Synthesis of Protodioscin

A Thesis

submitted to

Indian Institute of Science Education and Research, Pune

in partial fulfillment of the

requirements for the

BS-MS Dual Degree Programme

by

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April 2023

Certificate

This is to certify that this dissertation entitled "Synthesis of Protodioscin" towards the partial fulfilment of the BS-MS dual degree program at the Indian Institute of Science Education and Research, Pun represents study/work carried out by Aritra Das at Indian Institute of Science Education and Research, Pune under the supervision of Prof. Srinivas Hotha, Department of Chemistry during the academic year 2022-2023

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Declaration

I hereby declare that the matter embodied in the report entitled "Synthesis of Protodioscin" is the work carried out by me at the Department of Chemistry, Indian Institute of Science Education and Research, Pune, under the supervision of Prof. Srinivas Hotha and the same has not been submitted elsewhere for any other degree.

Aritra Das

To my father, Mr. Asit Kumar Das for his unwavering support, guidance, and inspiration throughout my academic journey, I dedicate this thesis.

Acknowledgements

The success and ultimate end of this project necessitated a great deal of direction and support from a large number of people, and I am lucky to have received this during the course of my project work. Everything I've accomplished has been made possible by their direction and aid, and I'll never forget to thank them. I owe a huge debt of gratitude to Prof. Srinivas Hotha, my project mentor, for allowing me to work in his lab. Through his constant support, counsel, and motivation, he has greatly inspired me throughout my work tenure. I owe a great deal of appreciation to my senior, Mr Sumit Sen, who took a personal interest in assisting me with my project work and guiding me through it to completion, as well as giving continuous information and assistance. He has been there for me through all the difficulties I faced. Moreover, through his guidance, I could gather keen knowledge and understanding of the project. I am grateful to my fellow labmates (Mr Bodhayan Biswas, Ms Jayashree Rajput, Mr Anish Das, Ms Ankita Sharma, Mr Prashant, Mr Kameshwar Prasad, Mr Pratim Das, Mr Suman Kundu and Ms Pooja Joshi) for assisting me and encouraging my interest in experimental work. I'd want to thank them for their dedication and guidance throughout the project and for planning, supporting, and implementing it. Without their help, I would not have been able to bring my project to a successful conclusion. I express my heartfelt gratitude and best wishes to all of my friends for their unwavering support and constant encouragement and for making my job more fascinating by creating a warm and welcoming atmosphere throughout my time at IISER Pune. Last but not least, I'd want to express my gratitude to all non-teaching personnel for providing me with the most pleasant environment throughout my time at IISER. Without the excellent scientific and learning environment offered by IISER Pune, I would not have been able to complete this study successfully. Above all, I'd like to express my gratitude to my parents and brother for their support throughout my career.

Contents

I.Abbreviations

NMR	Nuclear magnetic Resonance
Hz	Hertz
MHz	Mega Hertz
EtOAc	Ethyl acetate
AcCl	Acetyl Chloride
Bn	Benzyl
Bz	Benzoyl
$CDCl_3$	Chloroform-d
$CHCl_3$	Chloroform
DCM	Dichloromethane
cat.	catalytic
DMAP	N,N- Dimethylaminopyridine
DMF	N,N-Dimethyl Formamide
Et_3N	Triethyl Amine
g	gram

Kg	Kilogram
TBDPS	t-Butyldiphenylsilyl
DEPT	Distortion less Enhancement by Polarization Transfer
Py	Pyridine
mL	mililitre
mmol	milimolar

II. Abstract

Glycosylation is a fundamental reaction in organic chemistry that involves the attachment of a sugar moiety to a non-sugar molecule, such as a steroid or a protein. This ubiquitous biological process is observed across all living organisms and performs vital functions such as facilitating cell-cell recognition, signal transduction, and immune responses. Additionally, glycosylation can modulate the properties of biomolecules, such as their solubility, stability, and bioactivity, making it a valuable tool in drug discovery and development.

Protodioscin is a natural steroid saponin that has attracted attention for its pharmacological properties, which include anti-inflammatory, anti-cancer, and anti-diabetic effects. However, its isolation from natural sources is complex and inefficient, making chemical synthesis an attractive alternative. In this study, I developed a gold-catalyzed glycosylation approach for synthesising protodioscin from readily available starting materials. The synthesis involved using a gold(I) catalyst and a glycosyl donor to selectively activate the C-3 position of a steroid precursor, followed by regio- and stereoselective glycosylation at the C-26 position. The reaction was optimised using a range of reaction conditions. My results demonstrate an elementary, proficient, and scalable approach for the synthesis of protodioscin, which has the potential to facilitate its further investigation as a therapeutic agent.

1. Introduction

Protodioscin is a natural compound found in certain plants, particularly in the Tribulus Terrestris plant. It belongs to the class of steroidal saponins, which are known for their potential health benefits. Protodioscin has been studied for its numerous health-promoting effects, including its ability to boost testosterone levels, improve sexual function, enhance muscle growth and strength, and provide antioxidant and anti-inflammatory benefits. Due to its potential benefits, protodioscin is often used in dietary supplements and sports nutrition products. Nevertheless, further investigation is imperative to comprehensively comprehend its mode of operation and plausible adverse reactions

Structure:

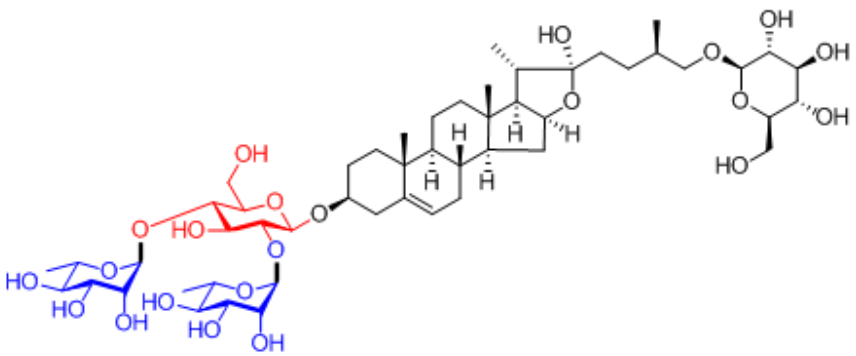


Figure 1.1

Chemical Properties:

Protodioscin is a steroidal saponin from the furostanol type. It has the chemical formula $C_{51}H_{84}O_{22}$ and a molecular weight of 1049.2 g/mol. The structure of protodioscin consists of a steroid backbone, which is attached to a carbohydrate chain. The carbohydrate chain comprises two glucose molecules, one rhamnose molecule, and one xylose molecule. The steroid backbone contains a furan ring and a lactone ring, which are essential for its biological activity.

Sources:

Protodioscin is found in several plants, including Tribulus Terrestris, Dioscorea villosa, and Trigonella foenum-graecum. However, Tribulus terrestris is the primary source of protodioscin. Tribulus Terrestris is a small leafy plant that grows in various regions worldwide, including Asia, Europe, and Africa. For centuries, it has been utilised in conventional medicine due to its potent aphrodisiac and fertility-amplifying characteristics.

Potential Health Benefits:

Protodioscin has been extensively studied for its potential health benefits. One of the most well-known effects of protodioscin is its ability to boost testosterone levels. Protodioscin is assumed to accomplish this mostly through increasing androgen receptor immunoreacivity, or the number of androgen receptors present in cells, making the body more susceptible to androgens like testosterone and dihydrotestosterone (DHT)(1). According to a research published in the Journal of Ethnopharmacology, researchers found that protodioscin could significantly increase testosterone levels in rats. The researchers suggested that this effect may be due to protodioscin's ability to stimulate the production of luteinising hormone, which is a hormone that signals the testes to produce testosterone (2).

Besides its capacity to enhance testosterone levels, protodioscin has demonstrated the potential to ameliorate sexual function. According to a research article published in the journal “Phytomedicine”, administering protodioscin supplements enhanced erectile function in men afflicted with mild to moderate erectile dysfunction. The researchers suggested that this effect may be due to protodioscin's ability to increase nitric oxide levels, which relaxes the blood vessels and improve blood flow to the penis. (3)

Protodioscin has also been studied for its potential effects on muscle growth and strength. A study published in the “Journal of Sport and Health Science” found that supplementation with protodioscin improved muscle strength and endurance in healthy men. The researchers suggested that this effect may be due to protodioscin's ability to increase protein synthesis and decrease muscle damage. (4)In a study on male athletes, supplementation with a fenugreek extract containing protodioscin resulted in improved muscle endurance and power output, which may be attributed to increased NO (nitric oxide) production (7).

In addition to its effects on testosterone levels, sexual function, and muscle growth, protodioscin has also been shown to have antioxidant and anti-inflammatory effects. A research article published in the "Journal of Agricultural and Food Chemistry" shows that protodioscin has potent antioxidant activity, which may help to protect cells against oxidative damage. (5) Another study published in the journal Planta Medica found that protodioscin had anti-inflammatory effects, which may help to reduce inflammation in the body. (6)

Overall, protodioscin is a natural compound with numerous potential health benefits. Although further investigation is necessary to comprehensively comprehend its mode of operation and plausible adverse reactions, the existing evidence proposes that it holds great potential as a compound to augment general health and well-being.

Glycosylation:

The process of glycosylation is characterised by the covalent attachment of carbohydrate molecules to a targeted macromolecule, commonly proteins or lipids (16). This process is one of the most ubiquitous, heterogenous, non-templated, and stereo-chemically complex post-translational modifications with many important structural and biophysical roles. (17,18). A essential step in numerous biological activities, including but not limited to the process of fertilization, viral replication, parasite infection, cell development, adhesion among cells, and inflammation, is the installation of glycans by glycosylation. It is a susceptible process, and any alteration will lead to abnormal glycosylation, which can result in various diseases, including cancer and other auto-immune diseases. One of the critical functions of glycosylation is in the recognition and clearance of proteins by the immune system. Many glycoproteins are recognised by lectins on the surface of immune cells, which can trigger phagocytosis or other immune responses. Alterations in glycosylation patterns results in various diseases, including cancer and autoimmune disorders.

Most glycans are generally attached to either protein (glycoprotein) or lipid (glycolipid), although they can be present in the cell without being attached to any other molecule.

Major types of glycosylations and their importance:

Glycosylation is mainly of two types: N-linked glycosylation and O-linked

glycosylation. (8)

a) N-linked glycosylation- The process of N-linked glycosylation involve a glycan adhesion to the nitrogen atom of an asparagine residue that is present within the protein sequence.(8) It is widespread in eukaryotic cells but scarce in bacterial cells. It has essential functions like providing components to the cell wall and ECM, modulation of protein properties (solubility and stability), trafficking of glycoproteins, and mediation of cell-cell signalling. This process entails the gradual incorporation of sugar residues into an expanding oligosaccharide chain that is linked to the protein in a stepwise manner(8). The initial step entails the transfer of an already assembled oligosaccharide from a lipid carrier to the protein present in endoplasmic reticulum (ER) by the oligosaccharyltransferase (OST) enzyme complex (9). The oligosaccharide then undergoes further processing by various glycosidases and glycosyltransferases to form a mature N-glycan (8). Subsequently, the fully formed N-glycan undergoes transportation to the Golgi apparatus, where a multitude of enzymes further alter it to produce a wide array of distinct glycan structures(8)(9).
b) O-linked glycosylation- This is a process where a glycan is linked to the oxygen atom of either a serine or threonine residue within the protein sequence(8). The process of adding single sugar molecules to a protein usually starts by attaching the first sugar to a serine or threonine amino acid residue, which is often marked (8) indicating the specific position in the protein sequence. This process is known as O-glycosylation, and it can result in the formation of complex sugar structures on the protein surface that may impact its stability, functionality, and interactions with other cellular molecules.The addition of subsequent sugar residues is catalysed by different glycosyltransferases, resulting in a diverse range of O-glycan structures (8).
c) Glypiation- It is a unique type of glycosylation where a protein is affixed to the lipid anchor by a glycan chain to form a GPI(glycosylphosphatidylinositol) anchor. A phosphoethanolamine linker part of GPI binds to the C-end of a particular designated protein. In contrast, the phospholipid tail present in the glycan core structure

anchors the protein to the membrane. It has various sports functions like cell adhesion, signal transduction and immune recognition.

In addition to the above-mentioned glycosylation processes, C-mannosylation is another type where a mannose sugar is covalently linked to a tryptophan residue by a C-C bond, as specified by notation (19). This type of glycosylation is unique and distinct from the N- and O-linked glycosylation, is also observed in living organisms. Another type of glycosylation called phospherine glycosylation (20) is also observed in living organisms.

Chemical Glycosylation:

There are several chemical methods for glycosylation, including direct glycosylation, glycosylation using glycosyl donors, and enzymatic glycosylation (10). Glycosylations using glycosyl donors can be described as a nucleophilic displacement of a leaving group attached to a glycosyl donor's anomeric carbon via certain promoters' action (Lewis acid, Bronsted acid, neutral reagents, inorganic salts etc.)(21). After the leaving group's displacement, an oxocarbenium ion forms,which is an intermediate then it is attacked by a glycosyl acceptor(nucleophile) to form a new glycosidic bond.

