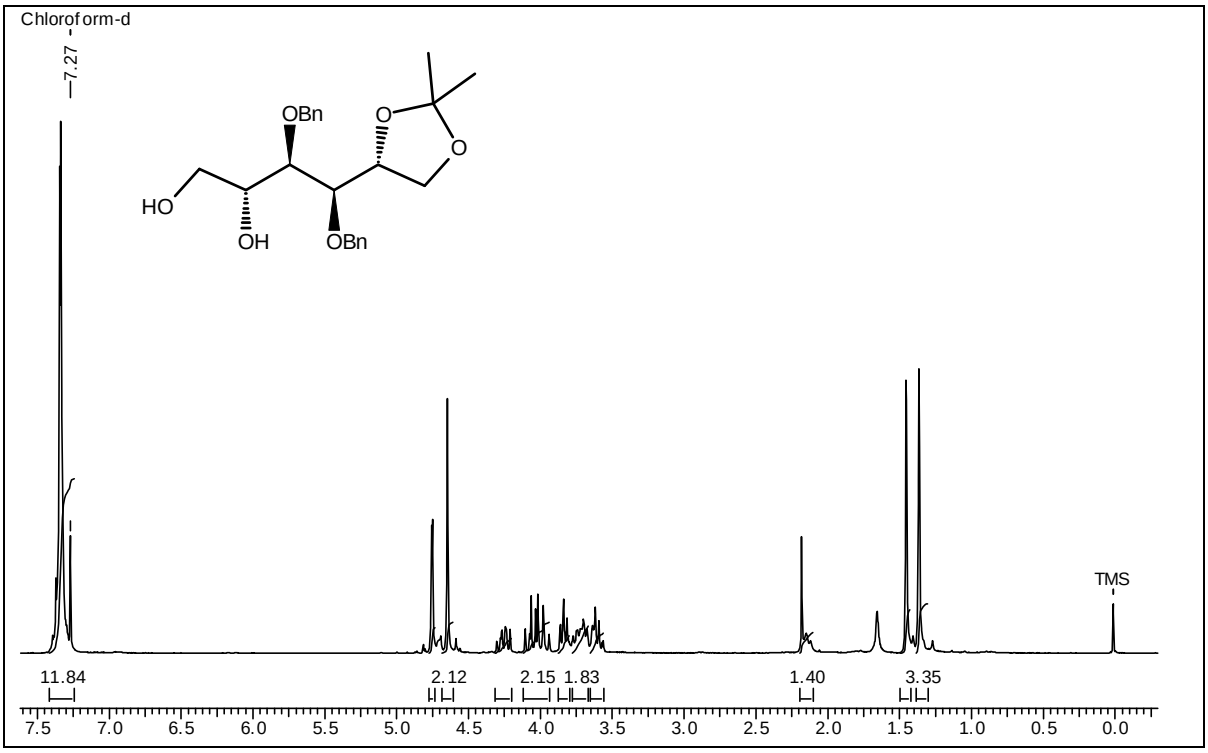
Spectra



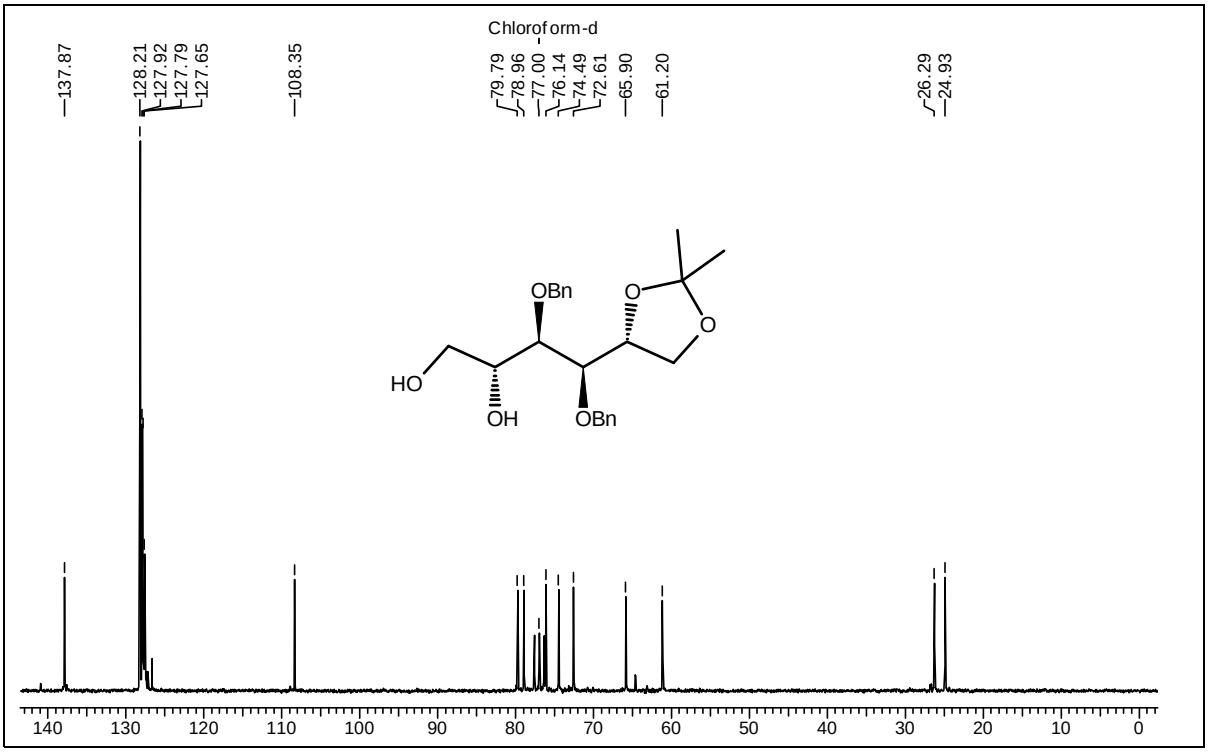
^{1}H NMR spectrum of compound 9 in $CDCl_3$



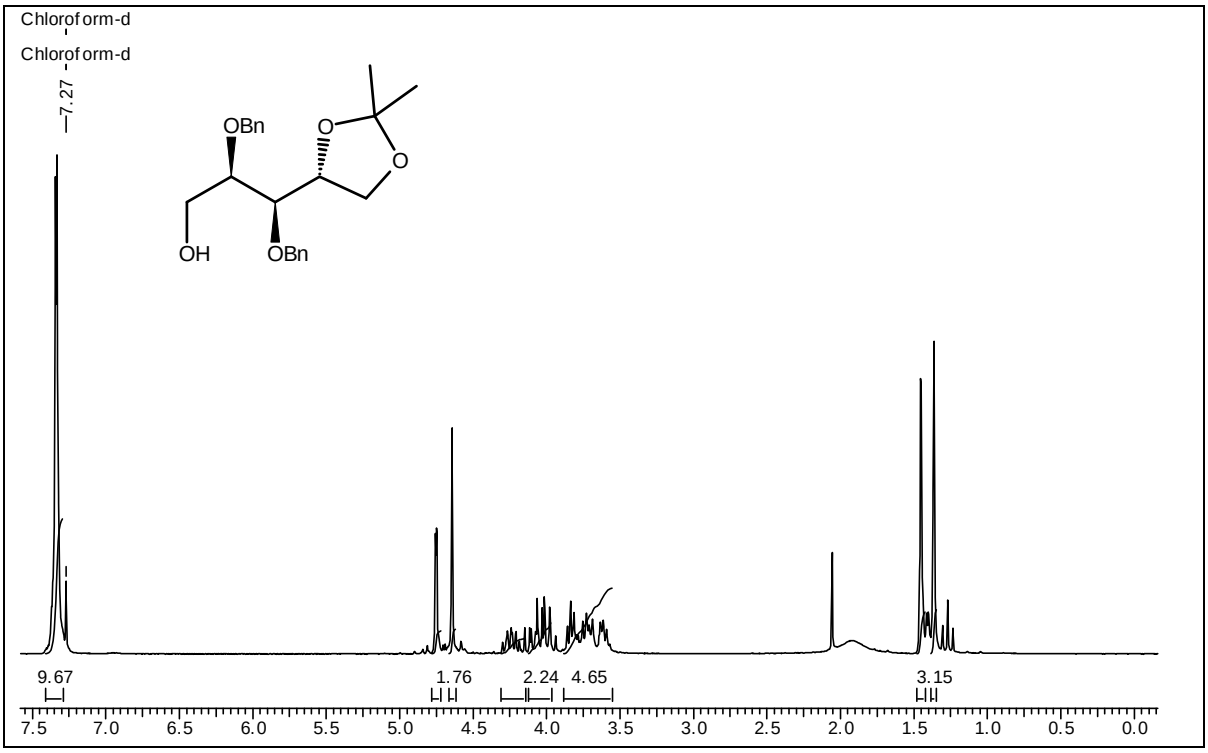
^{13}C NMR spectrum of compound 9 in $CDCl_3$



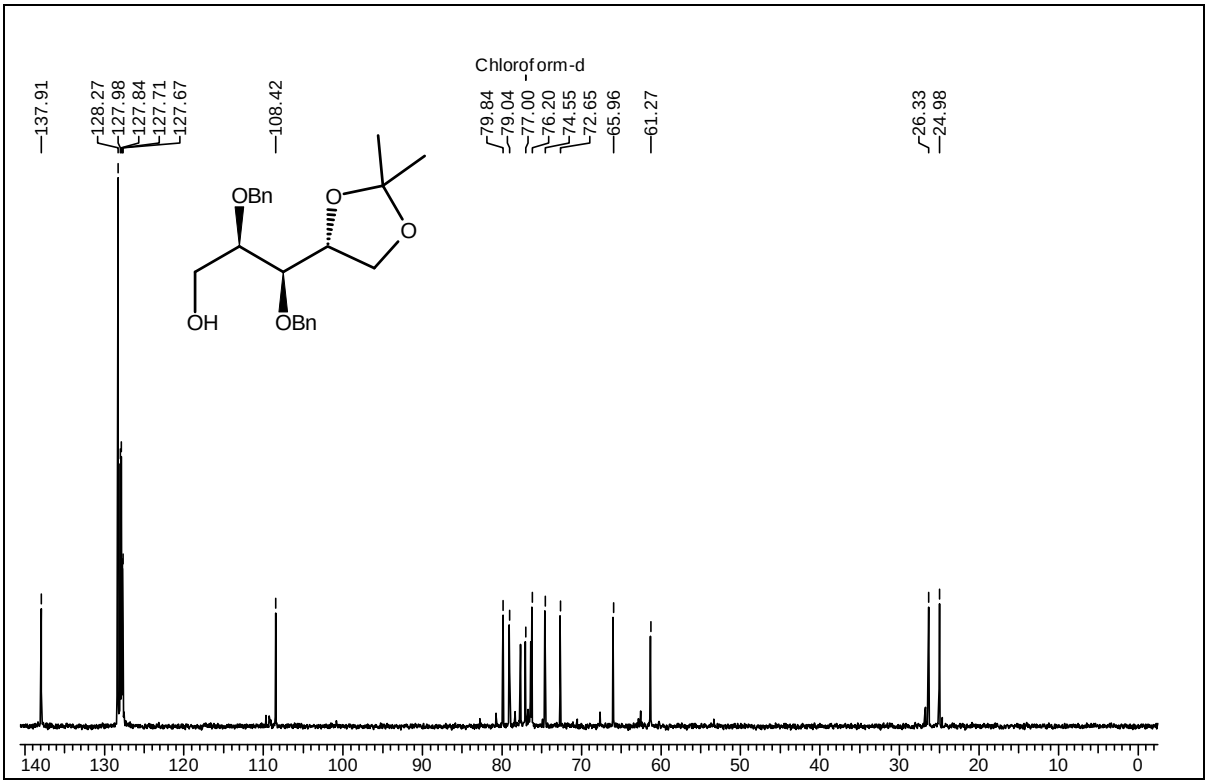
^{1}H NMR spectrum of compound 10 in $CDCl_3$



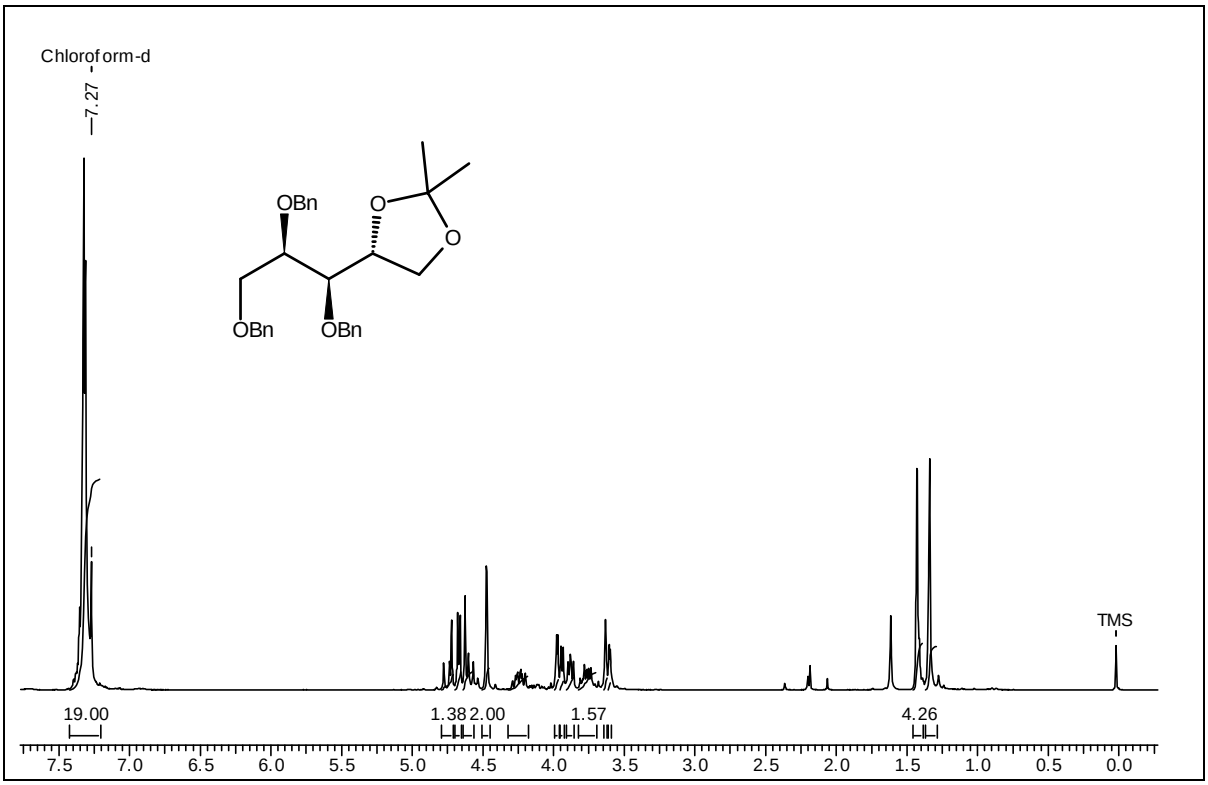
^{13}C NMR spectrum of compound 10 in $CDCl_3$



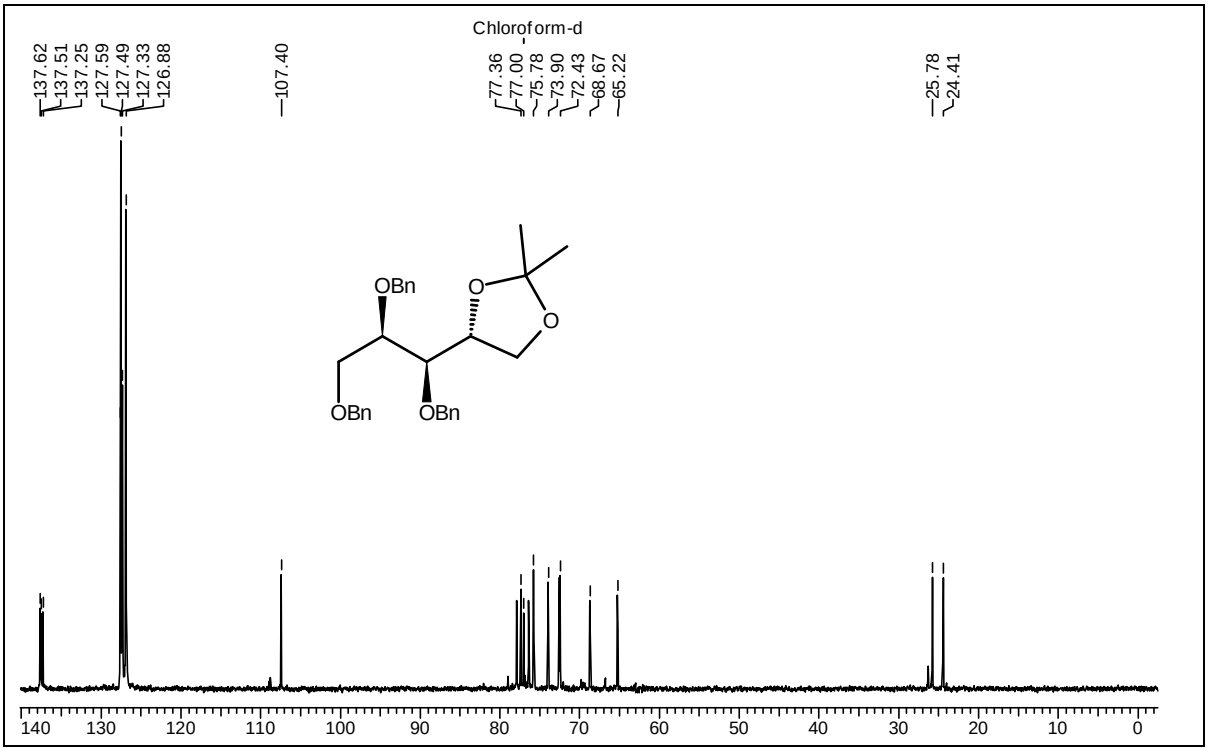
^{1}H NMR spectrum of compound 11 in $CDCl_3$



