SYNTHESIS OF POLYHYDROXY CYCLIC AMINES AS POTENT GLYCOSIDASES INHIBITORS

THESIS

Submitted To The

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For The Degree Of

DOCTOR OF PHILOSOPHY

In

CHEMISTRY

By

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Dedicated
To My
Parents

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CERTIFICATE

This is to certify that the work incorporated in the thesis entitled **"Synthesis of Polyhydroxy Cyclic Amines as Potent Glycosidases Inhibitors",** which is being submitted to the **University of Pune** for the award of **Doctor of Philosophy in Chemistry** by **Mr. Kishor Chandra Bharadwaj,** was carried out by him under my supervision at the **National Chemical Laboratory, Pune**.

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DECLARATION

I hereby declare that the work presented in the thesis entitled "**Synthesis of Polyhydroxy Cyclic Amines as Potent Glycosidases Inhibitors**" submitted for Ph. D. Degree to the University of Pune, has been carried out by me at the National Chemical Laboratory, Pune, under the supervision of Dr. Ganesh Pandey. The work is original and has not been submitted in part or full by me for any degree or diploma to this or any other University.

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Kishor Chandra Bharadwaj

CONTENTS

Abbreviations

aq.	aqueous	mp	Melting point
bp	boiling point	N	Normality
Bn	Benzyl	MS	Mass Spectrum
Boc	*t*-Butoxycarbonyl	MsCl	Methanesulfonyl chloride
DCM	dichloromethane	NMR	Nuclear magnetic resonance
DEPT	Distortionless enhancement by polarisation transfer	PET	Photoinduced electron transfer
DMSO	dimethylsulfoxide	*p*-TSA	*p*-Tolunesulfonic acid
g	gram	rt	Room temperature
GC	Gas Chromatography	TBAF	Tetrabutylammonium fluoride
h	hour	TBAI	Tetrabutylammonium iodide
$h\nu$	Ultraviolet light	THF	Tetrahydrofuran
Hz	Hertz	TLC	Thin layer chromatography
IBX	*o*-Iodoxybenzoic acid	TMEDA	Tetramethylethylenediamine
Ki	Inhibition constant	TMS	Trimethylsilyl
LAH	Lithium aluminium hydride	α-Gal	α-Galactosidase
M	Molarity	β-Gal	β-Galactosidase
mg	Milligram	α-Glc	α-Glucosidase
min	Minute(s)	β-Glc	α-Glucosidase
mL	Milliliter	α-Man	α-Mannosidase
mmol	Millimole	β-Man	α-Mannosidase
μM	Micromolar		

General Remarks

- All the solvents used were purified according to literature procedure.[1]
- Petroleum ether used in the experiments was of 60-80 °C boiling range.
- Column chromatographic separations were carried out by gradient elution with suitable combination of two solvents and silica gel (60-120 mesh/ 100-200 mesh/ 230-400 mesh).
- Reaction progress was monitored by TLC or GC. TLC was performed on manually prepared glass silica plates and E-Merck pre-coated 60 F_{254} plates and the spots were rendered visible by exposing to UV light, iodine, phosphomolybdic acid, *o*-Anisole, $KMnO_4$. 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, neat in case of liquid compounds or their solution in chloroform.
- NMR spectra were recorded on Brucker ACF 200 (200 MHz ^{1}H NMR and 50 MHz ^{13}C NMR), AV 400 MHz (400 MHz ^{1}H NMR and 100 MHz ^{13}C NMR) and DRX 500 (500MHz ^{1}H NMR and 125 MHz ^{13}C NMR). ^{13}C peak multiplicity assignments were made based on DEPT data.
- Mass spectra were recorded on PE SCIEX API QSTAR pulser (LC-MS)
- Microanalysis data were obtained using a Carlo-Erba CHNS-O EA 1108 Elemental Analyser. Elemental analyses observed for the entire newly synthesized compound were within the limit of accuracy (± 0.4 %).
- All the melting points recorded are uncorrected and were recorded using electrothermal melting point apparatus.
- Starting materials were obtained from commercial sources. *s*-BuLi, IBX, DCN and $NaBH(OAc)_3$ were prepared using known procedures.
- **Numbering of compounds, schemes, tables, referencing and figures for each section of each chapter and in abstract are independent.**

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

Abstract of thesis

Chapter-1:: An Introduction to Glycosidases

The oligosaccharides and glycoconjugates present in biological systems carry out a wide array of essential functions ranging from energy storage and utilization, to complex information display systems that modulate normal cell function. The number of possible permutations of assemblies of monosaccharides, both in the type and the manner in which individual units are connected, allows for a level of complexity which far exceeds that of DNA and even proteins. The glycosidic bond particularly that between two glucose residues, is the most stable of the linkages within naturally occurring biopolymers, with half-lives for spontaneous hydrolysis of cellulose and starch being in the range of 5 million years. Glycosidases responsible for the hydrolysis of these materials accomplish this with rate constants up to 1000 s^{-1}, earning them a reputation as some of the most proficient catalysts. They are involved in the biosynthesis and degradation of oligosaccharides and glycoconjugates (glycoproteins, glycolipids, proteoglycans) that are found in nearly all forms of life. Their inhibition can affect the digestion of polysaccharides and the maturation, transport and secretion of glycoproteins. Because cell surface carbohydrates are involved in various biological functions, such as cell-cell recognition, cell adhesion, and cell-growth regulation, their implication in the immune response, oncogenesis, tumor metastasis, and the differentiation of cells is no longer doubted. Therefore, search for inhibitors of glycosidases has become a hot area of research for the development of novel antiviral, anti-infective and anti-cancer agents. An attractive approach to potent inhibitors is to create compounds that 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. The search for still, more potent and selective inhibitors for various glycosidases is on the way.

Chapter-2:: Synthesis and Biological Evaluatuion of Piperidine 1-*N*-Iminosugars

A large number of sugar analogues having nitrogen atom in place of oxygen in the ring, such as NJ (**1**), DNJ (**2**) and fagomine (**3**) (Fig.1) are reported to be glycosidase inhibitors. Similarly, another class of sugar analogues in which nitrogen is placed at the anomeric

carbon such as isofagomine (**4**), isogalactofagomine (**5**), isofucofagomine (**6**), 5- hydroxy isofagomine (**7**), polyhdroxy piperidines (**8-11**), and their analogues, have also been shown to be potent glycosidase inhibitors. Here in we report our effort towards their synthesis.

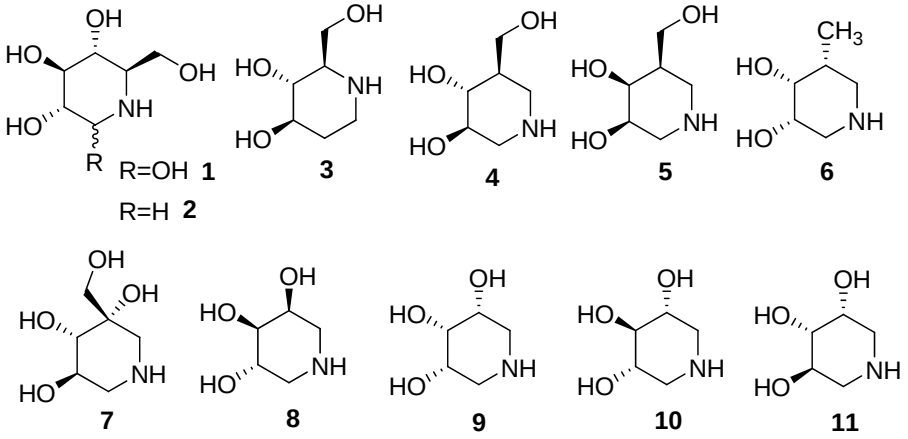
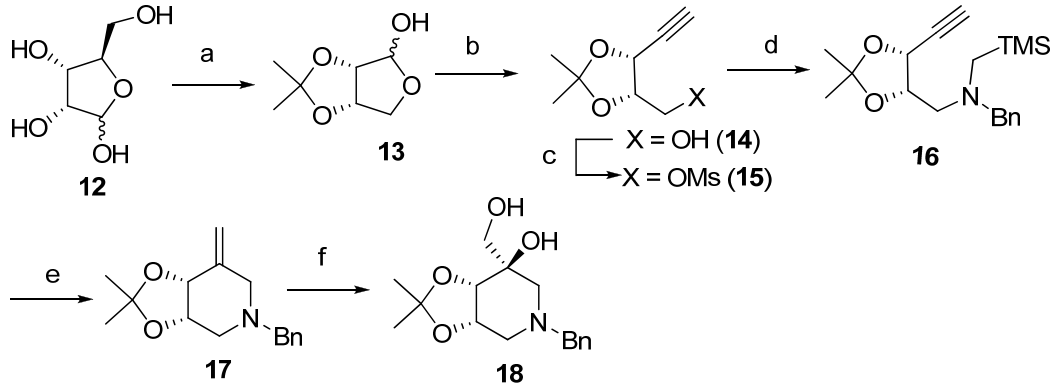


Figure 1.

We started with D-Ribose (Scheme 1). The lactol **13** obtained from D ribose was subjected to gradual addition of Ohira Bestamann reagent to obtain **14** whose free hydroxyl moiety was subsequently transformed to mesylate **15** which upon alkylation with $BnNHCH_2TMS$ gave **16**. Compound **16** upon photoinduced electron transfer (PET) underwent cyclization to give **17**. Dihydroxylation of double bond gave **18** as a single isomer.



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) $MeSO_2Cl$, Et_3N, DCM, 0 ^oC to rt, 5 h, 100%; (d) $BnNHCH_2TMS$, TBAI, K_2CO_3, CH_3CN, reflux, 96 h, 80%; (e) *hv*, DCN, 2-PrOH, 1 h, 60%; (f) OsO_4, NMO (50% aq. solution), t-BuOH, rt, 24 h, 90%.

However X- Ray analysis of **18** (Figure 2) established the *trans* stereochemistry between protons on C_{7A} and C_{3A} carbons as in **18a**. These observations led us to speculate that the

reaction of lactol **13** with Ohira-Bestmann reagent could have been the possible step where epimerization occurred. Careful literature scrutiny revealed the precedence of such epimeraization.

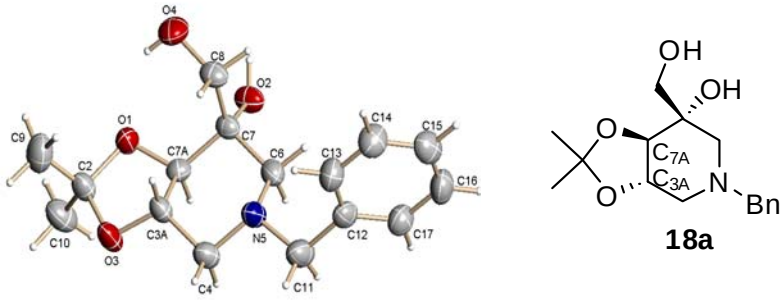
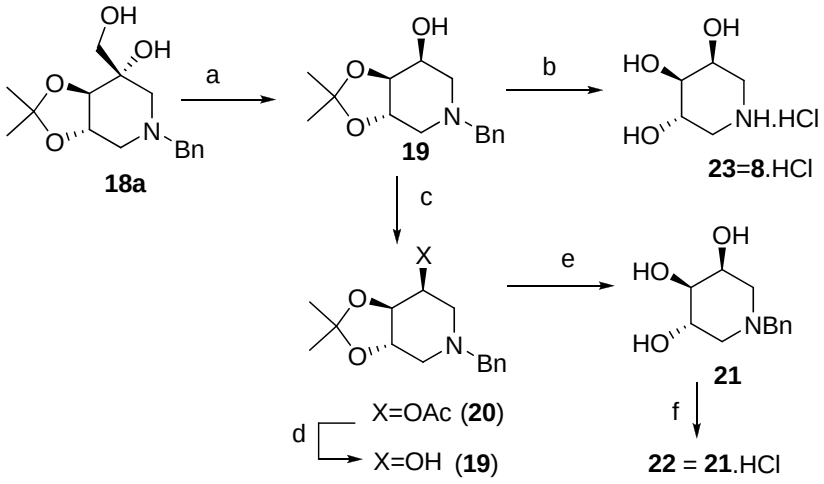


Figure 2.

Although, this unexpected result was disappointing, nevertheless protocol appeared more attractive for the synthesis of trans kind of systems. Hence, we decided to proceed with the existing schemes.

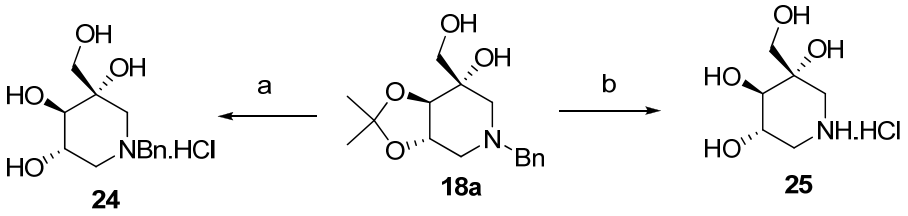


Scheme 2.

Reagents and Conditions:: (a) (i) $NaIO_4$, EtOH:H_2O (4:1) rt, 0.5 h; (ii) $NaBH_4$, MeOH, 0 ^oC to rt, 24 h, 85% over two steps, dr 9:1; (b) (i) $Pd(OH)_2$ on C, H_2 (1 atm), EtOH, rt 10 h; (ii) HCl, MeOH, rt, 4 h, 85% over two steps; (c) AcCl, Py, DCM, 30 h, rt; 80%; (d) K_2CO_3, MeOH, rt, 12 h, 90%; (e) HCl, MeOH, rt, 4 h, 90%; (f) HCl, MeOH, rt, 15 min, 100%.

To make piperidine triol, diol **18a** was converted to ketone (Scheme 2) followed by reduction of carbonyl moiety to get **19** (in 9:1 diastereomeric ratio as non separable diastereomers). Purification was achieved by either converting it to acetate **20** (for **22**), or by N-debenzylation and column separation (for **23**).

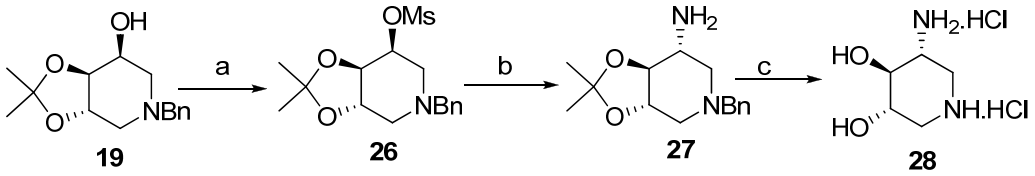
Removal of protecting groups from **18a** (Scheme 3) gave **24** and **25**.



Scheme 3.

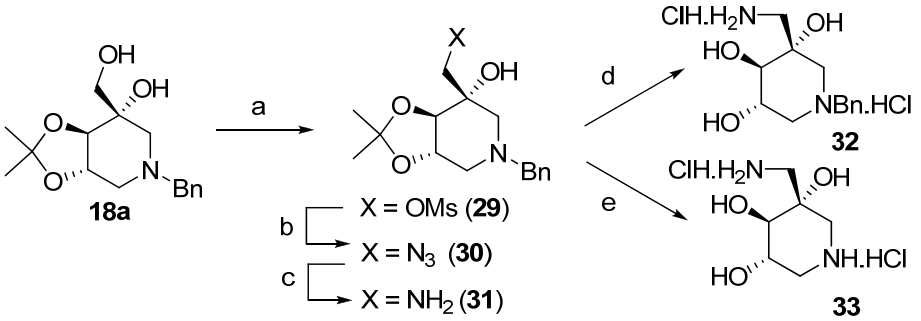
Reagents and Conditions:: (a) HCl, MeOH, rt, 4 h, 100%; (b) (i) $Pd(OH)_2$ on C, H_2 (1 atm), EtOH, rt 10 h; (ii) HCl, MeOH, rt, 4 h, 100% over 2 steps**.**

At this stage we also thought of introducing another basic site in the molecule and thus making the amine analogues of the above molecules. This was achieved by a three step sequence (Scheme 4), of converting alcohol **19** to mesylate **26** followed by replacement by azide and then LAH reduction, giving free amine **27**. Removal of protecting groups gave the required azasugar **28**. A similar sequence (Scheme 5) was followed for making of **32** & **33**.



Scheme 4.

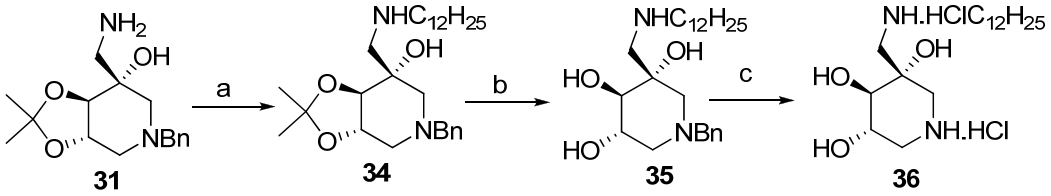
Reagents and Conditions:: (a) $MeSO_2Cl$, py, 0 ^oC to rt, 6 h, 85%; (b) (i) LiN_3, DMF, 110 ^oC, 20 h; (ii) LAH, THF, 12 h, 60%, over 2 steps; (c) (i) $Pd(OH)_2$ on C, H_2 (1 atm), EtOH, rt 10 h; (ii) HCl, MeOH, rt, 4 h, 100% over 2 steps.



Scheme 5.

Reagents and Conditions:: (a) $MeSO_2Cl$, Et_3N, DCM, 0 ^oC to rt, 6 h, 90%; (b) LiN_3, DMF, 110 ^oC, 20 h, 90%; (c) LAH, THF, 12 h, 90%; (d) HCl, MeOH, rt, 4 h, 100%; (e) (i) $Pd(OH)_2$ on C, H_2 (1 atm), EtOH, rt, 10 h; (ii) HCl, MeOH, rt, 4 h, 100% over 2 steps.

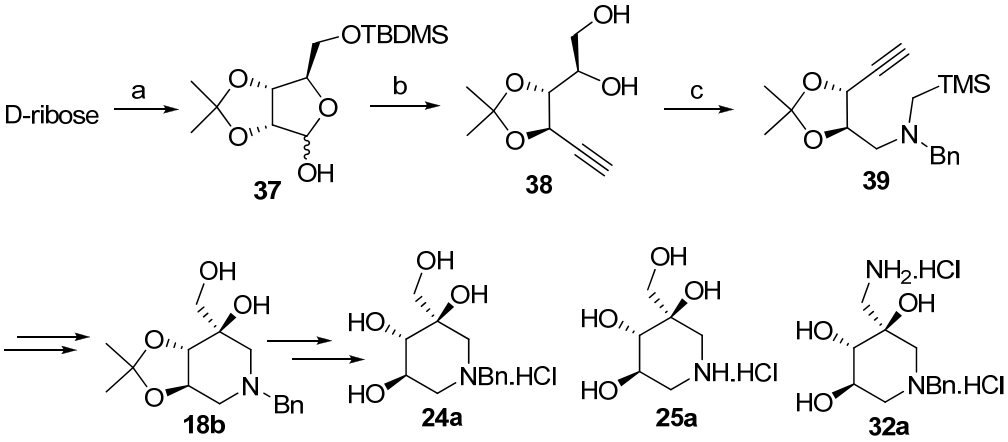
To alter the basisity of primary amine, a hydrocarbon chain too was attached to **31** to get **34** as shown in scheme 6. Removal of protecting groups gave **36**.



Scheme 6.

Reagents and Conditions:: (a) $C_{12}H_{25}Br$, K_2CO_3, CH_3CN:THF (3:1), rt to reflux, 6 h, 70%; (b) HCl, MeOH, rt, 4 h, 70%; (c) (i) $Pd(OH)_2$ on C, H_2 (1 atm), EtOH, rt 10 h; (ii) HCl, MeOH, rt, 15 min, 100% over 2 steps.

We also made enantiomers of few of the above azasugars starting from D ribose itself (Scheme 7). Thus acetylene diol **38** obtained from **37** was subjected to periodate cleavage. The reductive amination of resulting aldehyde gave **39** which after following similar sequence gave **24a**, **25a & 32a**.



Scheme 7.

Reagents and Conditions:: (a) (i) H_2SO_4, acetone (cat), 0 ^oC, 30 min; (ii) TBDMSCl, Et_3N, DCM, rt, 6 h, 80% over 2 steps; (b) $(MeO)_2P(O)C(N_2)COMe$, K_2CO_3, MeOH, 65 ^oC, 6 h, 55%; (c) (i) $NaIO_4$, EtOH:H_2O (4:1) rt, 0.5 h; (ii) $Na(OAc)_3BH$, EDC, $BnNHCH_2TMS$, rt, 24 h, 50% over 2 steps.

The epimerization at this stage too was confirmed by X ray of **18b** (Figure 3).

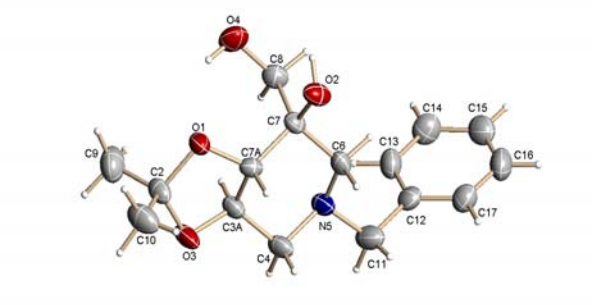


Figure 3.

The various inhibitor were tested for their biological properties and the result are summarized in table 1

Table1. Inhibition (*Ki* in μm) 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)	α-man (*A. Fischeri*)
24	967	ni	20%[a]	ni	ni	**33%[a]**	ni
25	ni	ni	27%[a]	ni	ni	**61%[a]**	217
24a	ni	ni	ni	ni	ni	**16.4**	41.4
25a	ni	ni	ni	ni	1066	**42%[a]**	ni
22	6%[a]	890	ni	23%[a]	ni	**210**	50%[a]
23	504	85	578	991	456	**1.07**	325
28	ni	ni	ni	ni	ni	**153**	35%[a]
32	ni	ni	ni	10%[a]	20%[a]	**36.8**	37.3
33	ni	ni	ni	14%[a]	ni	**217**	930
32a	ni	ni	ni	17%[a]	34%[a]	**471.3**	ni
36	ni	ni	ni	ni	ni	**72.3**	35%[a]

ni, no inhibition up to 1mm, , [a] percent inhibition at 1 mm level

As shown in the table the developed inhibitors proved to be selective inhibitors of α glucosidase, with few of them showing high inhibition.

Chapter-3:: Synthetic studies towards Pyrrolizidines and Pyrrolidines class of Azasugars

This chapter deals with our synthetic efforts towards synthesis of pyrrolizidine and pyrrolidine class of alkaloids.

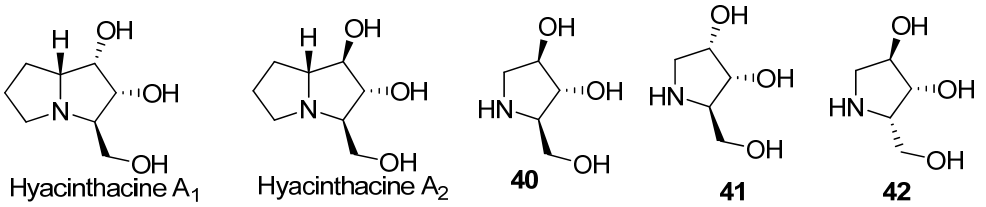


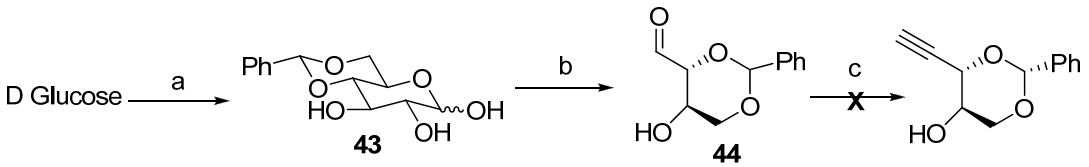
Figure 4.

Hyacinthacines **A_1**, **A_2** (a pyrrolizidine class of alkaloid), and pyrrolidines **40, 41** & **42** are naturally isolated alkaloids (Figure 4), which have been found to be active inhibitor of various glycosidases.

Hyacinthacine A_1 has been shown to be an effective inhibitor of rat intestinal lactase (IC_{50} 4.4 μm). Hyacinthacine A_2 has been shown to inhibit amyloglucosidase (aspergillus niger) (IC_{50} 8.6 μm). **40** is a much powerful inhibitor of yeast α- D- glucosidase and several mammalian α-glucosidase. It has also been identified as a potent inhibitor of muscle and liver glycogen phosphorylase (GPa). 46,47. In enzyme assay it was shown to be potent inhibitor of liver GPa (*Ki* = 0.4 μM). **41** a naturally occurring glycosidase inhibitor isolated from the tree Morus albaand is a potent inhibitor of α and β glucosidase. **42** isolated from Arachniodes Standishii and Angylocalyx boutiqueanus, has been proven to be a potential glycosidase inhibitor.

We took up the synthesis of hyacinthacine A_1, A_2 and the alkaloids **40**, **41**, and **42**. A close examination of the above alkaloids leads to basic similarities between them, *i.e*. the tri- substituted pyrrolidine ring, with only subtle stereochemical difference. So we thought of a strategy of constructing the ring in precise stereochemical fashion, with a flexibility of making the stereo isomers.

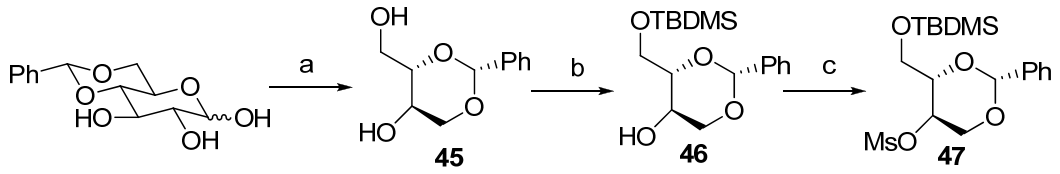
We started our synthesis with D- Glucose (Scheme 8). 1,3 diol protection of Glucose, gave the benzylidene protected compound **43**. The periodate cleavage of **43** gave the aldehyde **44**. However when **44**, (crude as such) was subjected for installation of triple bond, the reaction was found to be complicated and nothing could be isolated.



Scheme 8.

Reagents and Conditions:: (a) $PhC(OMe)_2H$, *p*TSA, DMF, 60 ^{0}C, 3 h, 40%; (b) $NaIO_4$, $NaHCO_3$, H_2O, 0 ^{0}C to rt 5 h; (c) $(MeO)_2P(O)C(N_2)COMe$, K_2CO_3, MeOH, rt, 24 h.

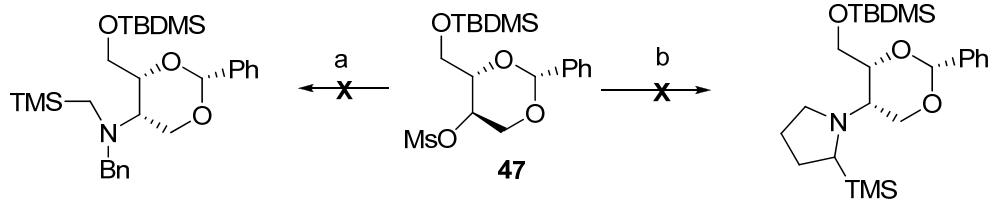
For the unknown reasons for the failure of reaction, we thought of proceeding further, and to go an extra step. We reduced the aldehyde and obtained diol **45** (Scheme 9) which upon TBDMS protection of primary hydroxyl gave **46**. This was mesyalted to give **47**.



Scheme 9.

Reagents and Conditions:: (a) (i) $NaIO_4$, $NaHCO_3$, H_2O, 0 ^{0}C to rt, 5 h; (ii) $NaBH_4$, H_2O, 85% over 2 steps; (b) TBDMSCl, Et_3N, DMAP (cat), DCM, rt, 6 h, 90%; (c) MsCl, Et_3N, DCM, rt, 6 h, 90%.

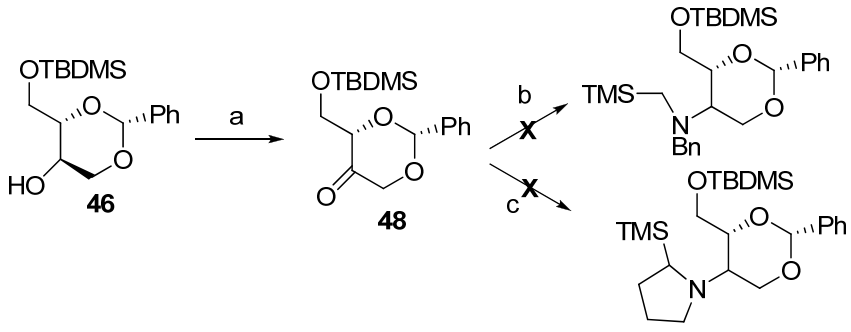
However mesylate **47**, when subjected to nucleophilic conditions by amine functionalities, failed to undergo any displacement, and starting material was obtained as such (Scheme 10).



Scheme 10.

Reagents and Conditions:: (a) $BnNHCH_2TMS$, K_2CO_3, CH_3CN, reflux; (b) 2-(trimethylsilyl)pyrrolidine, K_2CO_3, CH_3CN, reflux.

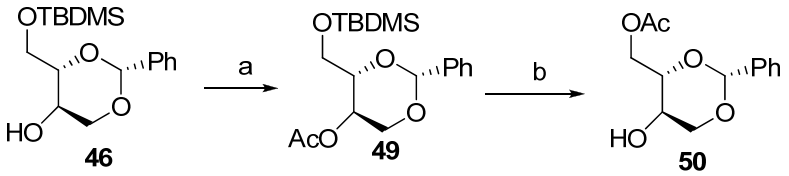
We also tried oxidation and reductive amination, for installation of amine functionality, but the ketone **48** obtained after oxidation of **46** failed to undergo reductive amination (Scheme 11).



Scheme 11.

Reagents and Conditions:: (a) IBX, EtOAc, reflux, 24 h; (b) $BnNHCH_2TMS$, $Na(OAc)_3BH$, EDC, rt, 24 h; (c) 2-(trimethylsilyl)pyrrolidine, $Na(OAc)_3BH$, EDC, rt, 24 h.

From the above set of reactions we realized that the reaction center was too sterically hindered, for the installation amine functionality. So we resorted to the installation of the compact acetylenic functionality first, which might relieve some strain.

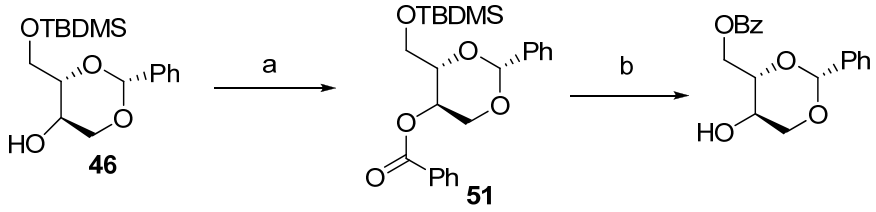


Scheme 12.

Reagents and Conditions:: (a) AcCl, py, DCM, rt, 20 h, 80%; (b) TBAF, THF, rt, 4 h, 90%.

Thus we thought of protecting the secondary hydroxyl in the form of acetate, which during the course of reaction for installation of triple bond would get cleaved.
Thus acetyl protection of **46** gave the acetate **49**. However during TBS deprotection, acetate moiety was found to be migrating to primary hydroxy, to give **50** (Scheme 12).

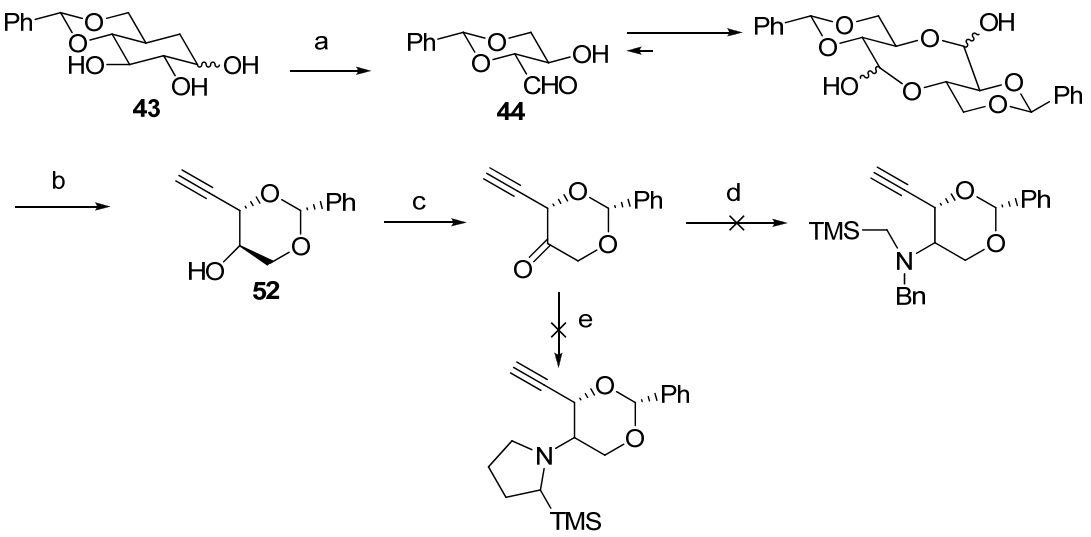
To prevent the migration we thought of using a bulky ester group, which would prevent the migration, during the course of deprotection. The secondary hydroxyl of **46** was converted to benzoate ester **51** but during the TBDMS deprotection, the benzoate also migrated to primary hydroxyl group (Scheme 13).



Scheme 13.

Reagents and Conditions:: (a) BzCl, py, rt, 48 h, 80%; (b) TBAF, THF, rt, 4 h 90%.

In the mean while we were trying to solve this problem we came across a literature report, which showed that the aldehye **44** existed in the form of lactol, as a dimmer (Scheme 14). This reasoned out the failure of reaction for the installment of acetylenic moiety earlier on **44**. Thus we proceeded with our usual protocol. Addition of Ohira-Bestmann reagent gradually over 6 hours to **44**, gave the required product **52**.
However when **52** was subjected to oxidation and then reductive amination, the reaction was not successful. The ketone was recovered back along with reduced product, alcohol.



Scheme 14.

Reagents and Conditions:: (a) $NaIO_4$, $NaHCO_3$, H_2O, 0 ^{0}C to rt 5 h; (b) $(MeO)_2P(O)C(N_2)COMe$, K_2CO_3, MeOH, 65 ^{0}C, 6 h, 40% over 2 steps; (c) IBX, EtOAc, reflux, 24 h; (d) $BnNHCH_2TMS$, $Na(OAc)_3BH$, EDC, rt; 24 h; (e) 2-(trimethylsilyl)pyrrolidine, $Na(OAc)_3BH$, EDC, rt, 24 h.

This again shows that the reaction center was too sterically hindered, for making C-N bond, and currently we are working over that.

Chapter-1

An Introduction to Glycosidases Inhibitors

Life exists in the universe only because the carbon atom possesses certain exceptional properties.-Sir James Jean, English astronomer, physicist and mathematician.

1.1-Glycosidases and their classification

Carbohydrates once considered being the storage house of energy only, have now been revealed to have wide range of functions. For example, the oligosaccharides and glycoconjugates present in biological systems are established to carry out a wide array of essential functions ranging from energy storage and utilization to complex information display systems that modulate normal cell function. The number of possible permutations of assemblies of monosaccharides, both in the type and the manner in which individual units are connected, allows for a level of complexity which far exceeds that of DNA and even proteins. Diversity further originates from the formation and breakdown of one of the most stable covalent linkages ($\Delta G^{\ddagger} \sim 30$ kcal mol^{-1}) found within natural biopolymers—the glycosidic bond.[1,2] 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 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 within naturally occurring biopolymers, with half-lives for spontaneous hydrolysis of cellulose and starch being in the range of 5 million years[3]. Enzymes, the glycosidases carrying out the hydrolyses 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.
Based on the mode of the action of glycosidases they are classified as follows:

1.1.A-α-Glycosidases and β-Glycosidases

Glycosidases are more importantly 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. Depicted below in Figure 1 is a proposed reaction mechanism.

(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.

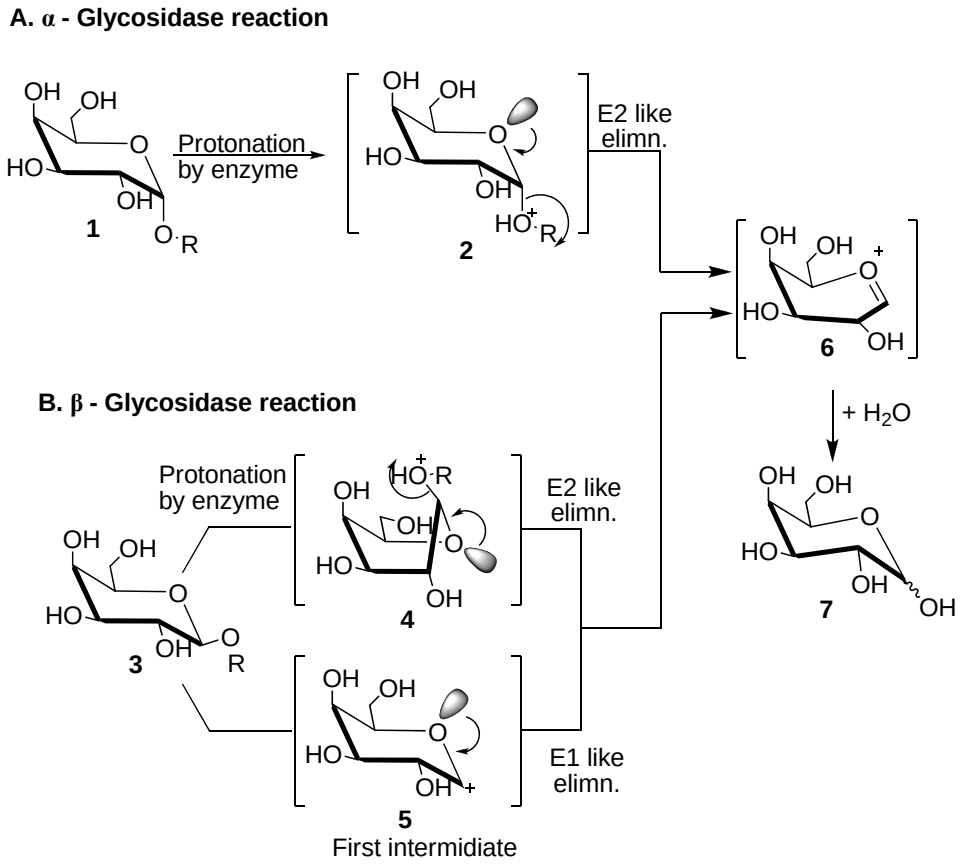


Figure 1. *Glycosidase reaction mechanism involved in α -glycosidases and β-glycosidases.*

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

1.1.B-Inverting and retaining Glycosidases

Glycosidases can also be classified depending upon the stereochemcial outcome of the anomeric bond formed. Hydrolysis of the glycosidic bond can occur with one of the two possible stereochemical outcomes e.g. inversion or retention of anomeric configuration. This observation demands (at least) two different mechanisms as shown in Figure 2.

Glycosidases have evolved with well defined cavities incorporating carboxyl/carboxylate groups.

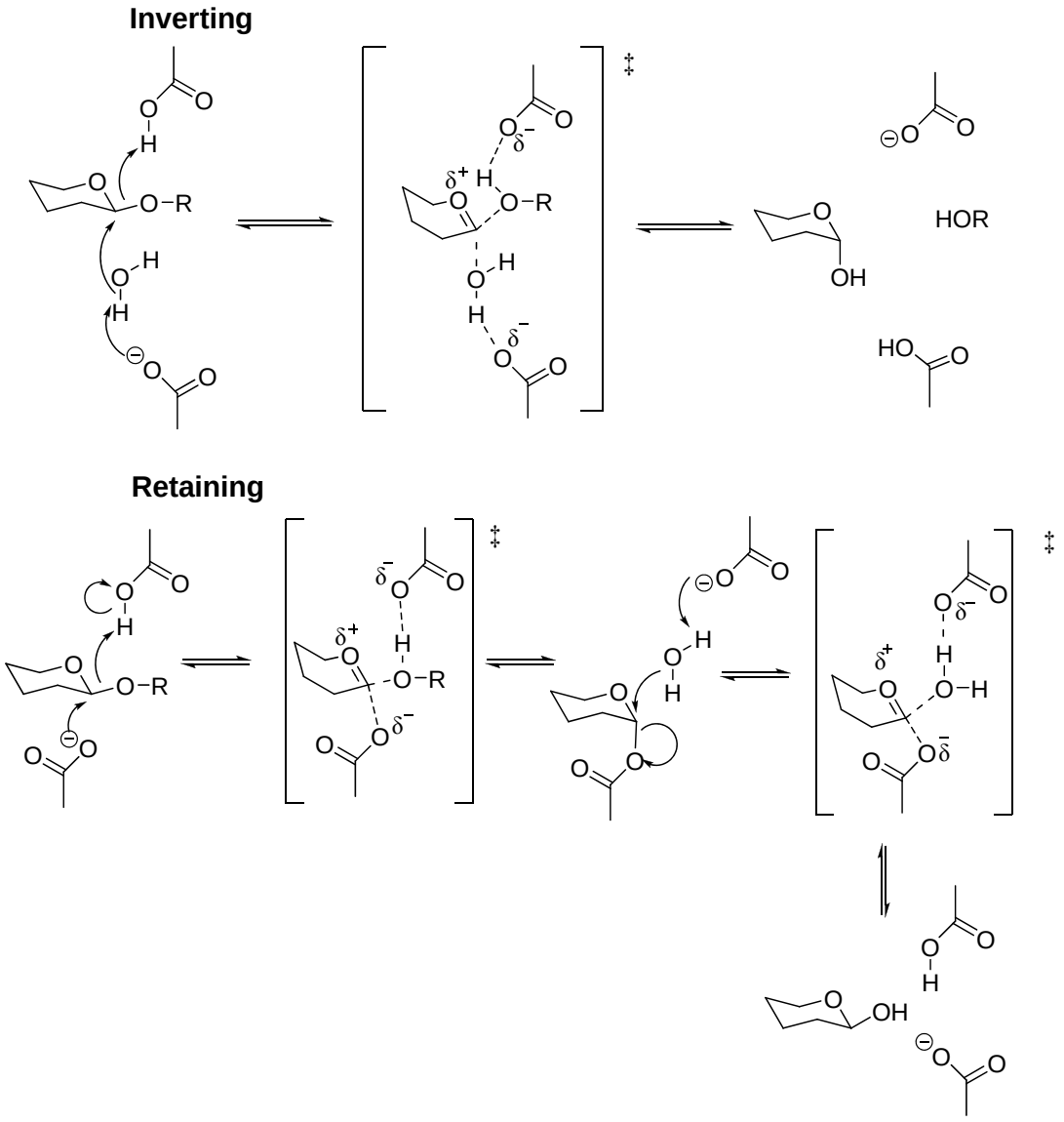


Figure 2*. General mechanism for inverting (a) and retaining (b) glycosidases.*

(a) *Inverting glycosidases* - The two carboxyl groups serve as general acid and general base catalysts and are suitably placed, about 10.5 Å apart on average,[3,5] to allow the

substrate and a water molecule to bind between them. Reaction occurs via a single-displacement mechanism involving an oxocarbenium ion-like transition state.

(b) *Retaining glycosidases* - By contrast, the carboxyl groups in the cavity are only 5.5 Å apart, consistent with a double displacement mechanism involving a covalent glycosylenzyme 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.C-Exoglycosidases and Endoglycosidases

Depending upon the position of the glycosidic bond that is cleaved by the enzyme, they can also be classified under two categories such as *exoglycosidases* and *endoglycosidases* (Figure 3).

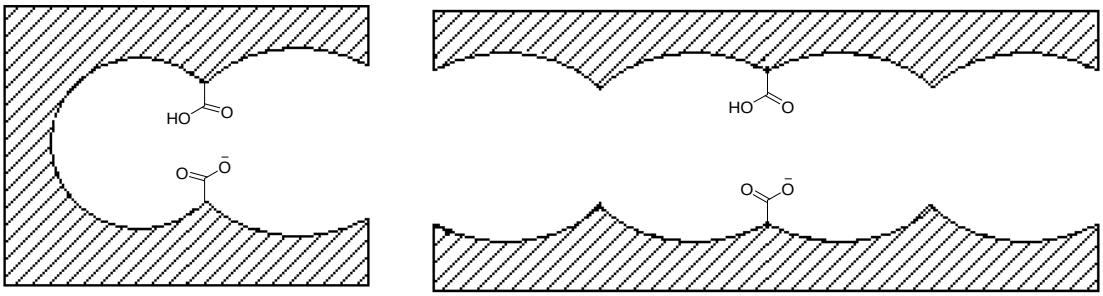


Figure 3*. Exo- and Endoglycosidases.*

(a) *Exoglycosidases* - They remove sugars one at a time, from the non-reducing end of an oligo- or polysaccharide and are involved in the breakdown of starch and glycogen, the processing of eukaryotic glycoproteins, the biosynthesis and modification of glycosphingolipids and the catabolism of peptidoglycans and other glycoconjugates.

(b) *Endoglycosidases* - They cleave interior glycosidic bond within polysaccharides and are involved in the catabolism and clearance of the aged glycoproteins. These enzymes also catalyze the alteration of bacterial and plant cell walls as well as the hydrolysis of highly insoluble structural polysaccharides like chitin and cellulose.[6]

1.1.D-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]

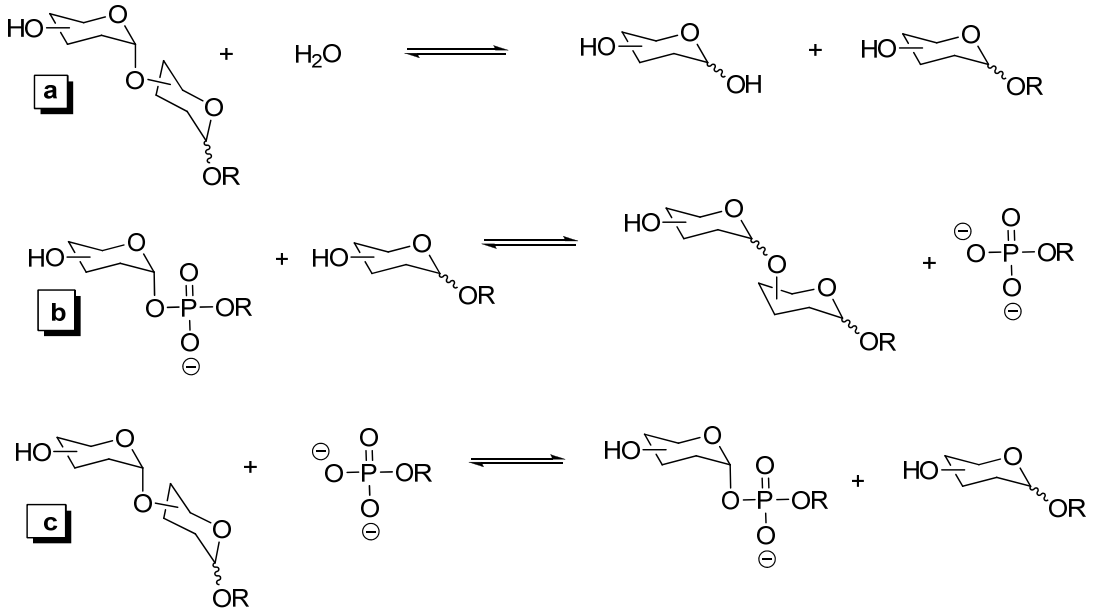


Figure 4. *Overall reactions catalyzed by (a) glycosidases, (b) glycosyltransferases, and (c) Phosphorylases*

(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.

(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

Any chemical entity which is capable of mimicking either the charge or shape (or both) of

the substrate or that of any of the transition states, can act as an inhibitor of that particular glycosidase. The term "***glycomimetics***" refers to the creation of molecules that mimic the activity of carbohydrates or simply carbohydrate mimics.

1.2-Azasugars as inhibitors of Glycosidases

Historically, the first glycosidase inhibitors were the families of the monosaccharide-derived δ-aldonolactones (such as D-gluconolactone **8**)[7], and glycosyl amines (1-amino-1-deoxy pyranoses such as D-glucosyl amine **9**)[8] (Figure 5).

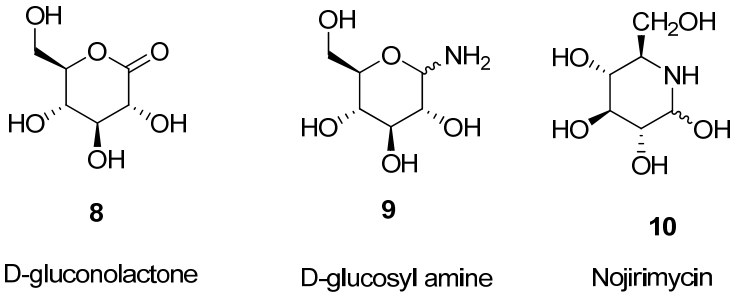


Figure 5. *The classical glycosidases inhibitors.*

Although, lacking long-term stability in aqueous solution, this family of compounds typically display competitive inhibition against glycosidases the substrates of which they closely resemble to. Ever since the pioneering work by Paulsen[9] 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**)[10], many other naturally occurring iminosugars have been identified and additional analogues and homologs have been synthesized, opening a dynamic research area[6,11].

Iminosugars, initially isolated from plants or microorganisms, and later synthesized are considered to have a high potential therapeutic value and are of interest to be applied in the elucidation of biological recognition processes, due to their glycosidase inhibition properties.[11h,11i] 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.[11a,11e,12] These iminosugars (polyhydroxylated alkaloids) are low molecular weight compounds, able to inhibit glycosidases because they mimic the conformation and charge of the oxocarbenium ion intermediate **6**.

Considering that 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.[13] 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.[4d,14] Thus, relevant structural factors for glycosidase inhibition may be related to the charge and/or shape, defined by the hybridization and conformation. These azasugars are widespread[15] in various forms and classes (Figures 6-8).

(a) The nitrogen heterocycles incorporating four to seven membered rings (Figure 6) (**11**-**17**). 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.

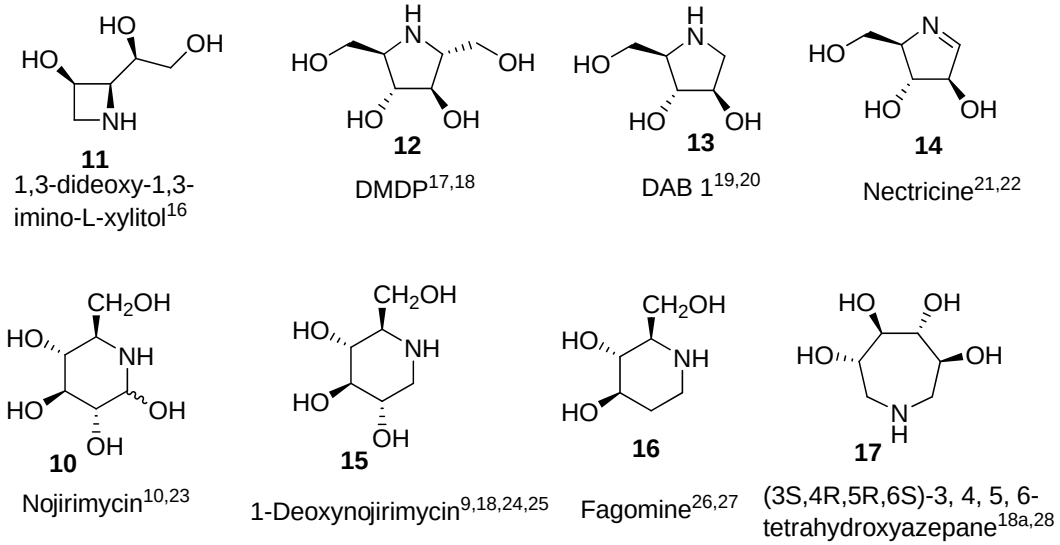


Figure 6*. Azasugars of various ring size.*

(b) Bicyclics like pyrrolizidines (Alexine **18**, Australin **19**) indolizidines Castenospermine **20**, Lentigenosine **21**, Swansonine **22**) and nortropanes (Calystegine A_3 **23**, Calystegine C_1 **24**) (Figure 7).

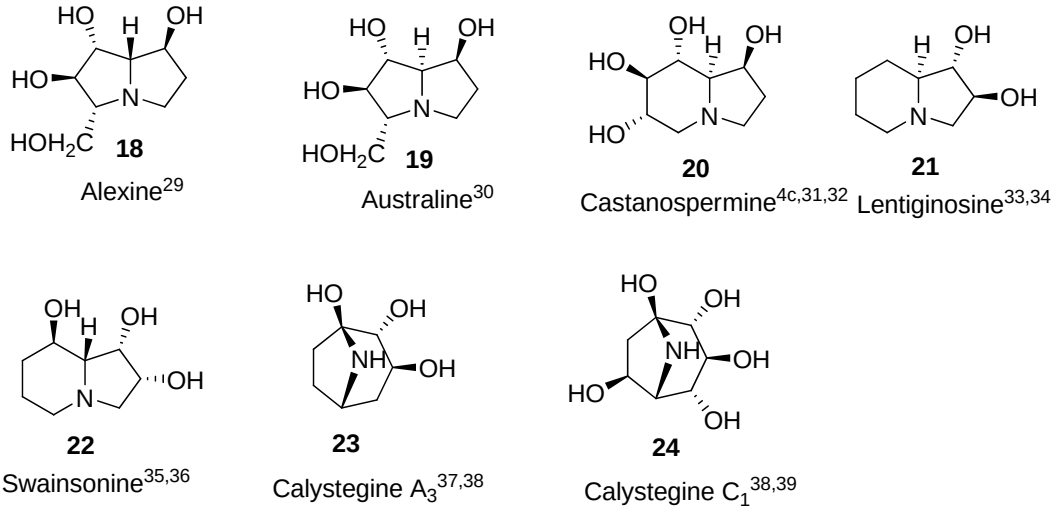


Figure 7. *Bicyclic azasugars.*

(c) Entities incorporating a nitrogen in more than one position, including the one in the ring, e.g. siastatins[40,41] **25**, nagstatins[42,43] **26** and aminocyclitols like mannostatins[44] **27** (Figure 8).

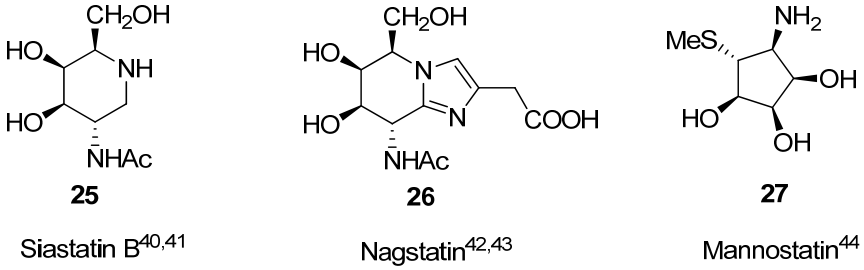


Figure 8.

Polyhydroxypiperidine derivatives comprise the main class of glycosidase inhibitors, with a great variety of compounds of natural origin, isolated from fungi, bacteria, and plants, besides the synthetic derivatives. Several of them show high inhibitory constants for both α- and β-glycosidases. Recently, these classes of compounds have attracted more attention owing to the inhibition of other enzymes such as glycosyl transferases, glycogen phosphorylase,[45] nucleoside phosphorylases[46] and sugar-nucleotide mutases[47].

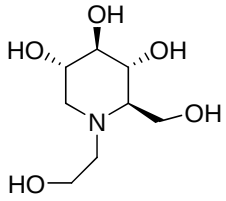
1.3-Therapeutic applications of Glycosidase inhibitors in the context of Biological activities

Carbohydrate branching or hydrolysis, catalyzed by enzymes, are widespread biological processes. The enzymes such as glycosyl transferases and glycosidases are involved in

the biosynthesis and degradation of oligosaccharides and glycoconjugates (glycoproteins, glycolipids, proteoglycans) and are found in nearly all forms of life. Their inhibition can affect the digestion of polysaccharides and the maturation, transport and secretion of glycoproteins. Because cell surface carbohydrates are involved in various biological functions such as cell-cell recognition, cell adhesion, cell-growth regulation, their implication in the immune response, oncogenesis, tumor metastasis, and differentiation of cells is no longer doubted. Therefore, search for inhibitors of glycosidases have been the hot area of research for the development of novel antiviral, anti-infective or anti-cancer agents. Few prominent among them are following.

1.3.A-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.



28

Figure 9. *Maglitol*

Deoxynojirimycin (DNJ) **15** was shown to have an inhibitory effect on mammalian α-glucosidases *in vitro* and *N*-alkylated-type analogs like Miglitol[48] (**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 (GLYSETTM).

1.3.B-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.[49] 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.[50] 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.

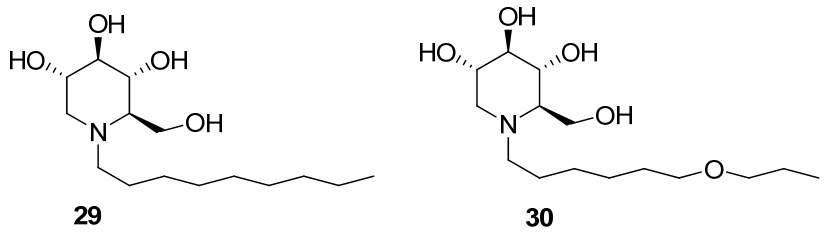


Figure 10. *DNJ derivatives*

1.3.C-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.[51]

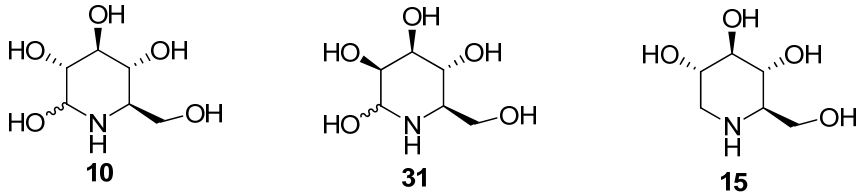
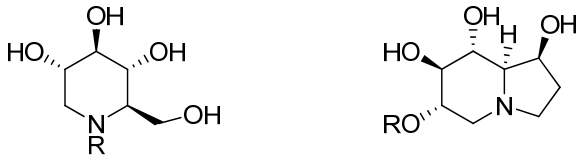


Figure 11. *Anti tumor agents*

A study of the inhibitory effect of imino sugars like nojirimycin **10**, mannonojirimycin **31** and deoxynojirimycin **15** (Figure 11) has been carried out by Tsuruoka and co-workers[51] in a model of pulmonary metastasis of mouse B16 melanoma.

