Synthesis and Biological Evaluation of Chitooligosaccharides and Related Derivatives

A Thesis Submitted for the Degree of

DOCTOR OF PHILOSOPHY

In Chemistry

by

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Statement

I hereby declare that the matter embodied in this thesis is the result of investigations carried out by me in the School of Chemistry, University of Hyderabad, Hyderabad, under the supervision of **Dr. Rengarajan Balamurugan**.

In keeping with the general practice of reporting scientific observations, due acknowledgement has been made wherever the work described is based on the findings of other investigators. Any omission, which might have occurred by oversight or error, is regretted.

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Certificate

Certified that the work embodied in this thesis entitled "**Synthesis and Biological Evaluation of Chitooligosaccharides and Related Derivatives**" has been carried out by **Mr. RAMANA NIDDANA** under my supervision and the same has not been submitted elsewhere for a degree.

Dr. R. BALAMURUGAN
(THESIS SUPERVISOR)

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Certificate

This is to certify that Mr. RAMANA NIDDANA has carried out the research work embodied in the present thesis under the supervision and guidance of Dr. R. Balamurugan for a full period prescribed under the Ph.D. ordinances of this University. I recommend his thesis entitled "**Synthesis and Biological Evaluation of Chitooligosaccharides and Related Derivatives**" for submission for the degree of Doctor of Philosophy of the University. Part of work (biological studies presented in Chapters 3 and 4) was done in the laboratory of **Prof. B. M. Moerschbacher** at the University of Muenster and **Prof. Stefan W. Schneider** at the University of Heidelberg (UMM), Germany in the framework of International Research Training Group in Molecular and Cellular Glycosciences (IRTG-MCGS). I, declare to the best of my knowledge that this work has not been submitted earlier for the award of degree or diploma from any other University or Institution.

Dr. R. BALAMURUGAN
(THESIS SUPERVISOR)

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Ramana

Table of contents

List of abbreviations used

Ac	acetyl
AcOH	acetic acid
Ac_2O	acetic anhydride
aq.	aqueous
Anal.	analysis
Ar	aryl
Bn	benzyl
br s	broad singlet
n-Bu	butyl
t-Bu	tertiary-butyl
calcd.	calculated
cat.	catalytic
DBU	1,8-diazabicyclo(5.4.0)undec-7-ene
DCC	*N,N'*-dicyclohexylcarbodiimide
DIPC	*N,N'*-diisopropylcarbodiimide
DCE	1,2-dichloroethane
DCM	dichloromethane
dd	doublet of doublets
DMAP	dimethylaminopyridine
DMF	*N,N*-dimethylformamide
dt	doublet of triplets
DTBMP	2,6-di-tert-butyl-4-methylpyridine
equiv.	equivalent (s)
Et	ethyl
ELISA	enzyme-linked immunosorbent assay
ESI	electronspray ionization
gm	gram (s)
h	hour (s)
HMPA	hexamethylphosphoramide
HPLC	high-performance liquid chromatography
HUVECs	human umbilical vein endothelial cells
Hz	hertz
KHMDS	potassium hexamethyldisilazane
NIS	*N*-iodosuccinimide
Lev	levulonyl
LCMS	liquid chromatography mass spectrometry
m	multiplet
M	molarity
MALDI	matrix-assisted laser desorption ionization
Me	methyl

mg	milligram (s)
mL	milliliter
mmol	millimole
NMR	nuclear magnetic resonance
Ph	phenyl
P	protecting group
Ppm	parts per million
py	pyridine
q	quartet
rt	room temperature
s	singlet
t	triplet
td	triplet of doublets
TBDMS	tertiary butyl dimethyl silyl
TES	triethylsilane
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
Tf_2O	trifluoromethanesulfonic anhydride
TfOH	trifluoromethanesulfonic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
p-TsCl	*p*-toluenesulphonyl chloride
p-TsOH	*p*-toluenesulfonic acid
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TNF-α	tumor necrosis factor alpha
ULVWF	ultra-large von Willebrand factor
UV	ultraviolet
WSTs	water soluble tetrazolium salts

Synopsis

Synopsis of the Ph. D. thesis entitled **"Synthesis and Biological Evaluation of Chitooligosaccharides and Related Derivatives"** is divided into five chapters. Chapter 1 presents general introduction to chitooligosaccarides synthesis and biological importance. Other four chapters account the synthesis and biological evaluation of chitooligosaccharides and their derivatives (chitobioses, chitotrioses, *p*-nitrophenyl chitobiosides). Thioglycosides and glycosyl trichloroacetimidates were utilized as glycosyl donors in the synthesis of chitooligosaccharides and their derivatives. Each chapter is subdivided into six sections namely introduction, results and discussion, conclusions, experimental section, references along with representative spectra.

Chapter 1: Introduction to Chitooligosaccharides

Chapter 1 presents the general introduction emphasizing poly- and oligosaccharides. Majorly, oligosaccharides related to chitin and chitosan have been discussed in detail. More specifically, the synthesis and biological importance of chitooligosaccharides have been reviewed. A brief discussion on glycosyl donors containing different leaving groups pertaining to the thesis work is provided. Finally, the construction of stereoslective glycosidic linkage for the synthesis of chitooligosaccharides is discussed.

Chapter 2: Synthesis and Biological Evaluation of Mono *N*-Acetylchitobioses

This chapter presents the chemical synthesis of mono *N*-acetylchitobioses **AD** and **DA** (Figure 1) and their binding studies on chitosan binding protein *Pe*CBM32. For the synthesis of mono *N*-acetylchitobioses, thioglycoside was used as glycosyl donor can be activated under NIS/TfOH reagent system. *N*-Phth group serve as amine protecting group and azide acts as masked amine group. Directing ability of phthalimide group was exploited for the formation of β-glycosides.

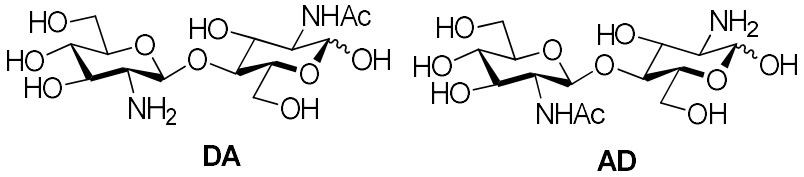


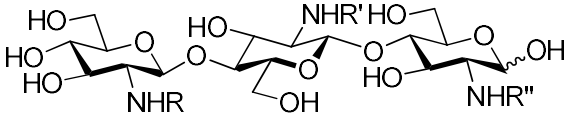
Figure 1: Synthesized mono *N*-acetylchitobioses (**AD**, **DA**).

To investigate the biological effects of the synthesized mono *N*-acetylated chitobioses, binding studies on chitosan binding protein *Pe*CBM32 were performed using isothermal

titration calorimetry (ITC). Results suggest the 1:1 binding of chitobiose having different pattern of *N*-acetylation (GlcN-GlcNAc and GlcNAc-GlcN) with *Pe*CBM32. Binding constant (K_b) for the GlcN-GlcNAc (**DA**) is greater (almost double) than that with GlcNAc-GlcN (**AD**) indicating stronger binding affinity of **DA** than **AD** on *Pe*CBM32.

Chapter 3: Synthesis of Partially *N*-Acetylated Chitotrioses and Their Biological Effects on Human Endothelial Cells

Synthesis of partially *N*-acetylated chitotrioses were achieved using chemoselective glycosylation strategy. Glycosyl trichloroactemidates and thioglycosides were used as glycosyl donors for the synthesis of partially *N*-acetylated chitotriose derivatives under chemoselective glycosylation conditions. Three kinds of D-glucosamine building blocks with different amine precursors (*N*-Phth, *N*-DMM, N_3) at C-2, were used in the synthesis. They were finally converted into free amino or acetamido groups. Synthesized chitotrioses having different *N*-acetylated pattern (**DDA**, **DAD**, **DAA**, **ADA**, and **AAD**) are shown in the Figure 2.



DDA: R = H, R' = H, R"= Ac
DAD: R = H, R' = Ac, R"= H
DAA: R = H, R' = Ac, R"= Ac
ADA: R = Ac, R' = H , R"= Ac
AAD: R = Ac, R' = Ac, R"= H

Figure 2: Synthesized partially *N*-acetylchitotrioses.

To investigate the biological effects of synthesized chitooligosaccharides [dimers (Chapter 2) and trimers] on human endothelium, studies were carried out in the context of coagulation and inflammation. Biological results suggest the impact of chitooligosaccharides (dimers and trimers) on the formation and immobilisation of ULVWF (Ultralarge von Willebrand factor) fibres on the luminal EC surface and chitooligosaccharide pre-treatment improves platelet binding to HUVECs (Human Umbilical Vein Endothelial Cells) under flow (Figure 3). This preliminary evidence of a non-toxic chitooligosaccharide-mediated change of the endothelial surface is most likely due to the direct interaction between the positively charged chitooligosaccharides and the negatively charged glycocalyx. This beneficial effect of VWF-mediated coagulation and biomedical application of chitoligosaccharides having different *N*-acetyl pattern might be valuable in treating bleeding disorders (Coagulation).

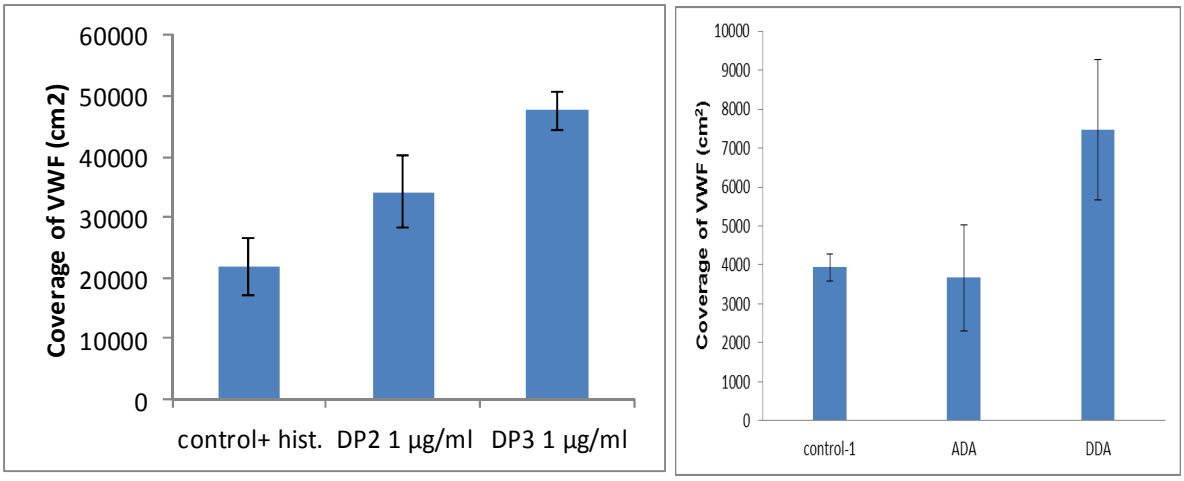


Figure 3: Entrapment of platelets to the endothelium under continuous and laminar flow conditions (shear stress = 6 dyne/cm^2) is amplified upon pre-treatment with chitooligosaccharides.

Chapter 4: Synthesis of *p*-Nitrophenyl Chitobiosides and Their Biological Evaluation

To study the activities of chitinolytic enzymes we developed synthetic chitooligosaccharide related chromogenic substrates, for example, *p*-nitrophenyl chitobiosides (Figure 4). Using thioglycoside donor, the *p*-nitrophenyl chitobiosides having different *N*-acetylated patterns were synthesised. To know the structure-activity relationships, substrate specificity of chitinolytic enzymes and glycosidases was studied using the synthesised *p*-nitrophenyl chitobiosides.

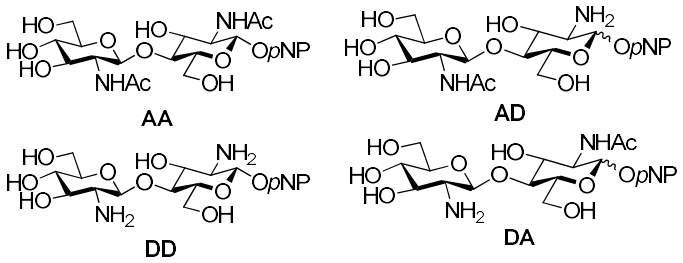


Figure 4: Synthesized of *p*-nitrophenyl chitobioside derivatives.

The results of biological studies suggest that the chitinolytic enzymes such as chitinases (ChiG and Bli), chitosanases (CSN) and glycosidases (GlcNAcase/GlcNase) have specificities with the novel *p*-nitrophenyl chitobioside derivatives (Figure 5 and 6)

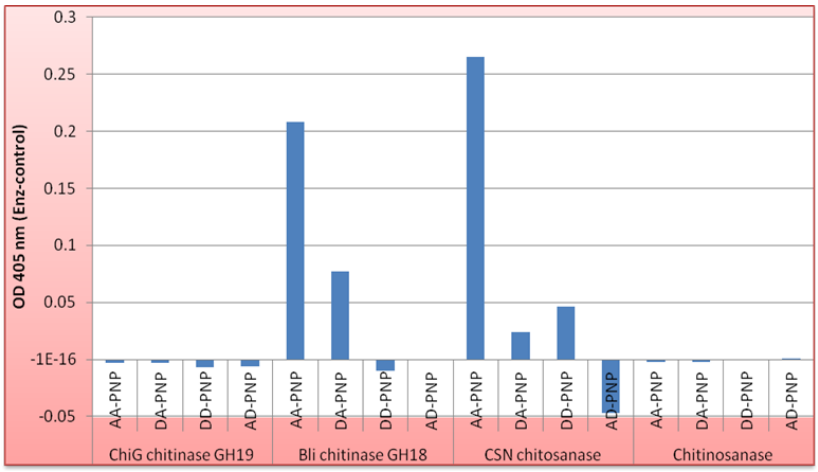


Figure 5: Substrate specificity of chitinolytic enzymes with *p*-nitrophenyl chitobiosides.

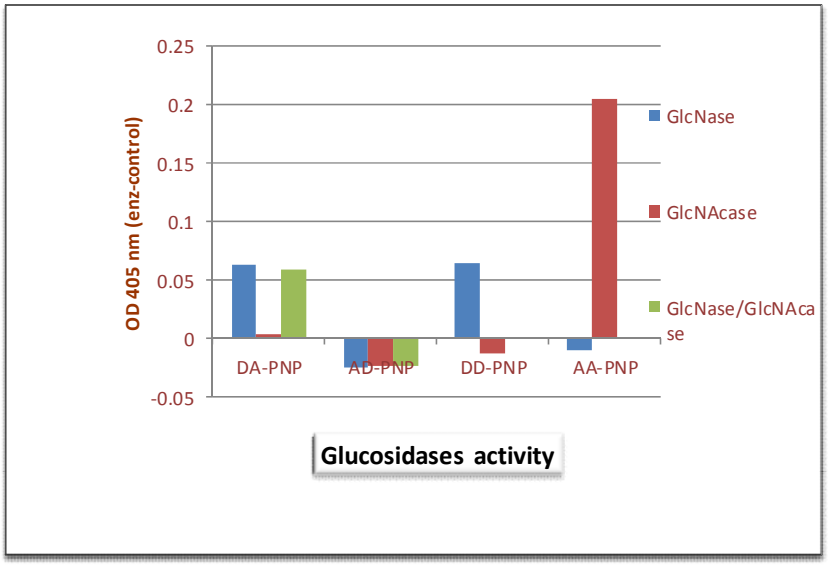


Figure 6: Glycosidases activity with different *p*-nitrophenyl chitobiosides.

Chapter 5: Towards the development of glycosidase inhibitors

With the aim to develop inhibitors for glycosidases, we designed molecules whose structure resembles the chitobioses having different *N*-acetylated patterns **A** (Figure 7). These compounds are expected to act as inhibitors by resisting the enzymatic hydrolysis due to the absence of anomeric carbon. However, all our attempts to synthesize the planed target chitobioside mimics failed. Later on we focussed on the synthesis of aryl esters having free or *N*-acetylated amine **B** as shown in the Figure **7**.

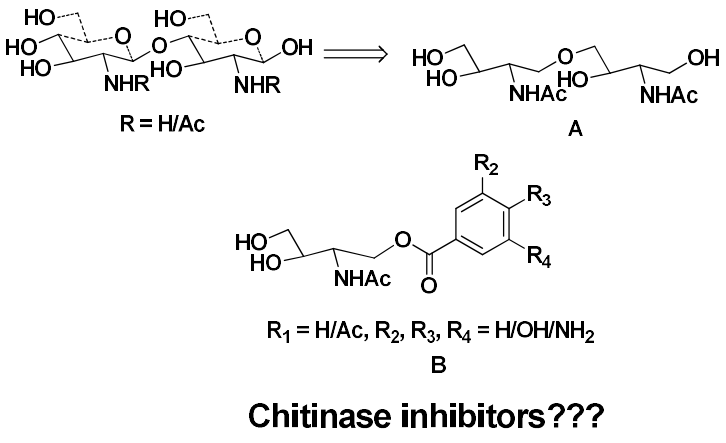


Figure 7: Target chitobioside mimics and aryl ester derivatives.

To check the chitinase inhibitor activity of the synthesized substituted aromatic esters **B**, we chose highly active chitinases *Pe*Chi3 from *Paenibacillus elgii* and *Sp*ChiD from *Serratia proteamaculans*. These results indicated that the synthesized aromatic esters were not active as they are smaller in size and hence may not bind to the catalytic cleft of chitinases (*Pe*Chi3 and *Sp*ChiD) to inhibit their enzymatic activities.

Chapter 1

Introduction to chitooligosaccharides

1.1 Importance of carbohydrates

Carbohydrates are one of the important class of biomolecules present in living system. Apart from acting as source of energy, they are involved in many vital functions of biological system. They are involved in cell and protein recognition. They are structurally defined as polyhydroxy aldehydes or ketones.[1] Carbohydrates can be classified into simple and complex carbohydrates. Examples of simple carbohydrates are monosaccharides such as glucose, fructose, galactose and mannose etc.

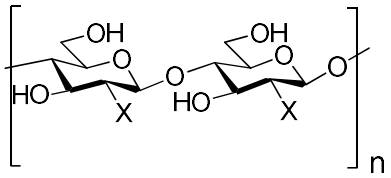
Complex carbohydrates can be classified as di- oligo- and polysaccharides based on the number of monosaccharide residues present in sugar. Glycoconjugates which are made of simple carbohydrates (mono, di, and oligosaccharides) linked with other biomolecules such as fatty acids, peptides involve in key biological functions.[2]

1.2 Carbohydrate polymers

Polysaccharides are large-sized carbohydrates made up of hundreds of monosaccharide residues. These carbohydrate polymers have potential application in the research areas such as chemistry, drug delivery, food, health, nanotechnology, bioenergy, bioplastics, biomaterials and biorefining etc.[3,4] These polymers can be isolated from natural sources. Polysaccharides consisting of similar or different monomer units are linked *via* glycosidic bonds such as α-(1→1), α-(1→2), α-(1→4), α-(1→6), β-(1→3), β-(1→4). Many polysaccharides are homopolymers such as starch, glycogen, cellulose and chitin (Figure 1.1). Starch and glycogen are storage polysaccharides consisting of a large number of glucose units and are found in plants and animals.[3] Starch is used in processed foods, and as thickening, stiffening or gluing agent and also adhesive in papermaking process. Other more common polysaccharide, cellulose consists of β-(1→4) glycosidic linkages between glucose residues. Cellulose **1** is an important structural component present in the cell walls of plants, algae and oomycetes. It is mainly obtained from cotton and wood pulp. Cellulose is mainly

used to produce paper. Using cellulosic ethanol as biofuel is under investigation as an alternative fuel source.[4]

Chitin **2**, another naturally occurring polymer, can be described as a homopolymer of a β-(1→4)-linked *N*-acetyl glucosamine (GlcNAc). Chitosan **3** which is a partially deacetylated derivative of chitin composed of randomly distributed β-(1→4)-linked glucosamine (GlcN) and GlcNAc units.[5] Chitin is a major constituent of the cell walls of fungi, exoskeletons of arthropods, such as crustaceans and insects and the beaks of cephalopods. Generally, chitin is obtained from the exoskeleton of crustaceans by industrial process.[6] Both have been found to have important role in many biological applications due to their low cost, nontoxicity, biocompatibility and biodegradability.[7] Especially, chitosan is present in skin-care creams, shampoos and hairsprays due to its antibacterial properties.[8,9] Also it is a main component in food additives[10] and environmental protecting agent.[11] Chitin and chitosan have poor solubility in water and most organic solvents under physiological pH conditions. This property makes chemical modification difficult and restricts their usage, particularly in medicine and food industry.[12]



1. X = OH (Cellulose)
2. X = NHAc (Chitin)
3. X = NHAc/NH_2 (Chitosan)

Figure 1.1: Structural representation of homopolysaccharides cellulose **1**, chitin **2** and chitosan **3**.

Heteropolysaccharides provide support to extracellular matrix that holds individual cells together in animal tissues and offer protection, shape and support to cells, tissues and organs in organisms from bacteria to humans. Fibrous proteins like collagen, elastin, fibronectin, laminin, hyaluronic acid, condroitin sulfate and dermatan sulfate are important heteropolysaccharides consist of amino sugar and acid sugar residues in the extracellular matrix. Another heteropolysaccharide namely heparin acts as a natural anticoagulant produced by basophils and mast cells.[13]

1.3 Importance of oligosaccharides

Glycoconjugates and oligosaccharides play a pivotal role in many biological processes.[14,15] Glycoconjugates can be subdivided into glycoproteins, glycolipids and proteoglycans. Glycan part in the glycoconjugates involves mainly in the antigen-antibody interactions, transport of toxins through the cell membrane and other molecular recognition. Cell membranes consisting of glycoconjugates involve in greater number of biological events such as cell-cell recognition, fertilization, cell growth and immune responses.[16,17] Oligosaccharides in the form of glycoconjugates such as glycolipids and glycoproteins involve in the transfer of information between the cells. This process is fundamental for life in all living systems.[18,19] There are two main classes of glycoproteins, namely *N*-linked and *O*-linked glycoproteins. The classification depends on whether the oligosaccharide side chain is linked to a protein *via* serine/threonine amino acid (*O*-linked) or asparagine (*N*-linked). Other glycoproteins such as *S*-linked, *P*-linked, *C*-linked are less abundant.[20] Examples of *O*-linked glycoproteins are glycophorin, mucin and thrombospondin etc. Sialyl-Lewis^X is a tetrasaccharide attached to *O*-glycans found on the cell surface, which plays an important role in cell-cell recognition and fertilization.[21]

1.3.1 Chitooligosaccharides and their derivatives

As already discussed, chitin/chitosan has poor solubility in water and most organic solvents which makes chemical modification on them difficult there by restricts their usage in medicine and food industry. Researchers have focused on partially hydrolyzed chitosans, i.e., chitooligosaccharides to improve the solubility for conducting biological studies. Chitooligosaccharides (COS) can be defined as β-(1→4) linked homo- or hetero-oligomers of GlcNAc and/or GlcN are highly soluble in neutral aqueous solutions and exhibit strong biological functions. They show biological activities such as antibacterial, antifungal, antitumor, anticoagulant, wound healing and elicitors of plant defence.[22-26] Fully and partially *N*-acetylated chitooligosaccharides are of interest for the study of the structure-activity relationships, determination of the substrate specificities and mechanism of action of chitinases and chitosanases which hydrolyze chitin and chitosan respectively.[27,28] Interestingly, these bio functions strongly depend on the degree of polymerization (DP), degree of *N*-acetylation (DA) and pattern of *N*-acetylation (PA) of the COS.[29] Due to the distinguished biological activities of chitooligosaccharides, different protocols have been developed to prepare different *N*-acetylated chitooligosaccharides.

Chitooligosaccharide congeners have also showed important role in many biological processes. These derivatives such as lipochitooligosaccharides **5**, thiochitooligosaccharides **6**,

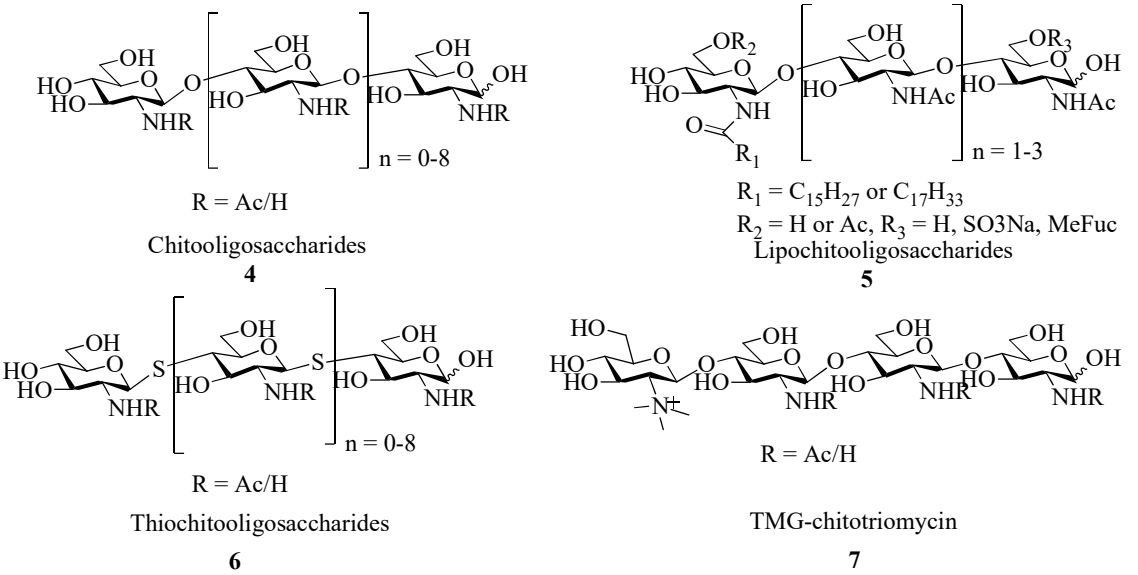
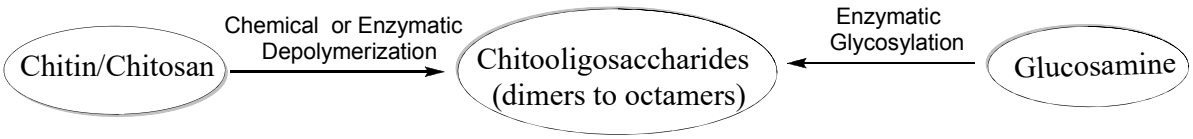


Figure 1.2: Structural representation of chitooligosaccharides and related derivatives (**4-7**).

TMGchitotriomycin **7** etc. are shown in Figure 1.2. Lipochitooligosaccharides acts as nod factors, i.e., nodulation signal molecules in the symbiosis of rhizobium bacteria in leguminous plants.[30,31] TMG-chitotriomycin **7** is highly selective inhibitor against the β-*N*-acetylglucosaminidases of insects and fungi.[32,33] Thiochitooligosaccharides show glycosidase inhibitory activity, which might be due to the resistance of thioglycosidic linkage enzymatic degradation.[34,35]

1.4 Chemical synthesis of chitooligosaccharides

Chitooligosaccharides can easily be produced by chemical and enzymatic depolymerization of chitin and chitosan. However, these methods produce heterogeneous COS mixtures.[22a,b] Disadvantages of these methods are the controlling of reaction, purification and characterization of products which are quite difficult task (Scheme 1.1). On the other hand glucosamine can be used as starting material to generate chitooligosaccharides by enzymatic glycosylation.[22c] Again the issue is the heterogeneity of the products.

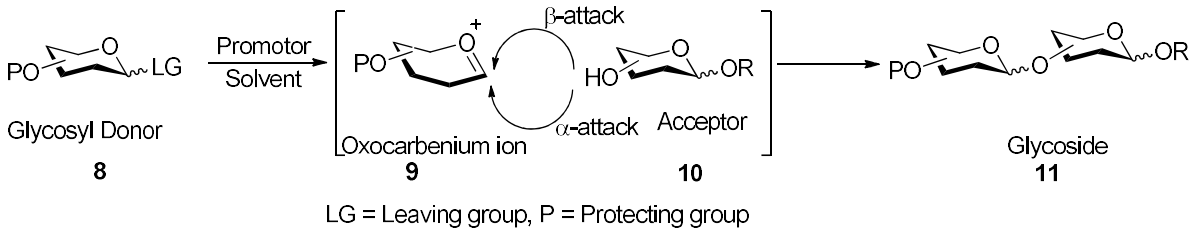


Scheme 1.1: Ways of preparation of chitooligosaccharides.

In order to study the biological activities such as the substrate specificity, characterization and mechanism of different enzymes, sufficient quantities of pure COS are required. For that efficient chemical strategies are required to synthesize well-defined fully and partially *N*-acetylated chitooligosaccharides. However, chemical synthesis of chitooligosaccharides is less explored area. Considering the importance of complex oligosaccharides,[36] well-defined oligosaccharides are good candidates to study many biological activities.

1.4.1 Glycosidic bond formation

The construction of glycosidic linkages are very important in the synthesis of complex oligosaccharides.[37,38] Glycosylation reactions involve formation of inter glycosidic linkage between glycosyl donor and glycosyl acceptor. A compound which donates a glycosyl moiety is called as glycosyl donor and the alcohol that receives it is known as acceptor. In these reactions glycosyl donors can be activated in the presence of promotors either in catalytic or stoichiometric amounts. Promoters initiate the formation of oxocarbenium ion intermediate **9** by activating the leaving group of glycosyl donor **8**. Subsequently the nucleophilic attack of acceptor alcohol **10** on the intermediate **9** leads to the formation of glycoside **11** (Scheme 1.2).[39,40]



LG = Leaving group, P = Protecting group

Scheme 1.2: Formation of glycosidic bond.

1.4.2 Glycosyl donors

Many glycosyl donors have been utilized for the synthesis of oligosaccharides and glycoconjugates. Among them, arguably, glycosyl halides, glycosyl trichloroacetimidates and thioglycosides have been extensively used because of the ease of preparation of them and their superior reactivity in the glycosylation reactions. Some of the glycosyl donors are given in Figure 1.3.

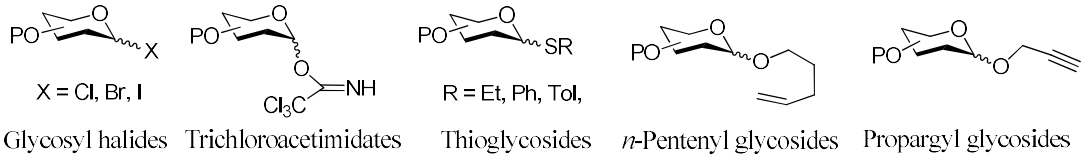


Figure 1.3: Different glycosyl donors.

1.4.2.1 Glycosyl halide donors

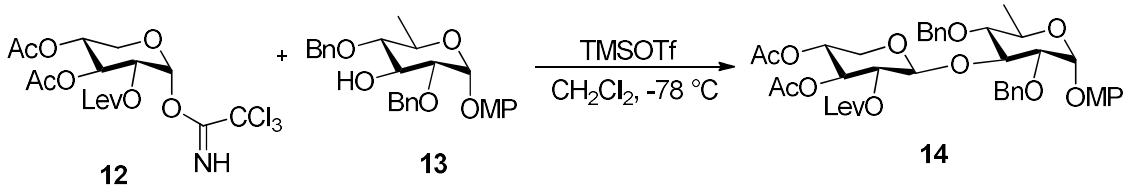
Glycosyl chlorides and bromides are effective donors which were introduced by Koenigs and Knorr in glycan synthesis.[41] This can be activated using heterogeneous catalysts such as Ag_2CO_3, Ag_2O or homogenous catalysts like $HgBr_2$, $Hg(CN)_2$ and AgOTf.[41,42]

To overcome the problems associated with glycosyl chlorides, Mukiyama *et al.* utilized the stable glycosyl fluorides in the preparation of *O*-glycosides.[43] Glycosyl fluorides can be activated using agents like $SnCl_2$-$AgClO_4$, TMSOTf,[44] $BF_3 \cdot Et_2O$,[45] Cp_2MCl_2-$AgClO_4$ (M = Zr, Hf).[46,47]

1.4.2.2 Glycosyl trichloroacetimidate donors

In 1980, Schmidt *et al.* reported glycosyl trichloroacetimidate as a very efficient alternative donor to the glycosyl halides.[48,49] This can be activated by catalytic amounts of TMSOTf,[50] $BF_3 \cdot Et_2O$,[48] AgOTf[51] under mild reaction conditions. Anomeric configuration of trichloroacetimidates is crucial for stereoselective glycosidic bond formation.[48]

Li *et al*. have successfully utilized the donor capability of trichloroacetimidate donor **12** in the synthesis of pentasaccharide moiety of Thornasterside A.[52] Formation of intermediate β-glycoside **14** depends on the anomeric configuration of acetimidate donor **12** and the participating ability of levulinate ester (Scheme 1.3).



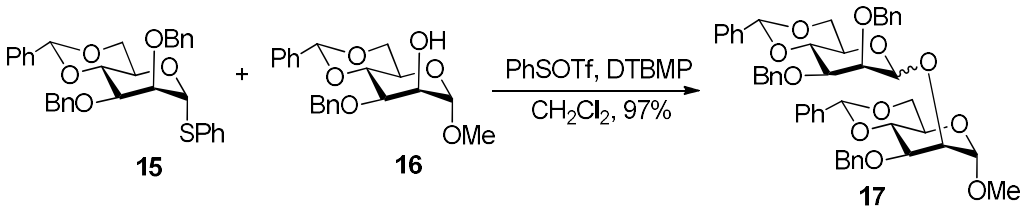
Scheme 1.3: Utilization of trichloroacetimidate donor **12** in the oligosaccharide synthesis.

1.4.2.3 Thioglycoside donors

Ferrier *et al.* used thioglycosides in glycosylation reactions for the first time.[53] In the preliminary stages, these donors were activated with heavy metal salts like $HgSO_4$, Ag(I),

Cu(II), Pd(II) etc.[54] Some non-metal compounds have also been found to activate these donors such as DMTST,[55] methylsulfonyl triflate,[56] benzenesulfenyl triflate,[57] NIS/TfOH[58,59] etc. Chemoselective oligosaccharides synthesis by using the activators like BSP/Tf_2O and DPSO/Tf_2O systems have also been reported.[60]

Crich *et al.* utilized thioglycoside donor for the synthesis of β-mannosides selectively. In their methodology, first thioglycoside **15** was treated with PhSOTf and DTBMP at -78 ºC to obtain α-mannosyl triflate intermediate and the reaction of this triflate with acceptor **16** resulted in β-anomeric glycoside **17** (β:α = 18:1) in good yield (Scheme 1.4).[60]



Scheme 1.4: Preparation of β-mannosides using thioglycoside **15** as a donor.

Other donors such as *n*-pentenyl glycosides,[61-63] phosphate/phosphite derivatives,[64,65] sulfoxides,[66] glycals,[67] 1-acyl,[68] 1-carbonates,[69] orthoesters,[70] 1,2-anhydro sugars,[71] 1-hydroxyl sugars,[72] and glycosyl thioimidates[73] have also been used in oligosaccharide synthesis.

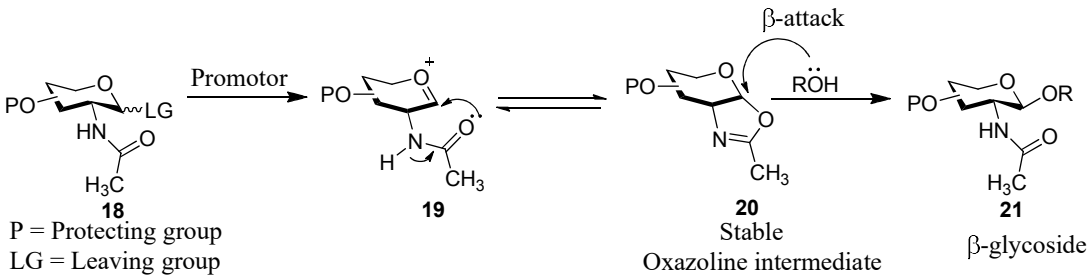
1.5 Amino protecting groups and stereoselective glycosidic bond formation

N-Acetylglucosamine/glucosamine derivative is present in many glycoconjugates and oligosaccharides such as chitin and chitosan through β-glycosidic linkages. Construction of β-glycosidic linkage is a major challenge in the oligosaccharide synthesis. Amino protecting group in the glycosyl donor can favour the stereoselective glycosidic bond formation through the neighbouring group participation and their steric crowding nature.[74,75]

1.5.1 Acetyl group (*N*-acetyl) as amine protecting group

In earlier days, acetyl group was used as an amine protecting group for the preparation of simple glycosides in a stereoselective manner. In the mechanism, first step involves the formation of oxocarbenium ion **19** by the activation of leaving group in donor **18** in the presence of promotor. Participating group at C-2 position in **19** assists the formation of *β*-glyosidic linkages through the oxazoline intermediate **20**. *N*-Acetyl derivative can serve as a glycosyl donor to construct the glycosidic linkage in a stereoselective manner.[74] But these

donors are not very effective in the oligosaccharide synthesis due to the stability of methyl oxazoline derivative **20** (Scheme 1.5). To avoid this problem, presence of electron withdrawing groups such as trifluoroacetyl, trichloroacetyl, and trichloroethoxycarbonyl groups in place of acetyl group offer better results.[75]

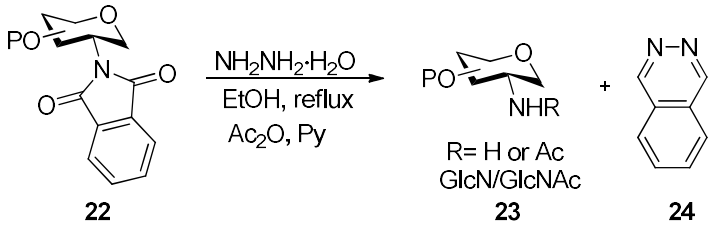


Scheme 1.5: Stereoselective glycosidic bond formation *via* neighbouring group participation.

1.5.2 Phthalimide group (*N*-Phth) as amine protecting group

Phthalimide group is widely used as an amine protecting group in carbohydrate chemistry.[76] Preparation of phthalimide derivative can be achieved by reacting the amine with phthalic anhydride in the presence of a base. This can be deprotected using methylamine/hydrazine/ethylenediamine in refluxing methanol/ethanol/butanol (Scheme 1.6).[77]

Conversion of *N*-Phth group into amine derivative followed by acetylation gives the *N*-acetyl derivatives in the oligosaccharides synthesis. *N*-Phth group is very useful for the formation of β-glycosidic linkages because of its both steric crowding nature and neighbouring group participation. 4,4,5,5-Tetrachlorophthaloyl (TCP) and 4,5-dichlorophthaloyl (DCPhth) groups have also been utilized in the stereoselective glycoside synthesis.[78]

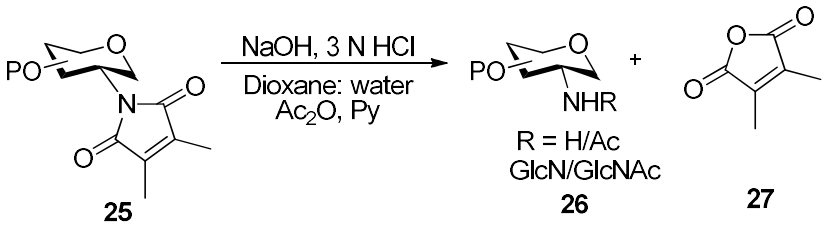


Scheme 1.6: Deprotection of *N*-Phth protecting group.

1.5.3 Dimethylmaleoyl (*N*-DMM) group as amine protecting group

Schmidt *et al.* used dimethylmaleoyl (DMM) group as an amine protecting group in the *N*-acetyl oligosaccharides synthesis. *N*-DMM derivative can be accessed by the reaction of

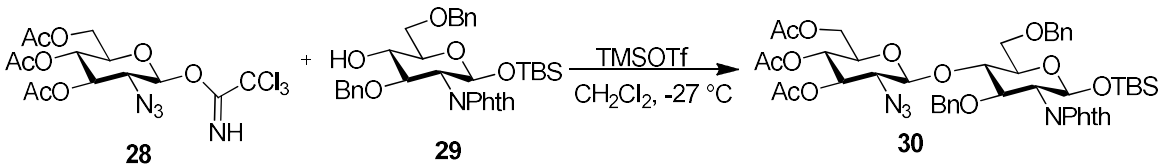
amine with dimethylmaleic anhydride. Removal of DMM group by treatment with base followed by acid gives the free amine. This is a good alternative for the phthalimide protecting group (Scheme 1.7).[79]



Scheme 1.7: Deprotection of DMM protecting group.

1.5.4 Azide (N_3) as masked amine group

Azides are versatile masked amine groups in the glycan synthesis. Azide containing trichloroacetimidates were widely used in α and β-glycoside synthesis.[80] Interestingly, β-linked disaccharide **30** was obtained in the glycosylation of N_3 containing trichloroacetimidate **28** with 4-hydroxy free acceptor **29** (Scheme 1.8).



Scheme 1.8: Utilization of azides as masked amine groups.

Under catalytic hydrogenation conditions azides can be reduced along with the removal of benzyl groups and alkene functionality.[81] Selective reduction of azido groups can also be achieved using PPh_3 in THF/water solvent mixture. Reductive acetylation of azide gives the *N*-acetyl derivatives in presence of AcSH/Py reagent system, which is useful in the synthesis *N*-acetyl oligosaccharides. *p*-Nitrobenzyloxycarbonyl,[82] trichloroethoxycarbonyl (Troc) group[83] are also utilized in the *N*-aminoglycoside synthesis.

1.6 Objective

Our object was to develop methods for the synthesis of chitooligosaccharides with a specific *N*-acetylated pattern and evaluate their biological activities. Chapters 2-5 describe our efforts on the synthesis of chitobioses, chitotrioses *p*NP-chitobiosides and related compounds. Biological studies have been carried out using the synthesized compounds.

1.7 References

1. Bochkov, A. F.; Zaikov, G. E.; Afanasiev, V. A. *Carbohydrates*, Vsp, Netherlands. **1991**.

2. Robyt, J. F. *Essentials of Carbohydrate Chemistry*, Springer, New york. **1998**.

3. Ball, S. G.; Morell, M. K. *Annu. Rev. Plant Biol*. **2003**, *54*, 207.

4. Gray, H. L.; Gtaud, C. J. *Chem. Rev.* **1928**, *4*, 355.

5. Zikakis, J. P. *Academic Press, Inc. New York*. **1984**.

6. Peter, M. G. *Biopolymers* **2002**, *6*, 481.

7. No, H. K.; Meyers, S. P. *J. Aquatic Food Product Technol.* **1995**, *4*, 27.

8. Kumar, M. N. V. R. *React. Funct. Polym.* **2000**, *46*, 1.

9. Anchisi, C.; Meloni, M. C.; Maccioni, A. M. *J. Cosmet. Sci.* **2006**, *57*, 205.

10. Agullo, E.; Rodriguez, M. S.; Ramos, V.; Albertengo, L. *Macromol. Biosci.* **2003**, *3*, 521.

11. Kittur, F. S.; Vishukumar, A. B.; Varadaraj, M. C.; Tharanathan, R. N. *Carbohydr. Res.* **2005**, *340*, 1239.

12. (a) Peter, M. G. *J. Macromol. Sci., Pure Appl. Chem*. **1995**, *A32*, 629. (b) Trombotto, S.; Ladaviere, C.; Delolme, F.; Domard, A. *Biomacromolecules* **2008**, *9*, 1731.

13. Sasisekharan, R.; Venkataraman, G. *Curr. Opin. Chem. Biol.* **2000**, *4*, 626.

14. Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357.

15. Rudd, P. M.; Elliott, T.; Cresswell, P.; Wilson, I. A.; Dwek, R. A. *Science* **2001**, *291*, 2370.

16. McAuliffe, J. C.; Hindsgaul, O. *Front. Mol. Biol*. **2000**, *30*, 249.

17. Varki, A. *Glycobiology* **1993**, *3*, 97.

18. Lis, H.; Sharon, N. *Eur. J. Biochem.* **1993**, *218*, 1.

19. Kolter, T.; Sandhoff, K. *Angew. Chem. Int. Ed.* **1999**, *38*, 1532.

20. Taylor, C. M. *Tetrahedron* **1998**, *54*, 11317.

21. Dwek, R. A. *Chem. Rev*. **1996**, *96*, 683.

22. (a) Aam, B. B.; Heggset, E. B.; Norberg, A. L.; Sorlie, M.; Vårum, K. M.; Eijsink, V. G. H. *Mar. Drugs*. **2010**, *8*, 1482. (b) Kim, S. K.; Rajapakse, N. *Carbohydr. Polym*. **2005**, *62*, 357.

23. Jeon, Y. J.; Park, P. J.; Kim, S. K. *Carbohydr. Polym*. **2001**, *44*, 71.

24. (a) Tokoro, A.; Tatewaki, N.; Suzuki, K.; Mikami, T.; Suzuki, S.; Suzuki, M. *Chem. Pharm. Bull*. **1988**, *36*, 784. (b) Liang, T. W.; Chen, Y. J.; Yen, Y. H.; Wang, S. L. *Process Biochem*. **2007**, *42*, 527.

25. Muraki, E.; Yaku, F.; Kojima, H. *Carbohydr. Res*. **1993**, *239*, 227.

26. (a) Artan, M.; Karadeniz, F.; Karagozlu, M. Z.; Kim, M. M.; Kim, S. K. *Carbohydr. Res*. **2010**, *345*, 656. (b) Fernandes, J. C.; Tavaria, F. K.; Soares, J. C.; Ramos, O. S.; Monteiro, M. J.; Pintado, M. E.; Malcata, F. X. *Food Microbiol*. **2008**, *25*, 922.

27. Honda, Y.; Tanimori, S.; Kirihata, M.; Kaneko, S.; Tokuyasu, K.; Hashimoto, M.; Watanabe, T.; Fukamizo, T. *FEBS Lett.* **2000**, *476*, 194.

28. Nordtveit, R. J.; Vårum, K. M.; Smidsrod, O. *Carbohydr. Polym.* **1996**, *29*, 163.

29. (a) Vander, P.; Vårum, K. M.; Domard, A. E. I.; Gueddari, N. E.; Moerschbacher, B. M. *Plant Physiol*. **1998**, *118*, 1353. (b) Akiyama, K.; Kawazu, K.; Kobayashi, A. *Z. Naturforsch. C* **1995**, *50*, 391.

30. Lerouge, P.; Roche, P.; Faucher, C.; Maillet, F.; Truchet, G.; Prome, J. C.; Denarie, J. *Nature* **1990**, *344*, 781.

31. Cooper, J. E. *J. Appl. Microbiol*. **2007**, *103*, 1355.

32. Usuki, H.; Nitoda, T.; Ichikawa, M.; Yamaji, N.; Iwashita, T.; Komura, H.; Kanzaki, H. *J. Am. Chem. Soc*. **2008**, *130*, 4146.

33. Yang, Y.; Li, Y.; Yu, B. *J. Am. Chem. Soc*. **2009**, *131*, 12076.

34. Wang, L. X.; Lee, Y. C. *J. Chem. Soc., Perkin Trans. 1* **1996**, 581.

35. Fettke, A.; Peikow, D.; Peter, M. G.; Kleinpeter, E. *Tetrahedron* **2009**, *65*, 4356.

36. Koeller, K. M.; Wong, C. -H. *Chem. Rev.* **2000**, *100*, 4465.

37. Ragupathi, G.; Koide, F.; Livingston, P. O.; Cho, Y. S.; Endo, A.; Wan, Q.; Spassova, M. K.; Keding, S. J.; Allen, J.; Ouerfelli, O.; Wilson, R. M.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2006**, *128*, 2715.

38. Seeberger, P. H.; Werz1, D. B. *Nature* **2007**, *446*, 1046.

39. Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503.

40. (a) Schmidt, R. R. *Angew. Chem. Int. Ed.* **1986**, *25*, 212. (b) Zhu, X.; Schmidt, R. R. *Angew. Chem. Int. Ed*. **2009**, *48*, 1900.

41. Koenigs, W.; Knorr, E. *Chem. Ber.* **1901**, *34*, 957.

42. Wulff, G.; Röhle, G. *Angew. Chem. Int. Ed.* **1974**, *13*, 157.

43. Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981**, *3*, 431.

44. Hashimoto, S.; Hayashi, M.; Noyori, R. *Tetrahedron Lett.* **1984**, *25*, 1379.

45. Nicolaou, K. C.; Chucholowski, A.; Dolle, R. E.; Randall, J. L. *J. Chem. Soc., Chem. Commun.***1984**, 1155.

46. Matsumoto, T.; Maeta, H.; Suzuki, K.; Tsuchihashi, G. *Tetrahedron Lett.* **1988**, *29*, 3567.

47. Nicolaou, K. C.; Caulfield, T. J.; Kataoka, H.; Stylianides, N. A. *J. Am. Chem. Soc.* **1990**, *112*, 3693.

48. Schmidt, R. R.; Michel, J. *Angew. Chem. Int. Ed.* **1980**, *19*, 731.

49. Schmidt, R. R.; Grundler, G. *Angew. Chem. Int. Ed.* **1983**, *22*, 776.

50. Schmidt, R. R. *Angew. Chem. Int. Ed.* **1986**, *25*, 212.

51. Robina, I.; LópezBarba, E.; Fuentes, J. *Tetrahedron* **1996**, *52*, 10771.

52. Xiong, J.; Lu, Z.; Ding, N.; Ren, S.; Li, Y. *Eur. J. Org. Chem.* **2013**, 6158.

53. Ferrier, R. J.; Hay, R. W.; Vethaviyasar, N. *Carbohydr. Res*. **1973**, *27*, 55.

54. Toshima, K.; Tatsuta, K. *Chem. Rev*. **1915**, *93*, 1503.

55. Fügedi, P.; Garegg, P. J. *Carbohydr. Res.* **1986**, *149*, C9.

56. Dasgupta, F.; Garegg, P. J. *Carbohydr. Res.* **1988**, *177*, C13.

57. Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1988**, *29*, 1061.

58. Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313.

59. Veeneman, G. H.; Leeuwen, S. H. V; Boom, J. H. V. *Tetrahedron Lett.* **1990**, *31*, 1331.

60. Crich, D.; Smith, M. *J. Am. Chem. Soc*. **2001**, *123*, 9015.

61. Fraser-Reid, B.; Konradsson, P.; Mootoo, D. R.; Udodong, U. *J. Chem. Soc., Chem. Commun.* **1988**, 823.

62. Mootoo, D. R.; Konradsson, P.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1989**, *111*, 8540.

63. Mootoo, D. R.; Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988**, *110*, 5583.

64. Hashimoto, S.; Honda, T.; Ikegami, S. *J. Chem. Soc., Chem. Commun*. **1989**, 613.

65. Kondo, H.; Ichikawa, Y.; Wong, C. H. *J. Am. Chem. Soc*. **1992**, *114*, 8748.

66. Kahne, D.; Walker, S.; Cheng, Y.; Engen, D. V. *J. Am. Chem. Soc*. **1989**, *111*, 6881.

67. Lemieux, R. U.; Levine, S. *Can. J. Chem*. **1962**, *40*, 1926.

68. Helferich, B.; Schmitz-Hillebrecht, E. *Chem. Ber*. **1933**, *66*, 378.

69. Inaba, S.; Yamada, M.; Yoshino, T.; Ishido, Y. *J. Am. Chem. Soc*. **1973**, *95*, 2062.

70. Kochetkov, N. K.; Bochkov, A. F.; Sokolovsakaya, T. A. *Carbohydr. Res*. **1971**, *16*, 17.

71. Lemieux, R. U.; Huber, G. *J. Am. Chem. Soc*. **1953**, *75*, 4118.

72. Nguyen, H. M.; Chen, Y.; Duron, S. G.; Gin, D. Y. *J. Am. Chem. Soc*. **2001**, *123*, 8766.

73. Demchenko, A. V.; Malysheva, N. N.; Meo, C. D. *Org. Lett*. **2003**, *5*, 455.

74. (a) Banoub, J.; Boullanger, P.; Lafont, D. *Chem. Rev*. **1992**, *92*, 1167. (b) Bongat, A. F. G.; Demchenko, A. V. *Carbohydr. Res*. **2007**, *342*, 374.

75. (a) Crich, D.; Dudkin, V. *J. Am. Chem. Soc*. **2001**, *123*, 6819. (b) Liao, L.; Auzanneau, F. I. *J. Org. Chem*. **2005**, *70*, 6265. (c) Enugala, R.; Carvalho, L. C. R.; Pires, M. J. D; Marques, M. M. B. *Chem. Asian J*. **2012**, *7*, 2482.

76. (a) Baker, B. R.; Joseph, J. P.; Schaub, R. E.; Williams, J. H. *J. Org. Chem*. **1954**, *19*, 1786. (b) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21. (c) Wang, A.; Auzanneau, F. I. *J. Org. Chem*. **2007**, *72*, 3585.

77. (a) Sato, S.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1988**, *29*, 5267. (b) Kanie, O.; Crawley, S. C.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **1993**, *243*, 139. (c) Srivastava, V.; Hindsgaul, O. *Carbohydr. Res.* **1989**, *185*, 163.

78. Debenham, J. S.; Fraser-Reid, B. *J. Org. Chem.* **1996**, *61*, 432.

79. Aly, M. R. E.; Castro-Palomino, J. C.; Ibrahim, E. -S. I.; El-Ashry, E. S. H.; Schmidt, R. R. *Eur. J. Org. Chem*. **1998**, 2305.

80. (a) Paulsen, H.; Kalar, C.; Stenzel, W. *Chem. Ber*. **1978**, *111*, 2358. (b) Tiruchinapally, G.; Yin, Z.; El-Dakdouki, M.; Wang, Z.; Huang, X. *Chem. Eur. J*. **2011**, *17*, 10106. (c) Paulsen, H. *Angew. Chem. Int. Ed.* **1982**, *21*, 155.

81. Sedinkin, S. L.; Zinin, A. I.; Malysheva, N. N.; Shashkov, A. S.; Torgov, V. I.; Shibaev, V. N. *Mendeleev Commun.* **2007**, *17*, 10.

82. Qian, X.; Hindsgaul, O. *Chem. Commun.* **1997**, 1059.

83. Dullenkopf, W.; Castro-Palomino, J. C.; Manzoni, L.; Schmidt, R. R. *Carbohydr. Res.* **1996**, *296*, 135.

Chapter 2

Synthesis and biological evaluation of mono *N*-acetylchitobioses

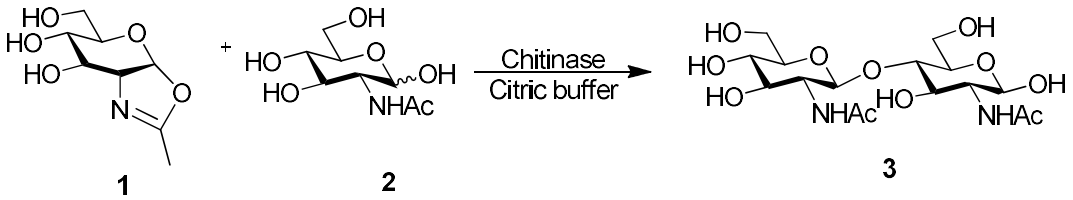
2.1 Introduction

Chitobioses are the simplest derivatives among the chitooligosaccharides. There are four chitobiose derivatives based on the degree and position of *N*-acetylation. Chitobiose building blocks have been widely used in the preparation of complex glycoconjugates and amino glycosides. Above all, they are used to study the structure–activity relationships, substrate specificity of chitinases and chitosanases.[1,2] Chitobioses are good substrates for the chitinolytic enzymes such as β-*N*-acetylglucosaminidases and lysozymes.[3,4] In addition, these are also useful in the biological studies of chitin binding proteins such as lectins.[5,6] Preparation of higher homochitooligosaccharides were achieved using *N*,*N*'-diacetylchitobiose *via* transglycosylation methodology. Synthesis of hexa-*N*-acetyl-chitohexaose and hepta-*N*-acetylchitoheptaose from *N,N'*-diacetylchitobiose have been achieved through lysozyme catalysis in the presence of ammonium sulfate buffer.[7,8] Due to these biological importance, several methods have been reported for the synthesis of fully or partially *N*-acetylated chitobioses. These derivatives can be obtained by chemical,[9] microbial or enzymatic degradation of chitin or chitosan.[10,11,12] Chemically, chitooligosaccharides can be obtained from depolymerization of chitin or chitosan polymers using acids such as HNO_2,[13] HF,[14] HCl,[15] sulfuric acid,[16] phosphoric acid,[17] and lactic acid.[18] These methods results in a mixture of chitooligosaccharides from dimers to chitooctamers. The homogeneity of the resulting chitooligosaccharide mixture makes characterization and purification of these mixtures very difficult to obtain pure form.[13,14] Substrate purity and sufficient quantities always matter for various biological studies. Only few methods are available in the literature to produce well defined structures of chitobiose derivatives either using chemical or enzymatic methods. They are documented below.

2.1.1 Enzymatic synthesis of fully and partially *N*-acetylated chitobioses

Kobayashi *et al*. reported a novel method to synthesize *N,N'*-diacetylchitobiose **3** *via* enzymatic glycosylation using oxazoline glycosyl donor **1** and *N*-acetylglucosamine **2**.[19] Here enzyme, chitinase from *bacillus sp*. is used for glycosylation reaction. Chitinase from *bacillus*

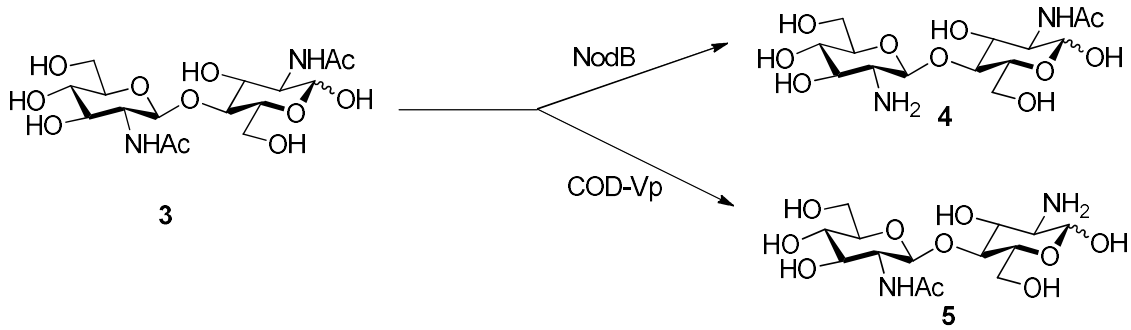
sp. has highly specific enzymatic activity at pH 7.8. This method involves the glycosidic bond formation which is highly stereo- and regioselective due to the high specificity of chitinase. Regioselective enzymatic glycosylation of the 4-hydroxy of *N*-acetyl glucosamine **2** with sugar oxazolinium intermediate **1** results the *N*,*N*'-diacetylchitobiose **3** *via* β-glycosidic bond formation (Scheme 2.1).



1 **2** **3**

Scheme 2.1: Synthesis of *N*,*N*'-diacetylchitobiose **3** *via* enzymatic glcosylation using oxazoline **1** as a glycosyl donor.

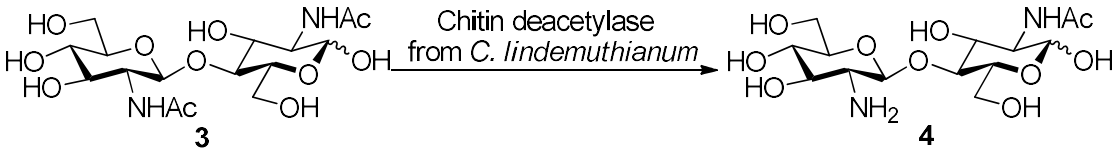
Partially *N*-acetylchitobioses **4** and **5** were synthesized from *N*,*N*'-diacetylchitobiose by regioselective enzymatic mono-deacetylation. John *et al*. investigated the activity of NodB deacetylase for the deacetylation of *N*,*N*'-diacetylchitobiose **3** at the non-reducing end (Scheme 2.2).[20] This enzyme is highly regioslective, deacetylates the *N*-acetyl group of the non-reducing end of the *N*,*N*'-diacetylchitobiose selectively. Chitin oligosaccharide deacetylase (COD) found in the species of *vibrio bacteria* is a class of enzyme that hydrolyses the amide bond at the reducing end of the chitooligosaccharides.[21] Hirano *et al*. reported the preparation of GlcNAc-β-(1,4)-GlcN **5** from *N,N'*-diacetylchitobiose **3** using chitin deacetylase (Scheme 2.2).



3 **4** **5**

Scheme 2.2: Synthesis of mono *N*-acetylchitobiose *via* enzymatic monodeacetylation.

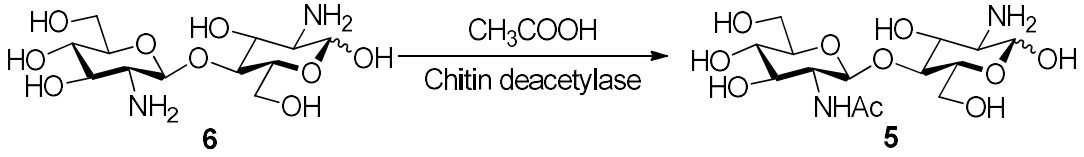
Tokuyasu *et al*. described the methods for the preparation of monoacetylated chitobioses **4** and **5** using chitin deacetylases from *Colletotrichum lindemuthianum via* selective enzymatic deacetylation (Scheme 2.3).[22]



3 **4**

Scheme 2.3: Preparation of mono *N*-acetylchitobiose (GlcN-GlcNAc) using chitin deacetylase.

Interestingly, the same group reported another chitin deacetylase for the enzymatic acetylation of chitobiose **6** for the preparation of GlcNAc-GlcN **5** (Scheme 2.4).[23]



6 **5**

Scheme 2.4: Preparation of mono *N*-acetyl chitobiose (GlcNAc-GlcN) *via* enzymatic acetylation.

There are advantages and disadvantages associated with enzymatic methods in producing fully and partially *N*-acetylated chitobioses. Several protection-deprotection steps which are usually encountered in chemical synthesis are not required and the concern about anomeric configuration of non-reducing sugar is not required as the starting chitobiose is synthesized from naturally occurring chitin or chitosan.

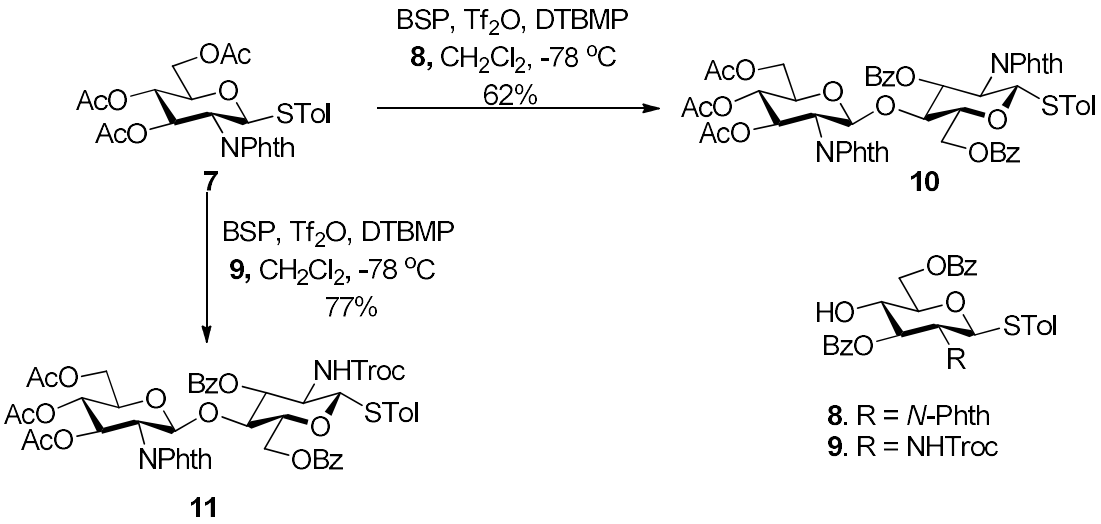
Disadvantages of enzymatic reactions are the high cost of hydrolytic enzymes, controlling the rate of enzymatic reaction which is not easy. Also, limited scope of substrates accepted by enzymes and the associated difficulties in purification and characterization of the heterogeneous product mixture.

2.1.2 Chemical synthesis of fully and partially *N*-acetylated chitobioses

To avoid the problems associated with the enzymatic methods efficient chemical strategies are required to produce fully and partially *N*-acetylated chitobioses. These are chemical probes employed in the biological studies such as evaluating the substrate specificities of chitinases and chitosanases. Chemical methods are good for making good amount of pure products. Only a few chemical methods are available in the literature for the synthesis of chitobiose derivatives with defined *N*-acetylated pattern. They are discussed below.

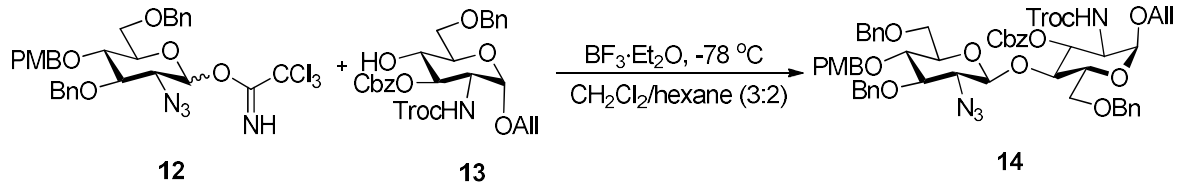
Hansen and Skrydstrup reported the synthesis of β-(1,4)-linked chitobiose derivatives by exploiting the Crich's *O*-glycosylation conditions involving glycosyl triflate intermediate.

These disaccharide building blocks are useful in the synthesis of higher chitooligosaccharides. Selective activation of thioglycoside donor **7** using Crich's methodology followed by reaction with the 4-hydroxy free thioglycoside acceptors **8** and **9** separately result in the formation of protected disaccharides **10** and **11** respectively (Scheme 2.5).[24]



Scheme 2.5: Synthesis of fully protected chitobioses **10** and **11** *via* chemical glycosylation.

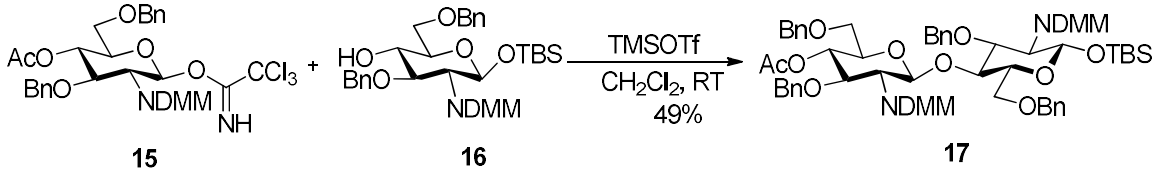
Cirillo *et al*. reported the synthesis of protected chitobiose derivative **14** by utilizing the N_3 containing trichloroacetimidate donor.[25] Generally, azide group acts as a masked amino group and plays a key role in the preparation of α-linked 2-amino glycosides. In some cases, it has been found that β-selectivity could also be attained with 2-azido glycosyl donor. Glycosylation of 4-hydroxy free sugar **13** with azide protected acetimidate donor **12** yielded the β-(1→4)-linked chitobiose derivative **14** (Scheme 2.6).[25] These building blocks are employed in the preparation of well-defined hetero-chitotetroses.



Scheme 2.6: Preparation of chitobiose derivative **14** using trichloroacetimidate donor **12**.

Schmidt and co-workers have reported the synthesis of fully protected chitobiose derivative **17**. They found that dimethylmaleoyl group as a versatile amino protective group in the glucosamine containing oligosaccharide synthesis. Utilization of participating capability of *N*-DMM group leads to the stereoselective β-glycosidic linkage. *N*-DMM

protected trichloroacetimidate donor **15** was activated with catalytic TMSOTf to react with 4-hydroxy free glucosamine derivative **16** to obtain the fully protected chitobiose derivative **17** (Scheme 2.7)**.**[26] This is a versatile building block for the preparation of fully *N*-acetylated chitotetroses.



Scheme 2.7: Preparation of *N*-DMM protected chitobiose derivative **17**.

2.2 Results and discussion

With the intention to develop efficient strategy for the synthesis of chitooligosaccharides with well-defined acetylated pattern, we focused on the synthesis of chitobioses, i.e., 2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose and 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-D-glucopyranose as shown in Figure 2.1. These chitobioses can serve as useful substrates to characterize the chitinolytic enzymes (*exo/endo*) as well as study the structure and activity relationships. For the synthesis of mono *N*-acetylchitobioses, thioglycosides were used as glycosyl donor along with NIS/TfOH as activator.[27] Phthalimide was used as the amine protecting group of the donor. The advantage of *N*-Phth is its ability to direct the acceptor to make β-glycosidic bond.[28] Azide glycoside was used as acceptor. The advantage of azide is that it can be converted into NH_2/NHAc group whenever it is required.

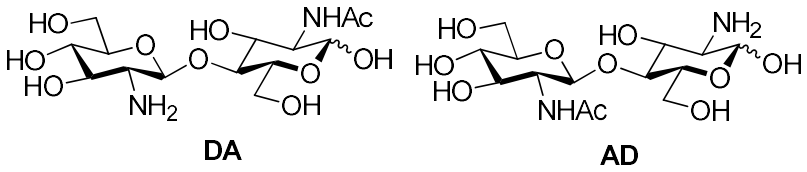
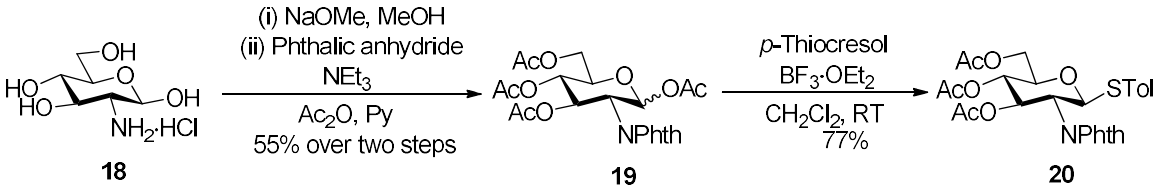


Figure 2.1: Structural representation of mono *N*-acetylated chitobioses (**AD** and **DA**).

2.2.1 Synthesis of *N*-Phth protected glycosyl donor

Thioglycoside donor **20** was synthesized in three steps from commercially available glucosamine hydrochloride **18** (Scheme 2.8). Glucosamine hydrochloride **18** was converted into 2-phthalimido derivative **19** by first neutralizing the glucosamine hydrochloride with NaOMe and then treating the free amine with phthalic anhydride in methanol as solvent. *In situ* peracetylation of the resulting product with Ac_2O in the presence of pyridine resulted

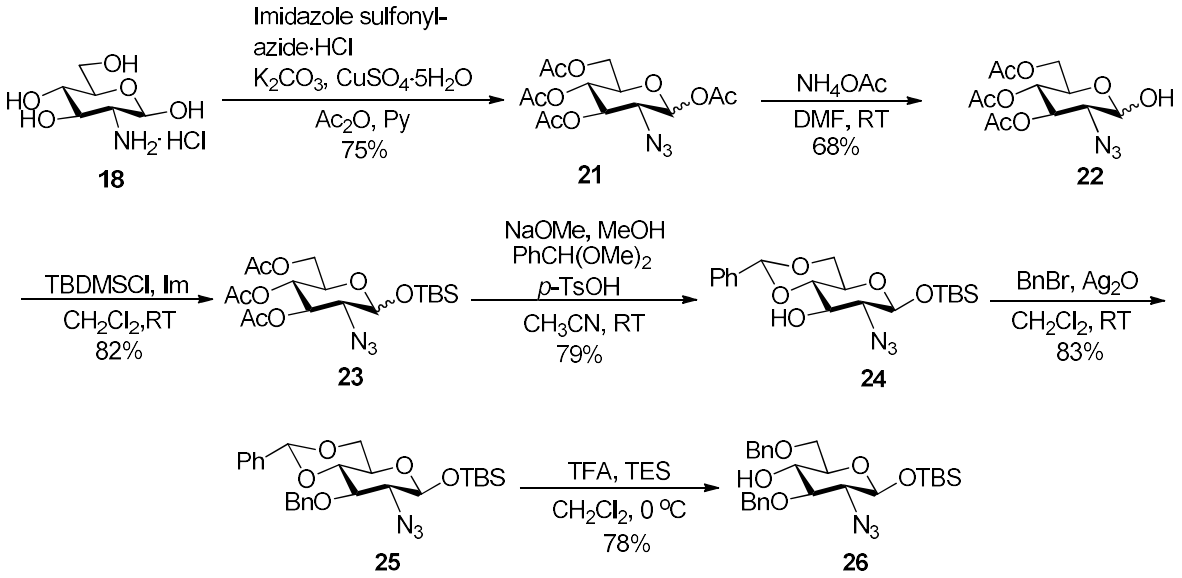
1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-α/β-D-glucopyranose **19** in 55% yield. Compound **19** was treated with *p*-thiocresol in the presence of $BF_3 \cdot OEt_2$ to get *p*-methylphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **20** in 77% yield as confirmed by NMR spectroscopy (Scheme 2.8).[29]



Scheme 2.8: Preparation of *p*-methylphenyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **20**.

2.2.2 Synthesis of azido glycosyl acceptor

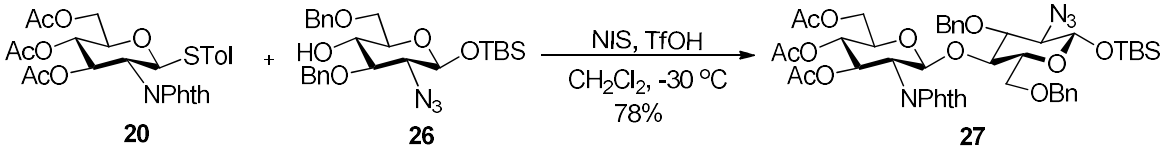
Glucosamine hydrochloride **18** was converted into azido glycoside by treating it with imidazole-1-sulfonyl azide hydrochloride in the presence of $CuSO_4 \cdot 5H_2O$ and base K_2CO_3 in methanol solvent. The resulting azido glycoside was peracetylated with Ac_2O in the presence of pyridine to obtain 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-D-glucopyranose **21** in 75% yield.[30] Compound **21** was treated with NH_4OAc in dry DMF to obtain anomeric unprotected sugar derivative **22**, which on treatment with TBDMS-Cl and imidazole gave *tert*-butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α/β-D-glucopyranoside **23**. Compound **23** was deacetylated with NaOMe in MeOH and the resulting triol was treated with benzaldehyde dimetyl acetal in the presence of *p*-TsOH to yield the *tert*-butyldimethylsilyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside **24** in 79% yield. Benzylidene derivative **24** was benzylated at the 3rd position with Ag_2O/BnBr reagent system in dry CH_2Cl_2 and the resulting product **25** was treated with TES and TFA to obtain *tert*-butyldimethylsilyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside **26** regioselectively in 78% yield (Scheme 2.9).[31] This compound **26** was utilized as glycosyl acceptor in the preparation of partially *N*-acetylchtobioses (**AD** and **DA**).



Scheme 2.9: Preparation of *tert*-butyldimethylsilyl 2-azido-2-deoxy-3,6-di-*O*-benzyl-β-D-glucopyranoside **26**.

2.2.3 Synthesis of disaccharide building block

After successfully synthesizing the well-defined glycosyl donor **20** and 4-hydroxy free acceptor **26**, we focused on the synthesis of disaccharide building block **27**. This disaccharide derivative **27** was used in the preparation of mono *N*-acetylchitobiose derivatives **4** and **5**. Reaction glycosyl acceptor **26** with glycosyl donor **20** in the presence of NIS/TfOH activator system[30] yielded fully protected chitobioside **27** in 78% yield (Scheme 2.10). Stereoslective glycosidic linkage formation is due to the neighboring group participation and steric crowding effect exerted by the bulky *N*-Phth protecting group. ^{1}H NMR data confirms the formation of β-linked disaccharide (^{1}H NMR: δ 5.61, J = 8.4 Hz, 1H).

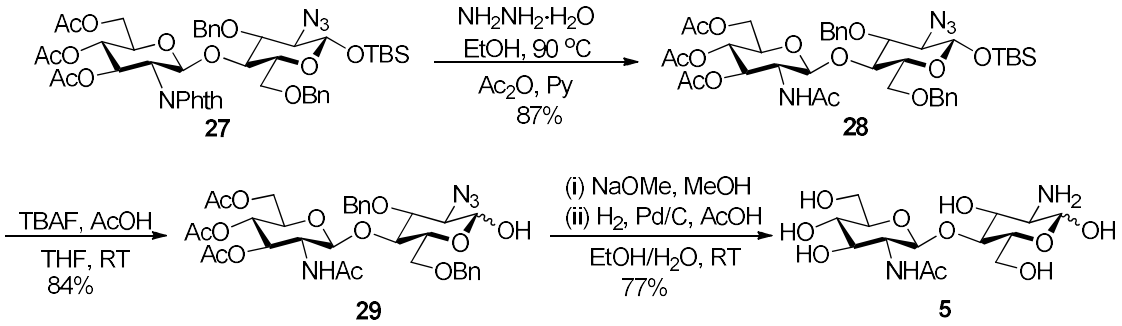


Scheme 2.10: Synthesis of fully protected chitobiose building block **27**.

2.2.3.1 Synthesis of mono *N*-acetylchitobiose derivative (AD)

Synthesis of *N*-acetylchitobiose derevative **5** (**AD**) was achieved by following a sequence of steps as shown in the Scheme 2.11. It starts with the removal of *N*-Phth group in compound **27** by treatment with NH_2NH_2·H_2O in EtOH at 90 ºC and the resulted amino

derivative was acetylated using Ac_2O/Py mixture (1:2) to get the *N*-acetyl derivative **28** in 87% yield. Desilylation of compound **28** was carried out using TBAF/AcOH in THF to provide the disaccharide derivative **29** in 84% yield. Finally *O*-deacetylation of compound **29** under Zemplén condition (NaOMe in MeOH) was performed. After completion of the reaction, the resulting mixture was neutralized with amberlite IR-120 resin. The filtrate was concentrated and the crude product was directly treated with H_2 under Pd/C conditions. Both reduction of azide and *O*-debenzylation occurred under the same condition (H_2, Pd/C) in one pot manner to give the *N*-acetylchitobiose **5** (**AD**) in 77% yield (Scheme 2.11).



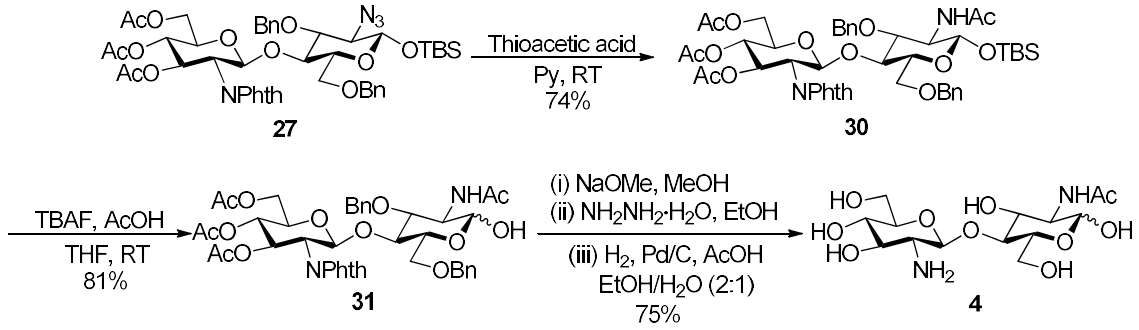
Scheme 2.11: Preparation of mono *N*-acetylchitobiose **5** (**AD**).

2.2.3.2 Synthesis of mono *N*-acetylchitobiose (DA)

N-Acetylchitobiose **4** (**DA**) was synthesized from disaccharide building block **27** by converting the azide function into *N*-acetyl followed by deprotection steps (Scheme 2.12). Reductive acetylation of azide in protected chitobioside **27** using thioacetic acid/Py reagent system gave the *N*-acetyl derivative **30** in 74% yield. Compound **30** was treated with TBAF/AcOH mixture to get the desilylated derivative **31** in 81% yield. To obtain the desired *N*-acetyl chitobiose **4** (DA), compound **31** was subjected to a sequence of deprotections as shown in Scheme 2.12. First *O*-deacetylation was carried out by NaOMe in MeOH. Then phthalimide was deprotected using $NH_2NH_2 \cdot H_2O$ followed by debenzylation using H_2 under Pd/C in the same pot.

The mono *N*-acetylchitobioses (AD and DA) were characterized by NMR spectroscopy and MALDI-MS analysis. Synthesized partially *N*-acetylated chitobioses was used in the binding studies on chitosan binding protein *Pe*CBM32 using isothermal titration calorimetry (ITC). Also, these mono *N*-acetylchitobioses were utilized in the biological studies such as blood coagulation and inflammation in human endothelium (Chapter 3). These are also important

chemical probes to know the structure and biological activity relationships, substrate specificity and mechanism of function of chitinolytic enzymes (chitinases and chitosanases).



Scheme 2.12: Preparation of *N*-acetyl chitobioside derivative **33** (**DA**).