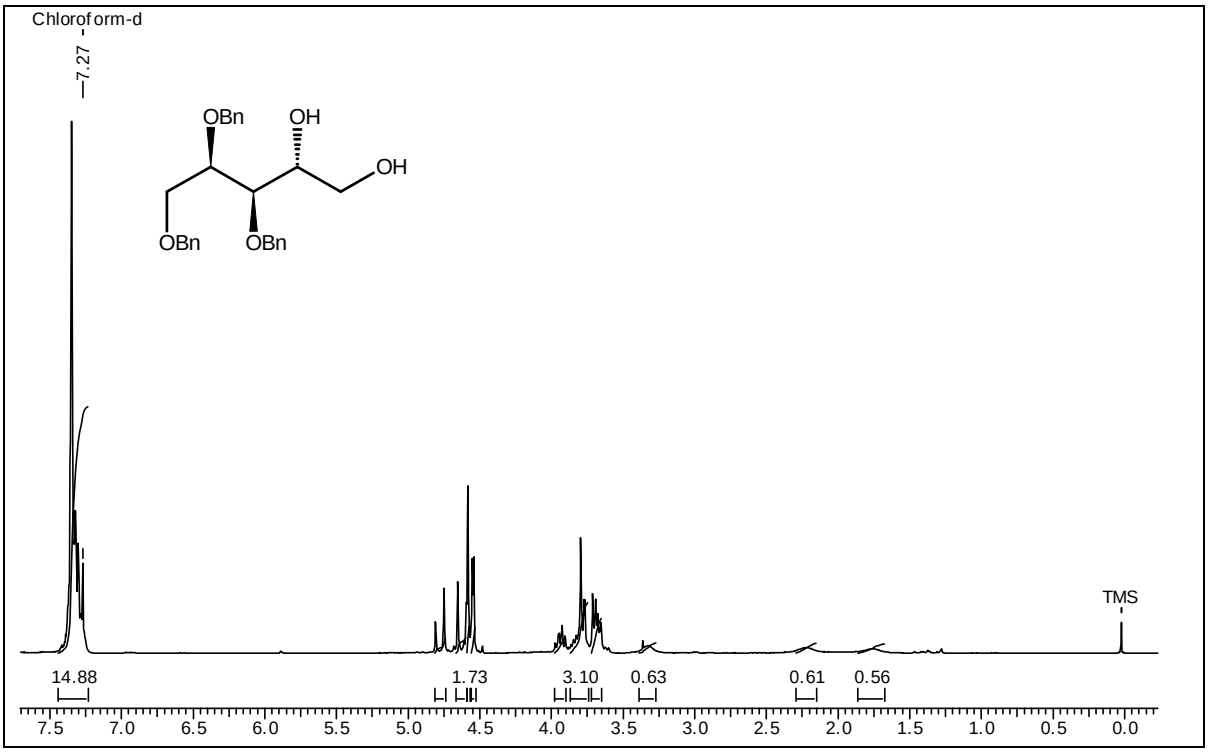
^{13}C NMR spectrum of compound 11 in $CDCl_3$



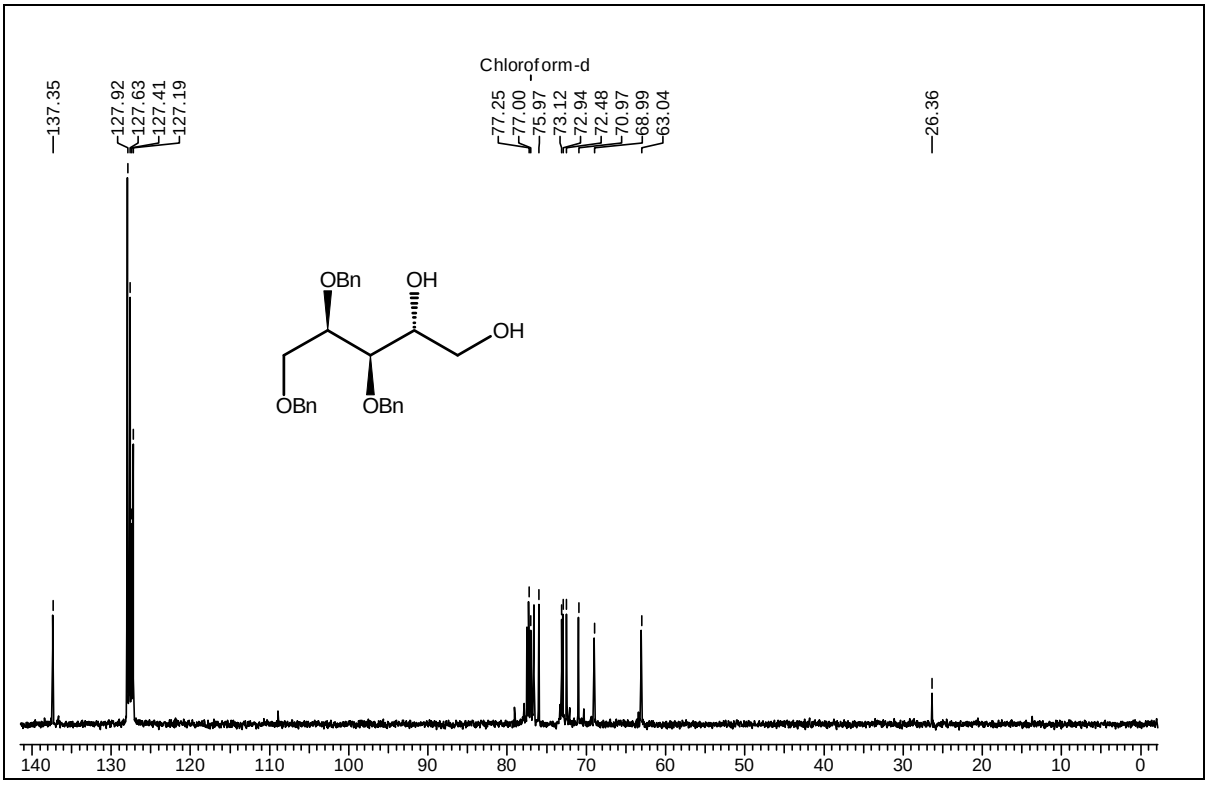
^{1}H NMR spectrum of compound 12 in $CDCl_3$



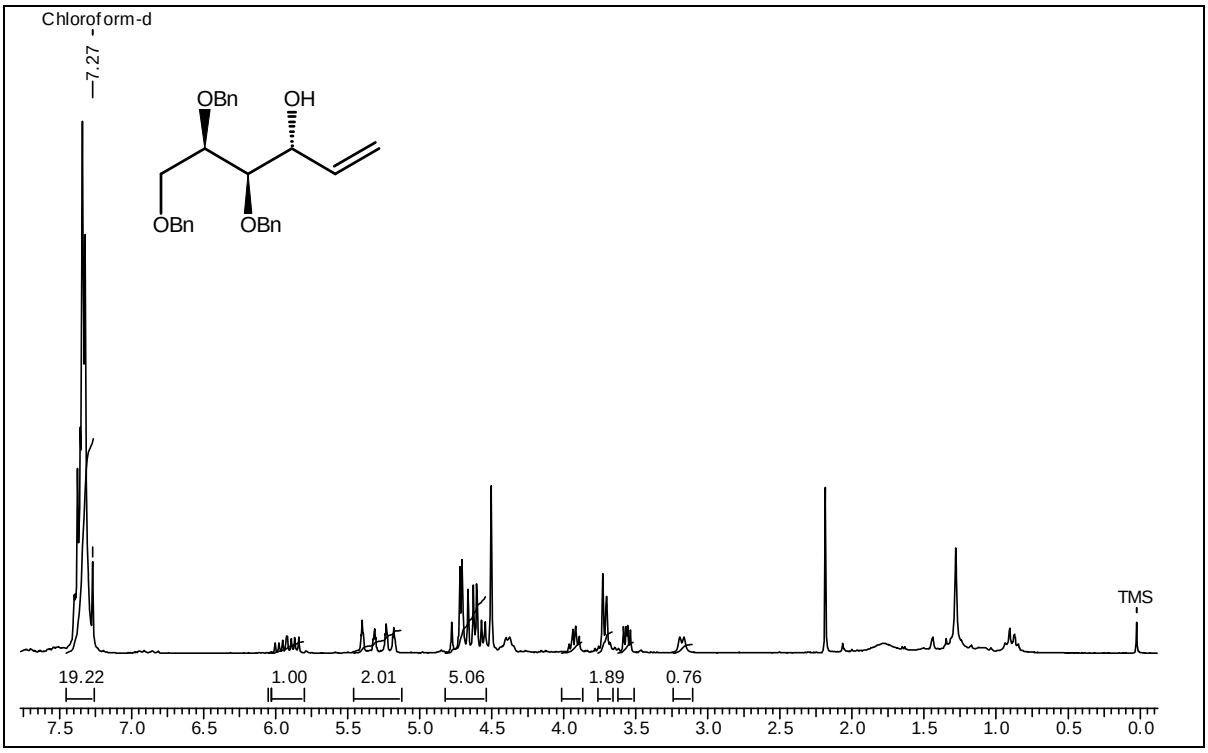
^{13}C NMR spectrum of compound 12 in $CDCl_3$



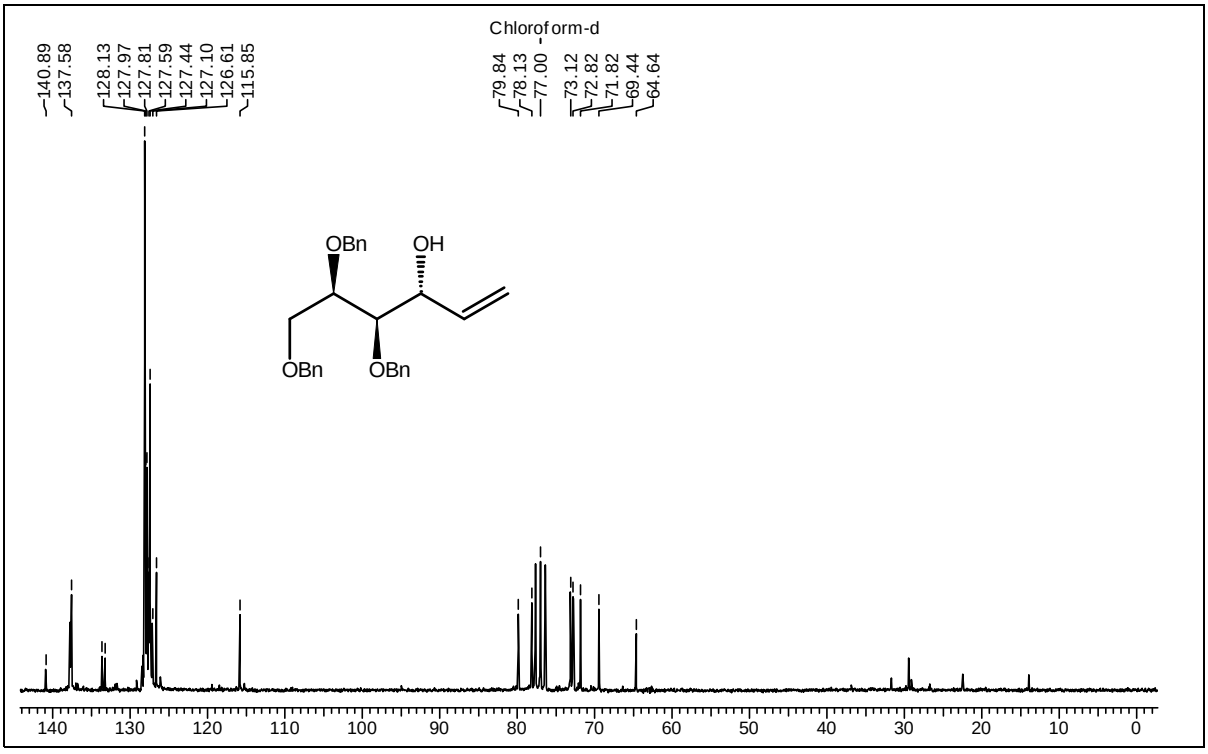
^{1}H NMR spectrum of compound 13 in $CDCl_3$



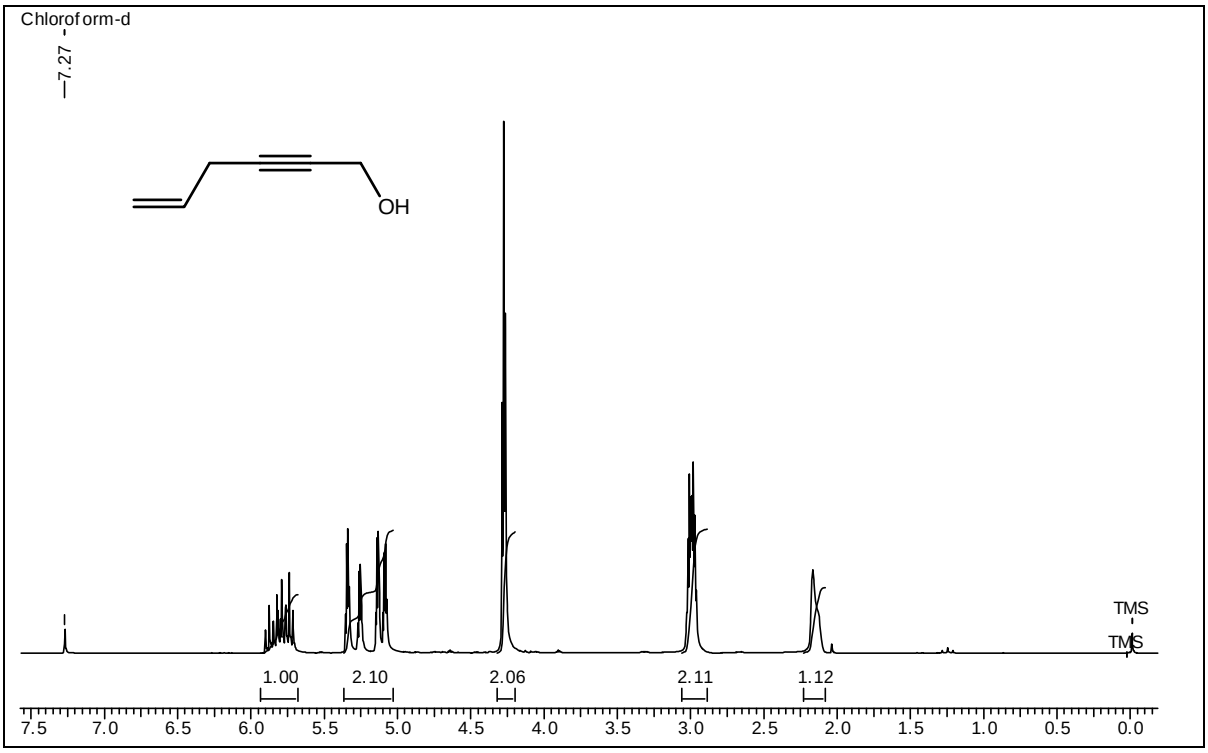
^{13}C NMR spectrum of compound 13 in $CDCl_3$



