Chapter 2

Rh(II)-Catalyzed Intramolecular N-H Insertion in D-Glucose derived δ-amino α-diazo β-ketoester: Synthesis of Pyrrolidine Iminosugars and Study of Glycosidase inhibitory and Immunomodulatory Activities
Chapter 2


2.1 Introduction

As discussed in chapter 1, polyhydroxylated pyrrolidines are promising glycosidase inhibitor and thus constitute an important class of natural and unnatural furanose sugar mimics having nitrogen in the ring. Especially, the naturally occurring (2R,3R,4R,5R)-2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP) 10,1 2,5-dideoxy-2,5-imino-D-glucitol (DGDP) 97,2 2,5-dideoxy-2,5-imino-α-L-glycero-D-manno-heptitol (homoDMDP) 11,3 and 2,5-dideoxy-2,5-imino-α-L-glycero-D-galacto-heptitol 1531b alkaloids are promising glycosidase inhibitors with potential antibacterial, antiviral, antitemetastic, and antidiabetic activity (Figure 7).2,4

![Figure 7. Natural pyrrolidine alkaloids](image)

These pyrrolidine iminosugars have also been used as starting materials for the preparation of other polyhydroxy alkaloids of the pyrrolizidine (the five-five fused ring system) family.5
Moreover, C₂ symmetrical derivatives of DMDP have been utilized as chiral ligands and catalyst for a variety of chemical transformations.

In case of naturally isolated pyrrolidine iminosugar 153, the absolute configuration at C-6 is unknown. In an attempt to assign the correct configuration at C-6 we are describing herewith synthesis of 2,5-dideoxy-2,5-imino-L-glycero-α-D-galacto-heptitol 154 and 2,5-dideoxy-2,5-imino-D-galactitol (DGADP) 84 and study of their glycosidase inhibitory and immunomodulatory activity. The core structure of target compounds is the substituted pyrrolidine ring skeleton with hydroxyl and hydroxymethyl substituent.

The common occurrence of the pyrrolidine ring in natural products, as well as it’s utility in asymmetric synthesis either as a chiral auxiliary or a ligand led to a number of inter-and intramolecular synthetic methodologies for its construction. In general, intramolecular methodologies have found wide utility that involved (a) metal assisted carbon-carbon (C-C) bond formation of aminoalkenes (b) carbon-nitrogen (C-N) bond formation including intramolecular amino/amido mercuration and (c) rhodium/copper carbenoid N-H insertion (C-N bond formation). A few examples of these strategies for the synthesis of pyrrolidine ring are depicted bellow:

(a) Carbon-carbon (C-C) bond formation

The C-C bond formation of γ-alkeneyl N-alkylamine 155 under samarium iodide induced reductive cyclization, which proceeds through the intermediacy of α-amino radicals, leads to the formation of substituted pyrrolidine ring skeleton 156.
(b) Carbon-Nitrogen (C-N) bond formation\textsuperscript{11c}

(i) Intramolecular nitrogen attack to carbenium ion:

Vinylsilane 157 bearing a amino group went acid-catalyzed C-N bond formation (and cyclization) that proceeds through a stepwise mechanism including protonation of the α-carbon to silyl group, leading to the \textit{insitu} formation of β-silyl carbenium ion intermediate which on intramolecular addition of the nitrogen nucleophile gave pyrrolidine ring skeleton 158. The reaction afforded high degree of diastereoselectivity in few cases.

\[
\begin{array}{c}
\text{NHMs} \\
\text{acid, CHCl}_3 \\
\end{array}
\] \quad \xrightarrow{\text{new C-N bond formation}} \quad \begin{array}{c}
\text{SiMe}_2Bn \\
\text{157} \\
\end{array}
\]

(ii) Intramolecular amino/ amido mercuration

Another method for C-N bond formation is stereoselective amido- and/or amino-mercuration reaction of δ-alkenylamines that afforded 2-substituted pyrrolidine ring through preferable 5-exo-trig cyclization in addition to the minor amount of 6-endo-trig cyclized piperidine ring.\textsuperscript{12}

\[
\begin{array}{c}
\text{R}_1 \quad \text{N} \\
\text{R}_2 \\
\end{array}
\] \quad \xrightarrow{\text{5-exo-trig}} \quad \begin{array}{c}
\text{NH} \\
\text{R}_1 \quad \text{N} \\
\text{δ-alkenylamine} \\
\text{R}_2 \\
\end{array}
\]

\[
\begin{array}{c}
\text{R}_1 \quad \text{N} \\
\text{R}_2 \\
\end{array}
\] \quad \xrightarrow{\text{6-endo-trig}} \quad \begin{array}{c}
\text{R}_1 \quad \text{N} \\
\text{R}_2 \\
\end{array}
\]

The δ-alkenylamine have been widely exploited by 5-exo trig fashion, however, only a single report is known on the mercury(II)- mediated 5-endo-trig cyclization of γ-alkenylamine leading to the formation of a pyrrolidine ring.\textsuperscript{12a}

\[
\begin{array}{c}
\text{R}_1 \quad \text{N} \\
\text{R}_2 \\
\end{array}
\] \quad \xrightarrow{\text{4-exo-trig}} \quad \begin{array}{c}
\text{NH} \\
\text{R}_1 \quad \text{N} \\
\text{γ-alkenylamine} \\
\text{R}_2 \\
\end{array}
\]

\[
\begin{array}{c}
\text{R}_1 \quad \text{N} \\
\text{R}_2 \\
\end{array}
\] \quad \xrightarrow{\text{5-endo-trig}} \quad \begin{array}{c}
\text{R}_1 \quad \text{N} \\
\text{R}_2 \\
\end{array}
\]
Although, an intramolecular exo-trig nucleophilic addition reaction to a double bond for rings smaller than five-membered ring is favored over the endo-trig, we have recently demonstrated that the aryl- as well as D-glucose derived α-alkenylamines led to the formation of the pyrrolidine ring via 5-endo-trig cyclization in the presence of mercury(II) salt in high yield. The methodology was further extended in the synthesis of pyrrolidine ring skeleton of (+)-castanospermine and 1-deoxy-castanospermine.

(c) One of the most attractive and promising method for C-N bond formation is the metal carbenoid induced intramolecular N-H insertion reaction of δ-amino α-diazo β-ketoester leading to the formation of pyrrolidine ring skeleton  

Although, this methodology is widely utilized in organic synthesis (vide supra) its applicability to a sugar substrate is unknown. This is probably due to the difficulty in getting the appropriate substrate with the combination of δ-amino α-diazo β-keto functionality in a sugar skeleton.
As a part of our continuing interest in the development of new methodologies for the synthesis of pyrrolidine ring and its utility to the iminosugars, we thought of exploiting rhodium acetate mediated N-H insertion (C-N bond formation) with D-glucose derived δ-amino α-diazo β-ketoester in the synthesis of 2,5-dideoxy-2,5-imino-L-glycero-α-D-galacto-heptitol 154 and 2,5-dideoxy-2,5-imino-D-galactitol (DGADP) 84.

2.2. Present work

2.2.1. Retrosynthetic analysis:

As shown in Scheme 18, we envisioned that the targeted pyrrolidine iminosugar 154 could be obtained from (I) by 1,2-acetonide cleavage followed by reduction of hemiacetal and deprotection. While, the pyrrolidine iminosugar 84 could also obtained from (I) by 1,2 acetonide cleavage and chopping of anomeric carbon and reduction followed by deprotection. Thus, the suitably protected bicyclic pyrrolidine (I) is the common intermediate and could be obtained from pyrrolidine (II) by ester and keto group reduction followed by protection. The formation of bicyclic pyrrolidinone ring (II) could be achieved by employing rhodium catalyzed N-H insertion of δ-amino α-diazo β-ketoester (III), easily prepared from β-ketoester (IV) that could be accessible from D-glucose. Thus, the pyrrolidinone (II) having ester and keto groups on one side are potent hydroxymethyl and hydroxyl groups, while sugar skeleton on other side of pyrrolidinone will provide the required hydroxy and hydroxymethyl substituent with defined stereochemistry.
Thus, our approach for the synthesis of target molecules hinges on the building of pyrrolidine ring skeleton with sugar appendage via rhodium carbenoid mediated N-H insertion pathway. The stereochemical outcome of the C-N bond formation will decide the orientation of the hydroxymethyl substituent in the target molecule.

As the key step in our synthesis of iminosugar is the rhodium carbenoid mediated NH insertion reaction, it is appropriate at this stage to discuss the use of rhodium carbenoid chemistry in the organic synthesis.

2.2.2. Rhodium carbenoid reactions in organic synthesis:

General Principles:–

The reaction of α-diazo carbonyl compound with rhodium (II) catalyst has now emerged as a prominent synthetic method for the rapid construction of polyfunctionalised, highly bridged carbon and heteroatom frameworks. One of the most attractive features of this process is the ability to predict the stereochemical outcome of the reaction at several centers. The combination of inter- and intra-molecular addition/cyclization reactions offer a number of interesting synthetic possibilities. The additional advantages gained from entropy, reactivity,
and diastereoselectivity also account for some of the explosive growth in this area as evident from the large number of review articles that has appeared in the past two decades. The diversity of reactions as well as unique bond forming capabilities are the impressive features of the rhodium-generated carbenes.

An electron deficient neutral species with six electrons surrounding the carbon, commonly called as carbene, have long been known as a highly energetic species with interesting properties. The carbene exists in two different configurations, namely, triplet- and singlet-carbene, depending on whether the nonbonding electrons are of the same or opposite spins, respectively.

![Singlet and Triplet Carbenes]

Carbenes are chiefly formed in two ways:

(a) \( \alpha \)-Elimination reactions with base: In \( \alpha \)-elimination, a carbon loses a group without its electron pair, usually a proton, and then a group with its pair, usually a halide ion. One of the best known \( \alpha \)-elimination occurs when chloroform is treated with base.

\[
\begin{align*}
\text{Cl} & \text{Cl} \\
\text{OH} &
\end{align*}
\]

(b) Disintegration of compounds containing certain types of double bonds

(i) Photolysis of ketene

\[
\begin{align*}
\text{H}_2\text{C} & \text{C} \\
\text{hv} &\rightarrow \text{CH}_2 + \text{C} \equiv \text{O}
\end{align*}
\]

(ii) Isoelectronic decomposition of diazomethane
(iii) Diazirines (isomeric with diazoalkanes) also give carbenes

\[
\begin{align*}
R_2C & \rightarrow CH_2 + N=NN
\end{align*}
\]

(c) Metal catalyzed decomposition of diazo compounds

Another common route to generate carbene is decomposition of diazo compounds with metals. The term carbenoid was applied to this process in 1966 to infer that the reacting species was neither a free carbene nor an activated diazo compound, but rather, a carbene bound to the transition metal. Interaction of divalent carbon with the transition metal forms metal-carbene complex which is more selective in its behavior relative to the free carbene. The decomposition of the α-diazoacarbonyl compounds by a variety of transition metals such as copper, rhodium, palladium, nickel, ruthenium and cobalt in the catalytic amount led to the formation of a metal carbenoid.

As shown in Figure 8, the overall process involves first formation of a diazo compound by reaction of a carbonyl compound with a sulfonyl azide in the presence of a base (diazo transfer), followed by a treatment with metal catalyst. The metal catalyzed decomposition of the diazo compound is believed to cause the loss of dinitrogen and production of an electrophilic metal-stabilized carbene entity which reacts with an electron rich substrate to give product and regeneration of catalyst thus, completes the catalytic cycle. Amongst a variety of
metal catalysts that are known for the diazo-decomposition, the use of rhodium(II) acetate is widely accepted.

This reaction has generated a wealth of novel and useful reactions in organic synthesis. For example, the metal carbenoid thus formed undergoes three major reaction pathways that include (a) cycloaddition, (b) X-H insertion and (c) ylide formation (Figure 9).

**Figure 8**

**Figure 9**

C=C (cyclopropanation)  
C≡C (cyclopropanation)  
Ar-H (Buchner reaction)

Addition  
Insertion  
X-H insertion reaction  
X = C, N, O, S, Si

Ylide formation

Oxonium Ylide  
Nitronium Ylide  
Sulphonium Ylide  
Carbonyl Ylide  
Thiocarbonyl Ylide
2.2.2.1 Cycloaddition Reactions:

The first application of rhodium(II) carbenoids in organic synthesis is the cycloaddition reaction. The α-diazocarbonyl compounds react with π-bonds of alkenes, alkynes and aromatic substrates, in the presence of rhodium(II) catalyst, to give cyclopropanes, cyclopropenes and the Buchner addition products, respectively. Although, the reaction takes place in both inter- as well as intra-molecular fashion, an intra-molecular pathway has found wide applications in the synthesis of polycyclic ring compounds which are otherwise difficult to synthesize.

\[
\begin{align*}
\text{CHN}_2 \text{ RhLn} & \rightarrow \text{CH=RhLn} \\
\end{align*}
\]

2.2.2.2. Ylide Formation:

Metallo carbenoids derived from α-diazocarbonyl compounds exhibit high electrophilic properties and function as a Lewis acids by interacting with a pair of nonbonding electrons contributed by a Lewis bases. If the Lewis base is an uncharged species, the end result of such an acid-base reaction is an ylide. Some nucleophilic species that are known to trap carbenes include ethers, thioethers, amines and halides. Intra- as well as inter-molecular formation of ylides and further chemical transformations from these ylides have shown great versatility in the synthesis of natural products as well as other complex molecules. The different types of known ylides are: (a) Nitronium ylides (b) Sulfonium ylides (c) Carbonyl and Thiocarbonyl ylides and (d) Oxonium ylides

In general, the ylide formation undergoes [2, 3]-sigmatropic and/or 1,2- rearrangement (Stevens) as well as dipolar cycloaddition reaction. An example of an oxonium ylide is given below.
Intramolecular generation of allylic oxonium ylide Y undergoes [2,3]-sigmatropic rearrangement to give useful oxygen heterocycle Z.

\[
\text{COOEt} \xrightarrow{\text{Rh}_2(\text{OAc})_4} Y \xrightarrow{[2,3]} Z
\]

The formation of 1,2 rearrangement product via the intermediacy of an oxonium ion is common. Our group has firmly established that in addition to the 2,3-sigmatropic and 1,2 rearrangement in oxonium ylide; the 1,4 migration is also a prominent process. (vide supra).

2.2.2.3. X-H Insertion Reactions.

a. C-H Insertion Reactions

The past 30 years have witnessed a significant increase in the utilization of α-diazocarbonyl compounds as precursors in carbon-carbon bond forming reactions. Intramolecular carbene insertion into the C-H bonds has its own strategic importance in C-C bond formation since the first discovery by Meerwein, Rathjen and Werner. Taber’s group has made use of an optically pure α-diazo β-keto ester 161, prepared by alkylation of chiral oxazolidone, that on treatment with rhodium(II) acetate undergoes C-H insertion to give 162 with retention of configuration. Further functional group manipulation gave (+)-α-cuparenone 163.
b. O-H Insertion Reactions

Since the pioneering work of Yates on the copper-catalyzed decomposition of diazoketones in alcohols and phenols, an insertion of carbenes and carbenoids into hydroxylic bonds has been extensively investigated. Ganem and co-workers faced a difficult task of attaching an enol pyruvate side chain to an already unstable diol in their synthesis of chorismic acid. Thus, treatment of hydroxyl ester 164 with diazomalonate in the presence of rhodium(II) acetate furnished the O-H insertion product 165, in an inter-molecular fashion, that was elaborated to the synthesis of natural product 166.

c. S-H Insertion Reaction

Intramolecular S-H insertion catalyzed by rhodium(II) acetate, in α-diazocarbonyl compounds has been reported by Moody and Taylor. Five-, six- and seven-membered cyclic adducts derived from carbenoid mediated S-H insertion are shown below.
d. Si-H Insertion Reaction

Insertion reaction of carbenoids into Si-H bond to form carbon-silicon bond is a synthetically useful procedure. Michael Doyle has recently published a general procedure for the formation of α-silyl carbonyl compounds by the rhodium(II) catalyzed decomposition of α-diazoketones in the presence of organosilanes. In general, the yields are good and this methodology offers an alternative to displacement of chloride in chlorosilanes with enolate anion.

\[
\begin{align*}
\text{Rh}_2(\text{OAc})_4 & \rightarrow \\
\text{R}_3\text{SiH} & \rightarrow \\
\text{SiR}_3
\end{align*}
\]

e. N-H Insertion Reactions

Intramolecular N–H insertion reactions of α-diazocarbonyl substrates catalyzed by metal complex have received considerable attention in recent years. This type of insertion reaction has been shown to be a mild, efficient and regiospecific route to four-, five-, and six-membered nitrogen heterocycle. The N-H insertion of metallocarbenoids was first described by Yates in 1952 and have found use in the synthesis of bicyclic β–lactum. Although N–H insertions by metallocarbenoids were originally promoted by copper-mediated diazo decomposition, most of the intramolecular N–H insertions recently reported are based on Rh(II)-catalyzed diazo decomposition, particularly with \( \text{Rh}_2(\text{OAc})_4 \) as the catalyst.

