Studies Toward the Total Synthesis of Radicamine B, Quercitols, Dolastatin 19 and Developing New Process for Sucralose.

A THESIS
SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
(IN CHEMISTRY)

TO

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BY

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(Research Guide)

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PUNE-411008

APRIL 2008

DEDICATED
TO
MY PARENTS,
BROTHER & SISTER.

DECLARATION

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

Organic Chemistry Division
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April 2008 **(Mr. Anuj Kumar Yadav)**

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CERTIFICATE

The research work presented in thesis entitled "**Studies Toward the Total Synthesis of Radicamine B, Quercitols, Dolastatin 19 and Developing New Process for Sucralose**" has been carried out under my supervision and is a bonafide work of **Mr. Anuj Kumar Yadav**. This work is original and has not been submitted for any other degree or diploma of this or any other University.

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- Anuj.

DEFINITIONS AND ABBREVIATIONS

Ac	-	Acetyl
AcOH	-	Acetic acid
Ac_2O	-	Acetic anhydride
AIBN	-	Azoisobutyronitrile
aq.	-	Aqueous
ATMS	-	Allyltrimethylsilane
Bn	-	Benzyl
BnBr	-	Benzyl bromide
$BH_3.Me_2S$	-	Boron dimethyl sulfide complex
BuLi	-	Butyl lithium
Dabco	-	1,4-Diazabicyclo[2.2.2]octane
DCC	-	Dicyclohexyl carbodiimide
DCM	-	Dichloromethane
DIBAL-H	-	Diisobutylaluminiumhydride
DIPEA	-	*N*,*N*-Diisopropylethylamine
DMDP	-	(2*R*,5*R*)-bis(Hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine
DMAP	-	4-Dimethylaminopyridine
DMP	-	2,2-Dimethoxypropane
DMF	-	*N*,*N*-Dimethylformamide
DMSO	-	Dimethyl sulfoxide
EDA	-	Ethyl diazoacetate
EtOH	-	Ethanol
Et	-	Ethyl
Et_2O	-	Diethyl ether
EtOAc	-	Ethyl acetate
Et_3N	-	Triethylamine
HMPA	-	Hexamethylphosphoramide
Im	-	Imidazole

LAH	-	Lithium aluminium hydride
m-CPBA	-	*meta*-Chloroperbenzoic acid
MeOH	-	Methanol
Me	-	Methyl
MeI	-	Methyl iodide
MEM	-	Methoxyethoxymethyl
MES	-	Mesitylenesulphonyl
MOM	-	Methoxymethyl
Ms	-	Methanesulfonyl
NIS	-	N-iodosuccinimide
NMO	-	4-Methyl morpholine *N*-oxide
NOESY	-	Nuclear overhauser effect spectroscopy
OD	-	Optical Density
ORTEP	-	Oak ridge thermal ellipsoid plot
Ph	-	Phenyl
Py	-	Pyridine
PTSA	-	*para*-Toluenesulfonic acid
r.t.	-	Room temperature
sat.	-	Saturated
TBDMS-Cl	-	*tert*-Butyldimethyl chlorosilane
TBME	-	*t*-Butyl methyl ether
TBTH	-	Tri-*n*-butyl tin hydride
TCDI	-	1,1′-Thiocarbonyldiimidazole
TES	-	Triethylsilane
TFA	-	Trifluoroacetic acid
THF	-	Tetrahydrofuran
TMSOTf	-	Trimethylsilyl trifluoromethanesulfonate
TPP	-	Triphenylphosphine
TsCl	-	*para*-Toluenesulphonyl chloride
WC	-	Wilkinson’s Catalyst

GENERAL REMARKS

- ^{1}H NMR spectra were recorded on AV-200 MHz, AV-400 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 AV-50 MHz, AV-100 MHz, and DRX-125 MHz spectrometer.
- EI Mass spectra were recorded on Finngan MAT-1020 spectrometer at 70 *eV* using a direct inlet system.
- The X-Ray Crystal data were collected on *Bruker SMART APEX* CCD diffractometer using Mo K_{α} radiation with fine focus tube with 50 kV and 30 mA.
- 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 developing agents.
- 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 Büchi rotary evaporator below 40 °C unless otherwise specified.
- Silica gel (60–120), (100-200), and (230-400) mesh were used for column chromatography.
- Different numbers were assigned for compounds in Abstract and Chapters.

CONTENTS

Abstract

Abstract

The thesis entitled **"Studies Toward the Total Synthesis of Radicamine B, Quercitols, Dolastatin 19 and Developing New Process for Sucralose"** consists of three chapters; and the third chapter is sub-divided into two parts. All the chapters (each part of chapter III also) in general are further sub-divided as Introduction, Present Work, Experimental, Spectroscopic data and References. Chapter I deals with the total synthesis of Radicamine B and its three possible diastereomers using a combination of chiral pool approach and Sharpless asymmetric dihydroxylation. Utilisation of quinic acid as a chiral building block for the syntheses of *quercitols and L-carbarhamnose* constitutes the Chapter II. Chapter III incorporates a new method for sucralose as the first part and the chiral pool synthesis of an advanced pyran intermediate in the process of total synthesis of dolastatin 19 as the second part.

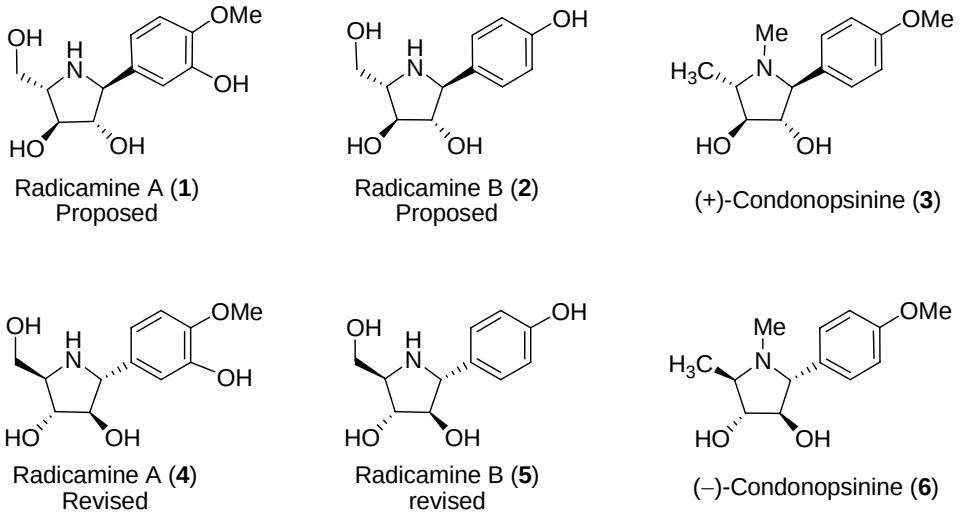
Chapter I:

Synthesis of Radicamine B and its Diastereomers

Radicamines belong to a family of polyhydroxy 2-aryl pyrrolidines that also include codonopsinines. These natural products share a common structural motif which is evident in their structures (Figure 1). Radicamines are isolated from the herb *Lobelia chinensis Lour*. which is prescribed in chinese folk medicine as a diuretic, an antidote, hemostat, and as carcinostatic agents for stomach cancer. All the substituents of the pyrrolidine ring are *trans* to each other. It shows inhibitory activities against α-glucosidase. When the present total synthesis is in advanced stage, the absolute structures of radicamines A and B have been revised as the enantiomers by total synthesis.

By virtue of the simple structural unit and promising biological activity, radicamines are attractive targets for total synthesis as there is a lot of scope for structural variation and subsequent biological activity dependence. Considering this we have devised a strategy for the total synthesis of radicamine B **2** with sufficient flexibility to procure all the possible diastereomers.

Figure 1. *Proposed Structures of Radicamines A (**1**) and B (**2**) & Revised Structures after the Synthesis*



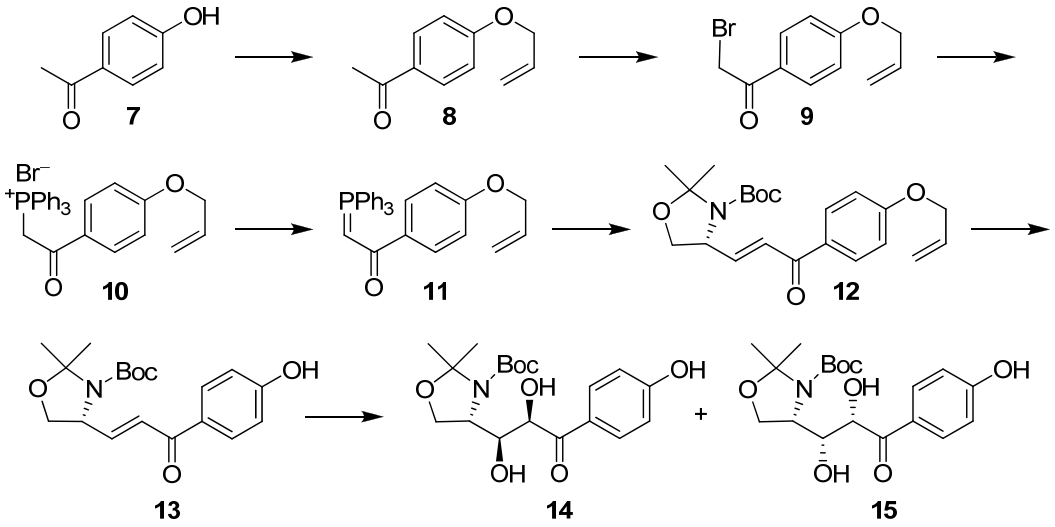
The intended total synthesis uses intramolecular reductive amination as the key step. Sharpless asymmetric dihydroxylation of an α,β-unsaturated ketone **13**, and a Wittig reaction of stable ylide **11** and Garner aldehyde to prepare the key intermediate **12** (Scheme 1).

According to known procedures, the L-Garner aldehyde was prepared from L-serine in four steps. The preparation of the key ylide **11** started with the allylation of *p*-hydroxy acetophenone **7** to give the allyl protected derivative **8**. The alpha-brominated product **9** was obtained on treatment with $CuBr_2$ which was further converted to its ylide **11** through the triphenylphosphonium salt **10**.

After a couple of experiments, the proposed Wittig reaction of Garner aldehyde and ylide **11** could be carried out successfully in anhydrous benzene and resulted in enone **12** with exclusive *E*-configuration. Again, for deallylation reaction after a substantial experimentation using different reagents and conditions it was successfully carried out by employing Wilkinson's catalyst and Dabco in refluxing ethanol:benzene:water (7:3:1) to afford the key substrate **13**. The dihydroxylation of **13** needs a special mention. Simple dihydroxylation by using OsO_4 and NMO in acetone:water (3:1) gave the dihydroxylated derivatives **14** and **15** in 55:45 ratio (determined from ^{1}H NMR), whereas dihydroxylation with AD-mix-α furnished

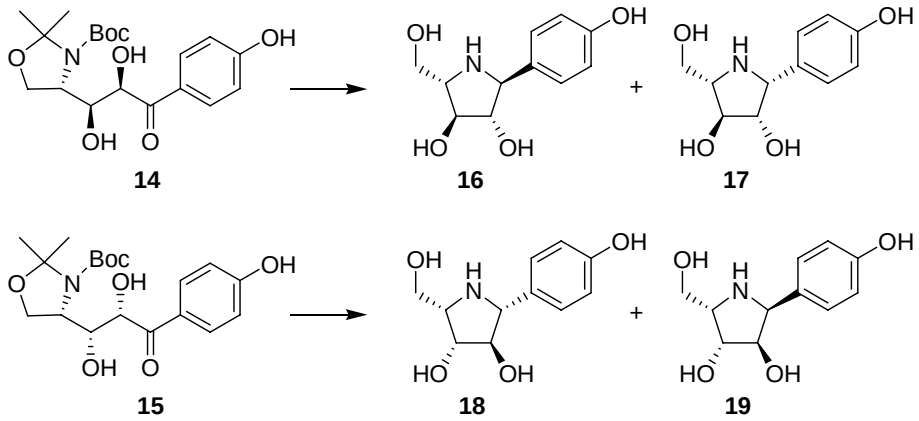
compound **14** selectively (80:20). Similarly, to procure **15,** AD-mix-β was employed but the selectivity was poor towards **15** (40:60). The relative stereochemistries of vicinal diol **14** was determined with the help of single crystal X-ray analyses.

Scheme 1



The final proposed intramolecular reductive amination of the diastereomers **14** and **15** were carried out by initial deprotection of acetonide/-Boc groups with 90% TFA followed by sodium cyanoborohydride in the presence of acetic acid to procure pyrrolidines **16** and **17** (65:35, determined by HPLC) from **14**, and **18** and **19** from **15** (85:15), which were separated by HPLC using C-18 column (Scheme 2). The structures of all the four diastereomers were assigned from NMR studies (COSY/NOESY).

Scheme 2



The relative glycosidase inhibitory activity of all the four diastereomers were studied against the enzymes α-glucosidase (yeast), β-glucosidase (almond), α-galactosidase (green coffee beans) and α-mannosidase (jack bean). Only **16** & **17** were found to show appreciable activity (micromolar range) against α-Glucosidase (Table 1).

Table 1

Compounds	α-Glucosidase IC_{50}/K_i (μM)	β-Glucosidase IC_{50}/K_i (μM)	α-Galactosidase IC_{50}/K_i (μM)	α-Mannosidase IC_{50}/K_i (μM)
16	64/64	NI	9.0%*	NI
17	386/390	NI	5.0%*	NI
18	13.1%*	NI	10.0%*	NI
19	32.3%*	NI	10.0%*	NI

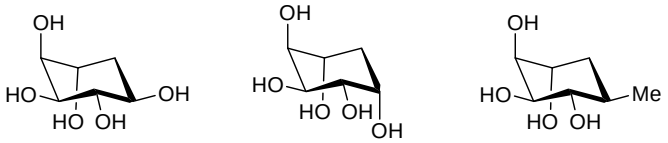
**) Percentage inhibition checked at 1mM inhibitor concentration;*
NI) No inhibition at 1mM concentration of inhibitors.

Chapter II:

Synthesis of (+)-*proto*, (–)-*gala* Quercitols and carba-L-rhamnose

Cyclitols (Figure 2) have attracted a great deal of attention from synthetic community due to their glycosidase inhibition activities and their versatility as synthetic intermediates. Quercitols are cyclohexanepentols, can exist in 16 diastereomeric forms, of which four are symmetric. Only (+)-*proto*, (–)-*proto* and (–)-*vibo*-quercitols are found in nature. In continuation of our current interest in the area of glycosidase inhibitors of natural origin, we undertook a program to address the simple and scalable synthesis of (+)-*proto* **20**, (–)-*gala* **21** quercitols and carba-L-rhamnose **22**, and D-(–)-quinic acid **23** has been identified as key chiral building block.

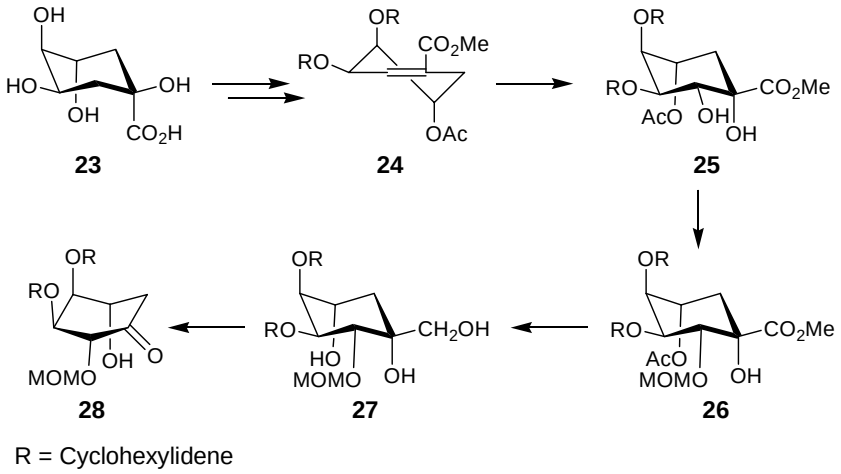
Figure 2. *Structures of quercitols and carba-L-rhamnose*



(+)-*proto*-quercitol (**20**) (–)-*gala*-quercitol (**21**) Carba-L-rhamnose (**22**)

The synthesis started from D-(–)-quinic acid **23** as shown in scheme 3. Quinic acid was converted to the shikimic ester derivative **24** by known literature procedures in four steps. Dihydroxylation of **24** with cat. OsO_4/NMO gave diol **25** in 70% yield as a single diastereomer. The secondary –OH group at C-2 was selectively protected as its MOM-ether and resulting compound **26** was subjected to LAH reduction followed by periodate cleavage to give the ketone **28** in quantitative yield.

Scheme 3



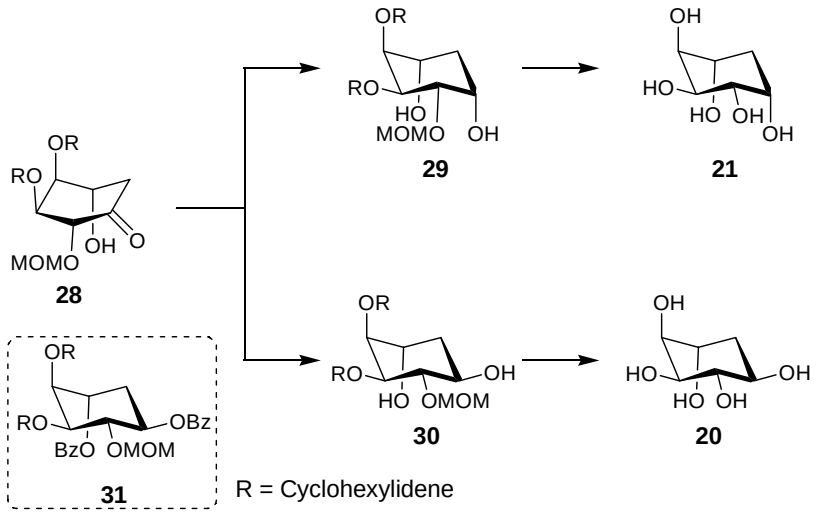
At this point, we anticipated that the presence of cyclohexylidene at C-3 and C-4 would direct the hydride attack from the *Si* face (Scheme 4) under non-chelation conditions on one hand and from the *Re* face if chelating (with C-5 hydroxyl group) reductant is employed on the other hand. We were, indeed, gratified to see that Sodium borohydride reduction of ketone **28** at –40 ^{o}C in methanol gave diol **30** as a single diastereomer whereas the reduction of **28** with K-Selectride in dry THF at –78 ^{o}C afforded diol **29** exclusively.

In order to establish the stereochemistry of newly formed chiral center at C-1, the diol **30** was converted to the corresponding dibenzoate **31** (Scheme 4). The configuration at C-1 was assigned based on the coupling constants of H-1 with H-2 and H-6a (J = 8.3 Hz and 12.6 Hz respectively). This was further confirmed by NOESY experiment.

The deprotection of the diol **30** was carried out with cat. HCl in MeOH at room temperature to give (+)-*proto* quercitol **20**. The analytical data of the synthetic material

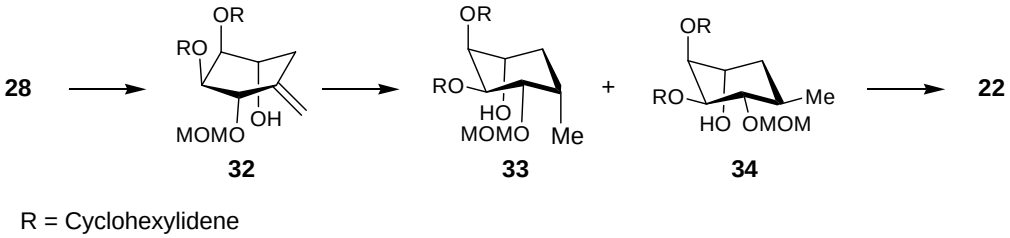
was in excellent agreement with the literature values. Deprotection of **29** with cat. HCl in MeOH furnished (–)-*gala* quercitol **21**, who's spectral and analytical data were found to be in excellent agreement with the reported values.

Scheme 4



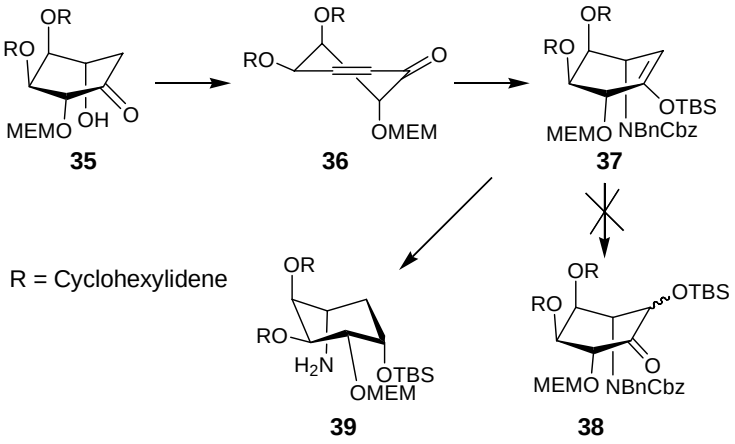
Having accomplished the synthesis of **20** & **21**, we turned our attention towards the synthesis of **22** from the keto derivative **28** (Scheme 5). The ketone **28** was converted to the exo-olefin **32** by employing Corey-Winter protocol (Scheme 5). Catalytic hydrogenation of **32** with 10% Pd/C at 40 psi in methanol afforded a diastereomeric mixture of **33** and **34** in the ratio 7:93. Global deprotection with cat. HCl in methanol gave **22** in excellent yield whose analytical data was in agreement with the reported data.

Scheme 5



Encouraged with the above results we tried to synthesize amino inositols (Scheme 6). The MEM protected derivative **35** was converted to the enone **36** by mesylation and elimination reaction with TEA. Michael type addition reaction was carried out with the anion of BnNHCbz and the enol formed was trapped with TBS protecting group to furnish **37**. All the attempts towards epoxidation of **37** employing various reagents and conditions were met with failure. Even the attempted dihydroxylation reaction with cat. OsO_4 and NMO was also unsuccessful. **37** was reduced with 10% Pd/C-H_2 to give amine tetrol **39**.

Scheme 6

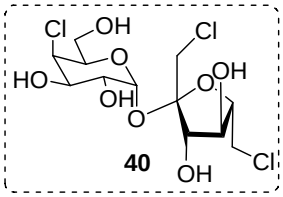


Chapter III:

Section I: A New Process for Sucralose

Sugar-free or reduced-sugar foods and beverages are very popular in the developed countries, and the sweeteners that make them possible are amongst the most conspicuous ingredients in the food supply. So, there is a need for an artificial, inexpensive, non-toxic and non-nutritive agent which is suitable for diabetics and also non-cariogenic. Sucralose **40** (Figure 3) qualifies all these parameters.

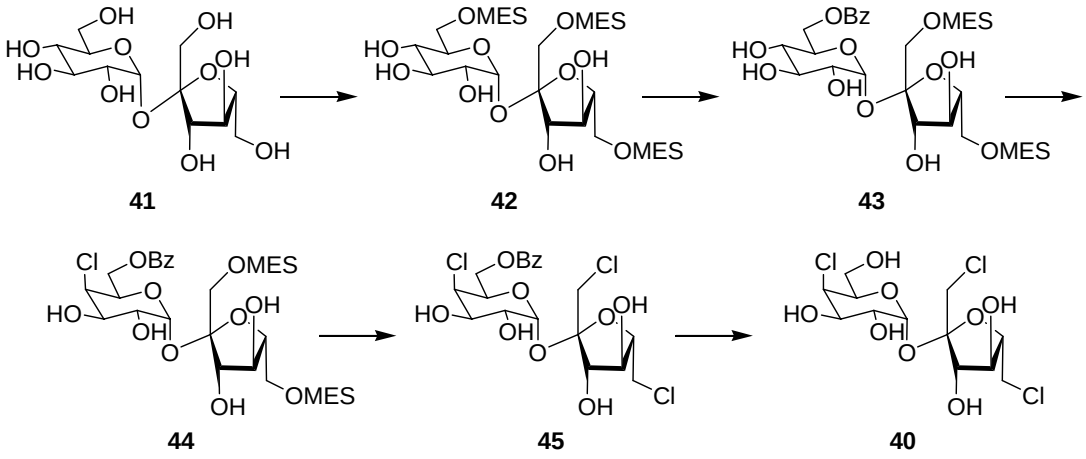
Figure 3. *Structure of Sucralose*



Although, there is considerable literature precedence on the synthesis of sucralose, we felt that scope still existed to develop a practical and efficient synthetic route to this compound in bulk quantities.

The synthesis started from commercially available sucrose **41** by taking advantage of the differences in reactivities amongst the hydroxyl groups (6 ~ 6′ > 4 > 1′ >> all other positions). Thus, all the three primary hydroxyl groups of sucrose **41** were converted to mesitylene sulfonate ester **42** on treatment with mesitylene sulphonyl chloride in pyridine. Selective displacement of C-6 mesitylsulfonate with benzoyl group was effected using sodium benzoate in DMF. Treatment of the resultant compound **43** with sulphuryl chloride in chloroform-pyridine mixture at −78 ^{o}C furnished monochloro compound **44**. The 1',6'-dimesityl sulfonate esters were substituted by chloride ion using lithium chloride and catalytic iodine in DMF to give **45**. The final C-6 benzoate group of **45** was deprotected by catalytic potassium carbonate in methanol to complete the synthesis of sucralose **40** (Scheme 7).

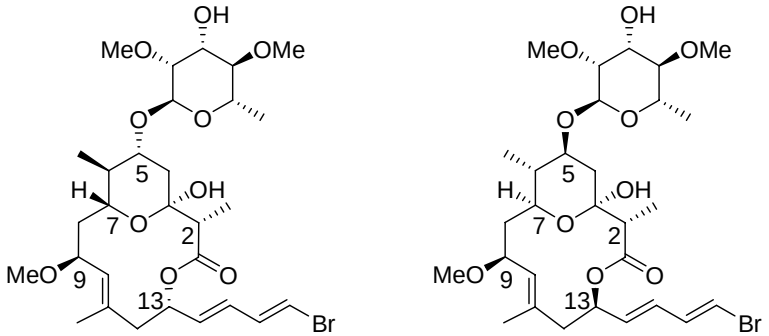
Scheme 7



Section II: Synthetic Studies Towards Dolastatin 19

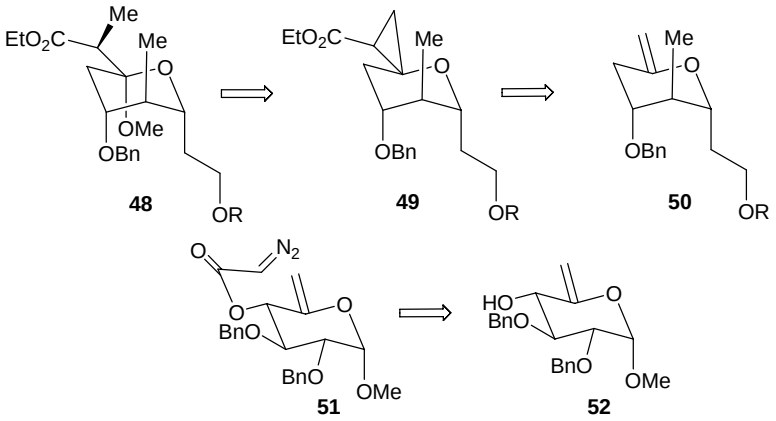
Dolastatin 19 (**46**) was isolated from the Gulf of California shell-less mollusc *Dolabella auricularia* and showed promising inhibition against cancer cell line growth. Considering its promising anticancer activity and complex molecular skeleton, we have initiated a program to address the synthesis of highly substituted hemiacetal pyran ring **48** of dolastatin 19 (Figure 4, before its structure was reassigned). During the initial stage of our work, Paterson et al. reported the first total synthesis of Dolastatin 19 revising the assigned structure.

Figure 4. *Proposed and Revised structures of Dolastatin 19*



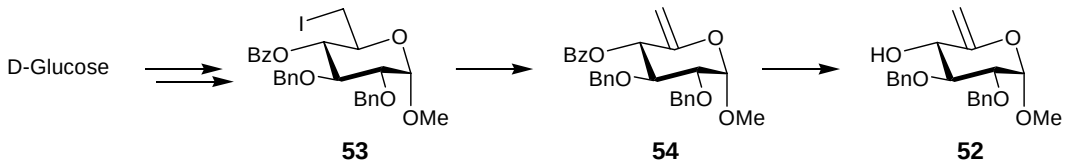
The intended total synthesis can be simplified by cleaving the molecule into sugar unit and aglycone moiety. The aglycone moiety containing the hemiacetal pyran ring is densely functionalized part **48** (Scheme 8). Our target was to synthesize it first and further assemble it with other parts by using known reactions.

Scheme 8. *Retrosynthetic strategy for advanced intermediate **48***



Hence, a model study was carried out to fix the methyl centre adjacent to ethyl ester (in **48)** by intramolecular cyclopropanation and regioselective ring opening. Thus, D-glucose was converted to the iodo derivative **53** according to the known literature procedures. The 5,6-ene **54** was obtained by treating **53** with silver fluoride in pyridine. **54** on treatment with cat. potassium carbonate in methanol gave **52** which was used for intramolecular cyclopropanation reaction (Scheme 9).

Scheme 9



52 was treated under different reaction conditions (Table 2) to obtain **51** but it did not furnish the desired product (Scheme 10).

Scheme 10

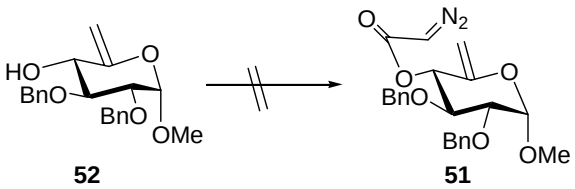
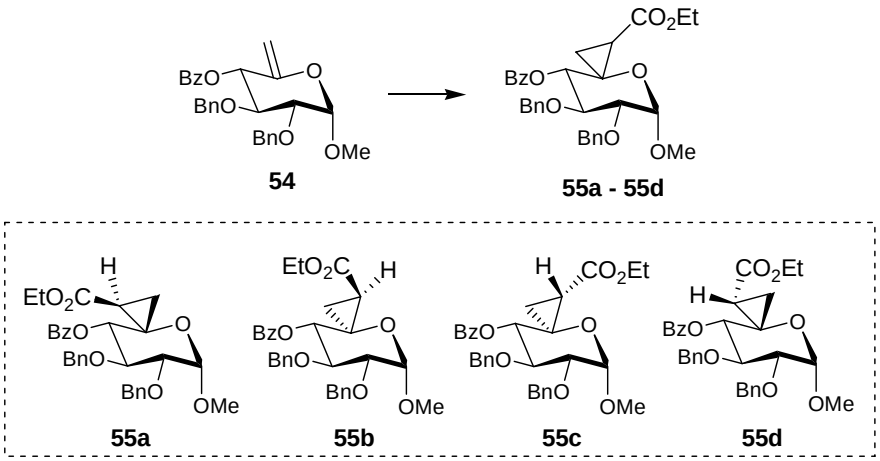


Table 2

S. No.	Reagents	Results
1.	TsNHNCHCOCl/$PhNMe_2$/TEA	Complex reaction mixture
2.	TsNHNCHCOCl/TEA	Complex reaction mixture
3.	TsNHNCHCOCl/$NaHCO_3$	Complex reaction mixture
4.	TsNHNCHCOCl/Collidine/TEA	Complex reaction mixture
5.	TsNHNCHCO_2H/DCC/DMAP	Starting material
6.	Succinimidyl diazoacetate/NaH	Starting material

The alternate intermolecular cyclopropanation of **54** with ethyl diazoacetate and copper powder in toluene at 100 ^{o}C gave cyclopropanated analog **55a-55d** (Scheme 11).

Scheme 11



The cyclopropane group of **55d** was opened in different ways (Table 3) but it did not result into the desired product **56**, instead a chain elongated product **57** was observed (Scheme 12).

Scheme 12

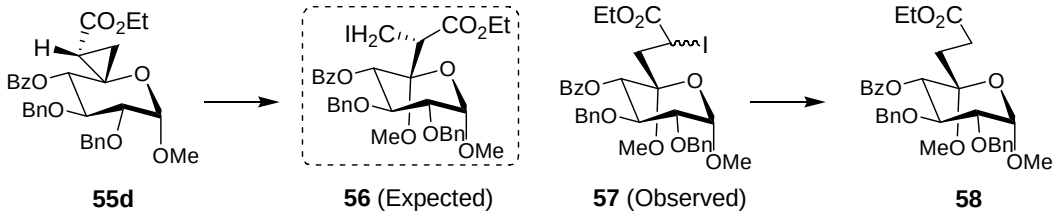
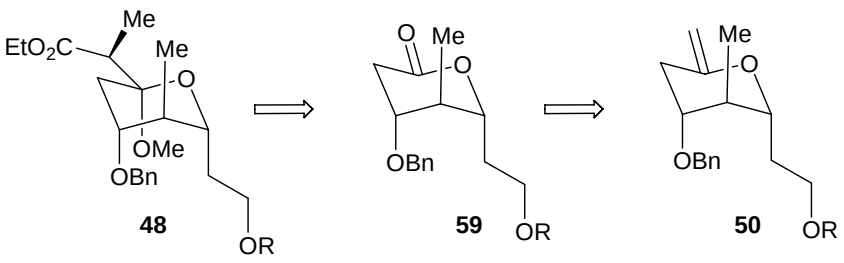


Table 3

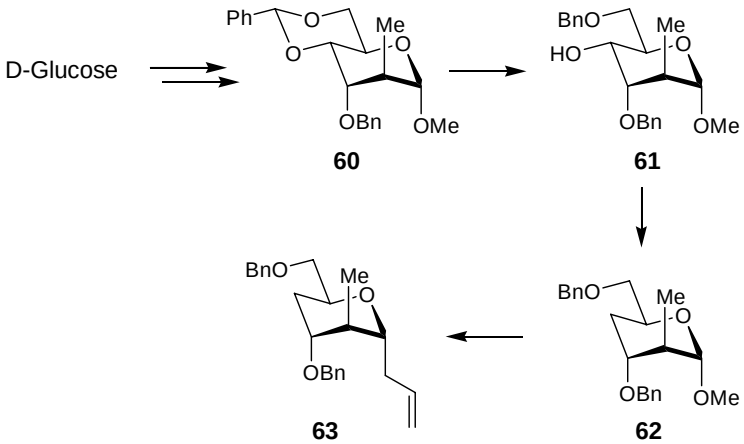
S. No.	Reagents (B)	Results
1.	$(HgTFA)_2$	Complex Mixture
2.	H^+/MeOH	Inseparable Mixture
3.	NIS	Chain Elongation

Scheme 13



The opposite regioselectivity in the cyclopropane ring opening reaction led us to modify our approach towards the Reformatsky reaction. Thus, we tried to focus on the synthesis of hemiacetal ring containing the three required stereocenters as in the proposed structure (Scheme 13). D-Glucose was converted to **60** by known literature procedure in six steps. The benzylidene group of **60** was reductively opened using triethylsilane and trifluoroacetic acid to furnish **61**. C-4 dehydroxylation was carried out by Barton – McCombie protocol to give **62**. C-glycosidation at C-1 of **62** was carried out with allyltrimethyl silane and trimethylsilyl trifluoromethanesulfonate to give **63** (Scheme 14). At this stage, the first total synthesis of Dolastatin 19 appeared in the literature by Paterson et al. with revision of all the three stereocenters synthesized by us. So, it requires complete revision of the strategy from the beginning.

Scheme 14



Conclusion:

All the three centers in the pyranose ring of the proposed Dolastatin 19 had been successfully synthesized. Intermolecular cyclopropanation accomplished the spirocyclic moieties. Further studies to achieve the hemiacetal containing pyranose ring with the desired stereocentres as in the revised structure of Dolastatin 19 are in progress.

Chapter I

Chapter I:

Total Synthesis of Putative Structures of Radicamine B and Evaluation of their Glycosidase Inhibitory Activity.

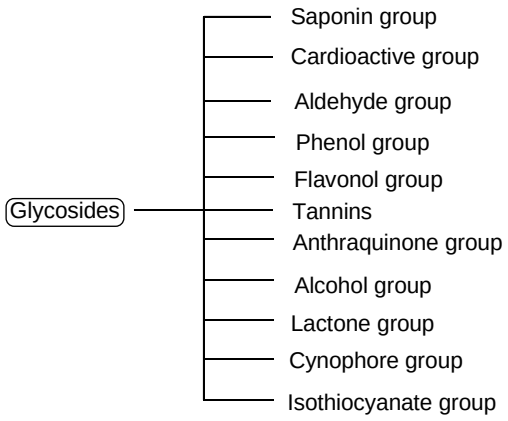
Introduction

The term alkaloid[1] or "alkali-like", which is usually applied to basic, nitrogen-containing compounds of plant origin, was first coined by the pharmacist, W. Meissner, in 1819. But, nowadays many compounds of this class are found to be present in animals and micro organisms. They are secondary metabolites whose absence may lead to improper functioning of vital life processes.

Polyhydroxy alkaloids are one of the diverse categories which encompass several structural types of compounds having potent activity as inhibitors of glycosidase. The presence of glycosidase inhibitory properties in this category of compounds led to extensive research for the past three decades.[2] Structurally, both monocyclic or bicyclic alkaloids exist which are classified into five different sub-classes like pyrrolidine, piperidine, pyrrolizidine, indolizidine, and nor-tropane classes bearing two or more hydroxyl groups.[3]

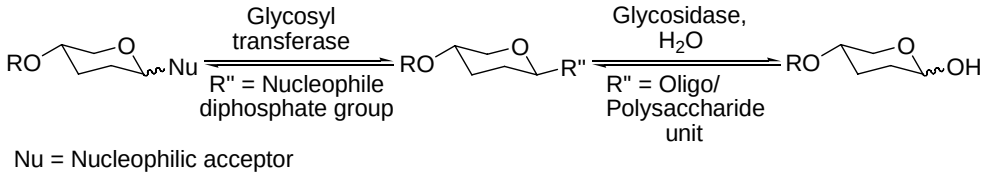
What are Glycosides and Glycosidases?[4]

Glycosides are compounds containing a carbohydrate and a non-carbohydrate unit attached by an acetal linkage, preferably at the anomeric carbon of carbohydrate unit. The carbohydrate (sugar) unit is called GLYCONE and the non-carbohydrate one as AGLYCONE. Aliphatic or aromatic alcohols which are used in the formation of glycosides are generally called aglycones. A simple example in which carbohydrate unit is glucose and the aglycone part is methanol is called methyl glucopyranoside. The glycosides can be classified on the basis of the chemical nature of the aglycone part as follows:



Glycosidases are enzymes which help in cleaving the glycosidic linkages in oligosaccharides or glycoconjugates. Enzymes are the biopolymers (consisting of proteins) which act as catalysts (required in minute amount and are generally recovered unchanged after the reaction) in cell for carrying out vital life processes smoothly. Generally, catalysts lower the energy gap between the transition state and substrate by coordinating with the substrate i.e. the reaction is funnelled through it via low energy states. Thus, the overall function of glycosidases is to cleave the glycosidic bond linking to the anomeric carbon of the sugars with an oligo- or polysaccharide or a nucleoside diphosphate group. The liberated glycosyl group is further transferred to water (by glycosidases) or to some other nucleophilic acceptor (by transferases) (Scheme 1).[5]

Scheme 1. *Schematic representation of glycosidase and glycosyl transferase functions*



Glycosidases have crucial role[6,7] in a broad range of metabolic pathways, including glycoprotein and glycolipid processing and carbohydrate digestion in the intestinal tract. Thus, the strong selective inhibitors of glycoside cleaving or synthesizing enzymes may be used in treatments of disorders that are affected by interfering with glycoprotein synthesis such as viral infections (HIV)[8] or inflammation or disorders related to carbohydrate metabolism (*e.g.*, diabetes).[9]

Classification of Glycosidases

Glycosidases have been classified based on different criterion as:

(i) on the nature of the glycosidic atom as O-, N- and S-glycosidases,

(ii) on the ring size of the glycosyl donor (as pyranosidases or furanosidases), most importantly as,

(iii) **α- and β-glycosidases based on the anomeric configuration of the glycosyl donor.** It may also be classified according to the

(iv) regioselectivity in the processing of oligosaccharides as *exo*-glycosidases (acting at a terminus of an oligosaccharide) and *endo*-glycosidases (acting within an oligosaccharide chain),

(v) as *syn*- or *anti*- protonators (based on the trajectory of protonation by the catalytic acid) and

(vi) on amino acid sequence *(vide infra)*.

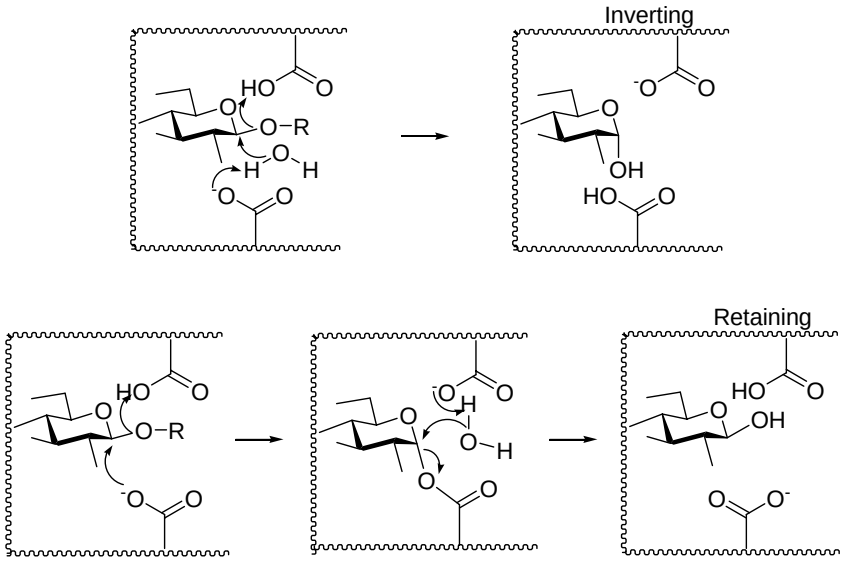
Importance of Glycosidases

Glycosidases are involved in a wide variety of functions[10] essential for the survival and existence of all living organisms. For example, in breaking down of food carbohydrates[11] by digestive glycosidases, glycoconjugate catabolism by lysosomal glycosidases, biosynthesis of the oligosaccharide portions of glycoproteins and glycolipids.[12] Their lack or dysfunction in biological systems may lead to a number of diseases. The wide application of naturally occurring glycosidases in enzyme replacement therapy; in food and pulp processing and as catalyst in oligosaccharide synthesis has encouraged the engineering of proteins with improved catalytic properties and stability.

Mechanism of glycoside hydrolysis[5]

Glycoside hydrolysis can take place either with retention or inversion of configuration,[4a,13,14] depending on the type of enzyme (glycosidases) taking part. In case of inverting glycosidases a combination of carboxylic acid and carboxylate group helps to achieve acid and base catalysis with a direct attack by water molecule at the anomeric centre, whereas in retaining glycosidases, the carboxylate functions as a nucleophile in a process involving glycosylenzyme intermediate. This was proved from X-ray crystallographic datas of several glycosidases which showed wide separation amongst the carboxyl groups of inverting enzymes than in retaining enzymes, presumably to create enough space for water molecule to participate in the reaction (Scheme 2).[15]

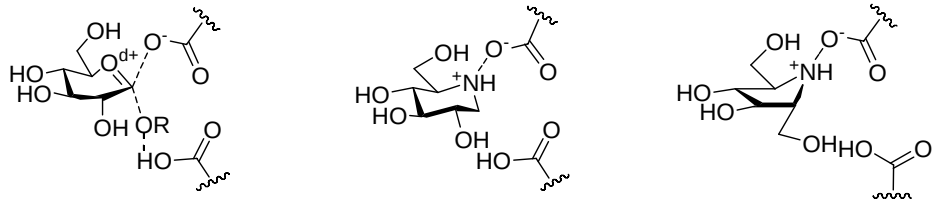
Scheme 2. *Glycosidases reactions involved in inverting and retaining pathways*



Inhibitors[16-19]

Inhibitors are chemical compounds that can bind to enzymes (glycosidases) and form stable complexes, thus, decreasing their activity. Structurally they either mimic the substrate's active site or can form transition state analogues to compete with the substrate. Blocking of an enzyme's activity can kill a pathogen or resurrect a metabolic imbalance. Hence, the role of inhibitors is crucial in biological processes. Generally, inhibitors are observed to be sugar (hexoses) mimics. Although pyrrolidines are five membered polyhydroxy compounds still they are found to show potent inhibition activities against glycosidases because of similar transition state analogues with hexoses. Moreover, the spatial arrangements of the hydroxyl groups in pyrrolidines are similar to hexoses (Scheme 3).[10]

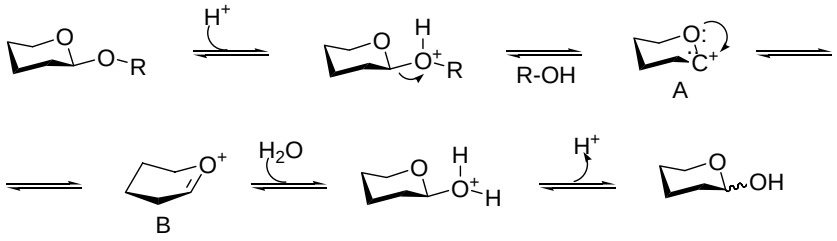
Scheme 3. *Transition state in reaction of D-glucose (**1**) with glucosidase, compared to those with inhibitors DNJ **(2)** and DMDP **(3)***



Natural substrate: D-glucose (**1**) 1-deoxy-nojirimycin (**2**) DMDP (**3**)

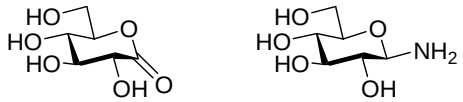
Theoretically, those compounds which mimic the transition state pyranosidic or furanosidic units of natural glucosidase substrates act as effective inhibitors for that glucosidase. Polyhydroxy alkaloids (iminosugars) are considered to have a high potential therapeutic value due to their glycosidase inhibition properties.

Scheme 4. *Carbocation (A) and oxycarbenium ion (B) intermediate formation during the cleavage of glycosidic bond catalyzed by β-glycosidases*



The glycosidase inhibition properties of these compounds may be due to their ability to mimic the conformation and charge of the oxycarbenium ion intermediate **(B)**, but not of the carbocation **(A)** normally generated in the transition state with sp^2 character during the glycosidic bond cleavage (Scheme 4).[10]

Figure 1. *D-gluconolactone **(4)** and D-glucosylamine **(5)***

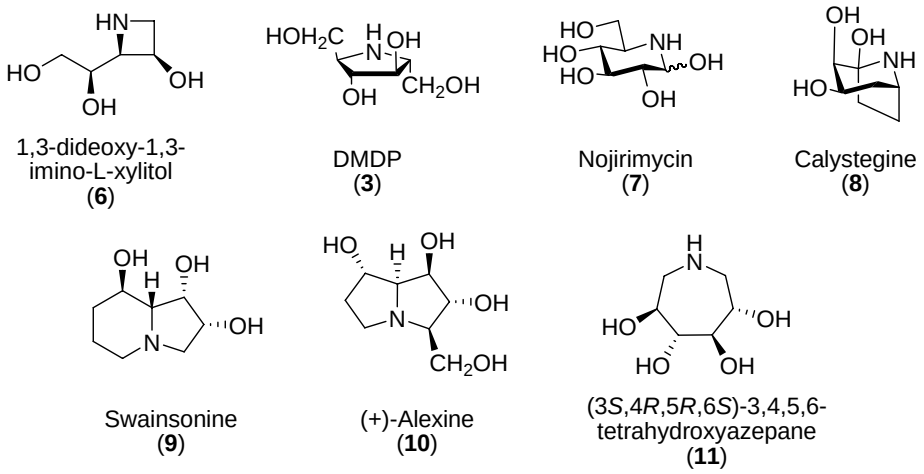


4 **5**

Historically, the first glycosidase inhibitors were D-gluconolactone **4**[20] and D-glucosylamine **5**[21] (Figure 1) of the families of monosaccharide derived δ-aldonolactones and glycosylamines, respectively. They lacked long term stability in

aqueous solutions. Thus, the continuing search for more stable inhibitors from natural sources led to the isolation of polyhydroxylated piperidines, pyrrolidines, and indolizine alkaloids from plants and micro organisms.

Figure 2. *Polyhydroxy iminosugars of 4, 5, 6 and 7-membered rings, and few bicyclic aza-sugars*



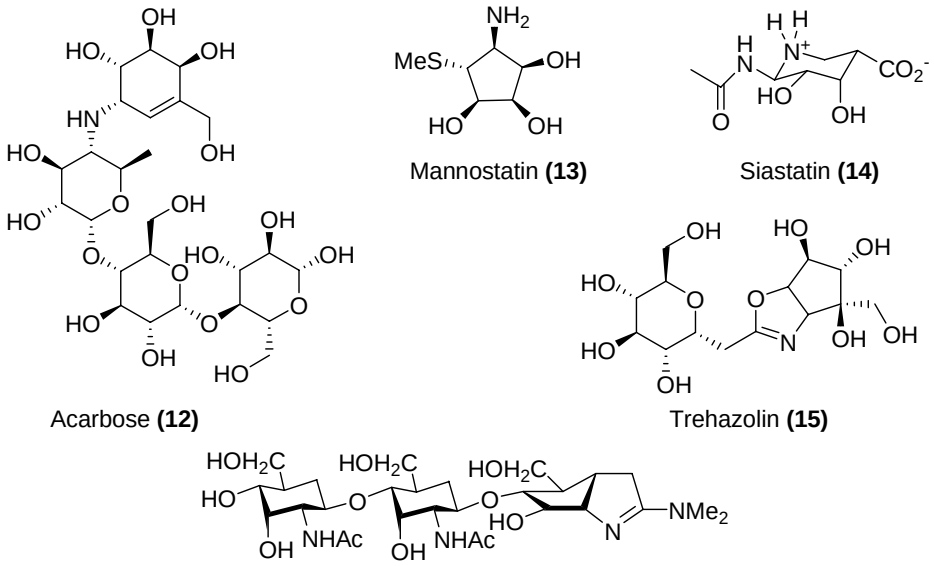
They may be classified as:

(a) The heterocycles containing nitrogen atom (four to seven membered rings) (Figure 2);

(b) Aminocyclitols like Acarbose **12**, Mannostatins **13**, Trehazolin **15** and Allosamidins **16** (Figure 3);

(c) Compounds containing nitrogen in more than one position, e.g. Siastatins **14**, Nagstatins (Figure 3), etc.

Figure 3. *Aminocyclitols*



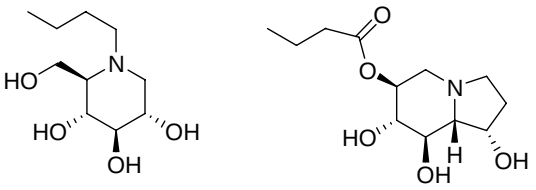
Therapeutic applications of glycosidase inhibitors

The polyhydroxy alkaloids which can mimic transition states formed during glycoside cleavage can compete and inhibit the processes. Thus, it has the potential of acting as therapeutic agents, and indeed it has been found that they show potent activities against viral infections (AIDS),[22] cancer,[23] lysosomal storage disorders,[24] diabetes,[25] etc.

AIDS

Acquired immune deficiency syndrome is a viral disease resulting from the specific damage to the immune system by the human immunodeficiency virus (HIV) in humans. It has spread all over the world like an epidemic because of the unavailability of any methods or medicine to cure it completely, and the ease in its transmission through direct contact of a mucous membrane or the blood stream with a fluid containing HIV, such as blood, semen, preseminal fluid, breast milk, etc.

Figure 4. *Iminosugars as anti HIV agents*



N-butyl deoxy-nojirimycin **(17)**

Celgosivir **(18)**

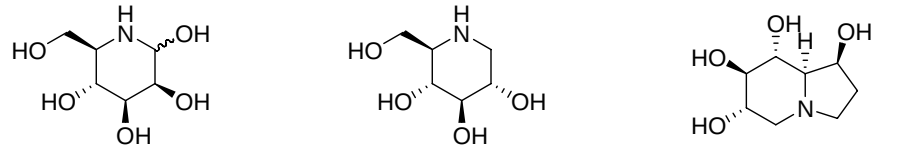
It was found from *in vitro* studies of azasugars, like DNJ **2** and castanospermine **20**, that they possess anti HIV activity i.e. they can alter the glycosylation patterns of viral coat glycoproteins (gp120 and gp41), and thus the interaction with CD4 receptor (membrane glycoprotein), found on the surface of immune system, is prevented. Thus they become non-infectious. But, structural and *in vivo* studies of various imino sugars have shown that N-butyl DNJ **17** is more active against HIV than naturally occurring DNJ **2**. Unfortunately, in phase II clinical trial it was observed that on oral administration it causes diarrhoea, abdominal pain and weight loss in human patients. Recently, Celgosivir **18** has displayed potent antiviral activity *in vitro* and *in vivo* studies against several viruses, including HIV-1, herpes simplex virus (HSV), bovine viral diarrhea virus (BVDV) and HCV, and the agent was chosen for further development. Moreover, Castanospermine **20** and 1-deoxynojirimycin **2** have been shown to be capable of suppressing the infectivity of a number of retroviruses, including the HIV responsible for AIDS. So, hectic researches on secondary metabolites derived from plants have increased the hope of generating HIV resistant vaccines from aza-sugar derivatives (Figure 4) as chemotherapeutic agents.

Cancer

It is one of the deadly diseases of the twenty first century, because it is caused by abnormalities in the genetic materials of the transformed cells. Particularly, cancer is a group of diseases in which cells are aggressive (grow and divide abnormally), invasive (invade and destroy adjacent tissues) and metastatic (spread to other locations in the body). It may affect people of all age groups. There are many ways to treat cancer, like chemotherapy, radiotherapy, etc. but none is a full proof at any stage

of cancer. Early stage detection of cancer may lead to complete cure. At the molecular level it was observed that both catabolic and glycoprotein processing glycosidases are responsible for the transformation of normal cells to cancer cells and in tumor cell invasion and migration. Thus, there is an abrupt increase in the level of glycosidases in the sera of cancer patients. So, finding a cure for cancer came as an extensive challenge to humans. But in the process of searching the curing agents amongst natural products, it was observed that polyhydroxylated alkaloids (Figure 5) have the property to prevent the formation of aberrant asparagine-linked oligosaccharides during glycoprotein processing and to inhibit catabolic glycosidases. So, they are actively pursued as a therapeutic strategy for cancer.

Figure 5. *Azasugars as anticancer agents*



Mannonojirimycin **(19)** Deoxynojirimycin **(2)** Castanospermine **(20)**

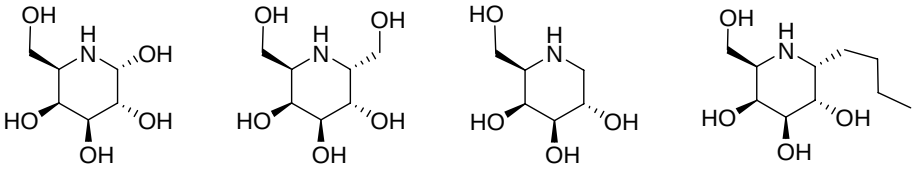
Many polyhydroxylated alkaloids (Figure 5) show anticancer activities prominent amongst them are nojirimycin **7**, mannojirimycin **19**, deoxynojirimycin **2**, castanospermine **20**, swansonine **9** (Figure 2), etc. Amongst them swansonine **9** in particular has been actively pursued because of its multiple characteristics against cancer cells (inhibition of the growth of tumor cells, prevention of dissemination of malignant cells and its stimulatory effect on immune systems). Moreover, it shows cell-specific effect, since it doesn't affect the processing of all glycoproteins equally. Thus, swansonine's anticancer property was first exhaustively studied on animal models which gave very encouraging results. Then it was tried on humans, and currently it is undergoing Phase II clinical trials.

Lysosomal storage diseases

It is classified as a group of diseases which results from defects in lysosomal function. Lysosomes are cytoplasmic organelles that contain enzymes (specifically,

acid hydrolases) which helps in breaking down macromolecules to peptides, amino acids, monosaccharides, nucleic acids and fatty acids. Gaucher's disease is the most common in this category. It is caused due to a deficiency of the enzyme glucocerebrosidase, leading to an accumulation of glucosylceramide. This fatty material gets collected in the spleen, liver, kidneys, lungs, brain and bone marrow resulting in enlarged spleen and liver, liver malfunction, skeletal disorders, neurologic complications, swelling of lymph nodes, distended abdomen, anaemia, etc. Lysosomal diseases are generally classified according to the accumulated substrate as sphingolipidoses, glycoproteinoses, mucolipidoses, mucopolysaccharidoses (MPSs), etc. An enzyme replacement therapy for Gaucher type I is there but it is not a complete solution since in glycosphingolipid degradation pathway there are diseases associated with every enzymes involved. However, it was observed that the mutated enzymes didn't completely stop working so it was thought of controlling the activity of the mutated enzyme by using drugs (Figure 6) in such a way that the amount of substrate which has to be catabolised by the defective enzyme is reduced to a level that matches the residual enzyme activity, thus balancing synthesis with degradation and preventing storage. This type of treatment is termed "substrate deprivation".

Figure 6. *Azasugars effective against Lysosomal diseases*

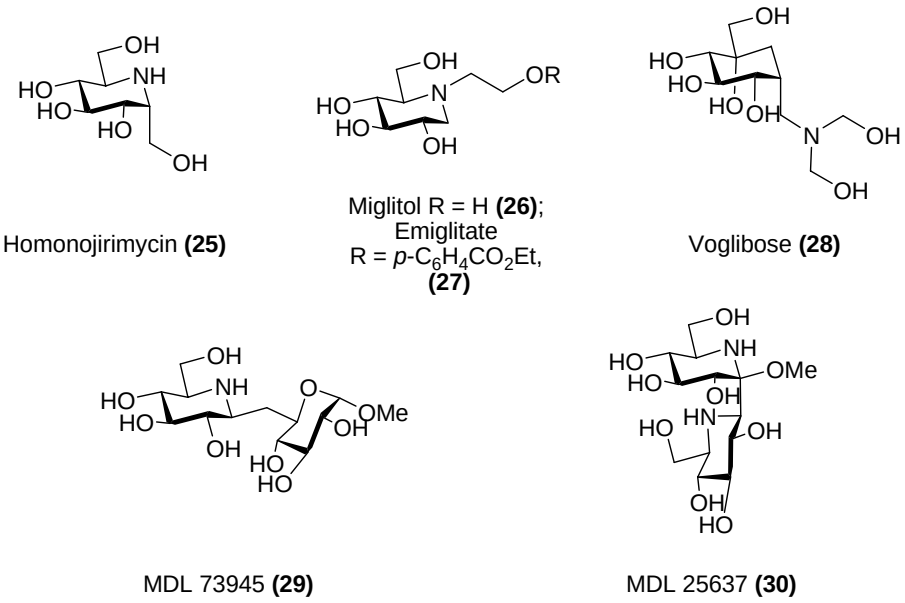


Fabry disease is one of the types of lysosomal diseases characterized by a deficiency in lysosomal α-galactosidase A (due to the genetic mutation which affects the folding of the enzyme). Fan et al. had demonstrated that DGJ **23** inhibits α-galactosidase A competitively by effectively enhancing the mutant enzyme activity (by binding it) in lymphoblasts of affected patients. In order to establish the concept of using competitive inhibitors as specific chemical chaperones a number of naturally occurring and chemically synthesized DGJ **23** derivatives were tested against α-galactosidases.

Diabetes

Diabetes is a type of disease characterized by disordered metabolism and inappropriately high blood sugar which results either due to low levels of hormone insulin or from abnormal resistance to insulin's effects.

Figure 7. *Azasugars effective against diabetes*



The main component of human food is carbohydrates which mainly consist of starch and sucrose. These complex carbohydrates are broken down into simple compounds like glucose, in small intestine by certain enzymes, to be used by body. In diabetes the uncontrolled action of these enzymes leads to undesirable elevation of blood glucose.

Naturally occurring glycosidase inhibitor like acarbose **12** (Figure 3) has been successfully used as an antidiabetic agent. Moreover, it has been observed that many polyhydroxylated alkaloids (Figure 7) are potent inhibitors of various α-glucosidase-specific disaccharides involved in mammalian digestion. In China, mulberry leaves have been used traditionally as a medicine to cure diabetes, as these leaves were found to contain DNJ **2** so the isolation and development of DNJ **2** as drug candidate was initiated. While screening its activity it was observed that DNJ **2** has lower activity *in vivo* against intestinal sucrase than observed *in vitro* studies. Thus, many of

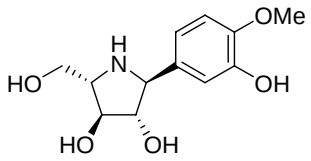
its synthetic derivatives were screened, and it was found that N-hydroxymethyl-deoxynojirimycin (Miglitol **26** or BAY m-1099) was highly effective and had been developed as a drug candidate. Due to improved retention in small intestine its potential *in vivo* antidiabetic activity is very high. Miglitol **26** are used as a substitute for Acarbose **12**. Another glycosidase inhibitor, voglibose **28**, a synthetic derivative of valiolamine is also being marketed as an antidiabetic. In addition to these MDL-25637 **30**, emiglitate **27**, MDL-73945 **29**, etc. are also found active against diabetes. These compounds effectively reduce postprandial elevation of blood glucose and plasma insulin in animals in loading tests with starch and sucrose.

Isolation of Radicamines[26]

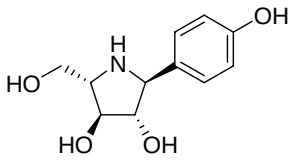
In 2001 Radicamines A & B (**31** & **32**) (Figure 8) were isolated by Kusano et al. from the herb *Lobelia chinensis Lour.* which is used as a diuretic, an antidote, haemostat, and as carcinostatic agents for stomach cancer in Chinese folk medicine. This herb is widely distributed in eastern asian countries like China, Taiwan, Korea and Japan.

After rigorous spectroscopic and analytical studies their structures were elucidated to be a highly substituted pyrrolidine rings having one aromatic group (Figure 8). It was also observed that both radicamines A & B are very active against α-glucosidases having IC_{50} values of 6.7 and 9.3 μM, respectively.

Figure 8. *Structure of Radicamines A & B*



Radicamine A (**31**)



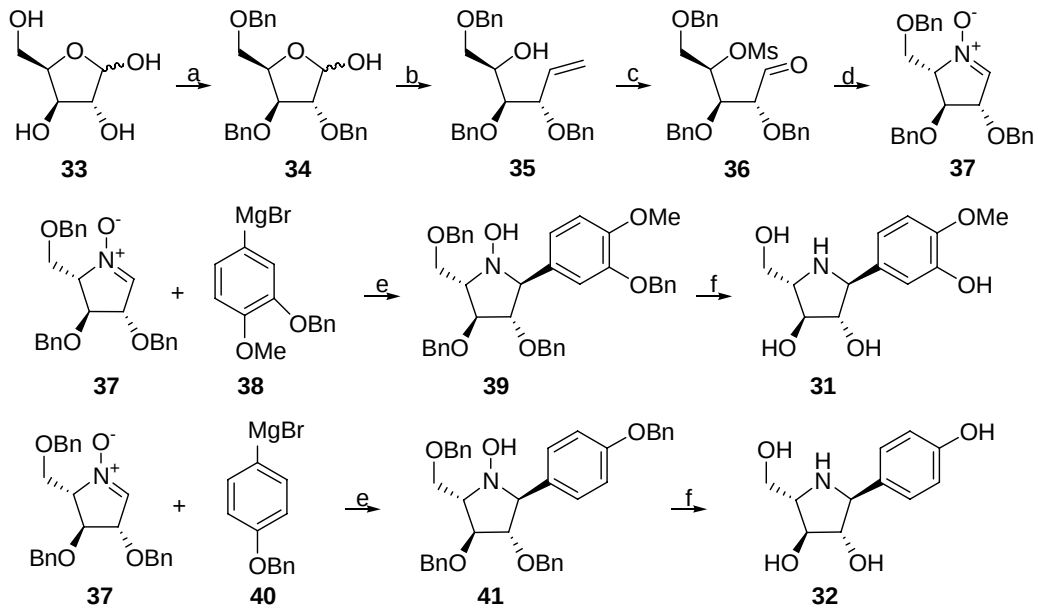
Radicamine B (**32**)

Other Synthetic Approaches of Radicamines

Yu et al.'s approach[27]

Yu et. al. were the first to report the total synthesis of radicamines with the revision of absolute structures. D-Xylose **33** was used as the starting material in their chiral pool approach (Scheme 5). The key reaction of the synthesis was Grignard reaction on nitrones **37** derived from D-Xylose **33**. The final deprotection was carried out using 10% Pd/C in methanol.

Scheme 5. *Total Synthesis of proposed structure of radicamine A & B*

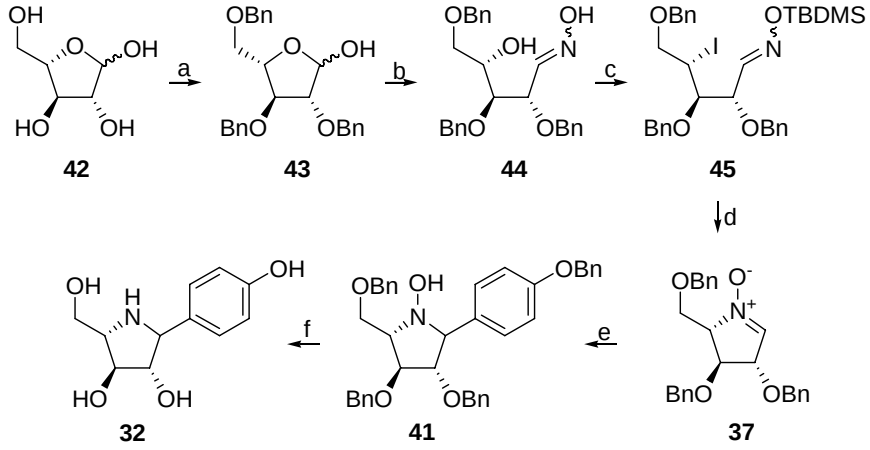


Reagents and Conditions: (a) ref. 28; (b) $Ph_3P^+CH_3Br^-$, *n*-BuLi, THF, 77%; (c) (i) MsCl, Py, DCM; (ii) O_3/O_2, DCM, –40 ^oC; (iii) Me_2S; (d) $NH_2OH.HCl$, Et_3N, H_2O-MeOH, r.t. to 60 ^oC, 58% for 3 steps; (e) THF, 0 ^oC; (f) H_2, 10% Pd/C, MeOH.

Gurjar et al.'s approach[29]

In a parallel approach Gurjar et. al. reported the synthesis of (–)-Radicamine B using similar strategy (i.e. Grignard reaction on nitrones) and proposed a similar structural revision (Scheme 6). The advanced nitrone intermediate **37** was prepared starting from L-Arabinose **42**.

Scheme 6. *Synthesis of the proposed structure of radicamine B*

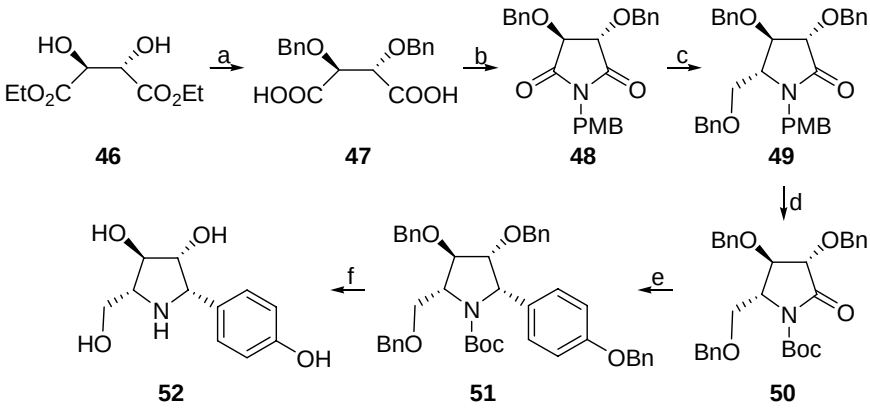


Reagents and Conditions: (a) ref 28; (b) $NH_2OH.HCl$, $NaHCO_3$, EtOH, reflux, 2 h; (c) (i) TBDMSCl, Py, r.t., 36 h; (ii) I_2, TPP, imidazole, toluene, reflux, 3 h; (d) TBAF, toluene, reflux, 3 h; (e) 4-$BnOC_6H_4MgBr$, Et_2O-THF (1:2), –78 ^oC, 2 h; (f) (i) Zn, aq. NH_4Cl, reflux, 3 h; (ii) H_2, $PdCl_2$, EtOH, r.t., 20 h.

Huang et al.'s approach[30]

Huang's group used tartaric acid as the starting material and a highly diastereoselective reductive benzyloxymethylation of protected tartarimide as the key step for the synthesis of 5-*epi*-radicamine B **52** (Scheme 7).

Scheme 7. *Synthesis of 5-epi-radicamine B*



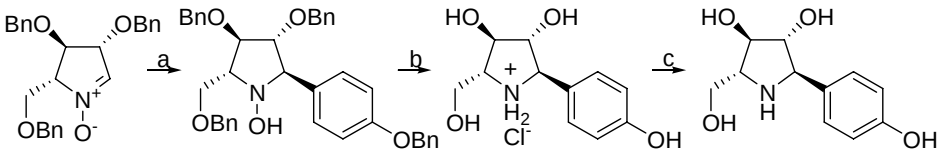
Reagents and Condition: (a) (i) NaH, BnBr, DMF, –20 to 0 ^oC; (ii) aq. LiOH, EtOH, 0-5 ^oC; (b) AcCl, reflux, $PMBNH_2$, DCM, AcCl, reflux; (c) (i) $BnOCH_2Cl$, THF, 0.1 M SmI_2 in THF, 0.01 equiv. $FeCl_3$, 0 ^oC-r.t.; (ii) Et_3SiH, $BF_3.OEt_2$, DCM, –78 ^oC to r.t.; (d) (i) CAN, CH_3CN:H_2O = 9:1, 0 ^oC, 1.5 h, r.t., 4

h; (ii) $(Boc)_2O$, DMAP, Et_3N/DCM, r.t., 4 h; (e) (i) *p*-$BnOC_6H_4MgBr$, DCM, –20 °C, 2 h; (ii) Et_3SiH, $BF_3.OEt_2$, DCM, –78 °C to r.t., 14 h; (f) $PdCl_2$, H_2, EtOH, r.t., 12 h.

Merino et al.'s approach[31]

Recently, Merino's group synthesized the revised structure of Radicamine B **56**. The key step of their synthesis was highly stereoselective nucleophilic additions and redox reactions of polyhydroxypyrroline N-oxides **53** (Scheme 8).

Scheme 8. *Synthesis of revised structure of radicamine B*



53 **54** **55** **56**

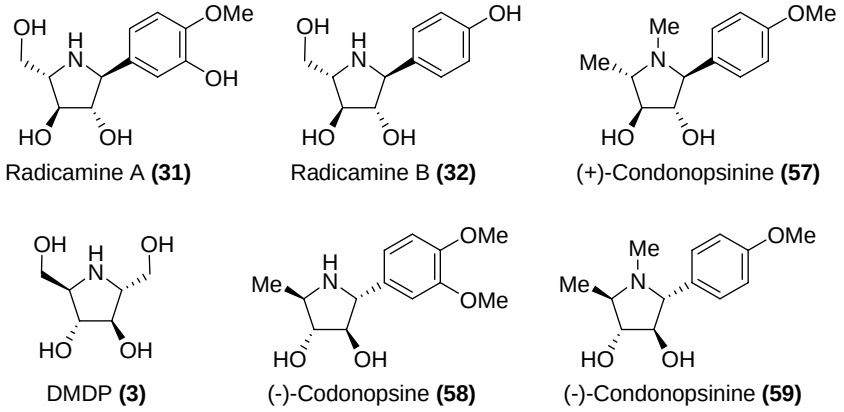
Reagents and Conditions: (a) 4-$BnOC_6H_4MgBr$, THF, 0 °C; (b) H_2 (5 atm), $Pd(OH)_2$-C, HCl-MeOH, r.t.; (c) Dowex 5WX8-200, NH_4OH (1N).

Present Work

Present Work

A wide variety of polyhydroxy pyrrolidines and piperidines broadly belonging to the glycosidase inhibitors family have become available as a result of extensive research over the past 30 years. In 2001 Radicamines A & B (Figure 9) were isolated[26] by Kusano et al. from the herb *Lobelia chinensis Lour.* widely distributed in China, Taiwan, Korea and Japan. This herb is used as a diuretic, an antidote, haemostat, and as carcinostatic agents for stomach cancer in Chinese folk medicine. Radicamines were isolated from this herb by extracting with 50% methanol and the structures of the pure products were determined by rigorous spectroscopic studies and analytical data interpretation. For example, radicamine B **32** was obtained as a pale yellow oil, $[\alpha]_D$ +72 (c = 0.10, H_2O). The molecular formula was determined to be $C_{11}H_{15}NO_4$ on the basis of pos. HR-SI-MS (*m/z*: 226.1073, $[M+H]^+$). After analysing its ^1H NMR, ^{13}C NMR, COSY, HSQC, HMBC, DEPT and NOESY spectrum its relative structure was determined. The absolute configuration of Radicamine B **32** was derived by comparing the $[\alpha]_D$ value of (+)-codonopsinine **57** with N-methyl radicamine B. This absolute stereostructure was supported by the result of the benzoate chirality method. The inhibitory activity of Radicamine B was assayed with respect to α-glucosidase and the IC_{50} value was found to be 9.3 μM. Because of its simple structure, rare availability and significant biological activity, we devised a program to synthesize all the possible diastereoisomers of radicamine B (Figure 10) and study structure – glycosidase inhibitory activity relationships hoping to derive a possible transition state for the observed glucosidase inhibitory activity.