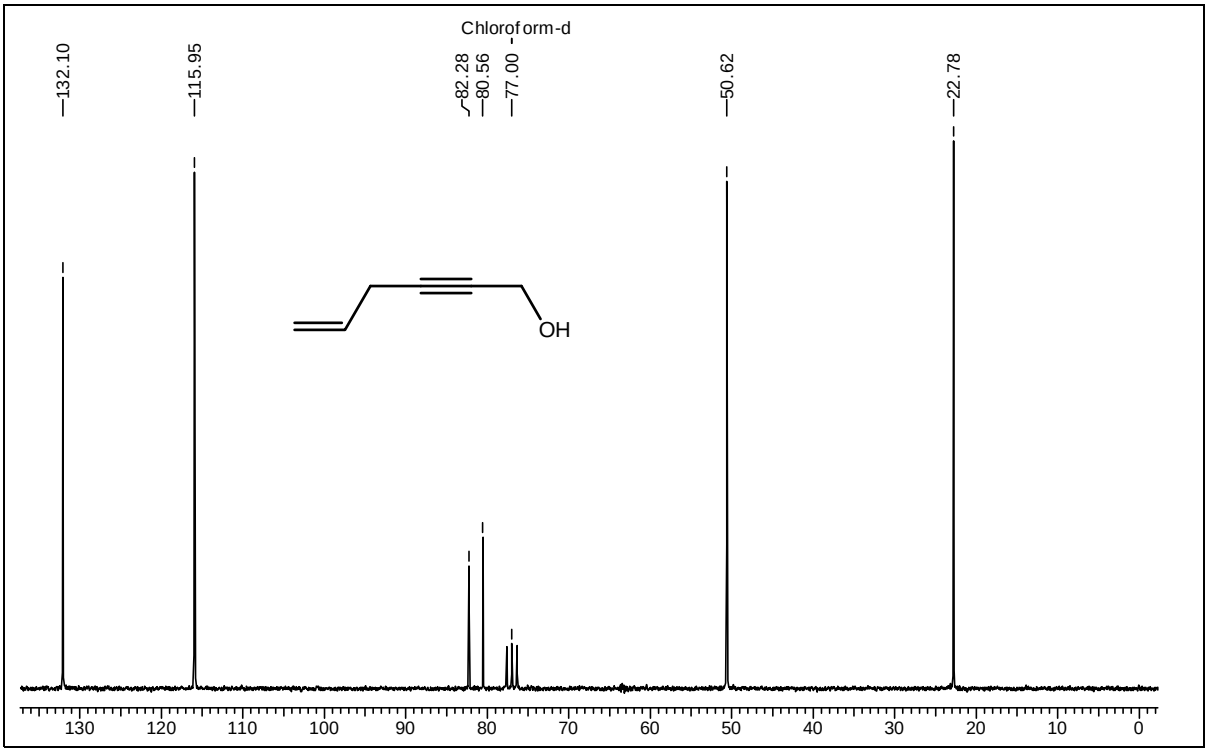
^{1}H NMR spectrum of compound 5 in $CDCl_3$



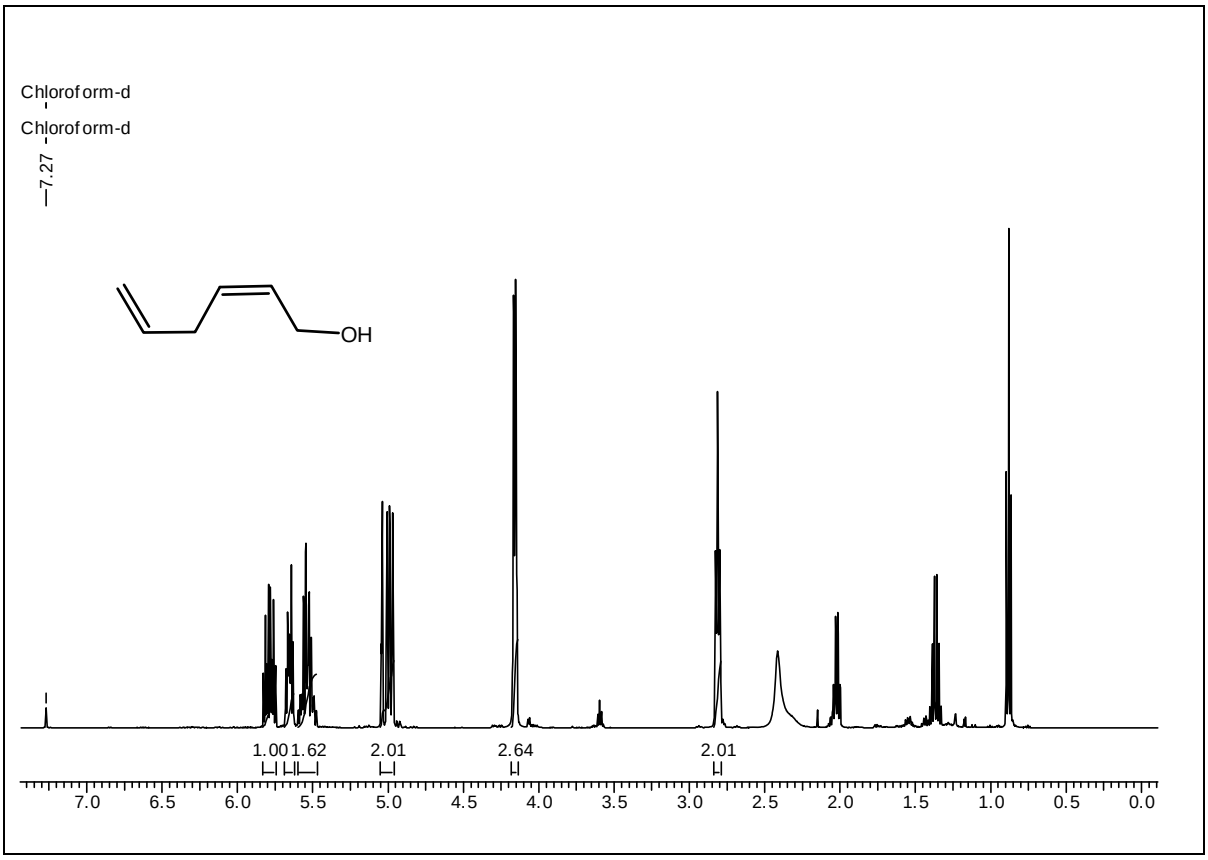
^{13}C NMR spectrum of compound 5 in $CDCl_3$



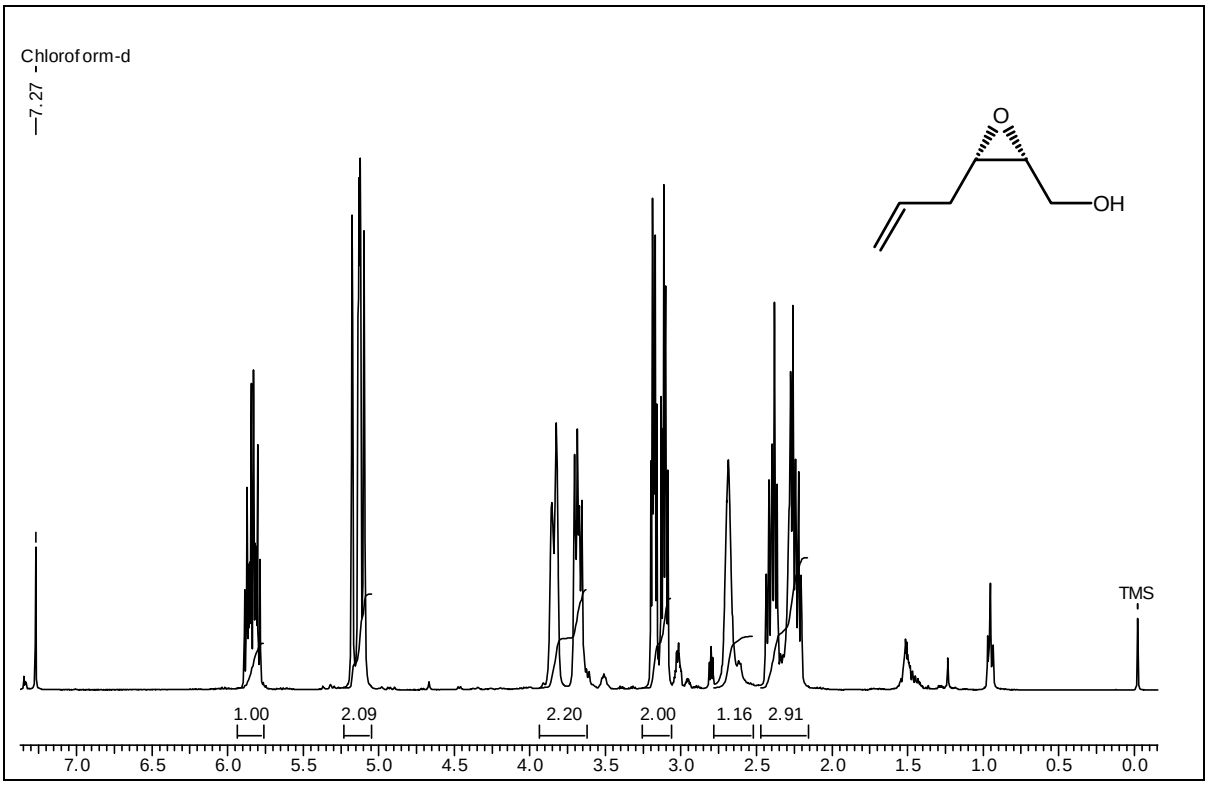
^{1}H NMR spectrum of compound 16 in $CDCl_3$



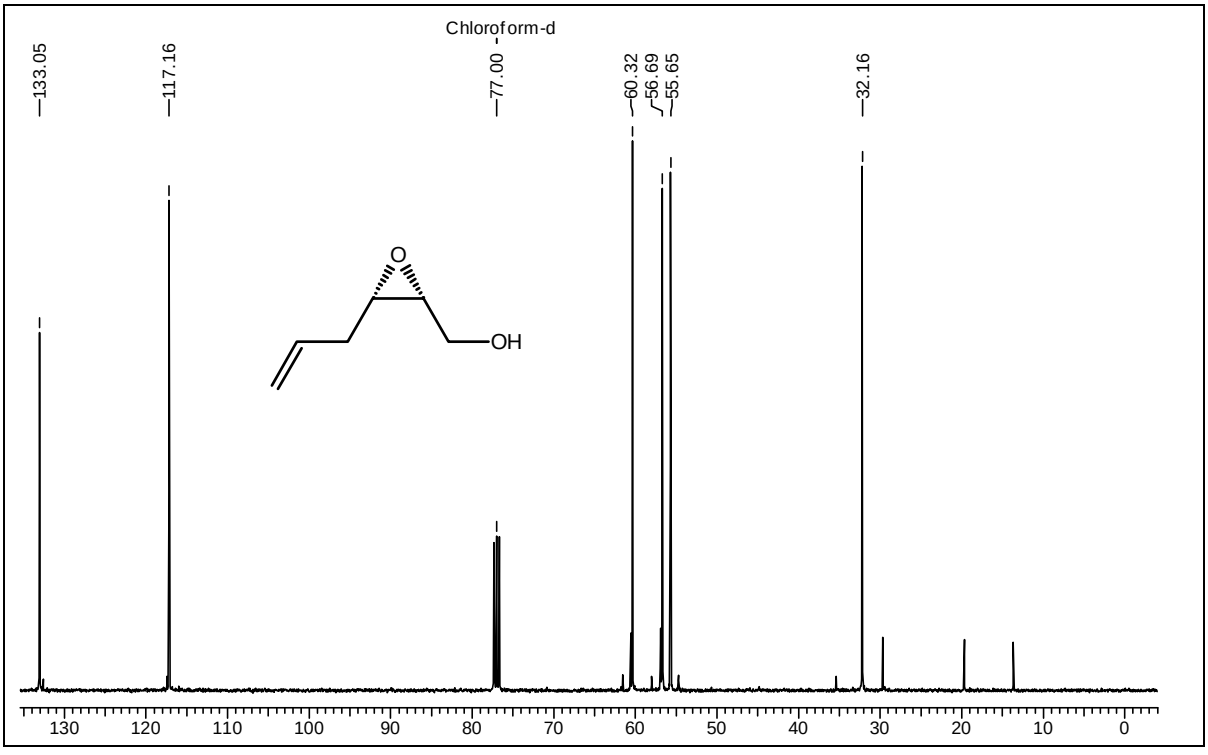
^{13}C NMR spectrum of compound 16 in $CDCl_3$



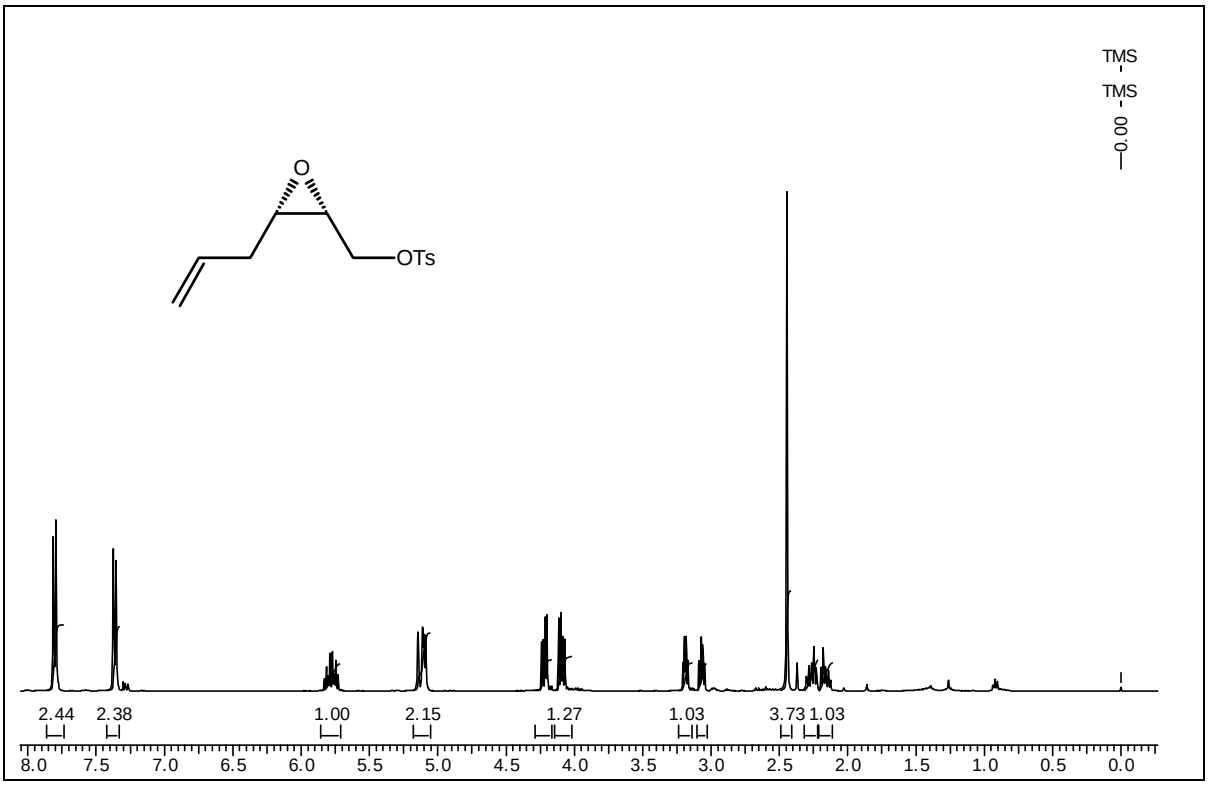
^{1}H NMR spectrum of compound 17 in $CDCl_3$



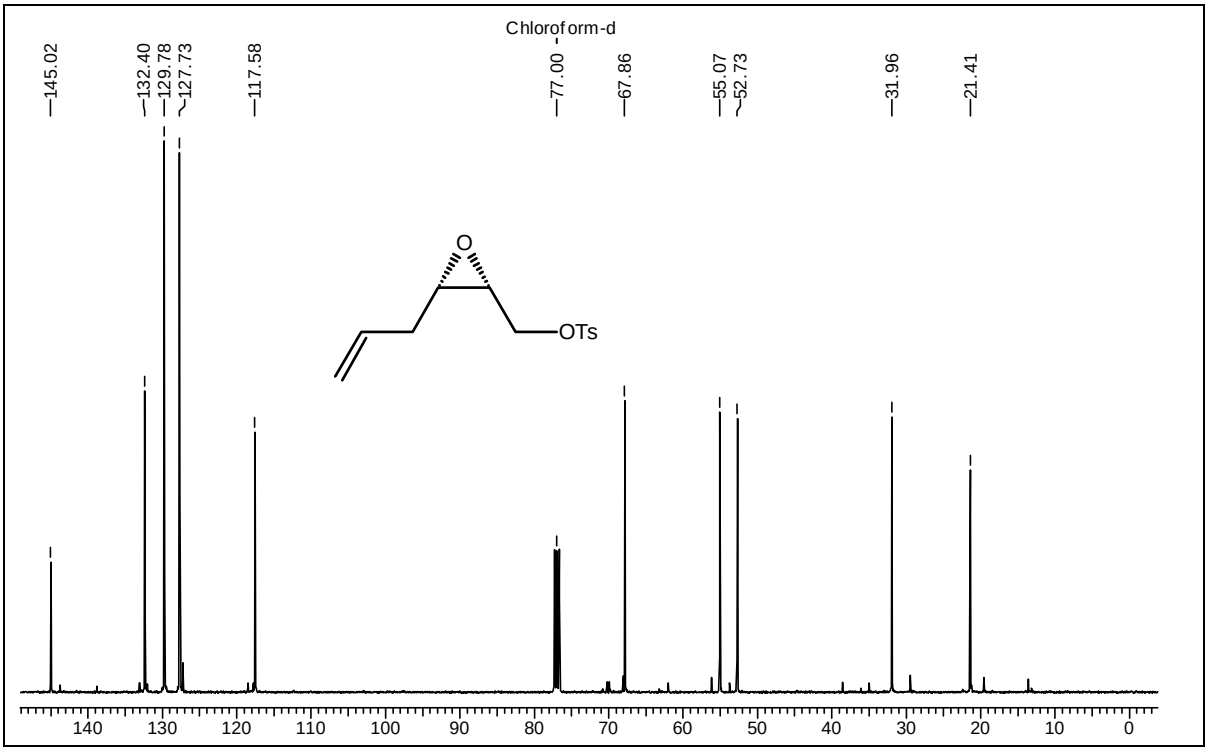
^{1}H NMR spectrum of compound 18 in $CDCl_3$