2.2.4. Biological activity profiles of mono *N*-acetylated chitobioses

Chitosanases which cleave chitosan have a single catalytic domain with no auxiliary domains, unlike chitinases and cellulases which are frequently associated with auxiliary domains in addition to carbohydrate binding modules (CBMs).[32] CBMs play an important role in hydrolytic enzymes that mediate the recycling of carbon and nitrogen in the biosphere. The diversity of CBMs in terms of their sequence, specificity and mechanism of binding with ligand, offers an attractive model for studying CBM-carbohydrate interaction.[33] The mechanism of chitosan binding and degradation by chitosanases, in comparison to chitin and cellulose degradations, is not fully understood due to lack of well-defined chitosan substrates in terms of their degree of *N*-acetylation (DA) and pattern of acetylation (PA).[33]

2.2.4.1 ITC binding studies of partially *N*-acetylated chitobioses with *Pe*CBM32[33,34]

To investigate the biological effects of the synthesized partially *N*-acetylated chitobioses, binding studies on chitosan binding protein *Pe*CBM32 was carried out using isothermal titration calorimetry (ITC). The binding affinity (k_b) and stoichiometry (n) and other parameters (ΔH and ΔS) of *Pe*CBM32 towards chitobiose derivatives were quantified by ITC (Figure 2.2). Thermodynamic parameters such as association constant (K_b), binding stoichiometry (n) and change in free energy (ΔG) were obtained by nonlinear least-squares fitting of experimental data using the *one set sites* binding model using ITC. Experimental data from ITC studies suggest that the difference in the binding affinity of different *N*-acetylated pattern of chitobioses with chitosan binding protein *Pe*CBM32.

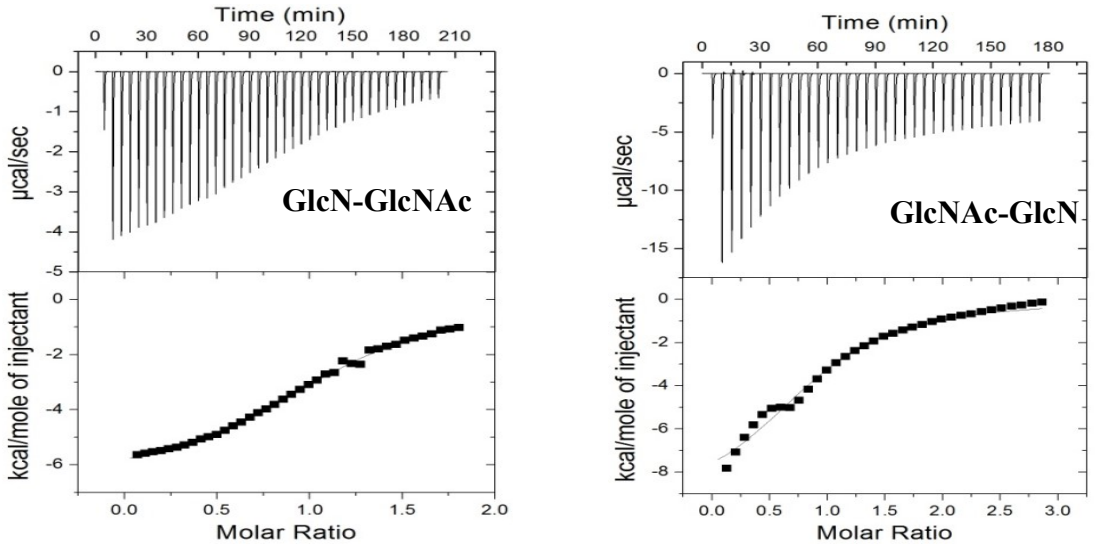


Figure 2.2: Upper panels show the raw ITC data obtained from successive automatic injections of the ligand from the syringe to the protein in the ITC cell. Lower panels show the integrated heats of binding obtained from raw data given in the upper panels together with binding isotherms to *one set sites* binding model. Results are shown for the binding of chitobiosides having different pattern of *N*-acetylation (GlcN-GlcNAc and GlcNAc-GlcN) to *Pe*CBM32. Titrations were performed at 25 ºC and pH 5.6 using a MicroCal VP-ITC System (Microcal, Northampton, MA, USA).

2.2.4.2 Thermodynamic parameters (n, Δ*H*, Δ*G* and K_b) obtained from ITC[34]

ITC data suggest the binding of partially *N*-acetylchitobioses on chitosan binding protein *Pe*CBM32. It, further, suggests the 1:1 binding of chitobiose derivative on *Pe*CBM32, i.e., binding stoichiometry (n) is one (Table 2.1). Binding constant (K_b) for the GlcN-GlcNAc (**DA**) is greater (almost double) than that for the GlcNAc-GlcN (**AD**) which indicates that **DA** has stronger binding affinity than GlcNAc-GlcN (**AD**) on *Pe*CBM32 (Table 2.1).[34] It is supported by free energy change (ΔG) shown in the table 2.1. In other words, the above data suggests that the chitobioses having different *N*-acetyl pattern have different binding efficiencies with the chitosan binding protein *Pe*CBM32.

Ligand	**N**	$K_b \times 10^{-5}$ **(M^1)**	**Δ*G*° (kcal.mol^{-1})**	**Δ*H*° (kcal.mol^{-1})**	**Δ*S*° (cal.mol^{-1}.K^1)**
GlcNAc-GlcN (**AD**)	1.13 (±0.32)	0.11 (±0.03)	-5.50	-8.58 (±1.68)	-10.33 (±5.76)
GlcNGlcNAc (**DA**)	1.01 (±0.10)	0.20 (±0.03)	-6.01	-7.53 (±0.51)	-5.75(±1.58)

Table 2.1: Thermodynamic parameters obtained from ITC.

2.3 Conclusions

We have developed efficient synthetic strategies for the preparation of mono *N*-acetylchitobioses (**AD** and **DA**) using thioglycosides as glycosyl donor. *N*-Phth group not only serves as amine protecting group, but also directs the formation of β-glycosides due to its bulky nature. To investigate the biological effects of the synthesized chitobioses, binding of them with chitosan binding module (PeCBM32) was studied. Biological data suggest that not only degree of *N*-acetylation but also the pattern of *N*-acetylation affects the binding of chitosan binding proteins.

2.4 Experimental section

2.4.1 General information

^{1}H and ^{13}C spectra were recorded on a Bruker 400 MHz NMR spectrometer using $CDCl_3$ and D_2O as solvents with reference to tetramethylsilane (TMS) as internal standard. Chemical shifts are reported in δ scale and coupling constants in Hz. All the reactions were carried out with freshly distilled solvents. Column chromatography was performed over silica gel (100-200 mesh) from Merck chemical company using ethyl acetate/hexanes and EtOH/$CHCl_3$ solvent mixtures as eluents. Organic extracts were dried over anhydrous Na_2SO_4. Solvents were removed under reduced pressure on a rotary evaporator. Thin Layer Chromatography was performed on commercial silica gel coated aluminum plates (Merck). The visualization of TLC plates was effected by 5% H_2SO_4 in methanol solution upon heating. Size-exclusion chromatography was carried out on Bio-Gel P-2 using water as eluent. MALDI-MS were recorded using 2,5-dihydroxybenzoic acid (DHB) as a matrix with a Bruker Reflex II mass spectrometer.

2.4.2 Experimental procedures, spectral data and analytical data

General procedure for synthesis of thioglycosides[29]

Peracetylated sugar (1 equiv.) was dissolved in dry CH_2Cl_2 (5 mL/mmol). It was treated with $BF_3{\cdot}OEt_2$ (1.4 equiv.) and then *p*-methyl thiophenol/thiophenol (1.5 equiv.) was added. The reaction mixture was stirred at room temperature for 22 h and diluted with CH_2Cl_2, washed with ice cold saturated $NaHCO_3$ solution. The organic extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The resulted residue was purified by column chromatography (SiO_2, hexanes/EtOAc) to obtain required thioglycosides.

General procedure for *O*-deacetylation followed by benzylidine acetal protection[31]

Starting material (1 equiv.) was dissolved in dry methanol (5 mL/mmol) and treated with sodium methoxide (0.1 equiv.) at room temprature. After 1 h, the reaction mixture was neutralized with amberlite IR-120, filtered and evaporated to get sugar triol derivatives. The crude triol was used directly for next step. The crude triol was dissolved in dry CH_3CN (6 mL/mmol). Benzaldehyde dimethylacetal (5 equiv.) and *p*-TsOH (0.1 equiv.) were added to the reaction mixture. The reaction mixture was stirred for overnight and it was quenched with NEt_3 then concentrated. The residue was purified by column chromatography (SiO_2, hexanes/EtOAc) to obtain benzylidene acetal derivatives.

General procedure for benzylation at 3rd position[31]

Benzylidine acetal (1 equiv.) was dissolved in dry CH_2Cl_2 (2 mL/mmol). Above solution was cooled to 0 ºC, treated with Ag_2O (3 equiv.) or NaH (1.5 equiv.). After 10 min, BnBr (1.5 equiv.) was added to the reaction mixture stirred for overnight. Reaction mixture was filtered and concentrated under reduced pressure in case of Ag_2O. Excess NaH was quenched with CH_3OH and dried in vacuo. The residue was purified by column chromatography (SiO_2, hexanes/EtOAc) to get the required products.

General procedure for regioselective benzylidine acetal opening[31]

Benzylidene acetal derivative (1 equiv.) was dissolved in CH_2Cl_2 (5 mL/mmol). To the above solution TEA (5 equiv.) was added at 0 ºC. After 10 min, TFAA (3 equiv.) and TFA (5 equiv.) were added dropwise at 0 ºC to the above reaction mixture. The reaction mixture was slowly warmed to room temperature and stirred for 3 h. After completion of the reaction, it was quenched with saturated $NaHCO_3$, extracted with dichloromethane. Organic extract was washed with brine solution, dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexanes/EtOAc) to get the 4-hydroxy free derivatives.

General procedure for deprotection of phthalimide protecting group followed by acetylation[35]

Hydrazine monohydrate or ethylenediamine (3 equiv.) was added to a stirred solution of the disaccharide (1 equiv.) in EtOH or *n*-BuOH (5 mL/mmol) at room temperature. The resultant reaction mixture was heated at 90 ºC for overnight. After completion of the reaction, the solvent was removed by co-evaporation with toluene (3 times). The crude product was treated

with 2:1 pyridine-acetic anhydride (10 mL/mmol) and allowed to stir for overnight at room temperature. The residue was dissolved in EtOAc and washed with HCl (3%), water, saturated $NaHCO_3$ and water successively. The organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuo. The residue was purified by column chromatography (SiO_2, hexanes/EtOAc) to get the required products.

General procedure for silyl ether deprotection[36]

A stirred solution of silyl ether (**28** and **30**) (1 equiv.) in dry THF (5 mL/mmol) was treated with glacial acetic acid (1.2 equiv.) and TBAF (1equiv.) at 0 ºC. After 1 h, the resultant mixture was diluted with aq. saturated NaCl and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by column chromatography (SiO_2, hexanes/EtOAc) to get required products (**29** and **31**).

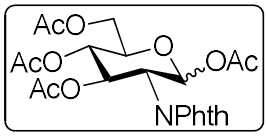
General procedure for the reductive acetylation of azide[37]

A solution of the compound (1 equiv.) in pyridine (5 mL/mmol) was treated with AcSH (2.5 mL/mmol) at room temperature under stirring. After overnight stirring, completion of the reaction was confirmed by TLC analysis and then the reaction mixture was co-evaporated with toluene (3 times). The crude product was purified by column chromatography (SiO_2, hexanes/EtOAc) to obtain the required derivatives.

General procedure for one pot deacetylation followed by debenzylation[38]

A solution of 1 M NaOMe solution was added dropwise to a solution of compound (**29** or **31**) in dry MeOH (5 mL/mmol) with stirring. After completion of the reaction as confirmed by TLC, it was neutralized with Amberlite IR 120 (H^+) then filtered and concentrated under vacuo. The crude product was dissolved in 2:1 EtOH-water and 10% Pd/C (25 mg) and 3 drops of AcOH was added under stirring. After 24 h, the resulting mixture was filtered through celite and concentrated under vacuo. The residue was purified by gel filtration on biogel P-2 column and then lyophilized to get the **5** and for **4** $NH_2NH_2 \cdot H_2O$ treatment also involves as a mixture of α:β anomers.

Preparation of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-α/β-D-glucopyranose 19[29]

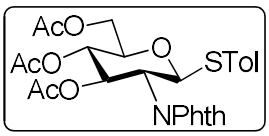


The compound **19** was prepared from glucosamine hydrochloride **18** (12.4 g, 57.3 mmol) by following the literature procedure.[29] Gummy liquid, yield = 15.1 g (55%), R_f = 0.56 in 1:1 EtOAc/hexanes: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90-7.84 (m, 2H), 7.79-7.74 (m, 2H), 6.52 (d, *J* = 8.8 Hz,

1H), 5.90 (m, 1H), 5.22 (dd, *J* = 10.2, 9.2 Hz, 1H), 4.51-4.45 (m, 1H), 4.38 (dd, *J* = 12.5, 4.5. Hz, 1H), 4.15 (dd, *J* = 12.3, 2.0 Hz, 1H), 4.07-4.01 (m, 1H), 2.12 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.87 (s, 3H, OAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.1, 169.6, 168.7, 167.4, 134.5, 133.1, 132.0, 131.1, 130.9, 130.2, 129.8, 128.7, 123.8, 90.6, 89.7, 72.6, 70.5, 70.5, 70.1, 68.2, 61.5, 53.4, 52.8, 20.8, 20.6, 20.4.

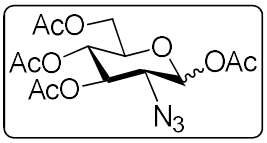
Preparation of *p*-methylphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside 20[29]

The compound **20** was prepared from **19** (10 g, 21.1 mmol) by following the literature procedure.[29] Colourless foam; Yield = 8.8 g (77%); R_f = 0.60 in 1:2 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89-7.86 (m, 2H), 7.77-7.76 (m, 2H), 7.30 (d, *J* = 7.6 Hz, 2H), 7.07 (d, *J* = 7.6 Hz, 2H), 5.78 (t, *J* = 10.4 Hz, 1H), 5.68 (d, *J* = 10.4 Hz, 1H), 5.13 (t, *J* = 9.2 Hz, 1H), 4.36-4.29 (m, 3H), 3.88 (br s, 1H), 2.32 (s, 3H), 2.11 (s, 3H), 2.02 (s, 3H), 1.83 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 169.9, 169.3, 167.7, 166.9, 138.6, 134.4, 133.8, 131.5, 129.5, 126.9, 123.6, 83.0, 77.3, 77.0, 76.7, 75.7, 71.6, 68.6, 62.1, 53.5, 21.1, 20.7, 20.5, 20.3.



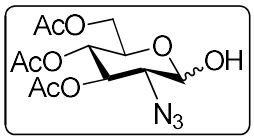
Preparation of 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-α/β-D-glucopyranoside 21[30]

The compound **21** was prepared from glucosamine hydrochloride **18** (9.3 g, 43.2 mmol)) by following the literature procedure.[30] Gummy liquid; Yield = 12 g (75%); R_f = 0.62 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.55 (d, *J* = 8.6 Hz, 1H), 5.13-5.01 (m, 2H), 4.31 (dd, *J* = 12.8, 4.4 Hz, 1H), 4.15-4.04 (m, 2H), 3.83-3.80 (m, 1H), 3.69-3.65 (m, 1H), 2.2 (s, 3H), 2.10 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.0, 169.8, 168.6, 92.5, 89.9, 77.4, 77.1, 76.7, 72.7, 72.6, 70 .7, 69.7, 67.8, 67.7, 62.5, 61.4, 60.3, 20.9, 20.7, 20.6, 20.5.



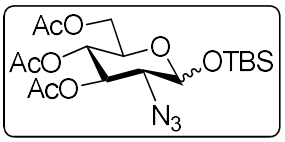
Preparation of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α/β-D-glucopyranoside 22[31]

The compound **22** was prepared from **21** (5 g, 13.4 mmol) by following the literature procedure.[31] Gummy yellow liquid; Yield = 3.02 g (68%); R_f = 0.41 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.53 (t, *J* = 9.7 Hz, 1H), 5.40 (s, 1H), 5.08-5.01 (m, 2H), 4.74 (d, *J* = 8.0 Hz, 1.6H), 4.31-4.10 (m, 5H), 3.74 (s, 0.5H), 3.52-3.48 (m, 0.6H), 3.41 (dd, *J* = 10.5, 2.1 Hz,



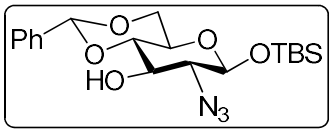
1H), 2.42 (br s, 1H), 2.10 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.0, 170.2, 169.8, 169.7, 96.0, 91.8, 72.5, 71.6, 70.4, 68.5, 68.3, 67.2, 64.7, 61.9, 61.3, 20.6, 20.5.

Preparation of *tert*-butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-α/β-D-glucopyranoside 23[31]



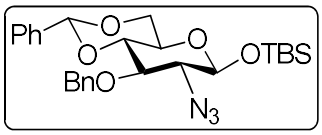
The compound **23** was prepared from **22** (3 g, 9.11 mmol) by following the literature procedure.[31] Colourless solid; Yield = 3.3 g (82%); R_f = 0.75 in 1:2 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.84 (d, *J* = 3.8 Hz, 0.3H), 5.39 (d, *J* = 4.4 Hz, 0.4H) 5.29-5.18 (m, 2H), 5.04 (s, 0.7H), 4.86 (dd, *J* = 7.6, 2.6 Hz, 2H), 4.53 (d, *J* = 7.6 Hz, 1H), 4.16 (d, *J* = 6.2 Hz, 0.8H), 4.11-3.99 (m, 4H), 3.59-3.55 (m, 1H), 3.33 (dt, *J* = 7.5, 2.7 Hz, 1H), 1.99-1.97 (m, 13H), 0.84 (s, 12H), 0.07 (s, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.5, 170.3, 169.9, 169.6, 140.1, 109.7, 107.3, 97.1, 89.2, 88.9, 72.2, 71.8, 68.8, 67.1, 65.9, 65.8, 62.9, 62.3, 25.6, 25.5, 20.9, 20.7, 20.6, 20.5, 17.9, -4.5, -5.3.

Preparation of *tert*-butyldimethylsilyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside 24[31]



The compound **24** was prepared from **23** (3 g, 6.79 mmol) by following the literature procedure.[31] Light yellow liquid; Yield = 2.2 g (79%); R_f = 0.52 in 1:3 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48-7.37 (m, 5H), 5.53 (s, 1H), 4.64 (d, *J* = 7.5 Hz, 1H), 4.29 (dd, *J* = 10.6, 4.8 Hz, 1H), 3.78 (t, *J* = 10.2 Hz, 1H), 3.64-3.54 (m, 2H), 3.41-3.31 (m, 2H), 2.70 (s, 1H), 0.94 (s, 9H), 0.16 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 136.8, 129.5, 128.4, 128.2, 126.7, 126.3, 103.1, 101.9, 97.5, 80.7, 71.7, 68.9, 68.5, 66.3, 52.7, 25.3, 17.9, -4.4, -5.2.

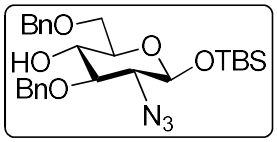
Preparation of *tert*-butyldimethylsilyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside 25[31]



The compound **25** was prepared from **24** (2.0 g, 4.87 mmol) by following the literature procedure.[31] Colourless solid; Yield = 2.1 g (83%); R_f = 0.72 in 1:5 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38-7.27 (m, 10H), 5.59 (s, 1H), 4.92 (d, *J* = 11.8 Hz, 1H), 4.80 (d, 11.4 Hz, 1H), 4.60-4.58 (m, 2H), 3.81 (t, *J* = 10.1 Hz, 1H), 3.73 (t, *J* = 9.4 Hz, 1H), 3.53 (t, *J* = 9.4 Hz, 1H), 3.40-3.36 (m, 2H), 0.95 (s, 9H), 0.17 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.2,

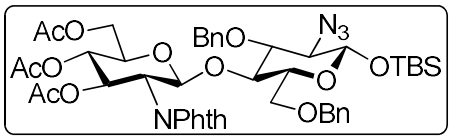
137.9, 137.2, 129.0, 128.4, 128.3, 128.2, 128.1, 127.8, 127.6, 126.0, 101.3, 97.5, 81.6, 78.8, 74.8, 72.1, 69.7, 68.7, 68.6, 66.3, 25.5, 17.9, -4.4, -5.2.

Preparation of *tert*-butyldimethylsilyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside 26[31]



The compound **26** was prepared from **25** (2.0 g, 4.04 mmol) by following the literature procedure.[31] Oily liquid; Yield = 1.57 g (78%); R_f = 0.63 in 1:2 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38-7.30 (m, 10H), 4.91 (d, *J* = 11.4 Hz, 1H), 4.76 (d, *J* = 11.3 Hz, 1H), 4.56 (d, *J* = 3.7 Hz, 2H), 4.52 (d, *J* = 7.7 Hz, 1H) 3.70 (d, *J* = 4.6 Hz, 2H), 3.63 (t, *J* = 8.9 Hz, 1H), 3.42-3.38 (m, 1H), 3.33-3.29 (m, 1H), 3.23-3.18 (m, 1H) 2.65 (br s, 1H), 0.94 (s, 9H), 0.16 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.2, 137.7, 128.6, 128.4, 128.0, 127.9, 127.7, 97.2, 82.3, 74.9, 73.9, 73.6, 71.9, 70.3, 68.1, 25.6, 17.9, -4.3, -5.2.

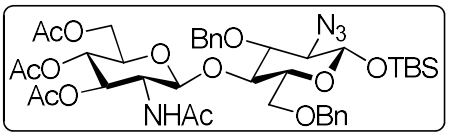
Preparation of *tert*-butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-D-glucopyranoside 27



Glycosyl donor **20** (1.57 g, 2.91 mmol) and glycosyl acceptor **26** (1.24 g, 2.48 mmol) were dissolved in dry CH_2Cl_2 (15 mL) and dried over 4 Å molecular sieves at room temperature for an hour. The suspension was cooled to -30 ºC and treated with NIS (1.1 g, 4.96 mmol) under N_2 atmosphere, and TfOH (11 µL, 0.124 mmol) was added slowly dropwise. After completion of reaction, the reaction mixture was quenched with saturated $Na_2S_2O_3$ solution and was extracted with dichloromethane. Organic layer was washed with saturated brine solution dried over anhydrous Na_2SO_4, filtered and then the solvent was evaporated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc) which gave the pure disaccharide **27** as colourless foam. Yield = 1.77 g (78%); R_f = 0.52 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.71 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.45-7.37 (m, 4H), 7.32-7.23 (m, 6H), 5.73 (dd, *J* = 10.6, 9.1 Hz, 1H), 5.61 (d, *J* = 8.4 Hz, 1H), 5.12 (t, *J* = 10.0 Hz, 1H), 4.96 (d, *J* = 11.6 Hz, 1H) 4.85 (d, *J* = 11.6 Hz, 1H), 4.43-4.34 (m, 3H), 4.26 (dd, *J* = 10.7, 8.4 Hz, 1H), 4.10-4.03 (m, 2H), 3.85 (dd, *J* = 12.4, 2.0 Hz, 1H), 3.45-3.40 (m, 3H), 3.31-3.28 (m, 2H), 3.19-3.17 (m, 1H), 1.99 (s, 6H), 1.83 (s, 3H), 0.88 (s, 9H), 0.07 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.1, 169.4, 138.6, 138.1, 134.3, 131.3, 128.3, 128.2, 127.4, 127.2, 123.6, 97.2, 97.0, 80.9, 75.1, 74.4, 74.3, 72.7, 71.6, 70.6, 68.5, 68.3, 67.7, 61.5, 55.2, 25.5, 20.7, 20.6, 20.4,

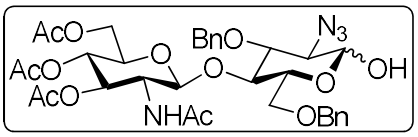
17.9, -4.4, -5.3. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{46}H_{56}N_4O_{14}Si$ 939.3460, found 939.3461.

Preparation of *tert*-butyldimethylsilyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-D-glucopyranoside 28



The compound **28** was prepared from disaccharide **27** (0.89 g, 0.97 mmol) using general procedure for deprotection of phthalimide protecting group followed by acetylation. Colourless solid; Yield = 0.7 g (87%); R_f = 0.42 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49-7.23 (m, 10H), 5.01-4.82 (m, 4H), 4.84 (d, *J* = 12.0 Hz, 1H), 4.71 (d, *J* = 11.4 Hz, 1H), 4.53 (d, *J* = 8.5 Hz, 1H), 4.46-4.41 (m, 2H), 4.09 (dd, *J* = 12.3, 4.5 Hz, 1H), 3.95-3.85 (m, 3H), 3.65 (dd, *J* = 11.1, 2.8 Hz, 1H), 3.57 (dd, *J* = 10.9, 1.3 Hz, 1H), 3.46-3.43 (m, 1H), 3.33-3.30 (m, 3H), 2.00 (s, 6H), 1.95 (s, 3H), 1.74 (s, 3H), 0.93 (s, 9H), 0.15 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.6, 169.8, 169.3, 138.7, 137.6, 128.8, 128.7, 128.6, 128.1, 127.4, 127.3, 100.6, 97.0, 80.7, 74.8, 74.3, 73.9, 72.9, 71.5, 68.4, 68.3, 67.9, 61.8, 54.4, 25.5, 23.1, 20.6, 17.9, -4.4, -5.2. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{40}H_{56}N_4O_{13}Si$ 851.3511, found 851.3515.

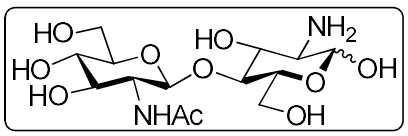
Preparation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-D-glucopyranoside 29



The azido derivative **29** was prepared from compound **28** (0.5 g, 0.6 mmol) using general procedure for silyl ether deprotection. Yellow liquid; Yield = 0.36 g (84%); R_f = 0.75 in 1:20 EtOH/$CHCl_3$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.51-7.22 (m, 10H), 5.27 (d, *J* = 2.1 Hz, 1H), 5.11 (d, *J* = 11.3 Hz, 1H), 5.03-4.94 (m, 2H), 4.87-4.82 (m, 2H) 4.75 (d, *J* = 9.4 Hz, 1H), 4.71-4.66 (m, 1H), 4.39-4.35 (m, 1H), 4.12-4.08 (m, 1H), 3.96-3.86 (m, 4H), 3.60 (t, *J* = 10.9 Hz, 1H), 3.49-3.42 (m, 2H), 3.40-3.33 (m, 1H), 2.02 (s, 3H), 1.99 (s, 3H), 1.92 (s, 3H), 1.74 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.7, 170.2, 169.3, 138.6, 138.4, 137.4, 129.1, 129.0, 128.9, 128.4, 128.1, 127.4, 100.6, 96.0, 91.8, 80.9, 77.8, 74.9, 74.1, 73.7, 72.6, 71.4, 69.7, 68.4, 68.2, 67.4, 67.1, 63.5, 61.8, 54.3, 23.0, 20.6. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{34}H_{42}N_4O_{13}Si$ 737.2646, found 737.2645.

Preparation of 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-D-glucopyranose 5[22]

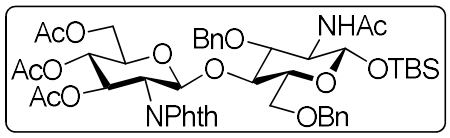
The mono *N*-acetylchitobiose **5** was prepared from compound **29** (40 mg, 0.056 mmol) by general procedure for one pot deacetylation followed by debenzylation.



Colourless amorphous solid; Yield = 15 mg (77%); ^{1}H NMR (400 MHz, D_2O): δ 5.07 (s, 0.5H), 4.83 (d, *J* = 4.1 Hz, 0.6H), 4.59 (dd, *J* = 2.0, 8.3 Hz, 1.4H), 3.83-3.34 (m, 18H), 1.93 (s, 4H). MALDI-MS (positive mode, DHB/MeOH matrix); $[M+Na]^+$ (m/z) 405.49.

Preparation of *tert*-butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-D-glucopyranoside 30

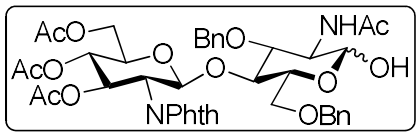
The compound **30** was prepared from disaccharide **27** (0.50 g, 0.54 mmol) using general procedure for the reductive acetylation of azide.



Light yellow solid; Yield = 0.37 g (74%); R_f = 0.42 in 10:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (dd, *J* = 5.1, 3.2 Hz, 2H), 7.75 (dd, *J* = 5.3, 2.9 Hz, 2H), 7.37-7.29 (m, 10H), 5.82 (t, *J* = 9.9 Hz, 1H), 5.58-5.52 (m, 2H), 5.15 (t, *J* = 9.8 Hz, 1H), 4.90-4.87 (m, 2H), 4.66 (d, *J* = 12.0 Hz, 1H), 4.48 (q, *J* = 12.1 Hz, 2H), 4.32 (dd, *J* = 10.5, 8.4 Hz, 1H), 4.20 (dd, *J* = 12.3, 4.1 Hz, 1H), 3.99-3.93 (m, 2H), 3.58-3.53 (m, 2H), 3.45-3.34 (m, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.87 (s, 3H), 1.84 (s, 3H), 0.82 (s, 9H), 0.02 (s, 3H), -0.02 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.1, 169.9, 169.5, 138.8, 138.3, 134.4, 131.3, 128.3, 128.2, 127.7, 127.4, 123.6, 96.9, 94.6, 77.7, 75.2, 74.0, 73.2, 72.7, 71.6, 70.5, 68.7, 68.4, 61.6, 57.7, 55.5, 25.5, 23.4, 20.7, 20.6, 20.4, 17.8, -4.5, -5.5. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{48}H_{60}N_2O_{15}Si$ 933.3841, found 933.3835.

Preparation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-D-glucopyranoside 31

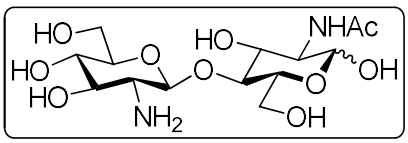
The compound **31** was prepared from compound **30** (200 mg, 0.21 mmol) by general procedure for silyl ether deprotection.



Yellow liquid; Yield = 139 mg (81%); R_f = 0.72 in 1:20 EtOH/$CHCl_3$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79-7.75 (m, 1H), 7.56-7.47 (m, 3H), 7.33-7.25 (m, 10H), 5.93 (d, *J* = 11.6 Hz, 1H), 5.33 (d, *J* = 8.3 Hz, 1H), 5.15-5.05 (m, 2H), 4.91-4.81 (m, 1H), 4.69-4.38 (m, 4H), 4.17-3.85 (m, 5H), 3.75 (d, *J* = 10.3 Hz, 1H) 3.59-3.32 (m, 3H), 2.04-1.94 (m, 9H), 1.77 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.5, 170.2, 168.1, 167.3, 143.9, 138.7, 132.8, 129.8, 128.4, 128.3, 128.2, 127.8, 127.6,

123.6, 123.3, 97.5, 91.0, 72.5, 71.2, 70.3, 69.8, 68.9, 68.6, 61.6, 61.1, 60.4, 52.3, 23.1, 20.6, 20.4. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{42}H_{46}N_2O_{15}$ 841.2796, found 841.2790.

Preparation of 2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose 4[23]



The compound **4** was prepared from compound **31** (48 mg, 0.057 mmol) using general procedure for deprotection of phthalimide and followed by one pot deacetylation and debenzylation. Colourless solid; Yield = 17 mg (75%); ^{1}H NMR (400 MHz, D_2O): δ 5.11 (s, 1H), 4.50-4.39 (m, 0.5H), 4.03-3.31 (m, 16H), 2.76-2.43 (m, 2H), 1.96 (s, 4H). MALDI-MS (positive mode, DHB/MeOH matrix); $[M+Na]^+$ (m/z) 405.73.

2.4.3 Isothermal titration calorimetry (ITC)[33]

The binding affinity and stoichiometry of *Pe*CBM32 with respect to the chitobioses were quantified by ITC. Titrations were performed on a VP-ITC isothermal titration calorimeter from MicroCal (Northampton, MA, USA). All purified proteins used in ITC measurements were dialysed extensively in a surplus of 50 mM sodium acetate buffer (pH 5.6). The buffer from the final dialysate was filtered through a 4 μM filter and used to dissolve ligands and also for titrations at 25 ºC. *Pe*CBM32 and chitosan disaccharide solutions were degassed under vacuum. The reaction cell contained *Pe*CBM32 at 80-340 μM and the syringe contained the chitobioside derivatives at a concentration of 2-15 mM. A constant stirring speed of 300 rpm was maintained. The data were analyzed using MicroCal Origin ITC software. Thermodynamic parameters such as change in enthalpy (ΔH), association constant (K_b) and binding stoichiometry (n) were obtained by nonlinear least-squares fitting of experimental data using the *one set sites* binding model in the MicroCal Origin software provided by the ITC manufacturer.

2.5 References

1. Mitsutomi, M.; Ohtakara, A.; Fukamizo, T.; Goto, S. *Agric. Biol. Chem*. **1990**, *54*, 871.

2. Honda, Y.; Tanimori, S.; Kirihata, M.; Kaneko, S.; Tokuyasu, K.; Hashimoto, M.; Watanabe, T.; Fukamizo, T. *FEBS Lett.* **2000**, *476*, 194.

3. Nordtveit, R. J.; Vårum, K. M.; Smidsrod, O. *Carbohydr. Polym.* **1996**, *29*, 163.

4. Kristiansen, A.; Vårum, K. M.; Grasdalen, H. *Biochim. Biophys. Acta* **1998**, *1425*, 137.

5. Bains, G.; Lee, R. T.; Lee, Y. C.; Freire, E. *Biochemistry* **1992**, *31*, 12624.

6. Kochibe, N.; Matta, K. L. *J. Biol. Chem.* **1989**, *264*, 173.

7. Usui, T.; Matsui, H.; Isobe, K. *Carbohydr. Res.* **1990**, *203*, 65.

8. Singh, S.; Gallagher, R.; Derrick, P. J.; Crout, D. H. G. *Tetrahedron: Asymmetry* **1995**, *6*, 2803.

9. Aam, B. B.; Heggset, E. B.; Norberg, A. L.; Sørlie, M.; Varum, K. M.; Eijsink, V. G. H. *Mar. Drugs.* **2010**, *8*, 1482.

10. Mourya, V. K.; Inamdar, M. N.; Choudhari, Y. M. *Polym. Sci., Ser. A* **2011**, *53*, 583.

11. Kim, S. K.; Rajapakse, N. *Carbohydr. Polym.* **2005**, *62*, 357.

12. Jeon, Y. J.; Shahidi, F.; Kim, S. K. *Food Rev. Int*. **2000**, *16*, 159.

13. Sashiwa, H.; Saimoto, H.; Shigemasa, Y.; Tokura, S. *Carbohydr. Res*. **1993**, *242*, 167.

14. Defaye, J.; Gadelle, A.; Pedersen, C. *Carbohydr. Res*. **1994**, *261*, 267.

15. Rupley, J. A. *Biochim. Biophys. Acta* **1964**, *83*, 245.

16. Nagasawa, K.; Tohira, Y.; Inoue, Y.; Tanoura, N. *Carbohydr. Res.* **1971**, *18*, 95.

17. Hasegawa, M.; Isogai, A.; Onabe, F. *Carbohydr. Polym*. **1993**, *20*, 279.

18. Ilina, A. V.; Varlamov, V. P. *Appl. Biochem. Microbiol*. **2004**, *40*, 300.

19. Kobayashi, S.; Kiyosada, T.; Shoda, S. *Tetrahedron Lett*. **1997**, *38*, 2111.

20. John, M.; Rdhrig, H.; Schmidt, J.; Wieneke, U.; Schell, J. *Proc. Natl. Acad. Sci*. **1993**, *90*, 625.

21. Hirano, T.; Uehara, R.; Shiraishi, H.; Hakamata, W.; Nishio, H. *J. Appl. Glycosci*. **2015**, *62*, 153.

22. Tokuyasu, K.; Ono, H.; Ohnishi-Kameyama, M.; Hayashi, K.; Mori, Y. *Carbohydr. Res*. **1997**, *303*, 353.

23. Tokuyasu, K.; Ono, H.; Hayashi, K.; Mori, Y. *Carbohydr. Res*. **1999**, *322*, 26.

24. Hansen, S. G.; Skrydstrup, T. *Eur. J. Org. Chem.* **2007**, 3392.

25. Cirillo, L.; Bedini, E.; Molinaro, A.; Parrilli, M. *Tetrahedron Lett*. **2010**, *51*, 1117.

26. Aly, M. R. E.; Castro-Palomino, J. C.; Ibrahim, E. S. I.; El-Ashry, E. S. H.; Schmidt, R. R. *Eur. J. Org. Chem*. **1998**, 2305.

27. Sato, S.; Ito, Y.; Ogawa, T. *Tetrahedron Lett*. **1988**, *29*, 5267.

28. Fuente, J. M.; Penade´s, S. *Tetrahedron: Asymmetry* **2002**, *13*, 1879.

29. Zhang, Z.; Ollmann, I. R.; Ye, X. S.; Wischnat, R.; Baasov, T.; Wong, C. H. *J. Am. Chem. Soc*. **1999**, *121*, 734.

30. Goddard-Borger, E. D.; Stick, R. V. *Org. Lett*. **2007**, *9*, 3797.

31. Orgueira, H. A.; Bartolozzi, A.; Schell, P.; Litjens, R. E. J. N.; Palmacci, E. R.; Seeberger, P. H. *Chem. Eur. J*. **2003**, *9*, 140.

32. Boraston, A. B.; Bolam, D. N; Gilbert, H. J.; Davies, G. J. *Biochem. J.* **2004**, *382*, 769.

33. Das, S. N.; Podile, A. R. Ph.D designation, University of Hyderabad, School of Life Sciences, **2016**.

34. Das, S. N.; Wagenknecht, M.; Nareddy, P. K.; Bhuvanachandra, B.; Niddana, R.; Balamurugan, R.; Swamy, M. J.; Moerschbacher, B. M; Podile, A. R. *J. Biol. Chem*. **2016**, DOI 10.1074/jbc.M116.727750.

35. Bowers, S. G.; Coe, D. M.; Boons, G. J. *J. Org. Chem.* **1998**, *63*, 4570.

36. Ogilvie, K. K.; Thompson, E. A.; Quilliam, M. A.; Westmore, J. B. *Tetrahedron Lett.* **1974**, 2865.

37. Ren, B.; Wang, M.; Liu, J.; Ge, J.; Zhang, X.; Dong, H. *Green Chem*. **2015**, *17*, 1390.

38. Rosen, T.; Lico, I. M.; Chu, D. T. W. *J. Org. Chem*. **1988**, *53*, 1580.

2.6 Representative spectra

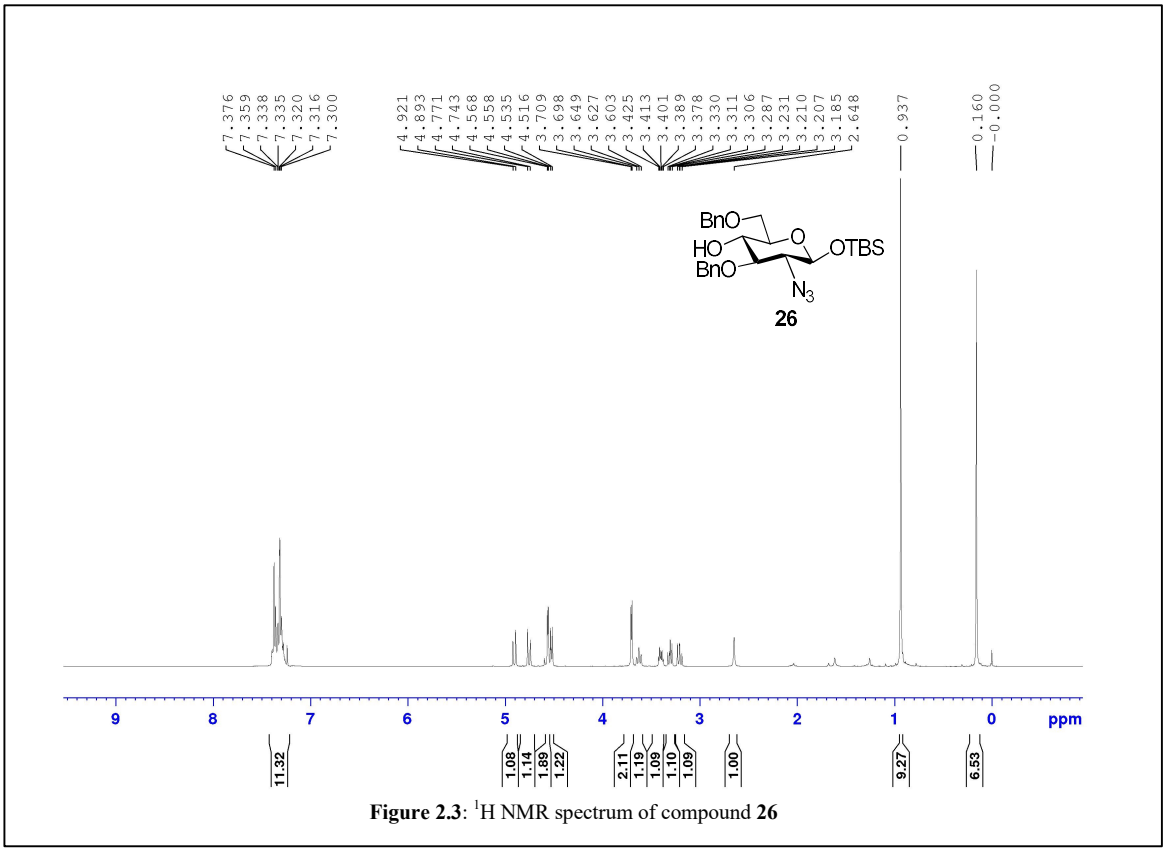


Figure 2.3: ^{1}H NMR spectrum of compound **26**

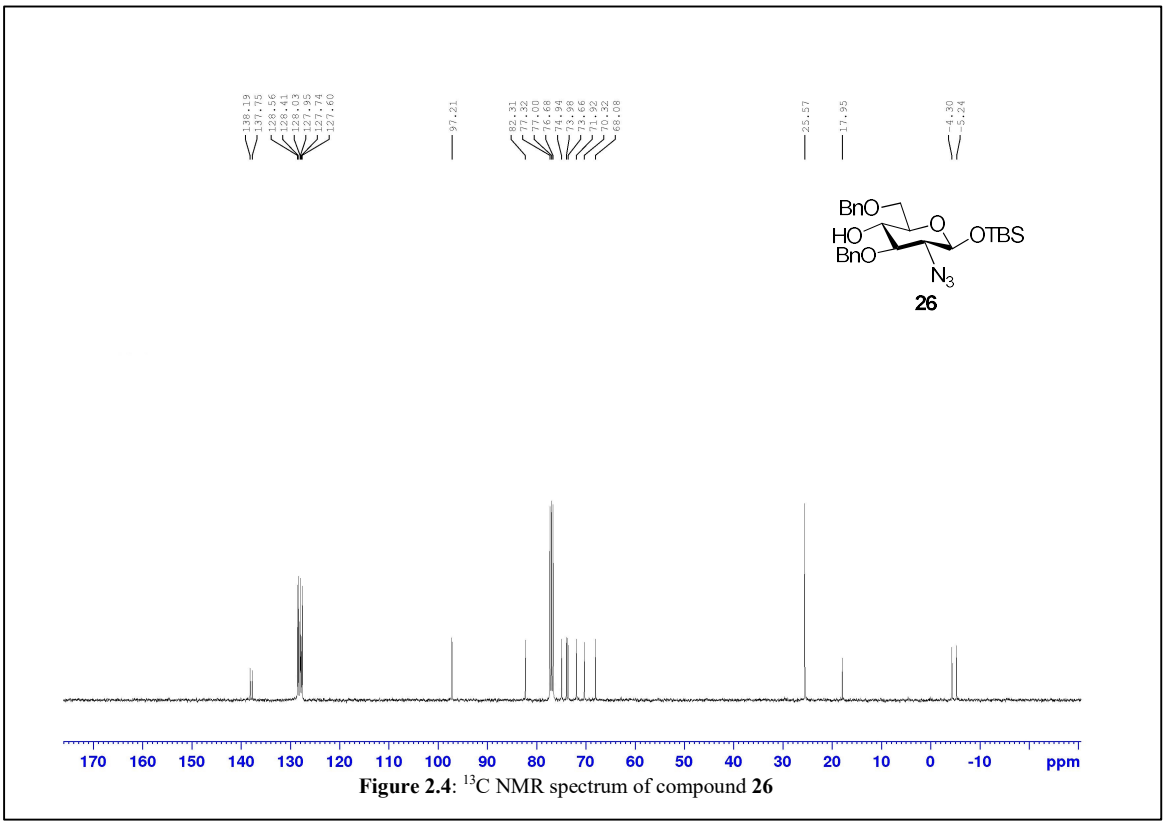


Figure 2.4: ^{13}C NMR spectrum of compound **26**

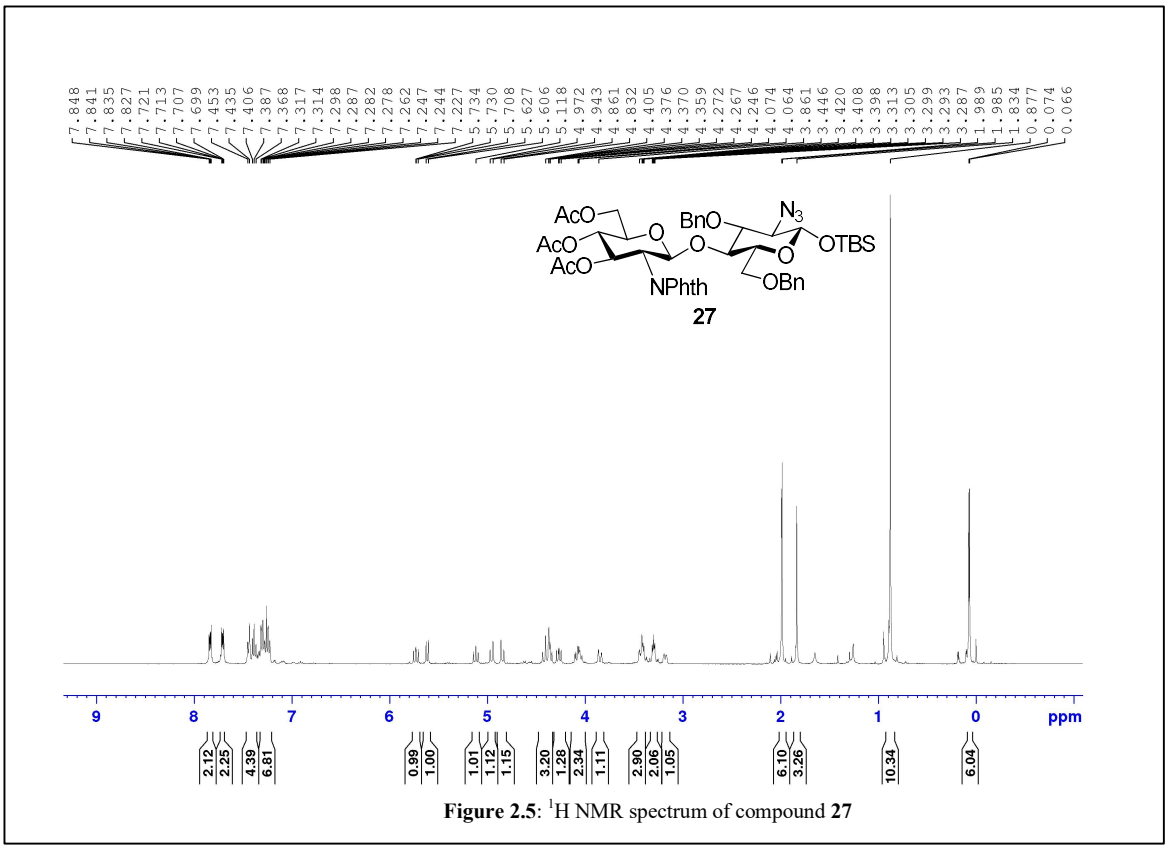


Figure 2.5: ^{1}H NMR spectrum of compound **27**

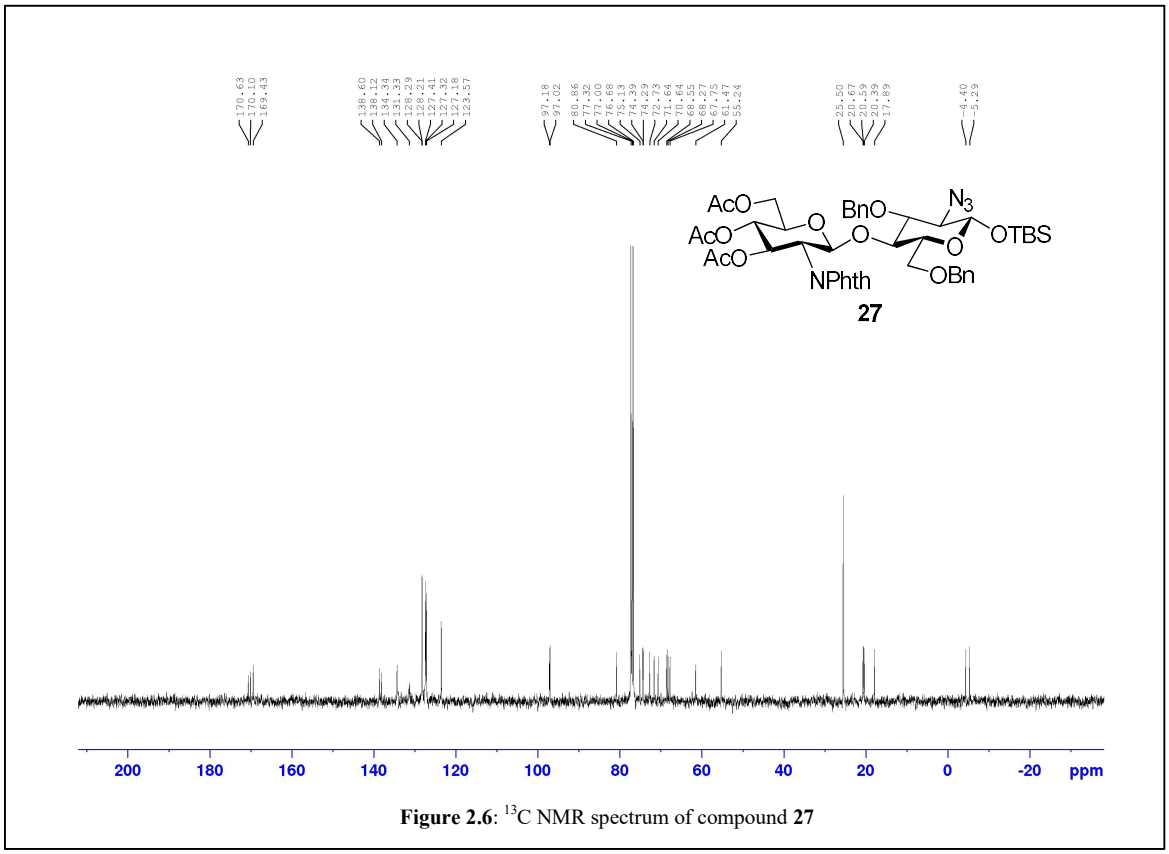


Figure 2.6: ^{13}C NMR spectrum of compound **27**

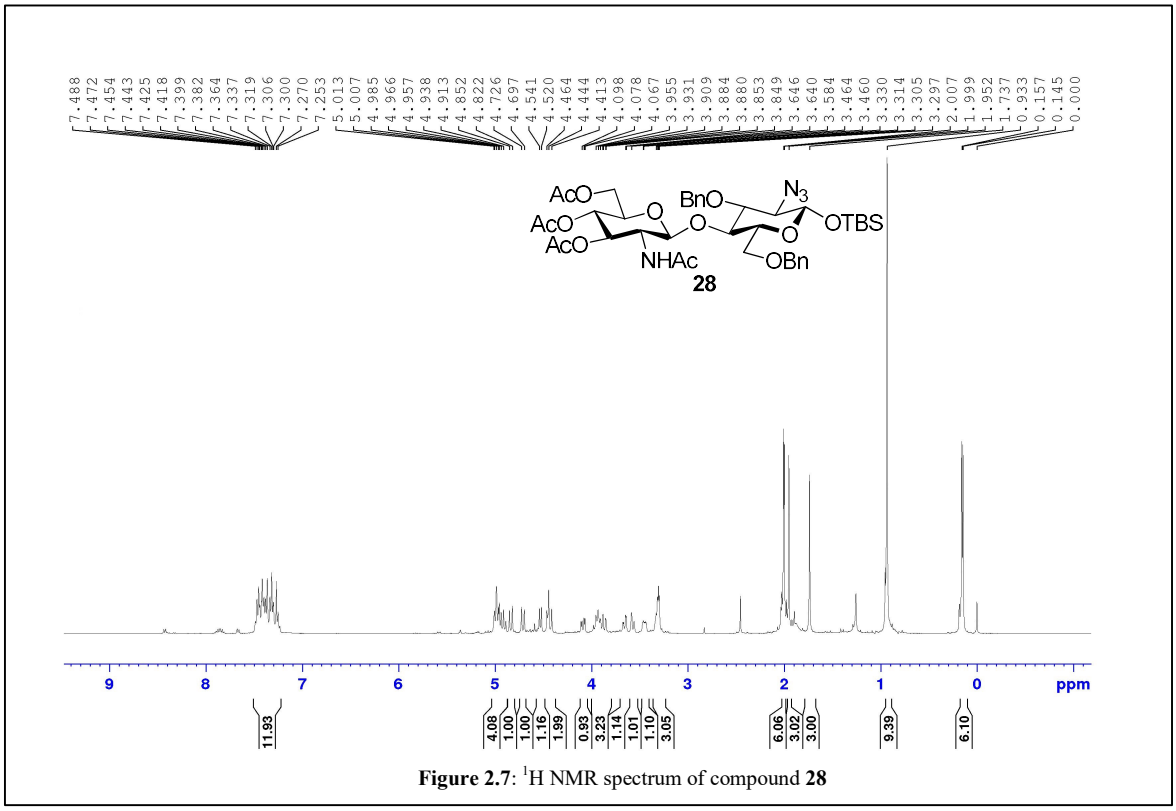


Figure 2.7: ^{1}H NMR spectrum of compound **28**

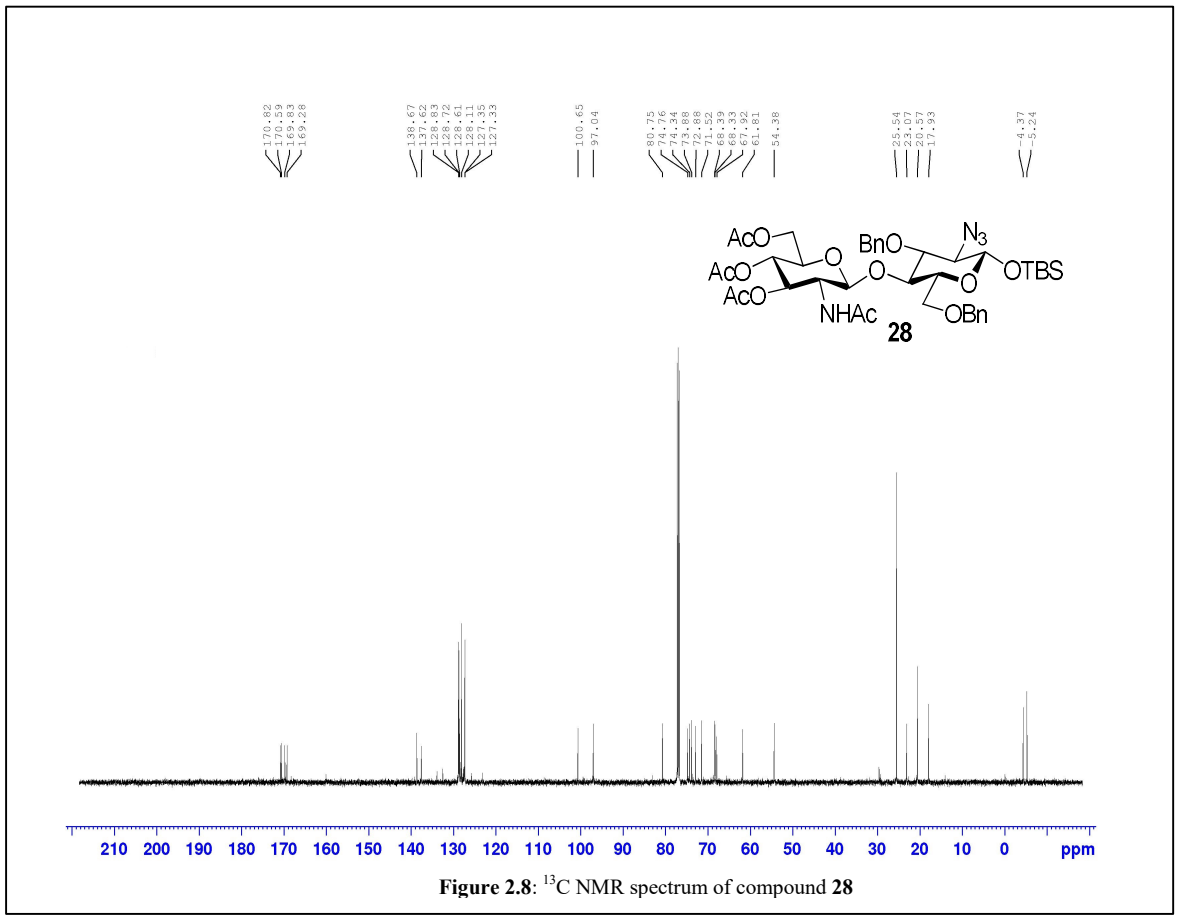


Figure 2.8: ^{13}C NMR spectrum of compound **28**

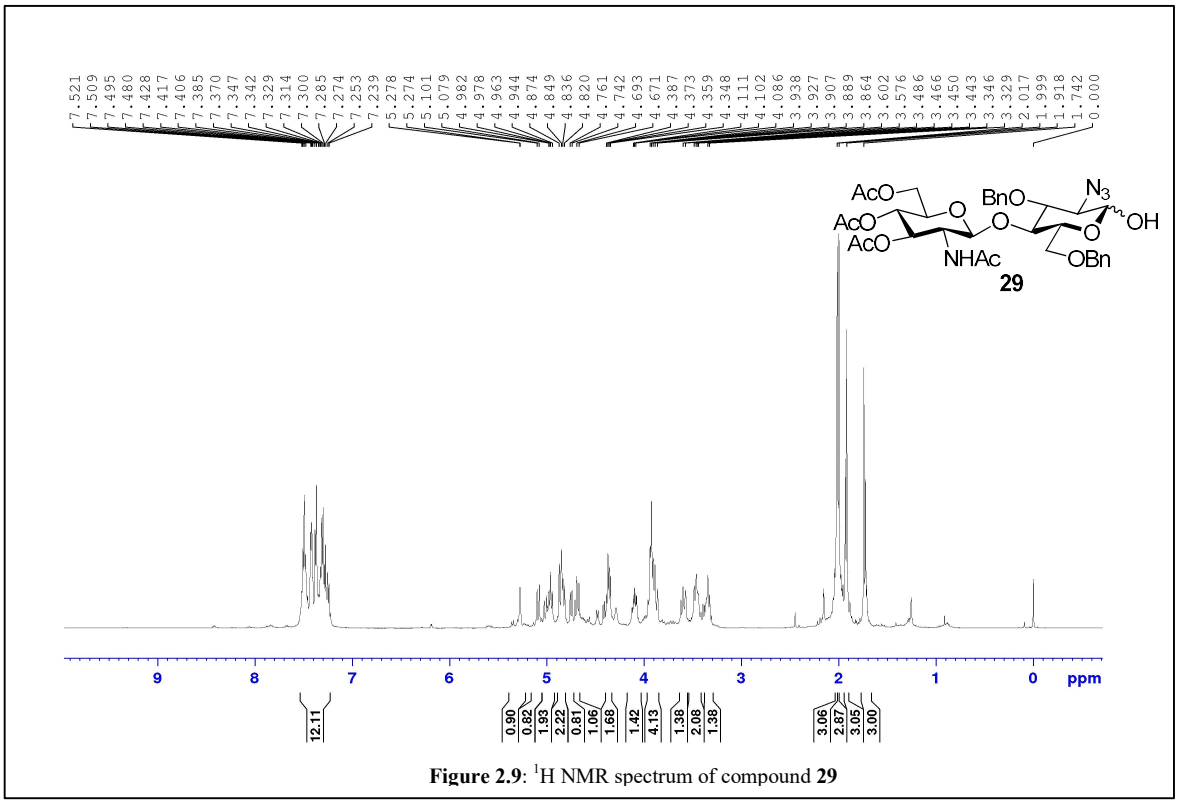


Figure 2.9: ^{1}H NMR spectrum of compound **29**

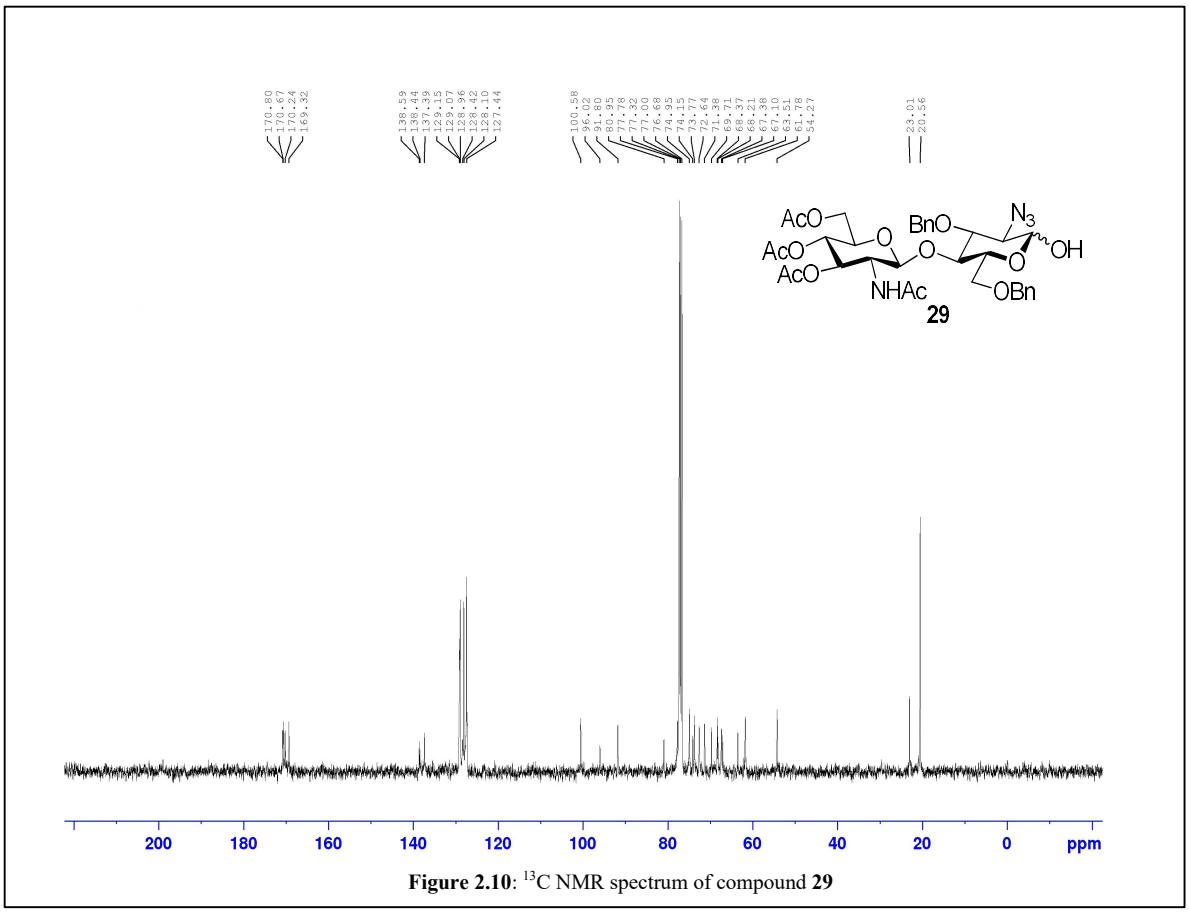


Figure 2.10: ^{13}C NMR spectrum of compound **29**

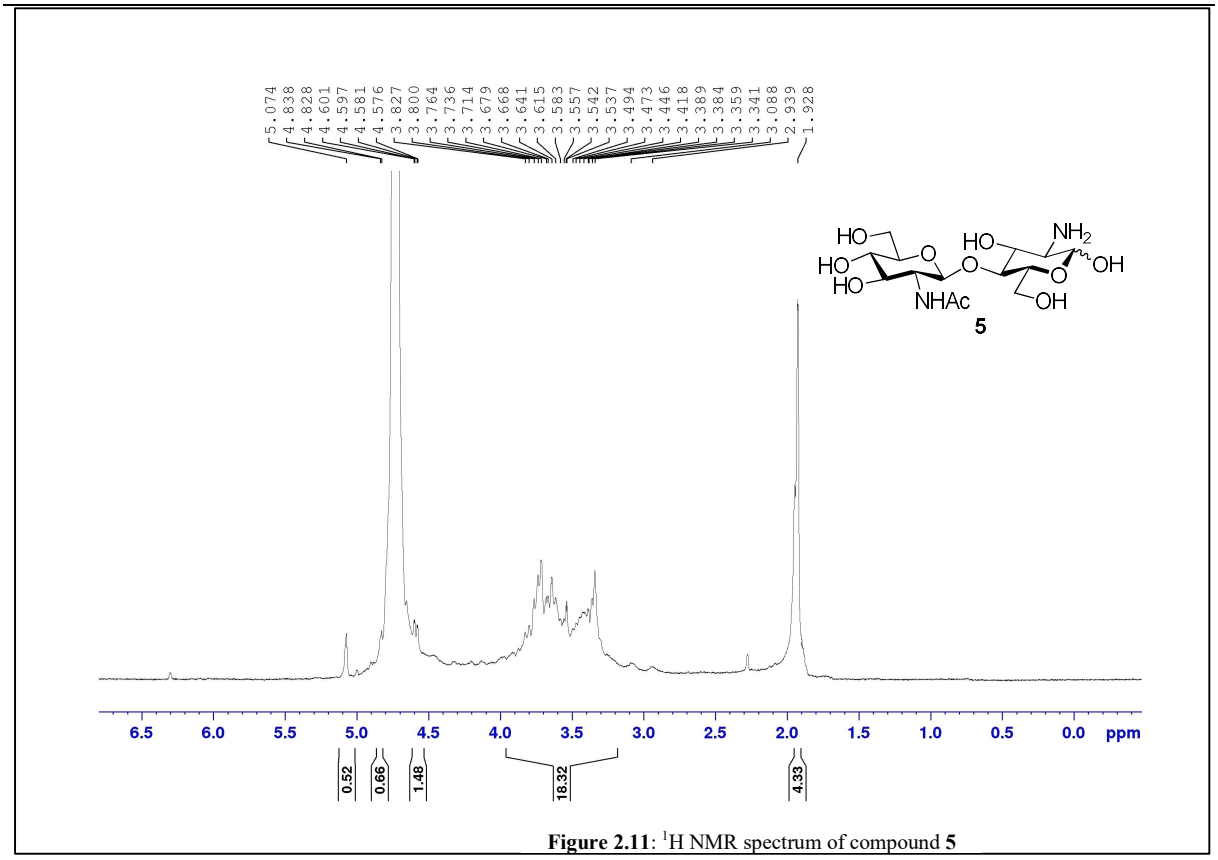


Figure 2.11: ^{1}H NMR spectrum of compound **5**

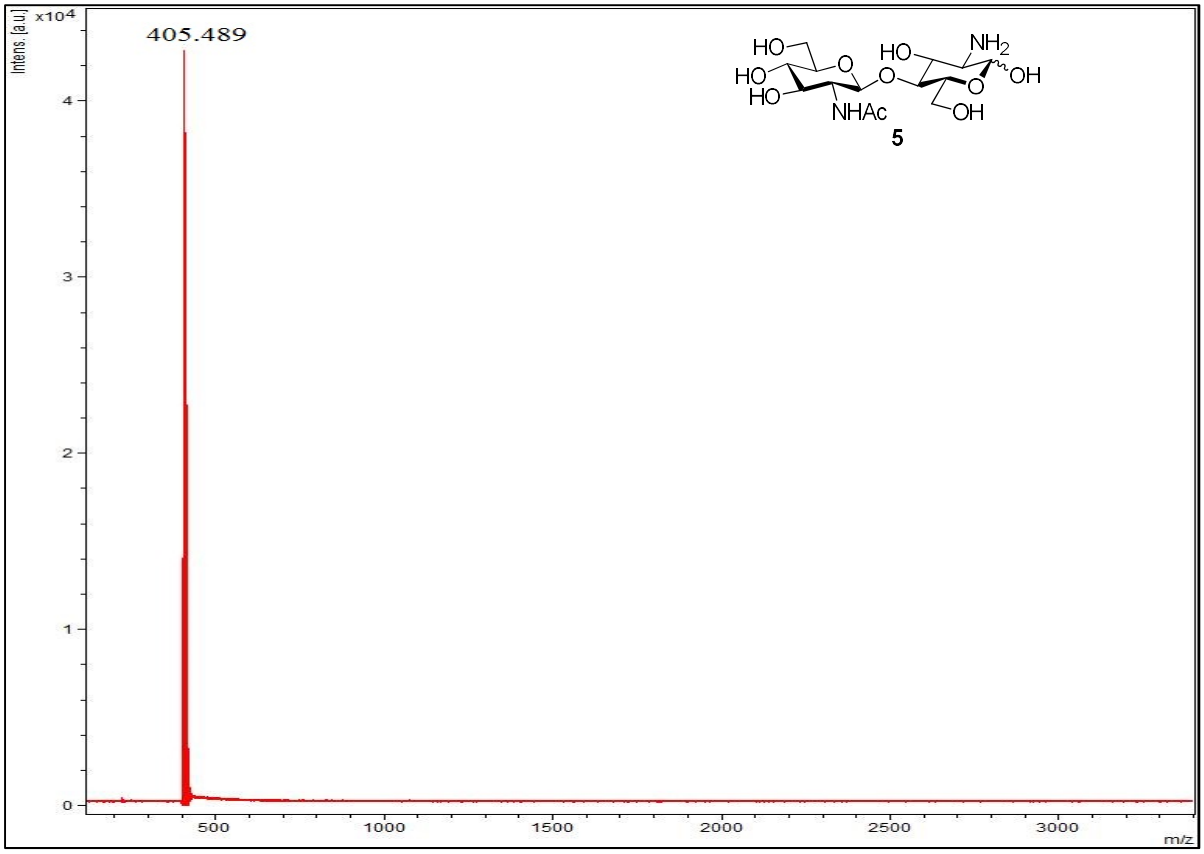


Figure 2.12: MALDI-MS spectrum of compound **5**

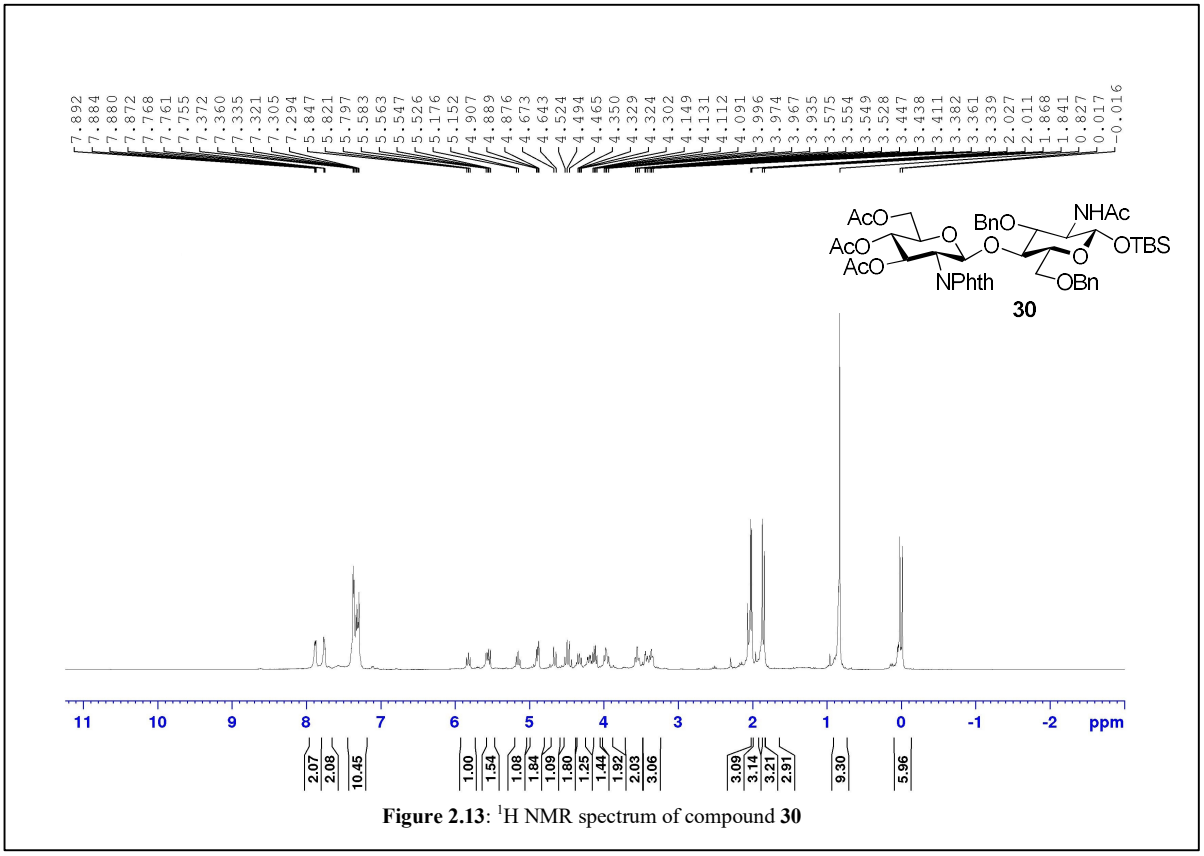


Figure 2.13: ^{1}H NMR spectrum of compound **30**

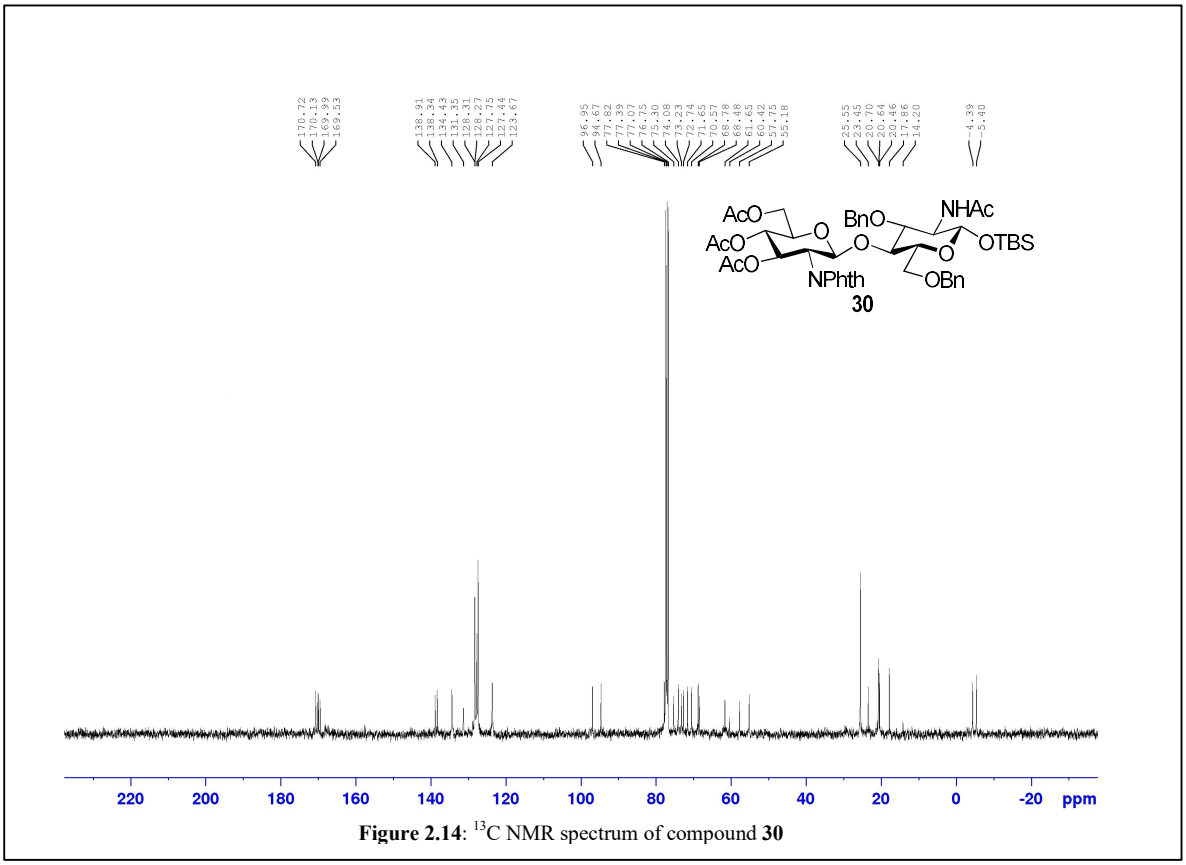


Figure 2.14: ^{13}C NMR spectrum of compound **30**

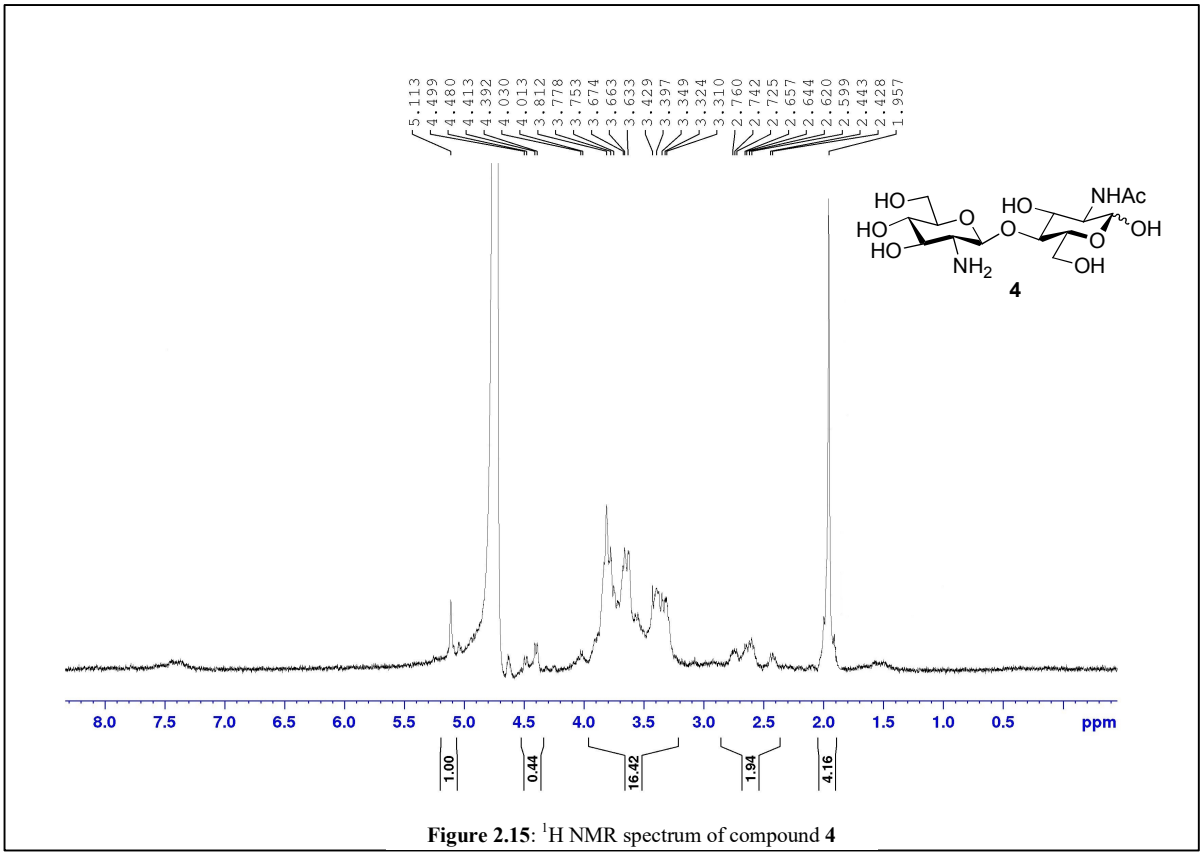


Figure 2.15: ^{1}H NMR spectrum of compound **4**

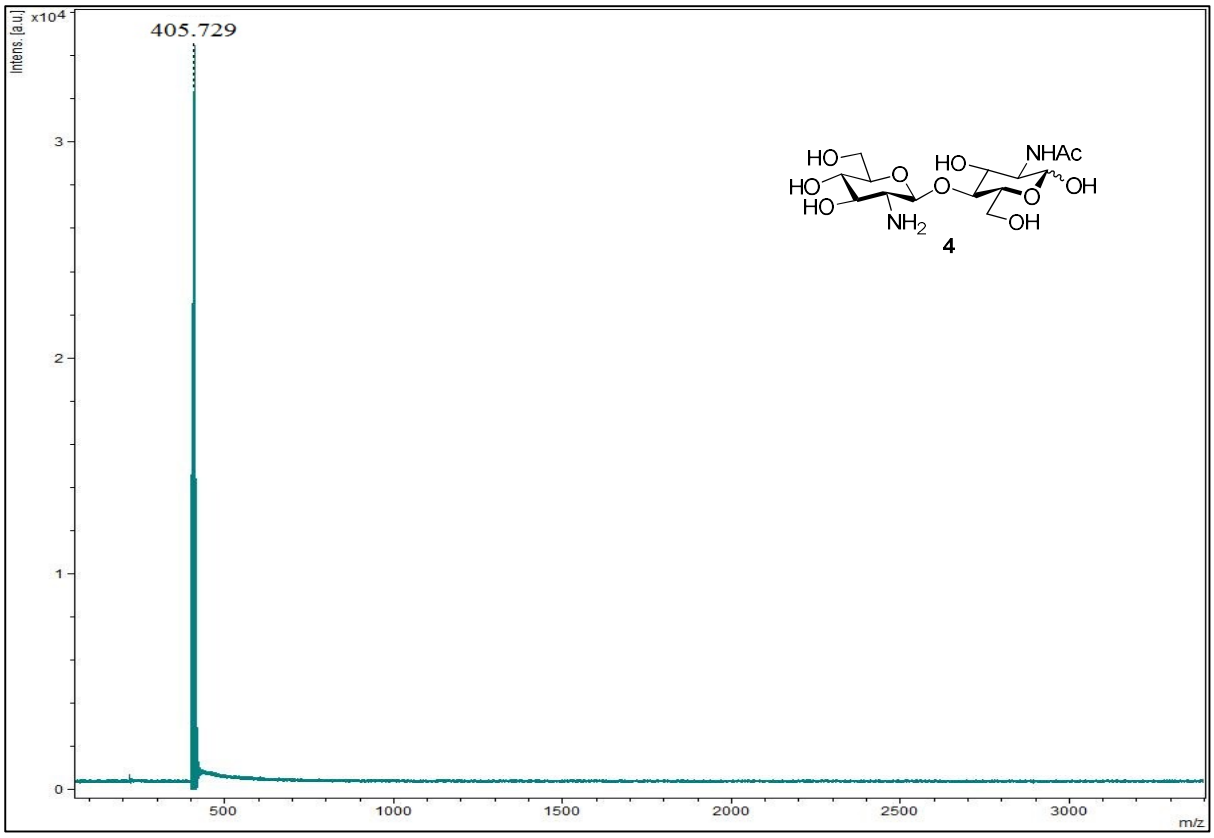


Figure 2.16: MALDI-MS spectrum of compound **4**

Chapter 3

Synthesis of partially *N*-acetylated chitotrioses and their biological effects on human endothelial cells

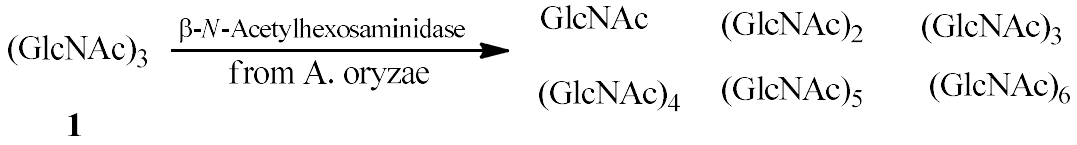
3.1 Introduction

Polycationic nature of chitosan makes it useful in a range of potential applications in medicine as a hemostatic agent[1,2] in tissue engineering[3] and drug delivery.[4,5] Several research groups have been working in the context of coagulation by utilizing the hemostatic properties of chitosan.[2] Chitosan has been suggested as a therapeutic agent to treat bleeding disorders, due to its haemostatic properties.[6,7] Blood coagulation is a process coordinated by contact activation on material surface, involving activation of a series of coagulatory factors. This results in thrombin generation followed by fibrin formation as well as EC and platelet activation at the site of injury.[8,9] Quite some amount work has been done on the effect of chitosan and its oligosaccharides on haemostasis. Various *in vitro* studies have been performed to understand the molecular effect of chitosan and its derivatives on haemostasis.[10,11] It was reported that platelets and leukocytes become activated *via* surface adsorption interactions of plasma and extracellular matrix proteins such as von Willebrand factor (vWF), fibrinogen, fibronectin, and collagen with the chitosan oligosaccharides.[12] This adsorption interactions mainly depends on the degree of hydrophobicity and the presence of positive charges on the biomaterial surface. This in turn influences the activation and adsorption of platelets as well as adsorption of proteins and leukocytes.[13,14]

Moerschbacher and co-workers studied plant elicitor activities of chitooligosaccharides in white leaves. They found that partially *N*-acetylated chitooligosaccharides show strong effects than homooligomers of *N*-acetyl glucosamine and glucosamine.[15] Akiyama and co-workers studied (+)-pisatin-inducing activities of oligomers of chitosan fully and partially *N*-acetyl chitooligosaccharides in pea epicotyl elicitor assay.[16] These studies clearly show that the partially *N*-acetyl chitooligosaccharide pentamer and hexamer shows strong elicitor activities than the other chitosan oligomers. Chitooligosaccharides have been used as probes in the biological studies on chitin binding lectins.[17] Furthermore, fully and partially *N*-acetylated chitooligosaccharides are useful substrates for the examination of the substrate

specificities and mechanism of action of chitinase, chitosanases and lysozymes.[18,19] These substrates have also been utilized in the preparation of higher chitooligosaccharides using lysozymes by transglycosylation strategy.[16] Chitooligosaccharides show significantly strong antimicrobial effect against bacteria and fungi than that of chitosan. Biological activities of chitooligosaccharides increase with increase of degree of deacetylation (DD), but decrease with increase of degree of polymerization (DP).