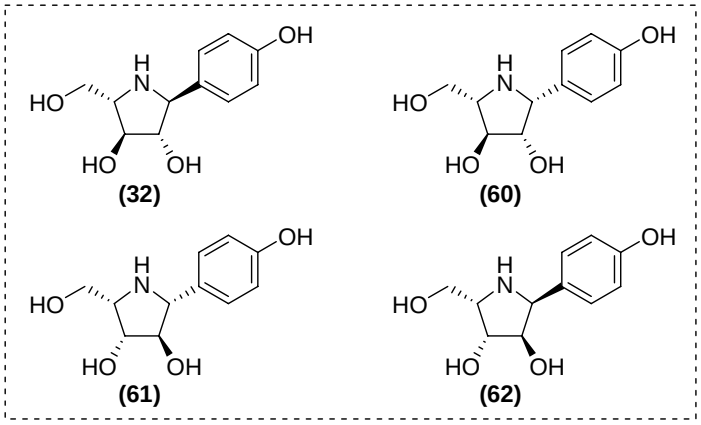
Figure 9. *Structures of C-aryl pyrrolodines and DMDP*



Synthetic Analysis

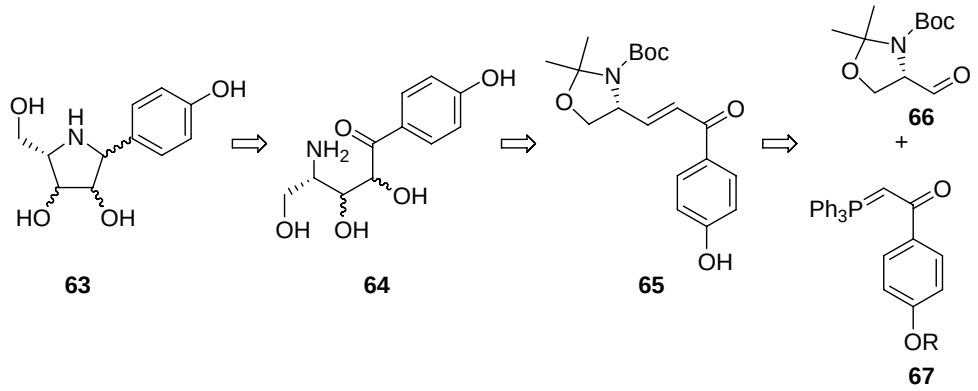
The structure of radicamine B is characterized by all trans-configuration. Considering this we have reduced the number of possible diastereomers to four by fixing the absolute stereochemistry at C-4 and the relative stereochemistry between the vicinal diols as *trans* (Figure 10).

Figure 10. *Four possible diastereomers of Radicamine B*



The basic disconnection of the radicamine B skeleton is between C(1)-Ar and secondary amine identifying an intramolecular reductive amination as the key transformation. As the complete control over the stereochemical out come may not be predictable, we anticipated this approach would address acquiring possible epimers at C(1) center. The next disconnection was between the C(2)-C(3) keeping "*E*-olefin" as a surrogate for the requisite *trans*-diol identifying the Horner-Wadsworth-Emmons reaction between the retrons **66** and **67** as the key reaction. Asymmetric dihydroxylation of the resulting *E*-enone by employing either of AD-mix reagents should address the requisite diastereoisomers synthesis (Scheme 9).

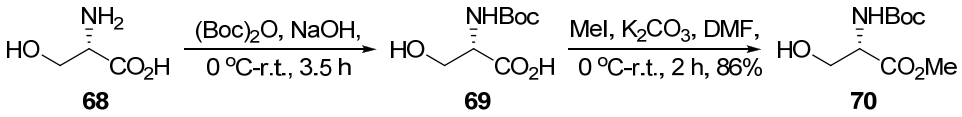
Scheme 9. *Retrosynthetic strategy for radicamine B and its diastereomers*



Synthesis of Garner Aldehyde[32] (66)

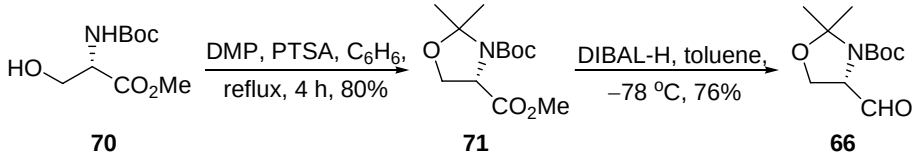
According to the proposed synthetic plan, the first coupling partner, Garner aldehyde **66** was prepared from commercially available L-serine **68** following the literature procedure in four steps. The amine group of L-serine was first protected as its Boc derivative **69**. Then the acid was converted to its methyl ester **70** (Scheme 10).

Scheme 10



The primary hydroxyl group and secondary amine group of compound **70** were protected with acetonide to give a cyclic compound **71** (Garner ester) which was subjected to controlled reduction with DIBAL-H to furnish Garner aldehyde **66** (Scheme 11). The spectral data of compound **66** was in agreement with the reported data for Garner aldehyde.

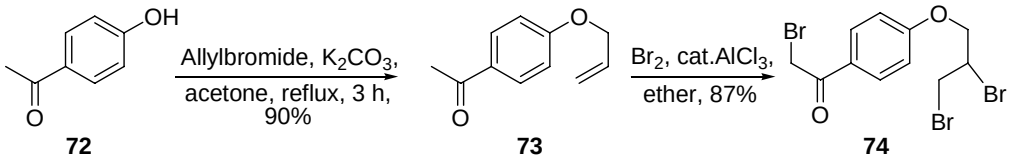
Scheme 11



Synthesis of ylide[33] (67)

Synthesis of the second fragment i.e. the ylide **67** was started from 4-hydroxyacetophenone **72**. The hydroxyl group of **72** was protected as its allyl ether using allyl bromide and potassium carbonate in acetone to give **73**. The selective bromination alpha- to carbonyl of **73** was found to be a difficult proposition under different reaction conditions. For example when tried under classical α-bromination conditions[34] like $Br_2/AlCl_3$ in anhydrous ether, we noticed the major product isolated was a tribromo- compound **74** resulting from the bromination of olefin in addition to the alpha- to carbonyl bromination (Scheme 12).

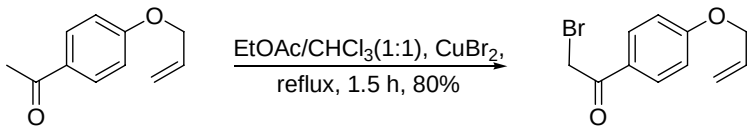
Scheme 12



72 **73** **74**

After examining various other heterogeneous bromine sources, finally we could obtain the alpha-bromo compound **75** (Scheme 13) selectively in excellent yield by employing $CuBr_2$ in ethylacetate/chloroform mixture (1:1).[35] In the ^1H NMR spectrum of compound **75**, the downfield shift of $-CH_2Br$ group protons (4.35 ppm (*s*)), clearly indicated the formation of desired product. Moreover, in ^{13}C NMR the presence of three methylene groups at 30.49, 68.71 and 118.04 ppm further confirmed its structure.

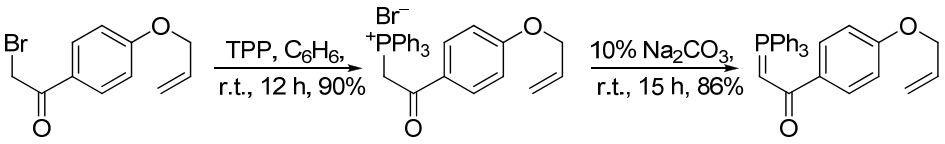
Scheme 13



73 **75**

After having a feasible method for the preparation of **75**, our next concern was the preparation of ylide **67**. The α-bromoketone **75** was converted to the corresponding triphenylphosphonium salt **76** by treating it with triphenylphosphine in benzene at room temperature. Finally, **76** on treatment with 10% aqueous sodium carbonate for 12 h gave the ylide **67** (Scheme 14).

Scheme 14



75 **76** **67**

Synthesis of triol 78 and 79

The proposed Wittig reaction[36] of Garner aldehyde **66** and ylide **67** could be carried out successfully in anhydrous benzene at room temperature for 15 h, and resulted in enone **77** with exclusive *E*-configuration (Scheme 15). The structure of the compound **77** was confirmed from its ^{1}H NMR, ^{13}C NMR and elemental analysis. Due to the merging of signals of olefinic and aromatic protons, the coupling constants amongst the former protons could not be determined. Deallylation[37] of phenylallyl ethers in general can be affected by several reagents. In the present case though one more olefin is present, however is electronically biased. Thus we anticipated the selective cleavage of allyl ether can be affected by screening the several of identified catalysts, for the above transformation. Table 1, gives a compilation of methods that have been attempted to carry out deallylation reaction.

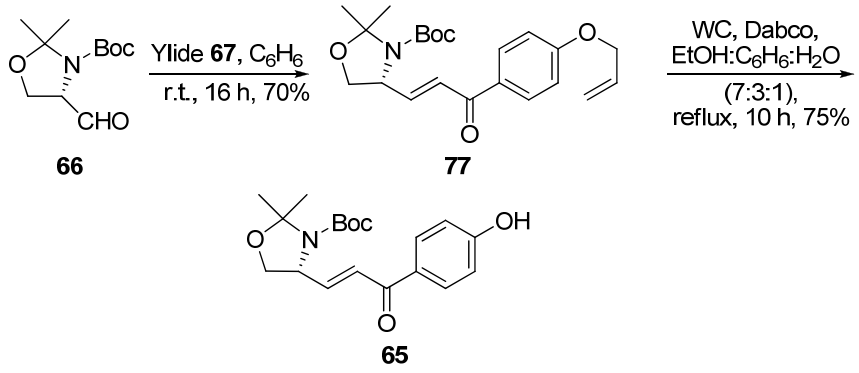
Table 1

S. No.	Reaction Conditions	Observations
1.	*t*-BuOK, DMSO, 100 ^{o}C; then after work-up $HgCl_2$/HgO	Complex reaction mixture
2.	Pd/C, PTSA, MeOH	Complex reaction mixture
3.	$PdCl_2$	Complex reaction mixture

4.	*trans*-$Pd(NH_3)_2Cl_2$, *t*-BuOH	Complex reaction mixture
5.	$(Ph_3P)_3RhCl$, Dabco, $(EtOH:C_6H_6:H_2O)$ 7:3:1	Required product obtained in 75% yield

Amongst the various catalysts employed, the deallylation was smooth with Wilkinson's catalyst[37e] when applied with Dabco and gave the phenol **65** in good yield. The structure of **65** was established with the help of spectral and analytical data. The ^1H NMR spectrum of compound **65** in DMSO-d_6 was well resolved and most of the H's can be seen separately. For example, the olefinic – H's were distinctly separated from the aromatic – H and the formers appeared at 6.72 (dd, J = 15.3, 6.8 Hz, 1H) and 7.07 (d, J = 15.3 Hz, 1H) ppm, clearly indicating the *E*-configuration. Further, the structure was confirmed from its combustion data analysis which also was found to be in line with the predicted structure **65**.

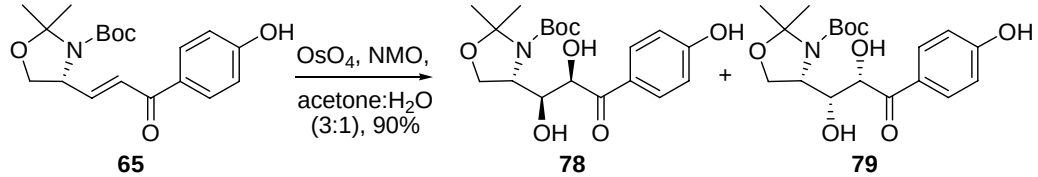
Scheme 15



66 **77** **65**

The next concern, asymmetric dihydroxylation[38] of **65** requires special mention. When dihydroxylation reaction was carried out by using catalytic osmium tetroxide and NMO in acetone:water (3:1), **78** and **79** were obtained in 55:45 ratio (Scheme 16).

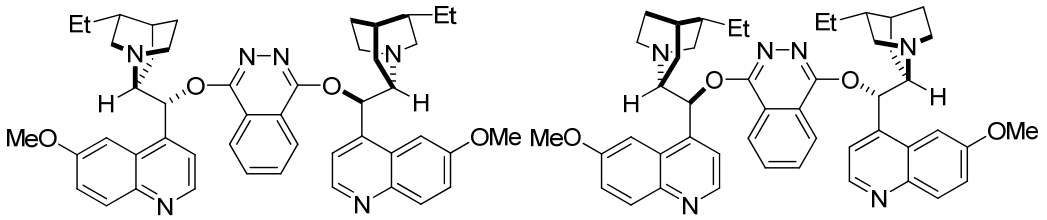
Scheme 16



65 **78** **79**

This has prompted us to employ Sharpless asymmetric dihydroxylation conditions. Thus, on using AD-mix-α, methanesulphonamide and 3 mol% $(DHQ)_2PHAL$ (**80**) ligand (Figure 11) in *t*-butanol:water (1:1) at 0 ^oC gave **78** & **79** in 80:20 ratio whereas the use of AD-mix-β and 3 mol% $(DHQD)_2PHAL$ (**81**) (Figure 11) with other conditions unchanged, **78** & **79** were obtained in 60:40 ratio as determined from their ^1H NMR spectrum.

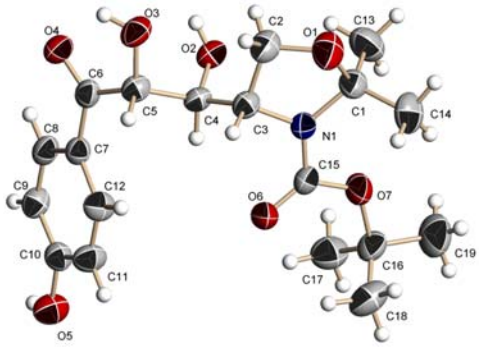
Figure 11. *Cinchona alkaloids derived ligands for asymmetric dihydroxylation*



$(DHQ)_2PHAL$ **(80)** $(DHQD)_2PHAL$ **(81)**

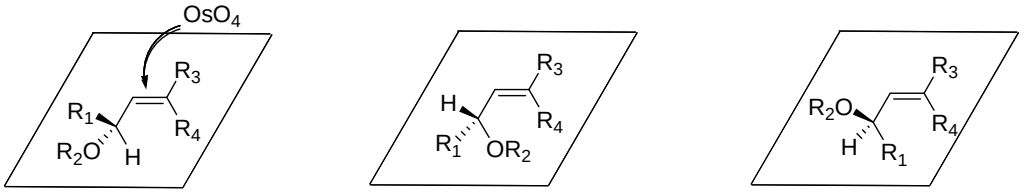
The diols were easily separable by column chromatography and characterized by ^1H, ^{13}C NMR and elemental analysis data. In ^1H NMR spectrum the peaks due to alkene protons were absent. Gratifyingly, one of the diol **78** was obtained as a crystalline solid and the stereochemistry of newly introduced vicinal diol unit was determined from X-ray crystal structure data as L-*arabino* (Figure 12). The details of crystal data and structure refinement (Table 2), bond lengths and bond angles (Table 3) and torsion angles (Table 4) are given at the end of this section (Page No. 46 to 48).

Figure 12. *ORTEP diagram of triol* ***78***



The stereochemical outcome of the major product of dihydroxylation reaction can be explained by Kishi rule[39] which states that the presence of *trans*-olefin does not lead to very high stereoselectivity in osmium tetroxide catalyzed dihydroxylation reaction and the vicinal diols obtained as major product has opposite orientation with respect to the hydroxyl or alkoxyl group (in this case protected amine group) already present in the substrate.

Figure 13. *Possible eclipse conformations of allylic hydroxyl groups containing systems*



An explanation based on the conformational analysis of sp^3-sp^2 single bond system was tried to address the problem. An eclipsed conformation is known to be preferred for such systems having allylic hydroxyl groups. Thus, amongst the three possible eclipsed conformations **A**, **B** and **C** (Figure 13) the conformation **A** is found to be sterically least compressed, so it is the most favored one. Now, if it is assumed that this conformational preference is exhibited in the transition state, then the stereochemistry of the major product can be shown to be arising from the preferential approach of osmium tetroxide to the face of the olefinic bond opposite to that of the pre-existing hydroxyl or alkoxyl group. So, the orientation of vicinal hydroxyl groups is observed to be opposite to that of the pre-existing hydroxyl or alkoxy group. Moreover, the stereoselectivity observed for *cis*-olefin is higher than that of *trans* one, since the preference for conformation **A** over **B** & **C** is expected to be more significant for *cis*-olefins than for corresponding *trans*-olefins.

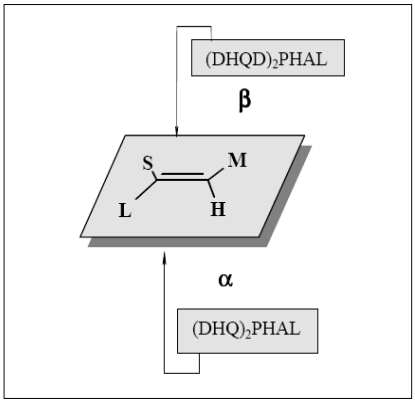
Finally, it was observed that the use of chiral ligands only helped in obtaining better stereoselectivity towards the desired product (from Kishi rule) while our attempt towards the enhancement of diastereoselectivity towards the other diastereomeric diol **79** with chiral ligand $(DHQD)_2PHAL$ **81** failed. In the case of

chiral ligands it has been observed that the geometry of incoming hydroxyl groups is controlled by the formation of stable transition states having enzyme like pockets.

A short account on Sharpless asymmetric dihydroxylation (AD)[40]

The development of asymmetric dihydroxylation (AD) reaction in the 90's of the twentieth century by Sharpless et al. is one of the most valued transformations in organic chemistry for the introduction of vicinal cis-hydroxyl groups in stereospecific manner. The process is crucially dependent on ligand acceleration effect (LAE), which ensures the reaction to pass through a pathway involving chiral catalyst. Initially the AD using cinchona alkaloids derivatives was performed under stoichiometric conditions. Later on exhaustive studies by Sharpless et al. led to the development of catalytic AD whose main features are: (i) use of two phase conditions with $K_3Fe(CN)_6$ *as reoxidant; (ii)* $MeSO_2NH_2$ *for rate acceleration and (iii) second generation ligands (phthalazine and diphenylpyrimidine, with two independent cinchona alkaloid units). The origin of the enantioselectivity in the AD have been demonstrated by ligand structure – activity studies which revealed the presence of enzyme- like binding pocket in the "dimeric" Cinchona alkaloids, e.g., the phthalazine ligands. The reaction rates are mainly influenced by the nature of O-9 substituent of the cinchona alkaloid. This rate enhancement is caused by a stabilization of the transition state due to aromatic stacking interactions. Though this kind of stabilization is operative in first generation ligands, it is most effective in the dimeric second generation ligands due to the presence of a binding pocket. Thus the almost perfect match between the phthalazine ligands and aromatic olefins with respect to rates and enantioselectivities can be readily explained by an especially good transition state stabilization resulting from offset-parallel interactions between the aromatic substituent of the olefin and the phthalazine floor of the ligand, as well as favorable edge-to-face interactions with the bystander methoxyquinoline ring.*

Figure 14. Mnemonic diagram *(S = small group, L = large group, M = medium group, H = proton)*

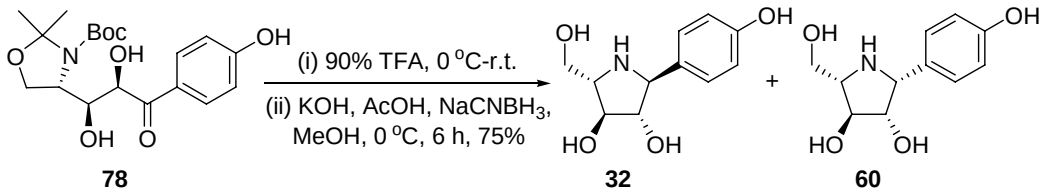


The above observations have led to a revised mnemonic device for predicting the enantio-facial selectivity in the reaction. An olefin positioned accordingly will be attacked either from the top face (β face) in the case of dihydroquinidine derivatives or from the bottom face (α face) in the case of dihydroquinine derived ligands.

Synthesis of pyrrolidines

After confirming the stereochemistry of both the compounds **78** & **79** our next concern was their intramolecular reductive amination or in other words completing the total synthesis of radicamine B and three of its possible diastereomers.

Scheme 17

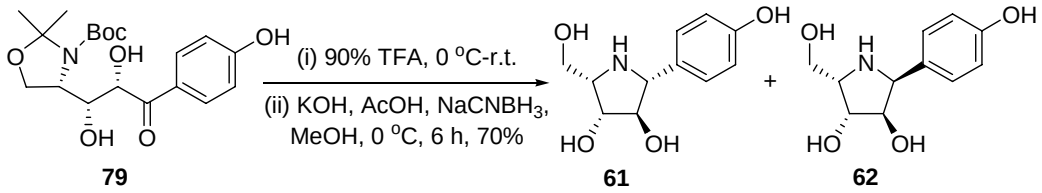


78 **32** **60**

So, after exploring several conditions for the above mentioned transformations by employing various acids and reducing agents, we concluded that the reductive amination of the diols **78** & **79** can be secured by a two step procedure, first acetonide

and –Boc deprotection with TFA followed by treatment with $NaCNBH_3$ as reducing agent.[41] For example, treatment of the diol **78** with 90% trifluoroacetic acid at 0 ^oC furnished the acetonide and –Boc deprotected derivative. TFA was removed on rotary evaporator by co-evaporating with toluene. The residue obtained was subjected to intramolecular reductive amination with sodium cyanoborohydride, sodium hydroxide and acetic acid in methanol at 0 ^oC to furnish the products **32** & **60** (Scheme 17). This mixture was analysed by HPLC and found that both **32** & **60** were present in 65:35 ratio. They were separated by semipreparative HPLC using C-18 achiral column and water:methanol:acetic acid (95:5:0.1) as mobile phase. Their structures were fully characterized by ^1H, ^{13}C NMR and elemental analysis data. The ESI-MS studies showed the molecular ion peak for both the compounds to be present at 226 $[M+H]^+$. In the ^1H NMR spectrum of both the pyrrolidines **32** & **60**, the peaks due to Boc and acetonide groups were found to be absent. Moreover, in ^{13}C NMR the peaks due to carbonyl carbon were absent and rest of the spectrum matches according to the designated structures of the compounds. The measured optical rotation value for **32**, shows that it is an enantiomer of natural Radicamine B.

Scheme 18



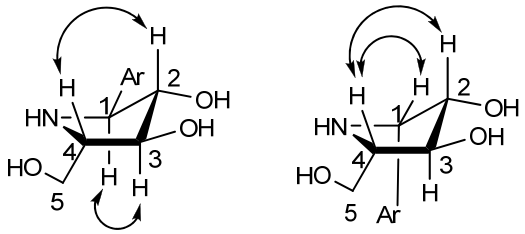
79 **61** **62**

Similarly, **79** on treatment with TFA and $NaCNBH_3$ under reductive amination conditions furnished **61** & **62** (Scheme 18) in 85:15 ratio as determined by HPLC. Using the same column and conditions as of the previous one, both **61** & **62** were separated. In ^1H NMR spectrum of **61** & **62**, the peaks due to –Boc and acetonide groups were found to be absent. Moreover, in ^{13}C NMR the peaks due to carbonyl carbon were absent and rest of the spectrum matches according to the designated structures of the compounds. Finally, the ESI-MS studies showed the molecular ion peaks for both of them to be present at 226 $[M+H]^+$.

COSY/NOESY studies were carried out to determine the stereochemistry of all the four synthesized pyrrolidines (**32**, **60**, **61** & **62**). In structure **32** strong coupling

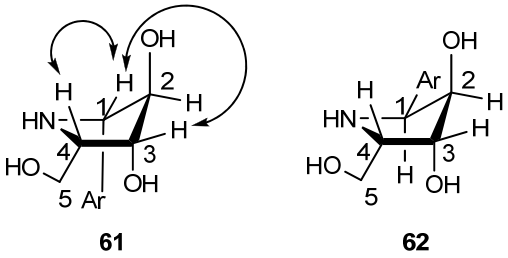
amongst H-1, H-3 and H-2, H-4 were observed from its NOESY spectrum, showing their *cis* orientations. Thus, all the substituents were found to be *anti* to each other. But, in structure **60** strong nOe's amongst H-1, H-4 and H-2, H-4 shows the *cis* orientation of C-1 aryl and C-2 hydroxyl group (Figure 15).

Figure 15. *NOE studies on **32** & **60** (Ar = p-HO-C_6H_4-)*



Similarly, the stereochemical orientations of the substituents in pyrrolidine ring of **61** and **62** were determined (Figure 16). In case of **61** strong nOe's amongst H-1, H-3 and H-1, H-4 were observed. Thus, confirming the assigned structure shown below. But, in **62** no, nOe signals were observed which was corroborated from its COSY spectrum.

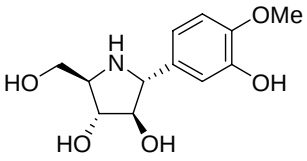
Figure 16. *NOE studies on **61** & **62** (Ar = p-HO-C_6H_4-)*



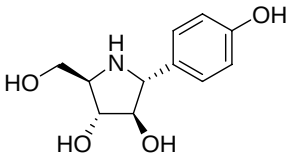
While our synthesis was in progress the first total synthesis appeared in the literature which warranted for revision of structure of Radicamine B and proposed it to be an enantiomer of the originally published structure, since the synthetic versions data matched well with the natural one but for the sign of optical rotation. Moreover, it was also observed that the sign and value of optical rotation of *N*-methyl radicamine B {$[\alpha]_D$ +8.3 (*c* = 0.05, MeOH)} (*N*-methyl derivative of natural product) was

matching well with *N*-methyl derivative of (+)-Codonopsinine {$[\alpha]_D$ +12.5 (*c* = 2.55, MeOH)}. Now both radicamines and codonopsinines can be regarded as DMDP (simplest naturally occurring polyhydroxy pyrrolidine) derivatives which usually possess (2*R*,3*R*,4*R*,5*R*)-pyrrolidine moiety whereas (+)-codonopsinine is the synthetic version having (2*S*,3*S*,4*S*,5*S*) orientations of the substituents in the pyrrolidine ring (Figure 8). Moreover, the results observed by Yu et al.[27] also corroborated the revision of Radicamine B structure after synthesizing the proposed structures of radicamines. Thus, the revised structures of radicamines are enantiomeric to the proposed ones (2*R*, 3*R*, 4*R*, 5*R*) configurations (Figure 17).

Figure 17. *Revised structures of Radicamines A & B*



Radicamine A **(82)**
(revised)

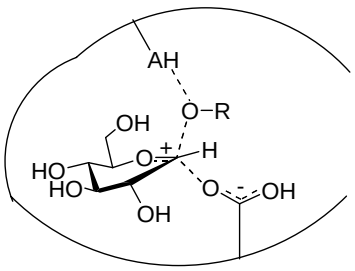


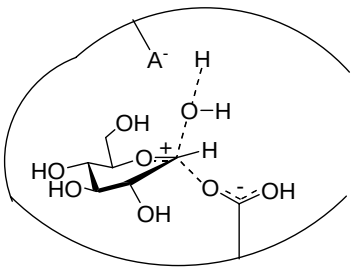
Radicamine B **(56)**
(revised)

Glycosidase Inhibition by Basic Sugar Analogs[42]

*Basic sugar analogs of 5 and 6-membered ring (e.g. Nojirimycin, isofagomine, 2,5-dideoxy-2,5-imino-D-mannitol) are found to be an effective inhibitors of glycosidases because it has been observed that structurally they mimic the transition states involved in the inhibition reactions (Figure **18** & **19**).*

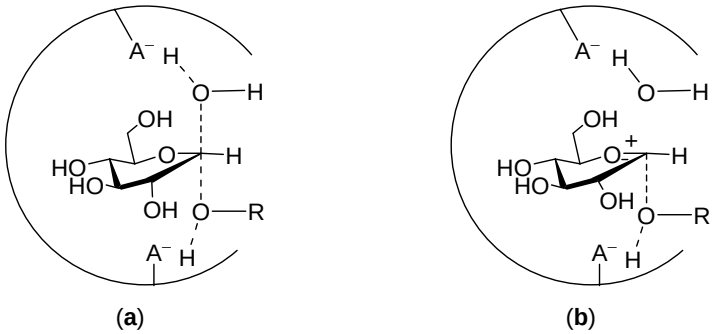
Figure 18. *Models of the first (a) and second transition states (b) of a retaining β-glucosidase. A- & AH represents base and acid, respectively. Dashed lines represent bonds being made or broken*





The transition state energies with an empiric model itself are very cumbersome to calculate. So, an indirect approach is used to visualize the transition state structures by comparing it with the substrate structures and their inhibitory strength. Thus, it has been found that a large number of hexopyranoses or their aza analogs are good inhibitors towards glycosidases. Hence, their transition states structure can be represented as an envelop-like in which the anomeric carbon has sp^2 character i.e. planar and there is also an electrostatic charge development due to bond fission and formation.

Figure 19. *Models for the transition state of an inverting-α-glucosidase. (a) The reaction proceeds by an S_N2-like mechanism. (b) Proton-assisted aglycon departure preceeds bond formation with water. A- & AH represents base and acid, respectively. Dashed lines represent bonds being made or broken*



In addition to that, many pyrrolidines (five membered rings) which do not have conformational similarities like hexopyranoses are also found to exhibit prominent inhibition against a number of glycosidases. These have been attributed to their hydroxyl group conformations (i.e. the better resemblance of hydroxyl group orientation in the half-chair furanose structures with the actual transition state than by the chair conformation of aza-pyranoses). They adopt conformations resembling an envelope or twisted half-chair.

Glycosidase Inhibition Studies for Radicamine B and its diastereomers

All the four diastereomers of Radicamine B were subjected to glycosidase inhibition studies against the enzymes α-glucosidase (yeast), β-glucosidase (almond), α-galactosidase (green coffee beans) and α-mannosidase (jack beans). It was observed

that **32** and **60** showed appreaciable activity against α-glucosidase (Table 2) while none of the compound showed any activity against β-glucosidase and α-mannosidase.

The inhibition rates (%) were calculated from the formula 100-100×(ODtest-ODblank)/(control ODtest-control ODblank). IC_{50} data were obtained over different concentrations of inhibitor and then plotted as percentage inhibition versus inhibitor concentration. K_i was determined under similar conditions to IC_{50} determinations, except that substrate concentrations were taken at two levels. Dixon plots were used to transform the kinetic data into K_i values.

Results of Inhibition Studies on Some Polyhydroxyamines

(a) α-Glucosidase inhibition:

Table 5

Inhibitors	IC_{50} (μM)	K_i (μM)
32	64	64
60	386	390
61	13.1% *	-
62	32.3% *	-

(b) β-Glucosidase inhibition:

No, inhibition at 1 mM concentration of all four compounds on enzyme.

(c) α-Galactosidase inhibition:

Table 6

Inhibitors	IC_{50} (μM)	K_i (μM)
32	9.0%*	-
60	5.0%*	-
61	10.0%*	-
62	10.0% *	-

(d) α-Mannosidase inhibition:

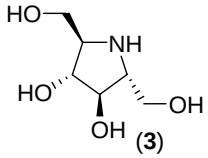
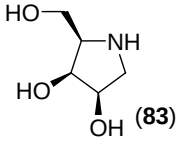
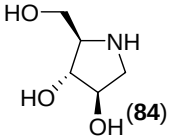
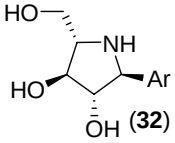
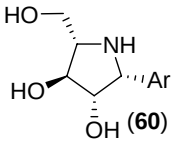
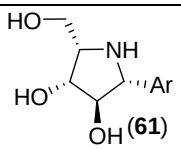
No inhibition at 1 mM concentration of all four compounds on enzyme.

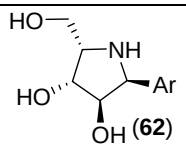
*** percentage inhibition checked at 1mM inhibitor concentration.**

Comparative Studies of K_i with few active Compounds[42]

Inhibition constants K_i (μM) of all the four synthetic diastereomeric Radicamine B are compared with the other known values for few pyrrolidines (**3**, **83** & **84**) having similar structures from the literature.

Table 7

Compounds	α-Glucosidase (yeast)	β-Glucosidase (almonds)	α-Galactosidase (coffee beans)	α-Mannosidase (jack bean)
 (**3**)	~ 1.6	~ 4	> 1000	
 (**83**)	> 1000	130	~ 0.1	~ 7
 (**84**)	~ 0.1	~ 100		~ 50
 (**32**)	64	NI	9.0% *	NI
 (**60**)	390	NI	5.0% *	NI
 (**61**)	13.1% *	NI	10.0% *	NI

 (62)	32.3% *	NI	10.0% *	NI

*~) approximate K_i estimated from IC_{50} assuming competitive inhibition and [S] = Km; *) percentage inhibition checked at 1 mM inhibitor concentration; NI) no inhibition at 1 mM inhibitor concentration; Ar) p-OH-C_6H_4.*

Thus, we can see from table 7 that the inhibitory activities against α-glucosidase (from yeast) for DMDP (**3**) and synthetic compound (**32**) (which are enantiomeric to each other with only one of the hydroxymethyl of DMDP is replaced by aromatic group in **32**) are in micromolar range (μM). Again for diastereomeric radicamine B (**60**) in which only the aromatic substituent's orientation is reversed the K_i value was found to be 390 μM. While the other two diastereomers of Radicamine B (**61**) and (**62**), showed poor activity i.e. 13.1% and 32.3%, respectively. This observation indicates that the relative orientations of all three substituents (except aromatic one) should be either like DMDP **3** or enantiomeric to it for being active against α-glucosidase. Now, when the inhibition studies against β-glucosidase were compared, it was observed that none of the synthetic compounds were active while for α-galactosidase the synthetic compounds exhibited poor inhibition activities in spite of (**83**) showing very good activity (0.1 μM). Moreover, for α-mannosidase, compounds (**83**) and (**84**) were observed to exhibit very good inhibitory activities while none of our synthetic compounds showed any activities.

Conclusions

In conclusion we have stereoselectively synthesized the proposed structure of Radicamine B, and observed in line with the reported syntheses that it is an enantiomer of the original natural product. Moreover, the flexibility of our approach has led to the synthesis of other three diastereomeric Radicamine B derivatives which helped us to carry out glycosidase inhibition studies for understanding the structure – activity relationships.

Table 2. Crystal data and structure refinement for compound 78

Empirical formula	$C_{19}H_{27}NO_7$
Formula weight	381.42
Temperature	297(2) K
Wavelength	0.71073 Å
Crystal system, space group	Triclinic, P-1
Unit cell dimensions	a = 9.293(4) Å , α = 101.226(7)^o
	b = 11.321(5) Å , β = 110.310(7)^o
	c = 11.772(5) Å , γ = 113.898(7)^o
Volume	977.3(7) Å^3
Z, Calculated density	2, 1.296 mg/m^3
Absorption coefficient	0.099 mm^{-1}
F(000)	408
Crystal size	0.36 x 0.17 x 0.05 mm
Theta range for data collection	2.00 to 25.00^o
Limiting indices	-11<=h<=10, -13<=k<=11, -13<=l<=13
Reflections collected / unique	7044 / 3425 [R(int) = 0.0334]
Completeness to theta = 25.00	99.3 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9951 and 0.9653
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3425 / 0 / 252
Goodness-of-fit on F^2	1.062
Final R indices [I>2sigma(I)]	R1 = 0.0608, wR2 = 0.1213
R indices (all data)	R1 = 0.1027, wR2 = 0.1355
Largest diff. peak and hole	0.377 and -0.176 e. Å $^{-3}$

Table 3. Bond lengths [Å] and angles [deg] for compound 78

O(1)-C(2)	1.408(3)	O(1)-C(1)-C(14)	104.7(3)
O(1)-C(1)	1.435(4)	N(1)-C(1)-C(14)	114.8(3)
O(2)-C(4)	1.424(3)	C(13)-C(1)-C(14)	112.1(3)

O(3)-C(5)	1.402(3)	O(1)-C(2)-C(3)	105.8(2)
O(4)-C(6)	1.212(3)	N(1)-C(3)-C(2)	101.0(2)
O(5)-C(10)	1.356(3)	N(1)-C(3)-C(4)	112.0(2)
O(6)-C(15)	1.214(3)	C(2)-C(3)-C(4)	115.8(2)
O(7)-C(15)	1.329(3)	O(2)-C(4)-C(5)	110.3(2)
O(7)-C(16)	1.479(4)	O(2)-C(4)-C(3)	112.1(2)
N(1)-C(15)	1.350(4)	C(5)-C(4)-C(3)	112.7(2)
N(1)-C(3)	1.468(3)	O(3)-C(5)-C(4)	108.0(2)
N(1)-C(1)	1.478(3)	O(3)-C(5)-C(6)	111.8(2)
C(1)-C(13)	1.489(5)	C(4)-C(5)-C(6)	111.2(2)
C(1)-C(14)	1.512(4)	O(4)-C(6)-C(7)	121.9(3)
C(2)-C(3)	1.516(4)	O(4)-C(6)-C(5)	117.8(3)
C(3)-C(4)	1.522(4)	C(7)-C(6)-C(5)	120.3(2)
C(4)-C(5)	1.518(4)	C(12)-C(7)-C(8)	117.9(3)
C(5)-C(6)	1.520(4)	C(12)-C(7)-C(6)	123.5(3)
C(6)-C(7)	1.474(4)	C(8)-C(7)-C(6)	118.6(3)
C(7)-C(12)	1.388(4)	C(9)-C(8)-C(7)	121.3(3)
C(7)-C(8)	1.390(4)	C(8)-C(9)-C(10)	119.6(3)
C(8)-C(9)	1.369(4)	O(5)-C(10)-C(11)	117.2(3)
C(9)-C(10)	1.383(4)	O(5)-C(10)-C(9)	122.6(3)
C(10)-C(11)	1.376(4)	C(11)-C(10)-C(9)	120.2(3)
C(11)-C(12)	1.368(4)	C(12)-C(11)-C(10)	119.7(3)
C(16)-C(17)	1.500(5)	C(11)-C(12)-C(7)	121.4(3)
C(16)-C(18)	1.506(5)	O(6)-C(15)-O(7)	125.7(3)
C(16)-C(19)	1.508(4)	O(6)-C(15)-N(1)	122.5(3)
C(2)-O(1)-C(1)	109.3(2)	O(7)-C(15)-N(1)	111.8(2)
C(15)-O(7)-C(16)	120.8(2)	O(7)-C(16)-C(17)	109.3(3)
C(15)-N(1)-C(3)	119.8(2)	O(7)-C(16)-C(18)	110.3(3)
C(15)-N(1)-C(1)	127.9(2)	C(17)-C(16)-C(18)	112.5(3)
C(3)-N(1)-C(1)	112.2(2)	C(17)-C(16)-C(18)	101.7(3)
O(1)-C(1)-N(1)	100.2(2)	C(17)-C(16)-C(19)	110.9(3)
O(1)-C(1)-C(13)	111.9(3)	C(18)-C(16)-C(19)	111.5(3)
N(1)-C(1)-C(13)	112.2(3)		

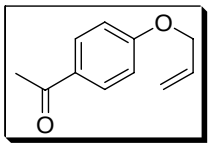
Table 4. Torsion angles [deg] for compound 78

C(2)-O(1)-C(1)-N(1)	-32.9(3)	O(3)-C(5)-C(6)-C(7)	-176.8(2)
C(2)-O(1)-C(1)-C(13)	86.2(3)	C(4)-C(5)-C(6)-C(7)	62.4(3)
C(2)-O(1)-C(1)-C(14)	-152.1(3)	O(4)-C(6)-C(7)-C(12)	-163.7(3)
C(15)-N(1)-C(1)-O(1)	-155.9(3)	C(5)-C(6)-C(7)-C(12)	17.0(4)
C(3)-N(1)-C(1)-O(1)	19.4(3)	O(4)-C(6)-C(7)-C(8)	16.1(4)
C(15)-N(1)-C(1)-C(13)	85.1(4)	C(5)-C(6)-C(7)-C(8)	-163.2(2)
C(3)-N(1)-C(1)-C(13)	-99.5(3)	C(12)-C(7)-C(8)-C(9)	-1.2(4)
C(15)-N(1)-C(1)-C(14)	-44.4(4)	C(6)-C(7)-C(8)-C(9)	179.0(2)
C(3)-N(1)-C(1)-C(14)	131.0(3)	C(7)-C(8)-C(9)-C(10)	0.3(4)
C(1)-O(1)-C(2)-C(3)	34.7(3)	C(8)-C(9)-C(10)-O(5)	-179.3(3)
C(15)-N(1)-C(3)-C(2)	175.6(3)	C(8)-C(9)-C(10)-C(11)	1.1(4)
C(1)-N(1)-C(3)-C(2)	-0.2(3)	O(5)-C(10)-C(11)-C(12)	178.8(3)
C(15)-N(1)-C(3)-C(4)	-60.5(3)	C(9)-C(10)-C(11)-C(12)	-1.6(5)
C(1)-N(1)-C(3)-C(4)	123.7(3)	C(10)-C(11)-C(12)-C(7)	0.7(5)
O(1)-C(2)-C(3)-N(1)	-19.9(3)	C(8)-C(7)-C(12)-C(11)	0.7(4)
O(1)-C(2)-C(3)-C(4)	-141.2(3)	C(6)-C(7)-C(12)-C(11)	-179.5(3)
N(1)-C(3)-C(4)-O(2)	-65.2(3)	C(16)-O(7)-C(15)-O(6)	-1.6(5)
C(2)-C(3)-C(4)-O(2)	50.0(3)	C(16)-O(7)-C(15)-N(1)	179.5(3)
N(1)-C(3)-C(4)-C(5)	169.7(2)	C(3)-N(1)-C(15)-O(6)	-7.0(4)
C(2)-C(3)-C(4)-C(5)	-75.1(3)	C(1)-N(1)-C(15)-O(6)	168.0(3)
O(2)-C(4)-C(5)-O(3)	-59.8(3)	C(3)-N(1)-C(15)-O(7)	171.9(2)
C(3)-C(4)-C(5)-O(3)	66.3(3)	C(1)-N(1)-C(15)-O(7)	-13.1(4)
O(2)-C(4)-C(5)-C(6)	63.1(3)	C(15)-O(7)-C(16)-C(17)	63.0(4)
C(3)-C(4)-C(5)-C(6)	-170.8(2)	C(15)-O(7)-C(16)-C(18)	-61.3(4)
O(3)-C(5)-C(6)-O(4)	3.9(4)	C(15)-O(7)-C(16)-C(19)	-179.7(3)
C(4)-C(5)-C(6)-O(4)	-116.9(3)		

Symmetry transformations used to generate equivalent atoms

Experimental

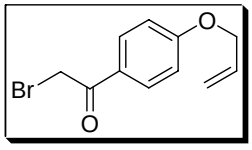
1-(4-(Allyloxy)phenyl)ethanone (73)



To a solution of 4-hydroxy acetophenone **72** (20 g, 146.8 mmol) in anhydrous acetone (200 mL), potassium carbonate (101.5 g, 734.4 mmol) and allyl bromide (25.4 mL, 293.5 mmol) were added and the contents were refluxed (3 h) until the complete consumption of starting material was indicated by TLC (30% ethyl acetate in pet-ether; R_f for product 0.5 and for starting material 0.2). The reaction mixture was cooled to room temperature and filtered through Celite, concentrated and the residue obtained was purified by column chromatography (10 % ethylacetate in pet-ether) to procure **73** as a colourless oil.

Yield	: 23.3 g; 90 %
Mol. Formula	: $C_{11}H_{12}O_2$
IR ($CHCl_3$) $\tilde{\nu}$	: 3080, 1676, 1600, 1456, 1359, 1075, 1018, 957, 932, 834 cm^{-1}
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.56 (s, 3H), 4.61 (td, *J* = 5.2, 1.4 Hz, 2H), 5.30-5.48 (m, 2H), 5.96-6.15 (m, 1H), 6.95 (d, *J* = 9.0 Hz, 2H), 7.94 (d, *J* = 9.0 Hz, 2H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: 25.83 (q), 68.40 (t), 113.98 (d), 117.58 (t), 130.00 (s), 130.15 (d), 132.21 (d), 162.06 (s), 195.91 (s) ppm.
Elemental Analysis	Calcd. : C, 74.98; H, 6.86 Found : C, 74.90; H, 6.78.
ESI-MS (*m/z*)	: 199 $[M+Na]^+$.

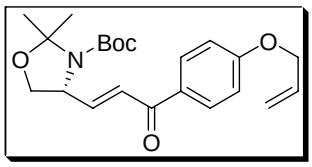
1-(4-(Allyloxy)phenyl)-2-bromoethanone (75)



To a refluxing suspension of copper(II) bromide (6.08 g, 27.3 mmol) in ethyl acetate (13.5 mL) was added slowly a solution of 4-*O*-allyl acetophenone **73** (3 g, 17.0 mmol) in chloroform and reflux was continued until the reaction was complete as judged by a colour change of the solution from green to amber, disappearance of all black solid, and cessation of hydrogen bromide evolution. The solution was cooled and filtered through Celite, washed with ethyl acetate, concentrated and purified over silica gel column (EtOAc:Petroleum Ether; 8:92 as eluent) to give **75** as a thick liquid.

Yield	: 3.5 g; 80 %
Mol. Formula	: $C_{11}H_{11}BrO_2$
IR ($CHCl_3$) $\tilde{\nu}$	: 3079, 1673, 1599, 1459, 1365, 1113, 989, 841, 822, 627, 592 cm^{-1}
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 4.40 (s, 2H), 4.61 (td, *J* = 5.3, 1.4 Hz, 2H), 5.31-5.48 (m, 2H), 5.96-6.15 (m, 1H), 6.98 (d, *J* = 9.0 Hz, 2H), 7.96 (d, *J* = 9.0 Hz, 2H) ppm.
^{13}C NMR ($CDCl_3$, 125 MHz)	: δ 30.49 (t), 68.71 (t), 114.53 (d), 118.04 (t), 126.83 (s), 131.11 (d), 132.19 (d), 162.89 (s), 189.35 (s) ppm.
Elemental Analysis	Calcd.: C, 51.79; H, 4.35; Br, 31.32 Found : C, 51.70; H, 4.25; Br, 31.31
ESI-MS (*m/z*)	: 277 $[M+Na]^+$.

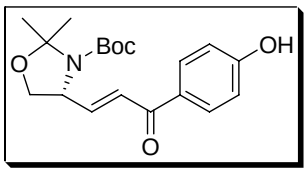
(*R*)-*Tert*-butyl 4-((*E*)-3-(4-(allyloxy)phenyl)-3-oxoprop-1-enyl)-2,2-dimethyloxazolidine-3-carboxylate (77)



To a homogeneous mixture of garner aldehyde **66** (4.1 g, 17.9 mmol) in benzene (138 mL), ylide **67** (9.4 g, 21.6 mmol) was added at room temperature. The mixture was stirred for 16 h at room temperature and progress in the reaction was monitored by TLC. After completion, the reaction mixture was quenched with saturated ammonium chloride. The organic layer was separated, and aqueous layer was extracted with ethylacetate (3 × 100 mL). The combined organic layers were dried over sodium sulphate, concentrated and purified by silica-gel column using 12% ethyl acetate in pet-ether as eluent to afford Wittig product **77** as light yellow solid.

Yield	: 4.8 g; 70 %
Mol. Formula	: $C_{22}H_{29}NO_5$
Melting Point	: 77 ^oC
Optical Rotation $[\alpha]_D^{25}$	: –33.8 (*c* 0.38, $CHCl_3$)
IR (Nujol) $\tilde{\nu}$	: 2924, 1708, 1666, 1615, 1461, 1422, 1264, 1252, 1086, 1059, 982, 972, 937, 877, 860, 850 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.42-1.68 (m, 15H), 3.86 (dd, *J* = 9.1, 2.4 Hz, 1H), 4.14 (dd, *J* = 9.0, 6.4 Hz, 1H), 4.50-4.58 (m, 1H), 4.62 (td, *J* = 5.3, 1.4 Hz, 2H), 5.29-5.49 (m, 2H), 5.97-6.16 (m, 1H), 6.90-6.99 (m, 4H), 7.94 (d, *J* = 9.0 Hz, 2H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 23.43 (q), 24.55 (q), 26.24 (q), 27.12 (q), 28.16 (q), 58.29 (d), 67.23 (t), 68.62 (t), 79.88 (s), 80.32 (s), 93.73 (s), 94.27 (s), 114.33 (d), 117.90 (t), 125.38 (d), 126.08 (d), 130.61 (d), 132.24 (d), 144.20 (d), 145.08 (d), 151.45 (s), 151.71 (s), 162.32 (s), 188.04 (s) ppm.
Elemental Analysis	Calcd.: C, 68.20; H, 7.54; N, 3.61 Found : C, 68.25; H, 7.48; N, 3.60.
ESI-MS (*m/z*)	: 410 $[M+Na]^+$.

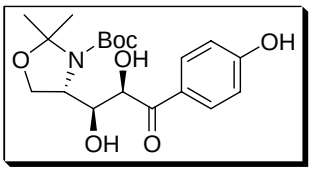
(*R*)-*Tert*-butyl 4-((*E*)-3-(4-hydroxyphenyl)-3-oxoprop-1-enyl)-2,2-dimethyloxazolidine-3-carboxylate (65)



The wittig product **77** (2.5 g, 6.4 mmol) was dissolved in a mixture of solvents (ethanol:benzene:water 28.6 mL:12.3 mL:4.1 mL) to which wilkinson's catalyst (137 mg, 0.15 mmol) and Dabco (0.159 g, 1.42 mmol) were added, and the resulting solution was refluxed for 10 h. Progress of reaction was monitored by TLC. Cooled, concentrated and partitioned between DCM and water. Aqueous layer was extracted with DCM (3 × 50 mL). The combined organic extracts were dried over sodium sulphate, concentrated and purified by column chromatography (EtOAc:Pet.Ether; 20:80) to give the deallylated product **65** as a solid.

Yield	: 1.75 g; 75 %
Mol. Formula	: $C_{19}H_{25}NO_5$
Melting Point	: 182 ^oC with decomposition (reddish colour)
Optical Rotation $[\alpha]_D^{25}$	: –9.7 (*c* 0.5, MeOH)
IR (Nujol) $\tilde{\nu}$	: 3294, 2924, 1661, 1651, 1617, 1464, 1367, 1238, 1170, 866, 842, 823 cm^{-1}
^{1}H NMR (DMSO-d_6, 400 MHz)	: δ 1.23-1.55 (m, 15H), 3.86 (d, *J* = 9.1 Hz, 1H), 4.11 (dd, *J* = 9.1, 6.6 Hz, 1H), 4.56 (br s, 1H), 6.72 (dd, *J* = 15.3, 6.8 Hz, 1H), 6.89 (d, *J* = 8.6 Hz, 2H), 7.07 (d, 15.3 Hz, 1H), 7.86 (d, 8.6 Hz, 2H), 10.48 (br s, 1H) ppm.
^{13}C NMR (DMSO-d_6, 50 MHz)	: δ 23.39 (q), 24.46 (q), 26.17 (q), 27.18 (q), 27.90 (q), 57.89 (d), 66.95 (t), 79.10 (s), 93.44 (s), 115.47 (d), 125.26 (d), 128.47 (s), 130.87 (d), 144.85 (d), 151.07 (s), 162.31 (s), 187.10 (s) ppm.
Elemental Analysis	Calcd.: C, 65.69; H, 7.25; N, 4.03 Found : C, 65.58; H, 7.04; N, 4.01.
ESI-MS (m/z)	: 370 $[M+Na]^+$.

(*S*)-*Tert*-butyl 4-((1*S*,2*R*)-1,2-dihydroxy-3-(4-hydroxyphenyl)-3-oxopropyl)-2,2-dimethyloxazolidine-3-carboxylate (78)

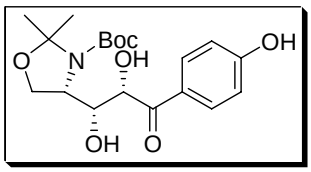


A toluene solution of OsO_4 (0.02 mL, 0.02 M, 0.4 mol %), $(DHQ)_2PHAL$ **80** (4.4 mg, 4 mol %), $NaHCO_3$ (36 mg, 0.43 mmol) and $MeSO_2NH_2$ (13 mg, 0.14 mmol) were sequentially added to a stirred solution of AD-mix-α (202 mg) in 1:1 *t*-butanol/water (1 mL each) at room temperature. The stirring was continued for 15 min., and the resulting clear solution was cooled to 0 ^oC. Ketone **65** (50 mg, 0.14 mmol) was added at once and the resulting mixture was stirred at 0 ^oC for 15 h. Ethyl acetate (1 mL) added and quenched by adding $Na_2S_2O_3$. The solution was allowed to attain room temperature with vigorous stirring for 1 h. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 × 5 mL). The combined organic layers were dried over sodium sulphate, concentrated and the

residue was purified by column chromatography (isopropanol:dichloromethane; 3:97) to give the diol **78** as a solid.

Yield	: 39 mg; 89 %
Mol. Formula	: $C_{19}H_{27}NO_7$
Melting Point	: squeezes at 90 ^oC and melts with decomposition at 170 ^oC (reddish colour)
Optical Rotation $[\alpha]_D^{25}$	: –51.5 (*c* 0.4, MeOH)
IR (Nujol) $\tilde{\nu}$	: 3337, 2924, 1679, 1654, 1603, 1464, 1378, 1282, 1171, 941, 864, 842 cm^{-1}.
^{1}H NMR (DMSO-d_6, 200 MHz)	: δ 1.42-1.45 (m, 15H), 3.85 (dd, *J* = 8.4, 6.0 Hz, 1H), 3.99-4.04 (m, 2H), 4.16 (d, *J* = 8.8 Hz, 1H), 4.63 (d, *J* = 7.6 Hz, 1H), 4.92 (dd, *J* = 13.6, 8.0 Hz, 2H), 6.85 (d, *J* = 8.7 Hz, 2H), 7.80 (d, *J* = 8.7 Hz, 2H), 10.43 (br s, 1H) ppm.
^{13}C NMR (DMSO-d_6, 50 MHz)	: 27.99 (q), 59.51 (d), 63.32 (t), 71.14 (d), 74.32 (d), 79.39 (s), 92.88 (s), 115.14 (d), 125.96 (s), 130.93 (d), 151.83 (s), 162.18 (s), 197.86 (s) ppm.
Elemental Analysis	Calcd.: C, 59.83; H, 7.14; N, 3.67 Found : C, 59.62; H, 6.99; N, 3.43.
ESI-MS (*m/z*)	: 404 $[M+Na]^+$.

(*S*)-*Tert*-butyl 4-((1*R*,2*S*)-1,2-dihydroxy-3-(4-hydroxyphenyl)-3-oxopropyl)-2,2-dimethyloxazolidine-3-carboxylate (79)

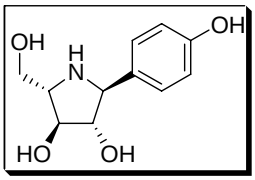


The reaction was performed in a similar way as described above for **78** but with different ligand i.e. $(DHQD)_2PHAL$ **81**. Selectivity in this case was poor (60:40 for **78** & **79**) and it was still towards **78**. The diastereomers were separated under same conditions as described above for **78**. **79** was obtained as a sticky solid.

Yield	: 19 mg; 90 %

Mol. Formula	: $C_{19}H_{27}NO_7$
Optical Rotation $[\alpha]_D^{25}$	: –45.2 (*c* 0.8, MeOH)
IR (Nujol) $\tilde{\nu}$	: 3356, 2923, 1673, 1604, 1584, 1461, 1378, 1259, 1170, 982, 863, 846 cm^{-1}.
^{1}H NMR (DMSO-d_6, 200 MHz)	: δ 1.35-1.54 (m, 15H), 3.88-4.04 (m, 3H), 4.36 (d, *J* = 8.5 Hz, 1H), 4.63 (br s, 1H), 5.05 (s, 1H), 6.84 (d, *J* = 8.5 Hz, 2H), 7.74 (d, *J* = 8.5 Hz, 2H), 10.44 (s, 1H) ppm.
^{13}C NMR (DMSO-d_6, 50 MHz)	: δ 27.91 (q), 59.88 (d), 63.27 (t), 71.00 (d), 72.12 (d), 79.87 (s), 93.36 (s), 115.20 (d), 125.59 (s), 130.81 (d), 153.00 (s), 162.12 (s), 197.73 (s) ppm.
Elemental Analysis	Calcd.: C, 59.83; H, 7.14; N, 3.67 Found : C, 59.73; H, 7.27; N, 3.65.
ESI-MS (*m/z*)	: 404 $[M+Na]^+$.

(2*S*,3*S*,4*S*,5*S*)-2-(Hydroxymethyl)-5-(4-hydroxyphenyl)pyrrolidine-3,4-diol (32)

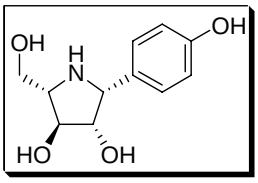


To the triol **78** (100 mg, 0.26 mmol) cooled at 0 ^oC, 90 % TFA (3 mL) was slowly added, and the reaction mixture was allowed to stir at room temperature for 0.5 h. Then, TFA was completely removed by coevaporating with toluene under vacuum to give a sticky compound which was dissolved in anhydrous methanol (1.2 mL) and slowly added to a mixture of KOH (15 mg, 0.27 mmol), anhydrous methanol (0.8 mL), glacial acetic acid (7.5 uL, 0.13 mmol) and sodium cyanoborohydride (16 mg, 0.25 mmol) precooled at 0 ^oC. This mixture was stirred for 6 h at 0 ^oC and allowed to attain room temperature slowly, solvent was evaporated under vacuum, and purified by column chromatography (using DCM:MeOH:(25%) aq.NH_3; 8:1.9:0.1 as eluent). Finally, pure **32** was obtained as a thick liquid after separating it from its diastereomer (65:35) through HPLC using C-18 column and water:methanol:acetic acid (95:5:0.1) as eluent.

Yield : 29 mg; 50 %

Mol. Formula	: $C_{11}H_{15}NO_4$
Optical Rotation $[\alpha]_D^{25}$	: –67.0 (*c* 0.22, H_2O)
^{1}H NMR	: δ 3.46-3.49 (m, 1H), 3.76 (dd, *J* = 12.5, 5.9 Hz, 1H), 3.80
(D_2O, 500 MHz)	(dd, *J* = 12.2, 3.7 Hz, 1H), 4.03 (t, *J* = 7.7 Hz, 1H), 4.20
	(d, *J* = 10.0 Hz, 1H), 4.30 (dd, *J* = 10.0, 7.7 Hz, 1H), 6.85
	(d, *J* = 8.6 Hz, 2H), 7.29 (d, *J* = 8.6, 2H) ppm.
^{13}C NMR	: δ 58.11 (t), 60.73 (d), 62.09 (d), 73.47 (d), 77.19 (d),
(D_2O, 125 MHz)	115.26 (d), 123.58 (s), 129.11 (d), 156.00 (s) ppm.
Elemental Analysis	Calcd.: C, 58.66; H, 6.71; N, 6.22
	Found : C, 58.49; H, 6.78; N, 6.14.
ESI-MS (*m/z*)	: 226 $[M+1]^+$.

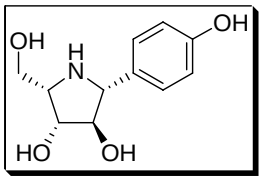
(2*S*,3*S*,4*S*,5*R*)-2-(Hydroxymethyl)-5-(4-hydroxyphenyl)pyrrolidine-3,4-diol (60)



Similarly, **60** was obtained as the minor diastereomer after HPLC separation of the above mixture as thick liquid.

Yield	: 15 mg; 27 %
Mol. Formula	: $C_{11}H_{15}NO_4$
Optical Rotation $[\alpha]_D^{25}$	: –18.0 (*c* 0.22, H_2O)
^{1}H NMR	: δ 3.35 (br s, 1H), 3.76 (dd, *J* = 12.1, 7.4 Hz, 1H), 3.84
(D_2O, 500 MHz)	(dd, *J* = 12.0, 4.6 Hz, 1H), 3.99 (d, *J* = 3.0 Hz, 1H), 4.19
	(br s, 1H), 4.51 (br s, 1H), 6.81 (d, *J* = 8.6 Hz, 2H), 7.23
	(d, 8.6 Hz, 2H) ppm.
^{13}C NMR	: δ 60.46 (t), 64.43 (d), 66.95 (d), 77.40 (d), 77.73 (d),
(D_2O, 100 MHz)	115.79 (d), 124.42 (s), 129.66 (d), 156.00 (s) ppm.
Elemental Analysis	Calcd.: C, 58.66; H, 6.71; N, 6.22
	Found : C, 58.44; H, 6.53; N, 6.20.
ESI-MS (*m/z*)	: 226 $[M+1]^+$.

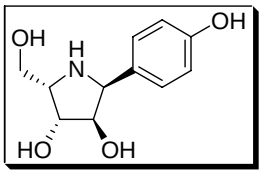
(2*S*,3*R*,4*R*,5*R*)-2-(Hydroxymethyl)-5-(4-hydroxyphenyl)pyrrolidine-3,4-diol (61)



61 was obtained by following the same reaction procedure described for **32**. It was also purified by using same conditions and column as described above to give pure diastereomer **61** as a thick liquid from the mixture of **61** & **62** (85:15).

Yield	: 35 mg; 60 %
Mol. Formula	: $C_{11}H_{15}NO_4$
Optical Rotation $[\alpha]_D^{25}$	: +38.6 (c 0.42, H_2O)
^{1}H NMR (D_2O, 500 MHz)	: δ 3.72 (dd, *J* = 11.3, 5.3 Hz, 1H), 3.80 (dd, *J* = 12.2, 5.0 Hz, 1H), 3.84 (dd, *J* = 12.2, 5.0 Hz, 1H), 4.16 (d, *J* = 6.0 Hz, 1H), 4.24 (dd, *J* = 5.1, 3.5 Hz, 1H), 4.31 (dd, *J* = 6.2, 3.5 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 2H), 7.28 (d, *J* = 8.5 Hz, 2H) ppm.
^{13}C NMR (D_2O, 125 MHz)	: δ 57.03 (t), 61.52 (d), 66.18 (d), 74.51 (d), 79.91 (d), 115.36 (d), 124.72 (s), 129.50 (d), 156.00 (s) ppm.
Elemental Analysis	Calcd.: C, 58.66; H, 6.71; N, 6.22 Found : C, 58.51; H, 6.51; N, 6.17.
ESI-MS (*m/z*)	: 226 $[M+1]^+$.

(2*S*,3*R*,4*R*,5*S*)-2-(Hydroxymethyl)-5-(4-hydroxyphenyl)pyrrolidine-3,4-diol (62)



62 was obtained as the minor diastereomer from the above mixture as a thick liquid.

Yield	: 6 mg; 11 %
Mol. Formula	: $C_{11}H_{15}NO_4$
Optical Rotation $[\alpha]_D^{25}$	: +43.5 (c 0.14, H_2O)

^{1}H NMR (D_2O, 500 MHz)	: δ 3.78 (dd, *J* = 12.0, 8.1 Hz, 1H), 3.86 (dd, *J* = 12.0, 5.1 Hz, 1H), 3.90-3.94 (m, 1H), 4.20 (d, *J* = 1.7 Hz, 1H), 4.35 (dd, *J* = 4.4, 1.5 Hz, 1H), 4.67 (br s, 1H), 6.82 (d, *J* = 8.6 Hz, 2H), 7.24 (d, *J* = 8.6 Hz, 2H) ppm.
^{13}C NMR (D_2O, 125 MHz)	: δ 58.19 (t), 62.23 (d), 63.59 (d), 74.98 (d), 77.21 (d), 115.50 (d), 123.69 (s), 129.69 (d), 156.00 (s) ppm.
Elemental Analysis	Calcd.: C, 58.66; H, 6.71; N, 6.22 Found : C, 58.42; H, 6.42; N, 6.30.
ESI-MS (*m/z*)	: 226 $[M+1]^+$.

Procedures for the enzyme inhibition assay

The residual hydrolytic activities of the glycosidases of the corresponding *p*-nitrophenyl glycosides in the presence of azasugars were measured spectrophotometrically (at 405 nm) for determining the inhibitory potencies of the synthesized azasugars (**32**, **60**, **61** & **62**).

(a) Assay of α-Glucosidase Inhibition:

α-Glucosidase (25 µl, 100 µg/mL) from yeast was incubated with inhibitors (**32**, **60**, **61** & **62**) of different concentrations (0.5 to 1 mM each) in 50 mM 6.8 pH citrate phosphate buffer (50 µl) and *p*-nitrophenyl-α-D-glucopyranoside (50 µl, 5mM) in a total volume of 500 µl at 37 ^{o}C for 20 min. The reaction was terminated by adding 1.0 mL of 1M Na_2CO_3. Optical density was measured at 405 nm on spectrophotometer. The respective blanks were taken for all enzymes and inhibitors during assay.

(b) Assay of β-Glucosidase Inhibition:

The enzyme β-Glucosidase (25 µl, 250 µg/mL) from almond was incubated with inhibitors (**32**, **60**, **61** & **62**) of different concentrations (0.5 to 1 mM each) in 50 mM 5.5 pH citrate phosphate buffer (25 µl) and *p*-nitrophenyl-β-D-glucopyranoside (25 µl, 5mM) in a total volume of 250 µl at 37 ^{o}C for 30 min. The reaction was terminated by adding 0.75 mL of 1M Na_2CO_3. Optical density was measured at 405 nm on spectrophotometer. The respective blanks were taken for all enzymes and inhibitors during assay.

(c) Assay of α-Galactosidase Inhibition:

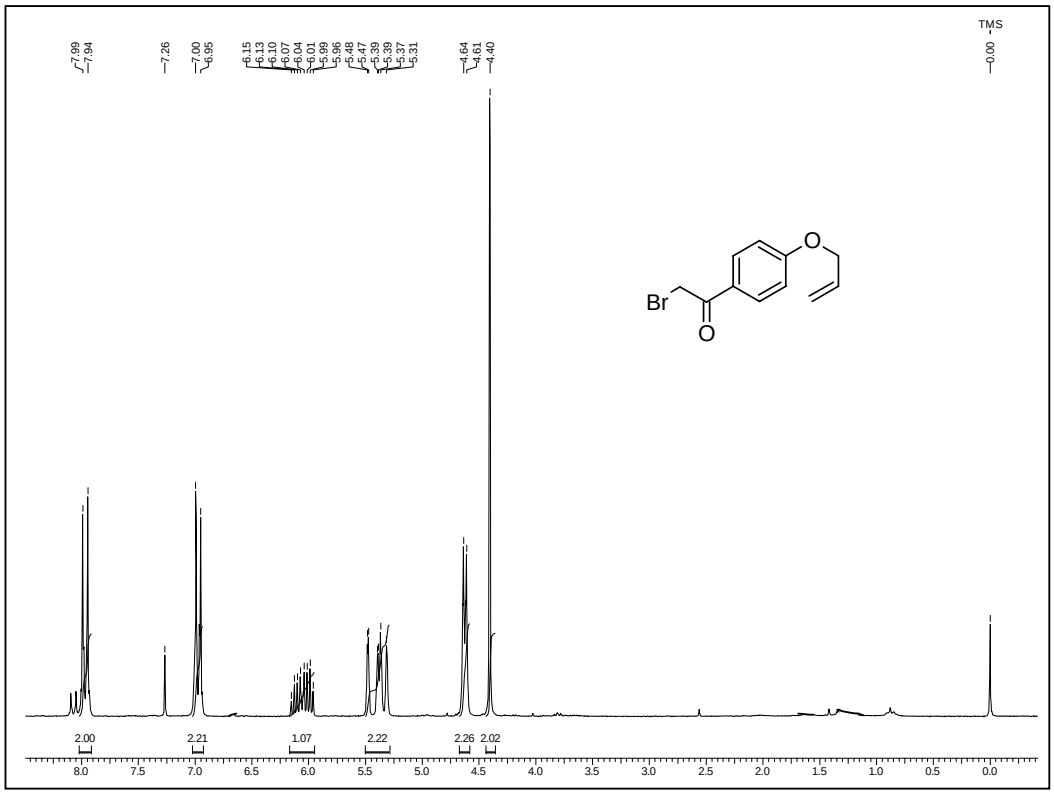
The enzyme α-Galactosidase (50 µl, 100 µg/mL) from green coffee beans was incubated with inhibitors (**32**, **60**, **61** & **62**) of different concentrations (0.5 to 1 mM each) in 50 mM 6.5 pH citrate phosphate buffer (50 µl) and *p*-nitrophenyl-α-D-galactopyranoside (50 µl, 1mM) in a total volume of 500 µl at 25 ^{o}C for 20 min. The reaction was terminated by adding 1.0 mL of 1M Na_2CO_3. Optical density was measured at 405 nm on spectrophotometer. The respective blanks were taken for all enzymes and inhibitors during assay.

(d) Assay of α-Mannosidase Inhibition:

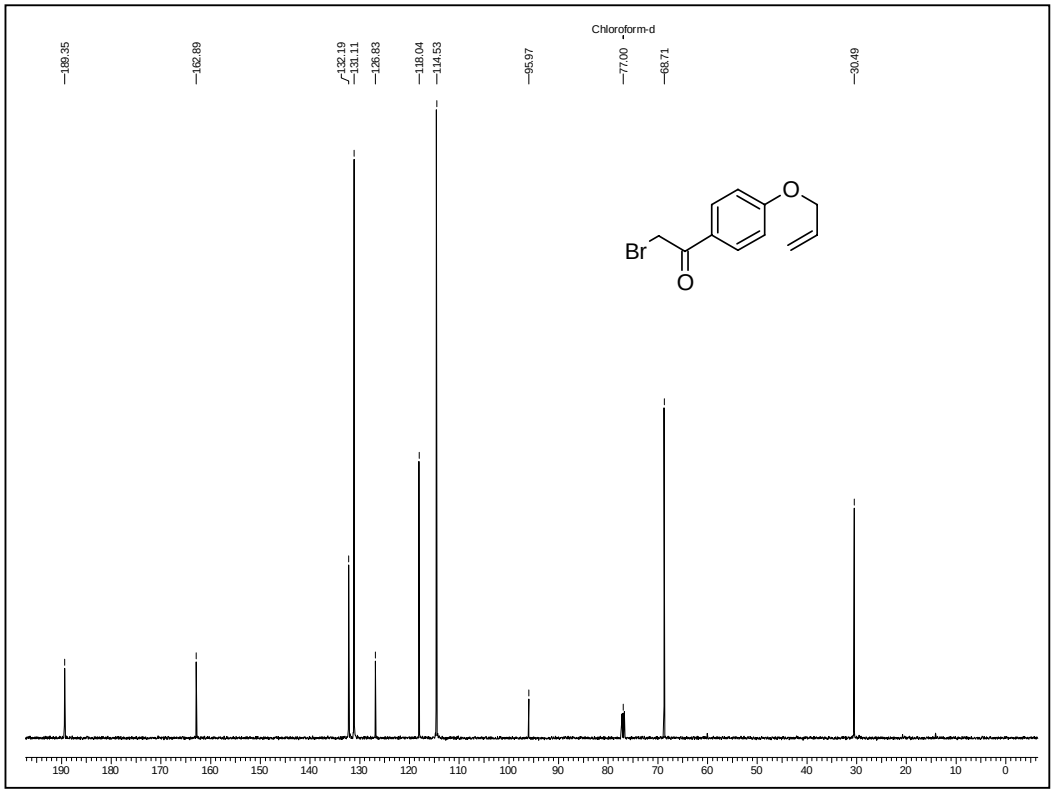
The enzyme α-Mannosidase (50 µl, 100 µg/mL) from jack bean was incubated with inhibitors (**32**, **60**, **61** & **62**) of different concentrations (0.5 to 1 mM each) in 50 mM 4.5 pH acetate buffer (50 µl) and *p*-nitrophenyl-α-D-mannopyranoside (50 µl, 1mM) in a total volume of 500 µl at 25 ^{o}C for 20 min. The reaction was terminated by adding 1.0 mL of 1M Na_2CO_3. Optical density was measured at 405 nm on spectrophotometer. The respective blanks were taken for all enzymes and inhibitors during assay.

The inhibition rates (%) were calculated from the formula 100-100×(ODtest-ODblank)/(control ODtest-control ODblank). IC_{50} data were obtained over different concentrations of inhibitor and then plotted as percentage inhibition versus inhibitor concentration. K_i was determined under similar conditions to IC_{50} determinations, except that substrate concentrations were taken at two levels. Dixon plots were used to transform the kinetic data into K_i values.