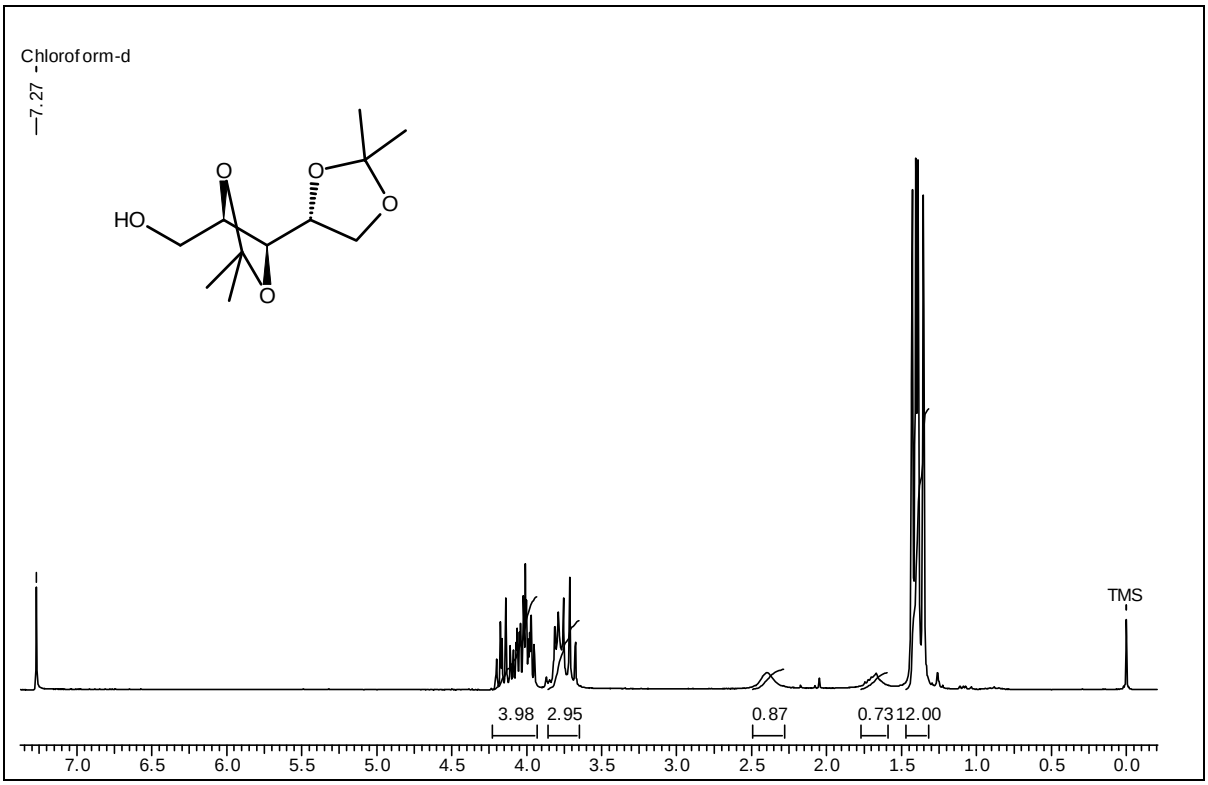
^{13}C NMR spectrum of compound 18 in $CDCl_3$



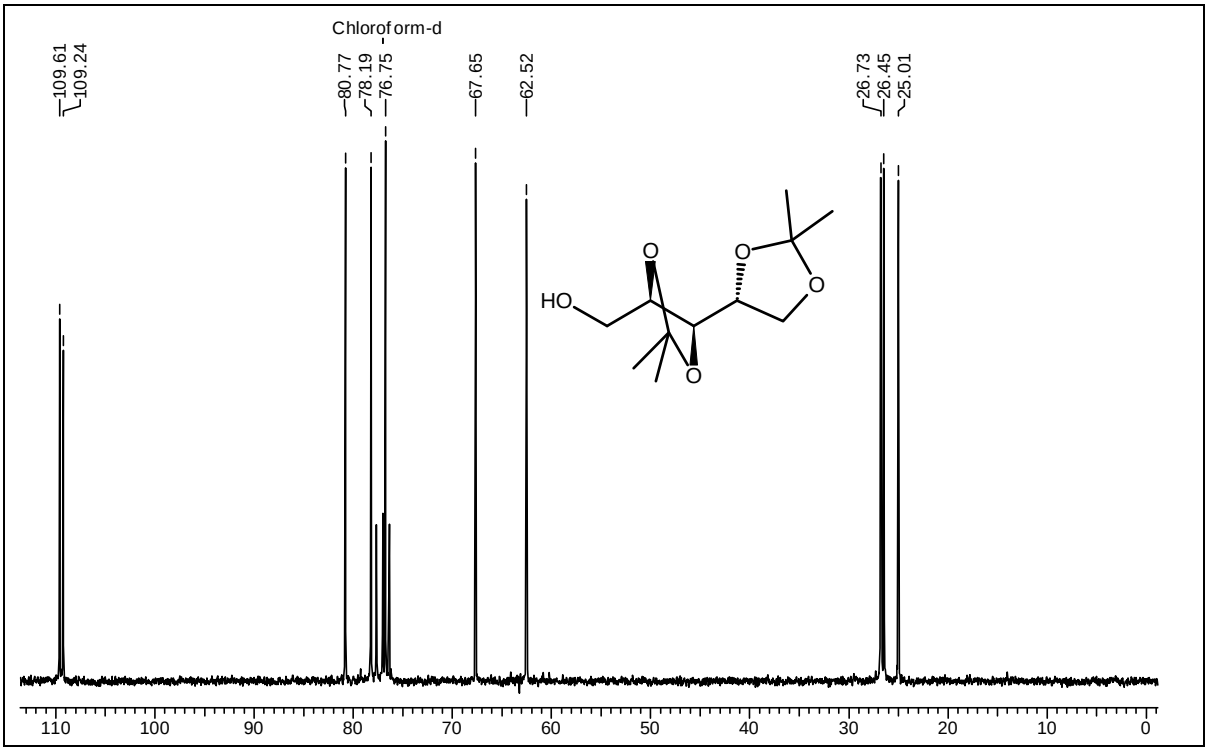
^{1}H NMR spectrum of compound 6 in $CDCl_3$



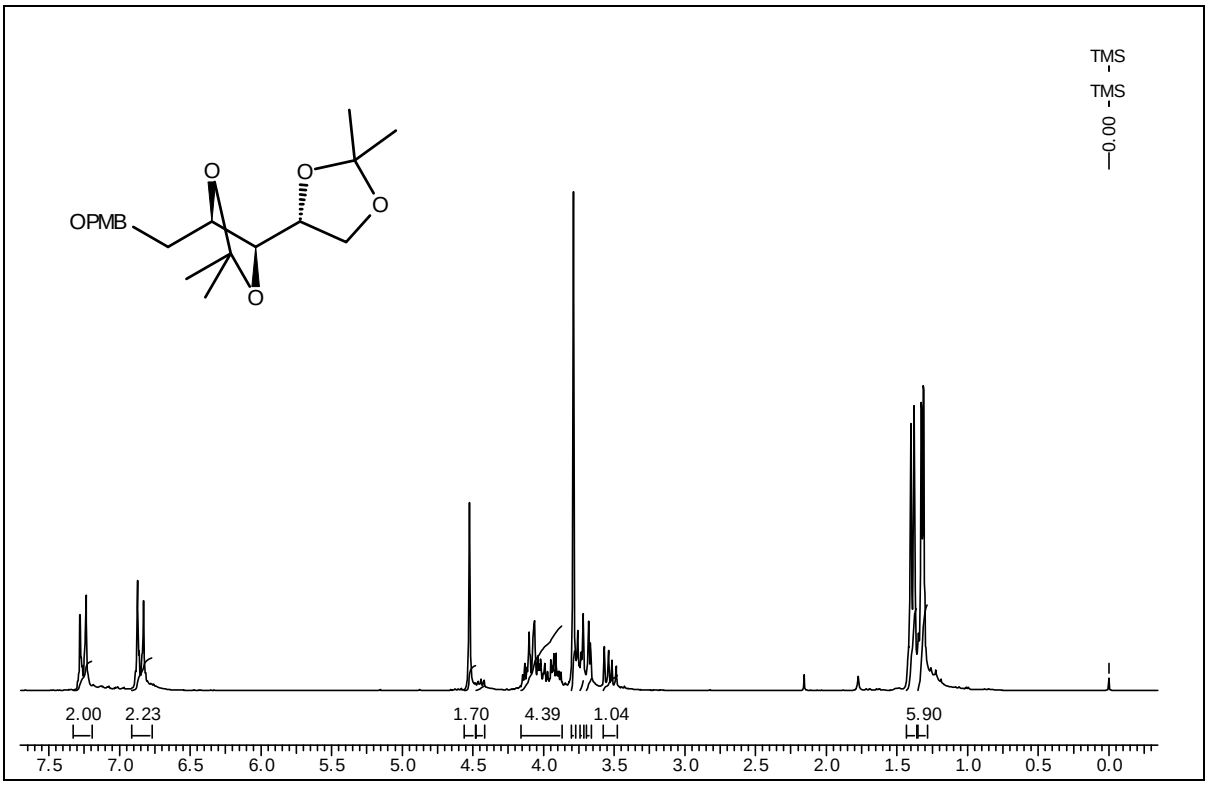
^{13}C NMR spectrum of compound 6 in $CDCl_3$



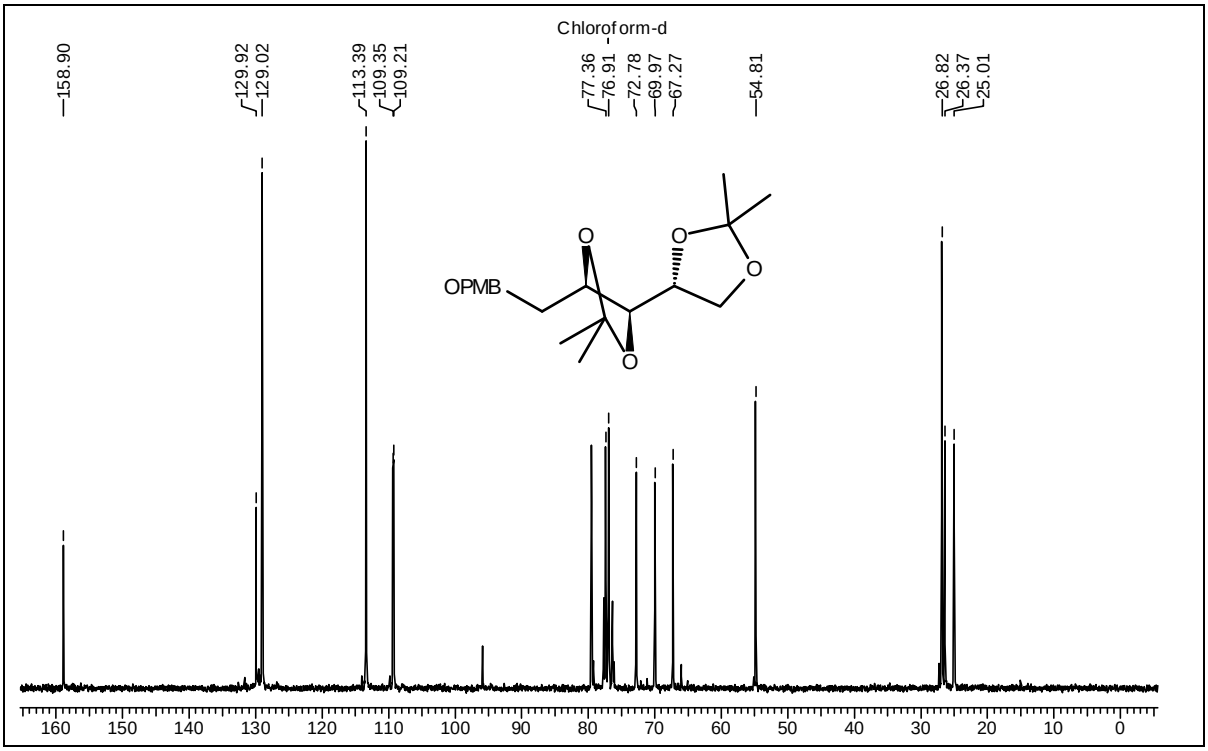
^{1}H NMR spectrum of compound 22 in $CDCl_3$



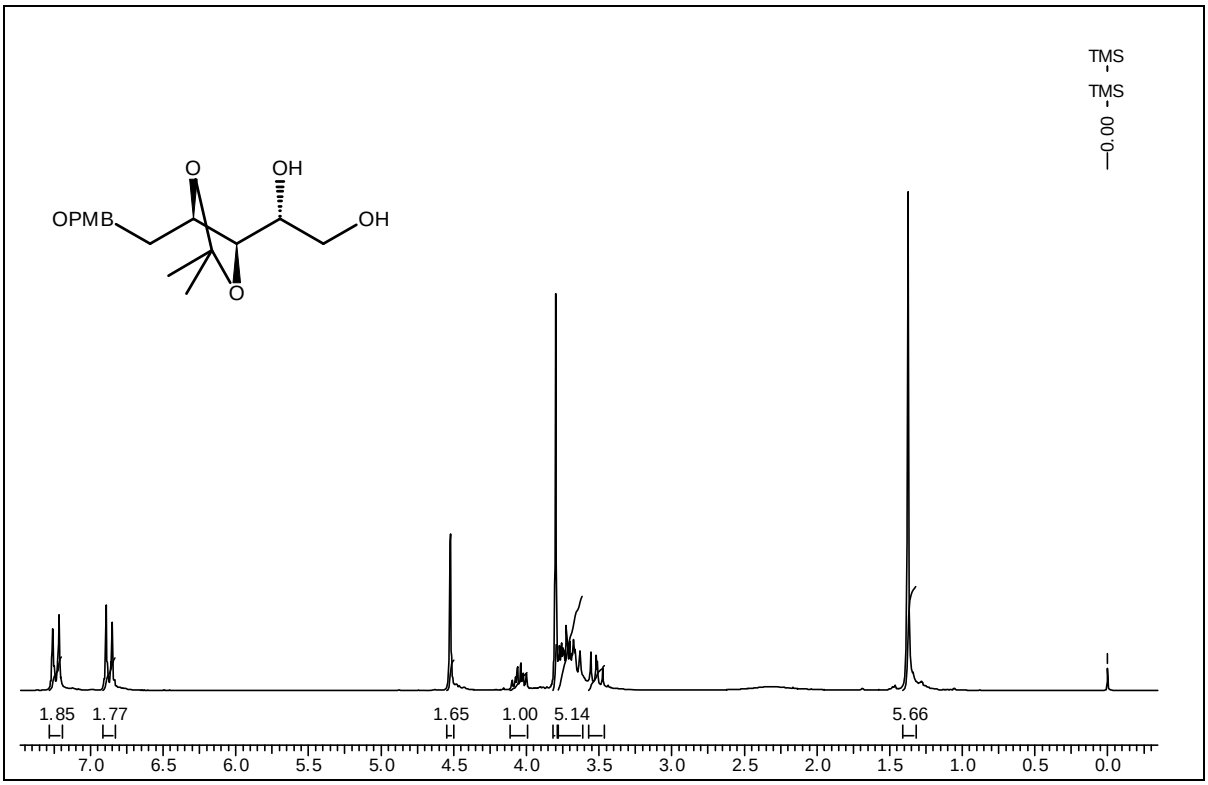
^{13}C NMR spectrum of compound 22 in $CDCl_3$



