SYNTHESIS AND EVALUATION OF QUINOLIZIDINE CLASS OF ALKALOIDS AS GLYCOSIDASE INHIBITORS AND STUDIES TOWARDS THE SYNTHESIS OF CONFORMATIONALLY RESTRICTED GLYCOMIMETICS

THESIS

Submitted To The

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For The Degree Of

DOCTOR OF PHILOSOPHY

In

CHEMISTRY

By

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To
Almighty, My Parents
And
The Loving Memories of My Grandmother

NCL

National Chemical Laboratory

Division of Organic Chemistry
Pune – 411 008, INDIA

Dr. Ganesh Pandey
FNA, FNASc, FASc

CERTIFICATE

This is to certify that the work incorporated in the thesis entitled "**Synthesis and Evaluation of Quinolizidine Class of Alkaloids as Glycosidase Inhibitors and Studies Towards the Synthesis of Conformationally Restricted Glycomimetics**" which is being submitted to the **University of Pune** for the award of **Doctor of Philosophy in Chemistry** by **Mr. Debasish Grahacharya** 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.

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DECLARATION

I hereby declare that the work presented in the thesis entitled **"Synthesis and Evaluation of Quinolizidine Class of Alkaloids as Glycosidase Inhibitors and Studies Towards the Synthesis of Conformationally Restricted Glycomimetics"** 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: (**Debasish Grahacharya**)

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Debasish Grahacharya

CONTENTS

Abbreviations

Ac	Acetate	min	Minute(s)
aq.	aqueous	mL	Milliliter
Atm	Atmospheric pressure	mmol	Millimole
bp	boiling point	MOM	Methoxymethyl
Bn	Benzyl	mp	Melting point
Boc	*tert*-Butoxycarbonyl	N	Normality
Bz	Benzoyl	NMO	*N*-Methylmorpholine-*N*-oxide
cat.	Catalytic	NMR	Nuclear magnetic resonance
Cbz	Benzyloxycarbonyl	NMU	*N*-Methyl-*N*-notrosourea
COSY	Correlation spectroscopy	NOESY	Nuclear overhauser
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene		enhancement spectroscopy
DCM	Dichloromethane	ORTEP	Orthogonal thermal ellipsoid plots
DEPT	Distortionless enhancement by	PET	Photoinduced electron transfer
	polarization transfer	*p*-TSA	*p*-Toluenesulfonic acid
DIPEA	*N*,*N*-Diisopropylethylamine	py	Pyridine
DMAP	*N*,*N*-Dimethylaminopyridine	rt	Room temperature
DMF	*N*, *N*-dimethylformamide	TBAF	Tetrabutylammonium fluoride
DMP	2,2-Dimethoxypropane	TBS	*tert*-Butyldimethylsilyl
DMS	Dimethyl sulfide	TEA	Triethylamine
DMSO	Dimethylsulfoxide	TFA	Trifluoroacetic acid
EDC	Ethylene dichloride	THF	Tetrahydrofuran
Eq	Equivalent	TLC	Thin layer chromatography
g	gram	TMEDA	Tetramethylethylenediamine
GC	Gas chromatography	TMS	Trimethylsilyl
h	hour	TS	Transition state
Hz	Hertz	Ts	Tosyl
IBX	2-Iodoxybenzoic acid	XRD	X-ray diffraction
Im	Imidazole	α-Gal	a-Galactosidase
LAH	Lithium aluminum hydride	β-Gal	β-Galactosidase
LHMDS	Lithium hexamethyldisilazide	α-Glc	α-Glucosidase
M	Molarity (molar)	β-Glc	α-Glucosidase
μg	Microgram	α-Man	α-Mannosidase
mg	Milligram	α-Man	α-Mannosidase

General Remarks

- All the solvents were purified according to literature procedure.[1]
- Petroleum ether used in the experiments was of 60–80 ^{o}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 silica gel 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 Bruker AV 200 (200 MHz ^{1}H NMR and 50 MHz ^{13}C NMR), Bruker AV 400 (400 MHz ^{1}H NMR and 100 MHz ^{13}C NMR) and Bruker DRX 500 (500 MHz ^{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 DBI column.
- Microanalysis data were obtained using a Carlo-Erba CHNS-O EA 1108 Elemental Analyser. Elemental analyses observed for all the newly synthesized compounds 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.
- **Numbering of compounds, schemes, tables, referencing and figures for each chapter, each section as well as abstract are independent.**

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

Research Guide	Dr. Ganesh Pandey
Title of the Thesis	Synthesis and Evaluation of Quinolizidine Class of Alkaloids as Glycosidase Inhibitors and Studies Towards the Synthesis of Conformationally Restricted Glycomimetics
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Thesis Abstract

The present dissertation is divided into three chapters. Chapter 1 gives an overview of glycosidases inhibitor, including the mechanistic aspect and the therapeutic application. Chapter 2 describes the synthesis of various polyhydroxy quinolizidine alkaloids and their exploration as inhibitors of glycosidases. Chapter 3 deals with the synthetic studies towards conformationally restricted glycomimetics.

Chapter 1

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

A number of polyhydroxy azabicyclic alkaloids such as castanospermine (**1**), swainsonine (**2**) and australine (**3**) are found to be potent glycosidase inhibitors (**Figure 1**). Considering the superfluous toxic effect of some of these alkaloids, there is a need to synthesize a comprehensive array of polyhydroxy analogs of these molecules to allow a better understanding of the structural requirements for glycosidase inhibitors and thus, to develop

more potent, selective and less toxic drugs. In this context, herein we report the synthesis of a variety of quinolizidine alkaloids and their evaluation as glycosidase inhibitors.

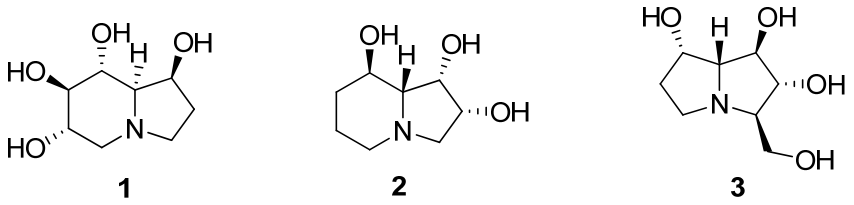
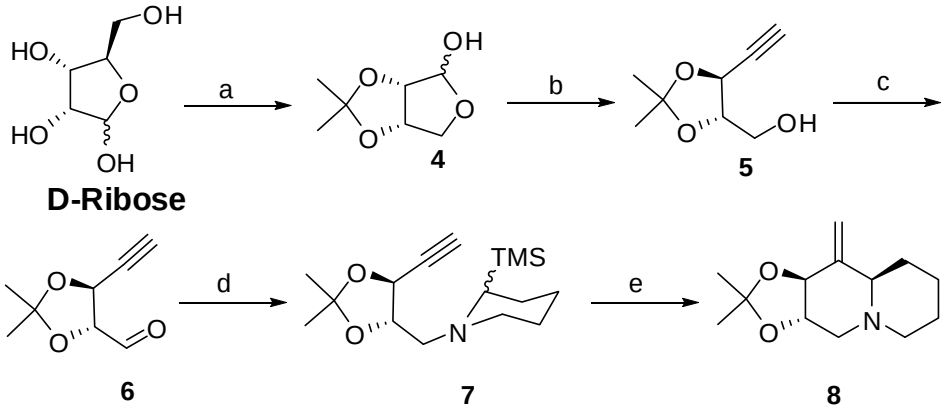


Figure 1: *Representative examples of polyhydroxy azabicyclic glycosidase inhibitors.*

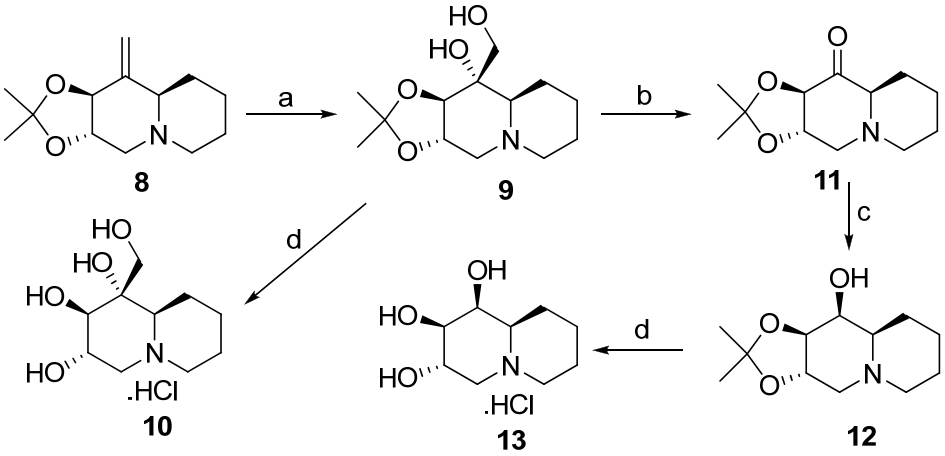
The lactol **4** obtained from D-ribose was subjected to gradual addition of Bestamann-Ohira reagent to obtain **5** whose hydroxyl moiety was oxidized to obtain aldehyde **6** which upon reductive amination with 2-(trimethylsily)-piperidine gave **7**. Compound **7** upon photoinduced electron transfer (PET) mediated cyclization gave **8** as a single diastereomer, confirmed by 1D and 2D NMR analysis (**Scheme 1**).



Reagents and Conditions:: (a) i) H_2SO_4 (cat.), acetone, 0 ^oC, 30 min; ii) $NaBH_4$, H_2O, 0 ^oC to rt 1 h; iii) $NaIO_4$, H_2O, 0 ^oC to rt 2 h (70% over 3 steps); (b) $(MeO)_2P(O)C(N_2)COMe$, K_2CO_3, MeOH, 65 ^oC, 6 h (70%); (c) IBX, EtOAc, reflux, 8 h (85%); (d) 2-(trimethylsilyl)piperidine, $NaBH(OAc)_3$, EDC, rt, 12 h (72%); (e) hν, DCN, 2-PrOH, 1 h (60%).

Scheme 1: *Synthesis of tricyclic template **8**.*

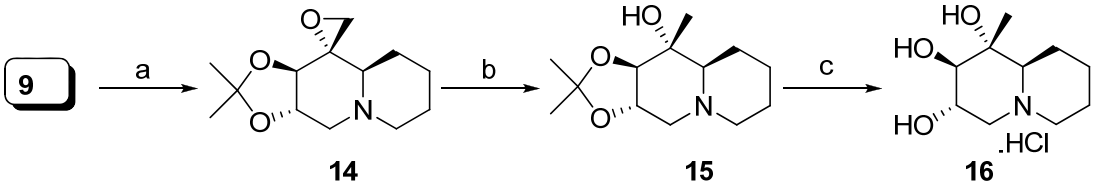
The synthetic potential of the chemical multitalent **8** was exploited by its conversion to various target molecules. In this connection, the exocyclic double bond was subjected to dihydroxylation, to get **9** as a single diastereomer. This diol **9** upon acetonide removal under acidic condition provided **10**. Further, the diol **9** was converted to azasugar **13** following the steps depicted in **Scheme 2**.



Reagents and Conditions:: (a) OsO_4, NMO (50% aq. solution), acetone, rt, 12 h (90%); (b) $NaIO_4$, silica gel, DCM, 10 min; (c) $NaBH_4$, MeOH, rt, 6 h (80% over two steps); (d) aq. (1N) HCl, rt, 4 h (100%).

Scheme 2: *Synthesis of polyhydroxyquinolizidine alkaloids* ***10*** *and* ***13****.*

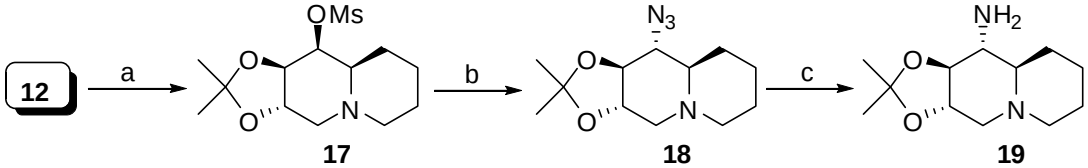
Synthesis of 1-deoxy-8-methylhomocastanospermine (**16**) from **9** was also initiated in order to study it as a potential glycosidase inhibitor. The diol **9** was converted to corresponding epoxide **14** via its primary mesylate. The regioselective reductive opening of epoxide and subsequent acetonide removal furnished **16** (**Scheme 3**).



Scheme 3: *Synthesis of 1-deoxy-8-methylhomocastanospermine* ***16****.*

Reagents and Conditions:: (a) $MeSO_2Cl$, Et_3N, DCM, 0 °C to rt, 12 h (90%); (b) LAH, THF, 12 h (90%); (c) aq. (1N) HCl, rt, 4 h (100%).

In order to obtain another class of azasugar having an additional basic site for binding to the enzyme, hydroxyl moiety at C-10 of **12** was converted to corresponding amine functionality. Further, to alter the basicity of the primary amine, a hydrocarbon chain was attached. These two aspects are incorporated in the synthesis of **20** and **22**, as shown in **Scheme 4**.



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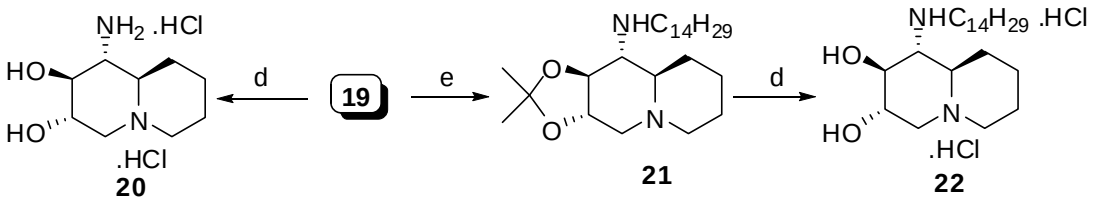
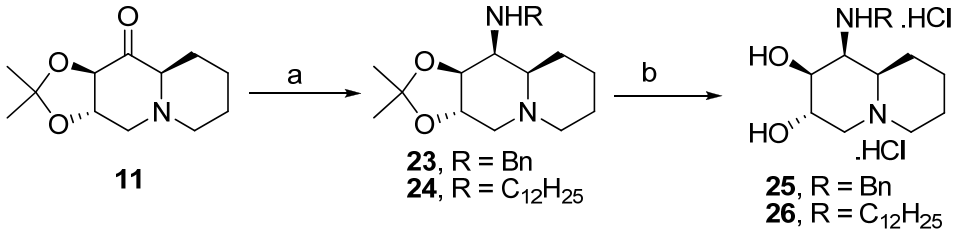


Reagents and Conditions:: (a) $MeSO_2Cl$, py, 0 °C to rt, 6 h (88%); (b) LiN_3, DMF, 110 °C, 20 h (82%); (c) 10% Pd/C, H_2 (1 atm), MeOH, rt, 2 h (85%); (d) aq. (1N) HCl, rt, 4 h (100%); (e) $C_{14}H_{29}Br$, K_2CO_3, CH_3CN–THF (3:1), reflux, 6 h (65%).

Scheme 4: *Synthesis of dibasic polyhydroxyquinolizidine alkaloids* ***20*** *and* ***22****.*

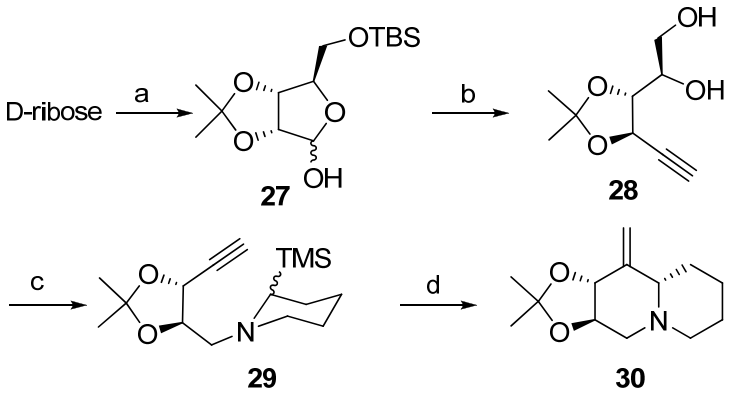
Since, it was not possible at this stage to predict which diastereomer will be the most biologically active, two other new analogs **25** and **26** were synthesized, through reductive amination of ketone **11** with appropriate amine, followed by acetonide removal (**Scheme 5**).



Reagents and Conditions:: (a) RNH_2, $NaBH(OAc)_3$, EDC, rt, 12 h (65–75%); (b) aq. (1N) HCl, rt, 4 h (100%).

Scheme 5: *Synthesis of dibasic polyhydroxyquinolizidine alkaloids* ***25*** *and* ***26****.*

Synthesis of the enantiomers of the above azasugars, starting from D-ribose and using even lesser number of steps were also carried out. Synthesis of the key tricyclic template **30** is depicted in **Scheme 6**.

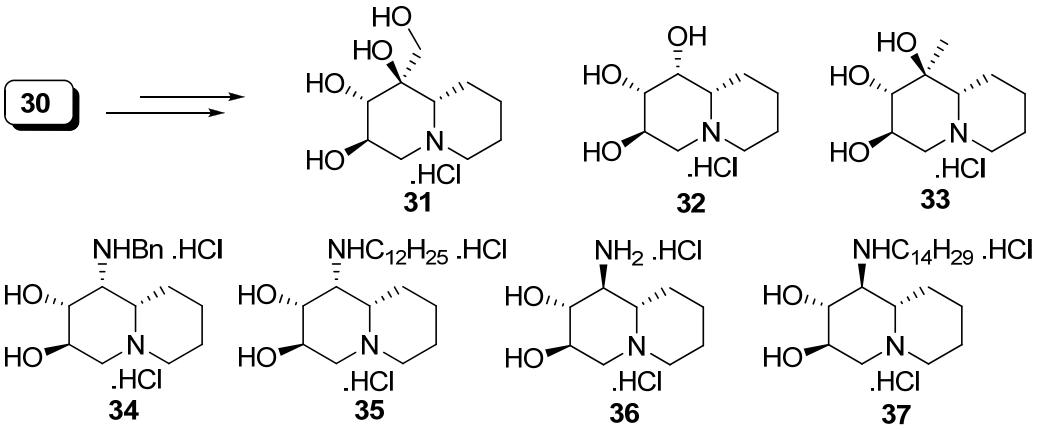


Reagents and Conditions:: (a) i) H_2SO_4, acetone, (cat.), 0 °C, 30 min; ii) TBSCl, Et_3N, DCM, rt, 6 h (80% over 2 steps); (b) $(MeO)_2P(O)C(N_2)COMe$, K_2CO_3, MeOH, 65 °C, 6 h (55%); (c) i) $NaIO_4$, silica gel, DCM,

10 min; ii) 2-(trimethylsilyl)piperidine, $NaBH(OAc)_3$, EDC, rt, 12 h (50% over 2 steps); (d) hν, DCN, 2-PrOH, 1 h (60%).

Scheme 6: *Synthesis of tricyclic template (**30**).*

The azasugars **31**–**37** were obtained by following identical reaction sequence as described earlier (**Scheme 7**)**.**



Scheme 7: *Synthesis of enantiomeric compounds **31**–**37**.*

For the synthesis of azasugars with general structure **38** and **39** (**Figure 2**) the aldehyde fragment **44** needed for reductive amination had a prerequisite of *cis*-stereochemical relationship between the hydroxyl groups at C-4 and C-5.

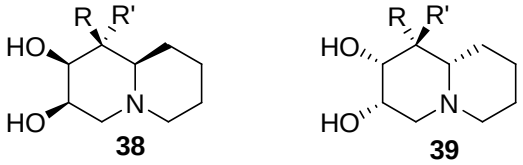
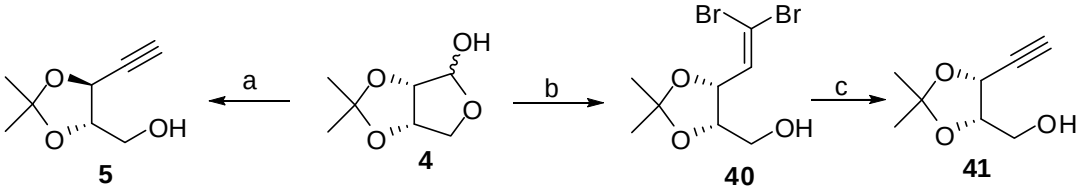


Figure 2: *General structure of two more series of quinolizidine alkaloids.*

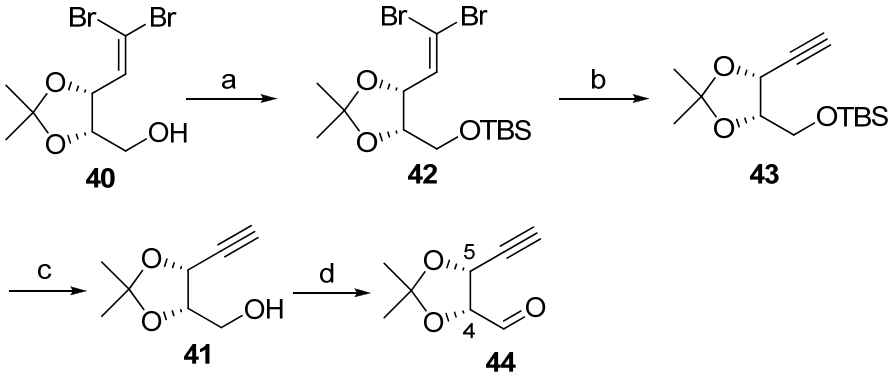
The initial effort to build this motif **41** from **4** using Bestmann-Ohira reagent resulted in thermodynamically more stable *trans* substituted compound **5**. However, a modified Corey's protocol provided the monosubstituted acetylene **41** (**Scheme 8**).



Reagents and Conditions:: (a) $(MeO)_2P(O)C(N_2)COMe$, K_2CO_3, MeOH, 65 °C, 6 h (70%); (b) Ph_3P^+ $CHBr_2$, Br^-, 0 °C, 1 h (65%); (c) *n*-BuLi, THF, −78 °C (25%).

Scheme 8: *Attempts for avoiding epimerization while installing acetylinic moiety over **4**.*

The conversion of **40** to acetylene **41** was low yielding, presumably due to the presence of free alcohol moiety. Thus, **40** after silyl protection provided **41** in good yield under usual Corey's condition which was converted to **43** (**Scheme 9**). Desilylation of the **43** followed by oxidation provided aldehyde **44**. Synthesis of the *ent*-**44** and subsequent synthesis of the azasugar series **38** and **39** are under progress.



Reagents and Conditions:: (a) TBSCl, Im, DMAP, DCM (90%); (b) *n*-BuLi, THF, −78 °C (80%); (c) 1M TBAF, THF (90%); (d) IBX, EtOAc, reflux, 8 h (85%).

Scheme 9: *Synthesis of aldehyde* ***44****.*

The quinolizidine alkaloids obtained were tested for their glycosidase inhibition properties and the results are summarized in **Table 1**. As shown in the table, the developed inhibitors proved to be selective inhibitors of α-glucosidase, with few of them showing high inhibition.

Table1. Inhibition (*Ki* in μm)[a] of glycosidases by inhibitors.

Inhibitor	β-gal *A. oryzaie*	α-gal Green coffee beans	β-man Snail	α-man Jack Beans	β-glu Almond	α-glu Yeast
10	33%[b]	NI	NI	11%[b]	NI	NI
13	**591**	**83.9**	NI	18%[b]	NI	NI
16	NI	NI	NI	22%[b]	NI	NI
20	NI	11%[b]	NI	NI	NI	**675**
22	NI	NI	**830**	**805**	**524**	**140**
25	NI	31%[b]	NI	NI	NI	**278**
26	NI	NI	NI	**293**	46%[b]	**120**
31	NI	NI	NI	NI	NI	42%[b]
32	28%[b]	13%[b]	18%[b]	NI	NI	**450**
33	NI	NI	14%[b]	29%[b]	NI	39%[b]
34	NI	NI	13%[b]	22%[b]	NI	**258**
35	NI	NI	NI	NI	NI	**222**
36	NI	NI	NI	**650**	43%[b]	**235**
37	NI	NI	NI	NI	NI	**28**

a) *Ki* in μm (in bold); b) percent inhibition at 1 mm level; NI, no inhibition up to 1mm.

Chapter 3

This chapter deals with our synthetic efforts towards the synthesis of conformationally restricted glycomimetics.

The stable compounds resembling the transition state for glycosidic cleavage or formation either by shape or charge or both are found to be potential inhibitors of the corresponding enzymes. Examples of naturally occurring inhibitors falling under this class are 1-deoxynojirimycin (**1**), castanospermine (**2**), *etc*. These enzymatic reactions are thought to proceed through a flattened half-chair (or twisted boat) oxocarbenium ion transition state (**3**), where substantial sp^2 character at the anomeric position reduces the degree of freedom of the species (**Figure 1**). This directly indicates the restriction of an inhibitors conformation to one that is recognized by the enzyme may increase the potency by lowering the entropic barrier for complex formation.

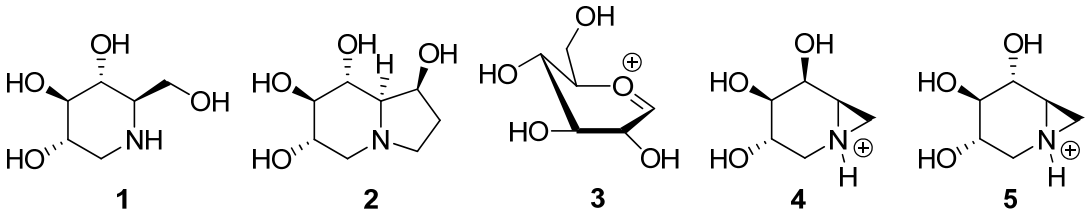
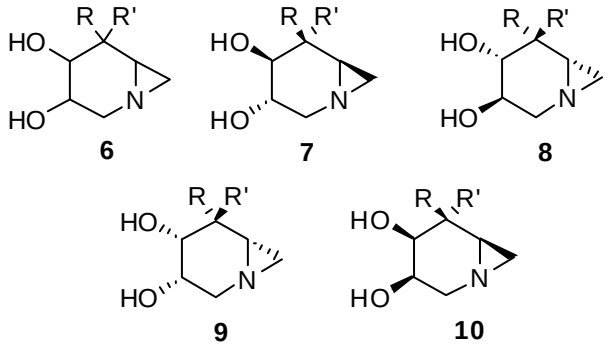


Figure 1.

Moreover, the conformational energy level calculations of **(4)** and **(5)** show that in protonated form, boat and twisted boat conformations are energetically more stable and therefore, these may bind more effectively to the active site of the enzyme as they now possess lesser degrees of freedom.

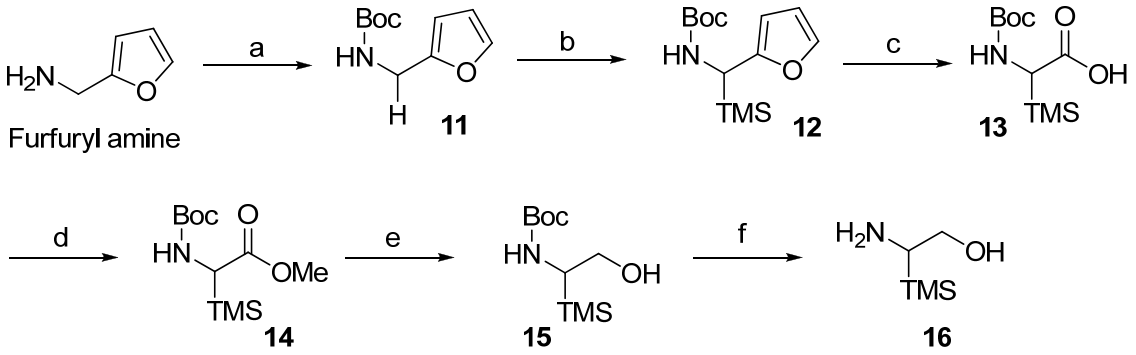


R	R’
−OH	−H
$-CH_2OH$	−OH
$-CH_2OH$	−H
$-CH_3$	−H
−COOH	−H
−H	$-NH_2$
−H	−NHR

Figure 2: *General structures of synthetic targets as conformationally restricted glycomimetics.*

Furthermore, it was speculated that protonated aziridine analog might preferentially interfere with α-glycoside hydrolysis by S_N2 esterification of the enzyme's β-face carboxylate anion. Considering these hypothesis in mind, four diastereomeric series of conformationally constrained azasugars **7**, **8**, **9** and **10**, were designed (**Figure 2**).

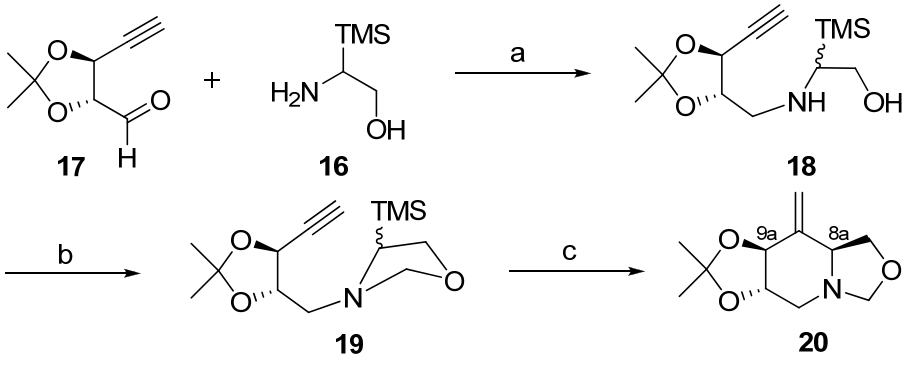
The synthesis of azasugar series with general structure **7** started with the synthesis of amine fragment **16** as given in **Scheme 1**.



Reagents and Conditions:: (a) Boc_2O, DCM, Et_3N, 0 °C, 24 h (90%); (b) TMSCl, *n*-BuLi, THF, −78 °C, 1 h, −78 °C to RT in 4 h then *n*-BuLi, −78 °C, 3 h (80%); (c) O_3, DCM, MeOH, −78 °C; (d) CH_2N_2, 0 °C, 0.5 h; (e) $LiAlH_4$, THF, −78 °C to RT, 12 h (50% over three steps); (f) aq. (1N) HCl, dioxan, 75 °C, 0.5 h (80%).

Scheme 1: *Synthesis of α-trimethylsilylaminoalcohol (**16**).*

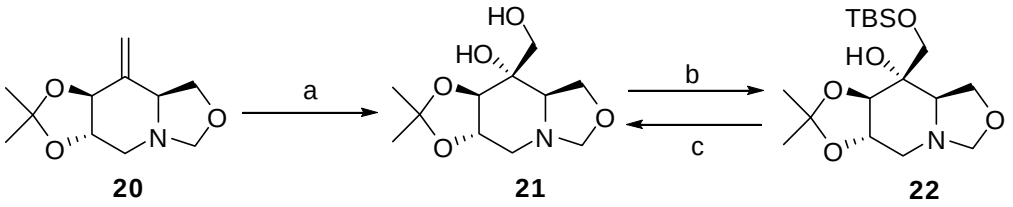
The reductive amination of aldehyde **17** with amine **16** produced **18**, which was transformed into **19** by using paraformaledyde. PET cyclization of **19** provided **20** as a single diastereomer (GC and NMR) (**Scheme 2**).



Reagents and Conditions:: (a) $NaBH(OAc)_3$, EDC, rt, 8 h, (70%); (b) $(H_2CO)_n$, benzene, reflux, 4 h, (87%); (c) hv, DCN, CH_3CN/2-PrOH (4 : 1), 2 h (55 %).

Scheme 2: *Synthesis of tricyclic template **20**.*

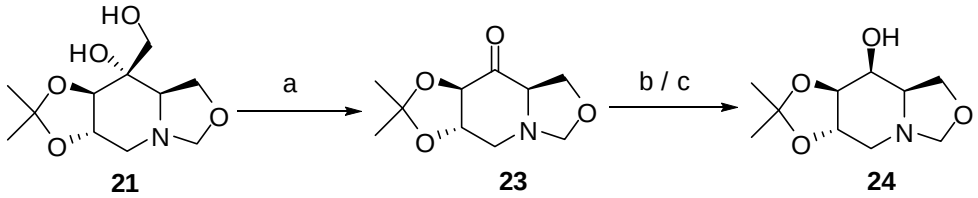
The functionalization of juxtaposed double bond of **20** was started with the dihydroxylation reaction. Unfortunately, the isolation of dihydroxy compound **21** was cumbersome, which compelled us to take two extra steps to proceed further in the scheme. Selective monosilyl protection of **21** resulted **22** which could be easily purified. Deprotection of TBS group produced back diol **21** (**Scheme 3**).



Reagents and Conditions:: (a) OsO_4, NMO, acetone, 15 h; (b) TBSCl, Et_3N, dry DCM, 0 °C to rt, 6 h; (c) TBAF, THF, 0 °C to RT, 4 h.

Scheme 3: *Functionalization of exocyclic double bond.*

Usual oxidative cleavage of **21** and subsequent reduction was attempted to obtain alcohol **24**. Unfortunately, we could not get the alcohol **24**, possibly due to the cleavage of protecting groups in acidic oxidative cleavage reaction which then forms stable complex with boron during reduction step (**Scheme 4**).

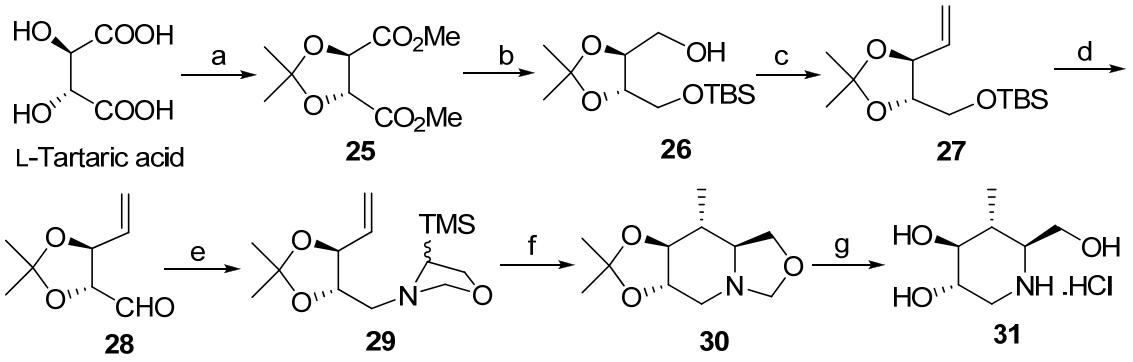


Reagents and Conditions:: (a) $NaIO_4$, silica gel, DCM/H_2O, rt; (b) $NaBH_4$, MeOH; (c) $LiBH_4$, THF.

Scheme 4: *Attempts towards the synthesis of **24**.*

The above observation led us to think an azasugar where no functionalization of double bond is required. Thus, the substrate of choice for PET cyclization was olefin tethered α-trimethylsilyl amine **29**, the synthesis of which is given in **Scheme 5**.

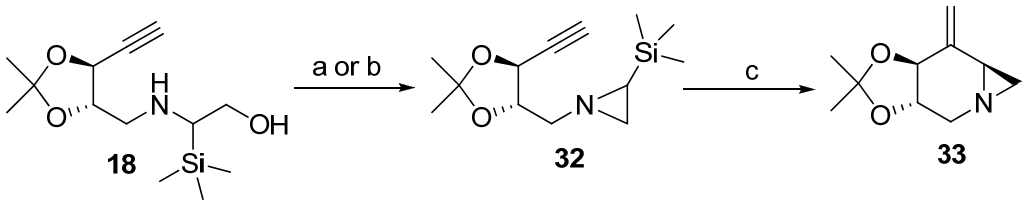
PET cyclization of **29** provided **30** as a single diastereomer. To our disappointment, due to the high polar nature of the product, formed by the removal of *N/O* methylene bridge of **30** using acidic reaction condition, could not allow this to be isolated (**Scheme 5)**.



Reagents and Conditions:: (a) i) 2,2-Dimethoxypropane, *p*-TSA, MeOH, cyclohexane (95%); (b) i) LAH, THF, reflux (90%); ii) TBSCl (1 eq), TEA, DCM (85%); (c) IBX, EtOAc, reflux (98%); ii) $P^{+}Ph_3$ CH_3I^{-}, *n*-BuLi, THF, −15 °C to rt, 16 h (70%); (d) i) TBAF, THF, 4 h, rt; ii) IBX, EtOAc, reflux (80%); (e) **14**, $NaBH(OAc)_3$, EDC, rt, 8 h (75%); ii) $(CH_2O)_n$, benzene, reflux, 4 h (93%); (f) hν, DCN, CH_3CN / 2-PrOH (4 :1), 4 h (50%); (g) 6N HCl, MeOH, reflux, 24 h.

Scheme 5: *Synthesis of template* ***30****.*

The above experience made us to explore another way to construct an aziridine **32** prior to PET cyclization. The 1, 2-amino alcohol **18** was converted to corresponding aziridine either by bromination or mesylation of alcohol functionality. However, the PET cyclization this time provided a complex reaction mixture (**Scheme 6**).



Reagents and Conditions:: (a) CBr_4, Ph_3P, Im, DCM, 0 °C, 15 min (40%); (b) MsCl, TEA, DCM, 0 °C, 15 min (65%); (c) hν, DCN, 2-PrOH, 3 h.

Scheme 6: *Attempt towards synthesis of template* ***33****.*

In summary, we have synthesized various azasugars which were found to be selective and potent inhibitors of α-glucosidase. Introduction of an additional basic site in the form of amino functionality proved to be useful, as it increased the inhibition. A study towards the synthesis of conformationally restricted glycomimetics is under progress.

Chapter 1

An Introduction to Glycosidases and their Inhibitors

"How on earth are you ever going to explain in terms of chemistry and physics so important a biological phenomenon as first love?"

-Albert Einsteine

1.1-Glycosidases and their classification

Carbohydrates, the sweet molecules of life, rightly called so because they have now been identified to play numerous vital roles in living things, including the storage and transport of energy (e.g., starch, glycogen) and structural components (e.g., cellulose in plants, chitin and cartilage in animals). Additionally, carbohydrates and their derivatives like the oligosaccharides and glycoconjugates present in biological systems play major roles in the working process of the immune system, fertilization, pathogenesis, blood clotting and complex information display system that modulates normal cell function. These oligosaccharides and glycoconjugates are composed of two glycosidic units linked through glycosidic bonds. The glycosidic bond is an acetal linkage between two glycosidic units.

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. while the sugar component is called glycon. The carbohydrate residue, the glycon part, is attached by glycosidic bond at the anomeric carbon to a non carbohydrate residue or aglycon. Glycosidic bond, particularly that between two glucose residues is the most stable of the linkages ($\Delta G^{\ddagger} \sim 30$ kcal mol^{-1}) within naturally occurring biopolymers, with half-lives for spontaneous hydrolysis of cellulose and starch being in the range of 5 million years[1]. Enzymes, the glycosidases carrying out the hydrolysis of these materials, therefore, face a challenging task yet they accomplish this with rate constants up to 1000 s^{-1}, earning them a reputation as some of the most proficient of catalysts. The hydrolysis of the glycosidic bond with the help of glycosidases continues to gain importance due to the vital roles played by the complex glycans in biological system, leading to concern over human health and disease.

Based on the mode of the action of glycosidases, they are classified as follows:

1.1.1-Inverting and retaining glycosidases

Glycosidases are more importantly classified based on the stereochemcial outcome of the hydrolysis of the glycosidic bond e.g. inversion or retention of anomeric configuration. The catalytic apparatus of both retaining and inverting enzymes feature bilateral carboxylic acid

group. Thus, the different stereochemical outcomes are achieved by mechanistically distinct pathways as described below (**Figure 1**).

(a) *Inverting glycosidases* - In this type of enzymes, the two carboxyl groups serve as general acid and general base catalysts and are suitably placed, about 10.5 Å apart on average,[1b,2] to allow the substrate and a water molecule to bind between them. This reaction occurs via a single-displacement mechanism involving an oxocarbenium ion-like transition state.

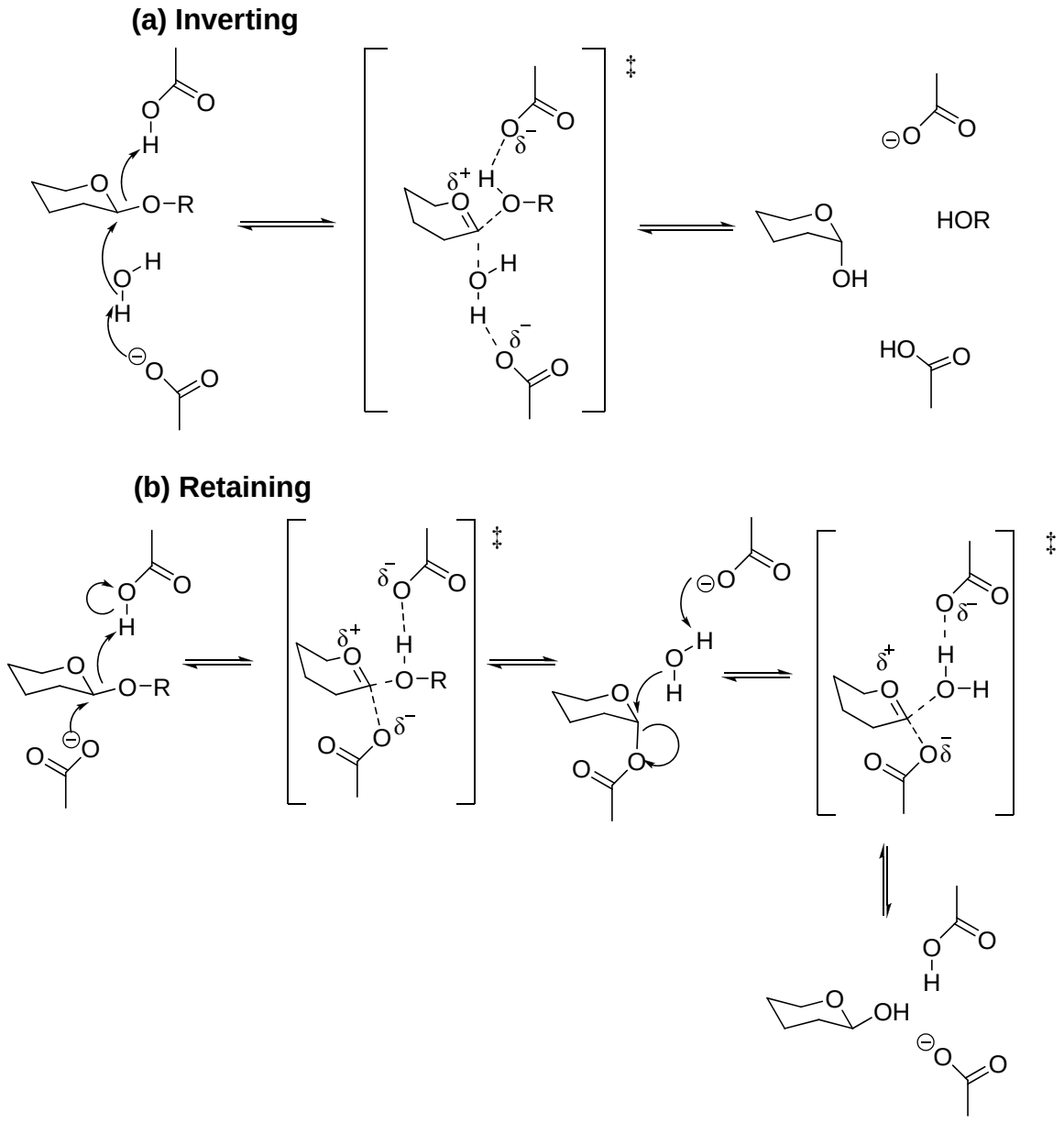


Figure 1*: Mode of action for inverting (a) and retaining (b) glycosidases.*

(b) *Retaining glycosidases* - By contrast, these enzymes contain the carboxyl groups in the cavity which are only 5.5 Å apart, consistent with a double displacement mechanism involving a covalent glycosyl-enzyme intermediate. In the first step, one of the carboxyl group functions as a general acid catalyst, protonating the glycosidic oxygen concomitantly with bond cleavage. The other acts as a nucleophile, forming a covalent glycosyl-enzyme intermediate. In the second step, the side-chain carboxylate deprotonates the incoming water molecule, which attacks at the anomeric center and displaces the sugar. Both steps occur via transition states with substantial oxocarbenium ion character.

1.1.2-α-Glycosidases and β-glycosidases

Glycosidases have also been classified based on the stereochemistry of the anomeric glycosidic bond that they cleave. Enzymes catalyzing the cleavage of α-glycosidic bond are termed as α-glycosidases while those cleaving a β-glycosidic bond are termed as β-glycosidases. **Figure 2** depicts the proposed reaction mechanism for both these classes of enzymes.

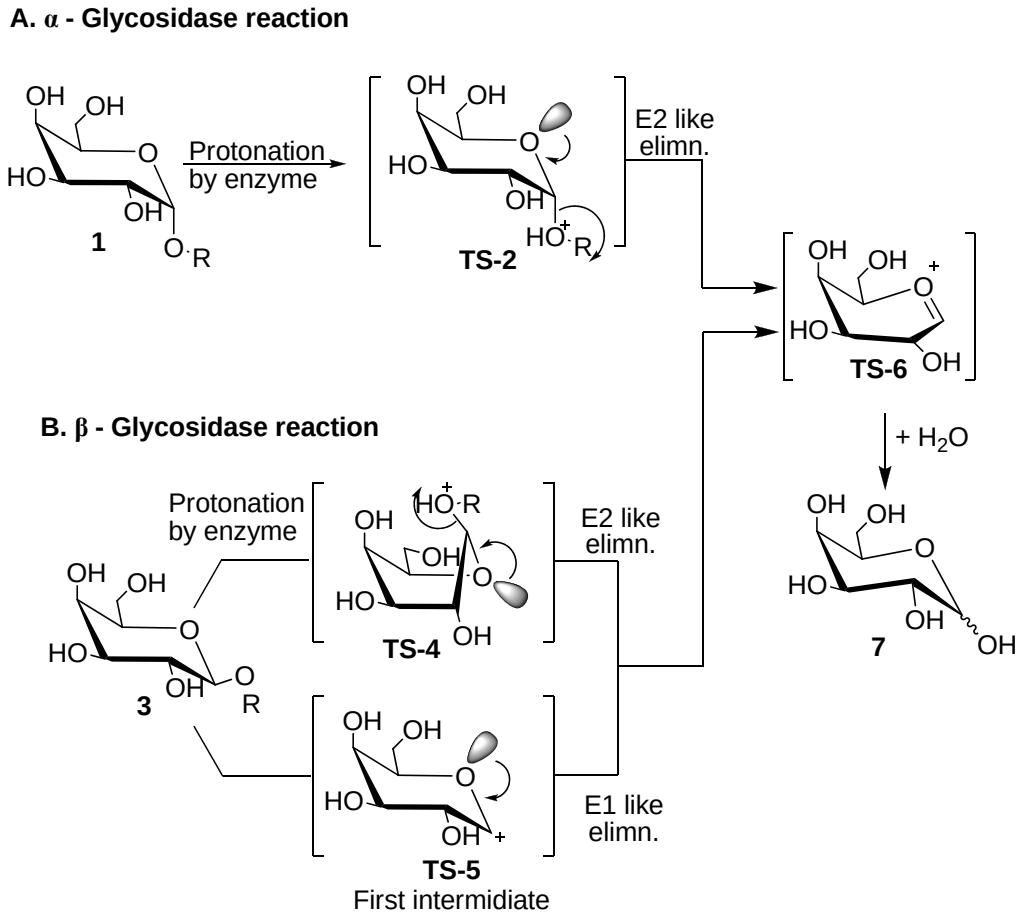


Figure 2*: Glycosidase reaction mechanism involved in α-glycosidases and β-glycosidases.*

(a) *α-Glycosidases* - They 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, co-operatively facilitating the cleavage of glycosidic bond.

(b) *β-Glycosidase* - The β-glycosidation reaction may proceed through either E1 or E2 like mechanism. If the reaction proceeds via an E2 like mechanism, similar to that of the α-glycosidases, the protonation of substrate **3** has to go through a highly strained species **TS-4** that may not favor further reaction. Therefore, in the case of a β-glycosidation reaction, the positively charged aglycon unit leaves via an E1 like mechanism, involving the glycosyl cation **TS-5**, further stabilized by the ring oxygen to give **TS-6**. Although the final reaction intermediate in both the reaction mechanisms is the same flattened, half chair oxocarbenium ion **TS-6**, the first intermediate in the case of β-glycosidase reaction differs with respect to the position of the charge development.[3]

1.1.3-Exoglycosidases and endoglycosidases

Depending upon the position of the glycosidic bond that is cleaved by the enzyme, they have also been classified under two categories: (a) *exoglycosidases* and (b) *endoglycosidases* (**Figure 3**).

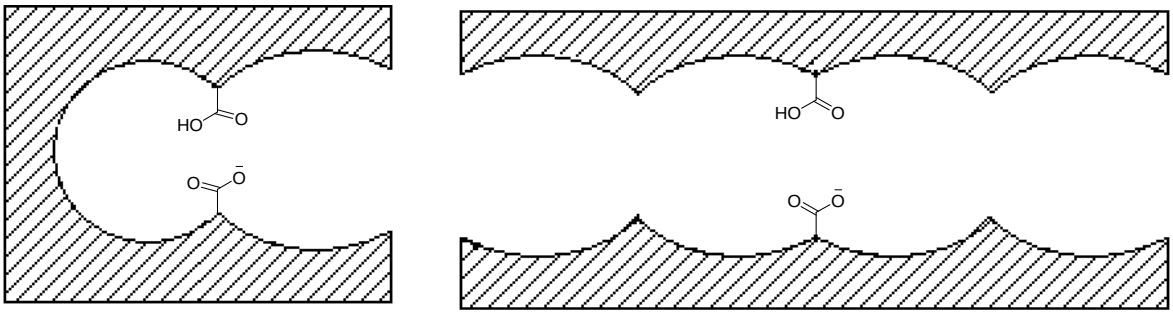


Figure 3*: Exo- and Endo-glycosidases.*

(a) *Exoglycosidases* - This class of enzymes remove sugars one at a time, from the non-reducing end of an oligo- or polysaccharide and are involved in various biological process such as the breakdown of starch and glycogen, the processing of eukaryotic glycoproteins, the catabolism of peptidoglycans and other glycoconjugates *etc*.

(b) *Endoglycosidases* - These enzymes cleave interior glycosidic bond within polysaccharides. They are involved in the catabolism and clearance of the aged glycoproteins, alteration of bacterial and plant cell walls as well as the hydrolysis of highly insoluble structural polysaccharides like chitin and cellulose *etc*.[4]

1.1.4-Glycosidases, glycosyltransferases and phosphorylases

Based on the kind of function the enzymes perform, they have also been categorized as glycosidases, glycosyltransferases and phosphorylases (**Figure 4**).[1]

(a) *Glycosidases* - They are the enzymes that simply carry out the hydrolysis of a glycosidic bond with the addition of a water molecule in between the two units.

(b) *Glycosyltransferases* - They catalyze the transfer of glycosyl moieties from activated donor sugars to an acceptor. The activating group of the donor is a nucleoside diphosphate (NDP) or monophosphate, phosphate, or a lipid phosphate and the acceptor is a hydroxyl group from another sugar, a lipid, a serine or threonine residue or the amide of an asparagine residue in a protein.

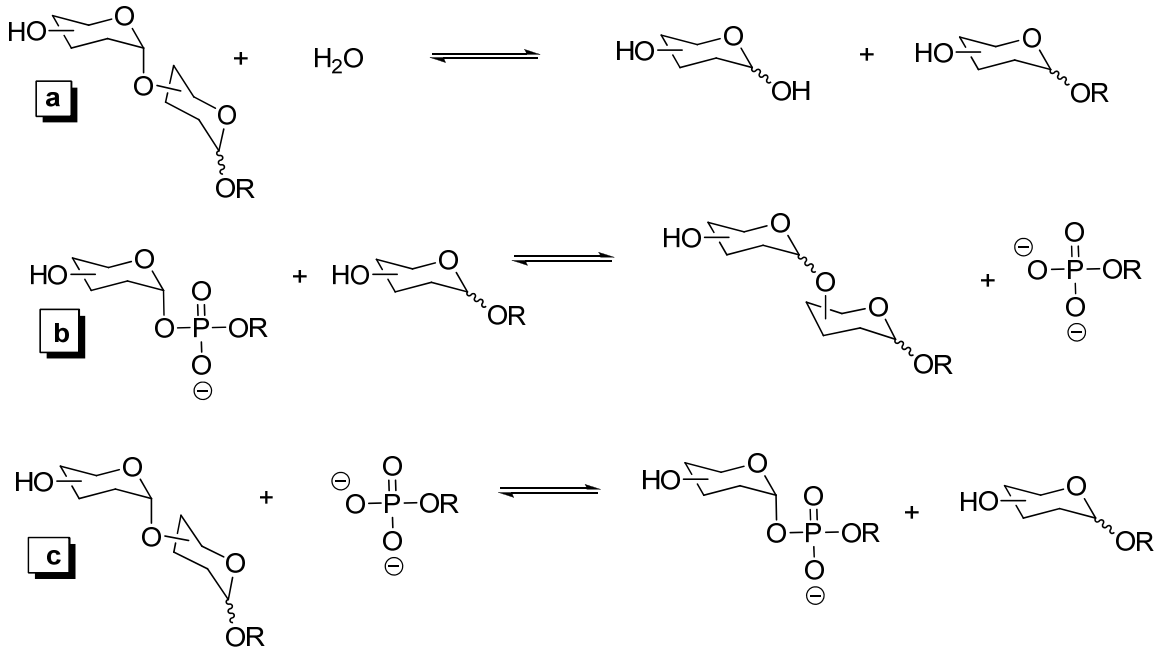


Figure 4*: Overall reactions catalyzed by (a) glycosidases, (b) glycosyltransferases, and (c) Phosphorylases.*

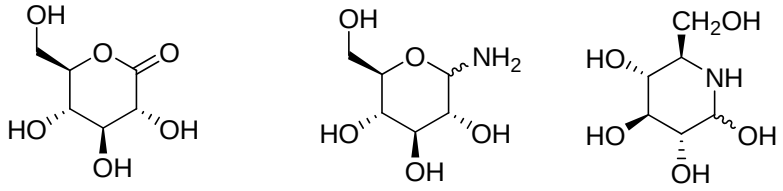
(c) *Phosphorylases* - Depending on the direction of the reaction being catalyzed, phosphorylase enzymes serve to either degrade or polymerize oligosaccharide substrates. The degradation process proceeds via phosphorolysis of a glycosidic linkage, while in the synthetic direction a sugar phosphate acts as the donor substrate

Sugar-mimicking alkaloids inhibit the glycosidases involved in a wide range of important biological processes, principally owing to their structural resemblance to the sugar moiety of the natural substrate. The possibility of modifying and blocking these processes by using such inhibitors for therapeutic applications has attracted a lot of attention, since some sugar-mimic alkaloids show potential antidiabetic[5], antiviral and anticancer effects[6]. These inhibitors are also being used to study the mechanism of action, topography of the active site and the purification of glycosidases [7].

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 of the reaction involving glycosidases, can act as an inhibitor of that particular enzyme. The term "***glycomimetics***" refers to the creation of molecules that mimic the activity of carbohydrates or simply carbohydrate mimics.

1.2-Azasugars as inhibitors of glycosidases

Historically, the first glycosidase inhibitors were the families of the monosaccharide-derived δ-aldonolactones (such as D-gluconolactone **8**)[8], and glycosyl amines (1-amino-1-deoxy pyranoses such as D-glucosyl amine **9**)[9] (**Figure 5**).



8 D-gluconolactone

9 D-glucosyl amine

10 Nojirimycin

Figure 5: *The classical glycosidases inhibitors.*

Although, the compounds of this family lack long-term stability in aqueous solution, they typically display competitive inhibition against glycosidases, the substrates of which they

closely resemble to. Ever since the pioneering work by Paulsen[10] and co-workers on sugar analogues with basic nitrogen instead of oxygen in the ring (also called the azasugars or iminosugars) and the discovery of such a natural product (nojirimycin **10**)[11], many other naturally occurring iminosugars have been identified and additional analogues and homologs have been synthesized, opening a dynamic research area.[4,12]

These azasugars are considered to have a high therapeutic potential and are applied in the elucidation of biological recognition processes, due to their glycosidase inhibition properties.[12g,12h] The great potency and specificity of these inhibitors are related to their ability to mimic transition state pyranosidic or furanosidic units of natural glucosidase substrates. Significant competitive inhibition is observed with many inhibitors, suggesting that both conformational (shape) and electrostatic (charge) influences may be important in the active site binding.[7,12d,13] Considering that, the partial cleavage of the glycosidic bond intensifies the positive charge generated in the oxygen or anomeric carbon of the natural glycoside, substitution of one of the two atoms by protonated nitrogen will mimic, in the transition state, the charge in these centers.[14] In fact, the main characteristics consisting of stabilization of the positive charge on the nitrogen atom, trigonal anomeric center, half-chair conformation, and specific configuration of the hydroxyl ions are crucial for activity in these alkaloids.[2d,15] Thus, relevant structural factors for glycosidase inhibition may be related to the charge and/or shape, defined by the hybridization and conformation. The natural azasugars are widespread[16] in various forms and classes (**Figures 6–8**).

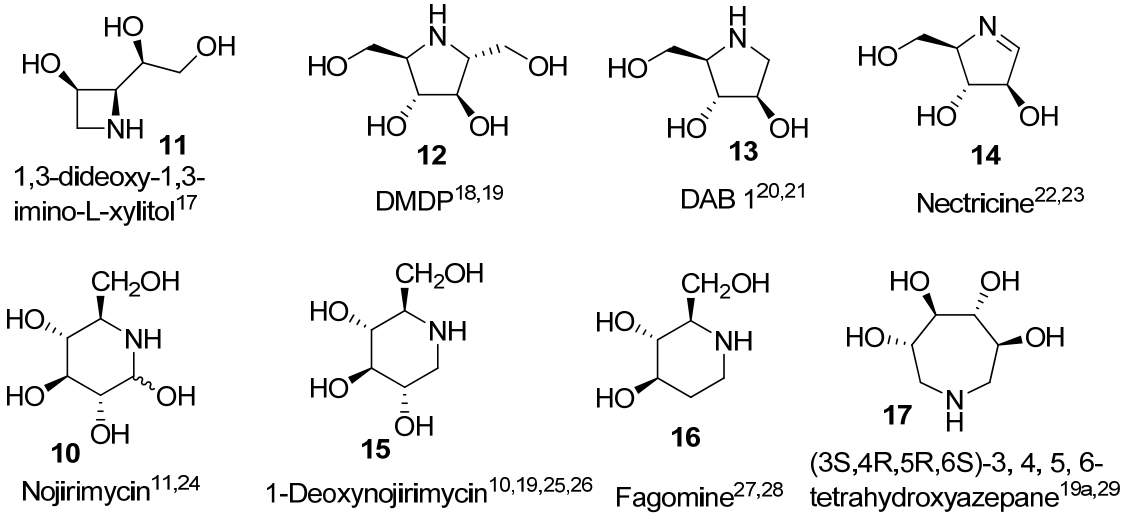


***Figure 6**: Azasugars of various ring size.*

(a) Representative examples of the nitrogen heterocycles incorporating four to seven membered rings (**11**–**17**) are shown in **Figure 6**.[11,17–29] While the small rings (like azitidine **11**) provide tight transition state upon protonation, the bigger rings (like azepane **17**) provide more flexibility to bind the active site of enzyme.

(b) The examples of bicyclic azasugars include pyrrolizidines (Alexine **18**,[30] Australin **19**[31]) indolizidines (Castenospermine **20**,[2c,32,33] Lentigenosine **21**,[34,35] Swansonine **22**[36,37]) and tropane/nortropanes (Calystegine A_3 **23**,[38,39] Calystegine C_1 **24**[39,40]) (**Figure 7**).

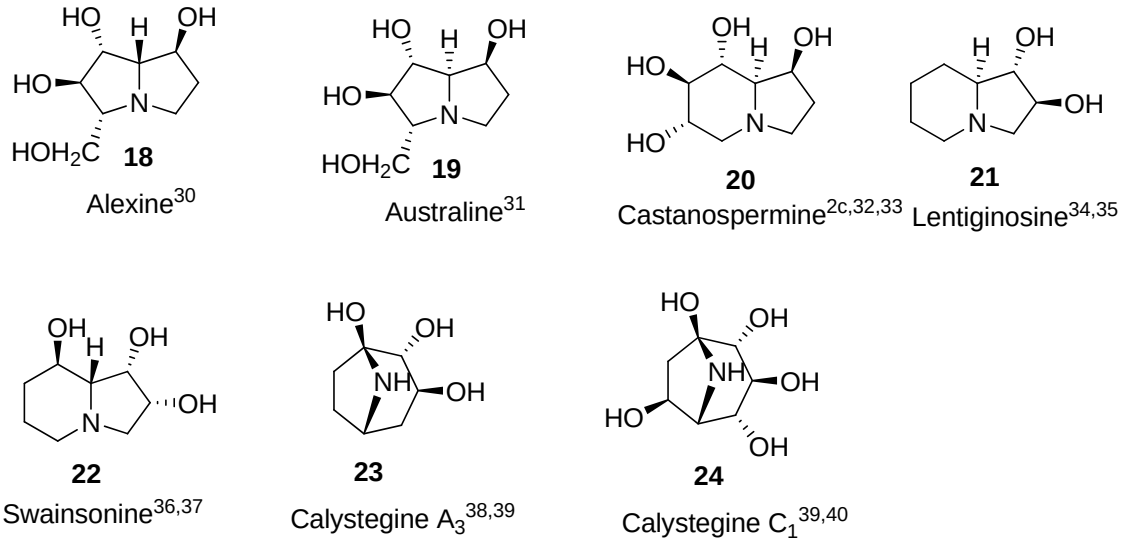


Figure 7: *Bicyclic azasugars.*

(c) Entities incorporating a nitrogen in more than one position, including the one in the ring, e.g. siastatins[41,42] **25**, nagstatins[43–46] **26** and aminocyclitols like mannostatins[47–48] **27** are given in **Figure 8**.

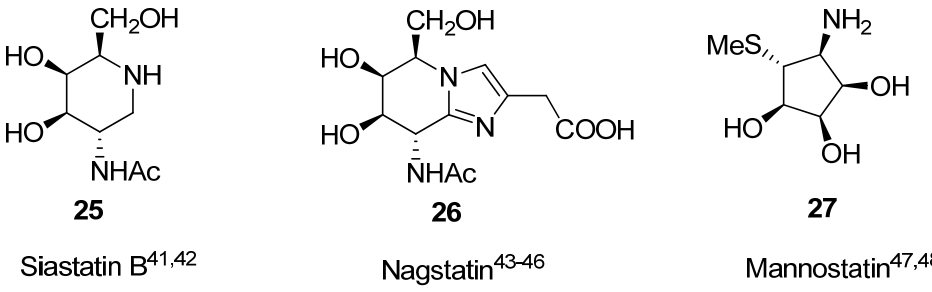


Figure 8: *Dibasic azasugars and aminocyclitols.*

1.3-Therapeutic applications of glycosidase inhibitors in the context of biological activities

According to our previous discussion, as the glycosidases play various fundamental roles in life processes, their malfunctioning leads to several diseases. Thus, the search for inhibitors of glycosidases has been the hot area of research for the development of novel antiviral, anti-infective or anti-cancer agents. Few prominent among them are as follows:

1.3.1-Antidiabetic agents

Digestive α-glucosidases, located in the small intestine, are enzymes that hydrolyze dietary carbohydrates to monosaccharides which are absorbed through the intestinal wall. Deoxynojirimycin (DNJ) **15** was shown to have an inhibitory effect on mammalian α-glucosidases *in vitro* and its *N*-alkylated-type analogs like Miglitol[49] (**28**; BAY m1099; **Figure 9**) was characterized as potent inhibitors of the glycogenolysis. Today, Miglitol is commercially available in the USA and Canada for the treatment of type II diabetes (GLYSET^{TM}).

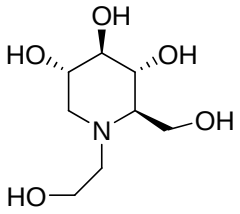


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Figure 9: *Miglitol*

1.3.2-Lysosomal storage disorders

In a similar approach, disorders in the biosynthesis or catabolism of glycolipids in the cell (glycosphingolipids) have an impact on the so-called lysosomal storage diseases like Type 1 Gaucher disease or Fabry disease.[50] In normal cells, there is a balance (homeostasis) between the degradation of glycosphingolipids (GSLs) in the lysosome and their biosynthesis in the ER/Golgi system. The rates of influx of GSLs and efflux of metabolites are equal. In a lysosomal storage disease cell, enzyme activity in the lysosome is so low that GSLs accumulate. However, although the catalytic activity of the enzymes is reduced, it is not totally eliminated. Thus, drugs that could regulate the biosynthesis of GSLs to a concentration that fits well in the residual enzymatic activity could prevent storage. Such a

therapeutic strategy has been carried out with *N*-alkylated DNJs, which are inhibitors of ceramide-specific glucosyl-transferases.[51] A structure activity relationship study on the inhibition of α-glucosidase and ceramide glucosyltransferase has been performed with *N*-alkylated compounds like *N*-nonyl-DNJ IC_{50} = 1.44 μM (**29**) or *N*-7-oxadecyl-DNJ (**30**) IC_{50} = 0.48 μM (**Figure 10**) and they have shown promising inhibitory activity.

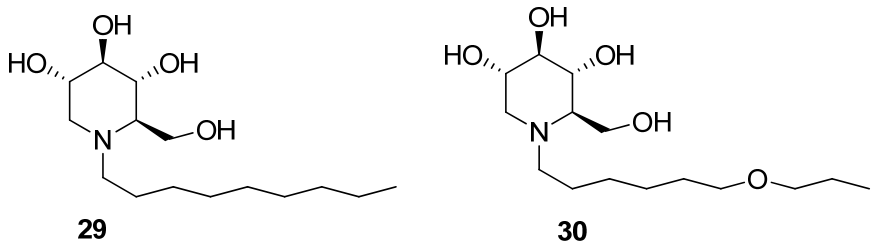


Figure 10: *DNJ derivatives*

1.3.3-Tumor metastasis

The membrane surfaces of malignant cells differ from normal ones in the structure and composition of their glycoproteins, glycolipids and proteoglycans. Consequently, the nature of the carbohydrates that participate in the complex process of metastasis is also specific and these sugars are sometimes altered.[52]

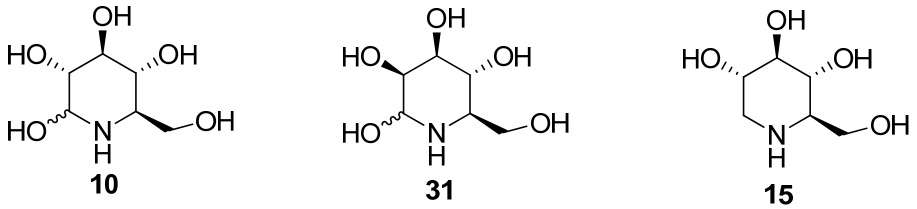


Figure 11: *Anti tumor agents*

A study of the inhibitory effect of imino sugars like NJ **10**, mannojirimycine (MJ) **31** and DNJ **15** (**Figure 11**) has been carried out by Tsuruoka and co-workers[52] in a model of pulmonary metastasis of mouse B16 melanoma.

1.3.4-Antiviral

It is now clear that modification or alteration of one or more biological events during the biosynthesis of *N*-linked and/or asparagine-linked glycoproteins could have an impact on viral infection or tumor invasion.[53] α-Glucosidase inhibitors, such as DNJ **15**, I-butyl-DNJ (**32**, *n*-Bu-DNJ), castanospermine **20** and celgosivi **33** (**Figure 12**) are potent inhibitors of HIV replication and HIV mediated syncytium formation *in vitro*.[54]

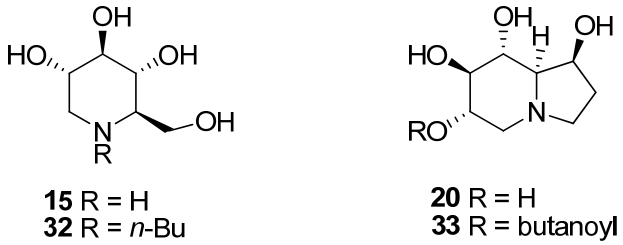


Figure 12.

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.[55] 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.[56–58]

The naturally occurring azasugar castanospermine **20** is a α-glucosidase I inhibitor with marked antiviral activity against a number of viruses. Unfortunately, this agent also inhibits intestinal sucrases and causes osmotic diarrhea. In contrast, celgosivir **33**, the 6-*O*-butanoyl derivative of castanospermine, (**Figure 12**) 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 under phase II development for the treatment of HCV infection.[59]

1.3.5-Anticancer

The malignant cells get altered in the structure and composition of their glycoproteins, glycolipids and proteoglycans. So if the enzymes involved in the synthesis of these complex glycoproteins, glycolipids and proteoglycans are inhibited then that would naturally lead to the reduction and growth of cancerous cells.

Although a number of azasugars have been reported to show anticancer activity such as, NJ **10**, MJ **31**, DNJ **15** and swainsonine **22**,[60] 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.[61]

1.4-Design of glycosidase inhibitors[15]

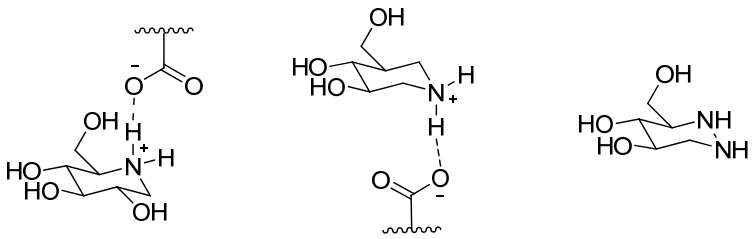
The design of glycosidase inhibitors through a computational method in which a molecule is virtually tied up into an enzyme pocket built on the coordinates obtained from the X-ray analysis of an inhibitor-enzyme complex[62–64] lead to successful design of a very potent sialidase inhibitor, Zanamivir™, which is marketed as an anti-influenza drug.[65,66] However, this method is not always successful and a number of glycosidase inhibitors have been designed as transition state (**Figure 2**) analogs of glycosidase hydrolysis.[7] Much attention has been put on mimicking the assumed geometry of the transition state or position of the assumed charge. Designs focusing on geometry have tried to create inhibitors with a half-chair conformation (shape of **TS-6**), while designs focusing on charge have tried to mimic charge build-up in a number of places. Many inhibitors have also been found by high-throughput screenings.[12g,12k] In this context, concepts involved in the design of glycosidase inhibitors are divided into six groups according to the 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.[67] 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.[68,69]

1.4.1-Ionic binding with a nucleophilic carboxylate

Azasugars are the most studied glycosidase inhibitors. 1-Deoxynojirimycin **(15)** (charge mimic **TS-5**, 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.[7,70,71] Isofagomine **34** (charge mimic **TS-5**), in which the location of the nitrogen atom corresponds to the anomeric center, shows strong and specific inhibition against β-glucosidase.[12b,72] 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 13**). Thus, the more efficient complexes with regard to the carboxylate-ammonium ion interaction are formed between α-glucosidase and **15**, and between β-glucosidase and **34**. This mechanism is strongly supported by the strong inhibitory activities against both α and β-glucosidases by the piperazine derivative **35** synthesized by Bols and co-workers.[73]



	15-H+	**34**-H+	**35**
Ki(μM) α-glucosidase (brewer's yiest)	25	86	3.9
β-glucosidase (almonds)	47	0.1	0.65

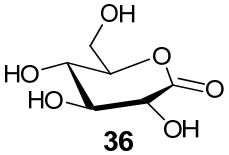
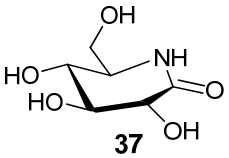
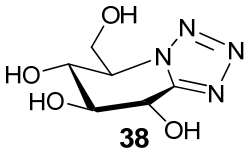
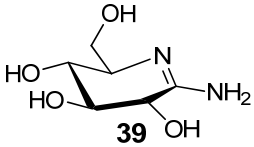
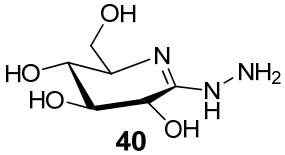
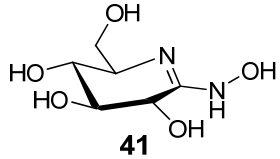
***Figure 13**: Structure and inhibition mechanism of **15, 34** and **35**.*

1.4.2-Mimicking the transition state structure (oxocarbenium ion)

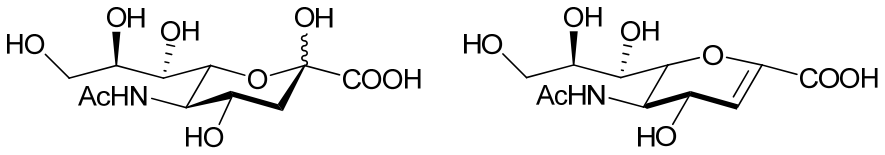
Enzymes promote reactions by lowering the activation energy, which is the result of the strong binding to the transition state structure among the other structures during the reaction course, so that the transition state binding governs the substrate specificity. The transition state structure in the hydrolysis of glycosides is believed to be the oxocarbenium ion intermediate **TS-6** with a flat shape. This means that mimicking this planar structure would

lead to a strong inhibitor of a glycosidase. As shown in **Table 1**, the planar structure of gluconolactone **36**, a classic glucosidase inhibitor, is thought to mimic the transition state structure.[7] δ-Glyconolactam **37** is proposed to inhibit through amide-iminol tautomer as it is showing good specificity for β-glucosidase. Bicyclic compound **38**, which is hybrid of deoxynojirimycin and tetrazole rings, has a half-chair structure that mimics the **TS-6** structure. Indeed, **38** 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.[7] Amidine **39**, amidrazone **40**, and amidoxime **41** were designed and synthesized as oxocarbenium ion analogs and are good inhibitors of β-glucosidases[9,74].

Table 1. Oxocarbenium analogues **36**–**41** and their Inhibitory activity against glucosidase.

Inhibitor	Enzyme Inhibition (*Ki* μM)		
	α-glucosidase (brewer's yeast)	β-glucosidase (almonds)	β-glucosidase (agrobacterum faecalis)
 **36**	2000	400	1.4
 **37**	1100	125	5.2
 **38**	1300	150	1.4
 **39**	—	10	—
 **40**	—	8.4	—
 **41**	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.[7]



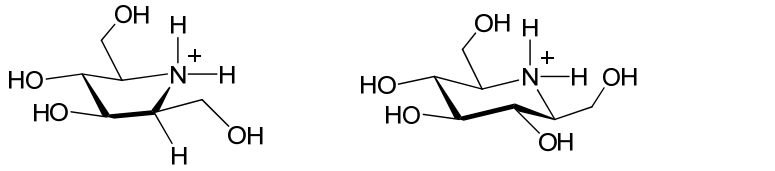
Sialic acid (**42**)

43; *Ki* 4μM
(Influenza A sialidase)

Figure14: *Sialic acid (**42**) and its 2,3-ene derivative **43***

However, it is also apparent that many planar analogs of glycopyranosides, like glycals, are glycosidase inhibitors. For instance, the 2,3-ene derivative **43** of sialic acid **(42)** is a good inhibitor of sialidases[75] (**Figure 14**).

Another approach to mimic the transition state structure is to construct a 5-membered ring as a basic structure. Pyrrolidine **44** was designed and synthesized as a glycosidase inhibitor mimicking the transition state structure and its positive charge (TS-**6**)[70,71] (**Figure 15**).



44-H^+

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

β-L-Homonojirimycin (45),
***Ki* = 900** mM for α-glucosidase (yeast)
***Ki* = 430** mM for β-glucosidase (almonds)

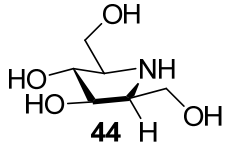
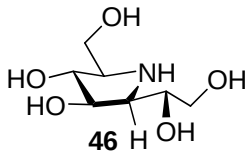
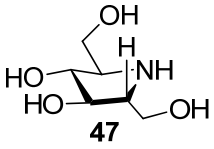
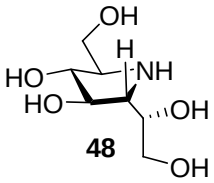
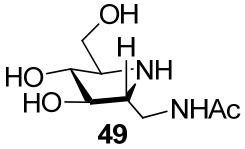
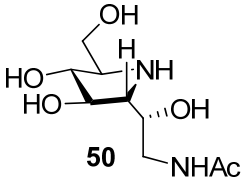
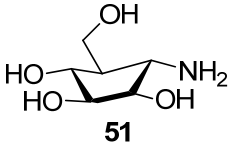
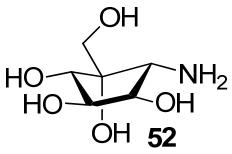
Mimic only the charge of transition state **9**
in chair conformation

Figure 15: *Structural comparison of pyrrolidine azasugar **44**-H^+ with **45***[76]

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**). Comparison of compound **44** and **47**, where the orientation of the side chain is different, revealed that the later (**47**) is a superior inhibitor against both α- and β-glucosidases.[77] Elongation of the side chain (**46**, **48**) 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 **(50)** is highly specific

toward β-glucosidase. Interestingly, compound **50** is a poor inhibitor of β-*N*-acetylhexosaminidase but compound **49** which carries a shorter side chain is a specific inhibitor against the enzyme.[78] Aminocyclopentitols such as compound **51** and **52** are similar to pyrrolidines with respect to their inhibitory mechanisms. [79,80]

Table 2. Relationship between structure and inhibitory activity for pyrrolidine compounds (**44, 46**–**50**) and aminocyclopentitol (**51** and **52** shape like **TS-6**)

Inhibitor	Enzyme Inhibition (*Ki* μM)		
	α-glucosidase (yeast)	β-glucosidase (almonds)	β-*N*-acetylhexosaminidase (human placenta p)
 **44**	80	52	—
 **46**	330	50	—
 **47**	0.73	1.7	—
 **48**	28	2.6	—
 **49**	380	poor	0.08
 **50**	poor	2.2	poor
 **51**	3.4	13	41 (for β-galactosidase, bovine liver)
 **52**	IC_{50} (μM) = 0.4	IC_{50} (μM) = 29	—

1.4.3-Hydrogen bonding with an acidic carboxylate

Triazole **53** and imidazole **54** are bicyclic compounds similar to compound **38** (**Figure 16**). 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 **55** against glucosidases.

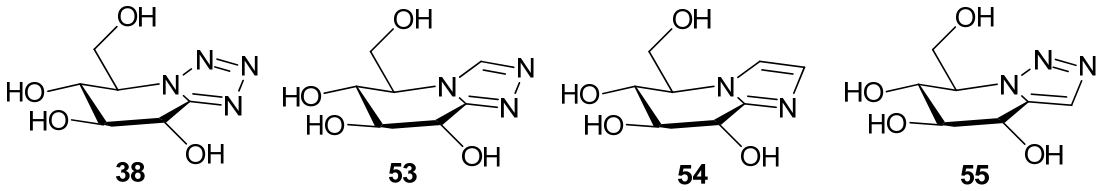


Figure 16: *Structure of azole-type glycosidase inhibitors* ***(38, 53–55)*** *and their inhibitory activities against β-glucosidase from almonds in K_i (μM).*

Thus, an inhibition mechanism where the acid carboxylate and the nucleophilic carboxylate synergistically operate has been proposed[7] (**Figure 17**).

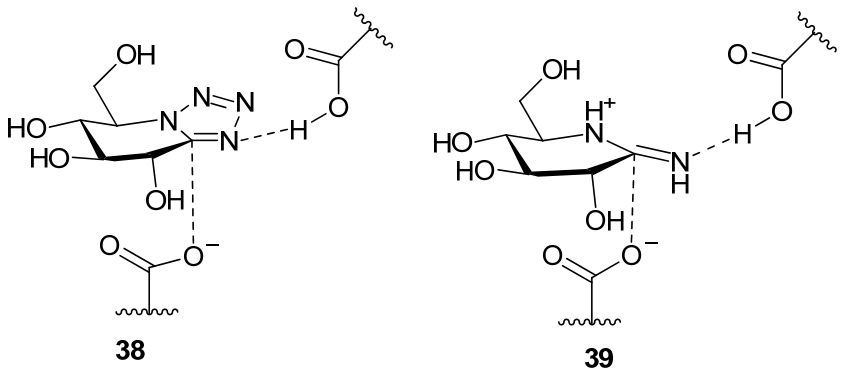


Figure 17: *Proposed mechanism involving a hydrogen bonding network among carboxylates and the inhibitors.*

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.[7] This model is also suggested to

be applicable for the amidine **39,** whose inhibition mechanism is explainable by cooperation between the two carboxylates.

1.4.4-Ionic binding with a non-catalytic group

Docking of **43** 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 **43** with enough room for their counter ion, a guanidino group. Thus, zanamivir **(56**, **Figure 18)**, the 4-guanidino derivative of **43**, was expected to show strong and specific inhibition against influenza sialidase and this indeed was found to be the case.[65]

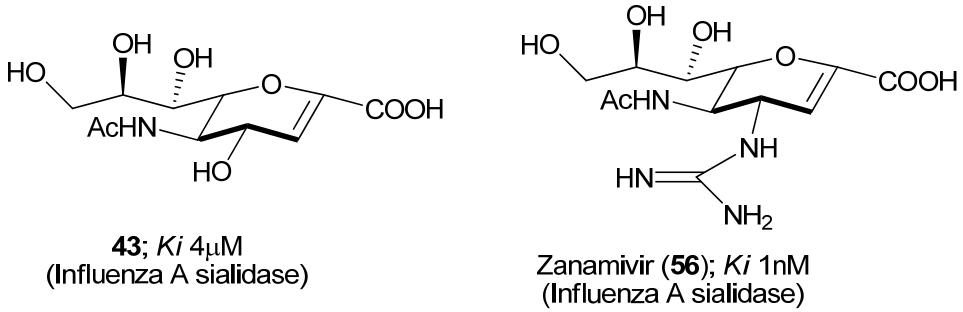
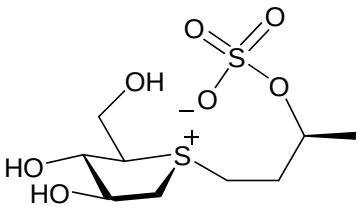


Figure 18: *Design of Zanamivir from coordination data of Sialic acid -sialidase complex.*

Salacinol **(57)**, 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.[81–85] The inhibition mechanisms of **56** have not been clarified. However, it is interesting to note that a similar tetrahydrothiophene sulfonium ion compound **58**, designed and synthesized as a transition state analogue inhibitor, shows weak glucosidase inhibition[86] (**Figure 19**).

It was proposed that the sulfonium ion interacted with the nucleophilic carboxylate in the catalytic site, as was suggested for inhibitory mechanism of pyrrolidine **44/47**, since the methyl sulfonium ion **59** showed good inhibition[87] while 1,4-epithioarabinitol **60** did not. Salacinol **(57)** has greater inhibitory activity and specificity against α-glucosidases than **59**. Hence, the sulfate group is believed to be important for the increased activity and specificity of **57**.[88] The specificity of **57** 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 (**56**) and salacinol (**57**) 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 designed synthesis of glucosidase inhibitors, as the coordination data for inhibitor-glycosidase complexes accumulate and computational chemistry improves.

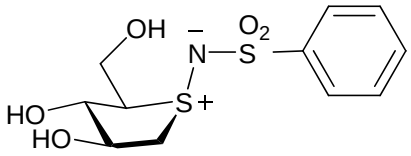


Salacinol (57)

IC_{50} (μM):

>5000 (yeast α-glucosidase)

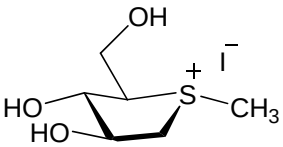
1.1 (rice α-glucosidase)



58

Ki (mM):

1.7 (almondsβ-glucosidase)

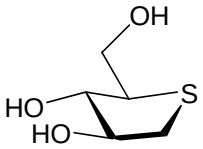


59

IC_{50} (μM):

360 (yeast α-glucosidase)

410 (rice α-glucosidase)



60

IC_{50} (μM):

>5000 (yeast α-glucosidase)

>5000 (rice α-glucosidase)

Figure 19: *Salacinol and other analogues for, establishing the significance of sulfate group in naturally occurring salacinol.*

1.4.5-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 view of this, it is apparent to put a hydrophobic group as an aglycon analog into a catalytic group targeting glycon analogs like azasugars.

The modification of aminocyclopentitol **(61)**,[89] calystegine B2 **(63)**,[90] and tetrahydroimidazopyridine **(54)**[91] by introducing a benzene ring into an appropriate position (compounds **62**, **64** and **65**) increased the glucosidase inhibition activity by 40-to 100-fold. The fact that, the introduction of *N*-phenyl cyclic isourea **(66)** into aminocyclopentitol **52**

caused a 50-fold increase in α-glucosidase inhibition [92] should be, at least in part, due to the hydrophobic effects (**Figure 20**).

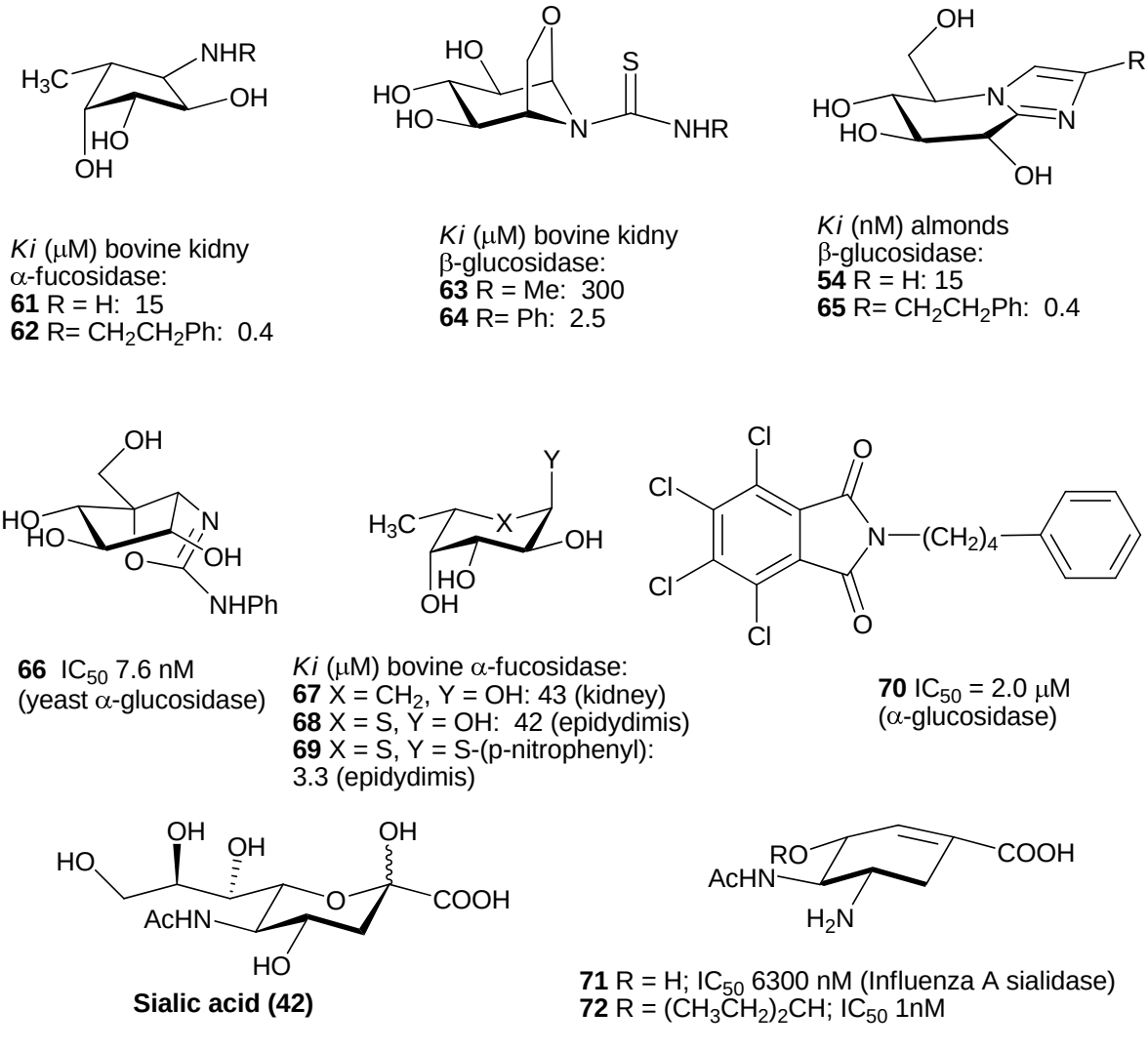


Figure 20: *Significance of hydrophobic interactions.*

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 **(67)** [93] and 5-thiofucose **(68)** [94] 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.[95] Further addition of a hydrophobic group into **68** at the aglycon site (**69**) caused a 10-fold increase in inhibitory activity.[96] The hydrophobic contact between the ring sulfur and Trp136 can be viewed in the crystal

structure of the 5-thioglucose-xylose isomerase complex.[97] Tetrachlorophthalimide **70**, 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.[98] Though it is not reminiscent of pyranose, the tetrachlorophthalimide portion may mimic the glycon and the pendant benzene the aglycon. Kim and coworkers[72] introduced alkyl chains, in place of the glycerol side chain of sialic acid, into a shikimic acid-analog **(71)** of the oxocarbenium ion intermediate in sialic acid hydrolysis, and studied their sialidase inhibition activities.[75] It was found that lengthening the alkyl chain up to three methylenes simply increased the inhibition activity, and the 3-pentyl compound **72** showed the best inhibition among the many alkyl chains tested, including branched chains. The crystal structure of the **72**-sialidase complex indicated that Glu276 was forced outward and the hydrophobic hydrocarbon chains of Glu276, Arg224, Ala246, and Ile222 were in close contact with the 3-pentyl group. These results represent an example of induced fitting, which will be discussed in the following section.

1.4.6-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.[99] High-throughput screening disclosed compound **74** (**Figure 21**) binding to the allosteric site 1000 times more strongly than the regular substrate glucose-6-phospate **73**. The crystal structure of the complex showed several hydrophobic interactions, including one with the chlorophenyl ring of compound **74** 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. The structure of compound **74** is totally different from that of glucose-6-phosphate, and this kind of unusual inhibitor could only be discovered by high-throughput screening and serendipity among the currently available methods. For example, compound **75**, isolated from *Umbelliferae* (a plant family) in Mongolia, is a strong inhibitor of α-glucosidase.[69] Compound **76** is also a strong inhibitor of α-glucosidase and was isolated from a marine sponge in Japan.[68] 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

76 may play the same role as those in the proposed mechanism of inhibition for salacinol **57**.

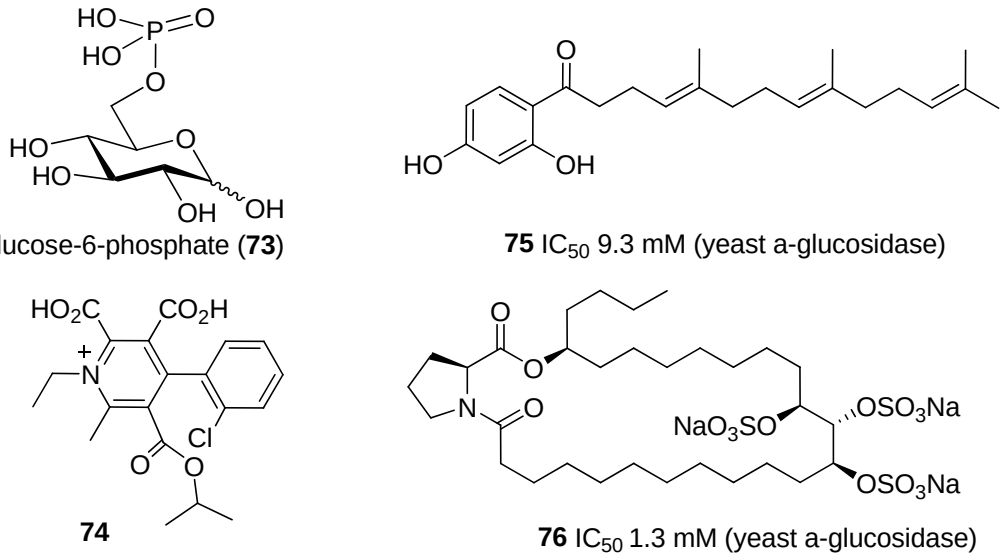


Figure 21: *Non carbohydrate-mimics acting through induced fitting by rearrangement of the Enzyme Loop Domain.*

1.5-Aim of this dissertation

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

Besides testing mechanistic hypotheses, the design, synthesis and testing of inhibitors may 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 of polyhydroxyquinolizidine alkaloids and some of their amino analogs as potential glycosidase inhibitor and enzyme inhibitory evaluation of their activity.
- Studies towards the synthesis of conformationaly restricted glycomimetics.

1.6-References

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Chapter 2

Synthesis and Biological Evaluation of Quinolizidine Class of Azasugars

"There is excitement, adventure, and challenge, and there can be great art in organic synthesis."

-R.B. Woodward

Section-A::An Introduction to Bicyclic Azasugars

2A.1-Polyhydroxy bicyclic alkaloids as glycosidase inhibitors

As discussed earlier[1], carbohydrates and their derivatives like oligosaccharides and glycoconjugates (glycoproteins, glycopeptides, glycolipids, lipopolysaccarides and peptidoglycans etc) play essential role in numerous biological processes such as cell-cell recognition, immune response and inflammation, fertilization, memory consolidation and many more.

The enzymes, glycosidases and glycosyltransferases, play pivotal role in a wide range of important biological processes, such as biosynthesis of polysaccharides and glycoconjugates,[2] controlling cell-surface carbohydrate structure and function, intestinal digestion, post-translational processing of glycoproteins and the lysosomal catabolism of glycoconjugates.[3] The possibility of modifying or blocking these processes by using glycosidase-inhibiting moieties for cell biological and therapeutic applications has attracted a great deal of attention, since some sugar-mimic alkaloids which inhibit the glycol-enzymes, show potential antidiabetic[4], antiviral and anticancer effects.[5]

Polyhydroxylated alkaloids mimicking the structures of sugars are widespread in plants as well as microorganisms and inhibit glycosidases because of the structural resemblance to the sugar moiety of the natural substrates. The nitrogen atom present in these molecules mimic the positive charge of the glycosyl cation (oxocarbenium ion) intermediate in the enzyme-catalyzed glycoside hydrolysis, as discussed in previous chapter.

The naturally occurring glycosidase inhibitors have been classified into five structural classes (**Figure 1**):

1) Polyhydroxylated piperidines (e.g., nojirimycin **1**),

2) Polyhydroxylated pyrrolidines [e.g., 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP) **2**],

3) Polyhydroxylated indolizidines and quinolizidines (e.g., swainsonine **3**),

4) Polyhydroxylated pyrrolizidines (e.g., australine **4**),

5) Polyhydroxylated tropanes and nortropanes (e.g., calystegine A_3 **5**).

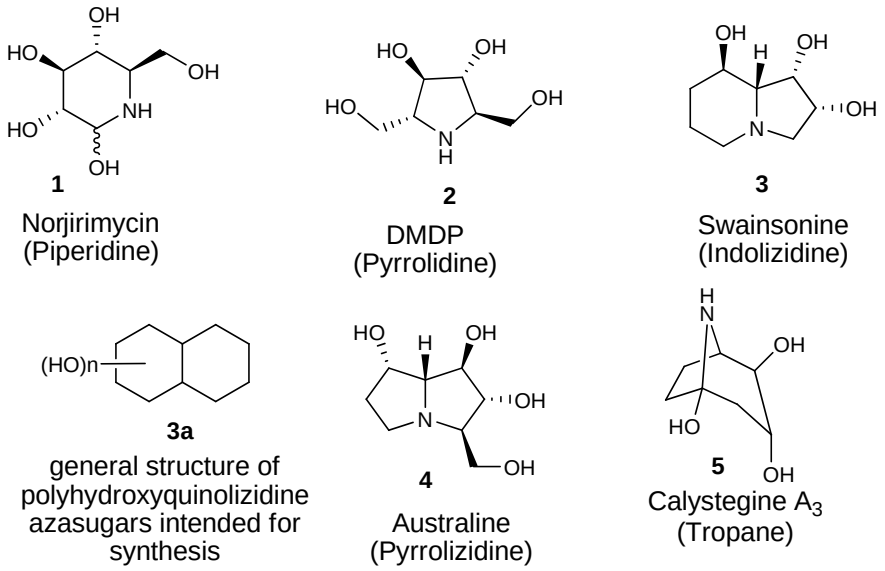


Figure 1: *Representative examples of various structural classes of glycosidase inhibitors.*

Many of these alkaloids are found to be potent inhibitors of glycosidases. Consequently, the last decade has witnessed a spurt in the synthetic organic chemistry for designing efficient route to many of these potent glycosidase inhibitors.

The scientific history of azasugars began in the early 1960s, with the pioneering works by Paulsen and co-workers [6] on sugar analogues with basic nitrogen instead of oxygen in the ring (although it was purely an academic exercise at that time). In 1966, Paulsen published the first synthesis of 1-deoxynojirimycin (DNJ).[6] The same year witnessed the isolation of nojirimycin from natural sources.[7] The first renaissance of azasugars came from the isolation of DNJ from natural sources along with the discovery of its α-glucosidase inhibition activity by Bayer chemists in 1976.

The isolation of the bicyclic iminosugars such as, castanospermine [8] (potent inhibitor of lysozomal α-glucosidase) [9] and swainsonine [10] (potent inhibitor of lysozomal α-mannosidase and Golgi α-mannosidase) [11] gave rise to a great impetus in research on these alkaloids and their applications, because of their high and specific glycosidase activity, which has been attributed, in part, to their rigid structure. Subsequently, these polyhydroxy azabicyclic alkaloids have emerged as chemotherapeutic potentials [12] for a variety of disease like HIV,[13] cancer [5] and several viral infections such as influenza.[14]

Despite their therapeutic potentials, these molecules have not found full clinical evaluation, largely due to the low natural abundance in conjunction with difficulties in preparing a

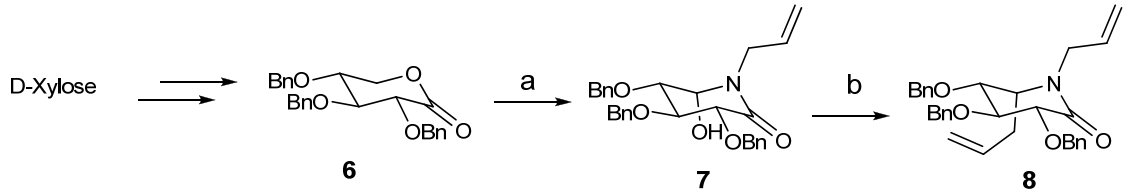
comprehensive array of variant structures. Escalating the snag, many of these molecules exhibit superfluous toxic effects e.g., castanospermine, although, known to display potent inhibitory activities against glucosidases and antiviral properties against a number of viruses,[13,14] is also found to inhibit intestinal sucrases causing osmic diarrhea [15] resulting in its withdrawal from the use as a clinical therapeutic. Thus, there is a need to synthesize a palette of polyhydroxylated analogs of these molecules to allow a better understanding of the structural requirements for glycosidase inhibitor and to develop more potent, selective and less toxic drugs.

As a result, considerable efforts have been directed towards the development of ring expansion analogs (polyhydroxyquinolizidines)[16,17] and stereoisomers[18] of alkaloids like castanospermine and swainsonine, resulting in interesting inhibitory activities. All these azasugars have led to the great enhancement in the research activities including the synthesis and their further biological evaluation. By contrast, examples involving ring expansion analogs of 1-deoxycastanospermine (general structure; **3a**), which can also be visualized as a bicyclic analog of another potent glycosidase inhibitor, 1-deoxynojirimycine (**1**),[16] are scarce in literature. In connection with this, intense research has also been done on changing the lipophilicity of **3** by attaching a hydrophobic group to its nitrogen atom, resulting in interesting inhibitory activities.[4,19] All these facts, coupled with our continuing interest in this area,[20] spurred us to synthesize azasugars of general structure **3a**. Before describing our synthetic efforts for these polyhydroxyquinolizidine alkanoids, it will be appropriate to have an overview of the synthetic approaches towards various known polyhydroxyquinolizidines azasugars.

2A.2-Synthetic approaches towards 1,2,3-trihydroxyquinolizidines

Pandit's approach (RCM**)**:[16a]

Starting from lactone **6** Pandit *et al.* have reported the first synthesis of **10** employing RCM as a key step. Lactone **6** in turn was derived from D-xylose in three steps (**Scheme 1**).