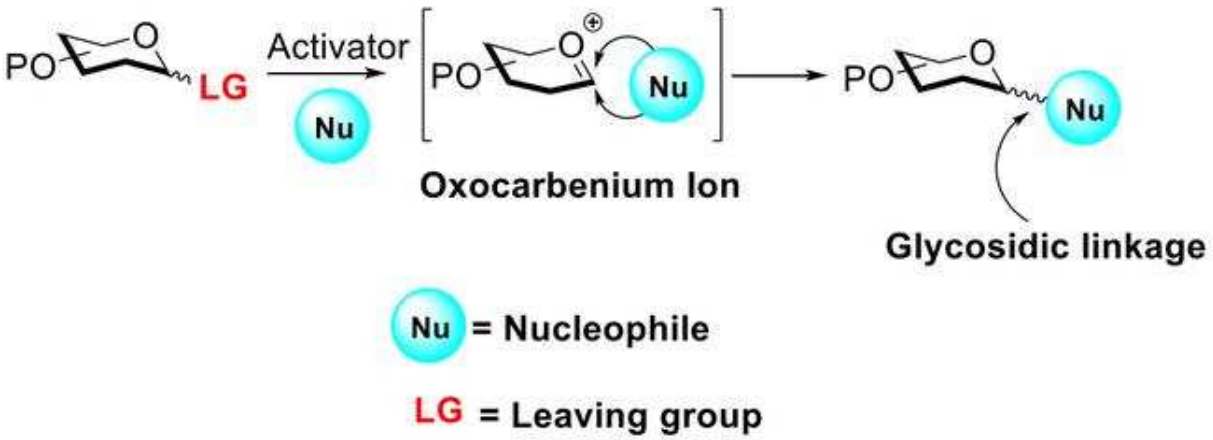


Figure 1.2

Chemical glycosylation has several advantages over enzymatic glycosylation, including the ability to synthesise glycoconjugates that are not accessible through enzymatic methods (11). Chemical glycosylation

also allows for the introduction of non-natural sugar molecules into glycoconjugates, which can be used to study the biological functions of specific sugar moieties(10)

Depending Factors for Chemical Glycosylation:

a) Anomeric effect: The cyclohexane system with chair conformation with all the substituents in the equatorial positions is the most stable conformation. It is true in the case of 1-hydroxy sugar moieties where **β**-conformer is more stable due to the intramolecular hydrogen bonding with O-5 ring oxygen. But if it gets replaced by OR/SR/halides or any other polar group, it prefers the axial conformation at the anomeric centre due to the dipole-dipole interaction.

b) Nature of glycosyl donors and acceptors: It can significantly impact the efficiency and selectivity of chemical glycosylation. Several kinds of glycosyl donors, for example glycosyl halides or sulfates, are used, and the choice of the donor can affect the reactivity and selectivity of the reaction. For example, glycosyl iodides are generally more reactive than glycosyl chlorides or bromides (12). The choice of glycosyl donor can also affect the regioselectivity of the glycosylation reaction, with some donors favouring the formation of α-linkages while others favour β-linkages (13). The direction of the leaving group is irrelevant when glycosylation reactions take place using an S_N1 mechanism but if it proceeds via S_N2 mechanism, an inversion of the stereo configuration occurs at the anomeric centre.

c) Protecting group: Protection and deprotection of the hydroxyl groups are very important for synthesising oligosaccharides. It is used to protect reactive functional groups and prevent unwanted reactions. It is also used to mask similar reactive sites. The intrinsic properties of the protecting group also affect glycosylation, which results in the formation of the concept of neighbouring group participation.

d) Reaction conditions: Solvents are very important for glycosylation reactions; polar solvents produce 1,2 trans-glycosides, whereas non-polar solvents favour 1,2 cis-glycosides. Along with this pressure, temperature, presence of chiral auxiliary protecting group, presence

of glycosyl acceptor tethering group, and addition of exogenous nucleophilic additive can also influence the outcome of glycosylation reaction. (22,23).

Different glycosidic linkages:

The formation of a glycosyl cation occurs once the leaving group is activated and released. The glycosyl cation becomes stable in molecules with non-participating groups by resonance originating from O-5. This leads to the development of an oxocarbenium ion (see figure 1.3) intermediate. The anomeric carbon in this intermediate is sp2 hybridised, which enables the nucleophile to attack from either side equally. The formation of trans, β-glycoside is facilitated by an attack from the top, while an attack from the bottom results in cis, α-glycoside. The participation of a neighbouring group provides anchimeric assistance, leading to a stereoselective 1,2-trans glycosidic linkage.

The production of the glycosyl cation is aided by an activator's assistance in removing the leaving group. The glycosyl cation is intramolecularly stabilised, creating an acyl oxonium ion (see figure 1.4), which is a bicyclic intermediate formed during the glycosidation process. The ring in this intermediate can only be attacked from the top side (route c), which results in the synthesis of 1,2-trans glycoside. However, minor products with 1,2-cis linkage can also be formed (pathway a,b)

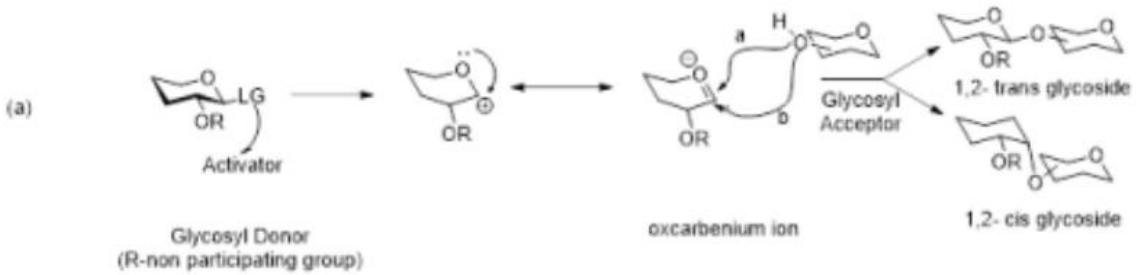


Figure 1.3

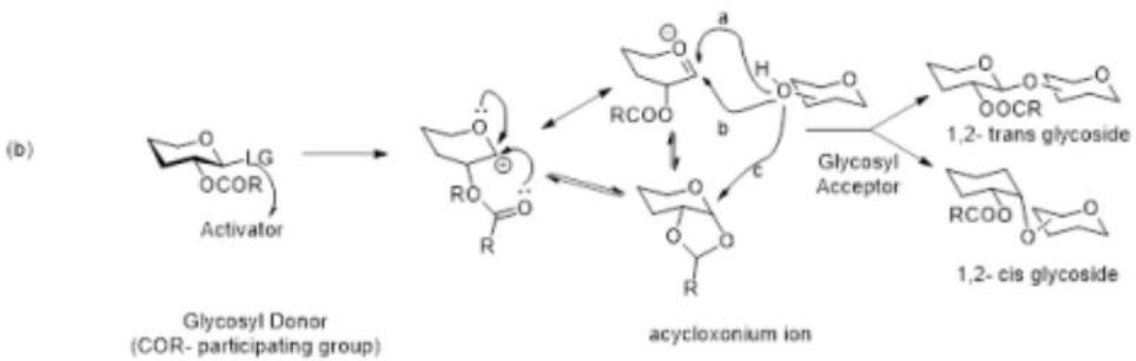


Figure 1.4

1,2-trans glycosides

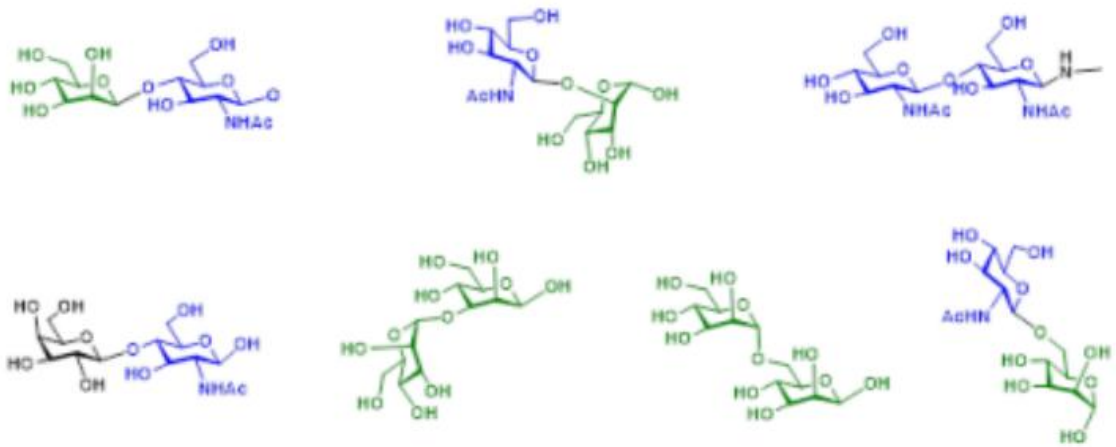


Figure 1.5

1,2-trans glycoside forms when C-1 and C-2 positions of both groups are non-coplanar. This kind of linkage is relatively simple because of the adjacent group participation at the C-2 position of the glycosyl donor.

1,2-cis glycosides

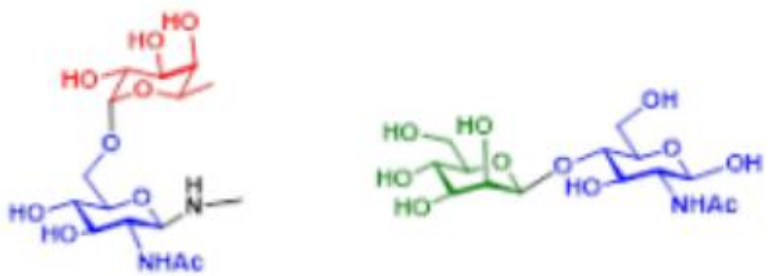


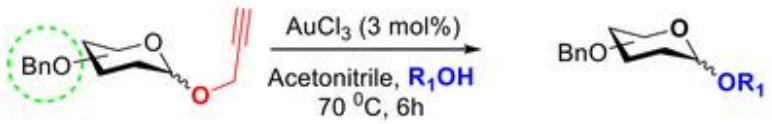
Figure 1.6

When the groups binded to both the positions of C-1 and C-2 are coplanar, the resulting linkage is known as a 1,2-cis linkage (as shown in fig. 1.6). However, the development of 1,2-cis glycosides is considerably more strenuous. One of many factors that can lead to the construction of 1,2-cis glycosides for glucosyl donors having a non-participating neighbouring group is the anomeric effect. Although achieving 1,2-cis stereoselectivity through variations in structural elements or specific

reaction conditions is possible, a comprehensive method for achieving 1,2-cis glycosidation has not yet been developed.

Gold catalysis in Glycosylation:

Hotha and Kashyap were motivated by Hashmi's work, which demonstrated the exceptional alkynophilicity of gold catalysts, and investigated the benefits of using propargyl glycosides as glycosyl donors. Propargyl glycosides are straightforward to prepare, stable, require only a small amount of activator, produce minimal side products, and are harmonious with an extensive variety of aglycons. These characteristics make propargyl glycosides an attractive candidate for serving as an optimal glycosyl donor. Using propargyl glycosides has opened up a fascinating new area of glycochemistry. The transglycosylation process, which utilises [Au] catalyst, is a reliable method for synthesising glycosides.



(Hotha, 2006)

R_1OH = Aliphatic, Alicyclic, Steroidal, Sugar alcohol

Armed system

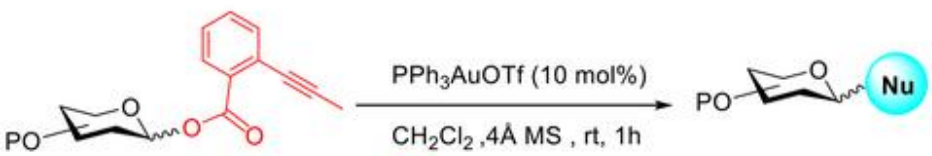
a) First report of gold ($AuCl_3$) catalyzed glycosylation using propargyl donor

(Hotha, 2007,2013)

R_1OH = Aliphatic, Alicyclic, Steroidal, Sugar alcohol

Disarmed system

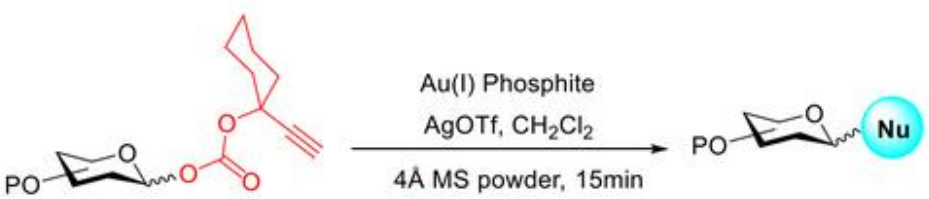
b) $AuBr_3$ catalyzed glycosylation using propargyl orthoester donor



(Yu, 2008)

Armed & Disarmed system Nu = O.N,C nucleophile

c) Au(I) catalyzed activation of *O*-alkynyl benzoate donor



(Hotha, 2016)

Armed & Disarmed system Nu = O.N,C nucleophile

d) Au/Ag catalyzed glycosylation using 1-ethynyl cyclohexyl carbonate donor

Figure 1.7

Retrosynthesis

The molecule can be divided into 3 parts.