The potential of an intra-molecular N-H insertion using rhodium (II) catalyst was not appreciated until Cama and Christensen at Merck reported the conversion of a penicillin analogue into the carbapenem nucleus via rhodium-catalyzed insertion of a keto carbenoid into the N-H bond of the β-lactam 167, a process which later became a key step in the Merck synthesis of the antibiotic, theinamycin 168. The N-H insertion proceeds in yields far
exceeding 90% on a production scale. The method has been widely applied in the synthesis of strained β-lactam bicyclic system, which is otherwise difficult to synthesize.

Wang and Zhu reported synthesis of polyfunctionalized β-fluoropyrrole using rhodium(II) acetate catalyzed intramolecular N-H insertion reaction as a key step. Thus, δ-amino-γ,γ-difluoro-α-diazo-β-ketoester 169 in the presence of 0.5 mol % rhodium (II) acetate, under toluene reflux afforded a mixture of N-H insertion product 170 that on HF elimination, gave β-fluoropyrrole 171.

Davis and Deng demonstrated efficient asymmetric synthesis of an antifungal pyrrolidine alkaloid (+)-preussin using δ-amino α-diazo β-ketoester 172 which on rhodium catalyzed N-H insertion gave 5-substituted 3-oxo praline 173 – an immediate precursor to natural product (+)-preussin 174.

Nakamura and Ukita developed a novel, mild and efficient synthetic method for the synthesis of 2,3-disubstituted indoles employing rhodium acetate catalyzed N-H insertion methodology.
Thus, rhodium(II)acetate-catalyzed reaction of 175 with α-diazophosphonate 176 in toluene at 80 °C gave the corresponding N-H insertion product 177 which on reaction with DBU afforded indol 178.

Recently F. A. Davis and coworkers reported an intramolecular N-H insertion in δ–amino α–diazo β–ketophosphonate 179 to give cis 2,5-disubstituted -3-oxo-pyrrolidine phosphonate 180 which was converted to the pyrrolidine 225C 181.

The above illustrative examples depict that the carbenoid mediated N-H insertion pathway is the most useful reaction in the synthesis of nitrogen heterocycles with high degree of regioselectivity which were otherwise difficult to synthesize.

For the last five years, our group is actively engaged in the application of sugar derived rhodium carbenoid in organic synthesis. In this direction, we have recently reported synthesis of D-glucose derived α-diazo β-keto ester A17,32a and demonstrated32b that the rhodium carbenoid generated bicyclic oxonium ylide undergoes [1,2] as well as [1,4] migration. The [1,4] migration pathway was substantially demonstrated with twelve examples of aromatic as well as sugar derived α-diazo β-ketoesters.
The [1,2] versus [1,4] migration product formation was found to be dependent on the migratory aptitude of the migratory group. This methodology was exploited in the formation of 1,5-dioxabicyclo[3.3.0]octane ring skeleton B—an intermediate for the synthesis of griseolic acid analogue (Scheme 19).

![Scheme 19: Synthesis of griseolic acid analogue.](image)

Inspired with these observations, we now investigated N-H insertion pathway of sugar derived δ-amino α-diazo β-ketoester in the building of pyrrolidine ring skeleton with sugar appendage—a chiral synthone for the target molecules. Our results are depicted below.

### 2.2.3 Synthesis of α-diazo β-ketoester:

As shown in Scheme 20, α-D-glucose was trapped in the furanose form by reacting with dry acetone in the presence of anhydrous copper sulphate and catalytic amount of conc. H₂SO₄ to give 1,2:5,6-di-O-isopropylidene-α-D-gluco-furanose 182. Diacetone-D-glucose 182 was oxidized with PCC in dichloromethane to give C3 keto derivative 183 which on reduction with sodium borohydride afforded diacetone-D-allose 184. The hydride delivery at C3 position is known to take place from the β-face as the 1,2-acetonide functionality hinders the α-attack giving 184 as the only product. The C-3 hydroxyl functionality in 184 was converted to tosyl
derivative using p-toluenesulfonyl chloride in pyridine to give 3-O-tosyl-α-D-allo-1,4-furanose 185 as a white solid in 87% yield. The spectral and analytical data as well as optical rotation value were found to be in good agreement with that literature data. The S_N2 displacement of tosyl group in 185 was achieved using sodium azide and TBAI in DMF to get 3-azido-3-deoxy-1,2,5,6-di-O-isopropylidene-α-D-gluco-1,4-furanose 186 in 80% yield. In the IR spectrum, the appearance of new characteristic signal at 2108 cm^{-1} indicated the formation of the C-3 azido derivative. This fact was supported by ^1H and ^13C NMR in which the aromatic signals for tosyl were absent. The spectral and analytical data was found to be in good agreement with that reported. Reduction of azide functionality in 186 with 10% Pd-C in methanol at 80 psi gave amine which was directly protected with benzylchloroformate to afford 3-deoxy-3-benzylxycarbonylamino-1,2,5,6-di-O-isopropylidene-α-D-gluco-1,4-furanose 187 in 85% yield. Selective deprotection of 5,6 acetonide functionality under controlled conditions using 10% H_2SO_4 in methanol at 25 °C afforded diol 188. The spectral and analytical data of diol 188 is found to be identical with that reported.\textsuperscript{33a}

\begin{equation}
\text{D-glucose} \quad \overset{a}{\rightarrow} \quad \text{182} \quad \overset{b}{\rightarrow} \quad \text{183} \quad \overset{c}{\rightarrow} \quad \text{186} \quad \overset{e,f}{\rightarrow} \quad \text{187} \quad \overset{d}{\rightarrow} \quad \text{184} \quad \text{185}
\end{equation}

Scheme 20. Reagent and conditions: (a) acetone, CuSO_4, H_2SO_4, 36 h, 80%; (b) PCC, 4 Å MS, CH_2Cl_2, 25 °C, 18 h; (c) NaBH_4, MeOH-H_2O, -10 °C, 2 h; (d) TsCl, pyridine, 0 to 25 °C, 6 h, 87%; (e) NaNO_3, DMF, 110 °C, 3 d, 80%; (f) (i) 10% Pd-C, MeOH, 80 psi, 6 h (ii) CbzCl, NaHCO_3, MeOH/H_2O, 4 h, 85%; (g) 10% H_2SO_4, MeOH, 25 °C, 3 h, 88%.
The reaction of diol 188 with sodium metaperiodate at 0 °C gave 3-benzyloxycarbonylamino-3-deoxy-1,2-O-isopropylidene-α-D-xylo-pentodialdose 189.

\[
\text{188} \xrightarrow{\text{NaIO}_4, \text{acetone-H}_2\text{O}, 0 \, ^\circ\text{C}, 2h} \text{189}
\]

R = Cbz

The literature survey indicated that aldehyde 189 is unknown although the corresponding C-3 epimer namely α-D-ribopentodialdose is known.33b

The IR spectrum of the compound showed broad band in the region 1722-1640 cm\(^{-1}\) corresponding to aldehyde and benzyloxy carbonyl functionality, respectively. The broad band at 3500-3300 cm\(^{-1}\) indicated the presence of N-H functionality.

The \(^1\)H NMR spectrum showed the doubling of signals due to the presence of N-Cbz functionality. This is due to the restricted rotation about the C-N bond which is invariably known in case of amide functionality.33c However, the appearance of two singlets at \(\delta 9.60\) and 9.68 indicated the presence of aldehyde functionality. The \(^13\)C NMR spectrum also showed doubling of signals, however, the appearance of two singlets at \(\delta 196.0\) and 199.0 indicated the presence of an aldehyde functionality.

In the subsequent step, aldehyde 189 was reacted with ethyl diazoacetate in the presence of catalytic amount of BF\(_3\).OEt\(_2\) in DCM that afforded a compound only in 30% yield.

\[
\text{OHC} \xrightarrow{\text{EDA, BF}_3\text{.OEt}_2, \text{DCM, 25 } ^\circ\text{C, 2h.}} \text{190 major, 30% yield}
\]

\[
\text{BF}_3\text{.OEt}_2, \text{ZnCl}_2 \xrightarrow{\text{DCM, 25 } ^\circ\text{C, 2h.}} \text{190a minor, 81% yield}
\]
The IR spectrum of the compound showed bands at 3400-3150 (broad), 1730 and 1650 cm$^{-1}$ which were assigned to the N-H group, ester and amide carbonyl stretching frequencies.

The $^1$H NMR spectrum showed the presence of keto-enol tautomers in the ratio 9:1.

The peaks due to major keto tautomers are (Figure 10):

$^1$H NMR (300 MHz, CDCl$_3$): δ 1.26 (t, $J = 7.4$ Hz, 3H, OCH$_2$CH$_3$), 1.30 (s, 3H, CH$_3$), 1.50 (s, 3H, CH$_3$), 3.40 (d, $J = 16.0$ Hz, 1H, H-6b), 3.75 (d, $J = 16.0$ Hz, 1H, H-6a), 4.18 (q, $J = 7.4$ Hz, 2H, OCH$_2$CH$_3$), 4.62-4.50 (br m, 2H, H-3, N-H), 4.89 (d, $J = 3.3$ Hz, 1H, H-2), 5.08 (AB quartet, $J = 12.0$ Hz, 2H, COOCH$_2$Ph), 5.86 (d, $J = 3.6$ Hz, 1H, H-4), 5.89 (d, $J = 3.3$ Hz, 1H, H-1), 7.20-7.40 (br s, 5H, Ar-H).

The appearance of a triplet at δ 1.26 with $J = 7.4$ Hz for CH$_3$ and a quartet at δ 4.18 with $J = 7.4$ Hz for CH$_2$ and AB quartet at δ 3.58 with $J = 16.0$ Hz for the methylene protons (flanked between two carbonyls) indicated the formation of β-ketoester.

The $^{13}$C NMR spectrum showed the following signals (Figure 11):

$^{13}$C NMR (75 MHz, CDCl$_3$) δ 14.0 (OCH$_2$CH$_3$), 26.1 (CH$_3$), 26.7 (CH$_3$), 46.8 (C-6), 58.0 (C-3), 61.7 (OCH$_2$CH$_3$), 66.9 (OCH$_2$Ph), 83.5 (C-2), 84.3 (C-4), 104.7 (C-1), 112.5 (OCO), 127.8, 127.9, 128.3, 128.4, 135.9 (Ar-C), 155.5 (NCOOCH$_2$Ph), 167.3 (COOEt), 199.4 (CO).

The $^1$H and $^{13}$C NMR spectrum showed additional signals (< 10%) corresponding to the enol form of β ketoester. The compound was analyzed for the molecular formula C$_{20}$H$_{25}$NO$_8$. Based on the spectral and analytical data, the structure of compound was assigned as 190.

A number of attempts were made to improve the yield of 190 by using different proportions of BF$_3$OEt$_2$ and under a variety of mild reaction conditions. However, no improvement in the yield was noticed. The use of other lewis acids such as SnCl$_4$, ZnBr$_2$ also gave poor yield of the product. However the use of catalytic amount of ZnCl$_2$ as a lewis acid in DCM at 0 °C afforded a 81% yield of the β-ketoester.
Figure 10: $^1$H NMR (300 MHz, CDCl$_3$) spectrum of compound 190
Figure 11: $^{13}C$ NMR (75 MHz, CDCl$_3$) spectrum of compound 190
In the next step, compound 190 was treated with methanesulphonyl azide (1.1 equiv) and triethylamine at room temperature that afforded a product in 87% yield.

\[
\begin{align*}
\text{EtO} & \quad \text{H} & \quad \text{NHR} & \quad \text{EtN$_3$} & \quad \text{CH$_3$CN}, \text{rt}, 2\text{h.}
\end{align*}
\]

In the IR spectrum, a strong band at 2146 cm$^{-1}$ was suggestive of the resonance form with cumulated double bonds of azide functionality. The bands at 1730 and 1658 cm$^{-1}$ which were assigned to the ester and amide carbonyl stretching frequencies.

The $^1$H NMR spectrum showed the following signals (Figure 12):

$^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.38 (t, $J = 6.9$ Hz, 3H, CH$_2$CH$_3$), 1.35 (s, 3H, CH$_3$), 1.59 (s, 3H, CH$_3$), 4.32 (q, $J = 6.9$ Hz, 2H, CH$_2$CH$_3$), 4.57 (d, $J = 3.3$ Hz, 1H, H-2), 4.67 (dd, $J = 8.7$, 3.6 Hz, 1H, H-3) on D$_2$O exchange becomes (d, $J = 3.6$ Hz), 5.05 (s, 2H, N-OOCPh), 5.22 (br d, $J = 8.7$ Hz, 1H, exchanges with D$_2$O, NH), 5.69 (d, $J = 3.6$ Hz, 1H, H-4), 5.97 (1H, d, $J = 3.3$ Hz, H-1), 7.20-7.40 (m, 5H, Ar-H).

The $^{13}$C NMR spectrum showed the following signals (Figure 13):

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 14.6 (OCH$_2$CH$_3$), 26.6 (CH$_3$), 27.1 (CH$_3$), 58.3 (C-3), 62.4 (OCH$_3$CH$_2$), 67.2 (OCH$_2$Ph), 80.0 (C-2), 84.8 (C-4), 104.6 (C-1), 112.8 (OCO), 128.1 (strong), 128.3, 128.7 (strong), 136.2, (Ar-C), 155.6 (N-COOCH$_2$Ph), 160.5 (COOEt), 186.1 (CO).

In the $^1$H NMR spectrum, the disappearance of a AB quartet at $\delta$ 3.57 due to methylene protons is suggestive of formation of a diazo compound. In the $^{13}$C NMR the C-5 carbon did not appear due to the presence of C=N$_2$ which is analogous to the earlier observation reported by Davis.$^{34}$

The compound was analysed for the molecular formula C$_{20}$H$_{23}$N$_3$O$_8$. Based on the spectral and analytical data, the structure of the compound was assigned as 191.
Figure 12. $^1$H NMR (300 MHz, CDCl$_3$) spectrum of compound 191
Having δ-amino α-diazo β-ketoester 191 with required sugar appendage in hand, we thought of performing rhodium acetate catalyzed N-H insertion reaction. Thus, in the key reaction the δ-amino α-diazo β-ketoester 191 was reacted with catalytic amount of Rh₂(OAc)₄ (2 mol%) in DCM at room temperature. The reaction did not show any progress even after 24 hrs. The use of different mole equivalent of Rh₂(OAc)₄ and change in solvent such as benzene did not show any change in the reaction. However, the use of Rh₂(OAc)₄ in benzene under reflux for 20 min followed by fast column separation afforded a compound in 78% yield.

