DESIGN AND SYNTHESIS OF NEW AZASUGARS: β-LACTAM-AZASUGAR HYBRIDS, 1-DEOXY-D-GALACTOHOMONOJIRIMYCIN AND 1-DEOXY-D-GLUCOHOMONOJIRIMYCIN AS GLYCOSIDASE INHIBITORS

THESIS

Submitted to the

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For the degree of

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in

CHEMISTRY

BY

SHRINIVAS G. DUMBRE

DIVISION OF ORGANIC CHEMISTRY (*SYNTHESIS*)

NATIONAL CHEMICAL LABORATORY

PUNE-411008, INDIA

Dedicated to my parents…..

NCL

National Chemical Laboratory

Division of Organic Chemistry (Synthesis)
Pune – 411 008, INDIA

Dr. Ganesh Pandey
FNA, FNASc, FASc

CERTIFICATE

This is to certify that the work incorporated in the thesis entitled **"Design and Synthesis of New Azasugars: β-Lactam-azasugar Hybrids, 1-Deoxy-D-galactohomonojirimycin and 1-Deoxy-D-glucohomonojirimycin as Glycosidase Inhibitors"** which is being submitted to the **University of Pune** for the award of **Doctor of Philosophy in Chemistry** by **Mr. Shrinivas G. Dumbre** was carried out by him under my supervision at the **National Chemical Laboratory, Pune**. A material that has been obtained from other sources has been duly acknowledged in the thesis.

Date:

Dr. Ganesh Pandey

(Research Guide)

Ph. 020-2590 2324(O), 2590 2417, 25883493 (R), Mobile: 9890335521, Fax: 020-25902624
E-mail: gp.pandey@ncl.res.in

DECLARATION

I hereby declare that the work presented in the thesis entitled **"Design and Synthesis of New Azasugars: β-Lactam-azasugar hybrids, 1-Deoxy-D-galactohomonojirimycin and 1-Deoxy-D-glucohomonojirimycin as Glycosidase Inhibitors"** submitted for Ph. D. degree to the **University of Pune**, has been carried out by me at the **National Chemical Laboratory, Pune**, under the supervision of **Dr. Ganesh Pandey**. The work is original and has not been submitted in part or full by me for any degree or diploma to this or any other University / Institute.

Date: (**Shrinivas G. Dumbre**)

Division of Organic Chemistry (Synthesis)

National Chemical Laboratory

Pune-411008

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Shrinivas G. Dumbre

CONTENTS

Abbreviations

aq.	aqueous	mp	Melting point
bp	boiling point	N	Normality (normal)
Bn	Benzyl	MS	Mass Spectum
Boc	*t*-Butoxycarbonyl	MsCl	Methanesulfonyl chloride
DCM	dichloromethane	NMR	Nuclear magnetic resonace
DCN	1,4-dicyanonaphthalene	NOE	Nuclear Overhauser effect/enhancement
DEAD	Diethyl azodicarboxylate	NOESY	Nuclear Overhauser enhancement spectroscopy
DIAD	Diisoprpyl azodicarboxylate	ORTEP	Orthogonal thermal ellipsoid plots
DEPT	Distortionless enhancement by polarisation transfer	PET	Photoinduced electron transfer
		PDC	Pyridinium dichromate
DMF	N,N-dimethylformamide	PPTS	Pyridinium *p*-tolunesulfonate
DMSO	dimethylsulfoxide	*p*-TSA	*p*-Tolunesulfonic acid
COSY	Correlated spectroscopy	py	Pyridine
g	gram	rt	Room temperature
GC	Gas Chromatography	TBAF	Tetrabutylammonium fluoride
h	hour	TBAI	Tetrabutylammonium iodide
HETCOR	Hetronuclear correlation	TBDPS	*t*-Butyldiphenylsilyl
HSQC	Hetronuclear single quantum coherence	TBS	*t*-Butyldimethylsilyl
		TEA	Triethylamine
HMPA	Hexamethylphospharamide	TFA	Trifluoroacetic acid
hν	Ultravialet light	THF	Tetrahydrofuran
Hz	Hertz	TLC	Thin layer chromatography
IBX	*o*-Iodoxybenzene acid	TMEDA	Tetramethylethylenediamine
Ki	Inhibition constant	TMS	Trimethylsilyl
LAH	Lithium aluminium hydride	α-Gal	a-Galactosidase
M	Molarity (molar)	β-Gal	β-Galactosidase
mg	Milligram	α-Glc	α-Glucosidase
min	Minute(s)	β-Glc	α-Glucosidase
mL	Milliliter	α-Man	α-Mannosidase
mmol	Millimole	α-Man	α-Mannosidase
μM	Micromaolar		

General Remarks

- All the solvents used were purified according to literature procedure.[1]
- Petroleum ether used in the experiments was of 60-80 °C boiling range.
- Column chromatographic separations were carried out by gradient elution with suitable combination of two solvents and silica gel (60-120 mesh/ 100-200 mesh/230-400 mesh).
- Reaction progress was monitored by TLC or GC. TLC was performed on manually prepared glass silica plates and E-Merck pre-coated 60 F_{254} plates and the spots were rendered visible by exposing to UV light, iodine, phosphomolibdic acid, *o*-Anisol, $KMnO_4$. GC analysis was performed on Perkin Elmer 8700 and Varian CP 3800 GCs using SGE BP1, BP20 and Varian Chromopack CP-Sil-5CB columns.
- IR spectra were recorded on FTIR instrument, for solid either as nujol mull, neat in case of liquid compounds or their solution in chloroform.
- NMR spectra were recorded on Brucker ACF 200 (200 MHz ^{1}H NMR and 50 MHz ^{13}C NMR), MSL 300 (300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR), AV 400 MHz (400 MHz ^{1}H NMR and 100 MHz ^{13}C NMR) and DRX 500 (500MHz ^{1}H NMR and 125 MHz ^{13}C NMR). ^{13}C peak multiplicity assignments were made based on DEPT data.
- Mass spectra were recorded on PE SCIEX API QSTAR pulser (LC-MS) and Shimadzu QP 5000 GC/MS coupled to Shimadzu 17A GC using a DB1 column.
- Microanalysis data were obtained using a Carlo-Erba CHNS-O EA 1108 Elemental Analyser. Elemental analyses observed for all the newly synthesized compound were within the limit of accuracy (± 0.4 %).
- All the melting points recorded are uncorrected and were recorded using electrothermal melting point apparatus.
- Starting materials were obtained from commercial sources. *n*-BuLi, *s*-BuLi, IBX, DCN and $NaBH(OAc)_3$ were prepared using known procedures.
- **Continuous numbering of compounds, schemes, tables and figures has been employed for all Chapters. However Independent referencing is used for each Chapter.**

1) Perin, D. D.; Armarego, W. L. F. Purification of Laboratory Chemicals, 4th ed.., Butterworth Heinemann, **1999**

Research Student	Shrinivas G. Dumbre
Research Guide	Dr. Ganesh Pandey
Title of Thesis	Design and Synthesis of New Azasugars: β-Lactam-Azasugar Hybrids, 1-Deoxy-D-galactohomonojirimycin and 1-Deoxy-D-glucohomonojirimycin as Glycosidase Inhibitors
Registration no.	PGS/Ph.D./ 15
Date of registration	31-12-2002
Place of work	Division of Organic Chemistry (Synthesis), National Chemical Laboratory, Pune-411008

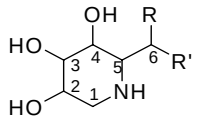
Abstract

The present dissertation is divided into four chapters.

Chapter 1 An Introduction to azasugars as Glycomimetics

This chapter gives an overview of glycosidases and their inhibitory mechanism, emergence of natural and synthetic azasugars as glycosidase inhibitors, biological importance of glycosidase inhibitors and brief elaboration of the concept for the design of new glycomimetics.

Chapter 2 Synthesis of the Stereoisomers of C-6 Homologues of 1-Deoxynojirimycin and their Analogues: Evaluation as Specific Glycosidase Inhibitors.



C-6 homo DNJ type iminosugar

R = H, R' = CH_2OH,
R = OH, R' = CH_2OH
R = H, R' = COOH

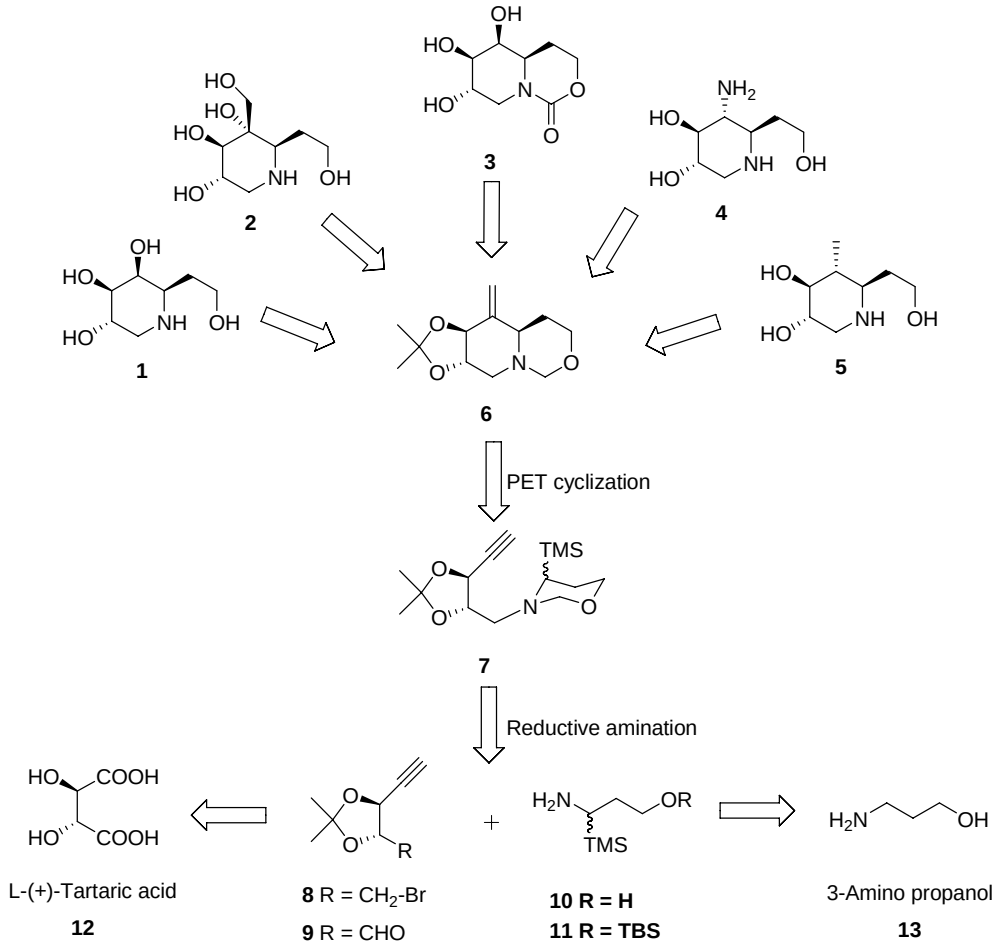
Figure I

This chapter starts with brief introduction elaborating the importance of nojirimycin class of azasugars and literature reports towards the synthesis of various analogues of C-6 homologues of 1-deoxynojirimycin (**Figure I**). The major challenge in designing a synthetic route for these types of azasugars has been the preparation of suitably polyhydroxylated piperidine moiety having a chiral alkyl group at C-5. Moreover, beyond the synthetic challenges, it was surprising to note that none of these studies have attempted the evaluation of the inhibitory potencies of the synthesized C-6 homologues of 1-deoxyazasugars.

Considering the unexplored potential of the C-6 homologues of 1-deoxy azasugars as glycosidase inhibitors, we ventured into developing an entirely new and versatile strategy

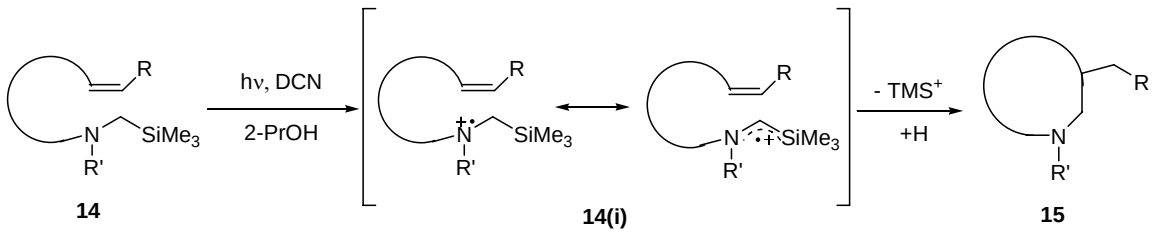
for the synthesis of C-6 homologues of 1-deoxy azasugars. The convergent synthetic approach towards the synthesis of the stereoisomers of C-6 homologues of 1-deoxynojirimycin such as 1-deoxy-D-galactohomonojirimycin **(1)**, 1-deoxy-4-hydroxymethyl-D-glucohomonojirimycin **(2)** and related analogues **3, 4** and **5** is depicted retrosynthetically in **Scheme I**.

Scheme I: Retrosynthetic analysis for various C-6 homo-1-deoxynojirimycin via template **6**



The crucial PET cyclization step for the preparation of key precursor **6** emerged from our earlier approach wherein it was shown that PET-generated α-trimethylsilylmethylamine radical cation of type **14(i)** cyclizes efficiently to the tethered π-functionality as depicted in **Scheme II**.

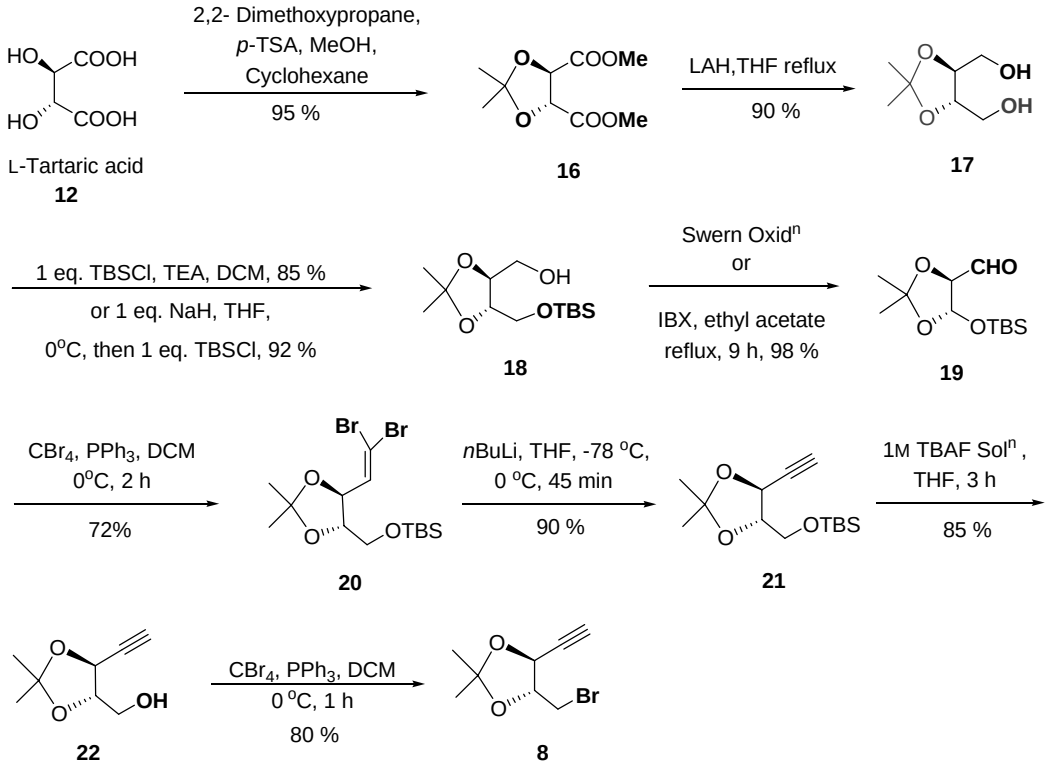
Scheme II: PET generated amine radial cation cyclization of π-tethered amine



As per our synthetic design, acetylene tethered amine **7** was proposed to be obtained by *N*-alkylation of amine **11** with compound **8**. The two components **8** and **11** are synthesized from L-(+)-tartaric acid **(12)** and 3-aminopropanol **(13)** respectively.

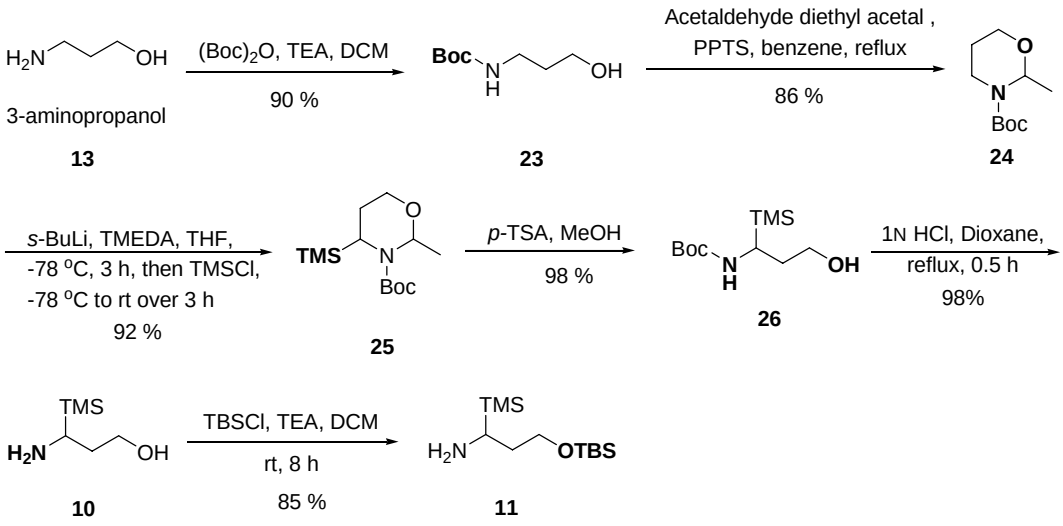
The synthesis of component **8** is depicted below in **Scheme III**

Scheme III: Synthesis of (4*R*,5*S*)-4-(bromomethyl)-5-ethynyl-2,2-dimethyl-1,3-dioxolane **(8)**



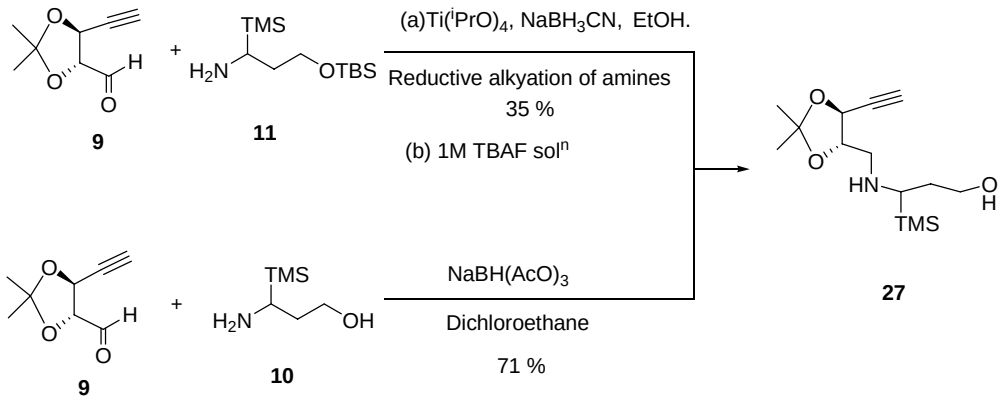
Synthesis of component **11** is depicted below in **Scheme IV**.

Scheme IV: Preparation of 3-(*t*-butyldimethylsilyloxy)-1-(trimethylsilyl)propan-1-amine **(11)**

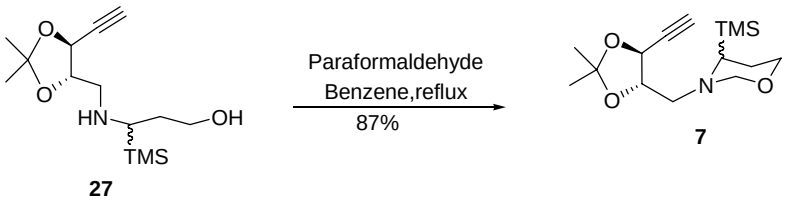


Initially, we tried to synthesize **27** by usual base promoted *N*-alkylation of the amine **11** with the primary halide **8** using large excess of K_2CO_3 or $CsCO_3$ in acetonitrile. However, the reaction was found to be very sluggish and the required *N*-alkylated product **27** was also obtained only in poor yield (~20 %). Therefore, we decided to utilize an alternative approach *via* reductive amination of aldehyde **9 (Scheme V)** which was obtained by IBX oxidation of **22**.

Scheme V: Reductive amination protocol



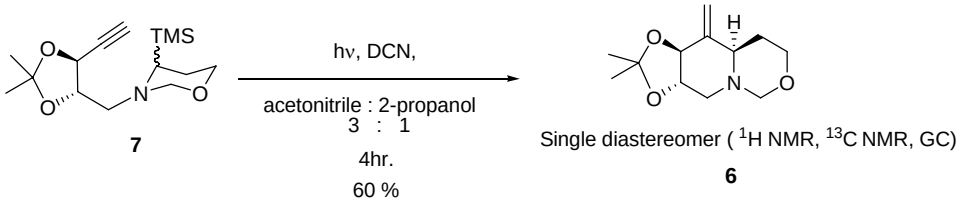
Scheme VI



The thought of utilizing open chain *N*-alkylated derivative of **27** for PET cyclization was abandoned considering our previous experience of poor diastereoselectivity, **27** was transformed into the cyclic 1,3-oxazine derivative **7** by refluxing with paraformaledyde (**Scheme VI**).

PET cyclization of 7 by irradiating a solution containing **7** (3 mmol), 1,4-dicyanonaphthalene (DCN, 0.4 mmol) in acetonitrile : 2-propanol (3:1) using 450 W medium pressure mercury lamp provided **6** as a single diastereomer (**Scheme VII**).

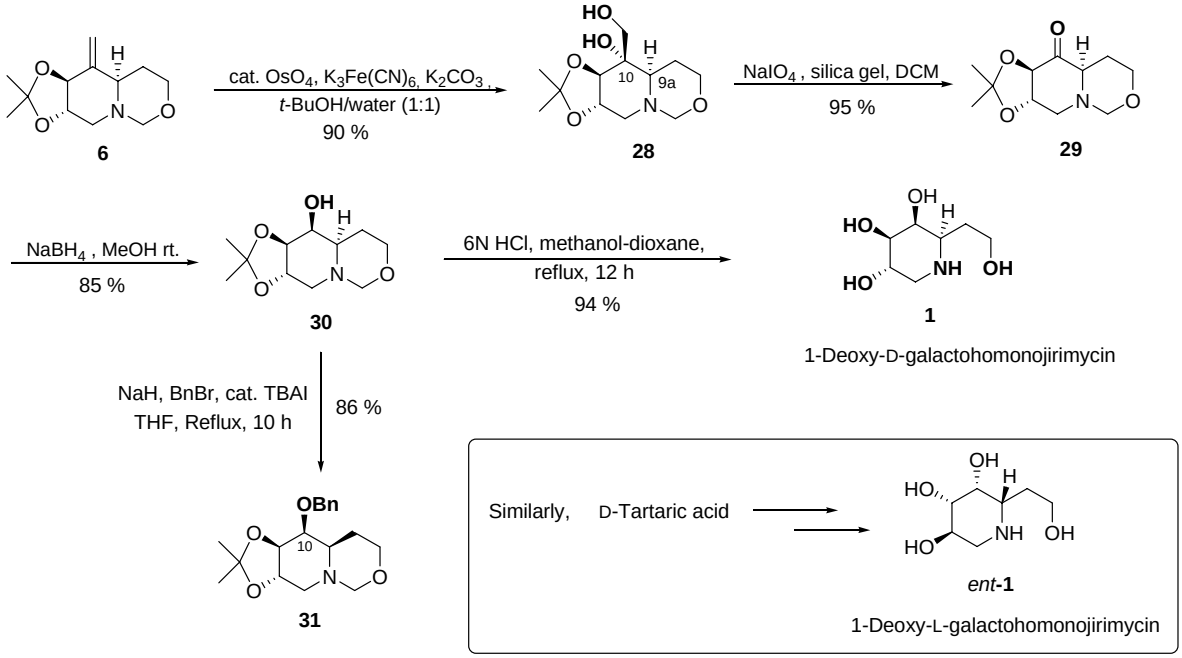
Scheme VII



1-Deoxy-galactohomonojirimycin (1) was obtained from PET cyclized product **6** as depicted below in **Scheme VIII**. The compound **6**, **28** and **31** were fully characterized by

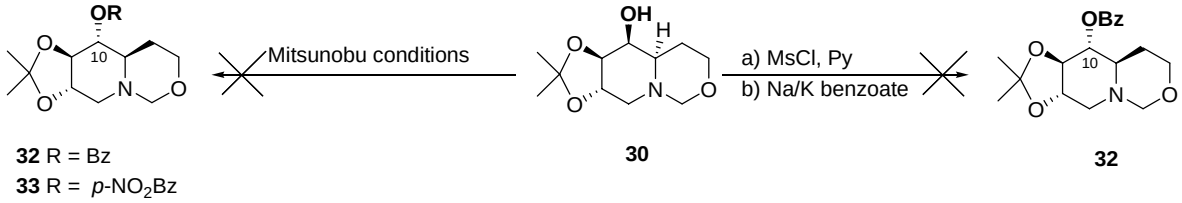
1D and 2D spectroscopy analysis. Further X-ray analysis of **28** and **31** confirms the stereochemistry at their C-10 and C-9a centers.

Scheme VIII: Synthesis of 1-deoxy-D-galactohomonojirimycin **(1)**



However, synthetic efforts towards 1-deoxy-D-glucohomonojirimycin by inverting the stereochemistry of hydroxyl group at C-10 of **30** under various permutations and combinations of reagents, solvents and temperature for Mitsunobu reaction met without success. Furthermore, we also tried nucleophilic S_N2 displacement of –OMs derivative of **30** with Na/K benzoate to obtain **32**, however, this effort also failed to give the required product (**Scheme IX**).

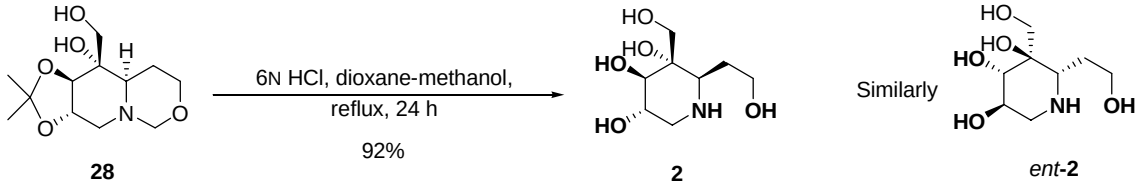
Scheme IX



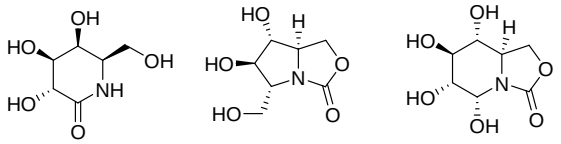
32 R = Bz
33 R = *p*-NO_2Bz

1-deoxy-4-hydroxymethyl-D-glucohomonojirimycin (**2**) was obtained by refluxing **28** with 6N HCl for 12 h (**Scheme X**).

Scheme X: Synthesis of 1-deoxy-4-hydroxymethylglucohomonojirimycin **(2)**



Nojirimycin, 1-deoxynojirimycin and their analogues are believed to exhibit their glycosidase inhibitory activities due to their binding with the glycosidases by mimicking the shape and charge of the postulated oxo-carbenium ion intermediate for the glycosidic bond cleavage reaction. However, there has been intense interest, recently, in evaluating non basic neutral glyconolactams such as **34**, **35** and **36** in which glycosidic oxygen is replaced by pseudo sp^2 ring nitrogen, as glycosidase inhibitors (**Figure II**) and considerable degree of inhibitory activities have been recorded. In these examples, where glycosidic oxygen is replaced by pseudo sp^2 ring nitrogen (e.g. **34,** *Ki* = 85 μM, β-glucosidase), mechanistically were originally believed to inhibit involving tautomeric iminol form. However, recent studies suggest that the glycosidase inhibition by these compounds may in fact be caused by the H-bonding of the lactam carbonyl moiety with the enzyme as the tautomerization energy for the amide-iminol conversion is of the order of 11 kcal mol^{-1} indicating the concentration of the corresponding iminol form in solution at a given time to be very low.

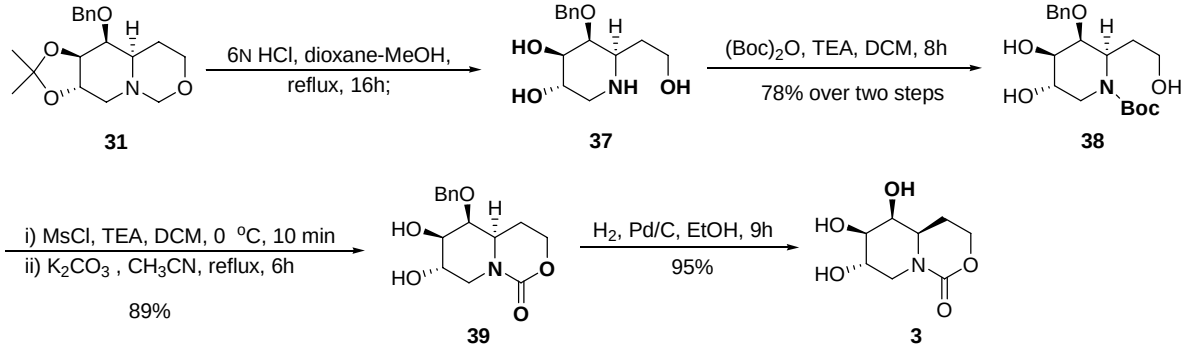


34 **35** **36**

Figure II: Non-basic azasugars

In this context, we synthesized a hitherto unknown non-basic neutral molecule **3** from **31** for evaluation as a new glycosidase inhibitor (**Scheme XI**).

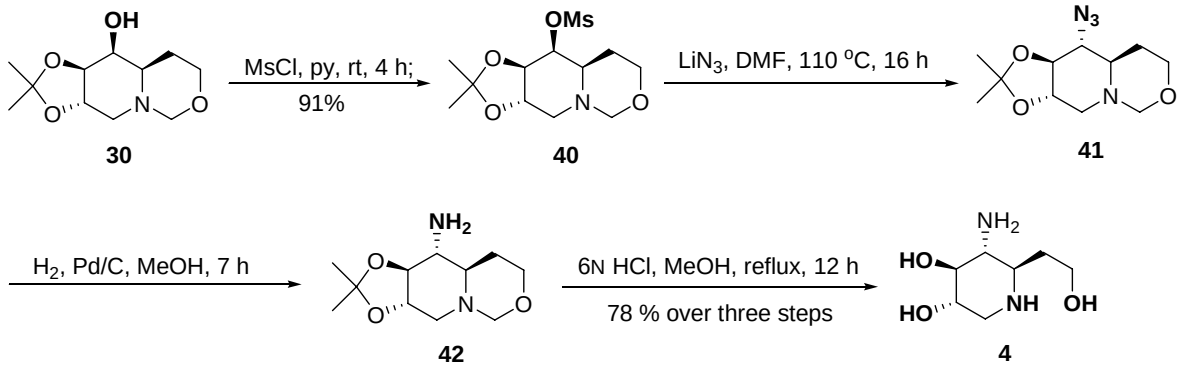
Scheme XI: Synthesis of pseudoamide type molecule **3**



31 **37** **38** **39** **3**

In continuation, another new analogue (3*S*,4*S*,5*R*,6*R*)-5-amino-6-(2-hydroxyethyl)piperidine-3,4-diol (**4**) having a basic amine moiety at C-4 was also visualized to be easily affordable from **30** for evaluation as a glycosidase inhibitor as shown in **Scheme XII.**

Scheme XII: Synthesis of (3*S*,4*S*,5*R*,6*R*)-5-amino-6-(2-hydroxyethyl)piperidine-3,4-diol **(4)**



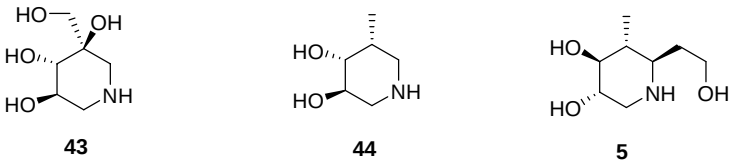
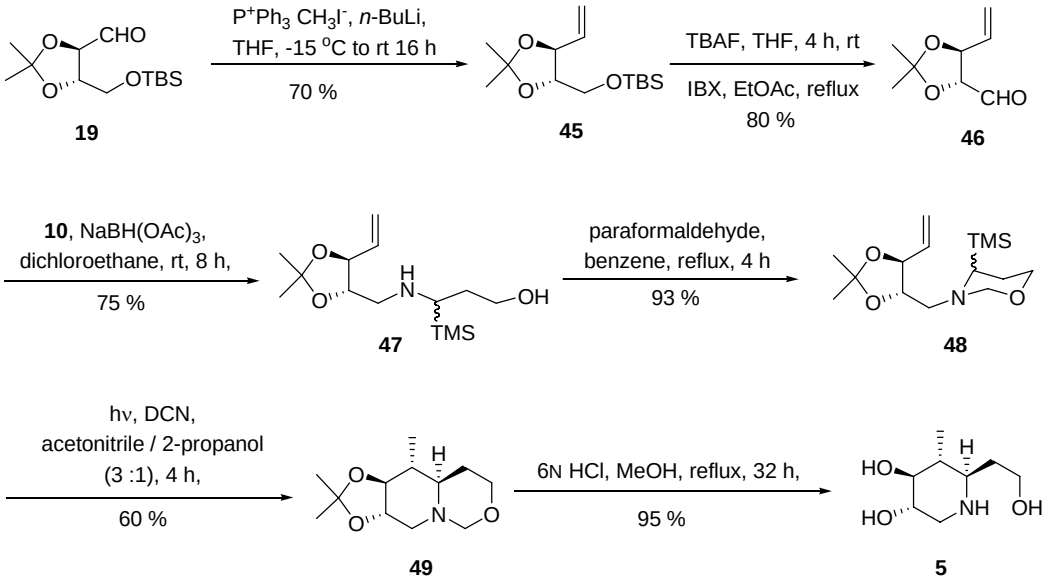


Figure III: 1-N-iminosugar (**43, 44**), DNJ-type iminosugar (**5**)

Since, we had earlier observed that 1-*N*-iminosugar **44** showed better inhibitory activity for the β-glucosidase (*Ki* = 30 μM) than **43** (*Ki* =90 μM), it also occurred to us that it would be pertinent to evaluate the enzyme inhibition activity of similar C-6 homoazasugar of 1-deoxynojirimycin type molecule **5** (**Figure III**). In this context, we synthesized compound **5** by following the analogous route as described for **1** starting from aldehyde **19** as shown in **Scheme XIII**. Since the direct hydrogenation of exocyclic double bond in **6** would have given diastereomeric mixture as observed in the case of the synthesis of **44**. The synthesis of **5** is outlined below in **Scheme XIII.** PET cyclized product **49** was obtained as a single diastereomer.

Scheme XIII: Synthesis of (3*S*,4*S*,5*R*,6*R*)-6-(2-Hydroxyethyl)-5-methylpiperidine-3,4-diol **(5)**



The inhibitory activities of **1**, **2, 3, 4, 5,** *ent*-**1** and *ent*-**2** were screened against β-galactosidase (*Aspergillus oryzae*), α-galactosidase (coffee beans), β-glucosidase / β-mannosidase (almonds), α-glucosidase (yeast) and α-mannosidase (jack beans).

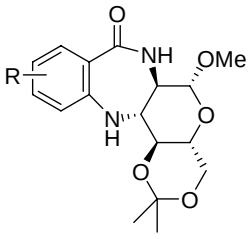
In conclusion, we have developed a general synthetic strategy to access stereoisomers of the C-6 homologue of 1-deoxynojirimycin. It was found that the molecules such as **1** (K_i = 1.7 μM, α-galactosidase), **2** (K_i = 28 μM, β-glucosidase), *ent*-**1** (K_i = 129 μM, β-glucosidase) and *ent*-**2** (K_i = 12 μM, β-glucosidase) exhibited enzyme specific inhibition against glycosidases.

Experimental section provides detail experimental procedures, tabulated analytical and spectral data for all new compounds and selected X-ray crystal data for compound **28** and **31**, enzyme inhibition assay general procedure.

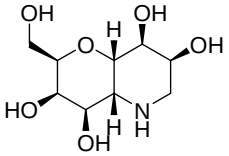
Spectra and Lineweaver-Burke plots section presents spectral characterization data (1D and 2D spectra) of all new compounds and Lineweaver-Burke plots.

Chapter 3 Synthesis of β-Lactam-Azasugar Hybrid

Recently, research activity in this field has evolved to evaluate hybrid molecules as glycosidase inhibitors and in this context hybrids of D-glucose with some heterocycles, D-galactose with 1-deoxynojirimycin and few more related structures have been synthesized and evaluated (**Figure IV**). The possibility of developing new glycosidase inhibitors through this approach is gaining an appreciable importance for future developments of this area.

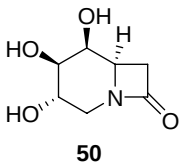


Hybrids of β-D-glucose-benzoheterodiazepine



Hybrid of D-Galactose and 1-DNJ

Figure IV: Hybrids of carbasugars



50

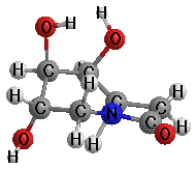


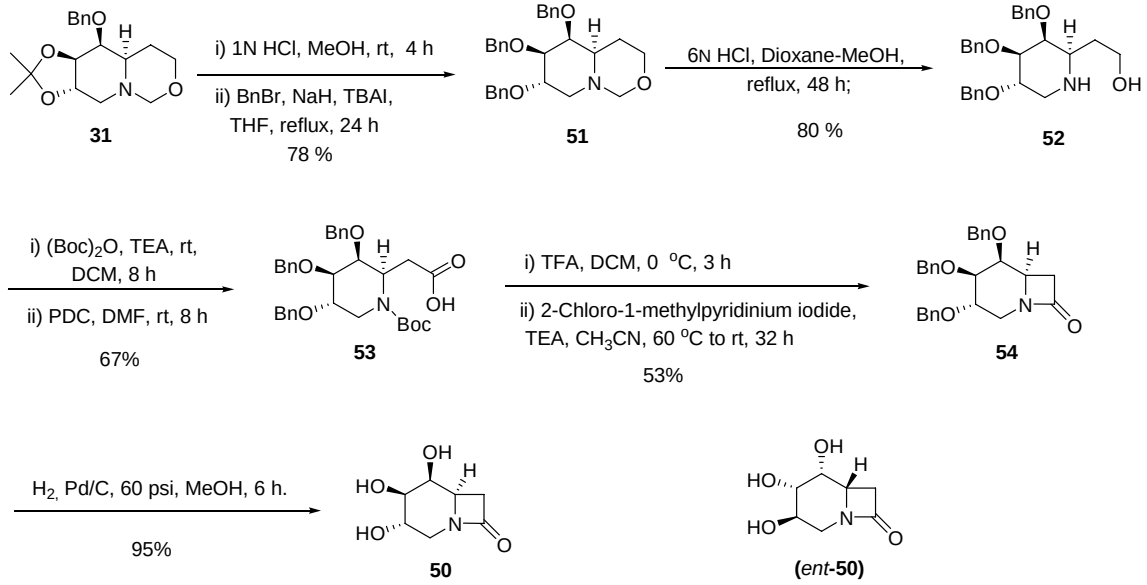
Figure V: β–Lactam-azasugar

We postulated that β-lactam-azasugar hybrid molecule of type **50** (**Figure V**) which could also be referred as polyhydroxylated carbacephem, may emerge as a potent glycosidase inhibitor due to its

ingenuous conformationally constrained structural features such as a) β-lactam ring compelling the polyhydroxylated piperidine ring to adapt nearly half chair conformation mimicking the shape of glycosidase inhibition transition state b) the carbonyl group in β-lactam ring may provide additional hydrogen bonding site for specific enzyme-substrate interactions.

With this hypothesis in mind, we have synthesized β-lactam-azasugar hybrid **50**. The synthesis of **50** began with the intermediate **31** as shown is **Scheme XIV**.

Scheme XIV: Synthesis of β-lactam- azasugar **50**.



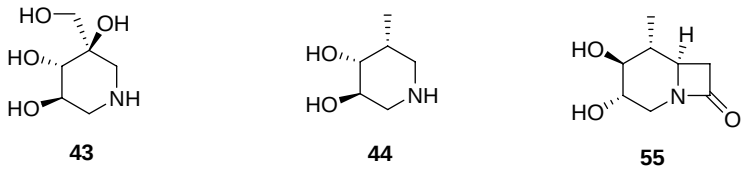
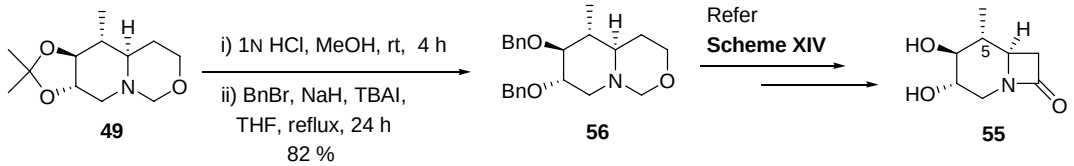


Figure VI: β-Lactam azasugar hybrid **55** and comparative structures

As described in Chapter 2, 1-*N*-iminosugar **44** showed better inhibitory activity for the β-glucosidase (*Ki* = 30 μM) than **43** (*Ki* =90 μM). In this context, β-lactam **55** was also synthesized using the similar procedure on compound **49** which was eventually transformed into (3*S*,4*S*,5*R*,6*R*)-3,4-dihydroxy-5-methyl-1-aza-bicyclo[4.2.0]octan-8-one **(55)** as shown in **Scheme XV**.

Scheme XV: Synthesis of β-lactam-azasugar **55**.



The inhibitory activities of **50,** *ent*-**50** and **55** were screened against β-galactosidase (*Aspergillus oryzae*), α-galactosidase (coffee beans), β-glucosidase / β-mannosidase (almonds), α-glucosidase (yeast), and α-mannosidase (jack beans). Compound **50** exhibited moderate inhibition in case of β-galactosidase (*Ki* = 172 μM)

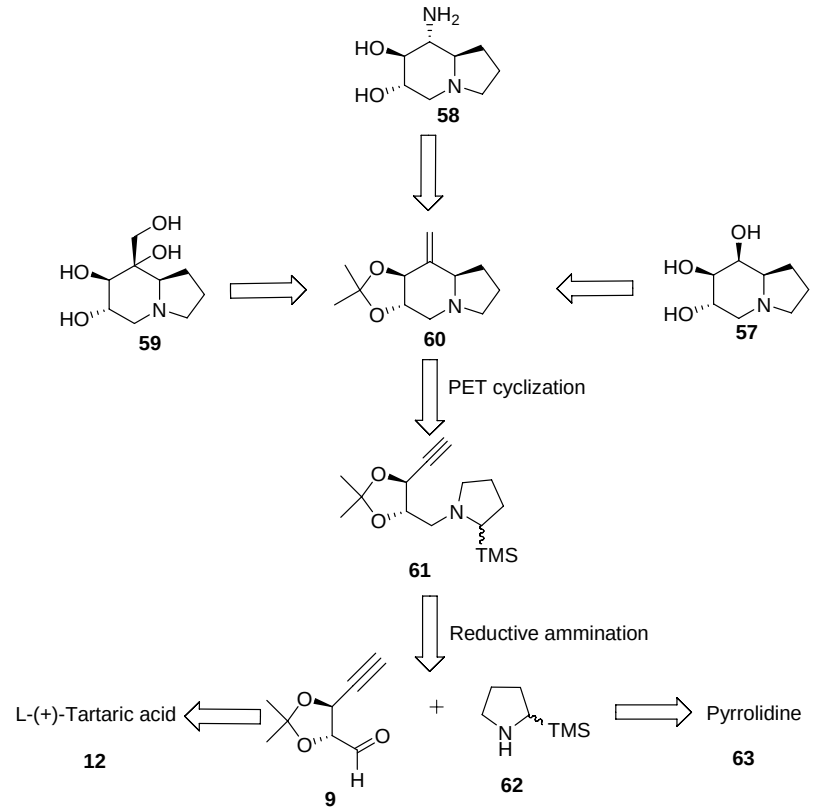
In conclusion, the fairly good and specific glycosidase inhibition exhibited by neutral β-lactam-azasugar hybrid molecule **50** points towards the possibility of improving its potency further by incorporating some minor structural variations to its core, such as incorporation of hydroxymethylene functionality at the C-7 of the β-lactam ring with a view that it may provide an additional H-bonding site for recognition and would also increase the polarity of the molecule.

Experimental section provides detail experimental procedures, tabulated analytical and spectral data for all new compounds.

Spectra and Lineweaver-Burke plots section presents spectral characterization data (1D and 2D spectrum) of all new compounds and Lineweaver-Burke plots.

Chapter 4 Synthesis of analogues of 1-Deoxy-castanospermine

Scheme XVI: Retrosynthetic analysis



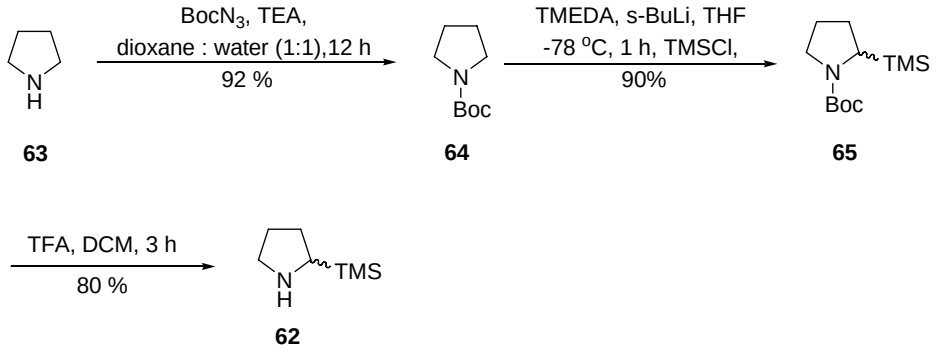
This chapter begins with the brief introduction on polyhydroxylated 1-azabicyclo[4.3.0]nonane skeleton comprising of indolizidine alkaloids such as (+)-castanospermine and their biological significance followed by two literature reports on the synthesis of 1-deoxy-8-*epi*-catanospermine (**57**).

Our synthetic approach towards 1-deoxy-8-*epi*-catanospermine (**57**) and other to analogues comprising of

polyhydroxylated 1-azabicyclo[4.3.0]nonane skeleton is depicted in retrosynthetic analysis (**Scheme XVI)**.

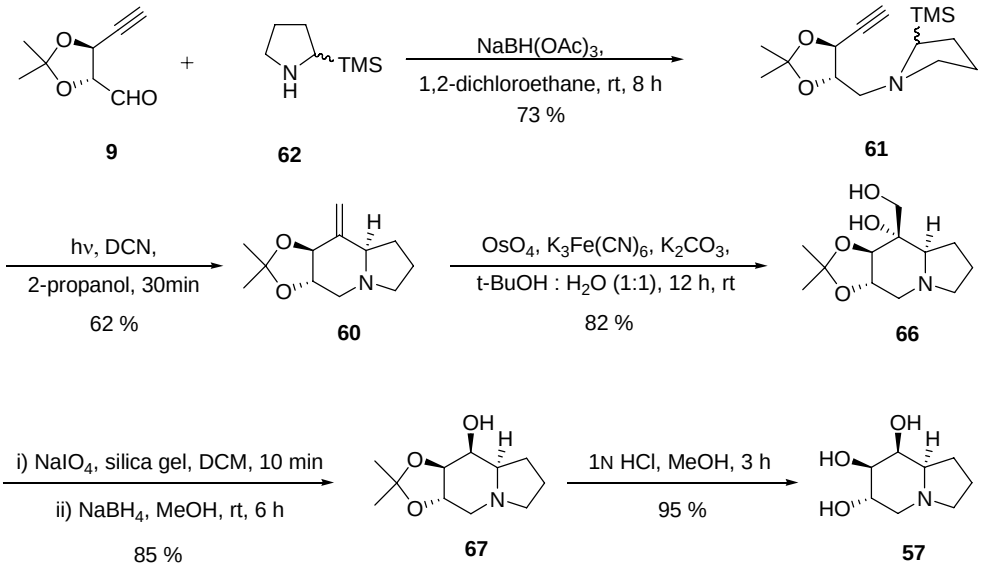
Synthesis of 2-(trimethylsilyl)pyrrolidine (**62**) is shown below in **Scheme XVII**.

Scheme XVII: Synthesis of 2-(trimethylsilyl)pyrrolidine **62**



Synthesis of 1-deoxy-8-*epi*-catanospermine (**57**) is depicted in **Scheme XVIII**. PET cyclized product **60** was obtained as an exclusive diastereomer.

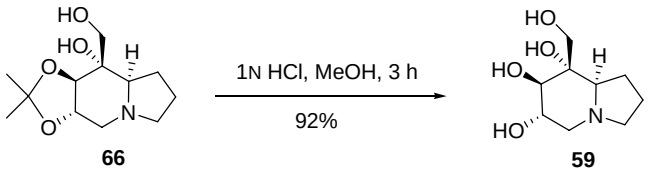
Scheme XVIII: Synthesis of 1-Deoxy-8-*epi*-castanospermine (**57)**



The intermediates **60**, **66** and 1-deoxy-8-*epi*-catanospermine (**57**) were extensively characterized by 1D and 2D spectroscopic analysis.

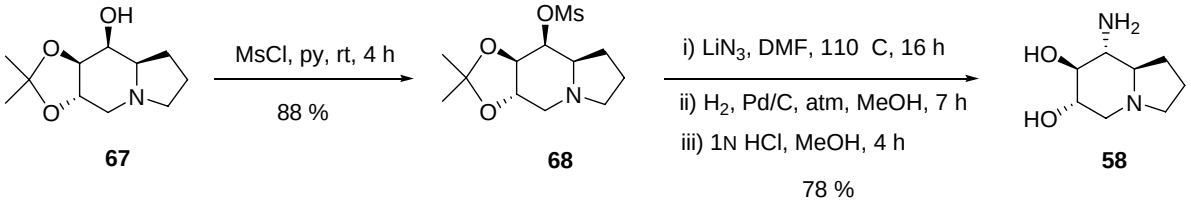
1-Deoxy-8-hydroxymethyl-castanospermine (**59**) was obtained by treating **66** with 1N HCl as shown in **Scheme XIX**.

Scheme XIX: Synthesis of 1-Deoxy-8-hydroxymethyl-castanospermine **59**



Furthermore, another new analogue **58** having more basic amine moiety at C-8 was also visualized to be easily affordable from **67** for evaluation as a new glycosidase inhibitor as shown in **Scheme XX.**

Scheme XX: Synthesis of (6*S*,7*S*,8*R*,8a*R*)-8-amino-octahydroindolizine-6,7-diol **(58)**



The inhibitory activities of **57, 58** and **59** were screened against β-galactosidase (*Aspergillus oryzae*), α-galactosidase (coffee beans), β-glucosidase / β-mannosidase (almonds), α-glucosidase (yeast), and α-mannosidase (jack beans).

None of these analogues of 1-deoxy-castanospermine inhibited α-glucosidase and α- / β-mannosidases. The 1-deoxy-8-*epi*-castanospermine (**57**) exhibited non-specific competitive inhibition against α-galactosidase (*Ki* = 71 μM), β-galactosidase (*Ki* = 73 μM) and β-glucosidase (*Ki* = 33 μM). To our dismay 1-deoxy-8-hydroxymethyl-castanospermine (**59**) in which an additional hydroxymethyl moiety is present at carbon C-8, showed non-competitive weak inhibition against β-glucosidase. Similarly compound **58** in which hydroxy group at C-8 of castanospermine is replaced by amino group showed weak inhibition against β-glucosidase

In conclusion, we have demonstrated a general synthetic strategy to access polyhydroxylated 1-azabicyclo[4.3.0]nonane skeleton such as **57**, **58** and **59**. Although, these synthesized molecules could not turn up as potent inhibitors, nevertheless, this study is useful in understanding the structure activity relationship of polyhydroxylated indolizidine alkaloids.

Experimental section provides detail experimental procedures, tabulated analytical and spectral data for all new compounds.

Spectra and Lineweaver-Burke plots section presents spectral characterization data (1D and 2D spectra) of all new compounds and Lineweaver-Burke plots.

Note: Compound numbers in the abstract are different from those in thesis.

Chapter 1 An Introduction to Azasugars as Glycomimetics

1.1. Glycosides, Glycosidases (Glycosyl Hydrolases) and their inhibitors

Glycosides are compounds containing a carbohydrate and a non-carbohydrate residue in the same molecule. The non-sugar component is called aglycon which may be methyl alcohol, glycerol, sterol, phenol, etc. The sugar component is called glycon. The carbohydrate residue is attached by an acetal linkage at the anomeric carbon to a non carbohydrate residue or aglycon. Glycosidase is an enzyme that cleaves this linkage between the aglycon and glycon and if the enzyme is specifically cleaving the linkage between glucose and aglycon then the enzyme is called glucosidase.

Enzymes are biocatalysts and are essential for life processes because most chemical reactions in living cells would occur too slowly, or would lead to different products without enzymes. Therefore, enzymes play fundamental roles in life's processes. Especially glycosidases (glycoside hydrolases/glycosyl hydrolases, E.C. number 3.2.1.x) and glycosyltransferases (EC 2.4) are omnipresent protein macromolecules which catalyze glycosyl group transfer reactions that assemble, trim and shape carbohydrates into bioactive glycoprotein and glycolipid conjugates. Largely, these processes involve cleavage of the glycosidic bond linking a sugars anomeric carbon with an oligo- or polysaccharide or a nucleoside diphosphate group. The liberated glycosyl group is further transferred to water (**2**, by glycosidases) or to some other nucleophilic acceptor (**3**, by transferases)[1] as shown in **Figure 1**. This means glycosidases and glycosyltransferases are responsible for the hydrolysis and formation, respectively, of glycosidic bond.

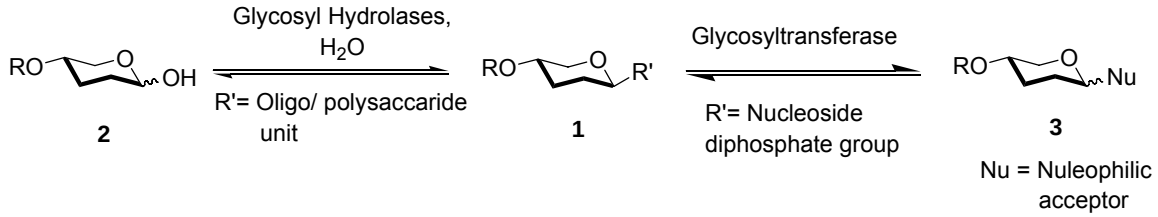


Figure 1: General Representation of function of glycosyl hydrolases and glycosyltransferase

Based on the above mentioned role of glycosidases, it would not be exaggeration to mention that glycosidases are essential for the survival and existence of all living organisms. For example digestive glycosidases break down large sugar-containing

molecules to release monosaccharides which can be more easily taken up and used metabolically by the organism. Lysosomal glycosidases catabolise glycoconjugates intracellularly and a wide range of glycosidases are involved in the biosynthesis of the oligosaccharide portions of glycoprotein's and glycolipids which play vital roles in mammalian cellular structure and functions.[2]

1.2 Glycosidases Classification and Mechanism

Gycosidases are classified according to,

- The nature of the glycosidic atom it is cleaving, e. g. O-, N-, and S-glycosidases (EC 3.2.1.x, EC 3.3.3.x, EC 3.2.3.x respectively)
- The ring size of glycosyl donors as pyranosides or furanosides
- The position of the glycosidic bond that is cleaved by the enzyme see **Figure 2**.

Exoglycosidases remove sugars one at a time, from the non-reducing end of an oligo or polysaccharide and are involved in the breakdown of starch and glycogen, the processing of eukaryotic glycoproteins, the biosynthesis and modification of glycosphingolipids and the catabolism of peptidoglycans and other glycoconjugates. Endoglycosidases cleave interior glycosidic bonds within polysaccharides and are involved in the catabolism and clearance of the aged glycoproteins. These enzymes also catalyze the alteration of bacterial and plant cell walls as well as the hydrolysis of highly insoluble structural polysaccharides like chitin and cellulose.[1]

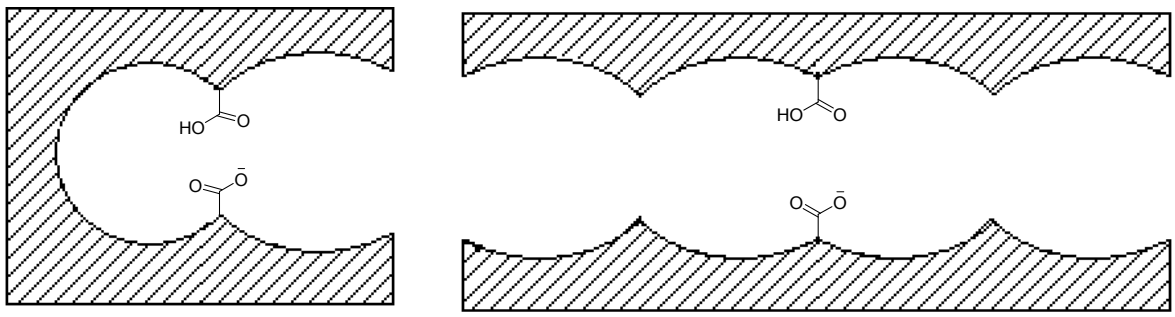


Figure 2: Schematic representation of exo- and endo-glycosidases

- Glycosidases are more rigorously classified based on the stereochemistry of the anomeric glycosidic bond that they cleave.[3] Enzymes catalyzing the cleavage of a α-glycosidic bond are termed as α-glycosidases while those cleaving a β-glycosidic bond are

termed as β-glycosidases. Depicted below in **Figure 3** is a typical glycosidase reaction mechanism. α-Glycosidases are generally believed to act through an E2 type elimination mechanism during which a positively charged aglycon (the leaving group) and the lone pair of the ring oxygen are positioned antiperiplanar, cooperatively facilitating the glycosidic bond cleavage reaction.[3]

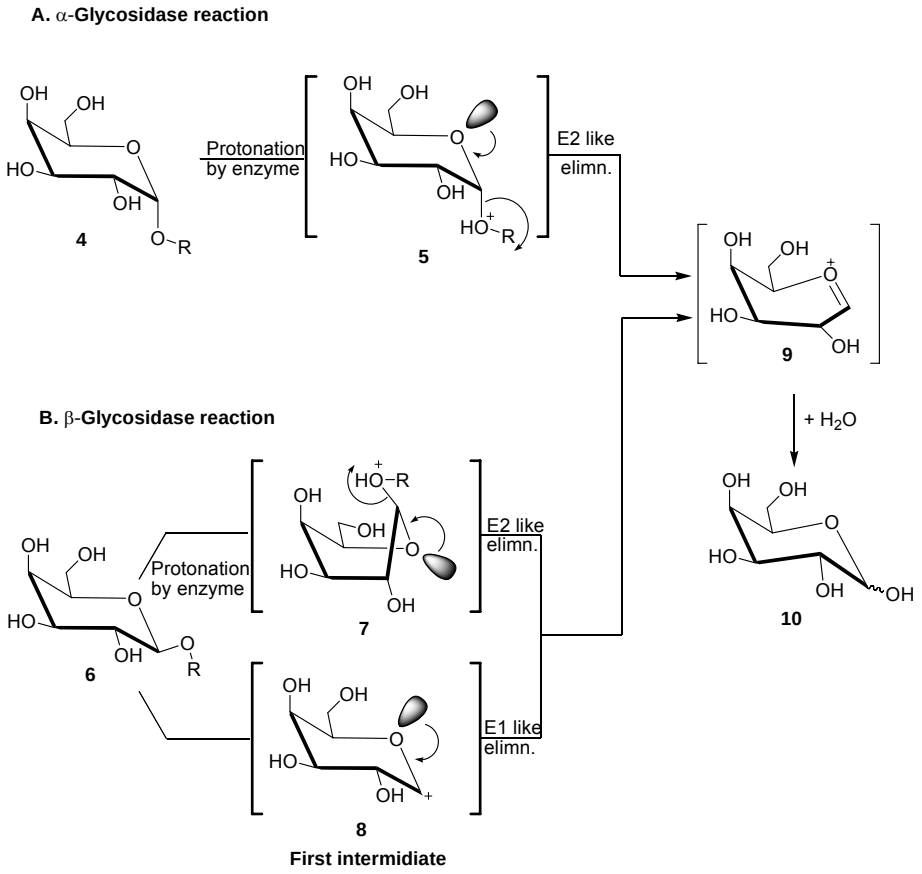


Figure 3: Glycosidase reaction mechanism involved in α-glycosidases and β-glycosidases.

In the case of the β-glycosidase reaction, if the enzyme proceeds via an E2 type mechanism, similar to that of the α-glycosidases, the protonation of substrate **6** has to go through a highly strained intermediate **7** that may not favor further reaction. Therefore, in the case of a β-glycosidase reaction, the positively charged aglycon leaves via an E1 like mechanism, involving the glycosyl cation **8**, further stabilized by the ring oxygen to give **9**. Thus, as seen in **Figure 3**, although the final reaction intermediate in both the reaction mechanisms is the same flattened, half chair oxocarbenium ion **9**, the first intermediate in the case of β-glycosidase reaction differs with respect to the position of charge development.

- Glycosidases are also classified on the basis of the stereochemical outcome of the newly formed anomeric bond. The enzymatic cleavage of the glycosidic bond liberates a sugar hemiacetal with either the same configuration as the substrate (retention), see **Figure 4** or less commonly, the opposite configuration (inversion), see **Figure 5**. Based on this criterion glycosidases are classified as retaining or inverting glycosidases.

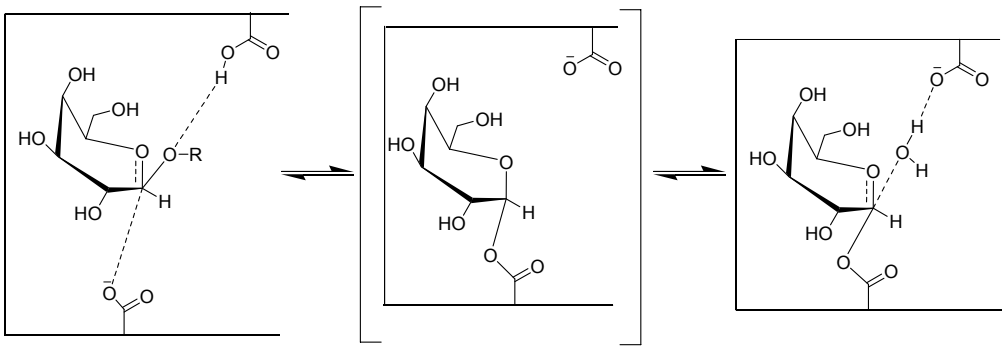


Figure 4: Transition state complexes involved in the retention enzyme

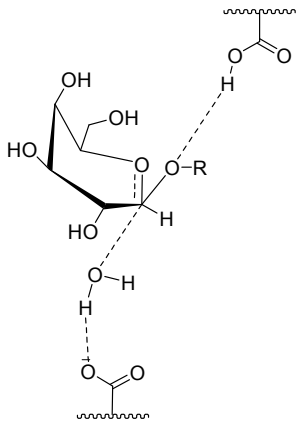


Figure 5: Hypothetical transition state of the inverting enzyme

Thus, ***glycosidase inhibitors*** are any chemical entity which is capable of mimicking either the charge or shape (or both) of the substrate or that of any of the transition states, can act as a reversible inhibitor of that particular glycosidase. The term "***glycomimetics***" refers to the creation of molecules that mimic the activity of carbohydrates or simply carbohydrate mimics.

1.3 Azasugars as glycosidase inhibitors

An important element of enzymatic catalysis is the ability of an enzyme to lower the energy of the transition state for the reaction it catalyses. The only real evidence for this theory is the fact that stable compounds that resemble the transition state, transition state (TS) analogues are competitive inhibitors of that particular enzyme. These understanding have lead to a keen interest in recent years for designing compounds that in terms of polarity and shape resemble the transition state of glycosidic cleavage or formation to create potent, selective enzyme inhibitors.

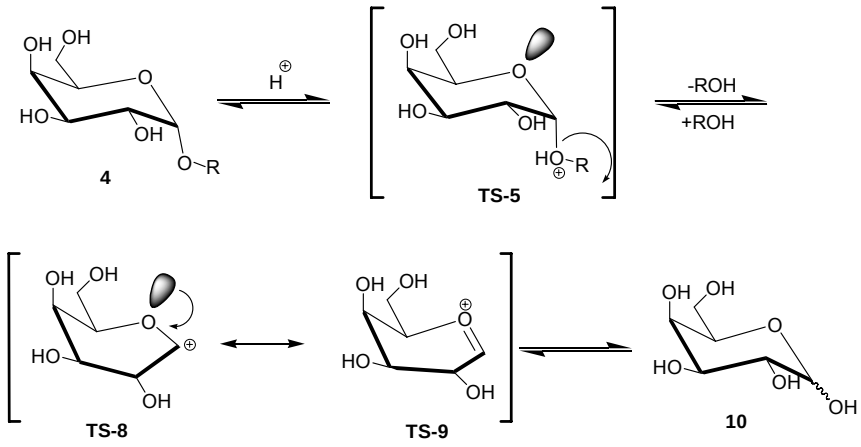


Figure 6: Schematic Presentation of Transition state Intermediates

As depicted in **Figure 6**, there are three important reaction intermediates TS-**5**, TS-**8** and TS-**9** depending upon the position of the charge build-up during the glycoside hydrolysis. It was observed that a compound that could resemble any of the transition state (TS) intermediates **5, 8** and **9** should be an inhibitor of the respective glycosidases.

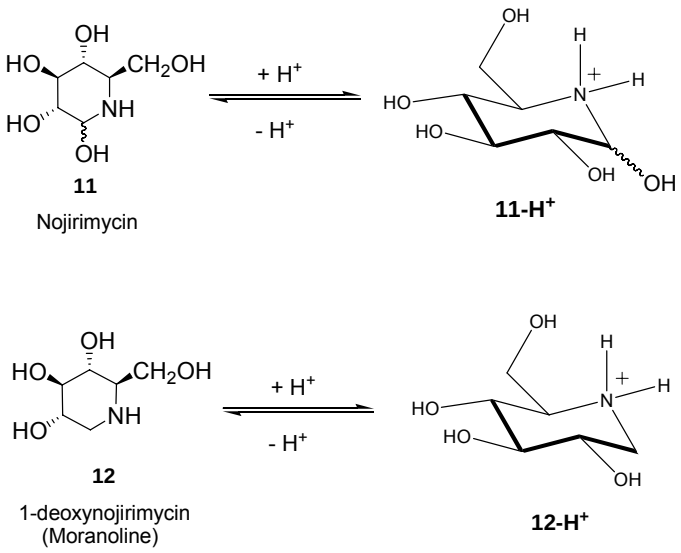


Figure 7: Protonated form of NJ and DNJ, a charge mimic of TS-**9**

Ever since the revolutionary work by Paulsen et al[4] on analogues of monosaccharides in which the ring oxygen is replaced by nitrogen are perhaps the best-known azasugar glycomimetics. This modification provides these analogues (polyhydroxylated azasugar alkaloids) metabolic inertness, retaining the recognition by glycosidases and other carbohydrate-recognizing proteins. Nojirimycin (NJ) **11** was the first polyhydroxylated piperidine alkaloid belonging to this class isolated in 1966.[5,6] However, in the same year 1-deoxynojirimycin (DNJ) **12** was synthesized and later in 1976 was isolated from Mulberry trees,[7] Bacillus[8] and

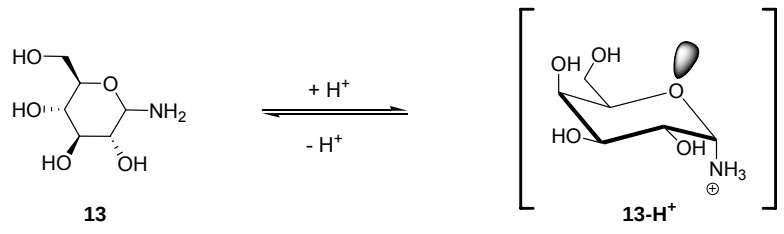
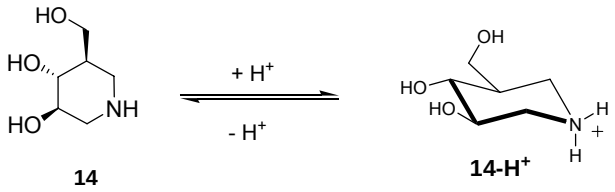


Figure 8: Protonated form of glycosyl amine (**13**), a charge mimic of TS-**5**

Streptomyces cultures.[9] Both NJ and DNJ, if protonated at the basic nitrogen atom they become the charge mimic of TS- **9**, see **Figure 7**.



14 **14-H$^+$**

Figure 9: *Protonated form of Isofagomine* **(14),** *a charge mimic of* TS-**8**

Another group of glycosidase inhibitors belongs to the glycosyl amine class, which upon protonation, resemble TS-5, see **Figure 8**. Analogues of TS-**8**; a creative chemical design of anomer selective β-glycosidase inhibitors led by the groups of Bols[14], Ichikawa[3] and Nishimura[19]. are also synthesized (e. g. Isofagomine **14**, **Figure 9**). This new class of designed molecules was termed as 1-azasugar or 1-*N*-iminosugar class of glycosidase inhibitors.

In a hope of discovering glycosidase-inhibiting alkaloidal analogs of sugars in nature, rigorous isolation and synthesis of many *pyrrolidine, piperidine, indolizidines, pyrrolizidines and Nortropane class of polyhydroxylated alkaloids*[10] lead to the identification of following (**Figure 10**) potent glycosidase inhibitors.

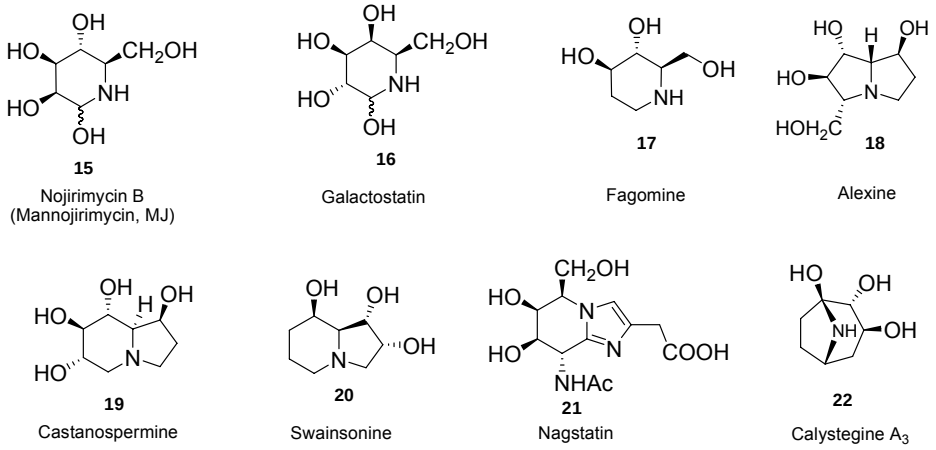


Figure 10: Structures of some of the naturally occurring azasugars.

In general a suitably polyhydroxylated nitrogen containing heterocyclic carbohydrate mimic is called as ***azasugar (iminosugar or azahexose***[11]***)***

These "sugar-shaped alkaloids" are widespread in plants and microorganisms[12,13] and are believed to bind to the active site of the glycosidases by closely mimicking the charge or shape or both of the transition state of the glycosidic cleavage reaction. Several comprehensive reviews and accounts on glycosidases and glycosidase inhibitors have been published, covering various subsections of the field.[10,14-23]

1.4 Therapeutic applications of Sugar–Mimicking (azasugars) Glycosidase inhibitors

Cell-surface carbohydrates are involved in various biological functions, such as cell-cell adhesion, cell-cell recognition and cell-growth regulation. Their implication in the immune response, oncogenesis, tumor metastasis and the differentiation of cells is well realized in glycobiology. Glycosyltranferases and glycosidases are involved in the biosynthesis and degradation of these oligosaccharides and glycoconjugates (glycoslipids, glycoproteins, proteoglycans) that are found in nearly all forms of life. Inhibition of these enzymes can influence the digestion of polysaccharides and the maturation, transport and secretion of glycoproteins. Therefore, hunting for inhibitors of glycosidases that play a significant role in the control of cell surface carbohydrate structure and function could lead to the emergence of novel antidiabetics, antiviral, anti-infective and anti-cancer agents.[2,24-27]

1.4.1 Antidiabetic

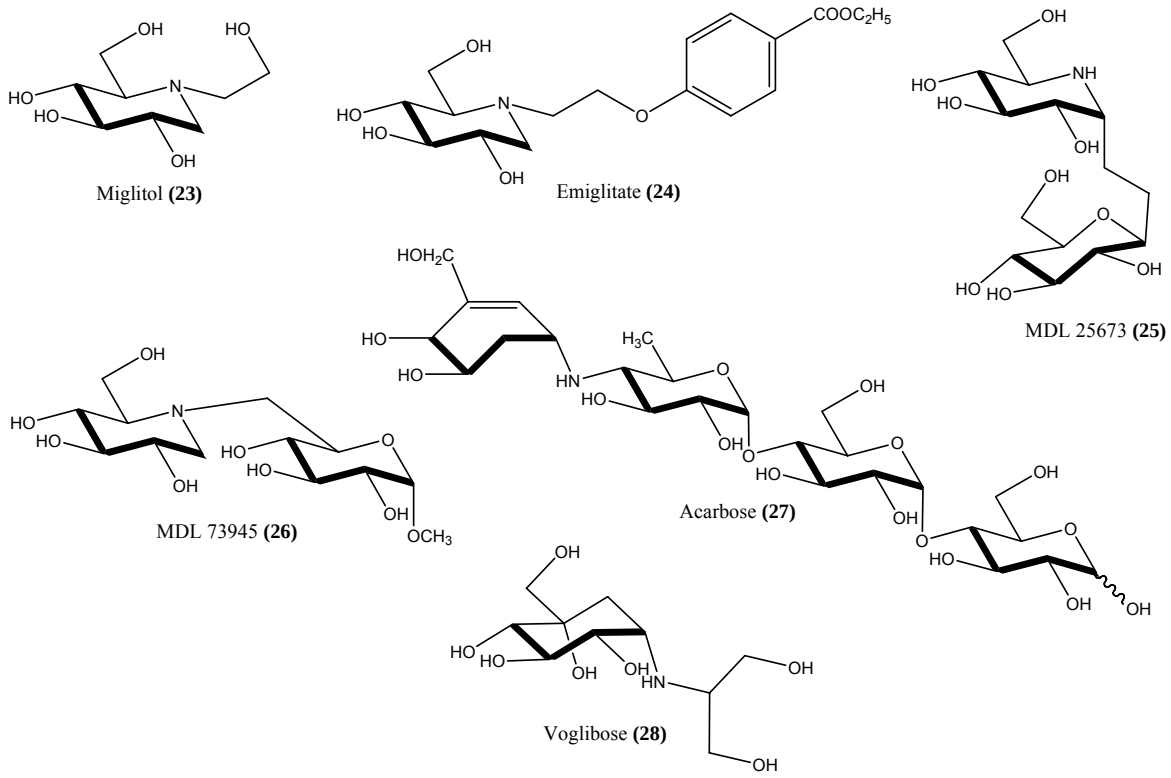


Figure 11: *Structures of some Designed α-glucomimetics as Antidiabetic agents.*

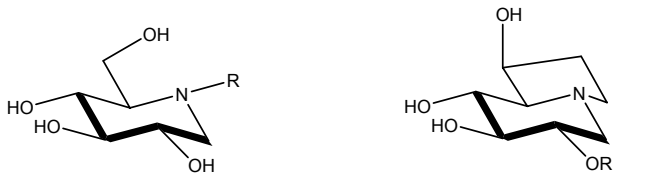
Mulberry leaves have traditionally been used to cure "Xiao-ke" (diabetes) in Chinese medicine. The original isolation of DNJ was prompted by the knowledge that extracts of mulberry were able to suppress the rise in blood glucose that follows eating. The discovery

of the in *vitro* inhibition of mammalian α-glucosidase by DNJ **(12)**, opened the possibility of therapeutic application of this compound. However, its efficacy in *vivo* was only moderate. Therefore, a large number of DNJ **(12)** derivatives were prepared in the hope of increasing the in vivo activity and were screened against α-glucosidase. This study lead to findings that miglitol[28,29] **(23)**, emiglitate **(24)** , MDL 25637 **(25)** and MDL 73945 **(26)** etc. (**Figure 11**) effectively reduce postprandial elevation of blood glucose and plasma insulin in animals in loading tests with starch and sucrose.[30-33]

Today, Miglitol (**23,** GLYSET™), Acarbose (**27**, GLUCOBAY™) and Voglibose (**28**, BASEN™) are commercially available in market for the treatment of type II diabetes.

1.4.2 Antiviral

It is now clear that modification or alteration of one or more biological events during the biosynthesis of *N*-linked, asparagine-linked glycoproteins could have an impact, such as on viral infection or tumor invasion.[24] α-Glucosidase inhibitors, such as DNJ **(12)**, *N*-butyl-DNJ (**29**, *n*-Bu-DNJ), castanospermine **(19)** and celgosivir **(30)** are potent inhibitors of HIV replication and HIV mediated syncytium formation in *vitro*.[34]



12 R = H, 1-deoxynojirimycin (DNJ)
29 R = butyl, *n*-Bu-DNJ

19 R = H, Castanospemine
30 R = Butanoyl, Celgosivir

Figure 12: Structures of N-*n*0Bu-DNJ and Celgosivir having potentials in treatment of HIV-1.

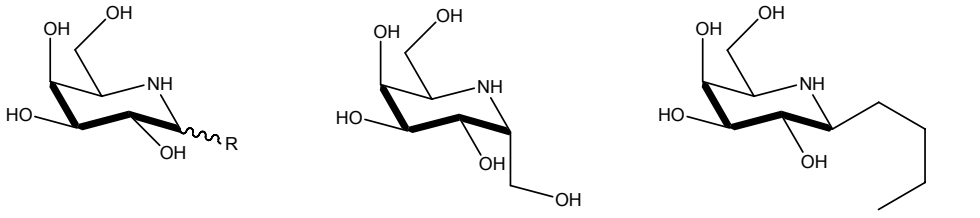
All sugar mimetics showing anti-HIV activity have the common property and it is presumed that the anti-HIV activity results from potent inhibition of processing glycosidase I, since there is a good correlation between the potency of the inhibition of this enzyme and viral control.[35] Treatment of HIV-1 infected cells with an inhibitors such as *n*-Bu-DNJ causes an inhibition of syncytium formation and a reduction in the release of the infectious virus.[36-38]

The naturally occurring azasugar castanospermine **(19)** is an α-glucosidase I inhibitor with marked antiviral activity against a number of viruses (**Figure 12**). Unfortunately, the agent also inhibits intestinal sucrases and causes osmotic diarrhea. In contrast, celgosivir

(30), the 6-*O*-butanoyl derivative of castanospermine, is a relatively inactive inhibitor of intestinal sucrase and appears to be nontoxic to the gastrointestinal tract. It possesses antiviral activity that is 30-fold greater than the parent compound, its active metabolite. Celgosivir has displayed potent antiviral activity in vitro and in vivo 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 as a treatment for HCV infection. The antiviral efficacy and safety of celgosivir were demonstrated in clinical trials in HIV-1-infected patients and it is currently undergoing phase II development for the treatment of HCV infection.[39]

1.4.3. Lysozomal Storage Disorder

Disorders in the biosynthesis or catabolism of glycosphingolipids in the cell have an impact on the so called lysozomal storage diseases like type I Gauchers disease or Fabry disease.[26] No effective treatment of this disorder is available at present. In normal cells, there is balance between the degradation of glycosphingolipids (GLSs) in the lysozomal and their biosynthesis in the ER/Golgi system. In a lysozomal storage disease cell, enzyme activity in the lysozomal cell is so low that GLSs accumulate. Thus, drugs that could regulate the biosynthesis of GLSs to concentration that fits well in the residual enzymatic activity could prevent storage.[40-43] Fabry disease is caused by deficiency of human lysozomal α-galactosidase A (α-Gal A), resulting in a renal failure along with premature myocardial infarction and strokes. Fan et al. demonstrated that DGJ **(31)** inhibits α-galalctosidase A in a competitive manner,[44] effectively enhanced the mutant enzyme activity in lymphoblasts established from Fabry patients [α-HGJ **(32)** and β-1-C-butyl-DGJ **(33)** also showed potent inhibition of α-Gal A] (**Figure 13**).



16 R = OH, Galactostatin
31 R = H, 1-deoxygalactonojirimycin (DGJ)

32 α-homogalactonojirimycin α-HGJ

33 β-1-butyl homoglactonojirimycin

Figure 13: Structures of Potent inhibitors of α-galactosidase, drugs of future in treatment of Fabry diseases

In order to establish the concept of using competitive inhibitors as specific chemical chaperones, a number of naturally occurring and chemically synthesized DGJ derivatives were tested for intracellular enhancement of mutant α-galactosidase A activity in Fabry lymphoblasts. This strategy can be extensively applicable to the lysosomal storage diseases.

1.4.4 Anticancer

The membrane cells of malignant cells differ from normal ones in the structure and composition of their glycoproteins, glycolipids and proteoglycans. As a result, the nature of the carbohydrates that participate in the complex process of metastasis is also specific and these sugars are sometimes modified. Although a number of azasugars have been reported to show anticancer activity such as, NJ **(11)**, MJ **(15)**, DNJ **(12)** and swainsonine **(20)**,[45] research has concentrated on developing swainsonine as a candidate for the management of human malignancies. It inhibits the growth of tumor cells and prevents the dissemination of malignant cells from primary tumor to secondary sites (a process known as metastasis) also there is considerable evidence that swainsonine enhances the natural antitumor defense of the body.[46]

1.5 Design of glycosidase inhibitors [47]

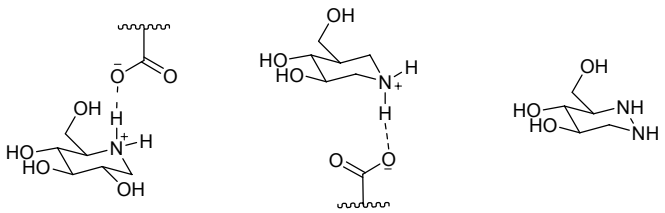
The design of glycosidase inhibitors through a computational method in which a molecule is virtually tied up into an enzyme pocket built on the coordinates obtained from the x-ray analysis of an inhibitor-enzyme complex[48-50] lead to successful design of a very potent sialidase inhibitor, Zanamivir™, which is marketed as an anti-influenza drug.[51,52] However, this method is not always successful and a number of glycosidase inhibitors have been designed based on the design of transition-state (**Figure 8**) analogues of glycosidase hydrolysis,[17] Much of the attention has been on mimicking the assumed geometry of the transition state or position of the assumed charge. Designs focusing on geometry have tried to create inhibitors that are in a half-chair conformation (shape of the oxocarbenium ion TS-**9**), which is commonly accepted to be the conformation of the transition state. Designs focusing on charge have tried to mimic charge build-up in a number of different places. Many lead inhibitors have also been found by high-throughput screenings.[12,20] In this context, aspects involved in design of glycosidase inhibitors are grouped in terms of the six known inhibition mechanisms according to mode of interaction between inhibitor-enzyme complex.

1) Ionic binding with a nucleophilic carboxylate
2) Mimicking the transition state structure
3) Hydrogen bonding with an acidic carboxylate
4) Ionic binding with a non-catalytic group
5) Hydrophobic binding
6) Induced fitting by rearrangement of the enzyme loop domain

If the coordinates of an inhibitor-enzyme complex are known, the computational design method can be used for mechanisms 1 to 5. Induced fitting, 6, however, is difficult to predict with current technologies.[53] On the other hand, many unknown inhibitors may be based on this mechanism, as suggested by the unusual structures recently discovered from nature as glycosidase inhibitors.[54,55]

1.5.1 *Ionic Binding with a Nucleophilic Carboxylate*

Azasugars are the most studied glycosidase inhibitors. 1-Deoxynojirimycin **(12)** (charge mimic **TS-9**, however, having a chair conformation instead of the expected half-chair conformation) is a ring-nitrogen analogue of 1-deoxyglucose and is an inhibitor of both α- and β-glucosidases.[17,56,57]



	12-H$^+$	**14-H$^+$**	**34**
	α-glucosidase	β-glucosidase	
Ki (μM) α-glucosidase (brewer's yeast)	25	86	3.9
β-glucosidase (almonds)	47	0.1	0.65

Figure 14: Structure, Inhibition constant and inhibition mechanism of **12, 14** and **34**.

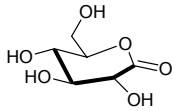
Isofagomine **14** (charge mimic **TS-8**), in which the location of the nitrogen atom corresponds to the anomeric center, shows strong and specific inhibition against β-glucosidase.[3,58] The difference among these inhibitors in the enzyme specificity against α- and β-glucosidases originate from the location of the nucleophilic carboxylate in the

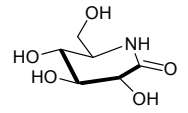
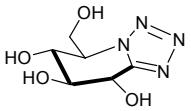
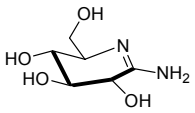
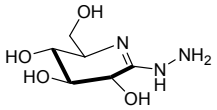
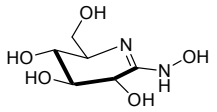
catalytic sites. In configuration-retaining glycosidases, the carboxylate of α-glucosidases resides above the β face of the pyranose ring, while that of β-glucosidases is below α face, in order to meet the steric requirements for the formation of intermediate glycosyl-enzyme complexes (**Figure 14**). Thus, the more efficient complexes with regard to the carboxylate-ammonium ion interaction are formed between α-glucosidase and **12**, and between β-glucosidase and **14**. This mechanism is strongly supported by the strong inhibitory activities against both α- and β-glucosidases by the piperazine derivative **34** synthesized by Bols and co-workers.[59]

1.5.2 Mimicking the Transition State Structure (Oxocarbenium Ion)

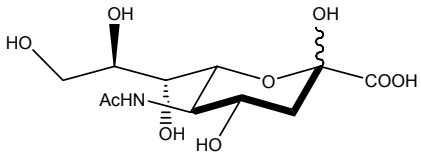
Enzymes promote reactions by lowering the activation energy, which is the result of the strong binding to the transition state structure among the other structures during the reaction course, so that the transition state binding governs the substrate specificity. The transition state structure in the hydrolysis of glycosides is believed to be the oxocarbenium ion intermediate **TS-9** with a flat shape. This means that mimicking this planar structure would lead to a strong inhibitor of a glycosidase. The planar structure of gluconolactone **35**, a classic glucosidase inhibitor, is thought to mimic the transition state structure.[17] δ-Glyconolactam **36** is proposed to inhibit through amide-iminol tautomer as it is showing good specificity for β-glucosidase. Bicyclic compound **37**, which is hybrid of deoxynojirimycin and tetrazole rings, has a half-chair structure that mimics the **TS-9** structure (**Table 1**). Indeed, **37** exhibited a very specific inhibition against a β-glucosidase among the glycosidases tested and a good correlation between its inhibitory activity against each glycosidase and the activation energy exhibited by each substrate, thereby supporting the inhibition mechanism by mimicking the transition state structure.[17] Amidine **38**, amidrazone **39**, and amidoxime **40** were designed and synthesized as oxocarbenium ion analogs and are good inhibitors of β-glucosidases[60,61].

Table 1. Oxocarbenium analogues **35-40** and their Inhibitory activity against glucosidase.

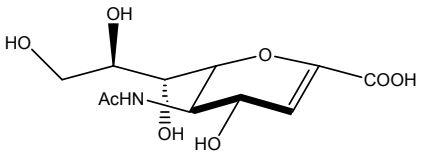
Inhibitor	Enzyme Inhibition (*Ki* μM)		
	α-glucosidase (brewer's yeast)	β-glucosidase (almonds)	β-glucosidase (agrobacterum faecalis)
 **35**	2000	400	1.4

 **36**	1100	125	5.2
 **37**	1300	150	1.4
 **38**	—	10	—
 **39**	—	8.4	—
 **40**	2.9	14	—

It was pointed out that these analogs do not perfectly mimic the transition state structure, because the enzyme specificities exhibited by these compounds are too low to be transition state analogs.[17]



Sialic acid **(41)**



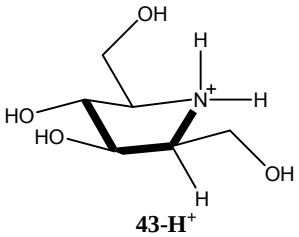
42; *Ki* 4μM (Influenza A sialidase)

Figure15: Sialic acid (**41**) and its 2,3-ene derivative **42**

However, it is also apparent that many planar analogs of glycopyranosides, like glycals, are glycosidase inhibitors. For instance, the 2,3-ene derivative **42** of sialic acid **(41)** is a good inhibitor of sialidases[62] (**Figure 15**).

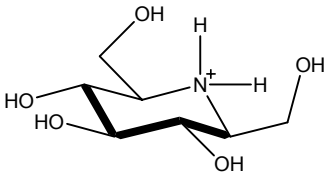
Another approach to mimic the transition state structure is to construct a 5-membered ring as a basic structure. Pyrrolidine **43** was designed and synthesized as a glycosidase inhibitor mimicking the transition state structure and its positive charge (TS-**9**).[56,57] (**Figure**

16).



43-H$^+$

Transition-state shape mimic as well as charge mimic **9**



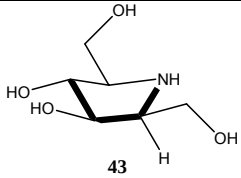
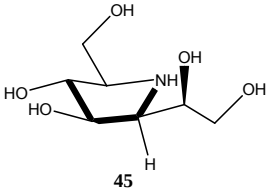
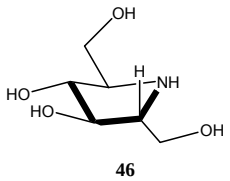
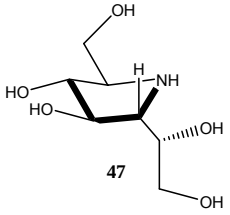
β-L-Homonojirimycin (44),
***Ki* = 900** μM for α-glucosidase (yeast)
***Ki* = 430** μM for β-glucosidase (almonds)

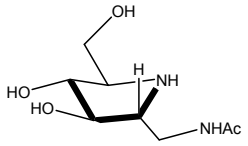
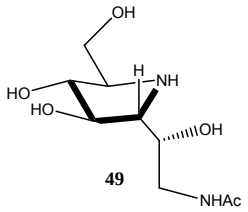
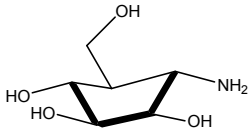
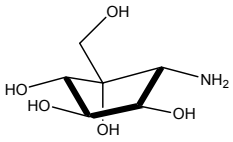
Mimic only the charge of transition state **9** in chair conformation

Figure 16: Structural comparison of pyrrolidine azasugar **43-H$^+$** with **44**[63]

These inhibitors generally show potent inhibitory activity against glycosidases, despite their low specificity. Recently, it was shown that modification of the side chain of pyrrolidine ring including changing stereochemistry enhances selectivity and inhibitory activity (**Table 2**).

Table 2. . Relationship between structure and inhibitory activity for pyrrolidine compounds (**43, 45-49**) and aminocyclopentitol (**50, 51** shape mimic like **9**)

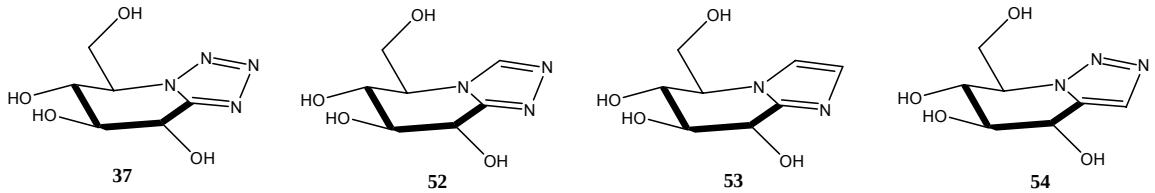
Inhibitor	Enzyme Inhibition (*Ki* μM)		
	α-glucosidase (yeast)	β-glucosidase (almonds)	β-*N*-acetylhexosaminidase (human placenta p)
 **43**	80	52	-
 **45**	330	50	—
 **46**	0.73	1.7	—
 **47**	28	2.6	—

 **48**	380	poor	0.08
 **49**	poor	2.2	poor
 **50**	3.4	13	41 (for β-galactosidase, bovine liver)
 **51**	IC_{50} (μM) = 0.4	IC_{50} (μM) = 29	—

Comparison of compound **43** and **46**, where the orientation of the side chain is different, revealed that the former is a superior inhibitor against both α- and β-glucosidases.[64] Elongation of the side chain (**45**, **47**) resulted in the inhibitory activity weakening only in α-glucosidase. This suggests that bulk substitution at the aglycon part may produce a specific inhibitor of β-glucosidase. In fact, compound with acetamide group **(49)** is highly specific toward β-glucosidase. Interestingly compound **49** is a poor inhibitor of β-*N*-acetylhexosaminidase but compound **48** which carries a shorter side chain is a specific inhibitor against the enzyme.[65] Aminocyclopentitols such as compound **50** and **51** are similar to pyrrolidines with respect to their inhibitory mechanisms.[66,67]

1.5.3 Hydrogen Bonding with an Acidic Carboxylate

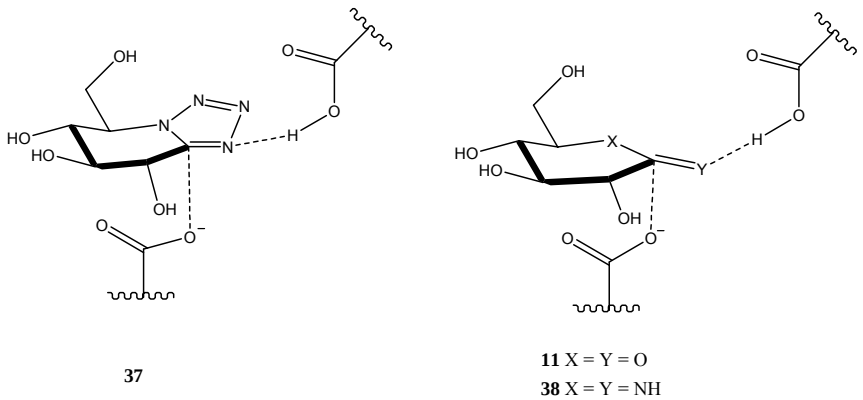
Triazole **52** and imidazole **53** are bicyclic compounds similar to compound **37** (**Figure 17**). These compounds require *exo*-pyranoid nitrogen atom adjacent to the anomeric center for their inhibitory activity, as suggested by the absence of an inhibitory effect of the *exo*-methylene type triazole **54** against glucosidases. Thus, an inhibition mechanism where the acid carboxylate and the nucleophilic carboxylate synergistically operate has been proposed[17] (**Figure 18**).



37 150 μM **52** 19 μM **53** 0.1 μM **54** >8000 μM

Figure 17: Structures of azole-type glycosidase inhibitors **(37, 52-54)** and their inhibitory activities against β-glucosidase from almonds in K_i (μM).

In this model, the hydrogen bonding between the carboxylate and the *exo*-pyranoid nitrogen atom causes an increase in the positive charge at the anomeric center, promoting the ion-ion interaction with the nucleophilic carboxylate. This model requires a coplanar arrangement of the pyranose ring and the acid carboxylate and it was confirmed that the all-available structures of β-glycosidases corresponded to this regime.[17] It was also suggested that lactone **11** and amidine **38** are inhibitors, whose inhibition mechanisms are explainable by cooperation between the two carboxylates.

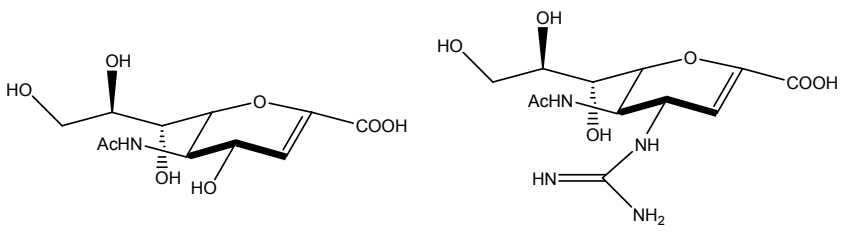


37

11 X = Y = O
38 X = Y = NH

Figure 18: Proposed mechanism involving a hydrogen bonding network among carboxylates and the inhibitors.

1.5.4 Ionic Binding with a Non-Catalytic Group



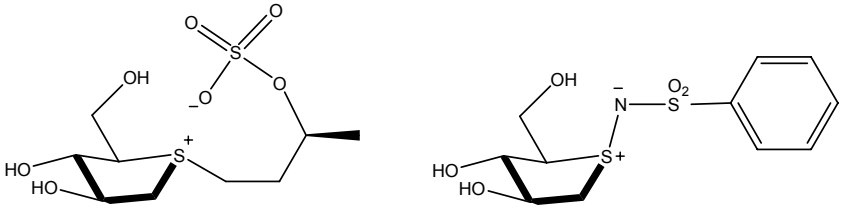
42; *Ki* 4μM (Influenza A sialidase)

Zanamivir (55)
Ki 1nM (Influenza A sialidase)

Figure 19: Design of Zanamivir from coordination data of Sialic acid -sialidase complex.