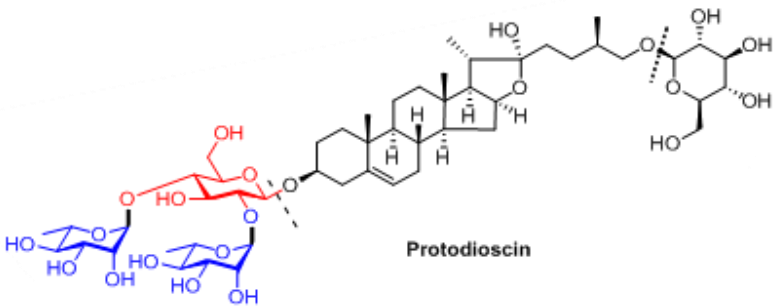


Figure 1.8

Now, in the next figure it is shown explicitly how it is divided into the 3 parts and my approach towards synthesising the molecule.

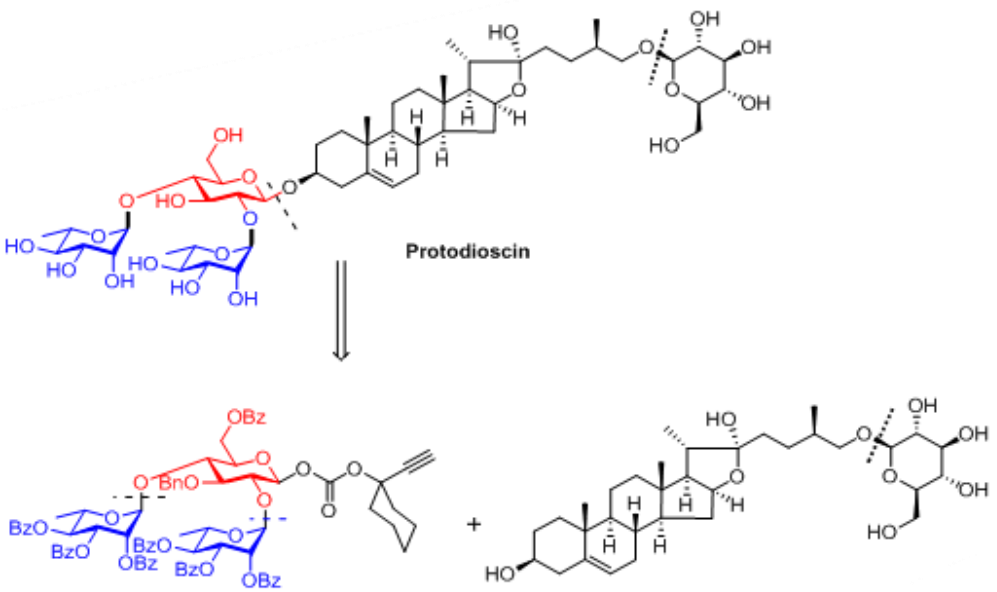


Figure1.9

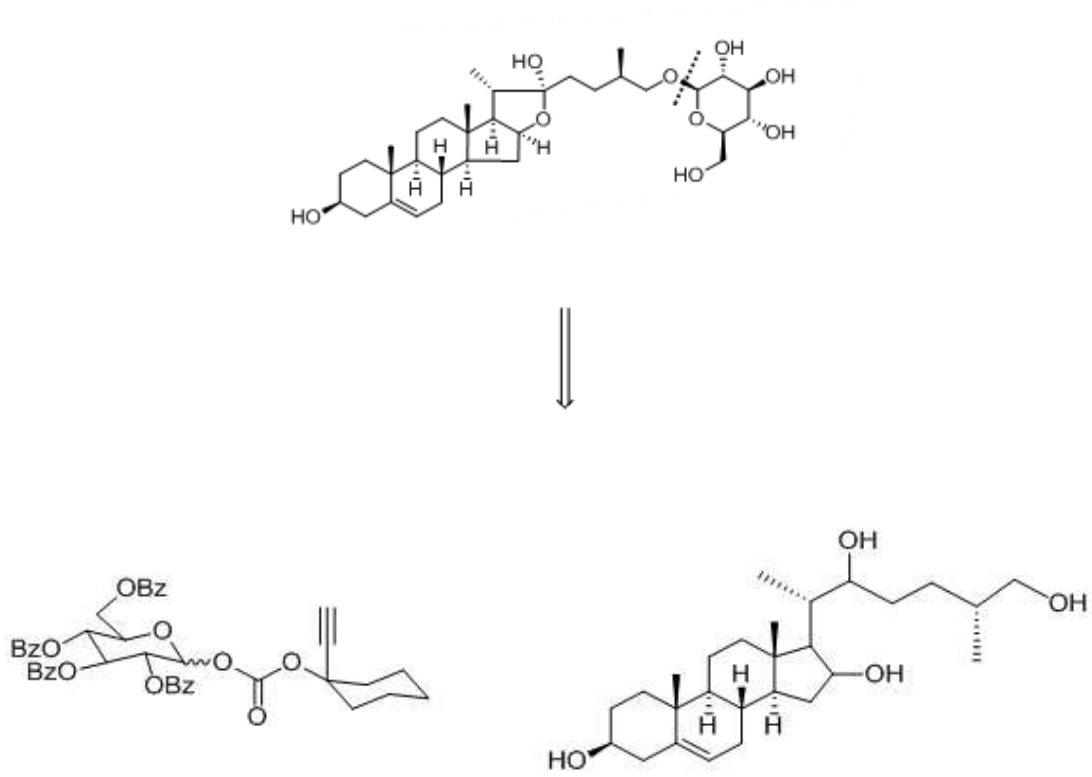


Figure 1.10

In the above figures that is Figure 1.9 and Figure 1.10 it is shown how I have divided the molecule into 3 parts. On the next chapters, it is shown how I have synthesized the motifs and attached them together to get the desired molecule finally.

2.Results and Discussion

As discussed in the earlier chapter, the molecule is divided into 3 parts, namely Motif A, B and C.

Motif A

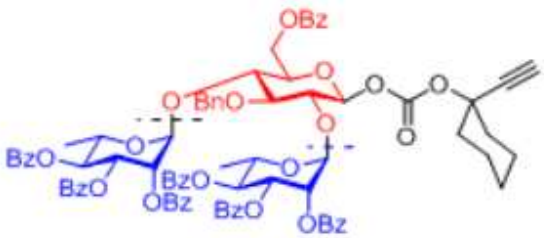
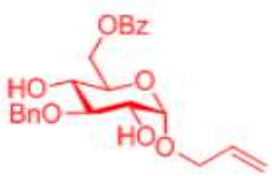


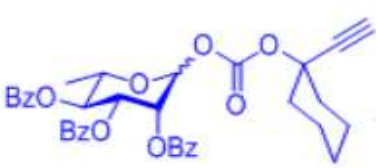
Figure 2.1

In order to get the final molecule, I had to synthesize this first (Motif A). It is basically a trisaccharide donor. This molecule is achieved after several steps of the reaction. The reaction scheme (Figure 1.12) is shown below. The trisaccharide is formed by three sugar units. As shown in the figure below one(Motif A.1) is achieved from Glucose diacetonide and the other two from L-Rhamnose (Motif A.2).

Motif A.1



Motif A.2



The unit derived from the Glucose diacetonide acts as an acceptor while the other two units that are derived from the L-Rhamnose unit are acting as a donor. At first Bn protection reaction is done on the Glucose Diacetonide. Going through various literatures(14) I have decided on the

scheme.

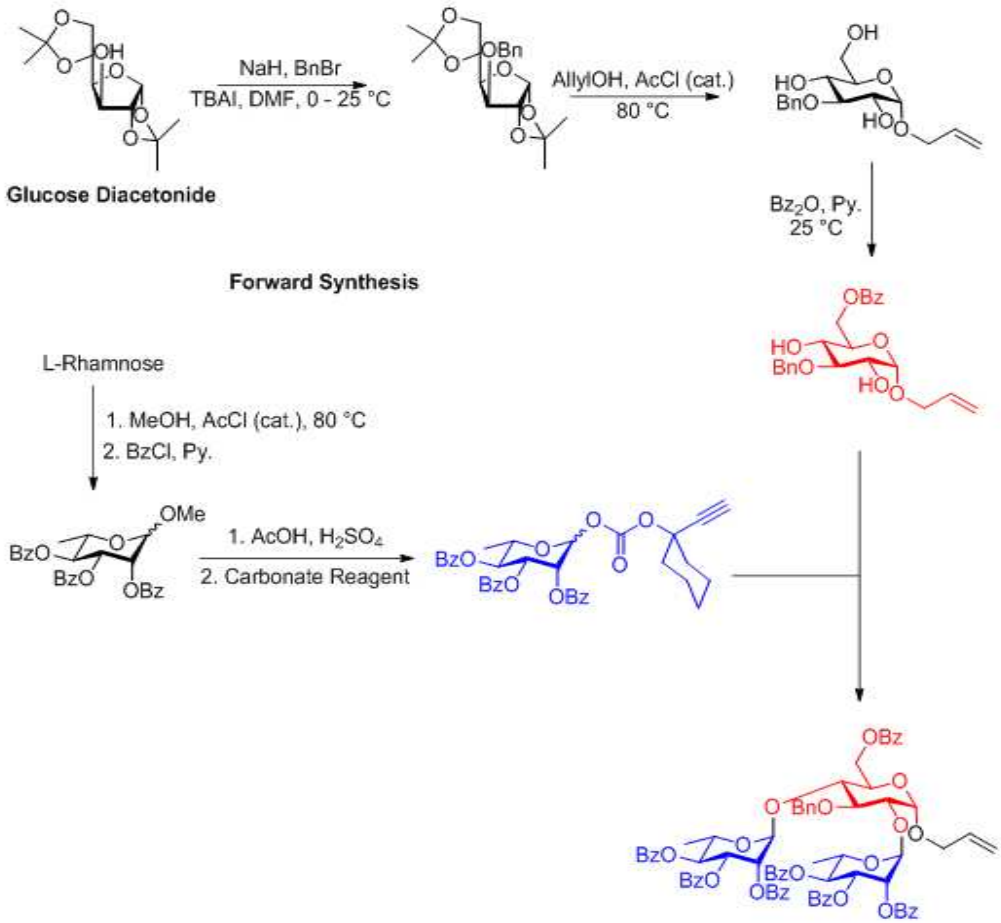


Figure 2.2

Next, I performed a reaction to ensure that the allyl group was in the anomeric position. Thus motif A.1 is prepared, which acts as an acceptor.

Next is the preparation of A.2 I have done the selective anomeric protection by OMe and the other positions are protected by OBz. Further, the OMe group is replaced by the carbonate reagent donor thus, I prepared motif A.2, which acts as a donor.

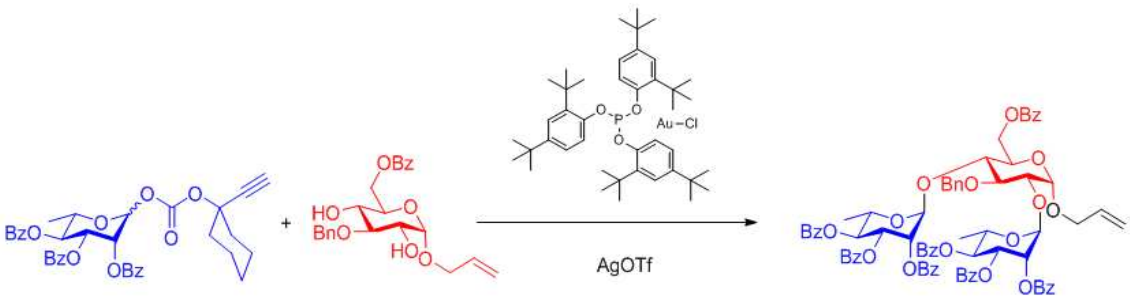


Figure 2.3

Next, I performed the Gold-catalyzed glycosylation, which finally generated the formation of a trisaccharide, the scheme is mentioned in the above figure (Figure 1.13).

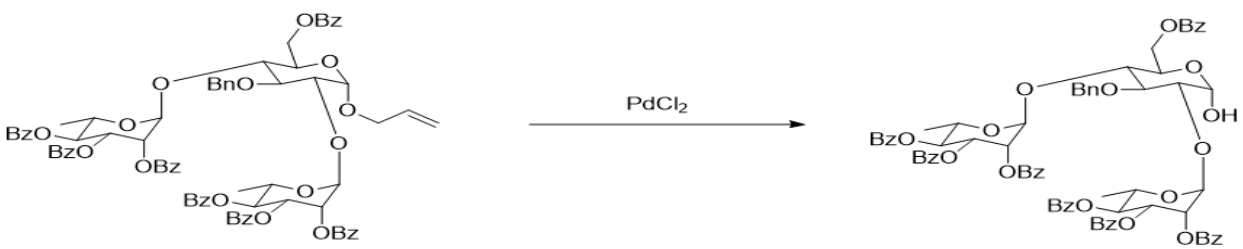


Figure 2.4

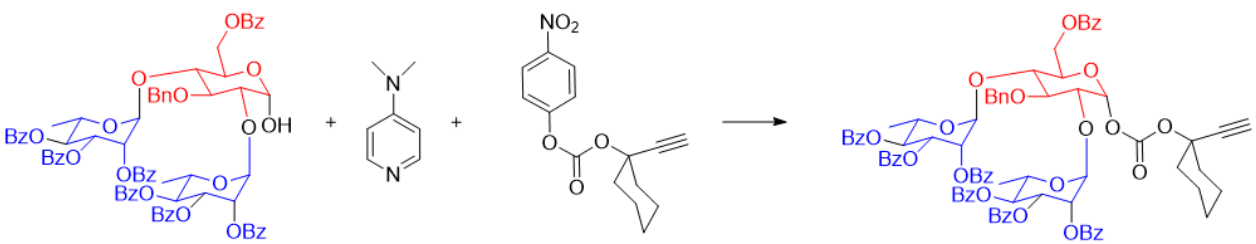


Figure 2.5

After that I performed the deprotection at the anomeric position and attach the carbonate donor with it making a trisaccharide donor which is the Motif A, one of the three parts of the desired final molecule.