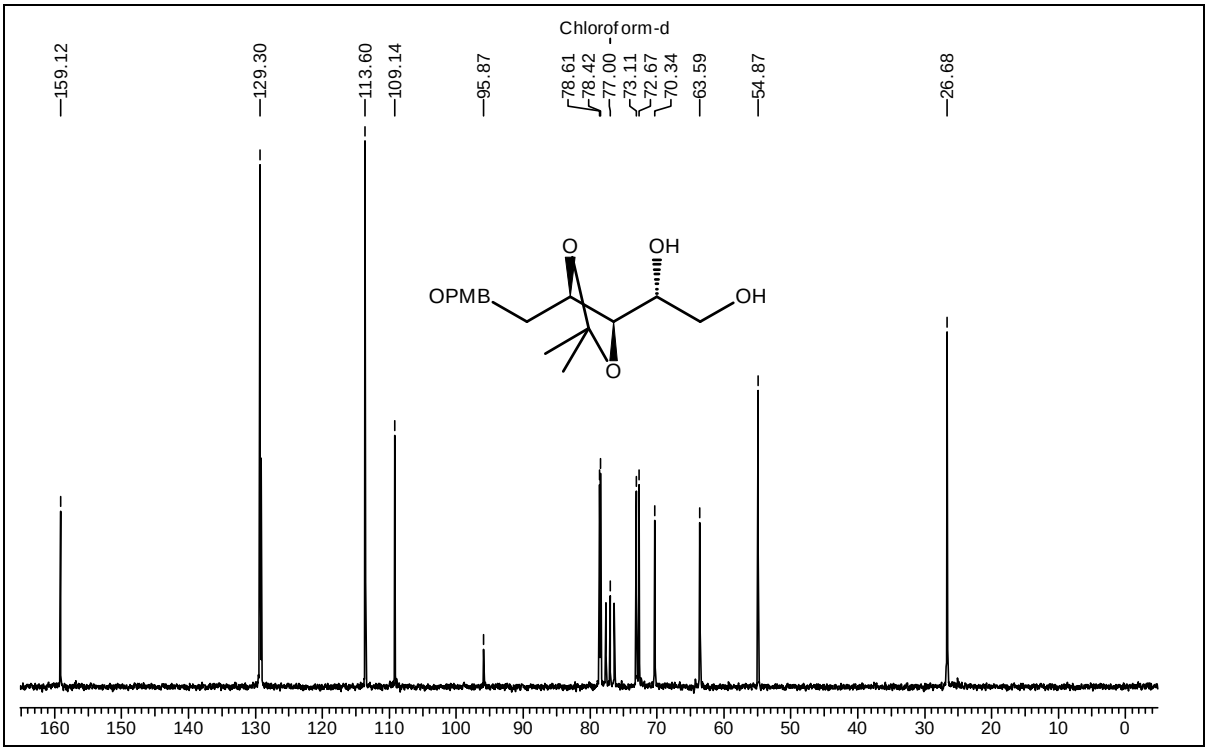
^{1}H NMR spectrum of compound 23 in $CDCl_3$



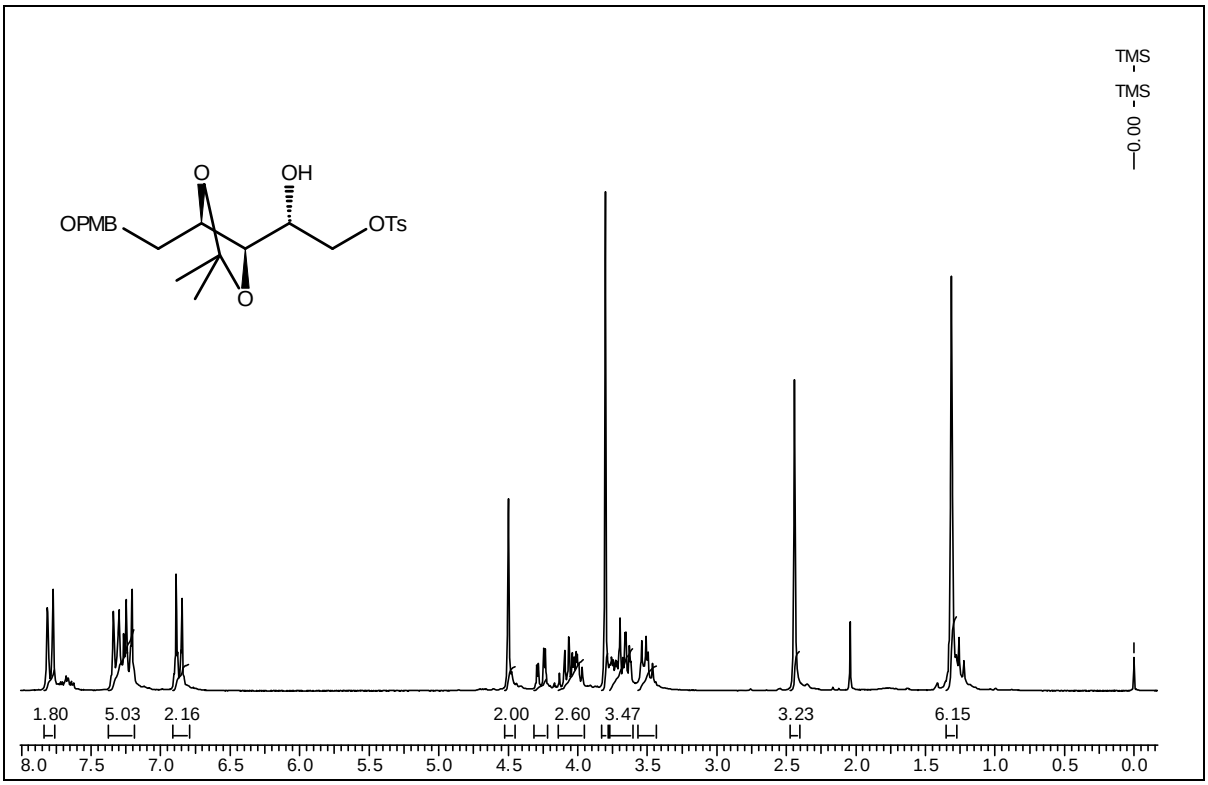
^{13}C NMR spectrum of compound 23 in $CDCl_3$



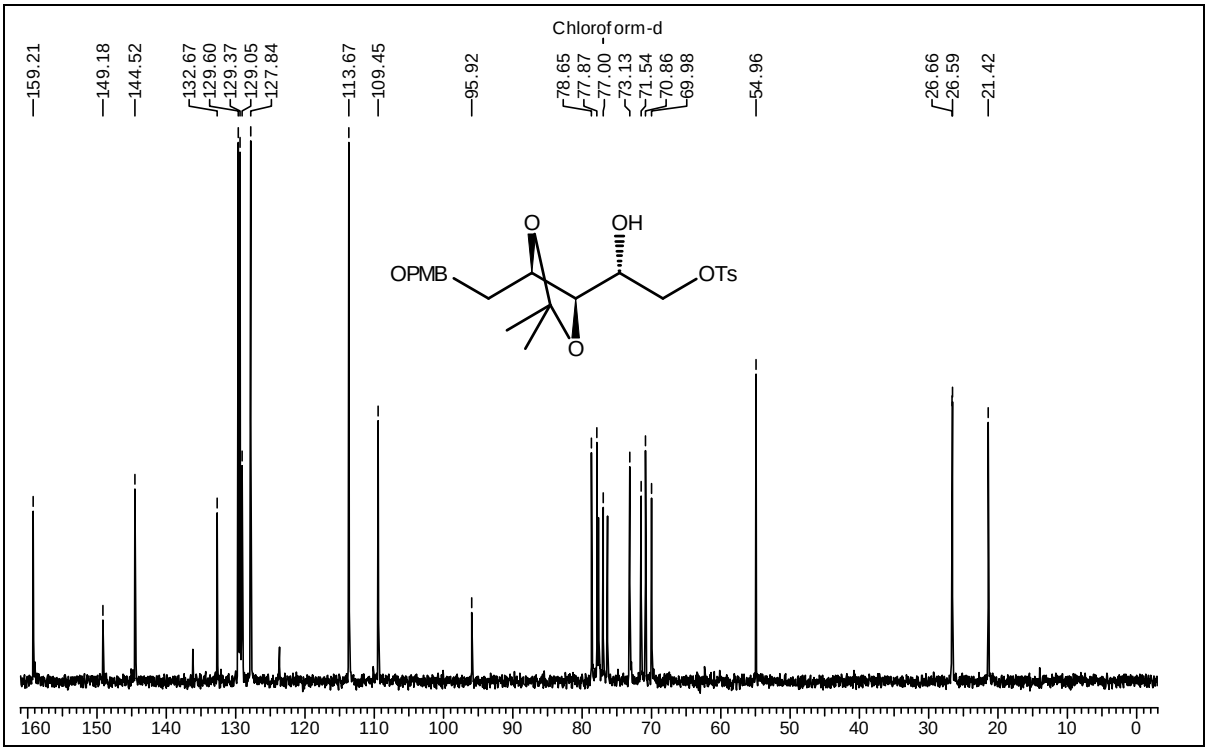
^{1}H NMR spectrum of compound 24 in $CDCl_3$



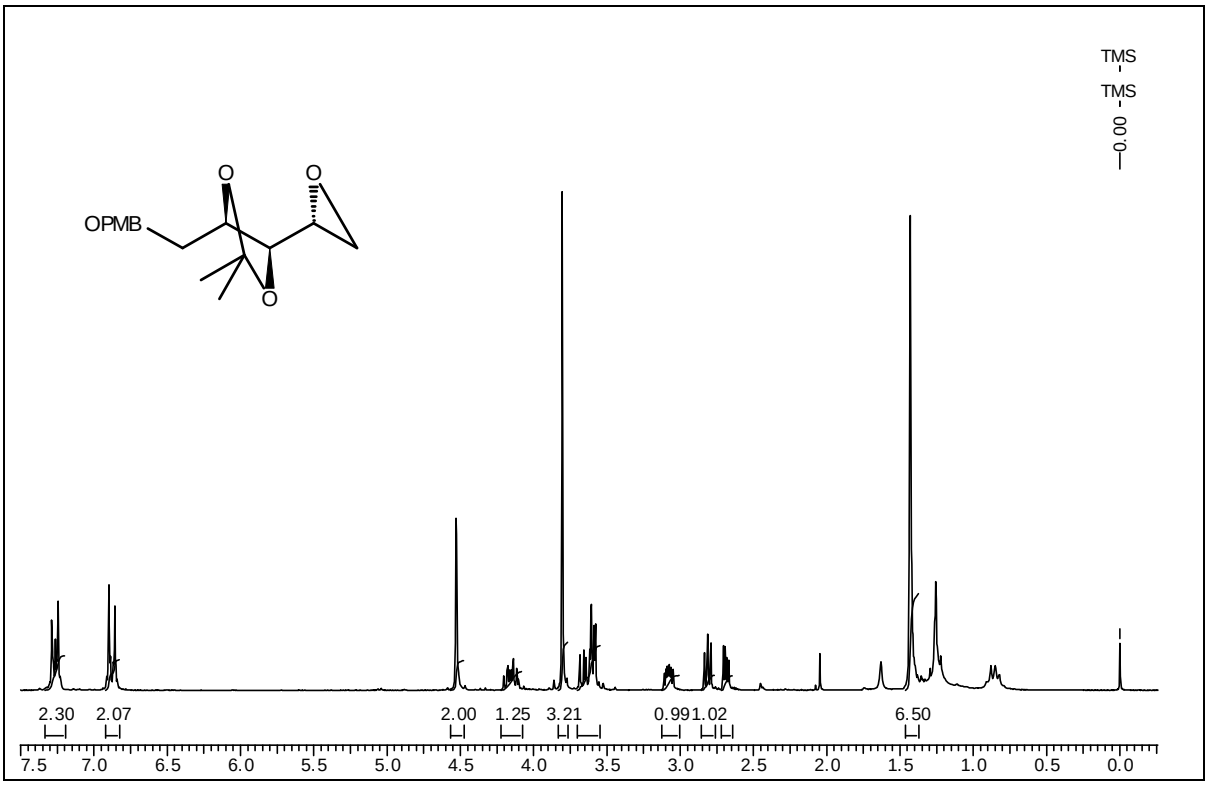
^{13}C NMR spectrum of compound 24 in $CDCl_3$



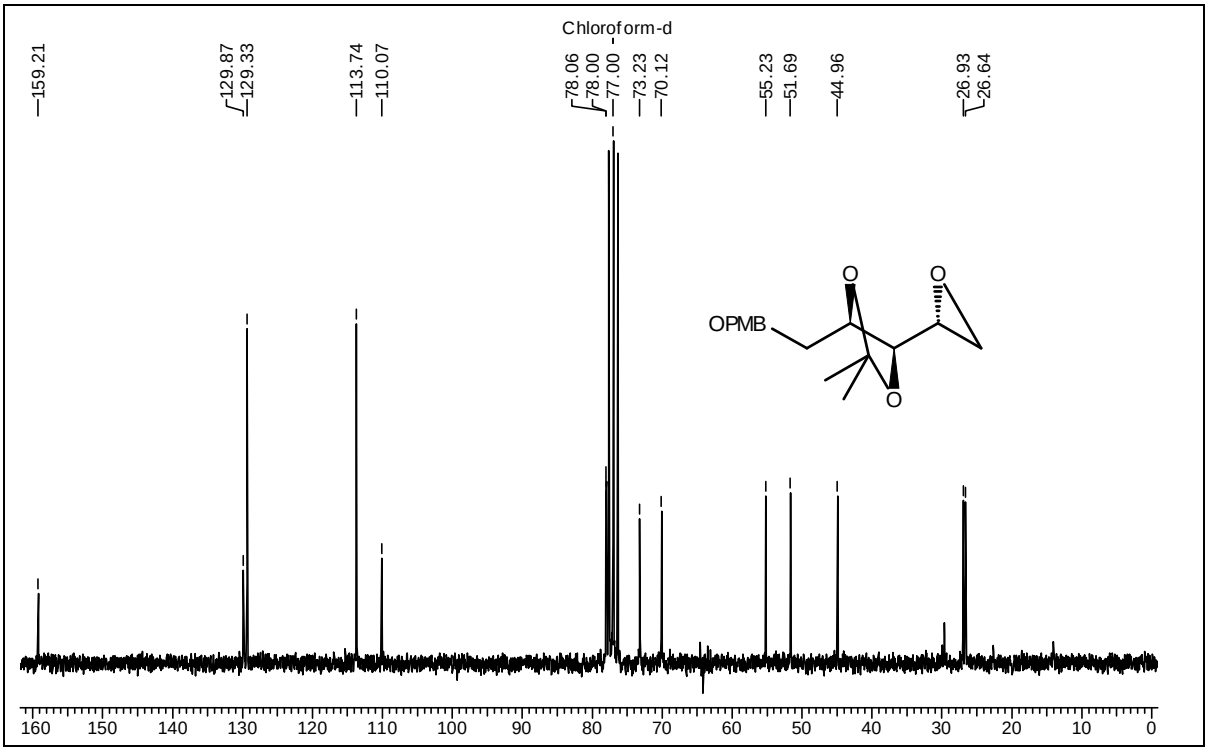
^{1}H NMR spectrum of compound 25 in $CDCl_3$



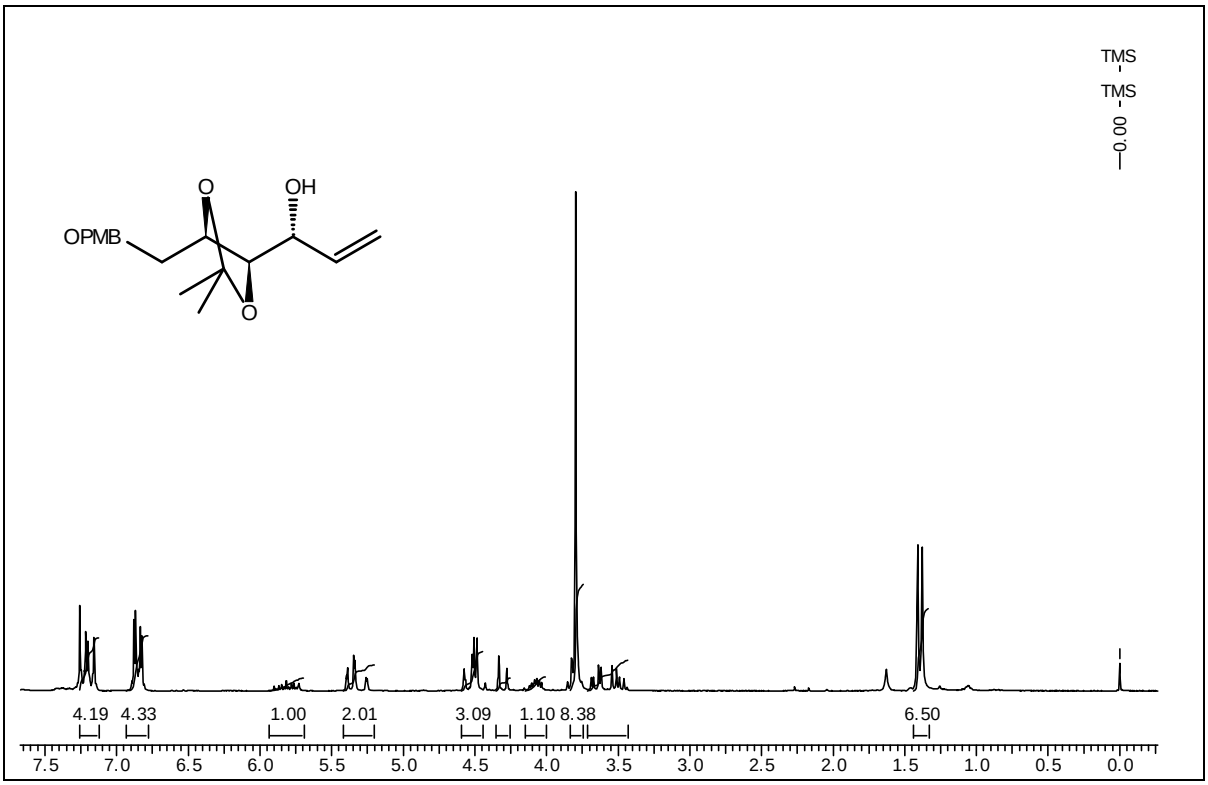
^{13}C NMR spectrum of compound 25 in $CDCl_3$