1.3.D-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.[52] α-Glucosidase inhibitors, such as DNJ **15**, N-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*.[53]



15 R = H
32 R = *n*-Bu

20 R = H
33 R = butanoyl

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.[54] 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.[55-57]

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 undergoing phase II development for the treatment of HCV infection.[58]

1.3.E-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 **22** and swainsonine **20**,[59] 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.[60]

1.4-References

1. Lairson, L. L.; Withers, S. G. *Chem. Commun*. **2004**, 2243.
2. Wolfenden, R.; Lu, X. D.; and Young, G. *J. Am. Chem. Soc*. **1998**, *120*, 6814.
3. Zechel, D. L.; Withers, S. G. *Acc. Chem. Res*. **2000**, *33*, 11.
4. (a) Kirby, A. J. *Acc. Chem. Res.* **1984**, *17*, 305. (b) Gorenstein, D. G. *Chem. Rev.* **1987**, *87*, 1047. (c) Legler, G. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 319. (d) Sinnot, M. L. *Chem. Rev*. **1990**, *90*, 1171.
5. (a) McCarter, J.; Withers, S. G. *Curr. Opin. Struct. Biol*. **1994**, *4*, 885. (b) Davies, G.; Henrissat, B. *Structure* **1995**, *3*, 853. (c) Wang, Q.; Graham, R. W.; Trimbur, D.; Warren, R. A. J.; Withers, S. G. *J. Am. Chem. Soc*. **1994**, *116*, 11594.
6. Ganem, B. *Acc. Chem. Res*. **1996**, *29*, 340.
7. Conchie, J.; Hay, A. J.; Strachan, I.; Levvy, G. A. *Biochem. J.* **1967**, *102*, 929.
8. Lai, H. –Y.; Axelrod, B. *Biochem. Biophys. Res. Commun*. **1973**, *54*, 463.
9. (a) Paulsen, H.; Sangster, I.; Heyns, K. *Chem. Ber.* **1967**, *100*, 802. (b) Paulsen, H.; Todt, K. *Adv. Carbohydr. Chem. Biochem*. **1968**, *23*, 116.
10. (a) Inouye, S.; Tsuruoka, T.; Niida, T. *J. Antibiot., Ser. A*. **1966**, *19*, 288. (b) Inouye, S.; Tsuruoka, T.; Ito, T.; Niida, T. *Tetrahedron* **1968**, *24*, 2125.
11. (a) Heightman, T. D.; Vasella, A. T. *Angew. Chem. Int. Ed*. **1999**, *38*, 750. (b) Sears, P.; Wong, C. –H. *Angew. Chem. Int. Ed*. **1999**, *38*, 2300. (c) Bols, M. *Acc. Chem. Res*. **1998**, *31*, 1. (d) Stütz, A. E. *Iminosugars as Glycosidase Inhibitors: Nojirimycin and Beyond*; Wiley-VCH: Weinheim, Germany, **1999**. (e) Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. *Chem. Rev*. **2002**, *102*, 515. (f) Nishimura, Y.; Shitara, E.; Adachi, H.; Toyoshima, M.; Nakajima, M.; Okami, Y.; Takeuchi, T. *J. Org. Chem.* **2000**, *65*, 2. (g) Afarinkia, K.; Bahar, A. *Tetrahedron: Asymmetry* **2005**, *16*, 1239. (h) Watson, A. A.; Fleet, G. W. J.; Asano, N.; Molyneux, R. J.; Nash, R. J. *Phytochemistry* **2001**, *56*, 265. (i) Whalem, L. J.; Wong, C.-H. *Aldrichimica Acta*, **2006**, *39*, 63. (j) Gravier-Pelletier, C.; Le Merrer, Y. *Curr. Org. Synth.* **2007**, *4*, 1. (k) Wrodnigg, T. M.; Steiner, A. J.; Bernhard, B. J. *Anti-Cancer Agents Med. Chem.*, **2008**, *8*, 77. (l) Pearson, M. S. M.; Mathé-Allainmat, M.; Fargeas, V.; Lebreton, J. *Eur. J. Org. Chem.* **2005**, 2159.
12. Krasikov, V. V.; Karelov, D. V.; Firsov, L. M. *Biochemistry (Moscow)* **2001**, *66*, 267.
13. Szczepina, M. G.; Johnston, B. D.; Yuan, Y.; Svensson, B.; Pinto, B. M. *J. Am. Chem. Soc.* **2004**, *126*, 12458.
14. (a) Lalégerie, P.; Legler, G. *Biochimie* **1982**, *64*, 977. (b)Yuasa, H.; Saotome, C.; Osamu, K. *Trends Glycosci. Glycotechnol*. **2002**, *14*, 231.
15. Asano, N.; Nash, R. J.; Molyneux, R. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2000**, *11*, 1645.

16. Krämer, B.; Franz, T.; Picasso, S.; Pruschek, P.; Jäger, V. *Synlett* **1997**, 295.
17. Welter, A.; Jadot, J.; Dardenne, G.; Marlier, M.; Casimir, J. *Phytochemistry* **1976**, *15*, 747.
18. (a) Merrer, Y. L.; Poitout, L.; Depezay, J-C.; Dosbaaa, I.; Geoffroy, S.; Foglietti, M J. *Bioorg. Med. Chem*. **1997**, *5*, 519. (b) Watanabe, S.; Kato, H.; Nagayama, K.; Abe, H. *Biosci. Biotech. Biochem.* **1995**, *59*, 936.
19. Nash, R. J.; Bell, E. A.; Williams, J. M. *Phytochemistry* **1985**, *24*, 1620.
20. Fleet, G. W. J.; Nicholas, S. J.; Smith, P. W.; Evans, S. V.; Fellows, L. E.; Nash, R. J. *Tetrahedron Lett*. **1985**, *26*, 3127.
21. Shibata, T.; Nakayama, O.; Tsurumi, Y.; Okuhara, M.; Terano, H.; Kohsaka, M. *J. Antibiot*. **1988**, *41*, 296.
22. Witte, J. F.; McClard, R. W. *Tetrahedron Lett.* **1991**, *32*, 3927.
23. Legler, G.; Jülich, E. *Carbohydr. Res*. **1984**, *128*, 61.
24. Yagi, M.; Kouno, T.; Aoyagi. Y.; Murai, H. *Nippon Nogei Kagaku Kaishi* **1976**, *50*, 571.
25. Andersen, S. M.; Ebner, M.; Ekhart, C. W.; Gradnig, G.; Legler, G.; Lundt, I.; Stütz, A. E.; Withers, S. G.; Wrodnigg, T. *Carbohydr. Res*. **1997**, *301*, 155.
26. Koyama, M.; Sakamura, S. *Agric. Biol. Chem*. **1974**, *38*, 1111.
27. Asano, N.; Oseki, K.; Kizu, H.; Matsui, K. *J. Med. Chem*. **1994**, *37*, 3701.
28. (a) Qian, X.; Moris-Varas, F.; Fitzgerald, M. C.; Wong, C.-H. *Bioorg. Med. Chem.* **1996**, *4*, 2055. (b) Qian, X.; Moris-Varas, F.; Wong, C.-H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 553.
29. (a) Nash, R. J.; Fellows, L. E.; Dring, J. V.; Fleet, G. W. J.; Derome, A. E.; Hamor, T. H.; Seafield, A. M.; Watkin, D. J. *Tetrahedron Lett*. **1988**, *29*, 2487. (b) Molyneux, R. J.; Pan, Y. T.; Tropea, J. E.; Benson, Kaushal, G. P.; Elbein, A. D. *Biochemistry* **1991**, *30*, 9981.
30. Molyneux, R. J.; Benson, M.; Wong, R. Y.; Tropea, J. E.; Elbein, A. D. *J. Nat. Prod.* **1988**, *51*, 1198.
31. Hohenschutz, L. D.; Bell, E. A.; Jewess, P. J.; Leworthy, D. P.; Pryce, R. J.; Arnold, E.; Clardy, J. *Phytochemistry* **1981**, *20*, 811.
32. Trugnan, G.; Rousset, M.; Zweibaum, A. *FEBS* **1986**, *199*, 28.
33. Pastuszak, I.; Molyneux, R. J.; James, L. F.; Elbin, A. D. *Biochemistry* **1990**, *29*, 1886.
34. Brandi, A.; Cicchi, S.; Cordero, F. M.; Frignoli, R.; Goti, A.; Picasso, S.; Vogel, P. *J. Org. Chem*. **1995**, *60*, 6806.
35. Colegate, S. M.; Dorling, P. R.; Huxtable, C. R. *Aust. J. Chem*. **1979**, *32*, 2257.
36. (a) Cenci di Bello, I.; Fleet, G. W. J.; Namgoong, S. K.; Tanado, K.-I.; Winchester, B. *Biochem. J*. **1989**, *259*, 855. (b) Winchester, B.; Al Daher, S.; Carpenter, N. C.; Cenci di Bello, I.; Choi, S. S.; Fairbanks, A. J.; Fleet, G. W. J. *Biochem. J.* **1993**, *290*, 743.

37. (a) Tepfer, D.; Goldmann, A.; Pamboukdjian, N.; Maille, M.; Lepingle, A.; Chevalier, D.; Denarie, J.; Rosenberg, C. *J. Bacteriol.* **1988**, *170*, 1153. (b) Goldmann, A.; Milat, M.–L.; Ducrot, P.–H.; Lallemand, J.–Y; Maille, M.; Lepingle, A.; Charpin, I.; Tepfer, D. *Phytochemistry* **1990**, *29*, 2125.
38. Asano, N.; Kato, A.; Oseki, K.; Kizu, H.; Matsui, K. *Eur. J. Biochem.* **1995**, *229*, 369.
39. (a) Asano, N.; Oseki, K.; Tomioka, E.; Kizu, H.; Matsui, K. *Carbohydr. Res*. **1994**, *259*, 243. (b) Asano, N.; Tomioka, E.; Kizu, H.; Matsui, K. *Carbohydr. Res.* **1994**, *253*, 235.
40. Umezawa, H.; Aoyagi, T.; Komiyama, T.; Morishama, H.; Hamada, M.; Takeuchi, T. *J. Antibiot*. **1974**, *27*, 963.
41. (a) Satoh, T.; Nishimura, Y.; Kondo, S.; Takeuchi, T. *J. Antibiot.* **1996**, *49*, 321. (b) Nishimura, Y.; Kondo, S.; Takeuchi, T.; Kudo, T. *J. Antibiot*. **1994**, *47*, 101.
42. Aoyama, T.; Naganawa, H.; Suda, H.; Uotani, K.; Aoyagi, T.; Takeuchi, T. *J. Antibiot*. **1992**, *45*, 1557.
43. (a) Tatsuta, K.; Miura, S.; Ohta, S.; Gunji, H. *J. Antibiot*. **1995**, *48*, 286. (b) Tatsuta, K. *Pure Appl. Chem*. **1996**, *68*, 1341. (c) Aoyagi, T.; Suda, H.; Uotani, K.; Kojima, F.; Aoyama, T.; Horiguchi, K.; Hamada, M.; Takeuchi, T. *J. Antibiot*. **1992**, *45*, 1404.
44. (a) Aoyagi, T.; Yamamoto, T.; Kojiri, K.; Morishima, H.; Nagai, M.; Hamada, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1989***, 42,* 883. (b) Morashima, H.; Kojiri, K.; Yamamoto, T.; Aoyagi, T.; Nakamura, H.; Iitaka, Y. *J. Antibiot.* **1989**, *42*, 1008.
45. (a) Schuster, M.; Blechert, S. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 1809. (b) Jakobsen, P.; Lundbeck, J. M.; Kristiansen, M.; Breinholt, J.; Demuth, H.; Pawlas, J.; Candela, M. P. T.; Andersen, B.; Westergaard, N.; Lundgren, K.; Asano, N. *Bioorg. Med. Chem*. **2001**, *9*, 733. (c) Compain, P.; Martin, O. R. *Bioorg. Med. Chem*. **2001**, *9*, 3077.
46. Fedorov, A.; Shi, W.; Kicska, G.; Fedorov, E.; Tyler, P. C.; Furneaux, R. H.; Hanso, J. C.; Gainsford, G. J.; Larese, J. Z.; Schramm, V. L.; Almo, S. C. *Biochemistry* **2001**, *40*, 853.
47. Lee, R. E.; Smith, M. D.; Pickering, L.; Fleet, G. W. J. *Tetrahedron Lett*. **1999**, *40*, 8689.
48. Bollen, M.; Stalmans, W. *Eur. J. Biochem.* **1989**, *181*, 775.
49. Butters, T. D.; Dwek, R. A.; Platt, F. M. *Curr. Top. Med. Chem.* **2003**, *3*, 561.
50. Platt, F. M.; Butters, T. D. *Exp. Rev. Mol. Med.* **2000**, 1.
51. Nishimura, Y. *Curr. Top. Med. Chem***. 2003**, *3*, 575 and references cited therein.
52. Greimel, P.; Spreitz, J.; Stütz, A. E.; Wrodnigg, T. M. *Curr. Top. Med. Chem.* **2003**, *3*, 513.
53. Fleet, G. W. J.; Karpas, A.; Dwek, R. A.; Fellows, L. E.; Tyms, A. S.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Smith, P. W.; Son, J. C.; Wilson, F.; Witty, D. R.; Jacob, G. S.; Rademacher, T. W. *FEBS Lett.* **1988**, *237*, 128.

54. Taylor, D. L.; Nash, R.; Fellows, L. E.; Kang, M. S.; Tyms, A. S. *Antiviral Chem. Chemother.* **1992**, *3*, 273.
55. Fischer, P. B.; Collin, M.; Karlsson, G. B.; James, W.; Butters, T. D.; Davis, S. J.; Gordon, S.; Dwek, R. A.; Platt, F. M. *J. Virol.* **1995**, *69*, 5791.
56. Fischer, P. B.; Karlsson, G. B.; Butters, T. D.; Dwek, R. A.; Platt, F. M. *J. Virol.* **1996**, *70*, 7143.
57. Fischer, P. B.; Karlsson, G. B.; Dwek, R. A.; Platt, F. M. *J. Virol.* **1996**, *70*, 7153.
58. Sorbera, L. A.; Castaũer, J.; García-Capdevila, L. *Drugs Fut.* **2005**, *30*, 545.
59. Gross, P. E.; Baptiste, J.; Fernandes, B.; Baker, M.; Dennis, J. W. *Cancer Res.* **1994**, *54*, 1450.
60. Humphries, M. J.; Matsumoto, K.; White, S. L.; Molyneux, R. J.; Olden, K. *Cancer Res.* **1988**, *48*, 1410.

Chapter-2

Synthesis and Biological Evaluation of Piperidine 1- N-Iminosugars

Science discovery is an intuition which turns out to be reality at the end of it. There is no difference between a scientist developing a marvelous discovery and an artist making a painting. – C. Rubbia (Italian physicist).

Section-A::An Introduction to 1-*N*-Iminosugars

2.1-Transition state analogue of glycosidase inhibitors: Development of 1-*N*-iminosugars

The search for selective and more potent glycosidase inhibitors has led to the extensive research interest in this area. An attractive approach to potent inhibitors is to create compounds that 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. To be able to design transition state analogues of glycosidases one has to analyze the mechanism for the cleavage of glycosidic bond.[1]

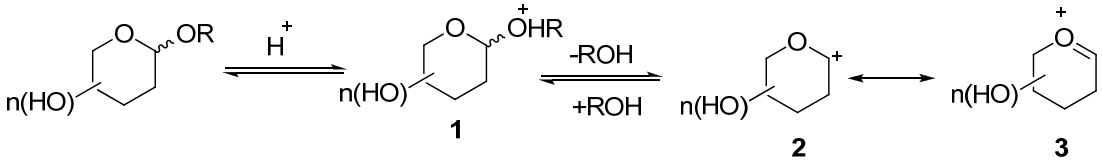


Figure 1. *Hydrolysis of Glycosidic bond*

As depicted (Figure 1), there are three important reaction intermediates **1**, **2**, and **3** depending upon the position of the charge build up during the glycoside hydrolysis. A compound that could resemble any of the intermediates **1-3** should be an inhibitor of the respective glycosidases.

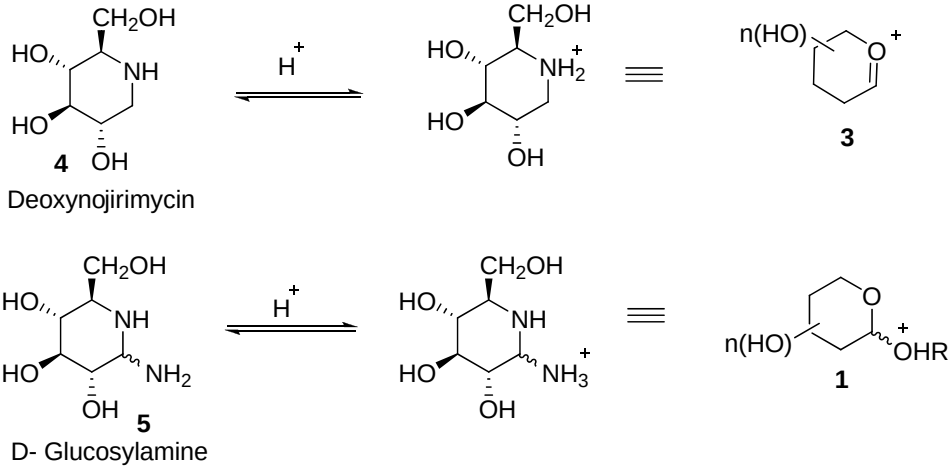


Figure 2. *Transition state resemblance of* ***4*** *and* ***5.***

There are a number of compounds that fulfill this criterion. One such group of compounds belongs to the deoxynojirimycin (**4**) class. These compounds resemble monosaccharides but the ring oxygen has been replaced with a nitrogen atom. Thus, if protonated at the basic nitrogen atom, these compounds become the analogues of **3**. Other class, the type D- glucosylamine **5**[2] is a group of glycosidase inhibitors which resemble **1** upon protonation (Figure 2).

Until the last decade, no glycosidase inhibitor that was an analogue of **2** was known. It was long interpreted that **2** was insignificant in the glycoside cleavage process and in many theoretical discussions of glycosidic cleavage, **2** was indeed ignored at the expense of **3**. In 1991, Reymond and co-workers reported isolating a catalytic antibody, (using transition state analogue **7** as a hapten) that could catalyze the hydrolysis of a tetrahydropyranyl ether, a simple model of a glycosidic bond (Figure 3).[3]

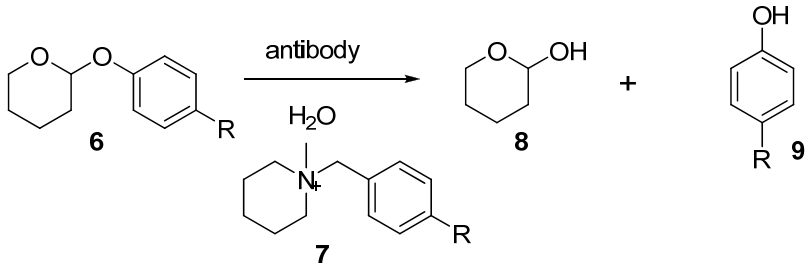


Figure 3.

Compound **7** was considered an analogue of a carbocation at the anomeric center and in principle, an analogue of ion **2**, except for the lack of hydroxyl groups. This report suggested that analogues of **2** could be transition state analogues of glycoside cleavage and since **2** was a significant transition state in the case of a β-glycosidase reaction, analogues of this would be inhibitors of β-glycosidases.

This finding led to a spurt of activity of creative chemical design of anomer selective β-glycosidase inhibitors led by the groups of Bols,[4] Ichikawa[5] and Nishimura.[6] These new class of designed molecules were termed as 1-azasugar or 1-*N*-iminosugar class of glycosidase inhibitors. The first 1-*N* iminosugar synthesized was the D-glucose type, isofagomine (**10**). Designed by Bols and co-workers,[4] this molecule turned out to be an

extremely potent inhibitor of β-glucosidase (sweet almonds, *Ki* = 0.11 μM). Subsequently, extremely potent and selective β glycosidase inhibitors were designed and synthesized. The most prominent amongst them are depicted in Figure 4 (**11**-**17**).

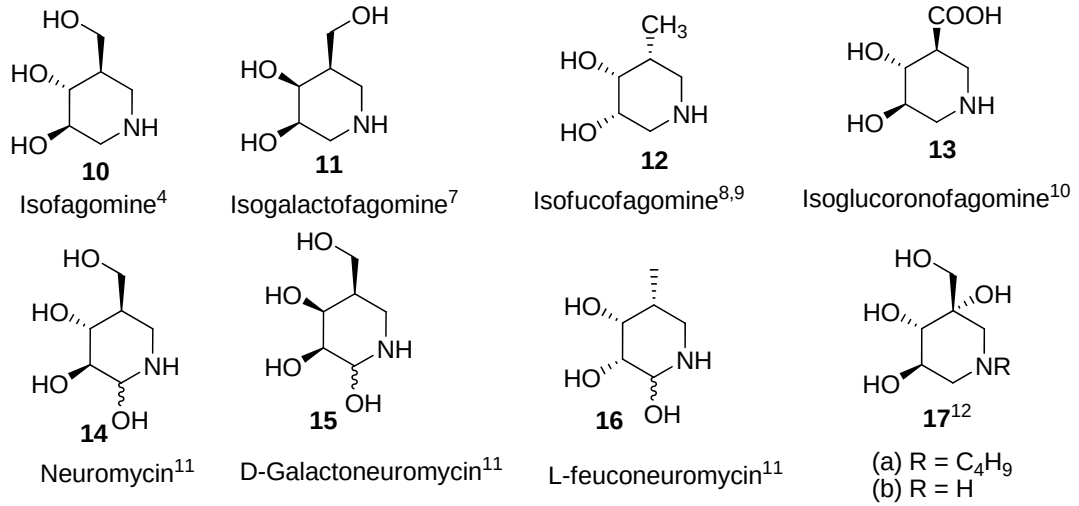


Figure 4. *Various 1-N Iminosugars.*

2.2-Piperidine triols as glycosidase inhibitors

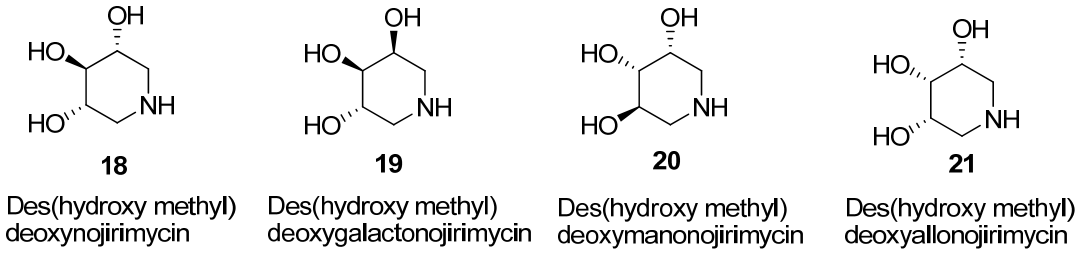


Figure 5. *Piperidine triols*

Dale and co-workers[13] systematically studied the inhibition of sweet almond β-glucosidase by a wide variety of normal and deoxy-sugars. While the stereochemical configurations of individual ring hydroxyls were important, removal of the C-6 hydroxymethyl substituent from type **4** sugars, altogether had remarkably little effect on enzyme substrate interactions. This surprising finding led Ganem and co-workers[14] to postulate that stereochemically simpler or nor-analogues of deoxynojirimycin **4** may also be good inhibitors of glycosidases.

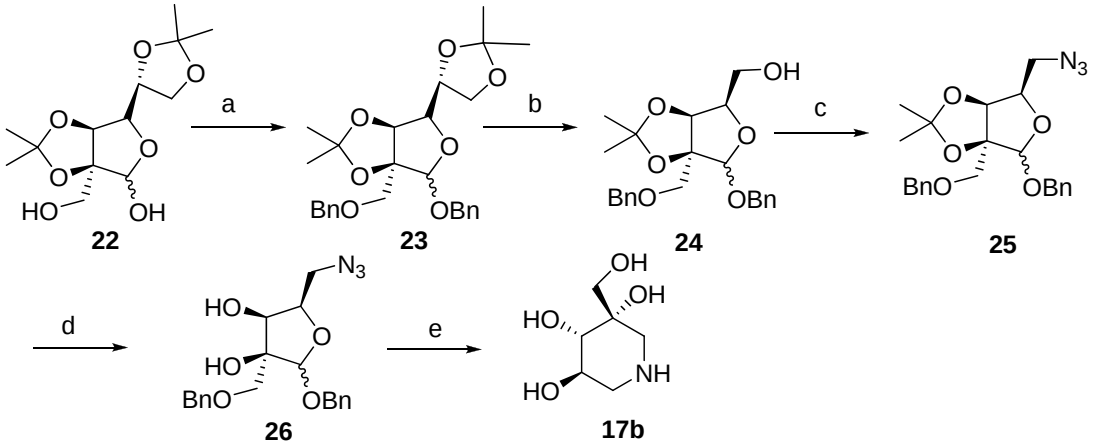
Thus, along with the gluco-analogue **18**, Ganem and co-workers[14] prepared and studied the galactose-**19** and mannose-analogues **20** of des(hydroxymethyl) deoxynojirimycin and

found that these molecules were almost as potent glycosidase inhibitors as their parent deoxynojirimycins (Figure 5).

Five years later, Kusano and co-workers[15] isolated triols **18**, **19** and **21** from *Eupatorium fortunei* TURZ and showed that these triols were active components of the extracts of this plant, used in traditional Chinese and Japanese folk medicine as a diuretic, antipyretic, emmenagogue and anti-diabetic agent. These structural analogues of the parent deoxynojirimycin can also be considered as 1-*N*-iminosugar type glycosidase inhibitors. These azasugars have led to the great enhancement in the research activity including the synthesis and their further biological evaluation. Given below are the few synthetic approaches towards these molecules.

2.3-Synthetic Approaches towards 5-hydroxy isofagomine

Ichikawa's approach:[13]



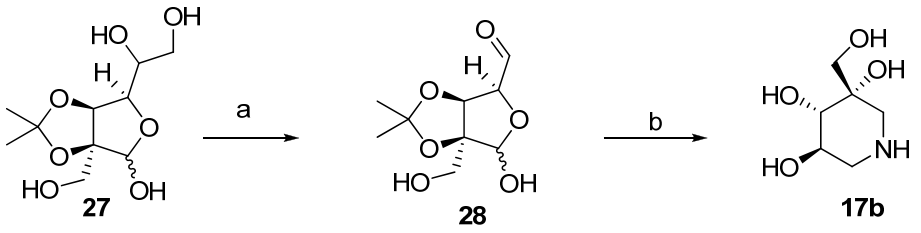
Reagents and conditions: (a) Ag_2O, BnBr, KI, DMF, rt, 10 h, 85 %; (b) (i) 70 % AcOH, rt, overnight, 80 %; (ii) $NaIO_4$, MeOH-H_2O, 0 °C, 30 min; (iii) $NaBH_4$, rt, overnight, 84 %; (c) (i) $(CF_3SO_2)_2O$, DCM, py, -40 °C to 0 °C, 30 min; (ii) NaN_3, DMF, 80 °C, overnight, 94 % over two steps; (d) 60 % TFA, rt, overnight, 83 %; (e) (i) 20 % $Pd(OH)_2$, H_2, aq. HCl, *pH* = 3; (ii) Dowex 50W-X8 [H^+] eluted with 5 % NH_4OH, 95 %.

Scheme 1.

Ichikawa and co-workers first designed and synthesized 5-hydroxy isofagomine **17b** in eight steps starting from **22** which was derived from D-mannose (Scheme 1).

Bols's approach:[16]

Bols and co-workers published a short three-step synthesis of 5-hydroxy isofagomine **17b** (Scheme 2) starting from **27** which in turn was derived from D-mannose in three steps.

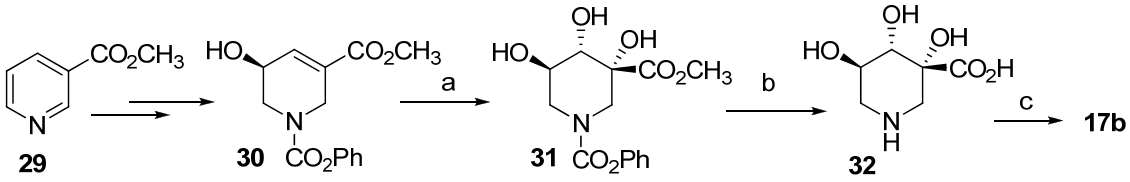


Reagents and conditions: (a) $NaIO_4$, MeOH-H_2O (1:1), 2 h, 0 °C, 93 %; (b) (i) NH_3, H_2, 37 atm, 5 % Pd/C, MeOH, 67 %; (ii) HCl, H_2O, MeOH, 50-55 °C, 2 h, ~100 %.

Scheme 2.

Ganem's approach:[17]

The approach of Ganem and co-workers for the synthesis of **17b** was based upon the use of selective Fowler reductions as the key step. The synthesis (Scheme 3) utilized nine steps and had an overall yield of 49 %.

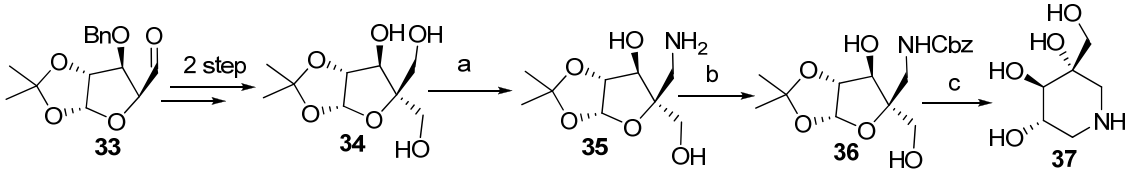


Reagents and conditions: (a) OsO_4, NMO, 81 %; (b) LiOH, 95 %; (c) (i) HMDS-py, 110 °C; (ii) LAH, THF, 83 %.

Scheme 3.

Dhavle's approach:[18]

This approach involved the aldol–Cannizzaro reaction of α-D xylopentodialdose followed by hydrogenolysis to afford the triol **34** which was converted to tetrol **37** (Scheme 4)**.**



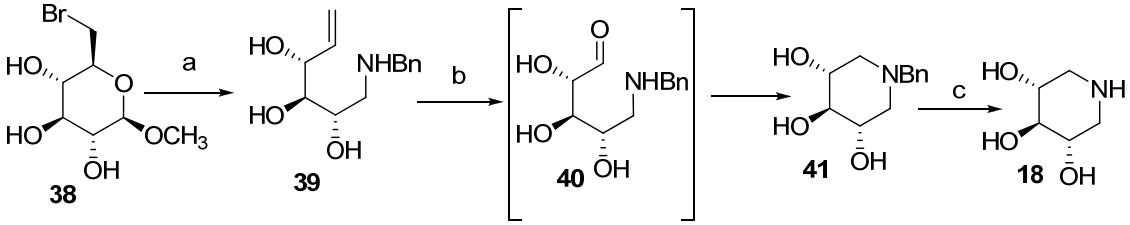
Reagents and conditions: (a) (i) TsCl, pyridine, -15 ^{0}C, 24 h, 57%; (ii) NaN_3, DMF, 110 ^{0}C, 60 h, 30%; (iii) LAH, THF, 0 ^{0}C – rt, 1.5 h; (b) CbzCl, $NaHCO_3$, EtOH–H_2O, 4 h, 83%; (c) (i) TFA–H_2O (3 : 2), 0 ^{0}C – rt, 2.5 h, 97%; (ii) $HCOONH_4$, 10% Pd/C, MeOH, reflux, 45 min, 91%.

Scheme 4.

2.4-Synthetic approaches towards 3,4,5-piperidine triols

Ganem's approach:[14]

Ganem and co-workers synthesized **18**, utilizing their strategy (Scheme 5) of reductive opening of bromopyranose **38** sugars.

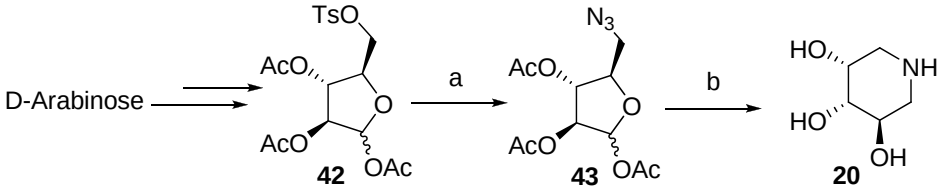


Reagents and conditions: (a) Zn, PrOH/H_2O (19:1), $NaBH_3CN$, $BnNH_2$, reflux, 2h; (b) (i) TFA, DCM; (ii) O_3, DCM, -78 ° C; (iii) Me_2S, $NaBH_3CN$, MeOH, rt, 3 h; (c) H_2, Pd/C, MeOH.

Scheme 5.

Legler's approach[19]

Legler's group synthesized des(hydroxymethyl)manonojirimycin **20** from D-arabinose, in seven steps (Scheme 6), with an overall yield of 41%.

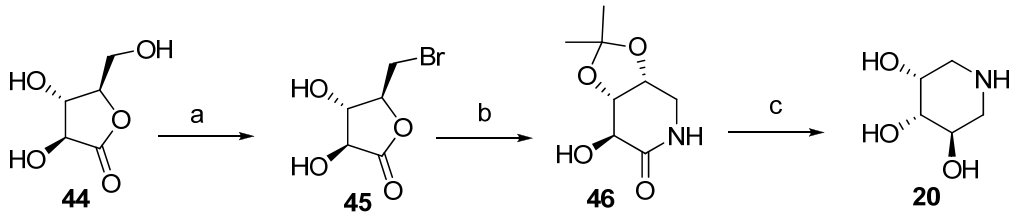


Reagents and conditions: (a) NaN_3, DMF, 90 °C, 94 %; (b) (i) NaOMe, MeOH, quant.; (ii) Pd/C, H_2, 1 atm, 16 h, 85 %.

Scheme 6.

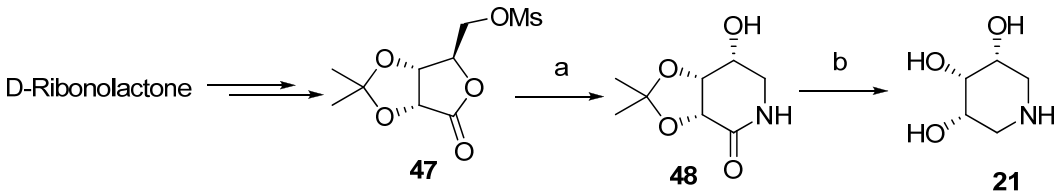
Lundt's approach:[20]

Lundt and co-workers derived various stereoisomers of 3, 4, 5-piperidine triols from aldonolactones by following the steps as shown in Schemes 7-10.



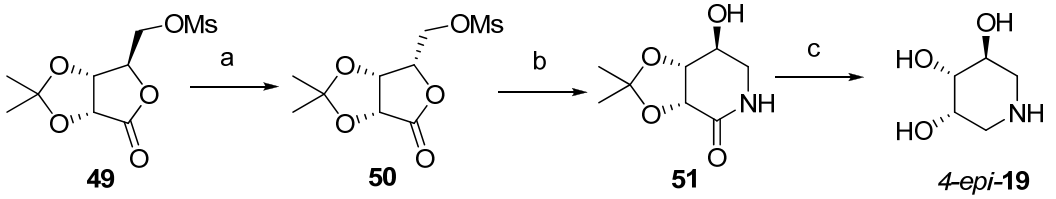
Reagents and conditions: (a) HBr-AcOH; (b) (i) 25 % aq. NH_3, 2 h, rt; (ii) 2,2- Dimethoxy propane, *p*-TSA, 50 % over three steps; (c) $NaBH_4$, TFA, dioxane, 100 °C, 3 h; IR 120 (H+); aq. HCl, 66 %.

Scheme 7.



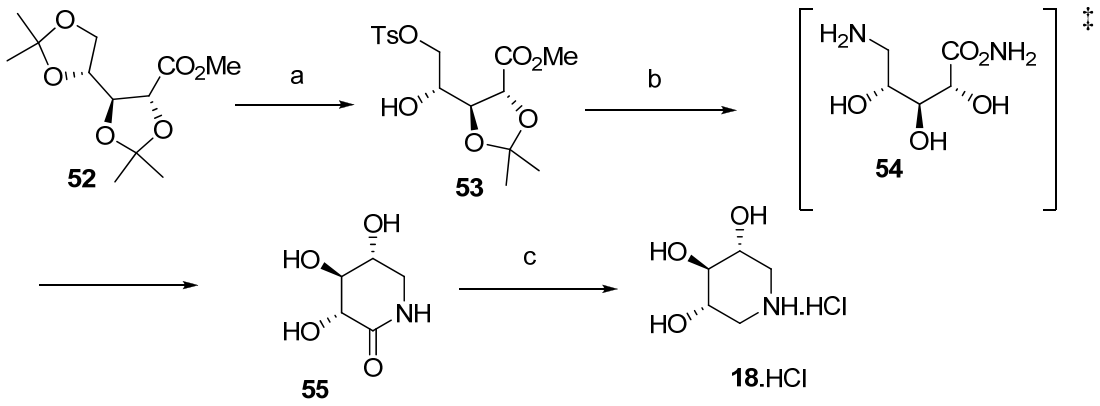
Reagents and conditions: (a) aq. NH_3, 84 %; (b) $NaBH_4$, AcOH, dioxane, 100 ^{0}C, 5 h; IR 120 (H+); aq. HCl, 50 %.

Scheme 8.



Reagents and conditions: (a) (i) KOH/ H_2O, 3 h; then aq. HCl to pH = 3, 84 %; (ii) MsCl, py, 1 h, 0 ^{0}C, 91 %; (b) 25 % aq. NH_3, 4h, rt, 70 %; (c) $NaBH_4$, AcOH, dioxane, 100 ^{0}C, 5h; IR 120 (H+); aq. HCl, 57 %.

Scheme 9.

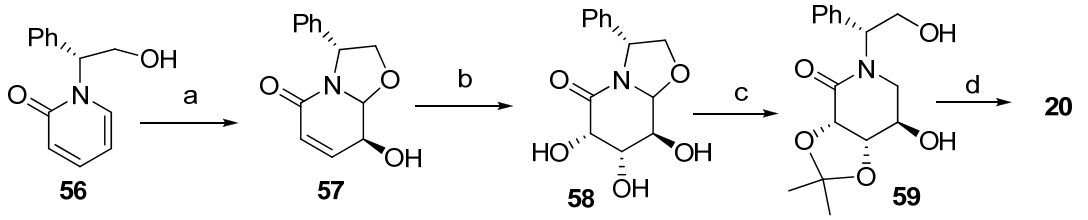


Reagents and conditions: (a) (i) CH_3OH, H_2O, IR 120 (H+) (ii) TsCl, py, 0 ^{0}C, 0.5 – 1.5 h, 38 %; (b) aq. NH_3, rt, 16 h; then 1 % HCl-MeOH, 60 °C, 4 h, IR 400 (HCO_3^-), 38 %; (c) (i) HMDS, TMSCl, CH_3CN, 1 h, 82 ^{0}C then $BH_3.SMe_2$, dioxane, 100 ^{0}C, 5 h; then 1M HCl, 100 ^{0}C, 1 h, 95%.

Scheme 10.

Amat's approach:[21]

Approach of Amat and co-workers was based (Scheme 11) on the stereoselective *m*-CPBA oxidation of phenylglycinol-derived 2-pyridone **57**. The synthesis of **20** involved six steps with an overall yield of 14%. The yield of the key step was rather low (~ 40%).

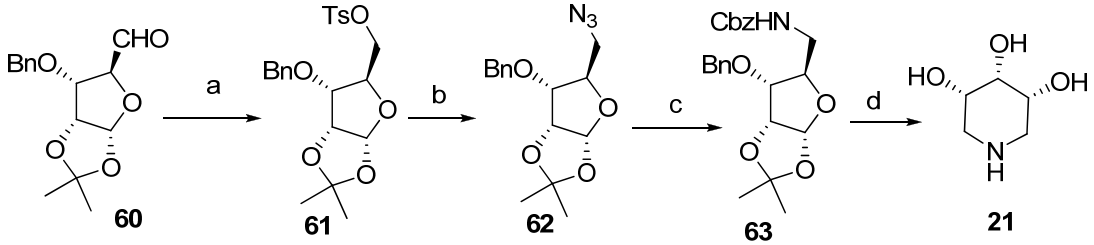


Reagents and conditions: (a) *m*-CPBA, DCM, 25 ^{0}C, 4 days, single diastereomer, 35 - 40 %; (b) OsO_4, NMO, aq. CH_3CN, 25 ^{0}C, 24 h, 78 %; (c) (i) 2,2-Dimethoxypropane, p TsOH, DCM, 25 ^{0}C, 24 h, 85 %; (ii) BH_3.THF, -78 ^{0}C (30 min) to 25 ^{0}C (3 h), 87 %; (d) (i) H_2, Pd/C, MeOH, 65 %; (ii) MeOH, HCl, 95 %.

Scheme 11.

Dhavale's approach:[22]

Dhavale's group utilized chiral pool derived aldehydes **60** in their approach (Scheme 12), towards the synthesis of *meso* triol **21**.

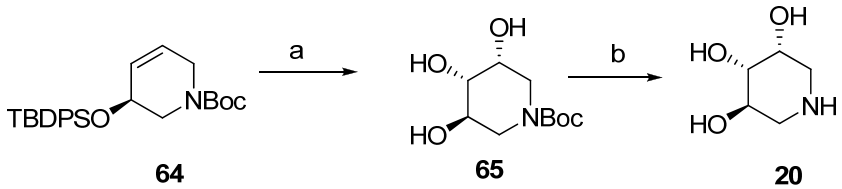


Reagents and conditions: (a) (i) $NaBH_4$, MeOH, rt, 15 min; (ii) TsCl, py, rt, 12 h, 85 %; (b) NaN_3, DMSO, 100 ^{0}C, 6 h, 86 %; (c) (i) 10 % Pd/C, H_2, 80 psi, MeOH, 12 h; (ii) CbzCl, $NaHCO_3$, EtOH-H_2O (8:2), rt, 2 h, 93 % over two steps; (d) (i) TFA-H_2O (3:2), rt, 2 h; (ii) 10 % Pd/C, H_2, 8 psi, MeOH, 12 h, 94 %.

Scheme 12.

Takhata's approach:[23]

Takhata carried out the synthesis of **20** in 9 steps through the key intermediate **64** which was obtained via enzymatic resolution (Scheme 13).

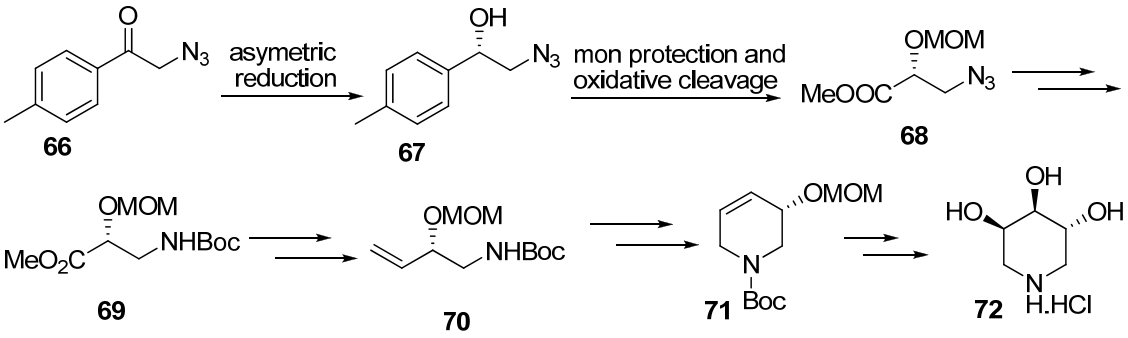


Reagents and conditions: (a) (i) cat. OsO_4, NMO, acetone, 74%; (ii) TBAF, THF, rt; (b) 10% HCl, dioxane, reflux.

Scheme 13.

Rama Rao's approach:[24]

The synthesis of another analogue of piperidinol **72** was obtained in 13 steps involving the synthesis of chiral intermediate **67** by chiral reduction of corresponding ketone **66** (Scheme 14).



Scheme 14.

From the above discussion it is quite clear that there are basically 3 kinds of approaches which are utilized for the synthesis of various 1-*N*-iminosugars (Figure 6).

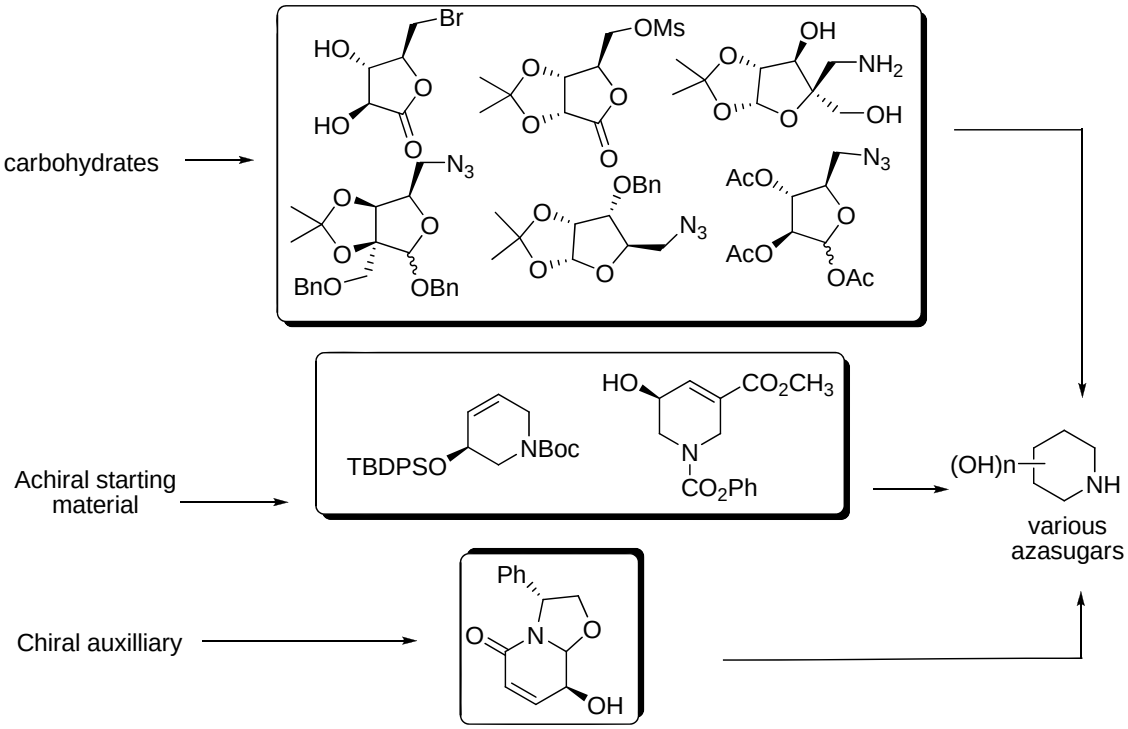


Figure 6.

One primarily utilizes carbohydrates as the building block material to fix the required stereochemistry in the required azaugar. 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 then 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 use of achiral material which are converted to chiral molecules by the use of asymmetric resolution (most common being enzymatic) and then carried forward for the synthesis. Use of chiral auxiliary has been another approach for installing requisite stereocentres which is removed finally at the latter stage of the synthesis.

2.5-References

1. (a) Sinnott, M. L. *Chem. Rev.* **1990**, *90*, 1171. (b) Zechel, D. L.; Withers, S. G. *Acc. Chem. Res*. **2000**, *33,* 11.
2. Ganem, B. *Acc. Chem. Res.* **1996**, *29*, 340.
3. Reymond, J.–L.; Janda, K. D.; Lerner, R. A. *Angew. Chem. Int. Ed.* **1991**, *30*, 1711.
4. (a) Jespersen, T. M.; Dong, W.; Sierks, M. R.; Skrydstrup, T.; Lundt, I.; Bols, M. *Angew. Chem. Int. Ed.* **1994**, *33*, 1778. (b) Jespersen, T. M.; Bols, M.; Sierks, M. R.; Skrydstrup, T. *Tetrahedron* **1994**, *50*, 13449.
5. Ichikawa, Y.; Igarashi, Y.; Ichikawa, M.; Suhara, Y. *J. Am. Chem. Soc.* **1998**, *120*, 3007.
6. Nishimura, Y.; Shitara, E.; Adachi, H.; Toyoshima, M.; Nakajima, M.; Okami, Y.; Takeuchi, T. *J. Org. Chem.* **2000**, *65*, 2 and references therein.
7. Ichikawa, Y.; Igarashi, Y. *Tetrahedron Lett*, **1995**, *36*, 4585.
8. Igarashi, Y.; Ichikawa, M.; Ichikawa, Y. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 553.
9. (a) Hansen, A.; Tagmose, T. M.; Bols, M. *Chem. Commun.* **1996**, 2649. (b) Hansen, A.; Tagmose, T. M.; Bols, M. *Tetrahedron* **1997**, *53*, 697.
10. Igarashi, Y.; Ichikawa, M.; Ichikawa, Y. *Tetrahedron Lett*. **1996**, *37*, 2707.
11. Liu, H.; Liang, X.; Søhoel, H.; Bülow, A.; Bols, M. *J. Am. Chem. Soc*. **2001**, *123*, 5116.
12. Ichikawa, M.; Igarashi, Y.; Ichikawa, Y. *Tetrahedron Lett*. **1995**, *36*, 1767.
13. Dale, M. P.; Ensley, H. E.; Kern, K.; Sastry, K. A. R.; Beyers, L. D. *Biochemistry* **1985**, *24*, 3530.
14. Bernotas, R. C.; Papandreou, G.; Urbach, J.; Ganem, B. *Tetrahedron Lett*. **1990**, *31*, 3393.
15. Sekioka, T.; Shibano, M.; Kusano, G. *Nat. Med*. **1995**, *49*, 332.
16. Bols, M. *Tetrahedron Lett.* **1996**, *37*, 2097.
17. Zhao, G.; Deo, U. C.; Ganem, B. *Org. Lett*. **2001**, *3*, 201.
18. Matin, M. M.; Sharma, T.; Sabharwal, S. G.; Dhavale, D. D. *Org. Biomol. Chem.* **2005**, *3*, 1702.
19. Legler, G.; Stütz, A. E.; Immich, H. *Carbohydr. Res*. **1995**, *272*, 17.
20. Godskesen, M.; Lundt, I.; Madsen, R.; Winchester, B. *Bioorg. Med. Chem*. **1996**, *4*, 1857.
21. Amat, M.; Llor, N.; Huguet, M.; Molins, E.; Espinosa, E.; Bosch, J. *Org. Lett*. **2001**, *3*, 3257.
22. Patil, N. T.; John, S.; Sabharwal, S. G.; Dhavale, D. D. *Bioorg. Med. Chem*. **2002**, *10*, 2155.
23. H. Ouchi, Y. Mihara and H. Takahata, *J. Org. Chem.* **2005**, *70*, 5207.
24. M. S. Reddy, M. Narender and K. R. Rao, *Tetrahedron* **2007**, *63*, 331.

Section-B::Result and Discussion

2.1 Methodology and retrosynthetic analysis

From the discussion appended in the previous chapter, it is quite clear that though there are many synthetic approaches known in the literature towards the synthesis of 1-*N*-iminosugars, most of them end up giving only a single product starting from a single carbohydrate substrate. 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 of 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 that 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 *K*i of a similar magnitude[1].

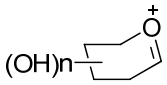


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Figure 1.

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.

In order to provide a versatile methodology and to discover new and more potent inhibitors, we took up the program of synthesizing polyhydroxypiperidine class of azasugars and evaluation of their enzyme inhibitory activities.

Towards this end, we envisioned a template of type **2** (Figure 2) in a bid to provide a versatile route for the synthesis of these 1-azasugars. The *exo*-cyclic double bond of this structure was further visualized to provide a handle to create multitude of functionalities. With this background in mind, the synthesis of enantiomeric precursors **3** and **4** were taken up as an immediate target.

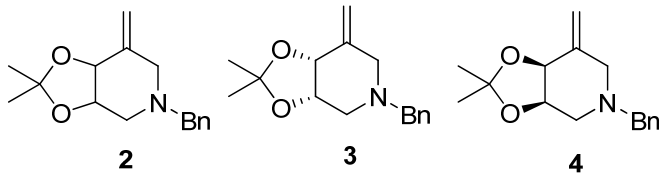


Figure 2.

Before going into the synthetic details, it would be pertinent to append a brief discussion on the methodology utilized in the synthesis of template **2**.

2.1-A-The concept

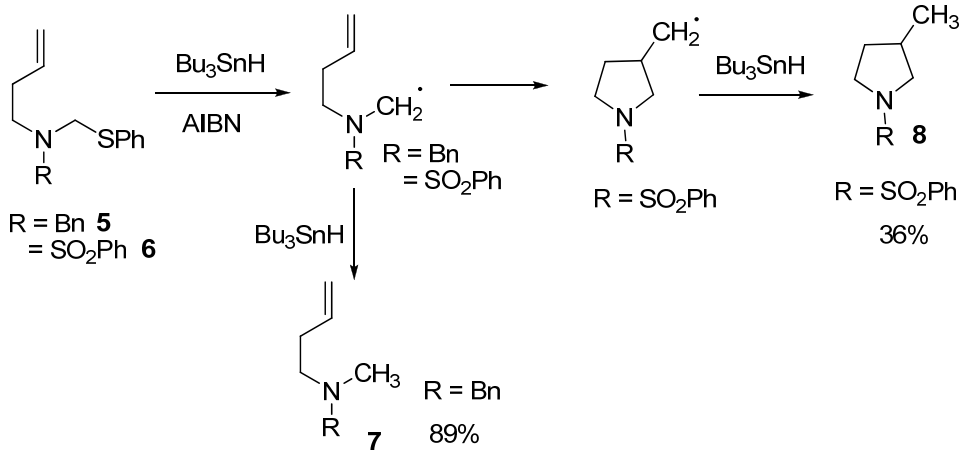
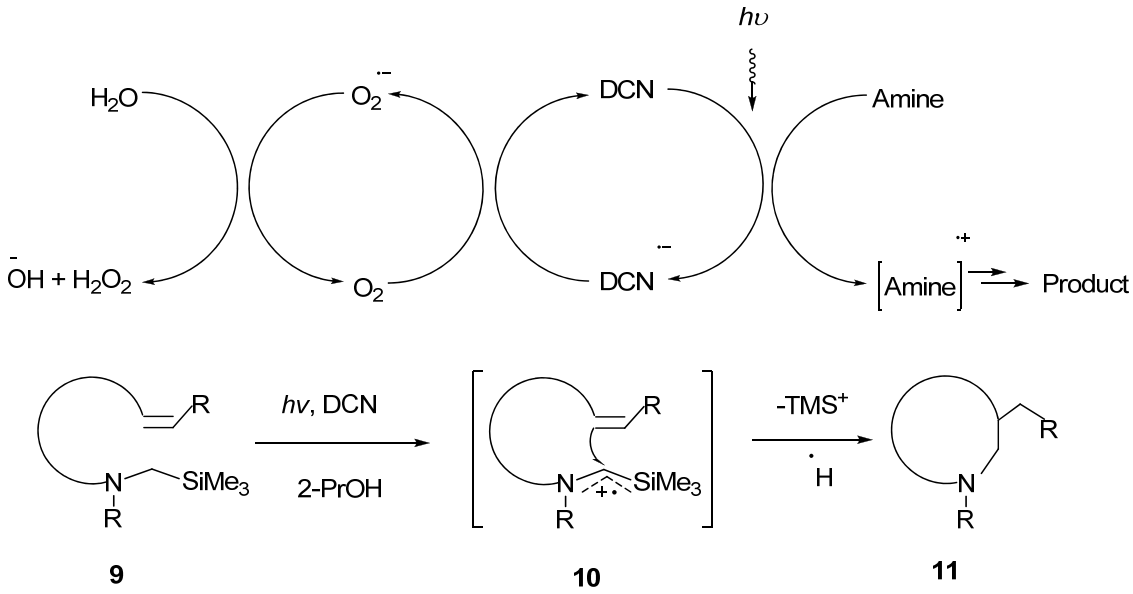


Figure 3.

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[2] but the use of α-amino radicals in the synthesis of nitrogen heterocycles is limited. Attempts by Padwa and co-workers (Figure 3) to cyclize free α-amino radicals,

generated by the conventional cleavage of C-S bond of *N*-alkenyl-*N*-(phenylthio) methylamine by Bu_3SnH failed, owing to the reduced radicaloid character[3] on the adjacent carbon α- to the nitrogen atom. This was a direct consequence of the electronic assistance provided by the amine lone pair to the radical center. This argument was substantiated by demonstrating the cyclization of α-amino radical generated from **6**, which had an electron-withdrawing sulfonyl group on the nitrogen.

Convinced by the fact that for cyclizable α-amino radicals, the electronic assistance of the amine group to the radical center must be reduced, Pandey and co-workers[4] developed a new concept for the synthesis of nitrogen heterocycles of the type **11** utilizing photoinduced electron transfer (PET) generated α-trimethylsilylmethylamine radical cations **10** as the reactive intermediate, as shown in Scheme 1.



Scheme 1.

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[5]. The photosystem to generate the reactive intermediate **10** employed 1,4-dicyanonaphthalene (DCN) as the light harvesting electron acceptor. DCN in its excited state upon irradiation, picks up an electron from the lone pair of nitrogen giving rise to the corresponding radical cationic species **10**. The electron from $DCN^{\cdot-}$, is ultimately transferred to water via

oxygen. The radical cation **10** eventually leads to the formation of cyclized product **11** as shown in Scheme 1.

A variety of substituted pyrrolidines, pyrrolidines, piperidines, pyrrolizidines, as well as indolizidine have been synthesized[4] using this methodology (Figure 4).

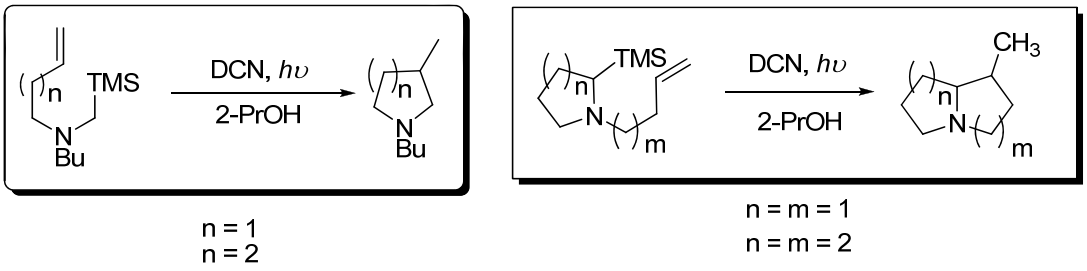


Figure 4.

With the above mentioned protocol in hand, we started our research program of synthesizing some 1-*N* iminosugars along with their enzyme inhibition studies.

2.1.B-Development of a General Strategy for the Synthesis of Polyhydroxy Piperidines

Initially, we took up the challenge of developing the synthesis of des(hydroxymethyl)deoxyallonojirimycin[6] **12**, isofucofagomine[7] **13**, isogalactofagomine[8] **14** and their analogues (Figure 5).

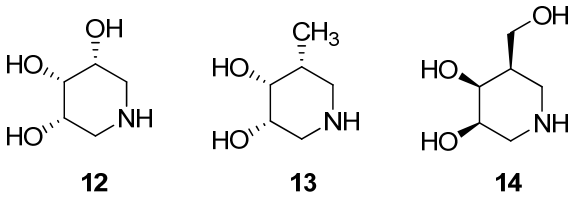


Figure 5.

If one takes a closer look at the molecular framework of these azasugars (Figure 6-A), it becomes apparent that these structures have many similarities with some subtle variations in the functionalities and stereochemistry. For example, they all are substituted at the same positions *i.e.* C_3, C_4 and C_5 with the hydroxy substitutions at C_3 and C_4. The functionality and stereochemistry at C_5 varies in all. The stereochemistry at C_3 and C_4 are

the same for **12** and **13** and exactly opposite for **14**. Keeping these facts in mind, we outlined a general strategy for the construction of these molecular framework as shown in Figure 6-B. We planned to obtain a suitable precursor starting from D-ribose which on PET cyclization would give **2** as the key template. Functionalization of the olefinic double bond and removal of protecting groups in the last stage was visualized to give the required azasugars.

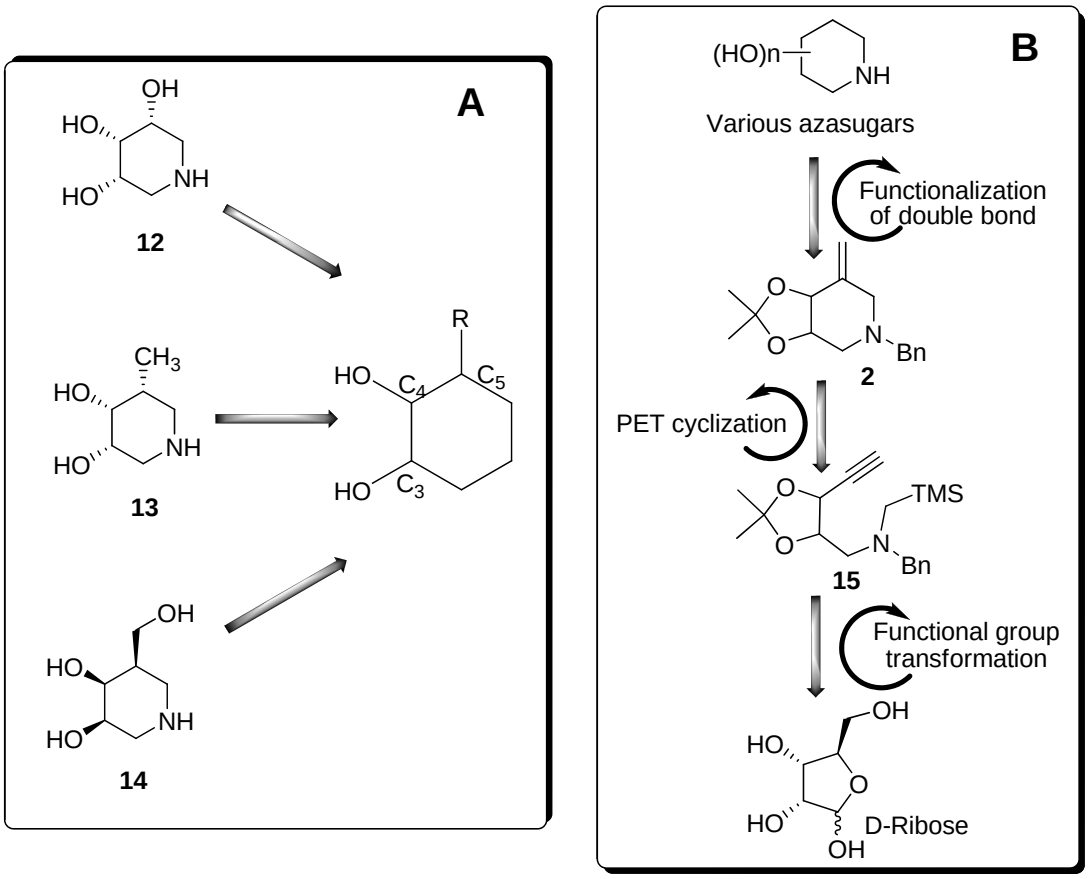


Figure 6.