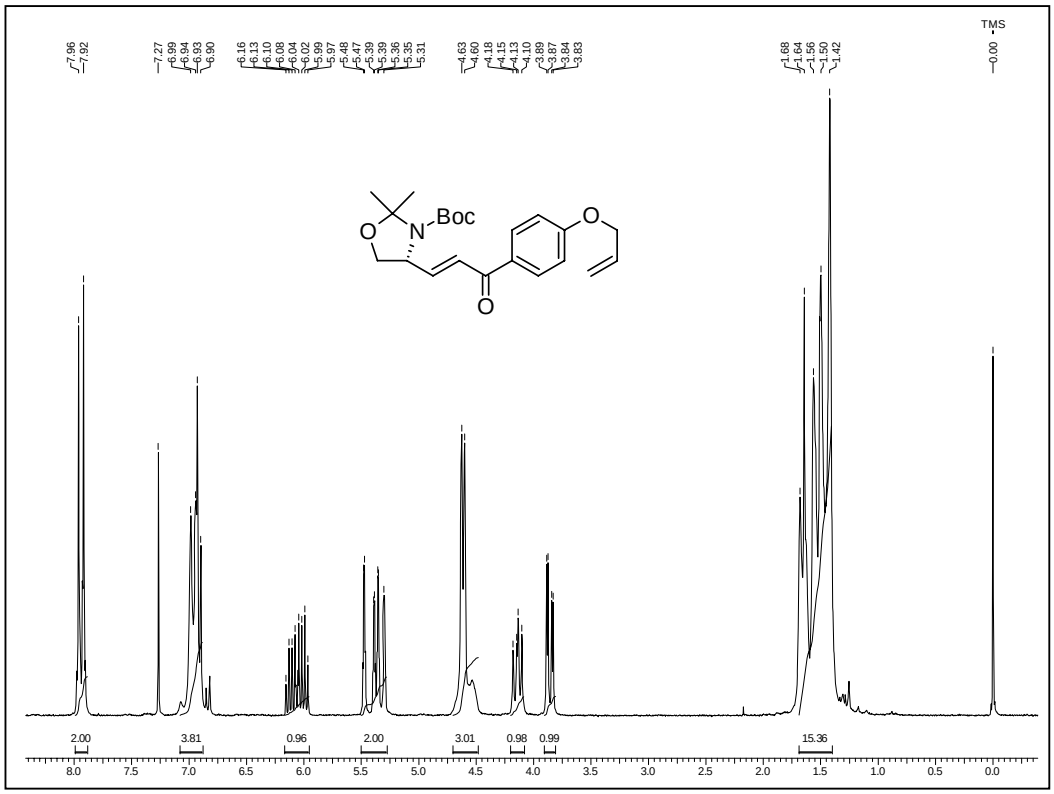
Spectra



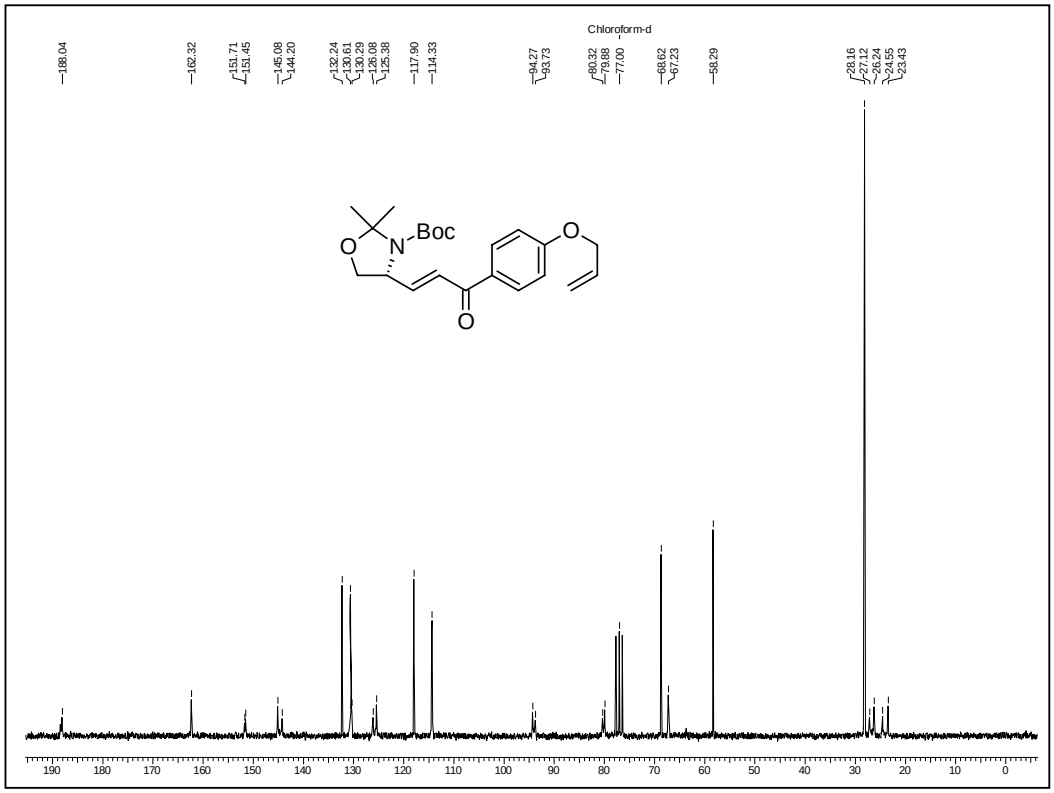
^{1}H NMR Spectrum of **75** in $CDCl_3$



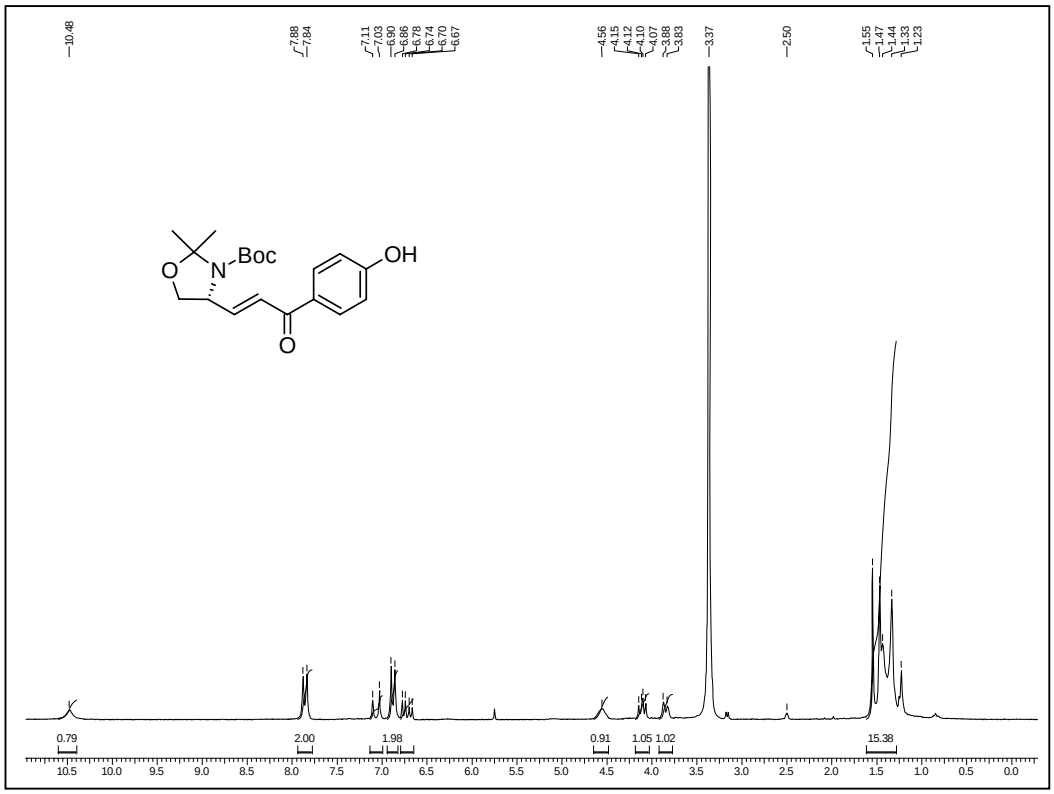
^{13}C NMR Spectrum of **75** in $CDCl_3$



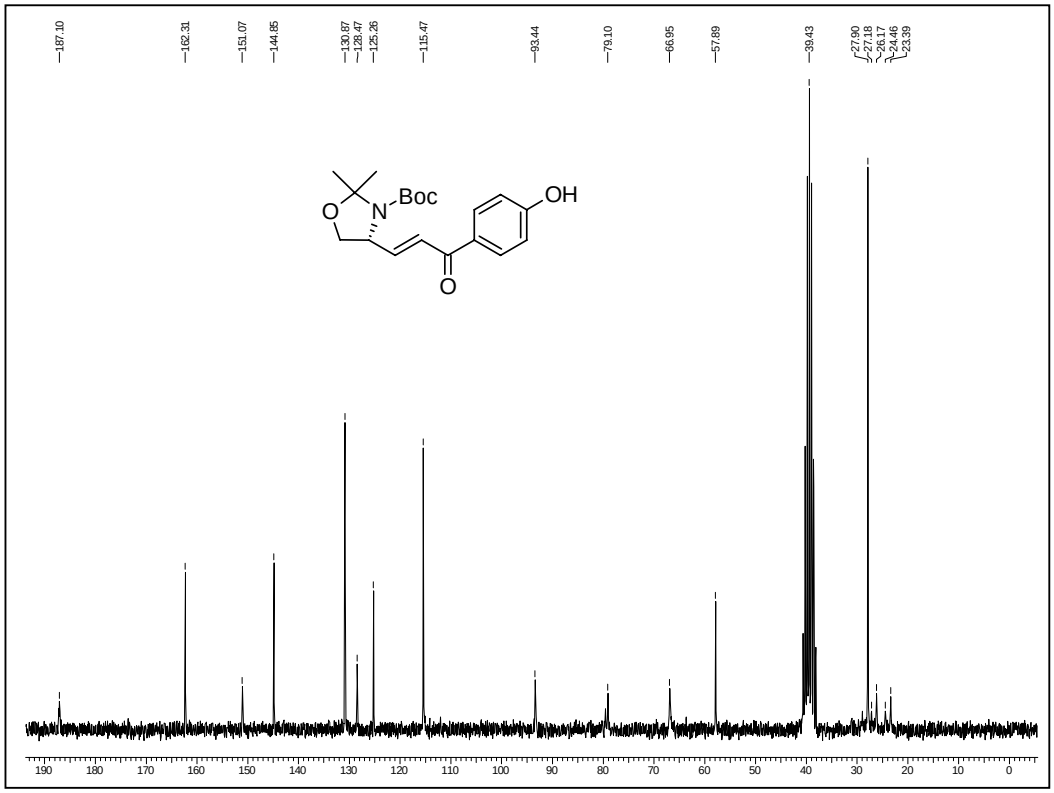
^{1}H NMR Spectrum of **77** in $CDCl_3$



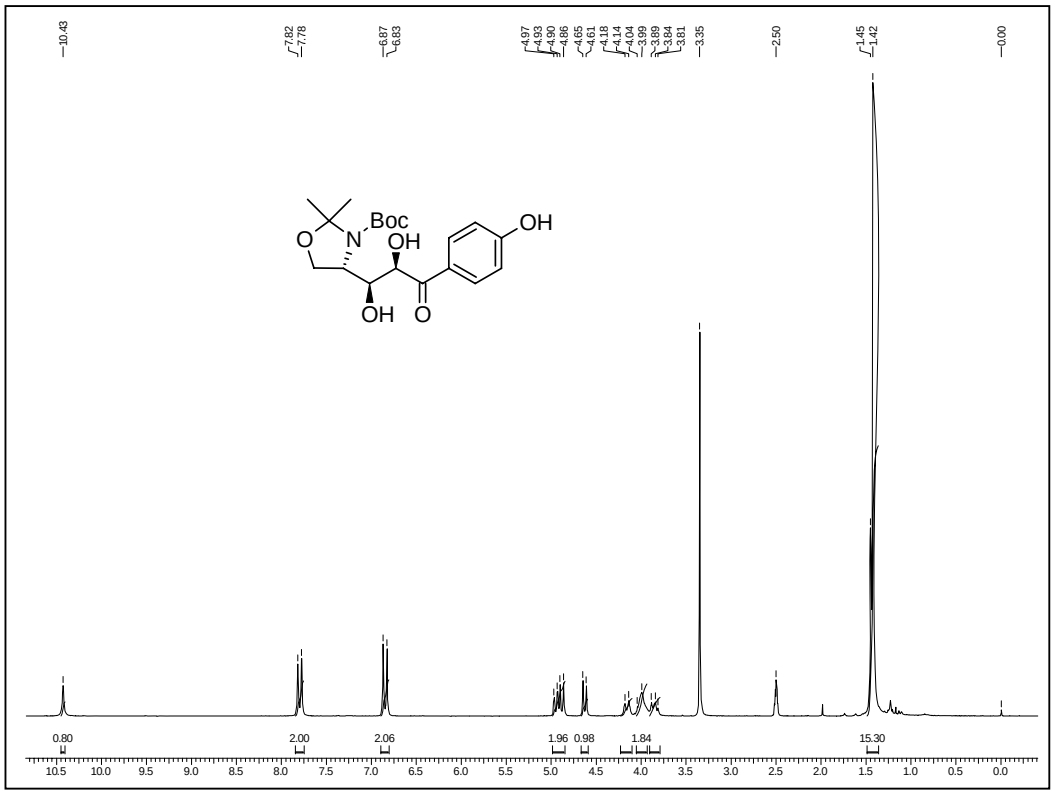
^{13}C NMR Spectrum of **77** in $CDCl_3$



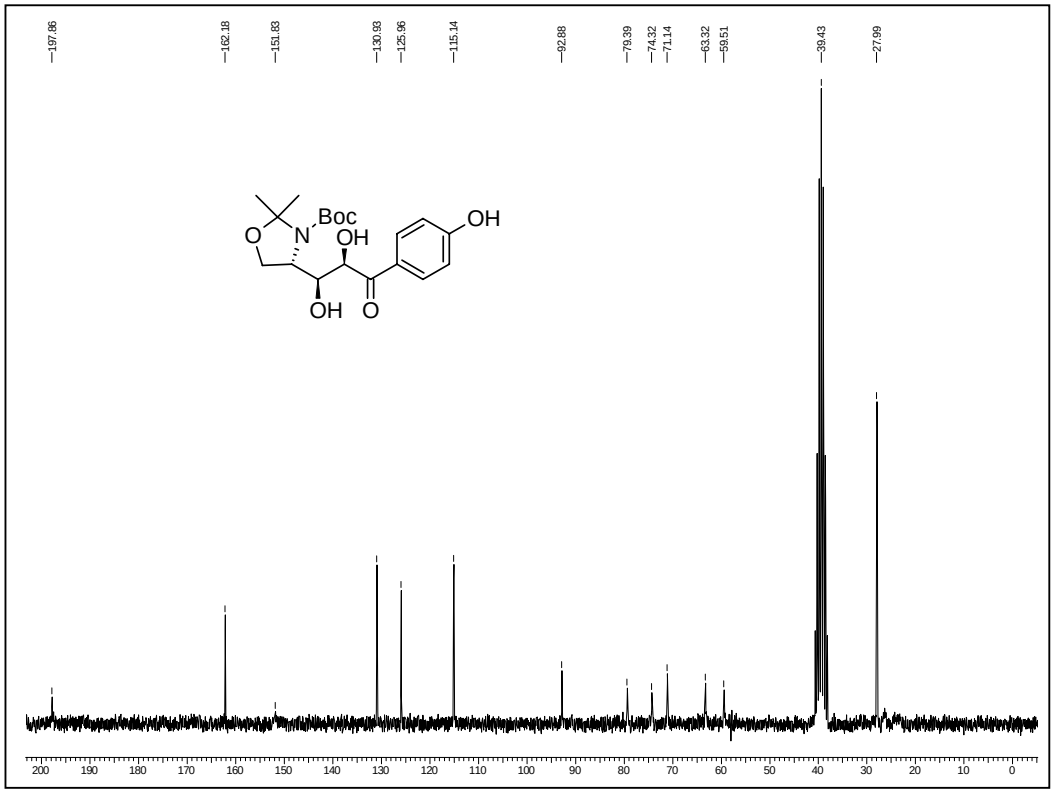
^{1}H NMR Spectrum of **65** in DMSO-d_6



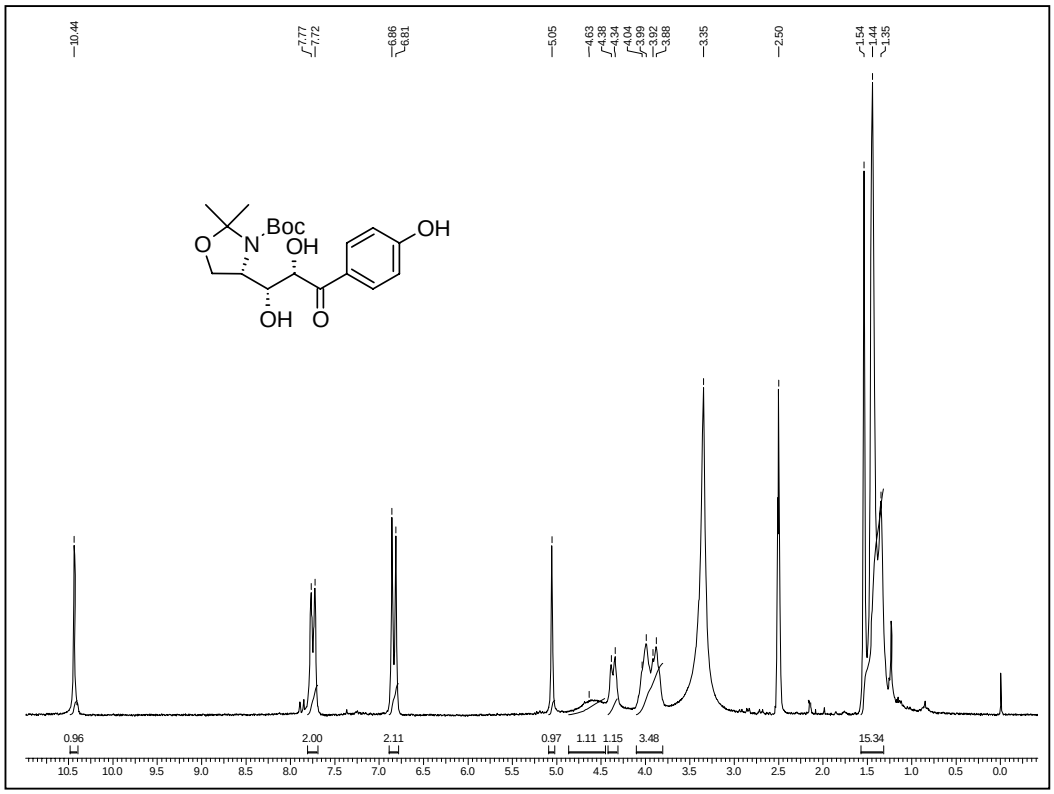
^{13}C NMR Spectrum of **65** in DMSO-d_6



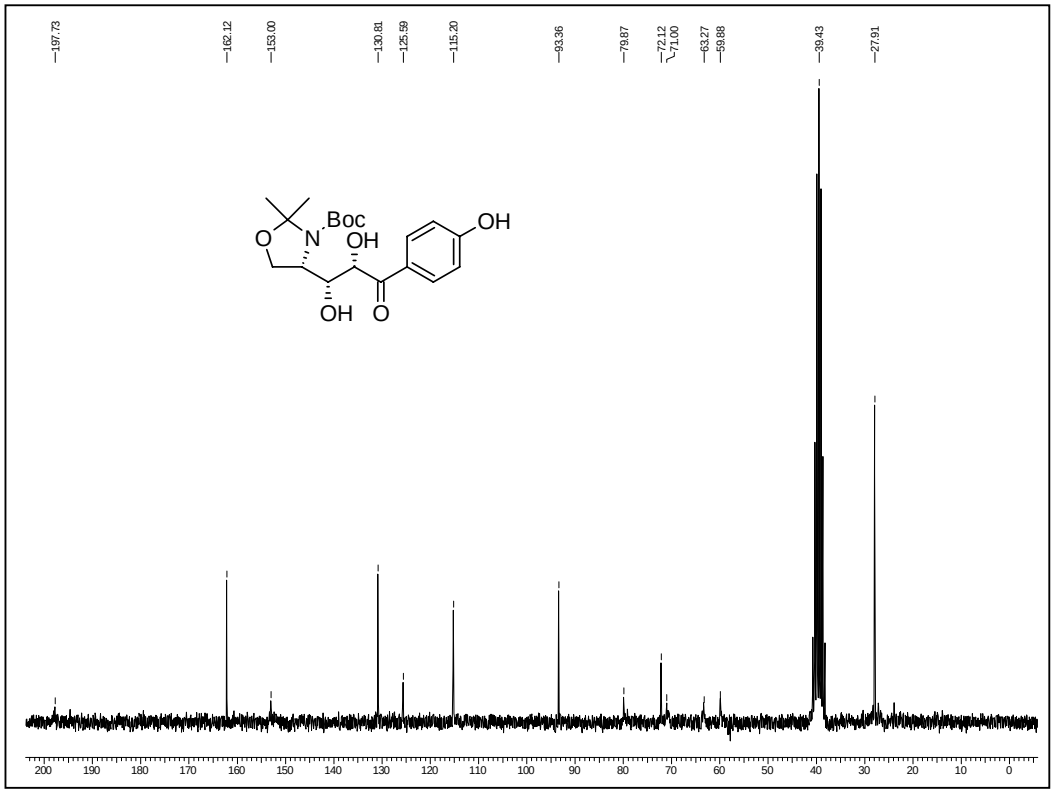
^{1}H NMR Spectrum of **78** in DMSO-d_6



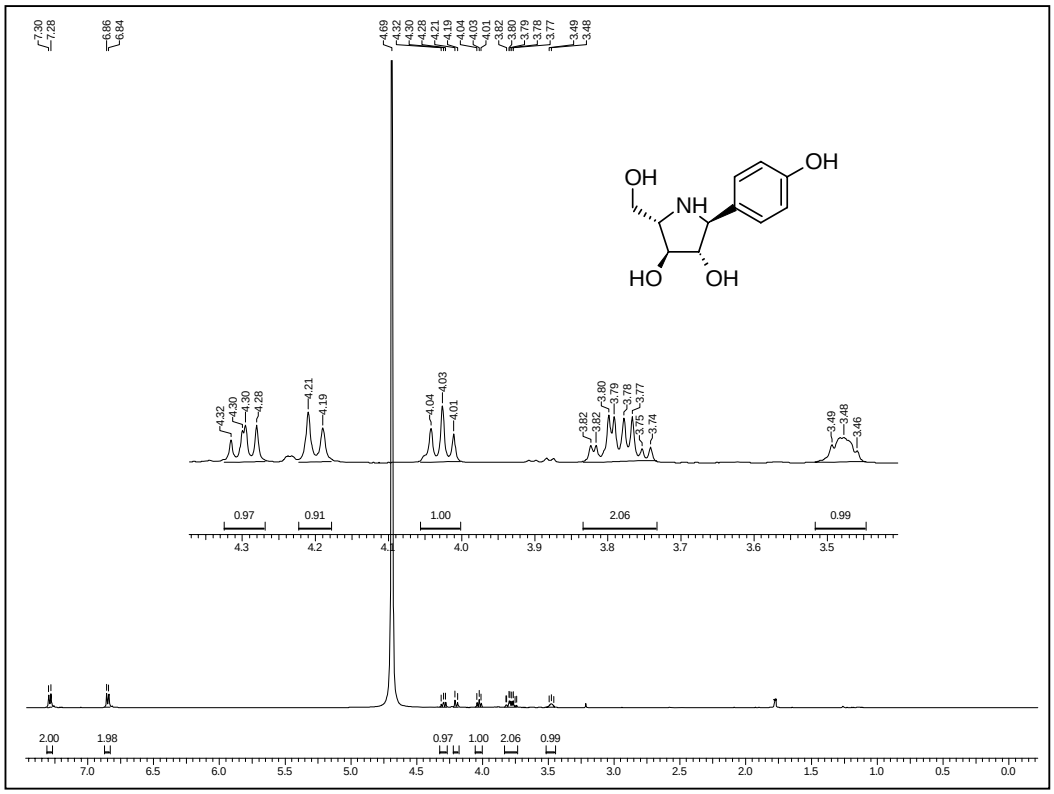
^{13}C NMR Spectrum of **78** in DMSO-d_6



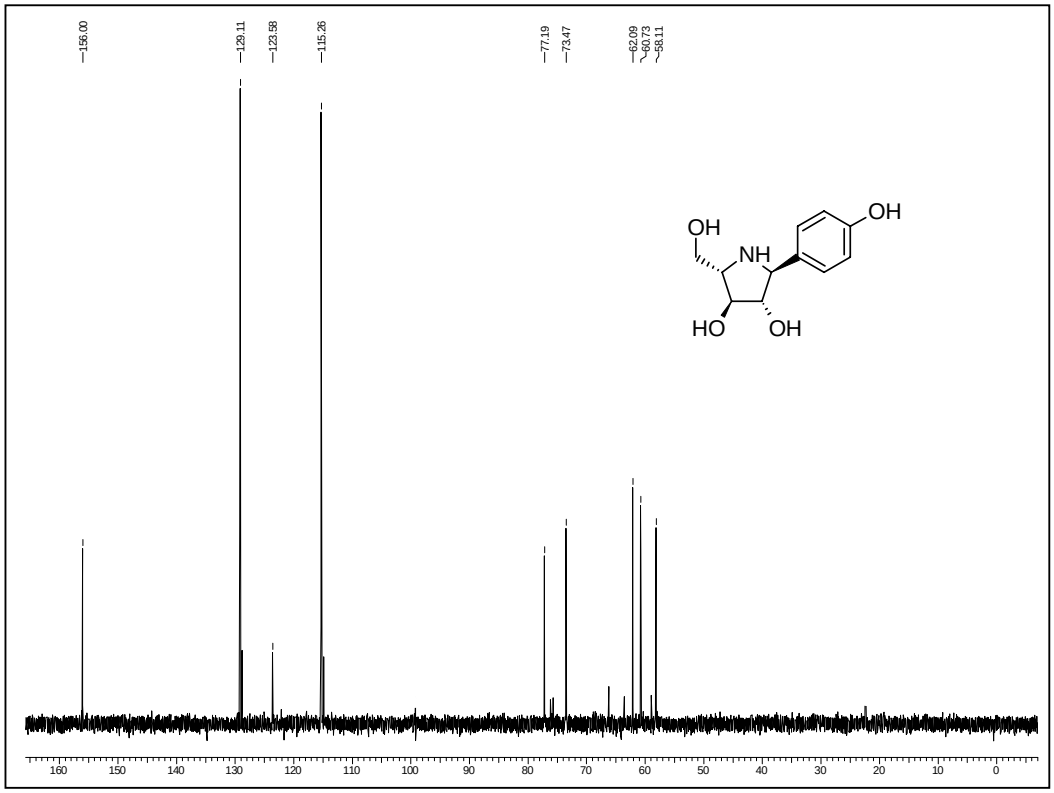
^{1}H NMR Spectrum of **79** in DMSO-d_6



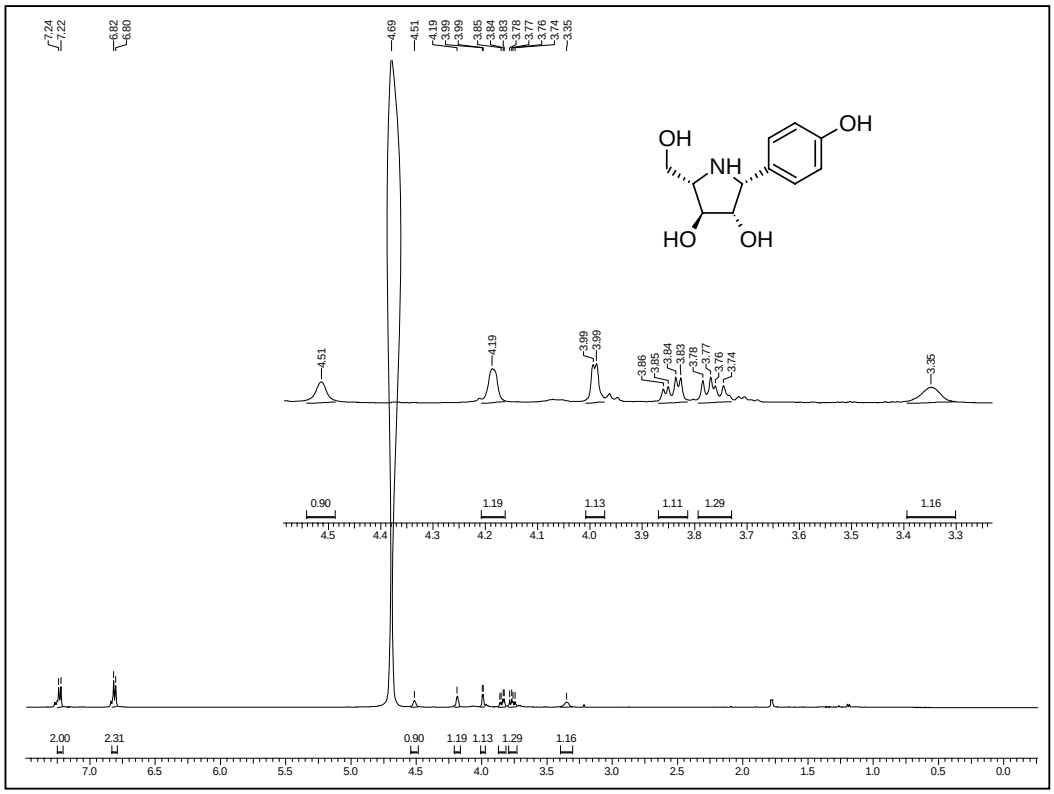
^{13}C NMR Spectrum of **79** in DMSO-d_6



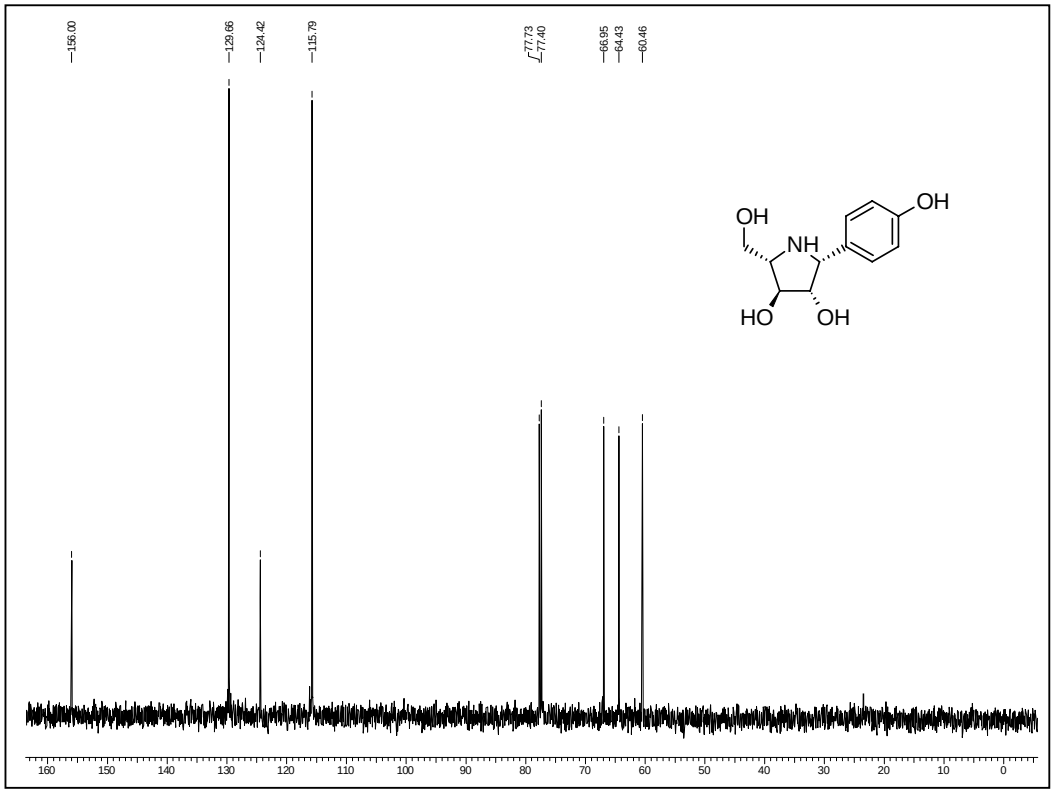
^{1}H NMR Spectrum of **32** in D_2O



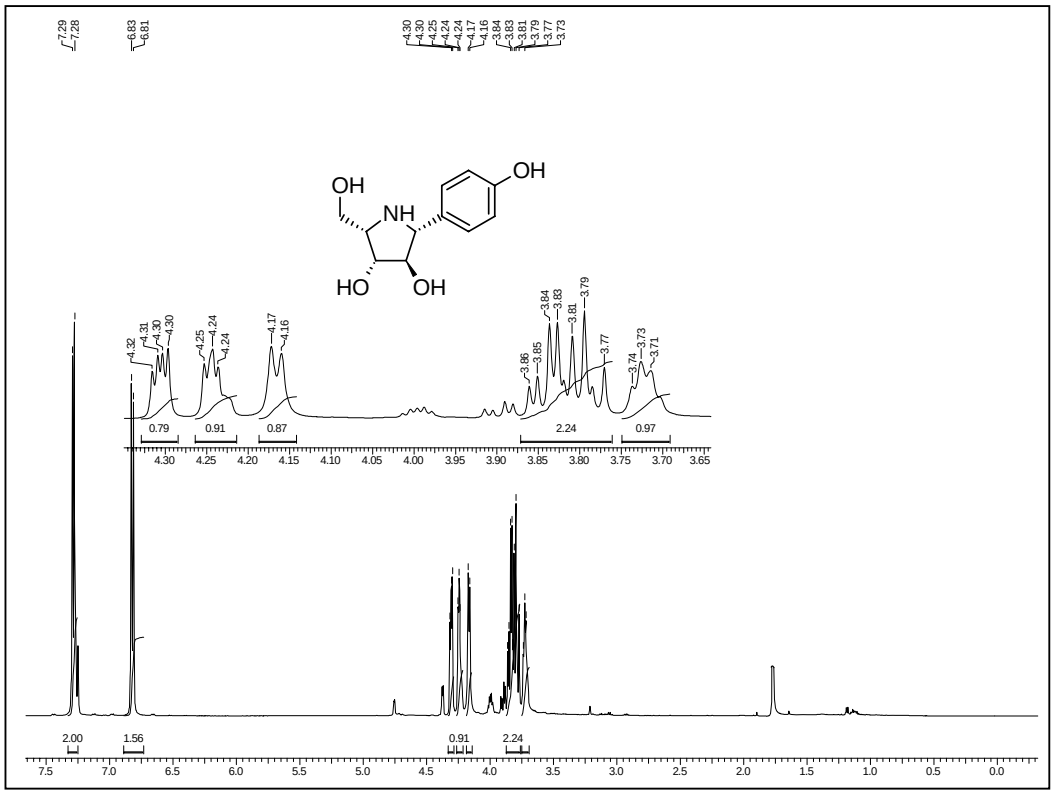
^{13}C NMR Spectrum of **32** in D_2O



^{1}H NMR Spectrum of **60** in D_2O



^{13}C NMR Spectrum of **60** in D_2O



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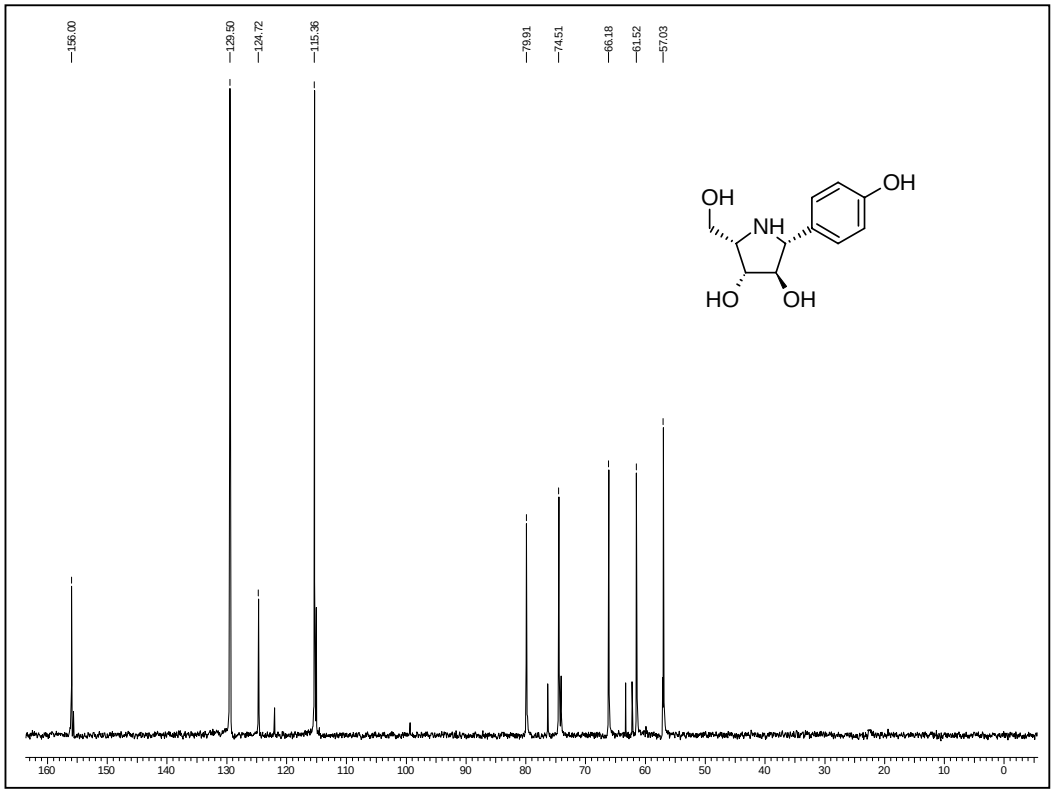

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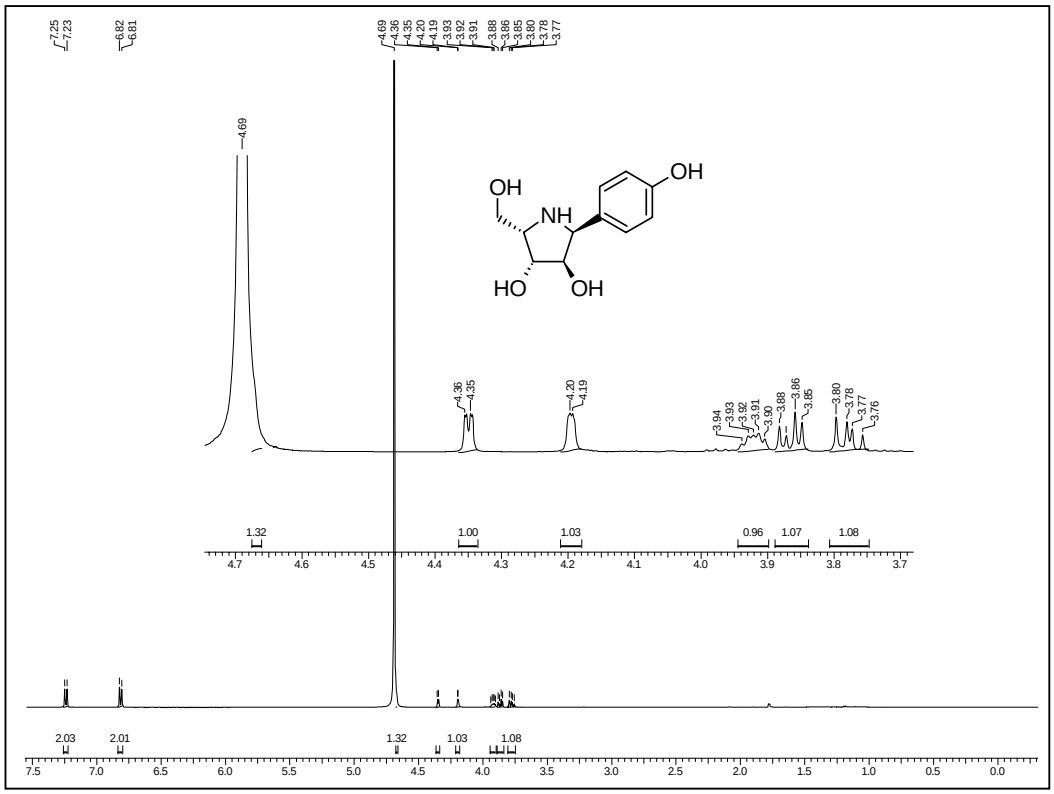

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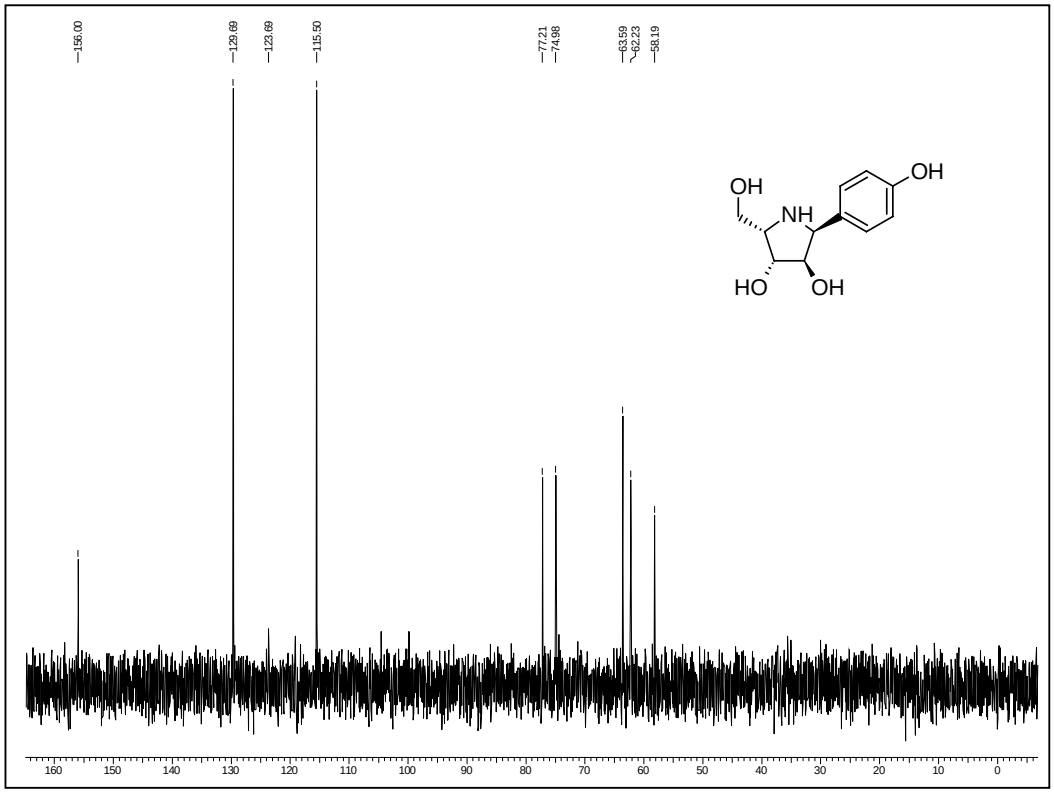

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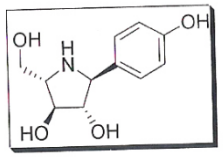

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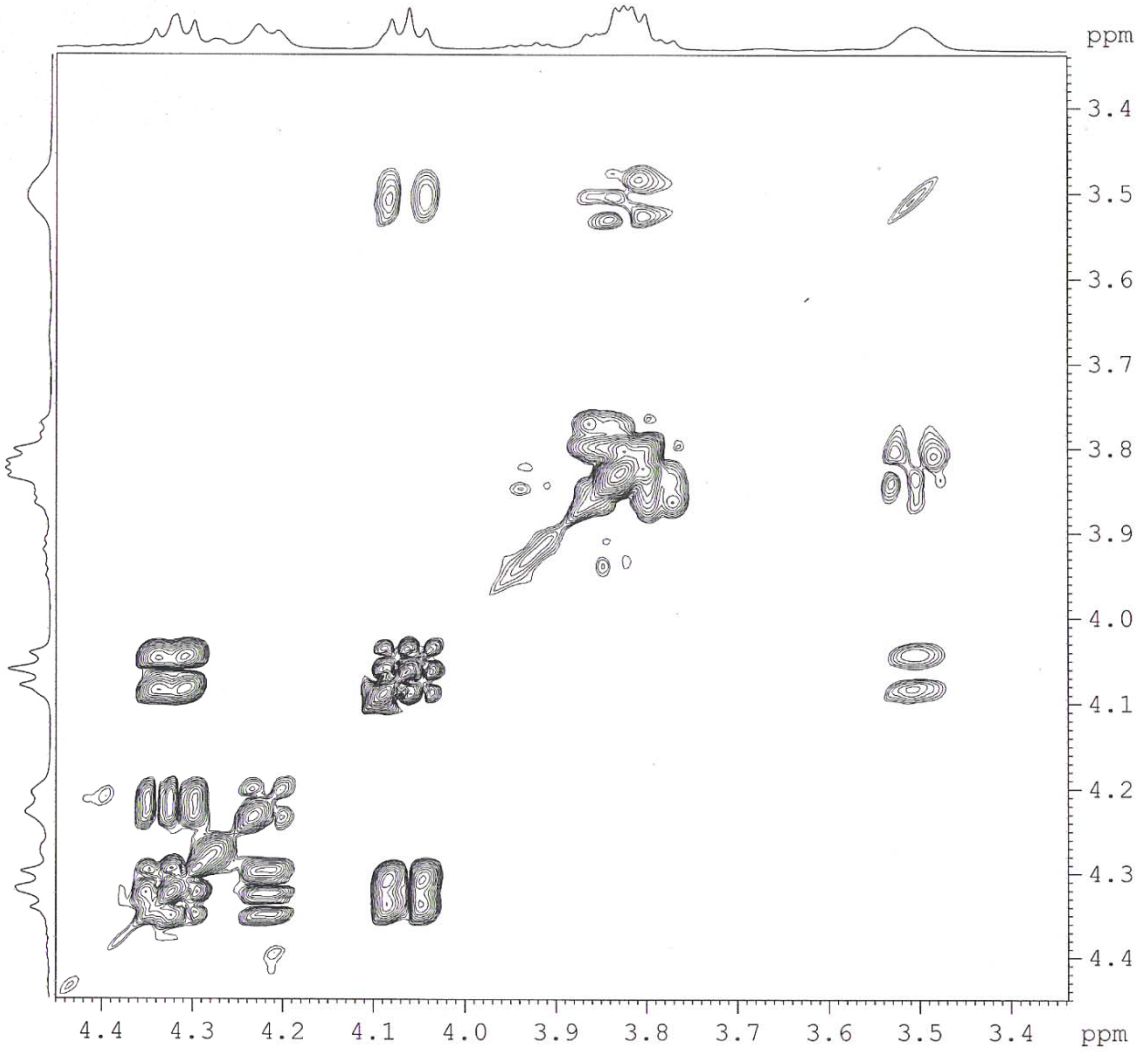


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RG                80.6
DW             302.933 usec
DE              20.00 usec
TE              298.4 K
d0          0.00000300 sec
D1          2.00000000 sec
d13         0.00000400 sec
D16         0.00010000 sec
IN0         0.00060585 sec

======== CHANNEL f1 ========
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P0                14.20 usec
P1                14.20 usec
PL1                1.00 dB
SFO1       400.1321609 MHz

====== GRADIENT CHANNEL =====
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GPNAM2          SINE.100
GPZ1              10.00 %
GPZ2              10.00 %
P16             1000.00 usec

F1 - Acquisition parameters
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FnMODE              QF

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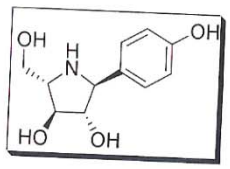

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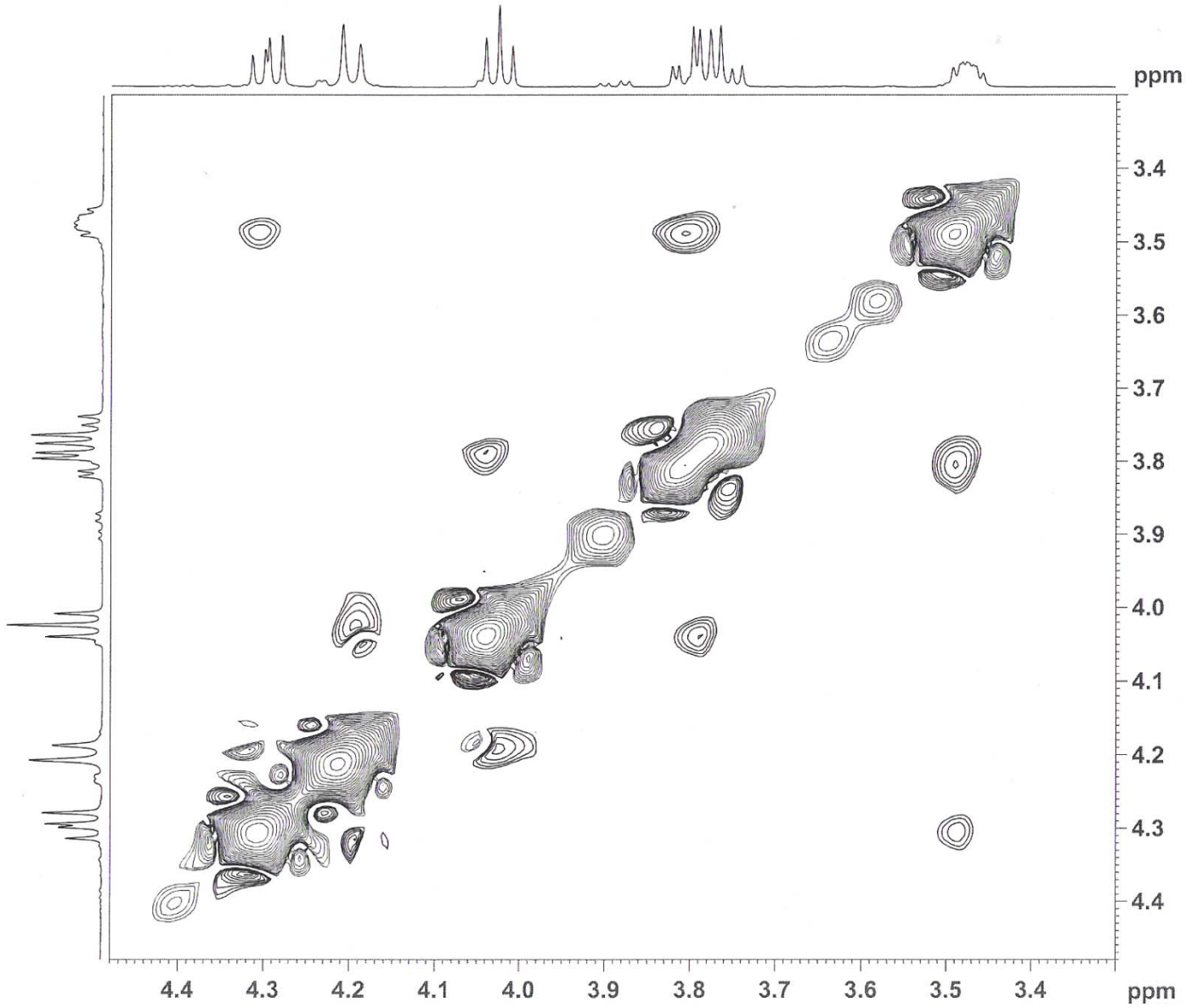

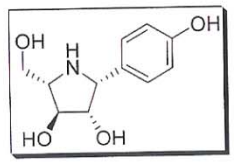

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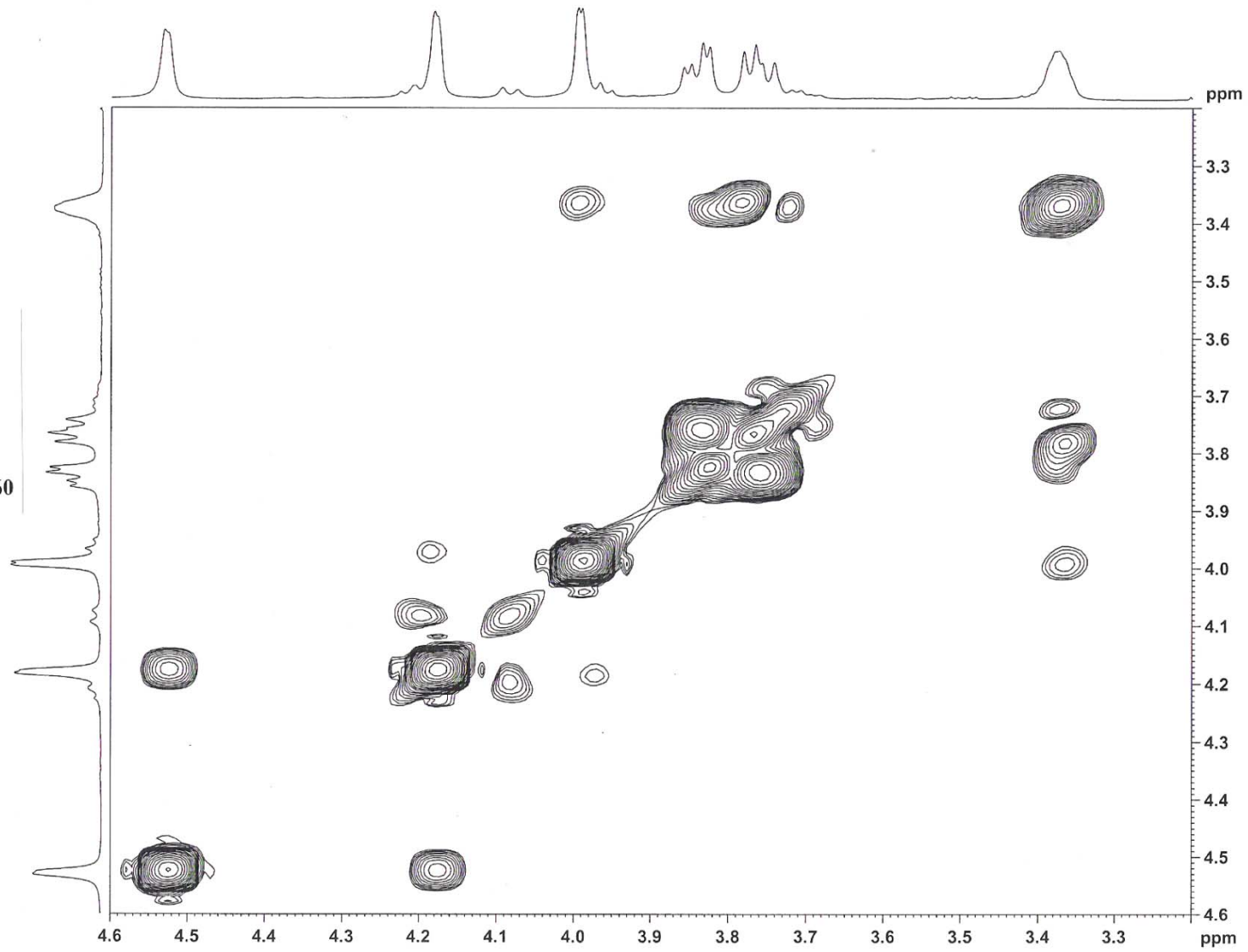

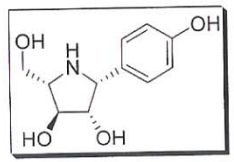

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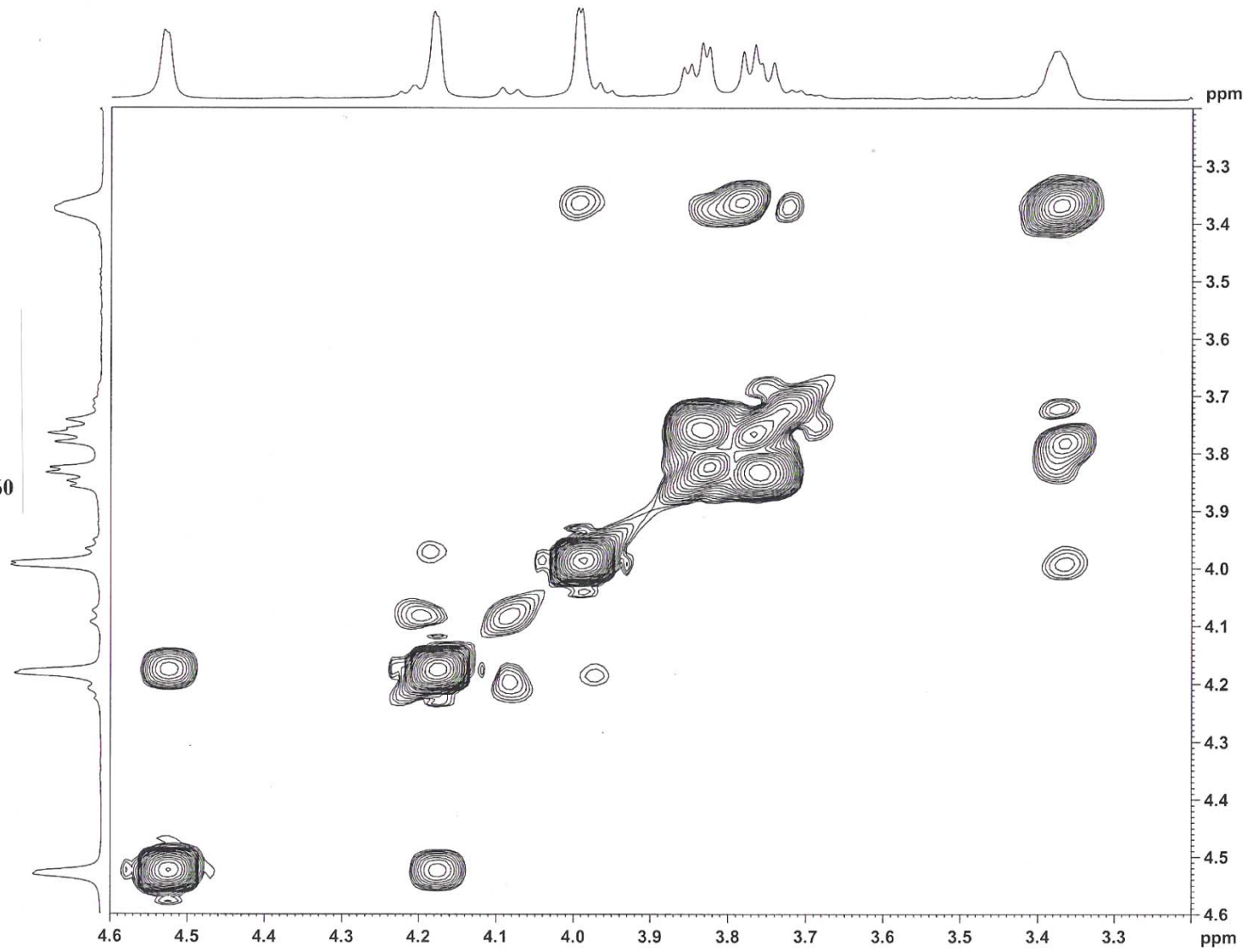

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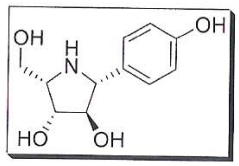

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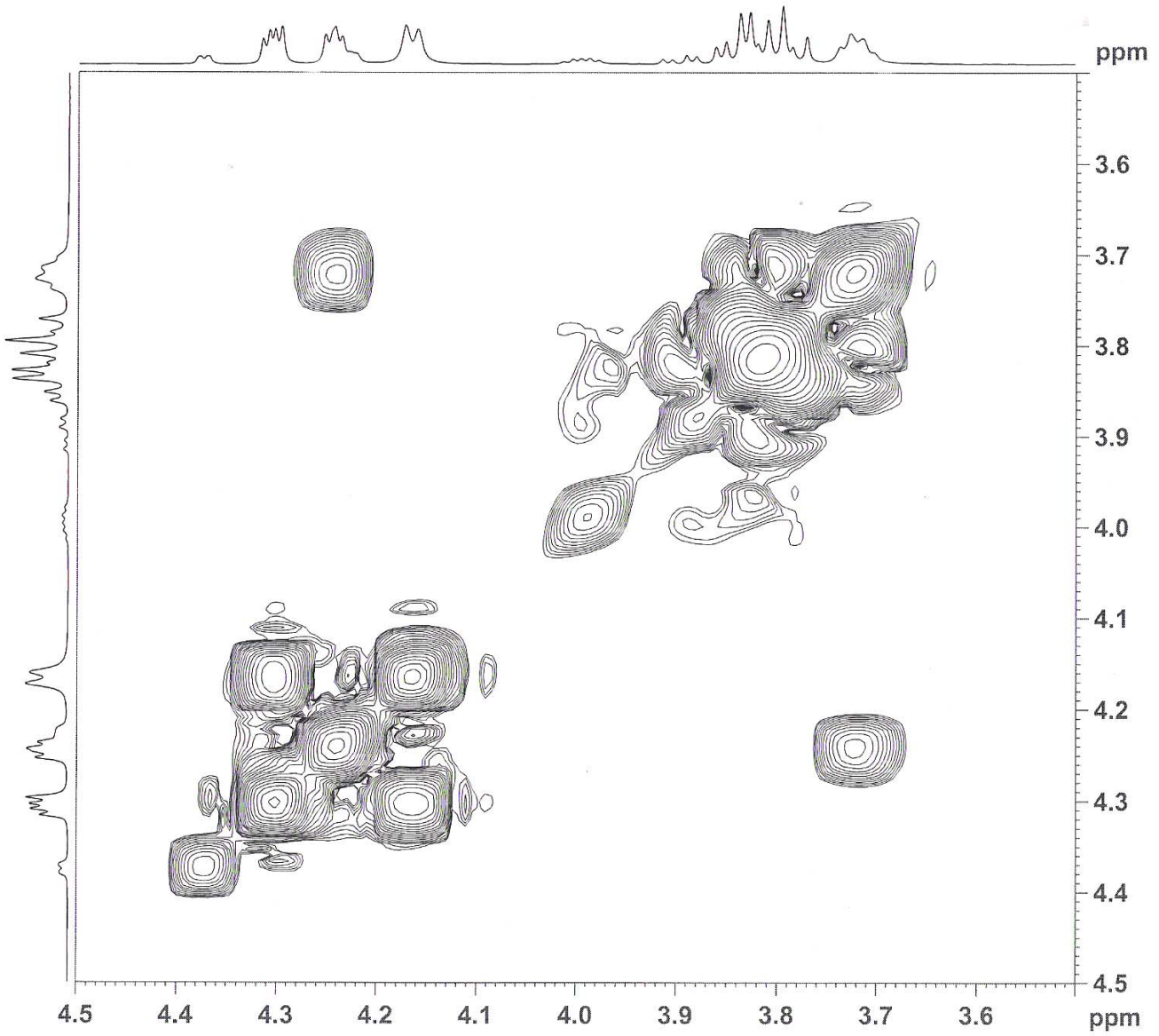

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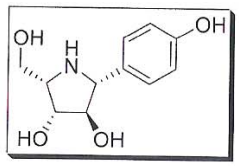

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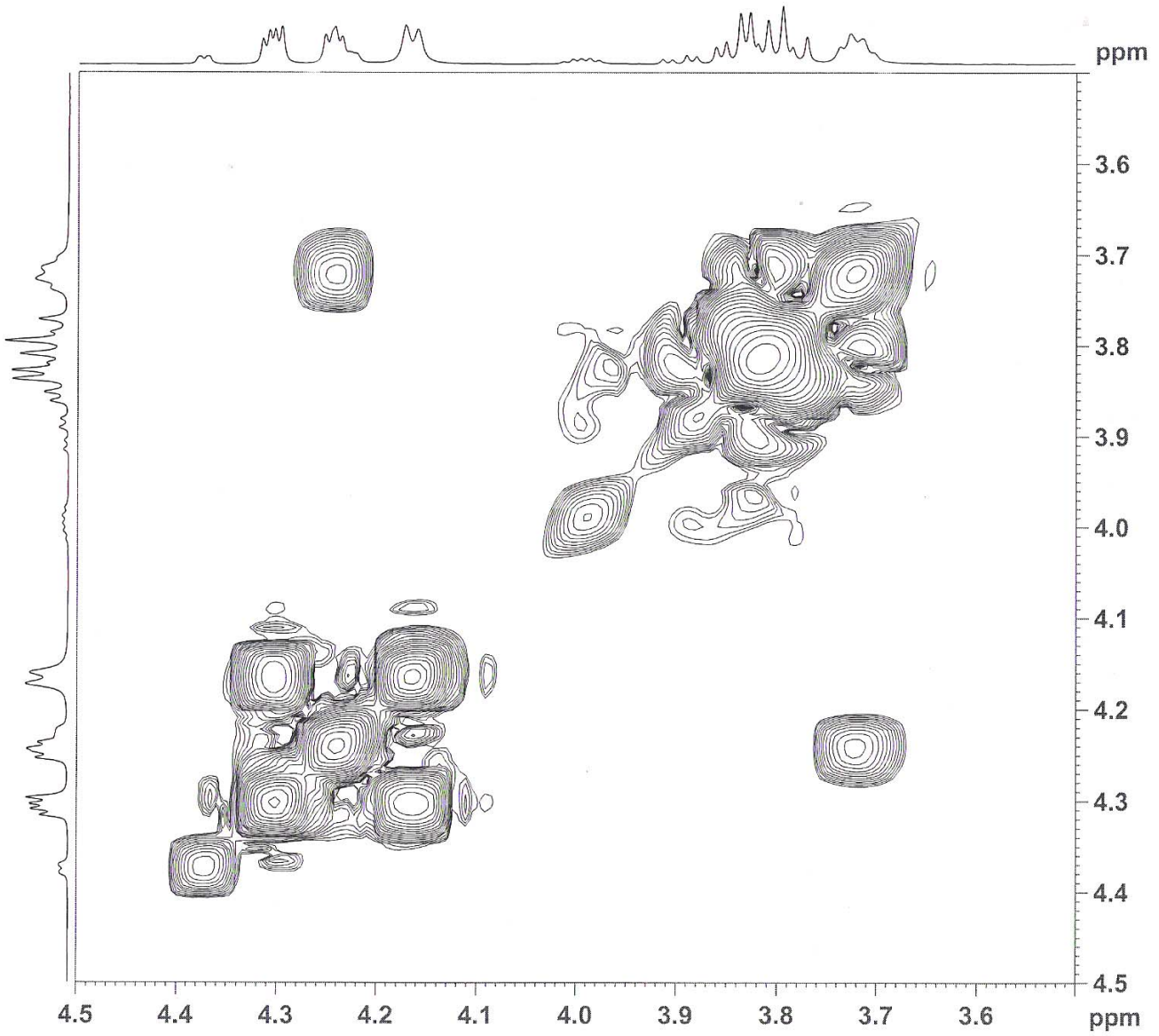

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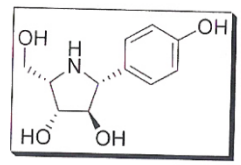

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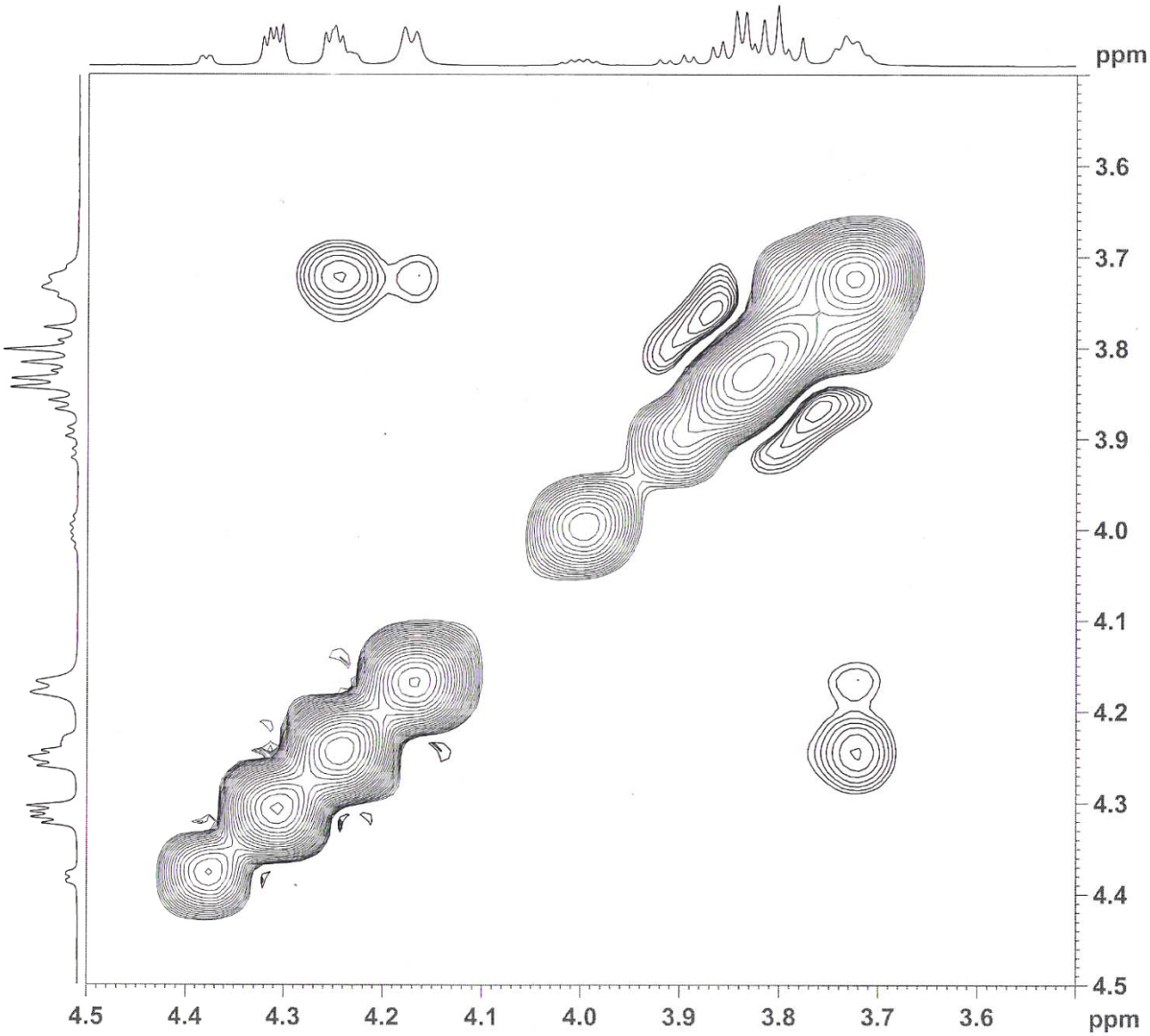
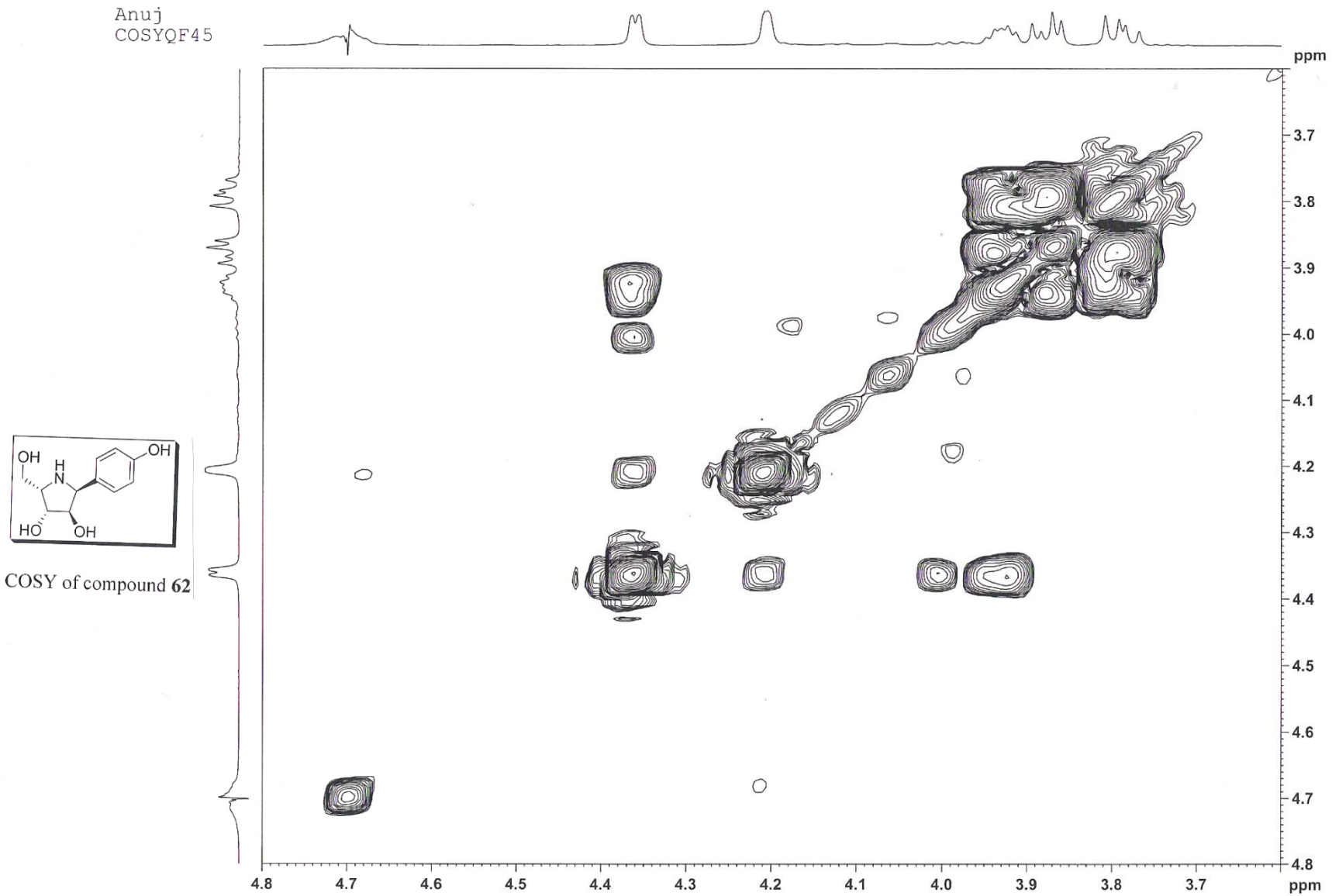
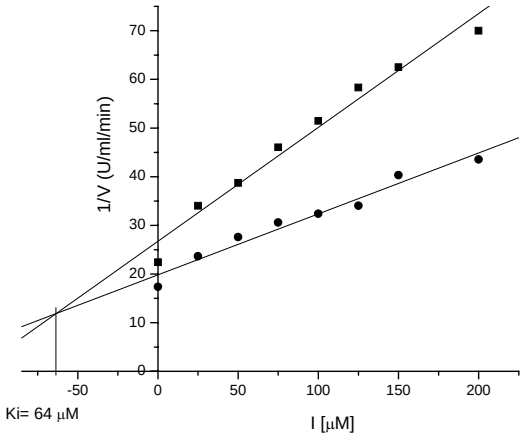




COSY of compound **62**

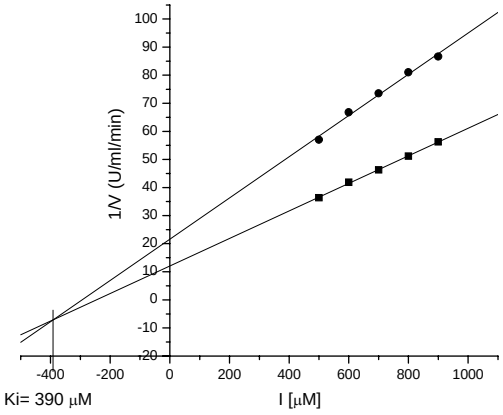
Lineweaver-Burke Plots:

Figure 20. *Plots showing K_i value of compound **32** for α-Glucosidase*



(▪) represent substrate (p-nitrophenyl-α-D-glucopyranoside) concentration of 500 μM; (•) represent substrate (p-nitrophenyl-α-D-glucopyranoside) concentration of 250 μM.

Figure 21. *Plots showing K_i value of compound **60** for α-Glucosidase*



(•) represent substrate (p-nitrophenyl-α-D-glucopyranoside) concentration of 500 μM; (▪) represent substrate (p-nitrophenyl-α-D-glucopyranoside) concentration of 250 μM.

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Chapter II

Chapter II:

Stereoselective Syntheses of Quercitols, carba-L-rhamnose and Aminocyclohexane tetrol.

Introduction

Nature consists of an amazingly diverse and countless species. Amongst them the most intellectual and curious is homo-sapiens, and for their survival plants are the indispensable assets because the main source of energy – carbohydrates (as food) are obtained from it. Moreover, these carbohydrates are also used by humans for their secondary purposes like wood (for furniture), pulp (for paper), etc. With the advancement of science it has been found that carbohydrates are not only used as food, they are also vital constituents for many biological processes.

Carbohydrates

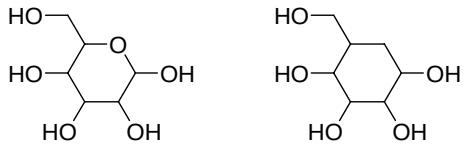
Carbohydrates are generally referred to as hydrates of carbon i.e. $C_x(H_2O)_y$. They are the major constituent of plants and synthesized from carbon dioxide and water in the presence of sunlight by a process known as photosynthesis. This is a very complex and difficult process but in presence of chlorophyll which catalyses the reaction, plants can easily make carbohydrates during sunlight.

The carbohydrates are also found to be the constituents of glycoproteins, glycolipids, and other conjugates. Thus, they are involved in a variety of vital processes like signalling, cell-cell communication, and molecular and cellular targeting. Even the other biological processes like blood clotting, fertilization, etc. involve carbohydrates. Thus, disturbance in their structure can cause/cure diseases such as cancer, diabetes or inflammatory processes.

Carba-sugars[1]

Carba-sugars/pseudo-sugars is the carbocyclic analogue of true sugars whose endocyclic oxygen atom is replaced by a methylene group (Figure 1). In recent years they have attracted the interest of a large number of researchers.