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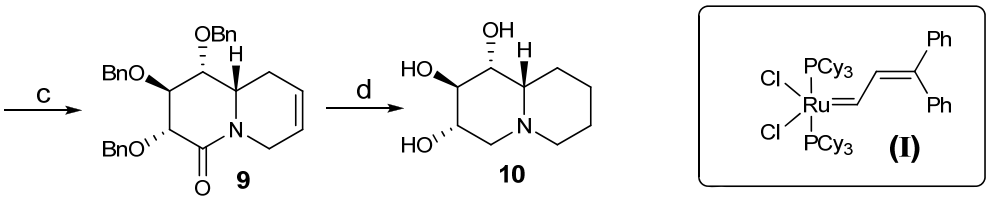
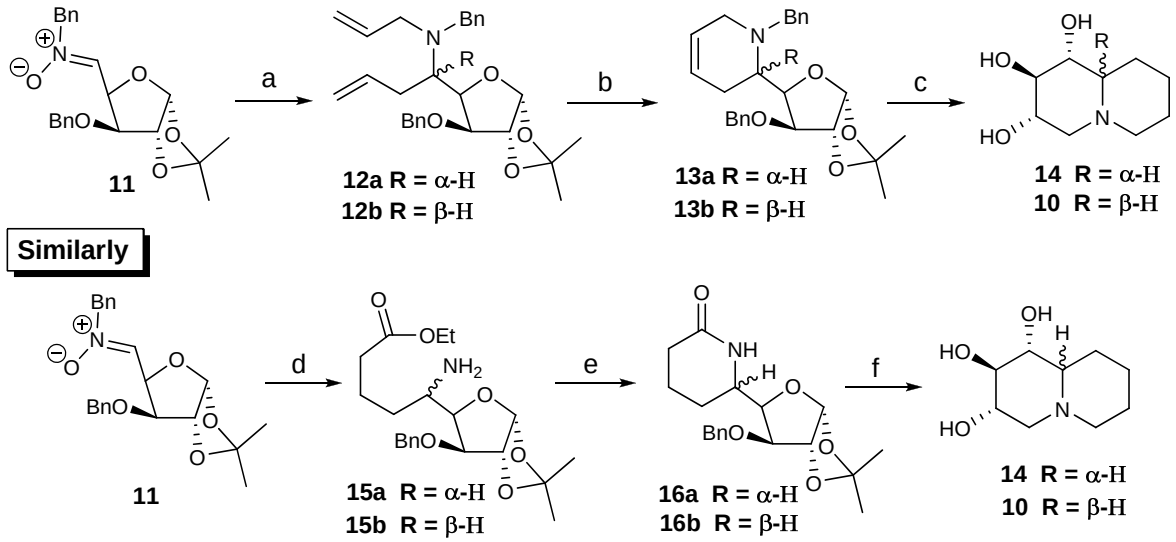


Reagents and conditions: (a) i) Allylamine, MeOH (98%); ii) Dess-Martin periodinane; iii) NH_3, MeOH (77%); (b) i) Ac_2O, py, DMAP (91%); ii) allyltrimethylsilane, $BF_3.OEt_2$ (32%); (c) **I** (1 mol %), toluene, rt (74% based on recovered starting material), *iii)* $LiAlH_4$, THF, then H_2, $Pd(OH)_2/C$, HCl, EtOH (56%).

Scheme 1.

Dhavale's approach (Cycloaddition on sugar nitrone / Regioselective ring opening of aziridine carboxylate):[16b–c]

The approach of Dhavale and co-workers for the synthesis of **10** and **14**, utilized 1,3-dipolar cycloaddition on sugar nitrtone as a key reaction (**Scheme 2**). The nitrone **11** was derived from D-glucose in three steps with 78% overall yield.

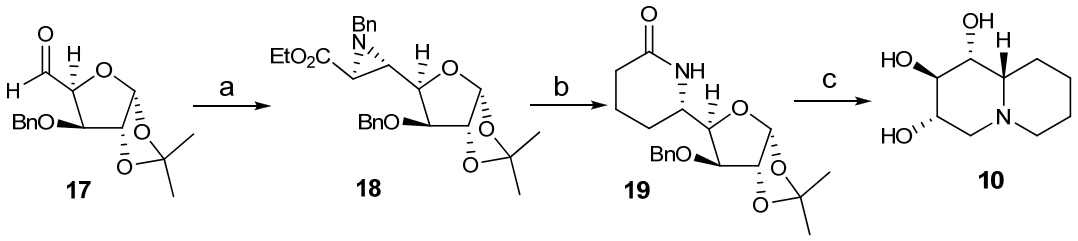


Reagents and conditions: (a) i) Allylmagnesium bromide (2.5 eq.), TMSOTf (1.0 eq), dry THF, −78 °C, 2h (93%); ii) Zn (2.0 eq), $Cu(OAc)_2$, HOAc, 70 °C, 1 h (80%); iii) allyl bromide (1.5 eq), K_2CO_3, (5 eq), dry DMF, 25 °C, 18 h (71%); (b) Grubb's catalyst (5% mol), dry benzene, reflux, 18 h (81%); (c) i) H_2, 10% Pd/C, MeOH, 25 °C, 12 h; ii) CbzCl (1.5 eq), $NaHCO_3$, aq. EtOH, 0 °C to rt, 2 h (78%); iii) TFA–H_2O (3:2), 0 °C to rt, 2.5 h; iv) H_2, 10% Pd/C, MeOH, 25 °C, 12 h (85%); (d) i) allylmagnesium bromide (2.5 eq), THF, −78 °C, 2 h (93%); ii) Zn (2 eq), $Cu(OAc)_2$, AcOH, 70 °C, 1 h (78%); iii) CbzCl (1.5 eq), $NaHCO_3$, aq. EtOH, 2 h (75%); iv) O_3, DCM, DMS, −40 °C, 1 h (90%); v) Ph_3P=CHCOOEt (1.5 eq), MeOH, rt, 2 h; vi) H_2, 10% Pd/C, MeOH, 25 °C, 12 h; (e) CH_3COONa (4 eq), MeOH, reflux, 6 h (69%); (f) i) LAH (5 eq), THF, 0 °C, 1

h; ii) CbzCl (1.5 eq), $NaHCO_3$, aq. EtOH, 2 h (74%); iii) TFA–H_2O (3:2), 0 °C to rt, 2.5 h; iv) H_2, 10% Pd/C, MeOH, 25 °C, 12 h (87%).

Scheme 2.

Another approach[16d] from the same group involved regioselective ring opening of aziridine carboxylate **18** under hydrogenation condition to obtain lactam **19** which was finally converted to **10**. The synthesis involved eight steps with 20% over all yield (**Scheme 3**).



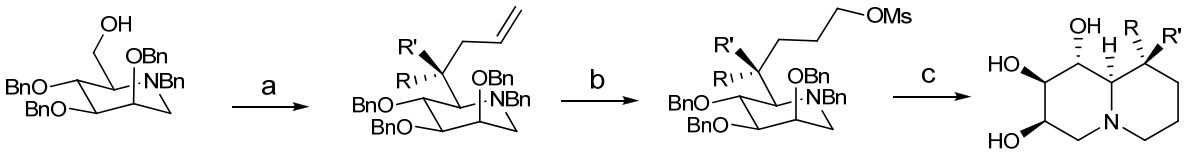
Reagents and conditions: (a) PPh_3=CBrCOOEt, DCM, 25 °C, 12 h (77%); (b) $BnNH_2$, benzene, 10 to 20 °C, 6 h (70%); (b) i) DIBAL-H, DCM, −78 °C, 2.5 h (79%); ii) Ph_3P=CHCOOEt, CH_3CN, 30 °C, 6 h (77%); iii) H_2 (80 psi), 10% Pd/C, MeOH, 30 °C, 12 h, then, NaOAc, MeOH, reflux, 1.5 h (74% over two steps); (c) i) NaH, BnBr, THF, 0 °C to reflux, 2.5 h (96%); ii) LAH, THF, 0 °C to reflux, 3 h (89%); iii) TFA–H_2O (7 : 3), 0 °C to 20 °C, 3 h; iv) H_2 (80 psi), 10% Pd/C, MeOH, 24 h (96%).

Scheme 3.

2A.3-Synthetic approaches towards other polyhydroxyquinolizidine azasugars

Ganem's approach (Chelation controlled Sakurai reaction):[17a]

For the synthesis of **23** and **24**, Ganem *et al.* utilized a highly selective chelation controlled Sakurai reaction on the aldehyde obtained from amino alditol **20** as a crucial step (**Scheme 4**). The amino alditol **20** was obtained from D-glucose in enantiomerically pure form in six steps in 29% overall yield.

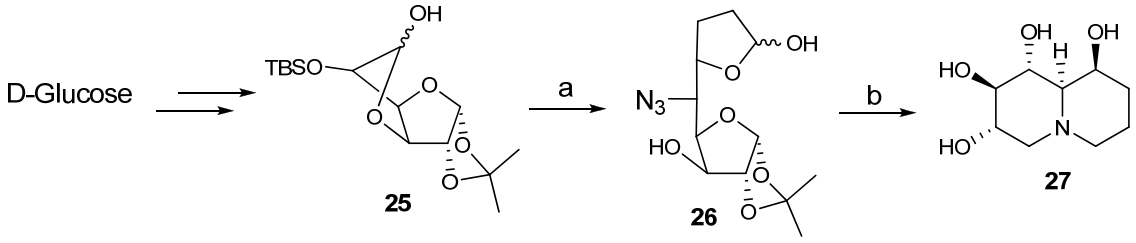


Reagents and conditions: (a) i) DMSO, $(COCl)_2$, Et_3N, DCM, −78 °C ii) allyltrimethylsilane (3.6 eq), $TiCl_4$ (2.4 eq), DCM, −85 °C; 15 h; iii) acetic anhydride, py; (b) i) BH_3.DMS, THF; ii) MsC1, Na_2CO_3, DCM; (c) i) H_2, Pd/C, EtOH–CH_3OH (3:l); ii) K_2CO_3, CH_3OH–H_2O (3:l).

Scheme 4.

Stutz's approach (Intramolecular double reductive amination):[17b]

Intramolecular double reductive amination of the primary amine derived from azidodeoxy sugar **26** with its suitably tethered lactol moieties gave **27** smoothly. The synthesis of **27** starting from D-glucose consisted of five steps and 8% overall yield (**Scheme 5**).

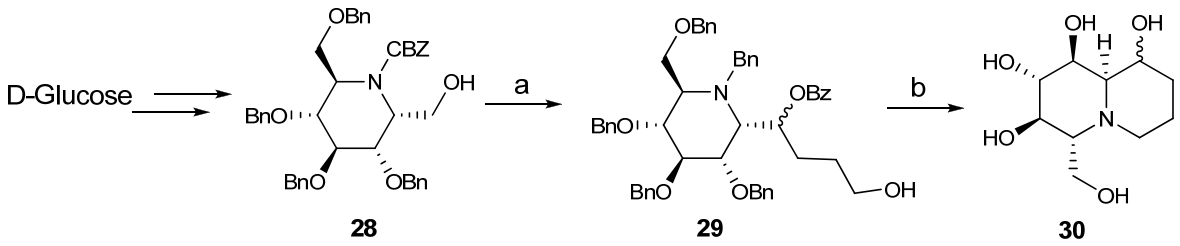


Reagents and conditions: (a) i) 2-(2-bromoethyl)-1,3-dioxolane, Mg, Et_2O, rt, 1.5 h then add **25**; ii) MOMCl, DIPEA, DCM (35% over two steps); iii) TBAF, THF (92%); iv) Tf_2O, then NaN_3, DMF (73%); (b) i) ion exchange resin, water–acetonitrile, 40 °C (69%); ii) 5% Pd/C, water–AcOH, 6d (51%).

Scheme 5.

Liu's approach:[17c]

Polyhydroxyquinolizidine **30** was synthesized from iminoheptitol **28**, obtained from D-glucose in seven steps with 23% overall yield, in seven steps with 11% overall yield as depicted in **Scheme 6**.

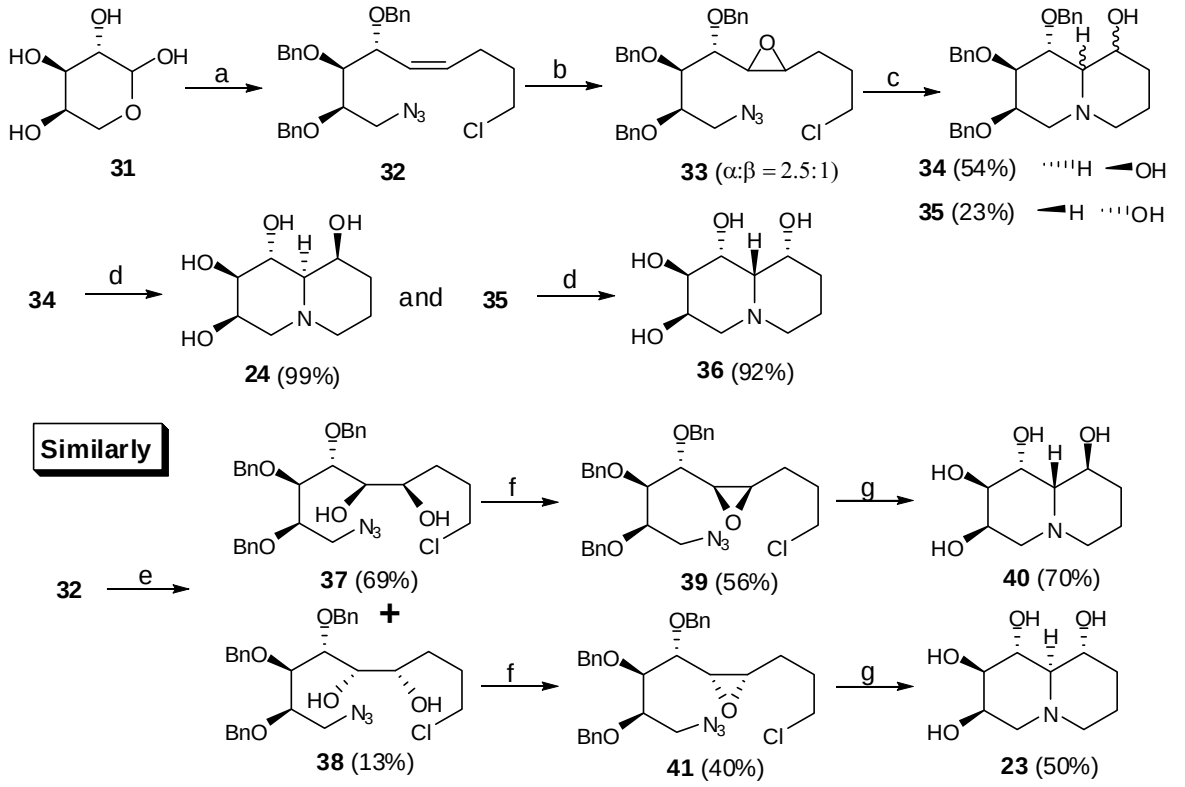


Reagents and conditions: (a) i) Pd/C, cyclohexene, MeOH (83%); ii,iii) PhCOCl, Et_3N, AlH_3, THF (87%); iv,v,vi) $(COCl)_2$, DMSO, allylmagnesium chloride, Et_2O, PhCOCl, DMAP (68%), vii) BH_3.DMS, then H_2O_2 (57%); (b) i) $Pd(OH)_2$, cyclohexene, MeOH, MsCl, py (50%); ii) Pd black, H_2 (85%); iii) NaOMe, MeOH (90%).

Scheme 6.

Pearson's approach (Intramoleculr double alkylation):[17d,17g]

Pearson and co-workers synthesized various stereoisomers of 1,2,3,9-tetrahydroxy-quinolizidines from ω-haloazidoalkene **32** and its derivatives following the steps as shown in **Scheme 7**.



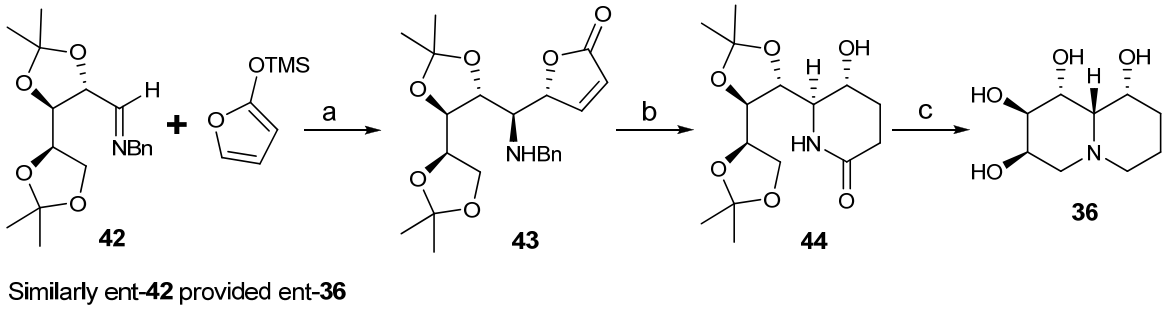
Reagents and conditions: (a) i) $BrPh_3P(CH_2)_4Cl$ (2.5 eq), KHMDS (2.5 eq), THF, 0 °C, 30 min, then **31** in DCM at −78 °C, and warm to rt, 5 h; ii) PPh_3 (1.2 eq) in PhH, HN_3 (1.2 eq, 1.2M soln in PhH), cool to 0 °C, DEAD (1.2 eq), warm to rt, 30 min; (b) *m*-CPBA (2 eq), DCM, 0 °C to rt for 12 h; (c) 10% w/w of 10% Pd/C, Et_2O–EtOH (2:1), H_2 (1 atm), rt for 24 h; filter Pd/C, then K_2CO_3 (6 eq), EtOH, reflux, 24 h, separate diastereomers. (d) 45% *w/w* of 10% Pd/C, 2% HCl in MeOH, H_2 (45 psi), rt, 48 h; (e) OsO_4, NMO, THF–*t*-BuOH, 72 h; (f) i) MsCl (1.1 eq), TEA, DCM, −50 °C (1 h), and warm slowly to 0 °C; ii) NaH, THF–DMSO, 0 °C, 12 h; (g) i) 10% w/w of 10% Pd/C, Et_2O–EtOH (2:1), H_2 (1 atm), rt for 24 h; filter Pd/C, then K_2CO_3 (6 eq), EtOH, reflux, 24 h; ii) 45% *w/w* of 10% Pd/C, 2% HCl in MeOH, H_2 (45 psi), rt, 48 h.

Scheme 7.

Rassu's approach (Condensation of 2-(trimethylsiloxy)furan with imines):[17e]

The approach utilized D- and L- arabinose derived **43** and *ent*-**43** to generate the skeleton and chirality of the target molecule **36** and *ent*-**36**. Precursor **43** (or *ent*-**43**) were derived by the Mannich type reaction of **42** (or *ent*-**42**) with 2-(trimethylsiloxy)furan catalyzed by a

Lewis acid. The procedure comprises five steps from **42** or *ent*-**42** and provides the polyhydroxy-quinolizidine **36** or its enantiomer in 36–37% overall yields (**Scheme 8**).

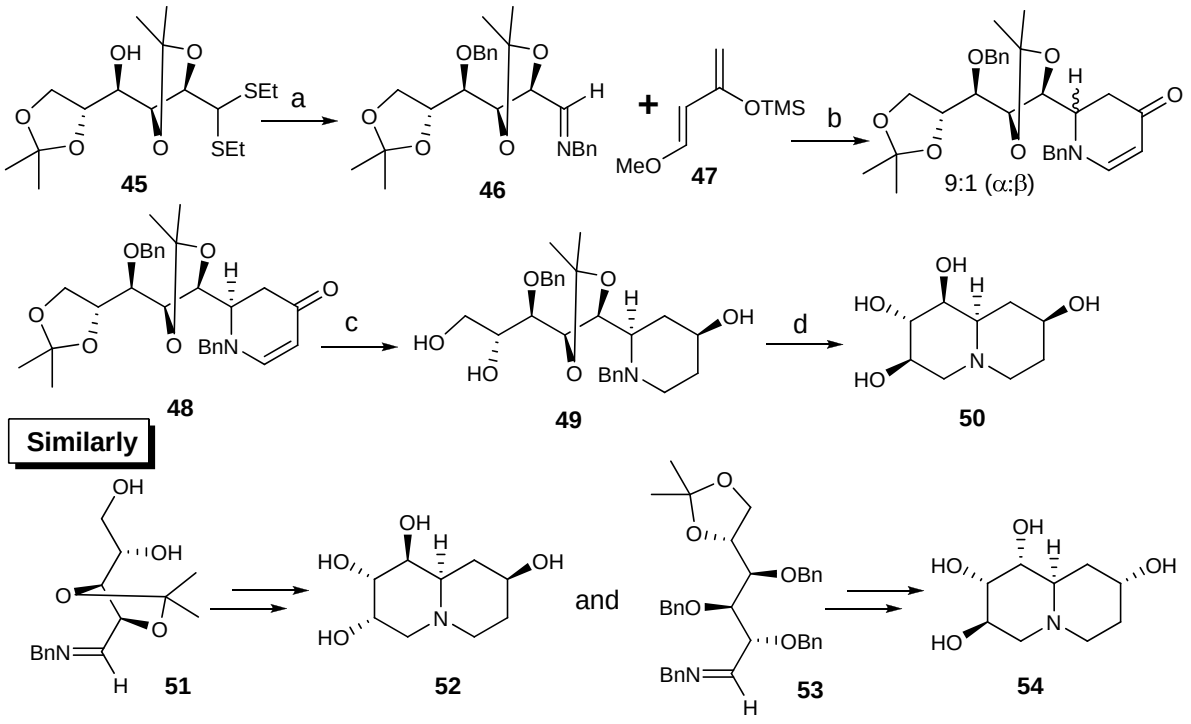


Reagents and conditions: (a) $BF_3.Et_2O$, DCM, −85 ºC (77%); (b) i) 10% Pd/C, H_2, THF, NaOAc (95%); ii) DBU, xylenes, 140 ºC, (96%); (c) i) BH_3.DMS, THF; ii) 70% CF_3COOH; iii) DOWEX-OH (74% over three steps); iv) TPP, CCl_4, TEA, DMF (69%).

Scheme 8.

Herczegh's approach (Hetero Diels-Alder reaction):[17f]

1,2,3,8-Tetrahydroxyquinolizidines **50**, **52** and **54**, were synthesized from D-glucose, L-arabinose and D-mannose, respectively, using hetero Diels-Alder reactions of sugar derived azomethines as the key step (**Scheme 9**).

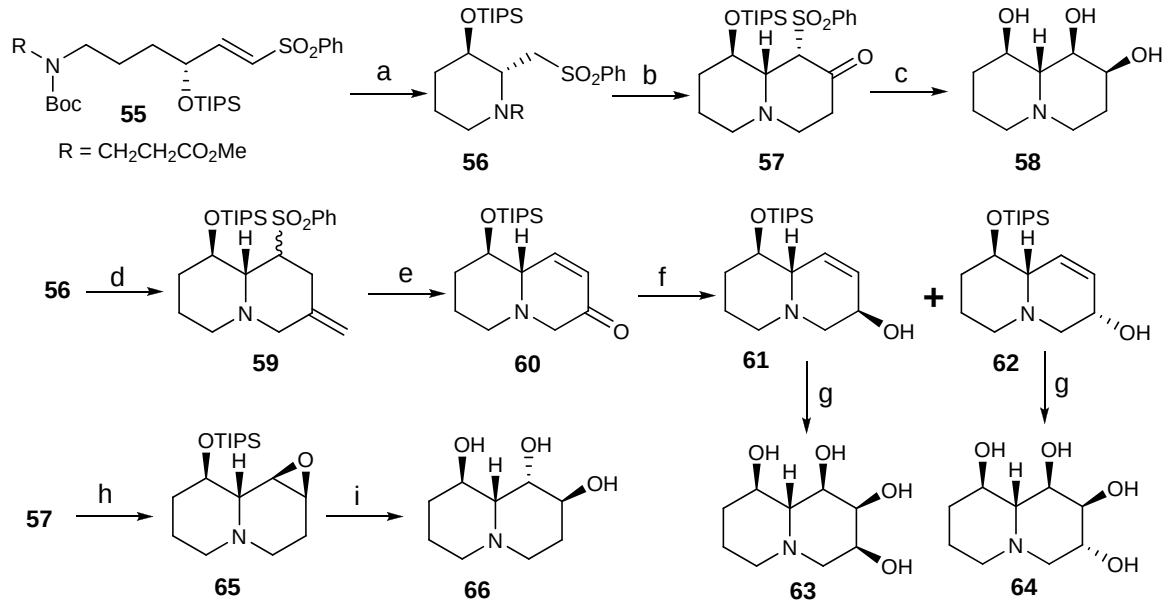


Reagents and conditions: (a) i) BnBr, NaH, DMF, rt, 16 h; ii) HgO, $Et_2O.BF_3$, H_2O–THF, rt; iii) $BnNH_2$, THF, rt, 1 h; (b) $ZnCl_2$, dioxane, 3 h; (c) i) $NaBH_4$, EtOH, 20 h; ii) AcOH, H_2O, 50 °C; (d) i) $Pb(OAc)_4$, PhH; ii) CF_3COOH, H_2O; iii) H_2, Pd(C), AcOH.

Scheme 9.

Carretero's approach (Use of γ-oxygenated-α,β-unsaturated sulfone):[17h]

Both 1,2,9-trihydroxylated and 1,2,3,9-tetrahydroxylated quinolizidines have been stereoselectively prepared in few steps and high overall yields from the readily available γ-oxygenated-α,β-unsaturated sulfone **55** (**Scheme 10**).



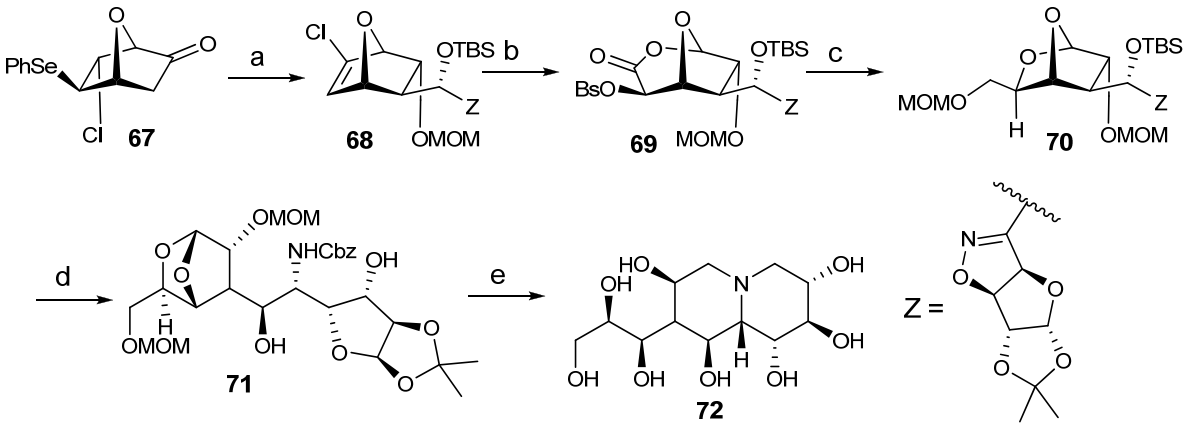
Reagents and conditions: (a) i) TFA, DCM; ii) TEA, −78 ºC (96%) (b) LHMDS, THF, 0 ºC (96%); (c) i) $NaBH_4$, EtOH, rt; ii) Na-Hg, MeOH, rt (66% over two steps); iii) OsO_4 (cat.), Me_3NO (82%); iv) HCl; v) OH^- (98%); (d) i) 3-chloro-2-chloromethylpropene, K_2CO_3, LiI; ii) LHMDS, THF, 0 ºC (80% over two steps); (e) i) O_3, TFA, −20 ºC; ii) TPP, rt; iii) TEA, rt (69%); (f) L-Selectride, THF (**61** : **62** = 32 : 68); (g) i) OsO_4 (cat.), Me_3NO; ii) HCl; iii) DOWEX-OH (66% and 75% over three steps for **63** and **64** respectively); (h) i) $(MeO)_3CMe$, PPTS, DCM; ii) TMSCl, DCM; iii) K_2CO_3, MeOH (60% over three steps); (i) i) TFA, rt (80%); ii) SiO_2.MeOH; iii) HCl; iv) DOWEX-OH (98% over three steps).

Scheme 10.

Vogel's approach (Diastereoselective cross-aldolization):[17i]

Synthesis of a polyhydroxyquinolizidine bearing a polyhydroxylated side-chain (**72)** featured the diastereoselective cross-aldolization of the enantiomerically pure 7-oxabicyclo-

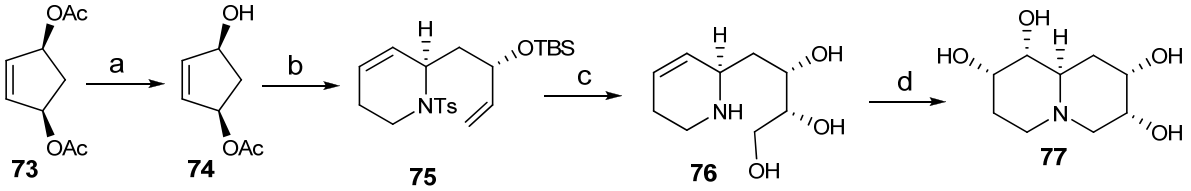
[2.2.1]heptan-2-one (–)-**67** with enantiomerically pure aldehyde (ZCHO) (**Scheme 11**). Compound **67** was derived from a "naked sugar of the first generation".



Reagents and conditions: (a) i) LHMDS, THF, −78 ºC, ZCHO, 3h (90%); ii) TBSOTf, 2,6-lutidine, DCM, 0 ºC, 1h then 25 ºC 5h (92%); iii) $NaBH_4$, MeOH, 5 ºC (91%); iv) $MeOCH_2Cl$, $(i\text{-}Pr)_2NEt$, DCM, 0 °C to 25 °C (86%); v) *m*-CPBA, DCM–THF, −78 °C to 20 °C, 14 h (95%); (b) i) OsO_4, $Me_3NO{\cdot}2H_2O$, ii) BsCl, Et_3N, 0 °C 1 h, then, 25 °C, 2 h (88%); iii) *m*-CPBA, $NaHCO_3$, DCM, 0 °C 5 h, then 25 ºC for 15 h (92%); (c) i) MeOH, K_2CO_3, DMF, 5 ºC for 30 min then 25 ºC, 90 min; ii) $LiAlH_4$, THF, 0 °C, 10 min; iii) MOMCl, DIPEA (92%); (d) $LiAlH_4$, Et_2O, 25 °C, 5 d, then protection as *N*-benzylcarbamate (88%); (e) i) TFA, H_2O, 25 °C; ii) H_2, Pd/C, H_2O, 25 °C, 15 h (60%).

Scheme 11.

Blechert's approach (Asymmetric desymmetrization through enzymatic hydrolysis):[17j] Blechert carried out the synthesis of **77** in eight steps involving key intermediate **74** which was obtained via enzymatic hydrolysis of *meso* diester **73** (**Scheme 12**).

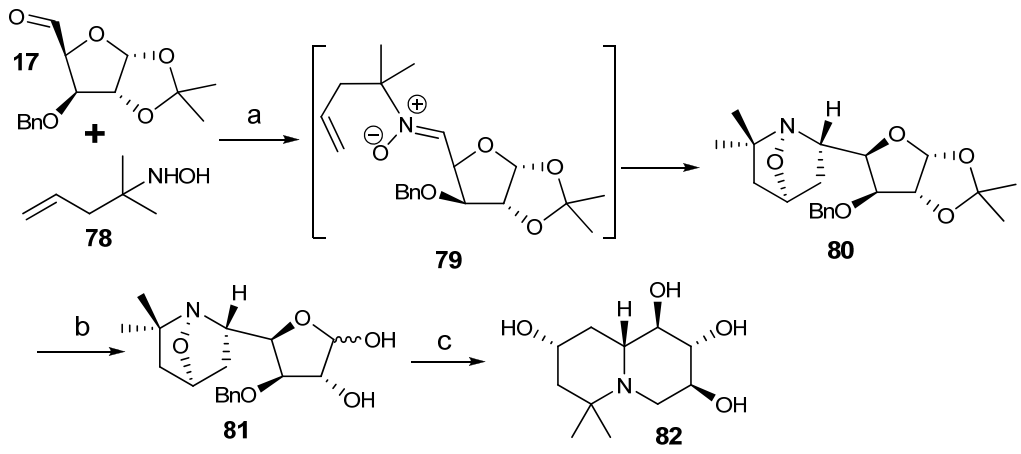


Reagents and conditions: (a) Lipase B (*Candida antarctica*), H_2O; (b) i) $Pd(OAc)_2$, PPh_3, *N*-nosylbutenylamine, DMF, rt to 40 °C (80%); ii) TBSCl, Im, DMF, rt (98%); iii) 1 mol % [Ru**]**, DCM, rt (95%); (c) i) TBAF, THF, rt (96%); ii) OsO_4 (cat.), NMO (1 eq), acetone–H_2O (1:1), 0 °C to rt (60%) 70% de; iii) K_2CO_3, PhSH, DMF, 40 °C (90%); (d) i) PPh_3, DEAD, py, 0 °C (53%); (i) OsO_4 (cat.), NMO (1.5 eq), acetone–H_2O (1:1) 0 °C to rt (81%) > 95% de.

Scheme 12.

Sas's approach (Intramolecular 1,3-dipolar cycloaddition):[17k]

Reaction of sugar derived carbaldehyde **17** with hydroxylamine derivative **78** followed by intramolecular 1,3-dipolar cycloaddition yielded 7-oxa-1-azabicyclo[2.2.1]heptane derivative **80**, which was easily converted into novel polyhydroxylatedquinolizidine **82** (**Scheme 13**).



Reagents and conditions: (a) Toluene, argon, 85─90 °C, 43 h (52%); (b) 5% aq. HCl, rt, 2 d (96%); (c) H_2 (10 bar), Raney-Ni, MeOH, 75─80 °C, 21 h (70% over two steps).

Scheme 13.

The above discussion regarding the strategies used for the synthesis of polyhydroxyquinolizidine alkaloids can be summarized as follows.

The known synthetic itineraries for 1,2,3-trihydroxyquinolizidines have involved chiron approach, with long reaction sequences producing maximum of two analogs. In some cases, a key reaction produces two diastereomers which were forwarded to get different analogs indicating difficulty in getting single required product in appreciable yield.[16b–c]

Basically, there are two kinds of approaches utilized for the synthesis of other polyhydroxyquinolizidine alkaloids (**Figure 2**). The first synthetic strategy mainly utilizes carbohydrates as the building block material to install the required stereochemistry in the azasugars. Most of the approaches utilizing sugars as starting material rely on the common methodology of making cyclic structures. The concept lies in generating amine functionality at suitable position, which is coupled with the anomeric carbonyl moiety of sugars.

Variation of sugars leads to variation of hydroxyl group stereochemistry in the end product. The other protocol employs the creation of chirality in achiral materials by the use of asymmetric desymmetrization (most common being enzymatic) which is carried forward for the synthesis of target azasugars.

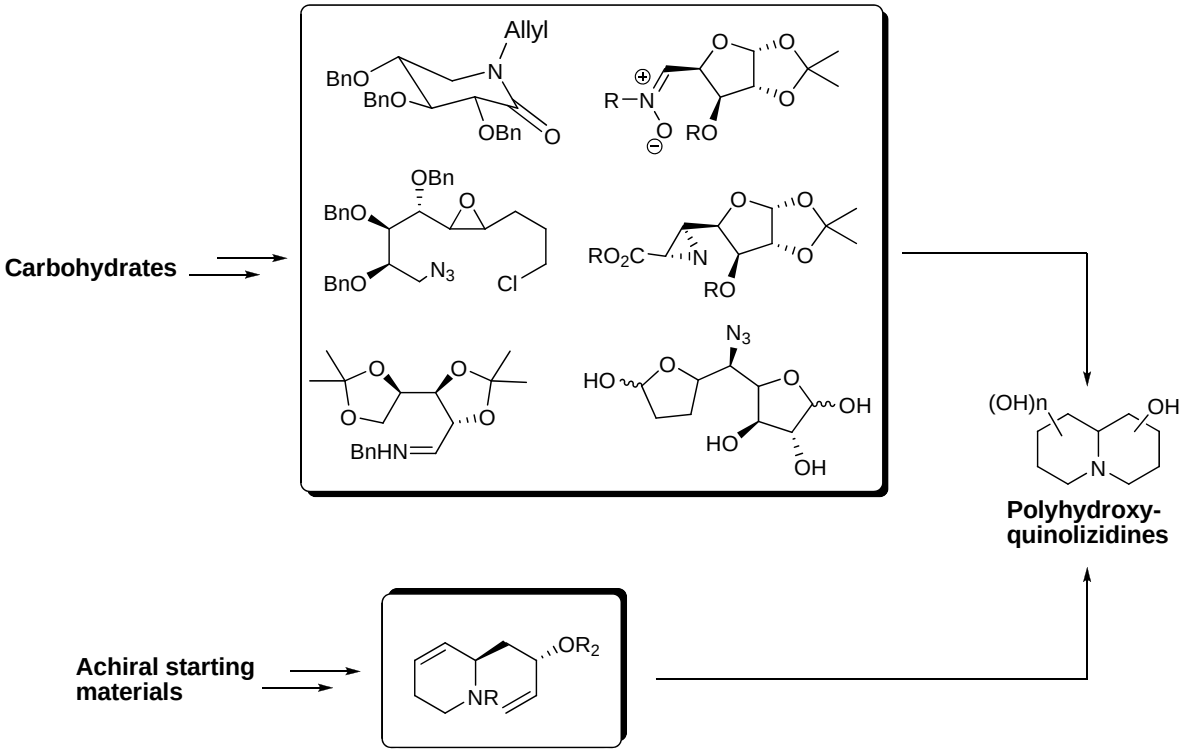


Figure 2: *Key intermediates used for the synthesis of various polyhydroxyquinolizidines.*

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Section-B:: Result and Discussion

2B.1-Methodology

From the discussion appended in the previous section, it is quite clear that though there are many synthetic approaches known in the literature towards the synthesis of polyhydxyquinolizidines; most of them end up giving only a single product starting from a single carbohydrate substrate. Therefore, a protocol which is diversity oriented and is capable of leading to a platform from where many azasugars could be synthesized is still worthwhile to explore. Furthermore, the search for new and potent inhibitors which are selective in their mode of action still constitutes a growing research area. An attractive approach to potent inhibitors is to create compounds those mimic the transition state of the enzyme-catalyzed reaction. The rationale behind this method is the belief that the transition state is likely to be the point on the reaction trajectory that has the highest degree of enzymatic stabilization. For glycosidases, it is believed that this stabilization is so huge that the transition state **1** (**Figure 1**) is bound with a dissociation constant of up to 10^{-20} M, which means that it is potentially possible to create transition-state analogue inhibitors with a *Ki* of a similar magnitude.[1]

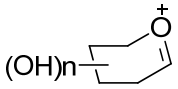

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Figure 1.

However, no inhibitor has yet been discovered that comes even closer to this value, due to the impossible task of precise mimicking of the shape and charge of the transition state. Most of the well known inhibitors operate in the range of nano molar (*i.e.* 10^{-9}) and so theoretically it is possible to develop an inhibitor which would be almost thousand billion (10^{11}) times more potent than the few of the best inhibitors known so far. Therefore, this challenging task makes this research area more interesting and significant. The possible ways to stretch out the limits and efforts of mimicking the transition state could be:

- Varying the stereochemistry on the periphery of ring.
- Varying the functionalities.
- Altering the ring size of inhibitors or
- A combination of all the above to render more suitable transition state analogues.

The current dissertation provides a combination of all the possible factors to render more suitable transition state analog. In order to provide a versatile methodology and to discover new and more potent inhibitors, we took up the program of synthesizing polyhydroxyquinolizidine (**2**) class of azasugars and evaluation of their enzyme inhibitory activities. The rationale behind the proposal to synthesize azasugars having a structural motif of polyhydroxyquinolizidines by assembling an additional saturated carbocyclic ring to the existing 1-deoxynojirimycine (**3**) ring system is that, the increased lipophilicity may lead to the interesting properties of *N*-alkyl-1-deoxynojirimycins.[2] At the same time, slight restriction in ring conformations created in the process, with structural similarity to **1**, may show the exciting properties of transition state analog glycosidase inhibitors.[3]

Towards this end, we envisioned a template **4** (**Figure 2**), in a bid to provide a versatile route for the synthesis of these azasugars. The *exo*-cyclic double bond of this structure was further visualized to provide a handle to create a multitude of functionalities. We envisaged four enantiomeric and/or diastereomeric structural motifs **5**–**8** from general template **4**. Out of these, the synthesis of precursors **5** was taken up as an immediate target. Before going into the synthetic details, it would be pertinent to append a brief discussion on the methodology utilized in the synthesis of template **5**.

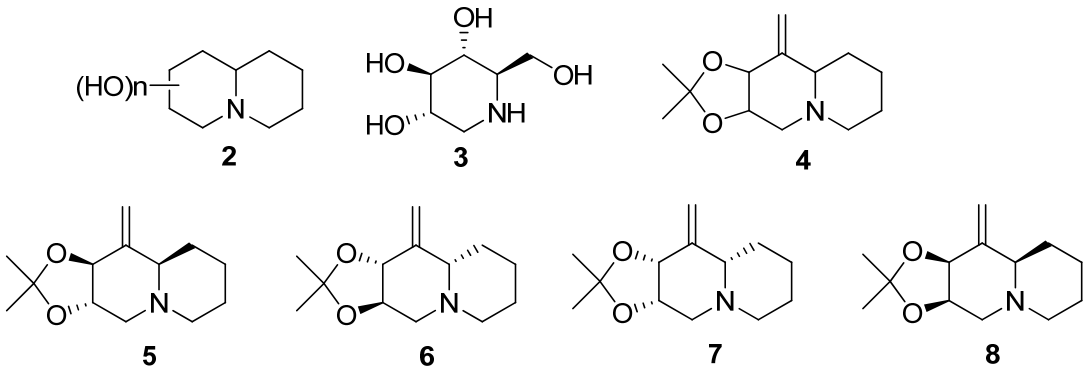
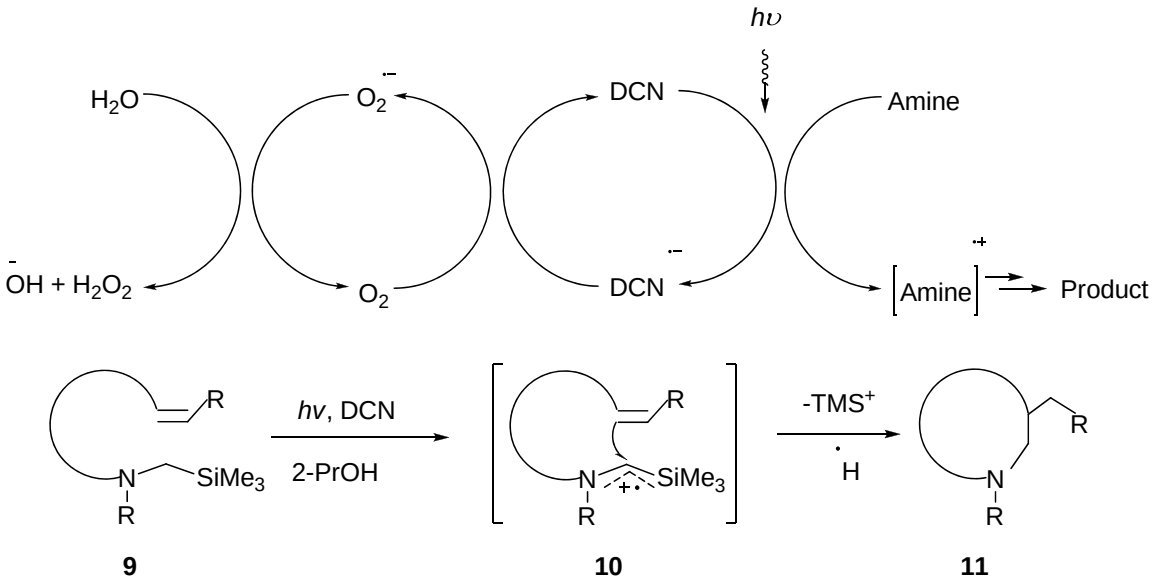


Figure 2.

2B.1.1-The concept

Radical ions generated by single electron transfer from neutral organic compounds have emerged as important intermediates in a variety of interesting chemical processes and reactions.[4] Pandey and co-workers[5] have developed a new concept for the synthesis of nitrogen heterocycles of the type **11** utilizing photoinduced electron transfer (PET)

generated α-trimethylsilylmethylamine radical cation **10** cyclization to tethered olefin, as shown in **Scheme 1**.



Scheme 1: *Mechanism for PET cyclization.*

The concept in such cyclizations involved a three centered amine radical cationic species **10**, where the radical cation is delocalized between nitrogen and silicon atom due to the vertical overlap of the filled C-Si orbital and the half vacant nitrogen orbital.[6] The photosystem to generate the reactive intermediate **10** employed 1,4-dicyanonaphthalene (DCN) as the light harvesting electron acceptor. DCN in its excited state, picks up an electron from the lone pair of nitrogen giving rise to the corresponding radical cationic species **10**. The electron from $DCN^{-\bullet}$, is finally transferred to water via oxygen. The radical cation **10** eventually leads to the formation of cyclized product **11**.

A variety of substituted pyrrolidines, piperidines, pyrrolizidines as well as indolizidines have been synthesized[5] using this methodology (**Figure 3**).

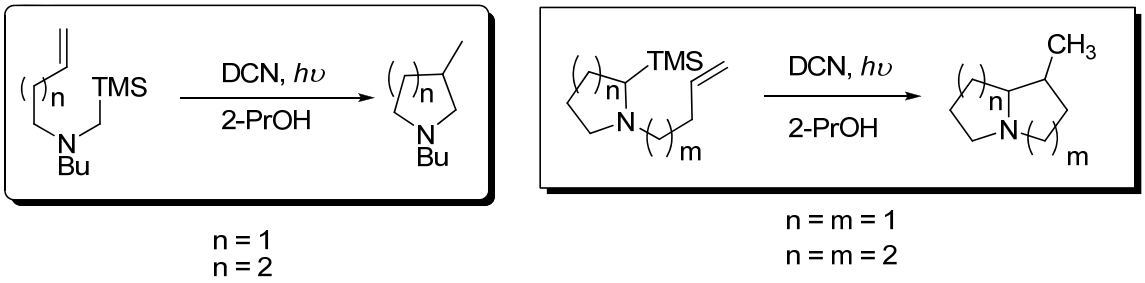
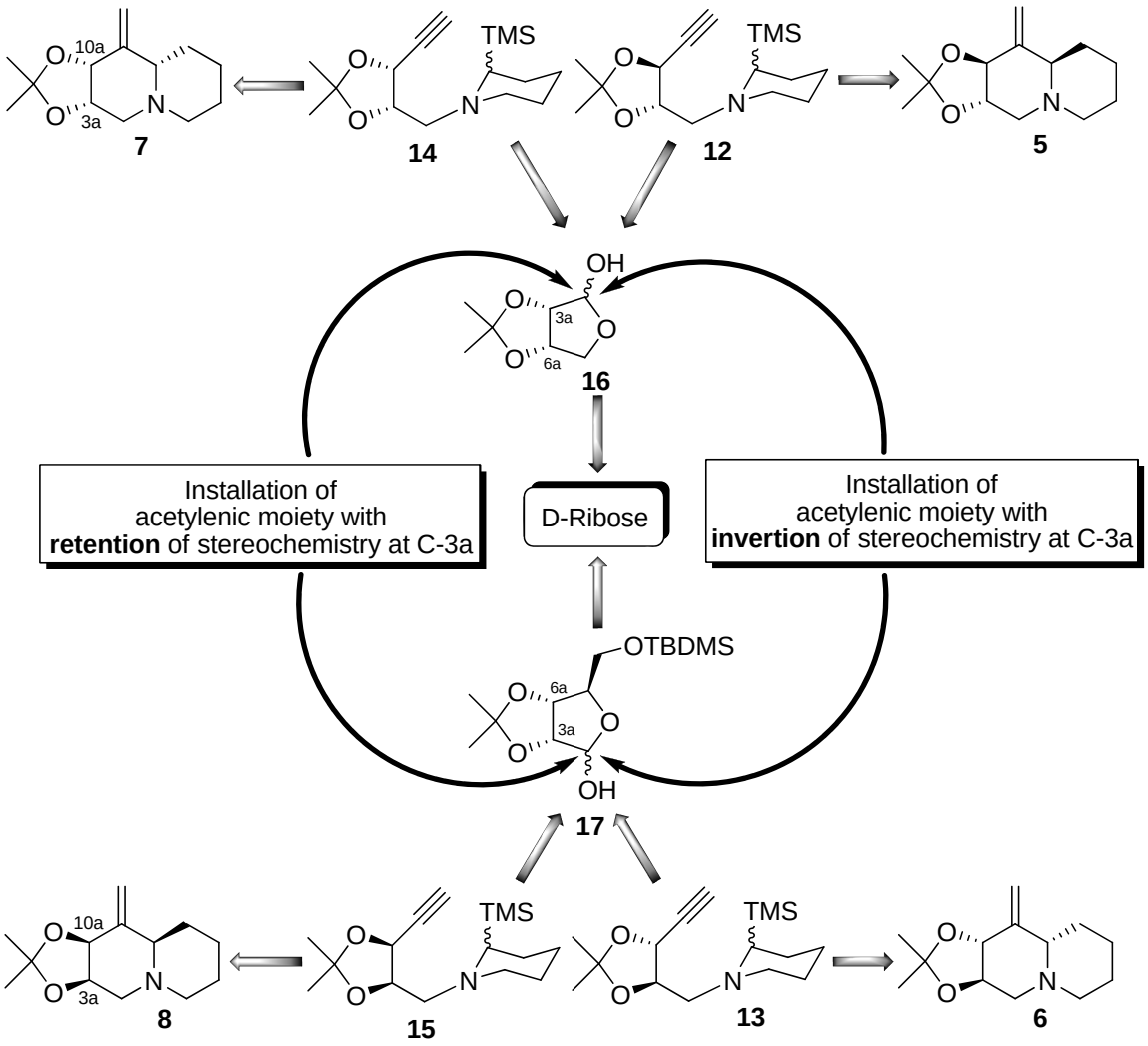


Figure 3: *Construction of various nitrogen-heterocyclics using PET cyclization.*

2B.2-Synthesis of tricyclic template 5

As per our synthetic strategy, the optically pure tricyclic templates **5**, **6**, **7** and **8** were designed to be obtained from acetylene tethered amines **12, 13**, **14** and **15**, respectively, utilizing aforesaid protocol (**Scheme 2**).



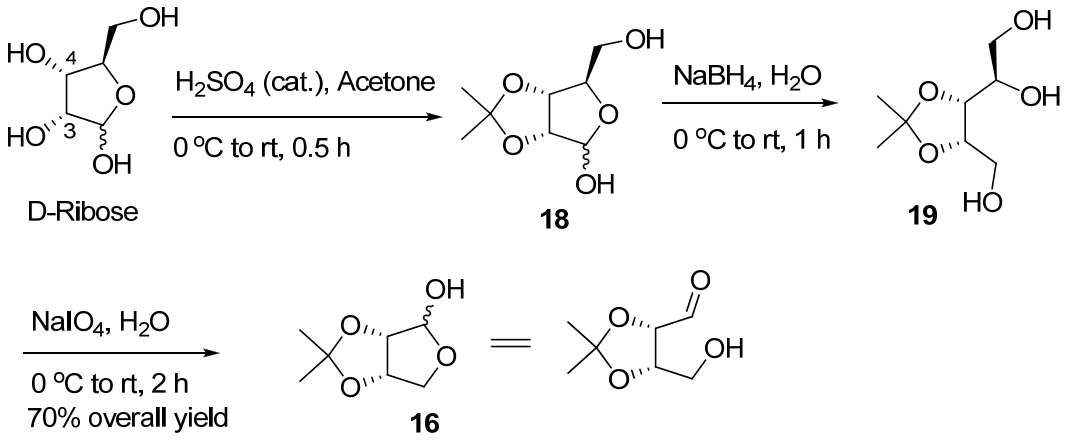
Scheme 2: *Retrosynthetic plan for various tricyclic templates.*

The amines, **12, 13**, **14** and **15** were planned to be obtained from the reductive aminaion of aldehydes having required stereochemistry with 2-(trimethylsilyl)piperidine. All the aldehydes required for reductive amination were proposed to be obtained from common starting material D-ribose through **16** and **17**. Substrate 2-(trimethylsilyl)piperidine in turn was envisaged to be synthesized from piperidine. Thus, the first task in our hand was to

synthesize the synthons necessary for reductive amination, which is described in the following segment.

2B.2.1-Synthesis of (4*R*,5*S*)-5-ethynyl-2,2-dimethyl-1,3-dioxolane-4-carb-aldehyde (26)

Towards synthesizing **26**, we designed our synthetic strategy starting with D-ribose as shown in **Scheme 3**. At first, C-3 and C-4 hydroxy functionalities of D-ribose were protected as acetonide by stirring its suspension in acetone with a few drops of H_2SO_4 for half an hour. Compound **18**, thus, formed was carried forward as such without any purification. Since, a hydroxyl moiety was required at the place of carbonyl in **18** at the later part of our proposed synthetic plan, **18** was reduced using $NaBH_4$ in water to obtain corresponding triol **19**.

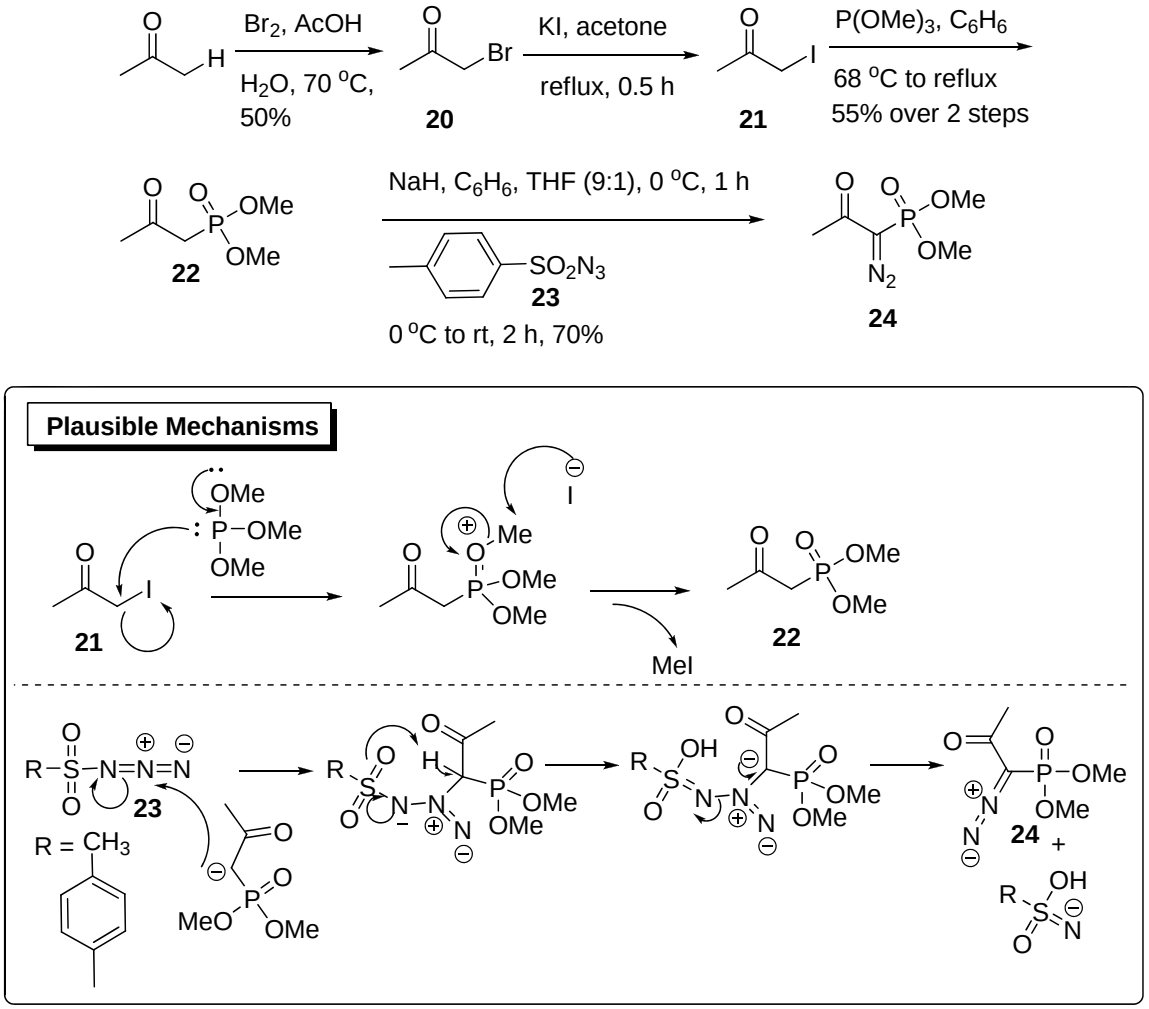


Scheme 3: *Synthesis of* ***16****.*

The oxidative cleavage of **19** using $NaIO_4$ gave required lactol **16**. It may be important to mention that all the three steps used in obtaining **16** through above reaction sequence, were carried out in a single pot without isolating any of the intermediates **18** and/or **19**. This strategy did not require any column chromatography except for the purification of **16**. The overall yield, for these three steps, was found to be 70%. The spectral data of **16** was in complete agreement with the one reported in the literature.[7]

In order to transform the masked aldehydic functionality of **16** to acetylene, we exploited a strategy using Bestmann-Ohira reagent **24**.[8] Although, this reagent is commercially available, it can also be prepared in large scale in the laboratory and can be stored over long

period of time at room temperature without any decomposition. The reagent **24** was prepared from acetone as shown in **Scheme 4**.



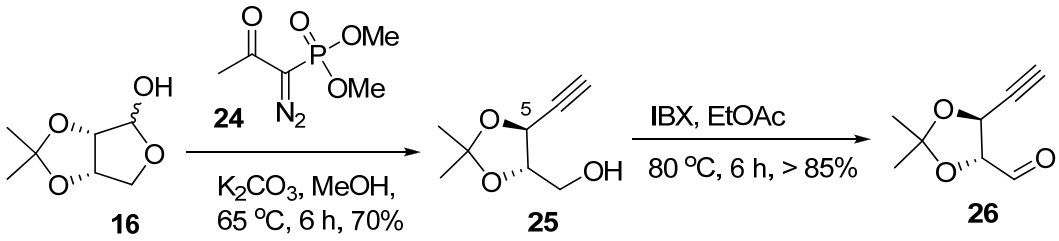
Scheme 4: *Synthesis of **24**.*

In the pursuance of the synthesis of **24**, initially bromoacetone[9a] was prepared by brominating acetone in acetic acid by adding bromine to a stirring solution of water, acetone and acetic acid while maintaining the temperature of the reaction mixture at 65–70 °C. Work up and fractional distillation (37–48 °C, 13 mm) of the reaction mixture gave bromoacetone in 50% yield. Bromoacetone **20** was transformed to iodoacetone **21** by refluxing with potassium iodide in acetone for 30 min.[a] The iodoacetone being highly unstable was carried forward as such without any purification. A solution of iodoacetone in benzene was refluxed with dropwise addition of trimethylphosphite and simultaneous

a *Since, bromoacetone and iodoacetone are highly lachrymatory, the reaction should be handled with extreme precaution in the efficient vacuum hood.*

distillation of methyl iodide (formed in situ). The crude reacrion mixture was distilled to get **22** in 55% yield (119–123 °C, 11 mm). Reaction of **22** with NaH in benzene–THF mixture (9:1) at 0 °C followed by subsequent quenching with tosyl azide[10] (**23**) (prepared in quantitative yield by treating a solution of tosyl chloride with NaN_3) gave **24**[8] in 70% yield. A plausible mechanism of the reaction is outlined in **Scheme 4**.

To a methanolic solution of **16** containing potassium carbonate, was added a solution of **24** dropwise while refluxing over a period of 6 h. Usual work up and purification gave **25** in good yield (70%)[11] (**Scheme 5**) which was characterized by IR spectroscopy by observing bands for acetylenic functionality at 3284 cm^{-1} and 2121 cm^{-1}, respectively. ^1H NMR spectrum also revealed acetylenic proton at 2.53 ppm (d, J = 2.15 Hz). Final confirmation of the formation of **25** came from its mass spectrum [*m/z* 157 (M^+ + H), 179 (M^+ + Na)].



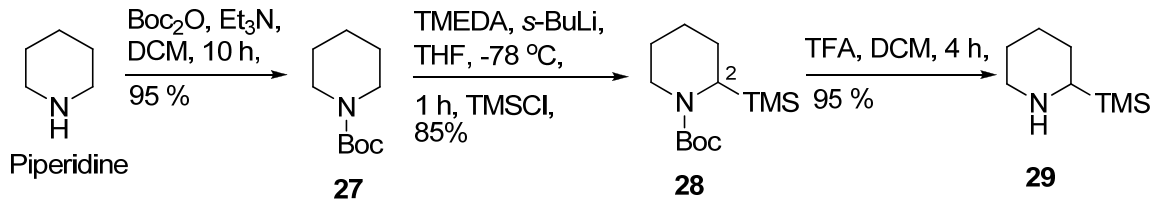
Scheme 5: *Synthesis of **26**.*

All the spectroscopic data along with optical rotation of **25** were also found to be in good agreement with the reported values.[12]

IBX oxidation[13] of **25** in ethyl acetate at 80 °C provided required aldehyde **26** in more than 85% yield (monitored by GC). Our next objective was to synthesize the amine fragment **29**, required for the reductive amination of aldehyde **26**. The synthesis of amine **29** was achieved as follows using the protocol developed in our laboratory.[14]

2B.2.2-Synthesis of 2-(trimethylsilyl)piperidine (29)

The synthesis of **29** started with the commercially available piperidine by following the steps as summarized in **Scheme 6**. The *N*-Boc protection of piperidine, carried out by stirring with $(Boc)_2O$ in the presence of TEA in anhydrous DCM, gave **27** in 95% yield. Amine **27** on α-metalation using *s*-BuLi / TMEDA in THF at −78 °C followed by the addition of the trimethylsilyl chloride provided **28** in 85% yield.



Scheme 6: *Synthesis of* ***62****.*

The ^{1}H NMR of **28** displayed a singlet at δ −0.07 integrating for nine protons indicating the introduction of TMS moiety in the piperidine ring. The nine protons appearing as a singlet at δ 1.43 represented the protons from the *N*-Boc moiety. The multiplets between δ 1.49–1.75 integrating for six protons were assigned to the protons on C-3, C-4 and C-5. Another multiplet between δ 2.53–2.99 integrating for one proton was characterized as H-2 proton, whereas the two H-6 protons appeared as a multiplet between δ 3.51–4.25.

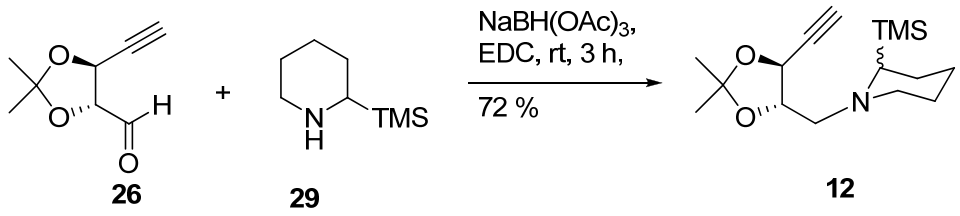
The ^{13}C spectrum displayed total nine signals, which were assigned as follows:
The methyl carbons on TMS group appeared at δ −0.8, whereas the methyl carbons of Boc moiety were found at δ 26.5. The methylenes C-3, C-4 and C-5 were observed at δ 22.5, 23.3 and 25.9, respectively. C-2 was located at δ 44.3 whereas the C-6 was found at δ 44.9. The signals at δ 78.8 and 154.9 were assigned to the quaternary carbons of tertiarybutyl and carbonyl group of Boc moiety, respectively.

The molecular ion peak was observed at *m/z* 258 (M^{+}+H) in the mass spectrum.

The deprotection of Boc moiety of **28** by TFA in anhydrous DCM afforded free amine **29** in 95% yield as a yellow liquid. The ^{1}H NMR and ^{13}C NMR spectra clearly indicated the absence of the protons and carbons corresponding to Boc moiety.

2B.2.3-Synthesis of 1-(((4*S*,5*S*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)-methyl)-2-(trimethylsilyl)piperidine (31)

Having both aldehyde **26** and amine **29** in hand, we proceeded further to synthesize the precursor for PET cyclization. In this context, reductive amination[15] of aldehyde **26** with amine **29** in the presence of sodium triacetoxyborohydride provided **12** in 72% yield (**Scheme 7**).



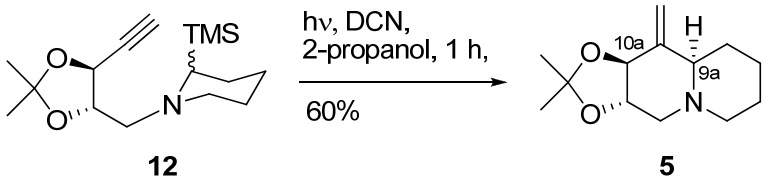
Scheme 7: *Synthesis of* ***31****.*

Based on the presence of two singlets for TMS group at δ 0.6 and 0.7 (nine protons), as well as four singlets at δ 1.40, 1.41, 1.46 and 1.46 (six protons), for acetonide protons of equal intensities, the product was characterized to be 1:1 diastereomeric mixture.

With the acetylene tethered α-trimethylsilylamine **12** in hand, the stage is now set for the crucial PET cyclization reaction.

2B.2.4-Synthesis of (3a*S*,9a*R*,10a*S*)-2,2-dimethyl-10-methyleneoctahydro-3aH-[1,3] dioxol o [4,5-b]quinolizine (5)

PET promoted cyclization[5] of **12** (1 g, 3.38 mmol) was carried out by irradiating its solution in 2-propanol (200 mL) containing catalytic amount of DCN (0.12 g, 0.67 mmol) (prepared by following the reported procedure[16]) in an open vessel using a 450-W Hanovia medium pressure mercury vapor lamp, kept in a pyrex vessel, as the light source. Usual work up and purification of the crude photolysate by column chromatography afforded D-*threo* tricyclic template **5** (0.453 g) in 60% yield (**Scheme 8**).



Scheme 8: *PET cyclization of* ***12****.*

The initial support for the formation of cyclized product **5** came by observing two broad singlets at δ 4.89 and 5.06, integrating for one proton each, for the olefinic moiety which was subsequently characterized by extensive analyses of NMR [^{1}H, ^{13}C, DEPT, COSY, NOESY and HETCOR], IR and mass spectroscopic data.

The stereochemistry of newly generated chiral centre C-9a is suggested based on the observation of the diagnostic cross peak in the NOESY spectum of **5** (**page no. 112**) between H-9a and H-10a (**Figure 4**).

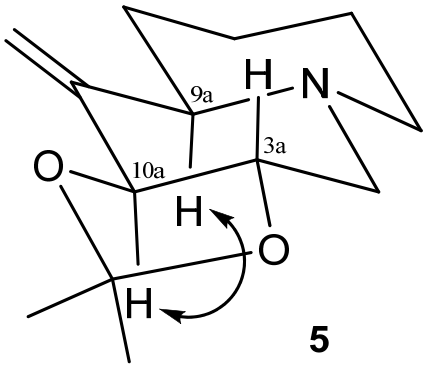


Figure 4: *nOe co-relation of compound* ***5****.*

The merit of this strategy may be highlighted considering the fact that it not only leads to the ring formation and gives the cyclized product but also introduces a suitably juxtaposed exocyclic double bond on a tricyclic framework. The double bond can be used precisely for generating different functionalities by simple functional group transformations. The chirality of α-carbons would govern the direction of the incoming functionality and the tricyclic nature would give the precise stereocontrol.

Successful implementation of our methodology in the synthesis of tricyclic enantiomeric template **5** encouraged us to synthesize various azasugars (**30**–**36**) as mentioned in **Figure 5**.

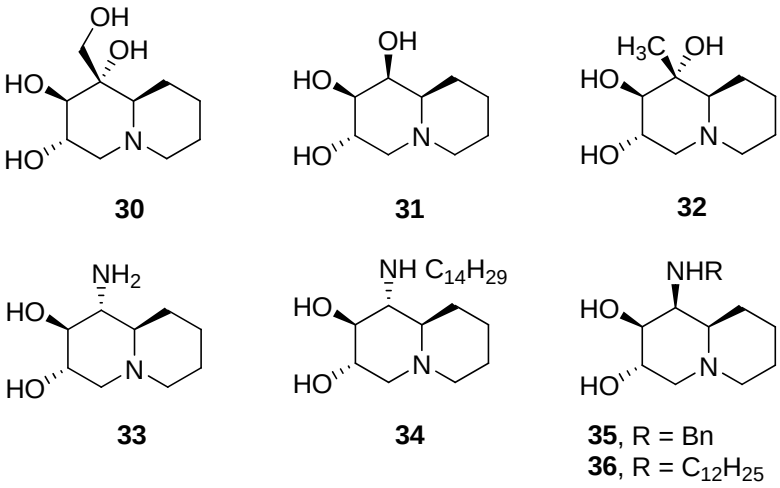


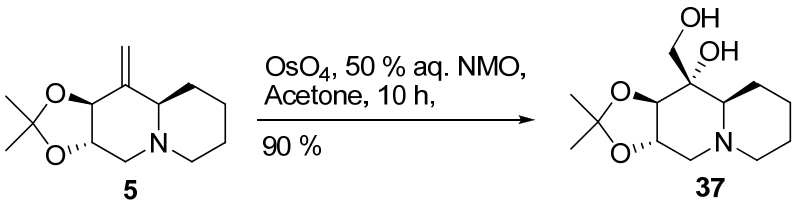
Figure 5: *Targeted quinolizidine alkaloids for synthesis.*

The synthetic potential of **5** was explored through simple chemical transformations leading to the quinolizidine alkaloids which are summarized as follows:

2B.3-Synthesis of polyhydroxyquinolizidine alkaloids (30, 31 and 32)

2B.3.1-Synthesis of 1-deoxy-8-hydroxymethylhomocastanospermine (30)

The synthesis of polyhydroxyquinolizidine alkaloids were started by the functionalization of olefinic moiety of **5**. To begin with, we performed OsO_4 mediated catalytic dihydroxylation of **5** using NMO as the oxidant, which yielded **37** as a single diastereomer, in the form of a colorless crystalline solid (mp 135–136 °C) (**Scheme 9**).



Scheme 9: *Dihydroxylation of **5**.*

The IR spectrum displayed a broad peak at 3432 cm^{-1} responsible for hydroxyl functionality. The ^1H NMR spectrum did not show any signal corresponding to olefinic protons. The mass spectrum also showed a molecular ion peak at *m/z* 258 (M^++H) suggesting the formation of **37**. Finally, the stereochemical outcome of dihydroxylation was confirmed by the single crystal XRD analysis (**Figure 6**).[b]

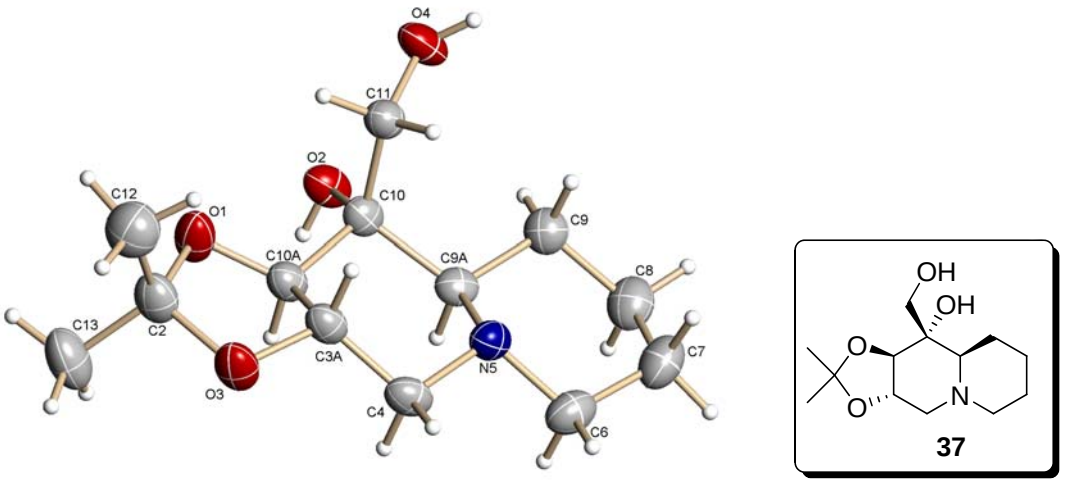
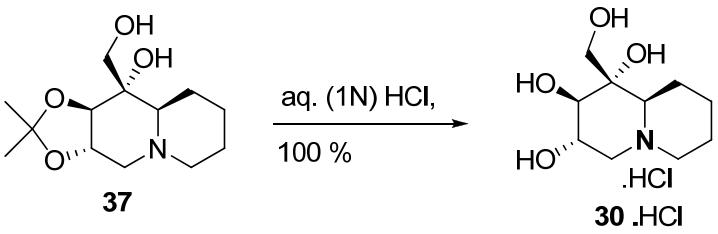


Figure 6: *ORTEP Diagram of **37** (Ellipsoids are drawn at 50% probability).* [b]

b *CCDC numbers for compounds **37** is 726212. X-ray crystal structure analysis of compound **37** is given in experimental section.*

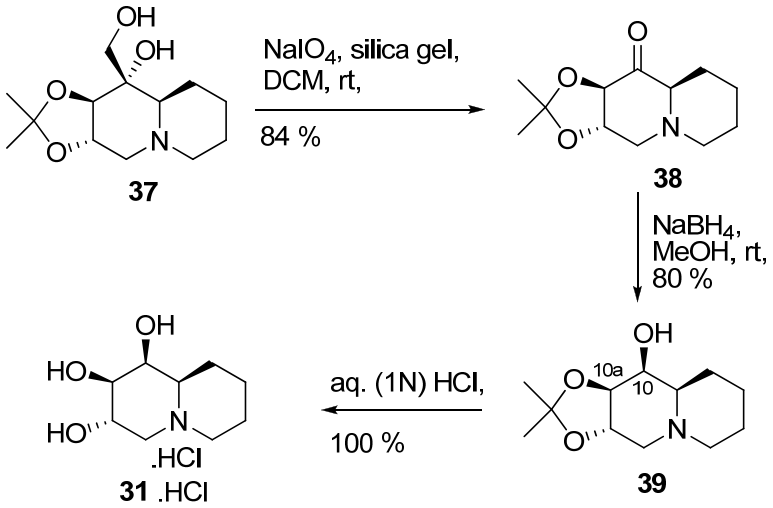
It was envisaged that, simple removal of acetonide group from diol **37** would provide a new molecule having all structural features of an azasugar. Thus, **37** upon acetonide deprotection using aq. (1N) HCl gave 1-deoxy-8-hydroxymethylhomocastanospermine (**30**) in quantitative yield, as its hydrochloride salt (**Scheme 10**). The ^{1}H NMR spectrum revealed the disappearance of acetonide protons. Mass spectrum showed molecular ion peak at *m/z* 218 (M^{+}+H) proving the formation of **30**.



Scheme 10: *Synthesis of* ***30****.*

2B.3.2-Synthesis of (1*S*,2*R*,3*S*,9a*R*)-octahydro-1H-quinolizine-1,2,3-triol (31)

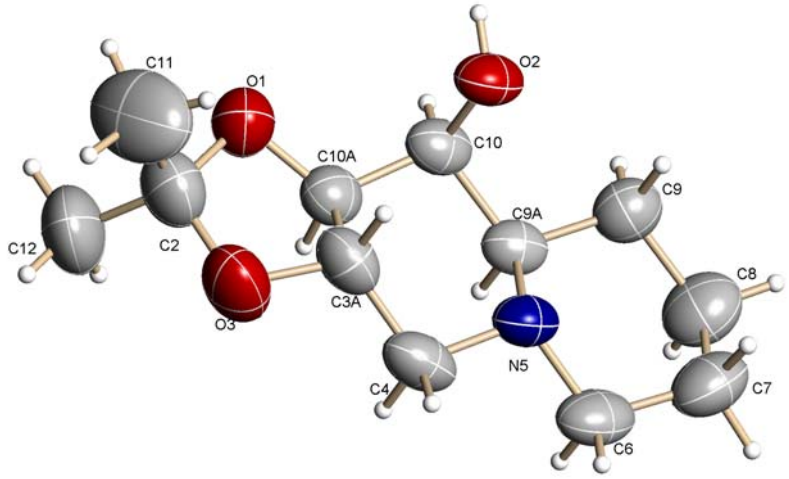
Another new analog **31** was visualized to be easily affordable from diol **37** (**Scheme 11**). Towards this endeavor, diol **37** was subjected to oxidative cleavage, using sodium periodate adsorbed on silica gel, which afforded corresponding ketone **38** as a white solid (mp 113–114 °C). The IR spectrum of **38** displayed absorption peak at 1750 cm^{-1}, accountable for carbonyl functionality. The molecular ion peak appearing at *m/z* 226 (M^{+}+H) in the mass spectra further confirmed the formation of the required compound.



Scheme 11: *Synthesis of* ***31****.*

The ketone **38** upon sodium borohydride reduction in methanol afforded **39** as a single diastereomer in the form of a colorless crystalline solid (mp 133–134 °C).

The stereochemistry of H-10 (3.96, dd, J = 2.01, 1.87 Hz) in **39** was deduced based on its coupling constant with H-10a (3.29, dd, J = 9.28, 2.01 Hz). The low values for the coupling constants of H-10 suggested its equatorial orientation and hence, *cis* spatial relationship with H-10a. The relative stereochemistries between various centers were also confirmed by NOESY spectrum (**page no. 120**). The mass spectrum gave the molecular ion peak at *m/z* 228 (M^++H). The structure of **39** was unambiguously confirmed by the single crystal XRD analysis (**Figure 7**).



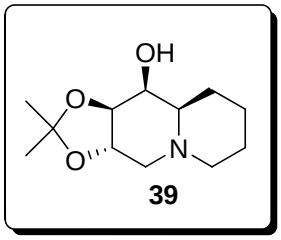


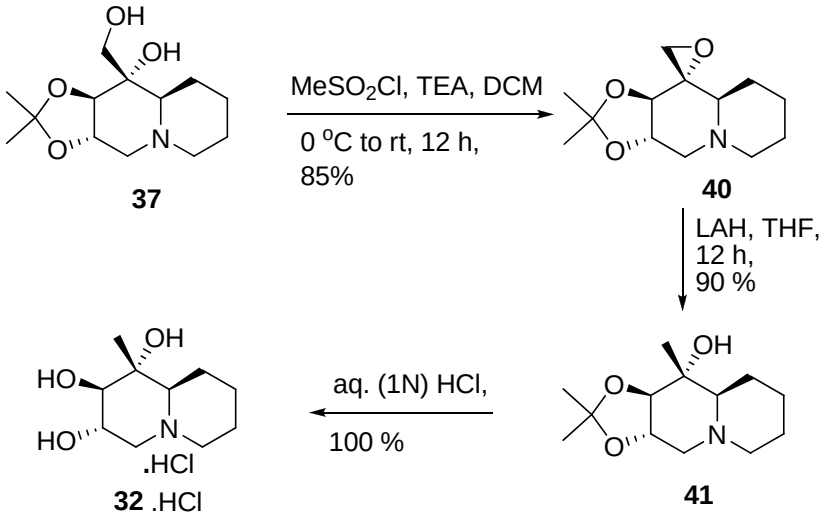
Figure 7: *ORTEP Diagram of* ***39*** *(Ellipsoids are drawn at 50% probability).* [c]

The acetonide moiety of **39** was removed as usual to provide the hydrochloride salt of azasugar **31** [*m/z* 188 (M^++H)].

2B.3.3-Synthesis of 1-deoxy-8-methylhomocastanospermine (32)

In continuation, another polyhydroxy azasugar 1-deoxy-8-methylhomocastanospermine **(32)** was envisaged from diol **37**, whose synthesis is shown in **Scheme 12**. Epoxide **40** was synthesized via monomesylation of diol **37** by using two equivalent of Et_3N in anhydrous DCM. The IR spectrum exhibited absorption peak at 3054 accountable for C-O stretching frequency of epoxide ring. The molecular ion peak appeared at *m/z* 240 (M^++H) confirming the formation of compound **40**.

c *CCDC numbers for compounds* ***39*** *is 726211. X-ray crystal structure analysis of compound* ***39*** *is given in experimental section.*



Scheme 12: *Synthesis of* ***19****.*

The regioselective reduction of oxirane ring of **40** provided **41**. The appearance of a singlet at δ 1.23, integrating for three protons suggested it to be of methyl group, formed by the regioselective epoxide opening. Mass spectrum revealed the molecular ion peak at *m/z* 242 (M^++H). The acetonide group of **41** was removed in regular manner to obtain 1-deoxy-8-methylhomocastanospermine **32** [*m/z* 202 (M^++H)], as its hydrochloride salt.

The effort, as outlined above, completed the successful synthesis of three polyhydroxy-quinolizidine azasugars.

2B.4-Synthesis of C_{10} amine analogs of 39 (33, 34, 35 and 36)

Siastatin B[17a–c] (**42**), a naturally occurring β-glucuronidase inhibitor (IC_{50} = 16 μM) has more than one nitrogen. Similarly, other azasugars consisting of more than one nitrogen such as **43**[1,17d–e] (IC_{50} = 0.48 μM against Bovine kidney α-L-fucosidase), **44**[1, 17d–e] (IC_{50} = 0.016 μM against same enzyme), and **45**[1, 17f] (IC_{50} = 4.7 μM against Bovine kidney β-glucuronidase) are also found to be potent inhibitors of glycosidases[17f] (**Figure 8**).

We have also observed previously that additional amine functionality in **46** (*Ki* 36.8 μM and 37.3 μM) results in approximately 30 fold increase in inhibitory activity against both α-glucosidase and α-mannosidase (A. fischeri) than its hydroxyl analog **47** (*Ki* 33%, and no inhibition at 1 mM).[12] Encouraged by these facts, we got interested to see the effect of an additional basic site in these synthesized quinolizidine class of azasugars. Towards fulfilling

above goal, we embarked upon the synthesis of various dibasic azasugars as delineated in this section as follows:

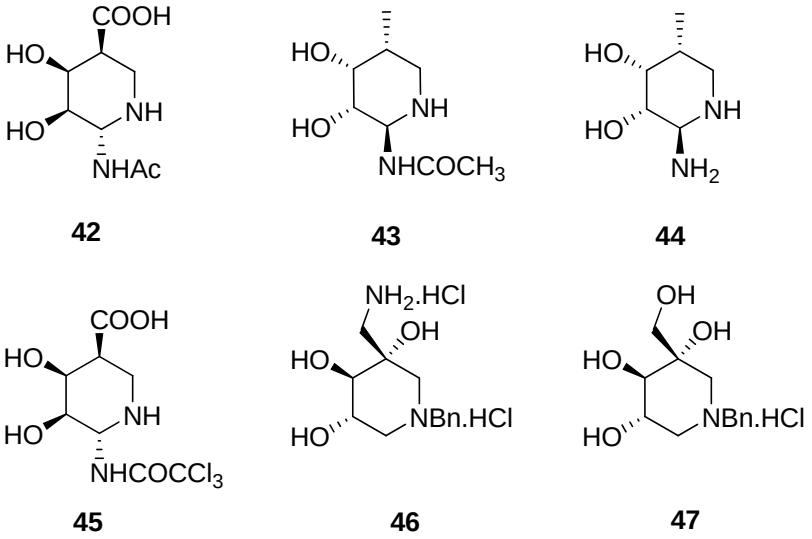


Figure 8: *Dibasic azasugars as potent glycosidase inhibitors.*

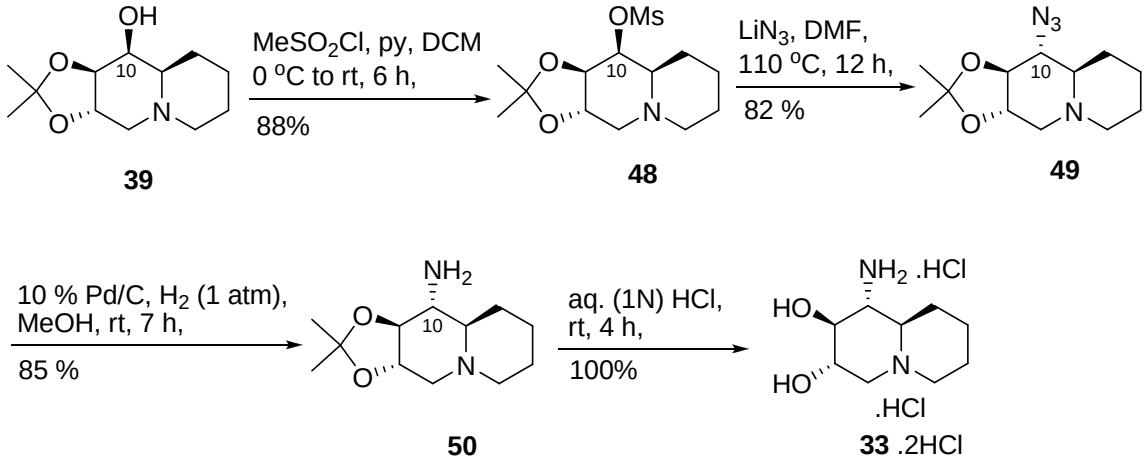
2B.4.1-Synthesis of (1*R*,2*S*,3*S*,9a*R*)-1-aminooctahydro-1H-quinolizine-2,3-diol (33)

At first, we envisaged the amine analog **33** through replacing the C-10 hydroxy moiety of **39** by amine functionality. This was accomplished by following the steps as elaborated in **Scheme 13**.

Alcohol **39** was converted to corresponding mesylate ester **48** with mesyl chloride using pyridine as a base in anhydrous DCM. The ^{1}H NMR spectrum of **48** displayed a singlet at δ 3.12 for the three protons of mesylate group indicating for the required transformation. The mass spectrum revealed the molecular ion peak at *m/z* 306 (M^++H) confirming the formation of **48**.

The S_N2 displacement of mesylate group of **48** with LiN_3 in DMF at 110 °C produced corresponding azide **49**, which was characterized by the appearance of a medium absorbance band of azide functionality at 2208 cm^{-1} in IR spectrum. The confirmation of the formation of **49** came from the mass spectrum which showed the molecular ion peak at *m/z* 253 (M^++H). The azide group was converted to amine functionality by catalytic hydrogenation using 10% Pd/C to obtain **50** as a white solid. IR spectrum displayed a broad peak at 3396 cm^{-1} confirming the formation of amine functionality. H-10 was shifted to

further upfield (between δ 3.20–3.28 for **49** in comparison to δ 2.66 for **50**) supporting the transformation of azide to amine. The molecular ion peak was found in mass spectrum at *m/z* 227 (M^++H), which established the formation of amine **50**.



Scheme 13: *Synthesis of* ***33****.*

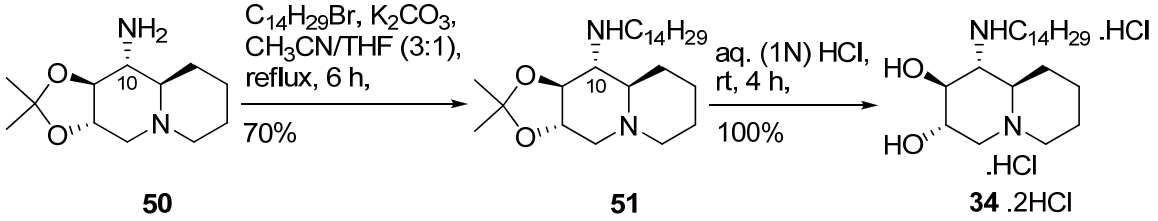
The inversion of stereocenter at C-10 by S_N2 displacement of the mesylate by azide was suggested based on the analysis of coupling constants of H-10 (2.66, dd, *J* = 10.09, 8.53 Hz) and H-10a (3.10, dd, *J* = 10.09, 9.07 Hz) from the ^{1}H NMR spectrum of **50** (in case of **39** this coupling constant was 2.01 Hz).

The removal of the acetonide moiety as usual provided the dihydrochloride salt of azasugar **33** [*m/z* 187 (M^++H)].

2B.4.2-Synthesis of (1*R*,2*S*,3*S*,9a*R*)-1-(tetradecylamino)octahydro-1H-quinolizine-2,3-diol (34)

Based on our previous research regarding structure activity relationship among glycosidase inhibitors,[12] we intended to increase the lipophilicity of primary amine functionality by attaching a long hydrocarbon chain[18] and evaluate its role in enzyme inhibition.

Towards this end, *N*-alkylation of **50** was carried out by refluxing with tetradecyl bromide in the presence of K_2CO_3 in 3:1 mixture of CH_3CN and THF which furnished corresponding alkylated product **51** (**Scheme 14**).



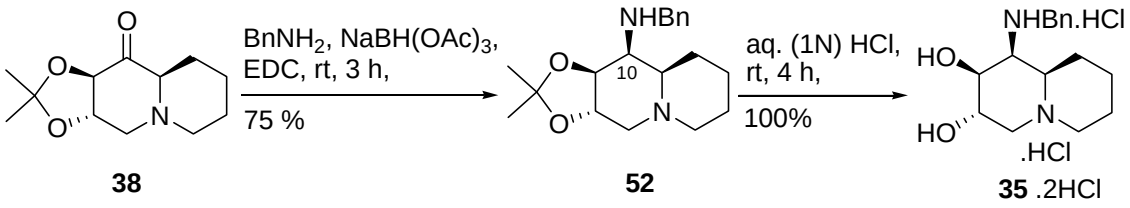
Scheme 14: *Synthesis of **34**.*

The triplet at δ 0.86 (J = 6.79 Hz) in the ^{1}H NMR of **51** was assigned to the methyl group of long carbon chain, whereas the broad singlet at δ 1.24 integrating for twentyfour protons represented the methylene protons of long hydrocarbon chain except those α to nitrogen, indicating the success of monoalkylation reaction. Further, the H-10 axial proton of **51** appeared as a triplet at a lower δ value (2.46) in comparison to that of **50** (2.66), proving that the *N*-alkylation of amine functionality has taken place. The mass spectrum displayed the molecular ion peak at *m/z* 423 (M^++H) confirming the formation of **51**. Removal of the acetonide moiety from **51** provided the azasugar **34** [*m/z* 383 (M^++H)], as its dihydrochloride salt, in quantitative yield.

2B.4.3-Synthesis of (1*S*,2*S*,3*S*,9a*R*)-1-(benzylamino)octahydro-1H-quinolizine-2,3-diol (35)

Since, it was not possible at this stage to predict which diastereomer will be the most biologically active, we felt this would be advantageous to make two other new amine analogs **35** and **36** for biological screening.

In this regard, ketone **38** upon reductive amination with benzyl amine produced corresponding amine derivative **52** as a single diastereomer in the form of a faint yellow solid (**Scheme 15**).



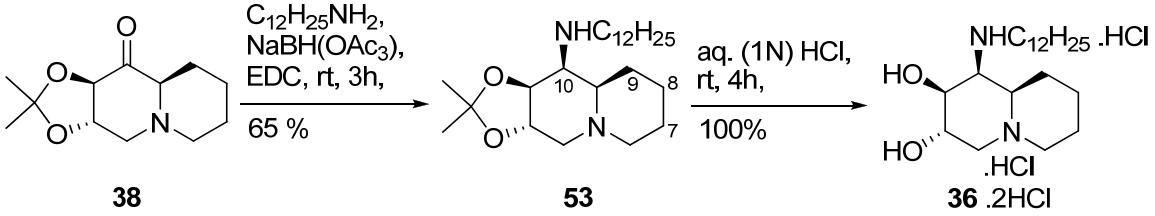
Scheme 15: *Synthesis of **35**.*

The stereochemistry of **52** at C-10 (the newly generated stereocentre) was ascertained by analyzing coupling constants for H-10 (δ 3.04, t, J = 2.91 Hz) establishing its equatorial orientation. This stereochemical outcome was further confirmed from NOESY spectrum

(**page no. 137**). The molecular ion peak in the mass spectrum at *m/z* 317 (M^++H) further confirmed the formation of **52**. Amine **52** upon acetonide deprotection following routine procedure provided dihydrochloride salt of azasugar **35** [*m/z* 277 (M^++H)], in quantitative yield.

2B.4.4- Synthesis of (1*S*,2*S*,3*S*,9a*R*)-1-(dodecylamino)octahydro-1H-quinolizine-2,3-diol (36)

In continuation, another diastereomeric amine analog **53** containing a lipophilic long hydrocarbon chain was synthesized by the reductive amination of ketone **38** with dodecyl amine (**Scheme 16**).



Scheme 16: *Synthesis of* ***36****.*

The diagnostic peaks in the ^{1}H NMR of **53** were as follows: The triplet at δ 0.86 (*J* = 6.84 Hz) accounted for the methyl group of long hydrocarbon chain. The multiplets between δ 1.24–1.27 integrating for twentyone protons accounted for twenty protons from the methylene carbons on long hydrocarbon chain excluding those two protons α to nitrogen. The mass spectrum displayed the molecular ion peak at *m/z* 395 (M^++H), confirming the formation of **53**. The acetonide moiety was removed quantitatively as usual to obtain azasugar **36** [*m/z* 355 (M^++H)] in the form of its dihydrochloride salt.

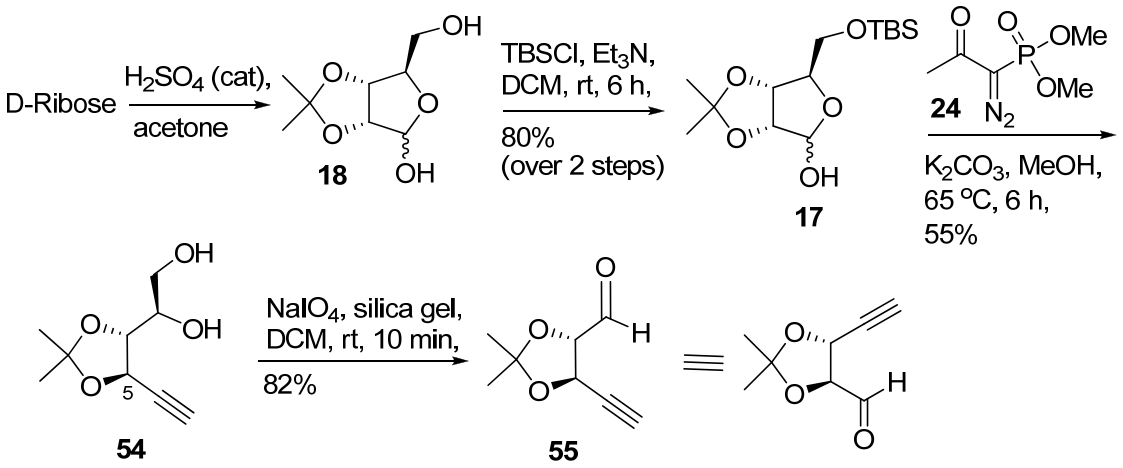
2B.5- Synthesis of D-Gluco type quinolizidine class of azasugars 56-62

2B.5.1- Synthesis of enantiomeric aldehyde 55

The success of our above described protocol for synthesizing various azasugars led us to extend this strategy to synthesize their enantiomers. In order to obtain the enantiomers, we had to just use the enantiomeric aldehyde **55** as discussed earlier in **Scheme 3**. The synthesis of aldehyde **55** required only the change in the sequence of reactions (with slight modification) compared to the one used in the synthesis of aldehyde **26** (**Scheme 3** and **5)**.

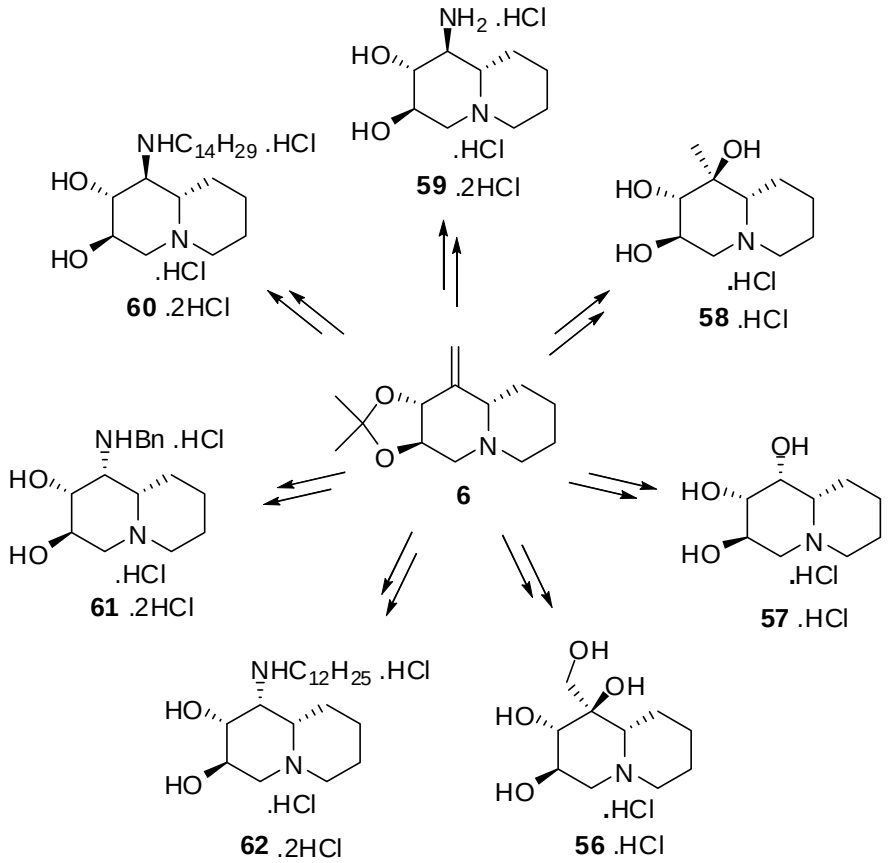
The reaction involving construction of acetylenic moiety using Bestmann-Ohira reagent on **17**[19] proceeded smoothly, producing required product **54** (**Scheme 17**). It was pleasing to

note that the silyl protecting group got knocked off during the acetylenation step itself due to strong basic reaction condition. The diol moiety of **54** upon oxidative cleavage with sodium periodate afforded corresponding aldehyde **55**.



Scheme 17: *Synthesis of **55**.*

2B.5.2-Synthesis of azasugars 56–62



Scheme 18: *Synthesis of **56–62**.*

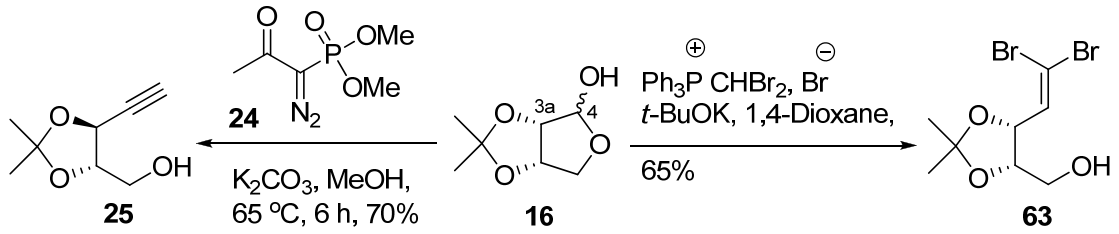
The common template **6** was synthesized from aldehyde **55**, following identical procedure as described in the synthesis of **5**. The polyhydroxy azasugars **56**–**58** were synthesized from **6**, using the identical reaction sequences as described earlier during the synthesis of **30**-**32**, respectively, where as the synthesis of the dibasic analogs **59**–**62** were achieved by following similar reaction sequences utilized in the synthesis of **33**–**36**, respectively (**Scheme 18)**.

2B.6-Attempt towards the Synthesis of L- and D-manno type quinolizidine alkaloids

After successful snthesis of L- and D-gluco type polyhydroxyquonolizidine alkaloids, we got interested in the synthesis of two other series i.e., L- and D-manno type polyhydroxyquonolizidine alkaloids and evaluate their potency as glycosidase inhibitors from template **7** and **8**, respectively (**Scheme 3)**. This exploratory work of synthesizing these diastereimeric analogs could also be advantageous for better understanding of the structural requirements for glycosidase inhibitor and to develop more potent, selective and less toxic drugs.