As discussed earlier, both enantiomeric precursors **3** and **4** were planned from the same protocol as outlined in Figure 7. Starting from D- ribose and depending on the choice of the reaction pathways and the stage at which the particular reaction is carried out, the synthesis of enantiomeric synthons was visualized. For example, if precursor **17,** prepared from D-ribose via **16** is used for the installation of triple bond (by Ohira Bestmann reagent[9]) at the place of carbonyl functionality and the rest two hydroxyl groups are used for the installation of amine functionality, we can obtain **18** whereas, if **16** is further

changed to corresponding lactol **19** followed by the Ohira Bestmann reaction, it would liberate the free hydroxyl group which can be used for the installation of amine functionality giving rise to **20**. In this manner, substrates **20** and **18** which are non-superimposable mirror images of each other (enantiomers), could be separately used as building blocks for the synthesis of azasugars such as **12**, **13**, **14** and their analogues.

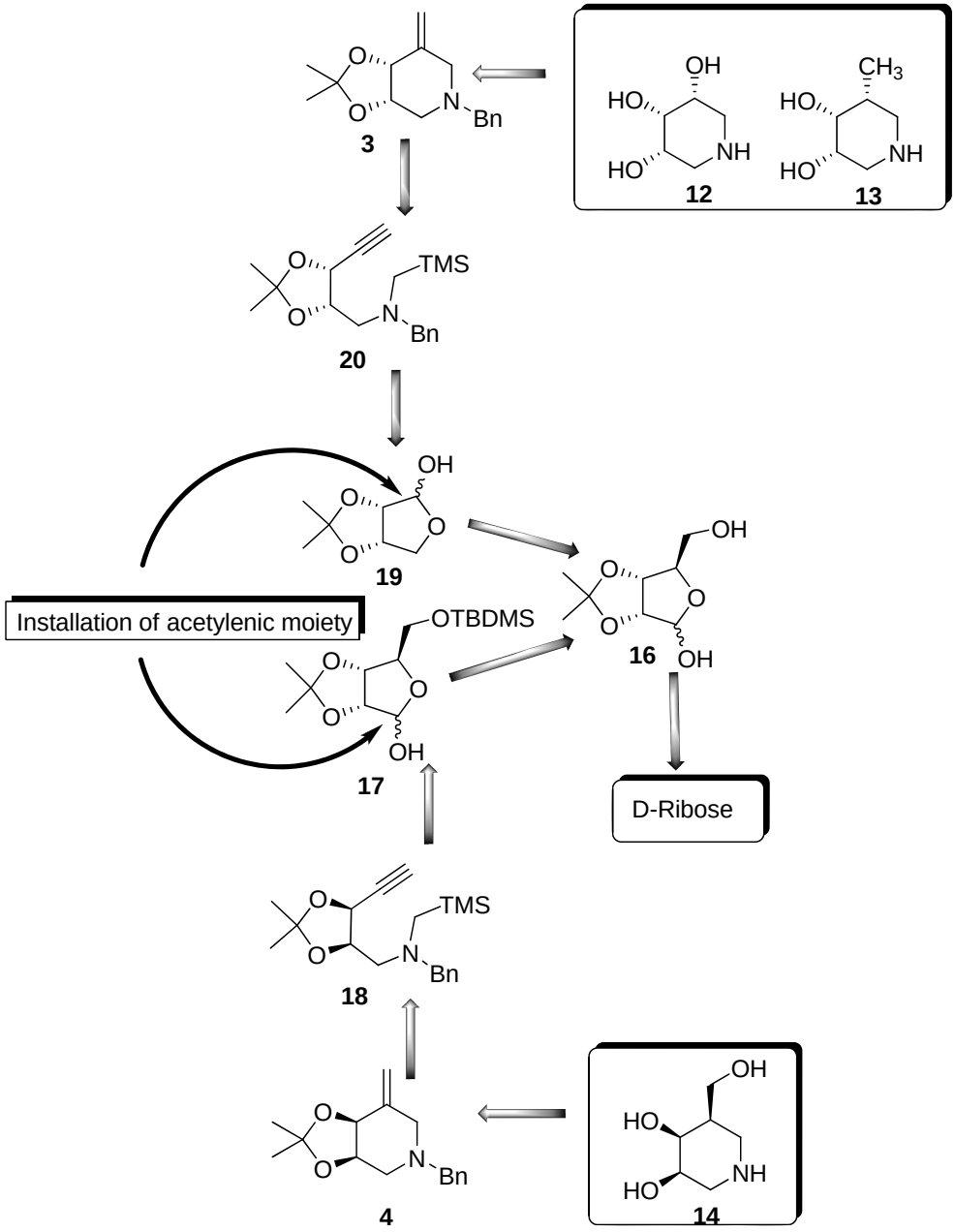
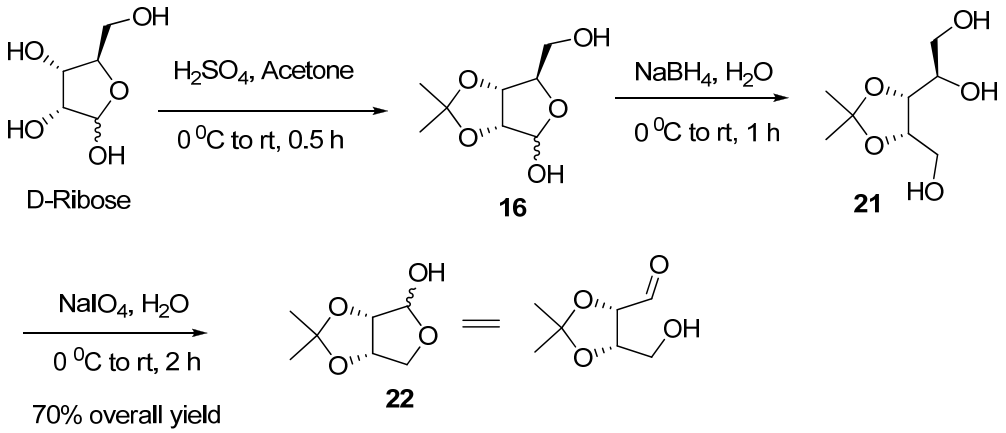


Figure 7.

2.2-Synthesis of (3aS,7S,7aR)-5-benzyl-7-(hydroxymethyl)-2,2-dimethyl-hexahydro [1,3]dioxolo[4,5-c]pyridin-7-ol (38)

2.2.A-Synthesis of ((4S,5R)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (32)



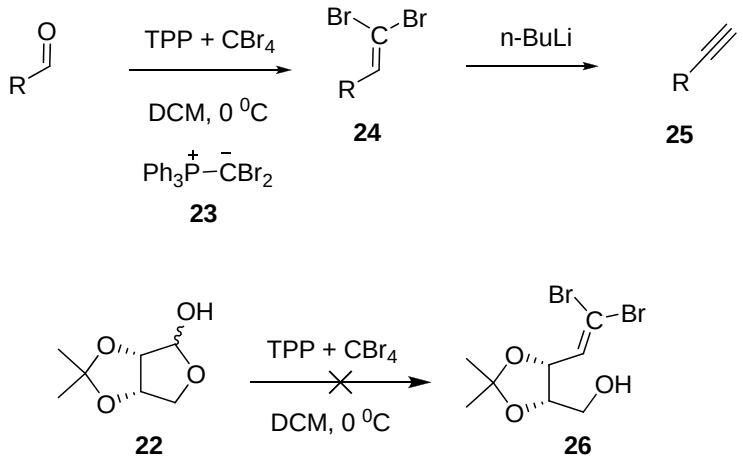
Scheme 2.

Towards this goal, we designed our synthetic scheme starting with D-ribose (Scheme 2). At first, C_3 and C_4 hydroxy functionalities were protected as acetonide by stirring a suspension of D-ribose in acetone with a few drops of H_2SO_4 for half an hour. Neutralization followed by filtration and concentration gave **16**, which was carried forward as such without any purification. The acetonide protecting group not only gave the advantage of selective protection of the secondary hydroxyl moieties but also imparted rigid cyclic framework which was envisaged to be useful in the stereochemical fuctionalization towards the later part of the synthesis.

Since a hydroxyl moiety was required at the place of carbonyl in **16** in the later part of our proposed scheme, **16** was reduced using $NaBH_4$ (182.3 mmol) in water, which on subsequent neutralization by drop wise addition of acetic acid gave the corresponding triol **21**. The cleavage of **21** using $NaIO_4$ (133.21 mmol) gave required lactol **22** in 70% yield. It may be important to mention that all the three steps required in obtaining **22** in the above reaction sequence, were carried out in a single pot without isolating any of the intermediates **16** and **21.** This strategy did'nt require any column chromatography except for the purification of **22**. The overall yield, over the three steps, was found to be 70%

over all yield. The spectral data of **22** was in perfect agreement with the one reported in the literature[10].

In order to transform the masked aldehydic functionality of **22** to acetylene**,** we tried at first the Corey's[11] protocol**.** The protocol involved simple steps as described in Scheme 3.



Scheme 3.

However unfortunately, we could not succeed in making the required precursor **26** necessary to obtain the penultimate acetylenic moiety. Therefore, another alternative strategy using Ohira-Bestmann reagent[9] **27** (Figure 8) was evaluated. 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 **27** can be prepared from acetone as shown in Fig. 8.

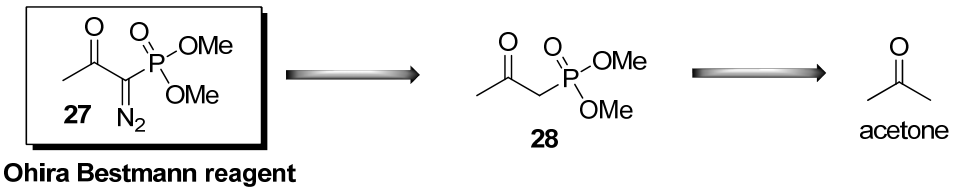
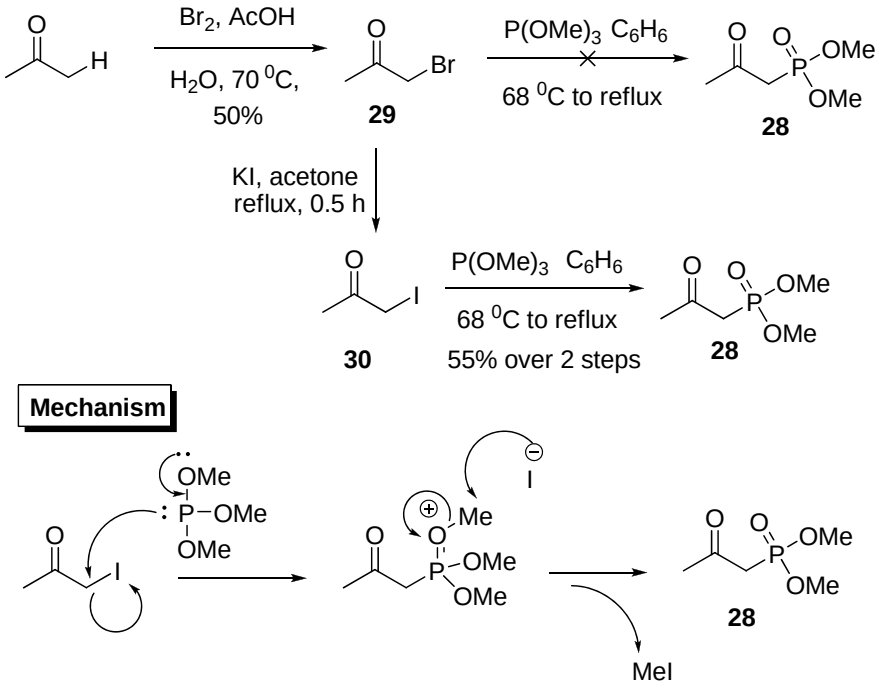


Figure 8.

In order to synthesize **27,** initially bromoacetone[12] was prepared by brominating acetone in acetic acid by adding bromine (1.38 mol) to a stirring solution of water (320 mL), acetone (1.36 mol) and acetic acid while maintaining the temperature of the reaction mixture at 65-70 ^{0}C. Work up and fractional distillation (37-48° C, 13 mm) of the reaction mixture gave bromo acetone (147 g) in 50% yield. The reaction temperature as

well as the rate of bromine addition was found to be critical for the optimal yield of bromoacetone. Higher temperature and extended hours of addition of bromine decreased the yield and led to the formation of 1,3-dibromo acetone as the side product. *It is cautioned that the reaction should be done carefully, as fast addition of bromine can lead to highly exothermic reaction (Scheme 4).*

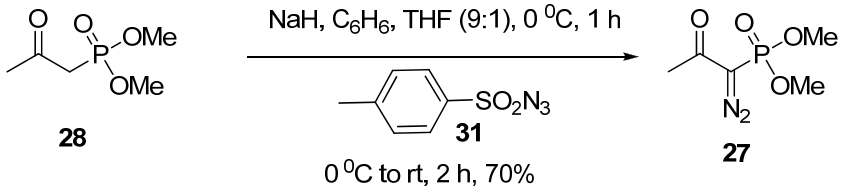
Bromoacetone, thus obtained was reacted with the trimethylphosphite (Scheme 4), however it failed to react. Literature [9, 13] scrutiny revealed that this reaction requires iodo acetone instead of bromo acetone. Therefore, bromoacetone (729.9 mmol) was transformed to corresponding iodo acetone **30** by refluxing **29** (0.5 h) with potassium iodide (948 mmol) in acetone. *Since, bromoacetone and iodoacetone are highly lachrymatory, the reaction should be handled with extreme precaution in the efficient vacuum hood.* The iodo acetone being highly unstable was carried forward as such without any purification.



Scheme 4.

Refluxing a benzene solution of iodoacetone with trimethyl phosphite (875.9 mmol) at 68 ^{0}C while distilling out methyl iodide crude liquid which was distilled to get **28** in 55% yield over two steps (119-123 ^{0}C, 11 mm).

Reaction of **28** (120 mmol) with NaH (132.53 mmol) in benzene/THF mixture (9:1) at 0 ^{0}C followed by subsequent quenching with tosyl azide[14] (prepared in 85% yield by treating a solution of tosyl chloride with NaN_3) gave **27**[15] in 70% yield (Scheme 5). The possible mechanism of the reaction is outlined in Figure-9.



Scheme 5.

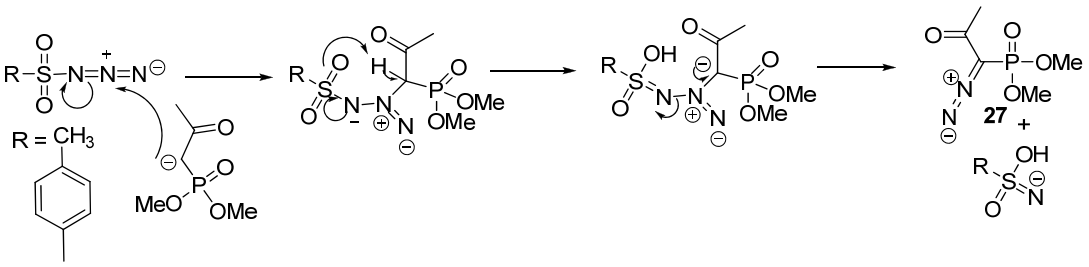
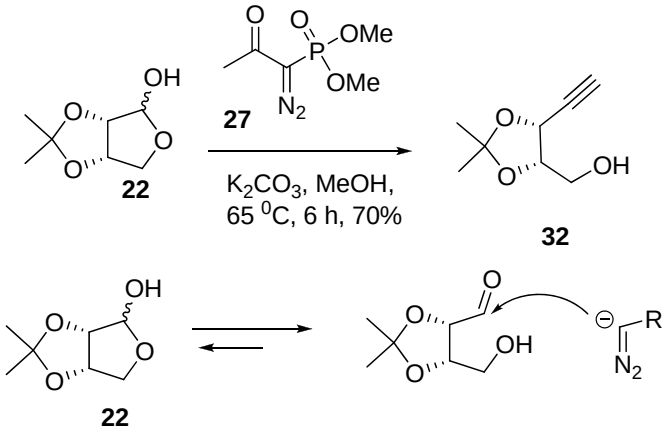


Figure 9.

Initially, stirring of a methanolic solution of **27** (81.25 mmol) and **22** (62.5 mmol) in the presence of potassium carbonate (81.25 mmol) at room temperature (Scheme 6) did not succeed to give **32**.



Scheme 6.

However, refluxing the same reaction mixture at 65 ^{0}C for a period of 6 h gave **32** in good yield (70%)[16]. Compound **32** exhibited characteristic IR stretching band for acetylenic C-

H at 3284 cm^{-1} and band for C≡C stretching at 2121 cm^{-1}. ^1H NMR spectrum revealed acetylenic proton at 2.53 ppm (d, J = 2.15 Hz). Final confirmation of the structure **32** was also obtained from its mass spectrum (M^+ + Na = 179).

A plausible explanation for the success of this reaction at higher temperature could be attributed to the rapid equilibrium between the lactol **22** and its corresponding hydroxy aldehyde moiety (Scheme 6). Drop wise addition of reagent **27** over 6 h was important for the gradual generation of ylide and to react further. The reaction conditions were further optimized for the lower consumption of reagent **27** to only 1.3 equivalents[16]. The mechanism for the formation of triple bond is discussed in Figure-10.

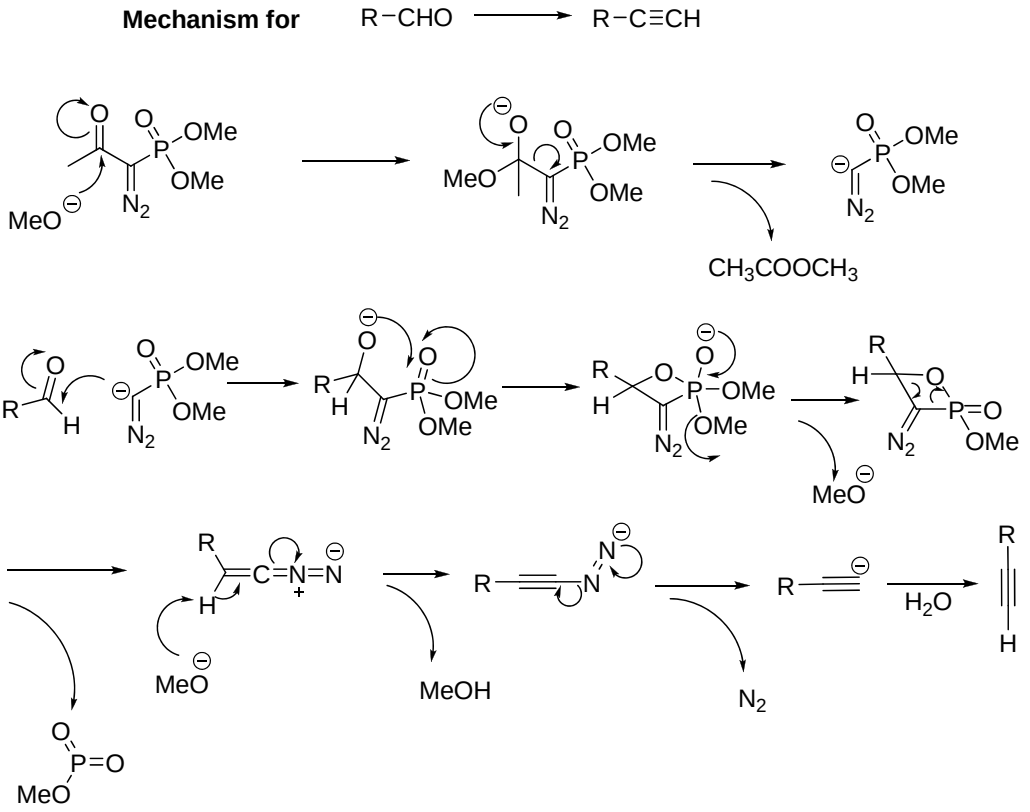
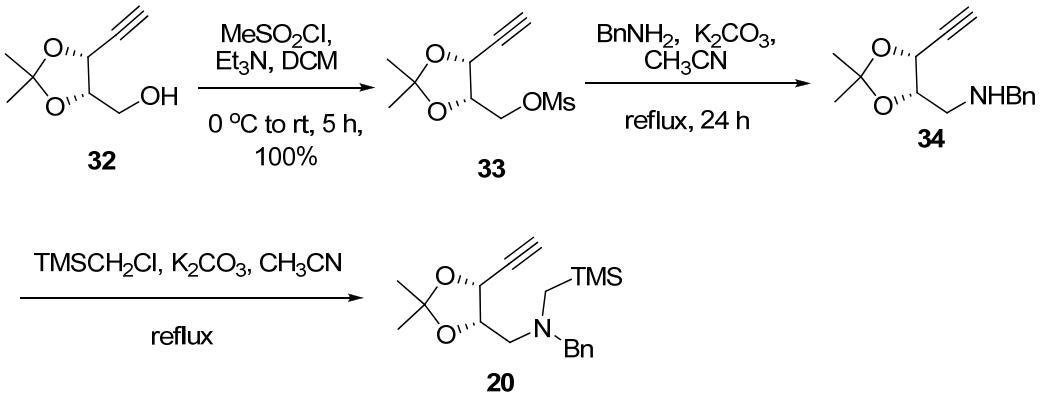


Figure 10.

2.2.B--N-benzyl-1-((4S,5R)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)-N-(trimethylsilyl methyl)methanamine (20)

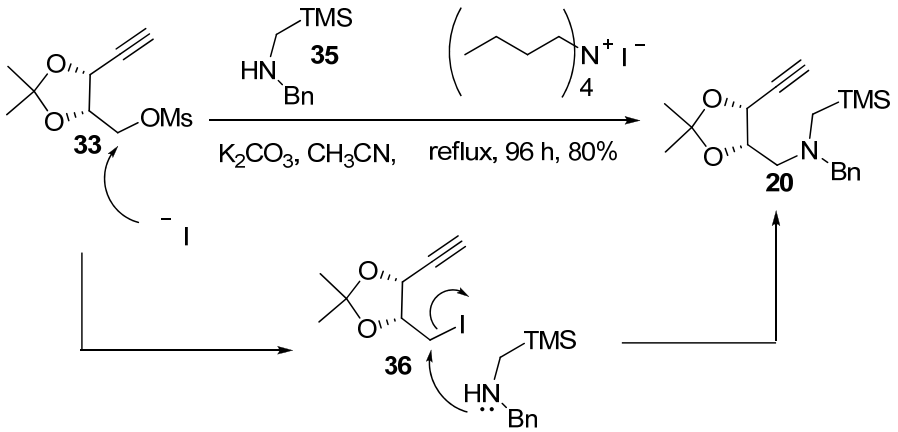
In order to transform **32** to precursor **20**, initially we proposed to convert the –OH group to corresponding N-benzyl amine derivative (Scheme 7) followed by N-alkylation using

$TMSCH_2Cl$. In this context, the –OH group was converted to corresponding mesylate **33** which upon refluxing with benzyl amine in the presence of anhydrous K_2CO_3 gave **34.** The reaction faced the problem of over alkylation and the yield was low. Further, our effort to alkylate **34** with chloromethyl trimethyl silane ($TMSCH_2Cl$) was only partially successful.



Scheme 7.

Therefore, we altered the course of the reaction and decided to alkylate **33** directly with $BnNHCH_2TMS$ **35,** (prepared by refluxing benzyl amine (429.93 mmol) with $TMSCH_2Cl$ (107.4 mmol) in the presence of K_2CO_3 (429.93 mmol) in acetonitrile). However, the alkylation of **33** with **35** did'nt go for completion resulting in poor yield of **20,** possibly due to poor leaving group ability of the mesylate.



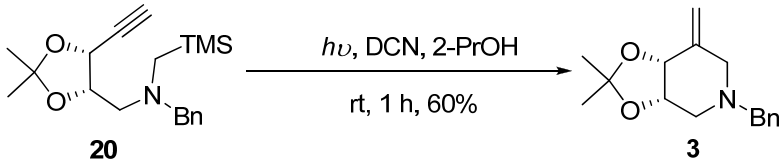
Scheme 8.

Therefore, we employed the *in situ* generated iodo precursor **36** by refluxing **33** (34.1 mmol) with amine **35** (68.3 mmol) in the presence of K_2CO_3 (136.7 mmol) and catalytic

tetrabutyl ammonium iodide (TBAI) (1 g) which gave the required product **20** in 80% yield (Scheme 8).

Compound **20** showed characteristic peaks for trimethylsilane (9 H) along with the two sets of N-CH_2TMS protons (apart from benzylic) at 2.08 - 2.43 and 2.51-2.56 in the ^{1}H NMR spectrum. The molecular ion peak was found at 332 (M^++H) in the mass spectrum.

2.2.C-Photoinduced Electron Transformation Cyclization of 20



20 **3**

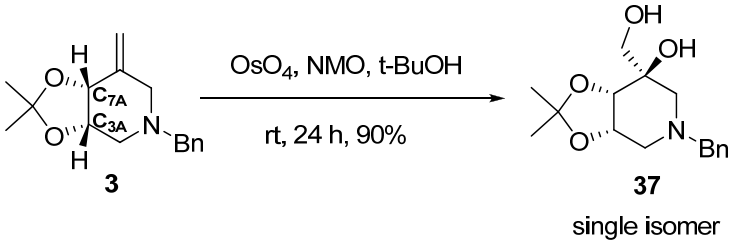
Scheme 9.

Photo-induced electron transfer (PET) mediated cyclization, carried out by irradiating a dilute solution of **20** (3.02 mmol) and 1,4-dicyanonapthalene (1.01 mmol) in *iso*-propanol (240 mL) in a pyrex vessel, using a 450-W Hanovia medium pressure lamp as the light source, produced cyclized product **3** in 60 % isolated yield (Scheme 9). The definite proof for the formation of **3** was ascertained from the ^{1}H NMR spectrum which exhibited two olefinic protons at 4.81 (doublet J = 1.25 Hz) and a singlet 4.94 ppm. ^{13}C NMR displayed olefinic CH_2 at 105.3 ppm. The molecular ion peak was obtained at 282 (M^++Na).

The merit of this strategy may be highlighted to 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 bicyclic framework. The double bond can be used precisely for generating different functionalities by simple functional group transformations. The chirality of the *alpha* carbon would govern the direction of the incoming functionality and the bicyclic nature would give the precise stereocontrol.

2.2.D-Stereochemistry revision

Careful analysis of the ^{1}H NMR of the cyclized product **3** indicated coupling constant between H-3 and H-7 unexpectedly higher (9.22 Hz) than the expected range for *cis* coupling (5-6 Hz), indicating the possibility of *trans*-relationship between the two.



Scheme 10.

However, it was not easy for us to visualize the stereochemical inversion at this stage. Therefore, we proceeded for the dihydroxylation of the olefinic bond of **3** (3.8 mmol), using catalytic osmium tetroxide (1% solution of OsO_4 in *t*-BuOH) and NMO (7.69 mmol) as co-oxidant, which gave diol **37** as a white crystalline solid (mp. 152-154 ^{0}C) in 90% yield (Scheme 10). X-Ray analysis of **37 (**Figure 11) confirmed the *trans*-stereochemistry between the H-7 and H-3**.** The structure of diol **37**, therefore, can now be re-written as **38**.

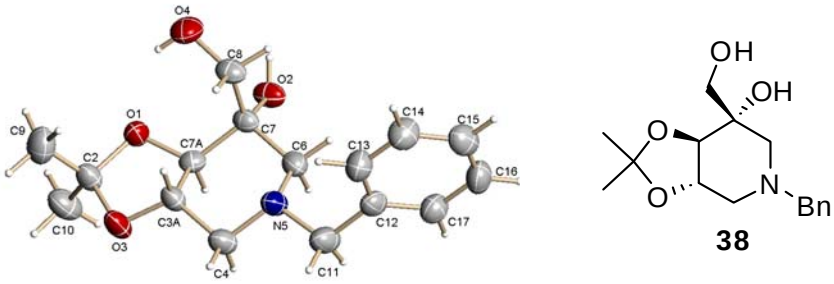


Figure 11.

This unexpected inversion in stereocenter compelled us to find out a possible explanation and/or the step at which the inversion could have occurred (Figure 12).

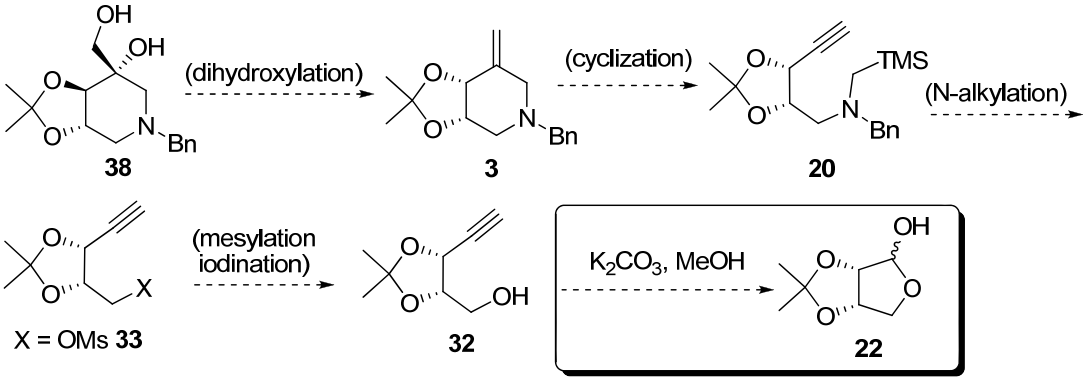


Figure 12.

Since PET cyclization step involved exclusively the trimethylsilylmethylamine and triple bond functionalities, it was not expected to affect the stereochemical inversion. Therefore, we suspected that the inversion might have occurred at the acetylenation step because the reaction involves refluxing with K_2CO_3 in MeOH. A careful literature survey[17] revealed the precedence of such epimerizations.

The whole scheme can now be re-written with the revised stereochemistry as shown in Figure 13.

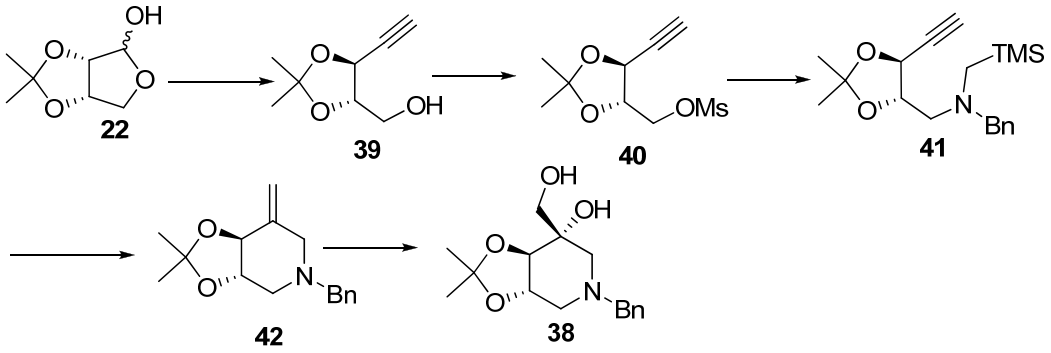


Figure 13.

Although, this inversion step was unexpected, it turned out to be effective in achieving the synthesis of compounds of type **38** as we have made such systems earlier[18] starting from tartaric acid (Figure 14) which required 11 steps.

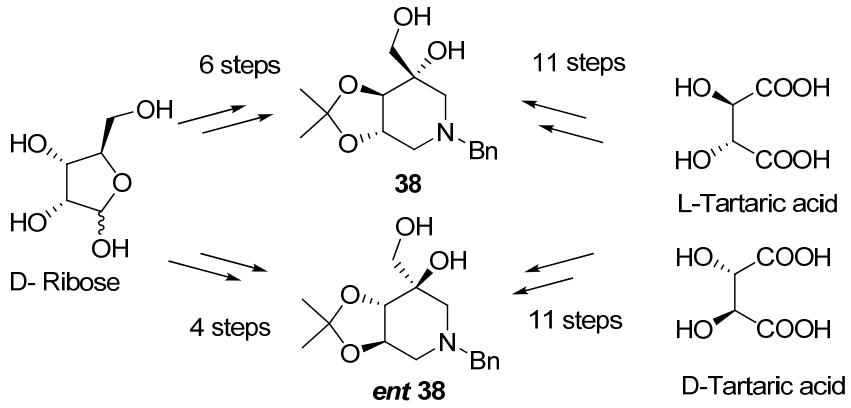


Figure 14.

However, the current protocol allowed the synthesis of similar product in just 6 easy steps. There was also possibility to synthesize its enantiomer in 4 steps (as proposed in Figure 7) starting from D-ribose (Figure 14).

Realizing this advantage, we immediately changed our protocol for synthesizing different azasugars as mentioned in Figure 15[18]

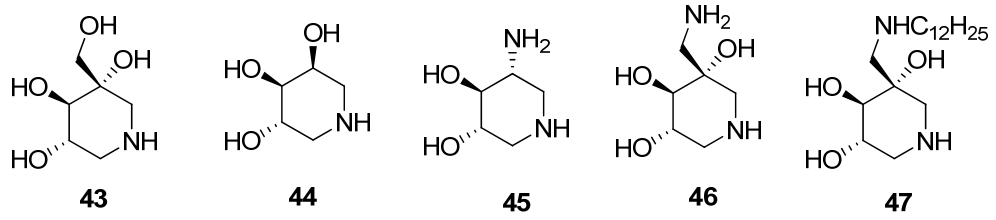
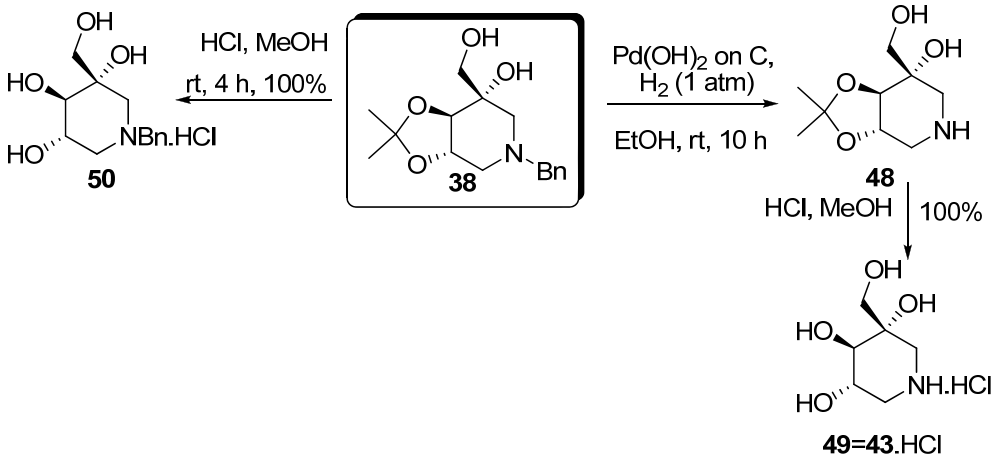


Figure 15.

2.3-Synthesis of (3S,4R,5S)-3-(hydroxymethyl)piperidine-3,4,5-triol hydrochloride (43.HCl)

Diol **38** was the key intermediate, from where we targeted different compounds. Immediate removal of the protecting groups from **38** seemed to be a short route for azasugars **49** (**43**.HCl) and **50** (Scheme 11).



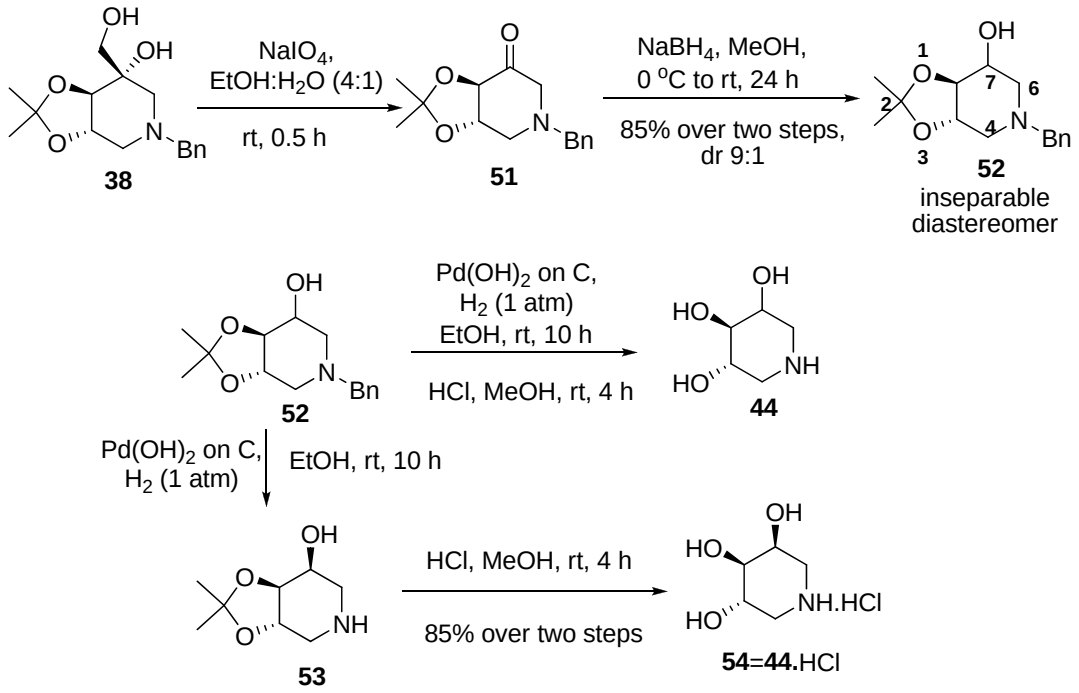
Scheme 11.

The *N*-debenzylation of **38** by hydrogenation over palladium hydroxide in ethanol gave **48**, which upon immediate acetonide deprotection using HCl/ MeOH, gave corresponding amine salt **49** in quantitative yield. The ^{1}H NMR spectrum revealed the disappearance of acetonide as well as the benzylic protons confirming the formation of **49**.

Corresponding *N*-alkylated amine salt **50** was also made for the biological evaluation as there are some reports in literature[19] wherein the *N*-alkylated analogue of the azasugar has been found to be a more potent and selective inhibitor as compared to their free amine analogues[19].

2.4-Synthesis of (3S,5S)-piperidine-3,4,5-triol-Hydrochloride (44.HCl)

$NaIO_4$ mediated cleavage of **38** in ethanol/water mixture (4:1) gave corresponding ketone **51** (Scheme 12) which upon immediate reduction using $NaBH_4$ in MeOH gave **52 (**85% yield) with a good diastereoselectivity of 9:1.



Scheme 12.

The ^1H NMR spectrum of **52** exhibited H_7 of newly generated chiral center, at 4.14 ppm. The IR spectrum showed a broad band corresponding to –OH at 3018 cm^{-1}. Final confirmation of the structure was obtained by observing molecular ion peak of 263 in the mass spectrum.

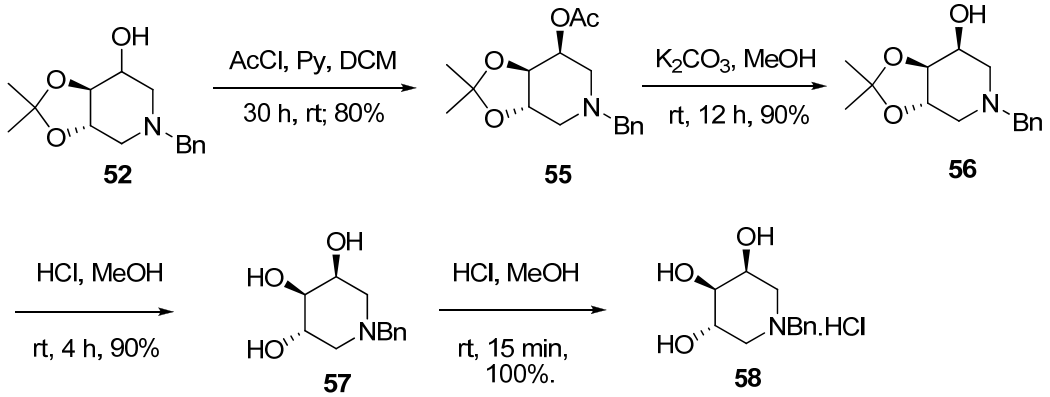
The precise stereo-control during $NaBH_4$ reduction as well as in dihydroxylation step could be attributed to the rigid bicyclic nature of the substrate.

The diastereomers formed after $NaBH_4$ reduction, were found difficult to separate. Therefore, we attempted diastereomeric separation of **44** after acetonide deprotection and

N-debenzylation of **52**. However, unfortunately, our effort remained unsuccessful. Therefore, we reverted back one step and separated pure diastereomer **53** (by *N*-debenzylating the alcohol **52**) which after acetonide cleavage gave **54**. The ^{1}H NMR spectrum revealed the disappearance of acetonide and benzylic protons. The rotation was in close agreement with the literature value +15 (c 0.3, MeOH), Lit.[6] $[\alpha]^{25}_D$ +16 (c 0.5, MeOH).

To compare the biological activity we also kept the *N*-benzylated analogue of **54**. In order to obtain pure diastereomer**,** we converted **52** to the corresponding acetate and obtained pure diastereomer **55** in 80% yield by column purification (Scheme 13). Compound **55** showed characteristic acetyl protons as a singlet at 2.13 ppm. The molecular ion peak appeared at 328 (M^++Na) in the mass spectrum.

Upon saponification **55** produced pure **56** in 90% yield. Acetonide deprotection of **56** gave **57** in 90% yield which was characterized by the absence of acetonide protons in the ^{1}H NMR spectrum and from molecular ion peak at 246 (M^++Na) observed in mass spectrum. For the purpose of studying enzyme inhibition properties, **57** was converted to its corresponding hydrochloride salt **58**.



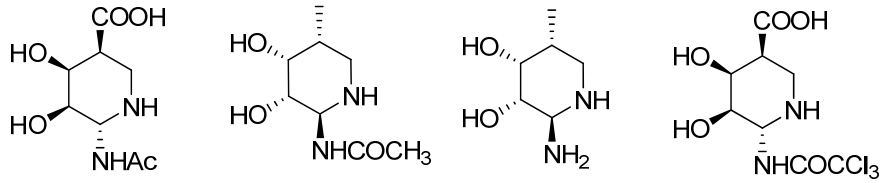
Scheme 13.

2.5 Synthesis of C_3 amine analogues of 49, 50 and 54

2.5.A-Synthesis of (3S,4S,5R)-5-aminopiperidine-3,4-diol dihydrochloride (67)

Siastatin B[20a-c] (**59**), a naturally occurring β-glucuronidase inhibitor (IC_{50} = 16 μM) has more than one nitrogen. Similarly other azasugars such as **60**[1, 20d-e] (IC_{50} = 0.48 μM

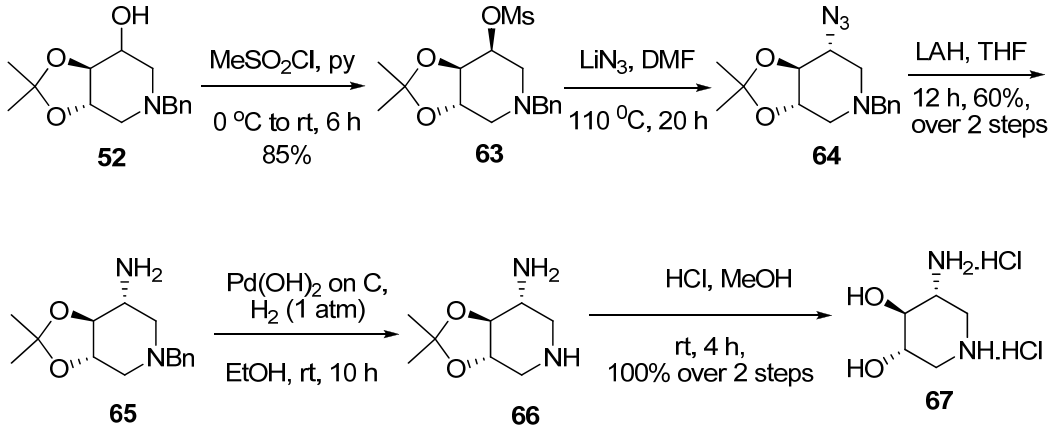
against Bovine kidney α-L-fucosidase), **61**[1, 20d-e] (IC_{50} = 0.016 μM against same enzyme), and **62**[1,20f] (IC_{50} = 4.7 μM against Bovine kidney β- glucuronidase) have shown to be potent inhibitors of glycosidases[20f].



59 **60** **61** **62**

Figure 16.

Therefore, we envisaged at first to see the effect of an additional basic site in these synthesized azasugars. It was assumed that the second basic site upon protonation at physiological pH may provide an extra hydrogen binding site and thus, may play a significant role in inhibition. To test this idea, we took up the synthesis of the corresponding amine analogues.



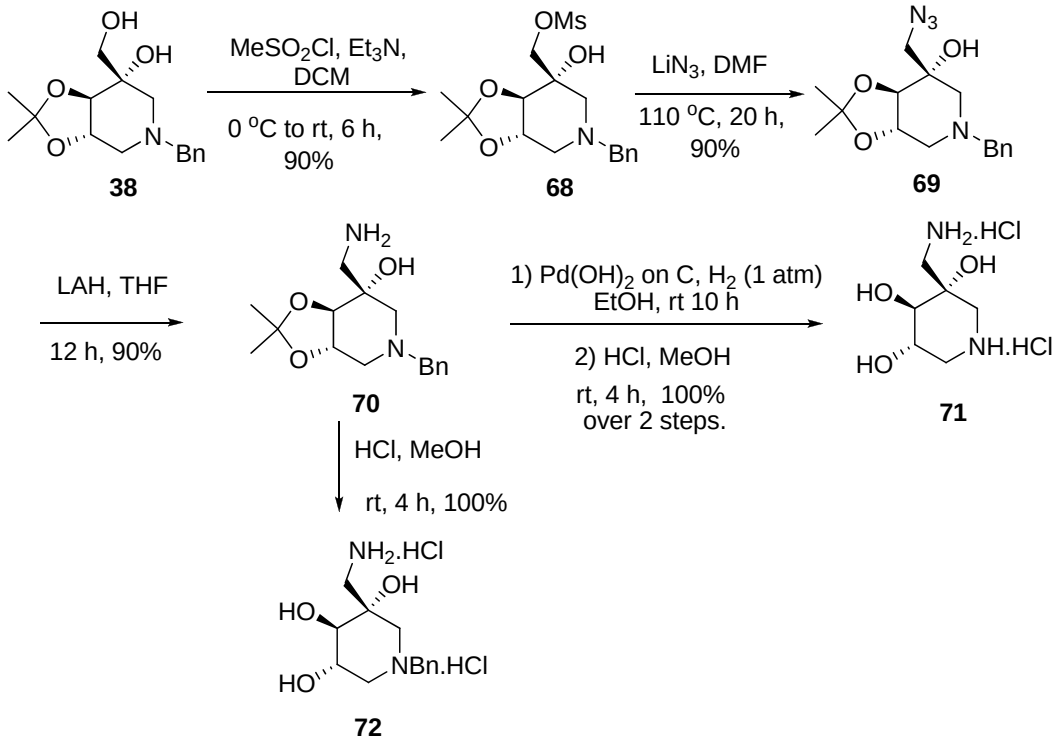
Scheme 14.

First, we planned to synthesize **65** by replacing the C-3-hydroxy moiety of **52** by an amine functionality. Towards this end, -OH functionality was transformed to corresponding mesylate **63** which was isolated as a pure diastereomers by using MsCl/Py in DCM (Scheme 14) in 85 %yield. Reaction of mesylate **63** with LiN_3 in refluxing DMF at 110 ^{0}C gave corresponding azide **64** which upon reduction with $LiAlH_4$ produced corresponding amine **65** in 60 % yield.

The IR spectrum of **65** exhibited a broad band at 3390 cm^{-1} characteristic of amine functionality. Other spectral data were intact in accordance with the structure of **65**. Further confirmation of the formation of **65** was obtained from the mass spectrum with molecular ion peak at 285 (M^++Na). Subsequent global deprotection of the acetonide as well as *N*-benzyl groups produced **67** as its dihydrochloride salt.

2.5.B-Synthesis of 71 & 72

In continuation, we also synthesized amine analogues **71** and **72** by following the same protocol as described for **67**. The details are shown schematically in scheme 15.

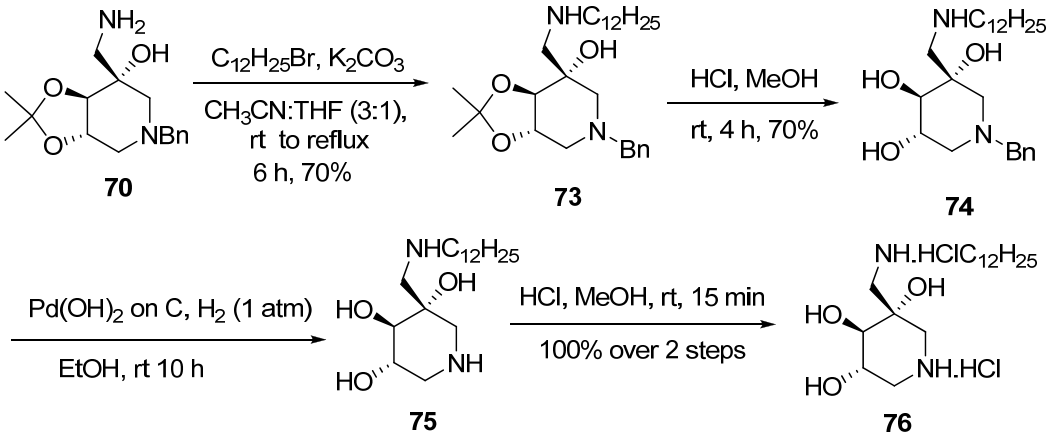


Scheme 15.

2.5.C-Synthesis of N- alkylated analogue

After synthesizing these amine analogues, we further thought of altering the lipophilicity of these compounds by N-alkylation using n-dodecyl bromide. Accordingly **69** was alkylated using n-dodecyl bromide in acetonitrile/THF (3:1) using K_2CO_3 as the base

which gave **73** in 70% yield (Scheme 16). The ^{1}H NMR of **73** revealed clearly the alkylated dodecyl functionality with a broad peak of 20 protons at 1.26 ppm. The corresponding CH_2 peaks of dodecyl group appeared in the ^{13}C spectrum from 29.3 to 29.7 ppm. The molecular ion peak was also observed at 461 (M^{+}+H) in the mass spectrum. Acetonide cleavage of **73** and purification by column chromatography gave **74** in 70% yield, which upon debenzylation and hydrochloride salt formation gave **76** in quantitative yield.

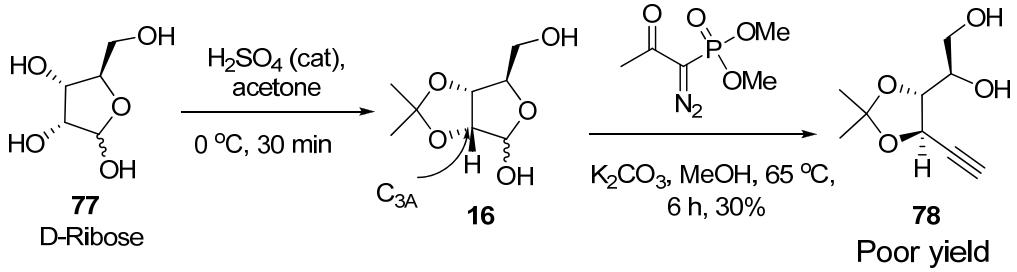


Scheme 16.

2.6- Synthesis of enantiomers of 49, 50 and 72

2.6.A-Synthesis of (3aR,7R,7aS)-5-benzyl-7-(hydroxymethyl)-2,2-dimethylhexahydro-[1,3]dioxolo[4,5-c]pyridin-7-ol (83)

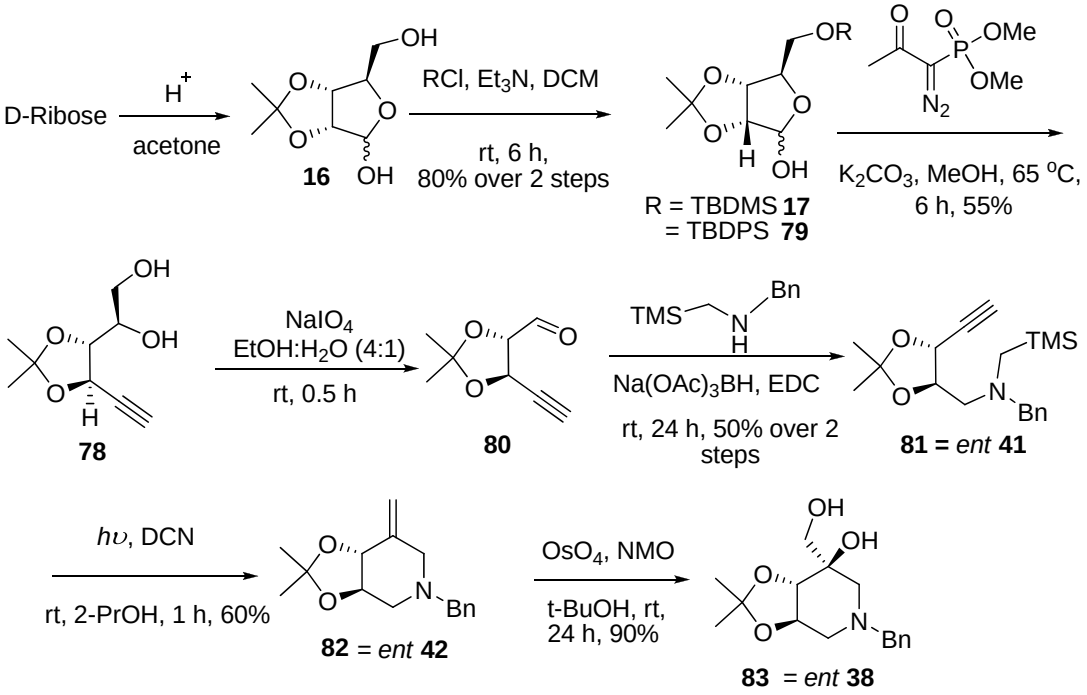
The success of our above described protocol for synthesizing various azasugars led us to extend this strategy for synthesizing corresponding enantiomers. In order to obtain the enantiomers, we had to just alter the sequence of the reaction (with slight modification) as discussed earlier in Figure 7. Obviously, the synthesis started from **16,** prepared from D-ribose (Scheme 17).



Scheme 17.

The **16** upon subjecting to acetylenation using Ohira-Bestmann reagent[9] gave expected acetylenic compound **78**, though, in poor yield (30% over two steps), possibly due to the presence of free primary hydroxyl group**.** This unexpected problem, led us to first protect the -CH_2OH as -OTBDMS ether (Scheme 18)[1].

The Ohira Bestmann reaction on **17**[21] proceeded quite smoothly producing required product **78** in 55% yield. The ^1H NMR spectrum of **78** indicated the presence of acetylenic proton at 2.56 ppm. While the two hydroxy groups showed a broad band in the IR spectrum at 3306 cm^{-1}, the ≡C-H stretching band was found at 3019 cm^{-1}. 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 stereochemistry at C_{3A} got inverted in this reaction too and the diastereomers were formed in 16:1 ratio (determined from ^1H NMR). The inversion in the stereochemistry during the formation of **78** was confirmed by the X-ray analysis of **83,** (Figure 17) prepared during the later part of the synthesis.

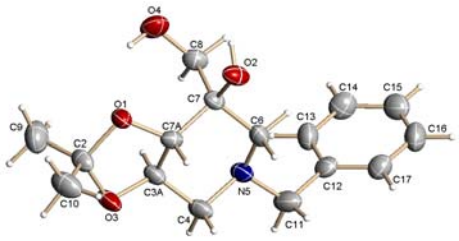


Scheme 18.

The cleavage of **78** using $NaIO_4$ in ethanol/water mixture gave corresponding aldehyde **80** which upon reductive amination with amine **35**[22] produced required precursor **81** in 50

[1] The corresponding TBDPS protected compound **79** is commercially available and the synthesis can be actually started from this stage.

% over all yield. Usual PET cyclization of **81** gave cyclized product **82** in 60% yield. The dihydroxylation of **82** by OsO_4 resulted in the formation of diol **83** in 90 % yield which was utilized as a common template for the synthesis of azasugars such as **85**, **86** and **90.**


O4
C8
O2
C7
O1
C9
C7A
C6
C13
C14
C15
C16
C2
C10
O3
C3A
C4
N5
C12
C17
C11

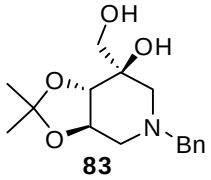
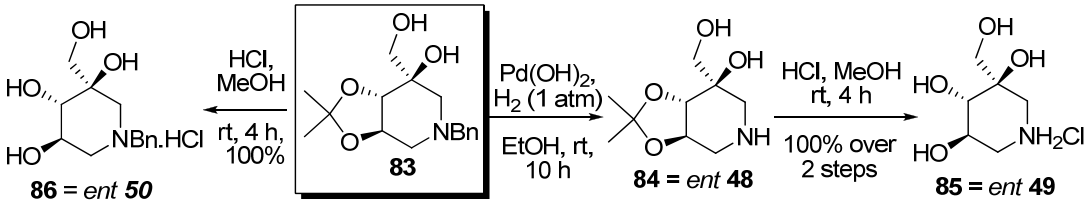


Figure 17. *ORTEP diagram of* ***83.***

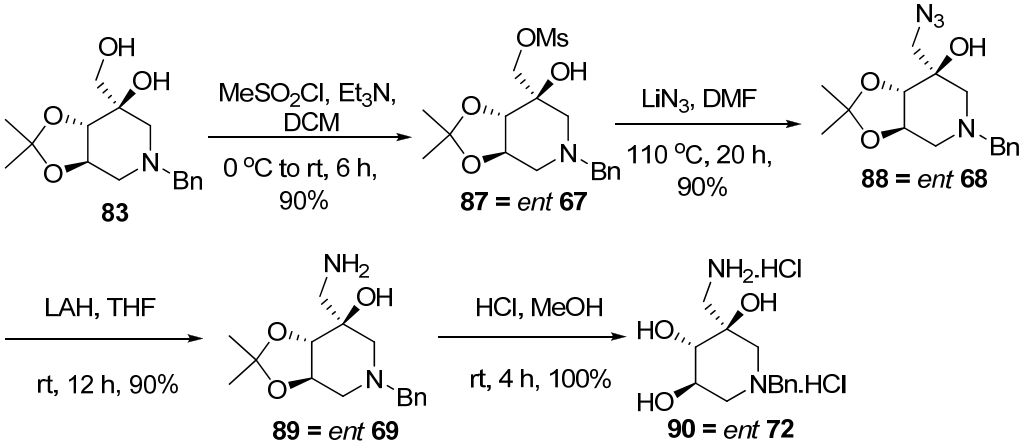
2.6.B-Synthesis of 85 and 86

Removal of protecting groups (Scheme 19) from **83,** as described earlier, gave azasugars **85** and **86.**



Scheme 19.

2.6.C- Synthesis of (3S,4S,5R)-3-(aminomethyl)-1-benzylpiperidine-3,4,5-triol dihydrochloride (90)



Scheme 20.

Enantiomer of **72** *i.e.*, **90** was prepared from **83** (Scheme 20) by following the reaction sequences described earlier during the synthesis of **72.**

2.7- Biological evaluation of 1-*N*-iminosugars and conclusion

With the successful synthesis of azasugars such as **49**, **50**, **54**, **58**, **67**, **71**, **72**, **76**, **85**, **86** and **90** we began to study their enzyme inhibitory efficiencies. In this context seven different enzymes were selected for the inhibition studies. β-Galactosidase (*A. oryzaie*), α-galactosidase (*Green coffee beans*), β-mannosidase (*Snail*), α-mannosidase (*Jack Beans*), β-glucosidase (*Almond*), α-glucosidase (*Yeast*), α-mannosidase (*A. fischeri*) the first six were commercially purchased, while the last one was isolated indigenously from *A. Fischeri*.[23]

2.7.A- 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 bonds 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 the 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:

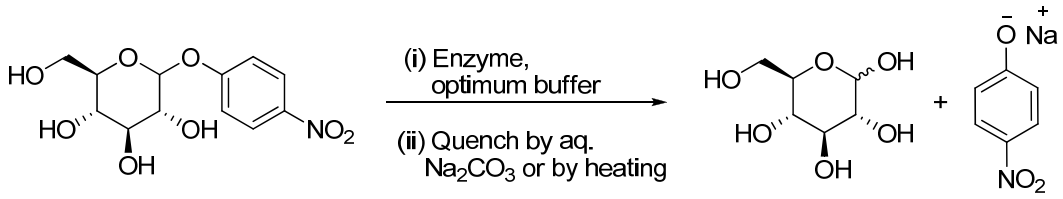
(a) Competitive
(b) Non-competitive
(c) Uncompetitive

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.