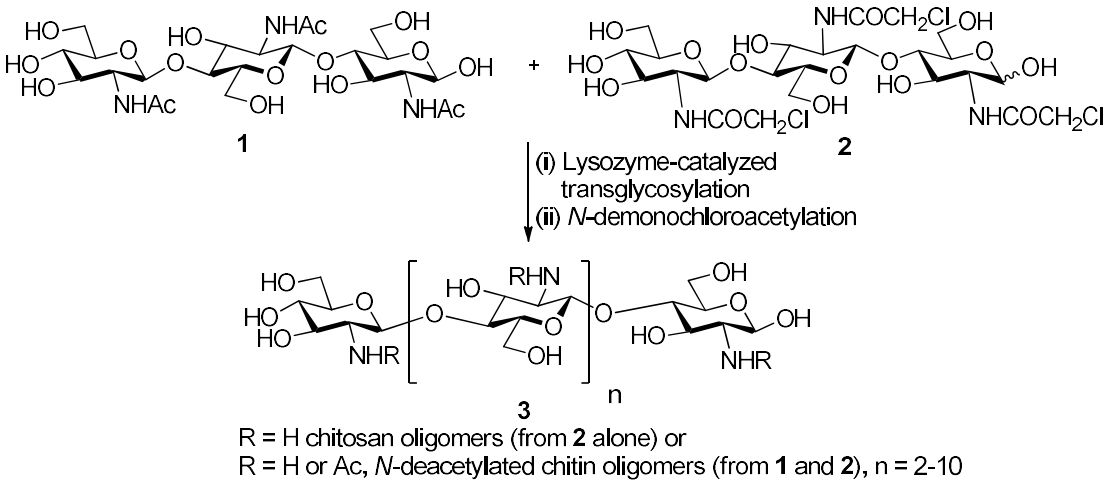
Singh *et al*. reported the synthesis of *N*-acetyl chitooligosaccharides using glycosidases. Production of *N*-acetyl chitooligosaccharides from *N*,*N*',*N*''-triacetylchitotriose $(GlcNAc)_3$ **1** was achieved using β-*N*-acetylhexosaminidase from Aspergillus oryzae (Scheme 3.1).[20]



1

Scheme 3.1: Production of *N*-acetyl chitooligosaccharides from chtitotriose **1** using β-*N*-acetylhexosaminidase.

Akiyama *et al*. developed a method for the synthesis of higher chitooligosaccharides *via* chemo-enzymatic transglycosylation strategy. This method involves the synthesis of homo- and heterochitooligosaccharides using lysozyme-catalyzed transglycosylation of *N*,*N*',*N*''-triacetylchitotriose **1** and *N*,*N*',*N*''-tri(monochloro)acetylchitotriose **2** followed by base-catalyzed removal of *N*-monochloroacetyl groups (Scheme 3.2). This method clearly shows that chitotrioses can act as good substrates for making higher chitooligosaccharides by transglycosylation method.[16]

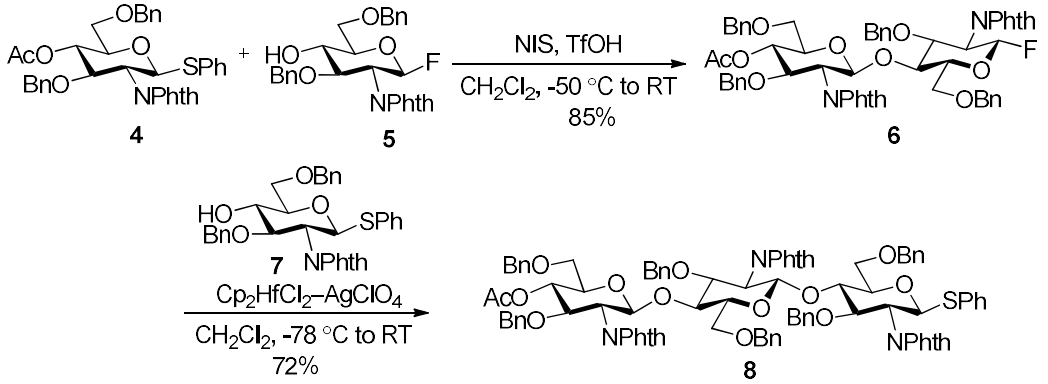


Scheme 3.2: Synthesis of homo- and heterochitooligosaccharides from chitotrioses **1** and **2** *via* enzymatic transglycosylation method.

3.1.1 Chemical synthesis of chitotriose derivatives

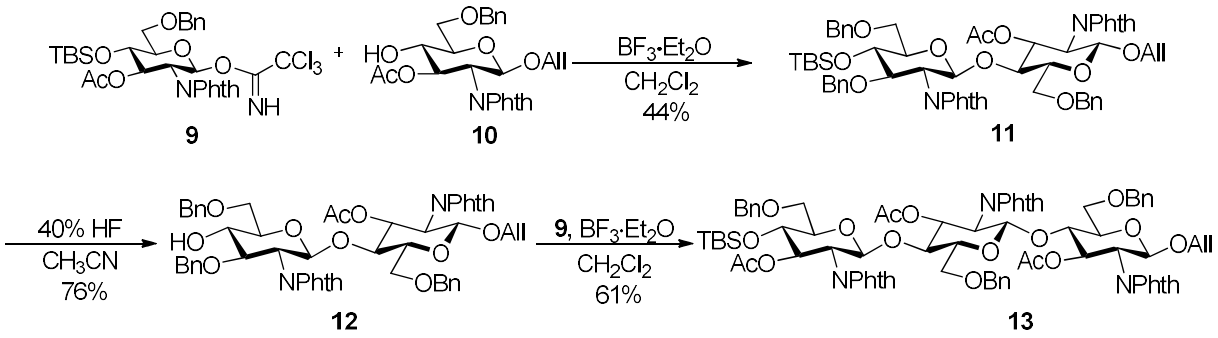
Currently different research groups have been developing strategies for the efficient synthesis oligosaccharides. The challenging task in oligosaccharide synthesis is the construction of stereoselective glycosidic linkage. At present researchers utilize the concept of chemoselective activation in glycosylation, which exploits the difference in reactivities of glycosyl donors which can be achieved by having different protecting groups (ether/ester) and leaving groups.[21] A wide range of glycosyl donors (leaving groups) such as glycosyl halides, glycosyl trichloroacetimidates and thioglycosides and *n*-pentenyl glycosides are commonly used in glycosylation reactions. Of them, one can selectively be activated over the other under mild conditions for the synthesis of oligosachharides especially the glucosamine containing oligosaccharides and glycoconjugates.[21,22]

Ogawa and co-workers described a method for the synthesis of fully protected chitotriose using orthogonal glycosylation strategy. Donor/acceptor capabilities of thioglycosides and glycosyl fluorides were successfully utilized in the oligosaccharide synthesis. Synthesis of fully protected chitotriose **8** was achieved by orthogonal activation of thioglycoside **4** and glycosyl fluoride **5** donors (Scheme 3.3). Synthesis of a heptasaccharide was also achieved using chitotriose building block **8** through the stereoselective β-glycoside formation.[21]



Scheme 3.3: Synthesis of fully protected chitotriose **8** by chemoselective activation in an orthogonal glycosylation strategy.

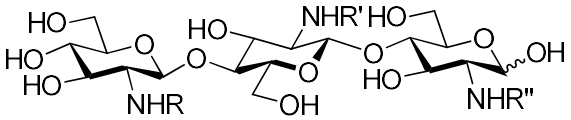
Bednarski and co-workers synthesised the protected chitotriose derivative **13** (Scheme 3.4). This trisaccharide **13** is a versatile building block in the amino oligosaccharide synthesis. Glycosylation of *N*-Phth protected trichloroacetimidate donor **9** with 4-free sugar alcohol **10** provided the disaccharide building block **11**. Fully protected chitotriose **13** was obtained from the desilylated derivative **12** by glycosylating it with the same glycosyl donor **9**.[22]



Scheme 3.4: Synthesis of fully protected chitotriose **13** using glycosyl trichloroacetimidate donor **9**.

3.2 Results and discussion

After achieving the synthesis of chitobioses using phthalimide and azide containing monomer building blocks (Chapter 2), we focused our attention on the synthesis of partially *N*-acetylated chitotrioses by using chemoselctive glycosylation strategy. Trichloroactemidates and thioglycosides were used as glycosyl donors for the synthesis of partially *N*-acetylated chitotriose derivatives. Three kinds of D-glucosamine based building blocks with different amine precursors (*N*-Phth, *N*-DMM, N_3) at C-2, were prepared and used in the glycosylation reactions. Finally they were converted into free amino or acetamido groups selectively. Due to neighbouring group participation and steric hindrance, the protecting groups *N*-Phth and *N*-DMM were expected to favour β-glycosylation. We have successfully achieved the synthesis of chitotrioses having different *N*-acetylated pattern **DDA**, **DAD**, **DAA**, **ADA**, **AAD** (Figure 3.1).



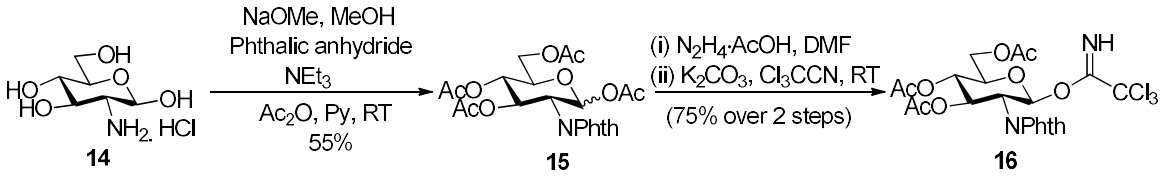
DDA R = R' = H, R"= Ac
DAD R = R"= H, R' = Ac
DAA R = H, R' = R"= Ac
ADA R = R"= Ac, R' = H
AAD R = R' = Ac, R"= H

Figure 3.1: Structural representation of partially *N*-acetylchitotriose derivatives.

3.2.1 Synthesis of *N*-Phth protected acetimidate donor

Acetimidate donor **16** was synthesized in three steps from commercially available glucosamine hydrochloride (Scheme 3.5). For the synthesis of *N*-Phth protected acetimidate

donor, glucosamine hydrochloride **14** was first converted into its *N*-Phth derivative by neutralization followed by treatment with phthalic anhydride in methanol as solvent. The crude product was peracetylated using excess of 1:2 mixture of Ac_2O and pyridine to get the 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-α/β-D-glucopyranose **15** in 55% yield. Then anomeric deacetylation of compound **15** by using hydrazine acetate yielded the anomeric free sugar derivative which was directly converted into the imidate donor **16** in 75% yield by treatment with trichloroacetonitrile in presence of K_2CO_3 base.[23] Trichloroacetimidate donor **16** was utilized further as glycosyl donor in the synthesis of partially *N*-acetylated chitotrioses.

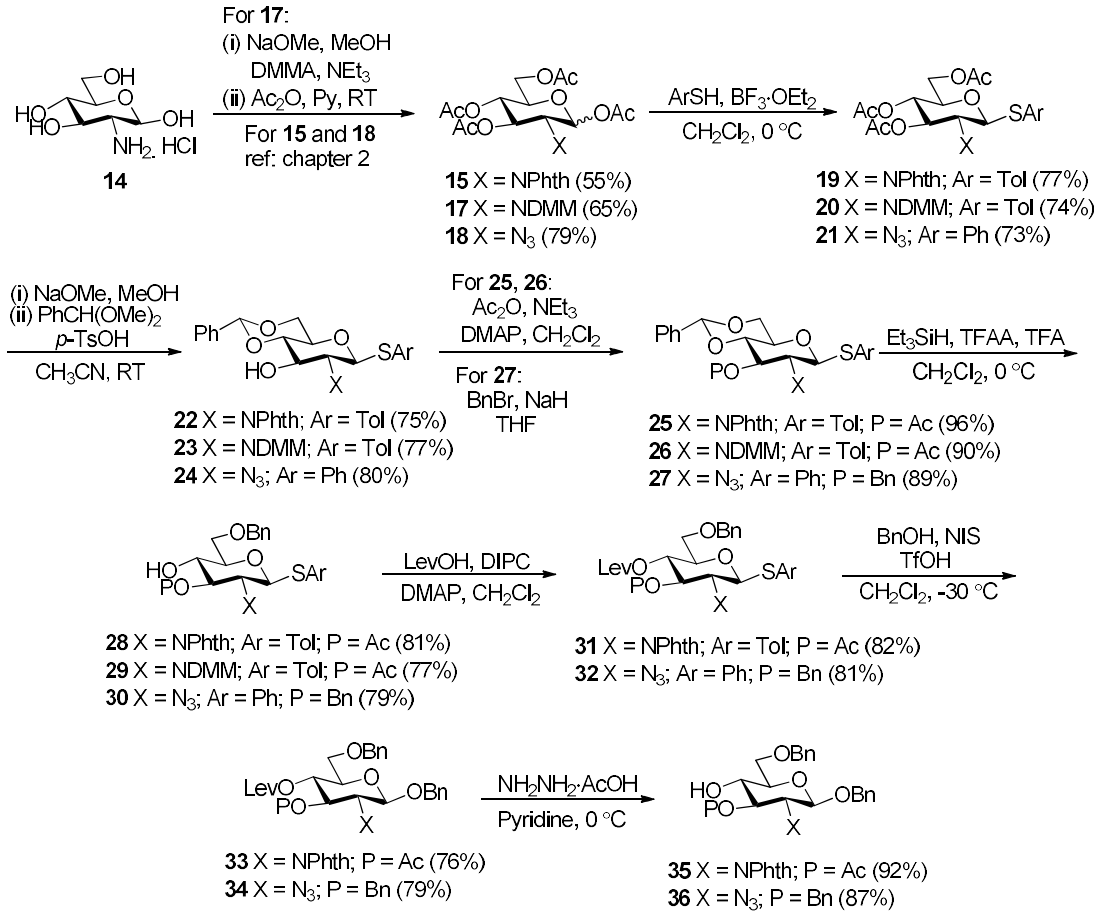


Scheme 3.5: Preparation of glycosyl trichloroacetimidate donor **16**.

3.2.2 Synthesis of monomer building blocks containing *N*-Phth, *N*-DMM and N_3 groups

The synthesis of peracetylated phthalimido and azido derivatives **15**, **18** have already been discussed in Chapter 2. For the preparation of dimethylmalemido derivative **17**, glucosamine hydrochloride **14** was converted into its free amine derivative by neutralization. Treatment of this with dimethylmaleic anhydride in methanol followed by peracetylation using a 1:2 mixture of Ac_2O/Py gave **17** in 65% yield (Scheme 3.6). Compounds **15**, **17** and **18** were glycosylated with thiophenol/*p*-methyl thiophenol in the presence of $BF_3{\cdot}OEt_2$ to get the corresponding thioglycoside derivatives **19**, **20** and **21** in 77%, 74%, 73% yields, respectively. These thioglycoside derivatives were deacetylated under basic conditions (NaOMe/MeOH) and then treated with $PhCH(OMe)_2$ in the presence of *p*-TsOH to get the corresponding benzylidene derivatives **22** (75%), **23** (77%) and **24** (80%). Acetylation of the 3-OH group of the benzylidene derivatives **22**, **23** were carried out using Ac_2O in the presence of NEt_3, DMAP in CH_2Cl_2 to get the acetyl benzylidene derivatives **25** (96%) and **26** (90%).[24] Benzyl benzylidene derivative **27** was prepared in 89% yield by the benzylation of azide protected derivative **24** using BnBr/NaH in THF.[25] Similar benzyl derivatives obtained from **22** and **23** resulted in low yields during the selective benzylidene ring-opening. Hence acetylation was done in these two cases. Regioselective benzylidene ring-opening in **25**, **26** and **27** using TES/TFAA/TFA system resulted in 4-OH free derivatives **28**, **29**, and **30**

respectively. The *N*-Phth, N_3 containing glycosyl acceptors **35** and **36** could be synthesized in a three steps sequence as shown in Scheme 3.6. Levulinoylation[26] of 4-free hydroxyl group in compounds **28** and **30** with levulinic acid, *N*,*N*'-diisopropylcarbodiimide (DIPC) and 4-(dimethylamino)pyridine (DMAP) afforded levulinoyl protected thioglycoside derivatives **31** and **32** respectively, which underwent glycosylation with BnOH under NIS/TfOH promoter system to give the fully protected derivatives **33** and **34** in good yields. Finally, glycosyl acceptors **35** and **36** were obtained from the compounds **33** and **34** respectively by the hydrolysis of levulinate ester[27] using $NH_2NH_2 \cdot H_2O$ in AcOH/Py mixture (Scheme 3.6). These compounds were utilized as glycosyl acceptors in the synthesis of chitotrioses having different *N*-acetylated pattern. *N*-Phth and *N*-DMM protecting groups were very useful in the stereoselctive synthesis of β-glycosides due to the neighbouring group participation ability and their steric bulkiness.[28-31]



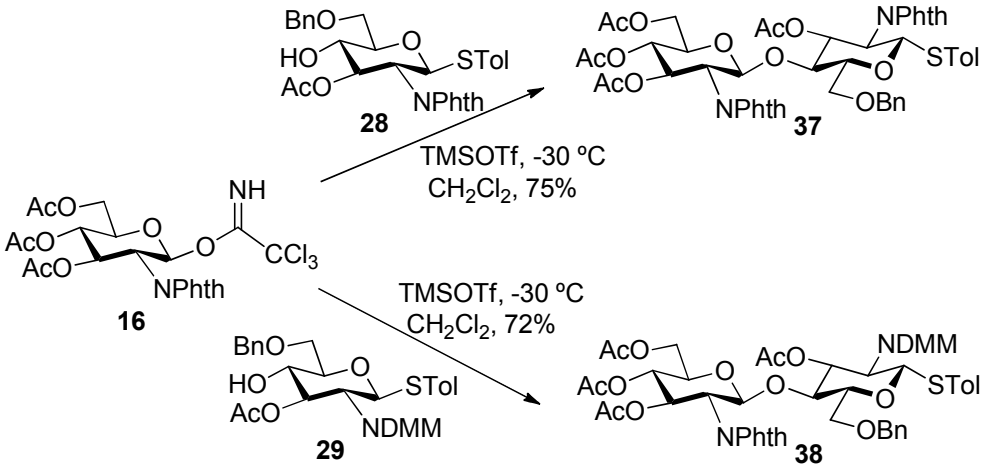
Scheme 3.6: Preparation of monomer building blocks **28**, **29**, **35** and **36**.

3.2.3 Synthesis of partially *N*-acetylated chitotrioses (DDA, AAD, DAA, DAD, ADA)

The monomer building blocks **16**, **28**, **29**, **35**, **36** were used in glycosylation reactions to make different chitotrioses. These chitotrioses were subjected to a series of deprotection steps in certain order to make chitotrioses with specific *N*-acetylated pattern. These synthetic details are discussed in the subsequent sections.

3.2.3.1 Preparation of disaccharide building blocks *via* chemoselective glycosylation

Disaccharide building blocks **37** and **38** were synthesized using chemoselective glycosylation[32] strategy by selective activation of trichloroacetimidate donor **16** in presence of *p*-methylphenyl thioglycoside donors **28** and **29** separately (Scheme 3.7). Glycosylation of the acceptors **28** and **29** with trichloroacetimidate donor **16** using TMSOTf (0.1 equiv.) as a promotor in CH_2Cl_2 at -30 ºC afforded the β-linked glycosides **37** (75%) and **38** (72%) respectively. Stereoslective formation of the β-anomer was observed, which was confirmed by ^{1}H NMR spectroscopy. Synthesized disaccharide thioglycosides **37** and **38** were utilized as glycosyl donors in the preparation of chitotrioses having different *N*-acetylated pattern.



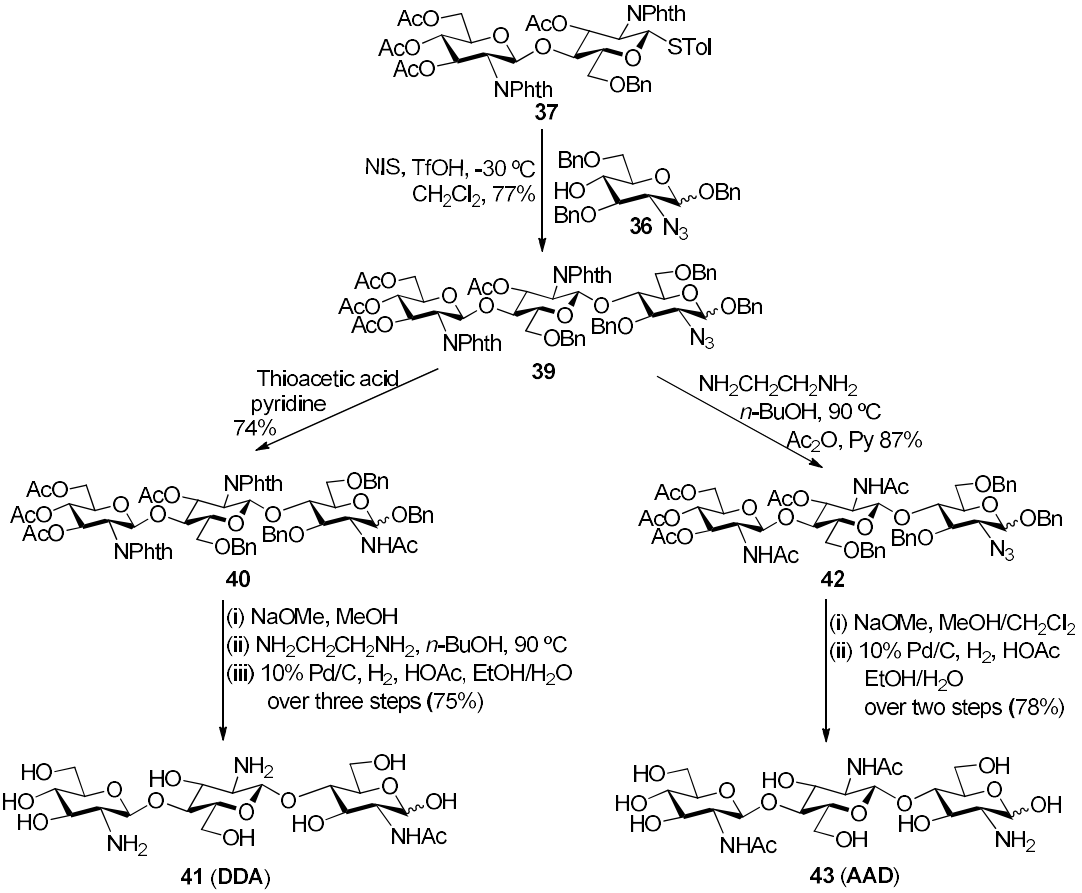
Scheme 3.7: Synthesis of disaccharide building blocks **37** and **38** *via* chemoselective glycosylation.

3.2.3.2 Synthesis of partially *N*-acetylated chitotriose derivatives (DDA and AAD)

After successfully synthesizing of disaccharide thioglycosides **37** and **38**, synthesis of partially *N*-acetylated chitotrioses **41** (**DDA**) and **43** (**AAD**) were undertaken (Scheme 3.8). Synthesis of fully protected trisaccharide precursor **39** was achieved from the glycosyl donor

37 by reacting it with glycosyl acceptor **36** under glycosylation conditions using NIS/TfOH as promoter system[33] at -30 ºC. Sequential deprotection steps were followed to obtain the partially *N*-acetyl chitotriose derivatives **41** and **43**. For the synthesis of chitotriose derivative **41** (**DDA**), the trisaccharide precursor **39** was first treated with 1:2 mixture of thioacetic acid and Py which furnished the **40** in 74% yield. Then, *O*-deacetylation of **40** under Zemplén conditions[34] and removal of *N*-Phth group by treatment with ethylenediamine at 90 ºC in *n*-BuOH were carried out. The crude product obtained at the end of the above two steps was subjected to catalytic hydrogenolysis to remove the benzyl groups[35] to provide the desired chitotriose derivative **41** (**DDA**) in 75% yield.

Synthesis of *N*,*N*'-diacetylchitotriose **43** (AAD) was achieved by the removal of *N*-Phth, *O*-acetyl and benzyl groups in a sequential manner from the trisaccharide derivative **39** (Scheme 3.8). Conversion of *N*-Phth groups of compound **39** into *N*-acetyl was achieved using ethylenediamine at 90 ºC in *n*-BuOH solvent followed by acetylation in presence of



Scheme 3.8: Preparation of partially *N*-acetylated chitotrioses **DDA** and **AAD**.

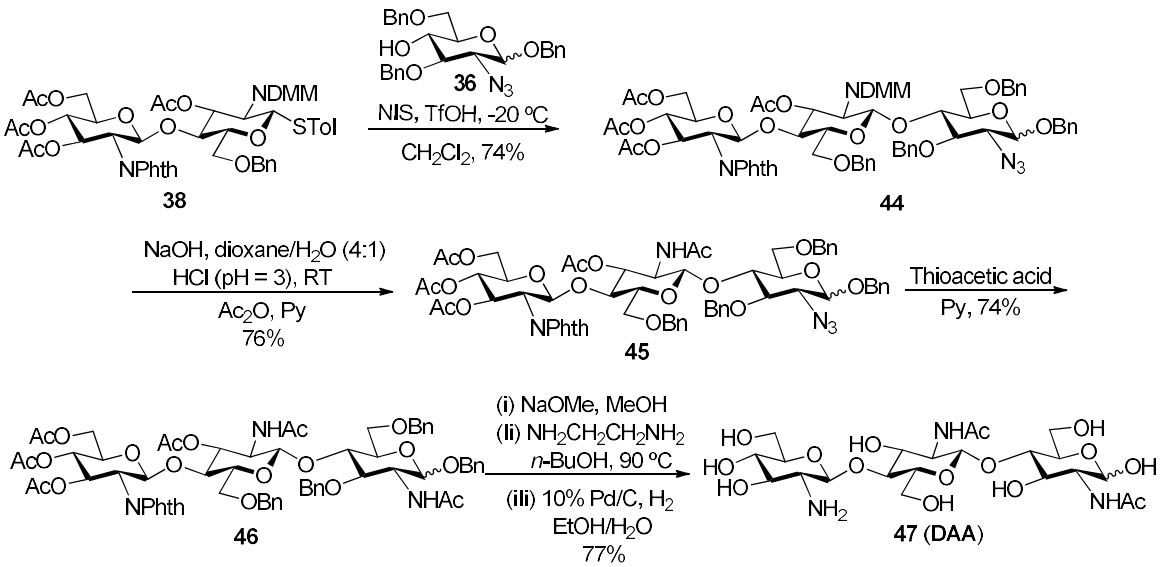
Ac_2O/Py (1:2) mixture to provide the *N,N'*-diacetyl derivative **42** in 87% yield. *O*-Deacetylation of compound **42** was carried out with NaOMe/MeOH. Finally the azide and

benzyl groups of the crude product were converted into free amine and hydroxy groups using H_2, 10% Pd/C to obtain the desired chitotriose derivative **43** (**AAD**) in 78% yield over two steps.

3.2.3.3 Synthesis of partially *N*-acetylated chitotriose derivative (DAA)

Trisaccharide precursor **44** was synthesized from the disaccharide thioglycosyl donor **38** and azide containing acceptor **36** under standard glycosylaton conditons (NIS/TfOH) in CH_2Cl_2 at -30 ºC (Scheme 3.9).

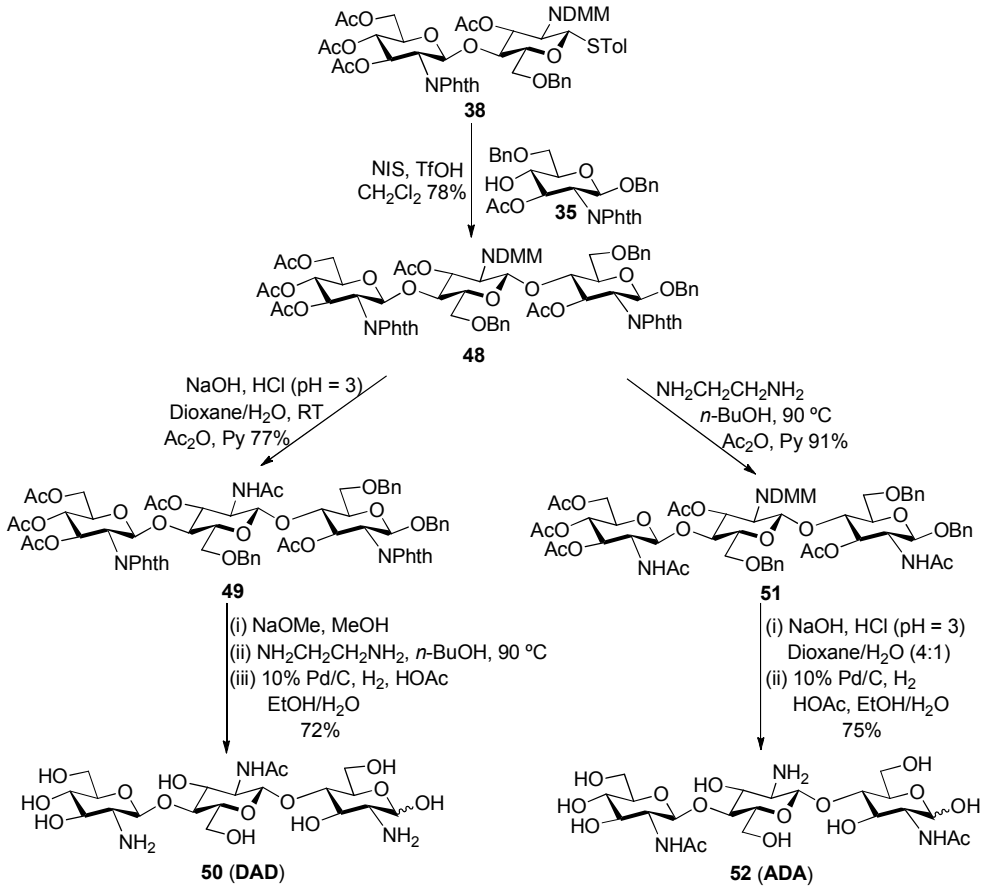
For the synthesis of *N,N′*-diacetylchitotriose derivative **47** (**DAA**), conversion of *N*-DMM group into required *N*-acetyl was achieved by treating **44** with 1.5 M NaOH in dioxane-water mixture (4:1), followed by addition of HCl to adjust pH to 3. Ethanolamine was used when the pH lowered below 3 to bring it back to 3. The crude product was *N*-acetylated using Ac_2O/Py (1:2) mixture to obtain *N*-acetylchitotriose derivative **45** in 76% yield. To convert azide into *N*-acetyl group, compound **45** was treated with thioacetic acid/Py (1:2) which resulted the *N,N′*-diacetylchitotrose derivative **46** in 74% yield. Finally, deprotection of *O*-acetyl groups and *N*-Phth groups were achieved by treating the trisaccharide derivative **46** with NaOMe in MeOH followed by ethylenediamine at 90 ºC in *n*-BuOH solvent. After the completion of reaction as indicated by TLC, the crude product was subjected to catalytic hydrogenolysis to deprotect the benzyl ethers using H_2 on Pd/C to obtain the desired partially *N,N′*-diacetylchitotriose **47** (**DAA**) in 77% yield.



Scheme 3.9: Preparation of *N,N′*-diacetylchitotriose **47** (**DAA**).

3.2.3.4 Synthesis of partially *N*-acetylated chitotriose derivatives (DAD and ADA)

Protected trisaccharide derivative **48** was first obtained by the glycosylation of *N*-Phth protected acceptor **35** with thioglycoside donor **38** using NIS/TfOH reagent system at -30 ºC in 78% yield. This fully protected trisaccharide building block was utilized for the synthesis of chitotriose derivatives **50** (**DAD**) and **52** (**ADA**) (Scheme 3.10). Synthesis of mono *N*-acetylchitotriose derivative **50** (**DAD**) was achieved by the sequential deprotection of protecting groups from the fully protected trisaccharide precursor **48** as follows. Conversion of *N*-DMM group in compound **48** into *N*-acetyl was done by treating **48** with NaOH followed by the addition of HCl/ethanolamine to adjust the pH to 3 and then acetylation using Ac_2O/Py (1:2) mixture to get the *N*-acetylchitotriose derivative **49** in 77% yield. Deacetylation of compound **49** using NaOMe and subsequent dephthalolation using ethylenediamine in *n*-BuOH under heating conditions gave the crude product which was subjected to catalytic hydrogenolysis using 10% Pd/C in EtOH:H_2O for the removal of benzyl groups to obtain the *N*-acetylchitotriose derivative **50** (**DAD**) in 72% yield.



Scheme 3.10: Preparation of partially *N*-acetylated chitotrioses **DAD** and **ADA**.

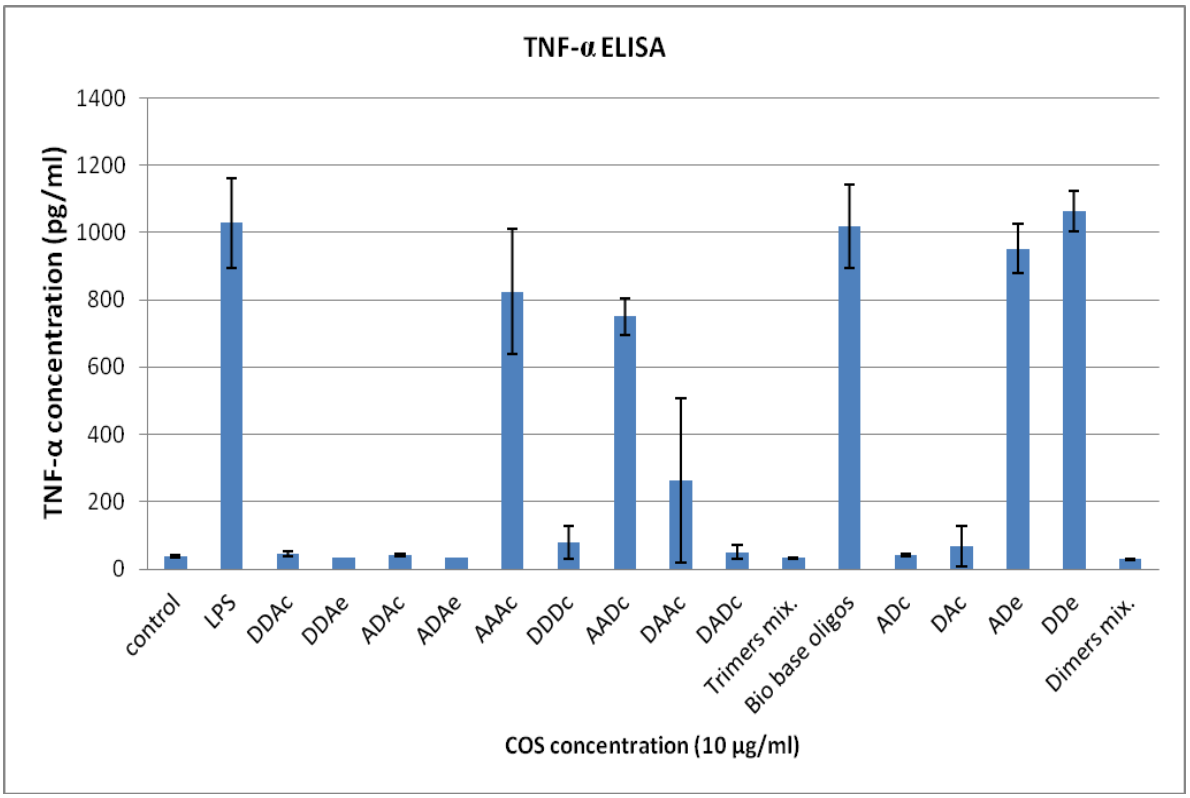
For the synthesis of *N,N′*-diacetylchitotriose derivative **52** (**ADA**), *N*-Phth groups in **48** were converted into *N*-acetyl group by treating it with ethylenediamine and Ac_2O:Py mixture (1:2) to obtain the intermediate **51** in 91% yield. The compound **51** was treated with NaOH/HCl in dioxane/water mixture (1:2) for the deprotection of *N*-DMM group. Finally, the desired *N,N′*-diacetylchitotriose **52** (**ADA**) was obtained in 75% yield by the removal of benzyl groups under hydrogenolytic conditions (H_2, 10% w/v Pd/C) (Scheme 3.10).

3.2.4 Partially *N*-acetylated chitotrioses mediated endothelial cell surface modifications

Endothelial surface is coated with glycocalyx which consists of a thick and dense meshwork of carbohydrates. The stability of glycocalyx depends on the type of endothelial cell and the extent of its presence on the cell surface.[37,38] Physiologically, the glycocalyx functions as a large storage for compounds such as growth factors or cytokines.[38] It mainly consists of polysaccharides such as negatively charged hyaluronan and heparan sulphate.[38] There may be charge-mediated interaction between the negatively charged glycocalyx and positively charged amino sugars at a physiological pH of 7.4.[39] Previous literature support the interaction of chitosan, COS and heparin with glycocalyx[40,41] In this context, we wished to study the effects of the synthesized chitotrioses with specific acetylated pattern on endothelial cell surface modification using fluorescence spectroscopy. The enzymatic generation of dimers and trimers was achieved by selective *N*-deacetylation of fully *N*-acetylated dimers and trimers by chitin deacetylases (Chapter 2). This part of work was carried out in the University of Heidelberg, Germany (UMM).

3.2.4.1 Analysis of COS purity: Inflammatory activity of chitooligosaccharides (COS) libraries on macrophages

Supernatants from human peripheral blood derived monocytes/macrophages were analyzed for the levels of TNF-α by using a sandwich ELISA. The obtained data suggest that few chitooligosaccharides (at 10 μg/mL) show very strong inflammatory effects on the macrophages. This may be due to contamination of COS by endotoxins or bacteria (Figure 3.2).



(c-chemically produced COS, e-enzymatically produced COS)

Figure 3.2: Quantification of cytokines: Supernatants from human peripheral blood derived monocytes/macrophages were analyzed for levels of TNF-α by using a sandwich ELISA according to manufacturer instruction using commercially available ELISA kit (R&D)

3.2.4.2 Cytotoxicity of chitooligosaccharides libraries on human endothelium cells

To know the potential toxic effects of chitooligosaccharides (dimers and trimers) on human ECs, HUVECs were treated with different concentrations of COS (50, 10, 2 µg/mL). Cell viability was quantified by measuring the mitochondrial activity (WST-1 assay) after incubating the mixtures for 24 h. There was not much change in the metabolic activity irrespective of the dose of chitooligosaccharides. Mitochondrial activity of untreated HUVECs was taken as control and normalised to 100% (Figure 3.3).

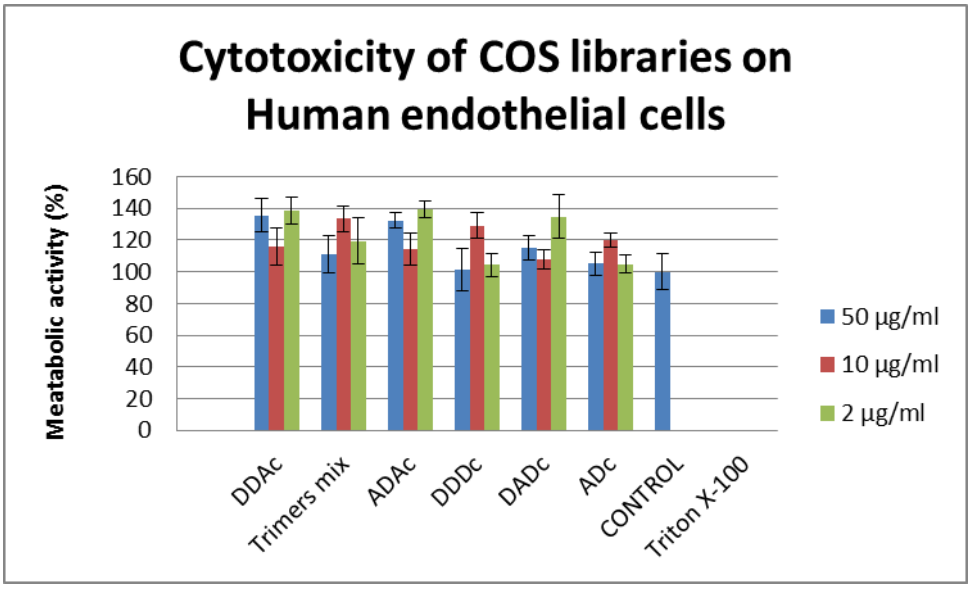


Figure 3.3: Metabolic activity of HUVEC upon treatment with 50, 10, 2 µg/mL chitooligosaccharides. Cellular metabolism was measured using the WST-1 assay and is shown relatively to untreated HUVECs (100%, control). Values are expressed as mean ± SD of three independent experiments.

3.2.4.3 EC activation and formation of ultra-large VWF fibers on the EC surface

Non-toxic concentrations of partially *N*-acetyl chitooligosaccharides (DP2 and DP3) were used to check the influence on the physiological behaviour of human ECs in the context of coagulation. HUVECs were treated with partially *N*-acetyl chitooligosaccharides (DP2 and DP3) and then stimulated with histamine. Histamine activates the cell surface and initiates a physiological response combined with a receptor. Then the effect on ECs was studied. Since the acute non-transcriptional regulated release of VWF to the luminal site of HUVECs is due to the rapid exocytosis of Weibel-Palade bodies, this is a characteristic physiological feature of ECs.[42]

The above data suggest that the impact of COS (dimers and trimers) on the formation and immobilisation of ULVWF fibres on the luminal EC surface. ULVWF deposition was completely absent on the non-stimulated HUVECs (without histamine) as shown in A (Figure 3.4). But only in the histamine stimulated cells, we found a strongly increased deposition of ULVWF fibres on the HUVECs after pre-treatment with 1µg/ml of either mixtures of chitobioses or trioses (Figure 3.4 A). Quantitative analyses of the ULVWF coverage revealed a statistically significant increase of amount of fibers on pre-treated HUVECs (Figure 3.4 B). Degree of polymerization has an effect on the formation and immobilisation of ULVWF

fibres, i.e., dimers mixture (DP2) show stronger effect than trimers mixture (DP3) of COS (Figure 3.4 B). These experiments were carried out in university of Heidelberg (UMM).

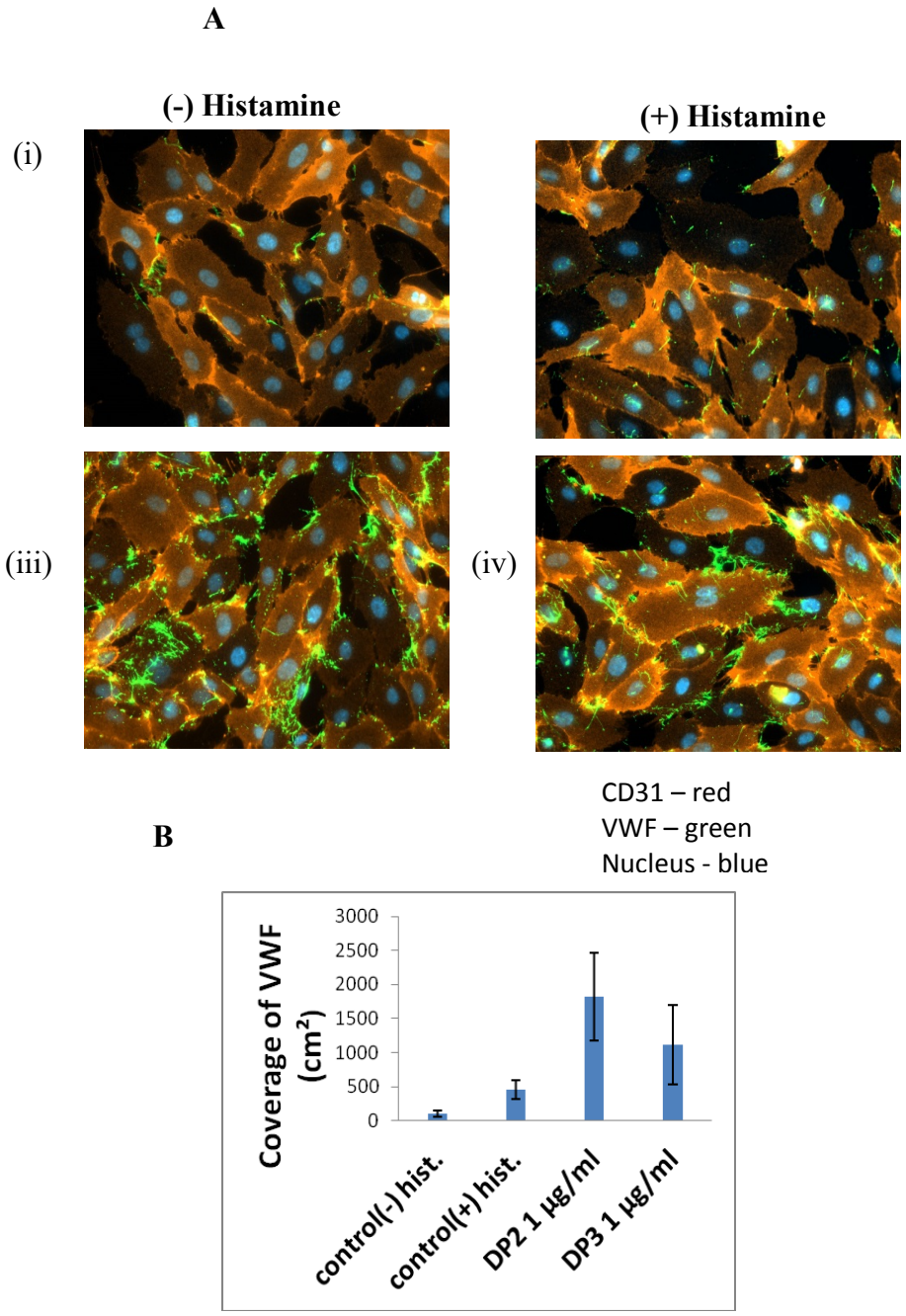


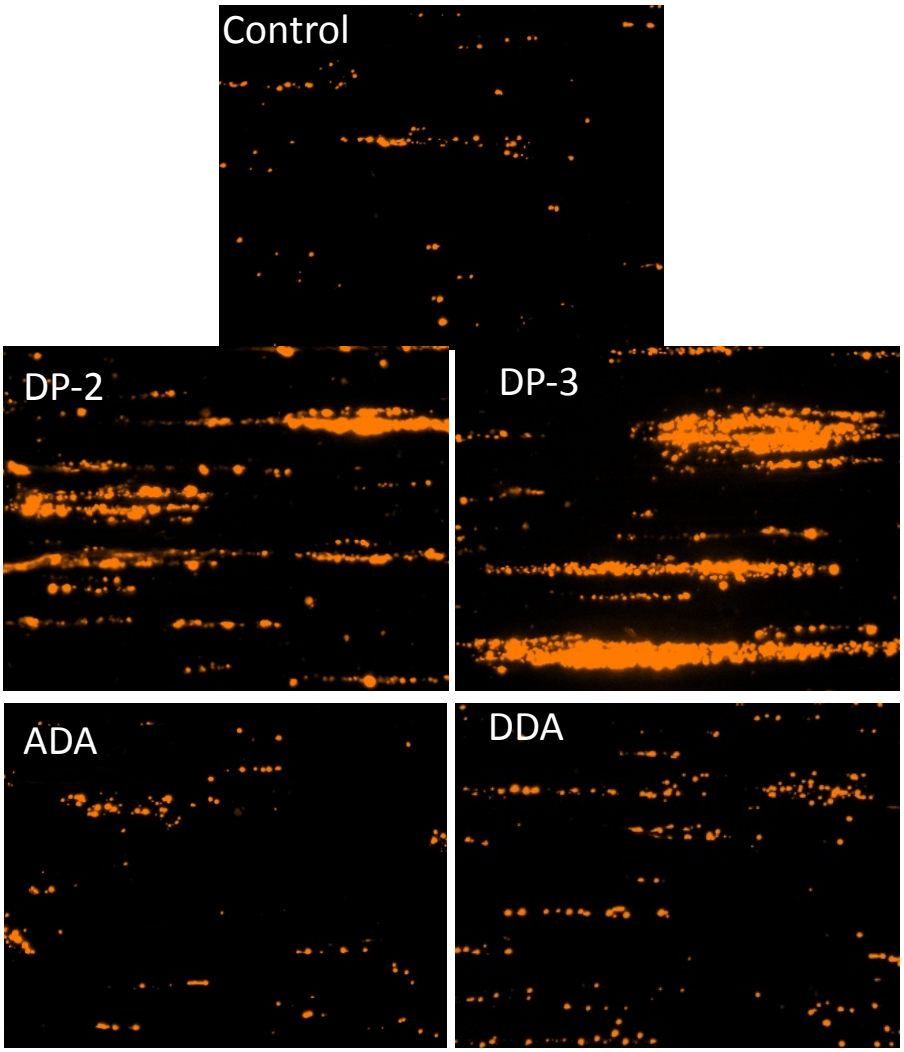
Figure 3.4: Pre-treatment of HUVEC with 1 µg/mL chitooligosaccharides (mixtures of DP2 and DP3) increases the formation of ULVWF at the cellular surface after histamine stimulation. Deposition of ULVWF on the surface of HUVECs was visualized by fluorescence microscopy (A). ULVWF was not detectable on non-treated EC (without histamine). ULVWF was only formed on histamine stimulated HUVECs (with histamine). Excessive ULVWF formation was found on the HUVECs pre-treated with COS mixtures for 16 h prior to the histamine stimulation. Scale bars correspond to 100 µm (A). Quantitative evaluation of ULVWF immobilized on HUVEC (B). Values are expressed as mean ± SD of three independent experiments.

3.2.4.4 Chitooligosaccharide (COS) pre-treatment improves platelet binding to HUVECs under flow

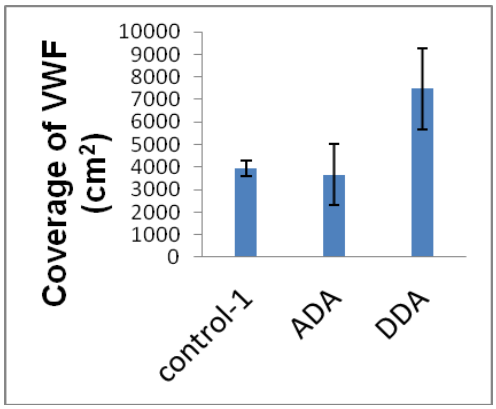
As seen in the last section chitooligosaccharides enhance the formation of ULVWF on the EC surface (Figure 3.5). This suggests an increased binding of platelets to the endothelium. Therefore, we further investigated the adhesion of platelets to ECs *via* ULVWF by applying a microfluidic device mimicking physiological blood flow conditions.[43] Adherence of fluorescence-labeled platelets to ULVWF was followed by fluorescence microscopy in real time. Extend of ultra-large VWF deposition on the EC surface and binding of platelets depend on the COS pre-treatment, and the DP and DA of the COS used. The data suggest that chitobioses or trioses mixture pretreatment is effective in supporting ULVWF formation (Figure 3.5). We measured an elevated platelet adhesion on histamine stimulated ECs pre-treated with 1µg/mL COS (dimers and mixtures) and also partially *N*-acetylated chitotrioses (**DDA** and **ADA**) in comparison to ECs stimulated with histamine alone (Figure 3.5). Quantification of the amounts of platelets strings revealed a two-fold increase in binding of platelets to the endothelium after COS treatment (Figure 3.5 B). Interestingly, adhesion of platelets to ECs depends on the degree of polymerization and degree of *N*-acetylation. It is clear that *N*-acetylchitotriose (**DDA**) shows strong effect than the *N*,*N*'-diacetylchitotriose (**ADA**).

These studies suggest that COS (mixtures of dimers or trimers) pre-treatment of HUVECs followed by an acute activation with histamine led to an increased deposition of VWF on the cellular surface under static conditions as well as under laminar flow conditions as indicated by an increased entrapment of platelets to the endothelium (Figure 3.5). Degree of *N*-acetylation also has an effect on the entrapment of platelets to the endothelium, i.e., mono *N*-acetyl chitotriose (**DDA**) shows stronger effect than the *N*,*N*'-diacetylchitotriose (**ADA**) (Figure 3.5 B).

A



B



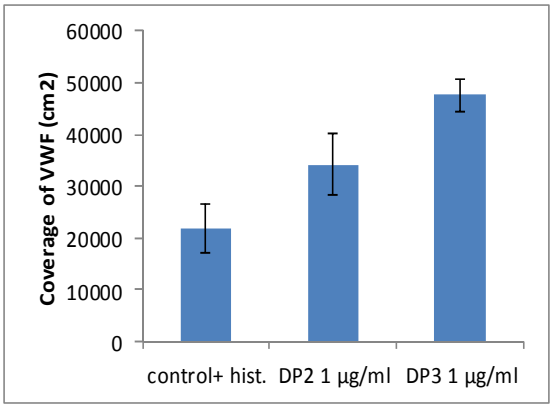


Figure 3.5: Entrapment of platelets to the endothelium under continuous and laminar flow conditions (shear stress = 6 dyne/cm^2) is amplified upon pre-treatment with COS. HUVECs were either pre-treated with COS (1µg/mL) for 16 h prior to histamine stimulation (COS), or only stimulated with histamine (control) (**A**). Binding of fluorescent-labelled platelets (red) was visualised by fluorescence microscopy (**A**) and quantitatively evaluated (10-20 fields of view/experiment) (**B**). Values are expressed as mean ± SD of three independent experiments.

3.3 Conclusions

We have successfully synthesized five partially *N*-acetylchitotriose derivatives by using chemoselective glycosylation strategy. To investigate biological effects of the synthesized chitooligosaccharides [dimers (Chapter 2) and trimers] on human endothelium was studied in the context of coagulation and inflammation (UMM, Mannheim). Biological results suggest the evidence of a non-toxic COS-mediated change of the endothelial surface most likely due to a direct interaction between the positively charged COS and the negatively charged glycocalyx. Interestingly, the increased deposition of ULVWF at the luminal site of HUVECs upon chitooligosaccharide treatment appears to merely be related to an alteration of the cellular surface and not due to an additional activation of the ECs. This beneficial effect of VWF-mediated coagulation, biomedical application of chitoligosaccharides having different *N*-acetyl pattern might be worthwhile in treating bleeding disorders (coagulation).

3.4 Experimental section

For general information refer Chapter 2, Section 2.4.1.

3.4.1. Experimental procedures, spectral and analytical data

General procedure for acetylation at 3rd position

To a solution of benzylidene acetal **22**/**23** (1 equiv.) in dry CH_2Cl_2 (2 mL/mmol), NEt_3 (3 equiv.), DMAP (5 mol%) and Ac_2O (1.5 equiv.) were added. After 5 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, hexanes/EtOAc) to get the required products **25**/**26**.

General procedure for chemoselective glycosylation with trichloroacetimidate donor[32]

A solution of trichloroacetimidate donor **16** (1.2 equiv.) and thioglycoside acceptor **28/29** (1 equiv.) in CH_2CI_2 (6 mL/mmol) dried over 4 Å molecular sieves powder was allowed to stir at room temperature. After 1 h, the reaction mixture was cooled to -30 ºC then TMSOTf (0.1 equiv.) was added slowly dropwise with continuous stirring. After completion of reaction as revealed by TLC, the reaction mixture was diluted with EtOAc and saturated $NaHCO_3$ was added to it. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated

in vacuo. The resulting syrup was purified by column chromatography (silica gel, hexanes/EOAc) to avail compounds **37** and **38**.

General procedure for glycosylation with thioglycosides

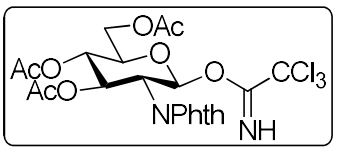
Glycosyl donor **37**/**38** (1.2 equiv.) and glycosyl acceptor **35**/**36** (1 equiv.) were dissolved in dry CH_2Cl_2 (9 mL/mmol). It was dried over 4 Å molecular sieves and allowed to stir at room temperature for an hour. The suspension was cooled to -30 ºC, treated with NIS (1.5 equiv.). After 10 min TfOH (0.1 M in CH_2Cl_2, 0.1 equiv.) was added dropwise. After completion of reaction, the reaction mixture was quenched with saturated $Na_2S_2O_3$ solution and was extracted with dichloromethane. The organic extract was washed with brine solution, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexanes/EOAc) to obtain compounds **39**, **44** and **48**.

General procedure for conversion of *N*-DMM to *N*-acetyl group

A suspension of *N*-DMM derivative (1 equiv.) in 4:1 dioxane-water (20 mL/mmol) was added NaOH (11 equiv.) at room temperature and allowed to stir for overnight. Then, the pH of the reaction mixture was adjusted to 5 using 3 N HCl in the presence of ethanolamine. After 1 day, the solution was neutralized with $HOCH_2CH_2NH_2$ and concentrated in vacuo. The crude product was directly treated with 2:1 pyridine-acetic anhydride (10 mL/mmol) and stirred at room temperature overnight. After completion of the reaction, the residue was purified by column chromatography (SiO_2, hexanes/EtOH) to obtain *N*-acetyl derivative.

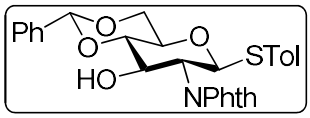
O-[3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl]trichloroacetimidate

16



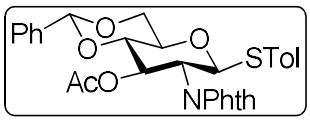
The compound **16** was prepared from **15** (2.74 g, 5.7 mmol) by following the literature procedure.[23] Light yellow foam; Yield = 2.5 g (75%); R_f = 0.70 in 1:1 EtOAc/hexanes ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.73 (s, 1H), 7.95–7.78 (m, 4H), 6.58 (d, *J* = 8.8 Hz, 1H), 5.95 (dd, *J* = 11.6, 3.5 Hz, 1H), 5.76 (d, *J* = 3.6 Hz, 1H), 4.93 (dd, *J* = 11.5, 9.1 Hz, 1H), 4.33–4.13 (m, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 1.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.1, 169.4, 167.4, 160.5, 134.7, 134.7, 131.1, 124.0 123.7, 93.5, 72.7, 70.4, 68.4, 61.5, 53.5, 20.8, 20.6, 20.5.

Preparation of *p*-methylphenyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside 22



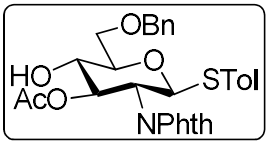
The compound **22** was prepared from **19** (4.4 g, 8.9 mmol) by general procedure for *O*-deacetylation followed by benzylidene acetal protection (Chapter 2). Colourless foam; Yield = 3.36 g (75%); R_f = 0.50 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85-7.81 (m, 2H), 7.70 (br s, 2H), 7.49-7.44 (m, 2H), 7.34-7.33 (m, 3H), 7.28-7.24 (m, 2H), 7.05 (d, *J* = 8.0 Hz, 2H), 5.59 (d, *J* = 10.8 Hz, 1H), 5.53 (s, 1H), 4.57 (t, *J* = 9.6 Hz, 1H), 4.37-4.33 (m, 1H), 4.27 (t, *J* = 10.4 Hz, 1H), 3.78 (t, *J* = 10.4 Hz, 1H), 3.65-3.60 (m, 1H), 3.59-3.51 (m, 1H), 2.78 (br s, 1H), 2.29 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.2, 167.5, 138.4, 136.9, 134.1, 133.2, 131.6, 129.7, 129.3, 128.3, 127.8, 126.8, 123.8, 123.3, 101.8, 84.4, 81.8, 77.3, 77.0, 76.7, 70.2, 69.6, 68.5, 55.6, 21.1. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{28}H_{25}NO_6S$ 526.1300, found 526.1294.

Preparation of *p*-methylphenyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside 25



The compound **25** was prepared from **22** (3.31 g, 6.56 mmol) by general procedure for acetylation at 3rd position. Light yellow foam; Yield = 3.44 g (96%); R_f = 0.50 in 1:1 EtOAc/hexanes ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87-7.85 (m, 2H), 7.74-7.71 (m, 2H), 7.45-7.42 (m, 2H), 7.34-7.32 (m, 3H), 7.28 (d, *J* = 8.4 Hz, 2H), 7.07 (d, *J* = 8.0 Hz, 2H), 5.88 (t, *J* = 9.6 Hz, 1H), 5.76 (d, *J* = 10.4 Hz, 1H), 5.52 (s, 1H), 4.43-4.40 (m, 1H), 4.34 (t, *J* = 10.4 Hz, 1H), 3.84-3.71 (m, 3H), 2.31 (s, 3H), 1.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 167.8, 167.2, 138.6, 134.4, 134.1, 133.6, 131.6, 131.2, 129.7, 129.1, 128.2, 127.2, 126.2, 123.6, 123.5, 101.6, 83.9, 78.9, 77.3, 77.0, 76.7, 70.6, 70.5, 68.5, 54.3, 21.1, 20.5. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{30}H_{27}NO_7S$ 568.1406, found 568.1408.

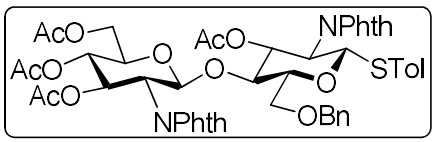
Preparation of *p*-methylphenyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside 28



The compound **28** was prepared from **25** (3 g, 5.49 mmol) by using general procedure for regioselective benzylidene ring opening (Chapter 2). Colourless oil; Yield = 2.44 g (81%); R_f = 0.60 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (dd, *J* = 7.2, 2.0 Hz, 2H), 7.66 (t, *J* =

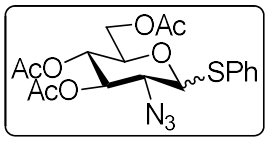
7.2 Hz, 1H), 7.55 (t, *J* = 6.8 Hz, 1H), 7.33-7.28 (m, 7H), 6.97 (d, *J* = 8.0 Hz, 2H), 5.84 (d, *J* = 10.4 Hz, 1H), 5.75 (t, *J* = 9.6 Hz, 1H), 4.58 (s, 2H), 4.30 (t, *J* = 10.4 Hz, 1H), 3.90-3.78 (m, 3H), 3.71 (t, *J* = 9.2 Hz, 1H), 3.45 (br s, 1H), 2.23 (s, 3H), 1.84 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1, 167.7, 167.1, 137.9, 134.2, 134.0, 133.0, 131.3, 130.9, 129.4, 128.2, 127.6, 127.4, 123.5, 123.3, 82.8, 78.9, 77.3, 77.0, 76.7, 74.2, 73.4, 70.2, 69.8, 53.6, 20.9, 20.4. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{30}H_{29}NO_7S$ 570.1567, found 570.1558.

Preparation of *p*-methylphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside 37



The compound **37** was prepared from **16** (0.69 g, 1.18 mmol) and **28** (0.54 g, 0.99 mmol) by following general procedure for the chemoslective glycosylation with trichloroacetimidate donor. Colourless solid; Yield = 0.72 g (75%); R_f = 0.40 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84 (br s, 4H), 7.74-7.71 (m, 4H), 7.37-7.23 (m, 7H), 6.96 (d, *J* = 7.9 Hz, 2H), 5.70 (q, *J* = 10.8 Hz, 2H), 5.53 (d, *J* = 10.5 Hz, 1H), 5.45 (d, *J* = 8.4 Hz, 1H), 5.10 (t, *J* = 9.4 Hz, 1H), 4.47 (q, *J* = 11.8 Hz, 2H), 4.37 (dd, *J* = 12.2, 3.7 Hz, 1H), 4.23-4.18 (m, 2H), 4.11 (d, *J* = 8.7 Hz, 1H), 3.93 (m, 1H), 3.60-3.52 (m, 3H), 3.50 (dd, *J* = 11.2, 3.3 Hz, 1H), 2.25 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H), 1.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.0, 169.9, 169.3, 167.7, 167.1, 138.3, 138.2, 134.3, 134.0, 133.7, 131.6, 131.2, 129.4, 128.2, 127.3, 127.1, 123.5, 96.9, 82.7, 78.4, 73.7, 72.6, 71.8, 71.5, 70.5, 70.0, 68.3, 68.0, 67.7, 61.7, 61.3, 54.8, 53.8, 21.04, 20.7, 20.5, 20.4, 20.3. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{50}H_{48}N_2O_{16}S$ 965.2803, found 965.2820.

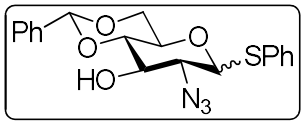
Preparation of phenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-α/β-D-glucopyranoside 21



The compound **21** was prepared from **18** (6.83 g, 18.3 mmol) by following general procedure for synthesis of thioglycosides (Chapter 2). Colourless foam; Yield = 5.64 g (73%); R_f = 0.43 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21-7.20 (m, 5H, Ar-H), 5.65 (d, *J* = 5.6 Hz, 1H), 5.32 (m, 1H), 4.97 (t, *J* = 9.8 Hz, 1H), 4.54 (ddd, *J* = 10.2, 5.2, 2.1 Hz, 1H), 4.30 (dd, *J* = 12.4, 5.2 Hz, 1H), 4.08 (dd, *J* = 10.5, 5.6 Hz, 1H), 3.96 (dd, *J* = 12.4, 2.2 Hz, 1H), 2.08 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.02 (s, 3H, OAc). ^{13}CNMR (100 MHz, $CDCl_3$): δ

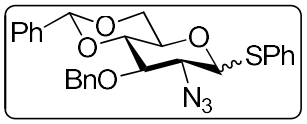
170.0, 169.9, 169.4, 169.2, 168.1, 133.6, 132.1, 131.5, 128.7, 128.6, 127.7, 89.6, 86.0, 85.2, 75.2, 73.9, 71.6, 68.4, 68.2, 62.2, 61.6, 61.2, 61.0, 59.8, 20.2.

Preparation of phenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-1-thio-α/β-D-glucopyranoside 24



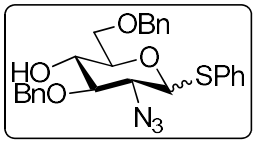
The compound **24** was prepared from **21** (2.34 g, 5.53 mmol) by general procedure for *O*-deacetylation followed by benzylidene acetal protection (Chapter 2). Colourless solid; Yield = 1.71 g (80%); R_f = 0.61 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59-7.30 (m, 10H, Ar-H), 5.54-5.47 (m, 2H), 4.34 (m, 1H), 4.20 (dd, *J* = 10.3, 5.0 Hz, 1H), 3.98 (t, *J* = 9.4 Hz, 1H), 3.84 (dd, *J* = 10.0, 5.6 Hz, 1H), 3.71 (t, *J* = 10.3 Hz, 1H), 3.52 (t, *J* = 9.3 Hz, 1H), 3.46-3.08 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 136.7, 133.6, 132.4, 131.8, 129.4, 129.1, 128.4, 127.9, 126.3, 102.1, 101.8, 87.7, 86.7, 81.6, 80.1, 73.9, 70.6, 65.0, 63.7, 63.4. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{19}H_{19}N_3O_4S$ 408.0994, found 408.0988.

Preparation of phenyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-1-thio-α/β-D-glucopyranoside 27



The compound **27** was prepared from **24** (1.7 g, 4.39 mmol) by general procedure for benzylation at 3rd position. Colourless solid; Yield = 1.86 g (89%); R_f = 0.69 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44-7.28 (m, 15H), 5.59 (s, 1H), 5.56 (d, *J* = 4.0 Hz, 1H), 4.98 (d, *J* = 10. 8 Hz, 1H, CH_2Ph), 4.83 (d, *J* = 10.8 Hz, 1H, CH_2Ph), 4.43 (m, 1H), 4.22 (dd, *J* = 10.4, 4.9 Hz, 1H), 4.00-3.93 (m, 2H), 3.80-3.72 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.5, 137.0, 133.9, 132.8, 132.4, 131.9, 129.1, 129.0, 128.4, 128.3, 128.2, 127.9, 127.9, 101.1, 87.7, 86.5, 82.6, 81.2, 77.8 ,75.2, 70.4, 68.5, 64.5, 63.7, 63.4. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{26}H_{25}N_3O_4S$ 498.1463, found 498.1457.

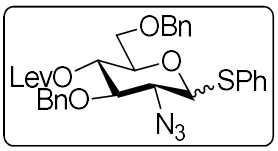
Preparation of phenyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-1-thio-α/β-D-glucopyranoside 30



The compound **30** was prepared from **27** (1.5 g, 3.15 mmol) by using general procedure for regioselective benzylidene ring opening (Chapter 2). Colourless oil; Yield = 1.19 g (79%); R_f = 0.63 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61-7.21 (m, 15H, Ar-H), 5.56 (d, *J* = 5.4 Hz, 1H), 4.94 (d, *J* = 11.1 Hz, 1H), 4.83 (d, *J* = 11.0 Hz, 1H), 4.57 (d, *J* = 12.1 Hz, 1H), 4.49 (d, *J* = 12.1 Hz, 1H), 4.33 (m, 1H), 3.89 (dd, *J* = 10.1, 5.4 Hz, 1H), 3.74 (dd, *J* = 10.3, 5.5

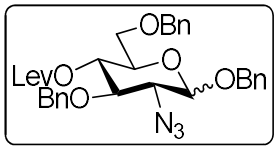
Hz, 2H), 3.70-3.62 (m, 2H), 2.59 (br s, 1H, OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.8, 137.6, 133.5, 133.3, 132.1, 131.9, 129.0, 128.6, 128.4, 128.2, 128.1, 127.8, 127.7, 87.2, 81.3, 75.4, 73.6, 72.2, 71.0, 69.6, 63.5. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{26}H_{27}N_3O_4S$ 500.1620, found 500.1617.

Preparation of phenyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-levulinoyl-1-thio-α/β-D-glucopyranoside 32



The compound **32** was prepared from **30** (0. 91 g, 1.88 mmol) by using literature procedure.[26] Gummy liquid; Yield = 0.88 g (81%); R_f = 0.60 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58-7.52 (m, 2.6H), 7.25-7.22 (m, 19H), 5.58 (d, *J* = 5.4 Hz, 1H), 5.17 (t, *J* = 9.4 Hz, 1H), 4.96 (t, *J* = 9.2 Hz, 0.3H), 4.84 (d, *J* = 11.2 Hz, 1.3H), 4.68 (d, *J* = 11.1 Hz, 1.4H), 4.52-4.46 (m, 4.3H), 3.96 (dd, *J* = 10.3, 5.5 Hz, 1H), 3.81 (t, *J* = 9.5 Hz, 1H), 3.58-3.56 (m, 3.4H), 3.38 (t, *J* = 9.5 Hz, 0.3H), 2.69-2.52 (m, 3.3H), 2.47-2.37 (m, 1.6H), 2.32-2.25 (m, 1.4H), 2.11 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.1, 171.4, 137.9, 137.8, 137.4, 133.3, 132.8, 132.3, 130.9, 129.0, 128.3, 128.2, 128.1, 127.9, 127.8, 127.6, 127.4, 86.8, 85.9, 82.3, 79.2, 77.5, 77.2, 75.1, 75.0, 73.4, 70.9, 70.6, 70.1, 69.2, 68.6, 64.6, 63.5, 37.6, 29.6, 27.7. LCMS (m/z): $[M+H]^+$ calcd for $C_{31}H_{33}N_3O_6S$ 576.22, found 576.65. Anal. calcd. for $C_{31}H_{33}N_3O_6$: C, 64.68; H, 5.78; N, 7.30, found C, 64.56; H, 5.71; N, 7.39.

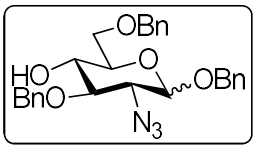
Preparation of benzyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-levulinoyl-α/β-D-glucopyranoside 34



The compound **34** was prepared from **32** (0.67 g, 1.16 mmol) by using general procedure for glycosylation with thioglycosides. Colourless liquid; Yield = 0.53 g (79%); R_f = 0.60 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39-7.25 (m, 29H), 5.15 (t, *J* = 9.1 Hz, 0.7H), 5.02-4.98 (m, 1.7H), 4.92 (d, *J* = 11.9 Hz, 1H), 4.80 (d, *J* = 9.4 Hz, 1H), 4.74 (d, *J* = 12.5 Hz, 1.3H), 4.69-4.59 (m, 5H), 4.53-4.50 (m, 3.4H), 4.34 (d, *J* = 8.0 Hz, 1H), 4.02 (t, *J* = 9.2 Hz, 0.8H), 3.94-3.90 (m, 0.8H), 3.61-3.56 (m, 2H), 3.51 (t, *J* = 8.6 Hz, 3.3H), 3.42-3.35 (m, 1.8H), 2.61-2.48 (m, 3.8H), 2.43-2.32 (m, 2H), 2.29 (m, 2H), 2.08 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.1, 206.0, 171.4, 171.3, 140.9, 138.0, 137.7, 136.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 126.8, 100.2, 96.4, 80.2, 77.7, 74.6, 74.5, 73.4, 73.4, 70.9, 70.8, 70.8, 69.5, 69.3, 69.2, 68.5, 65.8, 64.9, 62.8, 37.5, 29.6, 27.8,

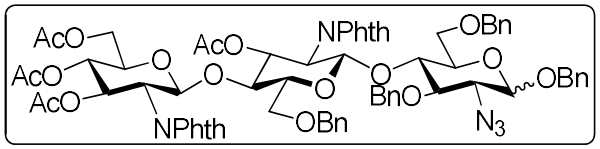
27.7. LCMS (m/z): $[M+H]^+$ calcd for $C_{32}H_{35}N_3O_7$ 574.26, found 574.40 Anal. calcd. for $C_{32}H_{35}N_3O_7$: C, 67.00; H, 6.15; N, 7.33, found C, 67.13; H, 6.08; N, 7.42.

Preparation of benzyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-α/β-D-glucopyranoside 36



The compound **36** was prepared from **34** (0.35 g, 0.61 mmol) by using literature procedure.[27] Colourless oil; Yield = 0.251 g (87%); R_f = 0.60 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38-7.27 (m, 2.7H), 4.95 (d, *J* = 3.6 Hz, 1H), 4.92-4.86 (m, 2.7H), 4.80 (d, *J* = 11.1 Hz, 0.7H), 4.74 (d, *J* = 11.2 Hz, 1.3H), 4.67 (t, *J* = 12.1 Hz, 1.5H), 4.60-4.50 (m, 5.5H), 4.32 (d, *J* = 8.1 Hz, 1H), 3.89 (dd, *J* = 10.1, 8.6 Hz, 0.7H), 3.73-3.58 (m, 3H), 3.63-3.59 (m, 1.7H), 3.43 (dd, *J* = 9.9, 8.1 Hz, 1H), 3.38-3.33 (m, 1H), 3.30 (dd, *J* =10.2, 3.6 Hz, 0.7H), 3.21 (t, *J* = 8.9 Hz, 1H), 2.83 (br s, 1H), 2.74 (br s, 0.6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 140.8, 138.0, 137.6, 136.6, 128.5, 128.4, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4, 126.8, 100.4, 96.8, 82.5, 79.8, 75.0, 74.1, 73.6, 73.5, 71.9, 71.6, 70.8, 70.3, 69.9, 69.5, 65.6, 65.1, 62.7. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{27}H_{29}N_3O_5$ 498.2005, found 498.2060.

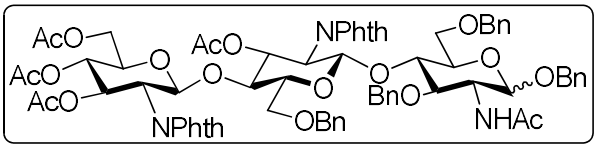
Preparation of benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α/β-D-glucopyranoside 39



The compound **39** was prepared from **37** (0.50 g, 0.51 mmol) and **36** (0.208 g, 0.42 mmol) by using general procedure for glycosylation with thioglycosides. Colourless solid; Yield = 0.43 g (77%); R_f = 0.34 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92-7.69 (m, 12H), 7.35-7.03 (m, 22H), 5.72-5.64 (m, 1.7H), 5.58 (t, *J* = 9.4 Hz, 1H), 5.48-5.35 (m, 2.5H), 5.27 (t, *J* = 6.0 Hz, 0.4H), 5.10 (q, *J* = 8.5 Hz, 1H), 4.98 (d, *J* = 11.1 Hz, 0.3H), 4.87-4.68 (m, 3H), 4.56-4.34 (m, 8H), 4.15 (q, *J* = 7.9 Hz, 5H), 4.04-3.98 (m, 1H), 3.92 (d, *J* = 12.9 Hz, 1H), 3.84 (t, *J* = 9.2 Hz, 0.5H), 3.51 (t, *J* = 11.5 Hz, 2H), 3.43-3.34 (m, 2.7H), 3.28-3.22 (m, 1.8H), 3.13 (d, *J* = 9.6 Hz, 0.6H), 3.07-3.04 (m, 1H), 2.03 (s, 4H), 1.98 (s, 3.6H), 1.91 (s, 4H), 1.81 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.4, 170.0, 169.9, 169.3, 168.1, 167.5, 138.3, 138.2, 138.0, 136.8, 136.6, 134.3, 134.0, 133.6, 131.3, 129.9, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 123.5, 123.4, 100.2, 97.0, 96.7, 96.5, 81.0, 77.9, 74.6, 74.3, 74.0, 73.4, 72.6, 72.5, 72.2, 71.5, 71.4, 70.9, 70.6, 70.1, 69.4, 68.2, 67.6, 66.8, 65.8, 62.7, 61.3, 60.3, 55.6, 54.9, 54.8, 29.6, 20.5, 20.4, 20.4, 20.3. HRMS-ESI (m/z):

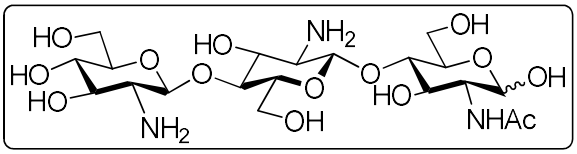
$[M+K]^+$ calcd for $C_{70}H_{69}N_5O_{21}$ 1354.4122, found 1354.4124. Anal. calcd. for $C_{70}H_{69}N_5O_{21}$: C, 63.87; H, 5.28; N, 5.32, found C, 63.97; H, 5.21; N, 5.26.