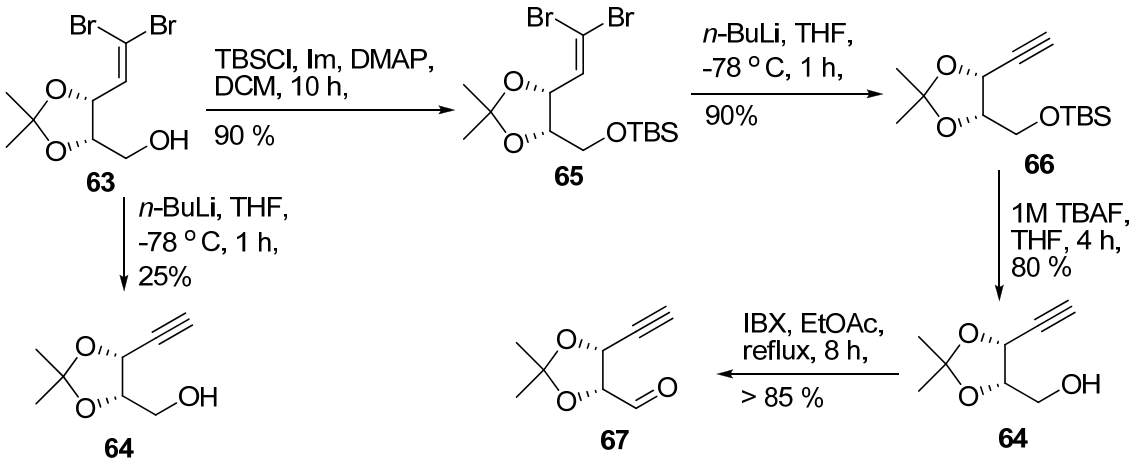
2B.6.1-Synthesis of (4R,5R)-5-ethynyl-2,2-dimethyl-1,3-dioxolane-4-carb-aldehyde (67)

In the two templates **7** and **8**, one can see *cis* relative stereochemistry of hydroxyl groups (acetonide protected) at C-3a and C-10a (**Scheme 3**), which require aldehydes **67** and *ent*-**67**, respectively. Thus, we initiated a synthetic itinerary for these two series with the synthesis of *cis* acetylene alcohols **64** and its enantiomer. Our previous effort for making alcohol **64** via installing an acetylene moiety on C-4 of lactol **16** using Bestmann-Ohira reagent resulted inversion of stereochemistry at C-3a producing *trans* acetylene alcohol **25**. The reason for this inversion was the strongly basic condition in conjunction with the protic media involved during the course of reaction. This prompted us to use milder Corey-Fuchs protocol[20] using carbon tetrabromide and triphenylphosphene in DCM for this transformation. To our dismay, the reaction gave a complex reaction mixture, probably due to the cleavage of acetonide moiety in acidic condition. However, the lactol was converted to the dibromo-olefin **63** using basic Lievre protocol (**Scheme 19**).[21]



Scheme 19: *Synthesis of dibromo-olefin (**63**).*

The direct exposure of the dibromo compound **(63)** to *n*-BuLi for conversion to the corresponding acetylene resulted in the product **64** with a lower yield, presumably due to the presence of free hydroxyl moiety. Hence, the free hydroxyl moiety was protected as silyl ether **65** with TBSCl, which upon subjecting to *n*-BuLi, provided the required monosubstituted acetylene **66** in good yield. The comound **66** upon TBAF mediated desilylation provided **64**. The IR spectrum of **64** displayed a broad peak at 3435 cm^{-1} corresponding to −OH stretching frequency. The molecular ion peak at *m/z* 157 (M^++H) in the mass spectrum confirmed its formation. IBX oxidation of **64** yielded the required aldehyde **67** (**Scheme 20**).[13]



Scheme 20: *Synthesis of **67**.*

The synthesis of templates **7** and **8** with their subsequent transformation to various quinolizidine alkaloids following similar reaction sequences as described earlier for the two enantiomeric series from **5** and **6** are under progress in our lab.

2B.7- Biological evaluation of quinolizidine alkaloids

With the successful synthesis of fourteen azasugars of quinolizidine class such as **17**–**30,** we embarked on the study of their enzyme inhibitory efficiencies. In this context six different enzymes were selected for the inhibition studies, which were β-galactosidase (*A. oryzaie*), α-galactosidase (*Green coffee beans*), β-mannosidase (*Snail*), α-mannosidase (*Jack Beans*), β-glucosidase (*Almond*) and α-glucosidase (*Yeast*).

2B.7.1-Enzyme Inhibition

A number of substances may cause a reduction in the rate of an enzyme catalyzed reaction. Some of these (eg. urea) are non-specific protein denaturants. Others, which generally act in a fairly specific manner, are known as inhibitors. Loss of activity may be either reversible, wherein activity may be restored by the removal of the inhibitor, or irreversible, wherein the loss of activity is time-dependent and cannot be recovered during the time scale of interest. In the case of irreversible inhibition, the inhibitor (**I**) forms stable covalent bond with the enzyme (**E**) (e.g. alkylation or acylation of an active site side chain). More important for most enzyme-catalyzed processes is the effect of reversible inhibitors. In case of reversible inhibition, the inhibitor binds to an enzyme and prevents the formation of the enzyme-substrate (**ES**) complex or its breakdown to **E** + **P**.

There are three basic mechanisms of reversible enzyme inhibition:
(a) Competitive
(b) Non-competitive
(c) Uncompetitive

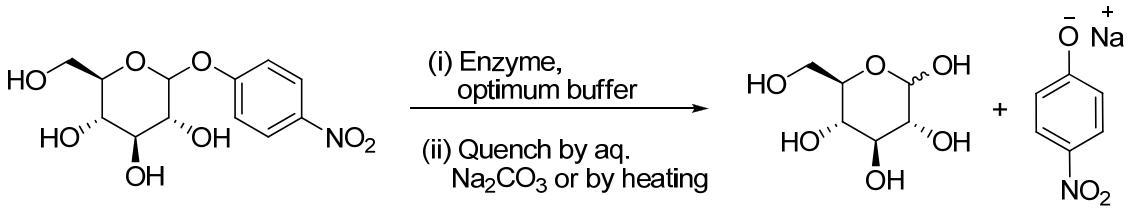
The difference between the three is in the nature of the binding of the enzyme and inhibitor and its effect on the enzyme substrate complex.

In competitive inhibition, the inhibitor **I**, binds with the enzyme at its active site, thus, making some of the enzyme unavailable to the substrate. This is the most common form of inhibition in single substrate enzyme systems. In non-competitive inhibition, the inhibitor **I**, and the substrate **S**, bind simultaneously with the enzyme rather than competing for the same site. The resulting complex **ESI** is unable to form the product. In the case of uncompetitive inhibition, the substrate binds with the active site to form the **ES** complex as

normal, but the inhibitor **I**, then binds to the **ES** complex to form an **ESI** complex, which as with non competitive inhibition, is unable to form the product. This particular form of inhibition is rare with single substrate enzyme systems.

In the case of some glycosidases, the rate of the reaction can be estimated by using the corresponding *o*- or *p*-nitrophenyl glycosides as substrates for the reaction. The amount of *o*- or *p*-nitrophenol released in the reaction can be estimated spectrometrically and the rate of the reaction (*v*) can be calculated, based on the optical density obtained.

Shown below, in **Scheme 21**, is an example of a β-glucosidase reaction using *p*- nitrophenyl β-D-glucoside as the substrate.



Scheme 21: *An example of β-glucosidase reaction.*

The same principle is applied for the inhibition assay and the type of inhibition obtained which is easily determined from the Dixon plots. [24]

In a typical inhibition assay, the reaction is initiated by addition of appropriately diluted enzyme to a solution of the requisite quantities of substrate and inhibitor in a buffer of optimum pH for the enzyme. The reaction is allowed to incubate at the temperature typical for that particular enzyme and at the end of a fixed reaction time, the reaction is quenched (the enzyme is inactivated) by chemical (aq. base) or thermal means (heating at high temperature). The optical density of this mixture is recorded and the reaction rate is determined.

The inhibitors were screened at different level of concentrations. Initially, the inhibition at concentration level of 1 mM was determined. The compounds showing no inhibition or inhibition less than 50% were not investigated further. Those showing activities more than 50% were taken and the screening was done at lower level of concentration. Thus optimum range of concentration was found where compound showed activity in the range of 50%. Several assays in a varying range of concentration at that level were performed and later the

experiment was repeated with a different concentration of substrate. The two sets of data were used to obtain Dixon plots[24], from which the inhibition constant *Ki* was determined. The *Ki* values determined were in good agreement with IC_{50} values. The results are summarized in the tabular format as follows:

2B.7.2-Results and Discussion

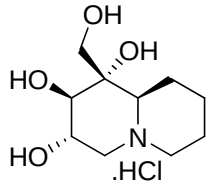
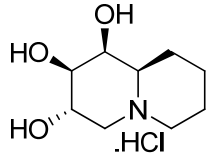
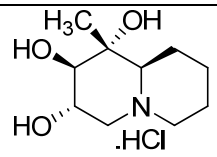
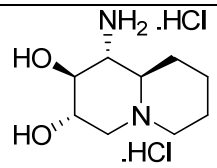
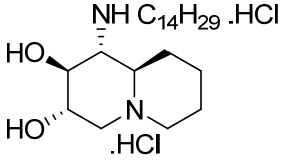
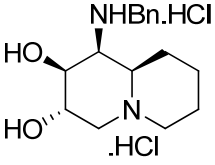
The inhibitory activities of all the final molecules **30–36** and **56–62** were tested against various enzymes [d] and the results are summarized in **Table 1**. Compound **36** and **60** were found to be anomer specific inhibitor of α-mannosidase (*Ki* = 293 μM and 650 μM, respectively). The other compounds **33**, **35**, **57**, **59**, **61** and **62** were found to be anomer specific inhibitor of α-glucosidase with *Ki* values (in μM) 675, 278, 450, 258, 28, and 222, respectively. Compound **61** and **62** in particular were found to be very selective potent inhibitors of α- glucosidase (*Ki* = 28 μM and 222 μM, respectively) as they did not show any inhibition against other enzymes under investigation.

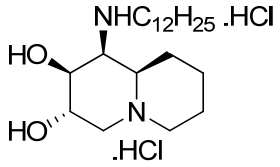
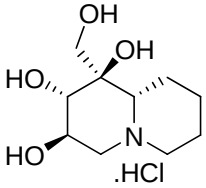
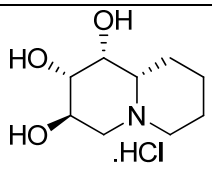
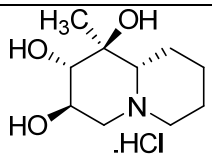
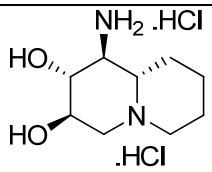
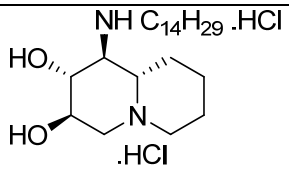
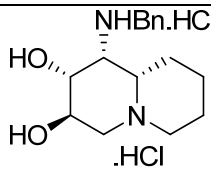
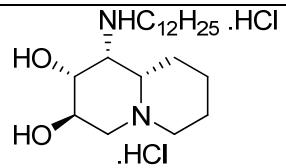
Among the polyhydroxy quinolizidine alkaloids, **31** was found to be a competitive inhibitor for α as well as β-galactosidase with *Ki* values 83.9 μM and 591 μM, respectively, whereas **57** was showing selective inhibitory activity for α-glucosidase, *Ki* = 450 μM. Barring these two cases none of the polyhydroxy analogs exhibited any significant inhibitions towards the enzymes under study.

Introduction of additional amine functionality was found to be advantageous as all the amine analogs afforded better inhibitory activity against α-glucosidase in general compared with polyhydroxy analogs. An increase in the inhibitory activity was successfully accomplished by increasing the lipophilicity. For example, **34** (*Ki* = 140 μM) was found to be five times more potent against α-glucosidase compared to its free amine counterpart **33** (*Ki* = 675 μM). This increased lipophilicity in compound **34** also exhibited better activity against β-glucosidase, α-mannosidase and β-mannosidase with *Ki* values (in μM) 524, 805 and 830, respectively, in comparison to no inhibition shown by **33** against these enzymes up to 1mM concentration.

d *Evaluation of inhibitory activities the compounds were done in collaboration with Dr. M.I. Khan, Division of Biochemical Sciences, National Chemical Laboratory, Pune..*

Table 1. (*Ki* in μM)

Inhibitors	**Enzymes**					
	β-gal (*A. oryzaie*)	**α-gal** *(Green coffee beans)*	**β-man** *(Snail)*	**α-man** *(Jack Beans)*	**β-glu** *(Almond)*	**α-glu** *(Yeast)*
	Ki	*Ki*	*Ki*	*Ki*	*Ki*	*Ki*
 **30**.HCl	33% [a]	NI	NI	11% [a]	NI	NI
 **31**.HCl	**591**	**83.9**	NI	18% [a]	NI	NI
 **32**.HCl	NI	NI	NI	22% [a]	NI	NI
 **33**.2HCl	NI	11% [a]	NI	NI	NI	**675**
 **34**.2HCl	NI	NI	**830**	**805**	**524**	**140**
 **35**.2HCl	NI	31% [a]	NI	NI	NI	**278**

 **36**.2HCl	NI	NI	NI	**293**	46% [a]	**120**
 **56**.HCl	NI	NI	NI	NI	NI	42% [a]
 **57**.HCl	28% [a]	13% [a]	18% [a]	NI	NI	**450**
 **58**.HCl	NI	NI	14% [a]	29% [a]	NI	39% [a]
 **59**.2HCl	NI	NI	13% [a]	22% [a]	NI	**258**
 **60**.2HCl	NI	NI	NI	**650**	43% [a]	**235**
 **61**.2HCl	NI	NI	NI	NI	NI	**28**
 **62**.2HCl	NI	NI	NI	NI	NI	**222**

NB: 1) *Ki* in μM (in bold); 2) [a]percent inhibition at 1 mM; 3) NI = no inhibition till 1 mM

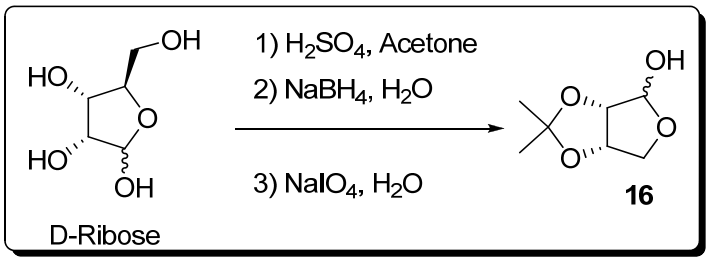
A similar observation was found in the case of alkylated amine **60** with respect to free amine **59**. To our pleasant surprise, the exploratory work to make another diastereomeric amine analog was also fruitful as **36** (*Ki* = 293 μM) was not only anomer specific for α-mannosidase but also found to be an inhibitor three fold stronger than **34** (*Ki* = 805 μM).

2B.8-Conclusion

In summary, we presented a general synthetic route for a variety of enantiopure polyhydoxy quinolizidine alkaloids and some of their amine analogs, utilizing PET mediated cyclizations of α-trimethylsilylmethylamine radical cation to a proximate tethered π-functionality. Fourteen new azasugars are synthesized and tested against six different enzymes. The acetonide protecting group was crucial in governing the outcome of PET cyclization reaction, which yielded the product as a single diastereomer. Further, the tricyclic framework of PET cyclization product and consequent compounds provided exclusively single diastereomeric products, in each case, due to their inherent rigid structural motif with a single face accessible to the reagents. The generality of the method developed, has been demonstrated by the synthesis of both the enantiomers of the potent glucosidase inhibitors. The simplicity of the steps involved in their syntheses make the route attractive for their preparation. As many of these compounds are selective inhibitors, efficient drug delivery may make them useful therapeutics. Moreover, the enzyme inhibitory study can provide an insight for the structure activity relationship for the development of newer analogs as drug. The polyhydroxy analogs synthesized were tested for their inhibitory activity and some of them were found to be moderate to good inhibitors of the enzymes tested. All the free and alkylated amine analogs produced promising activity. Most of the *N*-alkykated analogues showed better activity compared to the free amine analogue.

2B.9-Experimental

1. Preparation of (3aS,6aS)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-ol (16)[7]

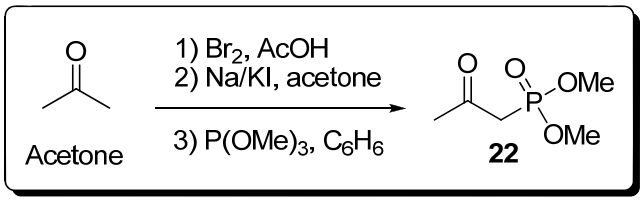


To an ice cold suspension of D-ribose (20.0 g, 133.21 mmol) in acetone (200 mL) was added conc. H_2SO_4 (1.0 mL). The clear solution was obtained after 30 minutes. The reaction mixture was neutralized by the addition of $Ca(OH)_2$ and the salt was removed by filtration. The filtrate was concentrated in *vacuo* to give the crude product (23.1 g) as colorless oil. To an ice cold solution of this product in H_2O (250 mL) was added a solution of $NaBH_4$ (6.93 g, 182.3 mmol) in H_2O (100 mL) and the reaction mixture was stirred for 1 h at rt. The *p*H of the mixture was adjusted to 6.0 by drop wise addition of acetic acid. It was cooled to 0 °C, and $NaIO_4$ (28.46 g, 133.21 mmol) was added gradually. After stirring for 2 h at rt, the aq. layer was extracted with ethyl acetate (2 × 200 mL), washed with sat. $NaHCO_3$ followed by brine. The combined organic extracts were dried over Na_2SO_4, concentrated under reduced pressure and purified by column chromatography (silica, pet. ether–ethyl acetate 10:3) to obtain 14.9 g (70%) of lactol **16**.

$[\alpha]^{27}_D$	+70.59, (*c* 1.2, $CHCl_3$), Lit[7] $[\alpha]^{27}_D$ = +74.8, (*c* 0.96, $CHCl_3$).
IR ν_{max} cm^{-1} in $CHCl_3$	3429, 1376, 1214, 1100.
^1H NMR (200 MHz, $CDCl_3$)[a]	δ 1.30 (s, 3H), 1.45 (s, 3H), 4.02 (d, *J* = 0.51 Hz, 1H), 4.04 (d, *J* = 3.16 Hz, 1H), 4.56 (d, *J* = 5.94 Hz, 1H), 4.82 (dd, *J* = 5.94, 3.16 Hz, 1H), 5.39 (s, 1H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ 24.5 (CH_3), 26.0 (CH_3), 71.5 (CH_2), 79.8 (CH), 85.0 (CH), 101.3 (CH), 112.1 (C).
Mass (ESI): *m/z*	178 (M^++NH_4), 183 (M^++Na).
Elemental analysis	Anal. Calcd for $C_7H_{12}O_4$: C, 52.49; H, 7.55. Found: C, 52.89; H, 7.84.

a = D_2O exchange

2. Preparation of dimethyl 2-oxopropylphosphonate (22)[9]

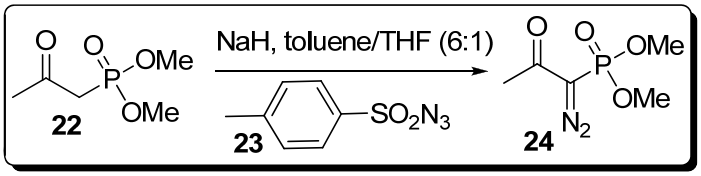


A solution of water (320 mL), acetone (100 mL, 1.36 mol) and acetic acid (74 mL, 1.29 mol) was heated to 65–70 °C. Bromine (71 mL, 1.38 mol) was added drop wise over a period of 1 h, taking care that unreacted bromine does not accumulate. After addition, the solution got decolorized in 20 min. Water (160 mL) was added to reaction mixture and cooled to 0 °C, neutralized by $NaHCO_3$ and extracted with DCM (2 × 200 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude oil was fractionally distilled (37–48 °C, 13 mm) to give bromo acetone (147 g, 50%).

The bromo acetone (100 g, 729.9 mmol) was refluxed with KI (157.5 g, 948 mmol) in 700 mL of dry acetone for 30 min. The reaction mixture was filtered and concentrated to obtain iodo acetone which was used as such without any purification. Trimethyl phosphite (103.2 mL, 875.9 mmol) was added drop wise to a solution of iodo acetone in benzene (75 mL) under argon atmosphere. Temperature of the reaction mixture was maintained at 65 °C while addition, and methyl iodide being formed was distilled out continuously. After addition, the reaction mixture was refluxed for 0.5 h. Solvent was removed under *vacuo* and the residue was fractionally distilled, discarding all the fractions except that boiling at (119–123 °C/11 mm) yielding **22** (66.7 g, 55%).

IR ν_{max} cm^{-1} in $CHCl_3$	1711, 1643, 1231.
^1H NMR (200 MHz, $CDCl_3$)	δ 2.28 (s, 3H), 3.01 (s, 1H), 3.12 (s, 1H), 3.72 (s, 3H), 3.78 (s, 3H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ 30.9 (CH_3), 40.3 (CH_2), 42.9 (CH_2), 52.5 (CH_3), 52.6 (CH_3), 199.2 (C), 199.3 (C).
Mass (ESI): *m/z*	167 (M^++H), 189 (M^++Na).
Elemental analysis	Anal. Calcd for $C_5H_{11}O_4P$: C, 36.15; H, 6.67. Found: C, 36.42; H, 6.86.

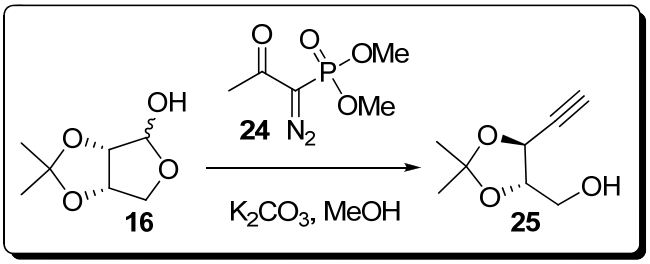
3. Preparation of dimethyl 1-diazo-2-oxopropylphosphonate (24)[8]



To an ice cold suspension of NaH (60% dispersion in mineral oil) (5.30 g, 132.53 mmol) in toluene (360 mL) and THF (60 mL) under argon atmosphere was added solution of phosphonate **22** (20.0 g, 120 mmol) in toluene (120 mL). After stirring for one hour, solution of tosyl azide **23** (26.1 g, 132 mmol) in toluene (60 mL) was added and the reaction mixture was allowed to warm to rt. After stirring for 2 h, the mixture was filtered, concentrated under reduced pressure and purified by column chromatography (silica, ethyl acetate–pet.ether, 7:3) to get the reagent **24** (16.19 g, 70%), as a yellow liquid.

IR ν_{max} cm^{-1} in $CHCl_3$	2125, 1659, 1459, 1273, 1028.
^1H NMR (200 MHz, $CDCl_3$)	δ 2.24 (s, 3H), 3.79 (s, 3H), 3.85 (s, 3H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ 26.8 (CH_3), 53.3 (CH_3), 53.4 (CH_3), 63.1 (C), 189.4 (C), 189.7 (C).
Mass (ESI): *m/z*	193 (M^++H), 215 (M^++Na).
Elemental analysis	Anal. Calcd for $C_5H_9N_2O_4P$: C, 31.26; H, 4.72; N, 14.58. Found: C, 30.98; H, 4.92; N, 14.89.

4. Preparation of ((4*S*,5*S*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (25)[12]

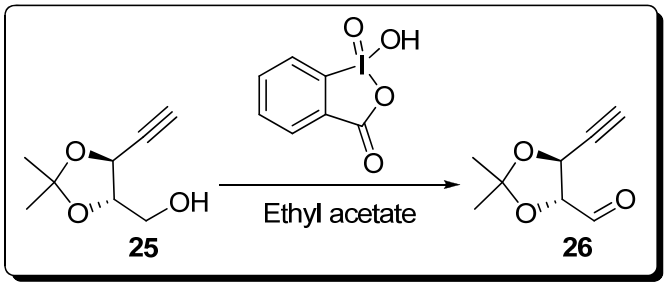


To a stirring mixture of **16** (10.0 g, 62.5 mmol) and anhydrous K_2CO_3 (11.37 g, 81.25 mmol) in anhydrous MeOH (240 mL) at 65 °C was added the solution of Bestmann-Ohira reagent (**24**, 15.6 g, 81.25 mmol) in anhydrous MeOH (80 mL) drop wise over a period of 6 h under argon atmosphere. Heating was continued further for 30 min. Cooled to a 0 °C and neutralized with acetic acid. Solvent was removed under *vacuo*, water was added and

mixture was extracted with ethyl acetate (2 × 100 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified by column chromatography (silica, pet. ether–ethyl acetate, 4:1) to obtain **25** (6.82 g, 70%) as a colorless liquid.

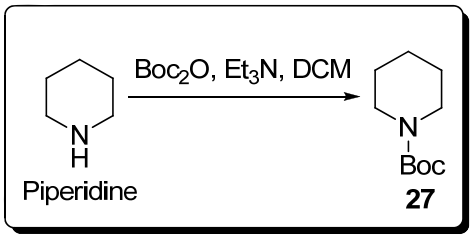
$[\alpha]^{26}_D$	−8.0 (*c* 1.0, MeOH); Lit.[12] $[\alpha]^{27}_{D.}$ −8.6 (*c* 2.0, MeOH).
IR (neat) ν_{max} cm^{-1}	3452, 3284 (≡C–H), 2121 (C≡C), 848, 665.
^1H NMR (200 MHz, $CDCl_3$)[a]	δ 1.42 (s, 3H), 1.48 (s, 3H), 2.53 (d, *J* = 2.15 Hz, 1H), 3.64 (dd, *J* = 12.25, 3.67 Hz, 1H,), 3.87 (dd, *J* = 12.25, 3.03 Hz, 1H), 4.16 (ddd, *J* = 7.58, 3.67, 3.03 Hz, 1H), 4.56 (dd, *J* = 7.57, 2.15 Hz, 1H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ 25.7 (CH_3), 26.4 (CH_3), 60.4 (CH_2), 66 (CH), 74.7 (CH), 80.5 (C), 81.7 (CH), 110.4 (C).
Mass (ESI): *m/z*	157 (M^++H), 179 (M^++Na).
Elemental analysis	Anal. Calcd for $C_8H_{12}O_3$: C, 61.52; H, 7.74. Found: C, 61.79; H, 7.84.

5. Preparation of (4*R*, 5*S*)-5-ethynyl-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde (26)[15]



To the solution of alcohol **25** (6.23 g, 39.93 mmol) in ethyl acetate (250 mL), IBX (19 g, 67.89 mmol) was added. The resulting suspension was immersed in an oil bath set at 80 °C and stirred vigorously open to the atmosphere. After 8 h (GC monitoring), the reaction mixture was cooled to room temperature and filtered through a short pad of celite. The filter cake was washed with 200 mL of ethyl acetate and concentrated to yield 5.8 g of **26** (85% yield, > 85% pure by GC). The unstable aldehyde was used immediately for the next step without any purification. ***Note****: IBA recovered from the reaction mixture was reoxidized to IBX and recycled.*

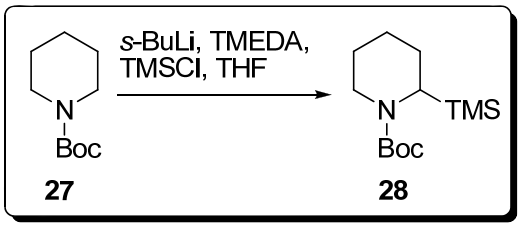
6. Preparation of tert-butyl piperidine-1-carboxylate (27)



To a solution of piperidine (20 g, 234.8 mmol) and Et_3N (35.64 g, 352.4 mmol) in DCM (600 mL) at 0 °C, di-*tert*-butyldicarbonate (51.26 g, 234.8 mmol) was added dropwise over a period of 30 min. The reaction mixture was allowed to warm to room temperature and further continued for 10 h. Reaction mixture was diluted with water and the DCM layer was separated, washed with brine (2 × 100 mL), dried and concentrated under reduced pressure. The resultant brown oil obtained, was purified by vacuum distillation (bp 60–65 °C/1mm) to obtain **27** (41.2 g, 95%) as a colorless liquid.

IR (neat) ν_{max} cm^{-1}	1694, 1421, 1270, 1148.
^1H NMR (400 MHz, $CDCl_3$)	δ 1.44 (s, 9H), 1.46–1.62 (m, 6H), 3.32–3.37 (m, 4H).
^{13}C NMR (125 MHz, $CDCl_3$)	δ 24.5 (CH_2), 25.7 (CH_2), 28.4 (CH_3), 44.3 (CH_2), 44.9 (CH_2), 79.1 (C), 154.9 (C).
Mass (ESI): *m/z*	186 (M^++H).

7. Preparation of tert-butyl 2-(trimethylsilyl)piperidine-1-carboxylate (28)[14]

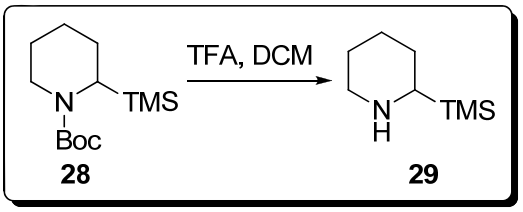


A solution of *tert*-butyl piperidine-1-carboxylate **27** (10 g, 54.0 mmol) in 100 mL of dry ether was charged into a 500 mL flask, equipped with a magnetic bar and argon gas balloon. Flask was cooled to −78 °C. TMEDA (7.54 g, 64.8 mmol) followed by *s*-BuLi (1.4 M solution in cyclohexane (46.4 mL 64.8 mmol) were introduced into the stirring mixture dropwise over 15 min. The mixture was further allowed to stir for 2 h at −78 °C. TMSCl (7.04 g, 64.8 mmol) was added dropwise into the flask. The reaction mixture was allowed to warm to room temperature and diluted with 40 mL of saturated aq. NH_4Cl solution. The

organic layer was concentrated and crude oily residue was purified by fractional distillation (bp 90–96 °C /1mm) to give **28** (11.8 g, 85%) as colorless oil.

IR (neat) ν_{max} cm^{-1}	1688, 1416, 1247,1159.
^1H NMR (400 MHz, $CDCl_3$)	δ −0.07 (s, 9H), 1.43 (s, 9H), 1.49–1.75 (m, 6H), 2.53–2.99 (m, 1H), 3.51–4.25 (m, 2H).
^{13}C NMR (125 MHz, $CDCl_3$)	δ −0.8 (CH_3), 22.5 (CH_2), 23.3 (CH_2), 25.9 (CH_2), 26.5 (CH_3), 44.3 (CH), 44.9 (CH_2), 78.8 (C), 154.9 (C).
Mass (ESI): *m/z*	258 (M^++H), 280 (M^++Na).

8. Preparation of 2-(trimethylsilyl)piperidine (29) [14]



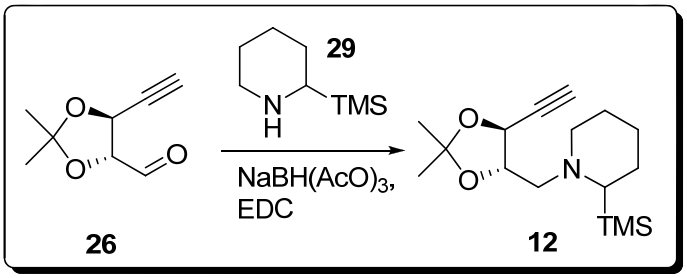
To a stirring solution of **28** (10 g, 38.9 mmol) in 60 mL of dry DCM at 0 °C, TFA (35.4 g, 311.2 mmol) was added drop-wise over a period of 30 min. The mixture was allowed to warm to room temperature and allowed to stir further for 4 h. The reaction mixture was re-cooled to 0 °C and was basified with 20% aq. NaOH solution (pH = 10). The organic layer was separated and the aq. layer was extracted with DCM (2 × 60 mL). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give crude amine **29** as a clear yellow liquid (5.9 g, 95%) which was utilized further without purification.

IR (neat) ν_{max} cm^{-1}	3391, 1440, 1247, 1111.
^1H NMR (400 MHz, $CDCl_3$)[a]	δ −0.02 (s, 9H), 1.21–1.36 (m, 2H), 1.38–1.49 (m, 1H), 1.55–1.62 (m, 2H), 1.78–1.81 (m, 1H), 2.01 (dd, *J* = 12.05, 2.26 Hz, 1H), 2.54 (dt, *J* = 11.89, 2.76 Hz, 1H), 3.06 (d, *J* = 11.54 Hz, 1H).
^{13}C NMR (100 MHz, $CDCl_3$)	δ −4.1 (CH_3), 26.3 (CH_2), 27.0 (CH_2), 27.7 (CH_2), 48.6 (CH), 49.1 (CH_2).

Mass (ESI): *m/z*	175 (M^++NH_4), 180 (M^++Na).

a = D_2O exchange

9. Preparation of 1-(((4*S*,5*S*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-2-(trimethylsilyl)piperidine (12)[15]

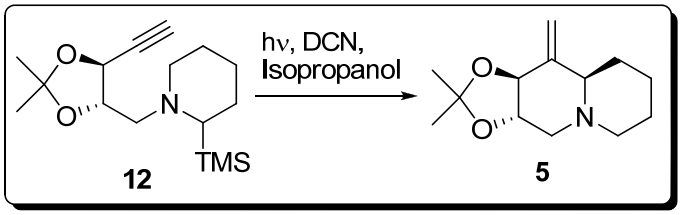


To the solution of **26** (5.76 g, approx. 31.82 mmol) (>85% pure by GC)) in dry 1,2-dichloroethane (90 mL), amine **29** (5.24 g, 33.41 mmol) followed by sodium triacetoxyborohydride (8.77 g, 41.36 mmol) was added. The mixture was stirred at rt under argon atmosphere for 3 h. The reaction mixture was ice cooled and quenched by adding (2N) NaOH till the aq. layer was basic. The reaction mixture was extracted with DCM (2 × 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 **12** (6.77 g, 72%) as a colorless oil.

$[\alpha]^{26}_D$	−17.20 (*c*, 1.25, $CHCl_3$).
IR ν_{max} cm^{-1} in $CHCl_3$	3311 (≡C–H), 2933, 2120 (C≡C), 1441, 1381, 1250, 1055.
^{1}H NMR (400 MHz, $CDCl_3$)	δ 0.6, 0.7 (s each, 4.5H each), 1.21–1.29 (m, 1H), 1.40, 1.41, 1.46, 1.46 (s each, 1.5H each), 1.50–1.63 (m, 4H), 1.71 (dt, *J* = 12.51, 3.76 Hz, 1H), 1.87 (app dt, *J* = 3.76 and 2.76, 11.54 and 11.29 Hz, 1H), 2.03–2.14 (m, 2H), 2.40–2.46 (m, 1H), 2.51 (app t, *J* = 2.01 and 2.26 Hz, 1H), 2.94 (dd, *J* = 13.29, 5.52 Hz, 1H), 3.12–3.18 (m, 1H), 4.22–4.32 (m, 2H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ −1.1, −1.0 (CH_3), 23.8, 24.2 (CH_2), 25.7 (CH_2), 26.0, 26.09 (CH_3), 26.03, 26.6 (CH_2), 27.06, 27.11 (CH_3),

	54.7, 54.9 (CH_2), 56.0, 56.1 (CH), 58.1, 58.2 (CH_2), 68.6, 68.8 (CH), 74.4, 74.5 (C), 80.3, 80.9 (CH), 81.0, 81.1 (CH), 110.5, 110.7 (C).
Mass (ESI): *m/z*	296 (M^++H).
Elemental analysis	Anal.Calcd. for $C_{16}H_{29}NO_2Si$: C, 65.03; H, 9.89; N, 4.74; Si, 9.50; Found: C, 65.12 ;H, 9.86; N, 4.76, Si, 9.44.

10. Preparation of (3a*S*, 9a*R*, 10a*S*)-2,2-dimethyl-10-methyleneoctahydro-3aH-[1,3]-dioxolo[4,5-b]quinolizine (5)

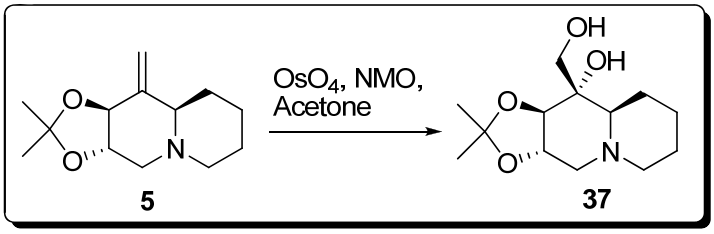


A solution containing **12** (1.0 g, 3.38 mmol) and 1, 4-dicyanonaphthalene (0.12 g, 0.67 mmol) in 2-propanol (250 mL) was irradiated in an open vessel using a 450 W Hanovia medium pressure mercury vapor lamp. The lamp was immersed in a Pyrex water-jacketed immersion well to allow only wavelengths greater than 280 nm to pass through. After about 1h 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, 6:1) to afford cyclized product **5** (0.453 g, 60%) as a yellow liquid.

$[\alpha]_D^{29}$	+55.80 (*c*, 0.85, CH_2Cl_2).
IR (neat) ν_{max} cm^{-1}	2985 (=C–H), 2858, 1806, 1667 (C=C), 1370, 1226.
^{1}H NMR (400 MHz, $CDCl_3$)	δ 1.24–1.33 (m, 1H), 1.44 (s, 3H), 1.47 (s, 3H), 1.48–1.65 (m, 3H), 1.82–1.92 (m, 2H), 2.27 (dt, *J* = 11.79, 3.01 Hz, 1H), 2.33 (d, *J* = 11.70 Hz, 1H), 2.38 (t, *J* = 10.29 Hz, 1H), 2.93 (d, *J* = 11.54 Hz, 1H), 3.23 (dd, *J* = 10.03, 3.97 Hz, 1H), 3.48 (dt, *J* = 10.02, 4.02 Hz, 1H), 3.74 (dt, *J* = 9.34, 1.79 Hz, 1H), 4.89 (broad s, 1H), 5.06 (broad s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$)	δ 24.0 (CH_2), 25.7 (CH_2), 26.7 (CH_3), 26.9 (CH_3), 27.9 (CH_2), 56.8 (CH_2), 57.7 (CH_2), 61.9 (CH), 77.1 (CH), 82.2 (CH), 103.2 (CH_2), 111.0 (C), 144.2 (C).
Mass (ESI): *m/z*	224 (M^++H).
Elemental analysis	Anal.Calcd for $C_{13}H_{21}NO_2$: C, 69.92; H, 9.48; N, 6.27; Found: C, 69.98; H, 9.51; N, 6.25.

11. Preparation of (3a*S*,9a*R*,10S,10a*R*)-10-(hydroxymethyl)-2,2-dimethyloctahydro-3aH-[1,3]dioxolo[4,5-b]quinolizin-10-ol (37)



To a solution of **5** (0.45 g, 2.02 mmol) in acetone (5 mL) was added *N*-methylmorpholine-*N*-oxide [(NMO) 50% aq solution, 1.41 g, 6.06 mmol]. The reaction mixture was cooled to 0 °C and to it was added a catalytic amount of osmium tetroxide (1 mL of 1% solution of OsO_4 in *t*-BuOH). The reaction mixture was allowed to come to rt and stirred for 10 h. Solid Na_2SO_3 was added to this reaction mixture. Stirring was continued for 30 min to quench excess NMO and OsO_4. It was filtered through a short pad of Celite and the solvent was evaporated off. The crude reaction mixture upon column chromatography (silica, pet ether–ethyl acetate, 3:2) afforded the diol **37** (0.466 g, 90%) as a colorless solid (mp 135–136 °C).

$[\alpha]^{29}_D$	+27.7 (*c* 1.2, DCM).
IR ν_{max} cm^{-1} in $CHCl_3$	3432 (OH), 2938, 2306, 2232, 1654, 1265.
^{1}H NMR (400 MHz, $CDCl_3$)[a]	δ 1.13–1.19 (m, 1H), 1.44 (s, 3H), 1.44 (apparent singlet, 4H), 1.59–1.62 (m, 1H), 1.79–1.80 (m, 1H), 1.93–1.95 (m,1H), 2.01–2.03 (m, 1H), 2.13 (dt, *J* = 12.55, 2.76 Hz, 1H), 2.21 (t, *J* = 10.04 Hz, 1H), 2.92 (d, *J* = 11.50 Hz,1H), 3.14 (dd, *J* = 9.88, 4.28 Hz, 1H) 3.43 (d, *J* = 9.54 Hz, 1H), 3.73 (d, *J* = 11.54 Hz, 1H), 3.80 (dt, *J* = 10.04, 4.27 Hz, 1H), 3.97 (d , *J* = 11.54

	Hz, 1H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ 24.2 (CH_2), 24.9 (CH_2), 25.7 (CH_2), 26.6 (CH_3), 56.9 (CH_2), 57.9 (CH_2), 62.9 (CH_2), 68.4 (CH), 71.9 (CH), 72.4 (C), 86.9 (CH), 110.6 (C).
Mass (ESI): *m/z*	258 (M^++H), 280 (M^++Na).
Elemental analysis	Anal.Calcd for $C_{13}H_{23}NO_4$: C, 60.68; H, 9.01; N, 5.44; Found: C, 60.51; H, 9.05; N, 5.45.
mp	135–136 °C (from EtOAc–pet ether)

a = D_2O exchange

X-ray crystal structure analysis for compound 37 and 39 Crystal Data:

Data for both the compounds **37** and **39** were collected at T = 296 K, on SMART APEX CCD single crystal X-ray diffractometer using Mo-Kα radiation (λ = 0.7107 Å) to a maximum θ range of 25.00°. The structures were solved by direct methods using SHELXTL. All the data were corrected for Lorentzian, polarisation and absorption effects. SHELX-97 (ShelxTL)[e] was used for structure solution and full matrix least squares refinement on F^2. Hydrogen atoms were included in the refinement as per the riding model. The refinements were carried out using SHELXL-97.

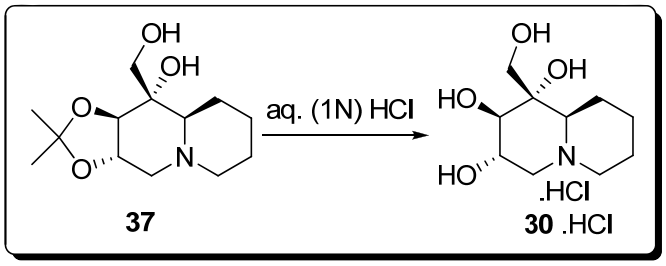
Compound no. 37 ($C_{13}H_{23}NO_4$): Single crystals of the complex were grown by slow evaporation of the solution a mixture of hexanes and ethyl acetate. Colorless needle crystal of approximate size 0.22 × 0.07 × 0.01 mm^3, was used for data collection. Crystal to detector distance 6.05 cm, 512 × 512 pixels / frame, multirun data acquisition. Total scans = 4, total frames = 2424, oscillation / frame −0.3º, exposure / frame = 20.0 sec / frame, maximum detector swing angle = −30.0º, beam center = (260.2, 252.5), in plane spot width = 1.24, SAINT integration, θ range = 1.68 to 25.0º, completeness to θ of 25.0º is 100.0%. SADABS correction applied, $C_{13}H_{23}NO_4$, M = 257.32. Crystals belong to orthorhombic, space group $P2_12_12_1$, a = 5.3743(4), b = 10.2506 (7), c = 24.311(2) Å, V = 1339.27(16) $Å^3$, Z = 4, D_c = 1.276 g/cc, μ (Mo–Kα) = 0.094 mm^{-1}, 12741, reflections measured, 2369 unique [I>2σ(I)], R value 0.0358, wR2 = 0.0823. Largest diff. peak and hole 0.153 and −0.200 e. $Å^{-3}$.

e *G. M. Sheldrick, SHELX-97 program for crystal structure solution and refinement, University of Gottingen, Germany, 1997.*

Compound no. 39 ($C_{12}H_{21}NO_3$): Single crystals of the complex were grown by slow evaporation of the solution a mixture of hexanes and ethyl acetate. Colorless needle of approximate size 0.20 × 0.05 × 0.01 mm^3, was used for data collection. Crystal to detector distance 6.05 cm, 512 × 512 pixels / frame, multirun data acquisition. Total scans = 5, total frames = 2101, oscillation / frame −0.3º, exposure / frame = 25.0 sec / frame, maximum detector swing angle = −30.0º, beam center = (260.2, 252.5), in plane spot width = 1.24, SAINT integration, θ range = 2.00 to 24.99º, completeness to θ of 24.99º is 100.0% SADABS correction applied, $C_{12}H_{21}NO_3$, M = 227.30.

Crystals belong to orthorhombic, space group $P2_12_12_1$, a = 9.725 (1), b = 6.4790(7), c = 20.325(2) Å, V = 1280.6(2) $Å^3$, Z = 4, D_c = 1.179 g/cc, μ (Mo–Kα) = 0.084 mm^{-1}, 10685 reflections measured, 2250 unique [I>2σ(I)], R value 0.0480, wR2 = 0.0864. Largest diff. peak and hole 0.101 and −0.101 e. $Å^{-3}$.

12. Preparation of (1*S*,2*R*,3S,9a*R*)-1,2,3-trihydroxy-1-(hydroxymethyl)decahydro-quinolizinium chloride (30)

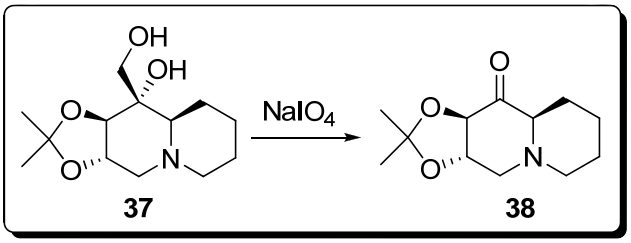


The amine **37** (0.040 g, 0.155 mmol) was taken in a 5 mL flask. To that 1.5 mL aq. solution of (1N) HCl was added and the reaction mixture was stirred at rt for 4 h. The solvent was removed under reduced pressure to afford **30** (0.039 g) quantitatively as a white solid.

$[\alpha]^{23}_D$	+24.1 (*c* 0.95, MeOH).
^{1}H NMR (400 MHz, D_2O)	δ 1.36–1.47 (m, 1H), 1.53–1.70 (m, 2H), 1.82–1.90 (m, 2H), 2.18, (dt, *J* = 14.38, 2.81 Hz, 1H), 2.82–2.90, (m, 2H), 3.01 (dd, *J* = 12.28, 2.03 Hz, 1H), 3.36–3.41 (m, 2H), 3.47, 3.52 (d, *J* = 10.55 Hz, 1H each), 3.98, (d, *J* = 10.80 Hz, 1H), 4.17 (ddd, *J* = 11.33, 10.25, 5.30 Hz, 1H).
^{13}C NMR (100 MHz, D_2O)	δ 21.3 (CH_2), 22.9 (CH_2), 23.0 (CH_2), 56.3 (CH_2), 56.8

	(CH_2), 58.7 (CH_2), 64.2 (CH), 67.9 (CH), 72.6 (C), 78.0 (CH).
Mass (ESI): *m/z*	218 (M^++H).
Elemental analysis	Anal.Calcd. for $C_{10}H_{20}ClNO_4$: C, 47.34; H, 7.95; N, 5.52; Found: C, 47.44; H, 7.99; N, 5.63.

13. Preparation of (3a*S*,9a*R*,10a*R*)-2,2-dimethylhexahydro-3aH-[1,3]dioxolo[4,5b]-quinolizin-10(4H)-one (38)

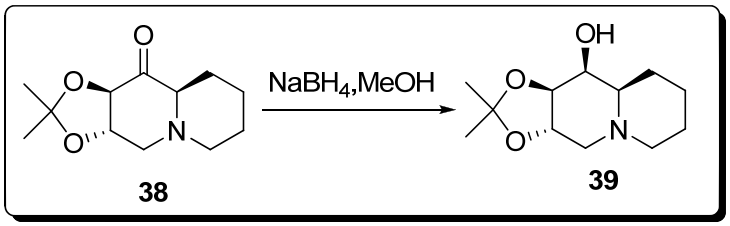


A solution of **37** (0.45 g, 1.75 mmol) in DCM (5 mL) was added to a suspension of silica gel supported sodium periodate [prepared by dissolving $NaIO_4$ (0.53 g, 2.62 mmol) in 1 mL water and 2.77 g of flash silica gel] in DCM (5 mL). The suspension was stirred for 10 min. and filtered. The solvent was evaporated off and the brownish pasty mass was extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure to give crude **38** (0.37 g, 95 %) which on crystallization from ethyl acetate–pet ether gave a white solid having mp113–114 °C (0.33 g, 84%).

$[\alpha]^{29}_D$	+48.37 (*c* 1.2, DCM).
IR ν_{max} cm^{-1} in $CHCl_3$	2989, 1750, 1265.
^{1}H NMR (400 MHz, $CDCl_3$)	δ 1.15–1.27 (m, 1H), 1.37–1.43 (m, 1H), 1.45 (s, 3H), 1.46 (s, 3H), 1.48–1.55 (m, 1H), 1.62 (broad singlet, 1H), 1.82 (dt, *J* = 13.14, 3.82 Hz, 1H), 1.94 (broad d, *J* = 13.58 Hz, 1H), 2.37 (dt, *J* = 11.76, 2.73 Hz, 1H), 2.58 (d, *J* =11.07 Hz, 1H), 2.67 (t, *J* = 10.09 Hz, 1H), 3.02 (d, *J* = 11.61 Hz, 1H), 3.35 (dd, *J* = 10.09, 3.79 Hz, 1H), 3.80 (dt, *J* = 10.24, 3.72 Hz, 1H), 4.10 (dd, *J* = 10.24, 1.25 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$)	δ 23.1 (CH_2), 25.1 (CH_2), 25.5 (CH_2), 26.2 (CH_3), 26.7 (CH_3), 56.3 (CH_2), 57.0 (CH_2), 67.0 (CH), 75.3 (CH), 83.6 (CH), 112.1 (C),198.5 (C).
Mass (ESI): *m/z*	226 (M^++H), 248 (M^++Na).
Elemental analysis	Anal.Calcd for $C_{12}H_{19}NO_3$: C, 63.98; H, 8.50; N, 6.22; Found: C, 63.66; H, 8.44; N, 6.35.
mp	113–114 °C (from EtOAc–pet ether)

14. Preparation of (3a*S*,9a*R*,10*S*,10a*S*)-2,2-dimethyloctahydro-3aH-[1,3]dioxolo[4,5b]quinolizin-10-ol (39)



38 **39**

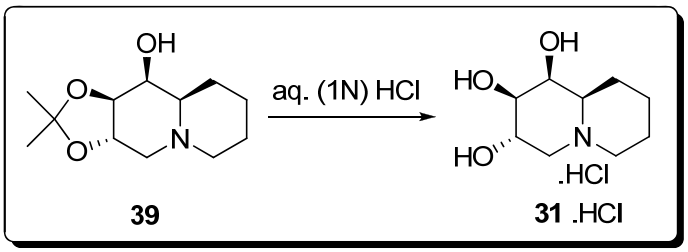
Sodium borohydride (0.118 g, 3.10 mmol) was added to a solution of ketone **38** (0.35 g, 1.55 mmol) in methanol (5 mL). The resulting mixture was stirred for 6 h at rt and then quenched by adding excess of the saturated solution of NaCl. This brownish suspension was stirred overnight and extracted with ethyl acetate (4 × 5 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, 1:9) to afford **39** (0.282 g, 80%) as a colorless solid (mp 133–134 °C).

$[\alpha]^{29}_D$	+16.77 (*c* 1.2, DCM).
IR ν_{max} cm^{-1} in $CHCl_3$	3436 (OH), 2940, 1653, 1265.
^{1}H NMR (400 MHz, $CDCl_3$)[a]	δ 1.23–1.29 (m, 1H), 1.43 (s, 3H), 1.43 (s, 3H), 1.56–1.58 (m, 3H) 1.77–1.80 (m, 2H), 1.98 (broad doublet, *J* = 11.04 Hz, 1H), 2.14–2.23 (m, 2H), 2.92 (d, *J* = 11.04 Hz, 1H), 3.17 (dd, *J* = 9.79, 4.02 Hz, 1H), 3.29 (dd, *J* = 9.28, 2.01 Hz, 1H), 3.96 (dd, *J* = 2.01, 1.87 Hz, 1H), 4.00 (dt, *J* = 10.29, 4.02 Hz, 1H).
^{13}C NMR (100 MHz, $CDCl_3$)	δ 24.1 (CH_2), 25.4 (CH_2), 26.5 (CH_3), 26.8 (CH_3), 28.4

	(CH_2), 56.4 (CH_2), 57.8 (CH_2), 63.8 (CH), 69.2 (CH), 70.2 (CH), 82.1 (CH), 110.2 (C).
Mass (ESI): *m/z*	228 (M^++H), 250 (M^++Na).
Elemental analysis	Anal.Calcd for $C_{12}H_{21}NO_3$: C, 63.41; H, 9.31,N, 6.16; Found: C, 63.44; H, 9.29; N, 6.19.
mp	133–134 °C (from EtOAc–pet ether).

a = D_2O exchange

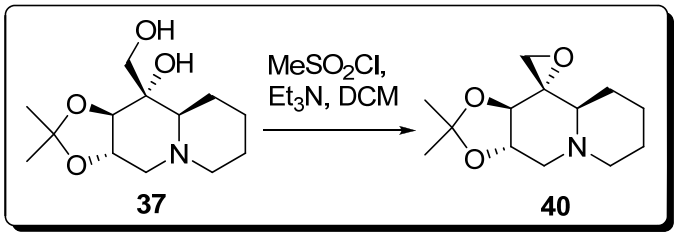
15. Preparation of (1*S*,2*R*,3*S*,9a*R*)-1,2,3-trihydroxydecahydroquinolizinium chloride (31)



Similar experimental procedure was used for the removal of acetonide moiety, as that described for the preparation of **30**.HCl, to afford **31**.HCl quantitatively as a white pasty mass.

$[\alpha]^{25}_D$	+7.75 (*c* 0.5, MeOH).
^{1}H NMR (400 MHz, D_2O)	δ 1.44–1.63 (m, 2H), 1.68–1.84 (m, 4H), 2.79 (t, *J* = 11.92 Hz, 1H), 2.95 (dt, *J* = 12.55, 2.5 Hz, 1H), 3.16 (dd, *J* = 9.79, 5.27 Hz, 1H), 3.33–3.42 (m, 2H), 3.56 (dd, *J* = 9.79, 3.03 Hz, 1H), 3.89 (d, *J* = 2.51Hz, 1H), 3.98 (ddd, *J* = 11.29, 9.78, 5.27 Hz, 1H).
^{13}C NMR (100 MHz, D_2O)	δ 20.9 (CH_2), 22.6 (CH_2), 25.7 (CH_2), 55.0 (CH_2), 56.1 (CH_2), 64.3 (CH), 64.8 (CH), 69.8 (CH), 72.9 (CH).
Mass (ESI): *m/z*	188 (M^++H).
Elemental analysis	Anal. Calcd. for $C_9H_{18}ClNO_3$: C, 48.32; H, 8.11; N, 6.26; Found: C, 48.43; H, 8.17; N, 6.21.

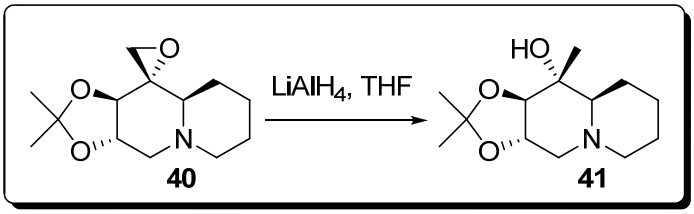
16. Preparation of (2'*S*,3a*S*,9a*R*,10a*R*)-2,2-dimethyloctahydrospiro[[1,3]dioxolo [4,5-b]quinolizine-10,2'-oxirane] (40)



To a solution of **37** (0.2 g, 0.777 mmol) in dry DCM (3 mL) at 0 °C under argon atmosphere was added triethyl amine (0.22 ml, 1.554 mmol) and methanesulphonyl chloride (0.066 mL, 0.855 mmol). The reaction mixture was stirred at room temperature for 12 h; water was added and extracted with DCM (2 × 5 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated under *vacuo* and purified by column chromatography (silica, pet. ether–ethyl acetate, 4:1) to get **40** (0.168 g, 90%) as a white solid.

$[\alpha]^{26}_D$	+ 49.16 (*c* 1.05, $CHCl_3$).
IR ν_{max} cm^{-1} in $CHCl_3$	3054 (C–O epoxide), 1421, 1265.
^1H NMR (500MHz, $CDCl_3$)	δ 1.01 (ddd, *J* = 15.96, 12.66, 3.31 Hz, 1H), 1.18 (tq, *J* = 12.93, 3.58 Hz, 1H), 1.39 (s, 3H), 1.43 (s, 3H), 1.50 (tq, *J* = 12.93, 3.85 Hz, 1H), 1.63–1.69 (m, 2H), 1.77 (d, *J* = 13.2 Hz, 1H), 2.26 (dt, *J* = 11.83, 2.48 Hz, 1H), 2.31 (dd, *J* = 11.22, 1.54 Hz, 1H), 2.37 (t, *J* = 10.18 Hz, 1H), 2.92 (d, *J* = 4.67 Hz, 1H), 2.99 (broad d, *J* = 11.56 Hz, 1H), 3.03 (d, *J* = 4.67 Hz, 1H), 3.23 (dd, *J* = 9.90, 3.85 Hz, 1H), 3.63 (d, *J* = 9.08 Hz, 1H), 3.72 (dt, *J* = 9.90, 3.85 Hz, 1H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ 23.6 (CH_2), 24.6 (CH_2), 25.8 (CH_2), 26.5 (CH_3), 26.7 (CH_3), 45.2 (CH_2), 56.7 (CH_2), 57.2 (CH_2), 60.3 (C), 62.3 (CH), 75.2 (CH), 78.4 (CH), 111.5 (C).
Mass (ESI): *m/z*	240 (M^++H), 262 (M^++Na).
Elemental analysis	Anal.Calcd. for $C_{13}H_{21}NO_3$: C, 65.25; H, 8.84; N, 5.85. Found: C, 65.34; H, 8.87; N, 5.83

17. Preparation of (3a*S*,9a*R*,10*R*,10a*R*)-2,2,10-trimethyloctahydro-3aH-[1,3]dioxolo-[4,5-b]quinolizin-10-ol (41)



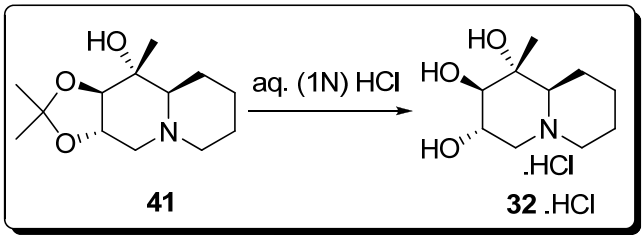
40 **41**

To a solution of **40** (0.1 g, 0.42 mmol) in dry THF (2 mL) was added LAH (0.031 g, 0.84 mmol) and stirred overnight at rt. Reaction mixture was quenched by drop wise addition of (2N) NaOH solution. Na_2SO_4, was added to the reaction mixture and it was filtered, concentrated and chromatographed (silica, pet. ether–ethyl acetate, 3:2) to get corresponding alcohol compound as a white solid **41** (0.091 g, 90%).

$[\alpha]^{25}_D$	+ 38.1 (*c* 1.0, CHCl3).
IR ν_{max} cm^{-1} in $CHCl_3$	3442 (OH), 2366, 1631, 1259.
^{1}H NMR (400 MHz, $CDCl_3$)[a]	δ 1.15–1.21 (m, 1H), 1.23 (s, 3 H), 1.24–1.27 (m, 1H), 1.42 (s, 3H), 1.44 (s, 3H), 1.46–1.52 (m, 1H), 1.61 (app d, *J* = 13.07, 12.93, 1H), 1.76–1.81 (m, 2H), 1.89–1.91 (m, 1H) 2.18 (dt, *J* = 12.38, 2.89 Hz, 1H), 2.21 (t, *J* = 10.18 Hz, 1H), 2.93 (app d, *J* = 11.50, 11.42 Hz, 1H), 3.12 (dd, *J* = 9.81, 3.96 Hz, 1H), 3.28 (d, *J* = 9.43 Hz, 1H), 3.53 (ddd, *J* = 10.31, 9.53, 4.13 Hz, 1H).
^{13}C NMR (125 MHz, $CDCl_3$)	δ 16.2 CH_3), 24.0 (CH_2), 24.3 (CH_2), 25.8 (CH_2), 26.5 (CH_3), 26.8 (CH_3), 56.8 (CH_2), 58.1 (CH_2), 69.4 (CH), 72.3 (C), 72.5 (CH), 86.9 (CH), 110.4 (C).
Mass (ESI): *m/z*	242 (M^++H).
Elemental analysis	Anal.Calcd. for $C_{13}H_{23}NO_3$: C, 64.70; H, 9.61; N, 5.80. Found: C, 64.75; H, 9.63; N, 5.75.

a = D_2O exchange

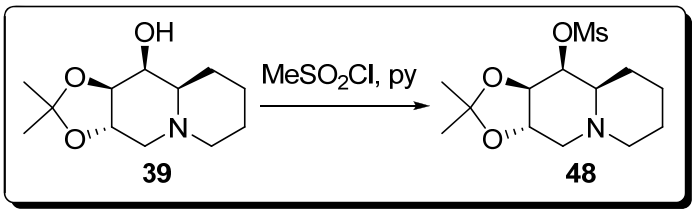
18. Preparation of (1*R*,2*R*,3*S*,9a*R*)-1,2,3-trihydroxy-1-methyldecahydro-quinolizin-ium chloride (32)



Usual procedure was used for the preparation of **32**.HCl (as that used for **30** .HCl).

$[\alpha]^{27}_D$	+ 27.8 (*c* 1.1, MeOH).
^{1}H NMR (400 MHz, D_2O)	δ 1.12 (s, 3H), 1.36–1.52 (m, 2H), 1.60 (tq, *J* = 13.64, 4.13 Hz, 1H), 1.85 (app s, 1H), 1.88 (app s, 1H), 2.13 (app d, *J* = 13.78, 13.50 Hz, 1H), 2.88 (t, *J* = 12.05 Hz, 1H), 2.95–3.03 (m, 2H), 3.39–3.48 (m, 3H), 3.65 (ddd, *J* = 11.29, 10.16, 5.14 Hz, 1H).
^{13}C NMR (100MHz, D_2O)	13.7 (CH_3), 21.2 (CH_2), 22.7 (CH_2), 22.8 (CH_2), 56.2 (CH_2), 56.7 (CH_2), 64.8 (CH), 68.2 (CH), 72.6 (C), 78.2 (CH).
Mass (ESI): *m/z*	202 (M^++H), 224 (M^++Na).
Elemental analysis	Anal. Calcd. for $C_{10}H_{20}ClNO_3$: C, 50.52; H, 8.48; N, 5.89; Found: C, 50.69; H, 8.39; N, 5.94.

19. Preparation of (3a*S*,9a*R*,10*S*,10a*R*)-2,2-dimethyloctahydro-3aH-[1,3]dioxolo [4,5 -b]quinolizin-10-yl methanesulfonate (48)

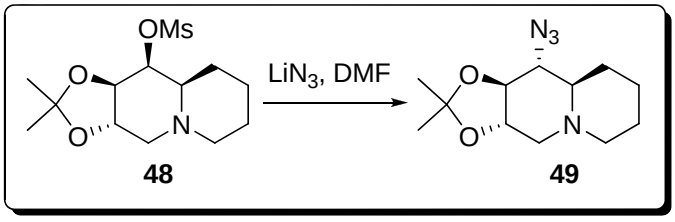


To a solution of **39** (70 mg, 0.309 mmol) in pyridine (2 mL) at 0 °C was added mesyl chloride (42 mg, 0.371 mmol, in 1mL DCM). The reaction mixture was stirred at room temperature for 6 h. When TLC revealed no starting material, the solution was diluted with

dichloromethane (10 mL) and washed with water (3 × 5 mL), brine solution (5 mL) and dried over anhydrous Na_2SO_4. Removal of the solvent followed by column chromatographic purification (silica, pet ether–ethyl acetat:, 7:3) gave **48** (83 mg, 88%) as a white solid (mp183–185 °C).

$[\alpha]^{26}_D$	+41.69 (*c* 1.15, $CHCl_3$).
IR ν_{max} cm^{-1} in $CHCl_3$	2947, 1346, 1266, 1175.
^1H NMR (400 MHz, $CDCl_3$)	δ 1.25–1.31 (m, 2H), 1.43 (s, 3H), 1.44 (s, 3H), 1.59–1.72 (m, 4H), 1.83 (d, *J* = 13.2 Hz, 1H), 2.17–2.23 (m, 2H), 2.26 (t, *J* = 10.18 Hz, 1H), 2.95 (broad d, *J* = 11.55, 1H), 3.12 (s, 3H), 3.23 (dd, *J* = 10.18, 4.18 Hz, 1H), 3.38 (dd, *J* = 9.35, 2.48 Hz, 1H), 3.99 (dt, *J* = 9.53, 3.08 Hz, 1H), 4.97 (t, *J* = 1.93 Hz, 1H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ 23.7 (CH_2), 25.2 (CH_2), 26.3 (CH_3), 26.8 (CH_3), 28.5 (CH_2), 39.1 (CH_3), 56.4 (CH_2), 57.5 (CH_2), 62.2 (CH), 70.7 (CH), 78.7 (CH), 79.4 (CH), 110.9 (C).
Mass (ESI): *m/z*	306 (M^++H), 328 (M^++Na).
Elemental analysis	Anal.Calcd for $C_{13}H_{23}NO_5S$: C, 51.13; H, 7.59; N, 4.59; Found: C, 51.21; H, 7. 63; N, 4.50.
mp	183–185 °C (from EtOAc–pet ether).

20. Preparation of (3a*S*,9a*R*,10*R*,10a*S*)-10-azido-2,2-dimethyloctahydro-3aH-[1,3]dioxolo[4,5-b]quinolizine (49)



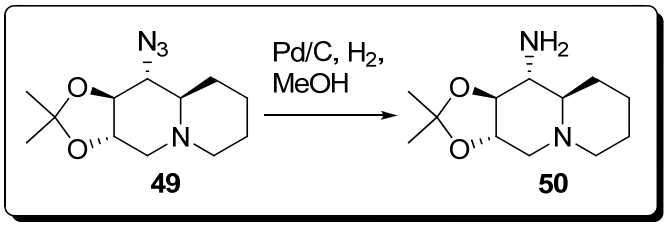
48 **49**

To a solution of **48** (77 mg, 0.251 mmol) in DMF (2 mL) was added LiN_3 (123 mg, 2.51 mmol) and heated to 110 °C for 12 h. When TLC revealed the absence of starting material, the reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The ethyl acetate layer was washed with water and dried over anhydrous Na_2SO_4. Solvent was removed and the crude product purified by column chromatography (silica, pet.

ether–ethyl acetate, 6:1) to give corresponding azide derivative **49** (52 mg, 82%) as a colorless liquid.

$[\alpha]^{25}_{D}$	+ 60.19 (*c* 1.1, $CHCl_3$).
IR ν_{max} cm^{-1} in $CHCl_3$	2936, 2208 (N_3), 2107, 1654, 1446, 1372, 1229.
^1H NMR (400 MHz, $CDCl_3$)	δ 1.19–1.25 (m, 2H), 1.44 (s, 3H), 1.45 (s, 3H), 1.48–1.54 (m, 1H), 1.61–1.64 (m, 1H), 1.74–1.81 (m, 2H), 2.07–2.11 (m, 1H), 2.22 (dt, *J* = 11.83, 2.76 Hz, 1H), 2.25 (t, *J* = 10.17 Hz, 1H), 2.90 (d, *J* = 11.28 Hz,1H), 3.10 (dd, *J* = 9.90, 3.85 Hz, 1H), 3.20–3.28 (m, 2H), 3.58 (ddd, *J* = 10.45, 8.53, 3.85 Hz, 1H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ 23.8 (CH_2), 25.6 (CH_2), 26.7 (CH_3), 29.4 (CH_2), 56.0 (CH_2), 56.9 (CH_2), 64.6 (CH), 65.2 (CH), 74.2 (CH), 81.9 (CH), 111.1 (C).
Mass (ESI): *m/z*	253 (M^++H), 275 (M^++Na).
Elemental analysis	Anal.Calcd for $C_{12}H_{20}N_4O_2$: C, 57.12; H, 7.99; N, 22.21 Found: C, 57.01; H, 7.90; N, 22.05.

21. Preparation of (3a*S*,9a*R*,10*R*,10a*S*)-2,2-dimethyloctahydro-3aH-[1,3]dioxolo[4,5-b]quinolizin-10-amine (50)



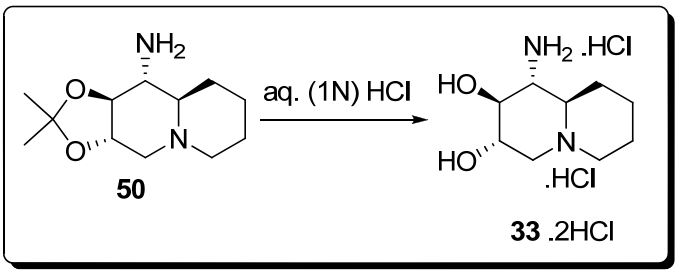
The azide derivative **49** (43 mg, 0.172 mmol) in methanol (2 mL) was hydrogenated for 7 h at atmospheric pressure in the presence of Pd on charcoal (10%, 4 mg). The reaction mixture was passed through a short pad of celite and the solvent was removed under reduced pressure. Column chromatography (silica, $CHCl_3$–MeOH, 24:1) afforded corresponding amine derivative **50**, (39 mg, 85%) as a white solid.

$[\alpha]^{25}_{D}$	+ 39.43 (*c* 0.7, $CHCl_3$).

IR ν_{max} cm^{-1} in $CHCl_3$	3396 (NH), 3054, 2305, 1598, 1265.
^1H NMR (500 MHz, $CDCl_3$)[a]	δ 1.13–1.23 (m, 2H), 1.42 (s, 6H), 1.50–1.56 (m, 1H), 1.58–1.64 (m, 2H), 1.80 (d, *J* = 12.9 Hz, 1H), 2.04–2.07 (m, 1H), 2.21 (dt, *J* = 11.83, 3.03 Hz, 1H), 2.27 (t, *J* = 10.18 Hz, 1H), 2.66 (dd, *J* = 10.09, 8.53 Hz, 1H), 2.89 (d, *J* = 11.56 Hz, 1H), 3.10 (dd, *J* = 10.09, 9.07 Hz, 1H), 3.13 (dd, *J* = 9.88, 4.01 Hz, 1H), 3.58 (ddd, *J* = 10.46, 9.08, 4.13 Hz, 1H).
^{13}C NMR (100 MHz, $CDCl_3$)	δ 24.1 (CH_2), 25.8 (CH_2), 26.7 (CH_3), 26.8 (CH_3), 28.6 (CH_2), 55.7 (CH), 56.2 (CH_2), 57.5 (CH_2), 67.6 (CH), 74.3 (CH), 84.4 (CH), 110.4 (C).
Mass (ESI): *m/z*	227 (M^++H), 249 (M^++Na).
Elemental analysis	Anal.Calcd. for $C_{12}H_{22}N_2O_2$: C, 63.68; H, 9.80; N, 12.38; Found: C, 63.80; H, 9.85; N, 12.35.

a = D_2O exchange

22. Preparation of (1*R*,2*S*,3*S*,9a*R*)-1-amino-2,3-dihydroxydecahydroquinolizinium chloride hydrochloride (33)

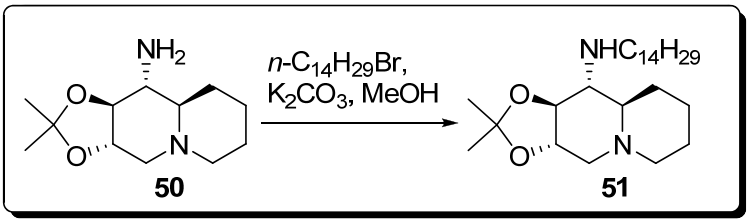


Usual procedure was used for the preparation of **33** .2HCl (as that used for **30** .HCl).

$[\alpha]^{28}_D$	+ 7.29 (*c* 1.4, MeOH).
^1H NMR (400 MHz, D_2O)	δ 1.43–1.72 (m, 3H), 1.88 (app s, 1H), 1.91 (app s, 1H), 2.14 (app d, *J* = 13.3 Hz, 1H), 3.01 (t, *J* = 12.05 Hz, 1H), 3.06 (dt, *J* = 12.90, 2.51 Hz, 1H), 3.22 (t, *J* = 10.79 Hz, 1H), 3.38 (dt, *J* = 11.54, 3.01 Hz, 1H), 3.50–3.54 (m, 2H), 3.61 (t, *J* = 10.04 Hz, 1H), 3.76 (ddd, *J* = 11.29, 9.29, 5.02 Hz,1H).

^{13}C NMR (100 MHz, D_2O)	δ 20.3 (CH_2), 22.2 (CH_2), 26.0 (CH_2), 53.8 (CH), 55.7 (CH_2), 61.7 (CH), 66.5 (CH), 71.8 (CH).
Mass (ESI): *m/z*	187 (M^++H), 209 (M^++Na).
Elemental analysis	Anal.Calcd. for $C_9H_{20}Cl_2N_2O_2$: C, 41.71; H, 7.78; N, 10.81; Found: C, 41.83; H, 7.83; N, 10.77.

23. Preparation of (3a*S*,9a*R*,10*R*,10a*S*)-2,2-dimethyl-N-tetradecyloctahydro-3aH-[1,3]dioxolo[4,5-b]quinolizin-10-amine (51)



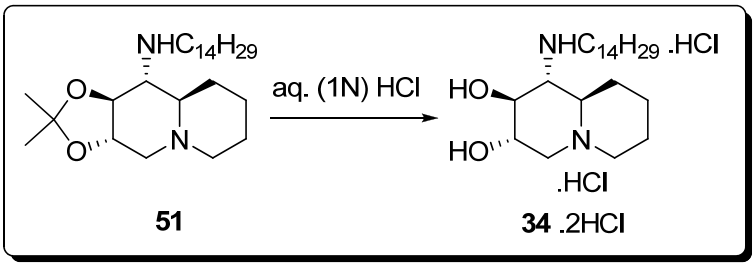
To a stirring solution of **50** (0.025 g, 0.11 mmol) in dry CH_3CN and THF (3:1), (2 mL) was added tetradecyl bromide (0.031 g, 0.11 mmol) followed by K_2CO_3 (0.029 g, 0.22 mmol) and the reaction mixture was refluxed for 12 h. Later water (2 mL) was added and mixture extracted in ethyl acetate (2 × 5 mL), dried over Na_2SO_4, concentrated and purified by column chromatography (silica, pet. ether–ethyl acetate, 7:3) to get **51** (0.030 g, 65%) as yellow liquid.

$[\alpha]^{25}_D$	+ 28.9 (*c* 1.5, CHCl3).
^{1}H NMR (500 MHz, $CDCl_3$)[a]	δ 0.86 (t, *J* = 6.79 Hz, 3H), 1.11–1.20 (m, 2H), 1.24 (s, 24H), 1.41 (s, 6H), 1.48–1.56 (m, 1H), 1.61 (d, *J* = 13.20 Hz, 1H), 1.70 (dt, *J* = 9.70, 2.07 Hz, 1H), 1.79 (d, *J* = 11.97 Hz, 1H), 2.12 (d, *J* = 11.97 Hz, 1H), 2.19 (dt, *J* = 11.96, 2.89 Hz, 1H), 2.23 (t, *J* = 10.18 Hz, 1H), 2.46 (t, *J* = 9.35 Hz, 1H), 2.62 (td, *J* = 11.14, 7.15 Hz, 1H), 2.77 (td, *J* = 11.28, 7.16 Hz, 1H), 2.89 (d, *J* = 11.42 Hz, 1H), 3.10 (dd, *J* = 9.77, 3.98 Hz, 1H), 3.19 (t, *J* = 9.59 Hz, 1H), 3.55 (ddd, *J* = 10.40, 9.08, 3.98 Hz, 1H).
^{13}C NMR (100MHz, $CDCl_3$)	14.1 (CH_3), 22.7 (CH_2), 24.3 (CH_2), 25.9 (CH_2), 26.7

	(CH_3), 26.9 (CH_3), 27.2 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 30.7 (CH_2), 31.9 (CH_2), 47.9 (CH_2), 56.4 (CH_2), 57.5 (CH_2), 62.0 (CH), 65.9 (CH), 74.7 (CH), 83.7 (CH), 110.0 (C).
Mass (ESI): *m/z*	423 (M^++H).
Elemental analysis	Anal.Calcd for $C_{26}H_{50}N_2O_2$: C, 73.88; H, 11.92; N, 6.63; O, 7.57; Found: C, 73.69; H, 11.75; N, 6.59.

a = D_2O exchange

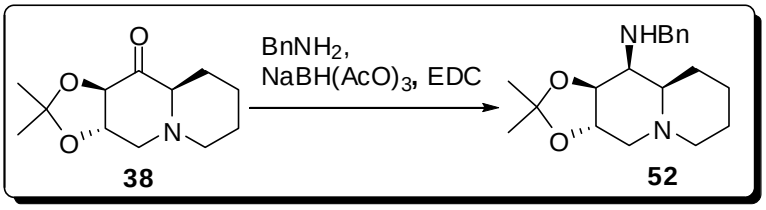
24. Preparation of (1*R*,2*S*,3*S*,9a*R*)-2,3-dihydroxy-1-(tetradecylamino)decahydro-quinolizinium chloride hydrochloride (34)



Usual procedure was used for the preparation of **34** .2HCl (as that used for **30** .HCl).

$[\alpha]^{26}_D$	+ 5.4 (*c* 0.3, MeOH).
^{1}H NMR (500 MHz, CD_3OD)	δ 0.81 (t, *J* = 6.90 Hz, 1H), 1.20–1.35 (m, 24H), 1.68–1.91 (m, 5H), 2.02–2.11 (m, 1H), 2.95–3.08 (m, 4H), 3.33–3.40 (m, 2H), 3.52 (br d, *J* = 11.87 Hz, 1H), 3.68–3.72 (m, 2H), 3.84–3.95 (m, 1H).
^{13}C NMR (125 MHz, CD_3OD)	δ 14.6 (CH_3), 22.2 (CH_2), 23.7 (CH_2), 23.9 (CH_2), 27.6 (CH_2), 27.7 (CH_2), 30.3 (CH_2), 30.4 (CH_2), 30.6 (CH_2), 30.7 (CH_2), 30.8 (CH_2), 30.90 (CH_2), 30.92 (CH_2), 33.2 (CH_2), 46.2 (CH_2), 57.0 (CH_2), 57.7 (CH_2), 61.2 (CH), 61.9 (CH), 68.2 (CH), 71.8 (CH).
Mass (ESI): *m/z*	383 (M^++H).
Elemental analysis	Anal.Calcd. for $C_{23}H_{48}Cl_2N_2O_2$: C, 60.64; H, 10.62; N, 6.15; Found: C, 60.78; H, 10.69; N, 6.11.

25. Preparation of (3a*S*,9a*R*,10*S*,10a*S*)-N-benzyl-2,2-dimethyloctahydro-3aH-[1,3]-dioxolo[4,5-b]quinolizin-10-amine (52)[15]



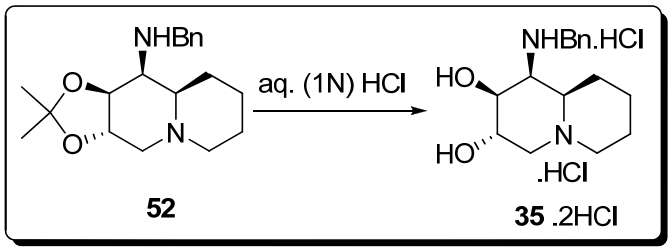
38 **52**

To the solution of **37** (0.078 g, 0.345 mmol) in dry 1,2-dichloroethane (2 mL), benzyl amine (0.045 g, 0.415 mmol) followed by sodium triacetoxyborohydride (0.147 g, 0.69 mmol) was added. The mixture was stirred at rt under argon atmosphere for 3 h. The reaction mixture was ice cooled and quenched by adding (2N) NaOH till the aq. layer was basic. The reaction mixture was extracted with ethyl acetate (2 × 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, pet. ether–ethyl acetate, 4:1) to afford **52** (0.082 g, 75%) as a single diastereomer in the form of a white solid.

$[\alpha]^{26}_D$	+18.41 (*c* 1.2, $CHCl_3$).
IR ν_{max} cm^{-1} in $CHCl_3$	3351 (NH), 2984 (CH arom), 1604(C=C arom), 1453, 1370, 1235.
^1H NMR (500 MHz, $CDCl_3$)	δ 1.20–1.27 (m, 1H), 1.44 (s, 3H), 1.45 (s, 3H), 1.48–1.59 (m, 3H), 1.76–1.84 (m, 2H), 1.97 (td, *J* = 11.14, 2.38 Hz, 1H), 2.11 (dt, *J* = 11.72, 3.53 Hz, 1H), 2.14 (t, *J* = 10.24 Hz, 1H), 2.91 (broad d, *J* = 11.37 Hz, 1H), 3.04 (t, *J* = 2.91 Hz, 1H), 3.17 (dd, *J* = 9.37, 4.10 Hz, 1H), 3.38 (dd, *J* = 9.45, 3.63 Hz, 1H), 3.88 (d, *J* = 13.11 Hz, 1H), 3.98 (dt, *J* = 10.11, 4.14 Hz, 1H), 4.01 (d, *J* = 13.11 Hz, 1H), 7.19–7.22 (m, 1H), 7.27–7.30 (m, 2H), 7.35–7.36 (m, 2H).
^{13}C NMR (125 MHz, $CDCl_3$)	δ 24.3 (CH_2), 25.4 (CH_2), 26.6 (CH_3), 26.9 (CH_3), 29.5 (CH_2), 54.4 (CH_2), 56.8 (CH_2), 58.3 (CH), 58.4 (CH_2), 64.9 (CH), 70.8 (CH), 82.9 (CH), 109.8 (C), 126.7 (CH), 128.10 (CH), 128.13 (CH), 140.9 (C).

Mass (ESI): *m/z*	317 (M^++H), 339 (M^++Na).
Elemental analysis	Anal.Calcd. for $C_{19}H_{28}N_2O_2$: C, 72.12; H, 8.92; N, 8.85; Found: C, 71.99; H, 8.97; N, 8.81.

26. Preparation of (1S,2S,3S,9a*R*)-1-(benzylamino)octahydro-1H-quinolizine-2,3-diol (35)

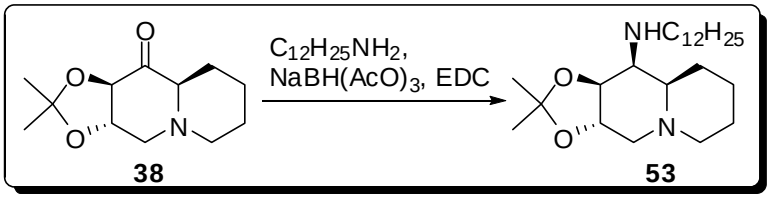


52 **35** .2HCl

Usual procedure was used for the preparation of **35** .2HCl (as that used for **30** .HCl).

$[\alpha]^{26}_D$	+ 5.2 (*c* 1.1, MeOH).
^{1}H NMR (400 MHz, D_2O)	δ 1.33–1.44 (m, 1H), 1.70–1.89 (m, 4H), 2.23–2.34 (m, 1H), 3.03 (d, *J* = 13.3 Hz, 1H), 3.23–3.32 (m, 2H), 3.73–3.81 (m, 2H), 3.97 (d, *J* = 13.30 Hz, 1H), 4.16–4.18 (m, 2H), 4.25 (d, *J* = 13.25 Hz, 1H), 4.35 (d, *J* = 13.25 Hz, 1H), 7.40–7.46 (m, 5H).
^{13}C NMR (100 MHz, D_2O)	δ 20.0 (CH_2), 22.0 (CH_2), 24.0 (CH_2), 48.2 (CH_2), 51.4 (CH_2), 55.3 (CH), 56.6 (CH_2), 59.1 (CH), 65.6 (CH), 68.5 (CH), 131.7 (CH), 131.9 (CH), 132.0 (C), 132.5 (CH), 132.7 (CH).
Mass (ESI): *m/z*	277 (M^++H).
Elemental analysis	Anal.Calcd. for : $C_{16}H_{26}Cl_2N_2O_2$: C, 55.02; H, 7.50; N, 8.02; Found: C, 54.89; H, 7.55; N, 8.09.

27. Preparation of (3a*S*,9a*R*,10*S*,10a*S*)-N-dodecyl-2,2-dimethyloctahydro-3aH-[1,3]dioxolo[4,5-b]quinolizin-10-amine (53)[15]

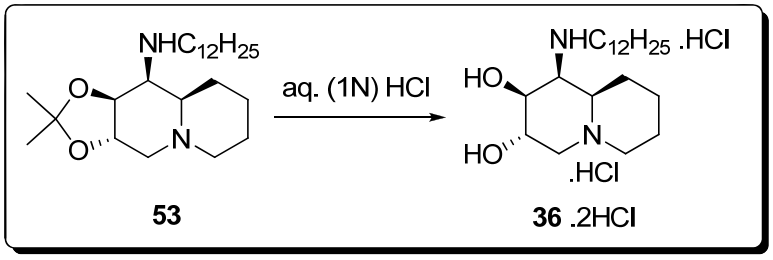


38 **53**

To the solution of **37** (0.084 g, 0.373 mmol) in dry 1,2-dichloroethane (2 mL), *n*-dodecyl amine (0.072 g, 0.391 mmol) followed by sodium triacetoxyborohydride (0.103 g, 0.487 mmol) was added. The mixture was stirred at rt under argon atmosphere for 3 h. The reaction mixture was ice cooled and quenched by adding 2N NaOH till the aq. layer was basic. The reaction mixture was extracted with ethyl acetate (2 × 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, pet. ether–ethyl acetate, 4:1) to afford **53** (0.096 g, 65%) as a single diastereomer in the form of a white solid.

$[\alpha]^{25}_D$	+ 17.19 (*c* 0.8, $CHCl_3$).
^{1}H NMR (500 MHz, $CDCl_3$)	δ 0.86 (t, *J* = 6.84 Hz, 3H), 1.24–1.27 (m, 21H), 1.42 (s, 3H), 1.43 (s, 3H), 1.50–1.57 (m, 4H), 1.67–1.79 (m, 1H), 1.75–1.79 (m, 1H), 1.92 (d, *J* = 11.01 Hz, 1H), 2.08–2.13 (m, 2H), 2.62–2.72 (m, 2H), 2.90–2.92 (m, 2H), 3.15 (dd, *J* = 9.94, 4.16 Hz, 1H), 3.37 (dd, *J* = 9.40, 3.52 Hz, 1H), 3.89 (dt, *J* = 9.73, 4.07 Hz, 1H).
^{13}C NMR, (100 MHz, $CDCl_3$)	δ 14.1 (CH_3), 22.7 (CH_2), 24.4 (CH_2), 25.5 (CH_2), 26.6 (CH_3), 26.9 (CH_3), 27.2 (CH_2), 29.3 (CH_2), 29.51 (CH_2), 29.58 (CH_2), 29.6 (CH_2), 30.5 (CH_2), 31.9 (CH_2), 51.6 (CH_2), 56.8 (CH_2), 58.3 (CH_2), 59.7 (CH), 65.2 (CH), 70.9 (CH), 82.4 (CH), 109.7 (C).
Mass (ESI): *m/z*	395 (M^++H), 417 (M^++Na).
Elemental analysis	Anal.Calcd. for : $C_{24}H_{46}N_2O_2$: C, 73.04; H, 11.75; N, 7.10; Found: C, 73.18; H, 11.69; N, 7.06.

28. Preparation of (1*S*,2*S*,3*S*,9a*R*)-1-(dodecylamino)-2,3-dihydroxydecahydro-quinolizinium chloride hydrochloride (36)

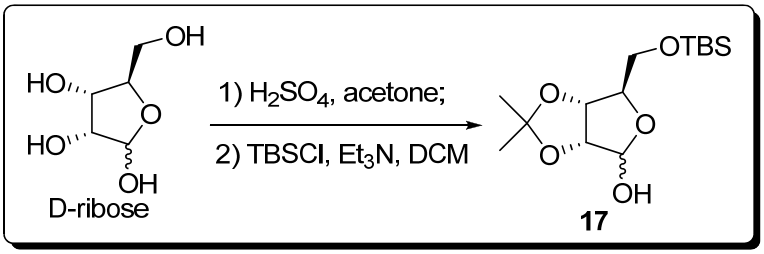


53 **36** .2HCl

Usual procedure was used for the preparation of **36** .HCl (as that used for **30** .HCl).

$[\alpha]^{25}_{D}$	+ 20.3 (*c* 0.3, MeOH).
^{1}H NMR (400 MHz, D_2O)	δ 0.76 (t, *J* = 6.23 Hz, 1H), 1.18–1.45 (m, 20H) 1.65–1.89 (m, 5H), 2.22-2.31 (m, 1H), 2.96–3.11 (m, 3H), 3.25–3.33 (m, 2H), 3.74–3.84 (m, 2H), 3.96 (d, *J* = 13.48 Hz, 1H), 4.11–4.16 (m, 2 H).
^{13}C NMR, (100 MHz, D_2O)	δ 13.4 (CH_3), 17.5 (CH_2), 19.4 (CH_2), 21.5 (CH_2), 22.0 (CH_2), 25.3 (CH_2), 25.7 (CH_2), 25.8 (CH_2), 28.1 (CH_2), 28.2 (CH_2), 28.5 (CH_2), 28.9 (CH_2), 31.2 (CH_2), 45.7 (CH_2), 53.5 (CH), 54.1 (CH_2), 56.5 (CH), 63.1 (CH), 66.0 (CH).
Mass (ESI): *m/z*	355 (M^++H).
Elemental analysis	Anal.Calcd. for $C_{21}H_{44}Cl_2N_2O_2$: C, 59.00; H, 10.37; N, 6.55; Found: C, 59.17; H, 10.41; N, 6.58.

29. Preparation of (3a*R*,6*R*,6a*R*)-6-((*tert*-butyldimethylsilyloxy)methyl) -2, 2 dimethyltetrahydro furo [3,4-d] [1,3]dioxol-4-ol (17)[19]



To an ice cold suspension of D-ribose (20.0 g, 133.21 mmol) in acetone (200 mL), was added conc H_2SO_4 (1.0 mL). The clear solution was obtained after 30 minutes. The reaction mixture was neutralized by the addition of $Ca(OH)_2$ and the salt was removed by filtration. The filtrate was concentrated in *vacuo* to give the crude product (23.1 g) as colorless oil and was passed through a short pad of silica gel and concentrated.

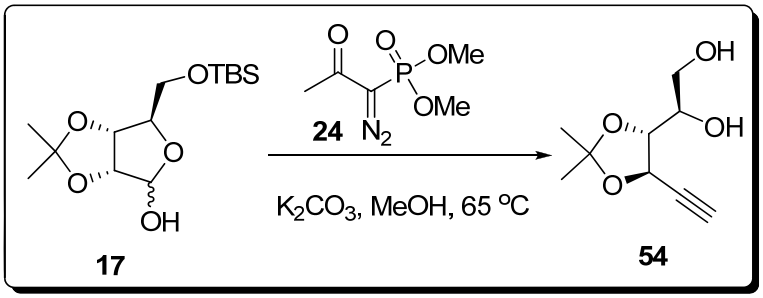
To a stirred solution of this acetonide protected precursor in dry DCM (500 mL) was added triethyl amine (20.4 mL, 146.6 mmol) and *tert*-butyldimethylsilylchloride (19.9 g, 133.3 mmol) at room temperature and the mixture stirred for 3 h. Water (100 mL) was added to the reaction mixture and was extracted with DCM (2 × 200 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified

by silica gel column chromatography (silica, pet ether–ethyl acetate, 7:3) to give **17** (32.4 g, 80%) as colorless oil (9:1 anomeric mixture).

IR ν_{max} cm^{-1} in $CHCl_3$	3411, 2935, 1074.
^1H NMR (200 MHz, $CDCl_3$)[a]	δ 0.11 (s, 3H), 0.12 (s, 3H), 0.90 (s, 9H), 1.30 (s, 3H), 1.46 (s, 3H), 3.73 (d, *J* = 1.39 Hz, 1H), 3.75 (d, *J* = 1.39 Hz, 1H), 4.33 (s, 1H), 4.48 (d, *J* = 5.94 Hz, 1H), 4.68 (d, *J* = 6.19 Hz, 1H), 5.25 (s, 1H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ −5.84 (CH_3), −5.80 (CH_3), 18.1 (C), 24.8 (CH_3), 25.6 (CH_3), 26.3 (CH_3), 64.7 (CH_2), 81.7 (CH), 86.8 (CH) 87.3 (CH), 103.1 (CH), 111.9 (C).
Mass (ESI): *m/z*	322 (M^++NH_4), 327 (M^++Na).

a = D_2O exchange

30. Preparation of (*R*)-1-((4*R*,5*R*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethane-1,2-diol (54)



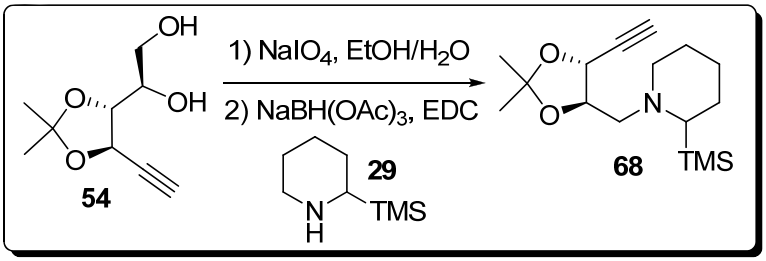
To a stirred mixture of **17** (20.0 g, 45.24 mmol) and anhydrous K_2CO_3 (8.23 g, 58.82 mmol) in dry MeOH (160 mL) at 65 °C was added the solution of Bestmann-Ohira reagent (**24**, 11.29 g, 58.82 mmol) in dry MeOH (80 mL) drop wise over a period of 6 h under argon atmosphere. Acetic acid was added drop wise to neutralize the reaction mixture. Latter, solvent was removed followed by addition of water (100 mL) to the residue. The reaction mixture was extracted by ethyl acetate (2 × 100 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4. Concentration under reduced pressure and purification by column chromatography (silica, pet. ether–ethyl acetate, 3:2) provided **54** (4.55 g, 55%) as white solid.

$[\alpha]^{30}_D$	+7.27 (*c* 0.55, $CHCl_3$).

IR ν_{max} cm^{-1} in $CHCl_3$	3306, 3019, 1215.
^1H NMR (200 MHz, $CDCl_3$)[a]	δ 1.40 (s, 3H), 1.48 (s, 3H), 2.56 (d, *J* = 2.01 Hz, 1H), 2.87 (bs, 1H), 3.26 (bs, 1H), 3.66 (dd, *J* = 11.54, 6.53 Hz, 1H), 3.76 (dd, *J* = 11.54, 3.26 Hz, 1H), 3.83–3.86 (m, 1H), 4.11 (t, *J* = 6.52 Hz, 1H), 4.68 (dd, *J* = 6.78, 2.01 Hz, 1H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ 5.8 (CH_3), 26.8 (CH_3), 63.1 (CH_2), 66.8 (CH), 71.7 (CH), 74.7 (C), 81.7 (CH), 110.9 (C).
Mass (ESI): *m/z*	187 (M^++H).
Elemental analysis	Anal. Calcd for $C_9H_{14}O_4$: C, 58.05; H, 7.58; Found: C, 58.15; H, 7.45.

a = D_2O exchange

31. Preparation of N-Benzyl((4*R*,5*R*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)-N-((trimethylsilyl) methyl) methanamine (68)



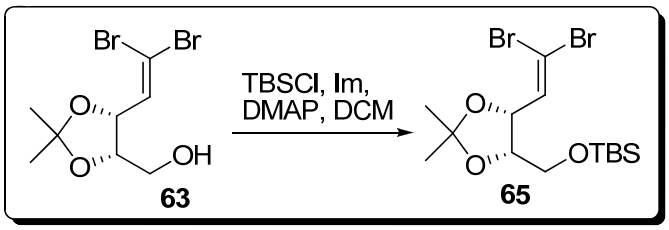
To a solution of **54** (5.0 g, 26.89 mmol) in ethanol–water (100 mL, 4:1) was added sodium periodate (6.9 g, 32.25 mmol) gradually. The white suspension was stirred for 0.5 h and filtered. The filtrate was concentrated and the white pasty mass was extracted with ethyl acetate (2 × 100 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure. To a solution of this aldehyde in EDC (ethylene dichloride, 90 mL), was added cyclic amine **29** (5.06 g, 32.25 mmol) and $Na(OAc)_3BH$ (7.98 g, 37.63 mmol) under argon atmosphere and stirred for 3 h. The reaction mixture was ice cooled and quenched by adding (2N) NaOH till the aq. layer was basic. After stirring for 0.5 h further, reaction mixture was extracted in DCM. The solvent was removed under reduced pressure and crude product was purified by column chromatography (silica, pet ether–ethyl acetate, 6:1) to furnish **68** (3.97 g, 50% over two steps). Spectral data was identical as reported for its enantiomer.

32. Preparation of ((4*S*,5*R*)-5-(2,2-dibromovinyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-methanol (63)[23]

Anhydrous 1,4-dioxane (200 mL) was added to a mixture of dibromomethyl-triphenylphosphonium bromide (25.76 g, 50 mmol) and *t*-BuOK (5.47 g, 48.8 mmol) under argon atmosphere. The mixture was stirred at rt and after 15 min, the lactol **16** (2 g, 12.5 mmol) was added. After the mixture was allowed to stir at 60 °C for 20 min, it was cooled to rt followed by filtration using Buchner funnel. After an extraction with DCM, the combined organic extracts was dried (Na_2SO_4) and concentrated. Column chromatography of reaction mixture (silica, pet. ether–EtOAc, 3:1) afforded **63** (2.8 g, 71%).

IR (neat) ν_{max} cm^{-1}	3381, 1642, 1217.
^1H NMR (400 MHz, $CDCl_3$)	δ 1.38 (s, 3H), 1.49 (3H), 1.94 (s, 1H), 3.52–3.88 (m, 2H), 4.33–4.41 (m, 1H), 4.84 (t, *J* = 7.43 Hz, 1H), 6.59 (d, *J* = 7.99 Hz, 1H).
^{13}C NMR, (100 MHz, $CDCl_3$)	24.9 (CH_3), 27.4 (CH_3), 61.4 (CH_2), 67.9 (CH), 77.6 (CH), 92.4 (C), 109.4 (C), 134.9 (CH).
Mass (ESI): *m/z*	317 (M^++H).

33. Preparation of *tert*-butyl(((4*S*,5*R*)-5-(2,2-dibromovinyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)dimethylsilane (65)

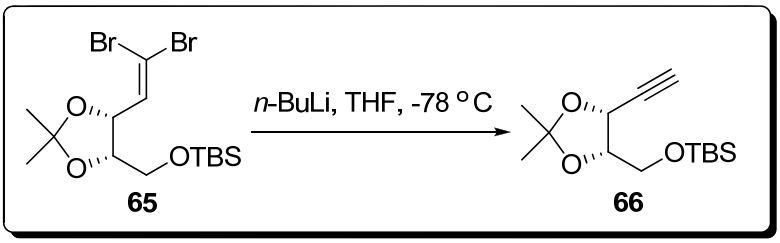


To a solution containing crude reaction mixture of **63** (2.5 g, 7.91 mmol) in DCM (25 mL) at 0 °C, was added imidazole (6.46 g, 9.5 mmol), TBSCl (1.19 g, 7.91 mmol) and catalytic amount of DMAP. The solution was stirred for 3 h, water (20 mL) was added, extracted

with DCM (3 × 25 mL), the combined organic phases were dried over anhydrous Na_2SO_4 and the solvent evaporated. Column chromatography (silica, pet. ether–ethyl acetate, 9:1) afforded **65** (3.20 g, 90%) as a viscous colorless oil.

IR (neat) ν_{max} cm^{-1}	1631, 1458, 1382, 1212.
^1H NMR (400 MHz, $CDCl_3$)	δ 0.06 (s, 3H), 0.89 (s, 9H), 1.35 (s, 3H), 1.45 (s, 3H), 2.16 (s, 1H), 3.64 (d, *J* = 5.43 Hz, 2H), 4.25 (dd, *J* = 5.55, 11.85), 4.82 (dd, *J* = 6.43, 8.39 Hz, 1H), 6.56 (d, *J* = 8.42 Hz, 1H).
^{13}C NMR, (100 MHz, $CDCl_3$)	δ −5.5 (CH_3), −5.4 (CH_3), 18.2 (C), 25.2 (CH_3), 25.80 (CH_3), 25.84 (CH_3), 27.6 (CH_3), 61.6 (CH_2), 73.7 (CH), 77.7 (CH), 92.4 (C), 109.2 (C), 134.9 (CH).
Mass (ESI): *m/z*	431 (M^++H), 453 (M^++Na).