The IR spectrum of the compound showed bands at 3421-3150 (br), 1750 and 1660 cm⁻¹ which were assigned to the OH group, ester group and amide carbonyl stretching frequencies, respectively. The presence of a broad band in the region 3421-3120 cm⁻¹ indicated the presence of enol form.

The ¹H NMR and ¹³C NMR spectra showed additional signals due to the presence of keto-enol tautomers as well as doubling of the signals due to N-Cbz functionality.

The compound was analysed for the molecular formula C₁₀H₁₈O₅N. Based on the spectral and analytical data, the structure 192 was tentatively assigned to the product.

In the next step, removal of N-Cbz group in 192 by hydrogenolysis (10% Pd/C in methanol) followed by reduction of –COOEt group and ketone group with LAH at 0 °C afforded a thick liquid.
In the IR spectrum of the crude product, absence of bands in the region 1750 and 1660 cm\(^{-1}\) indicated the reduction of COOEt and carbonyl groups.

The \(^1\)H NMR spectrum of crude product showed disappearance of the peaks in the region \(\delta 7.20-7.40\) for aromatic protons and also disappearance of a triplet at \(\delta 1.26\) supporting removal of N-Cbz group and reduction of -COOEt group. In order to confirm the the structure of amino diol, we have attempted the perbenzylation.

Perbenzylolation using sodium hydride and benzyl bromide in THF at room temperature under a variety of reaction conditions for prolonged time afforded a mixture of mono, di- and tribenzylated products. However, the same reaction at 80 °C for 48 h furnished a white solid (68% overall yield in three steps).

The IR spectrum of the compound did not show bands in the region 1750-1650 cm\(^{-1}\) indicating the absence of carbonyl groups.

The \(^1\)H NMR spectrum showed the following peaks (Figure 14):

\(^1\)H NMR (300 MHz, CDCl\(_3\)):
- \(\delta 1.23\) (s, 3H, CH\(_3\)), 1.45 (s, 3H, CH\(_3\)), 3.21 (ddd apparent q, \(J = 6.6, 6.3, 5.7\) Hz, 1H, H-6), 3.39 (d, \(J = 5.4\) Hz, 1H, H-3), 3.55 (dd, \(J = 9.3, 6.3\) Hz, 1H, H-7a), 3.80 (dd, \(J = 9.3, 5.7\) Hz, 1H, H-7b), 3.83 (d, \(J = 14.0\) Hz, 1H, N-CH\(_2\)Ph), 3.93 (dd, \(J = 6.6, 4.8\) Hz, 1H, H-5), 4.00 (d, \(J = 14.0\) Hz, 1H, N-CH\(_2\)Ph), 4.15 (d, \(J = 3.6\) Hz, 1H, H-2), 4.45 (AB quartet, \(J = 12.0\) Hz, 2H, O-CH\(_2\)Ph), 4.53 (d, \(J = 12.0\) Hz, 1H, O-CH\(_2\)Ph), 4.75 (d, \(J = 12.0\) Hz, 1H, O- CH\(_2\)Ph), 4.78 (dd, \(J = 5.4, 4.8\) Hz, 1H, H-4), 5.85 (d, \(J = 3.6\) Hz, 1H, H-1), 7.20-7.40 (m, 15H, Ar-H).
Figure 14: $^1$H NMR (300 MHz, CDCl$_3$) spectrum of compound 193
The $^{13}$C NMR spectrum showed the following signals (Figure 15):

$^{13}$C NMR (75 MHz, CDCl$_3$): δ 26.6 (CH$_3$), 27.5 (CH$_3$), 57.7 (N-CH$_2$Ph), 65.0 (C-3), 70.8 (C-6), 71.3 (C-7), 72.7 (OCH$_2$Ph), 73.2 (OCH$_3$Ph), 78.0 (C-5), 82.3 (C-4), 84.8 (C-2), 107.2 (C-1), 112.0 (OCO), 127.0, 127.3, 127.5, 127.7, 128.1, 129.2, 137.7, 138.0, 138.3 (Ar-C).

In the $^1$H NMR spectrum, the appearance of strong signals at δ 7.20 corresponding to aromatic protons, integrating for 15 protons suggested the formation of tribenzylated compound. This fact was supported by the $^{13}$C NMR spectrum wherein strong peaks at δ 127.0, 127.3, 127.5, 127.7, 128.1, 129.2 corresponding to aromatic carbons and three methylene carbons at 137.7, 138.0 and 138.3 were appeared. An additional signal at δ 71.3 was assigned to -CCH$_2$OBn.

The compound was analyzed for the molecular formula C$_{31}$H$_{35}$NO$_5$. Based on the spectral and analytical data, the structure of the compound was assigned as 193.

**Assignment of absolute configurations at C-5 and C-6 of compound 193:**

In the $^1$H NMR spectrum, the appearance of the H-5 as a doublet of doublet with a relatively high vicinal coupling constant value ($J_{5,6} = 6.6$ and $J_{4,5} = 4.8$ Hz) suggested the cis-relation between H-5 and H-6, and between H-5 and H-4. In the nOe experiment, an irradiation of H-5 showed nOe enhancement for H-4 and H-6 indicating the cis-relative orientation of H-5 with respect to H-6 and H-4. Similarly, irradiation of H-3 showed an nOe for H-4 indicating their cis relationship. As the orientation of H-3 and H-4 is α- in the substrate (D-glucose). The α-orientation of H-5 and H-6 was therefore assigned with 5R and 6S absolute configuration. This fact was further confirmed by the single crystal X-ray analysis of the compound (Figure 16). The single crystal of the compound was developed using chloroform/α-hexane (4:1) and the single crystal X-ray analysis of 193 indicated the formation of bicyclic pyrrolidine ring skeleton and confirmed the absolute configurations at the newly generated stereocentres as 5R and 6S.
Thus, in the above reaction sequence starting from \( \delta \)-amino \( \alpha \)-diazo \( \beta \)-ketoester 191, the rhodium acetate mediated \(-\text{NH}\) insertion occurred in a stereoselectively to the formation of pyrrolidine 192 with \( \beta \)-orientation of \(-\text{COOEt}\) group with \( 6R \) absolute configuration. The LAH reduction of 192 afforded ester and keto group reduced compound 193 as an exclusive product in 94 % yield. The formation of 193 with \( \beta \)-OH, could be explained on the basis of the hydride delivery in LAH reduction from the convex face of compound 192 giving 193 as the concave face LAH addition is hindered due to \(-\text{CH}_2\text{OH}\) functionality.
The pyrrolidine ring skeleton with sugar appendage 193 is thus an immediate precursor for the target molecule 2,5-dideoxy-2,5-imino-L-glycero-α-D-galacto-heptitol 160.

In the subsequent step, compound 193 was treated with TFA-water (3:2) at room temperature for 4 h (cleavage of 1,2 acetonide functionality) that afforded a thick liquid. The $^1$H NMR spectrum of the crude product showed absence of methyl signals of the isopropylidene functionality indicating formation of C-1 hemiacetal X as an anomic mixture. Hemiacetal X was observed to be unstable and therefore was directly treated with LAH in THF at 0 °C. The reaction was found to be sluggish even at room temperature (60% starting recovered after 24 h). However, the same reaction under reflux afforded a compound in 81% yield.

The IR spectrum showed a broad band at 3550-3100 cm$^{-1}$ due to the hydroxyl groups.

The $^1$H NMR spectrum showed the following signals (Figure 17):

$^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 3.02-3.12 (m, 2H, H-3, H-7a), 3.16 (ddd, $J = 8.1, 4.2, 2.1$ Hz, 1H H-6), 3.36 (dd, $J = 2.7, 9.3$ Hz, 1H, H-7b), 3.58 (d, $J = 13.5$ Hz, 1H, N-CH$_2$Ph), 3.66 (dd, $J = 11.4, 4.5$ Hz, 1H, H-1a), 3.77 (dd, $J = 11.4, 4.5$ Hz, 1H, H-1b), 3.96 (ddd apprant q, $J = 9.6, 4.8, 4.5$ Hz, 1H, H-2), 4.01 (d, $J = 13.5$ Hz, 1H, N-CH$_3$Ph), 4.05 (dd, $J = 4.8, 8.1$ Hz, 1H, H-5), 4.22 (dd, $J = 5.1, 4.8$ Hz, 1H, H-4), 4.44 (AB quartet, $J = 12.0$ Hz, 1H, O-CH$_2$Ph), 4.45 (d, $J = 11.7$ Hz, 1H, O-CH$_2$Ph), 4.68 (d, $J = 11.7$ Hz, 1H, O-CH$_2$Ph), 7.20-7.40 (m, 15H, Ar-H).
Figure 18: $^{13}$C NMR (75 MHz, CDCl$_3$ + D$_2$O) spectrum of compound 194
The $^{13}$C NMR spectrum showed the following signals (Figure 18):

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 60.3 ($N$-$\text{CH}_2$Ph), 63.3 (C-2), 64.8 (C-5), 67.1 (C-7), 68.4 (C-1), 69.4 (C-6), 69.9 (C-4), 71.9 (O-$\text{CH}_2$Ph), 73.5 (O-$\text{CH}_3$Ph), 77.7 (C-3), 127.2, 127.5, 127.6, 127.7, 128.3, 129.7, 137.0, 137.7, 138.5 (Ar-C).

The compound was analysed for the molecular formula C$_{28}$H$_{33}$NO$_5$. Based on the spectral and analytical data, the structure of the compound was assigned as 194.

In the final step, hydrogenolysis of compound 194 with 10% Pd/C in methanol at 80 psi for 24h afforded a compound which on purification by column chromatography gave a thick gum in 85% yield.

![Chemical structure of 194](image)

The IR spectrum of the compound showed a broad band at 3550-3200 cm$^{-1}$ due to the hydroxyl groups.

The $^1$H NMR spectrum showed the following signals (Figure 19):

$^1$H NMR (300 MHz, D$_2$O): $\delta$ 3.27 (dd, $J = 6.0, 5.7$ Hz, 1H, H-5), 3.46 (ddd prant q, $J = 6.6, 6.4, 5.8$ Hz, 1H, H-2), 3.63 (dd, $J = 12.0, 6.6$ Hz, 1H, H-7a), 3.72-3.78 (m, 2H, H-7b, H-1a), 3.83 (dd, $J = 11.4, 5.1$ Hz, 1H, H-1b), 3.97 (ddd, $J = 9.9, 6.3, 3.3$ Hz, 1H, H-6), 4.29 (dd, $J = 5.7, 4.8$ Hz, 1H, H-4), 4.35 (dd, $J = 6.6, 4.8$ Hz, 1H, H-3).

The $^{13}$C NMR spectrum showed the following signals (Figure 20):

$^{13}$C NMR (75 MHz, D$_2$O): $\delta$ 62.1 (C-1), 62.3 (C-7), 62.4 (C-2), 65.8 (C-5), 71.9 (C-6), 73.7 (C-3), 73.7 (C-4).
Figure 19: $^1$H NMR (300 MHz, D$_2$O) spectrum of compound 154
The compound was analysed for the molecular formula C$_7$H$_{15}$NO$_5$. Based on the spectral and analytical data, the structure of compound was assigned as 2,5-dIDEOXY-2,5-imino-L-glycero-$\alpha$-D-galacto-heptitol 154.

Asano and co-workers have isolated 2,5-dIDEOXY-2,5-imino-D/L-glycero-$\alpha$-D-galacto-heptitol 153$^{3b}$ however, the authors have not assigned the stereochemistry at C-6. We have compared our data with that reported for the isolated product. The $^1$H and $^{13}$C NMR data and specific rotation of 154 did not match with that of the naturally occurring 153 (in which the absolute configuration at C6 is unknown). As compounds 153 and 154 are C6-epimers and the latter 154 was found to have 6$R$ configuration, therefore, the 6$S$ absolute configuration was assigned to the natural product. Thus, we propose that the naturally occurring compound is 2,5-dIDEOXY-2,5-imino-D-glycero-$\alpha$-D-galacto-heptitol 195.

![Chemical structures](image-url)
2.3. Synthesis of meso pyrrolidine 2,5-dideoxy-2,5-imino-D-galactitol (DGADP) 84.

Thus, we have demonstrated that rhodium acetate catalyzed diazo decomoposition of D-glucose derived δ-amino α-diazo β-ketoester 191 led to stereoselective N-H insertion giving pyrrolidinone ring skeleton 192 which was elaborated to the synthesis of 2,5-dideoxy-2,5-imino-L-glycero-α-D-galacto-heptitol 154. We realized that tribenzylated chiral intermediate 193, obtained from 192, could be elaborated for the synthesis of another pyrrolidine iminosugar, namely 2,5-dideoxy-2,5-imino-D-galactitol (DGADP) 84. Pyrrolidine iminosugar 84 is a selective α-galactosidase inhibitor ($K_i = 5 \times 10^{-8}$ M) and to our knowledge it is the best pyrrolidine inhibitor of α-galactosidase among the pyrrolidine iminosugars known to date.

Although, DGADP 84 is a selective α-galactosidase inhibitor, only a few strategies are known for its synthesis due to its challenging structural features. DGADP 84 has four contiguous chiral centers with cis-orientation of all the substituents on the pyrrolidine ring. Most of the approaches are lengthy and having low selectivity. A brief account of known methods is described in chapter 1.

We envisioned that the pyrrolidine iminosugar 84 is a meso compound with hydroxymethyl groups at α and α'-position to the nitrogen in the pyrrolidine ring with the same orientation. As shown in the retro synthetic analysis (page 50) one of the hydroxymethyl group of 84 could be derived by the reduction of the ester functionality in 192 while, the other hydroxymethyl group could be obtained by chopping C-1 carbon atom followed by reduction. Our attempts in these direction are reported below.

Synthesis of 2,5-dideoxy-2,5-imino-D-galactitol (DALDP) 84:

Thus, tribenzylated compound 193 was treated with TFA-water (3:2) at room temperature for 4 h, which afforded a hemiacetal as a thick liquid as evident from the $^1$H NMR spectrum of the crude product. The crude product was immediately reacted with sodium metaperiodate in
acetone-water at room temperature for 2 h to get one carbon degraded α-amino aldehyde Y as indicated by the $^1$H NMR spectrum of the crude product. The α-amino aldehyde Y was found to be unstable and therefore, directly reacted with sodium borohydride in methanol at 0 °C to afford a thick oil in 52 % yield.

![Chemical structure](image)

The IR spectrum of the compound showed a broad band at 3550-3200 cm$^{-1}$ which was assigned to the amine and hydroxyl groups.

The $^1$H NMR spectrum showed the following signals (Figure 21):

$^1$H NMR (300 MHz, CDCl$_3$ + D$_2$O): δ 3.00-3.24 (m, 3H), 3.40 (dd, $J = 10.0$, 1.8 Hz, 1H), 3.60-3.90 (m, 2H), 3.90-4.15 (br s, 3H), 4.15-4.30 (br s, 1H), 3.30-4.50 (br s, 3H), 4.60-4.70 (d, $J = 12$ Hz, 1H), 7.05-7.45 (m, 15H, Ar-H).