Preparation of benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α/β-D-glucopyranoside 40



The compound **40** was prepared from **39** (210 mg, 0.16 mmol) using general procedure for reductive acetylation of azide (Chapter 2). Colourless solid; Yield = 160 mg (74%); R_f = 0.30 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (dd, *J* = 5.3, 3.1 Hz, 2H), 7.82 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.78 (dd, *J* = 5.5, 3.2 Hz, 2H), 7.70 (dd, *J* = 5.5, 3.2 Hz, 2H), 7.34-7.07 (m, 25H), 5.71-5.63 (m, 2H), 5.59 (dd, *J* = 10.4, 8.9 Hz, 0.5H), 5.54 (d, *J* = 8.3 Hz, 1H), 5.42 (d, *J* = 8.4 Hz, 1H), 5.31 (d, *J* = 8.3 Hz, 1H), 5.13 (d, *J* = 11.3 Hz, 0.6H), 5.09 (d, *J* = 9.9 Hz, 1H), 4.88 (d, *J* = 12.6 Hz, 0.3H), 4.81 (d, *J* = 3.8 Hz, 0.4H), 4.73 (d, *J* = 12.1 Hz, 1H), 4.69 (d, *J* = 12.1 Hz, 1H), 4.59 (d, *J* = 12.0 Hz, 1H), 4.55 (d, *J* = 6.6 Hz, 1H), 4.52 (t, *J* = 5.0 Hz, 1H), 4.49 (d, *J* = 5.8 Hz, 1H), 4.39-4.29 (m, 5H), 4.21-4.15 (m, 4H), 4.03 (t, *J* = 7.0 Hz, 1H), 3.94-3.91 (m, 1.3H), 3.78 (t, *J* = 7.3 Hz, 1H), 3.66 (q, *J* = 7.4 Hz, 1H), 3.59 (dd, *J* = 10.6, 3.7 Hz, 1H), 3.50-3.42 (m, 3.5H), 3.36-3.34 (m, 2H), 3.21 (dd, *J* = 11.2, 2.7 Hz, 1H), 3.05 (d, *J* = 9.8 Hz, 1H), 2.03 (s, 3.7H), 1.98 (s, 4H), 1.92 (s, 4H), 1.82 (s, 6.5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.1, 169.9, 169.8, 169.3, 167.9, 167.7, 139.0, 138.5, 138.3, 138.2, 138.1, 137.4, 134.4, 131.3, 128.9, 128.3, 128.2, 128.2, 128.1, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 127.0, 125.2, 123.5, 99.1, 96.8, 96.7, 96.5, 96.4, 77.6, 74.5, 74.2, 74.0, 73.7, 73.5, 73.1, 72.6, 72.4, 71.5, 70.7, 70.6, 70.4, 70.3, 69.4, 68.6, 68.3, 67.9, 67.1, 61.3, 55.4, 54.8, 53.9, 5, 29.6, 23.3, 20.6, 20.5, 20.4, 20.3. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{72}H_{73}N_3O_{22}$ 1354.4583, found 1354.4584.

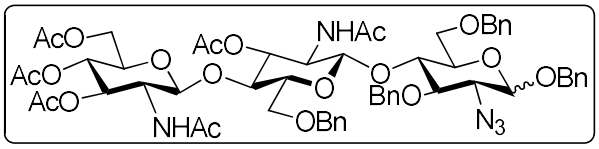
Preparation of 2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose 41



The compound **41** is prepared from **40** (41 mg, 0.033 mmol) by general procedure for general procedure for one pot *O*-deacetylation and phthalimide deprotection followed by debenzylation (Chapter 2). Colourless solid; Yield = 12.7 mg (75%); ^{1}H NMR (400 MHz,

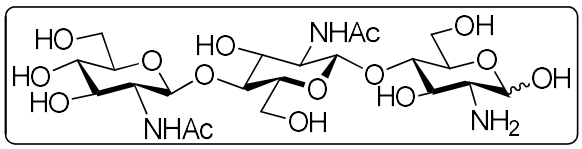
D_2O): δ 4.91-4.86 (m, 0.4H), 4.63-4.52 (s, 3H), 4.17-4.16 (m, 1H), 3.95-3.44 (m, 16H), 3.13-3.10 (m, 2.6H), 2.78 (d, *J* = 4.7 Hz, 1.8H), 1.95 (s, 3.8H). MALDI-MS (m/z): $[M+H]^+$ (positive mode, DHB/MeOH matrix); 544.24.

Preparation of benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α/β-D-glucopyranoside 42



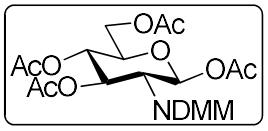
The compound **42** was prepared from **39** (184 mg, 0.14 mmol) by general procedure for deprotection of phthalimide protecting group followed by acetylation (Chapter 2). Colourless solid; Yield = 139 mg (87%); R_f = 0.34 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45-7.28 (m, 24H), 5.99 (d, *J* = 9.6 Hz, 0.2H), 5.39-5.28 (m, 1H), 5.17 (d, *J* = 11.4 Hz, 0.3H), 5.06 (t, *J* = 7.9 Hz, 1.3H), 4.96 (d, *J* = 9.6 Hz, 1H), 4.93-4.85 (m, 3H), 4.79 (d, *J* = 12.0 Hz, 1H), 4.73 (d, *J* = 12.0 Hz, 1H), 4.69-4.64 (m, 1.4H), 4.57 (dd, *J* = 12.2, 2.2 Hz, 1H), 4.53 (d, *J* = 11.9 Hz, 1H), 4.46 (d, *J* = 5.4 Hz, 1H), 4.34-4.29 (m, 4H), 4.27 (d, *J* = 8.0 Hz, 1H), 4.15-4.09 (m, 1.8H), 3.99 -3.76 (m, 6H), 3.65 (br s, 2H), 3.52-3.21 (m, 7.3H), 2.05 (s, 4H), 2.01-1.98 (m, 12H), 1.75 (s, 3.6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.6, 170.5, 170.4, 169.5, 169.8, 169.2, 141.3, 138.7, 137.5, 137.4, 137.3, 137.1, 136.6, 128.8, 128.7, 128.5, 128.4, 128.3, 128.0, 127.8, 127.7, 127.6, 127.4, 127.2, 127.2, 127.1, 101.1, 100.3, 100.0, 99.9, 96.7, 81.1, 78.0, 74.8, 74.6, 74.3, 74.2, 74.0, 73.6, 73.3, 73.2, 72.4, 71.3, 70.8, 70.2, 69.6, 68.1, 67.5, 65.9, 54.3, 54.0, 29.5, 22.9, 20.5, 20.4. LCMS (m/z): $[M+H]^+$ calcd for $C_{58}H_{69}N_5O_{19}$ 1140.47, found 1140. 64. Anal. calcd. for $C_{58}H_{69}N_5O_{19}$: C, 61.10; H, 6.10; N, 6.14, found C, 61.26; H, 6.05; N, 6.23.

Preparation of 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-D-glucopyranose 43



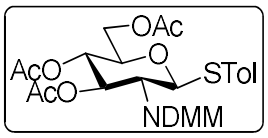
The compound **43** is prepared **42** (41 mg, 0.036 mmol) by general procedure for one pot deacetylation followed by debenzylation (Chapter 2). Colourless solid; Yield = 16 mg (78%); ^{1}H NMR (400 MHz, D_2O): δ 5.40 (s, 0.2H), 5.16 (s, 0.3H), 4.54-4.52 (m, 3H), 3.89-3.45 (m, 22H), 3.24 (s, 0.6H), 3.10-2.97 (m, 1.8H), 2.03 (s, 4H), 1.90 (s, 4H). MALDI-MS (m/z): $[M+Na]^+$ (positive mode, DHB/MeOH matrix); 608.21.

Preparation of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-dimethylmalemido-β-D-glucopyranose 17



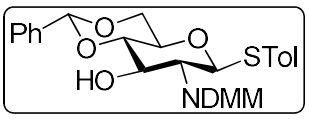
The compound **17** was prepared from **14** (4.51 g, 20.92 mmol) by following the literature procedure.[31] Gummy liquid; Yield = 6.2 g (65%); R_f = 0.44 in 1:1 EtOAc/hexanes ; 1H NMR (400 MHz , CDCl3); δ 6.25 (d, *J* = 8.9 Hz, 1H), 5.60 (dd, *J* = 10.4, 9.2 Hz, 1H), 5.20 (dd, *J* = 10.0, 9.2 Hz, 1H), 4.54 (dd, *J* = 9 5, 4.4 Hz, 1H), 4.10 (dd, *J* = 10.4, 8.9 Hz, 1H), 4.09 (dd, *J* = 12.5, 2.5 Hz, 1H), 3.95-3.93 (m, 1H), 2.10 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.96 (s, 3H), 1.82 (s, 6H). ^{13}C NMR (100 MHz, CDCl3): δ 171.9, 170.8, 169.8, 169.3, 168.8, 138.9, 90.8, 72.5, 70.8, 68.3, 63.5, 54.5, 20.8, 20.7, 20.5, 20.4, 8.8.

Preparation of *p*-methylphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-dimethylmalemido-1-thio-β-D-glucopyranoside 20



The compound **20** was prepared from **17** (6.2 g, 13.16 mmol) by general procedure for synthesis of thioglycosides (Chapter 2). Colourless solid; Yield = 5.06 g (74%); R_f = 0.60 in 1:1 EtOAc/hexanes ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31 (d, *J* = 8.0 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 2H), 5.60 (t, *J* = 10.1 Hz, 1H), 5.48 (d, *J* = 10.4 Hz, 1H), 5.05 (t, *J* = 9.6 Hz, 1H), 4.27-4.15 (m, 2H), 4.08 (t, *J* = 8.0 Hz, 1H), 3.83-3.78 (m, 1H), 2.34 (s, 3H), 2.09 (s, 3H), 2.00-1.97 (m, 9H), 1.89 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.4, 170.6, 170.1, 169.4, 138.6, 138.0, 137.0, 133.8, 129.6, 126.9, 83.0, 77.3, 77.0, 76.7, 75.7, 71.7, 68.6, 62.1, 53.4, 21.1, 20.7, 20.6, 20.4, 8.7.

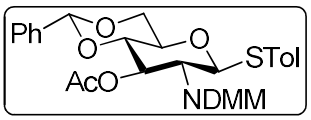
Preparation of *p*-methylphenyl 4,6-benzylidene-2-deoxy-2-dimethylmalemido-1-thio-β-D-glucopyranoside 23



The compound **23** was prepared from **20** (3 g, 5.77 mmol) by general procedure for *O*-deacetylation followed by benzylidene acetal protection (Chapter 2). Colourless foam; Yield = 2.14 g (77%); R_f = 0.50 in 1:1 EtOAc/hexanes ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47-7.44 (m, 2H), 7.36-7.34 (m, 3H), 7.28 (d, *J* = 8.0 Hz, 2H), 7.09 (d, *J* = 8.0 Hz, 2H), 5.51 (s, 1H), 5.43 (d, *J* = 10.4 Hz, 1H), 4.43 (t, *J* = 9.2 Hz, 1H), 4.33 (m, 1H), 4.05 (t, *J* = 10.4 Hz, 1H), 3.75 (t, *J* = 10.4 Hz, 1H), 3.60-3.54 (m, 1H), 3.50-3.46 (m, 1H), 2.61 (s, 1H), 2.33 (s, 3H), 1.96 (br s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.8, 171.2, 138.3, 137.4, 136.9, 133.2, 129.7, 129.3,

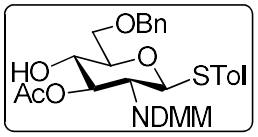
128.3, 127.9, 126.3, 101.8, 84.5, 81.8, 77.3, 77.0, 76.7, 70.1, 69.8, 68.5, 60.3, 55.4, 21.1, 14.1, 8.7. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{26}H_{27}NO_6S$ 504.1457, found 504.1456.

Preparation of *p*-methylphenyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-dimethylmalemido-1-thio-β-D-glucopyranoside 26



The compound **26** was prepared from **23** (1.03 g, 2.08 mmol) by general procedure for acetylation at 3rd position. Colourless foam; Yield = 0.98 g (90%); R_f = 0.53 in 1:1 EtOAc/hexanes ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41-7.40 (m, 2H), 7.32-7.28 (m, 5H), 7.10 (d, *J* = 8.0 Hz, 2H), 5.70 (t, *J* = 9.6 Hz, 1H), 5.60 (d, *J* = 10.4 Hz, 1H), 5.48 (s, 1H), 4.38-4.34 (m, 1H), 4.09 (t, *J* = 10.4 Hz, 1H), 3.79-3.62 (m, 3H), 2.32 (s, 3H), 1.95 (s, 6H), 1.90 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.2, 170.7, 169.9, 138.5, 138.0, 136.8, 133.5, 129.6, 128.9, 128.1, 127.2, 126.1, 101.4, 83.8, 78.7, 77.3, 77.2, 77.0, 76.7, 70.6, 70.3, 68.4, 54.0, 21.0, 20.4, 8.7. LCMS (m/z): $[M+Na]^+$ calcd for $C_{28}H_{29}NO_7S$ 546.16, found 546.50. Anal. calcd. for $C_{28}H_{29}NO_7S$: C, 64.23; H, 5.58; N, 2.68, found C, 64.13; H, 5.52; N, 2.61.

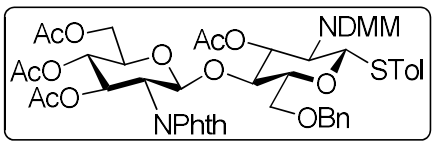
Preparation of *p*-methylphenyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-dimethylmalemido-1-thio-β-D-glucopyranoside 29



The compound **29** was prepared from **26** (0.91 g, 1.74 mmol) by using general procedure for regioselective benzylidene ring opening (Chapter 2). Colourless solid; Yield = 0.7 g (77%); R_f = 0.50 in 1:1 EtOAc/hexanes ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39-7.27 (m, 7H), 7.02 (d, *J* = 7.6 Hz, 2H), 5.57 (d, *J* = 10.4 Hz, 1H), 5.57 (d, *J* = 10.4 Hz, 1H), 5.51 (t, *J* = 8.4 Hz, 1H), 4.59-4.52 (m, 2H), 4.03 (t, *J* = 10.4 Hz, 1H), 3.84-3.75 (m, 2H), 3.68-3.64 (m, 2H), 3.25 (br s, 1H), 2.28 (s, 3H), 1.93-1.90 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.3, 170.9, 170.8, 137.9, 137.8, 136.7, 133.1, 129.4, 128.2, 127.6, 127.5, 82.9, 78.6, 77.3, 77.0, 76.7, 74.3, 73.4, 70.2, 69.7, 53.4, 20.9, 20.5, 8.7, 8.5. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{28}H_{31}NO_7S$ 548.1719, found 548.1712.

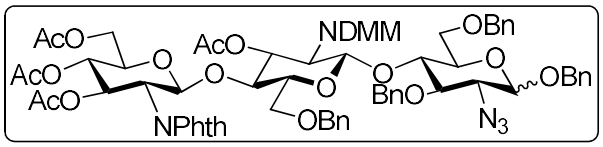
Preparation of *p*-methylphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside 38

The compound **38** was prepared from **16** (0.54 g, 0.92 mmol) and **29** (0.41 g, 0.77 mmol) by using general procedure for the chemoselective glycosylation with trichloroacetimidate



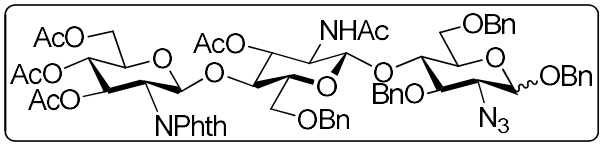
donor. Colourless foam; Yield = 0.52 g (72%); R_f = 0.47 in 1:1 EtOAc/hexanes ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.75 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.34-7.24 (m, 8H), 6.98 (d, *J* = 8.1 Hz, 1H), 5.71 (dd, *J* = 10.6, 9.1 Hz, 1H), 5.51 (t, *J* = 9.5 Hz, 1H), 5.43 (d, *J* = 8.3 Hz, 1H), 5.36 (d, *J* = 10.6 Hz, 1H), 5.15-5.05 (m, 1H), 4.51-4.46 (m, 1H), 4.40 (t, *J* = 3.6 Hz, 1H), 4.36 (d, *J* = 8.1, 4.2 Hz, 1H), 4.19 (dd, *J* = 10.6, 8.3 Hz, 1H), 4.11 (d, *J* = 7.1 Hz, 1H), 4.04 (d, *J* = 9.4 Hz, 1H), 3.96-3.91 (m, 1H), 3.57-3.54 (m, 2H), 3.45 (m, 2H), 2.26 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H), 1.93 (s, 3H), 1.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.1, 169.9, 169.4, 138.4, 138.3, 134.3, 133.8, 131.3, 129.5, 128.2, 127.8, 127.4, 127.2, 123.7, 96.8, 82.9, 78.4, 73.7, 72.7, 72.0, 71.6, 70.6, 68.4, 67.8, 61.4, 54.8, 53.8, 21.1, 20.7, 20.6, 8.9, 8.6. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{48}H_{50}N_2O_{16}S$ 965.2779, found 965.2781.

Preparation of benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α/β-D-glucopyranoside 44



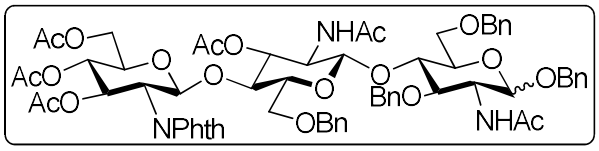
The compound **44** was prepared using **38** (0.39 g, 0.41 mmol) and **36** (0.170 g, 0.34 mmol) by using general procedure for the glycosylation with thioglycoside donor. Colourless liquid; Yield = 0.33 g (74%); R_f = 0.40 in 1:1 EtOAc/hexanes ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90-7.83 (m, 3H), 7.79-7.72 (m, 3H), 7.32-7.25 (m, 18H), 7.10 (t, *J* = 7.35 Hz, 4H), 5.66 (t, *J* = 9.7 Hz, 1H), 5.40 (d, *J* = 8.8 Hz, 2H), 5.26 (d, *J* = 8.4 Hz, 1H), 5.12-5.06 (m, 1.6H), 4.97-4.90 (m, 0.8H), 4.84 (dd, *J* = 12.3, 2.6 Hz, 1.5H), 4.77-4.65 (m, 1.8H), 4.60 (d, *J* = 12.3 Hz, 1H), 4.54-4.44 (m, 4.8H), 4.38-4.31 (m, 3H), 4.20- 4.06 (m, 4H), 3.99 (t, *J* = 9.1 Hz, 1.3H), 3. 93-3.83 (m, 3H), 3.59-3.35 (m, 5.7H), 3.29-3.18 (m, 2.8H), 3.04 (d, *J* = 9.7 Hz, 1H), 2.03 (s, 3.8H), 1.98 (s, 7H), 1.87 (br s, 8H), 1.81 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1, 170.3, 169.9, 169.8, 169.2, 167.5, 148.0, 138.3, 138.1, 138.0, 137.0, 136.6, 134.3, 133.4, 131.2, 129.9, 128.2, 128.1, 127.9, 127.9, 127.7, 127.6, 127.5, 127.3, 127.1, 127.1, 127.0, 123.5, 100.1, 97.1, 96.7, 96.6, 96.3, 80.9, 77.7, 74.5, 74.4, 74.3, 74.0, 73.9, 73.4, 73.2, 72.7, 72.6, 72.5, 72.1, 71.4, 71.3, 71.1, 71.0, 70.9, 70.8, 70.5, 70.1, 69.4, 68.3, 68.2, 67.8, 67.5, 66.8, 66.7, 65.7, 62.7, 61.3, 61.2, 55.4, 54.7, 52.6, 29.5, 20.5, 20.4, 20.2, 8.5. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{68}H_{71}N_5O_{21}$ 1316.4539, found 1316.4543.

Preparation of benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside 45



The compound **45** was prepared from **44** (0.28 g, 0.21 mmol) by general procedure for conversion of *N*-DMM to *N*-acetyl group. Colourless oil; Yield = 0.196 g (76%); R_f = 0.27 in 1:1 EtOAc/hexanes ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.77 (dd, *J* = 5.3, 3.0 Hz, 2H), 7.36-7.11 (m, 20H), 5.71 (t, *J* = 8.9 Hz, 1H), 5.34 (d, *J* = 8.4 Hz, 1H), 5.11 (t, *J* = 9.3 Hz, 1H), 5.04 (dd, *J* = 11.2, 8.9 Hz, 1H), 4.95-4.88 (m, 2H), 4.77 (dd, *J* = 11.0, 8.1 Hz, 1H), 4.72-4.54 (m, 3H), 4.43-4.29 (m, 3H), 4.27-4.11 (m, 4H), 4.06-3.80 (m, 4.7H), 3.67-3.55 (m, 2.4H), 3.42-3.34 (m, 3H), 3.27 (dd, *J* = 12.0, 8.8 Hz, 1H), 3.20-3.15 (m, 1H), 3.00 (d, *J* = 9.5 Hz, 1H), 2.07 (s, 7H), 2.01 (s, 4H), 1.84 (s, 3H), 1.75 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.6, 170.4, 170.0, 169.9, 169.8, 169.4, 167.2, 138.5, 138.4, 138.3, 138.2, 138.1, 137.3, 137.1, 137.0, 136.6, 136.5, 134.4, 131.2, 128.8, 128.7, 128.6, 128.3, 128.2, 128.1, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 127.3, 127.2, 127.1, 127.0, 126.9, 123.5, 100.6, 100.4, 99.5, 96.9, 96.8, 96.7, 80.9, 77.9, 77.2, 76.5, 74.8, 74.2, 74.1, 73.7, 73.4, 72.9, 72.2, 71.4, 70.8, 70.4, 69.6, 68.2, 67.6, 67.6, 65.9, 62.8, 61.2, 60.3, 54.8, 54.1, 29.6, 23.1, 20.6, 20.5, 20.3. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{64}H_{69}N_5O_{20}$ 1250.4434, found 1250.4439.

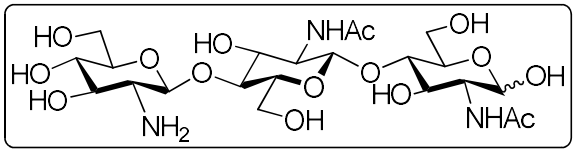
Preparation of benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-α/β-D-glucopyranoside 46



The compound **46** was prepared from **45** (152 mg, 0.124 mmol) using general procedure for reductive acetylation of azide to *N*-acetyl group (Chapter 2): Colourless oil; Yield = 114 mg (74%); R_f = 0.27 in 1:1 EtOAc/hexanes ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (d, *J* = 5.5, 3.1 Hz, 3H), 7.75 (dd, *J* = 5.5, 3.1 Hz, 3H), 7.38-7.16 (m, 24H), 5.76-5.71 (m, 1.5H), 5.60 (t, *J* = 7.7 Hz, 1H), 5.41 (dt, *J* = 13.4, 8.3 Hz, 1.5H), 5.14-5.09 (m, 2H), 4.88-4.77 (m, 3.6H), 4.70 (d, *J* = 12.1 Hz, 1H), 4.60-4.54 (m, 2H), 4.49 (d, *J* = 12.2 Hz, 2H), 4.46-4.30 (m, 6.5H), 4.21 (dd, *J* = 8.0, 2.5 Hz, 1.8H), 4.15 (dd, *J* = 12.4, 3.9 Hz, 1.7H), 4.06 (t, *J* = 9.3 Hz, 2H), 4.00-3.89 (m, 4H), 3.71 (d, *J* = 5.4 Hz, 1H), 3.66 (t, *J* = 5.0 Hz, 1H), 3.63-3.59 (m, 1.6H), 3.53-3.39 (m, 4.7H), 3.03-3.21

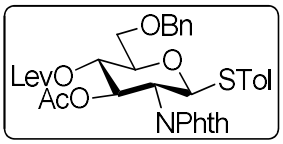
(m, 1.7H), 3.08-3.01 (m, 1H), 2.32 (s, 3H), 2.06 (s, 7H), 2.00 (s, 8H), 1.83 (s, 4.4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.2, 170.7, 170.6, 170.3, 170.2, 169.9, 169.8, 169.6, 169.3, 139.4, 138.4, 138.1, 137.9, 137.7, 137.5, 137.0, 134.4, 134.3, 131.2, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.5, 127.4, 127.3, 127.3, 127.2, 123.5, 100.6, 99.9, 99.7, 99.6, 97.0, 96.8, 77.7, 77.2, 74.3, 74.2, 73.9, 73.8, 73.6, 73.5, 73.3, 72.03, 72.7, 72.6, 72.4, 72.2, 71.5, 70.4, 70.0, 69.6, 69.5, 68.4, 67.4, 61.4, 54.8, 54.0, 53.6, 52.2, 50.5, 29.5, 22.5, 20.6, 20.5, 20.4, 20.2. LCMS (m/z): $[M+Na]^+$ calcd for $C_{66}H_{73}N_3O_{21}$ 1266.46, found 1266.42. Anal. calcd. for $C_{66}H_{73}N_3O_{21}$: C, 63.71; H, 5.91; N, 3.38, found C, 63.85; H, 5.81; N, 3.45.

Preparation of 2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose 47



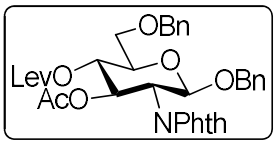
The compound **47** is prepared from **46** (41 mg, 0.032 mmol) by general procedure for one pot deacetylation and dephthaloylation followed by debenzylation. A colourless solid; Yield = 13.8 mg (77%); ^{1}H NMR (400 MHz, D_2O): δ 5.15 (s, 0.2H), 4.59 (s, 3H), 4.15-4.05 (m, 1.5H), 3.87-3.46 (m, 10H), 3.51-3.42 (m, 3.8H), 3.03-3.00 (m, 1.5H), 2.89-2.82 (m, 1H), 2.02 (s, 4H), 1.90 (s, 4H). MALDI-MS (m/z): $[M+H]^+$ (positive mode, DHB/MeOH matrix); 586.27.

Preparation of *p*-methylphenyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-levulinoyl-2-phthalimido-1-thi-β-D-glucopyranoside 31



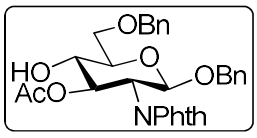
The compound **31** was prepared from **28** (720 mg, 1.31 mmol) by using literature procedure.[26] Colourless liquid; Yield = 693 mg (82%); R_f = 0.50 in 1:1 EtOAc/hexanes ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.74 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.35-7.30 (m, 7H), 7.00 (d, *J* = 8.1 Hz, 2H), 5.79 (t, *J* = 9.8 Hz, 1H), 5.68 (d, *J* = 10.5 Hz, 1H), 5.14 (t, *J* = 10.0 Hz, 1H), 4.54 (s, 2H), 3.33 (t, *J* = 10.4 Hz, 3.89-3.85 (m, 1H), 3.69-3.61 (m, 1H), 2.64 (q, *J* = 7.1 Hz, 2H), 2.40 (q, *J* = 7.0 Hz, 2H), 2.28 (s, 3H), 2.11 (s, 3H), 1.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 205.9, 171.2, 170.3, 167.7, 138.7, 137.9, 134.3, 134.1, 133.7, 133.4, 131.6, 131.1, 129.6, 128.2, 127.7, 127.5, 127.3, 123.5, 83.0, 73.4, 72.7, 71.4, 69.5, 69.1, 53.7, 37.5, 29.5, 27.7, 21.0, 20.3. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{35}H_{35}NO_9S$ 668.1930, found 668.1932.

Preparation of benzyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-levulinoyl-2-phthalimido-β-D-glucopyranoside 33



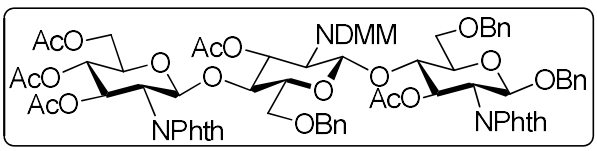
The compound **33** was prepared from **31** (600 mg, 0.93 mmol) by using general procedure for the glycosylation with thioglycoside donor. Gummy liquid; Yield = 443 mg (76%); R_f = 0.47 in 1:1 EtOAc/hexanes ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79-7.70 (m, 4H), 7.36-7.28 (m, 5H), 7.11-7.08 (m, 5H), 5.79 (t, *J* = 9.9 Hz, 1H), 5.38 (d, *J* = 8.4 Hz, 1H), 5.18 (t, *J* = 9.7 Hz, 1H), 4.85 (d, *J* = 12.2 Hz, 1H), 4.61-4.52 (m, 3H), 4.36 (dd, *J* = 10.6, 8.4 Hz, 1H), 3.87-3.82 (m, 1H), 3.70-3.62 (m, 2H), 2.64 (q, *J* = 6.3 Hz, 2H), 2.40 (q, *J* = 6.7 Hz, 2H), 2.12 (s, 3H), 1.89 (s, 3H), 1.61 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.1, 171.3, 170.4, 138.0, 136.8, 134.1, 131.4, 128.3, 128.2, 127.8, 127.7, 127.6, 123.5, 97.1, 73.6, 73.3, 71.1, 70.5, 69.9, 69.0, 54.7, 37.6, 29.6, 27.8, 20.5. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{35}H_{35}NO_{10}$ 652.2159, found 652.2161.

Preparation of benzyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside 35



The compound **35** was prepared from **33** (429 mg, 0.68 mmol) by using literature procedure.[27] Colourless oil; Yield = 334 mg (92%); R_f = 0.55 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (br s, 2H), 7.68 (dd, *J* = 5.3, 2.8 Hz, 2H), 7.38-7.29 (m, 5H), 7.10-7.04 (m, 5H), 5.67 (dd, *J* = 10.7, 8.4 Hz, 1H), 5.40 (d, *J* = 8.5 Hz, 1H), 4.84 (d, *J* = 12.2 Hz, 1H), 4.64 (q, *J* = 12.1 Hz, 2H), 4.54 (d, *J* = 12.2 Hz, 1H), 4.30 (dd, *J* = 10.7, 8.5 Hz, 1H), 3.86-3.74 (m, 4H), 3.19 (s, 1H), 1.90 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.2, 167.6, 137.7, 136.9, 134.0, 131.4, 128.4, 128.1, 127.7, 127.6, 127.5, 123.4, 97.2, 74.3, 73.6, 73.4, 71.1, 71.1, 70.0, 54.6, 20.6. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{30}H_{29}NO_8$ 554.1791, found 554.1793.

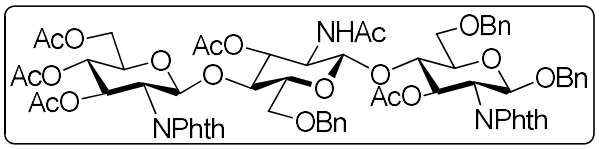
Preparation of benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2- phthalimido-β-D-glucopyranoside 48



The compound **48** was prepared from **38** (0.55 g, 0.58 mmol) and **35** (257 mg, 0.48 mmol) by using general procedure for the glycosylation with thioglycoside donor. Colourless solid; Yield = 0.51 g (78%); R_f = 0.37

in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (dd, *J* = 5.2, 3.2 Hz, 2H), 7.76 (dd, *J* = 5.3, 3.0 Hz, 2H), 7.71-7.65 (m, 4H), 7.36-7.24 (m, 10H), 7.11-7.02 (m, 5H), 5.65-5.59 (m, 2H), 5.36 (d, *J* = 7.0 Hz, 1H), 5.33 (d, *J* = 4.5 Hz, 1H), 5.25 (d, *J* = 8.7 Hz, 1H), 5.12 (d, *J* = 8.4 Hz, 1H), 5.06 (t, *J* = 9.5 Hz, 1H), 4.76 (d, *J* = 12.2 Hz, 1H), 4.55 (q, *J* = 12.1 Hz, 2H), 4.48 (d, *J* = 11.9 Hz, 1H), 4.42 (d, *J* = 9.2 Hz, 1H), 4.32 (dd, *J* = 12.5, 3.7 Hz, 1H), 4.22 (dd, *J* = 10.3, 8.7 Hz, 1H), 4.16-4.03 (m, 4H), 3.87 (d, *J* = 11.3 Hz, 1H), 3.78 (dd, *J* = 10.4, 8.6 Hz, 1H), 3.64 (d, *J* = 10.6 Hz, 1H), 3.57-3.44 (m, 4H), 3.30 (d, *J* = 11.1 Hz, 1H), 2.02 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.90 (br s, 6H), 1.79 (s, 3H), 1.74 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1, 170.3, 170.0, 169.9, 169.8, 169.2, 167.4, 148.4, 138.2, 138.0, 137.4, 136.8, 134.3, 133.9, 131.2, 128.1, 127.9, 127.8, 127.4, 127.3, 127.1, 127.0, 123.5, 123.2, 105.1, 97.0, 96.2, 96.2, 77.2, 76.5, 74.2, 73.5, 73.0, 72.7, 72.6, 72.1, 71.1,7, 71.3, 70.8, 70.6, 70.5, 68.1, 67.7, 67.3, 61.1, 60.2, 54.9, 54.7, 54.7, 20.5, 20.4, 20.3, 20.2, 8.7. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{71}H_{71}N_3O_{24}$ 1350.4506, found 1350.4563. Anal. calcd. for $C_{71}H_{71}N_3O_{24}$: C, 63.15; H, 5.30; N, 3.11, found C, 63.26; H, 5.23; N, 3.18.

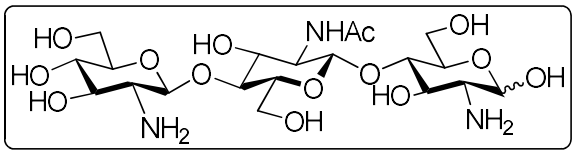
Preparation of benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-phthalimido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside 49



The compound **49** was prepared from **48** (210 mg, 0.15 mmol) by general procedure for conversion of *N*-DMM to *N*-acetyl group. Colourless oil; Yield = 148 mg (77%); R_f = 0.30 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (dd, *J* = 5.2, 3.1 Hz, 2H), 7.78 (dd, *J* = 5.2, 3.1 Hz, 2H), 7.72-7.67 (m, 4H), 7.37-7.26 (m, 10H), 7.09-7.05 (m, 5H), 5.69 (t, *J* = 10.0 Hz, 1H), 5.62 (t, *J* = 10.2 Hz, 1H), 5.31 (dd, *J* = 12.4, 8.4 Hz, 2H), 5.08 (t, *J* = 9.7 Hz, 1H), 5.01 (d, *J* = 9.2 Hz, 1H), 4.85-4.77 (m, 3H), 4.48 (dd, *J* = 12.3, 4.4 Hz, 2H), 4.41 (d, *J* = 12.1 Hz, 1H), 4.33 (d, *J* = 11.5 Hz, 2H), 4.29 (d, *J* = 8.4 Hz, 1H), 4.25-4.17 (m, 2H), 4.01 (t, *J* = 9.3 Hz, 1H), 3.91-3.86 (m, 2H), 3.69-3.64 (m, 3H), 3.60 (d, *J* = 9.9 Hz, 1H), 3.53 (d, *J* = 10.4 Hz, 1H), 3.32 (t, *J* = 11.0 Hz, 2H), 3.17 (d, *J* = 8.3 Hz, 1H), 2.05 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.83 (s, 3H), 1.75 (s, 3H), 1.72 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.4, 170.2, 170.0, 169.8, 169.4, 167.5, 137.9, 137.6, 136.9, 134.5, 134.0, 131.2, 128.6, 128.5, 128.3, 128.1, 127.6, 127.5, 127.5, 127.1, 123.6, 123.2, 114.8, 100.0, 97.3, 96.4, 75.1, 74.1, 73.7, 73.5, 72.8, 72.6, 72.2, 71.4, 71.1, 70.5, 70.5, 68.2, 67.9, 67.3, 61.2, 60.3, 54.8, 54.7, 54.3, 29.6, 23.1, 20.9, 20.6, 20.5, 19.5. LCMS (m/z): $[M+H]^+$ calcd for $C_{67}H_{69}N_3O_{23}$ 1284.44,

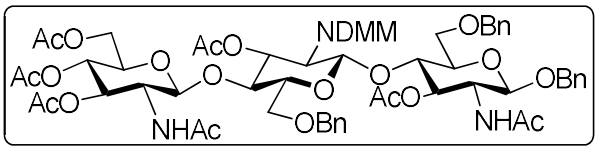
found 1284.72. Anal. calcd. for $C_{67}H_{69}N_3O_{23}$: C, 62.66; H, 5.42; N, 3.27, found C, 62.52; H, 5.48; N, 3.32.

Preparation of 2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-D-glucopyranose 50



The compound **50** is prepared from **49** (40 mg, 0.05 mmol) by general procedure for one pot deacetylation and dephthaloylation followed by debenzylation (Chapter 2). Colourless solid; Yield = 17.6 mg (72%); ^{1}H NMR (400 MHz, D_2O): δ 5.93 (s, 0.2H), 4.60-4.58 (m, 2H), 3.91-3.04 (m, 20H), 2.75-2.68 (m, 1.2H), 1.90 (s, 4H). MALDI-MS (m/z): $[M+H]^+$ (positive mode, DHB/MeOH matrix); 544.24.

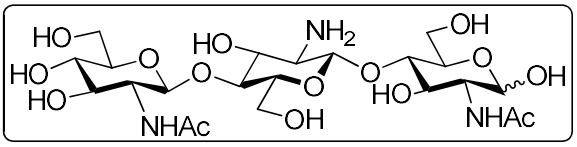
Benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside 51



The compound **51** was prepared from **48** (189 mg, 0.14 mmol) by general procedure for deprotection of phthalimide protecting group followed by acetylation (Chapter 2). Colourless solid; Yield = 146 mg (91%); R_f = 0.70 in 1:30 EtOAc/$CHCl_3$; ^{1}H NMR (400 MHz, $CDCl_3$+CD_3OD): δ 7.45-7.29 (m, 15H), 5.11 (t, *J* = 10.3 Hz, 1H), 4.98-4.93 (m, 2H), 4.87 (d, *J* = 12.2 Hz, 1H), 4.73 (d, *J* = 11.8 Hz, 1H), 4.66 (d, *J* = 11.6 Hz, 1H), 4.60 (d, *J* = 12.3 Hz, 2H), 4.56-4.41 (m, 4H), 4.34 (dd, *J* = 12.6, 3.8 Hz, 1H), 3.95-3.88 (d, *J* = 9.1 Hz, 4H), 3.76-3.68 (m, 5H), 3.62-3.59 (m, 1H), 3.49 (d, *J* = 9.1 Hz, 1H), 3.39-3.29 (m, 4H), 2.05 (s, 3H), 2.01-1.99 (m, 12H), 1.91 (s, 6H), 1.82 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$+CD_3OD): δ 171.2, 171.1, 170.9, 170.8, 170.7, 170.6, 170.5, 169.5, 137.5, 137.4, 136.9, 133.9, 128.4, 128.3, 128.0, 127.9, 127.8, 127.4, 122.9, 100.2, 99.6, 99.2, 74.4, 73.8, 73.6, 73.4, 72.9, 72.8, 71.9, 71.0, 70.2, 68.1, 67.6, 67.3, 61.4, 54.4, 53.8, 53.1, 29.3, 22.3, 22.2, 20.3, 20.1, 20.0, 8.1. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{59}H_{71}N_3O_{22}$ 1196.4427, found 1196.4428.

Preparation of 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose 52

The compound **52** is prepared from **51** (43 mg, 0.037 mmol) by general procedure for one pot *O*-deacetylation and conversion of *N*-DMM to *N*-acetyl followed by *O*-debenzylation. Colourless solid; Yield = 15.8 mg (75%); ^{1}H NMR (400 MHz, D_2O): δ 5.16 (s, 0.5H), 4.62-4.58 (m, 3H), 3.87-3.48 (m, 18H), 2.04-2.01 (s, 6H). MALDI-MS (m/z): $[M+Na]^+$ (positive mode, DHB/MeOH matrix); 608.21.



3.4.2 Materials and methods related to endothelial cells

Cell culture

ECs from collagenase-digested human umbilical veins (HUVECs) were isolated and cultured to confluency at 37 ºC with 5% CO_2 in gelatin coated flasks containing M199 medium (Invitrogen, Darmstadt, Germany) supplemented with 10% heat inactivated fetal calf serum (FCS) (PAA laboratories GmbH, Pasching, Austria), 5 U/mL heparin (Biochrom, Berlin, Germany), 1% penicillin and streptomycin, (PAA Laboratories GmbH, Pasching, Austria) and 1% growth supplement derived from bovine retina as described previously.[44]

Wst-1 Assay

To measure the influence of COS on the viability of HUVECs, 15000 cells/well were seeded and further cultivated for 24 h in gelatine coated 96-well plates (Becton Dickinson GmbH, Heidelberg, Germany) containing 100 µL of growth medium. Cell viability was determined by adding 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium salt (Wst-1 reagent) (Roche, Mannheim, Germany) to the cells treated with different concentrations (2, 10 and 50 µg/mL) of dimers and partially *N*-acetylchitotrioses for 24 h. Cell viability is related to the cellular reduction of Wst-1 reagent by mitochondrial dehydrogenases into an intensely coloured and soluble formazan.[45] Adsorption was measured at a wavelength of 440 nm.

HUVEC treatment

HUVECs were seeded in gelatine coated cover slips and cultivated to confluence in M199 medium. For pre-treatment, 1 µg/mL of COS was added to the medium and cells were incubated overnight at 37 ºC and 5% CO_2. Where indicated, ECs were stimulated with 100 µM histamine (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) for 15 min.

Subsequently, cells were prepared for immunofluorescence staining, and the supernatants were used for enzyme linked immunosorbent assay (ELISA).

VWF ELISA

VWF ELISA was performed as described previously.[46] In brief, supernatants of the HUVECs were added for 1 h at 37 ºC to a 96-well plate pre-coated with polyclonal rabbit anti-human VWF antibody (DAKO, Hamburg, Germany) at a final concentration of 6 µg/mL in phosphate buffered saline (PBS, pH 7.4). After washing the wells with washing buffer (PBS, 0.1% Tween 20, pH 7.4), samples were incubated for 1 h at 37 ºC with polyclonal rabbit anti-human VWF antibody/HRP (1.1 mg/mL) (DAKO, Hamburg, Germany) at a final dilution of 1:500. The enzyme reaction was started upon addition of ABTS (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt; Roche Diagnostics, Mannheim, Germany). The reaction mixture was incubated for 30 min and the absorption was measured at 405 nm. Serially diluted human plasma (800 ng/mL stock concentrations) was used as a standard. Concentrations of VWF measured in the supernatants of HUVECs were expressed in ng/mL.

Immunofluorescence staining

HUVECs were fixed with 4% paraformaldehyde dissolved in PBS. Cells were washed and blocked with 2% bovine serum albumin (BSA) in incubation buffer (100 mM sodium phosphate buffer pH 7.4 containing 4% sucrose) for 1 h at room temperature. Immunofluorescence staining was performed with a rabbit anti-human VWF antibody (3 mg/mL) (DAKO, Hamburg, Germany) at a final dilution of 1:200 in incubation buffer containing 0.5% BSA overnight at 4 ºC. After washing, cells were incubated with FITC-conjugated goat anti-rabbit antibody (0.5 mg/mL) (Becton Dickinson GmbH, Heidelberg, Germany) at a final dilution of 1:400 in incubation buffer containing 0.5% BSA at room temperature for 1 h. Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI) (1 mg/mL) with a dilution of 1:10,000 in PBS for 10 min. Finally, cover slips were embedded with mowiol-glycol solution which was freshly supplemented with 50 mg/mL DABCO (1,4-diazabicyclo-[2.2.2]-octane; Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Fluorescence microscopy was performed using a 40x oil-immersion objective and appropriate fluorescence filters (Zeiss, Jena, Germany). To quantify the amount of VWF fiber attached to the endothelium, 10-20 fields of view per experiment were analyzed using ImageJ software.

Preparation of platelets

Platelets were prepared as described previously.[47] In brief, blood was drawn on sodium citrate coated tubes (Sarstedt, Nuembrecht, Germany) and centrifuged at 120 x g for 15 min at room temperature to obtain platelet rich plasma (PRP). PRP was supplemented with apyrase (75 microunits/mL; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and prostaglandin E1 (100 nM; Sigma-Aldrich Chemie GmbH, Steinheim, Germany), and centrifuged at 1200 x g for 15 min. The platelet pellet was resuspended in wash buffer (36 mM citric acid, 5 mM glucose, 5 mM KCl, 1 mM $MgCl_2$, 103 mM NaCl, 2 mM $CaCl_2$, 75 microunits/mL apyrase, 100 nM PGE1, 3.5 mg/mL bovine serum albumin, pH 6.5) containing calcein red (5 µM) (life technologies, Darmstadt, Germany) at a final dilution of 1:1000 and incubated for 30 min in the dark at room temperature. Subsequently, platelets were centrifuged for 12 min at 1200 x g and washed twice with wash buffer. Finally, the pellet was resuspended in Hepes/Tyrode's buffer (137 mM NaCl, 2 mM KCl, 3 mM NaH_2PO_4, 1 mM $MgCl_2$, 5.5 mM glucose, 5 mM Hepes, 12 mM $NaHCO_3$, pH 7.4).

Platelet adhesion to HUVECs under continuous-unidirectional flow conditions

Setup and usage of the applied microfluidic device (IBIDI GmbH, Munich, Germany) has been described previously.[43] In brief, HUVECs (1 x 10^7 cells/cm²) were grown on gelatine coated µ-slide 0.2 Luer (IBIDI GmbH, Munich, Germany) for 48 h under slight flow (1 dyne/cm²). Cells were pre-treated with 1 µg/mL of COS (mixtures of DP2 and DP3) supplemented in EGM2 medium (Lonza, Basel, and Switzerland) over night. The slide was then connected to the fluidic unit and the perfusion set was filled with HEPES-buffered Ringer solution (10 mM HEPES, 5 mM glucose, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl, 140 mM NaCl) containing 100 µM histamine, 25% washed erythrocytes and fluorescent labelled platelets ($4x10^6$ cells/mL). Perfusion was performed with a shear stress of 6 dyne/cm^2 for 10 min. Calcein red labelled platelets were excited at a wavelength of 555 nm, allowing the visualisation of platelet decorated VWF strings in real time using fluorescence microscopy with 40 X objective (Zeiss, Jena, Germany). Adhesion was quantified at 10-20 fields of view for each microfluidic slide and analysed using ImageJ software.

3.5 References

1. Ong, S. Y.; Woochhala, S. M.; Tan, M. H.; Lu, J. *Biomaterials* **2008**, *29*, 4323.

2. Millner, R. W.; Lockhart, A. S.; Bird, H.; Alexiou, C. *Ann. Thorac. Surg*. **2009**, *87*, 13.

3. Zakhem, E.; Raghavan, S.; Gilmont, R. R.; Bitar, K. N. *Biomaterials* **2012**, *33*, 4810.

4. Lubben, I. M. V; Verhoef, J. C.; Borchard, G.; Junginger, H. E. *Eur. J. Pharm. Sci*. **2001**, *14*, 201.

5. Prabaharan, M.; Mano, J. F. *Drug Deliv*. **2005**, *12*, 41.

6. Malette, W. G.; Quigley, H. J.; Gaines, R. D.; Johnson, N. D.; Rainer, W. G. *Ann. Thorac. Surg*. **1983**, *36*, 55.

7. Ueno, H.; Yamada, H.; Tanaka, I.; Kaba, N.; Matsuura, M.; Okumura, M. *Biomaterials* **1999**, *20*, 1407.

8. Sperling, C.; Fischer, M.; Maitz, M. F.; Werner, C. *Biomaterials* **2009**, *30*, 4447.

9. Vogler, E. A.; Siedlecki, C. A. *Biomaterials* **2009**, *30*, 1857.

10. Janvikul, W.; Uppanan, P.; Thavornyutikarn, B.; Krewraing, J.; Prateepasen, R.; *J. Appl. Polym. Sci*. **2006**, *102*, 445.

11. Lee, K.Y.; Ha, W.S.; Park, W. H. *Biomaterials* **1995**, *16*, 1211.

12. Thatte, H. S.; Zagarins, S.; Khuri, S. F.; Fischer, T. H. *J. Trauma*. **2004**, *57*, S13.

13. He, Q.; Gong, K.; Ao, Q.; Ma, T.; Yan, Y.; Gong, Y.; *J. Biomater. Appl*. **2013**, *27*, 1032.

14. Weinbaum, S.; Tarbell, J. M.; Damiano, E. R. *Annu. Rev. Biomed. Eng*. **2007**, *9*, 121.

15. Vander, P.; Vårum, K. M.; Domard, A.; Gueddari, N. E. E.; Moerschbacher, B. M. *Plant Physiol.* **1998**, *118*, 1353.

16. Akiyama, K.; Kawazu, K.; Kobayashi, A. *Carbohydr. Res.* **1995**, *279*, 151.

17. Anantharam, V.; Patanjali, S. R.; Swamy, M. J.; Sanadi, A. R.; Goldstein, I. J.; Surolia, A. *J. Biol. Chem.* **1986**, *261*, 14621.

18. Nordtveit, R. J.; Vårum, K. M.; Smidsrod, O. *Carbohydr. Polym.***1996**, *29*, 163.

19. Kristiansen, A.; Vårum, K. M.; Grasdalen, H. *Biochim. Biophys. Acta.* **1998**, *1425*, 137.

20. Singh, S.; Gallagher, R.; Derrick, P. J.; Crout, D. H. G. *Tetrahedron: Asymmetry* **1995**, *6*, 2803.

21. Kanie, O.; Ito, Y.; Ogawa, T. *J. Am. Chem. Soc*. **1994**, *116*, 12073.

22. Lamberth, C.; Nagy, J. O.; Kasper, C.; Bednarski, M. D. *J. Carbohydr. Chem*. **1994**, *13*, 819.

23. Sarkar, S.; Roy, N. *J. Carbohydr. Chem.* **2000**, *22*, 285.

24. Shih, H.; Chen, K.; Cheng, T.; Wong, C.; Cheng, W. *Org. Lett*. **2011**, *13*, 4600.

25. Ngoje, G.; Li, Z. *Org. Biomol. Chem*. **2013**, *11*, 1879.

26. Ho, T. L.; Wong, C. M. *Synth. Commun.* **1975**, *5*, 91.

27. Melean, L. G.; Love, K. R.; Seeberger, P. H. *Carbohydr. Res.* **2002**, *337*, 1893.

28. Kanie, O.; Crawley, S. C.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **1993**, *243*, 139.

29. Sato, S.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1988**, *29*, 5267.

30. Debenham, J. S.; Maden, R.; Roberts, C.; Fraser-Reid, B. *J. Am. Chem. Soc*. **1995**, *117*, 3302.

31. Aly, M. R. E.; Castro-Palomino, J. C.; Ibrahim, E. S. I.; El-Ashry, E. S. H.; Schmidt, R. R. *Eur. J. Org. Chem.* **1998**, 2305.

32. Liew, S. -T.; Wei, A. *Carbohydr. Res*. **2002**, *337*, 1319.

33. Xia, J.; Xue, J.; Locke, R. D.; Chandrasekaran, E. V.; Srikrishnan, T.; Matta, K. L. *J. Org. Chem.* **2006**, *71*, 3696.

34. Zemplén, G. *Ber. Dtsch. Chem. Ges.***1927**, *60B*, 1555.

35. Donohoe, T. J.; Logan, J. G.; Laffan, D. D. P. *Org. Lett.* **2003**, *5*, 4995.

36. Nokami, T.; Isoda, Y.; Sasaki, N.; Takaiso, A.; Hayase, S.; Itoh, T.; Hayashi, R.; Shimizu, A.; Yoshida, J. *Org. Lett*. **2015**, *17*, 1525.

37. Ebong, E. E.; Macaluso, F. P.; Spray, D. C.; Tarbell, J. M. *Arterioscler. Thromb. Vasc. Biol.* **2011**, *31*, 1908.

38. Weinbaum, S.; Tarbell, J. M.; Damiano, E. R. *Annu. Rev. Biomed. Eng*. **2007**, *9*, 121.

39. Sorlier, P.; Denuziere, A.; Viton, C.; Domard, A. *Biomacromolecules* **2001**, *2*, 765.

40. Uygun, B. E.; Stojsih, S. E; Matthew, H. W. *Tissue Eng. Part A*. **2009**, *15*, 3499.

41. Chen, Y. L.; Chen, H. C.; Lee, H. P.; Chan, H. Y.; Hu, Y. C. *Biomaterials* **2006**, *27*, 2222.

42. Hamilton, K. K.; Sims, P. J. *J. Clin. Invest*. **1987**, *79*, 600.

43. Pappelbaum, K. I.; Gorzelanny, C.; Grassle, S.; Suckau, J.; Laschke, M. W.; Bischoff, M. *Circulation* **2013**.

44. Goerge, T.; Niemeyer, A.; Rogge, P.; Ossig, R.; Oberleithner, H.; Schneider, S. W. *J. Membr. Biol*. **2002**, *187*, 203.

45. Takamatsu, N. *Nihon Ronen Igakkai Zasshi*. **1998**, *35*, 535.

46. Desch, A.; Strozyk, E. A.; Bauer, A. T.; Huck, V.; Niemeyer, V.; Wieland, T. *Am. J Pathol*. **2012**, *181*, 693.

47. Ceunynck, K. D.; Rocha, S.; Feys, H. B.; Meyer, S. F. D.; Uji, H.; Deckmyn, H. *J. Biol. Chem*. **2011**, *286*, 36361.