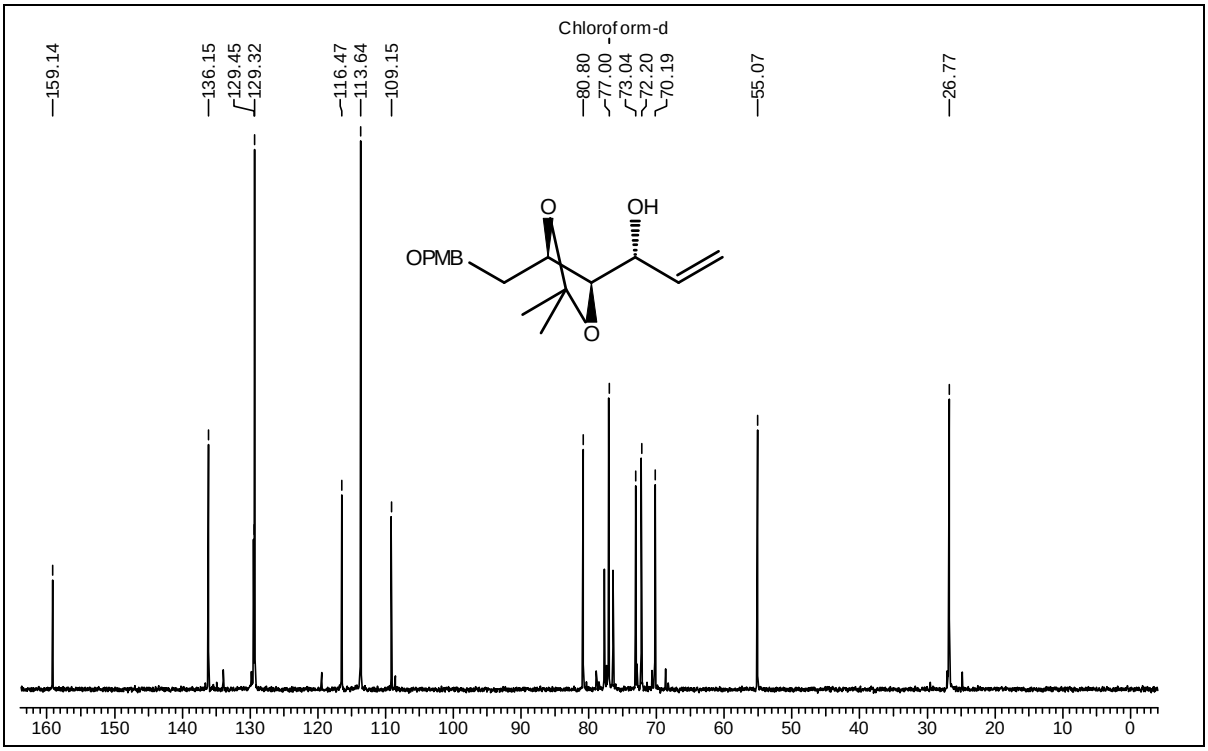
^{1}H NMR spectrum of compound 26 in $CDCl_3$



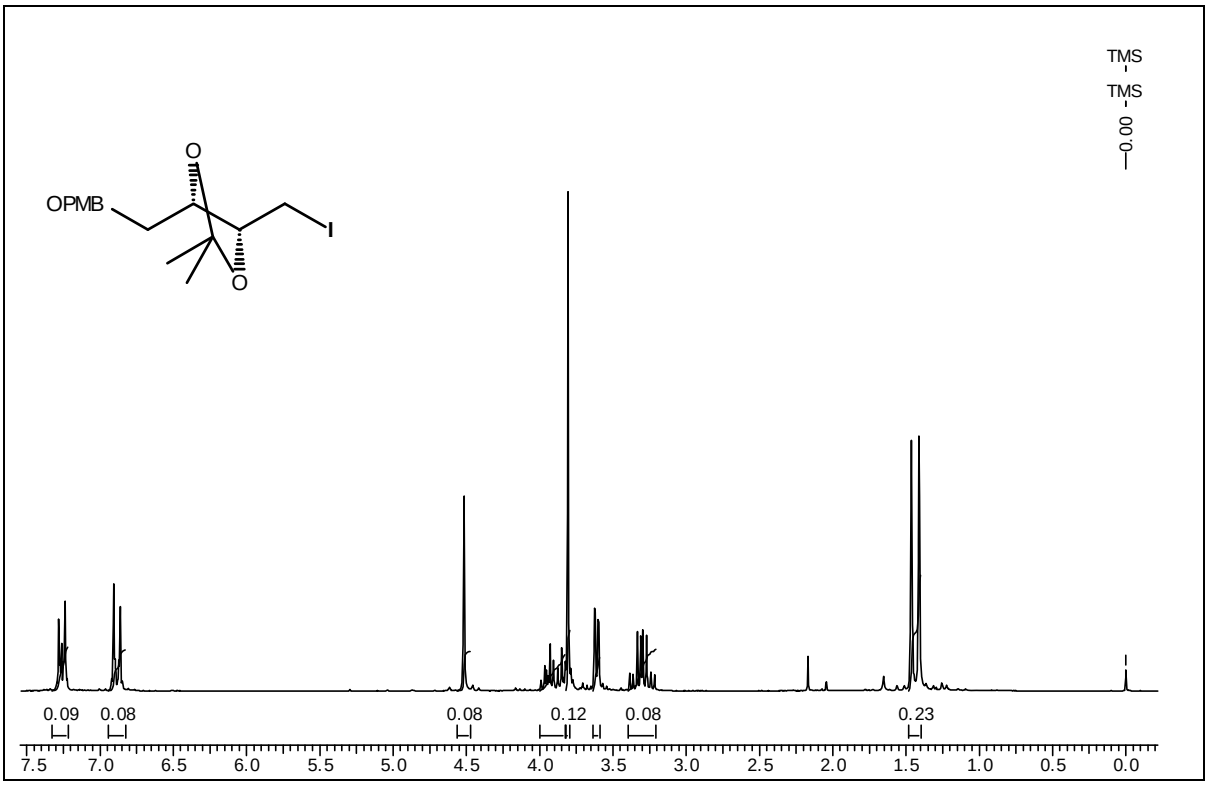
^{13}C NMR spectrum of compound 26 in $CDCl_3$



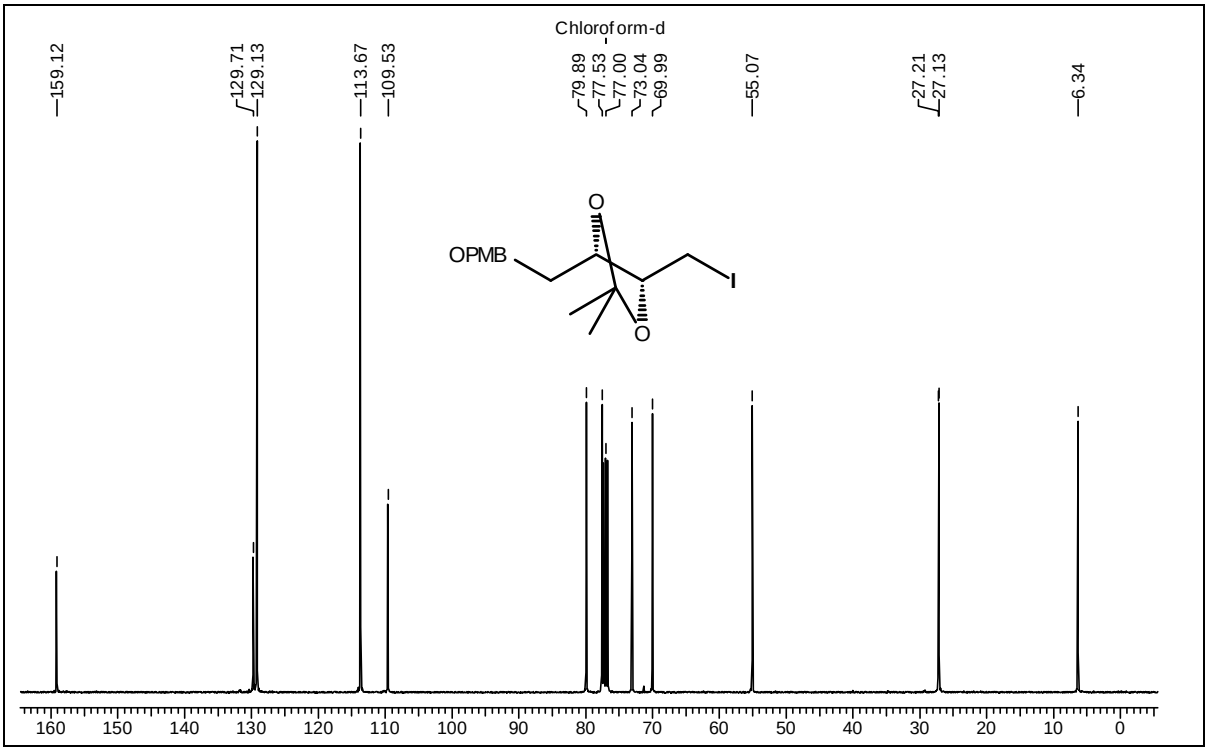
^{1}H NMR spectrum of compound 20 in $CDCl_3$



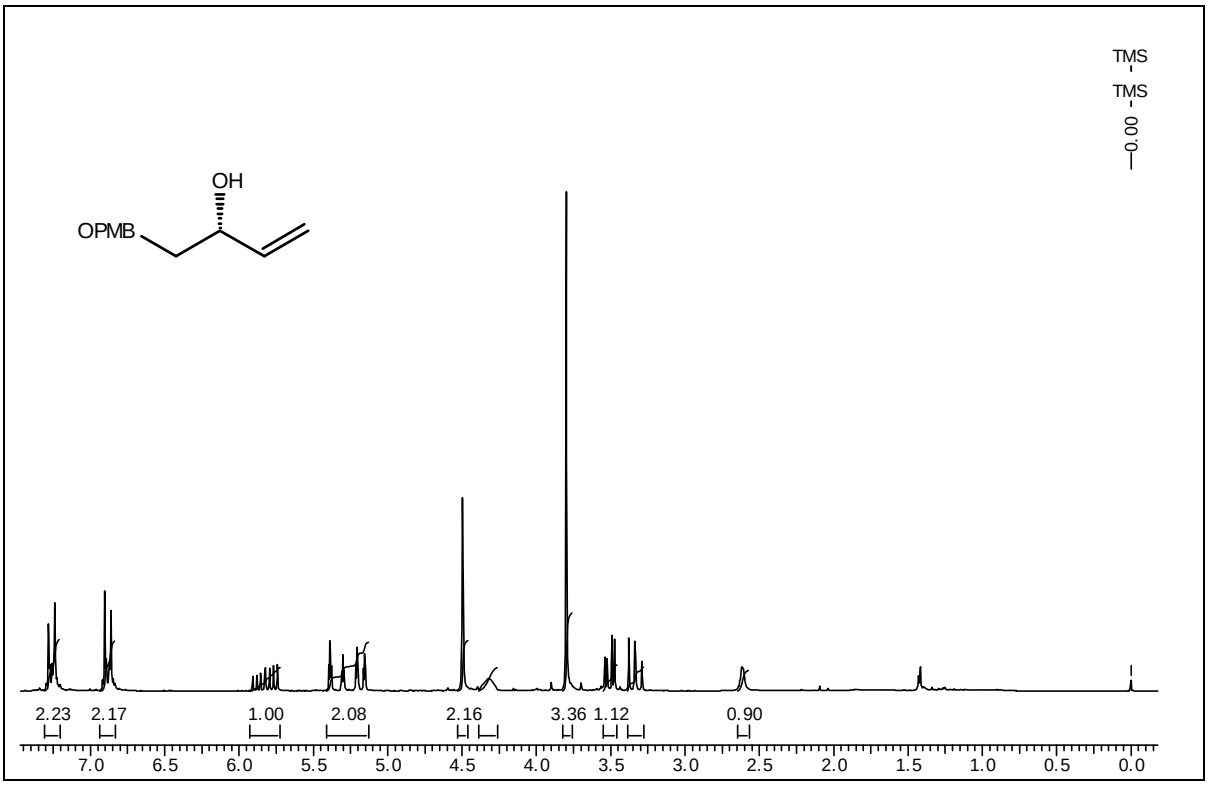
^{13}C NMR spectrum of compound 20 in $CDCl_3$



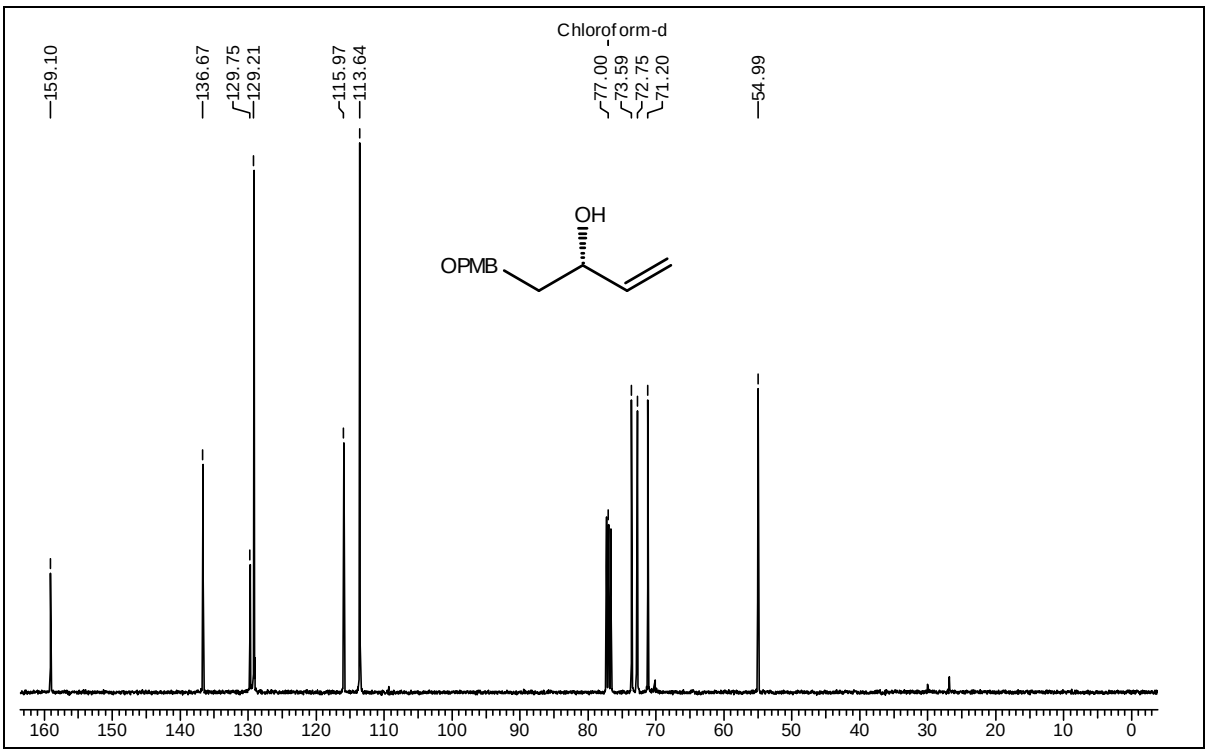
^{1}H NMR spectrum of compound 33 in $CDCl_3$



^{13}C NMR spectrum of compound 33 in $CDCl_3$



^{1}H NMR spectrum of compound 28 in $CDCl_3$



^{13}C NMR spectrum of compound 28 in $CDCl_3$

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12. Dependence of 2*J*C,H on the dihedral orientation of oxygen functions has been investigated (e.g., Schwarcz, J. A.; Cyr, N.; Perlin, A. S. *Can. J.Chem.* **1975**, *53*, 1872-1875).
13. The Δδ values of (**4b** - **4c**) were as follows: CDCl3, 500 MHz, H2- 37, -0.12/-0.06; H-38, -0.57; H-39, -0.14; H-40, +0.37; H2-41, +0.30/+0.37; H2-70, +0.33/+0.42. These data show that C39 has an *R* configuration, since the clear separation of

negative and positive values with respect to C39 shows that the Δδ values were predominately affected by the MTPA group on C39 rather than by other MTPA groups on relatively remote sites.