Docking of **42** into the virtual sialidase pocket built on the sialic acid-sialidase complex coordinates disclosed the existence of the polar groups, Glu119 and

Glu227, around 4-OH of **42** with an enough room for their counter ion, a guanidino group. Thus, zanamivir **(55, Figure 19)**, the 4-guanidino derivative of **42**, was expected to show strong and specific inhibition against influenza sialidase, and this indeed was found to be the case.[51] Salacinol**(56)**, a spirocyclic structure comprised of an intramolecular salt between a tetrahydrothiophene sulfonium ion and a sulfate tethered with an erythritol chain was recently isolated and synthesized.[68-72] The inhibition mechanisms of **56** have not been clarified. However, it is interesting to note that a similar tetrahydrothiophene sulfonium ion compound **57,** designed and synthesized as a transition state analogue inhibitor, shows weak glucosidase inhibition[73] (**Figure 20**).

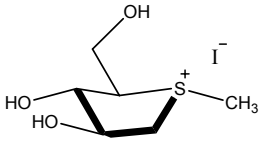


Salacinol (56)

IC_{50} (μM):
>5000 (yeast α-glucosidase)
1.1 (rice α-glucosidase)

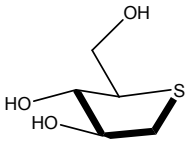
57

K_i (mM):
1.7 (almonds β-glucosidase)



58

IC_{50} (μM):
360 (yeast α-glucosidase)
410 (rice α-glucosidase)



59

IC_{50} (μM):
>5000 (yeast α-glucosidase)
>5000 (rice α-glucosidase)

Figure 20: Salacinol and other analogues for, establishing the significance of sulfate group in naturally occurring salacinol

It was proposed that the sulfonium ion interacted with the nucleophilic carboxylate in the catalytic site, as was suggested for the inhibitory mechanism of pyrrolidine **43/46**, since the methyl sulfonium ion **58** showed good inhibition [74] while 1,4-epithioarabinitol **59** did not. Salacinol **(56)** has greater inhibitory activity and specificity against α-glucosidases than **58**. Hence, the sulfate group is believed to be important for the increased activity and specificity of **56**.[75] The specificity of **56** towards certain glucosidases may be derived from the strong noncovalent binding of its sulfate group to such arginine residues in the binding sites. The above two glucosidase inhibitors, zanamivir (**55**) and Salacinol (**56**) represent good examples of the concept that an extra-functional group added to a non-specific inhibitor causes enhancement in activity and specificity.

This idea is being, increasingly applied to the design synthesis of glucosidase inhibitors,

as the coordination data for inhibitor-glycosidase complexes accumulate and computational chemistry improves.

1.5.5 Hydrophobic Interaction

The sugar-binding pocket of glycosidases is usually made up of several hydrophobic groups, such as tryptophan and tyrosine, surrounding sugar moieties. In the view of this, it is apparent to put a hydrophobic group as an aglycon analog into a catalytic group targeted glycon analogues like azasugars.

The modification of aminocyclopentitol **(60),**[76] calystegineB2 **(62)**,[77] and tetrahydroimidazopyridine **(53)**[78] by introducing a benzene ring into an appropriate position (compounds **61**, **63**, **64**) increased the glucosidase inhibition activity by 40-to 100-fold. The fact that the introduction of *N*-phenyl cyclic isourea **(65)** into aminocyclopentitol **51** caused a 50-fold increase in α-glucosidase inhibition [79] should be, at least in part, due to the hydrophobic effects. (**Figure 21**)

The hydrophobic groups in the glycon-binding sites are potential targets of glycosidase inhibitors, though in many cases, as in azasugars, the catalytic groups have been targeted for ionic binding. Carbafucose **(66)** [80] and 5-thiofucose **(67)** [81] are analogs in which the ring oxygen atom of fucose is replaced by less polar groups, and these analogs have shown good fucosidase inhibition. Replacement of the ring oxygen atom with a sulfur atom causes an approximate 1 kcal/mol gain in the binding free energy.[82] Further addition of a hydrophobic group into **67** at the aglycon site (**68**) caused a 10-fold increase in inhibitory activity.[83] The hydrophobic contact between the ring sulfur and Trp136 can be viewed in the crystal structure of the 5-thioglucose-xylose isomerase complex.[84] Tetrachlorophthalimide **69**, is electronically neutral and consists mainly of hydrophobic parts, except for the imide-nitrogen and carbonyl-oxygen atoms found to be a strong α-glucosidase inhibitor.[85] Though it is not reminiscent of pyranose, the tetrachlorophthalimide portion may mimic the glycon and the pendant benzene the aglycon. Kim and coworkers introduced alkyl chains, in place of the glycerol side chain of sialic acid, into a shikimic acid-analog **(70)** of the oxocarbenium ion intermediate in sialic acid hydrolysis, and studied their sialidase inhibition activities.[62] They found that lengthening the alkyl chain up to three methylenes simply increased the inhibition activity, and that the 3-pentyl compound **71** showed the best inhibition among the many alkyl chains tested, including branched chains. The crystal structure of the **71**-sialidase complex indicated that Glu276 was forced outward and that the

hydrophobic hydrocarbon chains of Glu276, Arg224, Ala246, and Ile222 were in close contact with the 3-pentyl group.

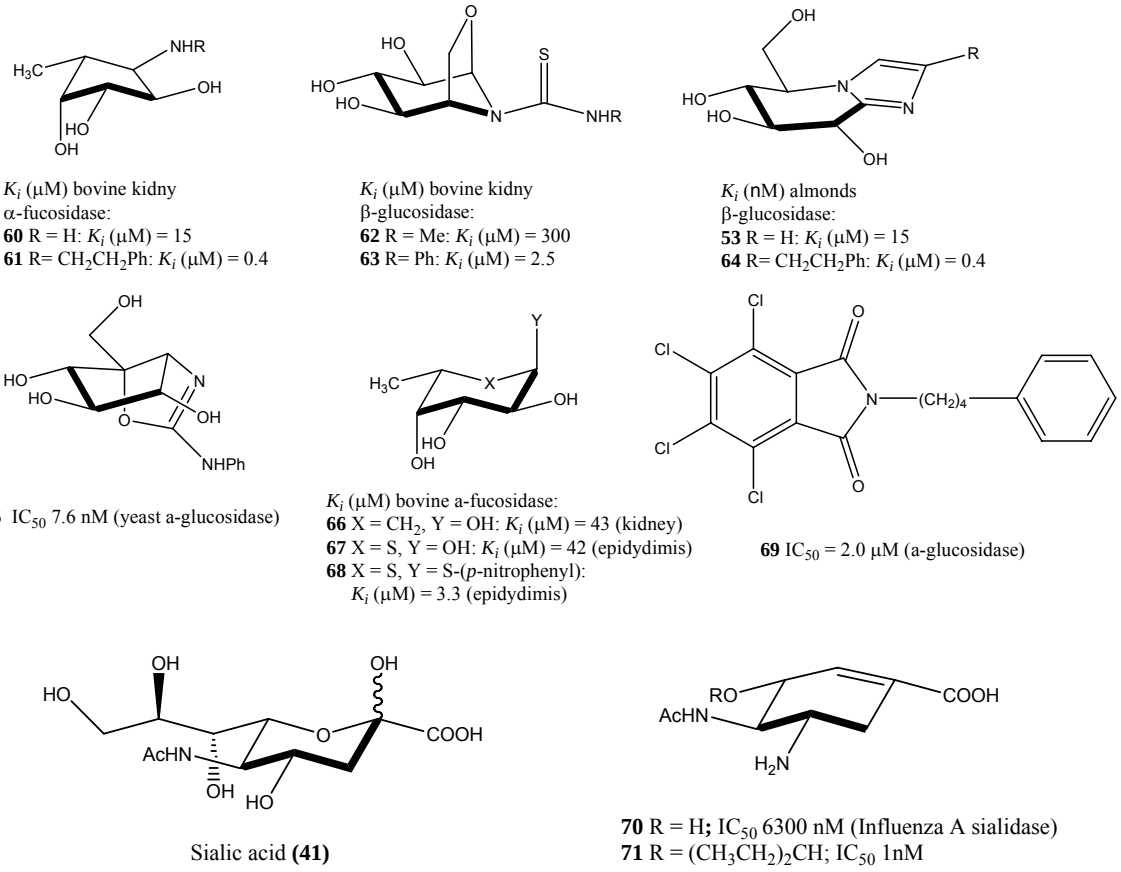


Figure 21: Significance of hydrophobic interactions

These results represent an example of induced fitting, which will be discussed in the following section.

1.5.6 Induced Fitting by Rearrangement of the Enzyme Loop Domain

An example of induced fitting can be seen in the recognition event of a glycogenphosphorylase, though it is not a glycosidase.[86] High-throughput screening disclosed compound **73** (**Figure 22**), which binds to the allosteric site 1000 times more strongly than the regular substrate glucose-6-phospate **72**. The crystal structure of the complex showed several hydrophobic interactions, including one with the chlorophenyl ring of compound **73** sandwiched by Phe196 and Val45'. This sandwiched structure was found to be the result of an induced fitting by shifts in the Phe196 side-chain atoms of up to 2.9 Å and Val45' side-chain atoms of 1.2 Å compared with those in the complex with glucose-6-phosphate.

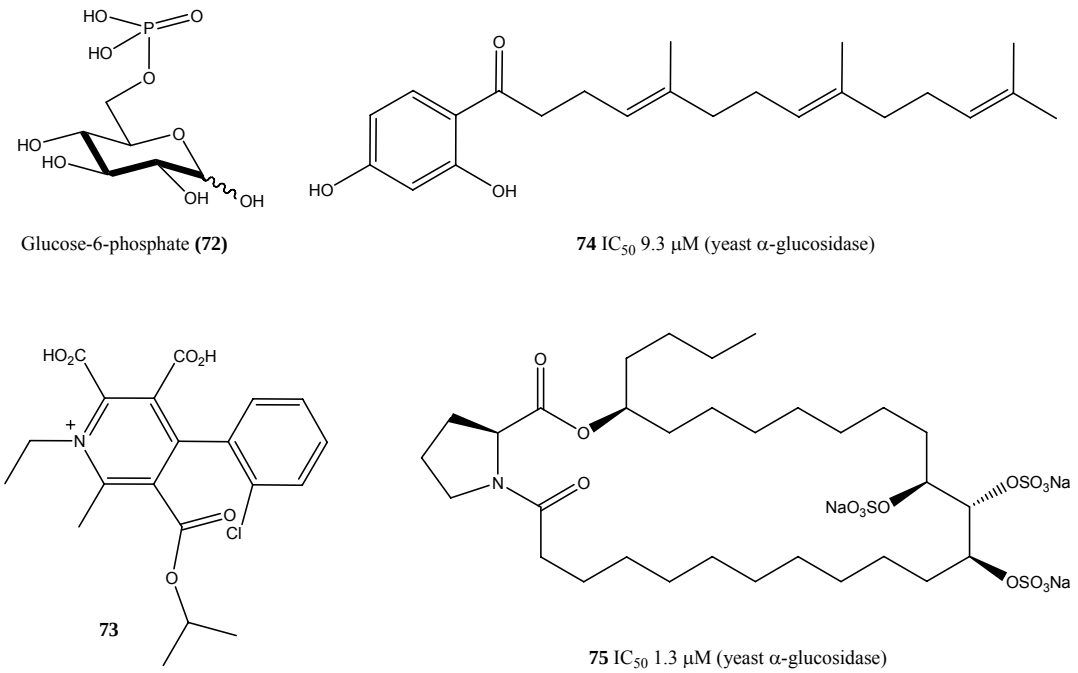


Figure 22: Non carbohydrate-mimics acting through induced fitting by rearrangement of the Enzyme Loop Domain

The structure of compound **73** is totally different from that of glucose-6-phosphate, and this kind of unusual inhibitor could only be discovered by high-throughput screening and serendipitically among the currently available methods. For example compound **74**, isolated from Umbelliferae (a plant family) in Mongolia, is a strong inhibitor of α-glucosidase.[55] Compound **75** is also a strong inhibitor of α-glucosidase and was isolated from a marine sponge in Japan.[54] Since the structures of these compounds do not bear the slightest resemblance to glucose derivatives, induced fitting *via* hydrophobic interactions would be the only plausible explanation for their mode of inhibition. The sulfate groups in **75** may play the same role as those in the proposed mechanism of inhibition for salacinol **56**.

Aims and Questions

As described in this chapter structure-activity relationship study of both substrate and transition state analogous inhibitors provide invaluable information about how an enzyme binds to the substrate and how it stabilizes the transition state. As the more and more data from kinetic, coordination data from inhibitor-glycosidase complexes, computational chemistry, theoretical, and inhibition studies accumulates effective speculations of the inhibition mechanism and probable design of potent inhibitors will emerge.

Besides testing mechanistic hypotheses, the design, synthesis and testing of inhibitors would lead to discovery of strong, selective inhibitors with potential enzymological or therapeutic applications and the purpose of studying inhibitor-enzyme interactions would progress in more fundamental way.

A prerequisite for any structure-activity study is extensive synthetic organic chemist's intellectual and manual labor in order to prepare new potential inhibitors.

The aims of the work presented in this dissertation are as follows:

- Synthesis C-6 homoazasugar analogues of 1-deoxynojirimycin and related analogues.
- Synthesis of β-lactam azasugar hybrid (neutral) molecules (new class of glycosidase) as a transition-state shape mimic with β-lactam carbonyl moiety providing an H-bonding site with acidic carboxylate of enzyme.
- Synthesis of analogues of 1-deoxycastanospermine

as a potential glycosidase inhibitor and enzyme inhibitory evaluation of their activity, with the hope to add valuable information in structural-activity relationship correlation study.

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Chapter 2 Synthesis of the Stereoisomers of C-6 Homologues of 1-Deoxynojirimycin and their Analogues: Evaluation as Specific Glycosidase Inhibitors.

2.1 Introduction

Immediately, after unraveling the potential of nojirimycin (NJ, **11**) as a strong α- and β-glucosidase inhibitor,[1,2] its 1-deoxy analogues such as 1-deoxynojirimycin (**12,** DNJ), 1-deoxygalactonojirimycin (**31,** DGJ**)** and other stereoisomers attracted attention of the synthetic chemists due to their enhanced stability (**Figure 23**).[3-5] Interestingly, intense research activities in this field have culminated in the launch of two new azasugar medicines like Miglitol (**23**, *N*-hydroxyethyl DNJ, Glyset® or Diastabol®)[6,7] for the treatment of type II diabetes and Miglustat (**29**, *N*-*n*-Bu DNJ, Zavesca®)[8-10] for the treatment of Gaucher's disease.

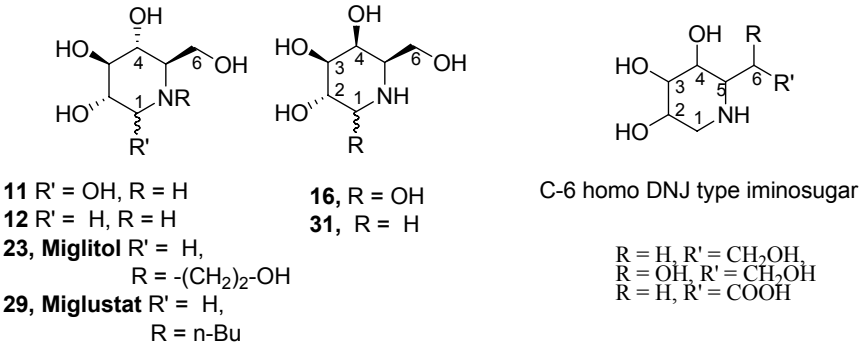


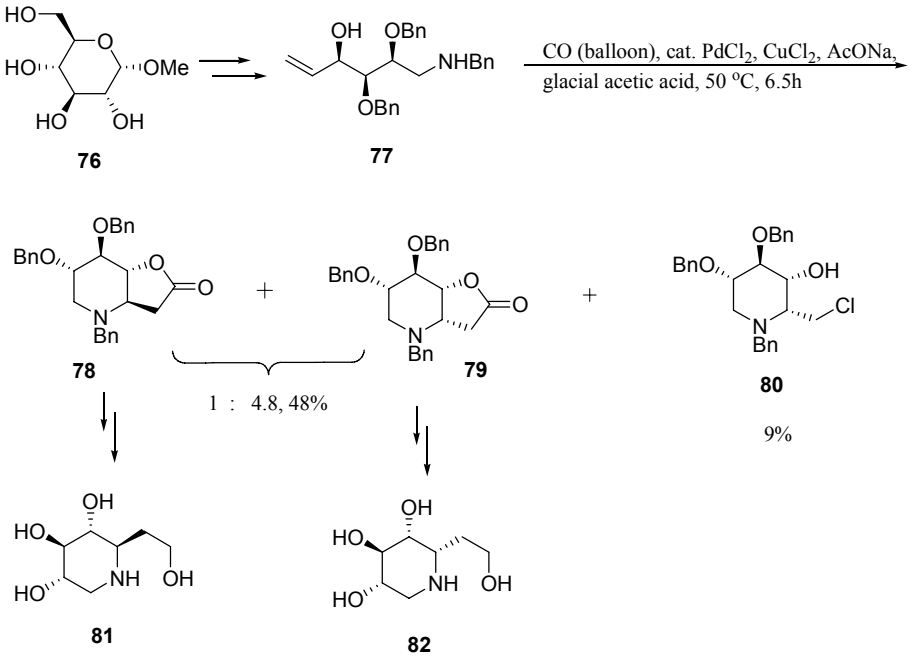
Figure 23: General Structure for C-6 homoazasugars of 1-deoxynojirimycin

In the search of the structure-activity relationship, strong synthetic efforts have also been directed towards the synthesis of the homoazasugars with (CH_2)-homologation at C-6 of such 1-deoxyazasugars.[11-19] The major challenge in designing a synthetic route for these types of azasugars has been the preparation of suitably polyhydroxylated piperidine moiety having a chiral alkyl group at C-5. Therefore, we took up this challenge of designing a suitable strategy for synthesizing C-6 homologues of 1-deoxynojirimycin. However, before we embark upon describing the details of our strategy, it would be imperative to discuss other synthetic strategies reported in the literature.

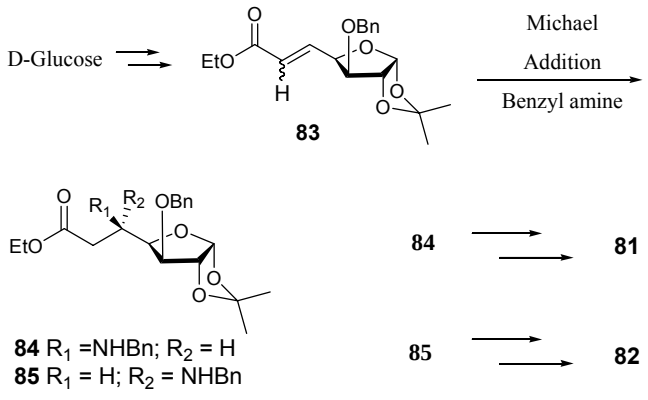
- **Pd(II) catalyzed aminocarbonylation**[19] (*Tetrahedron: Asymmetry* **2000**, *11*, 2579)

Szolcsányi, P. *et al* utilized Pd(II) catalyzed aminocarbonylation of an amine tethered allylic alcohol **77** (**Scheme 1**), derived from D-glucose for the synthesis of 1-deoxy-D-homonojirimycin **(81)** and 1-deoxy-L-homoidonojirimycin **(82)** in poor diastereoselectivity. (1:4.8 dr).

Scheme 1: Pd(II) catalyzed aminocarbonylation



- **Intermolecular Michael addition**[16] (*J. Org. Chem.* **2001**, *66*, 1065)



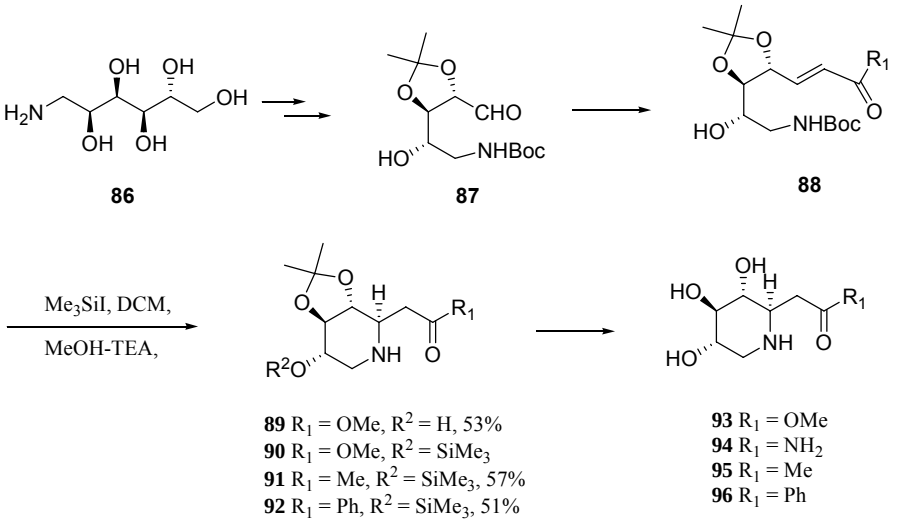
Scheme 2: Intermolecular Michael addition of benzyl amine onto α,β-unsaturated ester

An intermolecular Michael addition of benzyl amine to a sugar derived α,β-unsaturated ester **83** is utilized by Dhavale *et al* (**Scheme 2**). for fixing up the stereocenter at C-5 carbon, however, diastereomeric mixtures of **84** and **85** (25 : 75) were obtained during the synthesis of 1-deoxy-D-homonojirimycin (**81**) and 1-deoxy-L-homoidonojirimycin **(82)**. However, the authors have improved the diastereoselectivity in favor of **85** under kinetically controlled conditions using lithium *N*-benzyl amide as a Michael donor.

- **Intramolecular Michael addition**[15] (*Tetrahedron Lett.* **1994**, *35*, 9047)

Compernolle *et al* constructed C-6 homologues of DNJ (**93, 94, 95, and 96**) in moderate yield *via* intramolecular Michael addition of **88** using TMSI as a Lewis acid (**Scheme 3**). Since, the synthesis started with **86,** the overall sequence is lengthy and low yielding (50-70%).

Scheme 3: Intramolecular Michael addition



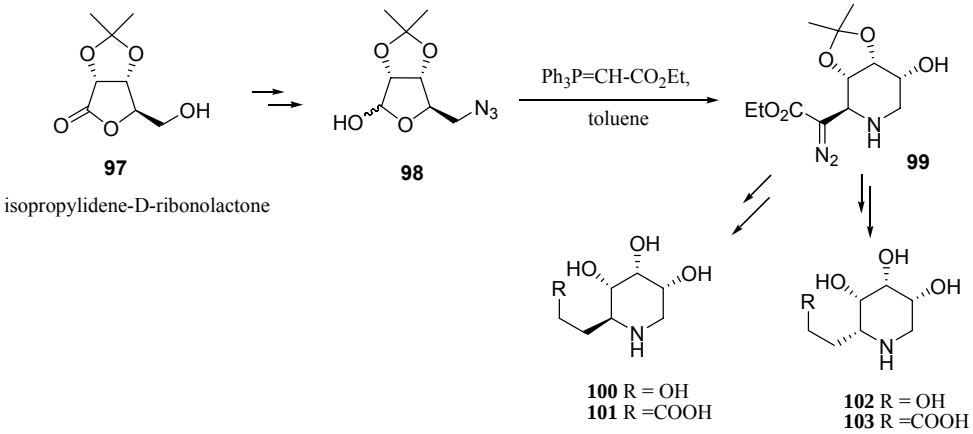
86 **87** **88**

89 R_1 = OMe, R^2 = H, 53%
90 R_1 = OMe, R^2 = $SiMe_3$
91 R_1 = Me, R^2 = $SiMe_3$, 57%
92 R_1 = Ph, R^2 = $SiMe_3$, 51%

93 R_1 = OMe
94 R_1 = NH_2
95 R_1 = Me
96 R_1 = Ph

- **Intramolecular tandem Wittig azide-olefin cycloaddition**[14] (*Tetrahedron* **1996**, *52*, 14745)

Scheme 4: Intramolecular tandem Wittig azide-olefin cycloaddition



97
isopropylidene-D-ribonolactone

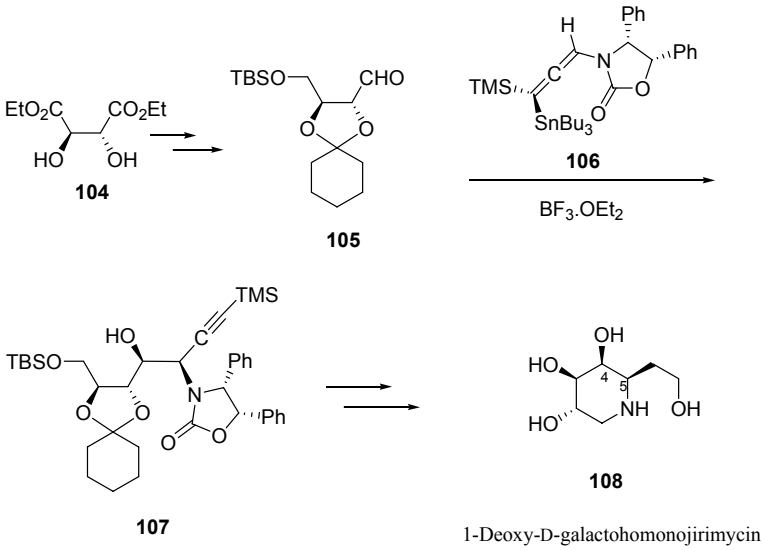
98 **99**

100 R = OH
101 R =COOH

102 R = OH
103 R =COOH

Herdeis *et al* have utilized crucial intramolecular tandem Wittig azide-olefin cycloaddition for the synthesis of D-talo and L-allo homo-1-deoxysugars starting from sugar derivative **97** (**Scheme 4**).

- **Utilization of optically active allenylstannane**[11] (*J. Org. Chem.* **2004**, *69*, 2229)



Hegedus *et al* have utilized an aldol reaction between an optically active allenylstannane **106** and chiral aldehyde **105** as the key step to synthesize 1-deoxy-D-galactohomonojirimycin **(108)** in seven steps (**Scheme 5**). However, the use of toxic tin reagent in the key step limits its application.

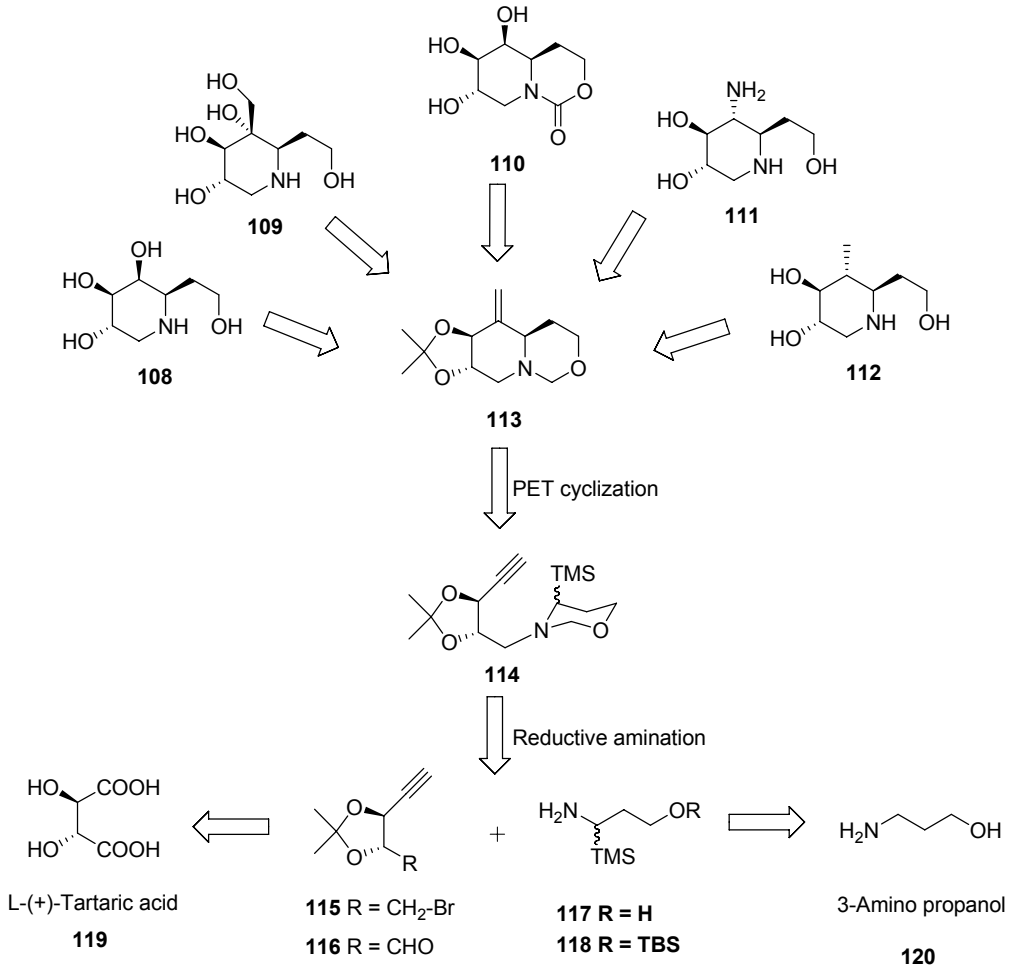
Scheme 5: Optically active allenylstanane for fixing up C-4 and C-5 stereocenters.

Beyond the synthetic challenges, it was surprising to note that none of these studies have attempted the evaluation of the inhibitory potencies of the synthesized C-6 homologues of 1-deoxyazasugars.

2.2 Present Work Plan and Retrosynthetic Analysis.

Considering the unexplored potential of the C-6 homologues of 1-deoxy azasugars as glycosidase inhibitors, we ventured into developing an entirely new and versatile strategy for the synthesis of C-6 homologues of 1-deoxy azasugars. The convergent synthetic approach towards the synthesis of the stereoisomers of C-6 homologues of 1-deoxynojirimycin such as 1-deoxy-D-galactohomonojirimycin **(108)**, 1-deoxy-4-hydroxymethyl-D-glucohomonojirimycin **(109)** and related analogues **110, 111** and **112** is depicted retrosynthetically in **Scheme 6**. The key step in designing template **113** involved cyclization of α-trimethylsilylmethylamine radical cation generated *via* photoinduced electron transfer (PET) reaction to the tethered π-functionality, a strategy developed earlier from our group.[20,21]

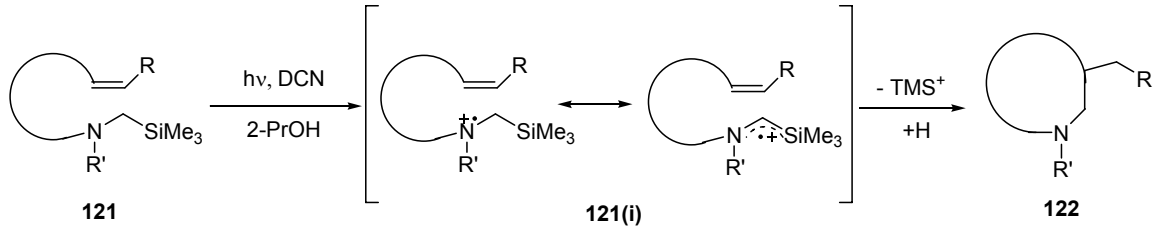
Scheme 6: Retrosynthetic analysis for various C-6 homo-1-deoxynojirimycin *via* template **113**



2.3 PET Initiated α-Trimethylsilymethylamine Radical Cation Cyclization Methodology

The crucial PET cyclization step for the preparation of key precursor **113** emerged from our earlier approach wherein it was shown that PET-generated α-trimethylsilymethylamine radical cation of type **121(i)** cyclizes efficiently to the tethered π-functionality as depicted in **Scheme 7**.

Scheme 7: PET generated amine radial cation cyclization of π-tethered amine



This concept originated from the well-known ability of a β-silicon moiety to participate in the stabilization of a cationic species. The radical cationic species on the nitrogen of intermediate **121(i)** is delocalized between the nitrogen and the silicon atom due to the vertical overlap of the filled C-Si σ-orbital and the half vacant hetero atom orbital of the cation radical intermediate.[21] Intramolecular addition of the π-electron of the tethered group on to the α-trimethylsilymethylamine radical cation and simultaneous elimination of TMS^+ followed by the termination of the resultant radical by H-abstraction from 2-propanol leads to the formation of the cyclic amine of the type **122**.

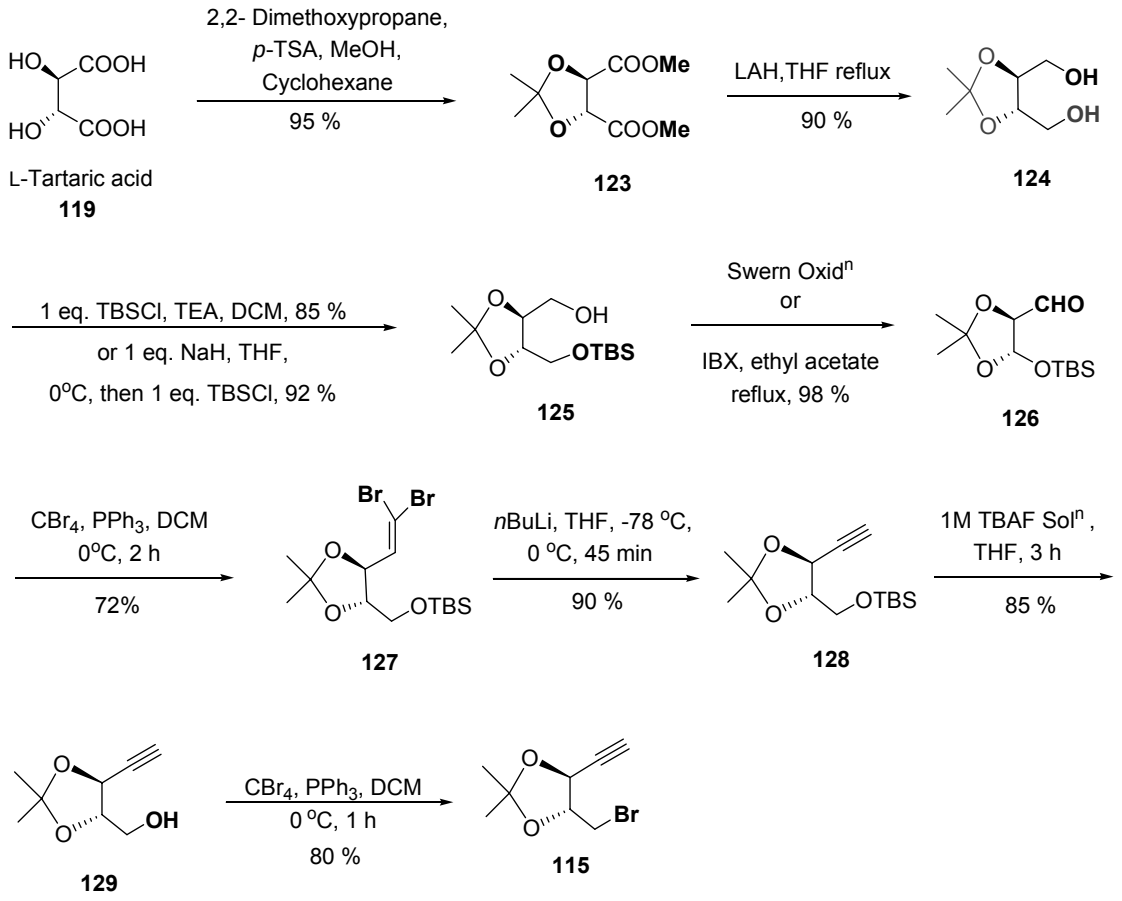
As per our synthetic design, acetylene tethered amine **114** was proposed to be obtained by *N*-alkylation of amine **118** with compound **115**. The two components **117** and **115** are synthesized from L-(+)-tartaric acid **(119)** and 3-aminopropanol **(120),** respectively.

2.4 Preparation of (4*R*,5*S*)-4-(bromomethyl)-5-ethynyl-2,2-dimethyl-1,3-dioxolane (115)

The synthesis of **115** commenced from L-tartaric acid as depicted in **Scheme 8**. One - pot acetonide protection and esterification[22] of L-tartaric acid was carried out by refluxing with methanol, 1,2-dimethoxypropane and *p*-TSA while removing methanol azeotropically. Simple work up and purification afforded **123** in 95 % yield.[22] LAH reduction of **123** resulted corresponding diol **124** in 90 % yield. Monosilylether protection of the diol **124** was chosen as it can be easily removed later under non-reductive and acidic conditions using mild reagent like 1M TBAF solution. The selective monosilylation of diol **124,** carried out by stirring with TBSCl at rt either in dilute solution of DCM / TEA or in THF/ NaH, gave **125** in 85 - 95 % yield.[23] The *t*-butyldimethylsilyl monoether **125** upon oxidation (Swern oxidation) gave corresponding aldehyde **126**. Aldehydic group of **126** was converted to corresponding acetylenic compound **128** using Corey-Fuch's[24] protocol. It would be worth mentioning that the oxidation of **125** using *o*-iodoxybenzoic acid (IBX)[25] followed by its conversion to **128** was found to give higher yield by 7-10 %. It was also noticed that the temperature (0 °C) and time (2 h) played important roles in enhancing the yield during preparation of **127**. Desilylation of **128** using TBAF afforded **129** in 85 % yield. The alcohol functionality in **129** was converted into corresponding bromo derivative **115** (80 % yield) by treating with CBr_4, PPh_3 in DCM.

The spectroscopic data and optical rotation of **115** was found to be in good agreement with the one reported in the literature.[26]

Scheme 8: Synthesis of (4*R*,5*S*)-4-(bromomethyl)-5-ethynyl-2,2-dimethyl-1,3-dioxolane **(115)**



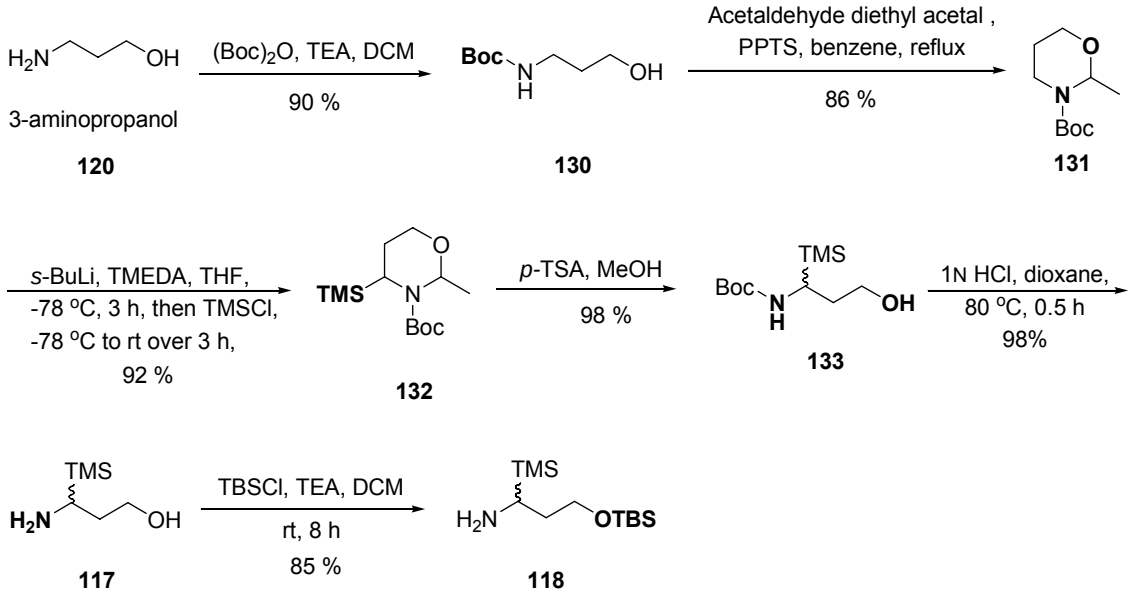
2.5 Synthesis of 3-(*t*-butyldimethylsilyloxy)-1-(trimethylsilyl)propan-1-amine (118)

The synthesis of **118** begins with the commercially available 3-amino propanol by following the steps as depicted in **Scheme 9**. The *N*-Boc protection of 3-aminopropanol (**119**) by stirring with $(Boc)_2O$ in the presence of TEA gave **130** in 90 % yields. The *N*-Boc protected amino alcohol **130** was converted into cyclic amine **131** by refluxing with acetaldehyde diethyl acetal in the presence of *p*-TSA. The cyclic amine **131** on α-metalation using *s*-BuLi / TMEDA in THF at -78 °C followed by the addition of the trimethylsilyl chloride gave **132** in 92 % yield. The ^1H NMR of **132** in $CDCl_3$ (without TMS) showed singlet at δ 0.08 integrating for 9 protons indicating the introduction of a TMS group in the moiety. A broad singlet at δ 1.52 corresponding to 12 protons was assigned to the nine protons of *N*-Boc and three protons of the methyl group. A quartet at δ 5.43 (J = 6.1 Hz)

integrating for 1 proton was assigned to (–N–C<u>H</u>–O–) proton. In the ^{13}C NMR spectrum, peaks at δ -1.1, 79.6 and 154.5 suggests the presence of –TMS, (–N–C<u>H</u>–O–) and *N*–Boc moieties, respectively.

The deprotection of *N*, *O*-acetal moiety of **132** by *p*-TSA in methanol at room temperature afforded amino alcohol **133** in quantitative yield. Heating **133** with 1N HCl in dioxane at 80 °C produced **117** in 98 % yield (crude). Although, direct treatment of **133** with 2N HCl also afforded **117**, the yield was found to be poorer compared to the above mentioned two steps sequence.

Scheme 9: Preparation of 3-(*t*-butyldimethylsilyloxy)-1-(trimethylsilyl)propan-1-amine **(118)**



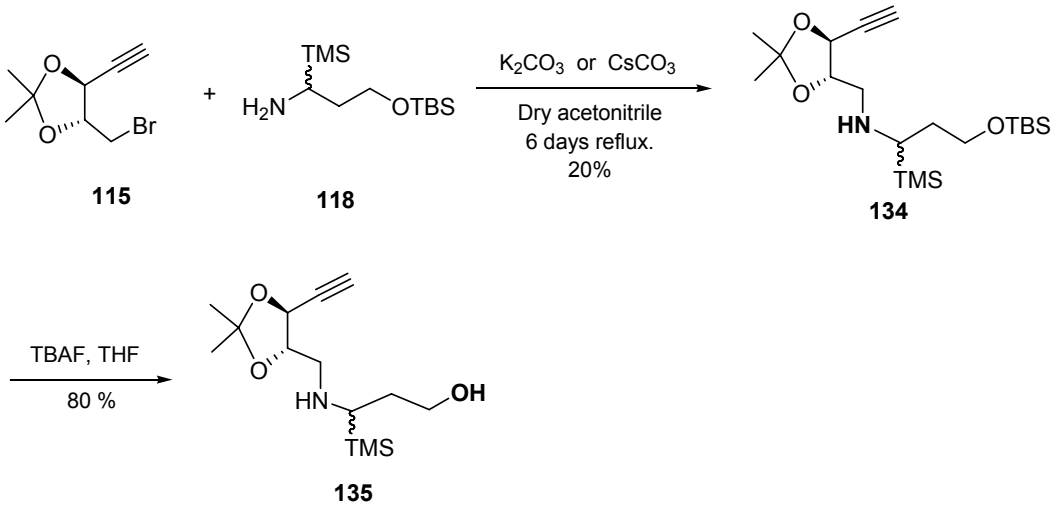
The ^{1}H NMR of **117** showed the presence of total 17 protons. TMS protons appeared at δ -0.04 (s, 9H). A quartet appearing at δ 1.59 (q, 2H, J = 4.9 Hz) corresponds to (-CH-C<u>H</u>$_2$-CH$_2$-OH). A doublet of doublet at δ 2.20 (dd, ^{1}H NMR, J = 4.9) is assigned to (-TMS(C<u>H</u>)-CH$_2$-), and a triplet for (-C<u>H</u>$_2$-OH) was observed at δ 3.62 (t, 2H, J = 5.8 Hz). ^{13}C NMR of **117** showed the presence of 4 non-equivalent carbon peaks at δ -4.1 [(<u>C</u>H$_3$)$_3$Si-], 2 CH$_2$ carbons at δ 34.4, 62.2 and one CH carbon at δ 40.3.

To avoid complications during alkylation of primary amine functionality in the presence of primary -OH, the primary -OH group was protected (85 %) as -OTBS ether **118**.

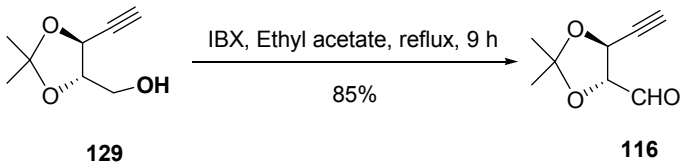
2.6 Preparation of PET precursor 114 and its cyclization to 113.

Initially, we tried to synthesize **134** by usual base promoted *N*-alkylation of the amine **118** with the primary halide **115** using large excess of K_2CO_3 or $CsCO_3$ in acetonitrile. However, to our frustration the reaction was found to be very sluggish and the required *N*-alkylated product **134** was also obtained only in poor yield (~20 %) (**Scheme 10**).Therefore, we decided to utilize an alternative approach *via* reductive amination of aldehyde **116**.

Scheme 10: Preparation of **135**

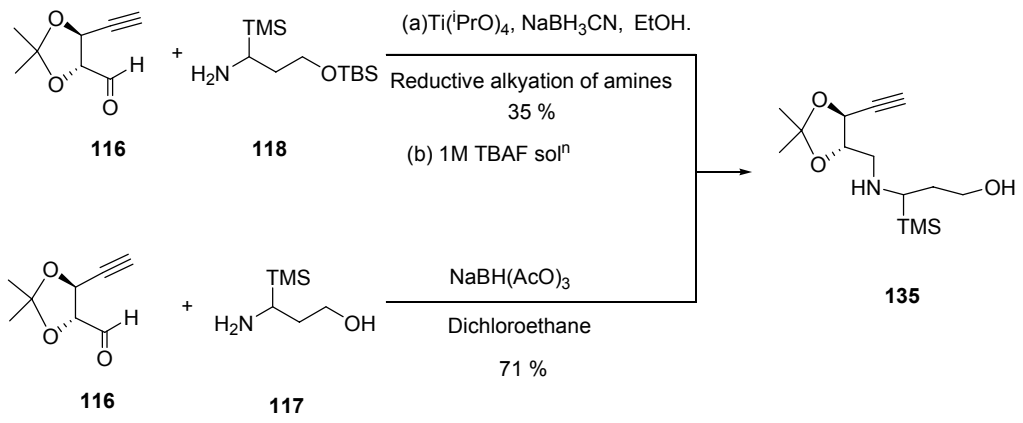


Scheme 11



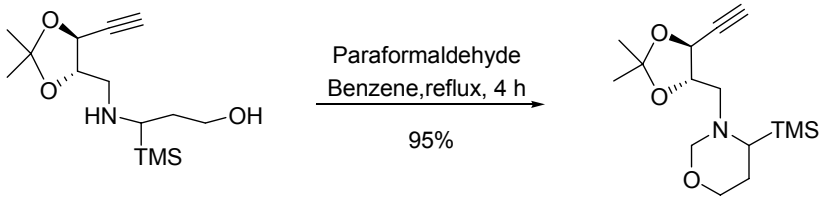
The aldehyde **116** which was obtained in 85 % yield by IBX oxidation[25] of **129** (**Scheme 11**) was found to be very labile and was quickly subjected to reductive amination with amine **118** using titanium isopropoxide and sodium cyanoborohydride.[27] Since the yield of the required product was just 35% better than the base promoted *N*-alkylation strategy, another protocol for the reductive amination was carried out using 2 equivalent of sodium triacetoxyborohydride[28] which afforded **135** in 71 % yield as a 1:1 diastereomeric mixture (**Scheme 12**).

Scheme 12: Reductive amination protocol



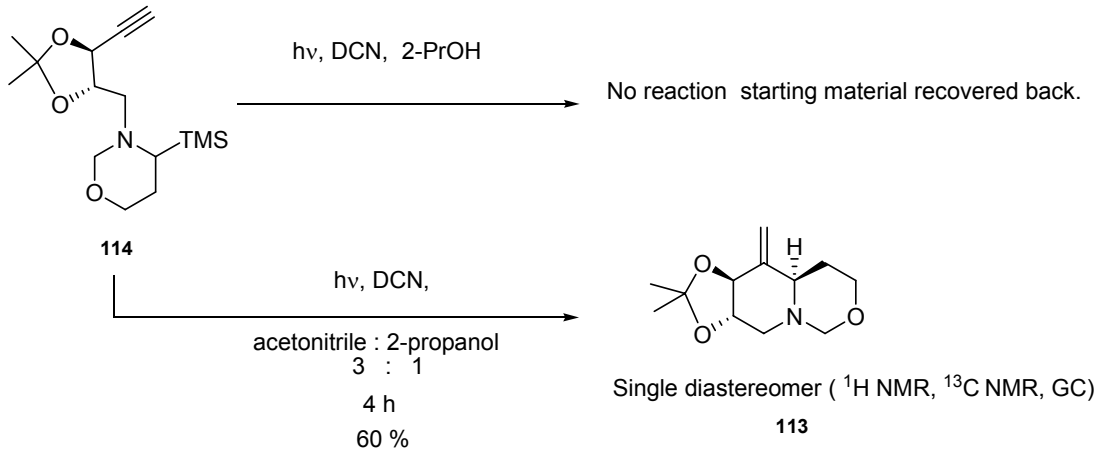
Since our PET cyclization strategy required *N*-alkylated α-trimethylsilylmethyl amine moiety, we transformed **135** into the cyclic 1,3-oxazine derivative **114** by refluxing with paraformaledyde (**Scheme 13**). The thought of utilizing open chain *N*-alkylated derivative of **135** for PET cyclization was abandoned considering our previous experience of poor diastereoselectivity.[20]

Scheme 13



The (1:1) diastereomeric mixture of compound **114** was fully characterized by ^{1}H NMR, ^{13}C NMR and COSY NMR spectroscopy.

Scheme 14: PET cyclization of **114**



Substrate **114** was attempted to cyclize, employing the PET reaction protocol reported from our group,[21] by irradiating a solution containing **114** (3 mmol), 1,4-dicyanonaphthalene (DCN, 0.4 mmol) in 2-propanol using 450 W medium pressure mercury lamp. However, to our great surprise no reaction could be observed even after 10 h of irradiation. Therefore, we evaluated various proportions of acetonitrile:2-propanol and finally 3:1 solvent mixture proved to the best for carrying out the cyclization reaction to obtain **113** as a single diastereomer in 60 % yield (**Scheme 14**).

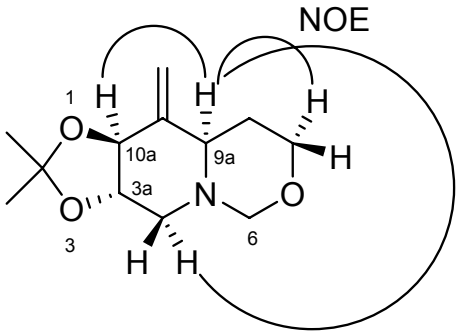


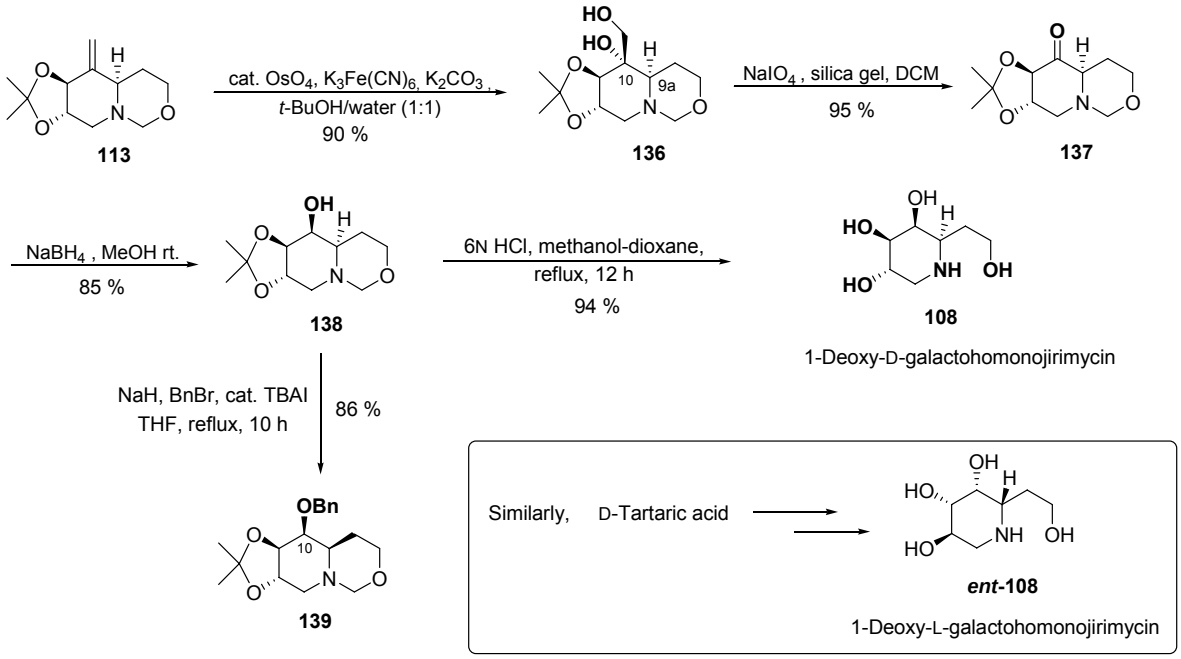
Figure 24: NOE correlation of cyclized product **113**

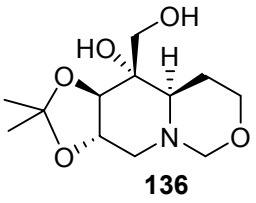
The cyclized product **113** was fully characterized by extensive ^{1}H NMR, ^{13}C NMR and 2D COSY, NOESY (**Figure 24**) spectral analyses. Based on the detailed 1D and 2D ^{1}H NMR study, the peaks at δ 4.9 (t, 1H, *J* = 1.7 Hz) and 5.1 (t, 1H, *J* = 1.7 Hz) suggest the presence of an exocyclic double bond in the product. The doublet at δ 2.65 (d, 1H) was assigned to H_{9a} proton whereas other doublets at 3.86 (d, 1H, *J* = 8.0 Hz) and 4.48 (d, 1H, *J* = 8.0 Hz) could be assigned to the methyelene protons of C-6. ^{13}C NMR indicated the presence of 12 non-equivalent carbons. DEPT spectrum characterized 2 acetonide methyls at δ 26.6, 26.8; 5 CH_2 carbons at δ 27.7, 51.2, 67.1, 86.5, 103.6; 3 CH carbons at δ 60.4, 76.5, 81.5; 2 quaternary carbons at δ 111.0, 142.7. The observed NOE between H-10a and H-9a proves the stereochemistry of H-9a to be *R* in **113.**

2.7 Synthesis of 1-Deoxy-galactohomonojirimycin (108)

In order to synthesize **108,** the PET product **113** was treated with catalytic osmium tetroxide along with $K_3Fe(CN)_6$ and K_2CO_3 in 1:1 mixture of *t*-BuOH and water. The usual work-up and purification gave **136** as a crystalline solid in 90 % yield as an exclusive diastereomer. The dihydroxylated product **136** was extensively characterized by ^{1}H NMR, ^{13}C NMR and 2D COSY, NOESY spectral studies confirming the stereochemical outcome of dihydroxylated product **136** (**Scheme 15**). The stereochemistry at C-10 and C-9a is further confirmed from the X-ray crystal structure (**Figure 25**). The diol **136** upon oxidative cleavage with sodium periodate adsorbed on silica gel afforded corresponding ketone **137** which was confirmed by observing a keto-carbonyl carbon in ^{13}C NMR at δ 197.6.

Scheme 15: Synthesis of 1-deoxy-D-galactohomonojirimycin **(108)**





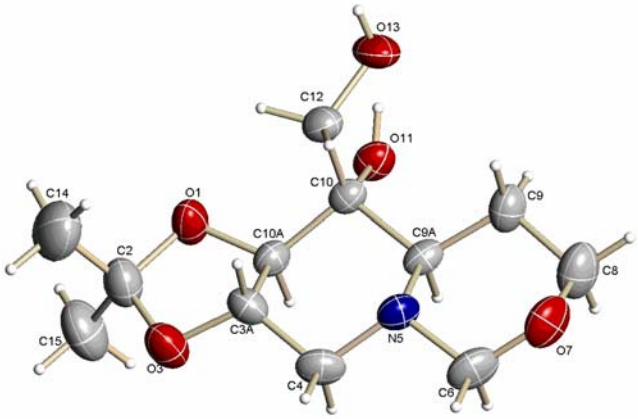
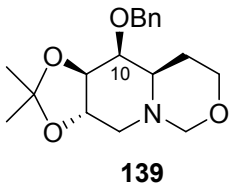


Figure 25: ORTEP Diagram for **136**

The sodium borohydride reduction of **137** in methanol gave **138** in 85 % yield as a single diastereoisomer. The stereochemistry of alcohol **138** was also confirmed from 1D as well as 2D ^{1}H NMR spectroscopy of the corresponding benzylated derivative **139**.

The stereochemistry at C-10 of **139** was ascertained by analyzing the coupling constants for H-3a (δ 4.24, dt, J = 4.2, 9.8 Hz), H-10a (δ 3.40, dd J = 2.2, 9.3 Hz), H-10 (δ 3.84, t, J = 2 Hz) and H-9a (δ 2.24, t, J = 3.3 Hz) which suggested the orientations for H-3a-axial, H-

10a-axial, H-10-equatorial and H-9a-axial. This stereochemical analysis was further confirmed by X-ray crystallography (**Figure 26**).



139

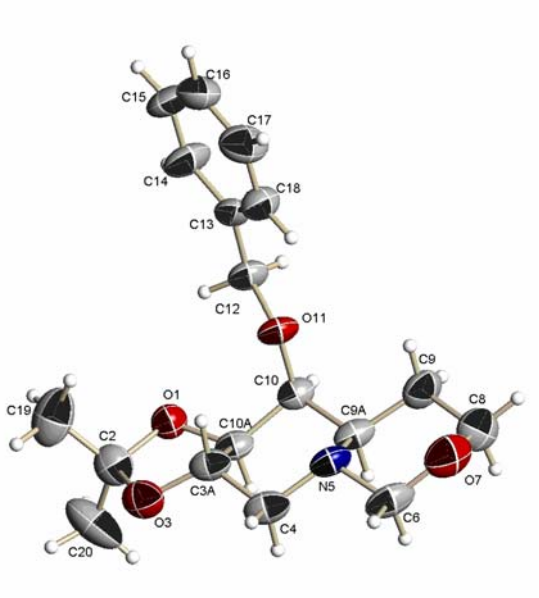
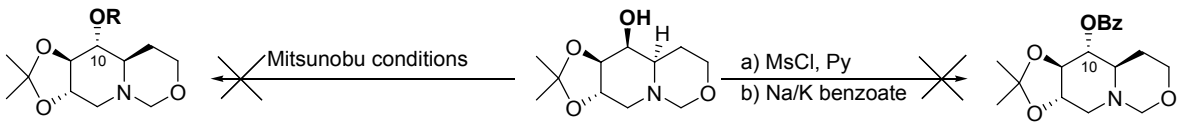


Figure 26: ORTEP diagram for **139**

The acetonide and 1,3-oxazine ring moieties of **138** were removed by refluxing with 6N HCl in methanol for 12 h to obtain the targeted compound 1-deoxy-D-galactohomonojirimycin **(108)** in 95% yield. Similarly, starting from D-tartaric acid 1-deoxy-L-galactohomonojirimycin **(*ent*-108)** was also synthesized. ^{1}H NMR of **108** in D_2O is in good agreement with the one reported in the literature[11] which was also characterized extensively by 2D NMR spectroscopy like COSY and HSQC.

Next, we attempted to synthesize 1-deoxy-D-glucohomonojirimycin by inverting the stereochemistry of hydroxyl group at C-10 of **138** under Mitsunobu conditions. However, various permutations and combinations of reagents, solvents and temperature met without success. Furthermore, we also tried nucleophilic S_N2 displacement of –OMs derivative of **138** with Na/K benzoate to obtain **140**, however, this effort also failed to give the required product (**Scheme 16**).

Scheme 16.



140 R = Bz
141 R = *p*-NO_2Bz

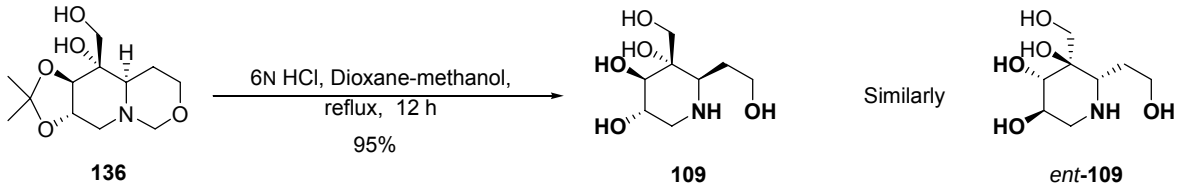
138

140

2.8 Synthesis of 1-deoxy-4-hydroxymethyl-glucohomonojirimycin

Having developed a novel strategy to obtain **136** in sufficiently good amount, it was visualized that removal of all the protecting groups from it, would provide a new molecule which would have all the structural features of an azasugar. Therefore, all the protecting groups from **136** and *ent*-**136** were removed by refluxing with 6N HCl in dioxane-methanol (1:1) to obtain 1-deoxy-4-hydroxymethyl-D-glucohomonojirimycin **(109)** and 1-deoxy-4-hydroxymethyl-L-glucohomonojirimycin **(*ent*-109),** respectively, in 95% yield.

Scheme 17: Synthesis of 1-deoxy-4-hydroxymethyl-glucohomonojirimycin

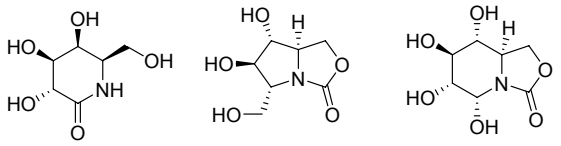


136 **109** ***ent*-109**

^{1}H NMR of **109** showed 11 protons while ^{13}C and DEPT indicated presence of 4 CH_2 carbons δ 30.6, 49.2, 60.0(for 2 CH_2); 3 CH carbons at δ 60.7, 68.9, 80.8; one quaternary carbon at δ 73.6 confirming the skeletal frame work of compound as **109**.

2.9 Synthesis of Pseudoamide type azasugar

As described earlier in Chapter I, generally NJ **(11)**, DNJ **(12)** and their analogues are believed to exhibit their glycosidase inhibitory activities due to their binding with the glycosidases by mimicking the shape and charge of the postulated oxo-carbenium ion intermediate for the glycosidic bond cleavage reaction.[29] However, there has been intense interest, recently, in evaluating non basic neutral glyconolactams such as **142**,[30,31] **143**[32] and **144**[33,34] in which glycosidic oxygen is replaced by pseudo sp^2 ring nitrogen, as glycosidase inhibitors (**Figure 27**) and considerable degree of inhibitory activities have been recorded. In these examples, where glycosidic oxygen is replaced by pseudo sp^2 ring nitrogen (e.g. **142,** *Ki* = 85 μM, β-glucosidase), mechanistically were originally believed to inhibit involving tautomeric iminol form. However, recent studies suggests that the glycosidase inhibition by these compounds may in fact be caused by the H-bonding of the lactam carbonyl[35] moiety with the enzyme as the tautomerization energy for the amide-iminol conversion is of the order of 11 kcal mol^{-1} indicating the concentration of the corresponding iminol form in solution at a given time to be very low.[36]

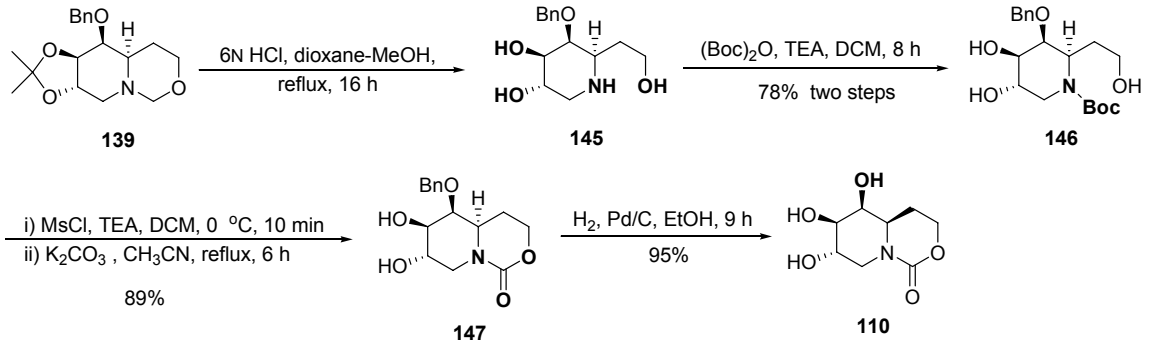


142 **143** **144**

Figure 27: Non-basic azasugars

In this context, we also envisioned that a hitherto unknown non-basic neutral molecule **110** could easily be realized from **139** for evaluation as a new glycosidase inhibitor. Towards this end, the acetonide as well as 1,3-oxazine ring moiety of **139** were deprotected by refluxing with 6N HCl dioxane-methanol (2:1) for 16 h to afford **145**. The secondary amine functionality in **145** was protected as *N*-Boc derivative **146**. Mesylation of the primary alcoholic moiety of **146** followed by reflux under basic conditions in acetonitrile[37] and debenzylation gave compound **110** in 85% yield over all three steps (**Scheme 18**).

Scheme 18: Synthesis of pseudoamide type molecule **110**



139 **145** **146** **147** **110**

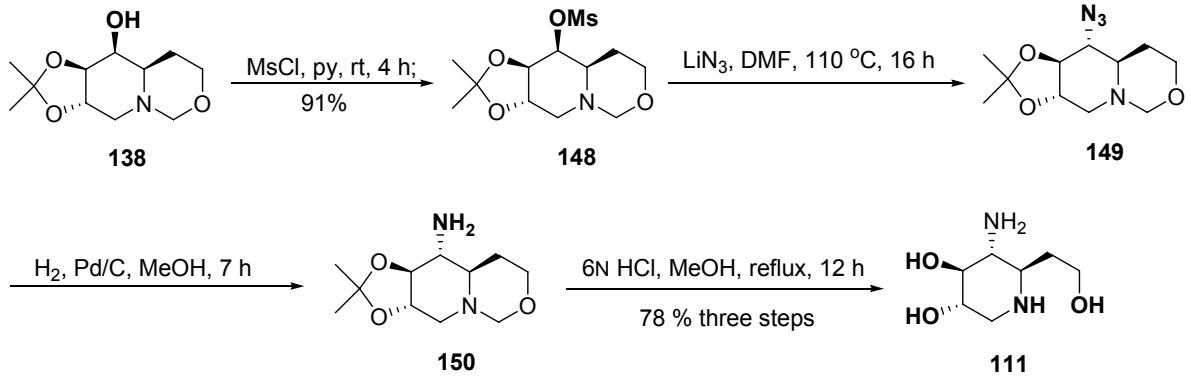
Structural characterization of compound **110** was carried out using ^{1}H, ^{13}C, COSY, NOESY and HETCOR spectroscopy. The ^{1}H NMR of compound **110** indicated the absence of *N*–Boc and -OMs moieties and the presence of total 10 protons. The presence of pseudoamide type carbonyl moiety was confirmed from ^{13}C NMR. ^{13}C and DEPT studies indicated the presence of 8 carbons; 3 CH_2 carbons at δ 23.2, 47.8, 65.4; 4 CH carbons at δ 55.0, 65.5, 70.5, 74.2; one quaternary carbon corresponding to pseudoamide type carbonyl carbon at δ 156.8. IR spectrum showed strong absorbance at 1682 cm^{-1} indicating the presence of carbonyl group in the molecule.

2.10 Synthesis of (3*S*,4*S*,5*R*,6*R*)-5-amino-6-(2-hydroxyethyl)piperidine-3,4-diol (111)

In continuation, another new analogue **111** having a basic amine moiety at C-4 was also visualized to be easily affordable from **138** for evaluation as a glycosidase inhibitor**.** To this end, alcohol **138** was treated with mesyl chloride in pyridine as a base and solvent as well to afford **148** in 91 % yield. The peak at δ 3.12 (s, 3H) in ^{1}H NMR confirms the

formation of **148**. The mesyl group was transformed in to an azide group by nucleophilic S_N2 displacement reaction using lithium azide in DMF at 110 °C. The presence of azide functionality in the product was confirmed from IR as a medium absorbance band at 2252 cm^{-1} was observed. The catalytic (Pd/C, 10%) hydrogenation of the azide derivative **149** afforded amine **150** almost quantitatively. The acetonide deprotection followed by 1,3-oxazine ring opening afforded **111** in 78% yield over three steps (**Scheme 19**).

Scheme 19: Synthesis of (3*S*,4*S*,5*R*,6*R*)-5-amino-6-(2-hydroxyethyl)piperidine-3,4-diol **(111)**



2.11 Synthesis of (3*S*,4*S*,5*R*,6*R*)-6-(2-hydroxyethyl)-5-methylpiperidine-3,4-diol (112)

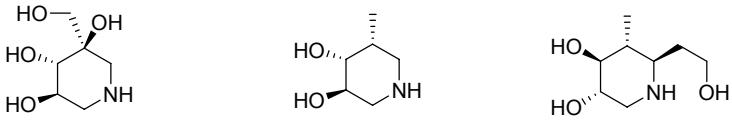


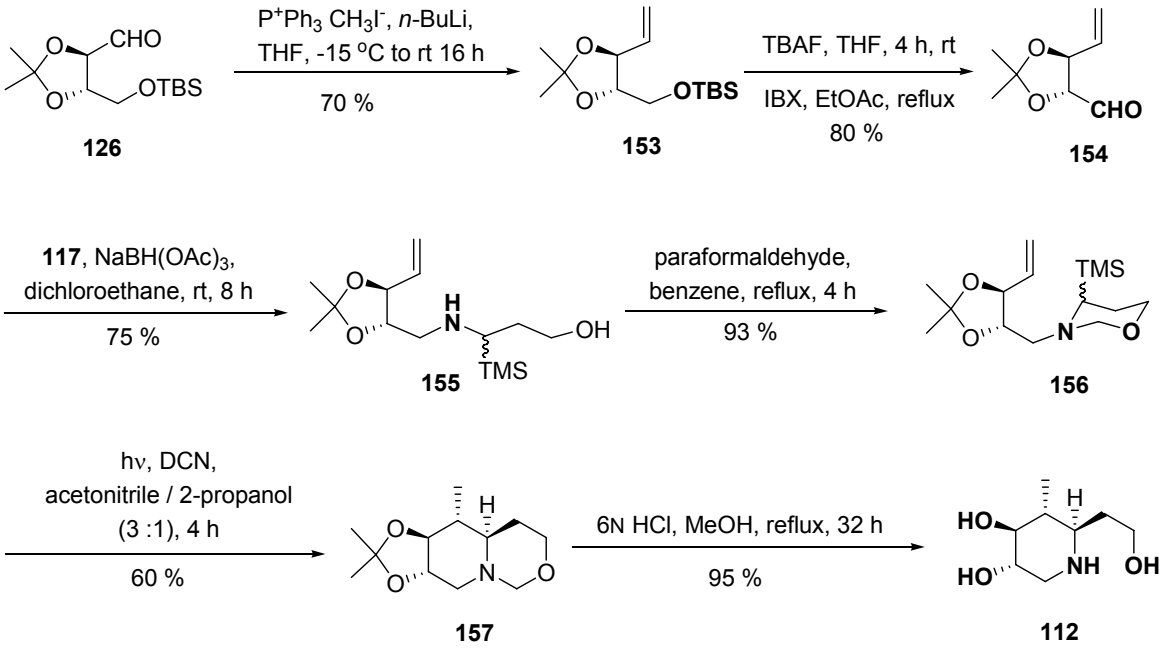
Figure 28: 1-*N*-iminosugar (**151, 152**), DNJ-type iminosugar (**112**)

Since, we had earlier observed that 1-*N*-iminosugar **152** showed better inhibitory activity for the β-glucosidase (*Ki* = 30 μM) than **151** (*Ki* =90 μM),[38] it also occurred to us that it would be pertinent to evaluate the enzyme inhibition activity of similar C-6 homoazasugar of 1-deoxynojirimycin type molecule **112** (**Figure 28**). In this context, we synthesized compound **157** by following the analogous route as described for **113** starting from aldehyde **126** as shown in **Scheme 20**. Since the direct hydrogenation of exocyclic double bond in **113** would have given diastereomeric mixture as observed in the case of the synthesis of **152**,[38] the aldehyde was subjected to one carbon Wittig reaction to obtain olefin **153.**

The olefin **153** on desilylation with 1M TBAF in THF afforded corresponding free alcohol which on IBX oxidation afforded aldehyde **154**. The aldehyde **154** on reductive amination with **117** afforded olefin tethered amino alcohol **155** in 75 % yield. Refluxing compound **155** with paraformaldehyde in benzene afforded olefin tethered cyclic amine

156. PET cyclization of **156** by following identical irradiation condition as described for **114** produced **157** as a single diastereomer in 60% yield. The cyclized product **157** was characterized by ^{1}H, ^{13}C, COSY, NOESY and HETCOR spectroscopic studies.

Scheme 20: Synthesis of (3*S*,4*S*,5*R*,6*R*)-6-(2-Hydroxyethyl)-5-methylpiperidine-3,4-diol **(112)**



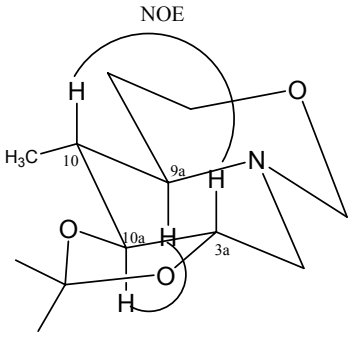


Figure 29: NOE correlation for PET cyclized product **157**

^{1}H NMR spectrum of **157** showed the presence of 21 protons. A doublet at δ 0.93 (d, J = 6.4 Hz) confirms the presence of a methyl group in the molecule. The stereochemistry at C-10 and C-9a of **157** was ascertained by analyzing the coupling constants for H-3a (δ 3.57, ddd, J = 4.1, 7.4, 9.5 Hz), H-10a (δ 2.96, dd, J = 8.7, 10.5 Hz) and by ^{1}H-^{1}H NOESY spectrum (**Figure 29**). The removal of the acetonide and 1,3-oxazine ring moiety from **157** gave **112** in 95% yield.

2.12 Enzyme inhibition study:

The inhibitory activities of **108**, **109, 110, 111, 112,** *ent*-**108** and *ent*-**109** were screened against β-galactosidase (*Aspergillus oryzae*), α-galactosidase (coffee beans), β-glucosidase / β-mannosidase (almonds), α-glucosidase (yeast) and α-mannosidase (jack beans). The results are summarized in the **Table 3**.

Table 3: Inhibition of various glycosidases by **108**, **109, 110, 111, 112, ent-108** and **ent-109.** (K_i in μM).*

Glycosidase → / Inhibitor↓	β- Gal	α-Gal	β-Glc	α-Glc	β-Man	α-Man
1-DNJ (12)[a]	—	—	47	25	—	270
1-DGJ (31)[b]	0.16	0.0016	540	1000	—	—
108	1100	1.7	n.i.	n.i.	n.i.	n.i.
109	1000	8% [c]	28	n.i.	n.i.	n.i.
111	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
110	1900	800	n.i.	n.i.	n.i.	n.i.
ent-109	n.i.	10% [d]	12	n.i.	n.i.	n.i.
ent-108	n.i.	n.i.	129	n.i.	n.i.	n.i.
112	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.

*, Detailed enzyme inhibition assay procedure in given in experimental section; n.i., no inhibition at 1 mM; —, means not measured; [a], The data for 1-DNJ is from ref.[39,40]; [b], The data for 1-DGJ is from ref.[41] ; [c], % inhibition at 0.6 mM; [d], % inhibition at 1 mM.

None of the prepared compounds inhibited α- and β-mannosidases. The compound **111** and **112** in which C-4 hydroxy functionality of 1-deoxy-D-glucohomonojirimycin is replaced by an amino group and methyl respectively, showed no inhibition against any of the enzymes under study. In the case of **111,** the non-inhibitory activity could be attributed to the higher basicity of the amino group at C-4 position than the ring nitrogen, thereby, forbidding it from binding to the active site of α-/β-glucosidase in correct orientation. Compound **112** is a structural analogue of DNJ-type azasugar whereas **152** represent the 1-*N*-iminosugar class.[42] Therefore, based on our observations, it suggests that there is a

necessity of a C-4 hydroxy functionality for DNJ-type azasugars for the substrate-enzyme interactions.

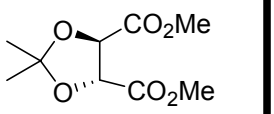
The compound **109** (D-gluco configured) exhibited anomer specificity for β-glucosidase (K_i = 28 μM) as it showed no inhibition against α-glucosidase and weak inhibition for β-galactosidase (K_i = 1000 μM). The corresponding enantiomer *ent*-**109** exhibited strong inhibition against β-glucosidase (K_i = 12 μM) but no inhibition against any other enzymes studied. 1-Deoxy-D-galactohomonojirimycin **(108)** exhibited potent inhibition (K_i = 1.7 μM) against α-galactosidase and 650 times weak inhibition (K_i = 1100 μM) against β-galactosidase, whereas its corresponding enantiomer *ent*-**108** (L-galacto/L-fuco- configured) showed moderate (K_i = 129 μM) inhibition against β-glucosidase and no inhibition against any of the enzyme under study. The compound **110** with pseudoamide type nitrogen exhibited weaker inhibition against both α- as well as β- galactosidases.

2.13 Summary

In conclusion, we have developed a general synthetic strategy to access stereoisomers of the C-6 homologue of 1-deoxynojirimycin in excellent diastereoselectivity. Some of the synthesized molecules such as **108**, **109**, ***ent*-108** and ***ent*-109** exhibited enzyme specific inhibition against glycosidases.

2.14 Experimental Section

1. Preparation of Dimethyl 2,3-O-isopropylidene-L-tartarate:

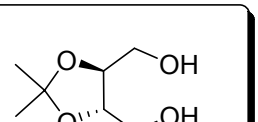


123

In a 1-L, one-necked, round-bottom flask fitted with a reflux condenser, a large magnetic stirring bar and argon balloon, a mixture of L-tartaric acid (101 g, 0.673 mol), 2,2-dimethoxypropane (190 mL, 161 g, 1.54 mol), dry methanol (40 mL) and *p*-toluenesulfonic acid monohydrate (0.4 g, 2.1 mmol) was charged and the whole contents were warmed for about 2 h on an oil bath at 60 ^{o}C with stirring until a dark-red homogeneous solution was obtained. Additional amounts of 2,2-dimethoxypropane (95 mL, 80.5 g, 0.77 mol) and cyclohexane (450 mL) were added and the reflux condenser was replaced with a 30-cm Vigreux column and distillation head. The mixture was heated to reflux and the azeotrope of the methanol-cyclohexane (53 °C) and acetone-cyclohexane (54.5 °C) were slowly removed over a period of 48 h (10-15ml/hr). After approximately 600 mL of distillate was collected, additional amount of 2,2-dimethoxypropane (6 mL, 5.1 g, 49 mmol) was added and the mixture was heated under reflux for 15 min. The reaction mixture was cooled to room temperature, anhydrous potassium carbonate (1 g, 7.2 mmol) was added and the mixture was stirred until the reddish color had disappeared. Volatile material is removed under reduced pressure (water aspirator) and the residue was fractionally distilled under vacuum to afford **123** (135 g, 95 % yield) as a pale-yellow oil, bp = 90–101°C (0.6 mm).

$[\alpha]^{27}_{D}$ (neat)	:	-46.3°, lit[22] $[\alpha]^{20}_{D}$ = -49.4°
IR (neat) ν_{max} cm^{-1}	:	2992, 2956, 1759, 1438, 1384, 1213, 1111.
^{1}H NMR (200 MHz, $CDCl_3$) δ	:	1.49 (s, 6H), 3.83 (s, 6H), 4.81 (s, 2H)
^{13}C NMR (50MHz, $CDCl_3$) δ	:	25.98(CH_3), 52.42(CH_2), 76.68(CH_2), 113.49(C), 169.75(C)

2. Preparation of 2,3-Di-O-isopropylidene-L-threitol:



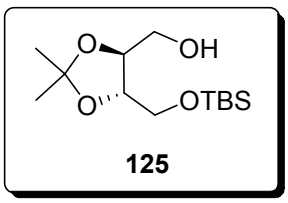
124

In a dry 500 mL two-neck round-bottom flask, equipped with a 250-mL pressure-equalized addition funnel, a reflux condenser and a magnetic stirring bar, was added lithium aluminum hydride (6 g, 158.1 mmol) in THF (100 mL) under argon. To this mixture a solution of **123** (34 g, 156 mmol) in THF (150 mL) was added drop-wise over a period of 2 h and then refluxed for 6 h. The mixture was cooled to 0–5 °C and *cautiously* treated with water (6 mL) followed by 2N sodium

hydroxide solution (12 mL) and water (12-18 mL). The mixture was then stirred at room temperature until the gray color of unquenched lithium aluminum hydride has completely disappeared. To the resulting white suspension was added anhydrous sodium sulphate. The slurry was filtered on a Büchner funnel and the inorganic precipitate was given a wash with (5 x 200ml) THF. The combined filtrate was dried over anhydrous sodium sulphate. The filtrate was concentrated under reduced pressure. This crude mixture was fractionally distilled under vacuum to afford the product **124** as a colorless to pale-yellow oil, bp 96-108 °C (0.6 mm); 23 g (90% yield).

$[\alpha]^{27}_D$	:	+2.9° (*c* 5, $CHCl_3$), lit.[22b] $[\alpha]^{20}_D$ = +4.1° (*c* 5, $CHCl_3$)
IR (neat) ν_{max} cm^{-1}	:	3413 (br), 2934, 1455, 1372, 1218, 1166, 1057
^{1}H NMR	:	1.42 (s, 6H), 2.73(br s, 2H, D_2O exchangeable), 3.73 (m, 4H),
(200 MHz, $CDCl_3$) δ		3.94 (m, 2H)
^{13}C NMR (50 MHz, $CDCl_3$) δ	:	26.8(CH_3), 62.1(CH_2), 78.3(CH_2), 109.1(C)

3. Preparation of [(4*S*,5*S*)-5-tert-butyldimethylsilyloxy- methyl)-2,2-dimethyl-1,3-dioxolan-4-yl]methanol:

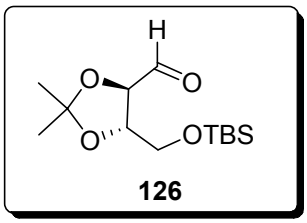


125

To a stirring solution of **124** (50g, 308.4 mmol) in dry DCM (1.25 L) at 0 °C was added triethyl amine (45.01 ml, 323 mmol). To this was added a solution of TBSCl (46.5g. 308.4 mmol) in dry DCM (200 ml) over a period of 1 h using a addition funnel. The reaction mixture was allowed to stir at rt for about 36 h. Usual work up and extraction of the reaction mixture in DCM (25 mL) followed by washing with water (3 x 100 mL), brine (100 mL) and drying over anhydrous Na_2SO_4 and removal of the solvent gave a crude product. Column chromatographic purification of crude product (silica, pet ether-ethyl acetate, 5:1) afforded pure **125** (72 g, 85 %) as a colorless liquid.

$[\alpha]^{27}_D$	:	-5.5° (*c* 2, MeOH) , lit[23] $[\alpha]^{25}_D$ = -5.4° (*c* 5, MeOH); *ent*-**125**: +5.9° (*c* 1.8, MeOH)
IR (neat) ν_{max} cm^{-1}	:	3463, 1253, 1379
^{1}H NMR	:	0.05 (s, 6H), 0.85 (s, 9H), 1.40 (s, 3H), 1.45 (s, 3H), 2.65 (bs, 1H),
(200 MHz, $CDCl_3$) δ		3,65 (m, 3H), 3.85 (m, 2H), 4.0 (m,1H)
^{13}C NMR	:	-5.3[-Si-($\underline{C}H_3)_2$], 18.4[-C-($\underline{C}H_3)_3$], 26.0(C), 27.1(CH_3), 27.2(CH_3),
(50 MHz, $CDCl_3$) δ		62.9(CH_2), 63.9(CH_2), 78.2(CH), 80.2(CH), 109.2(C)
Mass (m/z %)	:	261 (M^+-15) (6), 219 (8), 161 (23), 131 (67), 117 (36), 75 (100)

4. Preparation of (4*R*,5*S*)-5-[(*t*-butyldimethylsilyloxy) methyl]-2,2-dimethyl-1,3 dioxolane-4-carbaldehyde:

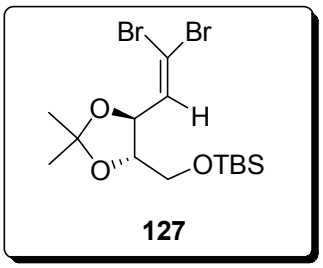


126

To a solution of alcohol **125** (20 g, 72.46 mmol) in ethyl acetate (250 mL) was added IBX (34.51g, 123 mmol). The resulting suspension was immersed in an oil bath set at 80 °C and stirred vigorously open to the atmosphere. After 9 h (GC monitoring), the reaction was cooled to room temperature and filtered through a Whatman filter. The filter cake was washed with 3 x 100 mL of ethyl acetate and the combined filtrates were concentrated to yield 19. 8 g of **126** (98 % yield, >98% pure by GC). ***Note:*** *IBX recovered from the reaction mixture was reoxidized and recycled three times.*

IR (neat) ν_{max} cm^{-1}	:	1735, 1255, 1373
^{1}H NMR (200 MHz, $CDCl_3$) δ	:	0.06 (s, 6H), 0.88 (s, 9H), 1.40 (s, 3H), 1.46 (s, 3H), 3.79 (d, 2H, *J* = 4.4 Hz), 3.65-4.30 (m, 4H), 9.65 (d, *J* = 1.5 Hz, 1H)
^{13}C NMR (50 MHz, $CDCl_3$) δ	:	-5.5(-Si-$\underline{C}H_3$), -5.4[-Si-($\underline{C}H_3)_3$], 18.3[-C($\underline{C}H_3)_3$], 25.8(C), 26.3(CH_3), 26.8(CH_3), 62.8(CH_2), 77.6(CH), , 81.9(CH), 111.5(C), 200.7(CH)
Mass (m/z %)	:	259 (M^+-15) (4), 259 (6), 217 (18), 159 (57), 129 (81), 117 (100)

5. Preparation of 5-[(*t*-butyldimethylsilyl)oxy]-1,1-dibromo-1,2-dideoxy-3,4-*O*-(1-methylethylidene)-L-*threo*-pent-1-ene:



127

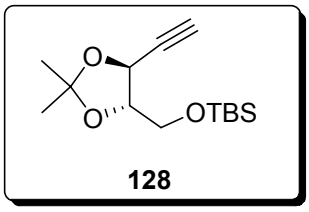
To a stirring solution of carbon tetrabromide (24.23 g, 72.99 mmol) in dry DCM (230 mL) in two-neck RB at 0 °C, triphenyl phosphine (38.39 g, 235.5 mmol) was added through a solid addition tube over a period of 10 min. Then the solution of **126** (10 g, 36.49 mmol) in DCM (50 mL) was slowly added through a cannula. The reaction mixture was stirred at 0 °C for 2 h, followed by addition of a large excess (750 mL) of hexane to precipitate the triphenyl phosphine oxide at 0 °C. The resulting mixture was quickly passed through a short pad of silica. The solvent was removed under reduced pressure and the residue was column chromatographed (silica, pet ether-ethyl acetate, 24:1) to afford pure **127** (11.4 g, 72 %) as a colorless liquid.

Caution: *To obtain good yield using this protocol needs to follow strict reaction conditions as these reaction conditions are also known to cleave acetal and ketal moiety*[43]

$[\alpha]^{27}_D$: -8.2° (*c* 0.44, $CHCl_3$); *ent*-**127**: +7.2° (*c* 0.6, $CHCl_3$)

IR (neat) ν_{max} cm^{-1}	:	1625, 1461, 1371, 1217
^{1}H NMR (200 MHz, $CDCl_3$) δ	:	0.10 (s, 6H), 0.90 (s, 9H), 1.40 (s, 6H), 3.80 (m, 3H), 4.65 (dd, 1H, *J* = 8.8, 7.3 Hz), 6.50 (d, 1H, *J* = 8.8 Hz)
^{13}C NMR (50 MHz, $CDCl_3$) δ	:	-5.4[-Si-($\underline{C}H_3)_3$], 18.1[-C($\underline{C}H_3)_3$], 25.7(C), 26.8(CH_3), 62.3(CH_2), 77.9(CH), 80.6(CH), 93.5(CH_2), 109.7(C), 136.1(C)
Mass (m/z %)	:	415 (M^+-15) (1), 343 (1), 315 (39), 285 (6), 256 (5), 200 (11), 137 (45), 73 (86), 57 (100)

6. Preparation of 5-[(*t*-butyldimethylsilyl)oxy]-1,2-dideoxy-3,4-*O*-(1-methyl ethylidene)-D-*threo*-pent-1-yne:

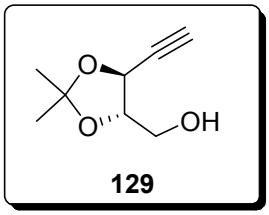


128

To a solution of **127** (22.5 g, 52.32 mmol) in dry THF (100 mL) was added a 2M hexane solution of *n*-BuLi (57.5 ml,115 mmol) at –78 °C over a period of 15 min. The reaction mixture was stirred at –78 °C for 45 min. and was quickly brought up to 0 °C. It was quenched by rapid addition of a large excess of water (30 mL). The reaction mixture was extracted with ethyl acetate (2 x 70 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed by rotary-evaporation and the residue was purified by column chromatography (silica, pet ether-ethyl acetate, 19:1) to afford **128** (12.7 g, 90 %) as a colorless oil.

$[\alpha]^{27}_D$	:	-11.02° (*c* 1.11, $CHCl_3$); *ent*-**128**: +11.1° (*c* 0.95, $CHCl_3$)
IR (neat) ν_{max} cm^{-1}	:	3300, 1460, 1380, 1260, 1220
^{1}H NMR (200 MHz, $CDCl_3$) δ	:	0.10 (s, 6H), 0.90 (s, 9H), 1.45 (s, 3H), 1.50 (s, 3H), 2.55 (d, 1H, *J* = 1.9 Hz), 3.80 (d, 2H, *J* = 4.4 Hz), 4.15 (m, 1H), 4.60 (dd, 1H, *J* = 7.4, 1.9 Hz)
^{13}C NMR (50 MHz, $CDCl_3$) δ	:	-5.6[-Si-($\underline{C}H_3)_3$], 18.1[-C($\underline{C}H_3)_3$], 25.6(C), 26.0(CH_3), 26.7(CH_3), 61.8(CH_2), 66.8(CH), 74.1(C), 81.1(CH), 82.0(CH), 110.4(C)
Mass (*m/z* %)	:	255 (M^+-15) (8), 213 (2), 197 (3), 155 (100), 125 (67)

7. Preparation of [(4*S*,5*S*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl]methanol:



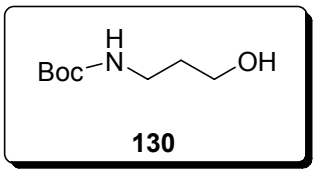
129

To a solution of **128** (12.7 g, 47 mmol) in dry THF (90 mL) was added a 1M solution of TBAF in THF (47 mL, 47 mmol) at 0 °C. The reaction mixture was stirred at rt for about 3 h and to this was added water (70 mL) followed by ethyl acetate (70 mL) and the layers were separated. The organic layer was dried over anhydrous

Na_2SO_4 and concentrated under reduced pressure. The crude mixture upon column chromatography (silica, chloroform-ethyl acetate, 9:1) afforded pure **129** (6.23 g, 85 %) as a colorless liquid.

$[\alpha]^{25}_D$	:	-10.91° (*c* 2.02, $CHCl_3$); *ent*-**129**: +11.2 (*c* 1.12, $CHCl_3$)
IR (neat) ν_{max} cm^{-1}	:	3446, 3290, 1460, 1379, 1215
^{1}H NMR (200 MHz, $CDCl_3$) δ	:	1.45 (s, 3H), 1.50 (s, 3H), 1.80 (bs, 1H), 2.55 (d, ^{1}H NMR, *J* = 1.9 Hz), 3.68 (dd, 1H, *J* = 12.3, 3.2 Hz), 3.94 (dd, 1H, *J* = 12.3, 3.0 Hz), 4.20 (m, 1H), 4.60 (dd, 1H, *J* = 7.9, 1.9 Hz)
^{13}C NMR (50 MHz, $CDCl_3$) δ	:	25.8(CH_3), 26.6(CH_3), 60.7(CH_2), 66.2(CH), 74.6(C), 80.7(CH), 82.0(CH), 110.6(C)

8. Preparation of *t*-butyl 3-hydroxypropylcarbamate:

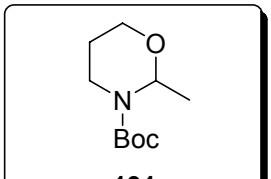


130

A solution of $(Boc)_2O$ (45.72 mL, 199 mmol) in 100 mL DCM was slowly added to a stirring solution of 3-amino propanol (15 g, 199 mmol) and Et_3N (30.51 mL, 218 mmol) in DCM (600 mL) at 0 °C. The reaction mixture was stirred for 36 h at room temperature. The reaction mixture was diluted with DCM (300 mL) and washed with water (3 x 100 mL) and brine (1 x 100 mL). The organic layer was dried over Na_2SO_4 before concentrating under reduced pressure. The resultant brown colored residue was purified by vacuum distillation (bp 100-105 °C/1 mm) to obtain **130** (31.5 g, 90%) as a colorless oil.

IR (neat) ν_{max} cm^{-1}	:	3357, 2975, 2935, 1691, 1529, 1367, 1367, 1278, 1253, 1172
^{1}H NMR (200 MHz, $CDCl_3$) δ	:	1.4 (s, 9H), 1.57-1.67 (m, 2H), 3.2 (q, 2H, *J* = 5.9, 6.6 Hz), 3.6 (app t, 2H, *J* = 5.5, 5.8 Hz)
^{13}C NMR (50 MHz, $CDCl_3$) δ	:	28.0(CH_3), 32.0(CH_2), 37.5(CH_2), 59.0 (CH_2), 79.0(C), 156.5(C).
Mass	:	176.22 (MH^+)
Elemental Analysis	:	Anal. Calcd for $C_8H_{17}NO_3$: C, 54.84; H, 9.78; N, 7.99. Found: C, 55.14; H, 9.66; N, 8.05

9. Preparation of *t*-butyl 2-methyl-1,3-oxazinane-3-carboxylate:



131

To a solution of **130** (31.5 g, 179.6 mmol) and PPTS (2.42 g, 9.65 mmol) in 550 mL of benzene, acetaldehyde diethyl acetal (24.40 mL, 171.42 mmol) was added slowly. The reaction mixture was subjected to azeotropic distillation for a period of a 10 h using

long Vigreux column maintaining distillation temperature between 67-71 °C. The brown colored mixture was washed with saturated $NaHCO_3$ (2 x 100 mL), water (2 x 100 mL), brine (1 x 200 mL), dried over Na_2SO_4 and concentrated. The residue was purified by vacuum distillation (bp 64-67 °C, 1 mm) to obtain **131** (31.1 g, 86 %) as a colorless oil.

IR (neat) ν_{max} cm^{-1} : 2975, 1703, 1477, 1407, 1278, 1155, 1107, 1024

^{1}H NMR (200 MHz, $CDCl_3$) δ : 1.24 (s, 2H) 1.27 (s, 2H), 1.31 (s, 3H), 1.41 (m, 1H), 3.06 (ddd, 1H, *J* = 1.2, 3.9, 12.3 Hz), 3.54 (m, 1H), 3.93 (dt, 2H *J* = 3.5, 11.3 Hz), 5.46 (q, 1H, *J* = 6.3 Hz)

^{13}C NMR (50 MHz, $CDCl_3$) δ : 16.0(CH_3), 25.5(CH_2), 28.5(CH_3), 36.7(CH_2), 59.7(CH_2), 79.2(CH), 80.1(C), 153.7(C)

Elemental Analysis : Anal. Calcd for $C_{10}H_{19}NO_3$: C, 59.68; H, 9.52; N, 6.96. Found: C, 59.91; H, 9.35; N, 7.08

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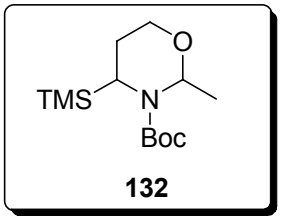
10. Preparation of *t*-butyl 2-methyl-4-(trimethylsilyl)-1,3-oxazinane-3-carboxylate:



132

TMEDA (16.15 mL, 109.45 mmol) was introduced to a solution of **131** (10 g, 49.75 mmol, 70 mL dry THF), charged into a 250 mL two necked RB flask equipped with magnetic stirring bar, under argon atmosphere at –78 °C. s-BuLi (1.6 M solution in cyclohexane, 68 mL,109.45 mmol) was added drop wise to the stirring solution over a period of 30 min. The mixture was further allowed to stir for 3 h at –78 °C. The anion generated was quenched by adding (13.89 mL, 109.45 mmol) TMSCl. The reaction mixture was allowed to come to room temperature on its own and stirring continued for another 2 h and was finally quenched by adding 40 mL of saturated aqueous NH_4Cl solution. The mixture was extracted with ethyl acetate (3 x 100 mL) and washed with brine (2 x 70 mL), dried over Na_2SO_4 and concentrated under vacuum. The yellowish colored mixture was purified by fractional distillation (b.p. 77-80 °C / 1 mm) to give **132** (12.6 g, 92 %) as a colorless oil.