3.6 Representative spectra

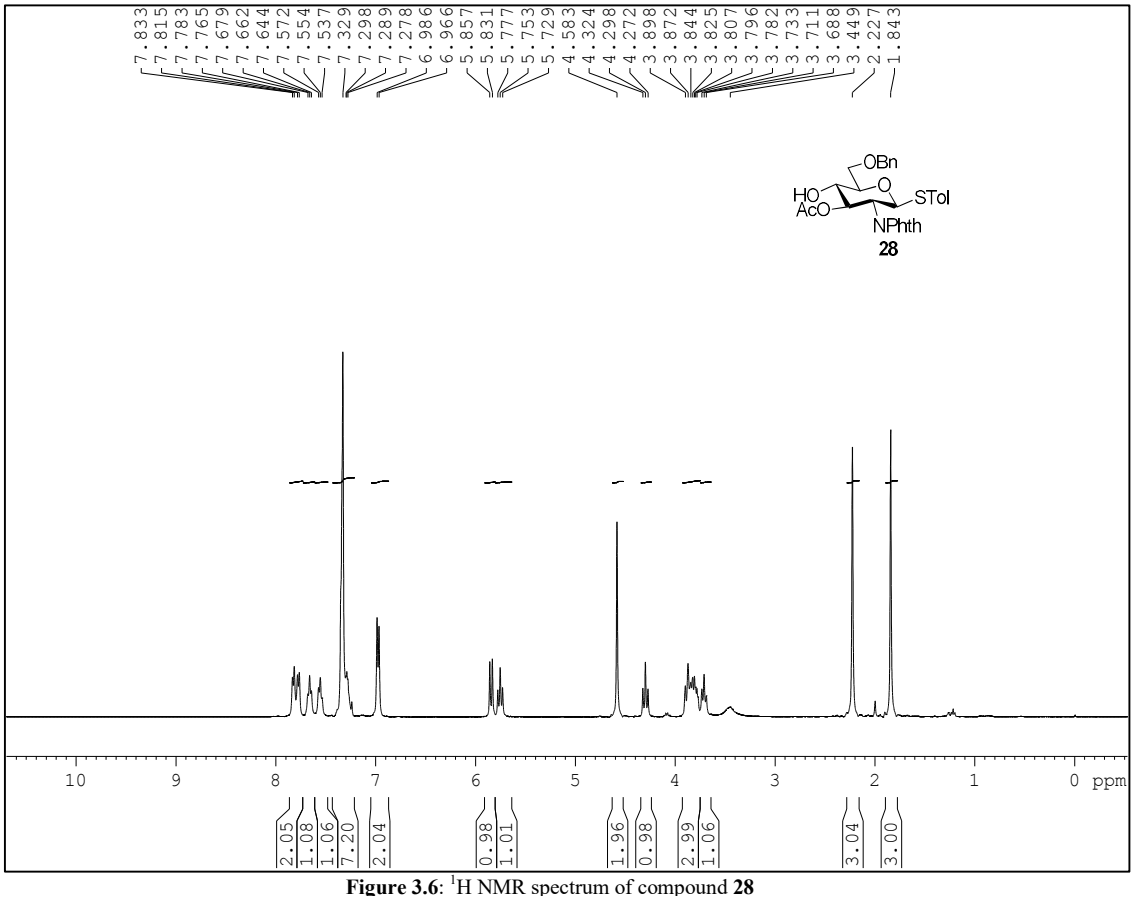


Figure 3.6: ^{1}H NMR spectrum of compound **28**

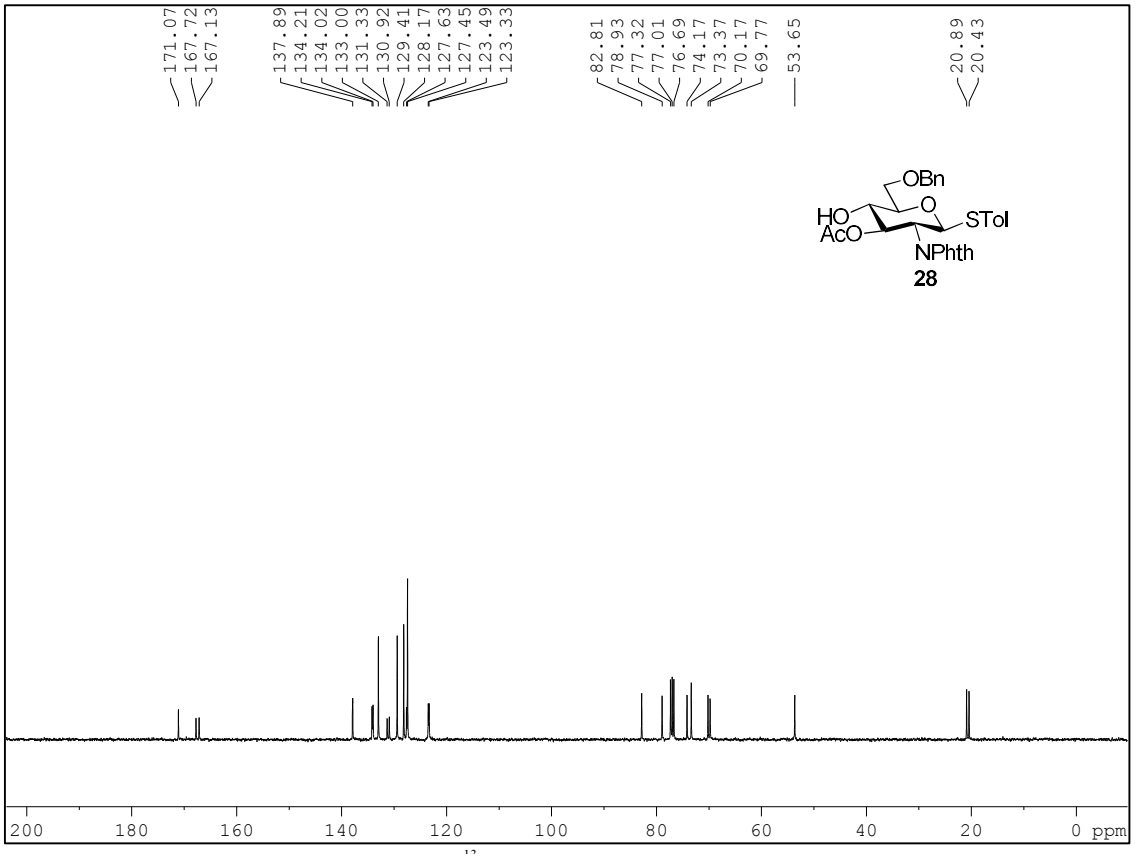


Figure 3.7: ^{13}C NMR spectrum of compound **28**

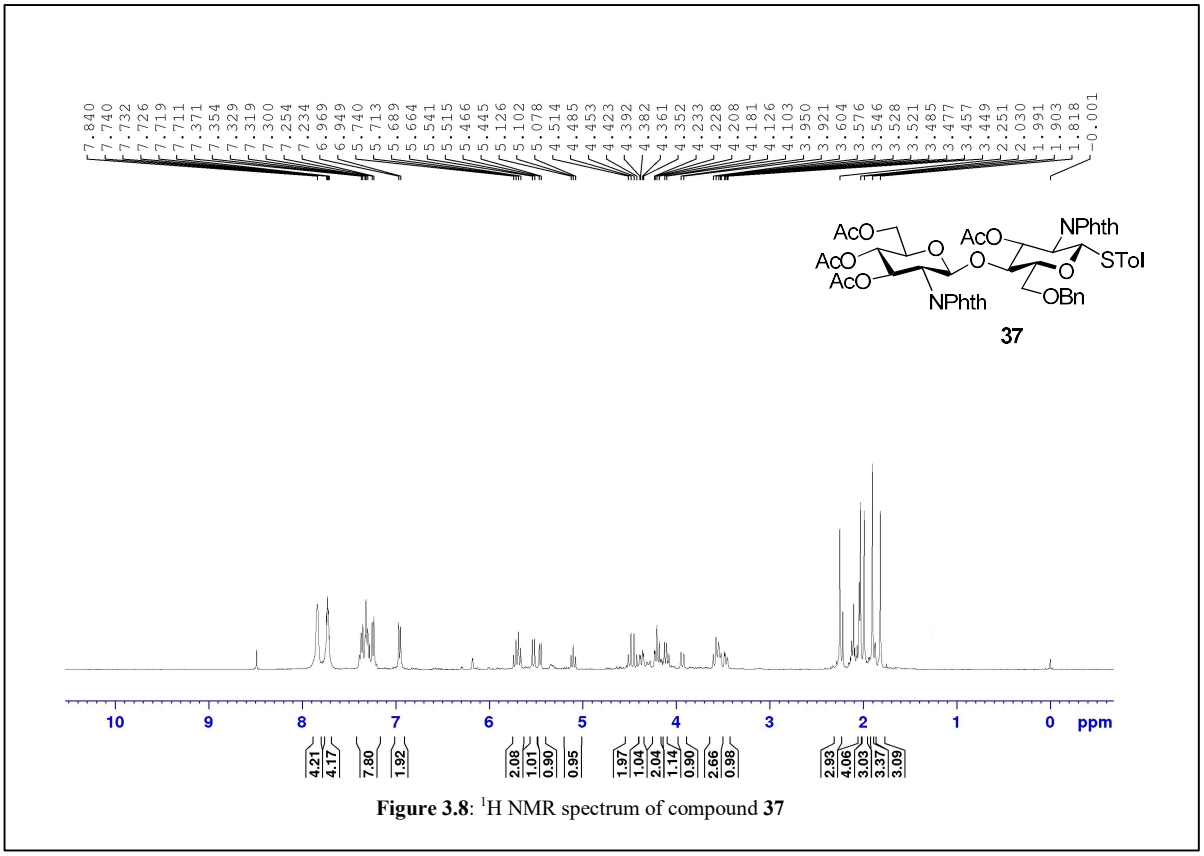


Figure 3.8: ^{1}H NMR spectrum of compound **37**

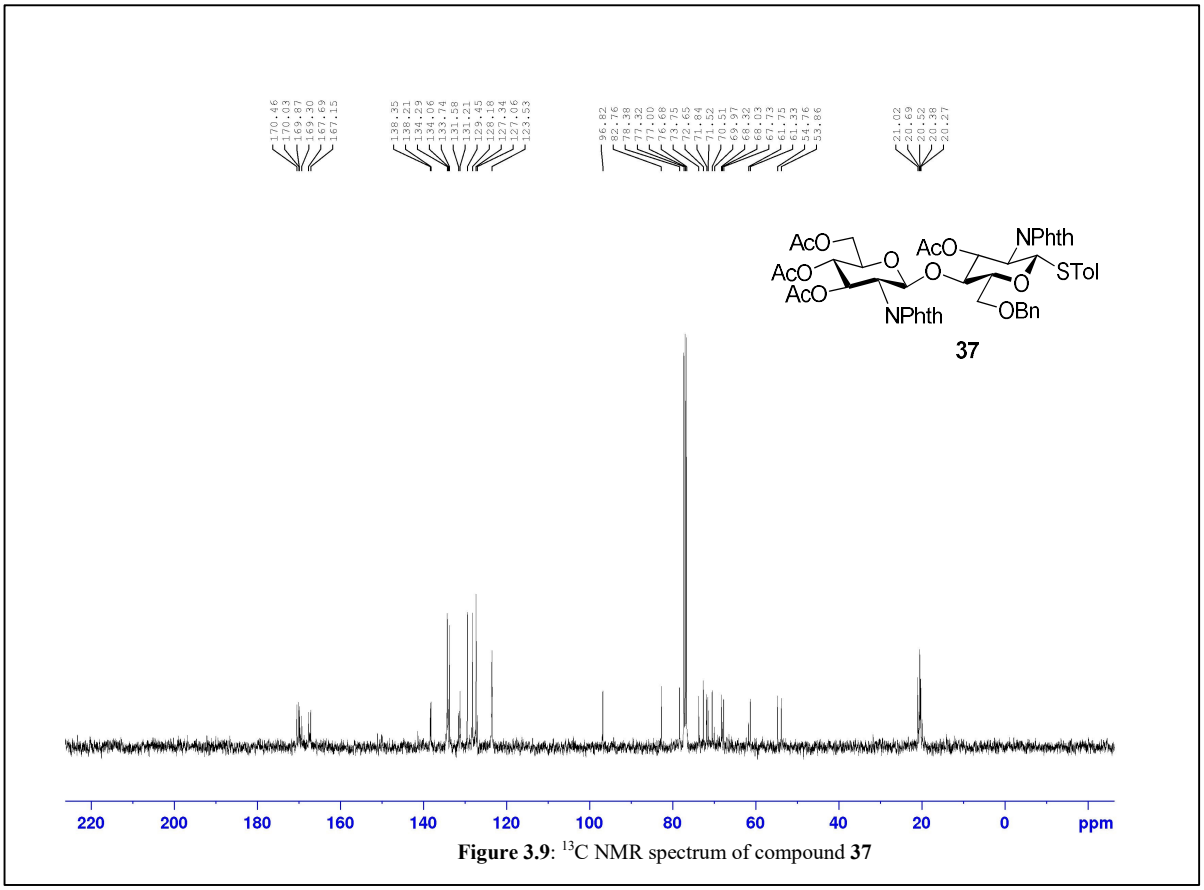


Figure 3.9: ^{13}C NMR spectrum of compound **37**

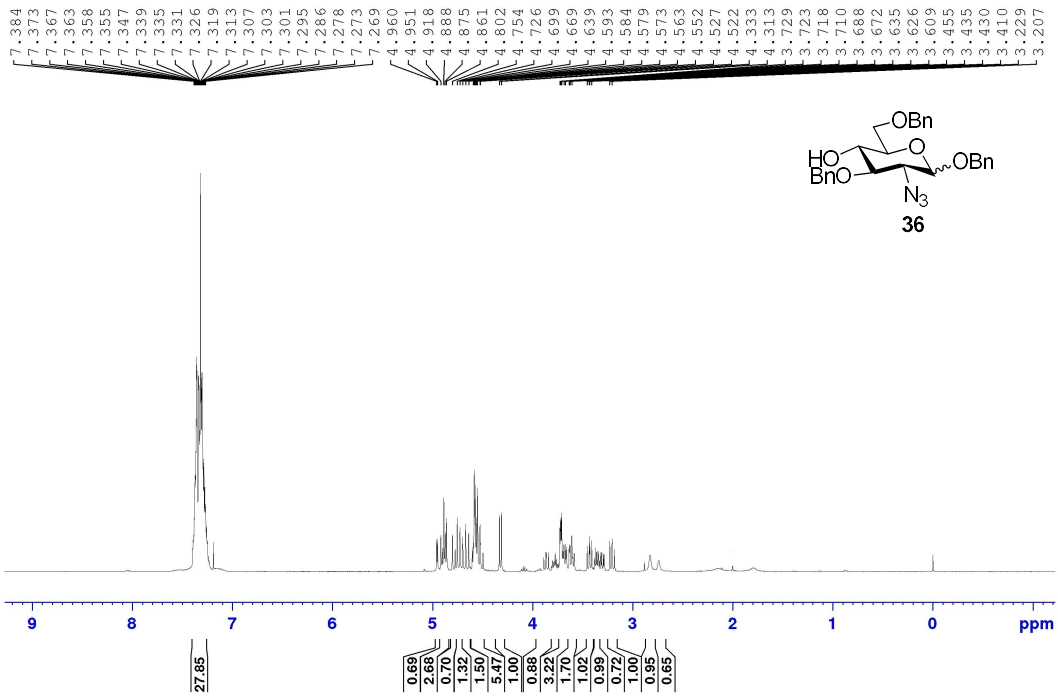


Figure 3.10: ^{1}H NMR spectrum of compound **36**

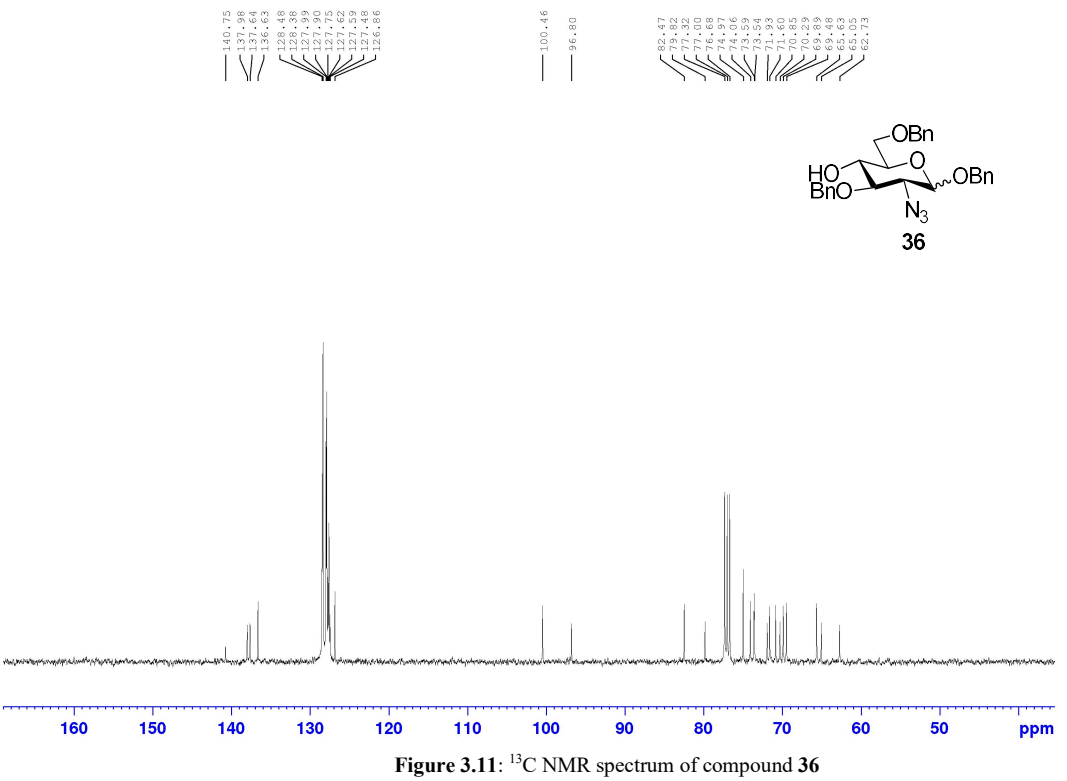


Figure 3.11: ^{13}C NMR spectrum of compound **36**

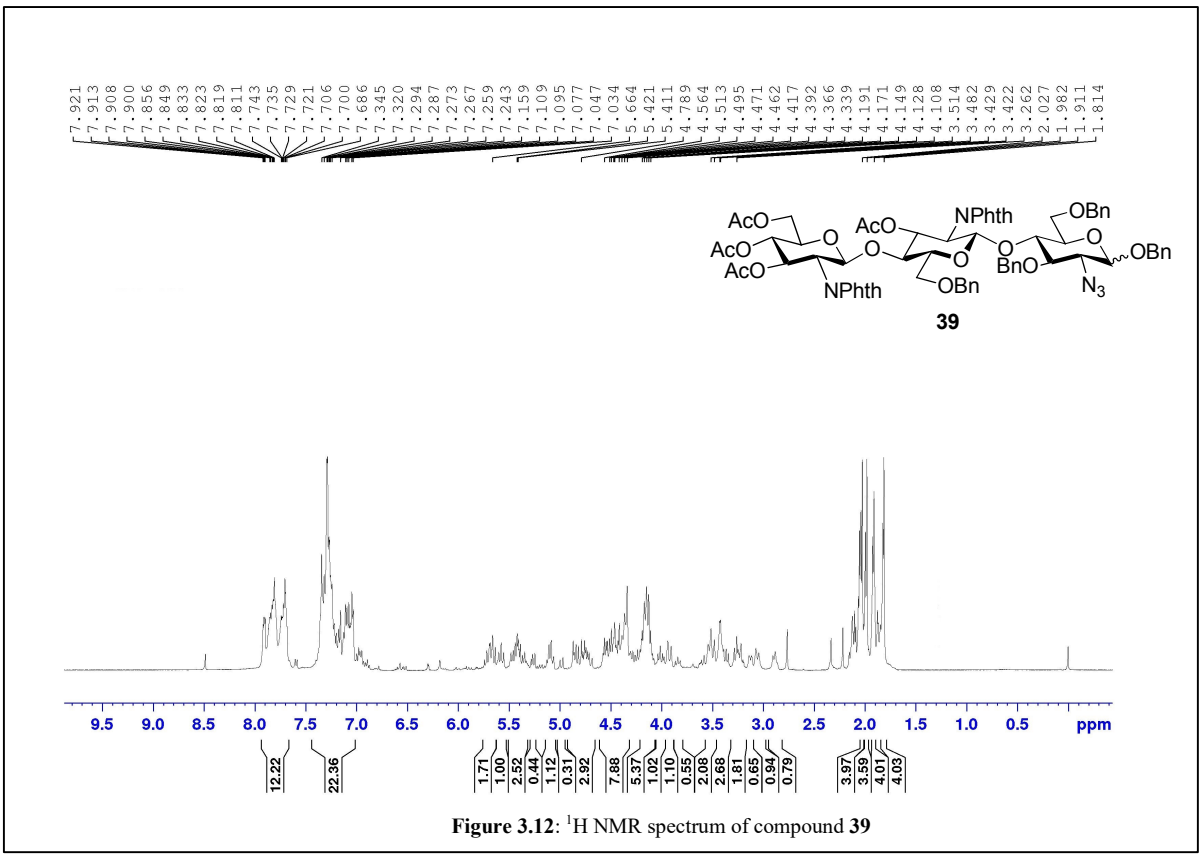


Figure 3.12: ^{1}H NMR spectrum of compound **39**

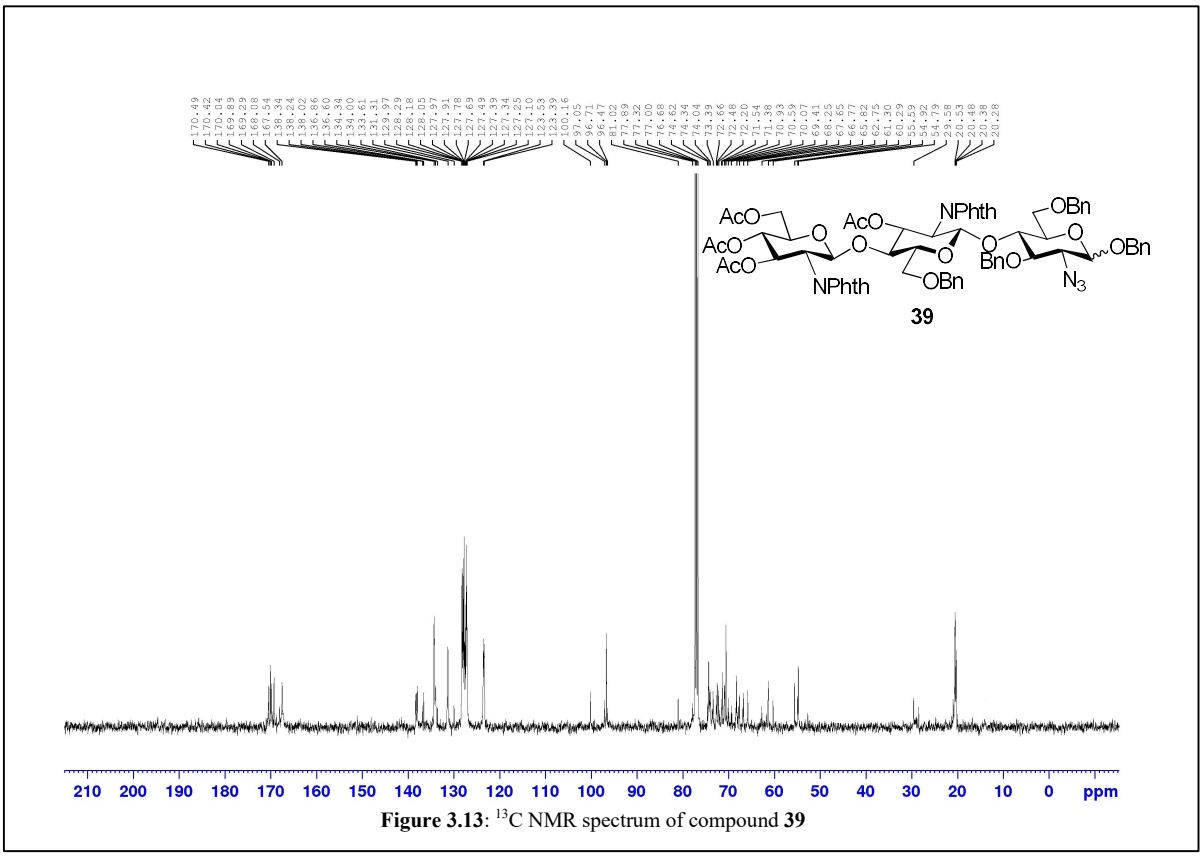


Figure 3.13: ^{13}C NMR spectrum of compound **39**

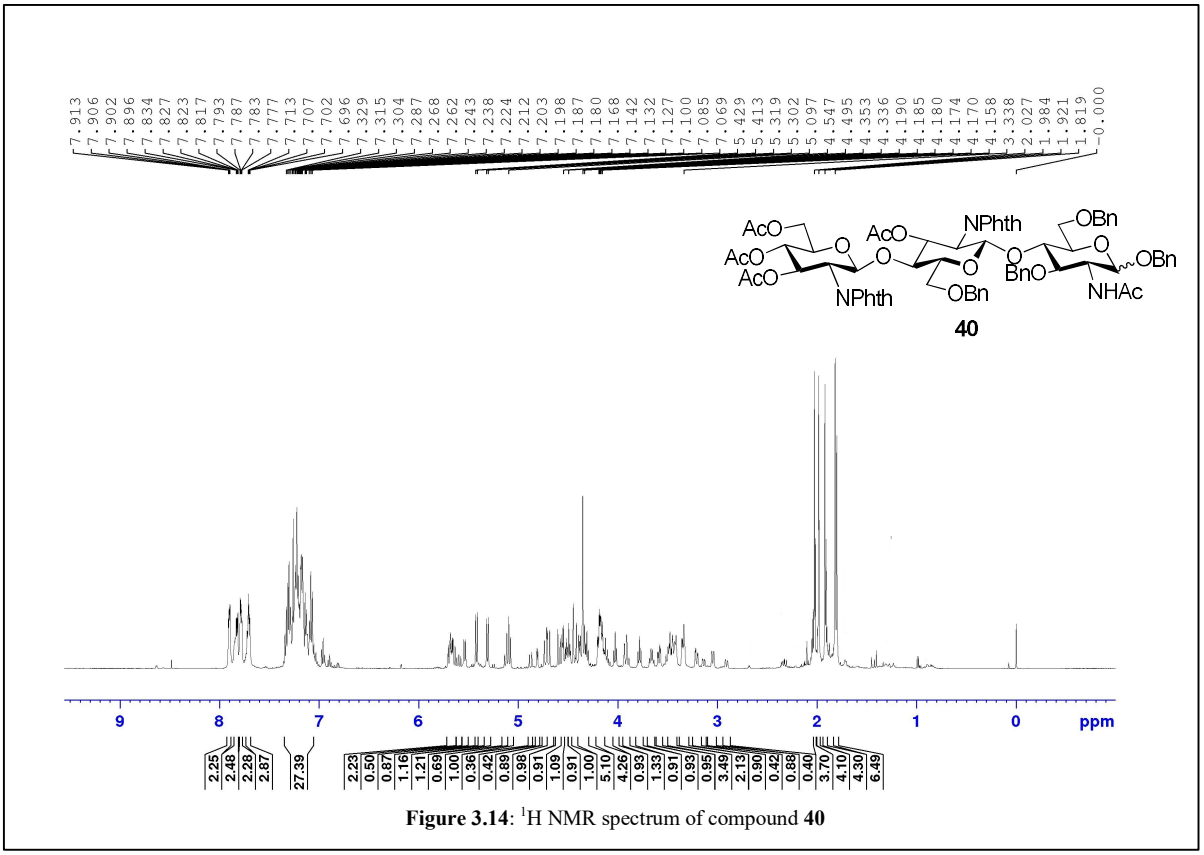


Figure 3.14: ^{1}H NMR spectrum of compound **40**

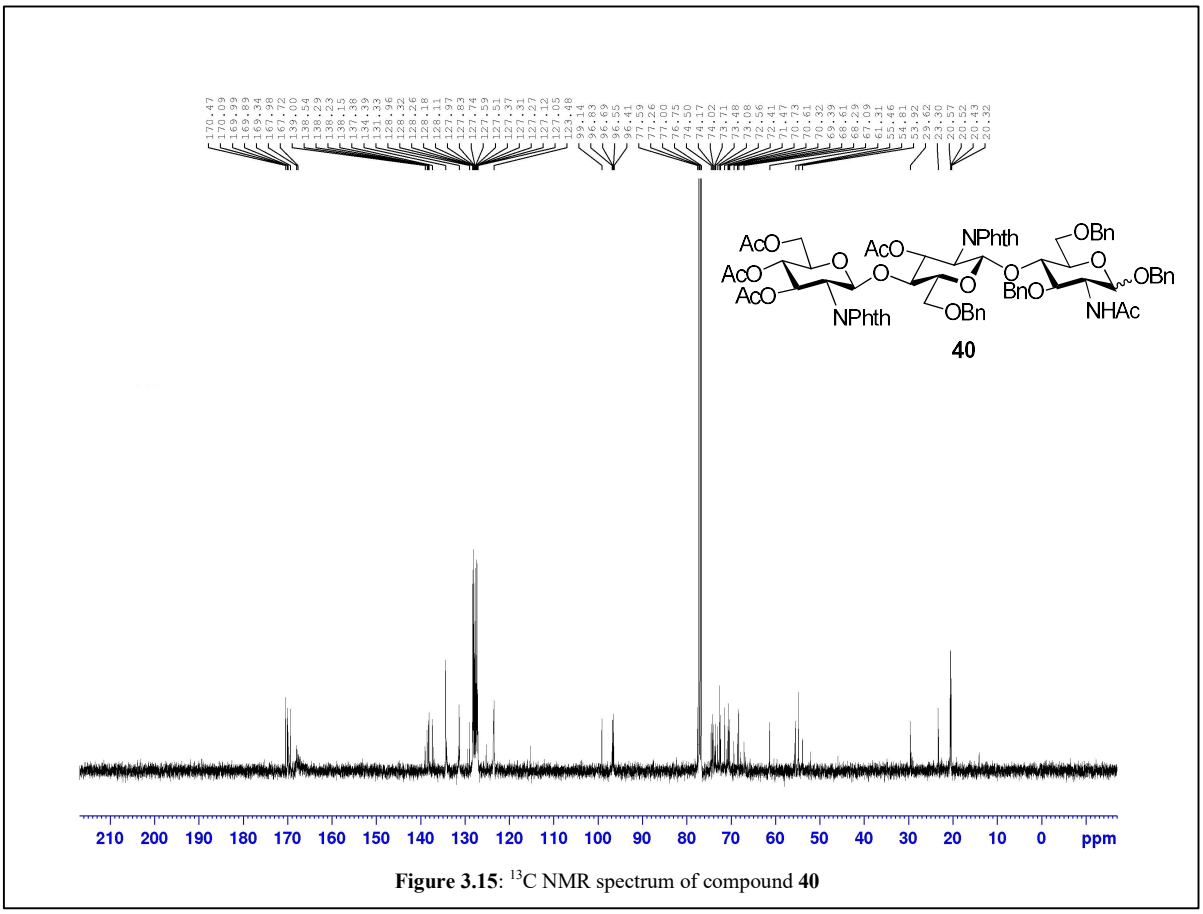


Figure 3.15: ^{13}C NMR spectrum of compound **40**

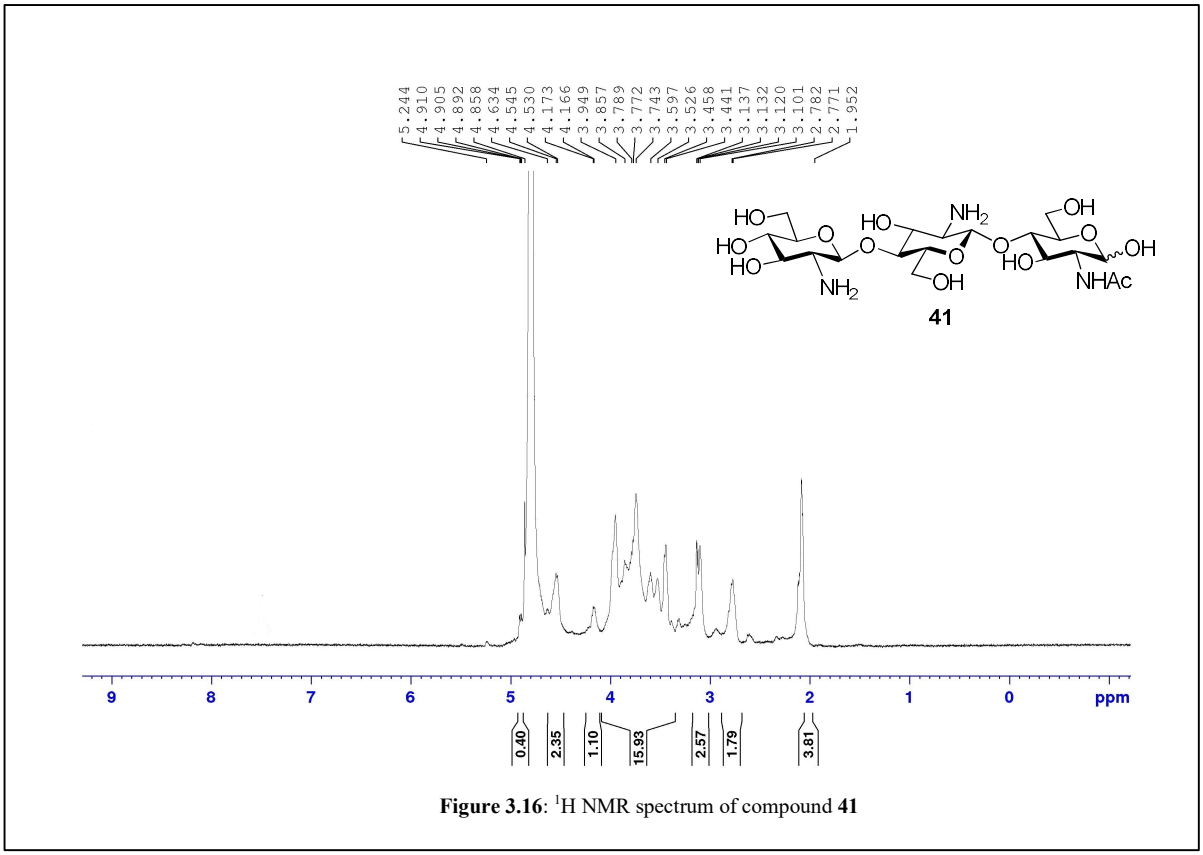


Figure 3.16: ^{1}H NMR spectrum of compound **41**

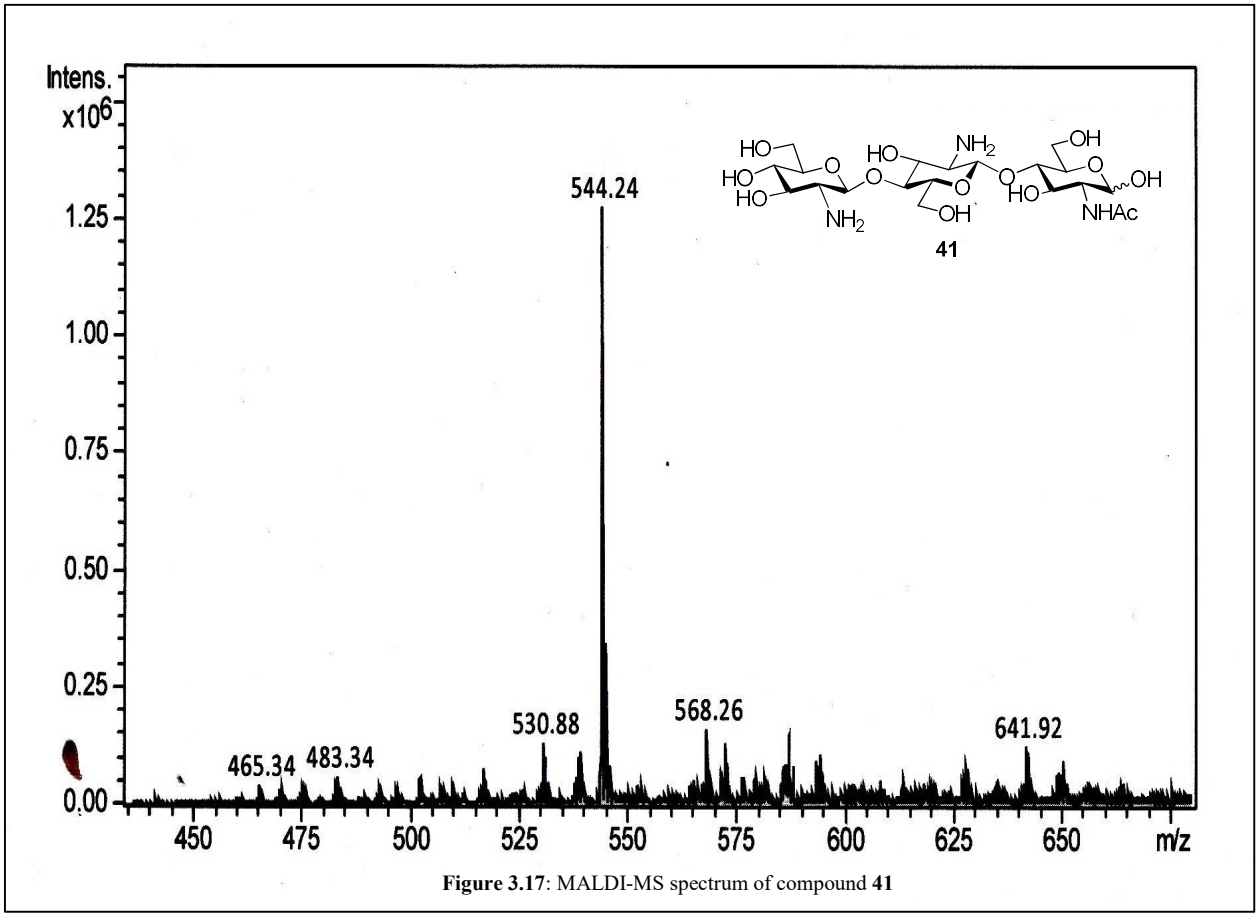


Figure 3.17: MALDI-MS spectrum of compound **41**

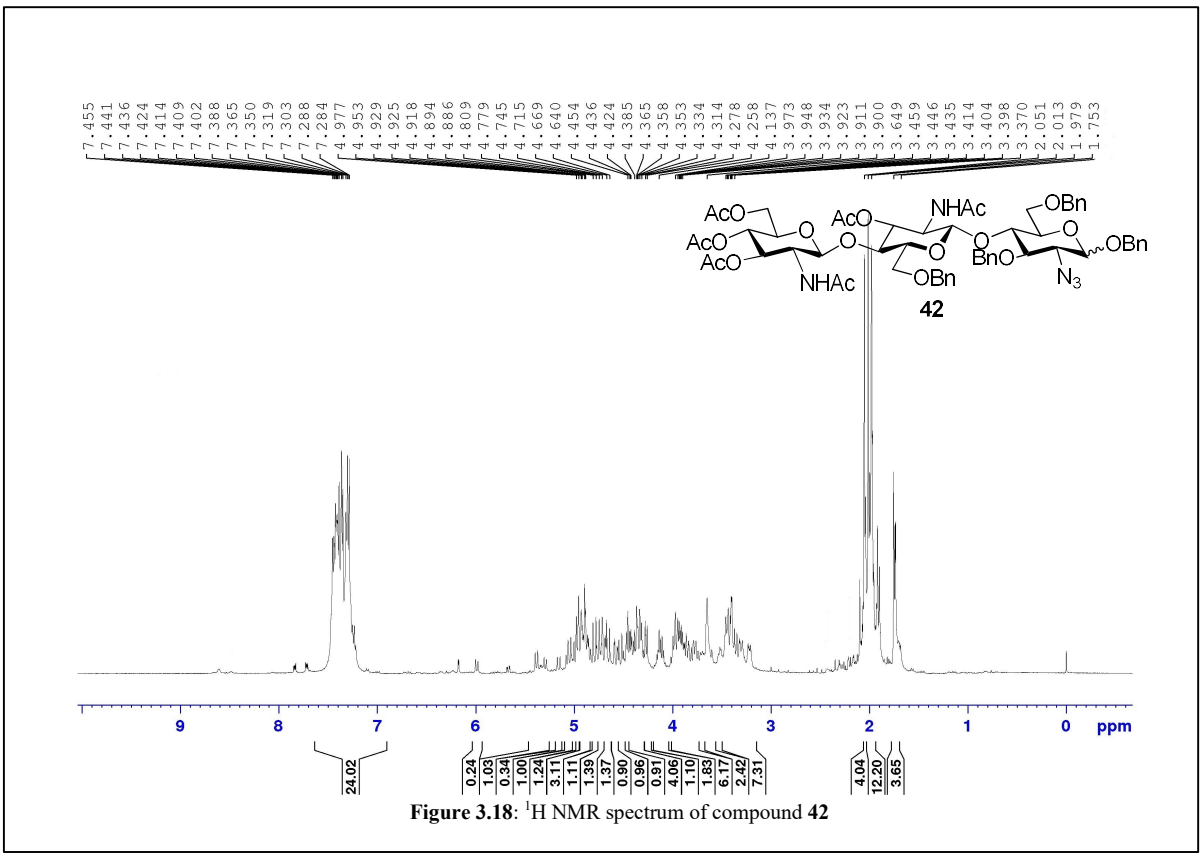


Figure 3.18: ^{1}H NMR spectrum of compound **42**

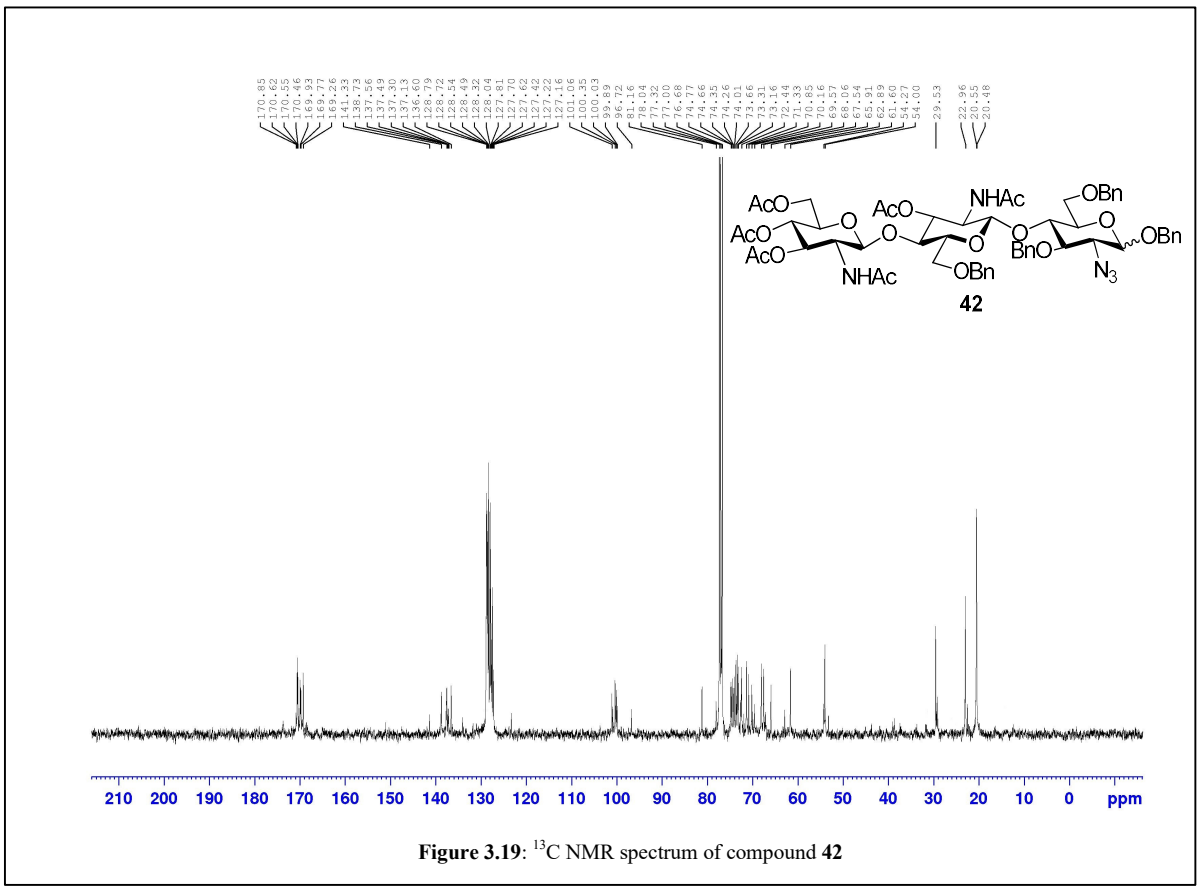


Figure 3.19: ^{13}C NMR spectrum of compound **42**

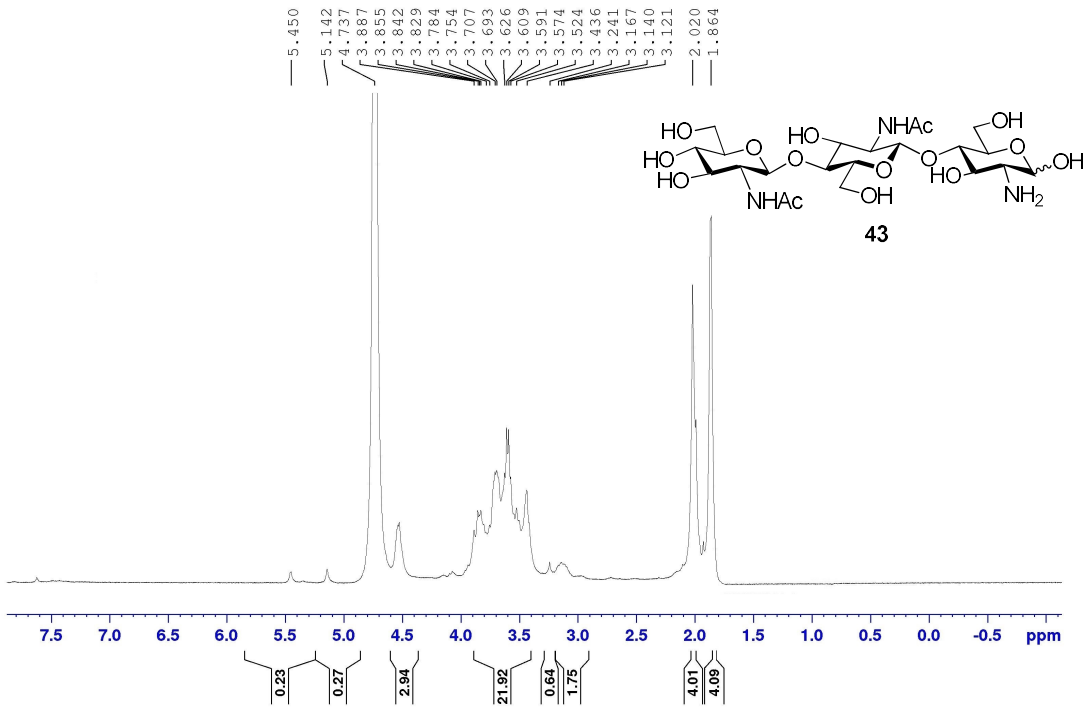


Figure 3.20: ^{1}H NMR spectrum of compound **43**

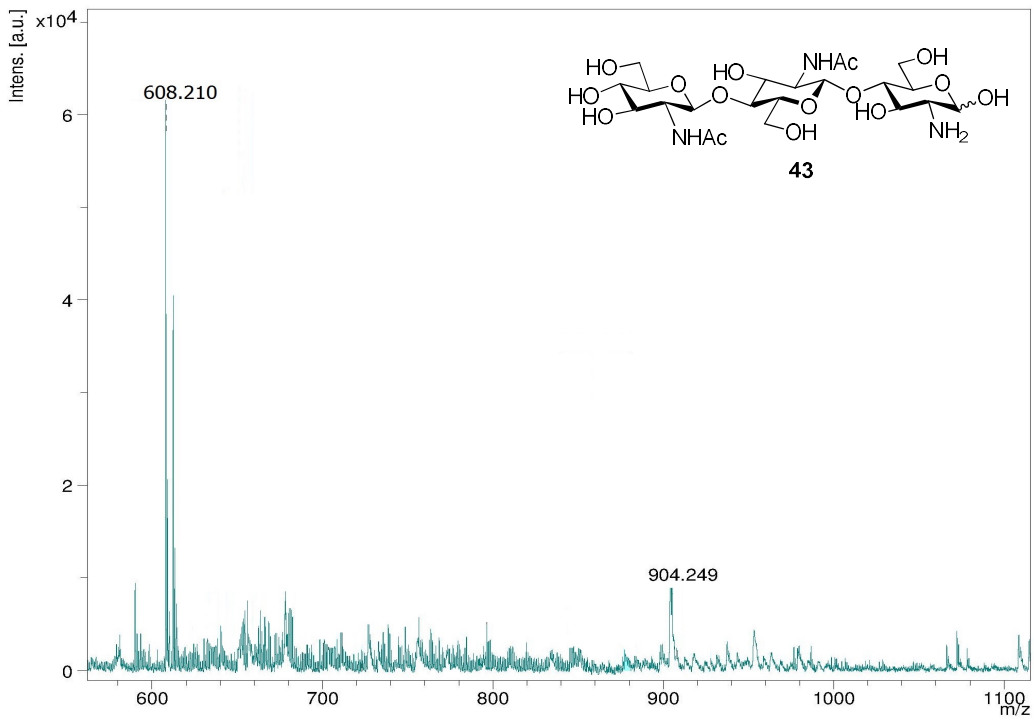


Figure 3.21: MALDI-MS spectrum of compound **43**

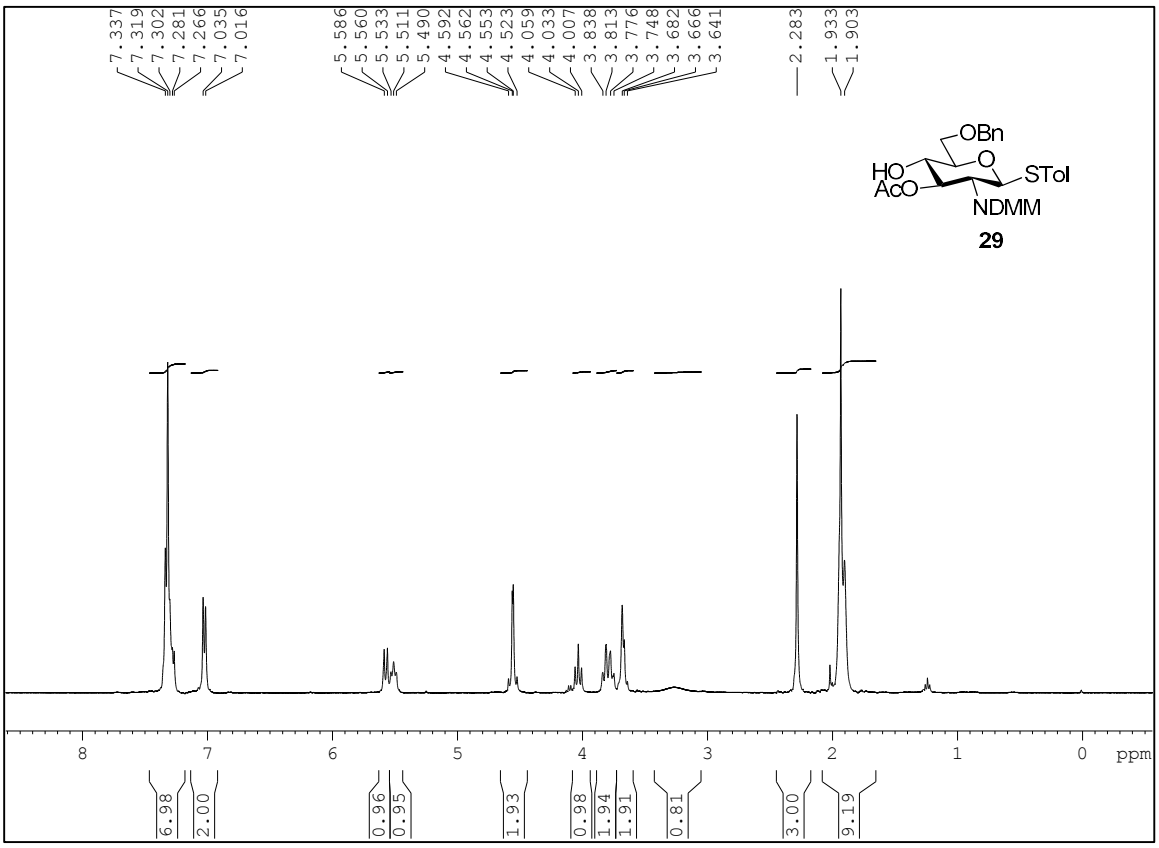


Figure 3.22: ^{1}H NMR spectrum of compound **29**

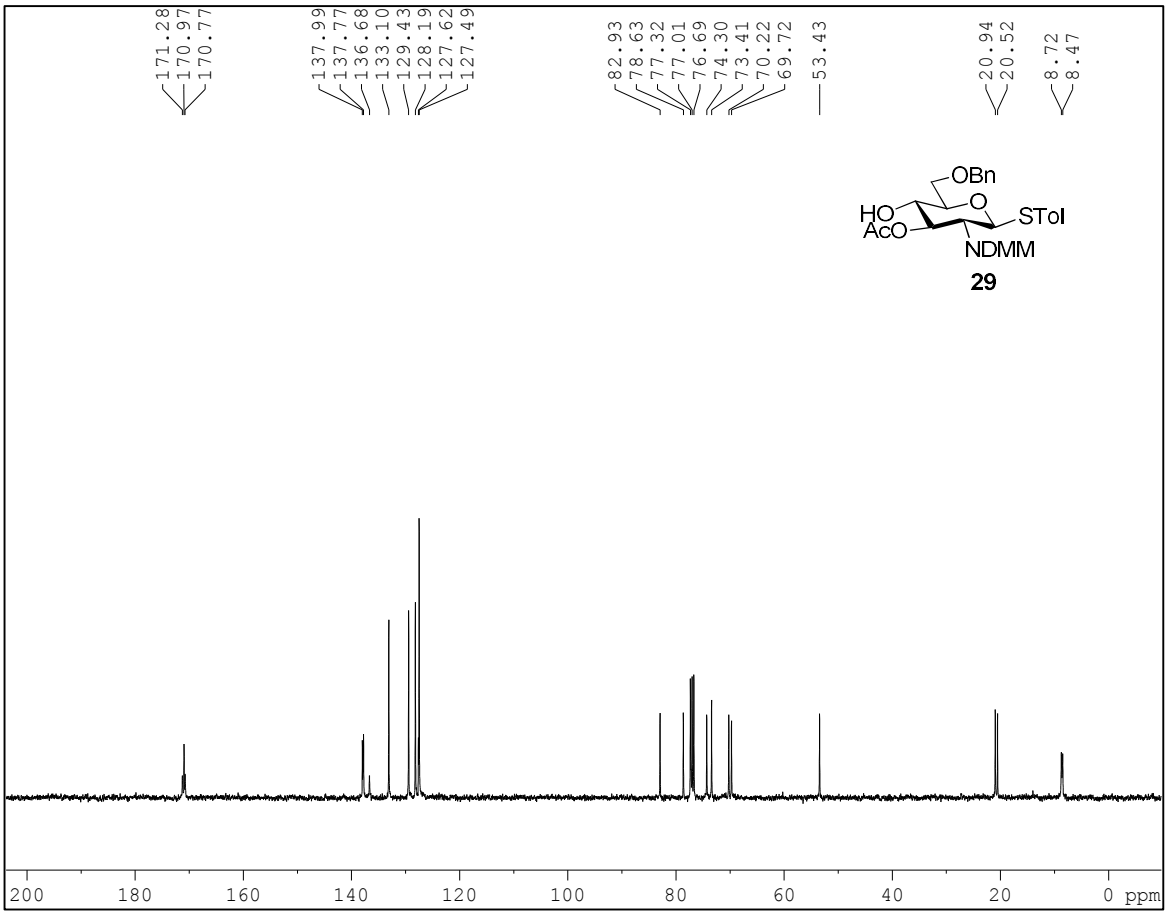


Figure 3.23: ^{13}C NMR spectrum of compound **29**

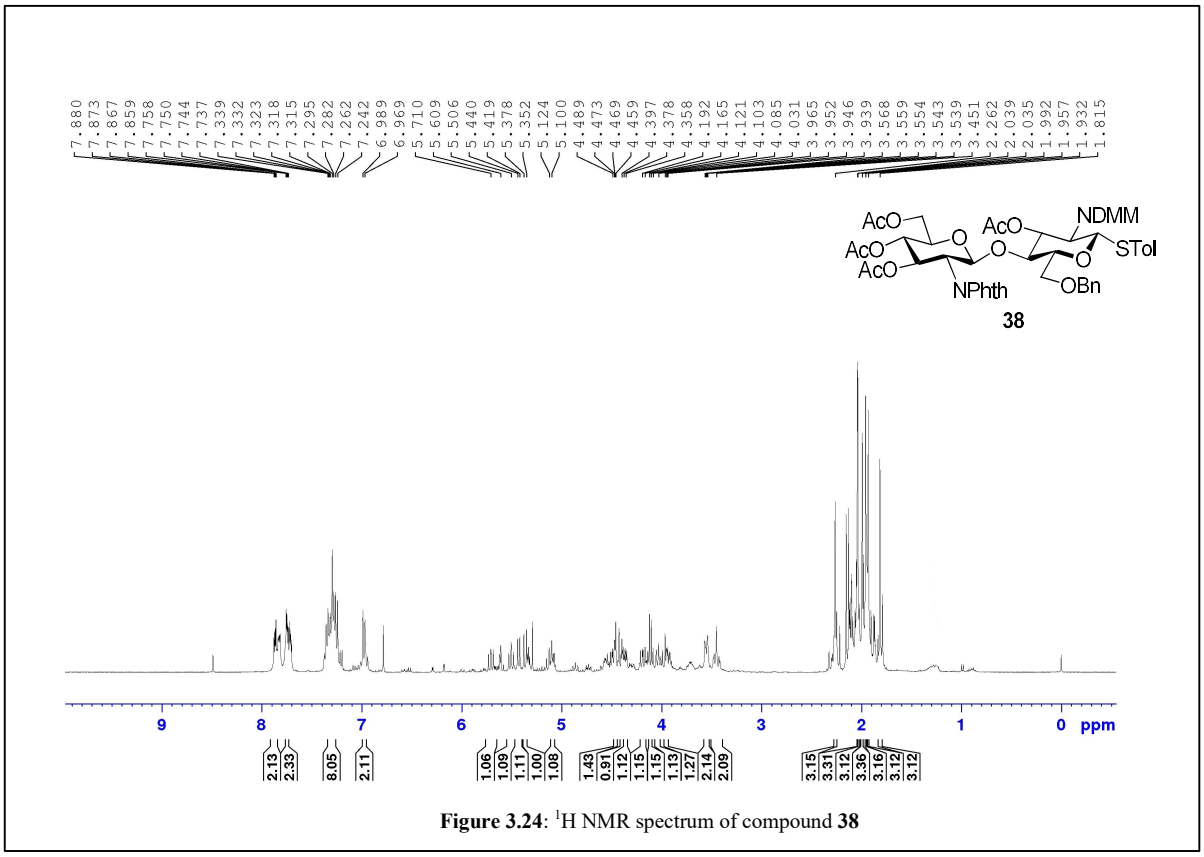


Figure 3.24: ^{1}H NMR spectrum of compound **38**

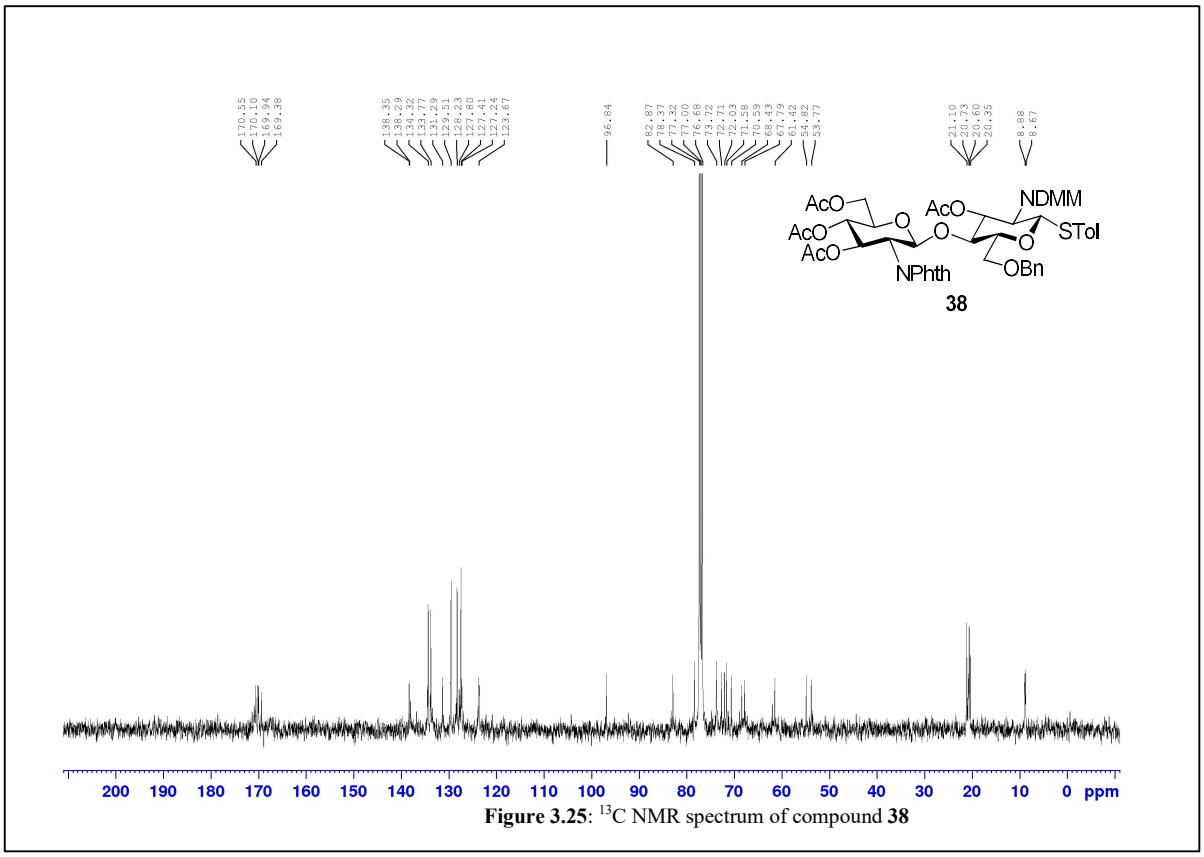


Figure 3.25: ^{13}C NMR spectrum of compound **38**

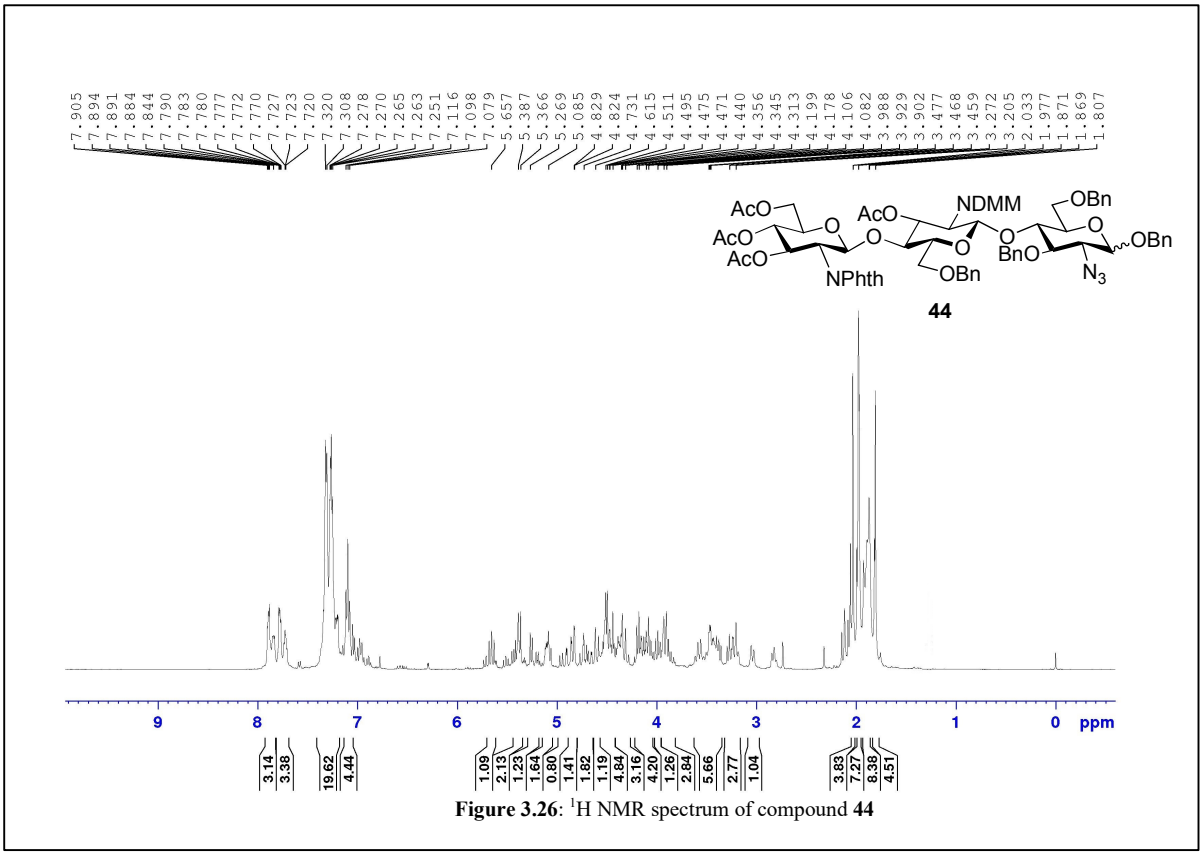


Figure 3.26: ^{1}H NMR spectrum of compound **44**

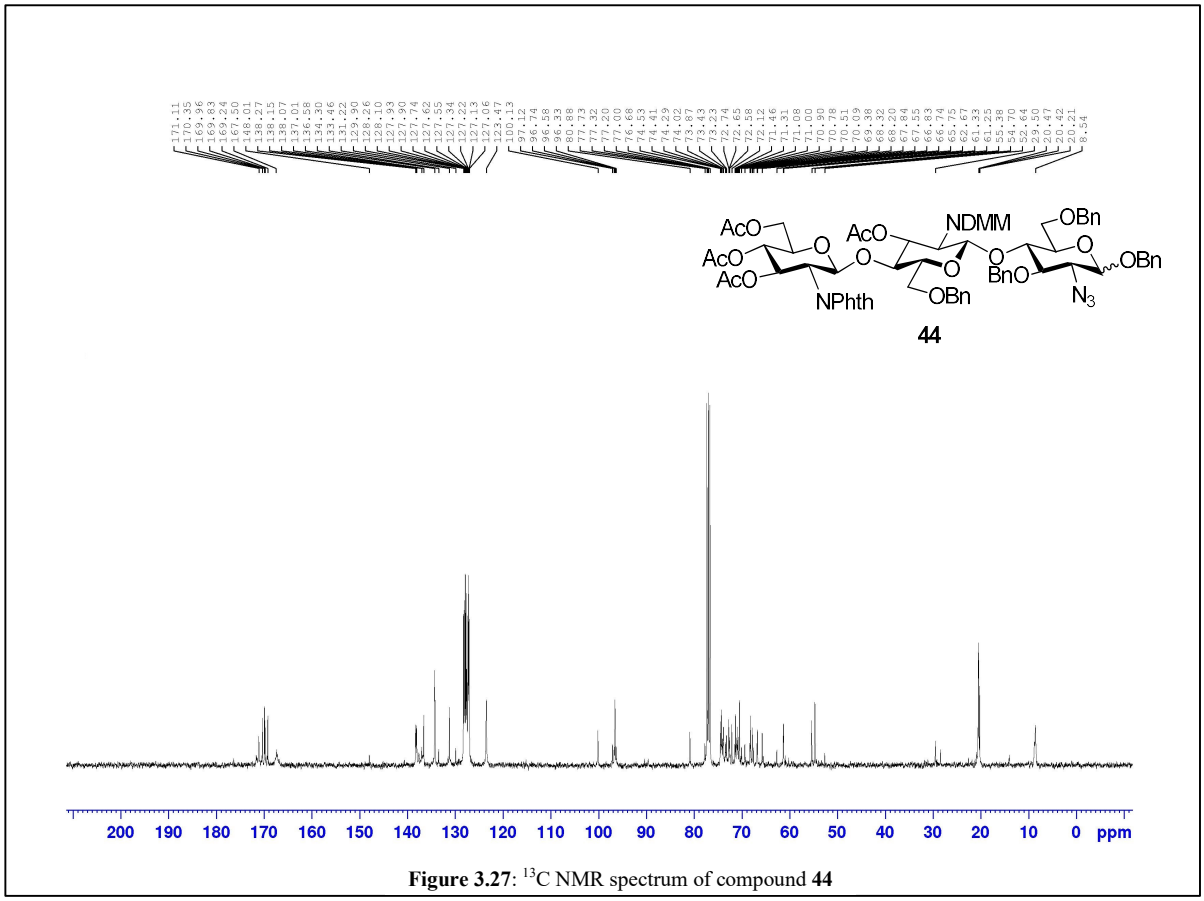


Figure 3.27: ^{13}C NMR spectrum of compound **44**

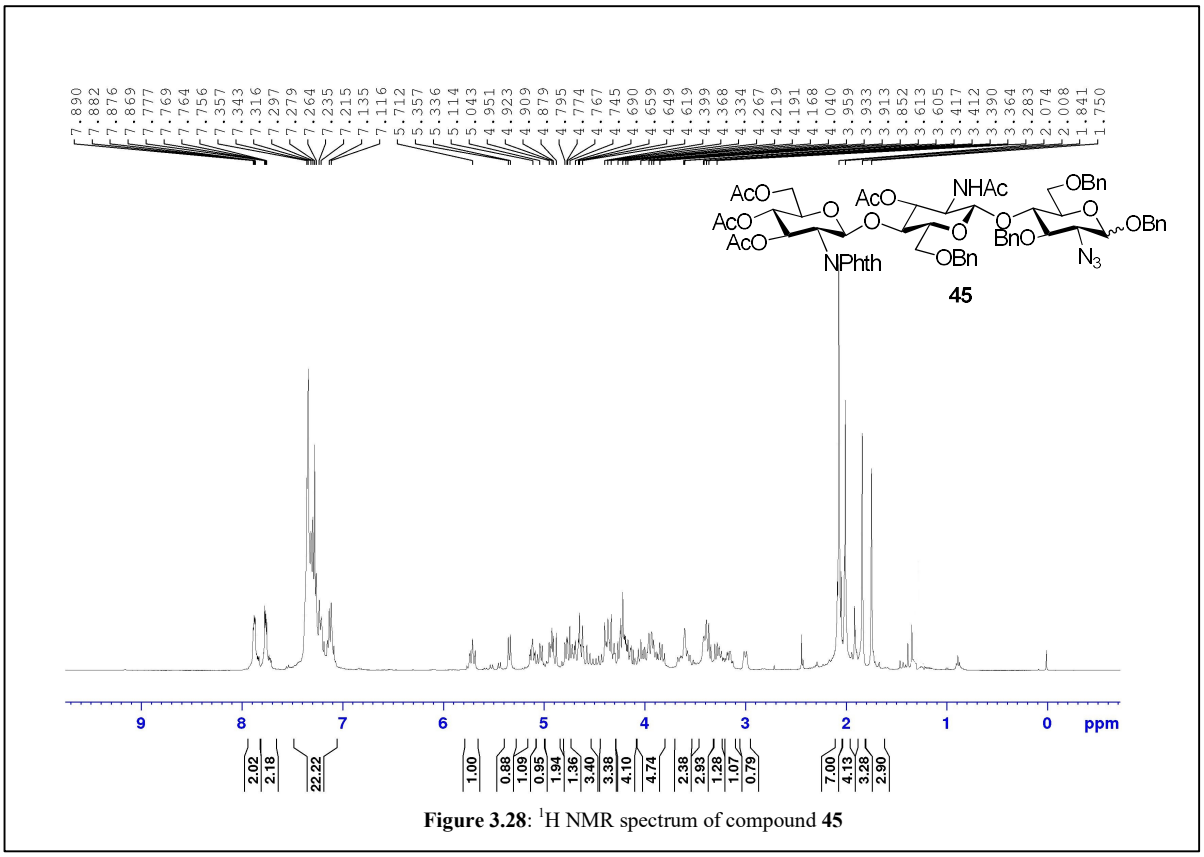


Figure 3.28: ^{1}H NMR spectrum of compound **45**

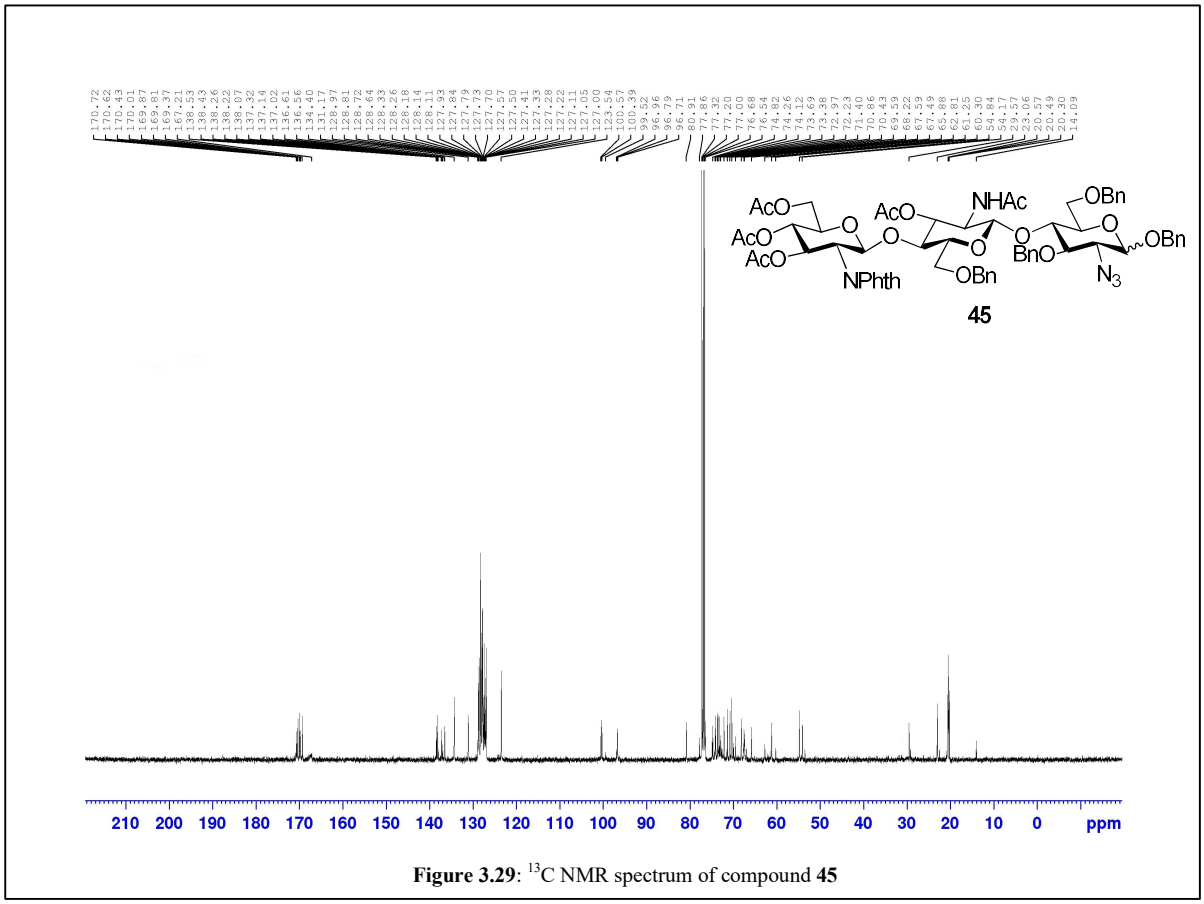


Figure 3.29: ^{13}C NMR spectrum of compound **45**

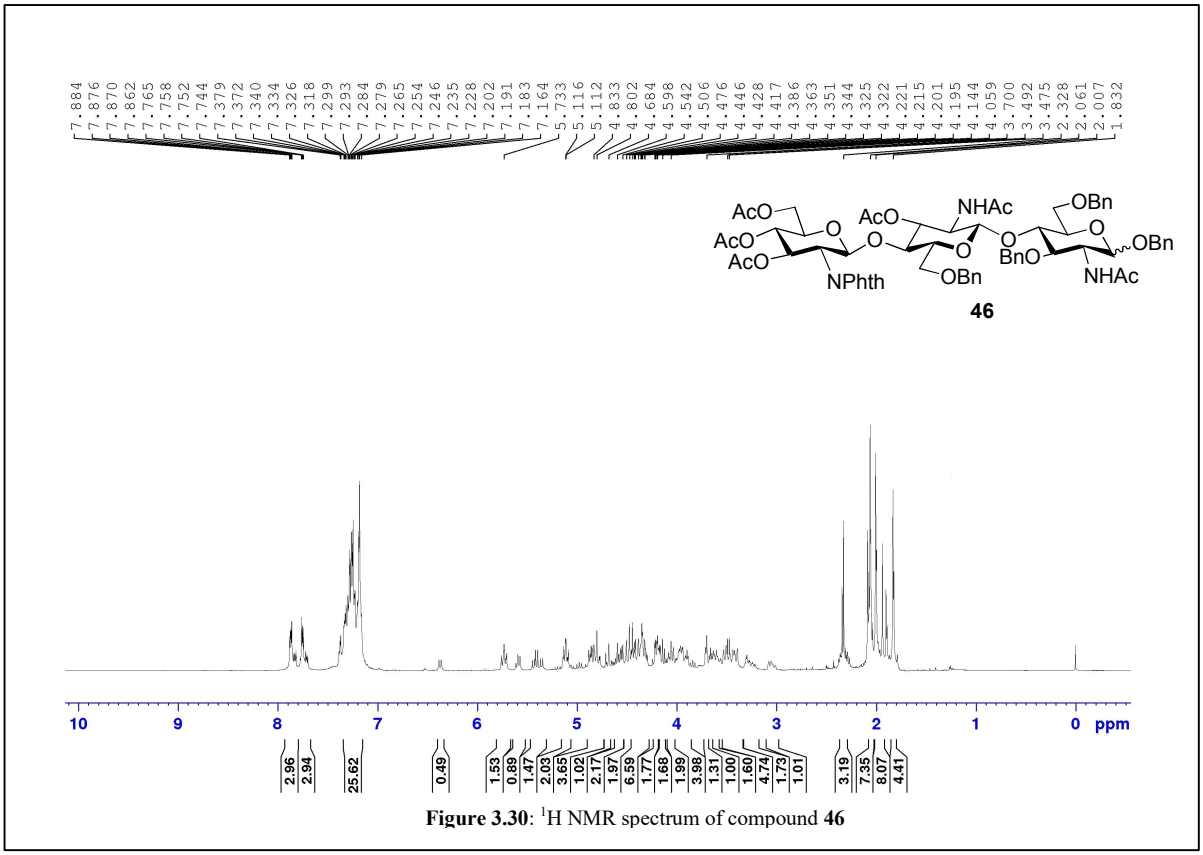


Figure 3.30: ^{1}H NMR spectrum of compound **46**

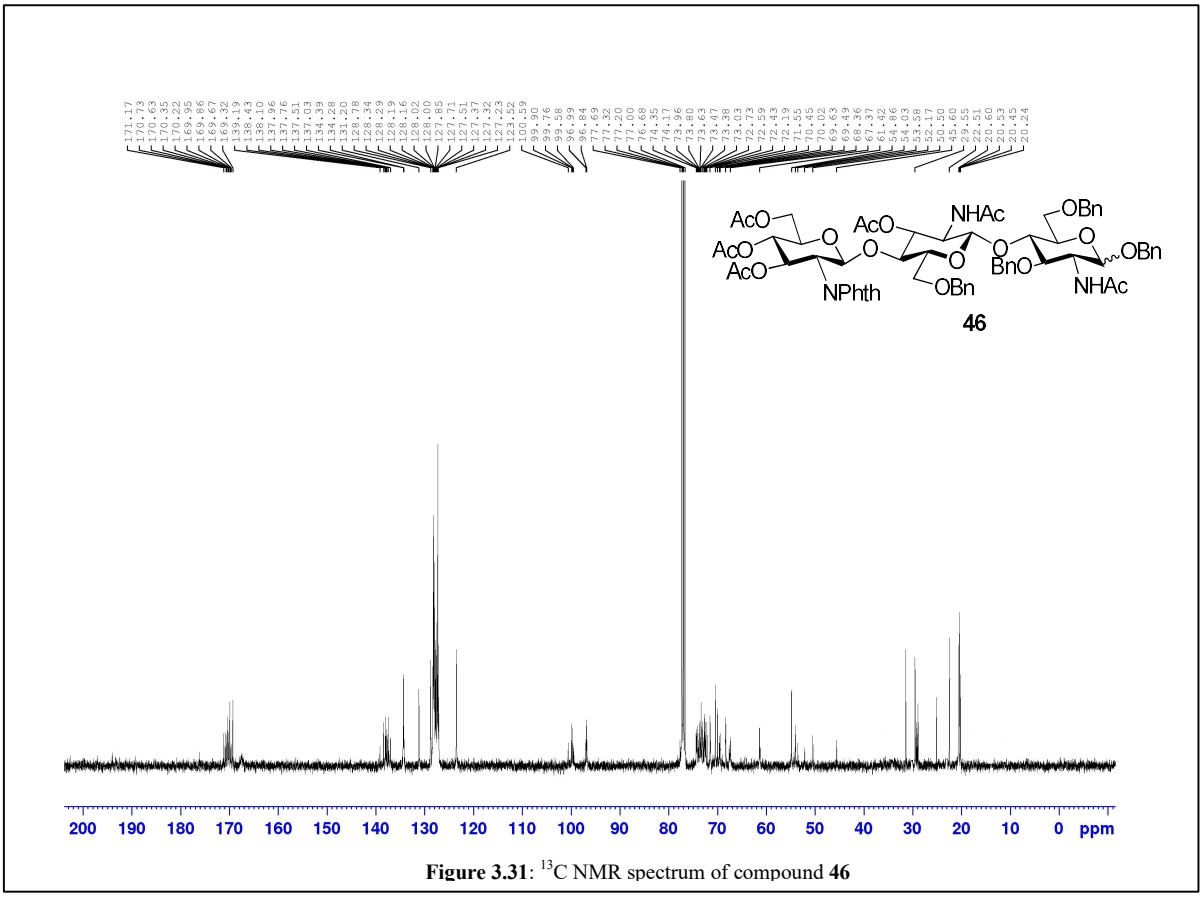


Figure 3.31: ^{13}C NMR spectrum of compound **46**

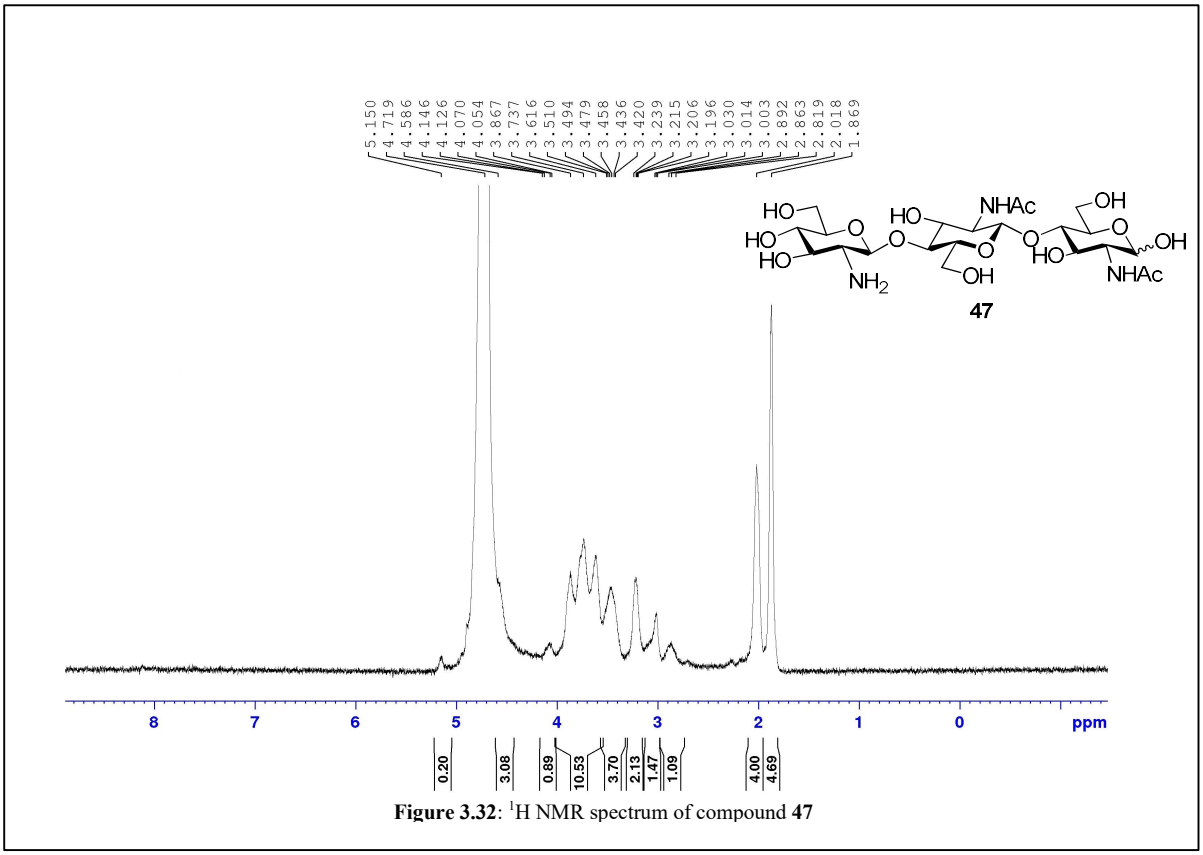


Figure 3.32: ^{1}H NMR spectrum of compound **47**

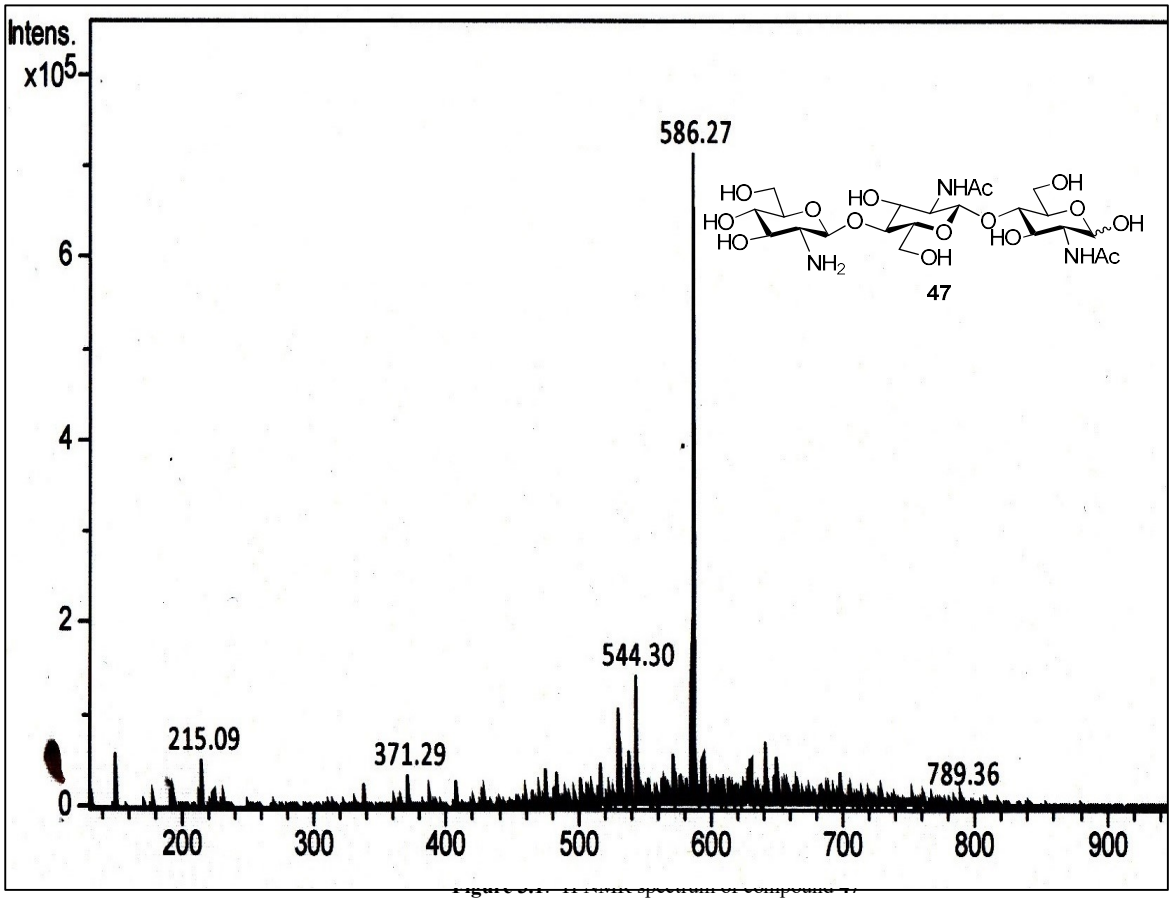


Figure 3.33: MALDI-MS spectrum of compound **47**

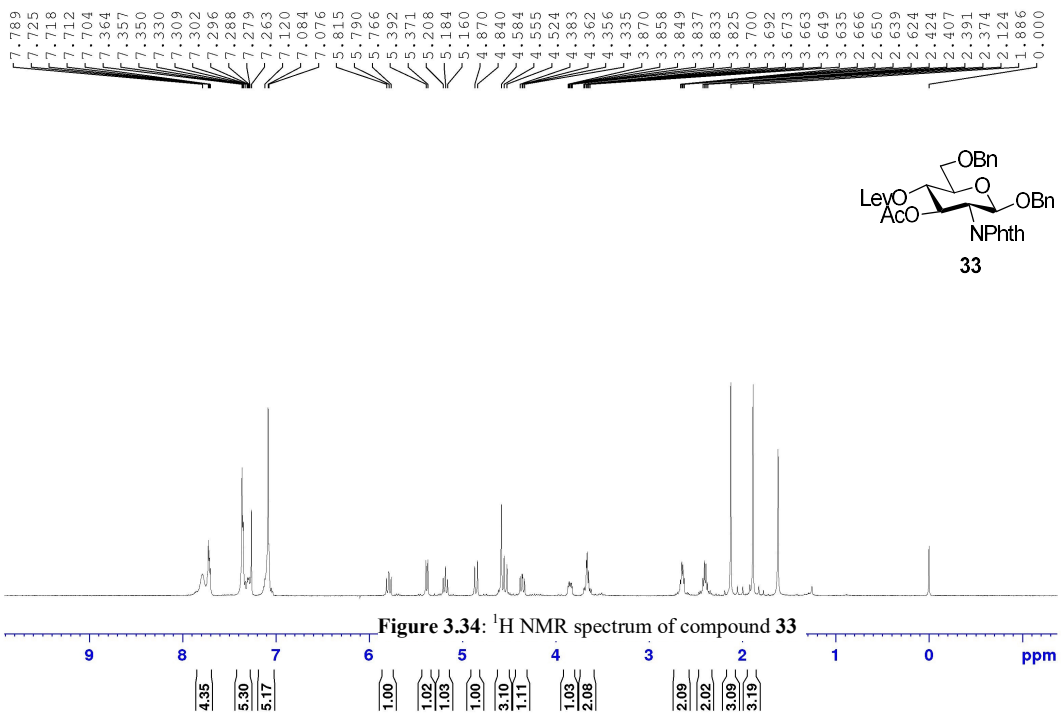


Figure 3.34: ^{1}H NMR spectrum of compound **33**

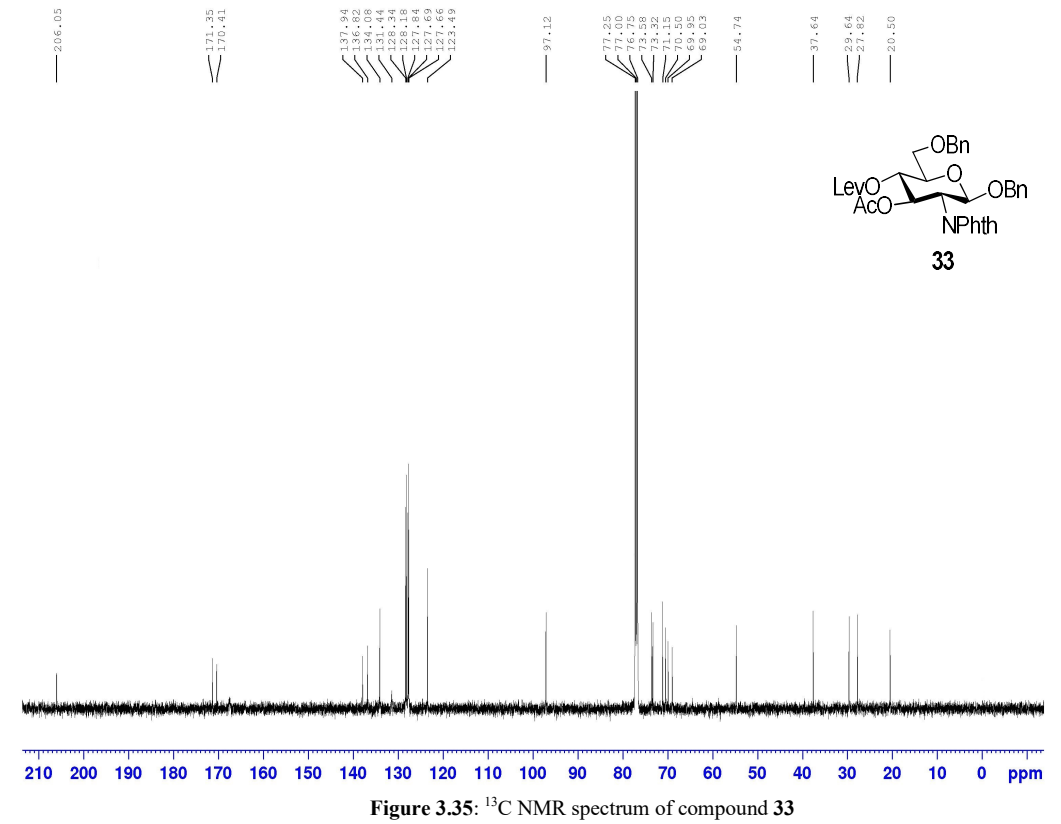


Figure 3.35: ^{13}C NMR spectrum of compound **33**

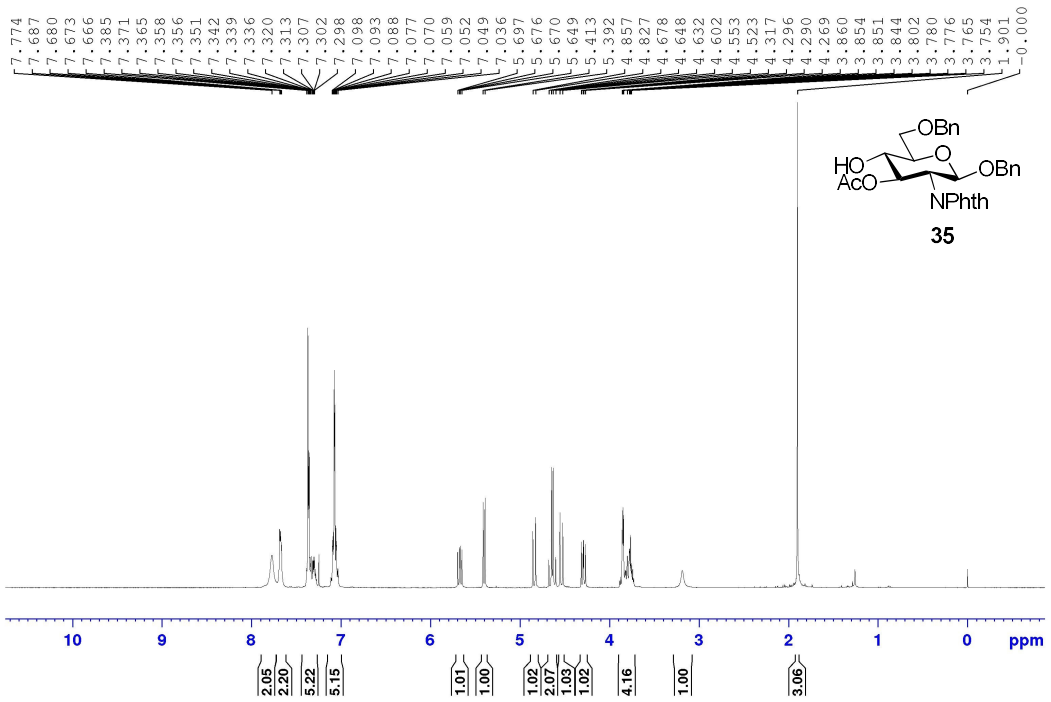


Figure 3.36: ^{1}H NMR spectrum of compound **35**

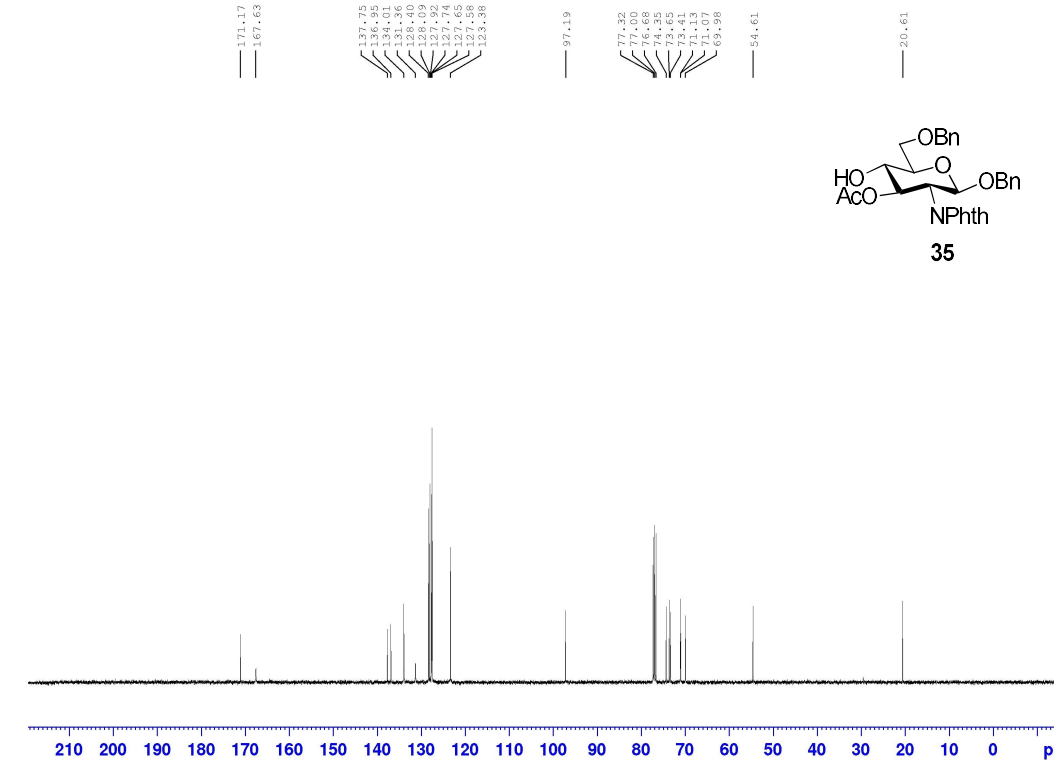


Figure 3.37: ^{13}C NMR spectrum of compound **35**

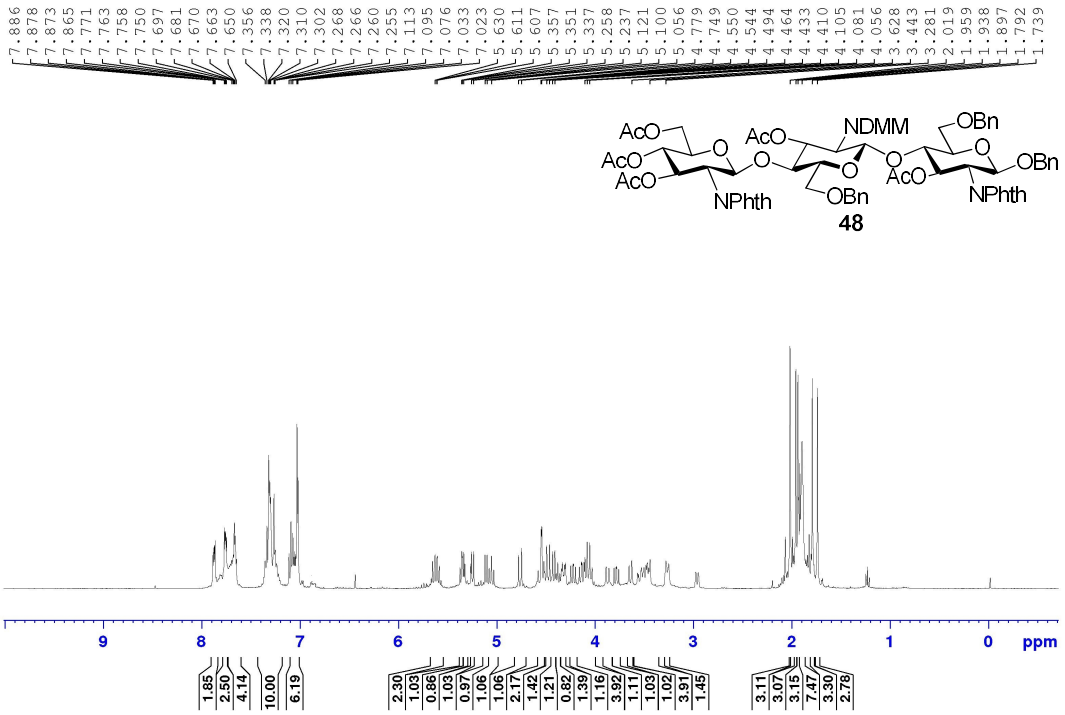


Figure 3.38: ^{1}H NMR spectrum of compound **48**

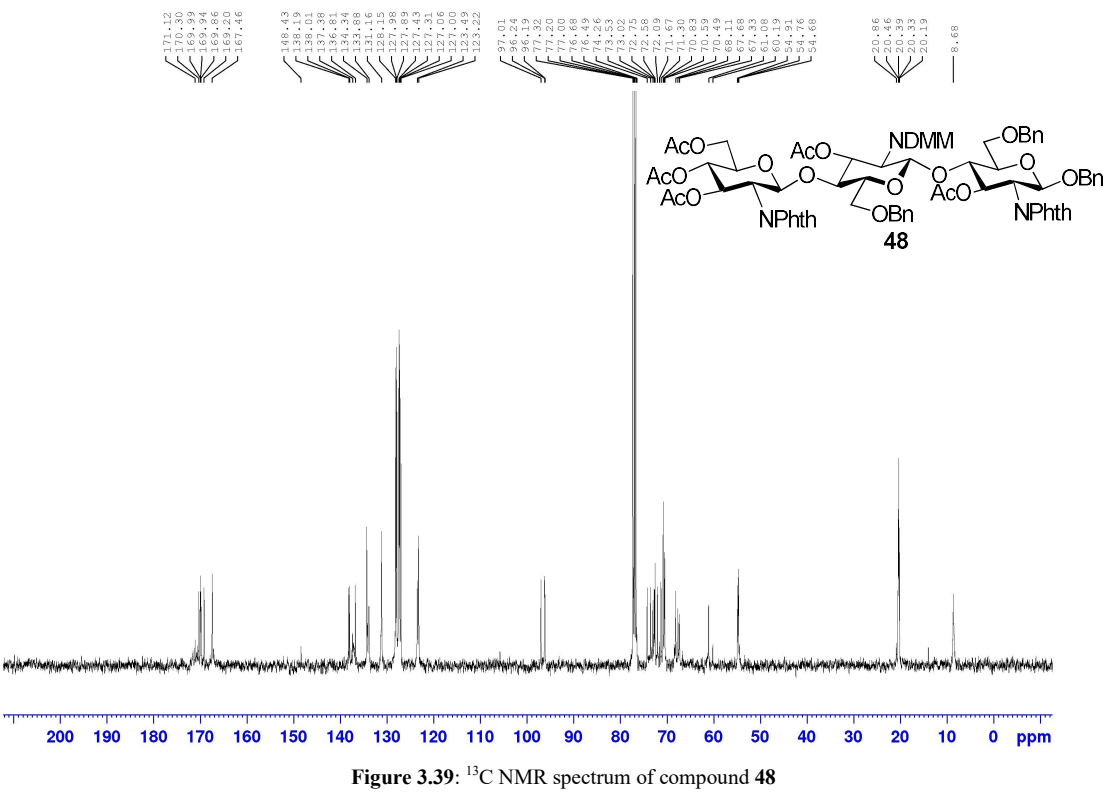


Figure 3.39: ^{13}C NMR spectrum of compound **48**

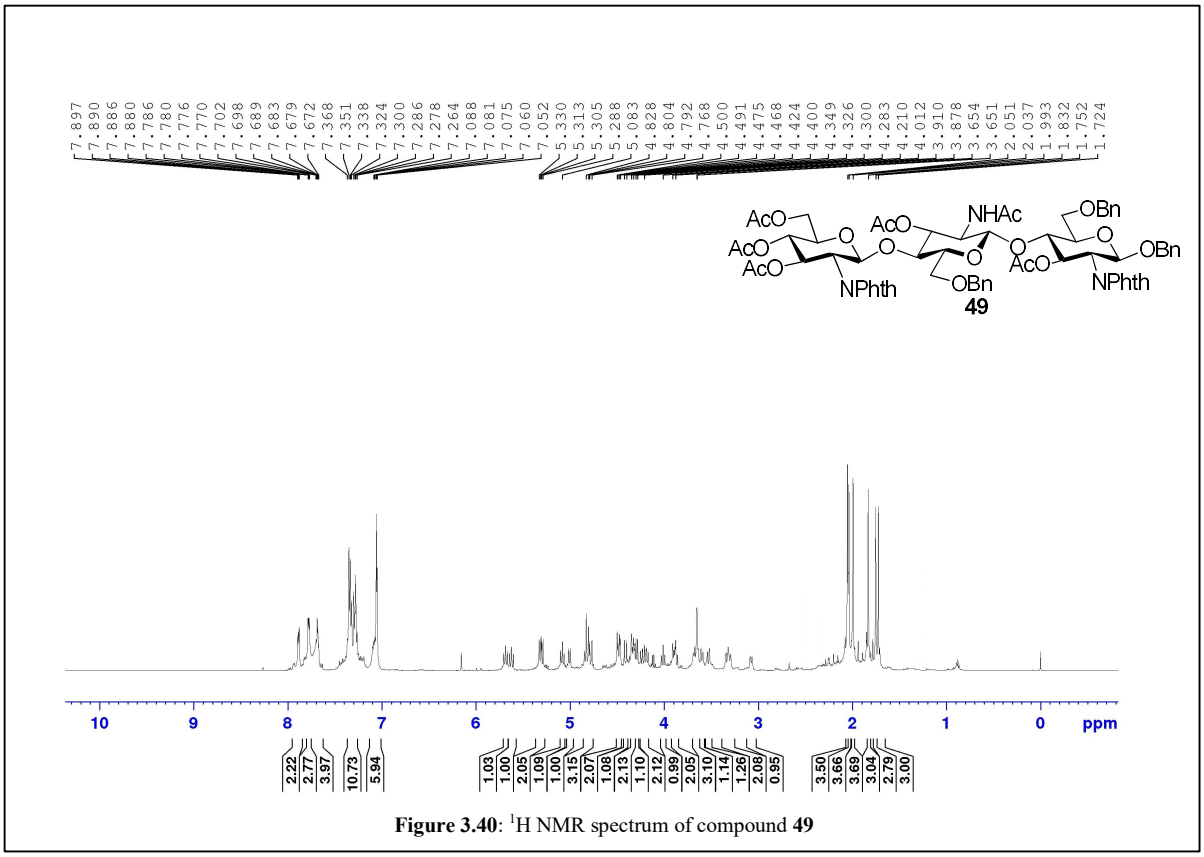


Figure 3.40: ^{1}H NMR spectrum of compound **49**

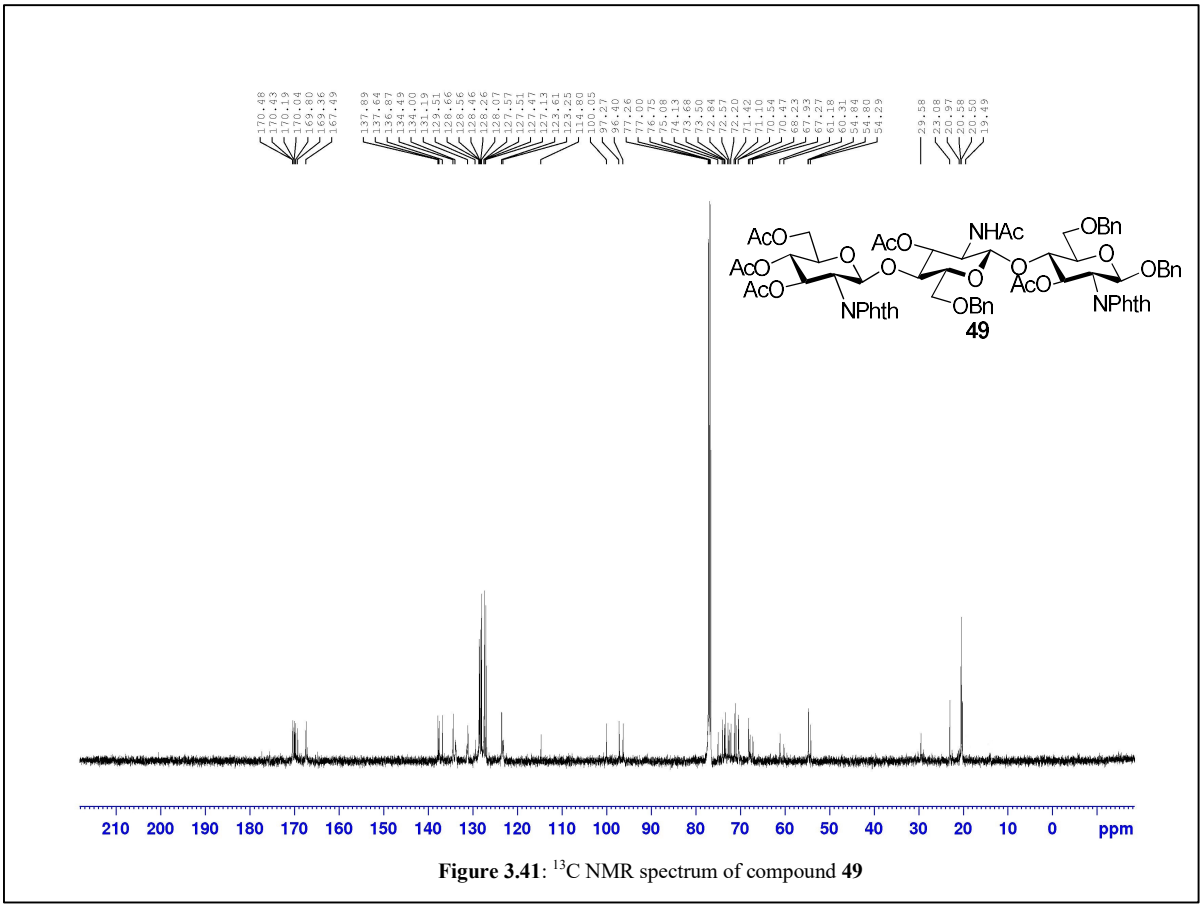


Figure 3.41: ^{13}C NMR spectrum of compound **49**

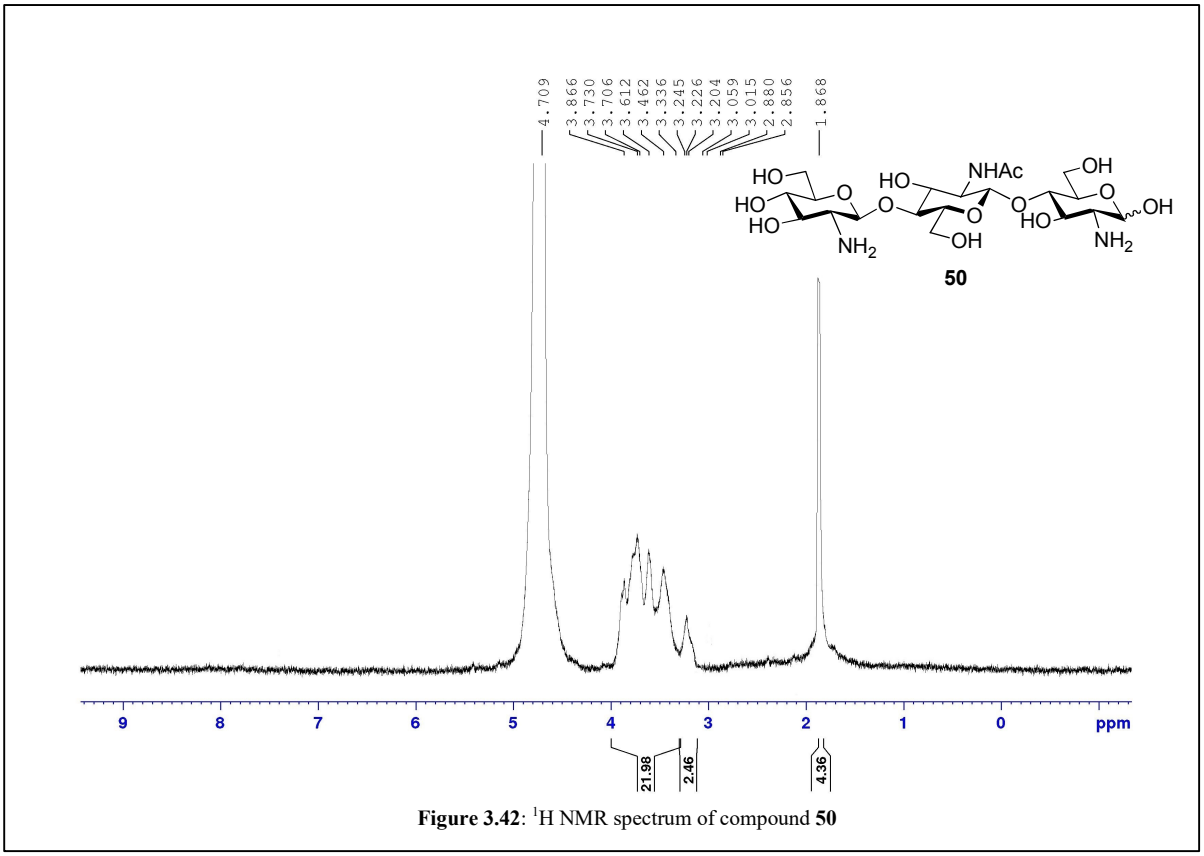


Figure 3.42: ^{1}H NMR spectrum of compound **50**

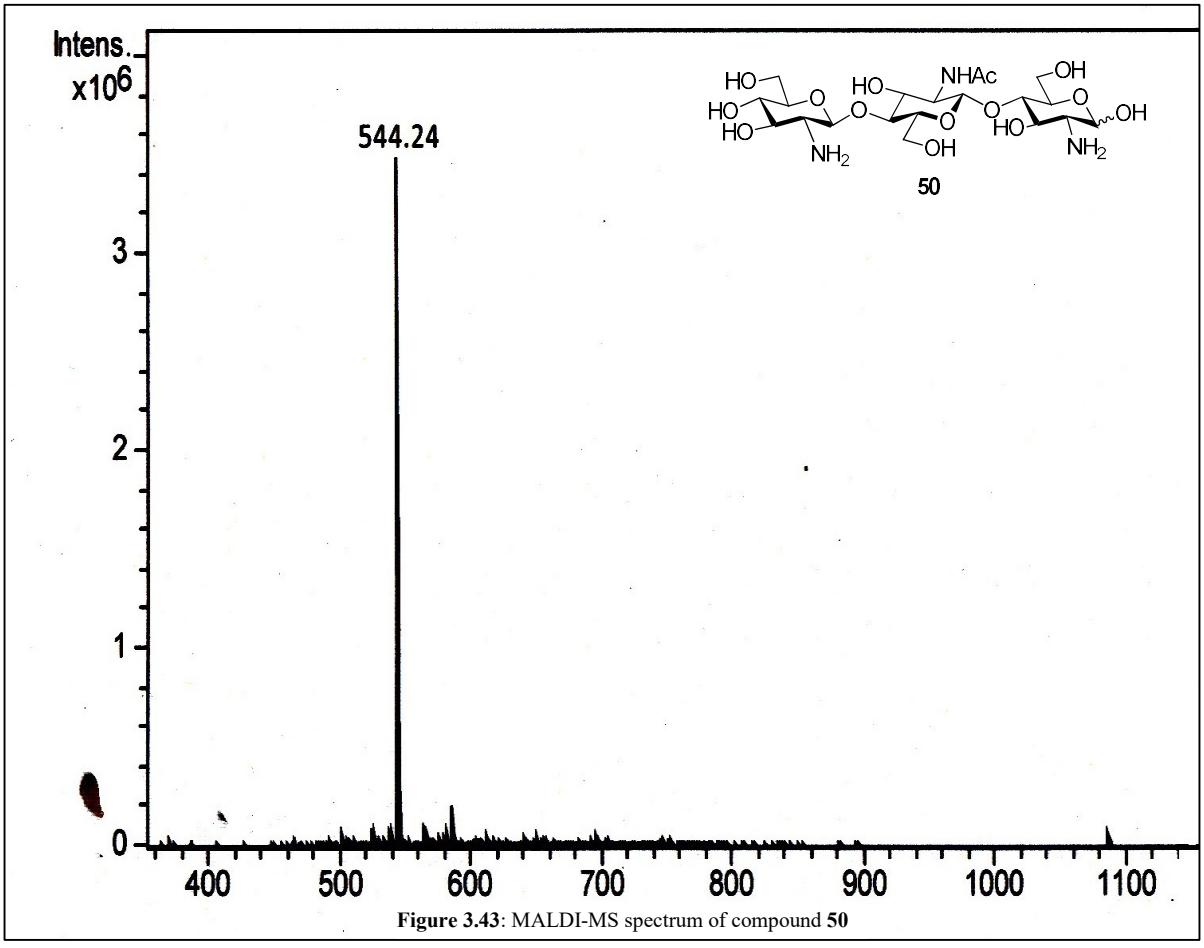


Figure 3.43: MALDI-MS spectrum of compound **50**

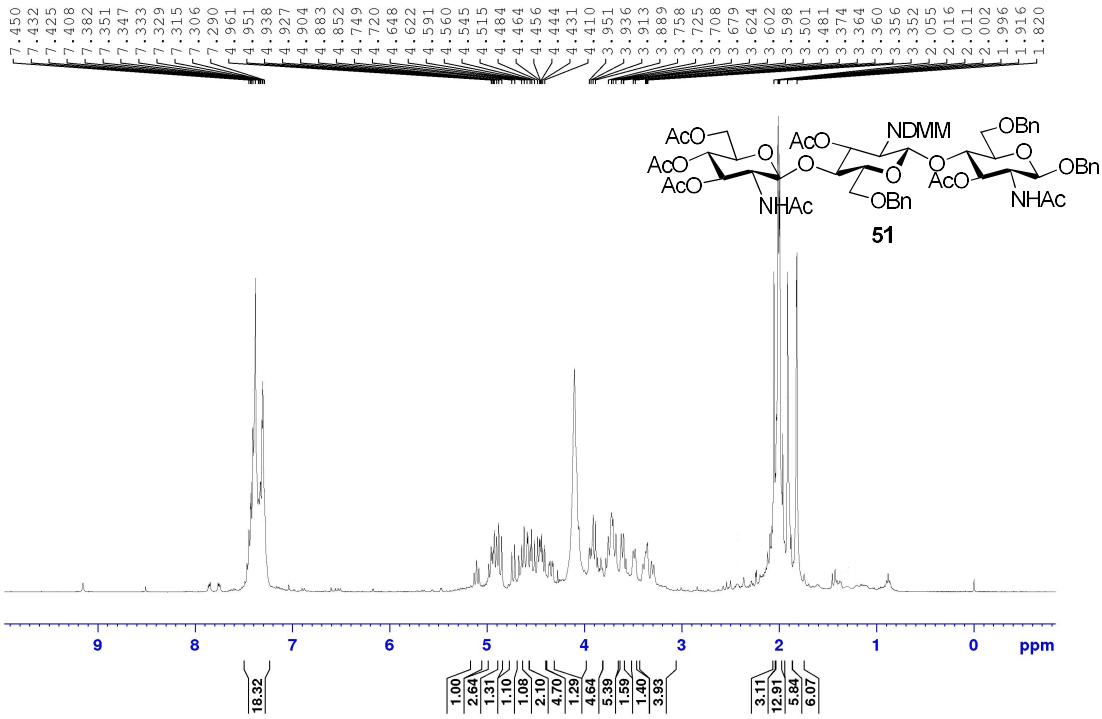


Figure 3.44: ^{1}H NMR spectrum of compound **51**

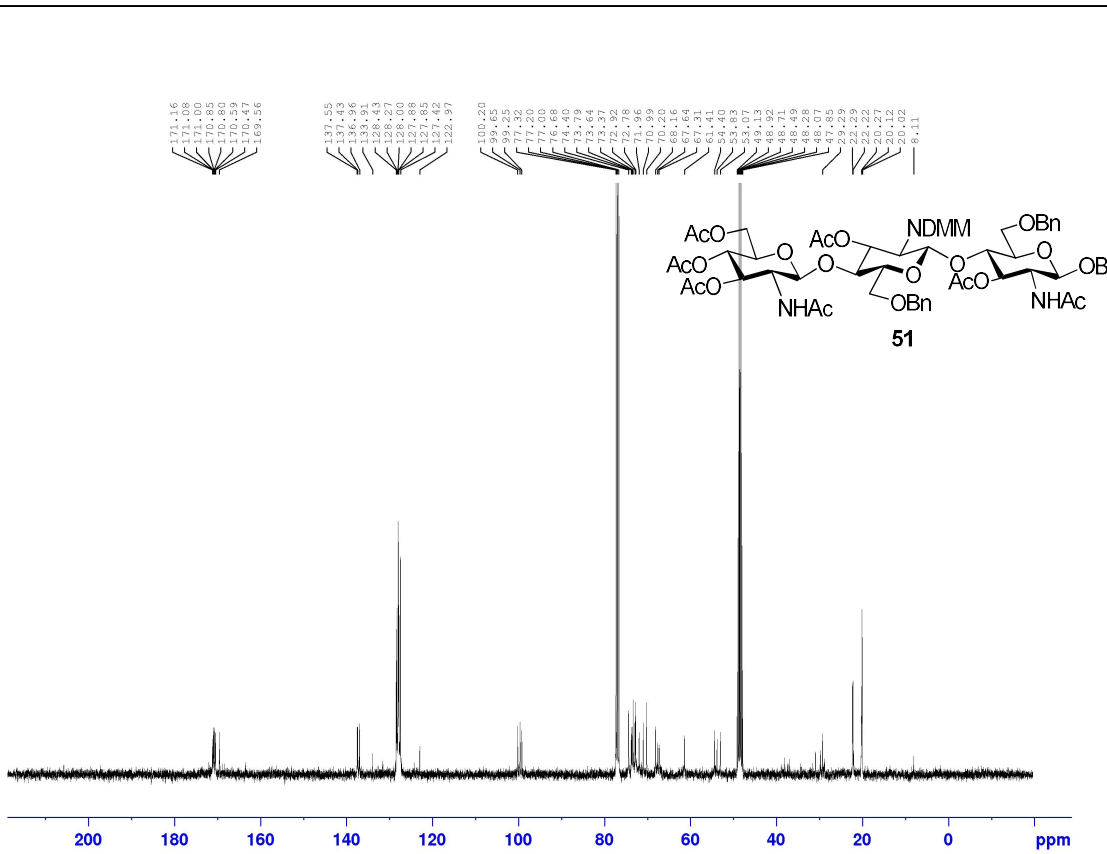


Figure 3.45: ^{13}C NMR spectrum of compound **51**

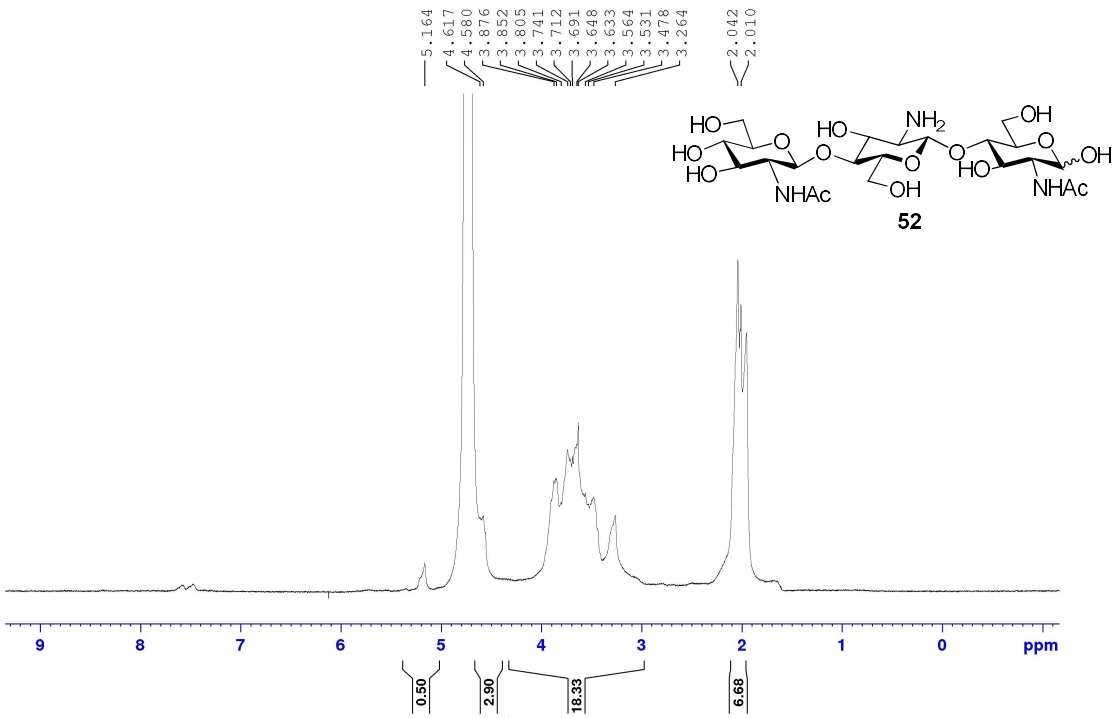


Figure 3.46: ^{1}H NMR spectrum of compound **52**

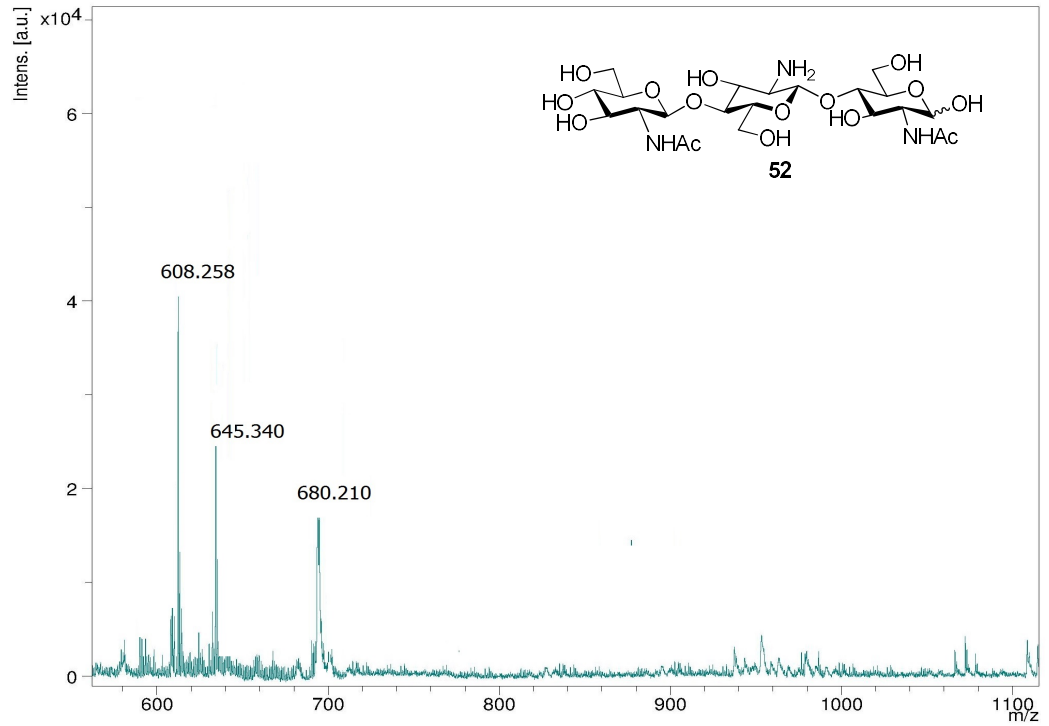


Figure 3.47: MALDI-MS spectrum of compound **52**

Chapter 4

Synthesis of *p*-nitrophenyl chitobiosides and their biological evaluation

4.1 Introduction

Glycosidases are a class of enzymes that involve in the metabolism of glycoconjugates, thereby playing a crucial role in many biological processes happening in living organisms. This activity is of great significance in the field of glycobiology and biotechnology.[1,2] Glycosidases can cleave the glycosidic bonds with high substrate specificity.[3] These enzymes can be categorised based on their substrate specificity, glycosidic cleavage site, and catalytic mechanism.[4] Based on the cleavage site of glycosidic bonds, these can be classified as *exo* glycosidases which specifically act on the termini of oligosaccharides, whereas *endo* glycosidases hydrolyze the internal glycosidic linkages. The cleavage may end up with either retention or inversion of configuration at the anomeric centre based on the mechanism of enzymatic hydrolysis (Figure 4.1).[4,5,6]

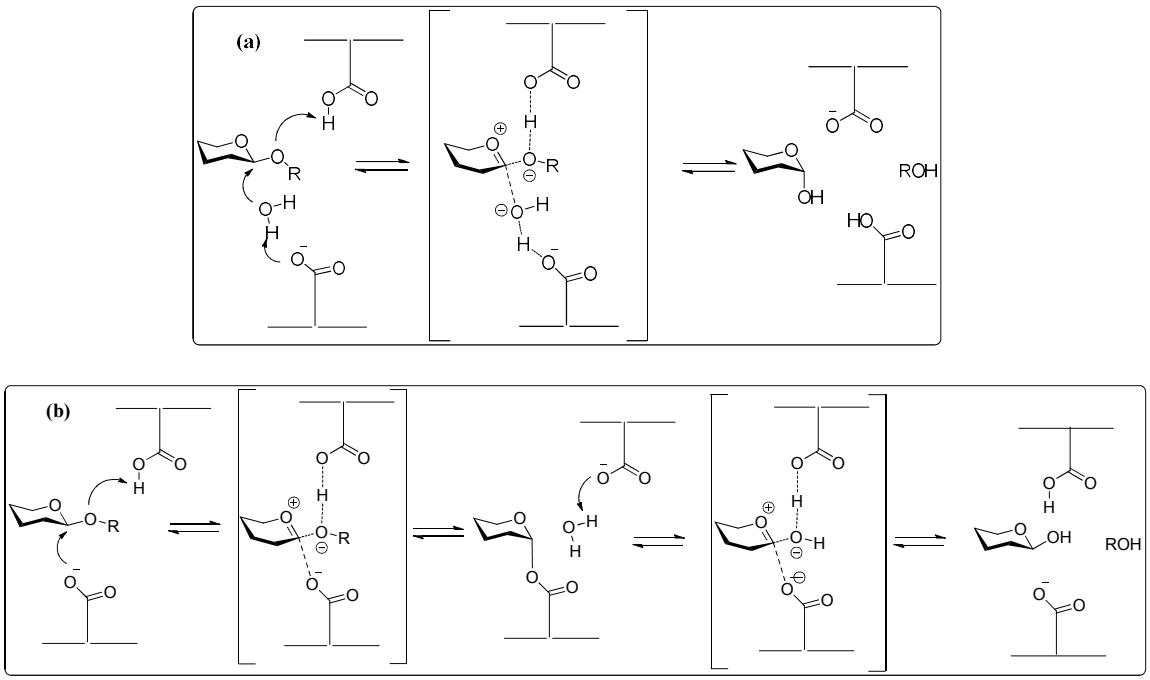
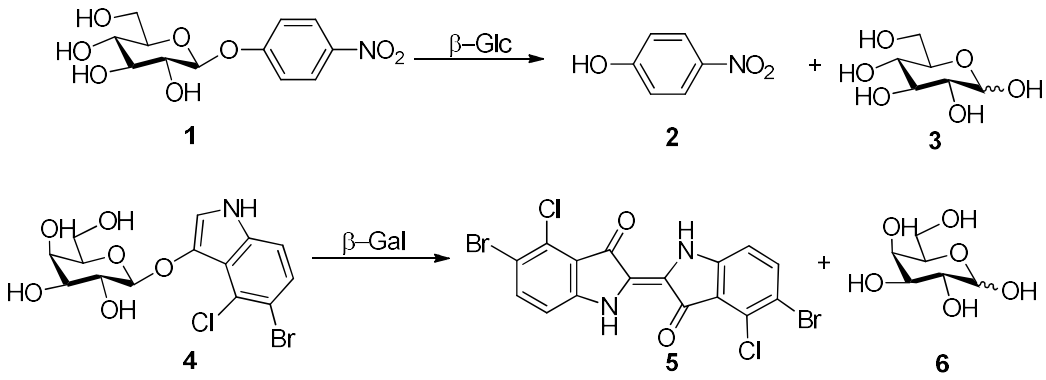


Figure 4.1: General mechanism of enzymatic hydrolysis of a glycosidic bond (a) inversion of anomeric configuration (b) retention of anomeric configuration.

Development of synthetic strategies for the synthesis of chemical probes such as glycoconjugates and oligosaccharides to study the function of glycosidases is of great importance. Particularly, these chemical probes are very useful in the detection and mechanism of glycosidase enzymes. These synthetic probes may be chromogenic substrates,[7] fluorogenic substrates,[8,9] lanthanide complexes,[10a] gels[10b] and gold nanoparticles.[11] Upon activation by the enzymes (glycosidases) these probes release the reporter molecule that can be detected. *p*-Nitrophenyl substrate **1** is a very good synthetic probe for the characterization of glycosidase enzymes. Here the enzyme acts on the substrate **1** and releases *p*-nitrophenol **2** which can be detected and quantified colorimetrically.[12] Measuring the absorbance at 405 nm gives the quantitative information about the extend of release of *p*-nitrophenol and thus the enzyme activity.

Ruan *et al*. reported that indolyl glycoside derivative **4** can also serve as chromogenic enzyme substrates through the formation of blue indigoid dye **5**. Hence, these substrates can be used for alkaline phosphatase enzyme assays (Scheme 4.1).[13]



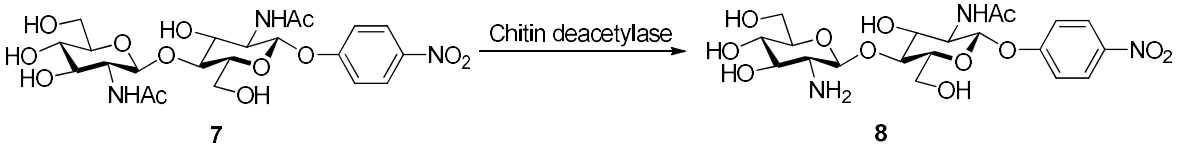
Scheme 4.1: Glycosidase activity on chromogenic substrates (**1** and **4**)

4.1.1 Biological significance of *p*-nitrophenyl chitobiosides

p-Nitrophenyl glycosides substrates generally serve as very good substrates for glycosidases. β-*N*-Acetylhexoaminidases are a class of enzymes which are highly specific and active towards the *p*-nitrophenyl N-acetylglucosamine and N, N′-diacetylchitobiose. This *p*-nitrophenyl glucosamine derivative is used extensively as glycosyl donors in the synthesis of higher chitooligosaccharides *via* enzymatic transglycosylation.[14]

Tokuyasu. *et al*. reported the chitin deacetylase isolated from *C*. *lindemuthianum* which can selectively deacetylate at the non-reducing end of the *p*-nitrophenyl *N*,*N*'-diacetylchitobioside **7**. Enzymatic deacetylation of $(GlcNAc)_2$-*p*NP **7** at the non-reducing end

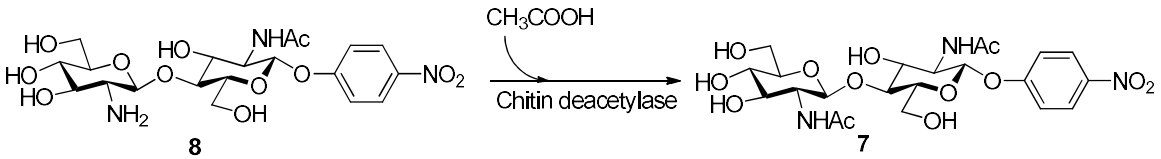
quantitatively yields the GlcNGlcNAc-pNP **8** (Scheme 4.2). These substrates are useful to differentiate chitin deacetylase from the chitinases which degrades $(GlcNAc)_2$-*p*NP based on their mode of action.[15]



7 **8**

Scheme 4.2: Mono deacetylation of *p*-nitrophenyl *N*,*N*'-diacetylchitobioside **7** using chitin deacetylase from *C. lindemuthianum*.

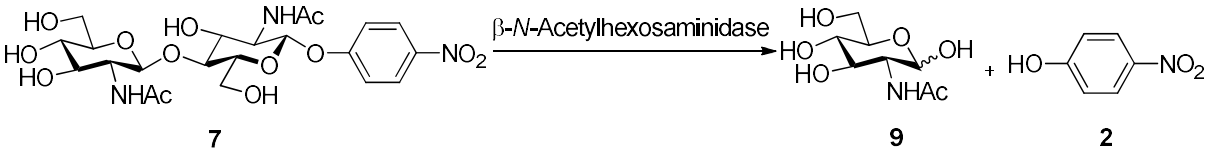
In 1999, the same research group reported the synthesis of GlcNAcGlcNAc-*p*NP **7** from the GlcNGlcNAc-pNP **8** by N-acetylation using another chitin deacetylase (Scheme 4.3).[16] These results suggest that *p*-nitrophenyl chitobiosides **7** and **8** can serve as useful substrates for the production of well-defined chitooligosacharides by using chitin deacetylases.



8 **7**

Scheme 4.3: Preparation of *p*-nitrophenyl chitobioside **7** using chitin deacetylase *via* enzymatic acetylation.

Substrate specificity of β-*N*-acetylhexosaminidase was evaluated using *p*-nitrophenyl chitobioside **7** as the substrate. These enzymes are specifically active on *N*,*N*'-diacetylchitobiose derivative by the cleavage of β-glycosidic linkages between the GlcNAc residues (Scheme 4.4).[16]



7 **9** **2**

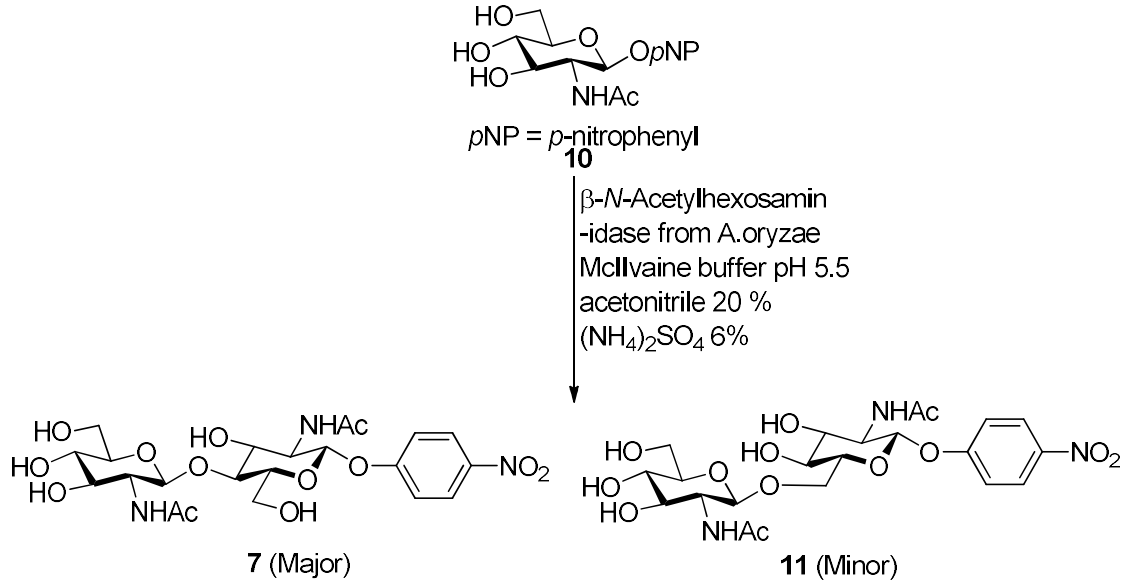
Scheme 4.4: Substrate specificity of β-*N*-acetylhexosaminidase on *p*-nitrophenyl chitobioside **7**.

4.1.2 Enzymatic and chemical synthesis of *p*-nitrophenyl chitobiosides

Development of synthetic strategies for the preparation of *p*-nitrophenyl chitobiosides with different *N*-acetylated pattern has great application in the characterization and mode of action of hydrolytic enzymes such as chitinases and glucosaminidases. Till now, there is no direct synthetic method available for the preparation of partially *N*-acetylated *p*-nitophenyl chitobiosides in the literature *via* chemical glycosylation. In that sense there is a need for the development of efficient synthetic strategies to make these chemical probes. In the following

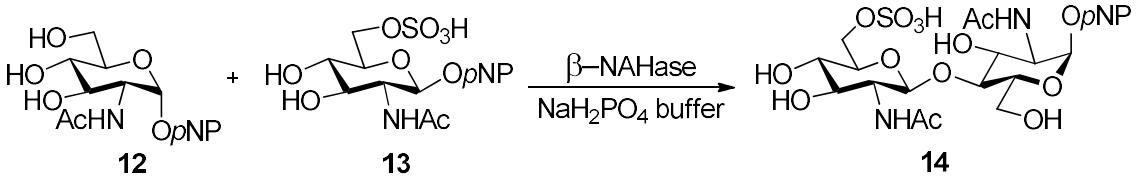
section different enzymatic and chemical methods available in the literature for the synthesis of related *p*-nitrophenyl disaccharides are discussed.

Kubisch *et al*. described a method for the synthesis of *p*-nitrophenyl chitobioside derivative **7** *via* enzymatic glycosylation.[17] β-*N*-Acetylhexoaminidases from *Aspergillus oryzae* acts on *p*-nitrophenyl 2-acetamido-2-*O*-deoxy-β-D-glucopyranoside **10** to generate *p*-nitrophenyl chitobioside **7**. In addition a β-(l→6)-linked regioisomer **11** was also obtained as a minor by-product (Scheme 4.5). Addition of organic co-solvents such as acetonitrile or dioxane and $(NH_4)_2SO_4$ was found to improve the yield.



Scheme 4.5: Synthesis of *N*,*N*'-diacetyl *p*-nitrophenyl chitobioside **7** using β-*N*-acetylhexosaminidase from *A.oryzae*.

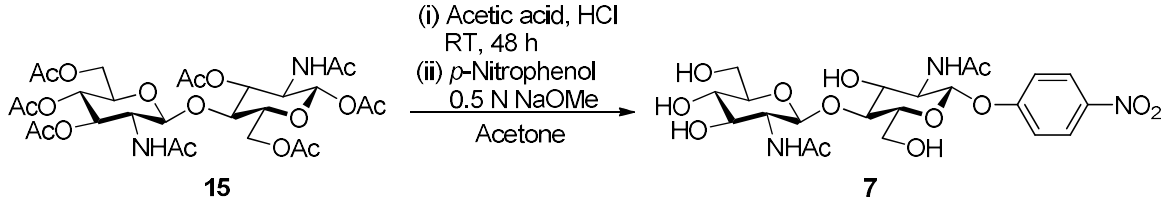
Uzawa and co-workers described a method for the synthesis of *p*-nitrophenyl 6-sulfated chitobiosides *via* enzymatic transglycosylation reaction.[18,19] β-*N*-acetylhexosaminidase from *Aspergillus oryzae* mediated transfer of β-D-(6-sulfo)-GlcNAc unit from **13** to **12** and afforded the *p*-nitrophenyl chitobioside derivative **14** (Scheme 4.6).



Scheme 4.6: Synthesis of *p*-nitrophenyl chitobioside derivative **14** *via* enzymatic transglycosylation raction.

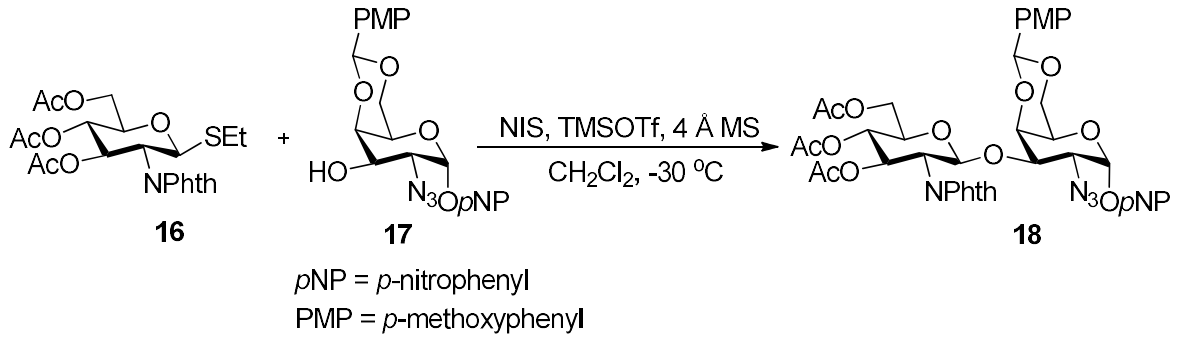
Few reports have been found in the literature for the chemical synthesis of *p*-nitrophenyl chitobiosides and its related derivatives. Osawa *et al*. reported the chemical synthesis of *p*-

nitrophenyl *N,N′*-diacetyl-β-D-chitobioside **7** using chitobiose octaacetate **15** (Scheme 4.7).[20] *N,N′*-diacetylchitobiose derived from chitin was hydrolyzed by acid and then peracetylated to get **15** which on conversion into glycosyl halide followed by treatment with sodium salt of *p*-nitrophenol in acetone yielded the chitobioside derivative **7**. This synthesized substrate was utilized to study the substrate specificities of egg-white lysozyme.



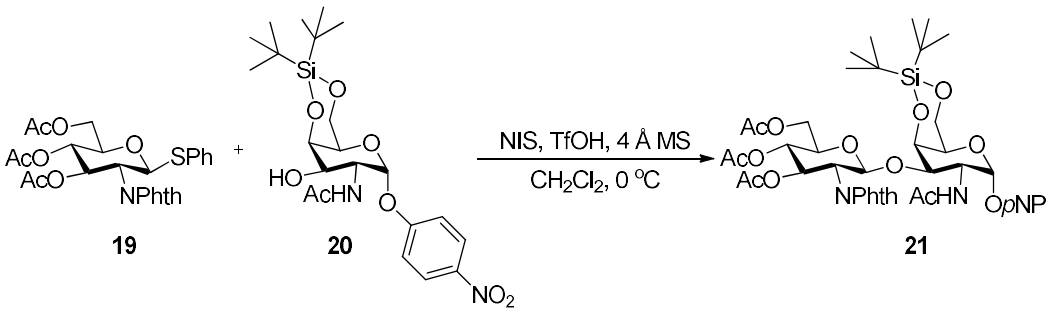
Scheme 4.7: Synthesis of *p*-nitrophenyl chitobioside **7** from chitobiose octaacetate **15**.

Hollinger *et al*. described a method for the synthesis of a related disaccharide building block having β-(1→3) linkage derivative. This *p*-nitrophenyl disaccharide is the core structure in the mucin *O*-glycan. Glycosylation of thioglycosyl donor **16** with 3-hydroxy free acceptor **17** under NIS/TMSOTf activation resulted in the β-(1→3) linked disaccharide building block **18** (Scheme 4.8).[21]



Scheme 4.8: Synthesis of *p*-nitrophenyl chitobiose related derivative **18** using thioglycoside donor **16**.

Sato and co-workers reported a simple method for the synthesis of *p*-nitrophenyl *T*-antigen analogue. *N*-Phth protected thioglycosyl donor **19** upon glycosylation with DTBS protected acceptor **20** gave the intermediate disaccharide **21** (Scheme 4.9).[22] Here the stereoselectivity achieved during the formation of exclusive β-linkage is due to neighbouring group participation from *N*-Phth group.



Scheme 4.9: Synthesis of *p*-nitrophenyl *T*-antigen precursor **21** using *N*-Phth protected thioglycoside donor **19**.

4.2 Results and discussion

After successful synthesis of partially *N*-acetylated chitooligosaccharides (chitobioses and trioses), we shifted our efforts to make chromogenic chitooligosaccharide derivatives, in particular *p*-nitrophenylchitobiosides. The main driving force for making such compounds is the unavailability of chemical methods for the synthesis of *p*-nitrophenyl chitobiosides with different *N*-acetylated pattern. Also they will be useful in evaluating the biological activities of glycosidases and chitinolytic enzymes.

4.2.1 Synthetic strategy for the preparation of *p*-nitrophenyl chitobiosides

For the synthesis of *p*-nitrophenyl chitobiosides having different *N*-acetylated pattern (**AA-*p*NP**, **DD-*p*NP**, **DA-*p*NP** and **AD-*p*NP**), thioglycoside **B** was employed as a glycosyl donor. *N*-Phth and azide containing *p*-nitrophenyl glycosyl derivatives (**A** and **C**) were used as glycosyl acceptors.

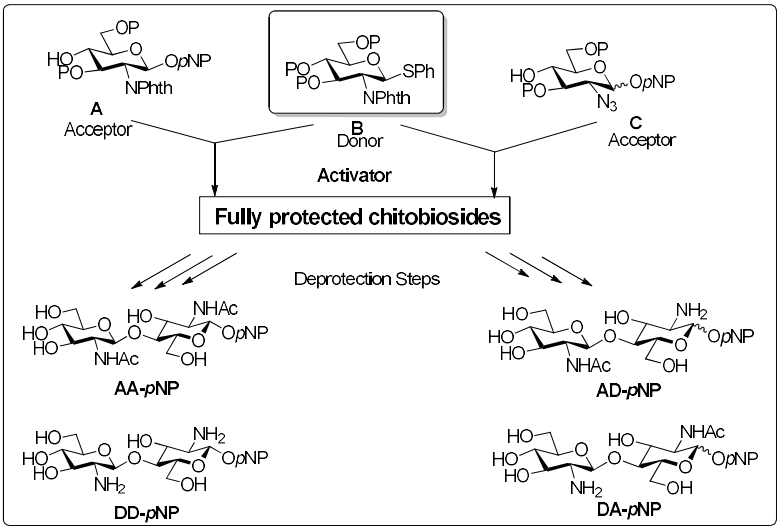
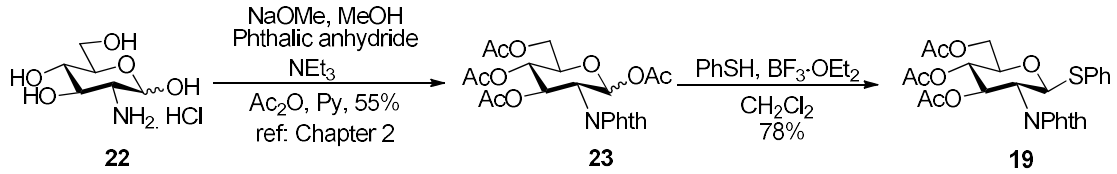


Figure 4.2: Synthetic strategy for the preparation of *p*-nitrophenyl chitobiosides having different *N*-acetylated pattern.

4.2.2. Synthesis of monomer building blocks

4.2.2.1 *N*-Phth protected thioglycoside donor

Compound **23** (1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose) was prepared in 55% yield from commercially available glucosamine hydrochloride **22** in two steps as described in chapter 2. Compound **23** was treated with thiophenol in the presence of $BF_3 \cdot OEt_2$ to obtain phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **19** in 78% yield (Scheme 4.10).[23]



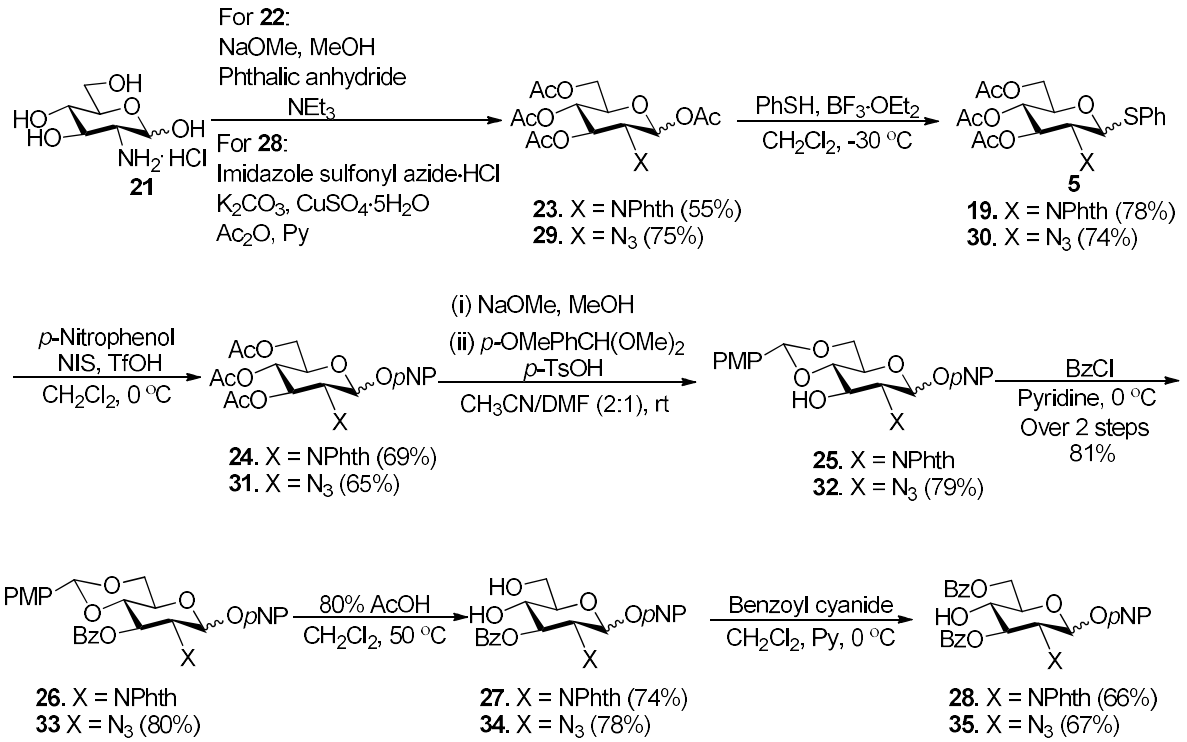
22 **23** **19**

Scheme 4.10: Preparation of phenyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **19**.

4.2.2.2 Synthesis of *N*-Phth and azide containing glycosyl acceptors

Synthesis of *N*-Phth protected and azido glycosyl acceptors **28** and **35** were accomplished from glucosamine hydrochloride **22** by using selective protection–deprotection strategy. Glucosamine hydrochloride was converted into peracetylated phthalimido **23** and azide derivative **29** as described in Chapter 2.[24,25] Compounds **23** and **29** were treated separately with thiophenol in the presence of $BF_3 \cdot OEt_2$ to get, respectively, 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **19**, phenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-α/β-D-glucopyranoside **30** (Scheme 4.11).[26] *p*-Nitrophenyl derivatives **24** and **31** were obtained by the glycosylation of thioglycosyl donors **19** and **30** respectively with *p*-nitrophenol under NIS/TfOH activator system at 0 ºC in CH_2Cl_2. Both the *p*-nitrophenyl derivatives **24** and **31** were treated separately with NaOMe in MeOH to get the corresponding *O*-deacetyl derivatives. The crude triols were treated separately with anisaldehyde dimethyl acetal in the presence of PTSA to get respective *N*-Phth and N_3 containing benzylidene protected glucosides **25** and **32** in 81% and 79% respectively. Benzoylation at 3rd position in compounds **25** and **32** using BzCl in dry pyridine and treatment of the resulting products **26** and **33** with 80% AcOH gave the mono benzoylated derivatives **27** and **34** in 74% and 78% yields respectively. Finally regioselective benzoylation at 6th position[27] of compounds **27** and **34** with benzoyl cyanide in Py/CH_2Cl_2 mixture provided the 4-hydroxy free glycosyl

acceptors **28** and **35** in moderate yields (Scheme 4.11). The products were analysed using different spectral techniques.



Scheme 4.11: Preparation of *N*-Phth and azide containing glycosyl acceptors **28** and **35**.

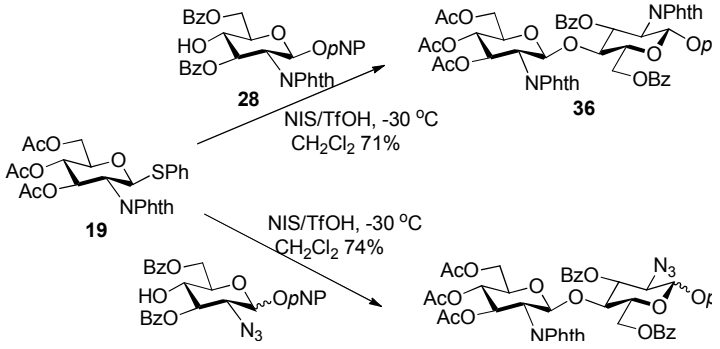
4.2.2.3 Synthesis of disaccharide building blocks

After successfully synthesizing the glycosyl donor **19** and acceptors **28** and **35**, we concentrated on the synthesis of disaccharide building blocks **36** and **37** which are the key intermediates for the synthesis of the desired *p*-nitrophenyl chitobiosides. In presence of NIS/TfOH activator system,[28] glycosylation was carried between glycosyl acceptors **28** and **35** separately with glycosyl donor **19** to obtain the fully protected disaccharides **36** and **37** in 71% and 74% yields respectively. These building blocks were subjected to sequential deprotection steps as described in the following schemes to prepare the desired *p*NP derivatives.