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

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

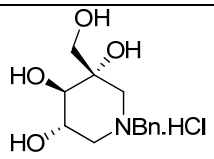
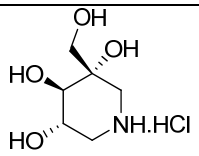
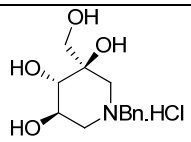
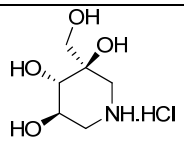
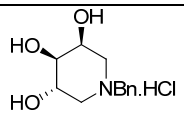
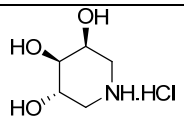
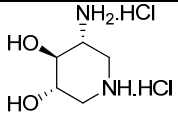
typical for that particular enzyme and at the end of a fixed reaction time, the reaction is quenched (the enzyme is inactivated) by chemical (aq. base) or thermal means (heating at high temperature). The optical density of this mixture is recorded and the reaction rate determined.

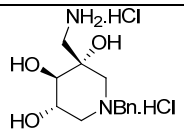
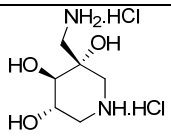
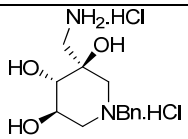
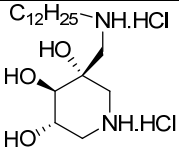
The inhibitors were screened at different level of concentrations. Initially, the inhibition at concentration level of 1 mM was determined. The compounds showing no inhibition or inhibition less than 50% were not investigated further. Those showing activities more than 50% were taken and the screening 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, 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 following.

2.7.B-Results and Discussion

The inhibitory activities of all the final molecules **50**, **49**, **86**, **85**, **58**, **54**, **67**, **72**, **71**, **90** and **76** were tested against various enzymes and the results are summarized in Table 1.

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)*	**α-man** (*A. fischeri*)
	Ki	*Ki*	*Ki*	*Ki*	*Ki*	*Ki*	*Ki*
 **50**	967	Ni	20%[a]	Ni	Ni	33%[a]	Ni
 **49**	Ni	Ni	27%[a]	Ni	Ni	88.2	217
 **86**	Ni	Ni	Ni	Ni	Ni	16.4	41.4
 **85**	Ni	Ni	Ni	Ni	1066	42%[a]	Ni
 **58**	6%[a]	890	Ni	23%[a]	Ni	210	50%[a]
 **54**	504	85	578	991	456	1.07	325
 **67**	Ni	Ni	Ni	Ni	Ni	153	35%[a]

 **72**	Ni	Ni	Ni	10%[a]	20%[a]	36.8	37.3
 **71**	Ni	Ni	Ni	14%[a]	Ni	217	930
 **90**	Ni	Ni	Ni	17%[a]	34%[a]	471.3	Ni
 **76**	Ni	Ni	Ni	Ni	Ni	72.3	35%[a]

[a] percent inhibition at 1 mM
Ni = no inhibition till 1 mM

None of the compounds (except **54**) showed any significant activity against both α- and β-galactosidase as well as mannosidases (from jack beans). However, all the compounds showed inhibitory activity against α-glucosidase. Compounds **49**, **86**, **54**, **72** and **76** were found to be anomer specific and inhibited α-glucosidase strongly, with K_i values (in μM) of 88.2, 16.4, 1.07, 36.8, 72.3, respectively, whereas there wasn't any significant inhibition of the corresponding β-glucosidase. Compounds **58**, **67**, **71** and **76** proved to be selective inhibitors of α-glucosidase. This reflects the fact that the current framework has the potential to inhibit this class of enzyme, and the results can be explored further and analyzed for further increase in inhibition and to gain an understanding of the mechanistic pathway. The introduction of a second basic site in the form of amine functionality proved to be fruitful since the amine analogue **72** (K_i 36.8 μM and 37.3 μM) proved to be approximately 30 times more potent against both α-glucosidase and α-mannosidase (*A. fischeri*) than its hydroxy analogue **50** (K_i 33%, and no inhibition at 1 mM). However, there was a slight reduction in the activity of the *N*-debenzylated analogue (**49** *vs* **71**) against both the enzymes and also a decrease in the activity against α-mannosidase (*A.*

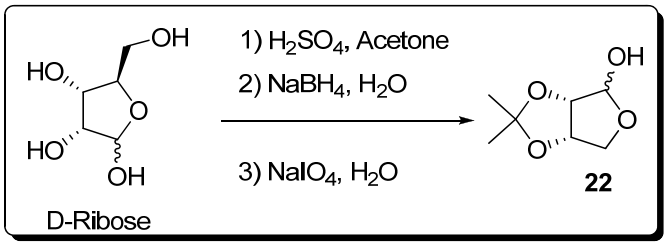
fischeri) when comparing **76** with **49**. Increasing the lipophilicity, was also fruitful as the alkylated product **76** was not only selective for α-glucosidase but also showed more activity (3 times) than its non alkylated form **71** (K_i 217 μM to K_i 72.3 μM). Compound **54** proved to be a very potent inhibitor, in particular, for α-glucosidases (K_i 1.07 μM) but also exhibited some inhibition properties for all the other enzymes tested. In addition, compounds **86** and **72** also displayed good activities (K_i 41.4 μM and 37.3 μM) against the α-mannosidase that was indigenously isolated from *Aspergillus fischeri*.

2.8-Conclusion

In short, we have designed and developed a new synthetic strategy for a general route towards 1-*N*-iminosugar type glycosidase inhibitors, utilizing PET mediated cyclizations of α-trimethylsilylmethylamine radical cation to a proximate tethered π-functionality. The generality of the method developed, has been demonstrated by the synthesis of both the enantiomers of the potent glucosidase inhibitors. Good to moderate diastereoselectivity was observed in the generation of the new stereocenters. The acetonide protecting group was crucial in governing the diastereomeric ratio. Any other acyclic protecting group, probably, would not have resulted in good selectivity in the generation of the new stereocenters. The utility of protocol lies in being diversity-oriented yet simple in nature. The reaction sequence for various azasugars remains almost similar and thus does'nt require a variety of reactions to be tried. The substrate **54** showed moderate to good activities against various enzymes tested. The new amine analogues of azasugars showed a promising activity introducing another area of research to be explored. Most of the *N*-benzylated analogues showed better activity compared to the free amine analogue. The other molecular entities synthesized were tested for their inhibitory activity and some of them were found to be moderate to good inhibitors of the enzymes tested.

2.9-Experimental

(3aS,6aS)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-ol (22)[10]

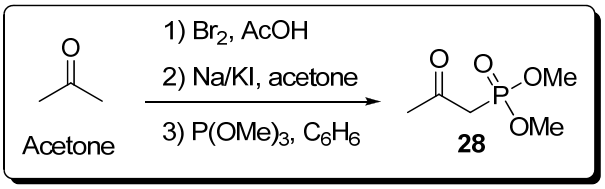


To an ice cold suspension of D-ribose (20.0 g, 133.21 mmol) in acetone (200 mL) was added conc. H_2SO_4 (1.0 mL). The clear solution was obtained after 30 minutes. The reaction mixture was neutralized by the addition of $Ca(OH)_2$ and the salt was removed by filtration. The filtrate was concentrated 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 X 200 mL) and was washed with sat. $NaHCO_3$ followed by brine. The combined organic extracts were dried over Na_2SO_4, concentrated under *vacuo* and purified by column chromatography (pet. ether/ethyl acetate 10:3) to obtain 14.9 g (70 %) of lactol **22**.

$[\alpha]^{27}_D$	+70.59, (*c* 1.2, $CHCl_3$), Lit[10] $[\alpha]^{27}_D$ = +74.8, (*c* 0.96, $CHCl_3$).
IR υ_{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, 1H, *J* = 0.51 Hz), 4.04 (d, 1H, *J* = 3.16 Hz), 4.56 (d, 1H, *J* = 5.94 Hz), 4.82 (dd, 1H, *J* = 5.94, 3.16 Hz), 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: *m/z* (%)	183 (M^++Na, 52), 178 (M^++NH_4, 12), 143 (100).
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

Dimethyl 2-oxopropylphosphonate (28)[12,13]



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.[2] After addition, the solution got decolorized in 20 min. Water (160 mL) was added to reaction mixture and it was cooled to 0 °C, neutralized by $NaHCO_3$ and extracted with DCM (2 X 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.[3]

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 ^{0}C, 11 mm) yielding **28** (66.7 g, 55%).

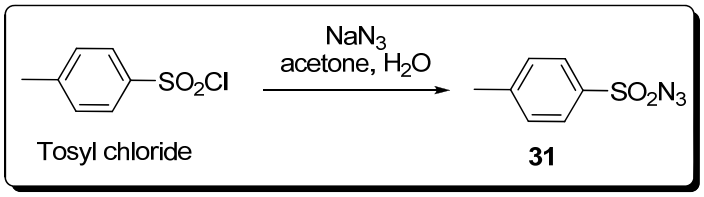
IR υ_{max} cm^{-1} in $CHCl_3$	1711, 1643, 1231.
^{1}H NMR (200 MHz, $CDCl_3$), δ	2.28 (s, 3H), 3.01 (s, 1H), 3.12 (s, 1H), 3.72 (s, 3H), 3.78 (s, 3H).
^{13}C NMR (50 MHz, $CDCl_3$), δ	30.9 (CH_3), 40.3 (CH_2), 42.9 (CH_2), 52.5 (CH_3), 52.6 (CH_3), 199.2 (C), 199.3 (C).

[2] *It is cautioned that the reaction should be done carefully, as fast addition of bromine can lead to highly exothermic reaction.*

[3] *Since, reaction mixture is highly lachrymatory, the reaction should be handled with extreme precaution in the efficient vacuum hood.*

Mass: *m/z* (%)	189 (M^++Na, 16.6), 167 (M^++H, 100), 135 (36.4).
Elemental analysis	Anal. Calcd for $C_5H_{11}O_4$: C, 36.15; H, 6.67. Found: C, 36.42; H, 6.86.

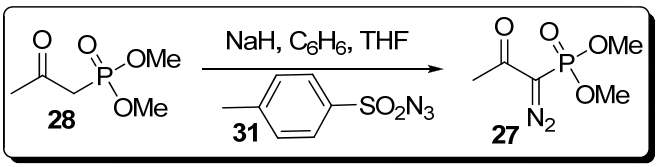
4-methylbenzenesulfonyl azide (31)[14]



To a stirred solution of NaN_3 (9.37 g, 144.2 mmol) in water (40 mL) and acetone (60 mL), was rapidly added solution of tosyl chloride (24.90 g, 131.0 mmol) in acetone (60 mL). The mixture was warmed to 56 °C. Color of the reaction mixture darkened and two phases were separated out. It was then brought to rt, and stirred for 2 h. Acetone was removed under reduced pressure, and the aq. layer was extracted by DCM (2 X 100 mL). The organic layer was dried over Na_2SO_4, concentrated under *vacuo* and chromatographed (petroleum ether/EtOAC, 95:5) to yield tosyl azide **31** (21.9 g) in 85 % yield.

IR υ_{max} cm^{-1} in $CHCl_3$	2128, 1595.
^{1}H NMR (200 MHz, $CDCl_3$), δ	2.47 (s, 3H), 7.37 (s, 1H), 7.42 (s, 1H), 7.81 (s, 1H), 7.85 (s, 1H).
^{13}C NMR (50 MHz, $CDCl_3$), δ	21.69 (CH_3), 127.49 (CH), 130.34 (CH), 135.47 (C), 146.36 (C).
Mass: *m/z* (%)	220 (M^++Na, 100).

Dimethyl 1-diazo-2-oxopropylphosphonate (27)[9,15]

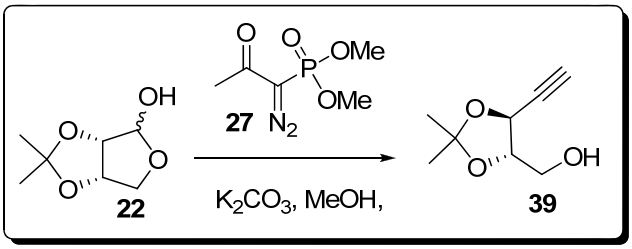


To an ice cold suspension of NaH (60% dispersion in mineral oil) (5.30 g, 132.53 mmol) in benzene (360 mL) and THF (60 mL) under argon atmosphere was added solution of phosphonate **28** (20.0 g, 120 mmol) in benzene (120 mL). After stirring for one hour, solution of tosyl azide **31** in benzene (60 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 (ethyl acetate/pet.ether, 7:3) to get reagent **27** (16.19 g, 70%) as a yellow liquid.

IR υ_{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: *m/z* (%)	215 (M^++Na, 20.49), 193 (M^++H, 38), 165 (100).
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*S*, 5*S*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (39)

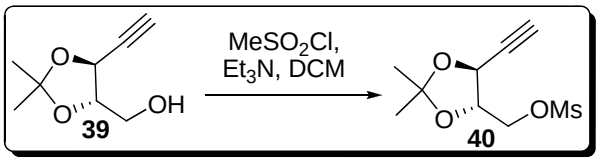


To a stirring mixture of **22** (10.0 g, 62.5 mmol) and anhydrous K_2CO_3 (11.37 g, 81.25 mmol) in dry MeOH (240 mL) at 65 °C was added the solution of Ohira reagent (15.6 g, 81.25 mmol) in dry MeOH (80 mL) drop wise over a period of 6 h under argon atmosphere. After neutralization with acetic acid, solvent was removed under *vacuo*, water was added and mixture was extracted with ethyl acetate (2 X 100 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified by column chromatography (pet. ether/ethyl acetate, 4:1) to obtain **39** (6.82 g, 70%) as a colorless liquid.

$[\alpha]^{27}_D$	-8.6 (*c* 1.0, MeOH); Lit.[18b] $[\alpha]^{20}_D$ -7.3 (*c* 2.0, MeOH).
IR (neat) υ_{max} cm^{-1}	3452, 3284, 2121, 848, 665.
^1H NMR (200 MHz, $CDCl_3$)[a], δ	1.42 (s, 3H), 1.48 (s, 3H), 2.53 (d, 1H *J* = 2.15 Hz), 3.64 (dd, 1H, *J* = 12.25, 3.67 Hz), 3.87 (dd, 1H *J* = 12.25, 3.03 Hz), 4.16 (ddd, 1H, *J* = 7.58, 3.67, 3.03 Hz), 4.56 (dd, 1H, *J* = 7.57, 2.15 Hz).

^{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: *m/z* (%)	179 (M^++Na, 100), 157 (M^++H, 32), 139 (57).
Elemental analysis	Anal. Calcd for $C_8H_{12}O_3$: C, 61.52; H, 7.74. Found: C, 61.79; H, 7.84.

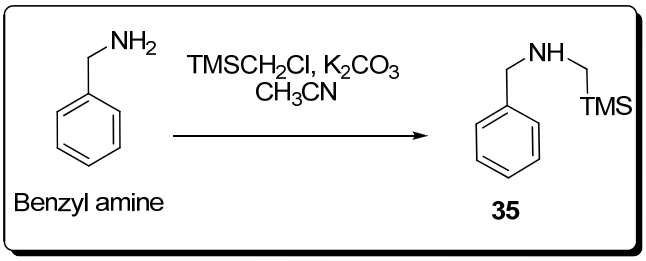
((4*S*,5*S*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)methyl methanesulfonate (40)



To a stirred solution of alcohol **39** (7.0 g, 44.87 mmol) and triethyl amine (6.87 mL, 49.35 mmol) in dry DCM (100 mL) under argon atmosphere, was added methane sulphonyl chloride (3.82 mL, 49.35 mmol) drop wise at 0 °C. The reaction mixture was allowed to come to the room temp. and stirred for additional 5 hours. Water was added and extracted with DCM (2 X 100 mL). The organic layer was given brine wash, dried over anhydrous Na_2SO_4, concentrated under *vacuo* and chromatographed (petroleum ether/EtOAc, 4:1) to get **40** (10.49 g) as a yellow liquid in quantitative yield.

$[\alpha]^{29}_D$	-29.36 (c 0.6, $CHCl_3$).
IR υ_{max} cm^{-1} in $CHCl_3$	3282, 2123, 1359, 1219.
^{1}H NMR (200 MHz, $CDCl_3$), δ	1.42 (s, 3H), 1.49 (s, 3H), 2.58 (d, 1H, *J* = 2.02 Hz), 3.06 (s, 3H), 4.25-4.44 (m, 3H), 4.54 (dd, 1H, *J* = 6.82, 2.02 Hz).
^{13}C NMR (100 MHz, $CDCl_3$), δ	26.0 (CH_3), 26.6 (CH_3), 37.6 (CH_3), 66.5 (CH), 67.1 (CH_2), 75.5 (CH), 79.1(CH), 79.7(C), 111.5 (C).
Mass: *m/z* (%)	234 (M^+, 8), 217 (71), 186 (64), 123 (100).
Elemental analysis	Anal. Calcd for $C_9H_{14}O_5S$; C, 46.14; H, 6.02; S, 13.69. Found: C, 46.24; H, 5.90; S, 13.66.

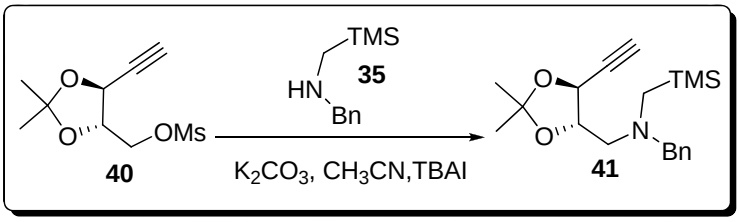
N-benzyl-1-(trimethylsilyl)methanamine (35)



A mixture of benzyl amine (47.11 mL, 429.93 mmol), chloromethyl trimethyl silane (15 mL, 107.4 mmol) and anhydrous K_2CO_3 (60.19 g, 429.93 mmol) in dry CH_3CN (26 mL) was refluxed for 8 h, under argon atmosphere. The reaction mixture was filtered and the filtrate concentrated. The crude mixture upon column chromatography (silica, pet ether-ethyl acetate, 7:3) afforded **35** (18.77 g, 90 %) as colorless oil.

IR υ_{max} cm^{-1} in $CHCl_3$	3366, 1216, 758.
^1H NMR (200 MHz, $CDCl_3$), δ	0.03 (s, 9H), 2.04 (s, 2H), 3.78 (s, 2H), 7.21-7.36 (m, 5H).
^{13}C NMR (50 MHz, $CDCl_3$), δ	-2.7 (CH_3), 39.3 (CH_2), 57.9 (CH_2), 126.7 (CH), 128.1 (CH), 128.2 (CH), 140.4 (C).
Mass: *m/z*	194 (M^++H, 100%).
Elemental analysis	Anal. Calcd for $C_{11}H_{19}NSi$: C, 68.33; H, 9.90; N, 7.24. Found: C, 68.56; H, 10.11; N, 7.77.

N-benzyl-1-((4S,5S)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)-N-((trimethylsilyl) methyl)methanamine (41)

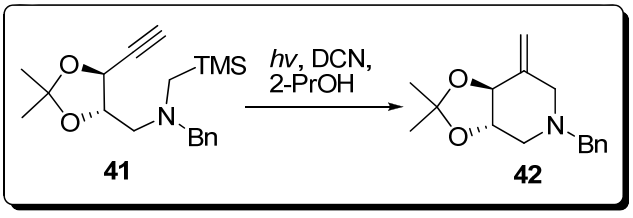


A mixture of **40** (8 g, 34.1 mmol), $PhCH_2NHCH_2TMS$ **35** (13.15 g, 68.3 mmol), anhydrous K_2CO_3 (19.15 g, 136.7 mmol) catalytic amount of TBAI (1.0 g, 4.1 mmol) in dry CH_3CN (60 mL) was refluxed for 96 h under argon atmosphere. Solvent was removed under reduced pressure, water was added and the reaction mixture was extracted with

EtOAc (2 X 100 mL). The combined organic extracts were dried over Na_2SO_4, concentrated in *vacuo* and the residue was column chromatographed (petroleum ether-ethyl acetate, 19:1) to afford pure **41** (7.96 g, 80 %) as a colorless liquid.

$[\alpha]^{27}_D$	-0.73, (c 0.5, $CHCl_3$); *ent* $[\alpha]^{28}_D$ = +0.72, (c 1.35, $CHCl_3$); Lit.[18b] $[\alpha]^{20}_D$ -0.7, (c 11.0, $CHCl_3$); $[\alpha]^{20}_D$ +1.2, (c 21.2, $CHCl_3$); respectively.
IR υ_{max} cm^{-1} in $CHCl_3$	3307, 1674, 757.
^1H NMR (200 MHz, $CDCl_3$), δ	0.00 (s, 9H), 1.29 (s, 3H), 1.39 (s, 3H), 1.95 (d, 1H, *J* = 14.66 Hz), 2.08 (d, 1H, *J* = 14.65 Hz), 2.43 (d, 1H, *J* = 2.02 Hz), 2.51-2.56 (m, 2H), 3.42 (d, 1H, *J* = 13.64 Hz), 3.64 (d, 1H, *J* = 13.51 Hz), 4.14-4.23 (m, 1H), 4.30 (dd, 1H, *J* = 7.33, 2.03 Hz), 7.12-7.30 (m, 5H).
^{13}C NMR (50 MHz, $CDCl_3$), δ	-1.4 (CH_3), 25.8 (CH_3), 26.8 (CH_3), 46.9 (CH_2), 58.1 (CH_2), 62.7 (CH_2), 68.6 (CH), 74.3 (CH), 80.4 (CH), 81.3 (C), 110.0 (C), 126.7 (CH), 127.9 (CH), 128.7 (CH), 139.3 (C).
Mass: *m/z*	332 (M^++H), 284 (22), 246 (19), 238 (33).
Elemental analysis	Anal. Calcd for $C_{19}H_{29}NO_2Si$: C, 68.83; H, 8.82; N, 4.22. Found: C, 68.63; H, 8.60; N, 4.38.

(3aS,7aS)-5-benzyl-2,2-dimethyl-7-methylenehexahydro-[1,3]dioxolo[4,5-c]pyridine (42)

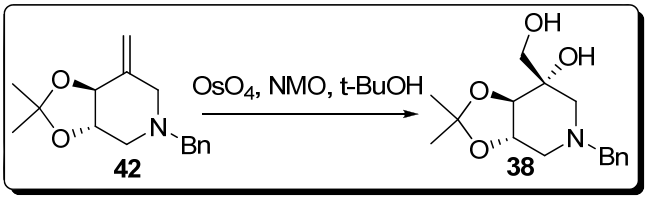


A solution containing the substrate **41** (1.0 g, 3.02 mmol) and 1,4-dicyanonaphthalene (DCN) (0.18 g, 1.01 mmol) in 2-propanol (80 mL per mmol of substrate) in an open vessel, was irradiated using a 450-W Hanovia medium pressure mercury vapor lamp as the light source. The lamp was housed in a Pyrex water-jacketed immersion well so as to allow only the wavelengths greater than 280 nm to pass through. The reaction was

monitored by TLC or GC till consumption of the starting material (> 90% ~ 1 h). The solvent was then removed under reduced pressure and the crude product was purified by column chromatography (petroleum ether-ethyl acetate, 92.5:7.5) to get the pure compound **42** (0.47g, 60%) as a white solid (mp 96-98 °C)

$[\alpha]^{22}_D$	+53 (c 0.75, $CHCl_3$); *ent* $[\alpha]^{28}_D$ = -52, (c 1.0, $CHCl_3$) Lit[18b] $[\alpha]^{20}_D$+49.2 (c 0.7, $CHCl_3$); $[\alpha]^{20}_D$ -50.9, (c 1.9, $CHCl_3$); respectively.
IR υ_{max} cm^{-1} in $CHCl_3$	3020, 1216, 757.
^1H NMR (200 MHz, $CDCl_3$), δ	1.39 (s, 6H), 2.24-2.34 (m, 1H), 2.70 (d, 1H, *J* = 12.63 Hz), 3.19 (s, 1H), 3.26 (s, 1H), 3.40-3.55 (m, 1H), 3.60 (s, 1H), 3.61 (s, 1H), 3.73 (td, 1H, *J* = 9.22, 1.77 Hz), 4.81 (d, 1H, *J* = 1.26 Hz), 4.94 (s, 1H), 7.19-7.29 (m, 5H).
^{13}C NMR (50 MHz, $CDCl_3$), δ	26.7 (CH_3), 26.9 (CH_3), 54.5 (CH_2), 57.2 (CH_2), 61.6 (CH_2), 77.4 (CH), 81.7 (CH), 105.3 (CH_2), 111.0 (C), 127.2 (CH), 128.3 (CH), 128.9 (CH), 137.7 (C), 140.3 (C).
Mass: *m/z* (%)	282 (M^++Na, 63), 260 (M^++H, 100), 250 (30).
Elemental analysis	Anal. Calcd for $C_{16}H_{21}NO_2$: C, 74.10; H, 8.16; N, 5.40. Found: C, 74.23; H, 8.56 N, 5.46.
mp	mp 96-98 °C (ethanol) Lit. 94-96 °C.

(3a*S*,7*S*,7a*R*)-5-benzyl-7-(hydroxymethyl)-2,2-dimethyl-hexahydro-[1,3]dioxolo[4,5-c]pyridin-7-ol (38)



To a suspension of **42** (1.0 g, 3.8 mmol) in *t*-BuOH (10 mL) was added N-methylmorpholine-N-oxide (50% aq. solution) (0.634 mL, 7.69 mmol) and osmium tetroxide (0.5 mL, 1% solution of OsO_4 in t-BuOH). The reaction mixture was stirred for

24 h. Solvent was removed under reduced pressure, water was added and the reaction mixture was extracted with EtOAc (3 X 25 mL). The combined organic extracts were dried over Na_2SO_4 and the residue was column chromatographed (petroleum ether-ethyl acetate, 10:3) to afford pure **38** (1.018g, 90%) as a white solid (mp152-154 °C).

$[\alpha]^{29}_D$	$[\alpha]^{29}_D$ +24.2 (c 0.6, MeOH), *ent* $[\alpha]^{29}_D$ -23.6, (c 1.2, MeOH); Lit.[18d,18e], $[\alpha]^{25}_D$ +23.1, (c 0.46, MeOH); $[\alpha]^{25}_D$ -21.6, (c 1.4, MeOH).
IR υ_{max} cm^{-1} in $CHCl_3$	3491, 1216.
^1H NMR (400 MHz, $CDCl_3$)[a], δ	1.35 (s, 6H), 2.02 (d, 1H, *J* = 11.8 Hz), 2.12 (dd, 1H, *J* = 10.04, 9.79 Hz), 2.78 (d, 1H, *J* = 11.80 Hz), 3.11 (dd, 1H, *J* = 9.54, 4.01 Hz), 3.42 (d, 1H, *J* = 9.54 Hz), 3.55 (s, 2H), 3.62 (d, 1H, *J* = 11.55 Hz), 3.64 (dt, 1H, *J* = 9.78, 4.01 Hz), 4.02 (d, 1H, *J* = 11.30 Hz), 7.16-7.26 (m, 5H).
^{13}C NMR (100 MHz, $CDCl_3$), δ	26.5 (CH_3), 26.6 (CH_3), 54.4 (CH_2), 59.1 (CH_2), 61.4 (CH_2), 64.7 (CH_2), 71.4 (C), 73.5 (CH), 86.0 (CH), 110.8 (C), 127.4 (CH), 128.4 (CH), 128.7 (CH), 137.7 (C).
Mass: *m/z* (%)	316(M^++Na, 77), 294 (M^++H, 100).
Elemental analysis	Anal. Calcd for $C_{16}H_{23}NO_4$: C, 65.51; H, 7.90; N, 4.77. Found: C, 65.43; H, 7.81 N, 4.97.
mp	152-154 °C (from EtOAc/pet ether) Lit. 150-152 °C.

a = D_2O exchange

X-ray Crystal Structure Analysis for compound 38 and 83

Crystal Data:

Data for both the compounds were collected at *T* = 293 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)[4]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 38: (CCDC number 673352)

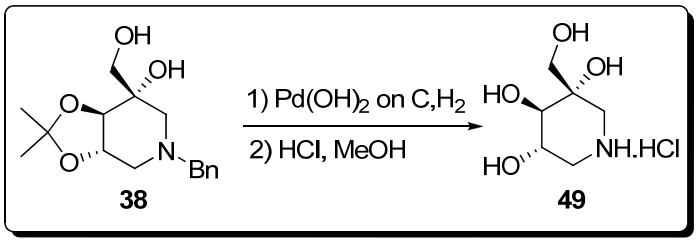
Single crystals of the complex were grown by slow evaporation of the solution mixture of ethyl acetate and pet ether. Colorless needle of approximate size 0.34 x 0.14 x 0.05 mm, was used for data collection. Crystal to detector distance 6.05 cm, 512 x 512 pixels / frame, Hemisphere data acquisition. Total scans = 3, total frames = 1283, 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 = 2.16 to 25.0 º, completeness to θ of 25.0 º is 98.8 %. SADABS correction applied, $C_{16}H_{23}NO_4$, M = 293.35. Crystals belong to Monoclinic, space group $P2_1$, a = a = 10.872 (1), b = 5.6676 (5), c = 12.916 (1) Å, β = 106.839 (1)º V = 761.75 (12) $Å^3$, Z = 2, Dc = 1.279 g/cc, μ (Mo–Kα) = 0.091 mm^{-1}, 3761 reflections measured, 2445 unique [I>2σ(I)], R value 0.0482, wR_2 = 0.1185. Largest diff. peak and hole 0.212 and -0.165 e. $Å^{-3}$.

Compound 83: (CCDC number 673353)

Single crystals of the complex were grown by slow evaporation of the solution mixture of ethyl acetate and pet ether. Colorless needle of approximate size 0.21 x 0.05 x 0.02 mm, was used for data collection. Crystal to detector distance 6.05 cm, 512 x 512 pixels / frame, multiscan data acquisition. Total scans = 5, total frames = 2545, 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 = 1.65 to 23.99 º, completeness to θ of 23.99 º is 92.7 %. SADABS correction applied, $C_{16}H_{23}NO_4$, M = 293.35. Crystals belong to monoclinic, space group $P2_1$, a = 10.9255(6), b = 5.6715(3), c = 12.9037(8) Å, beta = 107.143(1) deg, V = 764.04(8) $Å^3$, Z = 2, Dc = 1.275 g/cc, μ (Mo–Kα) = 0.091 mm^{-1}, 5981 reflections measured, 2186 unique [I>2σ(I)], R value 0.0565, wR_2 = 0.1142. Largest diff. peak and hole 0.201 and -0.224 e. $Å^{-3}$.

(3S,4R,5S)-3-(hydroxymethyl)piperidine-3,4,5-triol hydrochloride (49)

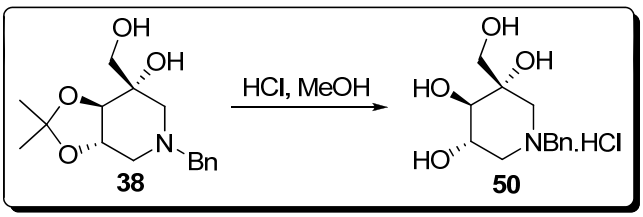
[4] 1 G. M. Sheldrick, SHELX-97 program for crystal structure solution and refinement, University of Gottingen, Germany, 1997



A solution of **38** (0.02 g, 0.068 mmol) in ethanol was hydrogenated (1 atm, rt) in the presence of $Pd(OH)_2$ on charcoal (20 %, 0.002 g) for 10 h. The reaction mixture was passed through a pad of Celite and the solvent was removed under reduced pressure. The crude hydrogenated product was dissolved in distilled methanol (1 mL) and conc. HCl (2 drops) was added to the solution and stirred at rt for 4 h. The solvent was removed under reduced pressure to afford **49** (0.013 g) quantitativly, as a white solid.

$[\alpha]^{28}_D$	-16.98 (c 0.5, MeOH), *ent* $[\alpha]^{28}_D$ = +15.47 (c 0.65, MeOH); Lit.[18d] $[\alpha]^{25}_D$ -12.1 (c 0.15, EtOH); Lit[18e] $[\alpha]^{25}_D$ +11.0 (c 0.2, EtOH).
^{1}H NMR (200 MHz, D_2O), δ	3.15 (d, 1H, *J* = 13.14 Hz), 3.25 (d, 1H, *J* = 5.56 Hz), 3.32 (d, 1H, *J* = 5.18 Hz), 3.43 (dd, 1H, *J* = 13.39, 2.91 Hz), 3.62 (d, 1H, *J* = 11.87 Hz), 3.71 (d, 1H, *J* = 11.88 Hz), 3.85 (d, 1H, *J* = 4.68 Hz), 4.11 (dd, 1H, *J* = 7.39, 4.11 Hz).
^{13}C NMR (100 MHz, D_2O), δ	45.3 (CH_2), 46.2 (CH_2), 63.7 (CH_2), 66.7 (CH), 68.0 (CH), 71.7 (C).
Mass: *m/z* (%)	164 (100), 146 (26), 128 (16).
Elemental analysis	Anal. Calcd for $C_6H_{14}ClNO_4$: C, 36.10; H, 7.07; N, 7.02; Found: C, 36.23; H, 7.27; N, 7.21.

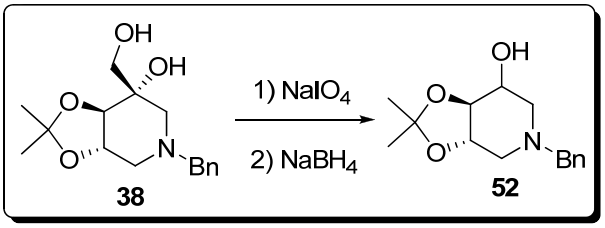
(3S,4R,5S)-1-benzyl-3-(hydroxymethyl)piperidine-3,4,5-triol hydrochloride (50)



To a solution of **38** (0.025 g, 0.085 mmol) in distilled methanol (1 mL) was added conc. HCl (2 drops) and the reaction mixture was stirred at rt for 4 h. The solvent was removed under reduced pressure to afford **50** (0.024 g,) quantitatively as a white solid.

$[\alpha]^{22}_D$	$[\alpha]^{22}_D$ -2.90, (c 1.05, MeOH), *ent* $[\alpha]^{27}_D$ +3.73 (c 0.75, MeOH).
^{1}H NMR (400 MHz, D_2O), δ	3.19 (d, 1H, *J* = 12.80 Hz), 3.30 (d, 1H, *J* = 12.80 Hz), 3.44 (d, 1H, *J* = 13.05 Hz), 3.52-3.56 (m, 2H), 3.69 (d, 1H, *J* = 12.04 Hz), 3.88 (s, 1H), 4.16 (d, 1H, *J* = 3.26 Hz), 4.45 (d, 1H, *J* = 13.05 Hz), 4.51 (d, 1H, *J* = 13.05 Hz), 7.57-7.60 (m, 5H).
^{13}C NMR (100 MHz, D_2O), δ	53.06 (CH_2), 53.13 (CH_2), 60.8 (CH_2), 64.1 (CH_2), 65.4 (CH), 67.8 (CH), 72.8 (C), 127.6 (C), 129.3 (CH), 130.4 (CH), 131.7 (CH).
Mass: *m/z* (%)	255 (33), 254 (100), 236 (13).
Elemental analysis	Anal. Calcd for $C_{13}H_{20}ClNO_4$: C, 53.89; H, 6.96; N, 4.83; Found: C, 53.69; H, 6.86 N, 4.97.

(3aS,7aS)-5-benzyl-2,2-dimethylhexahydro-[1,3]dioxolo[4,5-c]pyridin-7-ol (52)



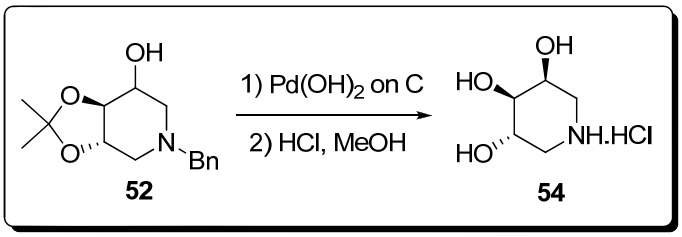
To a solution of **38** (0.50 g, 1.7 mmol) in ethanol-water (8 mL, 4:1) mixture was added sodium periodate (0.438 g, 2.04 mmol) gradually. The white suspension was stirred for 0.5 h and filtered. The filtrate was concentrated and the white pasty mass was extracted in ethyl acetate (2 X 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure. Sodium borohydride (0.077 g, 2.04 mmol) was added to a solution of ketone in methanol (5 mL). The resulting mixture was stirred for 24 h at room temperature and quenched by brine. This white suspension was stirred overnight and extracted with ethyl acetate (2 X 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent was removed under

reduced pressure. The residue was column chromatographed (pet ether-ethyl acetate, 3:2) to afford **52** (0.086 g, 85 %) as a colorless liquid.

$[\alpha]^{29}_{D}$	+38.38 (c 1.45, $CHCl_3$), Lit.[18d,18e] $[\alpha]^{25}_{D}$ +35.0 (c 0.2, $CHCl_3$).
IR υ_{max} cm^{-1} in $CHCl_3$	3018, 1216.
^{1}H NMR (400 MHz, $CDCl_3$)[a], δ	1.34 (s, 3H), 1.38 (s, 3H), 2.15 (t, 1H, *J* = 10.04 Hz), 2.23 (d, 1H, *J* = 12.80 Hz), 2.95 (d, 1H, *J* = 12.55 Hz), 3.18-3.22 (m, 2H), 3.61 (s, 2H), 3.92 (dt, 1H, *J* = 10.04, 4.02 Hz), 4.14 (s, 1H), 7.16-7.26 (m, 5H).
^{13}C NMR (100 MHz, $CDCl_3$), δ	26.5 (CH_3), 26.8 (CH_3), 54.8 (CH_2), 56.6 (CH_2), 61.7 (CH_2), 65.6 (CH), 70.8 (CH), 81.6 (CH), 110.3 (C), 127.3 (CH), 128.7 (CH), 129.0 (CH), 137.2 (C).
Mass: m/z (%)	264 (M^++H, 33), 263 (M^+, 100), 223 (27).
Elemental analysis	Anal. Calcd for $C_{15}H_{21}NO_3$: C, 68.42; H, 8.04; N, 5.32. Found: C, 68.82; H, 7.90; N, 5.66.

a = D_2O exchange

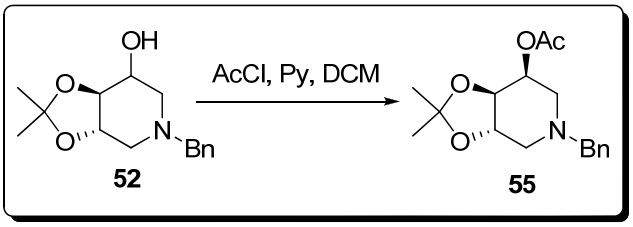
(3S,5S)-piperidine-3,4,5-triol hydrochloride (54)



A solution of **52** (0.05 g, 0.19 mmol) in ethanol was hydrogenated (1 *atm*, rt) in the presence of $Pd(OH)_2$ on charcoal (20 %) (0.008 g), for 10 h. The reaction mixture was passed through a pad of Celite and the solvent was removed under reduced pressure. The crude liquid was chromatographed by flash column chromatography (silica 260:400) (DCM/MeOH, 95:5)**.** To a solution of the resultant compound (0.029 g, 0.17 mmol) in distilled methanol (1 mL) was added conc. HCl (2 drops) and the reaction mixture was stirred at rt for 5h. The solvent was removed under *vacuo* to afford **54** (0.027 g) in 85 % yield as a white viscous liquid.

$[\alpha]^{27}_D$	+15 (c 0.3, MeOH), Lit.[6] $[\alpha]^{25}_D$ +16 (c 0.5, MeOH).
^{1}H NMR (400 MHz, D_2O), δ	δ 3.04 (dd, 1H, *J* = 12.80, 8.28 Hz), 3.28 (dd, 1H, *J* = 13.05, 2.76 Hz), 3.37 (dd, 1H, *J* = 13.05, 6.02 Hz), 3.49 (dd, 1H, *J* = 12.80, 4.01 Hz), 3.86 (dd, 1H, *J* = 7.78, 3.01 Hz), 4.18 (dt, 1H, *J* = 8.03, 4.01 Hz), 4.30-4.33 (m, 1H).
^{13}C NMR (100 MHz, D_2O), δ	45.5 (CH_2), 46.0 (CH_2), 64.7 (CH), 65.0 (CH), 70.8 (CH).
Mass: *m/z* (%)	132 (100), 107 (45).
Elemental analysis	Anal. Calcd for $C_5H_{12}ClNO_3$: C, 35.41; H, 7.13; N, 8.26; Found: C, 35.23; H, 7.27; N, 8.21.

(3aS,7S,7aR)-5-benzyl-2,2-dimethyl-hexahydro-[1,3]dioxolo[4,5-c]pyridin-7-yl acetate (55)

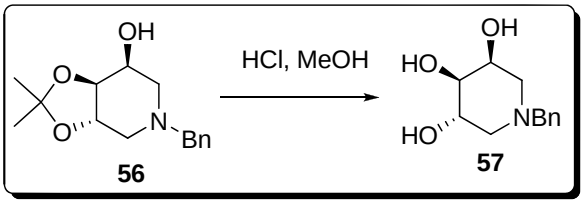


To a solution of alcohol **52** (0.1 g, 0.38 mmol) and pyridine (0.036 ml, 0.45 mmol) in dry DCM (2 mL) under argon atmosphere was added acetyl chloride (0.027 ml, 0.38 mmol) and the reaction mixture was allowed to stir at room temperature for 30 h. After adding few drops of water, it was extracted with DCM (2 X 5 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified by flash column chromatography (silica 260-400, pet. ether/ethyl acetate, 10:3) to get **55** (0.092 g, 80%) as a colorless liquid.

$[\alpha]^{31}_D$	+60.54 (c 1.85, $CHCl_3$).
IR (neat) υ_{max} cm^{-1}	1741, 1217, 756.
^{1}H NMR (200 MHz, $CDCl_3$), δ	1.42 (s, 6H), 2.13 (s, 3H), 2.27 (t, 1H, *J* = 9.85 Hz), 2.38 (dd, 1H, *J* = 13.13, 1.90 Hz), 3.08 (d, 1H, *J* = 13.39 Hz), 3.26-3.38 (m, 2H), 3.70 (s, 2H), 4.05 (dt, 1H, *J* = 9.72, 3.91 Hz), 5.34 (dd, 1H, *J* = 4.74, 2.46 Hz) 7.21 -7.37 (m, 5H).

^{13}C NMR (50 MHz, $CDCl_3$), δ	21.0 (CH_3), 26.4 (CH_3), 26.8 (CH_3), 53.9 (CH_2), 54.6 (CH_2), 61.5 (CH_2), 67.4 (CH), 71.4 (CH), 79.6 (CH), 110.5 (C), 127.3 (CH), 128.3 (CH), 128.9 (CH), 137.2 (C), 170.5 (C).
Mass: *m/z* (%)	328 (M^++Na, 75), 306 (M^++H, 100), 266 (64), 229 (95).
Elemental analysis	Anal. Calcd for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59. Found: C, 66.53; H, 7.89; N, 4.23.

(3S,5S)-1-benzylpiperidine-3,4,5-triol (57)



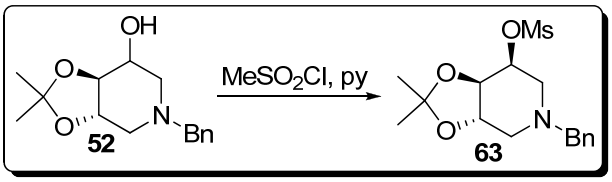
56 **57**

To a solution of **56** (0.022 g, 0.083 mmol) in distilled methanol (1 mL) was added conc. HCl (2 drops) and the reaction mixture was stirred at rt for 4 h. The pH of reaction mixture was made basic by $NaHCO_3$ solution. The solvent was removed under reduced pressure and the residue was chromatographed (DCM/MeOH, 92.5:7.5) to obtain **57** as a white solid (0.016 g, 90%).

$[\alpha]^{23}_D$	+26.66 (c 0.3, MeOH).
^{1}H NMR (400 MHz, $CDCl_3$)[a], δ	2.04-2.08 (m, 1H), 2.35 (d, 1H, *J* = 11.80 Hz), 2.91 (d, 1H, *J* = 9.03 Hz), 2.99 (d, 1H, *J* = 8.03 Hz), 3.36 (d, 1H, *J* = 5.02 Hz), 3.61 (s, 2H), 3.79 (dt, 1H, *J* = 8.78, 4.77 Hz), 3.91 (s, 1H), 7.28-7.36 (m, 5H).
^{13}C NMR (100 MHz, $CDCl_3$), δ	56.5 (CH_2), 56.8 (CH_2), 61.9 (CH_2), 68.3 (CH), 69.5 (CH), 127.5 (CH), 128.5 (CH), 129.1 (CH), 137.3 (C).
Mass: *m/z* (%)	246 (M^++Na, 42), 224 (M^++H, 100).
Elemental analysis	Anal. Calcd for $C_{12}H_{17}NO_3$: C, 64.55; H, 7.67; N, 6.27. Found: C, 64.44; H, 7.87; N, 6.37.

a = D_2O exchange

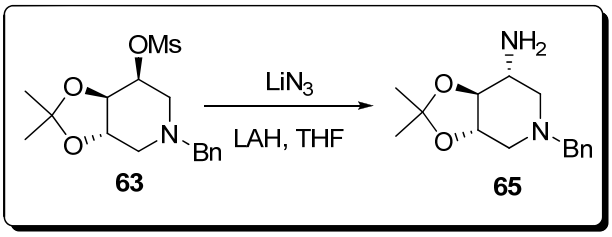
(3aS,7S,7aR)-5-benzyl-2,2-dimethyl-hexahydro-[1,3]dioxolo[4,5-c]pyridin-7-yl methanesulfonate (63)



To a solution of **52** (0.1g, 0.38 mmol) in pyridine (2 mL) at 0 °C was added methane sulphonyl chloride (0.032 mL, 0.42 mmol). The reaction mixture was stirred at room temperature for 6 h. After the removal of pyridine under reduced pressure, water was added and solution was extracted with DCM (2 X 5 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated under *vacuo*, and purified by column chromatography (pet. ether/ethyl acetate, 4:1) to get **63** (0.11 g) in 85 % yield as a colorless liquid.

$[\alpha]^{20}_D$	+46.66 (c 0.45, $CHCl_3$).
IR (neat) υ_{max} cm^{-1}	1361, 738.
^1H NMR (200 MHz, $CDCl_3$), δ	1.41 (s, 3H), 1.43 (s, 3H), 2.32 (t, 1H, *J* = 10.11 Hz), 2.52 (d, 1H, *J* = 13.64 Hz), 3.09, (s, 3H), 3.19-3.38 (m, 3H), 3.76 (s, 2H), 4.04 (dt, 1H, *J* = 9.86, 3.92 Hz), 5.13 (dd, 1H, *J* = 4.36, 2.34 Hz), 7.26-7.37 (m, 5H).
^{13}C NMR (125 MHz, $CDCl_3$), δ	26.3 (CH_3), 26.8 (CH_3), 38.9 (CH_3), 54.2 (CH_2), 55.0 (CH_2), 61.2 (CH_2), 71.1 (CH), 75.1 (CH), 79.2 (CH), 110.9 (C), 127.4 (CH), 128.4 (CH), 128.8 (CH), 137 (C).
Mass: *m/z* (%)	342 (M^++H, 93), 224 (100).
Elemental analysis	Anal. Calcd for $C_{16}H_{23}NO_5S$: C, 56.29; H, 6.79; N, 4.10; S, 9.39. Found: C, 56.18; H, 6.75; N, 4.58; S, 9.36.

(3a*S*,7*R*,7a*S*)-5-benzyl-2,2-dimethyl-hexahydro-[1,3]dioxolo[4,5-c]pyridin-7-amine (65)

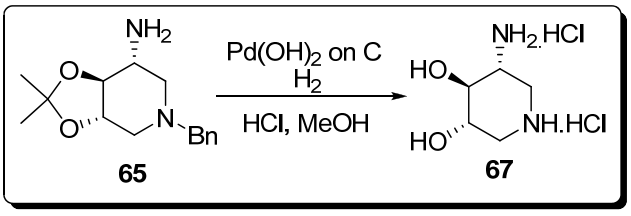


To a solution of **63** (0.05 g, 0.29 mmol) in DMF (2 mL) was added LiN_3 (0.072 g, 2.9 mmol) and heated to 110 °C for 20 h. The reaction mixture was extracted with EtOAc, water washed, dried over Na_2SO_4 and concentrated. The resultant azide was dissolved in dry THF to which LAH (0.022 g, 0.58 mmol) was added and stirred overnight. Reaction mixture was quenched by drop wise addition of 2N NaOH solution followed by solid Na_2SO_4, filtered, concentrated and chromatographed (DCM/MeOH, 95:5) to get amine **65** as a yellow liquid (0.023 g, 60% over two steps).

$[\alpha]^{22}_D$	+14.29 (c 1.4, $CHCl_3$).
IR (neat) υ_{max} cm^{-1}	3390, 2343, 736.
^1H NMR (400 MHz, $CDCl_3$)[a], δ	1.44 (s, 3H), 1.45 (s, 3H), 1.89 (dd, 1H, *J* = 11.29, 9.28 Hz), 2.22 (t, 1H, *J* = 10.04 Hz), 2.97 (dd, 1H, *J* = 11.34, 3.66 Hz), 3.04-3.14 (m, 2H), 3.21 (dd, 1H, *J* = 9.66, 3.89 Hz), 3.56-3.63 (m, 1H), 3.66 (s, 2H), 7.25-7.35 (m, 5H).
^{13}C NMR (100 MHz, $CDCl_3$), δ	26.7 (CH_3), 26.8 (CH_3), 50.2 (CH), 54.3 (CH_2), 59.4 (CH_2), 61.7 (CH_2), 75.4 (CH), 85.8 (CH), 110.6 (C), 127.3 (CH), 128.3 (CH), 128.9 (CH), 137.8 (C).
Mass: *m/z* (%)	285 (M^++Na, 5), 263 (M^++H, 100), 205 (44).
Elemental analysis	Anal. Calcd for $C_{15}H_{22}N_2O_2$: C, 68.67; H, 8.45; N, 10.68. Found: C, 68.52; H, 8.35; N, 10.77.

a = D_2O exchange

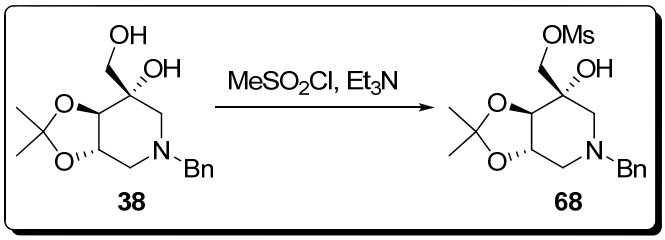
(3S,4S,5R)-5-aminopiperidine-3,4-diol dihydrochloride (67)



A solution of **65** (0.02 g, 0.075 mmol*)* in ethanol was hydrogenated (1 atm, rt) in the presence of $Pd(OH)_2$ on charcoal (20 %, 0.004 g) for 10 h. The reaction mixture was passed through a pad of Celite and the solvent was removed under reduced pressure. To a solution of this in distilled methanol (1 mL) was added conc. HCl (3 drops) and the reaction mixture was stirred at rt for 4 h. The solvent was evaporated to dryness to afford **67** (0.015 g) quantitatively as white viscous liquid.

$[\alpha]^{25}_D$	-1.05 (c 0.95, MeOH).
^{1}H NMR (400 MHz, D_2O), δ	3.11 (dd, 1H, *J* = 12.80, 10.29 Hz), 3.35 (dd, 1H, *J* = 10.67, 12.93 Hz), 3.58-3.67 (m, 2H), 3.80-3.85 (m, 2H), 3.93-3.99 (m, 1H).
^{13}C NMR (100 MHz, D_2O), δ	42.8 (CH_2), 46.1 (CH_2), 48.7 (CH), 66.8 (CH), 70.9 (CH).
Mass: *m/z* (%)	133 (100), 116 (81), 102 (20).
Elemental analysis	Anal. Calcd for $C_5H_{14}Cl_2N_2O_2$: C, 29.28; H, 6.88; N, 13.66, Found: C, 29.38; H, 6.56; N, 13.56.

(3a*S*,7*S*,7a*S*)-5-benzyl-7-hydroxy-2,2-dimethyl-hexahydro-[1,3]dioxolo[4,5-c]pyridin-7-yl)methyl methanesulfonate (68)



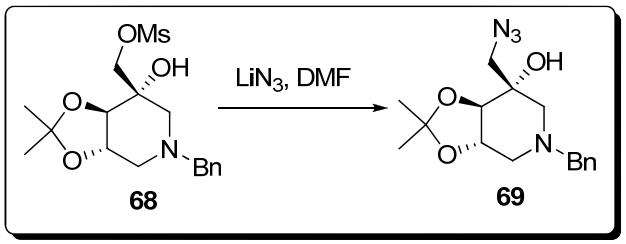
To a solution of **38** (0.2 g, 0.68 mmol) in dry DCM (3 mL) at 0 °C under argon atmosphere was added triethyl amine (0.114 ml, 0.82 mmol) and methanesulphonyl chloride (0.58 mL, 0.75 mmol). The reaction mixture was stirred at room temperature for 6 h, water was added and extracted with DCM (2 X 5 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated under *vacuo* and purified by column chromatography (pet. ether/ethyl acetate, 5:2) to get **68** (0.23 g, 90%) as a white solid (100-102 °C).

$[\alpha]^{30}_D$	$[\alpha]^{30}_D$ +46.52 (c 0.35, $CHCl_3$), *ent* $[\alpha]^{32}_D$ -43.47, (c

	1.40, $CHCl_3$).
IR υ_{max} cm^{-1} in $CHCl_3$	2933, 1359, 1228.
^1H NMR (200 MHz, $CDCl_3$)[a], δ	1.41(s, 3H), 1.42 (s, 3H), 2.03 (d, 1H, *J* = 11.87 Hz), 2.25 (dd, 1H, *J* = 9.72, 9.61 Hz), 2.95 (s, 3H), 3.00 (dd, 1H, *J* = 11.88, 1.01 Hz), 3.22 (ddd, 1H, *J* = 9.60, 3.92, 1.14 Hz), 3.49 (d, 1H, *J* = 9.60 Hz), 3.59 (dd, 1H, *J* = 9.6, 3.91 Hz), 3.65 (s, 1H), 3.67 (s, 1H), 4.49 (d, 1H, *J* = 10.49 Hz), 4.57 (d, 1H, *J* = 10.23 Hz), 7.21-7.37 (m, 5H).
^{13}C NMR (50 MHz, $CDCl_3$), δ	26.45 (CH_3), 26.5 (CH_3), 37.2 (CH_3), 54.6 (CH_2), 57.9 (CH_2), 61.3 (CH_2), 69.2 (CH_2), 71.1 (C), 73.1 (CH), 84.5 (CH), 111.1 (C), 127.4 (CH), 128.4 (CH), 128.8 (CH), 137.5 (C).
Mass: *m/z* (%)	394 (M^++Na, 75), 372 (M^++H, 42), 236 (59), 229 (100).
Mp	100-102 °C (from EtOAc/Pet ether).

a = D_2O exchange

(3a*S*,7*S*,7a*S*)-7-(azidomethyl)-5-benzyl-2,2-dimethyl-hexahydro-[1,3]dioxolo[4,5-c]pyridin-7-ol (69)

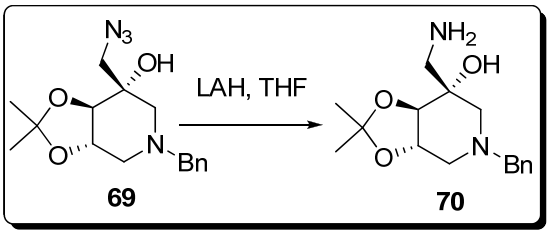


To a solution of **68** (0.1 g, 0.29 mmol) in DMF (2 mL) was added LiN_3 (0.132 g, 2.7 mmol) and heated to 110 °C for 20 h. It was extracted with EtOAc, water washed, dried over Na_2SO_4, concentrated and purified by column chromatography (pet. ether/ethyl acetate, 4:1), to get **69** (0.77 g, 90%) as a colorless liquid.

$[\alpha]^{29}_D$	+41.86 (c 0.35, $CHCl_3$), *ent* $[\alpha]^{30}_D$ -38.41, (c 1.55, $CHCl_3$).
IR (neat) υ_{max} cm^{-1}	2925, 2360, 2102.

^{1}H NMR (200 MHz, $CDCl_3$), δ	1.35 (s, 3H), 1.36 (s, 3H), 1.94 (d, 1H, *J* = 11.75 Hz), 2.15 (dd, 1H, *J* = 9.85, 9.60 Hz), 2.42 (s, 1H), 2.85 (dd, 1H, *J* = 11.75, 1.06 Hz), 3.13 (ddd, 1H, *J* = 9.73, 4.04, 1.27 Hz), 3.39 (d, 1H, *J* = 9.60 Hz), 3.50 (dd, 1H, *J* = 9.60, 3.92 Hz), 3.59 (s, 2H), 3.66 (s, 1H), 3.72 (d, 1H, *J* = 12.26 Hz), 7.13-7.31 (m, 5H).
^{13}C NMR (50 MHz, $CDCl_3$), δ	26.45 (CH_3), 26.5 (CH_3), 52.9 (CH_2), 54.5 (CH_2), 59.0 (CH_2), 61.3 (CH_2), 71.8 (C), 73.2 (CH), 84.6 (CH), 110.9 (C), 127.4 (CH), 128.3 (CH), 128.6 (CH), 137.5 (C).
Mass: *m/z* (%)	341 (M^++Na, 56), 319 (M^++H, 61), 301 (33), 279 (100), 229 (63).
Elemental analysis	Anal. Calcd for $C_{16}H_{22}N_4O_3$: C, 60.36; H, 6.97; N, 17.60. Found: C, 60.56; H, 7.04; N, 17.83.

(3aS,7R,7aS)-7-(aminomethyl)-5-benzyl-2,2-dimethyl-hexahydro-[1,3]dioxolo[4,5-c]pyridin-7-ol (70)

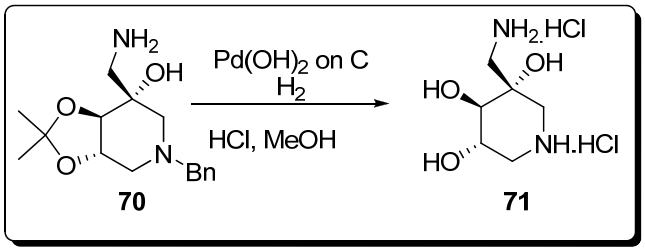


To a solution of **69** (0.1 g, 0.31 mmol) in dry THF (2 mL) was added LAH (0.023 g, 0.62 mmol) and stirred overnight at rt. Reaction mixture was quenched by drop wise addition of 2N NaOH solution. Na_2SO_4, was added to the reaction mixture and it was filtered, concentrated and chromatographed (DCM/MeOH, 4:1) to get corresponding amine compound as a white crystalline solid **70** (0.08 g, 90%) (mp 165-168 °C).

$[\alpha]^{28}_D$	+46.67 (c 0.3, $CHCl_3$), *ent* $[\alpha]^{28}_D$ -44.33, (c 0.55, $CHCl_3$).
^{1}H NMR (400 MHz, $CDCl_3$), δ	1.44 (s, 3H), 1.45 (s, 3H), 2.16-2.21 (m, 2H), 2.90 (d, 1H, *J* = 12.05 Hz), 3.16-3.21 (m, 2H), 3.40 (d, 1H, *J* =

	13.05 Hz), 3.52 (d, 1H, *J* = 9.53 Hz), 3.60-3.70 (m, 3H), 3.72-3.93 (bs, 3H), 7.26-7.34 (m, 5H).
^{13}C NMR (50 MHz, $CDCl_3$), δ	26.6 (CH_3), 42.4 (CH_2), 54.5 (CH_2), 60.1 (CH_2), 61.3 (CH_2), 70.0 (C), 73.3 (CH), 85.4 (CH), 110.5 (C), 127.2 (CH), 128.3 (CH), 128.6 (CH), 138 (C).
Mass: *m/z* (%)	315 (M^++Na, 6), 293 (M^++H, 86), 235 (100), 179 (13).
Elemental analysis	Anal. Calcd for $C_{16}H_{24}N_2O_3$: C, 65.73; H, 8.27; N, 9.58; Found: C, 65.63; H, 8.15; N, 9.70.
mp	165-168 °C (from EtOAc / pet. ether).