IR (neat) ν_{max} cm^{-1} : 2977, 2945, 2862, 1693, 1413, 1367, 1298, 1247

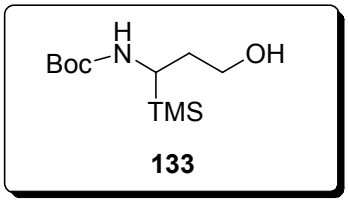
^{1}H NMR (200 MHz, $CDCl_3$) δ : 0.08 (s, 9H) 1.36-1.68 (br s, 12H), 1.76 (m, 2H), 2.65 (dd, 1H, *J* = 10.7, 4.7 Hz), 3.60 (m, 1H), 3.93 (m, 1H), 5.43 (q, 1H, *J* = 6.1 Hz)

^{13}C NMR (50 MHz, $CDCl_3$) δ : -1.08(Si-$\underline{Me}_3$), 17.5(CH-$\underline{Me}$), 26.7(CH_2), 28.3(C-$\underline{Me}_3$), 39.1(CH), 61.1(CH_2), 79.6(C), 81.1(CH), 154.5(C)

Mass (m/z %) : 274 (MH^+, 1), 258 (1), 244 (1), 230 (3), 216 (13), 202 (9), 186 (2), 158 (22), 73 (83), 57 (100)

Elemental analysis : Anal. Calcd for $C_{13}H_{27}NO_3Si$: C, 57.10; H, 9.95; N, 5.12. Found: C, 57.28; H, 10.15; N, 5.13

11. Preparation of *t*-butyl 3-hydroxy-1-(trimethylsilyl) propylcarbamate:



133

To a solution of **132** (14 g, 51.28 mmol) in 320 mL of methanol and 15 mL of water, *p*-TSA (0.25 g) was added and the mixture was stirred for 4 h at rt. Methanol was evaporated using rotary evaporator and the residue was diluted with ethyl acetate and washed with saturated $NaHCO_3$ solution (2 x 50 mL), water (2 x 50 mL), brine (2 x 50 mL), dried over Na_2SO_4 and concentrated under vacuum to give **133** as a white crystalline solid (12.6 g, 98 %.) which was sufficiently pure enough to proceed for the next step.

mp : 72-74 °C

IR (neat) ν_{max} cm^{-1} : 3440, 2979, 1683, 1500, 1367, 1253, 1215, 1045

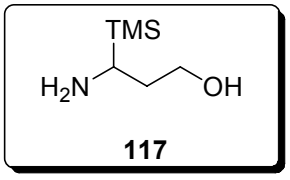
^{1}H NMR (200 MHz, $CDCl_3$) δ : 0.06 (s, 9H), 1.44 (s, 9H), 1.69 (m, 1H), 3.21 (broad dd, *J* = 2.74, 12.52 Hz, 1H), 3.58 (m, 2H)

^{13}C NMR (50 MHz, $CDCl_3$) δ : 3.9(Si-$\underline{C}H_3$), 28.0, 33.6, 35.6, 58.0, 79.2(C), 158.1(C)

GC-MS : 190 (M^+-57)

Elemental Analysis : Anal. Calcd for $C_{11}H_{25}NO_3Si$: C, 53.40; H, 10.19; N, 5.66. Found: C, 53.63; H, 10.13; N, 5.86

12. Preparation of 3-amino-3-(trimethylsilyl)propan-1-ol:



117

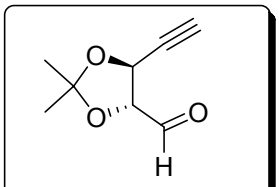
1 Lit. RB flask was charged with **133** (12.6 g, 51 mmol), 1,4-dioxane (306 mL) and 1N HCl (204 mL) and stirred while heating at 80 °C for 30 min. Dioxane was removed under reduced pressure and the residue was diluted with 300 mL DCM. This mixture was cooled to 0 °C and neutralized with (102 mL) 2N NaOH solution. The aqueous layer was extracted with DCM (3 x 150 mL), dried over Na_2SO_4 and concentrated under vacuo to give **117** (7.3 g, 98%) as an yellowish oil. This crude material was pure enough and used for the next step without further purifications. The analytically pure sample was obtained by passing through a small silica gel column.

IR (neat) ν_{max} cm^{-1} : 3353, 3284, 1431, 1369, 1249, 1053

^{1}H NMR (200 MHz, $CDCl_3$) δ : -0.04 (s, 9H), 1.59 (q, 2H, *J* = 4.9 Hz), 2.20 (dd, 1H, J = 4.9, 9.3 Hz), 2.87 (br s, 3H), 3.62 (t, 2H, *J* = 5.8 Hz)

^{13}C NMR (50 MHz, $CDCl_3$) δ	:	-4.1(CH_3), 34.4(CH_2), 40.3(CH), 62.2(CH_2)
GC-MS	:	147 (M^+)
Elemental Analysis	:	Anal. Calcd for $C_6H_{17}NOSi$: C, 48.93; H, 11.63; N, 9.51. Found: C, 48.61; H, 11.61; N, 9.73

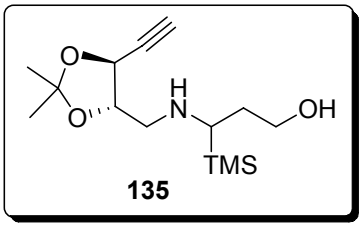
13. Preparation of (4*R*,5*S*)-5-ethynyl-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde:



116

To the solution of alcohol **129** (6.23 g, 39.93 mmol) in ethyl acetate (250 ml), IBX (19, 67.89 mmol) was added. The resulting suspention was immered in an oil bath set at 80 °C and stirred vigorously open to the atmosphere. After 9h (GC monitoring), the reaction was cooled to room temperature and filtered through a Whatman filter paper. The filter cake was washed with 3 x 100 mL of ethyl acetate and combined filtrates were concentrated to yield 5.8 g of **116** (85 % yield, >85% pure by GC). The aldehyde was found to be unstable and used immediately for the next step.

14. Preparation of 3-{[(4*S*,5*S*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl]methyl amino}-3-(trimethylsilyl)propan-1-ol:



135

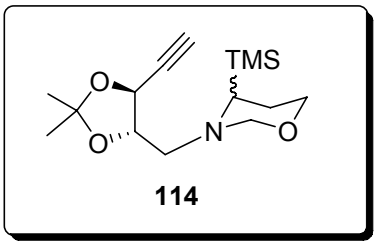
To the solution of **116** in dry 1,2-dichloroethane (180 mL), amine **117** (6.45 g, 43.92 mmol) was added followed by sodium triacetoxyborohydride (16.62 g, 78.78 mmol). This mixture was stirred at rt under argon atmosphere for 12 h. The reaction mixture was ice cooled and quenched by adding 1N NaOH till the aqueous layer was basic and stirred for additional 3 h. The reaction mixture was extracted with ethyl acetate (2 x 100 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed by rotary-evaporation and the residue was purified by column chromatography (silica, pet. ether-ethyl acetate, 7:3) to afford 1:1 diastereomeric mixture of **135** (8.2 g, 71 %) as a colorless oil.

IR (neat) ν_{max} cm^{-1}	:	3404, 3309, 2987, 2950, 2900, 2119, 1662, 1456, 1380, 1373, 1249
^{1}H NMR (200 MHz, $CDCl_3$) δ	:	0.00 (s, 9H), 0.98 (dd, 1H *J* = 6.2, 13.2 Hz), 1.32 (s, 3H), 1.36 (s, 3H), 1.45-1.85 (m, 2H), 2.19 (dt, 1H, *J* = 3.8, 8.6 Hz), 2.47 (d, 1H, *J* = 1.6 Hz), 2. 60-3.00 (m, 2H), 3.68-3.82 (m, 3H), 4.00-4.13 (m, 1H), 4.27 + 4.35 (dd, 1H, *J* = 2.0, 7.3 Hz)
^{13}C NMR (50 MHz, $CDCl_3$) δ	:	-2.8(CH_3), 25.9 + 26.0(C-$\underline{Me}_2$), 26.7 + 26.9(C-$\underline{Me}_2$), 31.1 + 31.3(CH_2), 48.8 + 49.8(CH), 49.6 + 51.5(CH_2), 64.0 + 64.4(CH_2), 67.6 + 68.2(CH), 74.8(CH), 80.5 + 81.1(CH), 80.5(C), 110.4 + 110.6(C)

Mass : 286 (MH^+)

Elemental Analysis : Anal. Calcd for $C_{14}H_{27}NO_3Si$: C, 58.91; H, 9.53; N, 4.91. Found: C, 58.65; H, 9.62; N, 4.81

15. Preparation 3-{[(4*S*,5*S*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl]methyl}-4-(trimethylsilyl)-1,3-oxazinane:



114

Paraformaldehyde (1.16 g, 38.94 mmol) and **135** (9.2 g, 32.45 mmol) in benzene (70 mL) were refluxed for 4 h under Dean-Stark condition. The reaction mixture was cooled and benzene was removed under reduced pressure. The crude mixture upon column chromatography (silica, pet ether-ethyl acetate, 9:1) afforded pure **114** (9.17g, 95 %) as a colorless liquid.

IR (neat) ν_{max} cm^{-1} : 3309, 3265, 2985, 2952, 2858, 2360, 1739, 1458, 1380, 1375, 1249, 1076

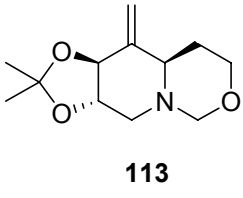
^{1}H NMR (500 MHz, $CDCl_3$) δ : 0.10 (two singlet's, 9H), 1.35-1.50 (three peaks 3:1.5:1.5 ,6H), 1.55-1.65 (bs, 1H), 1.75-1.85 (m, 1H), 2.53 (two d, 1H, *J* = 2 Hz), 2.60 (ddd, 1H, *J* = 3.9, 9.3, 13.2 Hz), 2.71-2.81 (two dd, 1H, *J* = 7.6, 13.7 Hz and *J* = 3.8, 13.7 Hz), 3.23-3.39 (two dd, 1H, *J* = 7.0, 13.7 Hz and *J* = 3.0, 13.7 Hz), 3.73-3.82 (m, 1H), 3.95-4.05 (m, 1H), 4.12-4.22 (m, 1H), 4.28 -4.36(m, 2H), 4.62 (app t, 1H, *J* = 9.9, 10.1 Hz)

^{13}C NMR (50 MHz, $CDCl_3$) δ : -1.9(CH_3), 22.2(CH_2), 25.7 + 26.2, 26.9 + 27.2, 51.6 + 52.1(CH_2), 52.7 + 53.0(CH), 68.2 + 68.3(CH_2), 68.4 + 68.5(CH), 74.5 + 74.7(CH), 80.7 + 82.5(CH_2), 81.0(C), 84.3 + 85.2(CH_2), 110.5 + 110.7(C)

GC-MS : 297 (M^+, 0.5%), 224 (80%), 166 (20%), 100 (65%), 73 (100%)

Elemental Analysis : Anal. Calcd for$C_{15}H_{27}NO_3Si$ C 60.57, H 9.15, N 4.71; Found C 60.62, H 9.25, N 4.55

16. Preparation of (3a*S*,9a*R*,10a*S*)-2,2-dimethyl-10-methylenehexahydro-4H-[1,3]dioxolo[4,5]pyrido [1,2-c][1,3]oxazine:



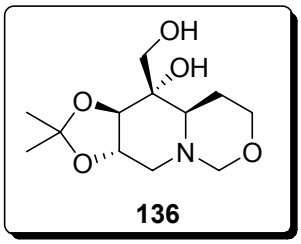
113

A solution containing **114** (1.0 g, 3.3 mmol) and 1,4-dicyanonaphthalene (0.12 g, 0.67 mmol) in acetonitrile:*iso*-propanol (3:1, 250 mL) mixture was irradiated in an open vessel using a 450 W Hanovia medium pressure mercury vapor lamp. The lamp was immersed in a Pyrex water-jacketed immersion well which allowed only wavelengths greater than 280 nm to pass through. After about 4 h of irradiation, the consumption of the starting material was found to be almost complete

(monitored by GC) and at this stage the irradiation was discontinued. The solvent was removed under reduced pressure and the residue was column chromatographed (silica, pet. ether-acetone, 9:1) to afford cyclized product **113** (0.450 g, 60%) as a yellow liquid.

$[\alpha]^{27}_D$	:	+52.4° (*c* 0.44, $CHCl_3$); *ent*-**113**: -44.3 (*c* 1.08, CH_2Cl_2)
IR ν_{max} cm^{-1} in $CHCl_3$	:	3097, 3053, 2985, 2931, 2846, 2732,1627 ,1456, 1371, 1226
^{1}H NMR (500 MHz, $CDCl_3$) δ	:	1.48 (s, 3H), 1.50 (s, 3H), 1.74 (dd, 1H, *J* = 1.6, 13.2 Hz), 1.90- 2.00 (m, 1H), 2.33 (t , 1H, *J* = 9.9 Hz), 2.66 (br d, 1H, *J* = 10.7 Hz), 3.19 (dd, 1H, *J* = 4.0, 9.4 Hz), 3.52-3.60 (m, 2H), 3.80 (td, 1H, *J* = 1.9, 9.1 Hz), 3.86 (d, 1H, *J* = 8.0 Hz), 4.17 (dd, 1H, *J* = 4.8, 11.4 Hz), 4.48 (d, 1H, *J* = 8.0 Hz), 4.88 (t, 1H, *J* = 1.7 Hz), 5.14 (t, 1H, *J* = 1.7 Hz).
^{13}C NMR (50 MHz, $CDCl_3$) δ	:	26.6(CH_3), 26.8(CH_3), 27.7(CH_2), 51.2(CH_2), 60.4(CH), 67.1(CH_2), 76.5(CH), 81.5(CH), 86.50(CH_2), 103.63(CH_2), 110.99(C), 142.71(C).
Elemental Analysis	:	Anal. Calcd for C 63.98, H 8.5, N 6.22; found C 63.83, H 8.63, N 6.39
GC-MS	:	225 (M^+, 5%), 196 (35%), 167 (100%), 149 (35%), 81 (90%)

17. Preparation of (3a*S*,9a*R*,10*S*,10a*R*)-10-(hydroxymethyl)-2,2-dimethylhydro-4H [1,3] dioxolo[4,5]pyrido[1,2-c][1,3]oxazine-10-ol:



136

To a mixture of potassium ferricyanide (2.61 g, 7.94mmol) and potassium carbonate (1.09 g, 7.94 mmol) in water (28 mL) at 5 °C, **114** (0.59 g, 2.64 mmol) dissolved in *t*-BuOH (28 mL) followed by osmium tetroxide (2 mL of 1 % solution of OsO_4 in *t*-BuOH) was added. The reaction mixture was allowed to warm to rt and stirred for 24 h. Solid 0.4 g of Na_2SO_3 was added to the stirring solution and clear separation of the two layers were noticed. Aqueous layer was extracted with ethyl acetate (5 x 20 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed by rotary-evaporation and the residue was purified by column chromatography (silica, pet. ether-ethyl acetate, 3:7) to afford **136** (0.59 g, 90 %) as a colorless crystalline solid.

$[\alpha]^{27}_D$	:	+36.2° (*c* 0.95, CH_2Cl_2); *ent*-**136**: -34.4 (*c* 1.0, CH_2Cl_2)
mp	:	165-168 °C
IR ν_{max} cm^{-1} in $CHCl_3$	:	3490, 2964, 2860, 1382, 1373, 1226, 1107
^{1}H NMR (400 MHz, $CDCl_3$) δ	:	1.42 (s, 3H), 1.43 (s, 3H), 1.64 (m, 1H), 1.82 (dd, 1H, *J* = 1.6, 13.1 Hz), 2.07 (t, 1H, *J* = 9.9 Hz), 2.15 (dd, 1H, *J* = 2.7, 11.3 Hz), 3.03 (dd, 1H, *J* = 4.3, 9.3 Hz), 3.38 (dt, 1H, *J* = 2.1, 12.0 Hz), 3.44 (d, 1H, *J* = 9.5 Hz), 3.64 (d, 1H, *J* = 8.0 Hz), 3.68-3.76 (m, 2H), 3.98 (d, 1H, *J* = 11.5 Hz),

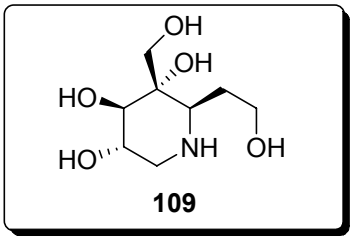
4.08 (dd, 1H, *J* = 4.3, 11.3 Hz)

^{13}C NMR (100 MHz, $CDCl_3$) δ : 24.7(CH_2), 26.6(CH_3), 51.5(CH_2), 62.3(CH_2), 66.5(CH), 67.9(CH_2), 72.0(CH), 72.1(C), 86.5(CH), 86.5(CH_2), 110.1(C)

Elemental analysis : Anal Calcd. for $C_{12}H_{21}NO_5$: C, 55.58; H, 8.16; N, 5.40. Found: C, 55.71; H, 8.36; N, 5.45

Mass : 260 (MH^+)

18. Preparation of 1-deoxy-4-hydroxymethyl-D-glucohomonojirimycin or (2*R*,3*S*,4*R*, 5*S*)-2-(2-hydroxyethyl)-3-(hydroxymethyl)piperidine-3,4,5-triol:



109

To a solution of **136** (0.025 g, 0.097 mmol) in distilled methanol (3 mL) was added 2 mL of 6N HCl and the reaction mixture was refluxed for 12 h The solvent was evaporated to dryness to afford **109**.HCl as a white foam which was further purified by column chromatography as a free base [(silica, chloroform-methanol-aq NH_3, 8.0:2:0.5), and finally eluting with (4:6) MeOH-Chloroform] to afford **109** (0.019 g, 95 %) as a white solid.

$[\alpha]^{27}_D$: +17.4° (*c* 1, MeOH), *ent*-**109**: -18.1° (*c* 0.8, MeOH)

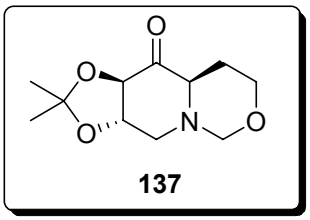
^{1}H NMR (200 MHz, D_2O) δ : 1.40-1.61 (m, 1H), 2.03 (dq, 1H, *J* = 2.5, 7.2 Hz), 2.41 (dd, 1H, *J* = 10.6, 12.5 Hz), 2.60 (dd, 1H, *J* = 2.4, 10.1 Hz), 3.10 (dd, 1H, *J* = 5.4, 12.5 Hz), 3.39 (d, 1H, *J* = 9.8 Hz), 3.96-3.86 (m, 5H)

^{13}C NMR (50 MHz, D_2O) δ : 30.6(CH_2), 49.2(CH_2), 60.0 (for two CH_2), 60.7(CH), 68.9(CH), 73.6(C), 80.8(CH)

Mass (m/z %) : 230 ($M+Na^+$, 32%), 208 (MH^+, 100%), 174 (20%)

Elemental Analysis : Anal. Calcd for $C_8H_{17}NO_5$: C, 46.37; H, 8.27; N, 6.76. Found: C, 46.191; H, 8.46; N, 6.39

19. Preparation of (3a*S*,9a*R*,10a*R*)-2,2-dimethyltetrahydro 4H-[1,3]dioxolo[4,5] pyrido[1,2-c][1,3]oxazin-10(8H)-one:



137

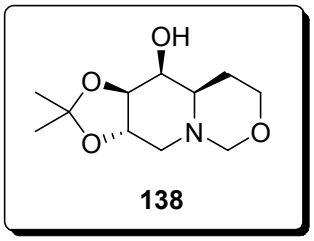
A solution of **136** (0.33 g, 1.28 mmol) in DCM (10 mL) was added to a suspension of silica gel supported sodium periodate [prepared by dissolving $NaIO_4$ (0.55 g, 2.56 mmol) in 1.3 mL water and 2.52 g of flash silica gel] in DCM (5 mL). This suspension was stirred for 15 min. and filtered. The solvent was evaporated off and the brownish pasty mass was extracted with ethyl acetate (3 x 3 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and solvent was removed under reduced

pressure. The crude mixture **137** (crude weight 0.28 g, 95%) was pure enough and was used as such for the next step.

Note: 137 *is unstable and should be used immediately for the next step.*

$[\alpha]^{27}_D$	:	+68.6° (*c* 0.42, CH_2Cl_2)
^{1}H NMR (200 MHz, $CDCl_3$) δ	:	1.40 (s, 3H), 1.41 (s, 3H), 1.67-1.87 (m, 2H), 3.45 (app t, 1H, *J* = 9.7, 9.9 Hz), 3.35 (dd, 1H, *J* = 4.4, 9.8 Hz), 3.45 (dt, 1H, *J* = 3.5, 11.6 Hz), 3.82 (dt, 1H, *J* = 4.4, 10.0 Hz), 3.93 (d, 1H, *J* = 8.3 Hz), 4.08 (two sets of dd, 1H, *J* = 1.5, 4.8; *J* = 2.2, 4.3 Hz and 11.4 Hz), 4.19 (dd, 1H, *J* = 1.5, 10.3 Hz), 4.42 (d, 1H, *J* = 8.3 Hz)
^{13}C NMR (50MHz, $CDCl_3$) δ	:	25.2(CH_2), 26.2(CH_3), 26.7(CH_3), 50.5(CH_2), 64.9(CH), 66.8(CH_2), 75.1(CH), 83.1(CH), 85.5(CH_2), 112.9(C), 197.6(C)

20. Preparation of (3a*S*,9a*R*,10*S*,10a*S*)-2,2-dimethyl hexahydro-4H-[1,3]dioxolo[4,5] pyrido[1,2-c][1,3] oxazin-10-ol:

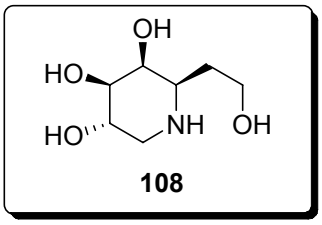


138

Sodium borohydride (0.09 g, 2.26 mmol) was added to a solution of **137** (0.257 g, 1.13 mmol) in dry methanol (4 mL). The resulting mixture was stirred for 6 h and then quenched by the addition of excess of the saturated solution of NaCl. This brownish suspension was stirred overnight and extracted with ethyl acetate (4 x 3 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent was evaporated off. The residue was column chromatographed (silica, pet. ether-ethyl acetate, 1:4) to afford **138** (0.22 g, 85 %) as a colorless oil.

$[\alpha]^{27}_D$	:	+28.4° (*c* 0.47, $CHCl_3$), *ent*-**138**: -24.4 (*c* 1.75, CH_2Cl_2)
IR ν_{max} cm^{-1} in $CHCl_3$	:	3438, 2987, 2929, 2856, 1674, 1456, 1382, 1215.
^{1}H NMR (500 MHz, $CDCl_3$) δ	:	1.42 (s, 4H), 1.43 (s, 3H), 2.12 (app t, 1H, *J* = 9.5, 9.9 Hz) 2.19-2.27 (m, 2H), 3.09 (dd, 1H, *J* = 4.3, 9.4 Hz), 3.29 (dd, 1H, *J* = 2.5, 9.2 Hz), 3.45 (dt, 1H, *J* = 2.8, 11.0 Hz), 3.72 (d, 1H, *J* = 7.9 Hz), 4.01 (dd, 1H, *J* = 4.0, 9.9 Hz), 4.02(s, 1H), 4.08 (dd, 1H, *J* = 4.4, 10.7 Hz), 4.44 (d, 1H, *J* = 7.5 Hz).
^{13}C NMR (50MHz, $CDCl_3$) δ	:	26.2(CH_3), 26.5(CH_3), 27.1(CH_2), 51.2(CH_2), 61.7(CH), 66.8(CH_2), 68.1(CH), 69.6(CH), 81.4(CH), 86.1(CH_2), 112.8(C).
Mass (m/z %)	:	228 (MH^+, 100%)
Elemental Analysis	:	Anal. Calcd for $C_{11}H_{19}NO_4$: C, 57.62; H, 8.35; N, 6.11. Found: C, 57.39; H, 8.34; N, 6.30.

21. **Preparation of 1-Deoxy-D-galactohomonojirimycin or (2*R*,3*S*,4*R*,5*S*)-2-(2-hydroxyethyl)piperidine-3,4,5-triol:**

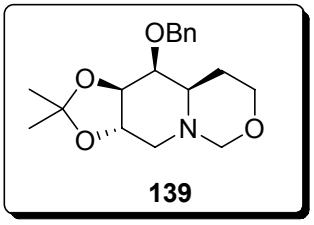


108

To a solution of **138** (25 mg, 0.109 mmol) in distilled dioxane-methanol (1:1, 3 mL) was added 2 mL of 6N HCl and the reaction mixture was refluxed for 12 h. The solvent was evaporated to dryness to afford **108**.HCl as a white foam which was further purified by column chromatography as a free base [(silica, chloroform-methanol-aq NH_3, 8.0:2:0.5) and finally eluted with (4:6) MeOH-chloroform to afford **108** (17 mg, 94%) as a white solid.

mp	:	166-168 °C, lit.[11] 167.5-168.5 °C
$[\alpha]^{27}_D$	:	+25.2° (*c* 1, MeOH), lit.[11] +24.8° (*c* 1, MeOH); *ent*-**108**, -24.6° (*c* 1.20, MeOH)
IR (neat) ν_{max} cm^{-1}	:	3360, 2958, 2825, 1077, 1024 cm^{-1}
^{1}H NMR (200 MHz, D_2O) δ	:	1.94-2.02 (m, 2H), 2.83 (dd, 1H, *J* = 11.6, 12.4 Hz), 3.36-3.49 (m, 2H), 3.61 (dd, 1H, *J* = 3.0, 9.7 Hz), 3.66-3.78 (m, 2H), 3.91-4.06 (m, 1H), 4.08 (dd, 1H, *J* = 1.2, 2.8 Hz)
^{13}C NMR (50MHz, D_2O) δ	:	30.4(CH_2), 46.2(CH_2), 56.9(CH), 57.5(CH_2), 64.2(CH), 68.1(CH), 72.8(CH)
Mass (m/z %)	:	178 (MH^+, 100%), 160 (10%), 142 (8%), 132.15 (12%).

22. Preparation of (3a*S*,9a*R*,10*S*,10a*S*)-2,2-dimethyl hexahydro-4H [1,3]dioxolo[4,5] pyrido[1,2-c][1,3]oxazin-10-ol:



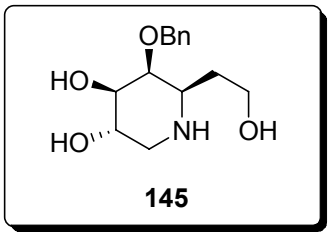
139

To a suspension of NaH (0.104 g, 2.5 mmol, 60 % granular coated with oil) in dry THF (12 mL) was added solution of **138** (0.5 g, 2.18 mmol) in dry THF (3 mL) at 0 °C. Benzyl bromide (0.297 mL, 2.5 mmol) was added drop wise to the reaction mixture followed by addition of catalytic amount of TBAI. The reaction mixture was refluxed for 10 h. Reaction was quenched at 0 °C by slowly adding water into it, aqueous layer was extracted with ethyl acetate (5 x 20 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed by rotary-evaporation and the residue was purified by column chromatography (silica, pet. ether-ethyl acetate, 7:3) to afford **139** (0.56 g, 86 %) as a colorless crystalline solid.

$[\alpha]^{27}_D$	:	+2.4° (*c* 1.14, CH_2Cl_2)
mp	:	84-87 °C

^{1}H NMR (500 MHz, $CDCl_3$) δ	:	1.22 (d, 1H, *J* = 9.5 Hz), 1.48 (s, 3H), 1.52 (s, 3H), 2.14 (app t, 1H, *J* = 9.5, 9.9 Hz), 2.22-2.29 (m, 2H), 3.17 (dd, 1H, *J* = 4.4, 9.1 Hz), 3.40 (dd, 1H, *J* = 2.2, 9.3 Hz), 3.42 (bd, 1H, *J* = 11.1 Hz), 3.71 (d, 1H, *J* = 7.6 Hz), 3.84 (s, 1H), 4.07 (dd, 1H, *J* = 3.7, 11.7 Hz), 4.24 (dt, 1H, *J* = 4.2, 9.8 Hz), 4.49 (d, 1H, *J* = 7.9 Hz), 4.65 (d, 1H, *J* = 11.9 Hz), 4.96 (d, 1H, *J* = 11.9 Hz), 7.20-7.40 (m, 5H)
^{13}C NMR (125 MHz, $CDCl_3$) δ	:	26.6(CH_3), 26.9(CH_3), 28.0(CH_2), 51.9(CH_2), 62.2(CH), 67.1(CH_2), 70.7(CH), 73.9(CH_2), 74.7(CH), 82.8(CH), 86.7(CH_2), 110.1(C), 127.6(CH), 128.2(CH), 128.4(CH), 138.3(C)
Mass (m/z %)	:	342 ($M+Na^+$, 40%), 320 (MH^+, 100%)
Elemental Analysis	:	Anal. Calcd for $C_{18}H_{25}NO_4$: C, 67.69; H, 7.89; N, 4.39; found C, 67.50; H, 8.01; N, 4.55

23. (3*S*,4*R*,5*S*,6*R*)-5-(benzyloxy)-6-(2-hydroxyethyl) piperidine-3,4-diol:

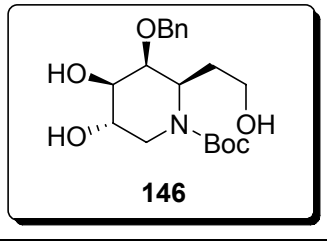


145

To a solution of the substrate **139** (0.150 g, 0.469 mmol) in dioxane: methanol (3:1, 8 mL) was added 3 mL of 6N HCl and the reaction mixture was refluxed for 16 h. The solvent was evaporated to dryness to afford hydrochloride salt of **145** as a white foam which was further purified by column chromatography as a free base [(silica, chloroform-methanol-aq NH_3, 8.0:2:0.5) and finally eluted with (4:6) MeOH-chloroform to afford **145** (0.110 g, 88%) as semisolid.

$[\alpha]^{27}_D$	:	+17.35° (*c* 0.65, MeOH)
^{1}H NMR (500 MHz, D_2O) δ	:	1.70-1.81 (m, 2H), 2.54 (t, 1H, *J* = 11.9 Hz), 3.01 (t, 1H, *J* = 6.8 Hz), 3.22 (dd, 1H, *J* = 5.4,12.9 Hz), 3.58 (t, 2H, *J* = 6.4 Hz), 3.66 (dd, 1H, *J* = 2.6, 9.7 Hz), 3.89-3.96 (m, 2H), 4.70 (d, 1H, *J* = 11.1 Hz), 4.96 (d, 1H, *J* = 11.1 Hz), 7.40-7.55 (m, 5H)
^{13}C NMR (125 MHz, D_2O) δ	:	32.5(CH_2), 48.2(CH_2), 55.6(CH), 58.3(CH_2), 67.2(CH), 75.3(CH), 75.4(CH_2), 78.4(CH), 128.2(CH), 128.5(CH), 128.6(CH), 137.8(C).
Mass (m/z %)	:	268 (MH^+, 100%)

24. (2*R*,3*S*,4*R*,5*S*)-*t*-butyl 3-(benzyloxy)-4,5-dihydroxy-2-(2-hydroxyethyl) piperidine-1-carboxylate:



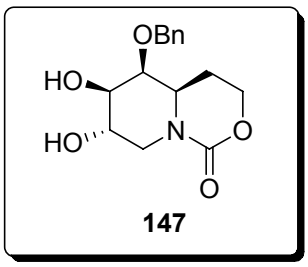
146

A solution of $(Boc)_2O$ (0.11 mL, 0.48 mmol) in 1 mL DCM was slowly added to a stirring solution of hydrochloride salt of **145** (0.12 g, 0.40 mmol) and Et_3N (0.13 mL, 1.00 mmol) in DCM (4 mL) at 0 °C. The reaction mixture was stirred for 8 h

at room temperature. The reaction mixture was diluted with DCM (5 mL) and washed with water (3 x 100 mL) and brine (1 x 100 mL). The organic layer was dried over Na_2SO_4 before concentrating under reduced pressure. The resultant colorless residue was purified by column chromatography (silica, 2:8, pet ether:ethyl acetate) to afford **146** (0.134 g, 92%) as a white solid.

$[\alpha]^{27}_D$	:	-8.5° (*c*, 2.25, $CHCl_3$)
mp	:	128-130 °C
^{1}H NMR (500 MHz, $CDCl_3$) δ	:	1.45 (s, 9H), 2.02 (br s, 1H), 2.67 (br s, 1H), 3.21 (d, 1H, *J* = 13.9 Hz), 3.42 (br s, 1H), 3.57-3.64 (m, 1H), 3.74-4.04 (m, 4H), 4.47-4.75 (m, 3H), 7.22-7.39 (m, 5H)
^{13}C NMR (125 MHz, $CDCl_3$) δ	:	28.3(CH_3), 29.6(CH_2), 40.4(CH_2), 48.2(CH), 58.5(CH_2), 68.7(CH), 70.2(CH), 71.3(CH_2), 73.2(CH), 80.9(C), 127.8(CH), 128.0(CH), 128.5(CH), 137.6(C), 157.0(C)
Mass (m/z %)	:	390 ($M+Na^+$, 100%), 368 (MH^+, 46%), 312 (20%), 268 (12%)
Elemental Analysis	:	Anal. Calcd for $C_{19}H_{29}NO_6$: C, 62.11; H, 7.96; N, 3.81. Found: C, 62.28; H, 8.05; N, 3.79

25. (4a*R*,5*S*,6*R*,7*S*)-5-(benzyloxy)-6,7-dihydroxy-hexahydropyrido[1,2-c][1,3]oxazin-1(3H)-one:



147

To a solution of **146** (0.12 g, 0.33 mmol) in DCM (5 mL) at 0 °C was added triethylamine (0.0314 g, 0.31 mmol, in 0.5 mL DCM) followed by drop wise addition of mesyl chloride (0.035 g, 0.31 mmol, in 0.5 mL DCM). The reaction was found to be complete in 10 min, monitored by TLC. The reaction mixture was diluted with dichloromethane (5 mL) and washed with water (3 x 5 mL), brine solution (5 mL) and then dried over Na_2SO_4. The solvent was removed by rotary-evaporation and the residue dissolved in dry acetonitrile followed by addition of solid K_2CO_3 (0.227 g, 1.65 mmol). The reaction mixture was refluxed for 6 h and cooled to room temperature. Reaction mixture was filtered through Whatman paper to separate solid K_2CO_3. Removal of solvent gave **147** (0.085g, 89%) as a white crystalline solid.

$[\alpha]^{27}_D$	:	+36.5° (*c*, 1.65, MeOH)
mp	:	80-83 °C
IR ν_{max} cm^{-1} in $CHCl_3$	:	3385, 2927, 1682, 1481, 1454, 1229
^{1}H NMR	:	1.95-2.10 (m, 2H), 2.71 (dd, 1H, *J* = 11.0, 13.2 Hz), 3.61 (app t, 1H,

(500 MHz, $CDCl_3$) δ		*J* = 6.4, 8.2 Hz), 3.69 (dd, 1H, *J* = 3.3, 9.9 Hz), 3.88 (dt, 1H, *J* = 5.5, 10.3 Hz), 3.96 (s, 1H), 4.15 (ddd, 1H, *J* = 3.3, 9.6, 11.3 Hz), 4.23-4.32 (m, 2H), 4.73 (d, 1H, *J* = 11.7 Hz), 4.95 (d, 1H, *J* = 11.7 Hz), 7.20-7.31 (m, 5H)
^{13}C NMR	:	23.4(CH_2), 40.0(CH_2), 54.9(CH), 65.4(CH_2), 65.9(CH), 75.3(CH),
(125 MHz, $CDCl_3$) δ		75.7(CH_2), 78.8(CH), 128.2(CH), 128.5(CH), 128.5(CH), 137.2(C), 156.7(C)
Mass (m/z %)	:	316 ($M+Na^+$, 100%), 294 (MH^+, 34%), 268 (27%), 242 (20%)
Elemental Analysis	:	Calcd for $C_{16}H_{19}NO_5$: C, 61.42: H, 6.53: N, 4.78. Found: C, 61.69: H, 6.81: N, 4.32

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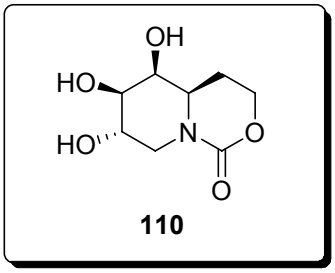
26. (4a*R*,5*S*,6*R*,7*S*)-5,6,7-trihydroxy-hexahydropyrido[1,2-c][1,3]oxazin-1(3H)-one:



110

A solution of **147** (0.085 g, 0.29 mmol) in methanol (5 mL) was hydrogenated at atomospheric pressure in the presence of Pd on charcoal (10%, 0.003 g) for 9 h. The reaction mixture was passed through a short pad of celite and the solvent was removed under reduced pressure to afford **110** (0.056 g, 95 %) as a white solid.

mp	:	201-203 °C decompose.
$[\alpha]^{27}_D$	:	+29.5° (*c*, 0.125, MeOH)
^{1}H NMR	:	2.11-2.27 (m, 2H), 2.72 (dd, 1H, *J* = 11.2, 13.0 Hz), 3.56 (dd, 1H, *J* =
(500 MHz, D_2O) δ		3.2, 9.6 Hz), 3.66 (app t, 1H, *J* = 7.3, 7.8 Hz), 3.79 (dt, 1H, *J* = 5.5, 10.5 Hz), 3.94 (d, 1H, *J* = 1.8 Hz), 4.22-4.34 (m,2H), 4.36-4.42 (m, 1H).
^{13}C NMR	:	23.3(CH_2), 47.8(CH_2), 55.0(CH), 65.4(CH_2), 65.5(CH), 70.5(CH),
(125 MHz, D_2O) δ		74.2(CH), 156.8(C)
Mass (m/z %)	:	609 ($M+M+M^+$, 15%), 204 (MH^+, 5%), 150 (100%)
Elemental Analysis	:	Calcd for $C_8H_{13}NO_5$: C, 47.25: H, 6.45: N, 6.89. Found: C, 46.99: H, 6.63: N, 6.79.

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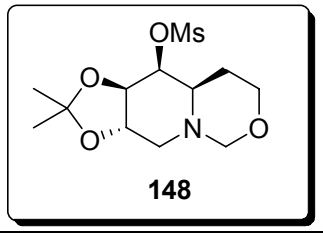
27. Preparation of (3a*S*,9a*R*,10*S*,10a*R*)-2,2-dimethylhexahydro-4H[1,3]dioxolo[4,5] pyrido[1,2-c][1,3]oxazin-10-yl methanesulfonate:



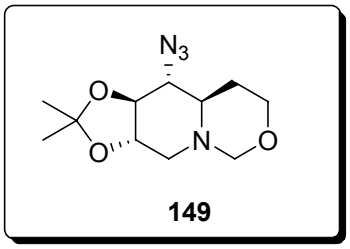
148

To a solution of **138** (0.7g, 3.040 mmol) in pyridine (6 mL) at 0 °C was added mesyl chloride (0.28 mL, 3.64 mmol). The reaction mixture was stirred at room temperature for 4 h. When TLC revealed no starting material, the solution was diluted with

dichloromethane (10 mL) and washed with water (3 x 5 mL), brine solution (5 mL) and then dried over Na_2SO_4. Removal of solvent followed by column chromatography purification (3:7, ethyl acetate: pet ether) gave **148** (850 mg, 91 %) as a crystalline solid.

mp	:	155-157 °C
$[\alpha]^{27}_D$		+20.08 (*c* 0.7, CH_2Cl_2)
IR (neat) ν_{max} cm^{-1}	:	1670, 1382, 1371,1363, 1352, 1215, 1174
^{1}H NMR (200 MHz, $CDCl_3$) δ	:	1.44 (s, 6H), 1.47-1.63 (m, 1H), 2.02-2.25 (m, 2H), 2.47 (td, 1H, *J* = 2.0, 10.0 Hz), 3.12 (s, 3H), 3.17 (dd, 1H, *J* = 4.2, 9.5 Hz), 3.37-3.56 (m, 2H), 3.78 (d. 1H, *J* = 8.0 Hz), 3.95-4.18 (m, 2H), 4.48 (d, 1H, *J* = 8.0 Hz), 5.02 (dd, 1H, *J* = 2.1, 2.3 Hz).
^{13}C NMR (50MHz, $CDCl_3$) δ	:	26.0(CH_3), 26.4(CH_3), 27.4(CH_2), 38.7(CH_3), 50.9(CH_2), 60.0(CH), 66.3(CH_2), 70.1(CH), 77.2(CH), 78.7(CH), 85.9(CH_2), 110.5(C).
Mass (m/z %)	:	330 (M+Na$^+$, 20%), 308 (MH$^+$, 100%), 212 (10%)

28. Preparation of (3a*S*,9a*R*,10*R*,10a*S*)-10-azido-2,2-dimethylhexahydro-4H-[1,3] dioxolo[4,5]pyrido[1,2-c][1,3]oxazine:

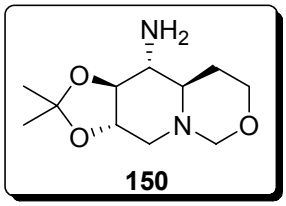


149

To a solution of **148** (110 mg, 0.358 mmol) in DMF (3 mL) was added LiN_3 (175 mg, 3.58 mmol) and heated to 110 °C for 16 h. When TLC revealed the absence of starting material, the reaction mixture was diluted with water (15 mL) and extracted with ethyl acetate (3 x15 mL). The ethyl acetate layer was washed with water, dried over Na_2SO_4 and concentrated to gave the title compound **149** (80 mg, 88%) after chromatographic (5:15, ethylacetate: pet ether) purification.

$[\alpha]^{27}_D$	:	+41.25 (c 1.8, $CHCl_3$)
IR ν_{max} cm^{-1}	:	2987, 2928, 2856, 2252, 2108, 1662, 1373, 1269, 1230, 1147.
^{1}H NMR (200 MHz, $CDCl_3$) δ	:	1.43 (s, 6H), 1.58-1.76 (m, 1H), 1.86-2.07 (m, 2H), 2.17 (dd, 1H, *J* = 9.8, 10.0 Hz), 3.03 (dd, 1H, *J* = 4.0, 9.5 Hz), 3.24-3.34 (m, 2H), 3.41 (dt, 1H, *J* = 2.4, 11.9 Hz), 3.52-3.66 (m, 1H), 3.74 (d, 1H, *J* = 7.8 Hz), 4.09 (dd, 1H, *J* = 4.8, 11.6 Hz), 4.41 (d, 1H, *J* = 7.9 Hz).
Mass (m/z %)	:	255 (MH$^+$, 100%), 212 (25%), 200 (50%), 180 (80%), 158 (25%)

29. Preparation of (3a*S*,9a*R*,10*R*,10a*S*)-2,2-dimethylhexa hydro-4H-[1,3]dioxolo[4,5]pyrido[1,2-c][1,3]oxazin-10-amine:



150

A solution of **149** (80 mg, mmol) in methanol (3 mL) was hydrogenated for 7 h at atmospheric pressure in the presence of Pd on charcoal (10%) (3 mg). The reaction mixture was passed through a short pad of Celite and the solvent was removed under reduced pressure to afford **150** (68 mg, 95 %) as a syrup.

$[\alpha]^{27}_D$	:	+16 (c 1.75, $CHCl_3$)
IR ν_{max} cm^{-1}	:	3285, 2987, 2928, 1665, 1373, 1270, 1234, 1024.
^{1}H NMR (200 MHz, $CDCl_3$) δ	:	1.42 (s, 6H), 1.50-1.71 (m, 1H), 1.75-2.03 (m, 2H), 2.17 (app t, 1H, *J* = 9.6, 10.0 Hz), 2.76 (dd, 1H, *J* = 8.3, 10.0 Hz), 3.02-3.17 (m, 2H), 3.44 (dt, 1H, *J* = 2.5, 12.0 Hz), 3.55 (ddd, 1H, *J* = 4.0, 7.5, 10.2 Hz), 3.75 (d, 1H, J = 7.8 Hz), 4.06-4.17 (dd, 1H, *J* = 4.8, 11.4 Hz), 4.42 (d, 1H, *J* = 7.8 Hz)
Mass (m/z %)	:	228 (M^+, 100%)

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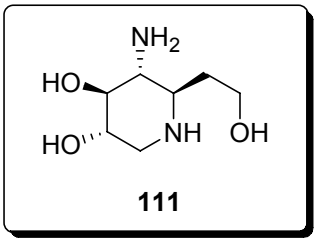
30. Preparation of (3*S*,4*S*,5*R*,6*R*)-5-amino-6-(2-hydroxy ethyl)piperidine-3,4-diol:

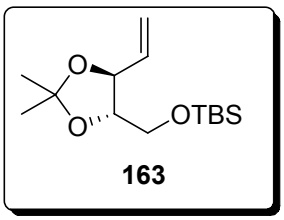


111

The title compound **111** was prepared by using the same procedure as that for **108.**

$[\alpha]^{27}_D$	:	+4.8 (c, 1.33, MeOH)
^{1}H NMR (400 MHz, D_2O) δ	:	1.96-2.06 (m, 1H), 2.13-2.22 (m, 1H), 3.00 (app t, 1H, *J* = 11.9, 12.4 Hz), 3.37 (app t, 1H, *J* = 10.4, 11.3 Hz), 3.56 (dd, 1H, *J* = 5.0, 12.8 Hz), 3.63 (ddd, 1H, *J* = 3.7, 9.27, 11.1 Hz) 3.71 (t, 1H, *J* = 9.6 Hz), 3.82-3.93 (m, 3H).
^{13}C NMR (100 MHz, D_2O) δ	:	30.9(CH_2), 46.2(CH_2), 53.3(CH), 55.4(CH), 57.4(CH_2), 67.1(CH), 72.2(CH).
Mass (m/z %)	:	176 (MH^+, 100%), 161 (22%), 127 (7%)
Elemental analysis	:	Anal. Calcd for $C_7H_{16}N_2O_3$: C, 47.71; H, 9.15; N, 15.90. Found: C, 47.57: H, 9.43: N, 16.23

31. Preparation of *t*-butyl{[(4*S*,5*S*)-2,2-dimethyl-5-vinyl-1,3-dioxolan-4-yl]methoxy} dimethylsilane:



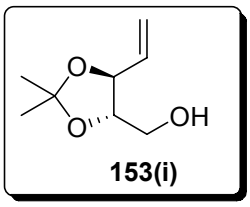
163

HMPA (4 mL) was added to a suspension of the Wittig salt (1.770 g, 4.38 mmol) in dry THF (10 mL) and the reaction mixture was cooled to 0 °C. To this was added *n*-BuLi (1.62M in hexane, 3.24 mL, 5.26 mmol). To the resultant clear brownish solution was added solution of **126** (1.0 g, 3.65 mmol) in THF (6 mL). The reaction mixture was allowed to stir at 0 °C for 2 h and then brought up to rt. After about 12

h, the reaction was found to be complete and was worked up by careful addition of water (15 mL) with external cooling. Extraction with DCM (2 x 10 mL) followed by drying of the combined organic extracts over anhydrous Na_2SO_4 and the removal of the solvent under reduced pressure afforded a semi-solid mass which was dissolved in a minimum amount of DCM. Pet-ether was added to precipitate out the phosphine oxide and the supernatant liquid was passed through a short pad of silica. The precipitated solid was dissolved in DCM and the procedure was repeated. The combined fractions were concentrated under reduced pressure and the resulting residue was column chromatographed (silica, pet ether-ethyl acetate, 98:2) to afford **153** (0.70 g, 70 %) as a colorless oil.

$[\alpha]^{27}_D$	:	+ 4.17° (*c* 1.92, $CHCl_3$)
IR (neat) ν_{max} cm^{-1}	:	1471, 1461
^{1}H NMR (200 MHz, $CDCl_3$) δ	:	0.05 (s, 6H), 0.90 (s, 9H), 1.40 (s, 6H), 3.75 (m,3H), 4.35 (m, 1H), 5.30 (m, 2H), 5.90 (m, 1H)
^{13}C NMR (50MHz, $CDCl_3$) δ	:	-5.6, -5.5, 18.2, 25.7, 26.7, 26.8, 62.3, 79.1, 81.1, 108.9, 117.8, 135.6
GC-MS	:	257 $(M-15)^+$ (3), 157 (67), 127 (71), 98 (31), 75 (100)

32. Preparation of [(4*S*,5*S*)-2,2-dimethyl-5-vinyl-1,3-dioxolan-4-yl]methanol:

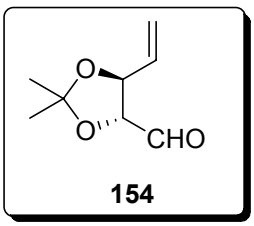


153(i)

Similar procedure was used as described for the preparation of **129**.

$[\alpha]^{27}_D$	:	-2.94° (*c* 1.36, $CHCl_3$).
IR (neat) ν_{max} cm^{-1}	:	3446 (broad), 1647, 1380.
^{1}H NMR (200 MHz, $CDCl_3$) δ	:	1.40 (s, 6H), 2.05 (dd, 1H, *J* = 8.0, 4.6 Hz), 3.60 (m, 1H), 3.85 (m, 2H), 4.30 (t, 1H, *J* = 8.3 Hz), 5.85 (m, 1H), 5.35 (m, 2H),
^{13}C NMR (50MHz, $CDCl_3$) δ	:	26.7, 60.6, 78.2, 80.9, 109.0, 118.8, 134.8.
GC-MS		143 (M+-15) (15), 127 (3), 98 (15), 83 (24), 59 (53), 43(100).

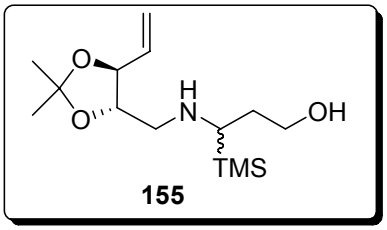
33. Preparation of (4*R*,5*S*)-2,2-dimethyl-5-vinyl-1,3-dioxolane-4-carbaldehyde:



154

Similar procedure was used as described for the preparation of **116.** The crude aldehyde **154** obtained was immediately used for the next step.

34. Preparation of 3-{[(4*S*,5*S*)-2,2-dimethyl-5-vinyl-1,3-dioxolan-4-yl]methylamino}-3-(trimethylsilyl) propan-1-ol:



155

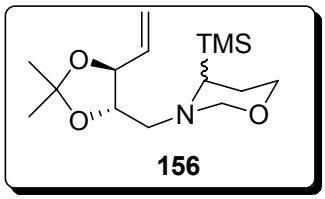
Similar procedure was used as described for the preparation of f **135.**

^{1}H NMR (200 MHz, $CDCl_3$) δ : 0.00 (s, 9H), 1.31(s, 3H), 1.32 (s, 3H), 1.45-1.79(m, 2H), 2.17(dt, 1H, *J* = 3.92, 8.5 Hz), 2.53- 2.9 (m, 2H), 3.39 (bs, 2H, -NH,-OH), 3.62-3.85 (m, 3H), 3.94-4.16 (m, 1H), 5.1-5.37(m, 2H), 5.6-5.82 (m, 1H)

^{13}C NMR (50MHz, $CDCl_3$) δ : -2.89 (CH_3), 26.69(CH_3), 26.90 (CH_3), 30.59 + 31.02 (CH_2), 48.77+49.66(CH), 48.90+51.16(CH_2), 63.17+64.17(CH_2), 79.23+79.60(CH), 79.77+80.37(CH), 108.96+108.96(C), 118.80+119.09(CH_2), 134.80+134.96(C)

Mass : 288 (MH^+)

35. Preparation of 3-{[(4*S*,5*S*)-2,2-dimethyl-5-vinyl-1,3-dioxolan-4-yl]methyl}-4-(trimethylsilyl)-1,3-oxazinane:



156

Similar procedure was used as described for the preparation of **114**

IR (neat) ν_{max} cm^{-1} : 3018, 2989, 2956, 2399, 1425, 1380, 1251, 1215, 1068

^{1}H NMR (200 MHz, $CDCl_3$) δ : 0.03+0.09 (singlets, 9H), 1.35-1.55 (m, 7H), 1.70-1.95 (m, 1H), 2.55-2.70 (m, 2H), 3.17-3.37 (m, 1H), 3.65-3.90 (m, 2H), 3.97-4.07 (m, 2H), 4.27 (d, 1H, *J* = 10.5 Hz), 4.62 (two set of d, 1H, *J* = 10.6 and 10.4 Hz), 5.20-5.40 (m, 2H), 5.71-5.91 (m, 1H)

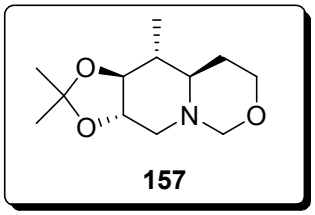
^{13}C NMR (50MHz, $CDCl_3$) δ : -2.31 and -2.28(Si-CH_3), 21.89+21.94(CH_2), 26.4+26.6(CH_3), 26.92+26.99(CH_3), 51.0+51.2(CH_2), 52.4(CH), 68.1+68.2(CH_2), 78.7+80.64(CH), 80.80+81.82(CH), 83.7+85.0(CH_2), 108.9(C), 118.4(CH_2), 135.0+135.3(CH)

Mass (m/z %) : 322 ($M+Na^+$, 45%), 300 (MH^+, 100%), 288 (63%)

Elemental Analysis : Anal. Calcd for $C_{15}H_{29}NO_3Si$: C, 60.16; H, 9.76; N, 4.68. Found: C, 60.28; H, 9.52; N, 4.60

---

36. Preparation of (3a*S*,9a*R*,10*S*,10a*S*)-2,2,10-trimethyl hexahydro-4H-[1,3] dioxolo [4,5]pyrido[1,2-c][1,3] oxazine:

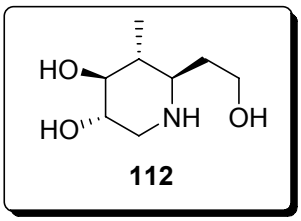


157

PET cyclization of **156** was carried out under similar conditions as that used for the cyclization of **114** to **113**.

$[\alpha]^{27}_D$	:	+11.8° (*c* 3.15, $CHCl_3$)
^{1}H NMR (200 MHz, $CDCl_3$) δ	:	0.93 (d, 3H, *J* = 6.42 Hz), 1.41(s, 3H), 1.42 (s, 3H), 1.50-1.70 (m, 2H), 1.75 (br d, 1H *J* = 13.3 Hz), 1.80 (dt, 1H, *J* = 2.7, 10.1 Hz), 2.15 (app t, 1H, *J* = 9.6, 10.1 Hz), 2.96 (dd, 1H, *J* = 8.7, 10.5 Hz), 3.08 (dd, 1H, *J* = 4.1, 9.2 Hz) 3.42 (dt, 1H, *J* = 2.7, 11.9 Hz), 3.57 (ddd, 1H, *J* = 4.1, 7.3, 10.4 Hz), 3.74 (d, 1H, *J* = 8.2 Hz), 4.08 (dd, 1H, *J* = 4.6, 11.7 Hz), 4.40 (d, 1H, *J* = 7.8 Hz)
^{13}C NMR (50MHz, $CDCl_3$) δ	:	13.3(CH_3), 26.7(CH_3), 26.9(CH_3), 29.4(CH_2), 39.4 (CH), 51.6(CH_2), 65.0(CH), 67.6(CH_2), 75.4(CH), 84.3(CH), 86.5(CH_2), 109.9(C)
Mass (m/z %)	:	228 (MH^+, 100%)
Elemental Analysis	:	Anal. Calcd for $C_{12}H_{21}NO_3$: C, 63.41; H, 9.31; N, 6.16. Found: C, 63.66; H, 9.51; N, 5.98

37. (3*S*,4*S*,5*S*,6*R*)-6-(2-hydroxyethyl)-5-methylpiperidine-3,4-diol:



112

PET cyclized product **157** was transformed into **112** by the identical procedure as used for **108**.

$[\alpha]^{27}_D$	:	+4.0° (*c* 1.15, MeOH)
^{1}H NMR (500 MHz, D_2O) δ	:	1.13 (d, 3H, *J* = 6.4 Hz), 1.71-1.80 (m, 1H), 1.81-1.89 (m, 1H), 2.12-2.20 (m, 1H), 2.93 (t, 1H, *J* = 11.9 Hz), 3.21 (ddd, 1H, *J* = 2.3, 9.7, 11.1 Hz), 3.31 (t, 1H, *J* = 9.6 Hz), 3.51 (dd, 1H, *J* = 5.0, 12.4 Hz), 3.72-3.88 (m, 3H)
^{13}C NMR (125 MHz, D_2O) δ	:	12.4(CH_3), 31.0(CH_2), 38.3(CH), 46.1(CH_2), 57.9(CH_2), 59.2(CH), 67.8(CH), 75.4(CH)
Mass (m/z %)		176 (MH^+, 100%), 149 (25%)
Elemental analysis	:	Anal. Calcd for $C_8H_{17}NO_3$: C, 54.84; H, 9.78; N, 7.99; Found C, 54.86; H, 9.58; N, 8.06

38. General procedure for enzyme inhibition assay:

Inhibition assay for the inhibitory potencies of the azasugars were determined by measuring the residual hydrolytic activities of the glycosidases of the corresponding *p*-nitrophenyl glycosides in the presence of azasugars spectrophotometrically.

In the case of β-galactosidase, each assay was performed in citrate buffer (50 mM, pH 4.5) with *p*-nitrophenyl β-D-galactosidase as the substrate. Varying concentrations of the substrate (50-150 μl, 10 mM) and β-lactam-azasugar hybrids were employed. The reaction

was initiated by the addition of 100 mL of appropriately diluted enzyme and the reaction mixture, which had a final volume of 1 mL was incubated for 20 min at 30 °C and then quenched by the addition of 2 mL of 1M Na_2CO_3 solution. The absorbance of the resulting solution was read at 405 nm.

In the case of α-galactosidase (Green coffee beans), the assay was performed in an citrate phosphate buffer (50 mM, pH 6.5) with *p*-nitrophenyl α-D-galactopyranoside as the substrate and the reaction was carried out at 25 °C for 20 min and then quenched by Na_2CO_3 solution.

In the case of β-glucosidase (Almond), the assay was performed in a citrate phosphate buffer (50 mM, pH 5.5) with *p*-nitrophenyl β-D-glucopyranoside as the substrate. The reaction was carried out at 37 °C for 30 min and then quenched by Na_2CO_3 solution.

In the case of α-glucosidase (Yeast), the assay was performed in a citrate phosphate buffer (50 mM, pH 6.8) with *p*-nitrophenyl α-D-glucopyranoside as the substrate. The reaction was carried out at 37 °C for 20 min and then quenched by Na_2CO_3 solution.

In the case of β-mannosidase (Snail acetone), the assay was performed in an acetate buffer (50 mM, pH 4.0) with *p*-nitrophenyl β-D-mannopyranoside as the substrate and the reaction was carried out at 25 °C for 20 min and then quenched by Na_2CO_3 solution.

In the case of α-mannosidase (Jack Bean), the assay was performed in an acetate buffer (50 mM, pH 4.5) with *p*-nitrophenyl α-D-mannopyranoside as the substrate and the reaction was carried out at 25 °C for 20 min and then quenched by Na_2CO_3 solution.

The *Ki* values were determined from the Lineweaver-Burke double reciprocal plots of 1/v vs. 1/[S]. *Ki* for competitive inhibition was determined using the formula:

$$Ki = \frac{[I]}{\left(\frac{K_{MI}}{K_M}\right) - 1}$$

2.15 References

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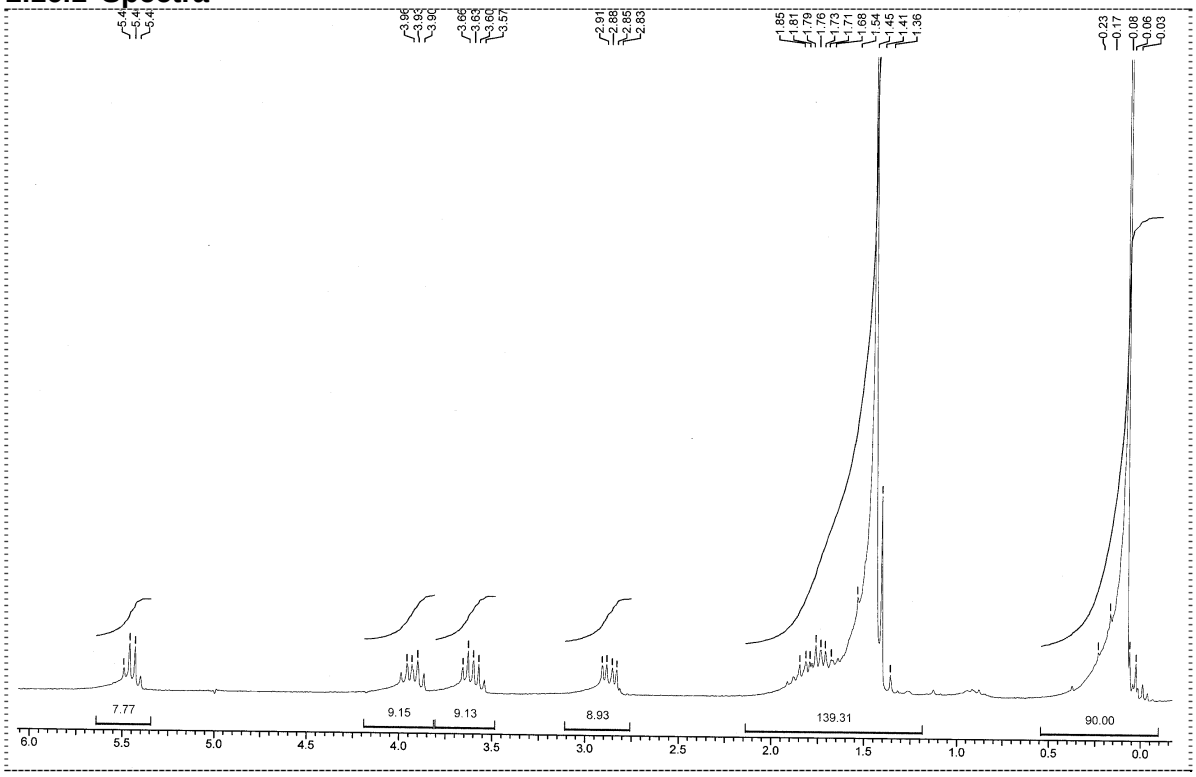
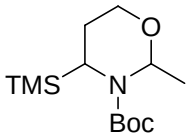
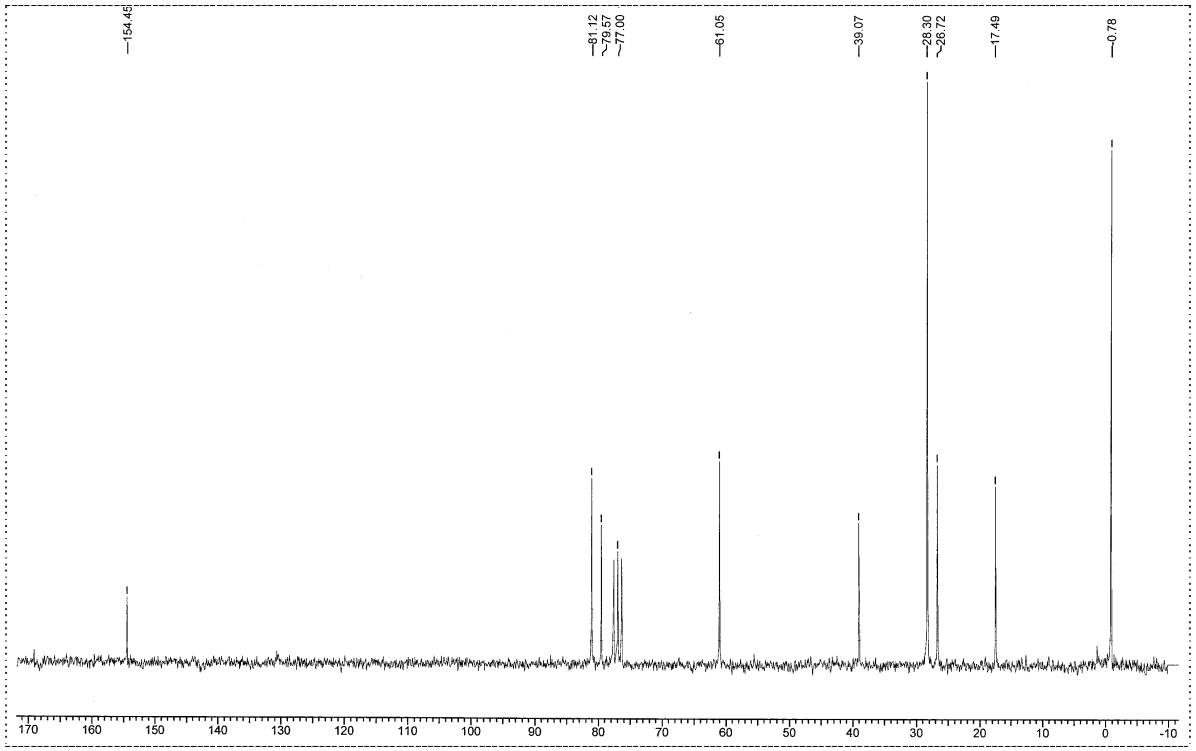
2.16 Spectra, X-ray data and Lineweaver-Burke plot

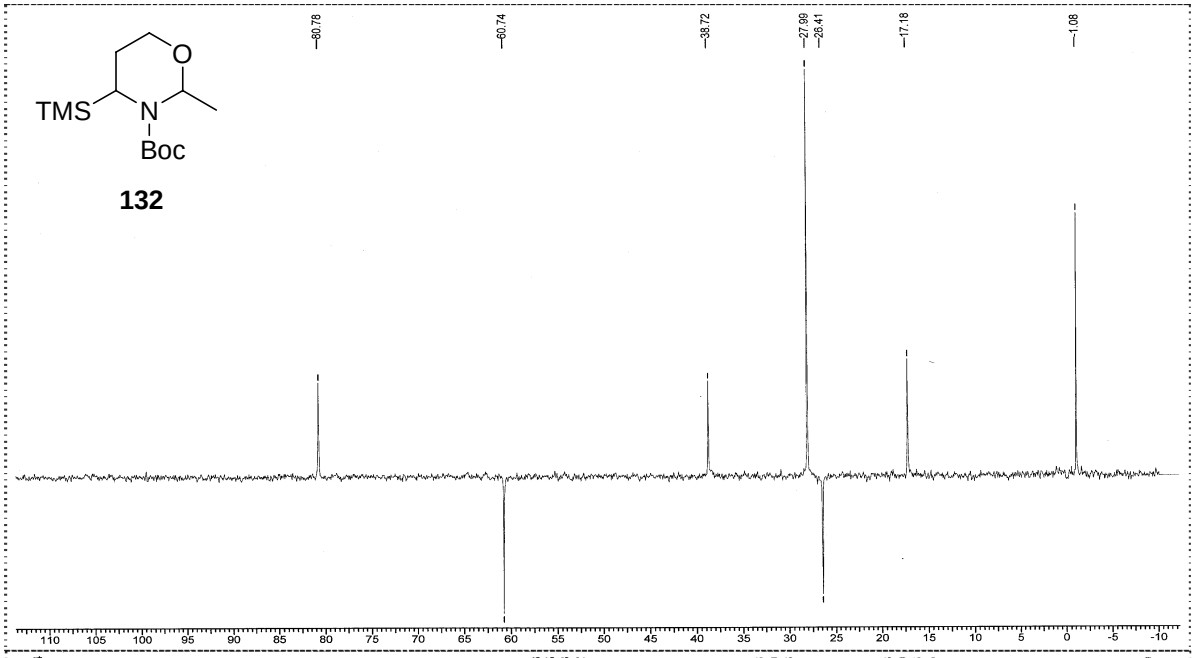
2.16.1 Spectra





132

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26.41
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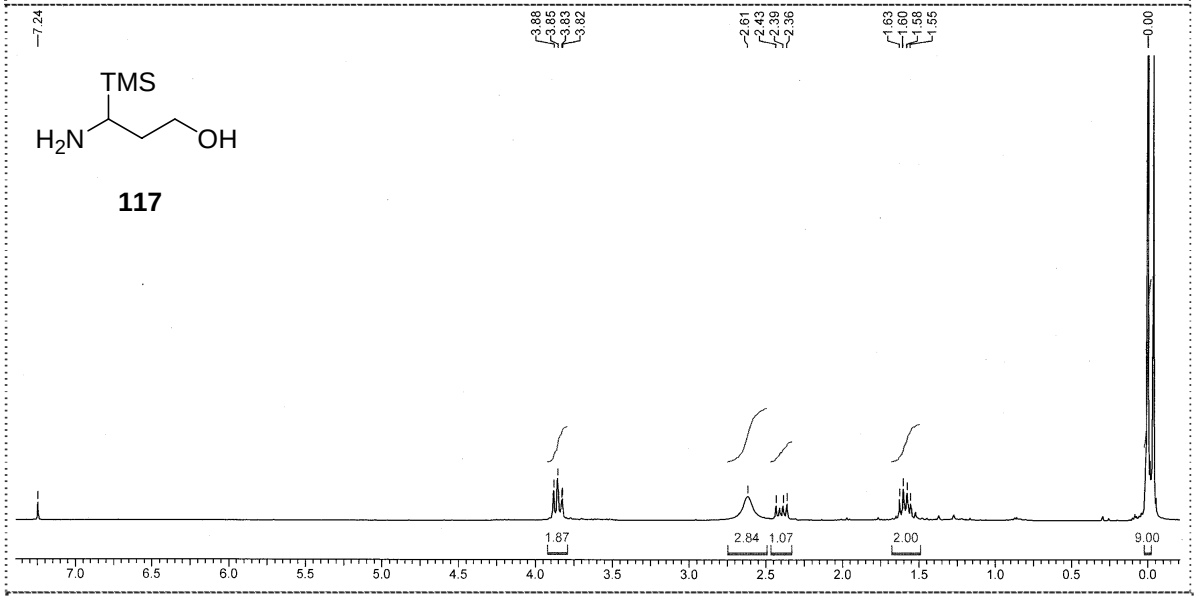
1.08

132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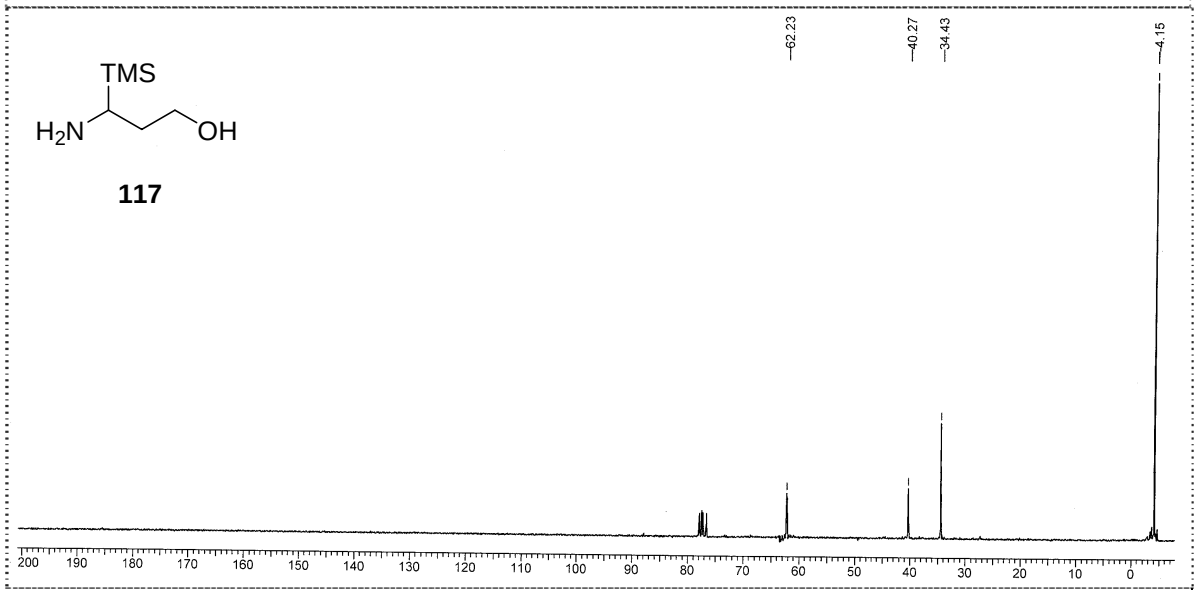
2.84
1.07
2.00
9.00

117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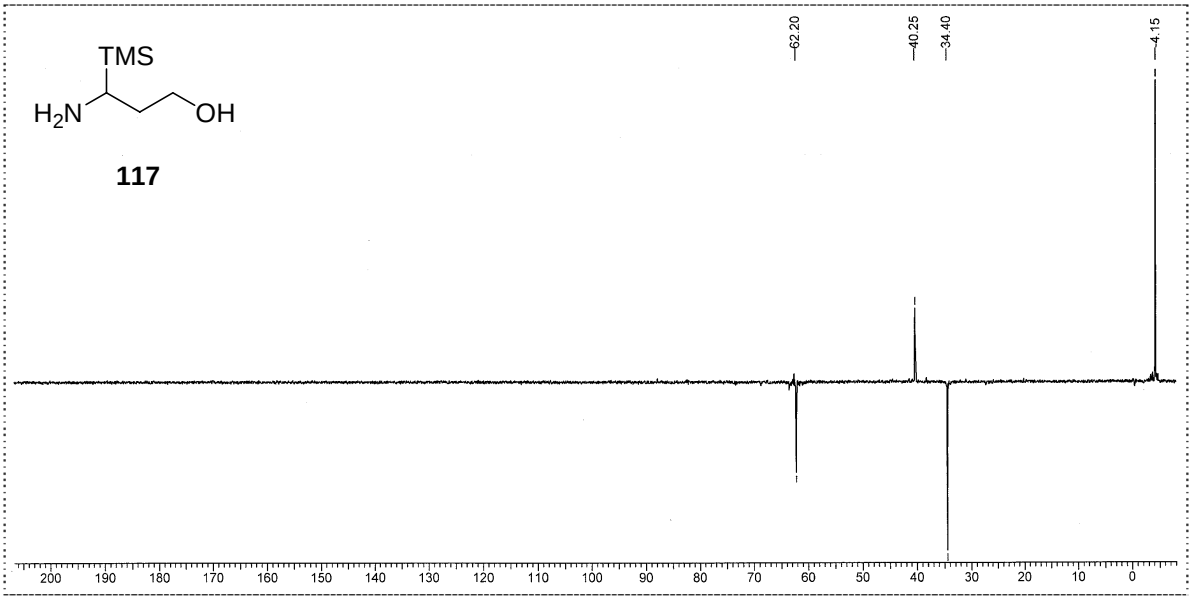
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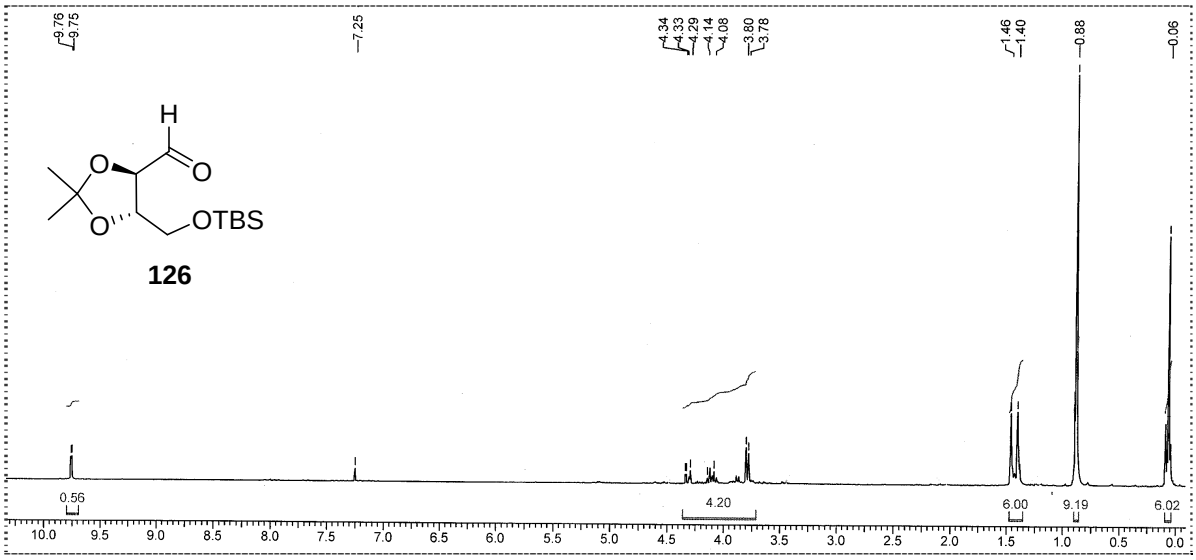
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4.08
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3.78
1.46
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H
O
O
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OTBS
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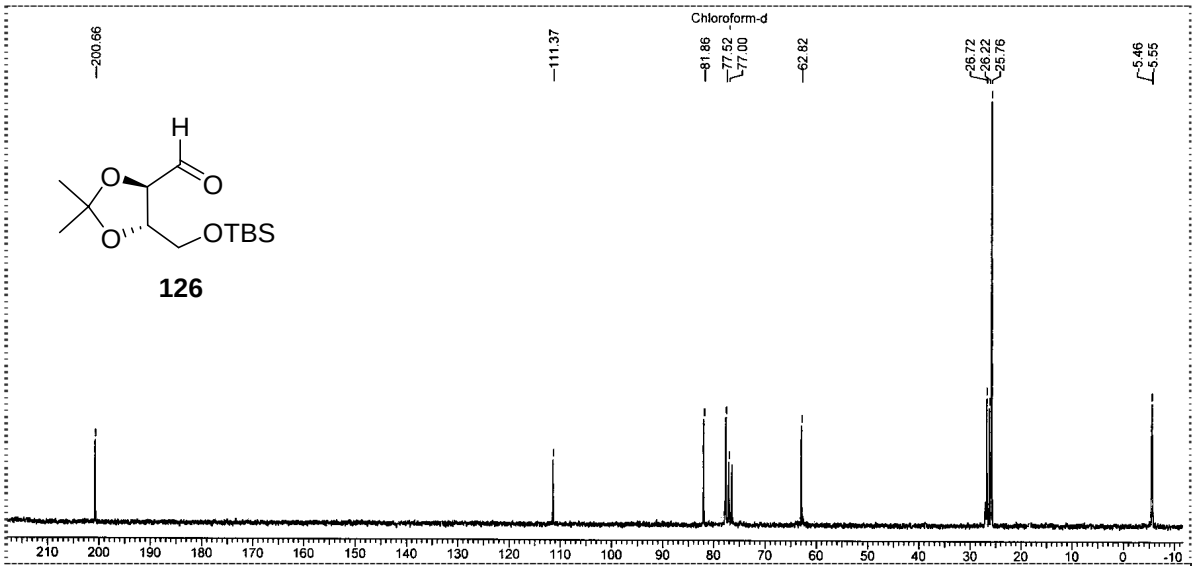
6.00
9.19
6.02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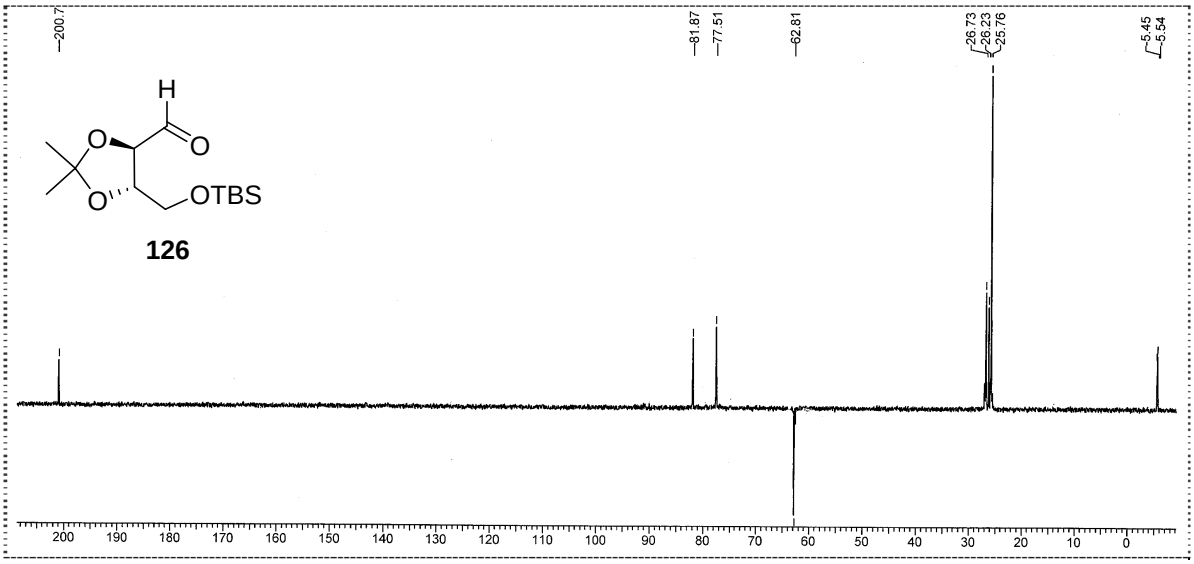
OTBS
126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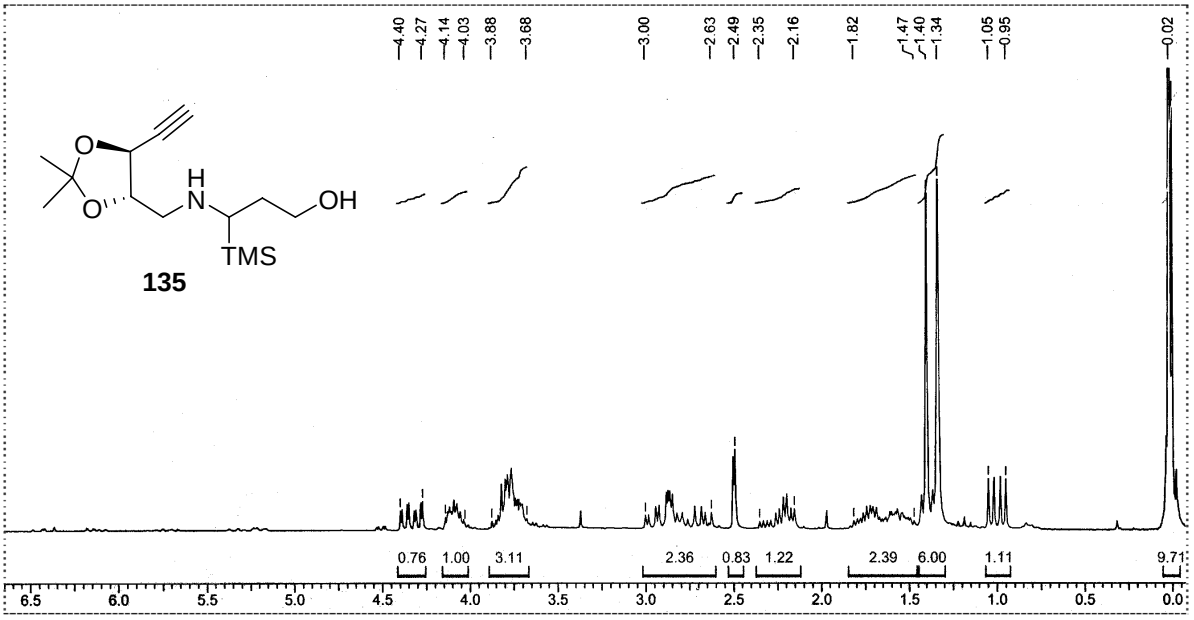
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1.40
1.34
1.05
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0.02
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O
H
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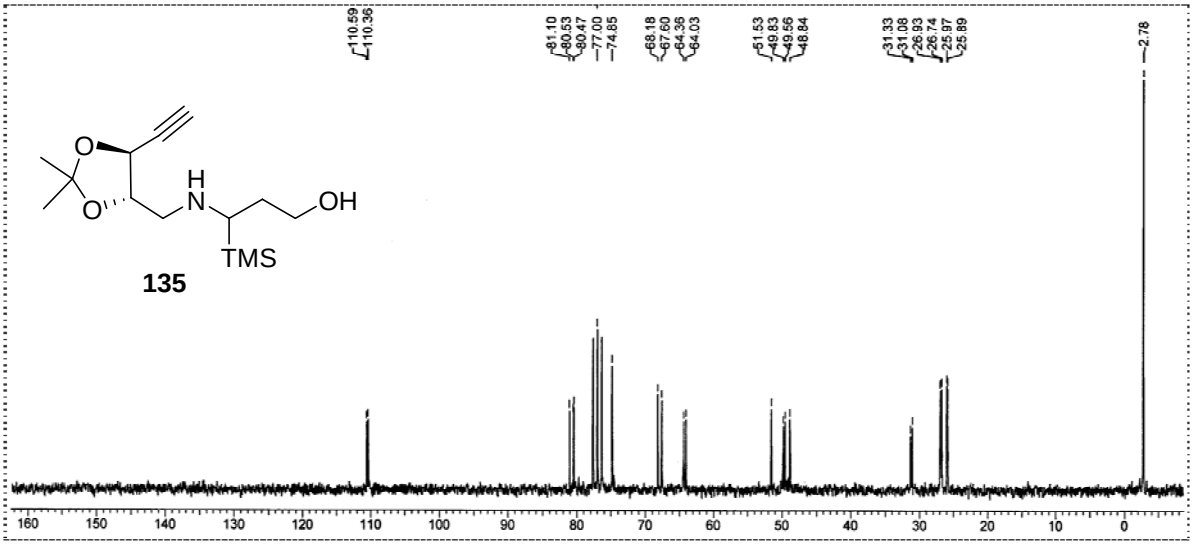
135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49.56
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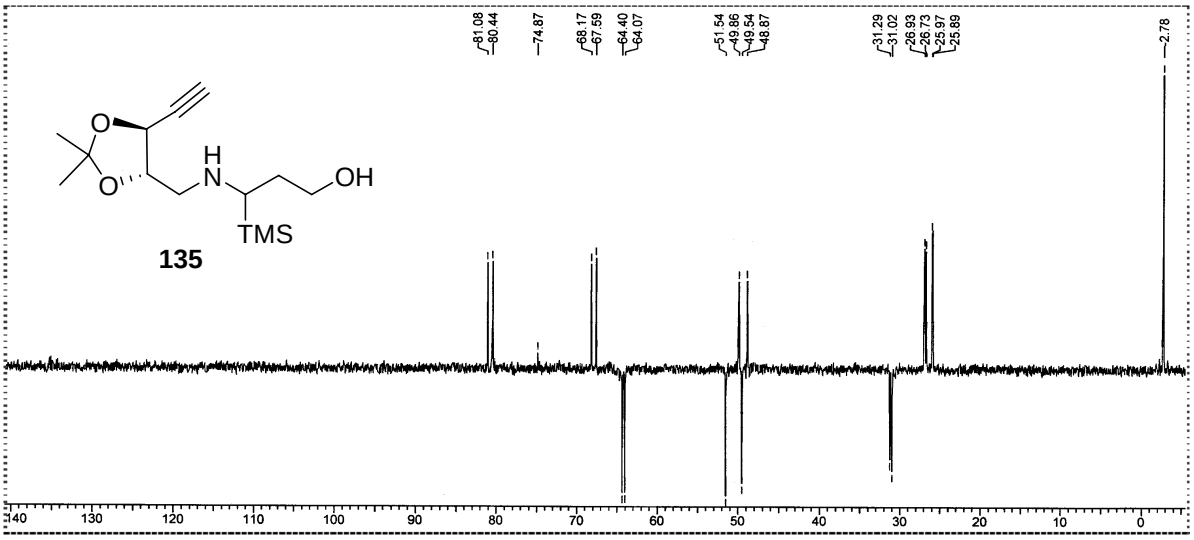
OH
TMS

135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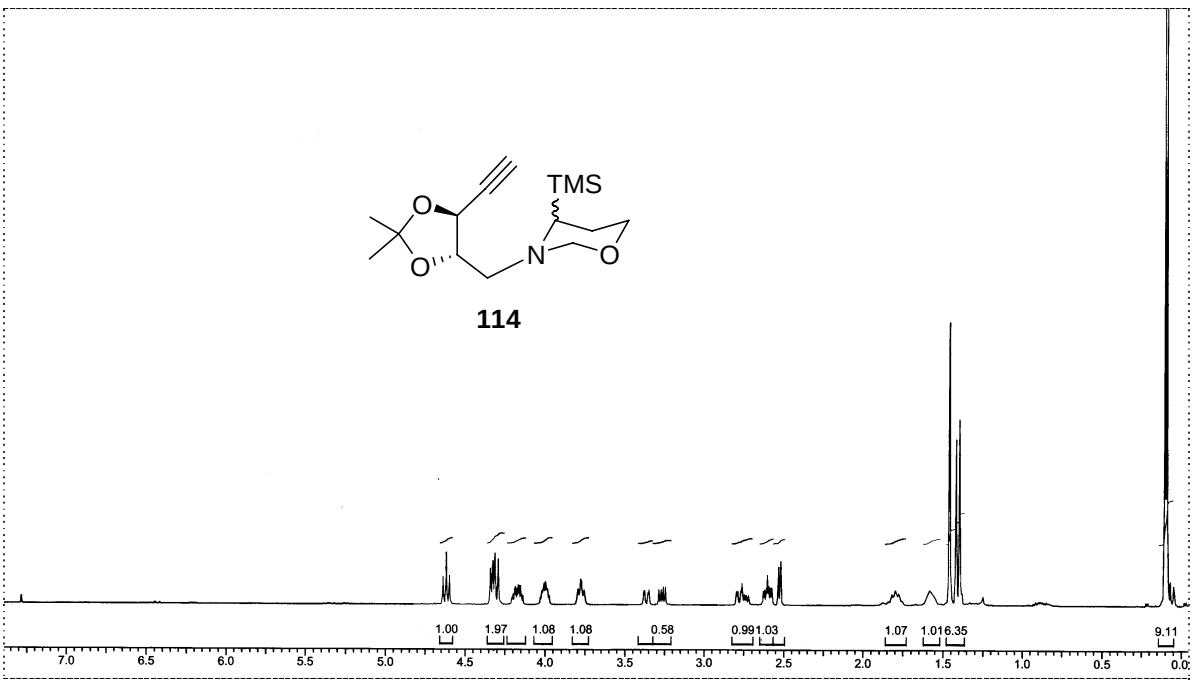
135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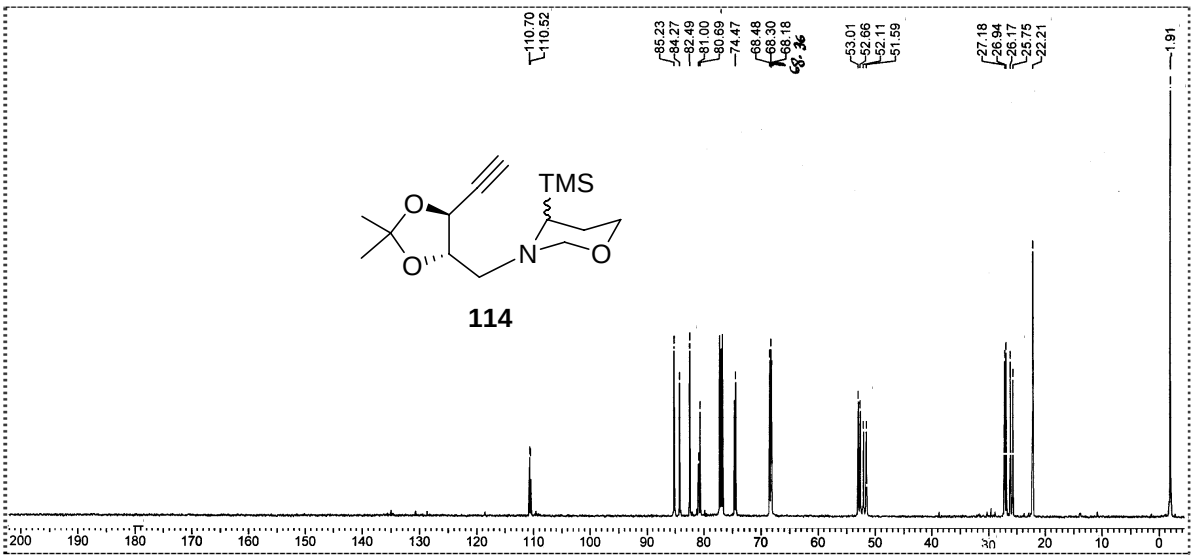
114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74.47
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53.01
52.66
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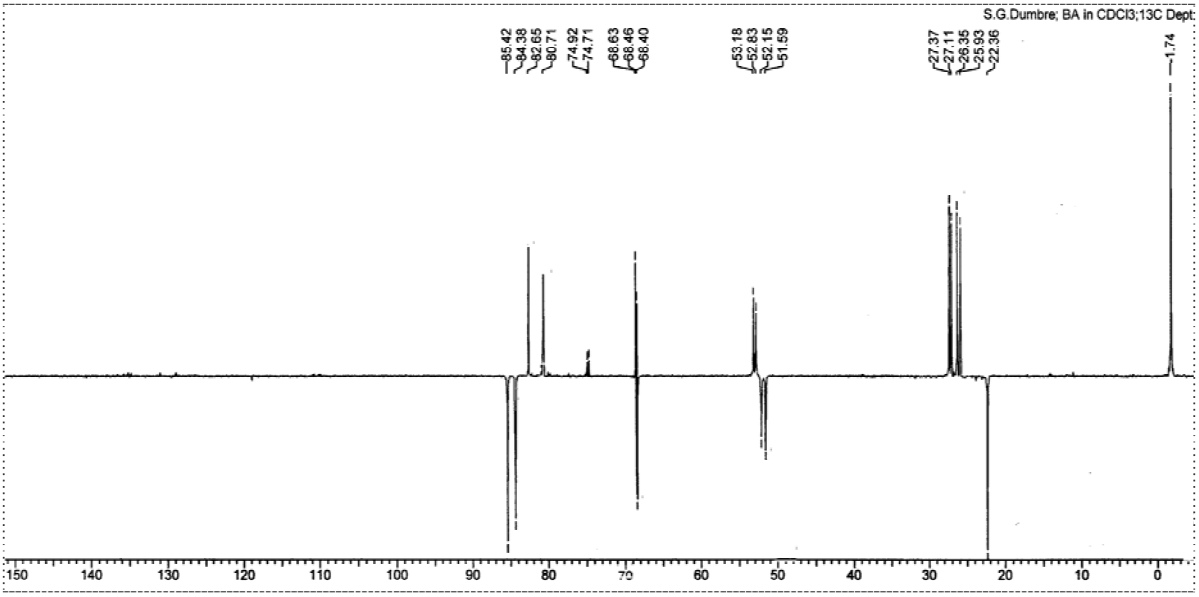
22.21
1.91
TMS
O
O
N
O

114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74.71
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68.46
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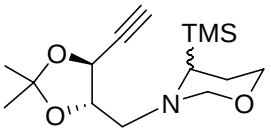
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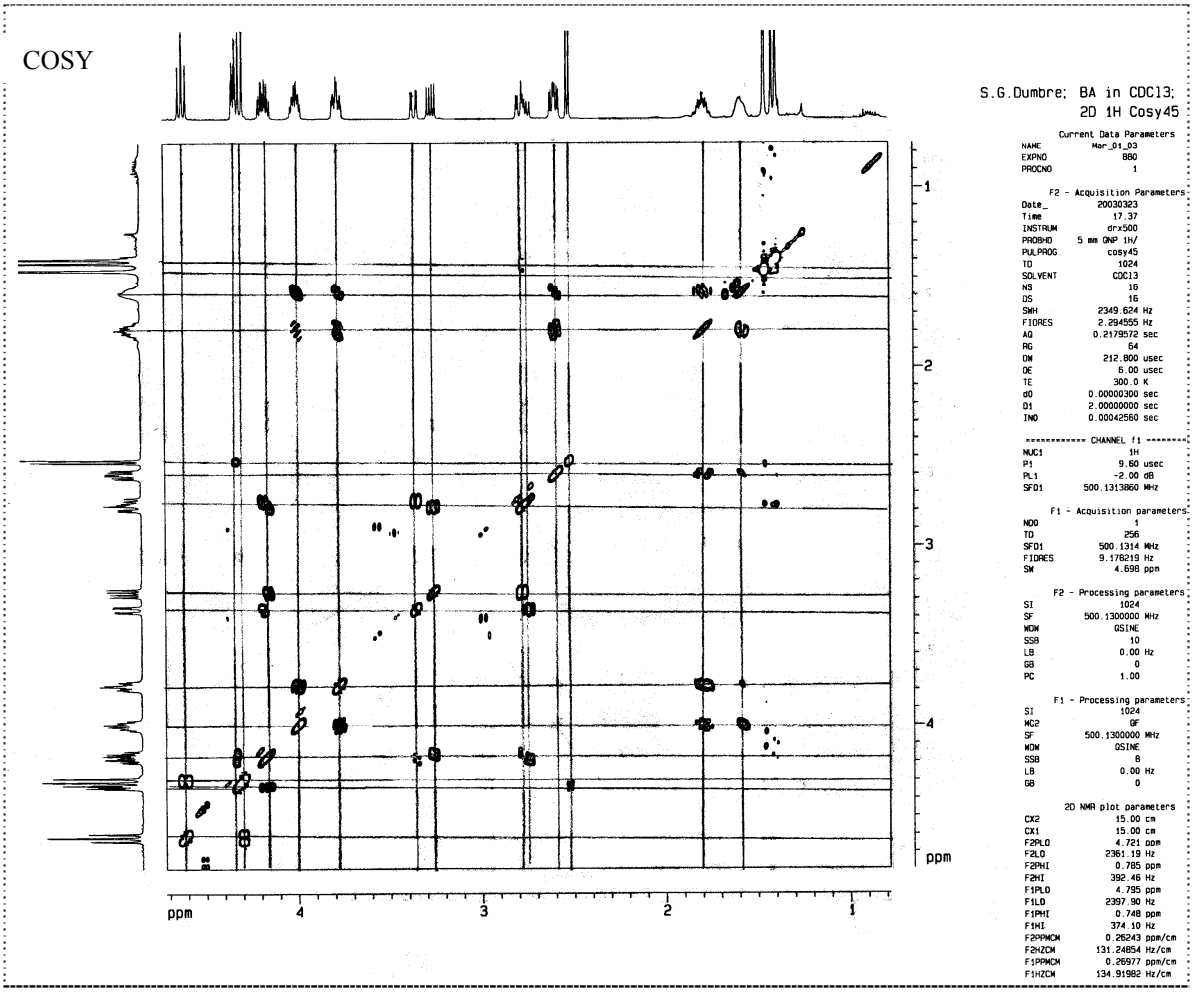
O
N
O