The $^{13}$C NMR (75 MHz, CDCl$_3$ + D$_2$O) showed the following signals (Figure 22):

$^{13}$C NMR (75 MHz, CDCl$_3$ + D$_2$O): δ 60.3 (N-CH$_2$Ph), 63.4/64.8 (C-2/C-5), 67.1 (C-6), 68.4 (C-1), 69.9 (C-4), 71.9 (O-CH$_2$Ph), 73.5 (O-CH$_2$Ph), 77.7 (C-3), 127.3, 127.5, 127.6, 127.6, 127.7, 127.7, 128.2 (strong), 128.8, 137.0, 137.7, 138.3 (Ar-C).
Figure 21: $^1$H NMR (300 MHz, CDCl$_3$ + D$_2$O) spectrum of compound 196
Figure 22. $^1$H NMR (75 MHz, CDCl$_3$+D$_2$O) spectrum of compound 196
The compound was analyzed for the molecular formula $\text{C}_{27}\text{H}_{31}\text{NO}_4$. Based on the spectral and analytical data, the structure of the compound was assigned as amino diol 196.

In the final step, hydrogenolysis of diol 196 with 10% Pd/C in methanol at 80 psi for 24 h afforded a compound which on purification by column chromatography gave a viscous liquid. The $^1\text{HNMR}$ (Figure 23), $^{13}\text{C NMR}$ (Figure 24) and analytical data was found to be identical with that reported\textsuperscript{35} for 2,5-dideoxy-2,5-imino-D-galactitol 84.

The structure 84 was further confirmed by converting it to hydrochloride salt. Thus, the reaction of 84 with MeOH.HCl afforded a sticky solid which was identified as hydrochloride salt of 84 on the basis of $^1\text{H NMR}$ (Figure 25) and $^{13}\text{C NMR}$ (Figure 26) data which were found to be in consonance with that reported.\textsuperscript{35}

Thus, we demonstrated the utility of rhodium carbenoid mediated -NH insertion pathway with δ-amino α-diazo β-ketoester, derived from D-glucose, in building the pyrrolidine ring with required potent hydroxy and hydroxymethyl substituents. The pyrrolidine ring with sugar appendage was then transformed to 2,5-dideoxy-2,5-imino-L-glycero-α-D-galacto-heptitol 154 and 2,5-dideoxy-2,5-imino-D-galactitol 84.

The glycosidase inhibitory and immunomodulatory activity of compounds is described in Part B.
Figure 13: $^1$H NMR (300 MHz, D$_2$O) spectrum of compound 84.
Figure 25: 1H NMR (300 MHz, D$_2$O) spectrum of compound 84A11.
2.4. Conclusions

1. The first example of the rhodium carbenoid mediated intramolecular N–H insertion in sugar substrates leading to the formation of the bicyclic pyrrolidinone ring skeleton.

2. We have successfully demonstrated the utility of δ-amino α-diazo β-ketoester in the synthesis of polyhydroxylated pyrrolidines 2,5-dideoxy-2,5-imino-L-glycero-α-D-galacto-heptitol 154 and 2,5-dideoxy-2,5-imino-D-galactitol (DGADP) 84.

3. We have assigned D-glycero-D-galactoheptitol configuration for the natural product.

4. We have tested compound 154 for their glycosidase inhibitory activity and found that it is selective α-mannosidase inhibitor.

5. We have also studied the immunomodulatory activity of compound 154.
2.5.1 Introduction

Most of the polyhydroxylated alkaloids have been shown to inhibit glycosidases in a reversible and competitive manner. Since the mode of action of glycosidases involves the cleavage of glycosidic bonds between sugar molecules, individual glycosidases show specificity for certain sugar molecules and for a specific anomeric configuration of that sugar. Polyhydroxylated alkaloids can be extremely potent and specific inhibitors of glycosidases by mimicking the pyranosyl or furanosyl moiety of their natural substrates. Therefore, the number, position and configuration of the hydroxyl groups of each alkaloid dictate the type of glycosidases which are inhibited. Although the spatial arrangement of the hydroxyl groups of polyhydroxylated alkaloids serves as a means of recognition by specific glycosidases, it is the influence of the endo-cyclic nitrogen atom on the conformation and electro-static properties of the molecule that is important for inhibition of enzyme activity. The exact mechanism of the glycosidase inhibition is discussed in chapter 1.

Amongst iminosugars, the five-membered iminosugars carrying hydroxyl groups with specific orientation to mimic the shape and charge of the transition state of the reacting sugar moiety have been shown to be potent inhibitors of glycosidase enzymes. Since a cation-like transition state is expected to be involved in glycosidase catalyzed reactions, five-membered iminosugars can be used as core components for the development of transition-state analog inhibitors of glycosidase enzymes.
During the course of our investigation toward this goal, we have synthesized five-membered iminosugars namely 2,5-dideoxy-2,5-imino-D-galactitol \(84\) and 2,5-dideoxy-2,5-imino-L-glycero-\(\alpha\)-D-galacto-heptitol \(154\). It is known in the literature\textsuperscript{35a} that the compound \(84\) is a selective \(\alpha\)-galactosidase inhibitor with \(K_i = 5 \times 10^{-8}\) M. Compound \(153\) was isolated by Asano (configuration at C-6 unknown) which did not show any glycosidase inhibitory activity.\textsuperscript{35b} To study the structure activity relationship we have studied the glycosidase inhibitory activity of compound \(154\) with different glycosidase enzymes. The glycosidase inhibition results are depicted below.

**Assay method:**

The seeds of *Erythrina indica*, jackbeans, *Amaranthus paniculatus* and sweet almond were used for glycosidase inhibitory activity study. Almond seeds were found to be a rich source of all the glycosidases namely \(\alpha\)-glucosidases (E.C. 3.2.1.2.3), \(\beta\)-glucosidases (E.C. 3.2.1.2.4), \(\alpha\)-galactosidases (E.C. 3.2.1.2.2), \(\beta\)-galactosidases (E.C. 3.2.1.2.3) and \(\alpha\)-mannosidases (E.C. 3.2.1.2.4). The seed meal obtained from powdered almond seeds was extracted in physiological (0.145 M NaCl) saline by mechanical stirring at 6 °C. The extract was then centrifuged and supernatant liquid was subjected to extensive dialysis for overnight. The glycosidase inhibitory activity in the dialysate was tested. \(2 \times 10^{-3}\) M substrates solutions of \(p\)-nitrophenyl \(N\)-acetyl-\(\beta\)-D-glucosamide, \(p\)-nitrophenyl \(N\)-acetyl-\(\beta\)-D-galactosamide, \(p\)-nitrophenyl \(N\)-acetyl-\(\alpha\)-D-galactosamide, \(p\)-nitrophenyl \(N\)-acetyl-\(\alpha\)-D-glucosamide, \(p\)-nitrophenyl \(N\)-acetyl-\(\alpha\)-D-mannoside were prepared in citrate buffer.

The test compound was preincubated with the enzyme (almond seed extract) for 1 h at 37 °C. The enzyme reaction was initiated by the addition of 100 \(\mu\)L substrate. Controls were run simultaneously in the substrate of test compound. The reaction was terminated at the end of 1.5
h by the addition of 0.05 M borate buffer (pH = 9.8) and absorbance of the liberated p-nitrophenol was measured at 420 nm. One unit of glycosidase activity is defined as the amount of enzyme that hydrolyzed 1 μM of p-nitrophenyl pyranoside per minute at 25 °C. IC\textsubscript{50} is the amount of inhibitor in μM concentration required for decreasing enzyme activity by 50% under assay conditions.\textsuperscript{39}

**Glycosidase inhibitory activity:**
The inhibitory activity of pyrrolidine 154 against glycosidases extracted from sweet almonds is summarized in Table 4. From the IC\textsubscript{50} values obtained for 154, we have observed that it is a selective inhibitor of α-mannosidase and showing no inhibition against α-galactosidase, α-glucosidase, β-galactosidase, and β-glucosidase under our assay conditions.

**Table 4. % Inhibition with different enzymes 154**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Enzyme</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>154</td>
<td>α-mannosidase</td>
<td>46.66</td>
</tr>
<tr>
<td></td>
<td>β-glucosidase</td>
<td>NIL</td>
</tr>
<tr>
<td></td>
<td>β-galactosidase</td>
<td>NIL</td>
</tr>
<tr>
<td></td>
<td>β-glucosidase</td>
<td>NIL</td>
</tr>
<tr>
<td></td>
<td>α-galactosidase</td>
<td>NIL</td>
</tr>
</tbody>
</table>

All above values are an average obtained from three sets of assay performed.

The IC\textsubscript{50} studies of pyrrolidine 154 were therefore carried out only against α-mannosidase and the nature of inhibition was determined from kinetic studies (Table 5).

**Table 5.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC\textsubscript{50}</th>
<th>Km</th>
<th>V\textsubscript{max}</th>
<th>K\textsubscript{i}</th>
<th>Vi</th>
</tr>
</thead>
<tbody>
<tr>
<td>154</td>
<td>162.5 μM</td>
<td>88.64 μM</td>
<td>1.428 mol/ml/min</td>
<td>88.64 mM</td>
<td>0.055</td>
</tr>
</tbody>
</table>
Thus, pyrrolidine iminosugar 154 showed selective inhibitory activity towards α-mannosidase with IC$_{50}$ value 163 μM. Kinetics studies revealed that pyrrolidine 154 is a competitive inhibitor of an enzyme α-mannosidase. From the glycosidase inhibition data it has been concluded that change of stereochemistry of hydroxyl group at C-6 enhances the glycosidase inhibitory activity.

The naturally isolated 153 did not show any glycosidase inhibitory activity, while 154 was found to be selective α-mannosidase inhibitor. It further supports our earlier observation that the synthetically prepared 154 is different from isolated compound 153. As the absolute configuration at 154 is $6R$ different from isolated compound 153 therefore absolute configuration in 153 should be $6S$.

The study of immunomodulatory activity of compound 154 is discussed in Chapter 3 Part B.
Part C: Synthesis of 3-epi-Hyacinthacine A₁ and Hyacinthacine A₄

2.6.1. Introduction

Amongst bicyclic iminosugars, polyhydroxylated five membered ring fused with five membered ring with a nitrogen atom at the ring junction are commonly known as pyrrolizidine alkaloids. Several members of this class of alkaloids, including australine 23 and alexine 24 have been isolated. More recently, Asano et al. have isolated a new class of polyhydroxylated pyrrolizidines from *Hyacinthoides non-scripta* as well as *Scilla campanulata* and grouped them under the name hyacinthacines. The unique feature of this class of compounds is that, they possess invariably a hydroxymethyl substituent at C-3 position and are basically characterized as 7αR-hydro-1,2-dihydroxy-3 hydroxymethylpyrrolizidines, with a methyl or hydroxymethyl group at C-5 and with hydroxyl substituents present at C-6 and/or C-7 in some cases (Figure 27).

![Figure 27](image)

In hyacinthacine, the pyrrolidine ring with more number of hydroxyl groups is called as first ring while, the pyrrolidine ring with the less number of hydroxyl groups is termed as second ring. Hyacinthacines are conformationally constrained structures and the glycosidase inhibitory activity mainly changes with the substitution pattern of the second pyrrolidine ring although, the first ring substituents also contribute to the activity. The known hyacinthacines
have been further classified into three groups A, B, and C on the basis of the number of hydroxyl and hydroxymethyl groups on the second ring. The distribution of hyacinthacines in nature appears to be restricted to the genus *Scilla* which is an especially rich source of these compounds. The known hyacinthacines A₁, A₂, A₃, B₃, and C₁ were isolated from *Muscari armeniacum* bulbs.⁴² and hyacinthacines A₄, A₅, A₆, A₇, B₃, B₄, B₅ and B₆ have been isolated from *Scilla sibirica* bulbs,⁴³ while hyacinthacine derivatives bearing a long side chain at C-5 have been isolated from *Scilla peruviana* bulbs.⁴⁴

Literature survey indicated that hyacinthacines are weak or moderate inhibitors of glycosidases.³⁷⁻³⁹ Hyacinthacine A₁ ²⁶ (Figure 28) is a potent inhibitor of the rat intestinal lactase with an IC₅₀ value of 4.4 µM. Hyacinthacine A₁ was, furthermore, a moderate inhibitor of α-L-fucosidase and amyloglucosidase with IC₅₀ values of 46 and 25 µM, respectively. The inversion of the hydroxyl group at C-1 in hyacinthacine A₁ ²⁶, to give hyacinthacine A₂ ¹⁹⁷ enhanced its inhibitory potential toward amyloglucosidase but abolished its inhibition towards α-L-fucosidase. Hyacinthacine A₃ ¹⁹⁸, which is the α-C-5-methyl derivative of hyacinthacine A₂ ¹⁹⁷, was a two-fold less effective inhibitor of rat intestinal lactase and amyloglucosidase than hyacinthacine A₂ ¹⁹⁷. Hyacinthacine B₂ ¹⁹⁹ is a weak inhibitor of bacterial β-glucosidase and bovine liver β-galactosidase, with IC₅₀ values of 490 and 160 µM, respectively.¹⁰ Hyacinthacine B₃ ²⁰⁰ proved to be a moderate inhibitor of lactase and amyloglucosidase, but had no significant activity toward other glycosidases. Hyacinthacine C₁ is weak inhibitors of β-glucosidase, β-galactosidase, and amyloglucosidase. Introduction of an OH group to C-7β in hyacinthacine B₂ ¹⁹⁹, to give hyacinthacine C₂ ²⁰² and to C-7α to give hyacinthacine C₃ ²⁰₃, enhanced their inhibitory potential towards bacterial β-glucosidase, which showed IC₅₀ values of 13 and 84 µM, respectively. Furthermore, hyacinthacine C₃ is also a good inhibitor of
bovine liver β-galactosidase (IC50 = 52 μM). Introduction of OH group at C-6β in
hyacinthacine B3 to give hyacinthacine C4 204 enhanced its inhibitory potential toward
intestinal maltase and amyloglucosidase, while hyacinthacine C4 205 was found to be a better
inhibitor of bacterial β-glucosidase than hyacinthacine C4. Although, alkaloid 206 also can be
regarded as the α-5-C-(3-hydroxybutyl) derivative of A3, it has lost inhibitory activity
completely. These results reveal that not only the presence of a long side chain at C-5α but also
the number of hydroxyl groups in the side chain play an important role in the inhibition of these
compounds towards β-glucosidase.

Figure 28. Naturally occurring hyacinthacines
Due to the importance of these compounds as glycosidase inhibitors, a number of natural and unnatural analogues of hyacinthacine were synthesized and evaluated for the biological activity. This fact inspired us to device an altogether different strategy utilizing N-H insertion of δ-amino α-diazo β-ketoesters a key step. First, brief account for selected synthesis of hyacinthacine and its stereoisomers using carbohydrate as well as noncarbohydrate substrates are summarized below.