(3*R*,4*R*,5*S*)-3-(aminomethyl)piperidine-3,4,5-triol dihydrochloride (71)



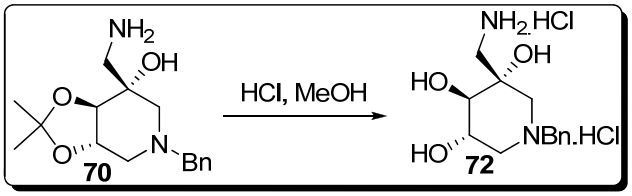
70 **71**

A solution of **70** (0.02 g, 0.068 mmol) in ethanol was hydrogenated (1 atm, rt) using $Pd(OH)_2$ on charcoal (20 %, 0.004 g) as catalyst for 10 h. The reaction mixture was passed through a pad of Celite and the solvent was removed under reduced pressure. To a solution of this mixture in distilled methanol (1 mL) was added conc. HCl (2 drops) and the reaction mixture was stirred at rt for 4 h. The solvent was removed under reduced pressure to afford **71** (0.016 g, >99 %) as a white solid.

$[\alpha]^{31}_D$	-8.27 (c 1.45, MeOH).
^{1}H NMR (400 MHz, D_2O), δ	3.14 (d, 1H, *J* = 13.55 Hz), 3.33 (d, 1H, *J* = 13.05 Hz), 3.37 (s, 1H), 3.41 (d, 1H, *J* = 2.51 Hz), 3.43 (d, 1H, *J* = 3.01 Hz) 3.52 (dd, 1H, *J* = 13.55, 2.51 Hz), 3.94 (d, 1H, *J* = 4.01 Hz), 4.22-4.25 (m, 1H).
^{13}C NMR (100 MHz, D_2O), δ	43.4 (CH_2), 44.6 (CH_2), 46.3 (CH_2), 66.3 (CH), 66.7 (CH), 69.3 (C).
Mass: *m/z* (%)	229 (98), 213 (59), 163 (100).
Elemental analysis	Anal. Calcd for $C_6H_{16}Cl_2N_2O_3$: C, 30.65; H, 6.86; N,

	11.92. Found: C, 30.66; H, 6.52; N, 11.72.

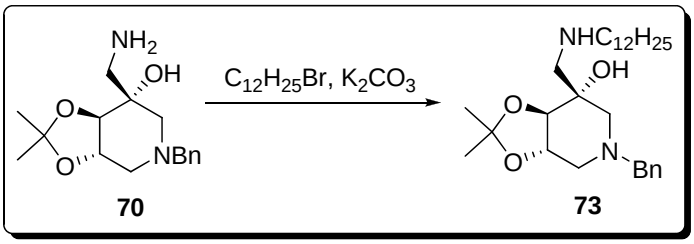
(3R,4R,5S)-3-(aminomethyl)-1-benzylpiperidine-3,4,5-triol dihydrochloride (72)



To a solution of **29** (0.025 g, 0.085 mmol) in distilled methanol (1 mL) was added conc. HCl (3 drops) and the reaction mixture was stirred at rt for 4 h. The solvent was evaporated to dryness to afford **30** (0.024 g,) quantitatively as a white solid.

$[\alpha]^{22}_{D}$	-3.00 (c 1.00, MeOH), *ent* $[\alpha]^{28}_{D}$ +4.00 (c 1.00, MeOH).
^{1}H NMR (400 MHz, D_2O), δ	3.07 (d, 1H, *J* = 13.30 Hz), 3.31-3.41 (m, 3H), 3.49-3.59 (m, 2H), 3.90 (s, 1H), 4.25 (s, 1H), 4.50 (d, 1H, *J* = 13.30 Hz), 4.55 (d, 1H, *J* = 13.30 Hz), 7.60 (s, 5H).
^{13}C NMR (100 MHz, D_2O), δ	43.4 (CH_2), 52.5 (CH_2), 53.5 (CH_2), 61.0 (CH_2), 65.3 (CH), 67.1 (CH), 70.4 (C), 127.4 (C), 129.4 (CH), 130.6 (CH), 131.8 (CH).
Mass: *m/z* (%)	254 (28), 253 (100), 206 (12).
Elemental analysis	Anal. Calcd for $C_{13}H_{22}Cl_2N_2O_3$: C, 48.01; H, 6.82; N, 8.61. Found: C, 48.11; H, 6.52; N, 8.41.

(3a*S*,7*R*,7a*R*)-5-benzyl-7-((dodecylamino)methyl)-2,2-dimethyl-hexahydro-[1,3]dioxolo[4,5-c]pyridin-7-ol (73)



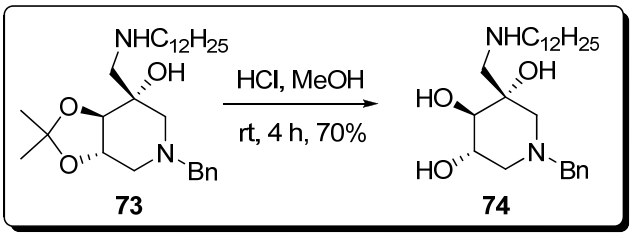
To a stirring solution of **70** (0.05 g, 0.17 mmol) in dry CH_3CN and THF (3:1), (2 mL) was added dodecyl bromide (0.04 mL, 0.17 mmol) and stirred for 3 h at rt. Then K_2CO_3 (0.047 g, 0.34 mmol) was added to the reaction mixture and refluxed for 3 h. Later water was added and mixture extracted in ethyl acetate (2 x 5 mL), dried over Na_2SO_4,

concentrated and purified by column chromatography (pet. ether/ethyl acetate, 5:2) , to get **73** (0.055 g, 70%) as yellow liquid.

$[\alpha]^{30}_D$	+26.66 (c 0.45, $CHCl_3$).
^{1}H NMR (400 MHz, $CDCl_3$)[a], δ	0.89 (t, 3H, *J* = 6.78 Hz), 1.26 (bs, 20H), 1.42-1.43 (m, 8H), 2.10 (d, 1H, *J* = 11.80 Hz), 2.21 (t, 1H, *J* = 9.79 Hz), 2.61 (t, 1H, *J* = 7.03 Hz), 2.69 (d, 1H, *J* = 11.80 Hz), 2.88 (d, 1H, *J* = 12.23 Hz), 3.06 (d, 1H, *J* = 12.30 Hz), 3.19 (dd, 1H, *J* = 9.29, 3.89 Hz), 3.49 (d, 1H, *J* = 9.29 Hz), 3.57 (d, 1H, *J* = 13.05 Hz), 3.65 (s, 1H), 7.25-7.33 (m, 5H).
^{13}C NMR (100 MHz, $CDCl_3$), δ	14.1 (CH_3), 22.7 (CH_2), 26.6 (CH_3), 26.7 (CH_3), 27.0 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 31.9 (CH_2), 50.2 (CH_2), 50.3 (CH_2), 54.9 (CH_2), 61.0 (CH_2), 61.5 (CH_2), 69.4 (C), 73.4 (CH), 85.4 (CH), 110.5 (C), 127.3 (CH), 128.3 (CH), 128.7 (CH), 138.1 (C).
Mass: *m/z* (%)	461 (M^++H, 100), 403 (34), 229 (12).

a = D_2O exchange

(3R,4R,5S)-1-benzyl-3-((dodecylamino)methyl)piperidine-3,4,5-triol (74)



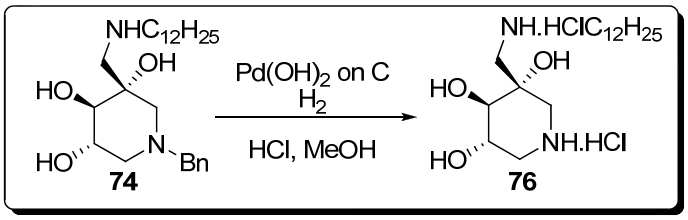
Conc. HCl (2 drops) was introduced to a solution of **73** (0.05 g, 0.083 mmol) in distilled methanol (2 mL) and reaction mixture was stirred at rt for 4 h. After neutralization by adding $NaHCO_3$, solvent was removed under reduced pressure and residue was chromatographed (silica, DCM/MeOH, 9:1)) to get **74** as a yellow liquid (0.032 g, 70%).

$[\alpha]^{29}_D$	+15.10 (c 0.55, $CHCl_3$).
^{1}H NMR (400 MHz, $CDCl_3$)[a], δ	0.88 (t, 3H, *J* = 6.78 Hz), 1.25 (bs, 20H), 1.69 (s, 2H), 2.23 (d, 1H, *J* = 11.54 Hz), 2.65 (d, 1H, *J* = 11.04 Hz), 2.84 (d, 1H, *J* = 12.80 Hz), 2.91-2.95 (m, 2H), 3.45-

	3.55 (m, 3H), 3.65 (d, 1H, *J* = 7.78 Hz), 3.72-3.80 (m, 1H), 7.23-7.31 (m, 5H).
^{13}C NMR (100 MHz, $CDCl_3$), δ	14.1 (CH_3), 22.7 (CH_2), 25.6 (CH_2), 26.7 (CH_2), 29.2 (CH_2), 29.4 (CH_2), 29.6 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 31.9 (CH_2), 49.1 (CH_2), 52.4 (CH_2), 61.7 (CH_2), 69.4 (CH), 70.9 (C), 127.4 (CH), 128.4 (CH), 129 (CH), 137.6 (C).
Elemental analysis	Anal. Calcd for $C_{25}H_{44}N_2O_3$: C, 71.39; H, 10.54; N, 6.66. Found: C, 71.55; H, 10.65; N, 6.75.

a = D_2O exchange

(3*R*,4*R*,5*S*)-3-((dodecylamino)methyl)piperidine-3,4,5-triol dihydrochloride (76)

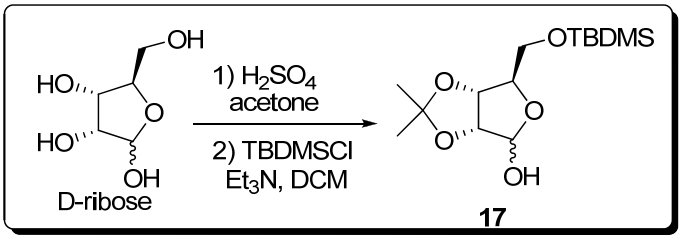


An ethanolic solution (2 mL) of **74** (0.025 g, 0.059 mmol) was hydrogenated (1 atm, rt) in the presence of $Pd(OH)_2$ on charcoal (20 %, 0.005 g) for 15 h. The reaction mixture was passed through a pad of Celite and the solvent was removed under reduced pressure. To a solution of this in distilled methanol (1 mL) was added conc. HCl (2 drops) and the reaction mixture was stirred at rt for 15 min. The solvent was removed under reduced pressure to afford **76** (0.023 g, quantitative) as a white solid.

$[\alpha]^{26}_D$	+7.27 (c 0.55, MeOH).
^{1}H NMR (400 MHz, D_2O), δ	0.90 (s, 3H), 1.32 (bs, 20H), 1.78 (s, 2H), 3.15 (s, 2H), 3.36-3.52 (m, 4H), 3.96 (s, 1H), 4.23 (s, 1H).
^{13}C NMR (100 MHz, D_2O), δ	13.6 (CH_3), 22.2 (CH_2), 25.1 (CH_2), 26.0 (CH_2), 28.5 (CH_2), 28.8 (CH_2), 29.1 CH_2), 31.5 (CH_2), 44.6 (CH_2), 46.4 (CH_2), 49 (CH_2), 51.1 (CH_2), 66.3 (CH), 66.8 (CH), 69.5 (C).
Mass: *m/z* (%)	345 (39), 331 (100).
Elemental analysis	Anal. Calcd for $C_{18}H_{40}Cl_2N_2O_3$: C, 53.59; H, 9.99; N,

	6.94. Found: C, 53.79; H, 10.09; N, 6.84;

(3a*R*,6*R*,6a*R*)-6-((*tert*-butyldimethylsilyloxy)methyl) -2, 2 dimethyltetrahydro furo [3,4-d] [1,3]dioxol-4-ol (17)[21]

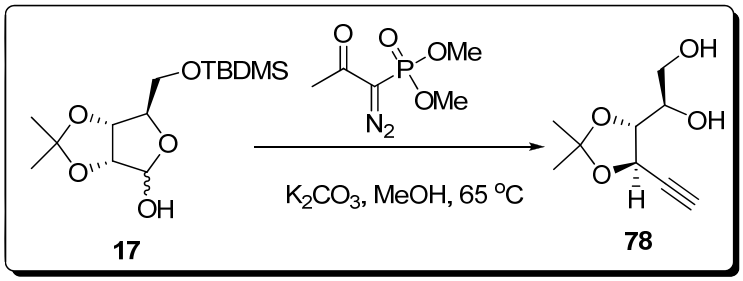


To an ice cold suspension of D-ribose (20.0 g, 133.21 mmol) in acetone (200 mL) was added conc H_2SO_4 (1.0 mL). The clear solution was obtained after 30 minutes. The reaction mixture was neutralized by the addition of $Ca(OH)_2$ and the salt was removed by filtration. The filtrate was concentrated in *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 was added to the reaction mixture and it was extracted. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography (pet ether/ethyl acetate, 70:30) to give **17** (32.4 g, 80%) as a colorless oil (9:1 anomeric mixture).

IR υ_{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, 1H, *J* = 1.39 Hz), 3.75 (d, 1H, *J* = 1.39 Hz), 4.33 (s, 1H), 4.48 (d, 1H, *J* = 5.94 Hz), 4.68 (d, 1H, *J* = 6.19 Hz), 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: *m/z* (%)	327 (M^++Na, 11), 322 (M^++NH_4, 100), 304 (M^+, 2.5), 287 (44.2%).

(*R*)-1-((4*R*,5*R*)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethane-1,2-diol (78)

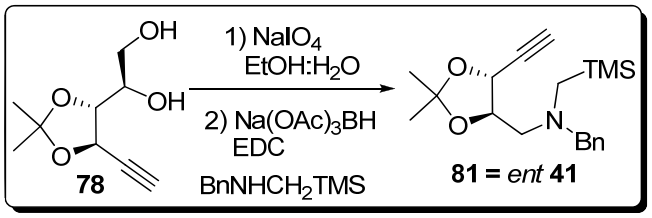


To a stirred mixture of **17** (20.0 g, 45.24 mmol) and anhydrous K_2CO_3 (8.23 g, 58.82 mmol) in dry MeOH (160 mL) at 65 °C was added the solution of Ohira reagent (11.29 g, 58.82 mmol) in dry MeOH (80 mL) drop wise over a period of 6 h under argon atmosphere. Acetic acid was added drop wise to neutralize the reaction mixture. Latter solvent was removed, water added to the residue and extracted by ethyl acetate (2 X 100 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified by column chromatography (pet. ether/ethyl acetate, 4:1, to get **78** (4.55 g, 55%) as white solid.

$[\alpha]^{30}_D$	+7.27 (c 0.55, $CHCl_3$).
IR υ_{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, 1H, *J* = 2.01 Hz), 2.87 (bs, 1H), 3.26 (bs, 1H), 3.66 (dd, 1H, *J* = 11.54, 6.53 Hz), 3.76 (dd, 1H, *J* = 11.54, 3.26 Hz), 3.83-3.86 (m, 1H), 4.11 (t, 1H, *J* = 6.52 Hz), 4.68 (dd, 1H, *J* = 6.78, 2.01 Hz).
^{13}C NMR (50 MHz, $CDCl_3$), δ	25.8 (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).
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

N-Benzyl((4R,5R)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)-N-((trimethylsilyl) methyl) methanamine (81)



To a solution of **78** (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 in ethyl acetate (2 X 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 $BnNHCH_2TMS$ (6.25 g, 32.25 mmol) and $Na(OAc)_3BH$ (7.98 g, 37.63 mmol) under argon atmosphere and stirred for 24 h. The reaction mixture was quenched by aq. saturated $NaHCO_3$ solution. 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 (pet ether/ethyl acetate, 95:5) to furnish **81** (4.45 g, 50% over two steps). Spectral data was as reported for its enantiomer

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. The absorbance of the resulting solution was read at 405 nm. In the case of β-galactosidase (*Aspergillus oryzaie*) each assay was performed in citrate phosphate buffer (pH 4.5) with *p*-nitrophenyl β-D-galactpyranooside as the substrate. Varying concentrations of the substrate (100 μM, 50 μM) were employed. The reaction was initiated by the addition of 25 μL of appropriately diluted enzyme. The reaction mixture (having inhibitor) which had a final volume of 0.5 mL was incubated for 20 min at 37 °C, and then quenched by the addition of 1.0 mL of 1M Na_2CO_3 solution.

In the case of α-galactosidase (Green coffee beans), the assay was performed in a potassium phosphate buffer (pH 6.5) with *p*-nitrophenyl α-D-galactopyranoside as the substrate. Varying concentrations of the substrate (100 μM, 50 μM) were employed. The reaction was initiated by the addition of 25 μL of appropriately diluted enzyme. The reaction mixture (having inhibitor) which had a final volume of 0.5 mL was incubated for 20 min at 25 °C, and then quenched by the addition of 1.0 mL of 1M Na_2CO_3 solution.

In the case of β-mannosidase (Snail), the assay was performed in a citrate phosphate buffer (pH 4.0) with *p*-nitrophenyl β-D-mannopyranoside as the substrate. Varying concentrations of the substrate (200 μM, 150 μM) were employed. The reaction was initiated by the addition of 50 μL of appropriately diluted enzyme. The reaction mixture (having inhibitor) which had a final volume of 0.5 mL was incubated for 20 min at 25 °C and then quenched by the addition of 1.0 mL of 1M Na_2CO_3 solution.

In the case of α-mannosidase (Jack Beans), the assay was performed in citrate phosphate buffer (pH 4.5) with *p*-nitrophenyl α-D-mannopyranoside as the substrate. Varying concentrations of the substrate (50 μM, 30 μM) were employed. The reaction was initiated by the addition of 25 μL of appropriately diluted enzyme. The reaction mixture (having inhibitor) which had a final volume of 0.5 mL was incubated for 20 min at 25 °C, and then quenched by the addition of 1.0 mL of 1M Na_2CO_3 solution.

In the case of β-glucosidase (Almond), the assay was performed in a potassium phosphate buffer (pH 5.5) with *p*-nitrophenyl β-D-glucopyranoside as the substrate. Varying concentrations of the substrate (100 μM, 50 μM) were employed. The reaction was initiated by the addition of 50 μL of appropriately diluted enzyme. The reaction mixture (having inhibitor) which had a final volume of 0.5 mL was incubated for 30 min at 37 °C, and then quenched by the addition of 1.0 mL of 1M Na_2CO_3 solution

In the case of α-glucosidase (Yeast), the assay was performed in a potassium phosphate buffer (pH 6.8) with *p*-nitrophenyl α-D-glucopyranoside as the substrate. Varying concentrations of the substrates (200 μM, 100 μM) for inhibitors **50, 58, 67, 71, 72, 76, 85, 86** and **90** were employed. For **49** (200 μM, 150 μM) and for **54** (300 μM, 200 μM) substrate concentration was used. The reaction was initiated by the addition of 50 μL of appropriately diluted enzyme. The reaction mixture (having inhibitor) which had a final volume of 0.5 mL was incubated for 20 min at 37 °C, and then quenched by the addition of 1.0 mL of 1M Na_2CO_3 solution.

In the case of α-mannosidase (*Aspergillus fischeri*), the assay was performed in a potassium phosphate buffer (pH 6.5) with *p*-nitrophenyl α-D-mannopyranoside as the substrate. Varying concentrations of the substrate (500 μM, 250 μM) were employed. The reaction was initiated by the addition of 25 μL of appropriately diluted enzyme. The reaction mixture (having inhibitor) which had a final volume of 0.5 mL was incubated for 15 min at 50 °C, and then quenched by the addition of 1.0 mL of 1M Na_2CO_3 solution.

Dixon method was employed for the determination of *Ki*. 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.

2.10-References

1. Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. *Chem. Rev*. **2002**, *102*, 515.
2. (a) Hintz, S.; Heidbreder, A.; Mattay, J. *Top. Curr. Chem.* **1996**, *177*, 77. (b) Pandey, G. *Top. Curr. Chem.* **1993**, *168*, 175. (c) Pandey, G. *Synlett* **1992**, 546.
3. Padwa, A.; Nimmesgern, H.; Wong, G. S. K. *J. Org. Chem.* **1985**, *50*, 5620.
4. (a) Pandey, G.; Kumaraswamy, G.; Bhalerao, U. T. *Tetrahedron Lett.* **1989**, *30*, 6059. (b) Pandey, G.; Reddy, G. D.; Kumaraswamy, G. *Tetrahedron* **1994**, *50*, 8185.
5. Yoshida, J, -i.; Maekawa, T.; Murata, T.; Matsunaga, S. –i.; Isoe, S. *J. Am. Chem. Soc.* **1990**, *112*, 1962.
6. Sekioka, T.; Shibano, M.; Kusano, G. *Nat. Med*. **1995**, *49*, 332.
7. (a) Igarashi, Y.; Ichikawa, M.; Ichikawa, Y. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 553. (b) Hansen, A.; Tagmose, T. M.; Bols, M. *Chem. Commun.* **1996**, 2649. (c) Hansen, A.; Tagmose, T. M.; Bols, M. *Tetrahedron* **1997**, *53*, 697.
8. Ichikawa, Y.; Igarashi, Y. *Tetrahedron Lett*. **1995**, *36*, 4585.
9. (a) Müller, S.; Liepold, B.; Roth, G. J.; Bestmann, H. J. *Synlett* **1996**, 521. (b) Roth, G. J.; Liepold, B.; Müller, S. G.; Bestmann, H. J. *Synthesis* **2004**, 59. (c) Pietruszka, J.; Witt, A. *Synthesis* **2006**, 4266.
10. Kotsuki, H.; Araki, T.; Miyazaki, A.; Iwasaki, M.; Datta, P. K. *Org. Lett*. **1999**, *1*, 499.
11. Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett.* **1972**, 3769.
12. Levene, P.A. *Org. Syn. Coll. Vol.* 2, page 88.
13. Cotton, F. A.; Schunn, R. A. *J. Am. Chem. Soc*. **1963**, *85*, 2394.
14. Curphey, T. J. Organic Preparation and Procedures International **1981**, 13, 112.
15. Gallant, P.; D'haenens, L.; Vandewallesyn, M. *Synth. Commun*. **1984**, *14*, 155.
16. (a) Thie´ry, J.-C.; Fre´chou, C.; Demailly, G. *Tetrahedron Lett.* **2000**, *41*, 6337. (b) Alca´zar, E.; Pletcher, J. M.; McDonald, F. E. *Org. Lett.* **2004**, *6*, 3877.
17. Dolhem, F.; Lie`vre, C.; Demailly, G. *Tetrahedron* **2003**, *59*, 155.
18. (a) Pandey, G.; Kapur, M. *Tetrahedron Lett.* **2000**, *41*, 8821. (b) Pandey, G.; Kapur, M. *Synthesis* **2001**, 1263. (c) Pandey, G.; Kapur, M. *Org. Lett.* **2002**, *4*, 3883. (d) Kapur, M.; *Ph. D. Thesis*, University of Pune. **2002**. (e) Pandey, G.; Kapur**,** M.; Khan, M. I.; Gaikwad, S. M. *Org. Biomol. Chem.* **2003,** *1*, 3321. (f) Pandey, G.; Dumbre, S. G.; Khan, M. I.; Shabab, M.; Puranik, V. G. *Tetrahedron Lett.* **2006,** *47*, 7923. (g) Pandey, G.; Dumbre, S. G.; Khan, M. I.; Shabab, M. *J. Org. Chem.* **2006**, *71*, 8481. (h) Pandey, G.; Dumbre, S. G.; Pal, S.; Khan, M. I.; Shabab, M.; *Tetrahedron* **2007**, *63*, 4756.

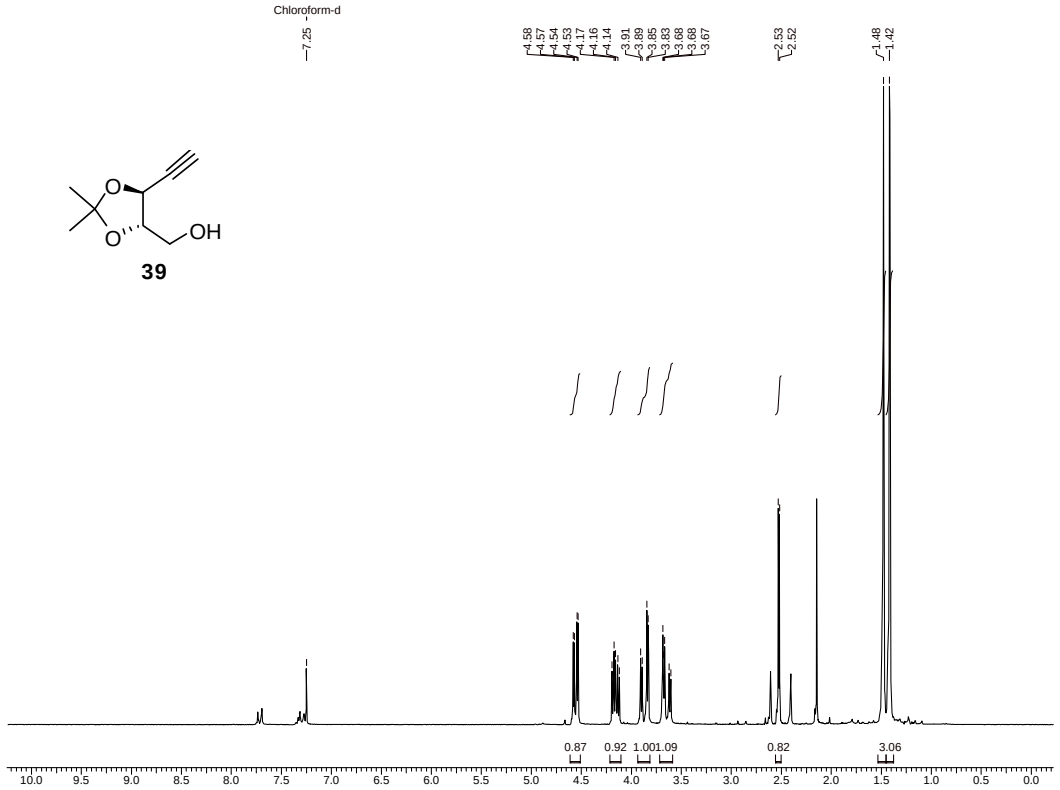
19. (a) Markad, S. D.; Karanjule, N. S.; Sharma, T.; Sabharwal, S. G.; Dhavale, D. D. *Bioorg. Med. Chem*. **2006,** *14* 5535. (b) Stutz, A. E., Ed.; Iminosugars as Glycosidase Inhibitors: Nojirimycin and Beyond; Wiley-VCH: Weinheim, Germany, **1999**; (c) Elbein, A. D. *FASEB J*. **1991**, *5,* 3055. (d) Asano, N.; Nash, R. J.; Molyneux, R. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2000**, *11*, 1645. (e) Platt, F. M.; Neises, G. R.; Dwek, R. A.; Butters, T. D. J. *Biol. Chem*. **1994,** *269*, 8362. (f) Block, X.; Lu, X.; Platt, F. M.; Foster, G. R.; Gerlich, W. H.; Blumberg, B. S.; Dwek, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 2235. (g) Rhinehart, B. L.; Robinson, K. M.; Liu, P.; Payne, A. J.; Wheatley, M. E.; Wagner, S. R. *J. Pharmacol. Exp. Ther*. **1987**, *241*, 915.

20. (a) Umezawa, H.; Aoyagi, T.; Komiyama, T.; Morishama, H.; Hamada, M.; Takeuchi, T. *J. Antibiot*. **1974**, *27*, 963. (b) Satoh, T.; Nishimura, Y.; Kondo, S.; Takeuchi, T. *J. Antibiot.* **1996**, *49*, 321. (c) Nishimura, Y.; Kondo, S.; Takeuchi, T.; Kudo, T. *J. Antibiot*. **1994**, *47*, 101. (d) Nishimura, Y.; Shitara, E.; Kojima, F.; Takeuchi, T. *Bioorg. Med. Chem*. **1999,** *7,* 1241. (e) Nishimura, Y.; Shitara, E.; Takeuchi, T. *Tetrahedron Lett.* **1999,** *40,* 2351. (f) Satoh, T.; Nishimura, Y.; Kondo, S.; Takeuchi, T. *J. Antibiot*. **1996,** *49,* 321.

21. Kumar, D. N.; Rao, B. V.; Ramanjaneyulu, G. S. *Tetrahedron: Asymmetry* **2005**, *16*, 1611.

22. Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849.

23. Gaikwad, S. M.; Keskar, S. S.; M.I. Khan, M. I. *Biochemic Biophys Acta*. **1995**, *1250*, 144.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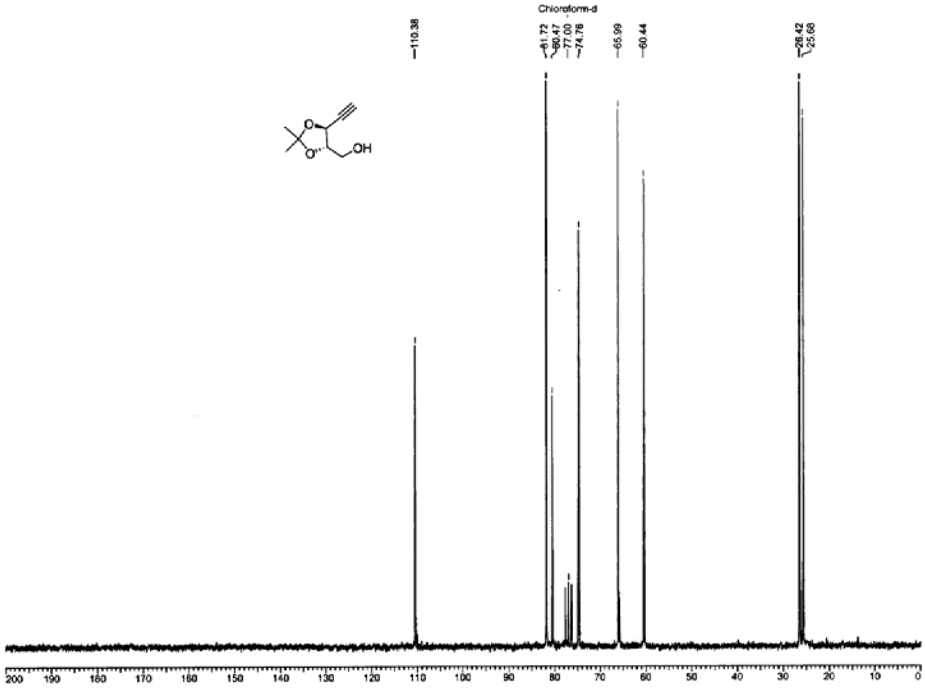
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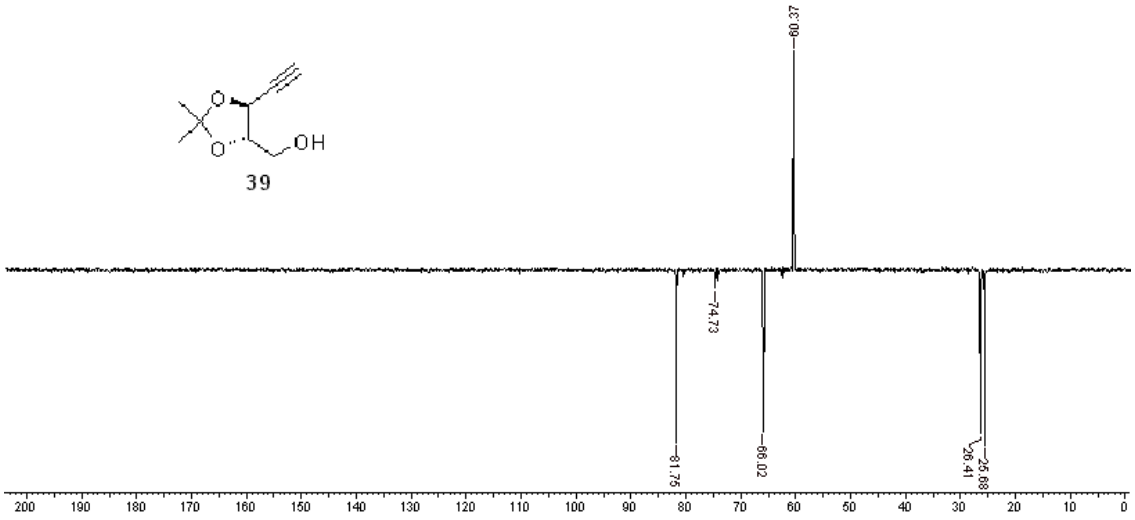
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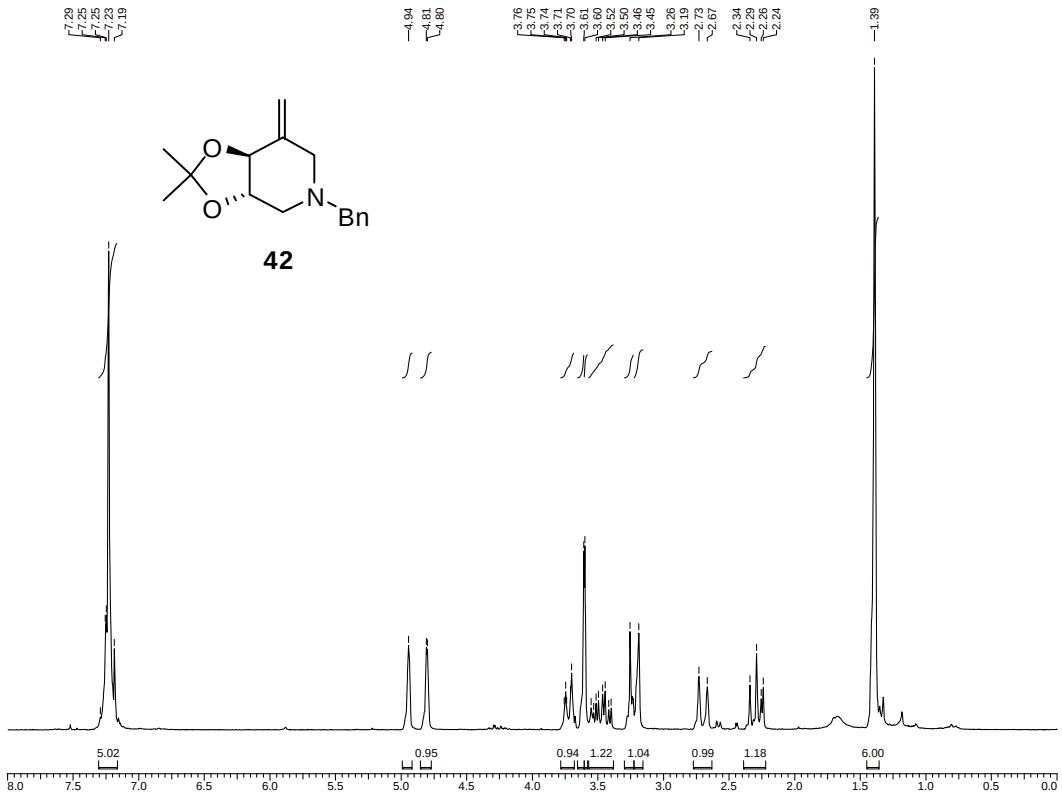
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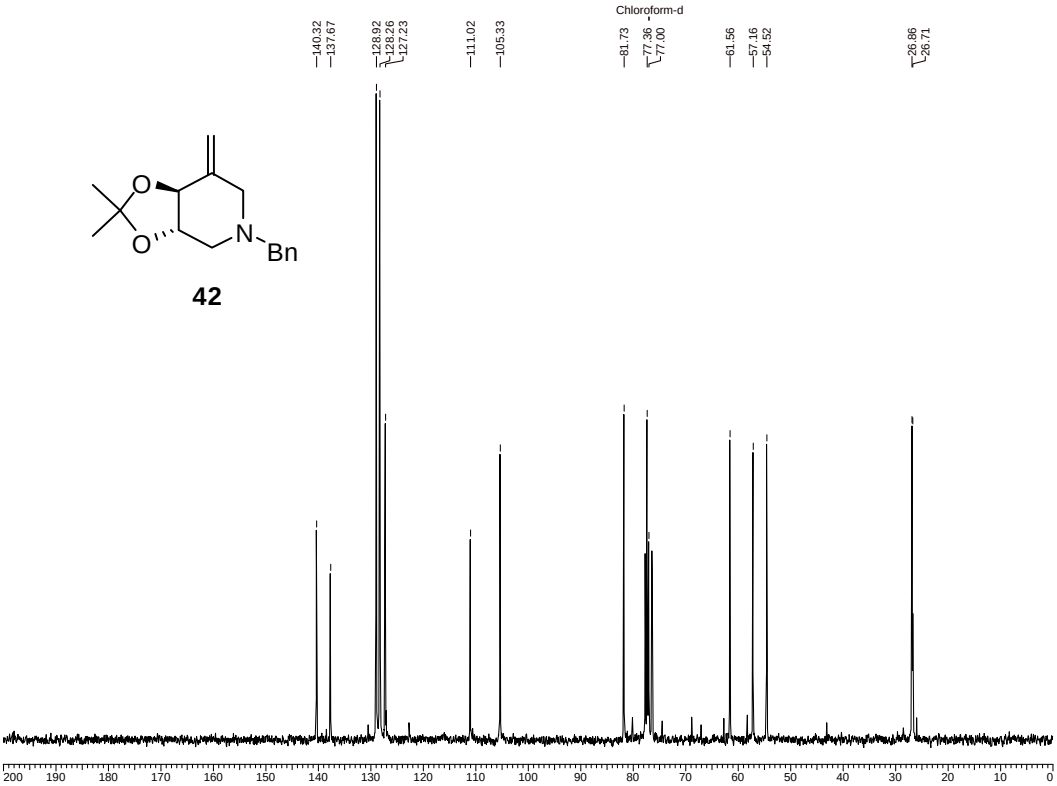
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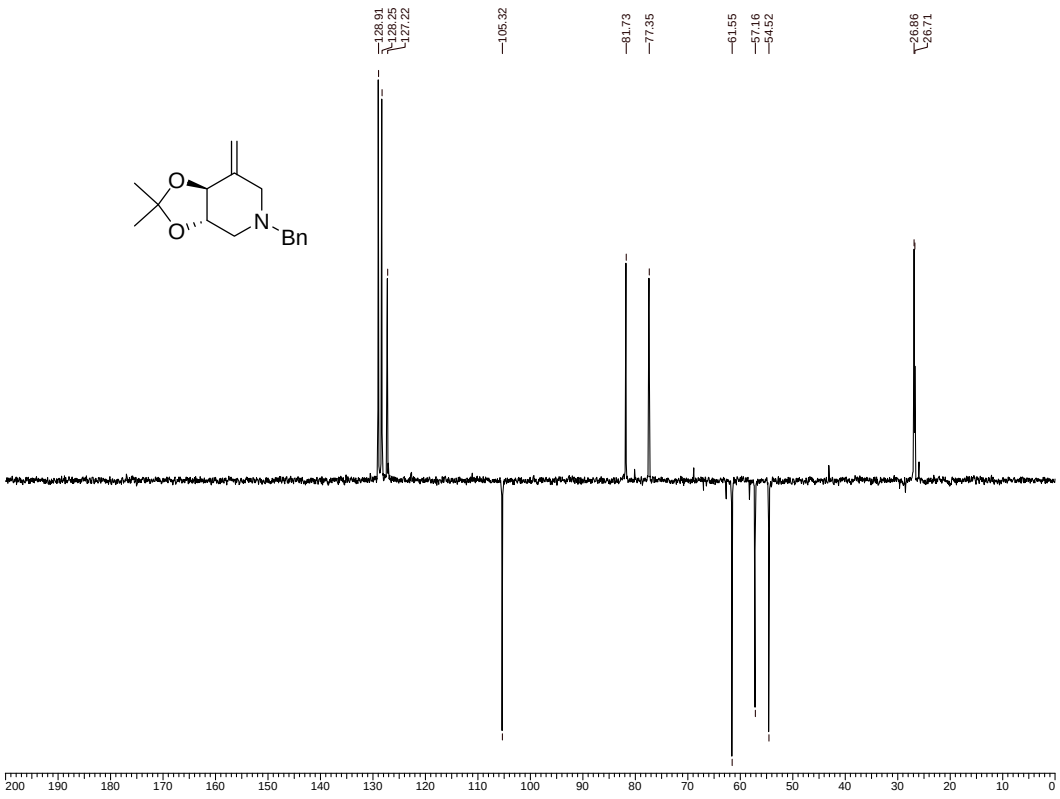
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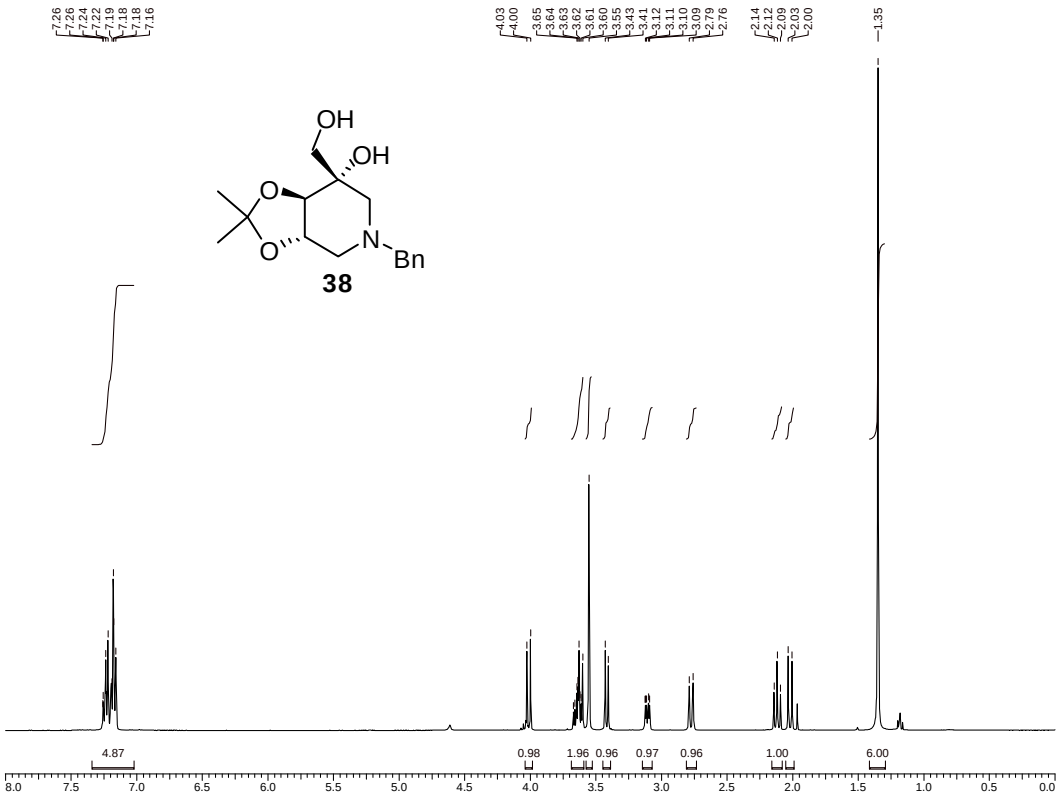
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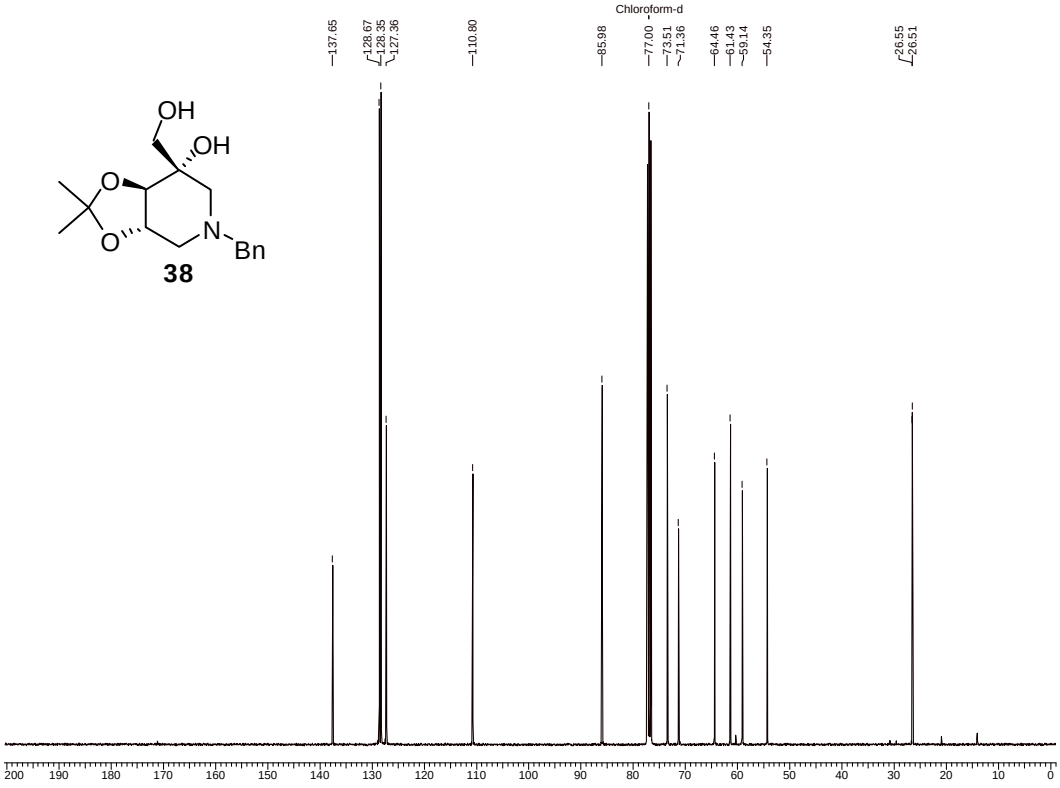
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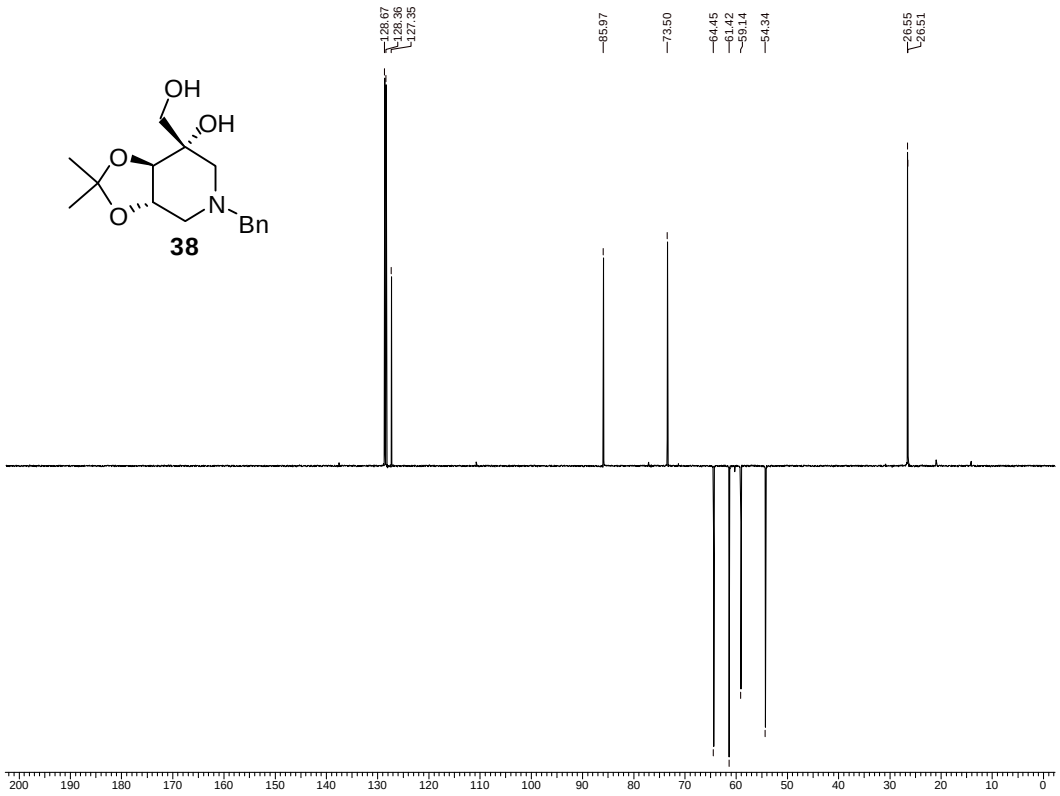
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O
O
N
Bn
38
128.67
128.36
127.35
85.97
73.50
64.45
61.42
59.14
54.34
26.55
26.51
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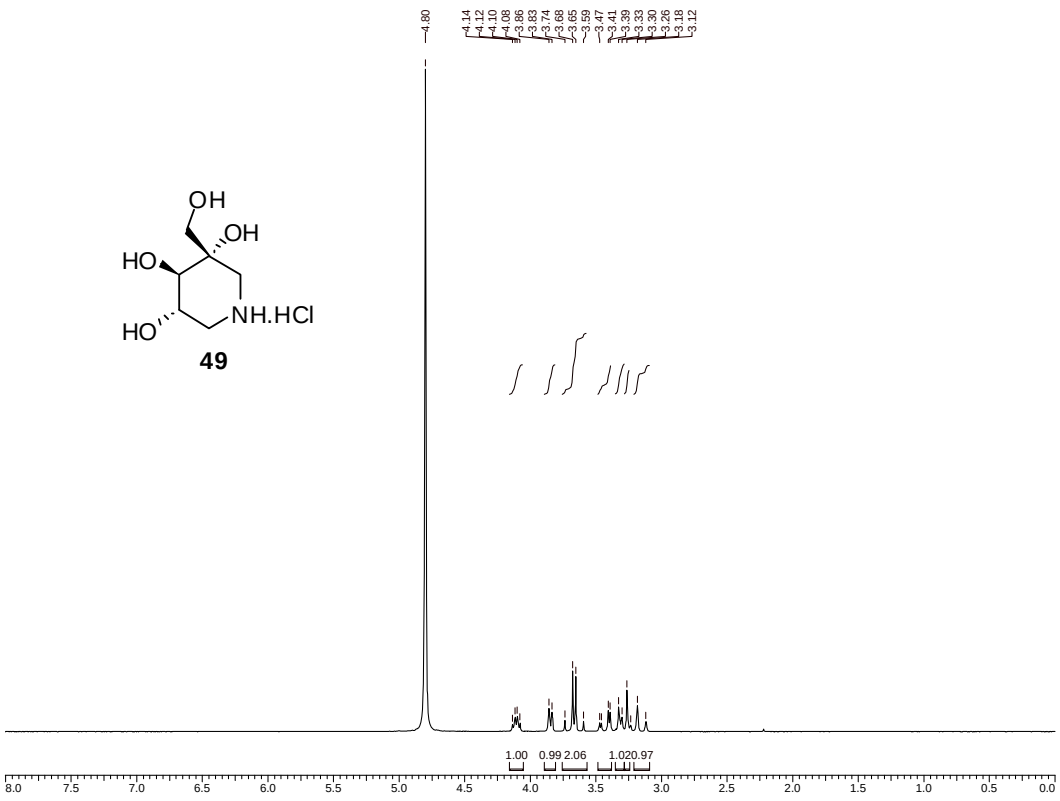
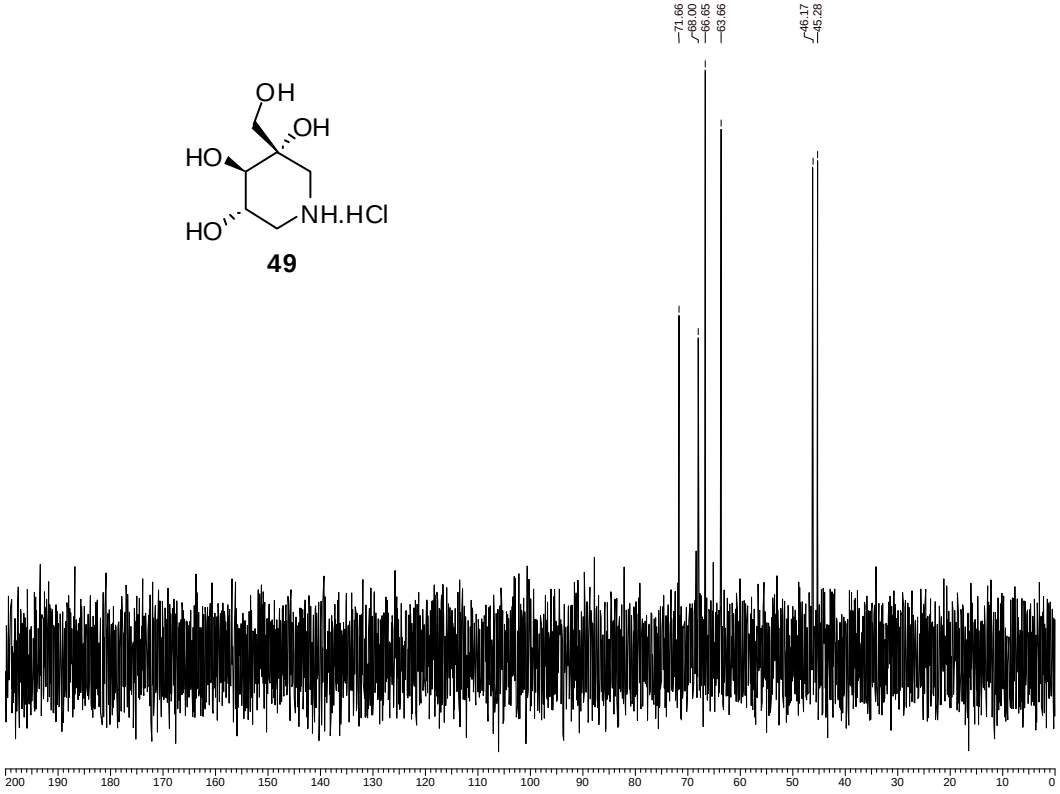
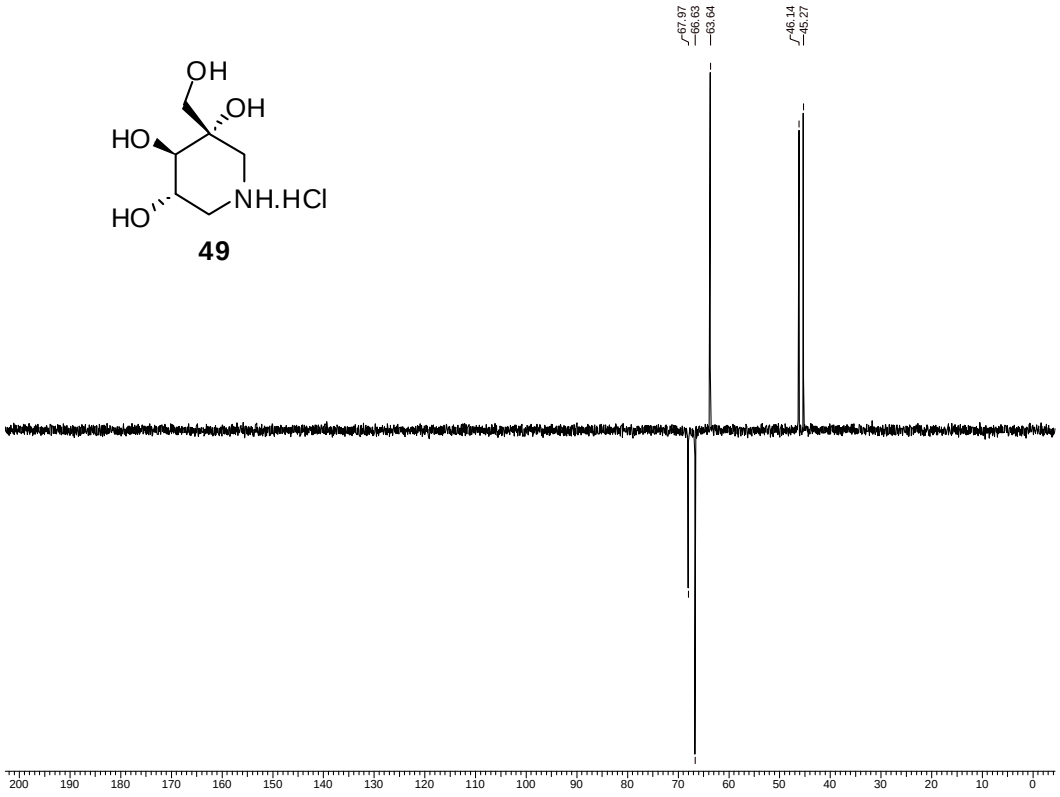
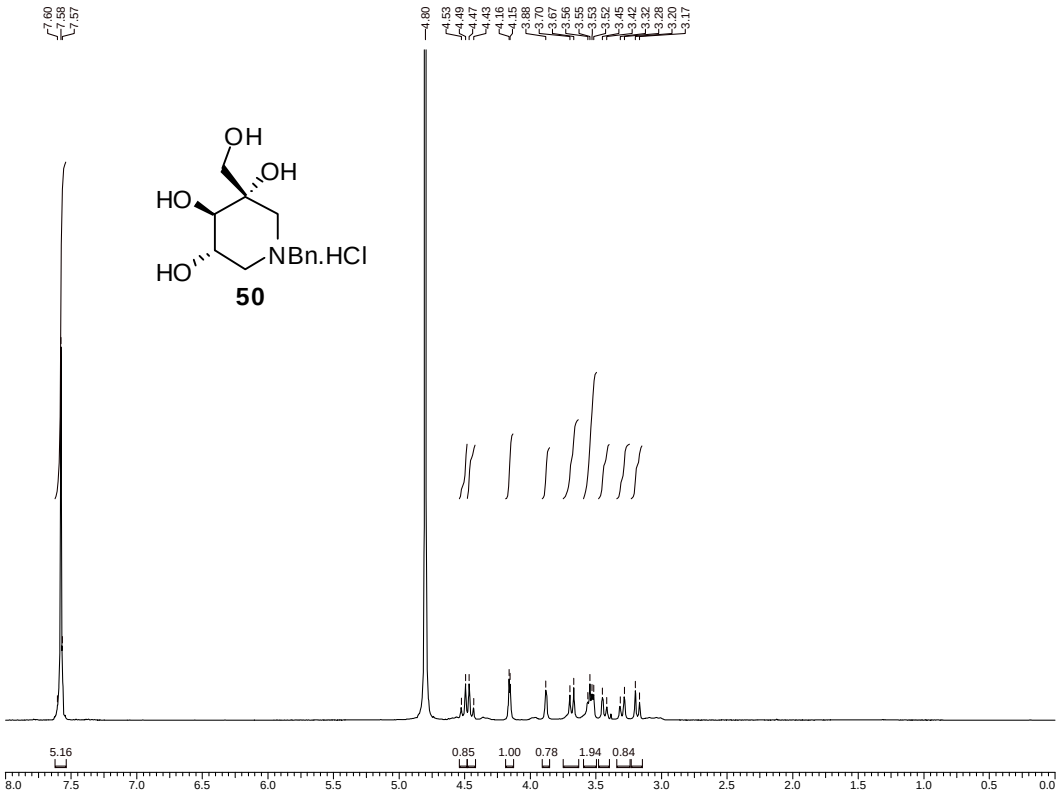
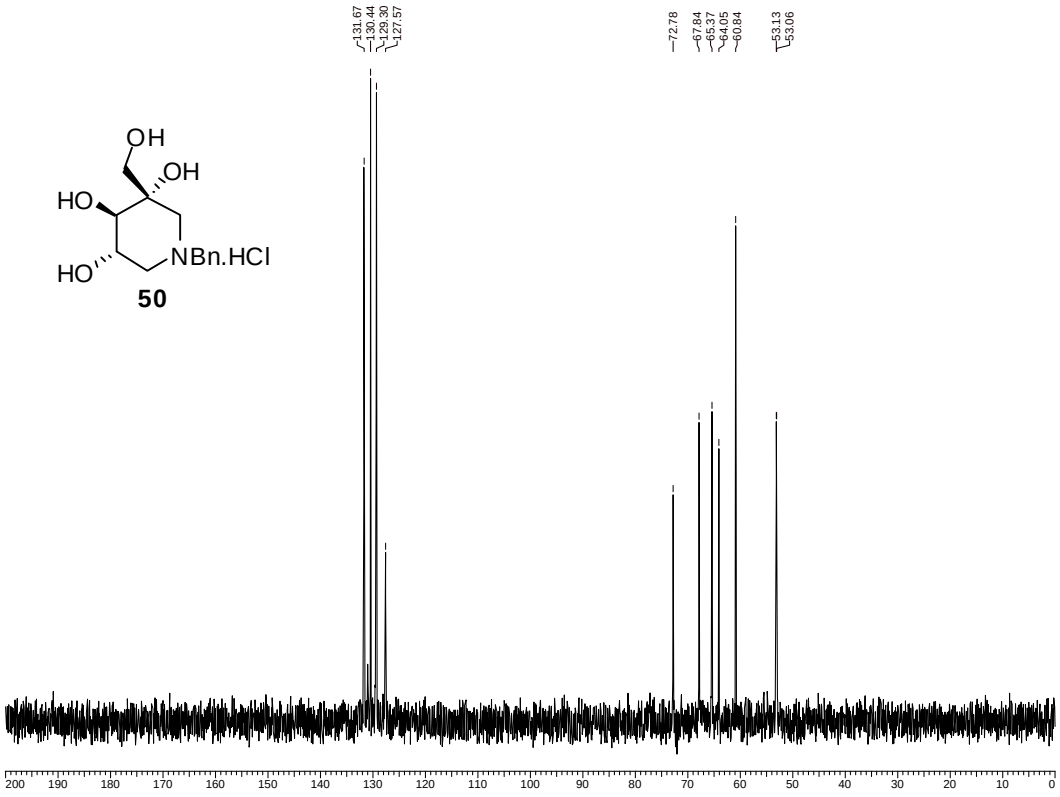
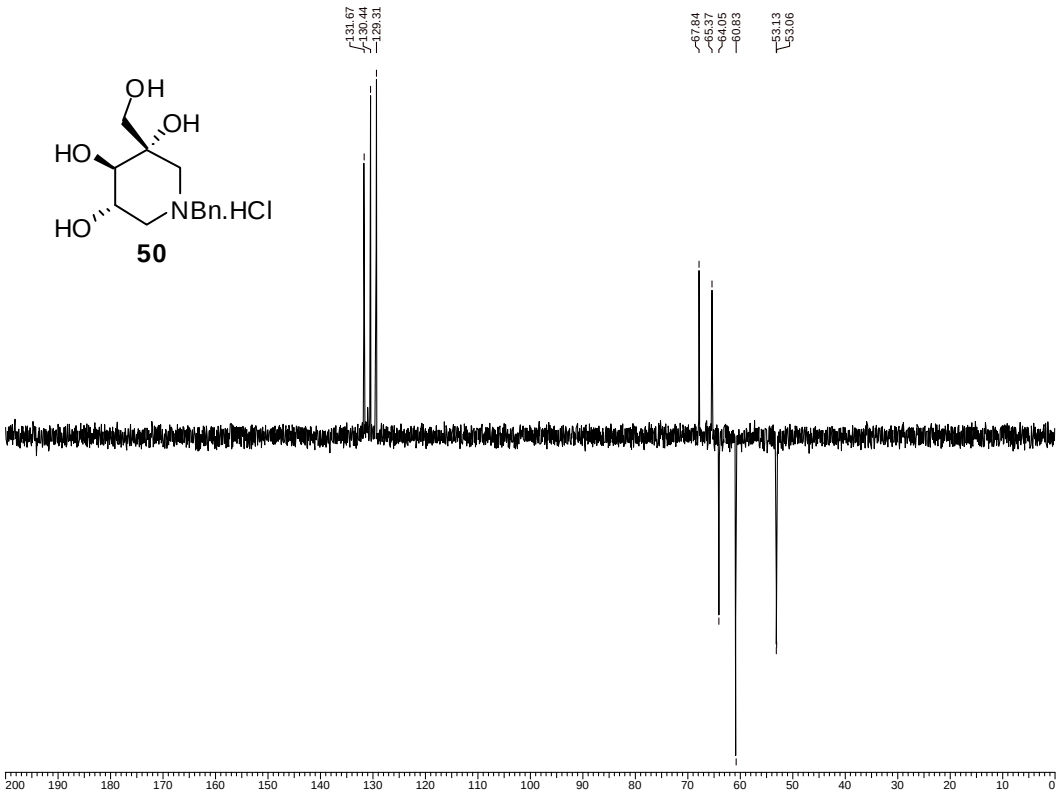