34. Preparation of *tert*-butyl(((4*S*,5*R*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy)dimethylsilane (66)

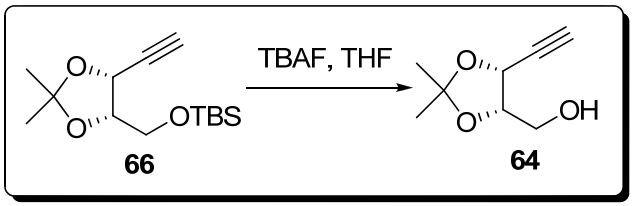


To a solution of **65** (2 g, 4.4 mmol) in dry THF (15 mL) at −78 °C was added a 2M hexane solution of *n*-BuLi (5.1 ml, 10.2 mmol) over a period of 15 min. The reaction mixture was stirred at −78 °C for 45 min. and was quickly brought up to 0 °C. It was quenched by rapid addition of a large excess of water (5 mL). The reaction mixture was extracted with ethyl acetate (2 × 15 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, 24:1) to afford **66** (1.08 g, 90%) as a colorless oil.

$[\alpha]^{27.2}_D$	+ 34.1 (*c* 1.4, MeOH).
IR (neat) ν_{max} cm^{-1}	3311, 1471, 1350, 1244.
^1H NMR (400 MHz, $CDCl_3$)	δ 0.07 (s, 6H), 0.88 (s, 9H), 1.34 (s, 3H), 1.52 (s, 3H),

	2.49 (d, J = 2.28 Hz, 1H), 3.89 (d, J = 6.06 Hz, 2H), 4.17 (dd, J = 6.11, 12.09 Hz, 1H), 2.27, 5.77 Hz, 1H).
^{13}C NMR, (100 MHz, $CDCl_3$)	δ −5.4 (CH_3), −5.4 (CH_3), 18.3 (C), 25.8 (CH_3), 26.0 (CH_3), 27.6 (CH_3), 62.8 (CH_2), 68.0 (CH), 75.5 (CH), 77.9 (C), 79.7 (CH), 110.3 (C).
Mass (ESI): *m/z*	271 (M^++H).
Elemental analysis	Anal.Calcd for $C_{14}H_{26}O_3Si$: C, 62.18; H, 9.69; Found: C, 62.29; H, 9.73.

35. Preparation of ((4*S*,5*R*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (64)



To a solution of **66** (1 g, 3.7 mmol) in dry THF (8 mL) was added a 1M solution of TBAF in THF (3.7 mL, 3.7 mmol) at 0 °C. The reaction mixture was stirred at rt for 3 h and to this was added water (4 mL). The reaction mixture was extracted with ethyl acetate (2 × 8 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Column chromatography (silica, chloroform–ethyl acetate, 9:1) afforded pure **64** (0.462 g, 80%) as a colorless liquid.

$[\alpha]^{25}_D$	+ 15.3 (*c* 0.75, MeOH).
IR (neat) ν_{max} cm^{-1}	3435, 3300, 1472, 1231.
^{1}H NMR (400 MHz, $CDCl_3$)	δ 1.33 (s, 3H), 1.50 (s, 3H), 1.55 (d, J = 2.23 Hz, 1H), 3.79–3.81 (m, 2H), 4.24 (dd, J = 6.22, 11.84 Hz, 1H), 4.82 (dd, J = 2.18, 6.27 Hz, 1H).
^{13}C NMR, (100 MHz, $CDCl_3$)	δ 25.5 (CH_3), 27.5 (CH_3), 62.6 (CH_2), 66.9 (CH), 76.1 (CH), 77.5 (CH), 78.7 (C), 110.4 (C).
Mass (ESI): *m/z*	157 (M^++H), 179 (M^++Na).
Elemental analysis	Anal.Calcd for $C_8H_{12}O_3$: C, 61.52; H, 7.74; Found: C, 61.41; H, 7.85.

General procedure for enzyme inhibition assay

Inhibition assay for evaluating 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.

General assay procedure

To a 10 mM solution of inhibitor in water was added appropriately diluted enzyme. 50 µL of buffer of appropriate pH was added to that. Double distilled water was added to make the final volume of the reaction mixture to 500 µL. Substrate of appropriate concentration and volume was added to that. The reaction was incubated for a suitable time period at appropriate temperature (on a water bath). It was quenched by the addition of 1.0 mL of (1M) Na_2CO_3 solution. The absorbance of the resulting solution was read at 405 nm.

Once the inhibitor was found to show inhibition to enzyme, IC_{50} (half maximal inhibitory concentration**)** was determined by checking the inhibition at various inhibitor concentrations with all other parameter remaining the same. Concentration of inhibitor was plotted against the percentage of inhibition it shows. IC_{50} was determined from the graph, which was the concentration of inhibitor showing 50% of inhibition. Dixon method was employed for the determination of *Ki* (dissociation constant of the enzymatic reaction). In this method, hydrolytic activity of enzyme was measured in the presence of two different concentrations of substrates and varying concentrations of inhibitors. The reciprocals of substrate hydrolysis (1/V) were plotted against the inhibitor concentration and the *Ki* was determined by fitting the data using ORIGIN 6.1.

2B.10-References

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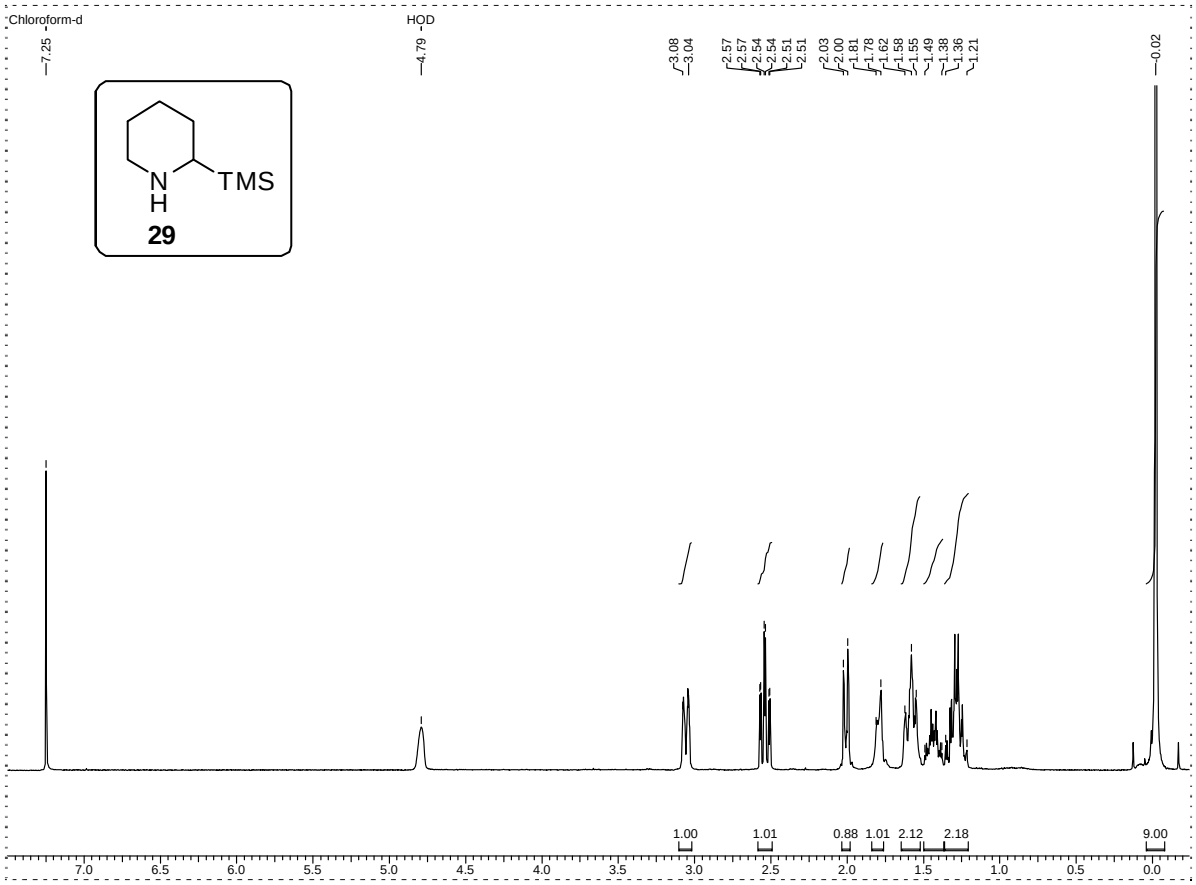
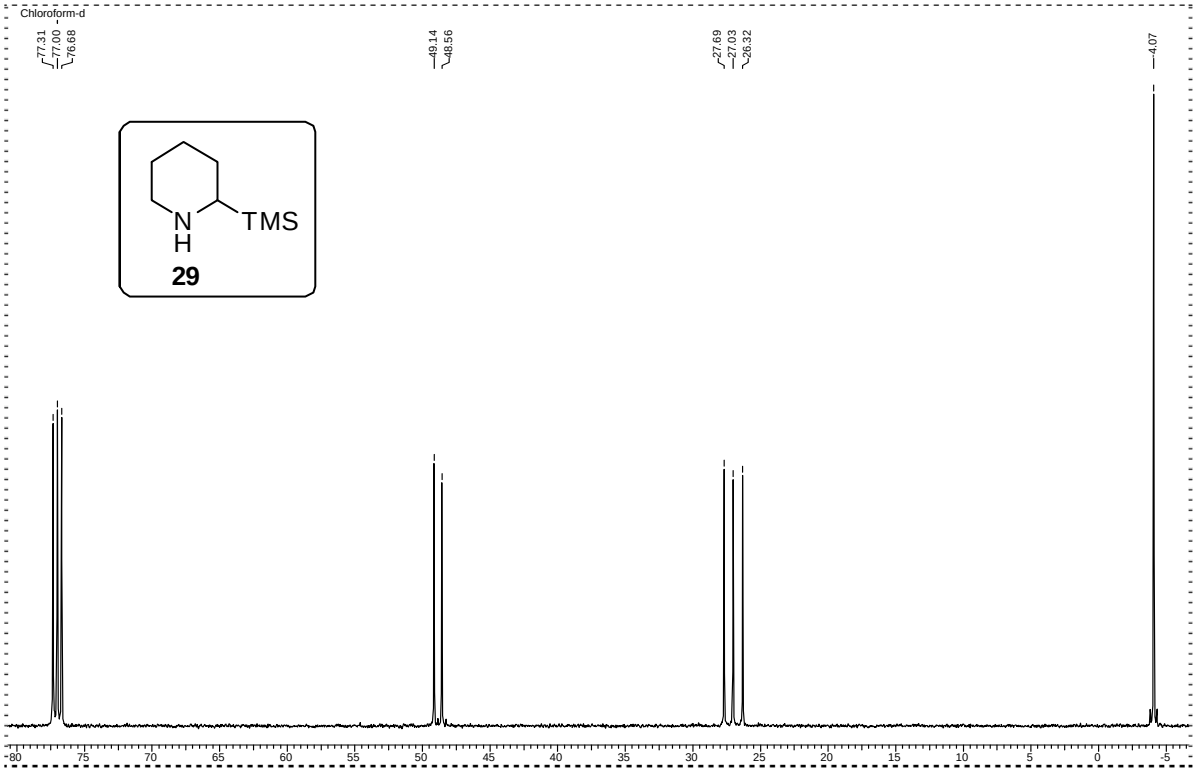
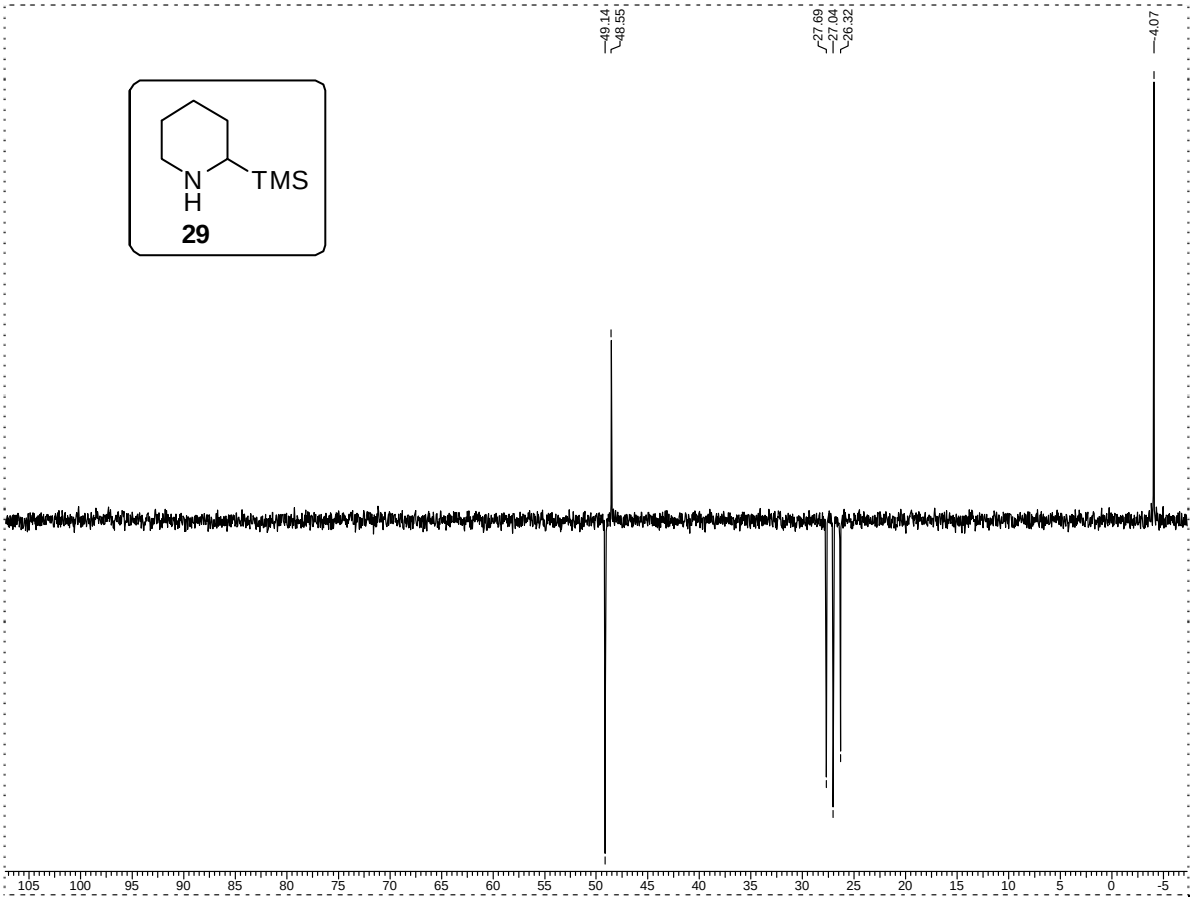
2B.11-Spectra and Dixon plots



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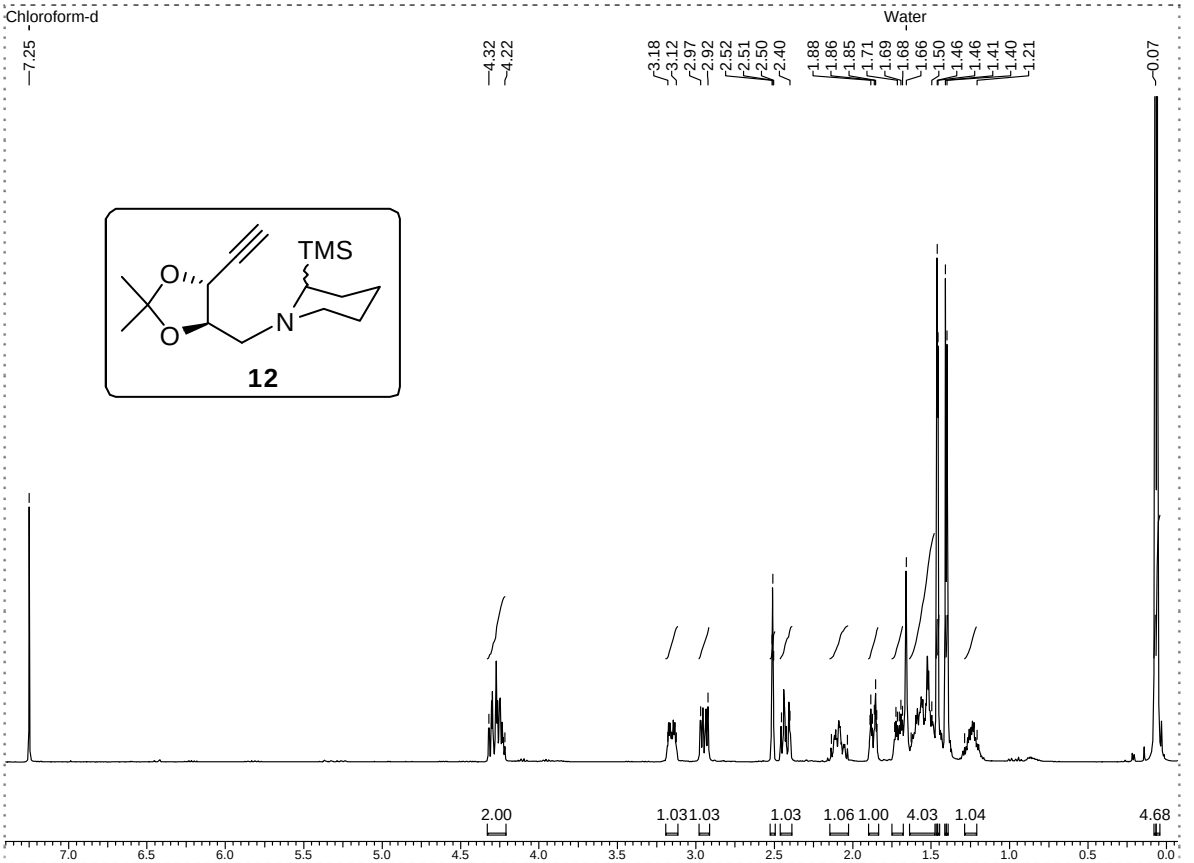
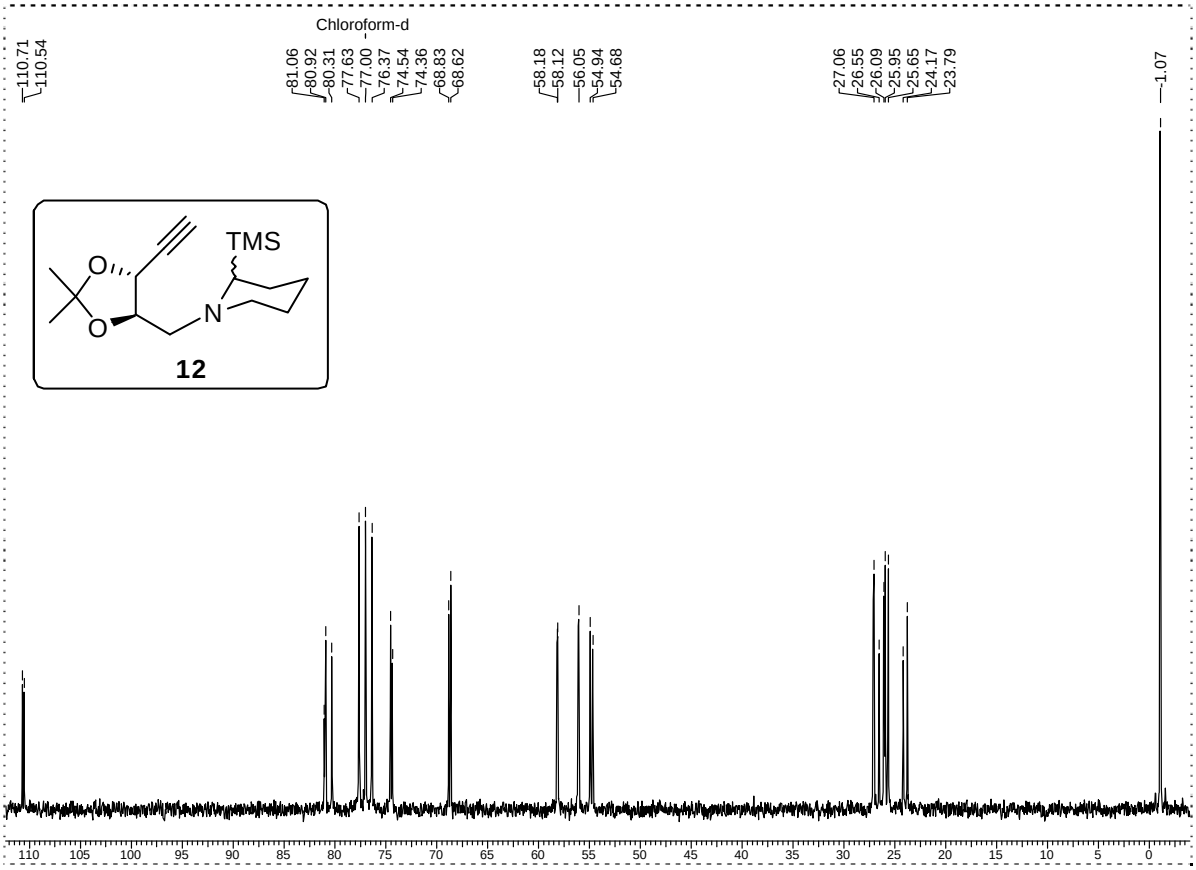
48.55
27.69
27.04
26.32
4.07
N
H
TMS
29


Chloroform-d
Water
7.25
4.32
4.22
3.18
3.12
2.97
2.92
2.52
2.51
2.50
2.40
1.88
1.86
1.85
1.71
1.69
1.68
1.66
1.50
1.46
1.46
1.41
1.40
1.21
0.07
TMS
O
O
N
12
2.00
1.03
1.03
1.03
1.06
1.00
4.03
1.04
4.68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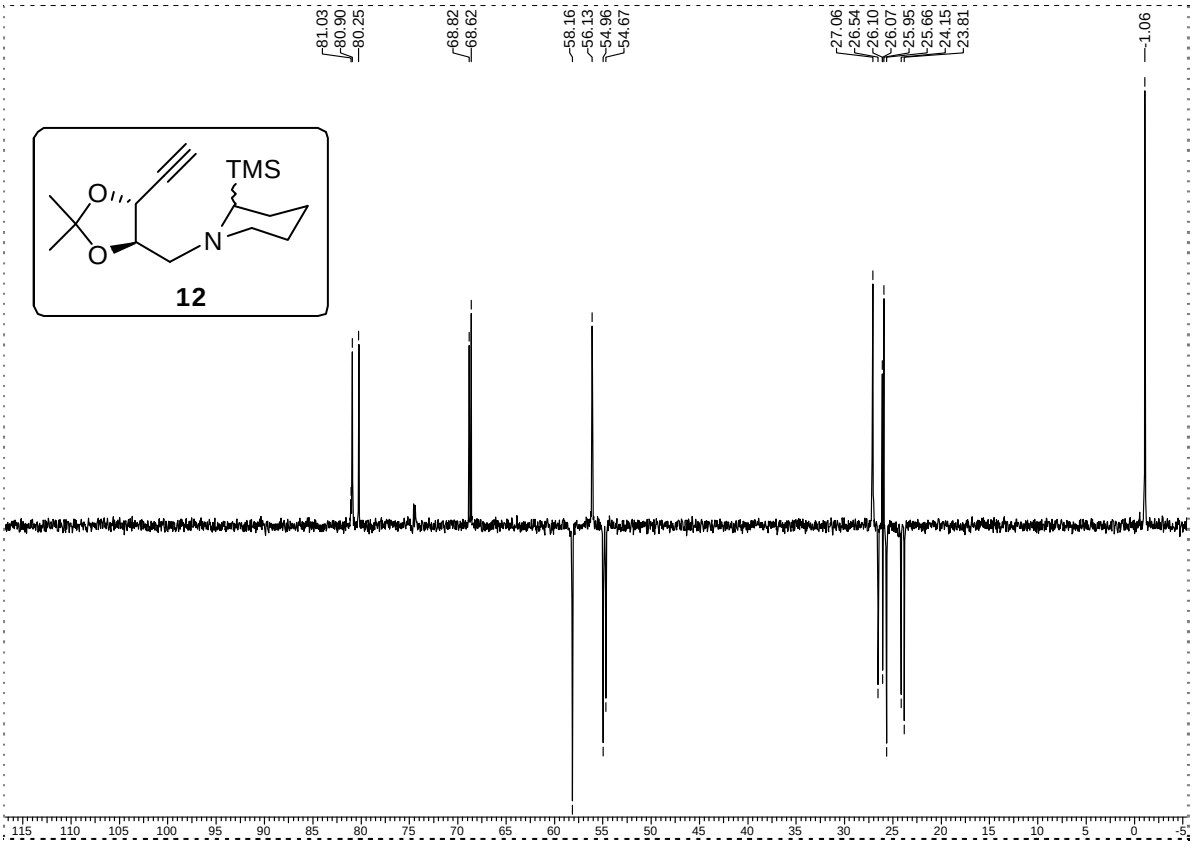
110.71
110.54
81.06
80.92
80.31
77.63
77.00
76.37
74.54
74.36
68.83
68.62
58.18
58.12
56.05
54.94
54.68
27.06
26.55
26.09
25.95
25.65
24.17
23.79
1.07
TMS
O
O
N
12


81.03
80.90
80.25
68.82
68.62
58.16
56.13
54.96
54.67
27.06
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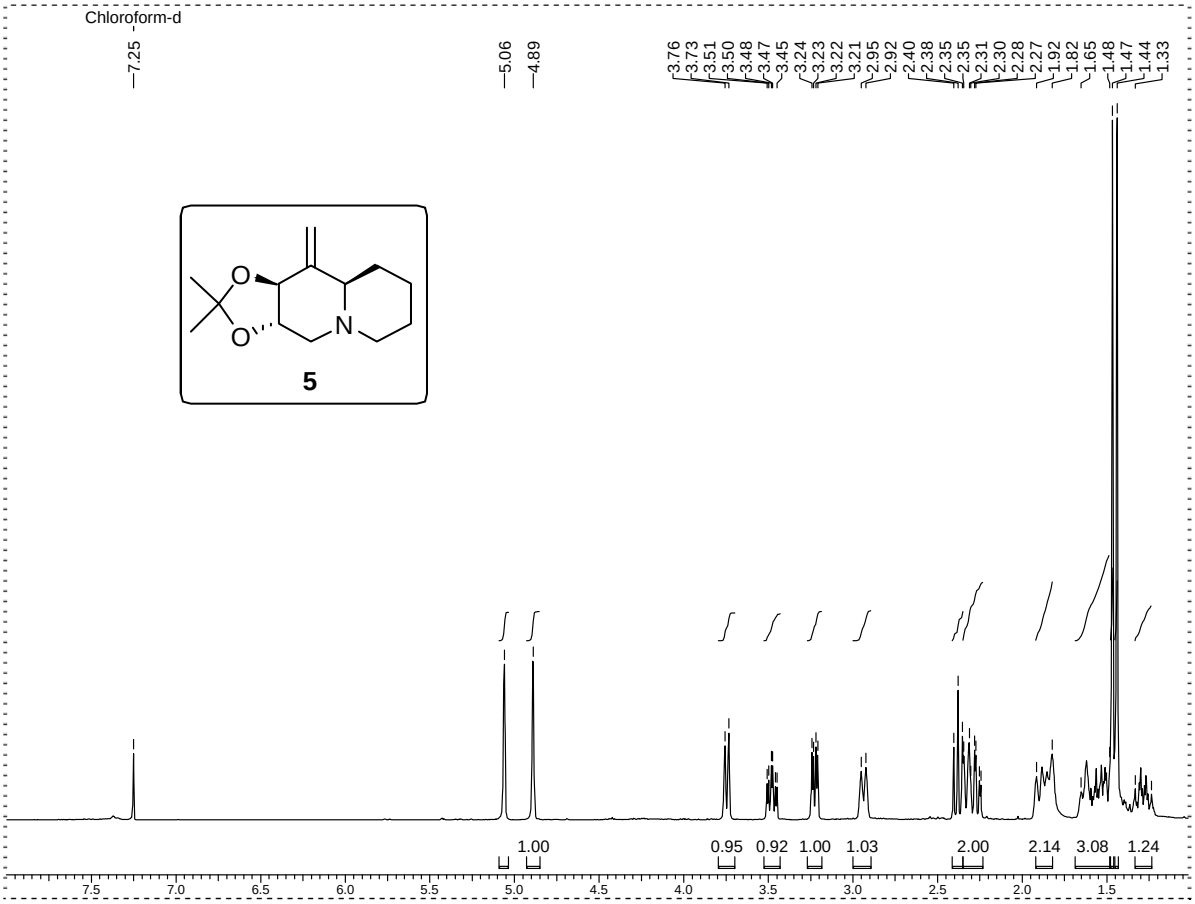
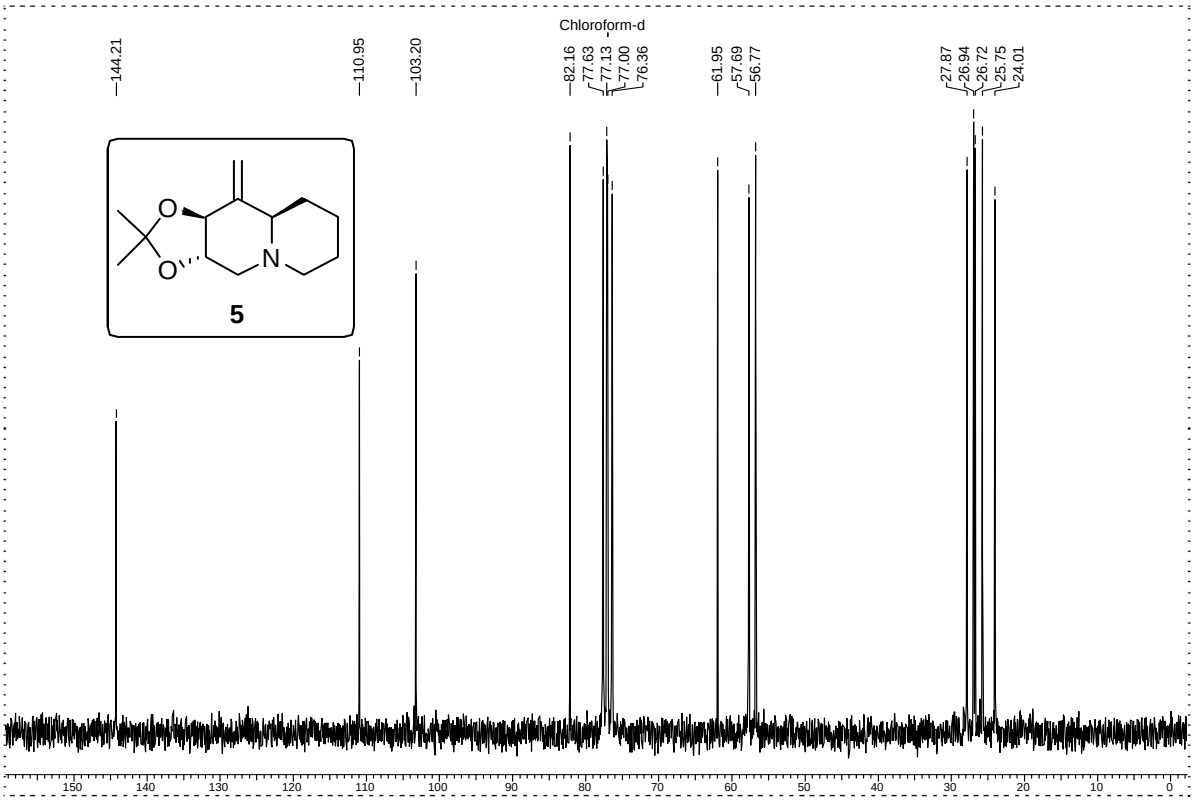
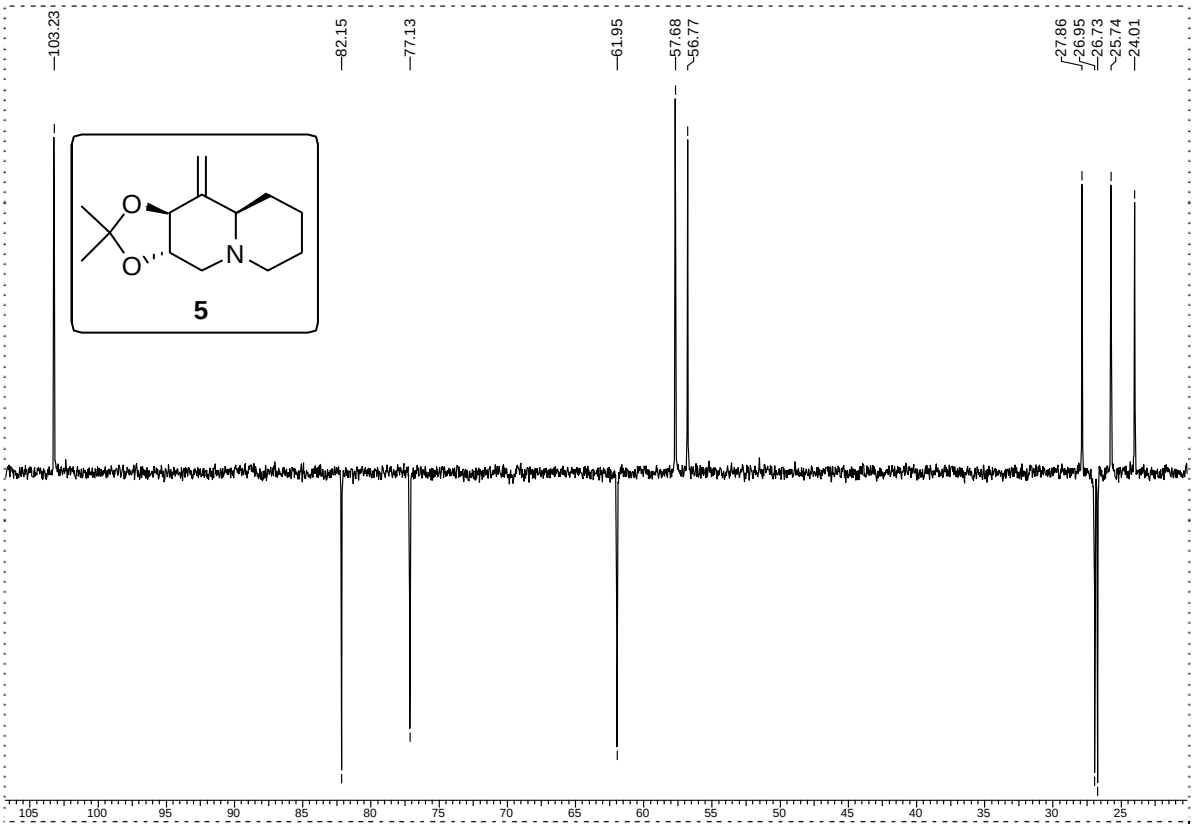
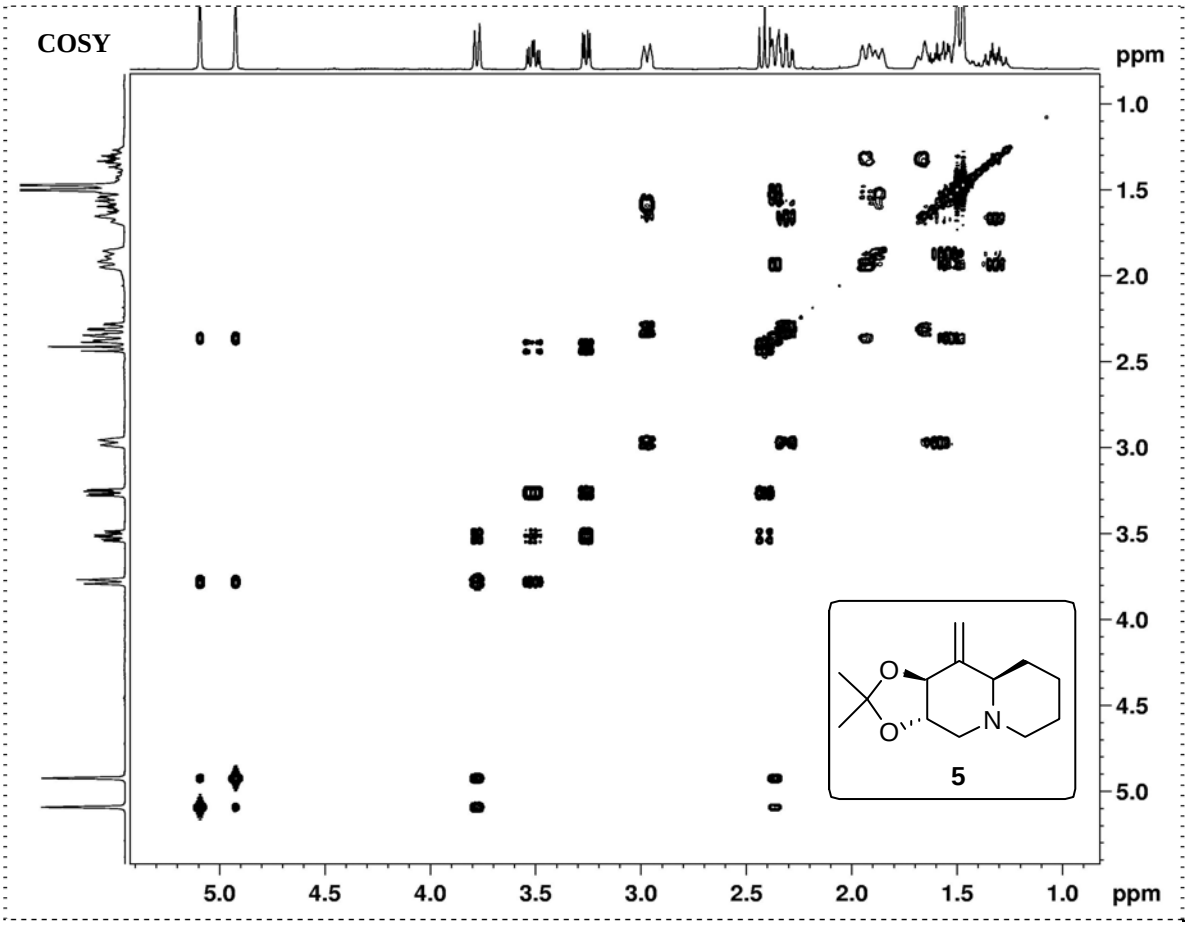
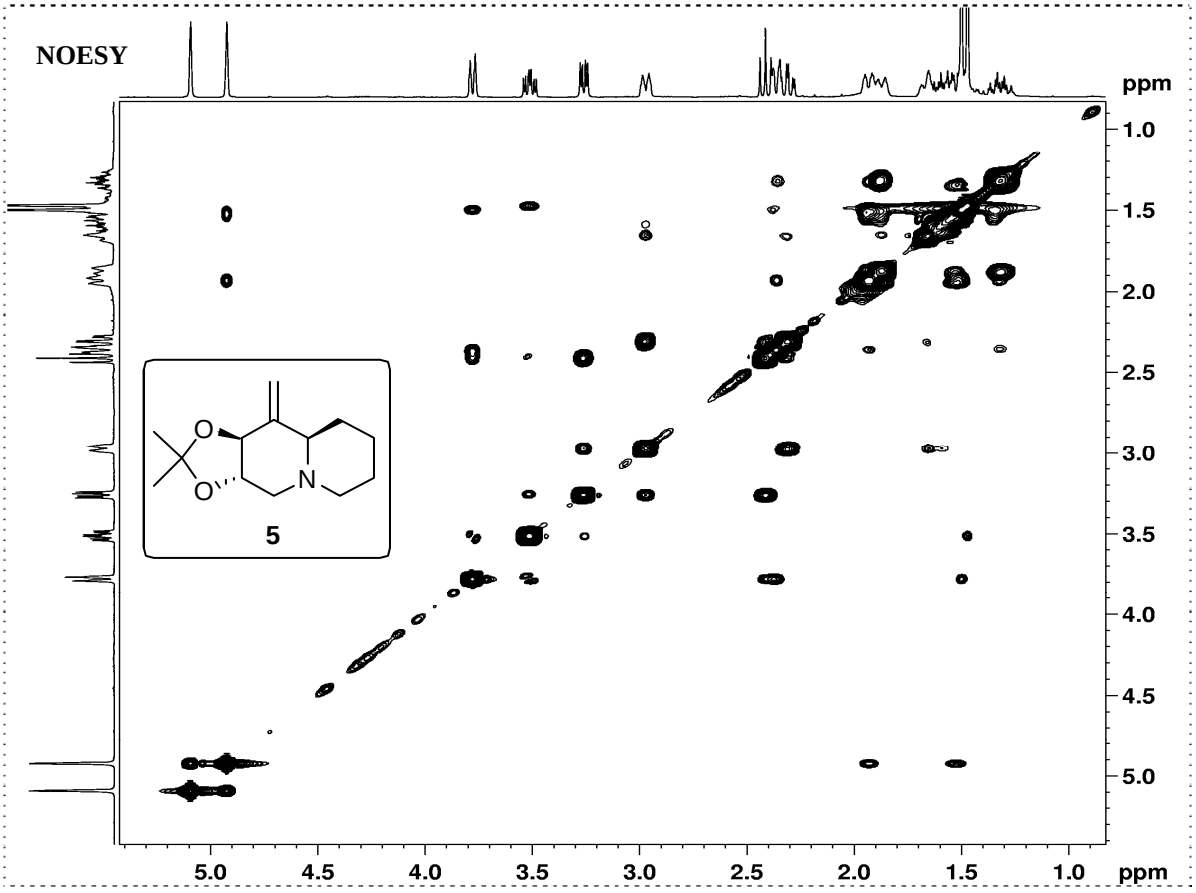
26.07
25.95
25.66
24.15
23.81
1.06
TMS
O
O
N
12


Chloroform-d
7.25
5.06
4.89
3.76
3.73
3.51
3.50
3.48
3.47
3.45
3.24
3.23
3.22
3.21
2.95
2.92
2.40
2.38
2.35
2.35
2.31
2.30
2.28
2.27
1.92
1.82
1.65
1.48
1.47
1.44
1.33
O
O
N
5
1.00
0.95
0.92
1.00
1.03
2.00
2.14
3.08
1.24
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


Chloroform-d
144.21
110.95
103.20
82.16
77.63
77.13
77.00
76.36
61.95
57.69
56.77
27.87
26.94
26.72
25.75
24.01
O
O
N
5
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


103.23
82.15
77.13
61.95
57.68
56.77
27.86
26.95
26.73
25.74
24.01
O
O
N
5
105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25


COSY
ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
O
O
N
5
5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm


NOESY
ppm
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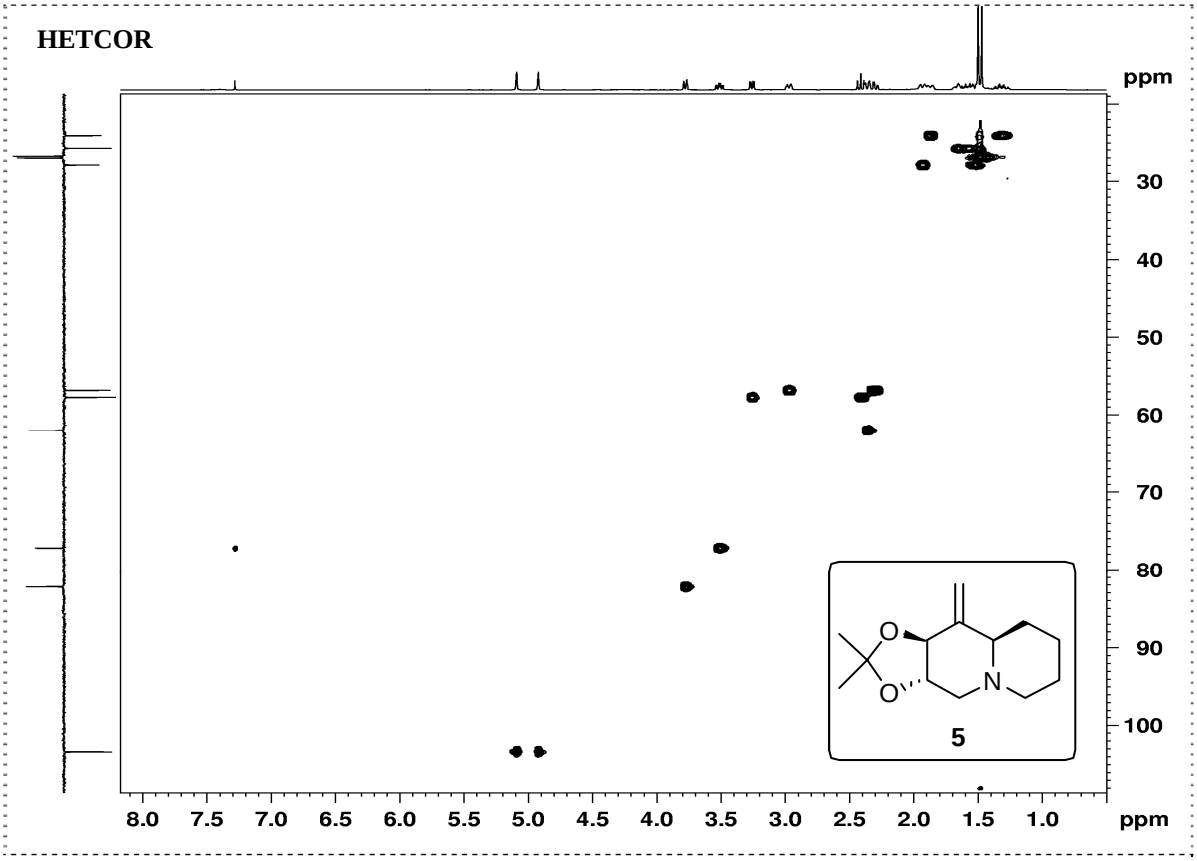
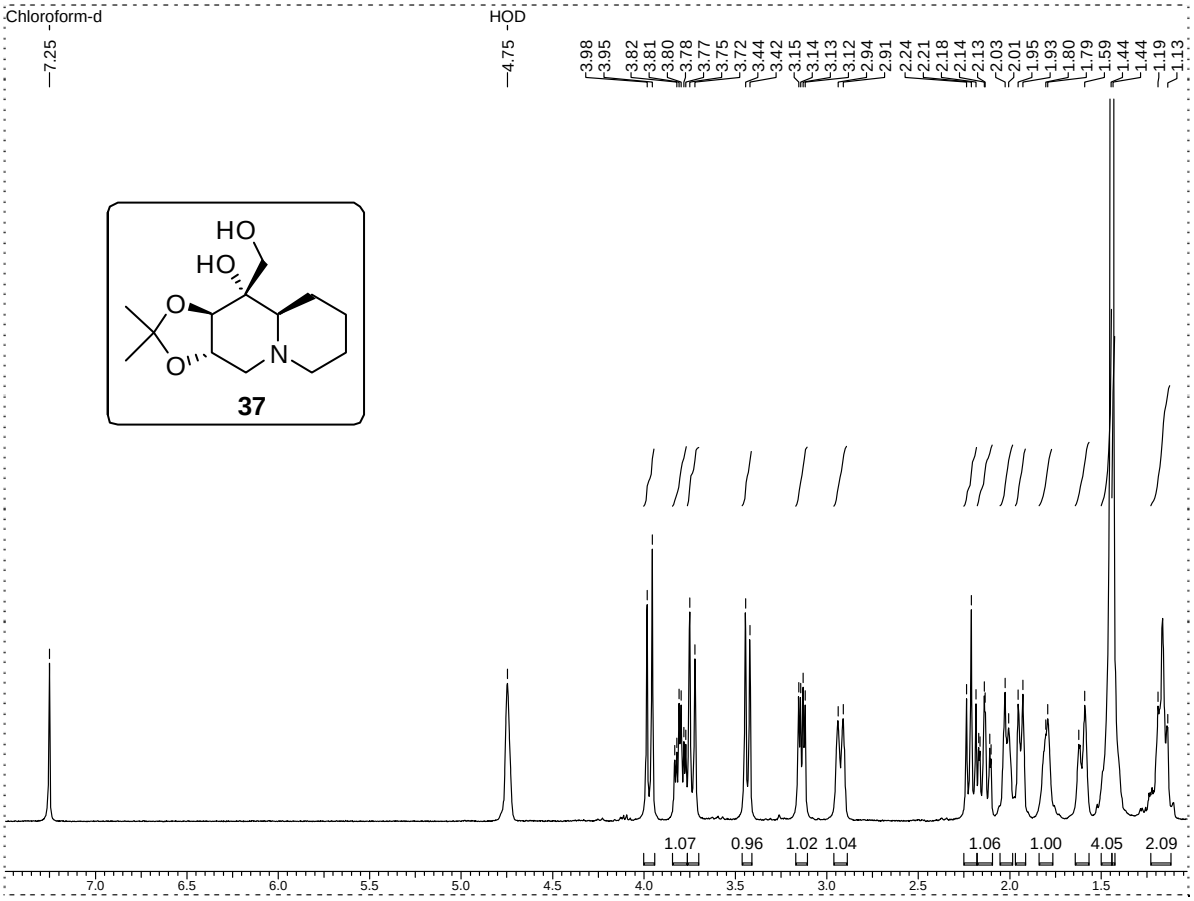
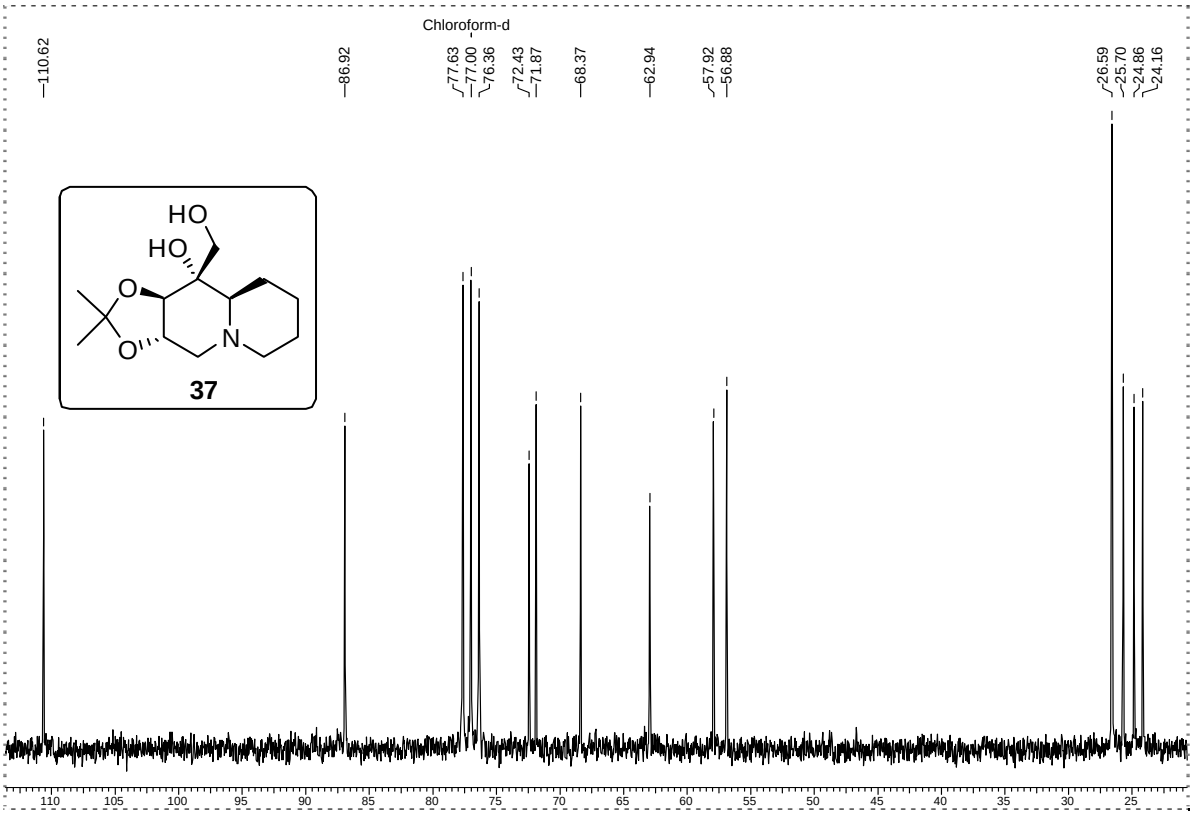
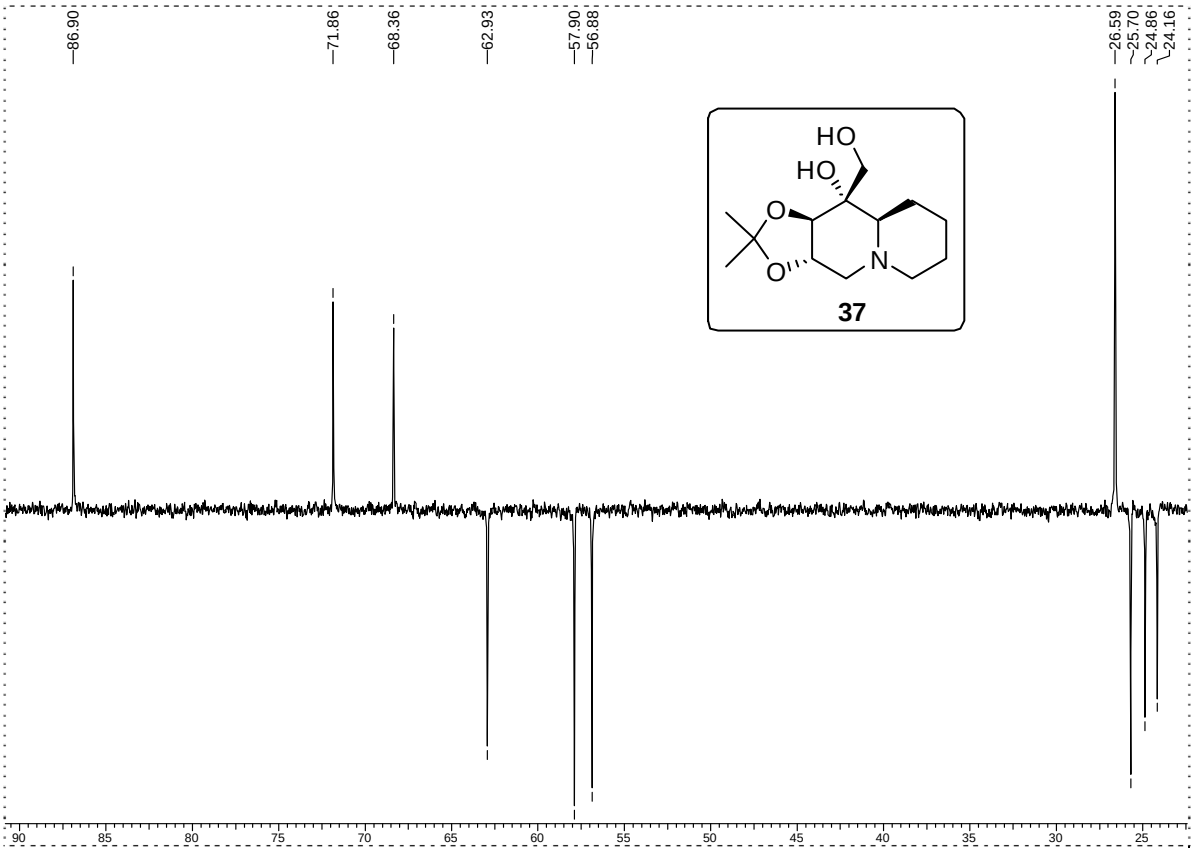
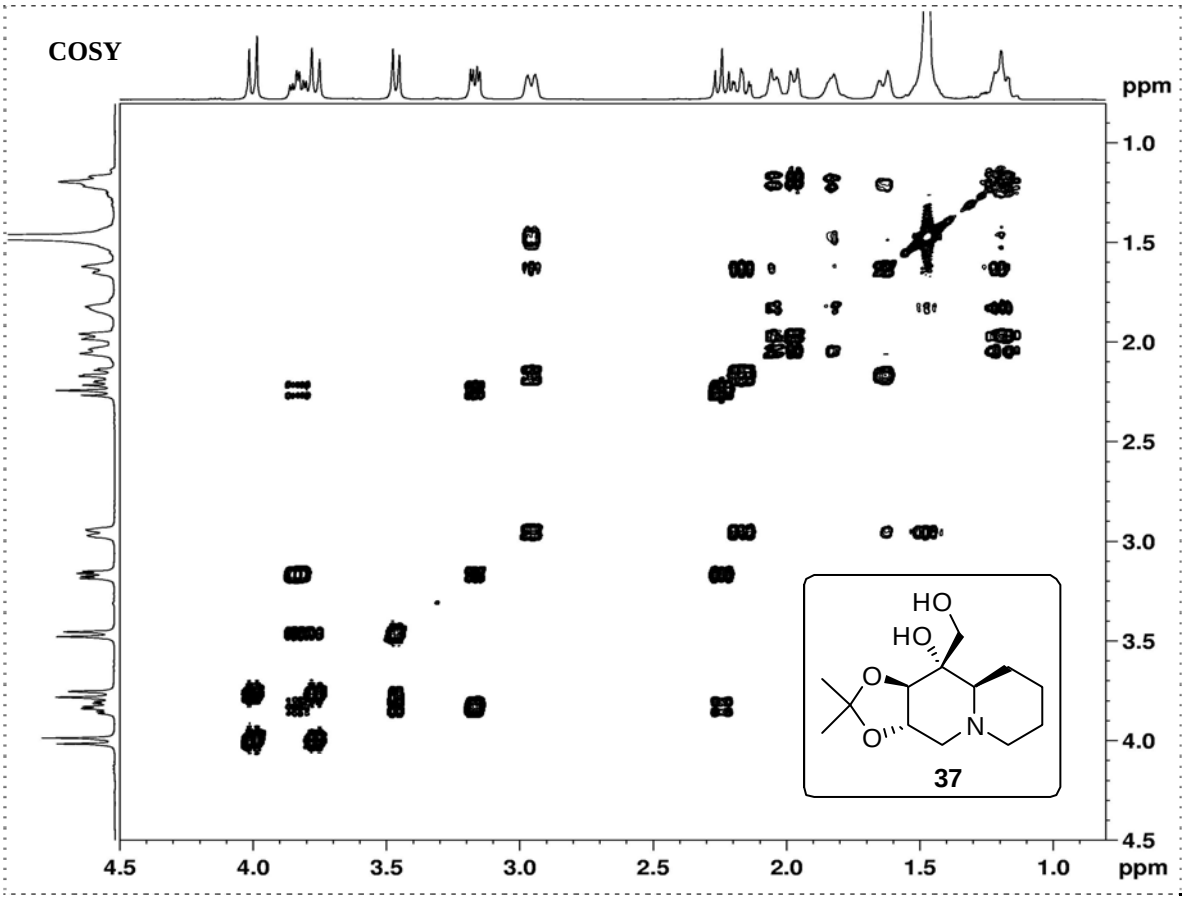
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
O
O
N
5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm


HETCOR
ppm
30
40
50
60
70
80
90
100
O
O
N
5
8.0
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
ppm


Chloroform-d
HOD
7.25
4.75
3.98
3.95
3.82
3.81
3.80
3.78
3.77
3.75
3.72
3.44
3.42
3.15
3.14
3.13
3.12
2.94
2.91
2.24
2.21
2.18
2.14
2.13
2.03
2.01
1.95
1.93
1.80
1.79
1.59
1.44
1.44
1.19
1.13
HO
HO
O
O
N
37
1.07
0.96
1.02
1.04
1.06
1.00
4.05
2.09
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5


Chloroform-d
110.62
86.92
77.63
77.00
76.36
72.43
71.87
68.37
62.94
57.92
56.88
26.59
25.70
24.86
24.16
HO
HO
O
O
N
37
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25


86.90
71.86
68.36
62.93
57.90
56.88
26.59
25.70
24.86
24.16
HO
HO
O
O
N
37


COSY
ppm
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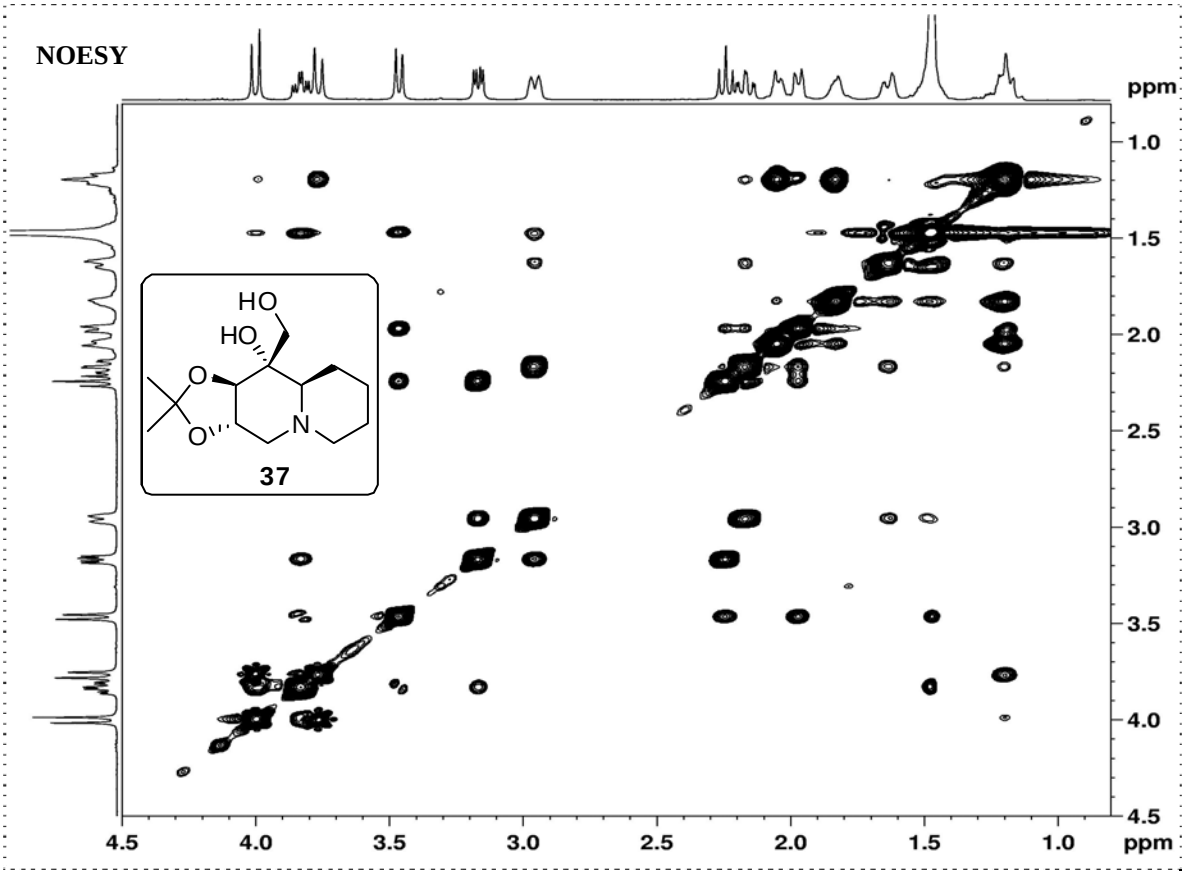
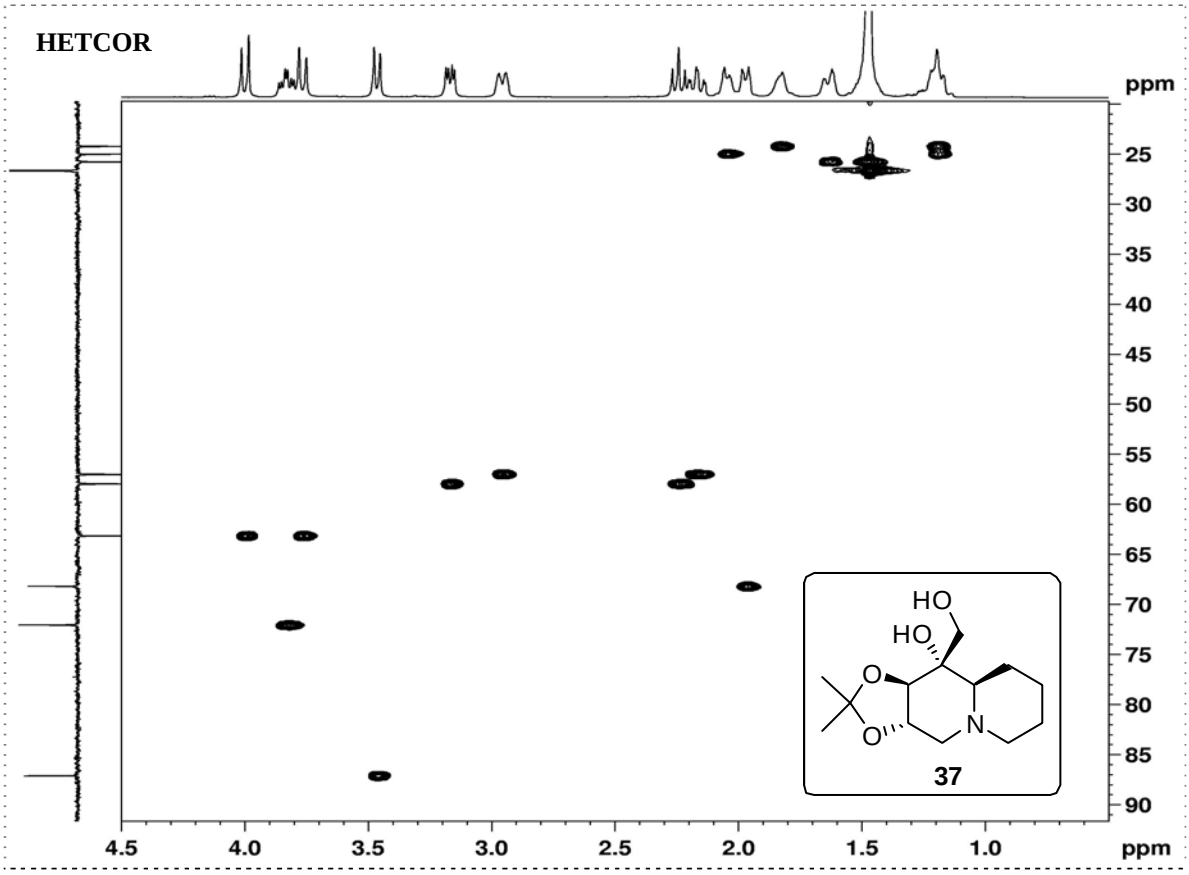
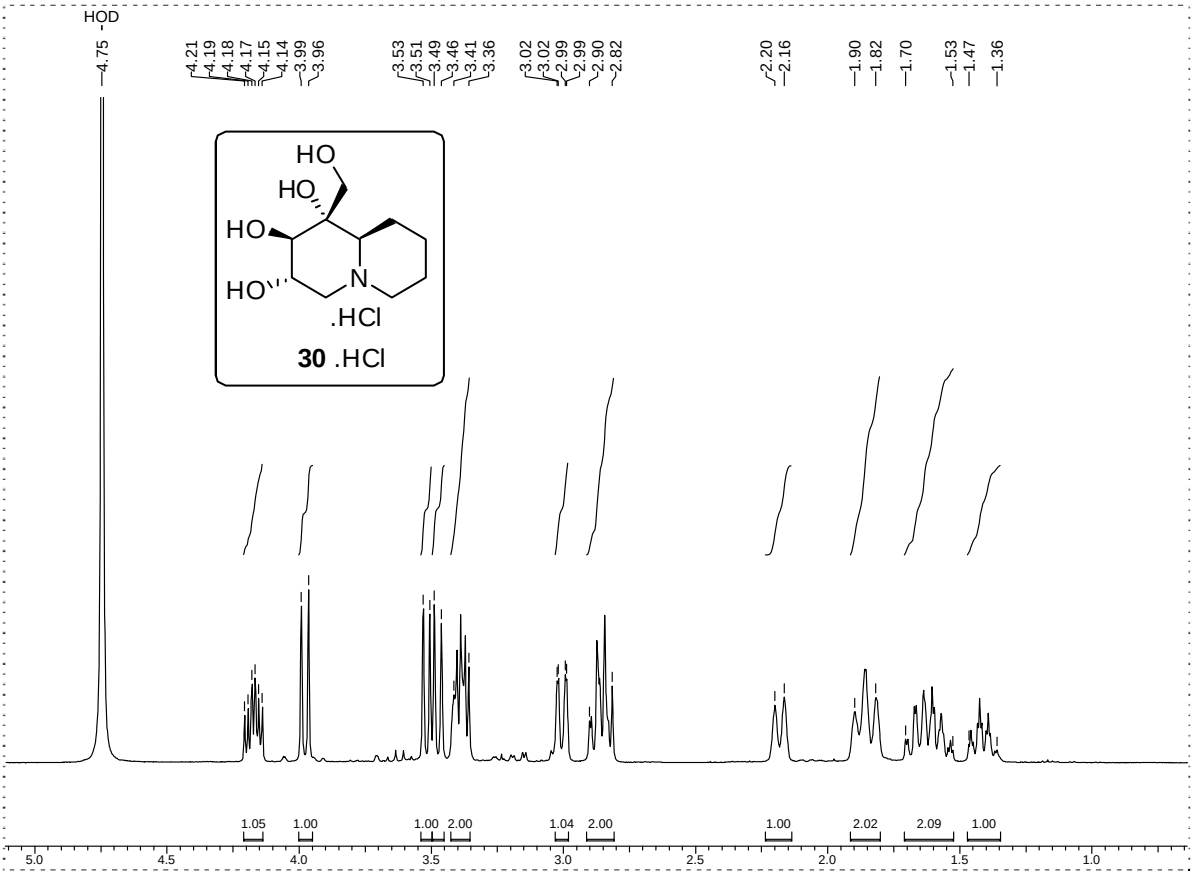
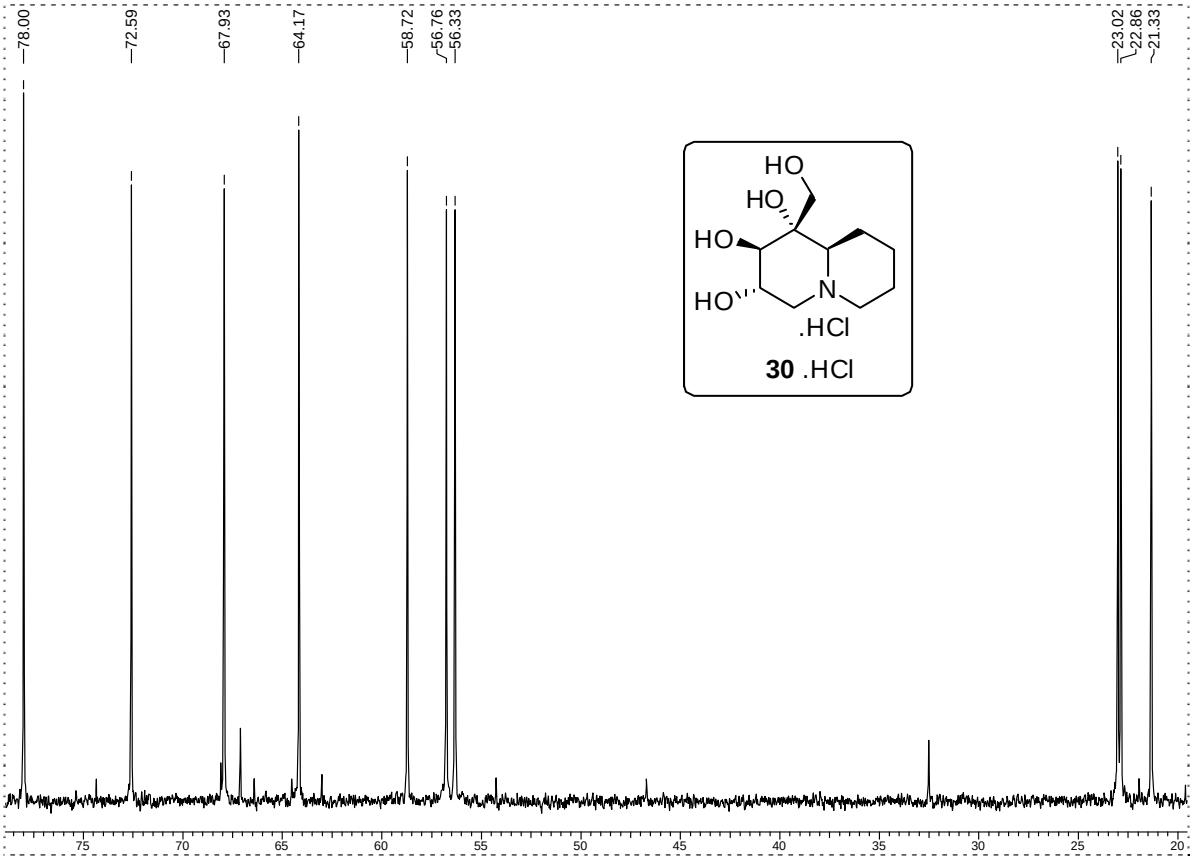
2.0
2.5
3.0
3.5
4.0
4.5
HO
HO
O
O
N
37


NOESY
ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
HO
HO
O
O
N
37
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm


HETCOR
ppm
25
30
35
40
45
50
55
60
65
70
75
80
85
90
HO
HO
O
O
N
37
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm


HOD
4.75
4.21
4.19
4.18
4.17
4.15
4.14
3.99
3.96
3.53
3.51
3.49
3.46
3.41
3.36
3.02
3.02
2.99
2.99
2.90
2.82
2.20
2.16
1.90
1.82
1.70
1.53
1.47
1.36
HO
HO
HO
HO
N
.HCl
30 .HCl
1.05
1.00
1.00
2.00
1.04
2.00
1.00
2.02
2.09
1.00
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0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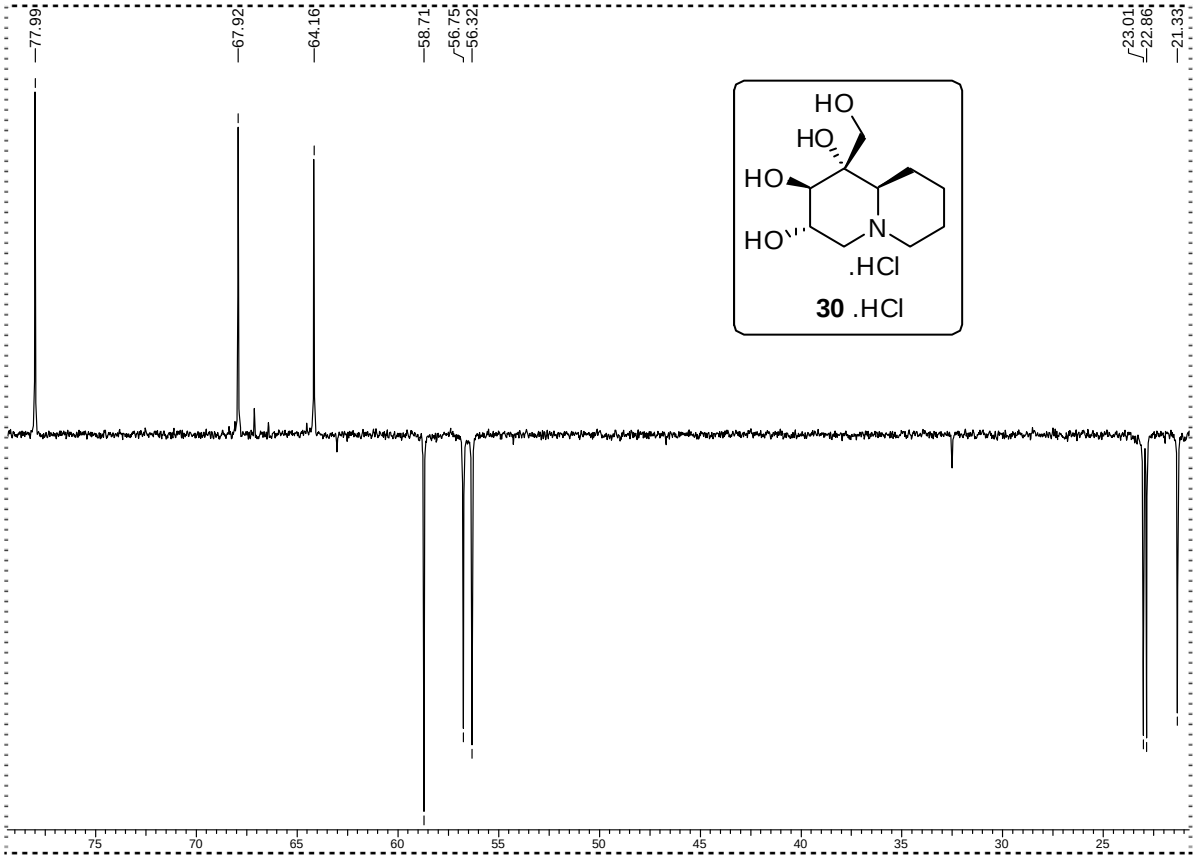
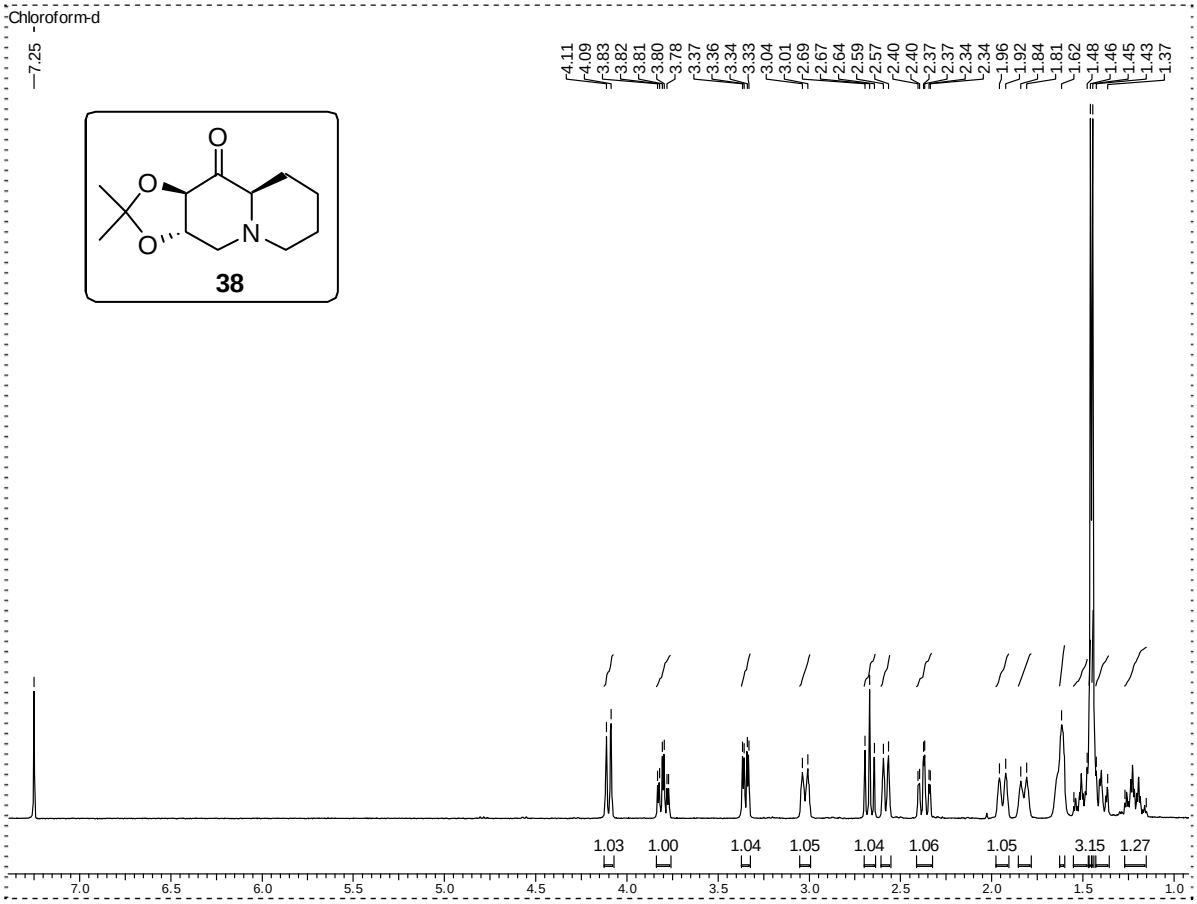
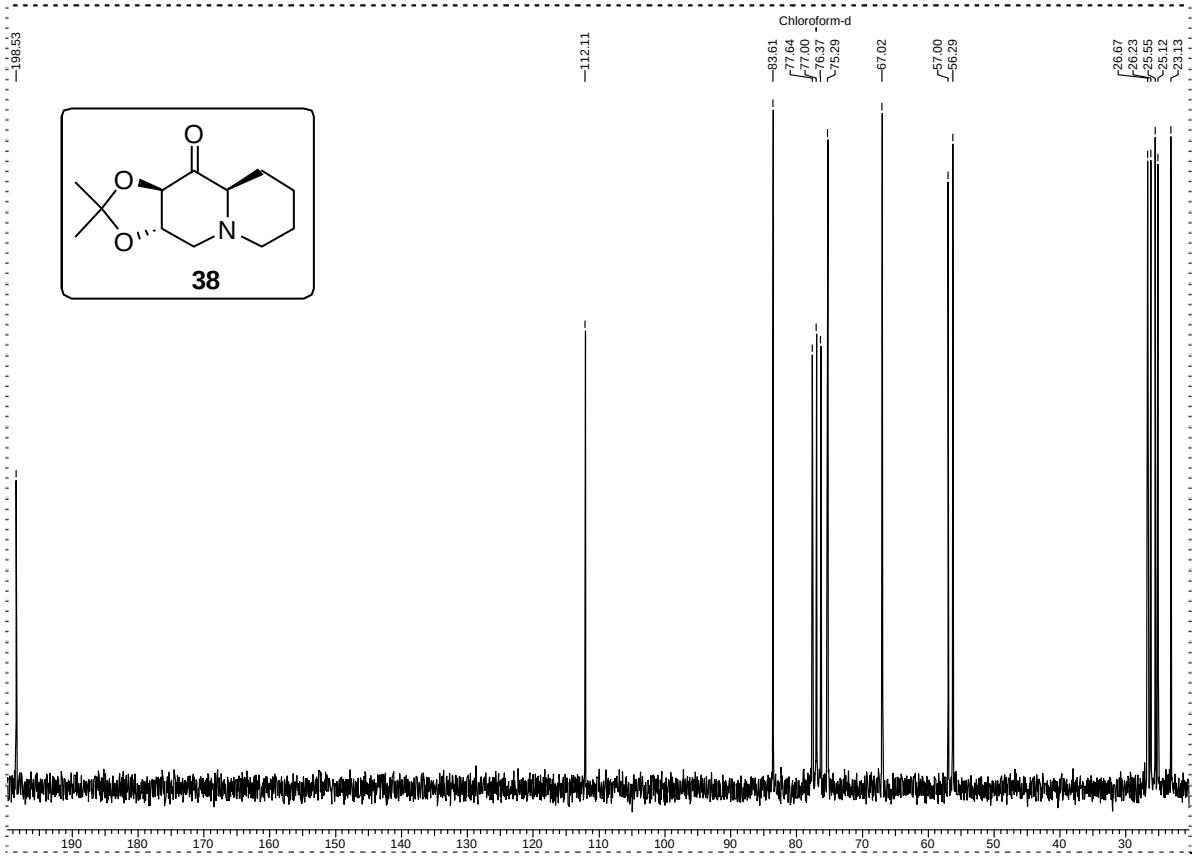
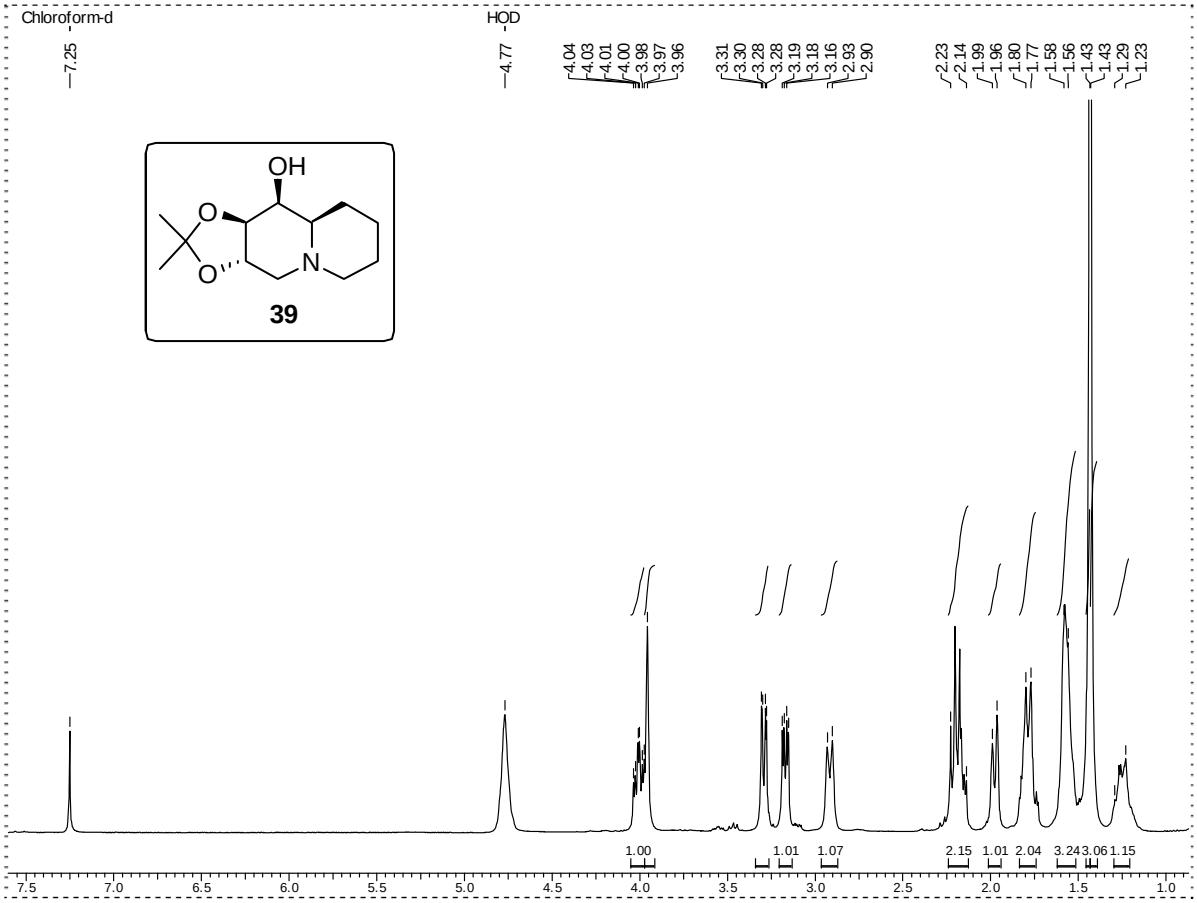
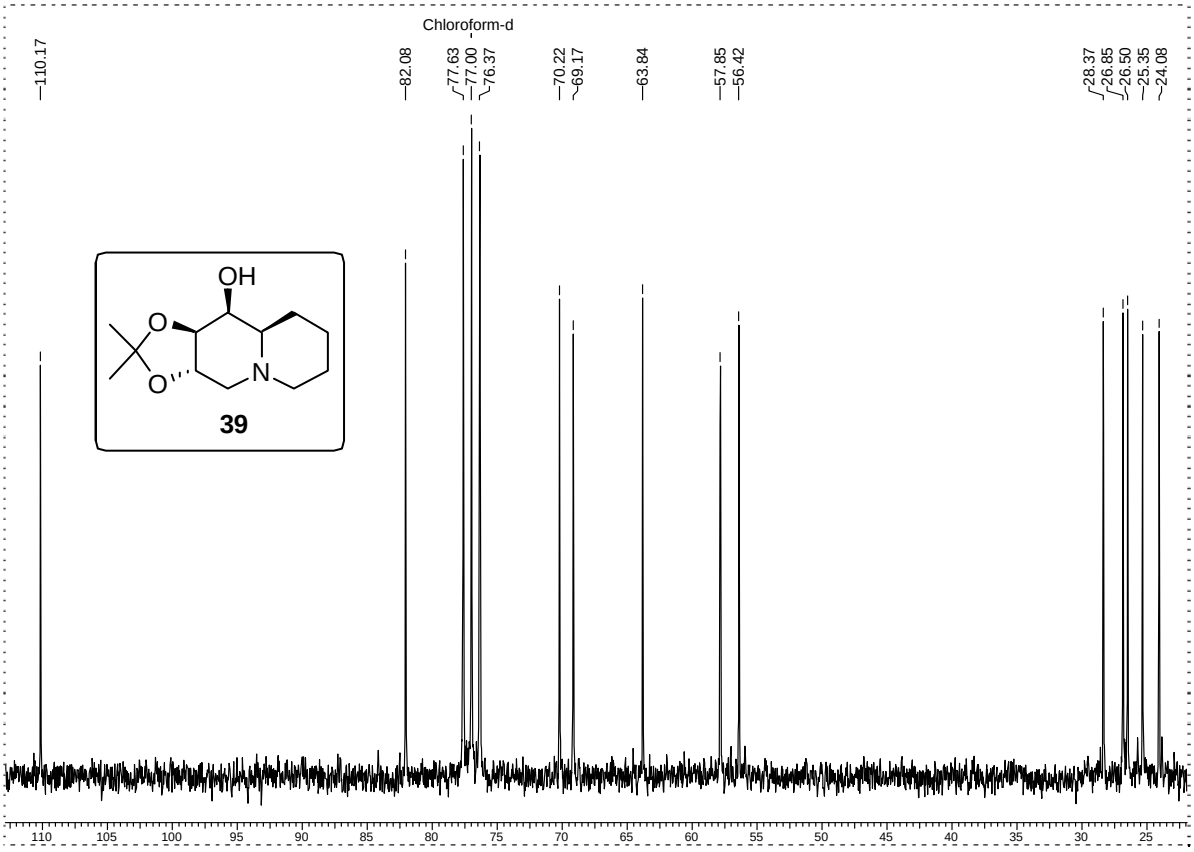
72.59
67.93
64.17
58.72
56.76
56.33
23.02
22.86
21.33
HO
HO
HO
HO
N
.HCl
30 .HCl
75
70
65
60
55
50
45
40
35
30
25
20

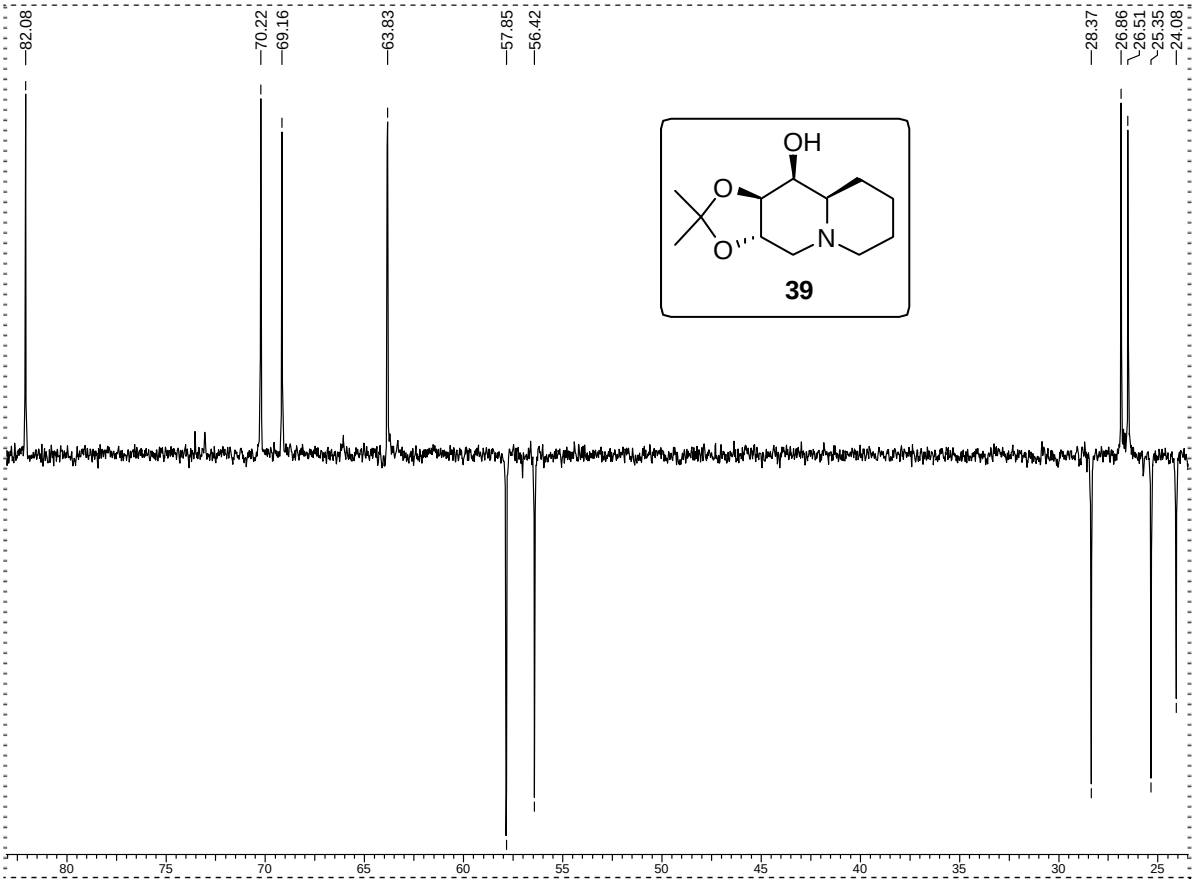

77.99
67.92
64.16
58.71
56.75
56.32
23.01
22.86
21.33
HO
HO
HO
HO
N
.HCl
30 .HCl

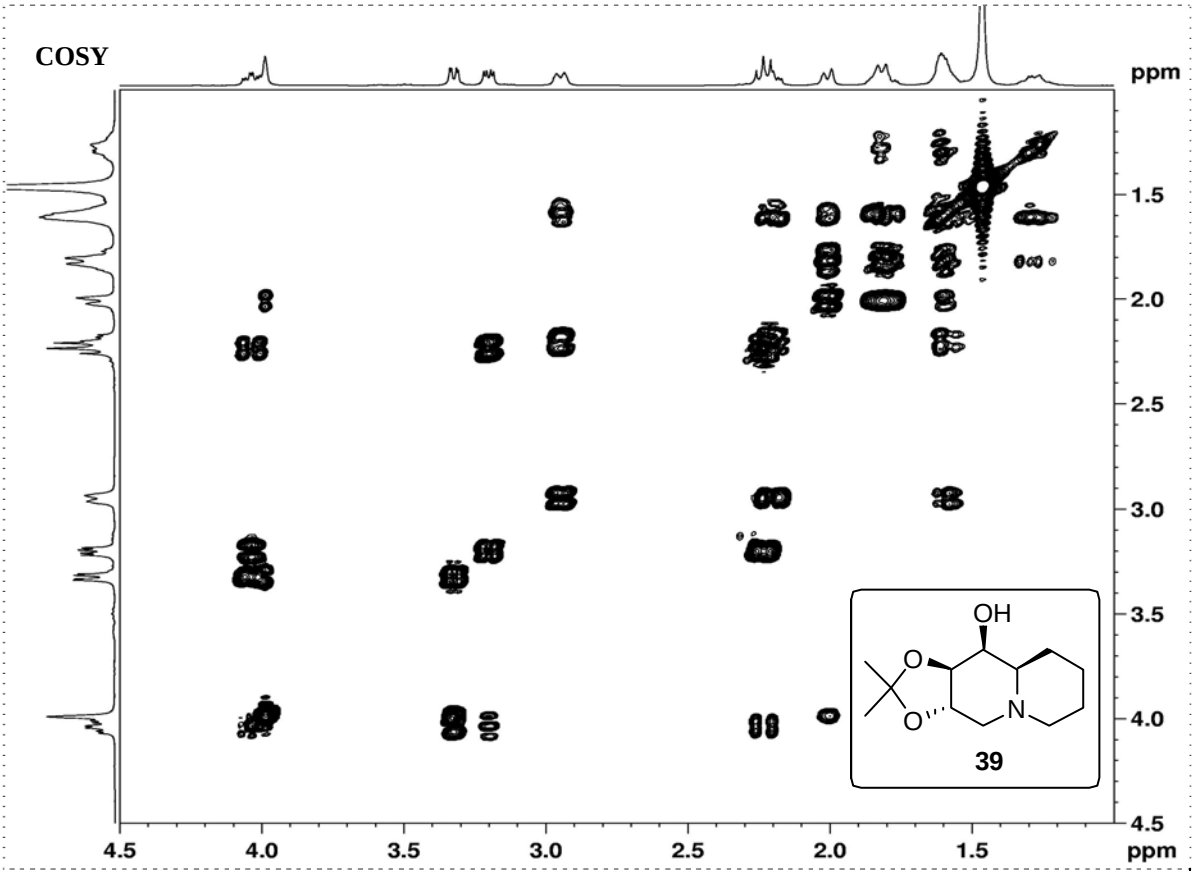

Chloroform-d
7.25
4.11
4.09
3.83
3.82
3.81
3.80
3.78
3.37
3.36
3.34
3.33
3.04
3.01
2.69
2.67
2.64
2.59
2.57
2.40
2.40
2.37
2.37
2.34
2.34
1.96
1.92
1.84
1.81
1.62
1.48
1.46
1.45
1.43
1.37
O
O
O
N
38
1.03
1.00
1.04
1.05
1.04
1.06
1.05
3.15
1.27

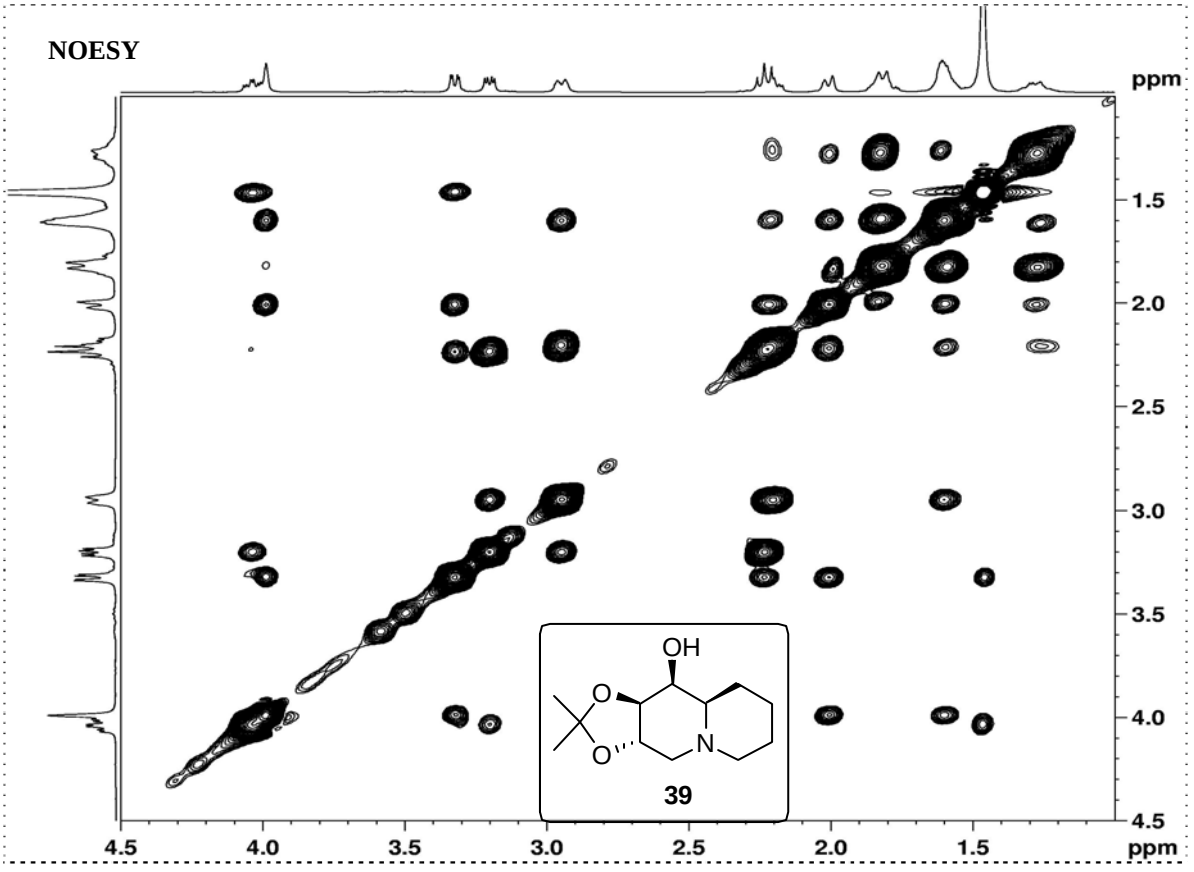

Chloroform-d
198.53
112.11
83.61
77.64
77.00
76.37
75.29
67.02
57.00
56.29
26.67
26.23
25.55
25.12
23.13
O
O
O
N
38