4.2.2.4 Synthesis of *p*-nitrophenyl chitobioside derivatives AA-*p*NP and DD-*p*NP

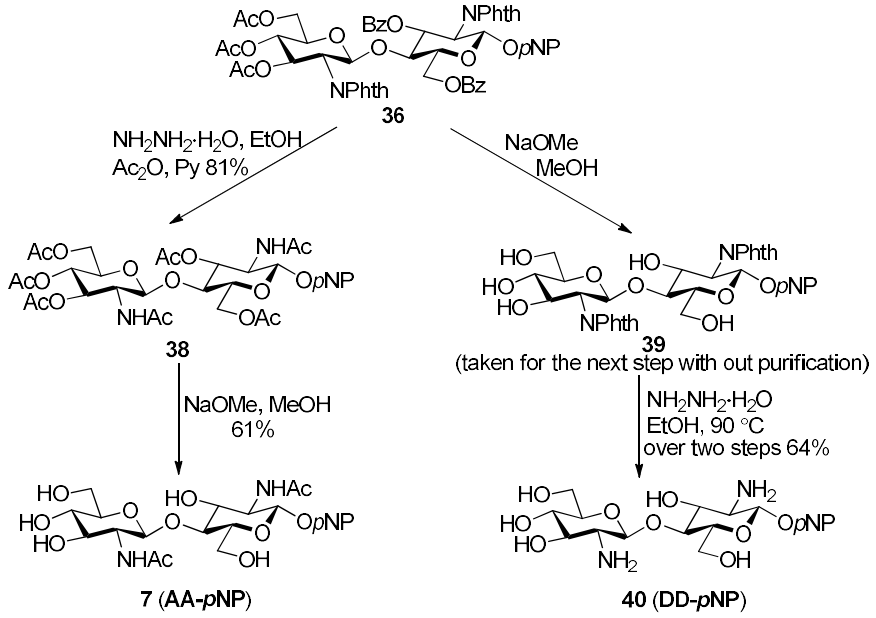
Synthesis of *p*-nitrophenyl *N,N′*-diacetylchitobioside **7** (**AA-*p*NP**) was achieved from **36** by following a sequence of steps as shown in the Scheme 4.12. Removal of two *N*-Phth groups in the disaccharide derivative **36** was done by treating it with hydrazine hydrate in EtOH at 90 ºC. The crude diamine derivative was acetylated using 1:2 Ac_2O/Py mixture to

get the *N*,*N*'-diacetyl derivative **38** in 81% yield. It has to be noted that during the deprotection of phthalimide group benzoyl group also got deprotected. However, subsequent acetylation protected the generated hydroxyl group into its acetate. Finally, deprotection of *O*-acetyl groups from compound **38** under Zemplén condition (NaOMe/MeOH) provided the desired *p*-nitrophenyl *N*,*N*'-diacetylchitobioside **7** (**AA-*p*NP**) in 61% yield (Scheme 4.12).



Scheme 4.11: Synthesis of disaccharide building blocks **36** and **37**.

For the synthesis of *p*-nitrophenyl chitobioside **40** (**DD-*p*NP**), deprotection of acetyl and benzoyl groups in compound **36** by treatment with NaOMe in MeOH solvent was performed first. The crude product **39** underwent smooth dephthalolation on treatment with hydrazine hydrate in EtOH under reflux conditions to give the desired *p*-nitrophenyl chitobioside **40** (**DD-*p*NP**) in 64% yield over two steps (Scheme 4.12). The final products **7** and **40** were characterized by ^{1}H NMR spectroscopy and HRMS-MS.

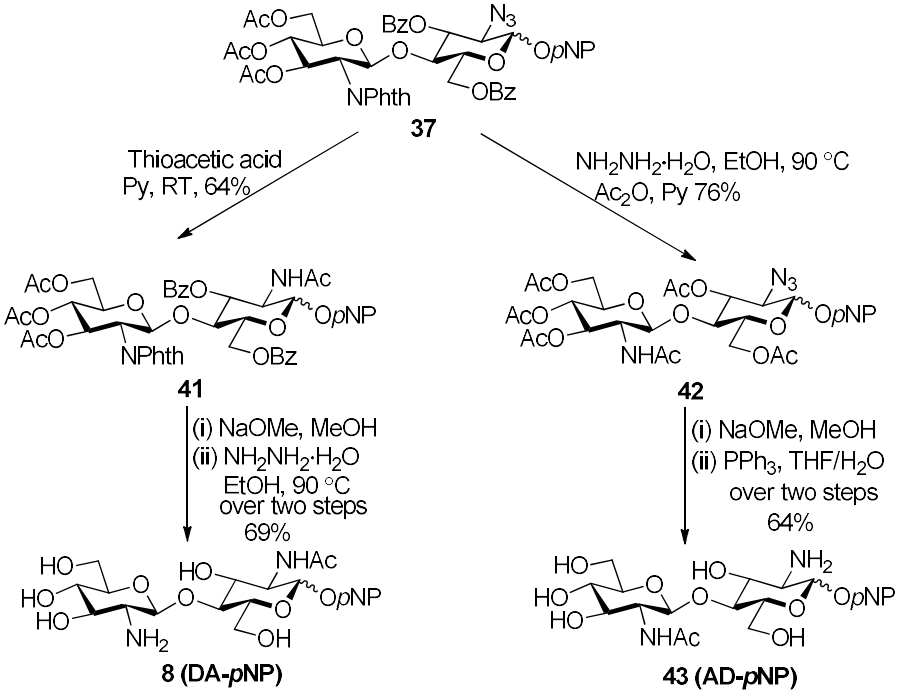


Scheme 4.12: Preparation of *p*-nitrophenyl chitobioside derivatives (**AA-*p*NP** and **DD-*p*NP**).

4.2.2.5 Synthesis of *p*-nitrophenyl chitobioside derivatives (DA-*p*NP and AD-*p*NP)

The synthesis of *p*-nitrophenyl *N*-acetylchitobioside **8** (**DA-*p*NP**) was started with the reductive acetylation of azide in disaccharide **37** by treating it with 1:2 mixture of thioacetic acid/Py[29] to get the *p*-nitrophenyl *N*-acetylated derivative **41** in 64% yield. Deprotection of acetyl and benzoyl groups in compound **41** was achieved under basic conditions (NaOMe in MeOH).[30] Finally, the *N*-Phth group was deprotected using hydrazine hydrate in EtOH at 90 ºC to get the *p*-nitrophenyl chitobioside derivative **8** (**DA-*p*NP**) in 69% (Scheme 4.13).

For the synthesis of mono *N*-acetyl *p*-nitrophenyl chitobioside **43** (**AD-*p*NP**), deprotection of *N*-Phth group in compound **37** using $NH_2NH_2 \cdot H_2O$[31] was carried out first followed by acetylation of the crude amine derivative using Ac_2O/Py (1:2) mixture which yielded the *N*-acetyl derivative **42** in 76% yield. It has to be noted that during the deprotection of phthalimide group, benzoyl groups also got deprotected. However, subsequent acetylation protected the generated hydroxyl groups into its acetate. Compound **42** was deacetylated under basic conditions (NaOMe in MeOH) and the hydrolysis of all the ester groups was confirmed by TLC analysis. Finally, the azide was converted into amine in the crude product by treatment with PPh_3[32] in THF/H_2O mixture to get the *p*-nitrophenyl *N*-acetylchitobiose **43** (**AD-*p*NP**) in 64% (Scheme 4.13).



Scheme 4.13: Preparation of *p*-nitrophenyl chitobiosides derivatives (**DA-*p*NP** and **AD-*p*NP**).

4.2.3 Biological activity profiles of *p*-nitrophenyl chitobiosides

Chitinolytic enzymes are generally two kinds of glycosyl hydrolases *viz*. chitinases and β-*N*-acetyl-hexosaminidases. In insect chitin catabolism, chitinases are responsible for chitin degradation into chitooligosaccharides, whereas glycosidases (GlcNAcase and GlcNase) are involved in chitooligosaccharide hydrolysis into GlcNAcs and GlcNs.[33] Chitinase activity has been estimated using colorimetric method on *p*-nitrophenyl *N*,*N*'-chitobiosides. Enzymatic activity was measured in terms of quantification of liberated yellow coloured *p*-nitrophenol which gives absorbance at 405 nm.[15]

4.2.3.1 Study of substrate specificities of chitinolytic enzymes

We then proceeded to study the structure-activity relationships of chitinases and glucosaminidases using the synthesized *p*-nitrophenyl chitobioside derivatives (AA-*p*NP, DD-*p*NP, AD-*p*NP and DA-*p*NP). For this study, two chitinases (ChiG and Bli), chitosanase (CSN) and chitinosanase which acts on both chitin and chitosan were chosen. Our data suggest that the ChiG chitinase from *streptomyces* (GH19) is inactive on all the four *p*-nitrophenyl chitobiosides as there was no absorbance at 405 nm. Interestingly, Bli chitinase from *bacillus licheniformis* (GH18) showed strong activity on the *p*-nitrophenyl *N*,*N*'-diacetylchitobioside AA-*p*NP and moderate activity on mono *N*-acetyl chitobioside DA-*p*NP. The same enzyme is inactive towards the other two substrates DD-*p*NP and AD-*p*NP as inferred from the Figure 4.3. These results summarise that substrates containing GlcNAc-*p*NP at the reducing end is highly specific for the chitinase GH18 family. CSN chitosanase from *bacillus sp* (GH8) showed strong activity on AA-*p*NP and weak activity towards DA-*p*NP and DD-pNP. No significant activity of chitinosanase from *Streptomyces* (GH46) on any of the *p*-nitrophenyl chitobioside derivatives was noted. These results suggest that the specificity of CSN chitosanase and Bli chitinase with the *p*-nitrophenyl derivatives can differentiate them from the ChiG and chitinosanase.

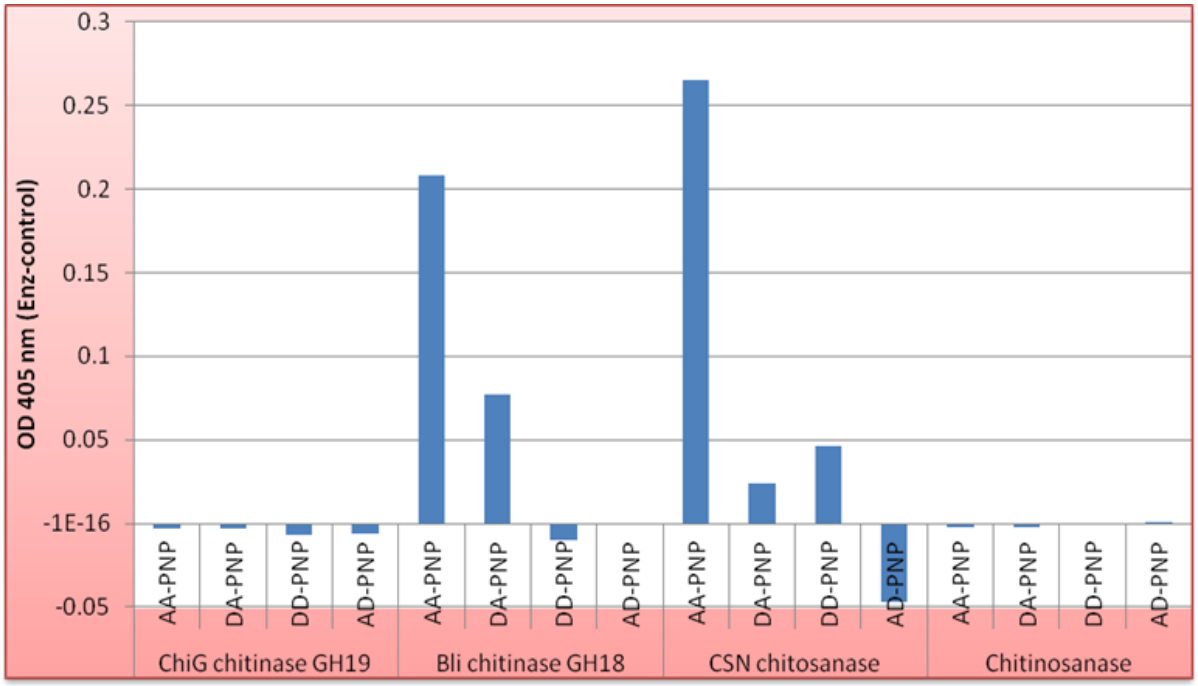


Figure 4.3: Substrate specificity of chitonolytic enzymes with *p*-nitrophenyl chitobiosides.

4.2.3.2 Glucosidase activity

Glucosidase activities of GlcNAcase and GlcNase were studied with the synthesized *p*NP probes. β-*N*-acetylglucosaminidase (GlcNAcase) was active on the substrate $(GlcNAc)_2$-*p*NP stepwise from its non-reducing end. In the same way glucosaminidases (GlcNase) degrade $(GlcN)_2$-pNP from non-reducing end side in a stepwise manner. Finally, the activity was quantified by measuring the absorbance at 405 nm due to the liberation of *p*-nitrophenol using a microplate reader.[15,16] GlcNAcase showed strong activity on the AA-*p*NP by the cleavage from the non-reducing end and eventually releases the *p*-nitrophenol and GlcNAc residues, whereas GlcNase was inactive on the AA-*p*NP (Figure 4.4). On the other hand DD-*p*NP was cleaved by GlcNase to release the GlcN residues and the liberation of *p*-nitrophenol as confirmed and quantified by UV-absorbance at 405 nm, while GlcNAcase showed no activity on DD-*p*NP (Figure 4.4). Interestingly, GlcNcase showed moderate activity on DA-*p*NP by cleaving finally the GlcNAc and pNP units at the reducing end. But it is inactive on AD-*p*NP substrate. In case of AD-*p*NP none of the glycosidase (GlcNAcase/GlcNase) showed any activity. A mixture of both glycosidases is active on DA-*p*NP but inactive on AD-*p*NP. These observations suggest that substrate specificities of glucosidases (GlcNAcase or GlcNase) with different *N*-acetylated *p*-nitrophenyl chitobiosides. Therefore these compounds might find application for the screening glycosidase inhibitors which can serve as a drugs and pesticides.

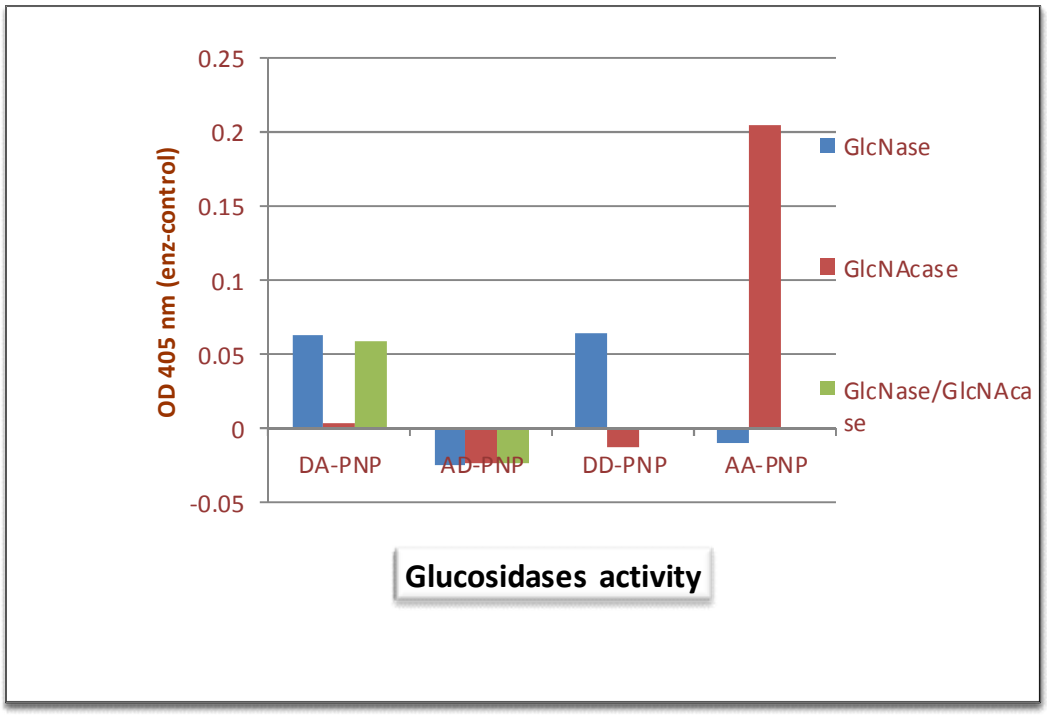


Figure 4.4: Glucosidases activity on *p*-nitrophenyl chitobiosides having different *N*-acetylated pattern.

4.3 Conclusions

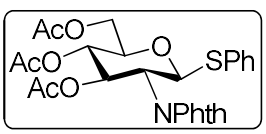
We have developed efficient synthetic strategies for the preparation of *p*-nitrophenyl chitobiosides having different *N*-acetylated pattern. These synthesized *p*NP chitobiosides were used to study the substrate specificities of chitinases/chitosanases and glucosidases. Biological data suggests that different *N*-acetylated *p*NP chitobiosides (AA-*p*NP, DA-*p*NP, DD-*p*NP) are good chromogenic substrates to differentiate chitinolytic enzymes and could in principle be used as substrates for screening glycosyl hydrolases inhibitors.

4.4 Experimental section

For general information refer Chapter 2, Section 2.4.1.

4.4.1 Experimental procedures, spectral and analytical data

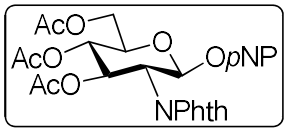
Preparation of phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside 19



The compound **19** was prepared from **23** (4.0 g, 7.75 mmol) by following the literature procedure.[20] Colourless foam; Yield = 6.8 g

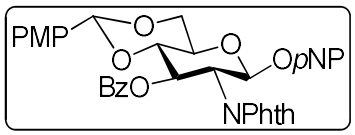
(78%); R_f = 0.65 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01-7.70 (m, 4H, *N*-Phth), 5.81 (t, *J* = 9.7 Hz, 1H), 5.72 (d, *J* = 10.5 Hz, 1H), 5.14 (t, *J* = 9.7 Hz, 1H), 4.35 (t, *J* = 10.3 Hz, 1H), 4.30 (dd, *J* = 12.3, 5.5 Hz, 1H), 4.21 (dd, *J* = 12.3, 2.3 Hz, 1H), 3.87 (m, 1H), 2.10 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.84 (s, 3H, OAc). ^{13}CNMR (100 MHz, $CDCl_3$): δ 170.7, 170.2, 169.5, 167.8, 134.5, 133.9, 133.3, 132.9, 132.2, 129.0, 128.9, 128.4, 123.7, 123.4, 83.0, 75.9, 71.6, 68.7, 62.2, 53.5, 20.7, 20.6, 20.4.

Preparation of *p*-nitrophenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside 24



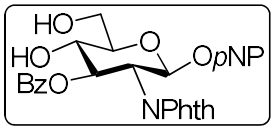
The compound **24** is prepared from **19** (6.0 g, 11.01 mmol) by using general procedure for glycosylation with thioglycosides (Chapter 3). Yellow solid; Yield = 4.2 g (69%); R_f = 0.31 in 1:3 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (m, 2H), 7.66 (dd, *J* = 7.0, 4.1 Hz, 2H) 5.81 (t, *J* = 9.7 Hz, 1H), 5.72 (d, *J* = 10.5 Hz, 1H), 5.14 (t, *J* = 9.7 Hz, 1H), 4.35 (t, *J* = 10.3 Hz, 1H), 4.30 (dd, *J* = 12.3, 5.5 Hz, 1H), 4.21 (dd, *J* = 12.3, 2.3 Hz, 1H), 3.87 (m, 1H), 2.10 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.84 (s, 3H, OAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.1, 169.4, 167.8, 138.7, 138.1, 134.4, 131.3, 129.0, 128.2, 128.1, 127.4, 127.3, 127.1, 97.2, 80.8, 75.1, 74.4, 74.2, 72.7, 71.6, 70.6, 68.5, 68.2, 61.4, 55.2, 20.7, 20.5, 20.3. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{26}H_{24}N_2O_{12}$ 579.1227, found 579.1229.

Preparation of *p*-nitrophenyl 3-*O*-benzoyl-4,6-*O*-*p*-methoxybenzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside 26



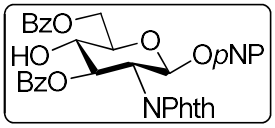
The compound **26** is prepared from **24** (3 g, 5.43 mmol) by following the literature procedure.[9] Light yellow solid; Yield = 2.87 g (81%); R_f = 0.54 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14-8.11 (m, 4H), 7.88 (d, *J* = 7.6 Hz, 1H), 7.77 (t, *J* = 7.5 Hz, 2H), 7.58 (t, *J* = 7.4 Hz, 2H), 7.47-7.44 (m, 2H), 7.37-7.32 (m, 3H), 7.04 (d, *J* = 9.2 Hz, 2H), 6.83 (d, *J* = 8.9 Hz, 2H), 6.31 (d, *J* = 8.4 Hz, 1H), 5.57-5.52 (m, 1H), 4.82 (dd, *J* =10.1, 8.4 Hz, 1H), 4.47 (dd, *J* = 10.9, 4.2 Hz, 1H), 4.05 (d, *J* = 8.3 Hz, 1H), 3.93-3.85 (m, 2H), 3.74 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.3, 168.0, 167.1, 166.8, 166.7, 161.0, 142.8, 134.4, 133.5, 133.4, 132.4, 131.5, 131.3, 131.0, 129.9, 129.7, 128.9, 128.6, 128.5, 128.3, 128.0, 125.6, 123.6, 116.4, 95.1, 83.1, 79.8, 76.3, 75.0, 73.7, 69.9, 69.7, 62.2, 61.8, 60.4, 54.2, 53.6. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{35}H_{28}N_2O_{11}$ 675.1591, found 675.1583.

Preparation of *p*-nitrophenyl 3-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside 27



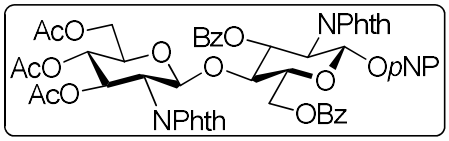
The compound **27** is prepared from **26** (1.0 g, 1.53 mmol) by following the literature procedure.[9] Light yellow liquid; Yield = 0.60 g (74%); R_f = 0.30 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (d, *J* = 9.0 Hz, 2H), 7.85-7.75 (m, 5H), 7.67-7.64 (m, 2H), 7.24-7.23 (m, 2H), 7.06 (d, *J* = 9.2 Hz, 2H), 6.35 (d, *J* = 8.5 Hz, 1H), 6.10 (dd, *J* = 10.4, 8.6 Hz, 1H), 6.00 (t, *J* = 9.9 Hz, 1H), 5.90 (d, *J* = 10.5 Hz, 1H), 4.75 (dd, *J* = 10.6, 8.5 Hz, 1H), 4.50 (t, *J* = 10.4 Hz, 1H), 4.10-3.90 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 166.9, 160.9, 142.9, 134.5, 133.6, 132.5, 131.1, 130.1, 129.8, 129.0, 128.4, 125.7, 123.6, 123.6, 116.4, 95.1, 83.1, 79.8, 76.3, 75.1, 73.9, 69.9, 61.9, 54.1, 53.6. LCMS (m/z): $[M+Na]^+$ calcd for $C_{27}H_{22}N_2O_{10}$ 557.12, found 557.24. Anal. calcd. for $C_{27}H_{22}N_2O_{10}$: C, 60.67; H, 4.15; N, 5.24, found C, 60.53; H, 4.19; N, 5.32.

Preparation of *p*-nitrophenyl 3,6-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside 28



The compound **28** is prepared from **27** (0.6 g, 1.13 mmol) by following the literature procedure.[9] Colourless solid; Yield = 0.48 g (66%); R_f = 0.42 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10-8.05 (m, 3H), 8.00 (d, *J* = 9.2 Hz, 2H), 7.87 (d, *J* = 7.6 Hz, 2H), 7.78 (t, *J* = 4.5 Hz, 2H), 7.50-7.45 (m, 5H), 7.34 (t, *J* = 7.6 Hz, 2H), 7.06 (d, *J* = 8.1 Hz, 2H), 6.33-6.30 (m, 1H), 6.13-6.08 (m, 1H), 4.86-4.78 (m, 2H), 4.72 (d, *J* = 12.2, 6.8 Hz, 1H), 4.31-4.27 (br s, 1H), 4.01 (t, *J* = 9.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.8, 166.5, 160.9, 142.9, 134.5, 133.7, 133.5, 132.9, 131.1, 130.1, 129.8, 129.8, 129.7, 129.4, 128.8, 128.5, 128.4, 125.5, 123.7, 116.6, 95.2, 83.0, 78.3, 74.8, 73.9, 70.4, 63.6, 60.4, 54.1. HRMS-ESI (m/z): $[M+K]^+$ calcd for $C_{34}H_{26}N_2O_{11}$ 677.1174, found 677.1134. Anal. calcd. for $C_{34}H_{26}N_2O_{11}$: C, 63.95; H, 4.10; N, 4.39, found C, 63.85; H, 4.03; N, 4.48.

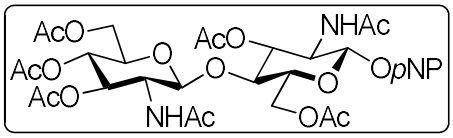
Preparation of *p*-nitrophenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside 36



The compound **36** was prepared from **19** (0.46 g, 0.84 mmol) and **28** (0.4 g, 0.70 mmol) by using general procedure for glycosylation with thioglycosides (Chapter

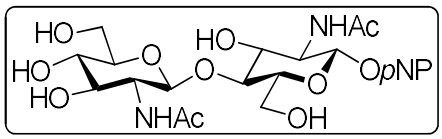
3). Colourless foam; Yield = 0.63 g (71%); R_f = 0.41 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (d, *J* = 7.5 Hz, 4H), 7.85-7.58 (m, 12H), 7.48-7.43 (m, 4H), 6.91 (d, *J* = 8.7 Hz, 1H), 6.27-6.14 (m, 2H), 5.66 (d, *J* = 8.2 Hz, 1H), 5.59 (t, *J* = 10.3 Hz, 1H), 5.01 (t, *J* = 9.3 Hz, 1H), 4.68-4.57 (m, 2H), 4.30-4.22 (m, 3H), 4.17-4.10 (m, 2H), 3.83 (d, *J* = 11.2 Hz, 1H), 3.60 (d, *J* = 11.9 Hz, 1H), 2.01 (s, 3H), 1.90 (s, 3H), 1.78 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.7, 170.5, 170.1, 169.2, 164.9, 160.7, 142.9, 134.4, 133.3, 131.2, 130.8, 129.6, 128.7, 128.4, 125.5, 123.7, 116.7, 114.0, 97.5, 95.1, 76.1, 72.9, 71.8, 71.6, 70.3, 67.9, 62.3, 60.8, 54.8, 54.5, 29.6, 20.6, 20.4, 20.3. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{54}H_{45}N_3O_{20}$ 1078.2494, found 1078.2488.

Preparation of *p*-nitrophenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside 38



The compound **38** is prepared from **36** (0.4 g, 0.38 mmol) by using general procedure for deprotection of phthalimide protecting group followed by acetylation (Chapter 2). Light yellow solid; Yield = 0.23 g (81%); R_f = 0.27 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (d, *J* = 8.9 Hz, 2H), 7.09 (d, *J* = 8.9 Hz, 2H), 5.26 (t, *J* = 6.4 Hz, 1H), 5.17 (t, *J* = 9.8 Hz, 1H), 5.02 (t, *J* = 10.1 Hz, 1H), 4.53 (d, *J* = 8.1 Hz, 1H), 4.45-4.30 (m, 5H), 4.07-4.04 (m, 2H), 3.83 (d, *J* = 7.2 Hz, 1H), 3.68 (d, *J* = 9.0 Hz, 2H), 2.01-1.97 (m, 21H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 177.5, 170.4, 169.3, 169.2, 166.6, 165.2, 142.9, 129.8, 129.1, 116.3, 98.1, 97.9, 75.4, 74.9, 72.8, 72.2, 71.9, 70.3, 69.3, 68.1, 67.9, 61.9, 60.8, 54.9, 52.3, 29.6, 23.3, 23.0, 21.1, 20.7, 20.5, 20.3. LCMS (m/z): $[M+Na]^+$ calcd for $C_{32}H_{41}N_3O_{18}$ 778.23, found 778.35. Anal. calcd. for $C_{32}H_{41}N_3O_{18}$: C, 50.86; H, 5.47; N, 5.56, found C, 50.72; H, 5.53; N, 5.48.

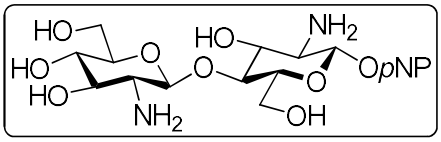
Preparation of *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-α/β-D-glucopyranoside 7



The compound **7** is prepared from **36** (35 mg, 0.059 mmol) by using general procedure for *O*-deacetylation (Chapter 2). The crude product was purified by flash column chromatography on RP C18 silica gel (H_2O–MeOH) afforded a colourless solid; Yield = 13 mg (61%); ^{1}H NMR (400 MHz, D_2O): δ 8.15 (d, *J* = 8.9 Hz, 2H), 7.07 (d, *J* = 8.8 Hz, 2H), 5.23 (d, *J* = 8.3 Hz, 1H), 4.53 (d, *J* = 8.3 Hz, 1H), 3.97 (t, *J* = 8.3 Hz, 1H), 3.86-3.79

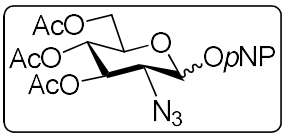
(m, 2H), 3.68-3.60 (m, 6H), 3.51-3.39 (m, 5H), 1.99 (s, 3H), 1.92 (s, 3H). HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{22}H_{31}N_3O_{13}$ 568.1755, found 568.1725.

Preparation of *p*-nitrophenyl 2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranoside 40



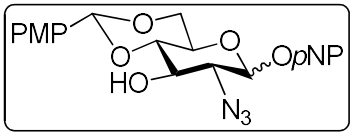
The compound **40** is prepared from **36** (40 mg, 0.056 mmol) by using general procedure for *O*-deacetylation and deprotection of phthalimide protecting group (Chapter 2). The crude product was purified by flash column chromatography on RP C18 silica gel (H_2O–MeOH) afforded as a colourless solid. Yield = 16 mg (64%); ^{1}H NMR (400 MHz, D_2O): δ 8.23 (d, *J* = 9.1 Hz, 2H), 7.20 (d, *J* = 9.2 Hz, 2H), 5.16 (d, *J* = 7.8 Hz, 1H), 4.45 (d, *J* = 7.9 Hz, 1H), 4.05 (d, *J* = 7.0 Hz, 1H), 3.83-3.57 (m, 8H), 3.47-3.43 (m, 2H), 3.37-3.33 (m, 3H). HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{18}H_{27}N_3O_{11}$ 484.1543, found 484.1524.