OH
OH
HO
HO
NH.HCl
49
4.80
4.14
4.12
4.10
4.08
3.86
3.83
3.74
3.68
3.65
3.59
3.47
3.41
3.39
3.33
3.30
3.26
3.18
3.12
1.00 0.99 2.06 1.02 0.97
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

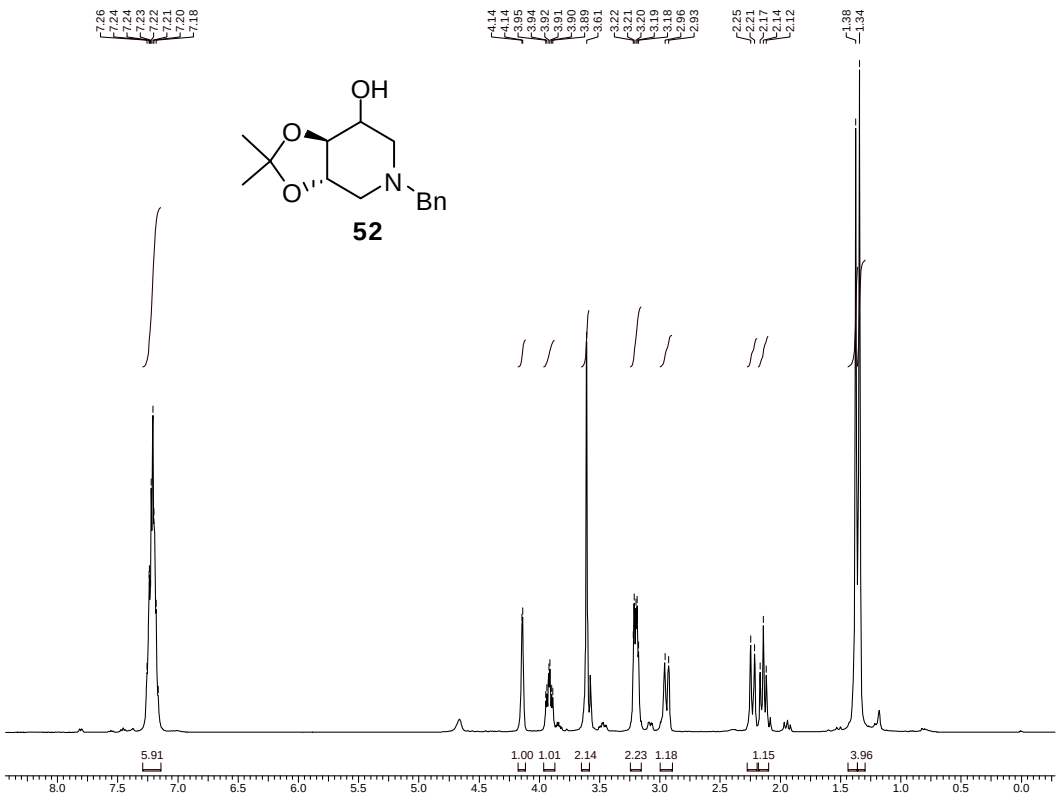

71.66
68.00
66.65
63.66
46.17
45.28
OH
OH
HO
HO
NH.HCl
49
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

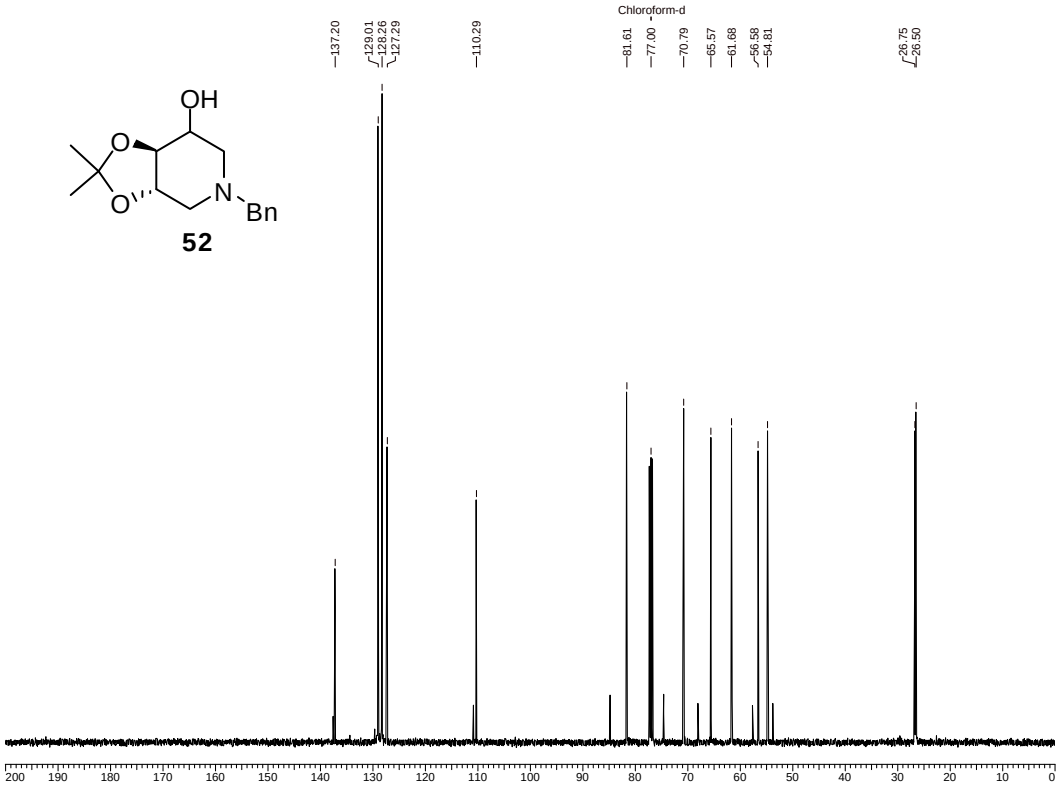

67.97
66.63
63.64
46.14
45.27
OH
OH
HO
HO
NH.HCl
49
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

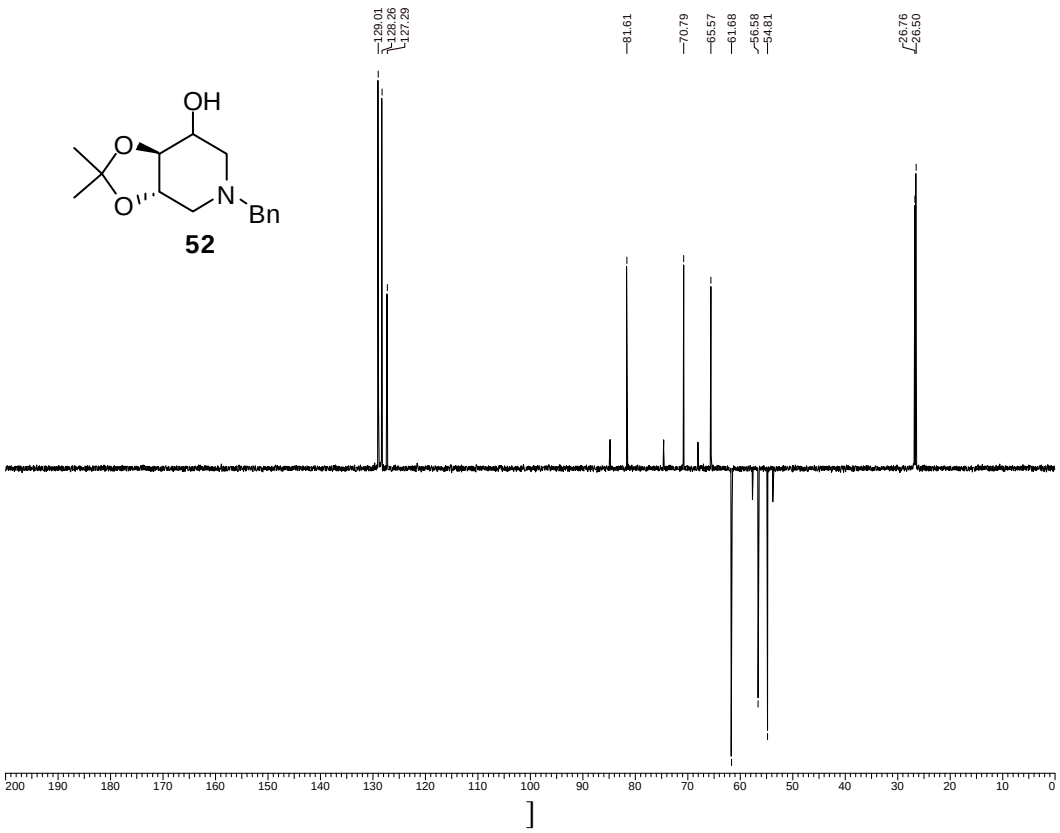

7.60
7.58
7.57
4.80
4.53
4.49
4.47
4.43
4.16
4.15
3.88
3.70
3.67
3.56
3.55
3.53
3.52
3.45
3.42
3.32
3.28
3.20
3.17
OH
OH
HO
HO
NBn.HCl
50
5.16
0.85
1.00
0.78
1.94
0.84


131.67
130.44
129.30
127.57
72.78
67.84
65.37
64.05
60.84
53.13
53.06
OH
OH
HO
HO
NBn.HCl
50

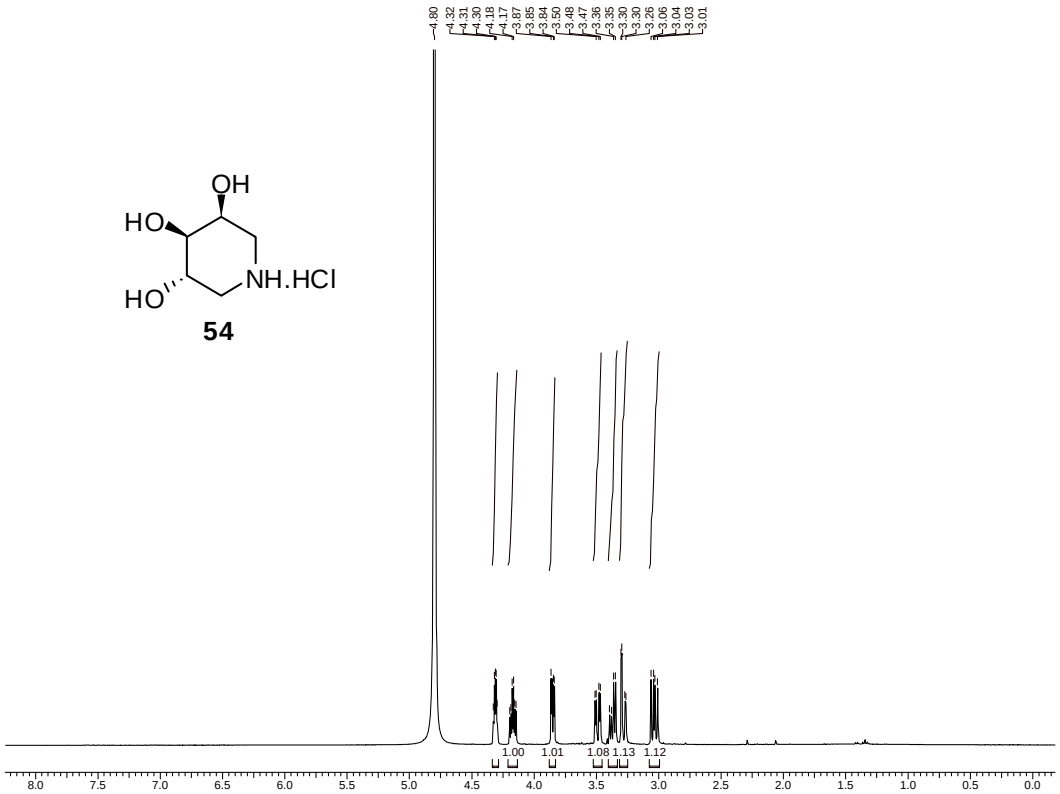

131.67
130.44
129.31
67.84
65.37
64.05
60.83
53.13
53.06
OH
OH
HO
HO
NBn.HCl
50

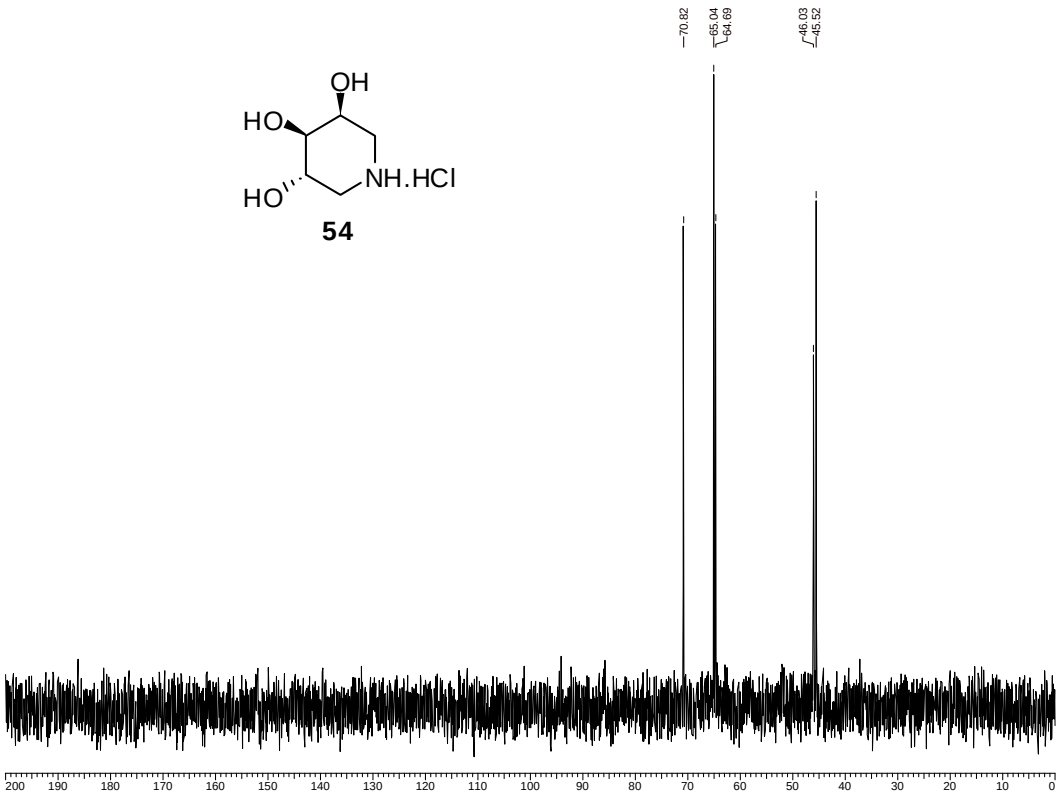

7.26
7.24
7.24
7.24
7.23
7.22
7.21
7.20
7.18
4.14
4.14
3.95
3.94
3.92
3.91
3.90
3.89
3.61
3.22
3.21
3.20
3.19
3.18
2.96
2.93
2.25
2.21
2.17
2.14
2.12
1.38
1.34
OH
O
O
N
Bn
52
5.91
1.00
1.01
2.14
2.23
1.18
1.15
3.96

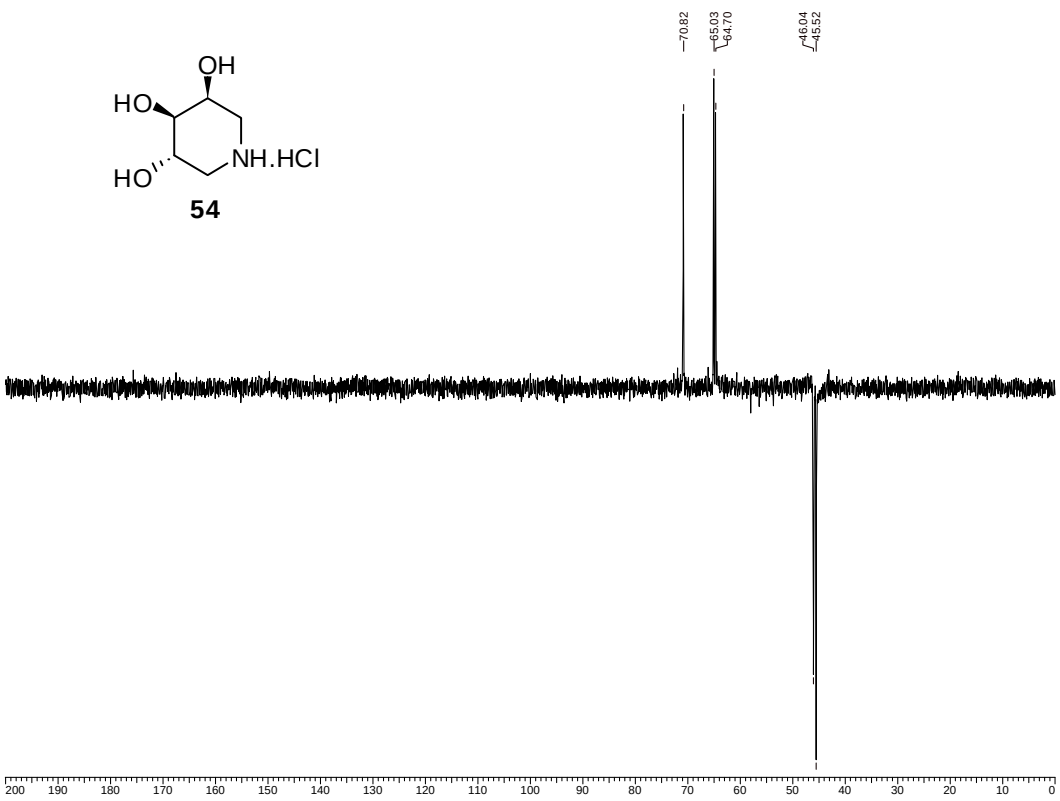

Chloroform-d
137.20
129.01
128.26
127.29
110.29
81.61
77.00
70.79
65.57
61.68
56.58
54.81
26.75
26.50
OH
O
O
N
Bn
52
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0


129.01
128.26
127.29
81.61
70.79
65.57
61.68
56.58
54.81
26.76
26.50
OH
O
O
N
Bn
52
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

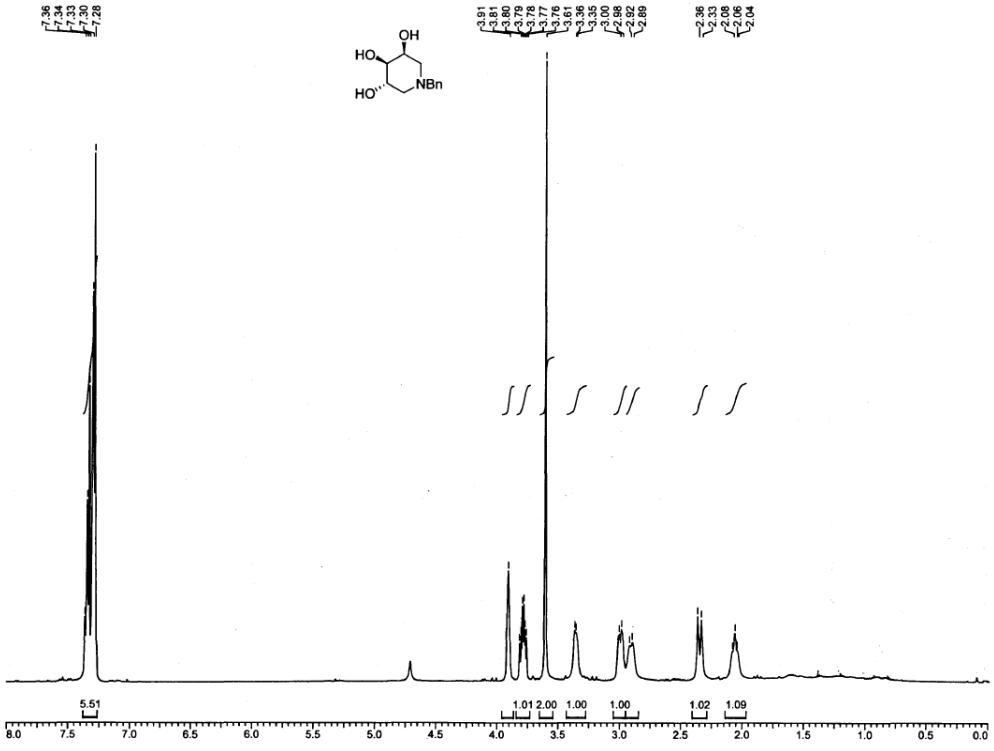
]

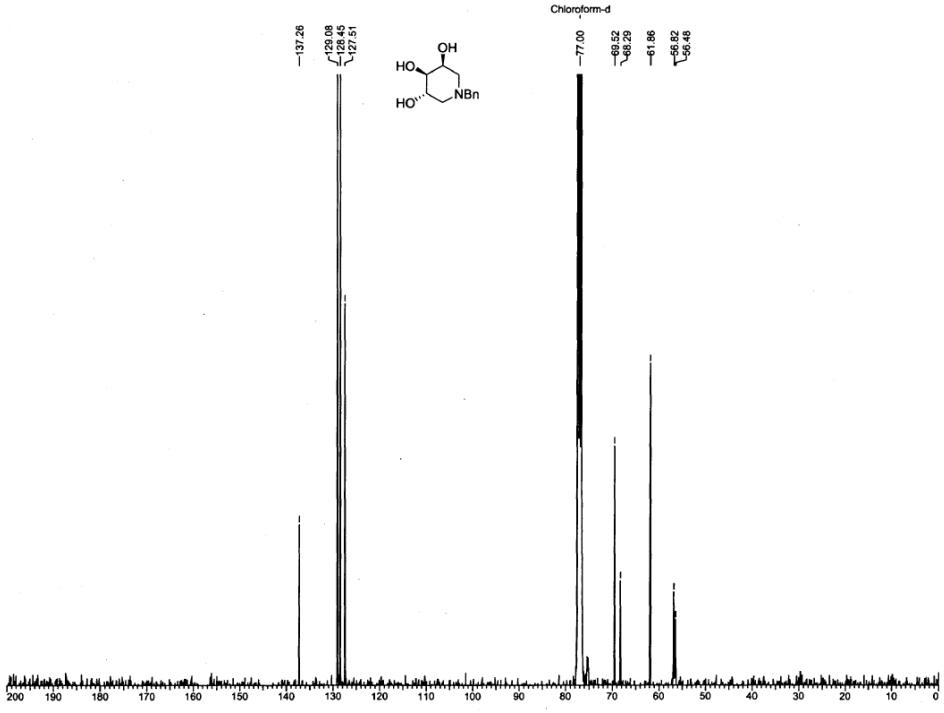

OH
HO
NH.HCl
HO
54
4.80
4.32
4.31
4.30
4.18
4.17
3.87
3.85
3.84
3.50
3.48
3.47
3.36
3.35
3.30
3.30
3.26
3.06
3.04
3.03
3.01
1.00
1.01
1.08
1.13
1.12

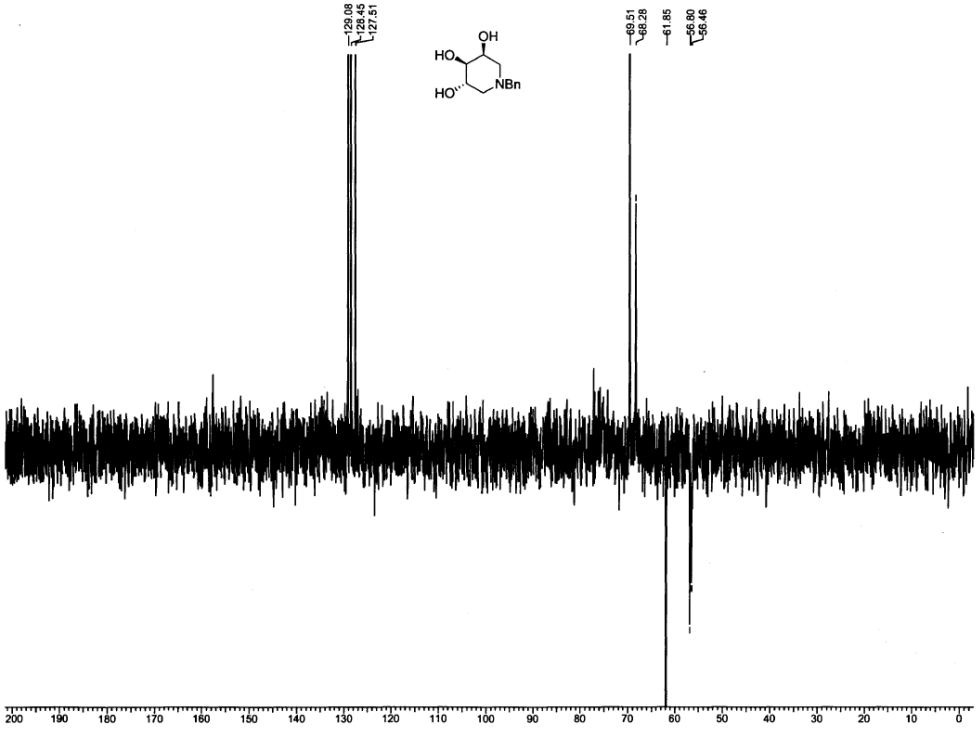

OH
HO
NH.HCl
HO
54
70.82
65.04
64.69
46.03
45.52

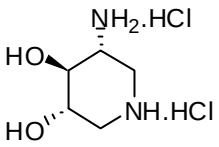

OH
HO
HO
NH.HCl
70.82
65.03
64.70
46.04
45.52

54


OH
HO
HO
NBn
7.36
7.34
7.33
7.30
7.28
3.91
3.81
3.80
3.79
3.78
3.77
3.76
3.61
3.36
3.35
3.00
2.98
2.82
2.89
2.36
2.33
2.08
2.06
2.04
5.51
1.01
2.00
1.00
1.00
1.02
1.09

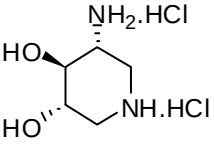

Chloroform-d
137.26
129.08
128.45
127.51
77.00
69.52
68.29
61.86
56.82
56.48
OH
HO
HO
NBn
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0


129.08
128.45
127.51
69.51
68.28
61.85
56.80
56.46
OH
HO
HO
NBn
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0


NH2.HCl
HO
NH.HCl
HO

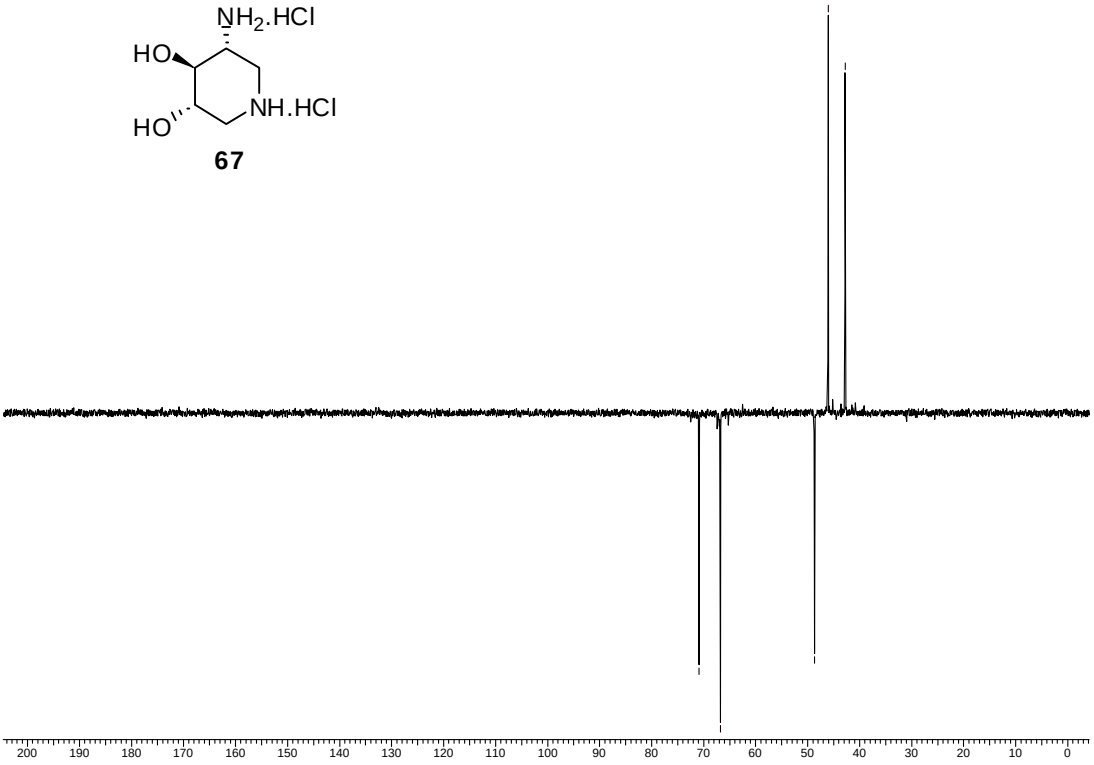
67

4.80
3.96
3.85
3.83
3.82
3.81
3.80
3.64
3.63
3.61
3.38
3.35
3.32
3.14
3.12
3.11
3.08
1.86 2.07 0.96 1.00
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 0.5 0.0

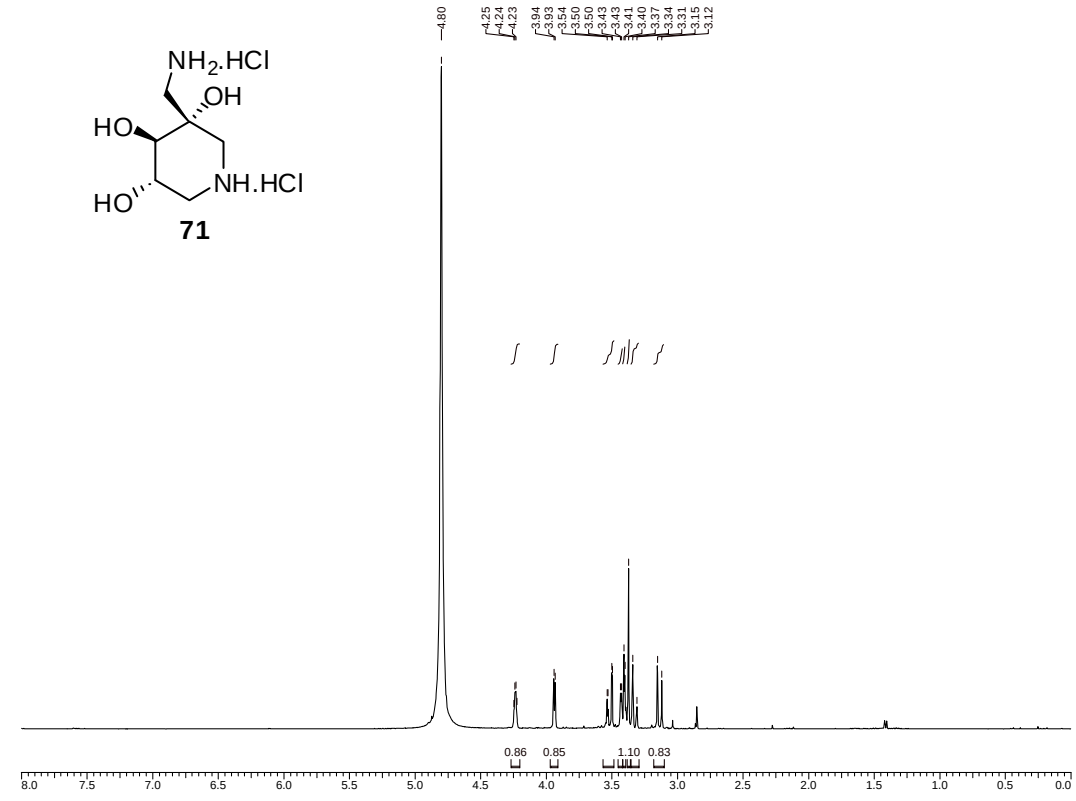

NH2.HCl
HO
NH.HCl
HO

67

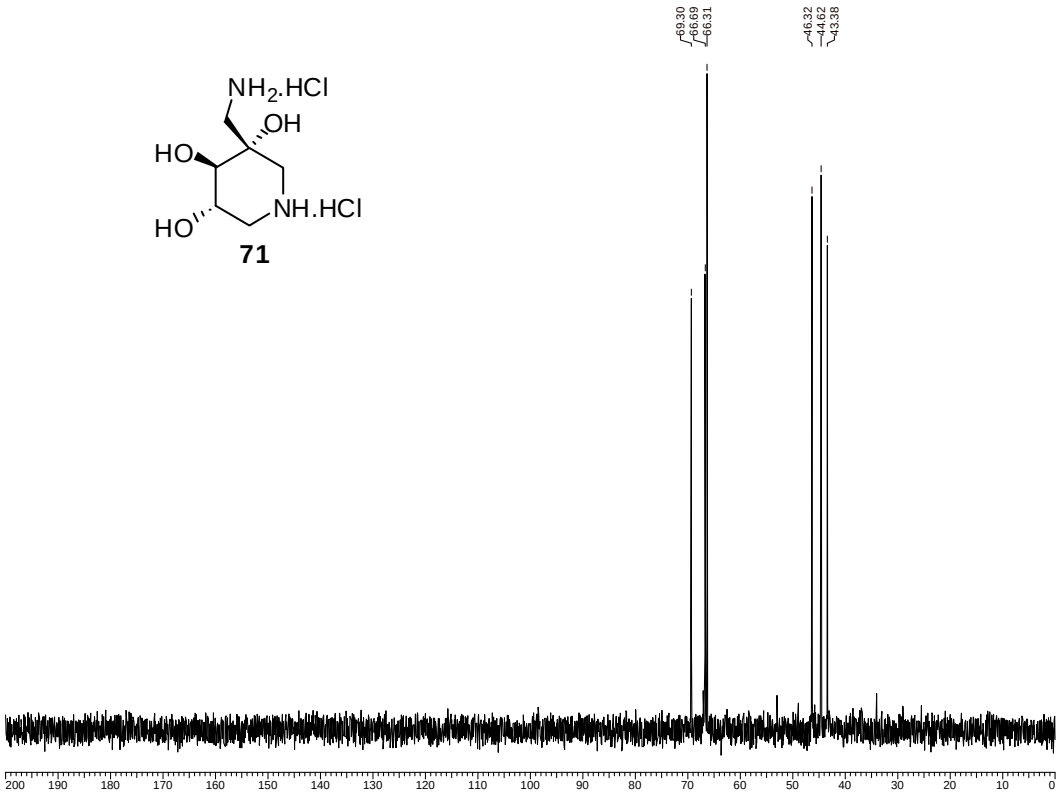
70.89
66.81
48.73
46.07
42.82
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

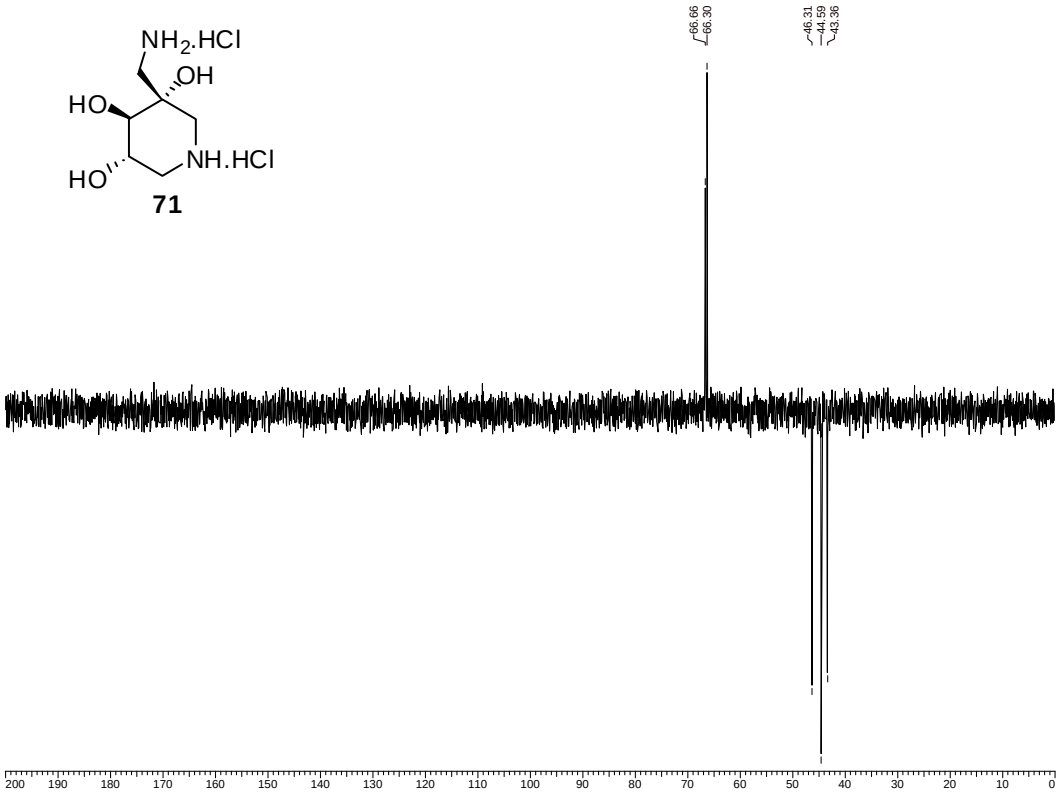

NH2.HCl
HO
NH.HCl
HO
70.88
66.80
48.71
46.06
42.81
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

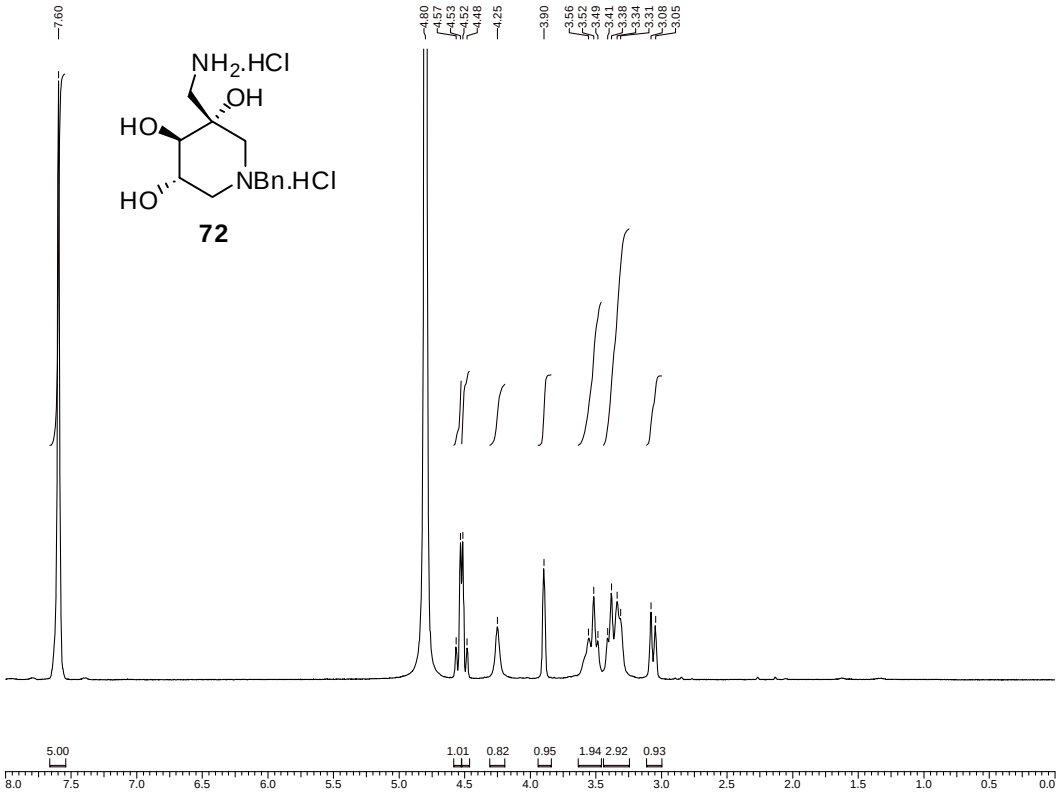
67

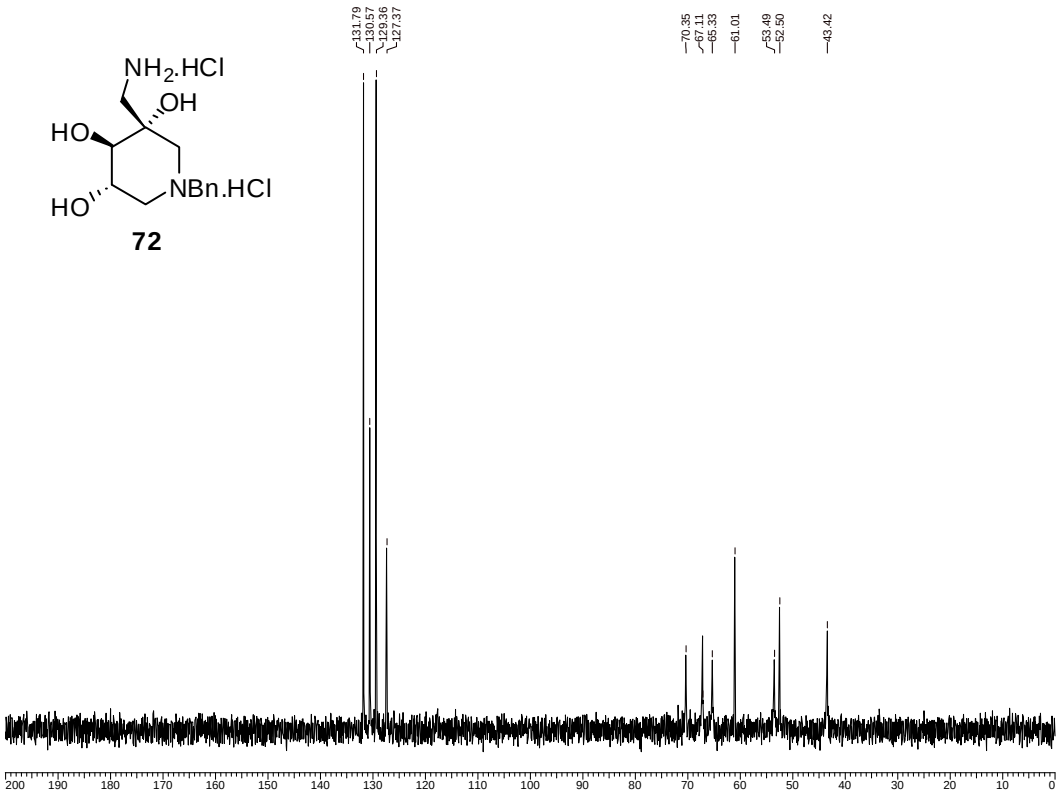

NH2.HCl
OH
HO
NH.HCl
HO
4.80
4.25
4.24
4.23
3.94
3.93
3.54
3.50
3.43
3.41
3.40
3.37
3.34
3.31
3.15
3.12
0.86
0.85
1.10
0.83
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

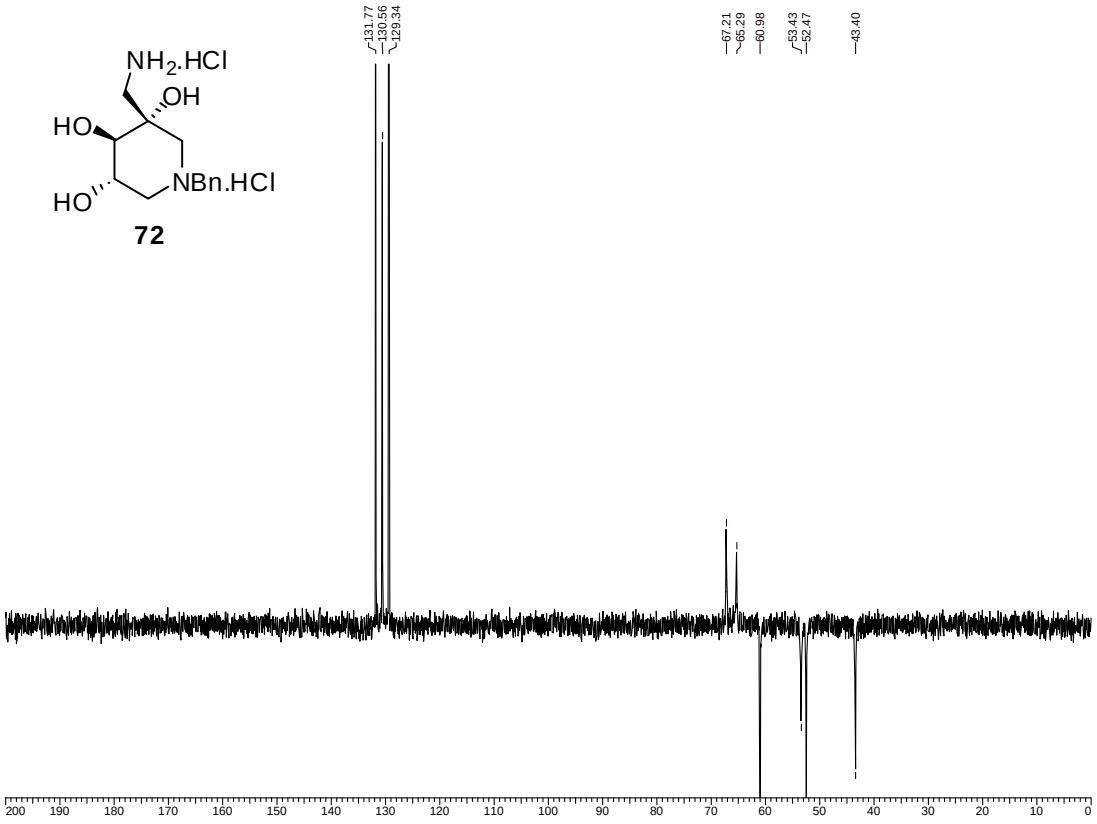
71

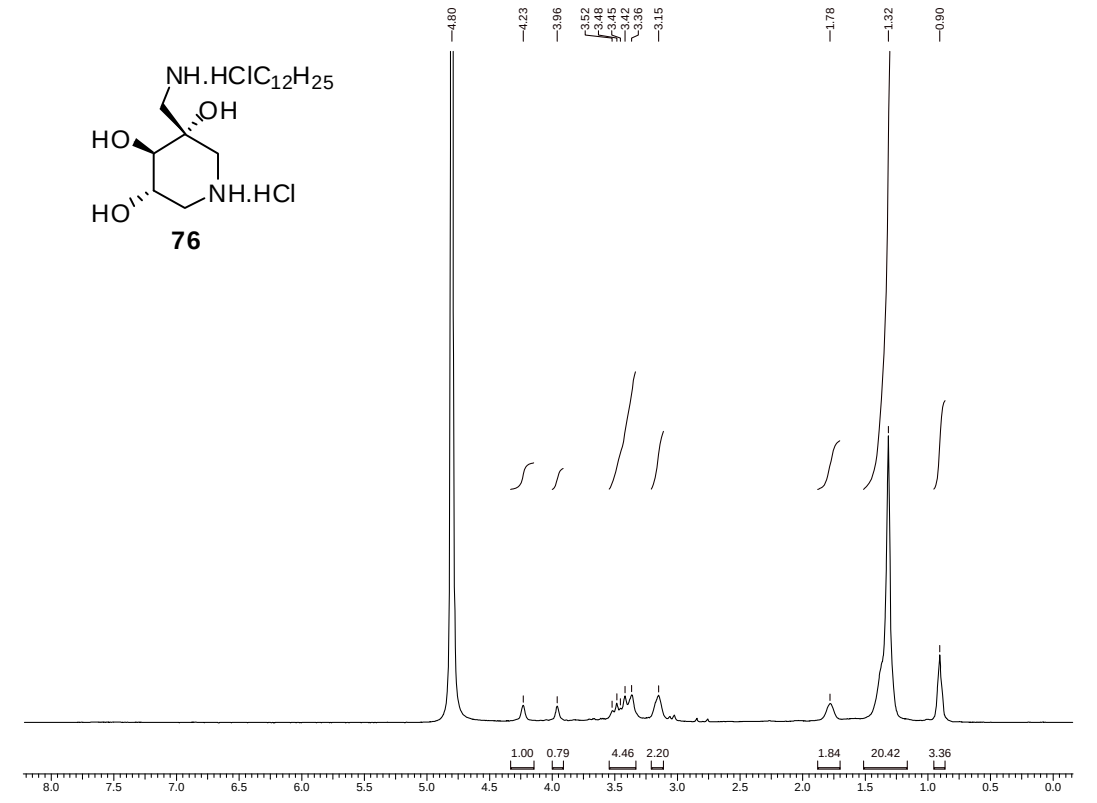

NH2.HCl
OH
HO
NH.HCl
HO
71
69.30
66.69
66.31
46.32
44.62
43.38
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

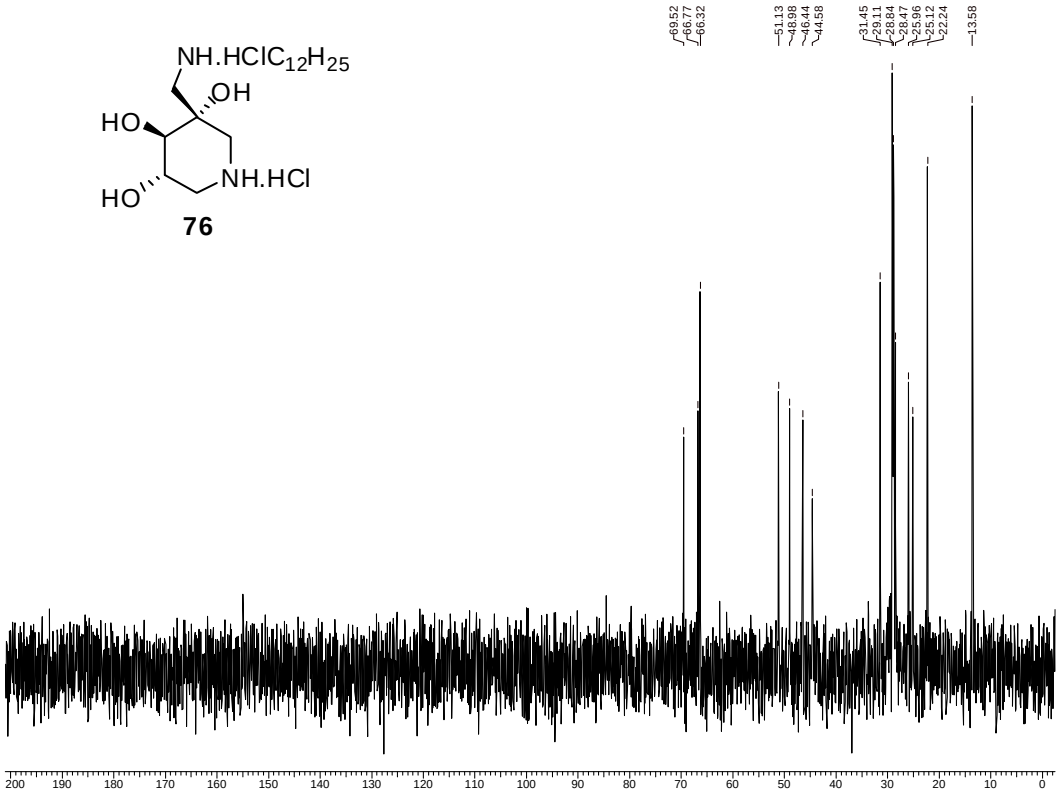

NH2.HCl
OH
HO
NH.HCl
HO
71
66.66
66.30
46.31
44.59
43.36
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0


7.60
4.80
4.57
4.53
4.52
4.48
4.25
3.90
3.56
3.52
3.49
3.41
3.38
3.34
3.31
3.08
3.05
NH2.HCl
OH
HO
HO
NBn.HCl
72
5.00
1.01
0.82
0.95
1.94
2.92
0.93

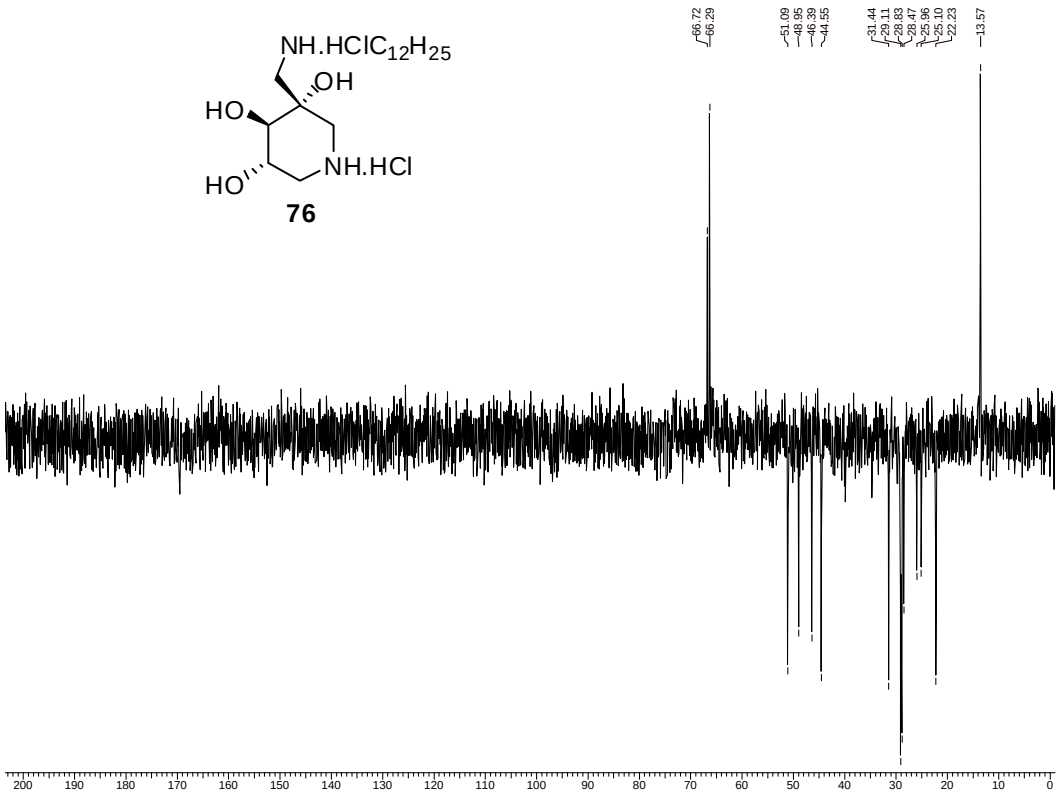

131.79
130.57
129.36
127.37
70.35
67.11
65.33
61.01
53.49
52.50
43.42
NH2.HCl
OH
HO
HO
NBn.HCl
72


NH2.HCl
OH
HO
HO
NBn.HCl
72
131.77
130.56
129.34
67.21
65.29
60.98
53.43
52.47
43.40

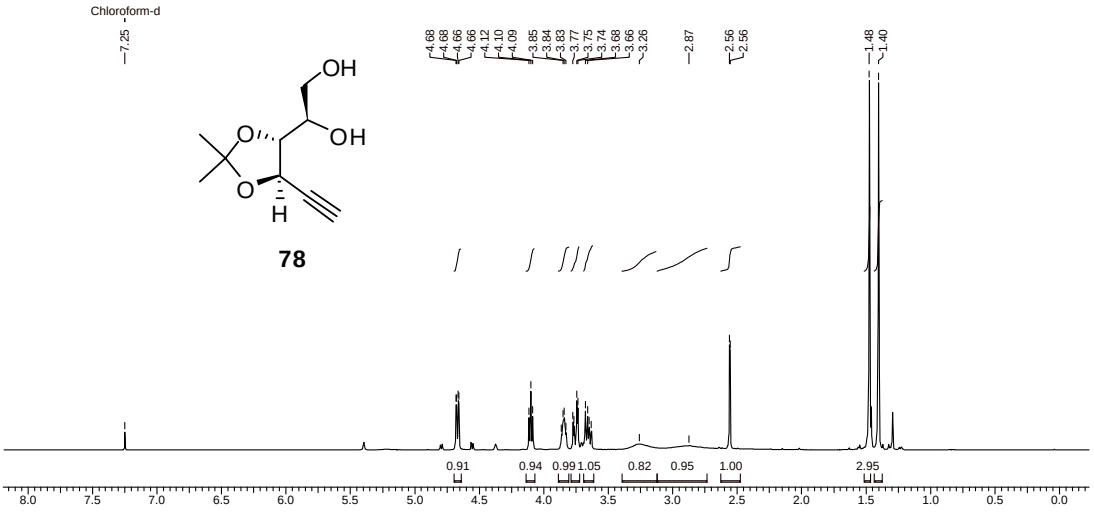

NH.HClC12H25
OH
HO
HO
NH.HCl
76
4.80
4.23
3.96
3.52
3.48
3.45
3.42
3.36
3.15
1.78
1.32
0.90
1.00
0.79
4.46
2.20
1.84
20.42
3.36


NH.HClC12H25
OH
HO
HO
NH.HCl
69.52
66.77
66.32
51.13
48.98
46.44
44.58
31.45
29.11
28.84
28.47
25.96
25.12
22.24
13.58
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