Motif B

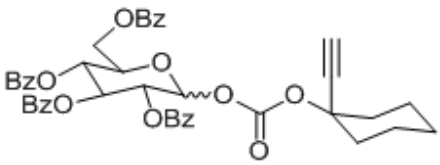


Figure 2.6

Motif B is actually a monosaccharide donor. After going through various literature I have made the scheme to synthesize this molecule. The scheme is mentioned in the following figures

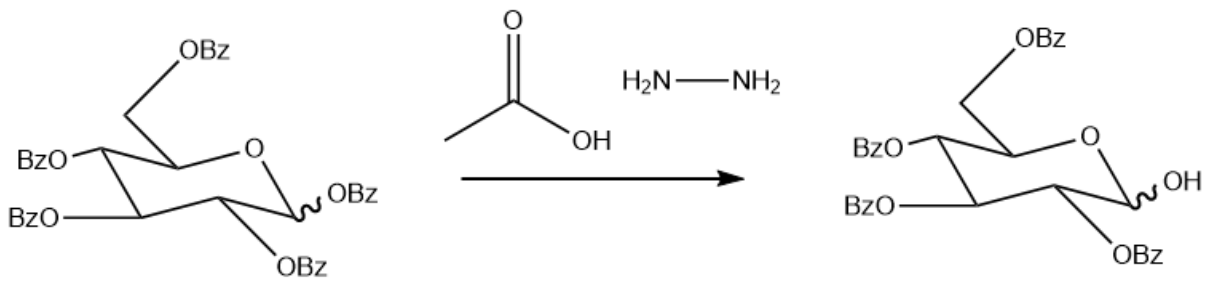


Figure 2.7

In this part I have done the selective deprotection of the anomeric Bz group. After that in the Figure 1.18 it is shown how I have attached the carbonate donor with it making it a monosaccharide donor.

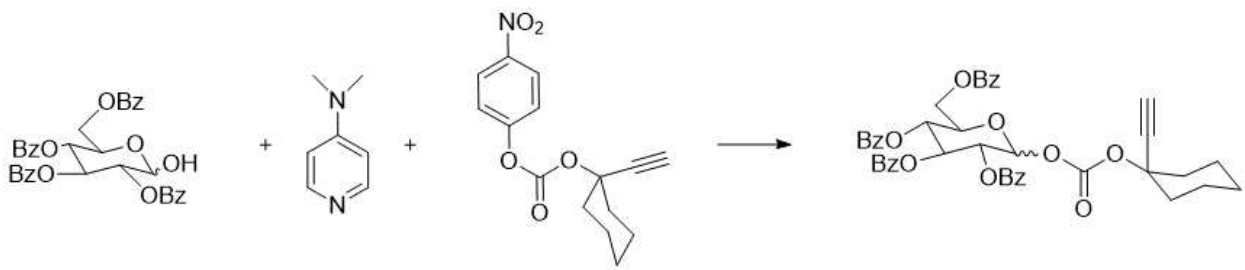


Figure 2.8

By following the above mentioned scheme I have synthesised the Motif B which is the second component of the desired final molecule.

Motif C

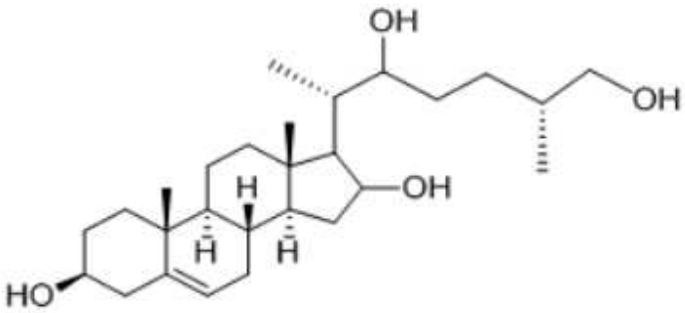


Figure 2.9

This unit is synthesised from Diosgenin. The reaction scheme to synthesise this motif is mentioned in the figure below.

Figure 2.10

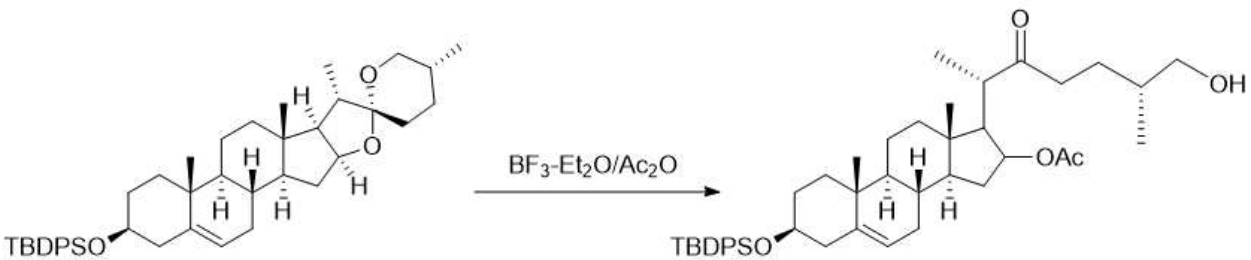


Figure 2.11

Here, at first I have protected the free OH group by TBDPS, then by using BF_3-$Et_2O$and acetic anhydride in dichloromethane.The direct opening of the E-ring resulted in a reduction of both the number of procedural steps and the time required to perform the process(15). In the E-ring opening reaction of the compound, the interaction between BF_3-Et_2O and acetic anhydride leads to the development of a complex, which aids in the generation of an oxo-carbenium-ion intermediate. This intermediate is then hydrolyzed by water, which is added during the quenching step and

attacks at C-22. Notably, the intramolecular cyclization from C26-OH to C22-oxo group is not observed under these conditions, resulting in the major product being diacetate from the residual acetic anhydride. Optimization of the reaction is necessary to minimize the by-product formation, and the optimal quantity required to maximize the desired product has been determined through literature review.

3. Materials and Methods

Compound 1

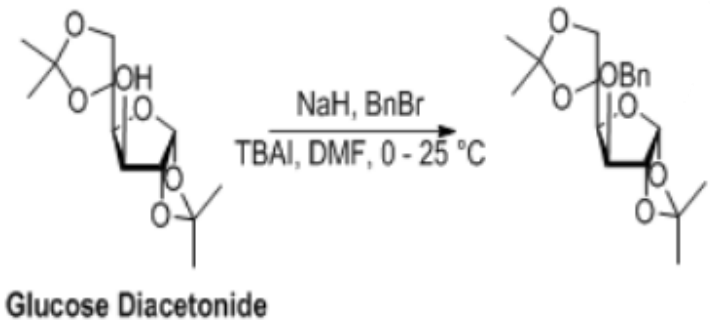


Figure 3.1

At first Glucose Diacetonide is dissolved in DMF and it is cooled down to 0°C. After that 1.2 eq. Of NaH is added slowly to the solution and stirred for 30 mins. Next, 1.5 eq. of BnBr is added dropwise. The mixture is then kept for 1 hr. Following that ice-cold water is added for quenching of NaH, and then the mixture is washed with brine solution (thrice) and ethyl acetate. Following that the volatile liquid is evaporated using a vacuum, and then purification is performed using silica gel chromatography with a mobile phase of ethyl-acetate and n-hexane. I have got an 80% yield.

Compound 2

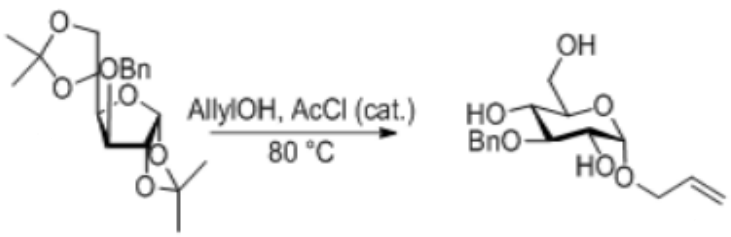


Figure 3.2

Compound 1 is taken, and it was dissolved in anhydrous allyl alcohol(50 eq.). It was then kept on ice to reduce the temperature to 0°C, subsequently, acetyl chloride was added slowly and stirred for some time

and was refluxed for 2 hrs at 80°C. Subsequently, the reaction mixture was quenched with Et_3N, and the volatile components were eliminated under lower pressure. The product is purified via silica gel column chromatography with a mobile phase of ethyl-acetate and n-hexane. I got compound 1 at 50% Ethyl-acetate and n-hexane solvent system. The yield I got is 80%.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.16 (m, 1H), 5.84 (ddddd, *J* = 17.0, 10.3, 6.3, 5.3, 3.7 Hz, 0H), 5.29 – 5.10 (m, 0H), 4.89 (dd, *J* = 11.7, 5.5 Hz, 0H), 4.69 (dd, *J* = 11.5, 4.5 Hz, 0H), 4.35 – 4.22 (m, 0H), 4.18 – 3.88 (m, 0H), 3.79 – 3.63 (m, 0H), 3.62 – 3.39 (m, 1H), 1.18 (s, 0H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 138.65, 138.59, 133.77, 133.60, 128.64, 128.62, 128.10, 128.06, 127.98, 127.94, 118.20, 118.15, 102.00, 97.78, 83.85, 82.79, 75.42, 75.04, 74.82, 74.29, 72.68, 71.45, 70.56, 69.90, 68.63, 62.02.

Compound A.1

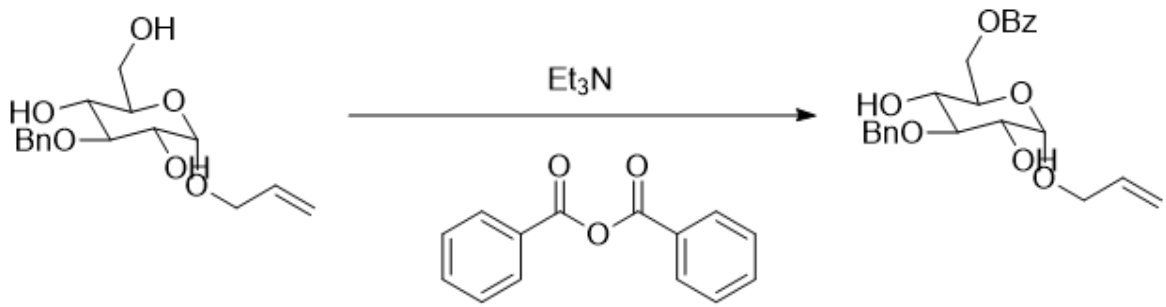


Figure 3.3

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 – 8.01 (m, 4H), 7.56 (ddd, *J* = 7.3, 4.1, 1.3 Hz, 2H), 7.49 – 7.26 (m, 14H), 5.93 (ddddd, *J* = 14.4, 10.4, 6.4, 5.4, 2.6 Hz, 2H), 5.35 – 5.19 (m, 4H), 5.03 – 4.93 (m, 3H), 4.82 (dd, *J* = 13.2, 7.5 Hz, 2H), 4.70 – 4.49 (m, 4H), 4.41 – 4.20 (m, 3H), 4.16 – 3.94 (m, 3H), 3.75 – 3.43 (m, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.03, 138.57, 133.74, 133.53, 133.34, 129.91, 129.86, 128.72, 128.70, 128.51, 128.48, 128.22, 128.16, 128.05, 118.32, 101.91, 97.76, 82.72, 75.32, 72.74, 70.19, 69.92, 68.80.

HRMS (ESI-MS): m/z Calculated: 414.45 Found: 414.4523

Compound 2 was dissolved in CH_2Cl_2 then Et_3N (5 eq.) and Bz_2O (1.5 eq) was added subsequently. I stirred the mixture at room temperature. I eliminate the volatile components under low pressure and I purified the product using silica-gel chromatography in which the mobile phase was ethyl-acetate and n-hexane.

Compound 4

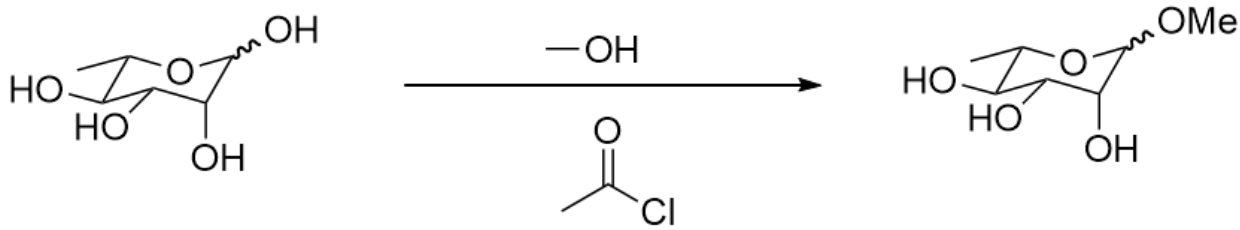


Figure 3.4

L-Rhamnose is taken, and it is diluted with MeOH (10 eq.). After that AcCl is added (1.5 eq). The mixture was refluxed at 80℃.