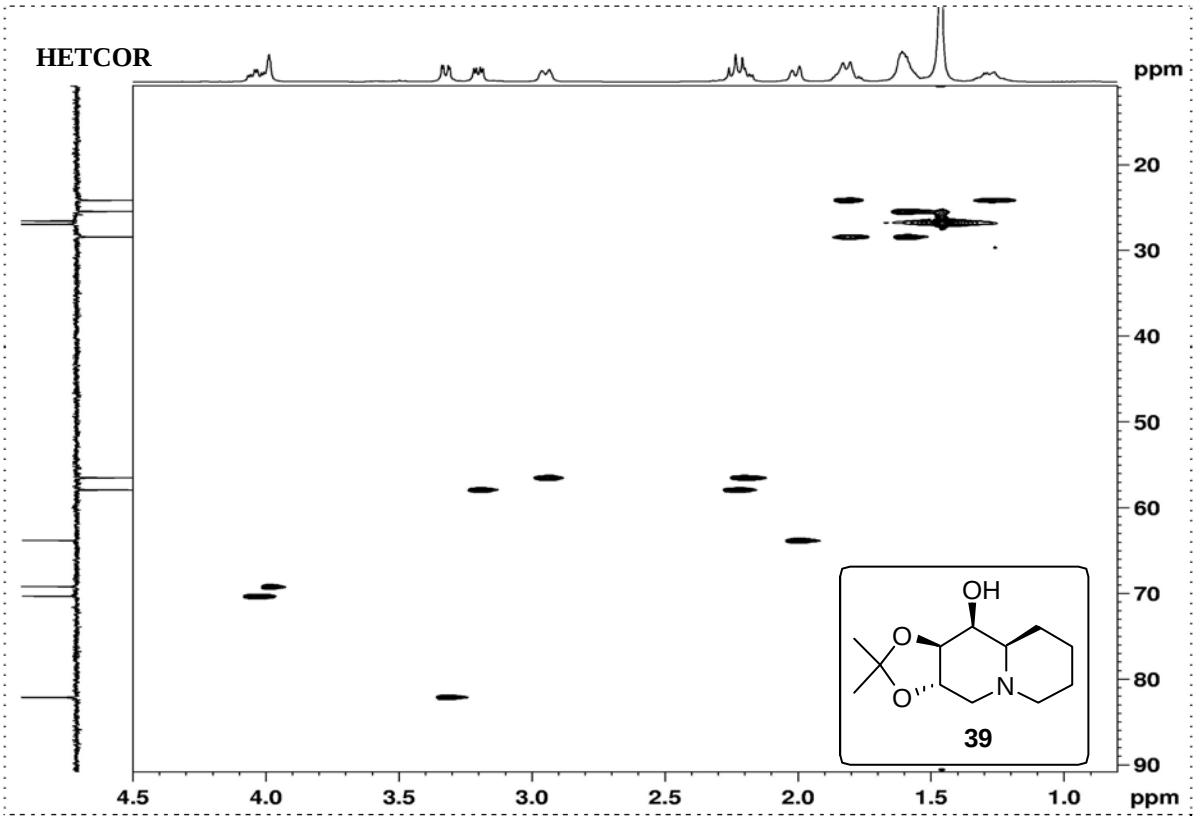

Chloroform-d
HOD
7.25
4.77
4.04
4.03
4.01
4.00
3.98
3.97
3.96
3.31
3.30
3.28
3.28
3.19
3.18
3.16
2.93
2.90
2.23
2.14
1.99
1.96
1.80
1.77
1.58
1.56
1.43
1.43
1.29
1.23
OH
O
O
N
39
1.00
1.01
1.07
2.15
1.01
2.04
3.24
3.06
1.15


Chloroform-d
110.17
82.08
77.63
77.00
76.37
70.22
69.17
63.84
57.85
56.42
28.37
26.85
26.50
25.35
24.08
OH
O
O
N
39


82.08
70.22
69.16
63.83
57.85
56.42
28.37
26.86
26.51
25.35
24.08
OH
O
O
N
39


COSY
ppm
1.5
2.0
2.5
3.0
3.5
4.0
4.5
OH
O
O
N
39
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
OH
O
O
N
39
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm


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
ppm
OH
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O
N
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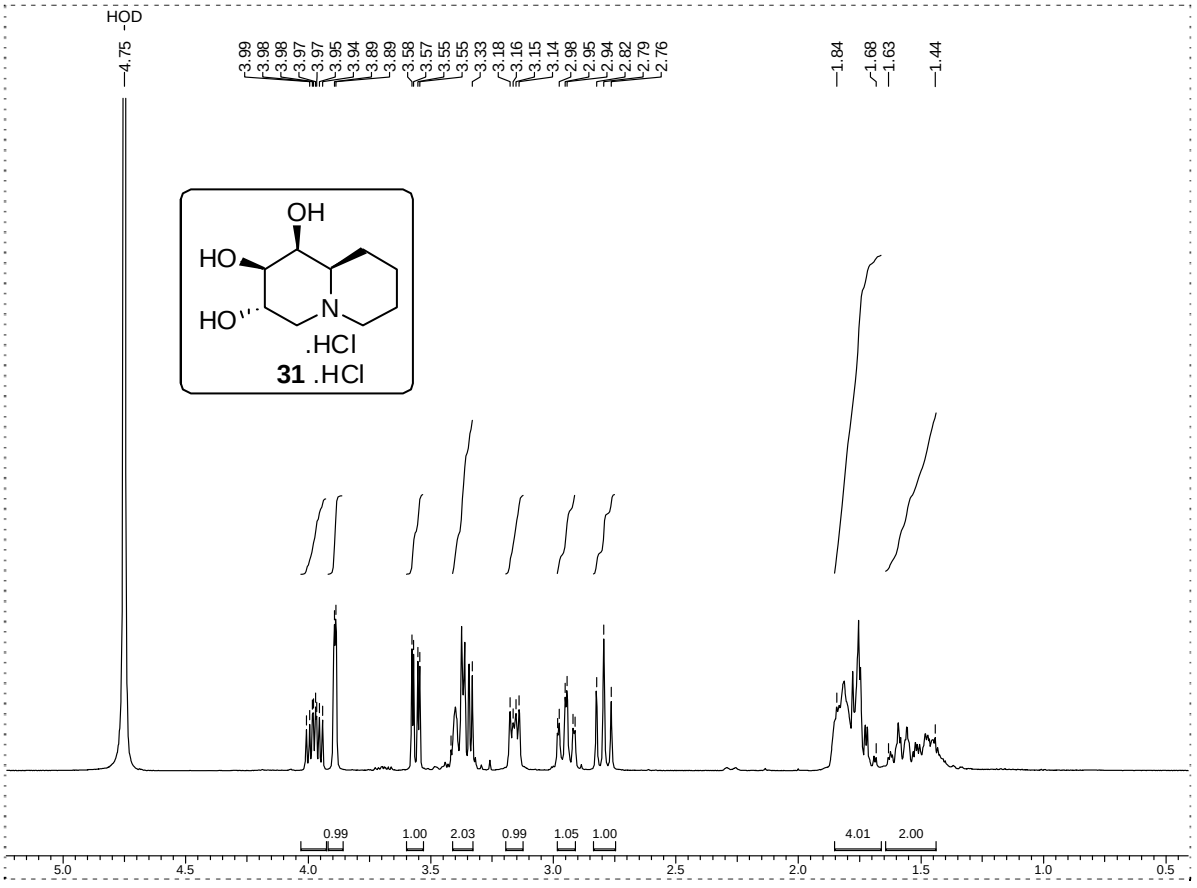
HOD
4.75
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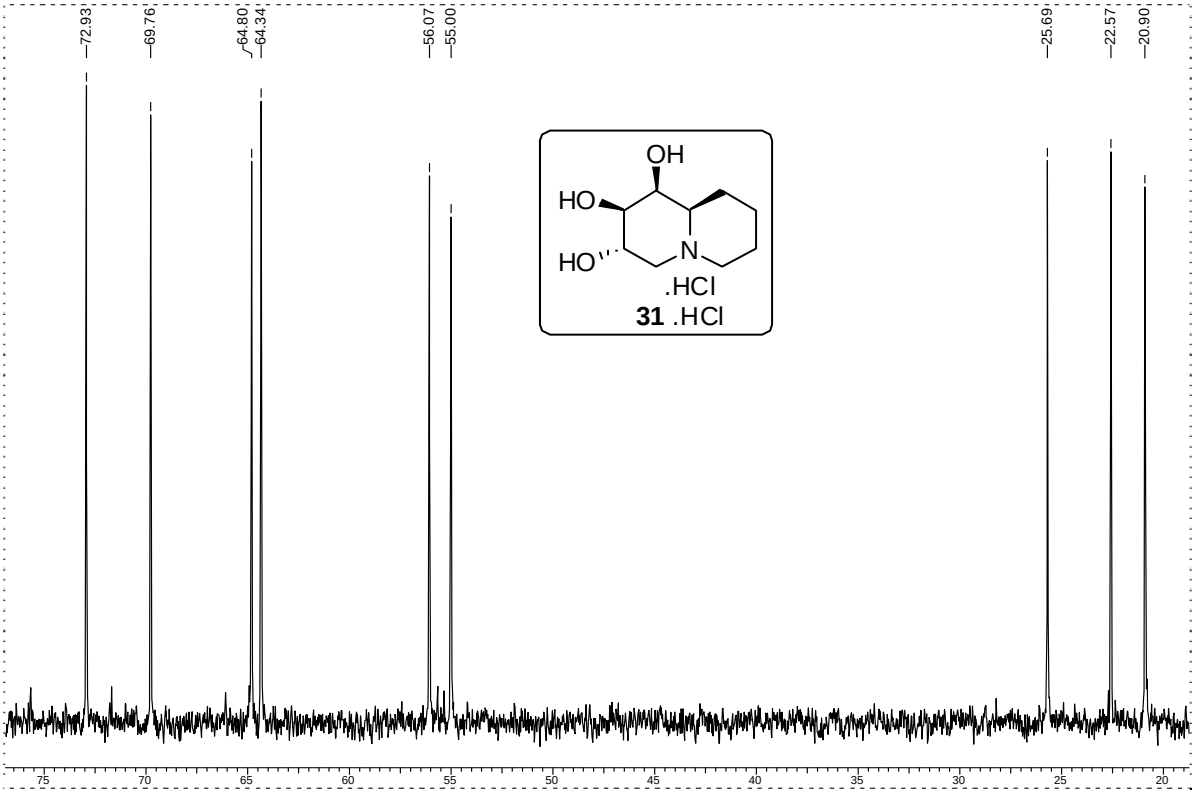
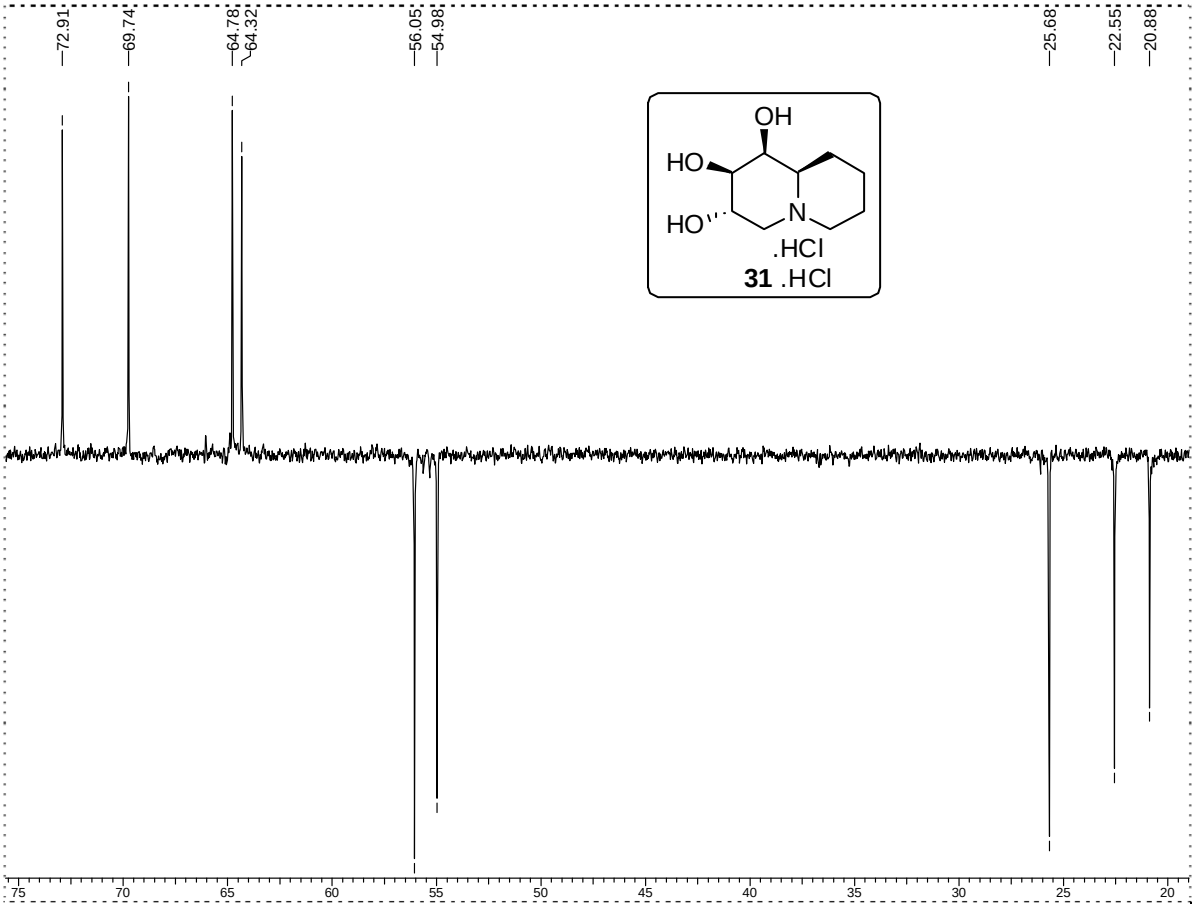
3.98
3.98
3.97
3.97
3.95
3.94
3.89
3.89
3.58
3.57
3.55
3.55
3.33
3.18
3.16
3.15
3.14
2.98
2.95
2.94
2.82
2.79
2.76
1.84
1.68
1.63
1.44
OH
HO
HO
N
.HCl
31 .HCl
0.99
1.00
2.03
0.99
1.05
1.00
4.01
2.00
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5


72.93
69.76
64.80
64.34
56.07
55.00
25.69
22.57
20.90
OH
HO
HO
N
.HCl
31 .HCl


72.91
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64.78
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25.68
22.55
20.88
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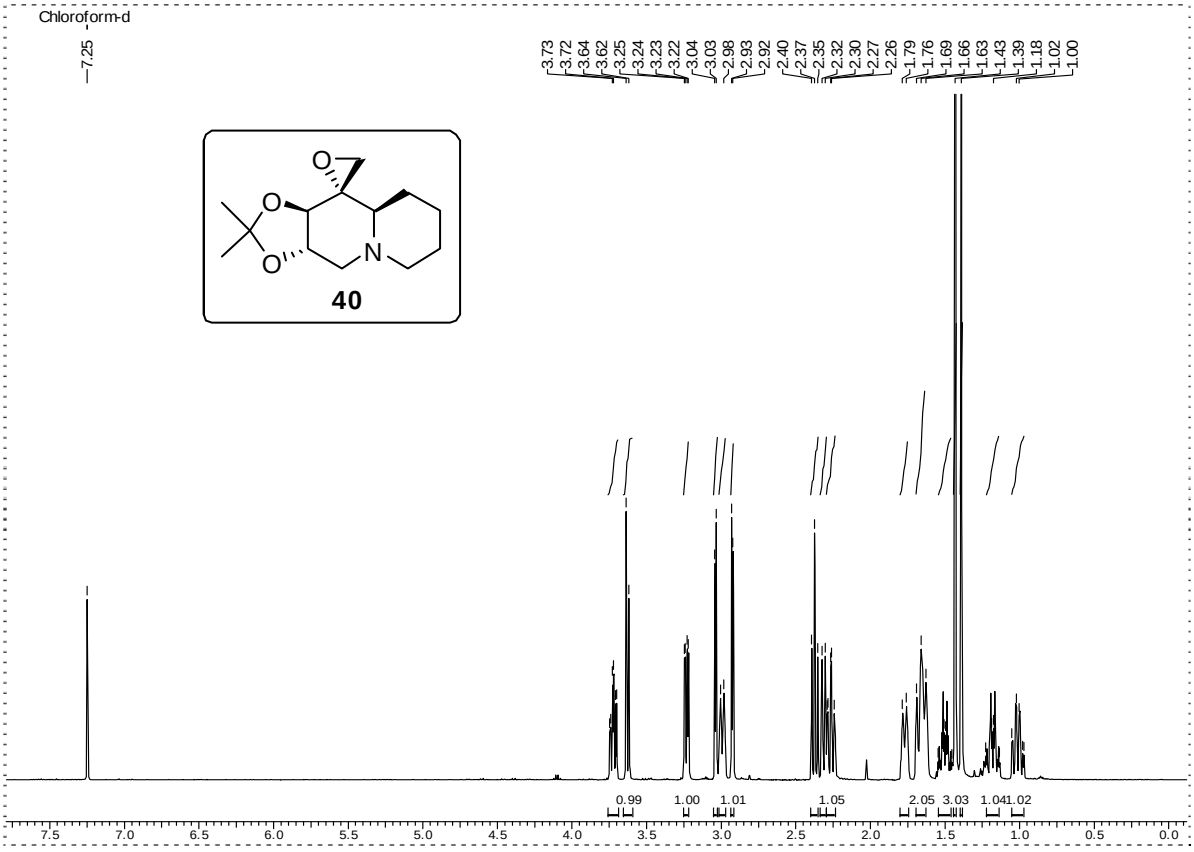
HO
HO
N
.HCl
31 .HCl


Chloroform-d
7.25
3.73
3.72
3.64
3.62
3.25
3.24
3.23
3.22
3.04
3.03
2.98
2.93
2.92
2.40
2.37
2.35
2.32
2.30
2.27
2.26
1.79
1.76
1.69
1.66
1.63
1.43
1.39
1.18
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1.00
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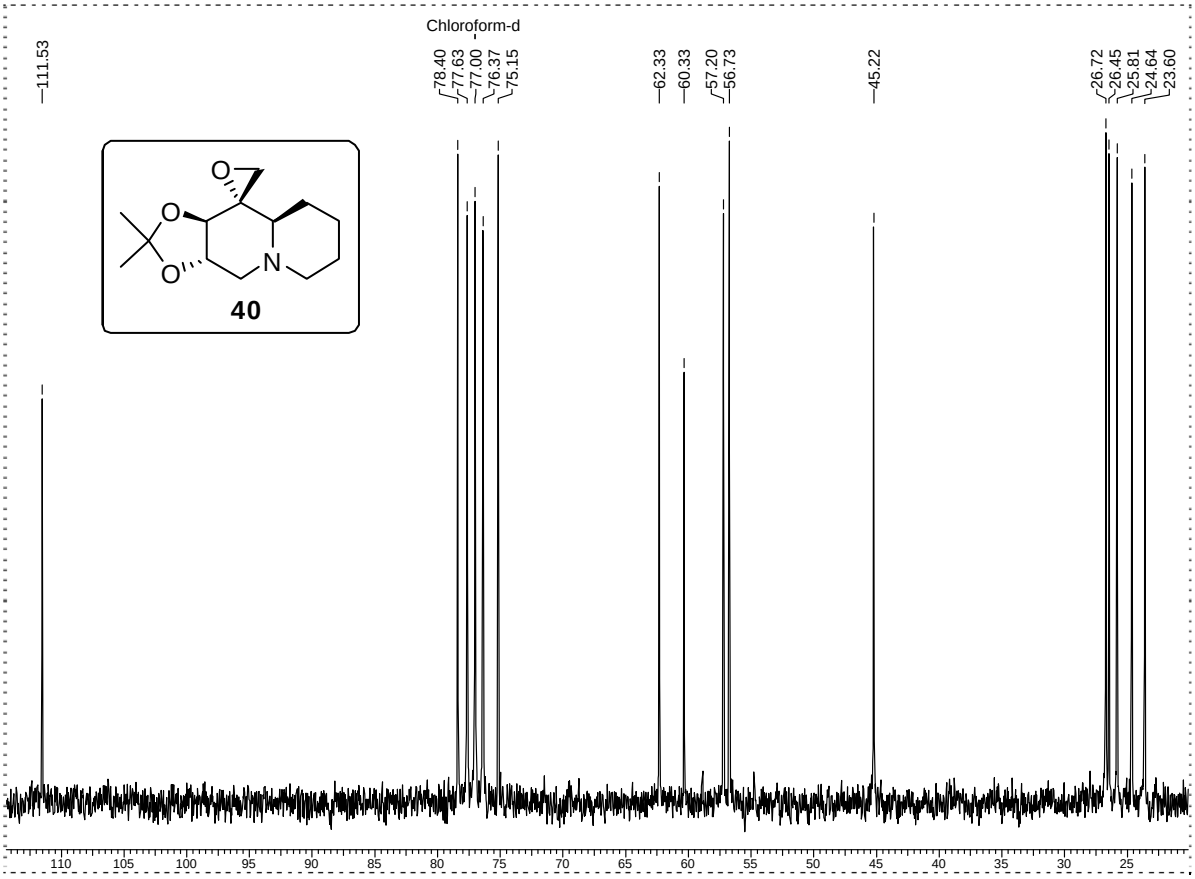
1.00
1.01
1.05
2.05
3.03
1.04
1.02
40


Chloroform-d
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78.40
77.63
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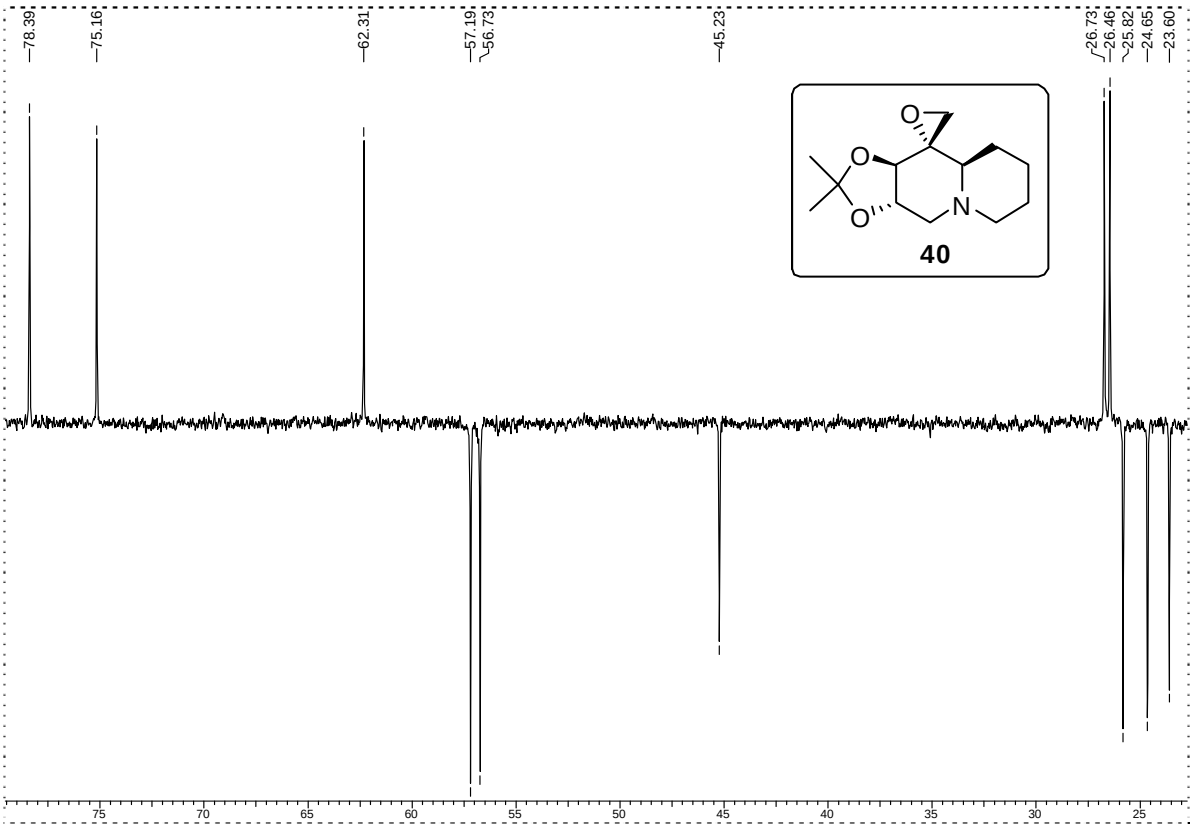
45.22
26.72
26.45
25.81
24.64
23.60
40


78.39
75.16
62.31
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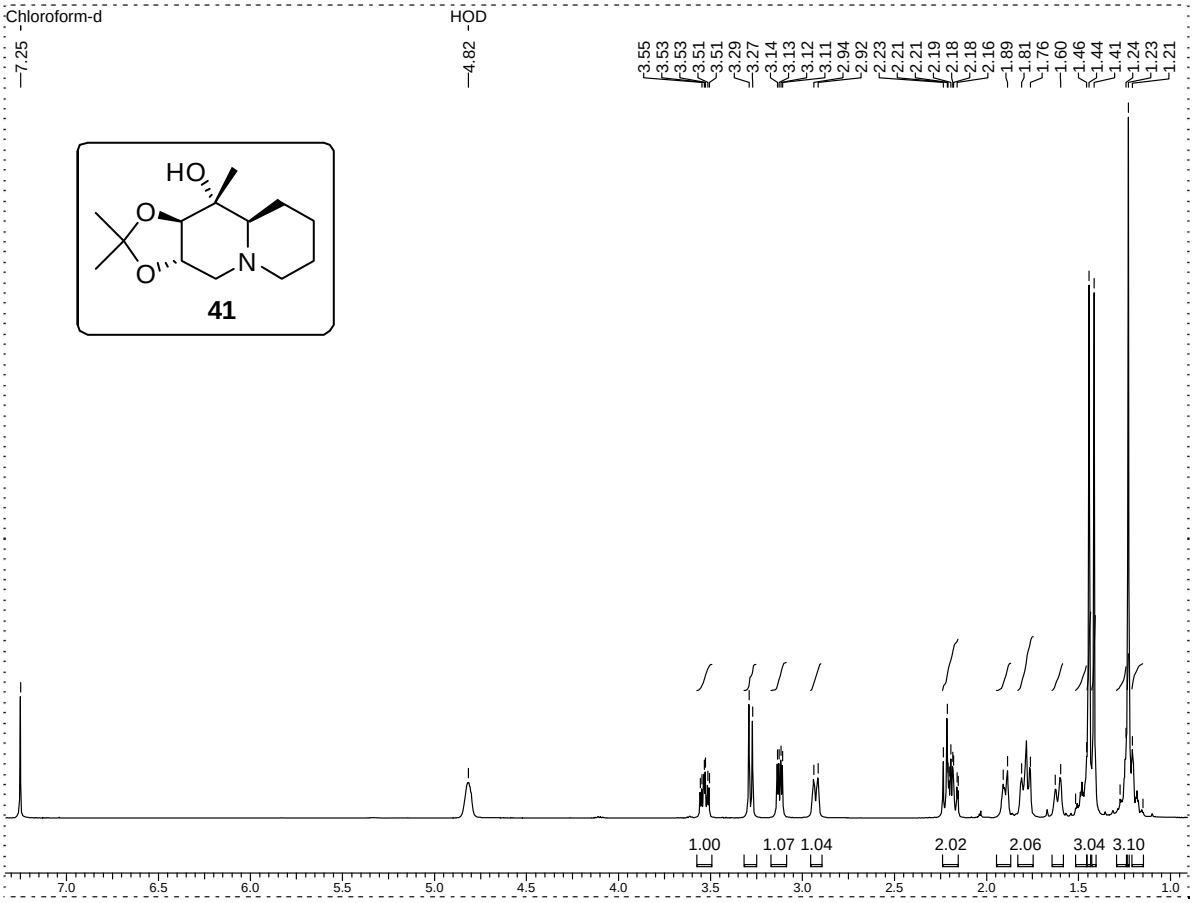
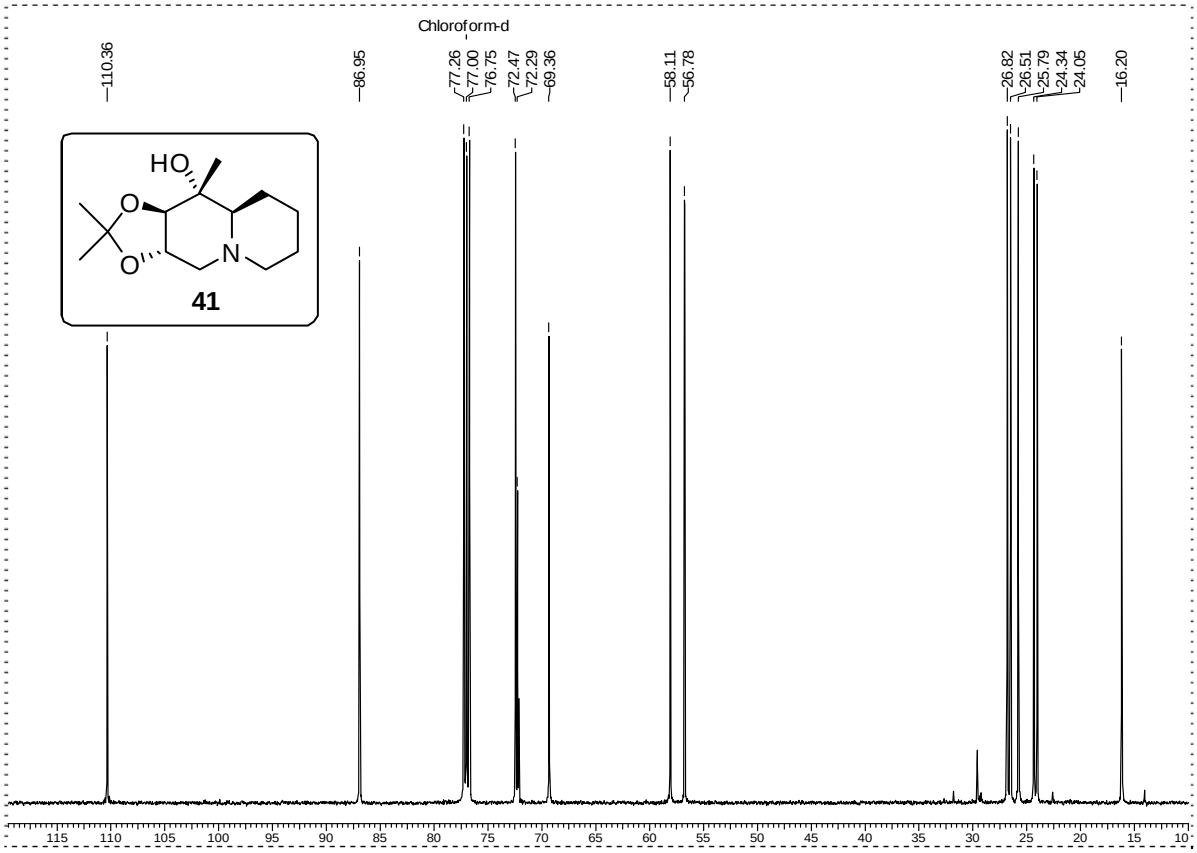
45.23
26.73
26.46
25.82
24.65
23.60
O
O
O
N
40


Chloroform-d
HOD
7.25
4.82
3.55
3.53
3.51
3.29
3.27
3.14
3.13
3.12
3.11
2.94
2.92
2.23
2.21
2.19
2.18
2.16
1.89
1.81
1.76
1.60
1.46
1.44
1.41
1.24
1.23
1.21
HO
O
O
N
41
1.00
1.07
1.04
2.02
2.06
3.04
3.10


Chloroform-d
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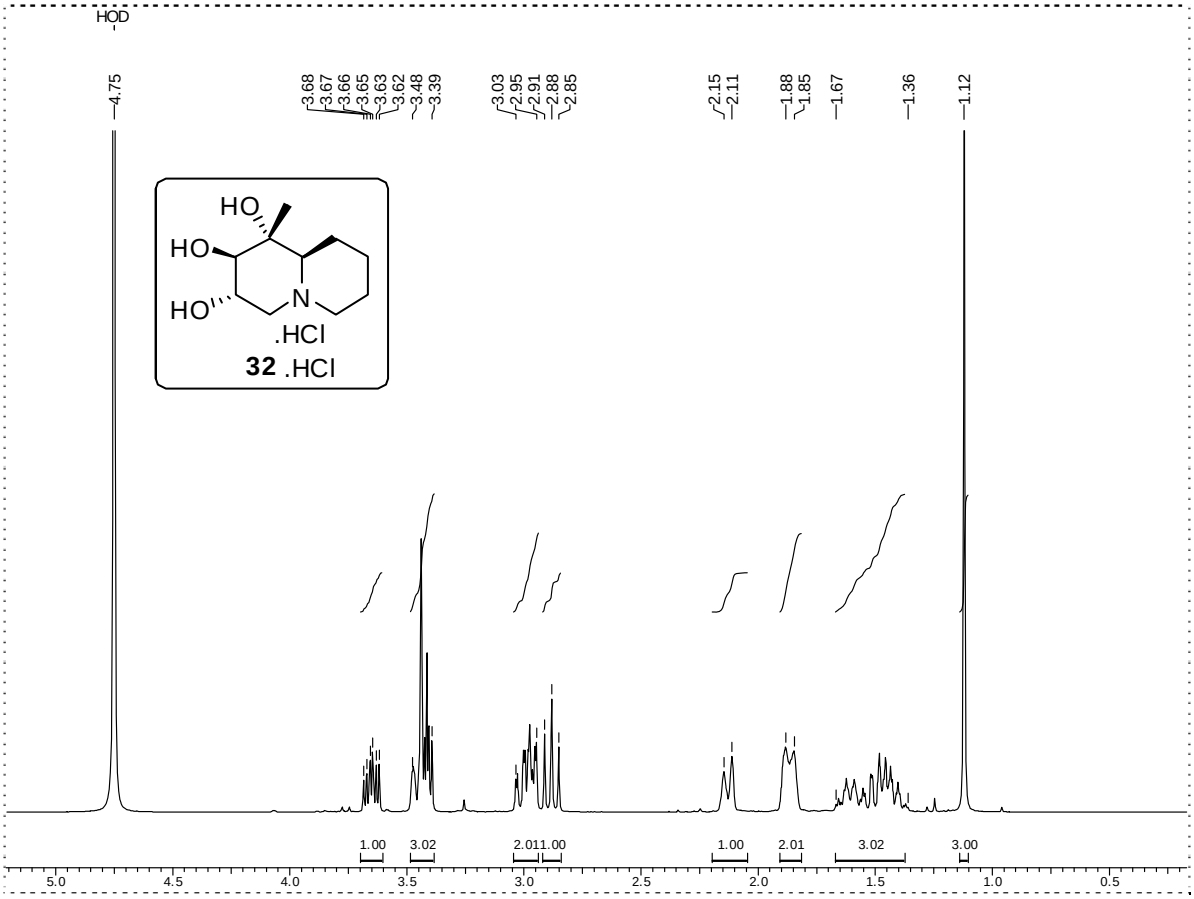
86.95
77.26
77.00
76.75
72.47
72.29
69.36
58.11
56.78
26.82
26.51
25.79
24.34
24.05
16.20
HO
O
O
N
41


86.94
72.46
69.35
58.10
56.77
26.82
26.51
25.78
24.33
24.04
16.20
HO
O
O
N
41


HOD
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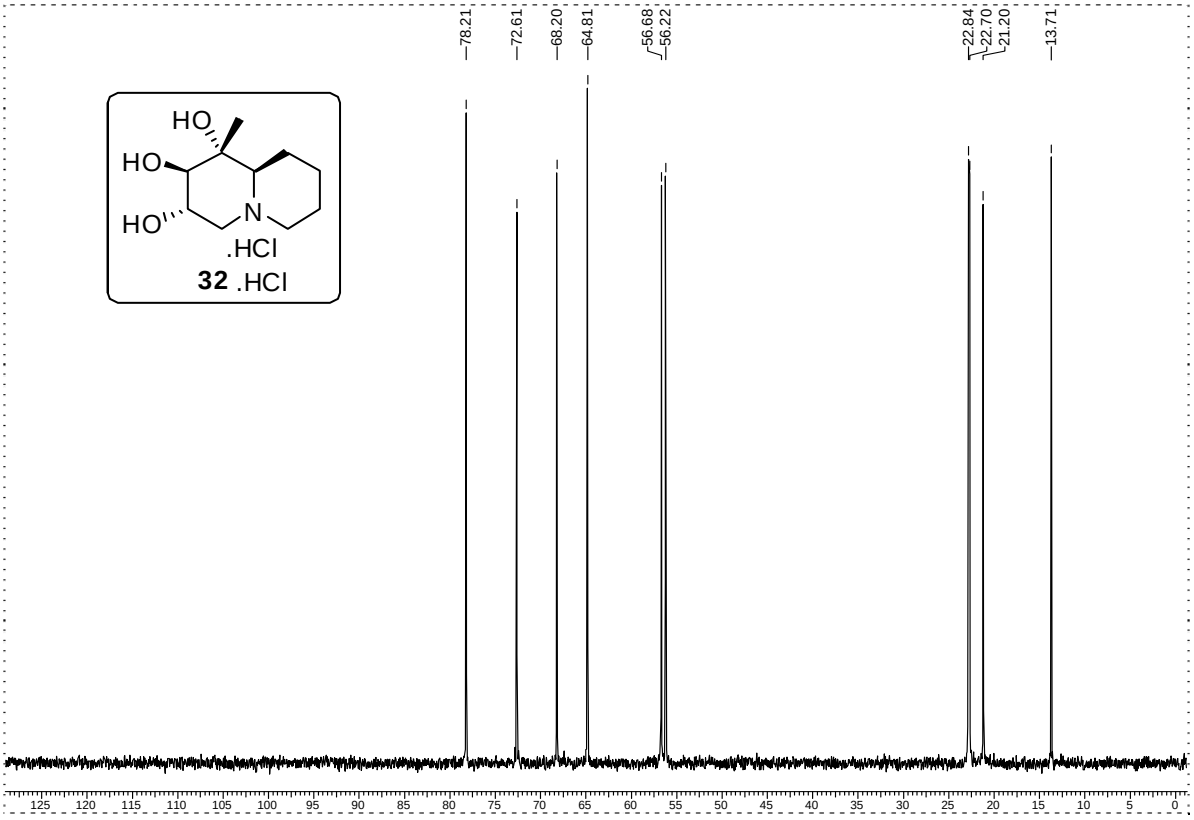
3.65
3.63
3.62
3.48
3.39
3.03
2.95
2.91
2.88
2.85
2.15
2.11
1.88
1.85
1.67
1.36
1.12
HO
HO
HO
N
.HCl
32 .HCl
1.00
3.02
2.01
1.00
1.00
2.01
3.02
3.00
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5


78.21
72.61
68.20
64.81
56.68
56.22
22.84
22.70
21.20
13.71
HO
HO
HO
N
.HCl
32 .HCl
125
120
115
110
105
100
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90
85
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75
70
65
60
55
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45
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35
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25
20
15
10
5
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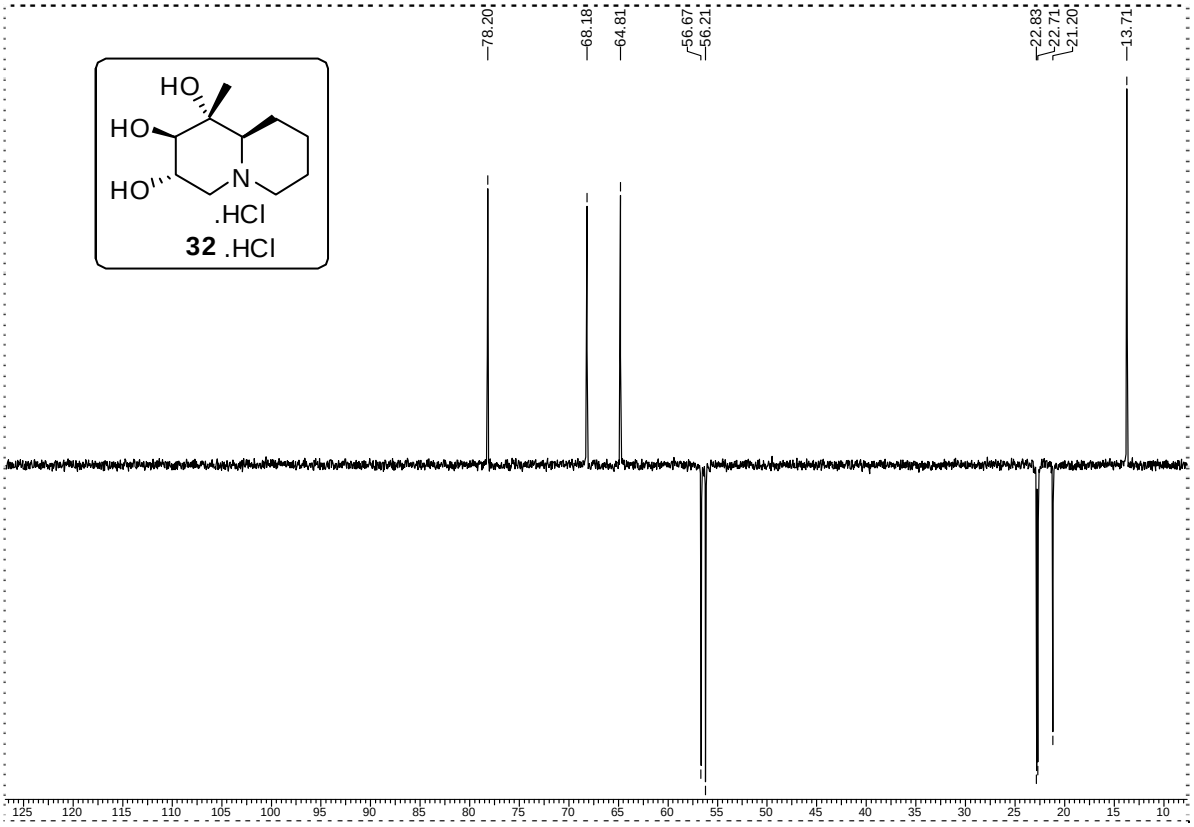
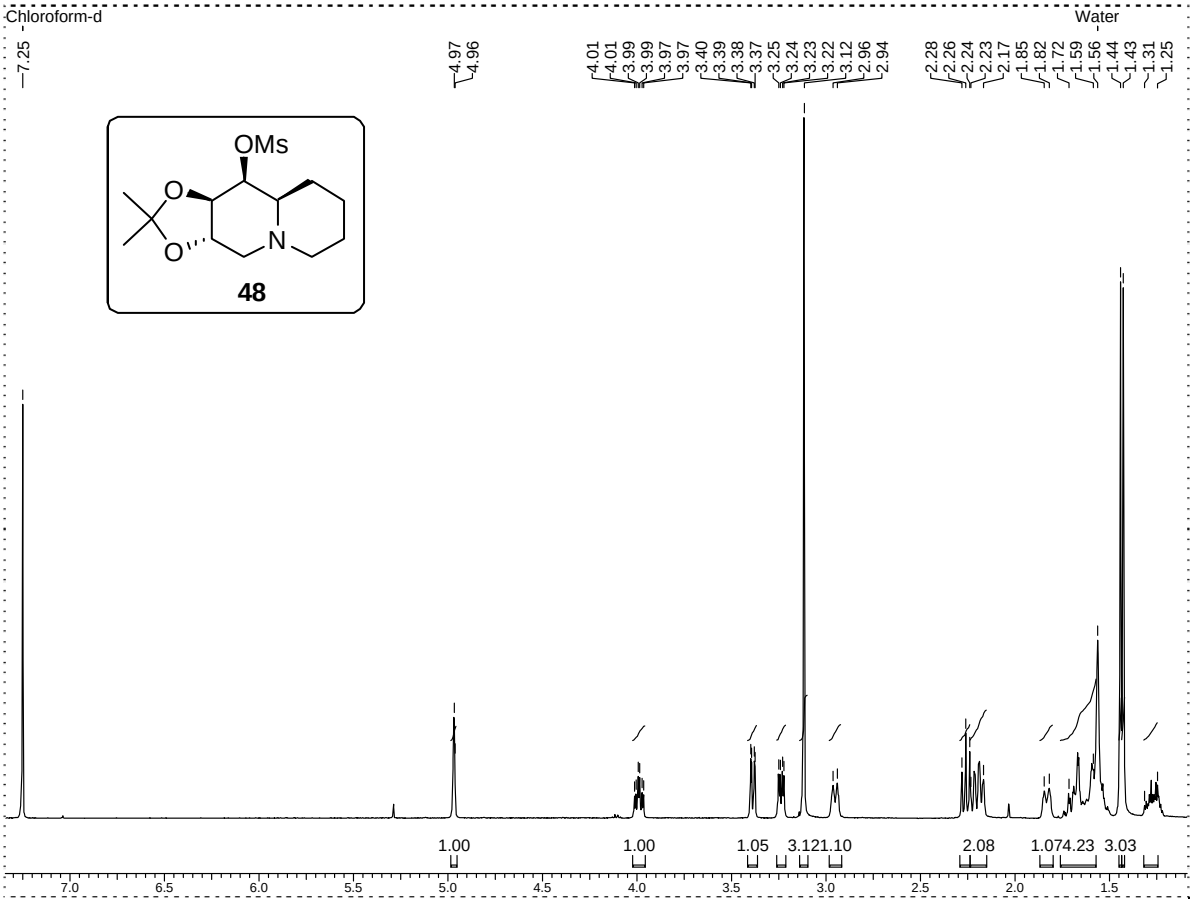

78.20
68.18
64.81
56.67
56.21
22.83
22.71
21.20
13.71
HO
HO
HO
N
.HCl
32 .HCl


Chloroform-d
Water
7.25
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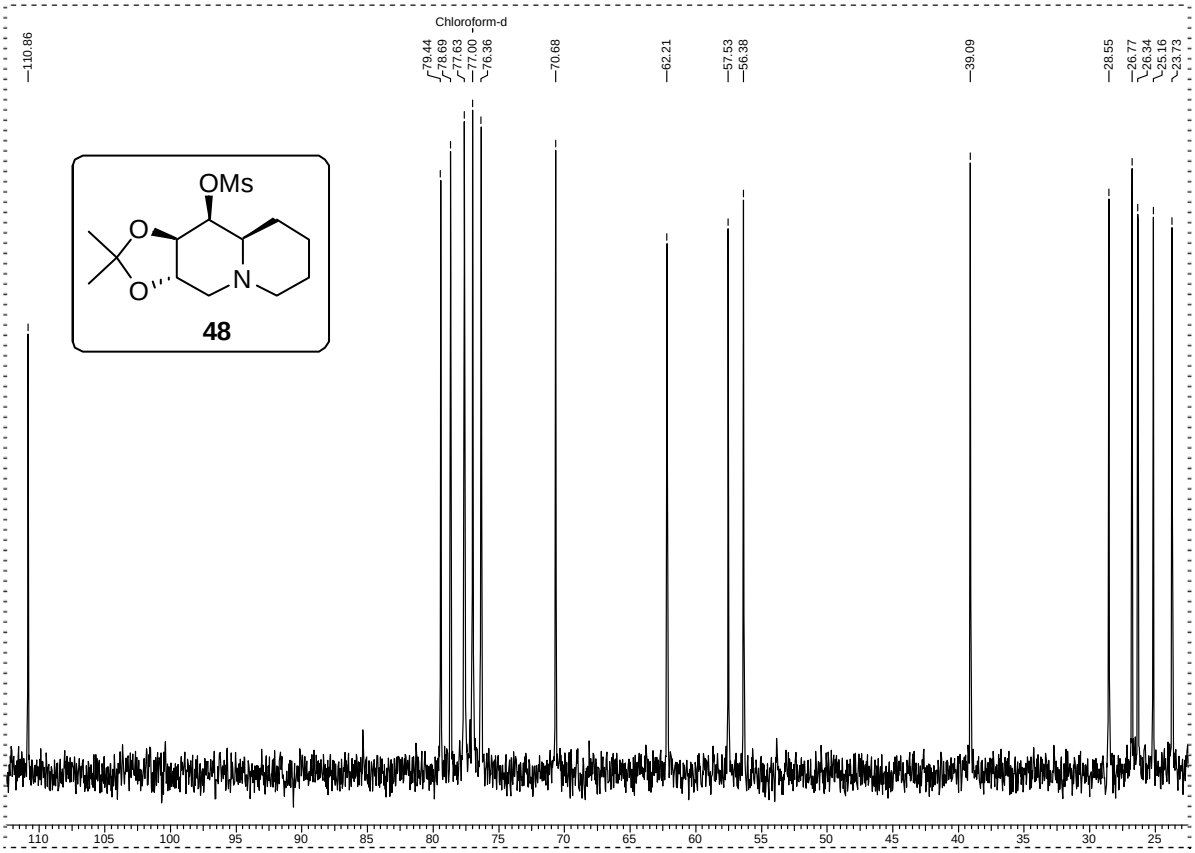
4.96
4.01
4.01
3.99
3.99
3.97
3.97
3.40
3.39
3.38
3.37
3.25
3.24
3.23
3.22
3.12
2.96
2.94
2.28
2.26
2.24
2.23
2.17
1.85
1.82
1.72
1.59
1.56
1.44
1.43
1.31
1.25
OMs
O
O
N
48
1.00
1.00
1.05
3.12
1.10
2.08
1.07
4.23
3.03


Chloroform-d
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79.44
78.69
77.63
77.00
76.36
70.68
62.21
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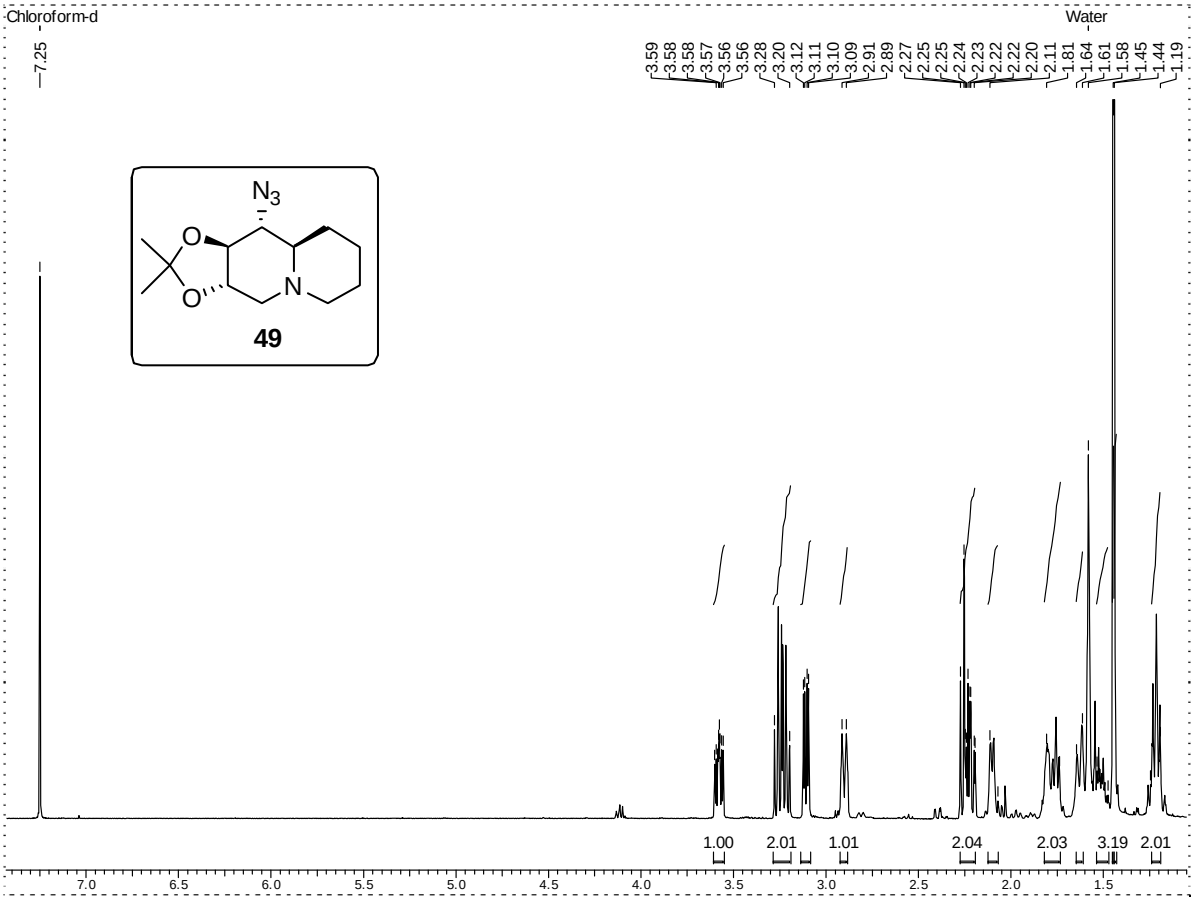
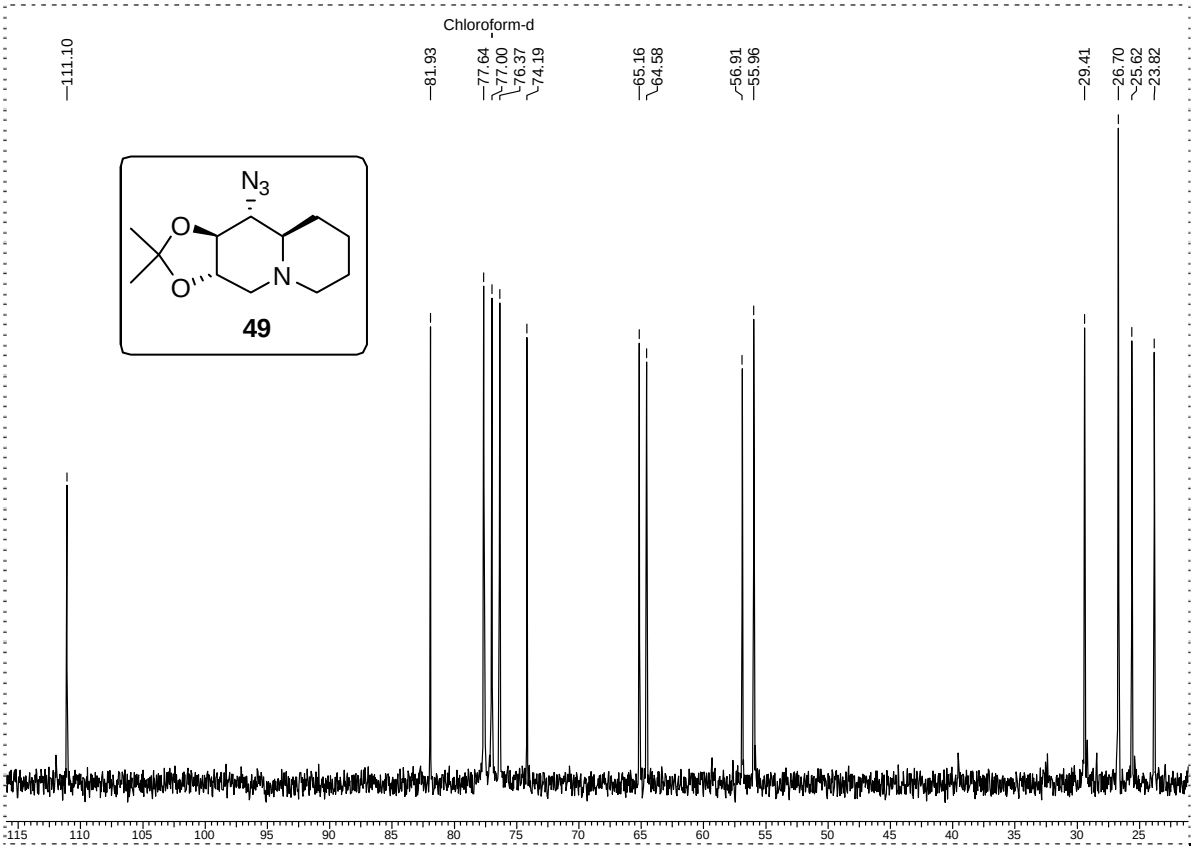
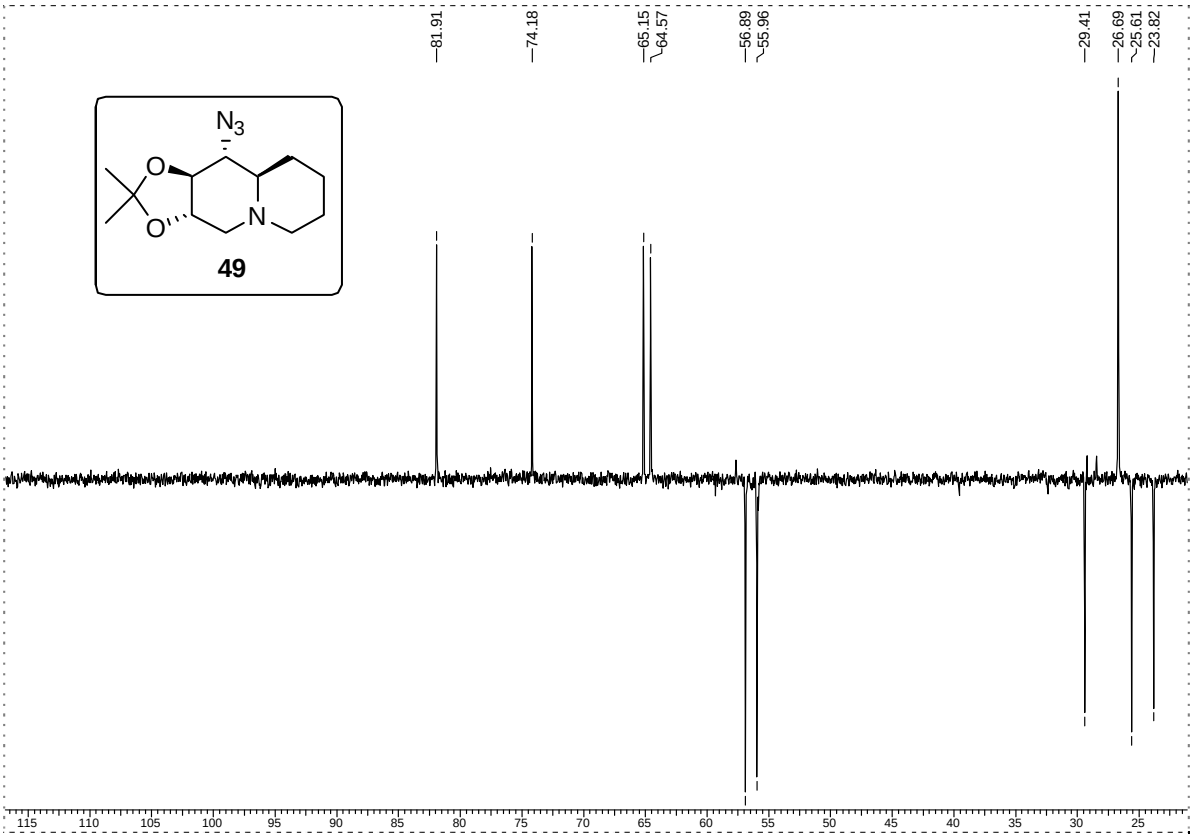
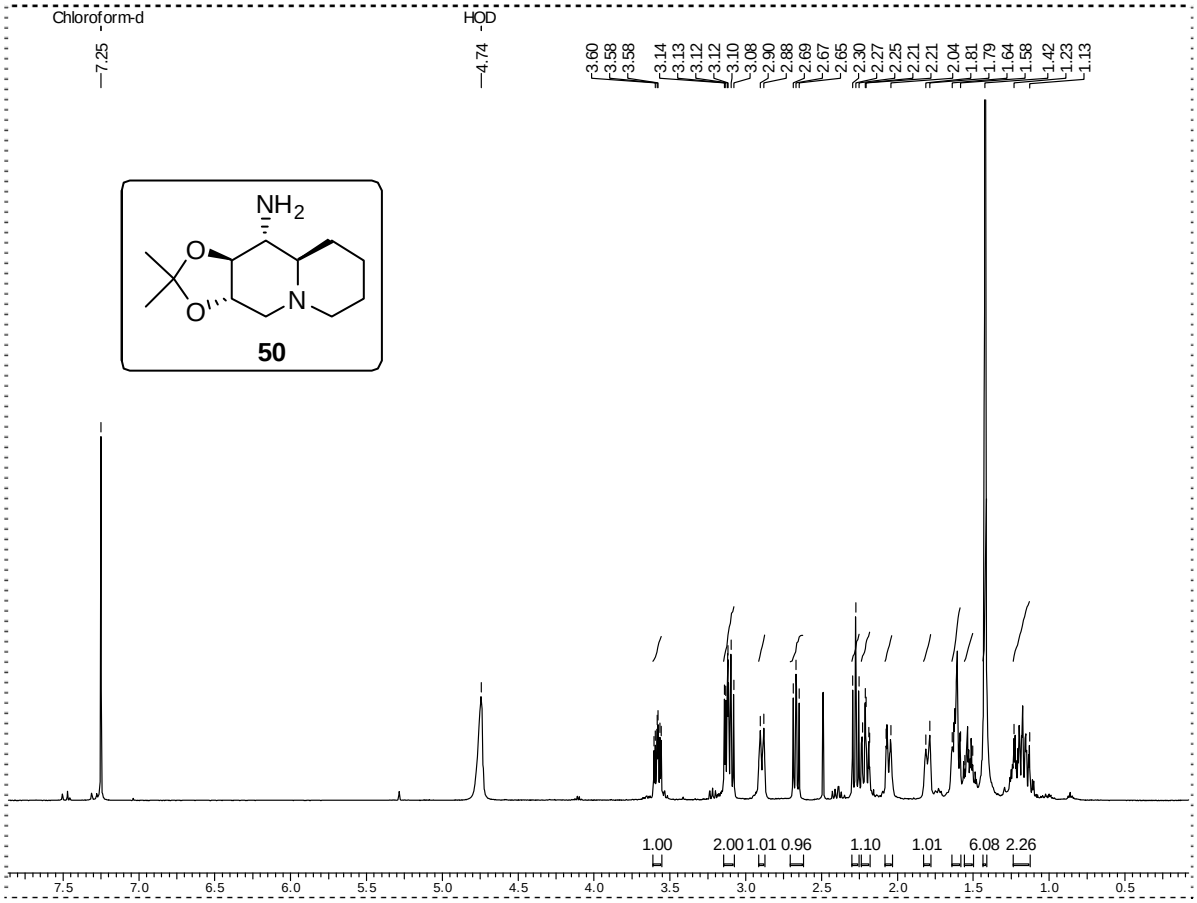
39.09
28.55
26.77
26.34
25.16
23.73
OMs
O
O
N
48


Chloroform-d
Water
7.25
3.59
3.58
3.58
3.57
3.56
3.56
3.28
3.20
3.12
3.11
3.10
3.09
2.91
2.89
2.27
2.25
2.25
2.24
2.23
2.22
2.22
2.20
2.11
1.81
1.64
1.61
1.58
1.45
1.44
1.19
N3
O
O
N
49
1.00
2.01
1.01
2.04
2.03
3.19
2.01


Chloroform-d
111.10
81.93
77.64
77.00
76.37
74.19
65.16
64.58
56.91
55.96
29.41
26.70
25.62
23.82
N3
O
O
N
49


81.91
74.18
65.15
64.57
56.89
55.96
29.41
26.69
25.61
23.82
N3
O
O
N
49


Chloroform-d
HOD
7.25
4.74
3.60
3.58
3.58
3.14
3.13
3.12
3.12
3.10
3.08
2.90
2.88
2.69
2.67
2.65
2.30
2.27
2.25
2.21
2.21
2.04
1.81
1.79
1.64
1.58
1.42
1.23
1.13
NH2
O
O
N
50
1.00
2.00
1.01
0.96
1.10
1.01
6.08
2.26
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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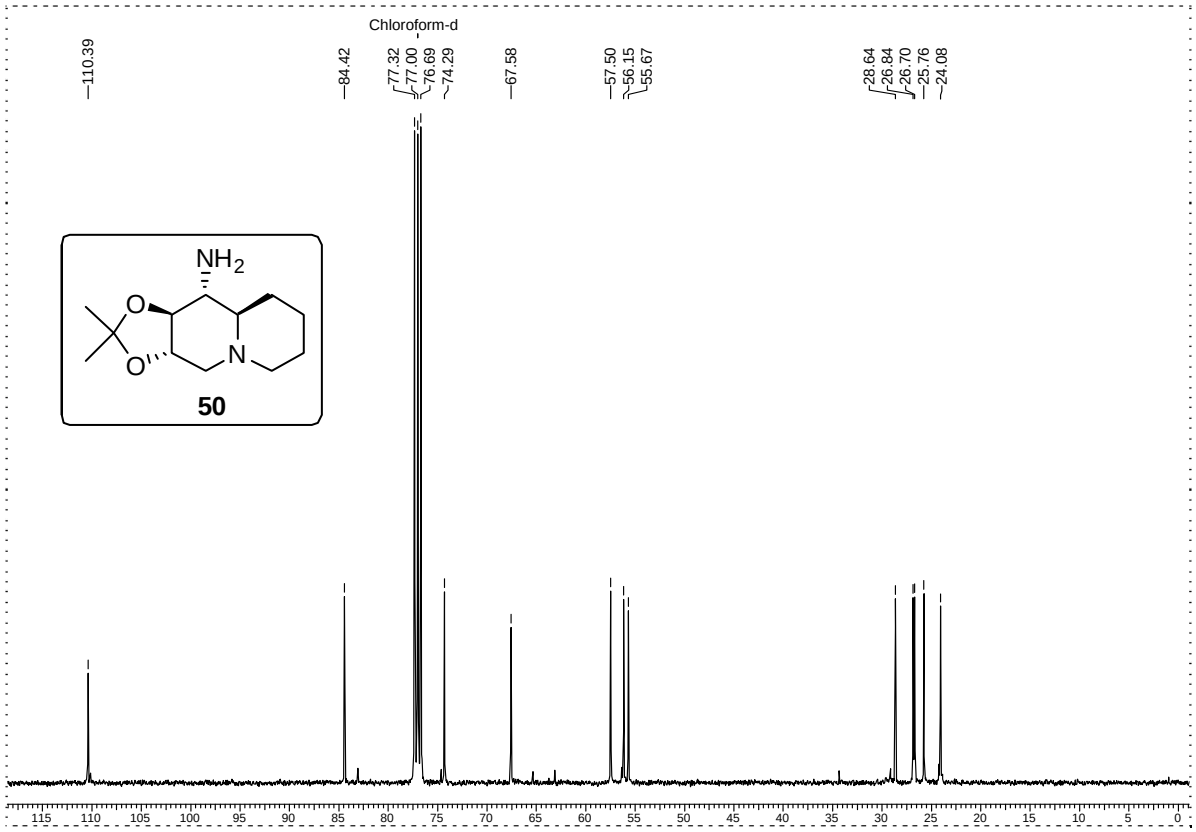
3.5
3.0
2.5
2.0
1.5
1.0
0.5


Chloroform-d
110.39
84.42
77.32
77.00
76.69
74.29
67.58
57.50
56.15
55.67
28.64
26.84
26.70
25.76
24.08
NH2
O
O
N
50
115
110
105
100
95
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75
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35
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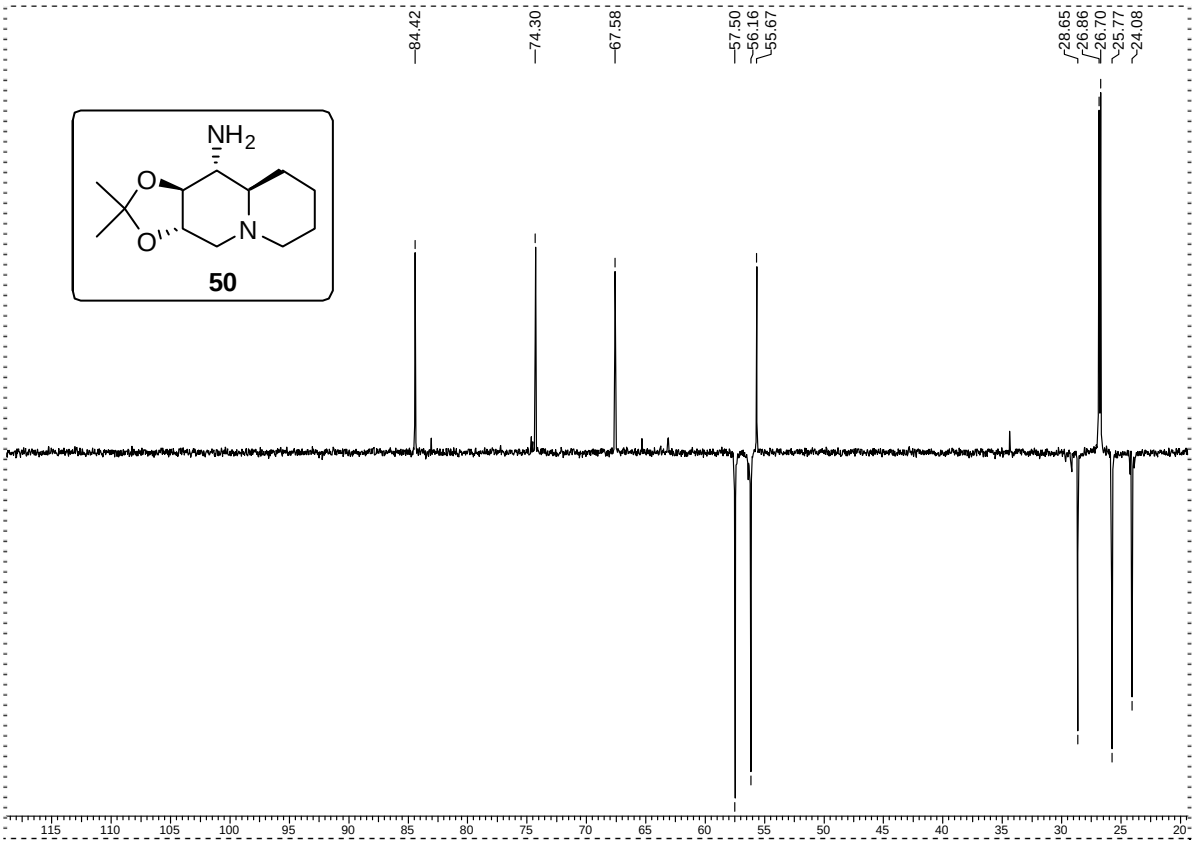
84.42
74.30
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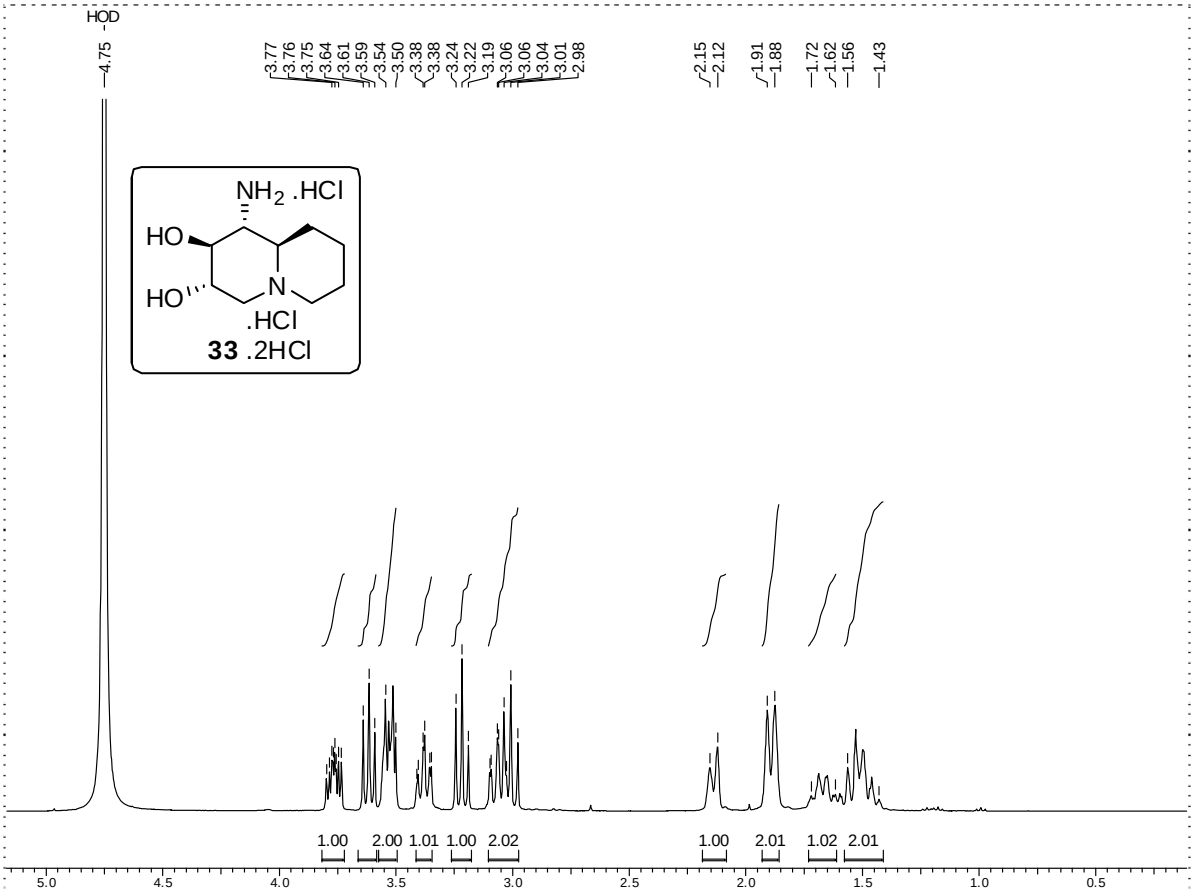
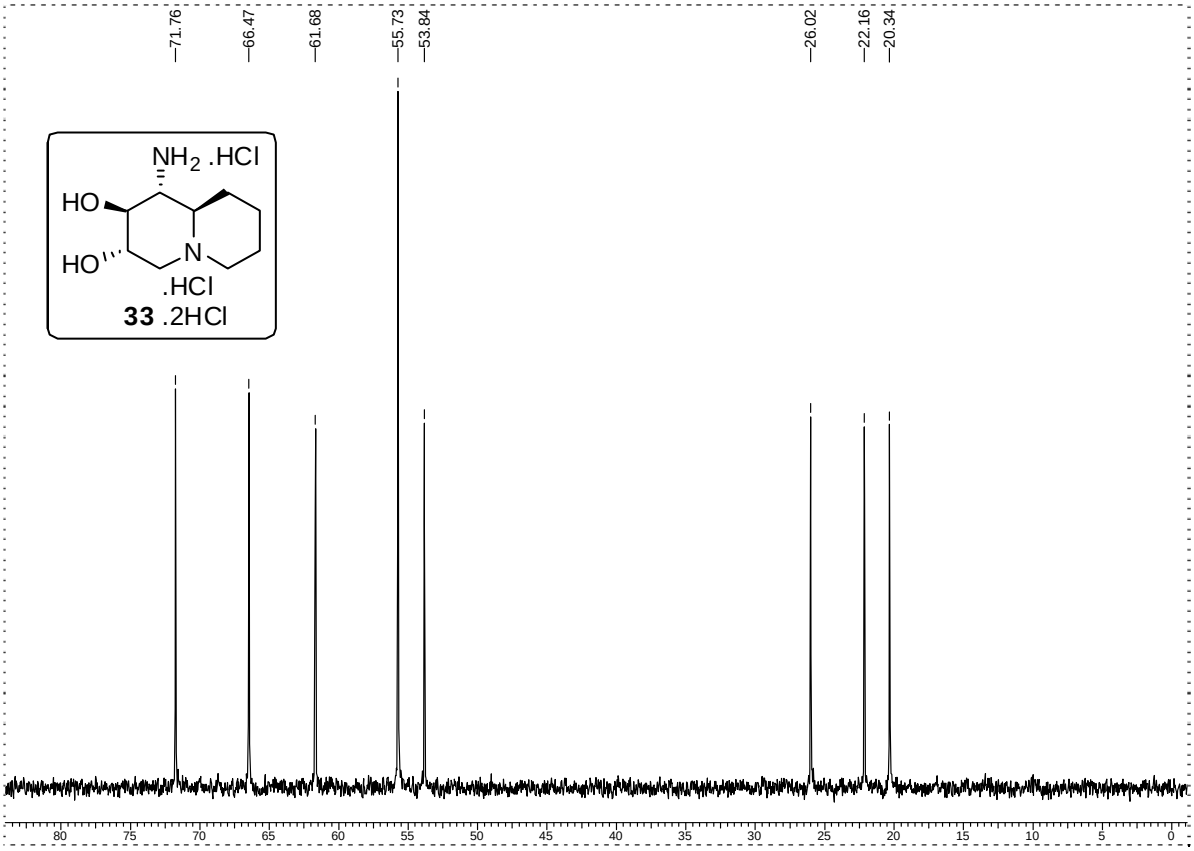
57.50
56.16
55.67
28.65
26.86
26.70
25.77
24.08
NH2
O
O
N
50


HOD
4.75
3.77
3.76
3.75
3.64
3.61
3.59
3.54
3.50
3.38
3.38
3.24
3.22
3.19
3.06
3.06
3.04
3.01
2.98
2.15
2.12
1.91
1.88
1.72
1.62
1.56
1.43
NH2 .HCl
HO
HO
N
.HCl
33 .2HCl
1.00
2.00
1.01
1.00
2.02
1.00
2.01
1.02
2.01


71.76
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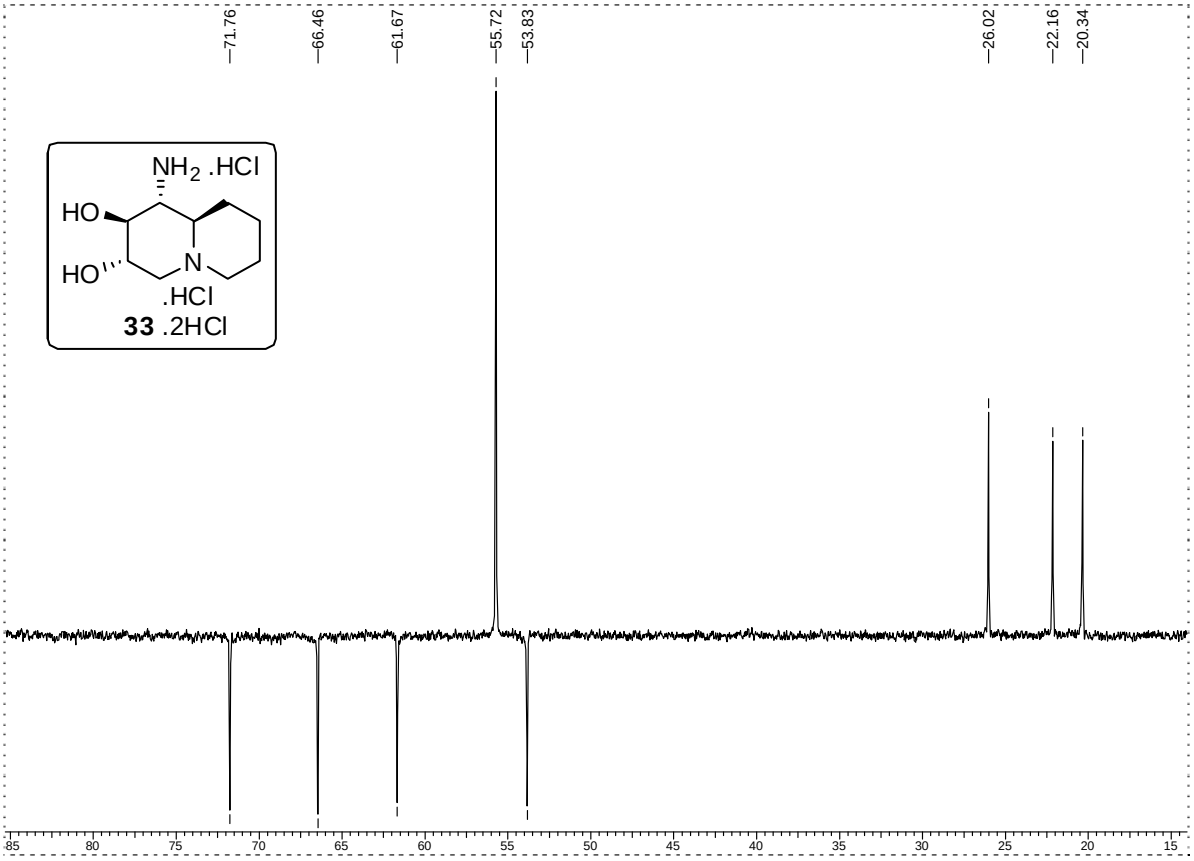
61.68
55.73
53.84
26.02
22.16
20.34
NH2 .HCl
HO
HO
N
.HCl
33 .2HCl


71.76
66.46
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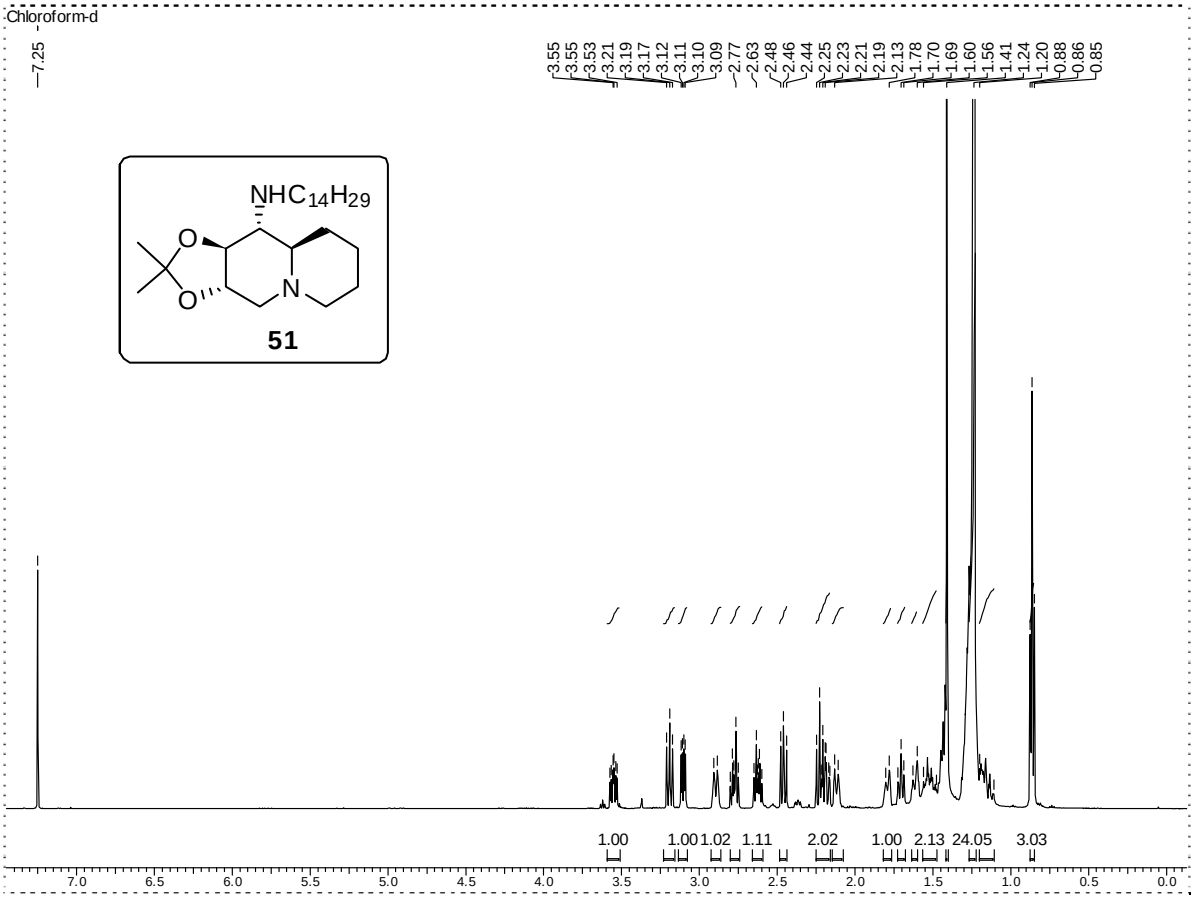
55.72
53.83
26.02
22.16
20.34
NH2 .HCl
HO
HO
N
.HCl
33 .2HCl


Chloroform-d
7.25
3.55
3.55
3.53
3.21
3.19
3.17
3.12
3.11
3.10
3.09
2.77
2.63
2.48
2.46
2.44
2.25
2.23
2.21
2.19
2.13
1.78
1.70
1.69
1.60
1.56
1.41
1.24
1.20
0.88
0.86
0.85
NHC14H29
O
O
N
51
1.00
1.00
1.02
1.11
2.02
1.00
2.13
24.05
3.03
7.0
6.5
6.0
5.5
5.0
4.5
4.0
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2.0
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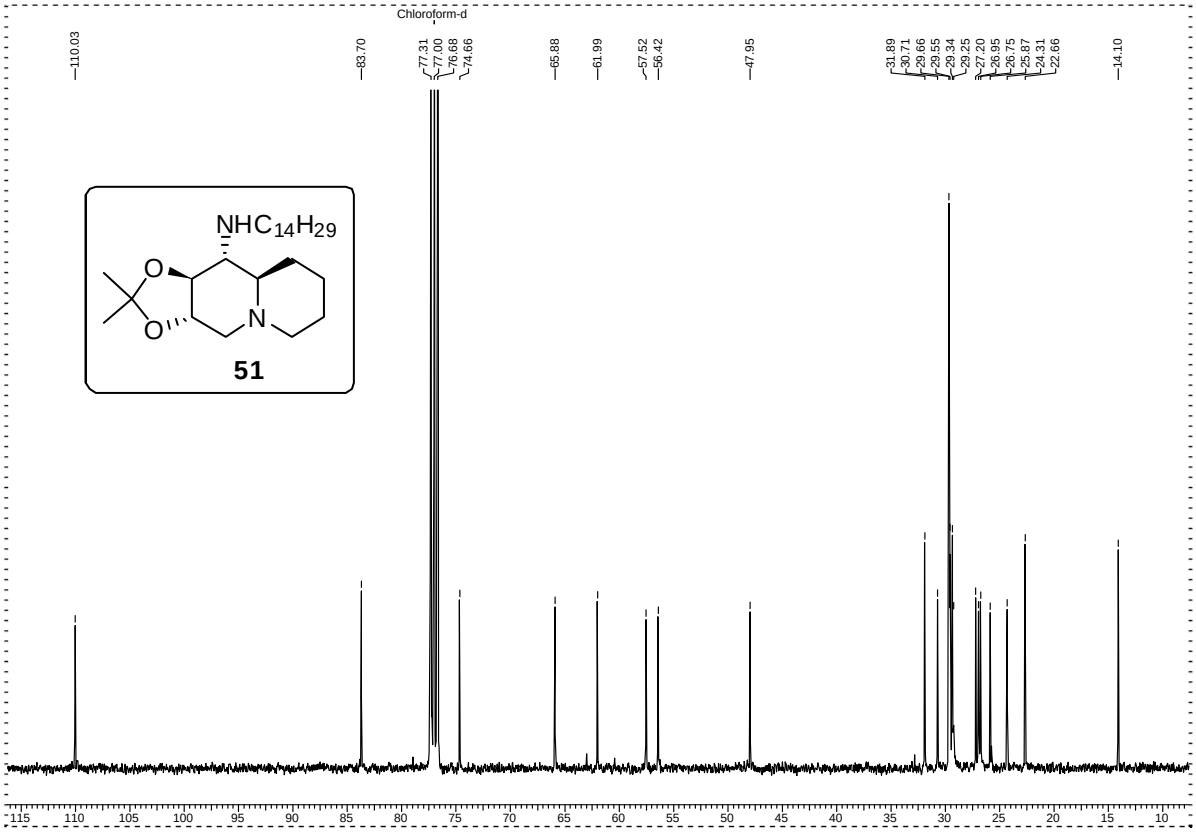
Chloroform-d
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83.70
77.31
77.00
76.68
74.66
65.88
61.99
57.52
56.42
47.95
31.89
30.71
29.66
29.55
29.34
29.25
27.20
26.95
26.75
25.87
24.31
22.66
14.10
NHC14H29
O
O
N
51
115
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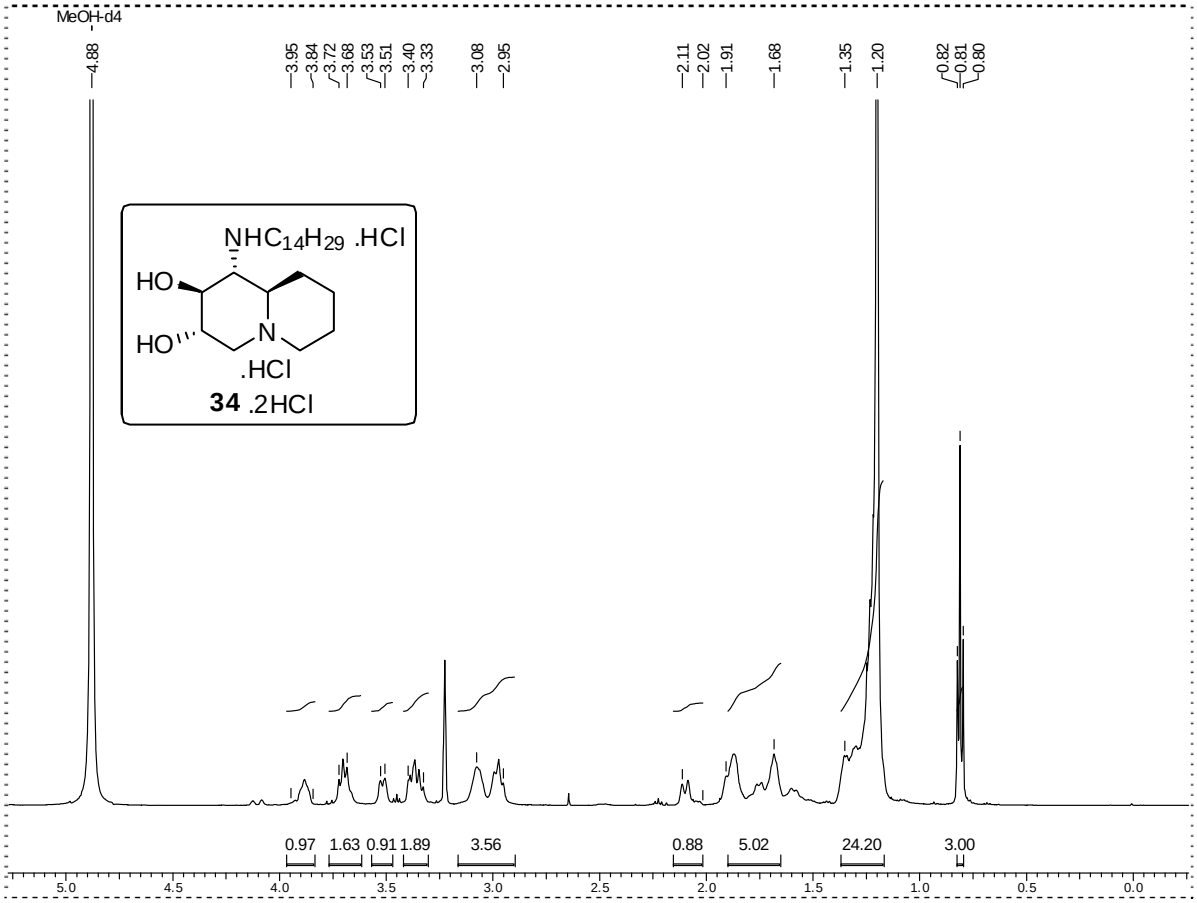
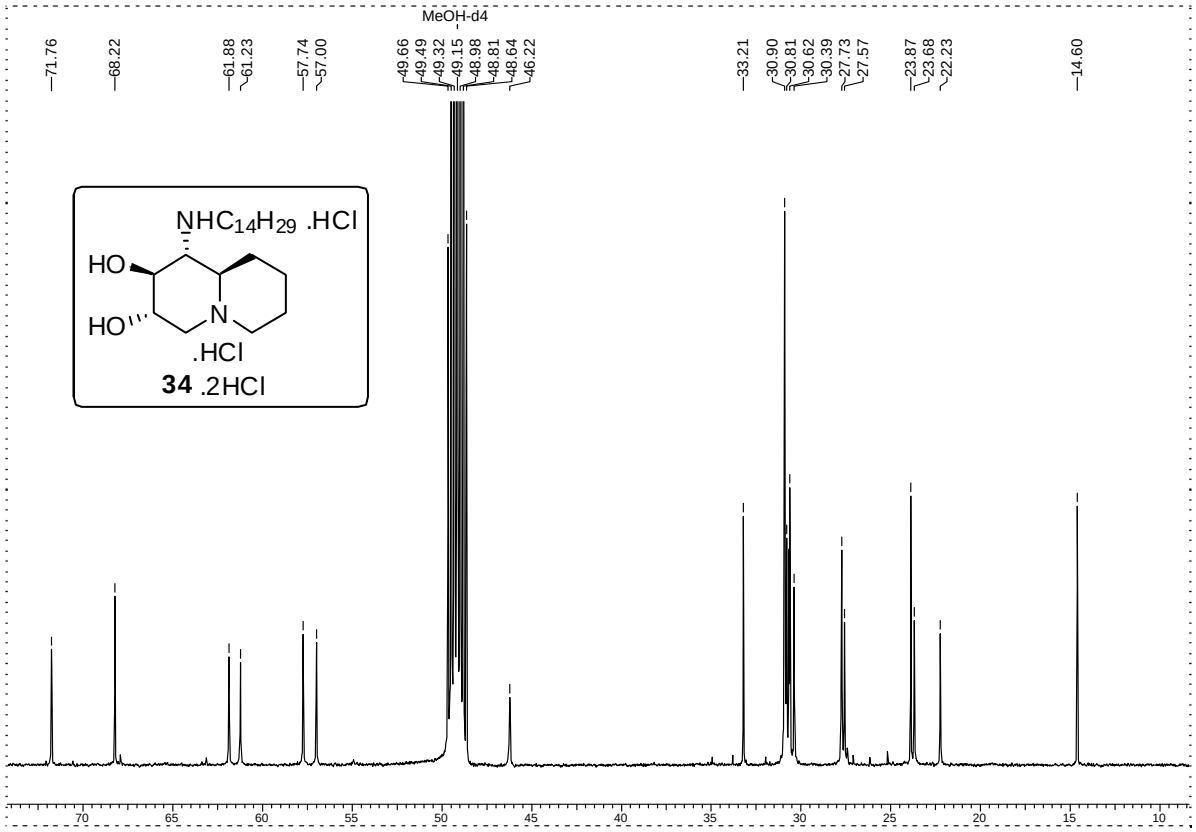
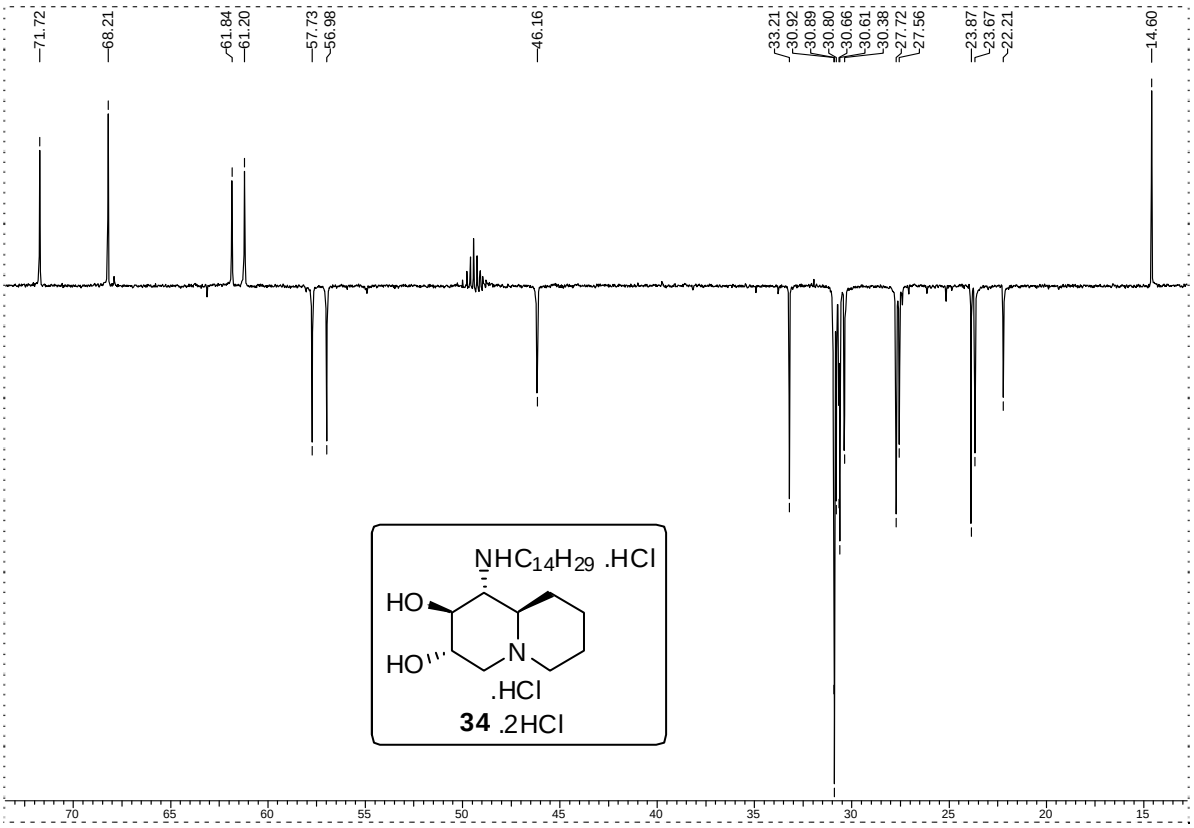
10


MeOH-d4
4.88
3.95
3.84
3.72
3.68
3.53
3.51
3.40
3.33
3.08
2.95
2.11
2.02
1.91
1.68
1.35
1.20
0.82
0.81
0.80
NHC14H29 .HCl
HO
HO
N
.HCl
34 .2HCl
0.97
1.63
0.91
1.89
3.56
0.88
5.02
24.20
3.00


MeOH-d4
71.76
68.22
61.88
61.23
57.74
57.00
49.66
49.49
49.32
49.15
48.98
48.81
48.64
46.22
33.21
30.90
30.81
30.62
30.39
27.73
27.57
23.87
23.68
22.23
14.60
NHC14H29 .HCl
HO
HO
N
.HCl
34 .2HCl


71.72
68.21
61.84
61.20
57.73
56.98
46.16
33.21
30.92
30.89
30.80
30.66
30.61
30.38
27.72
27.56
23.87
23.67
22.21
14.60
NHC14H29 .HCl
HO
HO
N
.HCl
34 .2HCl