2.6.1. Method due to Yoda and co-workers

The first synthesis of hyacinthacine was reported by Yoda’s group using 2,3,5-tri-β-208, prepared from arabinofuranose derivative 207 with 4-methoxybenzylamine, followed by oxidative degradation with PCC gave the lactam 209 (Scheme 21). This was transferred to N-Boc lactam followed by opening of lactam ring by NaBH₄ which afforded an acyclic compound 210 after silyl protection. The olefin 208 on dihydroxylation, oxidative cleavage followed by reduction gave alcohol 211. This was subjected to the successive reactions of mesylation and cyclization, leading to the key pyrrolidine intermediate 212 in 91% yield. The pyrrolidine 212 on desilylation followed by the Swern oxidation and the Grignard reaction in the presence of SmCl₃ afforded alcohol 213a and 213b in a 16: 84 ratio. Silyl protection of hydroxyl group in 213b, oxidative cleavage of the double bond via diol intermediate and reduction gave primary alcohol 214. The silyl-protecting group in 214 was replaced from the secondary to primary alcohol with DPSCl to give 215 which was subjected to the successive reactions of mesylation and BF₃OEt₂-induced Boc-deprotection, followed by the simultaneous cyclization under basic conditions, leading to the desired pyrrolizidine structure 216. Finally, removal of the protecting groups afforded (+)-7α-epi-hyacinthacine A₂ 217.
Scheme 21. Reagents and conditions: (a) MPMNH₂, benzene-CHCl₃ (1:1), MS 4A, reflux, quant; (b) butenylmagnesium bromide, THF, -78 to -20°C; (ii) PCC, MS 4A, CH₂C; (c) (i) CAN, CH₂CN-H₂O (9:1); 2, (Boc)₂O, DMAP, Et₃N, CH₂Cl₂; (d) (i) NaBH₄, EIOH; (ii) TBSCl, imidazole, DMF; quant.; (e) (i) OsO₄, NMO, acetone-H₂O (1:1); (ii) NaIO₄, Et₂O-H₂O (2:1); (iii) NaBH₄, EIOH; (f) (i) MsCl, Et₃N, CH₂Cl₂; 2, t-BuOK, THF, (g) (i) Bu₄NF, THF, quant.; (ii) (COCl)₂, DMSO, -78°C then Et₃N, -78 to 0°C; (h) vinylmagnesium bromide, SmCl₃, THF, -78°C, (i) TBSCl, imidazole, DMF, quant.; (ii) OsO₄, NMO, acetone-H₂O (1:1); quant.; (iii) NaIO₄, Et₂O-H₂O (2:1); 4, NaBH₄, EIOH; (i) (i) Bu₄NF, THF, quant.; (ii) DPSCI, imidazole, DMF, 86%; (k) (i) MsCl, Et₃N, CH₂Cl₂; (ii) BF₃·OEt₂, CH₂Cl₂, -20°C; (iii) KOH, MeOH; (l) (i) conc. HCl, MeOH, quant.; (ii) H₂, 10% Pd/C, EIOH.

2.6.1.2. Method due to Py and co-workers⁴⁶

Py and co-workers have reported use of cyclic nitrones derived from L-xylose in the synthesis of (+)-hyacinthacine A₂ (Scheme 22). Thus, 2,3,5-tri-O-benzyl-L-xylofuranose 218 was sequentially reacted with O-tert-butyldiphenylsilylhydroxylamine to give 219 which was treated with mesyl chloride and triethyl amine to afford compound 220. Silyl deprotection with TBAF in THF gave oxime 221. Mesyloxy oxime 221 in the presence of hydroxylamine hydrochloride and sodium hydrogen carbonate afforded nitrone 222. Nitrone 222 was treated...
with Sml₂ and ethyl acrylate in THF at -78 °C to give N-hydroxypyrrolidine 223 (dr = 90:10).

For the reduction of n-hydroxyamines, excess of Sml₂ was added to the reaction mixture which was then warmed to room temperature to afford a mixture of amine 224 and lactam 225. Treatment of 224 with potassium carbonate led to total conversion of 224 to 225. Reduction of lactam 81 with excess of LiAlH₄ followed by benzyl deprotection under hydrogenation condition afforded (+)-hyacinthacine A₂ 197.

Scheme 22. Reagents and conditions: (a) (i) 0.5% HCl, MeOH, r.t., 15h; (ii) NaH, BnBr, TBAI, DMF/THF, r.t. 48 h; (b) (i) AcOH/1M H₂SO₄, 100 °C, 1 h; (ii) TBDPSONH₂, cat. PPTS, MgSO₄, Toluene, 110 °C, 30 min; (c) MsCl, Et₃N, CH₂Cl₂, 0 °C, 30 min; (d) TBAF, THF, 0 °C, 5 min; (e) NH₃·HCl, NaHCO₃, MeOH/H₂O: 4/1, 65 °C, 15 h; (f) Ethyl acrylate Sml₂ (3 equi.), H₂O (8 equi.), THF, -78 °C, 3h; (g) Sml₂ (3 equi.) THF, -78 °C to r.t., 24h; (h) K₂CO₃, EtOH/H₂O; (i) Excess LiAlH₄, THF, 66 °C, 1h; (j) (i) H₂, Pd/C, MeOH, THF, 6N HCl, r.t., 4days; (ii) Dowex 1x 8

2.6.1.3. Method due to Donohoe and co-workers

Donohoe and co-workers reported the synthesis of hyacinthacine A₁ 26 starting from commercially available N-Boc pyrrole and using a partial reduction as the key step. Thus, pyrrole 226 was transformed into racemic trans-227 and then dihydroxylated under Poli’s
dihydroxylation conditions to give diol 228 (Scheme 23). Acetonide protection of 228 gave acetonide 229 that on chemoselective reduction with methanolic solution of NaBH₄ and silyl protection afforded 230. DIBAL-H reduction of 230 at -40 °C afforded aldehyde 231 which on the Wittig olefination gave trans α,β-unsaturated ester 232. Reduction of double bond with PtO₂ in methanol afforded saturated compound which on reduction with DIBAL-H afforded alcohol 233. Primary hydroxyl was activated by the reaction with methanesulfonyl chloride to give mesyl derivative 234. Deprotection of N-Boc in 234 with silyl triflate and subsequent cyclization gave bicyclic compound which on acetonide and silyl deprotection afforded hyacinthacine A₁ 26.

Scheme 23. Reagents and conditions: (a) Li, NH₃, -78 °C, isoprene, NH₄Cl; (b) cat. OsO₄, CH₂Cl₂, Me₃NO; (c) dimethoxypropane, cat. p-TsOH, acetone; (d) (i) NaBH₄, THF/MeOH; (ii) TBSCI, imidazol, DMF; (e) DIBAL-H, CH₂Cl₂, -40 °C; (f) PPh₃=CHCOOMe, toluene, 110 °C; (g) H₂/PtO₂, MeOH, r.t.; (h) DIBAL-H, CH₂Cl₂, -78 °C; (i) MsCl, py, 0 °C; (j) TESOTf, 2,6-lutidine, CH₂Cl₂, -78 °C to r.t.; (k) (COCl)₂, MeOH, r.t.
2.6.1.4. Method due to Renaud and co-workers

Renaud and co-workers reported synthesis of 3-epi-hyacinthacine A₁ 240 (Scheme 24) by stereocontrolled carboazidation of a chiral allylsilane as a key step. Thus, enantiomerically pure allylsilane 235 was easily obtained through the Roush allylation of D-mannitol derived aldehyde. The carboazidation reaction of allylsilane 235 with 3-pyridylsulfonyl azide at 60 °C afforded syn product 236 (dr 85: 15) as a major isomer. Azido silane 137 on the Tamao-Fleming oxidation of the silicon group gives mixture of acetates 237a,b due to 1,2 acetate migration. The acetates were saponified using a DOWEX 1-X-10 ion-exchange resin leading to a diol that was directly protected as an acetonide. Transesterification of the ethyl ester into a methyl ester occurred concomitantly. The selective deprotection of the bis-acetonide using Zn(NO₃)₂ at 50 °C provided diol 238. Selective protection of primary alcohol as a TBDMS ether followed by reduction of ester functionality with lithium borohydride and bis mesylation provided intermediate 239.

Scheme 24. Reagents and conditions (a) (Bu₃Sn)₂, t-BuON=NOt-Bu, Benzene, 60 °C; (b) AcOOH, KBr, AcONa; (c) (i) DOWEX 1X10 (ii) Me₂C(OMe)₂, p-TsOH (iii) Zn(NO₃)₂; (d) (i) TBDMSCl, Pyr. (ii) LiBH₄, THF (iii) MsCl, Pyr.; (e) Pd-C, H₂, AcOEt, rt, 12 h; (f) (i) HCl, MeOH (ii) DOWEX 1X10
Reduction of azide and concomitant cyclization gave pyrrolizidine ring skeleton which on acetonide and TBDMS deprotection in acidic conditions afforded 3-epi hyacinthacine A₁ 240. Although, synthesis of number of pyrrolizidine alkaloids are known in the literature only one synthesis of 3-epi-Hyacinthacine A₁ 240 is known in the literature while the synthesis of hyacinthacine A₄ 241 is not reported so far. Our attempts towards the synthesis of 240 and 241 are reported herein.

In the continuation of our attempts to demonstrate the utility of δ-amino α-diazo β-ketoester and N-H insertion pathway to build pyrrolidine ring, we now report our attempts towards the synthesis of 3-epi-hyacinthacine A₁ 240 and hyacinthacine A₄ 241.

2.6.2. Present work

Retrosynthesis:

In part A, we have discussed the preparation of benzyl substituted bicyclic pyrrolidine derivative III. We visualized that the tribenzylated derivative III is a common intermediate as the ring one of both the target molecules is already existing in III. While, the second ring of 240 could be built from III by 1,2 acetonide cleavage, one carbon chopping and Wittig olefination with Ph₃P=CHCOOEt to get II (Scheme 25). Lactamization of II will led to I which on reduction will give 240. Similarly, 1,2 acetonide cleavage, removal of anomeric carbon and the Wittig olefination (Ph₃P=CHCOCH₃) will give an access to α, β-unsaturated ketone IV with required methyl substituent that on lactamization and reduction will give 241. Our results in the execution of this scheme are described below.
2.6.2.1. Synthesis of 3-epi-hyacinthacine A₁:

The required tribenzylated derivative 193 was prepared as described earlier (Chapter 2, part A). In the next step, compound 193 was treated with TFA-water (3:2) at room temperature to give thick oil. The $^1$H NMR spectrum showed absence of methyl signals corresponding to isopropylidene group and indicated the formation of anomeric mixture of hemiacetal. The hemiacetal was subsequently treated with NaI in acetone-$H_2$O to give $\alpha$-amino aldehyde Y which was characterized by $^1$H and $^{13}$C NMR of the crude product. The isolation of Y as a pure compound was difficult. Therefore, compound Y was immediately reacted with Ph$_3$P=CHCOOEt in DCM to give a mixture of compounds in 88% yields. The appreciable difference between the $R_f$ values of two products allowed us to separate by column chromatography.
The first elution with n-hexane/ethyl acetate = 9.0/1.0 afforded a yellowish liquid in 62% yield. The IR spectrum of the compound showed the stretching frequencies at 3550-3100 (broad), 1721 and 1651 cm⁻¹ which was assigned to hydroxyl, α,β-unsaturated carbonyl ester and C = C, respectively.

The ¹H NMR spectrum showed the following signals (Figure 29):

¹H NMR (300 MHz, CDCl₃) δ 1.22 (t, 3H, J = 7.2 Hz OCH₃CH₂), 2.95 (dd, J = 9.6, 3.3 Hz, 1H, H-1a), 3.15 (ddd, J = 4.8, 3.3, 1.8 Hz, 1H, H-2), 3.40 (dd, J = 9.6, 1.8 Hz, 1H, H-1b), 3.58 (d, J = 13.8 Hz, 1H, N-CH₂Ph), 3.88 (d, J = 13.8 Hz, 1H, N-CH₂Ph), 4.10-4.22 (m, 3H, H-3, O-COCH₃), 4.30 (t, J = 3.9 Hz, 1H, H-4), 4.38 - 4.52 (m, 4H, O-COCH₂Ph, H-5), 4.74 (d, J = 11.4 Hz, 1H, O-COCH₂Ph), 5.92 (dd, J = 11.4, 0.9 Hz, 1H, H-7), 6.45 (dd, J = 11.4, 8.4 Hz, 1H, H-6), 7.10-7.40 (m, 15H, Ar-H).

In the ¹H NMR spectrum, the olefinic protons H-6 and H-7 were diagnostic and appeared at δ 6.45 (doublet of doublet with J = 11.4, 8.4 Hz) and at δ 5.92 (doublet of doublet with J = 11.4 and 0.9 Hz) respectively. The relatively low olefinic protons vicinal coupling constant (J = 11.4 Hz) the Z- geometry of the double bond. The small value of 0.92 Hz in H-7 was due to the
Figure 29: $^1$H NMR (300 MHz, CDCl$_3$) spectrum of compound 242a
Figure 30: $^{13}$C NMR (125 MHz, CDCl$_3$) spectrum of compound 242a
allylic coupling constant with H-5.

The $^{13}$C NMR spectrum showed the following signals (Figure 30):

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 14.2 (OCH$_2$CH$_3$), 57.3 (N-CH$_2$Ph), 60.3 (OCH$_2$CH$_3$), 63.7 (C-2), 67.6 (C-5), 67.9 (C-1), 71.3 (OCH$_2$Ph), 72.0 (C-4), 73.6 (OCH$_2$Ph), 78.0 (C-3), 123.7 (C-7), 127.2, 127.6, 128.2, 128.3, 129.1, 137.6, 138.1, 138.4 (Ar-C), 147.0 (C-6), 166.1 (COOEt).

The appearance of olefinic carbons at $\delta$ 147.0 and $\delta$ 123.7 (C-6/C-7) and a peak at $\delta$ 166.1 due to ester carbonyl indicated the presence of $\alpha,\beta$-unsaturated ester.

The compound was analyzed for the molecular formula C$_{31}$H$_{35}$NO$_5$. Based on the spectral and analytical data, structure of the compound was assigned as 242a.

Further elution with $n$-hexane/ethylacetate = 8.8/1.2 afforded a thick liquid in 26% yield. The IR spectrum showed the stretching frequencies at 3550-3100 (broad), 1717 and 1645 cm$^{-1}$ which were assigned to hydroxyl, $\alpha,\beta$-unsaturated ester carbonyl and double bond, respectively.

The $^1$H NMR spectrum showed the following signals (Figure 31):

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.42 (t, 3H, J = 7.2 Hz OCH$_2$CH$_3$), 2.98 (dd, $J$ = 9.6, 3.3 Hz, 1H, H-1a), 3.15-3.22 (m, 1H, H-2), 3.38 (dd, $J$ = 7.5, 3.9 Hz, 1H, H-5), 3.40 (dd, $J$ = 9.6, 1.8 Hz, 1H, H-1b), 3.50 (d, $J$ = 13.5 Hz, 1H, N-CH$_2$Ph), 3.88 (d, $J$ = 13.5 Hz, 1H, N-CH$_2$Ph), 4.08 (dd, $J$ = 5.1, 4.2 Hz, 1H, H-3), 4.12 (t, $J$ = 4.2 Hz, 1H, H-4), 4.20 (q, $J$ = 7.2 Hz, 2H, O-CH$_2$CH$_3$), 4.46 (d, $J$ = 11.7 Hz, 1H, O-CH$_2$Ph), 4.48 (ABq, $J$ = 12 Hz, 2H, O-CH$_2$Ph), 4.73 (d, $J$ = 11.7 Hz, 1H, O-CH$_2$Ph), 4.82 (broad, exchanges with D$_2$O, 1H, OH), 6.09 (dd, $J$ = 15.6, 7.5 Hz, 1H, H-7), 7.30 (dd, $J$ = 15.6, 0.9 Hz, 1H, H-6), 7.12-7.40 (m, 15H, Ar-H).

The olefinic protons H-6 and H-7 were appeared at $\delta$ 7.30 as a doublet of doublet with $J$ = 15.6, 7.5 Hz and at $\delta$ 6.09 as a doublet of doublet with $J$ = 15.6 and 0.9 Hz indicated $trans$-
geometry of the double bond. The presence of mutually coupled quartet at $\delta$ 4.20 and a triplet at $\delta$ 1.42 indicated the presence of $-$COOEt group.

The $^{13}$C NMR spectrum showed the following signals (Figure 32):

$^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 14.0 (OCH$_2$CH$_3$), 57.5 (N-CH$_2$Ph), 60.2 (OCH$_2$CH$_3$), 63.8 (C-2), 64.4 (C-5), 68.0 (C-1), 71.5 (OCH$_2$Ph), 72.0 (C-4), 74.2 (OCH$_2$Ph), 78.3 (C-3), 121.7 (C-7), 127.3, 127.7, 128.4, 128.9, 129.3, 137.5, 138.1, 139.4 (Ar-C), 151.0 (C-6), 171.2 (COOEt).