Compound 5

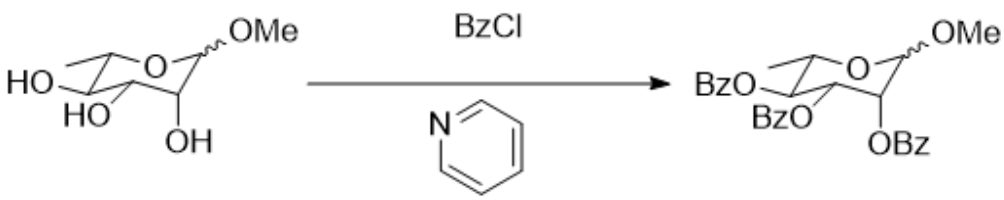


Figure 3.5

Compound 4 is taken and, at first, dissolved in pyridine(1 eq) and then chilled to 0℃ before adding BzCl to the reacting mixture after that, it was then kept to let it come to room temperature before it was stirred for 4 hrs. After that I add 1N HCl to quench the process. I isolate the product by extracting the mixture with ethyl acetate(twice). Afterwards, the mixed layers are washed with 1N HCL (twice) and then with brine solution (twice). The mixture is then dehydrated over dry Na_2SO_4 and next the resulting mixture was evaporated under vaccum to eliminate the volatile liquids present. Finally, I purified the product using silica-gel chromatography in which the mobile phase was ethyl-acetate and n-hexane.

Compound A.2

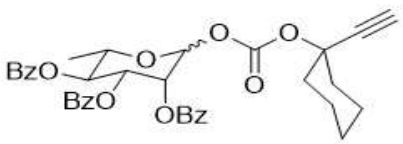


Figure 3.6

This compound can be achieved from Compound 5 but it was available in the lab, so I used that. I purified the compound using silica gel chromatography in which the mobile phase was ethyl- acetate in n-hexane.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 – 8.06 (m, 1H), 8.01 – 7.92 (m, 1H), 7.82 (dt, *J* = 8.3, 1.3 Hz, 1H), 7.63 (td, *J* = 7.4, 1.5 Hz, 0H), 7.57 – 7.35 (m, 3H), 7.26 (td, *J* = 7.8, 1.7 Hz, 1H), 5.88 (dd, *J* = 10.2, 3.4 Hz, 0H), 5.81 – 5.66 (m, 1H), 4.35 (dd, *J* = 9.9, 6.2 Hz, 0H), 2.70 (d, *J* = 1.1 Hz, 0H), 2.26 (t, *J* = 8.6 Hz, 1H), 2.01 – 1.90 (m, 1H), 1.80 – 1.54 (m, 3H), 1.40 (d, *J* = 6.3 Hz, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.78, 165.59, 165.39, 150.48, 133.77, 133.56, 133.34, 130.12, 129.88, 129.84, 129.25, 129.19, 129.12, 128.77, 128.60, 128.43, 93.88, 82.50, 79.10, 75.69, 71.33, 69.65, 69.60, 69.18, 37.00, 36.82, 25.07, 22.77, 17.80.

Compound 7

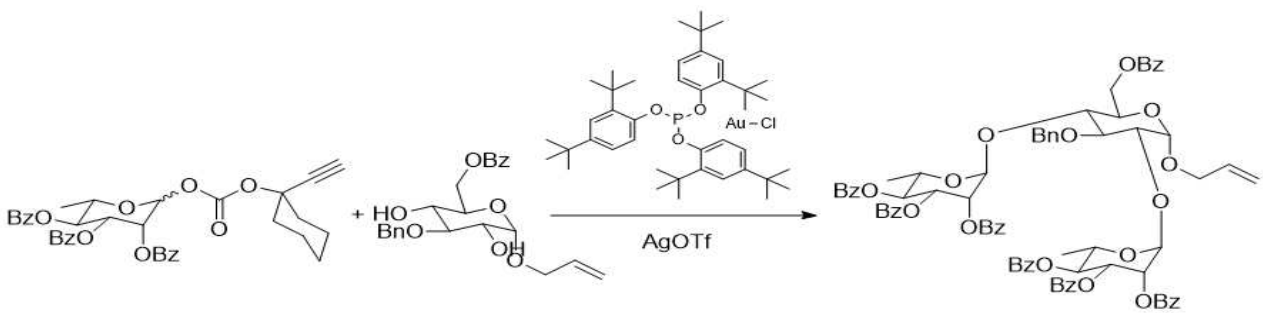


Figure 3.7

The donor(1 eq) and acceptor(0.4eq) were taken and dissolved in dehydrated CH_2Cl_2 containing 4 ÅM.S powder. It is stirred for some time before adding AgOTf (0.15 eq) and Au-phosphite (0.1eq). The stirring is continued at 25°C for 30 mins. After that the volatile liquid was evaporated using a vacuum and finally I purified the product using silica gel chromatography in which the mobile phase was ethyl- acetate in n-hexane.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 – 7.94 (m, 75H), 7.80 (ddd, *J* = 35.5, 21.0, 7.8 Hz, 38H), 7.63 – 7.32 (m, 131H), 7.32 – 7.18 (m, 6H), 7.18 – 7.10 (m, 1H), 7.10 – 6.81 (m, 1H), 6.18 – 4.97 (m, 79H), 4.73 – 3.83 (m, 25H), 1.37 – 1.29 (m, 21H), 1.06 (dd, *J* = 5.1, 2.9 Hz, 14H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.12, 166.05, 165.89, 165.80, 165.75, 165.51, 165.26, 137.92, 137.42, 133.67, 133.51, 133.47, 133.40, 133.31, 133.26, 133.16, 133.11, 129.99, 129.97, 129.95, 129.88, 129.86, 129.81, 129.77, 129.73, 129.51, 129.43, 129.38, 129.36, 129.31, 129.28, 129.24, 129.22, 129.17, 128.63, 128.57, 128.54, 128.46, 128.42, 128.39, 128.37, 128.32, 128.30, 128.20, 128.15, 127.68, 127.26, 126.42, 118.33, 100.16, 97.68, 97.33, 84.03, 82.33, 79.08, 76.27, 75.89, 71.83, 71.68, 71.24, 70.84, 69.94, 69.87, 69.24, 68.95, 67.70, 67.35, 17.88, 17.36.

HRMS (ESI-MS): m/z Calculated: 1331.39 Found: 1331.3964

Compound 8

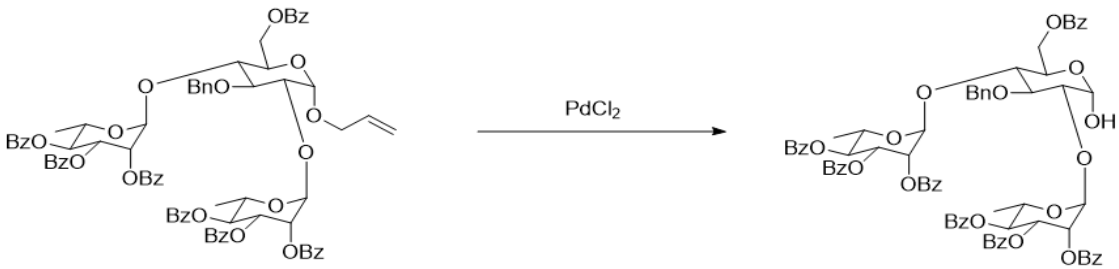


Figure 3.8

To obtain the desired compound, I dissolved the allyl glycoside (1.0 mmol) in a biphasic solution consisting of a 4:1 mixture of CH_3OH and CH_2Cl_2. To this reaction mixture, I add 0.3 equivalents of $PdCl_2$ and I stirred the solution for 4-8 hours at 25°C. It was then quenched using excess Et_3N, and the mixture was filtered through a Celite® bed. Finally I concentrated the substrate under low pressure and subsequently I purified the product using silica gel column chromatography in which the mobile phase was ethyl acetate.

Compound 9

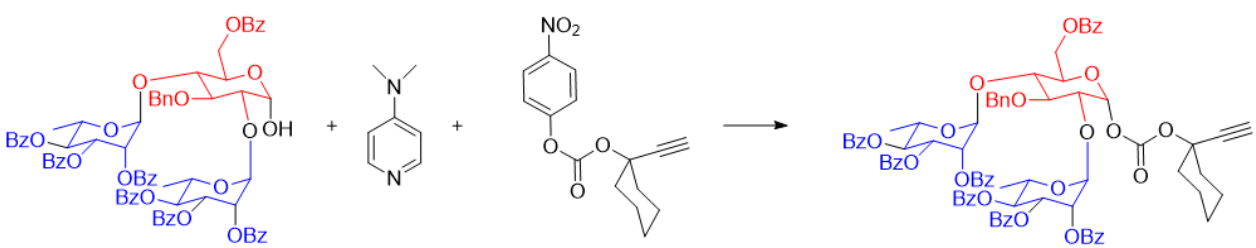


Figure 3.9

Compound 9 was taken and DMAP(1.25 eq.) and carbonate reagent (1.25

eq.) was added and I kept it at stirring for 12 hrs at 25°C. After that I evaporated the volatile components under lower pressure and I purify the product using silica gel column chromatography in which the mobile phase was ethyl acetate and n-hexane.

Compound 10

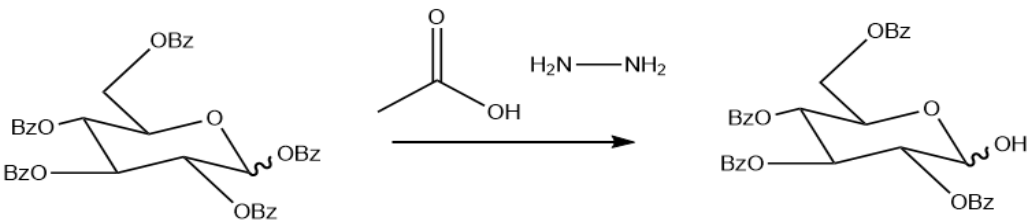


Figure 3.10

The starting compound was dissolved in DMF, hydrazine acetat(7 eq.) was introduced to the mixture. The resulting mixture was then stirred for 7 hrs. Henceforth, I diluted the mixture with EtOAc, and it was then washed with brine solution. Subsequently, I dried the organic layer by Na_2SO_4 , filter it, and evaporated in vaccum. I purified the compound by column chromatography in which the mobile phase was ethyl acetate and n-hexane.

Compound 11

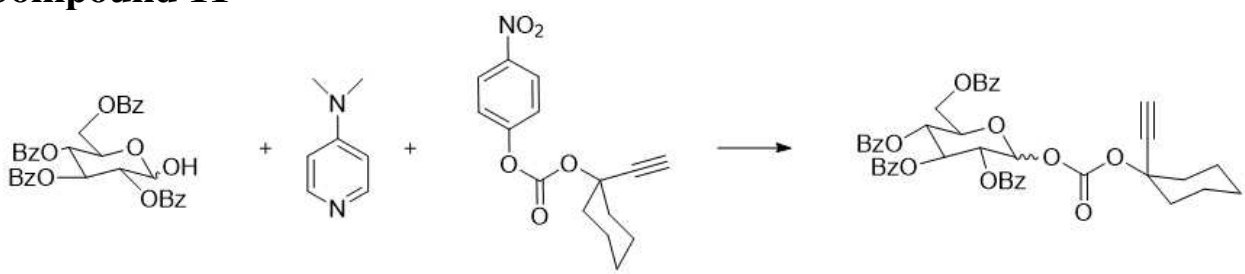


Figure 3.11

Compound 9 was taken and DMAP(1.25 eq.) and carbonate reagent (1.25 eq.) was added and it was stirred for 12 hrs at 25°C. I evaporated the volatile components at low pressure and purify the product using silica gel

column chromatography in which the mobile phase was ethyl acetate and n-hexane.

Compound 12

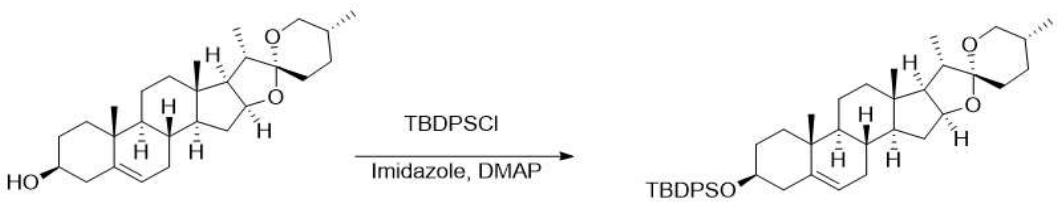


Figure 3.12

Following the dissolution of the compound in anhydrous CH_2Cl_2, a combination of imidazole (2 eq.), tert-Butyldiphenylsilyl chloride (TBDPSCl) (1.2 eq.), and DMAP (3 eq.) were introduced to the mixture. The reaction continued for 16 hours under stirring before ice-cold water was added to quench the mixture. The mixture that we get was then subjected to two washes with CH_2Cl_2 to extract the product. Afterward, the solution was dehydrated with dry $MgSO_4$ and the volatile liquid was removed using vacuum evaporation. Lastly, purification of the resulting material was performed using silica gel column chromatography, in which the mobile phase was ethyl acetate in n-hexane.