14. (*R*)-Methyl-1,4-butanediol was prepared from dimethyl-(*R*)-methylsuccinate by LiAlH4 reduction, followed by esterification with (*R*)- and (*S*)-MTPA. 1H NMR of **3b** was identical to that of the (*S*)-ester of the authentic diol; **3b** and (*S*)-ester (CDCl3, 500 MHz) *ä* 3.98, 3.92, 3.82, 3.73, 1.53, 1.38, 1.05, 0.52. (*R*)-ester *ä* 3.97, 3.93, 3.83, 3.70, 1.52, 1.38, 1.05, 0.52.
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16. Chiral resolution was performed on CHIRALPAK AD (*φ* 4.6 × 250 mm, DAICEL) eluted with hexane-1-propanol (25: 1). Authentic (*S*)-ester and the derivative from **1** (**5b**) were eluted at 16 min 50 sec and 16 min 59 sec, respectively, while (*R*)-ester appeared at 19 min 13 sec.
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List of Publications

LIST OF PUBLICATIONS

1. Mukund K. Gurjar, Bhargava Karumudi, and C. V. Ramana; *J. Org. Chem*, **2005**, *70*, 9658-9661. "Synthesis of Eupomatilone-6 and Assignment of Its Absolute Configuration "

2. Mukund K. Gurjar, Bhargava Karumudi and C. V. Ramana "First chemical synthesis of galactosyl maltobiono lactone LG-20: a potential mammalian β-amylase inhibitor" Manuscript to be submitted.

JOC*Note*

Synthesis of Eupomatilone-6 and Assignment of Its Absolute Configuration

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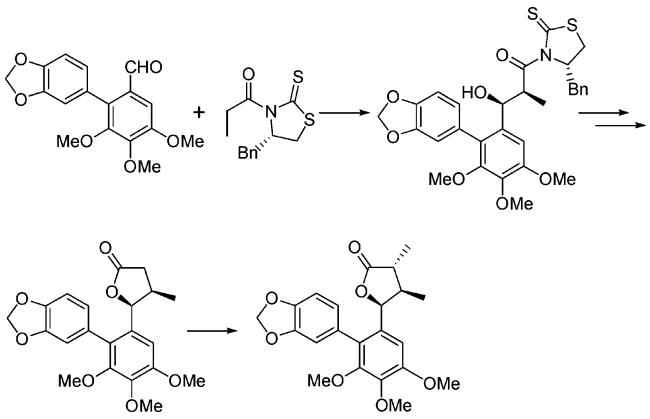

The Zn-mediated Barbier reaction of the biarylaldehyde **8** with crotyl bromide followed by hydroboration and oxidation provided the γ-butyrolactones **4** and **5**. The stereoselective installation of methyl group at C-3 by using LiHMDS and MeI completed the synthesis of racemic eupomatilone-6 (**2**) and its diastereomer **3**. The spectroscopic data of **2** was in full agreement with reported spectra of natural product, thus confirming the revised relative configuration of eupomatilone-6. Similarly, an optically active (3*R*,4*R*,5*S*)-isomer of eupomatilone-6 (**23**) was prepared in which the aldol reaction with thiazolidinethione as a chiral auxiliary was employed as a key step. On the basis of the spectroscopic data and optical rotation values of **23**, the absolute configuration of eupomatilone-6 was proposed.

In 1991, Taylor et al. isolated a series of novel lignans named as eupomatilones-1−7 from the shrubs *Eupomatia bennettii* F. Muell. Their relative configurations were elucidated by extensive NMR studies.[1] Eupomatilones are characterized by the biaryl system with a substituted γ-lactone ring attached to one of the aryl rings. Some of the eupomatilone molecules, including eupomatilone-6, exist as a mixture of fluctional atropisomers. Eupomatilones are the subject of intense synthetic activities.[2]

Earlier we reported the synthesis of a putative structure of eupomatilone-6 (**1**).[2b] The discrepancy in the spectral data of natural and synthetic eupomatilone-6 warranted revision of its relative stereochemistry as either 3α,4β,5β-configuration (**2**) or 3β,4α,5β-configuration (**3**). While the present work was in progress, Coleman and Gurrala[2d] reported the synthesis of racemic eupomatilone-6 and confirmed its relative stereochemistry as shown in structure **2**. Herein we disclosed a new synthetic strategy to prepare racemic eupomatilone-6 and then extended the protocol to synthesize optically active (+)-eupomatilone-6.

A retrosynthetic strategy for **2** and **3** is depicted in Figure 1. The stereoselective methylation at C-3 of the 4,5-disubstituted γ-butyrolactones **4** and **5** was envisaged to furnish 3,4,5-*trans-cis* and *trans-trans*-diastereomeric products (**2** and **3**).[3,4] The proposal to obtain both *cis*- and *trans*-4,5-disubstituted-γ-butyrolactones (**4** and **5**), respectively, from the *syn*- and *anti*-homoallylic alcohols **6** and **7** was envisioned as a straightforward proposition.[5] The proposed investigation on crotylation of the biaryl aldehyde **8**[2b] under Barbier[6]-type reaction conditions would give rise to both the requisite syn- and anti-products **6** and **7** (Scheme 1).

Treatment of **8** with crotyl bromide in the presence of zinc and ammonium chloride gave an inseparable 1:1 mixture of *syn*- and *anti*-homoallylic alcohols **6** and **7**. However, subsequent hydroboration−oxidation of **6/7** by using $BH_3{\cdot}SMe_2$ and H_2O_2 followed by treatment with 2,2-dimethoxypropane and PPTS gave the acetonide derivatives **11** and **12**, which were separated by silica gel chromatography. The ^1H NMR spectral analyses, particularly the characteristic chemical shifts and coupling constants of benzylic proton at C-5, were useful in assigning the relative stereochemistries as depicted in structures **11** and **12**. For example, **11** revealed signals of benzylic proton of the equilibrating atropisomers as singlets at 4.77 and 4.87 ppm, whereas it appeared as

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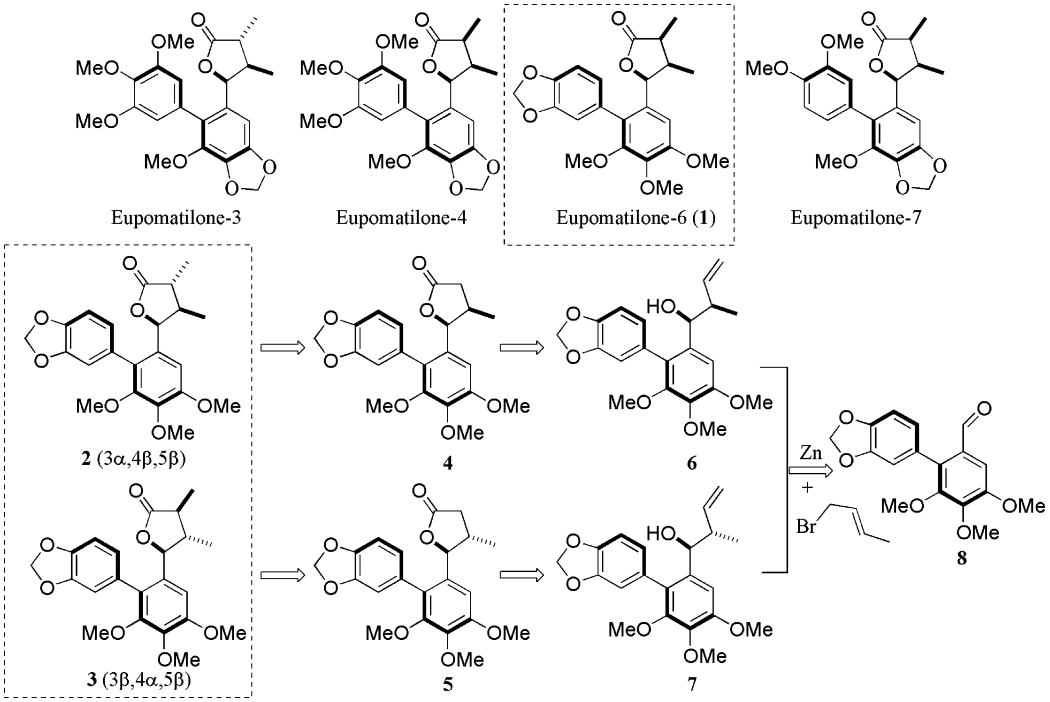
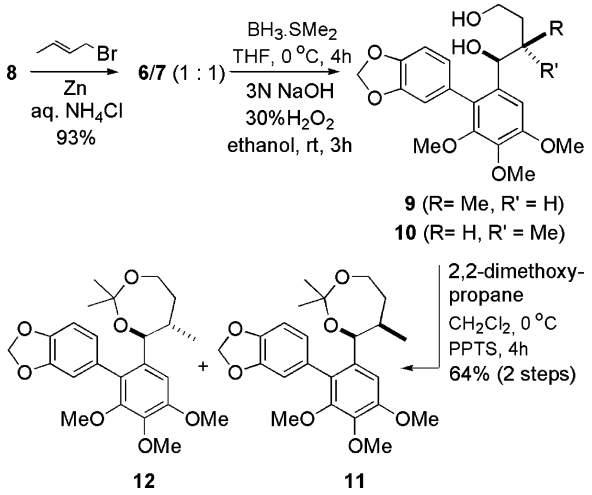


FIGURE 1. Representative natural eupomatilones and the alternative structures suggested for the eupomatilone-6 and the retrosynthetic strategy.

SCHEME 1. Synthesis of 1,4-Diols 9 and 10



doublets at 4.33 and 4.36 ppm with $J = 9.4$ Hz in the case of compound **12**.

The diol **9**, obtained by hydrolysis of **11**, was converted into the lactone derivative **4** by oxidation with NCS and TEMPO (Scheme 2).[2d,7] After screening several reagents, we observed that the diastereoselectivity of methylation at C-3 was highest with LiHMDS−MeI. The dialkylated product **13** was also isolated as a minor product. The spectral data of **2** was in complete agreement with the data published for the naturally occurring eupomatilone-6.[1,2d] Similarly, compound **12** was transformed by adopting the same strategy into diastereomeric eupomatilone **3** (Scheme 3). The ^{1}H NMR spectrum of **3** was substantially different from that of the natural eupomaltinone-6.

After successfully establishing the relative stereochemistry of racemic eupomatilone-6, we felt the need to design, on the basis of our new findings, the asymmetric synthetic protocol that would establish the absolute configuration of eupomatilone-6. It is pertinent to mention that eupomatilone-6 inherently exists in nature, as a mixture of atropisomers whose separation is still being precluded.

The titanium enolate of *N*-propionylthiazolidinethione **15** was reacted with the biarylaldehyde **8** to give **16**, whose free hydroxyl group was protected as TBS−ether **17**.[8] The reductive removal of the thiazolidinethione auxiliary from **17** with $NaBH_4$ in THF−EtOH gave **18**.[9] The Dess−Martin periodinane oxidation and consequent Wittig reaction with $PPh_3{=}CH_2$ gave the olefin derivative **19**, from which the TBS group was departed in the presence of 1 M solution *n*-Bu_4NF in THF to afford **20**. Compound **20** was subjected to successive hydroboration and oxidation to give the lactone derivative **22**. The chiral HPLC analysis (Chiracel-OD column) of **22** and **4** established the enantiomeric excess of 97%. The alkylation of **22** was executed by using essentially the same reaction (LiHMDS−MeI) as reported above to give (3*R*,4*R*,5*S*)-eupomatilone-6 (**23**), whose spectroscopic data was in full agreement with reported spectra (Scheme 4). The optical rotation observed {$[\alpha]^{25}_D$ 24.7 (*c* 0.5, $CHCl_3$)} for synthetic

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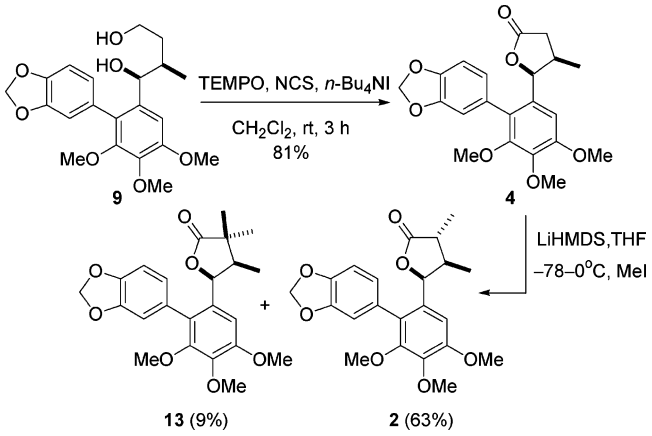
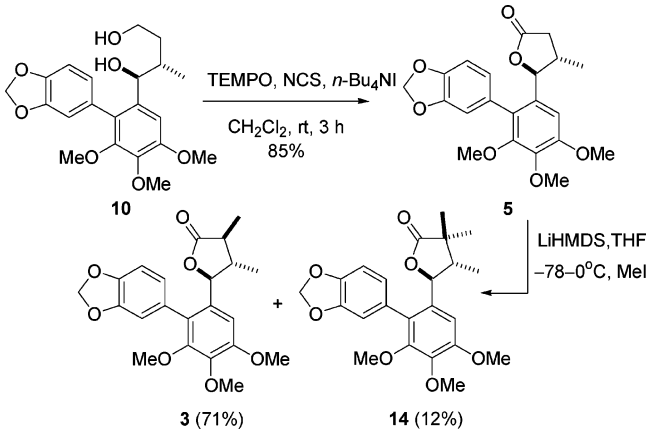
SCHEME 2. Synthesis of (±)-Eupomatilone-6 (2)



SCHEME 3. Synthesis of 3



product was in agreement with reported value {$[\alpha]^{25}_D$ −25.6 (*c* 0.5)}[1b] but with opposite sign. The minor quantity of dialkylated product (**24**) was also isolated and characterized.

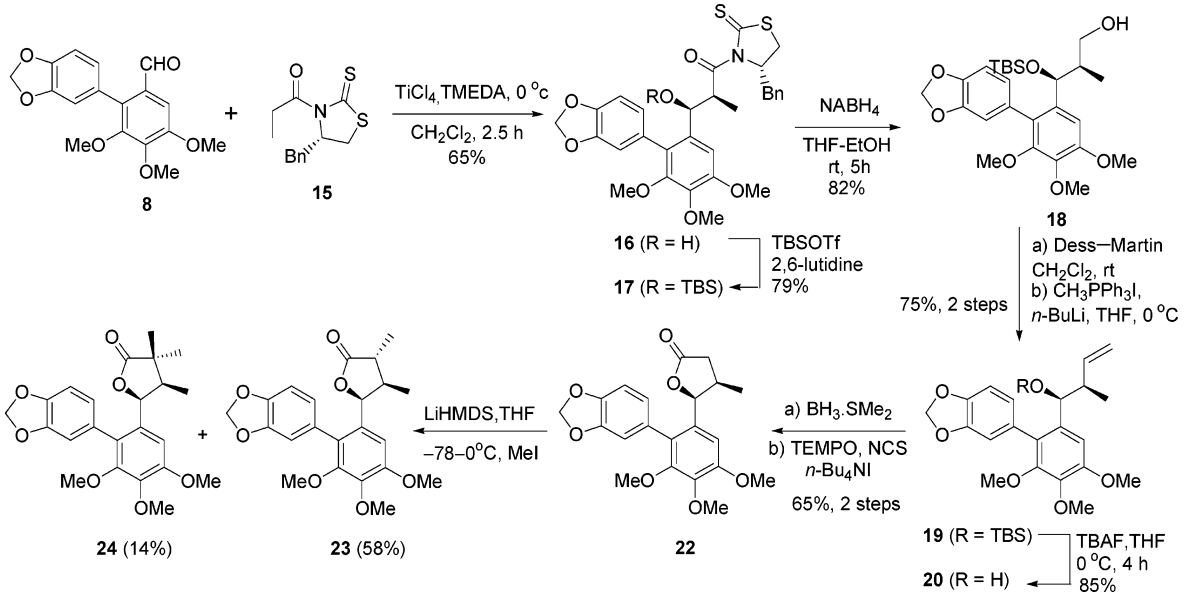
In summary, we have devised a four-step synthesis of eupomatilone-6 (**2**) and its diastereomer **3** that established the relative stereochemistry of the natural eupomatilone-6. Further studies on optically active (3*R*,4*R*,5*S*)-isomer (**23**) proposed the absolute configuration of naturally occurring eupomatilone-6 as (3*S*,4*S*,5*R*).