The appearance of peak at $\delta$ 171.2 for ester carbonyl and olefinic carbons at $\delta$ 151.0 and $\delta$ 121.7 (C-6/C-7) in the $^{13}$C NMR spectrum of the compound supported the presence of $\alpha,\beta$-unsaturated ester.

The compound was analyzed for the molecular formula C$_{31}$H$_{33}$NO$_5$. Based on the spectral and analytical data, structure of the compound was assigned as 242b.

With the success in getting $\alpha,\beta$-unsaturated ester 242a/b in hand, we attempted the selective reduction of C=C. Thus, a mixture of 242aA) was subjected to hydrogenation using 10% Pd/C at balloon pressure. The reaction was found to be sluggish and did not afford the product even after 48 h. Increasing the pressure (at 30, 40 and 80 psi) led to a complex mixture of products (Table 6). In an another attempt, the hydrogenation of 242 was performed in the presence of ammonium formate and 10% Pd-C in methanol at reflux. The reaction after chromatographic purification gave a thick oil in 17% yield.
Table 6. Reaction condition used for cyclization of 242a/b to 243

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Conditions</th>
<th>Solvent</th>
<th>Time</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pressure</td>
<td>Temp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% Pd-C, HCOONH₄</td>
<td>Atm.</td>
<td>80 °C</td>
<td>2 h</td>
<td>Complex mixture</td>
</tr>
<tr>
<td>10% Pd-C, HCOONH₄, HCl</td>
<td>Atm.</td>
<td>80 °C</td>
<td>4 h</td>
<td>Complex mixture</td>
</tr>
<tr>
<td>10% Pd-C, HCOONH₄, then CH₃COONa</td>
<td>Atm.</td>
<td>80 °C</td>
<td>4 h</td>
<td>Complex mixture</td>
</tr>
<tr>
<td>10% Pd-C, H₂</td>
<td>30 psi</td>
<td>30 °C</td>
<td>24 h</td>
<td>Complex mixture</td>
</tr>
<tr>
<td>10% Pd-C, H₂, cat. HCl</td>
<td>80 psi</td>
<td>30 °C</td>
<td>24 h</td>
<td>Complex mixture</td>
</tr>
<tr>
<td>10% Pd-C, H₂, then CH₃COONa</td>
<td>Atm.</td>
<td>80 °C</td>
<td>4 h</td>
<td>Complex mixture</td>
</tr>
</tbody>
</table>

Scheme 26

The IR spectrum showed bands at 3550-3100 (broad) and 1737 cm⁻¹ indicating the presence of hydroxyl and saturated ester functionality, respectively.
The $^1$H NMR spectrum showed the following signals (Figure 33):

$^1$H NMR (300 MHz, D$_2$O): $\delta$ 1.28 (t, 3H, $J = 6.9$ Hz OCH$_2$CH$_3$), 1.82-2.19 (m, 2H, H-6), 2.52 (t, $J = 7.5$ Hz, 2H, H-7), 3.26-3.80 (m, 1H, H-5), 3.51-3.61 (m, 1H, H-2), 3.79 (d, $J = 12.0$, 5.1 Hz, 1H, $J_a$), 3.87 (d, $J = 12.0$, 4.5 Hz, 1H, $J_a$), 4.18 (q, $J = 7.2$ Hz, 2H, O-CH$_2$CH$_3$), 4.25 (t, $J = 4.2$ Hz, 1H, H-4), 4.48 (dd, $J = 5.1$, 7.5 Hz, 1H, H-3).

In the $^1$H NMR spectrum the absence of aromatic and olefinic protons suggested the debenzylation and reduction of C=C. The appearance of mutually coupled triplet at $\delta$ 1.28 and a quartet at $\delta$ 4.17 showed the presence of --COOEt group. Based on the spectral and analytical data the structure was assigned as 244. Our attempts to cyclize 244 to lactam and further reduction to target molecule (Scheme 26) were unsuccessful under varity of reaction conditions of base, temperature and solvent.

As an alternative we thought of converting 242a/b to corresponding $\alpha,\beta$ unsaturated aldehyde using DIBAL reduction and perform the reductive amination under hydrogenation condition. The work in this direction is in progress.
Figure 33: $^1$H NMR (300 MHz, D$_2$O) spectrum of compound 244
2.6.2.2. Synthesis of Hyacinthacine A_4:

Targeting the synthesis of hyacinthacine A_4, the tribenzylated derivative 193 was treated with TFA-H_2O (3:2) at room temperature that afforded hemiacetal which was treated with NaIO_4 in acetone /H_2O to give α-amino aldehyde. In hyacinthacine A_4, the second ring contains methyl as a substituent at C-5 which we thought of deriving by treatment of α-aminal with suitable Wittig reagent namely 1-triphenylphosphoranylidene-2-propanone (Ph_3P=CHCOCH_3) 245. The required Wittig reagent was prepared as follows.

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{Br} \quad \text{pPh}_3 \\
\text{A} & \quad \text{B} \\
\end{align*}
\]

A solution of bromoacetone (5.0 g, 36.49 mmol) in benzene (15 mL) was added dropwise to a solution of triphenylphosphine (9.56 g, 36.49 mmol) in benzene (15 mL) under nitrogen. The mixture was stirred overnight and the resulting phosphonium salt was filtered. The precipitate was washed with benzene and collected to dry in vacuo. The dried phosphonium salt was suspended in a mixture of water (250 mL) and methanol (250 mL), and the mixture was stirred for 1 h. Aqueous sodium hydroxide (2.00 M) was added to the mixture until a pH between 7 and 8 was reached. The mixture was then stirred vigorously for 1 h. The phosphorane precipitate was filtered and washed water. After drying in vacuo, the phosphorane was recrystallized from ethyl acetate and dried under vacuum to obtain 7.54 g (19.1 mmol, yield 65%, white crystal) of pure product.

Having required Wittig reagent 245 in hand, the α-aminal obtained in earlier step was immediately treated with (Ph_3P=CHCOCH_3) in CH_3CN that afforded a thick liquid in 85% yield.
The IR spectrum of the compound showed bands at 3550-3100 (broad), 1704 and 1640 cm\(^{-1}\) which were assigned to the hydroxyl, \(\alpha,\beta\)-unsaturated ketone carbonyl and C=C stretching frequencies.

The \(^1\)H NMR spectrum showed the following signals (Figure 34):

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 2.20 (s, 3H, COCH\(_3\)), 3.1 (d, \(J = 9.6\), 1H, H-1a), 3.2 (d, \(J = 7.8\), 1H, H-5), 3.32 (m, 1H, H-2), 3.45 (d, \(J = 9.6\), 1H, H-1b), 3.59 (d, \(J = 13.8\) Hz, 1H, N-CH\(_2\)Ph), 3.80 (d, \(J = 13.8\) Hz, 1H, N-CH\(_2\)Ph), 4.08 (t, \(J = 4.5\) Hz, 1H, H-4), 4.10-4.15 (m, 1H, H-3), 4.48 (d, \(J = 11.7\) Hz, 1H, O-CH\(_2\)Ph), 4.49 (AB q, \(J = 11.7\) Hz, 1H, O-CH\(_2\)Ph), 4.72 (d, \(J = 11.7\) Hz, 1H, O-CH\(_2\)Ph), 6.13 (d, \(J = 16.2\) Hz, 1H, H-7), 6.72 (dd, \(J = 16.2, 7.8\) Hz, 1H, H-6), d 7.15-7.36 (m, 15H, Ar-H).

The \(^13\)C NMR spectrum showed the following signals (Figure 35):

\(^13\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 26.2 (COCH\(_3\)), 57.4 (C-2), 63.9 (N-CH\(_2\)Ph), 67.8 (C-5), 68.2 (C-1), 71.3 (C-3), 72.3 (OCH\(_2\)Ph), 73.6 (OCH\(_2\)Ph), 77.9 (C-4), 127.1, 127.3, 127.5, 127.7, 128.1, 128.2, 128.3, 128.8, 129.0 (Ar-C), 132.9 (C-7), 137.3, 137.7, 138.3 (Ar-C), 147.0 (C-6), 198.6 (CO).
In the $^1$H NMR spectrum, appearance of olefinic protons at $\delta$ 6.72 (doublet of doublet with $J = 15.9, 7.8$ Hz) and at $\delta$ 6.13 (doublet with $J = 15.9$ Hz) indicated the E-geometry of the double bond. The presence of the three proton singlet at 2.20 was assigned to –COCH$_3$. This fact was further supported by the appearance of olefinic carbons at $\delta$ 147.0 and $\delta$ 132.9 (C-6/C-7) as well as a peak at $\delta$ 198.6 for carbonyl carbon in the $^{13}$C NMR spectrum of the compound. The compound was analyzed for the molecular formula C$_{30}$H$_{33}$NO$_4$. Based on the spectral and analytical data structure of the compound was assigned as 246.

Having hydroxyl substituted pyrrolidine ring with $\alpha,\beta$-unsaturated ketone functionality on the $\alpha$-carbon to the ring nitrogen atom, we thought of building second ring under hydrogenation condition.

Thus, compound 246 was hydrogenated in the presence of 10% Pd-C in methanol under different reaction conditions as shown in Table 2. The TLC of the reaction mixture showed disappearance of the starting material and appearance of number of spots at low $R_f$ compare to the starting compound. Our attempts to separate the individual compound by column chromatography/ flash column were unsuccessful.

The hydrogenation of 246 using 10% Pd/C and ammonium formate under different reaction conditions (Table 5) were also unsuccessful.

Attempts are in progress to achieve the target from 242 and 243 under controlled reduction conditions. The results in this direction will be reported latter.
Table 7. Reaction condition used for cyclization

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Conditions</th>
<th>Solvent</th>
<th>Time</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pressure</td>
<td>Temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% Pd-C, H₂</td>
<td>balloon</td>
<td>30 °C</td>
<td>MeOH</td>
<td>48 h</td>
</tr>
<tr>
<td>10% Pd-C, H₂</td>
<td>80 psi</td>
<td>30 °C</td>
<td>MeOH</td>
<td>24 h</td>
</tr>
<tr>
<td>10% Pd-C, H₂</td>
<td>80 psi</td>
<td>30 °C</td>
<td>EtOAc</td>
<td>24 h</td>
</tr>
<tr>
<td>10% Pd-C, H₂</td>
<td>80 psi</td>
<td>30 °C</td>
<td>MeOH</td>
<td>24 h</td>
</tr>
<tr>
<td>cat. HCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% Pd-C, H₂</td>
<td>80 psi</td>
<td>30 °C</td>
<td>MeOH</td>
<td>4 h</td>
</tr>
<tr>
<td>HCOONH₄</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% Pd-C, H₂</td>
<td>Atm.</td>
<td>80 °C</td>
<td>EtOH</td>
<td>2 h</td>
</tr>
<tr>
<td>HCOONH₄, HCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In conclusion, our attempts to demonstrate the utility of δ-amino α-diazo β-ketoester with sugar appendage for synthesis of pyrrolizidine alkaloid namely 3-epi-hyacinthacine A₁ 240 and hyacinthacine A₄ 241 were unsuccessful. However, efforts are in progress to achieve the synthesis of target molecules.
Experimental

Expt. No. 2.7.1: Preparation of 1,2:5,6-di-O-isopropylidene-α-D-glucopyranose (182).

To a stirred solution of anhydrous copper sulphate (100 g, w/w) and D-glucose (100 g, w/w) in dry acetone was added conc. Sulphuric acid (5.0 mL) at 0 °C. The reaction mixture was stirred at room temperature for 36 h. Saturated solution of potassium carbonate was added slowly. Acetone was evaporated under reduced pressure; the residue was extracted with chloroform (150 mL x 3). The organic layer was dried and concentrated to afford white solid, which was recrystallised from chloroform-hexane to give 182 in 73 g, 77 % yield.

Expt. No. 2.7.2: Preparation of 1,2:5,6-di-O-isopropylidene-α-D-glucopyranose-3-one (183).

Pyridinium chlorochromate (20 g, 92.31 mmol) was added to a solution of diacetone D-glucose 182 (4.0 g, 15.38 mmol), molecular sieves (4A°, 13.2 g) in dichloromethane (100 mL). After stirring the reaction for 24 h, the reaction was diluted by adding diethyl ether (50 mL). The reaction mixture was then directly poured on a silica column and the eluents evaporated to afford the ketone 183 (3.9 g, 98%).
Expt. No. 2.7.3: Preparation of 1,2:5,6-di-O-isopropylidene-\(\alpha\)-D-\(\alpha\)-allo-1,4-furanose (184).

Sodium borohydride was added to a solution of ketone 183 (3.8 g, 14.73 mmol) in methanol-water (15 mL-5 mL) at \(-10\,\text{°C}\). After stirring the reaction mixture for 1 h, the reaction mixture was quenched by adding dilute HCl, methanol was evaporated and the residue extracted with chloroform to yield a diacetone D-allose 184 (3.4 g, 89%).

Expt. No. 2.7.4: Preparation of 1,2:5,6-di-O-isopropylidene-3-O-tosyl-\(\alpha\)-D-\(\alpha\)-allo-1,4-furanose (185).

To an ice-cooled solution of diacetone D-allose 184 (3.0 g, 11.5 mmol) in dry pyridine (10 mL) tosyl chloride (2.42 g, 12.6 mmol) followed by catalytic amount of DMAP was added. Reaction mixture was stirred for 6 h at 25 °C, usual workup and column purification provided 185 (4.2 g, 87%) as a white solid, mp. 109-111 °C.

Expt. No. 2.7.5: Preparation of 3-azido-3-deoxy-1,2:5,6-di-O-isopropylidene-\(\alpha\)-D-gluco-1,4-furanose (186).

Na\(\text{N}_3\), TBAI, DMF, reflux, 3 d.
To a solution of 185 (3.5 g, 8.45 mmol) in anhydrous DMF (15 mL) was added NaN₃ (1.3 g, 21.13 mmol) followed by TBAI (0.5 equiv.) and reaction mixture was stirred for 72 h at 110 °C. The reaction mixture was cooled to room temperature and DMF was removed using high vacuum, residue was extracted with ethyl acetate. The organic layer was dried, concentrated and purified by column chromatography on silica gel (n-hexane/ethyl acetate = 9/1) to furnish azido compound 186 (1.92 g, 80%) as a thick liquid. [α]₀ = -27.1 (c 0.5, CHCl₃).

H NMR (300 MHz, CDCl₃): δ 1.32 (s, 3H, CH₃), 1.36 (s, 3H, CH₃), 1.43 (s, 3H, CH₃), 1.50 (s, 3H, CH₃), 3.97 (dd, J = 4.6 and 8.5 Hz, 1H, H-4), 4.05-4.14 (m, 3H, H-2 and H-6), 4.20-4.24 (m, 1H, H-5), 4.60 (d, J = 3.6 Hz, 1H, H-2), 5.83 (d, J = 3.6 Hz, 1H, H-1).

Expt. No. 2.7.6: Preparation of 3-benzyloxy carbonylamino-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-gluco-1,4-furanose (187).

To an ice-cooled suspension of LAH (0.554 g, 14.60 mmol) in dry THF (4 mL) was added azido ester 186 (2.77 g, 9.73 mmol) in dry THF (15 mL) at 0 °C and stirred for 10 min. The reaction mixture was slowly warmed to room temperature and stirred for additional 2.5 h. The reaction mixture was quenched by adding excess of ethyl acetate (20 mL), followed by saturated solution of ammonium chloride (2 mL). The reaction mixture was filtered through a pad of celite and the filtrate was concentrated under reduced pressure. To a cooled solution of amine (0.2.52 g, 9.72 mmol) in methanol-water (10 mL, 9:1) was added benzyl chloroformate (1.98 g, 11.67 mmol) and sodium bicarbonate (3.26 g, 38.9 mmol) at 0 °C and stirred for 3.5 h. Methanol was evaporated under reduced pressure and the residue was extracted with
chloroform (3 \times 20 \text{ mL}). Usual workup and purification by column chromatography (n-hexane/ethyl acetate = 1/1) gave 187 (3.24 g, 85\% overall) as a thick liquid.