Compound 13

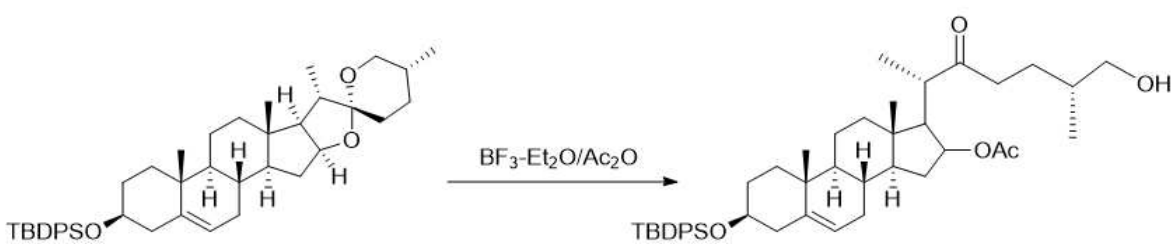


Figure 3.13

The compound was dissolved in CH_2Cl_2 and temperature of the mixture is lowered, after that a mixture of BF_3-Et_2O(12.8 eq) and Ac_2O (19.1 eq) was

introduced to the solution. Following the reaction, I stirred the mixture for 30 minutes after the addition of 30% MeOH in water. I purify the final product using silica-gel chromatography, in which the mobile phase was ethyl acetate and n-hexane.

^{1}H NMR (400 MHz, CHLOROFORM-D) δ 7.67 (dq, *J* = 8.6, 2.0 Hz, 4H), 7.43 – 7.32 (m, 7H), 5.11 (dd, *J* = 4.7, 2.9 Hz, 1H), 4.96 (td, *J* = 7.9, 4.7 Hz, 1H), 3.53 (tt, *J* = 10.8, 4.4 Hz, 1H), 3.41 (d, *J* = 5.9 Hz, 2H), 2.94 (dq, *J* = 10.7, 7.0 Hz, 1H), 2.62 (ddd, *J* = 18.0, 8.6, 6.5 Hz, 1H), 2.43 – 2.27 (m, 4H), 2.13 (ddd, *J* = 13.3, 4.5, 1.8 Hz, 1H), 2.04 (t, *J* = 3.8 Hz, 1H), 1.93 – 1.83 (m, 3H), 1.73 – 1.63 (m, 4H), 1.62 – 1.52 (m, 2H), 1.46 (td, *J* = 8.6, 7.2, 4.0 Hz, 4H), 1.30 – 1.23 (m, 4H), 1.12 (d, *J* = 7.1 Hz, 3H), 1.06 (s, 10H), 0.98 (s, 5H), 0.94 – 0.87 (m, 5H), 0.87 – 0.82 (m, 5H).

HRMS (ESI-MS): m/z Calculated: 713.09 Found: 713.0972

4. Future plan of work

I have planned the future in order to get the final product. Here I have proposed the plan in course of action in order to get the final product.

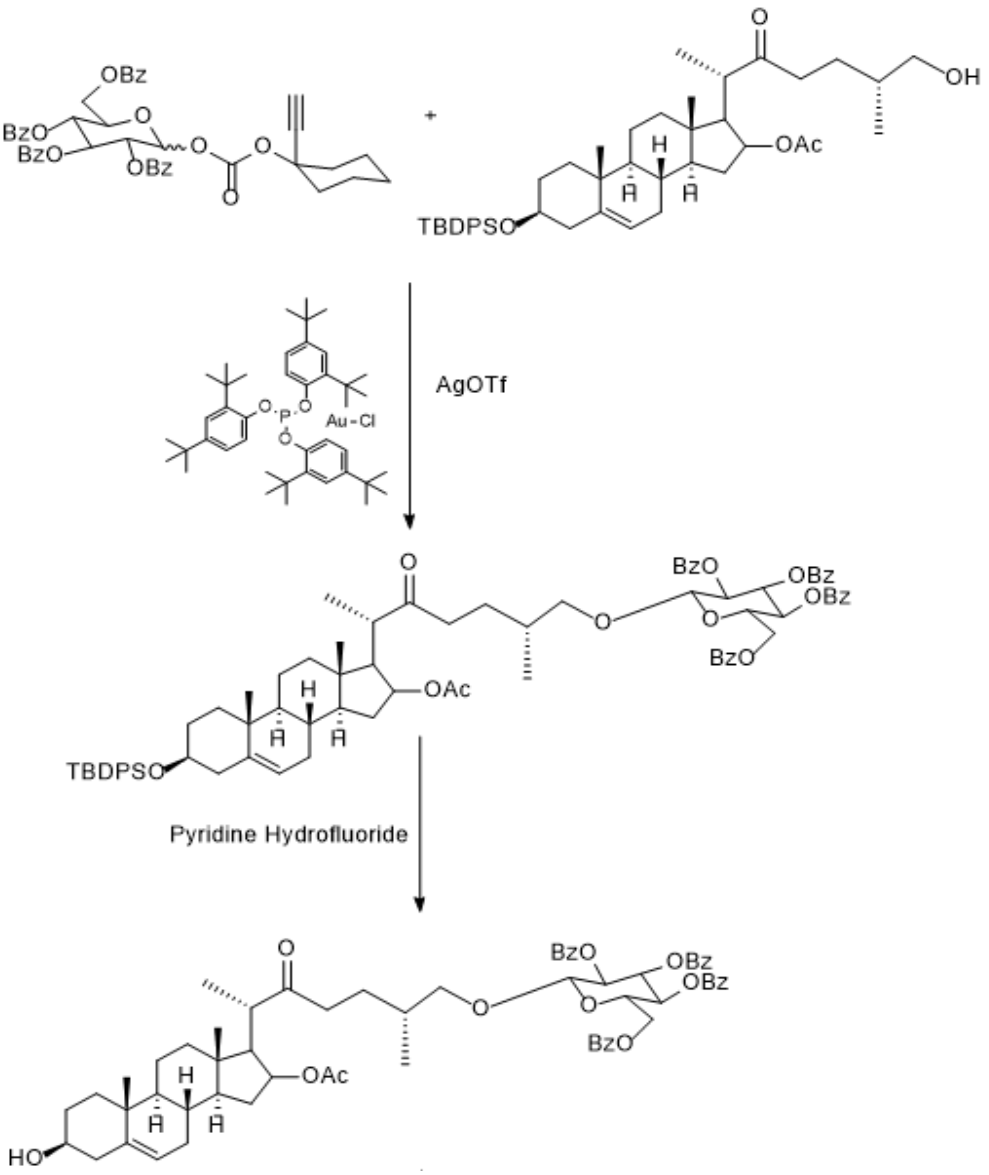


Figure 4.1

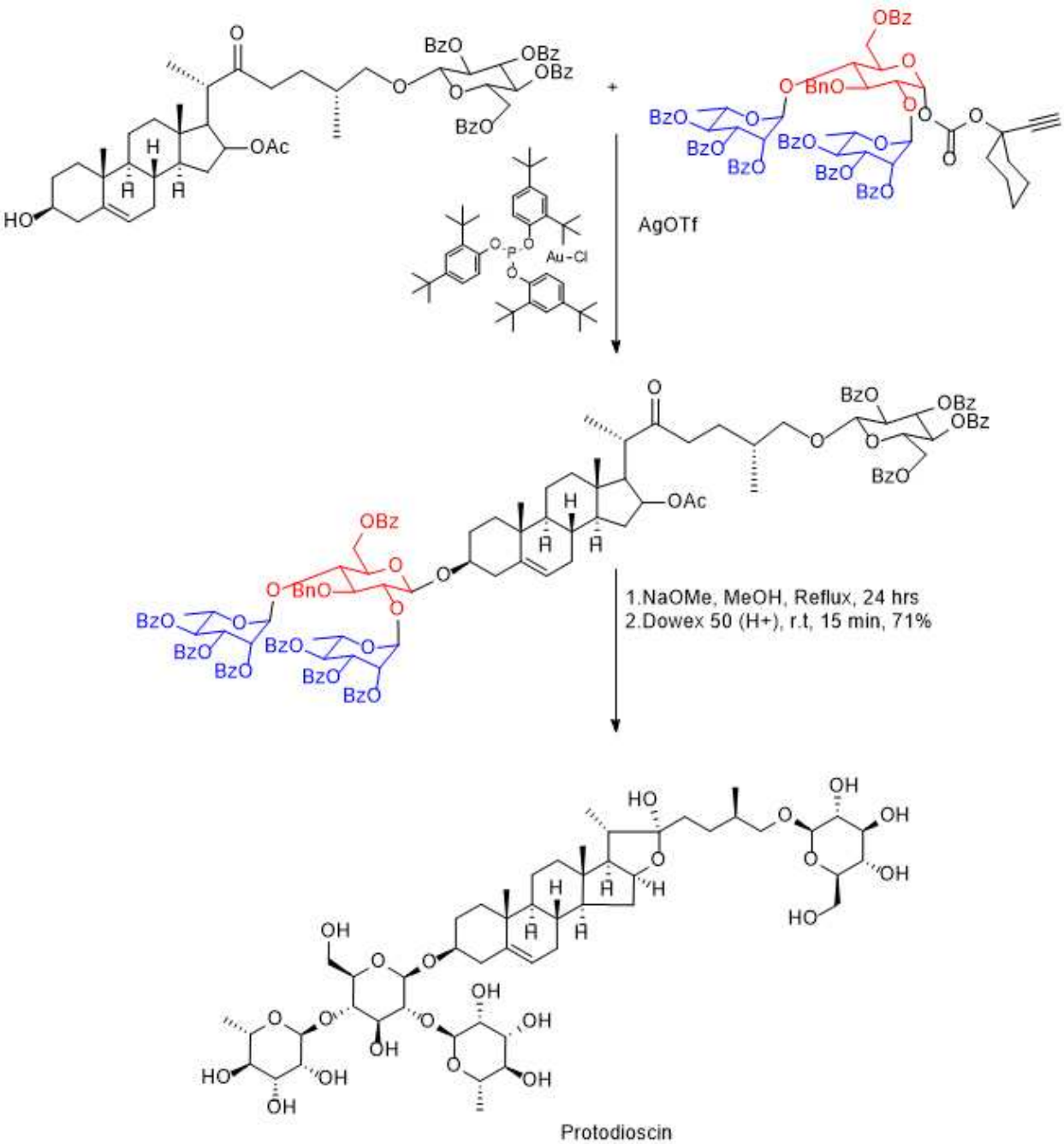

AgOTf
1.NaOMe, MeOH, Reflux, 24 hrs
2.Dowex 50 (H+), r.t, 15 min, 71%
Protodioscin

Figure 4.2

As described in the above schemes (Figure 4.1 and Figure 4.2) first glycosylation needs to done at the primary position, followed by TBDPS deprotection then again glycosylation at the secondary position. At last whole deprotection needs to be performed which will automatically results into cyclization and we will get the final desired product **Protodioscin.**

5. Spectral Charts

Compound 7

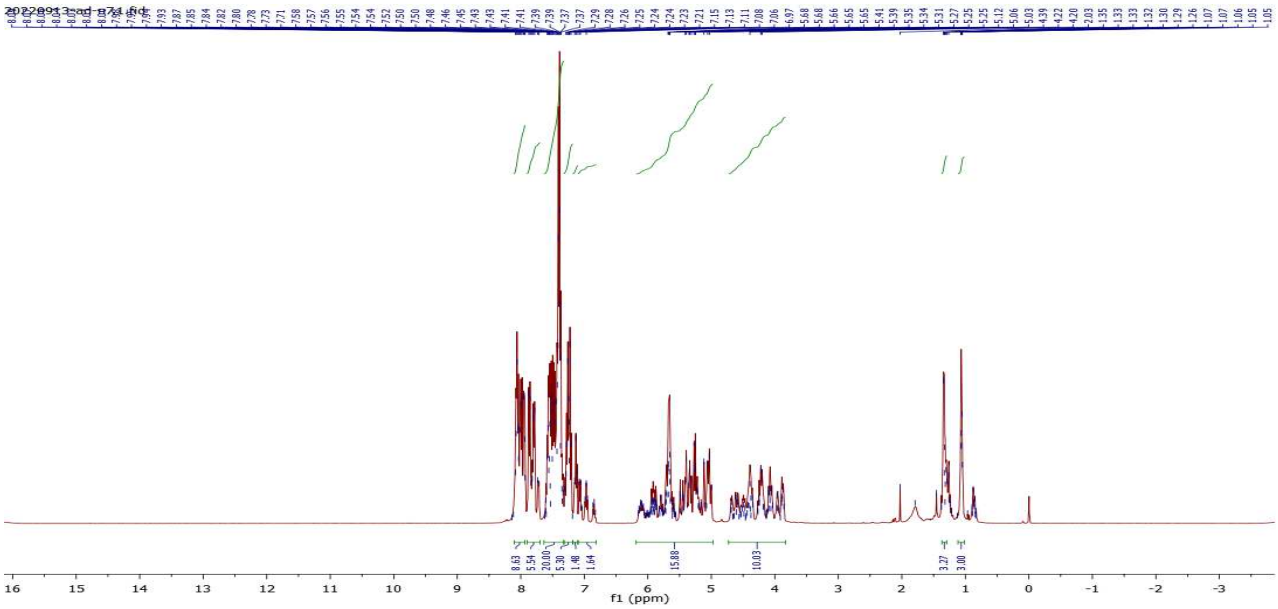


Figure 5.1(^{1}H NMR)