114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Time 17.37
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PULPROG cosy45
TD 1024
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DS 16
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FIDRES 2.294555 Hz
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RG 64
DW 212.800 usec
DE 6.00 usec
TE 300.0 K
D0 0.00000300 sec
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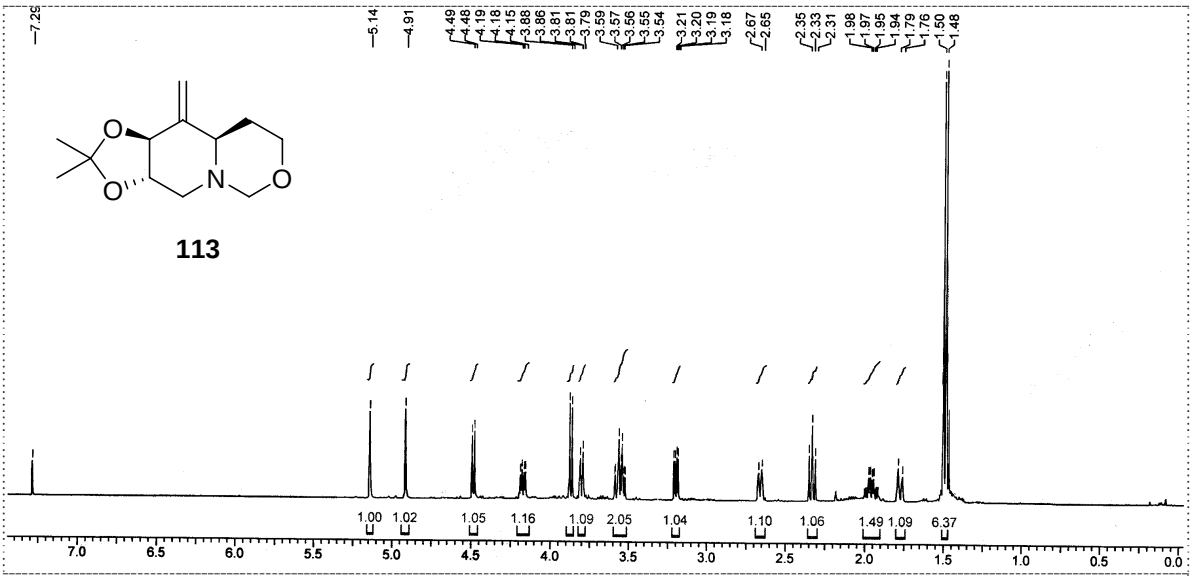
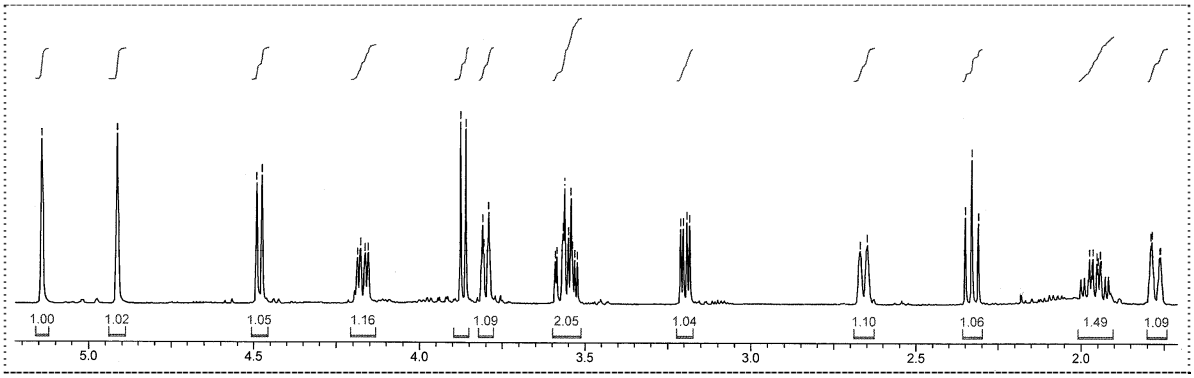
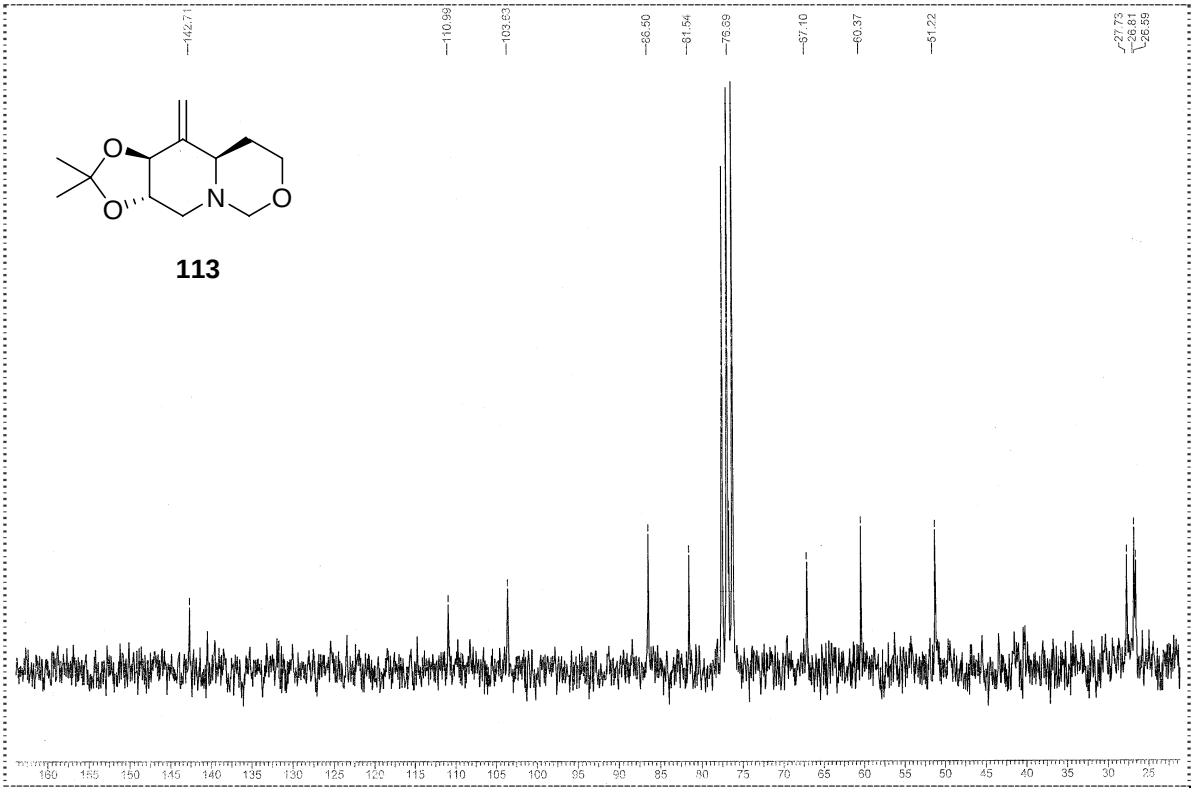
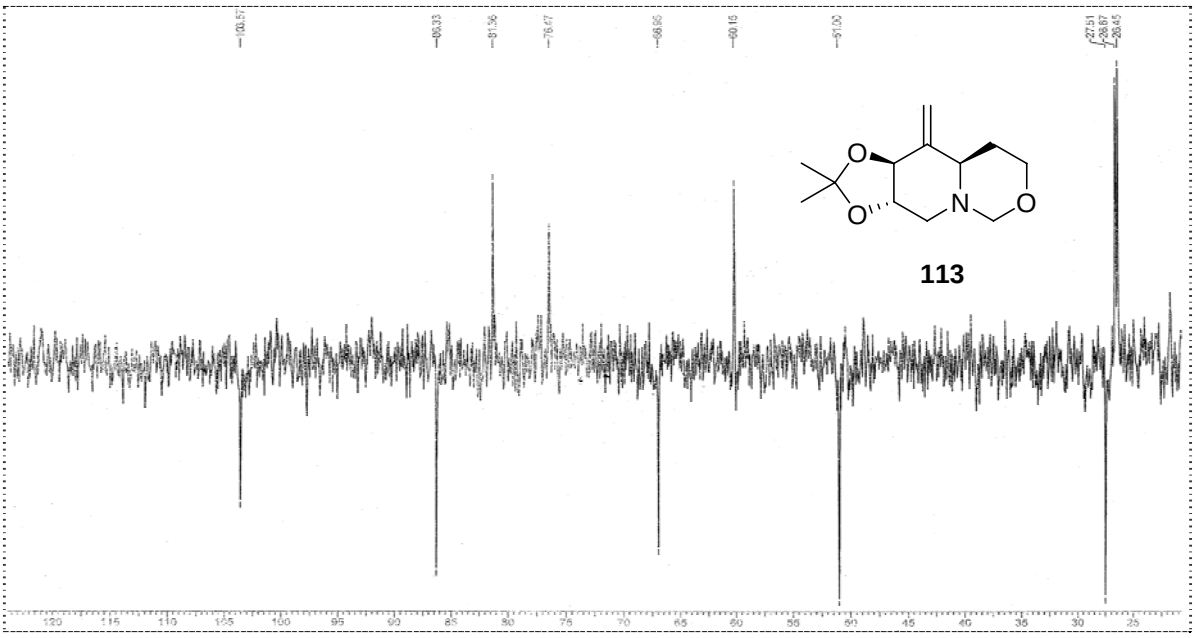
NUC1 1H
P1 9.60 usec
PL1 -2.00 dB
SFO1 500.1313860 MHz
F1 - Acquisition parameters
NDO 1
TD 256
SFO1 500.1314 MHz
FIDRES 9.176219 Hz
SW 4.698 ppm
F2 - Processing parameters
SI 1024
SF 500.1300000 MHz
WDW QSINE
SSB 10
LB 0.00 Hz
GB 0
PC 1.00
F1 - Processing parameters
SI 1024
MC2 QF
SF 500.1300000 MHz
WDW QSINE
SSB 8
LB 0.00 Hz
GB 0
2D NMR plot parameters
CX2 15.00 cm
CX1 15.00 cm
F2PLO 4.721 ppm
F2LO 2361.19 Hz
F2PHI 0.785 ppm
F2HI 392.46 Hz
F1PLO 4.795 ppm
F1LO 2397.90 Hz
F1PHI 0.748 ppm
F1HI 374.10 Hz
F2PPMCM 0.26243 ppm/cm
F2HZCM 131.24854 Hz/cm
F1PPMCM 0.26977 ppm/cm
F1HZCM 134.91982 Hz/cm
ppm
4
3
2
1


7.29
5.14
4.91
4.49
4.48
4.19
4.18
4.15
3.88
3.86
3.81
3.81
3.79
3.59
3.57
3.56
3.55
3.54
3.21
3.20
3.19
3.18
2.67
2.65
2.35
2.33
2.31
1.98
1.97
1.95
1.94
1.79
1.76
1.50
1.48
O
O
N
O
113
1.00 1.02 1.05 1.16 1.09 2.05 1.04 1.10 1.06 1.49 1.09 6.37
7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

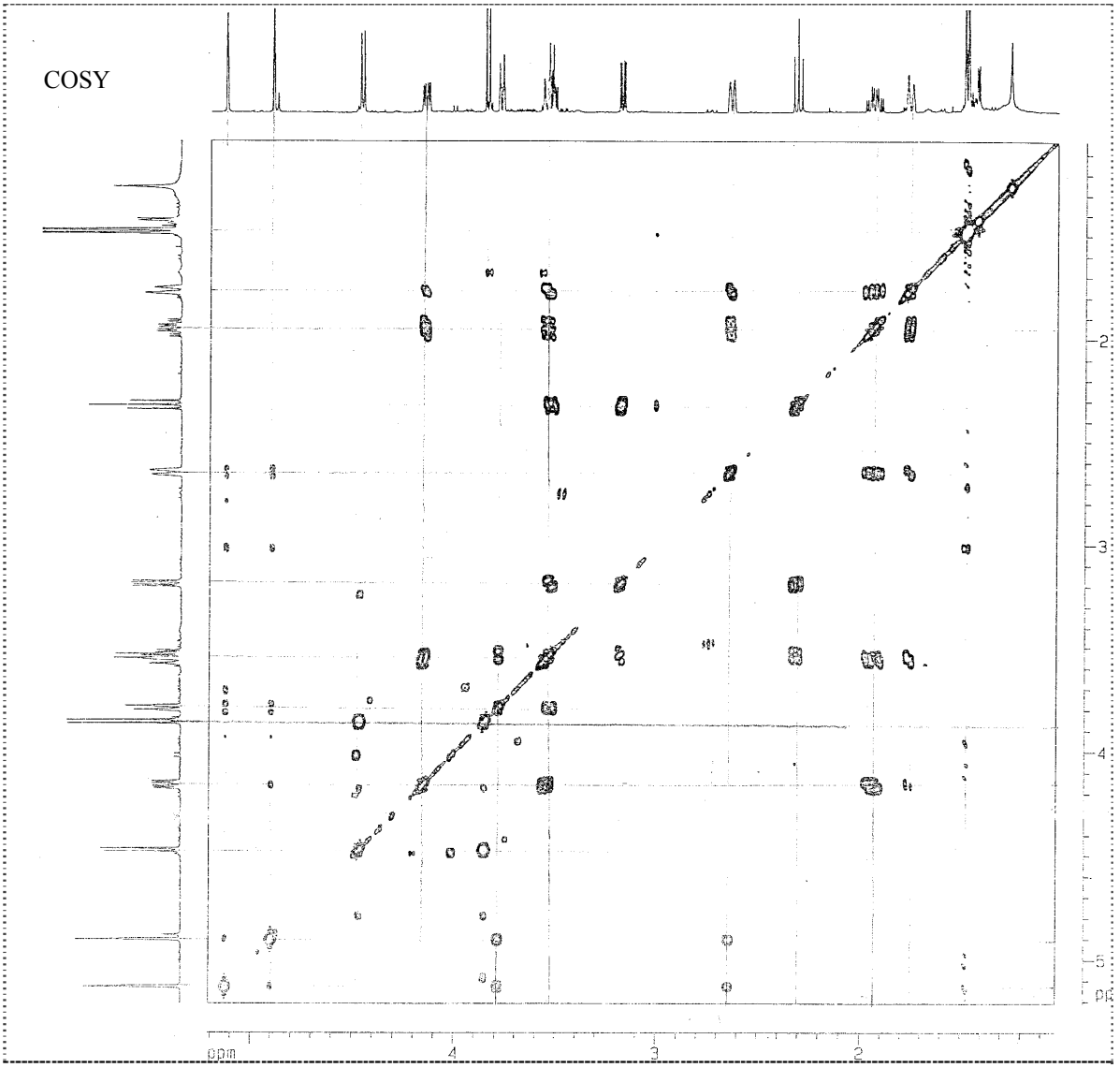

1.00 1.02 1.05 1.16 1.09 2.05 1.04 1.10 1.06 1.49 1.09
5.0 4.5 4.0 3.5 3.0 2.5 2.0


142.71
110.99
103.63
86.50
81.54
76.89
67.10
60.37
51.22
27.73
26.81
26.59
O
O
N
O
113

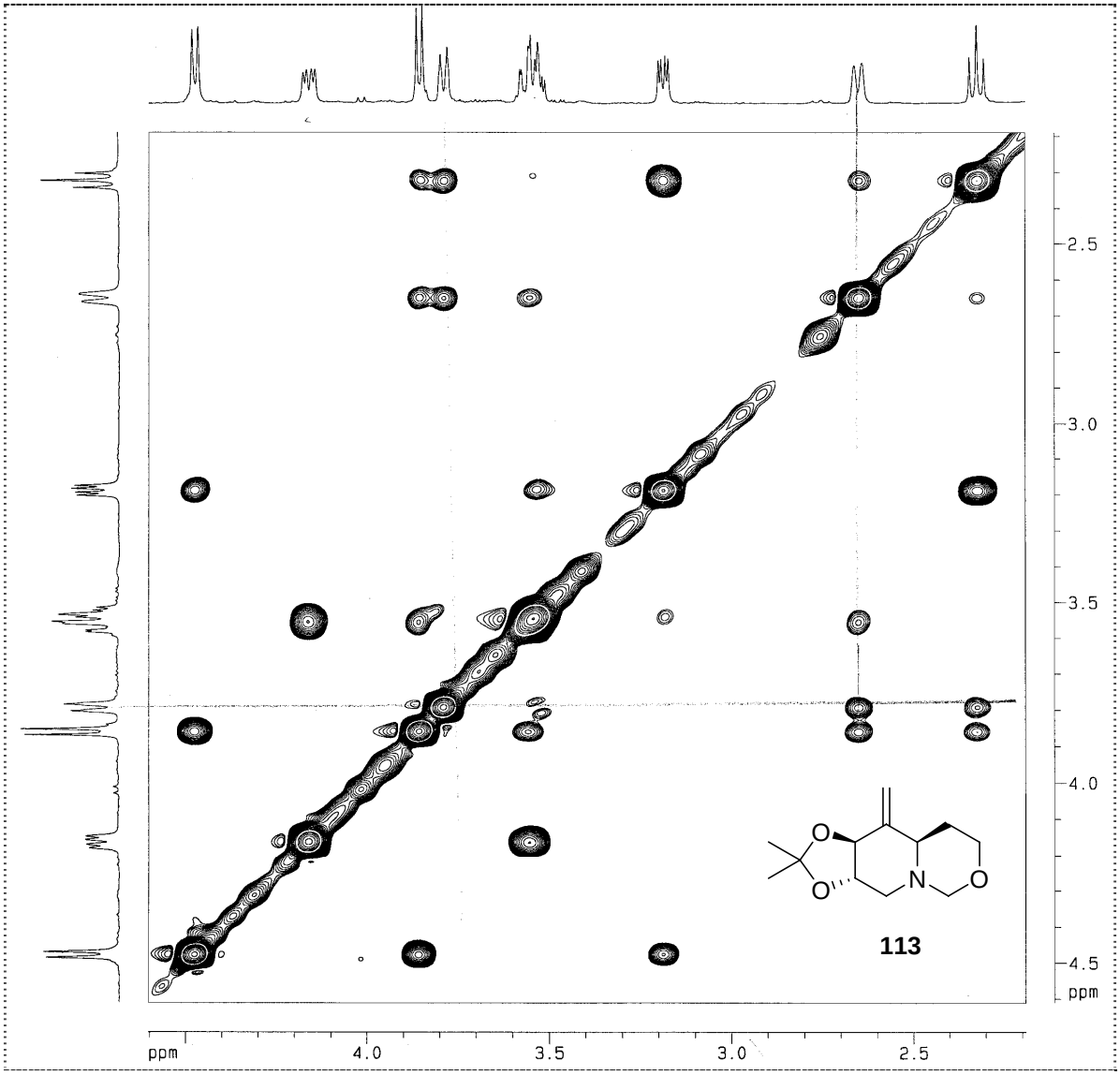


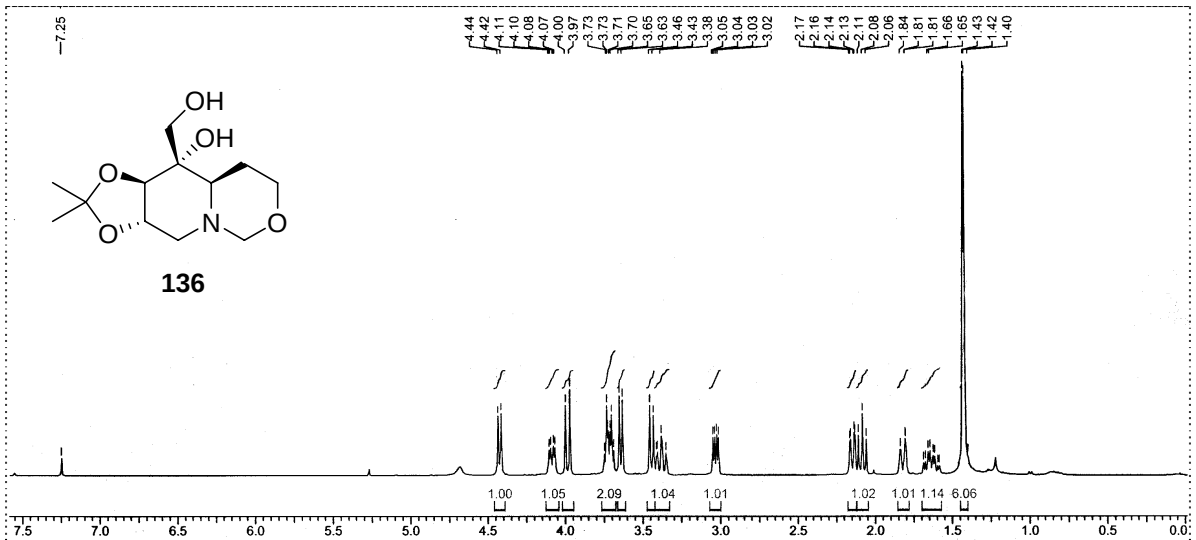
113

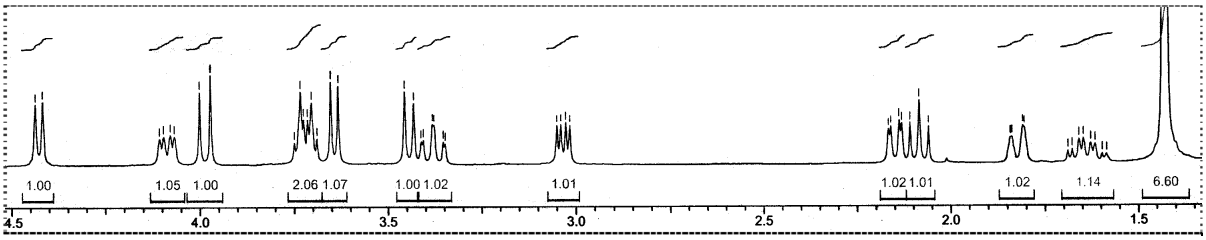
COSY

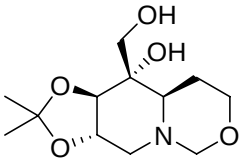


NOESY

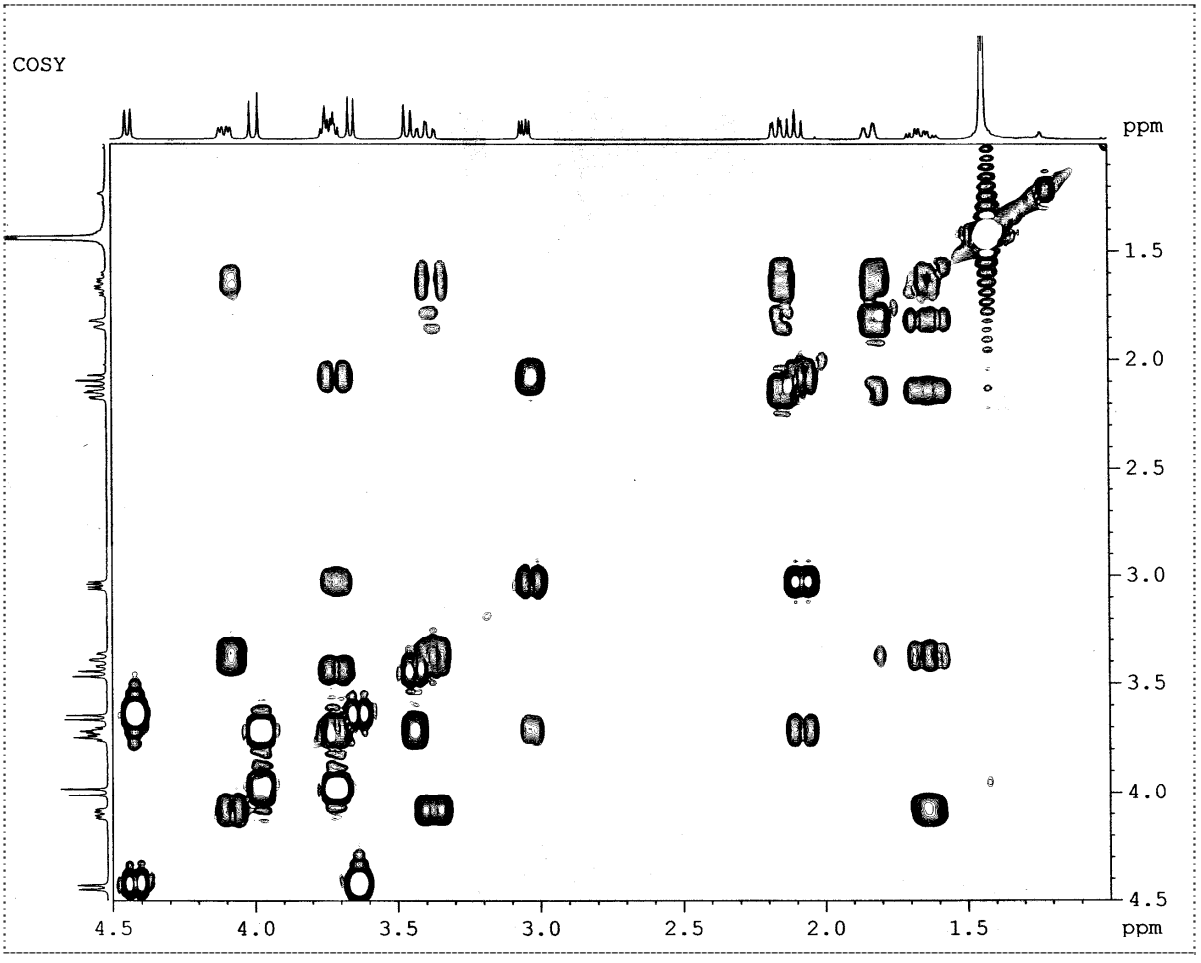

2.5
3.0
3.5
4.0
4.5
ppm
ppm
4.0
3.5
3.0
2.5
O
O
O
N
O
113


7.25
4.44
4.42
4.11
4.10
4.08
4.07
4.00
3.97
3.73
3.71
3.70
3.65
3.63
3.46
3.43
3.38
3.05
3.04
3.03
3.02
2.17
2.16
2.14
2.13
2.11
2.08
2.06
1.84
1.81
1.81
1.66
1.65
1.43
1.42
1.40
OH
OH
O
O
N
O
136
1.00
1.05
2.09
1.04
1.01
1.02
1.01
1.14
6.06
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

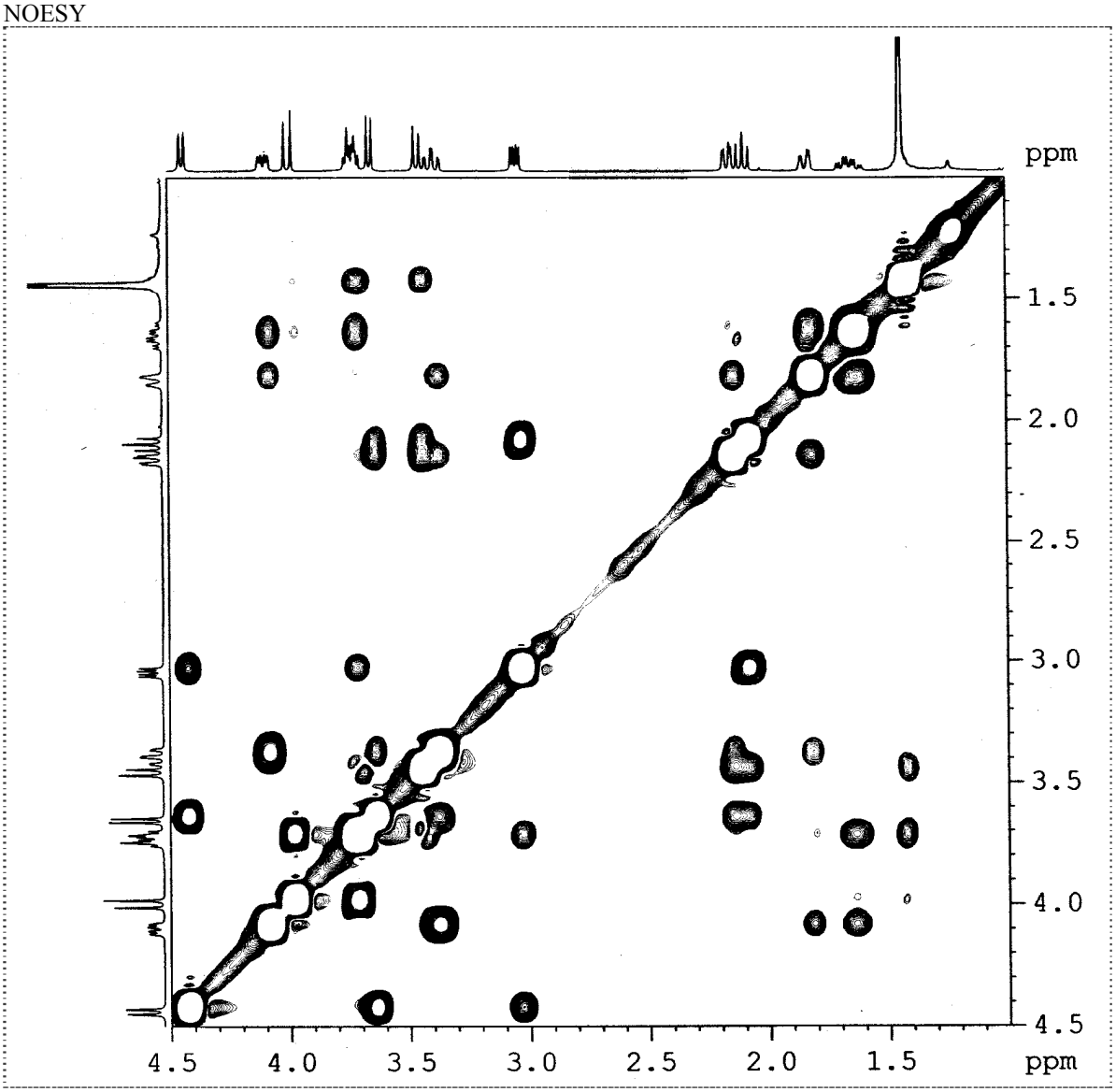

1.00
1.05 1.00
2.06 1.07
1.00 1.02
1.01
1.02 1.01
1.02
1.14
6.60
4.5
4.0
3.5
3.0
2.5
2.0
1.5

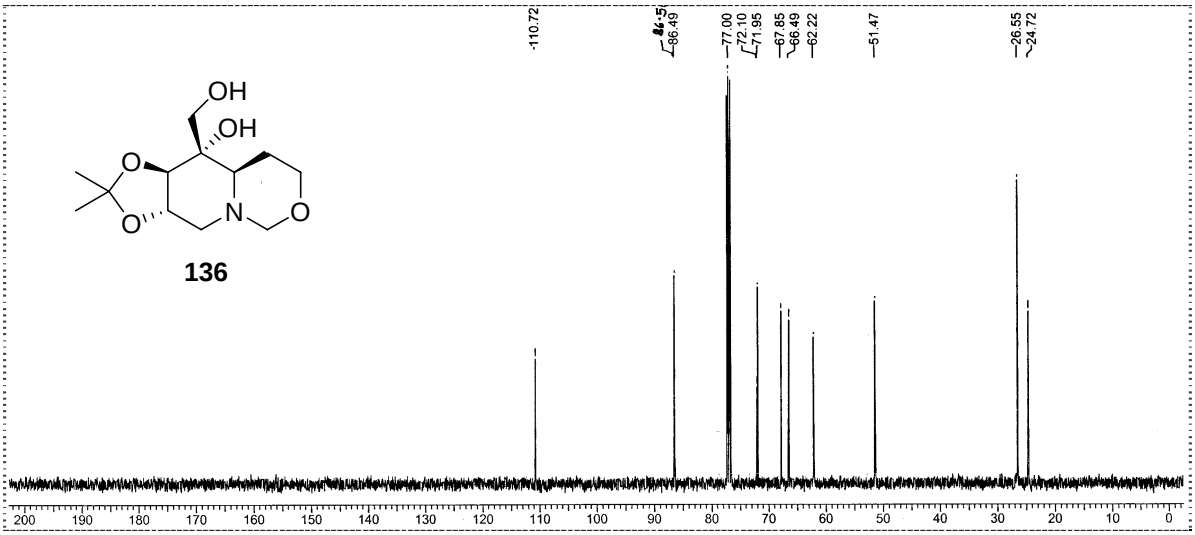

OH
OH
O
O
N
O

136

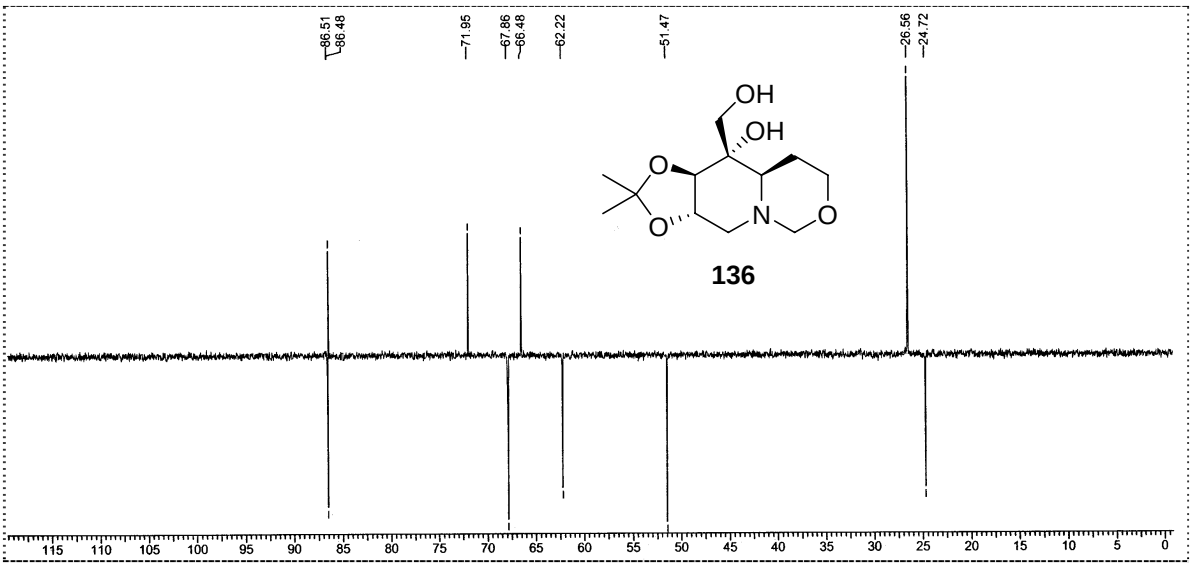

COSY
ppm
1.5
2.0
2.5
3.0
3.5
4.0
4.5
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm

NOESY

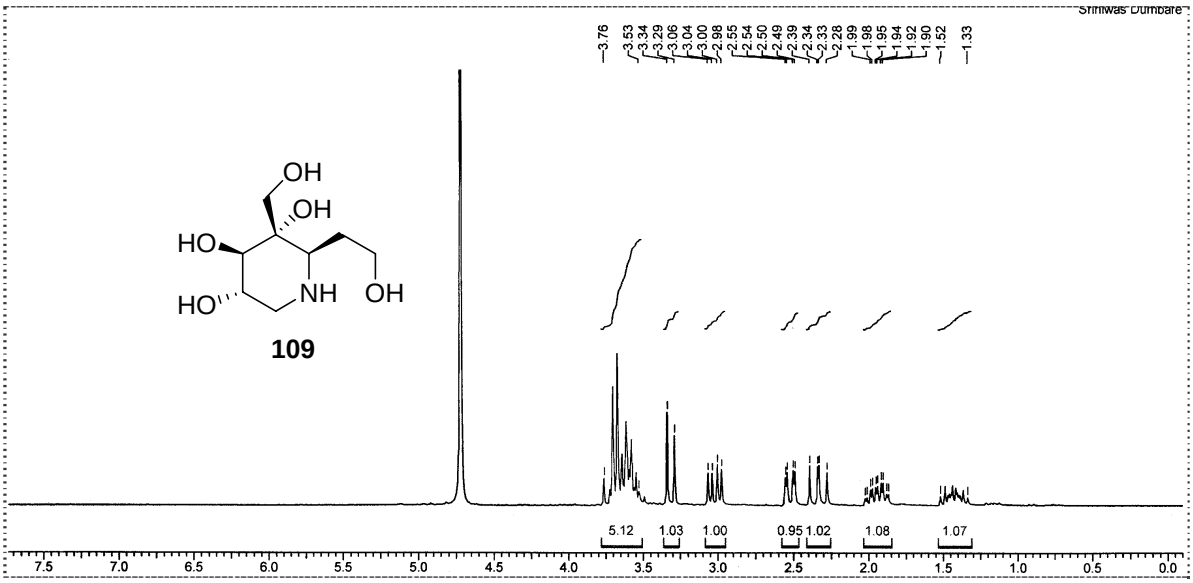

ppm
1.5
2.0
2.5
3.0
3.5
4.0
4.5
4.5 4.0 3.5 3.0 2.5 2.0 1.5
ppm


110.72
86.49
77.00
72.10
71.95
67.85
66.49
62.22
51.47
26.55
24.72
OH
OH
O
O
N
O
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

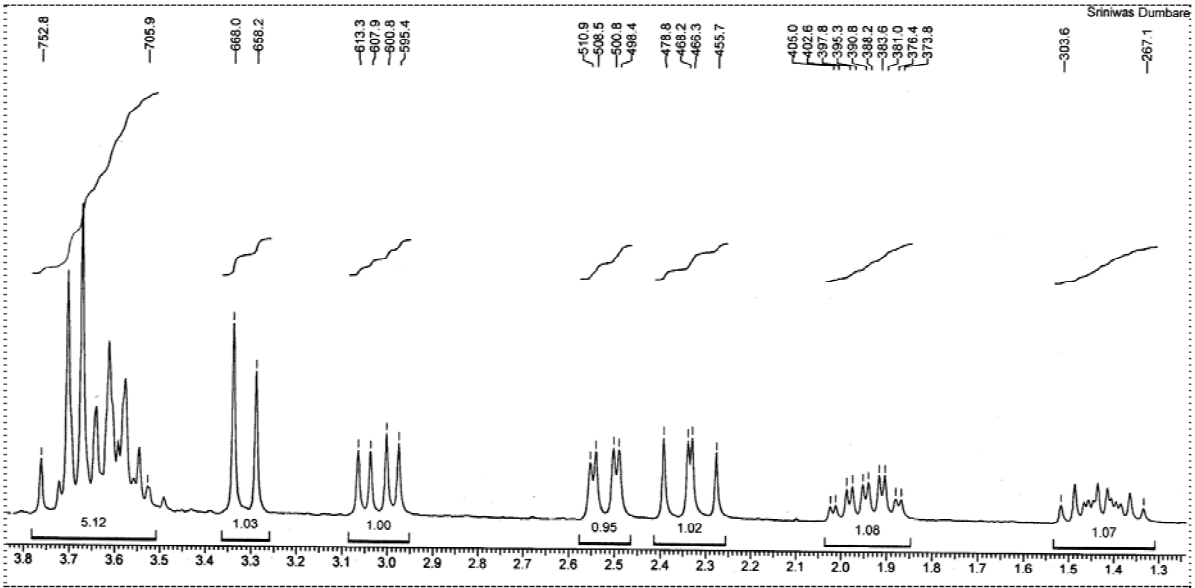
136

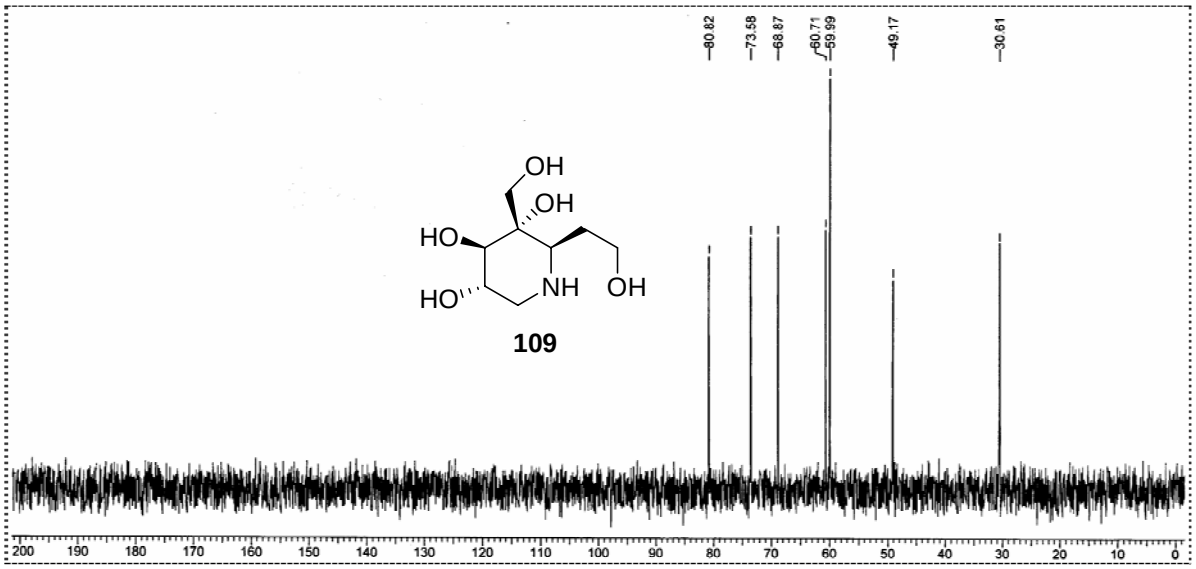

86.51
86.48
71.95
67.86
66.48
62.22
51.47
26.56
24.72
OH
OH
O
O
N
O

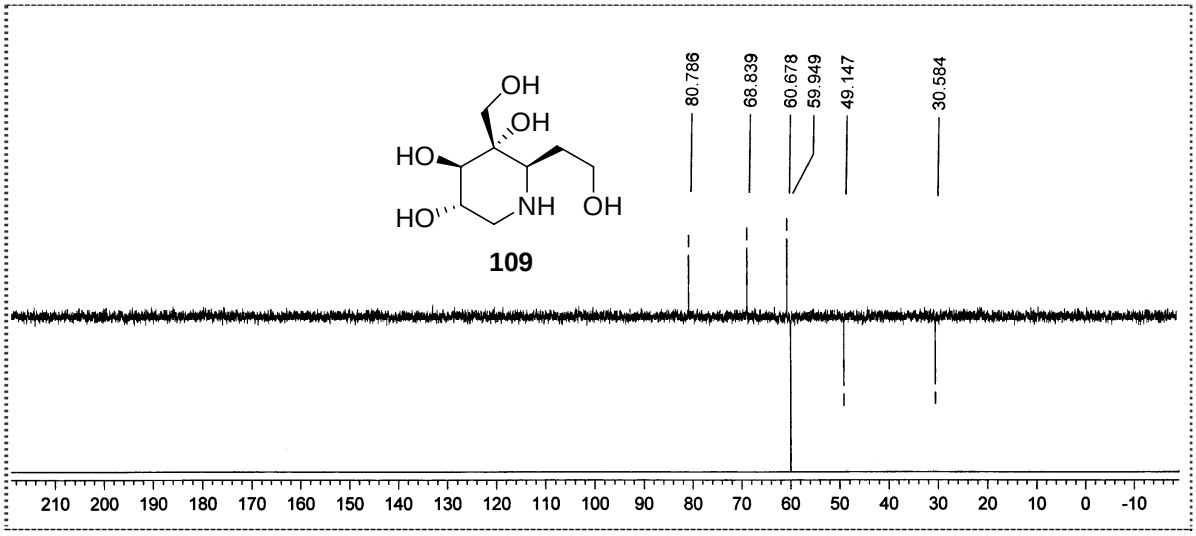
136

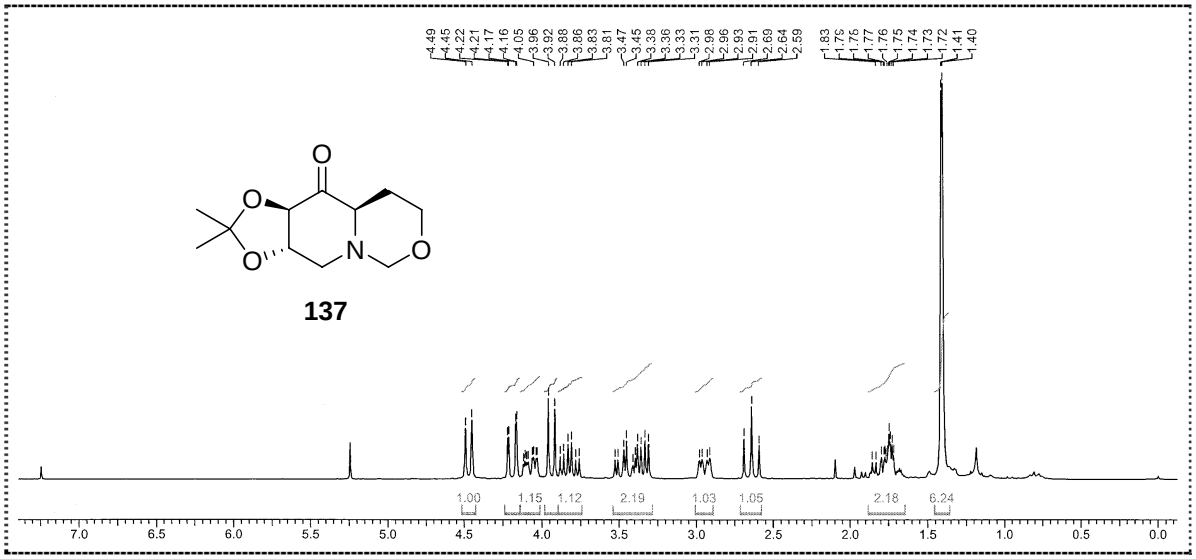

Srinivas Dumbare
3.76
3.53
3.34
3.29
3.06
3.04
3.00
2.98
2.55
2.54
2.50
2.49
2.39
2.34
2.33
2.28
1.99
1.98
1.95
1.94
1.92
1.90
1.52
1.33
OH
OH
HO
HO
NH
OH
5.12
1.03
1.00
0.95
1.02
1.08
1.07

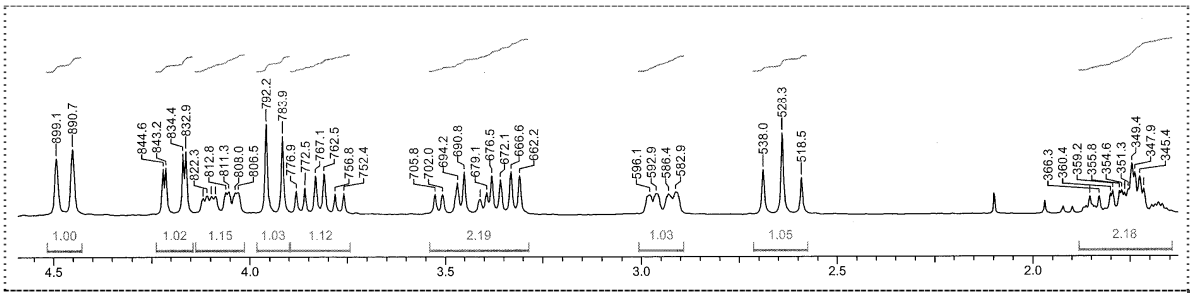
109

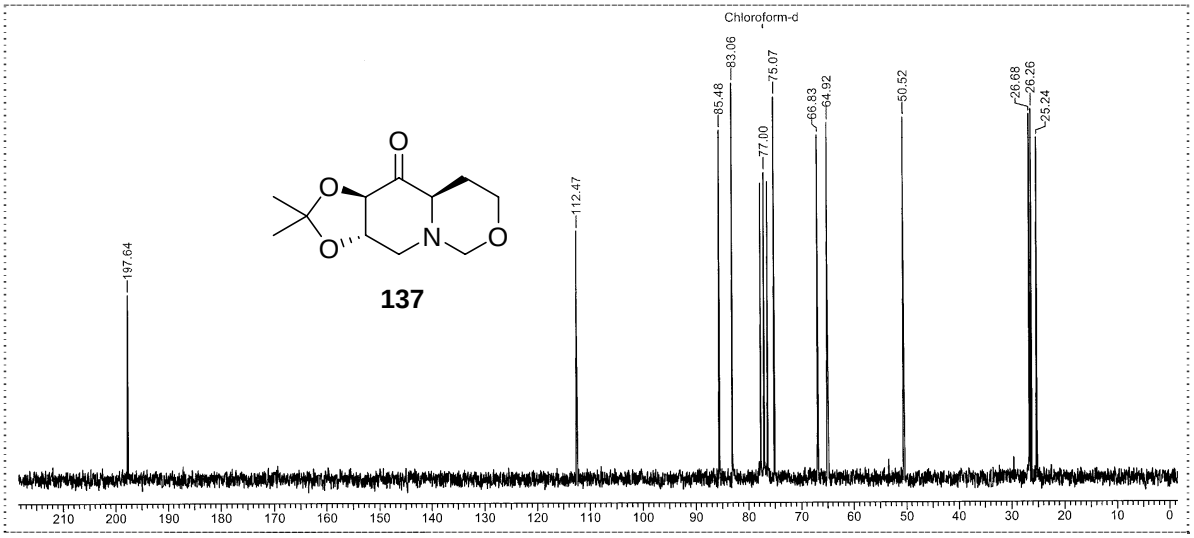

Srinivas Dumbare
752.8
705.9
668.0
658.2
613.3
607.9
600.8
595.4
510.9
508.5
500.8
498.4
478.8
468.2
466.3
455.7
405.0
402.6
397.8
395.3
390.8
388.2
383.6
381.0
376.4
373.8
303.6
267.1
5.12
1.03
1.00
0.95
1.02
1.08
1.07

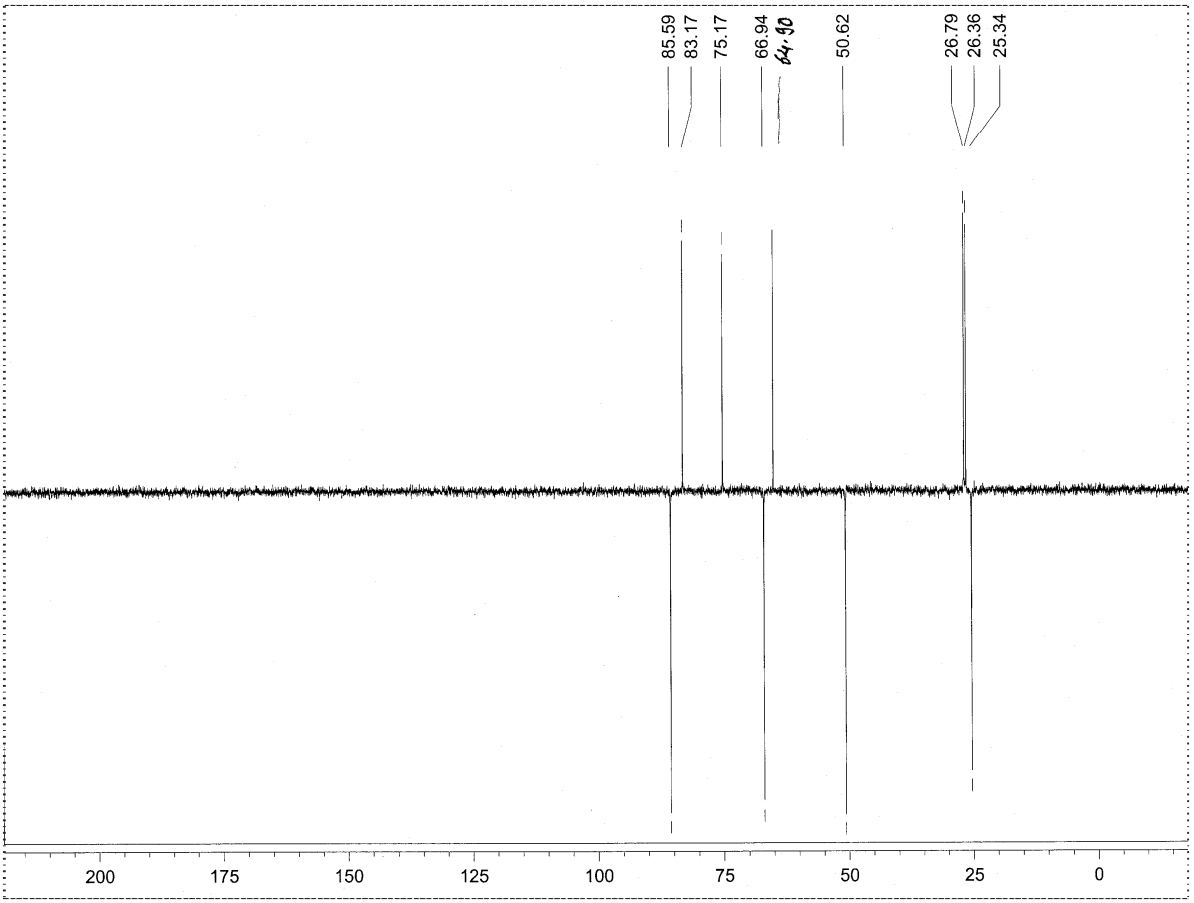

80.82
73.58
68.87
60.71
59.99
49.17
30.61
OH
OH
HO
HO
NH
OH
109

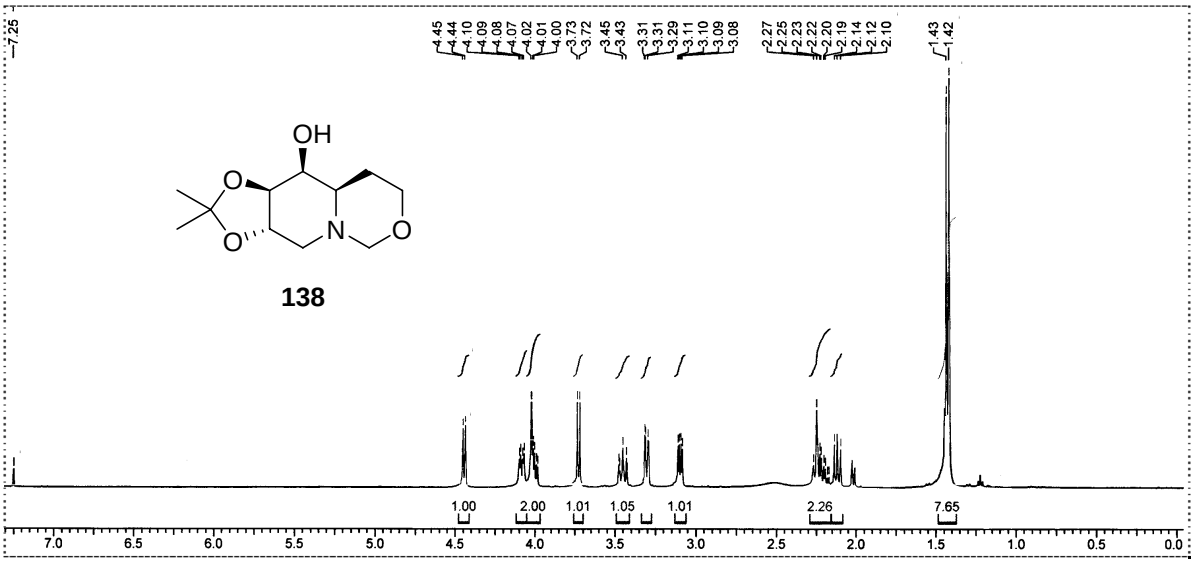

80.786
68.839
60.678
59.949
49.147
30.584
OH
OH
HO
HO
NH
OH
109


O
O
O
N
O
137
1.00
1.15
1.12
2.19
1.03
1.05
2.18
6.24

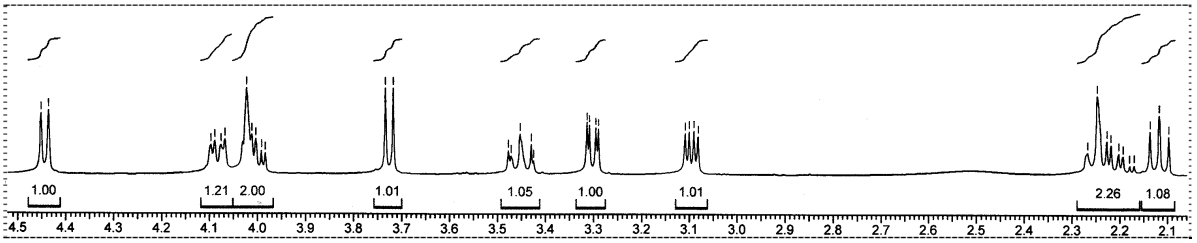

899.1
890.7
844.6
843.2
834.4
832.9
822.3
812.8
811.3
808.0
806.5
792.2
783.9
776.9
772.5
767.1
762.5
756.8
752.4
705.8
702.0
694.2
690.8
679.1
676.5
672.1
666.6
662.2
596.1
592.9
586.4
582.9
538.0
523.3
518.5
366.3
360.4
359.2
355.8
354.6
351.3
349.4
347.9
345.4
1.00
1.02
1.15
1.03
1.12
2.19
1.03
1.05
2.18
4.5
4.0
3.5
3.0
2.5
2.0

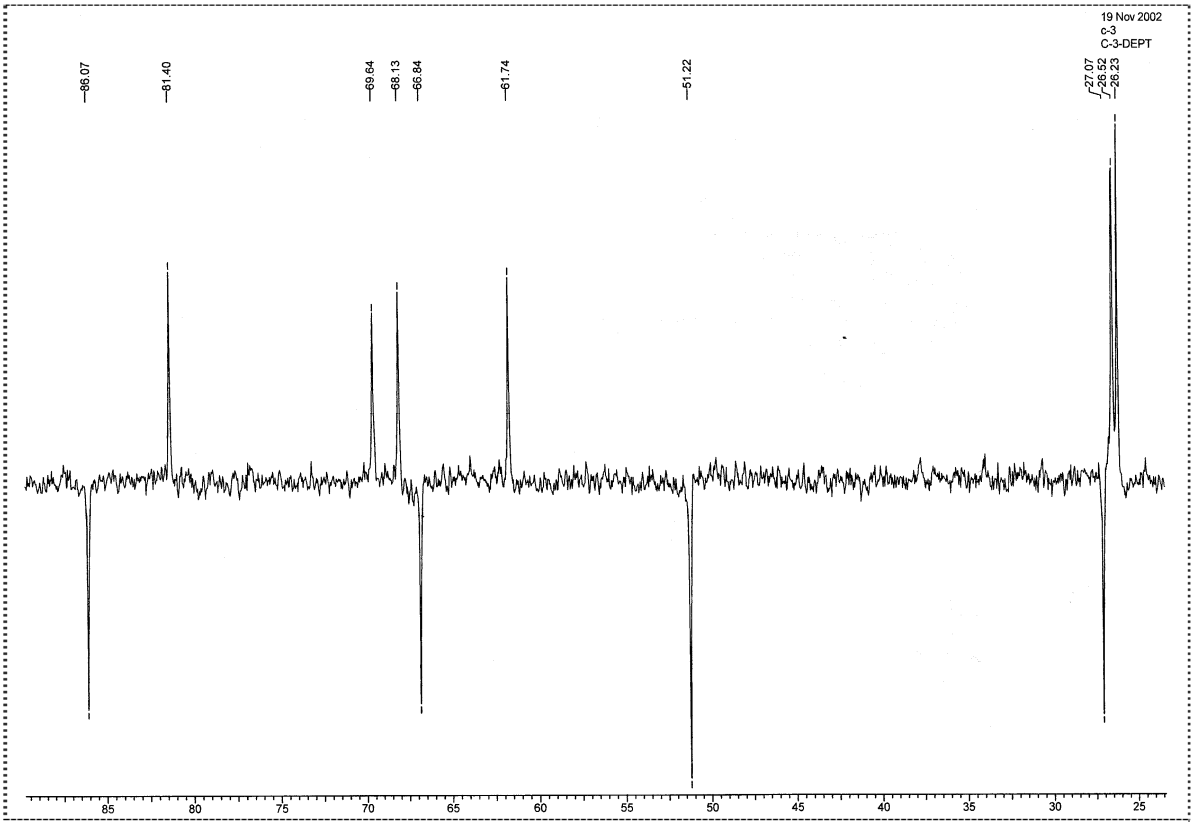

Chloroform-d
197.64
112.47
85.48
83.06
77.00
75.07
66.83
64.92
50.52
26.68
26.26
25.24
O
O
O
N
O
137
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

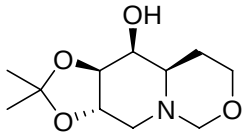

85.59
83.17
75.17
66.94
64.90
50.62
26.79
26.36
25.34
200
175
150
125
100
75
50
25
0


7.25
4.45
4.44
4.10
4.09
4.08
4.07
4.02
4.01
4.00
3.73
3.72
3.45
3.43
3.31
3.31
3.29
3.11
3.10
3.09
3.08
2.27
2.25
2.23
2.22
2.20
2.19
2.14
2.12
2.10
1.43
1.42
OH
O
O
N
O
1.00
2.00
1.01
1.05
1.01
2.26
7.65

138

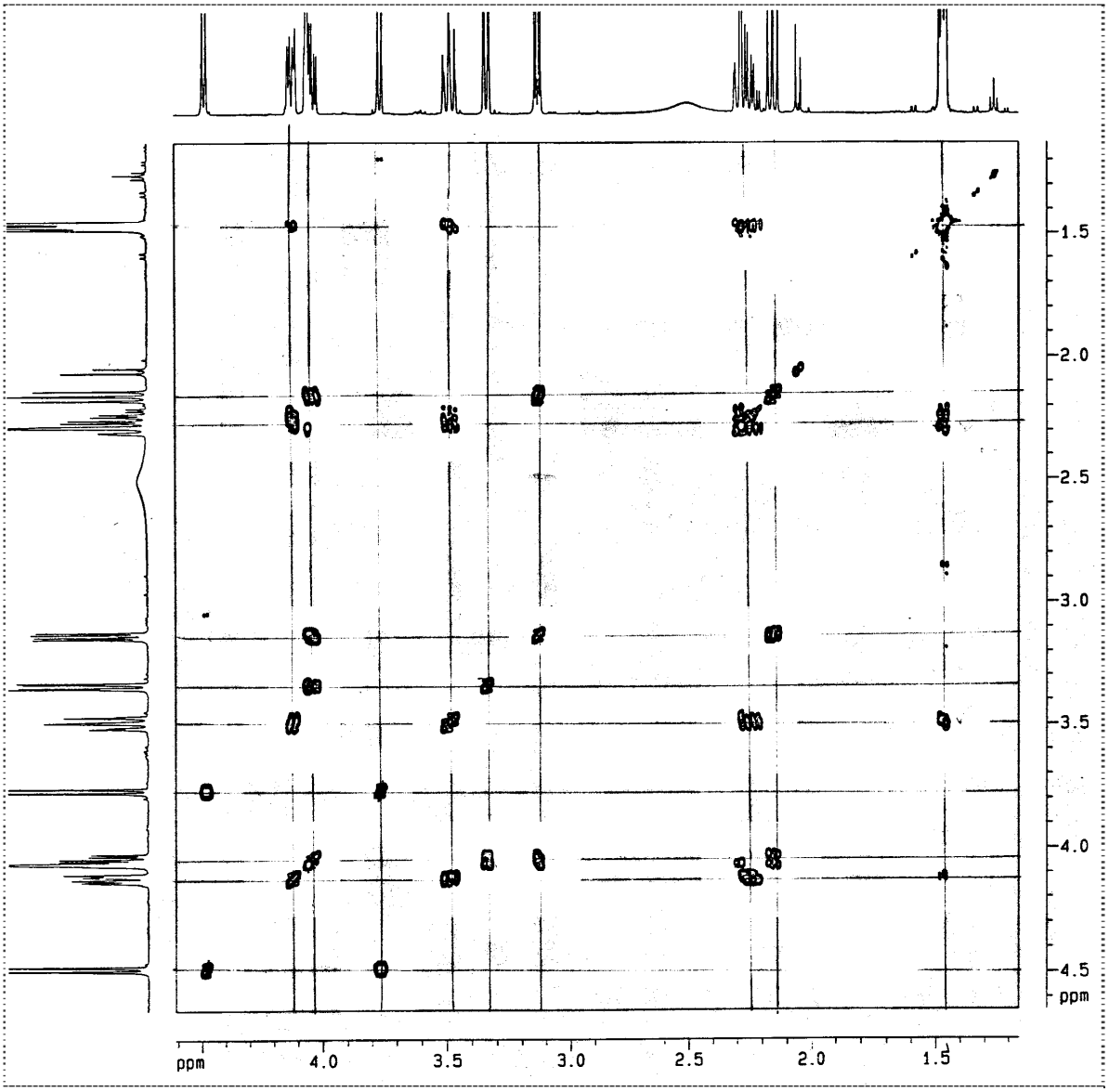

1.00
1.21
2.00
1.01
1.05
1.00
1.01
2.26
1.08

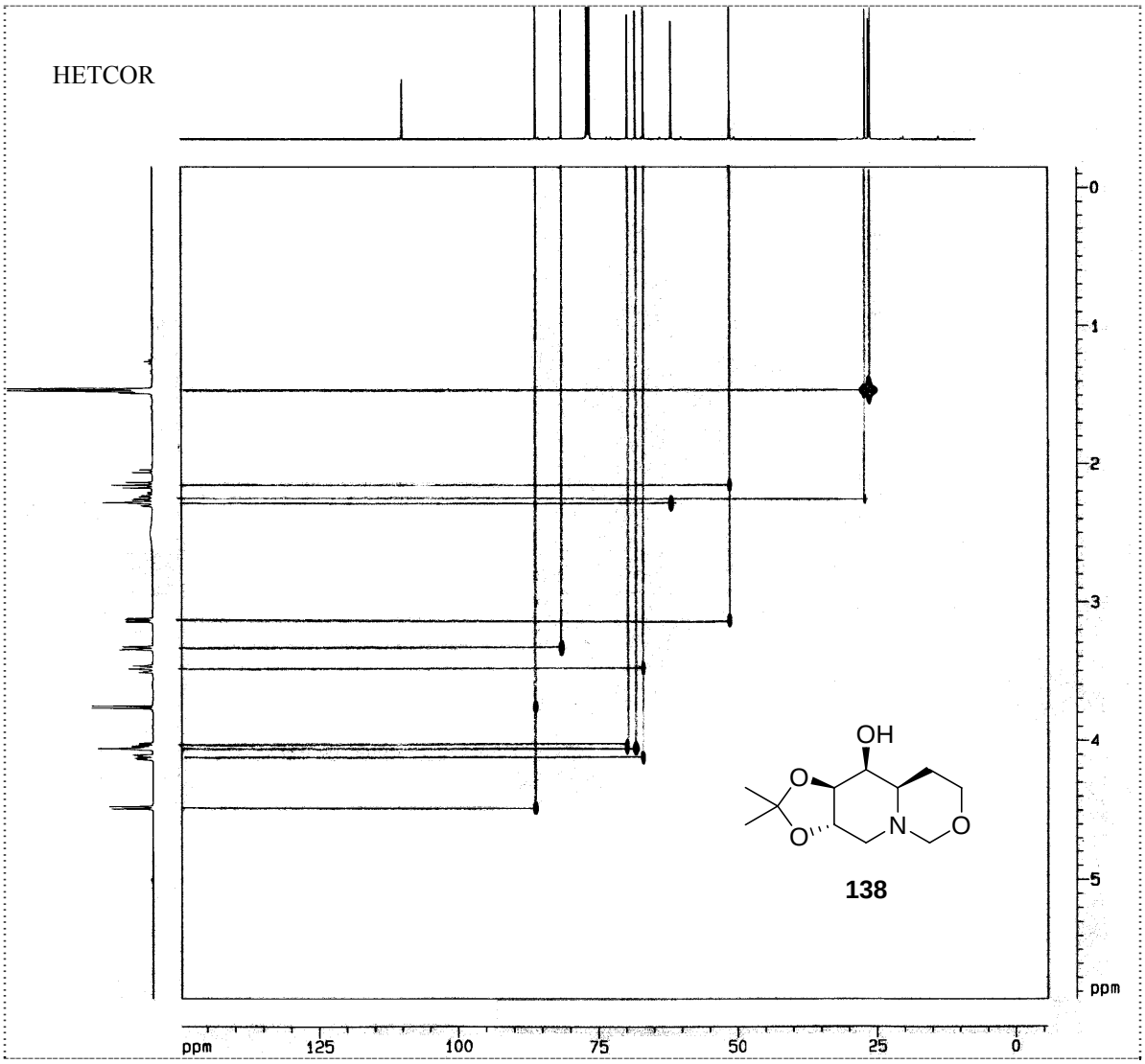

19 Nov 2002
C-3
C-3-DEPT
86.07
61.40
69.64
68.13
66.84
61.74
51.22
27.07
26.52
26.23

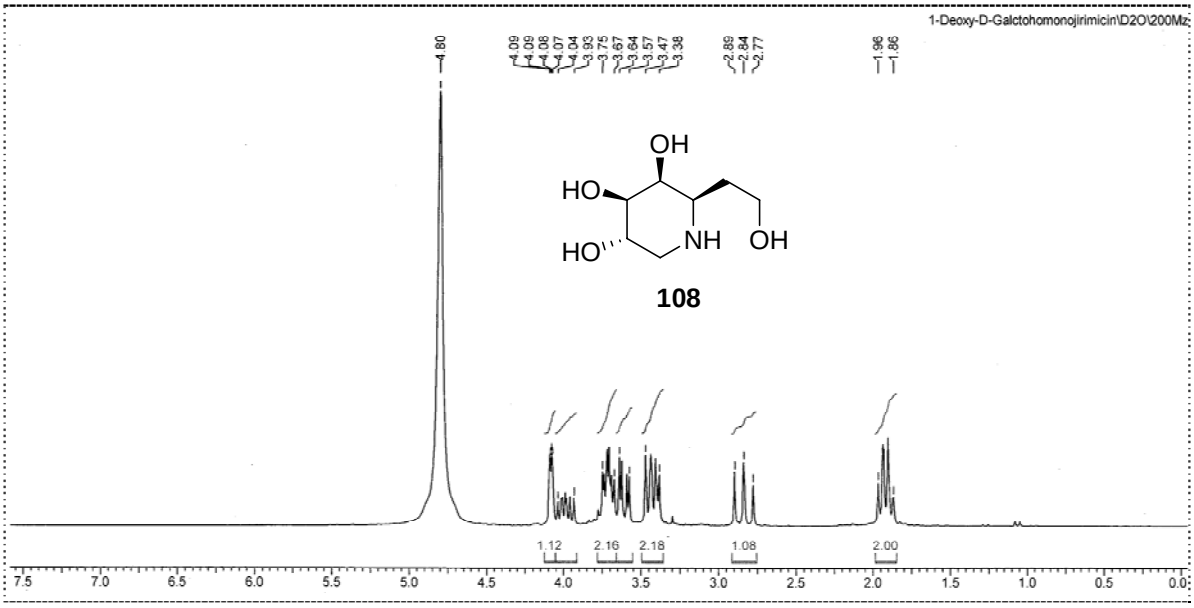


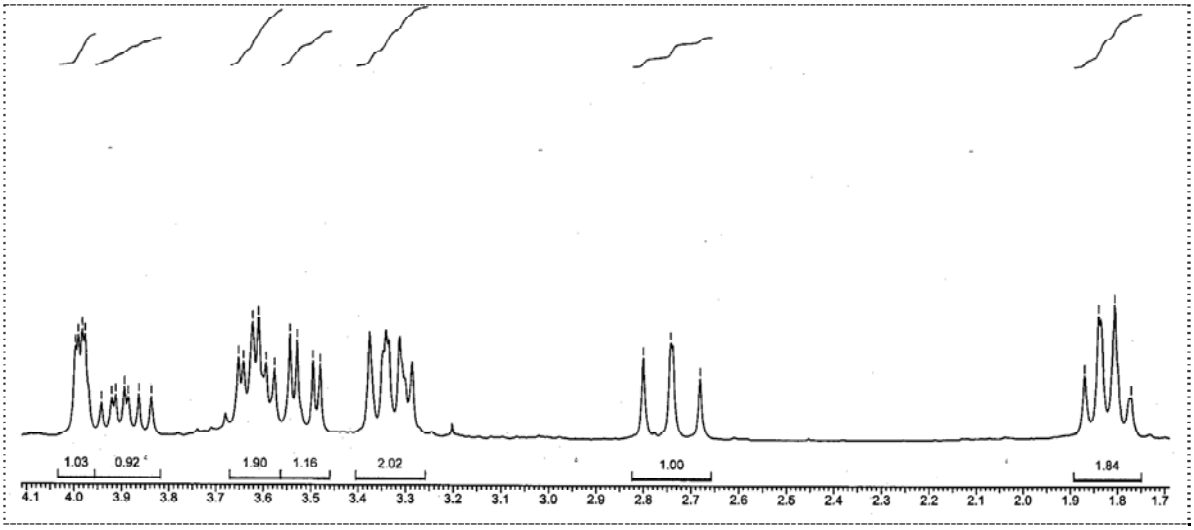
138

COSY

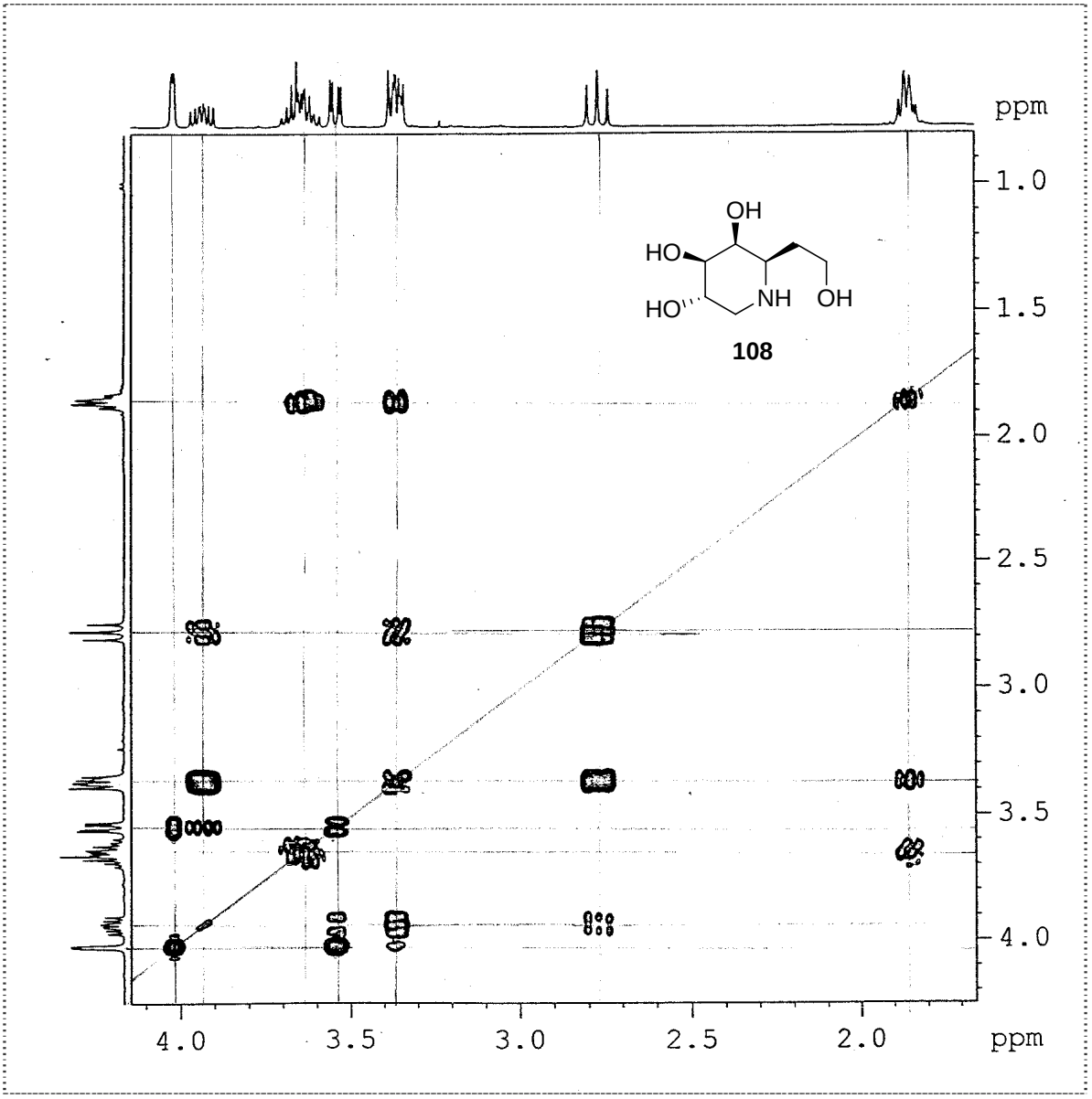


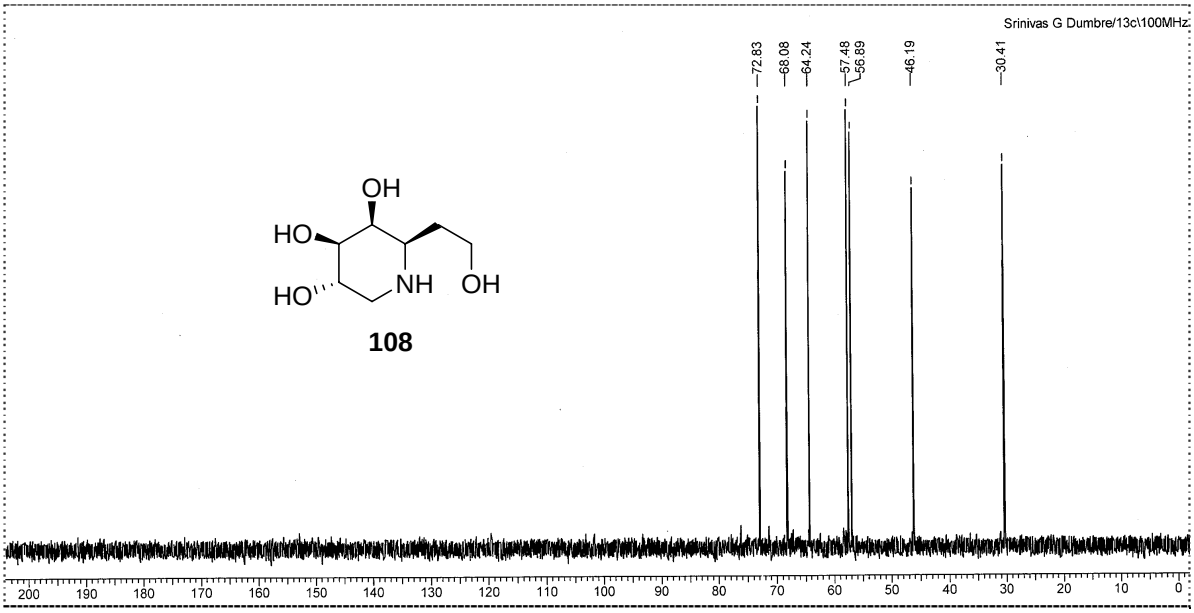

HETCOR
OH
O
O
N
O
138
ppm
125
100
75
50
25
0
0
1
2
3
4
5
ppm


1-Deoxy-D-Galctohomonojirimicin\D2O\200Mz
OH
HO
HO
NH
OH
108
1.12
2.16
2.18
1.08
2.00
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


1.03
0.92
1.90
1.16
2.02
1.00
1.84
4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7

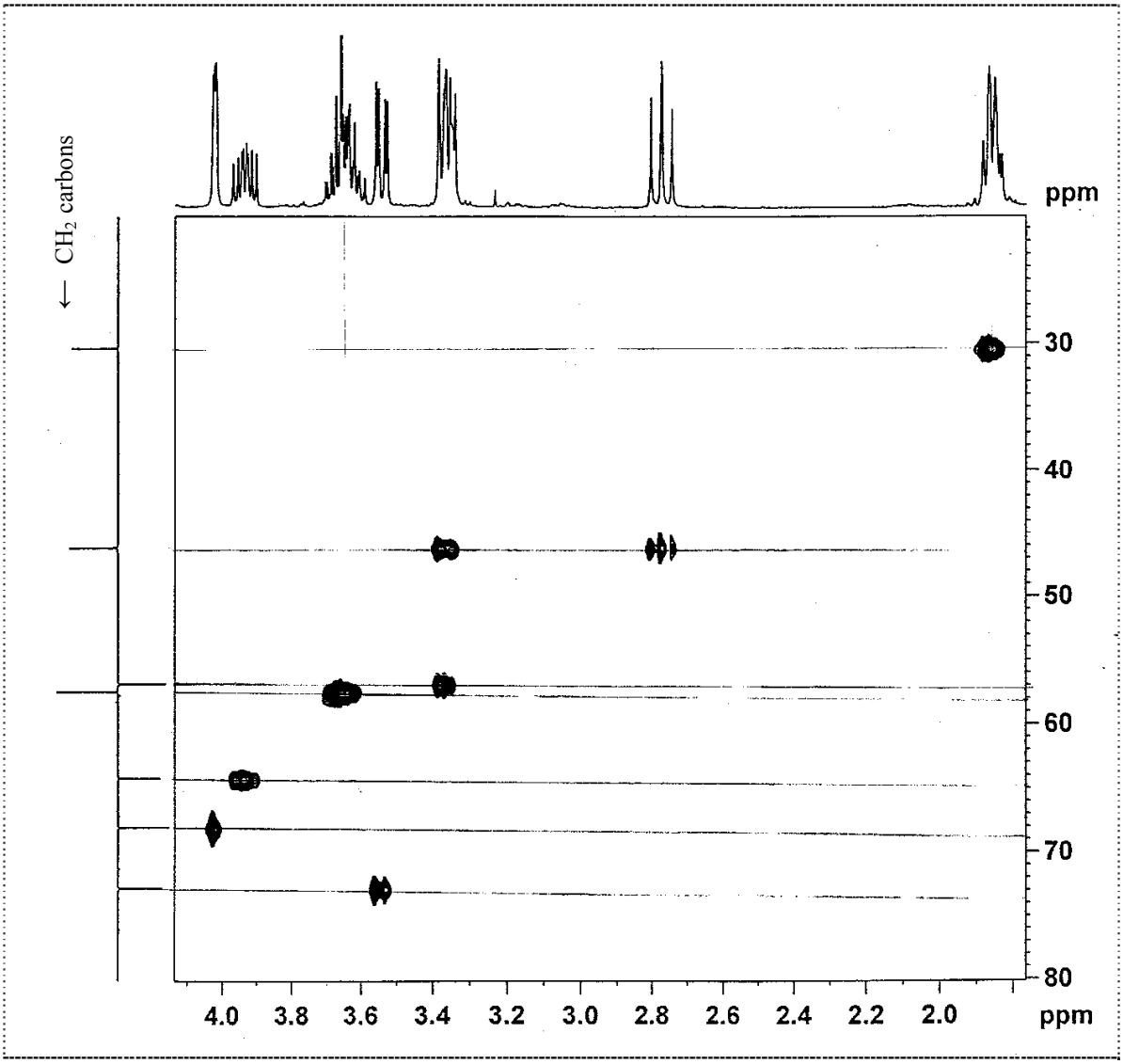
COSY

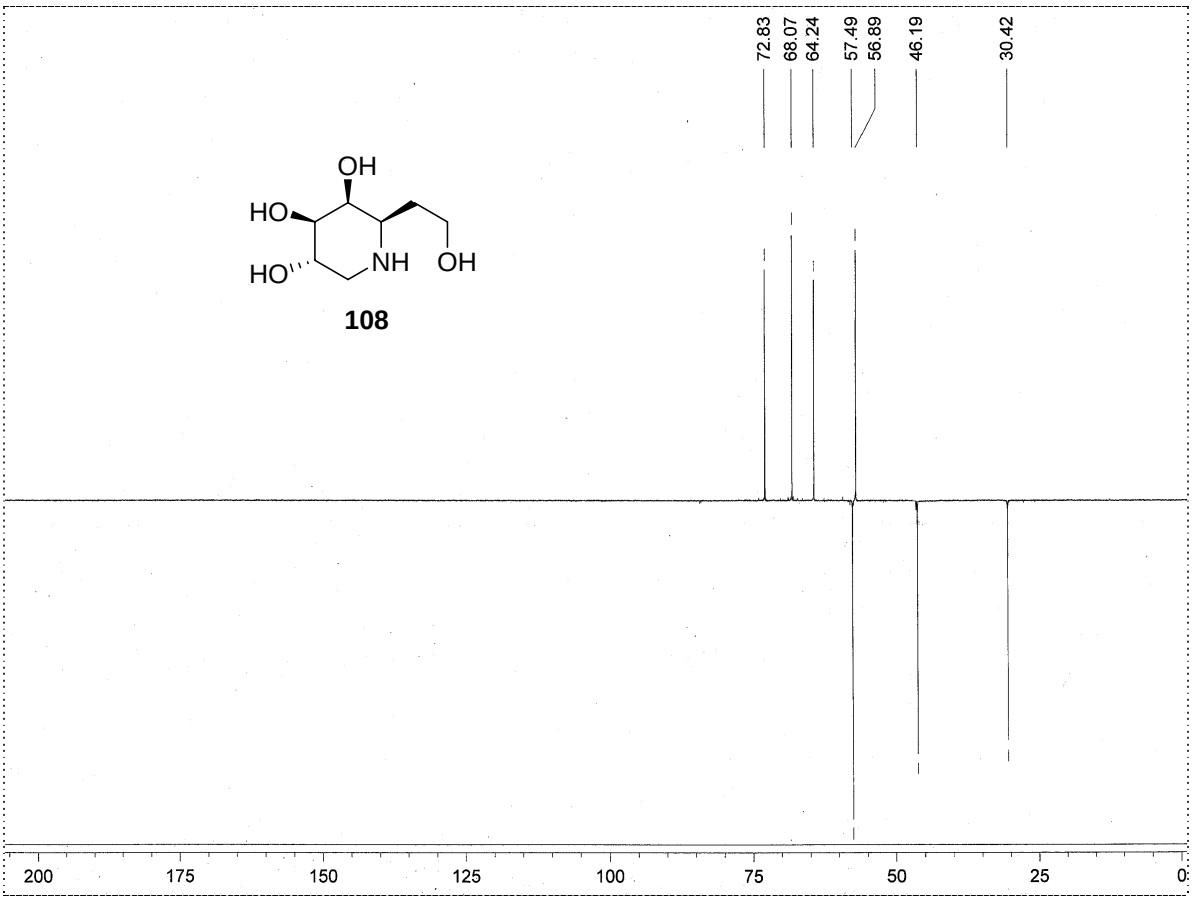

ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.0
3.5
3.0
2.5
2.0
ppm
OH
HO
HO
NH
OH
108


Srinivas G Dumbre/13c\100MHz
72.83
68.08
64.24
57.48
56.89
46.19
30.41
OH
HO
HO
NH
OH
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

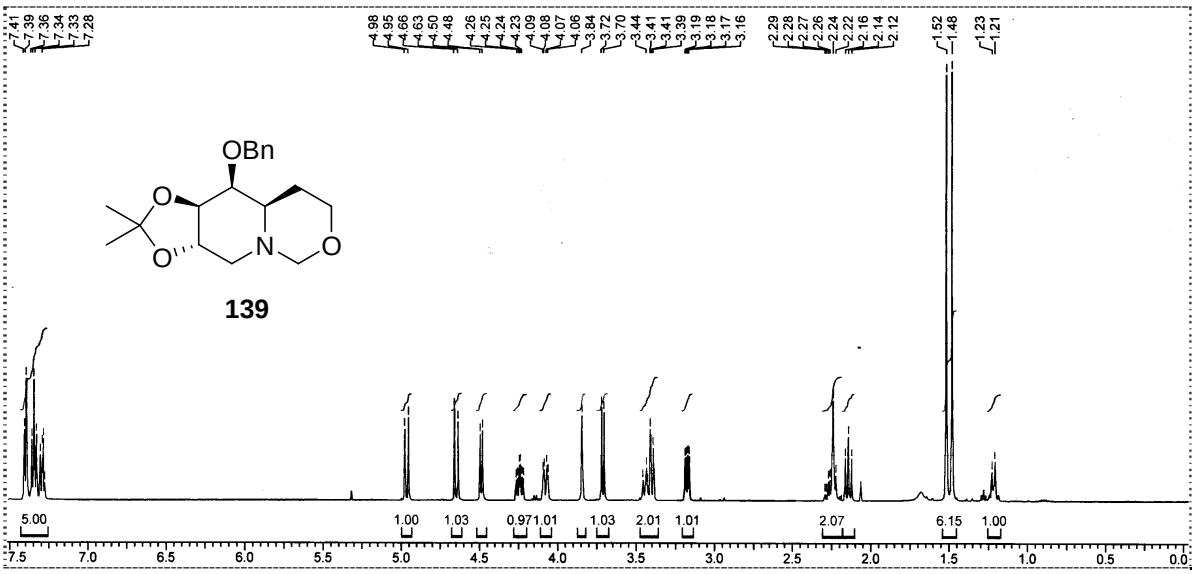
108

HETCOR

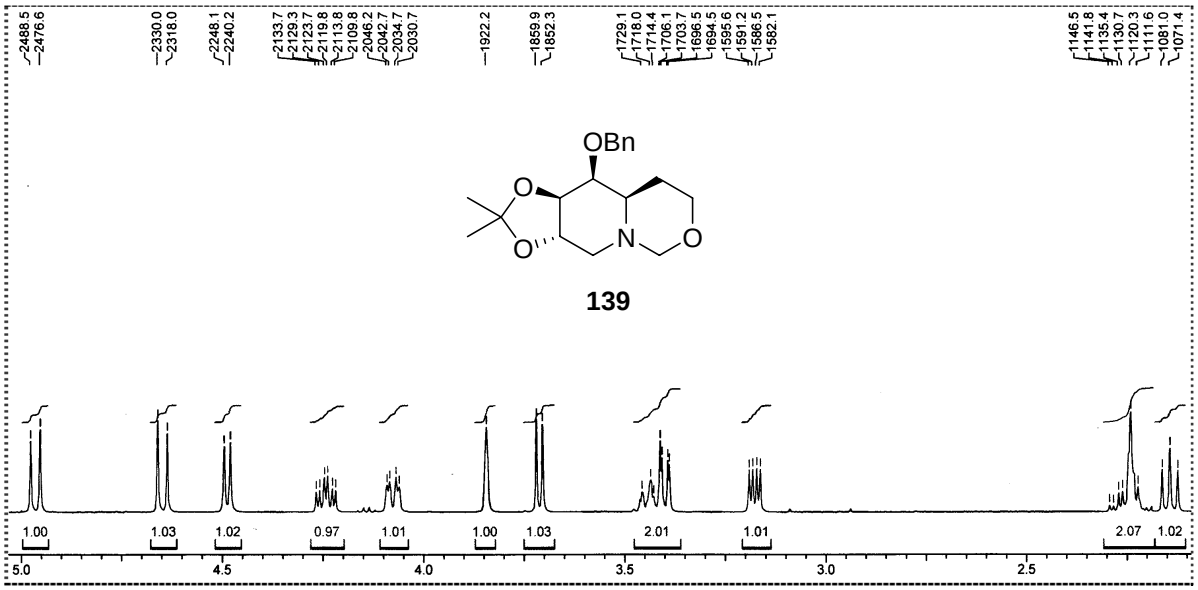

← CH2 carbons
ppm
30
40
50
60
70
80
4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0
ppm