Chloroform-d
7.36
7.35
7.30
7.28
7.27
7.25
7.22
7.21
4.02
4.00
3.99
3.89
3.86
3.40
3.39
3.38
3.37
3.19
3.18
3.17
3.16
3.04
3.03
2.92
2.90
2.16
2.14
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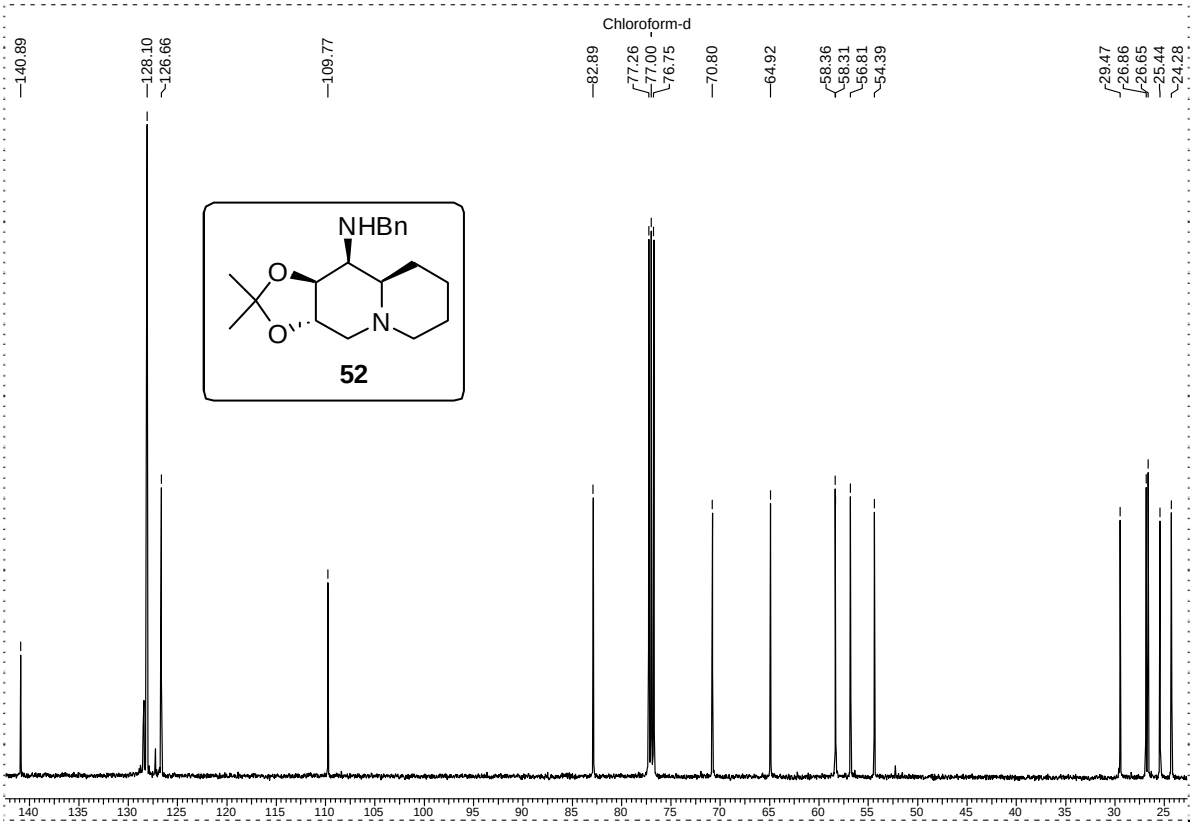
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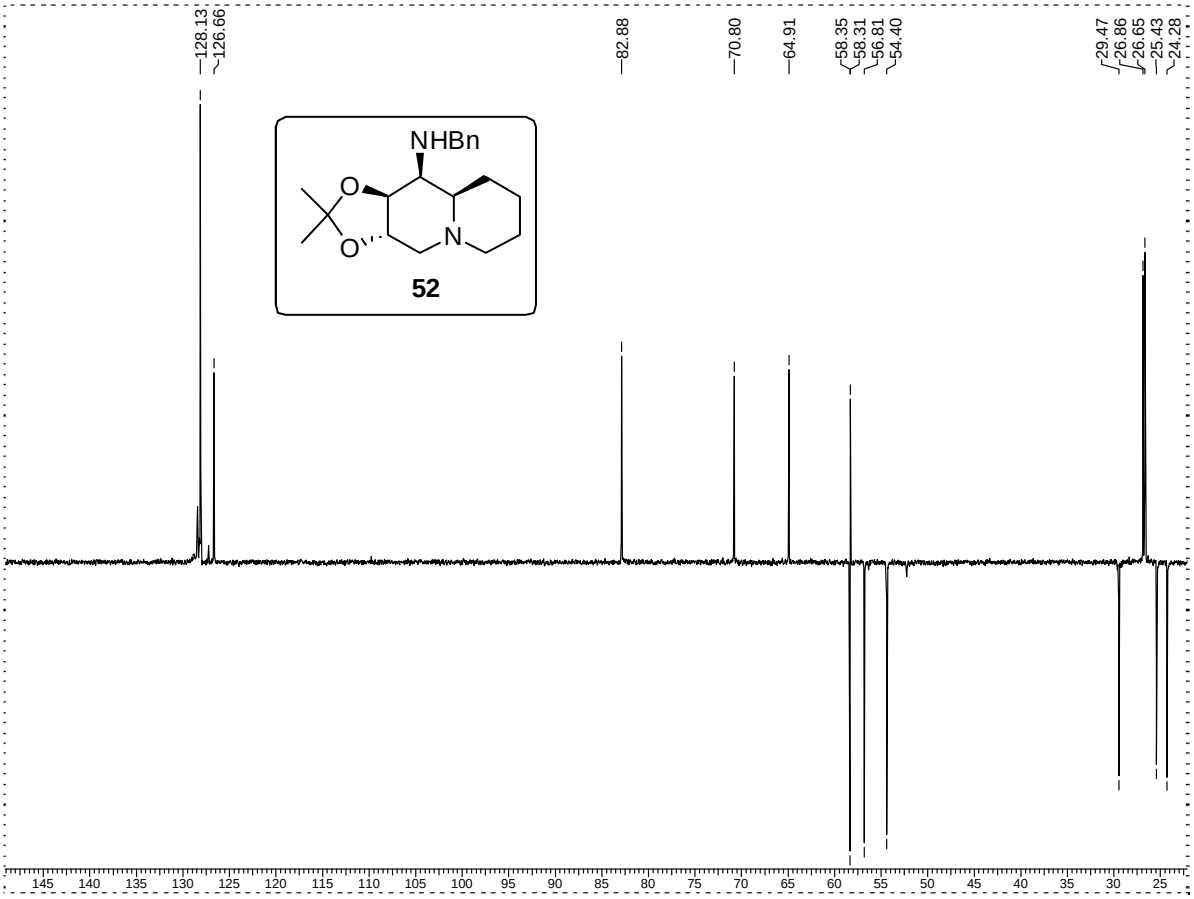
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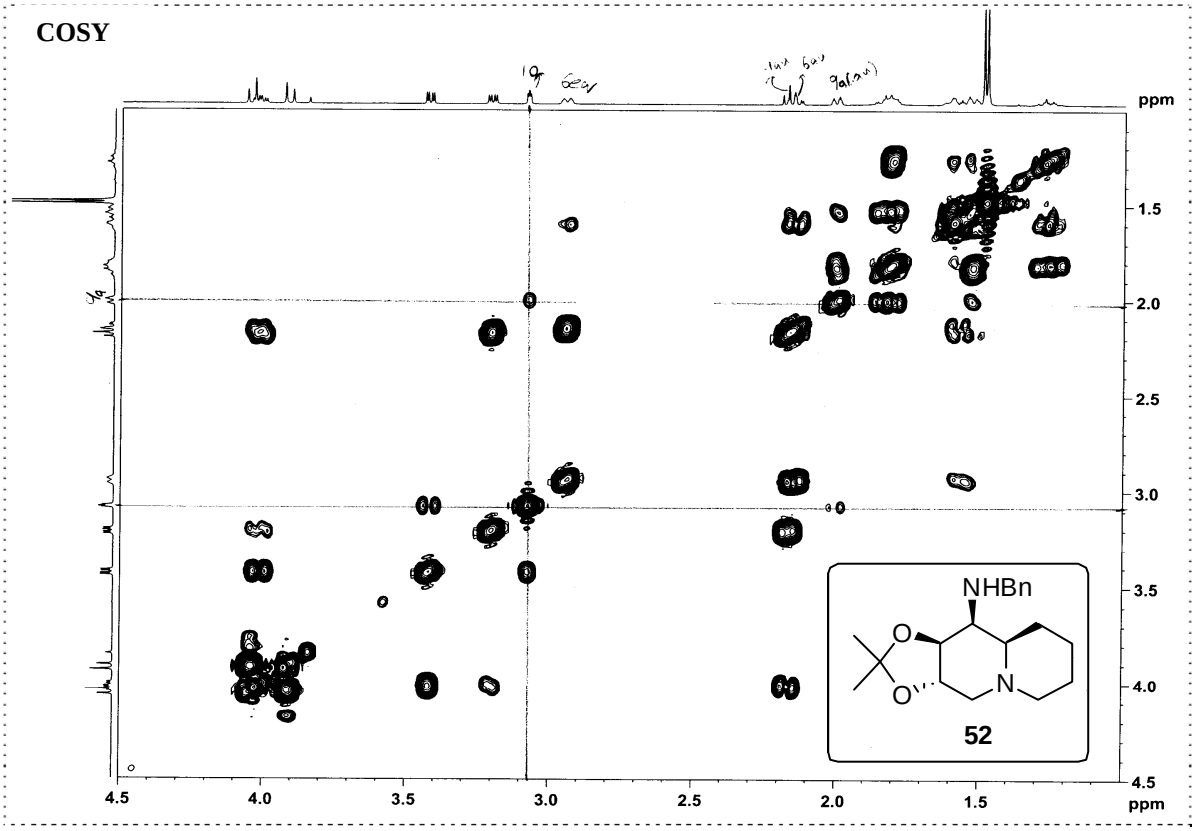
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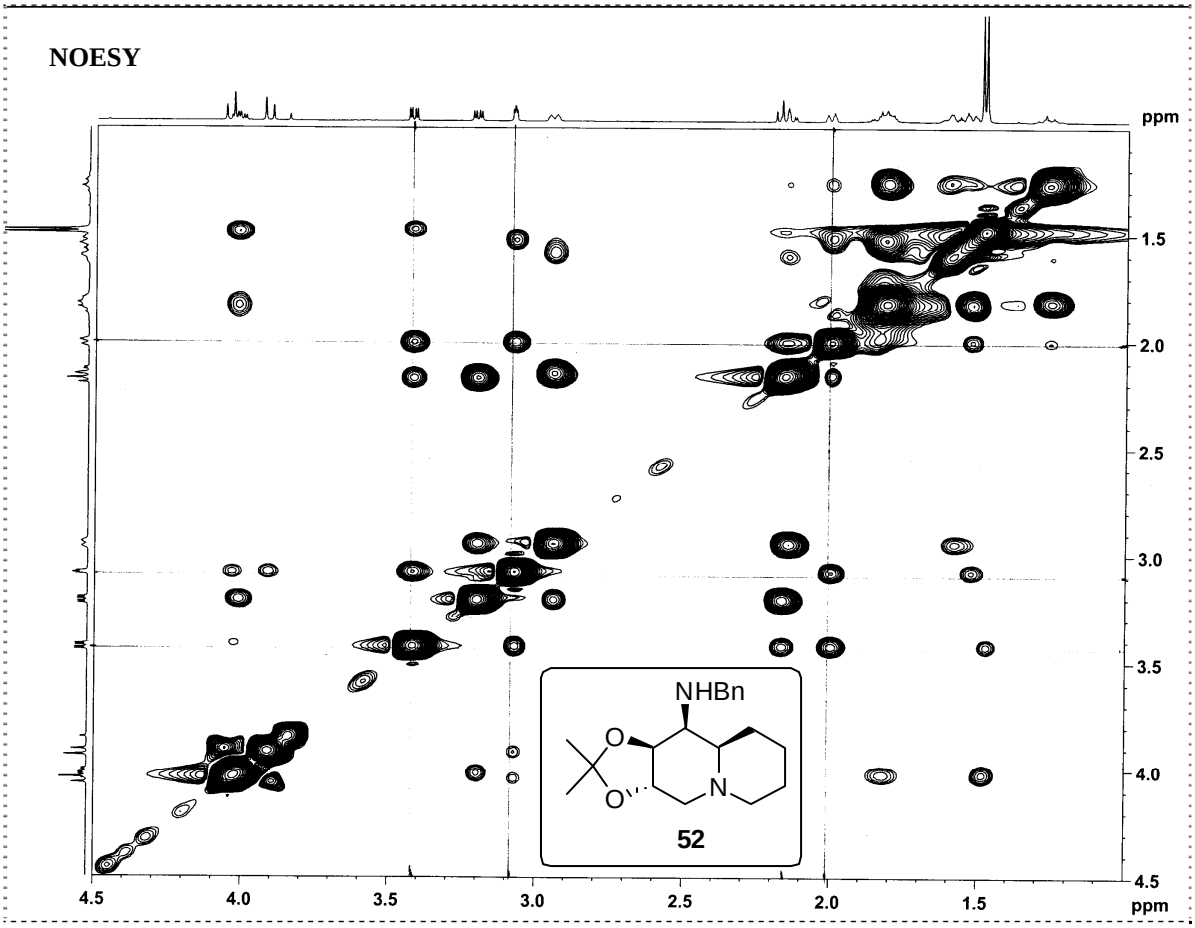
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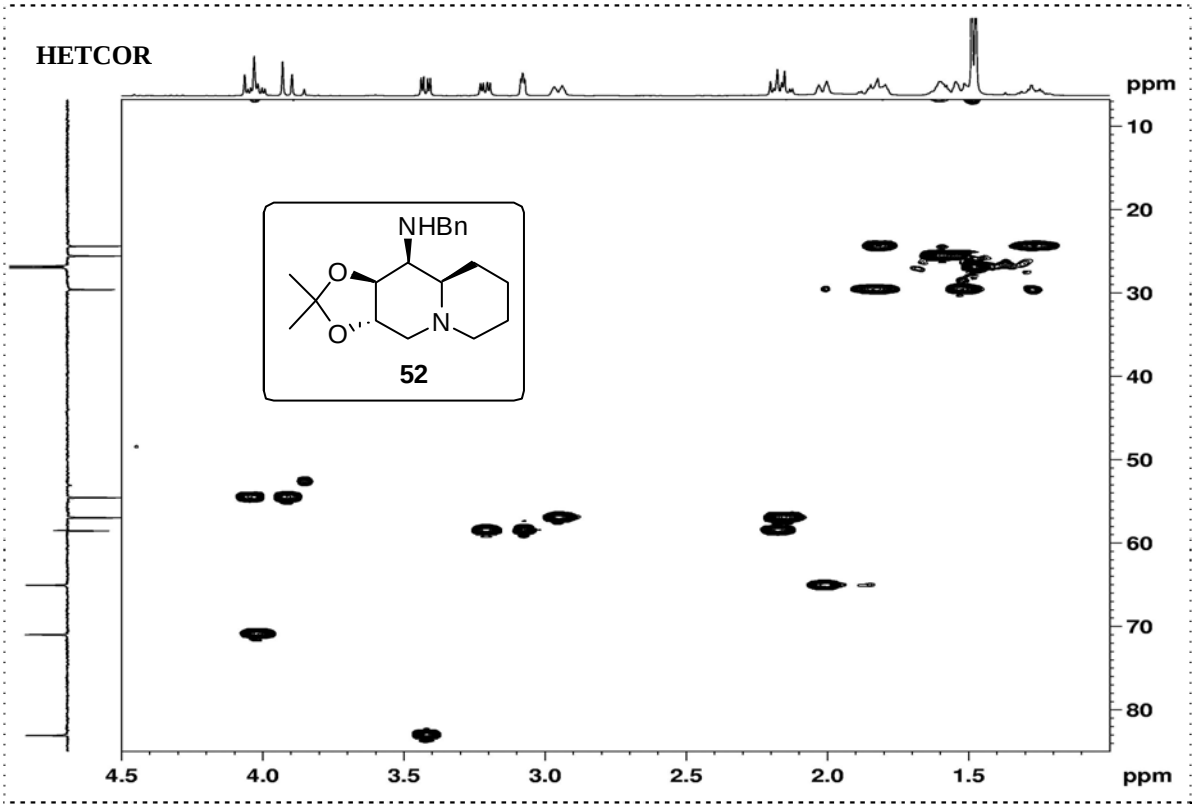
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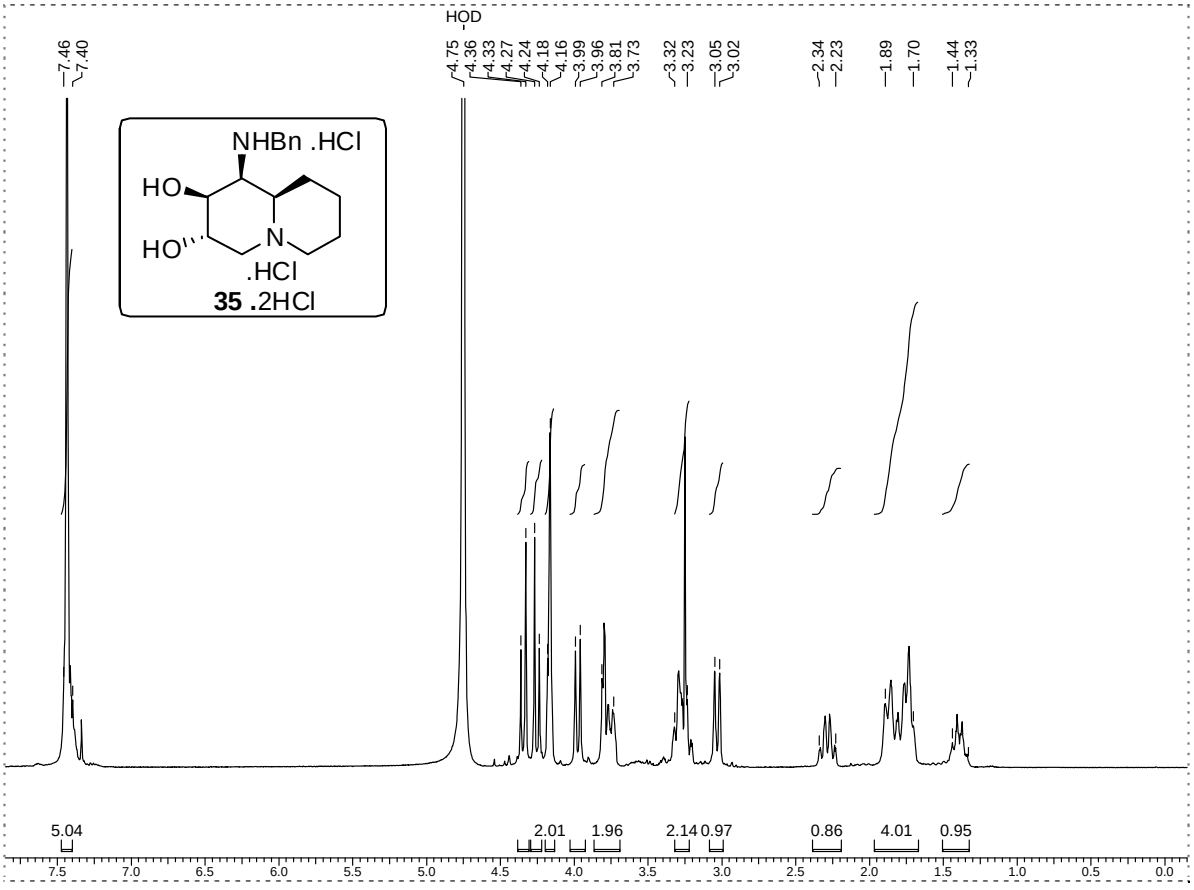
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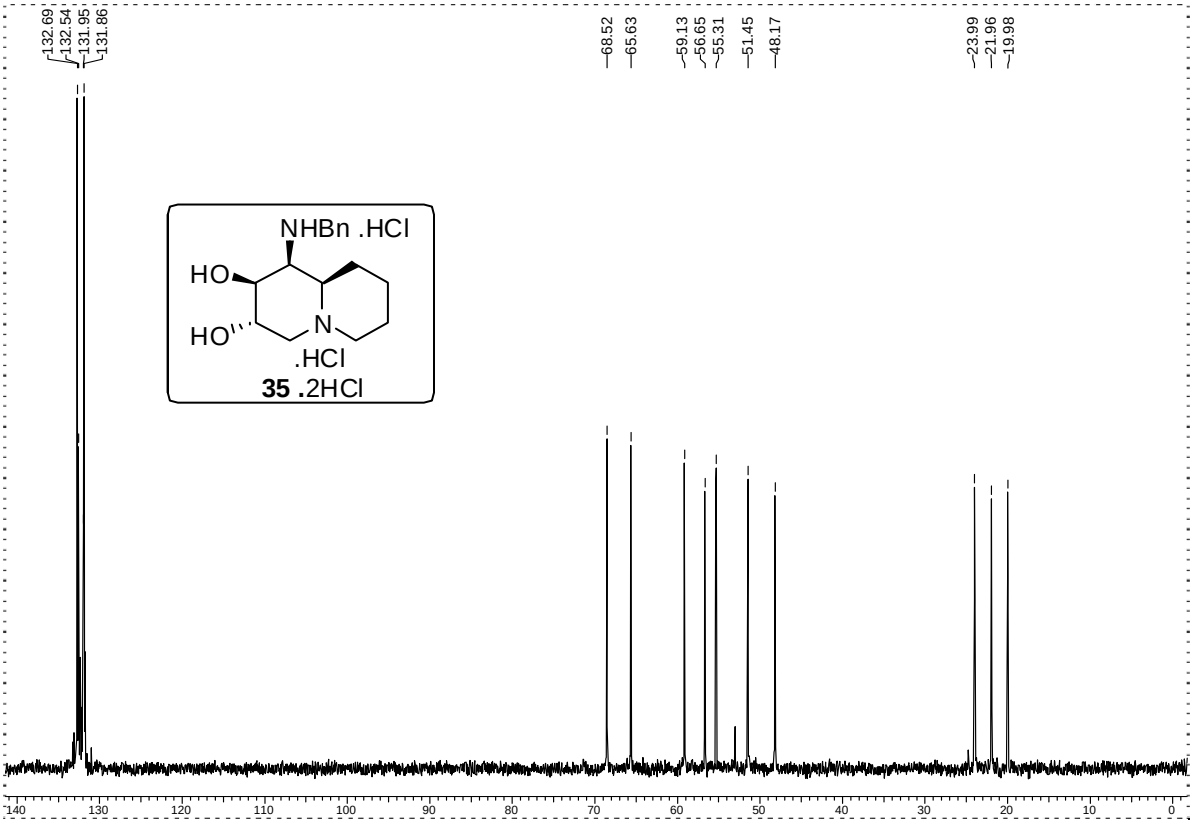
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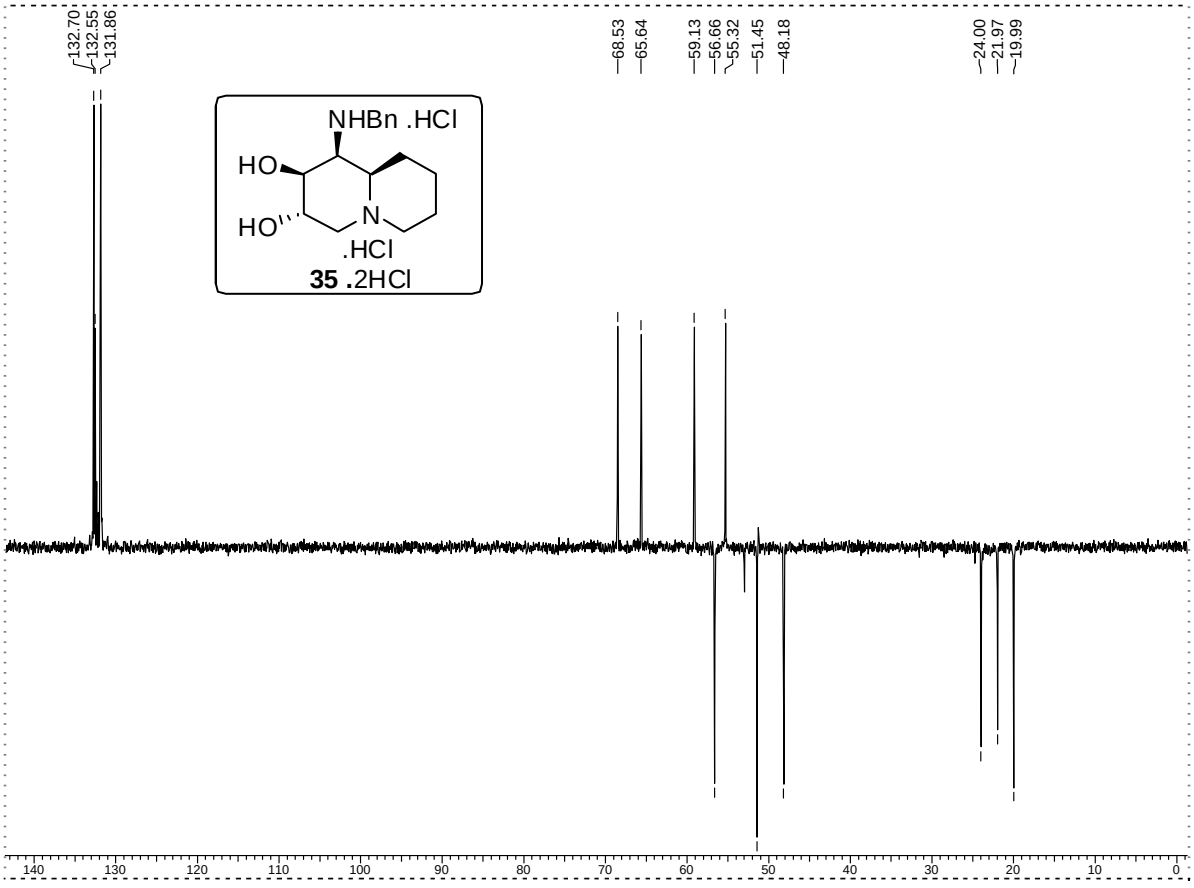
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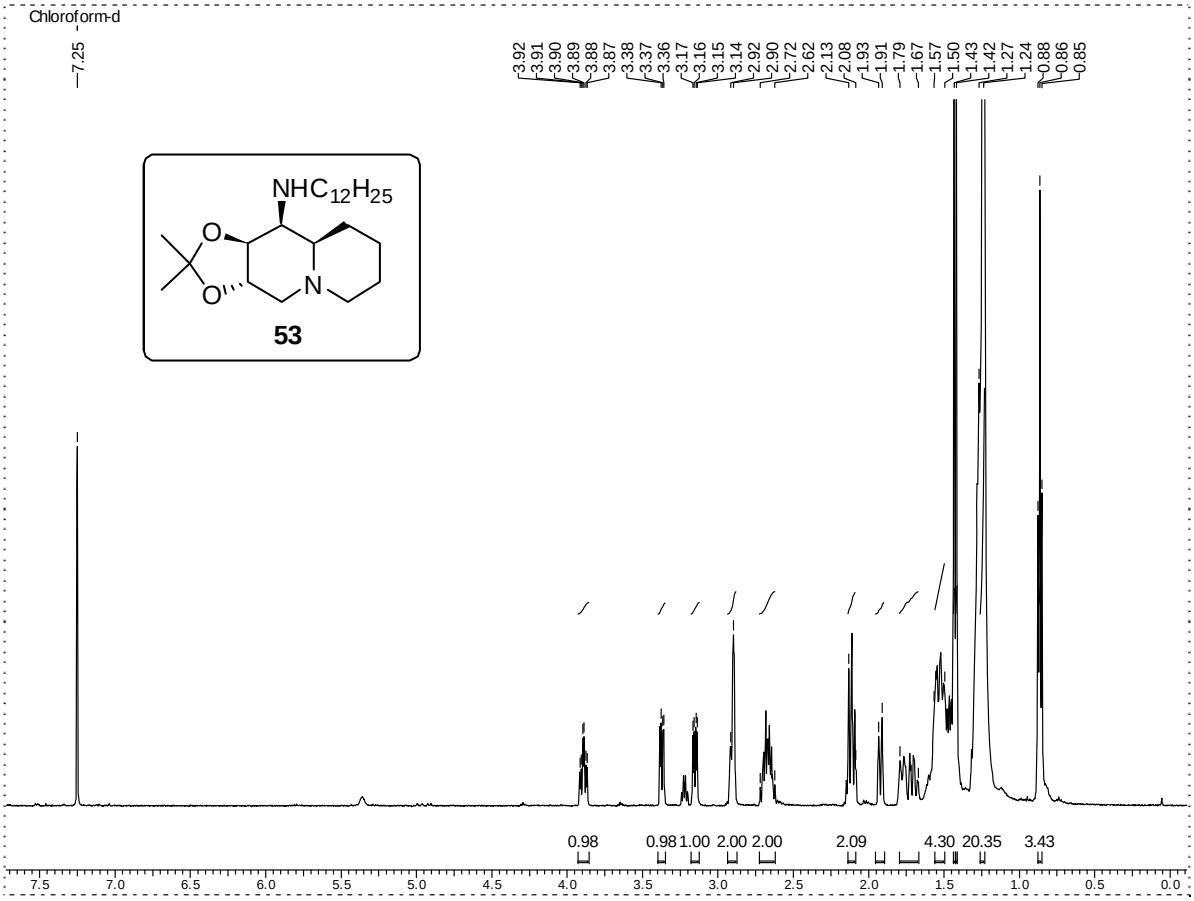
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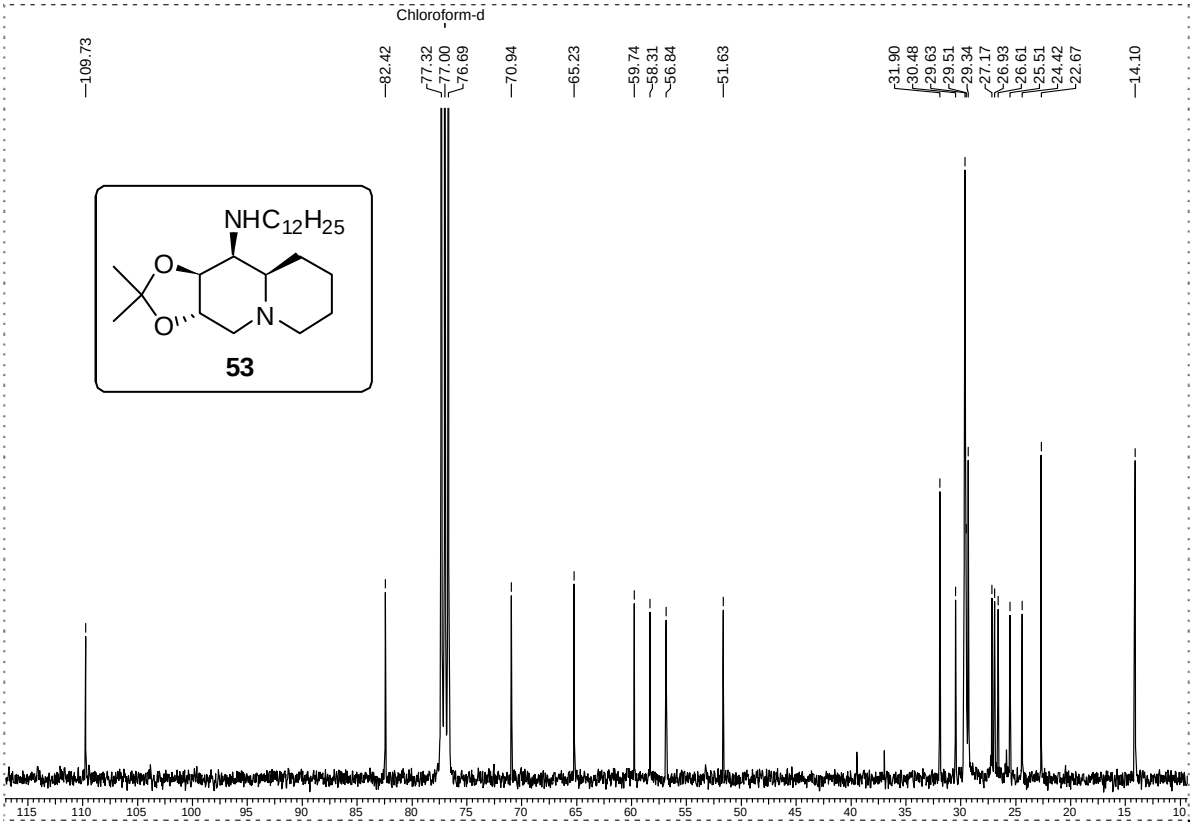
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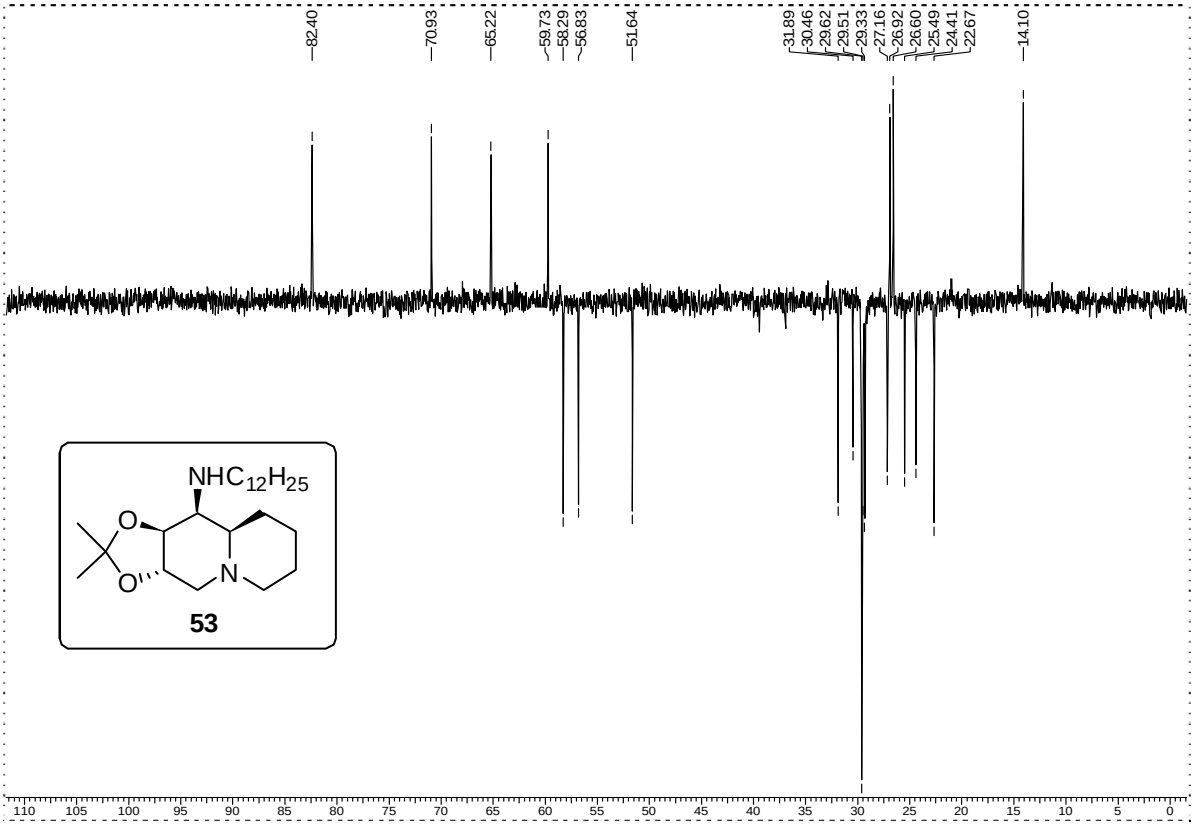
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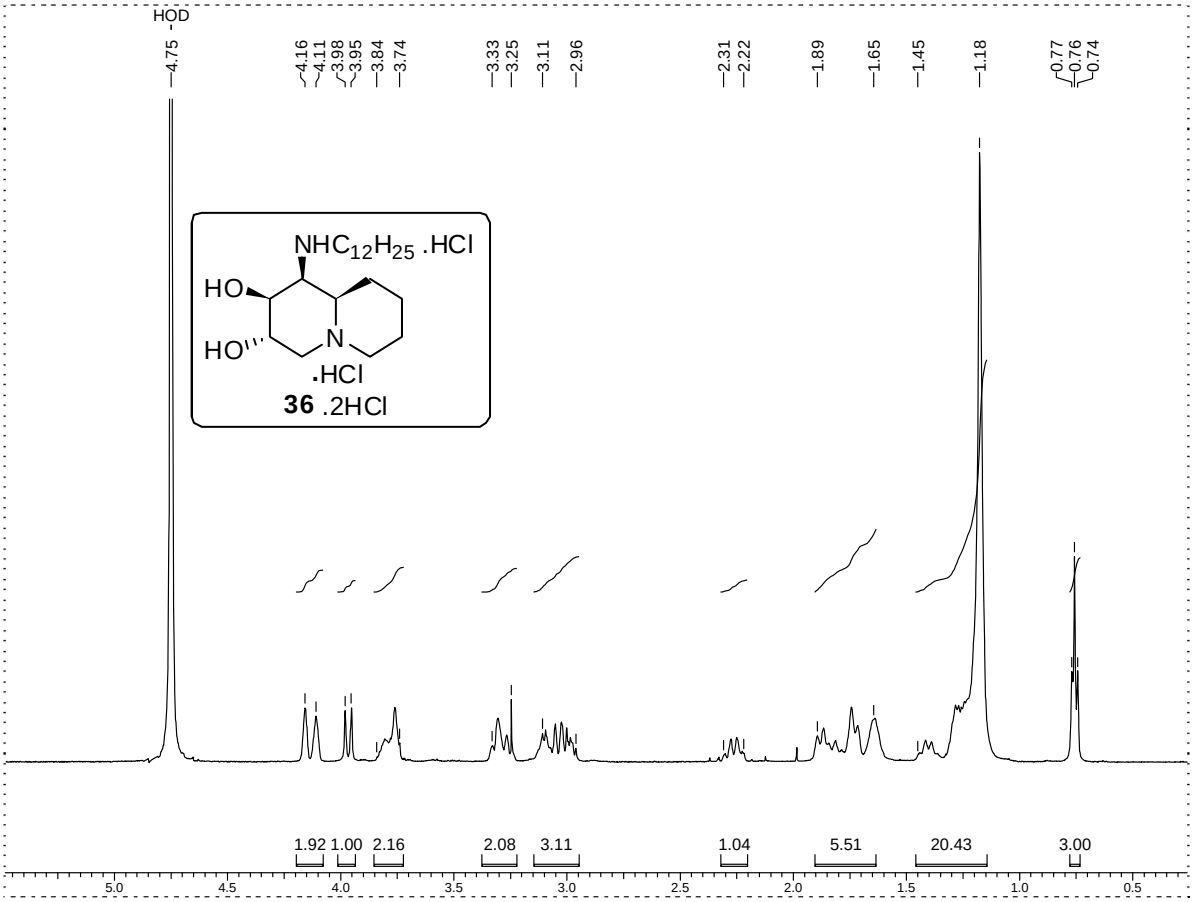
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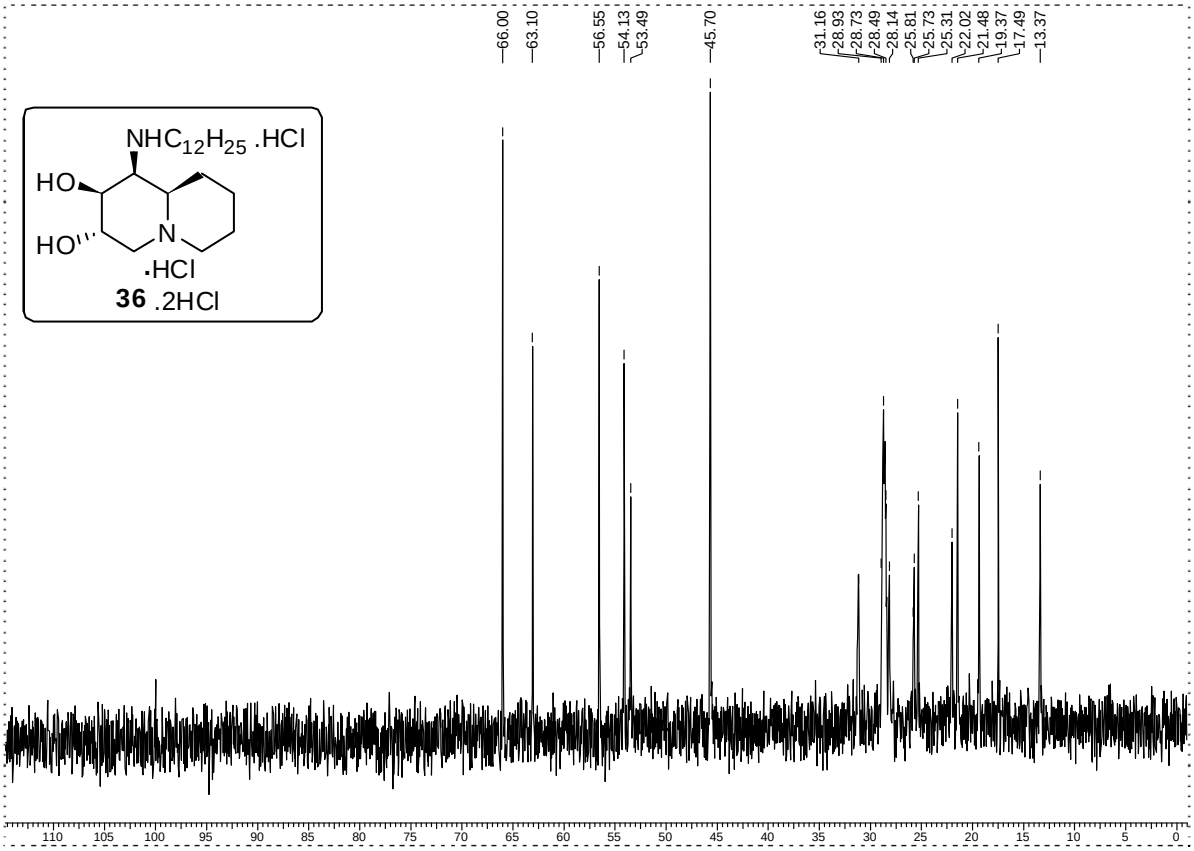
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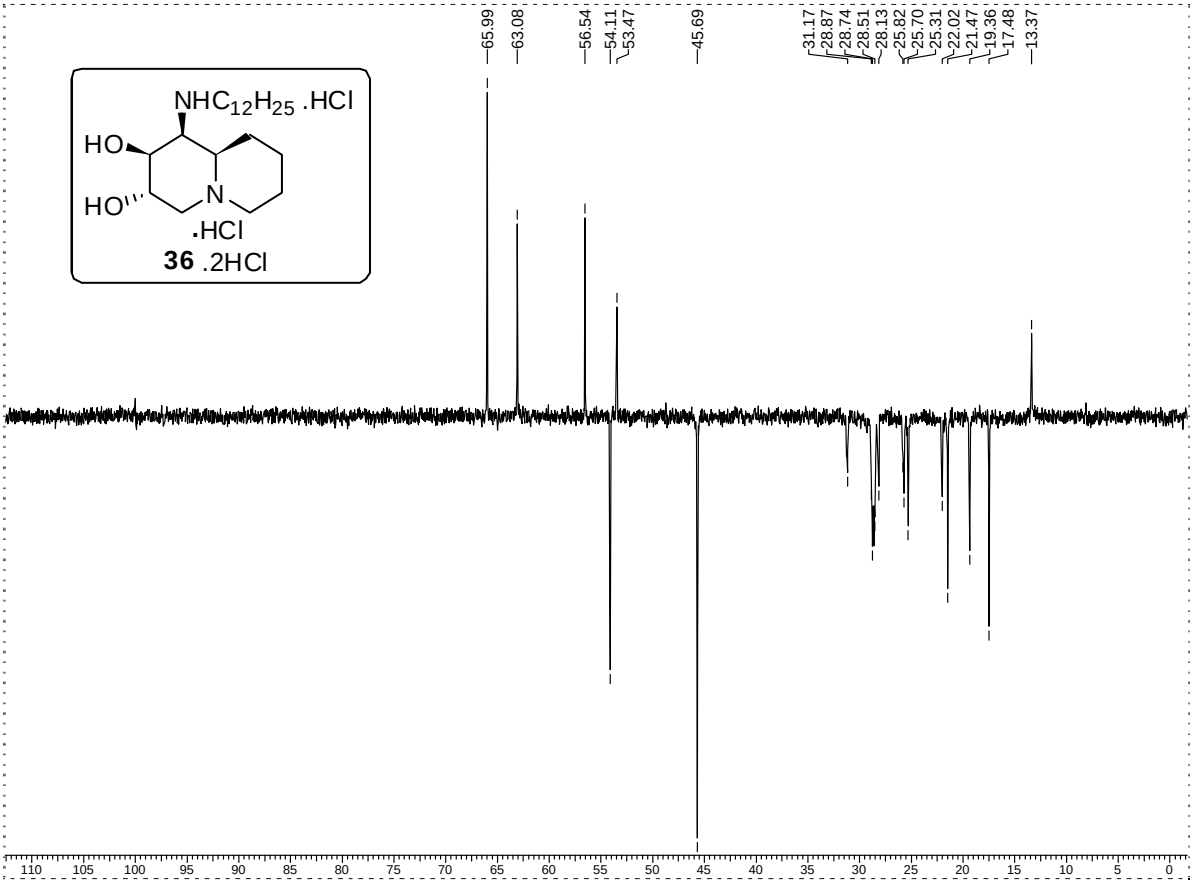
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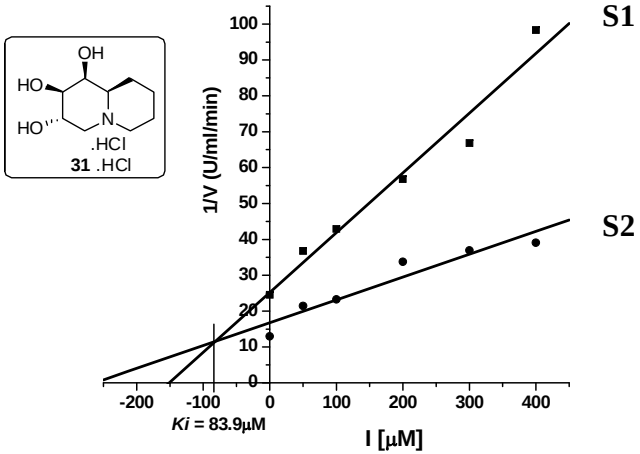
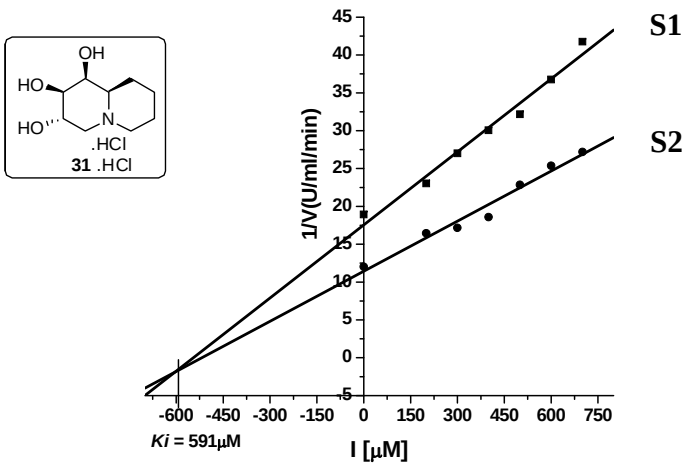
Dixon plots

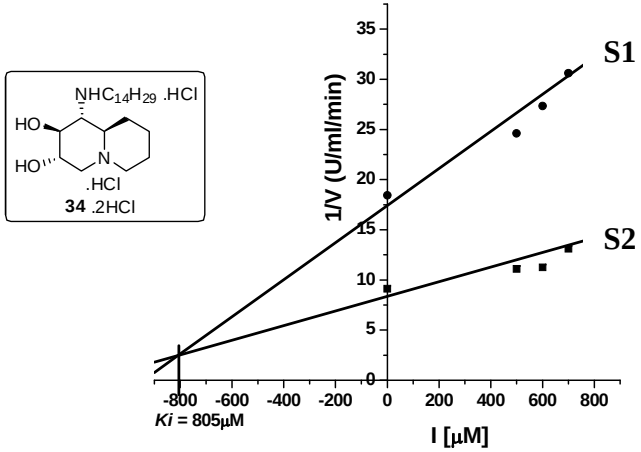
Compound **31 (*Ki* = 83.9 μM** for α-galactosidase**)**



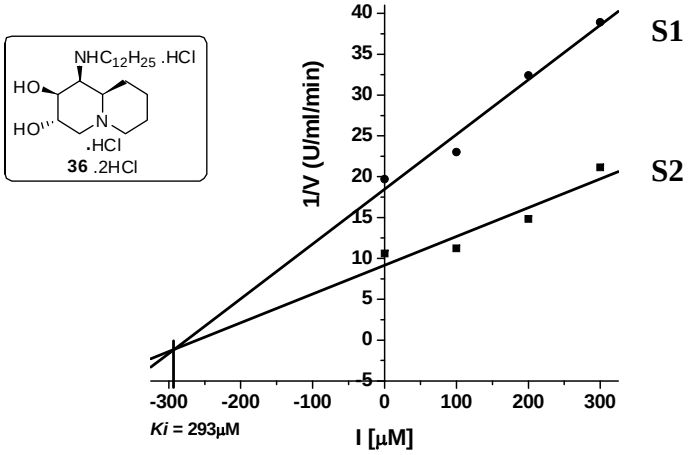
Compound **31 (*Ki* = 591 μM** for β-galactosidase**)**



Compound **34 (*Ki* = 805 μM** for α-mannosidase**)**

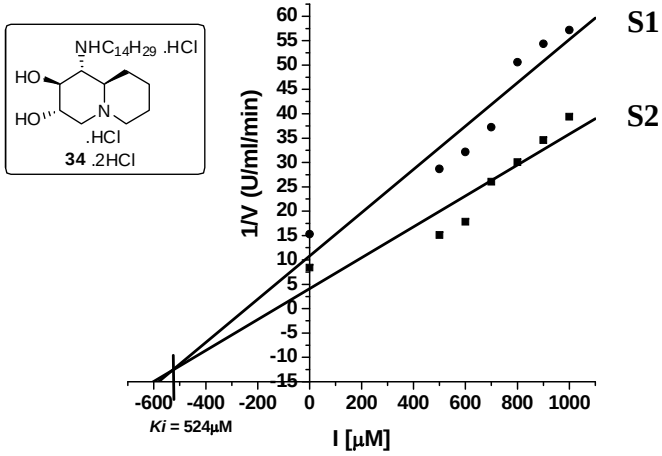


Compound **36 (*Ki* = 293 μM** for α-mannosidase)

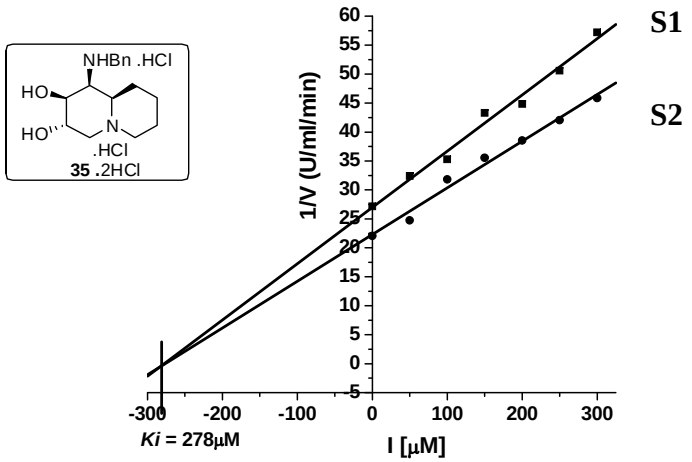


Dixon plots

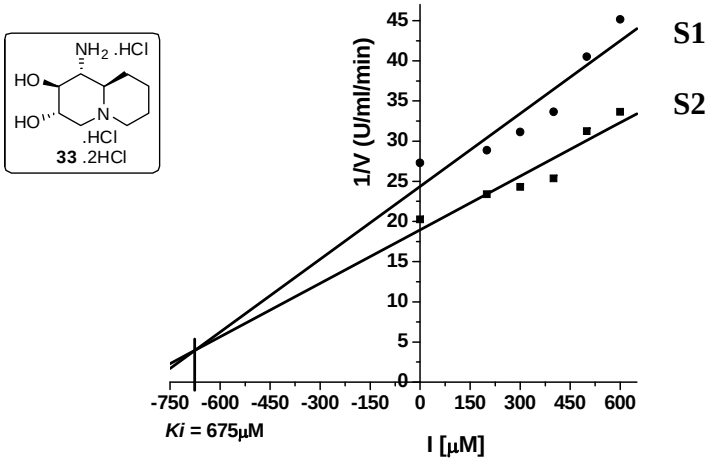
Compound **34 (*Ki* = 524 μM** for β-glucosidase**)**



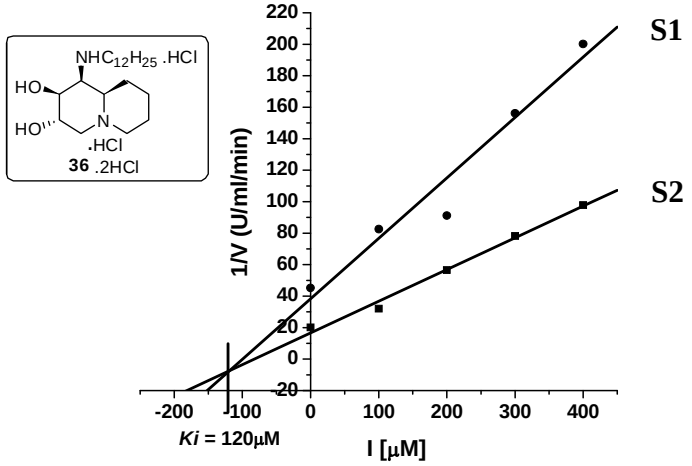
Compound **35 (*Ki* = 278 μM** for α-glucosidase**)**



Compound **33 (*Ki* = 675 μM** for α-glucosidase**)**

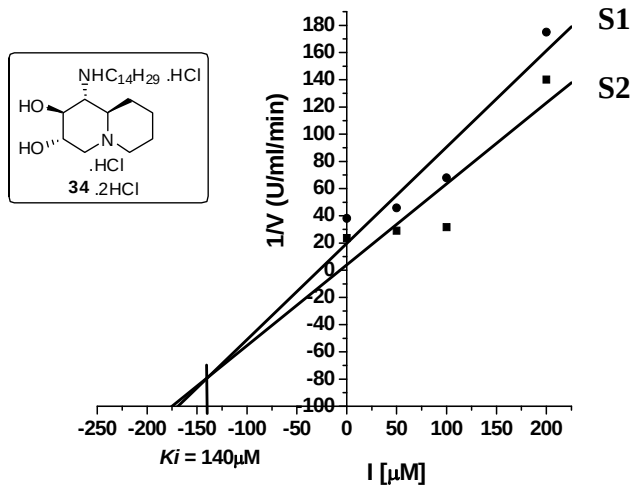


Compound **36 (*Ki* = 120 μM** for α-glucosidase**)**

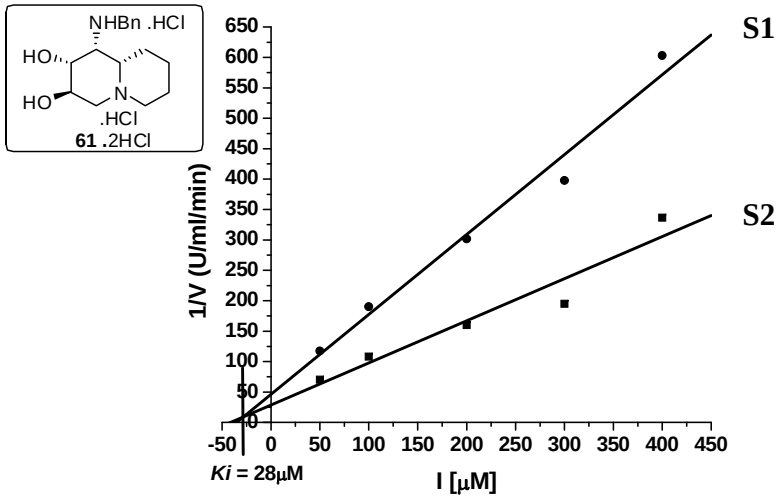


Dixon plots

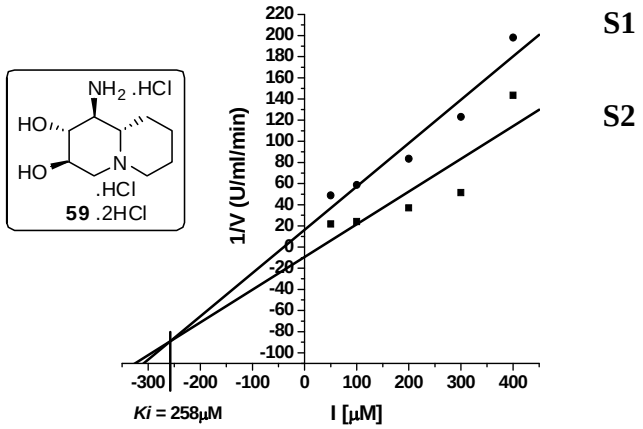
Compound **34 (*Ki* = 140 μM** for α-glucosidase**)**



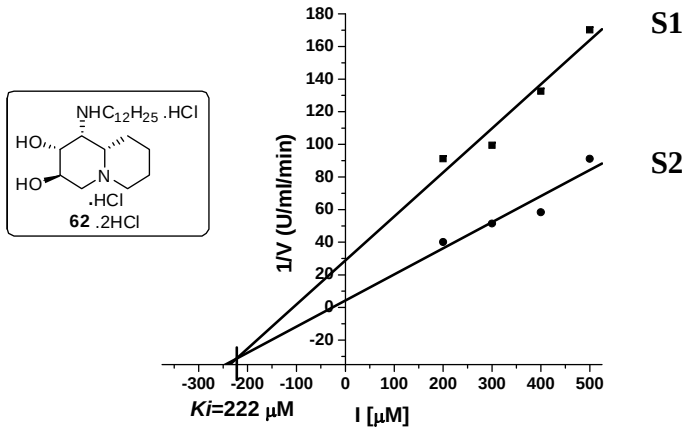
Compound **61 (*Ki* = 28 μM** for α-glucosidase**)**



Compound **59 (*Ki* = 258 μM** for β-glucosidase**)**



Compound **62 (*Ki* = 222 μM** for β-glucosidase**)**



Chapter 3

Synthetic Studies Towards Conformationally Restricted Glycomimetics

"Where nature finishes producing its own spices, man begins, using natural things and in harmony with this very nature, to create an infinity of spices."

-Leonardo da Vinci

Section A :: An Introduction to Conformationally Restricted Glycomimetics

3A.1-Introduction

As discussed earlier (***Chapter 2, Section A***), stable compounds those resemble the transition state and transition state analogues of enzymatic reactions may be the competitive inhibitors of the corresponding enzymes.[1] These enzymatic reactions are thought to proceed through a flattened half-chair (or twisted boat) transition state (**1**), where substantial sp^2 character at the anomeric position reduces the degree of freedom of the species. Thus, it is believed that when an entity (binding species) has lesser degree of freedom, it would bind more effectively to the active site of the enzyme, resulting enhanced inhibition of corresponding enzyme.[2] This understanding has led to a keen interest in recent years for designing compounds that in terms of shape[3] and polarity[4] resemble the transition state of glycosidic cleavage or formation reaction to create potent, selective enzyme inhibitors.[1]

In fact, the designed glycosidase inhibitors have a general structural homology with natural glycosides inhibitors, which are traditionally polyhydroxylated six and five-membered heterocyclic rings, often azacylic rings e.g. 1-deoxynojirimycin (**2**), castanospermine (**3**), etc (**Figure 1**). These polyhydroxy azacyclics, which act as inhibitors of the glyco-enzymes, mimic the electronic character and conformation of the transition state (**1**) of the enzymatic glycoside hydrolysis.[4c] These conformationally restricted compounds, in which the six-member ring is forced into a half-chair conformation, are found to be extremely potent glycosidase inhibitors.[2] This knowledge of the structural requirements for glycosidase inhibitors in conjunction with our continuous efforts towards the development of more potent and selective glycomimetics encouraged us to create a new collection of conformationally restricted azasugars and evaluate their potential as inhibitors against various glyco-enzymes.

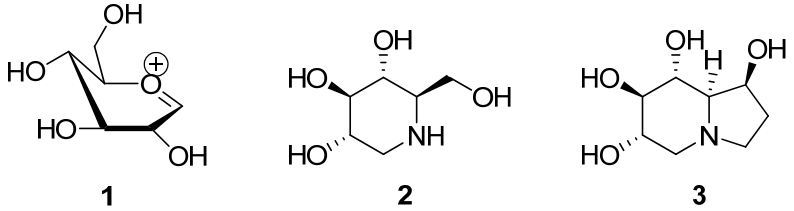


Figure 1

3A.2-Design of conformationally restricted azasugars

Based on above fact, we have designed a library of molecules in which the six-membered ring is forced into a half-chair conformation, thus, supposed to be better glycomimetics (**Figure 2**). All these designed molecules resemble sugars in terms of polarity and size accompanying additional conformational restriction, thus, believed to come out as potential inhibitors of glycosidases.

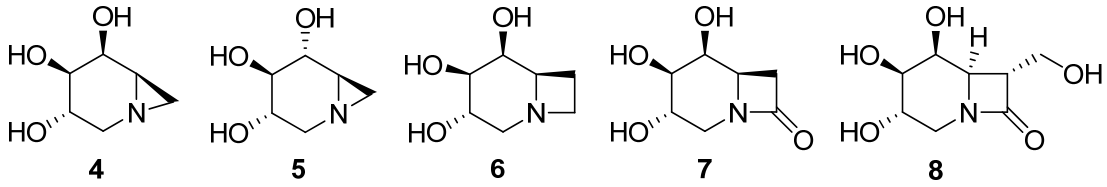


Figure 2: *Some designed conformationally restricted molecules*

In order to have a better insight, we made the conformational energy level calculation[a] for the protonated forms of aziridine fused bicyclics **4** and **5**. These advanced level calculations indicated that in protonated form, twisted boat (TB) conformations are energetically more stable (**Table 1**) and therefore, these may bind more effectively to the active site of the enzymes, as they now possess lesser degree of freedom. Thus, these molecules could prove to be better inhibitors of glycosidases. In addition, the restriction of an inhibitors conformation to one that is recognized by the enzyme may increase the potency by lowering the entropic barrier for complex formation.

Table 1: Conformational energy calculations of azabicyclo[4.1.0] systems **4** and **5**.

Molecule	Conformer	B3LYP/6-31g**Opt. (a.u.)
4H$^+$	TB[i]	−515.9814
4H$^+$	C[ii]	−515.9797
5H$^+$	TB[i]	−515.9771
5H$^+$	C[ii]	−515.9671

i) TB = Twist boat conformation, ii) C = chair conformation.

Moreover, the interesting structural motif, aziridine's proton accepting properties along with its rigidity and potential reactivity can all contribute to specific molecular interactions with enzymes. Indeed, several important natural products such as Mitomycin C,[5] Porfiromycin[6]

a *Calculation of conformational energy of protonated **4** and **5** was done in collaboration with Prof. S. R. Gadre, University of Pune.*

and Carzinophilin A[7] contain the aziridine functionality. A number of saccharide derivatives containing the aziridine group have been made, not only as intermediates,[8] but also as glycosidase inhibitors.[9]

Besides, it is speculated that protonated aziridine analog might preferentially interfere with α-glycoside hydrolysis by S_N2 esterification of the enzyme's β-face carboxylate anion. Thus, the design is expected to alkylate the key catalytic carboxylate group involved in most currently accepted mono- or bilateral mechanism of enzyme-assisted glycoside hydrolysis (**Figure 3**). [8b,10]

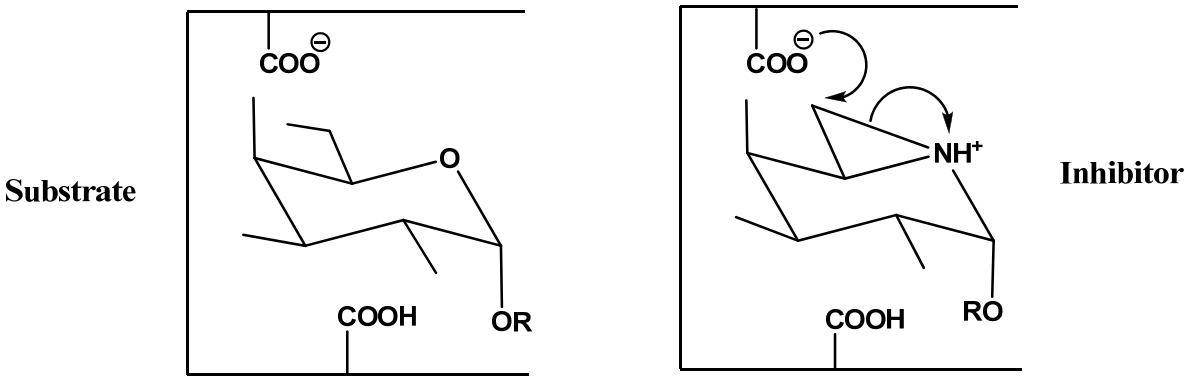
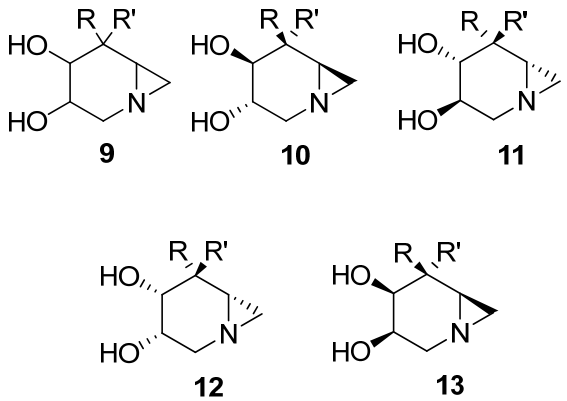


Figure 3: *Active-site-directed glycosidase inhibitors capable of alkylating the key catalytic carboxylate group of the glyco-enzymes*

Banking on all the above insight, we took up the synthesis of conformationally restricted molecules of general structure **9**, as an attempt to develop new selective and potent glycosidase inhibitors. In this connection, we designed structural motifs consisting of four enantiomeric and/or diastereomeric series **10**, **11**, **12** and **13** derived from the general structural framework **9** (**Figure 4**).



R	R'
−OH	−H
$-CH_2OH$	−OH
$-CH_2OH$	−H
$-CH_3$	−H
−COOH	−H
−H	$-NH_2$
−H	−NHR
	(R = Bn/long alkyl chain)

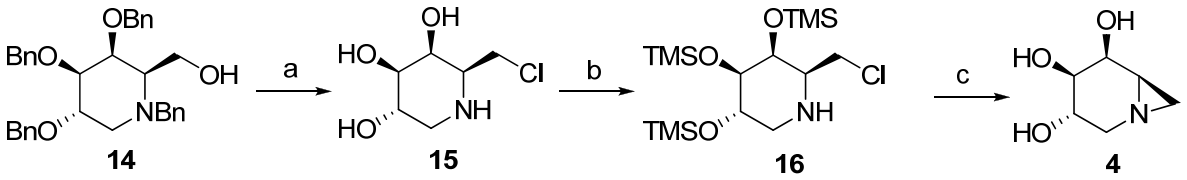
Figure 4: *Library of conformationally restricted molecules as glycosidase inhibitors*

Further, the evaluation of their potential as inhibitors against a group of glyco-enzymes was planned. Final goal was to explore the use of these compounds, the glycosidase inhibitors as possible therapeutics for a variety of carbohydrate-mediated diseases. Before going into the details of our synthetic efforts towards these molecules, it will be pertinent to update the synthetic approaches available in literature for such conformationally restricted glycosidase inhibitors (where a five or six membered ring is fused to aziridine ring).

3A.3-Synthetic approaches towards conformationally restricted azasugars (where an aziridine ring is fused to five/six member ring)

Ganem's approach:[9a]

The synthesis of aziridinyltriol **4** from the known piperidine **14**[11] was achieved by Ganem *et al.* in five steps with 27% overall yield (**Scheme 1**).

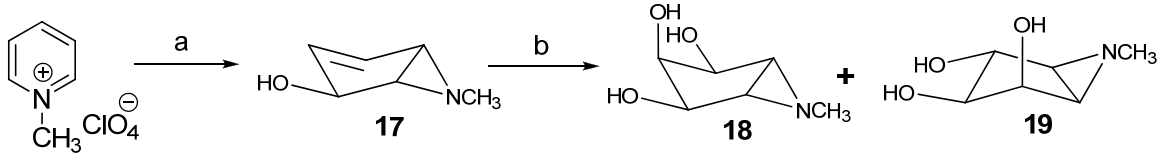


Reagents and conditions: (a) i) Mesylation and displacement (76%, *conditions are not provided*); ii) hydrogenolysis (100%, *conditions are not provided*); (b) $(TMS)_2NH$, TMSCl, py then H_2O (88%); (c) i) *n*-BuLi (1 eq), THF, −78 °C (36–40%); ii) K_2CO_3, MeOH, rt (100%).

Scheme 1.

Photohydration of *N*-alkylpyridinium salts:[9c]

Another approach from the same group involved synthesis of *meso* diastereomer **18** and (±)-**19** using photohydration of *N*-alkylpyridinium salts as a key reaction **(Scheme 2)**. The two step sequence provided *meso*-**18** and (±)-**19** in 83% and 9% yield, respectively.



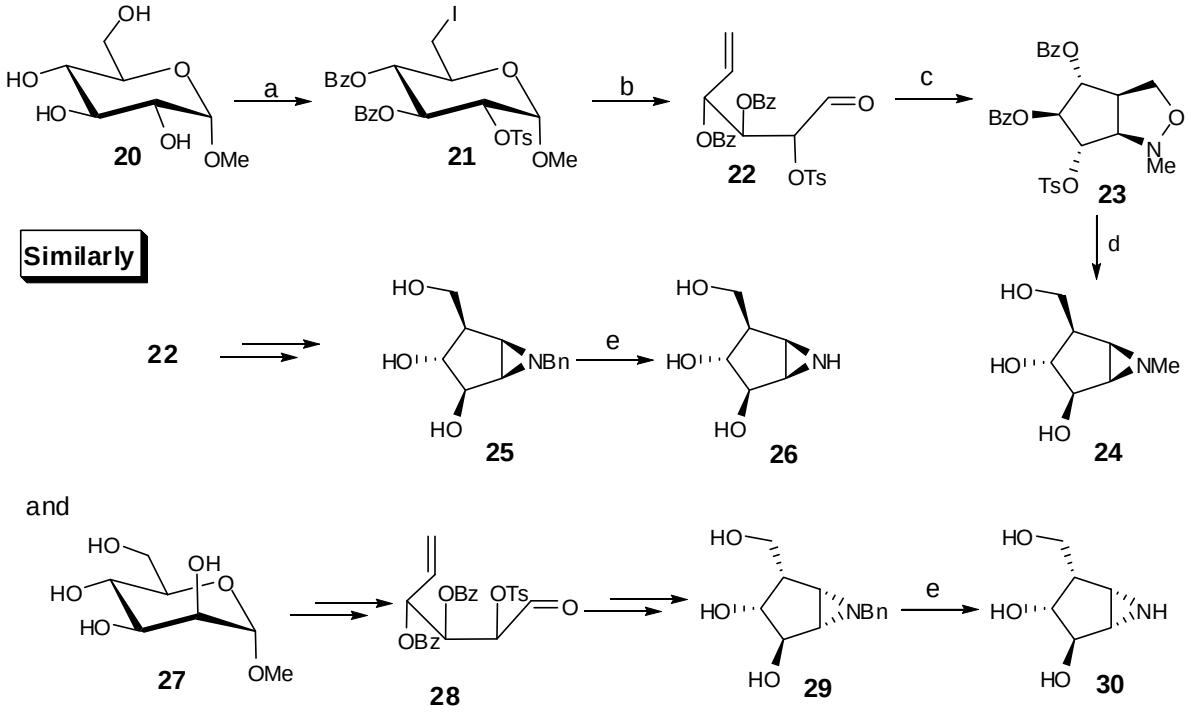
Reagents and conditions: (a) hν (254 nm) (98%); (b) OsO_4 (cat.), NMO (83% for **18** and 9% for **19**).

Scheme 2.

Bols's approach:[9d]

Bols group synthesized some more conformationally restricted molecules, such as **24**, **25** and **26** from methyl D-glucopyranoside (**20**) by following the route outlined in **Scheme 3** [b]. The compound **21** was converted to zinc mediated reductive elimination product **22** which upon 1,3-dipolar cycloaddition with *N*-methylhydroxylamine followed by aziridination involving N-O bond cleavage and benzoyl deprotection sequence provided **24**. A similar route using *N*-benzyl hydroxyl amine in place of *N*-methylhydroxylamine also resulted **25** and **26**.

Also, in a similar manner, **29** and **30** were derived from methyl D-mannoside (**27**).



Reagents and conditions: (a) i) TsCl, py; ii) BzCl, py; iii) NaI, Ac_2O, reflux; (b) Zn, EtOH, reflux; (c) MeNHOH, EtOH, py, 45 °C; (d) i) H_2, Ni, 1 atm; ii) NaOMe (78% over two steps); (e) H_2, 1 atm, Pd/C (57% for **29** and 83% for **30**).

Scheme 3.

[b] *Yields are not provided in many of the reactions in the Scheme 3.*

3A.4-References

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Section-B::Result and Discussion

3B.1-Retrosynthetic analysis

In line with our goal, a library of conformationally restricted azasugars of general structure **1**–**4** was designed for the synthesis with the subsequent evaluation of their potency as the inhibitors of glycosidases (**Figure 1**).

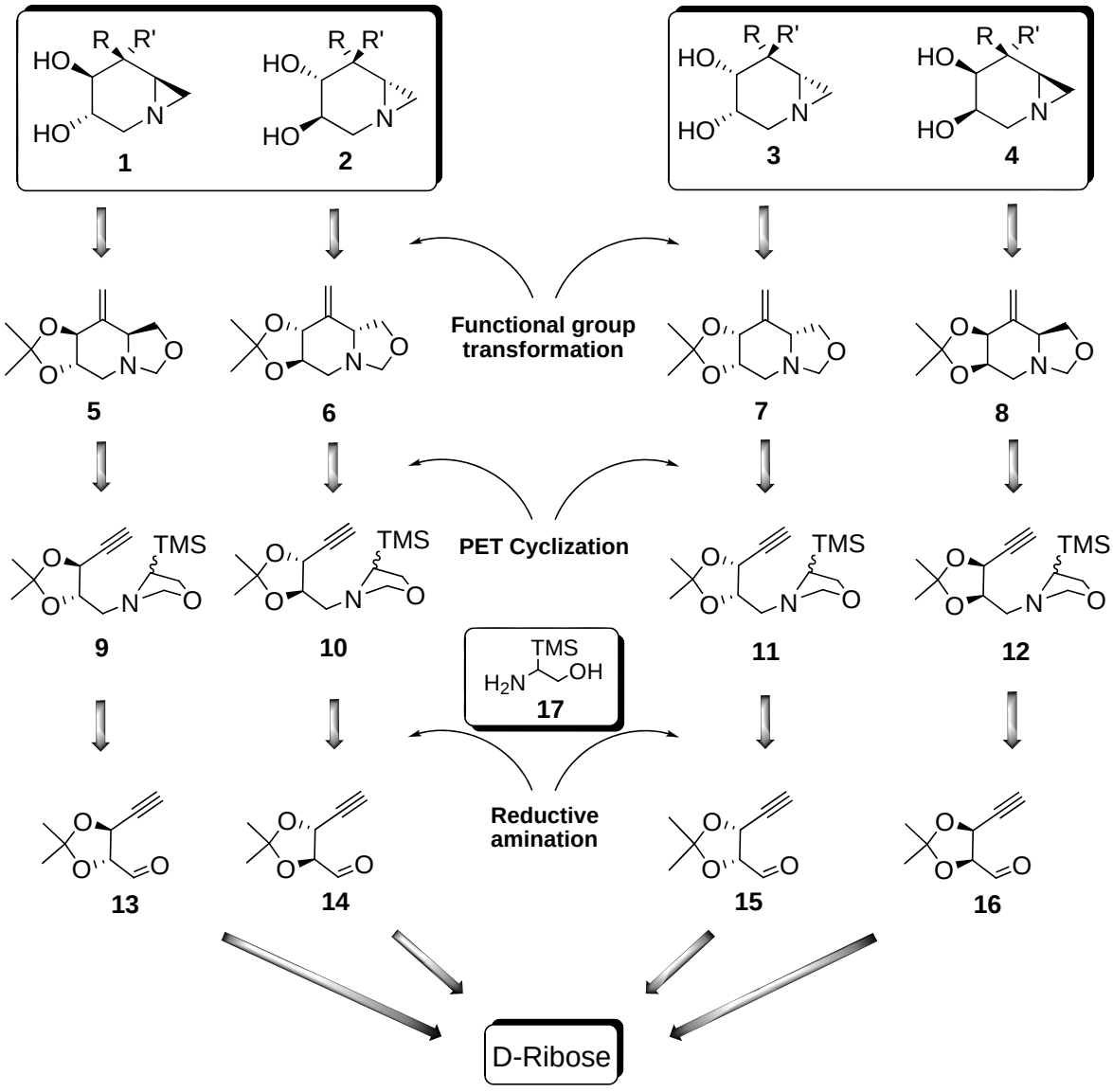


Figure 1: *A Retrosynthetic plan for few conformationally restricted glycosidase inhibitors.*

Those four series of azasugars **1**–**4** were visualized from the corresponding enantiopure tricyclic frameworks **5**–**8**, respectively, which in turn were planned to be obtained from the acetylene tethered α-trimethylsilyl amine **9**–**12**, respectively, using PET cyclization strategy.

These precursors for PET cyclization were visualized to be made from the reductive amination of corresponding enantiopure aldehydes (**13**–**16**) with amine **17**. The syntheses of the aldehydes **13**, **14** and **15** have already been described in earlier chapter. Aldehyde **16** could be synthesized by changing the sequence of installation of acetylenic moiety and oxidative cleavage reaction utilized for the synthesis of aldehyde **15** (***Chapter 2, Section B***), starting from D-ribose. The synthesis of amine fragment (**17**) which was central to the design of all of these series had been planned to be synthesized from furfuryl amine. Herein, we are presenting the efforts for the synthesis of these conformationally restricted glycomimetics. We initiated the synthetic scheme with the synthesis of tricyclic template **5**, which was proposed to lead to D-gluco type conformationally restricted azasugars (**1**).

3B.2-Synthesis of key tricyclic template 5 and its application towards conformationally restricted glycomimetics

3B.2.1-Synthesis of 2-amino-2-(trimethylsilyl)ethanol (17)

The amine **17** required for the construction of structural motif **5** was synthesized from the commercially available furfuryl amine, by following the steps as depicted in **Scheme 1**. The *N*-Boc protection of furfuryl amine was effected by stirring with $(Boc)_2O$ in the presence of TEA to give **18** in 90% yield as a colorless solid (mp 82–84 °C). *N*-Boc-*N*-trimethylsilyl furfurylamine, generated in situ from **18** in the presence of *n*-BuLi and trimethylchlorosilane underwent reverse-aza-Brook rearrangement with another equivalent of *n*-BuLi to provide **19** as a colorless solid (mp 55–57 °C). The spectral data were in close agreement with reported values.[1] The electron rich furan moiety of **19** upon ozonolysis provided oxidative cleavage product in the form of a carboxylic acid, which was *in situ*, converted to the corresponding methyl ester with the help of diazomethane. The TMS group α to carbonyl moiety of the ester was found to be labile and thus, the ester was used as such for reduction. The methyl ester was reduced in the presence of $LiAlH_4$ to provide alcohol **20**.

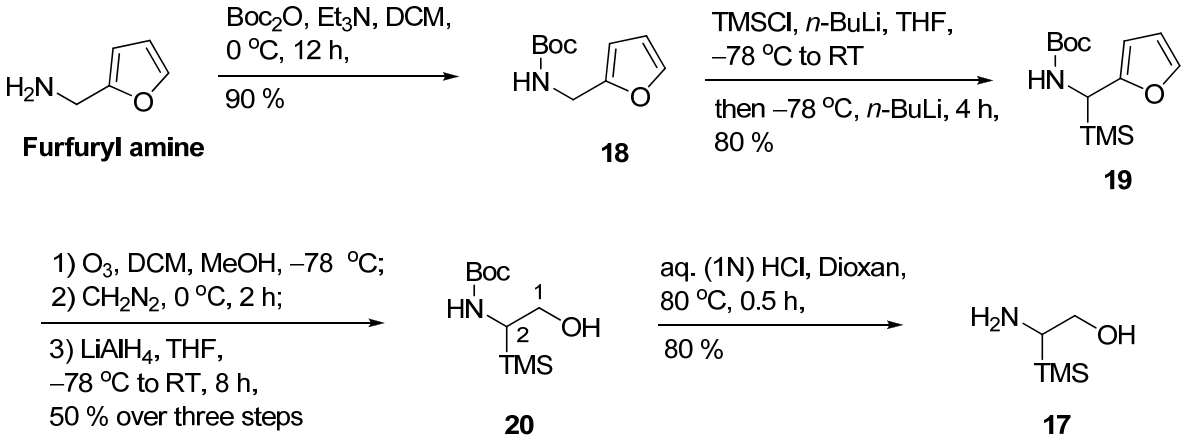
The compound **20** was characterized as follows:

The singlet at δ 0.07 in the ^{1}H NMR accounted for the TMS protons. The singlet at δ 1.43 represented the protons on Boc moiety. The doublet of triplet at δ 3.19 (J = 8.21, 2.83 Hz) was assigned to H-2. The two sets of doublet of doublets at δ 3.60 (J = 11.37, 8.34 Hz) and 3.78 (J = 11.37, 2.90 Hz) were accounted for the two H-1 protons. The doublet at δ 4.65 (J

= 7.67 Hz) was assigned to N-H proton, whereas the multiplet between δ 4.76–4.78 was accountable for −OH proton.

The six signals in ^{13}C spectrum were interpreted as follows:
The signals at δ −3.1 and 28.3 were assigned to primary carbons of TMS and Boc moiety, respectively. C-2 and C-1 appeared at δ 45.1 and 65.2, respectively. The quaternary carbons in *t*-Bu and carbonyl group of Boc moiety appeared at δ 79.8 and 156.4, respectively.

The molecular ion peak at *m/z* 234 (M^++H) confirmed the formation of **20**.



Scheme 1: *Synthesis of aminoalcohol **17**.*

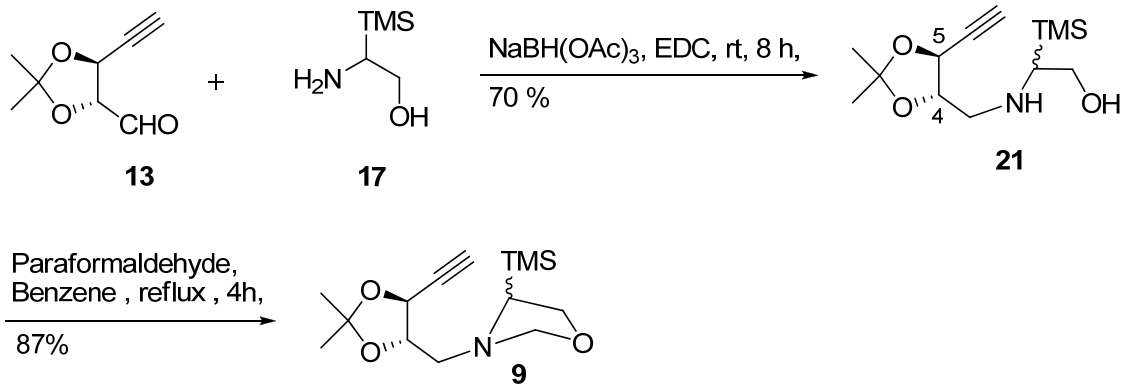
The acid catalyzed removal of *N*-Boc moiety [2] from **20** gave the free amine **17** [*m/z* 134 (M^++H)].

3B.2.2-Synthesis of (3aS,8aS,9aS)-2,2-dimethyl-9-methylenehexahydro-3aH-[1,3]dioxolo[4,5-d]oxazolo[3,4-a]pyridine (5)

The aldehyde **13** (whose synthesis has already been described in ***Chapter 2, Section B***) was subjected to reductive amination[3] with 2-amino-2-(trimethylsilyl)ethanol (**17**), in presence of $NaBH(OAc)_3$ to give **21** as a 1:1 diasteriomeric mixture in good yield (**Scheme 2**).

The characteristic spectral data for compound **21** are as follows:
The ^1H NMR showed a singlet at δ 0.06 for nine TMS protons. The acetylenic proton appeared as a triplet at δ 2.53 (app. t, *J* = 1.97, 1.83 Hz). The signals in the ^{13}C spectrum at δ −2.79 and −2.76 were accountable for TMS moiety. The quaternary carbon of acetelynic group was found at δ 74.7 and 74.8, whereas the signals at δ 110.4 and 110.6 represented

the quaternary carbon of acetonide moiety. The mass spectrum of **21** displayed molecular ion peak at *m/z* 272 (M^++H).



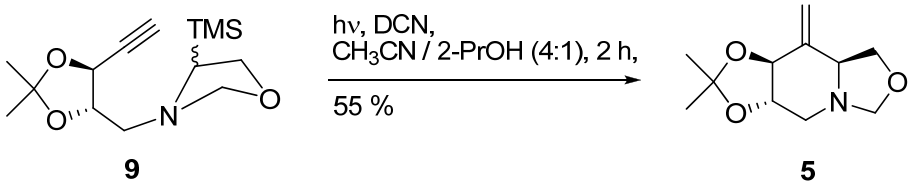
13 **17** **21** **9**

Scheme 2: *Synthesis of **9**.*

The thought of utilizing this open chain reductive amination product **21** for PET cyclization was abandoned considering our previous experience of poor diastereoselectivity.[4] Thus, we transformed **21** into the cyclic 1,3-oxazine derivative **9**, by refluxing with paraformaledyde (**Scheme 2**), anticipating that *N*-substituted α-trimethylsilylmethyl amine moiety would provide superior stereoselectivity.

The ^{1}H NMR spectrum of **9** showed protons on *N/O* methylene bridge as two apparent triplets at δ 4.18 (*J* = 5.95, 5.81 Hz) and 4.31 (*J* = 5.93, 5.81 Hz). The mass spectrum showed the molecular ion peak at *m/z* 284 (M^++H) confirming the formation of **9**.

Substrate **9** (1 g, 3.53 mmol) was attempted to cyclize, employing the PET reaction protocol reported from our group,[5] by irradiating its solution containing DCN (0.12 g, 0.67 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 and 2-propanol.[6] Finally, 4:1 ratio of the solvent mixture proved to be the best for carrying out the cyclization reaction to obtain **5** as a single diastereomer in 55% yield (**Scheme 3**).



9 **5**

***Scheme** 3:PET cyclization.*

The initial support for the formation of cyclized product **5** came from IR spectrum, which displayed a weak absorption peak at 1672 cm^{-1}, characteristic of olefin functionality. Furthermore, the ^{1}H NMR spectrum showed two olefinic protons as two triplets at δ 4.74 (*J* = 1.70 Hz) and 5.08 (*J* = 1.70 Hz). The cyclized product **5** was subsequently characterized by extensive analyses of other NMR (^{13}C, DEPT, COSY, NOESY and HETCOR) techniques. The molecular ion peak was found in mass spectrum at *m/z* 212 (M^{+}+H).

The appearance of cross peak between H-9a and H-8a in the NOESY spectrum (**page no. 186**) confirmed the stereochemistry in the newly generated stereocentre at C-8a (**Figure 2**).

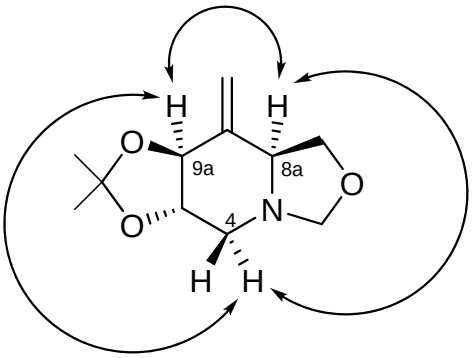
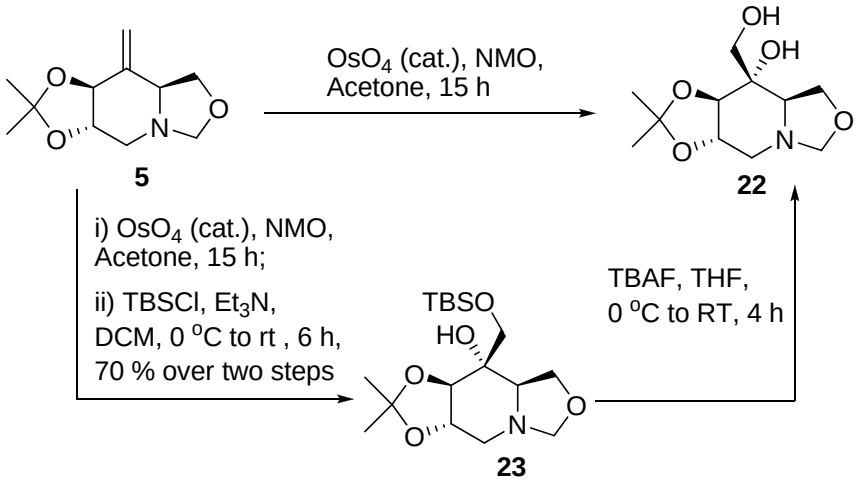


Figure 2: *nOe correlation of cyclized product* ***5.***

With the successful synthesis of the tricyclic template **5**, we planned the functionalization of the exocyclic double bond before the installation of aziridine moiety, leading to the completion of the synthesis of azasugars of general structure **1**.

3B.2.3-Utilization of 5 towards the synthesis of comformationally restricted glycomimetics

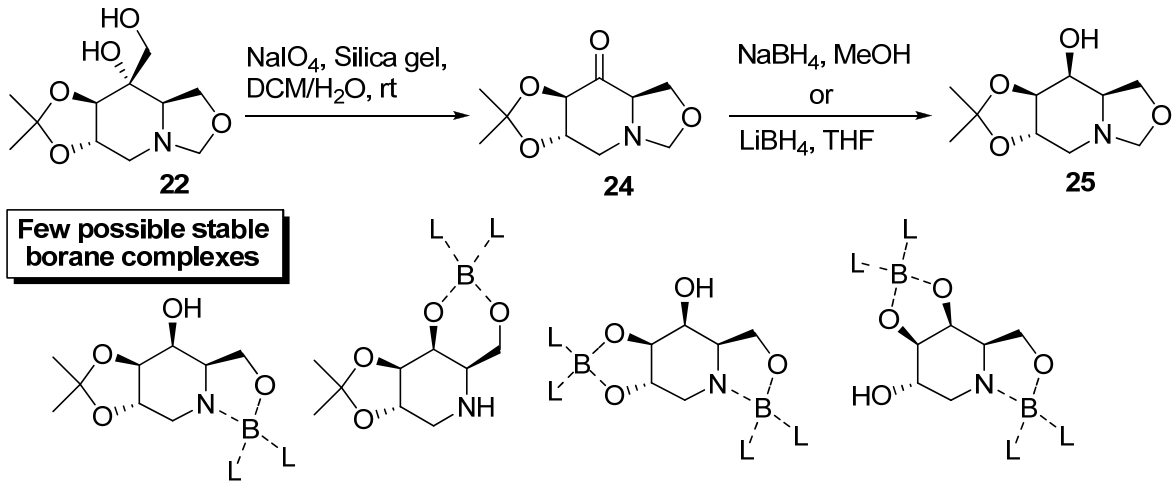
In this context, catalytic dihydroxylation of olefine **5** using OsO_4 (cat.) and NMO provided **22**. Non-appearance of the olefinic protons in the PMR spectrum of **22** combined with emergence of broad peak at 3354 cm^{-1} in the IR spectrum proved its formation (**Scheme 4**). To our dismay, the isolation of this dihydroxy compound was found to be cumbersome, due to high polarity. Thus, we planned to proceed further with an extra step to protect the diol selectively at the primary position as monosilylether with TBSCl. Selective mono protection of the diol **22** was effected with TBSCl to obtain **23**, which was isolated easily in pure form by means of column chromatography.



Scheme 4: *Synthesis of **32**.*

The compound **23** was characterized from following diagnostic spectral data. The singlets at δ 0.11, 0.12 and 0.89 totally integrating for fifteen protons in the ^{1}H NMR spectrum were accounted for the protons of TBS moiety. The signals in the ^{13}C NMR at δ −5.7 and −5.5 were assigned two methyl carbons attached to silicon in TBS moiety, whereas the signal at δ 18.0 was accountable for the quaternary carbon of TBS group. The remaining methyl carbon of TBS group appeared at δ 25.7. The mass spectrum showed the molecular ion peak at *m/z* 360 (M^{+}+H), confirming the formation of the monosilylether **23**.

After successful isolation of **23**, it was necessary to remove the TBS group. In this regard, TBAF mediated desilylation seemed to be the best reaction condition for this purpose. Stirring a solution of **23** in THF with TBAF resulted in its smooth conversion to diol **22**. Next, we attempted the conversion of diol **22** to alcohol **25**. In this context, **22** as usual was subjected to $NaIO_4$ cleavage followed by borohydride reduction (**Scheme 5**).

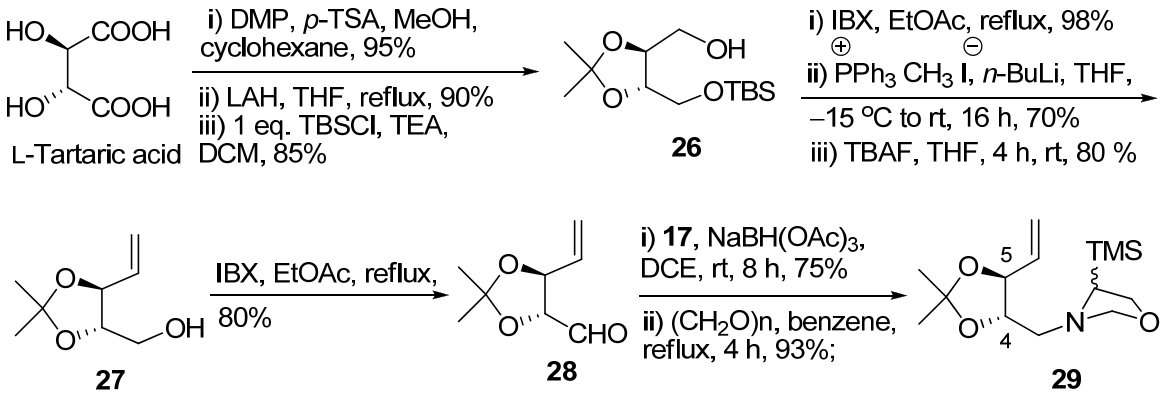


Scheme 5: *Synthetic effort for **25**.*

However, unfortunately, we could not isolate the alcohol **25**, possibly due to the cleavage of protecting groups in acidic oxidative cleavage reaction condition and possible formation of stable complexes with boron during reduction step.

3B.3-Synthesis of key tricyclic template 30 and its application towords conformationally restricted glycomimetics

The above set back, led us to think an azasugar where no functionalization of such exocyclic double bond is required. Thus, we visualized that PET cyclization of olefin tethered α-trimethylsilyl amine **29** would go well with the criterion. The synthesis of **29** is given in **Scheme 6**.



Scheme 6. *Synthesis of precursorforPET cyclization (**29**).*

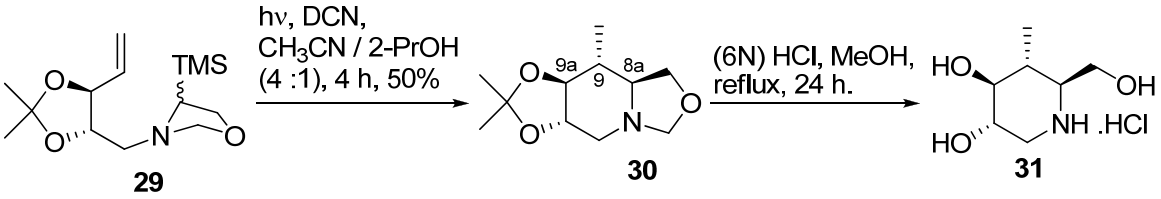
Acid mediated acetonide protection of L-tartaric acid followed by esterification and subsequent reduction of diester with LAH provided corresponding diol, which was converted to monosilylether (**26**) using TBSCl. The free hydroxyl group of **26** was oxidized to corresponding aldehyde which was subjected to one carbon Wittig homologation reaction, followed by removal of monosilylether to obtain **27**. The spectral data for **27** were in good agreement with literature value.[7] IBX oxidation of **27** provided aldehyde **28,** which was utilized for reductive amination with **17** in the presence of $NaBH(OAc)_3$ to afford 1,2-aminoalcohol required for the formation of cyclic 1,3-oxazine derivative **29**.

The diagnostic spectral data for **29** was observed as follows:

^{1}H NMR showed a singlet at δ 0.04 for nine TMS protons. The two doublet of doublets at δ 5.23 (J = 10.55, 5.27 Hz) and 5.34 (J = 17.02, 6.52 Hz) were accountable for the two terminal olefin protons, whereas the multiplet between δ 5.76–5.87 was assigned to the

internal olefinic proton. The ^{13}C spectrum revealed the signals for TMS carbons at δ −3.1 and −3.0. The terminal and internal carbons of olefinic moiety were observed at δ (118.1, 118.5) and (135.2, 135.3), respectably. The molecular ion peak was found in mass spectrum at *m/z* 286 (M^++H) and 308 (M^++Na).

PET cyclization of **29,** carried out by using an identical procedure as described for compound **5**, provided **30** as a single diastereomer (**Scheme 7**).



Scheme 7: *Attempted synthesis for* ***31*****.**

^1H NMR of **30** showed C-9 methyl protons as a doublet at δ 1.02 (*J* = 6.42 Hz). The mass spectrum displayed the molecular ion peak at *m/z* 214 (M^++H) and 236 (M^++Na) confirming the formation of **30**.

The higher value of coupling constant for H-9a (3.61, dd, *J* = 7.43, 7.16 Hz) and H-9 (2.29–2.34, *J* ~ 6.5 – 7.9) accounted the axial orientation for both these protons. This 1,2-diaxial relationship suggested the *trans* spatial relationship between H-9 and H-9a.

Our next attempt was the installation of an adjacent aziridine ring, which required the removal of *N/O* methylene bridge. To our disappointment, the isolation of high polar product formed by the removal of *N/O* methylene bridge using of acidic reaction condition was found to be unsuccessful.

3B.4-Revised strategy for key tricyclic template 32

The above experience made us to explore another way to construct the fused bicyclic system. Our previous approach was based on the construction of ring **A** prior to the formation of adjacent aziridine ring **B** of azasugar series **1**. As the construction of ring **B** in the later stage of the scheme was found to be difficult, we resorted back to the installation of aziridine ring prior to PET cyclization for construction of ring **A** (**Figure 3**).

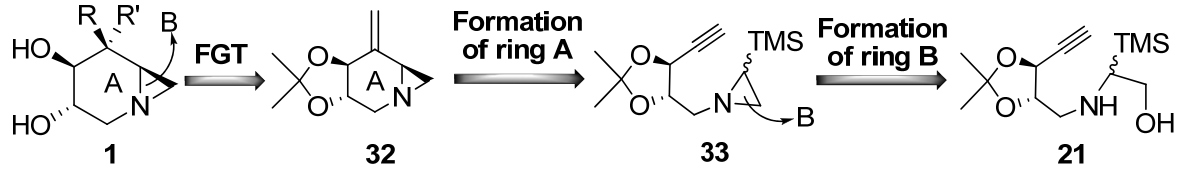
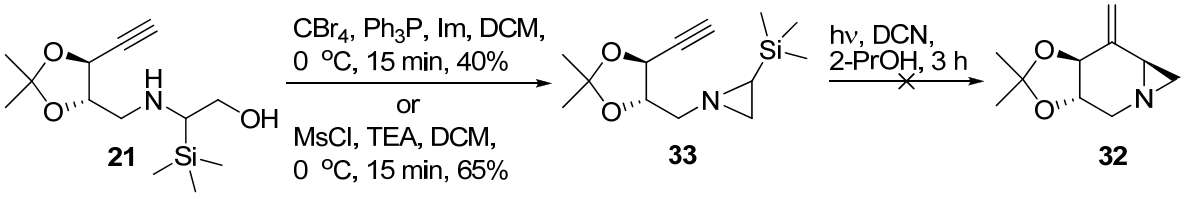


Figure 3: *Alternative strategy.*

3B.4.1-Synthesis of 1-(((4*S*,5*S*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) -2(trimethylsilyl)aziridine (33)

In this regard, the available 1, 2-amino alcohol **21** was converted to corresponding aziridine **33**, either by bromination or mesylation of alcohol functionality under basic condition (**Scheme 8**). The IR spectrum of **33** did not show any peak corresponding to NH or OH stretching frequency. The mass spectrum revealed the molecular ion peak at *m/z* 254 (M^++H) confirming its formation.