Figure 1. *Representative structure of sugar and pseudo-sugar*



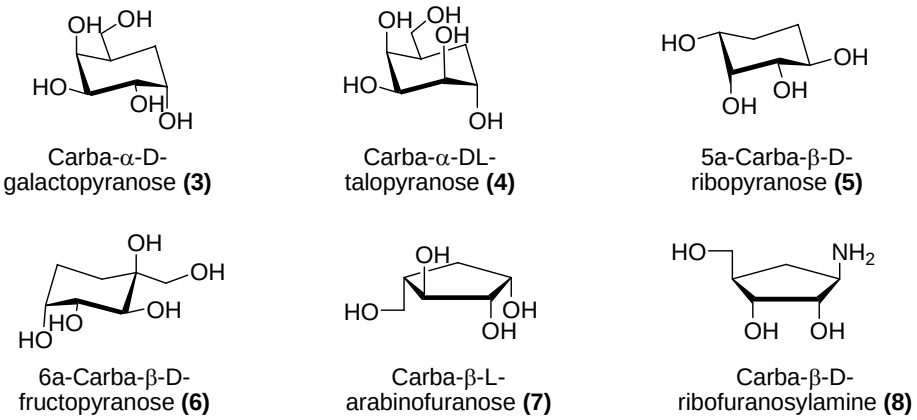
Aldopyranose **(1)** Carba-sugar **(2)**

The absence of keto/aldehyde group in their structure results in different stability and reactivity (i.e. they do not exhibit any characteristic reaction of a reducing sugar, such as reduction of heavy metal salts in alkaline solution, mutarotation, and formation of osazones or hydrazones) whereas their structural resemblance to natural sugars is responsible for their interesting biological properties. This important characteristic property has resulted in the stability of carbaoligosaccharides to hydrolysis *in vivo*. Therefore, studies on carba-sugars will help in development of biologically active carbohydrate mimics as well as useful research tools for glycobiology.

Carba-monosaccharides

In 1966 McCasland[2] first synthesized a pseudo-sugar and proposed that due to its resemblance to sugar it may be accepted by some enzymes and may play vital role in inhibiting malignant or pathogenic cells. Seven years later his prediction became true with the discovery of a free carba-hexopyranose with α-D-*gala*cto configuration **3** which was isolated from the fermentation broth of a certain streptomyces showing weak antibiotic activity.

Figure 2. *Some 5 and 6 - membered pseudo-sugars*

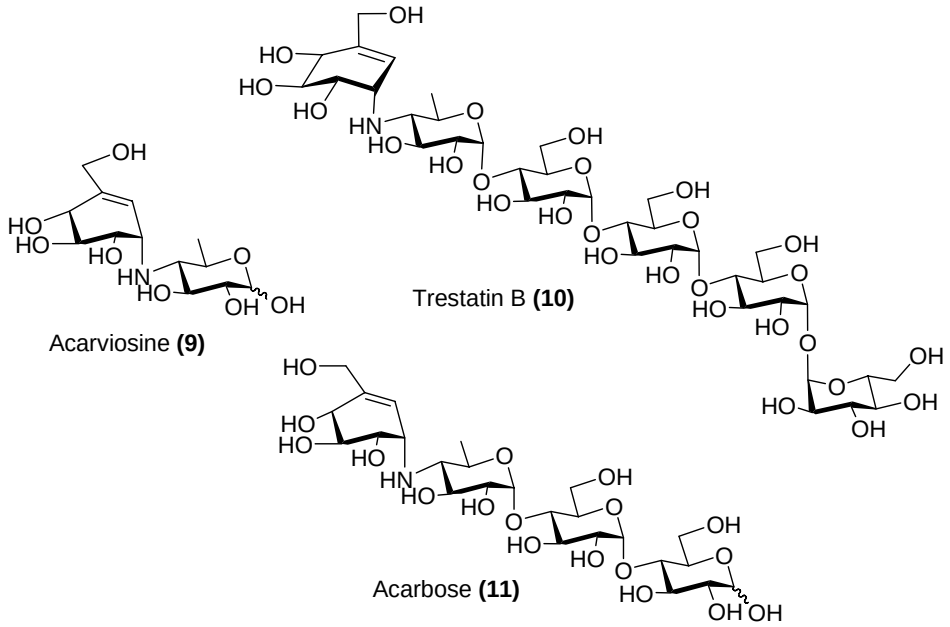


There are two forms of carba-sugars:[3] carbafuranoses and carbapyranoses (Figure 2). But only carbapyranoses have been extensively studied for the last four decades ever since their derivatives have been found in nature in many antibiotics. However, very little is known about carbafuranoses, except for carba-β-L-arabinofuranose **7** and carba-β-D-ribofuranosylamine **8** moiety of the antibiotic aristeromycin. The first recognized carbasugar was carba-α-DL-talopyranose **4**[2a] which was first synthesized by McCasland.

Carba-oligosaccharides

The cell surface is covered with numerous types of oligosaccharide chains, which are anchored covalently to lipids and proteins, having a vital part of biological activity and cellular function. In processing these cell-surface oligosaccharides various glycosidases and glycosyltransferases play important roles.[4] Oligosaccharides are found to have promising therapeutic properties. But they have poor stability *in vivo* due to their easy hydrolysis. So, a search for more stable chemical entity which can mimic the oligosaccharides structurally and in biological activities led to the development of carba-oligosaccharides (Figure 3).

Figure 3. *Pseudo-oligosaccharides*



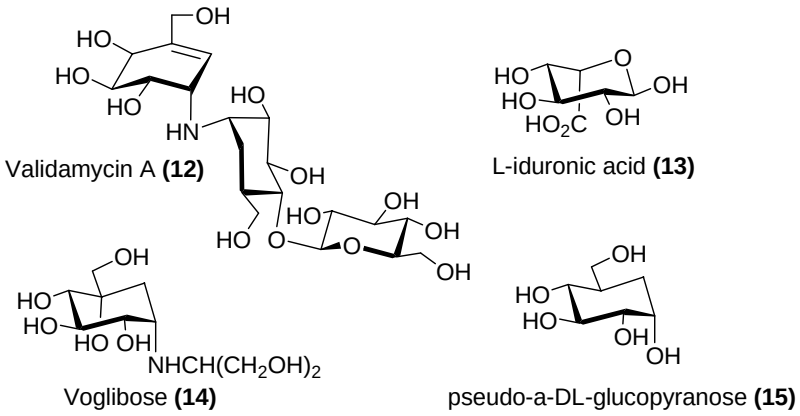
These are more stable under similar conditions. Thus, in recent years extensive studies are carried out in designing and chemically modifying potent inhibitors to obtain highly strong and specific glycoprocessing inhibitors. This strategy has helped in the development of synthetic products like voglibose **14**[5] to control diabetes. Moreover, some carba-oligosaccharides are found in nature like carba-trisaccharidic antibiotics, validamycins,[6] had been discovered in 1970 from a fermentation beer of *Streptomyces hygroscopicus var. limoneus*. Amongst them validamycin A **12** is the most active component which exhibits strong activity against the sheath blight of rice plants and "damping off" of cucumber seedlings caused by an infection of *Pellicularia sasakii* and *Rhizoctania solani*.[6] Validamycins have been widely used in Japan as an agricultural chemical. Some more carba-oligosaccharidic antibiotics like acarbose **11**,[7] adiposin,[8] trestatins **10**[9] and oligostatins have been isolated from fermentation broths.

Biological Utility of Carba-sugars

The close resemblance of pseudo-sugar to true sugar structurally may be the reason for their biological activity (Figure 4). They are stable to enzymatic hydrolysis

in biological systems and exhibit potent glycosidase inhibition activities.[10] Pseudo-α-D-galactopyranose **3** has been found in a fermentation broth *Streptomyces* sp. MA-4145 as a weak antibiotic.[9] A concentration of about 125 μg/l is required to produce a standard inhibition zone of 25 mm (diameter) against *Klebsiella pneumonia* MB-1264, using 13 mm assay discs in a discplate assay. A sample of synthetic pseudo-α-DL-galactopyranose was found to be half as potent as natural product – in the same assay system, indicating the high probability of inactivity of L-enantiomer.

Figure 4. *Biologically active pseudo-sugars*



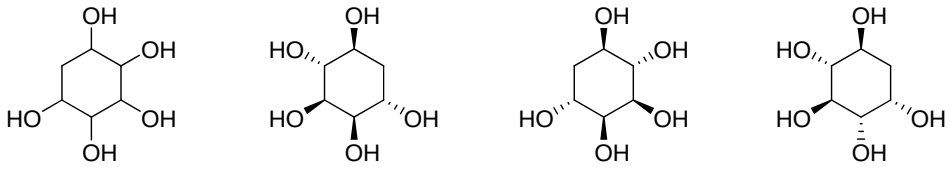
It has been observed from biological activity studies that pseudo-α-DL-glucopyranose **15** act as an inhibitor of glucose-stimulated insulin release because of the inhibition of islet glucokinase by them. This shows the essential roles of islet glucokinase in glucose-stimulating insulin.[11] Moreover, it was found that idose residues (as L-iduronic acid **13**) play a crucial role in determining the biological activity of glycosaminoglycans.[12] The critical importance of L-iduronic acid **13** in the antithrombin III binding sequence of heparin[13] and FGF-2 binding of heparin sulphate[14] has been specifically demonstrated.

The synthesis of carba-sugars started four decades ago. Since then a large number of compounds belonging to this category have been synthesized by using different starting materials and various synthetic pathways. The synthetic strategies have been classified in a broad way according to the type of precursors used like carbohydrate or non-carbohydrate precursors.

Quercitols

Quercitol is a generic term for cyclohexane pentols or deoxyinositols. Theoretically sixteen diastereomers[15] are possible out of which four are symmetric and six pairs of enantiomers are there. Only, three of them (+)-*proto* **18**, (–)-*proto* **17** and (–)-*vibo* **19** are found in nature[16] (Figure 5). They have attracted a great deal of attention from synthetic chemists because of their potential glycosidase inhibition activities and also their versatility as synthetic intermediates.[17] (+)-*proto*-Quercitol **18** was discovered initially and its first synthesis was completed in 1968 by McCasland et al.[18] using (–)-chiro-inositol as starting material. Till recently, ten possible diastereomers, *proto*, *allo*, *talo*, *epi*, *vibo*, *gala*, *scyllo*, *neo*, *cis* and *muco*-quercitols have been synthesized by different approaches to give either their racemic or chiral forms.[19]

Figure 5. *Naturally occurring quercitols*

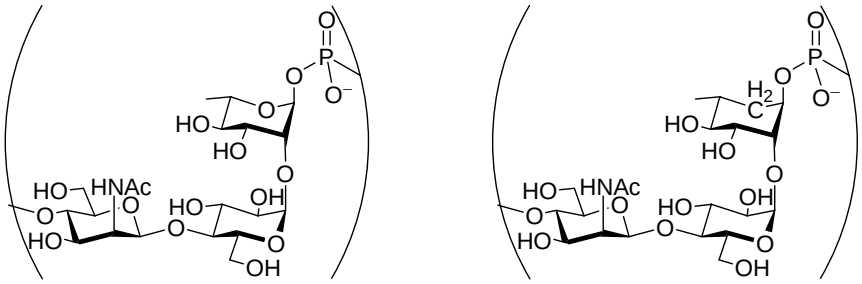


Cyclohexane pentol **(16)** | (-)-*proto*-quercitol **(17)** | (+)-*proto*-quercitol **(18)** | (-)-*vibo*-quercitol **(19)**

Carba-L-rhamnose

It is a pseudo sugar in which the substituents are oriented like in rhamnose with the endocyclic oxygen atom replaced by a methylene group.[20] So, it is a hydrolytically stable analogue of sugar and can be replaced for rhamnose in biologically-active oligosaccharides to enhance their hydrolytic stability and keep the biological activity intact due to their characteristic property of mimicking (due to similar structures).

Figure 6. *Structure of S. pneumoniae 19F repeating unit (**20a**) and its carba-L-rhamnose[21] containing analogue (**20b**)*



20a **20b**

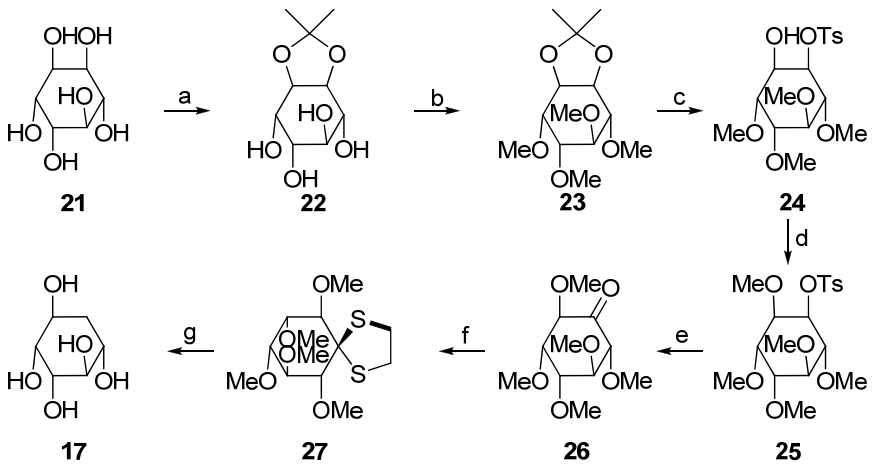
For example in case of glycoconjugate vaccines from *S. pneumoniae* 19F whose CPS is composed of trisaccharide repeating units [→4-β-D-ManpNAc-(1→4)-α-D-Glcp-(1→2)-α-LRhap-1→] **78a** (Figure 6) joined by phosphodiester bridges. It has been observed that the presence of the phosphate on the anomeric position makes the polysaccharide somewhat labile and the preservation of such vaccine during the storage more problematic. Moreover, the lability of anomeric phosphate group and the possibility to form two anomers for each phosphodiester bridge makes the chemical synthesis of oligomer of the repeating unit very difficult.

Past Work

(a) McCasland et al.[18]

They synthesized (−)-*proto*-quercitol **17** from inositol **21** by selectively isolating one of its hydroxyl groups and eliminating it further (Scheme 1).

Scheme 1

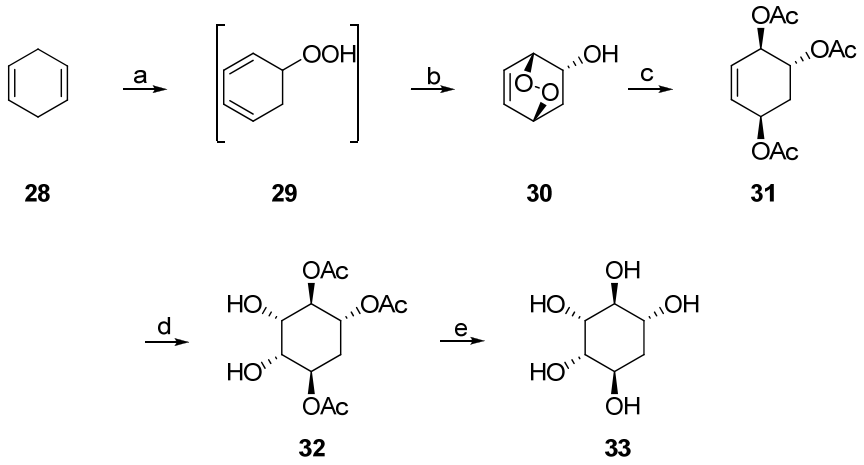


Reagents and Conditions: (a) see back references in ref. 18; (b) MeI, Ag_2O; (c) (i) H^+; (ii) Tosylation; (d) Methylation; (e) (i) NaOMe, EtOH; (ii) RuO_2, $NaIO_4$; (f) 1, 2-ethanedithiol; (g) (i) Raney Ni, ethanol, reflux; (ii) HBr in glacial acetic acid.

(b) Balci et al.[22]

(i) They used singlet oxygen for functionalizing cyclohexadiene **28** which was further converted to DL-*proto*-quercitol **33**. This is one of the shortest syntheses of quercitols (Scheme 2).

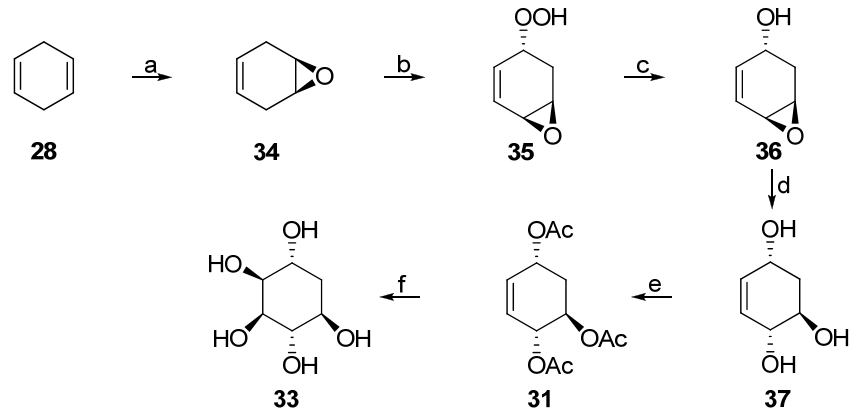
Scheme 2



Reagents and Conditions: (a) $^1O_2$, TPP, r.t., 48 h; (b) $^1O_2$; (c) (i) $LiAlH_4$; (ii) Ac_2O, Py; (d) $KMnO_4$, MeOH, $-5\ ^oC$; (e) NH_3, MeOH, r.t.

(ii) The photo-oxygenation reaction was carried out on epoxy-ene **34** to give **35**, which was finally converted to DL-*proto*-quercitol **33** (Scheme 3).[23]

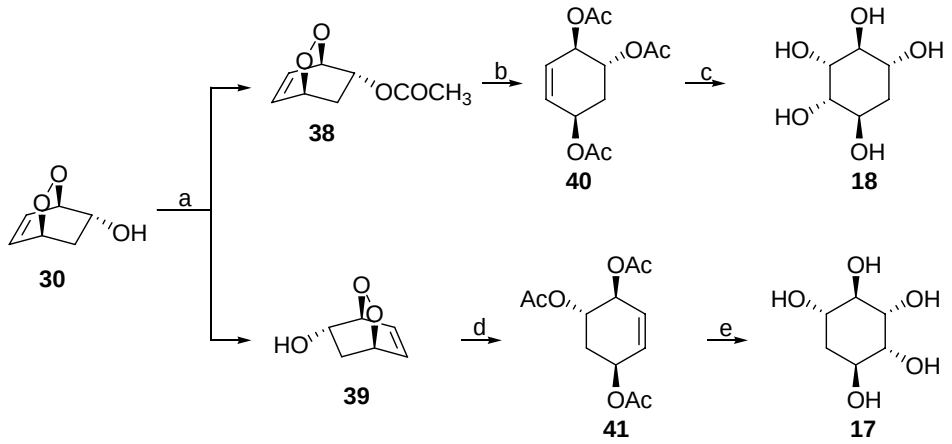
Scheme 3



Reagents and Conditions: (a) *m*-CPBA, CH_2Cl_2, $NaHCO_3$; (b) TPP, hν, O_2, CH_2Cl_2, 90%; (c) $P(OEt)_3$; (d) H^+, H_2O, 30 min.; (e) Ac_2O, Py; (f) (i) $KMnO_4$, $MgSO_4$; (ii) NH_3, MeOH, r.t.

(iii) In this case the oxygen adducts **30** formed after photo-oxygenation reaction were separated enzymatically to give chiral (+) and (–)-*proto*-quercitols **18** and **17**, respectively (Scheme 4).[24]

Scheme 4

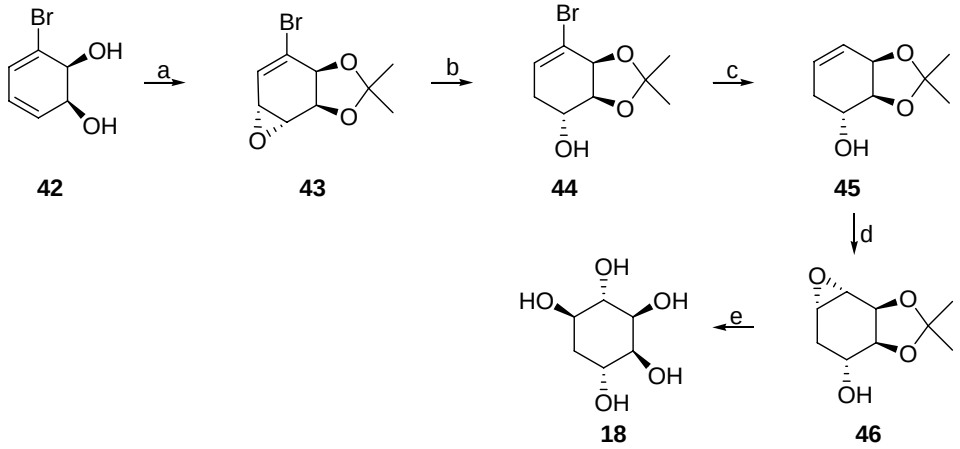


Reagents and Conditions: (a) enzyme; (b) (i) Thiourea; (ii) AcCl; (c) (i) OsO_4, NMO; (ii) NH_3, MeOH.

(c) Hudlicky et al.[25]

Aromatic halides were converted to dihydro *cis*-diol **42** by using enzyme which was further used as a precursor for the synthesis of (+)-*proto*-quercitol **18** (Scheme 5).

Scheme 5

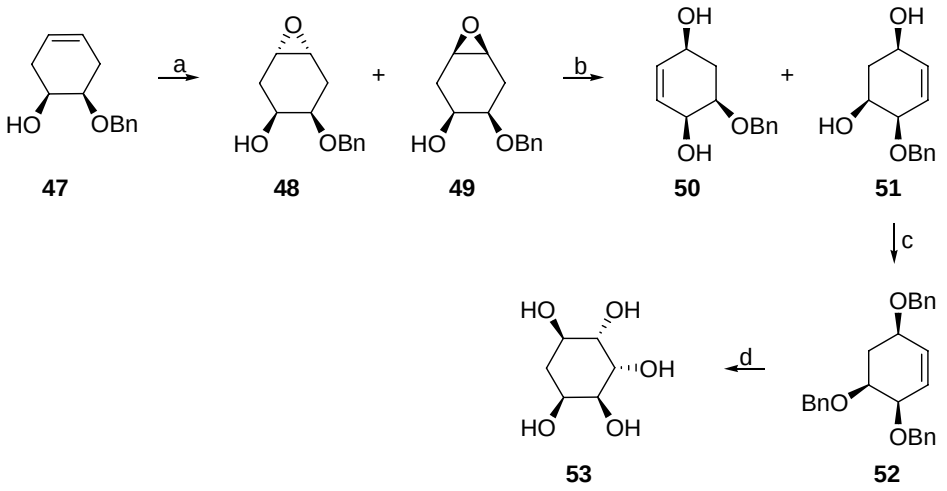


Reagents and Conditions: (a) *m*-CPBA; (b) $LiAlH_4$, ether; (c) Bu_3SnH, AIBN, toluene; (d) *m*-CPBA, CH_2Cl_2, 74%; (e) $PhCO_2Na$, H_2O, Δ, 88%.

(d) Tanaka et al.[26]

The transformation of a C_6-chiron, 6-(benzyloxy)-3-cyclohexen-1-ol **47** into (−)-*gala*-quercitol **53** involving a stereodivergent epoxide rearrangement is explored (Scheme 6).

Scheme 6

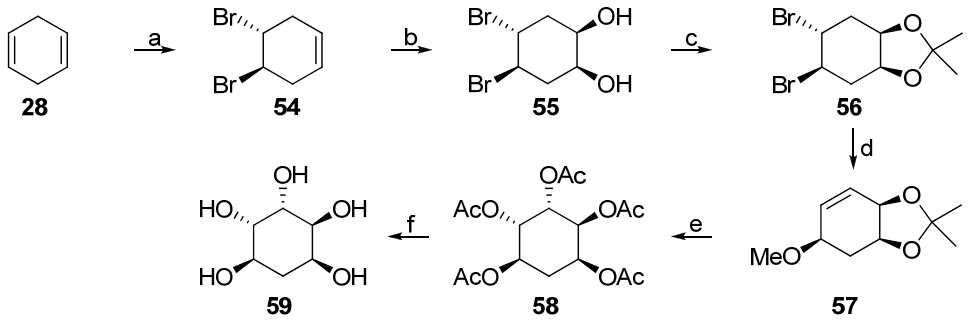


Reagents and Conditions: (a) TBHP, $VO(acac)_2$, CH_2Cl_2, r.t.; (b) *n*-Pr_2NLi, HMPA, THF, −78 °C; (c) BnBr, NaH, n-Bu_4NI, THF, −78 °C to r.t., 83%; (d) (i) cat. OsO_4, NMO, acetone:water (30:1), r.t.; (ii) H_2, $Pd(OH)_2$-C, MeOH, 3 atm., r.t.

(e) Balci et al.[27]

1,4-cyclohexadiene **28** is converted to DL-*gala*-quercitol **59** by using different sets of conditions (Scheme 7).

Scheme 7

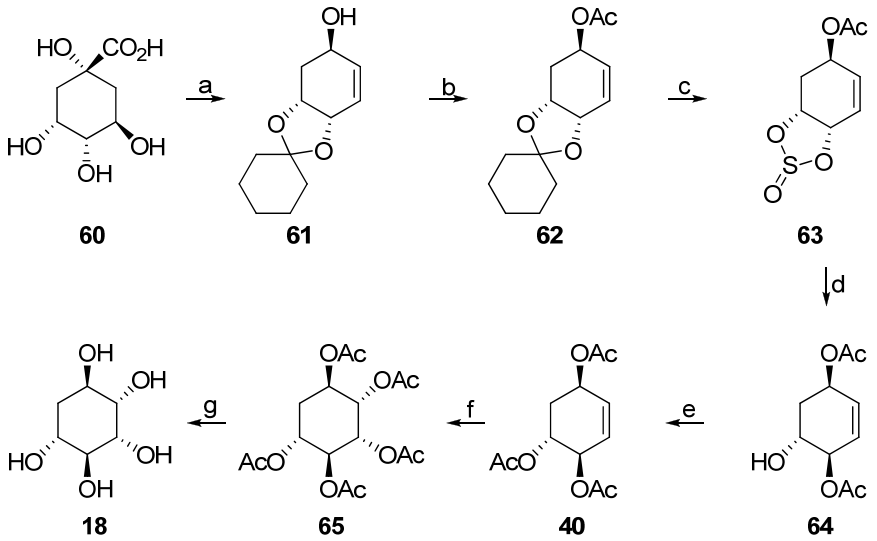


Reagents and Conditions: (a) Br_2, hexane, –45 °C, 95%; (b) OsO_4, NMO, acetone-water, 90%; (c) 2,2-Dimethoxypropane, TsOH, 97%; (d) NaOMe, MeOH, r.t., 96%; (e) OsO_4, NMO, acetone-water; then AcCl, 95%; (f) 47% HBr, r.t., 97%.

(f) Shih et al.[28]

They used quinic acid **60** as the chiral source to synthesize (+)-*proto*-quercitol **18** (Scheme 8).

Scheme 8



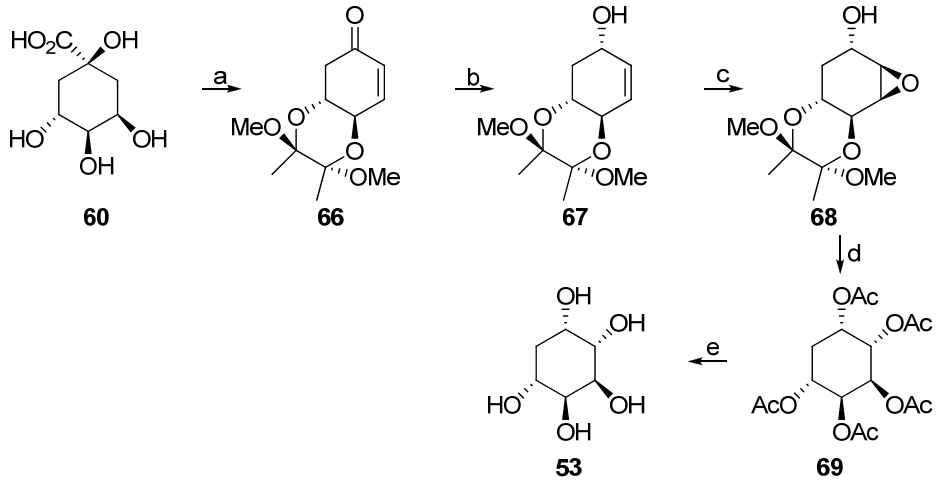
60 **61** **62** **63** **64** **40** **65** **18**

Reagents and Conditions: (a) Luche reduction in ref. 28; (b) Ac_2O, Py, 93%; (c) (i) 80% AcOH; (ii) $SOCl_2$, Et_3N, 70% (two steps); (d) NaOAc, DMF, 90 °C, 72%; (e) Ac_2O, Py, 90%; (f) (i) $KMnO_4$, $MgSO_4$; (ii) Ac_2O, Py, 34% (two steps); (g) NH_3/MeOH, quantitative.

(g) Shih et al.[29]

Here again quinic acid **60** was used as a chiral source but under different sets of conditions to fetch (–)-*gala*-quercitol **53** (Scheme 9).

Scheme 9



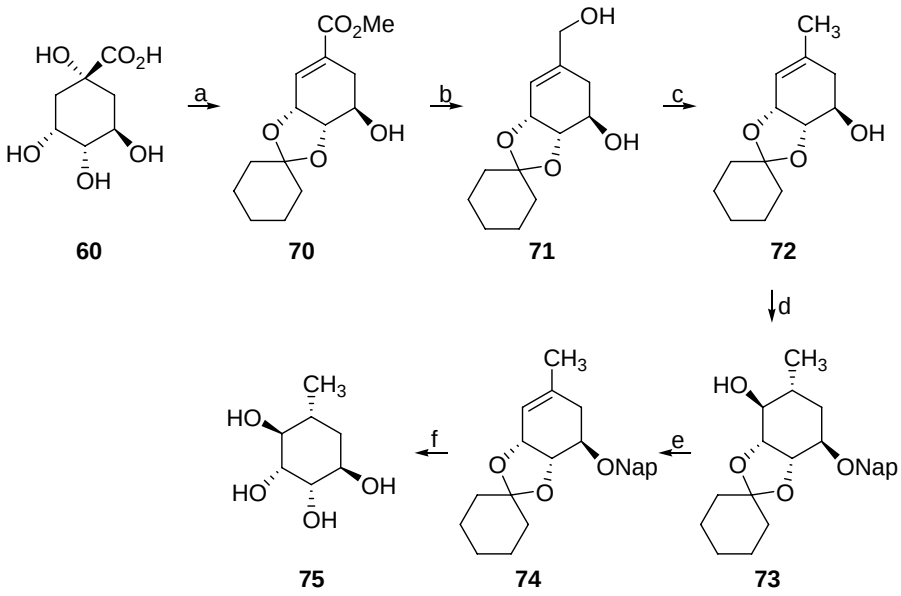
60 **66** **67** **68** **69** **53**

Reagents and Conditions: (a) ref. 29; (b) ref. 19; (c) *m*-CPBA, $NaHPO_4.12H_2O$, CH_2Cl_2; (d) Ac_2O, H_2SO_4 (cat.), reflux; (e) 7N NH_3/MeOH.

Synthesis of carba-L-rhamnose[21b]

Only one report for the synthesis of carba-L-rhamnose **75** was found in the literature where quinic acid **60** has been used as the starting material. In this case the remaining hydroxyl group was introduced by hydroboration reaction (Scheme 10).

Scheme 10



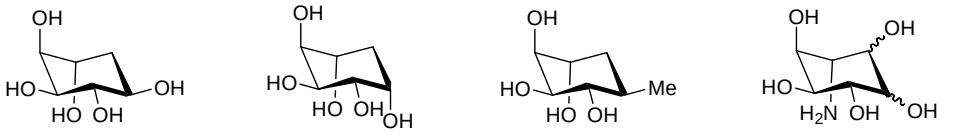
Reagents and Conditions: (a) ref. 21b; (b) DIBALH, THF, 0 ^oC, 78%; (c) (i) Ph_3P, CBr_4, *syn*-collidine, r.t., 93%; (ii) SuperHydride®, THF, 0 ^oC to r.t., 92%; (d) NapBr, KOH, 18-crown-6, THF, r.t., 85%; (e) 9-BBN, reflux, then NaOH, H_2O_2, 0 ^oC to r.t., 90%; (f) Deprotection.

Present Work

Present Work

Cyclohexane polyols and their amino analogues are biologically very important molecules as they are involved in many biological processes. Amongst them cyclohexane pentols or deoxyinositols having resemblance to the parent carbohydrates are also known by the generic term quercitols (Figure 7). They exist in sixteen diastereomeric forms of which four are symmetric. Only, (+)-proto **18**, (–)-proto **17** and (–)-vibo **19** quercitols are found in nature. (+)-proto quercitol **18** was first isolated and synthesized by McCasland[18] in 1968. The revelations of their glycosidase inhibition activities and versatility as synthetic intermediates have attracted a great deal of attention from synthetic community. Besides quercitols, carbasugars are cyclic monosaccharide analogues in which the endocyclic oxygen atom is replaced by a methylene group (e.g. carba-L-rhamnose **75** (Figure 7) is an analogue of L-rhamnose). As a consequence, carbasugars are hydrolytically stable analogues to their parent sugars towards degradation by glycosidases.[3,30] Finally, amino-inositols[31] (Figure 7) having one amino group as one of the substituents are very important molecules in cell-cell communications and signal transductions. By permutations and combinations in the orientations of the amine group with respect to the hydroxyl groups a large number of diastereomers of amino-inositols may be obtained which can be used for structure activity relationship (SAR) studies.

Figure 7. *Representative examples of cyclohexane polyols and amino-inositols*



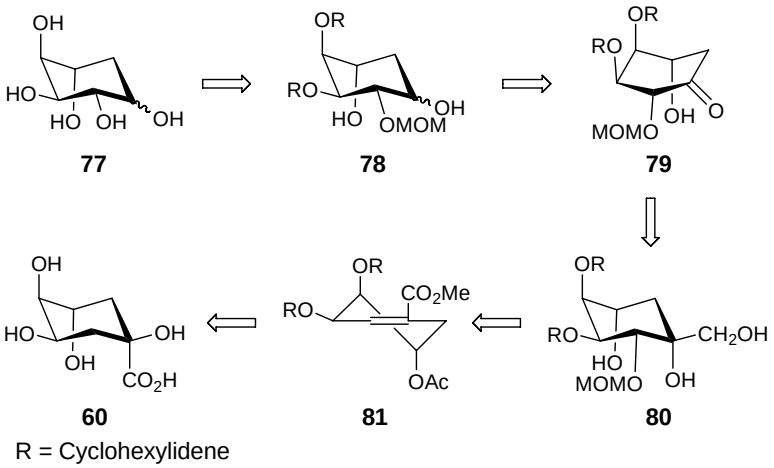
(+)-*proto*-quercitol (**18**) | (–)-*gala*-quercitol (**53**) | Carba-L-rhamnose (**75**) | Amino-cyclohexane-pentol (**76**)

Retrosynthetic Strategy

Thus, because of the significant biological properties of these compounds we planned a simple and scalable strategy for synthesizing these compounds from easily available starting material D-(–)-quinic acid **60**[32] as the key chiral building block. As

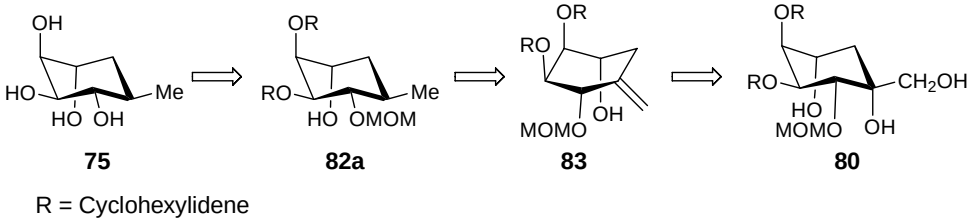
shown in scheme 11, the intended total synthesis of quercitol uses stereoselective reduction reaction of the keto derivative **79** as the key step.[33] This keto derivative **79** can be easily obtained from triol **80**. The *cis*- hydroxyl group of **80** were obtained in high diastereoselectivity from the shikimate ester derivative **81**[34] by osmium tetroxide catalyzed dihydroxylation which in turn can be obtained from naturally occurring D-(−)-quinic acid **60** by known literature procedures.[35]

Scheme 11. *Retrosynthetic strategy for quercitols*



The carba-L-rhamnose **75** can be obtained by stereoselective reduction of exocyclic methylene derivative **83** which in turn can be obtained from triol **80** through Corey-Winter olefination[36] reaction (Scheme 12).

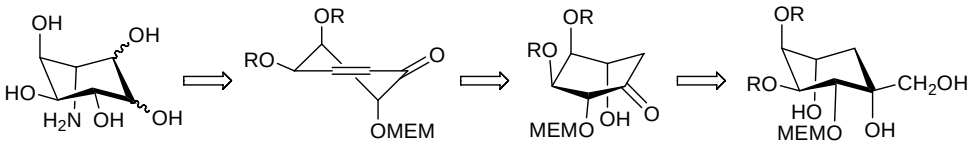
Scheme 12. *Retrosynthetic strategy for carba-L-rhamnose*



The amino-inositols **76** are assumed to be obtained by Michael addition of secondary amine on α,β-unsaturated ketone derivative **84** and trapping the enol

formed,[37] which is subsequently epoxidized, ring opened and reduced. The α,β-unsaturated ketone **84** is obtained from the keto derivative **79a** (an analogue of **79** having MEM protecting group instead of MOM) which in turn can be easily obtained from the triol **80a** (Scheme 13).

Scheme 13. *Retrosynthetic strategy towards aminoinositols*



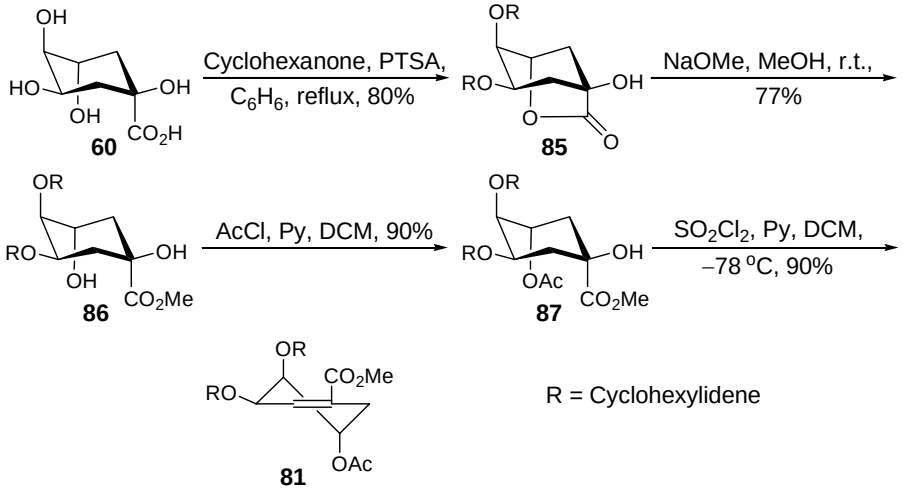
76 **84** **79a** **80a**

R = Cyclohexylidene

Synthesis of shikimate ester derivative 81

Synthesis of the key intermediate **81** commenced from commercially available D-(−)-quinic acid **60** by known literature procedures (Scheme 14).[35] In the first step the 3,4-dihydroxyl groups of Quinic acid were protected as its cyclohexylidene derivative with concurrent lactonisation of 1-carboxylic acid group with C-5 hydroxyl group to obtain **85**. The lactone moiety was opened with the conversion of carboxylic acid functionality into its methyl ester derivative **86**, and the free C-5 hydroxyl group so formed was protected as its acetate derivative **87**. Finally, the C-1 tertiary hydroxyl group was dehydrated to furnish α,β-unsaturated ester (shikimate ester) derivative **81** (Scheme 14). The spectral data of compound **81** were found to be in complete agreement with the reported values.

Scheme 14

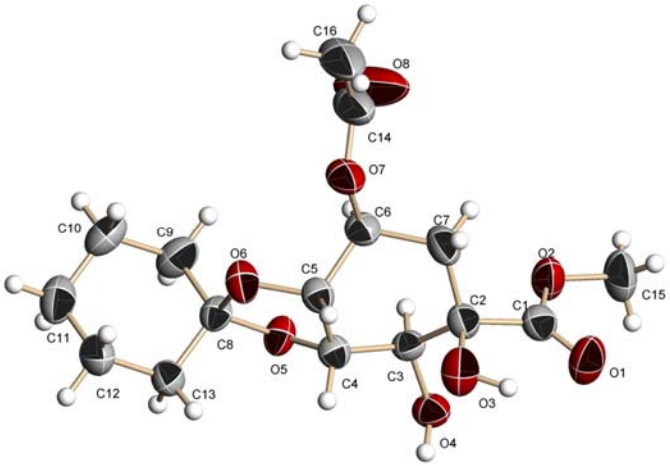


60 **85** **86** **87** **81**

Synthesis of keto derivative

The shikimate ester derivative **81** was subjected to dihydroxylation reaction condition using catalytic osmium tetroxide, 4-methylmorpholine N-oxide as oxidant and pyridine as base in *t*-butanol to give the diol derivative **88** in high diastereoselectivity. The reason for such high *de* was assumed to be due to the presence of favourable steric crowding adjacent to olefin in form of cyclohexylidene group and thus ensuring dihydroxylation from the less hindered α-face. The structure of diol was confirmed from its ^{1}H, ^{13}C NMR and elemental data analyses. In ^{1}H NMR the olefinic proton was found to be absent (most deshielded proton was observed at 5.17 ppm, for H-5) and rest of the spectrum matches according to the assigned structure. Further the conformation and stereochemistry of the newly inserted vicinal hydroxyl groups were confirmed from its X-ray crystallographic data analysis (Figure 8). The details of crystal data and structure refinement (Table 1), bond lengths and bond angles (Table 2) and torsion angles (Table 3) are given at the end of this section (Page No. 103 to 105).

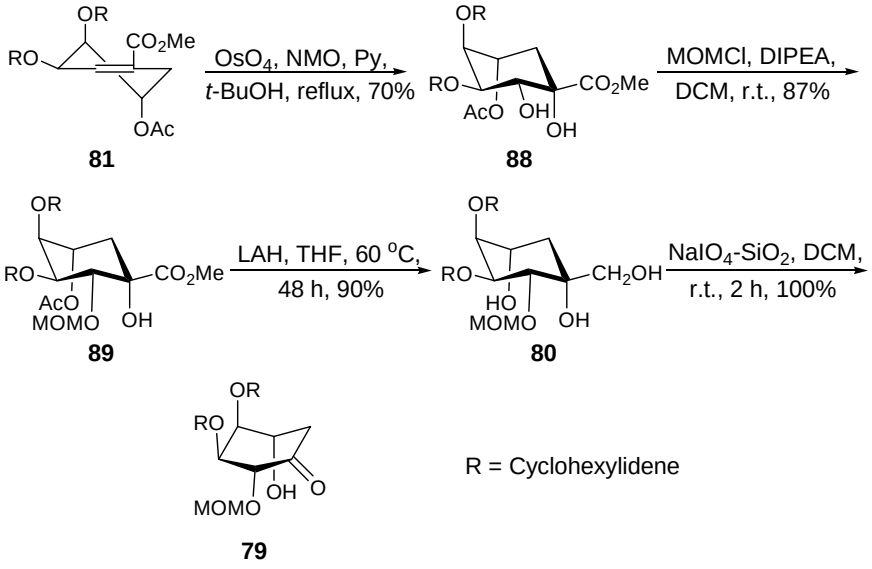
Figure 8. *ORTEP diagram of diol* ***88***



After getting the vicinal diol **88** with required stereochemistry our next task was to protect the secondary hydroxyl group selectively. When it was treated with MOMCl and diisopropylethylamine in DCM at r.t. the secondary hydroxyl was selectively protected as its MOM ether to furnish **89**. In ^{1}H NMR the presence of an

additional peak at 3.32 ppm (s, 3H) accounts for the methoxy protons of –MOM group whereas in DEPT spectrum presence of one more methylene carbon at 95.93 ppm confirms the presence of –MOM group. Rest of the spectrum was found to be in line with the proposed structure. The reduction of **89** with lithium aluminium hydride in dry THF gave triol **80**. The absence of signals for both the methyl protons of –CO_2Me and –OAc in ^1H NMR spectrum, and their carbonyl carbons signals from ^{13}C NMR in addition to the presence of an extra methylene carbon signal at 66.83 ppm for (–CH_2OH) in its DEPT spectrum clearly indicated the proposed structure to be **80**. Moreover, the elemental analysis data confirmed the structure. Finally, the periodate cleavage of triol **80** gave the ketone **79** in quantitative yield whose structure was confirmed from its spectroscopic (^1H and ^{13}C NMR) data analyses. The presence of a signal at 204.33 ppm in ^{13}C NMR spectrum clearly indicates the keto group. Moreover, the combustion study was also found to be in favour of the compound **79** having molecular formula $C_{14}H_{22}O_6$ (Scheme 15).

Scheme 15

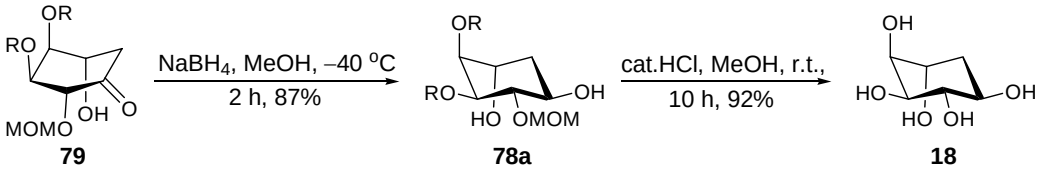


Synthesis of quercitols[33]

At this point, it was anticipated that the presence of cyclohexylidene at C-3 and C-4 would direct the hydride attack from *Si* face under non-chelation conditions

and indeed it was observed that the reduction with sodium borohydride of ketone **79** at –40 ^{o}C furnished diol **78a** as a single diastereomer (Scheme 16). The structure was confirmed from its ^{1}H, ^{13}C NMR and elemental analysis data. In ^{13}C NMR spectrum the absence of carbonyl carbon's peak clearly indicates the formation of alcohol.

Scheme 16

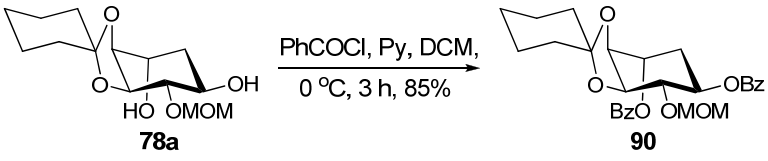


79 **78a** **18**

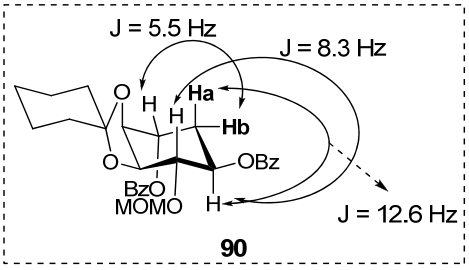
R = Cyclohexylidene

In order to determine the relative stereochemistry of **78a**, the diol was converted to its dibenzoate **90** (on treatment with benzoyl chloride and pyridine in DCM at 0 ^{o}C) because the assignment of the configuration of newly introduced stereocenter has become complicated in **78a**. Thus, it was observed that the downfield shift of H-1 and H-5 protons made the spectrum simplified. Moreover, the coupling constants of H-1 with H-2 and H-6a (J = 8.3 and 12.6 Hz, respectively), clearly indicates the *trans*-diaxial relationships (Scheme 17). This was further confirmed from COSY experiment.

Scheme 17



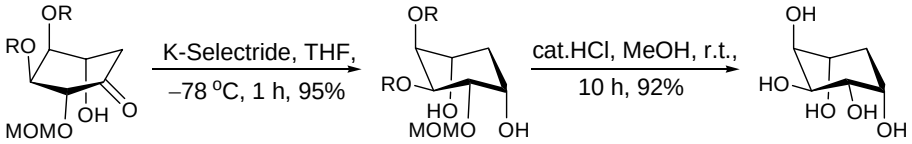
78a **90**



90

On getting one diastereomer our next target was to achieve the other one. So, **79** was treated with $NaBH_4/CeCl_3$ to give the same diol **78a** but in poor yield. Hence, after a couple of experiments, the other diastereomeric diol **78b** was obtained as a single diastereomer on reduction with K-Selectride (Scheme 18). The selectivity was attributed to chelation of a potassium ion with the C-1 carbonyl oxygen and the C-5 hydroxyl group from the *Si* face, which directed the hydride attack from the *Re* face to furnish the required diol **78b**. The structure of the diol obtained was confirmed from its ^1H, ^{13}C NMR and elemental analysis data.

Scheme 18



79 **78b** **53**

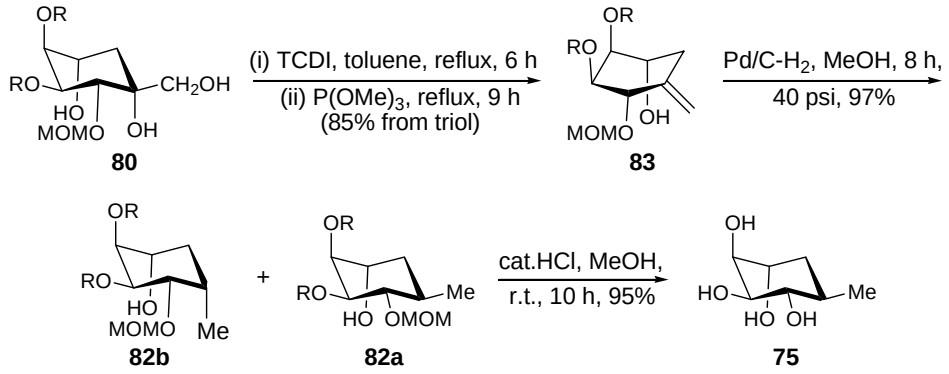
R = Cyclohexylidene

Compounds **78a** and **78b** were deprotected using catalytic HCl in methanol at room temperature to give (+)-*proto* **18** and (–)-*gala* **53** quercitols whose data were found to be in excellent agreement with the reported values.[38,39]

Synthesis of Carba-L-rhamnose[33]

Having accomplished the synthesis of **53** and **18** we turned our attention towards the synthesis of **75** from the triol **80**. The triol **80** was converted to the *exo*-olefin **83** by employing Corey-Winter protocol.[36] In ^1H NMR spectrum of **83**, the presence of a peak at 5.16 (d, J = 16.2 Hz, 2H) ppm corresponds to the exocyclic methylene group's protons, and in ^{13}C NMR spectrum the downfield shift of the olefinic methylene carbon at 115.07 ppm clearly indicates the formation of **83**. We anticipated that the reduction of the exo-olefin would proceed in a similar manner as in the case of carbonyl reduction. Catalytic hydrogenation of **83** with 10% Pd/C at 40 psi in methanol afforded a diastereomeric mixture of **82b** and **82a** in the ratio 7:93 (as determined by HPLC), which were separated by flash column chromatography (Scheme 19).

Scheme 19



R = Cyclohexylidene

The reduction reaction was also carried out by using different reagents and conditions. In all cases both the diastereomeric mixtures were obtained and their ratios were determined by GC-MS analyses (Table 4).

Table 4

S. No.	Reducing Agents	Ratios (82a:82b)
(i)	Ra/Ni	84:16
(ii)	$Pd/BaSO_4$	81:19
(iii)	[40]Rh/Al_2O_3	72:28
(iv)	Wilkinson's catalyst[41]	73:27
(v)	[42]$BH_3.SMe_2$	91:9*
(vi)	Crabtree catalyst[43]	Rearrangement**

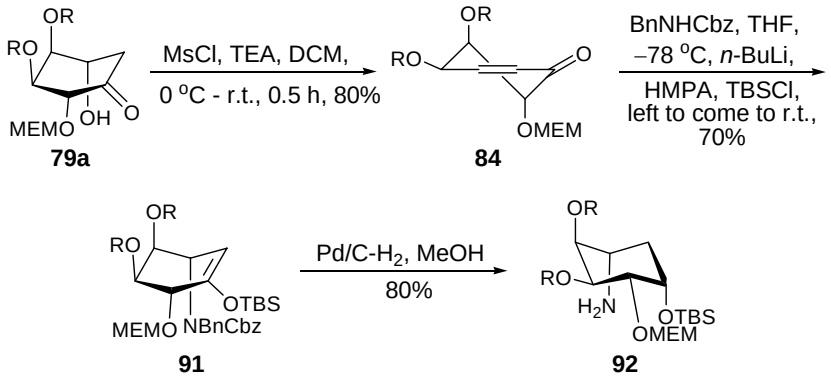
**hydroxyl methyl was obtained; **double bond was inside the ring.*

Only, with Pd/C the *de* observed was the highest compared to other conditions applied. Finally, global deprotection of **82a** with catalytic HCl in methanol furnished carba-L-rhamnose **75**[21a] (Scheme 19).

Synthetic Studies towards aminoinositols

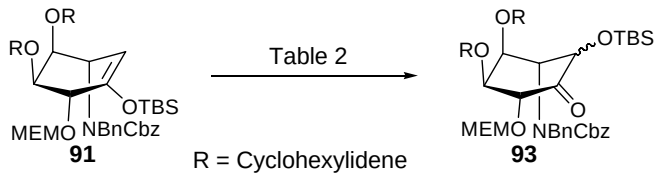
After successfully synthesizing **79a** (MEM protected analogue of **79**), our next target was to synthesize amino-inositols. Thus, **79a** was converted to the α,β-unsaturated keto- derivative **84**[37] on treatment with mesyl chloride and triethyl amine in anhydrous CH_2Cl_2. The structure was confirmed from its spectroscopic data analyses. In ^{1}H NMR the presence of peaks at 6.79 (dd, J = 3.9, 10.2 Hz, 1H) and 6.09 (d, J = 10.2 Hz, 1H) clearly indicates the presence of olefinic protons. Then 1,4-addition (Michael addition) of BnNHCbz on **84** was carried out. So, on treating **84** with the anion of BnNHCbz at −78 ^{o}C, and trapping the resultant enol formed with TBS group furnished **91** (Scheme 20). In its ^{1}H NMR spectrum the presence of peaks at 0.02 (br s, 6H) and 0.87 (s, 9H) indicates the presence of TBS group whereas peaks at 7.37-7.26 (m, 10 H) are for the aromatic ones from −Bn and −Cbz groups. Rest of the spectrum was in line with the mentioned structure. Moreover, the mass spectrum data also confirmed the structure of the compound, as represented by **91**.

Scheme 20



Then, we tried to convert **91** to **93** by epoxidation and cleavage of the epoxide ring (Scheme 21). But all our attempts towards epoxidation reaction itself failed (Table 2).

Scheme 21



Then the enolic double bond was subjected to dihydroxylation reaction condition but that also led to complex reaction mixtures (Table 5).

Table 5

S. No.	Reaction Conditions	Observations
1.	*m*-CPBA[44]	Decomposition of starting material
2.	[45]*m*-CPBA/$NaHCO_3$	Decomposition of starting material
3.	Oxone[46]	Complex reaction mixture
4.	H_2O_2/CH_3CN[47]	No reaction
5.	OsO_4/NMO	Complex reaction mixture

Finally, **91** was reduced with Pd/C-H_2 in MeOH to furnish aminocyclohexane-tetrol **92** (Scheme 20). Its structure was determined from the NMR and mass spectrum data analyses while the stereochemistry of the newly introduced amine functionality could not be determined by COSY/NOESY studies because of the broadness of its ^{1}H NMR spectrum.

Conclusions

In conclusion we have successfully synthesized (+)-*proto* **18**, (–)-*gala* **53** quercitols and carba-L-rhamnose **75** from D-(–)-quinic acid **60** in ten linear steps by employing readily available reagents and chemicals. The other diastereomers of quercitols can also be synthesized using suitable transformations. We have also shown that by employing stereoselective 1,4-conjugate addition of amine nucleophiles, one can synthesize aminocyclohexanetetrols.

Table 1. Crystal data and structure refinement for compound 88

Empirical formula	$C_{16}H_{24}O_8$
Formula weight	344.35
Temperature	297(2) K
Wavelength	0.71073 Å
Crystal system, space group	Orthorhombic, P2(1)2(1)2(1)
Unit cell dimensions	a = 5.675(3) Å, α = 90°
	b = 16.707(10) Å, β = 90°
	c = 18.140(10) Å, γ = 90°
Volume	1719.9(17) $Å^3$
Z, Calculated density	4, 1.330 mg/m^3
Absorption coefficient	0.107 mm^{-1}
F(000)	736
Crystal size	0.21 x 0.06 x 0.05 mm
Theta range for data collection	1.66 to 25.00°
Limiting indices	-6<=h<=6, -19<=k<=19, -21<=l<=20
Reflections collected / unique	11947 / 3028 [R(int) = 0.0674]
Completeness to theta = 25.00	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9943 and 0.9779
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3028 / 0 / 227
Goodness-of-fit on F^2	1.042
Final R indices [I>2sigma(I)]	R1 = 0.0449, wR2 = 0.0958
R indices (all data)	R1 = 0.0890, wR2 = 0.1123
Absolute structure parameter	1.4(16)
Largest diff. peak and hole	0.138 and -0.138 e. $Å^{-3}$

Table 2. Bond lengths [Å] and angles [deg] for compound 88

O(2)-C(1)	1.327(4)	O(1)-C(1)-O(2)	123.7(3)
O(2)-C(15)	1.455(3)	O(1)-C(1)-C(2)	123.2(3)

O(5)-C(8)	1.434(3)	O(2)-C(1)-C(2)	113.1(3)
O(5)-C(4)	1.439(3)	O(4)-C(3)-C(4)	112.8(2)
O(4)-C(3)	1.420(3)	O(4)-C(3)-C(2)	111.0(2)
O(3)-C(2)	1.428(4)	C(4)-C(3)-C(2)	109.2(2)
O(6)-C(5)	1.424(3)	O(3)-C(2)-C(1)	107.9(2)
O(6)-C(8)	1.439(3)	O(3)-C(2)-C(3)	107.6(2)
O(7)-C(14)	1.341(5)	C(1)-C(2)-C(3)	110.7(2)
O(7)-C(6)	1.442(3)	O(3)-C(2)-C(7)	109.7(3)
C(4)-C(3)	1.518(4)	C(1)-C(2)-C(7)	111.0(2)
C(4)-C(5)	1.531(4)	C(3)-C(2)-C(7)	109.9(2)
C(1)-O(1)	1.209(3)	O(5)-C(8)-O(6)	105.0(2)
C(1)-C(2)	1.514(4)	O(5)-C(8)-C(9)	109.1(2)
C(3)-C(2)	1.528(4)	O(6)-C(8)-C(9)	109.6(2)
C(2)-C(7)	1.540(4)	O(5)-C(8)-C(13)	110.8(2)
C(8)-C(9)	1.494(4)	O(6)-C(8)-C(13)	109.8(2)
C(8)-C(13)	1.521(4)	C(9)-C(8)-C(13)	112.3(3)
C(6)-C(5)	1.502(4)	O(7)-C(6)-C(5)	108.5(2)
C(6)-C(7)	1.528(4)	O(7)-C(6)-C(7)	108.0(2)
C(13)-C(12)	1.525(4)	C(5)-C(6)-C(7)	111.5(3)
C(14)-O(8)	1.184(5)	C(8)-C(13)-C(12)	111.6(2)
C(14)-C(16)	1.486(6)	O(6)-C(5)-C(6)	112.5(2)
C(11)-C(10)	1.509(5)	O(6)-C(5)-C(4)	104.3(2)
C(11)-C(12)	1.514(5)	C(6)-C(5)-C(4)	109.8(2)
C(9)-C(10)	1.538(4)	C(6)-C(7)-C(2)	113.3(2)
C(1)-O(2)-C(15)	116.4(2)	O(8)-C(14)-O(7)	122.5(4)
C(8)-O(5)-C(4)	107.2(2)	O(8)-C(14)-C(16)	126.3(4)
C(5)-O(6)-C(8)	109.73(19)	O(7)-C(14)-C(16)	111.2(4)
C(14)-O(7)-C(6)	117.5(3)	C(10)-C(11)-C(12)	110.3(3)
O(5)-C(4)-C(3)	109.0(2)	C(8)-C(9)-C(10)	111.3(3)
O(5)-C(4)-C(5)	101.6(2)	C(11)-C(10)-C(9)	111.3(3)
C(3)-C(4)-C(5)	115.0(2)	C(11)-C(12)-C(13)	111.3(3)

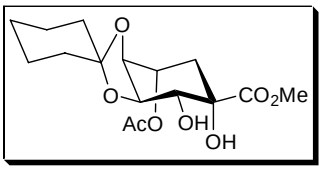
Table 3. Torsion angles [deg] for compound 88

C(8)-O(5)-C(4)-C(3)	-157.0(2)	O(5)-C(8)-C(13)-C(12)	-175.4(3)
C(8)-O(5)-C(4)-C(5)	-35.2(3)	O(6)-C(8)-C(13)-C(12)	69.0(3)
C(15)-O(2)-C(1)-O(1)	-1.8(5)	C(9)-C(8)-C(13)-C(12)	-53.1(3)
C(15)-O(2)-C(1)-C(2)	178.6(3)	C(8)-O(6)-C(5)-C(6)	106.9(3)
O(5)-C(4)-C(3)-O(4)	-86.9(3)	C(8)-O(6)-C(5)-C(4)	-12.1(3)
C(5)-C(4)-C(3)-O(4)	159.8(2)	O(7)-C(6)-C(5)-O(6)	63.7(3)
O(5)-C(4)-C(3)-C(2)	149.1(2)	C(7)-C(6)-C(5)-O(6)	-177.4(3)
C(5)-C(4)-C(3)-C(2)	35.8(3)	O(7)-C(6)-C(5)-C(4)	179.5(2)
O(1)-C(1)-C(2)-O(3)	-1.2(4)	C(7)-C(6)-C(5)-C(4)	-61.7(3)
O(2)-C(1)-C(2)-O(3)	178.4(3)	O(5)-C(4)-C(5)-O(6)	28.5(3)
O(1)-C(1)-C(2)-C(3)	-118.7(3)	C(3)-C(4)-C(5)-O(6)	146.1(2)
O(2)-C(1)-C(2)-C(3)	60.9(3)	O(5)-C(4)-C(5)-C(6)	-92.3(3)
O(1)-C(1)-C(2)-C(7)	119.0(4)	C(3)-C(4)-C(5)-C(6)	25.3(4)
O(2)-C(1)-C(2)-C(7)	-61.4(4)	O(7)-C(6)-C(7)-C(2)	152.8(3)
O(4)-C(3)-C(2)-O(3)	-69.4(3)	C(5)-C(6)-C(7)-C(2)	33.7(4)
C(4)-C(3)-C(2)-O(3)	55.7(3)	O(3)-C(2)-C(7)-C(6)	-89.8(3)
O(4)-C(3)-C(2)-C(1)	48.3(3)	C(1)-C(2)-C(7)-C(6)	151.0(3)
C(4)-C(3)-C(2)-C(1)	173.4(2)	C(3)-C(2)-C(7)-C(6)	28.2(4)
O(4)-C(3)-C(2)-C(7)	171.3(2)	C(6)-O(7)-C(14)-O(8)	3.4(6)
C(4)-C(3)-C(2)-C(7)	-63.7(3)	C(6)-O(7)-C(14)-C(16)	-173.9(3)
C(4)-O(5)-C(8)-O(6)	28.7(3)	O(5)-C(8)-C(9)-C(10)	176.5(2)
C(4)-O(5)-C(8)-C(9)	146.1(3)	O(6)-C(8)-C(9)-C(10)	-69.1(3)
C(4)-O(5)-C(8)-C(13)	-89.8(3)	C(13)-C(8)-C(9)-C(10)	53.2(4)
C(5)-O(6)-C(8)-O(5)	-9.3(3)	C(12)-C(11)-C(10)-C(9)	57.1(4)
C(5)-O(6)-C(8)-C(9)	-126.3(2)	C(8)-C(9)-C(10)-C(11)	-55.6(4)
C(5)-O(6)-C(8)-C(13)	109.9(2)	C(10)-C(11)-C(12)-C(13)	-56.8(4)
C(14)-O(7)-C(6)-C(5)	-152.5(3)	C(8)-C(13)-C(12)-C(11)	54.6(4)
C(14)-O(7)-C(6)-C(7)	86.5(4)		

Symmetry transformations used to generate equivalent atoms

Experimental

(3a*R*,4*R*,5*S*,7*R*,7a*S*)-Methyl 7-acetoxy-4,5-dihydroxyhexahydrospiro[benzo[*d*][1,3]dioxole-2,1′-cyclohexane]-5-carboxylate (88)



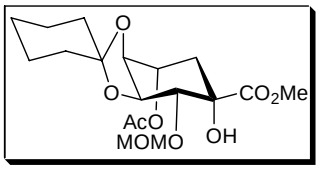
A solution of **81** (6 g, 19.35 mmol), NMO (6.8 mL, 29.03 mmol), pyridine (9.4 mL, 116.13 mmol), water (1.9 mL, 106.45 mmol) and OsO_4 (100 mg) in *t*-BuOH (180 mL) was refluxed in the dark for 3 h. After cooling, *t*-BuOH was removed on rota-vapor. The reaction mixture was quenched with saturated solution of sodium sulfite and extracted with ethyl acetate (3 × 100 mL). The combined ethyl acetate extracts were dried over sodium sulphate, concentrated in vacuo and purified by column chromatography (eluent Pet. Ether:Ethylacetate 6:4) to afford the diol **88** as a white solid.

Yield	: 4.7 g, 70 %
Mol. Formula	: $C_{16}H_{24}O_8$
Melting Point	: 128.8 ^oC
Optical Rotation $[\alpha]_D^{25}$	: +9.7 (*c* 0.6, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3020, 2864, 1738, 1640, 1450, 1369, 1333, 1243, 1216, 1163, 1135, 1114 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.41 (br s, 2H), 1.59-1.69 (m, 8H), 1.84 (dd, *J* = 14.9, 7.1 Hz, 1H), 2.09 (s, 3H), 2.48 (dd, *J* = 14.9, 6.1 Hz, 1H), 2.99-3.01 (m, 1H), 3.69 (s, 1H), 3.83 (s, 3H), 3.92-3.97 (m, 1H), 4.23-4.36 (m, 2H), 5.15 (dd, *J* = 12.7, 6.1 Hz, 1H) ppm.
^{13}C NMR ($CDCl_3$, 125 MHz)	: δ 21.03 (q), 23.46 (t), 23.82 (t), 24.85 (t), 34.63 (t), 35.06 (t), 37.41 (t), 53.17 (q), 69.34 (d), 73.60 (d), 75.86 (d), 76.34 (s), 77.06 (d), 110.24 (s), 170.24 (s), 174.36 (s) ppm.
Elemental Analysis	Calcd.: C, 55.81; H, 7.02

Found: C, 55.59; H, 7.21

ESI-MS (*m/z*) : 367.1 $[M+Na]^+$.

(3a*S*,4*R*,5*S*,7*R*,7a*S*)-Methyl 7-acetoxy-5-hydroxy-4-(methoxymethoxy)hexahydrospiro[benzo[*d*][1,3]dioxole-2,1′-cyclohexane]-5-carboxylate (89)



To a solution of **88** (3.5 g, 10.17 mmol) in dry DCM (40 mL) at 0 ^oC was added Hünig's base (2.1 mL, 12.21 mmol) and MOMCl (1.5 mL, 20.35 mmol), and stirred at room temperature for 12 h. Washed with water twice, once with brine, dried over sodium sulphate, concentrated in vacuo and purified by silica-gel column chromatography (eluent Pet. Ether:Ethylacetate 8:2) to afford **89** as a thick liquid.

Yield : 3.4 g, 87 %

Mol. Formula : $C_{18}H_{28}O_9$

Optical Rotation $[\alpha]_D^{25}$: –47.6 (*c* 1.45, $CHCl_3$)

IR ($CHCl_3$) $\tilde{\nu}$: 3020, 2858, 1742, 1449, 1438, 1370, 1334, 1242, 1216, 1160, 1134, 1106 cm^{-1}.

^{1}H NMR ($CDCl_3$, 200 MHz) : δ 1.32-1.42 (m, 2H), 1.58-1.71 (m, 8H), 1.80 (dd, *J* = 14.8, 7.3 Hz, 1H), 2.09 (s, 3H), 2.59 (dd, *J* = 14.8, 6.6 Hz, 1H), 3.32 (s, 3H), 3.82 (s, 3H), 4.03-4.06 (m, 1H), 4.33-4.43 (m, 2H), 4.61 (d, *J* = 6.7 Hz, 1H), 4.98 (d, *J* = 6.7 Hz, 1H), 5.07-5.17 (m, 1H) ppm.

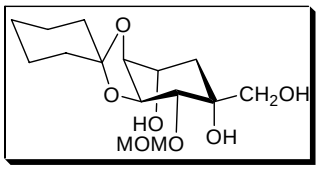
^{13}C NMR ($CDCl_3$, 75 MHz) : δ 20.97 (q), 23.65 (t), 23.96 (t), 24.99 (t), 34.82 (t), 35.71 (t), 37.51 (t), 52.80 (q), 55.88 (q), 69.43 (d), 76.39 (s), 76.73 (d), 77.15 (d), 77.58 (d), 96.07 (t), 110.14 (s), 170.08 (s), 173.99 (s) ppm.

Elemental Analysis Calcd.: C, 55.66; H, 7.27

Found: C, 55.41; H, 7.05

ESI-MS (*m/z*) : 411.2 $[M+Na]^+$.

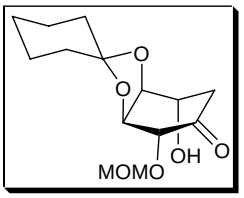
(3a*S*,4*R*,5*R*,7*R*,7a*S*)-5-(Hydroxymethyl)-4-(methoxymethoxy)hexahydrospiro[benzo[*d*][1,3]dioxole-2,1′-cyclohexane]-5,7-diol (80)



In a 100 mL RB flask (1.62 g, 42.52 mmol) LAH was taken and chilled to 0 ^{o}C. To it (30 mL) dry THF added and after some time a solution of **89** (3 g, 7.73 mmol) in 20 mL dry THF added slowly. The flask was allowed to warm to room temperature and kept for 10 min. Then it was heated at 60 ^{o}C for 2 days. Cooled to room temperature and quenched with little ethyl acetate and moist globular salt, filtered, concentrated in vacuo and purified by silica-gel column chromatography (eluent; Pet. Ether:Ethylacetate 3:7) to afford **80** as a pasty mass.

Yield	: 2.2 g, 90 %
Mol. Formula	: $C_{15}H_{26}O_7$
Optical Rotation $[\alpha]_D^{25}$	: –10.9 (*c* 0.8, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3419, 3014, 2861, 1596, 1449, 1368, 1278, 1251, 1216, 1158, 1092, 1036 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.34-1.44 (m, 2H), 1.60-1.67 (m, 8H), 1.86-2.09 (m, 2H), 2.96 (br s, 2H), 3.44 (s, 3H), 3.52 (d, *J* = 6.3 Hz, 1H), 3.71 (d, *J* = 7.0 Hz, 1H), 3.84 (d, *J* = 9.2 Hz, 1H), 4.14-4.24 (m, 1H), 4.30-4.42 (m, 2H), 4.76 (d, *J* = 6.1 Hz, 1H), 5.06 (d, *J* = 6.1 Hz, 1H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 23.52 (t), 23.83 (t), 24.81 (t), 33.71 (t), 35.25 (t), 37.95 (t), 56.02 (q), 66.83 (t), 67.40 (d), 75.99 (s), 77.55 (d), 78.53 (d), 78.91 (d), 97.38 (t), 109.62 (s) ppm.
Elemental Analysis	Calcd.: C, 56.59; H, 8.23 Found: C, 56.43; H, 8.12
ESI-MS (*m/z*)	: 341.2 $[M+Na]^+$.

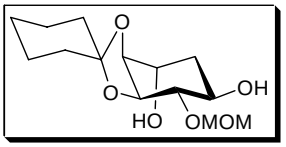
(3a*S*,4*R*,7*R*,7a*S*)-7-Hydroxy-4-(methoxymethoxy)tetrahydrospiro[benzo[*d*][1,3]dioxole-2,1′-cyclohexan]-5(6*H*)-one (79)



To a solution of **80** (2.5 g, 7.86 mmol) in DCM was added $NaIO_4$ (4 g, 18.70 mmol) adsorbed on silica gel portion−wise. The reaction mixture was stirred for 2 h, filtered, washed with DCM and concentrated in vacuo to afford a white solid **79**.

Yield	: 2.2 g, 100 %
Mol. Formula	: $C_{14}H_{22}O_6$
Melting Point	: 80 ^oC
Optical Rotation $[\alpha]_D^{25}$	: +34.3 (*c* 1.05, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3019, 2863, 1735, 1464, 1450, 1370, 1334, 1282, 1216, 1155, 1111 cm^{-1}.
^{1}H NMR ($CDCl_3$, 300 MHz)	: δ 1.40-1.42 (m, 2H), 1.59-1.68 (m, 8H), 2.47 (dd, *J* = 18.3, 7.3 Hz, 1H), 2.86 (dd, *J* = 18.3, 4.4 Hz, 1H), 2.85 (br s, 1H), 3.43 (s, 3H), 4.27-4.35 (m, 3H), 4.43 (t, *J* = 6.6 Hz, 1H), 4.78 (br s, 2H) ppm.
^{13}C NMR ($CDCl_3$, 75 MHz)	: δ 23.53 (t), 23.90 (t), 24.96 (t), 34.21 (t), 37.08 (t), 42.15 (t), 55.82 (q), 67.97 (d), 77.18 (d), 77.58 (d), 79.17 (d), 95.80 (t), 110.88 (s), 204.33 (s) ppm.
Elemental Analysis	Calcd.: C, 58.73; H, 7.74 Found: C, 58.81; H, 7.53
ESI-MS (*m/z*)	: 309.1 $[M+Na]^+$.

(3a*R*,4*S*,5*R*,7*R*,7a*S*)-4-(Methoxymethoxy)hexahydrospiro[benzo[*d*][1,3]dioxole-2,1′-cyclohexane]-5,7-diol (78a)

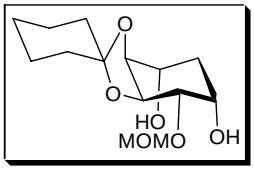


A solution of **79** (0.5 g, 1.75 mmol) in MeOH (5 mL) was cooled to −40 ^oC using acetonitrile-dry ice bath, and $NaBH_4$ (0.079 g, 2.10 mmol) was added portion wise. The reaction was continued at this temperature for 2 h. Quenched with acetic

acid (0.1 mL, 1.92 mmol) and little water; extracted in ethyl acetate, dried over Na_2SO_4, concentrated under vacuum and purified by flash column chromatography (eluent Isopropanol:Chloroform 4:96) to afford **78a** as a white solid.

Yield	: 0.44 g, 87%
Mol. Formula	: $C_{14}H_{24}O_6$
Melting Point	: 97 ^oC
Optical Rotation $[\alpha]_D^{25}$	: −12.6 (*c* 0.7, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3020, 2940, 1449, 1216, 1105 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.39-1.41 (m, 2H), 1.59-1.74 (m, 8H), 1.87 (ddd, *J* = 13.2, 9.5, 3.7 Hz, 1H), 2.05 (dt, *J* = 14.1, 5.1 Hz, 1H), 3.45 (br s, 4H), 3.62 (br s, 1H), 3.84 (ddd, *J* = 14.1, 9.2, 4.9 Hz, 1H), 4.15 (m, 2H), 4.26 (dd, *J* = 5.0, 3.7 Hz, 1H), 4.83 (s, 2H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: 23.60 (t), 23.89 (t), 24.89 (t), 34.57 (t), 35.13 (t), 37.80 (t), 55.80 (q), 66.45 (d), 66.70 (d), 77.71 (d), 78.19 (d), 85.99 (d), 97.61 (t), 109.96 (s) ppm.
Elemental Analysis	Calcd.: C, 58.32; H, 8.39 Found: C, 58.04; H, 8.51
ESI-MS (*m/z*)	: 311.1 $[M+Na]^+$.