72.83
68.07
64.24
57.49
56.89
46.19
30.42
OH
HO
HO
NH
OH
200
175
150
125
100
75
50
25
0

108


OBn
O
O
N
O
5.00
1.00
1.03
0.97
1.01
1.03
2.01
1.01
2.07
6.15
1.00

139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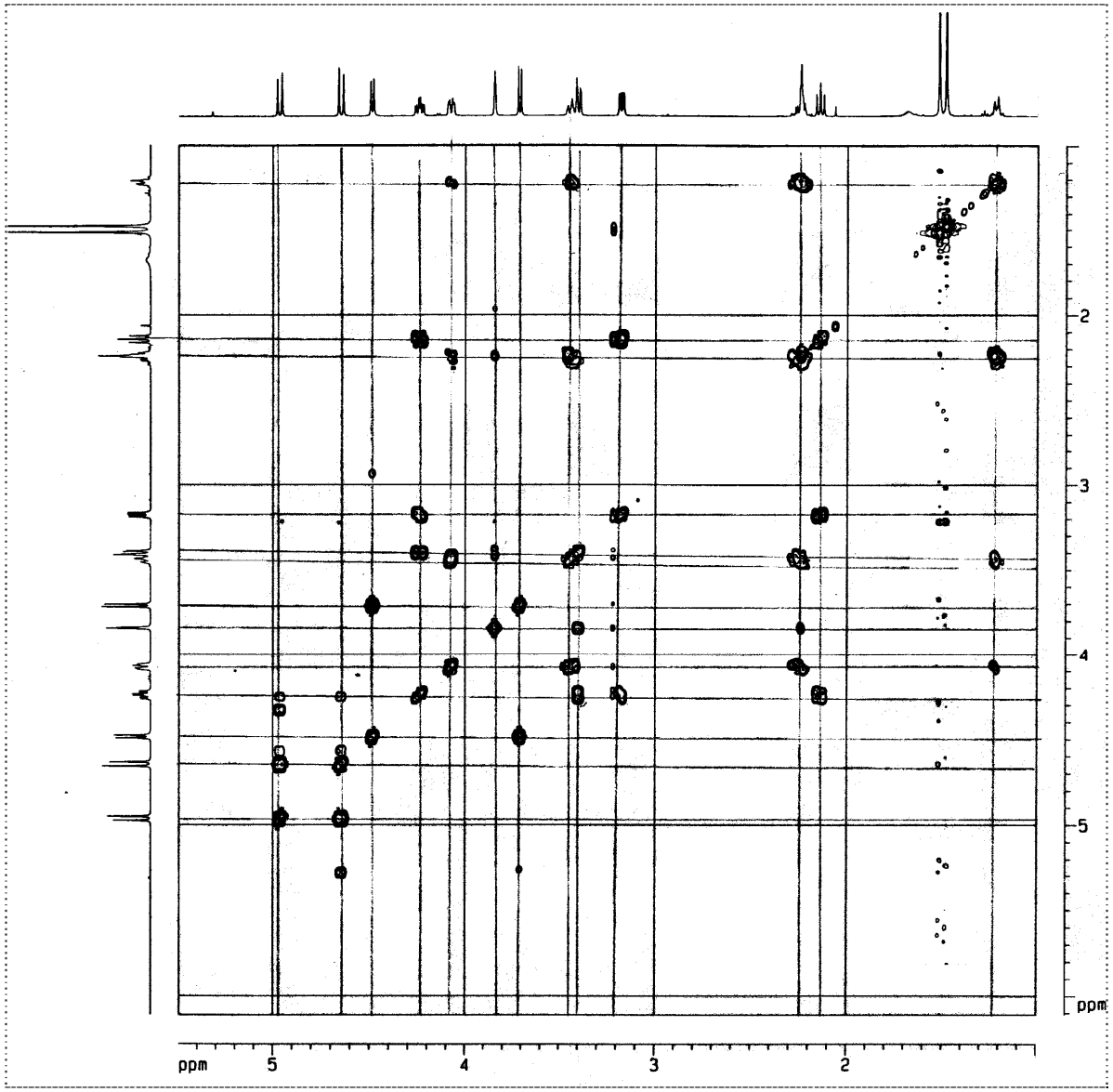
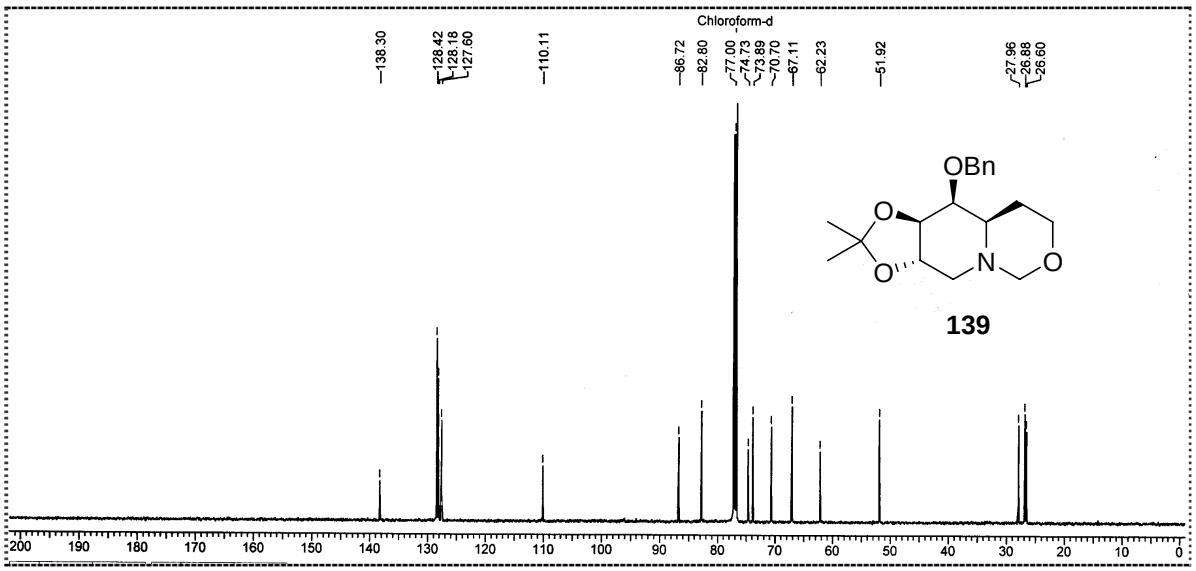
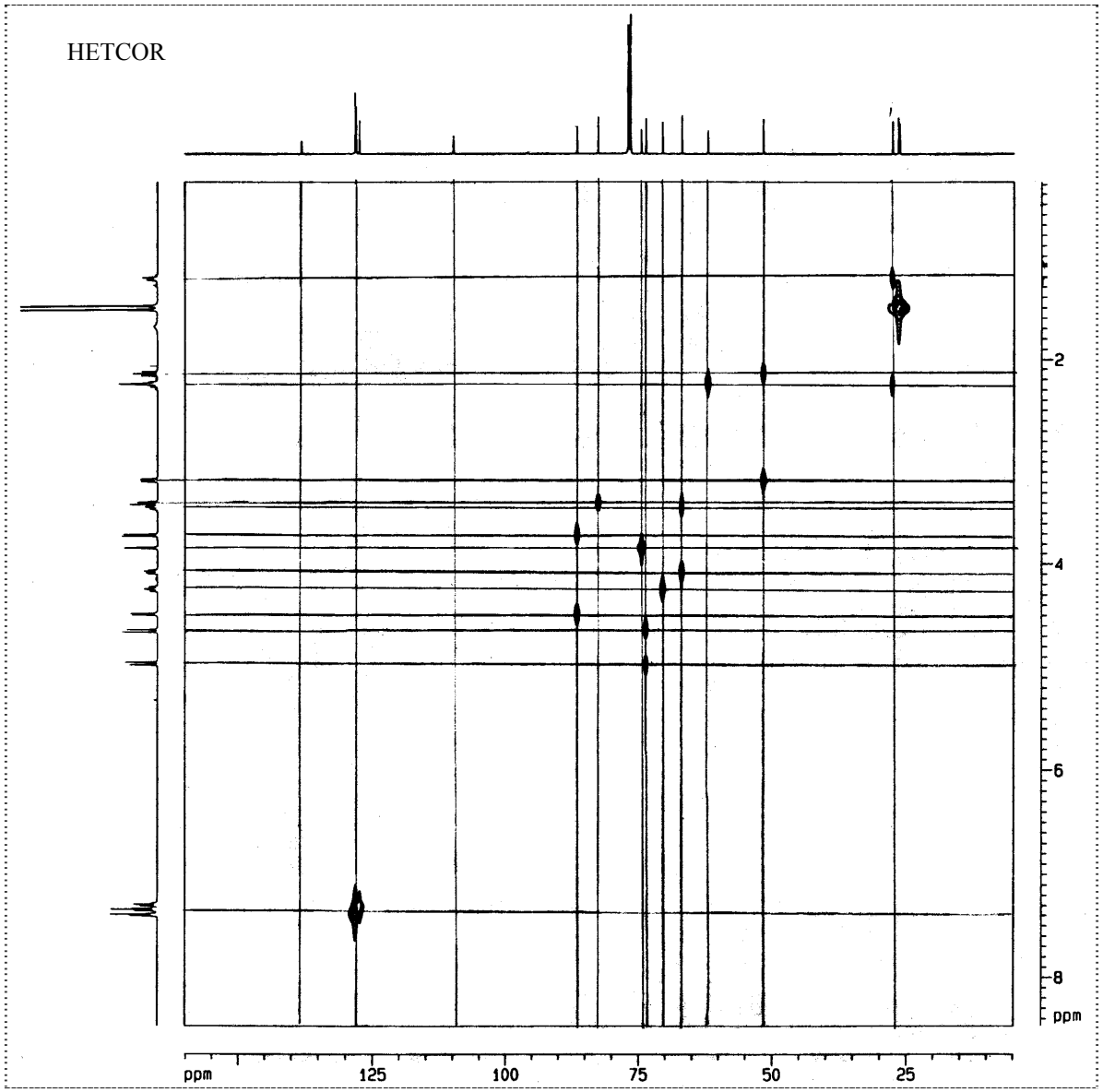
2476.6
2330.0
2318.0
2248.1
2240.2
2133.7
2129.3
2123.7
2119.8
2113.8
2109.8
2046.2
2042.7
2034.7
2030.7
1922.2
1859.9
1852.3
1729.1
1718.0
1714.4
1706.1
1703.7
1696.5
1694.5
1595.6
1591.2
1586.5
1582.1
1146.5
1141.8
1135.4
1130.7
1120.3
1111.6
1081.0
1071.4
OBn
O
O
O
N
O
1.00
1.03
1.02
0.97
1.01
1.00
1.03
2.01
1.01
2.07
1.02
5.0
4.5
4.0
3.5
3.0
2.5

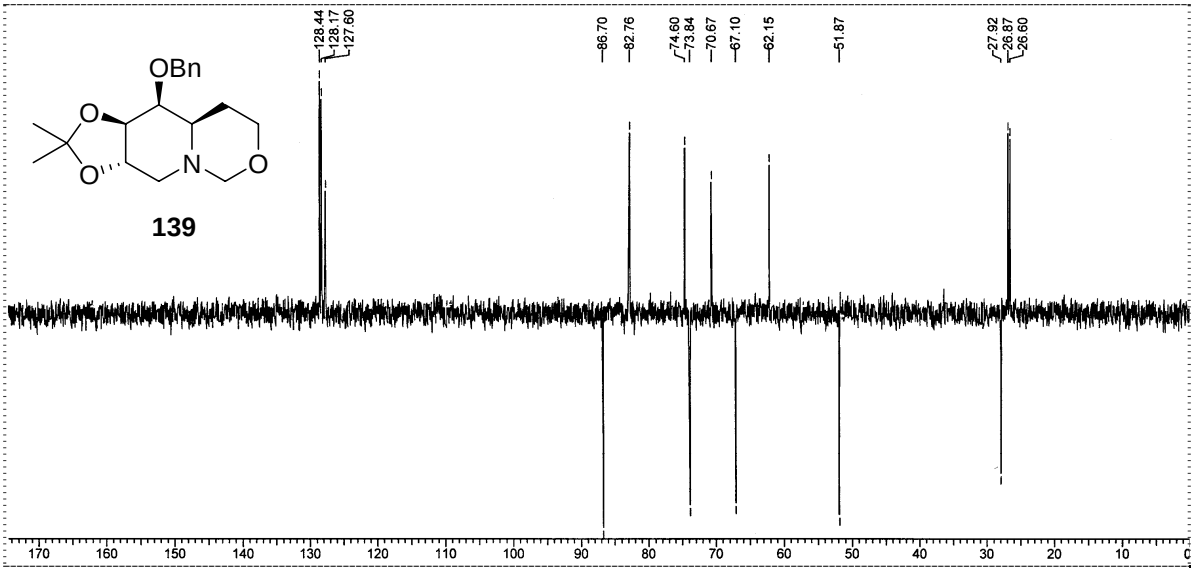
139

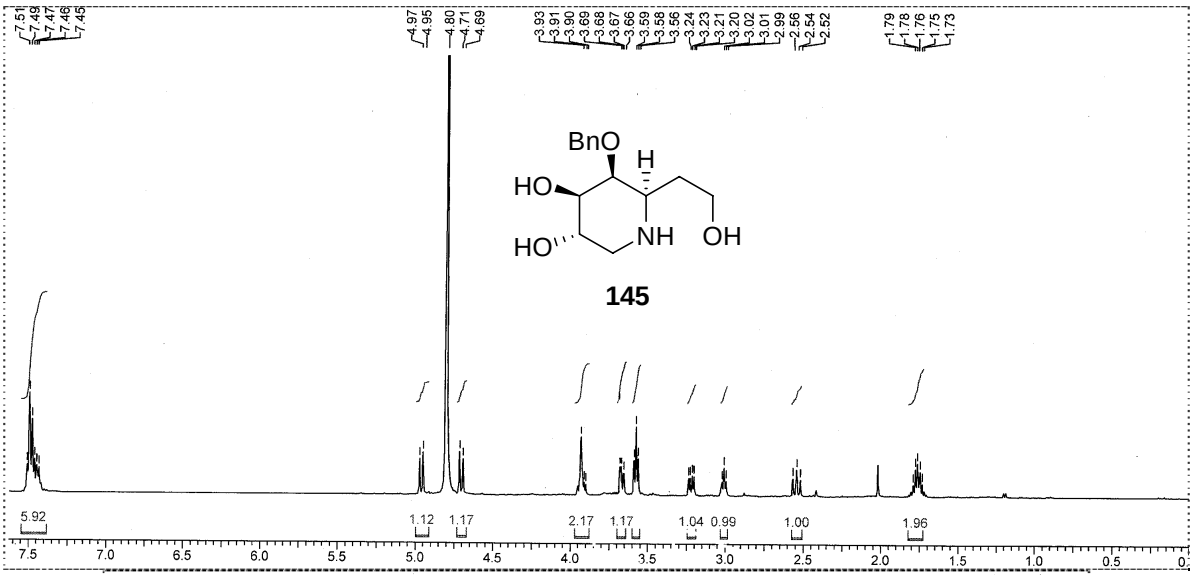
COSY

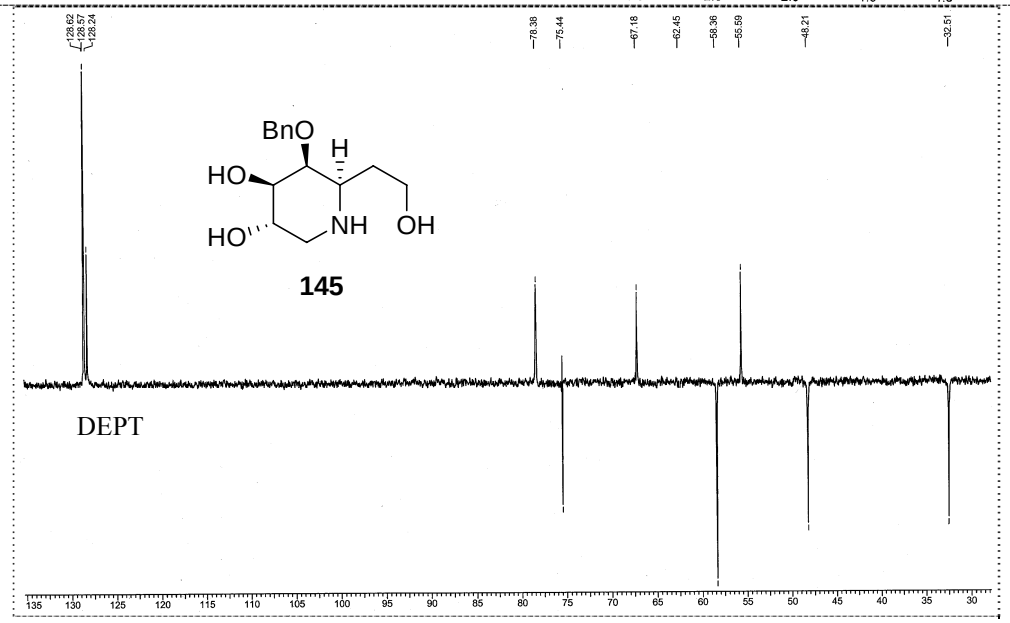

2
3
4
5
ppm
ppm
5
4
3
2

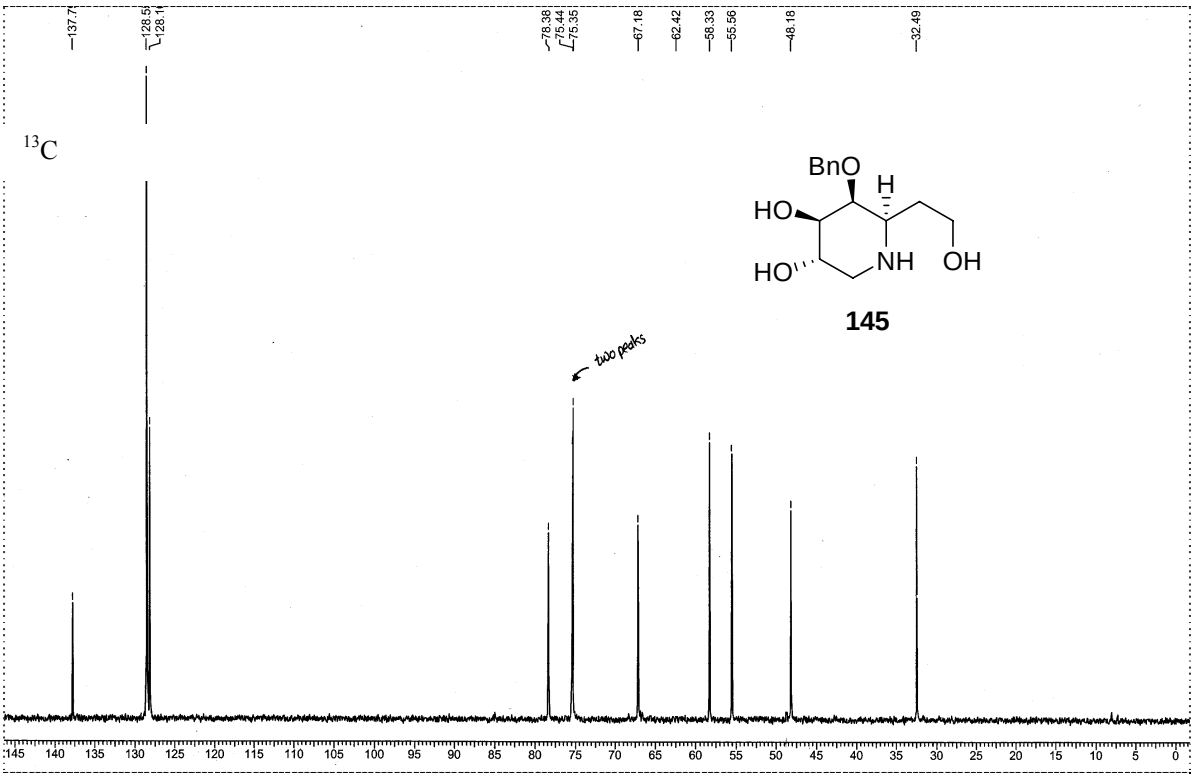

Chloroform-d
138.30
128.42
128.18
127.60
110.11
86.72
82.80
77.00
74.73
73.89
70.70
67.11
62.23
51.92
27.96
26.88
26.60
OBn
O
O
N
O
139
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

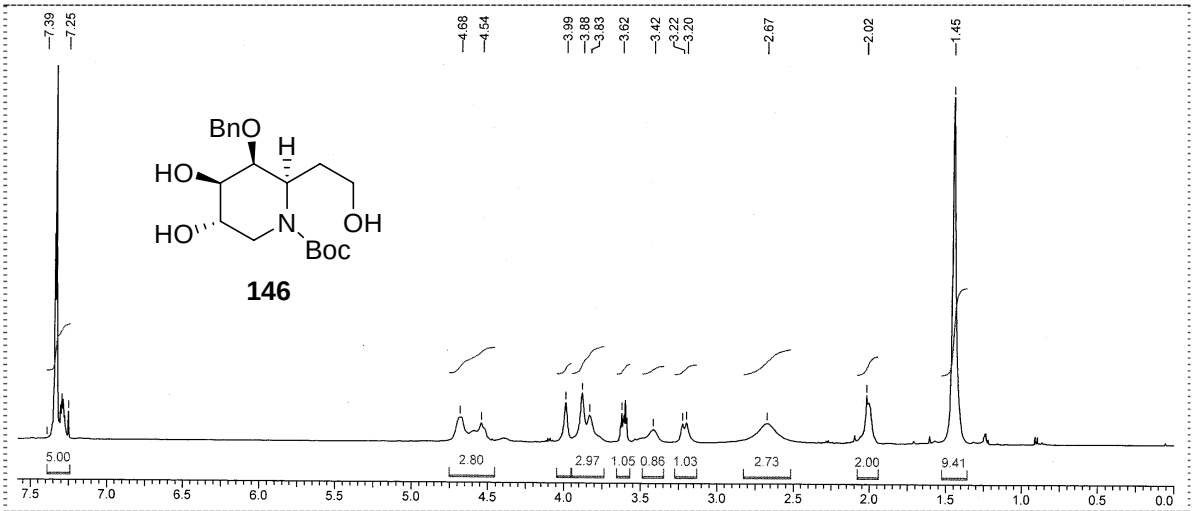

HETCOR
2
4
6
8
ppm
ppm
125
100
75
50
25

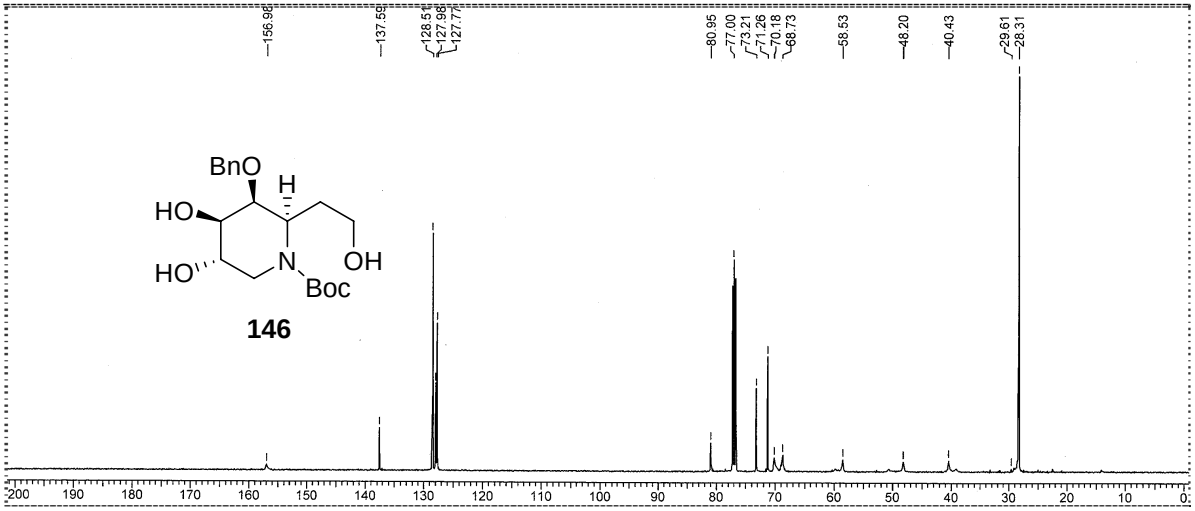

OBn
O
O
N
O
139

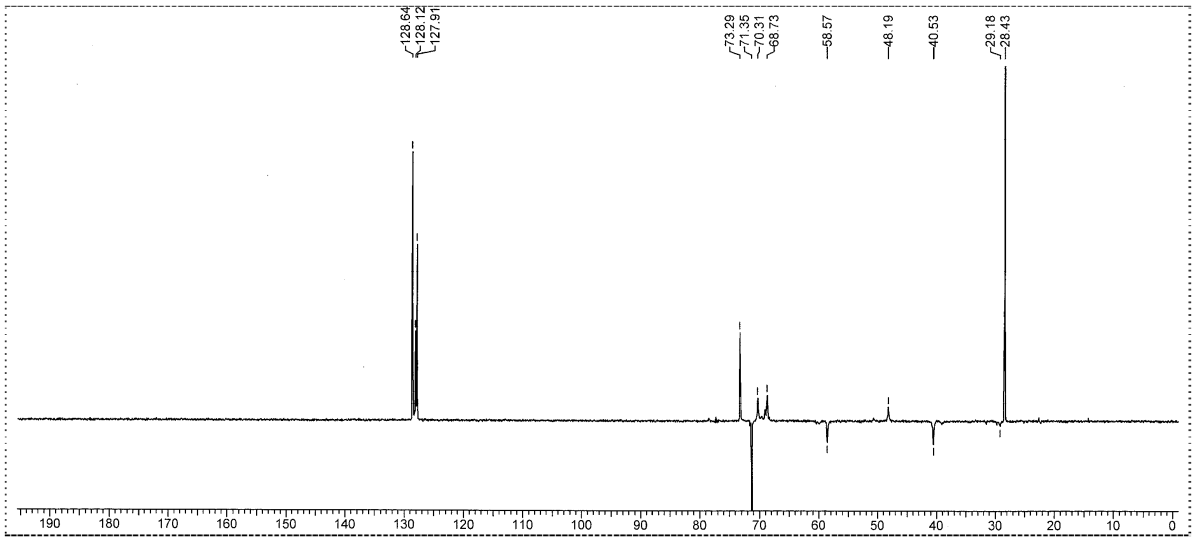

BnO
H
HO
HO
NH
OH
145


BnO
H
HO
HO
NH
OH
145
DEPT

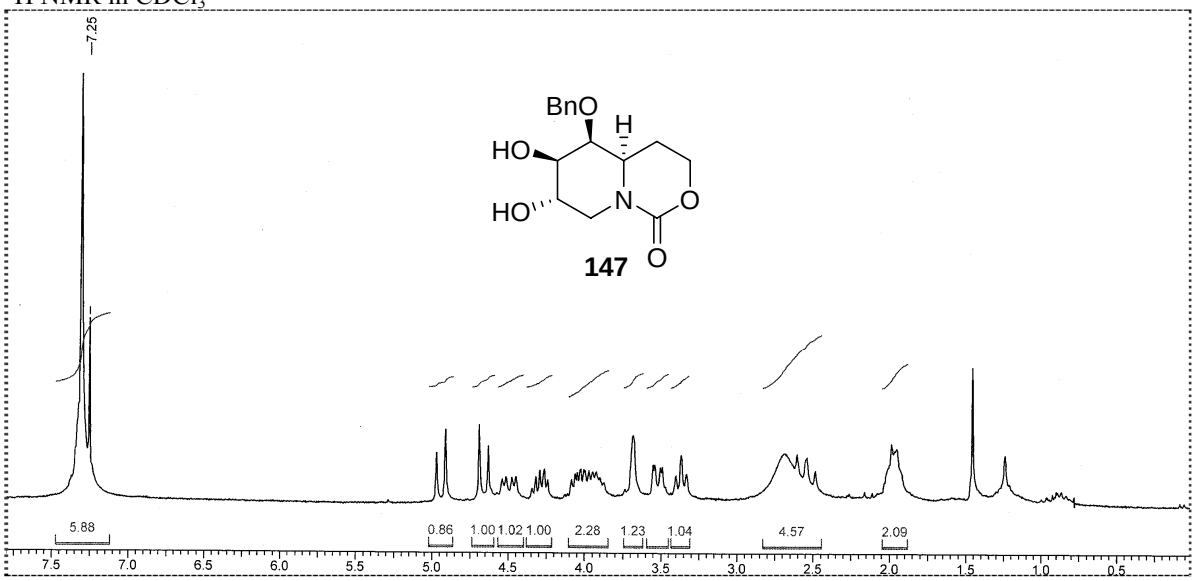

137.77
128.55
128.11
78.38
75.44
75.35
67.18
62.42
58.33
55.56
48.18
32.49
13C
BnO
H
HO
HO
NH
OH
145
two peaks


7.39
7.25
4.68
4.54
3.99
3.88
3.83
3.62
3.42
3.22
3.20
2.67
2.02
1.45
BnO
H
HO
HO
N
OH
Boc
146
5.00
2.80
2.97
1.05
0.86
1.03
2.73
2.00
9.41

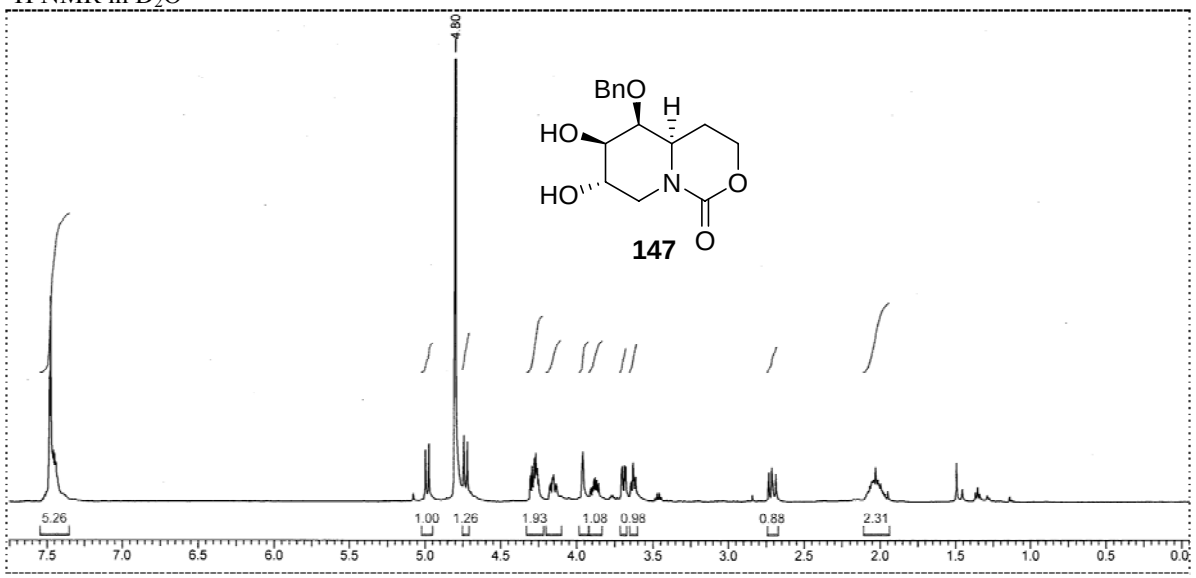

156.96
137.59
128.61
127.96
127.77
80.95
77.00
73.21
71.26
70.18
68.73
58.53
48.20
40.43
29.61
28.31
BnO
H
HO
HO
N
OH
Boc
146

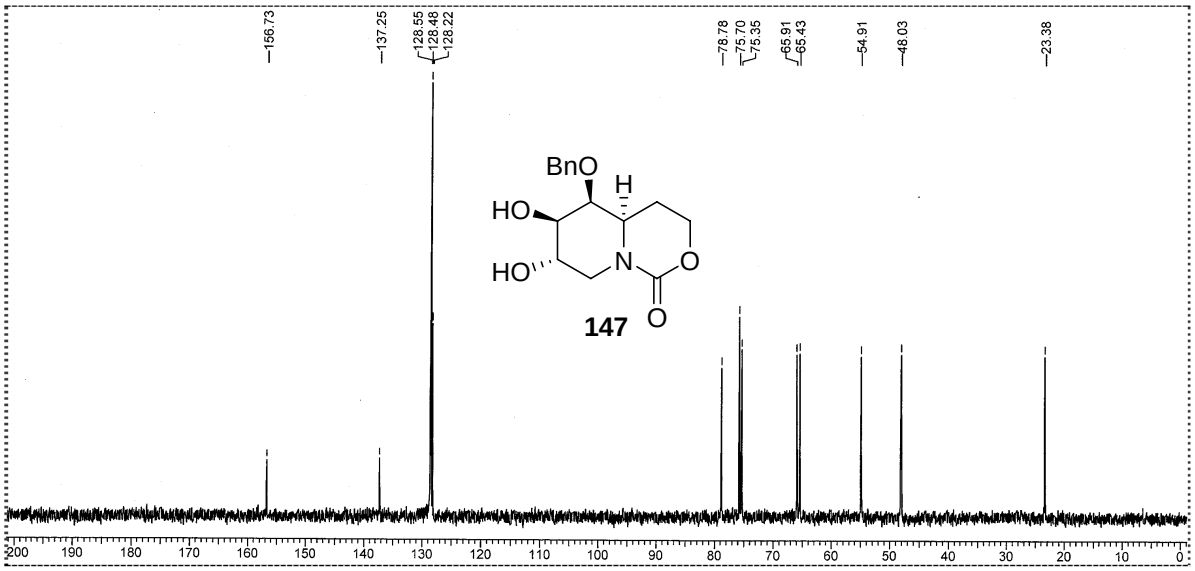


^{1}H NMR in $CDCl_3$

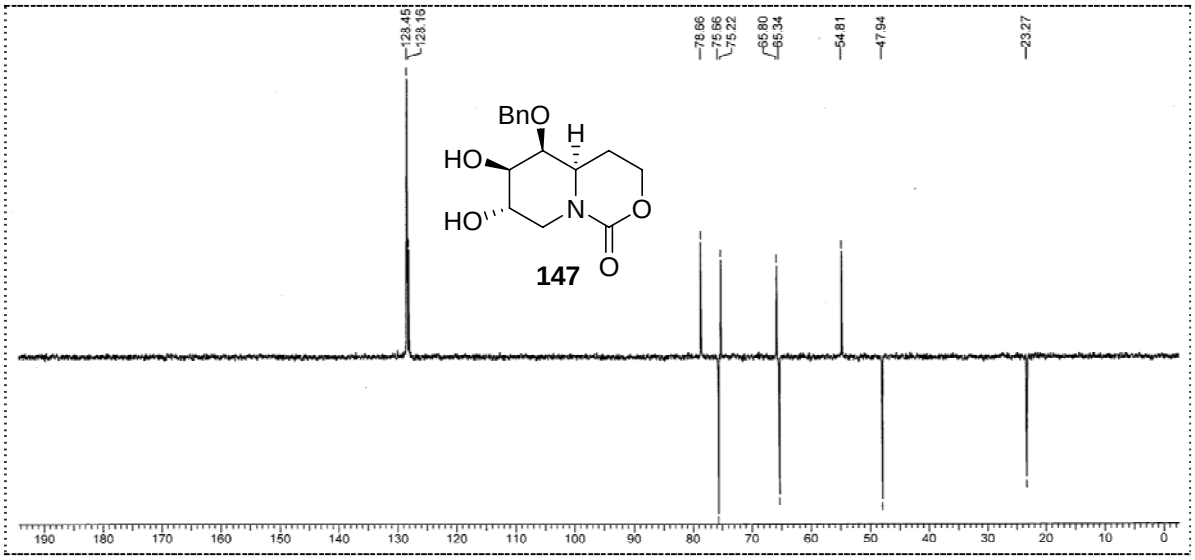


^{1}H NMR in D_2O

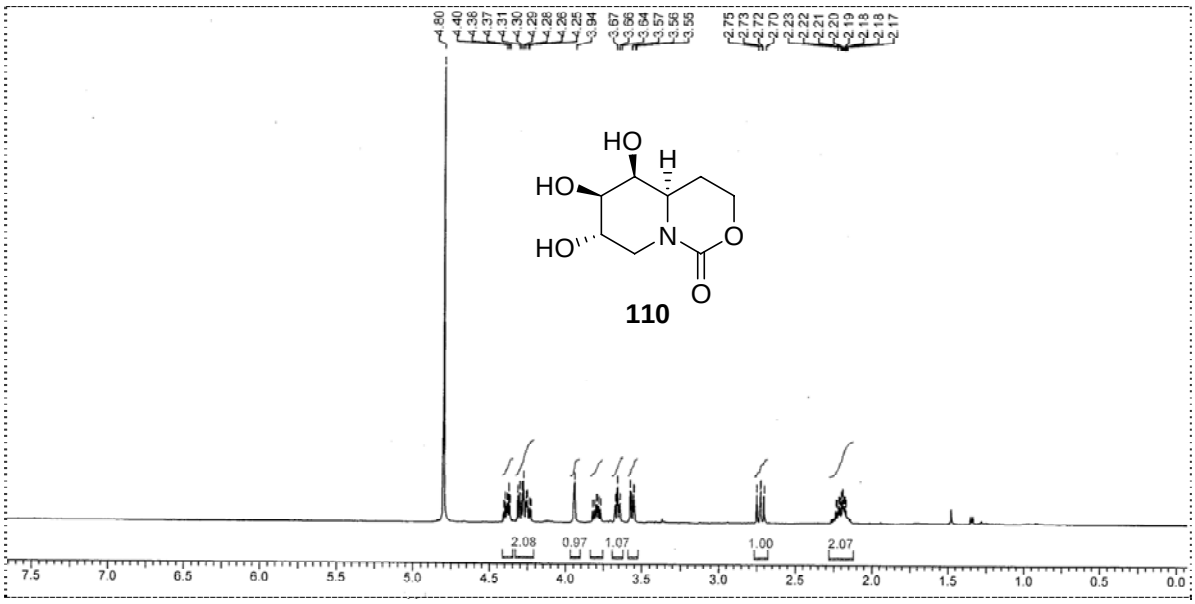



156.73
137.25
128.55
128.48
128.22
78.78
75.70
75.35
65.91
65.43
54.91
48.03
23.38
BnO
H
HO
HO
N
O
O
O

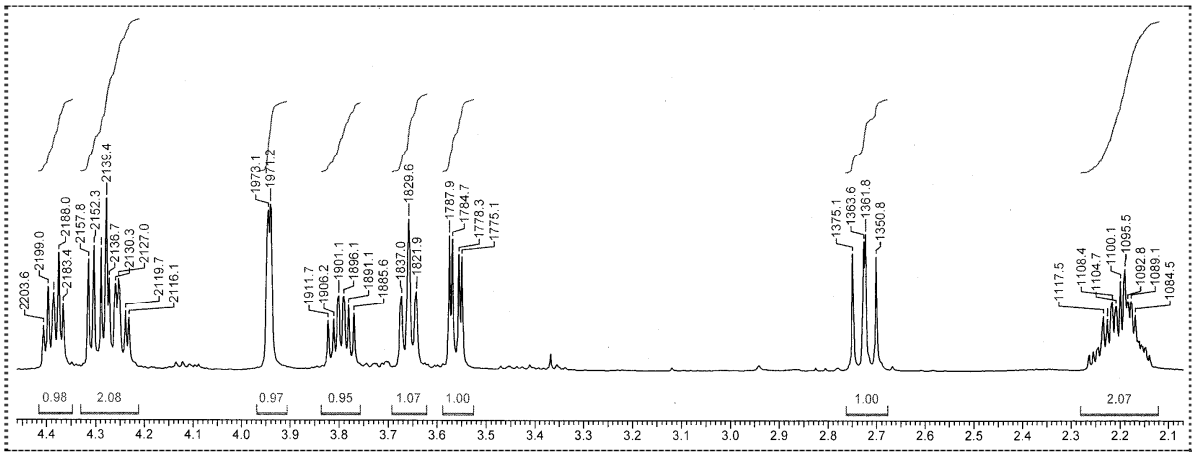
147


123.45
123.16
78.66
75.66
75.22
65.80
65.34
54.81
47.94
23.27
BnO
H
HO
HO
N
O
O
O

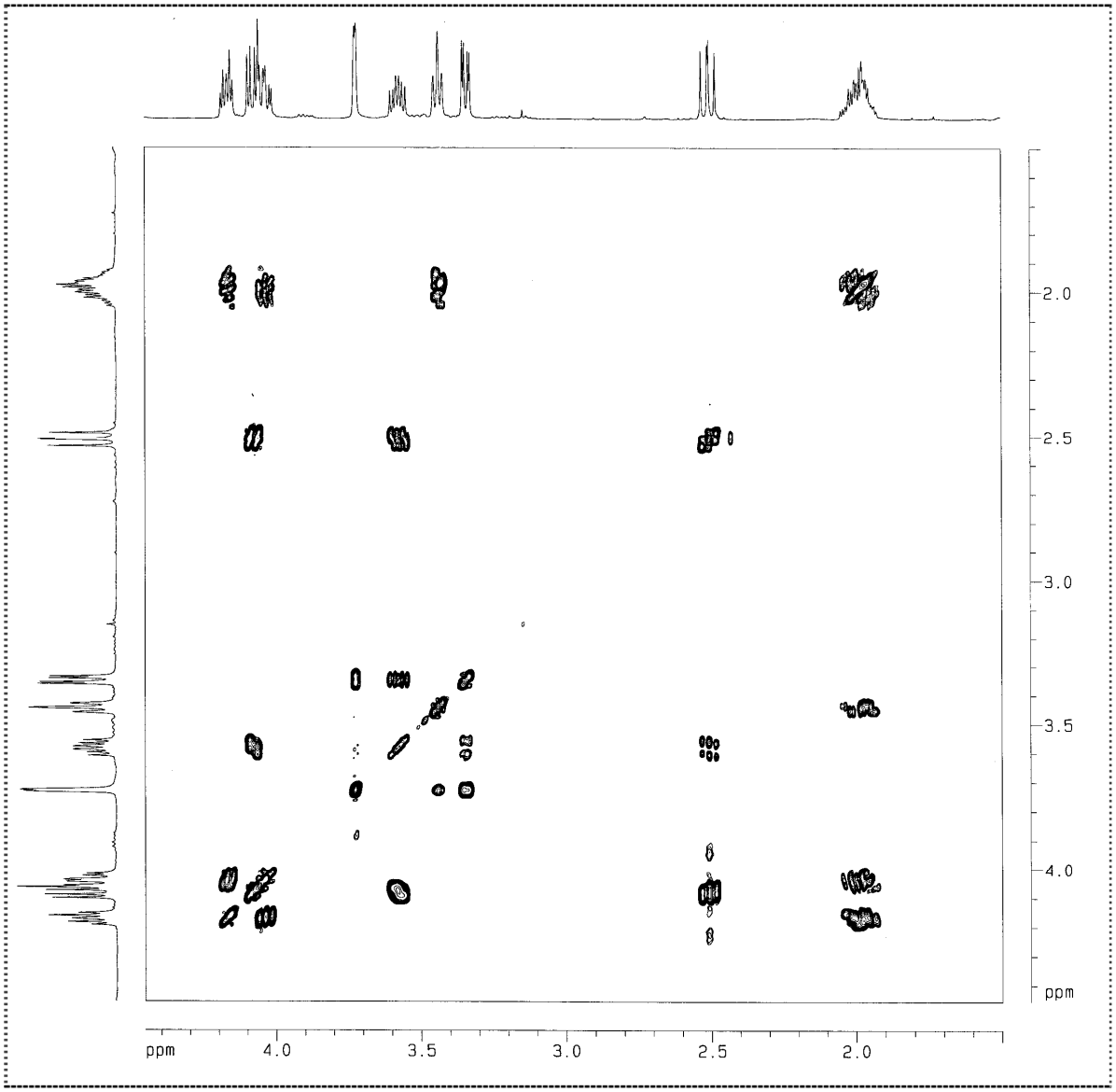
147


HO
H
HO
HO
N
O
O
2.08
0.97
1.07
1.00
2.07

110


2203.6
2199.0
2188.0
2183.4
2157.8
2152.3
2136.4
2136.7
2130.3
2127.0
2119.7
2116.1
1973.1
1971.2
1911.7
1906.2
1901.1
1896.1
1891.1
1885.6
1837.0
1829.6
1821.9
1787.9
1784.7
1778.3
1775.1
1375.1
1363.6
1361.8
1350.8
1117.5
1108.4
1104.7
1100.1
1095.5
1092.8
1089.1
1084.5
0.98
2.08
0.97
0.95
1.07
1.00
1.00
2.07
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1

COSY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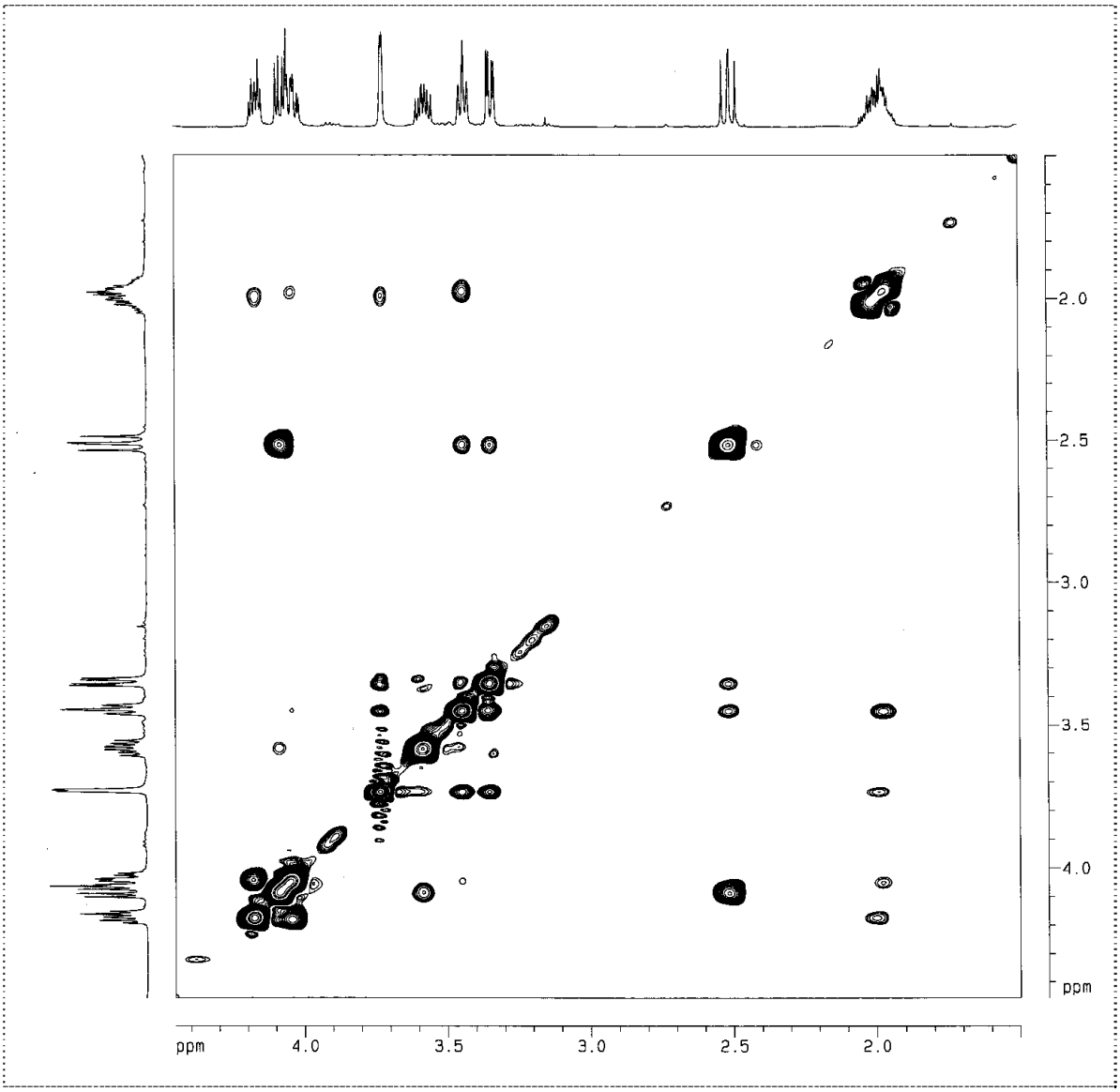
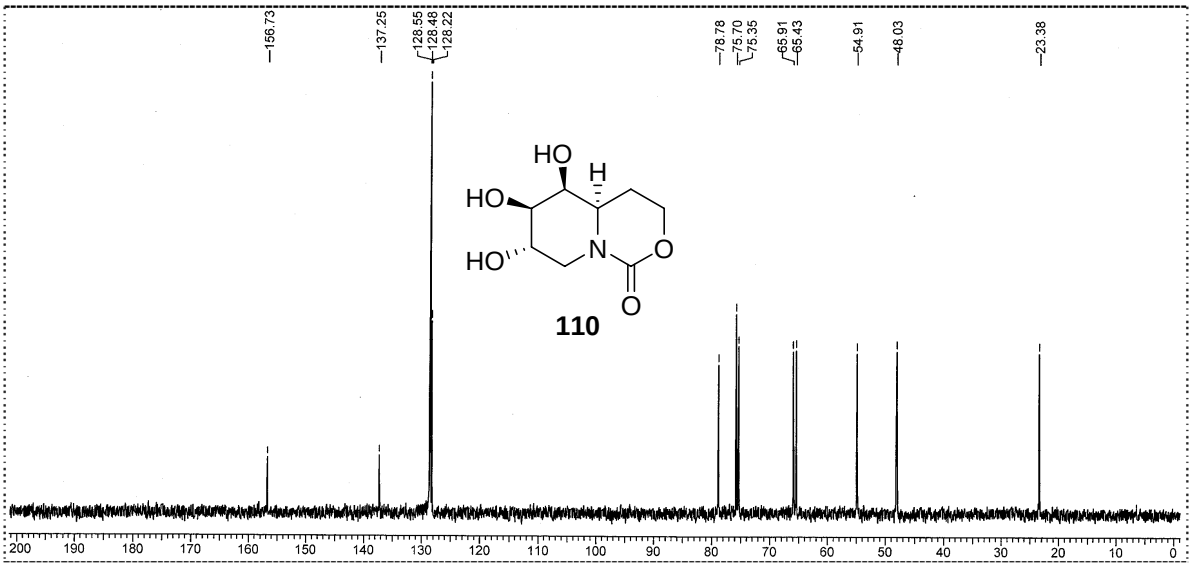
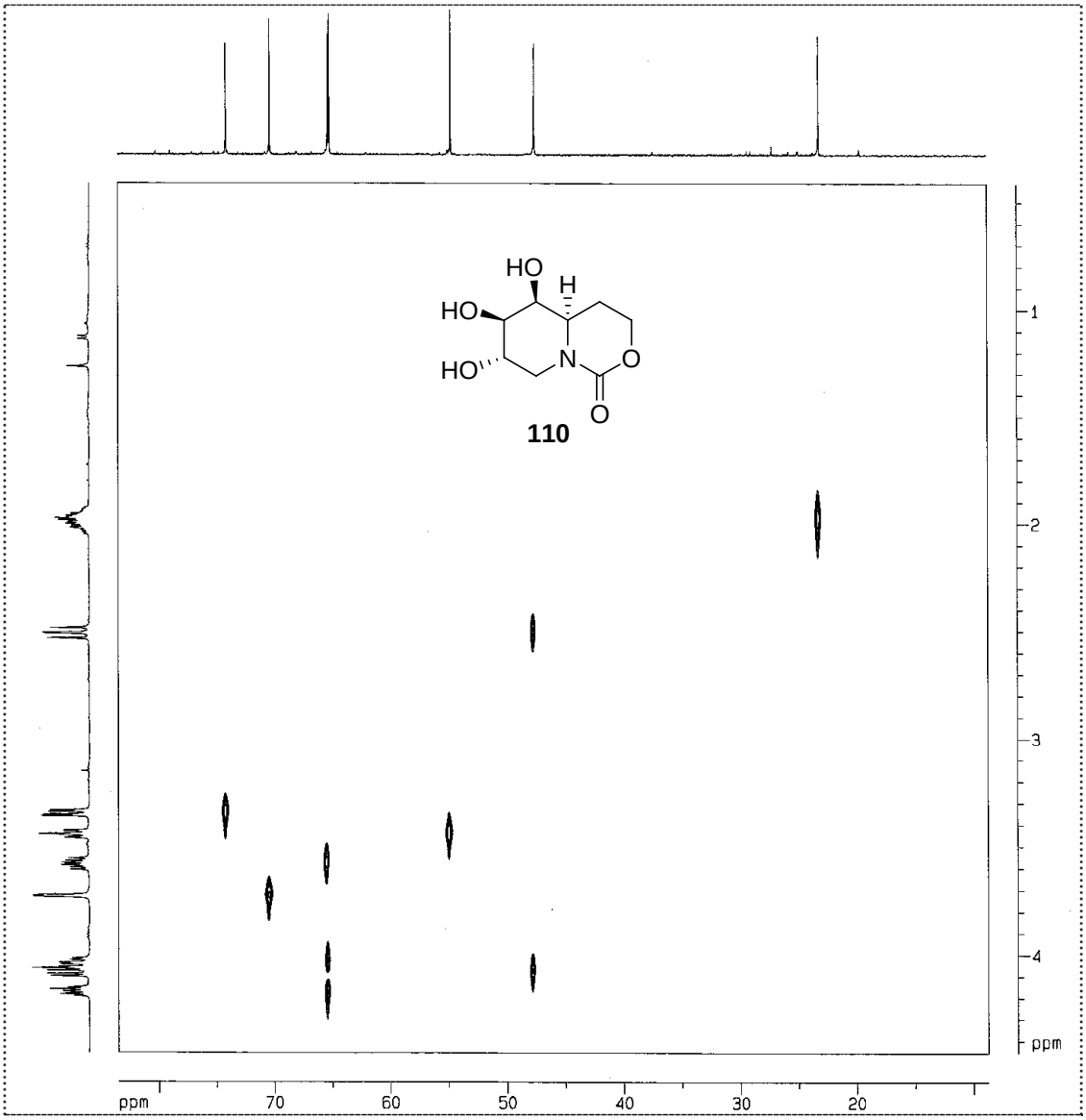
2.5
3.0
3.5
4.0
ppm
ppm
4.0
3.5
3.0
2.5
2.0

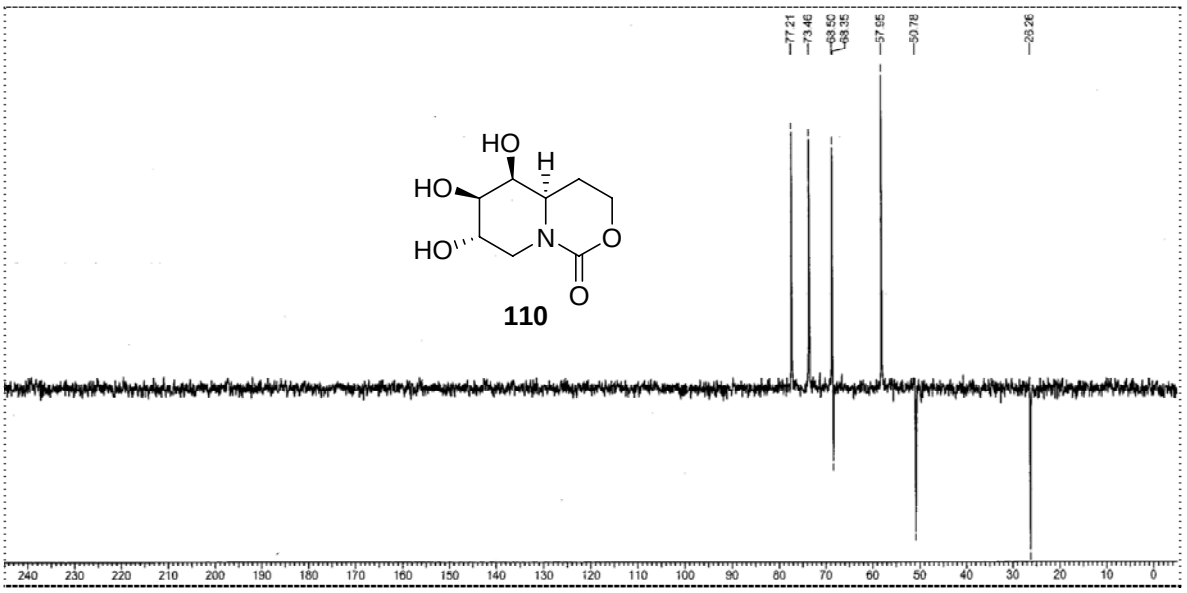
NOESY

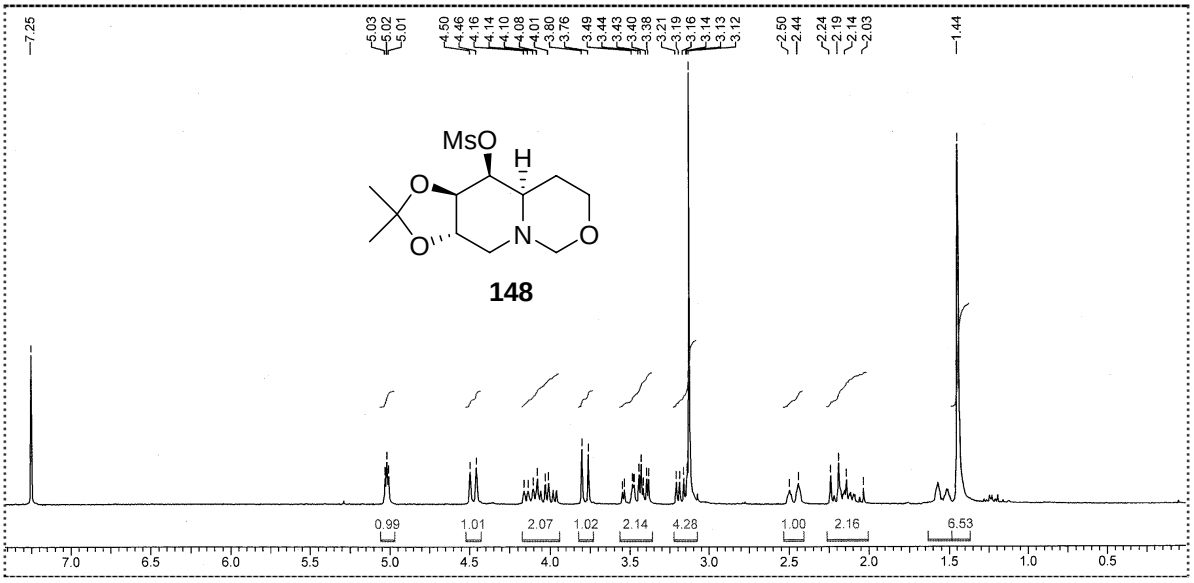

2.0
2.5
3.0
3.5
4.0
ppm
ppm
4.0
3.5
3.0
2.5
2.0


156.73
137.25
128.55
128.48
128.22
78.78
75.70
75.35
65.91
65.43
54.91
48.03
23.38
HO
H
HO
HO
N
O
O
110
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

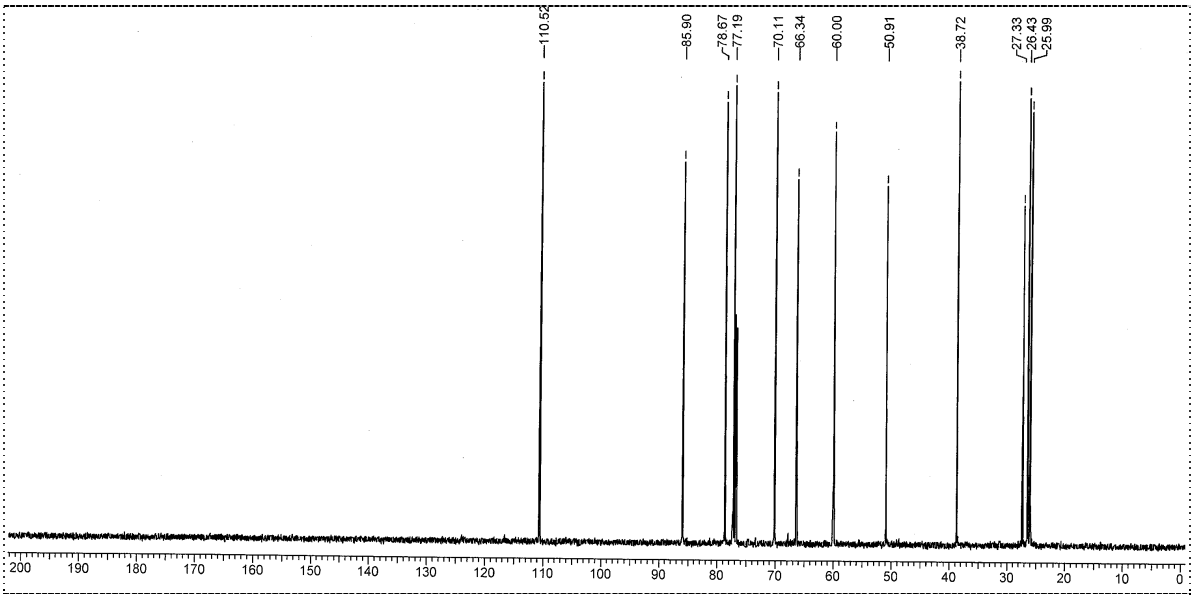
HETCOR


HO
H
HO
HO
N
O
O
110
1
2
3
4
ppm
ppm
70
60
50
40
30
20


77.21
73.46
68.50
68.35
57.65
50.78
28.26
HO
H
HO
HO
N
O
O
110
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


7.25
5.03
5.02
5.01
4.50
4.46
4.16
4.14
4.10
4.08
4.01
3.80
3.76
3.49
3.44
3.43
3.40
3.38
3.21
3.19
3.16
3.14
3.13
3.12
2.50
2.44
2.24
2.19
2.14
2.03
1.44
MsO
H
O
O
N
O
0.99
1.01
2.07
1.02
2.14
4.28
1.00
2.16
6.53

148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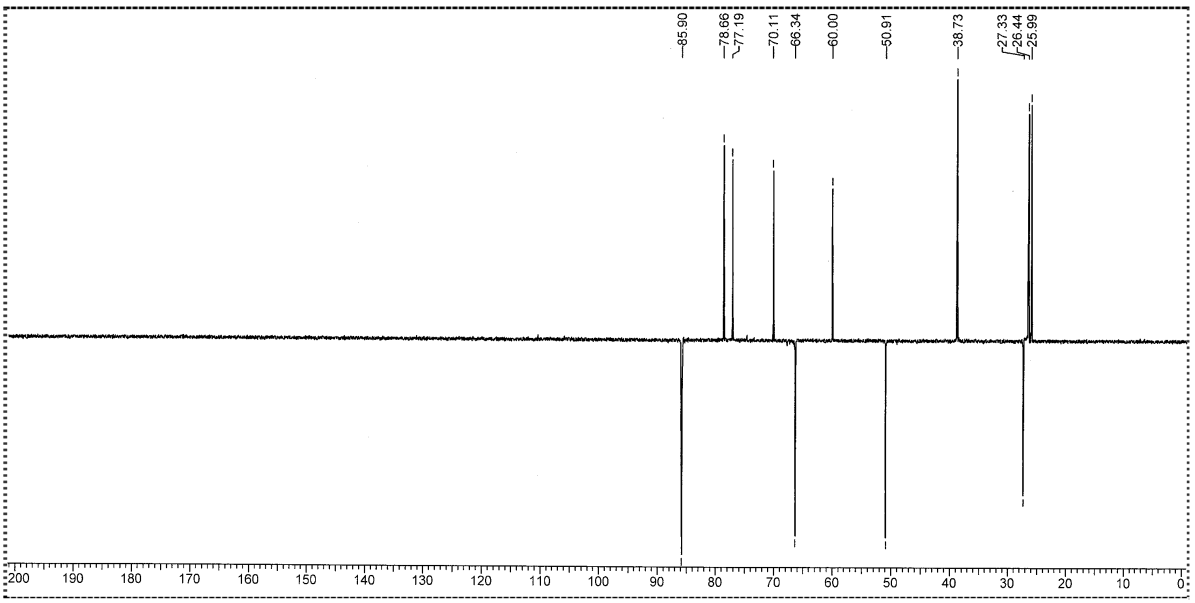
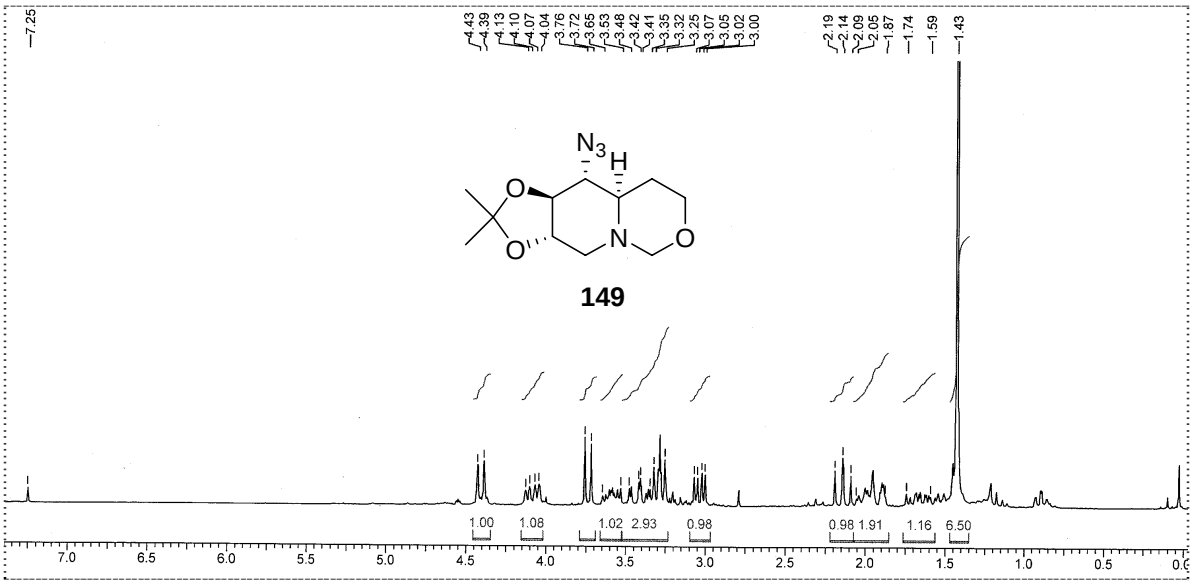
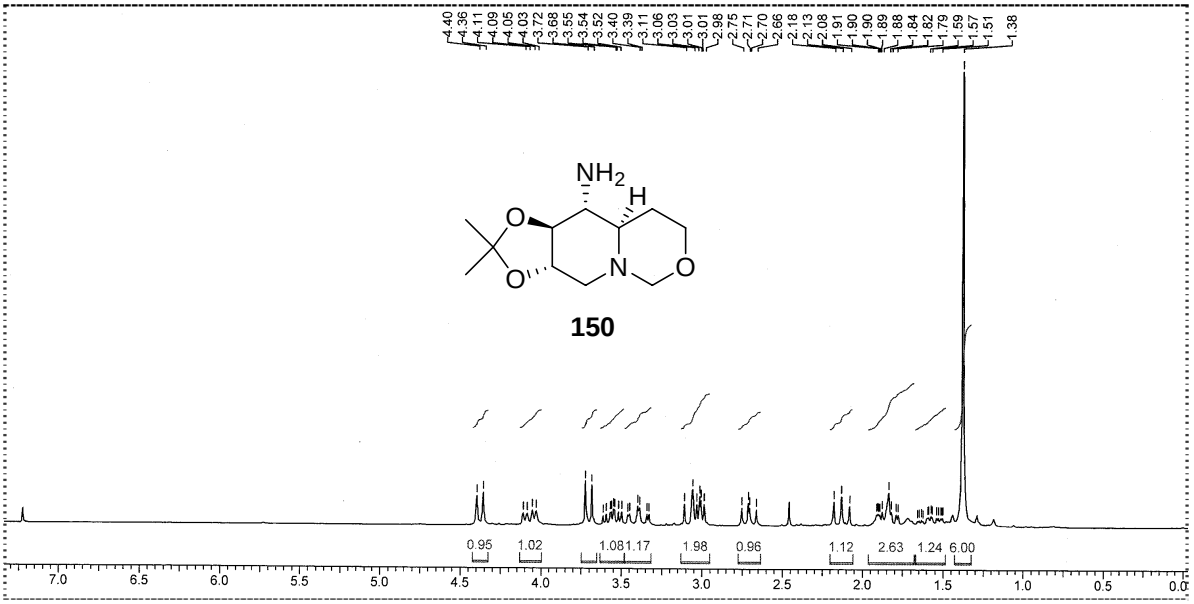
85.90
78.67
77.19
70.11
66.34
60.00
50.91
38.72
27.33
26.43
25.99

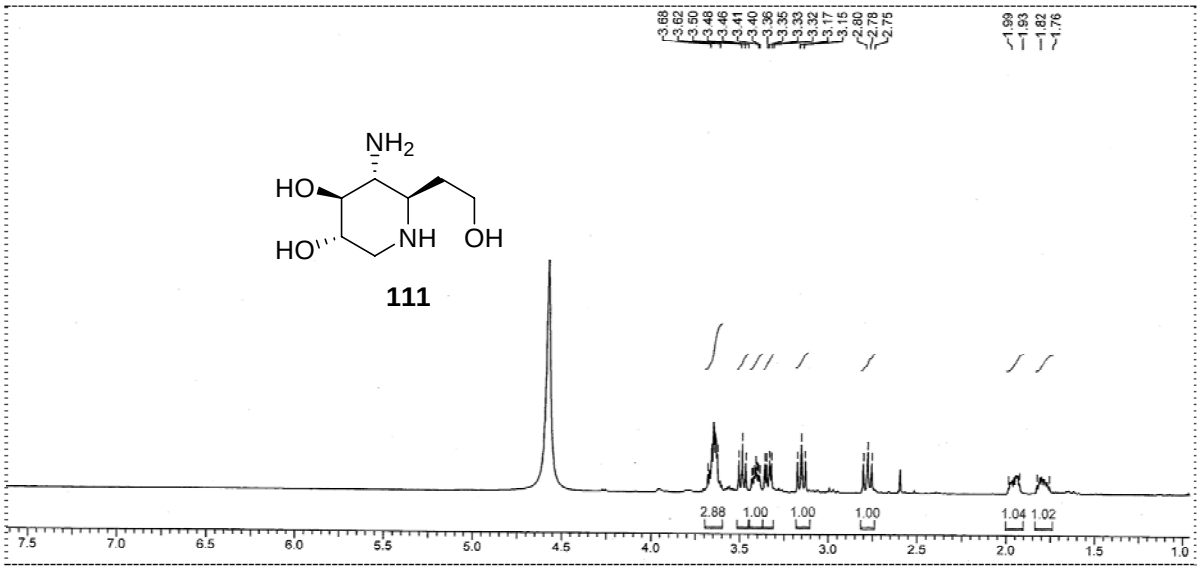

85.90
78.66
77.19
70.11
66.34
60.00
50.91
38.73
27.33
26.44
25.99


7.25
4.43
4.39
4.13
4.10
4.07
4.04
3.76
3.72
3.65
3.53
3.48
3.42
3.41
3.35
3.32
3.25
3.07
3.05
3.02
3.00
2.19
2.14
2.09
2.05
1.87
1.74
1.59
1.43
N3
H
O
O
N
O
1.00
1.08
1.02
2.93
0.98
0.98
1.91
1.16
6.50

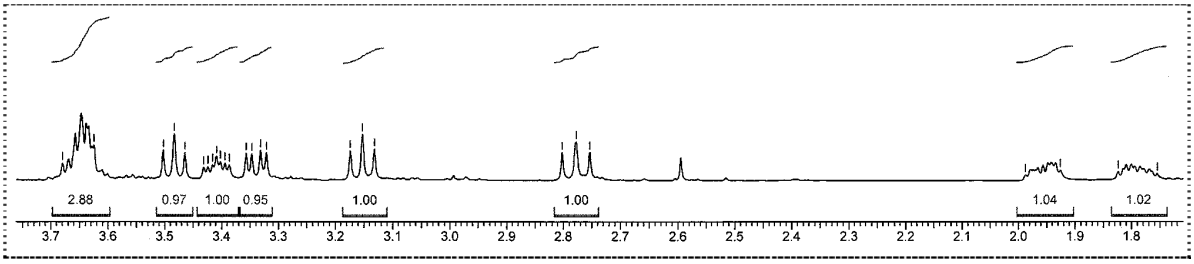
149

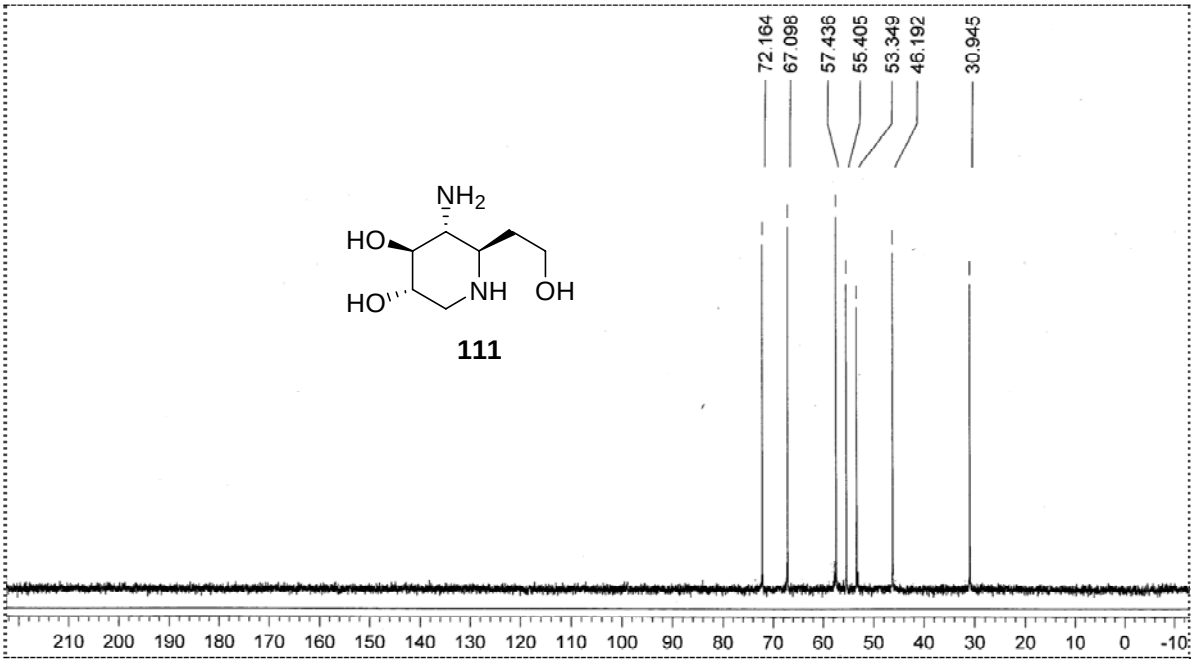

4.40
4.36
4.11
4.09
4.05
4.03
3.72
3.68
3.55
3.54
3.52
3.40
3.39
3.11
3.06
3.03
3.01
3.01
2.98
2.75
2.71
2.70
2.66
2.18
2.13
2.08
1.91
1.90
1.90
1.89
1.88
1.84
1.82
1.79
1.59
1.57
1.51
1.38
NH2
H
O
O
N
O
0.95
1.02
1.08
1.17
1.98
0.96
1.12
2.63
1.24
6.00

150

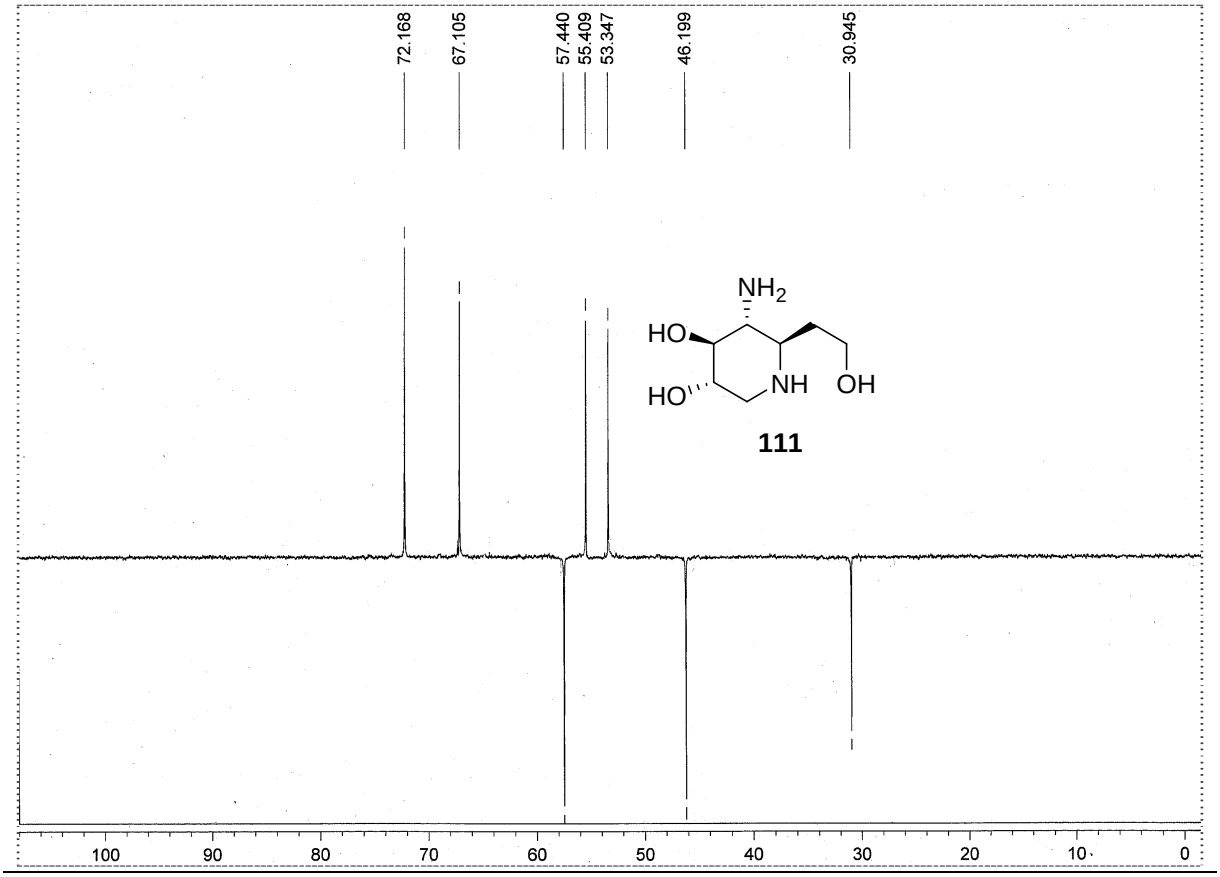

3.68
3.62
3.50
3.48
3.46
3.41
3.40
3.36
3.35
3.33
3.32
3.17
3.15
2.80
2.78
2.75
1.99
1.93
1.82
1.76
NH2
HO
HO
NH
OH
2.88
1.00
1.00
1.00
1.04
1.02

111

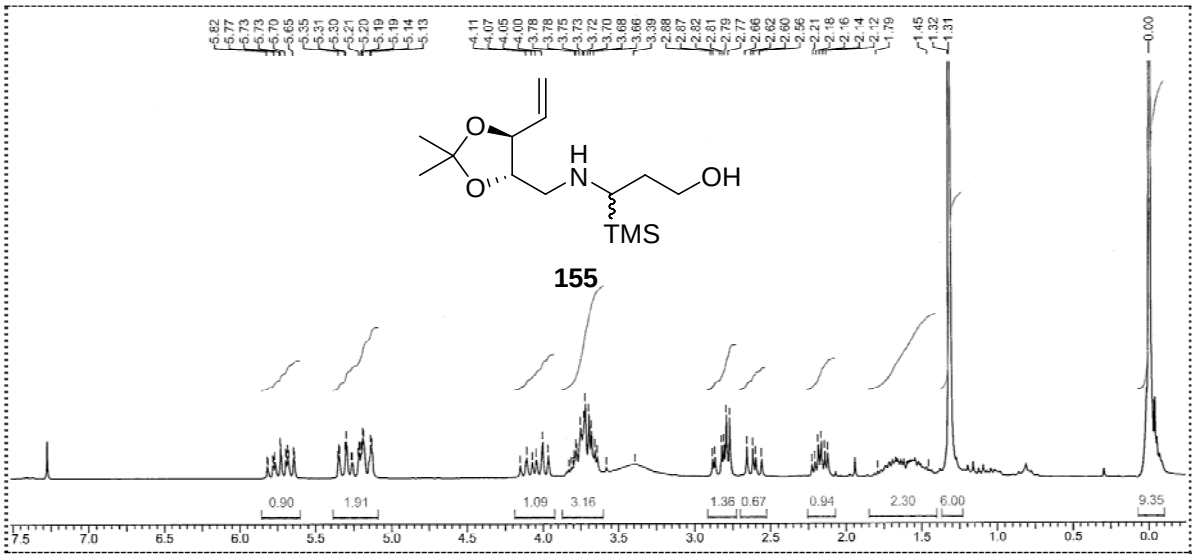

2.88
0.97
1.00
0.95
1.00
1.00
1.04
1.02


72.164
67.098
57.436
55.405
53.349
46.192
30.945
NH2
HO
HO
NH
OH

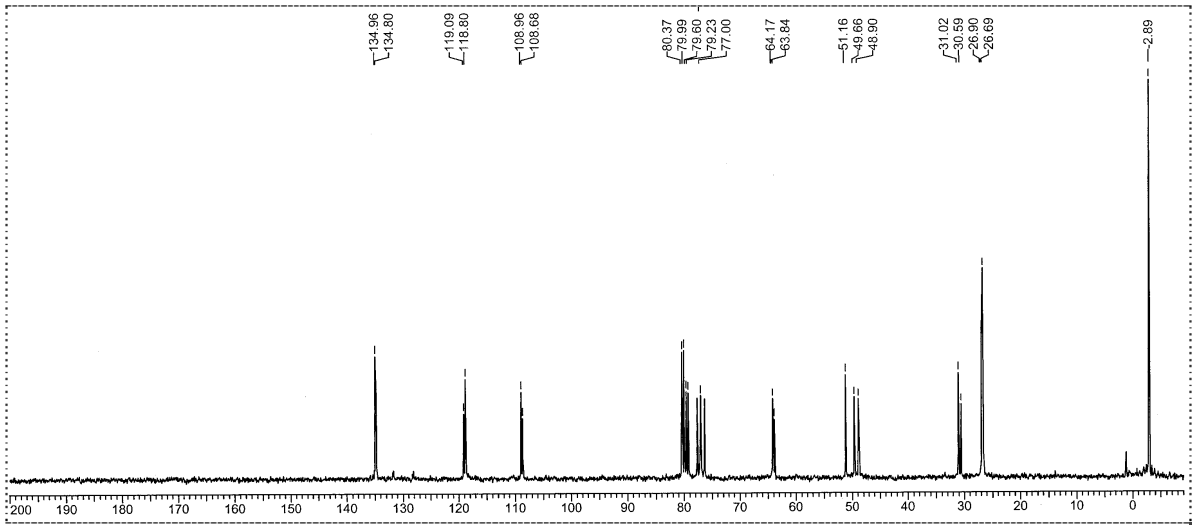
111

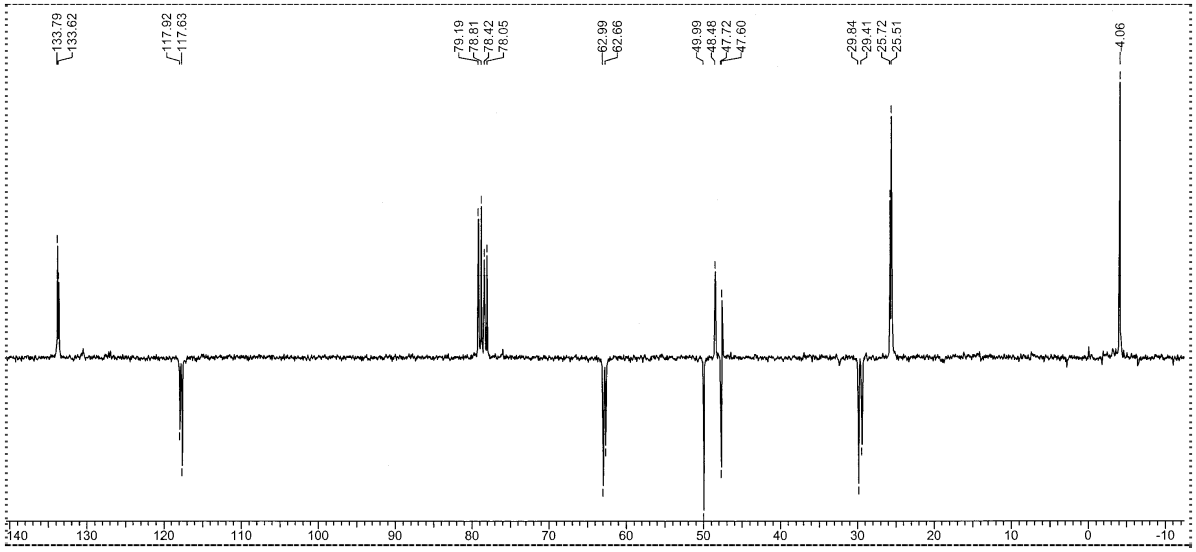

72.168
67.105
57.440
55.409
53.347
46.199
30.945
NH2
HO
HO
NH
OH

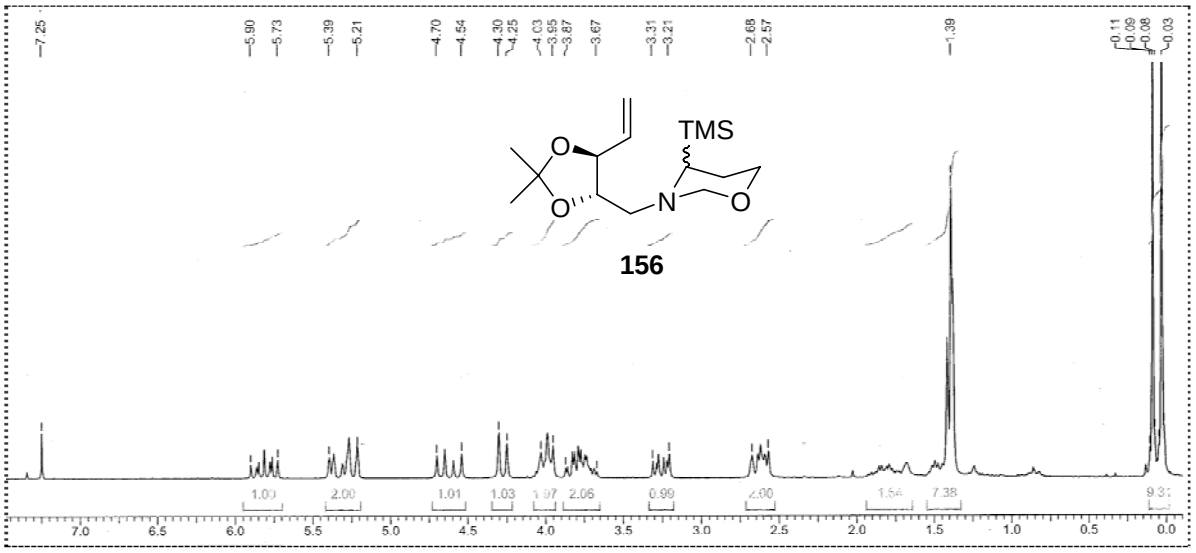
111

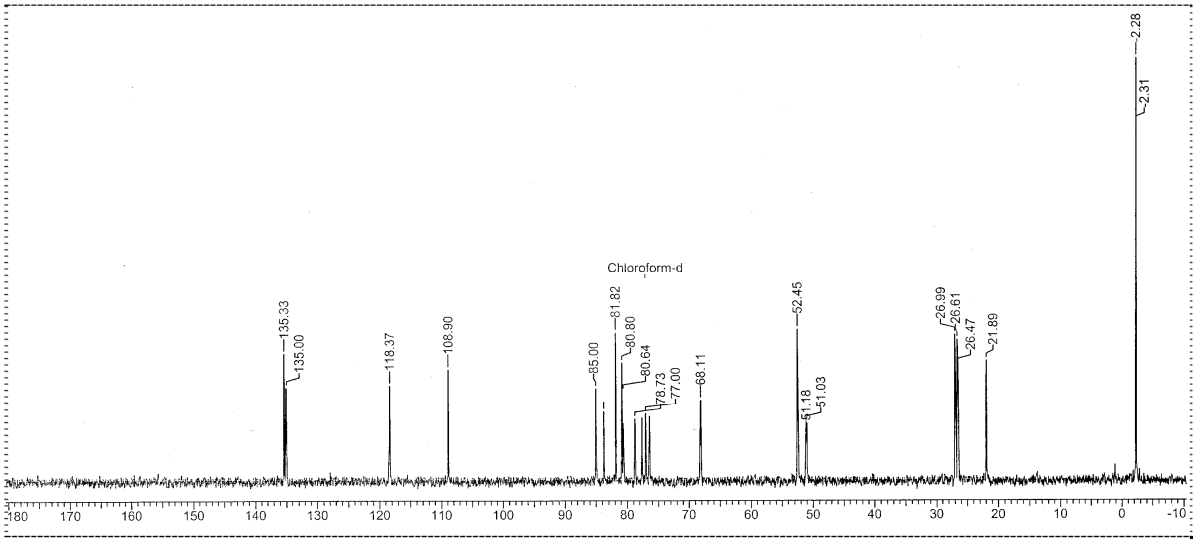


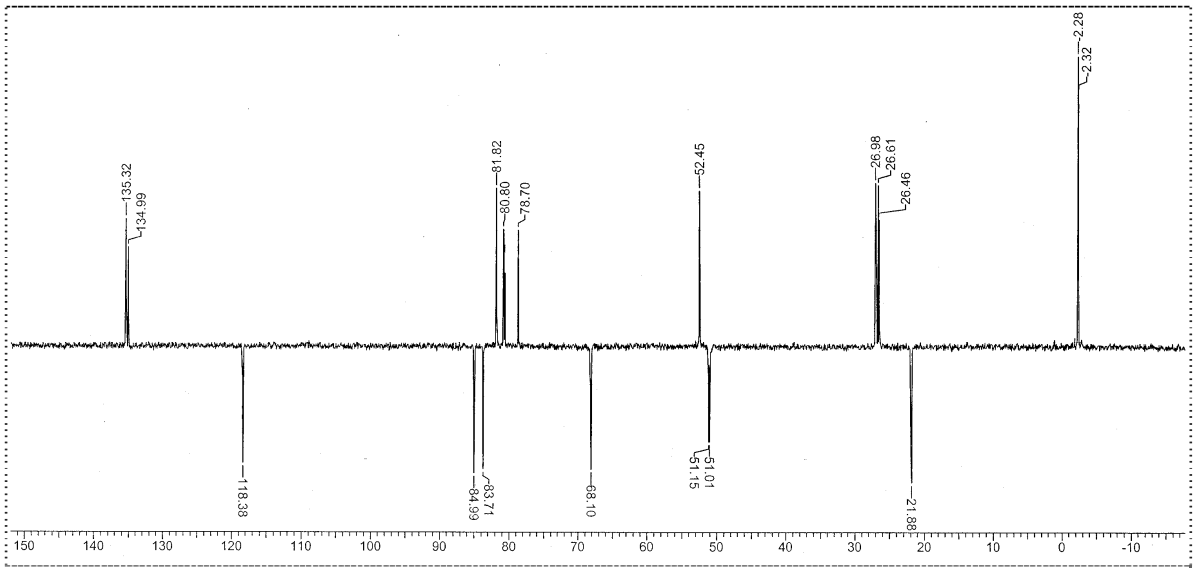
155

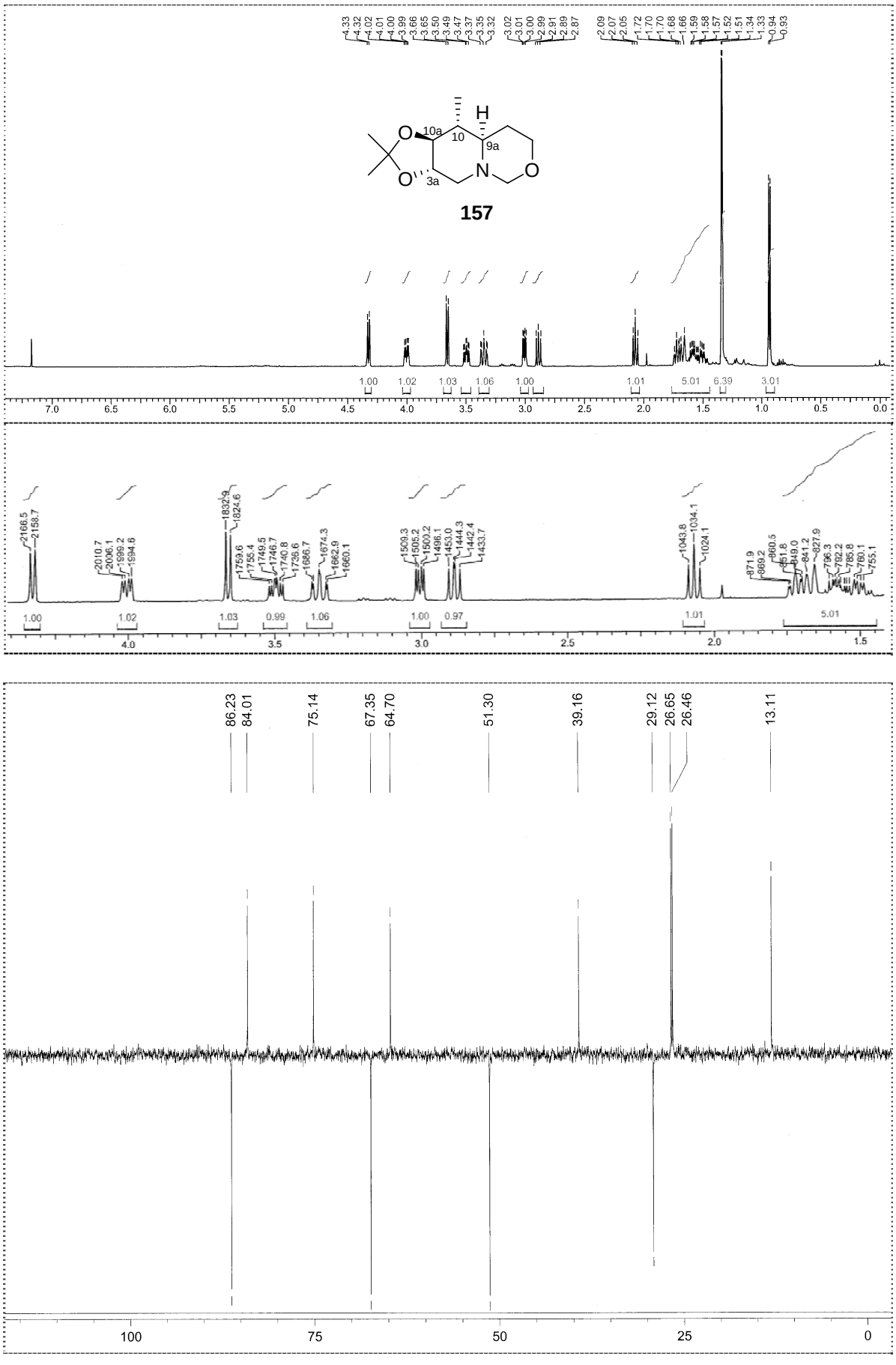





TMS
O
O
O
N
O
156


Chloroform-d

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10a
10
9a
N
O
O
3a
157
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75.14
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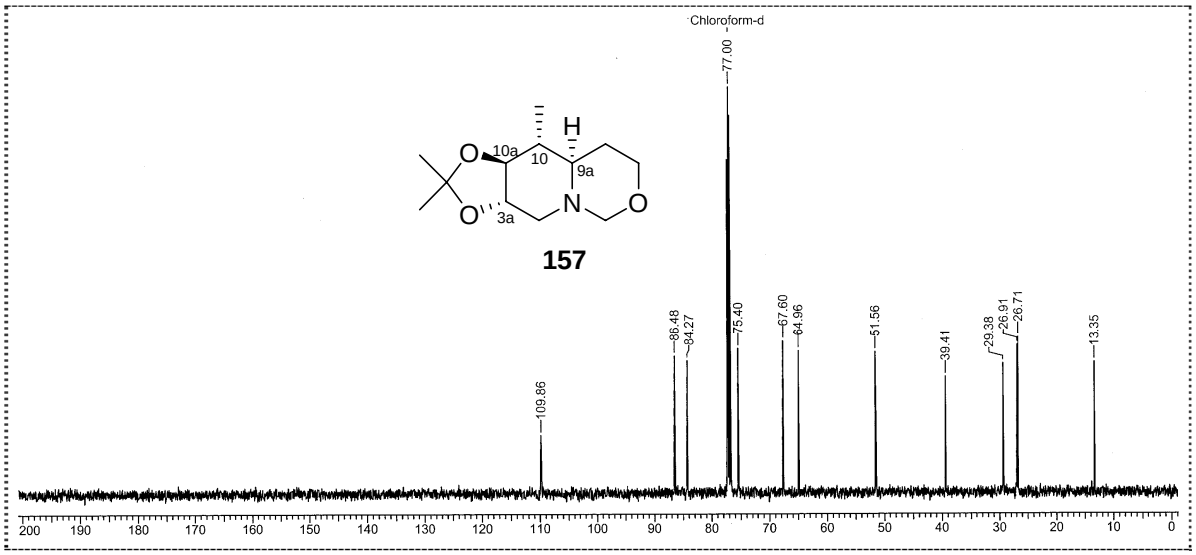
51.30
39.16
29.12
26.65
26.46
13.11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3a
N
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13.35
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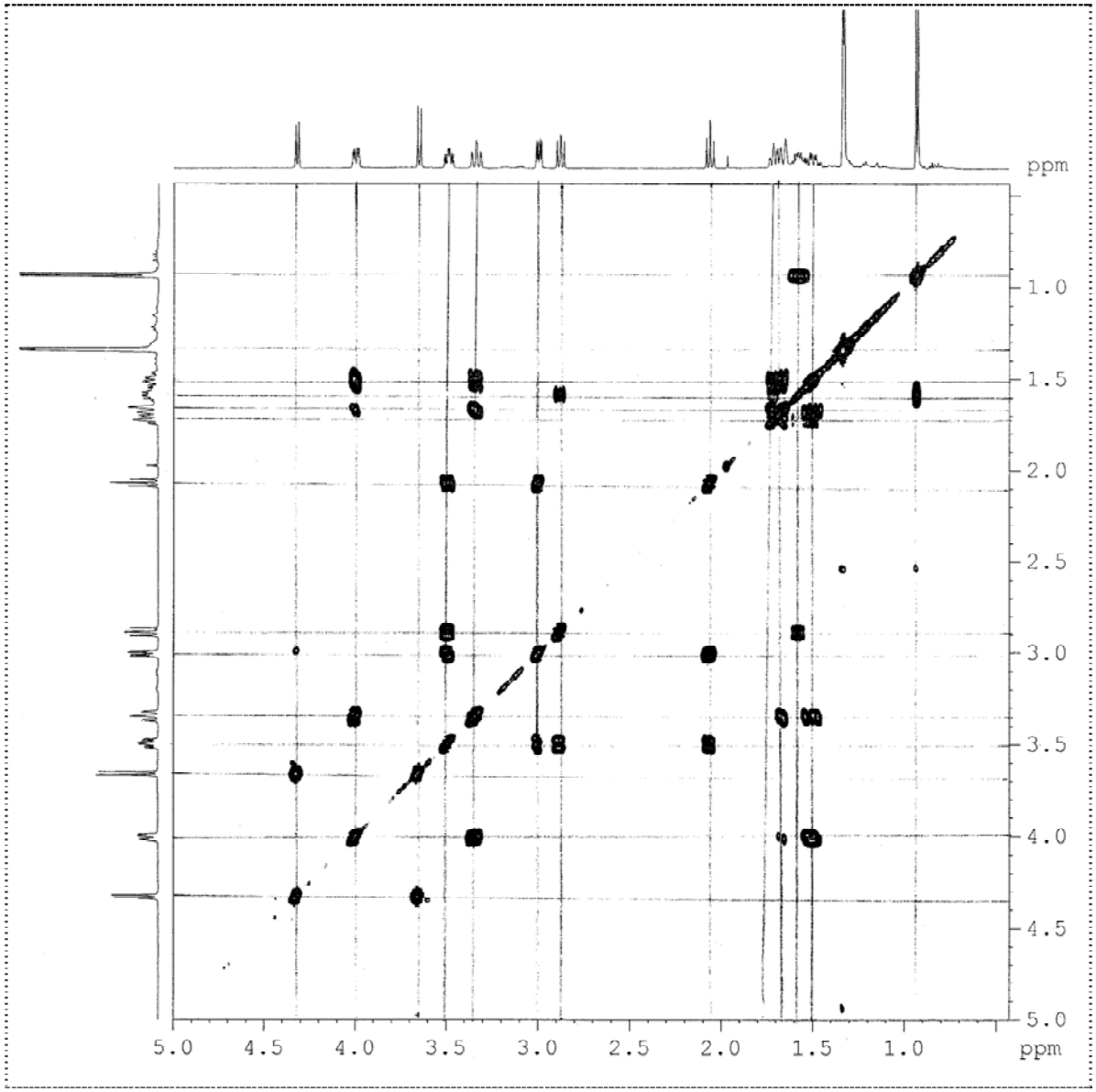
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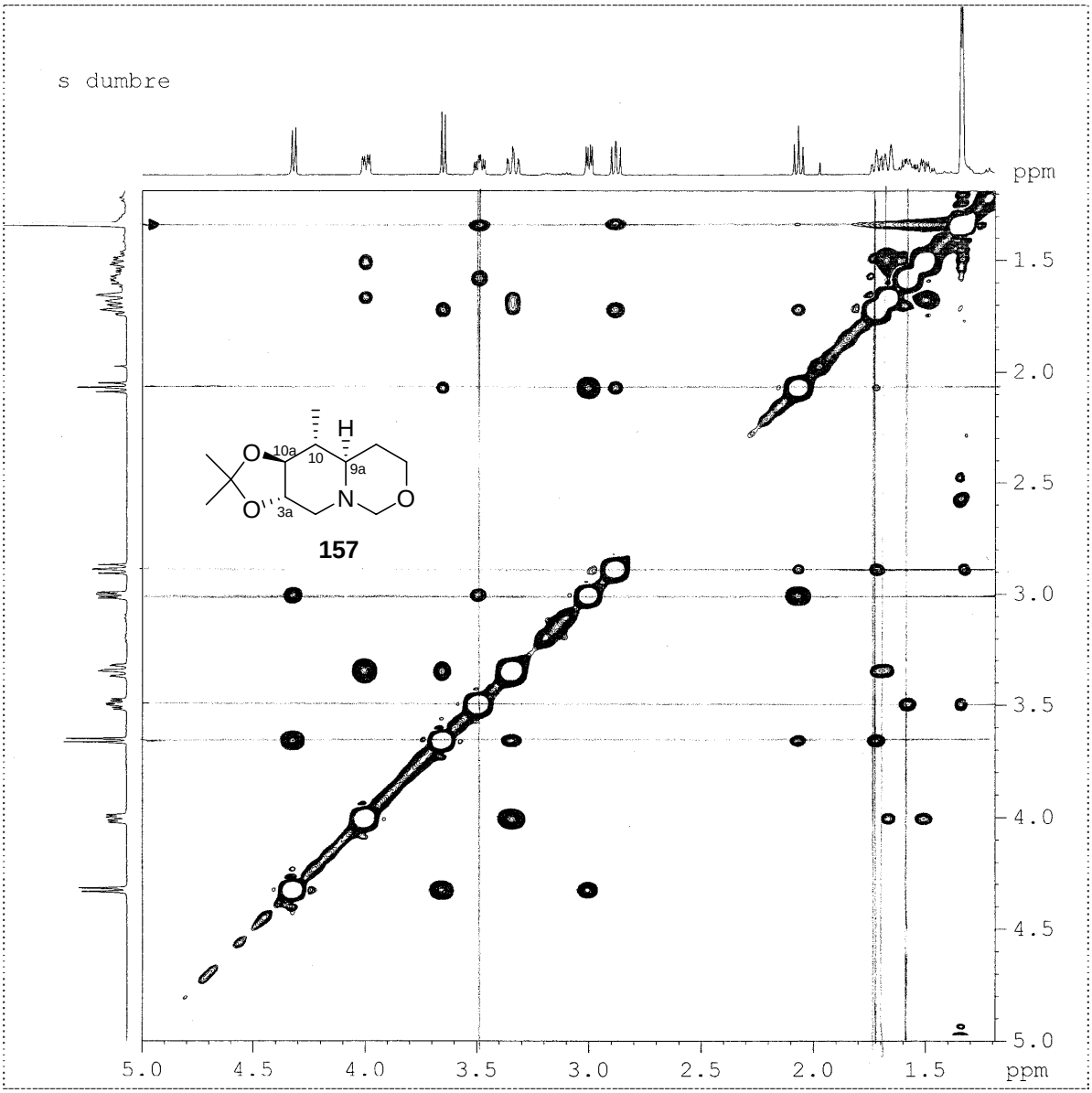
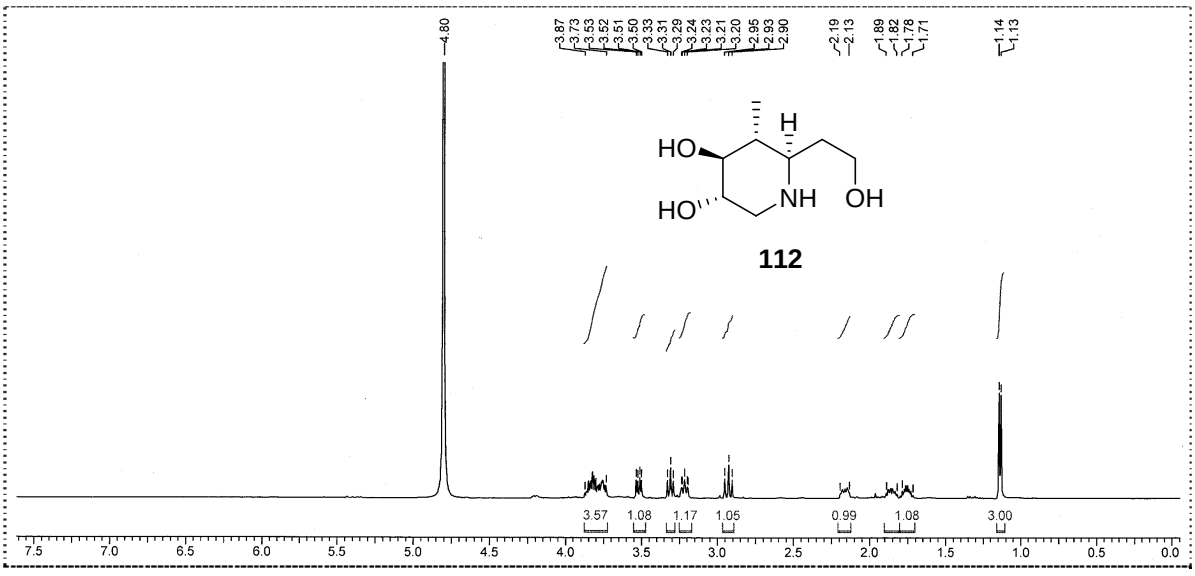
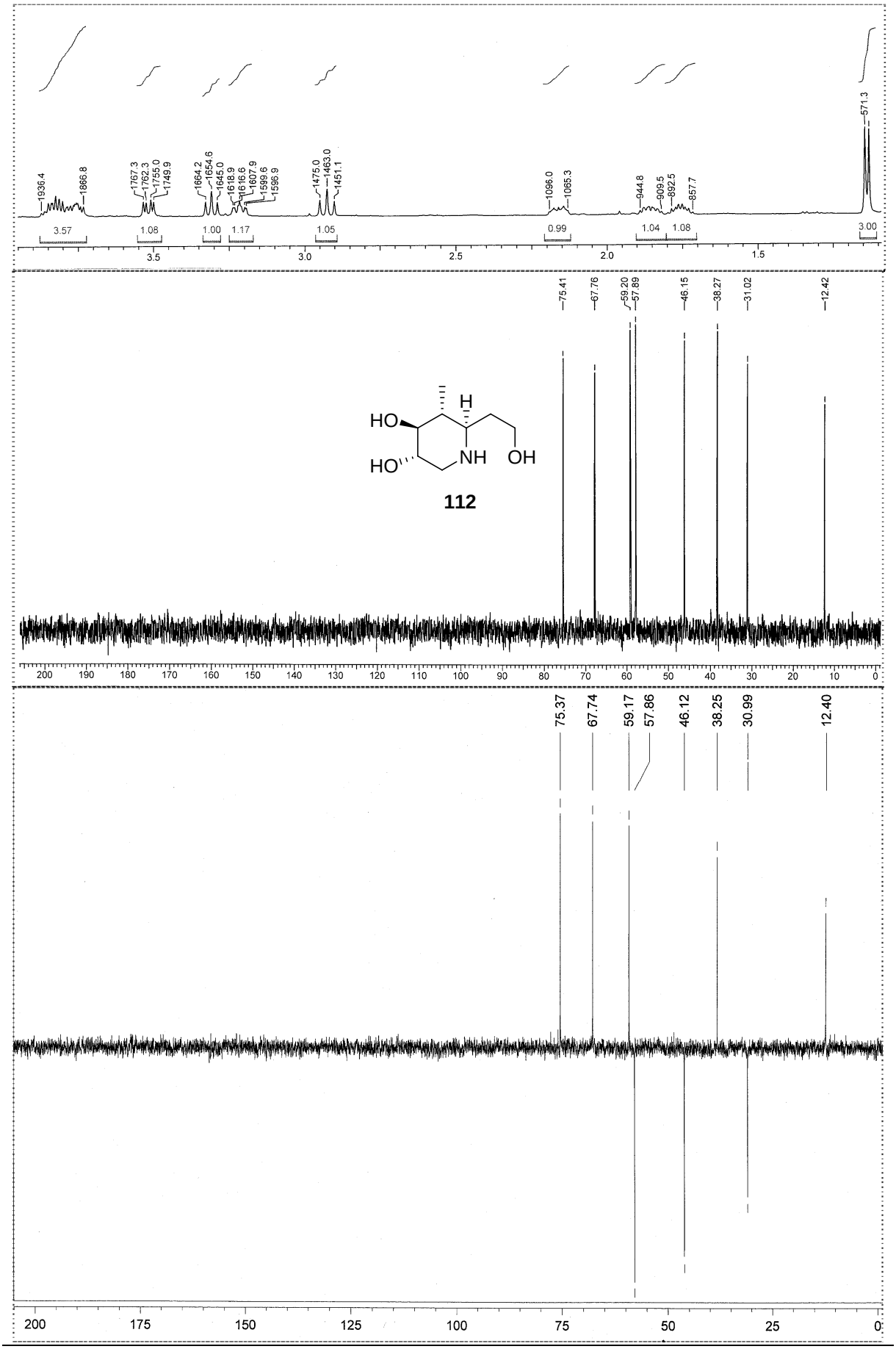