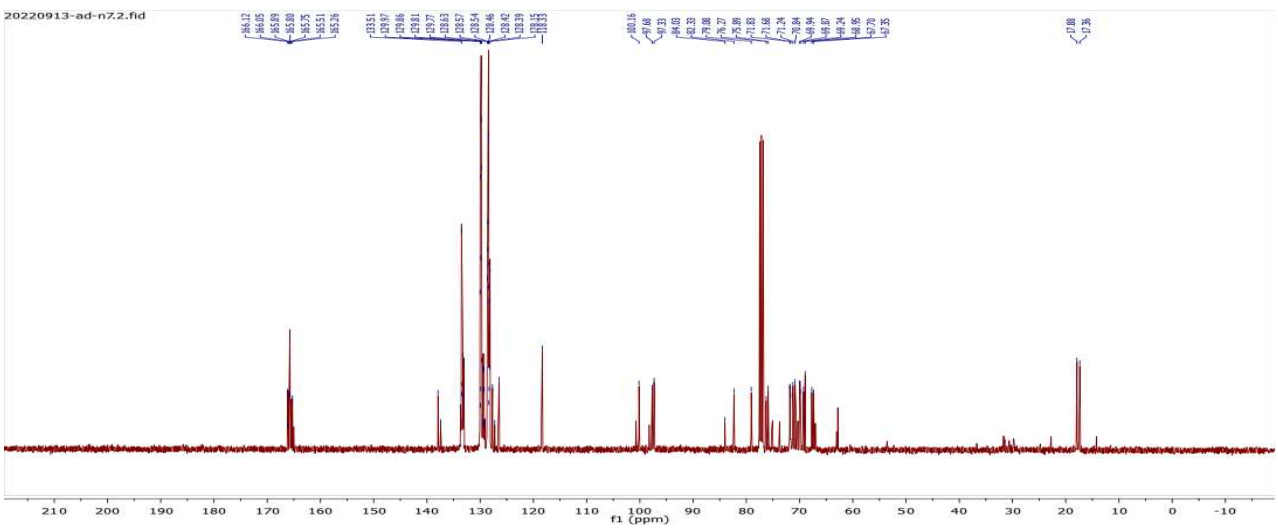


Figure 5.2 (^{13}C NMR Spectrum)

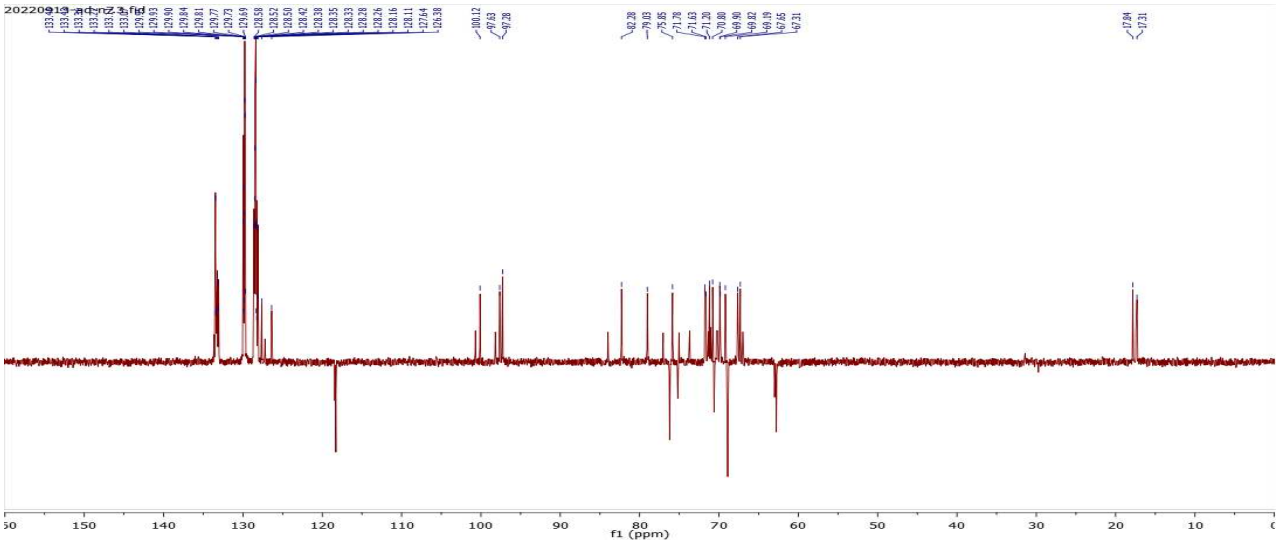


Figure 5.3 (DEPT-135 NMR Spectrum)

Compound A.1

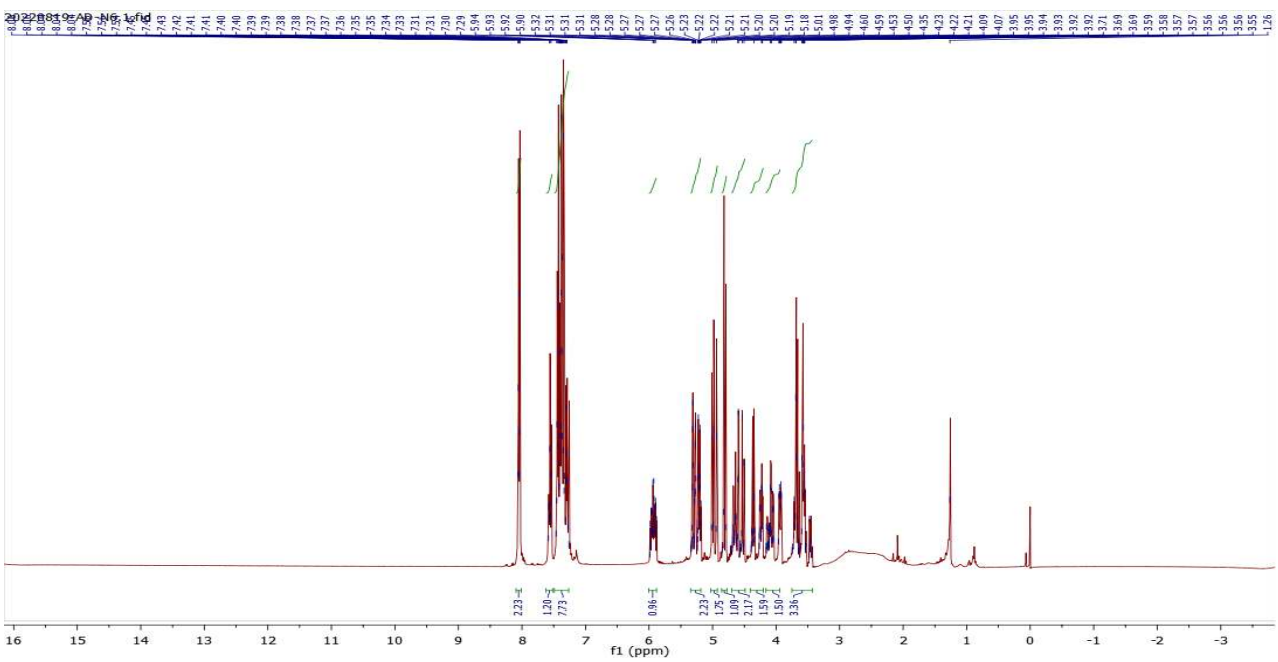


Figure 5.4 (^{1}H NMR)

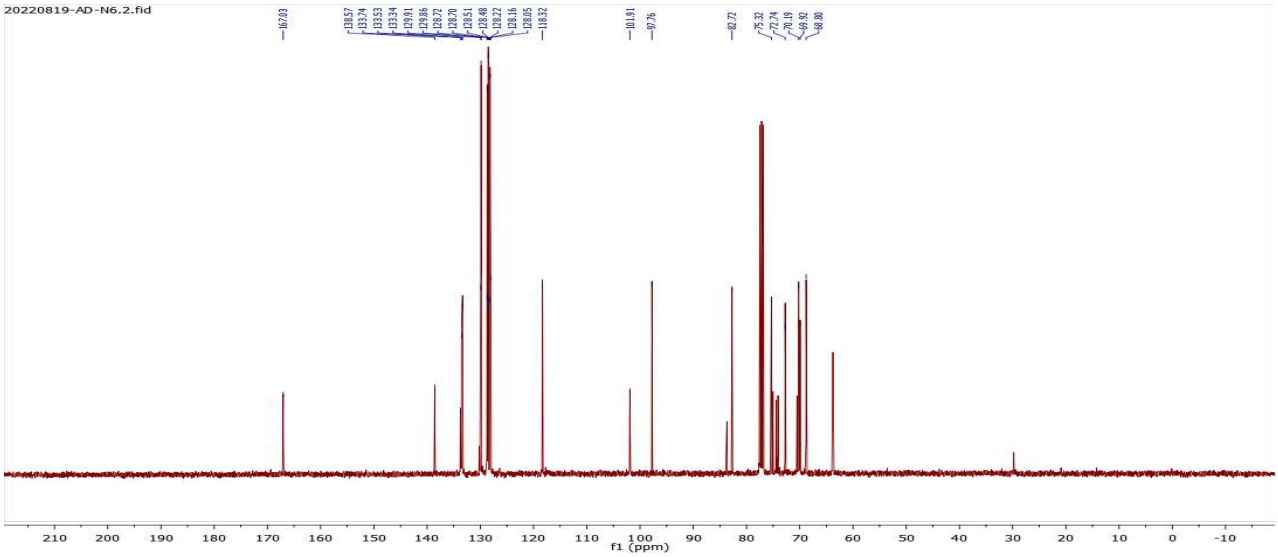


Figure 5.5 (^{13}C NMR Spectrum)

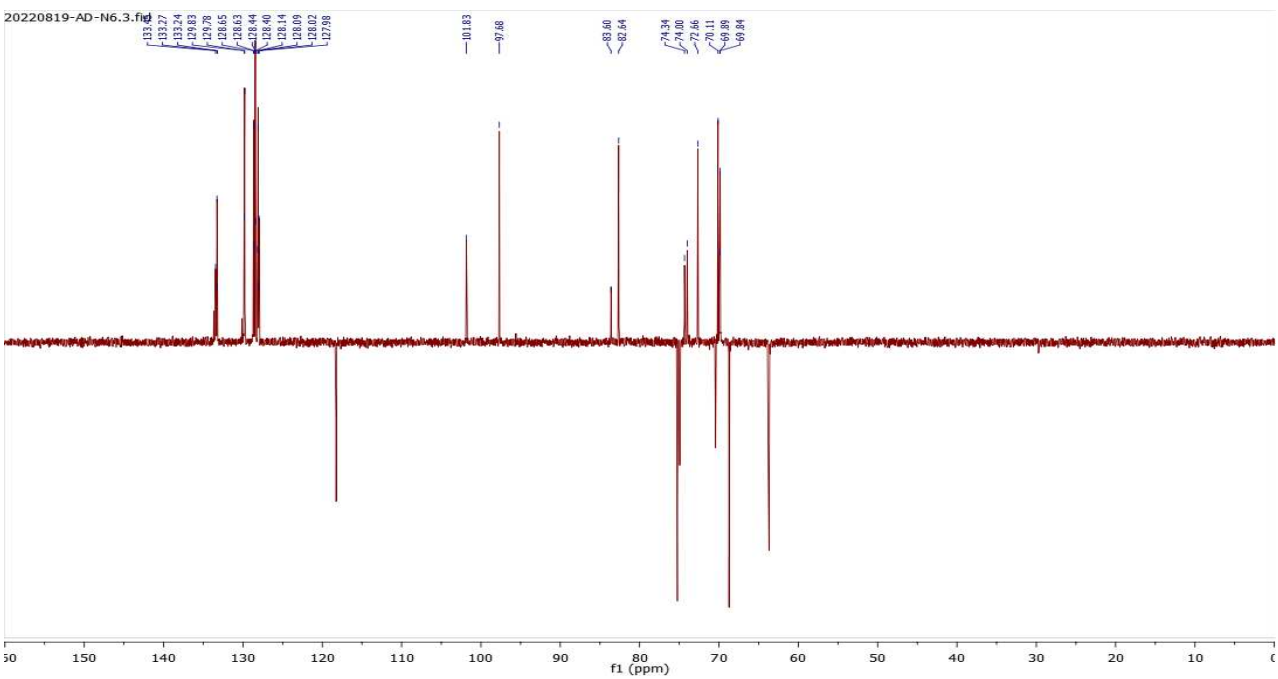


Figure 5.6 (DEPT-135 NMR Spectrum)

Compound A.2

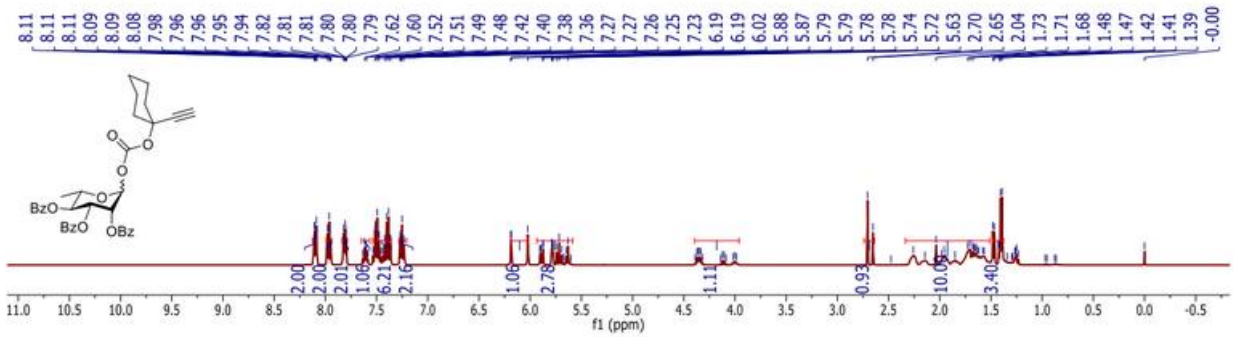


Figure 5.7 (^{1}H NMR)