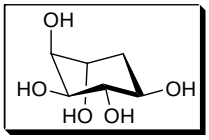
(3a*R*,4*S*,5*S*,7*R*,7a*S*)-4-(Methoxymethoxy)hexahydrospiro[benzo[*d*][1,3]dioxole-2,1′-cyclohexane]-5,7-diol (78b)



To a solution of **79** (0.25 g, 0.87 mmol) in dry THF (15 mL) at −78 ^oC was added K-Selectride (1.48 ml, 1.48 mmol) dropwise. After half an hour the reaction mixture was quenched with MeOH (1.48 mL) followed by 10 % NaOH (7.4 mL) at −78 ^oC only. Then it was kept at 0 ^oC for half an hour and finally at room temperature for 1 h. Extracted in diethylether, dried over Na_2SO_4, concentrated and purified by flash column chromatography (eluent; Isopropanol:Chloroform 4:96) to afford **78b**. as a clear syrup.

Yield	: 0.2 g, 95%
Mol. Formula	: $C_{14}H_{24}O_6$
Optical Rotation $[\alpha]_D^{25}$	: +32.9 (*c* 1.5, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3017, 2861, 1449, 1370, 1334, 1276, 1216, 1165, 1153, 1101 cm^{-1}.
^{1}H NMR ($CDCl_3$, 500 MHz)	: δ 1.38-1.40 (m, 2H), 1.58-1.70 (m, 8H), 1.93 (ddd, *J* = 2.6, 3.9, 14.6 Hz, 1H), 2.14 (td, *J* = 15.1, 4.4 Hz, 1H), 3.43 (s, 3H), 3.69 (dd, *J* = 2.9, 6.4 Hz, 1H), 4.11 (dd, *J* = 6.5, 4.0 Hz, 1H), 4.18-4.20 (m, 1H), 4.30-4.34 (m, 2H), 4.77 (d, *J* = 6.4, 1H), 4.85 (d, *J* = 6.8, 1H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 23.63 (t), 23.92 (t), 24.93 (t), 31.25 (t), 35.37 (t), 38.02 (t), 55.63 (q), 67.90 (d), 69.36 (d), 75.59 (d), 78.35 (d), 78.57 (d), 95.66 (t), 109.66 (s) ppm.
Elemental Analysis	Calcd.: C, 58.32; H, 8.39 Found: C, 58.23; H, 8.56
ESI-MS (*m/z*)	: 311.1 $[M+Na]^+$.

(+)-*proto*-Quercitol (18)

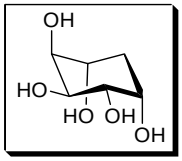


A solution of **78a** (70mg, 0.43 mmol) in MeOH (4 mL) was treated with catalytic conc. HCl and stirred at room temperature for 10 h. Then it was concentrated and recrystallized from methanol – ethyl acetate mixture to give **18** as a solid.

Yield	: 37 mg, 92%
Mol. Formula	: $C_6H_{12}O_5$
Optical Rotation $[\alpha]_D^{25}$	: +25.1 (*c* 0.5, H_2O)
Melting Point	: 237 ^oC
^{1}H NMR (D_2O, 200 MHz)	: δ 1.80 (ddd, *J* = 3.0, 11.4, 14.3, 1H), 1.93-2.04 (m, 1H), 3.50-3.59 (m, 1H), 3.67-3.81 (m, 2H), 3.90-3.94 (m, 1H), 4.01 (dd, *J* = 6.6, 3.2 Hz, 1H) ppm.

^{13}C NMR	: δ 46.29 (t), 81.54 (d), 81.91 (d), 84.01 (d), 85.27 (d),
(D_2O, 75 MHz)	87.55 (d) ppm.
Elemental Analysis	Calcd.: C, 43.90; H, 7.37
	Found: C, 43.82; H, 7.35
ESI-MS (*m/z*)	: 187.1 $[M+Na]^+$.

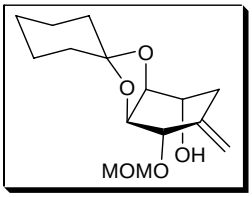
(–)-*gala*-Quercitol (53)



Compound **78b** (50 mg, 0.30 mmol) was treated in same way as **78a** to furnish **53** as a solid.

Yield	: 26 mg, 92%
Mol. Formula	: $C_6H_{12}O_5$
Optical Rotation $[\alpha]_D^{25}$	: –48.9 (*c* 0.5, H_2O)
Melting Point	: 256-258 ^oC
^{1}H NMR	: δ 1.80 (quartet, *J* = 11.8, 1H), 2.04-2.09 (m, 1H), 3.74
(D_2O, 500 MHz)	(dd, *J* = 9.2, 3.4 Hz, 1H), 3.86 (ddd, *J* = 14.9, 10.0, 4.3
	Hz, 1H), 3.99 (t, *J* = 3.4 Hz, 1H), 4.06-4.10 (m, 2H)
	ppm.
^{13}C NMR	: δ 34.26 (t), 67.11 (d), 68.62 (d), 72.51 (d), 72.71 (d),
(D_2O, 125 MHz)	72.95 (d) ppm.
Elemental Analysis	Calcd.: C, 43.90; H, 7.37
	Found: C, 43.75; H, 7.29
ESI-MS (*m/z*)	: 187.1 $[M+Na]^+$.

(3a*R*,4*S*,7*R*,7a*S*)-4-(Methoxymethoxy)-5-methylenehexahydrospiro[benzo[*d*][1,3]dioxole-2,1′-cyclohexan]-7-ol (83)

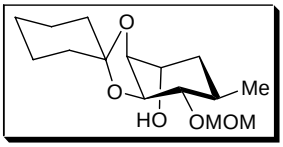


To a solution of triol **80** (100 mg, 0.31 mmol) in toluene (3 mL) at 0 ^{o}C was added 1,1′-thiocarbonyldiimidazole (84 mg, 0.47 mmol), and the solution was refluxed for 6 h. Then cooled to room temperature, water was added, extracted in ethyl acetate (3×5 mL). The combined organic layer was dried over sodium sulfate, concentrated and column was done using pet. ether:ethylacetate (3:1) ratio as eluent to give the thiocarbonate derivative.

The thiocarbonate derivative was treated with trimethyl phosohite (6 mL) and refluxed for 9 h. Then the solvent was distilled out under high vacuum and the residue was fractionated over silica-gel column (eluent 20% ethylacetate in pet ether) to furnish exocyclic olefin **83** as a thick liquid.

Yield	: 84 mg, 85%
Mol. Formula	: $C_{15}H_{24}O_5$
Optical Rotation $[\alpha]_D^{25}$	: +72.4 (*c* 0.8, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3018, 2857, 1657, 1464, 1449, 1369, 1333, 1282, 1216, 1154, 927 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.33-1.41 (m, 2H), 1.59-1.66 (m, 8H), 2.38 (dd, *J* = 15.2, 5.4 Hz, 1H), 2.66 (tdd, *J* = 15.2, 4.7, 1.4 Hz, 2H), 3.41 (s, 3H), 4.00 (br s, 1H), 4.22-4.26 (m, 3H), 4.70 (dd, *J* = 15.2, 6.6 Hz, 2H), 5.12 (br s, 1H), 5.20 (s, 1H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 23.52 (t), 23.87 (t), 24.93 (t), 34.60 (t), 34.70 (t), 37.27 (t), 55.53 (q), 68.83 (d), 76.05 (d), 77.91 (d), 93.72 (t), 109.69 (s), 115.07 (t), 139.30 (s) ppm.
Elemental Analysis	Calcd.: C, 63.36; H, 8.51 Found: C, 63.31; H, 8.45
ESI-MS (*m/z*)	: 307.2 $[M+Na]^+$.

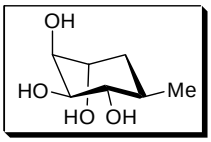
(3a*R*,4*S*,5*R*,7*R*,7a*S*)-4-(Methoxymethoxy)-5-methylhexahydrospiro[benzo[*d*][1,3]dioxole-2,1′-cyclohexan]-7-ol (82a)



A solution of **83** (50 mg, 0.18 mmol) in MeOH (6 mL) was treated with 10% Pd/C (catalytic) at 40 psi H_2 pressure in parr shaker for 8 h. Then it was filtered, washed with MeOH, concentrated and purified by column chromatography (eluent: 18% ethylacetate in pet ether) to afford **82a** as clear syrup.

Yield	: 49 mg, 97%
Mol. Formula	: $C_{15}H_{26}O_5$
Optical Rotation $[\alpha]_D^{25}$	: –30.4 (*c* 0.85, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 2936, 1450, 1369, 1332, 1280, 1229, 1157 cm^{-1}.
^{1}H NMR ($CDCl_3$, 500 MHz)	: δ 1.06 (d, *J* = 6.8 Hz, 3H), 1.39-1.40 (m, 2H), 1.56-1.75 (m, 10H), 1.85-1.93 (m, 1H), 3.39 (dd, *J* = 9.8, 6.8 Hz, 1H), 3.42 (s, 3H), 4.07-4.11 (m, 2H), 4.15 (dd, *J* = 6.5, 5.6 Hz, 1H), 4.67 (d, *J* = 6.6, 1H), 5.00 (d, *J* = 6.6 Hz, 1H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 18.42 (q), 23.69 (t), 23.98 (t), 25.01 (t), 29.14 (d), 35.20 (t), 35.23 (t), 37.75 (t), 55.89 (q), 67.32 (d), 78.77 (d), 79.31 (d), 81.06 (d), 96.38 (t), 109.44 (s) ppm.
Elemental Analysis	Calcd.: C, 62.91; H, 9.15 Found: C, 62.71; H, 9.52
ESI-MS (*m/z*)	: 309.2 $[M+Na]^+$.

Carba-L-rhamnose (75)

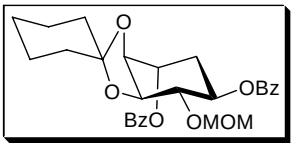


A solution of **82a** (40 mg, 0.25 mmol) in MeOH (2.5 mL) was treated with one drop of conc. HCl and stirred overnight at room temperature for 10 h. Filtered, concentrated and purified by chromatography (eluent: 10% methanol in ethylacetate) to give **75** as a thick liquid.

Yield	: 21 mg, 95%
Mol. Formula	: $C_7H_{14}O_4$

Optical Rotation $[\alpha]_D^{25}$	: +5.9 (*c* 0.65, MeOH)
^{1}H NMR	: δ 1.00 (d, *J* = 6.3 Hz, 3H), 1.55-1.61 (m, 2H), 1.70-1.86
(Methanol-d_4, 200 MHz)	(m, 1H), 3.25 (t, *J* = 9.6 Hz, 1H), 3.61 (dd, *J* = 9.3, 2.9 Hz, 1H), 3.80-3.88 (m, 2H) ppm.
^{13}C NMR	: δ 18.49 (q), 33.01 (d), 35.82 (t), 71.00 (d), 74.23 (d),
(Methanol-d_4, 125 MHz)	74.88 (d), 76.75 (d) ppm.
Elemental Analysis	Calcd.: C, 51.84; H, 8.70
	Found: C, 51.75; H, 8.67
ESI-MS (*m/z*)	: 185.1 $[M+Na]^+$.

(3a*R*,4*S*,5*R*,7*R*,7a*S*)-4-(Methoxymethoxy)hexahydrospiro[benzo[*d*][1,3]dioxole-2,1′-cyclohexane]-5,7-diyl dibenzoate (90)

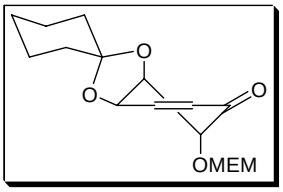


The diol **78a** (100 mg, 0.35 mmol) was taken into CH_2Cl_2 (2 ml) and cooled to 0 ^oC. Then pyridine (0.08 mL, 1.04 mmol) and benzoyl chloride (0.12 mL, 1.04 mmol) were added, and stirred for 3 h. After the completion of the reaction as judged by TLC little cold water added and extracted in ethylacetate (3 × 5 mL). The combined organic layers were dried over sodium sulfate, concentrated and purified by column chromatography (eluent: 10% ethylacetate in pet ether) to furnish **90** as clear syrup.

Yield	: 0.15 g, 85%
Mol. Formula	: $C_{28}H_{32}O_8$
Optical Rotation $[\alpha]_D^{25}$	: +45.0 (*c* 0.2, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3020, 2858, 1721, 1603, 1492, 1451, 1366, 1316, 1269, 1216, 1177, 1149, 1110 cm^{-1}.
^{1}H NMR	: δ 1.40-1.42 (m, 2H), 1.59-1.68 (m, 6H), 1.85 (t, J = 6.2
($CDCl_3$, 500 MHz)	Hz, 2H), 2.22 (ddd, *J* = 13.5, 9.5, 3.7 Hz, 1H), 2.30 (ddd, *J* = 9.8, 5.3, 4.4 Hz, 1H), 3.33 (s, 3H), 4.12 (dd, *J* = 8.2, 5.7 Hz, 1H), 4.31-4.37 (m, 2H), 4.78 (d, *J* = 6.6 Hz, 1H), 4.90 (d, *J* = 6.6 Hz, 1H), 5.46 (ddd, *J* = 12.6, 8.9, 3.9 Hz, 1H), 5.68-5.71 (m, 1H), 7.43-7.48 (m, 4H), 7.55-7.60

	(m, 2H), 8.06-8.09 (m, 4H) ppm.
^{13}C NMR	: δ 23.71 (t), 23.91 (t), 24.90 (t), 30.03 (t), 35.40 (t),
($CDCl_3$, 50 MHz)	37.90 (t), 55.82 (q), 69.36 (d), 70.02 (d), 75.31 (d), 77.01 (d), 78.54 (d), 96.52 (t), 110.47 (s), 128.37 (d), 128.46 (d), 129.67 (d), 129.76 (d), 129.91 (s), 133.13 (d), 133.30 (d), 165.34 (s), 165.61 (s) ppm.
Elemental Analysis	Calcd.: C, 67.73; H, 6.50
	Found: C, 67.65; H, 6.25
ESI-MS (*m/z*)	: 519.2 $[M+Na]^+$.

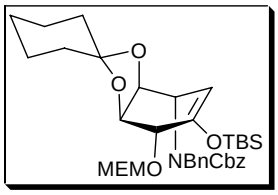
(3a*S*,4*R*,7a*S*)-4-((2-Methoxyethoxy)methoxy)-3a,4-dihydrospiro[benzo[*d*][1,3]dioxole-2,1′-cyclohexan]-5(7aH)-one (84)



To solution of **79a** (1.02 g, 3.09 mmol) in CH_2Cl_2 (15 mL) cooled to 0 ^oC was added MsCl (0.3 mL, 3.71 mmol). Then a mixture of triethyl amine (1.0 mL, 7.11 mmol) in CH_2Cl_2 (6 mL) was added slowly over 1.5 h and the reaction mixture was kept at r.t. for half an hour. The reaction mixture was washed with water twice, once with brine and dried over sodium sulphate. Finally it was concentrated and purified by column chromatography using 30% ethylacetate in pet ether as eluent to give **84** as a pasty mass.

Yield	: 0.77 g, 80%
Mol. Formula	: $C_{16}H_{24}O_6$ (MW 312.2)
^{1}H NMR	: δ 1.40-1.64 (m, 10H), 3.38 (s, 3H), 3.54 (t, J = 4.6 Hz,
($CDCl_3$, 200 MHz)	2H), 3.70-3.92 (m, 2H), 4.41-4.52 (m, 2H), 4.79-4.83 (m, 1H), 4.87-4.96 (m, 2H), 6.10 (d, J = 10.2 Hz, 1H), 6.80 (dd, J = 10.2, 3.9 Hz, 1H) ppm.
ESI-MS (*m/z*)	: 335.2 $[M+Na]^+$.

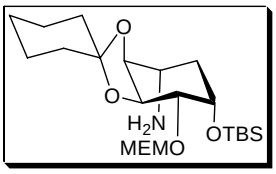
Benzyl benzyl((3a*S*,4*R*,7*R*,7a*S*)-5-(*tert*-butyldimethylsilyloxy)-4-((2-methoxyethoxy)methoxy)-3a,4,7,7a-tetrahydrospiro[benzo[*d*][1,3]dioxole-2,1′-cyclohexane]-7-yl)carbamate (91)



A solution of benzyl benzylcarbamate (115 mg, 0.48 mmol) in THF (1.5 mL) was cooled to −78 ^{o}C and to it *n*-BuLi (0.2 mL, 0.5 mmol) was added, and the reaction mixture was stirred for 30 min. Then HMPA (0.4 mL) was added and again kept for 20 min stirring. After that a solution of **84** (135 mg, 0.43 mmol) in THF (0.5 mL) was added, and kept for 30 min. Finally TBSCl (78 mg, 0.52 mmol) in THF (0.5 mL) was added and the reaction mixture was left to come to r.t. slowly. Then it was quenched with a solution of saturated NH_4Cl, diluted with ethyl acetate, washed with water twice, concentrated and purified by column chromatography (eluent 12% ethyl acetate in pet ether).

Yield	: 0.20 g, 70%
Mol. Formula	: $C_{37}H_{53}NO_8Si$ (MW 667.4)
Optical Rotation $[\alpha]_D^{25}$	: +7.8 (*c* 1, $CHCl_3$)
^{1}H NMR ($CDCl_3$, 300 MHz)	: δ 0.00 (br s, 6H), 0.86 (s, 9H), 1.53-1.65 (m, 10H), 3.37 (s, 3H), 3.51-3.69 (m, 4H), 3.76-3.86 (m, 1H), 4.08-4.20 (m, 3H), 4.40-4.59 (m, 4H), 4.81 (d, *J* = 6.6 Hz, 1H), 4.88 (d, *J* = 7.3 Hz, 1H), 5.13-5.20 (m, 1H), 7.20-7.38 (m, 10H) ppm.
^{13}C NMR ($CDCl_3$, 75 MHz)	: δ –4.61 (q), 18.04 (s), 23.74 (t), 24.02 (t), 25.15 (t), 25.64 (q), 29.66 (t), 34.85 (t), 37.39 (t), 57.47 (d), 58.93 (q), 67.11 (t), 67.33 (t), 71.75 (t), 73.76 (d), 74.99 (d), 77.98 (d), 95.49 (t), 103.61 (d), 109.35 (s), 126.84 (d), 126.96 (s), 127.05 (s), 127.88 (d), 128.36 (d), 136.67 (s), 139.05 (s) ppm.
ESI-MS (*m/z*)	: 690.4 $[M+Na]^+$.

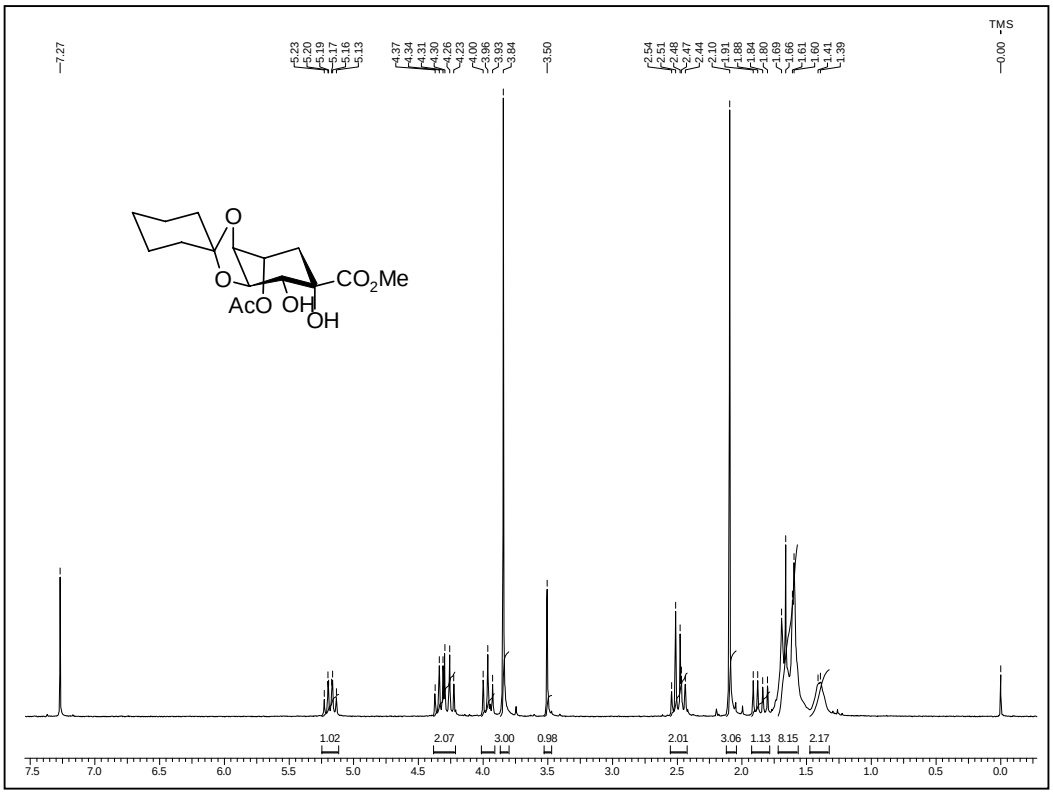
(3a*S*,4*R*,5*S*,7*R*,7a*S*)-5-(*Tert*-butyldimethylsilyloxy)-4-((2-methoxyethoxy)methoxy)hexahydrospiro[benzo[*d*][1,3]dioxole-2,1′-cyclohexan]-7-amine (92)



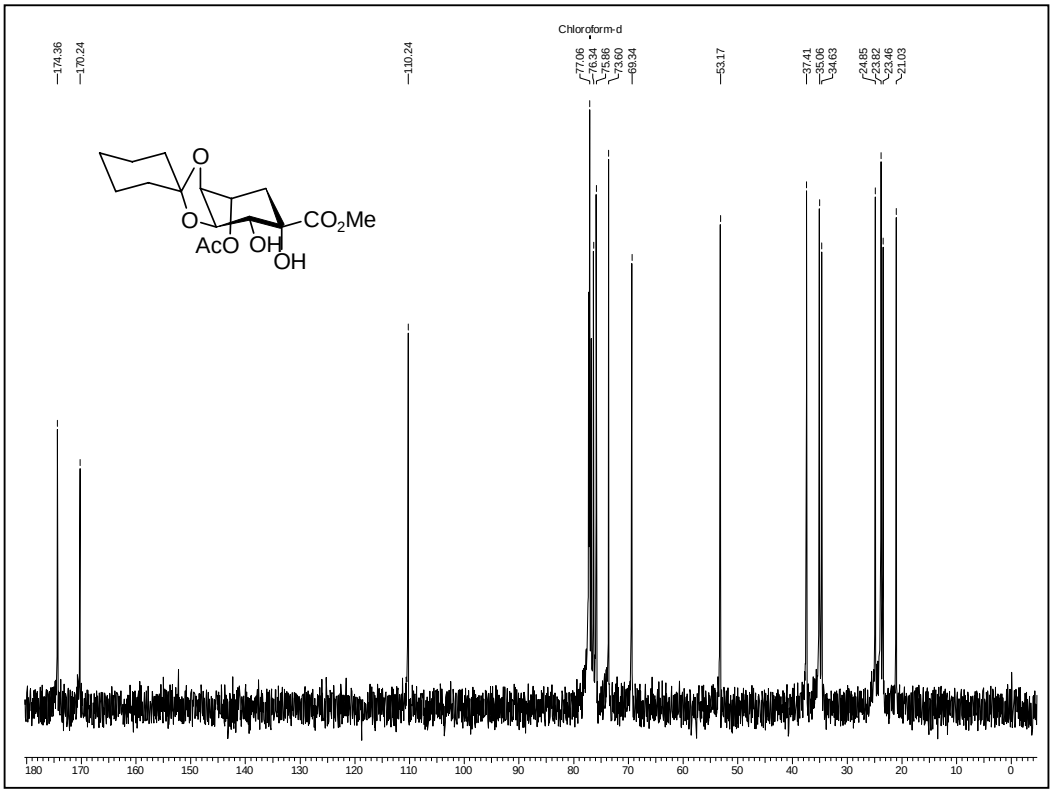
A solution of **91** (50 mg, 0.07 mmol) in MeOH (3 mL) was treated with 10% Pd/C (catalytic) at 40 psi H_2 pressure in parr shaker for 12 h. Then it was filtered, washed with MeOH, concentrated and purified by column chromatography (eluent: 20% ethylacetate in pet ether) to afford **92** as a thick liquid.

Yield	: 27 mg, 80%
Mol. Formula	: $C_{21}H_{41}NO_7Si$ (MW 447.3)
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 0.08 (s, 6H), 0.88 (s, 9H), 1.39-1.66 (m, 10H), 1.88-2.04 (m, 3H), 3.39 (s, 3H), 3.56 (t, J = 4.4 Hz, 2H), 3.66-3.80 (m, 2H), 3.84-4.04 (m, 2H), 4.12-4.22 (m, 2H), 4.78-4.84 (m, 2H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ −5.03 (q), −5.01 (q), 11.77 (s), 23.72 (t), 23.89 (t), 24.93 (t), 25.60 (q), 31.83 (t), 34.95 (t), 37.93 (t), 48.61 (d), 58.93 (q), 67.08 (t), 68.25 (d), 71.58 (t), 75.71 (d), 76.43 (d), 95.24 (t), 110.10 (s) ppm.
ESI-MS (*m/z*)	: 457.1 $[M+H]^+$; 470.3 $[M+Na]^+$.

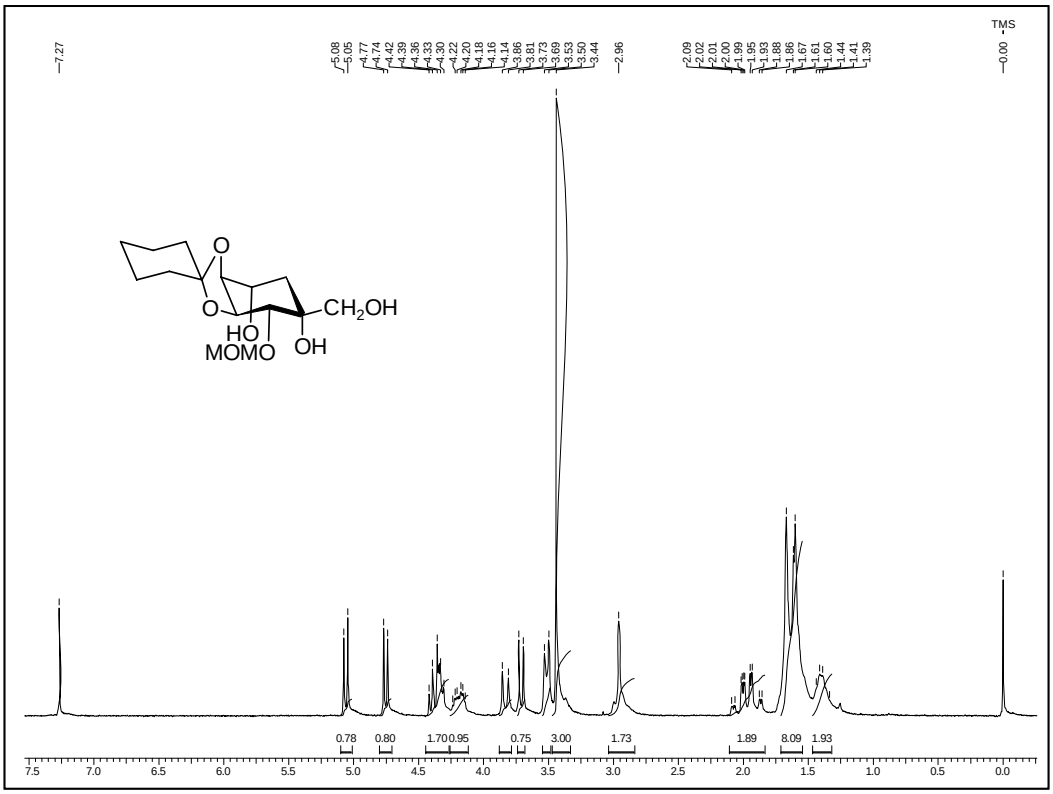
Spectra



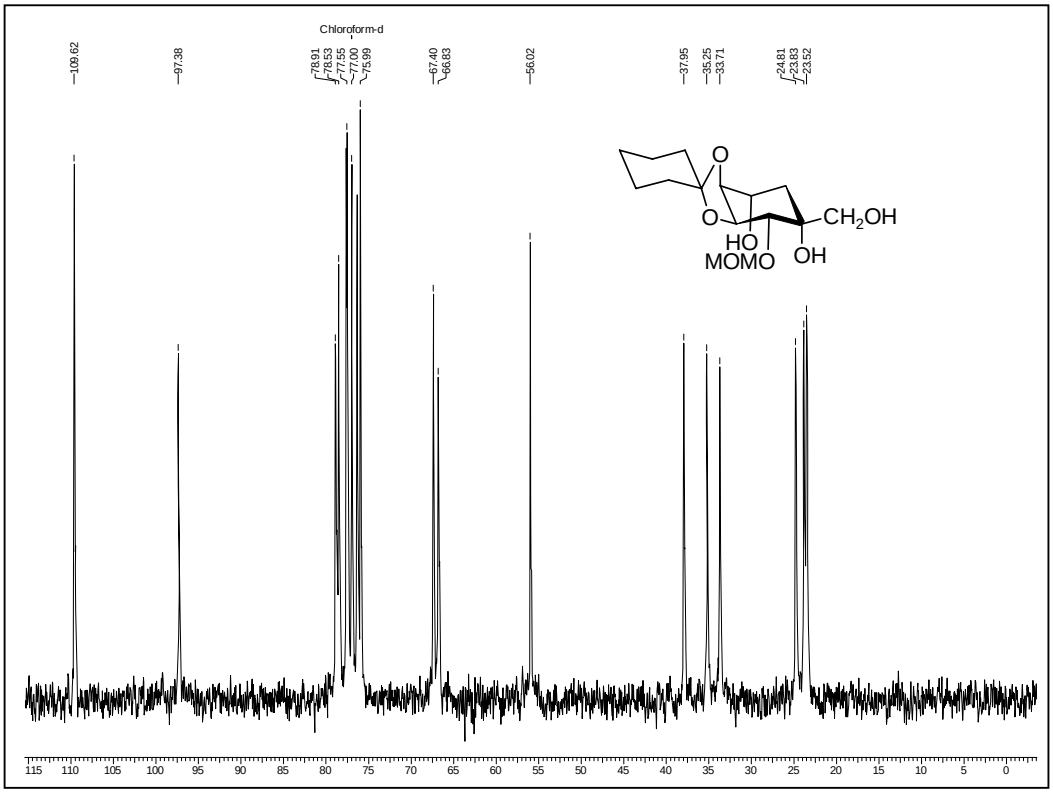
^{1}H NMR Spectrum of **88** in $CDCl_3$



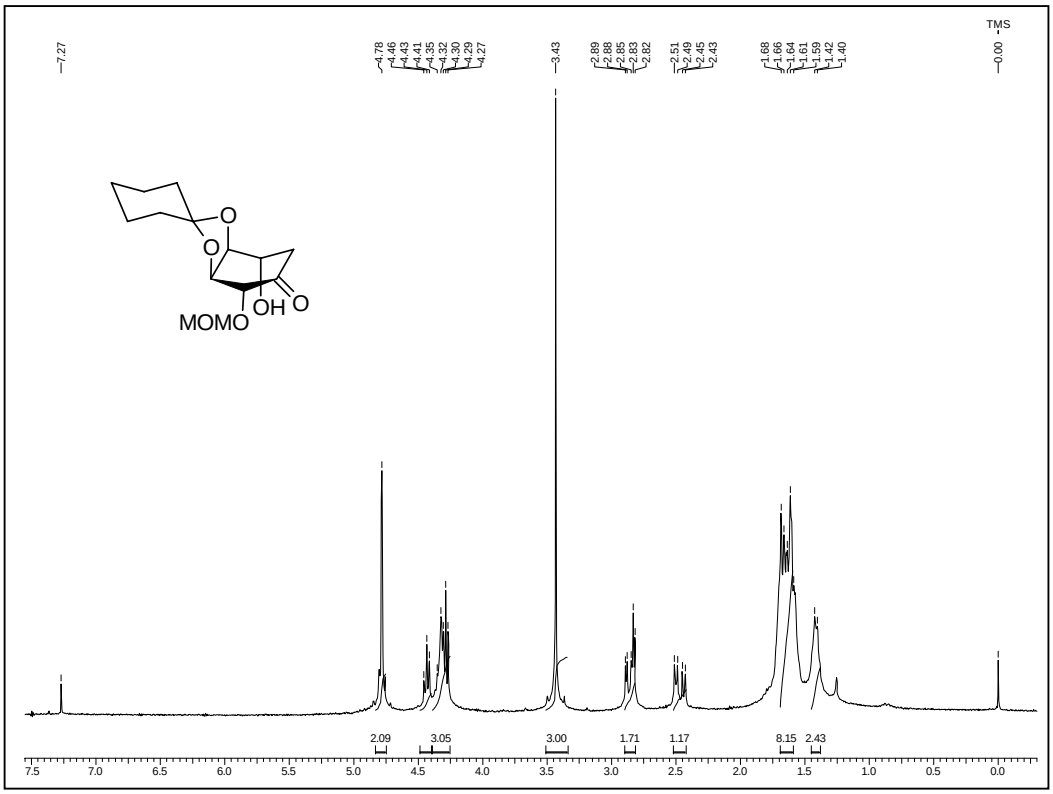
^{13}C NMR Spectrum of **88** in $CDCl_3$



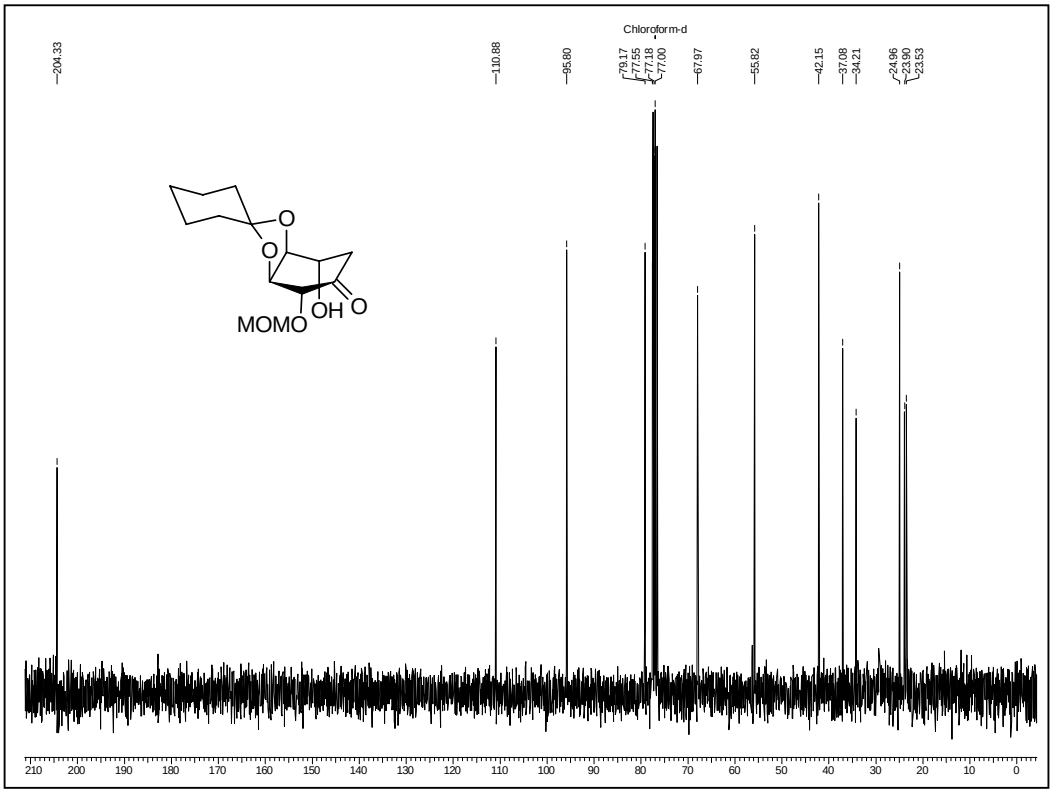
^{1}H NMR Spectrum of **80** in $CDCl_3$



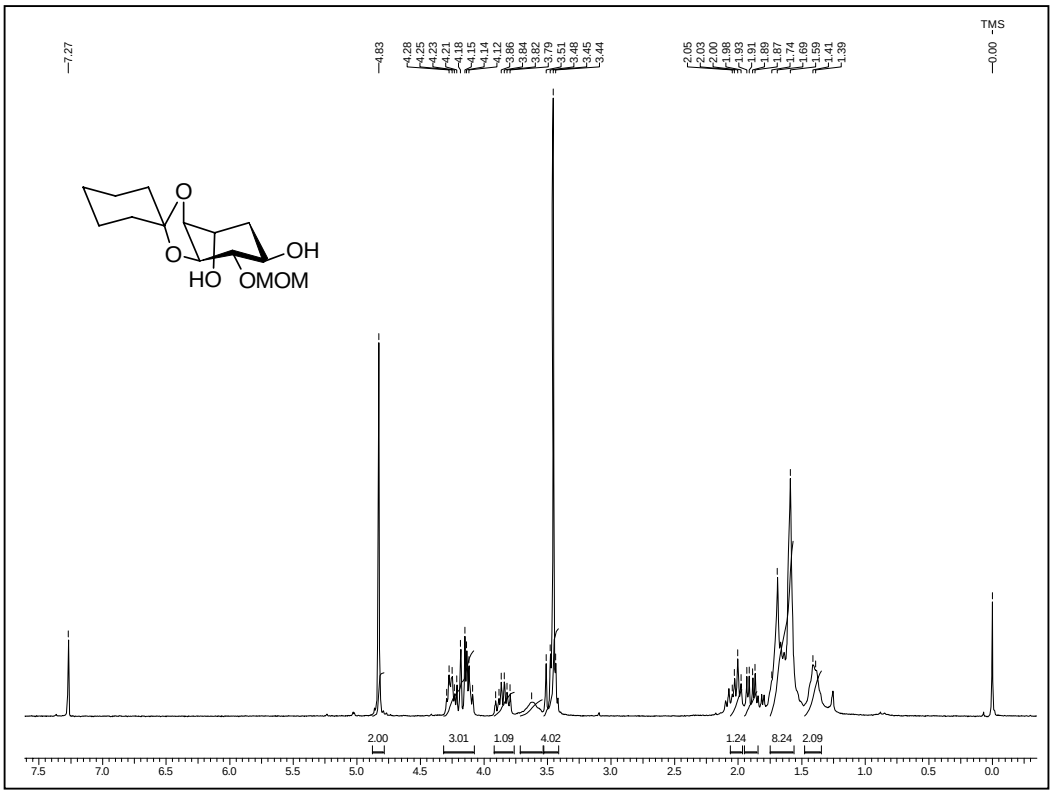
^{13}C NMR Spectrum of **80** in $CDCl_3$



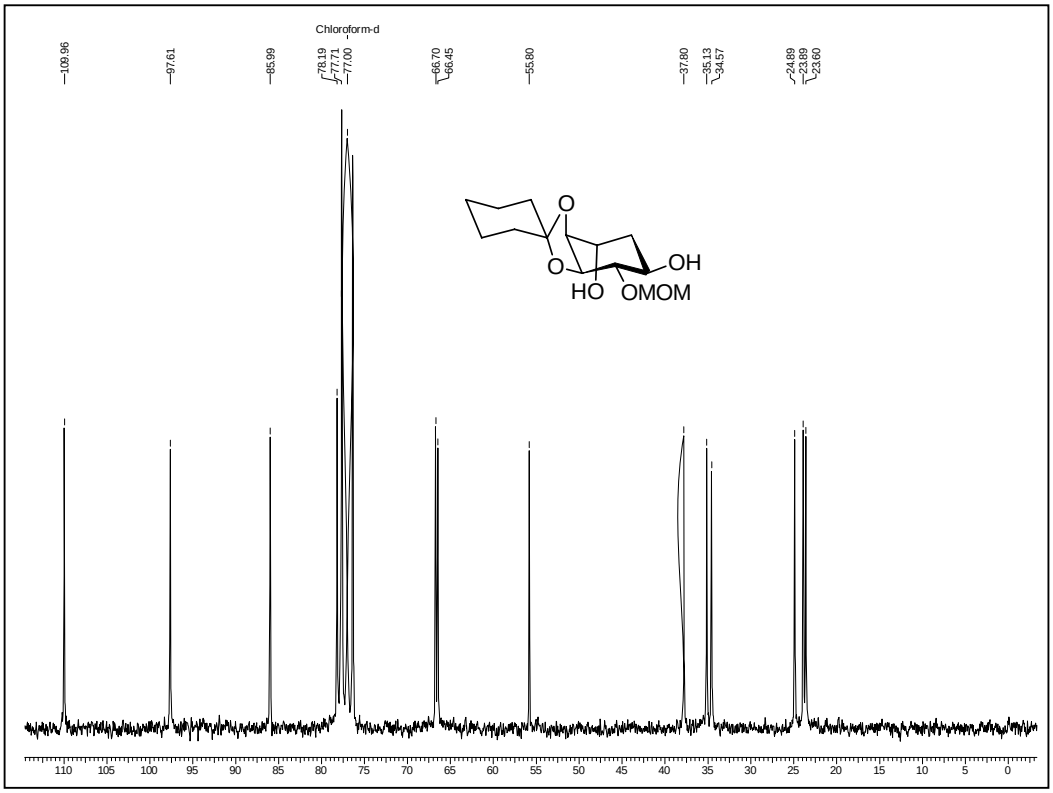
^{1}H NMR Spectrum of **79** in $CDCl_3$



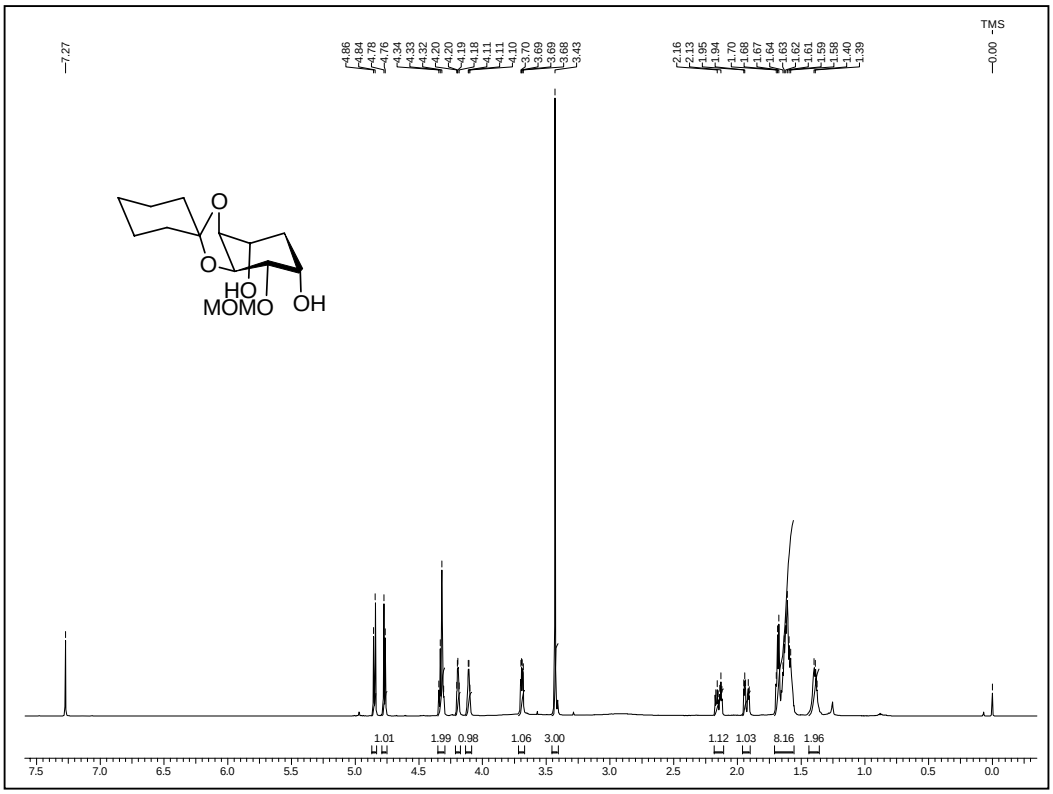
^{13}C NMR Spectrum of **79** in $CDCl_3$



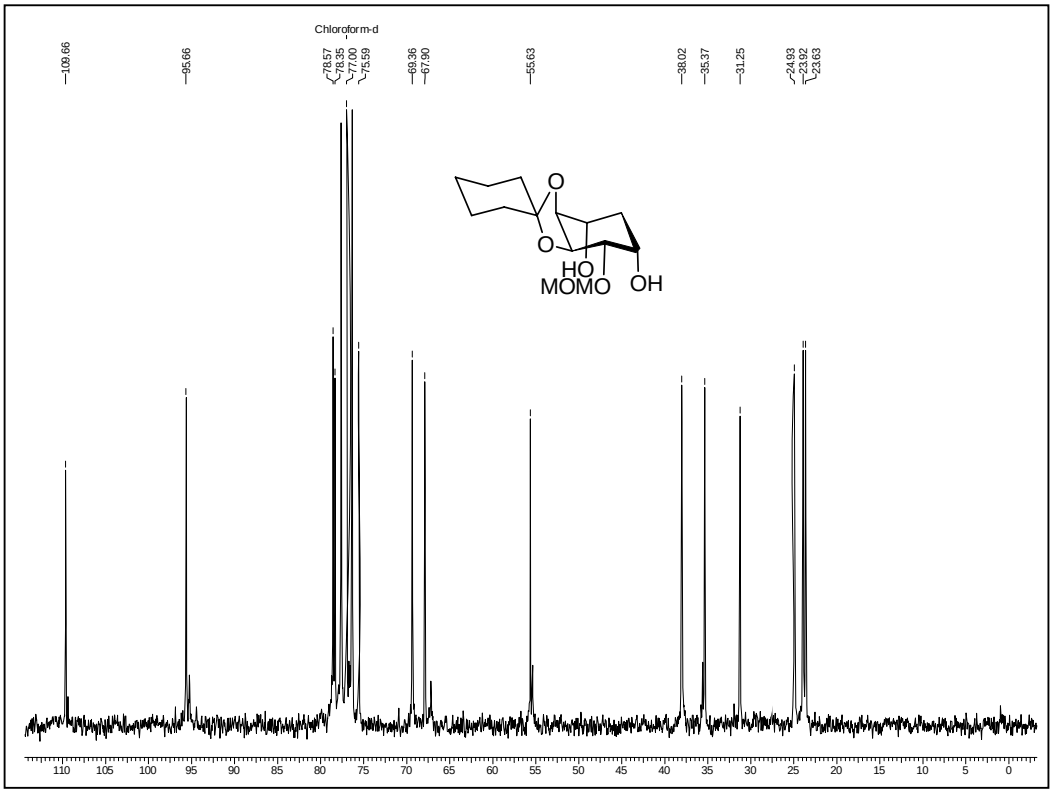
^{1}H NMR Spectrum of **78a** in $CDCl_3$



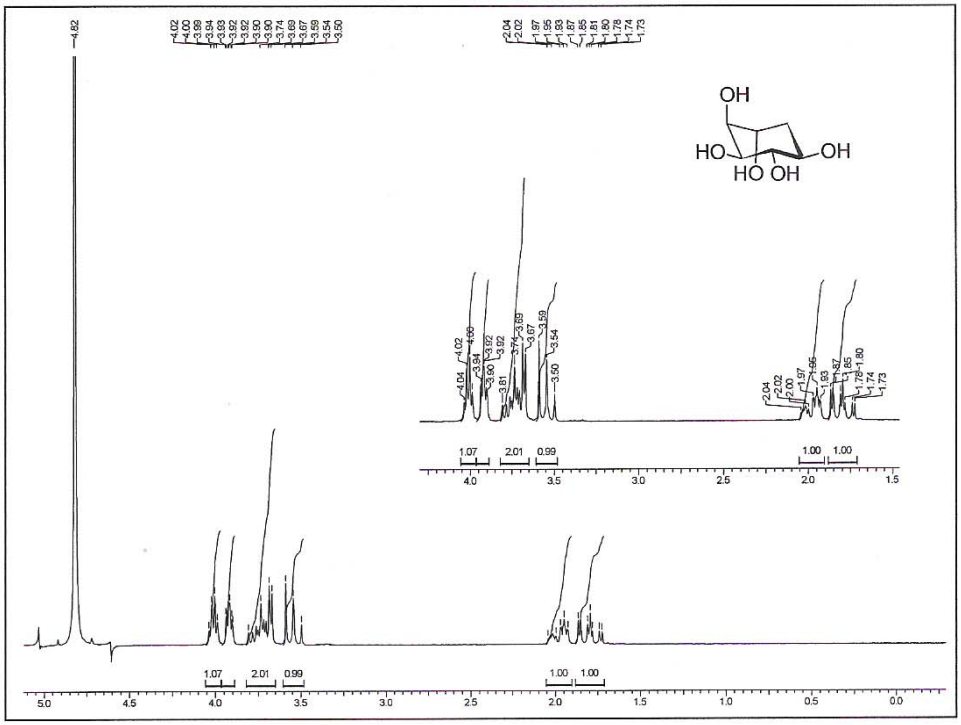
^{13}C NMR Spectrum of **78a** in $CDCl_3$



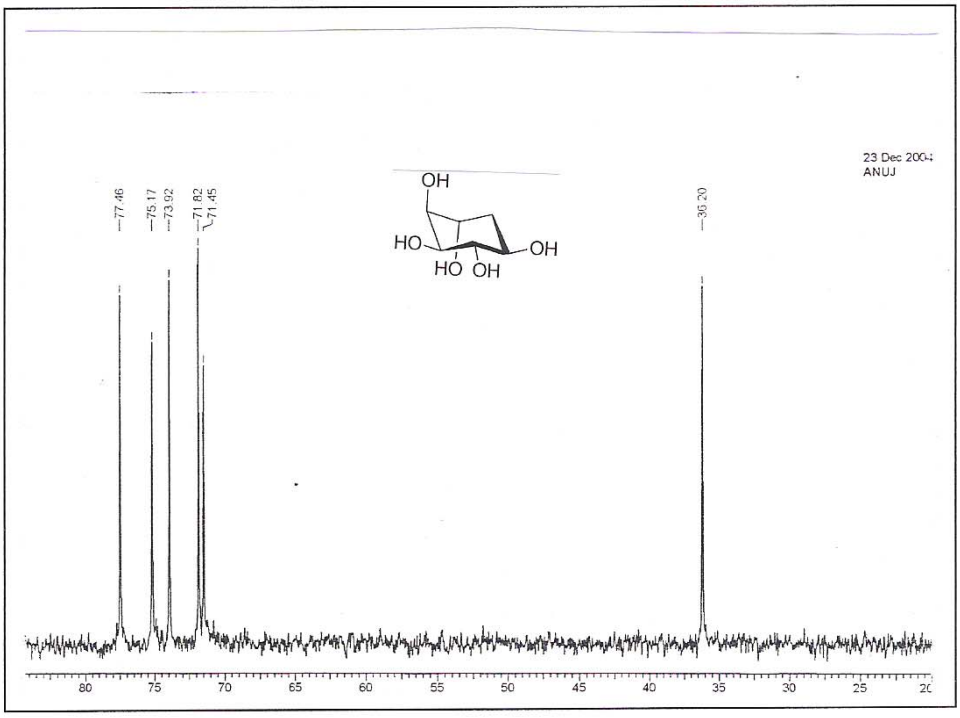
^{1}H NMR Spectrum of **78b** in $CDCl_3$



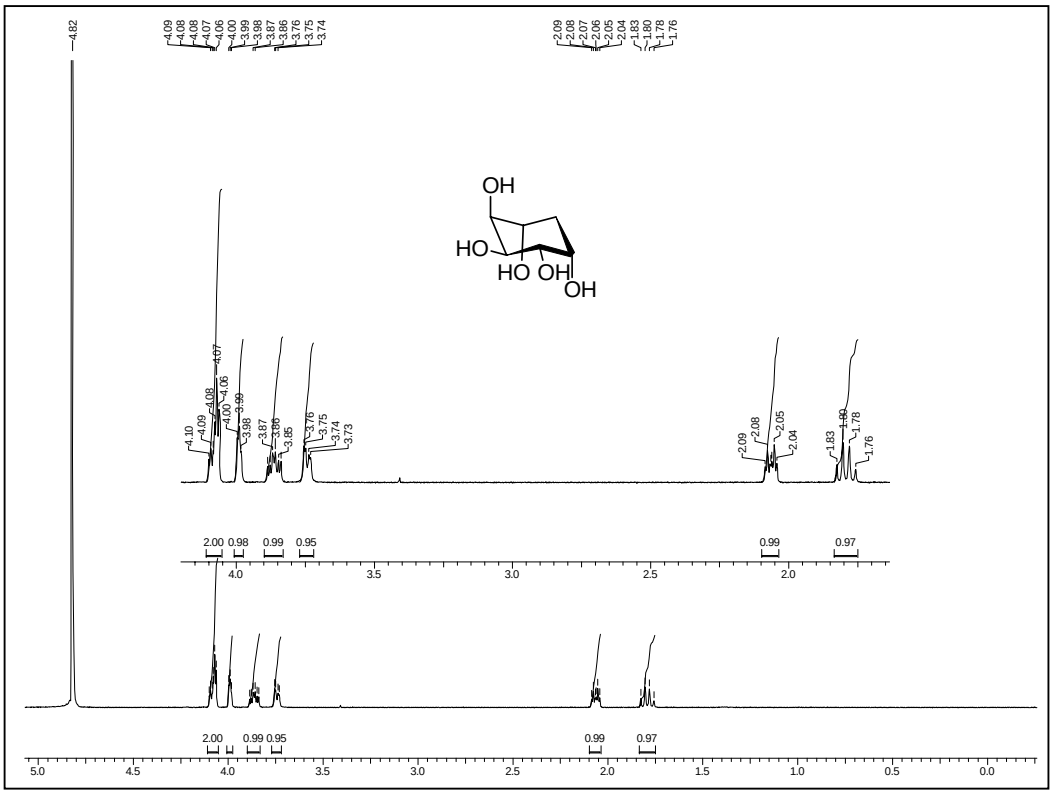
^{13}C NMR Spectrum of **78b** in $CDCl_3$



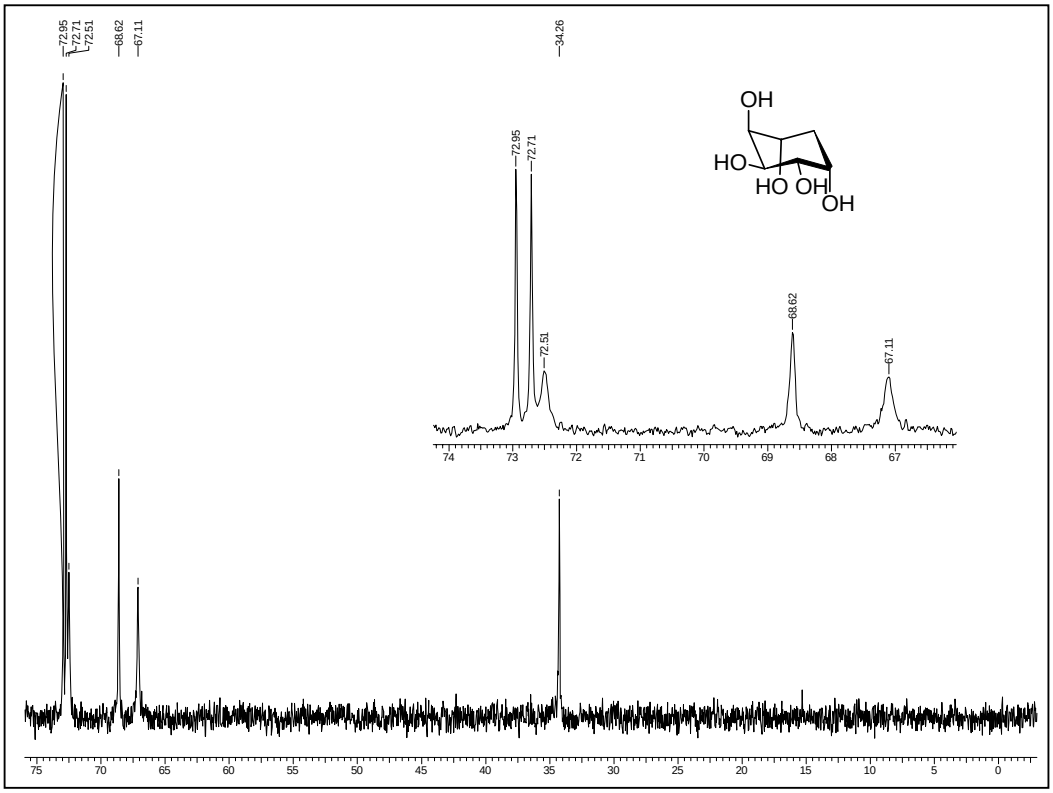
^{1}H NMR Spectrum of **18** in D_2O



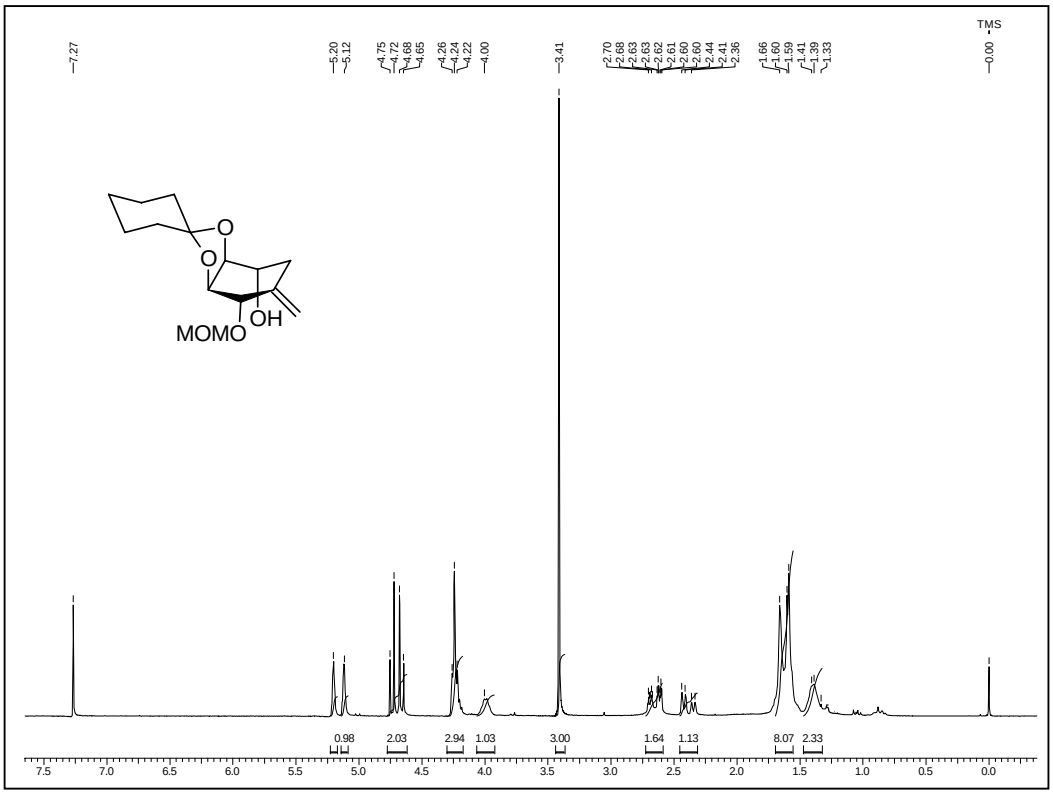
^{13}C NMR Spectrum of **18** in D_2O



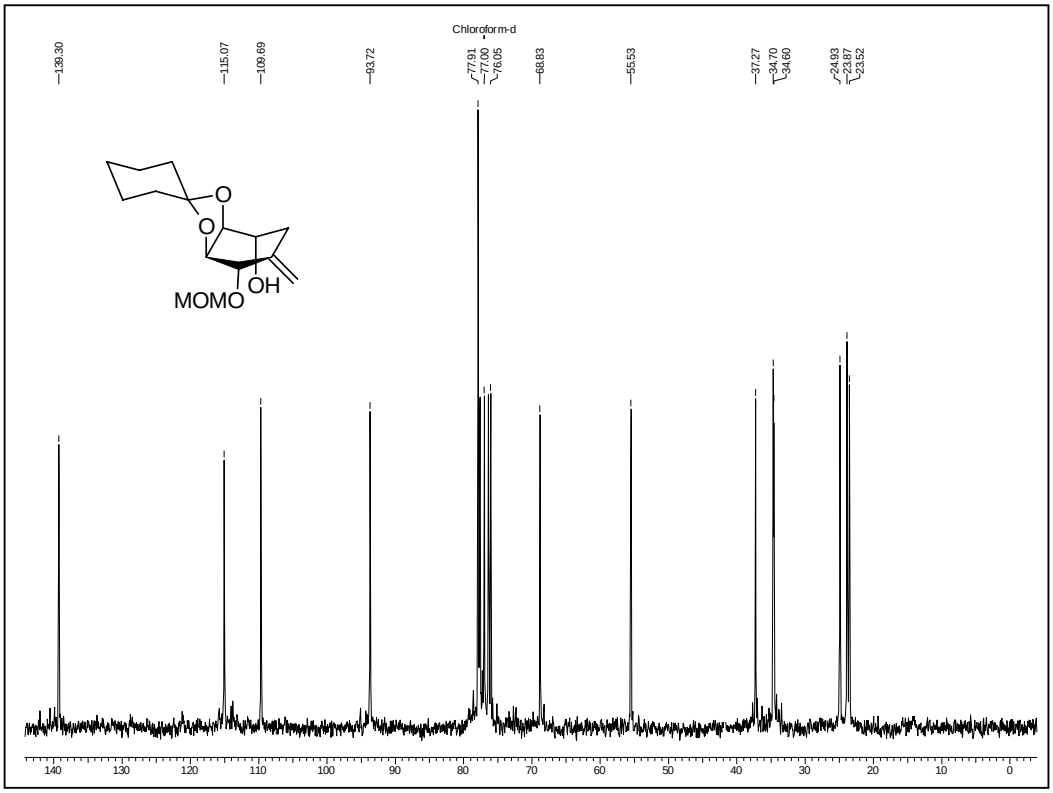
^{1}H NMR Spectrum of **53** in D_2O



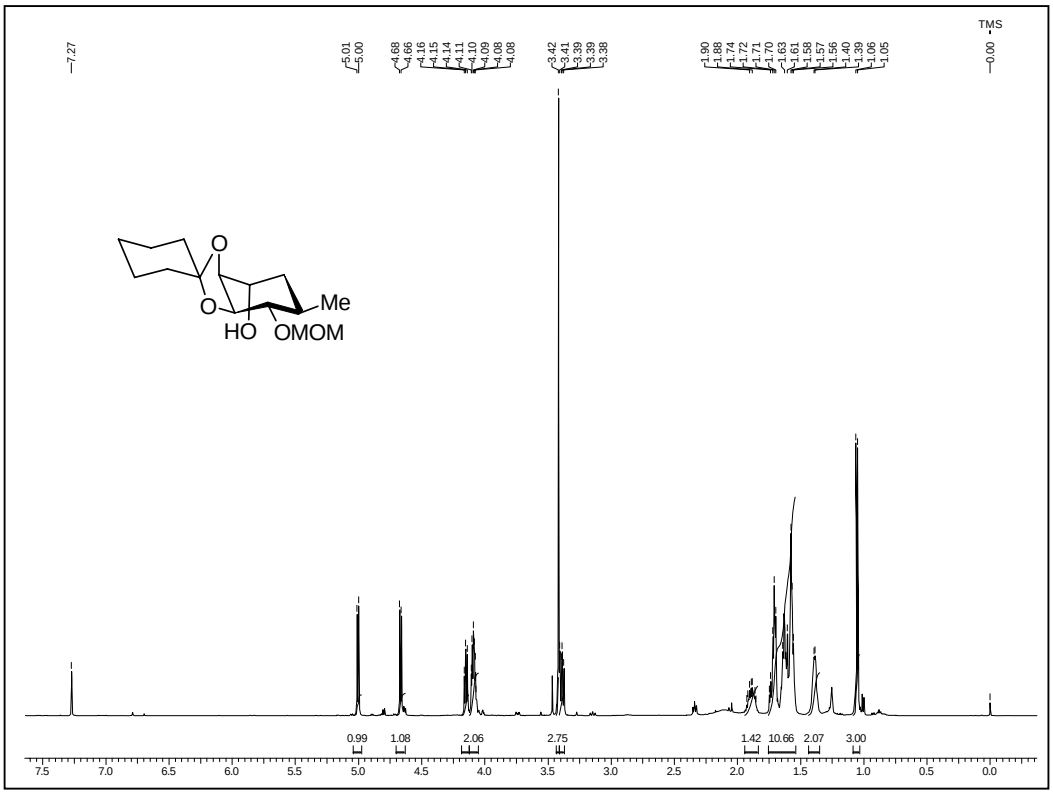
^{13}C NMR Spectrum of **53** in D_2O



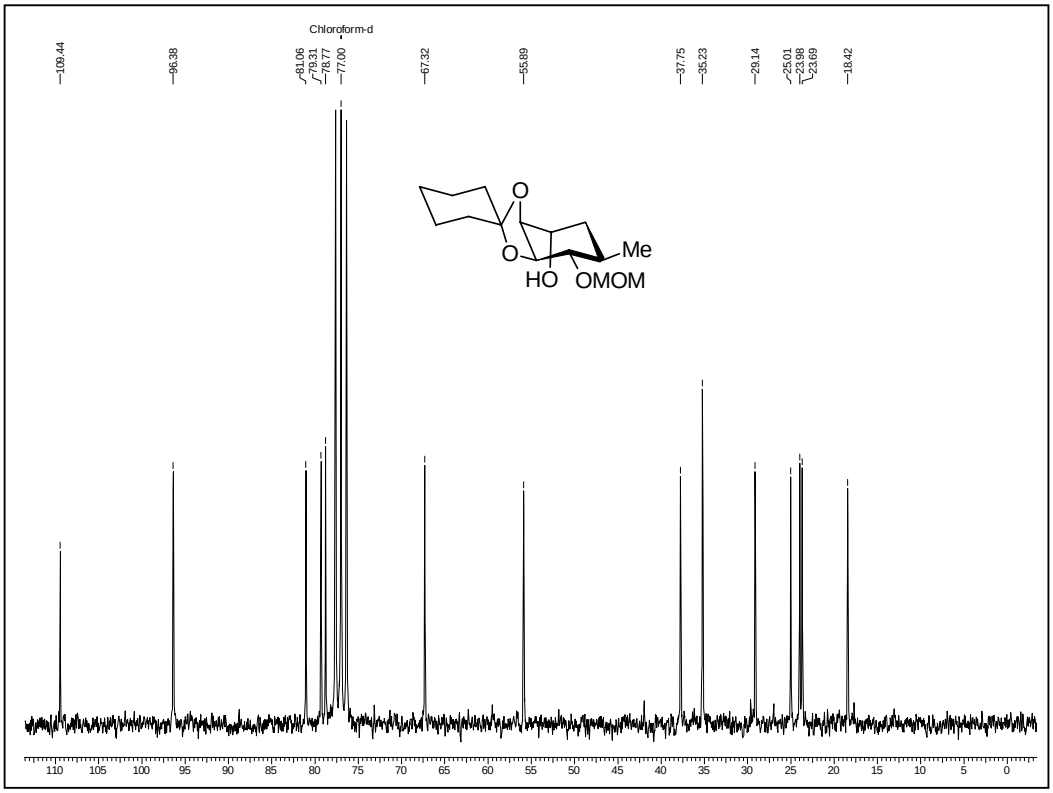
^{1}H NMR Spectrum of **83** in $CDCl_3$



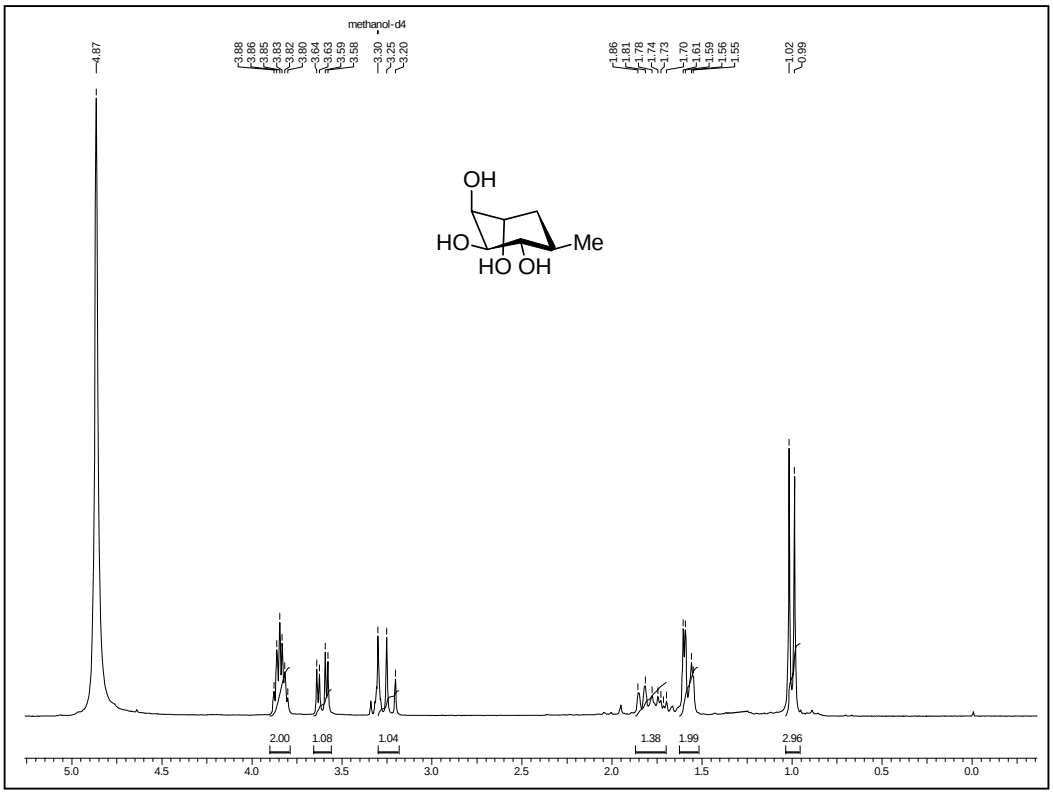
^{13}C NMR Spectrum of **83** in $CDCl_3$



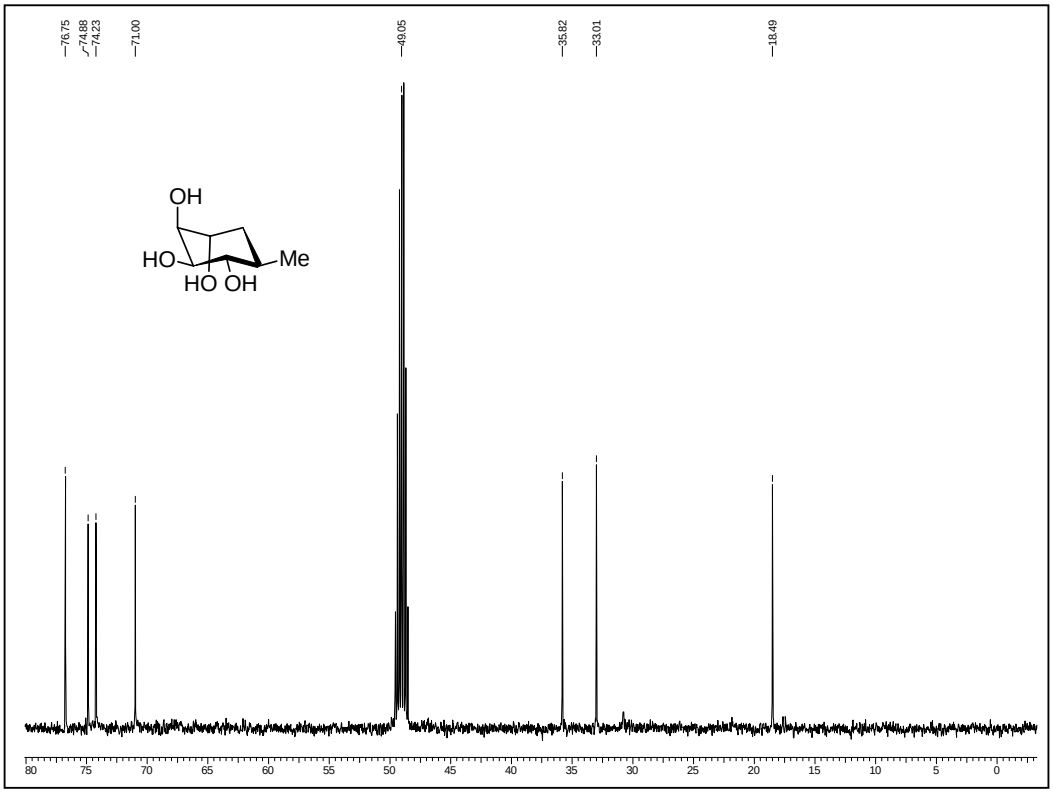
^{1}H NMR Spectrum of **82a** in $CDCl_3$



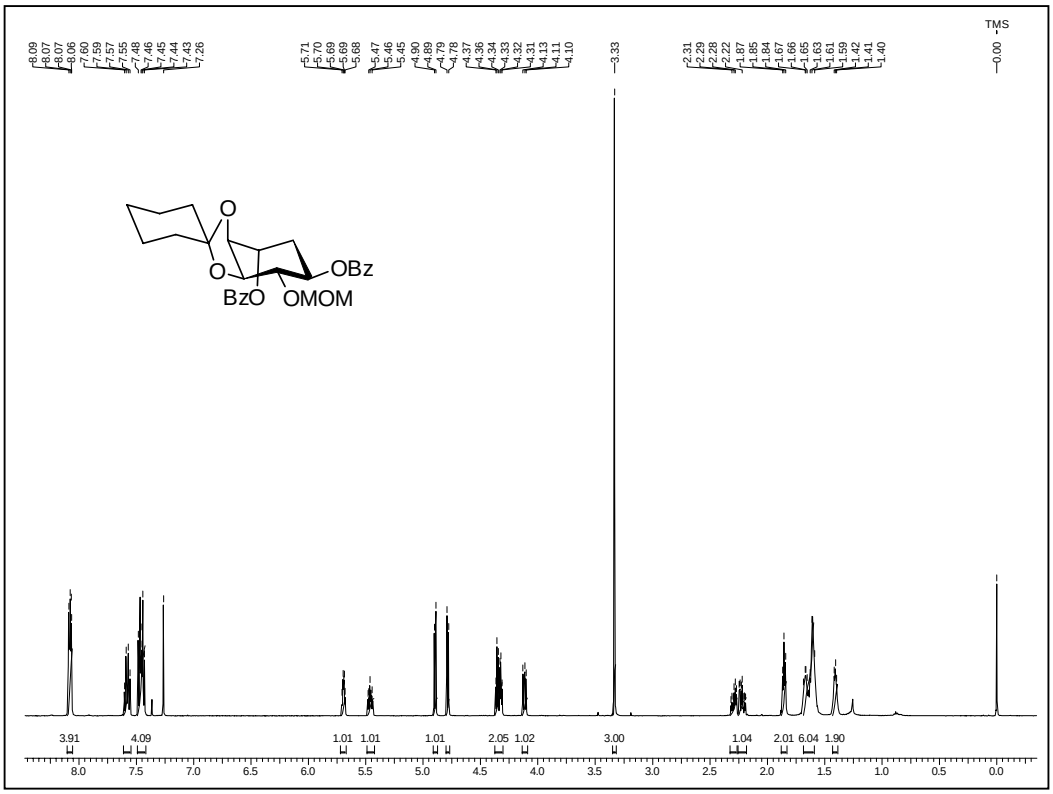
^{13}C NMR Spectrum of **82a** in $CDCl_3$