157



112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1762.3
1755.0
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1664.2
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1616.6
1607.9
1599.6
1596.9
1475.0
1463.0
1451.1
1096.0
1065.3
944.8
909.5
892.5
857.7
571.3
3.57
1.08
1.00
1.17
1.05
0.99
1.04
1.08
3.00
3.5
3.0
2.5
2.0
1.5
75.41
67.76
59.20
57.89
46.15
38.27
31.02
12.42
HO
H
HO
NH
OH
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200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
75.37
67.74
59.17
57.86
46.12
38.25
30.99
12.40
200
175
150
125
100
75
50
25
0

2.16.2 X-Ray Crystal data for Compound 138 and 139

Table 4. Selected crystal data crystal data and structure refinement for **138**

Empirical formula	$C_{12}H_{21}NO5$
Formula weight	259.30
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system, space group	Orthoprhombic, $P2_12_12$
Unit cell dimensions	a = 19.017(3) Å
	b = 7.7976(11) Å
	c = 8.8937(12) Å
Volume	1318.8(3) $Å^3$
Z, Calculated density	4, 1.306 Mg/m^3
Crystal size	0.53 x 0.19 x 0.12 mm
θ range for data collection	2.29 to 23.28 deg.
Completeness to θ = 23.28	99.7 %
Data / restraints / parameters	1899 / 0 / 167
Goodness-of-fit on F^2	1.215
Final R indices [I>2sigma(I)]	R1 = 0.0385, wR2 = 0.0836
R indices (all data)	R1 = 0.0412, wR2 = 0.0845

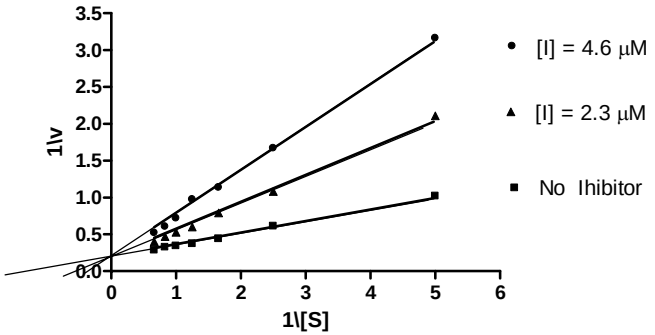
Table 5. Selected crystal data and structure refinement for compound **139**

Empirical formula	$C_{18}H_{25}NO_4$
Formula weight	319.39
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, $P2_1$
Unit cell dimensions	a = 5.3217(7) Å
	b = 9.7758(13) Å β = 92.897(2) deg.
	c = 16.549(2) Å
Volume	859.8(2) $Å^3$
Z, Calculated density	2, 1.234 Mg/m^3
Crystal size	0.43 x 0.26 x 0.22 mm

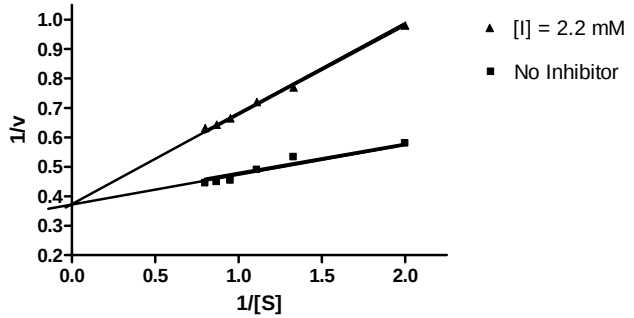
θ range for data collection	2.42 to 23.35 deg.
Reflections collected / unique	4783 / 2373 [R(int) = 0.0255]
Completeness to θ = 23.35	98.3 %
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2373 / 1 / 210
Final R indices [I>2sigma(I)]	R1 = 0.0724, wR2 = 0.2217
R indices (all data)	R1 = 0.0746, wR2 = 0.2235

2.16.3 Lineweaver-Burke Plots

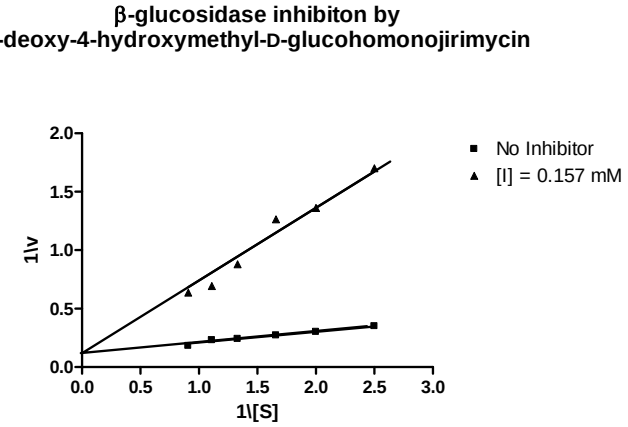
Compound **108 (*Ki* = 1.7 μM)**



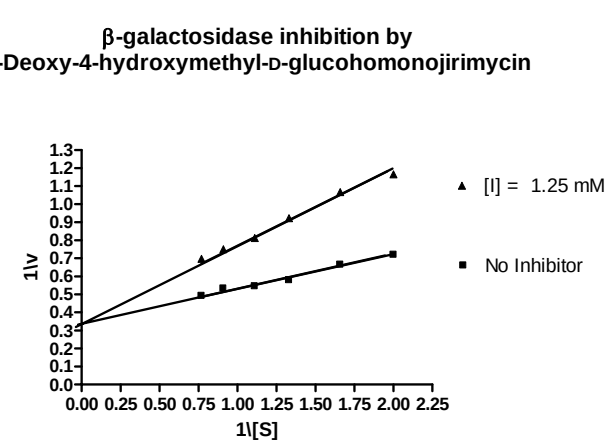
Compound **108 (*Ki* = 1100 μM)**



Compound **109 (*Ki* = 28 μM)**

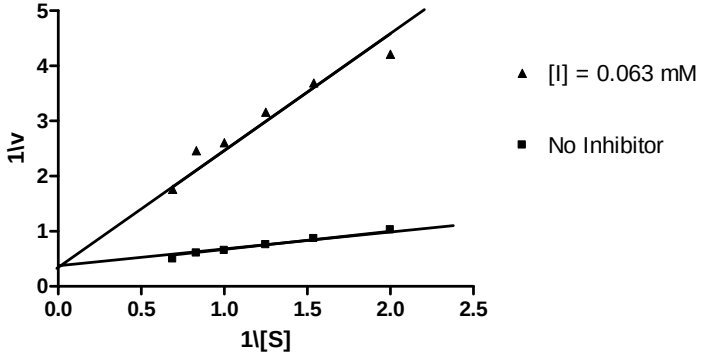


Compound **109 (*Ki* = 1000 μM)**



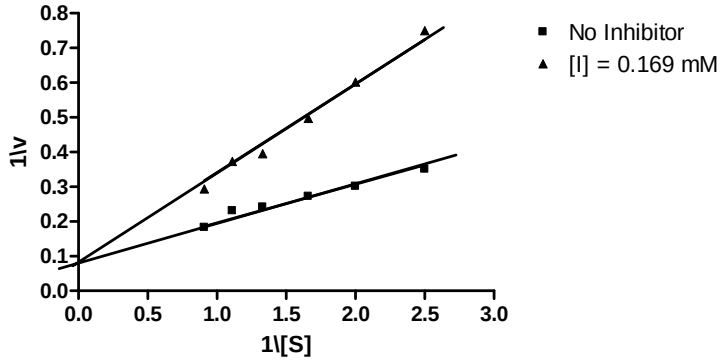
Compound *ent*-**109 (*Ki* = 12 μM)**

β-glucosidase inhibition by 1-Deoxy-4-hydroxymethyl-L-glucohomonojirimycin



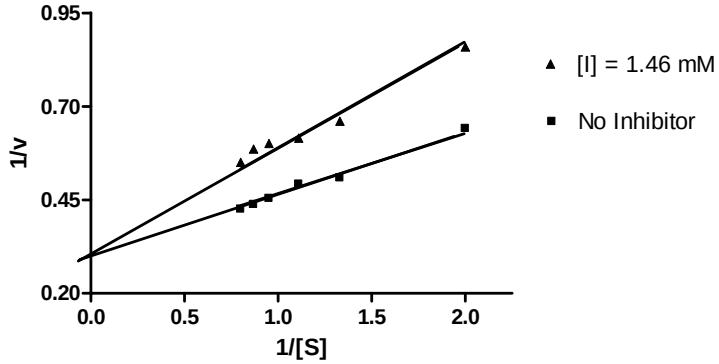
Compound **21** *ent*-**108 (*Ki* = 129 μM)**

β-glucosidase inhibition by 1-Deoxy-L-galactohomonojirimycin



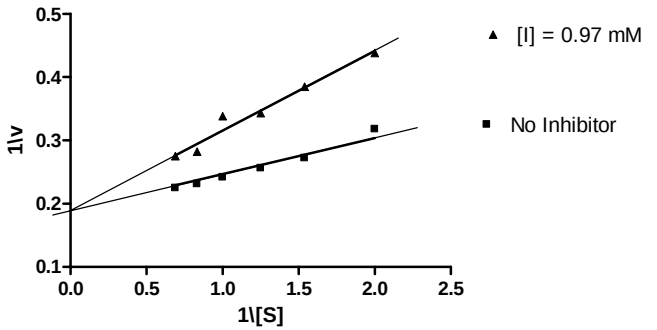
Compound **110 (*Ki* = 1900 μM)**

β-galactosidase inhibition by 110



Compound **110 (*Ki* = 800 μM)**

α-galactosidase inhibition by compound 110



Chapter 3 Synthesis of β-Lactam-Azasugar Hybrid

3.1 Introduction

Design and synthesis of hybrid molecules i.e., structural motifs developed through domain assimilation of two or more different class of biologically active compounds of natural and/or synthetic origin, has attracted the attention of synthetic chemists in the past few years owing to enhanced possibility of discovering new biologically active therapeutic agents. In this perspective, several hybrid molecules[1,2] of natural products such as steroids, taxoids,[3] carbohydrates[4] and peptides with counterparts such as β-lactams,[5] C_{60}-fullerenes,[6] anthraquinones,[7] enediyne[5] and porphyrin[8] have been synthesized and their properties well evaluated (**Figure 30**).

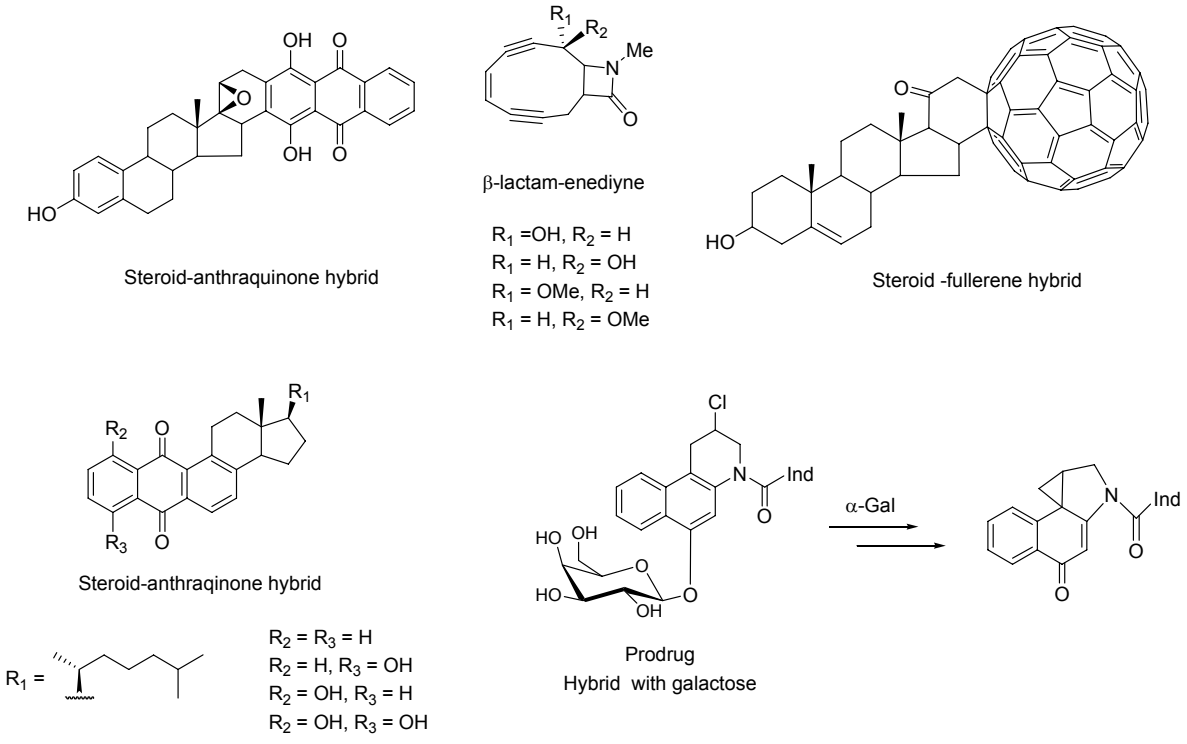


Figure 30: Hybrid molecules

As described in previous chapter azasugar inhibitors of glycosidases and related enzymes are subject of intense current research interest due to their potential clinical applications as anti-diabetic, anti-cancer, anti-HIV, and anti-influenza agents. These low molecular weight entities are believed to exhibit their inhibitory activities due to their

binding with the glycosidases by mimicking the shape and charge of the postulated oxo-carbenium ion intermediate for the glycosidic bond cleavage reaction. Some of the potent azasugar based glycosidase inhibitors (**Figure 31**), such as **12**,[9] **14**,[10] **34**,[11] **158**,[12] and **38**[13] which become positively charged on protonation due to the presence of basic amino, amidine and hydrazine moieties, are suggested to derive their inhibitory activities either by mimicking the charge or shape or both of the glycosidase transition state. In contrast, neutral glyconolactams, such as **36** (*Ki* = 85 μM, β-glucosidase) where glycosidic oxygen is replaced by pseudo sp^2 ring nitrogen is found to be potent inhibitor of β-glycosidases. Wither *et al*[14] found that the lactam **161**, an analogue of xylobiose type 1- azasugar **163** is as potent (*Ki* = 340 nM) as **163** (*Ki* = 130 nM) against a β-xylosidase (**Figure 32**).

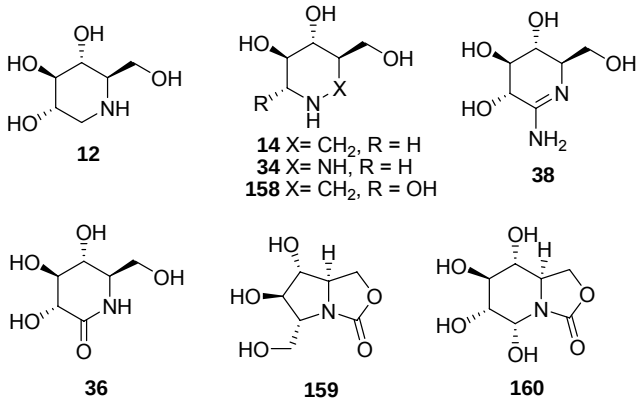


Figure 31: Structures of some potent glycosidase inhibitors

The affinity of **161** was explained by considering the tautomeric form of the amide **162** which is able to act both as an amine as well as 2-hydroxy group. However, recent research by Withers *et al* suggests that glycosidase inhibition by **161** and similar compounds are to a large extent caused by the H-bonding to the carbonyl group of the lactam.[14]

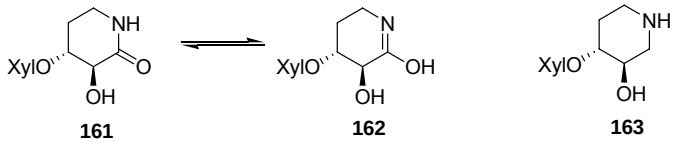
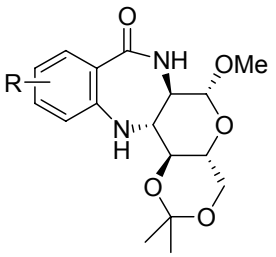


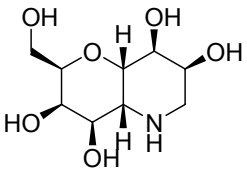
Figure 32

In analogy, this study suggests that the glycosidase inhibition by **36** and similar other compounds such as **159**[15] and **160**[16] may in fact be caused by the H-bonding of the lactam carbonyl moiety with enzyme as the tautomerisation energy for the amide-iminol conversion is of the order of 11 kcal mol^{-1},[17] indicating the concentration of the corresponding iminol form in solution at a given time to be very low. Azasugars **159** and **160** in which sp^3-nitrogen is replaced by a pseudoamide type nitrogen with substantial sp^2-character, had lead to a new group of non basic glycosidase inhibitor (sp^2-azasugar) with high anomer specificity.[15,18]

Recently, research activity in this field has evolved to evaluate hybrid molecules as glycosidase inhibitors and in this context hybrids of D-glucose with some heterocycles,[19,20] D-galactose with 1-deoxynojirimycin[21] and few more related structures have been synthesized and evaluated (**Figure 33**). The possibility of developing new glycosidase inhibitors through this approach is gaining an appreciable importance for future developments of this area.



Hybrids of β-D-glucose-benzoheterodiazepine

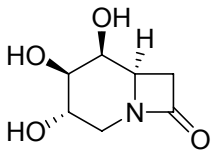


Hybrid of D-Galactose and 1-DNJ

Figure 33: Hybrids of carbasugars

3.2 Design and synthesis of β-lactam azasugar

With aforementioned background, we postulated that β-lactam-azasugar hybrid molecule of type **164** (**Figure 34**) which could also be referred as polyhydroxylated carbacephem, may emerge as a potent glycosidase inhibitor due to its ingenuous conformationally constrained structural features such as a) β-lactam ring compelling the polyhydroxylated piperidine ring to adapt nearly half chair conformation mimicking the shape of glycosidase inhibition transition state b) the carbonyl group in β-lactam ring may provide additional hydrogen bonding site for specific enzyme-substrate interactions.



164

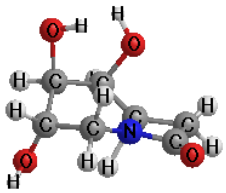
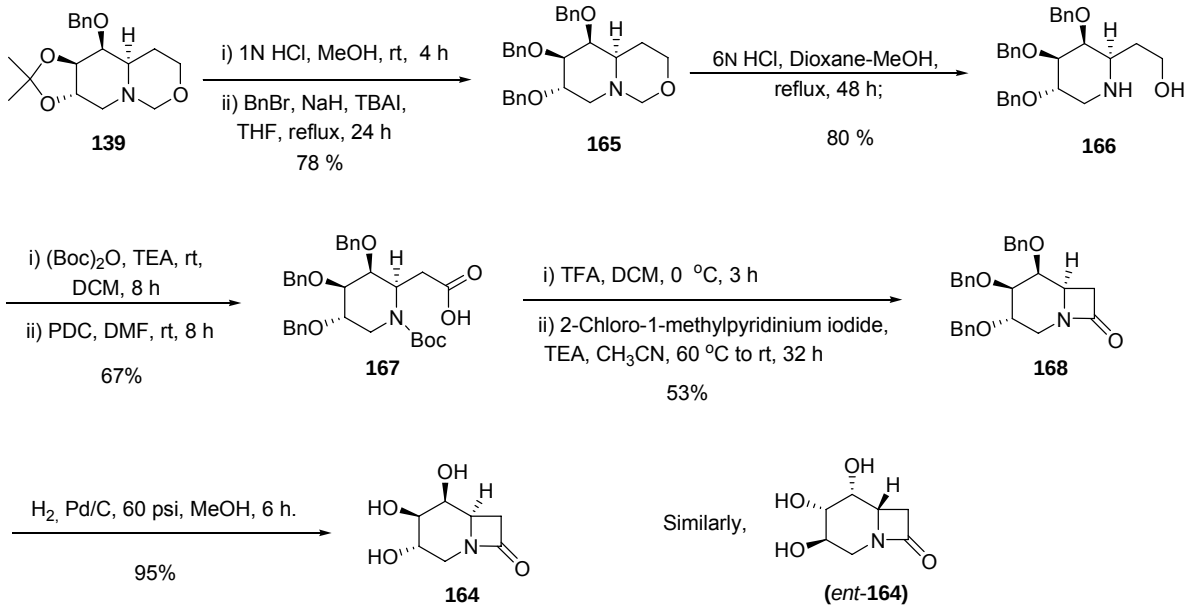


Figure 34: β–Lactam-azasugar

With this hypothesis in mind, we have synthesized β-lactam-azasugar hybrid **164**. The synthesis of **164** began with the intermediate **139** (synthesized earlier, see **Scheme 15,** *Chapter 1*) as shown is **Scheme 21**. Acetonide moiety of **139** was selectively deprotected by stirring with 1N HCl at room temperature for 4 h to afford the corresponding diol. This diol was re-protected with benzyl group to obtain tribenzylated compound **165** in 78 % yield. Subsequently, the 1,3-oxazine ring moiety of tribenzylated compound **165** was cleaved by

refluxing with 6N HCl in dioxane-methanol for 48 h to afford tribenzylated amino alcohol **166.** The structure of **166** was confirmed by the disappearance of 1,3-oxazine methylene protons in the ^{1}H NMR spectrum and the presence of only three methylene (CH_2) carbons in comparison with the ^{1}H NMR and in ^{13}C NMR of **165**. The resultant secondary amine moiety was reprotected as *N*-Boc derivative prior to its PDC oxidation[22] to corresponding acid **167**. The presence of carboxylic acid was confirmed by ^{13}C NMR and DEPT, which indicated two quaternary carbon peaks at δ 155.6 corresponding to *N*–Boc carbonyl and another one at δ 177.4 corresponding to a carbonyl carbon of carboxylic acid. Furthermore, the mass spectrum also showed mass peak at 584 ($M+Na^+$, 100%). Deprotection of *N*-Boc moiety of **167** by stirring with TFA in DCM at 0 °C for 3 h followed by treating it with 2-chloro-1-methylpyridinium iodide (Mukaiyama's reagent)[23] in the presence of the excess of the triethylamine afforded β-lactam **168** in 53% yield. The tribenzylated β-lactam azasugar **168** was confirmed by MS spectrum 466 ($M+Na^+$, 20%). The IR spectrum showed peak at 1752 cm^{-1} corresponding to carbonyl stretching.

Scheme 21. Synthesis of β-lactam-azasugar **164**.



^{13}C NMR and DEPT indicated the presence of a quaternary carbon peak at δ 166.3 confirming amide carbonyl moiety. Removal of the *O*-benzyl protecting groups by the hydrogenation at 60 psi afforded β-lactam-azasugar hybrid molecule **164** in 95 % yield. In order to correlate the enzyme specific inhibition property of **164**, we also synthesized its (L-

galacto configurated) enantiomer-**164 (*ent*-164)** in a similar manner starting with the D-(-)-tartaric acid as shown in **Scheme 21**.

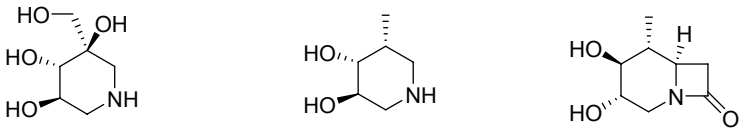
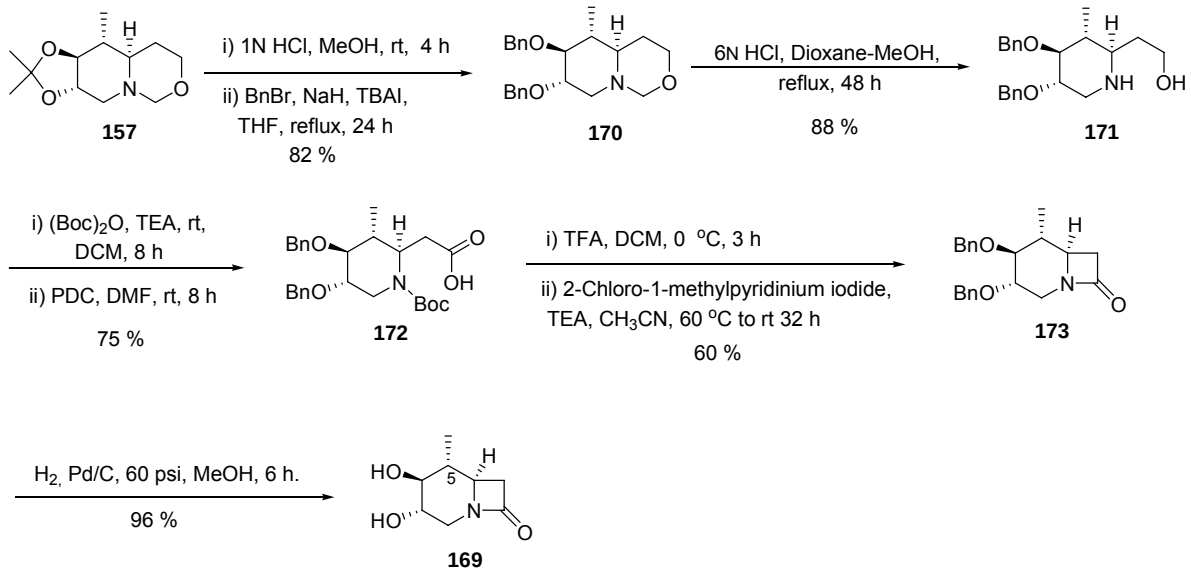


Figure 35: β-Lactam azasugar hybrid **169** and comparative structures

As described in Chapter 2, 1-*N*-iminosugar **152** showed better inhibitory activity for the β-glucosidase (*Ki* = 30 μM) than **151** (*Ki* =90 μM).[24] In this context, β-lactam **169** (**Figure 35**) was also synthesized using the similar procedure on compound **157** which was eventually transformed into (3*S*,4*S*,5*R*,6*R*)-3,4-dihydroxy-5-methyl-1-aza-bicyclo[4.2.0]octan-8-one **(169)** as shown in **Scheme 22**.

Scheme 22. Synthesis of β-lactam-azasugar **169**.



3.3 Enzyme inhibitory activity of β-Lactam-azasugars

The inhibitory activities of **164, *ent*-164** and **169** were screened against β-galactosidase (*Aspergillus oryzae*), α-galactosidase (coffee beans), β-glucosidase / β-mannosidase (almonds), α-glucosidase (yeast), and α-mannosidase (jack beans). The results are summarized in **Table 6**.

Table 6: Enzyme inhibition (*Ki* in μM) for **164**, *ent*-**164**, **169***

Enzyme	**164**	*ent*-**164**	**169**
β- galactosidase	172	n.i.	n.i.
α-galactosidase	900	n.i.	n.i.
β-glucosidase	n.i.	n.i.	n.i.
α-glucosidase	n.i.	n.i.	n.i.
β-mannosidase	n.i.	n.i.	n.i.
α-mannosidase	n.i.	n.i.	n.i.

*, general enzyme inhibition assay procedure is detailed in *Chapter 1* experimental section; n.i = no inhibition up to 1 mM

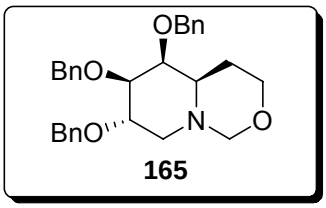
From the above results, it is apparent that the D-galacto-configured β-lactam **169** exhibited moderate, competitive and specific inhibition against β-galactosidase only. It inhibited α-galactosidase very poorly and no inhibition against α- and β-glucosidase as well as α- and β- mannosidase. This enzyme specific inhibition of **164** is in good agreement with its D-galacto-configured structure. Similarly, compound ***ent*-164**, which is L-galacto / L-fuco-configured showed no inhibition against any of the enzymes studied suggesting that it might be specific to fucosidase. Furthermore, β-lactam **169** which lacks hydroxy functionality at C-5 (loss of polarity and a binding site), unfortunately, did not inhibit any of the enzymes studied.

3.4 Summary

In conclusion we have designed, synthesized and evaluated β-lactam-azasugar as a glycosidase inhibitor. The fairly good and specific glycosidase inhibition exhibited by neutral β-lactam-azasugar hybrid molecule **164** points towards the possibility of improving its potency further by incorporating some minor structural variations to its core, such as incorporation of hydroxymethylene functionality at the C-7 of the β-lactam ring with a view that it may provide an additional H-bonding site for recognition and would also increase the polarity of the molecule.

3.5 Experimental Section

1. Preparation of (4a*R*,5S,6*R*,7*S*)-5,6,7-tris(benzyloxy)-octahydropyrido[1,2c][1,3] oxazine:



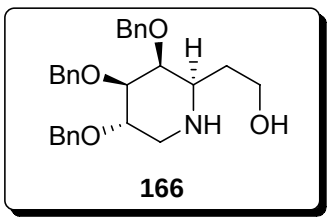
165

To a methanolic (6 mL) solution of **139** (0.56 g, 1.75 mmol) was added 1N HCl (2.5 mL) and mixture was stirred at room temperature for 4 h. The solvent was evaporated to dryness to afford acetonide deprotected diol hydrochloride salt of **139**. Ethyl acetate (10 mL) followed by 2N NaOH (2 mL) was added to the mixture to make the resultant diol free. Two layers were separated and aqueous layer was extracted with ethyl acetate (5 x 5 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed by rotary-evaporation and the residue was pure enough to carry out the next step.

To a suspension of NaH (0.22 g, 5.04 mmol, 60 % granular coated with oil) in dry THF (15 mL) was added solution of acetonide deprotected diol (0.47 g, 1.68 mmol) in dry THF (3mL) at 0 °C and stirred at rt for 15 min. The reaction mixture was cooled to 0 °C and benzyl bromide (0.60 ml, 5.04 mmol) was added dropwise followed by catalytic amount of TBAI and the reaction mixture was refluxed for 24 h. Reaction was quenched at 0 °C by slowly adding water into it. Aqueous layer was extracted with ethyl acetate (5 x 20 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed by rotary-evaporation and the residue was purified by column chromatography (silica, pet ether-ethyl acetate, 7:3) to afford **165** (0.63 g, 78 %) as a colorless liquid.

$[\alpha]^{27}_D$	:	+4.7° (*c* 1.73, CH_2Cl_2)
^{1}H NMR (500 MHz, $CDCl_3$) δ	:	1.07 (d, 1H, *J* = 8.25 Hz), 1.70-2.30((m, 3H), 3.08 (S, 1H), 3.36 (t, 1H, *J* = 9.8 Hz), 3.46 (s, 1H), 3.65 (s, 1H), 3.95 (d, 2H, *J* = 2.8, 10.2 Hz), 3.40-4.80 (m, 8H), 7.20-7.40 (m, 15H)
^{13}C NMR (75 MHz, $CDCl_3$) δ	:	24.9, 53.01, 62.6, 66.8, 72.0, 72.6, 73.2, 74.8, 75.7, 85.8, 127.0, 127.0, 127.2, 127.7,127.8, 138.1, 138.2, 138.3
Mass (m/z %)	:	482 ($M+Na^+$, 90%), 460 (MH^+, 100%)

2. 2-[(2*R*,3*S*,4*R*,5*S*)-3,4,5-tris(benzyloxy)piperidin-2-yl]ethanol:



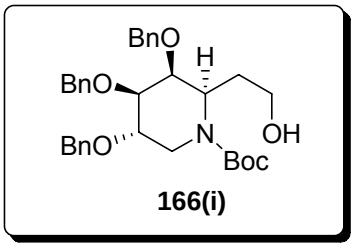
166

To a solution of the **165** (0.16 g, 0.35 mmol) in dioxane:methanol (4:1, 5 mL) was added 2 mL of 6N HCl and

the reaction mixture was refluxed for 48 h. The solvent was evaporated to dryness to afford amino alcohol hydrochloride salt of **166**. Ethyl acetate (10 mL) followed by 2N NaOH was added to the mixture to make the resultant aminol free. Two layers were separated and aqueous layer was extracted with ethyl acetate (5 x 5 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed by rotary-evaporation and the residue was purified by column chromatography (silica, methanol: chloroform, 0.5:9.5) to afford **166** (0.13 g, 80 %)

^{1}H NMR (200 MHz, $CDCl_3$) δ	:	1.30-1.47 (m, 1H), 1.70-1.95 (m, 1H), 2.44 (dd, 1H, *J* = 10.4, 13.4 Hz), 2.75 (dd, 1H, *J* = 2.2, 10.7 Hz), 3.18 (dd, 1H, *J* = 5.2, 13.6 Hz), 3.45 (dd, 1H, *J* = 2.4, 9.1 Hz), 3.62 (m, 4H), 4.50-4.95 (m, 6H), 7.15-7.40 (m, 15H)
^{13}C NMR (50 MHz $CDCl_3$) δ	:	33.7(CH_2), 48.4(CH_2), 59.0(CH), 61.5(CH_2), 72.8(CH_2), 73.12CH_2), 74.6(CH_2), 76.8(CH), 77.8(CH), 84.5(CH), 127.6(CH), 127.7(CH), 127.8(CH), 128.0(CH), 128.3(CH), 128.4(CH), 128.5(CH), 128.6(CH), 138.8(C), 138.9(C), 139.0(C)
Mass (m/z %)	:	448 (MH^+, 100%), 384 (5%)

3. (2*R*,3*S*,4*R*,5*S*)-*t*-butyl-3,4,5-tris(benzyloxy)-2-(2-hydroxyethyl)piperidine-1-carboxylate:



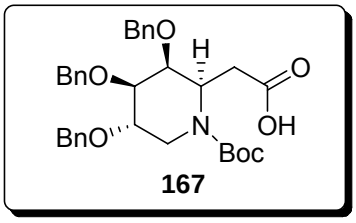
166(i)

A solution of $(Boc)_2O$ (0.07 mL, 0.31 mmol, in 1 mL DCM) was slowly added to a stirring solution of **166** (0.13 g, 0.28 mmol) and Et_3N (0.04 mL, 0.31 mmol) in DCM (4 mL) at 0 °C. The reaction mixture was stirred for 8 h at room temperature. The reaction mixture was diluted with DCM (5 mL) and washed with water (3 x 100 mL) and brine (1 x 100 mL). The organic layer was dried over Na_2SO_4 before concentrating under reduced pressure. The resultant colorless residue was purified by column chromatography (silica, pet ether:ethyl acetate, 6:4) to afford **166(i)** (0.14 g, 88 %) as a colorless oil.

$[\alpha]^{27}_D$	:	+28.3° (*c* 1,$CHCl_3$); *ent*-**166(i)**: -30.5° (*c* 1,$CHCl_3$)
IR ν_{max} cm^{-1} in $CHCl_3$	:	3440, 3016, 2977, 2873, 1654, 1454, 1423, 1367, 1217
^{1}H NMR (500 MHz, $CDCl_3$) δ	:	1.49 (s, 9H), 1.63 (bs, 1H), 2.14 (bs, 1H), 3.16 (d, 1H, *J* = 14.3 Hz), 3.52 (bs, 1H), 3.62 (s, 1H), 3.62 (bs, 1H), 3.89 (s, 1H), 3.98 (d, 1H, *J* = 3.6 Hz), 4.15 (bd, 1H, *J* = 14.7 Hz), 4.42 (d, 1H, *J* = 11.9 Hz), 4.54-4.85 (m, 6H), 7.25-7.40 (m, 15H)
Mass (m/z %)	:	570 ($M+Na^+$, 73%), 547 (M^+, 47%), 447 (MH^+-100, 100%)

Elemental analysis : Anal Calcd. for $C_{33}H_{41}NO_6$: C, 72.37; H, 7.55; N, 2.56. Found C, 72.57; H, 7.53; N, 2.42

4. 2-[(2*R*,3*S*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-1-(*t*-butoxycarbonyl)piperidin-2-yl]acetic acid:



167

To a solution of **166(i)** (0.14 g, 0.25 mmol) in 1.5 mL of DMF was added PDC (0.45g, 1.23 mmol) and mixture was stirred at room temperature for 9 h. The reaction mixture was diluted with 5 mL ethyl acetate followed by 5 mL of water. The organic layer was washed with (2 X 5 mL) water. The solvent was evaporated and the residue was purified by column chromatography (silica, 5:5, pet ether: ethyl acetate) to afford **167** (0.11 g, 76 %).

$[\alpha]^{27}_D$: +27.7° (*c* 0.44, $CHCl_3$); *ent*-**167**: -28.9° (*c* 2, $CHCl_3$)

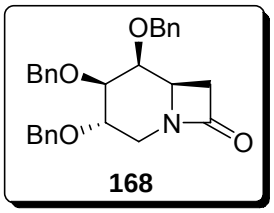
IR (neat) ν_{max} cm^{-1} : 3400-2500, 3020, 1710, 1419, 1365, 1215

^{1}H NMR (200 MHz, $CDCl_3$) δ : 1.35 (s, 9H), 2.6-3.2 (m, 3H), 3.53 (bs, 1H), 3.70-3.90 (broad multiplate, 2H), 4.27 (d, 1H, *J* = 11.7 Hz), 4.4-4.9 (m, 5H). 7.10-7.30 (m, 15H)

^{13}C NMR (75 MHz, $CDCl_3$) δ : 28.3(CH_3), 32.6(CH_2), 35.7(CH_2), 50.4(CH), 70.5(CH_2), 71.4(CH_2), 73.5(CH_2), 74.0(CH), 74.6(CH), 76.2(CH) 80.2(C), 127.5(CH), 128.3(CH), 138.1(C), 138.2(C), 138.5(C), 155.2(C), 177.4(C)

Mass (m/z %) : 584 ($M+Na^+$, 100%), 562 (MH^+, 20%), 462 (MH^+-100, 80%)

5. Preparation of (3*S*,4*R*,5*S*,6*R*)-3,4,5-tris(benzyloxy)-1-aza-bicyclo[4.2.0]octan-8-one



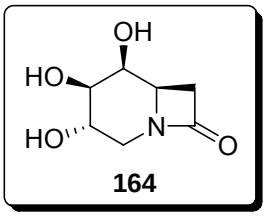
168

To a solution of **167** (0.11 g, 0.186 mmol) in DCM (1 mL) was added TFA (0.15mL. 1.88 mmol) and mixture stirred for 3 h at room temperature. The solvent was evaporated and the residue was vacuum dried and dissolved in dry acetonitrile (15 mL).

To a two neck round bottom flask fitted with reflux condenser containing 2-chloro-1-methylpyridinium iodide (0.15g, 0.56 mmol) in 50 mL was added dry acetonirile (15 mL) and triethyl amine(0.26 mL, 1.86 mmol). The resulting solution was heated at 65-70 °C (oil bath) and to this was slowly added an acetonitrile (15 mL) solution of TFA salt of *N*-Boc-deprotected **167** over a period of 5 h. The deep-red reaction solution was cooled to room temperature and stirred for an additional 24 h. The solvent was removed by rotary-evaporation and the residue was purified by column chromatography (silica, 5: 5, per ether: ethyl acetate) to afford **168** (0.043 g, 53%).

$[\alpha]^{27}_D$	:	+47.8° (*c* 1.25, MeOH); *ent*-**168**: -42.3° (*c* 0.46, MeOH)
IR ν_{max} cm^{-1} in $CHCl_3$	:	3018, 1751, 1363, 1215
^{1}H NMR (500 MHz, $CDCl_3$) δ	:	2.58 dd, 1H, *J* = 1.4, 9.6 Hz, 2.75 [two set of dd, 1H, *J* = (1.8, 4.6 Hz) for first dd, *J* = (1.4, 4.6 Hz) for second dd and *J* = 14.2 Hz]. 2.94 [two set of d, 1H, *J* = 1.4 Hz for first d, *J* = 1.8 Hz for second d, and *J* = 14.2 Hz], 3.35-3.38 (m, 1H), 3.85 (app t, 1H, *J* = 1.8, 2.3 Hz), 3.60-4.10 (m, 2H), 4.6 (app t, *J* = 11.4, 11.9 Hz), 4.64 (d, 1H, *J* = 11.9 Hz), 4.67 (d, 1H, *J* = 11.4 Hz), 4.71 (d,1H, *J* = 11.9 Hz), 4.87 (d, 1H, *J* = 11.9 Hz), 7.10-7.25 (m, 15H)
^{13}C NMR (125 MHz, $CDCl_3$) δ	:	38.8(CH_2), 42.7(CH_2), 50.0(CH), 73.1(CH_2), 73.3(CH_2), 73.7(CH), 73.9(CH_2), 77.0(CH), 82.9(CH), 127.3(CH), 127.5(CH), 128.2(CH), 137.8(C), 138.0(C), 138.1(C), 166.3(C)
Mass (m/z %)	:	909 ($M+M+Na^+$, 8%) 466 ($M+Na^+$, 20%), 444 (MH^+, 3%), 308 (45%), 208 (100%)

6. Preparation of (3*S*,4*R*,5*S*,6*R*)-3,4,5-trihydroxy-1-aza-bicyclo[4.2.0]octan-8-one

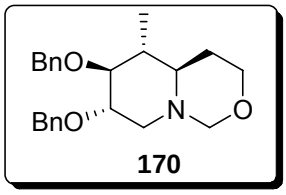


164

A solution of **168** (0.043 g, 0.096 mmol) in methanol (3 mL) was hydrogenated at 60 *psi* pressure in the presence of Pd on charcoal (10%, 0.003 g) for 6 h. The reaction mixture was passed through a short pad of celite and the solvent was removed under reduced pressure to afford **164** (0.016 g, 95 %) as a gummy liquid.

$[\alpha]^{27}_D$	:	+19.7° (*c* 0.25, MeOH); ent-164: -22.3° (c 0.1, MeOH)
^{1}H NMR (200 MHz, D_2O) δ	:	2.82 (app t, 1H, *J* = 11.2, 11.6 Hz), 3.05-3.12 (broad multiplate, 2H), 3.76 (dd, 1H, *J* = 2.3, 9.7 Hz), 3.81-3.85 (m, 1H), 3.90-3.96 (m, 1H), 4.70 (dd, 1H, *J* = 6.8, 13.0 Hz), 4.24 (app t, 1H, *J* = 1.9, 2.3 Hz).
^{13}C NMR (50 MHz, D_2O) δ	:	37.3(CH_2), 43.2(CH_2), 50.3(CH), 64.6(CH), 68.2(CH), 73.4(CH), 169.5(C).
Mass (m/z %)	:	196 ($M+Na^+$, 100%), 174 (MH^+, 20%), 155 (18%)

7. Preparation of (4a*R*,5*S*,6*S*,7S)-6,7-bis(benzyloxy)-5-methyl-octahydropyrido[1,2-c][1,3]oxazine:



170

Compound **157** was converted to **170** using procedure similar to the one used for **165**.

$[\alpha]^{27}_D$	:	+7.1° (*c* 1.2, CH_2Cl_2)
^{1}H NMR (200 MHz, $CDCl_3$) δ	:	0.89 (d, 3H, *J* = 6.3 Hz), 1.30-1.70 (m, 4H), 1.83(app t, 1H, *J* =10.5, 10.6 Hz), 2.80-2.98(m, 2H), 3.33 (dt, 1H, *J* = 2.3, 11.4 Hz), 3.46 (d, 1H, *J* = 7.8

Hz), 3.50-3.74 (m, 1H), 3.98 (dd, 1H, *J* = 4.3, 11.4 Hz), 4.27 (d, 1H, *J* = 7.7 Hz) 4.45-4.62 (m, 3H), 4.89 (d, 1H, *J* =10.9 Hz)

^{13}C NMR (50 MHz, $CDCl_3$) δ : 13.0(CH_3), 29.9(CH_2), 41.5(CH), 52.1(CH_2), 64.6(CH), 67.5(CH_2), 72.5(CH_2), 75.3(CH_2), 79.9(CH), 85.7(CH), 86.2(CH_2), 127.0(CH), 127.6(CH), 127.7(CH), 127.9(CH), 127.9(CH), 128.1(CH), 128.4(CH), 128.5(CH), 128.6(CH), 138.5(C), 138.8(C).

Mass (m/z %) : 390 ($M+Na^+$, 88%), 368 (MH^+, 90%)

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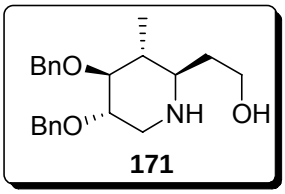
8. Preparation of 2-[(2*R*,3*S*,4*S*,5*S*)-4,5-bis(benzyloxy)-3-methylpiperidin-2-yl]ethanol

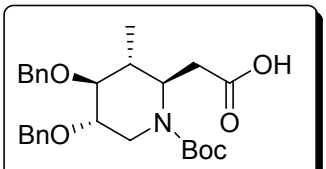


171

Compound **170** was converted to **171** using procedure similar to the one used for **166**.

^{13}C NMR (50 MHz, $CDCl_3$) δ : 14.1(CH_3), 32.6(CH_2), 40.6(CH), 46.8(CH_2), 60.5(CH_2), 61.2(CH), 72.7(CH_2), 75.4(CH_2), 79.4(CH), 84.7(CH), 127.7(CH), 127.8(CH), 128.4(CH), 138.1(C), 138.5(C).

9. Preparation of 2-((2*R*,3*S*,4*S*,5*S*)-4,5-bis(benzyloxy)-1-(*t*-butoxycarbonyl)-3-methylpiperidin-2-yl)acetic acid



172

Compound **171** was converted to **172** using procedure similar to the one used for **167**.

$[\alpha]^{27}_D$: +22.7° (*c* 1.1, $CHCl_3$)

^{1}H NMR (200 MHz, $CDCl_3$) δ : 1.07 (d, 3H, *J* = 7.2 Hz), 1.88-2.00 (m, 1H), 2.76 (dd, 2H, *J* = 4.17, 6.57 Hz) 3.11 (d, 1H, *J* = 2.2, 14.9 Hz), 3.31 (app t, 1H, *J* = 3.7, 3.8 Hz), 3.50 (d, 1H, *J* = 2.5 Hz), 4.10- 4.36 (m, 3H), 4.44 (s, 2H), 4.65 (d, 1H, *J* = 11.62 Hz)

^{13}C NMR (50 MHz, $CDCl_3$) δ : 17.5(CH_3), 26.3(CH_3), 35.4(CH), 37.5(CH_2), 52.8(CH), 59.7(CH_2), 70.3(CH_2), 71.9(CH_2), 75.5(CH), 79.9(CH), 127.5(CH), 127.5(CH), 127.6(CH), 128.3(CH), 128.4(CH), 138.2(C), 155.6(C), 177.2(C)

Mass (m/z %) : 492 ($M+Na^+$ 100%), 470 (MH^+, 43%)

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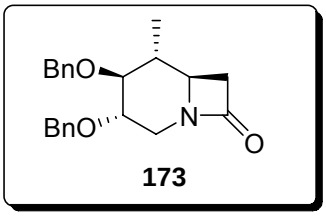
10. Preparation of (3*S*,4*S*,5*S*,6*R*)-3,4-bis(benzyloxy)-5-methyl-1-aza-bicyclo[4.2.0]octan-8-one



173

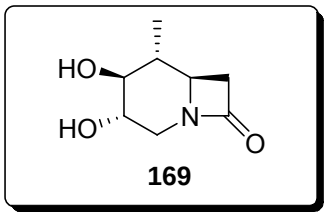
Compound **172** was converted to **173** using the procedure similar to the one used for **168**.

$[\alpha]^{27}_D$: +13.8° (*c* 1.3, MeOH)

IR (neat) ν_{max} cm^{-1} : 3020, 1753, 1365, 1210.

^{1}H NMR (500 MHz, $CDCl_3$) δ	:	1.04 (d, 3H, *J* 6.4), 146-1.56 (m,1H), 2.56 (two sets of d, app dd, 1H, *J* = 1.4, 14.5 Hz), 2.70 (ddd, 1H, *J* = 1.4, 10.2, 13.1 Hz), 3.01 (ddd, 1H, *J* = 1.4, 4.4, 9.7 Hz), 3.12 [two sets of dd, 1H, *J* = (1.4, 4.6 Hz), (1.4, 4.6 Hz) and 14.5 Hz], 3.20 (app t, 1H, *J* = 9.2, 10.1 Hz), 3.49-3.55 (m, 1H), 4.15 (dd, 1H, *J* = 6.4, 13.3 Hz), 4.60-4.70 (m, 3H), 4.96 (d, 1H, *J* = 10.5 Hz).
^{13}C NMR (125 MHz, $CDCl_3$) δ	:	13.38(CH_3), 42.35(CH), 42.49(CH_2), 43.52(CH_2), 51.64(CH), 72.68(CH_2), 75.45(CH_2), 79.21(CH), 83.65(CH), 127.44(CH), 127.50(CH), 127.62(CH), 127.82(CH), 128.13(CH), 128.23(CH), 137.68(C), 138.05(C), 165.70(C).
Mass (m/z %)	:	374 ($M+Na^+$, 100%), 352 (MH^+, 20%)

11. Preparation of (3*S*,4*S*,5*S*,6*R*)-3,4-dihydroxy-5-methyl-1-aza-bicyclo[4.2.0]octan-8-one



169

Compound **173** was converted to **169** using the procedure similar to the one used for **164**.

$[\alpha]^{27}_D$	:	+15.8° (*c* 0.18, MeOH)
IR ν_{max} cm^{-1} in $CHCl_3$	:	3440, 1750, 1212
^{1}H NMR (500 MHz, D_2O) δ	:	0.84 (d, 3H, *J* = 6.6 Hz), 1.27-1.36 (m, 1H), 2.46-2.59 (m, 2H), 2.96 [two set of dd, 1H, *J* = (2.2, 4.4), (1.6, 4.4) Hz and 14.8 Hz], 3.03 (dd, 1H, *J* = 4.4, 9.9 Hz), 3.08 (app t, 1H, *J* = 9.3, 10.4 Hz), 3.30-3.36 (m, 1H), 3.78 (dd, 1H, *J* = 6.0, 13.2 Hz)
^{13}C NMR (125 MHz, D_2O) δ	:	12.2(CH_3), 41.7(CH), 42.2(CH_2), 43.8(CH_2), 52.2(CH), 70.5(CH), 76.2(CH), 169.8(C)
Mass (m/z %)	:	194 ($M+Na^+$, 100%), 172 (MH^+, 15%)

3.6 References

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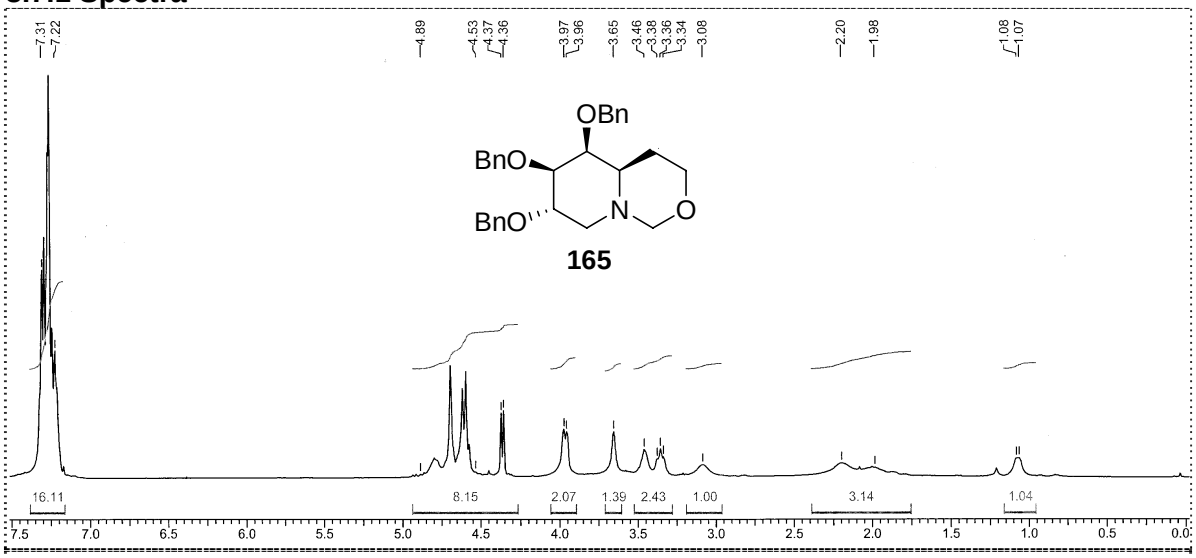
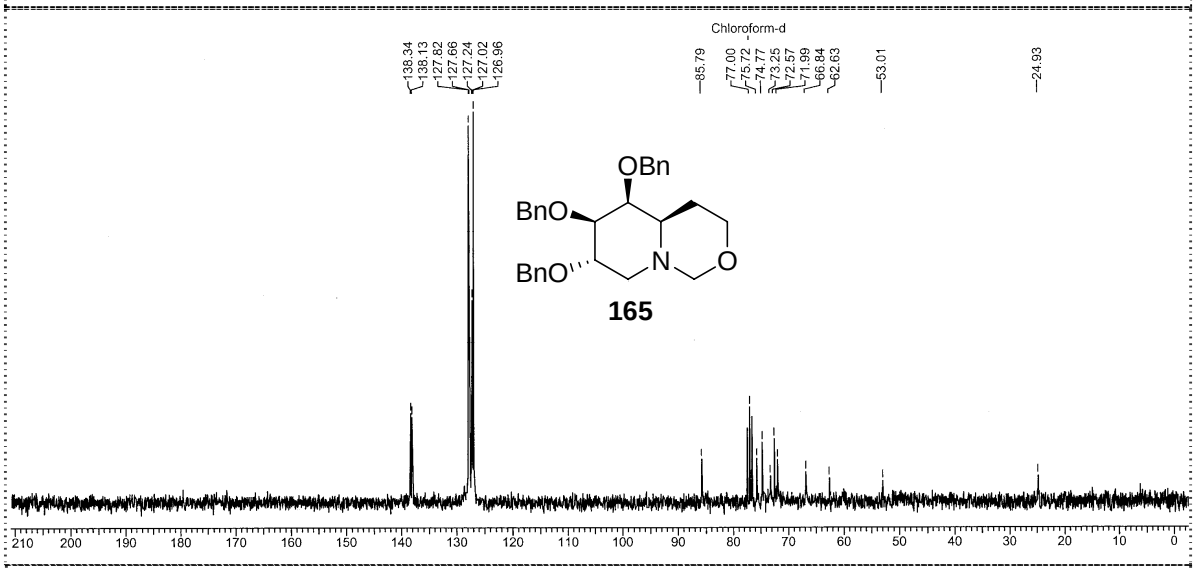
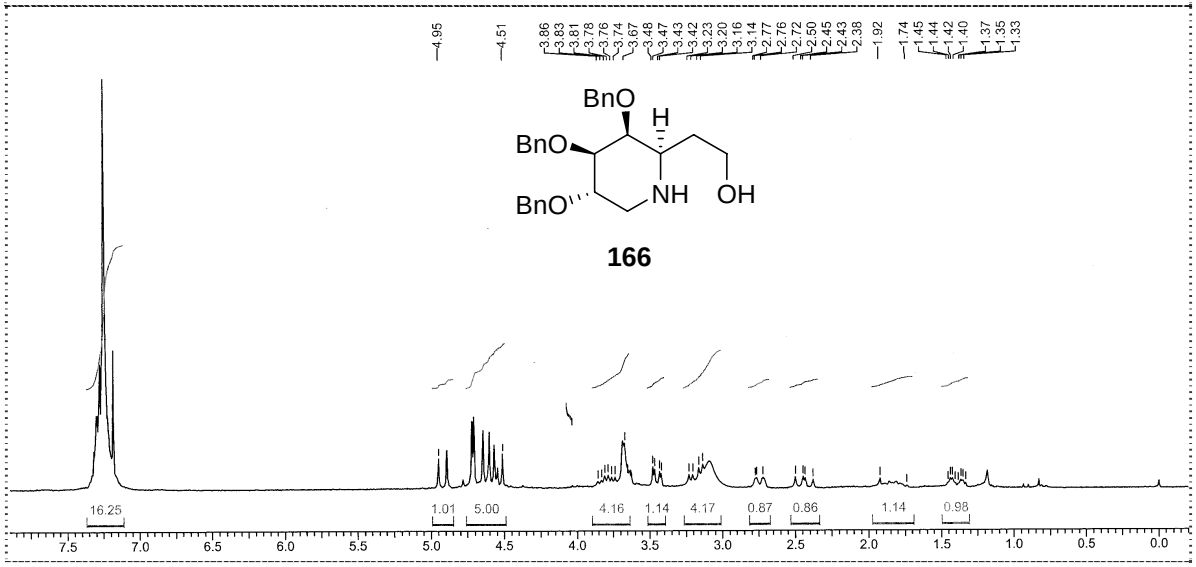
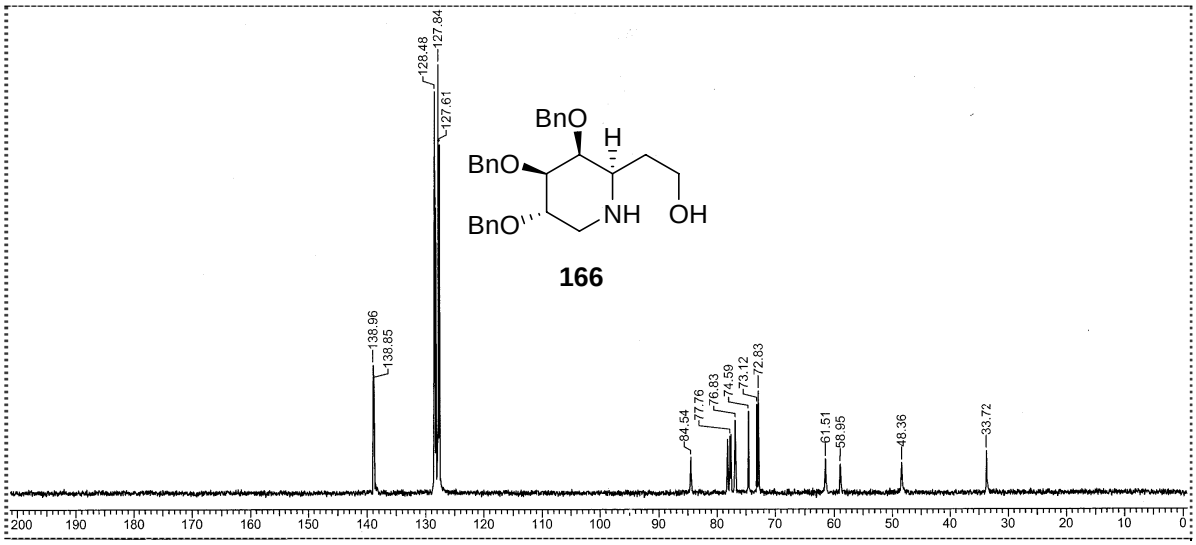
3.7 Spectra and Lineweaver-Bruke plots

3.7.1 Spectra





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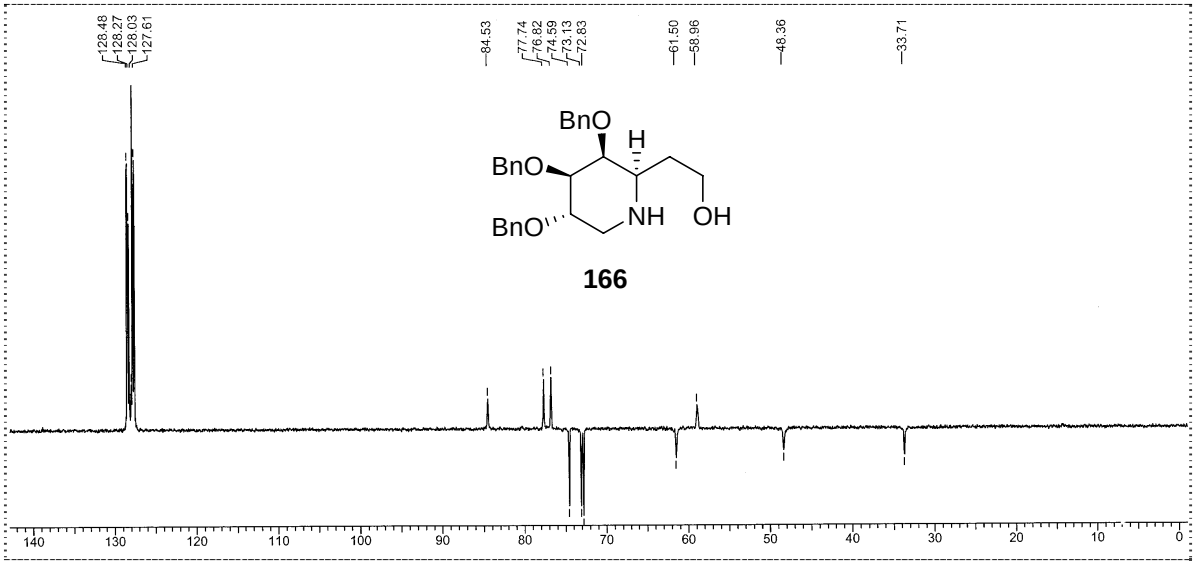
33.72
BnO
H
BnO
NH
OH
BnO

166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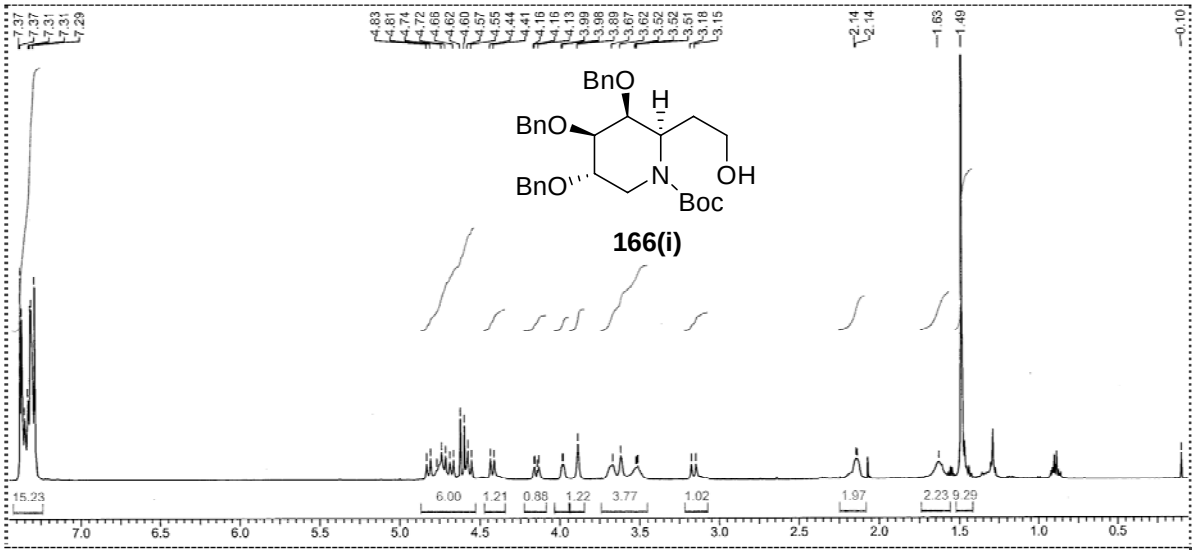
33.71
BnO
H
BnO
NH
OH
BnO

166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4.57
4.55
4.44
4.41
4.16
4.13
3.99
3.98
3.89
3.67
3.62
3.52
3.51
3.18
3.15
2.14
2.14
1.63
1.49
0.10
BnO
H
BnO
N
OH
Boc
BnO
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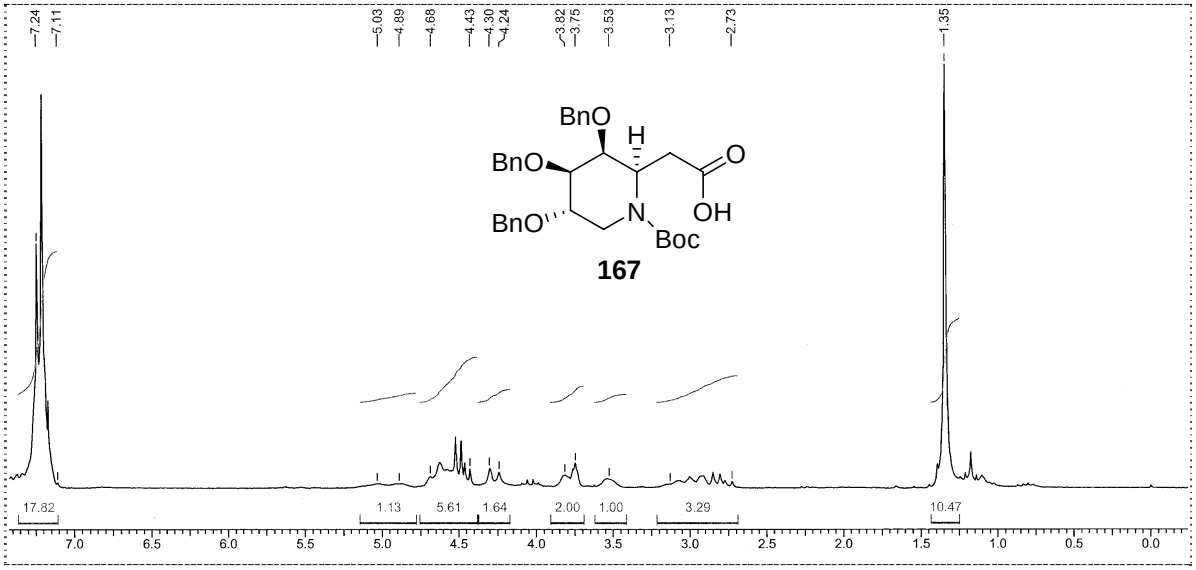
1.22
3.77
1.02
1.97
2.23
9.29

166(i)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4.24
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3.53
3.13
2.73
1.35
BnO
H
BnO
O
OH
N
BnO
Boc
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1.13
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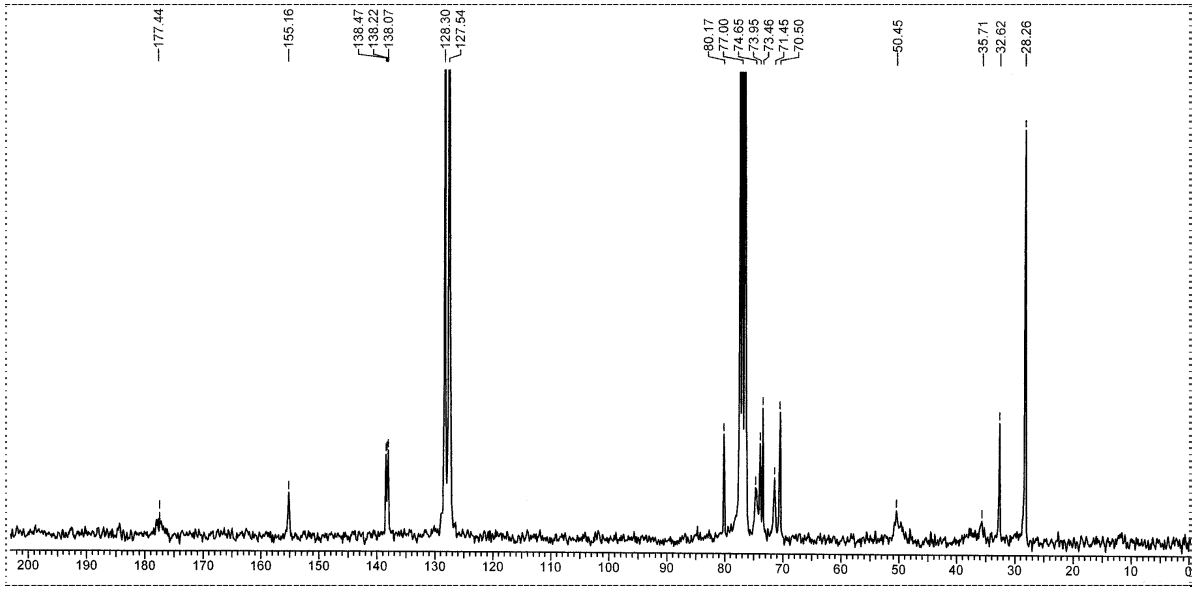
1.64
2.00
1.00
3.29
10.47

167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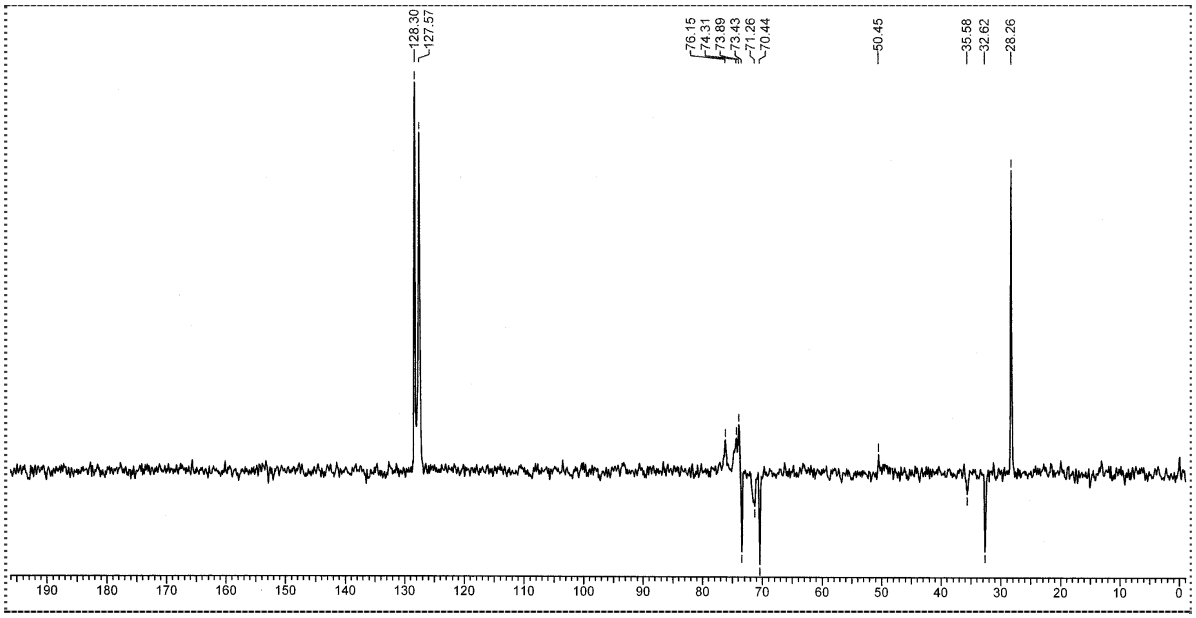
73.46
71.45
70.50
50.45
35.71
32.62
28.26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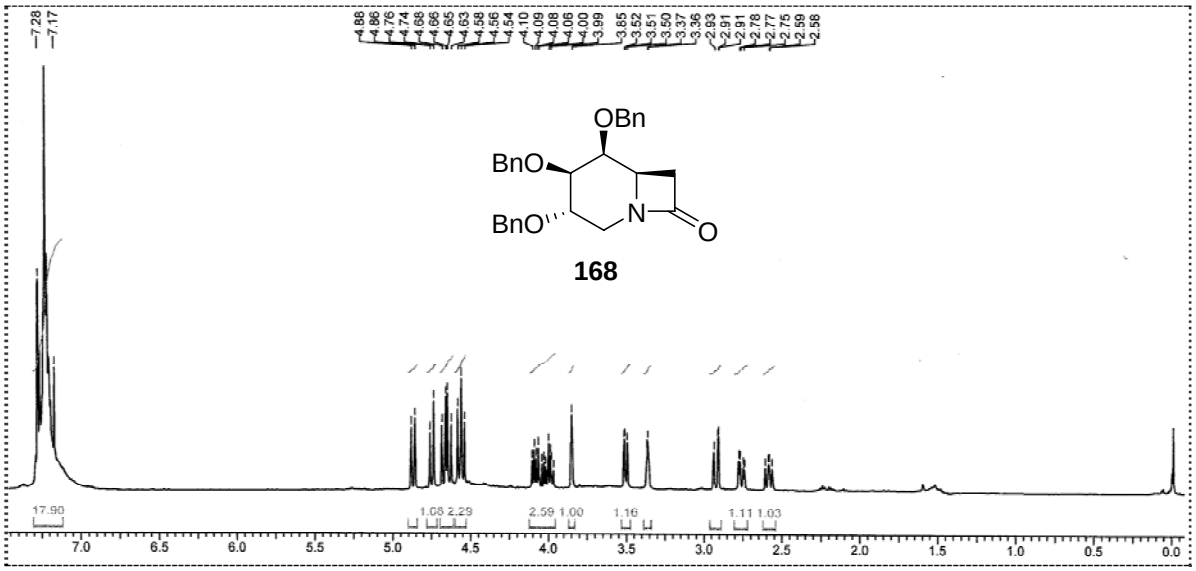
35.58
32.62
28.26



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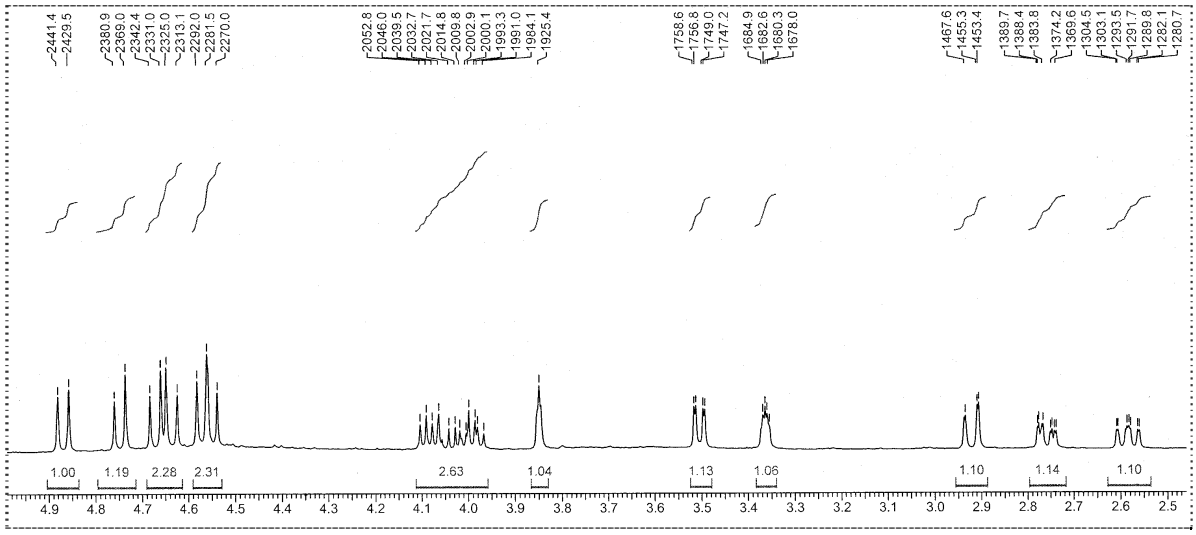

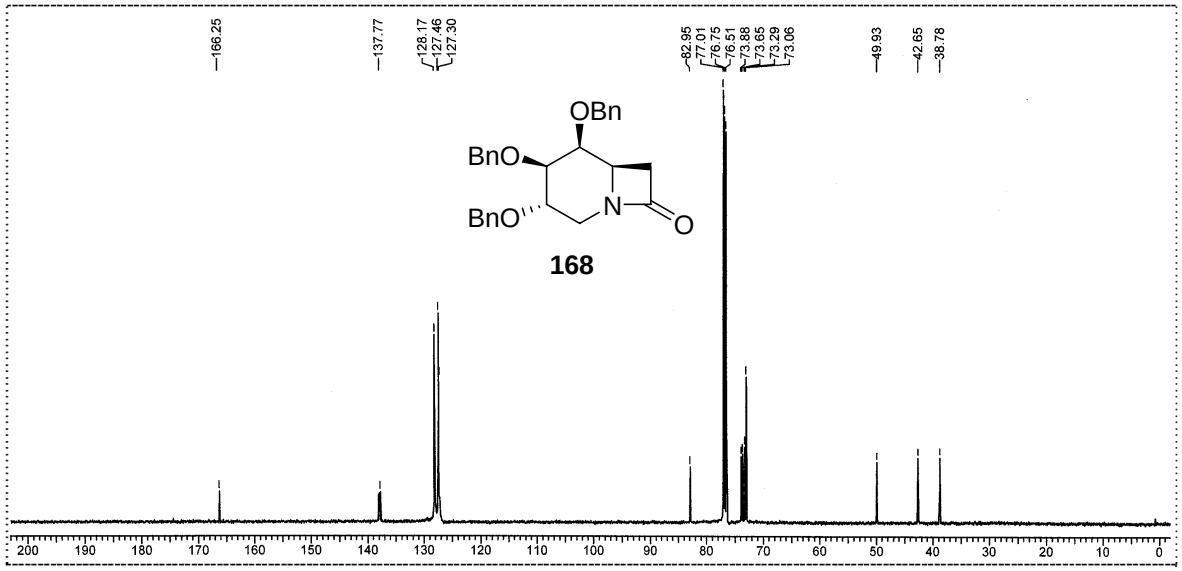

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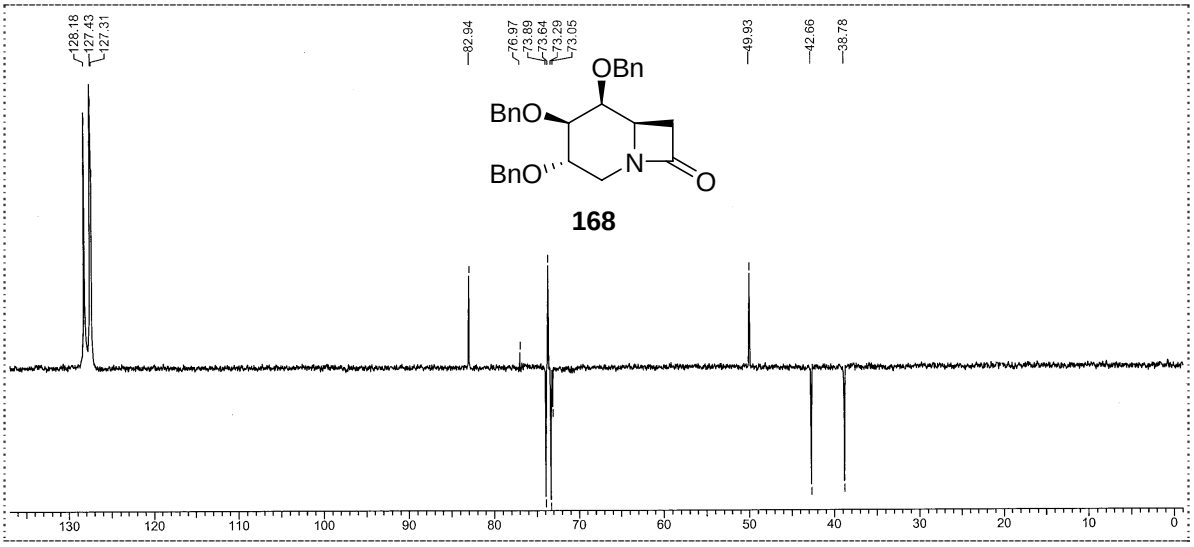
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127.31
82.94
76.97
73.89
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73.29
73.05
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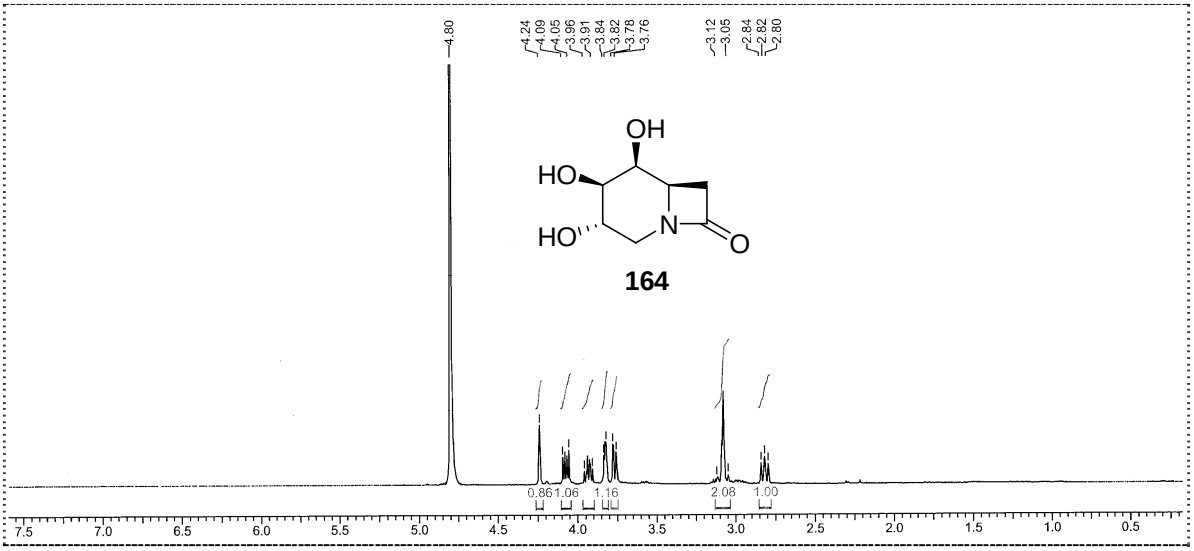
42.66
38.78
OBn
BnO
BnO
N
O
168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4.24
4.09
4.05
3.96
3.91
3.84
3.82
3.78
3.76
3.12
3.05
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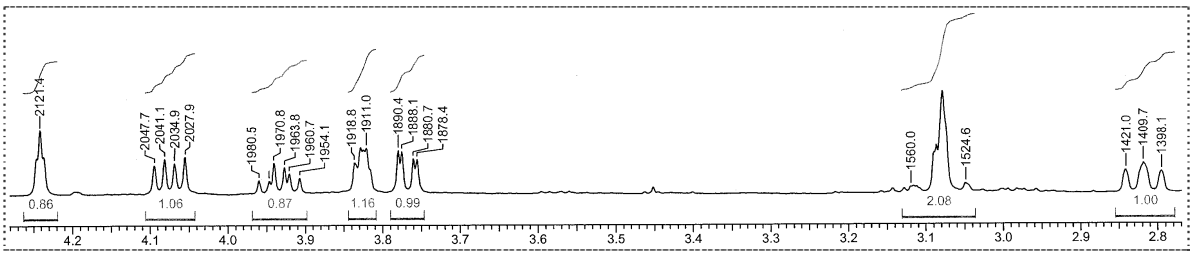
HO
HO
N
O
164
0.86
1.06
1.16
2.08
1.00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1980.5
1970.8
1963.8
1960.7
1954.1
1918.8
1911.0
1890.4
1888.1
1880.7
1878.4
1560.0
1524.6
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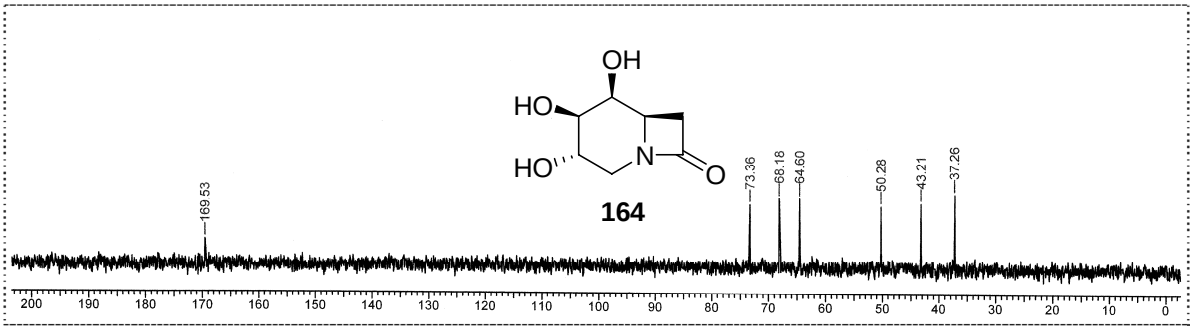
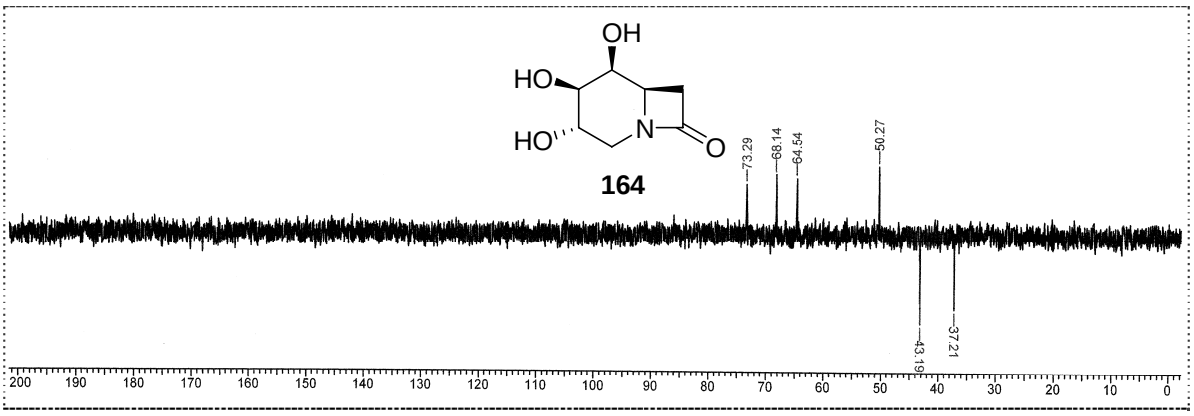
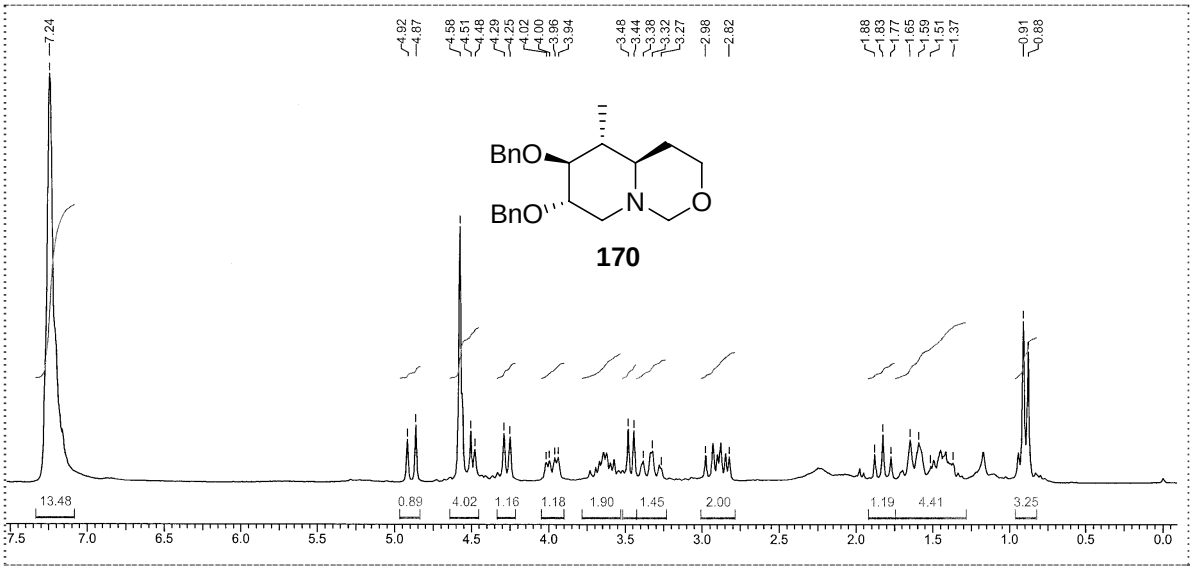
1409.7
1398.1
0.86
1.06
0.87
1.16
0.99
2.08
1.00