**Expt. No. 2.7.7: Preparation of 3-benzyloxy carbonylamino-3-deoxy-1,2-\text{O}-isopropylidene-\alpha-D-gluc-1,4-furanose (188).**

![Diagram of 187 and 188](image)

To a solution of 187 (2.85 g, 7.25 mmol) in methanol/water (49 mL, 5:1) was added 10\% H$_2$SO$_4$ (2.5 mL) slowly at room temperature and the resulting reaction mixture was stirred for 5 h. The reaction was neutralized (pH = 7–8) by adding saturated potassium carbonate and methanol was removed under reduced pressure. The residue was extracted with chloroform (4 \times 20 \text{ mL}), organic layer was dried (Na$_2$SO$_4$) and evaporated to give a thick liquid of diol, which after column purification (n-hexane/ethyl acetate = 8.5/1.5) gave diol 188 (2.24 g, 88\%) as a thick liquid.

**Expt. No. 2.7.8: Preparation of 3-benzyloxy carbonylamino-3-deoxy-1,2-\text{O}-isopropylidene-\alpha-D-xylo-pentodialdose (189).**

![Diagram of 188 and 189](image)

To a solution of diol 188 (5.00 g, 14.16 mmol) in acetone-water (5:1) at 0 °C was added NaIO$_4$ (4.52 g, 21.24 mmol) in portion wise. Reaction mixture was stirred for 30 min. and allow to attend room temperature. After 2 h ethylene glycol (1 mL) was added and acetone was evaporated and residue extracted with CH$_2$Cl$_2$. Usual workup and purification by column...
chromatography on silica gel (n-hexane/ethyl acetate = 13/7) afforded aldehyde 189 (4.17 g, 92%) as a thick liquid.

Rf 0.45 (n-hexane/ethyl acetate = 3/2).

[α]D + 3.33 (c 1.2, CHCl3).

IR (neat) 3550-3100, 2856 weak, 1640 cm⁻¹.

The ¹H and ¹³C NMR showed doubling of signals and is uninterpretable however this compound is further characterized in the next step.

Expt. No. 2.7.9: Preparation of ethyl-3,6-dideoxy-3-benzyloxycarbonylamino-1,2-O-isopropylidene-α-D-xylo-hept-5-ulosuranonate (190).

To a stirred solution of aldehyde 189 (6.30 g, 19.60 mmol) and ethyl diazoacetate (2.69 mL, 25.50 mmol) in dichloromethane (50 mL) at 0 °C under nitrogen atmosphere, was added zinc chloride (0.26 g, 1.96 mmol) and the solution was stirred for 2 h allowing to warm it to room temperature. After nitrogen evolution had stopped, the reaction mixture was decomposed with brine solution (10 mL). Usual workup and purification by column chromatography on silica gel (n-hexane/ethyl acetate = 88/12) afforded β-ketoester 190 (6.50 g, 81%) as a thick liquid.

Rf 0.65 (n-hexane/ethyl acetate = 5/1).

[α]D = +3.33 (c 0.60, CHCl3).

IR (neat) 3400-3150 (broad), 1730, 1650 cm⁻¹.

¹H NMR (300 MHz, CDCl3): δ 1.26 (t, J = 7.4 Hz, 3H, OCH₂CH₃), 1.30 (s, 3H, CH₃), 1.50 (s, 3H, CH₃), 3.40 (d, J = 16.0 Hz, 1H, H-6b), 3.75 (d, J = 16.0 Hz, 1H, H-6a), 4.18 (q, J = 7.4 Hz, 2H, OCH₂CH₃), 4.62-4.50 (br m, 2H, H-3, N-H), 4.89 (d, J = 3.3 Hz, 1H, H-2), 5.08 (AB
quartet, $J = 12.0 \text{ Hz, } 2\text{H, COOCH}_2\text{Ph}$, 5.86 (d, $J = 3.6 \text{ Hz, } 1\text{H, } H-4$), 5.89 (d, $J = 3.3 \text{ Hz, } 1\text{H, } H-1$), 7.20-7.40 (br s, 5H, Ar-H).

$^{13}$C NMR (75 MHz, CDCl$_3$): δ 14.0 (OCH$_2$CH$_3$), 26.1 (CH$_3$), 26.7 (CH$_3$), 46.8 (C-6), 58.0 (C-3), 61.7 (OCH$_2$CH$_3$), 66.9 (OCH$_3$Ph), 83.5 (C-2), 84.3 (C-4), 104.7 (OCO), 127.8, 127.9, 128.3, 128.4, 135.9 (Ar-C), 155.5 (NCOOCH$_2$Ph), 167.3 (COOEt), 199.4 (CO).

The $^1$H and $^{13}$C NMR spectrum showed additional signals (< 5%) corresponding to the enol form of $\beta$ ketoester.

Anal. Calcd. for C$_{20}$H$_{25}$NO$_8$: C, 58.96; H, 6.18. Found: C, 58.82; H, 6.12.

Expt. No. 2.7.10: Preparation of ethyl-3,6-dideoxy-6-diazo-3-benzyloxycarbonylamino-1,2-O-isopropylidene-α-D-xylo-hept-5-ulosuranuronate (191).

In a two-necked round bottom flask (100 mL) equipped with a magnetic stirring bar and nitrogen inlet was placed $\beta$-ketoester 190 (3.75 g, 9.21 mmol), triethylamine (2.56 mL, 18.42 mmol) and methanesulphonyl azide (1.22 g, 10.13 mmol) in dry acetonitrile (40 mL). The reaction mixture was stirred at room temperature for 2 h under nitrogen atmosphere and decomposed with aqueous 2M sodium hydroxide solution (1 mL). Usual workup and column chromatography on silica gel (n-hexane/ethyl acetate = 85/15) afforded diazo compound 191 (3.46 g, 87%) as a thick liquid.

$R_f$ 0.60 ($n$-hexane/ethyl acetate = 5/1).

$[\alpha]_D^0 = +44.00$ (c 0.5, CHCl$_3$).

IR (neat) 3330-3150 (broad NH), 2146 (N$_2$), 1730, 1658 (NHCOCOCH$_2$Ph) cm$^{-1}$.
$^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.38 (t, $J = 6.9$ Hz, 3H, CH$_2$CH$_3$), 1.35 (s, 3H, CH$_3$), 1.59 (s, 3H, CH$_3$), 4.32 (q, $J = 6.9$ Hz, 2H, CH$_2$CH$_3$), 4.57 (d, $J = 3.3$ Hz, 1H, H-2), 4.67 (dd, $J = 8.7$, 3.6 Hz, 1H, H-3) on D$_2$O exchange become (d, $J = 3.6$ Hz), 5.05 (s, 2H, N-OOCCH$_2$Ph), 5.22 (br d, $J = 8.7$ Hz, 1H, exchanges with D$_2$O NH), 5.69 (d, $J = 3.6$ Hz, 1H, H-4), 5.97 (1H, d, $J = 3.3$ Hz, H-1), 7.20-7.40 (m, 5H, Ar-H).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 14.6 (OCH$_2$CH$_3$), 26.6 (CH$_3$), 27.1 (CH$_3$), 58.3 (C-3), 62.4 (OCH$_2$CH$_3$), 67.2 (OCH$_2$Ph), 80.0 (C-2), 84.8 (C-4), 104.6 (C-1), 112.8 (OCO), 128.1 (strong), 128.3, 128.7 (strong), 136.2, (Ar-C), 155.6 (N-COOCH$_2$Ph), 160.5 (COOEt), 186.1 (CO).

Anal. Calcd. for C$_{20}$H$_{23}$N$_3$O$_8$: C, 55.42; H, 5.35 Found: C, 55.32; H, 5.32.

Expt. No. 2.7.11: Preparation of ethyl-3,6-dideoxy-3,6-benzylloxycarbonylamino-1,2-O-isopropylidine-a-D-xylo-hept-5-ulofuranonate (192).

To a solution of the diazo compound 191 (1.00 g, 2.30 mmol) in dry benzene (5 mL) was added Rh$_2$(OAc)$_4$ (0.03g, 0.04 mmol) under nitrogen atmosphere and the solution was refluxed for 20 min. On cooling, the reaction mixture was directly loaded on silica gel column and eluted (n-hexane/ethyl acetate = 7/3) to give 192 (0.73g, 78%) as a thick liquid.

R$_f$ = 0.50 (n-hexane/ethyl acetate = 3/2).

[\alpha]_D$ = + 10.00 (c 0.8, CHCl$_3$).

IR (neat) 3421-3150 (broad), 1750, 1660 (NHCOOEt) cm$^{-1}$.

Anal. Calcd. for C$_{20}$H$_{23}$NO$_5$: C, 59.25; H, 5.72. Found: C, 59.27; H, 5.68.
The $^1$H and $^{13}$C NMR spectrum of this compound showed complex pattern due to keto-enol tautomerism and doubling of signals associated with N-Cbz functionality.

**Expt. No. 2.7.12: Preparation of 3,6-dideoxy-3,6-(N-benzylamino)-5,7-di-O-benzyl-1,2-O-isopropylidene-α-D-glycer-D-gluco-hepto-1,4-furanose (193).**

A solution of compound 192 (0.70 g, 1.72 mmol) and 10 % Pd/C (0.07 g) in dry methanol (7 mL) was hydrogenolyzed under 80 psi at room temperature for 12 h. The catalyst was filtered through celite and washed with methanol (20 mL). The filtrate was concentrated and the residue (0.50 g) was dissolved in dry THF (10 mL). This solution was added drop wise to an ice cooled suspension of LAH (0.09 g, 2.58 mmol), in dry THF (3 mL) over a period of 10 min. under nitrogen atmosphere. The reaction mixture was stirred at 0 °C for 2h, ethyl acetate (3 mL) was added and the reaction mixture was stirred for further 10 min. and quenched with saturated solution of NH$_4$Cl (2 mL). The solution was filtered through celite and concentrated. The crude residue (0.65 g) dissolved in dry THF (10 mL) was added to a suspension (60% in oil) of sodium hydride (0.27 g, 6.92 mmol) in dry THF (5 mL ) at 0 °C for 10 min followed by addition of benzyl bromide (0.81 mL, 6.92 mmol) and tetrabutylammonium iodide (0.12 g, 0.34 mmol). The reaction mixture was warmed to room temperature, stirred for 30 min., refluxed at 80 °C for 48 h and decomposed with saturated solution of NH$_4$Cl (1 mL). Usual workup and column chromatography on silica gel afforded 193 (1.09 g, 68%) as a white solid. mp 96-97 °C.

$R_f = 0.70$ (n-hexane ethyl/acetate= 19/1).
[α]₀ = +48.57 (c 0.7, CHCl₃).

IR (KBr) 1452, 1369, 1124, 1074, 698 cm⁻¹.

¹H NMR (300 MHz, CDCl₃): δ 1.23 (s, 3H, CH₃), 1.45 (s, 3H, CH₃), 3.21 (ddd apparent q, J = 6.6, 6.3, 5.7 Hz, 1H, H-6), 3.39 (d, J = 5.4 Hz, 1H, H-3), 3.55 (dd, J = 9.3, 6.3 Hz, 1H, H-7a), 3.80 (dd, J = 9.3, 5.7 Hz, 1H, H-7b), 3.83 (d, J = 14.0 Hz, 1H, N-CH₂Ph), 3.93 (dd, J = 6.6, 4.8 Hz, 1H, H-5), 4.00 (d, J = 14.0 Hz, 1H, N-CH₂Ph), 4.15 (d, J = 3.6 Hz, 1H, H-2), 4.45 (AB quartet, J = 12.0 Hz, 2H, O-CH₂Ph), 4.53 (d, J = 12.0 Hz, 1H, O-CH₂Ph), 4.75 (d, J = 12.0 Hz, 1H, O-CH₂Ph), 4.78 (dd, J = 5.4, 4.8 Hz, 1H, H-4), 5.85 (d, J = 3.6 Hz, 1H, H-1), 7.20-7.40 (m, 15H, Ar-H).

¹³C NMR (75 MHz, CDCl₃): δ 26.6 (CH₃), 27.5 (CH₃), 57.7 (N-CH₂Ph), 65.0 (C-3), 70.8 (C-6), 71.3 (C-7), 72.7 (OCH₂Ph), 73.2 (OCH₂Ph), 78.0 (C-5), 82.3 (C-4), 84.8 (C-2), 107.2 (C-1), 112.0 (OCO), 127.0, 127.3, 127.5, 127.7, 128.1, 129.2, 137.7, 138.0, 138.3 (Ar-C).

Anal. Calcd. for C₃₁H₅₅NO₇: C, 74.23; H, 7.03. Found: C, 74.22; H, 7.00.

Expt. No. 2.7.13: Preparation of 2,5-dideoxy-2,5-imino-1,3-di-O-benzyl-L-glycero-α-D-galacto-heptitol (194).

A cooled solution of compound 193 (0.60 g, 1.19 mmol) in TFA-H₂O (5 mL, 3:2) was stirred at 0°C for 1h and allowed to warm up to 25 °C and stirred for 2 h. TFA was evaporated at high vacum to yield crude hemiacetal (0.63 g). A solution of crude hemiacetal in dry THF (5 mL) was added to an ice cooled suspension of LAH (0.09 g, 2.39 mmol) in dry THF (3 mL) over a period of 10 min. under nitrogen atmosphere. The reaction mixture was stirred at room
temperature for 6 h. Work up as in case of 193 (LAH reduction) and column chromatography on silica gel (n-hexane/ethyl acetate = 3/7) afforded 194 as a thick liquid (0.45 g, 81%).

Rf 0.40 (n-hexane/ethyl acetate = 9/1).

[α]D = +3.07 (c 0.65, CHCl3).

IR (neat) 3550-3100 (broad OH), 1639, 1456, 1369 cm⁻¹.

1H NMR (300 MHz, CDCl3 + D2O): δ 3.02-3.12 (m, 2H, H-3, H-7a), 3.16 (ddd, J = 8.1, 4.2, 2.1 Hz, 1H H-6), 3.36 (dd, J = 2.7, 9.3 Hz, 1H, H-7b), 3.58 (d, J = 13.5 Hz, 1H, N-CH2Ph), 3.66 (dd, J = 11.4, 4.5 Hz, 1H, H-1a), 3.77 (dd, J = 11.4, 4.5 Hz, 1H, H-1b), 3.96 (ddd apprant q, J = 9.6, 4.8, 4.5 Hz, 1H, H-2), 4.01 (d, J = 13.5 Hz, 1H, N-CH2Ph), 4.05 (dd, J = 4.8, 8.1 Hz, 1H, H-5), 4.22 (dd, J = 5.1, 4.8 Hz, 1H, H-4), 4.44 (AB quartet, J = 12.0 Hz, 1H, O- CH2Ph), 4.45 (d, J = 11.7 Hz, 1H, O- CH2Ph), 4.68 (d, J = 11.7 Hz, 1H, O- CH2Ph), 7.20-7.40 (m, 15H, Ar-H).

13C NMR (75 MHz, CDCl3 + D2O): δ 60.3 (N-CH2Ph), 63.3 (C-2), 64.8 (C-5), 67.1 (C-7), 68.4 (C-1), 69.4 (C-6), 69.9 (C-4), 71.9 (O-CH2Ph), 73.5 (O-CH2Ph), 77.7 (C-3), 127.2, 127.5, 127.6, 127.8, 129.7, 137.0, 137.7, 138.5 (Ar-C).