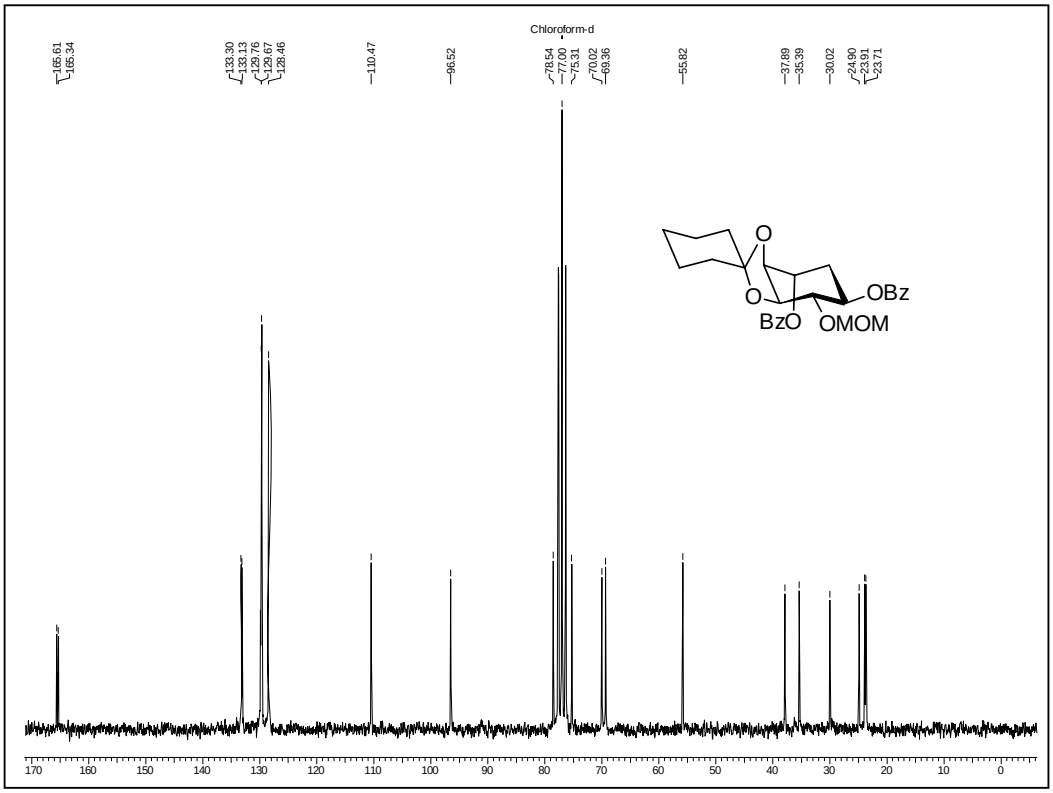
^{1}H NMR Spectrum of **75** in Methanol-d_4



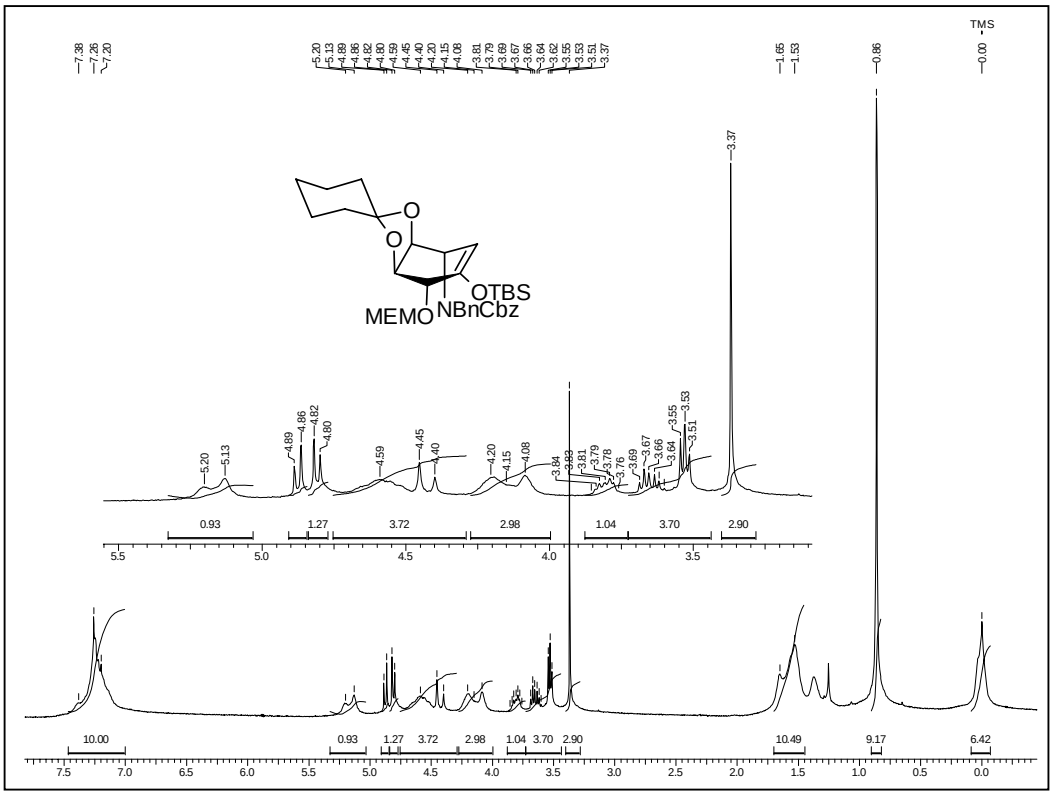
^{13}C NMR Spectrum of **75** in Methanol-d_4



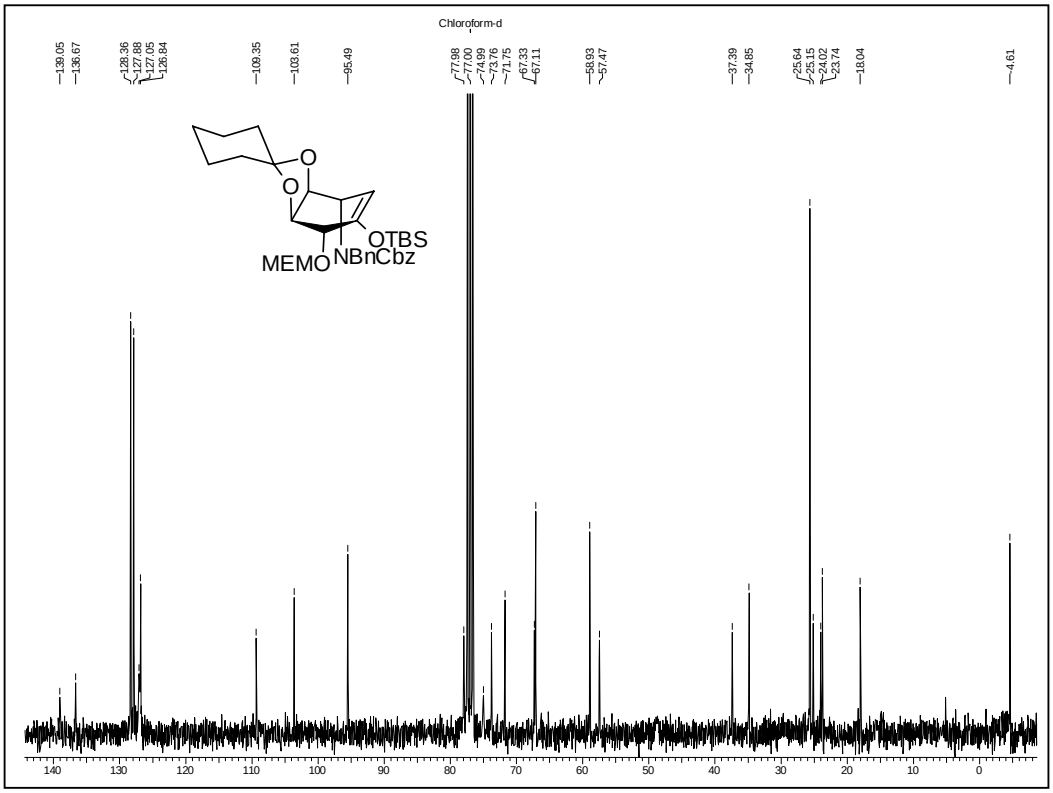
^{1}H NMR Spectrum of **90** in $CDCl_3$



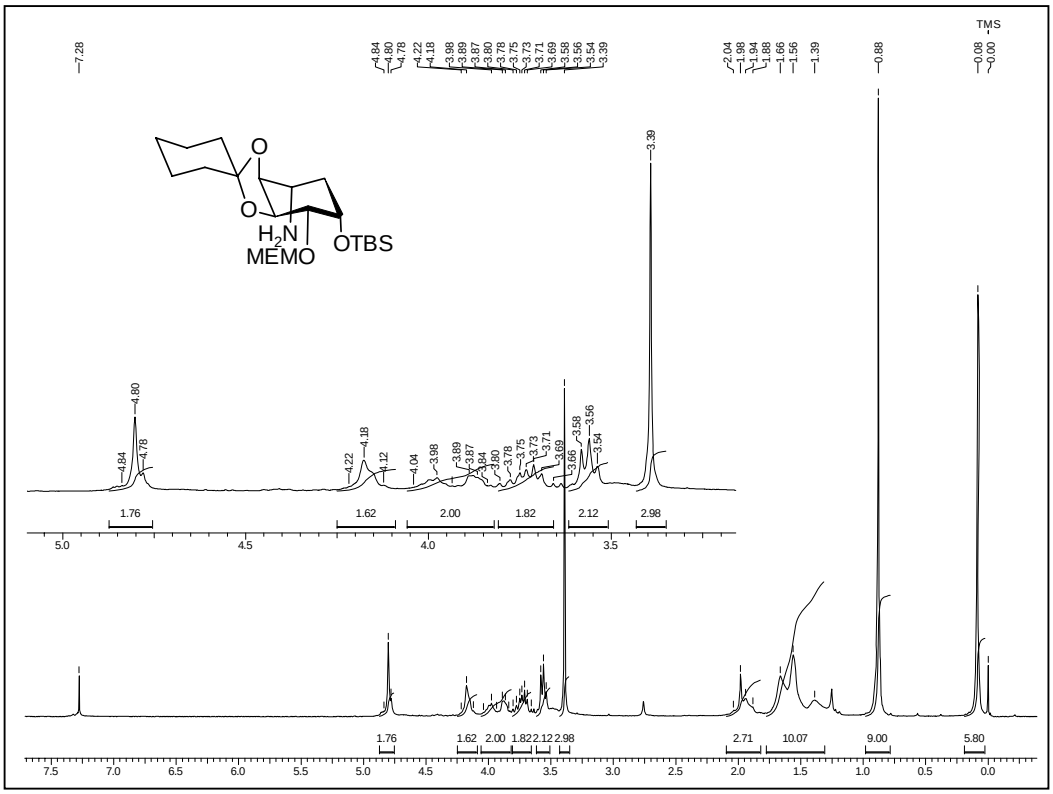
^{13}C NMR Spectrum of **90** in $CDCl_3$



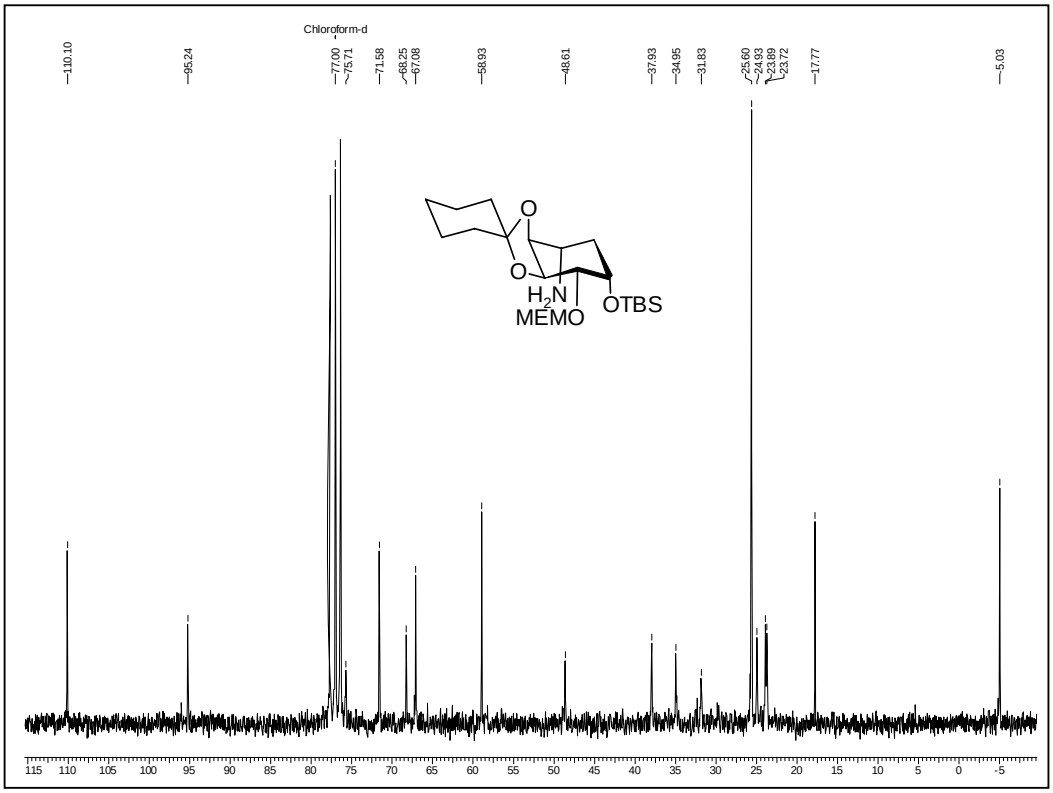
^{1}H NMR Spectrum of **91** in $CDCl_3$



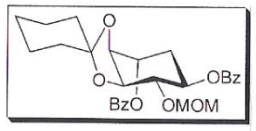
^{13}C NMR Spectrum of **91** in $CDCl_3$



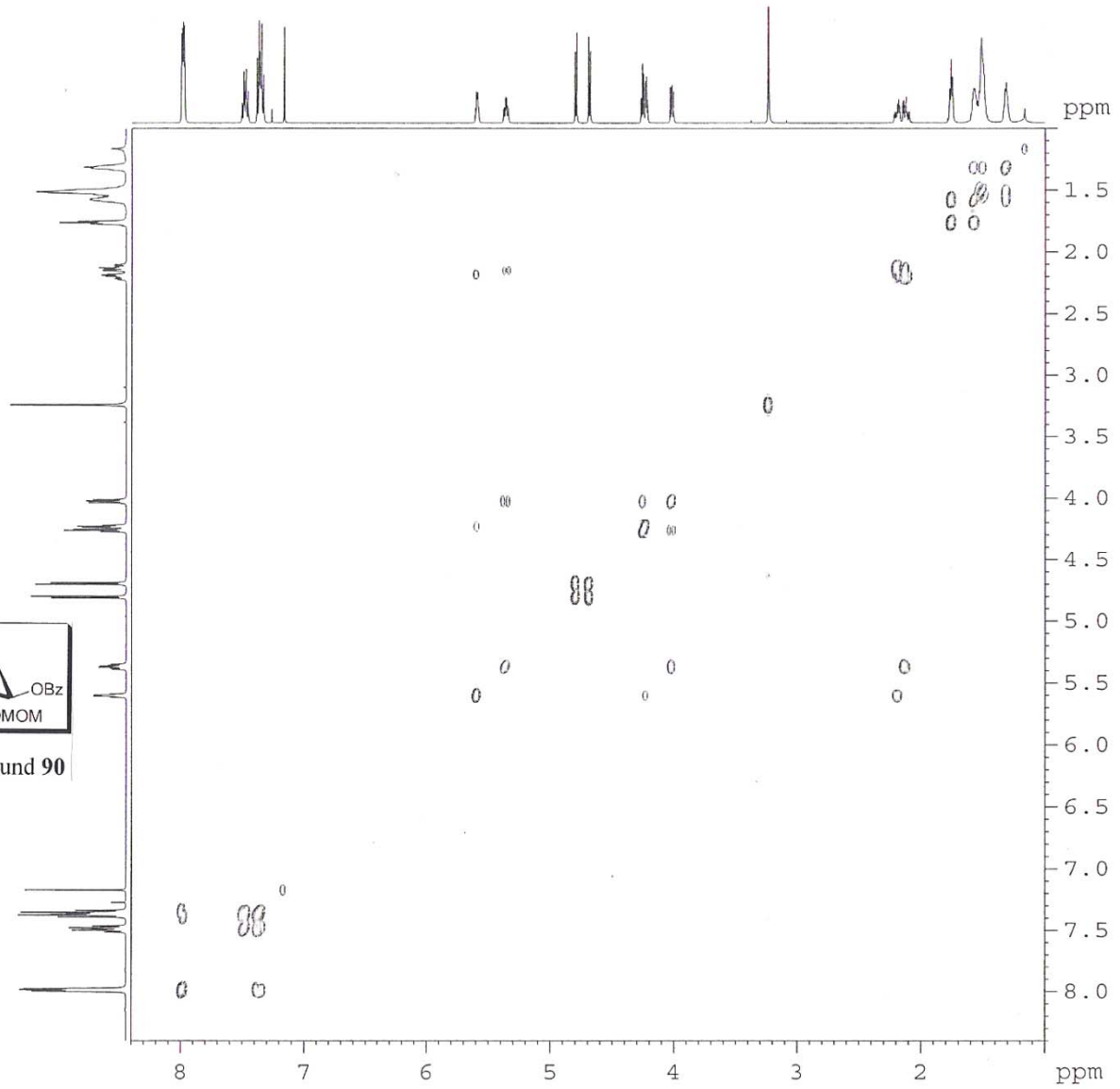
^{1}H NMR Spectrum of **92** in $CDCl_3$



^{13}C NMR Spectrum of **92** in $CDCl_3$



COSY of compound **90**

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PROCNO                 1

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PULPROG         cosyqf45
TD                1024
SOLVENT           CDCl3
NS                  16
DS                  16
SWH           4084.967 Hz
FIDRES        3.989226 Hz
AQ            0.1255100 sec
RG                256
DW              122.400 usec
DE                6.00 usec
TE               666.5 K
d0            0.00000300 sec
D1            2.00000000 sec
IN0           0.00024480 sec
MCREST        0.00000000 sec
MCWRK         2.00000000 sec

======== CHANNEL f1 ========
NUC1                1H
P1                9.60 usec
PL1              -2.00 dB
SFO1        500.1323730 MHz

F1 - Acquisition parameters
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TD                226
SFO1        500.1324 MHz
FIDRES     18.075077 Hz
SW            8.168 ppm
FnMODE           QF

F2 - Processing parameters
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SSB                 0
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GB                  0
PC               1.00

F1 - Processing parameters
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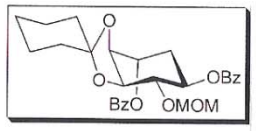
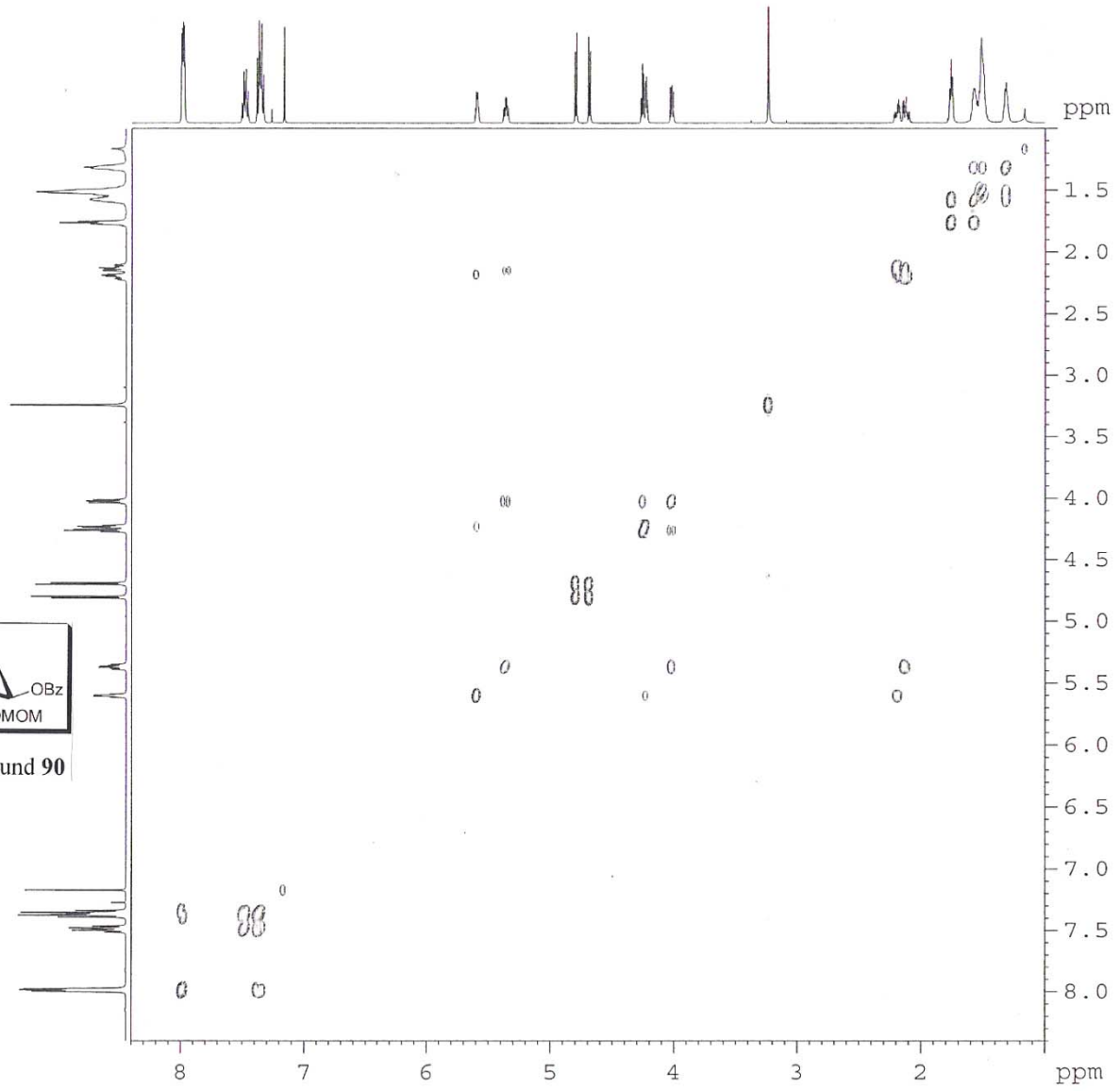


COSY of compound **90**



Anuj; NBH-40-MBZ;COSY 45

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PROCNO                1

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TD                1024
SOLVENT           CDCl3
NS                  16
DS                  16
SWH           4084.967 Hz
FIDRES        3.989226 Hz
AQ            0.1255100 sec
RG                  256
DW             122.400 usec
DE               6.00 usec
TE              666.5 K
d0            0.00000300 sec
D1            2.00000000 sec
IN0           0.00024480 sec
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MCWRK         2.00000000 sec

======== CHANNEL f1 ========
NUC1                1H
P1               9.60 usec
PL1             -2.00 dB
SFO1       500.1323730 MHz

F1 - Acquisition parameters
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FIDRES    18.075077 Hz
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FnMODE           QF

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LB               0.00 Hz
GB                  0
PC               1.00

F1 - Processing parameters
SI                1024
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SF         500.1300570 MHz
WDW             QSINE
SSB                 0
LB               0.00 Hz
GB                  0
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References

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Chapter III

Chapter III, Section I:

Development of a New Process for Artificial Sweetener Sucralose.

Introduction

Large and complex molecules (biopolymers) are vital for life processes in higher living organisms. Amongst the four most important classes of natural biomolecules[1] i.e. carbohydrates, proteins, nucleic acids and lipids; carbohydrates are the most abundant and participate in a wide range of processes. Their hydrophilic nature and structural complexities make them difficult to study. As the name suggests, carbohydrates are hydrates of carbon with the general formula $C_x(H_2O)_y$ except for few compounds which do not follow this general rule and few in which this formula is applicable but they are not carbohydrates. Starch and glycogen are used for the storage and transport of energy; whereas cellulose and chitin find utility as structural components. Moreover, carbohydrates and their derivatives are also responsible for the working processes of the immune systems, fertilization, blood clotting, pathogenesis, etc.

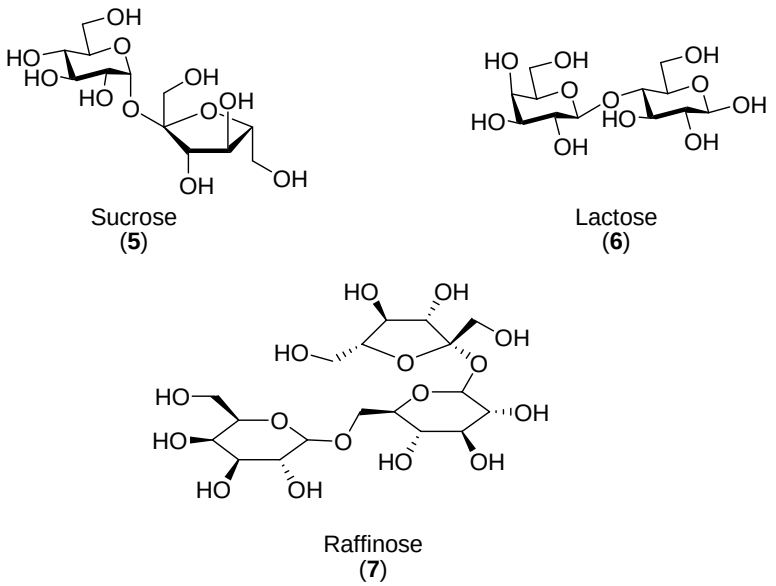
Figure 1. *Representative examples of monosaccharides*

β-Ribose (**1**) β-D-Glucopyranoside (**2**) β-D-Galactopyranoside (**3**) D-Fructose (**4**)

Carbohydrates can be classified as monosaccharides, oligosaccharides and polysaccharides. The simplest monosaccharides cannot be hydrolyzed into smaller carbohydrates and are represented by furanose or pyranose (5 or 6 membered ring) forms e.g. ribose **1**, glucose **2**, etc. (Figure 1). They are used as energy source (via metabolism) in biosynthesis. Oligosaccharides (Figure 2) generally contain 2 to 9 monosaccharides linked by glycosidic bonds formed by a loss of water molecules. Sucrose **5** is a disaccharide constituting of D-glucose **2** and D-fructose **4** units; similarly lactose **6** (obtained from milk) is a disaccharide consisting of D-galactose **3** and D-glucose **2**. Raffinose **7** is a trisaccharide. Polysaccharides, consisting of ten or more monosaccharides linked by glycosidic bonds are useful in structure and storage

related issues in living organisms. Cellulose is an example of structural polysaccharide used in plants whereas chitin is used in animals.

Figure 2. *Few disaccharides and trisaccharide*



Sucrose

It is the most important chemical obtained from natural sources in very high purity, abundantly available and cheap. It is a disaccharide adorned with eight hydroxyl groups with varying reactivities. Besides a sweetening agent,[2] it finds utility in preparation of fine chemicals, pharmaceuticals, polymers, building and structural materials, fermentation or enzyme substrates for chemicals, new food products and sweeteners, cogeneration/fuel/biodiesel/ethanol.

Use in Food Products

(a) Olestra is sucrose based fat substitute made by reacting sucrose with fatty acids to produce a liquid sucrose polyester. It has similar properties to liquid vegetable oil but is not imbued with calories.

(b) Fructo-oligosaccharides, made by fermentation or enzymatic transformation of sucrose are in between food additives and nutraceuticals. They are good for

abdominal health because they promote the growth of beneficial *Bifidobacteria* in the gut. They have also been used to control swine odour and protecting them from *E. coli* infection.

Use in pharmaceuticals

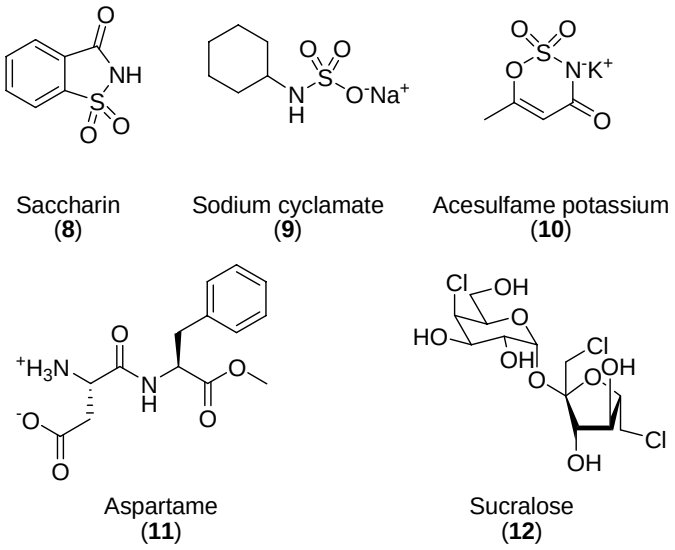
(a) sucralfate is a sucrose aluminium hydroxide sulphate complex used as an ulcer medication for humans and animals. It acts like an ulcer bandage without being absorbed by the body.
(b) Polysucrose is a copolymer of sucrose and epichlorohydrin that is used in making density gradients for cell separation and as diagnostic.
(c) Specialty sucrose esters:[3] The eight hydroxyl groups of sucrose can react with many fatty acid groups to give different sucrose esters with many food and non-food uses like surfactants and emulsifiers. They are found to be biodegradable, non-toxic and mild to the skin leading to utility in pharmaceuticals, cosmetics, detergents and food. Sucrose acetate isobutyrate (SAIB) is used in automotive paints, nail polishes, hair sprays and as a clouding and stabilizing agent in beverages.
(d) Sucrose epoxy:[4] A large number of epoxy allyl sucroses have been discovered which do not show mutagenic or cytotoxic properties like petrochemical derived diepoxides.
(e) Sucrogels:[5,6] The properties of these "sucrose hydrogels" can be altered, over wide range by adjusting the crosslink ratios and initial monomer concentrations. They have very unique properties like very high porosity and fast swelling characteristics which make them amenable for use in controlled release drug delivery.
(f) Biodegradable plastics: Some bacteria produce such storage polymers within their cells: the technology has not yet advanced and they are expensive. The current goal is to engineer bacteria designed to use sucrose and molasses as carbon source to make polyhydroxyalkanoates (PHAs) and other polymers.[7]

Sucralose[1,8]

Constantly changing life styles and food habits necessitate the use of artificial sweeteners that is widely acceptable and has low calorific value, high tolerance level, are suitable for diabetics and are non-cariogenic. Currently, artificial sweeteners like

saccharin **8**, sodium cyclamate **9**, acesulfame-K **10**, aspartame **11**, sucralose **12** etc. (Figure 3) are available in the market.

Figure 3. *Artificial sweeteners*



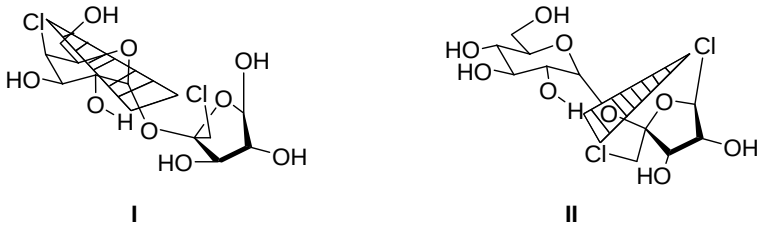
Amongst them, sucralose **12** with sweetness 600 times more than sucrose, no unpleasant aftertaste, no discernible side effects has been found to be very safe. Structurally, it is similar to sucrose **5** i.e. a disaccharide, in which 4-chloro galactose 1α unit is linked to 2β of 1′,6′-dichloro-1′,6′-dideoxyfructose by a glycosidic bond. Though it is derived from carbohydrates, the human body does not recognise it as such and hence it is not metabolized. Therefore, it does not provide any calories. It has also been found to be stable under a broad range of pH and temperature conditions. So, it has been widely used in processed foods by food industries all over the world.

Theory of Sweetness[9]

The sensory responses to sweet substances are related to the chemical structures as a slight modification may lead to complete loss of sweetness or a development of bitterness. For example D-glucose is sweet but L-glucose has a slightly salty taste. Thus, considering these characteristics Shallenberger and Acree[10] proposed AH, B theory of hydrogen bonding of the sweet substances to the reception

site. They proposed that certain hydroxyl groups in polyols act as a hydrogen bond donor (AH) and other as hydrogen bond receptor (B) with a distance of separation amongst the proton of (AH) and electronegative group (B) to be 3Å, and the presence of similar groups on receptor site also help in bonding to take place and thus initiating a sweet response. Later Kier[11] suggested for the presence of a third feature i.e. a hydrophobic site (X) located 3.5 Å from A of (AH) and 5.5 Å from B. Thus, when this theory was applied in case of chloro derivatives of sucrose it was observed that 1′-, 4- and 6′- monochlorinated sucroses are 20, 5 and 20 times sweeter than sucrose whereas the 6-chloro-6-deoxy sucrose is bitter. Moreover, the dichloride and trichloride at the above mentioned positions exhibited high sweetness except the one at 6-position was substituted (substitution at 6-position reduces the sweetness drastically in case of di- or trichlorides also).

Figure 4. *Representative examples for Kier triangle formation*



In case of 1′-chloro substituent the chloro group act as acceptor whereas the equatorial 2-hydroxyl as hydrogen bond donor (Fig 4). An axial lipophilic group (H or Cl) at C-4 is the third site (X) (**I**). Similarly, for the 6′-chloro substituted derivative like 1′,6′-dichloro-1′,6′-dideoxy sucrose the 6′-chloro group function as a lipophilic centre (X) in the Kier triangle (**II**). The adverse effect of 6-chloro substituent was assumed to be due to the competition of the axial group at C-4 (X) and thus preventing 2-hydroxyl group to act as the corresponding donor.

Previous Syntheses

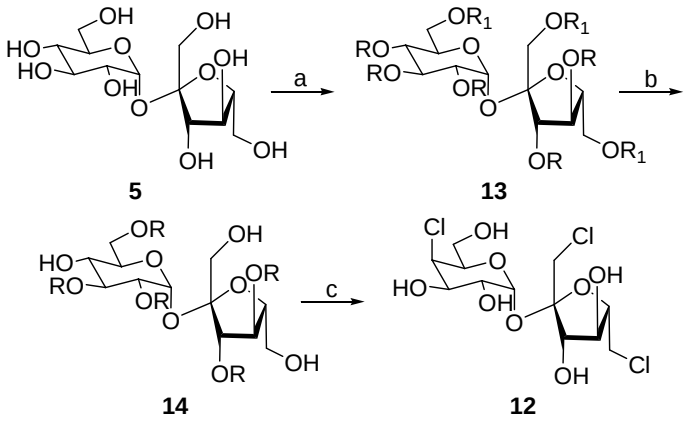
Since the discovery of sucralose **12** in 1976 and the demonstration of its sweetness (600 times sweeter than sucrose), many new patented processes and

techniques for its syntheses and purifications have been developed by various groups. Some of the methods are discussed below:

(i) Methods involving selective 6-position protection of sucrose:

(a) A five step process starting from sucrose having 4,6 acetyl migration[12] as the key step (Scheme 1):

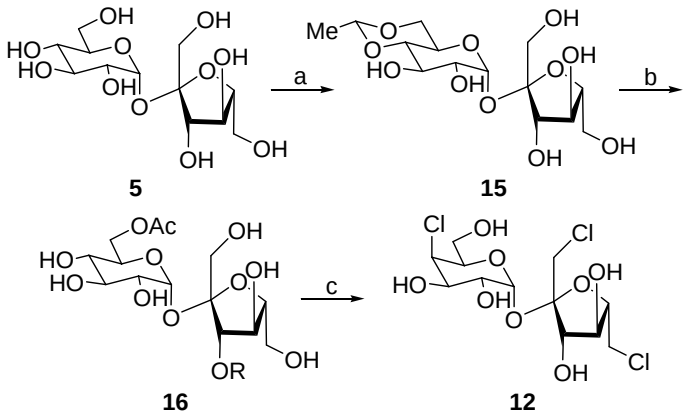
Scheme 1



Reagents and Conditions: (a) (i) Tritylchloride, Py, 48 h, r.t., 58%; (ii) Ac_2O, Py, r.t.; (b) Boiling aq. AcOH; (c) (i) SO_2Cl_2, Py:$CHCl_3$ (2:1); (ii) NaOMe, MeOH.

(b) The key step in this method is the conversion of sucrose methyl 4,6-orthoacetate[13] **15** to sucrose-6-acetate **16** (Scheme 2):

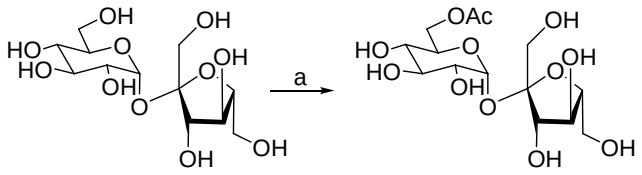
Scheme 2



Reagents and Conditions: (a) Trimethyl orthoacetate, DMF, PTSA, r.t.; (b) H_2O, pH 5, 2 h then Py, 4 h; (c) (i) $SOCl_2$, TPPO, Py, 95 ^oC; (ii) Ac_2O, Py; (iii) NaOMe, MeOH.

(c) Tin compounds[14] like, dioctyltinoxide, distannoxane diacetate, etc. are used to convert sucrose **5** to sucrose-6-acetate **16** (Scheme 3):

Scheme 3



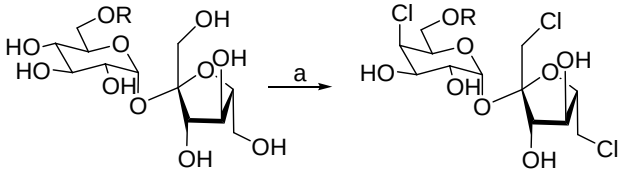
5 **16**

Reagents and Conditions: (a) Dioctyltin oxide, DMF, isooctane, refluxed then cooled and acetic anhydride in DMF added.

(d) An electrolysis method[15] was developed for the preparation of sucrose 6-acetate (anode chamber: DMF, distilled H_2O, sucrose, acetaldehyde, KBr and Et_4NBF_4; cathode chamber: 10% aq. NaCl solution) which was then chlorinated using SO_2Cl_2 to give sucralose 6-acetate.

(ii) Effecting chlorination:[16] Chloromethylene dimethyliminium chloride (Vilsmeier reagent) is used as the chlorinating agent (R = suitable protecting group) (Scheme 4):

Scheme 4

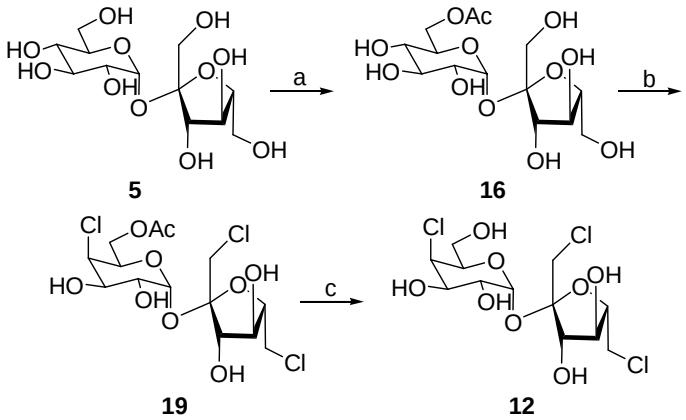


17 **18**

Reagents and Conditions: (a) $COCl_2$, DMF, heating 75-100 ^oC.

(iii) A recent short synthesis[17] involves selective esterification of the 6-position of sucrose **5**, chlorination of the 4,1′,6′-positions and finally deacetylation to furnish sucralose **12** (Scheme5):

Scheme 5



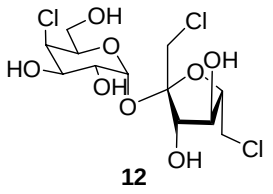
Reagents and Conditions: (a) Azo dicarboxylic diethylmaleate, AcOH, DMF, r.t., 5 h; (b) PCl_5, DMF, Cl_3CCN, 85 ^oC; (c) KOH, MeOH, 45 ^oC.

We were interested in developing a technically simple and high yielding synthesis of sucralose by exploiting the differences in reactivities of the hydroxyl groups for selective chlorination reactions.

Present Work

Modern and sometimes sedentary life styles, food habits (particularly the propensity to consume fast foods), and a variety of causative factors have led to increased cardiovascular risks and increasing patient populations with clinical obesity and diabetes. A search for low calorie sweeteners resulted in the widespread use of sucralose **12** (Figure 5); a synthetic compound i.e. 600 times sweeter than sucrose and is not systemically absorbed into the body resulting in low calorific value. It is stable over a wide range of pH and temperature conditions and widely used in the food industry worldwide. Chemically named as 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside, its structure and commercial importance has attracted attention of a large number of chemists interested in developing shorter and efficient routes for commercial scale synthesis.

Figure 5. *Structure of Sucralose*



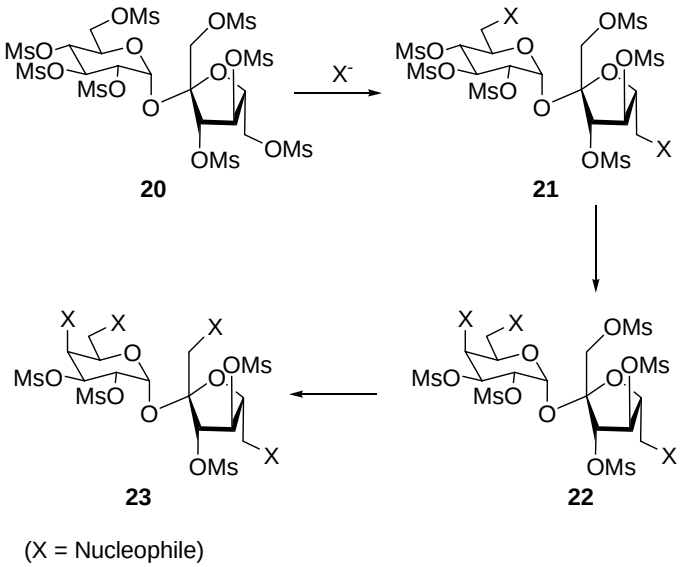
12

Several syntheses have been reported but in all of them the hydroxyl groups were completely protected at some stages. In our synthetic endeavours, only the three primary hydroxyl groups were protected, thereby facilitating the synthesis by avoiding the complete protection – deprotection steps.

There is a distinct difference in the reactivities of the three primary hydroxyl groups[18] of sucrose. The nucleophilic (iodo, bromo, chloro, azo, etc.) displacement of the mesyl groups in sucrose octamesylate in aprotic solvents, such as DMF or HMPT have revealed the specific mesyloxy substituents to undergo nucleophilic substitution to give 6,6′-di-substituted (**21**), 4,6,6′-tri-substituted (**22**) and 1′,4,6,6′-tetra-substituted (**23**) products that could readily be isolated by careful control of the reaction conditions and subsequent purification by column chromatography (Scheme 6). Thus,

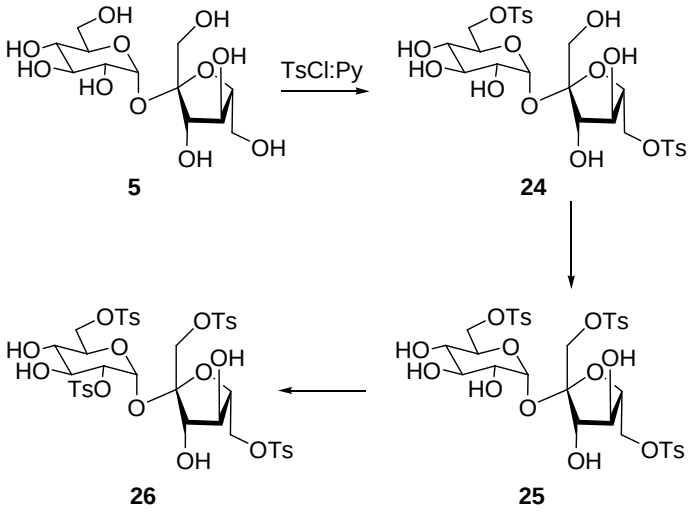
it was observed that the order of replacement with nucleophiles is 6 ~ 6′ > 4 > 1′ >> all other positions.

Scheme 6



Partially substituted sulphonate esters of sucrose can be prepared by selective esterification with sulphonyl halides. Treatment with limited quantities of tosyl chloride in pyridine gave 6,6′-ditosylate (**24**), 1′,6,6′-tritosylate (**25**) and 1′,2,6,6′-tetratosylate (**26**) (Scheme 7)[18] whereas the bulkier sulphonyl halides (tripsylchloride and mesitylene sulphonyl chloride) exhibited increased selectivity in the direct formation of 1′,6,6′-trisulphonate esters in good yield. These partially substituted sulphonate esters can also be displaced by nucleophiles to give 6,6′-di- and 1′,6,6′-tri-substituted sucrose derivatives. Moreover, it has also been observed that sucrose octa-acetate undergoes selective de-esterification upon the passage of its chloroform solution through a column of alumina. Thus, three heptaacetates were obtained with free hydroxyl groups at C-6′, C-4′ and C-4 which is due to the acetyl migration from C-4 and C-4′. This also demonstrates that the primary hydroxyl/ester groups of sucrose are more active than all other positions.

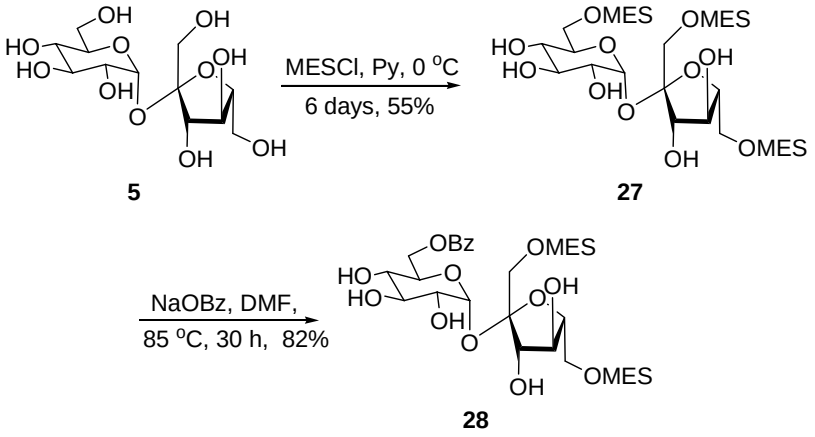
Scheme 7



It is evident from the above examples that the primary hydroxyl groups of sucrose are more reactive than all others. Further, there is a distinct variation of the reactivities amongst these groups also as shown earlier.

The three primary hydroxyl groups of sucrose **5** were protected as its mesitylene sulphonate ester derivative **27** in 55% yield by using known literature procedures[19] (on treatment with mesitylenesulphonyl chloride[20] and pyridine at 0 ^{o}C for 6 days). Again, the slight difference in reactivities of the three mesitylene sulphonate protected primary hydroxyl groups was exploited. Thus, tri-mesitylenesulphonate ester derivative **27** on treatment with sodiumbenzoate in DMF at 85 ^{o}C for 30 h furnished 6-benzoate derivative **28**[9] (Scheme 8). The structure of compound **28** was confirmed from its NMR studies and analytical data analyses. In ^{1}H NMR spectrum the protons for benzyl groups were observed at 7.47-7.68 (m, 3H) and 7.95-8.00 (m, 2H) ppm, and the protons for one mesitylene group were found to be absent. Moreover, in ^{13}C NMR spectrum, a prominent signal at 157.73 (s) ppm for the carbon of benzoate's carbonyl group was observed which clearly indicated the formation of **28**. The ESI-MS measurement showed the presence of prominent peak at m/z 833.2 corresponding to $[M+Na]^{+}$.

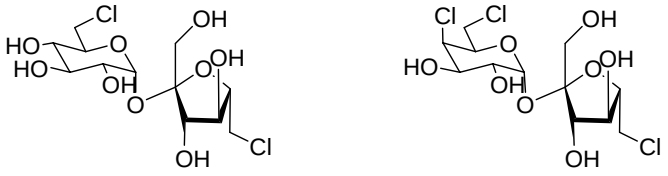
Scheme 8



5 **27** **28**

Our next task was to chlorinate the C-4 hydroxyl group with inversion of configuration. It was observed that under controlled condition sucrose **5** reacts with sulphuryl chloride in pyridine in a highly selective manner to give either 6,6′-dichloro-6,6′-dideoxysucrose **29** or 4,6,6′-trichloro-4,6,6′-trideoxy-galacto-sucrose **30**, after dechlorosulphation (Figure 6). Thus, the order of reactivities for chlorination reactions with sulphuryl chloride[18] is 6 ~ 6′ > 4 > 1′, in line with the steric bulk of the substituents in sucrose.

Figure 6. *di and tri chloro derivatives of sucrose*



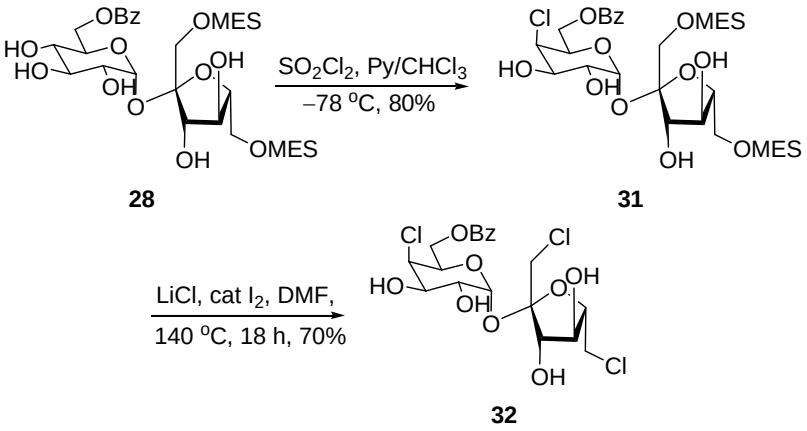
29 **30**

The reaction of C-4 hydroxyl with sulphuryl chloride in pyridine involves an initial formation of a chlorosulphate ester which then undergoes an intramolecular nucleophilic substitution reaction with the insertion of chloride and inversion of configuration of the monochloro derivative obtained (Scheme 9).[21]

The higher reactivity of C-4 hydroxyl of **28** (as other highly active positions were blocked) was exploited for selective replacement by chloro substituent.[22]

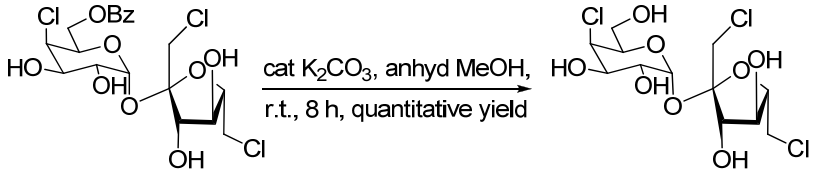
Treatment of **28** with sulphuryl chloride in chloroform pyridine mixture at −78 ^{o}C, furnished **31**. The analytical data analysis clearly indicates the introduction of chloro group. The ESI-MS measurement showed the presence of a prominent peak at *m/z* 850.7 corresponding to the adduct of molecular ion for **31** i.e. $[M+Na]^{+}$. But the orientation of 4Cl group could not be determined at this stage because of the complexity of its ^{1}H NMR signals. Then, **31** was treated with LiCl and catalytic iodine in DMF for 18 h at 140 ^{o}C to furnish trichloro derivative **32**.[9] The absence of both the mesitylene groups protons and carbons from its ^{1}H & ^{13}C NMR spectrum (C-6′, C-1′ at 44.70 (t) & 46.24 (t) ppm, respectively), and the similarity in its analytical data with the theoretically calculated values clearly demonstrates the formation of trichloro derivative **32** (Scheme 10).

Scheme 10



Finally, the benzoyl group was hydrolysed using catalytic potassium carbonate in methanol[12] to furnish sucralose **12** having spectral and analytical data in full agreement with the reported values (Scheme 11). This indirectly proves the orientation of 4Cl to be axial.

Scheme 11



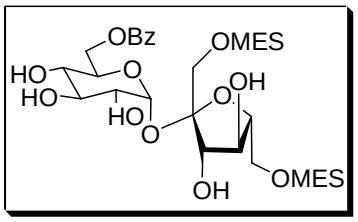
32 **12**

Conclusions

In conclusion, we have successfully exploited the varying degrees of reactivities of primary hydroxyl groups and C-4 hydroxyl (due to the electronic and steric effects) to prepare the highly polar synthetic target molecule (sucralose **12**) without resorting to complete protection and deprotection for all the 8-hydroxyl's present in the naturally occurring sucrose **5** molecule.

Experimental

6-*O*-Benzoyl-α-D-glucopyranosyl 1,6-di-*O*-mesitylenesulphonyl-β-D-fructofuranoside (28)

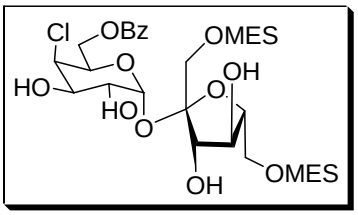


6,1',6'-Trimesitylenesulphonyl sucrose **27** (4.6 g, 5.2 mmol) and sodium benzoate (1.38 g, 9.6 mmol) were taken in dry DMF (69 mL). The mixture was warmed to 85 ^{o}C and maintained at that temperature for 30 hours. TLC in chloroform:methanol (7:1) showed a slow moving product and the absence of any starting material. Reaction mixture was cooled to r.t. and DMF was removed on a rotary evaporator. Column chromatography with 4% methanol in CH_2Cl_2, yielded the benzoate derivative **28** that was recrystallized from CH_2Cl_2 with a little added methanol to furnish a white solid.

Yield	: 3.4 g; 82 %
Mol. Formula	: $C_{37}H_{46}O_{16}S_2$
Melting Point	: 120 ^{o}C
Optical Rotation $[\alpha]_D^{25}$	: +19.8 (*c* 0.56, CH_3COCH_3)
IR (Nujol) $\tilde{\nu}$	: 3404, 2924, 1686, 1604, 1568, 1410, 1377, 1354, 1190, 1172, 1121, 1098, 1057, 1030, 993, 849, 835, 816, 757, 737, 714 cm^{-1}.
^{1}H NMR (Acetone-d_6, 200 MHz)	: δ 2.27 (s, 3H), 2.34 (s, 3H), 2.41 (s, 6H), 2.63 (s, 6H), 3.20-3.28 (m, 1H), 3.50-3.61 (m, 1H), 3.92 (br s, 1H), 3.99 (d, *J* = 4.6 Hz, 2H), 4.01-4.07 (m, 3H), 4.17-4.29 (m, 2H), 4.43 (dd, *J* = 9.1 and 4.4 Hz, 1H), 4.49-4.58 (m, 2H), 4.90 (d, *J* = 5.4 Hz, 1H), 6.88 (s, 2H), 7.13 (s, 2H),

	7.47-7.68 (m, 3H), 7.95-8.00 (m, 2H) ppm.
^{13}C NMR	: δ 12.00 (q), 13.51 (q), 13.79 (q), 57.18 (t), 58.24 (t),
(Acetone-d_6, 50 MHz)	62.28 (d), 62.47 (d), 62.64 (t), 62.87 (d), 65.05 (d), 66.17 (d), 68.24 (d), 71.08 (d), 83.23 (d), 93.60 (s), 120.39 (d), 121.27 (d), 121.67 (s), 122.12 (s), 122.41 (s), 123.21 (d), 123.64 (d), 124.91 (d), 131.56 (s), 131.74 (s), 135.09 (s), 135.38 (s), 157.73 (s) ppm.
Elemental Analysis	Calcd. : C, 54.80; H, 5.72; S, 7.91
	Found: C, 54.70; H, 5.80; S, 7.88.
ESI-MS (*m/z*)	: 833.2 $[M+Na]^+$.

6-*O*-benzoyl 4-chloro-α-D-galactopyranosyl 1,6-di-*O*-mesitylenesulphonyl-β-D-fructofuranoside (31)

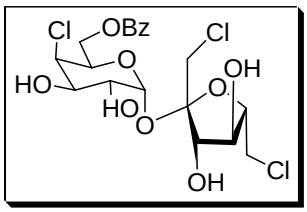


6-Benzoyl 1', 6'-dimesitylenesulphonyl sucrose **28** (3 g, 3.7 mmol) was taken into a mixture of pyridine (30 mL) and chloroform (15 mL), and cooled to –78 ^oC. Sulphuryl chloride (3.6 ml, 44.8 mmol) was added to this mixture over 10 mins. The resulting mixture was stirred at –78 ^oC for 4 hours and then allowed to attain r.t. slowly. The reaction mixture was filtered into a stirred suspension of sodium carbonate (3.18 g, 30 mmol) and methanol (31.8 mL) containing a catalytic amount of sodium iodide. Stirring for half an hour, filtration of the mixture, concentration in vacuo and column chromatography using 10% acetone in CH_2Cl_2 gave **31** as a white solid.

Yield	: 2.4 g; 80 %
Mol. Formula	: $C_{37}H_{45}ClO_{15}S_2$
Melting Point	: 128 ^oC
Optical Rotation $[\alpha]_D^{25}$	: +37.4 (*c* 0.6, $CHCl_3$)

IR (Nujol) $\tilde{\nu}$	: 3436, 2924, 1722, 1603, 1404, 1377, 1356, 1189, 1174, 1094, 1028, 994, 848, 816, 756, 715, 664 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.27 (s, 3H), 2.31 (s, 3H), 2.49 (s, 6H), 2.54 (s, 6H), 3.78-3.84 (m, 2H), 3.94-4.09 (m, 4H), 4.13-4.25 (m, 5H), 4.44-4.52 (m, 2H), 4.80 (d, *J* = 3.5 Hz, 1H), 6.86(s, 2H), 6.96 (s, 2H), 7.31-7.39 (m, 2H), 7.45-7.52 (m, 1H), 7.88-7.92 (m, 2H) ppm.
^{13}C NMR $CDCl_3$, 50 MHz)	: δ 21.00 (q), 22.30 (q), 22.54 (q), 63.23 (d), 64.49 (t), 66.14 (t), 67.77 (d), 68.14 (d), 68.40 (d), 69.44 (t), 73.80 (d), 76.75 (d), 78.49 (d), 92.45 (d), 101.73 (s), 128.47 (d), 128.93 (s), 129.41 (s), 129.56 (d), 129.68 (s), 131.60 (d), 131.94 (d), 133.40 (d), 139.87 (s), 140.10 (s), 143.55 (s), 143.96 (s), 166.43 (s) ppm.
Elemental Analysis	Calcd.: C, 53.59; H, 5.47; Cl, 4.27; S, 7.73 Found: C, 53.67; H, 5.45; Cl, 4.18; S, 7.48.
ESI-MS (*m/z*)	: 850.7 $[M+Na]^+$.

6-*O*-Benzoyl 4-chloro-α-D-galactopyranosyl 1,6-dichloro-β-D-fructofuranoside (32)

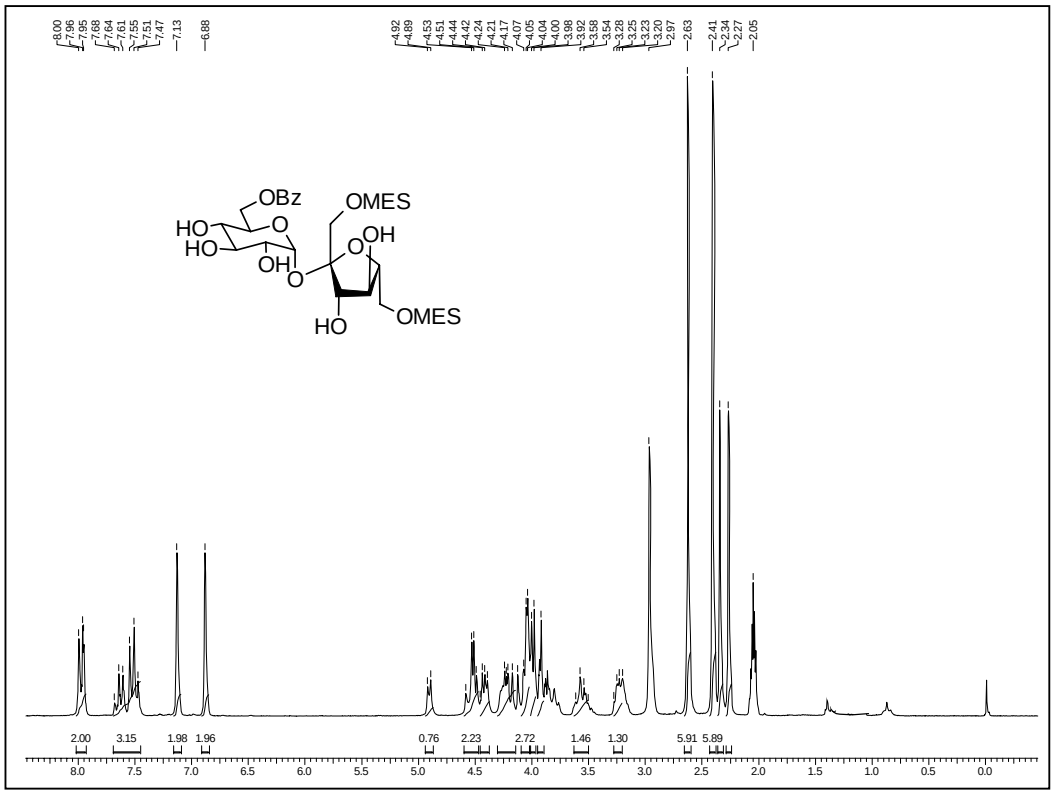


4-Chloro 6-benzoyl 1', 6'-dimesitylene sulphonyl sucrose **31** (0.5 g, 0.6 mmol) was taken into DMF (12.5 mL) and to it lithium chloride (1.6 g, 37.7 mmol), and catalytic iodine (0.04 g, 0.2 mmol) were added. Then, the reaction mixture was heated at 140 ^{o}C for 18 hours cooled to r.t., poured into ice-cooled water and extracted with chloroform. The organic solution was dried over sodium sulfate, concentrated and column chromatographed using 8% methanol in CH_2Cl_2 to furnish **32** as a white solid.

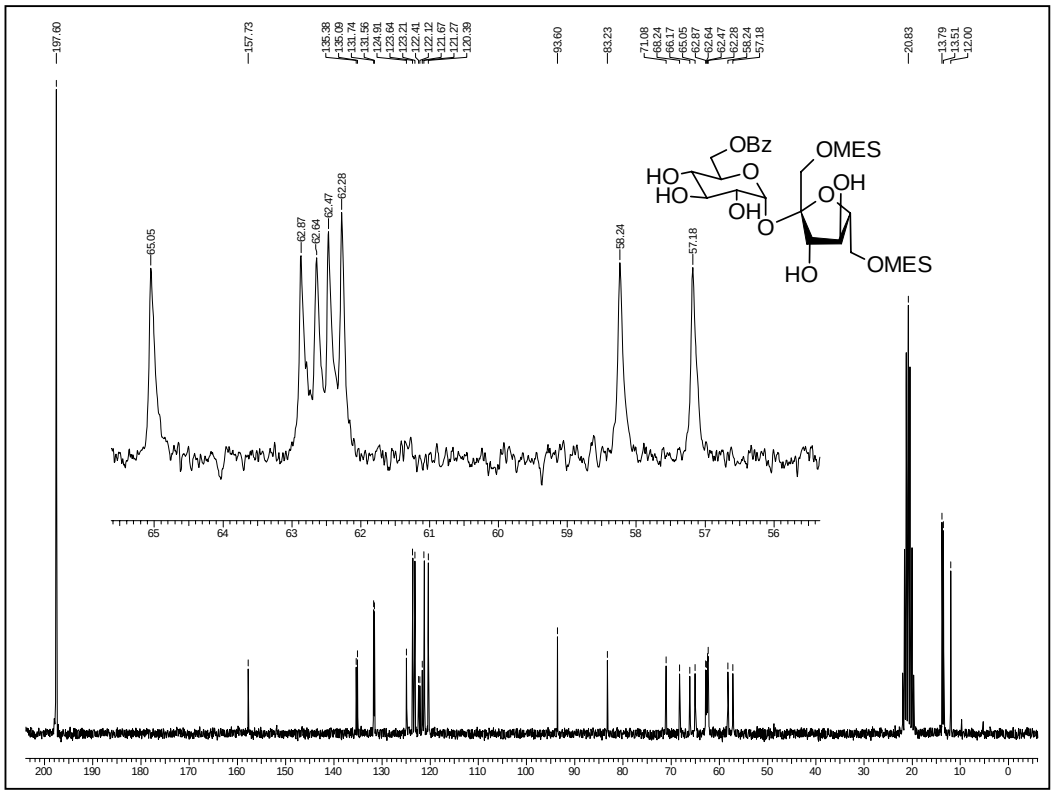
Yield : 0.2 g; 70 %

Mol. Formula	: $C_{19}H_{23}C_{l3}O_9$
Melting Point	: 132 ^{o}C
Optical Rotation $[\alpha]_D^{25}$	: + 68.9 (*c* 0.25, MeOH).
IR ($CHCl_3$) $\tilde{\nu}$	: 3392, 3019, 2926, 1719, 1178, 1091, 1027, 991, 759, 713, 669 cm^{-1}.
^{1}H NMR (Methanol-d_4, 400 MHz)	: δ 3.51 (dd, *J* = 11.3 and 2.8 Hz, 1H), 3.72-3.79 (m, 3H), 3.86 (dd, *J* = 10.0 and 3.8 Hz, 1H), 4.00 (t, *J* = 7.8 Hz, 1H), 4.11 (dd, *J* = 10.2 and 3.7 Hz, 1H), 4.26 (d, *J* = 8.3 Hz, 1H), 4.38 (dd, *J* = 11.5 and 4.8 Hz, 1H), 4.47-4.52 (m, 2H), 4.56 (s, 1H), 4.78 (br t, *J* = 6.3 Hz, 1H), 5.40 (d, *J* = 4.0 Hz, 1H), 7.47 (t, *J* = 7.6 Hz, 2H), 7.60 (t, *J* = 7.6 Hz, 1H), 8.00-8.02 (m, 2H) ppm.
^{13}C NMR (Methanol-d_4, 100 MHz)	: δ 44.70 (t), 46.24 (t), 65.19 (d), 66.20 (t), 69.25 (d), 69.51 (d), 69.58 (d), 77.14 (d), 77.98 (d), 83.55 (d), 94.53 (d), 105.18 (s), 129.79 (d), 130.77 (d), 130.99 (s), 134.54 (d), 167.77 (s) ppm.
Elemental Analysis	Calcd.: C, 45.48; H, 4.62; Cl, 21.20 Found: C, 45.41; H, 4.53; Cl, 21.29.
ESI-MS (*m/z*)	: 522.7 $[M+Na]^+$.

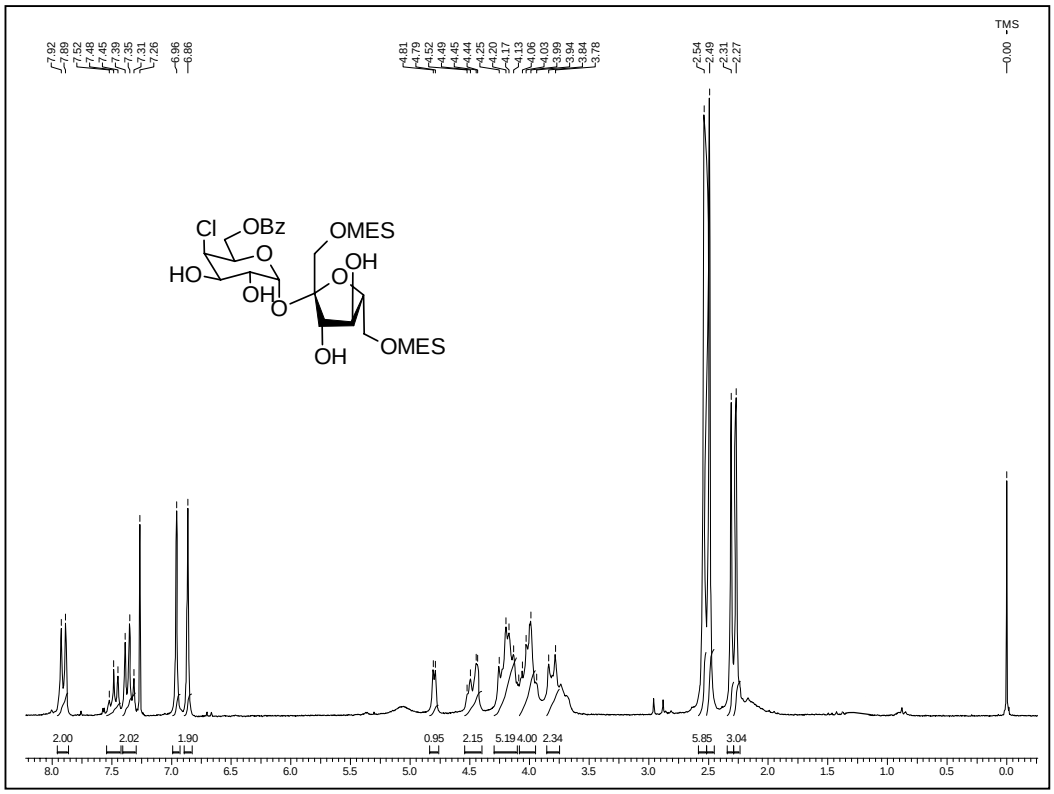
Spectra



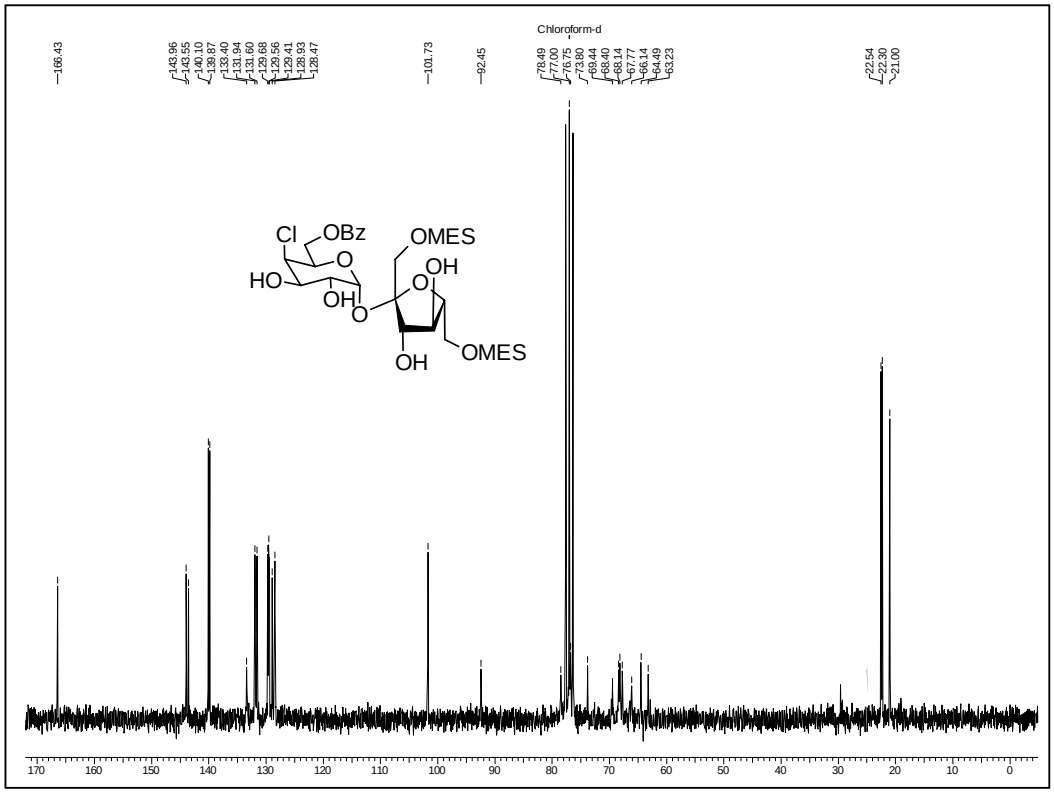
^{1}H NMR Spectrum of **28** in Acetone-d_6



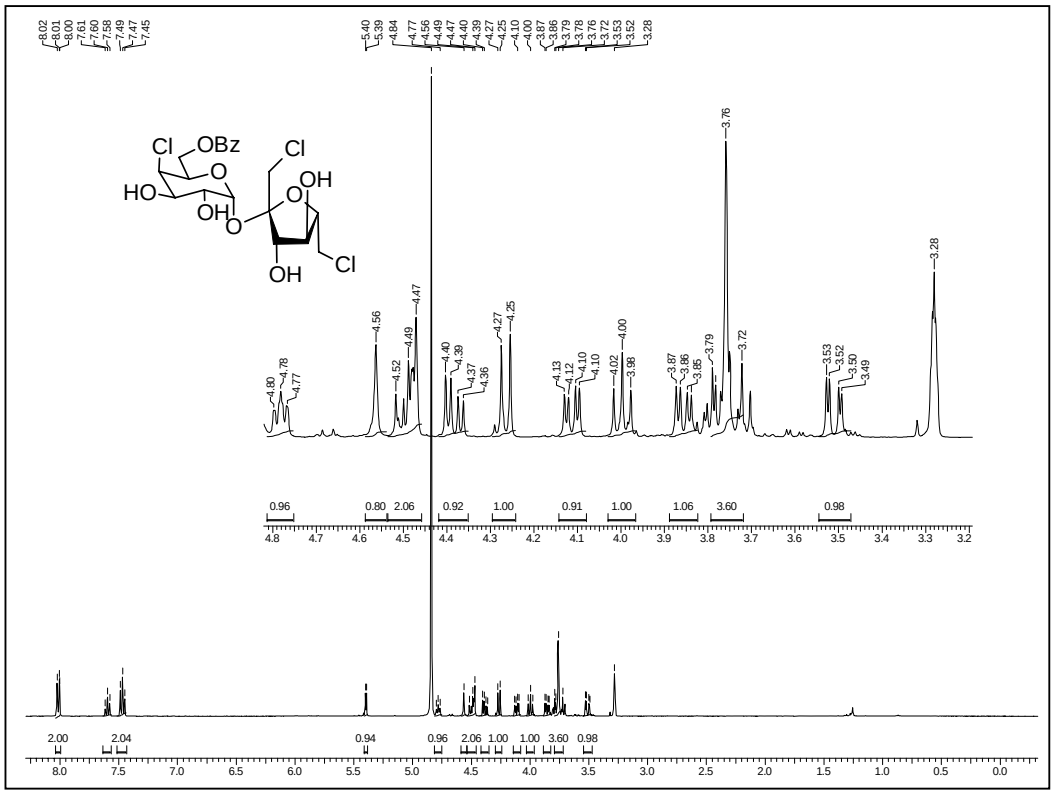
^{13}C NMR Spectrum of **28** in Acetone-d_6



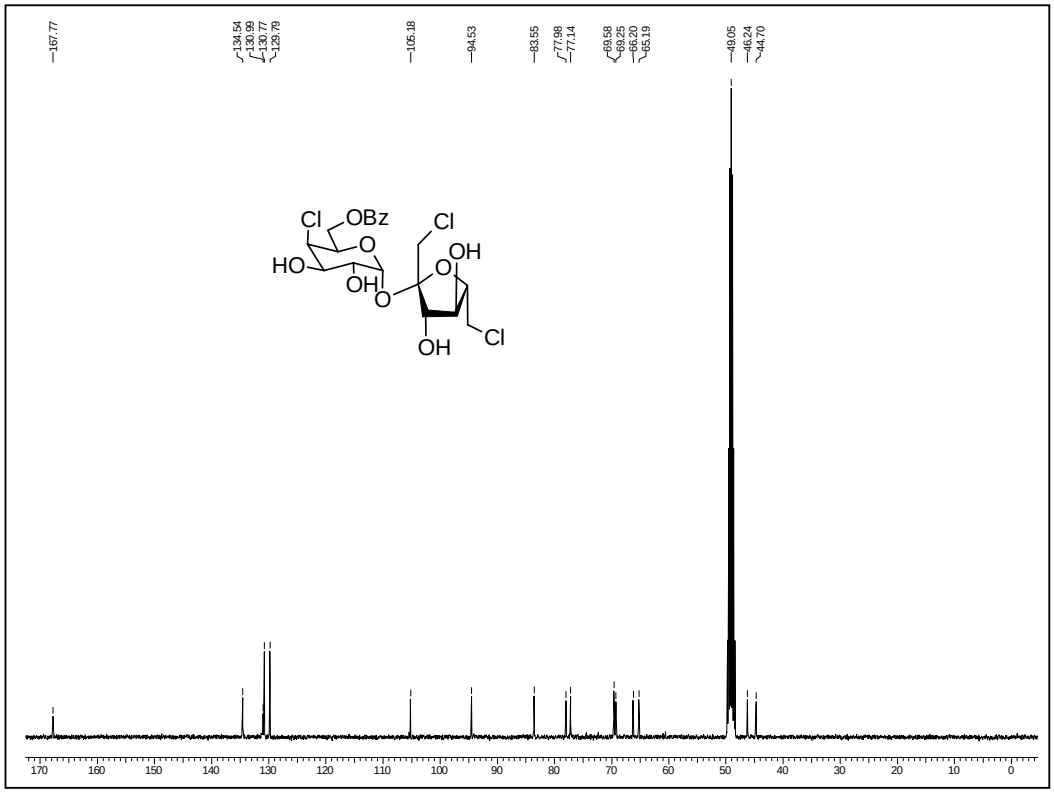
^{1}H NMR Spectrum of **31** in $CDCl_3$



^{13}C NMR Spectrum of **31** in $CDCl_3$



^{1}H NMR Spectrum of **32** in Methanol-d_4



^{13}H NMR Spectrum of **32** in Methanol-d_4

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Chapter III, Section II:

Synthetic Studies Towards Dolastatin 19.