OH
HO
HO
N
O
164
169.53
73.36
68.18
64.60
50.28
43.21
37.26


OH
HO
HO
N
O
164
73.29
68.14
64.54
50.27
43.19
37.21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4.25
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4.00
3.96
3.94
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3.44
3.38
3.32
3.27
2.98
2.82
1.88
1.83
1.77
1.65
1.59
1.51
1.37
0.91
0.88
BnO
BnO
N
O
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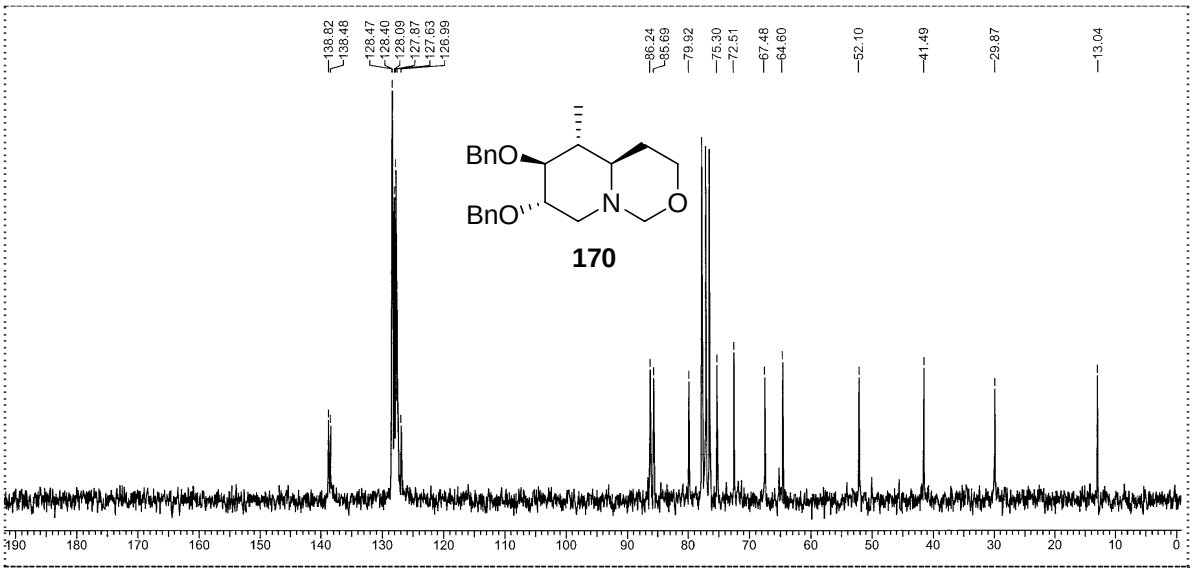
0.89
4.02
1.16
1.18
1.90
1.45
2.00
1.19
4.41
3.25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79.92
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67.48
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41.49
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13.04
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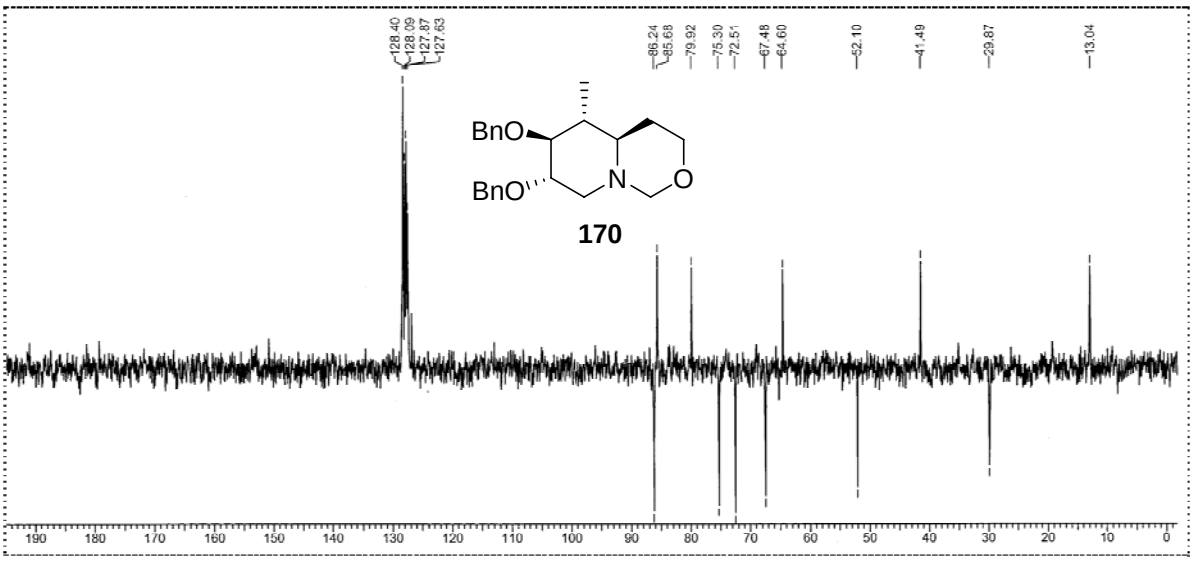
BnO
N
O
170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79.92
75.30
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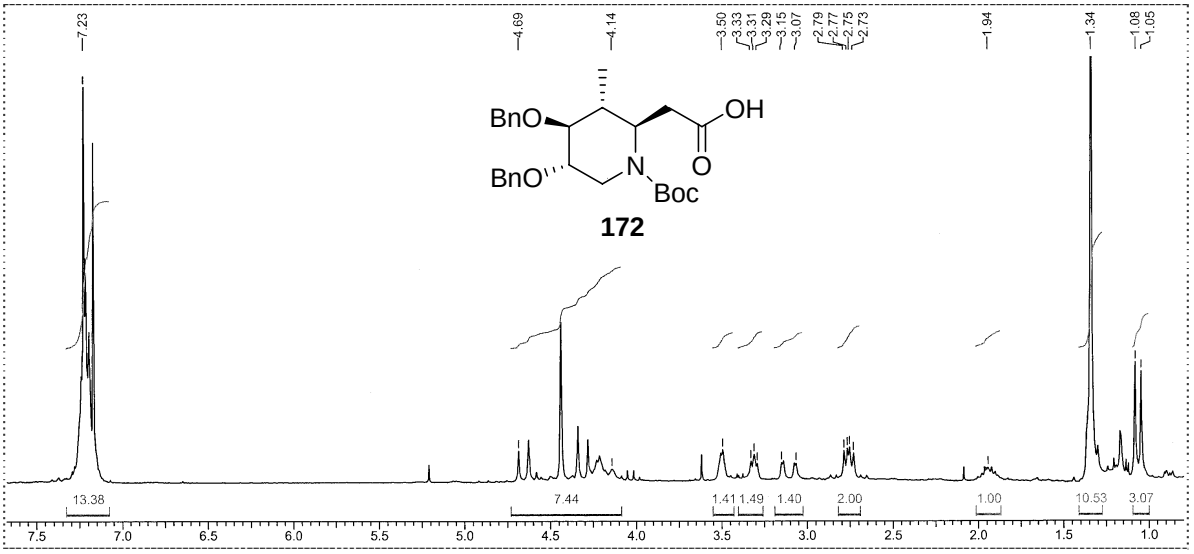
67.48
64.60
52.10
41.49
29.87
13.04
BnO
BnO
N
O
170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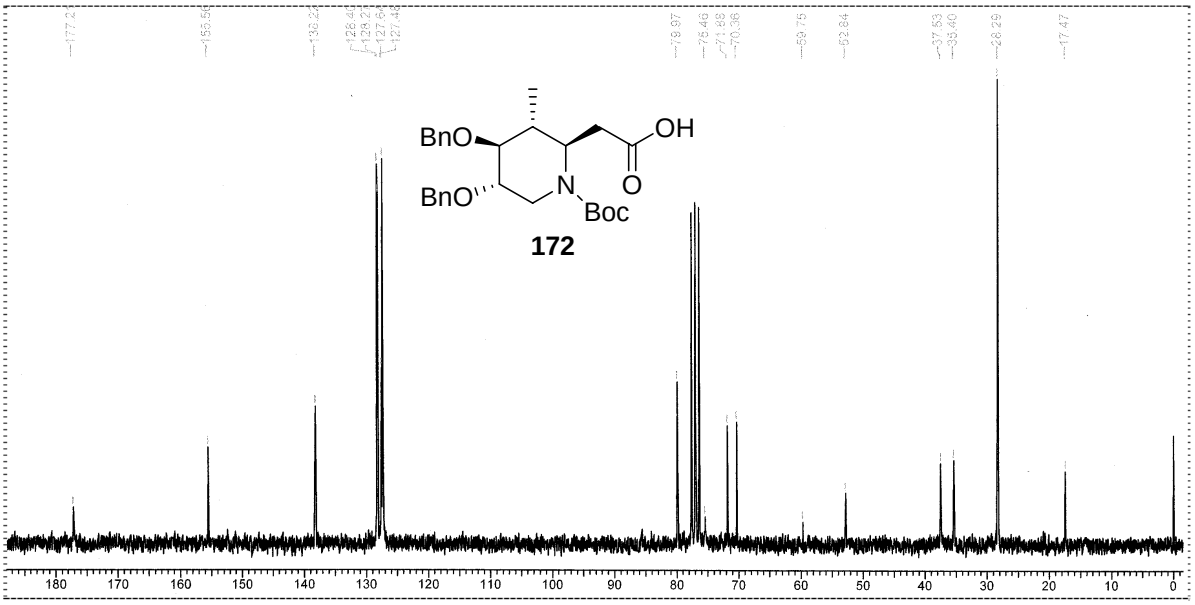
3.31
3.29
3.15
3.07
2.79
2.77
2.75
2.73
1.94
1.34
1.08
1.05
BnO
BnO
N
Boc
O
OH
172
13.38
7.44
1.41
1.49
1.40
2.00
1.00
10.53
3.07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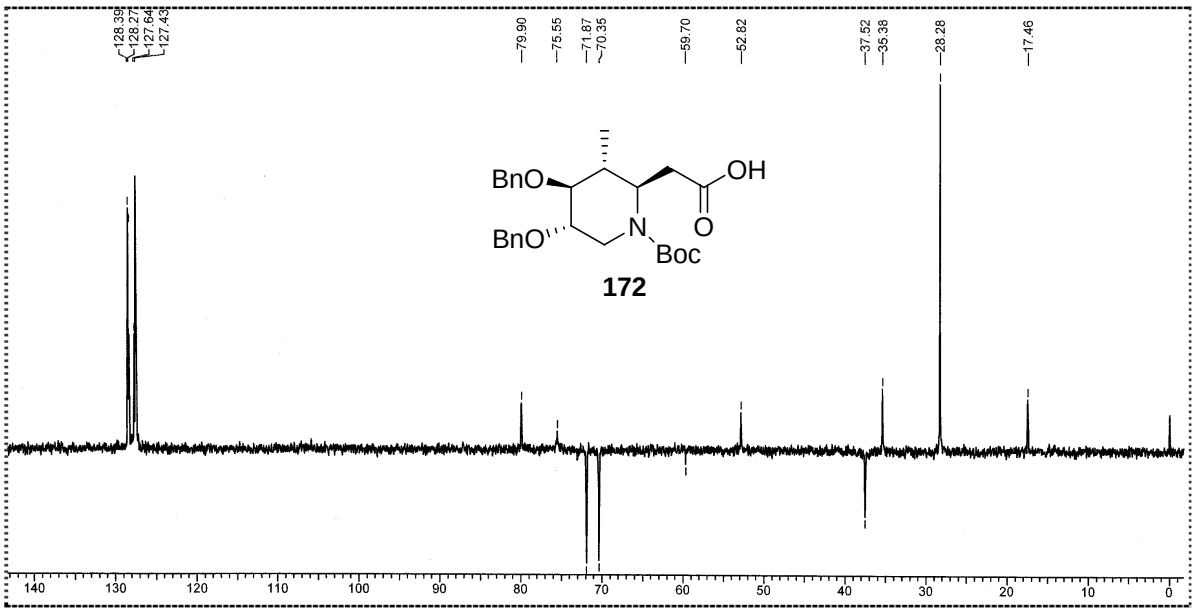
128.23
127.64
127.48
78.97
75.46
71.68
70.36
69.75
62.84
37.53
35.40
28.29
17.47
BnO
BnO
N
Boc
O
OH
172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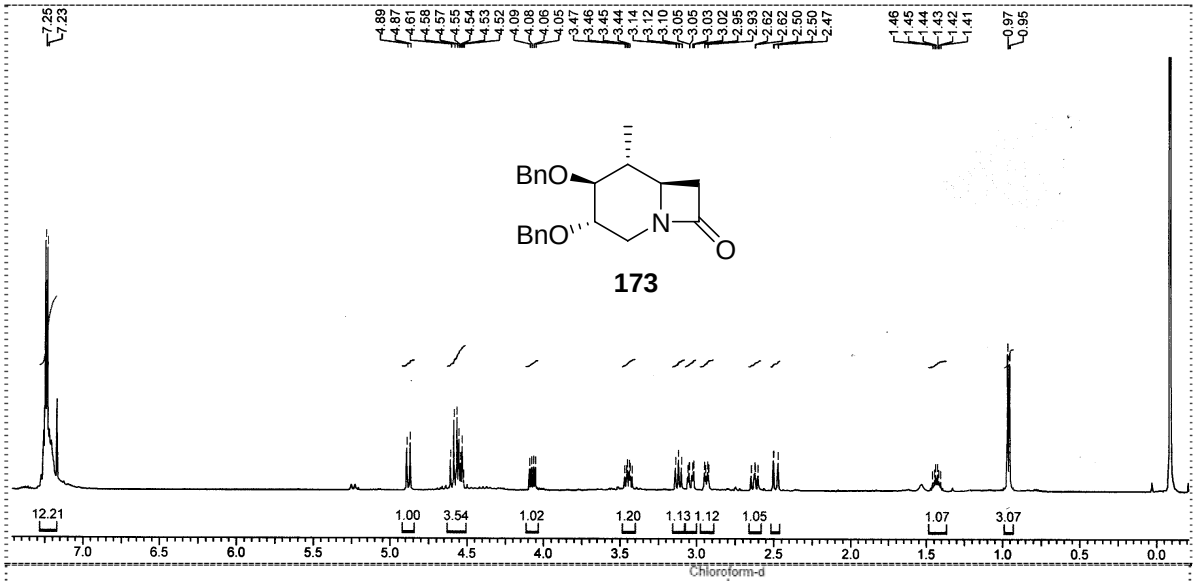
128.27
127.64
127.43
79.90
75.55
71.87
70.35
59.70
52.82
37.52
35.38
28.28
17.46
BnO
BnO
OH
O
N
Boc

172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7.23
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4.87
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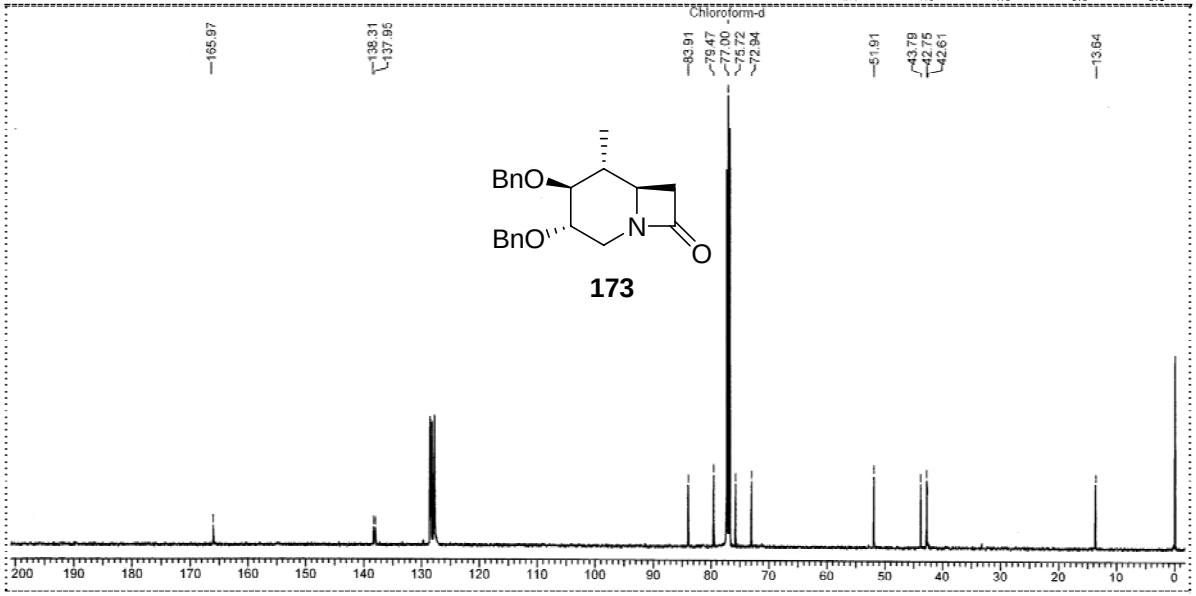
4.58
4.57
4.55
4.54
4.53
4.52
4.09
4.08
4.06
4.05
3.47
3.46
3.45
3.44
3.14
3.12
3.10
3.05
3.05
3.03
3.02
2.95
2.93
2.62
2.62
2.50
2.50
2.47
1.46
1.45
1.44
1.43
1.42
1.41
0.97
0.95
BnO
BnO
N
O
12.21
1.00
3.54
1.02
1.20
1.13
1.12
1.05
1.07
3.07

173


Chloroform-d
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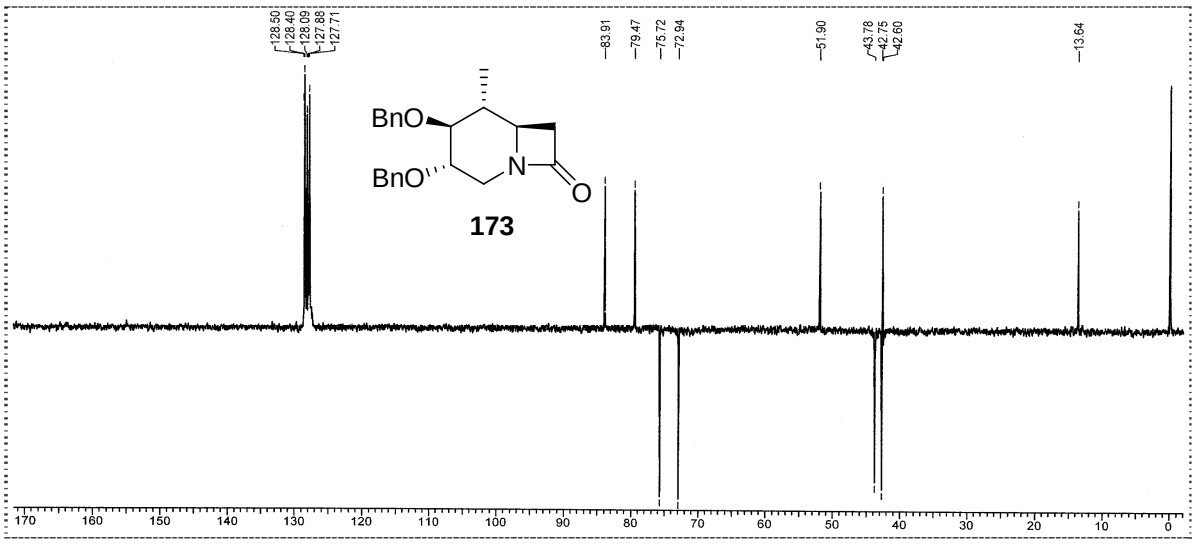
83.91
79.47
77.00
75.72
72.94
51.91
43.79
42.75
42.61
13.64
BnO
BnO
N
O

173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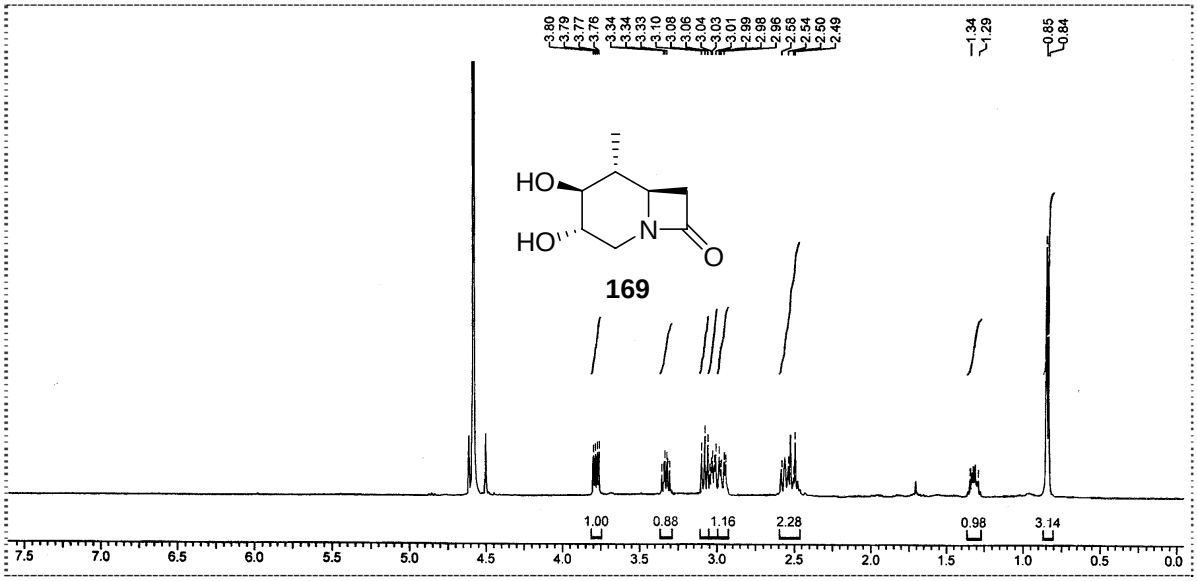
128.40
128.09
127.88
127.71
83.91
79.47
75.72
72.94
51.90
43.78
42.75
42.60
13.64
BnO
BnO
N
O
173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3.77
3.76
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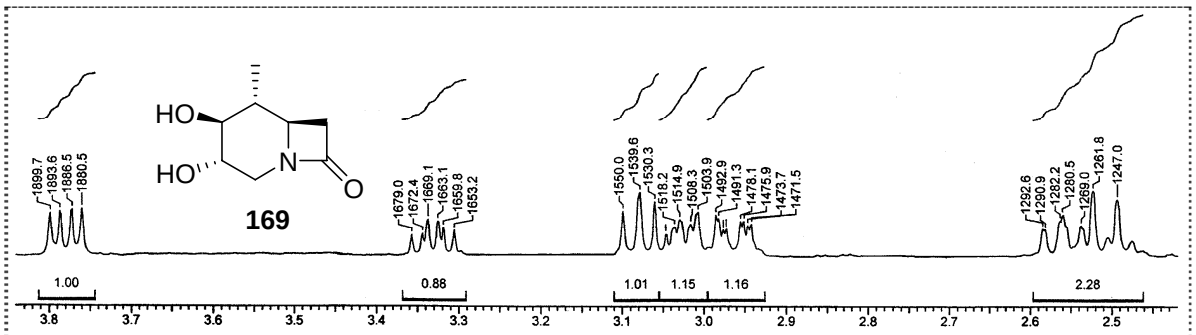
3.34
3.33
3.10
3.08
3.06
3.04
3.03
3.01
2.99
2.98
2.96
2.66
2.64
2.50
2.49
1.34
1.29
0.85
0.84
HO
HO
N
O
169
1.00
0.88
1.16
2.28
0.98
3.14


1999.7
1893.6
1886.5
1880.5
HO
HO
N
O
169
1679.0
1672.4
1669.1
1663.1
1659.8
1653.2
1550.0
1539.6
1530.3
1518.2
1514.9
1508.3
1503.9
1492.9
1491.3
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1471.5
1292.6
1290.9
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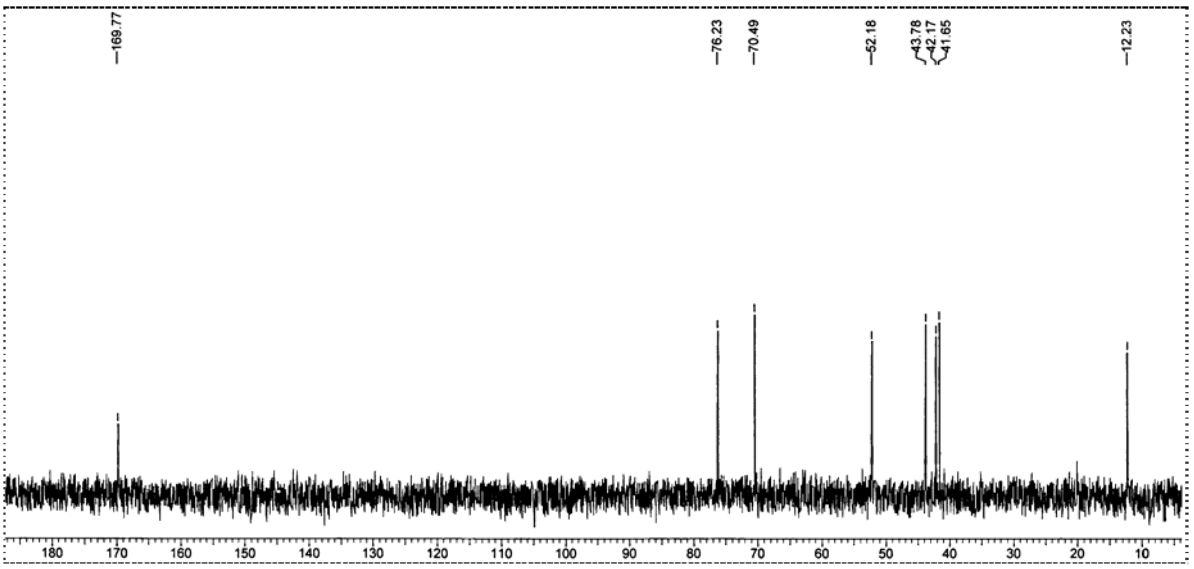
1247.0
1.00
0.88
1.01
1.15
1.16
2.28


169.77
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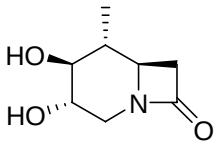
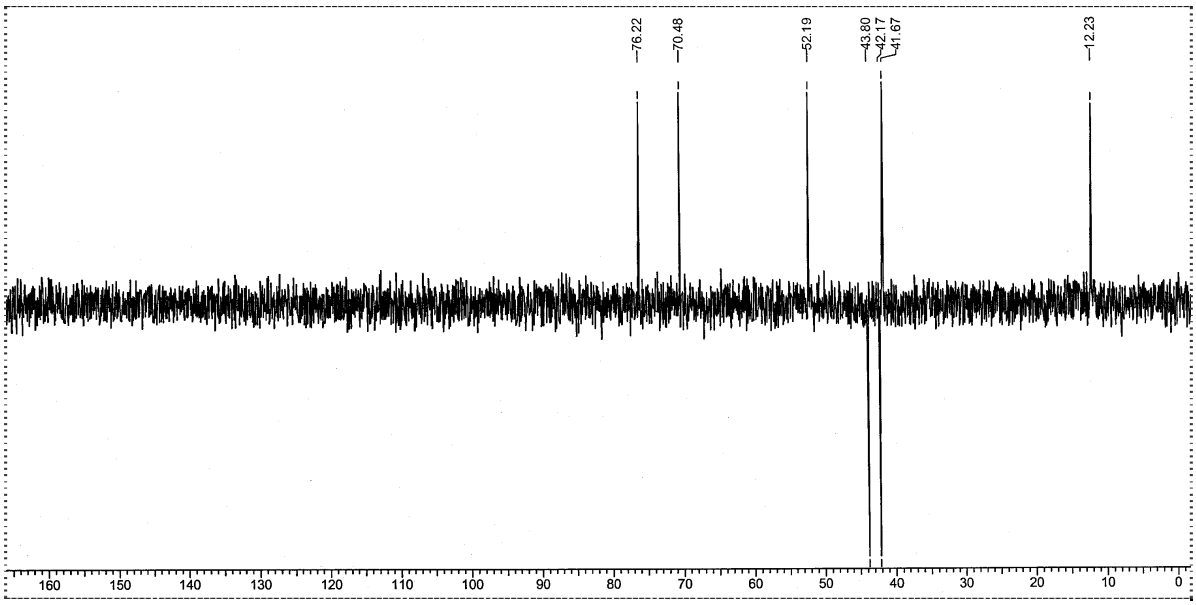
70.49
52.18
43.78
42.17
41.65
12.23
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10


HO
HO
N
O

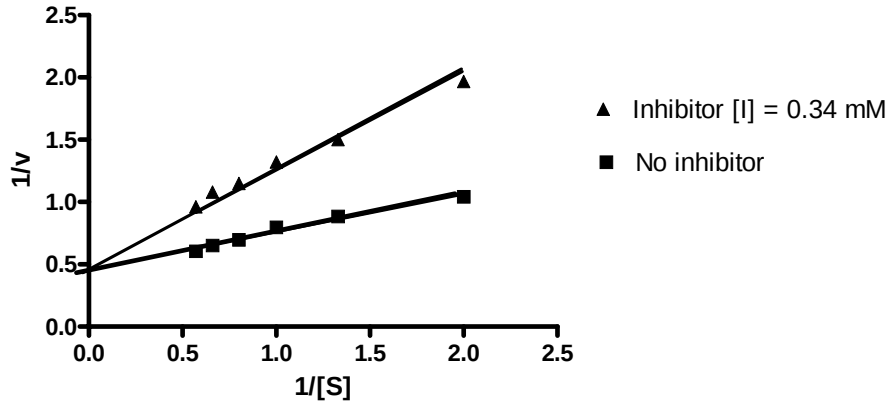
169


76.22
70.48
52.19
43.80
42.17
41.67
12.23
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

3.7.2 Lineweaver-Burke plots

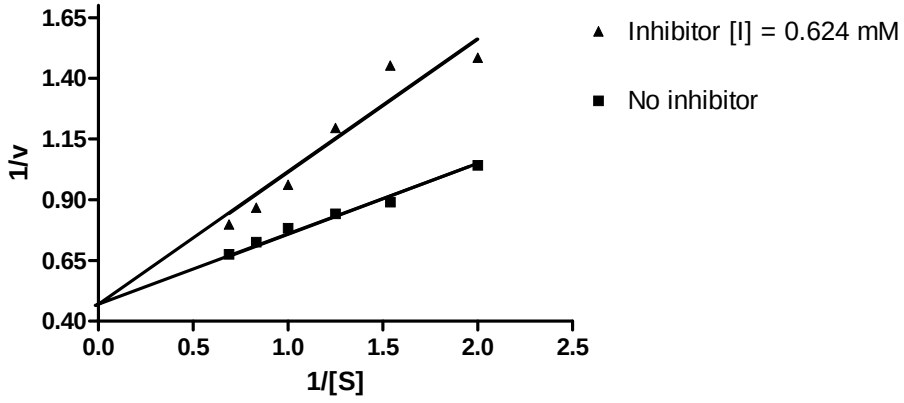
Compound **164** (*Ki* = 172 μM)

β-galactosidase inhibiton by compound **164**



Compound **164** (*Ki* = 900 μM)

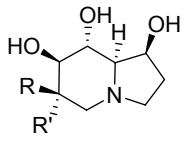
α-galactosidase inhibition by compound **164**



Chapter 4 Synthesis of analogues of 1-Deoxycastanospermine

4.1 Introduction

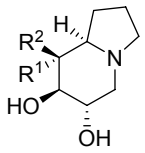
Polyhydroxylated 1-azabicyclo[4.3.0]nonane skeleton comprising of indolizidine alkaloids such as (+)-castanospermine[1] (**19**), (+)-6-*epi*-castanospermine[2] (**174**) and swainsonine[3] (**20**) (**Figure 35**) which are regarded as a bicyclic derivative of 1-deoxynojirimycin (1-DNJ) with an ethylene bridge between the hydroxymethyl group and the nitrogen at the ring junction, are known to exhibit potent activity against glucosidases and antiviral properties against a number of viruses.[4-7] However, unfortunately, castanospermine (**19**) is also found to inhibit intestinal sucrases causing osmotic diarrhea.[8] In an attempt to minimize the side effects of castanospermine and to establish possible structure-activity relationship, various stereoisomers of **19**,[9-11] and its derivatives such as *O*-acyl[9-10] and several analogues of deoxy-castanospermines (**175**)[10,12-27] are synthesized and evaluated. To the success of these studies, Celgosivir (6-*O*-butanoyl derivative of castanospermine, **30**) is currently under clinical phase II trials[28] (**Figure 36**).



19. (+)-Castanospermine
R = H, R' = OH

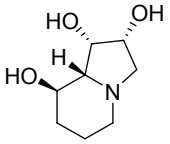
174. (+)-6-*epi*-Castanospermine
R = OH, R' = H

30. Celgosivir
R = H, R' = -OCO*n*-Bu



175. 1-Deoxycastanospermine
R^1= OH, R^2= H

176. 1-Deoxy-8-*epi*-castanospermine
R^1 = H, R^2 = OH



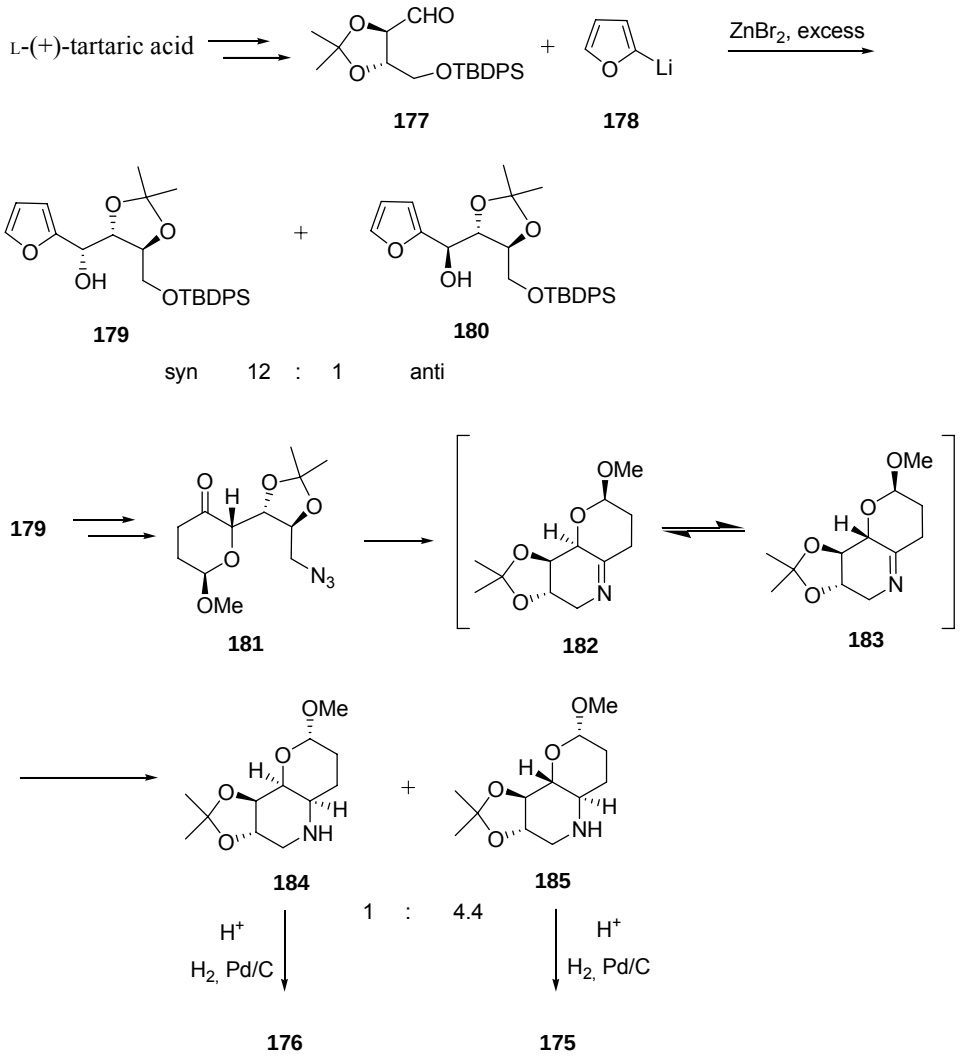
20. Swainsonine

Figure 36: Polyhydroxylated indolizidine alkaloids

In particular, 1-deoxy-8-*epi*-castanospermine (**176**) was, though, synthesized by Martin *et al*[19,20] and Majewski *et al*,[18] no effort was made to study its enzyme inhibition properties. The synthetic efforts by the authors are summarized below.

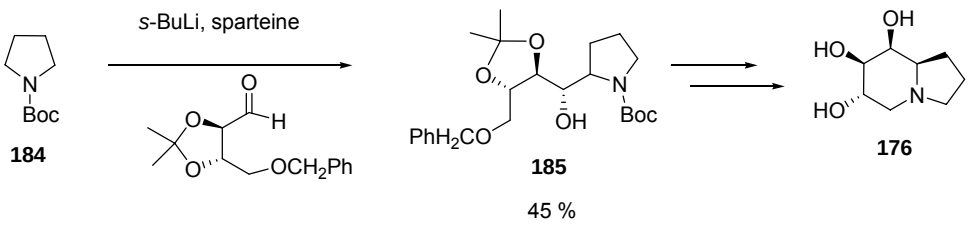
- Martin *et al*[19,20] synthesized **176** as depicted below in **Scheme 23**. Intermediate **181** on treatment with PPh_3 in refluxing benzene followed by hydride reduction afforded **184** and **185** in (1:4.4, dr). Compound **184** on hydrogenation under acidic condition afforded 1-deoxy-8-*epi*-castanospermine **(176)** which was initially wrongly characterized as 1-deoxy-8a-*epi*-castanospermine. However, in subsequent year they reported catalytic hydrogenation of **181** affording **176** as a single product.

Scheme 23



- Majewski *et al*[18] synthesized **176** as depicted below in **Scheme 24**. The key step in this synthesis involved reaction of lithiated *N*-Boc-pyrrolidine in the presence of sparteine with a chiral aldehyde derived from L-(+)-tartaric acid to obtain diastereomeric mixture in 9:1. The major diastereomer **185** was obtained in 45 % yield.

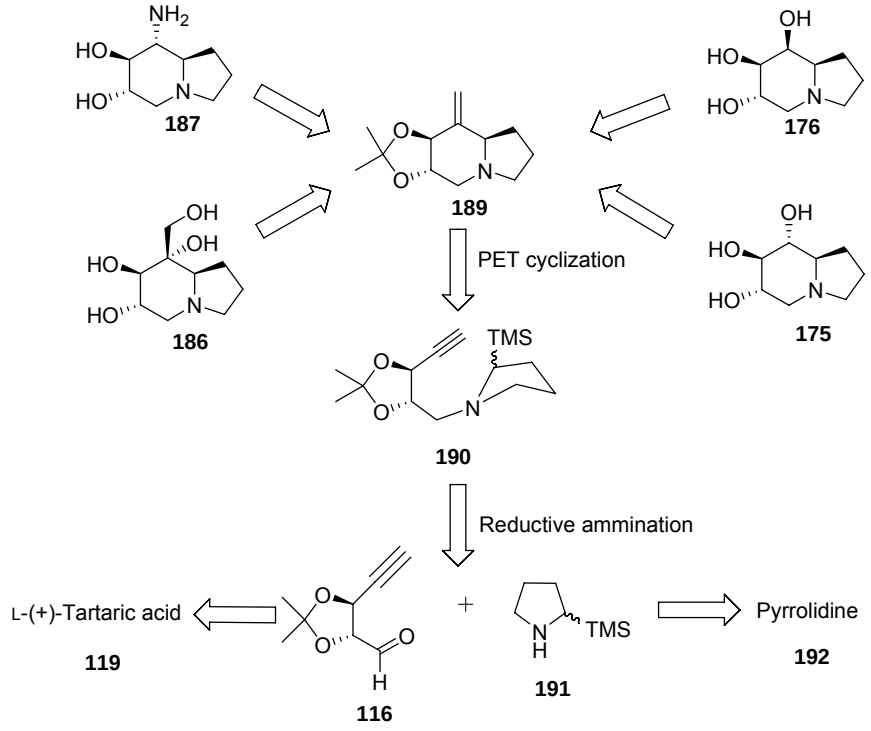
Scheme 24



4.2 Retrosynthetic plan

Considering the unexplored biological significance of this molecule, based on the similar strategy as depicted in **Scheme 6** (*Chapter 1*), a new and versatile synthetic route for the stereoselective synthesis of polyhydroxylated 1-azabicyclo[4.3.0]nonane skeleton comprising of indolizidine alkaloids such as deoxy-castanospermine (**176** and **175**) and some structural analogues (**186** and **187**) from a common intermediate **190** is shown in **Scheme 25**.

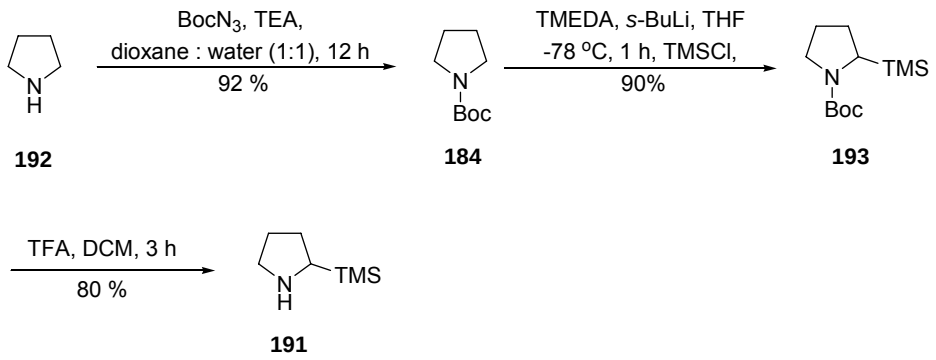
Scheme 25: Retrosynthetic analysis



4.3 Synthesis of 1-Deoxy-8-*epi*-castanospermine (176)

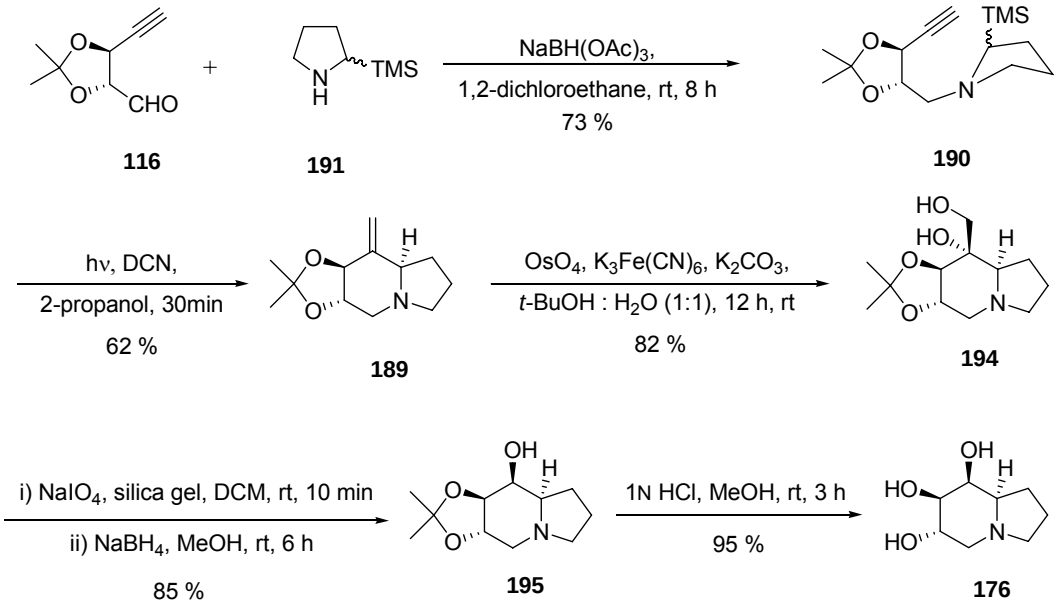
We carried out the synthesis of 1-deoxy-8-*epi*-castanospermine **176** as outlined in **Scheme 27**. The key precursor **189** was obtained by the photoinduced electron transfer (PET) cyclizaton of acetylene tethered amine **190** by following a PET initiated cyclization of amine on the tethered π functionality. The acetylene tethered amine **190** was prepared *via* reductive amination of aldehyde **116** with amine **191** using sodium triacetoxyborohydride as a reducing agent. The synthesis of 2-(trimethylsilyl)pyrrolidine **191** was carried out in three steps from pyrrolidine by following the literature reported[29,30] procedure as shown in **Scheme 26**.

Scheme 26: Synthesis of 2-(trimethylsilyl)pyrrolidine **191**

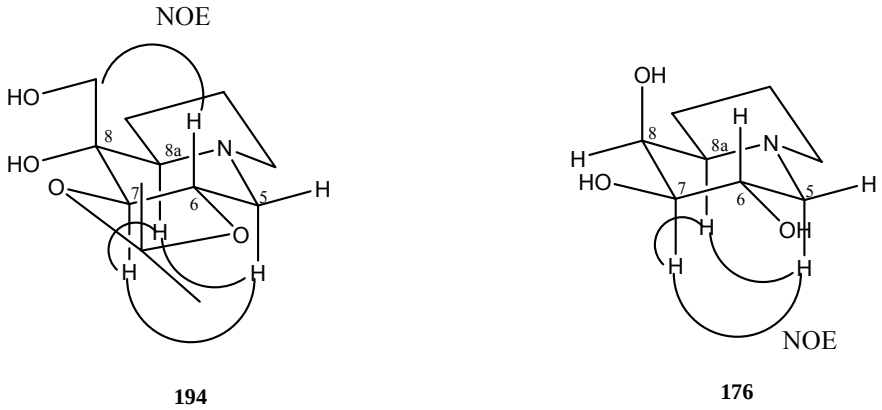


The PET cyclization of **190** essentially involved the irradiation of a dilute solution of **190** (3 mmol) and 1,4-dicyanonaphthalene (0.4 mmol) in 2-propanol (250 mL) through a Pyrex filtered light emanating from a 450 W Hanovia medium pressure lamp. Usual work-up and purification by column chromatography produced **189** as a single diastereomer in 62% yield which was fully characterized by extensive ^{1}H NMR, ^{13}C NMR and ^{1}H-^{1}H NOESY and ^{1}H-^{13}C HETCOR spectral analysis. ^{1}H NMR showed the presence of double bond at δ 4.83 (s, 1H), 5.02 (s, 1H). ^{13}C and DEPT NMR indicated presence of 2 CH_3 carbon at δ 26.7, 26.9; 5 CH_2 carbons at δ 22.0, 24.6, 52.6, 52.7, 102.7; 3 CH carbons at δ 64.5, 78.2, 82.6; and 2 quaternary carbons at δ 110.6, 142.7 confirming the structure of the PET cyclized product **189.**

Scheme 27: Synthesis of 1-Deoxy-8-*epi*-castanospermine (**176)**



Dihydroxylation of **189** using osmium tetroxide afforded **194** in 82% yield. The stereochemical outcome of **194** was adjudged by ^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR, and ^{1}H-^{1}H NOESY spectral analysis as shown in **Figure 37** and **Table 7**. The diol **194** upon sodium periodate oxidation afforded corresponding ketone which on sodium borohydride reduction gave **195** in 85% yield as a single diastereomer. The ^{1}H NMR data for **195** is in full harmony with the literature data.[18]



194 **176**

Figure 37: Graphical summary of NOESY spectrum observed for **194** and **176**

Removal of acetonide protecting group from **195** afforded 1-deoxy-8-*epi*-castanospermine (**176**) in 95% yield. Although, the ^{1}H NMR data of **176** matched well with the values reported by Martin *et al*,[19,20] to complete the stereochemical assignments we carried out extensive ^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR, and ^{1}H-^{1}H NOESY spectral studies and details are summarized in **Figure 37** and **Table 7**.

Table 7. Selective coupling constants *J* (Hz) for **194** and **176**

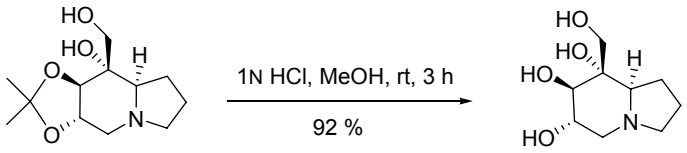
	H_5 -axial	H_5-equatorial	H_6-axial	H_7- axial	H_8-equatorial	H_{8a}-axial
194	2.16 (app t), $J_{5\alpha,5\beta}$=$J_{5\alpha,6\beta}$= 9.8	3.26 (dd) $J_{5\beta,5\alpha}$ = 9.6 $J_{5\beta,6\beta}$ = 4.1	3.80 (dt) $J_{6\beta,7\alpha}$ = 9.8	3.49 (d) $J_{7\alpha,6\beta}$ = 9.5	No Hydrogen	2.19-2.26 merged with H_3
176	2.94 (app. t) $J_{5\alpha,5\beta}$=$J_{5\alpha,6\beta}$= 11.5	3.74 (dd) $J_{5\beta,5\alpha}$ = 9.8 $J_{5\beta,6\beta}$ = 5.3	4.10 (ddd) $J_{6\beta,7\alpha}$ = 7.7 $J_{6\beta,5\alpha}$ = 11.4 $J_{6\beta,5\beta}$ = 5.4	3.76 (dd), $J_{7\alpha,6\beta}$ = 9.5 $J_{7\alpha,8\alpha}$ = 3.0	4.30 (dd) $J_{8\alpha,7\alpha}$= 3.0 $J_{8\alpha,8a\alpha}$= 1.0	3.53 (ddd) $J_{8a\alpha,8\alpha}$= 1.0

Towards the synthesis of 1-deoxycastanospermine (**175**), our efforts to invert the C-8 hydroxy functionality in **195** by Mitsunobu inversion on **195** or nucleophilic displacement by Na/K benzoate on the corresponding mesyl derivative of **195** were not successful.

4.4 Synthesis of 1-Deoxy-8-hydroxymethyl-castanospermine (186)

Having developed a simple protocol for synthesizing **194** as an intermediate for the synthesis of **186**, we realized that it would be interesting to obtain a new compound **186**, hitherto unknown, for evaluation as glycosidase inhibitor. In this context, acetonide deprotection of **194**, brought about by stirring with 1N HCl in methanol for 3 h, gave **186** in 92 % yield (**Scheme 28**).

Scheme 28: Synthesis of 1-Deoxy-8-hydroxymethyl-castanospermine **186**



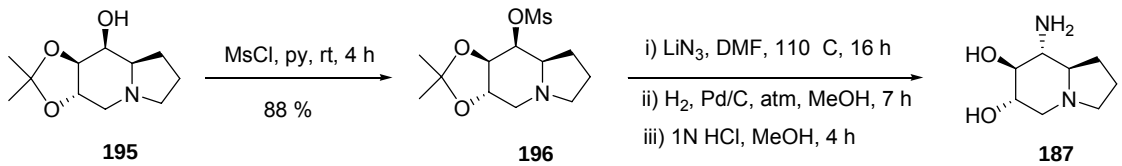
194 **186**

^{1}H NMR of **186** in D_2O showed presence of total 13 protons. ^{13}C and DEPT NMR indicated presence of 5 CH_2 carbons at δ 20.9, 22.9, 53.6, 55.2, 60.4; 3 CH carbons at δ 68.5, 70.1, 80.7 and one quaternary carbon at δ 72.3.

4.5 Synthesis of (6*S*,7*S*,8*R*,8a*R*)-8-amino-octahydroindolizine-6,7-diol (187)

Furthermore, another new analogue **187** having more basic amine moiety at C-8 was also visualized to be easily affordable from **195** for evaluation as a new glycosidase inhibitor**.** Towards this end, C-8 hydroxy moiety of **195** was first converted to corresponding mesylate derivative **196** which upon nucleophilic S_N2 displacement with azide followed by catalytic (Pd/C, 10%) hydrogenation and acetonide deprotection afforded (6*S*,7*S*,8*R*,8a*R*)-8-amino-octahydroindolizine-6,7-diol (**187**) in 78% yield.

Scheme 29: Synthesis of (6*S*,7*S*,8*R*,8a*R*)-8-amino-octahydroindolizine-6,7-diol **(187)**



195 **196** **187**

^{1}H NMR of **187** in D_2O showed presence of total 12 protons. ^{13}C and DEPT NMR indicated the presence of 4 CH_2 carbons at δ 20.6, 26.5, 52.3, 53.4; 4 CH carbons at δ 54.9, 63.7, 69.3, 74.0 confirming the structural features of **187**.

4.6 Enzyme inhibitory activity of analogues of 1-Deoxy-castanospermine

The inhibitory activities of **176, 186** and **187** were screened against β-galactosidase (*Aspergillus oryzae*), α-galactosidase (coffee beans), β-glucosidase / β-mannosidase (almonds), α-glucosidase (yeast), and α-mannosidase (jack beans). The results are summarized in **Table 8**.

None of these analogues of 1-deoxy-castanospermine inhibited α-glucosidase and α- / β-mannosidases. The 1-deoxy-8-*epi*-castanospermine (**176**) exhibited potent non-specific competitive inhibition against α-galactosidase (*Ki* = 71 μM), β-galactosidase (*Ki* = 73 μM) and β-glucosidase (*Ki* = 33 μM). To our dismay 1-deoxy-8-hydroxymethyl-castanospermine (**186**) in which an additional hydroxymethyl moiety is present at carbon C-8, showed non-competitive weak inhibition against β-glucosidase. Similarly compound **187** in which hydroxy group at C-8 of castanospermine is replaced by amino group showed weak inhibition against β-glucosidase. In the case of **187,** the weak inhibitory activity could be attributed to the more basicity of the amino group at C-8 position than the ring nitrogen thereby forbidding it from binding to active site of α- / β-glucosidase in correct orientation.

Table 8: Enzyme inhibition (Ki in μM) for **176, 186** and **187***

Glycosidase→ / Inhibitor↓	β- Gal	α-Gal	β-Glc	α-Glc	β-Man	α-Man
19[a]			1.5	0.015		
175[b]			31	7		21
176	71	73	33	n.i.	n.i.	n.i.
186	4000	n.i.	942	n.i.	n.i.	n.i.
187	n.i.	n.i.	1400	n.i.	n.i.	n.i.

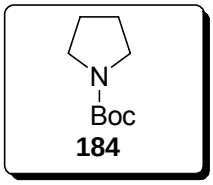
*, general enzyme inhibition assay procedure is detailed in *Chapter 1* experimental section; n.i. No Inhibition at 1mM; — means not meseasured; [a] Inhibition value *Ki,* α-glucosidase (rice) and β-glucosidase (almonds) for castanospermine are referred from ref. [31]. [b] % inhibition at 1mM for α-/ β-glucosidase (lysosomal) and α-mannosidase (lysosomal), the data for 1-deoxycastanospermine is from ref. [32].

4.7 Summary

In conclusion, we have demonstrated a general synthetic strategy to access polyhydroxylated 1-azabicyclo[4.3.0]nonane skeleton such as **176**, **186** and **187**. Although, these synthesized molecules could not turn up as potent inhibitors, nevertheless, this study is useful in understanding the structure activity relationship of polyhydroxylated indolizidine alkaloids.

4.8 Experimental Section

1. Preparation of tert-butyl pyrrolidine-1-carboxylate



184

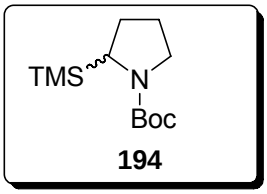
To a solution of pyrrolidine (6.56 g, 92.3 mmol) and Et_3N (11.6 g, 115.3 mmol) in dioxane (50 mL), *t*-butyl azidoformate (11.0 g, 76.9 mmol) was added drop-wise over a period of 15 min. The pH of the reaction mixture was maintained at 12 by the addition of excess Et_3N if required. The reaction mixture was stirred until a clear solution resulted. After the evaporation of dioxane, the residue was taken up in ether, washed twice with water (75 mL) followed by brine (75 mL). Ether was evaporated and the resultant brown oil obtained was purified by vacuum distillation (bp 55-60 °C/1 mm) to obtain **184** (15 g, 95 %) as colorless oil.

IR (neat) ν_{max} cm^{-1} : 1700, 1400, 1160, 1110

^{1}H NMR (200 MHz, $CDCl_3$) δ : 1.45 (s, 9H), 1.80-1.95 (m, 4H), 3.37 (t, 4H, *J* = 7.3 Hz)

Mass (m/z %) : 171 (M+, 11), 114 (100), 57 (82)

2. Preparation of *t*-butyl 2-(trimethylsilyl)pyrrolidine-1 carboxylate:



194

A solution of *N*-Boc derivative of cyclic amine **184** (3.44 g, 20.1 mmol) in 40 mL of dry ether was charged into a 250 mL flask, equipped with a magnetic bar and argon gas balloon and was cooled to –78 °C. TMEDA (2.79 g, 24.12 mmol) followed by *s*-BuLi (1.5 M solution in cyclohexane, 16.1 mL, 24.12 mmol) were introduced to the stirring mixture dropwise over 15 min. The mixture was further allowed to stir for 2 h at –78 °C. TMSCl (2.61 g, 24.12 mmol) was added dropwise into the flask. The reaction mixture was allowed to warm to room temperature and diluted with 15 mL of saturated aqueous NH_4Cl solution. The organic layer was concentrated and the crude oily residue was purified by fractional distillation (bp 55-60 °C/0.5 mm) to give **193** (4.3 g, 88 %) as colorless oil.

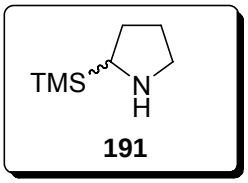
IR (neat) ν_{max} cm^{-1} : 1692, 1478, 1365, 1246, 1170

^{1}H NMR (200 MHz, $CDCl_3$) δ : 0.05 (s, 9H), 1.45 (s, 9H), 1.75-1.95 (m, 3H), 1.95-2.05 (m, 1H, 3.15-3.30 (m, 2H), 3.35-3.60 (m, 1H).

^{13}C NMR (50 MHz, $CDCl_3$) δ : -2.3, 27.8, 28. 4, 46.7, 47.5, 78.0, 154.5

Mass (m/z %) : 243 (M+, 1), 186 (43), 172 (100), 142 (94)

3. Preparation of 2-(trimethylsilyl)pyrrolidine



191

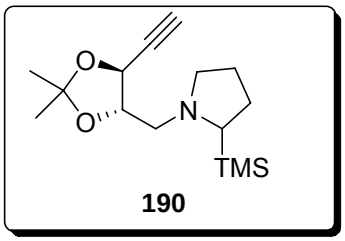
To a stirring solution of **193** (3.39 g, 13.95 mmol) in 20 mL of dry DCM at 0 °C, TFA (5.7 g, 50.0 mmol) was added drop-wise over a period of 30 min. The mixture was allowed to warm to room temperature and allowed to stir further for 4 h. The reaction mixture was re-cooled to 0 °C and was basified with 20 % aqueous NaOH solution (pH = 10). The organic layer was separated and the aqueous layer was extracted with DCM (2 x 30 mL). The combined extracts were washed with brine, dried over Na_2SO_4 and concentrated to give crude amine **191**, which was utilized as such without further purification for the next step.

^{1}H NMR (200 MHz, $CDCl_3$) δ : 0.00 (s, 9H), 1.48-2.07 (m, 4H), 2.50 (dd, 1H, J = 6.9, 12.1 Hz), 2.96-3.23 (m, 2H)

^{13}C NMR (50 MHz, $CDCl_3$) δ : -3.8(CH_3), 24.9(CH_3), 27.2(CH_2), 46.6(CH_2), 49.1(CH)

Mass : 143 (M^+)

4. Preparation of 1-{[(4*S*,5*S*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl]methyl}-2-(trimethylsilyl)pyrrolidine:



190

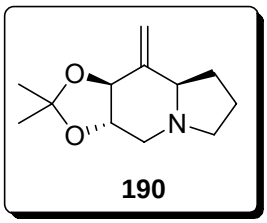
To the solution of **116** (4.5 g, 29.09 mmol) in dry 1,2-dichloroethane (180 mL), amine **191** (4.57 g, 32.00 mmol) followed by sodium triacetoxyborohydride (8.15 g, 38.46 mmol) was added. The mixture was stirred at rt under argon atmosphere for 8 h. The reaction mixture was ice cooled and quenched by adding 1N NaOH till the aqueous layer was basic. The reaction mixture was extracted with ethyl acetate (2 x 100 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed by rotary-evaporation and the residue was purified by column chromatography (silica, pet. ether-ethyl acetate, 9:1) to afford 1:1 diastereomeric mixture of **190** (6.6 g, 73 %) as a colorless oil.

IR (neat) ν_{max} cm^{-1} : 3311, 2985, 2956, 2119, 1730, 1691, 1456, 1380, 1371, 1247, 1060

^{1}H NMR : 0.00+0.01 (two s, 9H), 1.37+1.39+1.43+1.44 (four s for acetonide

(200 MHz, $CDCl_3$) δ		methyl, 6H), 1.50-1.85 (m, 5H), 1.90-2.12 (m, 1H), 2.28-2.40 (m, 1H), 2.42-2.51 (m, 1H), 2.80-3.02 (m, 1H), 3.12-3.34 (m, 1H), 4.09-4.27 (m, 1H), 4.32-4.47 (two sets of dd, 1H, *J* = 2.2, 7.8 Hz)
^{13}C NMR	:	-3.0(CH_3), 24.2+24.5(CH_2), 25.5+25.9(CH_3), 26.6+26.9(CH_3), 26.7(CH_2),
(50 MHz, $CDCl_3$) δ		55.6+56.1(CH), 56.2+56.7(CH_2), 57.0+58.6(CH_2), 67.5(CH), 68.9(CH), 73.6+74.2(C), 80.7+81.0(CH), 109.9+110.1(C)
MS (m/z %)	:	304 ($M+Na^+$, 15%), 282 (MH^+, 100%)
Elemental analysis	:	Anal. Calcd for $C_{15}H_{27}NO_2Si$: C, 64.01; H, 9.67; N, 4.98; Found C, 64.12; H, 9.71; N, 5.01

5. Preparation of (3a*S*,8a*R*,9a*S*)-2,2-dimethyl-9-methylene-octahydro-[1,3]dioxolo [4,5-f]indolizine:

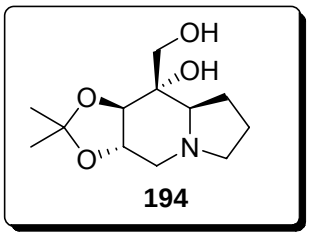


190

A solution containing **190** (1.0 g, 3.55 mmol) and 1,4-dicyanonaphthalene (0.12 g, 0.67 mmol) in 2-propanol (250 mL) was irradiated in an open vessel using a 450 W Hanovia medium pressure mercury vapor lamp. The lamp was immersed in a Pyrex water-jacketed immersion well to allow only wavelengths greater than >280 nm to pass through. After about 20 min of irradiation, the consumption of the starting material was found to be almost complete (monitored by GC) and at this stage the irradiation was discontinued. The solvent was removed under reduced pressure and the residue was column chromatographed (silica, pet. ether-acetone, 9:1) to afford cyclized product **189** (0.460 g, 62%) as a yellow liquid.

$[\alpha]^{27}_D$	:	+56.1 (*c* 2.3, CH_2Cl_2)
IR (neat) ν_{max} cm^{-1}	:	3093, 2985, 2935, 1674, 1461, 1380, 1371, 1228.
^{1}H NMR	:	1.44 (s, 3H), 1.46 (s, 3H), 1.76-1.84 (m, 3H), 1.85-1.99 (m, 1H), 2.33-
(500 MHz, $CDCl_3$) δ		2.41 (m, 2H), 2.59 (app t, 1H, *J* = 6.4, 8.9 Hz), 3.04 (dt, 1H, *J* = 1.9, 9.2 Hz), 3.40 (dd, 1H, *J* = 4.1, 9.6 Hz), 3.60 (dt, 1H, *J* = 4.1, 9.6 Hz), 3.85 (td, 1H, *J* = 1.8, 9.2 Hz) 4.83 (s, 1H), 5.02 (s, 1H)
^{13}C NMR	:	22.0(CH_2), 24.6(CH_2), 26.7(CH_3), 26.9 (CH_3), 52.6 (CH_2), 52.7(CH_2),
(125 MHz, $CDCl_3$) δ		64.5(CH), 78.2(CH), 82.6(CH), 102.7(CH_2), 110.6(C), 142.7(C)
Mass (m/z %)	:	209 (M^+, 100%)
Elemental Analysis	:	Anal. Calcd for $C_{12}H_{19}NO_2$: C, 68.87; H, 9.15; N, 6.69; Found C, 68.97; H, 9.20; N, 6.54

6. Preparation of (3a*S*,8a*R*,9*S*,9a*R*)-9-(hydroxymethyl)-2,2-dimethyl-octahydro-[1,3] dioxolo[4,5-f]indolizin-9-ol:

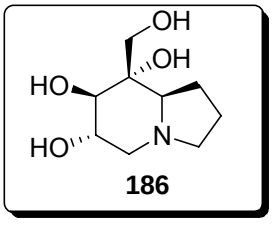


194

To a mixture of potassium ferricynide (2.83 g, 8.61 mmol) and potassium carbonate (1.19 g, 8.61 mmol) in water (36 mL) at 5 °C, **189** (0.45 g, 2.15 mmol) dissolved in *t*-BuOH (28 mL) followed by osmium tetroxide (2 mL of 1 % solution of OsO_4 in *t*-BuOH) was added. The reaction mixture was allowed to warm to rt and stirred for 12 h. Solid 0.4 g of Na_2SO_3 was added to the stirring solution and clear separation of two layers were noticed. Aqueous layer was extracted with ethyl acetate (5 x 20 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed by rotary-evaporation and the residue was purified by column chromatography (silica, pet. ether-ethyl acetate, 3:7) to afford **194** (0.43 g, 82 %) as a white gummy liquid.

$[\alpha]^{27}_D$	:	+30.7 (*c* 1.35, CH_2Cl_2)
IR (neat) ν_{max} cm^{-1}	:	3448, 2985, 2937, 2912, 2810, 1460, 1373, 1234, 1130, 1114
^{1}H NMR (500 MHz, $CDCl_3$) δ	:	1.39 (s, 6H), 1.61-1.86 (m, 4H), 2.16 (app t, 1H, *J* = 9.6, 11.2 Hz), 2.19-2.26 (m, 2H), 2.96 (dt, 1H, *J* = 2.3, 8.2 Hz), 3.26 (dd, 1H, *J* = 4.3, 9.3 Hz), 3.49 (d, 1H, *J* = 9.5 Hz), 3.75 (d, 1H, *J* = 11.0 Hz), 3.80 (dt, 1H, *J* = 4.3, 9.8 Hz), 3.92 (d, 1H, *J* = 11.4 Hz)
^{13}C NMR (125 MHz, $CDCl_3$) δ	:	22.1(CH_2), 22.9(CH_2), 26.5(CH_3), 26.6(CH_3), 52.8(for two CH_2), 61.8(CH_2), 70.0(CH), 71.2(C), 73.7(CH), 87.1(CH), 111.4(C)
HRMS (M^+)	:	Anal Calcd. for $C_{12}H_{21}NO_4$ 243.1470, Found 243.1475

7. Preparation of 1-deoxy-8-hydroxymethyl-castanospermine or (6*S*,7*R*,8*S*,8a*R*)-8-(hydroxymethyl)-octahydroindolizine-6,7,8-triol:



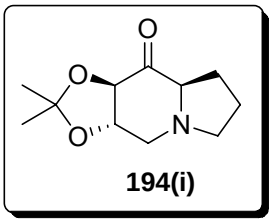
186

To a solution of **194** (25 mg, 0.102 mmol) in distilled methanol (3 mL), was added 1 mL of 1N HCl and the reaction mixture was stirred at rt for 3 h. The solvent was evaporated to dryness to afford **186** as a HCl salt which was further purified by column chromatography as a free base [(silica, chloroform-methanol-aq.NH_3, 8.0:2:0.5) and finally eluted with MeOH-chloroform (4:6)] to afford **186** (19 mg, 92 %) as a colorless gummy liquid.

$[\alpha]^{27}_D$	:	+31.7 (*c* 1.35, MeOH)
^{1}H NMR	:	1.82-2.03 (m, 4H), 2.20 (appt t, 1H, J 10.3, 11.9), 2.30-2.41 (m, 1H),

(400 MHz, D_2O) δ		2.42-2.52 (m, 1H), 3.12 (appt t, 1H, 8.4, 10.1), 3.31(dd, 1H, J 5.3, 11.0), 3.49 (d, 1H J 9.3) 3.87 (s, 2H), 3.88 (dt, 1H J 5.3, 9.8)
^{13}C NMR	:	20.9(CH_2), 22.9(CH_2), 53.6(CH_2), 55.2(CH_2), 60.4(CH_2), 68.5(CH),
(100 MHz, D_2O) δ		70.1(CH), 72.3(C), 80.7(CH)
Mass	:	226 ($M+Na^+$, 30%), 203 (M^+, 100%), 121 (37%)
Elemental Analysis	:	Anal. Calcd for $C_9H_{17}NO_4$: C, 53.19; H, 8.43; N, 6.89; Found C, 53.32; H, 8.48; N, 6.74

8. Preparation of (3a*S*,8a*R*,9a*R*)-2,2-dimethyl-hexahydro-[1,3]dioxolo[4,5-f] indolizin-9(9aH)-one:

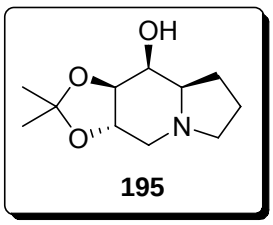


194(i)

A solution of **194** (0.162 g, 0.666 mmol) in DCM (10 mL) was added to a suspension of silica gel supported sodium periodate [prepared by dissolving $NaIO_4$ (0.285 g, 1.33 mmol) in 0.65 mL water and 1.31 g of flash silica gel] in DCM (5 mL). The suspension was stirred for 10 min. and filtered. The solvent was evaporated off and the brownish pasty mass was extracted with ethyl acetate (3 x 3 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure. The crude mixture, keto **194(i)** (crude weight 0.130 g, 95%) was pure enough and was used as such for the next step.

$[\alpha]^{27}_D$	:	+70.6 (*c* 0.80, CH_2Cl_2)
^{1}H NMR	:	1.43 (s, 3H), 1.44 (s, 3H), 1.65-1.90 (m, 3H), 2.00-2.25 (m, 1H), 2.58-2.75
(200 MHz, $CDCl_3$) δ		(m, 1H), 2.78-2.96 (m, 2H), 3.13 (app t, 1H, *J* = 9.2, 9.8 Hz), 3.54 (dd, 2H, *J* = 4.2, 9.6 Hz), 3.87 (dt, 1H, *J* = 4.0, 9.6 Hz), 4.29 (d, 1H, *J* = 9.6 Hz)
^{13}C NMR	:	21.9(CH_2), 22.2(CH_2), 26.0(CH_3), 26.4(CH_3), 50.7(CH_2), 52.1(CH_2),
(50MHz, $CDCl_3$) δ		67.1(CH), 762(CH), 83.7(CH), 112.1(C), 199.3(C)

9. Preparation of (3a*S*,8a*R*,9*S*,9a*S*)-2,2-dimethyl-octahydro-[1,3]dioxolo[4,5-f] indolizin-9-ol:



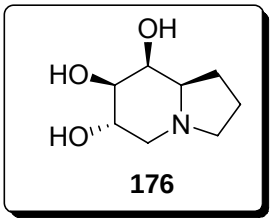
195

Sodium borohydride (47 mg, 1.24 mmol) was added to a solution of ketone **194(i)** (0.13 g, 0.622 mmol) in methanol (4 mL). The resulting mixture was stirred for 6 h at rt and then quenched by adding excess of the saturated solution of NaCl. This brownish suspension was stirred overnight and extracted with ethyl acetate (4 x 3 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent was removed under

reduced pressure. The residue was purified by column chromatography (silica, pet ether-ethyl acetate, 1:4) to afford **195** (0.119 g, 85 %) as a colorless oil.

$[\alpha]^{27}_D$	:	+17.2 (*c* 0.45, $CHCl_3$), lit.[18] $[\alpha]^{27}_D$ = +25 (*c* 1.2 $CHCl_3$)
IR (neat) ν_{max} cm^{-1}	:	3406, 2995, 2923, 2810, 1419, 1363, 1215
^{1}H NMR (200 MHz, $CDCl_3$) δ	:	1.42(s, 3H), 1.44(s, 3H), 1.62-1.97 (m, 4H), 2.14-2.36 (m, 3H), 3.01 (dt, 1H, *J* = 1.9, 8.1), 3.31-3.43 (m, 2H), 4.01 (dt, 1H, *J* = 4.2, 9.8 Hz), 4.11 (app t, 1H, *J* = 1.9, 2.7)
^{13}C NMR (50MHz, $CDCl_3$) δ	:	22.4(CH_2), 23.7(CH_2), 26.5(CH_3), 26.8(CH_3), 52.6(CH_2), 52.8(CH_2), 65.6(CH), 65.5(CH), 71.2(CH), 83.1(CH), 110.9(C)
Elemental Analysis	:	Anal. Calcd for $C_{11}H_{19}NO_3$: C, 61.95; H, 8.98; N, 6.57; Found C, 62.02; H, 8.91; N, 6.50

10. Preparation of 1-deoxy-8-*epi*-castanospermine or (6*S*,7*R*,8*S*,8a*R*)-octahydro indolizine-6,7,8-triol:

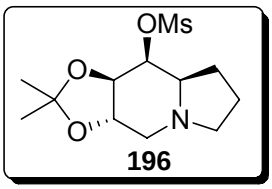


176

To a solution of **195** (20 mg, 0.093 mmol) in distilled methanol (3 mL) was added 1 mL of 1N HCl and the reaction mixture was stirred at rt for 3 h. The solvent was evaporated to dryness to afford **176**.HCl as a white foam which was further purified by column chromatography as a free base [(silica, chloroform-methanol-aq NH_3, 8.0:2:0.5) and finally eluted with MeOH-chloroform (4:6)] to afford **176** (15 mg, 95%) as a white solid.

mp	:	149-151 °C, lit[20] mp 149-150 °C
$[\alpha]^{27}_D$	:	+26.1 (*c* 0.45, MeOH), lit.[20] $[\alpha]^{27}_D$ = +41.8 (*c* 0.0057, MeOH)
^{1}H NMR (400 MHz, D_2O) δ	:	1.99-2.30 (m, 4H), 2.94 (t, 1H, *J* = 11.5, 11.5 Hz), 3.14-3.23 (m, 1H), 3.53 (ddd, 1H, *J* = 1.0, 6.6, 11.6 Hz), 3.67 (ddd, 1H, *J* = 3.0, 8.3, 11.4 Hz), 3.74 (dd, 1H, *J* = 5.3, 9.8 Hz),3.76 (dd, 1H, *J* = 3.0, 9.5 Hz) 4.10 (ddd, 1H, *J* = 5.4, 7.7, 11.4 Hz), 4.27 (dd, 1H, *J* = 1.0, 3.0 Hz)
^{13}C NMR (100 MHz, D_2O) δ	:	22.2(CH_2), 24.3(CH_2), 53.9(CH_2), 54.3(CH_2), 66.8(CH), 67.8(CH), 69.6(CH), 75.0(CH)
Mass	:	174 (MH^+, 100%), 118 (5%), 99 (55%)
Elemental Analysis	:	Anal. Calcd for $C_8H_{15}NO_3$: C, 55.47; H, 8.73; N, 8.09; Found C, 55.52; H, 8.77; N, 8.15

11. Preparation of (3a*S*,8a*R*,9*S*,9a*R*)-2,2-dimethyl-octahydro-[1,3]dioxolo[4,5-f] indolizin-9-yl methanesulfonate:



196

To a solution of 195 (70 mg, 0.33 mmol) in pyridine (2 mL)

at 0 °C was added mesyl chloride (40 mg, 0.35 mmol, in 1ml DCM). The reaction mixture was stirred at room temperature for 4 h. When TLC revealed no starting material, the solution was diluted with dichloromethane (10 mL) and washed with water (3 x 5 mL), brine solution (5 mL) and then dried over Na_2SO_4. Removal of the solvent followed by column chromatographic purification (3:7, ethyl acetate: pet ether) gave **196** (83 mg, 88 %) as a semi-solid.

IR (neat) ν_{max} cm^{-1} : 1670, 1382, 1371, 1363, 1352, 1215.

^{1}H NMR (400 MHz, $CDCl_3$) δ : 1.42 (s, 3H), 1.43 (s, 3H), 1.72-1.99 (m, 4H), 2.26-2.39(m, 2H), 2.48 (app t, 1H *J* = 6.4, 9.1 Hz), 2.98-3.08 (m, 1H), 3.10 (s, 3H), 3.42 (dd, 1H, *J* = 4.0, 9.6 Hz), 3.49 (dd, 1H, *J* = 2.2, 9.6 Hz), 4.02 (dt, 1H, *J* = 4.2, 9.8 Hz), 5.09 (app t, 1H, *J* = 1.6, 2.4 Hz).

^{13}C NMR (100 MHz, $CDCl_3$) δ : 22.2(CH_2), 24.3(CH_2), 26.4(CH_3), 26.8(CH_3), 39.0(CH_3), 52.1(CH_2), 52.3(CH_2), 63.6(CH), 71.5(CH), 75.4(CH), 80.5(CH), 111.5(C).

Mass (m/z %) : 314 ($M+Na^+$, 18%), 291 (MH^+, 100%), 252 (50%).

Elemental Analysis : Anal. Calcd for $C_{12}H_{21}NO_5S$: C, 49.47; H, 7.26; N, 4.81. Found: C, 49.52; H, 7.30; N, 4.74.

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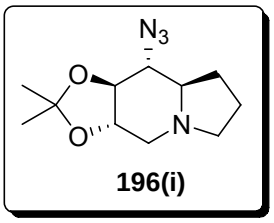
13. Preparation of (3a*S*,8a*R*,9*R*,9a*S*)-9-azido-2,2-dimethyl-octahydro-[1,3]dioxolo [4,5-f]indolizine:



196(i)

To a solution of **196** (70 mg, 0.24 mmol) in DMF (3 mL) was added LiN_3 (117 mg, 2.44 mmol) and heated to 110 °C for 16 h. When TLC revealed the absence of starting material, the reaction mixture was diluted with water (15 mL) and extracted with ethyl acetate (3 x 15 mL). The ethyl acetate layer was washed with water, dried over Na_2SO_4 and concentrated to give corresponding azide derivative **196(i)**

IR (neat) ν_{max} cm^{-1} : 2989, 2928, 2856, 2254, 2106, 1662, 1363, 1215, 1147.

^{1}H NMR (200 MHz, $CDCl_3$) δ : 1.45 (s, 6H), 1.60-1.257 (m, 7H), 3.00-3.22 (bs, 1H), 3.27-3.58 (m, 3H), 3.65-3.92 (bs, 1H).

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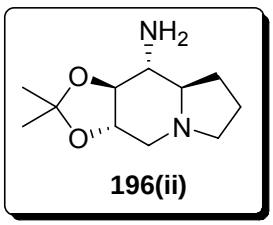
14. Preparation of (3a*S*,8a*R*,9*R*,9a*S*)-2,2-dimethyl-octahydro-[1,3]dioxolo[4,5-f]indolizin-9-amine:



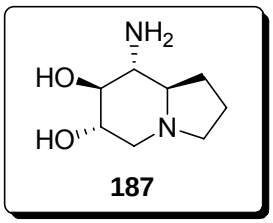
196(ii)

The azide derivative **196(i)** in methanol (3 mL) was hydrogenated for 7 h at atmospheric pressure in the presence of Pd on charcoal (10%) (3 mg). The reaction mixture was passed through

a short pad of celite and the solvent was removed under reduced pressure to afford corresponding amine derivative **196(ii)** (43 mg, 85 %) as a syrup.

$[\alpha]^{27}_D$	:	+14 (*c* 0.3, MeOH).
IR (neat) ν_{max} cm^{-1}	:	3410, 2985, 2935, 2800, 1642, 1419, 1363, 1249.
^{1}H NMR (400 MHz, $CDCl_3$) δ	:	1.43 (s, 6H), 1.53-1.66 (m, 1H), 1.74-1.86 (m, 1H), 1.87-1.98 (m, 1H), 1.99-2.26 (m, 2H), 2.31 (app t, 1H, *J* = 9.9, 10.7 Hz), 2.35-2.42 (m, 1H), 2.87 (app t, 1H, *J* = 9.0, 10.1 Hz), 3.05 (dt, 1H, *J* = 2.5, 9.6 Hz) 3.29 (app t, 1H, *J* = 9.5, 10.1 Hz), 3.34 (dd, 1H, *J* = 4.0, 9.8 Hz), 3.71 (ddd, 1H, *J* = 4.0, 8.8, 10.0 Hz).
^{13}C NMR (100 MHz, $CDCl_3$) δ	:	22.3(CH_2), 26.7(CH_3), 26.8(CH_3), 27.2(CH_2), 52.1(CH_2), 52.5(CH_2), 55.1(CH), 68.5(CH), 75.6(CH), 84.8(CH), 111.5(C).
Mass	:	212 (M^+, 100%).

15. Preparation of (6*S*,7*S*,8*R*,8a*R*)-8-amino-octahydroindolizine-6,7-diol:



187

To a solution of amine **196(ii)** (43 mg, 0.20 mmol) in distilled methanol (3 mL) was added 1 mL of 1N HCl and the reaction mixture was stirred at rt for 4 h. The solvent was evaporated to dryness to afford **7**.HCl as a white foam which was further purified by column chromatography as a free base [(silica, chloroform-methanol-aq.NH_3, 8.0:2:0.5) and finally eluted with MeOH-chloroform, 4:6] to afford **187** (32 mg, 92%) a light yellow gummy liquid.

$[\alpha]^{27}_D$	:	+4.5 (*c* 0.3, CH_2Cl_2).
^{1}H NMR (400 MHz, D_2O) δ	:	1.62-1.74 (m, 1H), 1.96-2.05 (m, 2H), 2.16-2.26 (m, 1H), 2.43 (app t, 1H, *J* = 11.0, 12.3 Hz), 2.54-2.73 (m, 2H), 3.05 (app t, 1H, *J* = 10.2, 11.2Hz), 3.19-3.27 (m, 1H), 3.41 (ddd, 1H, *J* = 5.0, 11.4 Hz), 3.53 (app t, 1H *J* = 9.2, 10.1 Hz), 3.72-3.79 (m, 1H)
^{13}C NMR (100 MHz, D_2O) δ	:	20.6(CH_2), 26.5(CH_2), 52.3(CH_2), 53.4(CH_2), 54.9(CH), 63.7(CH), 69.3(CH), 74.0(CH).
Mass (m/z %)	:	173 (M^+, 100%).
Elemental Analysis	:	Anal. Calcd for $C_8H_{16}N_2O_2$: C, 55.79; H, 9.36; N, 16.27; Found C, 55.81; H, 9.31; N, 16.38.

4.9 References

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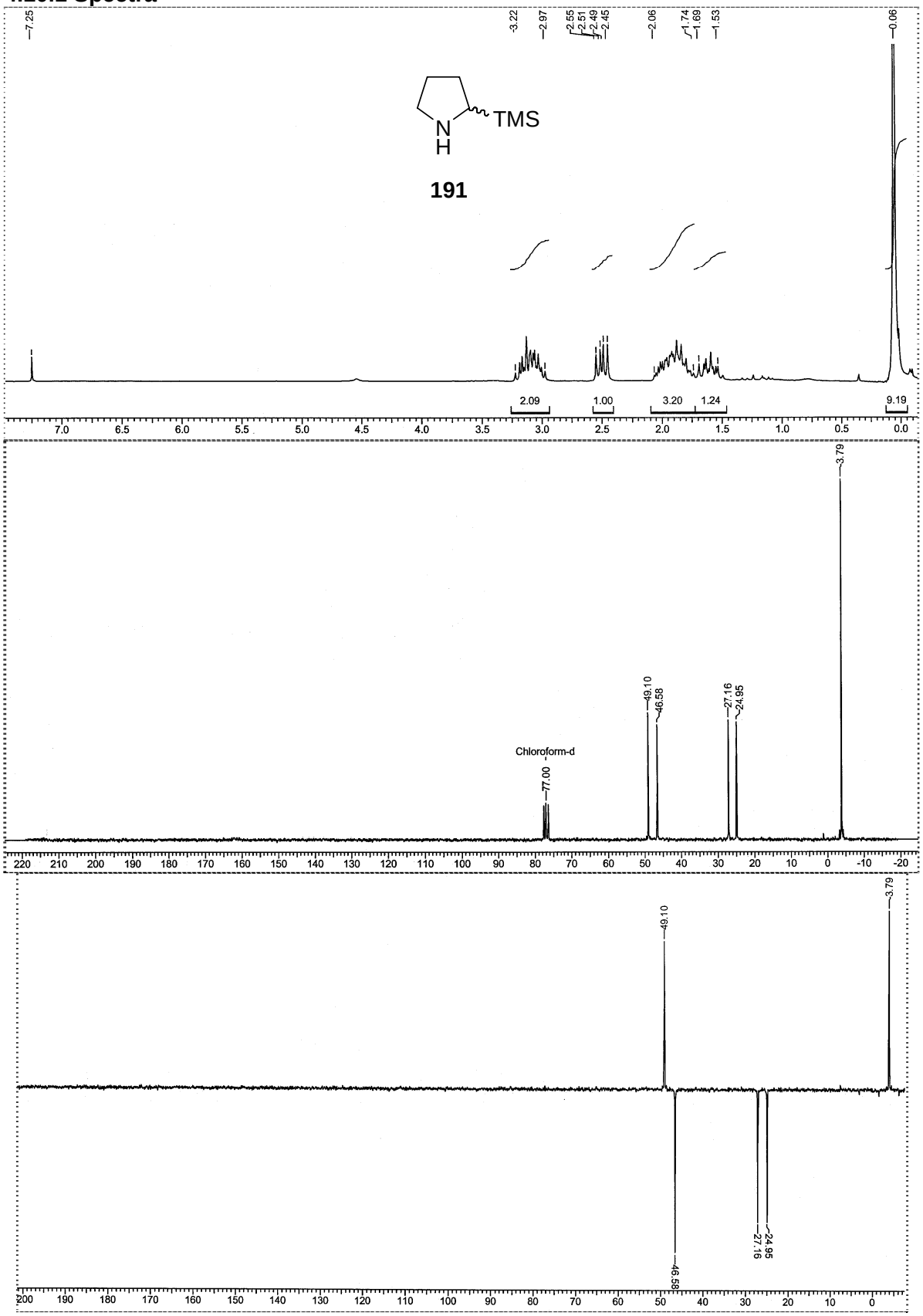
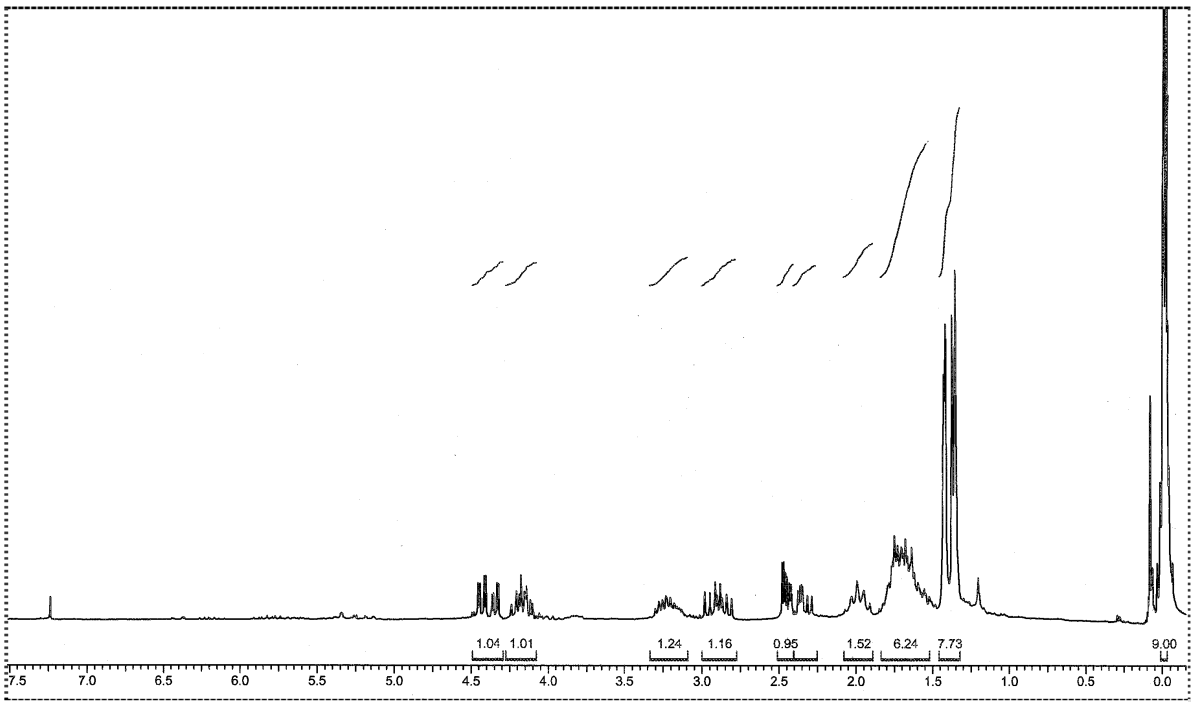
4.10 Spectra and Lineweaver-Bruke plots

4.10.1 Spectra

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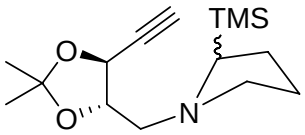
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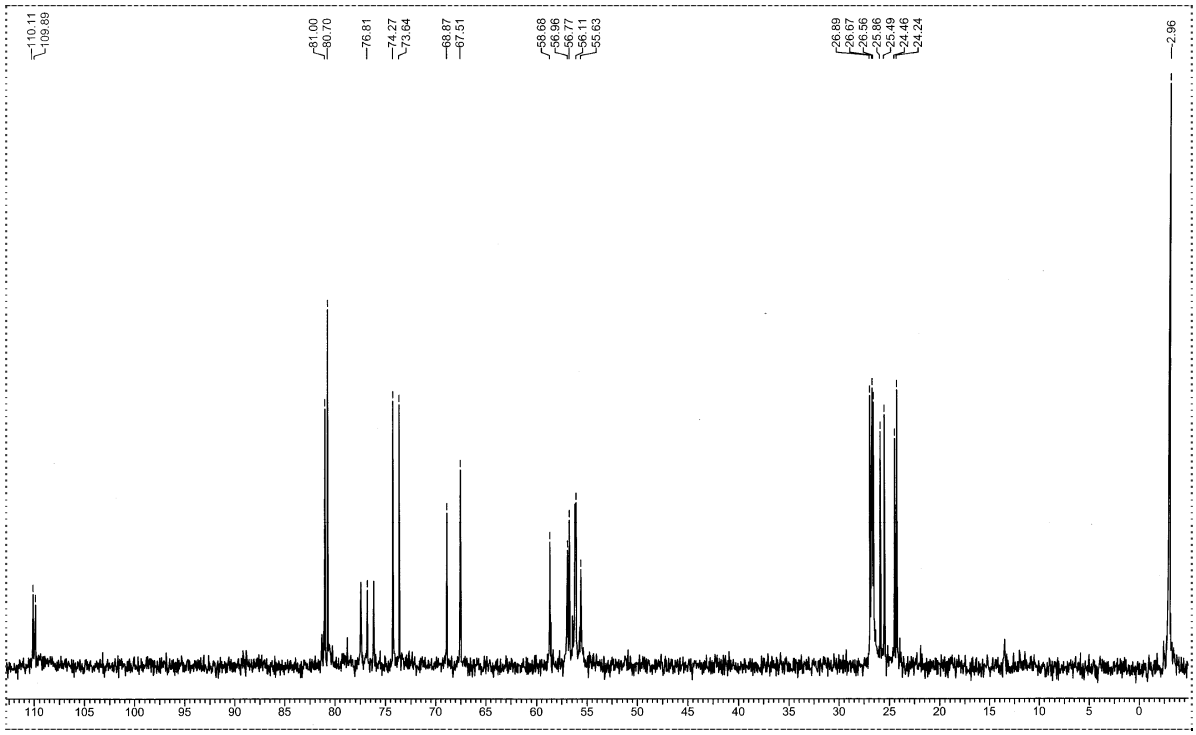
190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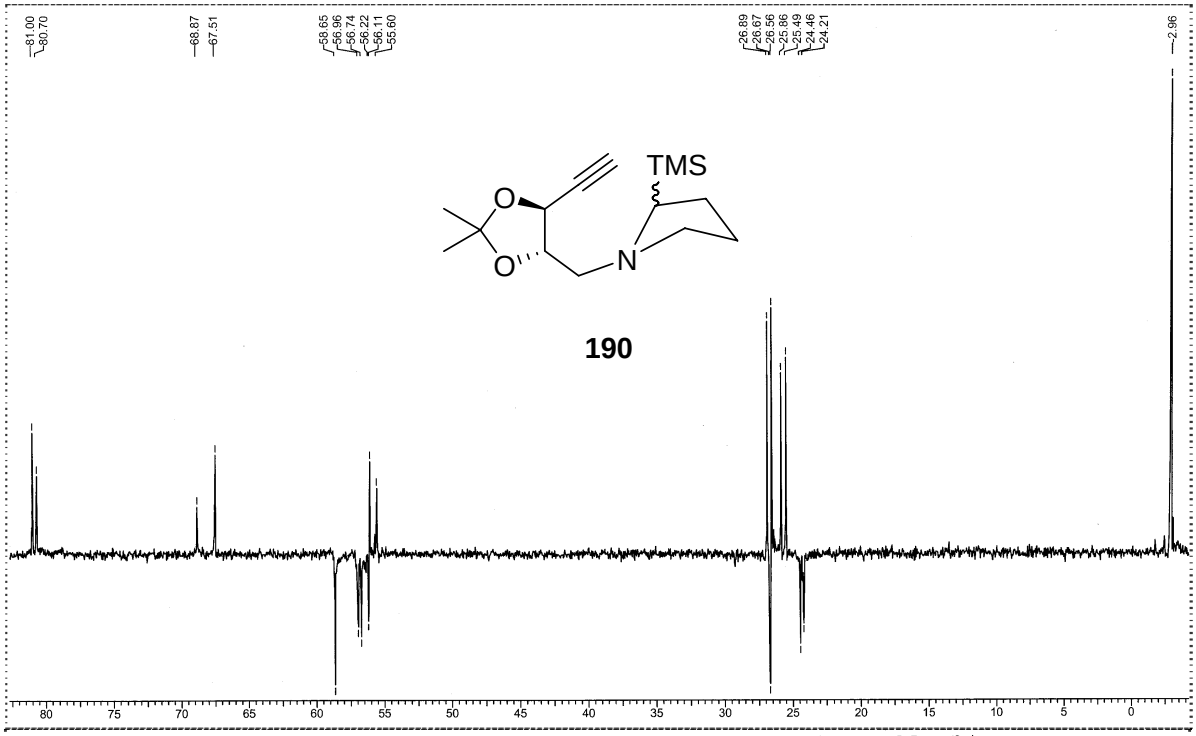
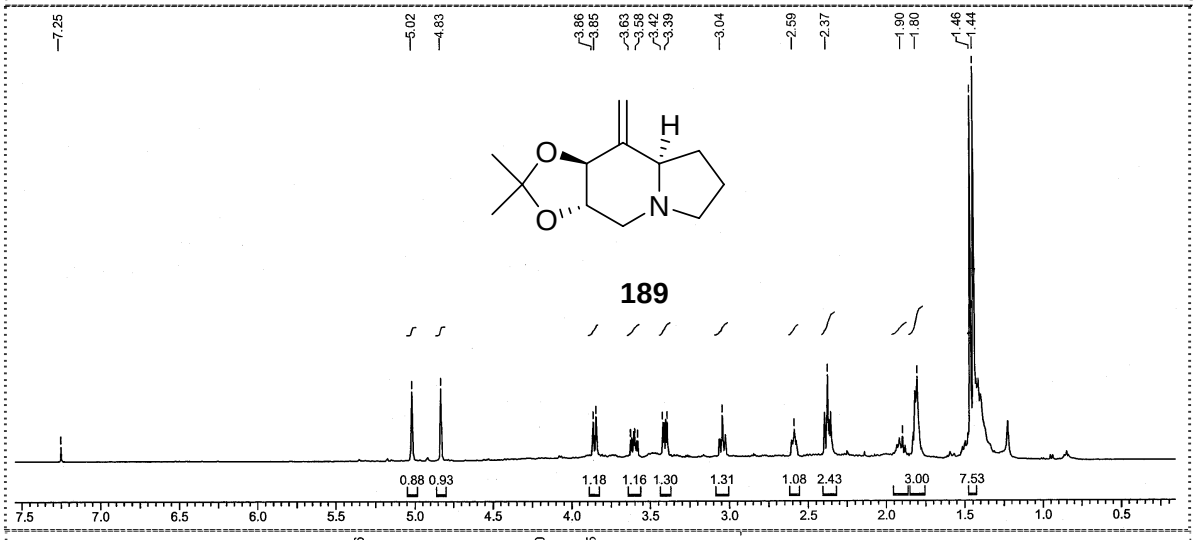
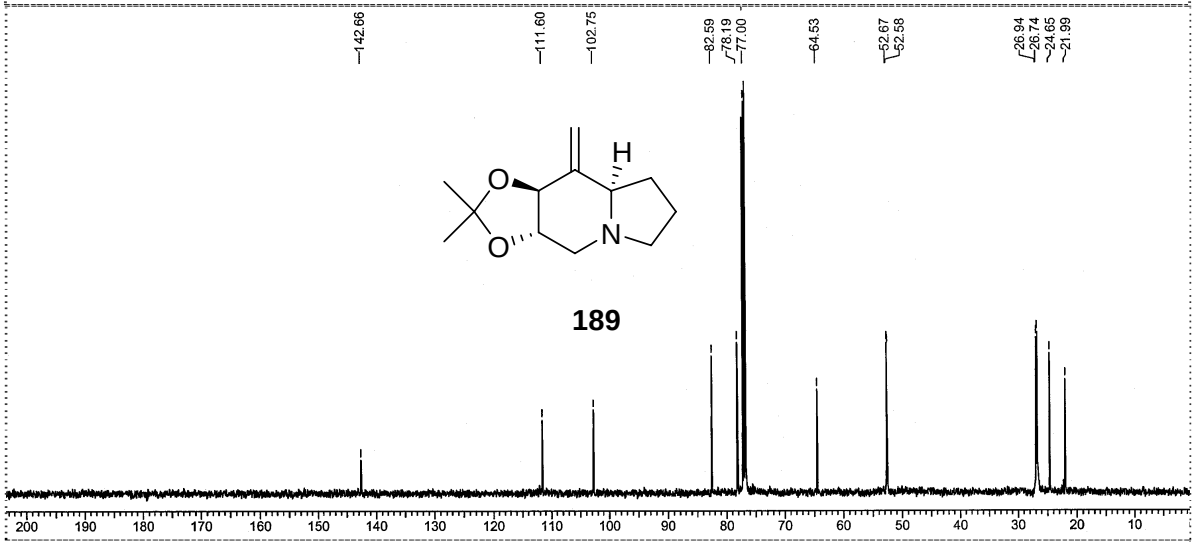
5
0