Preparation of *p*-nitrophenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α/β-D-glucopyranoside 31



The compound **31** is prepared from **30** (4 g, 9.45 mmol) by following the literature procedure.[9] Yellow solid; Yield = 2.78 g (65%); R_f = 0.47 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, *J* = 9.1 Hz, 2.6H), 7.23 (d, *J* = 9.2 Hz, 2.3H), 6.28 (d, *J* = 3.4 Hz, 1H), 5.71 (d, *J* = 3.3 Hz, 1H), 5.68-5.63 (m, 1H), 5.58-5.54 (m, 0.3H), 5.15 (t, *J* = 10.3, 1H), 5.10-5.05 (m, 1.2H), 4.29-4.20 (m, 2.4H), 4.11-4.03 (m, 3H), 3.71-3.64 (m, 0.8H), 3.60 (dd, *J* = 10.6, 3.3 Hz, 1H), 2.04 (s, 4H), 2.03 (s, 7H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.3, 170.3, 169.9, 169.5, 160.3, 143.3, 132.2, 129.9, 129.3, 125.9, 116.6, 96.5, 70.0, 69.0, 67.9, 62.5, 61.3, 60.5, 28.5, 20.6, 20.5. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{18}H_{20}N_4O_{10}$ 475.1077, found 475.1071.

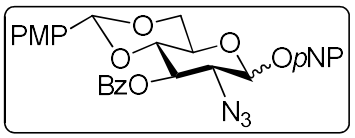
Preparation of *p*-nitrophenyl 2-azido-4,6-*O*-*p*-methoxybenzylidene-2-deoxy-α/β-D-glucopyranoside 32



The compound **32** is prepared from **31** (2.5 g, 5.52 mmol) by following the literature procedure.[9] Light yellow liquid; Yield = 1.94 g (79%); R_f = 0.57 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (d, *J* = 9.1 Hz, 1.5H), 7.81 (s, 1.6H), 7.40-7.25 (m, 5H), 7.08-7.06 (m, 2H), 6.77 (d, *J* = 8.6 Hz, 1H), 5.53-5.41 (m, 2H), 4.42 (dd, *J* = 10.3, 3.9 Hz,

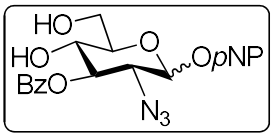
0.3H), 4.30 (m, 1H), 4.12-4.04 (m, 1H), 3.81-3.77 (m, 0.7H), 3.67 (s, 3H), 3.57-3.52 (m, 1.5H), 3.43 (dt, *J* = 8.1, 3.8 Hz, 0.7H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.3, 142.5, 136.6, 132.9, 129.1, 128.8, 128.7, 128.5, 127.9, 127.3, 126.0, 125.4, 124.1, 116.2, 115.9, 113.2, 101.6, 96.6, 81.0, 80.9, 68.1, 67.9, 63.6, 62.5, 54.9. HRMS-ESI (m/z): $[M+K]^+$ calcd for $C_{20}H_{20}N_4O_8$ 483.0918, found 483.0953.

Preparation of *p*-nitrophenyl 2-azido-3-*O*-benzoyl-4,6-*O*-*p*-methoxybenzylidene-2-deoxy-α/β-D-glucopyranoside 33



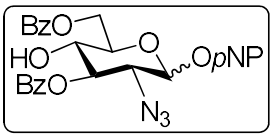
The compound **33** is prepared from **32** (1.90 g, 4.27 mmol) by following the literature procedure.[9] Colourless solid; Yield = 1.87 g (80%); R_f = 0.55 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (d, *J* = 9.2 Hz, 2H), 8.13 (d, *J* = 7.3 Hz, 3H), 7.63-7.57 (m, 2H), 7.49-7.41 (m, 5H), 7.36-7.27 (m, 3H), 7.22 (d, *J* = 9.1 Hz, 2H), 6.10 (dt, *J* = 9.9, 4.1 Hz, 1H), 5.81 (s, 1H), 5.58-5.54 (m, 1H), 4.30-4.24 (m, 1H), 4.27 (dt, *J* = 9.8, 4.9 Hz, 1H), 4.15-4.08 (m, 1H), 3.97 (q, *J* = 9.2 Hz, 1H), 3.87-3.80 (m, 1.4H), 3.73 (s, 3.7H).^{13}C NMR (100 MHz, $CDCl_3$): δ 165.3, 160.3, 143.0, 133.5, 133.3, 129.9, 129.7, 129.0, 128.3, 128.0, 127.3, 125.9, 125.7, 116.4, 113.7, 101.6, 97.2, 78.8, 69.1, 68.2, 68.1, 61.3, 55.0. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{27}H_{24}N_4O_9$ 571.1441, found 571.1447.

Preparation of *p*-nitrophenyl 2-azido-3-*O*-benzoyl-2-deoxy-α/β-D-glucopyranoside 34



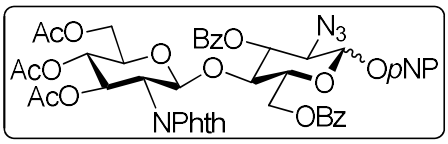
The compound **34** is prepared from **33** (1.8 g, 3.29 mmol) by following the literature procedure.[9] Colourless solid; Yield = 1.1 g (78%); R_f = 0.33 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, CD_3OD+$CDCl_3$): δ 8.34 (d, *J* = 9.3 Hz, 2H), 8.22-8.19 (m, 2H), 7.72 (t, *J* = 6.1 Hz, 1H), 7.63-7.57 (m, 2H), 7.49-7.44 (m, 2H), 7.29-7.17 (m, 1H), 6.02 (dd, *J* = 5.9, 3.8 Hz, 1H), 5.89-5.83 (m, 1H), 4.67 (d, *J* = 5.4 Hz, 0.3H), 3.97-3.93 (m, 1H), 3.86-3.80 (m, 4H), 3.40-3.38 (m, 3H), 2.39 (br s, 1H). ^{13}C NMR (100 MHz, CD_3OD+$CDCl_3$): δ 166.0, 160.7, 142.5, 133.0, 129.3, 129.0, 127.9, 125.4, 116.3, 96.7, 73.3, 72.6, 67.4, 60.6, 59.9. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{19}H_{18}N_4O_8$ 453.1022, found 453.1032.

Preparation of *p*-nitrophenyl 2-azido-3,6-di-*O*-benzoyl-2-deoxy-α/β-D-glucopyranoside 35



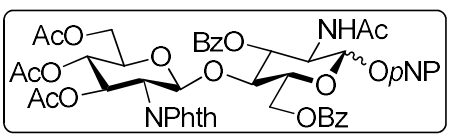
The compound **35** is prepared from **34** (0.9 g, 2.1 mmol) by following the literature procedure.[9] Colourless solid; Yield = 0.75 g (67%); R_f = 0.58 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (d, *J* = 8.9 Hz, 0.6Hz), 8.13 (t, *J* = 7.4 Hz, 2H), 8.00-7.89 (m, 1.9H), 7.78 (t, *J* = 7.8 Hz, 1.8H), 7.64-7.49 (m, 3H), 7.44-7.36 (m, 7.4H), 7.29-7.27 (m, 1H), 6.18 (t, *J* = 9.7 Hz, 0.2H), 5.29-5.83 (m, 1.1H), 5.62 (t, *J* = 9.6 Hz, 0.2H), 3.90-3.81 (m, 1H), 3.71-3.65 (m, 2H), 3.36 (br s, 1.9H), 2.71 (dd, *J* = 10.6, 4.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.8, 167.1, 160.9, 143.0, 134.5, 133.8, 133.6, 131.2, 129.9, 129.5, 128.6, 125.6, 123.8, 116.7, 95.3, 90.6, 74.9, 74.1, 70.5, 63.5, 54.1, 53.5. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{26}H_{22}N_4O_9$ 535.1465, found 535.1461.

Preparation of *p*-nitrophenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzoyl-2-deoxy-D-glucopyranoside 37



The compound **37** is prepared **19** (0.62 g, 1.13 mmol) and **35** (0.5 g, 0.94 mmol) by using general procedure for glycosylation with thioglycosides (Chapter 3). Light yellow solid; Yield = 0.66 g (74%); R_f = 0.48 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (d, *J* = 7.7 Hz, 2H), 8.13-8.09 (m, 2H), 7.74-7.66 (m, 6H), 7.60-7.55 (m, 5H), 7.44-7.39 (m, 4H), 7.12 (d, *J* = 9.0 Hz, 2H), 6.91 (d, *J* = 9.1 Hz, 1H), 6.19 (d, *J* = 8.9 Hz, 0.5H), 6.12 (d, *J* = 9.1 Hz, 1H), 5.87-5.82 (m, 1H), 5.71 (d, *J* = 8.3 Hz, 1H), 5.64-5.56 (m, 2H), 5.28 (d, *J* = 6.6 Hz, 0.5H), 5.08-5.00 (m, 1H), 4.57-4.51 (m, 2H), 4.31-4.11 (m, 3H), 3.73 (d, *J* = 11.3 Hz, 1H), 3.55-3.49 (m, 1H), 3.26 (d, *J* = 10.1 Hz, 0.7H), 2.02 (s, 4H), 1.90 (s, 5H), 1.83 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.4, 170.4, 169.9, 169.1, 166.5, 165.1, 161.1, 160.2, 142.8, 134.3, 134.2, 133.3, 129.8, 129.5, 129.0, 128.3, 125.8, 125.5, 123.6, 116.2, 98.0, 97.8, 95.3, 75.3, 74.9, 72.7, 72.1, 71.8, 70.2, 69.3, 68.1, 67.7, 61.7, 60.7, 54.9, 52.2, 29.5, 22.9, 20.7. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{46}H_{41}N_5O_{18}$ 974.2344, found 974.2378. Anal. calcd. for $C_{46}H_{41}N_5O_{18}$: C, 58.04; H, 4.34; N, 7.36, found C, 58.16; H, 4.30; N, 7.45.

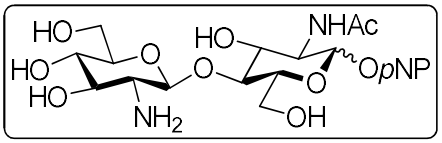
Preparation of *p*-nitrophenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-D-glucopyranoside 41



The compound **41** is prepared from **37** (0.3 g, 0.32 mmol) by using general procedure for reductive

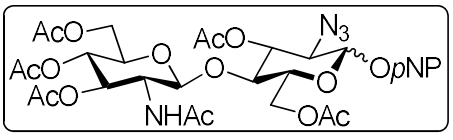
acetylation of azide group (Chapter 2). Yellow solid; Yield = 0.198 g (64%); R_f = 0.32 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19-8.04 (m, 4H), 7.96 (d, *J* = 9.2 Hz, 1.3H), 7.72 (t, *J* = 8.3 Hz, 5.7H), 7.58-7.53 (m, 6.5H), 7.43-7.40 (m, 3H), 7.12 (d, *J* = 8.9 Hz, 1H), 6.91 (d, *J* = 9.1 Hz, 1H), 6.21 (d, *J* = 9.4 Hz, 0.6H), 6.10 (d, *J* = 8.9 Hz, 0.4H), 6.00 (d, *J* = 8.6 Hz, 0.3H), 5.86-5.81 (m, 1H), 5.70 (t, *J* = 8.6 Hz, 1H), 5.65-5.56 (m, 2.6H), 5.28 (d, *J* = 6.5 Hz, 0.5), 5.07-4.96 (m, 1.5H), 4.58-4.39 (m, 2H), 4.33-4.17 (m, 4H), 3.98-3.91 (m, 2.5H), 2.03 (s, 3.6H), 1.95 (s, 3.8H), 1.90 (s, 3.4H), 1.8 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.4, 169.2, 167.3, 160.1, 132.3, 129.9, 129.4, 128.8, 128.4, 128.6, 125.9, 125.7, 123.8, 116.5, 98.0, 96.7, 89.7, 72.5, 71.9, 71.7, 71.0, 70.4, 70.3, 69.5, 68.2, 67.9, 61.8, 60.8, 54.8, 29.6, 28.5, 20.7, 20.4. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{48}H_{45}N_3O_{19}$ 990.2545, found 990.2540.

Preparation of *p*-nitrophenyl 2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranoside 8



The compound **8** is prepared from **41** (40 mg, 0.04 mmol) by using general procedure for *O*-deacetylation and deprotection of phthalimide protecting group (Chapter 2). The crude product was purified by flash column chromatography on RP C18 silica gel (H_2O–MeOH) afforded as a colourless solid. Yield = 13.3 mg (69%); ^{1}H NMR (400 MHz, D_2O): δ 8.14 (d, *J* = 8.5 Hz, 2H), 7.16 (d, *J* = 8.9 Hz, 2H), 5.68 (s, 1H), 4.41 (d, *J* = 7.0 Hz, 1H), 4.12-4.05 (m, 2H), 3.85-3.68 (m, 7H), 1.93 (s, 3H). HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{20}H_{29}N_3O_{12}$ 504.1829, found 504.1819.

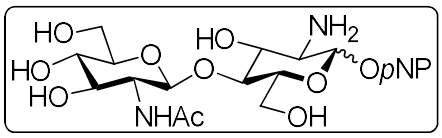
Preparation of *p*-nitrophenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-azido-3,6-di-*O*-acetyl-2-deoxy-D-glucopyranoside 42



The compound **42** is prepared from **37** (0.25 g, 0.27 mmol) by using general procedure for deprotection of phthalimide protecting group followed by acetylation (Chapter 2). Light yellow solid; Yield = 0.15 g (76%); R_f = 0.34 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, *J* = 8.3 Hz, 2.8H), 7.10 (d, *J* = 9.0 Hz, 2H), 6.08 (d, *J* = 8.6 Hz, 1H), 5.95 (d, *J* = 8.4 Hz, 1H), 5.67-5.64 (m, 11H), 5.33 (td, *J* = 9.8, 5.0 Hz, 2H), 5.07-5.20 (m, 3H), 4.76 (t, *J* = 8.0 Hz, 1H), 4.46-4.39 (m, 3H), 4.25-4.19 (m, 2H), 4.07-4.05 (m, 2H), 3.81-3.68 (m, 5H), 3.55 (dd, *J* = 10.9, 3.5 Hz, 1H), 2.06-1.88 (m, 25H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.5, 169.8, 169.4, 160.4, 143.2, 125.9, 116.6, 100.5, 99.2, 96.4, 71.8, 68.1, 61.7, 60.6, 29.6, 29.3, 23.1, 20.8, 20.7, 20.6. LCMS (m/z): $[M+Na]^+$ calcd

for $C_{30}H_{37}N_5O_{17}$ 762.21, found 762.60. Anal. calcd. for $C_{30}H_{37}N_5O_{17}$: C, 48.72; H, 5.04; N, 9.47, found C, 48.65; H, 5.12; N, 9.36.

Preparation of *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-2-deoxy-D-glucopyranose 43



The compound **42** is prepared from **41** (41 mg, 0.056 mmol) by using general procedure for *O*-deacetylation (Chapter 2) and reduction of azide using PPh_3 in THF/H_2O.[33] The crude product was purified by flash column chromatography on RP C18 silica gel (H_2O–MeOH) afforded as a colourless solid. Yield = 17 mg (64%); ^1H NMR (400 MHz, D_2O): δ 8.18 (br s, 2H), 7.20 (d, J = 6.8 Hz, 2H), 5.73-5.68 (m, 1H), 4.51 (br s, 1H), 4.07-3.66 (m, 10H), 3.51-3.33 (m, 4H), 1.92 (s, 3H). HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{20}H_{29}N_3O_{12}$ 526.1649, found 526.1631.

4.4.2 Enzyme assays for chitinolytic enzymes and glycosidases using *p*NP-chitobiosides as the substrates[15]

p-Nitrophenyl chitobioside derivatives were used as substrates for chitinolytic enzymes (chitinases/chitosanases) and glycosidases (GlcNAcase/GlcNase) assays at the final concentration of 2 mM. The reaction mixture (25 µL) composed of the substrate (2 mM), 20 mM TEA buffer (pH 8.0) in case of ChiG and Bli chitinase and an aliquot of the enzyme was incubated for 60 min at 37 ºC. NaOAc buffer (pH 6.0 and 4.2) was used for the CSN and chitosanases at 30 ºC. Both the buffers have been used to know the activity of glycosidases (GlcNAcase/GlcNase). Finally 25 mL of 0.1 M NaOH was added to stop the reaction and to increase the pH. Measurement of absorbance of the liberated *p*-nitrophenol was measured at 405 nm using a microplate reader (model 3550, Bio Rad).

4.5 References

1. Thuan, N. H.; Sohng, J. K. *J. Ind. Microbiol. Biotechnol*. **2013**, *40*, 1329.

2. Hers, H. G. *Biochem. J*. **1963**, *86*, 11.

3. Wolfenden, R.; Lu, X.; Young, G. *J. Am. Chem. Soc*. **1998**, *120*, 6814.

4. Stubbs, K. A. *Carbohydr. Res*. **2014**, *390*, 9.

5. Zechel, D. L.; Withers, S. G. *Acc. Chem. Res*. **2000**, *33*, 11.

6. Rye, C. S.; Withers, S. G. *Curr. Opin. Chem. Biol*. **2000**, *4*, 573.

7. Aamlid, K. H.; Lee, G.; Smith, B. V.; Richardson, A. C.; Price, R. G. *Carbohydr. Res*. **1990**, *205*, C5.

8. Kiyose, K.; Kojima, H.; Nagano, T. *Chem. Asian J*. **2008**, *3*, 506.

9. Wagner, B. D. *Molecules* **2009**, *14*, 210.

10. (a) Mcmahon, B. K.; Gunnlaugsson, T. *Tetrahedron Lett*. **2010**, *51*, 5406. (b). Ochi, R.; Kurotani, K.; Ikeda, M.; Kiyonaka, S.; Hamachi, I. *Chem. Commun*. **2013**, *49*, 2115.

11. Zeng, Z.; Mizukami, S.; Kikuchi, K. *Anal. Chem*. **2012**, *84*, 9089.

12. Tangkuaram, T.; Gerlach, J. Q.; Xiang, Y.; Kawde, A. -N.; Dai, Z.; Bhavanandan, V. P.; La-Belle, J. T.; Veerasai, W.; Joshi, L.; Wang, J. *Analyst* **2006**, *131*, 889.

13. Ruan, C.; Wang, W.; Gu, B. *Anal. Chem*. **2006**, *78*, 3379.

14. Singh, S.; Packwood, J.; Samuel, C. J.; Critchley, P.; Crout, D. H. G. *Carbohydr. Res.* **1995**, *279*, 293.

15. Tokuyasu, K.; Ono, H.; Kitagawa, Y.; Ohnishi-Kameyama, M.; Hayashi, K.; Mori, Y. *Carbohydr. Res.* **1999**, *316*, 173.

16. Tokuyasu, K.; Ono, H.; Hayashi, K.; Mori, Y. *Carbohydr. Res.* **1999**, *322*, 26.

17. Kubisch, J.; Weignerova, L.; Ktter, S.; Lindhorst, T. K.; Sedmera, P.; Kfen, V. *J. Carbohydr. Chem*. **1999**, *18*, 975.

18. Zeng, X.; Sun, Y.; Ye, H.; Liu, J.; Uzawa, H. *Biotechnol. Lett.* **2007**, *29*, 1105.

19. Uzawa, H.; Toba, T.; Nishida, Y.; Kobayashi, K.; Minoura, N.; Hiratani, K. *Chem. Commun.* **1998**, 2311.

20. Osawa, T. *Carbohydr. Res*. **1966**, *1*, 435.

21. Hollinger, M.; Abraha, F.; Oscarson, S. *Carbohydr. Res.* **2011**, *346*, 1454.

22. Sato, T.; Imamura, A.; Ando, H.; Ishida, H.; Kiso, V. *Glycoconj. J.* **2009**, *26*, 83.

23. Fuente, M.; Penade, S. *Tetrahedron: Asymmetry* **2002**, *13*, 1879.

24. Zhang, Z.; Ollmann, I. R.; Ye, X. -S.; Wischnat, R.; Baasov, T.; Wong, C. H. *J. Am. Chem. Soc*. **1999**, *121*, 734.