Introduction

Nature is a vast store house of a very large number of molecules having diverse structures and bioactivities. The two third part of earth is covered by the ocean which hosts infinite number of organisms, and hence it's like a mermaid's grotto of useful chemicals.[1] Since oceans are considered to be very hostile and inhospitable place so it remained untouched for a long period of time. But, with the advancement of technology (like, sample collection techniques by scuba diving, more sensitive biological tests, etc.), the quest for exploring marine organisms *viz*, soft corals, sponges, tunicates, algae and bacteria for extracting pharmacologically active drugs increased.

Marine natural products are found to be attractive sources of secondary metabolites (i.e. they are not essential for the growth and development of an organism but are responsible in protecting the organisms from hostile environments, can help in making reproduction more probable, etc.). It has been observed that in most of the cases these compounds are not generated by the organisms but the microbes which reside in them. So, scientists are also trying to identify the microbes or the genes responsible for it so that they can be inserted into an organism which is easier to work with like, *Escherichia coli*.

Hence, the painstaking effort in this field has led to the discovery of more than 16,000 marine natural products. Many of these compounds have shown very promising biological activity like, Prialt a pain reliever derived from the hunting venom of the cone snail and Vidarabin which is an antiviral drug derived from nucleosides of sponge are already in market.

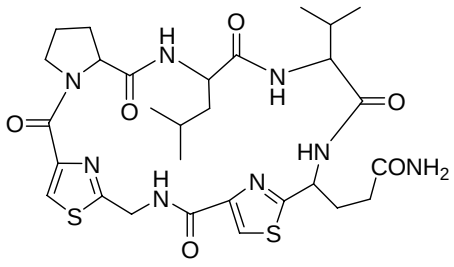
Dolastatins

Marine molluscs[2] are a source of large number of secondary metabolities derived from amino acids. Many of them are poisonous, but few are found to be useful in regulating sexual and digestive functions of the organisms. These are found to be a linear peptides comprised of (*S*) – amino acids whereas the Indian Ocean sea-hare *Dolabella auricularia* is found to be a source of few cyclic peptides – dolastatins (Figure 1). They are generally antineoplastic and cytotoxic agents. Dolastatins are

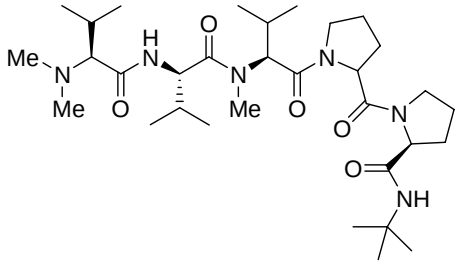
also observed to be a powerful cell growth inhibitors of the P388 lymphocytic leukaemia cell line with dolastatin 3 (**1**) having ED_{50} 1×10^{-4} to 1×10^{-7}. Dolastatin 10 (**3**) contains many unusual amino acids including a modified proline and *O*-methylisostatine, and was claimed to be the most potent antineoplastic agent known (17-67%). It is a tubulin interactive agent, which had entered into Phase II clinical trial but unfortunately it did not show significant antitumor activity against prostate cancer and melanoma in man. Still some of the dolastatins derivatives are in clinical and preclinical trials. Due to the potent anticancer activity, a large number of dolastatin derivatives have been synthesized.

Synthadotin [ILEX 651] (**2**) is an analogue of dolastatin 15 (**4**) which is a synthetic version and is found to be an orally active pentapeptide. It targets tubulin with a unique mechanism of action. Currently the Phase II clinical trials for ILEX 651 (**2**) against recurrent or metastatic melanoma are going on. Preliminary results have shown that the compound is active in a range of solid tumors.

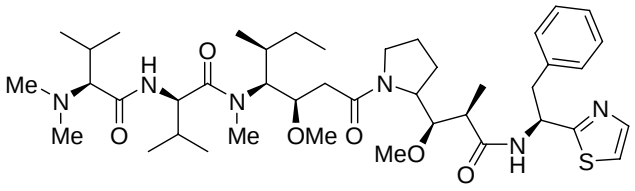
Figure 1. *Structural representation of few Dolastatins*



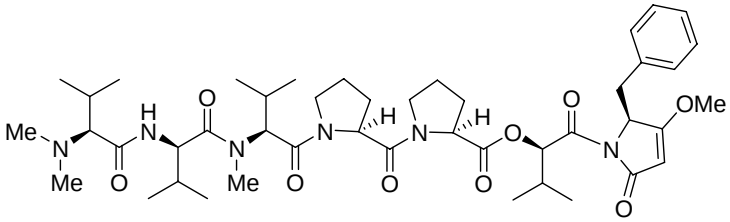
Dolastatin 3 (**1**)



ILEX 651 (**2**)



Dolastatin 10 (**3**)

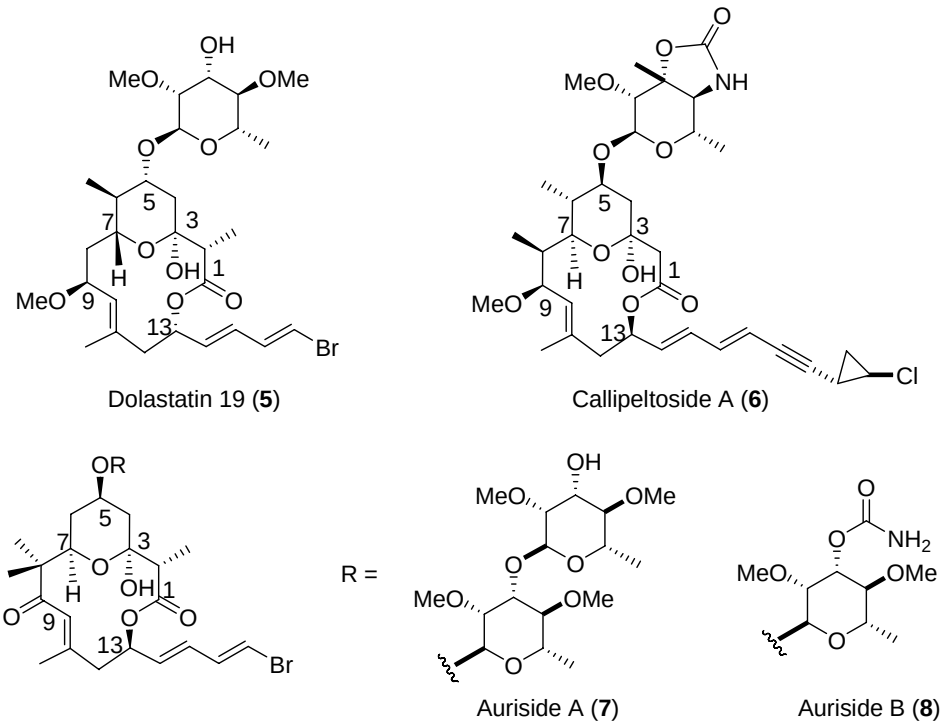


Dolastatin 15 (**4**)

Glycosidic Macrolides

Glycosidic macrolides have got very interesting structural features i.e. the macrolactone moieties are appended with a sugar unit by a glycosidic bond (Figure 2). Dolastatin 19 (**5**) is one such compound isolated[3] from *D. auricularia*. Many more compounds having these unique structural features are aurisides (**7** & **8**)[4] (from *D. auricularia*), lyngbouilloside[5] (from *Lyngbya bouillonii*), callipeltoside A (**6**)[6] (from *Callipelta sp.*), etc.

Figure 2. *Representative structures of few glycosidic macrolides*



Isolation[3]

The shell-less mollusc *Dolabella auricularia* has been found to be a very interesting organism since it is responsible for producing many structurally complex series of molecules (Dolastatin 1-19). In 2004, Pettit et al. isolated Dolastatin 19 (**5**) from these species (600 kg) collected in the Gulf of California in 1996. It was first extracted with MeOH-CH_2Cl_2 (1:1) and then the successive partitioning of organic

fraction in suitable solvents followed by bioassay in the P388 lymphocytic leukaemia cell line led to the isolation of some bioactive compounds. They were finally separated and purified by using reversed phase (C8 and C18) HPLC (6.5:3.5 MeOH-H_2O and 4.5:5.5 CH_3CN-H_2O) to yield 0.5 mg of a new compound as a colourless amorphous powder in addition to two known macrolides, debromoaplysiatoxin (5.5 mg) and anhydrodebromoaplysiatoxin (1.2 mg).

Structural Interpretation

The structure of this newly obtained compound was determined by exhaustive spectroscopic and analytical data analyses. The FABMS spectral data analysis led to the derivation of molecular weight to be 634 and hence the molecular formula was found to be $C_{29}H_{45}O_{10}Br$. The complete structural elucidation was carried out by rigorous studies of its ^1H, APT, ^1H–^1H COSY, TOCSY, HMQC and HMBC at 500 MHz. Thus, it was found to have one carbonyl, five olefinic methines, one olefinic quaternary carbon which was observed at 119.13 ppm and the oxygenated one at 98.24 ppm. Other different units like 11 methines, 3 methylenes, 4 methyls, 3 methoxy groups and 2 hydroxy protons were also observed at 2.97 and 4.47 ppm. Furthermore, the two dimensional NMR studies and their rigours analyses helped in determining the three dimensional structure to be as represented in Figure 2.

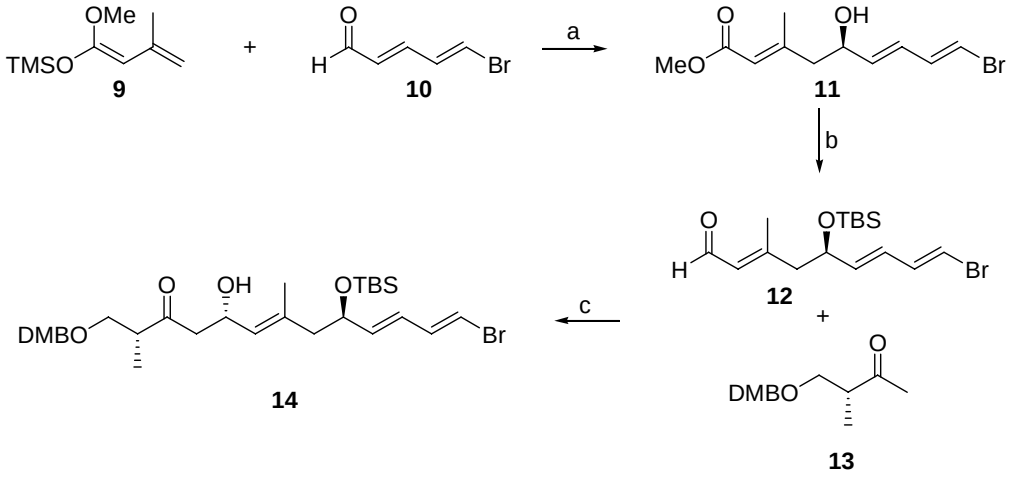
Biological Activity

This newly obtained compound **5** displayed a remarkable cancer cell growth inhibitory activity (GI_{50} 0.72 μg/mL for breast MCF-7 and 0.76 μg/mL for colon KM20L2).

The remarkable biological activity, low isolation yield and interesting molecular structure put forth a challenge to the synthetic chemists to synthesize it in appreciable amount so that its further biological activity can be unravelled. In 2006, Paterson et al. first reported the total synthesis of Dolastatin 19 (**5**) with revision of its structure.[7] They used asymmetric vinylogous aldol reaction in a judicial way to generate the C-13 stereocenter and the (*E*) trisubstituted alkene. The other centers were successfully generated by 1,4-*syn* boron mediated aldol reactions. Finally, the

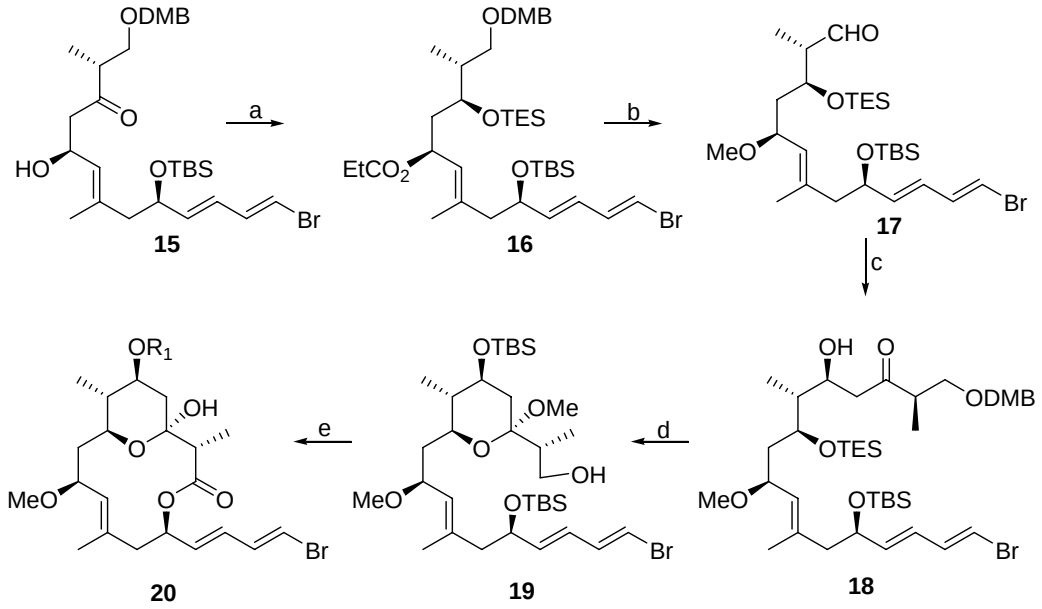
Mukaiyama glycosylation approach was used to append the L-rhamnose derived pyranoside. The synthetic strategy is shown below (Schemes 1, 2 & 3). But a revision of its structure was predicted on the basis of its same cyanobacterial origin with aurisides and considering the stability of the diamond lattice like arrangement of the hemiacetal pyranoside ring of aurisides compared with dolastatin 19. The hypothesis was also proved by total synthesis.

Scheme 1. *Synthesis of fragment **14***



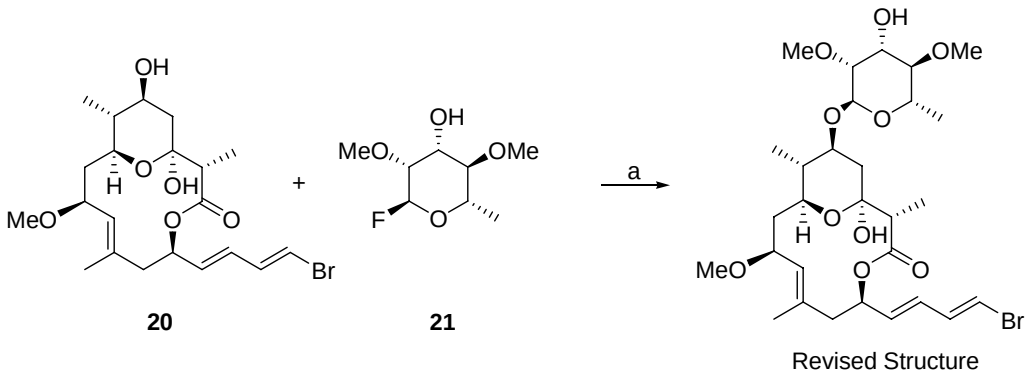
Reagents and Conditions: (a) (*R*)-BINOL, Ti(O*i*-Pr)$_4$, THF, −78 ^{o}C, 93%, 94% *ee*; (b) (i) TBSCl, imid., CH_2Cl_2; (ii) DIBAL, CH_2Cl_2, −78 ^{o}C; (iii) MnO_2, Et_2O, overall yield 75%; (c) (+)-Ipc$_2$BCl, Et_3N, Et_2O, −78 to 0 ^{o}C, 88%, >95:5 *dr*.

Scheme 2. *Synthesis of macrolactone **20***



Reagents and Conditions: (a) (i) EtCHO, SmI_2, THF, –10 ^oC; (ii) TESOTf, 2,6-lut., CH_2Cl_2, –78 ^oC, 72% overall yield, > 95:5 *dr*; (b) (i) DIBAL, CH_2Cl_2, –78 ^oC; (ii) Me_3OBF_4, Proton Sponge, CH_2Cl_2, (iii) DDQ, pH7 buffer, CH_2Cl_2, 60 ^oC; (iv) Dess-Martin, $NaHCO_3$, CH_2Cl_2; (c) **13**, *c*-Hex_2BCl, Et_3N, Et_2O, –78 to 0 ^oC; (d) (i) PPTS, MeOH; (ii) TBSOTf, 2,6-lut., CH_2Cl_2, –78 ^oC, 70% overall yield; (iii) DDQ, pH9 buffer, CH_2Cl_2, 0 ^oC, 53%; (e) (i) Dess-Martin, $NaHCO_3$, CH_2Cl_2; (ii) $NaClO_2$, ^{t}BuOH; (iii) TBAF, THF; (iv) 2,4,6-$(Cl_3C_6H_2)COCl$, Et_3N, DMAP, PhMe, 60 ^oC; (v) TBAF, THF; (vi) PPTS, wet MeCN.

Scheme 3. *Glycosidation reaction*



Reagents and Conditions: (a) (i) $SnCl_2$, $AgClO_4$, Et_2O; (ii) HF.Py, THF, 39% overall yield.

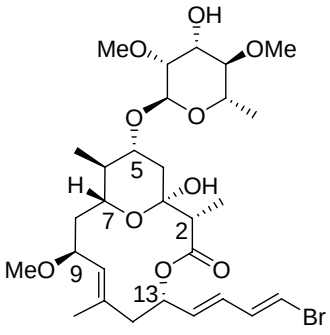
The unusual structure of dolastatin 19 (**5**) also attracted us to devise a new strategy (by using carbohydrate chemistry with spirocyclic cyclopropanation reaction and its opening) towards the synthesis because this type of chemistry has not been explored (as observed from the literature survey) in synthesizing pyranosidic hemiacetal containing macrolactones.

Present Work

Present Work

The natural products obtained from marine sources have wide structural diversities and pharmacological activities. One such diverse marine source is *Dolabella auricularia* which is a shell-less mollusc and is responsible for a large number of compounds (of Dolastatin series 1-18) having interesting structural and pharmacological properties. In 2004, Pettit et al. isolated[3] Dolastatin 19 (**5**) from these organisms of the Gulf of California, which is generally believed to be of cyanobacterial origin rather than directly produced by these organisms. It is a 14-membered glycosidic macrolide with a six-membered hemiacetal ring and bromine substituted conjugated diene side chain (Figure 3). Other compounds having similar structures are aurisides and lyngbyalosides. Preliminary biological studies have shown significant cancer cell growth inhibitory activity (GI_{50} values of 0.72 μg/mL and 0.76 μg/mL for breast MCF-7 and colon KM20L2 cell lines respectively). But, due to very poor yield (0.5 mg from 600 kg of *D. auricularia*) further biological studies could not be carried out. Hence, considering its important biological activity, complex molecular skeleton and poor isolation yield, we undertook the initiative to address its total synthesis.

Figure 3. *Structure of Dolastatin 19*

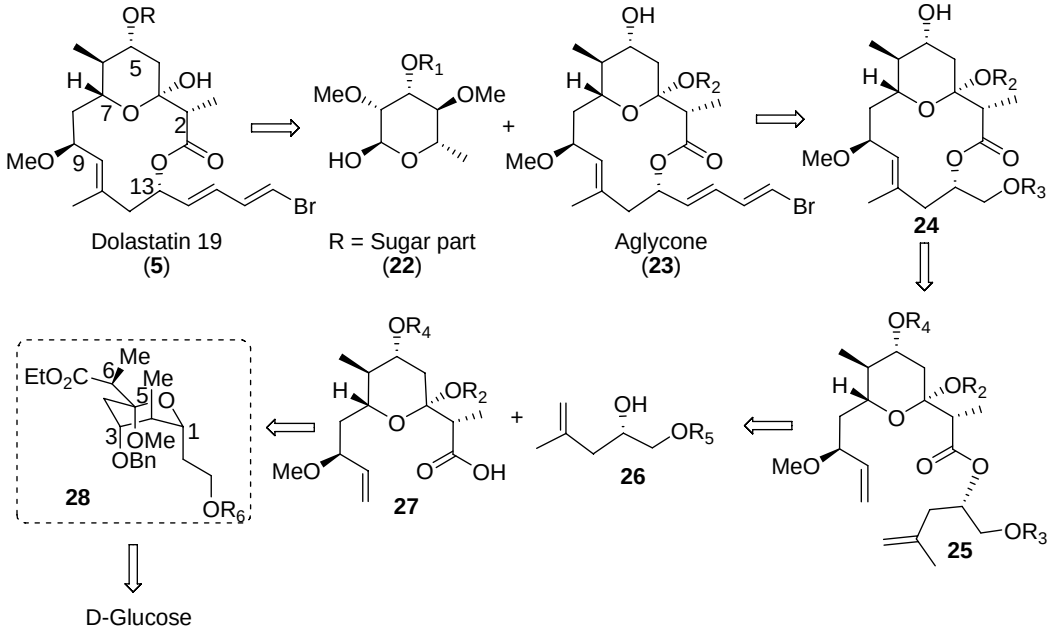


Original Structure (**5**)

The key retrosynthetic disconnections for the total synthesis of dolastatin 19 (**5**) are given in Scheme 4. The initial disconnection of dolastatin core is between the sugar and central macrocyclic cores projecting a glycosidation of the macrolactone **23** and appropriately activated/protected glycosyl donor **22** at the end of the total

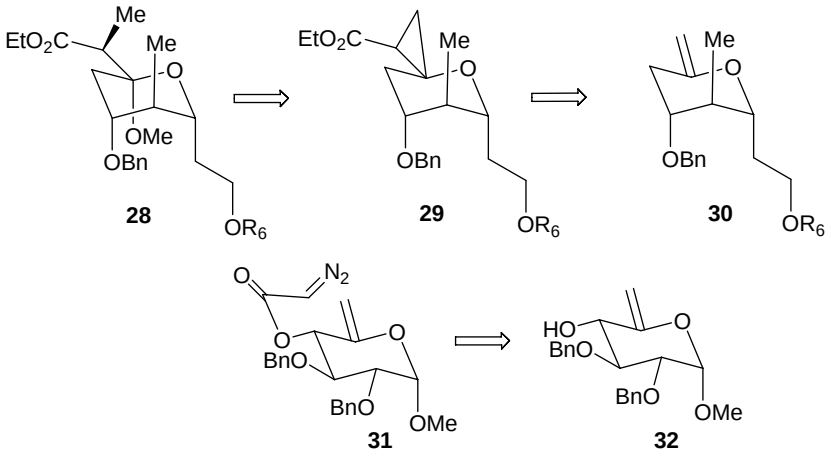
synthesis. The principle C-C coupling event in the synthesis of macrocyclic lactone **23** is between C-10 and C-11 and the ring closing metathesis being selected as appropriate complex transformation. Synthesis of one of the coupling partner **26** is planned by the established routes and for the other coupling partner **27** (hemiacetal pyranose ring), D-glucose has been selected after considering the stereochemical similarity between **27** and D-glucose.

Scheme 4. *Retrosynthetic analysis (R_1, R_2, R_3, R_4, R_5 & R_6 are suitable protecting groups)*



The key features of the retrosynthetic strategy for the advanced intermediate **28** were given in Scheme 5. One of the bottlenecks in the proposed synthetic strategy is the diastereoselective cyclopropanation of advanced *exo*-glycal **30** and the regioselectivity in electrophilic opening of the resulting product **29**.

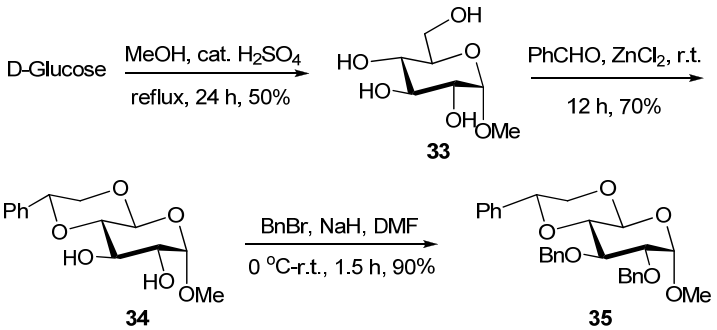
Scheme 5



In order to address the regioselectivity in the opening of cyclopropane ring, we initially planned for a model study selecting the *exo*-glycal **32** and its intramolecular cyclopropanation followed by cyclopropane ring opening.

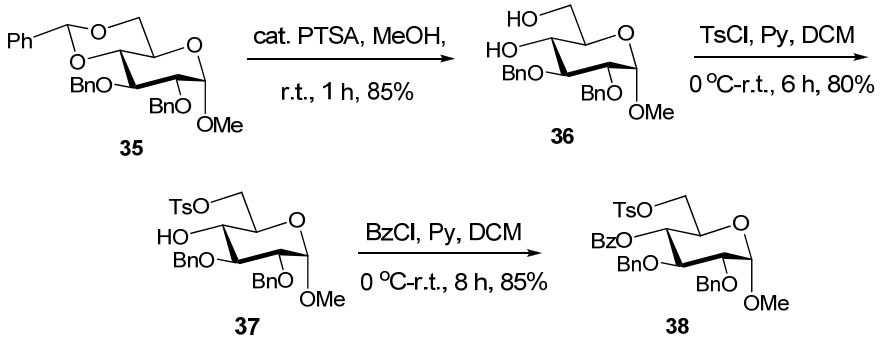
The synthesis of the derivative **32** was started from D-glucose following established procedures. Treatment of D-glucose with methanol and catalytic sulphuric acid furnished methylglucopyranoside **33** which was subsequently subjected to benzaldehyde and zinc chloride to furnish 4,6-benzylidene acetal derivative **34**. Then C-2, C-3 hydroxyl functionalities of **34** were protected as its benzyl ethers to furnish **35** (Scheme 6).[8]

Scheme 6



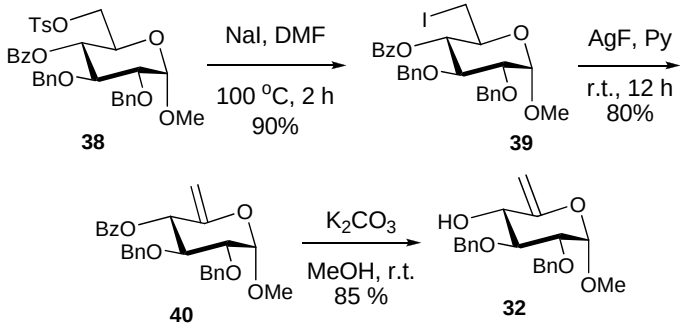
The 4,6-benzylidene acetal unit of **35** was first cleaved by catalytic PTSA in methanol to furnish diol **36**. Selective 1°-OH tosylation of **36** followed by benzoylation gave **38** (Scheme 7).

Scheme 7



Nucleophilic displacement of tosyl group with NaI in DMF furnished the iodo–derivative **39** and was subsequently transformed to the 5,6-ene derivative **40** by treating it with AgF in pyridine.[9] Hydrolysis of the benzoate derivative **40** with catalytic potassium carbonate in methanol gave the *exo*-glycal **32** (Scheme 8).

Scheme 8



The next endeavour, i.e. to convert **32** into **31** was studied thoroughly under different reaction conditions as summarized in Table 1. In all the cases either a complex reaction mixture was obtained or the starting material was recovered (Scheme 9). When TsNHNCHCOCl[10] was used with different bases complex reaction mixtures were obtained (entries 1, 2, 3 & 4).[11] Our effort to directly couple enol **32**

with acid $TsNHNCHCO_2H$ also met with failure (entry 5).[12] Finally, N-hydroxy succinimide was converted to the succinimidyl diazoacetate[13] and its attempted coupling[14] with **32** did not procure any **31** but the starting material was recovered back (entry 6).

Scheme 9

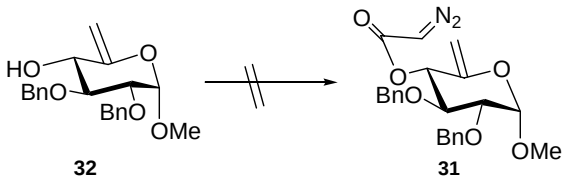
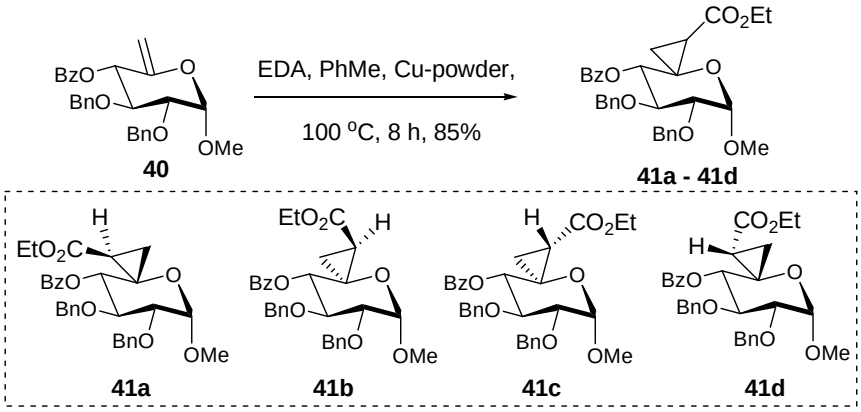


Table 1. *Various protocols followed for conversion **32** to **31***

S. No.	Reagents	Results
1.	$TsNHNCHCOCl/PhNMe_2/TEA$	Complex reaction mixture
2.	TsNHNCHCOCl/TEA	Complex reaction mixture
3.	$TsNHNCHCOCl/NaHCO_3$	Complex reaction mixture
4.	TsNHNCHCOCl/Collidine/TEA	Complex reaction mixture
5.	$TsNHNCHCO_2H$/DCC/DMAP	Starting material
6.	Succinimidyl diazoacetate/NaH	Starting material

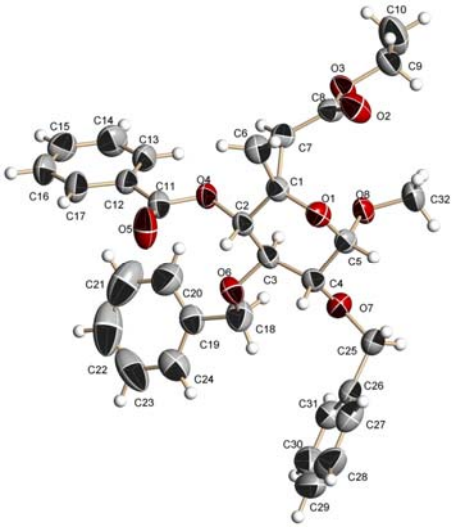
Since the attempted preparation of key substrate **31** for the projected intramolecular cyclopropanation strategy was found to be futile, we subjected the available benzoate **40** for an intermolecular cyclopropanation employing commercially available ethyl diazoacetate.[15] Exposure of **40** to ethyl diazoacetate in presence of copper powder at 100 ^oC in toluene furnished all the four possible diastereomeric cyclopropane derivatives **41a-d** as a mixture (Scheme 10). The ratio of the four diastereomers were found to be 1:0.7:1.8:1 (as determined by HPLC, column:- μ–Porasil, 3.9 × 300 mm; Mobile Phase:- TBME:pet. ether 20:80).

Scheme 10



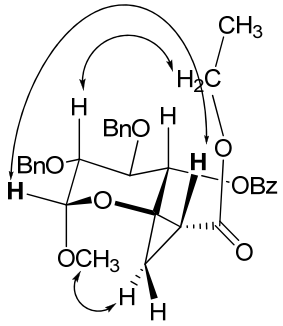
Two (**41c** & **41d**) more polar ones among the four diastereomeric cyclopropane derivatives were successfully separated by flash column chromatography (15% ethyl acetate in pet. ether) whereas the first two non-polar ones could not be separated by flash column chromatography and varying combinations of solvents (eluents). **41d** which happened to be the most polar product was obtained as a crystalline solid, and its relative configuration was assigned from single crystal X-ray diffraction studies (Figure 4). The details of crystal data and structure refinement (Table 2), bond lengths and bond angles (Table 3) and torsion angles (Table 4) are given at the end of this section (Page No. 180 to 183).

Figure 4. *ORTEP diagram of* ***41d***



COSY/NOESY studies were carried out to determine the stereochemistry of the cyclopropane ring and its substituents in **41c**. Strong nOe's amongst anomeric proton (H-1) and one of the cyclopropane ring proton was observed which was found to be the proton geminal to the carboxylate group. The methylene protons of ethyl ester group also showed nOe with H-2 of the pyran ring indicating a spacial proximity between the ethyl ester and H-2. Moreover, one of the methylene protons in cyclopropane ring also exhibited strong nOe's with methoxy protons at C-1 suggesting the orientation of these protons on the same side of pyranose ring i.e. *trans* to the carboxylate group. All these observations suggest that the stereochemical orientations of different substituents are as depicted in Figure 5.

Figure 5



41c

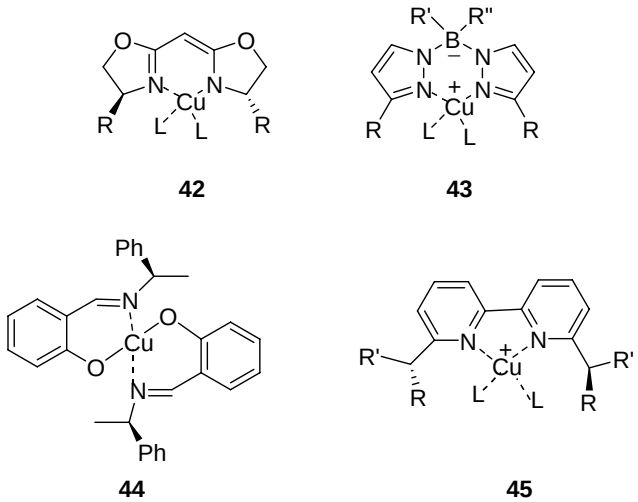
A Short Account on Cyclopropanation Reaction[16]

Cyclopropanes were first synthesized more than 100 years ago, way back in the latter part of the nineteenth century (i.e. 1885) by Curtius and Barton. Since then the reactions of diazoacetates with various unsaturated compounds have been studied under thermal, catalytic and photochemical conditions. Chiral cyclopropanes are an important class of strained small ring compounds because they are found as a basic structural unit in a wide range of naturally occurring compounds both in plants and in microorganisms and they can also lead to many useful intermediates by variety of chemical transformations while the catalytic asymmetric cyclopropanation reaction was first reported by Nozaki et al. only four decades ago (in 1966) for synthesizing chiral cyclopropanes. As the carbenes are very active and short lived entities so, it is

difficult to obtain selective products in normal cyclopropanation reactions. However, the catalyzed decomposition of diazo compounds leads to the formation of "carbenoids" which are highly selective due to their better stability. The catalysts used for such transformations are the transition metals like, Cu, Rh, Co and Ru. These metals when coordinated to chiral ligands show extraordinary stereoselectivity in cyclopropanation reactions. As these transition metals are coordinatively unsaturated, so they act as electrophiles (Lewis acids) with diazo groups. Different types of ligands which can form a variety of complexes are available. Anionic ligands from neutral Cu(I) complexes are semicorrins, polypyrazolylborates, etc. With bipyridines, phenanthrolines, etc. cationic complexes are obtained (Figure 6). The reactivity of cationic complexes are effective if the counter ions are (TfO^-) and (PF_6^-) whereas halides, cyanides, acetates and perchlorates show little or no catalytic activity. After such a long period of its inception little is known about the nature of these carbenoid complexes till to this date.

The reaction intermediates or transition states formed in these reactions are not fully characterized, though from computational studies the pathways have been predicted. But, they are not reliable in predicting the results obtained due to small differences in the energy of diastereomeric transition states. Therefore, till now the progress in these reactions are dependent largely on experience and intuition of the chemists dealing.

Figure 6. *Structures of copper complexes with different ligands*



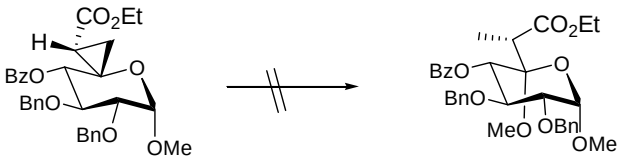
42 **43**

44 **45**

Ring Opening Reactions of Cyclopropane[17]

The cyclopropane derivative **41d** was now tried to open under different reaction conditions. But in all cases (Table 5) the required product could not be isolated.

Scheme 11



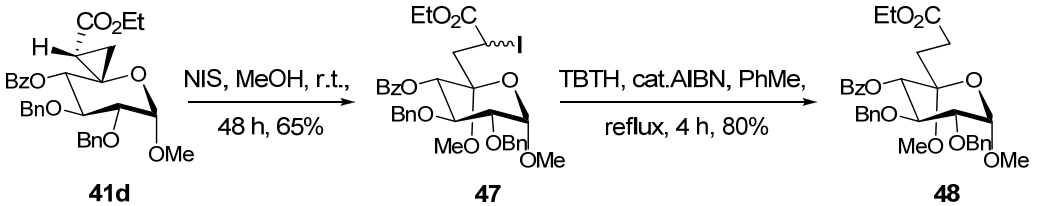
41d **46**

Table 5

S. No.	Reagents	Results
1.	Mercuric trifluoroacetate	Complex mixture
2.	H^+/MeOH	Inseparable mixture
3.	N-iodosuccinimide	Chain elongation

When **41d** was treated with mercuric trifluoroacetate a complex reaction mixture resulted whereas H^+/MeOH led to inseparable reaction mixture. Finally, with NIS the ring opening product **47** was observed (Scheme 12). But it was obtained as the chain elongated iodo derivative rather than the desired one having methyl branching **46** (Scheme 11). The iodo group was reduced with tri-*n*-butyl tinhydride to give **48** (Scheme 12). The structure of **48** was confirmed from its NMR spectroscopic and analytical data analyses. There were two triplets in the upfield region (i.e. 27.91 & 28.77 ppm) for the two methylene carbons at C-5.

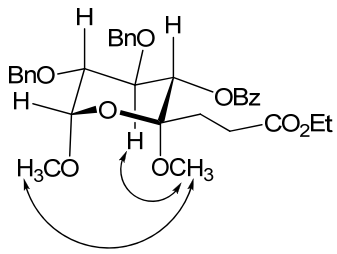
Scheme 12



41d **47** **48**

The stereochemistry of the newly introduced substituents in **48** was confirmed from its COSY/NOESY studies. Strong nOe's were observed amongst C-5 methoxy protons, and H-3 & C-1 methoxy protons. Moreover, other nOe interactions for H-6 & H-7 protons were not observed (Figure 7).

Figure 7

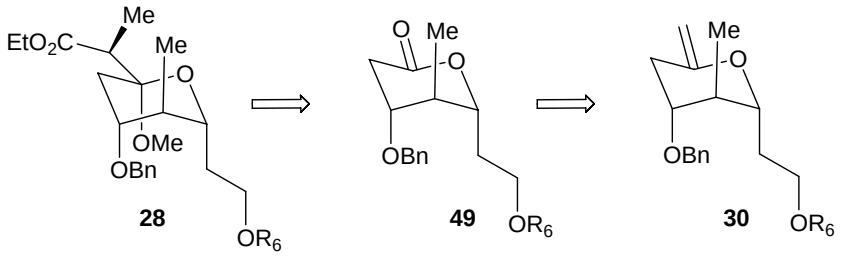


48

Retrosynthetic strategy involving lactone

Since, the cyclopropane ring opening reaction exhibited undesired regioselectivity we have modified our retrosynthetic strategy, identifying Reformatsky reaction[18] on lactone **49** to achieve the key intermediate **28** (Scheme 13).

Scheme 13

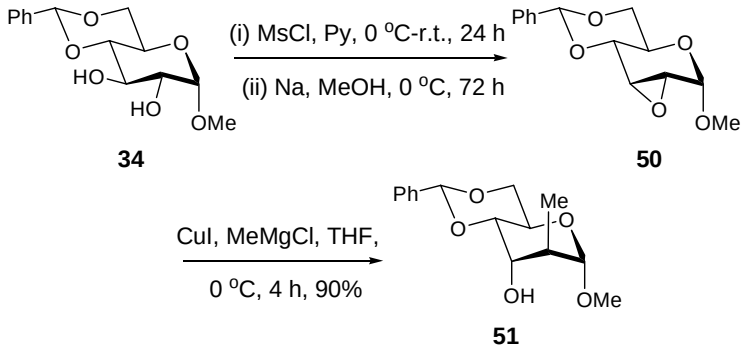


Synthesis of the three stereogenic centers of the hemiacetal pyranose ring

Synthesis of the intermediate lactone **49** was planned from the *exo*-glycal **30**, containing the requisite stereogenic centers at C-1, C-2 and C-3 in the pyran ring. The synthesis of *exo*-glycal **30** was commenced from one of the intermediate of the

previous scheme 6 i.e. **34**. Thus 4,6-benzylidene methylglucopyranoside **34** was di-mesylated and the 2,3-epoxide derivative **50** was obtained on treatment with base (sodium in methanol). The epoxide ring of **50** was opened by using MeMgCl and CuI complex generated *in situ* at 0 ^{o}C in THF solvent to furnish **51** (Scheme 14).[19]

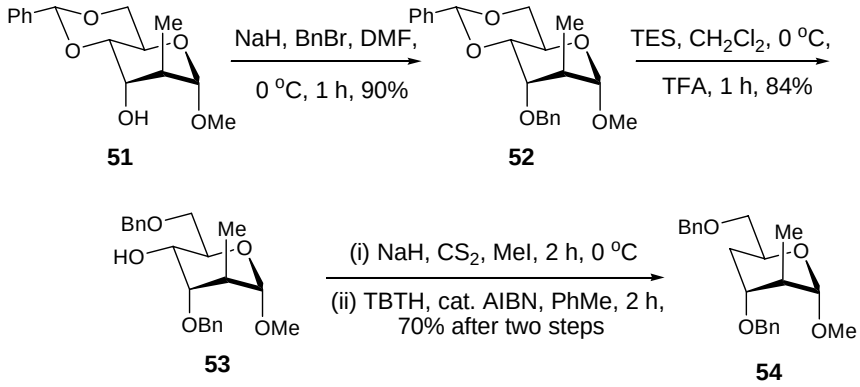
Scheme 14



34 **50** **51**

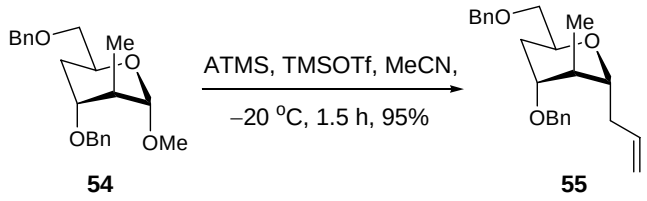
The hydroxyl group of **51** was protected as its benzyl ether on treatment with BnBr and NaH in DMF solvent to give **52**. The benzylidene group of **52** was reductively opened[20] using triethylsilane and trifluoroacetic acid to furnish **53**. The structure of **53** was confirmed by analysing its spectral and analytical data. In ^{1}H NMR spectrum the signal due to the methine proton of benzylidene acetal unit was absent whereas in ^{13}C NMR spectrum a signal at 70.93 ppm was observed which corresponds to the methylene group of the benzyl protecting unit on C-6 hydroxyl. Following Barton-McCombie protocol,[21] deoxygenation at C-4 of **53** was carried out in two steps by first converting **53** into its xanthate derivative, and then treating the intermediate xanthate with tri-*n*-butyl tin hydride to furnish **54**. In ^{13}C NMR spectrum a triplet was observed at 28.24 ppm corresponding to C-4 methylene carbon. The ESI-MS plot showed the presence of the sodiated molecular ion peak at 379.2 $[M+Na]^{+}$ for the molecular weight of **54** (Scheme 15).

Scheme 15



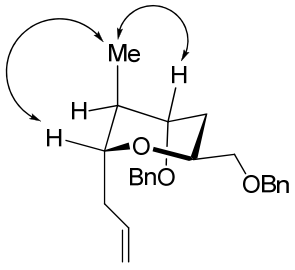
C-Glycosidation at the anomeric carbon (C-1) of **54** was carried out with allyltrimethyl silane and trimethylsilyl trifluoromethanesulfonate at –20 °C to furnish **55** (Scheme 16).[22] In ^{1}H NMR spectrum signals at 5.85-5.93 (m, 1H), 5.03-5.10 (m, 2H) and 2.36-2.49 (m, 2H) ppm corresponding to the allyl group appeared, whereas in ^{13}C NMR spectrum two more signals (w.r.t. **54**'s spectrum) at 37.27 & 116.37 ppm were observed corresponding to the methylene carbons of the allyl unit.

Scheme 16



Finally, the orientation of C-1 allyl group in **55** was confirmed from COSY/NOESY studies. In their NOESY spectrum strong interactions of C-2 methyl protons with H-3 & H-1 were observed which indicates that both are *cis* to it and are also equatorially oriented. This implies that C-1 allyl group is axially oriented as shown in Figure 8.

Figure 8

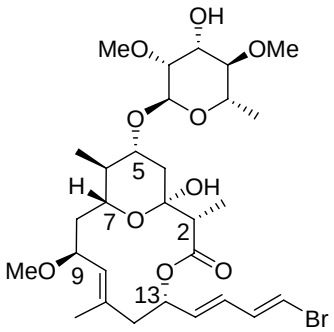


55

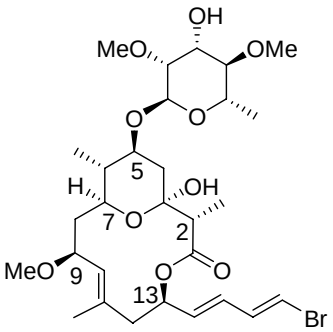
Revision of Structure of Dolastatin 19[7a]

After successfully synthesizing **55** having three stereogenic centers (C-1, C-2 & C-3) as in the original molecule of Dolastatin 19 (**5**), a total synthesis of Dolastatin 19 was reported by Paterson et al. with the revision of structure (**56**). They argued that both Dolastatin 19 and aurisides are from the same bacterial biogenesis and have a similar diamond lattice arrangement for the macrolide with the six membered hemiacetal ring adopting a chair conformation which facilitates the formation of a stabilizing hydrogen bond between the axial C-3 OH and the lactone carbonyl oxygen, thus minimizing steric interactions. Whereas the proposed structure of Dolastatin 19 (**5**) predicts a boat conformation of the pyran ring and so a distortion in the macrolactone will be there. Hence, they revised the stereocentres at C-5 to C-7 array and the C-13 carbinol in **5** (Figure 9). This was proved by the total synthesis. At this stage we stopped our synthetic work as it demanded to be started from the very beginning for fixing the revised stereocenters generated by us.

Figure 9



Original Structure (**5**)



Reassigned Structure (**56**)

Conclusions

In conclusion, we have successfully synthesized **55** having all the three stereocenters (C-1, C-2 & C-3) of the hemiacetal pyranose ring **28** of the original Dolastatin 19 (**5**). Spirocyclic cyclopropane rings **41a-41d** were also successfully synthesized by intermolecular cyclopropanation reaction and two of them (**41c** & **41d**) were separated and their stereochemistry were determined. Moreover, the cyclopropane ring of **41d** was successfully cleaved but it resulted into the product having undesired regioselectivity.

Table 2. Crystal data and structure refinement for compound 41d

Empirical formula	$C_{32}H_{34}O_8$
Formula weight	546.59
Temperature	297(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, P 21
Unit cell dimensions	a = 11.4247(16) Å, α = 90°
	b = 7.9495(12) Å, β = 95.716(2)°
	c = 15.880(2) Å, γ = 90°
Volume	1435.1(4) $Å^3$
Z, Calculated density	2, 1.265 mg/m^3
Absorption coefficient	0.090 mm^{-1}
F(000)	580
Crystal size	0.98 x 0.15 x 0.13 mm
Theta range for data collection	2.31 to 25.00°
Limiting indices	-13<=h<=13, -9<=k<=9, -18<=l<=18
Reflections collected / unique	13943 / 5051 [R(int) = 0.0213]
Completeness to theta = 25.00	99.8 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9883 and 0.9166
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5051 / 1 / 363
Goodness-of-fit on F^2	1.049
Final R indices [I>2sigma(I)]	R1 = 0.0326, wR2 = 0.0822
R indices (all data)	R1 = 0.0356, wR2 = 0.0849
Absolute structure parameter	0.7(7)
Largest diff. peak and hole	0.198 and -0.099 e. $Å^{-3}$

Table 3. Bond lengths [Å] and angles [deg] for compound 41d

O(1)-C(1)	1.4057(19)	C(6)-C(1)-C(2)	119.83(15)
O(1)-C(5)	1.433(2)	O(1)-C(1)-C(7)	119.99(13)

O(2)-C(8)	1.2004(19)	C(6)-C(1)-C(7)	60.64(12)
O(3)-C(8)	1.339(2)	C(2)-C(1)-C(7)	120.15(13)
O(3)-C(9)	1.448(2)	O(4)-C(2)-C(3)	107.02(13)
O(4)-C(11)	1.349(2)	O(4)-C(2)-C(1)	109.80(13)
O(4)-C(2)	1.4387(19)	C(3)-C(2)-C(1)	113.90(13)
O(5)-C(11)	1.193(2)	O(6)-C(3)-C(2)	105.16(13)
O(6)-C(3)	1.4133(18)	O(6)-C(3)-C(4)	113.59(13)
O(6)-C(18)	1.423(2)	C(2)-C(3)-C(4)	110.14(13)
O(7)-C(4)	1.417(2)	O(7)-C(4)-C(3)	108.27(13)
O(7)-C(25)	1.432(2)	O(7)-C(4)-C(5)	110.62(14)
O(8)-C(5)	1.390(2)	C(3)-C(4)-C(5)	109.98(13)
O(8)-C(32)	1.414(2)	O(8)-C(5)-O(1)	111.73(14)
C(1)-C(6)	1.469(3)	O(8)-C(5)-C(4)	108.64(14)
C(1)-C(2)	1.520(2)	O(1)-C(5)-C(4)	110.18(14)
C(1)-C(7)	1.526(2)	C(1)-C(6)-C(7)	61.56(12)
C(2)-C(3)	1.514(2)	C(8)-C(7)-C(6)	116.98(15)
C(3)-C(4)	1.523(2)	C(8)-C(7)-C(1)	120.37(14)
C(4)-C(5)	1.532(2)	C(6)-C(7)-C(1)	57.80(12)
C(6)-C(7)	1.513(3)	O(2)-C(8)-O(3)	123.46(16)
C(7)-C(8)	1.480(2)	O(2)-C(8)-C(7)	125.42(17)
C(9)-C(10)	1.462(3)	O(3)-C(8)-C(7)	111.10(13)
C(11)-C(12)	1.483(2)	O(3)-C(9)-C(10)	109.39(17)
C(12)-C(13)	1.383(3)	O(5)-C(11)-O(4)	123.38(16)
C(12)-C(17)	1.394(3)	O(5)-C(11)-C(12)	125.29(16)
C(13)-C(14)	1.380(3)	O(4)-C(11)-C(12)	111.33(15)
C(14)-C(15)	1.381(4)	C(13)-C(12)-C(17)	119.53(17)
C(15)-C(16)	1.357(4)	C(13)-C(12)-C(11)	122.90(16)
C(16)-C(17)	1.377(3)	C(17)-C(12)-C(11)	117.49(17)
C(18)-C(19)	1.508(3)	C(14)-C(13)-C(12)	119.6(2)
C(19)-C(20)	1.362(3)	C(13)-C(14)-C(15)	120.4(2)
C(19)-C(24)	1.373(3)	C(16)-C(15)-C(14)	120.0(2)
C(20)-C(21)	1.371(4)	C(15)-C(16)-C(17)	120.7(2)
C(21)-C(22)	1.362(6)	C(16)-C(17)-C(12)	119.7(2)

C(22)-C(23)	1.366(6)	O(6)-C(18)-C(19)	110.35(17)
C(23)-C(24)	1.384(4)	C(20)-C(19)-C(24)	118.8(2)
C(25)-C(26)	1.495(3)	C(20)-C(19)-C(18)	121.7(2)
C(26)-C(27)	1.383(3)	C(24)-C(19)-C(18)	119.4(2)
C(26)-C(31)	1.385(3)	C(19)-C(20)-C(21)	121.1(3)
C(27)-C(28)	1.384(4)	C(22)-C(21)-C(20)	119.7(3)
C(28)-C(29)	1.354(4)	C(21)-C(22)-C(23)	120.7(3)
C(29)-C(30)	1.360(4)	C(22)-C(23)-C(24)	119.0(3)
C(30)-C(31)	1.372(3)	C(19)-C(24)-C(23)	120.8(3)
C(1)-O(1)-C(5)	113.84(12)	O(7)-C(25)-C(26)	113.22(15)
C(8)-O(3)-C(9)	116.43(13)	C(27)-C(26)-C(31)	117.49(18)
C(11)-O(4)-C(2)	118.08(13)	C(27)-C(26)-C(25)	122.14(19)
C(3)-O(6)-C(18)	117.23(14)	C(31)-C(26)-C(25)	120.34(18)
C(4)-O(7)-C(25)	115.35(16)	C(26)-C(27)-C(28)	120.6(2)
C(5)-O(8)-C(32)	115.00(14)	C(29)-C(28)-C(27)	120.4(2)
O(1)-C(1)-C(6)	116.22(14)	C(28)-C(29)-C(30)	120.1(2)
O(1)-C(1)-C(2)	111.44(13)	C(29)-C(30)-C(31)	120.2(2)
		C(30)-C(31)-C(26)	121.2(2)

Table 4. Torsion angles [deg] for compound 41d

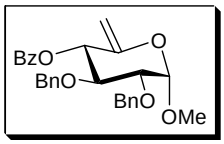
C(5)-O(1)-C(1)-C(6)	-161.37(14)	C(6)-C(7)-C(8)-O(2)	15.4(3)
C(5)-O(1)-C(1)-C(2)	56.52(17)	C(1)-C(7)-C(8)-O(2)	-51.3(3)
C(5)-O(1)-C(1)-C(7)	-91.66(18)	C(6)-C(7)-C(8)-O(3)	-163.35(15)
C(11)-O(4)-C(2)-C(3)	112.89(16)	C(1)-C(7)-C(8)-O(3)	129.90(17)
C(11)-O(4)-C(2)-C(1)	-123.02(16)	C(8)-O(3)-C(9)-C(10)	-172.7(2)
O(1)-C(1)-C(2)-O(4)	-169.67(13)	C(2)-O(4)-C(11)-O(5)	12.2(3)
C(6)-C(1)-C(2)-O(4)	49.75(19)	C(2)-O(4)-C(11)-C(12)	-166.93(14)
C(7)-C(1)-C(2)-O(4)	-21.6(2)	O(5)-C(11)-C(12)-C(13)	-174.8(2)
O(1)-C(1)-C(2)-C(3)	-49.69(18)	O(4)-C(11)-C(12)-C(13)	4.3(3)
C(6)-C(1)-C(2)-C(3)	169.73(15)	O(5)-C(11)-C(12)-C(17)	2.0(3)
C(7)-C(1)-C(2)-C(3)	98.43(18)	O(4)-C(11)-C(12)-C(17)	-178.94(17)
C(18)-O(6)-C(3)-C(2)	159.88(17)	C(17)-C(12)-C(13)-C(14)	-1.5(3)

C(18)-O(6)-C(3)-C(4)	-79.6(2)	C(11)-C(12)-C(13)-C(14)	175.2(2)
O(4)-C(2)-C(3)-O(6)	-67.77(14)	C(12)-C(13)-C(14)-C(15)	0.6(4)
C(1)-C(2)-C(3)-O(6)	170.69(12)	C(13)-C(14)-C(15)-C(16)	0.4(4)
O(4)-C(2)-C(3)-C(4)	169.49(11)	C(14)-C(15)-C(16)-C(17)	-0.5(4)
C(1)-C(2)-C(3)-C(4)	47.94(18)	C(15)-C(16)-C(17)-C(12)	-0.3(4)
C(25)-O(7)-C(4)-C(3)	-160.89(13)	C(13)-C(12)-C(17)-C(16)	1.3(3)
C(25)-O(7)-C(4)-C(5)	78.53(17)	C(11)-C(12)-C(17)-C(16)	-175.6(2)
O(6)-C(3)-C(4)-O(7)	70.04(17)	C(3)-O(6)-C(18)-C(19)	-139.12(16)
C(2)-C(3)-C(4)-O(7)	-172.32(13)	O(6)-C(18)-C(19)-C(20)	61.8(3)
O(6)-C(3)-C(4)-C(5)	-168.99(14)	O(6)-C(18)-C(19)-C(24)	-121.1(2)
C(2)-C(3)-C(4)-C(5)	-51.34(17)	C(24)-C(19)-C(20)-C(21)	0.2(4)
C(32)-O(8)-C(5)-O(1)	99.30(18)	C(18)-C(19)-C(20)-C(21)	177.3(3)
C(32)-O(8)-C(5)-C(4)	-138.94(17)	C(19)-C(20)-C(21)-C(22)	-0.8(5)
C(1)-O(1)-C(5)-O(8)	59.34(17)	C(20)-C(21)-C(22)-C(23)	0.7(6)
C(1)-O(1)-C(5)-C(4)	-61.52(16)	C(21)-C(22)-C(23)-C(24)	0.1(5)
O(7)-C(4)-C(5)-O(8)	54.68(17)	C(20)-C(19)-C(24)-C(23)	0.7(3)
C(3)-C(4)-C(5)-O(8)	-64.87(18)	C(18)-C(19)-C(24)-C(23)	-176.5(2)
O(7)-C(4)-C(5)-O(1)	177.38(12)	C(22)-C(23)-C(24)-C(19)	-0.8(4)
C(3)-C(4)-C(5)-O(1)	57.83(17)	C(4)-O(7)-C(25)-C(26)	89.4(2)
O(1)-C(1)-C(6)-C(7)	111.23(15)	O(7)-C(25)-C(26)-C(27)	-121.6(2)
C(2)-C(1)-C(6)-C(7)	-109.98(16)	O(7)-C(25)-C(26)-C(31)	60.4(3)
C(1)-C(6)-C(7)-C(8)	-110.48(16)	C(31)-C(26)-C(27)-C(28)	1.0(3)
O(1)-C(1)-C(7)-C(8)	-0.5(3)	C(25)-C(26)-C(27)-C(28)	-177.08(19)
C(6)-C(1)-C(7)-C(8)	104.62(19)	C(26)-C(27)-C(28)-C(29)	-0.8(3)
C(2)-C(1)-C(7)-C(8)	-145.91(17)	C(27)-C(28)-C(29)-C(30)	-0.7(4)
O(1)-C(1)-C(7)-C(6)	-105.11(18)	C(28)-C(29)-C(30)-C(31)	2.1(4)
C(2)-C(1)-C(7)-C(6)	109.47(18)	C(29)-C(30)-C(31)-C(26)	-1.9(3)
C(9)-O(3)-C(8)-O(2)	0.1(3)	C(27)-C(26)-C(31)-C(30)	0.4(3)
C(9)-O(3)-C(8)-C(7)	178.87(16)	C(25)-C(26)-C(31)-C(30)	178.5(2)

Symmetry transformations used to generate equivalent atoms

Experimental

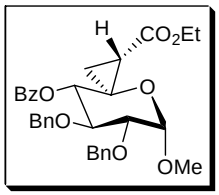
4,5-Bis(benzyloxy)-tetrahydro-6-methoxy-2-methylene-2H-pyran-3-yl benzoate (40)



To a solution of **39** (4 g, 6.8 mmol) in pyridine (40 mL), AgF (3.3 g, 26.0 mmol) was added and the reaction mixture was stirred for approximately 6 h at r.t. The reaction was monitored by TLC, and after completion it was diluted with diethyl ether, filtered and the solvents were evaporated under vacuum. The mixture was purified by column chromatography using 10% EtOAc in pet. ether to furnish **40** as a thick liquid.

Yield	: 2.5 g, 80%
Mol. Formula	: $C_{28}H_{28}O_6$
Optical Rotation $[\alpha]_D^{25}$	: –33.3 (*c* 0.6, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3064, 3031, 1729, 1666, 1602, 1496, 1453, 1315, 1269, 1162, 1096, 1071, 1028, 935, 711, 698 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 3.46 (s, 3H), 3.74 (dd, *J* = 9.5, 3.3 Hz, 1H), 4.10 (t, *J* = 9.4 Hz, 1H), 4.52 (t, *J* = 1.8 Hz, 1H), 4.66-4.71 (m, 4H), 4.80-4.87 (m, 2H), 5.64 (td, *J* = 9.3, 2.1 Hz, 1H), 7.13 (br s, 5H), 7.31-7.37 (m, 5H), 7.41-7.48 (m, 2H), 7.54-7.63 (m, 1H), 8.03-8.08 (m, 2H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 55.57 (q), 71.51 (d), 73.59 (t), 75.19 (t), 78.64 (d), 79.06 (d), 96.34 (t), 99.00 (d), 127.45 (d), 127.87 (d), 127.96 (d), 128.03 (d), 128.10 (d), 128.41 (d), 129.38 (s), 129.76 (d), 133.25 (d), 137.70 (s), 137.90 (s), 151.17 (s), 165.07 (s) ppm.
Elemental Analysis	Calcd.: C, 73.03; H, 6.13 Found : C, 73.32; H, 6.00
ESI-MS (*m/z*)	: 483.2 $[M+Na]^+$.

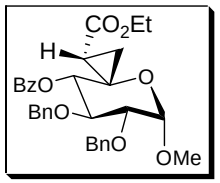
(1*S*,3*R*,6*S*,7*R*,8*S*)-Ethyl 8-(benzoyloxy)-6,7-bis(benzyloxy)-5-methoxy-4-oxaspiro[2.5]octane-1-carboxylate (41c)



The exocyclic methylene compound **40** (3.5 g, 7.6 mmol) was dissolved into anhydrous toluene (20 mL) and Cu-powder (0.39 g, 6.09 mmol) was added to it. This heterogeneous reaction mixture was heated at 100 ^{o}C, and to it a solution of ethyl diazoacetate (4.0 mL, 38.0 mmol) in toluene (18 mL) was added for 8 h by using syringe pump. After completion of the reaction it was concentrated and purified by column chromatography to furnish (**41a**, **41b**, **41c** & **41d**), collectively in 85% yield. **41c** was separated by flash column chromatography using 15% ethylacetate in pet. ether and obtained as a thick liquid.

Yield	: 1.4 g, 34%
Mol. Formula	: $C_{32}H_{34}O_8$
Optical Rotation $[\alpha]_D^{23}$	: +22.7 (*c* 1.5, $CHCl_3$)
^{1}H NMR ($CDCl_3$, 400 MHz)	: δ 1.24 (t, *J* = 7.2 Hz, 3H), 1.40 (dd, *J* = 8.9, 5.9 Hz, 1H), 1.65-1.68 (m, 1H), 1.72 (dd, *J* = 8.8, 7.0 Hz, 1H), 3.48 (s, 3H), 3.72 (dd, *J* = 8.3, 3.3 Hz, 1H), 4.03 (t, *J* = 8.0 Hz, 1H), 4.07-4.22 (m, 2H), 4.60-4.71 (m, 3H), 4.76-4.80 (m, 2H), 5.39 (d, *J* = 7.8 Hz, 1H), 7.14 (br s, 5H), 7.28-7.35 (m, 5H), 7.37-7.41 (m, 2H), 7.54-7.58 (m, 1H), 7.92-7.95 (m, 2H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 14.24 (q), 14.71 (t), 21.02 (d), 56.76 (q), 60.16 (s), 60.73 (t), 70.48 (d), 73.68 (t), 74.67 (t), 77.97 (d), 78.40 (d), 100.06 (d), 127.49 (d), 127.72 (d), 127.88 (s), 128.07 (d), 128.15 (d), 128.40 (d), 129.38 (s), 129.78 (d), 133.27 (d), 137.89 (s), 137.98 (s), 165.14 (s), 168.78 (s) ppm.
Elemental Analysis	Calcd.: C, 70.31; H, 6.27 Found : C, 70.21; H, 6.05
ESI-MS (*m/z*)	: 569.2 $[M+Na]^+$.

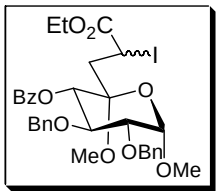
(1*R*,3*S*,6*S*,7*R*,8*S*)-Ethyl 8-(benzoyloxy)-6,7-bis(benzyloxy)-5-methoxy-4-oxaspiro[2.5]octane-1-carboxylate (41d)



41d was obtained as a crystalline solid by using the same procedure as described above for **41c**.

Yield	: 0.79 g, 18.9%
Mol. Formula	: $C_{32}H_{34}O_8$
Melting Point	: 135 ^oC
Optical Rotation $[\alpha]_D^{23}$	: –137.5 (*c* 0.8, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3064, 3029, 2925, 1729, 1718, 1600, 1454, 1377, 1315, 1287, 1257, 1180, 1152, 1099, 1074, 754, 738, 720, 707, 695 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.02 (dd, *J* = 9.2, 6.4 Hz, 1H), 1.30 (t, *J* = 7.2 Hz, 3H), 1.56 (t, *J* = 6.8 Hz, 1H), 2.08 (dd, *J* = 9.0, 7.0 Hz, 1H), 3.38 (s, 3H), 3.68 (dd, *J* = 8.3, 3.3 Hz, 1H), 4.04 (t, *J* = 8.3 Hz, 1H), 4.08 (dd, *J* = 10.8, 7.3 Hz, 1H), 4.26 (dd, *J* = 10.8, 7.0 Hz, 1H), 4.61-4.68 (m, 3H), 4.76 (d, *J* = 4.8 Hz, 1H), 4.78 (d, *J* = 4.3 Hz, 1H), 5.37 (d, *J* = 8.3 Hz, 1H), 7.11-7.15 (m, 5H), 7.27-7.31 (m, 5H), 7.42 (t, *J* = 7.8 Hz, 2H), 7.55-7.59 (m, 1H), 7.97-7.99 (m, 2H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 14.00 (t), 14.29 (q), 22.67 (d), 58.90 (q), 60.56 (t), 61.72 (s), 70.74 (d), 73.85 (t), 74.80 (t), 77.64 (d), 78.76 (d), 101.28 (d), 127.53 (d), 127.65 (d), 127.92 (d), 128.05 (d), 128.19 (d), 128.43 (d), 129.38 (s), 129.84 (d), 133.33 (d), 137.99 (s), 165.24 (s), 169.32 (s) ppm.
Elemental Analysis	Calcd.: C, 70.31; H, 6.27 Found : C, 70.45; H, 6.17
ESI-MS (*m/z*)	: 569.4 $[M+Na]^+$.

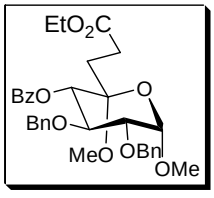
2-(2-(Ethoxycarbonyl)-2-iodoethyl)-4,5-bis(benzyloxy)-tetrahydro-2,6-dimethoxy-2H-pyran-3-yl benzoate (47)



To a cooled solution of **41d** (0.5 g, 0.9 mmol) in MeOH (1 mL) at 0 ^{o}C was added NIS (1.03 g, 4.6 mmol) and the reaction mixture was kept at r.t. for 48 h stirring. After the consumption of the starting material it was concentrated and purified by column chromatography (12% EtOAc in pet. ether as eluent) to give a thick liquid (**47**).

Yield	: 0.4 g, 65%
Mol. Formula	: $C_{33}H_{37}IO_9$
Optical Rotation $[\alpha]_D^{23}$	: –4.4 (*c* 0.9, $CHCl_3$)
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.08 (t, J = 7.1 Hz, 3H), 2.70 (dd, J = 14.3, 1.6 Hz, 1H), 3.18 (dd, J = 14.3, 11.8 Hz, 1H), 3.21 (s, 3H), 3.61 (s, 3H), 3.66 (dd, J = 9.2, 3.9 Hz, 1H), 3.94 (t, J = 9.0 Hz, 1H), 4.00-4.10 (m, 2H), 4.52 (dd, J = 11.7, 1.6 Hz, 1H), 4.61-4.83 (m, 5H), 5.38 (d, J = 8.7 Hz, 1H), 7.08-7.19 (m, 5H), 7.27-7.35 (m, 5H), 7.41-7.49 (m, 2H), 7.54-7.63 (m, 1H), 8.00-8.05 (m, 2H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 13.61 (d), 14.58 (q), 42.50 (t), 48.79 (q), 57.76 (q), 61.30 (t), 69.87 (d), 73.77 (t), 75.09 (t), 76.57 (d), 79.07 (d), 99.64 (d), 102.49 (s), 127.59 (d), 127.87 (d), 128.03 (d), 128.19 (d), 128.50 (d), 128.53 (d), 129.57 (s), 129.83 (d), 133.28 (d), 137.82 (s), 137.85 (s), 165.18 (s), 171.62 (s) ppm.
ESI-MS (*m/z*)	: 727.3 $[M+Na]^+$.

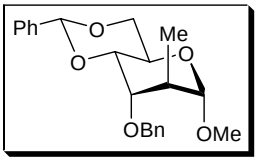
2-(2-(Ethoxycarbonyl)ethyl)4,5-bis(benzyloxy)-tetrahydro-2,6-dimethoxy-2H-pyran-3-yl benzoate (48)



A solution of **47** (0.1 g, 0.14 mmol) in anhydrous toluene (2 mL) was properly degassed under argon, and to it TBTH (0.08 mL, 0.28 mmol) and catalytic AIBN were added by simultaneously degassing. Then the reaction mixture was refluxed for 3 h and after completion of the reaction it was concentrated and purified by column chromatography (eluent: 20% EtOAc in pet. ether) to furnish **48** as a thick liquid.

Yield	: 65 mg, 80%
Mol. Formula	: $C_{33}H_{38}O_9$
Optical Rotation $[\alpha]_D^{23}$	: +11.4 (*c* 2.1, $CHCl_3$)
^{1}H NMR ($CDCl_3$, 400 MHz)	: δ 1.18 (t, *J* = 7.2 Hz, 3H), 2.19-2.35 (m, 2H), 2.37-2.40 (m, 2H), 3.28 (s, 3H), 3.55 (s, 3H), 3.76 (dd, *J* = 7.7, 3.3 Hz, 1H), 3.96 (dd, *J* = 7.4, 5.8 Hz, 1H), 4.06 (quartet, *J* = 7.2 Hz, 2H), 4.66 (d, *J* = 12.1 Hz, 1H), 4.71-4.78 (m, 4H), 5.37 (d, *J* = 5.8 Hz, 1H), 7.18-7.20 (m, 5H), 7.28-7.30 (m, 5H), 7.41-7.44 (m, 2H), 7.57 (t, *J* = 7.4 Hz, 1H), 8.04 (d, *J* = 7.4 Hz, 2H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 14.10 (q), 27.91 (t), 28.77 (t), 48.32 (q), 57.07 (q), 60.30 (t), 70.18 (d), 73.59 (t), 73.82 (t), 77.29 (d), 77.64 (d), 98.30 (d), 101.73 (s), 127.57 (d), 127.82 (d), 127.94 (d), 128.00 (d), 128.22 (d), 128.36 (d), 128.41 (d), 129.64 (s), 129.90 (d), 133.24 (d), 137.92 (s), 138.01 (s), 165.19 (s), 173.43 (s) ppm.
Elemental Analysis	Calcd.: C, 68.50; H, 6.62 Found : C, 68.32; H, 6.44
ESI-MS (*m/z*)	: 601.2 $[M+Na]^+$.