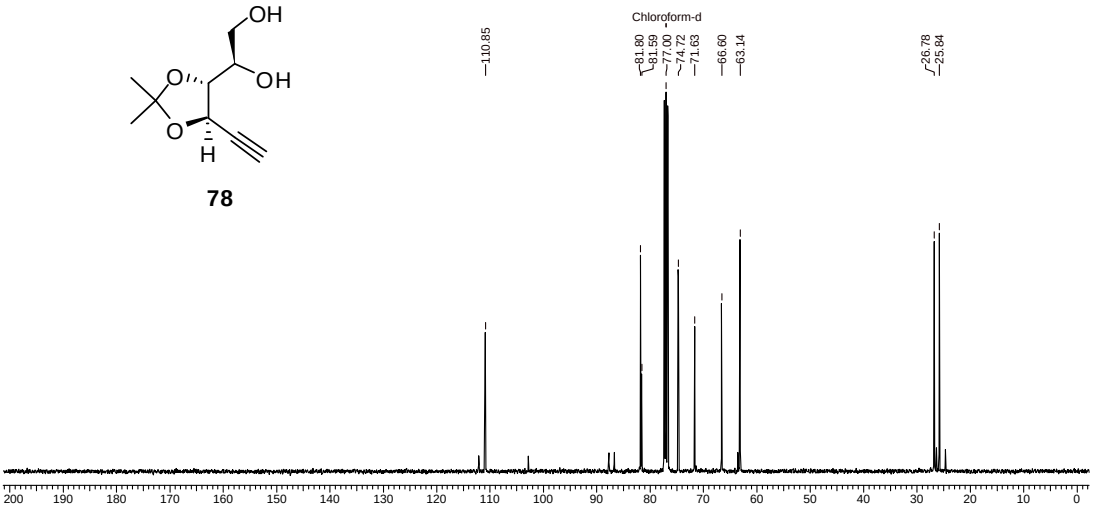
76


NH.HClC12H25
OH
HO
HO
NH.HCl
66.72
66.29
51.09
48.95
46.39
44.55
31.44
29.11
28.83
28.47
25.96
25.10
22.23
13.57
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

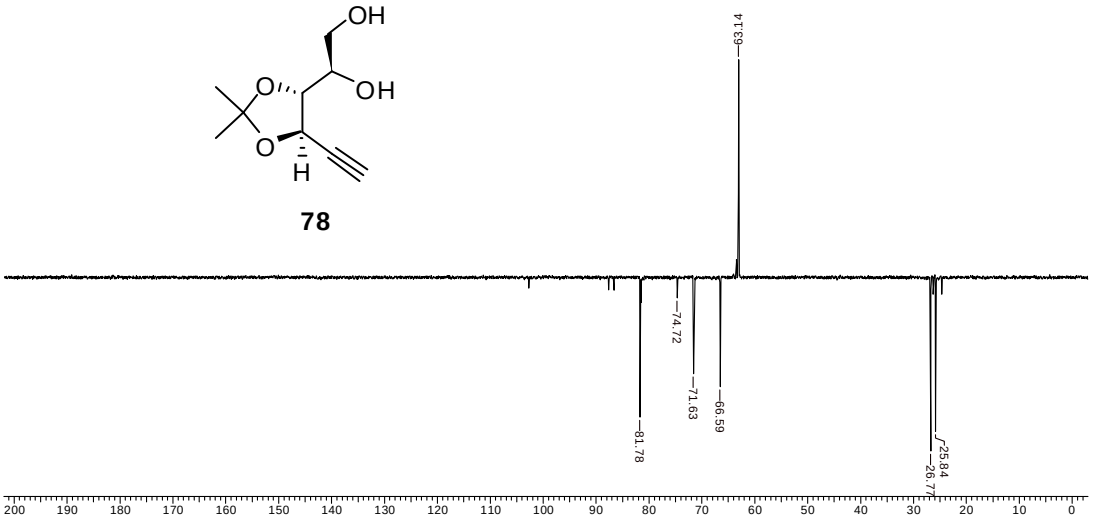
76


Chloroform-d
7.25
4.68
4.68
4.66
4.66
4.12
4.10
4.09
3.85
3.84
3.83
3.77
3.75
3.74
3.68
3.66
3.26
2.87
2.56
2.56
1.48
1.40
OH
OH
O
O
H
0.91
0.94
0.99
1.05
0.82
0.95
1.00
2.95

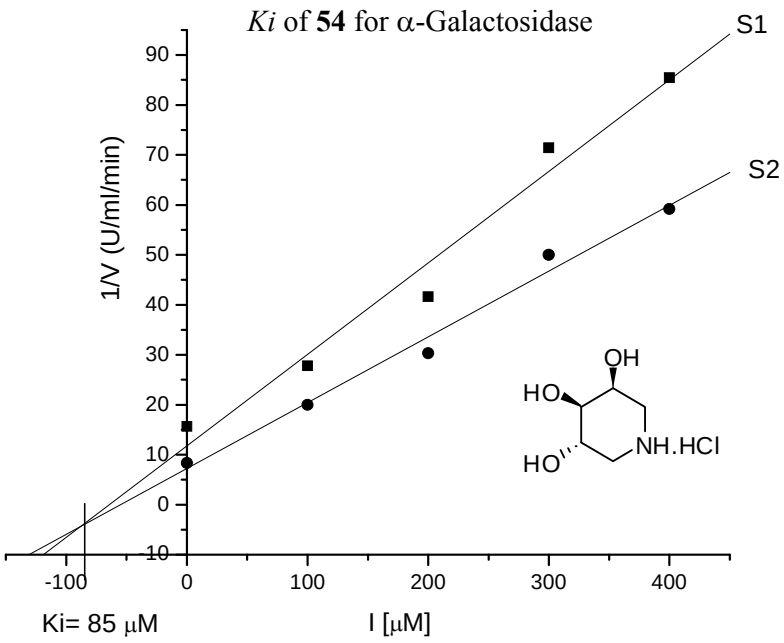
78

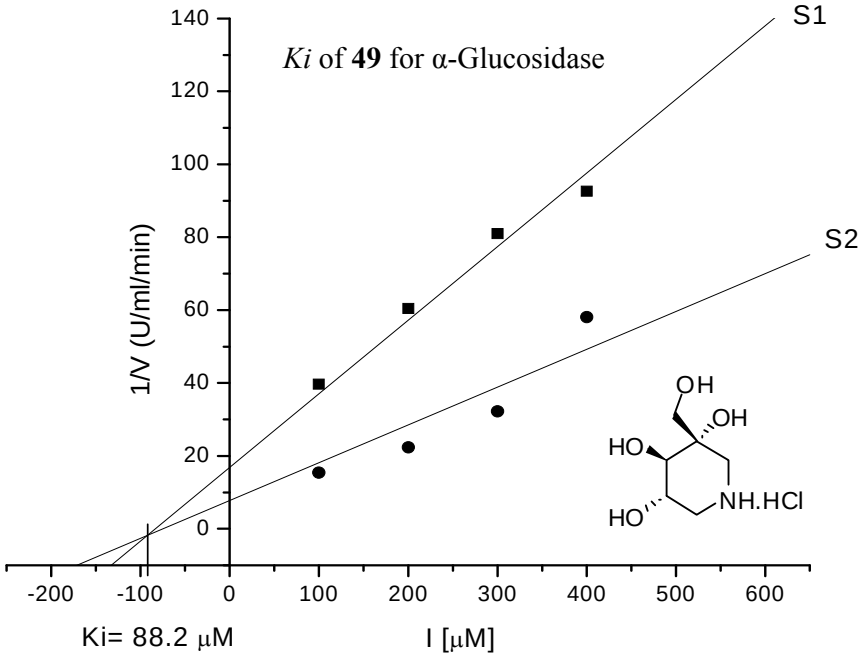

Chloroform-d
110.85
81.80
81.59
77.00
74.72
71.63
66.60
63.14
26.78
25.84
OH
OH
O
O
H

78

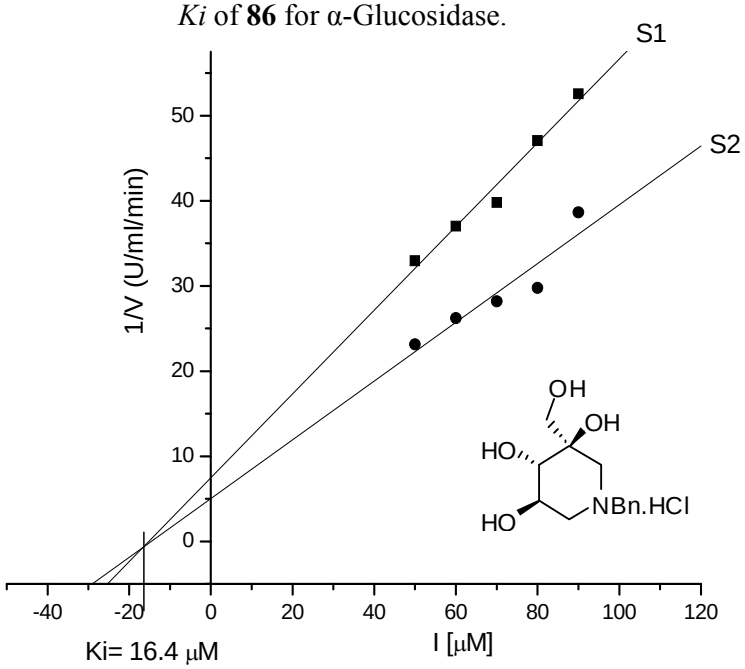

63.14
74.72
71.63
66.59
81.78
25.84
26.77
OH
OH
O
O
H

78

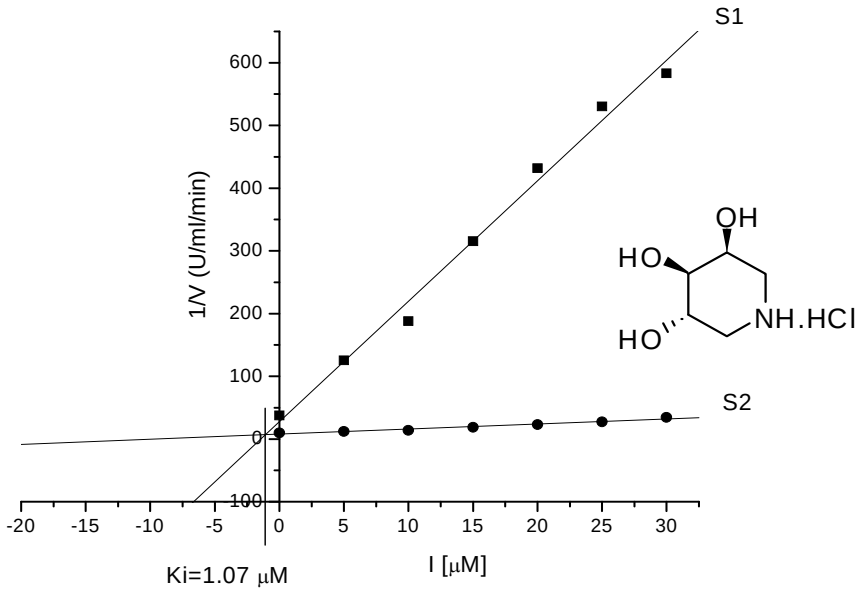

Ki of **54** for α-Galactosidase
S1
S2
1/V (U/ml/min)
I [μM]
Ki= 85 μM
OH
HO
HO
NH.HCl


Ki of **49** for α-Glucosidase
S1
S2
1/V (U/ml/min)
I [μM]
Ki= 88.2 μM
OH
OH
HO
HO
NH.HCl

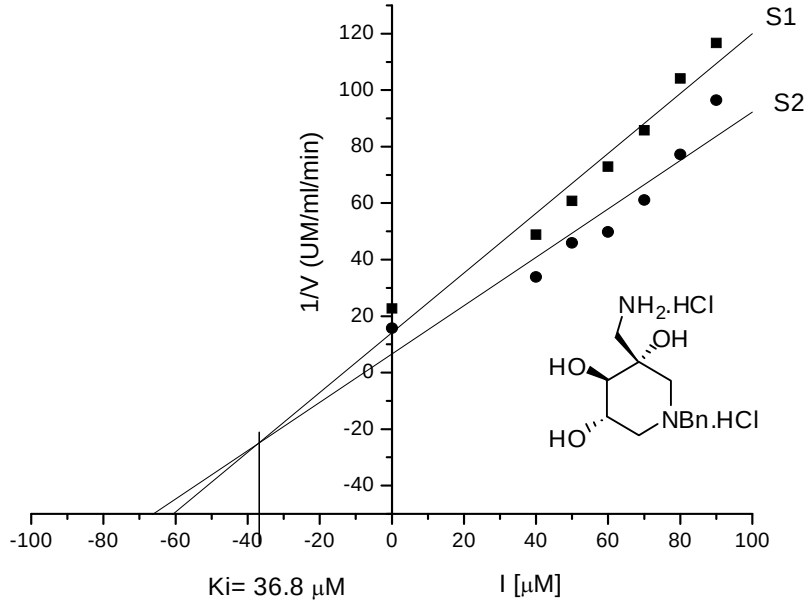
Ki of **86** for α-Glucosidase.



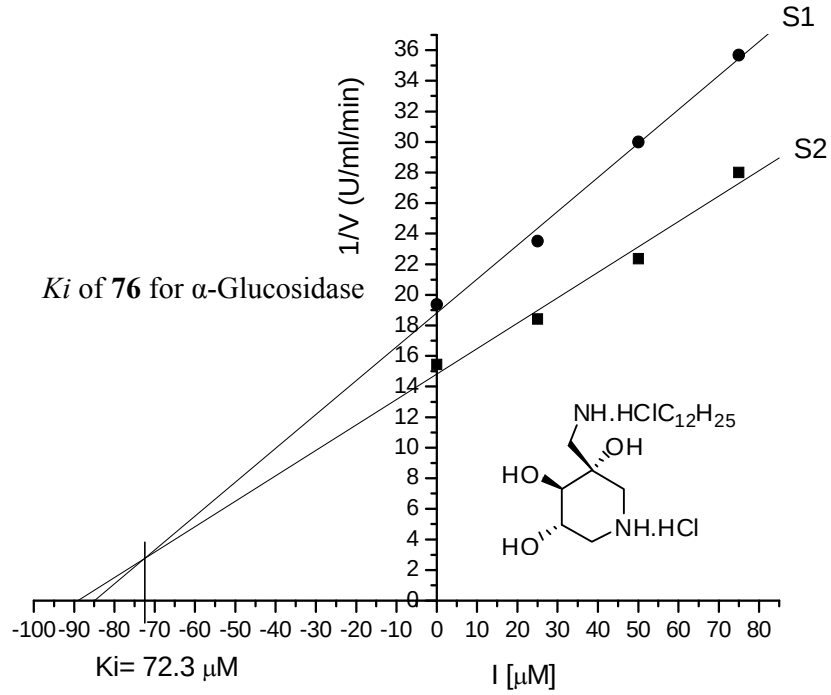
Ki of **54** for α-Glucosidase

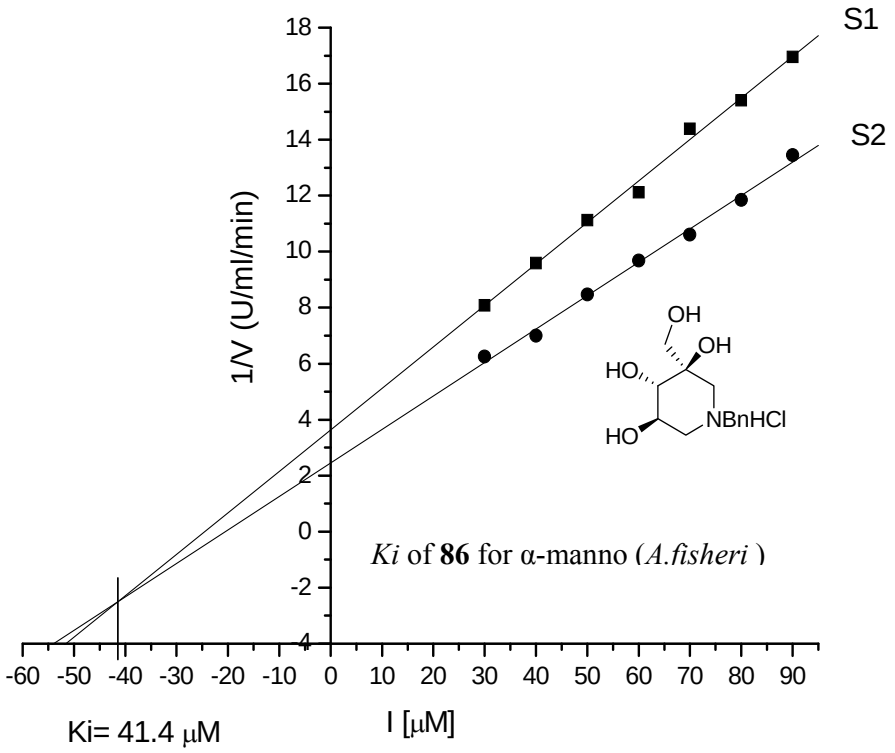


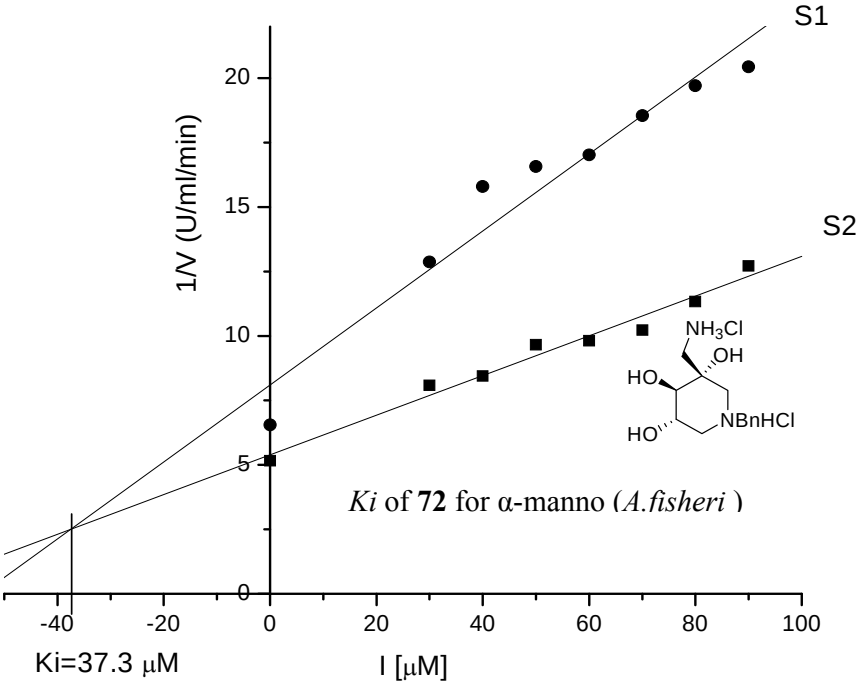
Ki of **72** for α-Glucosidase



Ki of **76** for α-Glucosidase




S1
S2
1/V (U/ml/min)
I [μM]
OH
OH
HO
HO
NBnHCl
Ki of 86 for α-manno (A.fisheri)
Ki= 41.4 μM


S1
S2
1/V (U/ml/min)
I [μM]
NH3Cl
OH
HO
HO
NBnHCl
Ki of 72 for α-manno (A.fisheri)
Ki=37.3 μM

Chapter-3

Synthetic studies towards pyrrolizidines and pyrrolidines class of azasugars

The Voyage of discovery lies not in seeking new horizons, but in seeing with new eyes – Marcel Proust (French novelist).

Section-A:: 3-Hydroxymethyl Pyrrolizidines and 2-Hydroxymethyl Pyrrolidines alkaloids

3.1-Introduction

As discussed earlier[1], glycosidases are the enzymes which are involved in the wide range of important biological processes, such as intestinal digestion of carbohydrates, post-translational processing of glycoproteins and the lysosomal catabolism of glycoconjugates. Polyhydroxylated alkaloids that mimic the structures of sugars are widespread in plants and have been shown to inhibit glycosidase activities. Some of these compounds have been shown as potential anti-cancer, antiviral and anti-retroviral agents[1].

At physiological pH, these polyhydroxylated alkaloids are protonated. These protonated compounds are potent glycosidase inhibitors because of their structural resemblance to the oxocarbenium ion intermediate that is generated in the active site of glycosidase enzymes during the processing of carbohydrates and glycoproteins.

The naturally occurring glycosidase inhibitors have been classified into five structural classes: polyhydroxylated piperidines (e.g. nojirimycin **1**), pyrrolidines (e.g. 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP) **2**), indolizidines (e.g. swainsonine **3**), pyrrolizidines (e.g. australine **4**) and nortropanes (e.g. calystegine A_3 **5**) (Fig. 1).

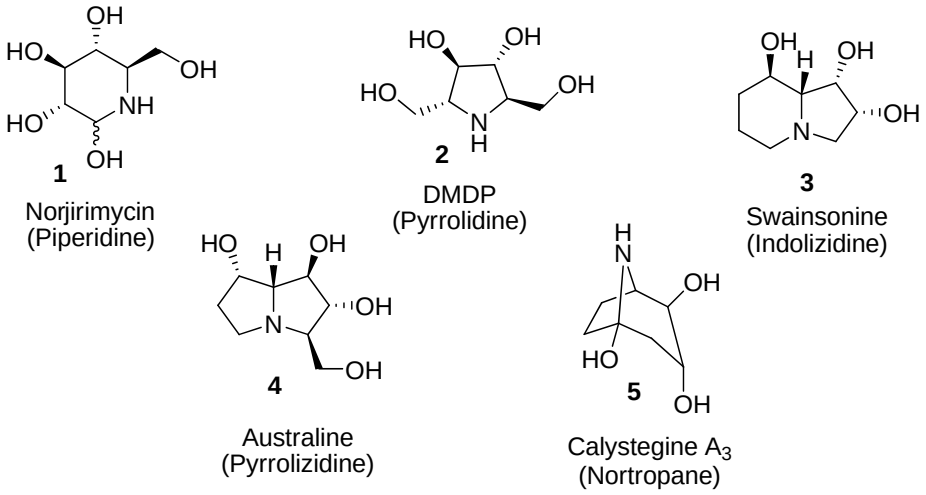


Figure 1.

Earlier in this dissertation, we have discussed the successful synthesis and evaluation of the inhibitory properties of various azasugars which are of piperidine class. In continuation, we got interested in attempting the synthesis of some known pyrrolizidine and pyrrolidine class of azasugars and would like to delineate our endeavor in this Chapter. However, before highlighting our attempts in this regard, it would be pertinent to append a short introduction on pyrrolidine and pyrrolizidines class of azasugars to put the current study in proper perspectives.

3.2-Natural Occurrence and Biological Activities of 3-Hydroxymethyl pyrrolizidine azasugars

Although, the broad class of pyrrolizidine alkaloids bearing a carbon branch at C-1 (necines) are well documented (e.g.dihydroxyheliotridane **6**), a relatively new group of 3-hydroxymethyl substituted pyrrolizidine alkaloids have been found since the isolation of alexine (**7)** and australine (**4)**[2,3].

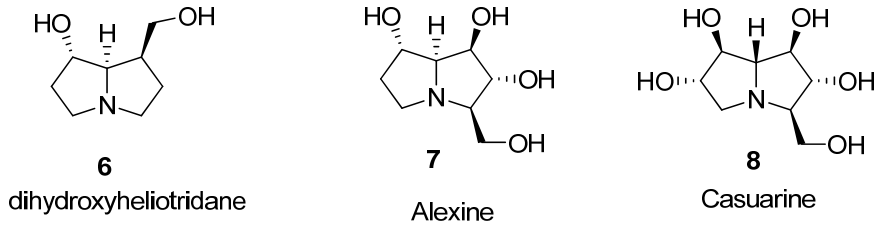
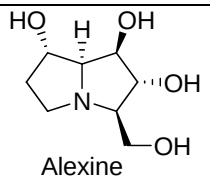
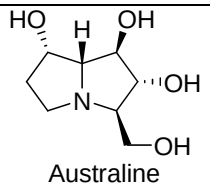
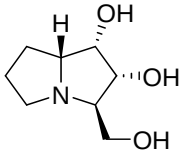
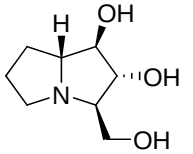
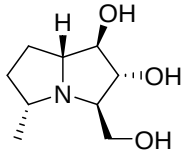
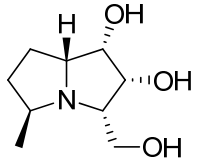
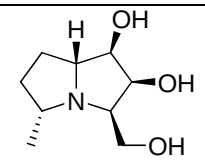
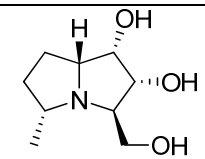
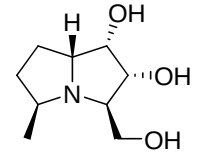
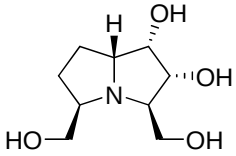
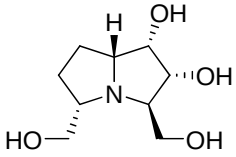
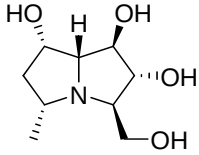


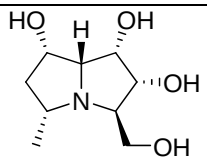
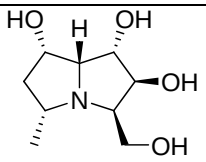
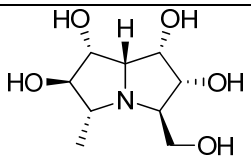
Figure 2.

These new groups of pyrrolizidine alkaloids (alexines or australines) have been thought to be of very restricted natural occurrence. These compounds have only been reported in two small genera of the Leguminosae (*Castanospermum* and *Alexa*). Similarly, Casuarine (**8,** Figure 2) another pyrrolizidine alkaloid is known to occur only in the related genera of the *Casuarinaceae* and *Myrtaceae.*[4,5] Recently, some new 3-hydroxymethylpyrrolizidines, with carbon branches both at C-3 and C-5, have also been isolated from *Hyacinthoides non-scripta* and *Scilla campanulate* (both *Hyacinthaceae*) and named hyacinthacines[6,7,8] The representative example of this class of azasugars along with their enzyme inhibitory activities are highlighted in Table 1

Table 1.

Inhibitor	Source	Biological Activity
 Alexine **7**	Isolated from legume *Alexa leiopetala*, a leguminous tree.[2]	Strong inhibitor of fungal amyloglucosidase[9].
 Australine **4**	Isolated from the seeds of *Castanospermum Australae*.[3]	Inhibits amyloglucosidase from *aspergillus niger*. (IC_{50} = 5.8 μM).[3]
 Hyacinthacines A_1 **9**	Isolated from bulbs of *Muscari armeniacum* (Hyacinthaceae).[7]	Inhibits rat intestinal lactase (IC_{50} = 4.4 μM).[7]
 Hyacinthacines A_2 **10**	Isolated from bulbs of *Muscari armeniacum* (Hyacinthaceae).[7]	Inhibits strongly amyloglucosidase (IC_{50} = 8.6 μM).[7]
 Hyacinthacines A_3 **11**	Isolated from bulbs of *Muscari armeniacum* (Hyacinthaceae).[7]	Good inhibitor of amyloglucosidase (IC_{50} = 17 μM).[7]
 Hyacinthacines A_4 **12**	Isolated from bulbs of *Scilla siberica*.[8]	Not significant

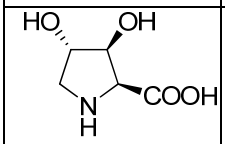
 Hyacinthacines A_5 **13**	Isolated from bulbs of *Scilla siberica.*[8]	Modest inhibitors of amyloglucosidase (IC_{50} = 110 μM)[8]
 Hyacinthacines A_6 **14**	Isolated from bulbs of *Scilla siberica.*[8]	Not significant
 Hyacinthacines A_7 **15**	Isolated from bulbs of *Scilla siberica.*[8]	Not significant
 Hyacinthacines B_1 **16**	Isolated from the immature fruits and stalks of bluebells, *Hyacinthoides non-scripta* (Hyacinthaceae).[6]	Weak inhibitors of b-glucosidase[6]
 Hyacinthacines B_2 **17**	Isolated from the immature fruits and stalks of bluebells, *Hyacinthoides non-scripta* (Hyacinthaceae).[6]	Potent inhibitor towards rat intestinal lactase (IC_{50} = 3.6 μM).[6]
 Hyacinthacines B_4 **18**	Isolated from the bulbs of *Scilla siberica.*[8]	Inhibitors of amyloglucosidase (IC_{50} = 89 μM).[8]

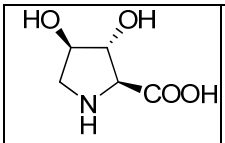
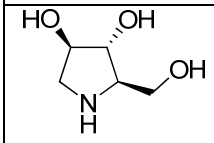
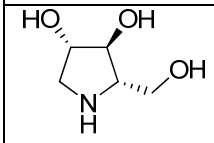
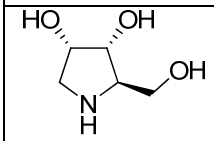
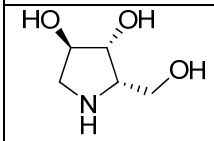
 Hyacinthacines B_5 **19**	Isolated from the bulbs of *Scilla siberica.*[8]	Inhibitors of amyloglucosidase (IC_{50} = 110 μM).[8]
 Hyacinthacines B_6 **20**	Isolated from the bulbs of *Scilla siberica.*[8]	Not significant
 Hyacinthacines C_1 **21**	Isolated from the immature fruits and stalks of bluebells, *Hyacinthoides non-scripta* (Hyacinthaceae).[6]	Inhibitor of amyloglucosidase (IC_{50} = 84 μM).[6]

3.3-Natural Occurrence and Biological Activities of 2-Hydroxymethyl pyrrolidine azasugars

Hydroxylated pyrrolidines and pyrroles constitute one of the main class of naturally occurring sugar mimics having nitrogen in the ring. As a consequence of their role as glycosidase inhibitors, much attention has been focused on these classes of compounds because of their potentials for cell-biological studies and therapeutic applications. Some of the active and most studied members of this family are summarized in the Table 2.

Table 2

Inhibitor	Source	Biological Activity
 **22**	Isolated from diatom cell walls[10]	Believed to act in plants as defense agents against predators and parasites[11]

 **23**	Isolated from Amanita vitosa mushrooms[12].	do
 **24**	Found in both Arachniodes standishii[13] and Angylocalyx boutiqueanu[14]	Potent inhibitor of yeast α-glucosidase (IC_{50} = 0.18 μM)[15]
 **25**	Occurs as a component of bacterial lipopolysaccharides[16]	Inhibition of α-glucosidase (IC_{50} = 10 μM)[17]
 **26**	Isolated from mulberry trees (Morus alba)[18]	Potent inhibitor of glucosidase and of eukaryotic DNA polymerases.[19]
 **27**	Isolated from *Arachniodes Standishii* and *Angylocalyx boutiqueanus*[15b,20]	Potential glycosidase inhibitor[20d]

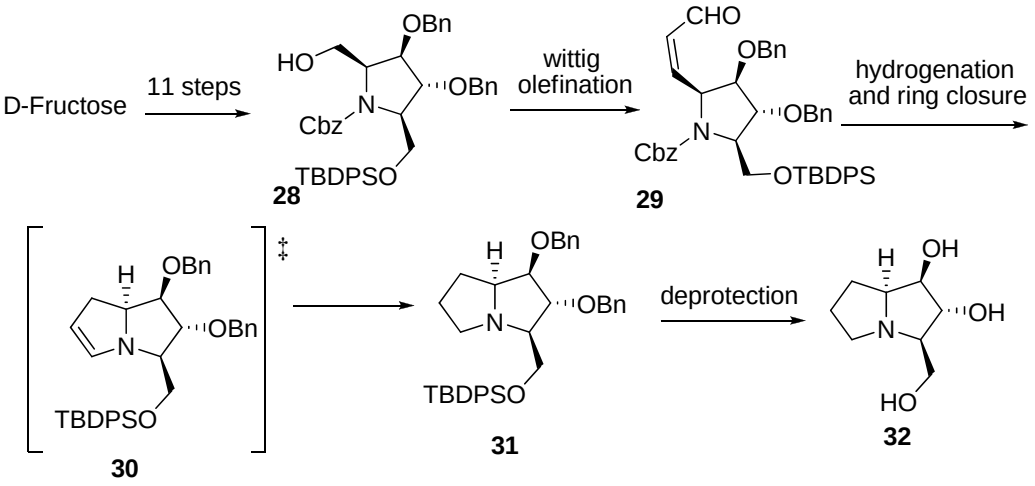
The hyacinthacines are the new classes of alkaloids and not many reports on their syntheses have been reported so far. The reports available don't have the advantage of being diversity oriented and generally end up in one product. Described below are few of the literature reports of their synthesis.

3.4-Synthetic approaches towards Hyacinthacine class of azasugars[21]

Izquierdo's approach[22]

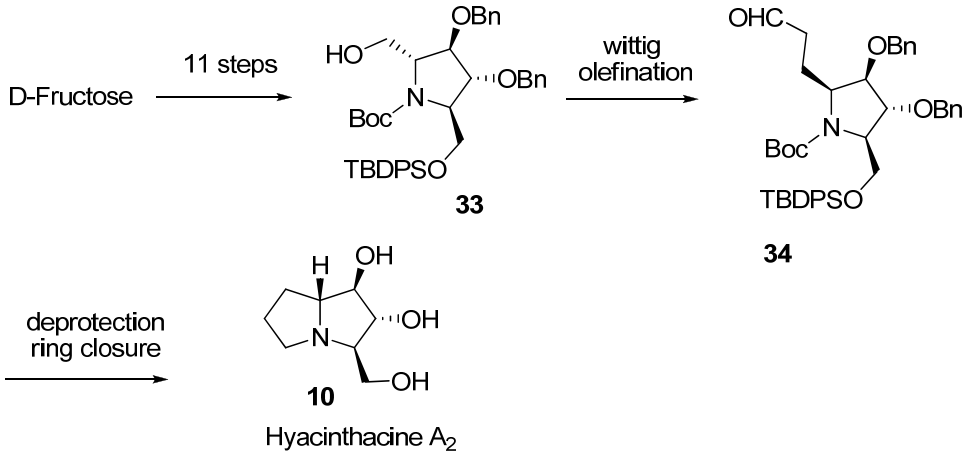
Izquierdo's group have prepared 7a-epi-hyacinthacine A_2 (**32**) from the differentially protected 2, 5-dihydroxymethyl-3,4-dihydroxypyrrolidine **28** obtained in 11 steps from D-fructose[23] (Scheme 1). Hydrogenation of **29** leads to the generation of free amine

which undergoes *in situ* enamine formation and reduction to give **31**. Deprotection of **31** gave **32**.



Scheme 1.

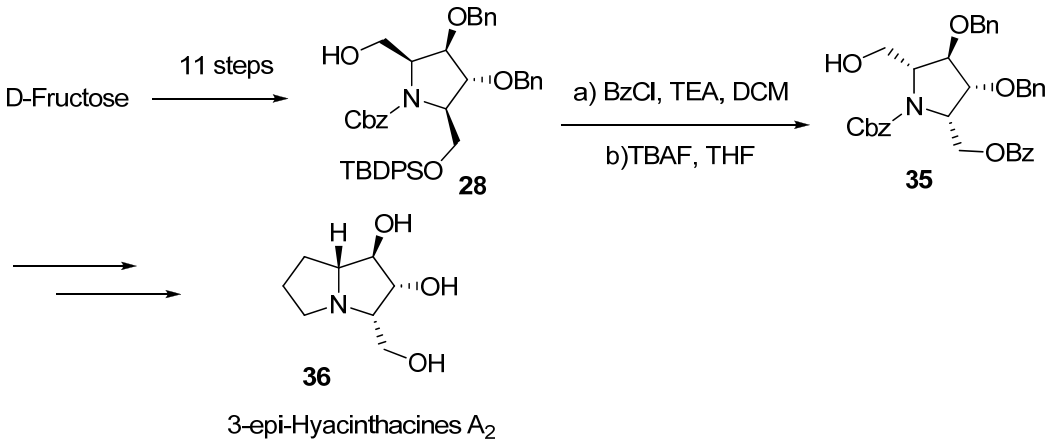
This group have also reported the total synthesis of (+) - Hyacinthacine A_2 (**10**) using similar chemistry (Scheme 2).



Scheme 2.

More recently, the same group have also employed compound **28** in the synthesis of 3-epi hyacinthacines A_2 (**36**) using related chemistry (Scheme 3). The 5- hydroxymethyl group of **28** in this approach was protected as its benzoate ester followed by cleaving of the primary silyl ether moiety to obtain the primary alcohol **35** resulting opposite

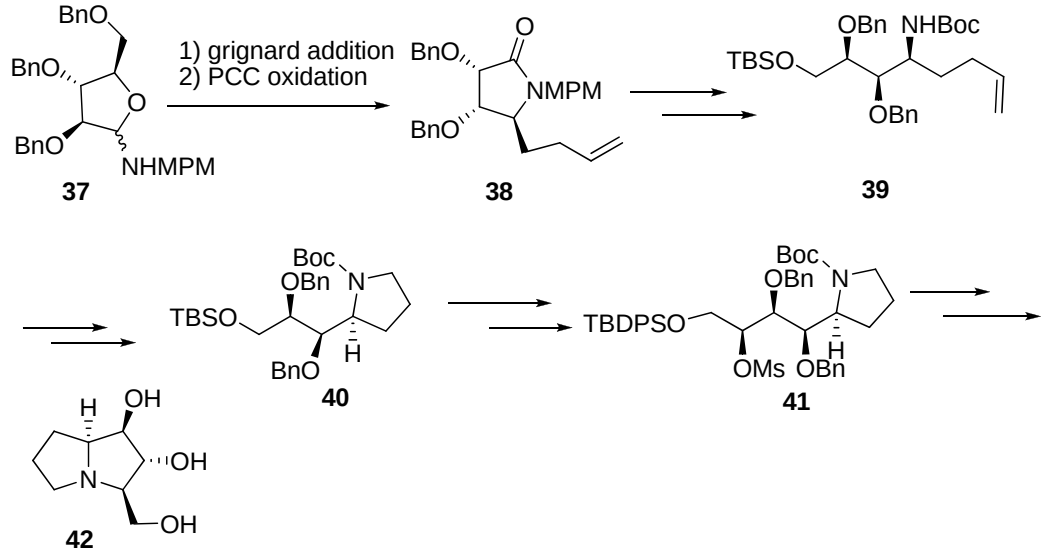
stereochemistry with respect to **28** at C-2 and C-5.[24] Similar set of reactions later produced **36**.



Scheme 3.

Yoda's approach[25]

The stereocontrolled synthesis of 7-deoxyalexine (**42**) from functionalized lactum **38**, prepared from D-arabinofuranose tribenzylether **37**, was obtained as described in Scheme- 4.

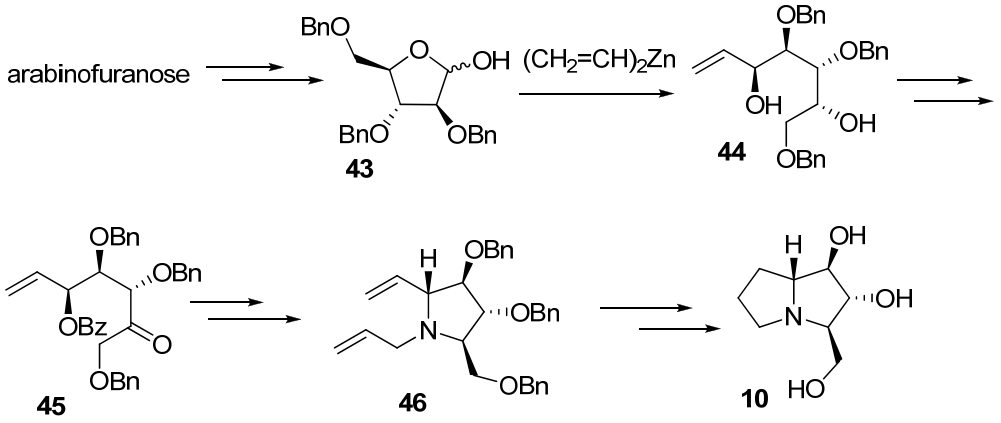


Scheme- 4.

Martin's approach[26]

The first synthesis of (+)-Hyacinthacine A_2 (**10**) was achieved by Martin *et al*. in six steps (Scheme 5) from 2,3,5-tri-*O*-benzyl- D-arabinofuranose **43** in an overall yield of 11%

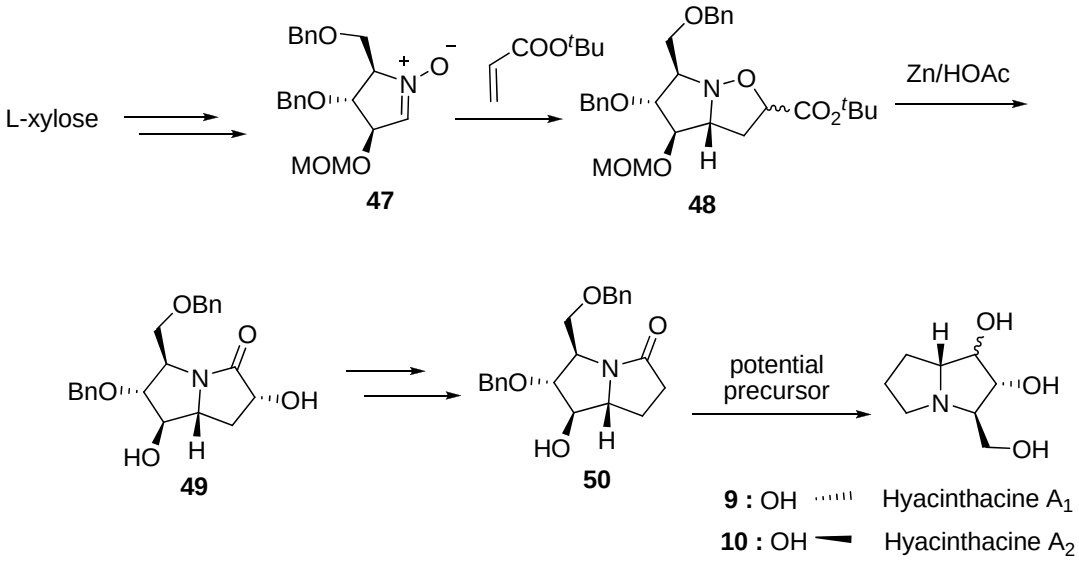
employing RCM reaction as the key step. The synthesis utilized highly stereoselective addition of divinylzinc to the commercially available D-arabinofuranose derivative **43** affording heptenitol **44** in 95% yield (Scheme 5). Regioselective benzoylation of the less hindered allylic hydroxyl group of **44** followed by Swern oxidation gave **45** which was later transformed to target molecule **10**.



Scheme 5.

Ishibashi's approach[27]

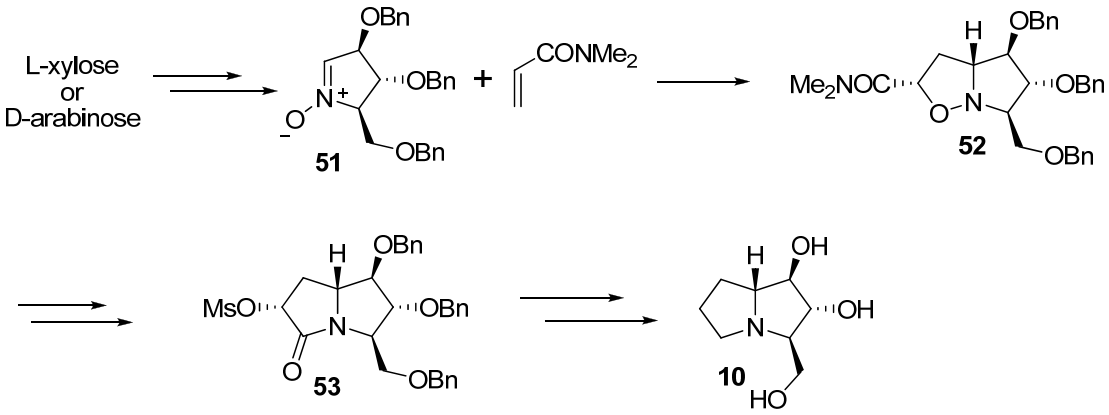
Ishibashi's group obtained potential pyrrolizidine precursor **50** for the synthesis of hyacinthacine A_1 and A_2 **(9** and **10)** from polyhydroxylated cyclic nitrone **47.** The 1, 3-dipolar cycloaddition of nitrone **47** with *tert*-butyl acrylate gave **48**. The N-O bond of **48** was cleaved under reductive conditions. Latter Barton-McCombie deoxygenation followed by removal of the MOM gave **50** (Scheme 6).



Scheme 6.

Goti's approach[28]

This group reported the total synthesis of hyacinthacine A_2 (**10**) by utilizing 1,3-dipolar cycoaddtion reactions of the carbohydrate derived nitrone **51** (Scheme 7). Cycloadduct **52** on reductive cleavage of the N-O bond (using Zn/HOAc) bond and mesylation of resulting alcohol produced **53**. LAH reduction followed by deprotection gave **10**.

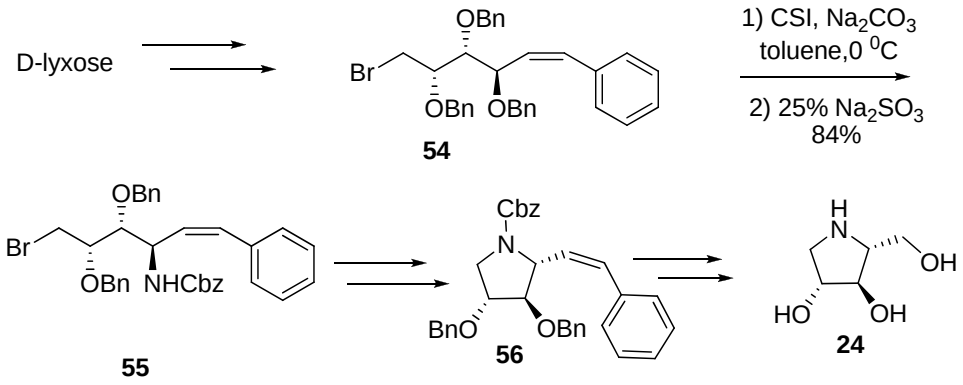


Scheme 7.

3.5-Synthetic approaches towards pyrrolidine class of azasugars[29]

Jung's approach [30]

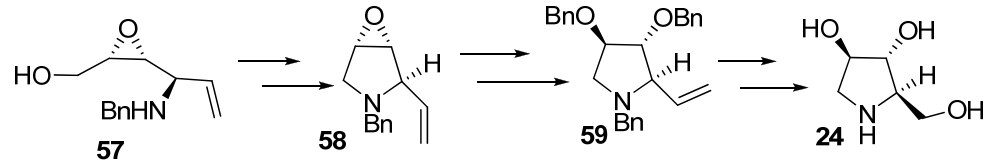
The synthesis of **24** was accomplished in 10 steps (Scheme 8) by involving a key step of introducing regio-and diastereoselective NHCbz group in cinnamyl polybenzyl ether **54** using CSI (chlorosulfonyl isocyanate) followed by its cyclization.



Scheme 8.

Gorrichon's approach[31]

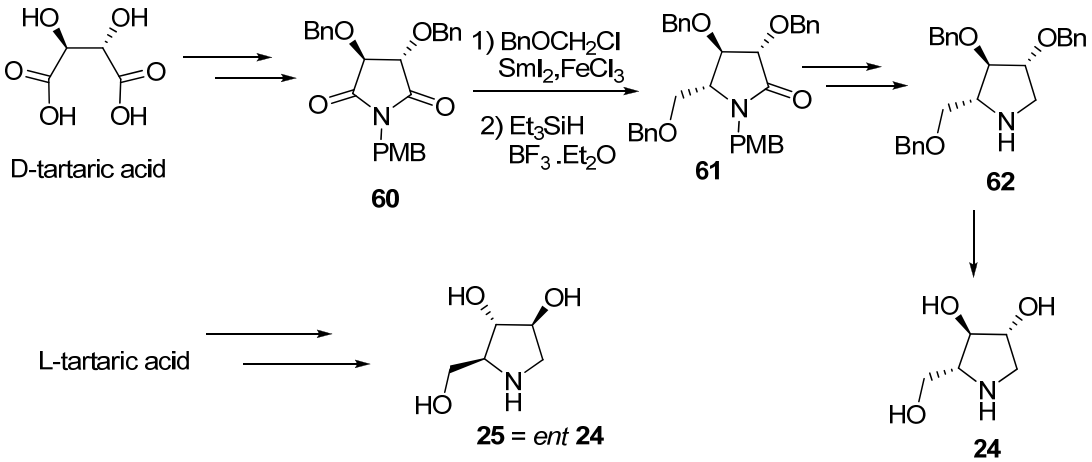
This approach relied on the regiocontrolled intramolecular opening of the epoxy moiety by tethered amine group of the intermediate **58** (Scheme 9).



Scheme 9.

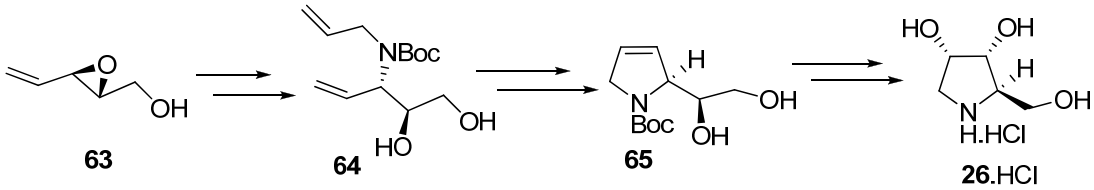
Huang's approach[32]

Both L- as well as D-tartaric acids were utilized to synthesize enantiomeric **24** and **25** in 9 steps each (Scheme 10). Their approach was based upon SmI_2-mediated benzyloxymethylation of *O,O′*-dibenzyltartarimide **60** followed by $BF_3.Et_2O$ mediated reduction using Et_3SiH.



Scheme 10.

Riera's approach[33]

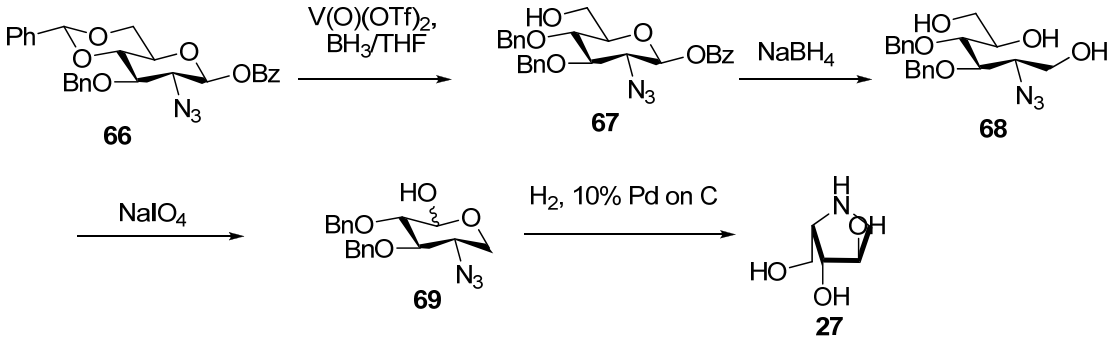


Scheme 11.

The strategy used RCM as the key step to obtain key intermediate dihydropyrrolidine **65** (Scheme 11) for the synthesis of **26**.

Hung's approach[34]

Hung capitalized (Scheme 12) borane mediated reductive ring opening of the 4,6-*O*-benzylidene-D-glucoyranosides **66** to obtain **67.** Sodium borohydride reduction of **67** followed by periodate cleavage of resulting triol **68** gave lactol **69** which upon reductive cyclization gave **27**.



Scheme 12.

3.6-References

1. (a) Ganem, B. *Acc. Chem. Res.* **1996**, *29*, 340. (b) de Raadt, A.; Ekhart, C. W.; Ebner, M.; Stütz, A. E. *Top. Curr. Chem.* **1997**, *187*, 157. (c) Ichikawa, Y.; Igarashi, M.; Ichikawa, M.; Suhara, Y. *J. Am. Chem. Soc.* **1998**, *120*, 3007. (d) Stütz, A. E. *Iminosugars as Glycosidase Inhibitors: Nojirimycin and Beyond*, Wiley-VCH, Weinheim, Germany, **1999**. (e) Asano, N.; Nash, R. J.; Molyneux, R. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2000**, *11*, 1645. (f) Lillelund, V. H.; Jensen, H. H.; Laing, X.; Bols, M. *Chem. Rev.* **2002**, *102*, 515. (g) Pearson, M. S. M.; Mathé-Allainmat, M.; Fargeas, V.; Lebreton, J. *Eur. J. Org. Chem.* **2005**, 2159. (h) Afarinkia, K.; Bahar, A. *Tetrahedron: Asymmetry* **2005**, *16*, 1239. (i) Whalem, L. J.; Wong, C.-H. *Aldrichimica Acta* **2006**, *39*, 63. (j) Gravier-Pelletier, C.; Le Merrer, V. *Current Organic Synthesis* **2007**, *4*, 1. (k) Wrodnigg, T. M.; Steiner, A. J.; Bernhard, B. J. *Anti-cancer agents in medicinal chemistry* **2008**, *8*, 77.
2. Nash, R. J.; Fellows, L. E.; Dring, J. V.; Fleet, G. W. J.; Derome, A. E.; Hamor, T. A.; Scofield, A. M.; Watkin, D. J. *Tetrahedron Lett.* **1988**, *29*, 2487.
3. Molyneux, R. J.; Mabry, B.; Wong, R. Y.; Tropea, J. E.; Elbein, A. D. *J. Nat. Prod.* **1988**, *51*, 1198.
4. Wormald, M. R.; Nash, R. J.; Watson, A. A.; Bhadoria, B. K.; Langford, R.; Sims, M.; Fleet, G. W. J. *Carbohydr. Lett.* **1996**, *2*, 169.
5. Nash, R. J.; Thomas, P. I.; Waigh, R. D.; Fleet, G. W. J.; Wormald, M. R.; de Q. Lilley, P. M.; Watkin, D. J. *Tetrahedron Lett.* **1994**, *35*, 7849.
6. Kato, A.; Adachi, I.; Miyauchi, M.; Ikeda, K.; Komae, T.; Kizu, H.; Kameda, Y.; Watson, A. A.; Nash, R. J.; Wormald, M. R. *Carbohydr. Res.* **1999**, *316*, 95.
7. Asano, N.; Kuroi, H.; Ikeda, K.; Kizu, H.; Kameda, Y.; Kato, A.; Adachi, I.; Watson, A. A.; Nash, R. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2000**, *11*, 1.
8. Yamashita, T.; Yasuda, K.; Kizu, H.; Kameda, Y.; Watson, A. A.; Nash, R. J.; Fleet, G. W. J.; Asano, N. *J. Nat. Prod.* **2002**, *65*, 1875.
9. Nash, R. J.; Fellows, L. E.; Dring, J. V.; Fleet, G. W. J.; Girdhar, A.; Ramsden, N. G.; Peach, J. M.; Hegarty, M. P.; Scofield, A. M. *Phytochemistry* **1990**, *29*, 111.
10. Nakajima, T.; Volcani, B. E. *Science* **1969**, *164*, 1400.
11. Fellows, L. E. *Chem. Ber*. **1987**, 842.

12. (a) Buku, A.; Faulstich, H.; Wieland, T.; Dabrowski, J. *Proc. Natl. Acad. Sci. USA* **1980**, *77*, 2370. (b) Karl, J.-U.; Wieland, T. *Liebigs Ann. Chem*. **1981**, 1445.
13. (a) Furukawa, J.; Okuda, S.; Saito, K.; Hatanaka, S.-I. *Phytochemistry* **1985**, *24,* 593. (b) Jones, D. W. C.; Nash, R. J.; Bell, E. A.; Williams, J. M. *Tetrahedron Lett.* **1985**, *26,* 3125.
14. Nash, R. J.; Bell, E. A.; Williams, J. M. *Phytochemistry* **1985,** *24*, 1620.
15. (a) Fleet, G. W. J.; Karpas, A.; Dwek, R. A.; Fellows, L. E.; Tyms, A. S.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Smith, P. W.; Son, J. C.; Wilson, F.; Witty, D. R.; Jacob, G. S.; Rademacher, T. W. *FEBS Lett.* **1988**, *237*, 128. (b) Meng, Q.; Hesse, M. *Helv. Chim. Acta* **1991**, *74*, 445.
16. (a) Volk, W. A.; Galanos, C.; Luderitz, O. *Eur. J. Biochem.* **1970**, *17*, 223. (b) Galanos, C.; Lűdertiz, O.; Rietschel, E. T.; Westphal, O. *In Biochemistry of Lipids II ;* Goodwin, T. W., Ed.; *Int. Rev. Biochem*. **1977**, *14*, 239.
17. (a) Fleet, G. W. J.; Nicholas, S. J.; Smith, P. W.; Evans, S. V.; Fellows, L. E.; Nash, R. J. *Tetrahedron Lett.* **1985**, *26*, 3127. (b) Fleet, G. W. J.; Smith, P. W. *Tetrahedron* **1986**, *42*, 5685.
18. (a) Asano, N.; Tomioka, E.; Kizu, H.; Matsui, K. *Carbohydr. Res*. **1994**, *253,* 235. (b) Asano, N.; Oseki, K.; Tomioka, E.; Kizu, H.; Matsui, K. *Carbohydr. Res.* **1994**, *259*, 243.
19. (a) Kumareswaran, R.; Hassner, A. *Tetrahedron: Asymmetry* **2001**, *12*, 3409. (b) Goli, D. M.; Cheesman, B. V.; Hassan, M. E.; Lodaya, R.; Slama, J. T. *Carbohydr. Res.* **1994**, *259*, 219. (c) Mizushina, Y.; Xu, X.; Asano, N.; Kasai, N.; Kato, A.; Takemura, M.; Asahara, H.; Linn, S.; Sugawara, F.; Yoshida, H.; Sakaguchi, K. *Biochem. Biophys. Res. Commun.* **2003**, *304*, 78. (d) Yasuda, K.; Kizu, H.; Yamashita, T.; Kameda, Y.; Kato, A.; Nash, R. J.; Fleet, G. W. J.; Molyneux, R. J.; Asano, N. *J. Nat. Prod.* **2002**, *65*, 198.
20. (a) Buchanan, J. G.; Lumbard, K. W.; Sturgeon, R. J.; Thompson, D. K.; Wightman, R. H. *J. Chem. Soc., Perkin Trans. 1* **1990**, 699. (b) Huang, Y.; Dalton, D. R. *J. Org. Chem*. **1997**, *62,* 372. (c) Kim, S.; Lee, W. S.; Park, K. H. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2877. (d) Lee, B. W.; Jeong, I.; Yang, M. S.; Choi, S. U.; Park, K. H. *Synthesis* **2000**, 1305.
21. Pyne, S. G.; Tang, M. *Curr. Org. Chem.* **2005,** *9,* 1393.
22. Izquierdo, I.; Plaza, M. T.; Franco, F. *Tetrahedron: Asymmetry* **2002**, *13*, 1503.

23. Isidoro Izquierdo Cubero, I. I.; Lo´pez-Espinosa, M. T. P.; Dı´az, R. R.; Montalba´n, F. F. *Carbohydr. Res.* **2001**, *330*, 401.
24. Izquierdo, I.; Plaza, M. T.; Franco, F. *Tetrahedron: Asymmetry* **2004**, *15*, 1465.
25. Yoda, H.; Asai, F.; Takabe, K. *Synlett* **2000**, 1001.
26. Rambaud, L.; Compain, P.; Martin, O. R. *Tetrahedron: Asymmetry* **2001**, *12*, 1807.
27. Toyao, A.; Tamura, O.; Takagi, H.; Ishibashi, H. *Synlett* **2003**, 35.
28. Cardona, F.; Faggi, E.; Liguori, F.; Cacciarini, M.; Goti, A. *Tetrahedron Lett.* **2003**, *44*, 2315.
29. (a) Watson, A. A.; Fleet, G. W. J.; Asano, N.; Molyneux, R. J.; Nash, R. J. *Phytochemistry* **2001**, *56*, 265. (b) El-Ashry, E.-S. H.; El Nemr, A. *Carbohydr. Res.* **2003**, *338,* 2265. (c) Ayad, T.; Genisson, Y.; Baltas, M. *Curr. Org. Chem.* **2004**, *8*, 1211.
30. Kim, I. S.; Zee, O. P.; Jung, Y. H. *Org. Lett*. **2006**, *8,* 4101.
31. Ayad, T.; Génisson, Y.; Broussy, S.; Baltas, M.; Gorrichon, L. *Eur. J. Org. Chem.* **2003**, 2903.
32. Zhou, X.; Liu, W-J.; Ye, J-L.: Huang, P-Q. *Tetrahedron* **2007**, *63*, 6346.
33. Murruzzu, C.; Riera, A. *Tetrahedron: Asymmetry* **2007**, *18*, 149.
34. Wang, C-C.; Luo, S-Y.; Shie, C-R.; Hung, S-C. *Org. Lett*. **2002**, *4*, 847.

Section-B:: Result and Discussion

3.1-Retroanalysis

A critical survey of current literature elaborated in Section A indicates the lack of diversified and essentially multi-targeted synthetic protocols towards pyrrolidine and pyrrolizidine class of azasugars till date. An approach which leads to the parallel synthesis of both these classes of alkaloids would be of great synthetic importance. In this context, we explored the utility of our protocol to synthesize these azasugars which vary not only in structure but also in their classes. Accordingly, we have devised a strategy which consists of an inexpensive chiral starting material (D-Glucose), elucidated by an appropriate retrosynthetic analysis as shown in figure 1.