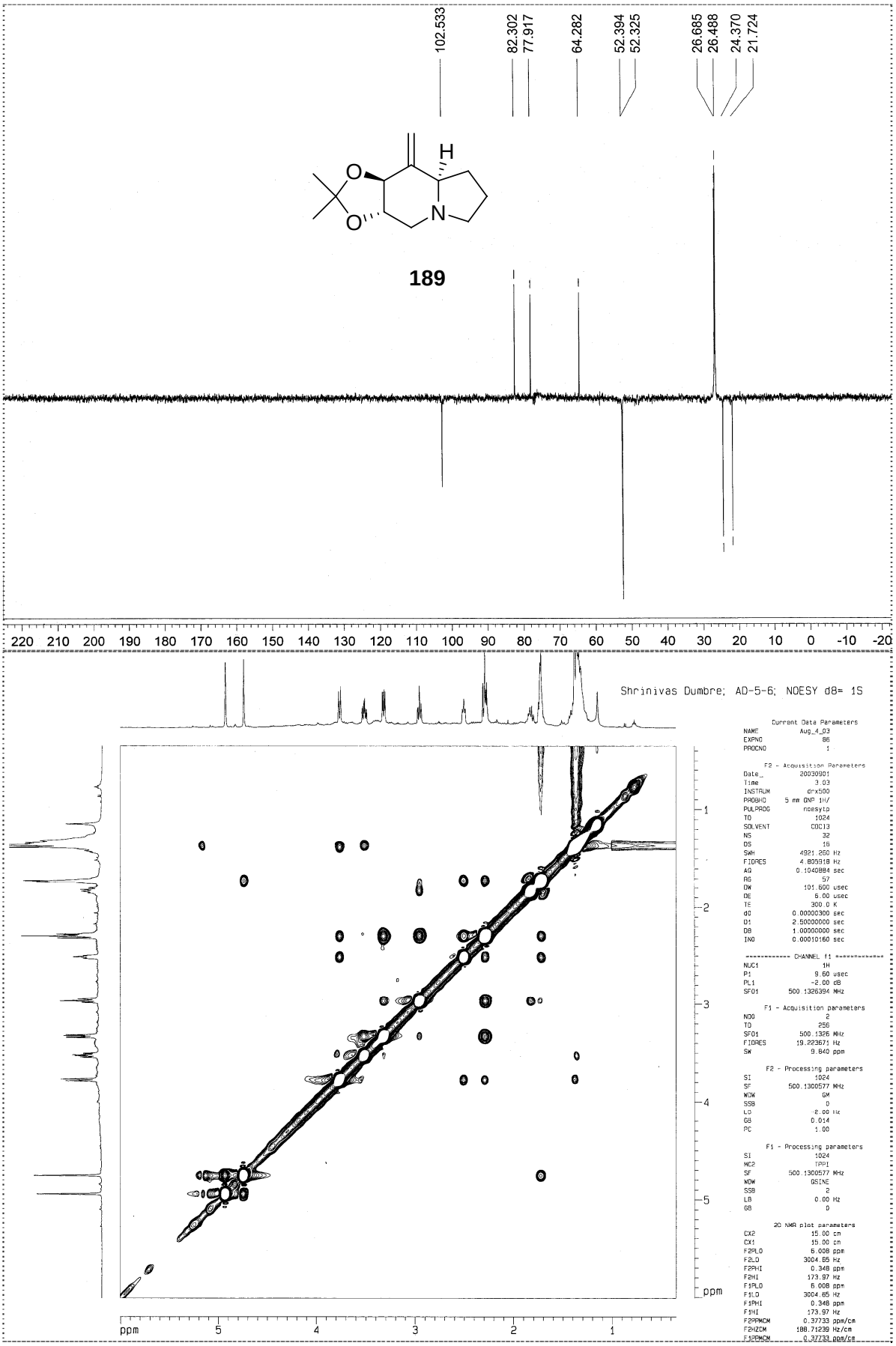
190



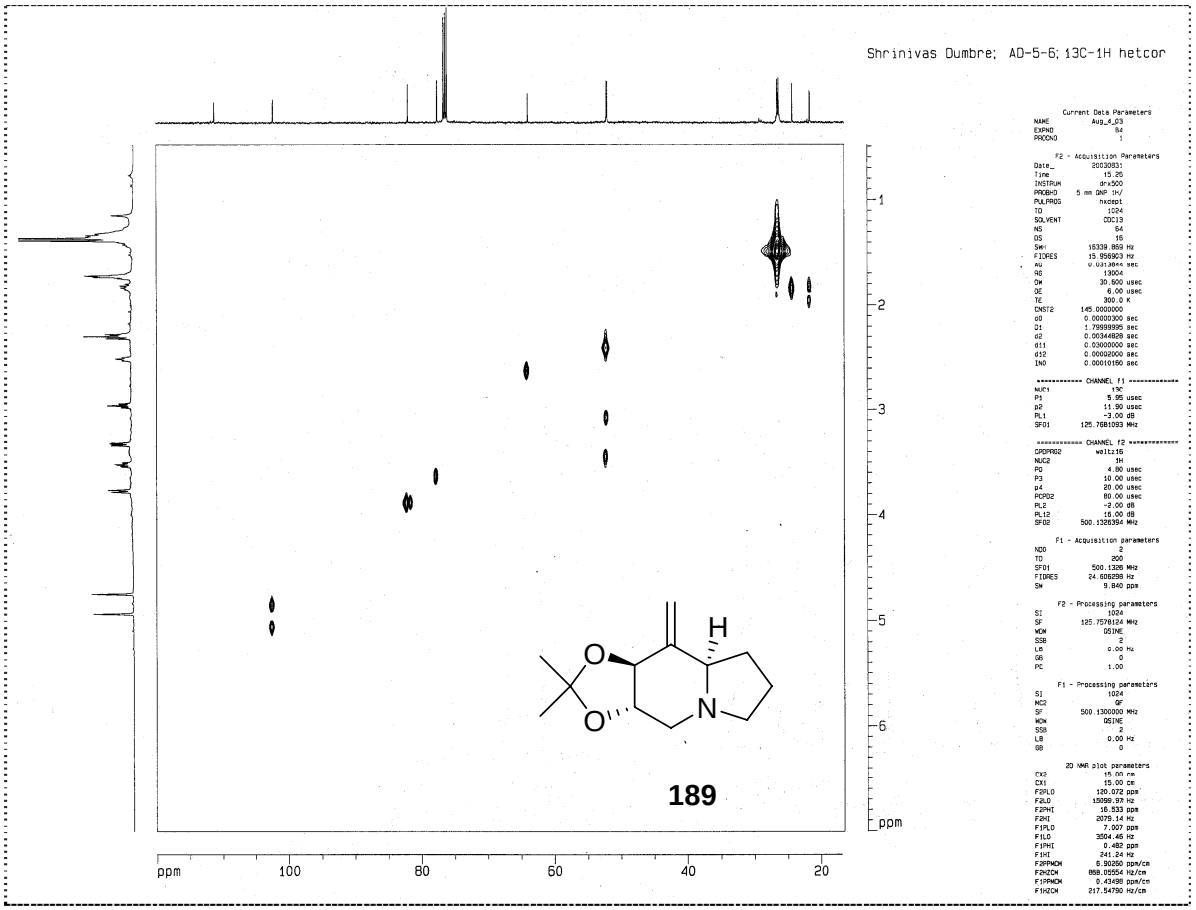
189

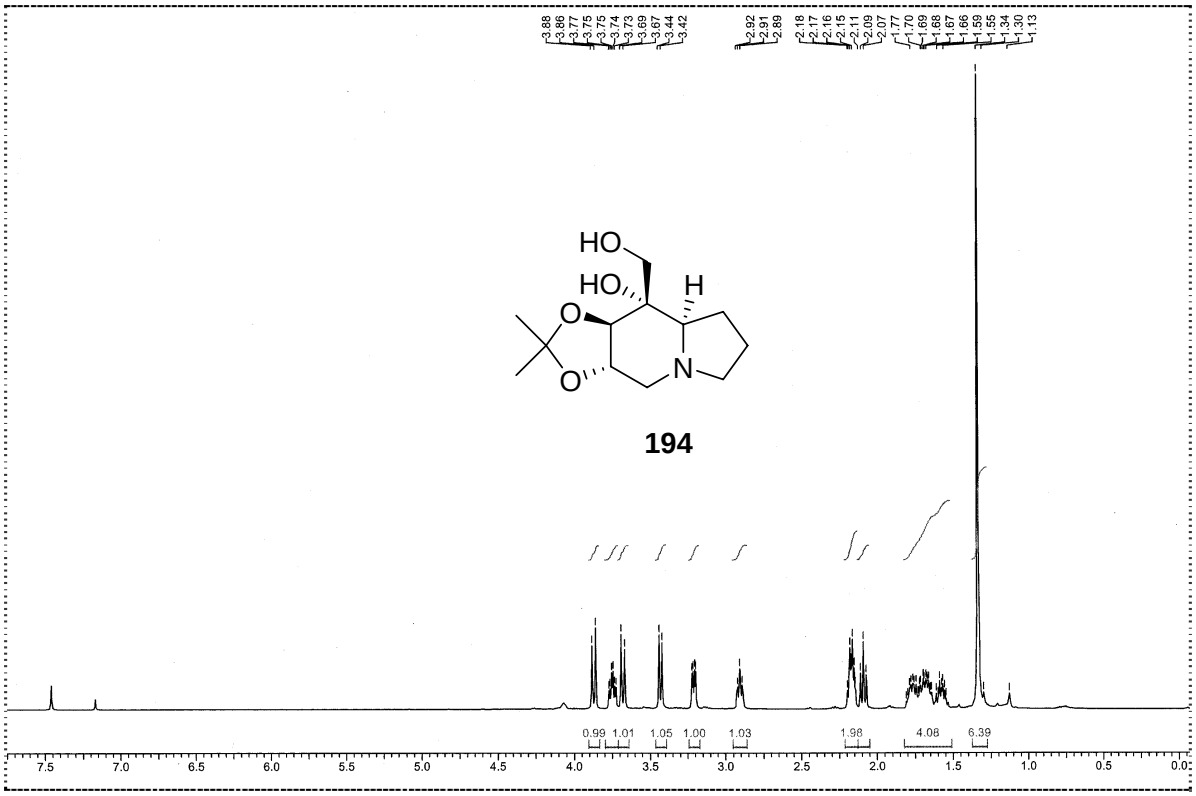


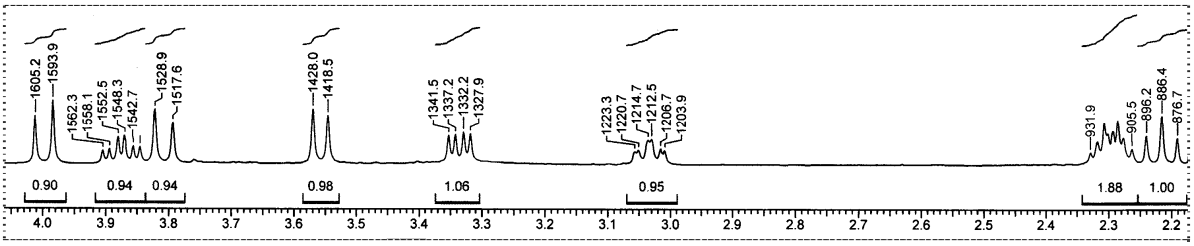
189

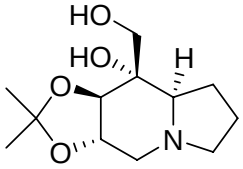

102.533
82.302
77.917
64.282
52.394
52.325
26.685
26.488
24.370
21.724
H
O
O
N
189
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
Shrinivas Dumbre; AD-5-6; NOESY d8= 1S
ppm
5
4
3
2
1

HETCOR


Shrinivas Dumbre; AD-5-6; 13C-1H hetcor
189
ppm
100
80
60
40
20

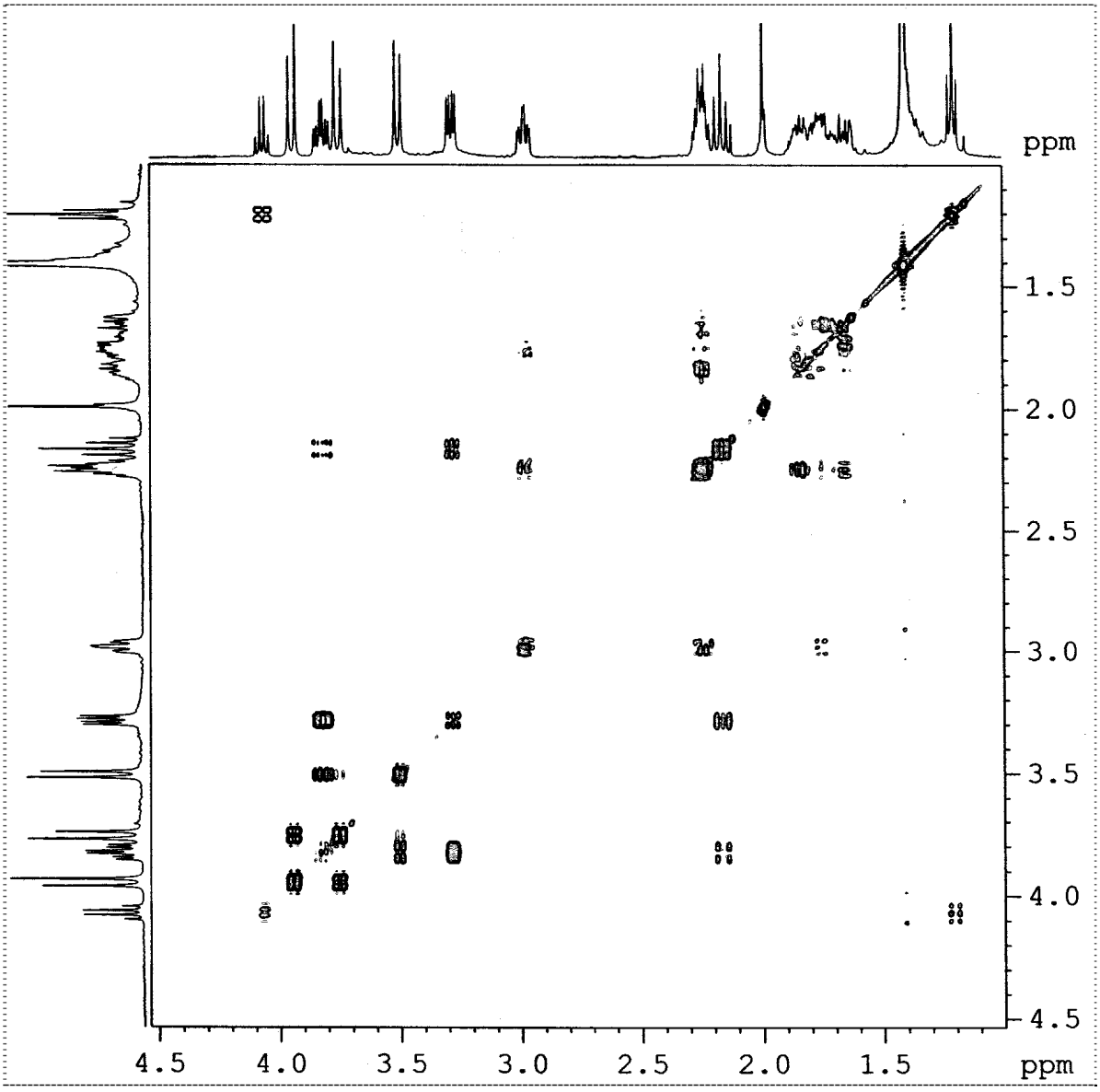

194
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

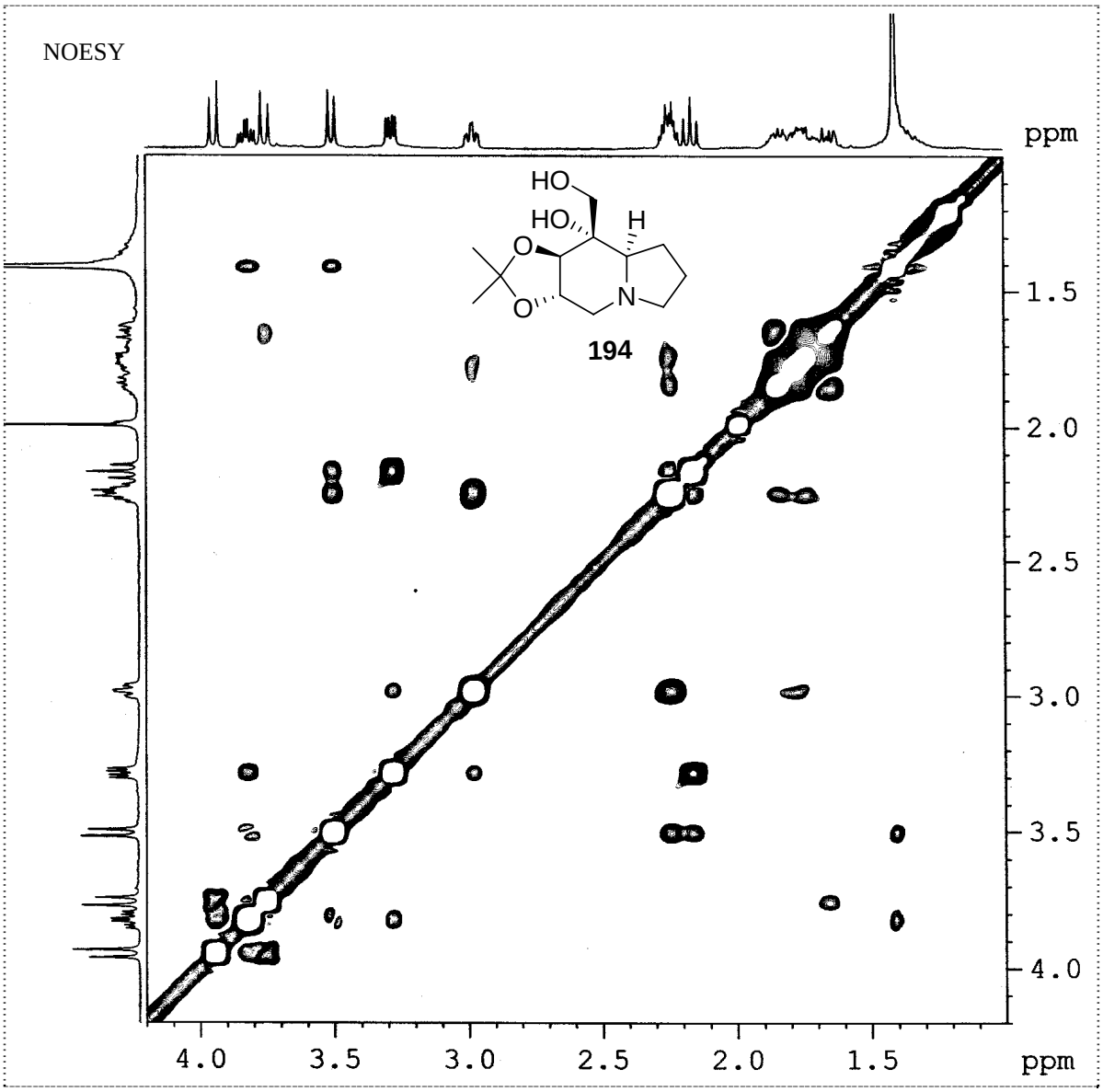

1605.2
1593.9
1562.3
1558.1
1552.5
1548.3
1542.7
1528.9
1517.6
1428.0
1416.5
1341.5
1337.2
1332.2
1327.9
1223.3
1220.7
1214.7
1212.5
1206.7
1203.9
931.9
905.5
896.2
886.4
876.7
0.90
0.94
0.94
0.98
1.06
0.95
1.88
1.00
4.0
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2

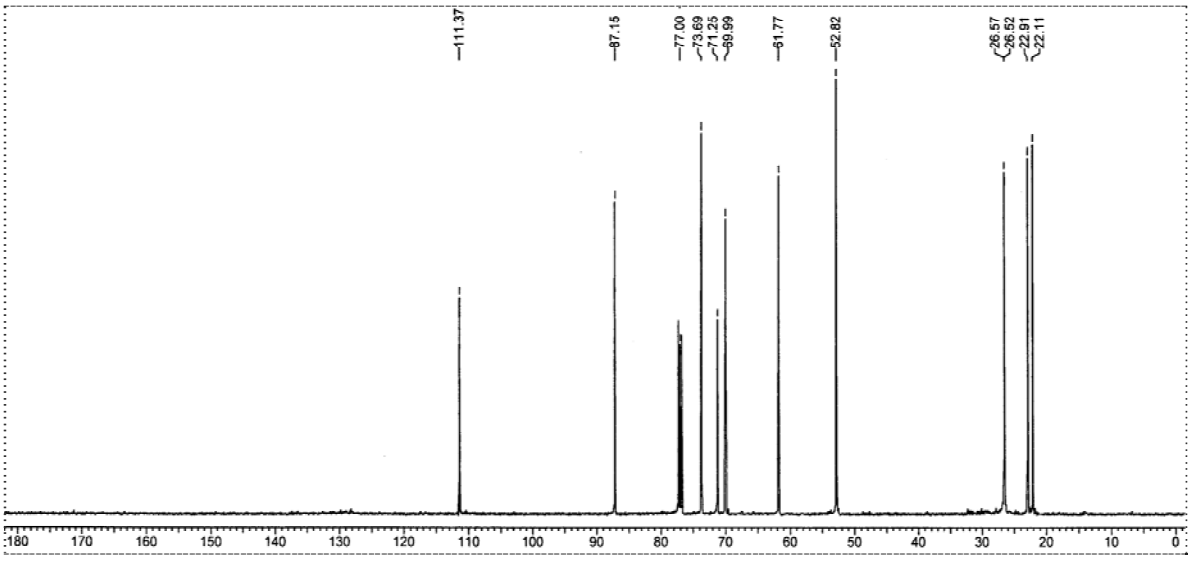

HO
HO
H
O
O
N

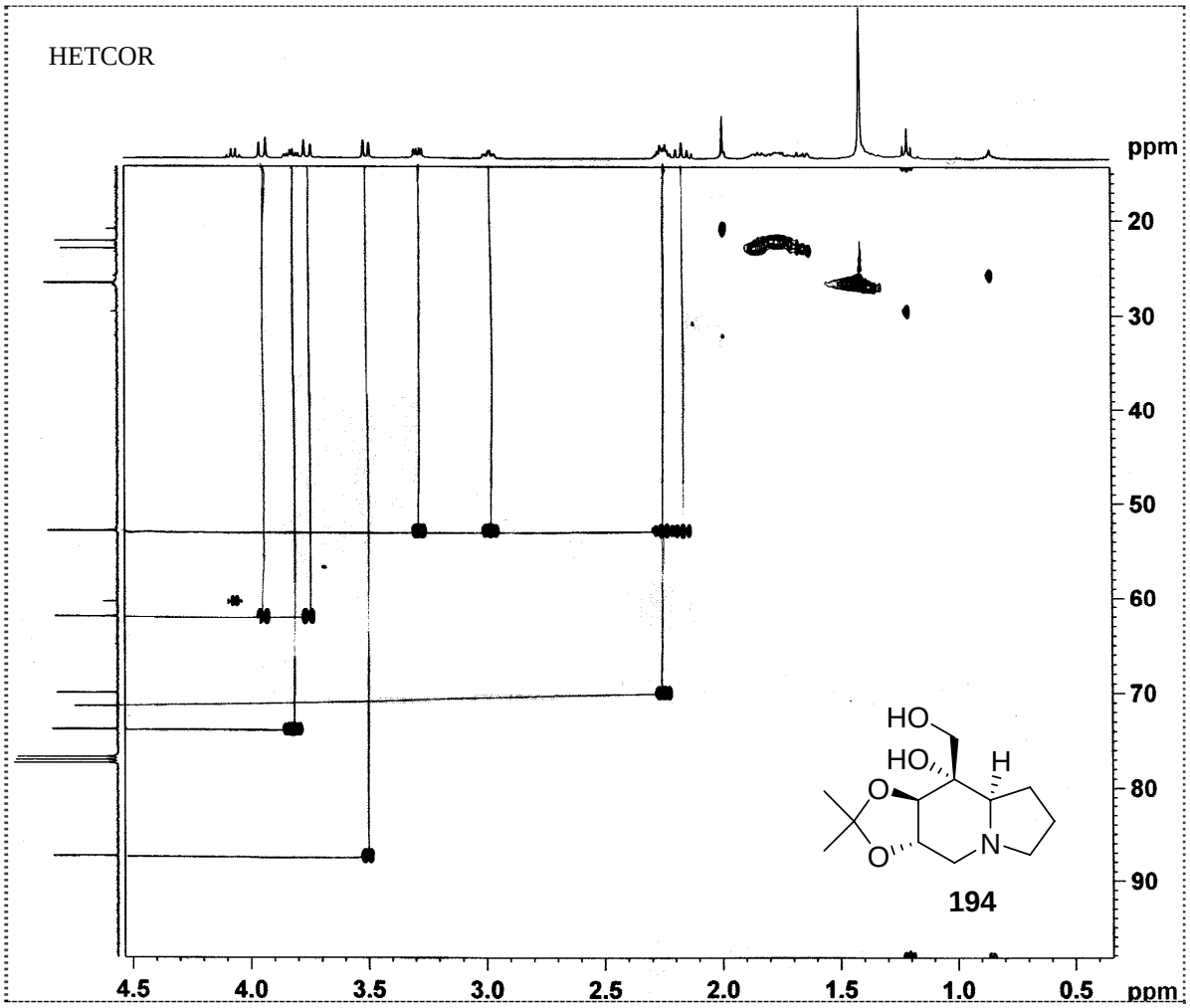
194

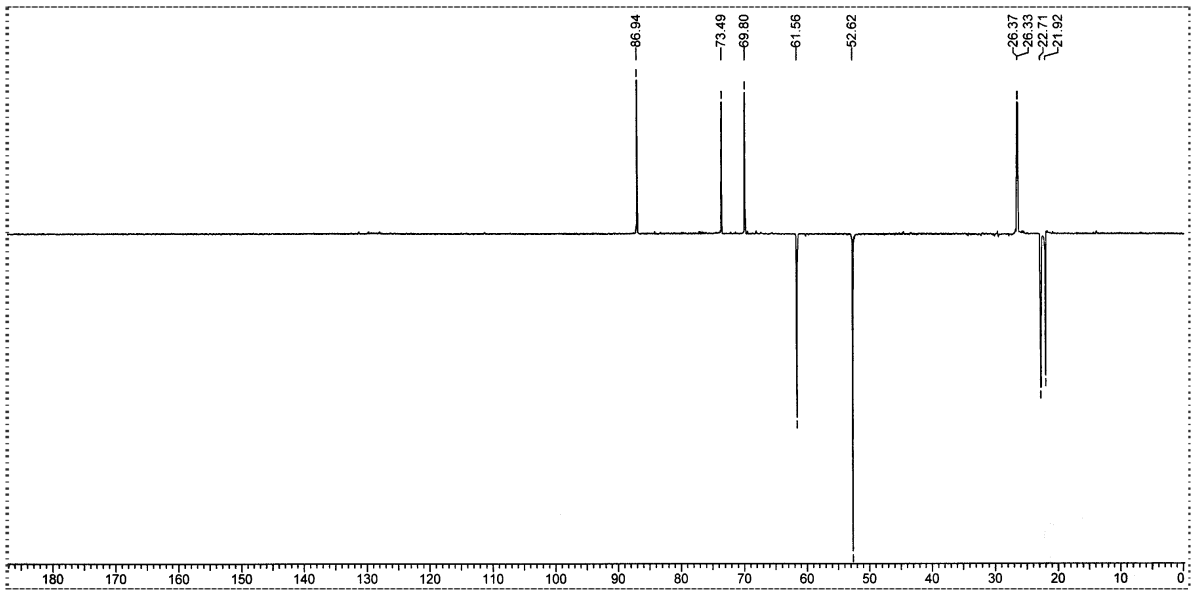
COSY

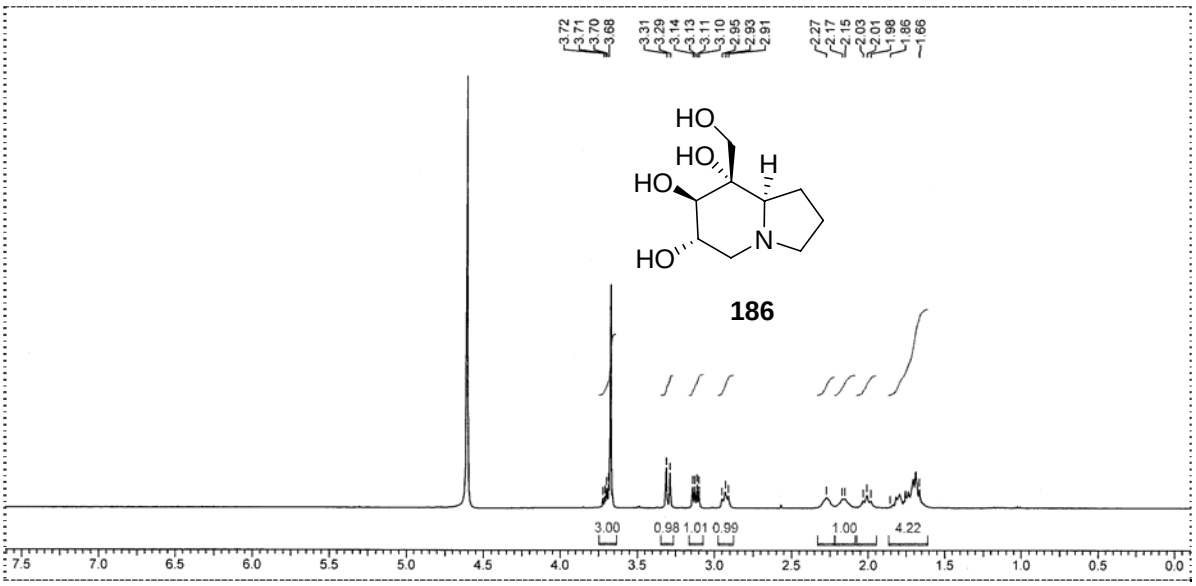

ppm
1.5
2.0
2.5
3.0
3.5
4.0
4.5
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm


NOESY
ppm
HO
HO
H
O
O
N
194
1.5
2.0
2.5
3.0
3.5
4.0
4.0
3.5
3.0
2.5
2.0
1.5
ppm

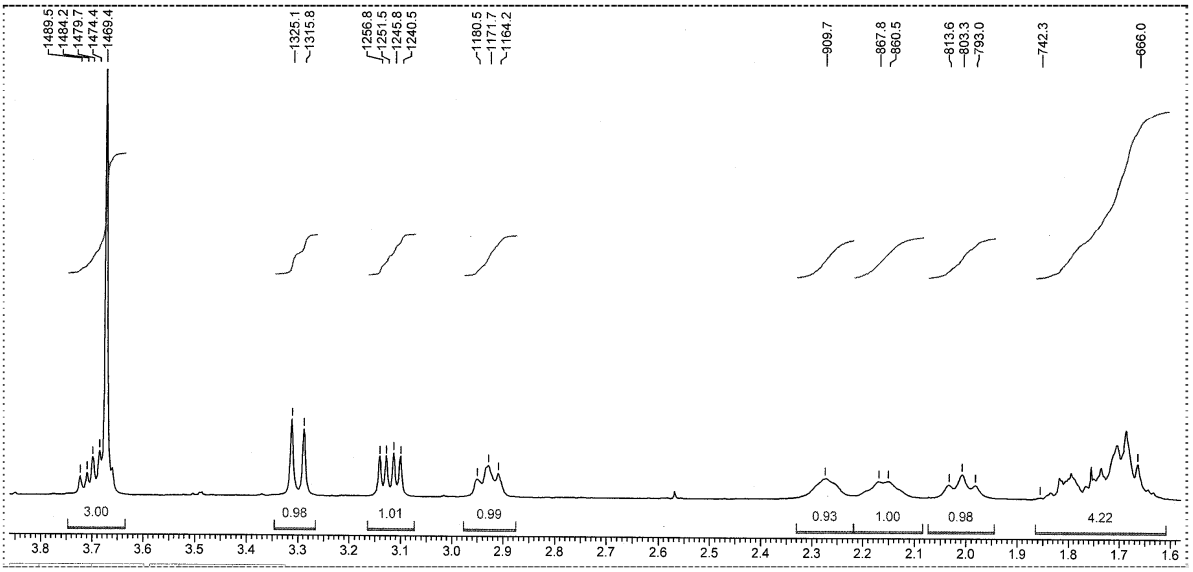

111.37
87.15
77.00
73.69
71.25
69.99
61.77
52.82
26.57
26.52
22.91
22.11
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

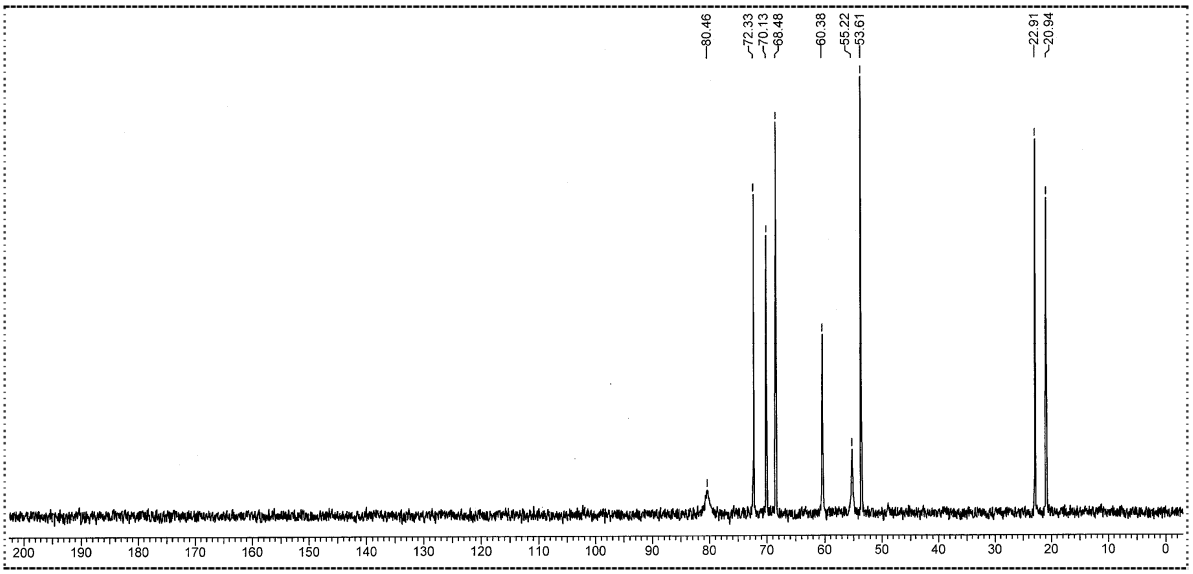

HETCOR
ppm
20
30
40
50
60
70
80
90
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
HO
HO
H
O
O
N
194

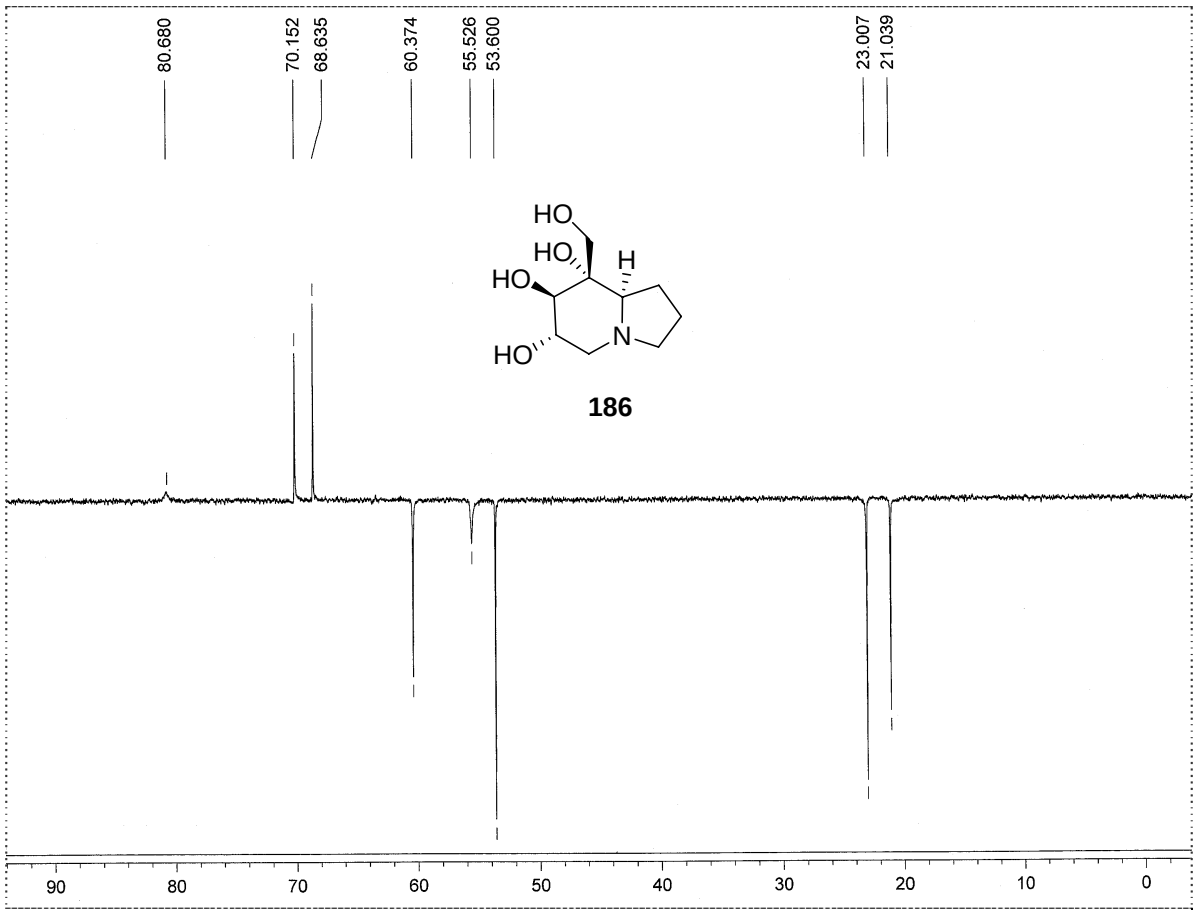

86.94
73.49
69.80
61.56
52.62
26.37
28.33
22.71
21.92
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0



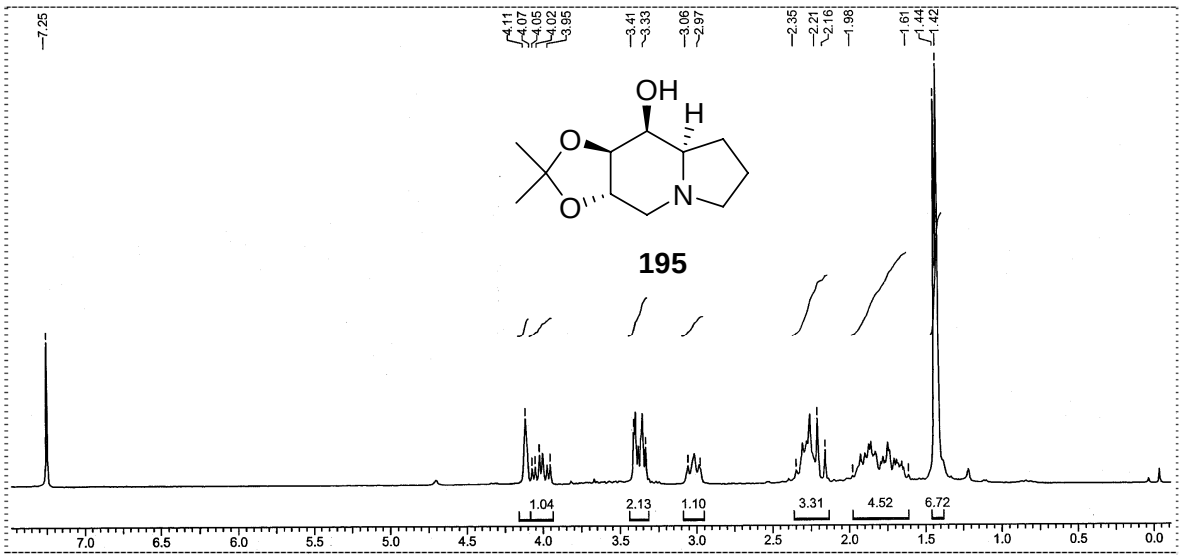
186



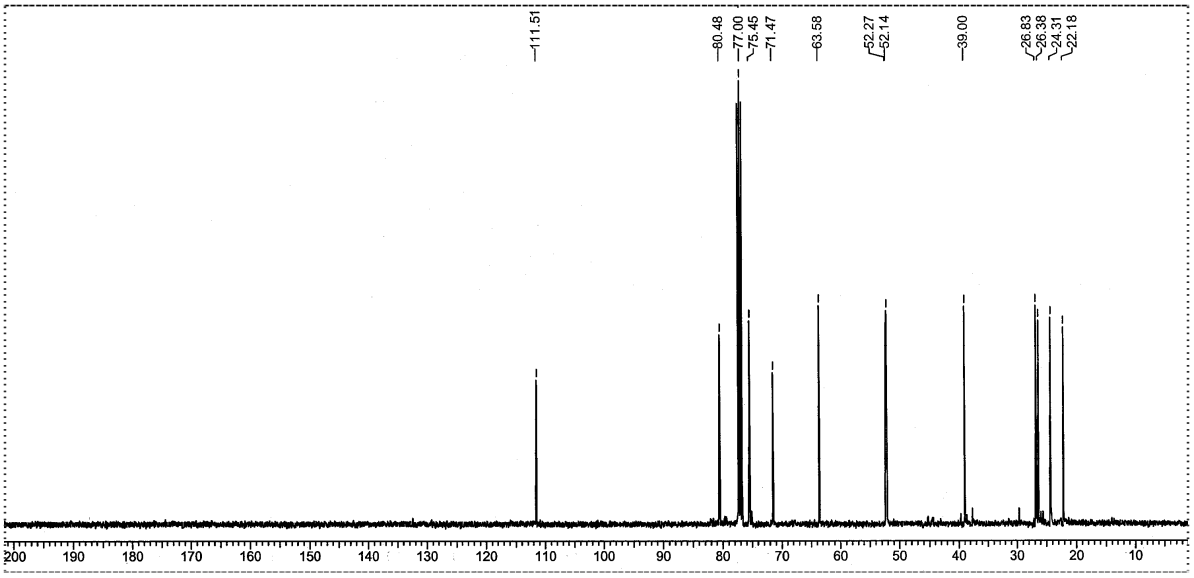


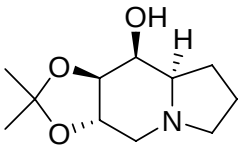

80.680
70.152
68.635
60.374
55.526
53.600
23.007
21.039
HO
HO
HO
HO
H
N
90
80
70
60
50
40
30
20
10
0

186

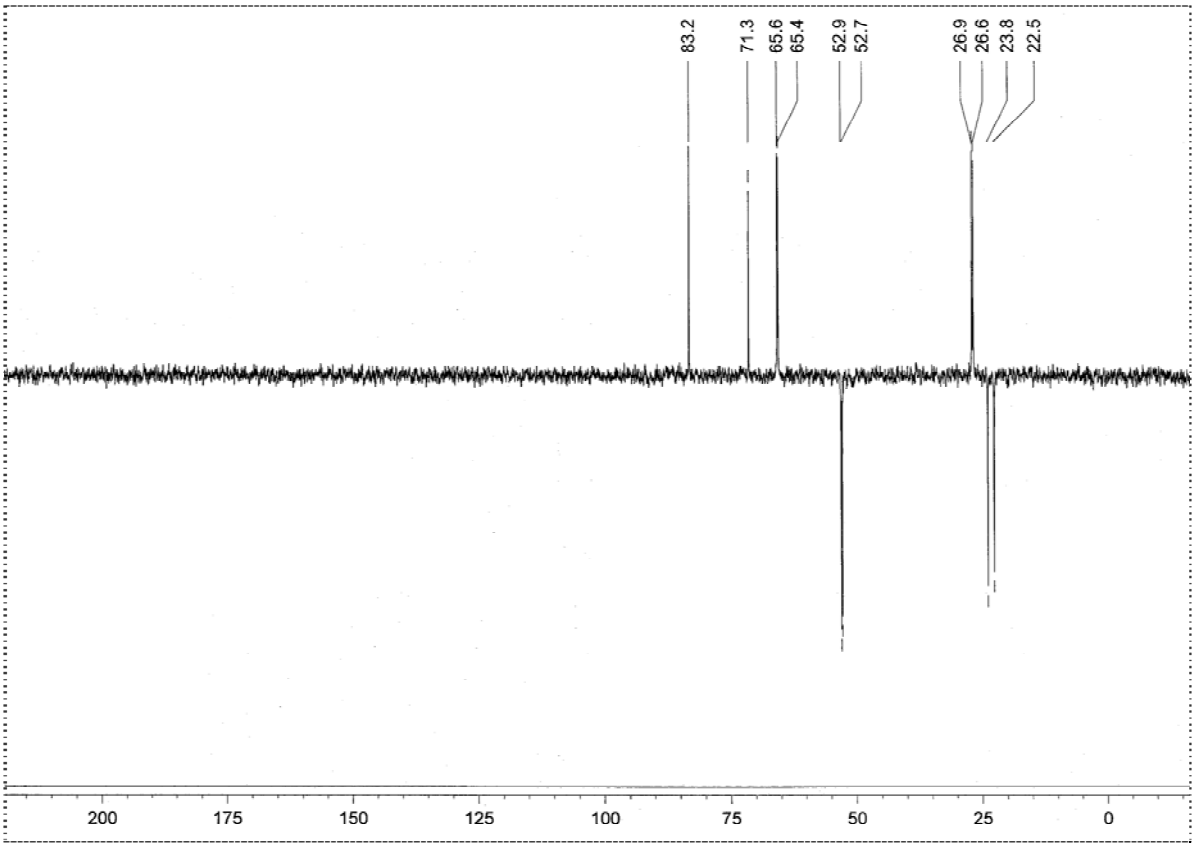

7.25
4.11
4.07
4.05
4.02
3.95
3.41
3.33
3.06
2.97
2.35
2.21
2.16
1.98
1.61
1.44
1.42
OH
H
O
O
N
1.04
2.13
1.10
3.31
4.52
6.72
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

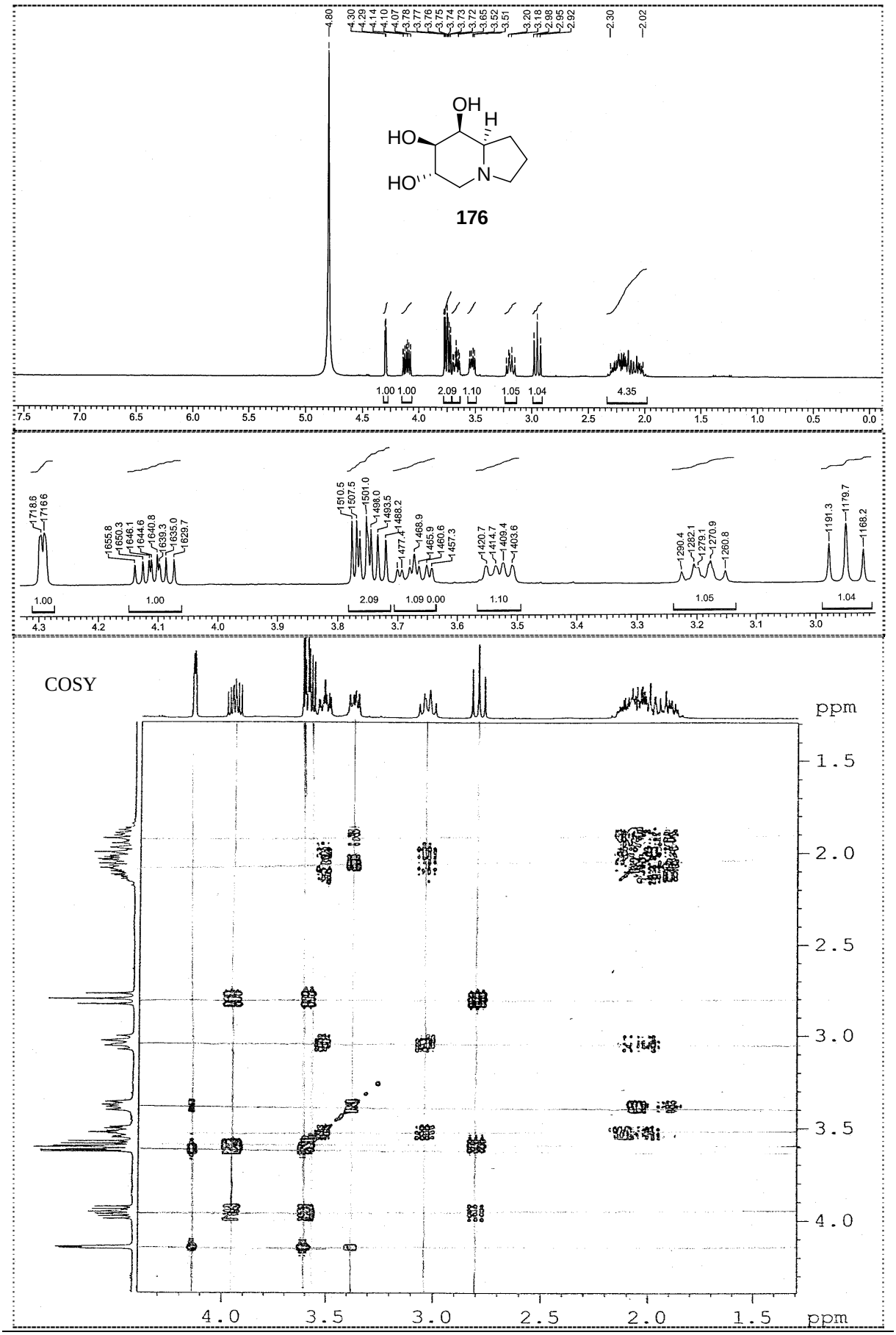
195

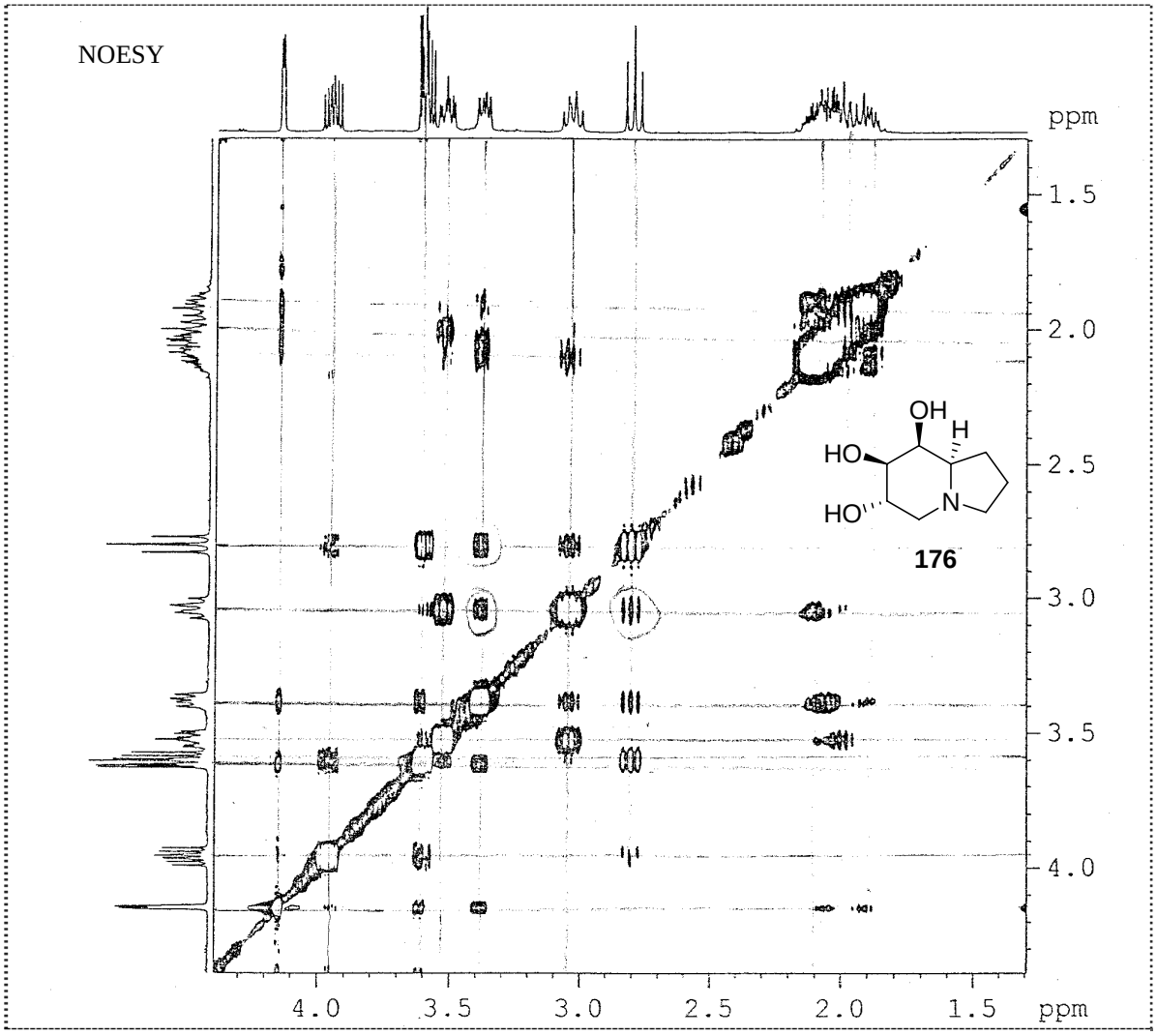

111.51
80.48
77.00
75.45
71.47
63.58
52.27
52.14
39.00
26.83
26.38
24.31
22.18
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

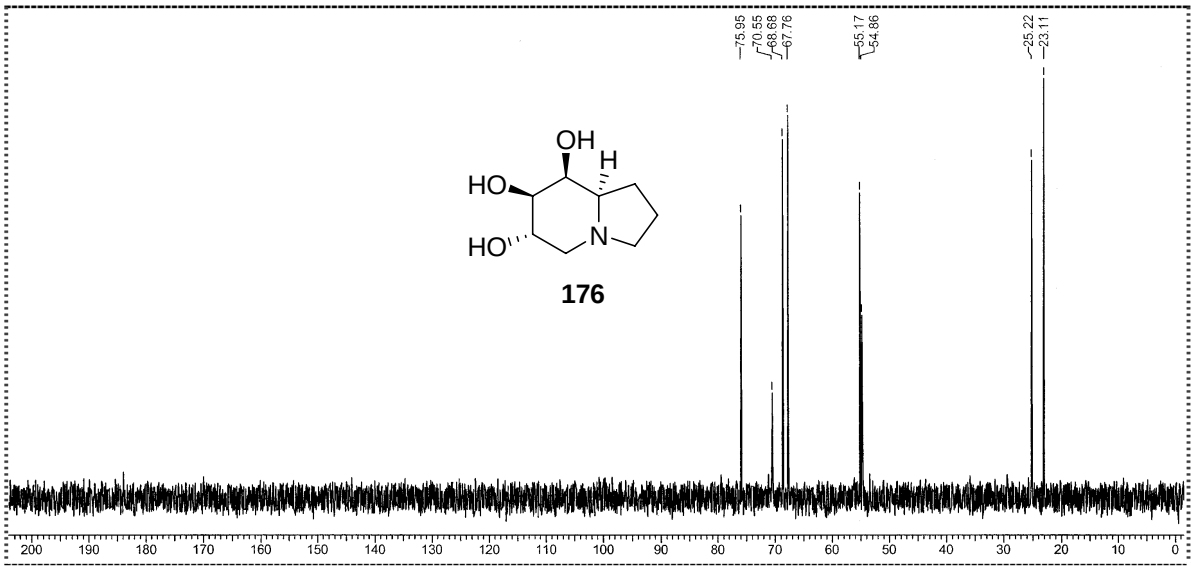

OH
H
O
O
N

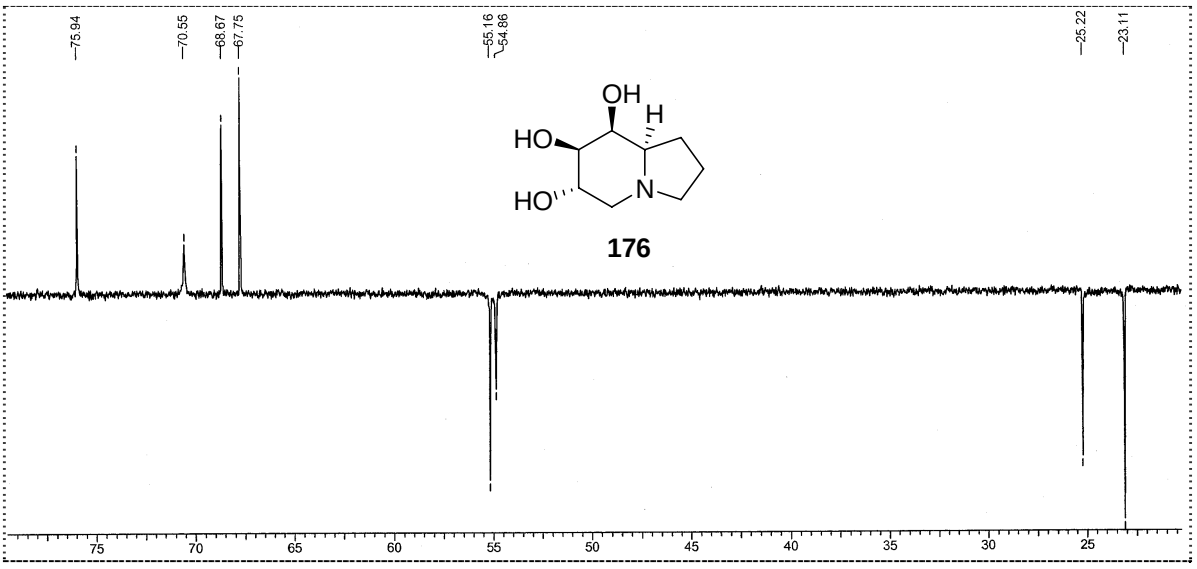
195


83.2
71.3
65.6
65.4
52.9
52.7
26.9
26.6
23.8
22.5
200
175
150
125
100
75
50
25
0

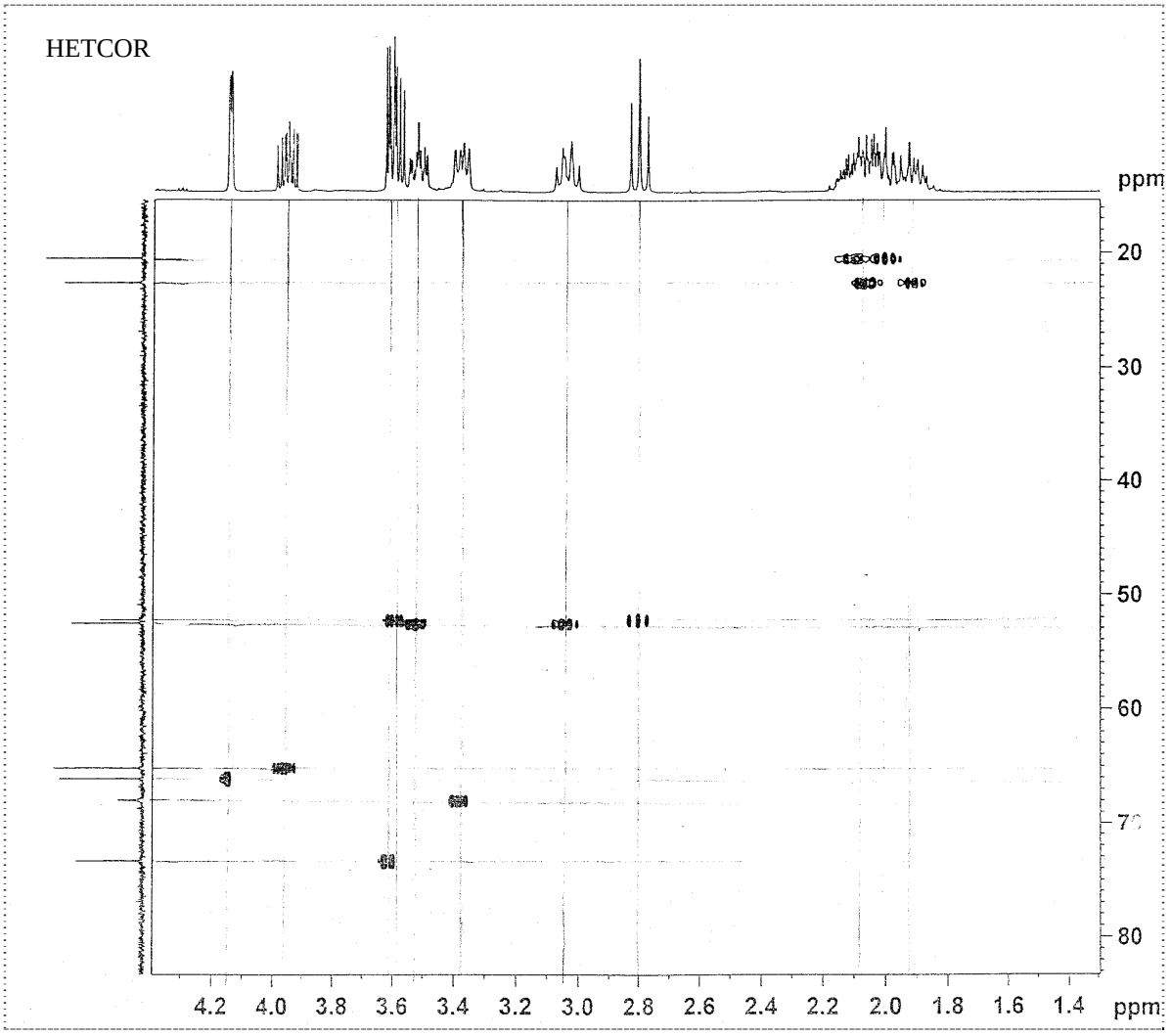

OH
H
HO
HO
N
176
COSY
ppm

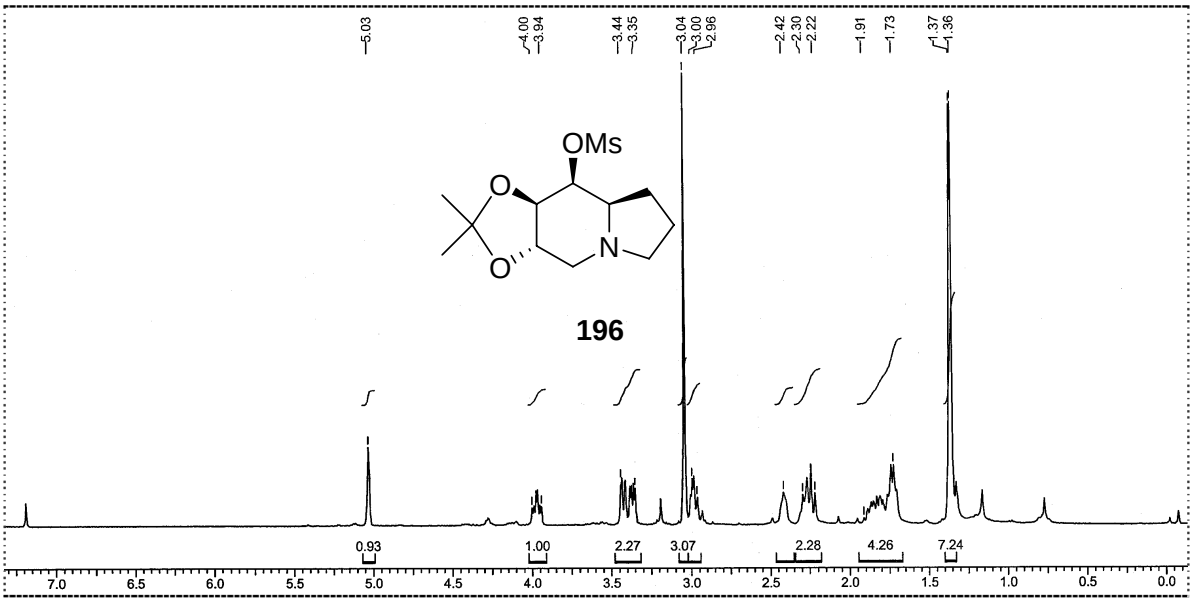

NOESY
ppm
1.5
2.0
2.5
3.0
3.5
4.0
4.0
3.5
3.0
2.5
2.0
1.5
ppm
OH
H
HO
HO
N
176

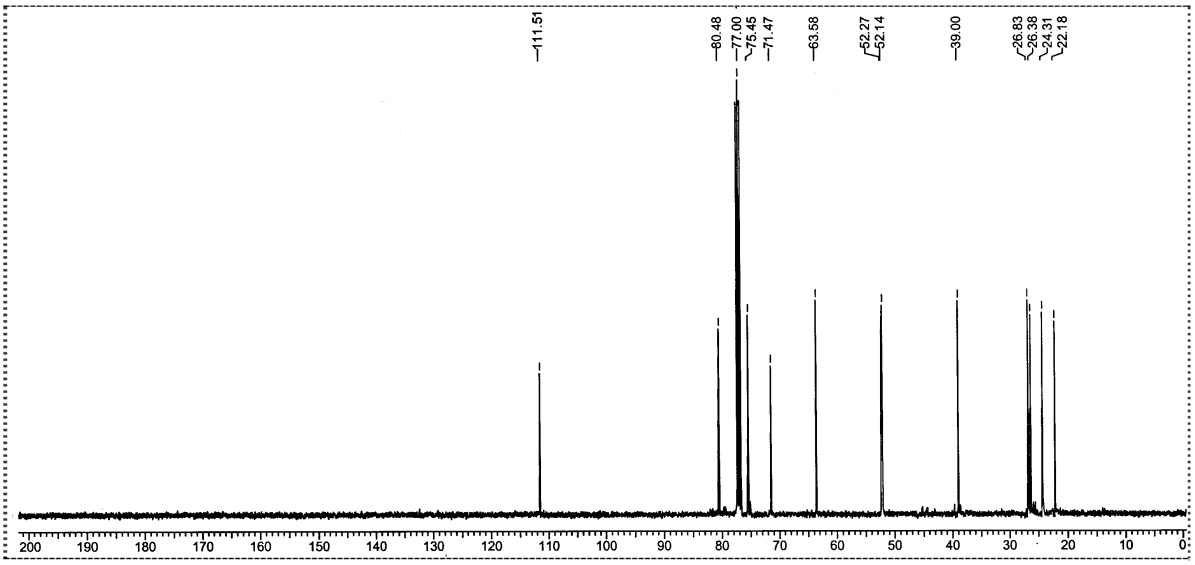

75.95
70.55
68.68
67.76
55.17
54.86
25.22
23.11
OH
H
HO
HO
N
176
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

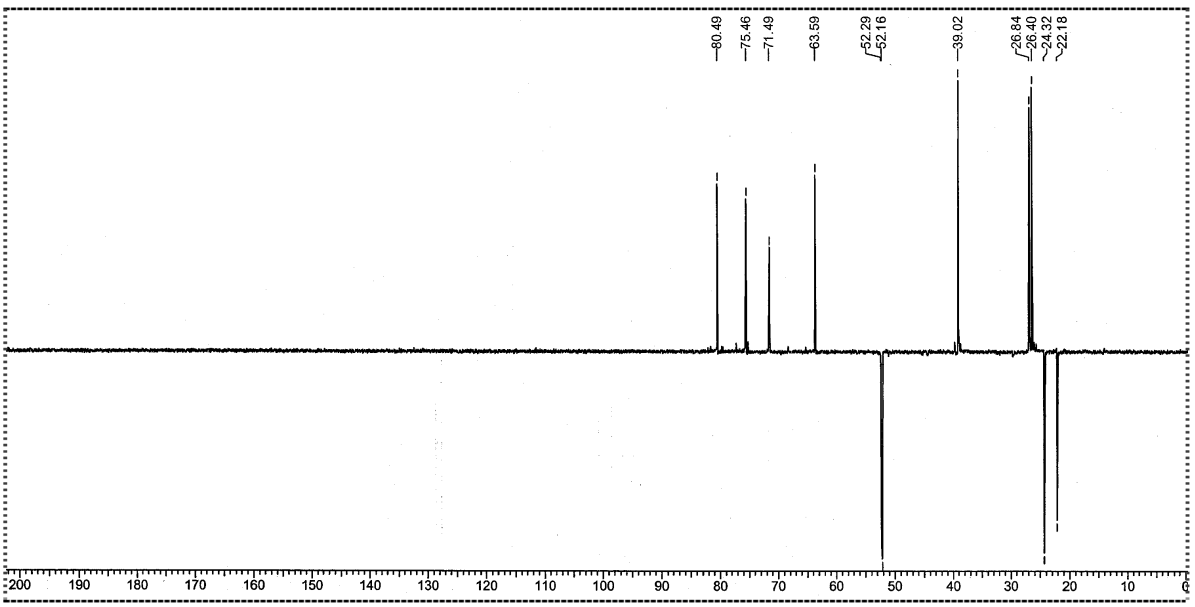

75.94
70.55
68.67
67.75
55.16
54.86
25.22
23.11
OH
H
HO
HO
N
75
70
65
60
55
50
45
40
35
30
25

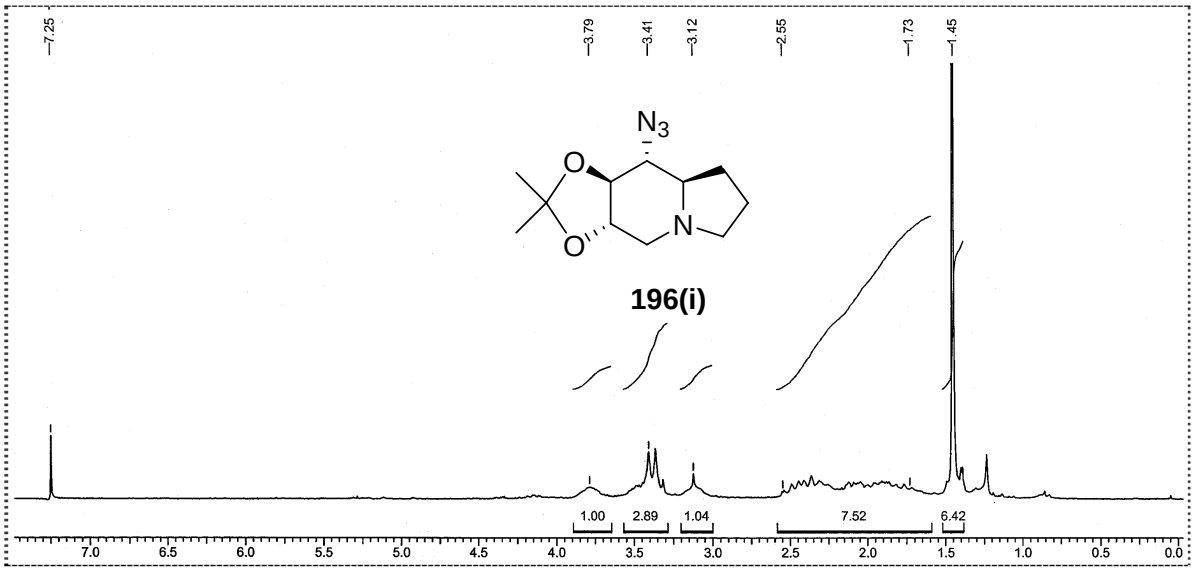
176


HETCOR
ppm
20
30
40
50
60
70
80
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
ppm

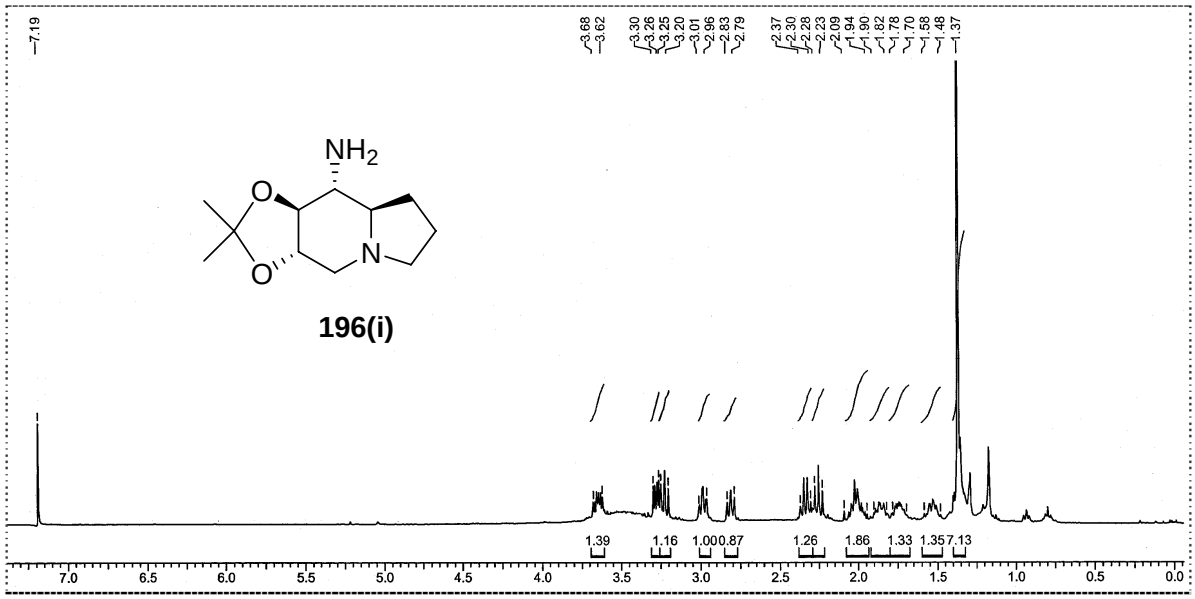

5.03
4.00
3.94
3.44
3.35
3.04
3.00
2.96
2.42
2.30
2.22
1.91
1.73
1.37
1.36
OMs
O
O
N
196
0.93
1.00
2.27
3.07
2.28
4.26
7.24


111.51
80.48
77.00
75.45
71.47
63.58
62.27
62.14
39.00
26.83
26.38
24.31
22.18

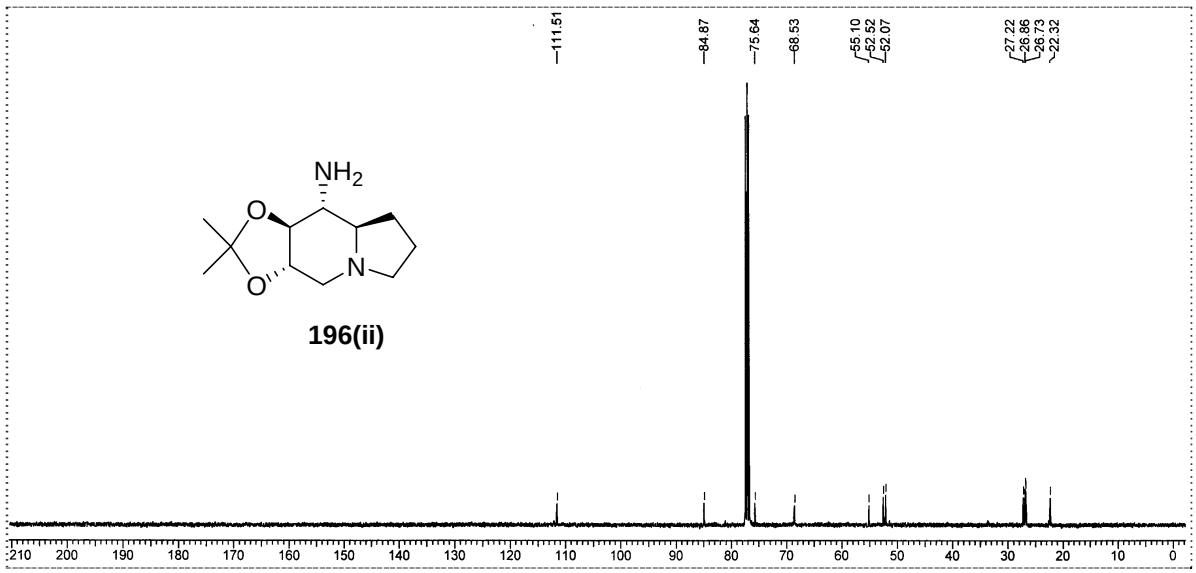

80.49
75.46
71.49
63.59
52.29
52.16
39.02
26.84
26.40
24.32
22.18



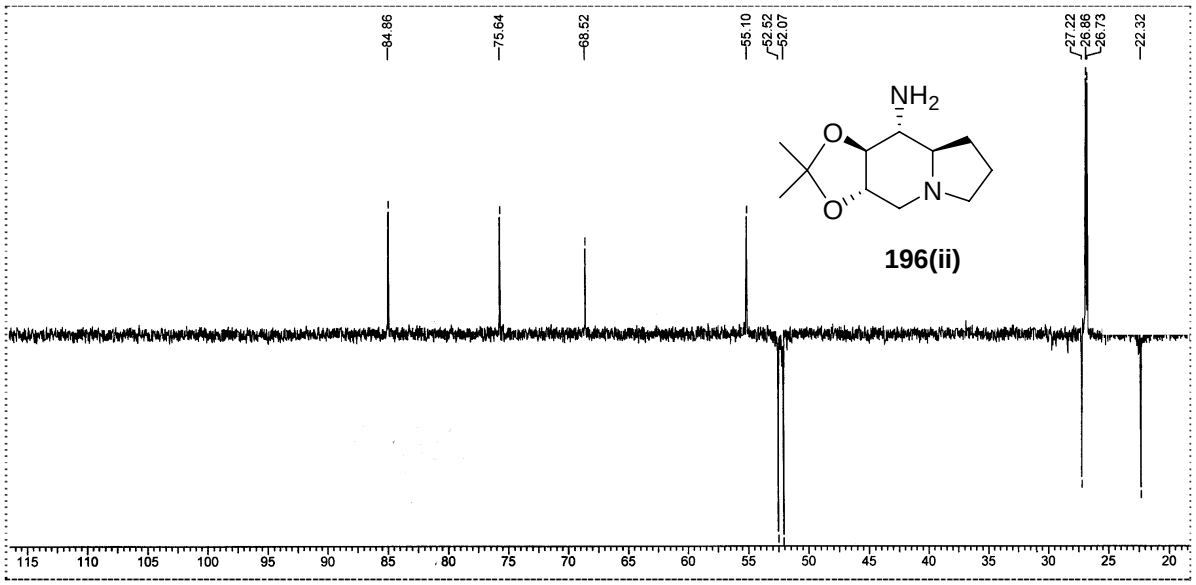
196(i)

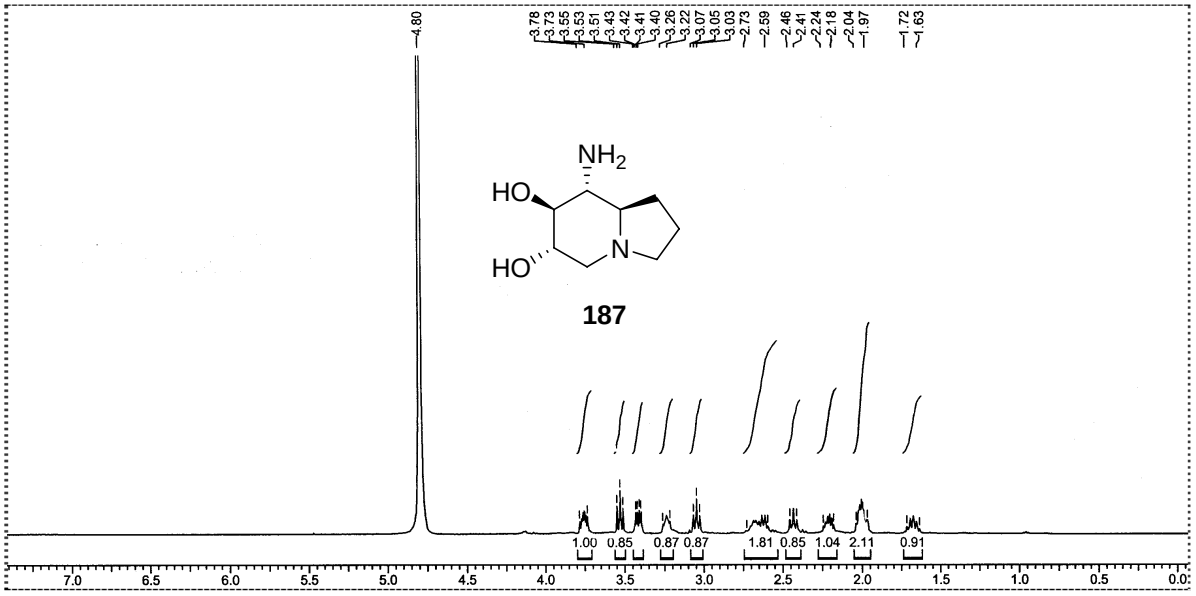


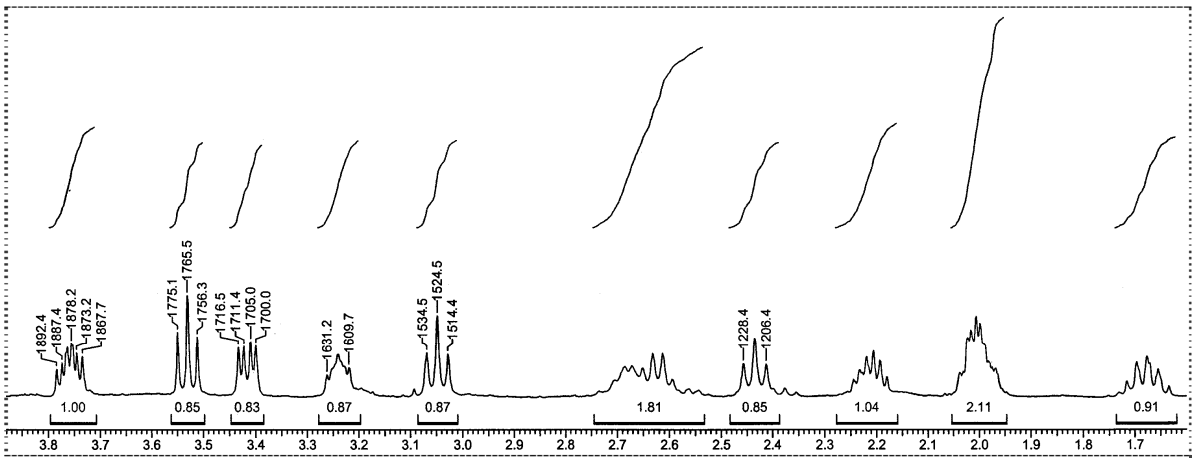
196(i)

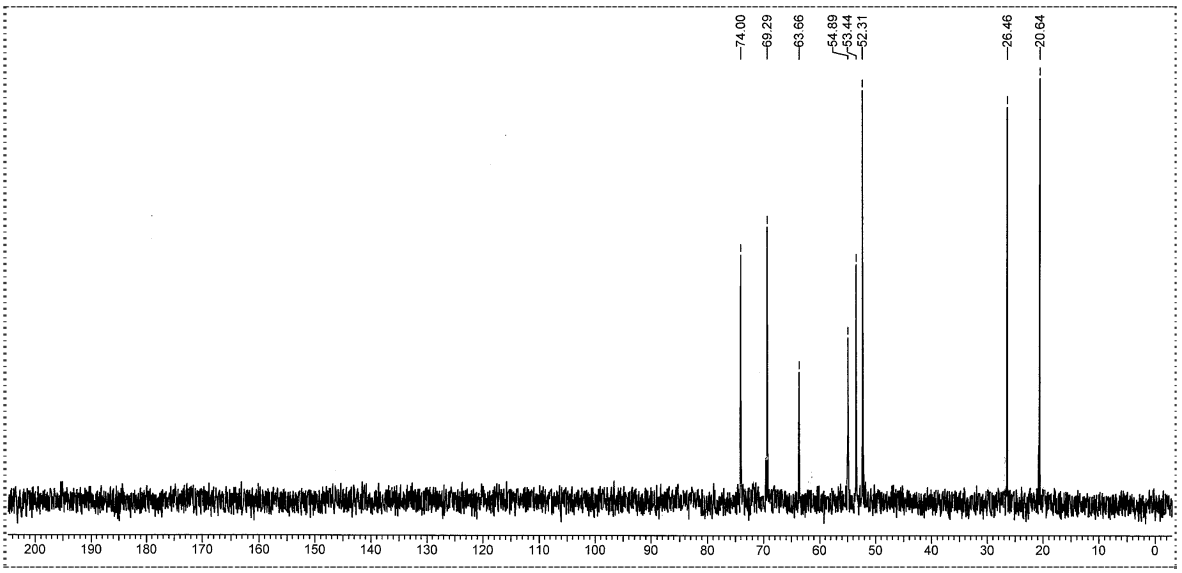


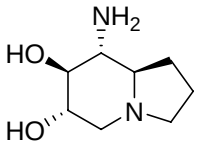
196(ii)


84.86
75.64
68.52
55.10
52.52
52.07
27.22
26.86
26.73
22.32
NH2
O
O
N
196(ii)

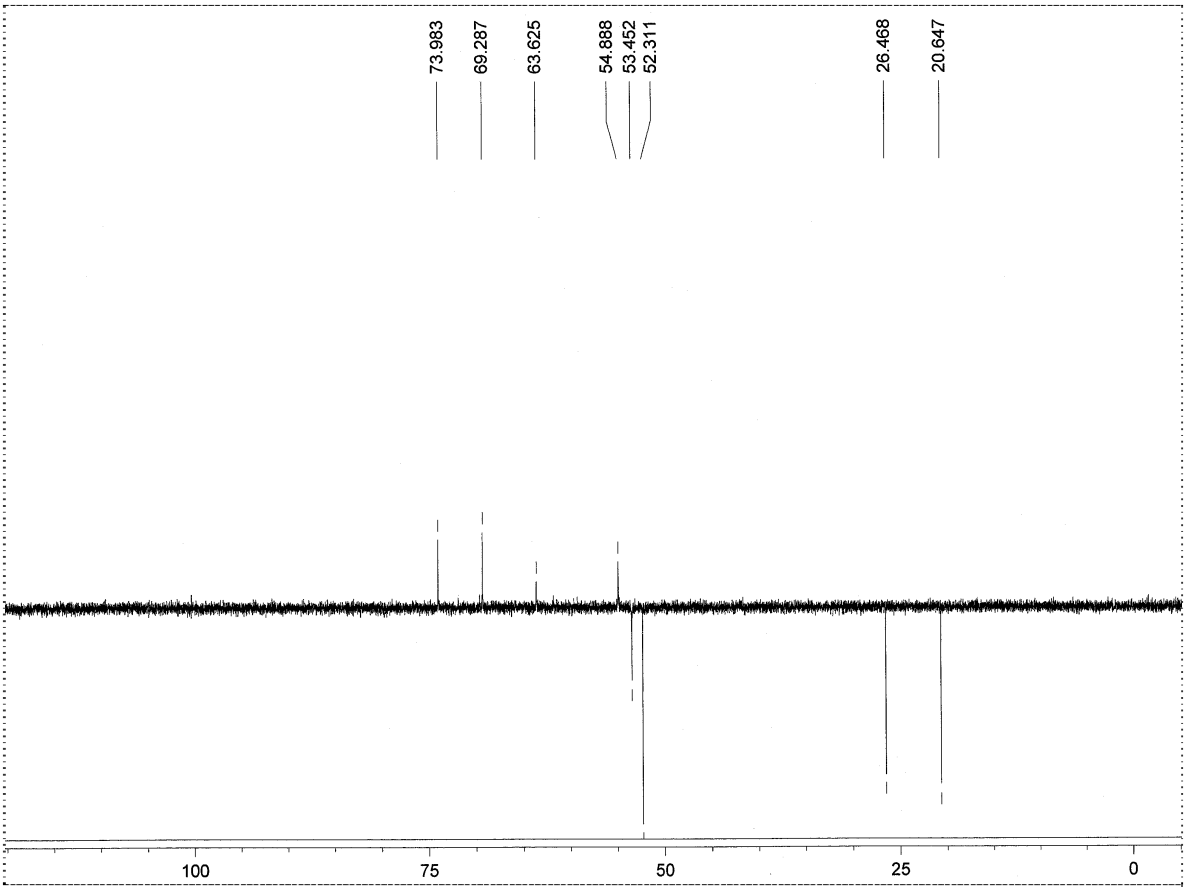

4.80
3.78
3.73
3.55
3.53
3.51
3.43
3.42
3.41
3.40
3.26
3.22
3.07
3.05
3.03
2.73
2.59
2.46
2.41
2.24
2.18
2.04
1.97
1.72
1.63
NH2
HO
HO
N
187
1.00 0.85 0.87 0.87 1.81 0.85 1.04 2.11 0.91


1892.4
1887.4
1878.2
1873.2
1867.7
1775.1
1765.5
1756.3
1716.5
1711.4
1705.0
1700.0
1631.2
1609.7
1534.5
1524.5
1514.4
1228.4
1206.4
1.00 0.85 0.83 0.87 0.87 1.81 0.85 1.04 2.11 0.91


74.00
69.29
63.66
54.89
53.44
52.31
26.46
20.64
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

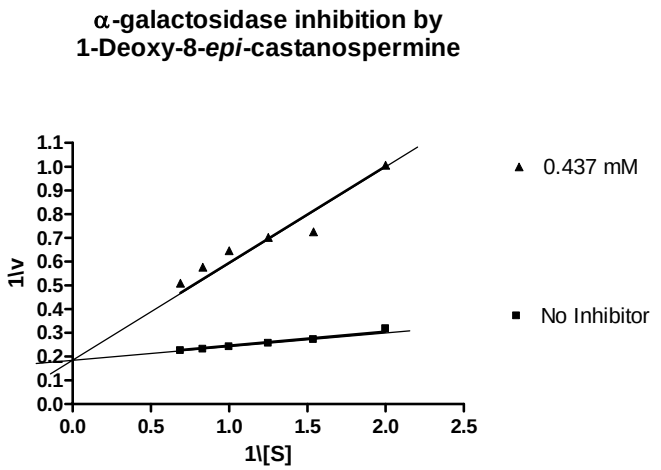

NH2
HO
HO
N

187

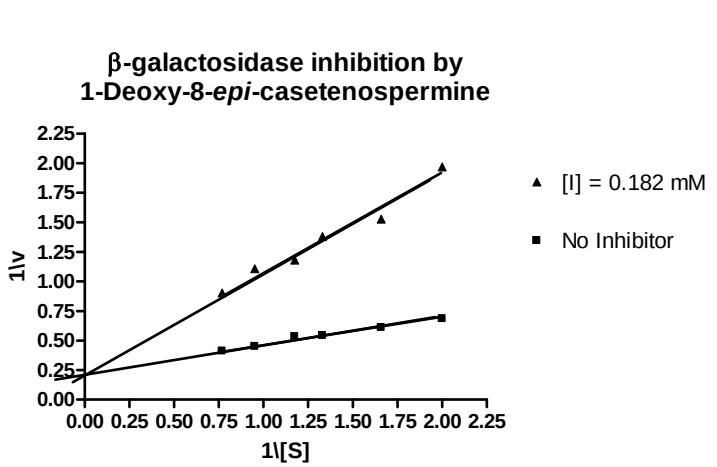

73.983
69.287
63.625
54.888
53.452
52.311
26.468
20.647
100 75 50 25 0

4.10.2 Lineweaver-Burke Plots:

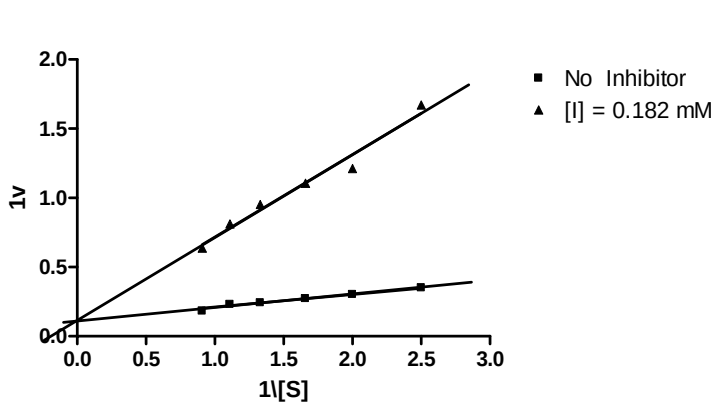
Compound **176 (*Ki* = 71 μM)**



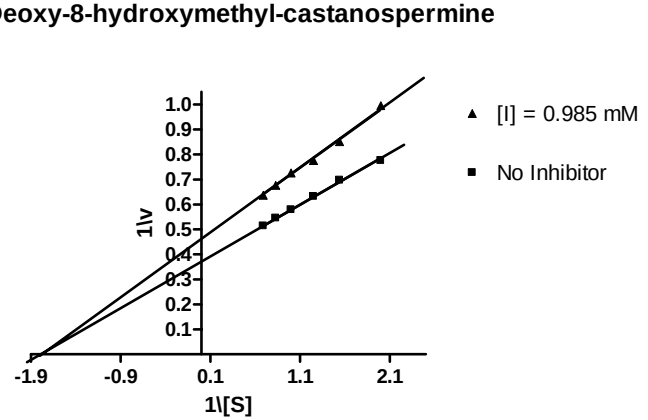
Compound **176 (*Ki* = 73 μM)**



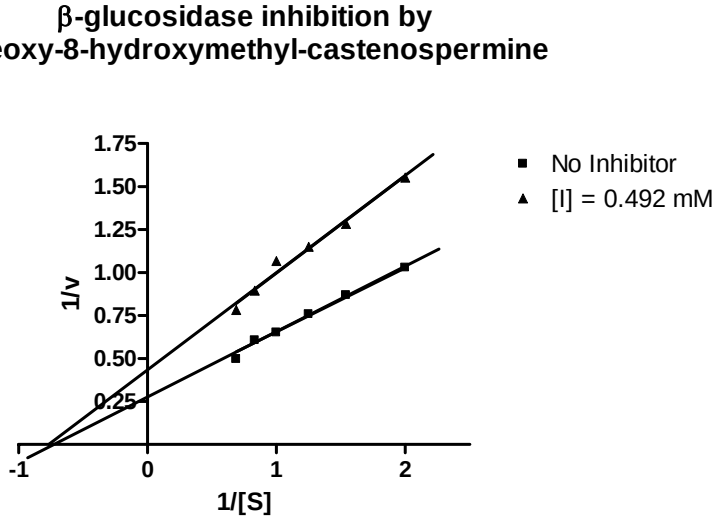
Compound **176 (*Ki* = 33 μM)**



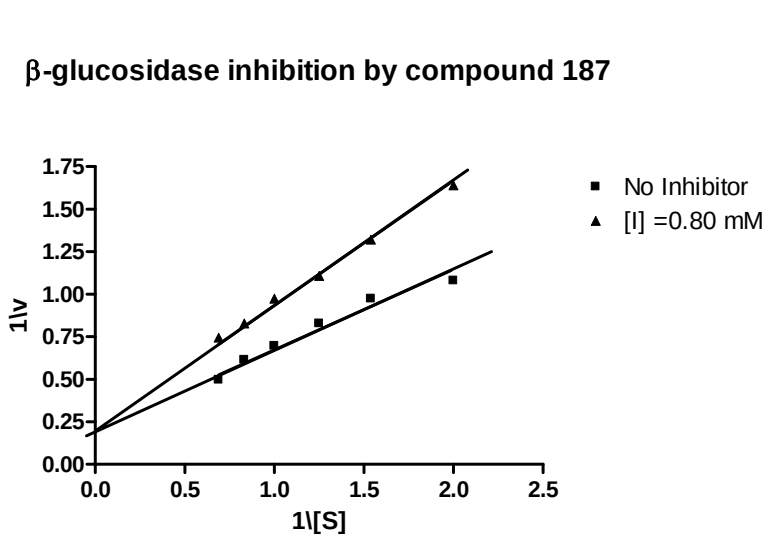
Compound **186 (*Ki* = 4 mM)**



Compound **186 (*Ki* = 942 μM)**



Compound **187 (*Ki* = 1400 μM)**



List of publications

- Convergent approach towards the synthesis of the stereoisomers of C-6 homologues of 1-deoxynojirimycin and their analogues: Evaluation as specific glycosidase inhibitors

  Pandey, G.; **Dumbre, S. G**.; Khan, M. I.; Shabab, M.; Puranik V. G. (*J. Org. Chem.* **2006**, *71*, 8481-8488)

- A β-lactam azasugar hybrid as a competitive potent galactosidase inhibitor

  Pandey, G.; **Dumbre, S. G**.; Khan, M. I.; Shabab, M.; Puranik V. G. (*Tetrahedron Lett.* **2006**, *47*, 7923-7926)

- Synthesis and evaluation of 1-deoxy-8-*epi*-castanospermine, 1-deoxy-8-hydroxymethyl castanospermine and (6*S*,7*S*,8*R*,8a*R*)-8-amino-octahydroindolizine-6,7-diol

  Pandey, G.; **Dumbre, S. G**.; Pal, S.; Khan, M. I.; Shabab, M. (Communicated)

Erratum