8-(Benzyloxy)-hexahydro-6-methoxy-7-methyl-2-phenylpyrano[3,2-d][1,3]dioxine (52)

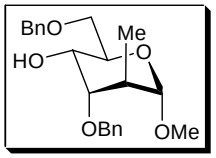


Compound **51** (5 g, 17.8 mmol) was dissolved in 5 mL anhydrous DMF and cooled to 0 ^oC. To this reaction mixture NaH (1.07 g, 26.8 mmol) was added slowly, after

sometime benzyl bromide (2.3 mL, 19.6 mmol) was added and stirred for 1 h. The reaction was monitored by TLC and after its completion it was quenched with ice water; organic layer was separated and the aqueous layer was extracted with EA. Combined organic layers were dried over sodium sulphate, concentrated and purified by column chromatography using 12% EtOAc in pet. ether as eluent to furnish **52** as a solid.

Yield	: 5.9 g, 90%
Mol. Formula	: $C_{22}H_{26}O_5$
Melting Point	: 106 ^oC
Optical Rotation $[\alpha]_D^{25}$	: +40.3 (*c* 0.79, $CHCl_3$)
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.10 (d, *J* = 7.7 Hz, 3H), 2.36 (ddd, *J* = 9.8, 7.7, 2.2 Hz, 1H), 3.40 (s, 3H), 3.69-3.79 (m, 2H), 3.82 (dd, *J* = 9.5, 2.9 Hz, 1H), 4.32 (dd, *J* = 10.1, 5.3 Hz, 1H), 4.38-4.51 (m, 2H), 4.76 (d, *J* = 12.9 Hz, 1H), 4.84 (d, *J* = 12.9 Hz, 1H), 5.56 (s, 1H), 7.26-7.30 (m, 3H), 7.33-7.42 (m, 5H), 7.47-7.52 (m, 2H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 16.56 (q), 38.74 (d), 55.45 (q), 58.37 (d), 69.50 (t), 72.21 (t), 75.98 (d), 77.25 (d), 102.18 (d), 103.00 (d), 126.16 (d), 127.01 (d), 127.29 (d), 128.02 (d), 128.14 (d), 128.88 (d), 137.72 (s), 139.07 (s) ppm.
Elemental Analysis	Calcd.: C, 71.33; H, 7.07 Found : C, 71.59; H, 6.85
ESI-MS (*m/z*)	: 393.4 $[M+Na]^+$.

4-(Benzyloxy)-2-((benzyloxy)methyl)-tetrahydro-6-methoxy-5-methyl-2H-pyran-3-ol (53)

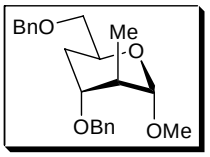


Compound **52** (3 g, 8.1 mmol) was dissolved in CH_2Cl_2 (22.5 mL) and to it triethylsilane (6.5 mL, 40.5 mmol) was added. Then the reaction mixture was cooled to 0 ^oC and TFA (3.1 mL, 40.5 mmol) was added dropwise. The reaction was monitored by TLC and after its completion it was diluted with EtOAc, washed with

satd. $NaHCO_3$, brine and then dried over sodium sulphate. The filtrate was concentrated and purified by column chromatography using 15% EtOAc in pet. ether as eluent to give **53** as a pasty mass.

Yield	: 2.5 g, 84%
Mol. Formula	: $C_{22}H_{28}O_5$
Optical Rotation $[\alpha]_D^{26}$	: +83.3 (*c* 1.2, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3546, 3088, 3065, 3012, 2930, 1605, 1497, 1454, 1390, 1364, 1276, 1106, 1066, 1027, 965, 756, 698 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.06 (d, *J* = 7.3 Hz, 3H), 2.29-2.42 (m, 1H), 3.39 (s, 3H), 3.56 (t, *J* = 3.9 Hz, 1H), 3.71-3.88 (m, 3H), 3.98-4.07 (m, 1H), 4.44 (d, *J* = 11.5 Hz, 1H), 4.46 (s, 1H), 4.62 (br s, 2H), 4.76 (d, *J* = 11.5 Hz, 1H), 7.32-7.38 (m, 10H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 15.27 (q), 34.78 (d), 55.30 (q), 64.04 (d), 69.31 (d), 70.22 (t), 70.94 (t), 73.34 (t), 78.43 (d), 102.80 (d), 127.39 (d), 127.42 (d), 127.82 (d), 127.95 (d), 128.24 (d), 128.43 (d), 137.97 (s), 138.42 (s) ppm.
Elemental Analysis	Calcd.: C, 70.94; H, 7.58 Found : C, 70.64; H, 7.85
ESI-MS (*m/z*)	: 395.2 $[M+Na]^+$.

4-(Benzyloxy)-6-((benzyloxy)methyl)-tetrahydro-2-methoxy-3-methyl-2H-pyran (54)



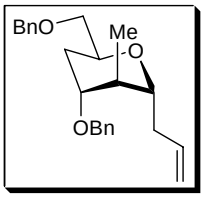
NaH (0.26 g, 6.4 mmol) was added slowly to a solution of **53** (2 g, 5.4 mmol) in anhydrous THF (20 mL) cooled at 0 ^oC, and the reaction mixture was stirred for 30 mins. Then CS_2 (0.8 mL, 13.4 mmol) was added and again stirred for 15 mins. Finally, MeI (0.84 mL, 13.4 mmol) was added and the reaction mixture was stirred for 1-2 h. After completion of the reaction it was quenched with cold water, extracted in EA, concentrated and column was done fastly (15% EtOAc in pet. ether as eluent).

The xanthate derivative (2.3 g, 5.0 mmol) was dissolved in anhydrous toluene (25 mL) and degassed with argon. To it TBTH (2.0 mL, 7.5 mmol) and catalytic

AIBN were added followed with proper degassing again. Finally, this reaction mixture was refluxed for 6 h, cooled, concentrated and purified by column chromatography (eluent 12% EtOAc in pet. ether) to give **54** as a thick liquid.

Yield	: 1.3 g, 70% after two steps.
Mol. Formula	: $C_{22}H_{28}O_4$
Optical Rotation $[\alpha]_D^{23}$	: +31.4 (*c* 0.7, $CHCl_3$)
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.03 (d, *J* = 7.3 Hz, 3H), 1.63-1.69 (m, 1H), 1.84 (ddd, *J* = 14.2, 10.8, 3.8 Hz, 1H), 2.04-2.11 (m, 1H), 3.41 (s, 3H), 3.46 (d, *J* = 3.8 Hz, 1H), 3.51 (d, *J* = 4.9 Hz, 2H), 4.25-4.36 (m, 1H), 4.48-4.49 (m, 1H), 4.56-4.59 (m, 4H), 7.29-7.36 (m, 10H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 15.63 (q), 28.04 (t), 35.69 (d), 55.07 (q), 63.81 (d), 69.83 (t), 72.76 (t), 72.95 (t), 75.06 (d), 103.21 (d), 127.11 (d), 127.21 (d), 127.24 (d), 128.03 (d), 128.05 (d), 138.20 (s), 138.68 (s) ppm.
Elemental Analysis	Calcd.: C, 74.13; H, 7.92 Found : C, 74.01; H, 8.19
ESI-MS (m/z)	: 379.3 $[M+Na]^+$.

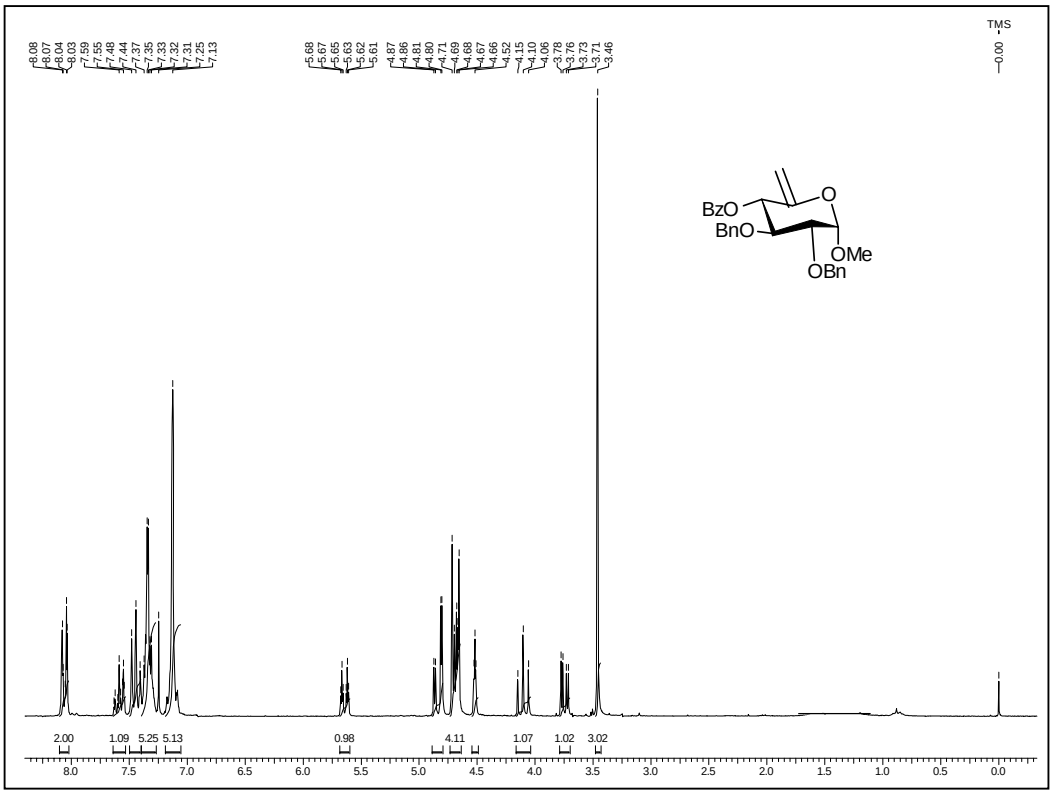
2-Allyl-4-(benzyloxy)-6-((benzyloxy)methyl)-tetrahydro-3-methyl-2H-pyran (55)



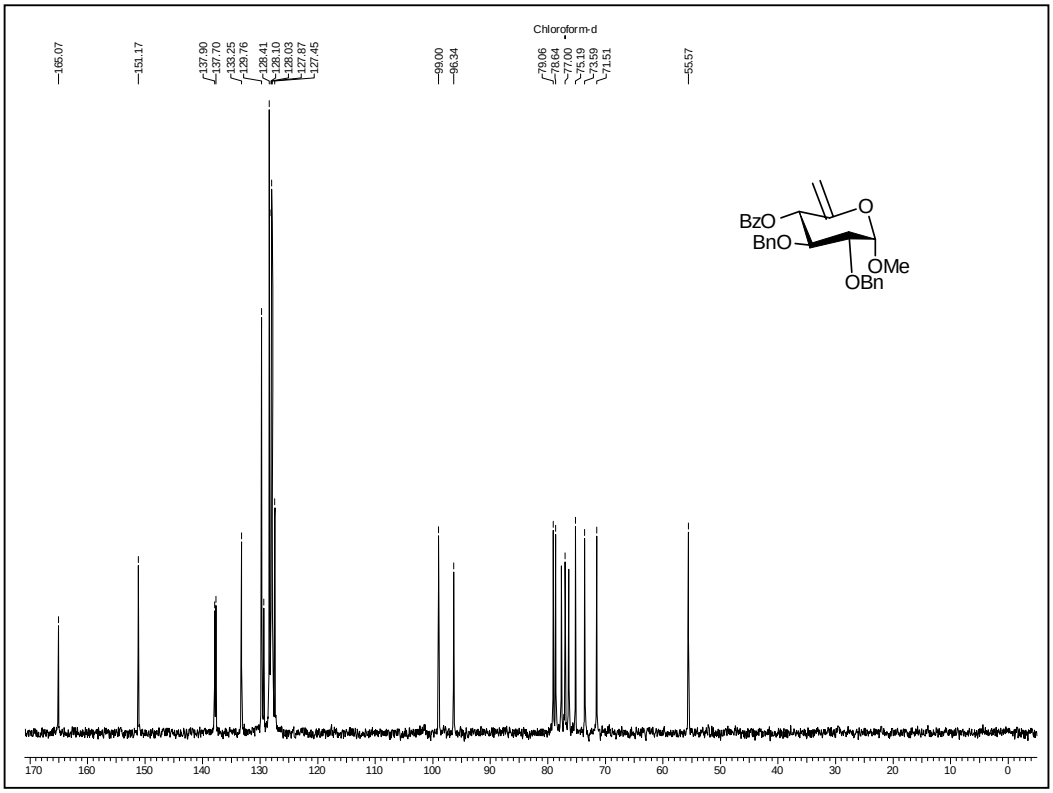
To a solution of **54** (1 g, 2.8 mmol) in anhydrous acetonitrile (10 mL) allyltrimethyl silane (1.8 mL, 11.2 mmol) was added. Then TMSOTf (0.24 mL, 1.4 mmol) was added to this reaction mixture after cooling to –20 ^oC (dry ice CCl_4 bath), and stirred for 1.5 h. After completion of the reaction the reaction mixture was quenched with aqueous satd. $NaHCO_3$ solution. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over sodium sulphate, concentrated and column was done (8% EtOAc in pet. ether as eluent) to furnish **55** as sticky mass.

Yield	: 0.98 g, 95%
Mol. Formula	: $C_{24}H_{30}O_3$
Optical Rotation $[\alpha]_D^{23}$	: −12.5 (*c* 0.8, $CHCl_3$)
^{1}H NMR ($CDCl_3$, 400 MHz)	: δ 0.98 (d, *J* = 6.8 Hz, 3H), 1.62-1.71 (m, 2H), 2.02 (td, *J* = 13.3, 4.5 Hz, 1H), 2.36-2.50 (m, 2H), 3.36 (ddd, *J* = 12.3, 8.3, 4.3 Hz, 1H), 3.41-3.45 (m, 1H), 3.48 (dd, *J* = 10.0, 5.5 Hz, 1H), 3.60 (dd, *J* = 10.0, 6.3 Hz, 1H), 4.23 (quintet, *J* = 5.5 Hz, 1H), 4.42, (d, *J* = 11.8 Hz, 1H), 4.54-4.59 (m, 3H), 5.03-5.10 (m, 2H), 5.84-5.94 (m, 1H), 7.24-7.36 (m, 10H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 14.62 (q), 30.32 (t), 37.27 (t), 38.64 (d), 69.40 (d), 70.45 (t), 71.10 (t), 73.11 (t), 75.95 (d), 76.90 (d), 116.35 (t), 127.43 (d), 127.50 (d), 128.27 (d), 128.29 (d), 135.37 (d), 138.29 (s), 138.64 (s) ppm.
Elemental Analysis	Calcd.: C, 78.65; H, 8.25 Found : C, 78.87; H, 8.17
ESI-MS (*m/z*)	: 389.3 $[M+Na]^+$.

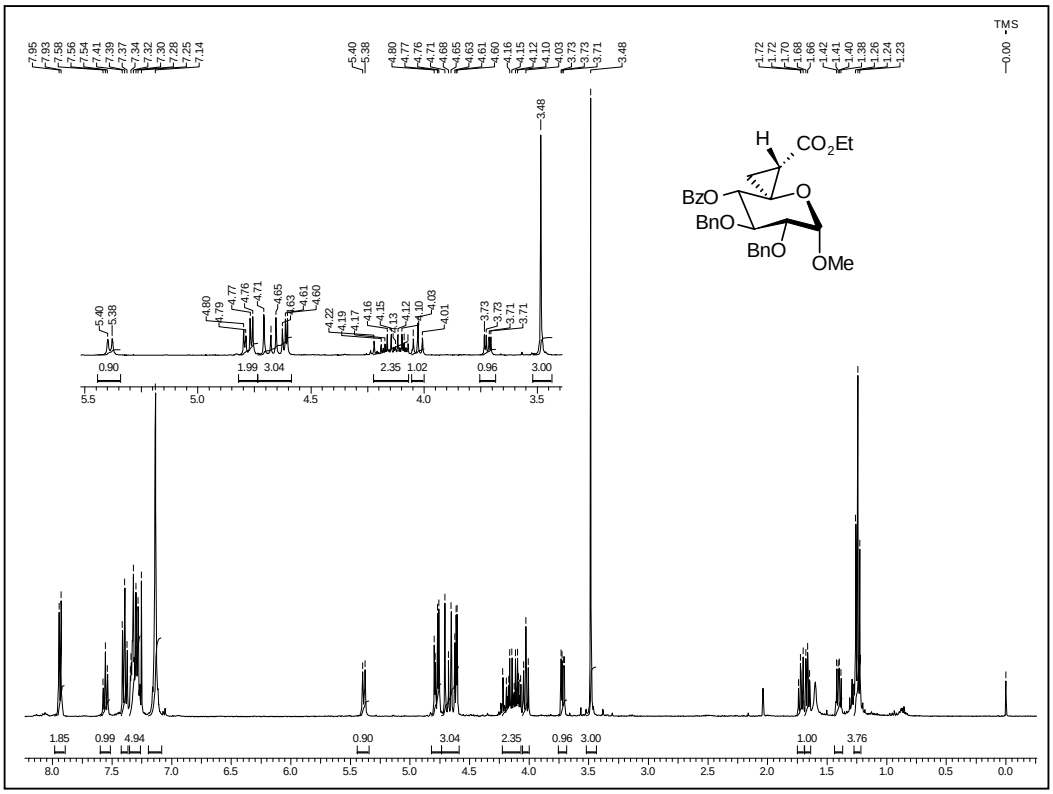
Spectra



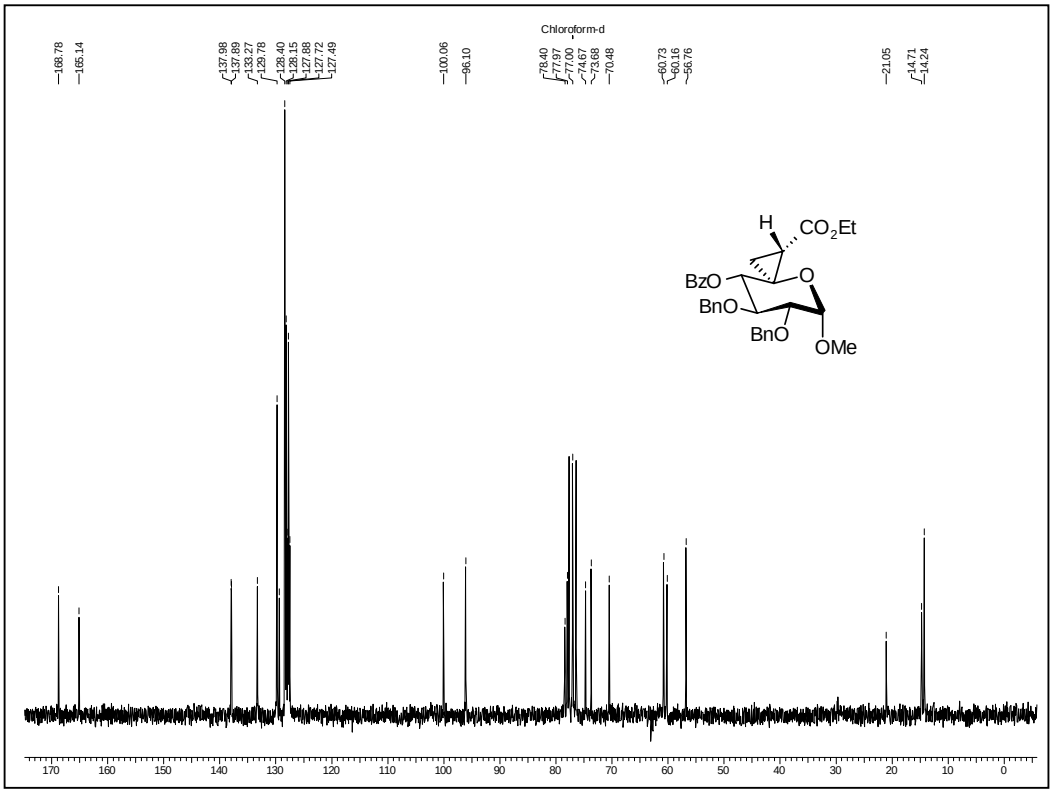
^{1}H NMR Spectrum of **40** in $CDCl_3$



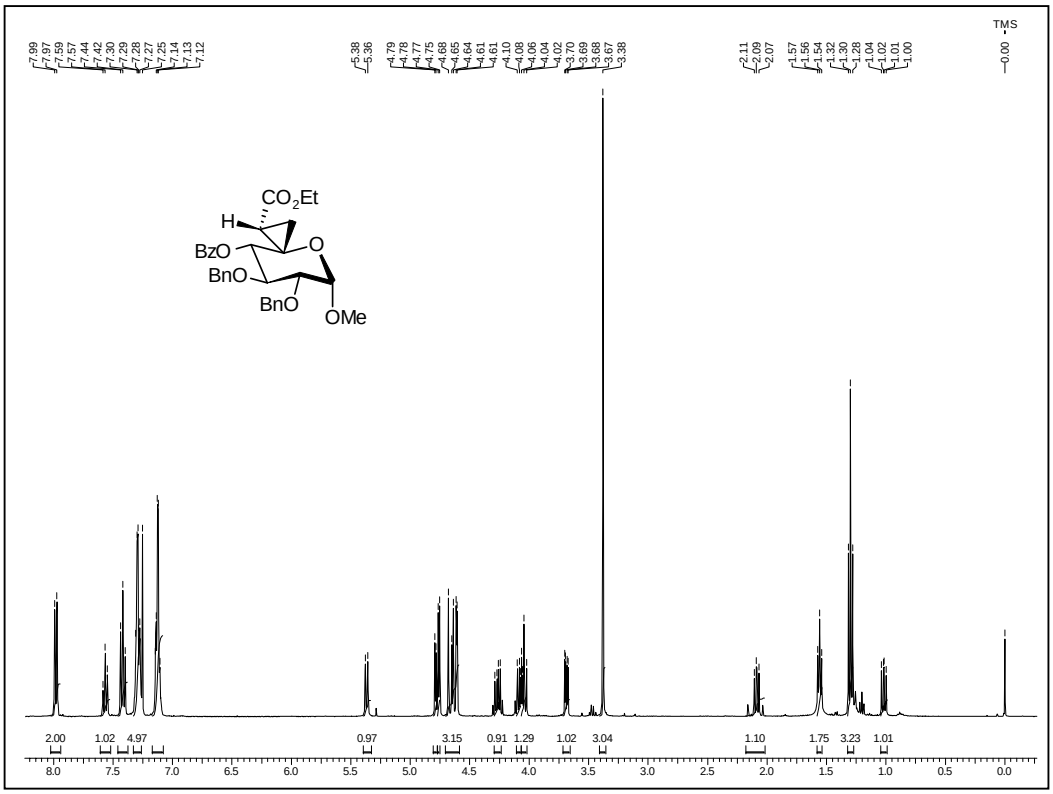
^{13}C NMR Spectrum of **40** in $CDCl_3$



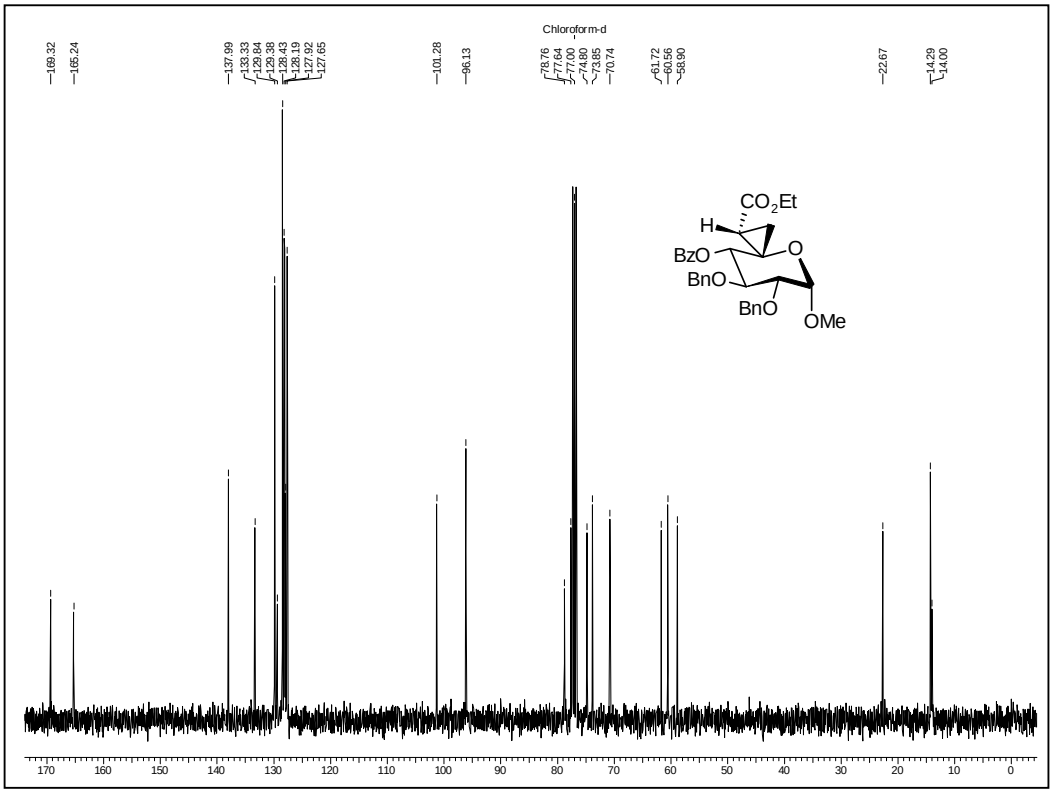
^{1}H NMR Spectrum of **41c** in $CDCl_3$



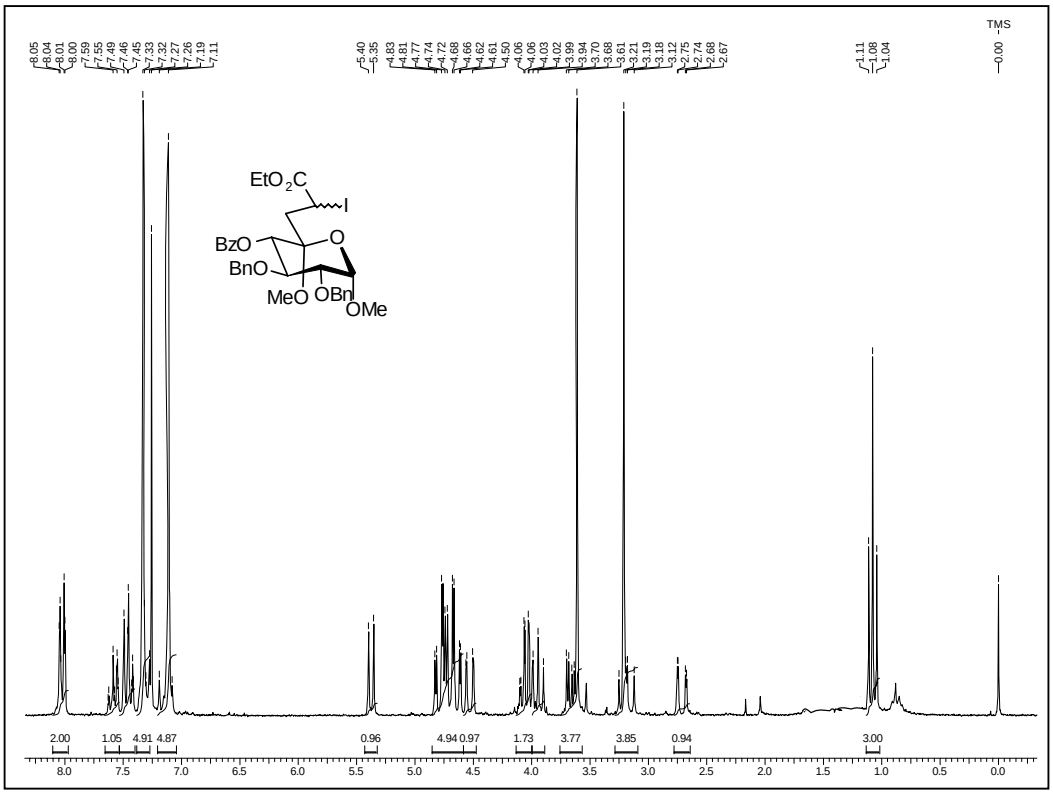
^{13}C NMR Spectrum of **41c** in $CDCl_3$



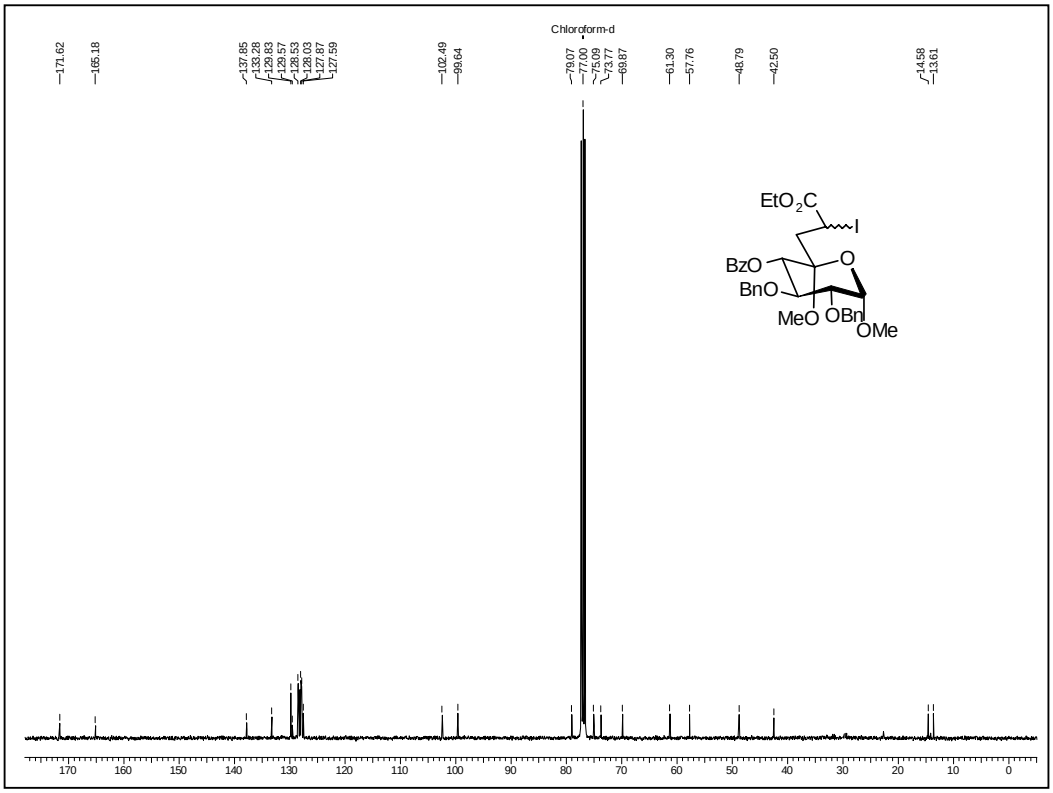
^{1}H NMR Spectrum of **41d** in $CDCl_3$



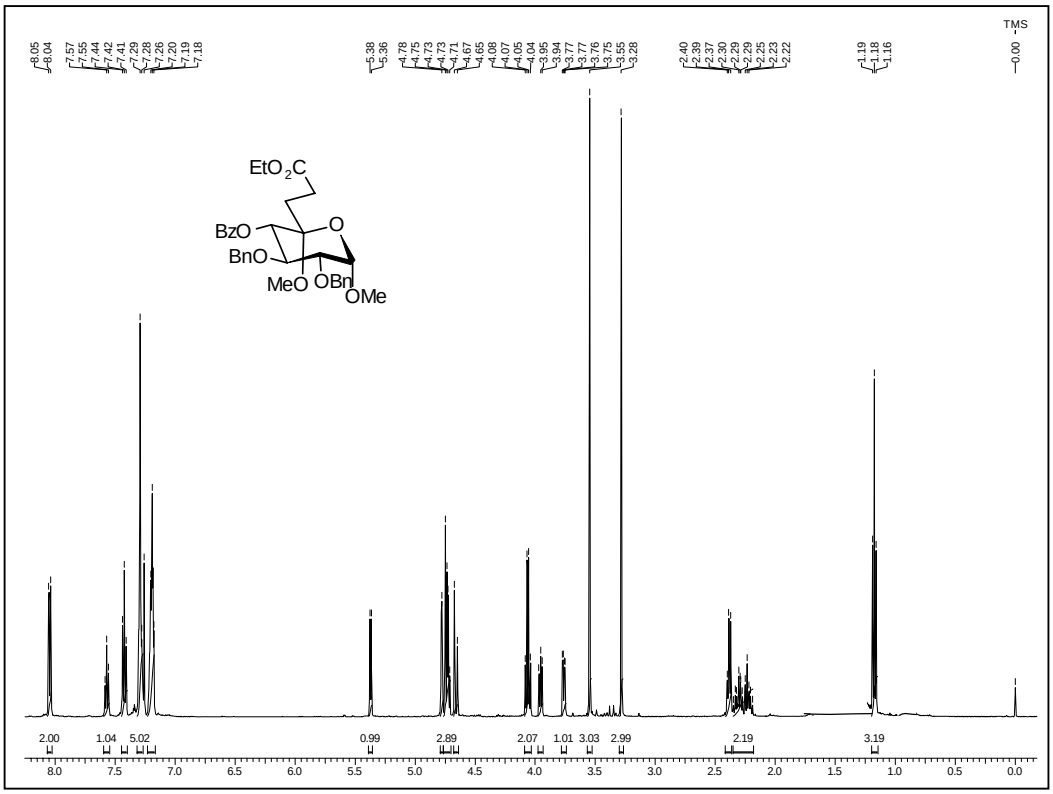
^{13}C NMR Spectrum of **41d** in $CDCl_3$



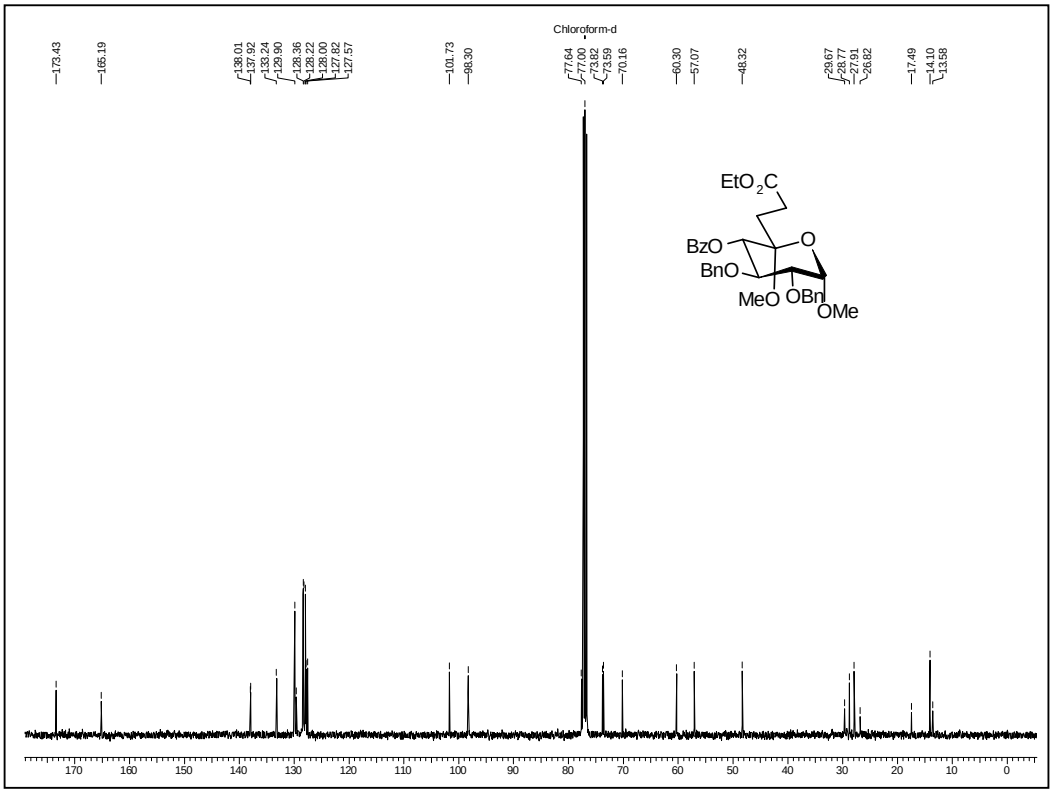
^{1}H NMR Spectrum of **47** in $CDCl_3$



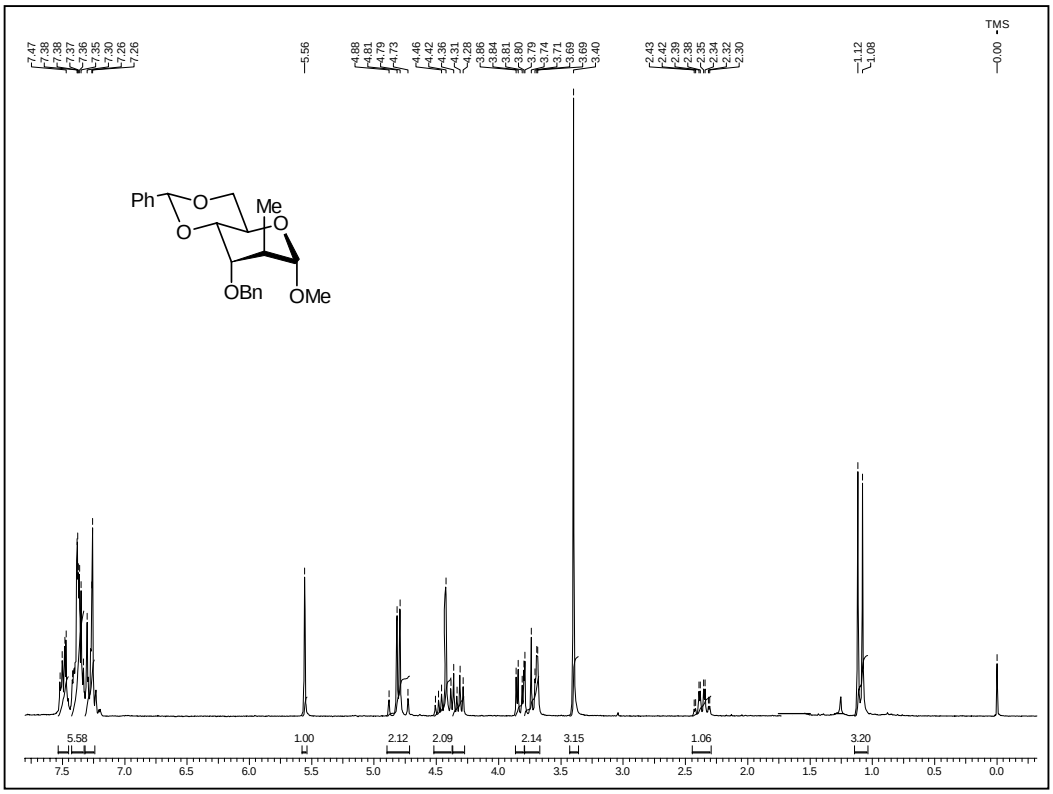
^{13}C NMR Spectrum of **47** in $CDCl_3$



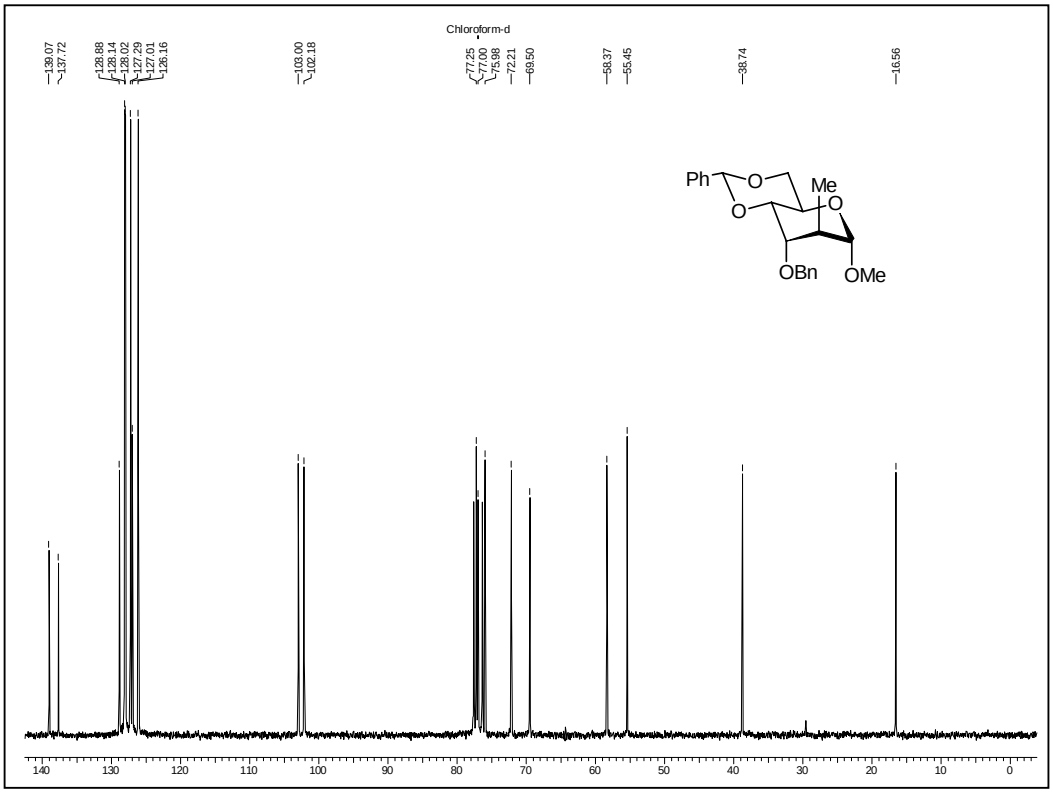
^{1}H NMR Spectrum of **48** in $CDCl_3$



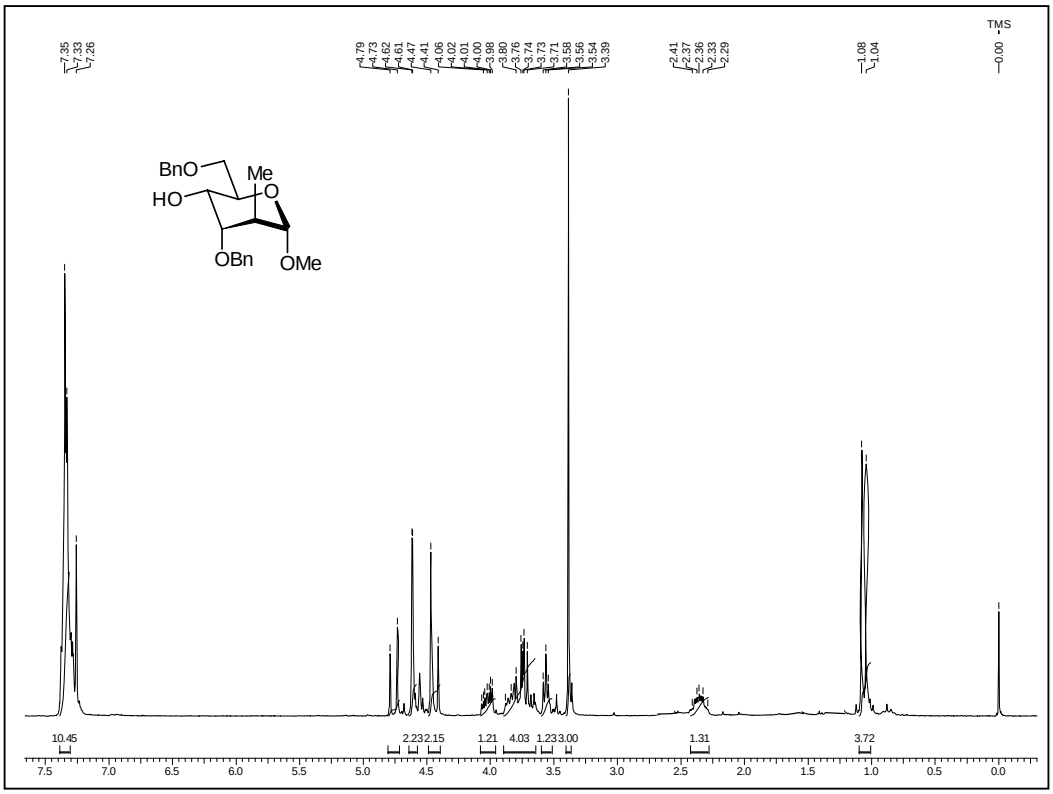
^{13}C NMR Spectrum of **48** in $CDCl_3$



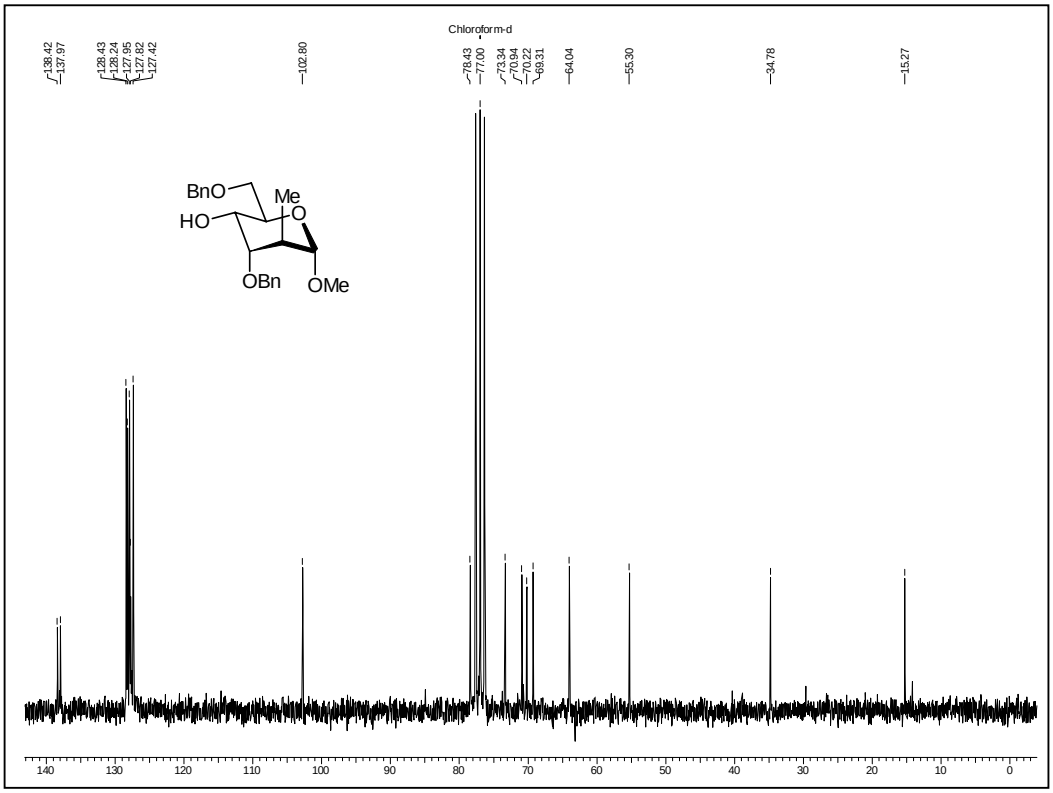
^{1}H NMR Spectrum of **52** in $CDCl_3$



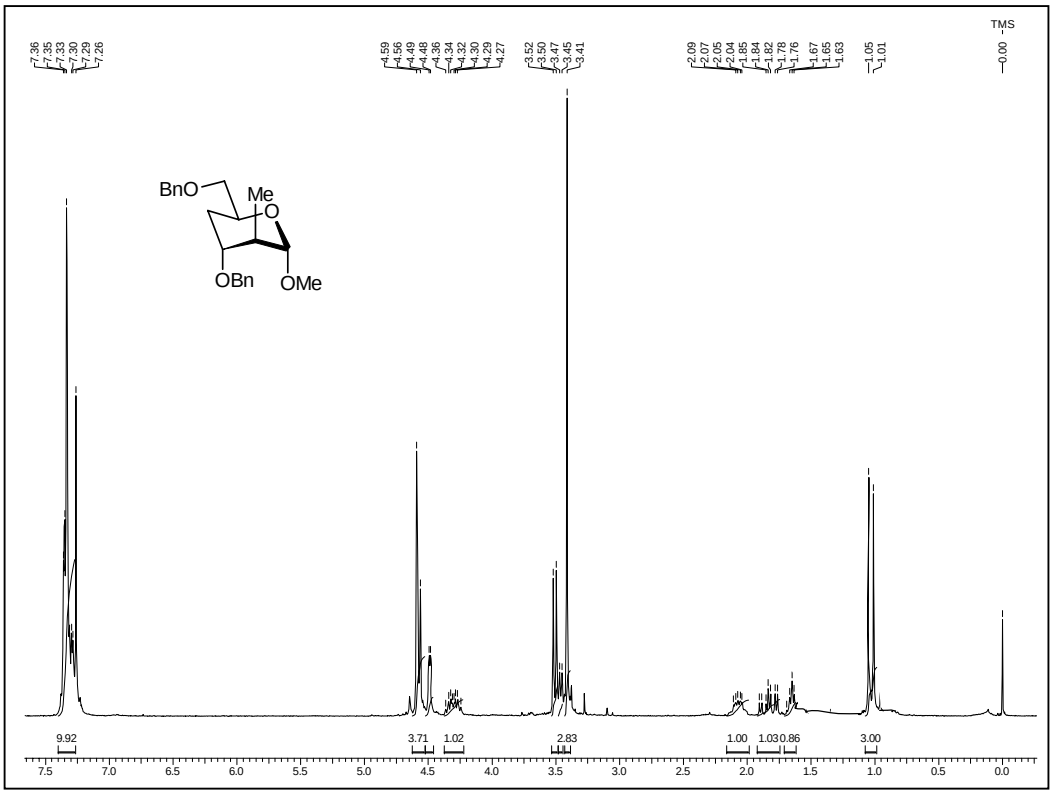
^{13}C NMR Spectrum of **52** in $CDCl_3$



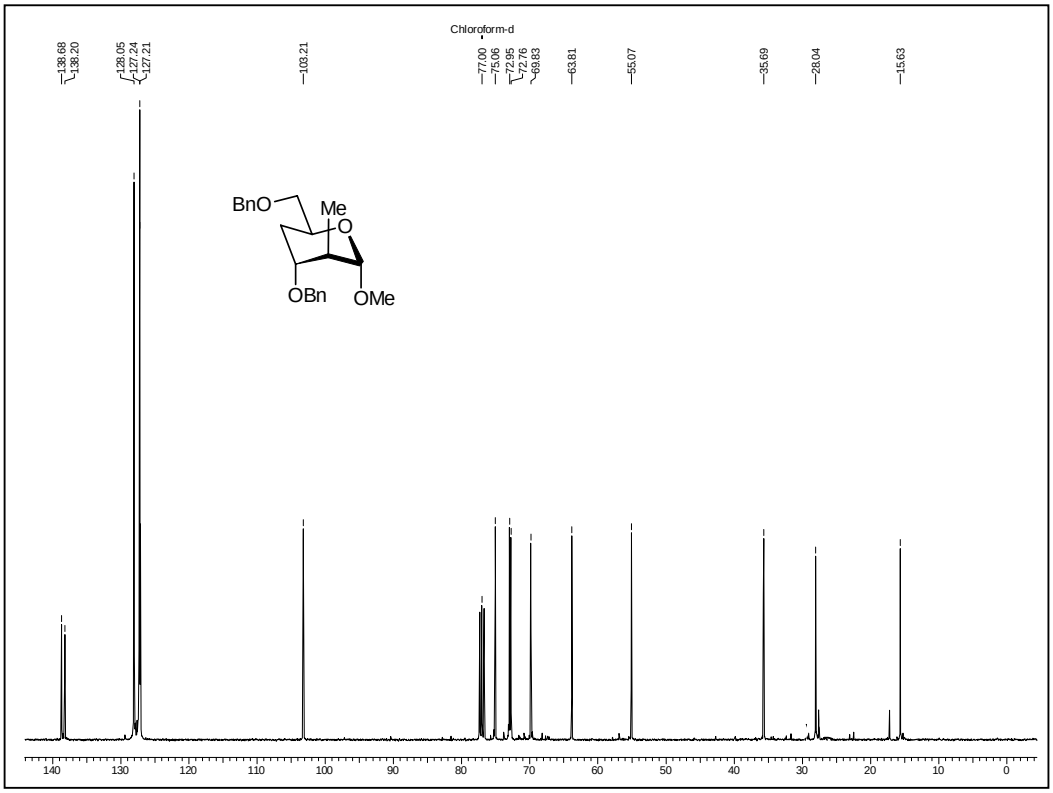
^{1}H NMR Spectrum of **53** in $CDCl_3$



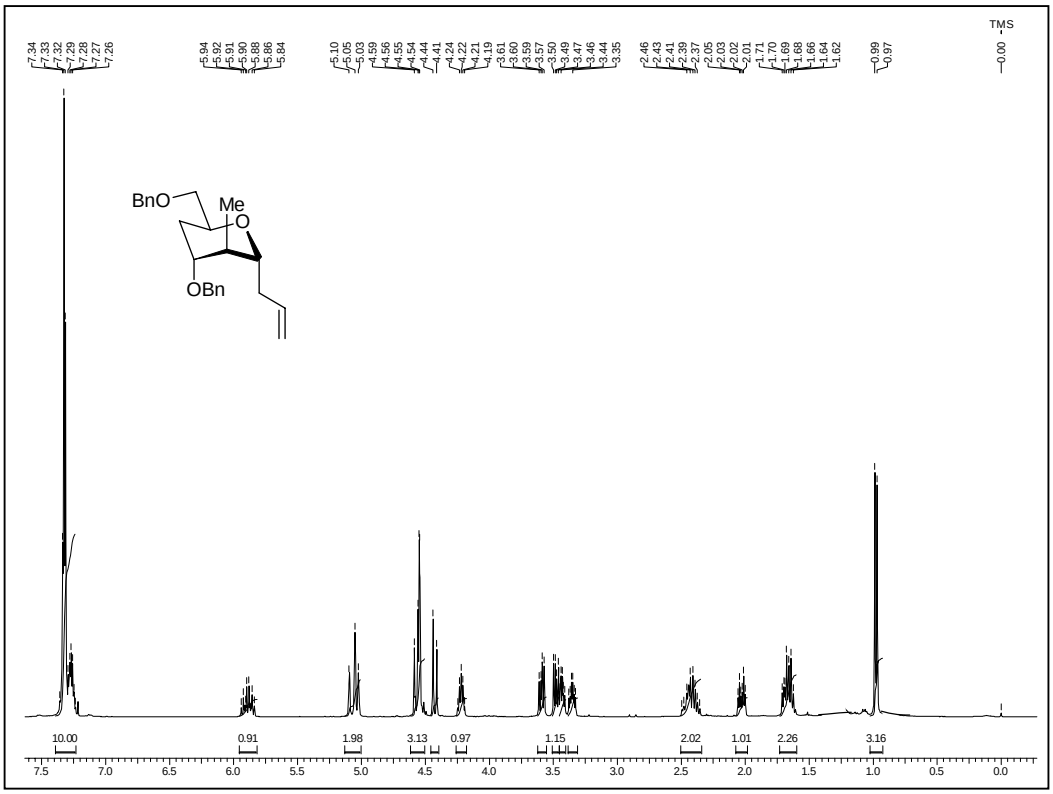
^{13}C NMR Spectrum of **53** in $CDCl_3$



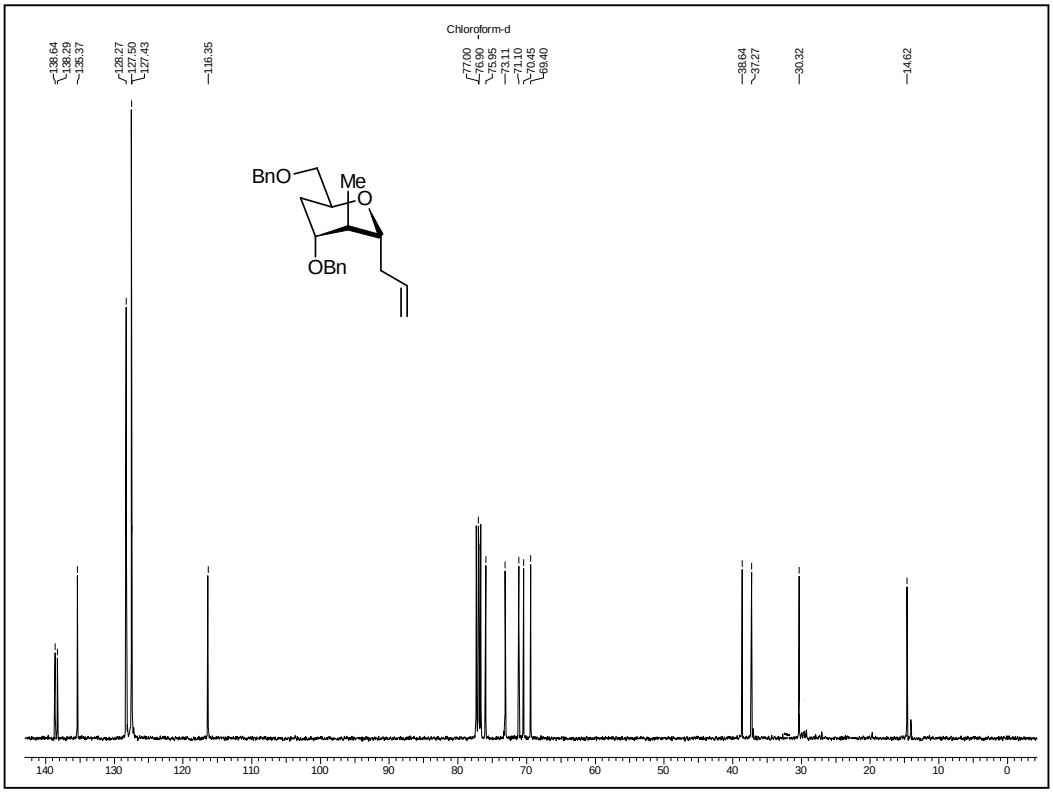
^{1}H NMR Spectrum of **54** in $CDCl_3$



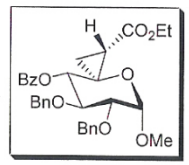
^{13}C NMR Spectrum of **54** in $CDCl_3$



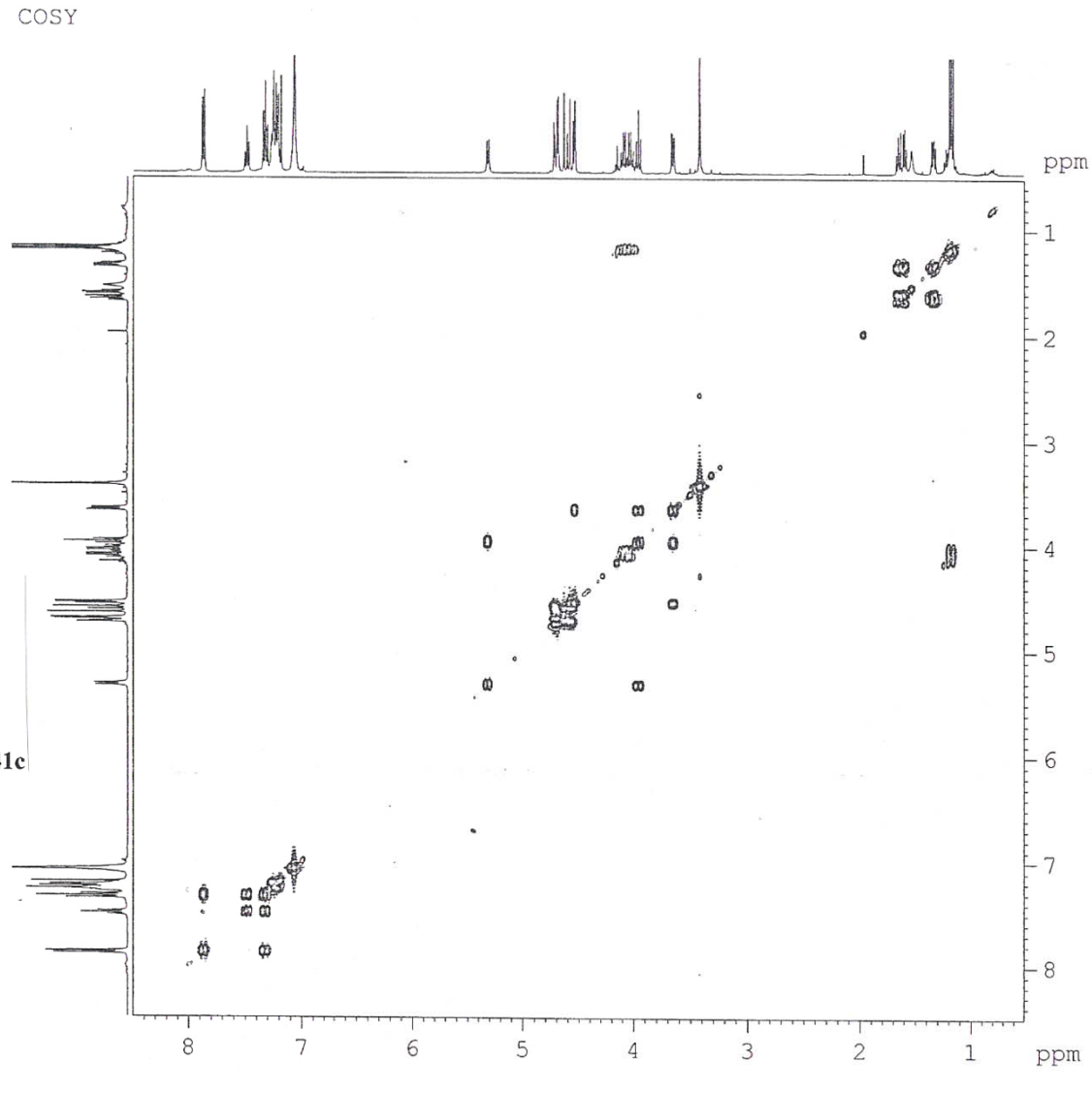
^{1}H NMR Spectrum of **55** in $CDCl_3$



^{13}C NMR Spectrum of **55** in $CDCl_3$



COSY of compound **41c**



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Current Data Parameters
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EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
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Time                13.35
INSTRUM             spect
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PULPROG          cosygpqf
TD                  1024
SOLVENT             CDCl3
NS                      4
DS                      2
SWH              3360.215 Hz
FIDRES           3.281460 Hz
AQ               0.1524212 sec
RG                    128
DW                148.800 usec
DE                 20.00 usec
TE                 299.7 K
d0             0.00000300 sec
D1             2.00000000 sec
d13            0.00000400 sec
D16            0.0001 000 sec
IN0            0.00 752 sec

======== CHANNEL f1 ========
NUC1                   1H
P0                  14.20 usec
P1                  14.20 usec
PL1                  1.00 dB
SFO1          400.1317500 MHz

====== GRADIENT CHANNEL =====
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GPNAM2            SINE.100
GPZ1                10.00 %
GPZ2                10.00 %
P16               1500.00 usec

F1 - Acquisition parameters
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SW                 8.400 ppm
FnMODE                 QF

F2 - Processing parameters
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SSB                     0
LB                   0.00 Hz
GB                      0
PC                   1.00

F1 - Processing parameters
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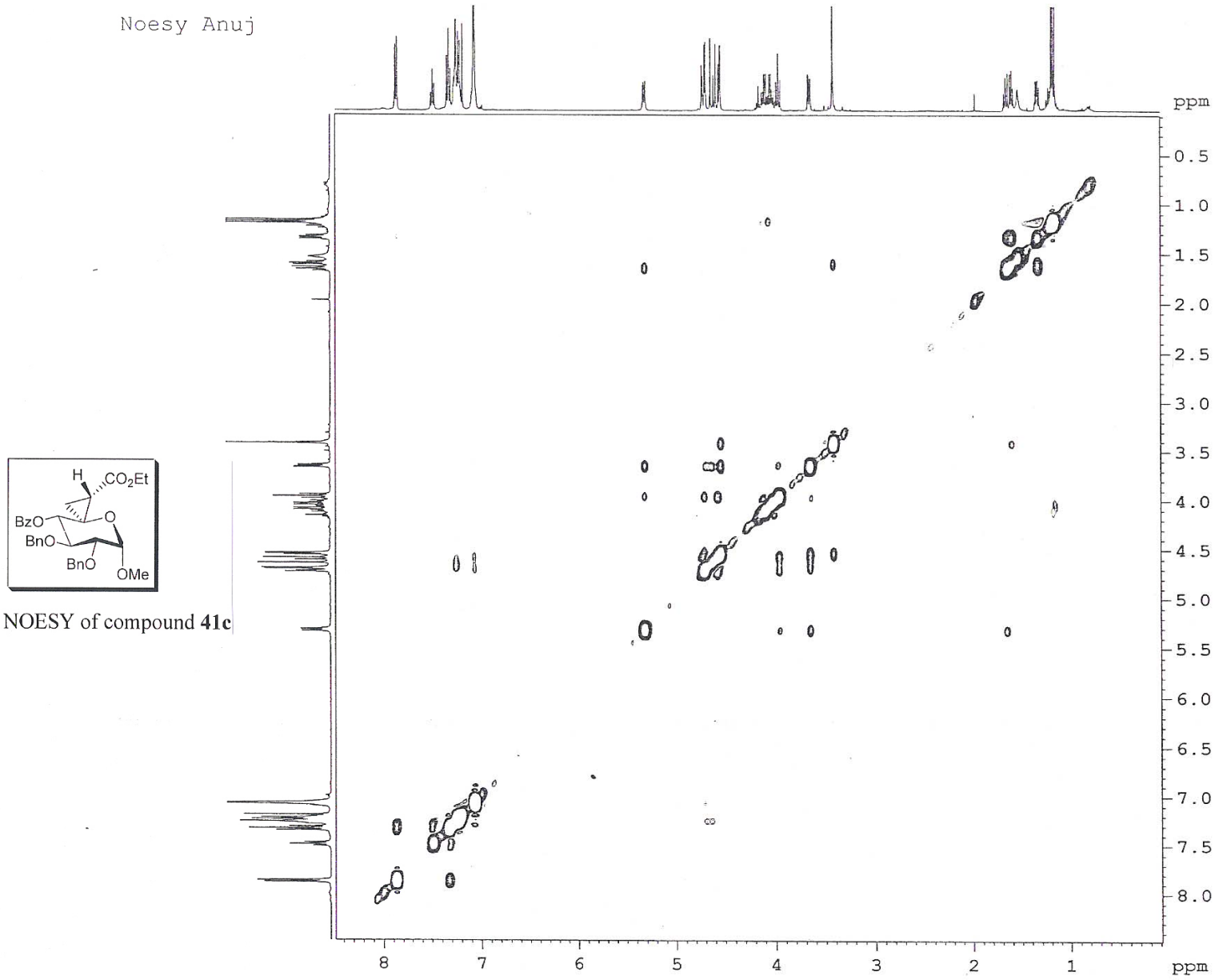

NOESY of compound **41c**

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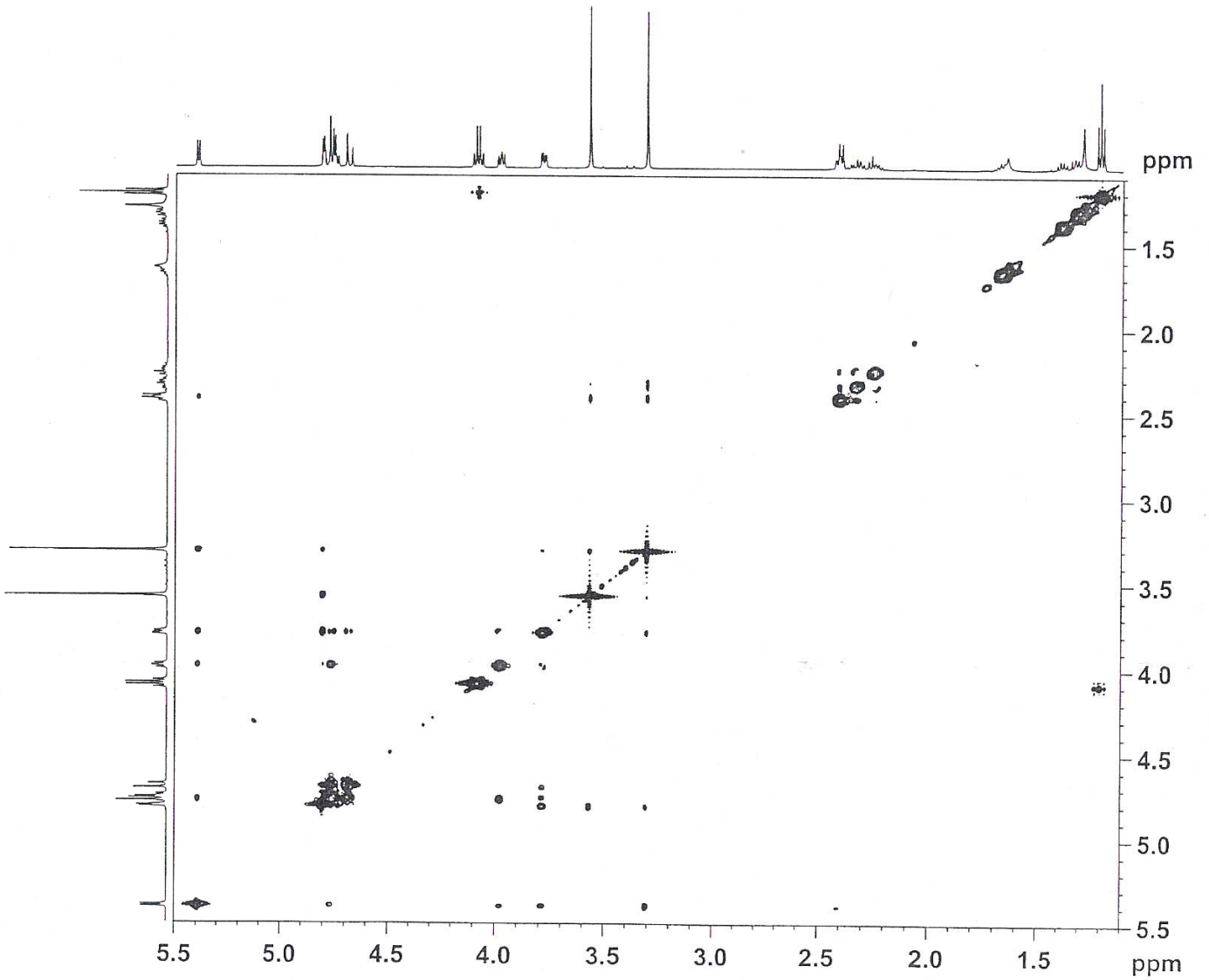
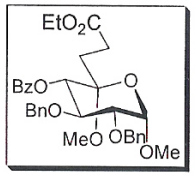
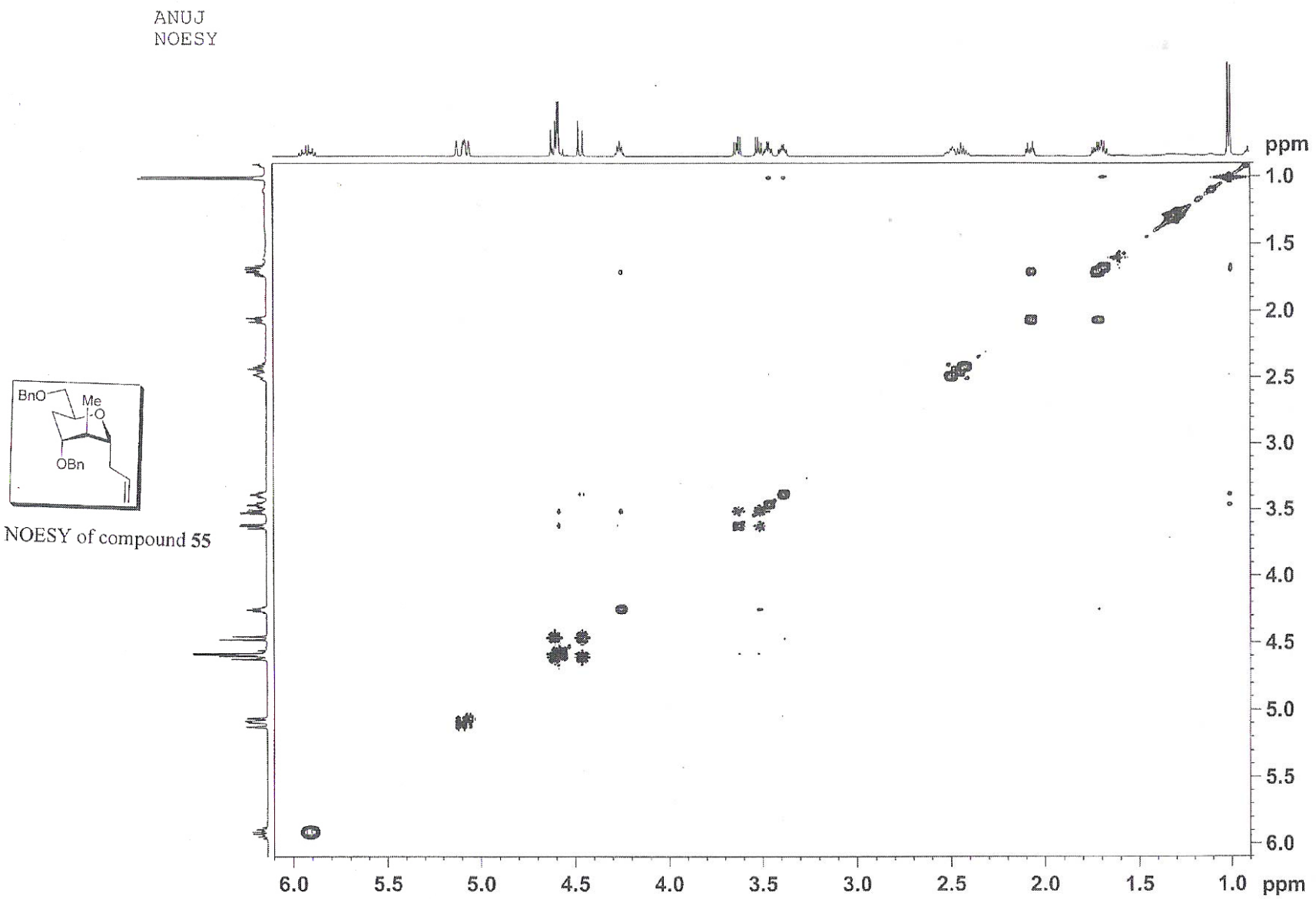
noesy





NOESY of compound **48**



NOESY of compound **55**

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List of Publications

List of Publications:

1. “Stereoselective syntheses of (+)-*proto*, (–)-*gala* quercitols and carba-L-rhamnose from D-(–)- quinic acid” Andiappan Murugan,* Anuj K. Yadav and Mukund K. Gurjar, *Tetrahedron Lett.* **2005**, 46, 6235-6238.
2. “A concise synthesis of putative structures of Radicamine B and its diastereomers and evaluation of their glycosidase inhibitory activity” *Manuscript under preparation.*
3. “Development of a new process for artificial sweetner sucralose” *Manuscript under preparation.*