Scheme 8: *Attempted synthesis for **32**.*

We attempted the cyclization of **33** to **32** by usual PET reaction. However, to our great disappointment, we did not get the required compound, instead, a mixture of compounds were obtained. The possible reason may be the amine radical cation (**34**) formed during the course of PET condition instead of losing a TMS^+, essential for cyclization, might be getting cleaved due to the extreme ring strain involved with aziridine ring system (**Figure 4**).

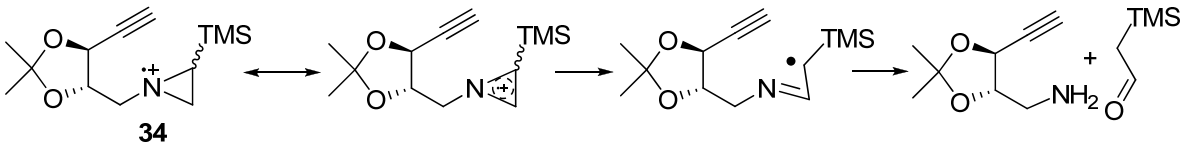
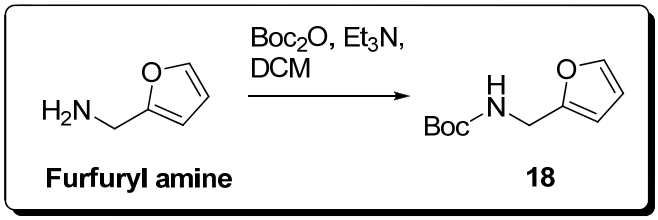


Figure 4: *Possible cleavage of aziridine ring prior to loss of TMS moiety.*

With these frustrating results in hand, we abandoned this scheme momentarily and work is being carried out to transform **25** itself to corresponding aziridine compound.

3B.5- Experimental

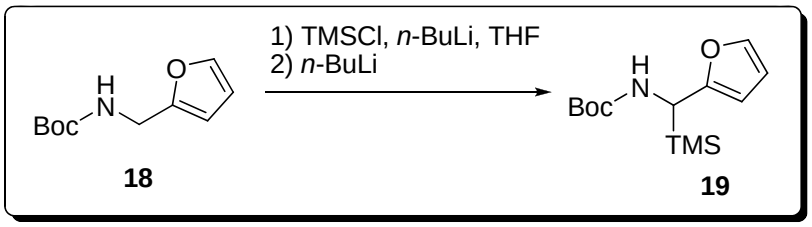
1. Preparation of *tert*-butyl furan-2-ylmethylcarbamate (18)



A solution of $(Boc)_2O$ (56.2 g, 257.5 mmol) in 100 mL DCM was slowly added to a stirring solution of furfuryl amine (25 g, 257.5 mmol) and Et_3N (31.2 g, 308.9 mmol) in DCM (600 mL) at 0 °C. The reaction mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with DCM (300 mL) and washed with water (2 × 150 mL) and brine (1 × 150 mL). The organic layer was dried over anhydrous 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 **18** (45.7 g, 90%) as a colorless oil.

^{1}H NMR, 200 MHz, $CDCl_3$	δ 1.44 (s, 9H), 4.28 (d, *J* = 5.68Hz, 1H), 4.84 (br s, 1H), 6.19 (d, *J* = 2.7 Hz, 1H), 6.30 (dd, *J* = 3.25, 1.90 Hz, 1H), 7.33 (dd, *J* = 1.90, 0.89 Hz, 1H).
^{13}C NMR, 50 MHz, $CDCl_3$	δ 28.3 (CH_3), 37.7 (CH_2), 79.6 (C), 106.9 (CH), 110.3 (CH), 142.0 (CH), 152.0 (C), 155.5 (C).
Mass (ESI): *m/z*	198 (M^++H).
Elemental analysis	Anal. Calcd for $C_{10}H_{15}NO_3$: C, 60.90; H, 7.67; N, 7.10; Found: C, 60.79; H, 7.71; N, 6.90.

2. Preparation of *tert*-butyl furan-2-yl(trimethylsilyl)methylcarbamate (19)

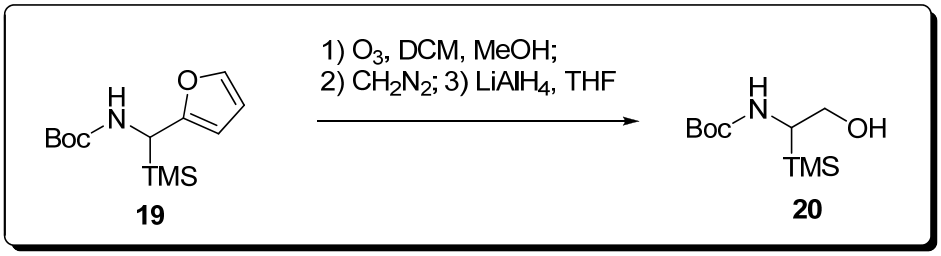


To a solution of **18** (12.0 g, 60.9 mmol) and trimethylsilyl chloride (8.3 g, 76.1 mmol) in THF (400 mL) at −78 °C was added dropwise *n*-BuLi (40.1 mL, 1.9 M in hexane, 76.1 mmol). After one hour, the mixture was warmed to rt and allowed to stir for 4 h. After

recooling to −78 °C, a second portion of *n*-BuLi (40.1 mL, 1.9 M in hexane, 76.1 mmol) was slowly added. After stirring for 3 h at −78 °C, the mixture was poured into saturated ammonium chloride (100 mL) and the aqueous phase was extracted with ethyl acetate (3 × 200 mL), the combined organic layers were dried over Na_2SO_4 and concentrated. Column chromatography (20:1 hexanes/diethyl ether) gave **19** as a colorless solid (13.1 g, 80%).

IR ν_{max} cm^{-1} in $CHCl_3$	3461, 3211, 1698, 1446.
^1H NMR (200 MHz, $CDCl_3$)	δ 0.05 (s, 9H), 1.41 (s, 9H), 4.34 (d, *J* = 9.34 Hz, 1H), 4.78 (d, J = 9.22 Hz, 1H), 6.00 (d, *J* = 2.78 Hz, 1H), 6.26 (dd, *J* = 3.16, 1.78 Hz, 1H), 7.27 (dd, *J* = 1.77, 0.81 Hz, 1H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ −3.4 (CH_3), 28.3 (CH_3), 40.8 (CH), 79.4 (C), 104.6 (CH), 110.2 (CH), 141.0 (CH), 155.1 (C), 155.8 (C).
Mass (ESI): *m/z*	270 (M^++H), 292 (M^++Na).
Elemental analysis	Anal. Calcd for $C_{13}H_{23}NO_3Si$: C, 57.96; H, 8.60; N, 5.20; Found: C, 58.21; H, 8.51; N, 5.27.

3. Preparation of *tert*-butyl 2-hydroxy-1-(trimethylsilyl)ethylcarbamate (20)

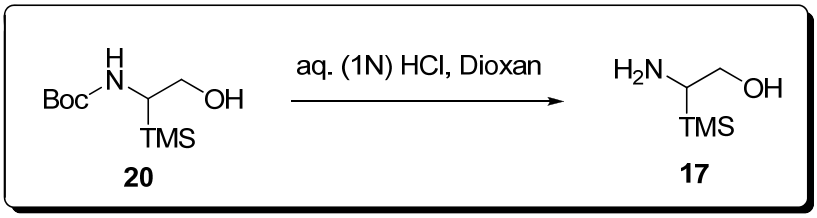


The solution of **19** (8.0 g, 29.74 mmol) at −78 °C in a mixture of DCM (200 mL) and methanol (40 mL) was bubbled with a stream of ozone until the blue color of ozone intensified. The mixture was warmed to 0 °C. A solution of diazomethane in diethyl ether [prepared by the addition of *N*-methylnitrosourea (9.0 g, 87.3 mmol) to the aqueous KOH (6.0 g, 106.9 mmol)] was added to the above reaction mixture. After 1 h of stirring, the reaction mixture was concentrated. The crude reaction mixture in THF (50 mL) was added through a cannula to a suspension of LAH (1.13 g, 29.74 mmol) in THF (50 mL) at −78 °C. The mixture was allowed to warm to rt and stirred additionally for 8 h. The excess LAH was quenched by the addition of ethyl acetate at 0 °C followed by addition of ice. Anhydrous Na_2SO_4 was added to the reaction mixture and the solid was filtered out through

a Buchner funnel. Concentration and subsequent column chromatography (3:2 hexanes/ethyl acetate) of the organic portion gave **20** as colorless solid (3.465 g, 50%).

IR ν_{max} cm^{-1} in $CHCl_3$	3457, 3391, 1321.
^1H NMR (200 MHz, $CDCl_3$)	δ 0.07 (s, 9H), 1.43 (s, 9H), 3.19 (dt, *J* = 8.21, 2.83 Hz, 1H), 3.6 (dd, *J* = 11.37, 8.34 Hz, 1H), 3.78 (dd, *J* = 11.37, 2.9 Hz, 1H), 4.65 (d, *J* = 7.67 Hz, 1H), 4.76–4.78 (m, 1H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ −3.1 (CH_3), 28.3 (CH_3), 45.1 (CH), 65.2 (CH_2), 79.8 (C), 156.4 (C).
Mass (ESI): *m/z*	234 (M^++H).
Elemental analysis	Anal. Calcd for $C_{10}H_{23}NO_3Si$: C, 51.46; H, 9.93; N, 6.00; Found: C, 51.31; H, 9.99; N, 6.12.

4. Preparation of 2-amino-2-(trimethylsilyl)ethanol (17)

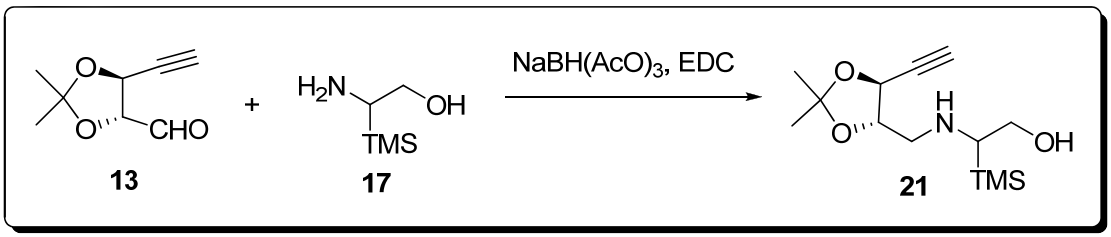


1 Lit. RB flask containing 1,4-dioxane (190 ml) and aq. (1N) HCl (150 mL) was charged with **20** (9 g, 38.63 mmol) and the contents stirred while heating at 80 °C for 30 min. Dioxane was removed under reduced pressure and the residue was diluted with 250 mL DCM. This mixture was cooled to 0° C and neutralized by adding (approximately 95 mL) (2N) NaOH solution. The aqueous layer was extracted with DCM (3 × 125 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **17** (3.85 g, 75%) as 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 (15:1 DCM/methanol).

^1H NMR (200 MHz, D_2O)	δ −0.06 (s, 9H), 2.29 (dd, *J* = 10.09, 3.29 Hz, 1H), 3.44 (dd, *J* = 11.56, 10.09 Hz, 1H), 3.69 (dd, *J* = 11.60, 3.33 Hz, 1H).
^{13}C NMR (50 MHz, D_2O)	δ −4.5 (CH_3), 43.5 (CH), 64.7 (CH_2).
Mass (ESI): *m/z*	134 (M^++H).

Elemental analysis	Anal. Calcd for $C_5H_{15}NOSi$: C, 45.06; H, 11.35; N, 10.51; Found: C, 44.90; H, 11.29; N, 10.67.

5. Preparation of 2-(((4*S*,5*S*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)methylamino)-2-(trimethylsilyl)ethanol (21)[3]

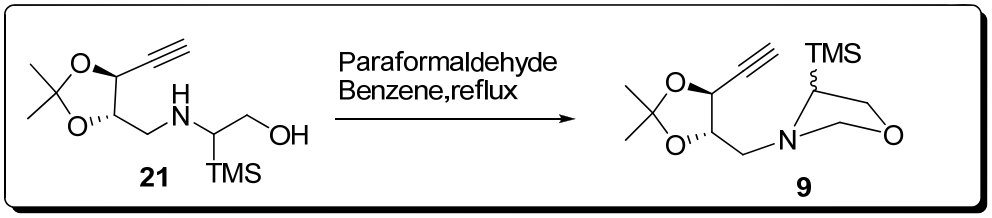


Identical experimental procedure was used as described for the preparation of **12** (*Chapter 2, Section B*)**.**

IR ν_{max} cm^{-1} in $CHCl_3$	3421, 3375, 3317, 1325.
^1H NMR (400 MHz, $CDCl_3$)[a]	δ 0.06 (s, 9H), 1.41 (s, 3H), 1.47 (s, 3H), 2.09–2.13 (m, 1H), 2.53 (apparent t, *J* = 1.97, 1.83 Hz, 1H), 2.77–2.87 (m, 1H), 2.92–2.96 (m, 1H), 3.48–3.53 (m, 1H), 3.72 (dt, *J* = 10.79, 4.01 Hz, 1H), 4.13–4.20 (m, 1H), 4.40, 4.52 (dd each, *J* = 7.53, 2.01 Hz, 1H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ −2.79, −2.76 [Si(CH_3)$_3$], 25.9, 26.0, 26.8, 27.0 [C(CH_3)$_2$], 49.6, 50.8 (CH_2), 51.0, 51.7 (CH), 61.6, 61.8 (CH_2), 67.7, 68.1 (CH), 74.7, 74.8 (C), 80.8, 81.4 (CH), 110.4, 110.6 (C).
Mass (ESI): *m/z*	272 (M^++H).
Elemental analysis	Anal. Calcd for $C_{13}H_{25}NO_3Si$: C, 57.53; H, 9.28; N, 5.16; Found: C, 57.67; H, 9.01; N, 5.26.

a = D_2O exchange

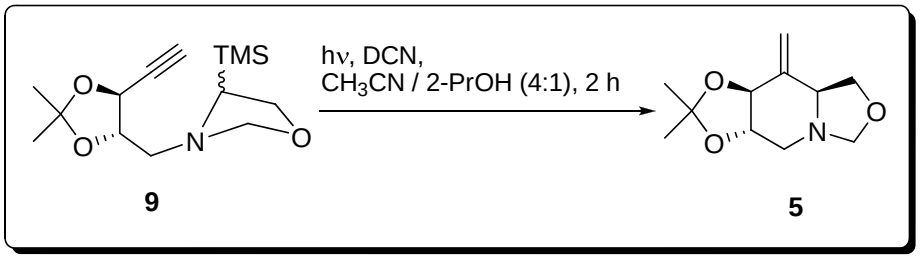
6. Preparation of 3-(((4*S*,5*S*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-4-(trimethylsilyl)oxazolidine (9)



Paraformaldehyde (0.509 g, 16.98 mmol) and **21** (2.3 g, 8.49 mmol) in benzene (50 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 **9** (2.28, 95%) as a colorless liquid.

IR ν_{max} cm^{-1} in $CHCl_3$	3319, 2922, 2102, 1235.
^1H NMR (200 MHz, $CDCl_3$)	δ 0.05, 0.06 (s each, 4.5H each), 1.39 (s, 3H), 1.44 (s, 3H), 2.32 (dd, *J* = 17.43, 8.59 Hz, 1H), 2.51 (dd, *J* = 2.02, 0.88 Hz, 1H), 2.70–2.82 (m, 2H), 3.45–3.55 (m, 1H), 3.95–4.12 (m, 2H), 4.18 (app t, *J* = 5.95, 5.81 Hz, 1H), 4.31 (app t, *J* = 5.93, 5.81 Hz, 1H), 4.48 (two dd, *J* = 7.83, 2.02 Hz, 1H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ −3.0 [Si(CH_3)$_3$], 25.9, 26.0, 26.9, 27.0 [C(CH_3)$_2$], 55.2, 55.3 (CH), 55.7, 57.7 (CH_2), 66.2, 66.3 (CH_2), 67.2, 68.6 (CH), 74.5, 74.8 (C), 80.5 (≡CH), 81.4, 81.7 (CH), 87.7, 88.7 (CH_2), 110.2, 110.6 (C).
Mass (ESI): *m/z*	284 (M^++H).
Elemental analysis	Anal. Calcd for $C_{14}H_{25}NO_3Si$: C, 59.32; H, 8.89; N, 4.94; Found: C, 59.44; H, 8.79; N, 4.83.

7. Preparation of (3aS,8aS,9aS)-2,2-dimethyl-9-methylenehexahydro-3aH-[1,3]dioxolo-[4,5-d]oxazolo[3,4-a]pyridine (5)

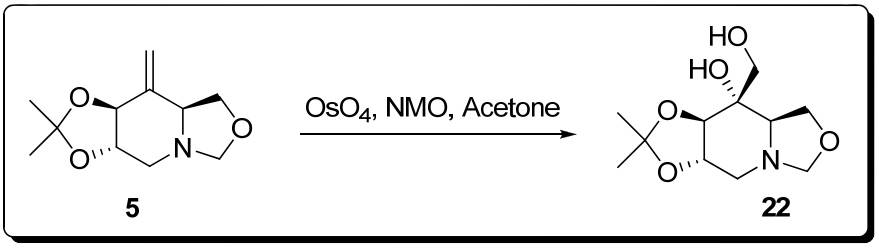


A solution containing **9** (1 g, 3.53 mmol) and 1,4-dicyanonaphthalene (0.12 g, 0.67 mmol) in acetonitrile/2-propanol (4:1, 250 mL) mixture was irradiated in an open vessel using a 450 W Hanovia medium pressure mercury vapor lamp. The lamp was immersed in a Pyrex water-jacketed immersion well which allowed only wavelengths greater than 280 nm to pass through. After about 2 h of the starting material was found to be almost disappeared

(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 **5** (0.410 g, 55%) as a yellow liquid.

$[\alpha]^{27}_D$	+114.47 (*c* 1.0, DCM)
IR ν_{max} cm^{-1} in $CHCl_3$	1672 (C=C stretch), 1431, 1381, 1236.
^1H NMR (400 MHz, $CDCl_3$)	δ 1.44 (s, 3H), 1.45 (s, 3H), 2.57 (t, *J* = 10.19 Hz, 1H), 3.12 (tdd, *J* = 7.71, 6.32, 1.59 Hz, 1H), 3.39 (dd, *J* = 10.24, 4.21 Hz, 1H), 3.62 (ddd, *J* = 10.11, 9.23, 4.22 Hz, 1H), 3.89 (td, *J* = 9.22, 1.94 Hz, 1H), 3.92 (t, *J* = 7.73 Hz, 1H), 4.03(dd, *J* = 7.23, 6.08 Hz, 1H), 4.10 (d, *J* = 1.19 Hz, 1H), 4.52 (d, *J* = 1.19 Hz, 1H), 4.74 (t, *J* = 1.70 Hz, 1H), 5.08 (t, *J* = 1.70 Hz, 1H)
^{13}C NMR (100 MHz, $CDCl_3$)	δ 26.7 [$C(CH_3)_2$], 26.9 [$C(CH_3)_2$], 47.6 (CH_2), 61.4 (CH), 67.0 (CH_2), 77.3 (CH), 82.0 (CH), 84.0 (CH_2), 103.8 (=CH_2), 111.7 (C), 139.6 (C)
Mass (ESI): *m/z*	212 (M^++H).
Elemental analysis	Anal. Calcd for $C_{11}H_{17}NO_3$: C, 62.54; H, 8.11; N, 6.63; Found: C, 62.39; H, 8.00; N, 6.74.

8. Preparation of (3a*S*,8a*R*,9*S*,9a*R*)-9-(hydroxymethyl)-2,2-dimethylhexahydro-3aH-[1,3]dioxolo[4,5-d]oxazolo[3,4-a]pyridin-9-ol (22)



5 **22**

To a solution of **5** (0.3 g, 1.42 mmol) in acetone (4.5 mL) was added NMO (50% aq solution, 0.66 g, 2.84 mmol). The reaction mixture was cooled to 0 °C and to it was added a catalytic amount of osmium tetroxide (0.3 mL of 1% solution of OsO_4 in *t*-BuOH). The reaction mixture was allowed to come to rt and stirred for 10 h. Solid Na_2SO_3 was added to this reaction mixture. Stirring was continued for 30 min to quench excess NMO and OsO_4.

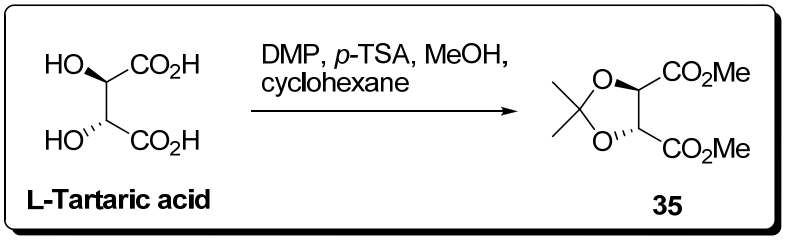
It was passed through a short pad of celite and the solvent was evaporated off. The crude reaction mixture was used as such for next step.

9. Preparation of (3a*S*,8a*R*,9*S*,9a*R*)-9-((*tert*-butyldimethylsilyloxy)methyl)-2,2-dimethylhexahydro-3aH-[1,3]dioxolo[4,5-d]oxazolo[3,4-a]pyridin-9-ol (23)

To a solution containing crude reaction mixture of **22** (considering approx. 90% yield 0.314 g, 1.28 mmol) in DCM (3.5 mL) at 0 °C, was added TEA (0.11 mL, 1.54 mmol), TBSCl (0.192 g, 1.28 mmol) and a catalytic amount of DMAP. The solution was stirred for 24 h and finally quenched by adding water (1 mL). The aqueous phase was extracted with DCM (3 × 10 mL), the combined organic phases were dried over anhydrous Na_2SO_4 and the solvent evaporated. Column chromatography (silica, pet ether–ethyl acetate, 9:1) of the crude afforded **23** (0.357 g, 70% over two steps) as a viscous colorless oil.

$[\alpha]^{26}_D$	+71.14 (*c* 1.0, $CHCl_3$)
^{1}H NMR (500 MHz, $CDCl_3$)	δ 0.11 (s, 3H), 0.12 (s, 3H), 0.89 (s, 9H), 1.42 (s, 3H), 1.46 (s, 3H), 2.26 (t, *J* = 9.53 Hz, 1H), 2.61 (dd, *J* = 9.60, 6.39 Hz, 1H), 3.27 (dd, *J* = 9.21, 4.28 Hz, 1H), 3.57–3.61 (m, 1H), 3.67 (d, *J* = 9.75 Hz, 1H), 3.81 (d, *J* = 9.53 Hz, 1H), 3.85 (dd, *J* = 9.66, 7.66 Hz, 1H), 3.91 (d, *J* = 9.38 Hz, 1H), 3.98–4.01 (m, 2H), 4.58 (s, 1H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ −5.7 (CH_3), −5.5 (CH_3), 18.0 (C), 25.7 (CH_3), 26.5 (CH_3), 26.7 (CH_3), 48.2 (CH_2), 58.2 (CH_2), 66.8 (CH_2), 68.5 (CH), 70.9 (C), 73.0 (CH), 84.3 (CH), 84.4 (CH_2), 111.6 (C).
Mass (ESI): *m/z*	360 (M^++H).
Elemental analysis	Anal. Calcd for $C_{17}H_{33}NO_5Si$: C, 56.79; H, 9.25; N, 3.90; Found: C, 56. 95; H, 9.38; N, 3.73.

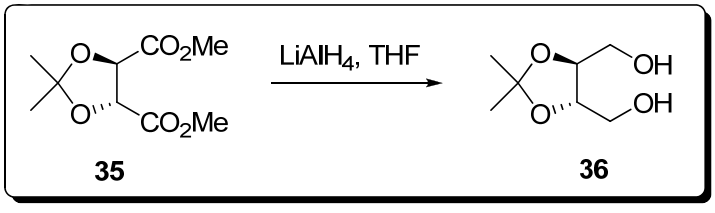
10. Preparation of (4*R*,5*R*)-dimethyl 2,2-dimethyl-1,3-dioxolane-4,5-dicarboxylate (35)[8]



In a 1-L, RB 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*-TSA .H_2O (0.4 g, 2.1 mmol) was charged and the whole contents were warmed to 60 °C and stirred for about 2 h 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 azeotrop 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 and the residue was fractionally distilled under vacuum (bp 90–101°C /0.6 mm) to afford **35** (135 g, 95% yield) as a pale-yellow oil.

$[\alpha]^{27}_D$ (neat)	−46.3, lit.[8] $[\alpha]^{20}_D$ = −49.4
IR ν_{max} (neat) cm^{-1}	2992, 2956, 1759, 1438, 1384, 1213, 1111.
^1H NMR (200 MHz, $CDCl_3$)	δ 1.49 (s, 6H), 3.83 (s, 6H), 4.81 (s, 2H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ 25.98 (CH_3), 52.42 (CH_2), 76.68 (CH_2), 113.49 (C), 169.75 (C).

11. Preparation of ((4*S*,5*S*)-2,2-dimethyl-1,3-dioxolane-4,5-diyl)dimethanol (36)[8b]



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 **35** (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) NaOH solution (12 mL) and water (12–18 mL). The mixture was 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 × 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 **36** as a colorless to pale-yellow oil, bp 96–108 °C (0.6 mm); 23 g (90% yield).

$[\alpha]^{27}_D$	+2.9 (*c* 5.0, $CHCl_3$), lit.[8b] $[\alpha]^{20}_D$ = +4.1 (*c* 5, $CHCl_3$)
IR ν_{max} cm^{-1} (neat)	3413 (br), 2934, 1455, 1372, 1218, 1166, 1057
^1H NMR (200 MHz, $CDCl_3$)	δ 1.42 (s, 6H), 2.73(br s, 2H, D_2O exchangeable), 3.73 (m, 4H), 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).

12. Preparation of ((4*S*,5*S*)-5-((*tert*-butyldimethylsilyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (26)[9]

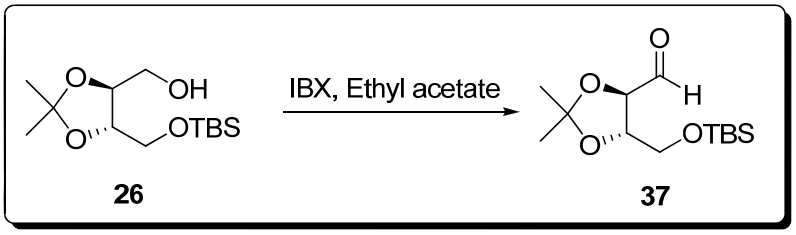
36 **26**

To a stirring solution of **36** (50 g, 308.4 mmol) in dry DCM (1.25 L) at 0 °C was added triethyl amine (45.01 mL, 323 mmol). To this mixture was added a solution of TBSCl (46.5 g. 308.4 mmol) in dry DCM (200 ml) over a period of 1 h using an 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 × 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 **26** (72 g, 85%) as a colorless liquid.

$[\alpha]^{27}_D$	−5.5 (*c* 2, MeOH) , lit.[9] $[\alpha]^{25}_D$ = −5.4 (*c* 5, MeOH).
IR ν_{max} cm^{-1} (neat)	3463, 1253, 1379.
^{1}H NMR (200 MHz, $CDCl_3$)	δ 0.05 (s, 6H), 0.85 (s, 9H), 1.40 (s, 3H), 1.45 (s, 3H), 2.65 (bs, 1H), 3,65 (m, 3H), 3.85 (m, 2H), 4.0 (m,1H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ −5.3 [Si(*C*H_3)$_2$], 18.4 [C(*C*H_3)$_3$], 26.0 (C), 27.1 (CH_3), 27.2 (CH_3), 62.9 (CH_2), 63.9 (CH_2), 78.2 (CH), 80.2 (CH), 109.2 (C).
Mass (ESI): *m/z*	277 (M^++H).

13. Preparation of (4*R*,5*S*)-5-((*tert*-butyldimethylsilyloxy)methyl)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde (37)

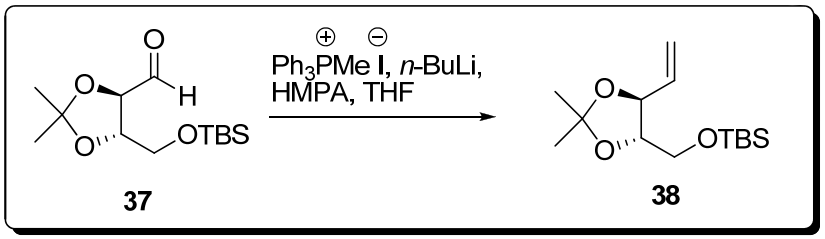


To a solution of alcohol **26** (20 g, 72.46 mmol) in ethyl acetate (250 mL) was added IBX (34.51 g, 123 mmol). The resulting suspension was immersed in an oil bath set at 80 °C and stirred vigorously open to the atmosphere. After 8 h (GC monitoring), the reaction was cooled to room temperature and passed through a short pad of celite. The filter cake was washed with 3 × 100 mL of ethyl acetate and the combined filtrates were concentrated to yield 19. 8 g of **37** (98% yield, >98% pure by GC). The crude aldehyde **37** obtained was immediately used for the next step. ***Note:*** *IBX recovered from the reaction mixture was reoxidized and recycled.*

IR ν_{max} cm^{-1} (neat)	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, −5.4 [−Si(*C*H_3)$_2$], 18.3 [−C(*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 (ESI): *m/z*	275 (M^++H).

14. Preparation of *tert*-butyl(((4*S*,5*S*)-2,2-dimethyl-5-vinyl-1,3-dioxolan-4-yl)methoxy)-dimethylsilane (38)

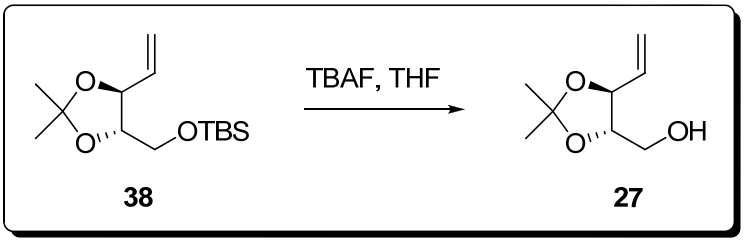


37 38

Hexamethylphosphoramide (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 solution was added *n*-BuLi (1.62M in hexane, 3.24 mL, 5.26 mmol). To the resultant clear brownish solution was added solution of **37** (1.0 g, 3.65 mmol) in THF (6 mL). The reaction mixture was allowed to stir at 0 °C for 2 h and 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 × 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. Excess of 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 **38** (0.70 g, 70%) as a colorless oil.

$[\alpha]^{27}_D$	+4.17 (*c* 1.92, $CHCl_3$).
IR ν_{max} cm^{-1} (neat)	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 (50 MHz, $CDCl_3$)	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.
Mass (ESI): *m/z*	273 (M^++H).

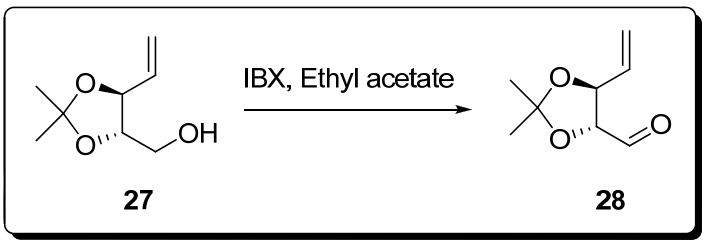
15. Preparation of ((4*S*,5*S*)-2,2-dimethyl-5-vinyl-1,3-dioxolan-4-yl)methanol (27)



To a solution of **38** (0.7 g, 2.57 mmol) in dry THF (5 mL) was added 1M solution of TBAF in THF (2.6 mL, 2.6 mmol) at 0 °C. The reaction mixture was stirred at rt for about 3 h and to this was added water (4 mL) followed by ethyl acetate (5 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 **27** (0.325 g, 80%) as a colorless liquid.

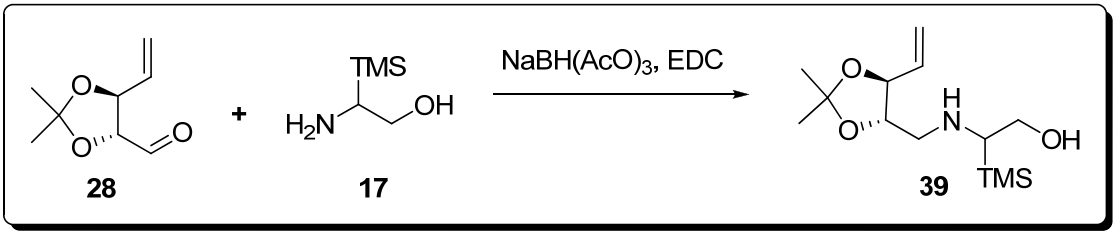
$[\alpha]^{27}_D$	−2.94 (*c* 1.36, $CHCl_3$).
IR ν_{max} cm^{-1} (neat)	3446 (broad), 1647, 1380.
^1H NMR (200 MHz, $CDCl_3$)	δ 1.40 (s, 6H), 2.05 (dd, 1H, *J* = 8.0, 4.6 Hz), 3.60 (m, 1H), 3.85 (m, 2H), 4.30 (t, 1H, *J* = 8.3 Hz), 5.85 (m, 1H), 5.35 (m, 2H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ 26.7, 60.6, 78.2, 80.9, 109.0, 118.8, 134.8.
Mass (ESI): *m/z*	169 (M^++H).

16. Preparation of (4*R*,5*S*)-2,2-dimethyl-5-vinyl-1,3-dioxolane-4-carbaldehyde (28)



Similar experimental procedure was used as described earlier for the preparation of **37.** The crude aldehyde (**28)** obtained was immediately used for the next step.

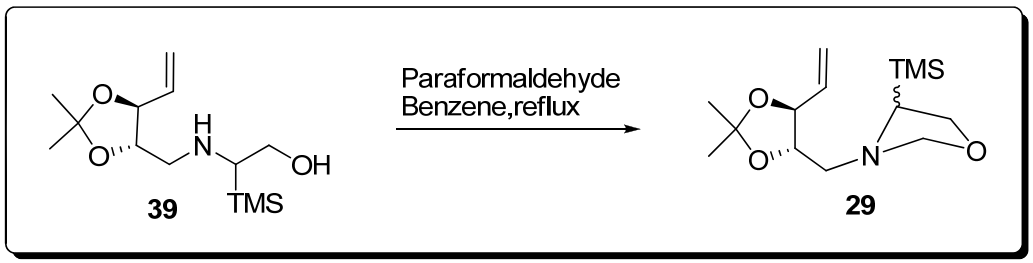
17. Preparation of 2-(((4*S*,5*S*)-2,2-dimethyl-5-vinyl-1,3-dioxolan-4-yl)methylamino)-2-(trimethylsilyl)ethanol (39)[3]



Similar experimental procedure was used as described for the preparation of **21**.

IR ν_{max} cm^{-1} in $CHCl_3$	3489, 3349, 1644, 1446, 1388, 1236.
^1H NMR (400 MHz, $CDCl_3$)	δ 0.06 (s, 9H), 1.40 (s, 6H), 2.09–2.12 (m, 1H), 2.73–2.89 (m, 2H), 3.47–3.53 (m, 1H), 3.69–3.74 (m, 1H), 3.78–3.82 (m, 1H), 4.11, 4.24 (t each, *J* = 7.92 Hz, 0.5H each), 5.22–5.25 (m, 1H), 5.31–5.39 (m, 1H), 5.77–5.86 (m, 1H).
^{13}C NMR (50 MHz, $CDCl_3$)	δ −2.71, −2.68 (CH_3), 26.9 (CH_3), 27.0, 27.1 (CH_3), 49.5, 51.0 (CH_2), 51.7 (CH), 61.4, 61.7 (CH_2), 79.8, 80.1 (CH), 80.5, 80.6 (CH), 109.2, 109. 7 (C), 118.8, 118.9 (CH_2), 135.3, 135.4 (CH).
Mass (ESI): *m/z*	274 (M^++H).
Elemental analysis	Anal. Calcd for $C_{13}H_{27}NO_3Si$: C, 57.10; H, 9.95; N, 5.12; Found: C, 57.01; H, 10.03; N, 5.22.

18. Preparation of 3-(((4*S*,5*S*)-2,2-dimethyl-5-vinyl-1,3-dioxolan-4-yl)methyl)-4-(trimethylsilyl)oxazolidine (29)

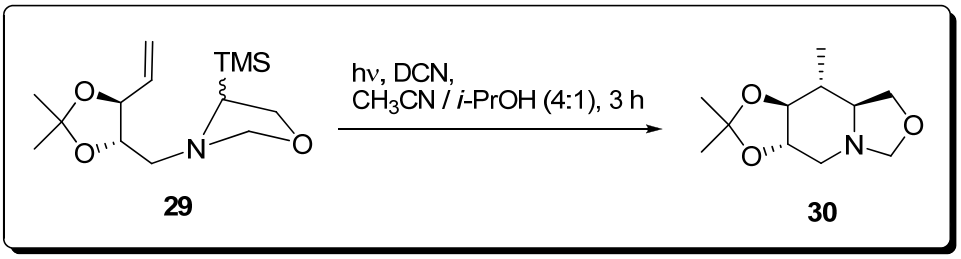


Similar procedure was used as described for the preparation of **9**.

IR ν_{max} cm^{-1} in $CHCl_3$	1651, 1474, 1377.
^1H NMR (400 MHz, $CDCl_3$)	δ 0.04 (s, 9H), 1.38 (s, 3H), 1.39 (s, 3H), 2.24, 2.44 (t

	each, *J* = 8.79 Hz, 0.5H each), 2.61–2.74 (m, 2H), 3.48–3.54 (m, 1H), 3.73–3.78 (m, 1H), 3.96–4.22 (m, 3H), 4.29, 4.34 (d each, *J* = 5.02 Hz, 0.5H each), 5.23 (dd, *J* = 10.55, 5.27 Hz, 1H), 5.34 (dd, *J* = 17.02, 6.52 Hz, 1H), 5.76–5.87(m, 1H).
^{13}C NMR (100 MHz, $CDCl_3$)	δ −3.1, −3.0 (Si(*C*H_3)$_3$), 26.6, 26.9, 27.00 (C(*C*H_3)$_2$), 54.8, 54.9 (CH), 56.4, 57.6 (CH_2), 65.7, 66.3 (CH_2), 79.8, 80.4 (CH), 80.8, 80.9 (CH), 87.7, 88.4 (CH_2), 108.8, 109.0 (C), 118.1, 118.5 (CH_2), 135.2, 135.3 (CH).
Mass (ESI): *m/z*	286 (M^++H), 308 (M^++Na).
Elemental analysis	Anal. Calcd for $C_{14}H_{27}NO_3Si$: C, 58.91; H, 9.53; N, 4.91; Found: C, 58.83; H, 9.39; N, 4.86.

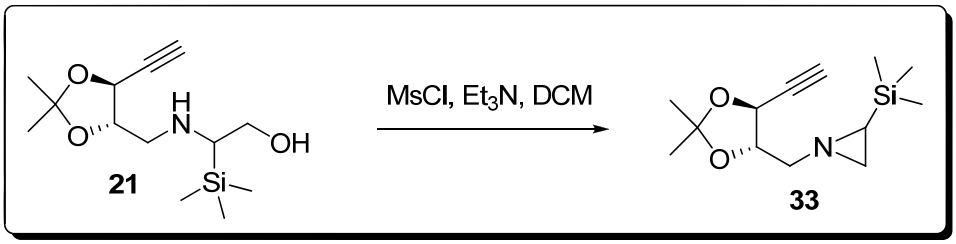
19. Preparation of (3a*S*,8a*S*,9*R*,9a*S*)-2,2,9-trimethylhexahydro-3aH-[1,3]dioxolo[4,5-d] oxazolo[3,4-a]pyridine (30)



Similar experimental procedure was used as described for the preparation of **5**.

$[\alpha]^{28}_D$	+38.86 (*c* 1.15, $CHCl_3$)
IR ν_{max} cm^{-1} in $CHCl_3$	2964, 1461, 1392, 1176.
^{1}H NMR (500 MHz, $CDCl_3$)	δ 1.02 (d, *J* = 6.42 Hz, 3H), 1.42 (s, 3H), 1.43 (s, 3H), 2.29–2.34 (m, 1H), 2.39 (t, *J* = 10.08 Hz, 1H), 3.14 (dd, *J* = 10.54, 8.71 Hz, 1H), 3.33 (dd, *J* = 9.63, 4.13 Hz, 1H), 3.61 (dd, *J* = 7.43, 7.16 Hz, 1H), 3.68 (ddd, *J* = 10.03, 8.94, 4.21 Hz, 1H), 4.01–4.04 (m, 2H), 4.08–4.10 (m, 1H), 4.54 (s, 1H).
Mass (ESI): *m/z*	214 (M^++H), 236 (M^++Na).
Elemental analysis	Anal. Calcd for $C_{11}H_{19}NO_3$: 61.95; H, 8.98; N, 6.57; Found: C, 61.99; H, 9.11; N, 6.45.

20. Preparation of 1-(((4*S*,5*S*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-2-(trimethylsilyl)aziridine (33)



To a stirring solution of **21** (0.25 g, 0.921 mmol) in dry DCM (5mL) at 0° C was added mesyl chloride (0.106 g, 0.921 mmol). The reaction was found to be complete within 5–7 min. The mixture was diluted with water (3 mL) and extracted with DCM (monitored by TLC). The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated under *vacuo* and purified by column chromatography (pet. ether/ethyl acetate, 9:1) to get **33** (0.152 g, 65%) as a colorless liquid.

IR ν_{max} cm^{-1} in $CHCl_3$	3317, 2117, 1362, 1266, 1151.
^1H NMR (400 MHz, $CDCl_3$)	δ 0.02, 0.03 (s each, 4.5H each), 1.40 (s, 3H), 1.47 (s, 3H), 2.08, 2.12 (dd, *J* = 6.27, 3.77 Hz, 0.5H each), 2.49, 2.52 (t each, *J* = 2.26 Hz, 0.5H each), 2.63, 2.79 (dd each, *J* = 12.30, 4.27 Hz, 0.5H each), 2.95, 3.10 (dd each, *J* = 12.55, 4.27 Hz, 0.5H each), 3.57–3.63 (m, 1H), 3.77–3.83 (m, 1H), 4.14–4.19 (m, 1H), 4.50, 4.63 (dd each, *J* = 7.28, 2.01 Hz, 0.5H each).
^{13}C NMR (100 MHz, $CDCl_3$)	δ −2.6, −2.5 (CH_3), 26.0, 26.2 (CH_3), 26.9, 27.0 (CH_3), 49.3, 50.2 (CH_2), 51.3, 51.5 (CH), 62.2, 62.5 (CH_2), 67.5, 68.1 (CH), 74.32, 74.34 (C), 81.2, 81.3 (CH), 81.4, 81.6 (CH), 110.2, 110.3 (C).
Mass (ESI): *m/z*	254 (M^++H).
Elemental analysis	Anal. Calcd for $C_{13}H_{23}NO_2Si$: C, 61.61; H, 9.15; N, 5.53; Found: C, 61.51; H, 9.09; N, 5.69.

3B.6- References

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2. Pandey, G.; Banerjee, P.; Kumar, R.; Puranik, V. G. *Org. Lett.* **2005**, *7*, 3713.
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6. (a) Pandey, G.; Dumbre, S. G.; Khan, M. I.; Shabab, M.; Puranik, V. G. *Tetrahedron Lett.* **2006**, *47*, 7923. (b) Pandey, G.; Dumbre, S. G.; Khan, M. I.; Shabab, M. *J. Org. Chem.* **2006**, *71*, 8481. (c) Pandey, G.; Dumbre, S. G.; Pal, S.; Khan, M. I.; Shabab, M. *Tetrahedron* **2007**, *63*, 4756.
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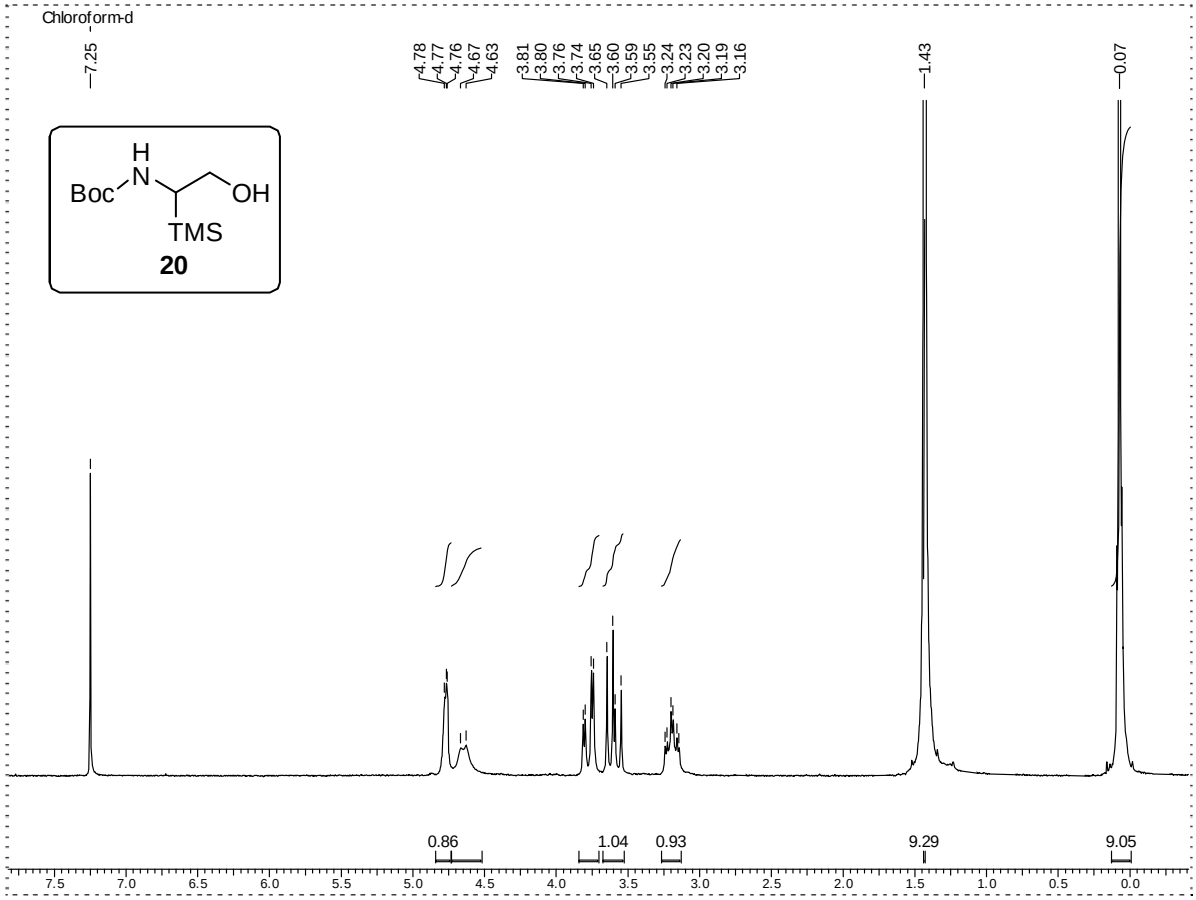
3B.7- Spectra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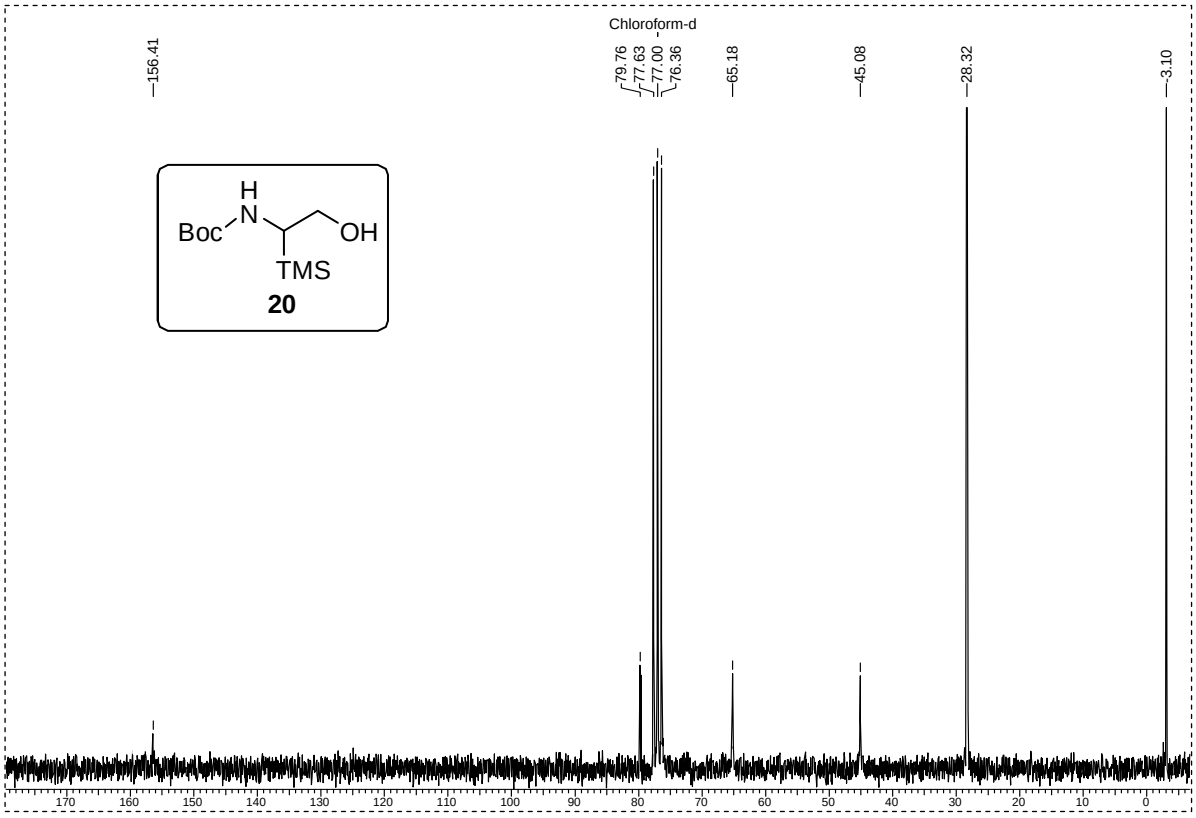
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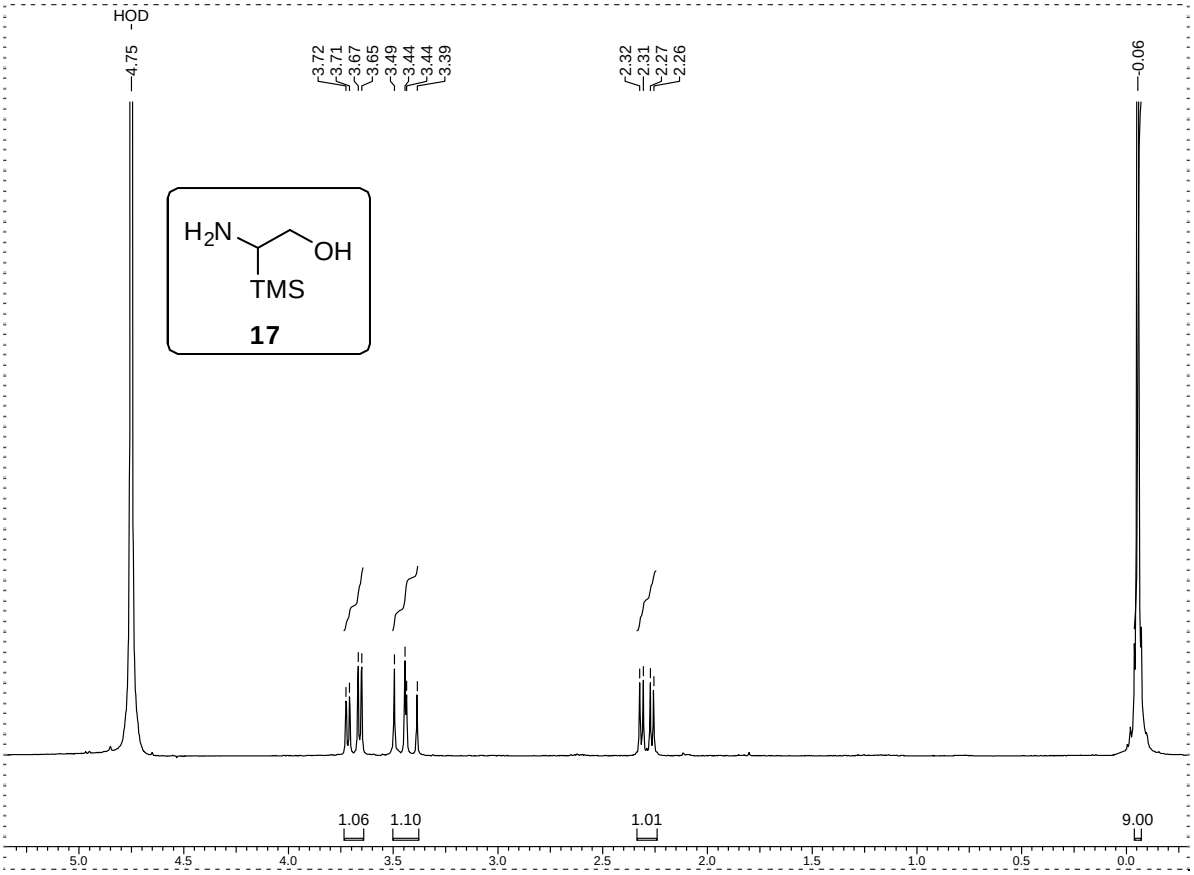
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OH
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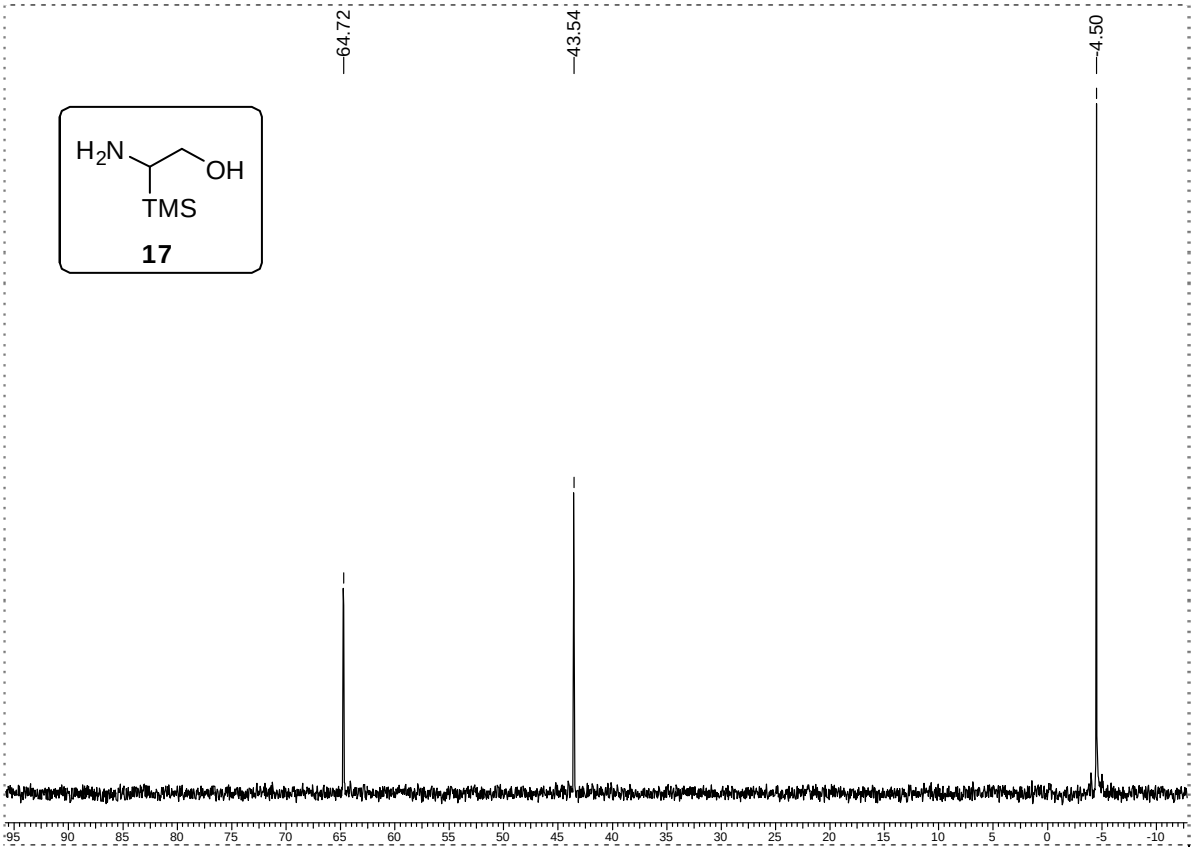
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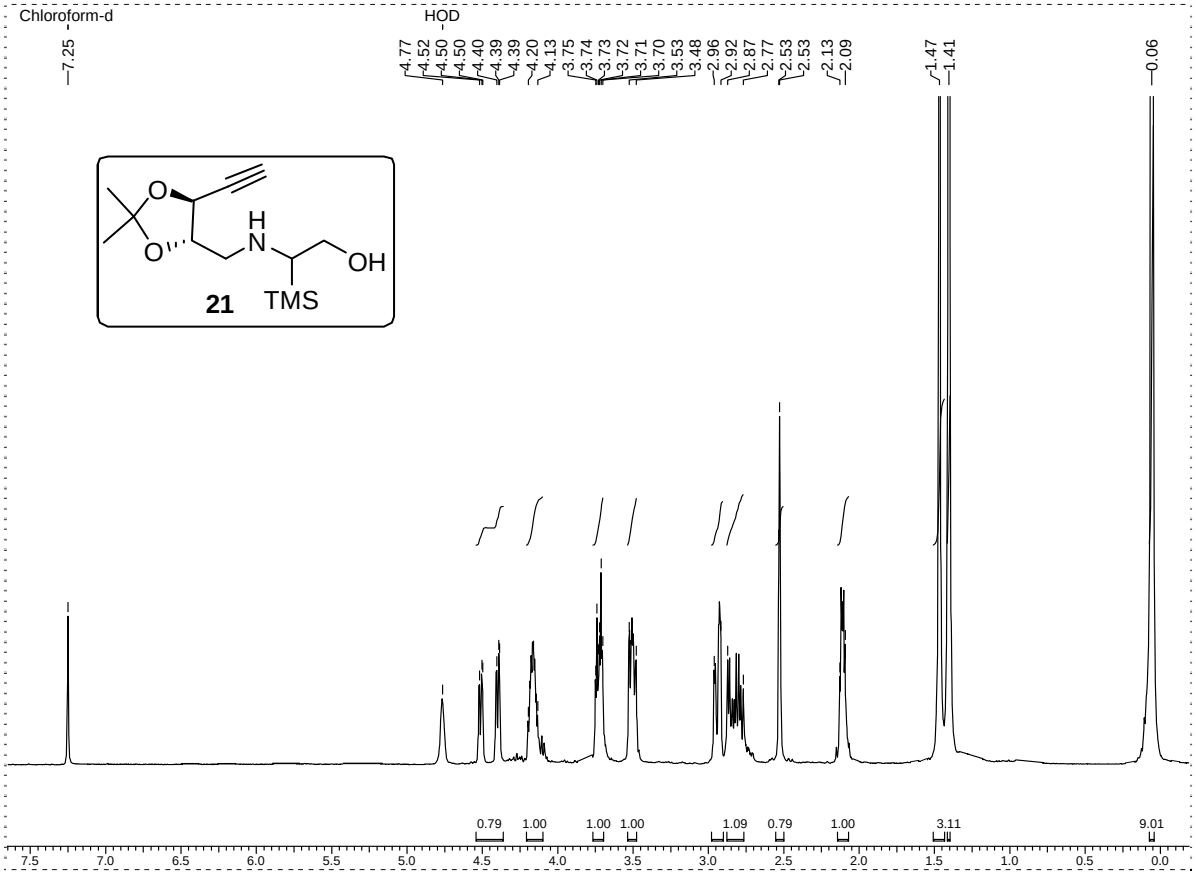
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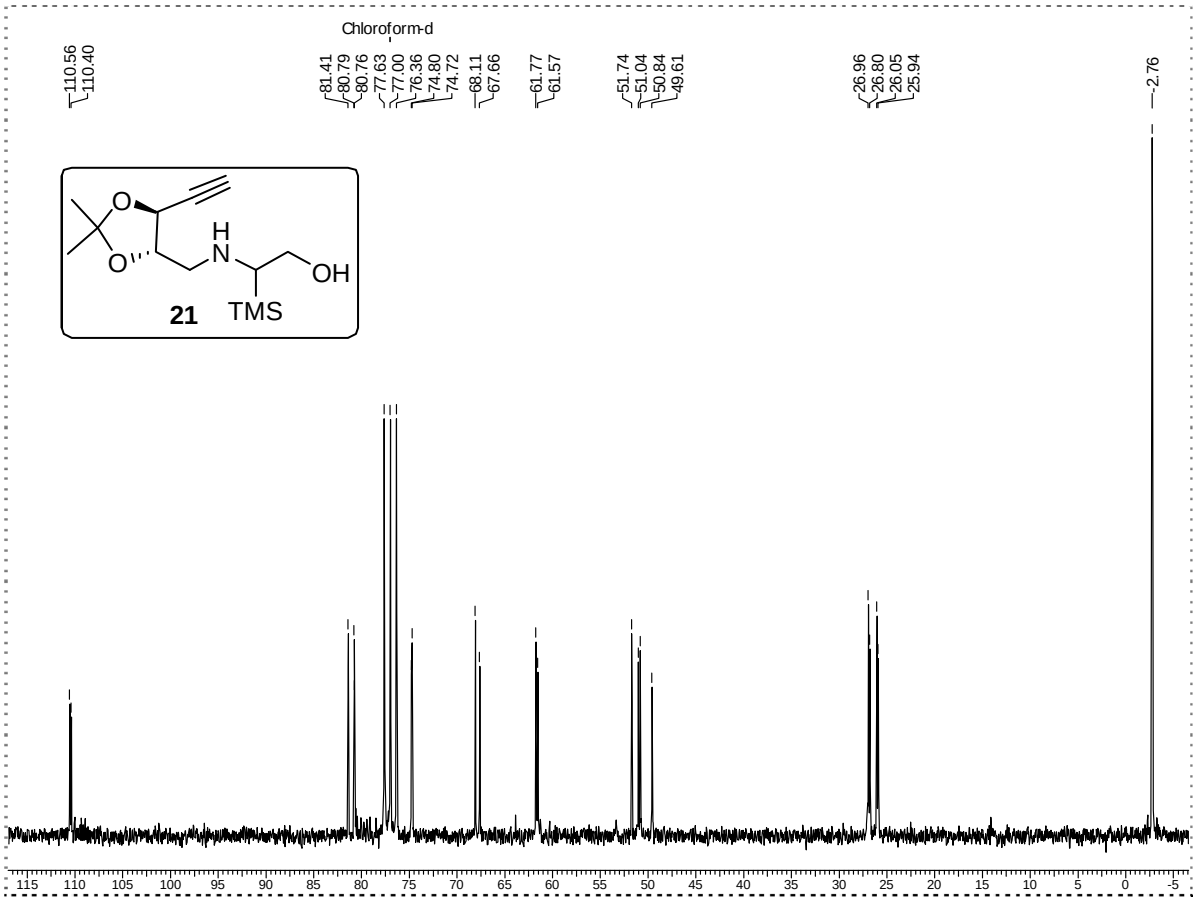
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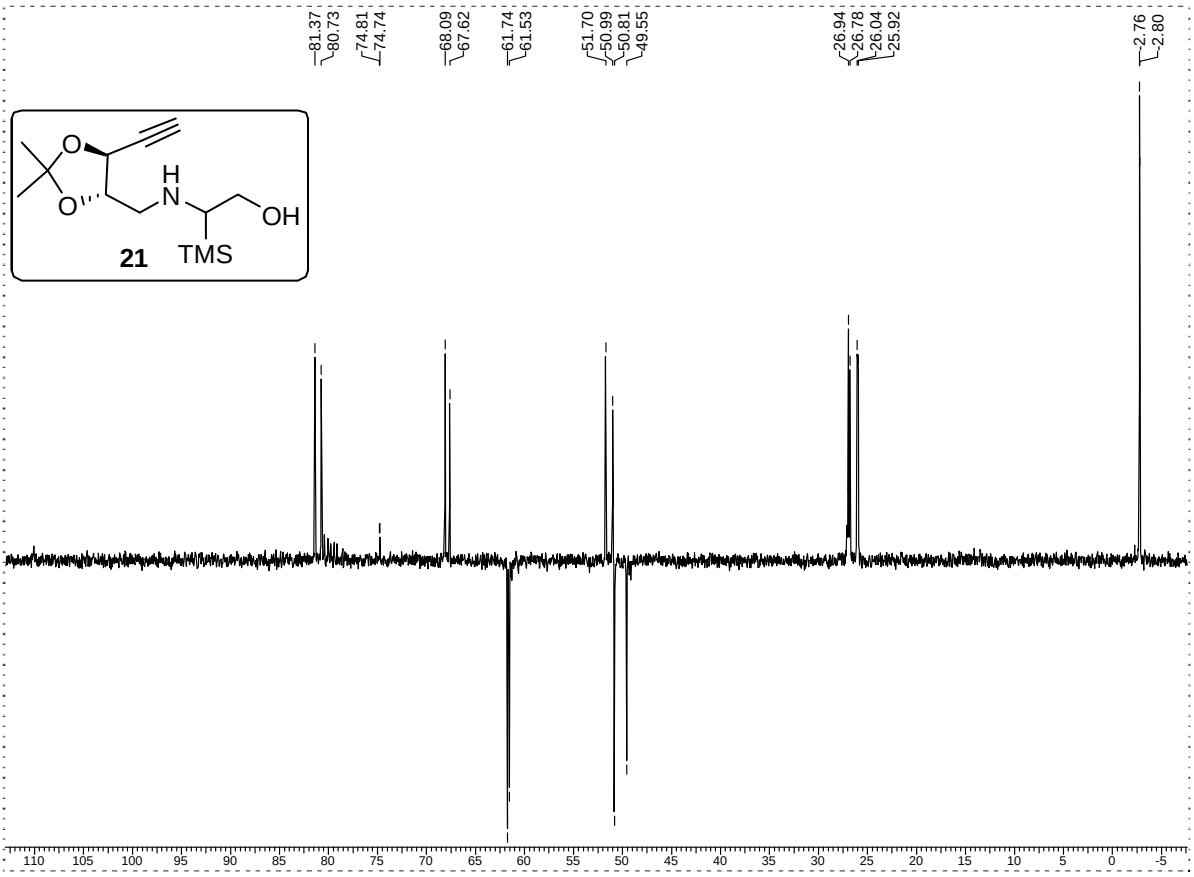
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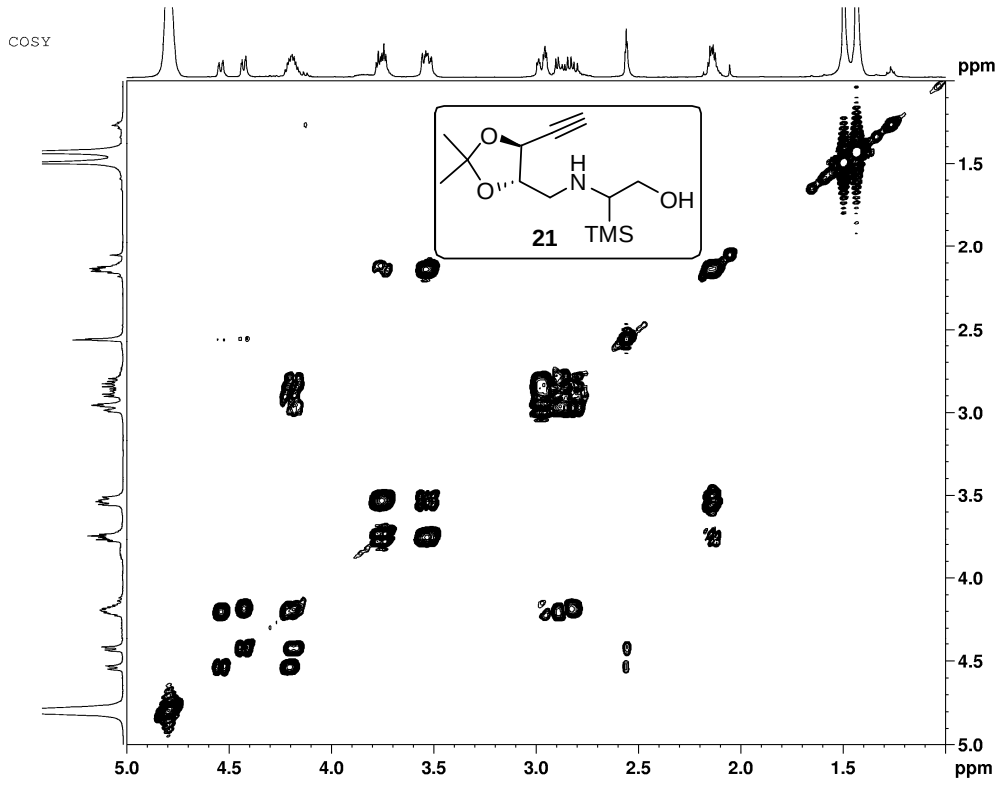
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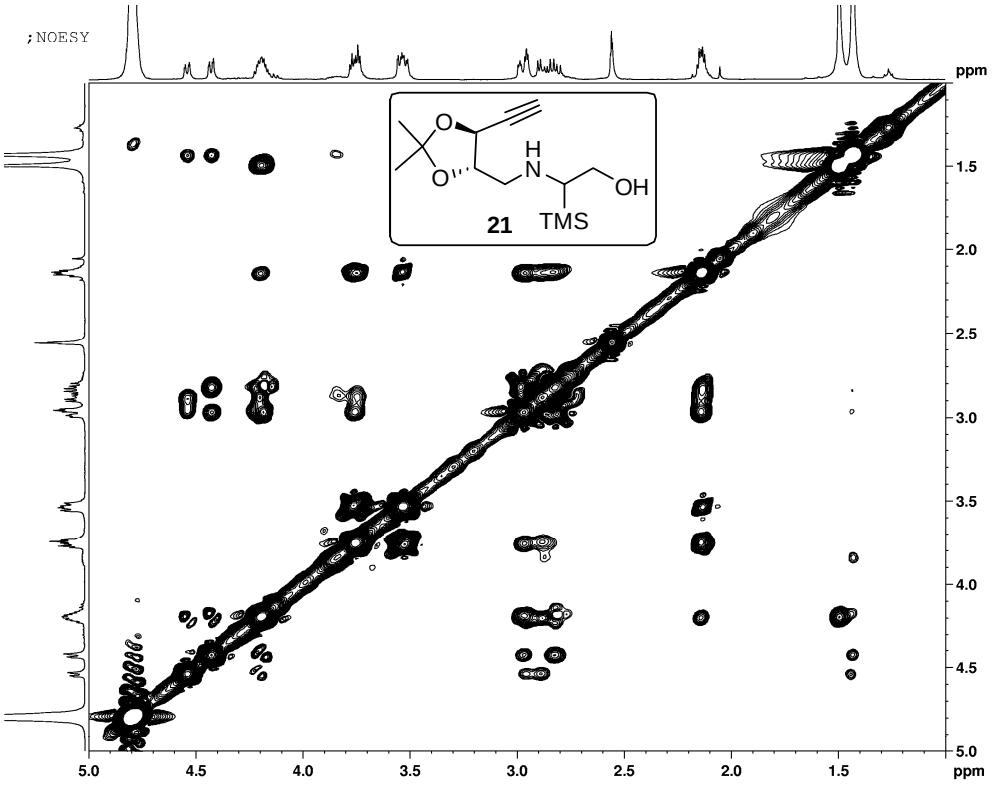
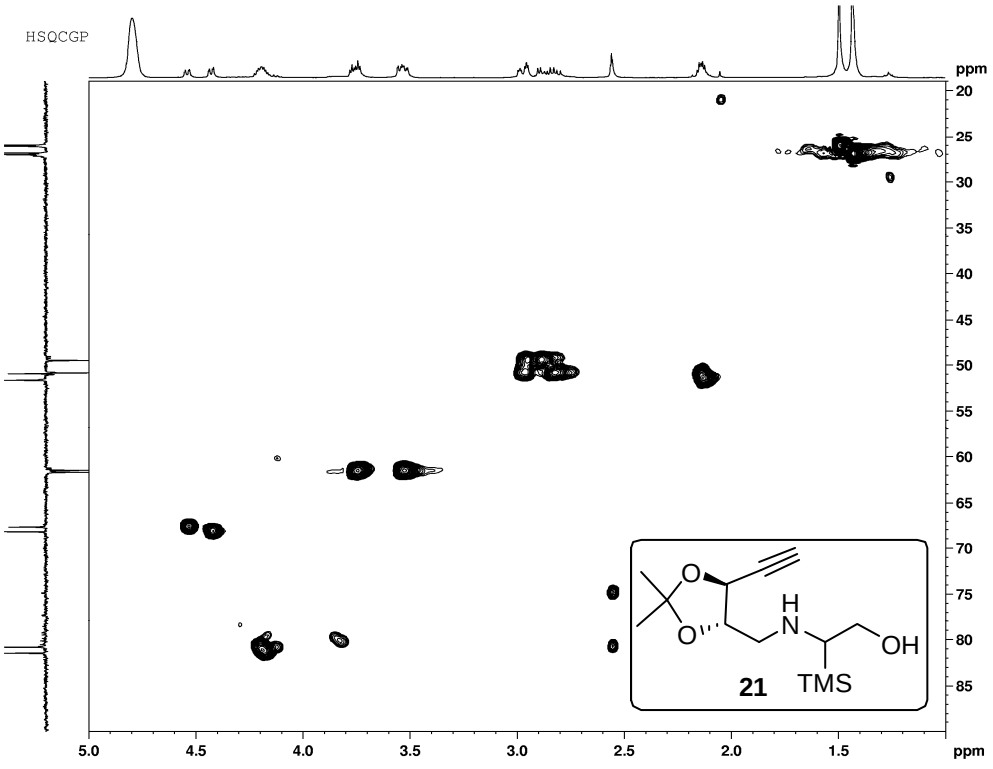
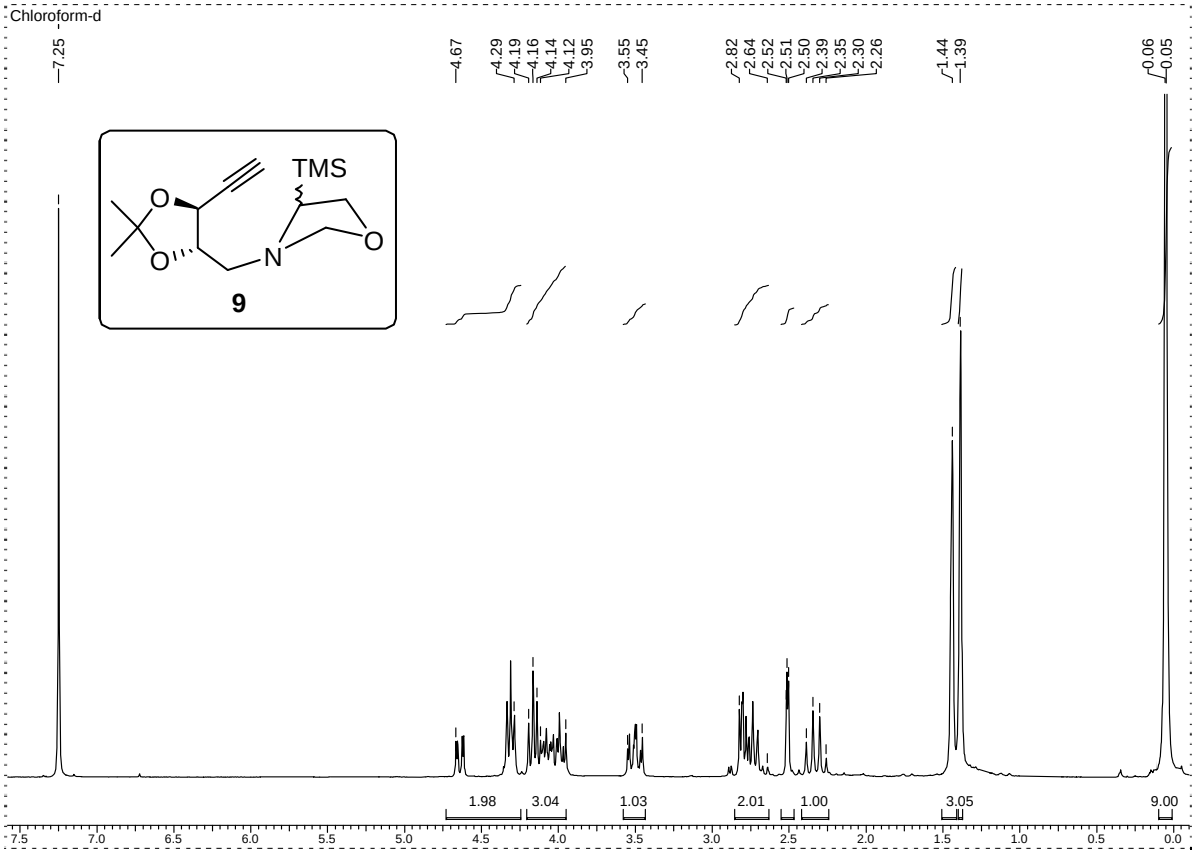