Expt. No. 2.7.14: Preparation of 2,5-Dideoxy-2,5-imino-L-glycero-α-D-galacto-heptitol (154).

A solution of 194 (0.10 g, 0.21 mmol) and 10% Pd/C (0.01 g) in dry MeOH (2 mL) was hydrogenolyzed at 80 psi for 24 h. The solution was filtered through celite-540, evaporated and
purified by column chromatography on silica gel (methanol/chloroform = 9/1) to give 154 (0.035 g, 85%) as a thick liquid.

R_f = 0.10 (MeOH).

[α]_D = 86.66 (c 0.6, H_2O).

IR (nujol) 3200-3600 (broad) cm⁻¹.

^1H NMR (300 MHz, D_2O): δ 3.27 (dd, J = 6.0, 5.7 Hz, 1H, H-5), 3.46 (ddd apprapt q, J = 6.6, 6.4, 5.8 Hz, 1H, H-2), 3.63 (dd, J = 12.0, 6.6 Hz, 1H, H-7a), 3.72-3.78 (m, 2H, H-7b, H-1a), 3.83 (dd, J = 11.4, 5.1 Hz, 1H, H-1b), 3.97 (ddd, J = 9.9, 6.3, 3.3 Hz, 1H, H-6), 4.29 (dd, J = 5.7, 4.8 Hz, 1H, H-4), 4.35 (dd, J = 6.6, 4.8 Hz, 1H, H-3).

^13C NMR (75 MHz, D_2O): δ 62.1 (C-1), 62.3 (C-7), 62.4 (C-2), 65.8 (C-5), 71.9 (C-6), 73.7 (C-3), 73.7 (C-4).

Anal. Calc. for C_{17}H_{23}NO_5 (193.2): C, 43.52; H, 7.83. Found: C, 43.50; H, 7.81.

Expt. No. 2.7.15: Preparation of 2,5-dideoxy-2,5-imino-(N-benzyl)4,5-di-O-benzyl-D-galactitol (196).

A reaction of 194 (0.40 g 0.79mmol) with TFA-water (3:2, 4 mL) as described for 194 afforded a thick liquid. To the cooled solution of crude hemiacetal (0.35 g) at 0 °C in acetone: water (5:1, 10 mL) was added sodium metaperiodate (0.25 g, 1.19 mmol). After stirring for 1.5 h at 0 °C the excess sodium metaperiodate was decomposed using ethylene glycol (0.20 mL). Usual workup and purification by column chromatography on silica gel (n-hexane/ethyl acetate = 3/2) afforded aldehyde as a thick liquid. A solution of aldehyde (0.32 g) in methanol (6 mL) was
reacted with NaBH₄ (0.04 g 1.19 mmol) at 0 °C. for 2 h. Usual workup and column chromatography (n-hexane/ethyl acetate = 1/1) afforded 196 (0.17 g, 52%) as a thick liquid. 

Rᵣ = 0.45 (n-hexane/ethyl acetate = 3/2).

[α]₀ = + 8.88 (c 0.45, CHCl₃).

IR (neat) 3550-3100, (broad OH), 1639, 1456 cm⁻¹.

¹H NMR (300 MHz, CDCl₃ + D₂O): δ 3.00-3.24 (m, 3H), 3.40 (dd, J = 10.0, 1.8 Hz, 1H), 3.60-3.90 (m, 2H), 3.90-4.15 (br s, 3H), 4.15-4.30 (br s, 1H), 4.30-4.50 (br s, 3H), 4.60-4.70 (d, J = 12 Hz, 1H), 7.05-7.45 (m, 15H, Ar-H).

¹³C NMR (75 MHz, CDCl₃): δ 60.3 (N-CH₃Ph), 63.4/64.8 (C-2/C-5), 67.1 (C-6), 68.4 (C-1), 69.9 (C-4), 71.9 (O-CH₂Ph), 73.5 (O-CH₂Ph), 77.7 (C-3), 127.3, 127.5, 127.6, 127.9, 128.2 (strong), 128.8, 137.0, 137.7, 138.3 (Ar-C).

Anal. Calcd. for C₂₇H₃₁NO₄ (433.54); C, 74.80; H, 7.21. Found; C, 74.74; H, 7.20.

The assignment of protons was difficult due to broadening of the signals.

Expt. No. 2.7.16: Preparation of 2,5-dideoxy-2,5-imino-D-galactitol (84).

The hydrogenolysis of compound 196 (0.12 g, 0.28 mmol) in dry MeOH (2 mL) with 10% Pd/C (0.03 g) in MeOH (1 mL) as in case of 154 and column chromatography on silica gel (methanol/chloroform = 9/1) afforded 84 (0.039 g, 83%) as a thick liquid.

Rᵣ = 0.10 (MeOH).

IR (nujol) 3200-3600 (broad) cm⁻¹.

133
$^1$H NMR (300 MHz, D$_2$O): $\delta$ 3.75-3.83 (m, 2H, H-2, H-5), 3.92 (dd, $J = 12.3$, 8.4 Hz, 2H, H-1a, H-6a), 4.01 (2H, dd, $J = 12.3$, 5.1 Hz, H-1b, H-6b) 4.50 (dd, $J = 1.5$, 4.5 Hz, 2H, H-3, H-4);

$^{13}$C NMR (75 MHz, D$_2$O): $\delta$ 60.2 (C-1, C-6), 63.8 (C-2, C-5), 72.3 (C-3, C-4).

Anal. Calcd. for C$_6$H$_{13}$NO$_4$ (163): C, 44.16; H, 8.03. Found: C, 44.17; H, 8.05.

Expt. No. 2.7.17: Preparation of 2,5-Dideoxy-2,5-imino-D-galactitol. Hydrochloride (84.HCl).

![Chemical structure of 84](image)

A solution of 84 (0.015 g, 0.09 mmol) in methanolic hydrochloric acid (5 mL) was stirred under nitrogen at 0 °C for 3 h. The reaction mixture was concentrated on vacuum afforded 84.HCl (0.016 g, 89%) as a semi solid.

IR (nujol) 3200-3600 (broad) cm$^{-1}$.

$^1$H NMR (300 MHz, D$_2$O): $\delta$ 3.73-3.81 (m, 2H, H-2, H-5), 3.90 (dd, 2H, $J = 8.1$, 12.0 Hz, H-1a, H-6a), 4.01(dd, $J = 4.8$, 12.0 Hz, 2H, H-1b, H-6b), 4.48 (d, $J = 5.1$ Hz, 2H, H-3, H-4).

$^{13}$C NMR (75 MHz, D$_2$O): $\delta$ 57.8 (C-3, C-4), 61.4 (C-2, C-5), 70.0 (C-1, C-6).


A reaction of 193 (0.50 g 0.99 mmol) with TFA-water (3:2, 5 mL) afforded hemiacetal as thick liquid which on oxidative cleavage with NaI\textsubscript{2}O (0.32 g 1.50 mmol) as described for 196 gave amino aldehyde. To a stirred solution of aldehyde (0.43 g, 0.99 mmol) in dry dichloromethane (5 mL) was added PPh\textsubscript{3}=CHCOOEt (0.52 g, 1.50 mmol) and stirred for 2 h at 25 °C under nitrogen atmosphere. The reaction mixture was purified by column chromatography on silica (n-hexane/ethyl acetate = 9/1) afforded major cis- isomer 242a (0.27 g, 55%) as thick oil. R\textsubscript{f} = 0.70 (n-hexane ethyl acetate= 4/1).

[\alpha] \textsubscript{D} = -24.0 (c 1.0, CHCl\textsubscript{3}).

IR neat 3550-3100 (broad), 1721, 1651 cm\textsuperscript{-1}.

\textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) δ 1.22 (t, 3H, J = 7.2 Hz OCH\textsubscript{2}CH\textsubscript{3}), 2.95 (dd, J = 9.6, 3.3 Hz, 1H, H-1a), 3.15 (ddd, J = 4.8, 3.3, 1.8 Hz, 1H, H-2), 3.40 (dd, J = 9.6, 1.8 Hz, 1H, H-1b), 3.58 (d, J = 13.8 Hz, 1H, N-CH\textsubscript{2}Ph), 3.88 (d, J = 13.8 Hz, 1H, N-CH\textsubscript{2}Ph), 4.10-4.22 (m, 3H, O-CH\textsubscript{2}Ph), 4.30 (t, J = 3.9 Hz, 1H, H-4), 4.38 - 4.52 (m, 4H, O-CH\textsubscript{2}Ph, H-5), 4.74 (d, J = 11.4 Hz, 1H, O-CH\textsubscript{2}Ph), 5.92 (dd, J = 11.4, 0.9 Hz, 1H, H-7), 6.45 (dd, J = 11.4, 8.4 Hz, 1H, H-6), 7.10-7.40 (m, 15H, Ar-H).

\textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}): δ 14.2 (OCH\textsubscript{2}CH\textsubscript{3}), 57.3 (N-CH\textsubscript{2}Ph), 60.3 (OCH\textsubscript{2}CH\textsubscript{3}), 63.7 (C-2), 67.6 (C-5), 67.9 (C-1), 71.3 (OCH\textsubscript{2}Ph), 72.0 (C-4), 73.6 (OCH\textsubscript{2}Ph), 78.0 (C-3), 123.7 (C-135)
Further elution (n-hexane/ethyl acetate = 87/13) afforded minor trans isomer 242b (0.10 g, 21%) as thick liquid.

ref 0.68 (n-hexane ethyl acetate = 4/1).

[α]D = -15.38 (c 0.65, CHCl3).

IR neat 3550-3100 (broad), 1717, 1645 cm⁻¹.

¹H NMR (300 MHz, CDCl₃) δ 1.42 (t, 3H, J = 7.2 Hz OCH₂CH₃), 2.98 (dd, J = 9.6, 3.3 Hz, 1H, H-1a), 3.15-3.22 (m, 1H, H-2), 3.38 (dd, J = 7.5, 3.9 Hz, 1H, H-5), 3.40 (dd, J = 9.6, 1.8 Hz, 1H, H-1b), 3.50 (d, J = 13.5 Hz, 1H, N-CH₂Ph), 3.88 (d, J = 13.5 Hz, 1H, N-CH₂Ph), 4.08 (dd, J = 5.1, 4.2 Hz, 1H, H-3), 4.12 (t, J = 4.2 Hz, 1H, H-4), 4.20 (q, J = 7.2 Hz, 2H, O-CO₂CH₃), 4.46 (d, J = 11.7 Hz, 1H, O-CH₂CH₂), 4.48 (ABq, J = 12 Hz, 2H, O-CH₂Ph), 4.73 (d, J = 11.7 Hz, 1H, O-CH₂Ph), 4.82 (broad, exchanges with D₂O, 1H, OH), 6.09 (dd, J = 15.6, 7.5 Hz, 1H, H-7), 7.30 (dd, J = 15.6, 0.9 Hz, 1H, H-6), 7.12-7.40 (m, 15H, Ar-H).

¹³C NMR (75 MHz, CDCl₃) δ 14.0 (OCH₂CH₃), 57.5 (N-CH₂Ph), 60.2 (OCH₂CH₃), 63.8 (C-2), 64.4 (C-5), 68.0 (C-1), 71.5 (OCH₂Ph), 72.0 (C-4), 74.2 (OCH₂Ph), 78.3 (C-3), 121.7 (C-7), 127.3, 127.7, 128.4, 128.9, 129.3, 137.5, 138.1, 139.4 (Ar-C), 151.0 (C-6), 171.2 (COOEt).

Anal. Calcd. for C₃₁H₃₅NO₅: C, 74.23; H, 7.03. Found: C, 74.47; H, 7.24.

Expt. No. 2.7.19: Preparation of ethyl 3-((2R,3S,4R,5S)-3,4-dihydroxy-5 (hydroxymethyl) pyrrolidin-2-yl)propanoate (245).
To a solution of 242a/b (0.35 g, 0.69 mmol) in methanol (5 mL) was added ammonium formate (0.264 g, 4.19 mmol) and 10% Pd/C (0.05 g). The reaction mixture was heated at 80 °C for 3 h, filtered through celite and the filtrate was evaporated. The reaction mixture was purified by column chromatography on silica (methanol/ chloroform = 40/60) afforded 245 (0.034 g, 21%) as thick liquid.

R_f = 0.30 (Methanol/chloroform = 3/2).

IR neat 3550-3100 (broad), 1737 cm⁻¹.

^1H NMR (300 MHz, D₂O): δ 1.28 (t, 3H, J = 6.9 Hz OCH₂CH₃), 1.82-2.19 (m, 2H, H-6), 2.52 (t, J = 7.5 Hz, 2H, H-7), 3.26-3.80 (m, 1H, H-5), 3.51-3.61 (m, 1H, H-2), 3.79 (dd, J = 12.0, 5.1 Hz, 1H, Jα), 3.87 (dd, J = 12.0, 4.5 Hz, 1H, Jα), 4.18 (q, J = 7.2 Hz, 2H, O-CH₂CH₃), 4.25 (t, J = 4.2 Hz, 1H, H-4), 4.48 (dd, J = 5.1, 7.5 Hz, 1H, H-3).


A reaction of 193 (0.50 g 0.99 mmol) with TFA-water (3:2, 5 mL) afforded hemiacetal as thick liquid which on oxidative cleavage with NaIO₄ (0.32 g 1.50 mmol) as described for 194 gave amino aldehyde. To a stirred solution of crude aldehyde (0.43 g, 0.99 mmol) in dry acetonitrile (5 mL) was added PPh₃CHOCH₃ (0.480 g, 1.50 mmol) and stirred for 4 h at 25 °C under nitrogen atmosphere. The crude reaction mixture was purified by column chromatography on
silica (n-hexane/ethyl acetate = 90/10) afforded α,β-unsaturated methyl ketone 246 (0.32 g, 70%) as thick liquid.

Rf = 0.7 (n-hexane ethyl/acetate= 3/2).

IR neat 3550-3100 (broad), 1704, 1640 cm⁻¹.

$^1$H NMR (300 MHz, CDCl₃) δ 2.20 (s, 3H, COCH₃), 3.1 (d, J = 9.6, 1H, H-1a), 3.2 (d, J = 7.8, 1H, H-5), 3.32 (m, 1H, H-2), 3.45 (d, J = 9.6, 1H, H-1b), 3.59 (d, J = 13.8 Hz, 1H, N-CH₂Ph), 3.80 (d, J = 13.8 Hz, 1H, N-CH₂Ph), 4.08 (t, J = 4.5 Hz, 1H, H-4), 4.10- 4.15 (m, 1H, H-3), 4.48 (d, J = 11.7 Hz, 1H, O-CH₂Ph), 4.49 (AB q, J = 11.7 Hz, 1H, O-CH₂Ph), 4.72 (d, J = 11.7 Hz, 1H, O-CH₂Ph), 6.13 (d, J = 16.2 Hz, 1H, H-7), 6.72 (dd, J = 16.2, 7.8 Hz, 1H, H-6), d 7.15-7.36 (m, 15H, Ar-H).

$^{13}$C NMR (75 MHz, CDCl₃): δ 26.2 (COCH₃), 57.4 (C-2), 63.9 (N-CH₂Ph), 67.8 (C-5), 68.2 (C-1), 71.3 (C-3), 72.3 (OCH₂Ph), 73.6 (OCH₃Ph), 77.9 (C-4), 127.1, 127.3, 127.5, 127.7, 128.1, 128.2, 128.3, 128.8, 129.0 (Ar-C), 132.9 (C-7), 137.3, 137.7, 138.3 (Ar-C), 147.0 (C-6), 198.6 (CO).

2.8 References