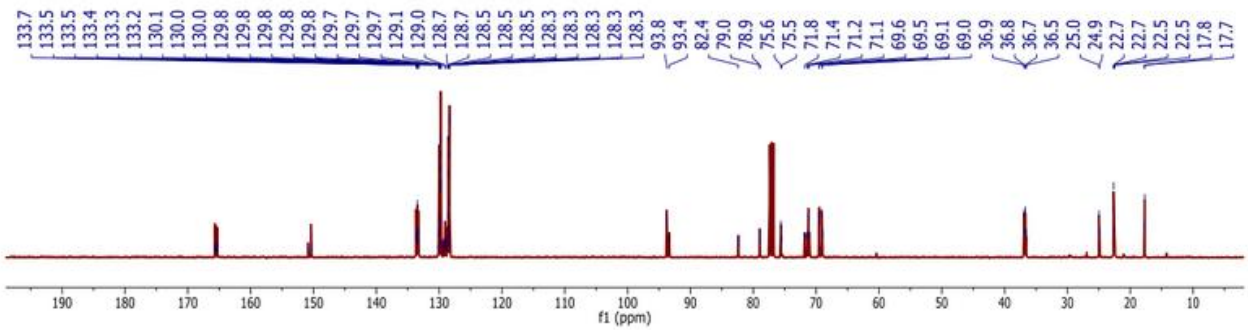


Figure 5.8 (^{13}C NMR Spectrum)

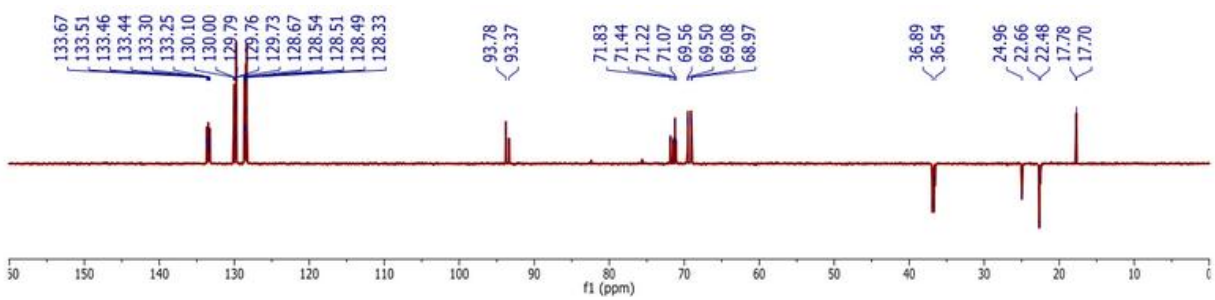


Figure 5.9 (DEPT-135 NMR Spectrum)

Compound 13

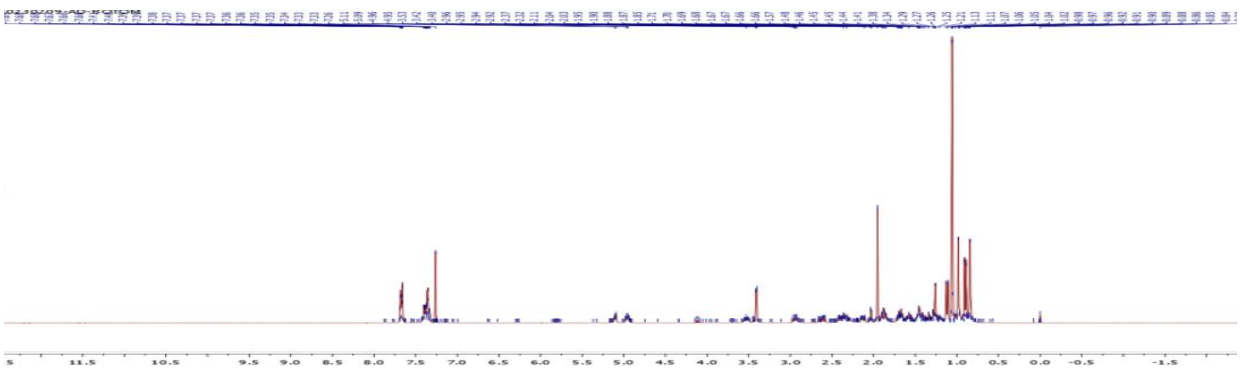


Figure 5.10 (^{1}H NMR)

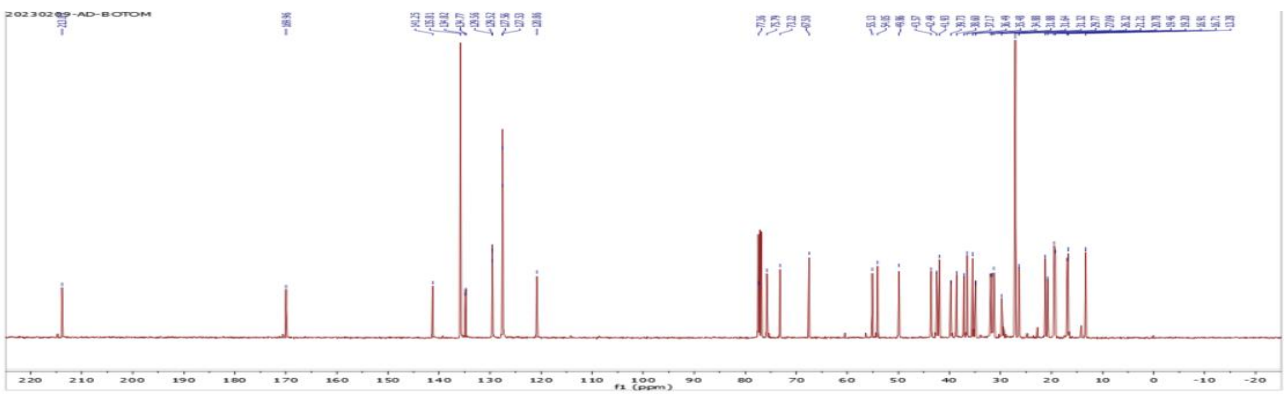


Figure 5.11 (^{13}C NMR Spectrum)

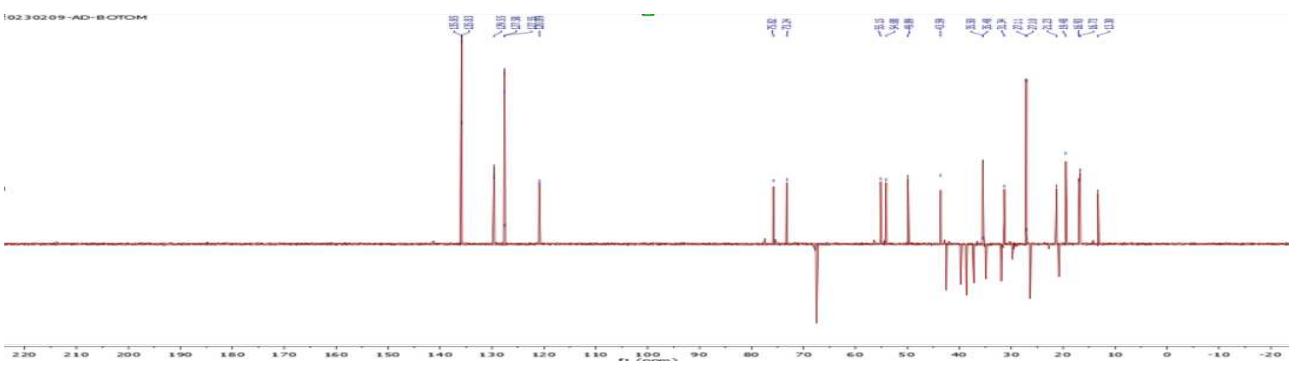


Figure 5.12 (DEPT-135 NMR Spectrum)

Compound 2

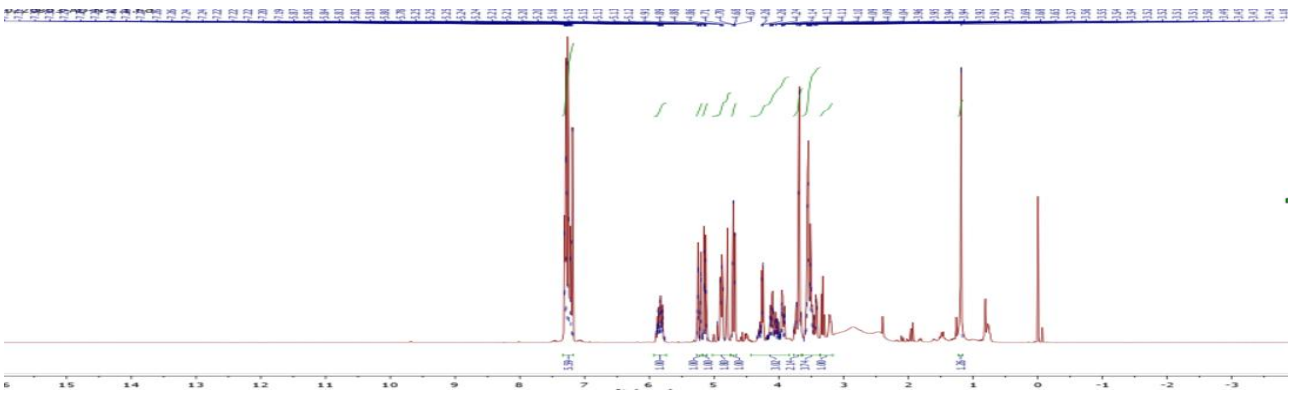


Figure 5.13 (^{1}H NMR)

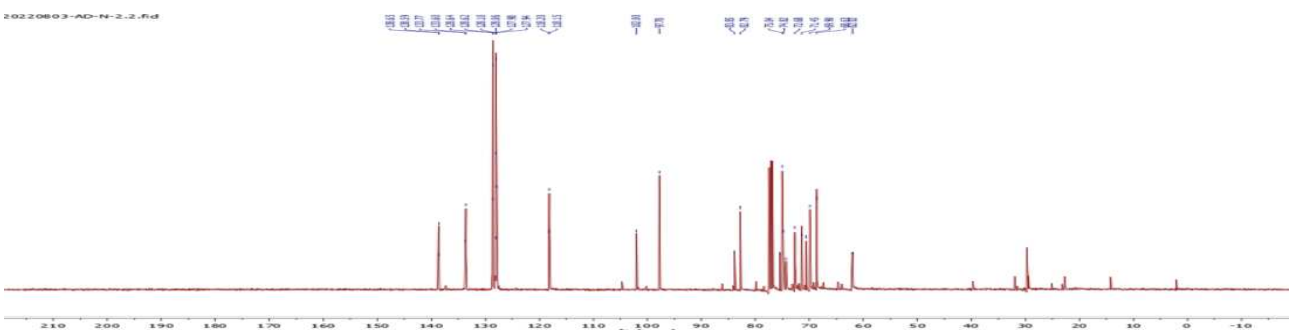


Figure 5.14 (^{13}C NMR Spectrum)

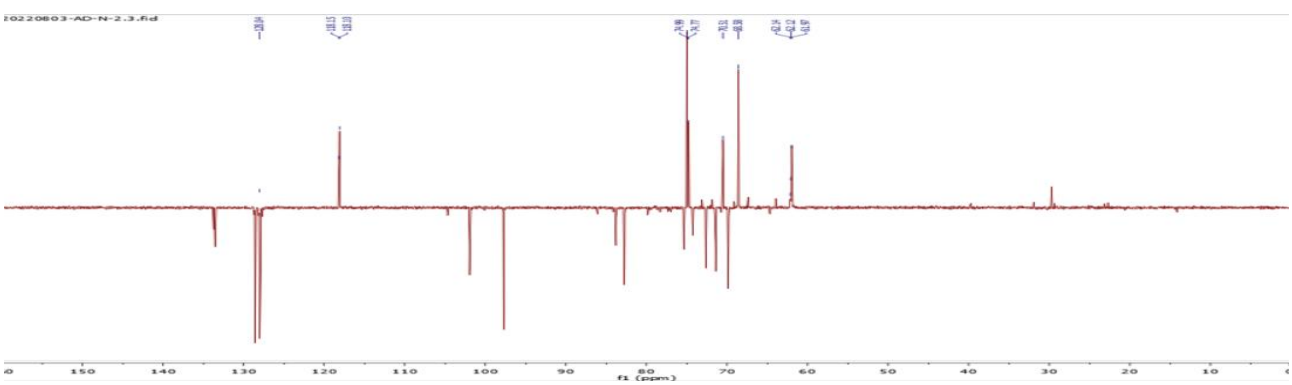


Figure 5.15 (DEPT-135 NMR Spectrum)

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