Experimental Section

Synthesis of *syn*-Lactone 4. A biphasic mixture of diol **9** (2 g, 5.1 mmol), TEMPO (80 mg, 0.51 mmol), *n*-Bu_4NI (190 mg, 0.51 mmol) and *N*-chlorosuccinimide (2.05 g, 15.4 mmol), aqueous $NaHCO_3$ (0.5M, 10 mL), and aqueous K_2CO_3 (0.05M, 10 mL) and CH_2Cl_2 (20 mL) was vigorously stirred for 3 h at room temperature. The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2. The organic layer was washed with brine, dried, and evaporated. The residue was purified by silica gel column chromatography by eluting with light petroleum/ethyl acetate (4:1) to give **4** (1.6 g, 81%). IR ($CHCl_3$): 3020, 1776, 1599, 1483, 1324, 1215, 1080, 1042, 929, 757, 669 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.68 (d, J = 7.3 Hz, 3H), 0.70 (d, J = 7.3 Hz, 3H), 2.20−2.30 (m, 4H), 2.69 (dd, J = 7.8, 6.3 Hz, 1H), 2.72 (dd, J = 7.8, 6.3 Hz, 1H), 3.65 (s, 3H), 3.66 (s, 3H), 3.91 (s, 12H), 5.42 (d, J = 5.6 Hz, 1H), 5.51 (d, J = 5.6 Hz, 1H), 6.02−6.03 (m, 2H), 6.04−6.05 (m, 2H), 6.58 (dd, J = 7.9, 1.6 Hz, 1H), 6.62 (d, J = 1.6 Hz, 1H), 6.70 (dd, J = 7.9, 1.6 Hz, 1H), 6.73 (d, J = 1.6 Hz, 1H), 6.81 (s, 1H), 6.82 (s, 1H), 6.85 (d, J = 7.9 Hz, 1H), 6.88 (d, J = 7.9 Hz, 1H). ^{13}C NMR (75 MHz): 15.2 (q), 15.3 (q), 33.6 (d), 33.8 (d), 37.7 (t), 56.0 (q), 60.6 (q), 60.9 (q), 81.9 (d), 101.0 (t), 104.8 (d), 108.0 (d), 108.2 (d), 109.5 (d), 110.5 (d), 122.3 (d), 123.3 (d), 126.3 (s), 126.4 (s), 128.9 (s), 130.0 (s), 141.6 (s), 146.8 (s), 146.9 (s), 147.5 (s), 151.4 (s), 152.7 (s), 176.1 (s) ppm. Maldi top Calcd for $C_{21}H_{22}O_7$: 386.14, obsd: 387.16 (M^+ + 1), 409.13 (M^+ + Na). Anal. Calcd for $C_{21}H_{22}O_7$: C, 65.28; H, 5.74%. Found: C, 65.21; H, 5.53%.

Synthesis of Eupomatilone-6 (2) and Its 3-Methylated Derivative 13. To a solution of **4** (0.50 g, 1.3 mmol) in anhydrous THF (5 mL) at −78 °C was added LiHMDS (1 M solution in THF, 1.55 mL). After 1 h, MeI (0.09 mL, 1.45 mmol) was added and stirring was continued for an additional 1 h at −78 °C. The reaction mixture was quenched with saturated ammonium chloride, extracted with ethyl acetate, dried over sodium sulfate, and concentrated. The crude residue was purified by silica gel chromatography by eluting with light petroleum/ethyl acetate (9:1) to give **13** (0.05 g, 9%) and **2** (0.33 g, 63%). Spectral data of eupomatilone-6 (**2**). IR ($CHCl_3$): 3429, 2932, 1773, 1596, 1457, 1406, 1326, 1226, 1197, 1127, 1040, 937, 754, 667 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.70 (d, J = 7.1 Hz,

SCHEME 4. Synthesis of (+)-Eupomatilone-6 (23)



3H), 0.73 (d, J = 7.1 Hz, 3H), 1.19 (d, J = 7.4 Hz, 3H), 1.20 (d, J = 7.4 Hz, 3H), 1.93−2.04 (m, 2H), 2.37 (sextet, J = 7.2 Hz, 2H), 3.63 (s, 3H), 3.64 (s, 3H), 3.89 (s, 6H), 3.90 (s, 6H), 5.53 (d, J = 6.9 Hz, 1H), 5.64 (d, J = 6.9 Hz, 1H), 6.01−6.03 (m, 4H), 6.59 (dd, J = 7.9, 1.6 Hz, 1H), 6.64 (d, J = 1.5 Hz, 1H), 6.66 (2s, 2H), 6.70 (dd, J = 7.9, 1.6 Hz, 1H), 6.72 (d, J = 1.5 Hz, 1H), 6.86 (d, J = 7.9 Hz, 1H), 6.88 (d, J = 7.9 Hz, 1H). ^{13}C NMR (125 MHz): 14.8 (q), 14.9 (q), 15.3 (q), 15.5 (q), 41.2 (d), 41.5 (d), 42.5 (d), 42.6 (d), 56.1 (q), 60.8 (q), 61.0 (q), 61.1 (q), 79.8 (d), 79.8 (d), 101.1 (t), 101.2 (t), 104.6 (d), 104.7 (d), 108.1 (d), 108.3 (d), 109.9 (d), 110.9 (d), 122.6 (d), 123.7 (d), 127.0 (s), 127.1 (s), 128.9 (s), 129.0 (s), 130.3 (s), 141.7 (s), 146.8 (s), 146.9 (s), 147.6 (s), 147.6 (s), 151.5 (s), 152.8 (s), 179.7 (s) ppm. Maldi top Calcd for $C_{22}H_{24}O_7$: 400.15, obsd: 400.57 (M^+), 401.57 (M^+ + 1), 423.57 (M^+ + Na). Anal. Calcd for $C_{22}H_{24}O_7$: C, 65.99; H, 6.04%. Found: C, 65.85; H, 5.91%.

Methylation of *anti*-Lactone 5. Compound **5** (0.5 g, 1.29 mmol) was subjected to essentially the same reaction conditions to produce **14** (0.068 g, 12%) and **3** (0.37 g, 71%). Spectral data of compound **3**. IR ($CHCl_3$): 3429, 2932, 1772, 1596, 1457, 1406, 1326, 1226, 1197, 1127, 1040, 937, 754 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (d, J = 6.0 Hz, 3H), 0.89 (d, J = 6.0 Hz, 3H), 1.23 (s, 3H), 1.26 (s, 3H), 1.92−2.24 (m, 4H), 3.62 (s, 3H), 3.63 (s, 3H), 3.91 (s, 12H), 4.78 (d, J = 9.4 Hz, 1H), 4.80 (d, J = 9.4 Hz, 1H), 6.01−6.03 (m, 4H), 6.61−6.73 (m, 6H), 6.83−6.87 (m, 2H). ^{13}C NMR (50 MHz): 12.9 (q), 14.3 (q), 43.3 (d), 47.6 (d), 56.1 (q), 60.8 (q), 60.9 (q), 61.0 (q), 82.5 (d), 82.6 (d), 101.1 (2t), 105.1 (d), 105.1 (d), 107.8 (d), 108.1 (d), 110.5 (d), 111.4 (d), 123.3 (d), 124.3 (d), 128.8 (s), 128.9 (s), 129.9 (s), 130.6 (s), 130.8 (s), 142.6 (s), 146.8 (s), 146.9 (s), 147.3 (s), 147.5 (s), 151.2 (s), 151.2 (s), 153.3 (s), 178.4 (s), 178.5 (s) ppm. Maldi top Calcd for $C_{22}H_{24}O_7$: 400.15, obsd: 400.57 (M^+), 401.57 (M^+ + 1), 423.57 (M^+ + Na). Anal. Calcd for $C_{22}H_{24}O_7$: C, 65.99; H, 6.04%. Found: C, 66.15; H, 6.07%.

Synthesis of 16 by Aldol Reaction between Biaryl Aldehyde (8) and Thiazolidine Thione (15). To a solution of *N*-propionylthiazolidinethione (**15**, 9.2 g, 34.7 mmol) in dry CH_2Cl_2 (90 mL) at 0 °C were added $TiCl_4$ (6.3 g, 33.4 mmol) and TMEDA (8.9 g, 87 mmol) and stirred for 20 min. To this dark red enolate solution was added biaryl aldehyde **8** (10.0 g, 31.6 mmol) in dry CH_2Cl_2 (30 mL) and stirring was continued for an additional 2 h at 0 °C. The reaction was quenched with aqueous NH_4Cl (60 mL), and the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layer was dried over sodium sulfate, filtered, and concentrated. Purification of the residue by column chromatography on silica gel with light petroleum/ethyl acetate (4:1) as eluent gave **16** (12 g, 65%). $[\alpha]^{25}_D$ +133 (c 1.0, $CHCl_3$). IR ($CHCl_3$): 3400, 3019, 1672, 1597, 1456, 1341, 1215, 1041, 938, 759, 668 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.23 (d, J = 7.0 Hz, 3H), 1.27 (d, J = 7.0 Hz, 3H), 2.79 (d, J = 11.4 Hz, 1H), 2.80 (d, J = 11.4 Hz, 1H), 2.87−3.19 (m, 8H), 3.56 (s, 3H), 3.58 (s, 3H), 3.86 (s, 6H), 3.94 (s, 6H), 4.31−4.39 (m, 2H), 4.72−4.89 (m, 4H), 5.94 (d, J = 1.3 Hz, 1H), 5.96 (d, J = 1.3 Hz, 1H), 6.02 (d, J = 1.5 Hz, 1H), 6.08 (d, J = 1.5 Hz, 1H), 6.59−6.66 (m, 4H), 6.78 (d, J = 7.7 Hz, 1H), 6.99 (d, J = 7.8 Hz, 1H), 6.98 (s, 1H), 6.99 (s, 1H), 7.19−7.39 (m, 10H). ^{13}C NMR (50 MHz): 11.3 (q), 11.5 (q), 32.4 (t), 32.5 (t), 36.2 (t), 44.4 (d), 55.8 (q), 60.5 (q), 60.7 (q), 60.8 (q), 68.7 (d), 68.8 (d), 71.5 (d), 71.7 (d), 100.7 (t), 101.0 (t), 105.2 (d), 105.3 (d), 107.4 (d), 108.2 (d), 110.0 (d), 111.6 (d), 122.4 (d), 124.1 (d), 127.0 (d), 127.2 (s), 128.7 (d), 128.8 (s), 129.1 (d), 134.7 (s), 134.8 (s), 136.1 (s), 141.3 (s), 141.3 (s), 146.4 (s), 146.5 (s), 147.0 (s), 147.3 (s), 151.0 (s), 151.1 (s), 152.5 (s), 152.5 (s), 178.2 (s), 178.3 (s), 200.2 (s), 200.5 (s) ppm. Anal. Calcd for $C_{30}H_{31}NO_7S_2$: C, 61.94; H, 5.37; N, 2.41; S, 11.02%. Found: C, 62.02; H, 5.54; N, 2.40; S, 11.29%.

Synthesis of 18. Compound **17** (5 g, 7.2 mmol) and $NaBH_4$ (540 mg, 14.3 mmol) in THF (25 mL) and EtOH (5 mL) were stirred at room temperature for 5 h. Excess borohydride was quenched at 0 °C with diluted HCl and concentrated. The residue was partioned between water−ether, and the organic layer was separated and washed with 1 M NaOH, H_2O, and brine, dried over sodium sulfate, and concentrated. The crude was purified on silica gel by using light petroleum/ethyl acetate (4:1) as an eluent to give **18** (2.91 g, 82%). $[\alpha]^{25}_D$ −8.5 (c 1.05, $CHCl_3$). IR ($CHCl_3$): 3389, 2930, 1598, 1481, 1402, 1384, 1322, 1232, 1125, 1040, 939, 872, 838, 757 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ −0.15 (s, 3H), −0.15 (s, 3H), 0.01 (s, 6H), 0.75 (d, J = 6.9 Hz, 3H), 0.78 (d, J = 6.9 Hz, 3H), 0.92 (s, 18H), 1.53−1.68 (br m, 2H), 3.33 (s, 2H), 3.36 (s, 2H), 3.62 (s, 3H), 3.63 (s, 3H), 3.89 (s, 6H), 3.90 (s, 6H), 4.80 (d, J = 2.7 Hz, 1H), 4.89 (d, J = 2.7 Hz, 1H), 6.01−6.04 (m, 4H), 6.60 (dd, J = 7.8, 1.7 Hz, 1H), 6.63−6.67 (m, 3H), 6.85 (d, J = 7.8 Hz, 1H), 6.87 (d, J = 8.1 Hz, 1H), 6.93 (s, 2H). ^{13}C NMR (50 MHz): −5.2 (q), −4.5 (q), 9.6 (q), 18.0 (s), 25.8 (q), 42.4 (d), 42.6 (d), 55.6 (q), 60.7 (q), 60.8 (q), 60.9 (q), 65.8 (t), 70.7 (d), 100.9 (t), 106.3 (d), 107.8 (d), 107.9 (d), 109.9 (d), 111.4 (d), 122.5 (d), 124.0 (d), 126.0 (s), 129.6 (s), 129.7 (s), 137.7 (s), 137.8 (s), 140.6 (s), 146.4 (s), 146.5 (s), 147.3 (s), 147.4 (s), 150.9 (s), 152.0 (s) ppm. Maldi top Calcd for $C_{26}H_{38}O_7Si$: 490.24, obsd: 513.57 (M^+ + Na). Anal. Calcd for $C_{26}H_{38}O_7Si$: C, 63.64; H, 7.81%. Found: C, 63.81; H, 7.76%.

Synthesis of 19. A suspension of alcohol **18** (2.0 g, 4.1 mmol) and Dess−Martin periodinane (2.08 g, 4.9 mmol) in CH_2Cl_2 (10 mL) was stirred at 0 °C for 1.5 h, filtered through Celite, and concentrated to procure the aldehyde (1.8 g, 90%). To a suspension of methyltriphenylphosphonium iodide (7.4 g, 18.3 mmol) in anhydrous THF (30 mL) at 0 °C was added *n*-BuLi (23 mL of 1.6 M in hexane). The mixture was stirred for 1 h at room temperature and then transferred into the above prepared aldehyde solution in THF (10 mL) maintained at 0 °C. The reaction mixture was allowed to attain room temperature and was further stirred for 12 h. The reaction was quenched with saturated aqueous NH_4Cl, extracted with ethyl acetate, dried (Na_2SO_4), and concentrated. Purification of the residue on silica gel by using light petroleum/ethyl acetate (19:1) furnished **19** (1.49 g, 75%). $[\alpha]^{25}_D$ −8.1 (c 1, $CHCl_3$). IR ($CHCl_3$): 3379, 2932, 2856, 1597, 1481, 1401, 1322, 1195, 1157, 1083, 1040, 938, 837, 775 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ −0.19 (s, 3H), −0.18 (s, 3H), −0.06 (s, 6H), 0.88−0.91 (m, 6H), 0.89 (s, 18H), 2.11−2.25 (m, 2H), 3.63 (s, 6H), 3.89 (s, 6H), 3.90 (s, 6H), 4.52 (d, J = 3.6 Hz, 1H), 4.57 (d, J = 3.6 Hz, 1H), 4.67 (ddd, J = 17.2, 1.8, 1.3 Hz, 2H), 4.82 (br. ddd, J = 10.2, 1.7, 0.9 Hz, 2H), 5.58 (ddd, J = 17.2, 10.2, 7.3 Hz, 1H), 5.60 (ddd, J = 17.2, 10.2, 7.3 Hz, 1H), 6.01−6.04 (m, 4H), 6.60−6.69 (m, 4H), 6.84−6.95 (m, 4H). ^{13}C NMR (50 MHz): −4.9 (q), −4.6 (q), 12.3 (q), 12.4 (q), 18.2 (s), 25.8 (q), 44.7 (d), 44.8 (d), 55.7 (q), 60.7 (q), 60.9 (q), 61.0 (q), 74.3 (d), 100.9 (t), 106.2 (d), 106.3 (d), 107.8 (d), 107.9 (d), 109.9 (d), 111.8 (d), 113.6 (t), 115.3 (d), 120.0 (s), 122.5 (d), 124.4 (d), 126.4 (s), 129.4 (d), 129.7 (s), 129.9 (s), 138.2 (s), 140.6 (s), 142.5 (d), 142.6 (d), 146.4 (s), 146.5 (s), 147.3 (s), 150.8 (s), 152.0 (s) ppm. Anal. Calcd for $C_{27}H_{38}O_6$ Si: C, 66.63; H, 7.87%. Found: C, 66.87; H, 7.78%.

Synthesis of (+)-Eupomatilone-6 (23) and 3-Methyl Derivative 24. To solution of **22** (0.10 g, 0.258 mmol) in anhydrous THF (1 mL) at −78 °C, 1 M LiHMDS solution in THF (0.3 mL) was added. After 1 h, MeI (0.02 mL, 0.32 mmol) was added and stirring was continued for an additional 1 h. The reaction mixture was quenched with saturated ammonium chloride, extracted with ethyl acetate, dried over sodium sulfate, and concentrated. The crude residue was purified by silica gel chromatography by eluting with light petroleum/ethyl acetate (9:1) to give **24** (0.015 g, 14%) and **23** (0.06 g, 58%).

23 $[\alpha]^{25}_D$ +24.7 (c 0.5, $CHCl_3$) Lit.[1b] {$[\alpha]^{25}_D$ −25.6 (c 0.5)}.

24 $[\alpha]^{25}_D$ −40.8 (c 0.5, $CHCl_3$).

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Supporting Information Available: Experimental procedures, analytical and/or spectral data for all new compounds (**5**−**7**, **9**−**14**, **17**), NMR spectra of **2**−**5**, **11**−**14**, and HPLC chromatograms for **2**, **23**, **4**, **22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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