HETCOR

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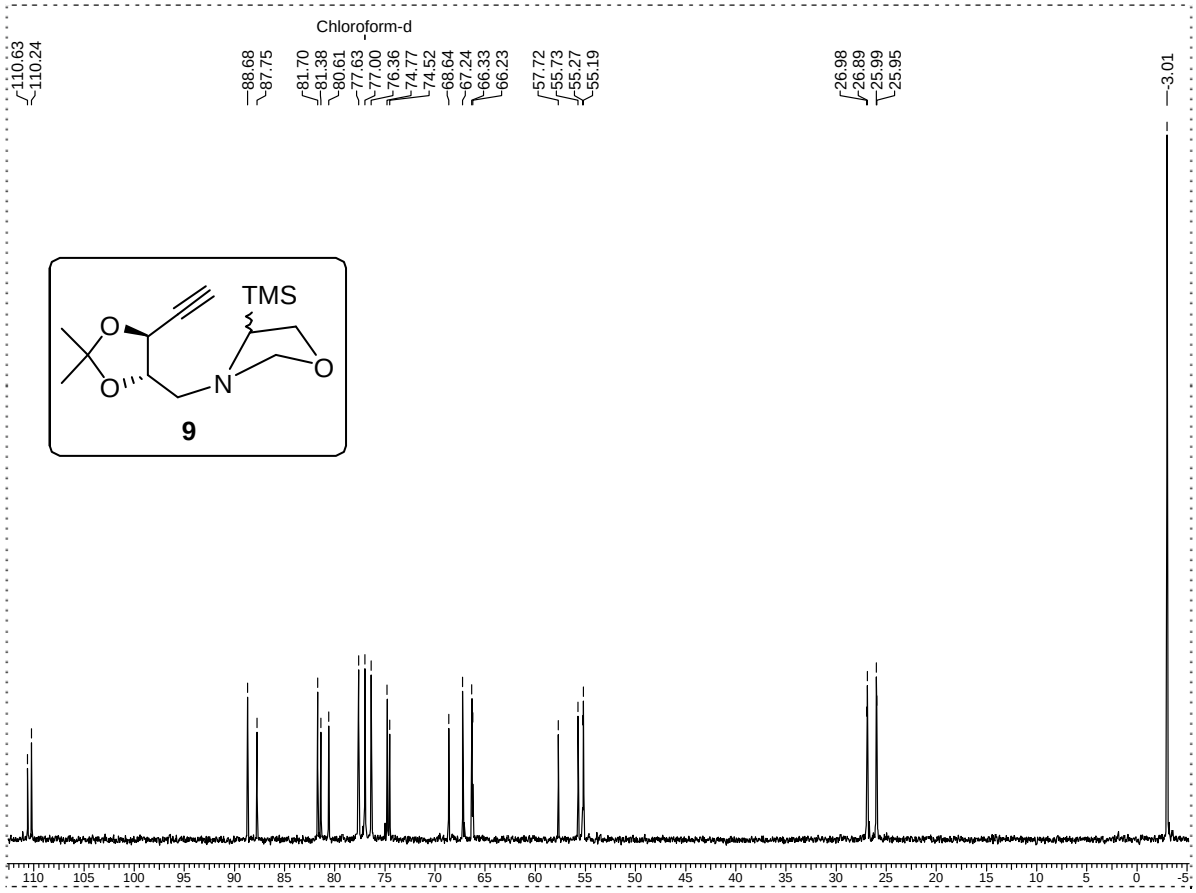
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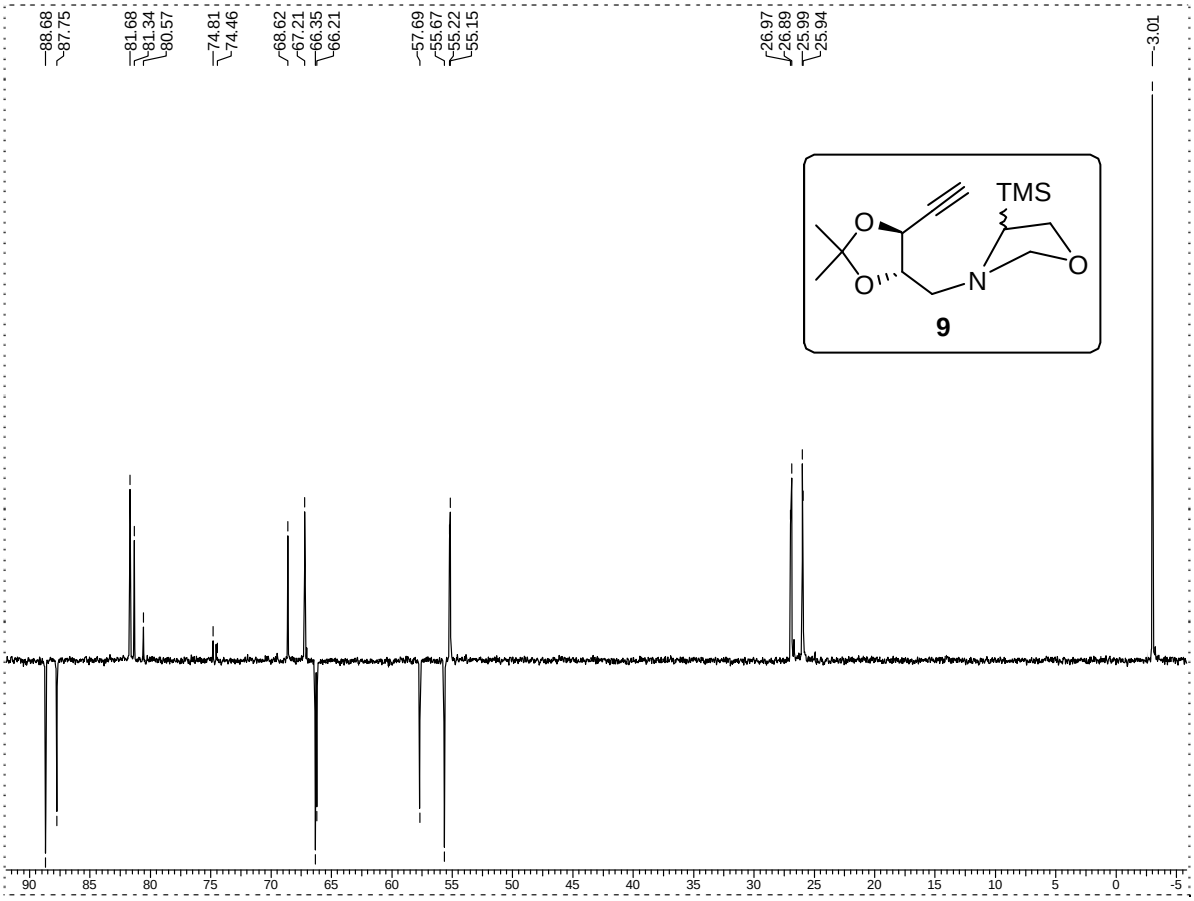
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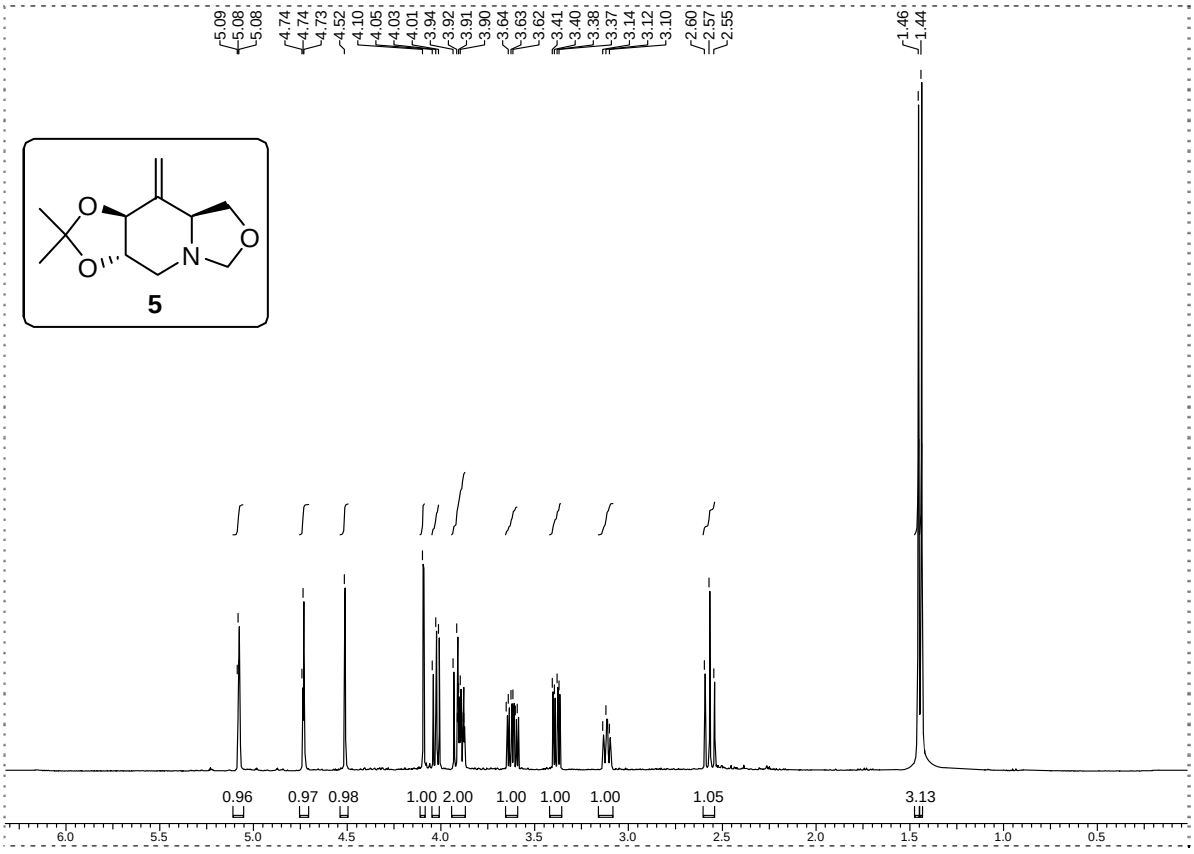
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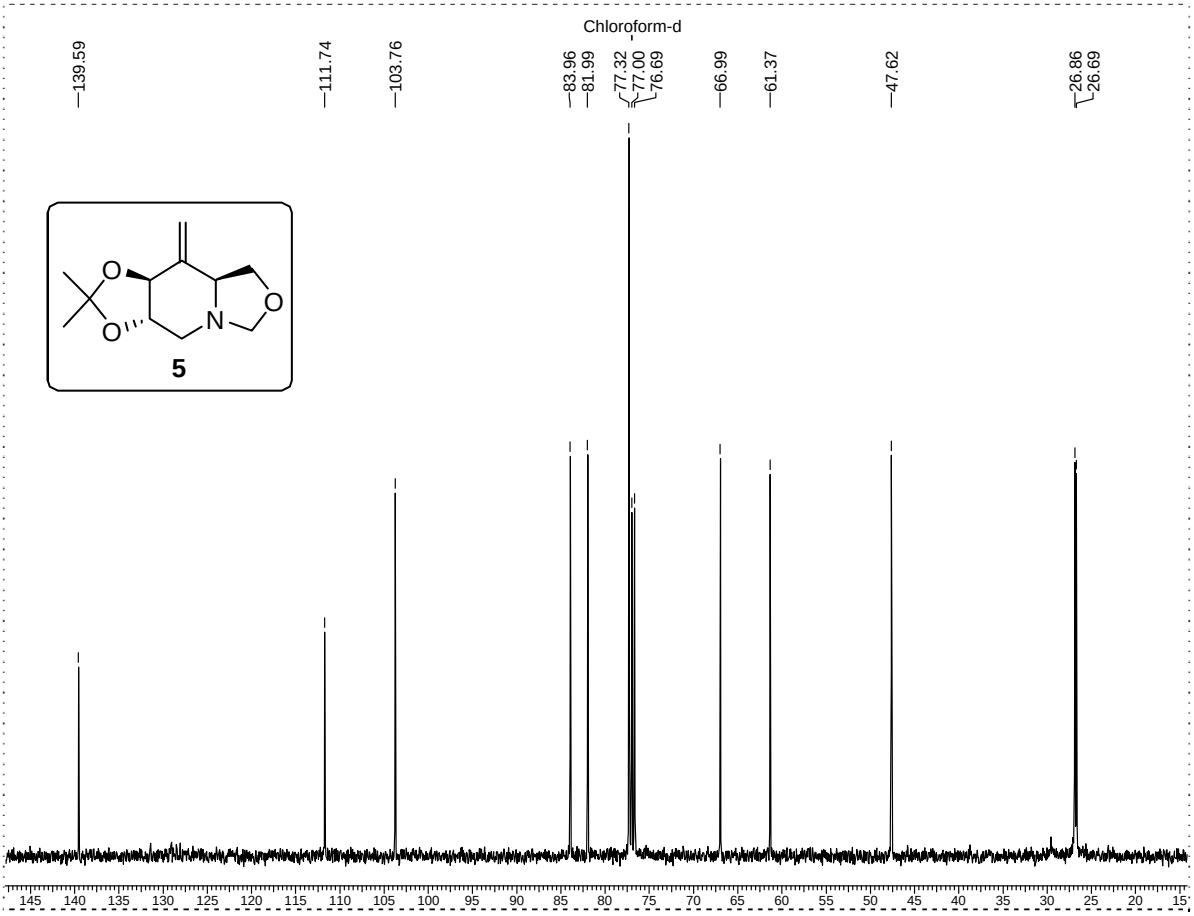
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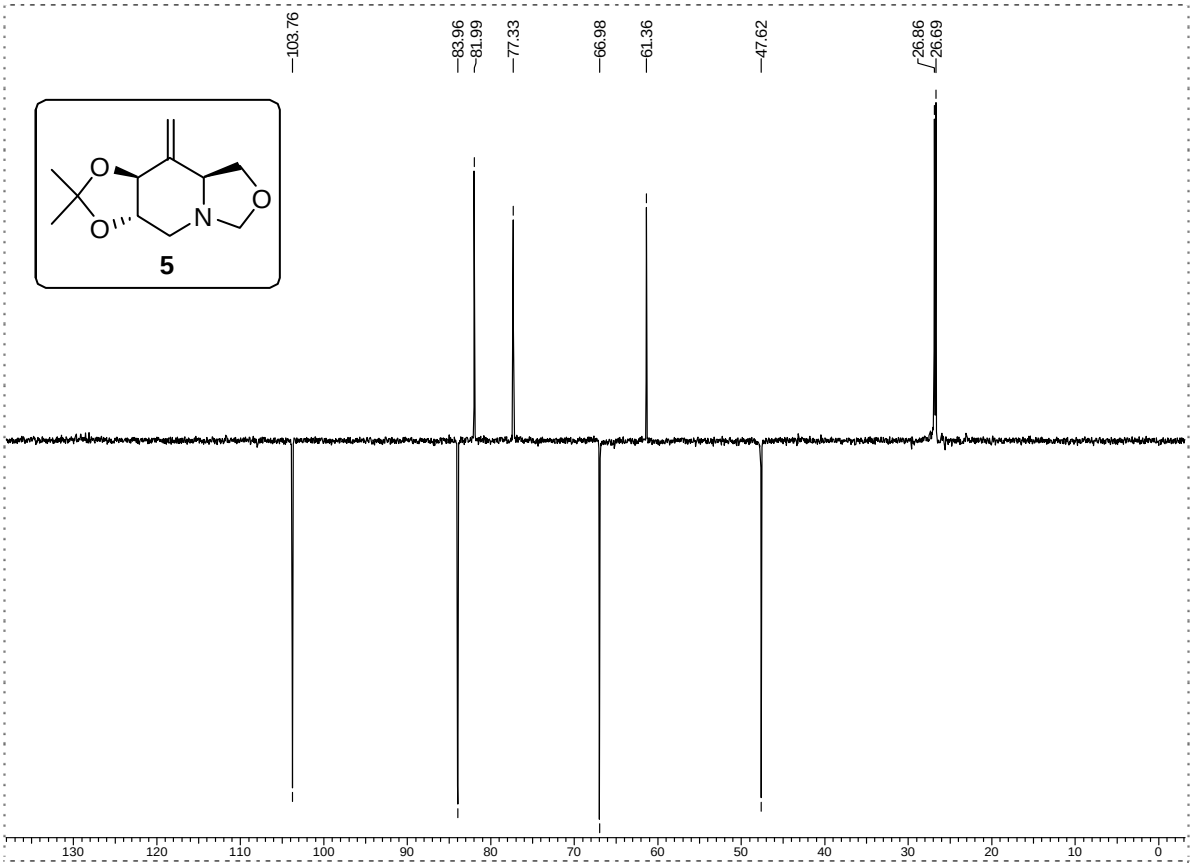
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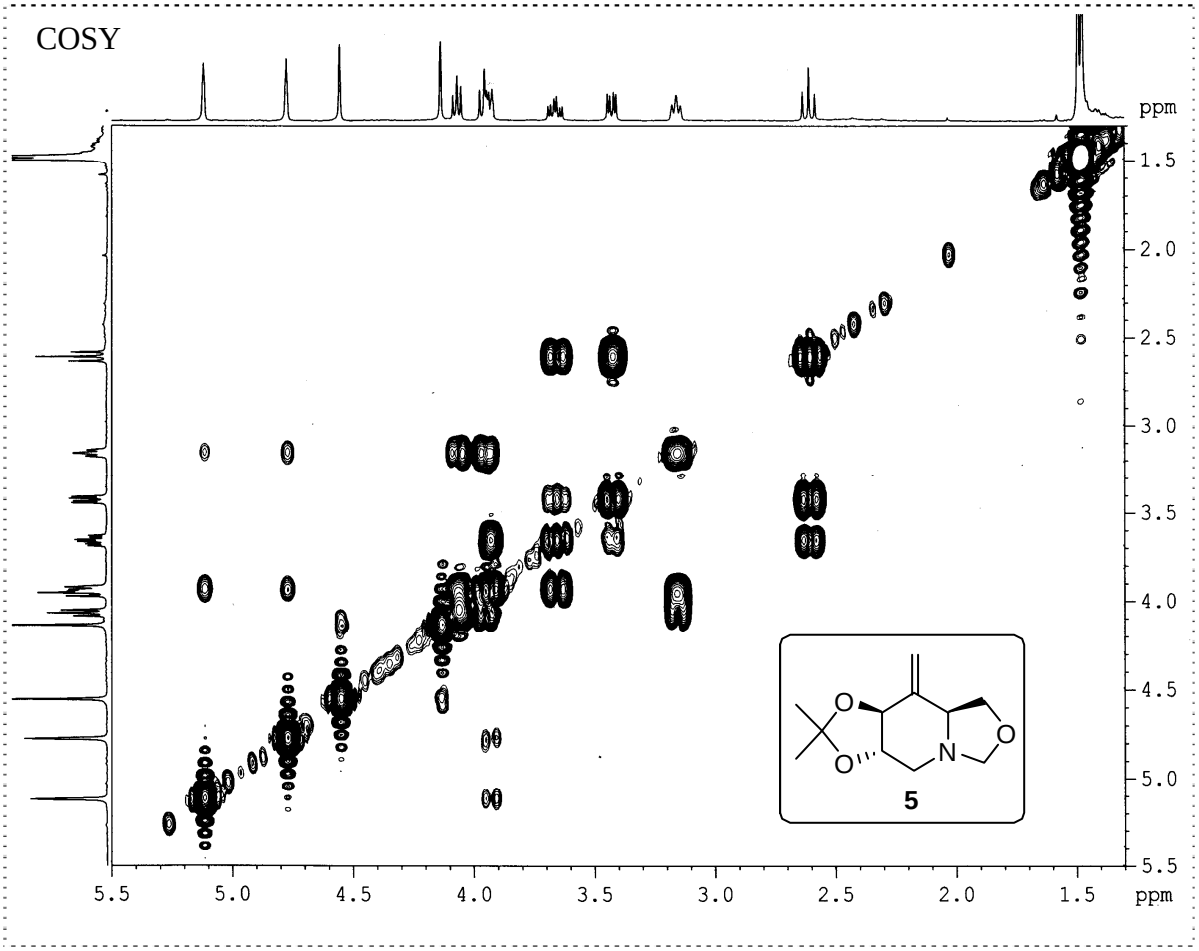
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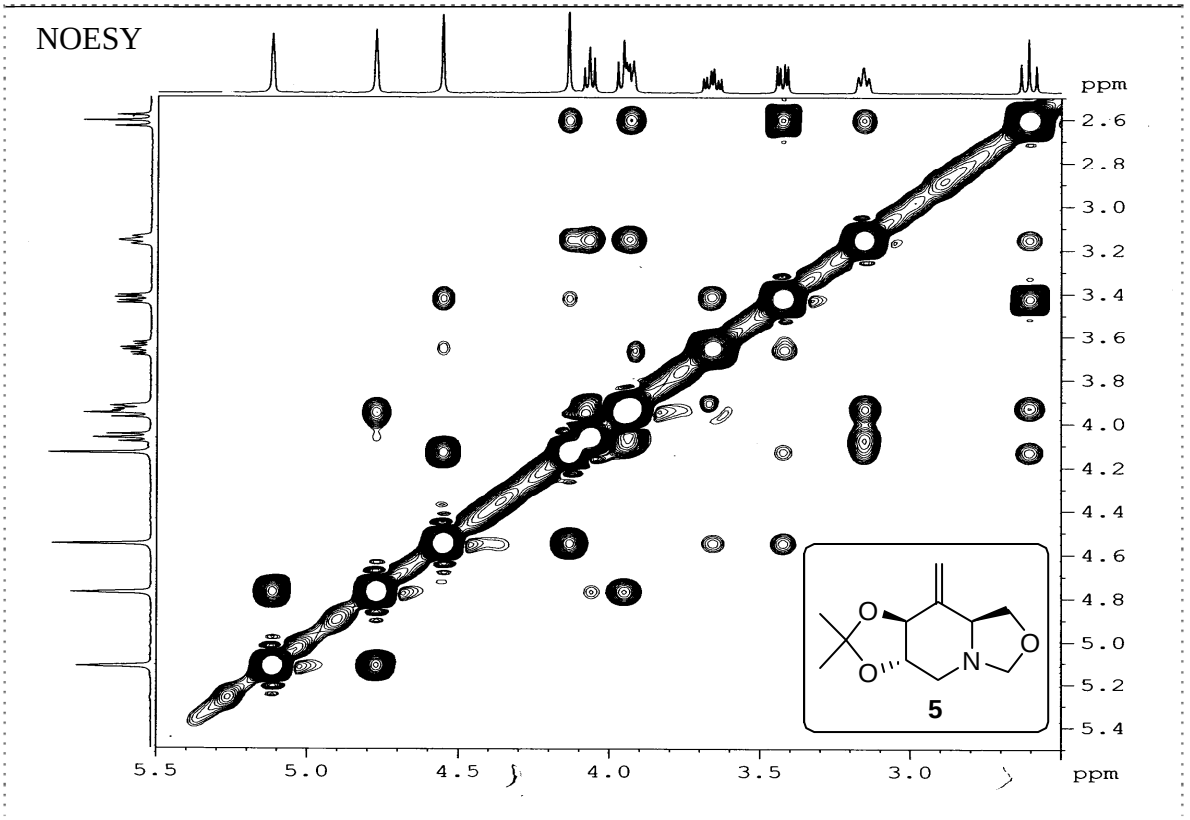
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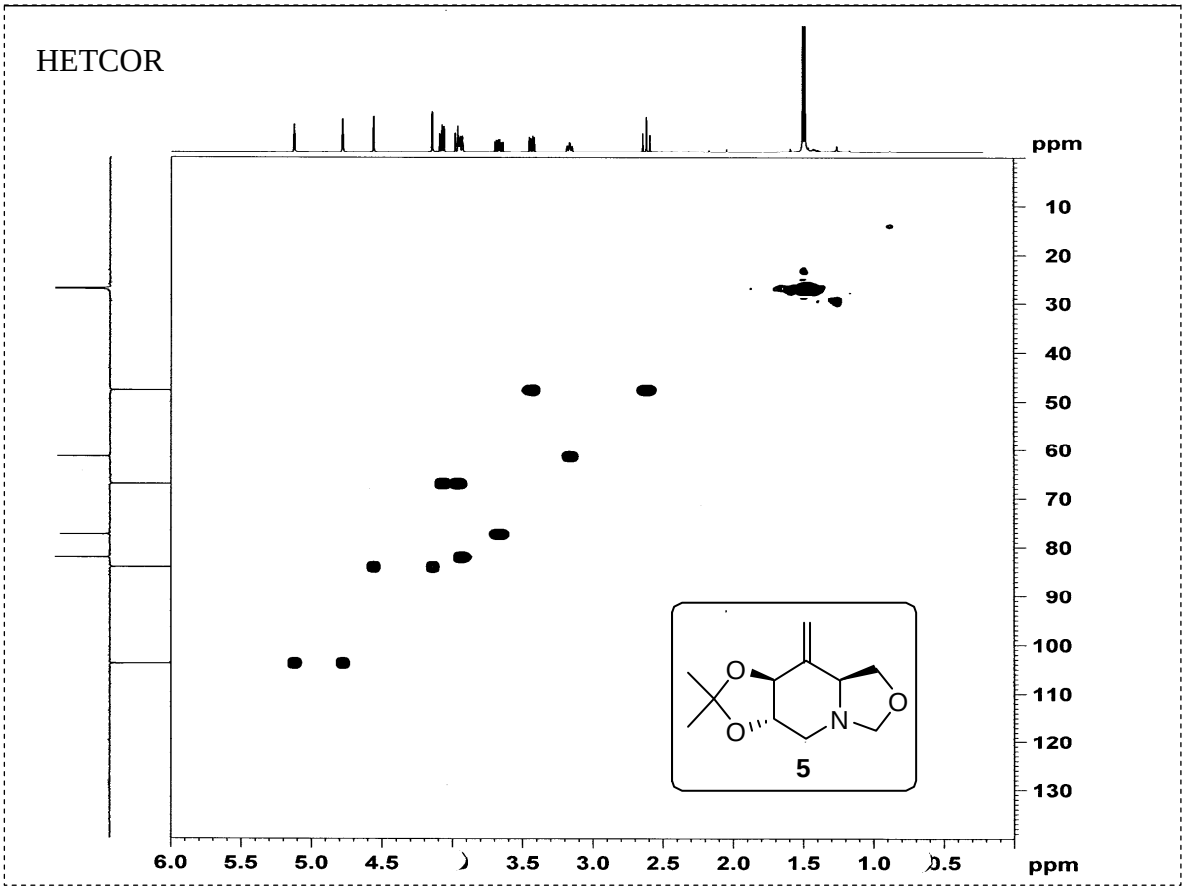
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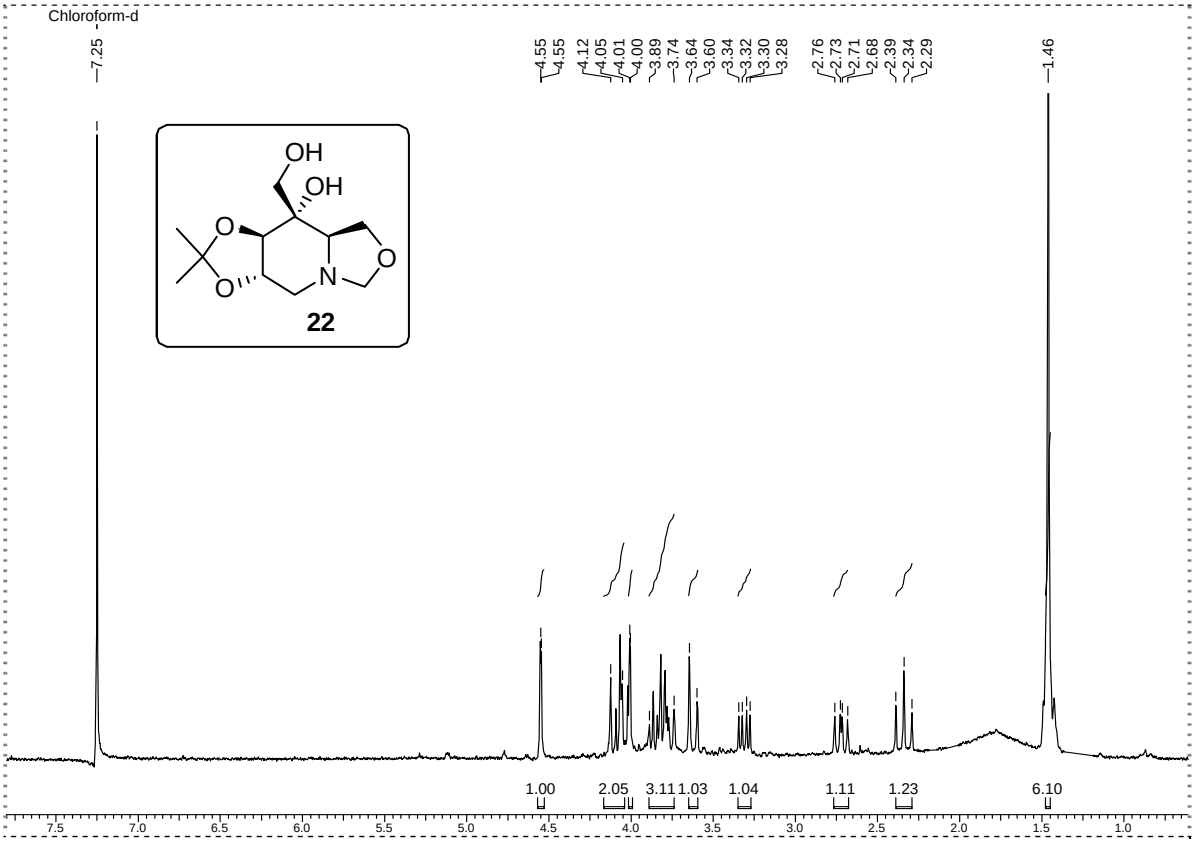
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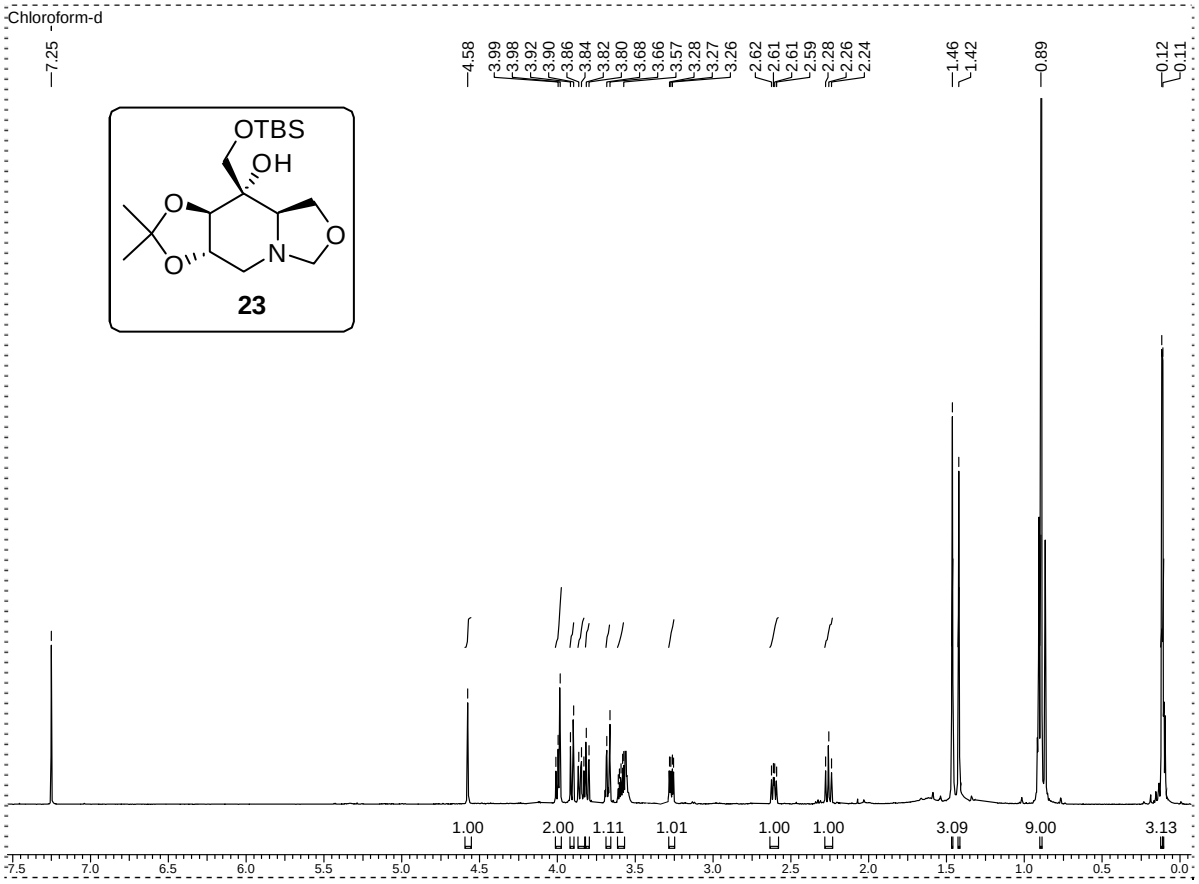
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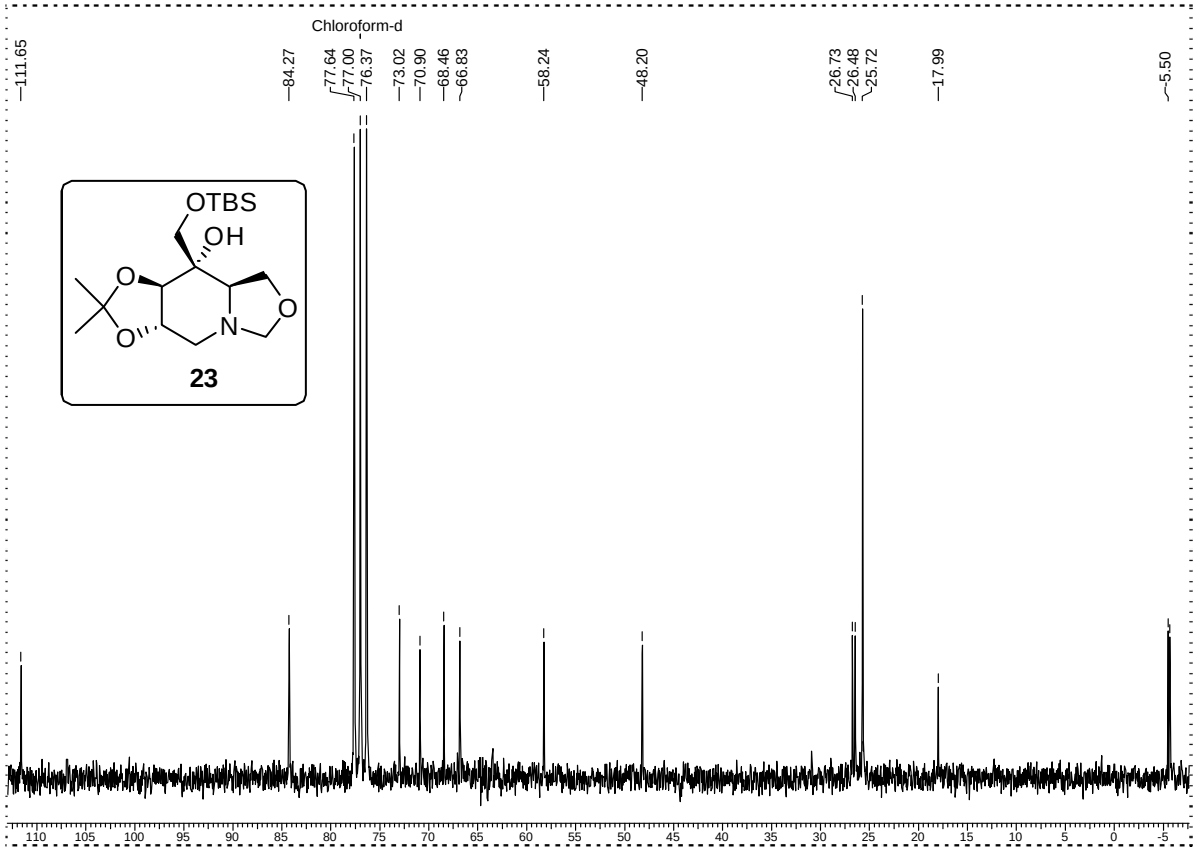
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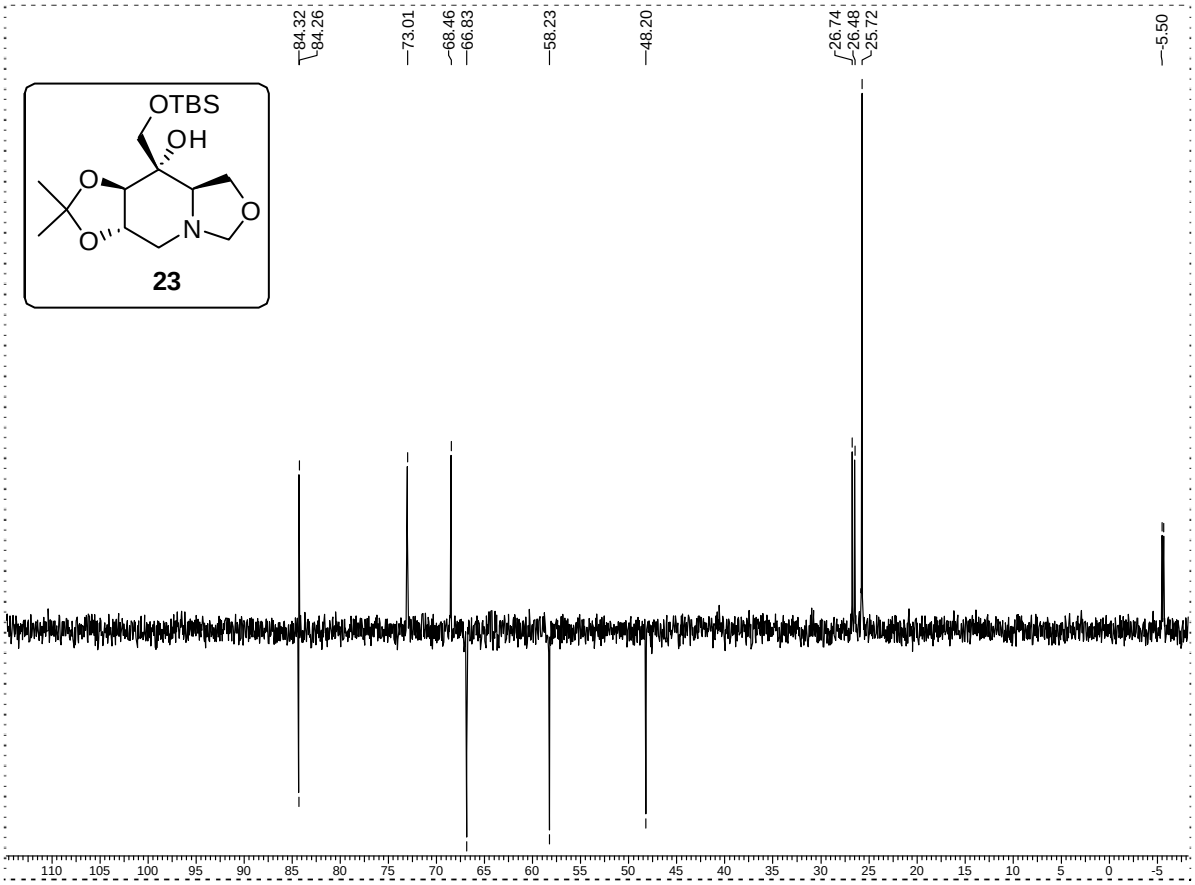
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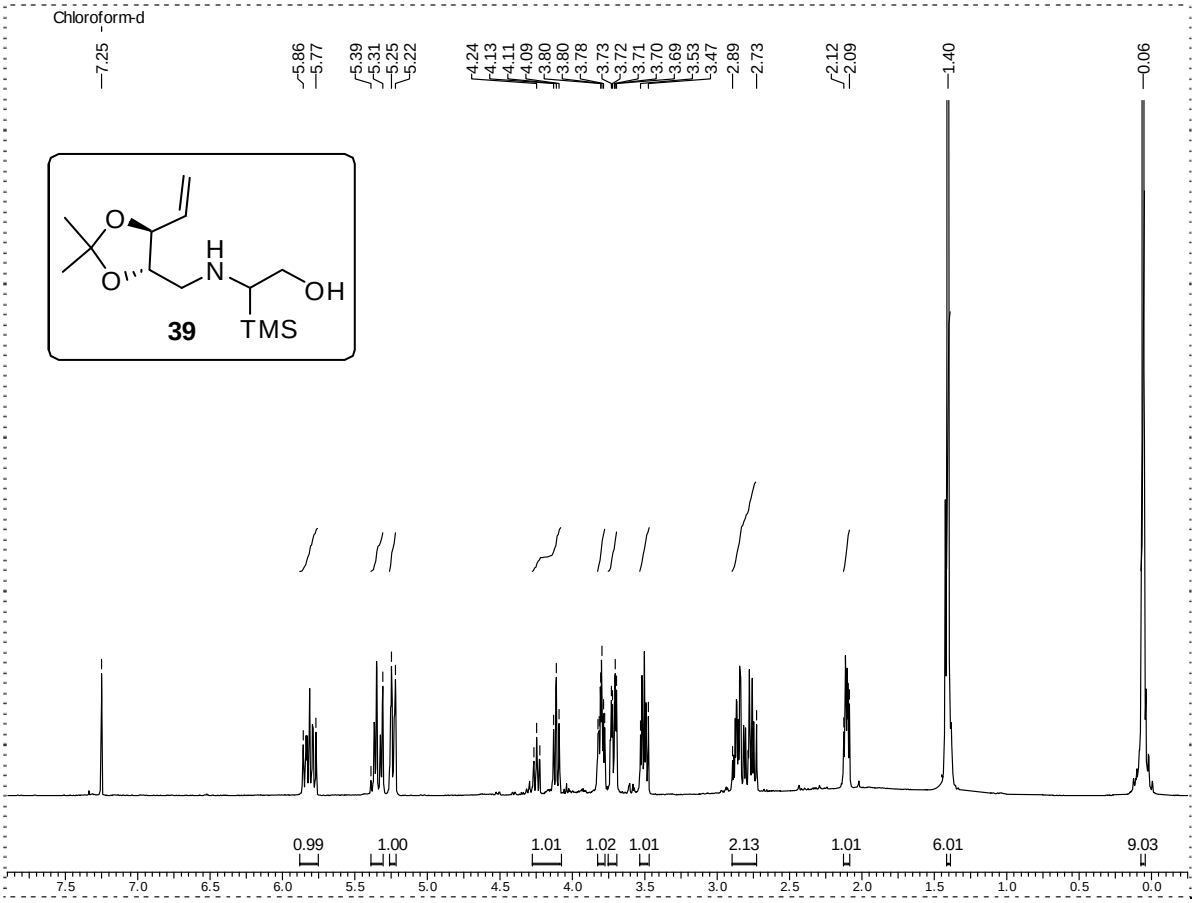
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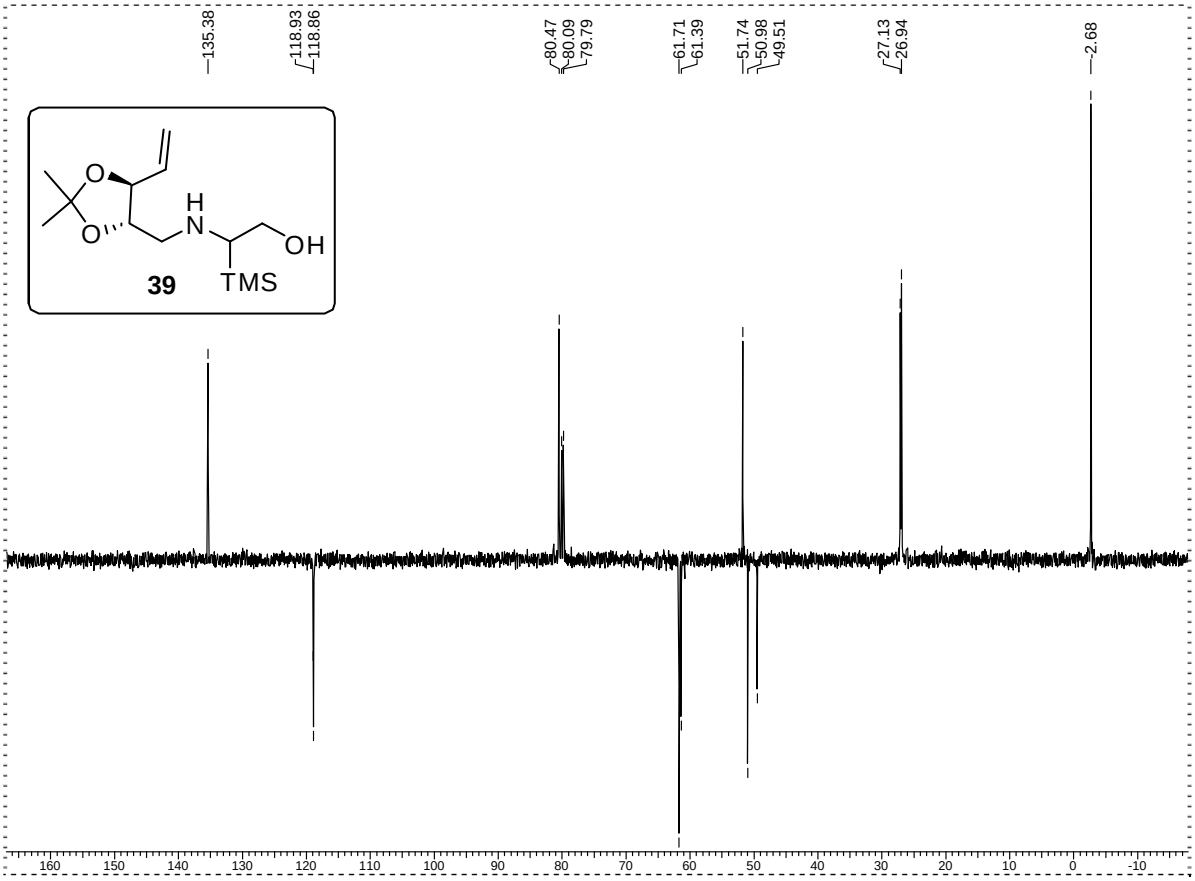
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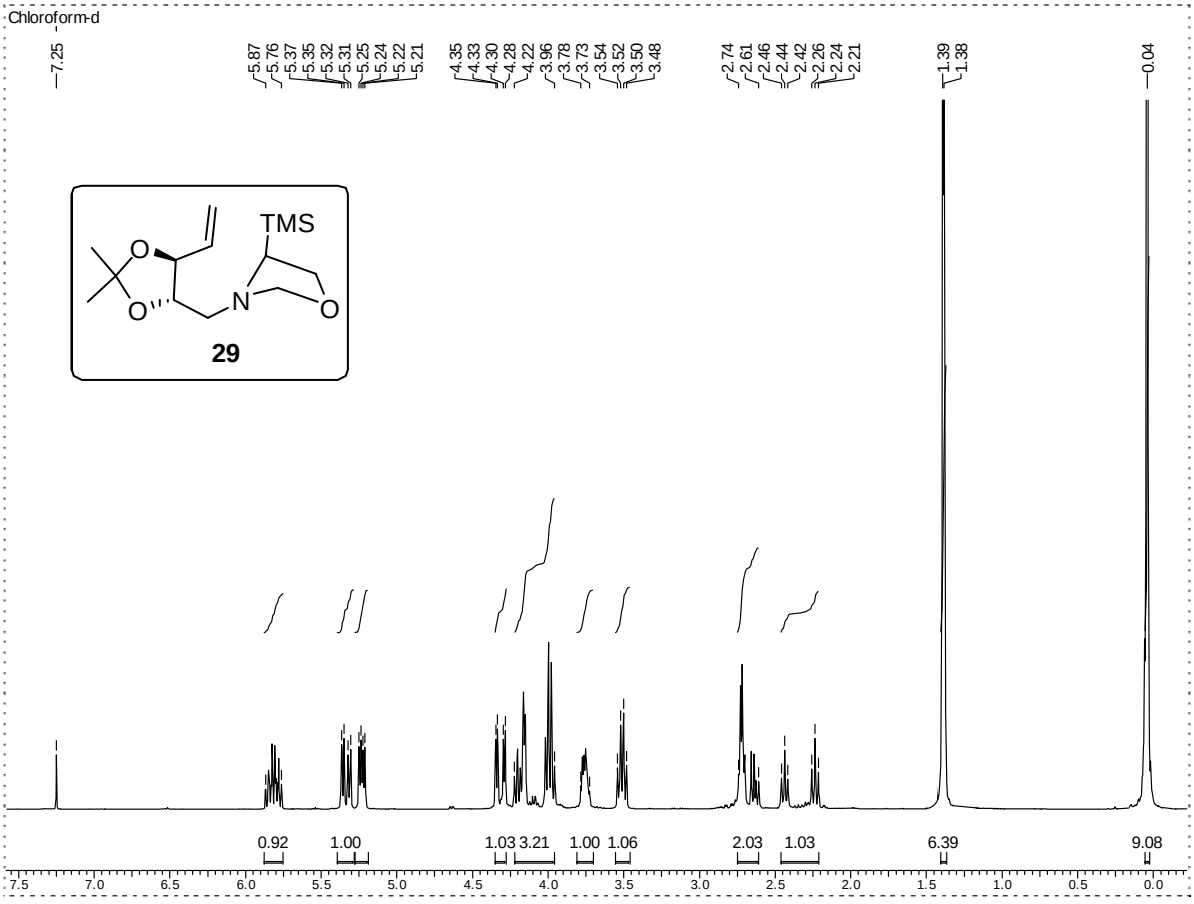
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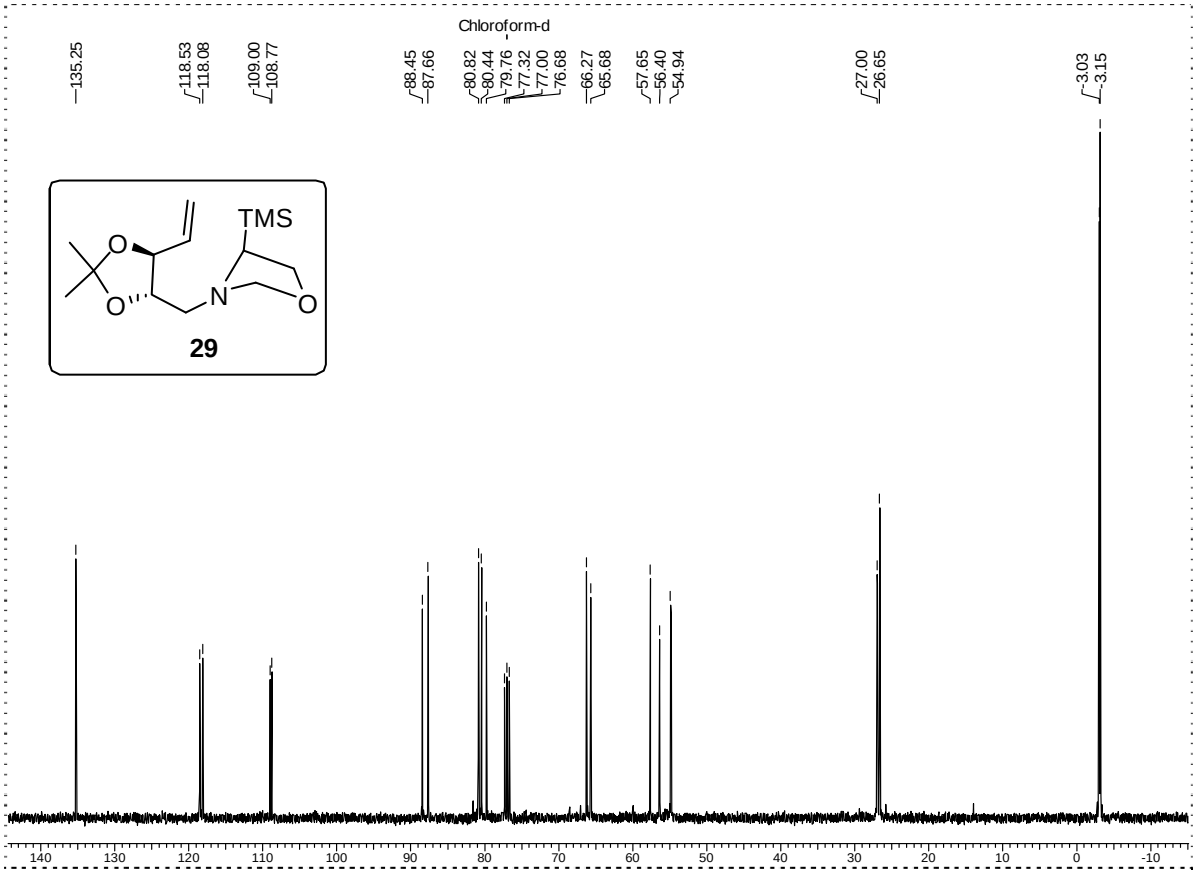
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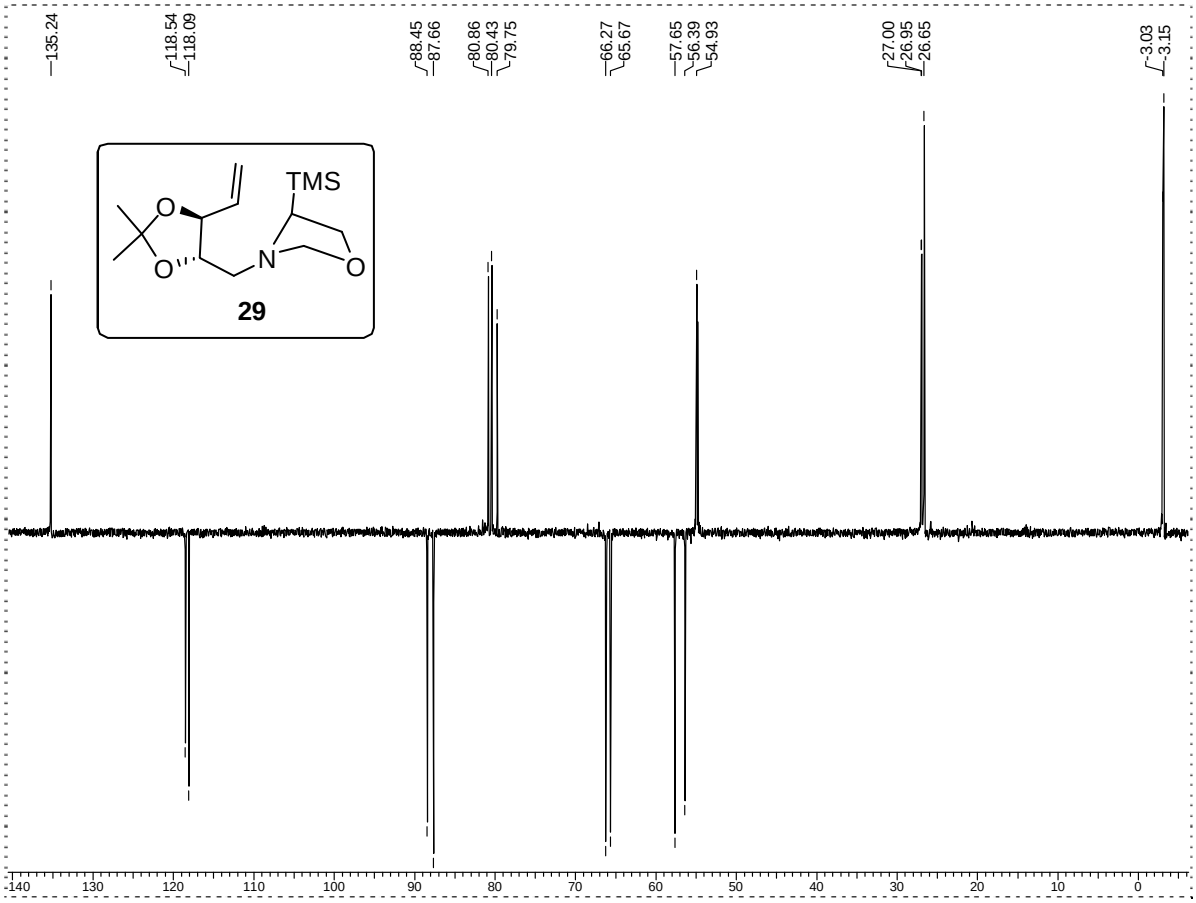
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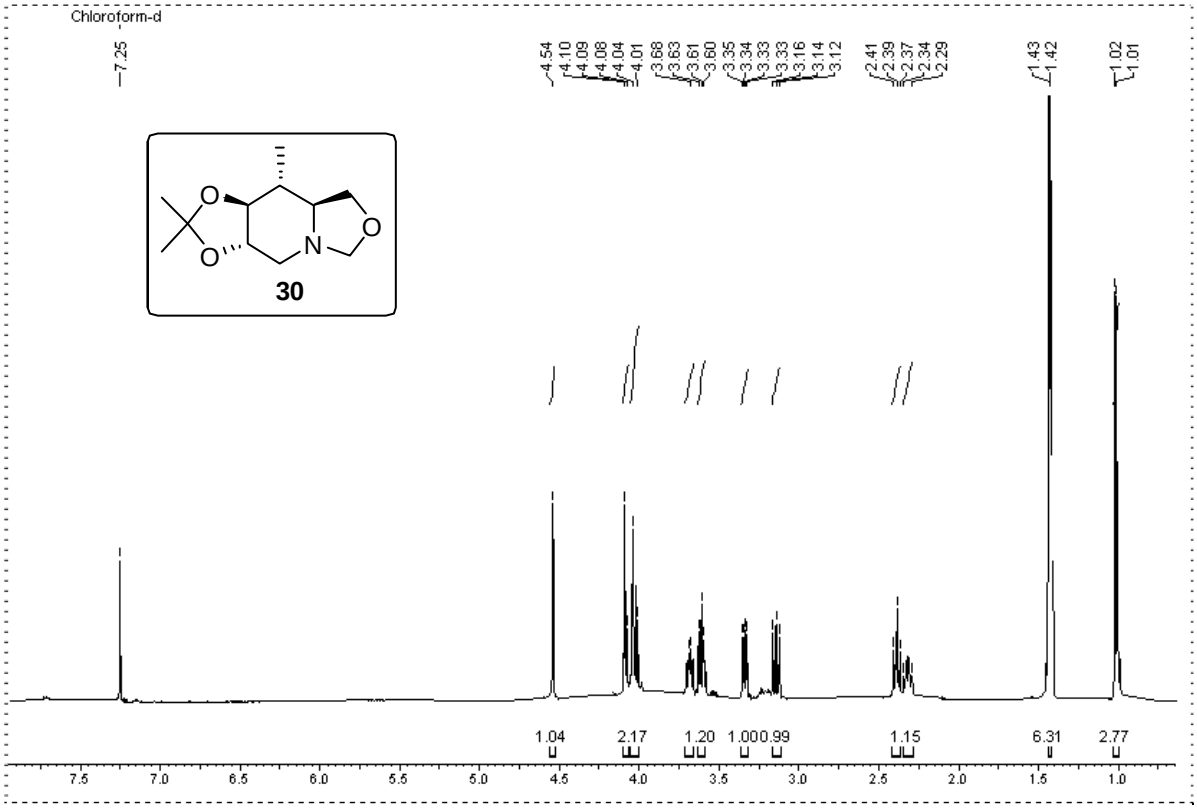
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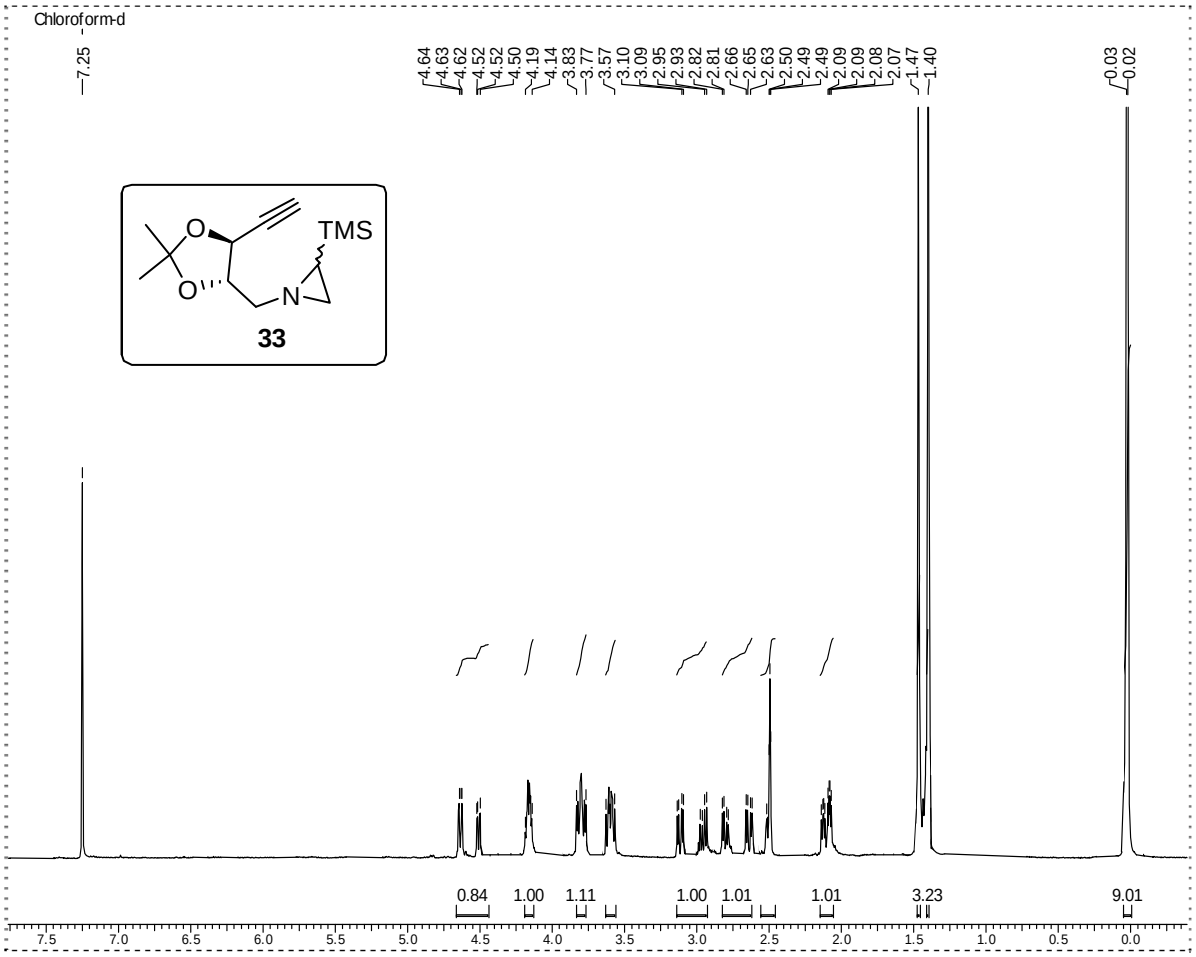
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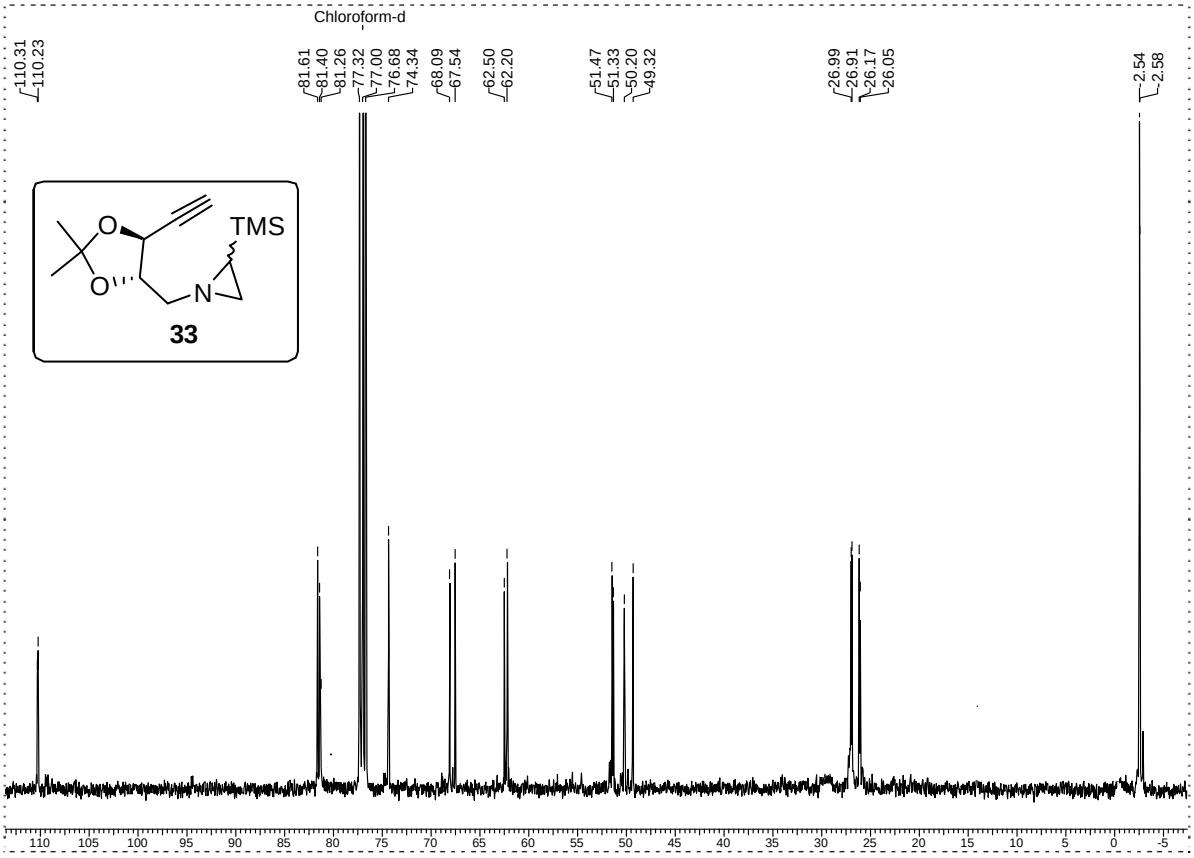
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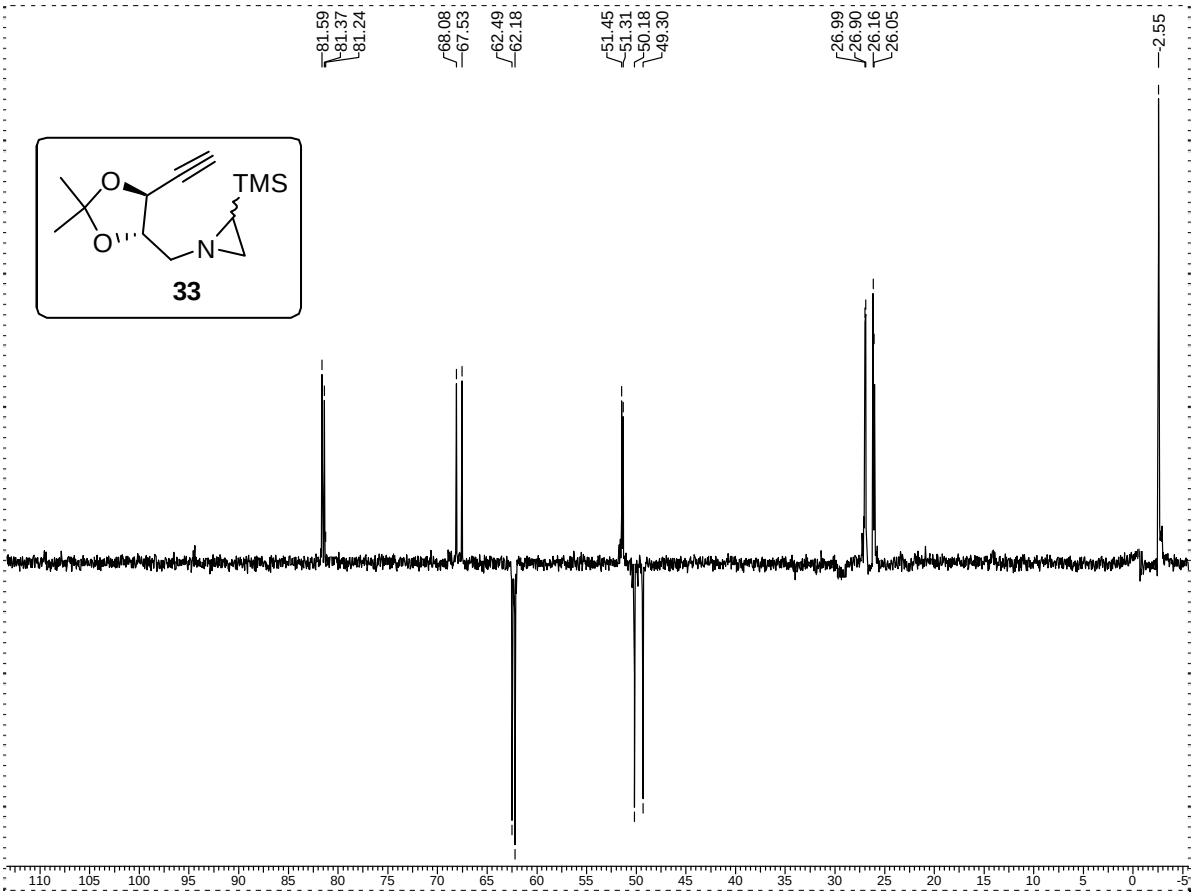
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O
N
33

Synthesis of polyfunctional quinolizidine alkaloids: development towards selective glycosidase inhibitors†

Ganesh Pandey,*[a] Debasish Grahacharya,[a] K. S. Shashidhara,[b] M. Islam Khan[b] and Vedavati G. Puranik[c]

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A highly divergent route to a variety of quinolizidine alkaloids is described. The enantiomeric precursors **22a** and **22b** utilized for the synthesis of these alkaloids were constructed stereospecifically from the PET cyclization of the corresponding acetylene tethered α-trimethylsilyl amine moieties **21a** and **21b**, respectively, both of which were synthesised from D-ribose. The polyhydroxy quinolizidine alkaloid **7** was found to be a selective inhibitor of α-galactosidase with *Ki* 83.9 μM. The amine analogs **18**, **12** and **10** are found to be selective and potent inhibitors of α-glucosidase with *Ki* 28, 120 and 140 μM, respectively.

Introduction

Polyhydroxy azabicyclic alkaloids like castanospermine[1] (**1**) and swainsonine[2] (**2**) have emerged as an important family of glycosidase inhibitors having chemotherapeutic potential[3] for a variety of diseases such as HIV,[4] cancer[5] and several viral infections such as influenza (Fig. 1).[6] Despite their therapeutic potential, these molecules have not had a full clinical evaluation, largely due to their low natural abundance and the difficulty in preparing a comprehensive array of variant structures. In addition, many of these molecules exhibit superfluous toxic effects *e.g.* castanospermine, although known to display potent inhibitory activity against glucosidases and antiviral properties against a number of viruses[6], is also found to inhibit intestinal sucrases causing osmic diarrhea[7] resulting in its withdrawal from use as a clinical therapeutic. Thus, there is a need to synthesize a palette of polyhydroxylated analogs of these molecules to allow a better understanding of the structural requirements for a glycosidase inhibitor and to develop more potent, selective and less toxic drugs.

As a result, considerable efforts have been directed towards the development of ring expansion analogs[8] and stereoisomers[9] of **1** and **2**. By contrast, examples involving ring expansion analogs of 1-deoxycastanospermine (**4**), which can also be visualized as a bicyclic analog of the potent glycosidase inhibitor 1-deoxynojirimycine (**3**), are scarce in literature.[10]

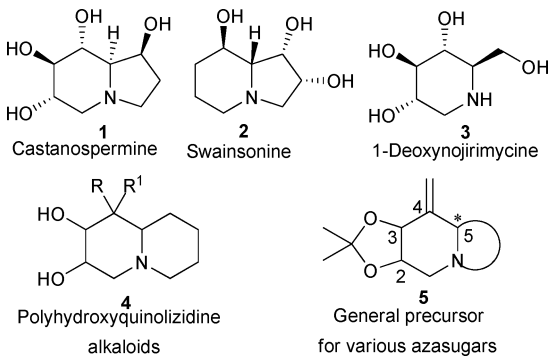

Fig. 1

In connection with this, intense research has also been done on changing the lipophilicity of **3** by attaching a hydrophobic group to its nitrogen atom, resulting in interesting inhibitory activities.[11] All these facts, coupled with our continuing interest in this area,[12] spurred us to synthesize azasugars of general structure **4**. The known synthetic efforts towards molecules of type **4** have involved a chiron approach with long reaction sequences producing a maximum of two analogs. In some cases, a key reaction produces two diastereomers which were further reacted to get different analogs indicating a difficulty in getting a single required product in an appreciable yield.[10b,c] Recently, we have demonstrated the construction of azabicyclic system **5** where the bridge head stereocentre is constructed in a stereospecific manner along with subsequent application to the syntheses of various classes of azasugars.[12e–g] Thus, we envisaged the synthesis of azasugars **6–12** from a common intermediate **22a** and similarly azasugars **13–18** from **22b**. Both **22a** and **22b** were traced back to a common starting material, D-ribose, through **21a** and **21b**, respectively (Scheme 1). We wish to report herein the synthesis of a variety of quinolizidine alkaloids having general structure **4** along with their enzyme inhibition study.

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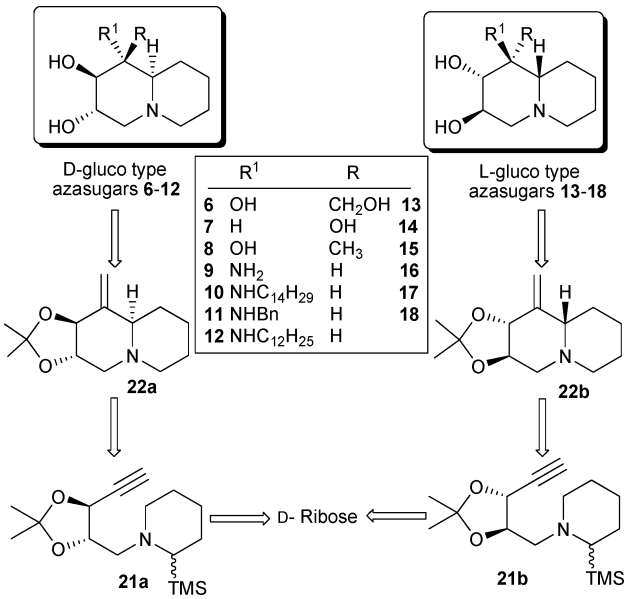
[b]*Division of Biochemical Sciences, National Chemical Laboratory, Pune, 411008, India*
[c]*Center for Material characterization, National Chemical Laboratory, Pune, 411008, India*

† Electronic supplementary information (ESI) available: Experimental and spectroscopic data of compounds **7**·HCl, **8**·HCl, **9**·2HCl, **10**·2HCl, **11**·2HCl, **12**·2HCl and **29**, X-ray crystal structure analysis of **23** and **25**, optical rotation data for all enantiomeric compounds, copies of ^{1}H and ^{13}C-NMR spectra for compounds **6–12**, **21a**, **22a** and **23–31**, COSY, NOESY, HETCOR spectra for **22a**, **23**, **25** and **28**, general procedure for the enzyme inhibition assay, Lineweaver–Burke plots for selected compounds. CCDC reference numbers 726211 and 726212. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b907007a

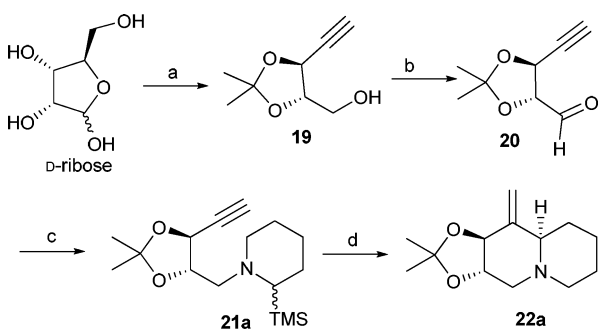


Scheme 1 Retrosynthetic plan.

Results and discussion

Stereospecific synthesis of azabicyclic template 22a

The synthesis of azasugars **6–12** began with the preparation of the key precursor **21a** (72% yield), which was achieved by the reductive amination[13] of aldehyde **20** in the presence of 2-(trimethylsilyl)piperidine.[14] The aldehyde **20** was obtained by the IBX oxidation[15] of **19** (85% yield) synthesized from D-ribose by following literature procedures.[12h,16] The photoinduced electron transfer (PET) mediated cyclization[17] of **21a** (3.38 mmol) was carried out by irradiating its dilute solution containing 1,4-dicyanonaphthalene (DCN) (0.67 mmol) in isopropanol (200 ml) in a pyrex vessel using a 450 W Honovia medium pressure lamp as the light source. Usual workup and purification of the photolysate by column chromatography gave **22a** as a single diastereomer in 65% yield (Scheme 2).



Scheme 2 *Reagents and conditions:* (a) Ref. 12h; (b) IBX, EtOAc, reflux, 8 h, 85%; (c) 2-(trimethylsilyl)piperidine, $NaBH(OAc)_3$, 1,2-dichloroethane (DCE), rt, 3 h, 72%; (d) hv, DCN, 2-PrOH, 1 h, 60%.

The cyclized product **22a** was fully characterized by extensive ^{1}H NMR, ^{13}C NMR, COSY, NOESY and HETCOR spectral analyses.

Transformation of 22a to various polyhydroxy quinolizidine alkaloids 6, 7 and 8

The olefinic moiety of **22a** upon dihydroxylation with OsO_4 in the presence of 50% aqueous NMO using acetone as a solvent produced **23** in 90% yield (crystalline solid, m.p. 135–136 °C) as a single diastereomer. X-Ray diffraction analysis unambiguously confirmed the relative stereochemistry of the newly generated stereocentre (Fig. 2).[18] Acetonide deprotection from **23** using 1 N HCl gave **6**·HCl in quantitative yield.

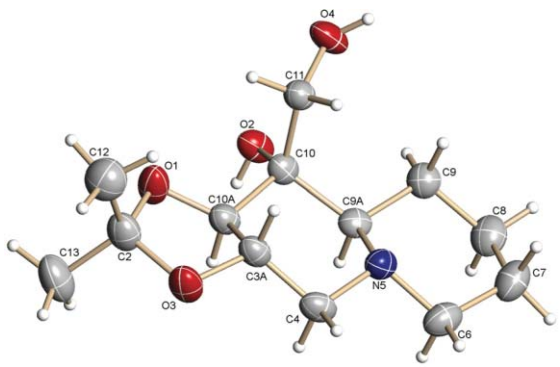


Fig. 2 ORTEP diagram of **23**.[18] Ellipsoids are drawn at 50% probability.

$NaIO_4$ cleavage of **23** afforded the corresponding ketone **24**, which on sodium borohydride reduction provided **25** in 82% yield as an exclusive diastereomer (Scheme 3). The stereochemistry of **25** was ascertained from its ^{1}H NMR coupling constants, in addition to COSY, NOESY and HETCOR spectral analyses. The relative stereochemical outcome was further confirmed by X-ray crystallography (Fig. 3).[18] The acetonide moiety was removed to give **7**·HCl in quantitative yield.

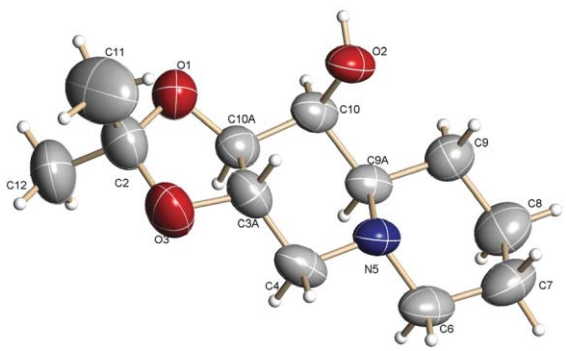
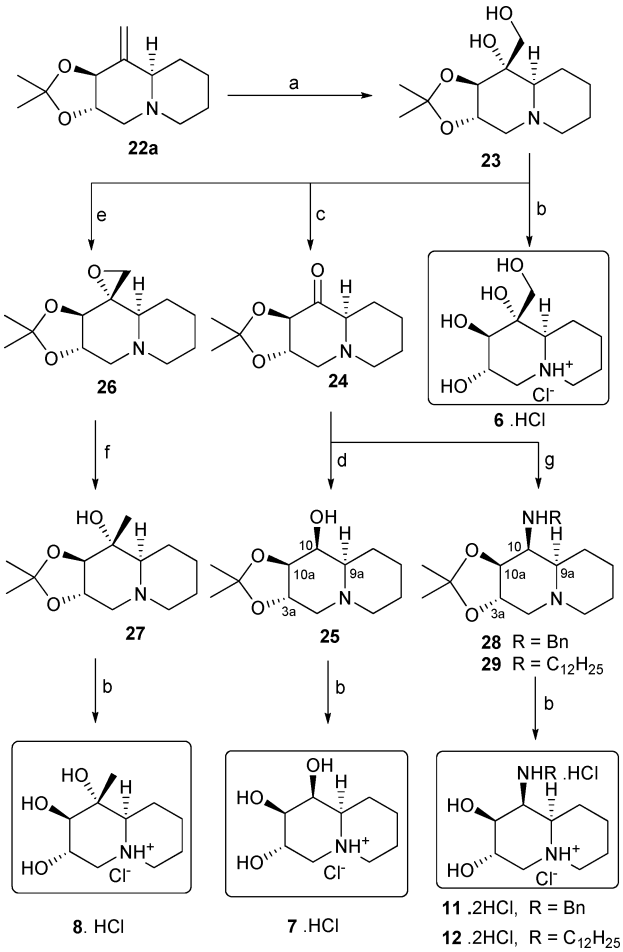


Fig. 3 ORTEP diagram of **25**.[18] Ellipsoids are drawn at 50% probability.

At this stage, we also synthesized 1-deoxy-8-methylhomocastanospermine (**8**) from **23** and studied its potential as a glycosidase inhibitor. In this context, the diol **23** was converted to the corresponding epoxide **26** (via its primary mesylate) in 90% yield using mesylchloride and triethylamine. The regioselective reductive opening of the epoxide ring using $LiAlH_4$ gave **27** as an exclusive product in 90% yield, which upon acetonide removal furnished **8**·HCl quantitatively.



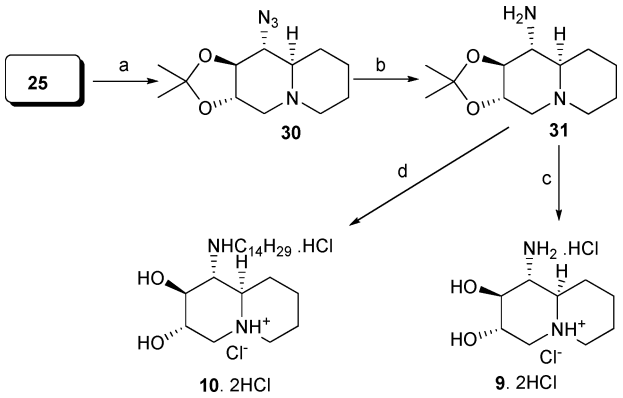
Scheme 3 *Reagents and conditions:* (a) OsO_4, NMO (50% aq. solution), acetone, rt, 12 h, 90%; (b) 1 N HCl, rt, 4 h, 100%; (c) $NaIO_4$, silica gel, DCM, 10 min; (d) $NaBH_4$, MeOH, rt, 6 h, 80% over two steps; (e) $MeSO_2Cl$, Et_3N, DCM, 0 °C to rt, 12 h, 90%; (f) $LiAlH_4$, THF, 12 h, 90%; (g) $NaB(OAc)_3H$, EDC, R NH_2, rt, 3 h, 65–75%.

Synthesis of amine analogs 9, 10, 11 and 12

At this juncture, we also visualized the creation of another class of azasugars with an additional basic site[12h,19] for binding to the enzyme. In this regard, a hydroxyl moiety at C-10 was converted to corresponding amine functionality. The mesylate obtained from alcohol **25** was directly subjected to S_N2 displacement with LiN_3 to produce azide **30** in 72% yield, which on catalytic hydrogenation over 10% Pd/C furnished the corresponding free amine in 85% yield. Removal of the acetonide group gave **9**·2 HCl in quantitative yield (Scheme 4).

Based on our previous research experiences regarding structure activity relationships among glycosidase inhibitors,[12e–h] we intended to increase the lipophilicity of amine functionalities by attaching a long hydrocarbon chain[20] and evaluating its role in enzyme inhibition. Towards this end, *N*-alkylation of **31** was carried out by refluxing with tetradecyl bromide in the presence of K_2CO_3 in CH_3CN–THF (3:1) to furnish the corresponding alkylated product in 65% yield which, upon acetonide removal, produced **10**·2 HCl quantitatively (Scheme 4).

Since it was not possible at this stage to predict which diastereomer would be the most biologically active, we felt that



Scheme 4 *Reagents and conditions:* (a) (i) $MeSO_2Cl$, Et_3N, DCM, 0 °C to rt, 6 h, (ii) LiN_3, DMF, 110 °C, 20 h, 72% over two steps; (b) H_2, 10% Pd/C, MeOH, 2 h, 85%; (d) 1 N HCl, rt, 4 h, 100%; (d) (i) $C_{14}H_{29}Br$, K_2CO_3, CH_3CN–THF (3:1), reflux, 6 h, 65%, (ii) 1 N HCl, rt, 4 h, 100%.

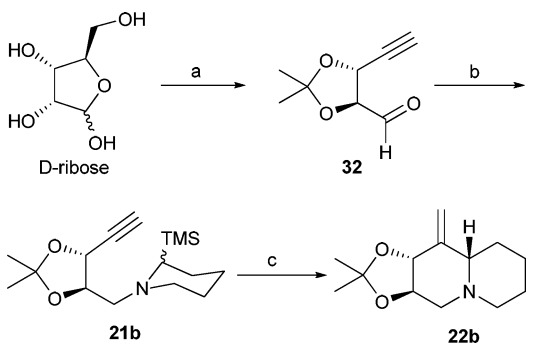
it would not be a disadvantage to make two other new analogs **11** and **12**. In this regard, ketone **24**, upon reductive amination with benzyl amine, furnished **28** as a single diastereomer in 75% yield.

The stereochemistry at C-10 of **28** was ascertained by analyzing coupling constants for 3a-H (δ_H 3.98, dt, J = 10.07, 4.10 Hz), 10a-H (δ_H 3.38, dd, J = 9.45, 3.63 Hz), 10-H (δ_H 3.04, t, J = 2.91 Hz) and 9a-H (δ_H 1.97, td, J = 11.14, 2.34 Hz) suggesting the orientations for 3a-H-axial, 10a-H-axial, 10-H-equatorial and 9a-H-axial, respectively. This stereochemical outcome was further confirmed from COSY, NOESY and HETCOR experiments. Amine **28** upon acetonide deprotection gave **11**·2 HCl in quantitative yield.

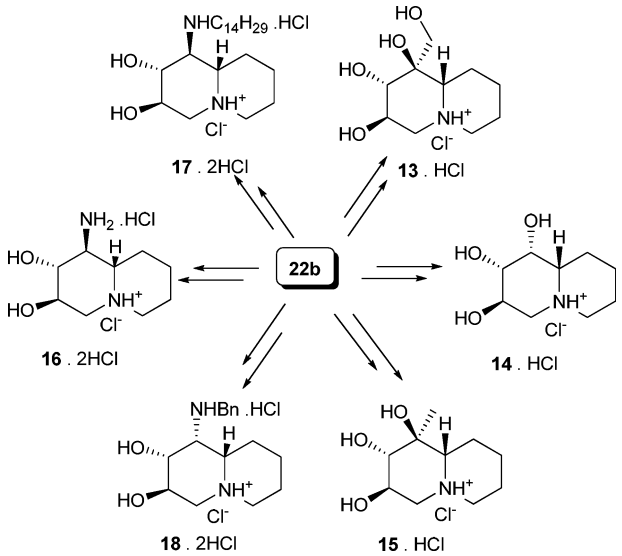
Similarly, **12**·2 HCl was obtained as a single diastereomer by reductive amination of keto **24** with dodecyl amine (65% yield) followed by acetonide removal (Scheme 3).

Synthesis of enantiomers 13–18. The syntheses of **13–18** were envisaged from synthon **22b** which in turn we planned to obtain from **21b** in a similar manner to that discussed in Scheme 2. Synthon **21b** was obtained by the reductive amination of aldehyde[12h] **32** with 2-(trimethylsilyl)piperidine[14] in 65% yield.

Following similar steps as described above, the enantiomeric template **22b** was obtained (Scheme 5), which was transformed to hydrochloride salts of **13–18** (Scheme 6) in a parallel manner as described for **6–12**.



Scheme 5 *Reagents and conditions:* (a) Ref. 12h; (b) 2-(trimethylsilyl)-piperidine, $NaBH(OAc)_3$, 1,2-dichloroethane, rt, 3 h; (c) hν, DCN, 2-PrOH, 1 h, 60%.



Scheme 6 Syntheses of **13–18**.

Enzyme inhibition studies

The enzyme inhibitory activities of all the final molecules **6–18** were tested against various enzymes and the results are summarized in Table 1. Compound **12** and **17** were found to be anomer specific inhibitors of α-mannosidase (*Ki* = 293 μM and 650 μM, respectively). The other compounds **9**, **11**, **14**, **16** and **18** were found to be anomer specific inhibitors of α-glucosidase with *Ki* values (in μM) of 675, 278, 450, 258 and 28, respectively.

Compound **18** in particular was found to be a very selective potent inhibitor of α-glucosidase (*Ki* = 28 μM) as it did not show any inhibition against other enzymes under investigation.

Among the polyhydroxy quinolizidine alkaloids, **7** was found to be a competitive inhibitor for α as well as β-galactosidase with *Ki* values 83.9 μM and 591 μM, respectively, whereas **14** was showing selective inhibitory activity for α-glucosidase, *Ki* = 450 μM. Barring these two cases, none of the polyhydroxy analogs exhibited any significant inhibitions towards the enzymes under study. Introduction of an additional amine functionality was found to be advantageous as all the amine analogs afforded better inhibitory activity against α-glucosidase in general compared with their polyhydroxy analogs. An increase in the inhibitory activity was successfully accomplished by increasing the lipophilicity. For example, **10** (*Ki* = 140 μM) was found to be 5 times more potent against α-glucosidase compared to its free amine counterpart **9** (*Ki* = 675 μM). This increased lipophilicity in compound **10** also exhibited better activity against β-glucosidase, α-mannosidase and β-mannosidase with *Ki* values (in μM) of 524, 805 and 830, respectively, in comparison to no inhibition shown by **9** against these enzymes up to 1 mM concentration. A similar observation was found in the case of alkylated amine **17** with respect to free amine **16**. To our pleasant surprise, the exploratory work to make another diastereomeric amine analog was also fruitful as **12** (*Ki* = 293 μM) was not only anomer specific for α-mannosidase but was also found to be an inhibitor 3 fold stronger than **10** (*Ki* = 805 μM).

Conclusions

In summary, we present a general synthetic route for a variety of enantiopure polyhydoxy quinolizidine alkaloids and some of their amine analogs. Thirteen new azasugars are synthesized and tested against six different enzymes. The simplicity of the steps involved in their syntheses make the route attractive for their preparation. As many of these compounds are selective inhibitors, efficient drug delivery may make them useful therapeutics. Moreover, the enzyme inhibitory study can provide an insight into structure activity relationships for the development of newer analogs as drugs. All the free and alkylated amine analogs produced promising activity, demanding more research in this area.

Experimental section

General experimental methods

Unless mentioned, all reactions were performed under an argon atmosphere. All commercially available reagents were used without further purification unless otherwise noted. Enzymes were purchased from commercial sources. Tetrahydrofuran was freshly distilled from benzophenone ketyl radical under argon prior to

Table 1 Inhibition of glycosidases (*Ki* in μM)[a]: a comparative study

Inhibitor[b]	Enzyme (source)					
	β-Gal. (*Aspergills oryzaie*)	α-Gal. (Green coffee beans)	β-Man. (Snail)	α-Man. (Jack Beans)	β-Glu. (Almond)	α-Glu. (Yeast)
6	33%[c]	NI	NI	11%[c]	NI	NI
7	*591*	*83.9*	NI	18%[c]	NI	NI
8	NI	NI	NI	22%[c]	NI	NI
9	NI	11%[c]	NI	NI	NI	*675*
10	NI	NI	*830*	*805*	*524*	*140*
11	NI	31%[c]	NI	NI	NI	*278*
12	NI	NI	NI	*293*	46%[c]	*120*
13	NI	NI	NI	NI	NI	42%[c]
14	28%[c]	13%[c]	18%[c]	NI	NI	*450*
15	NI	NI	14%[c]	29%[c]	NI	39%[c]
16	NI	NI	13%[c]	22%[c]	NI	*258*
17	NI	NI	NI	*650*	43%[c]	*235*
18	NI	NI	NI	NI	NI	*28*

[a] *Ki in* μM (in italics). [b] Hydrochloride salts of the compounds **6–18** were used for inhibitory activity tests. [c] Percent inhibition at 1 mM; NI, no inhibition up to 1 mM.

use. Column chromatography was performed with silica gel (100–200 and 230–400 mesh). The combined organic layers were dried over $NaSO_4$. Solvents were evaporated under reduced pressure. All yields given refer to isolated yields. Melting points are reported uncorrected. Optical rotations were measured on a precision automated polarimeter JASCO P-1030. NMR spectra were recorded on 200, 400, and 500 MHz spectrometers. Chemical shifts are reported in ppm. Coupling constants (J values) are reported in Hertz. ^{13}C peak multiplicity assignments were made based on DEPT data. IR spectra were recorded on a FT-IR spectrometer. MS experiments were performed on a low resolution magnetic sector mass spectrometer. GC analysis was performed on a Varian CP 3800 GC using a CP-Sil 5CB column. Microanalysis data were obtained using a Carlo-Erba CHNS-O EA 1108 Elemental Analyser. The optical density measurements were carried out on a Varian CARY-50 BIO UV-vis spectrophotometer. The crystal data for compounds **23** and **25** were collected at $T = 296$ K, on SMART APEX CCD Single Crystal X-ray diffractometer using Mo-Kα radiation ($\lambda = 0.7107$ Å) to a maximum θ range of 25.00°.

General procedure for the enzyme inhibition assays

Inhibition assays to determine the inhibitory potencies of the azasugars were carried out spectrophotometrically measuring the residual hydrolytic activities of the glycosidases on the corresponding *p*-nitrophenyl glycosides in the presence of the azasugars. The absorbance of the resulting solution was read at 405 nm.

1-(((4*S*,5*S*)-5-Ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-2-(trimethylsilyl)piperidine (21a). To a solution of crude aldehyde **20** (5.76 g, 31.82 mmol) (>85% pure by GC) in DCE (90 ml), was added 2-(trimethylsilyl)piperidine (5.24 g, 33.41 mmol) and $NaB(OAc)_3H$ (8.77 g, 41.36 mmol) under argon atmosphere and the mixture was stirred for 3 h. The reaction mixture was cooled in an ice bath and quenched by adding 2 N NaOH until the aqueous layer was basic. After stirring for 0.5 h, the reaction mixture was extracted in DCM. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (pet ether/ethyl acetate, 6:1) to furnish **21a** (6.77 g, 72%). $[\alpha]_D^{26} = -17.20$ (*c* 1.25, $CHCl_3$). Anal. Calcd. for $C_{16}H_{29}NO_2Si$: C, 65.03; H, 9.89; N, 4.74; Si, 9.50; Found: C, 65.12; H, 9.86; N, 4.76, Si, 9.44%. IR ν_{max} cm^{-1} in $CHCl_3$ 3311 (≡C–H), 2933, 2120 (C≡C), 1441, 1381, 1250, 1055. δ_H (400 MHz, $CDCl_3$) 0.6, 0.7 (4.5 H each, 2 s, $Si(CH_3)_3$), 1.21–1.29 (1H, m, 4ax-H), 1.40, 1.41, 1.46 1.46 (1.5H each, 4 s, $C(CH_3)_2$), 1.50–1.63 (4H, m, 3ax-H, 3eq-H, 4eq-H, 5ax-H), 1.71 (1H, dt, $^2J_{H,H}$= 12.51, $^3J_{5eq,6ax}$ = 3.76, 5eq-H), 1.87 (1H, dt, $^3J_{2ax,3ax} = 11.54$, $^3J_{2ax,3eq} = 3.76$, 2ax-H), 2.03–2.14 (1H, m, 6ax-H), 2.40–2.46 (1H, m, NCH_2CH), 2.51 (1H, t, $^4J_{H,H} = 2.14$, C≡C*H*), 2.92–2.97 (1H, m, 6eq-H), 3.12–3.18 (1H, m, NCH_2CH), 4.22–4.32 (2H, m, H-4 dioxolane, H-5 dioxolane). δ_C (50 MHz, $CDCl_3$) −1.1, −1.0 ($Si(CH_3)_3$), 23.8, 24.2 (C-4), 25.7 (C-5), 26.0, 26.09 ($C(CH_3)_2$), 26.03, 26.6 (C-3), 27.06, 27.11 ($C(CH_3)_2$), 54.7, 55.0 (NCH_2CH), 56.0, 56.1 (C-2), 58.1, 58.2 (C-6), 68.6, 68.8 (C-5, dioxolane), 74.4, 74.5 (C≡CH), 80.3, 80.9 (C-4, dioxolane), 81.0, 81.1 (*C*≡CH), 110.5, 110.7 ($C(CH_3)_2$). Mass (ESI): *m*/*z* 296 (M^+ + H).

(3a*S*,9a*R*,10a*S*)-2,2-Dimethyl-10-methyleneoctahydro-3aH-[1,3]dioxolo[4,5-b]quinolizine (22a). A solution containing **21a** (1.0 g, 3.38 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 1 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, 6:1) to afford cyclized product **22a** (0.453 g, 60%) as a yellow liquid. $[\alpha]_D^{29} = +55.80$ (*c* 0.85, CH_2Cl_2). Anal. calcd. for $C_{13}H_{21}NO_2$: C, 69.92; H, 9.48; N, 6.27; Found: C, 69.98; H, 9.51; N, 6.25%. IR (neat) ν_{max} cm^{-1} 2985(=C–H), 2858, 1806, 1667(C=C), 1370, 1226. δ_H (400 MHz, $CDCl_3$) 1.24–1.33 (1H, m, 8ax-H), 1.44, 1.47 (3H each, 2 s, $C(CH_3)_2$), 1.48–1.65 (3H, m, 7ax-H, 7eq-H, 9ax-H), 1.82–1.92 (2H, m, 8eq-H, 9eq-H), 2.27 (1H, dt, $^3J_{6ax,7ax} = 11.79$, $^3J_{6ax,7eq} = 3.01$, 6ax-H), 2.33 (1H, d, $^3J_{9ax,9a\text{-}ax} = 11.70$, 9a-ax-H), 2.38 (1H, t, $^3J_{4ax,3ax} = 10.29$, 4ax-H), 2.93 (1H, d, $^2J_{H,H} = 11.54$, 6eq-H), 3.23 (1H, dd, $J_{4eq,3ax}$ = 3.97, 4eq-H), 3.48 (1H, dt, $^3J_{3a\text{-}ax,10a\text{-}ax} = 10.02$, 3a-ax-H), 3.74 (1H, dt, $^4J_{H,H} = 1.79$, 10a-ax-H), 4.89, 5.06 (1H each, 2 s, C=CH_2). δ_C (100 MHz, $CDCl_3$) 24.0 (C-8), 25.7 (C-7), 26.7, 26.9 $C(CH_3)_2$, 27.8 (C-9), 56.8 (C-6), 57.7 (C-4), 61.9 (C-9a), 77.1 (C-3a), 82.2 (C10a), 103.2 (C=CH_2), 111.0 ($C(CH_3)_2$), 144.2 (*C*=CH_2). Mass (ESI): *m*/*z* 224 (M^+ + H).

(3a*S*,9a*R*,10S,10a*R*)-10-(Hydroxymethyl)-2,2-dimethyloctahydro-3aH-[1,3]dioxolo[4,5-b]quinolizin-10-ol (23). To a solution of **22a** (0.45 g, 2.02 mmol) in acetone (5 ml) was added *N*-methylmorpholine-*N*-oxide (50% aq solution, 1.41 g, 6.06 mmol). The reaction mixture was cooled to 0 °C and to that was added a catalytic amount of osmium tetroxide (1 mL of 1% solution of OsO_4 in *t*-BuOH). The reaction mixture was allowed to come to rt and stirred for 10 h. Solid Na_2SO_3 was added to this reaction mixture. Stirring was continued for 30 min to quench excess *N*-methylmorpholine-*N*-oxide and OsO_4. The mixture was filtered through a short pad of celite and the solvent was evaporated off. The crude reaction mixture upon column chromatography (silica, pet ether–ethyl acetate, 3:2) afforded **23** (0.466 g, 90%) as a colorless solid (mp. 135–136 °C (from ethyl acetate/hexanes)). $[\alpha]_D^{29} = +27.7$(*c* 1.2, DCM). Anal. Calcd. for $C_{13}H_{23}NO_4$: C, 60.68; H, 9.01; N, 5.44; Found: C, 60.51; H, 9.05; N, 5.45%. IR ν_{max} cm^{-1} in $CHCl_3$ 3432 (OH), 2938, 2306, 2232, 1654, 1265. δ_H (400 MHz, $CDCl_3$, D_2O exchange) 1.13–1.19 (2H, m, 8ax-H, 9ax-H), 1.44 (3H, s, $C(CH_3)_2$), 1.44 (4H, apparent s, $C(CH_3)_2$, 7ax-H), 1.59–1.62 (1H, m, 7eq-H), 1.79–1.80 (1H, m, 8eq-H), 1.93–1.95 (1H, m, 9a-ax-H), 2.01–2.03 (1H, m, 9eq-H), 2.13 (1H, dt, $^3J_{9ax,8ax}$ = 12.55,$^3J_{9ax,8eq} = 2.76$, 9ax-H), 2.21 (1H, t, $^3J_{4ax,3a\text{-}ax} = 10.04$, 4ax-H), 2.92 (1H, d, $^2J_{H,H} = 11.50$, 6eq-H), 3.14 (1H, dd, $^2J_{H,H} = 9.88$, $^3J_{4eq,3a\text{-}ax} = 4.28$, 4eq-H), 3.43 (1H, d, $^3J_{10a\text{-}ax,3a\text{-}ax} = 9.54$, 10a-ax-H), 3.73 (1H, d, $^2J_{H,H} = 11.54$,CH_2OH), 3.80 (1H, dt, 3a-ax-H), 3.97 (1H, d, CH_2OH). δ_C (50 MHz, $CDCl_3$) 24.2 (C-8), 24.9 (C-9), 25.7 (C-7), 26.6 $C(CH_3)_2$, 56.7 (C-4), 57.9 (C-6), 62.9 (CH_2OH), 68.4 (C-9a), 71.9 (C-3a), 72.4 (C-10), 86.9 (C-10a), 110.6 $C(CH_3)_2$. Mass (ESI): *m*/*z* 257 (M^+ + H), 280 (M^+ + Na).

(1*S*,2*R*,3*S*,9a*R*)-1,2,3-Trihydroxy-1-(hydroxymethyl) decahydroquinolizinium chloride (6·HCl). Compound **23a** (0.025 g, 0.097 mmol) was subjected to acetonide deprotection using aqueous 1 N HCl (1 ml) to provide the hydrochloride salt of **6** quantitatively. $[\alpha]_D^{23} = +24.1$ (*c* 0.95, MeOH). Anal. Calcd. for

$C_{10}H_{20}ClNO_4$: C, 47.34; H, 7.95; N, 5.52; Found: C, 47.44; H, 7.99; N, 5.63%. δ_H (400 MHz, D_2O) 1.36–1.47 (1H, m, 8ax-H), 1.53–1.70 (2H, m, 7ax-H, 9ax-H), 1.82–1.90 (2H, m, 7eq-H, 8eq-H), 2.18 (1H, dt, $^2J_{H,H}$ = 14.38, $^3J_{9eq,8ax}$ = 2.81, 9eq-H), 2.82–2.90 (2H, m, 4ax-H, 6ax-H), 3.01 (1H, dd, $^3J_{9a\text{-}ax,9ax}$ = 12.28, $^3J_{9a\text{-}ax,9eq}$ = 2.03, 9a-ax-H), 3.36–3.41 (2H, m, 4eq-H, 6eq-H), 3.47, 3.52 (1H each, d, $^2J_{H,H}$ = 10.55, CH_2OH), 3.98 (1H, d, $^3J_{2ax,3ax}$ = 10.80, 2ax-H), 4.17 (1H, ddd, $^3J_{4ax,3ax}$ = 10.25, $^3J_{4eq,3ax}$ = 5.30, 3ax-H). δ_C (100 MHz, D_2O) 21.3, 22.9, 23.0 (C-7, C-8, C-9), 56.3, 56.8 (C-4, C-6), 58.7 (CH_2-OH), 64.2 (C-3), 67.9 (C-9a), 72.6 (C-1), 78.0 (C-2). Mass (ESI): *m/z* 218 (M^+ + H).

(3a*S*,9a*R*,10*S*,10a*S*)-2,2-Dimethyloctahydro-3aH-[1,3]dioxolo-[4,5-b]quinolizin-10-ol (25). A solution of **23** (0.45 g, 1.75 mmol) in DCM (5 ml) was added to a suspension of silica gel supported sodium periodate [prepared by dissolving $NaIO_4$ (0.53 g, 2.62 mmol) in 1 mL water and 2.77 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 × 10 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. To the solution of that crude ketone (**24**) (0.35 g, 1.55 mmol) in methanol (5 ml) was added $NaBH_4$ (0.118 g, 3.10 mmol). The resulting mixture was stirred at rt for 6 h and then quenched by adding an excess of the saturated solution of NaCl. This brownish suspension was stirred overnight and extracted with ethyl acetate (4 × 5 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, 1:9) to afford **25** (0.282 g, 80%) as a colorless solid (mp 133–134 °C (from ethyl acetate/hexanes)). $[\alpha]_D^{29}$ = +16.77 (*c* 1.2, DCM). Anal. Calcd. for $C_{12}H_{21}NO_3$: C, 63.41; H, 9.31; N, 6.16; Found: C, 63.44; H, 9.29; N, 6.19%. IR ν_{max} cm^{-1} in $CHCl_3$ 3436 (OH), 2940, 1653, 1265. δ_H (400 MHz, $CDCl_3$, D_2O exchange) 1.23–1.29 (1H, m, 8ax-H), 1.43, 1.43 (3H each, 2 s, C(CH_3)$_2$, 1.56–1.58 (3H, m, 7ax-H, 7eq-H, 9ax-H), 1.77–1.80 (2H, m, 8eq-H, 9eq-H), 1.98 (1H, d, $^3J_{9ax,9a\text{-}ax}$ = 11.04, 9a-ax-H), 2.14–2.23 (2H, m, 6ax-H, 4ax-H), 2.92 (1H, d, $^2J_{H,H}$ = 11.04, 6eq-H), 3.17 (1H, dd, $^2J_{H,H}$ = 9.79, $^3J_{4eq,3a\text{-}ax}$ = 4.02, 4eq-H), 3.29 (1H, dd, $^3J_{3a\text{-}ax,10a\text{-}ax}$ = 9.28, $^3J_{10eq,10a\text{-}ax}$ = 2.51, 10a-ax-H), 3.96 (1H, t, 10-H), 4.00 (1H, dt, 3a-ax-H). δ_C (100 MHz, $CDCl_3$) 24.1 (C-8), 25.4 (C-7), 26.5, 26.8 C(CH_3)$_2$, 28.4 (C-9), 56.4 (C-6), 57.8 (C-4), 63.8 (C-9a), 69.2 (C-10), 70.2 (C-3a), 82.1 (C-10a), 110.2 (*C*(CH_3)$_2$). Mass (ESI): *m/z* 228 (M^+ + H), 250 (M^+ + Na).

(2′*S*,3a*S*,9a*R*,10a*R*)-2,2-Dimethyloctahydrospiro[[1,3]dioxolo-[4,5-b]quinolizine-10,2′-oxirane] (26). To a solution of **23** (0.2 g, 0.777 mmol) in dry DCM (3 ml) at 0 °C under argon atmosphere was added triethyl amine (0.22 ml, 1.554 mmol) and methanesulfonyl chloride (0.066 mL, 0.855 mmol). The reaction mixture was stirred at room temperature for 12 h; water was added and extracted with DCM (2 × 5 ml). The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified by column chromatography (silica, pet. ether/ethyl acetate, 4:1) to get **26** (0.168 g, 90%) as a white solid. $[\alpha]_D^{26}$ = +49.16 (*c* 1.05, $CHCl_3$). Anal. Calcd. for $C_{13}H_{21}NO_3$: C, 65.25; H, 8.84; N, 5.85 Found: C, 65.34; H, 8.87; N, 5.83%. IR ν_{max} cm^{-1} in $CHCl_3$ 3054 (C–O epoxide), 1421, 1265. δ_H (500 MHz, $CDCl_3$) 1.01 (1H, ddd, $^2J_{H,H}$ = 15.96, $^3J_{8ax,9ax}$ 3 = 12.66, $^3J_{7eq,8ax}$ = 3.31, 8ax-H), 1.18 (1H, tq, $^3J_{9ax,9a\text{-}ax}$ = 12.9, $^3J_{8eq,9ax}$ = 3.58, 9ax), 1.39, 1.43 (3H each, 2 s, C(CH_3)$_2$), 1.50 (1H, tq, $^3J_{7ax,8ax}$ = 12.93, $^3J_{6eq,7ax}$ = 3.85, 7ax), 1.63–1.69 (2H, m, 7eq, 8eq), 1.77 (1H, d, $^2J_{H,H}$ = 13.2, 9eq), 2.26 (1H, dt, $^3J_{6ax,7ax}$ = 11.83, $^3J_{6ax,7eq}$ = 2.48, 6ax-H), 2.31 (1H, dd, $^3J_{9ax,9a\text{-}ax}$ = 11.22, $^3J_{9eq,9a\text{-}ax}$ = 1.54, 9a-ax-H), 2.37 (1H, t, $^3J_{4ax,3a\text{-}ax}$ = 10.18, 4ax-H), 2.92 (1H, d, $^2J_{H,H}$ = 4.67, 3′-H), 2.99 (1H, br d, $^2J_{H,H}$ = 11.56, 6eq-H), 3.03 (1H, d, 3′-H), 3.23 (1H, dd, $^2J_{H,H}$ = 9.90, $^3J_{4eq,\ 3a\text{-}ax}$ = 3.85, 4eq-H), 3.63 (1H, d, $^3J_{10a\text{-}ax,3a\text{-}ax}$ = 9.08, 10a-H), 3.72 (1H, dt, 3a-ax-H). δ_C (50 MHz, $CDCl_3$) 23.6 (C-8), 24.6 (C-7), 25.8 (C-9), 26.5, 26.7 (C(CH_3)$_2$), 45.2 (C-3′), 56.7 (C-6), 57.2 (C-4), 60.3 (C-2′), 62.3 (C-9a), 75.2 (C-3a), 78.4 (C-10a), 111.5 (*C*(CH_3)$_2$). Mass (ESI): *m/z* 240 (M^+ + H), 262 (M^+ + Na).

(3a*S*,9a*R*,10*R*,10a*R*)-2,2,10-Trimethyloctahydro-3aH-[1,3]dioxolo[4,5-b]quinolizin-10-ol (27). To a solution of **26** (0.1 g, 0.42 mmol) in dry THF (2 ml) at 0 °C was added $LiAlH_4$ (0.031 g, 0.84 mmol), which was allowed to come to rt and stirred overnight. After recooling to 0 °C the reaction mixture was quenched by drop wise addition of 2 N NaOH solution. It was dried with Na_2SO_4 and was filtered through a short celite pad. The reaction mixture was concentrated and purified by column chromatography (pet. ether/ethyl acetate, 3:2) to give corresponding alcohol **27** (0.091 g, 90%) as a white solid. $[\alpha]_D^{25}$ = +38.1 (*c* 1.0, $CHCl_3$). Anal. Calcd. for $C_{13}H_{23}NO_3$: C, 64.70; H, 9.61; N, 5.80. Found: C, 64.75; H, 9.63; N, 5.75%. IR ν_{max} cm^{-1} in $CHCl_3$ 3442 (OH), 2366, 1631, 1259. δ_H (500 MHz, $CDCl_3$, D_2O exchange) 1.15–1.21 (1H, m, 8ax-H), 1.23 (3H, s, 10-Me), 1.24–1.27 (1H, m, 9ax-H), 1.42, 1.44 (3H each, 2 s, C(CH_3)$_2$), 1.46–1.52 (1H, m, 7ax-H), 1.61 (1H, br d, $^2J_{H,H}$ = 13.07, 7eq-H), 1.76–1.81 (2H, m, 8eq-H, 9eq-H), 1.89–1.91 (1H, m, 9a-ax), 2.18 (1H, dt, $^3J_{6ax,7ax}$ = 12.38, $^3J_{6ax,7eq}$ = 2.89, 6ax-H), 2.21 (1H, t, $^3J_{4ax,3a\text{-}ax}$ = 10.18, 4ax-H), 2.93 (1H, br d, $^2J_{H,H}$ = 11.50, 6eq-H), 3.12 (1H, dd, $^2J_{H,H}$ = 9.81, $^3J_{3a\text{-}ax,4eq}$ = 3.96, 4eq-H), 3.28 (1H, d, $^3J_{3a\text{-}ax,10a\text{-}ax}$ = 9.43, 10a-ax-H), 3.53 (1H, ddd, 3a-ax-H). δ_C (125 MHz, $CDCl_3$) 16.2 (10-Me), 24.0, 24.3, 25.8 (C-7, C-8, C-9), 26.5, 26.8 (C(CH_3)$_2$), 56.8 (C-6), 58.1 (C-4), 69.4 (C-9a), 72.3 (C-10), 72.5 (C-3a), 86.9 (C-10a), 110.4 (*C*(CH_3)$_2$). Mass (ESI): *m/z* 242 (M^+ + H).

(3a*S*,9a*R*,10*S*,10a*S*)-*N*-Benzyl-2,2-dimethyloctahydro-3aH-[1,3]dioxolo[4,5-b]quinolizin-10-amine (28). The reductive amination procedure used for synthesizing **21a** was applied to ketone **24** (0.078 g, 0.345 mmol) in the presence of benzyl amine (0.045 g, 0.415 mmol) to produce **28** (0.082 g, 75%) as a faint yellow solid. $[\alpha]_D^{26}$ = +18.41 (*c* 1.2, $CHCl_3$). Anal. Calcd. for $C_{19}H_{28}N_2O_2$: C, 72.12; H, 8.92; N, 8.85; Found: C, 71.99; H, 8.97; N, 8.81%. IR ν_{max} cm^{-1} in $CHCl_3$ 3351 (NH), 2984 (C–H arom), 1604 (C=C arom), 1453, 1370, 1235. δ_H (500 MHz, $CDCl_3$) 1.20–1.27 (1H, m, 8ax-H), 1.44, 1.45 (3H, 2 s, C(CH_3)$_2$), 1.48–1.59 (3H, m, 7ax-H, 7eq-H, 9ax-H), 1.76–1.84 (2H, m, 8eq-H, 9eq-H), 1.97 (1H, td, $^3J_{9ax,9a\text{-}ax}$ = 11.14, $^3J_{9eq,9a\text{-}ax}$ = 2.38, 9a-ax-H), 2.11 (1H, dt, $^2J_{H,H}$ = 11.72, $^3J_{6ax,7eq}$ = 3.53, 6ax-H), 2.14 (1H, t, $^3J_{3a\text{-}ax,4ax}$ = 10.24 Hz, 4ax-H), 2.91 (1H, d, 6eq-H), 3.04 (1H, t, $^3J_{10eq,10a\text{-}ax}$ = $^3J_{10eq,9a\text{-}ax}$ = 2.91 Hz, 10eq-H), 3.17 (1H, dd, $^3J_{3a\text{-}ax,4eq}$ = 4.10, 4eq-H), 3.38 (1H, dd, $^3J_{10a\text{-}ax,3a\text{-}ax}$ = 9.45, $^3J_{10a\text{-}ax,10eq}$ = 3.63, 10a-ax-H), 3.88 (1H, d, $^2J_{H,H}$ = 13.11, CH_2Ph), 3.98 (1H, dt, 3a-ax-H), 4.01 (1H, d, CH_2Ph), 7.19–7.22 (1H, m, H-arom), 7.27–7.30 (2H, m, H-arom), 7.35–7.36 (2H, m, H-arom). δ_C (125 MHz, $CDCl_3$) 24.3 (C-8), 25.4 (C-7), 26.6, 26.9 (C(CH_3)$_2$), 29.5 (C-9), 54.4 (*C*H_2Ph), 56.8

(C-6), 58.3 (C-10), 58.4 (C-4), 64.9 (C-9a), 70.8 (C-3a), 82.9 (C-10a), 109.8 ($C(CH_3)_2$), 126.7, 128.1, 128.1, 140.9 (4 C-arom). Mass (ESI): m/z 317 (M^+ + H), 339 (M^+ + Na).

(3a*S*,9a*R*,10*R*,10a*S*)-10-Azido-2,2-dimethyloctahydro-3aH-[1,3]dioxolo[4,5-b]quinolizine (30). To a solution of **26** (70 mg, 0.309 mmol) in pyridine (2 ml) at 0 °C was added mesyl chloride (42 mg, 0.371 mmol, in 1 mL DCM). The reaction mixture was stirred at room temperature for 6 h. When TLC revealed no starting material, the solution was diluted with dichloromethane (10 ml) and washed with water (3 × 5 ml) followed by brine solution (5 ml). It was then dried over anhydrous Na_2SO_4. After removal of solvent the crude mesylate (77 mg, 0.251 mmol) was taken up in DMF (2 ml). LiN_3 (123 mg, 2.51 mmol) was added and the mixture was heated at 110 °C for 12 h. The reaction mixture was diluted with water (10 ml) and extracted with ethyl acetate (3 × 10 ml). The ethyl acetate layer was washed with water and dried over anhydrous Na_2SO_4. Solvent removal followed by column chromatography (silica, pet. ether/ethyl acetate, 6:1) gave **30** (56 mg, 72%) as a colorless liquid. $[\alpha]_D^{25}$ = +60.19 (*c* 1.1, $CHCl_3$). Anal. Calcd. for $C_{12}H_{20}N_4O_2$: C, 57.12; H, 7.99; N, 22.21; Found: C, 57.01; H, 7.90; N, 22.05%. IR ν_{max} cm^{-1} in $CHCl_3$ 2936, 2208 (N_3), 2107, 1654, 1446, 1229. δ_H (400 MHz, $CDCl_3$) 1.19–1.25 (2H, m, 8ax-H, 9ax-H), 1.44, 1.45 (3H each, 2 s, C(CH_3)$_2$), 1.48–1.54 (1H, m, 7ax-H), 1.61–1.64 (1H, m, 8eq-H), 1.74–1.81 (2H, m, 7eq-H, 9eq-H), 2.07–2.11 (1H, m, 9a-ax-H), 2.22 (1H, dt, $^3J_{6ax,7ax}$ = 11.83, $^3J_{6ax,7eq}$ = 2.76, 6ax-H), 2.25 (1H, t, $^3J_{3a\text{-}ax,4ax}$ = 10.17, 4ax-H), 2.90 (1H, d, $^2J_{H,H}$ = 11.28, 6eq-H), 3.10 (1H, dd, $^2J_{H,H}$ = 9.90, $^3J_{3a\text{-}ax,4eq}$ = 3.85, 4eq-H), 3.20–3.28 (2H, m, 10ax-H, 10a-ax-H), 3.58 (1H, ddd, 3a-ax-H). δ_C (50 MHz, $CDCl_3$) 23.8 (C-8), 25.6 (C-7), 26.7 (C(CH_3)$_2$), 29.4 (C-9), 56.0 (C-6), 56.9 (C-4), 64.6, 65.2 (C-9a, C-10), 74.2 (C-3a), 81.9 (C-10a), 111.1 ($C(CH_3)_2$). Mass (ESI): m/z 253 (M^+ + H), 275 (M^+ + Na).

(3a*S*,9a*R*,10*R*,10a*S*)-2,2-Dimethyloctahydro-3aH-[1,3]dioxolo-[4,5-b]quinolizin-10-amine (31). The azide **30** (43 mg, 0.172 mmol) in methanol (2 ml) was hydrogenated for 7 h at atmospheric pressure in the presence of 10% Pd on charcoal (4 mg). The reaction mixture was passed through a short pad of celite. Solvent removal followed by column chromatography (silica, $CHCl_3$/MeOH, 24:1) afforded **31** (39 mg, 85%) as a white solid. $[\alpha]_D^{25}$ = +39.43 (*c* 0.7, $CHCl_3$). Anal. Calcd. for $C_{12}H_{22}N_2O_4$: C, 63.68; H, 9.80; N, 12.38; Found: C, 64.80; H, 9.85; N, 12.35%. IR ν_{max} cm^{-1} in $CHCl_3$ 3396 (NH), 3054, 2305, 1598, 1265. δ_H (500 MHz, $CDCl_3$, D_2O exchange) 1.13–1.23 (2H, m, 8ax-H, 9ax-H), 1.42 (6H, s, C(CH_3)$_2$), 1.50–1.56 (1H, m, 7ax-H), 1.58–1.64 (2H, m, 7eq-H, 8eq-H), 1.80 (1H, br d, $^2J_{H,H}$ = 12.9, 9eq-H), 2.04–2.07 (1H, m, 9a-ax-H), 2.21 (1H, dt, $^3J_{6ax,7ax}$ = 11.83, $^3J_{6ax,7eq}$ = 3.03, 6ax-H), 2.27 (1H, t, $^3J_{3a\text{-}ax,4ax}$ = 10.18, 4ax-H), 2.66 (1H, dd, $^3J_{10ax,10a\text{-}ax}$ = 10.18, $^3J_{10ax,9a\text{-}ax}$ = 8.53, 10ax-H), 2.89 (1H, br d, $^2J_{H,H}$ = 11.56, 6eq-H), 3.10 (1H, dd, $^3J_{3a\text{-}ax,10a\text{-}ax}$ = 9.07, 10a-ax-H), 3.13 (1H, dd, $^3J_{4eq,3a\text{-}ax}$ = 4.01, 4eq-H), 3.58 (1H, ddd, 3a-H). δ_C (100 MHz, $CDCl_3$) 24.1 (C-8), 25.8 (C-7), 26.7, 26.8 (C(CH_3)$_2$), 28.6 (C-9), 55.7 (C-9a), 56.2 (C-6), 57.5 (C-4), 67.6 (C-10), 74.3 (C-3a), 84.4 (C-10a), 110.4 ($C(CH_3)_2$). Mass (ESI): m/z 227 (M^+ + H), 249 (M^+ + Na).

Crystal structure determination of compound 23

Crystal data

$C_{13}H_{23}NO_4$, M = 257.32, orthorhombic, a = 5.3743(4), b = 10.2506 (7), c = 24.311(2) Å, V = 1339.27(16) Å^3, space group $P2_12_12_1$, Z = 4, D_c = 1.276 g/cc, μ (Mo–Kα) = 0.094 mm^{-1}, 12741 reflections measured, 2369 unique (R_{int} = 0.0398) [$I>2\sigma(I)$], R value 0.0358, the final w$R(F_2)$ = 0.0823 [$I>2\sigma(I)$].

Crystal structure determination of compound 25

Crystal data

$C_{12}H_{21}NO_3$, M = 227.30, orthorhombic, a = 9.725 (1), b = 6.4790(7), c = 20.325(2) Å, V = 1280.6(2) Å^3, space group $P2_12_12_1$, Z = 4, D_c = 1.179 g/cc, μ (Mo–Kα) = 0.084 mm^{-1}, 10685 reflections measured, 2250 unique (R_{int} = 0.0745) [$I>2\sigma(I)$], R value 0.0480, the final w$R(F_2)$ = 0.0864 [$I>2\sigma(I)$].

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Erratum