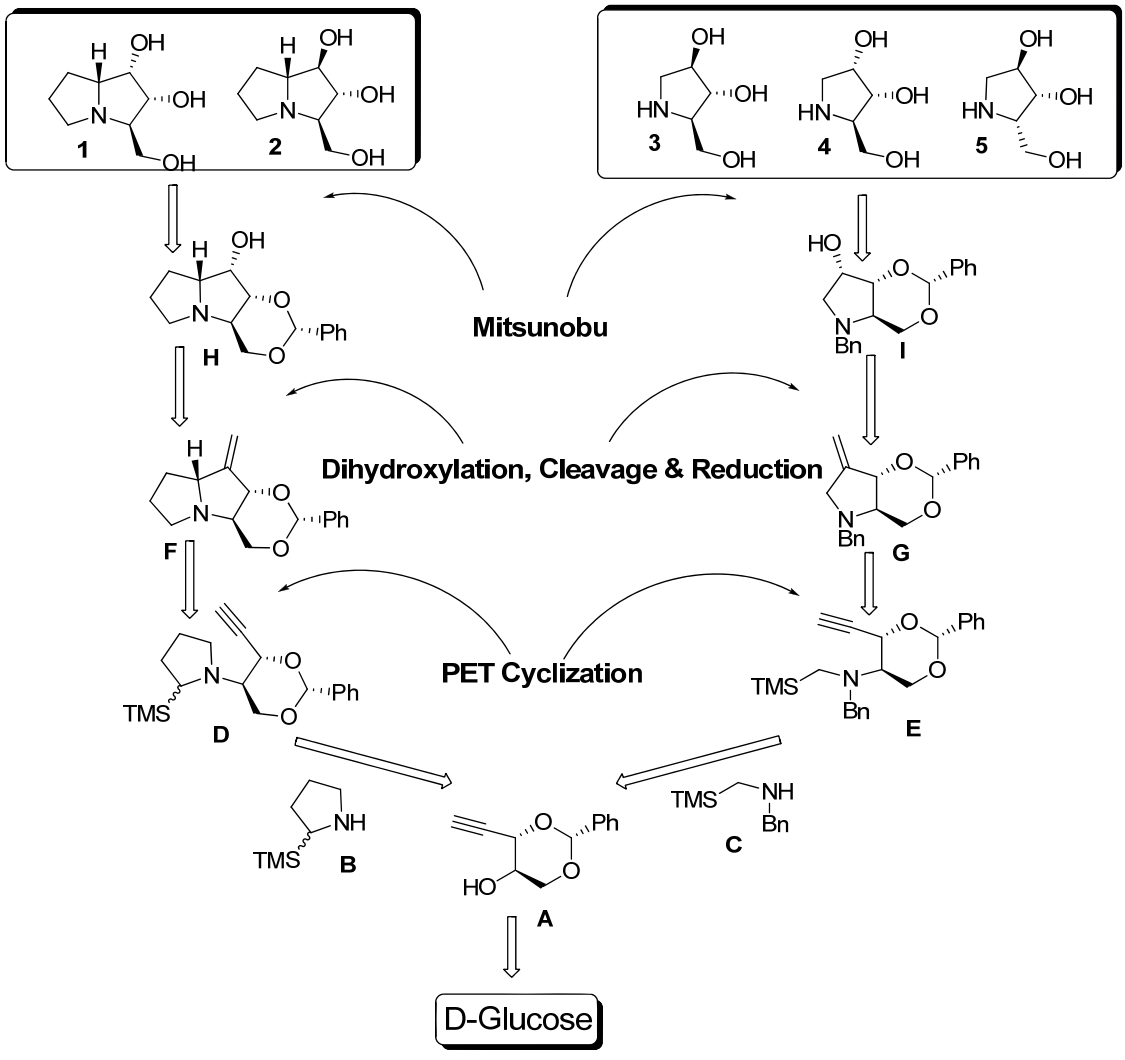
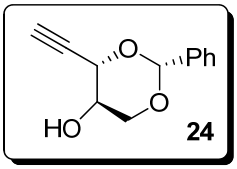


Figure 1.

A precursor of type **A** with free hydroxyl group was required for the synthesis of azasugars hyacinthacines A_1 (**1**) A_2 (**2**) **3**, **4** and **5.** The hydroxyl group could be used for the installation of amine functionality. It can be visualized that just by varying the amine functionalities i.e. **B** and **C**, we could access both the classes of azasugars. If **A** is coupled with the pyrrolidine trimethylsilane **B**, we could get precursor **D**. However, if benzyl aminomethyl trimethylsilane **C** is used for coupling with **A**, **E** can be obtained**.** PET cyclization reaction of **D** and **E**, followed by the manipulation of double bond of the corresponding cyclized product **F** and **G** would give advanced intermediates **H** and **I** from where removal of protecting groups would give rise to azasugars **1** and **4**. The opportunity to perform Mitsunobu reaction at various stages of the scheme could also enable a convenient control over the stereochemistry. For example, inversion of free hydroxyl group of **H** and **I** could give azasugars **2** and **3.** Inverted stereochemistry of hydroxymethylene group of **5** can be achieved if Mitsunobu reaction is done in the early stage of the scheme *i.e.* at **A**. Our efforts in this area are described below.

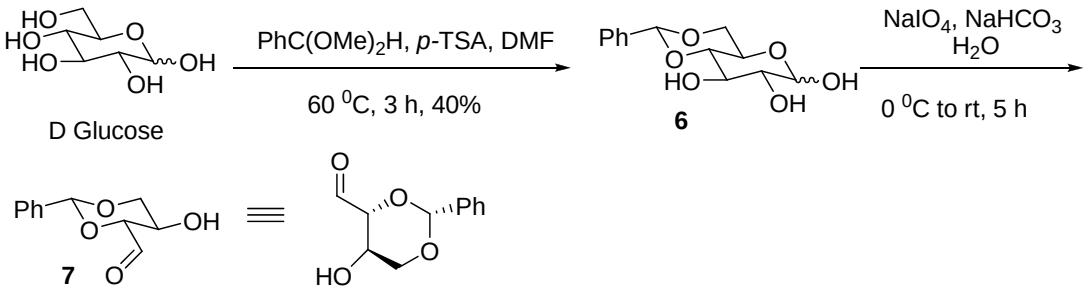
3.2-Synthesis of key precursor (2R,4S,5R)-4-ethynyl-2-phenyl-1,3-dioxan-5-ol (24)



24

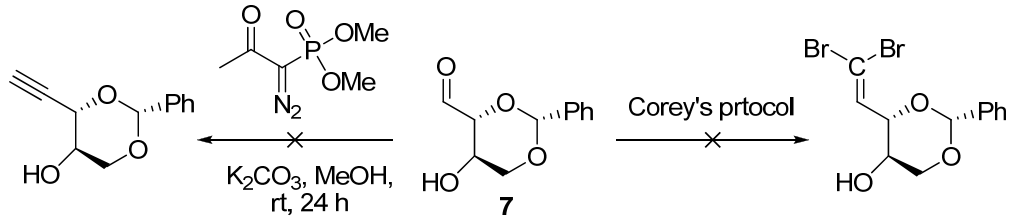
At the outset, we visualized **A** as the key precursor for our synthetic plan in mind and towards fulfilling our goal, we started the synthesis with D-glucose (Scheme 1). Since this polyhydroxy compound had to be selectively protected for the successful manipulation of other hydroxyl groups, we resorted to benzylidene protection. The cyclic 1, 3-dioxan moiety was not only expected to give better diastereoselectivity during the advanced PET reaction of the proposed step, but was also expected to control the stereoselectivity during the installation of the third hydroxyl functionality.

The benzylidene protection was achieved by stirring the suspension of D-glucose, benzaldehyde dimethyl acetal, catalytic amount of *p*-toluenesulfonic acid in DMF at 60 ^{0}C while distilling out MeOH continuously from the reaction mixture[1] (Scheme 1). The amount of *p*-TSA was critical, as the excess use of *p*-TSA gave other hydroxyl groups also protected, thus, lowering the yield.



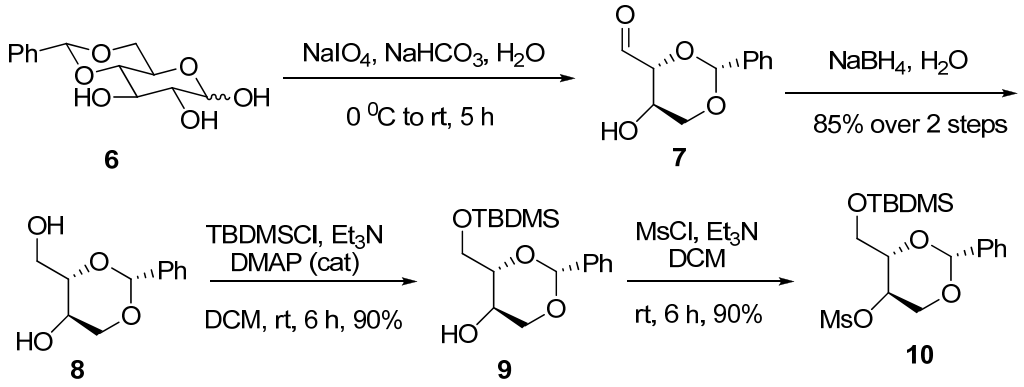
Scheme 1.

The benzylidene protected glucose **6** was subjected to sodium periodate cleavage of the 1,2-dihydroxy moiety to obtain aldehyde **7**. As the aldehyde was unstable, it was directly used as such for the next step. The aldehydic moiety of compound **7** was initially subjected to transformation to an acetylenic functionality using Corey's[2] protocol, which however did not succeed (Scheme 2).



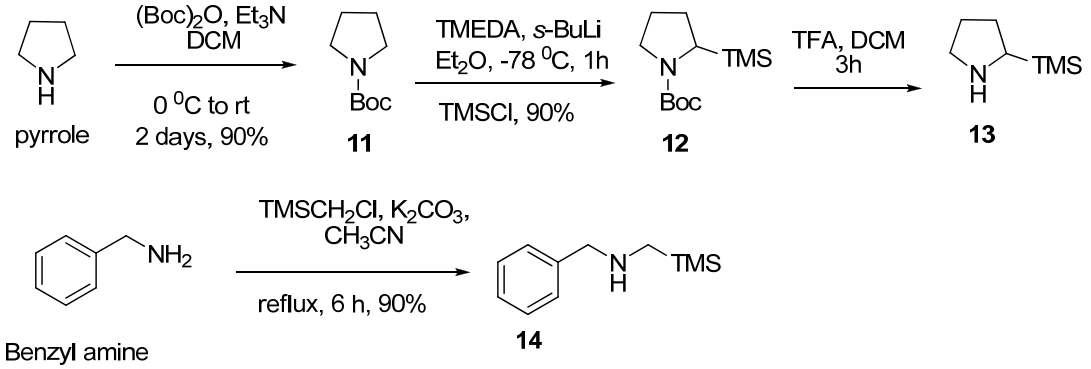
Scheme 2.

As an alternative option, we also attempted this reaction utilizing the Ohira Bestmann reagent[3]. However, unfortunately, this also failed to give the expected acetylenic product. For reasons not known at that time, though, we came to know later which would be described latter in the Results & Discussions Section, we thought of going ahead by reducing the aldehyde followed by selectively handling the primary and secondary hydroxyl groups. Concomitantly, the aldehyde **7** was reduced without purification by sodium borohydride (Scheme 3) to obtain corresponding diol[4] **8** in 85% yield over two steps. Selective silyl protection of primary hydroxyl group of **8**, achieved by stirring a solution of **8** in DCM with TBDMSCl, triethyl amine and catalytic DMAP, produced **9** in 90% yield. The free secondary hydroxyl group of **9** was converted to a good leaving group, mesylate by stirring a solution of **9** with mesyl chloride and triethyl amine in a solution of DCM at room temperature for 6 h which gave **10** in 90% yield.



Scheme 3.

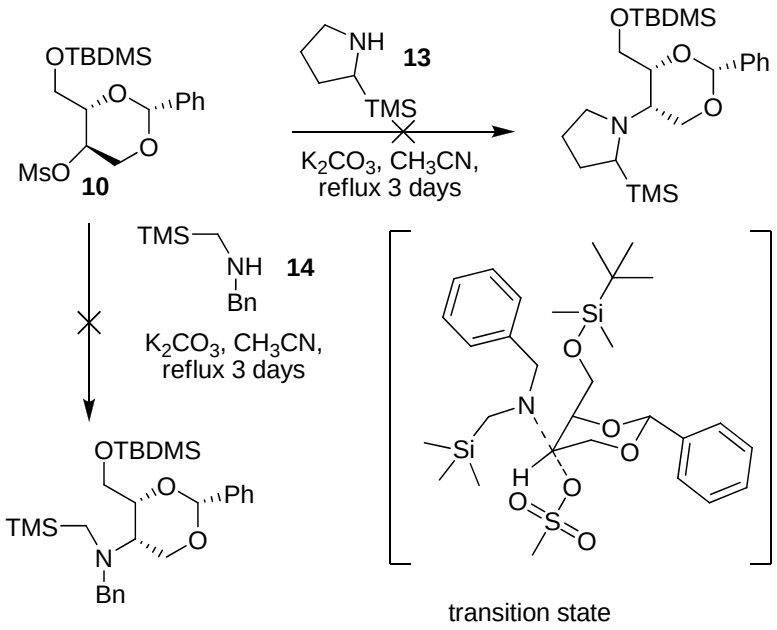
The required coupling amine precursors **13** and **14** were obtained as shown is in Scheme 4. For synthesizing **13**[5], pyrrole was protected as *N*-Boc derivative by stirring a solution of pyrrolidine with $(Boc)_2O$ in the presence of Et_3N in DCM, which gave **11** in 90 % yield. Next *N*-Boc protected pyrrole **11**, was subjected to lithiation at C_2 carbon using *s*-BuLi / TMEDA in Et_2O at -78 ^{0}C which upon subsequent quenching with TMSCl gave the corresponding silylated product **12** in 90 % yield. Finally, *N*-Boc deprotection, carried out by TFA in DCM produced **13,** which was used as such after work up. The other amine precursor **14** was prepared in the usual way as described earlier in chapter 2.



Scheme 4.

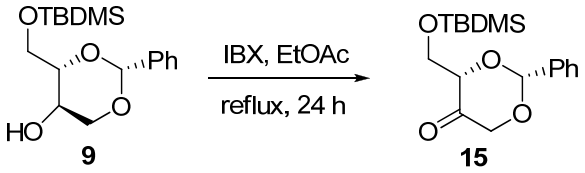
Mesylate **10** was subjected to coupling with **13** and **14** by refluxing in acetonitrile in the presence of anhydrous K_2CO_3. However, neither of the reactions was successful to give the required product (Scheme 5). Even addition of TBAI to the reaction mixture for the *in situ* generation of corresponding iodo compound from mesylate **10** and continued refluxing for 3-4 days was also not found to be effective. A possible explanation for the

failure of this reaction could be steric crowding of the mesylate moiety at the secondary carbon C_5 on a ring, which itself was substituted with bulky TBDMS and phenyl groups. Moreover, the incoming nucleophile **13** and **14** could be considered to be bulky due to the presence of trimethyl silane functionality. These arguments could be explicitly visualized from a model of the possible transition state as shown in scheme 5.



Scheme 5.

Owing to such a sluggish nature of the reaction, an alternative pathway involving reductive amination was visualized for the installation of amino group.

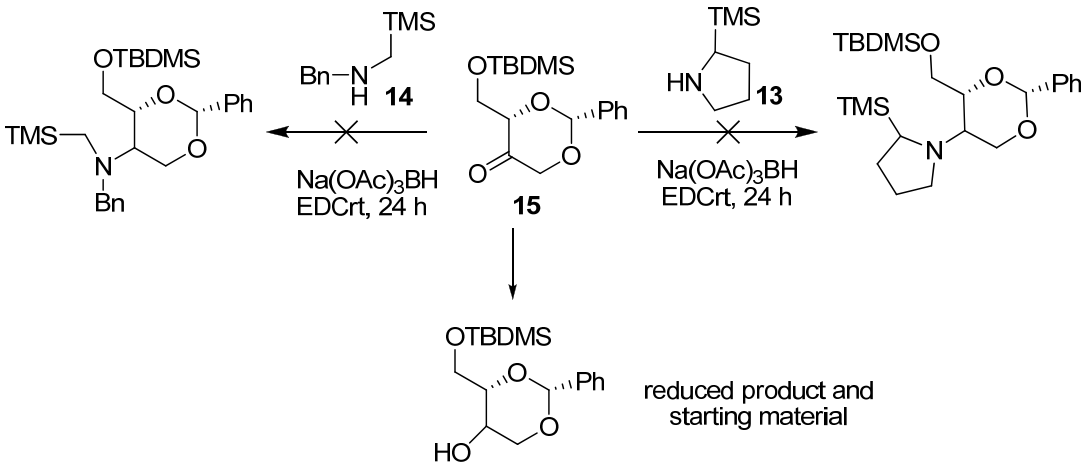


Scheme 6.

Towards this end, alcohol **9** was subjected to IBX oxidation to obtain the corresponding ketone **15** (Scheme 6) which was used as such for the next step without any purification. However when ketone **15** was subjected to reductive amination with either of the amines **13** or **14**, using sodium triacetoxyborohydride (Scheme 7) as reducing agent, none of them resulted in the expected aminated product. However, work up and analysis of the

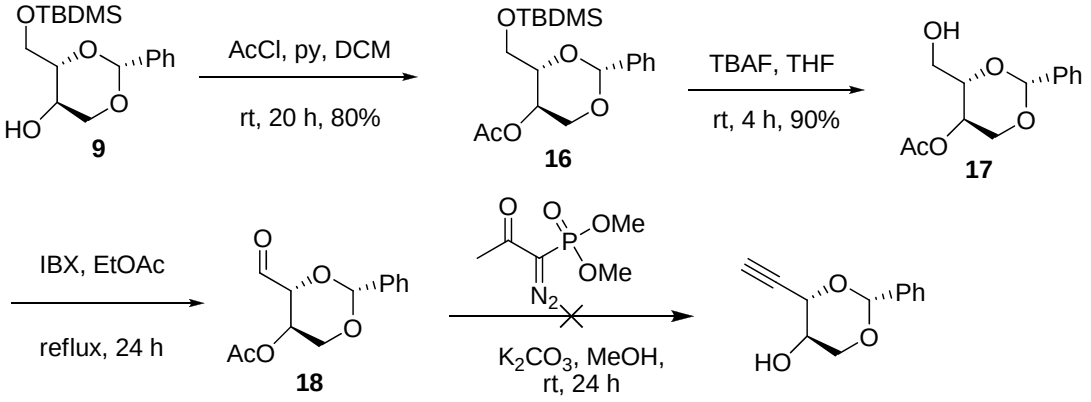
reaction mixture revealed the formation of reduced product, **9** (as revealed by GC analysis) in this reaction.

The failures of the above two sets of reactions *i.e.*, both the alkylative coupling and reductive amination reveal that the reaction center at C_5 was too hindered. To reduce the steric crowding, we decided to remove the bulky TBDMS functionality and install triple bond at first before attempting the installation of amine functionality.



Scheme 7.

Accordingly, alcohol **9** was protected with acetyl grouping to obtain corresponding acetate **16** in 80% yield (Scheme 8).



Scheme 8.

O-Acetyl protection was chosen particularly so that it could be knocked off easily in basic conditions during acetylene bond formation from Ohira Bestmann reagent, thus, saving an extra step of deprotection. In this context, deprotection of TBDMS from **16** by TBAF gave the expected desilylated product **17** in 90% yield which was further oxidized by IBX

to obtain the corresponding aldehyde **18.** The Ohira Bestmann reaction of **18** was, however, not successful and resulted in a complex reaction mixture.

The failure of this reaction led us to investigate the authenticity of the starting material **17**. In the ^{1}H NMR spectrum of **16** (*i.e.*, before TBDMS deprotection) the proton on C_5 (Figure 2) appeared distinctively downfield at δ 4.9-5.17 Hz. Similar deshielding was expected for the same proton in the case of **17**. However, the ^{1}H NMR spectrum of **17** revealed a change and did not show any signal in the expected range. The C_5 proton appeared instead, up field and got merged with other ring protons. These observations revealed that during deprotection there was migration of the acetate moiety from secondary hydroxyl to primary hydroxyl. Therefore, deprotection actually led to the generation of **19** instead of **17**. The oxidation by IBX, thus, resulted in the formation of the corresponding ketone **20** instead of the aldehyde **18**, which eventually gave a complex reaction mixture on reacting with Ohira Bestmann reagent (Figure 2).

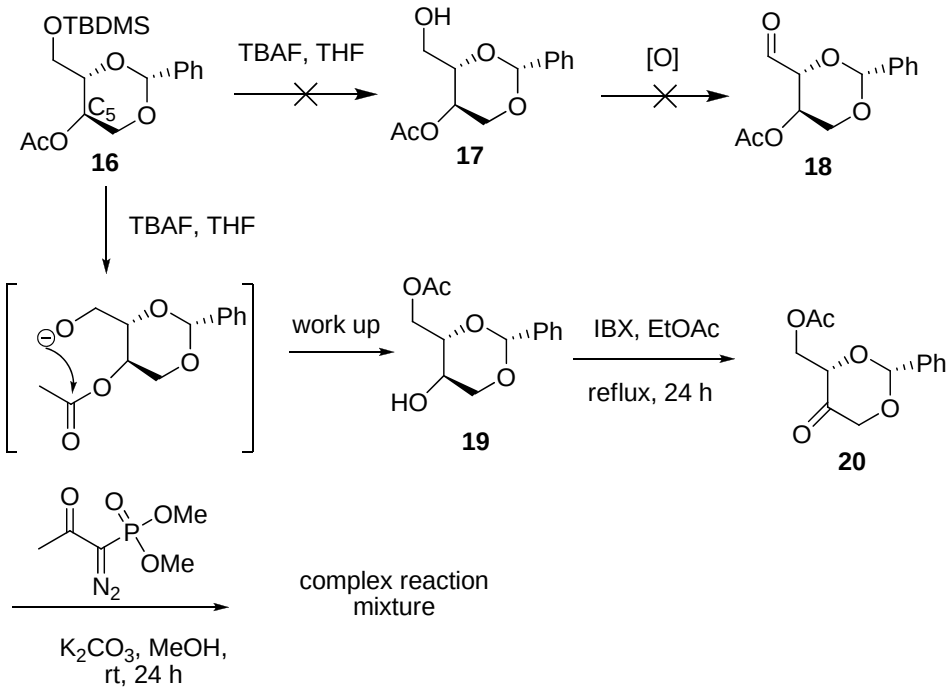
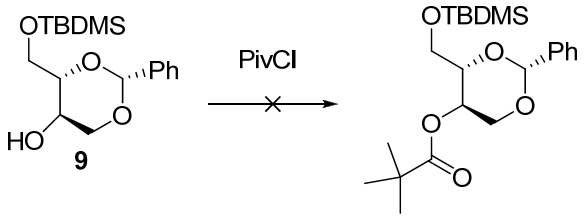


Figure 2.

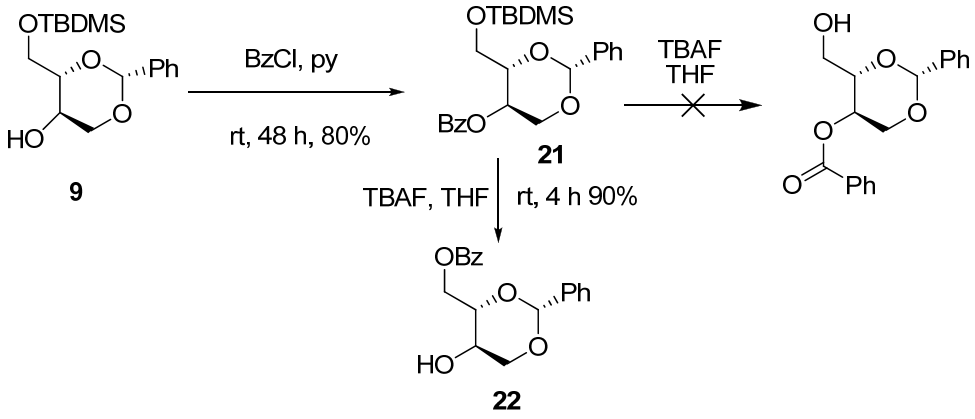
To overcome this difficulty, we expected that the migration could be precluded by using a protecting group bulkier than the acetyl group, for example, pivolate group. However, when we tried reacting the alcohol **9** (Scheme 9) with pivolyl chloride, the reaction was

not successful. The pivolyl chloride proved to be too bulky to react at C_5 position. We tried different conditions but all of them resulted in starting material only. Hence, we adopted our strategy with yet another reagent *viz*., benzoyl chloride.



Scheme 9.

The reaction of **9** with benzoyl chloride was smooth (though it also required 48 h and pyridine as a solvent for completion), giving rise to the benzoate **21** in 80% yield (Scheme 10). However, when the deprotection of TBDMS was attempted, the problem of migration of benzoyl group continued. Immediately after the TBAF deprotection, there was a migration of benzoyl from secondary to primary hydroxyl.



Scheme 10.

Because of all these challenges, both the reacting centers of the molecule proved difficult to be handled. In the mean time, we came across a literature report[6] which indicated (Figure 3) that the aldehyde **7** formed after periodate cleavage of **6** did not exist as such, but existed in the form of a dimmer. The dimmer actually was a lactol **23** which did not allow the carbonyl to exist freely. We attributed finally to the existence of the dimeric **23**, for the failure of reactions during the installation of the triple bond on **7** (Scheme 2).

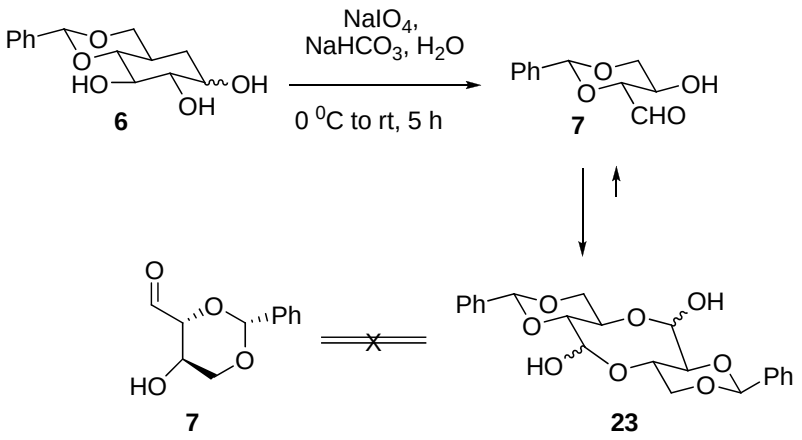
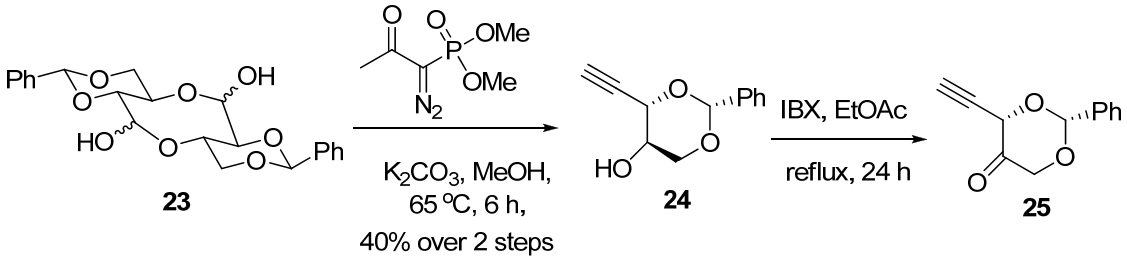


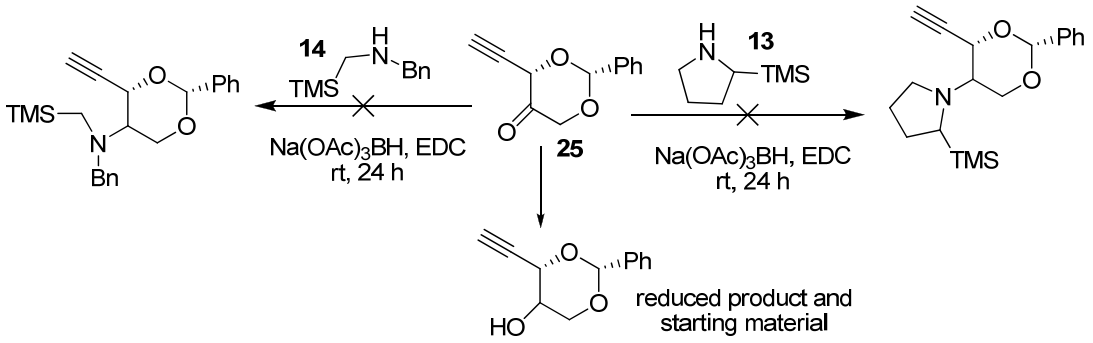
Figure 3.

Realizing this fact, we immediately attempted the usual protocol of making the triple bond from lactol. Subjecting **23** to the Ohira Bestmann reagent at higher temperature (refluxing methanol) and gradual addition of reagent over a period of 6 h, gave required product **24** in 40% yield (Scheme 11). With the acetylenic precursor in hand, we tried installing the amine functionality which would give the required precursor for photochemical reaction. For this purpose, we oxidized alcohol **24** to the corresponding ketone by IBX for trying reductive coupling with amine precursors **13** and **14**.



Scheme 11.

When ketone **25** was subjected to reductive coupling mediated by sodium triacetoxy borohydride with the amine precursors **13** and **14**, the reaction failed again and did not give any of the required products. As usual, we obtained only the starting material and the reduced alcohol as product (Scheme 12).



Scheme 12.

With so many failures, we realized that the C_5 center was too sterically hindered to undergo any kind of reaction and the steric strain should be reduced in order to proceed further. The only possible way was to open the ring and make the structure acyclic and then try for the installation of amine functionality. To serve this purpose, we designed a different synthetic route as shown in Figure 4.

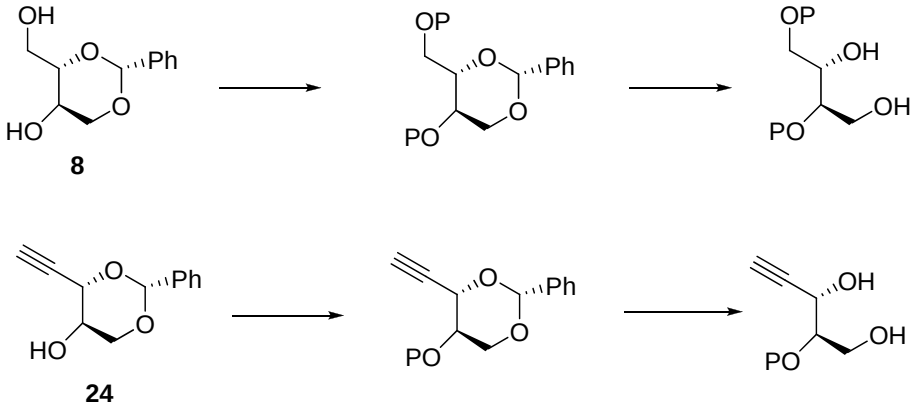
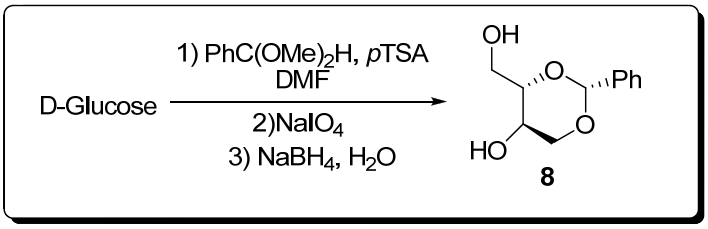


Figure 4.

The scheme can be carried forward either from **8** or **24.** The present work is now being continued in the lab.

3.3-Experimental

(4S,5R)-4-(hydroxymethyl)-2-phenyl-1,3-dioxan-5-ol (8)[1,4]

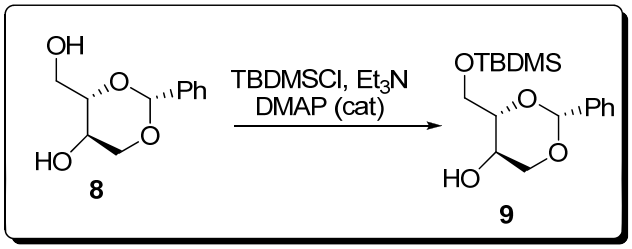


A mixture of D-Glucose (10.0 g, 55.5 mmol), benzaldehyde dimethyl acetal (9.3 g, 61 mmol) and catalytic amount of *p*-toluenesulfonic acid (12 mg) in 40 mL dry DMF was heated at 60 ^{0}C under vacuum to remove methanol formed during the reaction as a bye product. The heating was stopped after 3 h and 0.3 mL of triethyl amine was added to the reaction mixture at 0 ^{0}C, and the solvent was removed under high vacuum. The crude mixture was purified by column chromatography eluting with EtOAc giving rise to white solid **6** (6 g) in 40% yield. To a solution of 4,6-*O*-benzylidene-D-glucose[1] **6** (10.0 g, 37.4 mmol) in 70 mL of water at 0 ^{0}C was added a solution of $NaIO_4$ (16.1 g, 75.4 mmol) and $NaHCO_3$ (3.17 g, 37.7 mmol) in 130 mL of water. The pH was adjusted to 6-7 by adding few drops of saturated $NaHCO_3$ solution. The mixture was stirred at room temperature for 5 h. Latter a solution of $NaBH_4$ (2.00 g, 52.9 mmol) in 20 mL of water was added drop wise at 0 ^{0}C. The reaction was stirred at rt for 30 min, and neutralized with acetic acid. The precipitate formed was filtered and washed with ethyl acetate. The filtrate was extracted with ethyl acetate (3 X 100 mL), the organic phases were washed with 1 N $Na_2S_2O_3$ (75 mL), with brine (75 mL) and dried over $MgSO_4$. The concentration of the organic layer under vacuum and purification by column chromatography (cyclohexane/AcOEt: 3/7) produced **8**[1a,4] as a white solid (6.65 g, 85% yield over two steps) (mp 136-138 °C).

$[\alpha]^{22}_D$	-41 (*c* 1.0, MeOH). Lit.[4b] $[\alpha]^{23}_D$ -43 (*c* 2.0, MeOH).
^{1}H NMR (200 MHz, CD_3OD), δ	3.54-3.80 (m, 4H), 3.93 (d, 1H, *J* = 11.62 Hz), 4.15-4.23 (m, 1H), 5.54 (s, 1H), 7.32-7.35 (m, 3H), 7.49-7.53 (m, 2H).

^{13}C NMR (50 MHz, CD_3OD), δ	62.6 (CH), 62.8 (CH_2), 72.3 (CH_2), 84.2 (CH), 102.4 (CH), 127.6 (CH), 129.1 (CH), 129.9 (CH), 139.5 (C).
Mass: *m/z* (%)	233 (M^++Na, 100%).
Elemental analysis	Anal. Calcd for $C_{11}H_{14}O_4$: C, 62.85; H, 6.71. Found: C, 62.45; H, 6.53.
mp	136-138 °C, Lit.[4b] mp 135-136 °C.

(4*S*,5*R*)-4-((tert-butyldimethylsilyloxy)methyl)-2-phenyl-1,3-dioxan-5-ol (9)

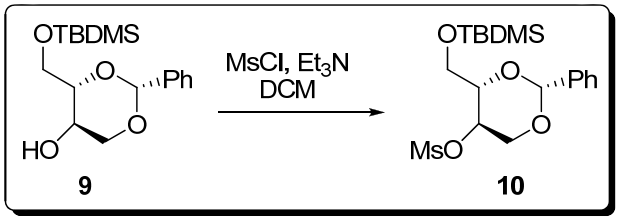


To a stirring suspension of **8** (10.0 g, 47.6 mmol) in DCM (120 mL) at 0 ^{0}C was added triethyl amine (7.3 mL, 52.3 mmol), TBDMSCl (7.16 g, 47.6 mmol) and catalytic DMAP (0.23 g). The reaction mixture gradually turned to a clear solution and was stirred at rt for additional 6 h. Water was added and reaction mixture and extracted with DCM. The organic phase was dried over Na_2SO_4 concentrated and chromatographed (pet ether/ethyl acetate 4:1) to obtain **9** (13.9 g, 90%).

$[\alpha]^{25}_D$	+3.2 (*c* .75 $CHCl_3$).
IR (neat) υ_{max} cm^{-1}	3450, 2900, 2825.
^{1}H NMR (200 MHz, $CDCl_3$), δ	0.11 (s, 6H), 0.91 (s, 9H), 1.62 (bs, 1H), 3.58-3.96 (m, 4H), 4.04 (dd, 1H, *J* = 9.54, 4.49 Hz), 4.32 (dd, 1H, *J* = 10.68, 5.11 Hz), 5.49 (s, 1H), 7.32-7.48 (m, 5H).
^{13}C NMR (50 MHz, $CDCl_3$), δ	-5.6 (CH_3), 18.1 (C), 25.7 (CH_3), 65.9 (CH_2), 66.0 (CH), 70.5 (CH_2), 79.2 (CH), 101.0 (CH), 126.1 (CH), 128.2 (CH), 128.9 (CH), 137.5 (C).
Mass: *m/z* (%)	363 (M^++K, 83%), 347 (M^++Na, 67%), 325 (M^++H, 15%), 259 (100%).
Elemental analysis	Anal. Calcd for $C_{17}H_{28}O_4Si$: C, 62.92; H, 8.70. Found:

	C, 62.42; H, 8.40.

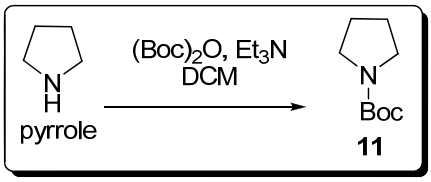
(4*S*,5*R*)-4-(*tert*-butyldimethylsilyloxy)methyl)-2-phenyl-1,3-dioxan-5-yl methanesulfonate (10)



To a stirred solution of **9** (1 g, 3.1 mmol) in DCM (10 mL) was added triethylamine (0.47 mL, 3.3 mmol), mesyl chloride (0.26 mL, 3.3 mmol), at 0 ^{0}C. The reaction mixture was stirred at rt for 8 h. Water was added to the reaction mixture and extracted with DCM. The organic phase was dried over Na_2SO_4, concentrated and chromatographed (pet ether/ethyl acetate 4:1) to get **10** (1.12 g) in 90% yield.

$[\alpha]^{26}_D$	-44.2 (*c* 1.2, $CHCl_3$).
IR (neat) υ_{max} cm^{-1}	2954, 2931, 2853, 1363.
^{1}H NMR (200 MHz, $CDCl_3$), δ	0.05 (s, 3H), 0.08 (s, 3H), 0.89 (s, 9H), 3.12 (s, 3H), 3.72-3.88 (m, 2H), 3.93 (s, 1H), 3.94 (s, 1H), 4.56 (dd, 1H, *J* = 10.61, 5.43 Hz), 4.83 (dt, 1H, *J* = 10.1, 5.43 Hz), 5.5 (s, 1H), 7.34-7.38 (m, 3H), 7.43-7.48 (m, 2H).
^{13}C NMR (50 MHz, $CDCl_3$), δ	-5.5 (CH_3), -5.1 (CH_3), 18.2 (C), 25.7 (CH_3), 37.3 (CH_3), 61.5, (CH_2), 68.4 (CH), 68.6 (CH_2), 78.9 (CH), 101.3 (CH), 126.1 (CH), 128.1 (CH), 129.0 (CH), 136.9 (C).
Mass: *m/z* (%)	403 (M^++H, 10%) 304 (100%).
Elemental analysis	Anal. Calcd for $C_{18}H_{30}O_6SSi$; C, 53.70; H, 7.51. Found: C, 53.60; H, 7.91.

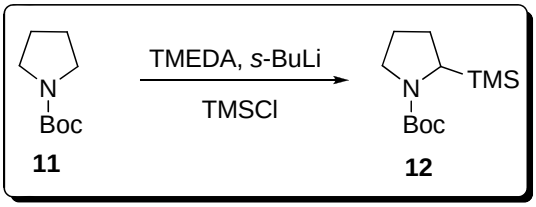
tert-butyl pyrrolidine-1-carboxylate (11)



To a stirring solution of pyrrolidine (6.56 g, 92.3 mmol) and Et_3N (11.6 g, 115.3 mmol), in DCM (150 mL) at 0 ^{0}C was added $(Boc)_2O$ (22.1 g, 101.4 mmol) in DCM (50 mL) and the reaction was stirred at rt for 2 days. Latter water was added and the mixture was extracted with DCM. Organic layer was dried, concentrated and resultant brown oil obtained was purified by vacuum distillation (55-60 °C/1 mm) to give **11** (14.2 g, 90 %) as colorless oil.

IR (neat) υ_{max} cm^{-1}	1700, 1400, 1160, 1110.
^{1}H NMR (200 MHz, $CDCl_3$), δ	1.45 (s, 9H), 1.80-1.95 (m, 4H), 3.37 (t, 4H, *J* = 7.3 Hz).
Mass: *m/z* (%)	171 (M^+, 11), 114 (100), 57 (82).

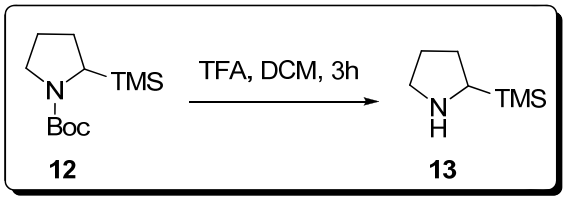
tert-butyl 2-(trimethylsilyl)pyrrolidine-1-carboxylate (12)



To a solution of *N*-Boc derivative of cyclic amine **11** (3.44 g, 20.1 mmol) in 40 mL of dry ether at –78 °C was added TMEDA (2.79 g, 24.12 mmol) followed by *s*-BuLi (1.5 M solution in cyclohexane, 16.1 mL, 24.12 mmol) dropwise over a period of 15 min. The mixture was further allowed to stir for 2 h at –78 °C. TMSCl (2.61 g, 24.12 mmol) was added dropwise into the flask. The reaction mixture was allowed to warm to room temperature and diluted with 15 mL of saturated aqueous NH_4Cl solution. The organic layer was concentrated and the crude oily residue was purified by fractional distillation (bp 55-60 °C/0.5 mm) to give **12** (4.39 g, 90 %) as a colorless oil.

IR (neat) υ_{max} cm^{-1}	1692, 1478, 1365, 1246, 1170.
^{1}H NMR (200 MHz, $CDCl_3$), δ	0.05 (s, 9H), 1.45 (s, 9H), 1.75-1.95 (m, 3H), 1.95-2.05 (m, 1H), 3.15-3.30 (m, 2H), 3.35-3.60 (m, 1H).
^{13}C NMR (50 MHz, $CDCl_3$), δ	-2.3, 27.8, 28. 4, 46.7, 47.5, 78.0, 154.5.
Mass: *m/z* (%)	243 (M^+, 1), 186 (43), 172 (100), 142 (94).

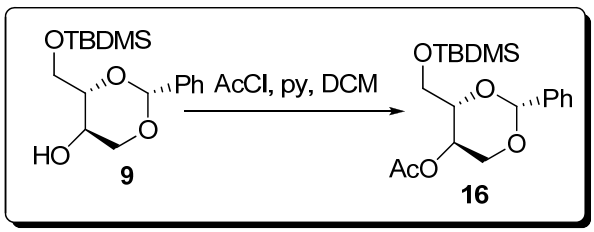
2-(trimethylsilyl)pyrrolidine (13)



To a stirring solution of **12** (3.39 g, 13.95 mmol) in 20 mL of dry DCM at 0 °C, TFA (5.7 g, 50.0 mmol) was added drop-wise over a period of 30 min. The mixture was allowed to warm to room temperature and allowed to stir further for 4 h. The reaction mixture was re-cooled to 0 °C and was basified with 20 % aqueous NaOH solution. The organic layer was separated and the aqueous layer was extracted with DCM (2 x 30 mL). The combined extracts were washed with brine, dried over Na_2SO_4 and concentrated to give crude amine **13** which was utilized as such without further purification for the next step.

^{1}H NMR (200 MHz, $CDCl_3$), δ	0.00 (s, 9H), 1.48-2.07 (m, 4H), 2.50 (dd, 1H, J = 6.9, 12.1 Hz), 2.96-3.23 (m, 2H).
^{13}C NMR (50 MHz, $CDCl_3$), δ	-3.8 (CH_3), 24.9 (CH_3), 27.2 (CH_2), 46.6 (CH_2), 49.1 (CH).
Mass: *m/z* (%)	143 (M^+).

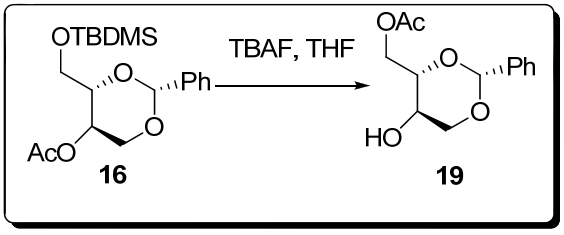
(4*S*,5*R*)-4-(*tert*-Butyldimethylsilyloxy)methyl)-2-phenyl-1,3-dioxan-5-yl acetate (16)



To a stirred solution of **9** (1.0 g, 3.1 mmol) in DCM (10 mL) was added acetyl chloride (0.24 mL, 3.3 mmol), pyridine (0.14 mL, 4.6 mmol) at 0 ^{0}C. The reaction mixture was stirred at rt for 20 h. Water was added to the reaction mixture and extracted with DCM. The organic phase was dried over Na_2SO_4, concentrated and chromatographed (pet ether/ethyl acetate 9:1) to get **16** in 80% yield.

^{1}H NMR (200 MHz, $CDCl_3$), δ	0.03 (s, 3H), 0.05 (s, 3H), 0.89 (s, 9H), 2.06 (s, 3H), 3.62 (t, 1H, *J* = 10.36 Hz), 3.76-4.28 (m, 3H), 4.40 (dd, 1H, *J* = 10.49, 5.43 Hz), 4.90-5.17 (m, 1H), 5.51 (s, 1H), 7.31-7.51 (m, 5H).
^{13}C NMR (50 MHz, $CDCl_3$), δ	-5.4 (CH_3), 18.3 (C), 20.8 (CH_3), 25.8 (CH_3), 63.0 (CH_2), 63.4 (CH), 67.8 (CH_2), 80.1 (CH), 101.2 (CH), 126.2 (CH), 128.1 (CH), 128.9 (CH), 137.4 (C), 169.7 (C).
Mass: *m/z* (%)	389 (M^++Na, 52%), 384 (M^++NH_4, 12%), 366 (M^+, 14%), 301 (100%).

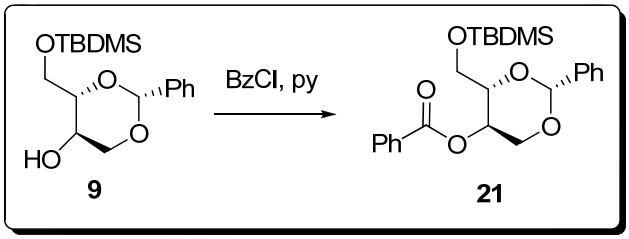
(4*S*,5*R*)-5-Hydroxy-2-phenyl-1,3-dioxan-4-yl)methyl acetate (19)



To a stirred solution of **16** (0.1 g, 0.27 mmol) in THF (1 mL) was added 1M solution of TBAF (0.3 mL, 0.3 mmoL) (in THF) at 0 ^{0}C. The reaction mixture was stirred at rt for 4 h. Solvent was removed from the reaction mixture at reduced pressure. Water was added to the reaction mixture and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4, concentrated and chromatographed (pet ether/ethyl acetate 3:2) to get **19** (0.61 g) in 90 % yield.

IR υ_{max} cm^{-1} in $CHCl_3$	3500, 1720, 1225.
^1H NMR (200 MHz, $CDCl_3$), δ	2.13 (s, 3H), 2.88 (d, 1H *J* = 3.66 Hz), 3.57-3.80 (m, 3H), 4.26-4.33 (m, 2H), 4.62 (dd, 1H, *J* = 12.38, 3.79 Hz), 5.49 (s, 1H), 7.32-7.39 (m, 3H), 7.44-7.51 (m, 2H).
^{13}C NMR (50 MHz, $CDCl_3$), δ	20.8 (CH_3), 61.4 (CH), 63.6 (CH_2), 70.6 (CH_2), 79.9 (CH), 101.0 (CH), 126.0 (CH), 128.2 (CH), 129.0 (CH), 137.1 (C), 172.1 (C).
Mass: *m/z* (%)	291 (M^++K, 17%), 275 (M^++Na, 100%).

(4*S*,5*R*)-4-(*tert*-Butyldimethylsilyloxy)methyl)-2-phenyl-1,3-dioxan-5-yl benzoate (21)

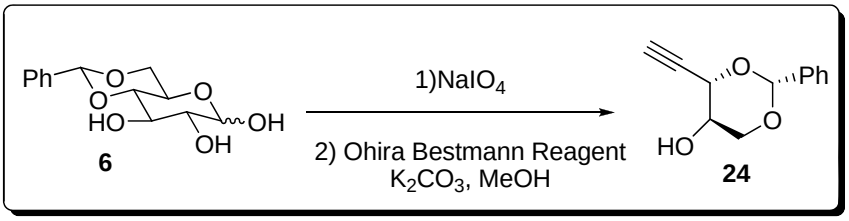


To a stirring solution of **9** (0.1 g, 0.3 mmol) in pyridine (1 mL) was added bezoyl chloride (0.04 mL, 0.33 mmol) at 0 ^{0}C. The reaction mixture was allowed to stir at rt for 48 h. Water was added to the reaction mixture and extracted with ethyl acetae. The organic phase was dried over Na_2SO_4, concentrated and chromatographed (pet ether/ethyl acetate 9:1) to get **21** in 80% yield.

$[\alpha]^{25}_D$	-53.7 (*c* 1.0, $CHCl_3$).
^1H NMR (200 MHz, $CDCl_3$), δ	-0.02 (s, 3H), 0.00 (s, 3H), 0.85 (s, 9H), 3.76 (t, 1H, *J* = 10.36 Hz), 3.88-3.90 (m, 2H), 3.99-4.08 (m, 1H), 4.55 (dd, 1H, *J* = 10.61, 5.43 Hz), 5.21 (dt, 1H, *J* = 9.85, 5.43 Hz), 5.59 (s, 1H), 7.35-7.64 (m, 8H), 8.01-8.05 (m, 2H).
^{13}C NMR (50 MHz, $CDCl_3$), δ	-5.4 (CH_3), 18.3 (C), 25.8 (CH_3), 63.1 (CH_2), 63.8 (CH), 68.0 (CH_2), 80.1 (CH), 101.3 (CH), 126.2 (CH),

	128.1 (CH), 128.4 (CH), 128.9 (CH), 129.4 (C), 129.6 (CH), 133.3 (CH), 137.4 (C), 165.2 (C).
Mass: *m/z* (%)	467 (M^++K, 5%), 451 (M^++Na, 100%), 429 (M^++H, 2%).
Elemental analysis	Anal. Calcd for $C_{24}H_{32}O_5Si$; C, 67.26; H, 7.53. Found: C, 67.53; H, 8.10.

(4*S*,5*R*)-4-ethynyl-2-phenyl-1,3-dioxan-5-ol (24)



To a solution of 4,6-*O*-benzylidene-D-glucose **6** (0.5 g, 1.8 mmol) in 4 mL of water at 0 ^{0}C was added a solution of $NaIO_4$ (0.8 g, 3.7 mmol) and $NaHCO_3$ (0.16 g, 1.8 mmol) in 5 mL of water. The pH was maintained to 6–7 by adding few drops of a saturated $NaHCO_3$ solution. The mixture was stirred at room temperature for 5 h. Ethyl acetate was added to the reaction mixture and *aq* layer was extracted with ethyl acetate (3 X 10 mL). The combined organic extracts were dried over Na_2SO_4 and the concentrated to give **23**.

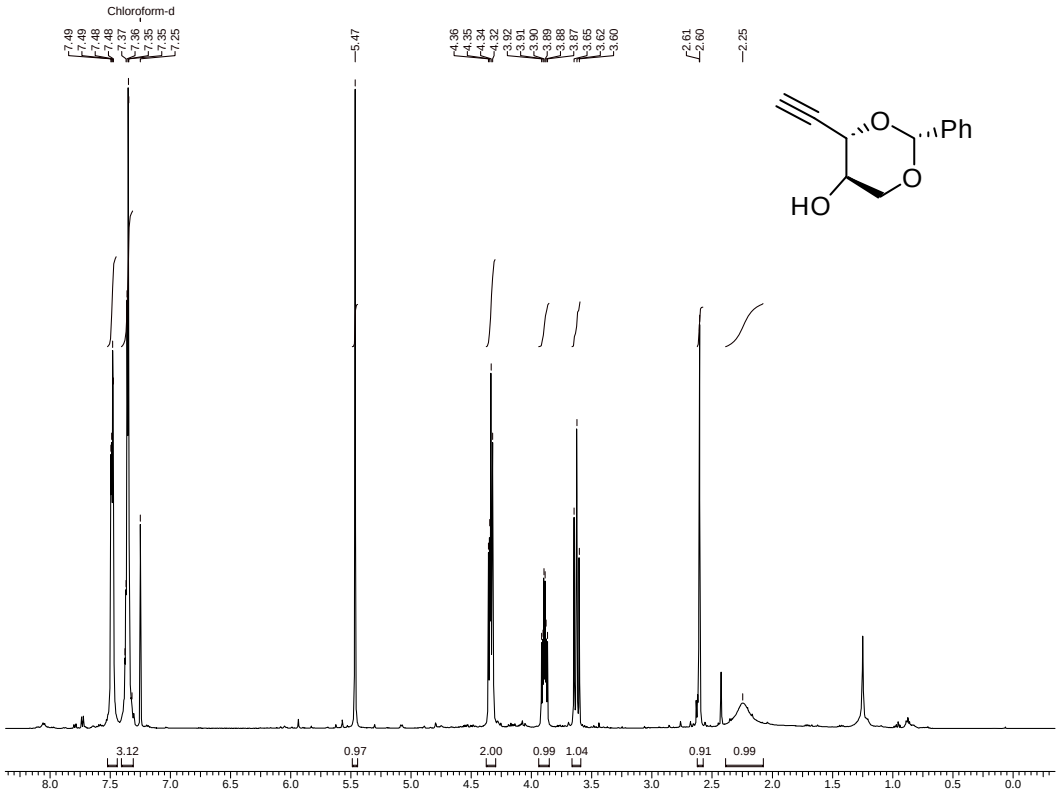
To a stirring mixture of **23** (crude itself) and anhydrous K_2CO_3 (0.34 g, 2.4 mmol) in dry MeOH (6 mL) at 65 °C was added the solution of Ohira reagent (0.47 g, 2.4 mmol) in dry MeOH (6 mL) dropwise over a period of 6 h under argon atmosphere. After neutralization with acetic acid, solvent was removed under *vacuo*, water was added and mixture was extracted with ethyl acetate (2 X 100 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified by column chromatography (pet. ether/ethyl acetate, 4:1) to obtain **24** (0.15 g, 40% over two steps) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$), δ	2.25 (bs, 1H), 2.61 (d, 1H, *J* = 1.91 Hz), 3.63 (t, 1H, *J*

	= 10.68 Hz), 3.90 (dt, 1H, *J* = 9.92, 5.34 Hz), 4.32-4.36 (m, 2H), 5.47 (s, 1H), 7.32-7.38 (m, 3H), 7.48-7.49 (m, 2H).
^{13}C NMR (50 MHz, $CDCl_3$), δ	65.8 (CH), 70.5 (CH_2), 73.0 (CH), 75.5 (C), 79.6 (CH), 101.4 (CH), 126.2 (CH), 128.3 (CH), 129.2 (CH), 136.8 (C).

3.4-References

1. (a) Ghavami, A.; Johnston, B. D.; Pinto, B. M. *J. Org. Chem.* **2001,** *66,* 2312. (b) Baker, S. R.; Clissold, D. W. *Tetrahedron Lett.* **1988**, *29*, 991. (c) Wood Jr, H. B.; Diehl, H. W.; Fletcher Jr, H. G. *J. Am. Chem. Soc.* **1957**, *79*, 1986. (d) Barili, P. L.; Berti, G.; Catelani, G.; Cini, C.; D'Andrea, F.; Mastrorilli, E. *Carbohydr. Res.* **1995**, *278*, 43.
2. Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett.* **1972**, *13*, 3769.
3. (a) Müller, S.; Liepold, B.; Roth, G. J.; Bestmann, H. J. *Synlett* **1996,** 521. (b) Roth, G. J.; Liepold, B.; Müller, S. G.; Bestmann, H. J. *Synthesis* **2004**, 59. (c) Pietruszka, J.; Witt, A. *Synthesis* **2006**, 4266.
4. (a) Gallienne, E.; Benazza, M.; Demailly, G.; Boltea, J.; Lemairea, M. *Tetrahedron* **2005**, *61*, 4557. (b) MacDonald, D. L.; Hermann; Fisher, O. L.; Ballou, C. E. *J. Am. Chem. Soc.* **1956**, *78*, 3720.
5. Pandey, G.; Reddy, G. D.; Chakrabarti, D. *J. Chem. Soc., Perkin Trans. 2* **1996**, 219.
6. Rhee, J. U.; Bliss, B. I.; RajanBabu, T. V. *J. Am. Chem. Soc.* **2003**, *125*, 1492.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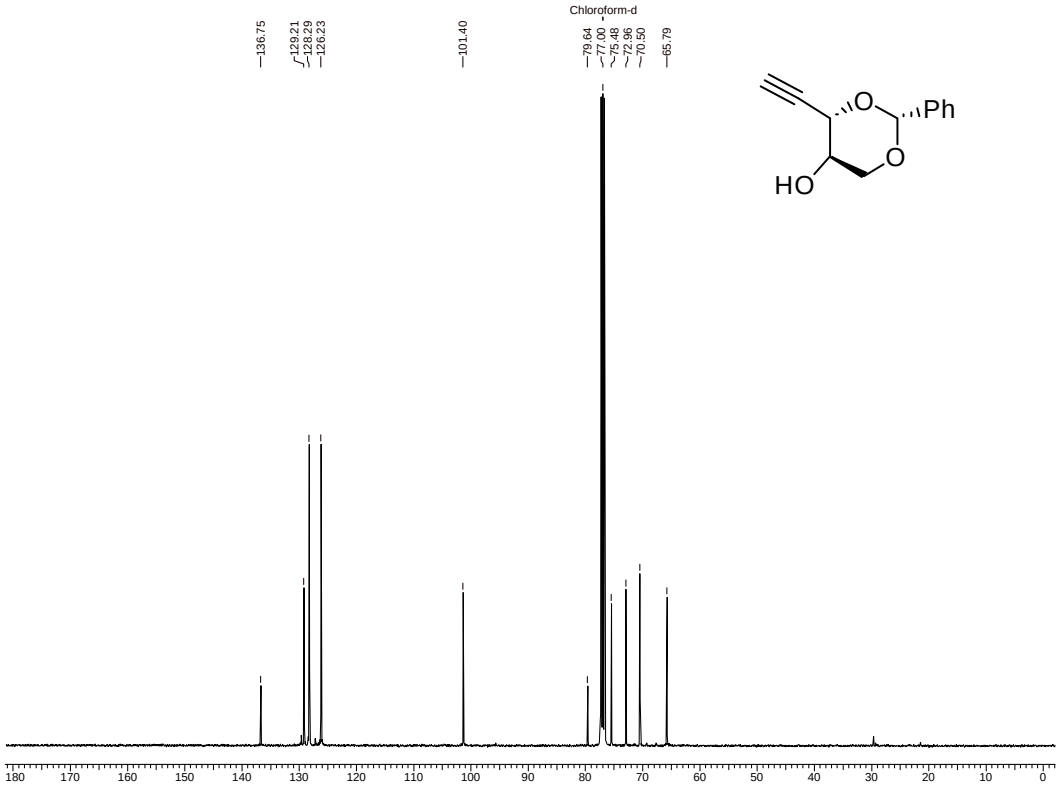
7.49
7.48
7.48
7.37
7.36
7.35
7.35
7.25
5.47
4.36
4.35
4.34
4.32
3.92
3.91
3.90
3.89
3.88
3.87
3.65
3.62
3.60
2.61
2.60
2.25
O
Ph
O
HO
3.12
0.97
2.00
0.99
1.04
0.91
0.99
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


136.75
129.21
128.29
126.23
101.40
Chloroform-d
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77.00
75.48
72.96
70.50
65.79
O
Ph
O
HO
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

Publication

Synthesis of polyhydroxy piperidines and their analogues: a novel approach towards selective inhibitors of α-glucosidase

Ganesh Pandey, Kishor Chandra Bharadwaj, M. Islam Khan, K. S. Shashidhara and Vedavati G. Puranik (*Org.Biomol.Chem*. DOI: 10.1039/b804278k)

Erratum