25. Goddard-Borger, E. D.; Stick, R. V. *Org. Lett*. **2007**, *9*, 3797.

26. Chang, S.; Lin, C.; Li, Y.; Mong, K. T. *Carbohydr. Res.* **2009**, *344*, 432.

27. Chen, H.; Withers, S. G. *Carbohydr. Res.* **2010**, *345*, 2596.

28. Robina, I.; López-Barba, E.; Fuentes, J. *Tetrahedron* **1996**, *52*, 10771.

29. Rosen, T.; Lico, I. M.; Chu, D. T. W. *J. Org. Chem*. **1988**, *53*, 1580.

30. Ren, B.; Wang, M.; Liu, J.; Ge, J.; Zhang, X.; Dong, H. *Green Chem.* **2015**, *17*, 1390.

31. Bowers, S. G.; Coe, D. M.; Boons, G. J. *J. Org. Chem.* **1998**, *63*, 4570.

32. Kramer, K. J.; Koga, D. *Insect Biochem*. **1986**, *16*, 851.

4.6 Representative spectra

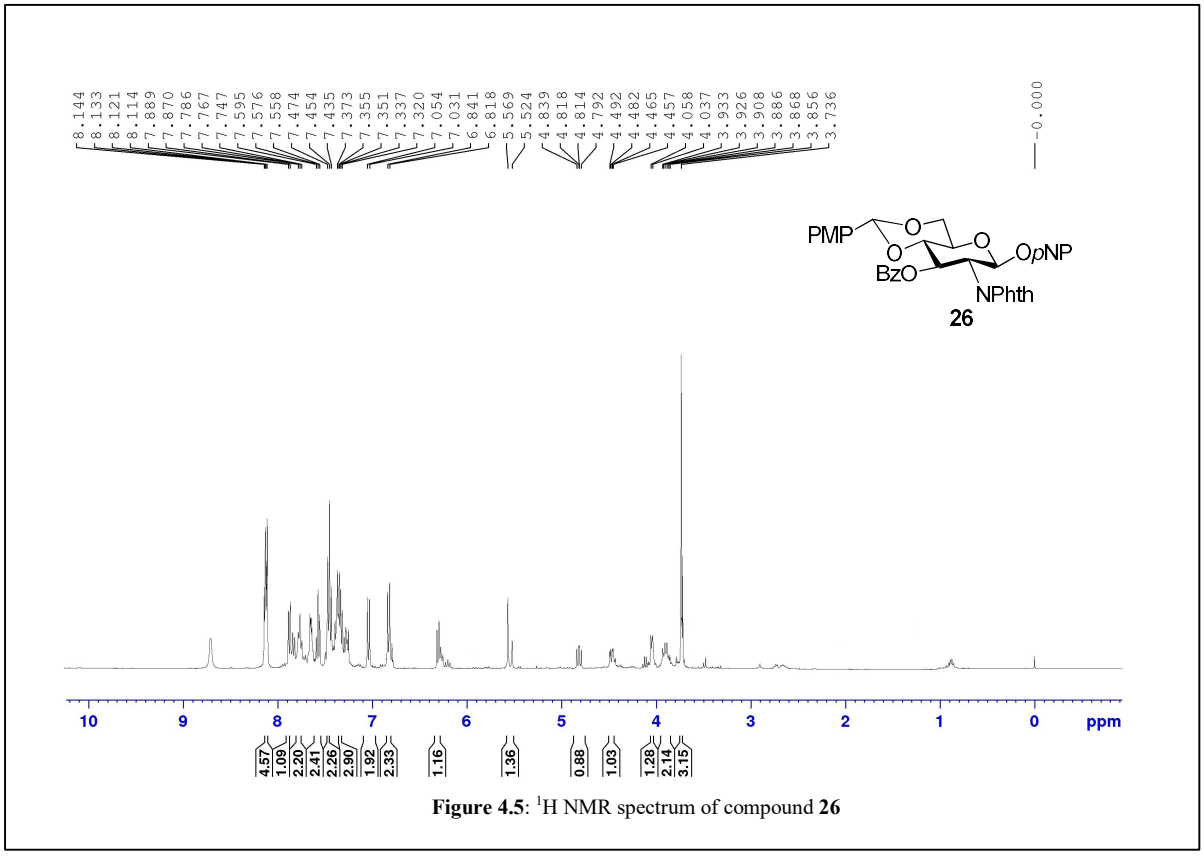


Figure 4.5: ^{1}H NMR spectrum of compound **26**

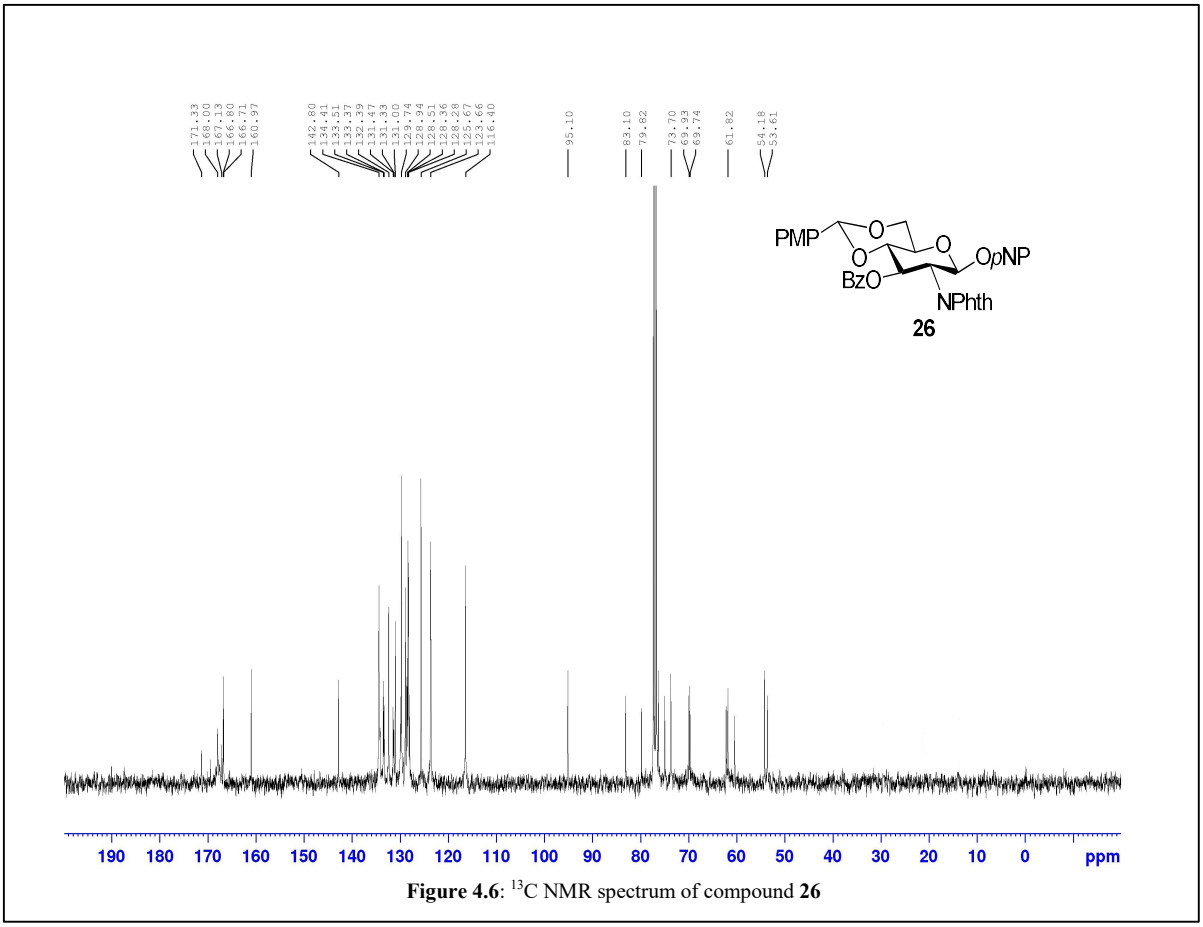


Figure 4.6: ^{13}C NMR spectrum of compound **26**

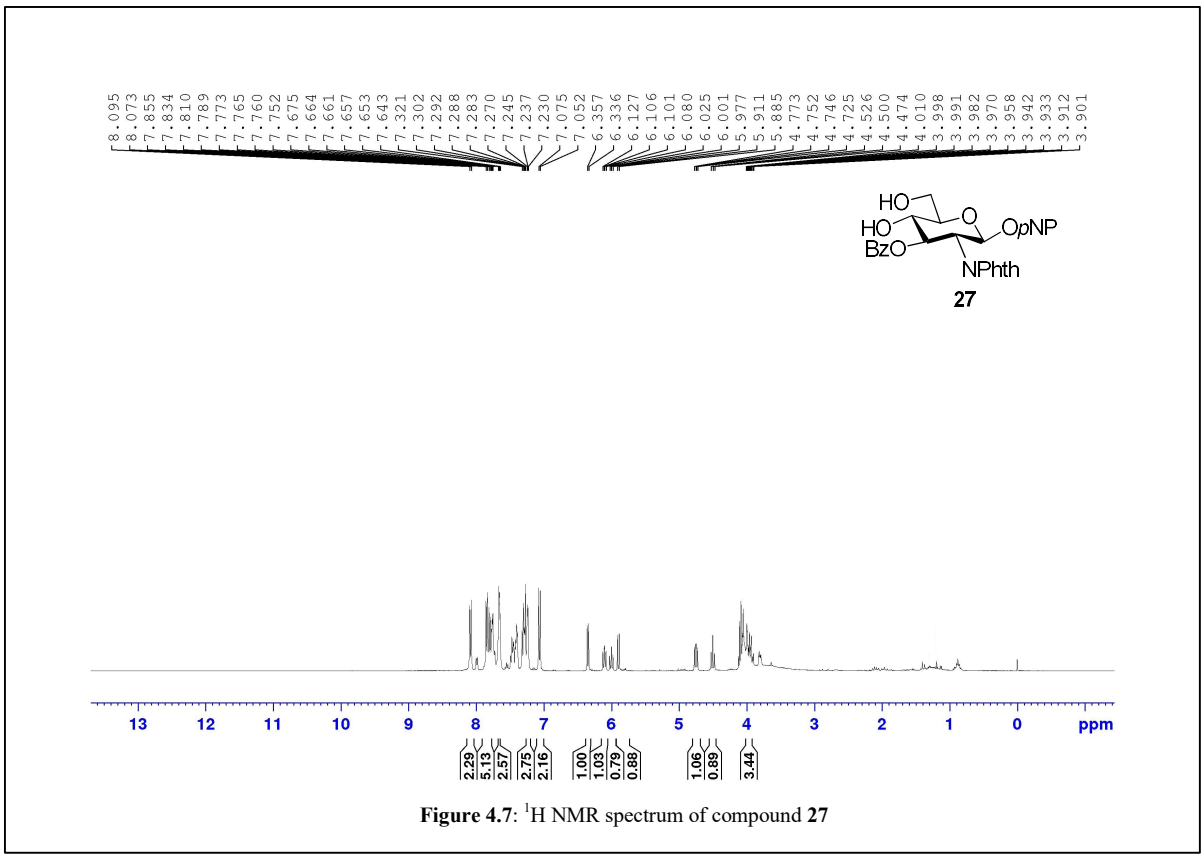


Figure 4.7: ^{1}H NMR spectrum of compound **27**

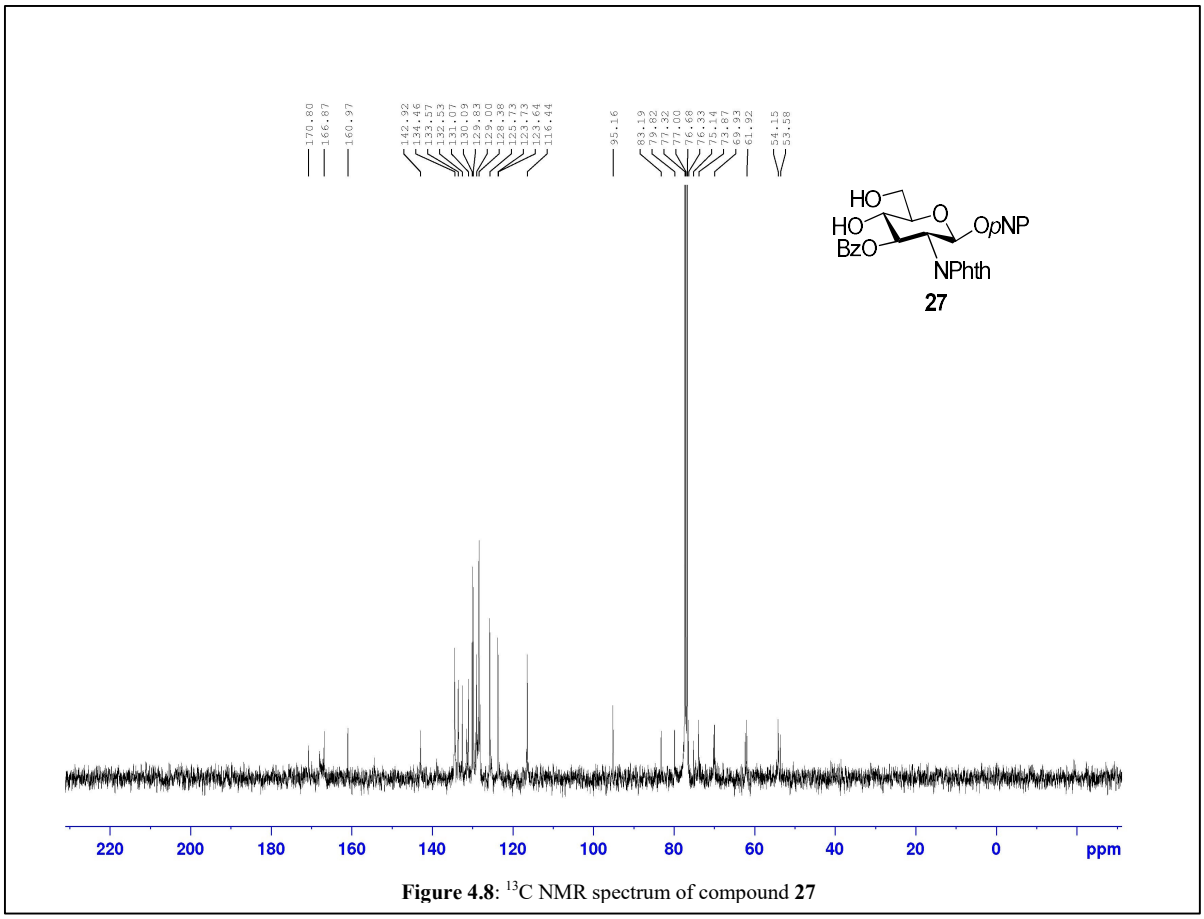


Figure 4.8: ^{13}C NMR spectrum of compound **27**

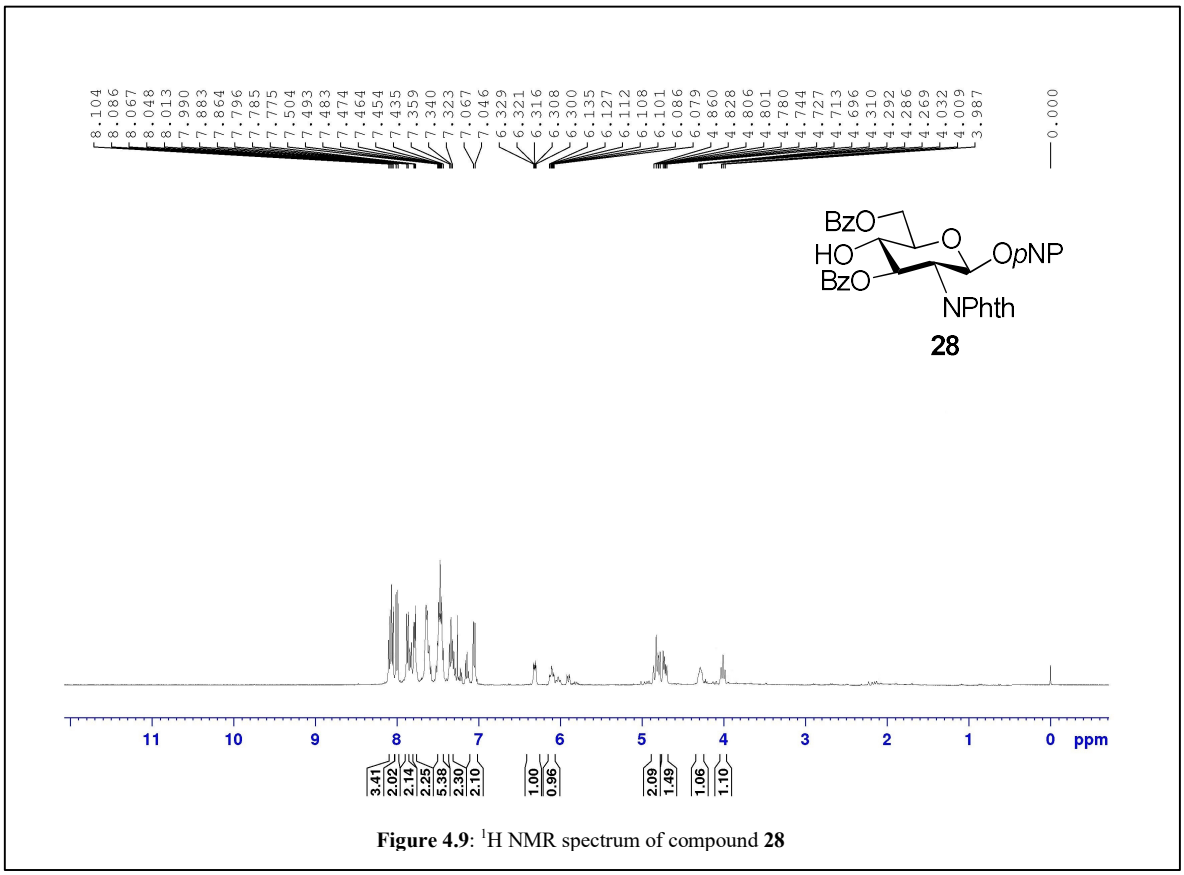


Figure 4.9: ^{1}H NMR spectrum of compound **28**

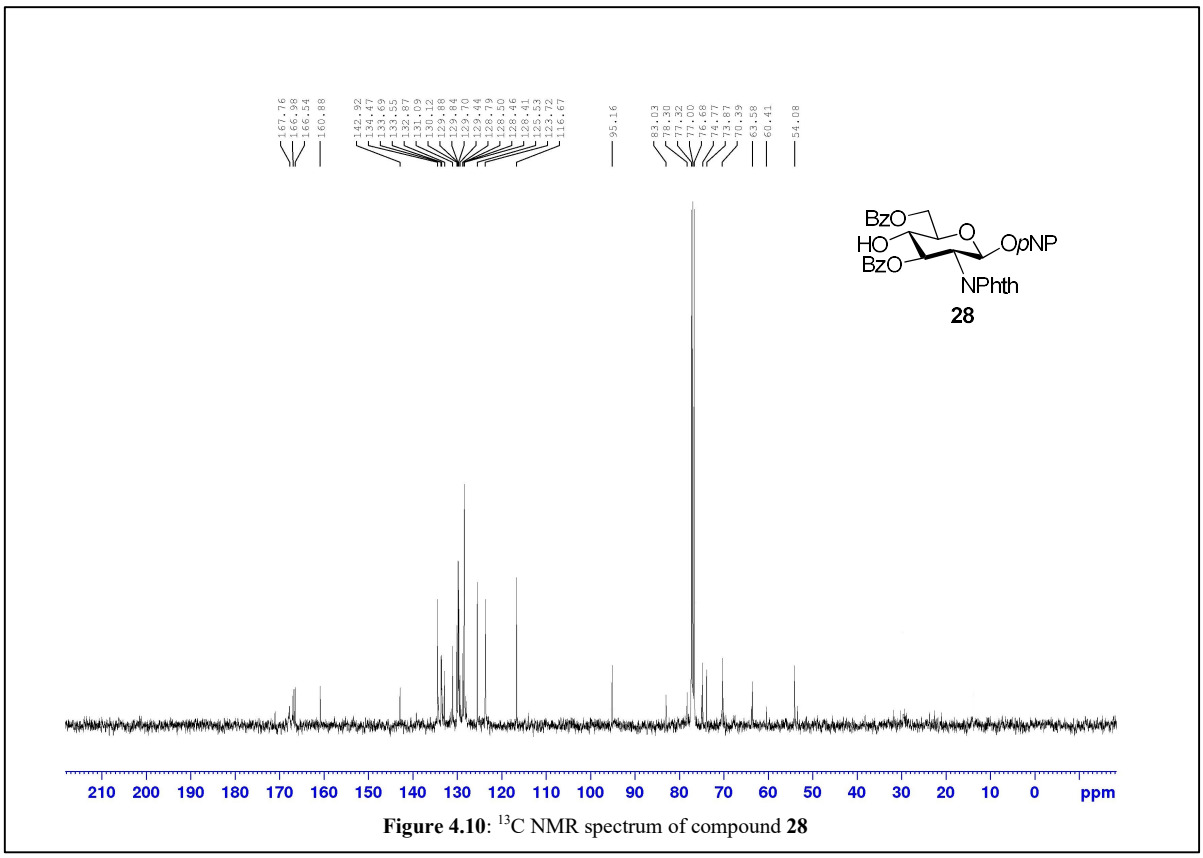


Figure 4.10: ^{13}C NMR spectrum of compound **28**

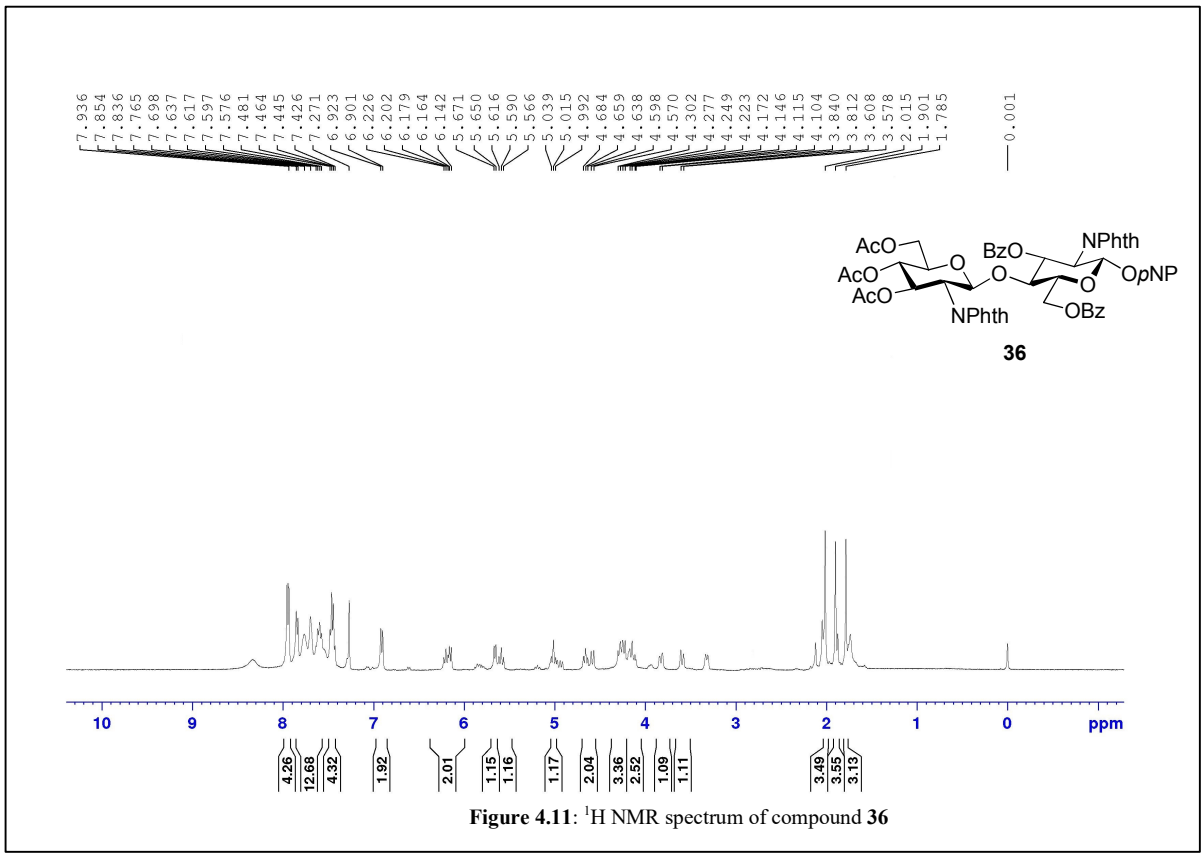


Figure 4.11: ^{1}H NMR spectrum of compound **36**

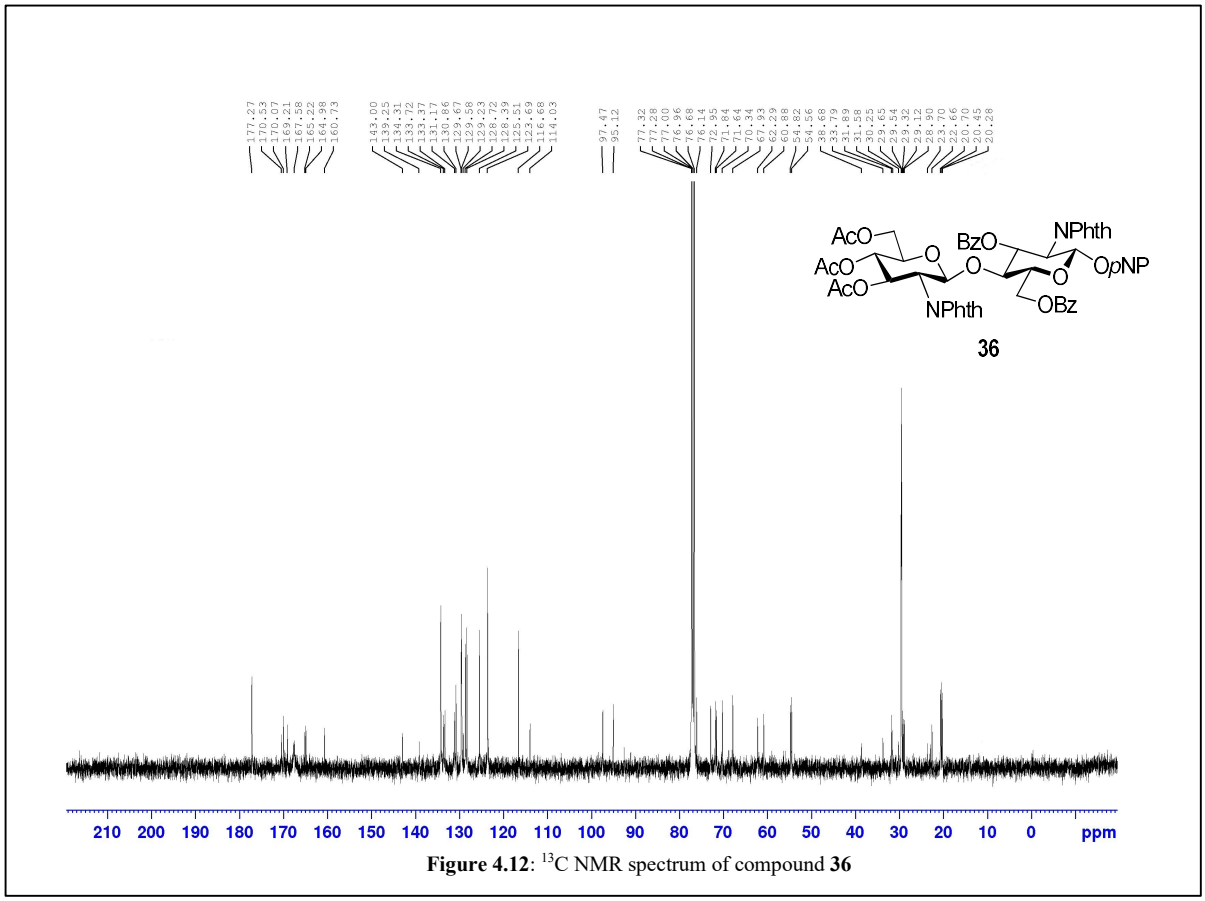


Figure 4.12: ^{13}C NMR spectrum of compound **36**

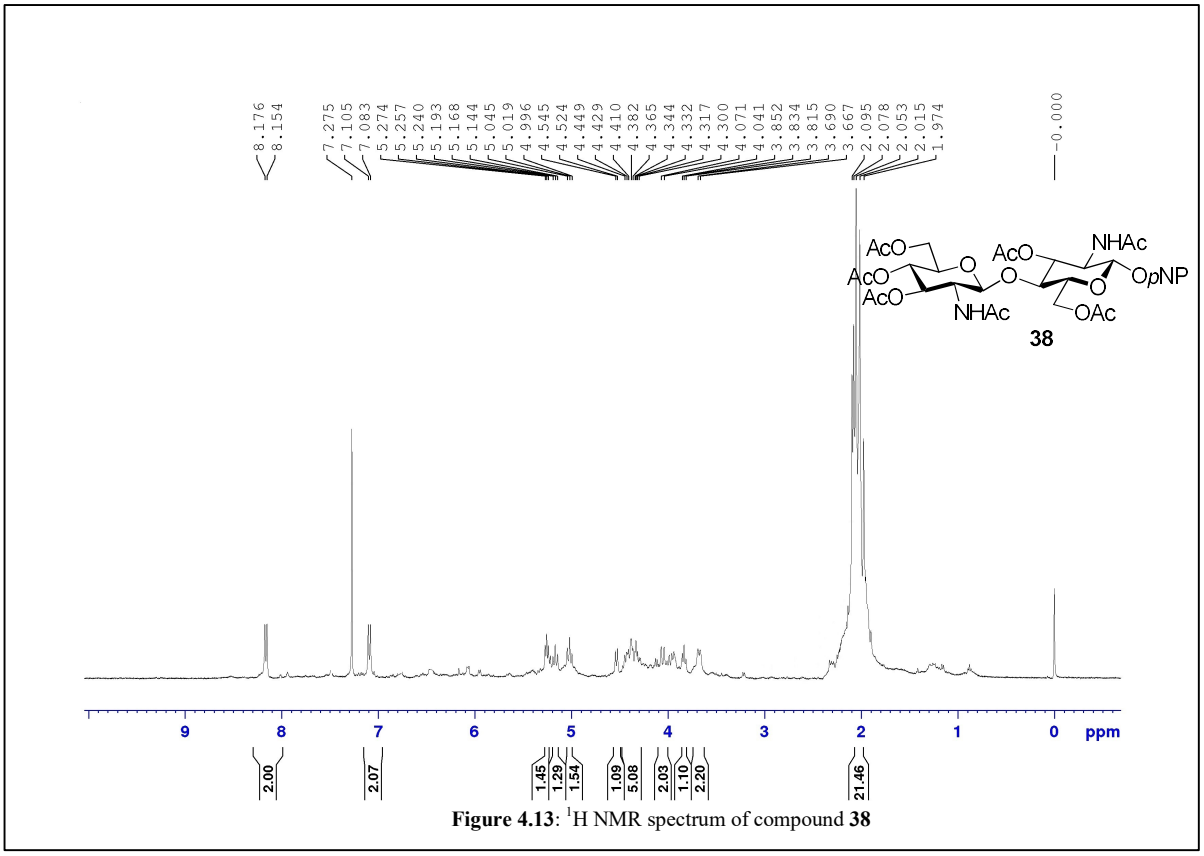


Figure 4.13: ^{1}H NMR spectrum of compound **38**

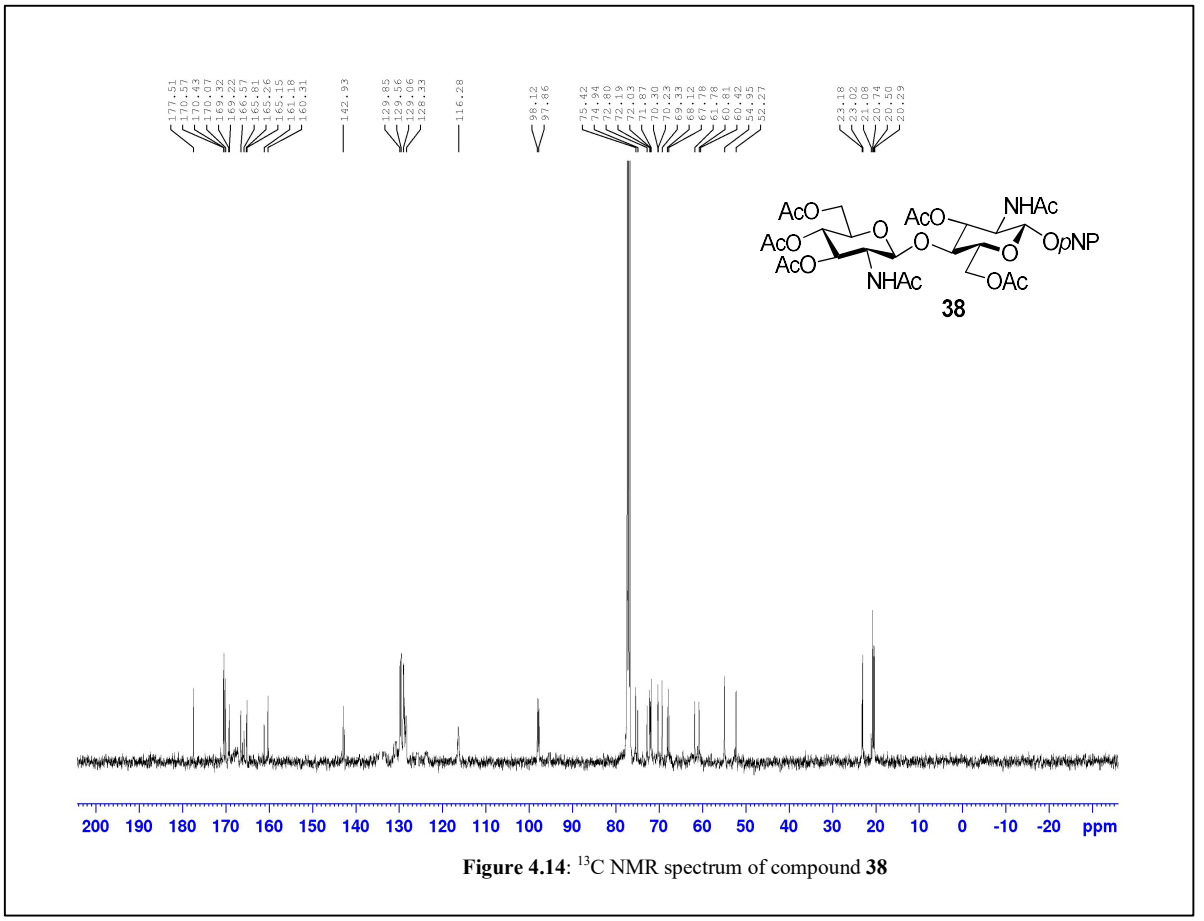


Figure 4.14: ^{13}C NMR spectrum of compound **38**

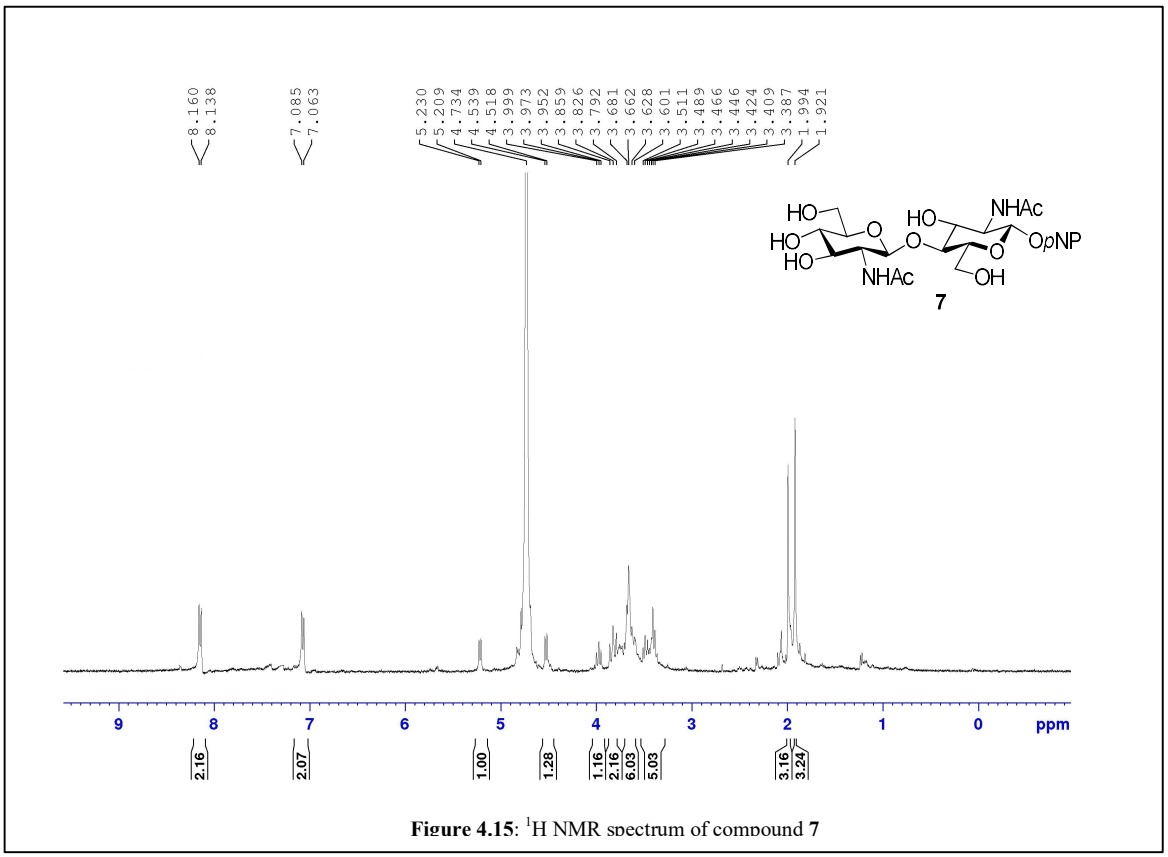


Figure 4.15: ^{1}H NMR spectrum of compound **7**

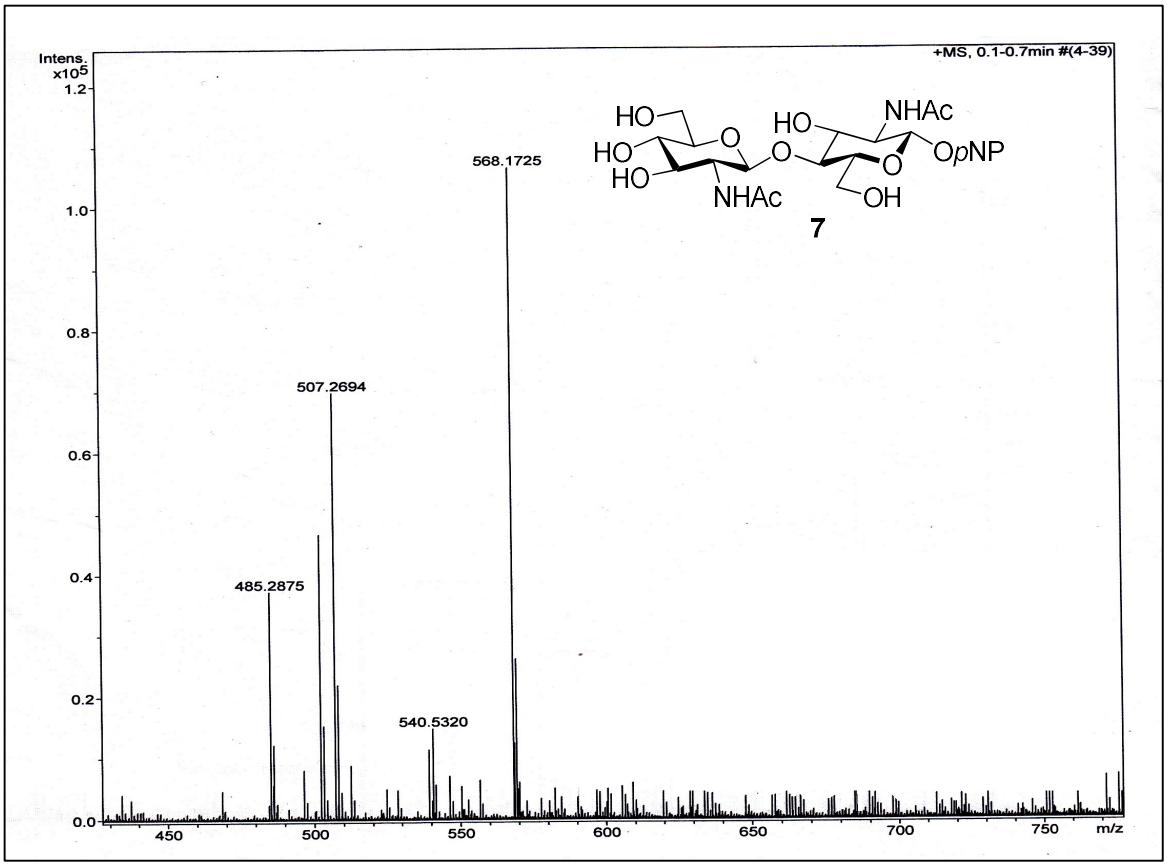


Figure 4.16: HRMS spectrum of compound **7**

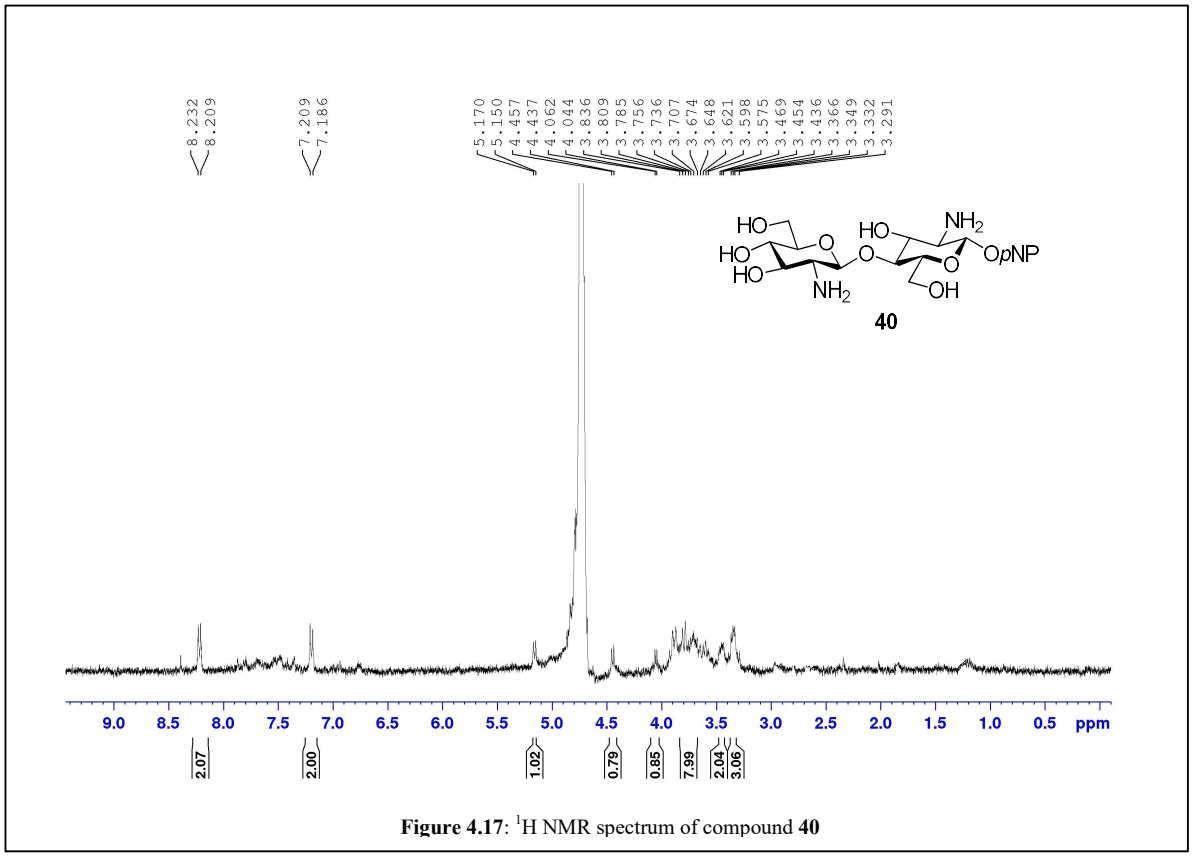


Figure 4.17: ^{1}H NMR spectrum of compound **40**

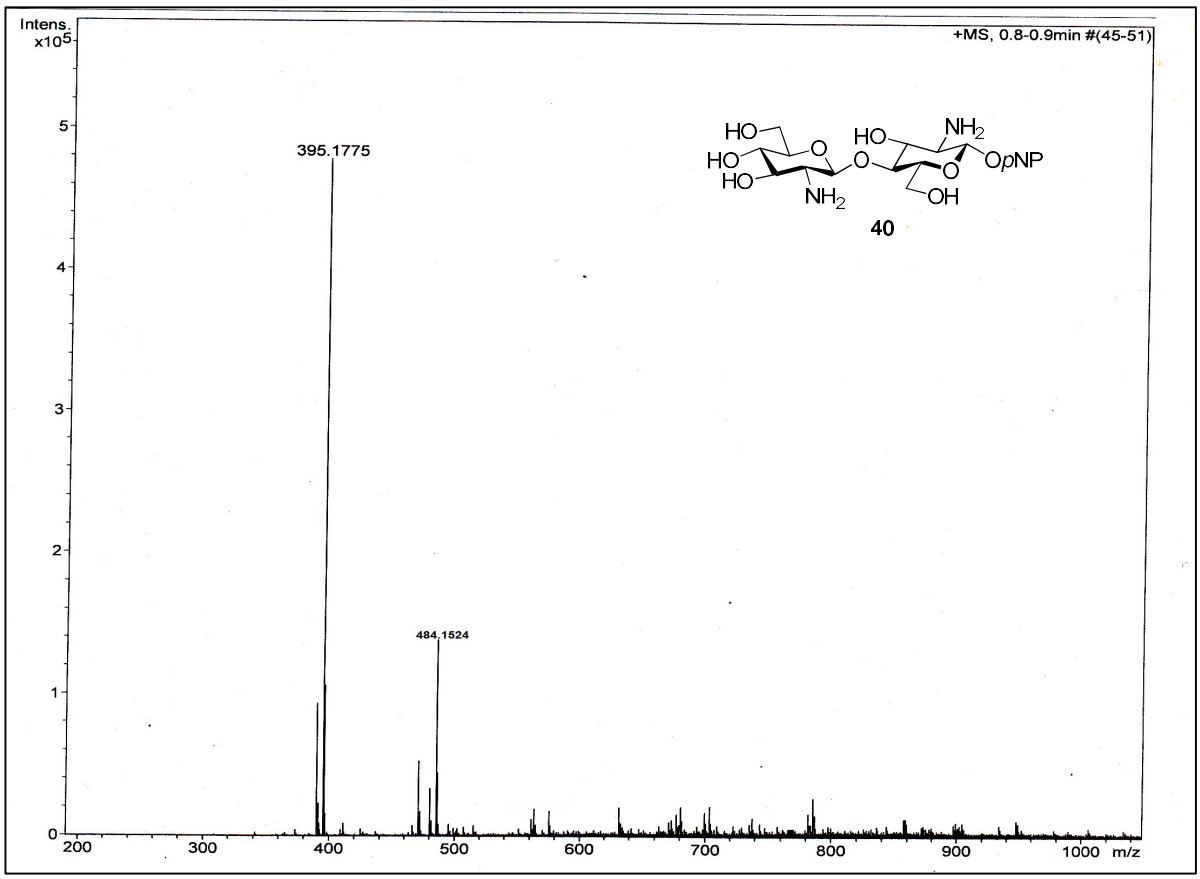


Figure 4.18: HRMS spectrum of compound **40**

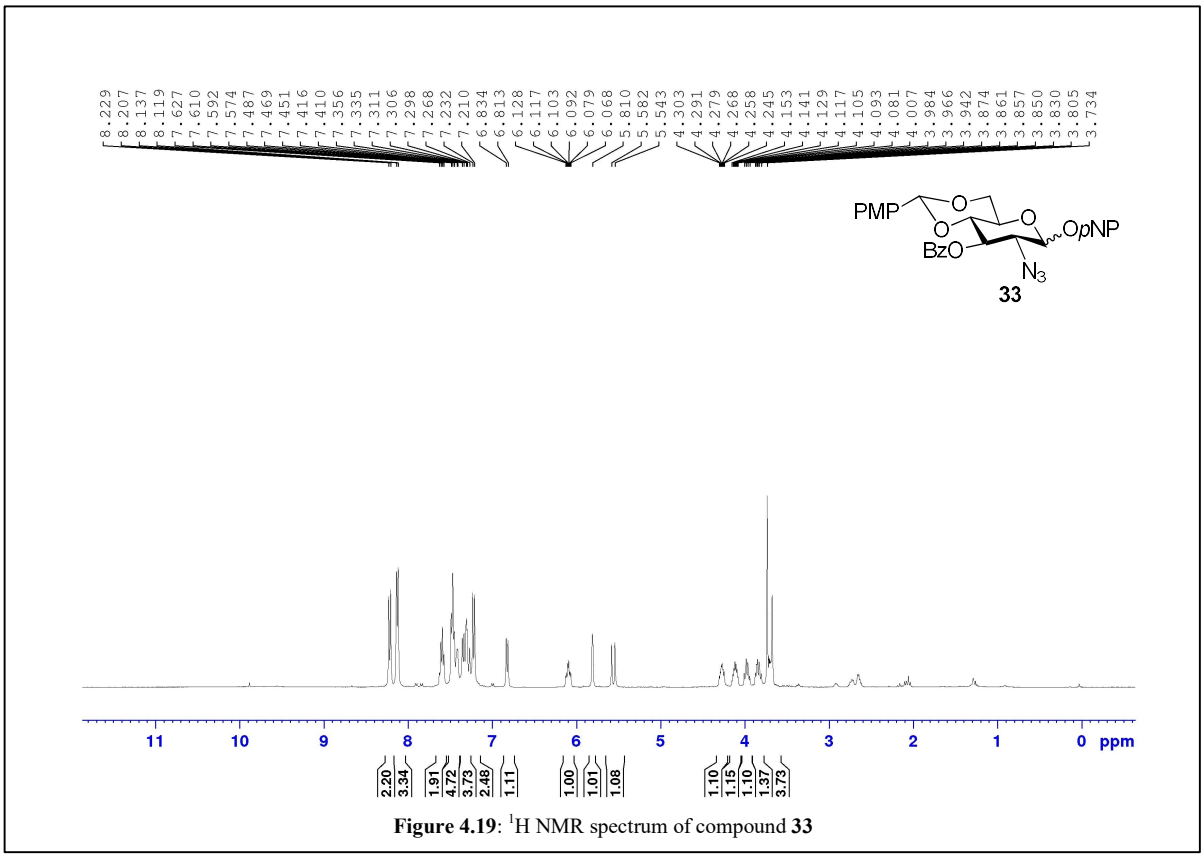


Figure 4.19: ^{1}H NMR spectrum of compound **33**

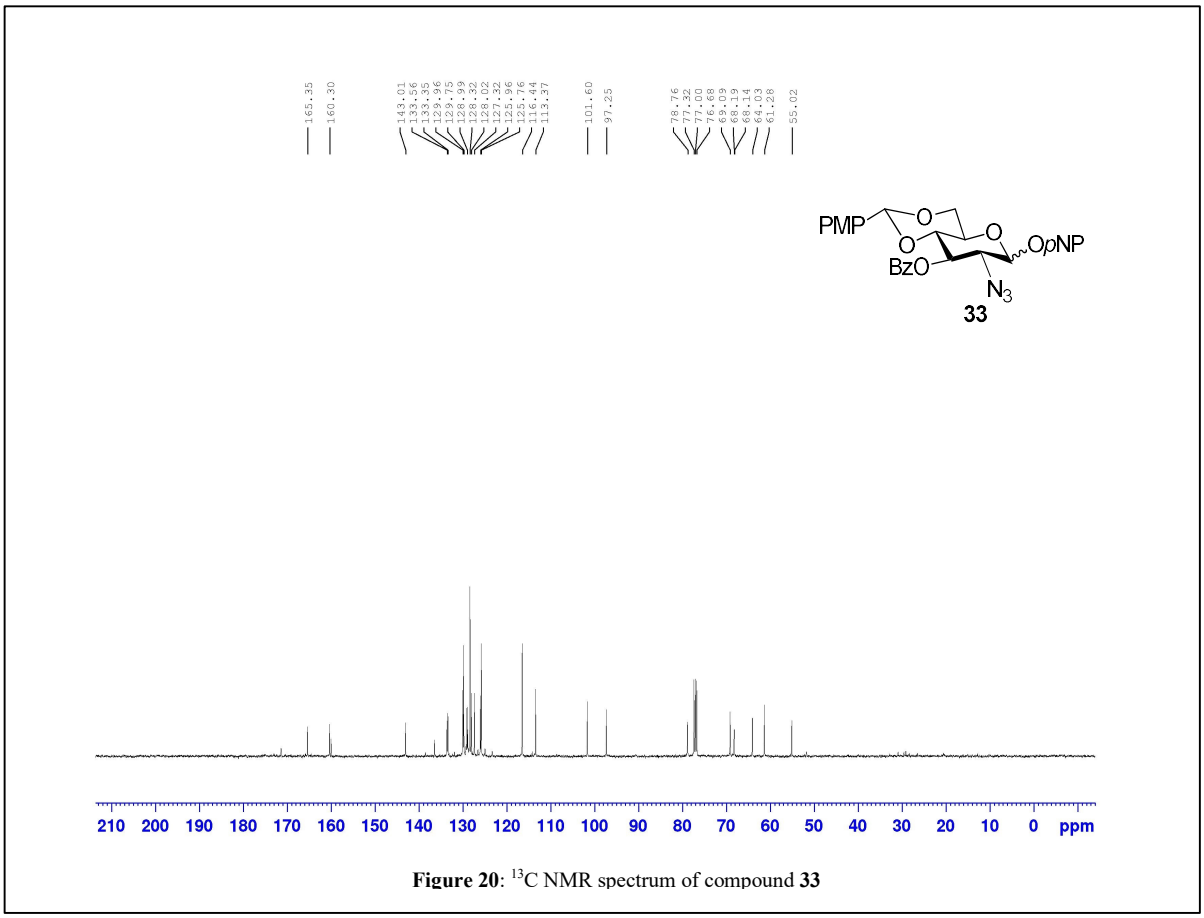


Figure 20: ^{13}C NMR spectrum of compound **33**

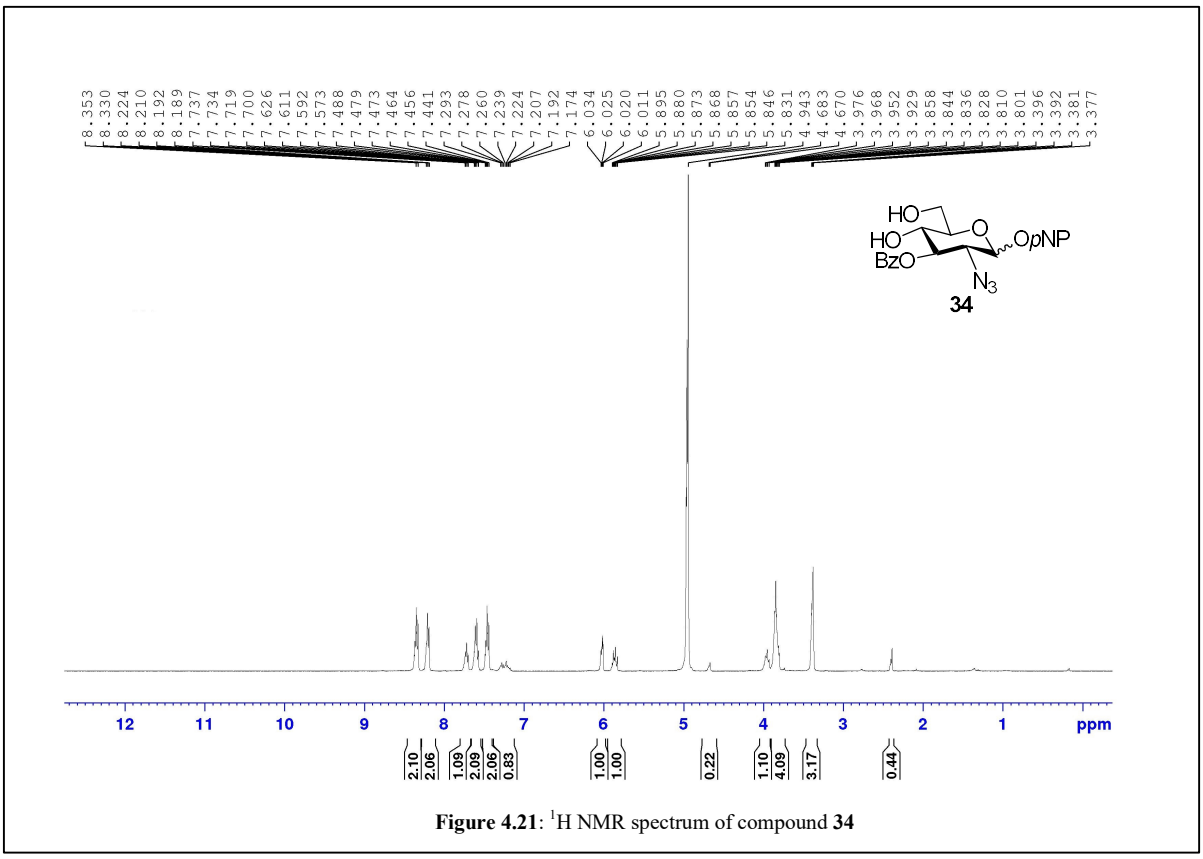


Figure 4.21: ^{1}H NMR spectrum of compound **34**

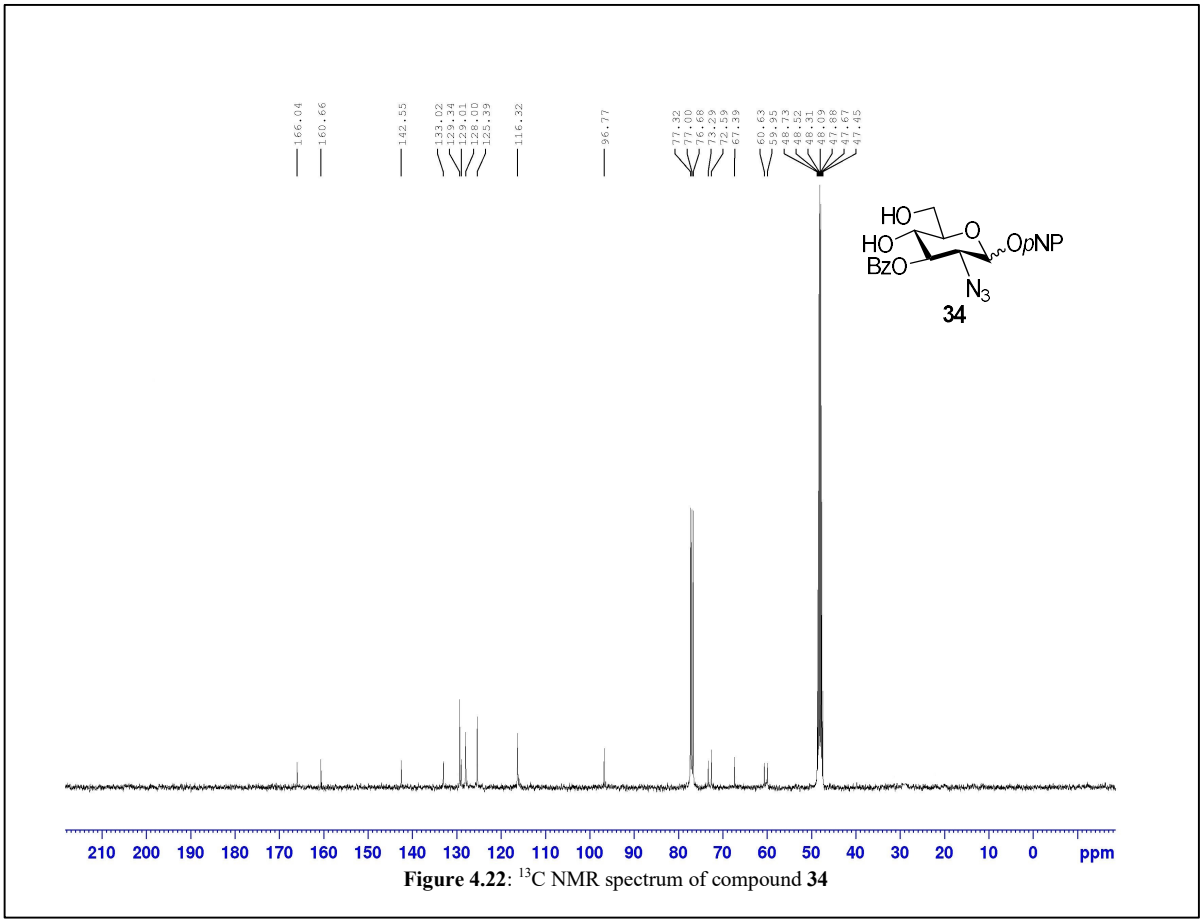


Figure 4.22: ^{13}C NMR spectrum of compound **34**

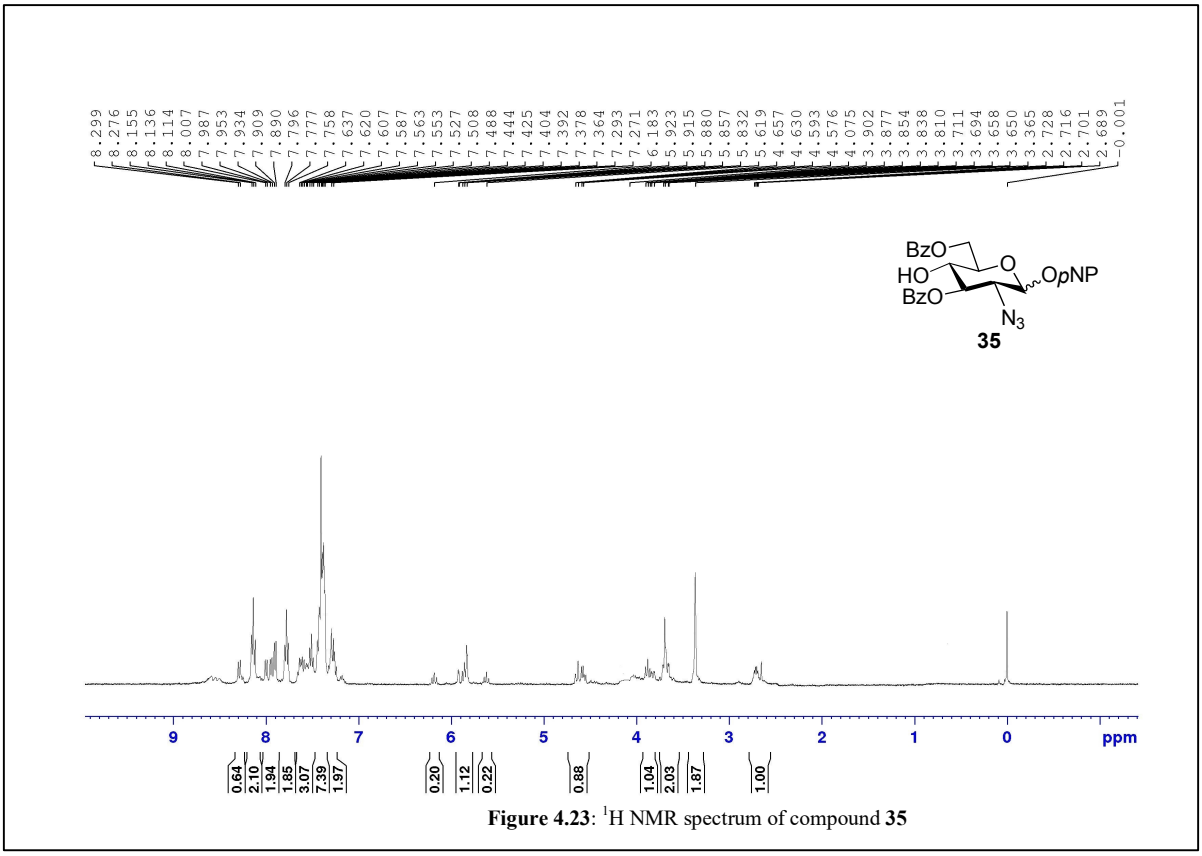


Figure 4.23: ^{1}H NMR spectrum of compound **35**

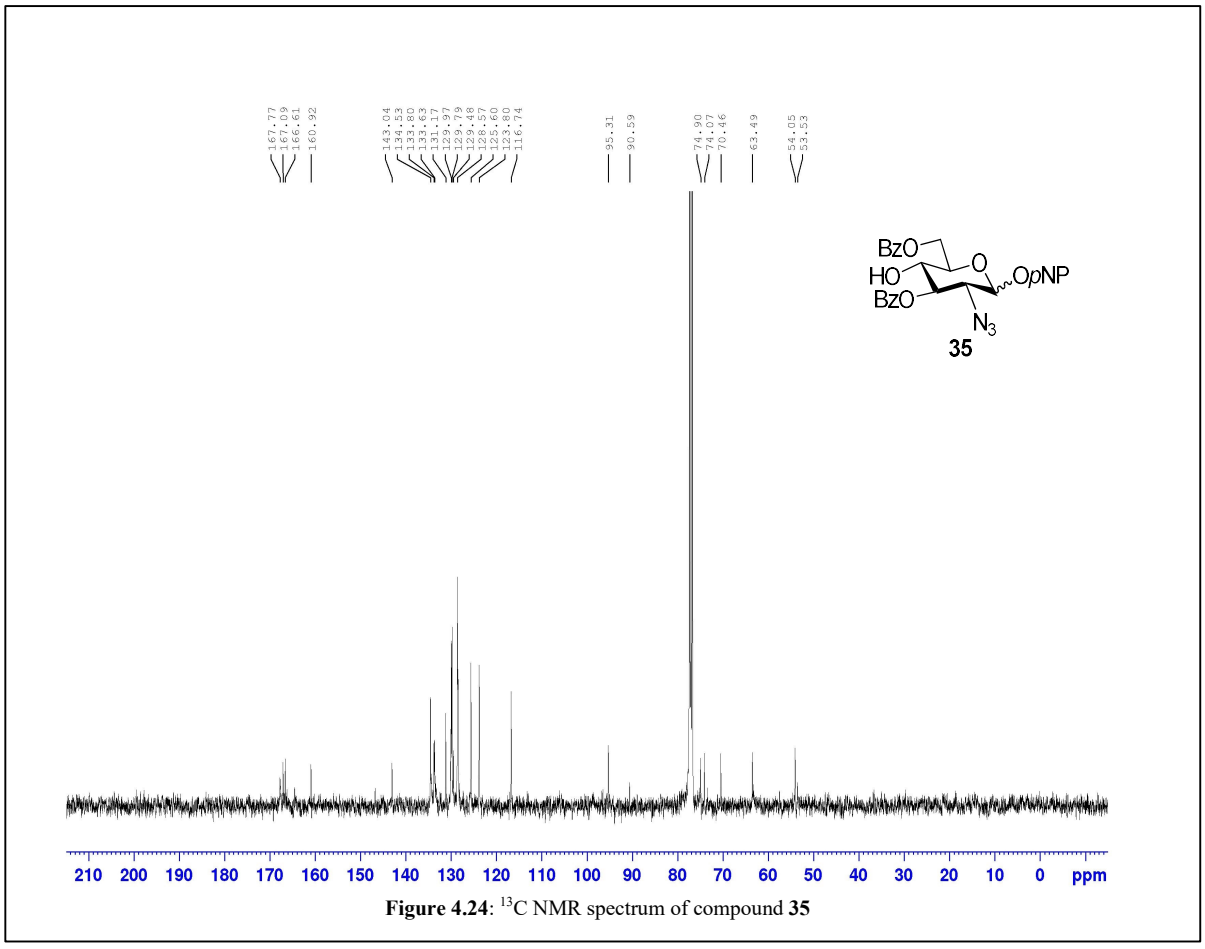


Figure 4.24: ^{13}C NMR spectrum of compound **35**

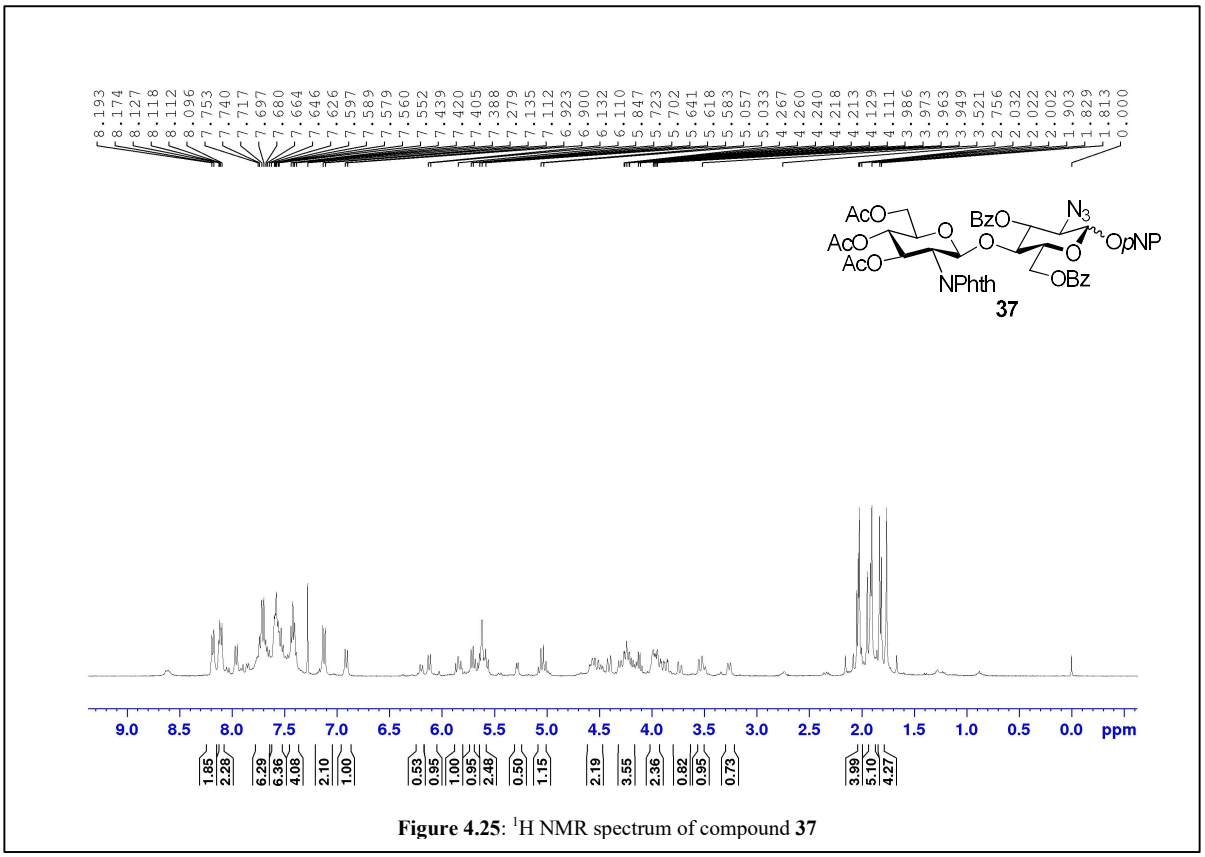


Figure 4.25: ^{1}H NMR spectrum of compound **37**

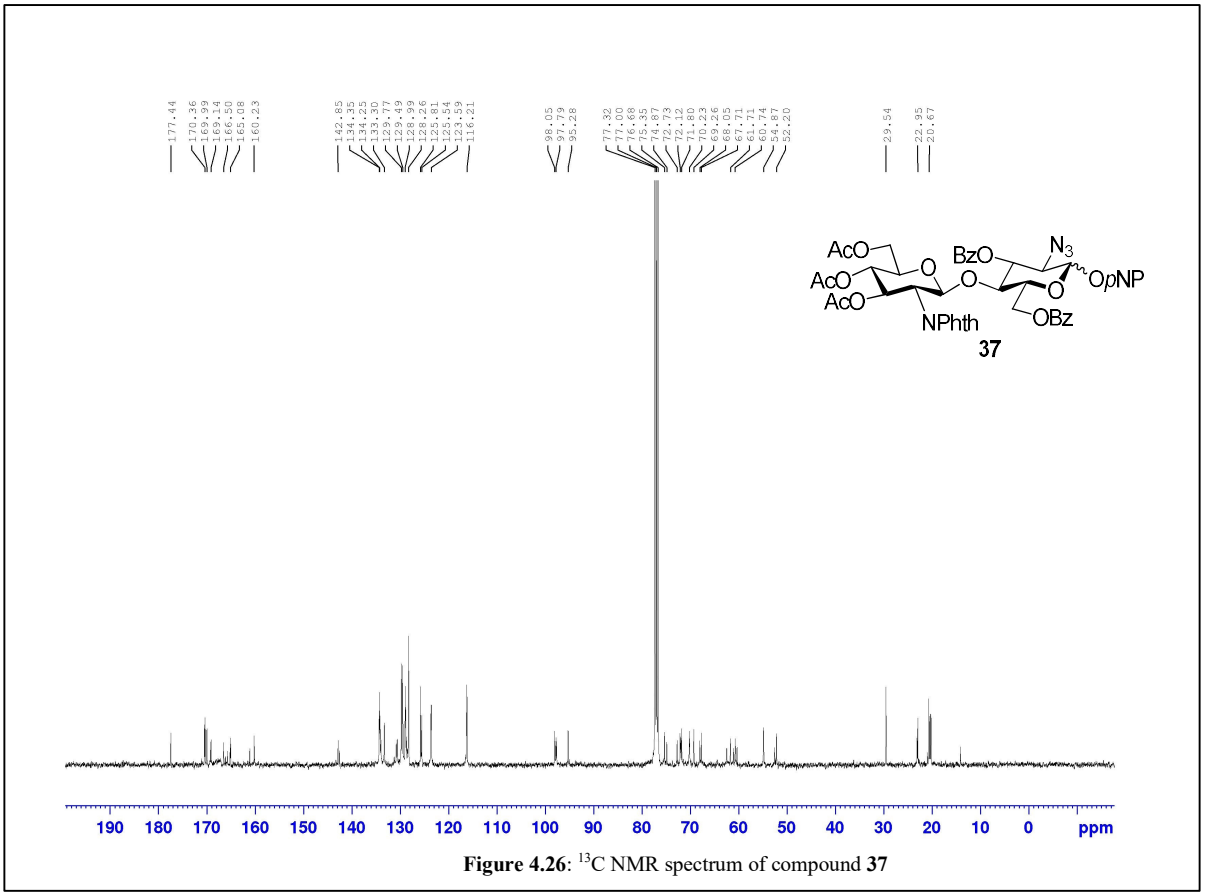


Figure 4.26: ^{13}C NMR spectrum of compound **37**

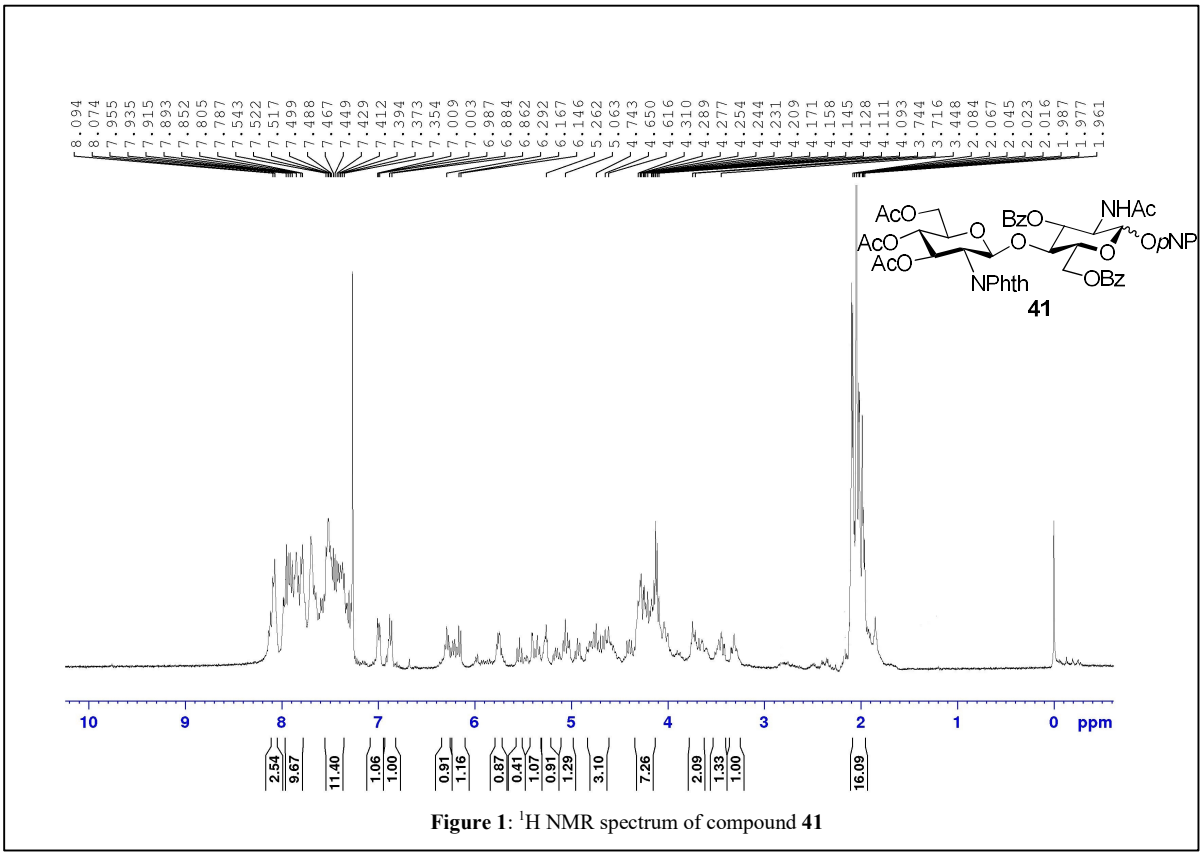


Figure 1: [1]H NMR spectrum of compound **41**

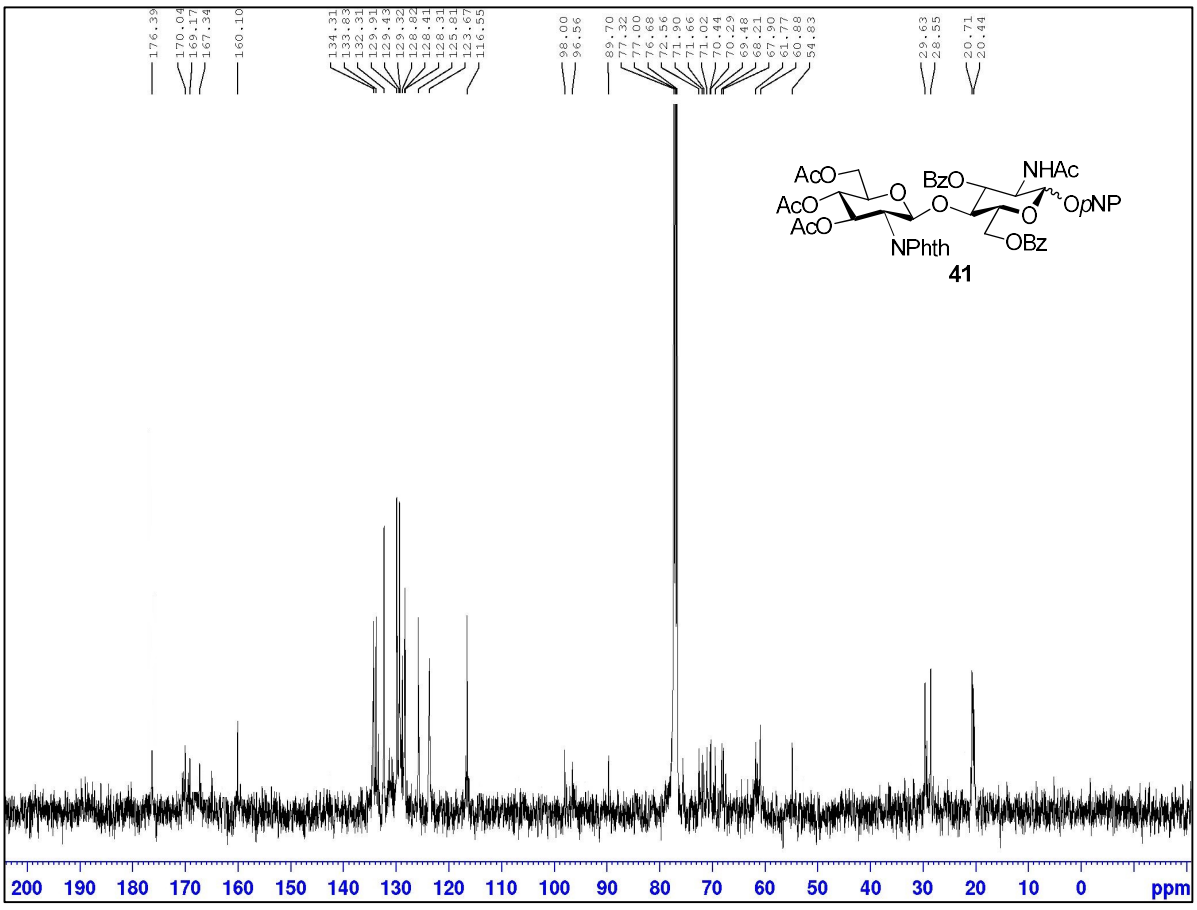


Figure 1: [13]C NMR spectrum of compound **41**

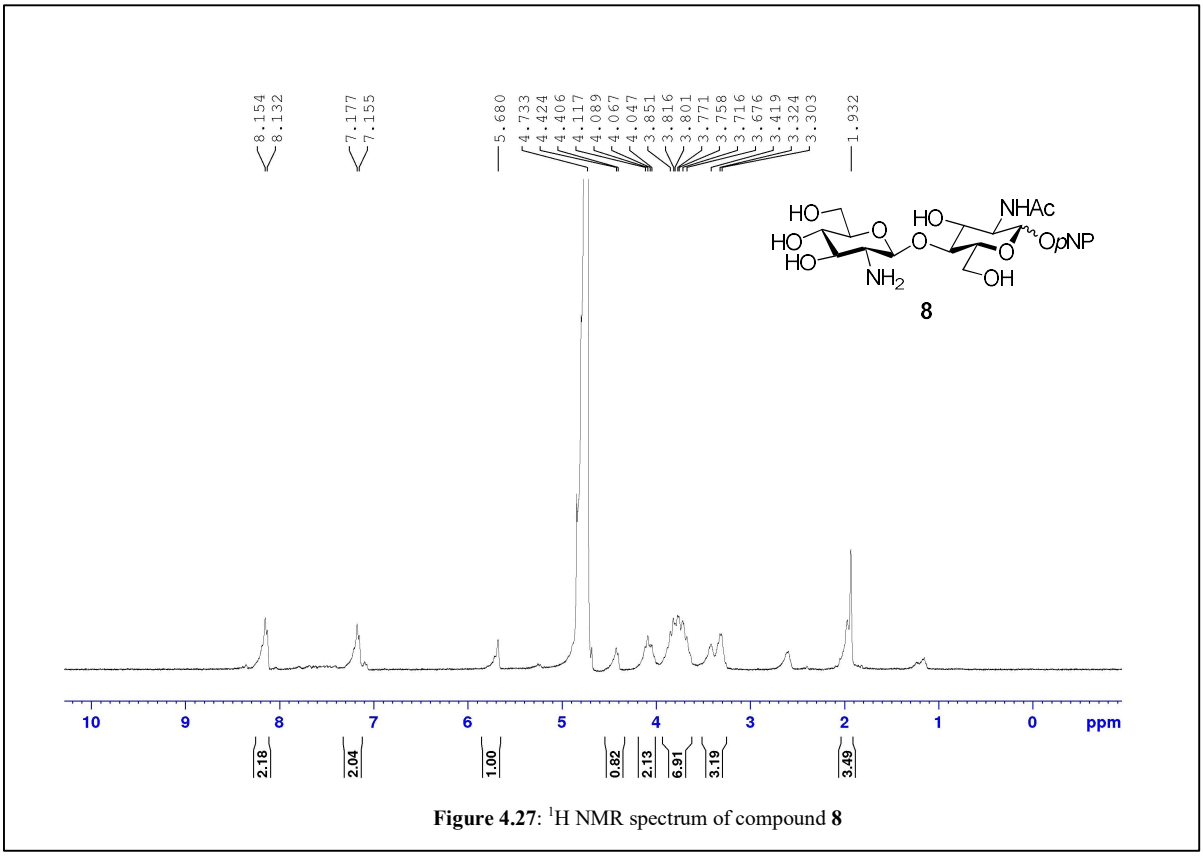


Figure 4.27: ^{1}H NMR spectrum of compound **8**

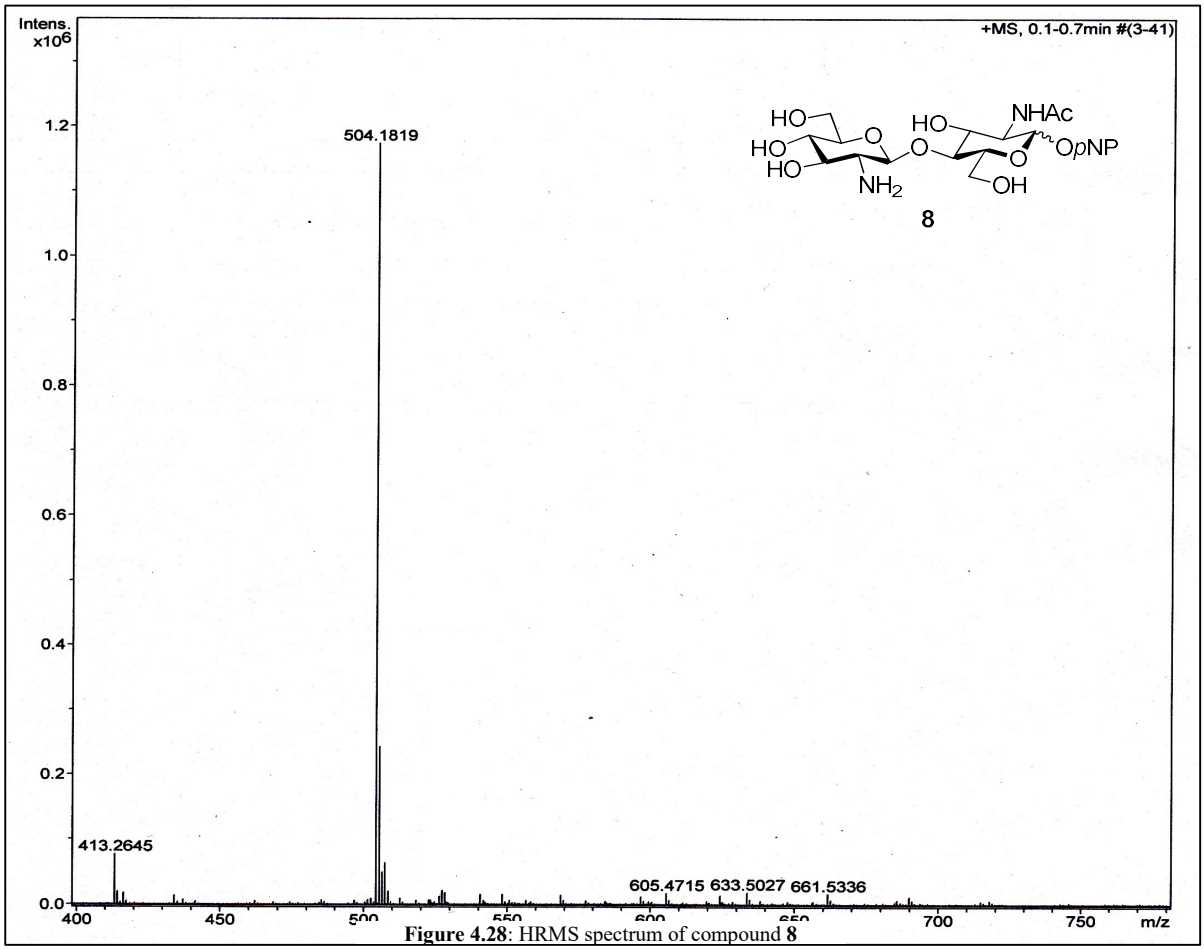


Figure 4.28: HRMS spectrum of compound **8**

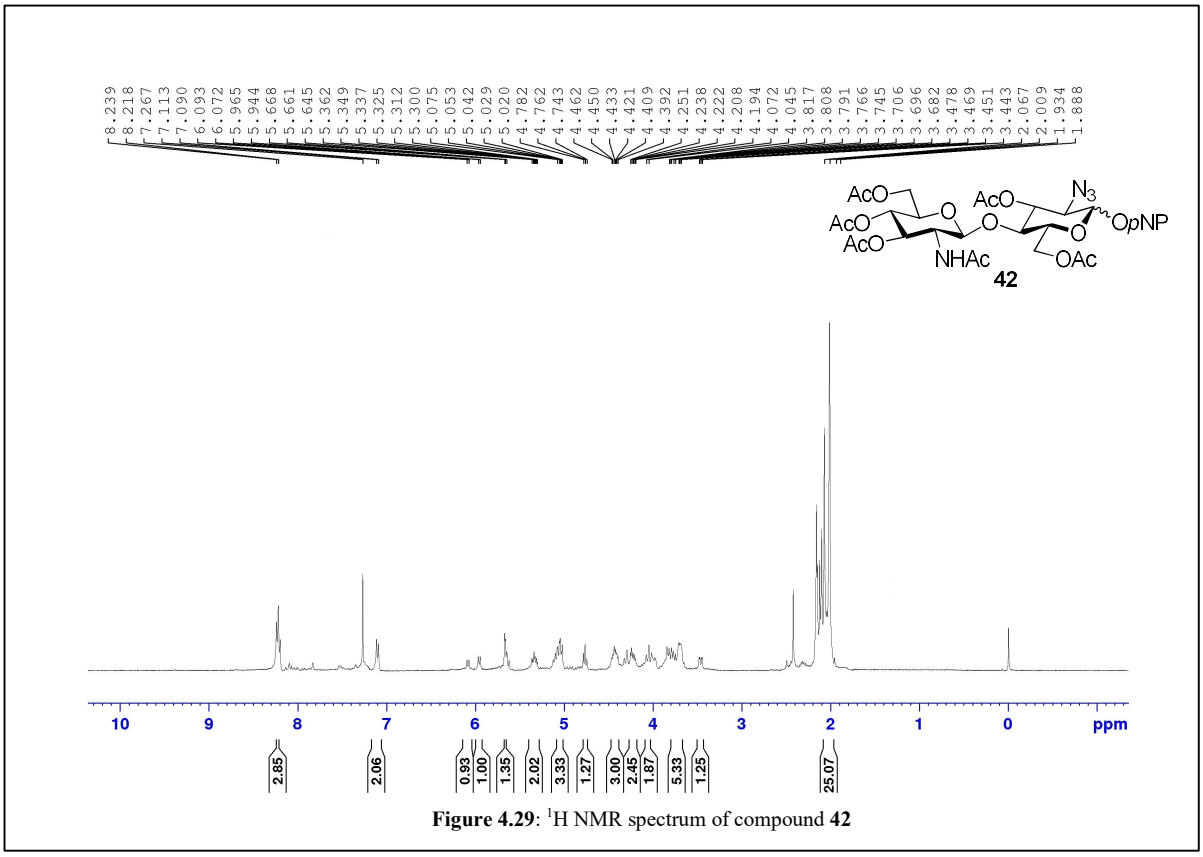


Figure 4.29: ^{1}H NMR spectrum of compound **42**

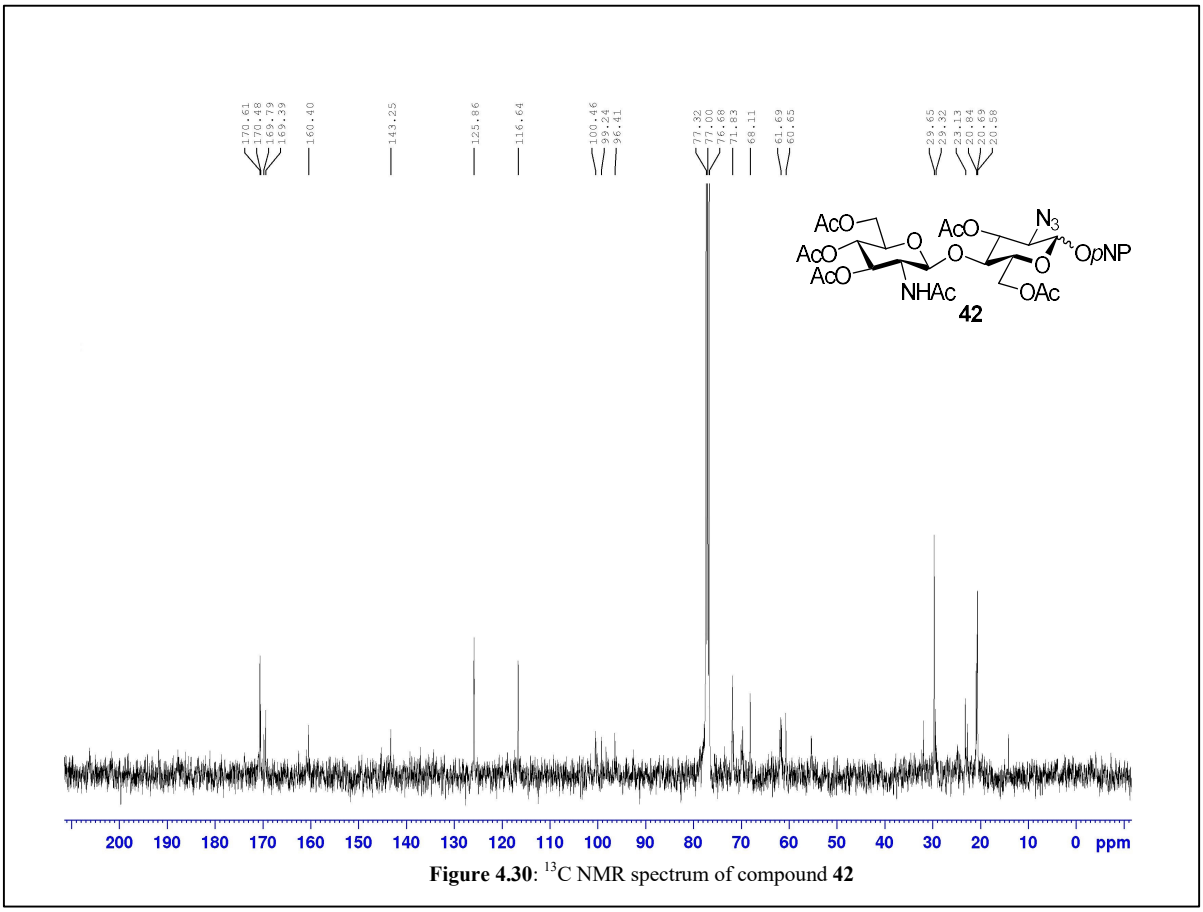


Figure 4.30: ^{13}C NMR spectrum of compound **42**

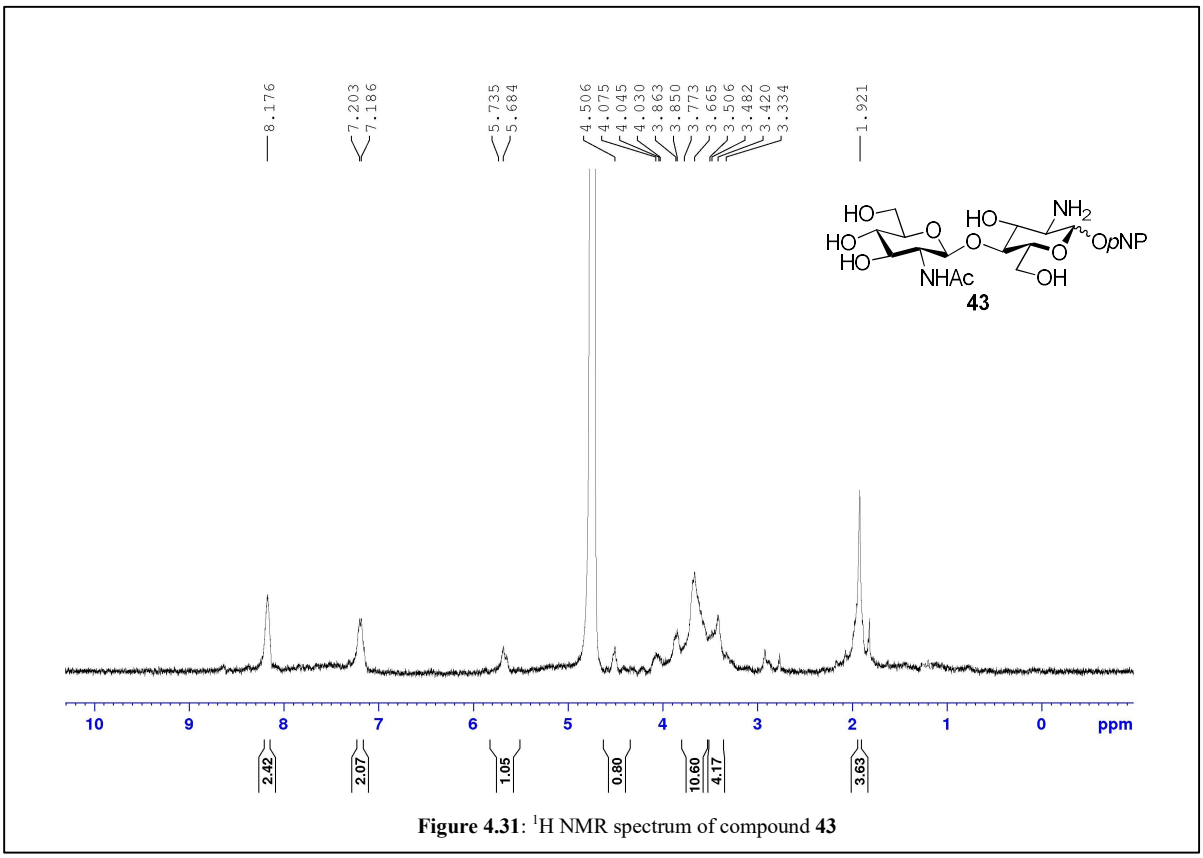


Figure 4.31: ^{1}H NMR spectrum of compound **43**

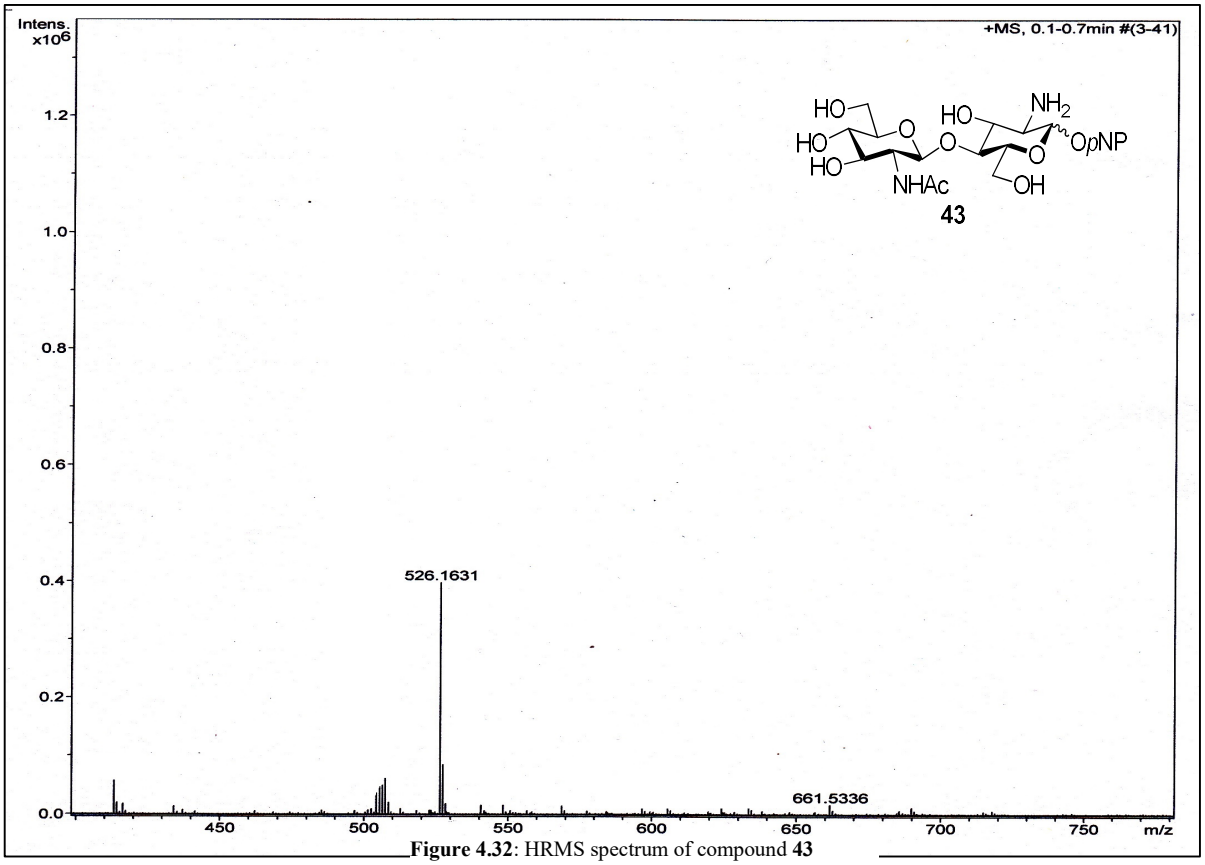


Figure 4.32: HRMS spectrum of compound **43**

Chapter 5

Towards the development of glycosidase inhibitors

5.1 Introduction

Glycosidases are a class of enzymes that hydrolyze the glycosidic linkages in glycans such as glycoconjugates and oligosaccharides. Glycosidase inhibitors play an important role in the investigation and treatment of many diseases.[1-7] Stable and selective structures that can inhibit therapeutically important glycosidase enzymes are desired for this purpose.[1] Many synthetic and biomedical research groups have been doing intensive research on the design and biological evaluation of glycosidase inhibitors. These inhibitors find application in the treatment of HIV,[2] cancer,[3] diabetes,[4,5] viral infections,[6] and lysosomal storage diseases[7] by modifying cellular functions. *N*-acetyl- glucosamine and galctosamine are present in certain glycoconjugates and amino glycosides which play impotant roles in many biologial processes. These substrates are highly active towards the hexosaminidases. Chitinase inhibitors acts as selective insecticides[8] and fungicides.[9]

5.1.1 Allosamidins and aza sugars as glycosidase inhibitors

Allosamidins (**1**-**3**) and aza sugars (**7**-**13**) are good inhibitors of glycosidases due to their structural similarity with the *N*-acetyl glucosamine derivatives. Allosamidin **2** acts as a powerful insect chitinase inhibitor[10] due to its structural similarities with β-(1→4) linked chitobiose derivatives (Figure 5.1). Recently, several allosamidin derivatives having structural similarities with chitooligosaccharides were reported to exhibit strong inhibition towards the activities chitinases.[11,12]

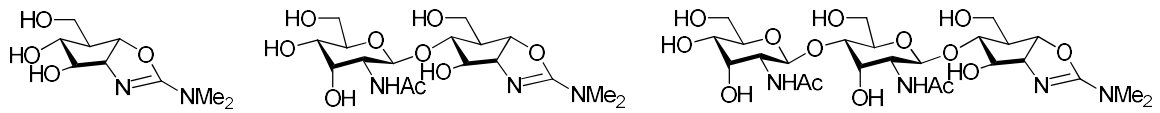
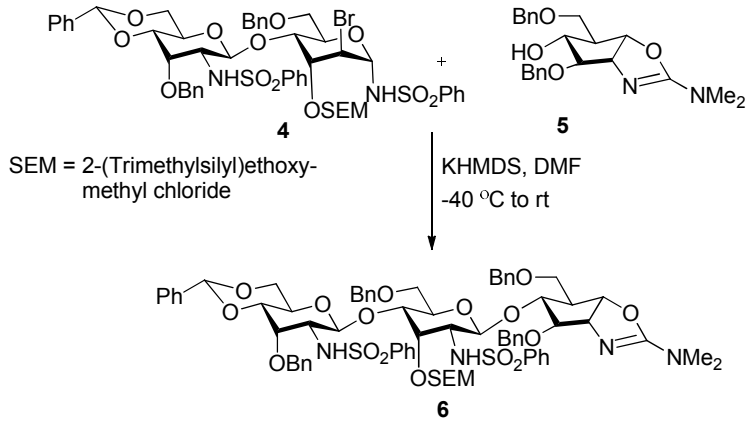


Figure 5.1: Structures of allosamidins (**1**-**3**) as potential chitinase inhibitors.

Griffith *et al*. reported an efficient total synthesis of allosamidin derivatives and explored their biological activities.[13] Coupling of chitobioside derivative **4** with oxazoline derivative **5** through the formation of 1,2-sulfonylaziridine intermediate in the prsence of strong base

KHMDS in DMF solvent is the key step in the preparation of allosamidin derivative **3** (Scheme 5.1).



Scheme 5.1: Synthesis of allosamidin derivative **6** using chemical protection-deprotection strategy.

Iminosugars and their derivatives are well studied substrates as glycosides inhibitors. Their strong activities make them good candidates for the treatment of a broad spectrum of carbohydrates related diseases and disorders.[14,15] These are analogues of sugars in which nitrogen atom replaces the endocyclic oxygen atom of the ring. Well known azasugar alkaloids such as 1-deoxy nojirimycin and its derivatives **7**-**13** serve as glycosidase inhibitors (Figure 5.2).[16,17] Castanospermine iminosugar **13** shows strong inhibitory activity towards α-glucosidases, thereby exhibits antitumoral and antiviral activities but with lack of selectivity.[18] Iminosugars based glycosidase inhibitors are generally six- or seven-membered rings in size.

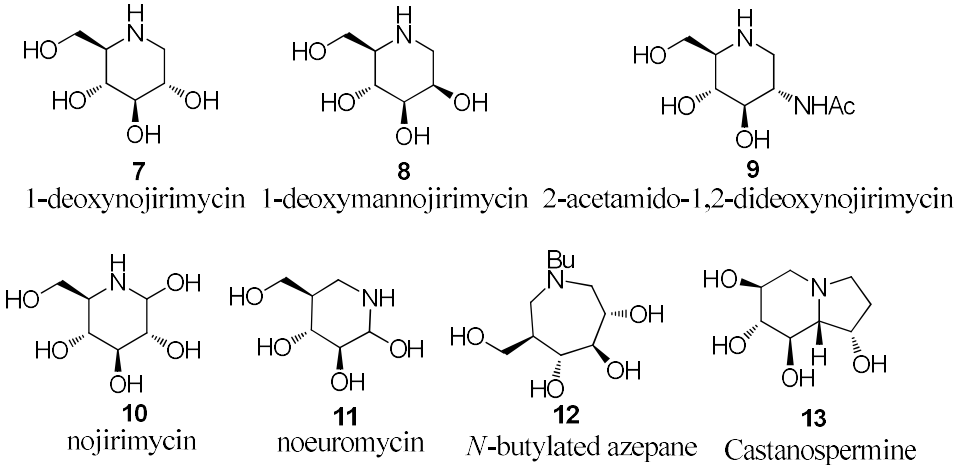


Figure 5.2: structural representation of iminosugars **7**-**13**.

A strong inhibitory activity of azasugars was explained based on the transition state structures of the enzyme with the active substrate and inhibitors (Figure 5.3). They act as strong and stable inhibitors due to the formation of transition state similar to the stable oxacarbenium ion with regular glycosidase substrates.

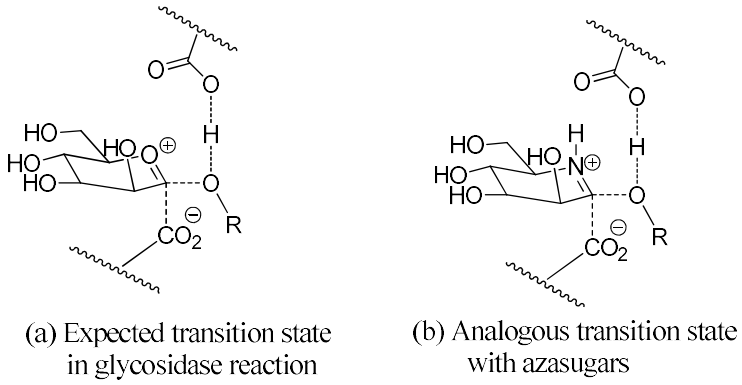
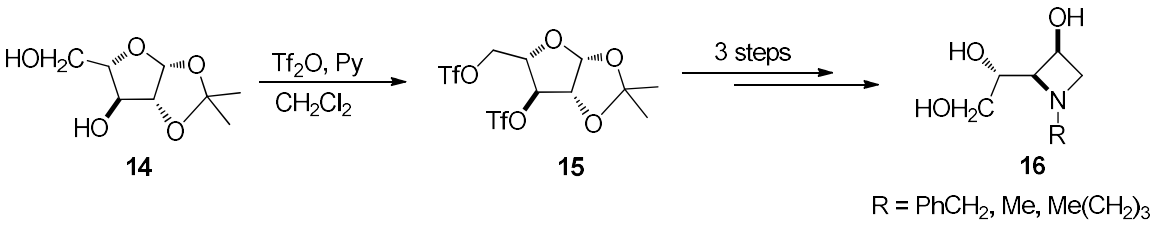


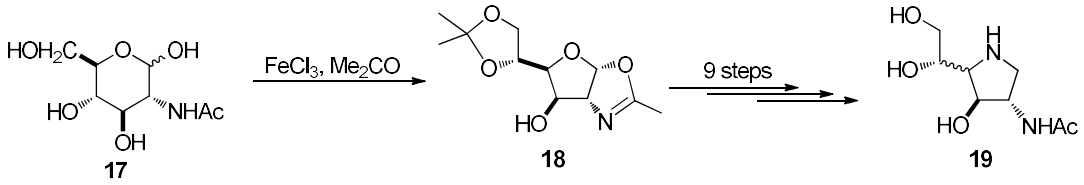
Figure 5.3: Comparison of transition states of (a) active substrate and (b) inhibitor in glycosidase catalyzed hydrolysis.

Fleet and co-workers synthesized the azetidine iminosugars from L-arabinose and ribose sugars. This synthetic strategy involves ring-closure of stable 3,5-di-*O*-triflyl pentose **15** with the alkyl and aralkyl amines to give the four-membered iminosugar **16** (Scheme 5.2). Preliminary studies suggest that these substrates show significant and specific inhibition of non-mammalian α-glucosidases.[19]



Scheme 5.2: Synthesis of azetidine iminosugar **16** from protected L-arabinose **14**.

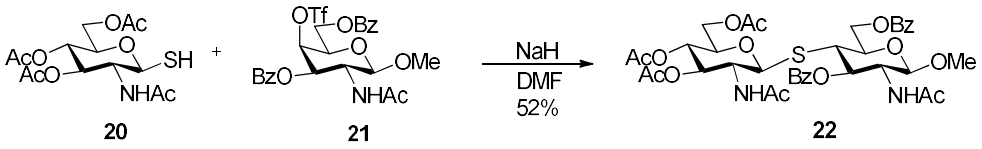
Croucher *et al*. reported an efficient method for the synthesis of 2-acetamido-1,2,4-trideoxy-1,4-imino-D-galactitol **19** and its epimer from *N*-acetyl glucosamine **17** (Scheme 5.3). Syntheses of these compounds were achieved using the Fleet's methodology of making imino-alditols. In the context of biological evaluation, these compounds show strong inhibitory activities towards hexosamindases.[20]



Scheme 5.3: Synthesis of 2-acetamido-1,2,4-trideoxy-1,4-imino-D-galactitol **19** from *N*-acetyl glucosamine **17**.

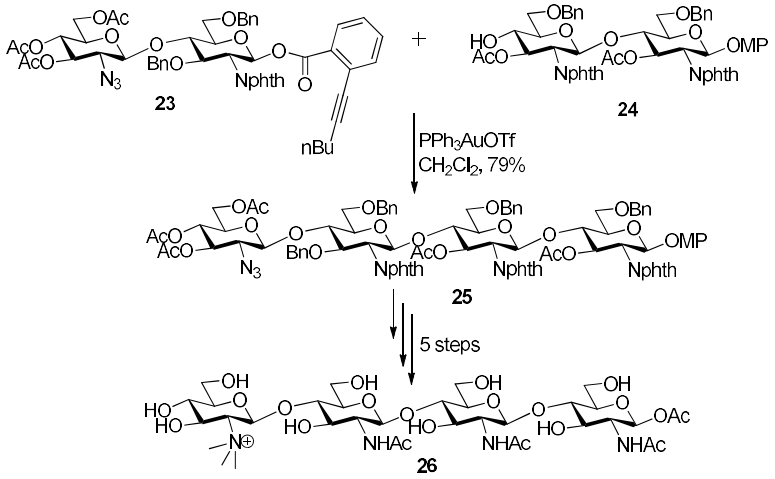
5.1.2 Chitooligosaccharide related glycosidase inhibitors

Thiochitooligosaccharides have the potential to resist the enzymatic hydrolysis due to the presence of stronger thioglycosidic linkage.[21] Wang *et al*. reported a stereoselective synthesis of thiochitobioside **22** by the coupling of triflate **21** with *N*-acetylglucosamine thiol derivative **20** in presence of NaH in DMF solvent (Scheme 5.4).[22]



Scheme 5.4: Synthesis of thiochitobiose derivative **22**.

TMG-chitotriomycin is a structural mimic of chitotetrose having *N,N′,N″*-trimethyl-D-glucosamine (TMG) linked to *N,N′,N″*-triacetylchitotriose at its non-reducing end. It is a potent and selective inhibitor of *N*-acetyl-*β*-glucosaminidases (GlcNAcases) of insects and fungi. Interestingly it does not show inhibition towards the GlcNAcases of plants and mammals.[23] Yang *et al*. achieved the total synthesis of TMG-chitotriomycin *via* Au(I)-catalyzed glycosylation of glycosyl *ortho*-hexynylbenzoate donors (Scheme 5.5).[24]



Scheme 5.5: Synthesis of TMG-chitotriomycin using glycosyl *ortho*-hexynylbenzoates as donors.

5.2 Results and discussion

With the aim to prepare glycosidase inhibitors, we have designed the target molecules which mimic the structures of chitobiosides having different *N*-acetylated patterns. These molecules are expected to show inhibitor activity by resisting the enzymatic hydrolysis due to absence of anomeric carbon. The planed target chitobioside mimics **A** are shown in the Figure 5.4.

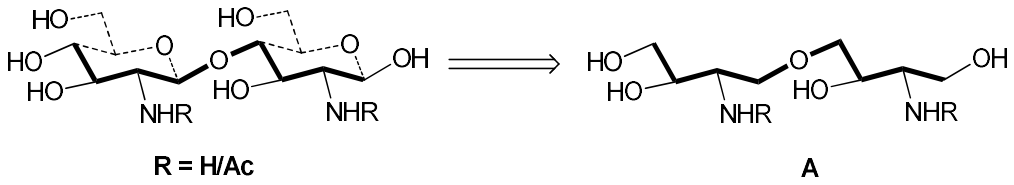


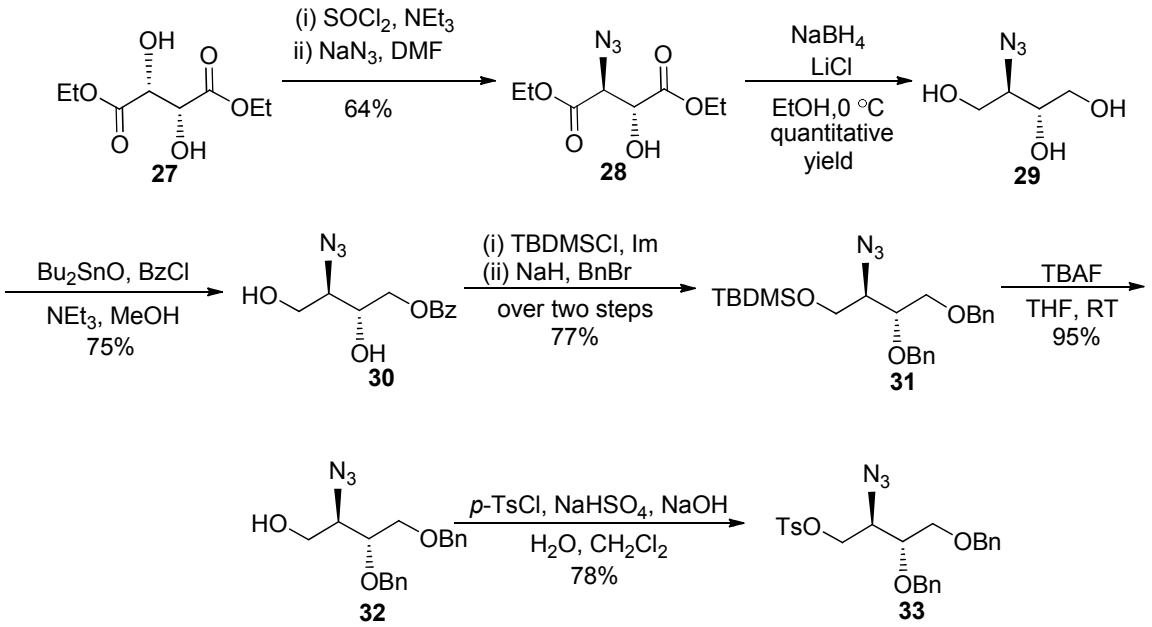
Figure 5.4: Structural representation of planed target chitobioside mimics **A**.

5.2.1 Preparation of building blocks for the synthesis of target chitobioside mimics

5.2.1.1 Synthesis of azido tosyl derivative

For the synthesis of proposed glycosidase inhibitors, we concentrated first on the synthesis of tosylate building block **33** and alcohol derivatives **35** and **37**. Synthesis of tosyl derivative **33** was achieved from the commercially available (+)-diethyl L-tartrate in 7 steps. To begin the synthesis, (+)-diethyl tartrate **27** was converted in to its azido derivative **28** by treating it with thionyl chloride followed by NaN_3 in DMF. Reduction of the ester groups in **28** was carried out in presence of $NaBH_4$/LiCl conditions which yielded the azido triol **29** under literature reported condition.[25] Selective benzoylation of azido triol **29** with *n*-Bu_2SnO/BzCl reagent system gave the mono benzoyl azide derivative **30** *via* the formation of stannylene acetal intermediate in 75% yield.[26] This is the key intermediate for the preparation of building block **33** utilized in the subsequent steps. Silylation of the primary alcohol function in **30** followed by debenzoylation and benzylation with BnBr in presence of NaH in one pot yielded the fully protected silyl derivative **31** in 77% yield.[27]

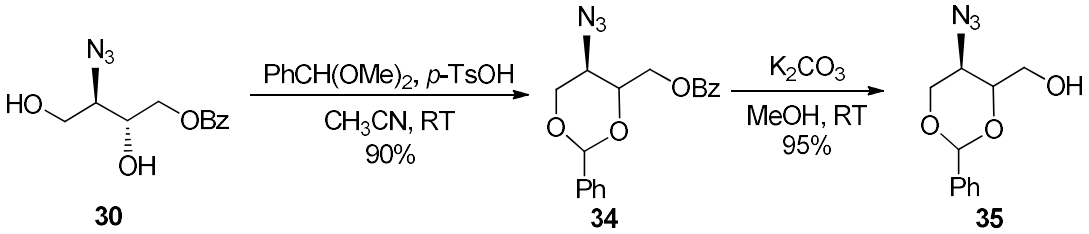
The tosyl building block **33** was finally obtained by desilylation[28] of compound **31** using TBAF in THF solvent and subsequent tosylation using *p*-TsCl/NaOH[29] in 78% yield (Scheme 5.6). This was utilized for the coupling (etherification[30]) reactions.



Scheme 5.6: Preparation of azido tosyl derivative **33**.

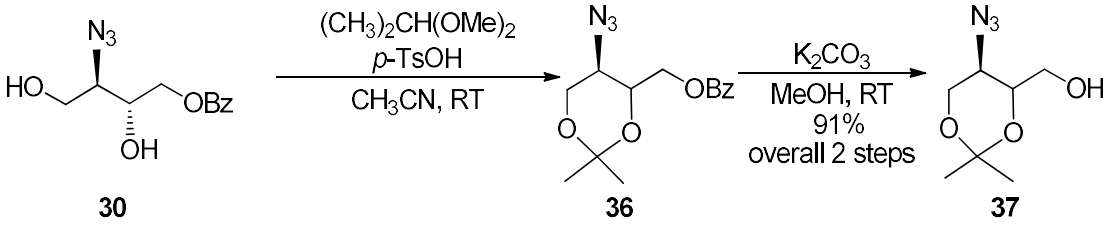
5.2.1.2 Synthesis of azido alcohol derivatives 35 and 37

Benzylidene derivative **35** was synthesized from the key benzoylated intermediate **30** in 2 steps. Initially, compound **30** was treated with $PhCH(OMe)_2$ in presence of *p*-TsOH to get benzylidene acetal derivative **34** in 90% yield. Debenzoylation of **34** was carried out using K_2CO_3 in MeOH solvent to get the required **35** in 95% (Scheme 5.7).[27]



Scheme 5.7: Preparation of azido alcohol derivative **35**.

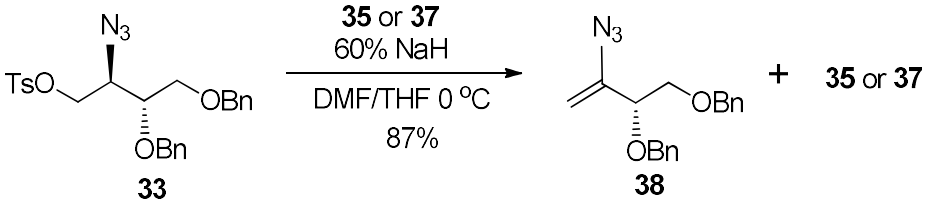
For the synthesis of acetonide derivative **37**, mono benzoylated derivative **30** was treated with 2,2-dimethoxypropane in CH_3CN solvent to get the intermediate derivative **36**.[31] Debenzoylation of compound **36** yielded the derivative **37** in 91% yield (Scheme 5.8). Primary alcohol derivatives **35** and **37** were employed in the etherification reaction with tosylate derivative **33**.



Scheme 5.8: Preparation of acetonide derivative **37**.

5.2.1.3 Etherification (coupling) of alcohol derivatives 35 and 37 with tosyl derivative 33

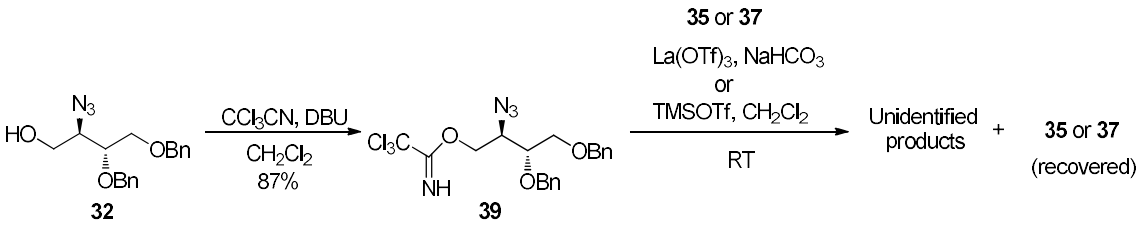
Having successfully made the building blocks tosylate **33** and alcohol derivatives **35** and **37**, we focused on the synthesis of protected mimics of chitobiose. Unfortunately, when the oxyanion derived from **35** or **37** was treated with the tosylate **33** in DMF or THF solvent it underwent elimination instead of substitution to yield the vinyl azide derivative **38**. Reason for the elimination may be the acidic nature of proton adjacent to the azide group. Several attempts were made by changing the reaction conditions. However, all the reactions resulted in the product **38** along with quantitative recovery of the alcohol derivative **35**/**37** (Scheme 5.9).



Scheme 5.9: Attempted etherification reaction using tosyl derivative **33**.

5.2.1.4 Preparation of acetimidate derivative

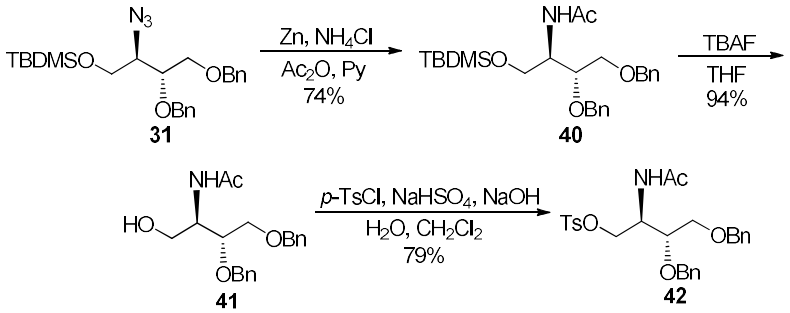
Due to the unsuccessful attempts with tosylate **33**, acetimidate building block **39** was synthesized for the coupling reaction. The reason for choosing acetimidate leaving group is that the coupling reaction could be carried out under Lewis acidic conditions thereby base which created problem with **33** is avoided. The reaction of azido alcohol derivative **32** with CCl_3CN in presence of DBU yielded the acetimidate derivative **39** in 87% yield.[32,33] The activation of acetimidate derivative **39** in presence of alcohol derivatives **35** and **37** under different Lewis acid catalyst resulted in a mixture of unidentified products (Scheme 5.10). The alcohol derivatives were recovered quantitatively.



Scheme 5.10: Attempted etherification *via* acetimidate derivative **39**.

5.2.1.5 Synthesis of *N*-acetyl protected tosylate derivative

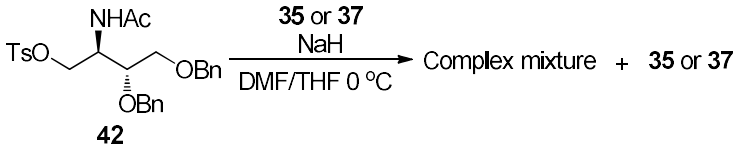
The failures of the above attempts were believed to be due to the presence of azide group next to the leaving group. Therefore, it was planned to convert it into NHAc group before coupling. Accordingly the synthesis of required **42** was started from intermediate **31**. Reduction of azide in compound **31** using Zn dust under neutral conditions followed by acetylation of the resulting free amine gave the *N*-acetyl derivative **40** in 74% yield. Desilylation of silyl group in derivative **40** using TBAF in THF solvent at room temperature yielded the primary alcohol derivative **41** in 94% yield. Finally tosylation of the primary alcohol **41** under standard tosylation conditions yielded the tosyl derivative **42** in 79% yield (Scheme 5.11).



Scheme 5.11: Preparation of *N*-acetyl protected tosyl derivative **42**.

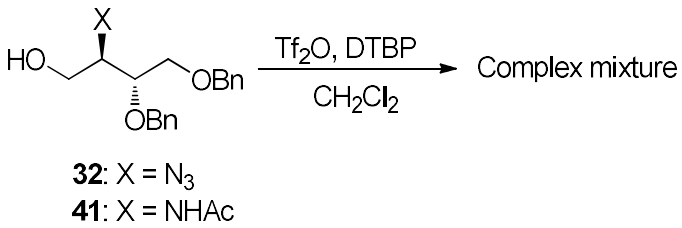
5.2.1.6 Attempted etherification of *N*-acetyl tosylate 42 with 35 and 37

We tested the etherification of alcohol derivatives **35** and **37** with the *N*-acetyl protected tosyl derivative **42** separately (Scheme 5.12). Under basic conditions tosylate **42** decomposed to give a complex product mixture along with the unreacted alcohol derivatives **35** or **37**.



Scheme 5.12: Attempted etherification of *N*-acetyl tosylate **42**.

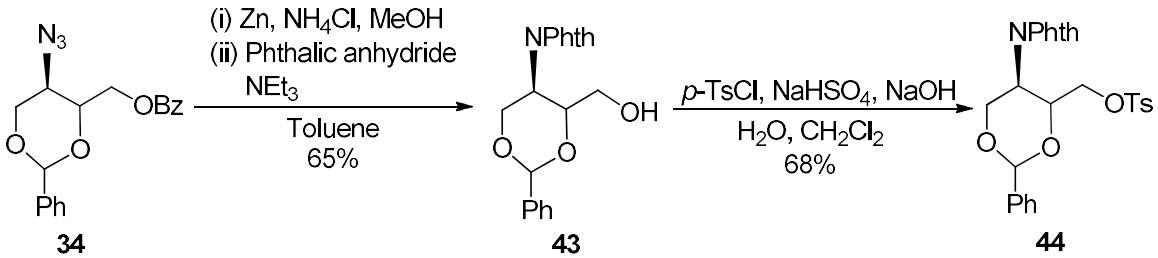
After unsuccessful etherification using tosyl derivative **42**, we wished to use the triflate derived from **32** or **41** for etherification reaction considering the higher reactivity of triflates. But preparation triflate derivative itself was problematic using Tf_2O/DTBP as it resulted in a complex mixture of products (Scheme 5.13).



Scheme 5.13: Attempts to prepare N_3 or *N*-acetyl containing triflate derivatives.

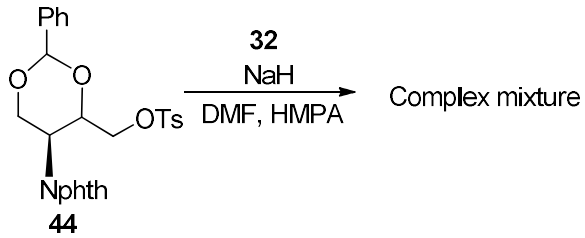
5.2.1.7 Etherification with azido alcohol derivatives 32 of *N*-Phth protected tosylte derivative 44

After unsuccessful etherification with azido and *N*-acetyl derivatives, we looked on to the synthesis of *N*-Phth protected derivative **44** from the benzylidene derivative **34**. This way the roles of the coupling partners are reversed, i.e., leaving group is placed on the hydroxyl derivative used in the earlier attempts. First step involves the reduction of azide in compound **34** under Zn/NH_4Cl conditions followed by phthalimide protection of the free amine to get protected primary alcohol derivative **43** in 65% yield. Tosylation of the primary alcohol function in **43** yielded the required *N*-Phth protected tosyl derivative **44** in 68% yield (Scheme 5.14). Synthesised tosyl derivatives **44** and azido alcohol **32** were utilized in the coupling reactions.



Scheme 5.14: Preparation of *N*-Phth protected tosyl derivative **44**.

Under etherification conditions (NaH in DMF), decomposition of both the tosyl derivative **44** and alcohol derivative **32** was observed. Even in the presence of HMPA which can selectively solvate the cation (Na^+) to accelerate the reaction did not help (Scheme 5.15).



Scheme 5.15: Etherification attempt with *N*-Phth protected tosylate derivative **44**.

5.2.2 Synthesis of aryl esters having free or *N*-acetylated amine

Since all our efforts to make the designed substrates failed we turned our attention to make simple analogues having the core unit. With this intention we planned the synthesis of the benzoic ester derivatives **B** as shown in Figure 5.5. In this structure aryl ring will provide certain rigidity and when it has hydroxyl or amino groups H-bonding interaction can be facilitated.

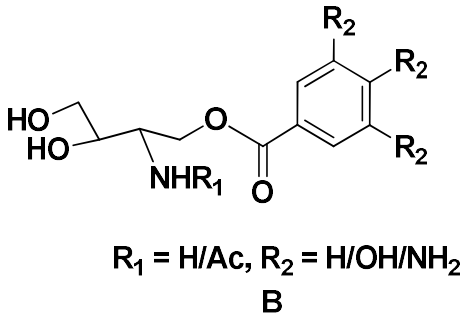
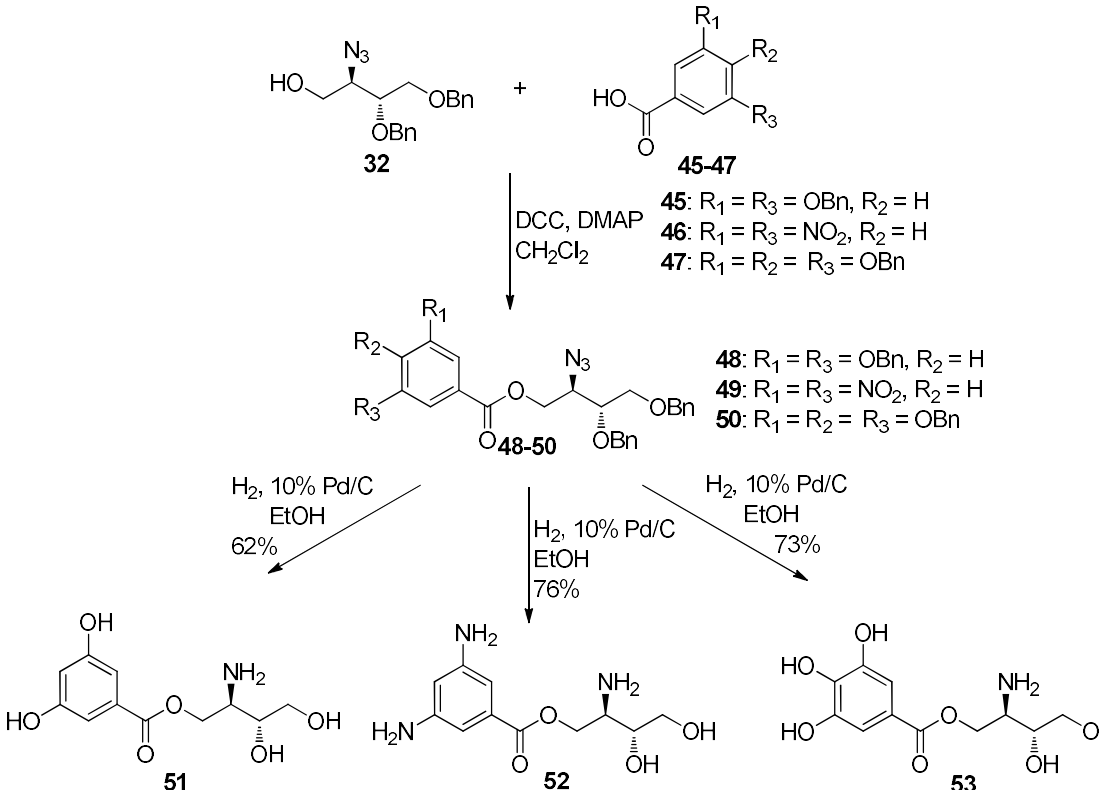


Figure 5.5: Structural representation of target aryl esters **B**.

5.2.2.1 Preparation of aryl esters having free amine (51-53)

Building block **32** was used for the syntheses of aryl esters **51**-**53**. Firstly substituted benzoic acids **45**-**47** were esterified with the azido alcohol **32** separately in presence of DCC and DMAP to get the protected aryl esters **48**-**50** in 78%, 82% and 81% yields respectively.[35,36] Treatment of **48**-**50**, with H_2 in the presence of 10% Pd/C resulted the desired aryl esters **51**-**53** (Scheme 5.16). Nitro groups in the substrate **49** also got reduced to primary amine group under the reaction condition to give the expected **52**.



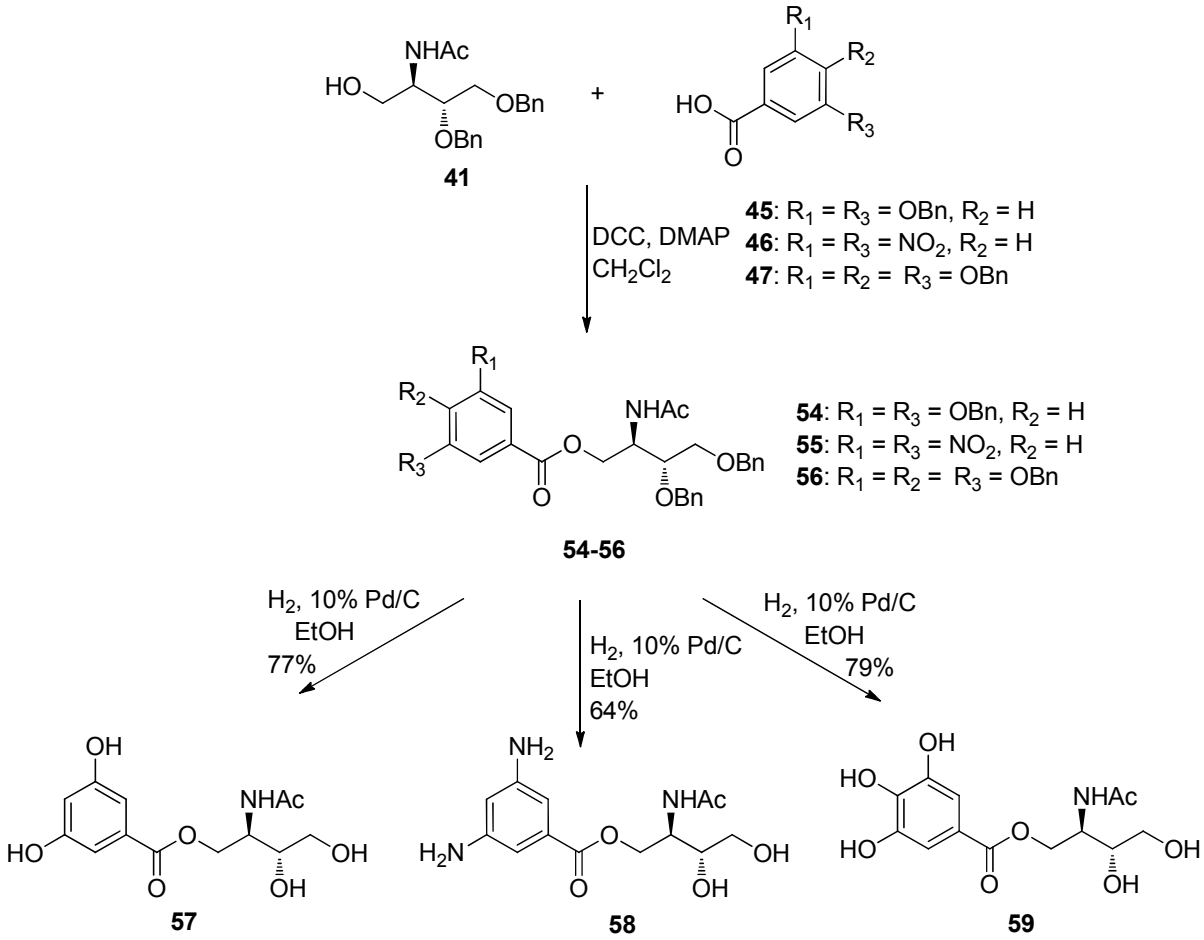
Scheme 5.16: Preparation of aryl esters **51**-**53**.

5.2.2.2 Preparation of aryl esters having *N*-acetyl amine

For the synthesis of *N*-acetyl aryl esters **57**-**59** the syntheses were started from *N*-acetamido primary alcohol **43**. Like in the previous case, esterification followed by treatment with H_2 in the presence of 10% Pd/C resulted the desired aryl esters **57**-**59** in good yields (Scheme 5.18).

5.2.3 Screening of aryl esters for chitinase inhibitor activities

To check the inhibitory activities of the synthesized esters **51**-**53** and **57**-**59** we chose highly active chitinases *Pe*Chi3 from *Paenibacillus elgii* and *Sp*ChiD from *Serratia proteamaculans*. In the presence of free amino/*N*-acetyl containing phenyl esters (**51** and **53**), *Pe*Chi3 activity was measured using colloidal chitin polymer (1%) as substrate. There was no loss of activity of *Pe*Chi3 on colloidal chitin in the presence of substituted phenyl esters (Figure 5.6). The absence of any observable effect on the activity of *Pe*Chi3 may be due to the aryl ester derivatives are smaller in size, thereby, not fitting in the extended catalytic binding cleft of *Pe*Chi3 enzyme.



Scheme 5.18: Preparation of aryl esters having *N*-acetyl amine (**57**-**59**).

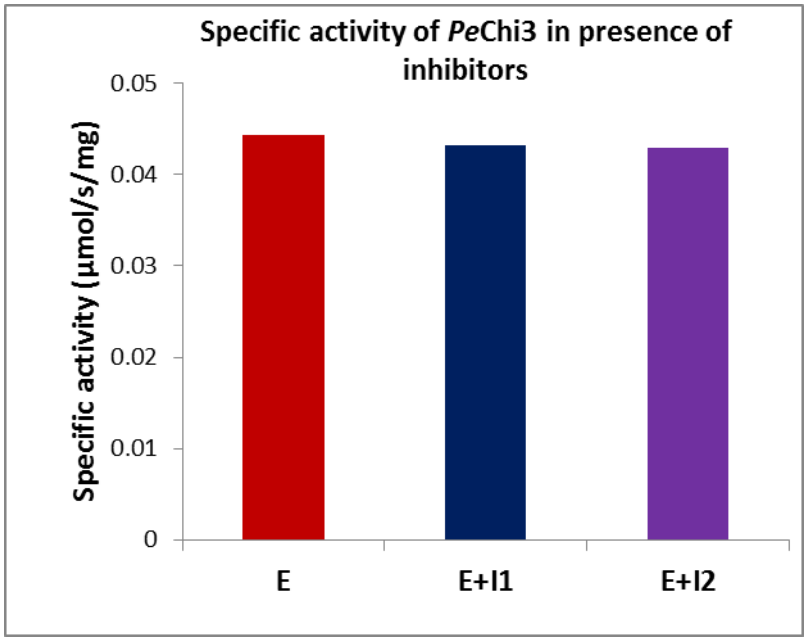


Figure 5.6: Specific activity of *Pe*Chi3 from *Paenibacillus elgii* on colloidal chitin in presence of aryl esters I1 = **51** and I2 = **53**.

As *Pe*Chi3 requires the chitin oligomer of minimum chain length of three, we wanted to test the inhibitory activity on an enzyme which has chitobiase activity. Therefore, we checked the chitinase inhibitory activity on *Sp*ChiD using the aryl esters (**51**-**53** and **57**-**59**). *Sp*ChiD is active on the higher chitooligosaccharides and also exhibited chitobiase activity by hydrolysing $(GlcNAc)_2$ to GlcNAc. Activity of *Sp*ChiD on *p*-nitrophenyl tetra *N*-acetylchitotetrose was tested in the presence of aryl esters. Absorption at 405 nm due to the release of *p*-nitrophenol from the *p*-nitrophenyl tetra *N*-acetylchitotetrose was the base for the measurement of activity of *Sp*ChiD. Here also no significant reduction of the activity of *Sp*ChiD in cleaving the *p*NP tetramer was observed in presence of phenyl esters (Figure 5.7). These results clearly indicate that the synthesized aryl esters do not bind to the catalytic cleft of *Sp*ChiD to affect its enzymatic activity significantly. These experiments were carried out in the lab of Prof. Appa Rao, School of Lifesciences, University of Hyderabad with the enzymes generated in their lab. With this we can conclude that phenyl esters are not showing inhibitory activities on chitinases having larger catalytic cleft. However, they might show activity on glycosidases such as glucosaminidases and *N*-acetyl glucosaminidases having smaller catalytic cleft.

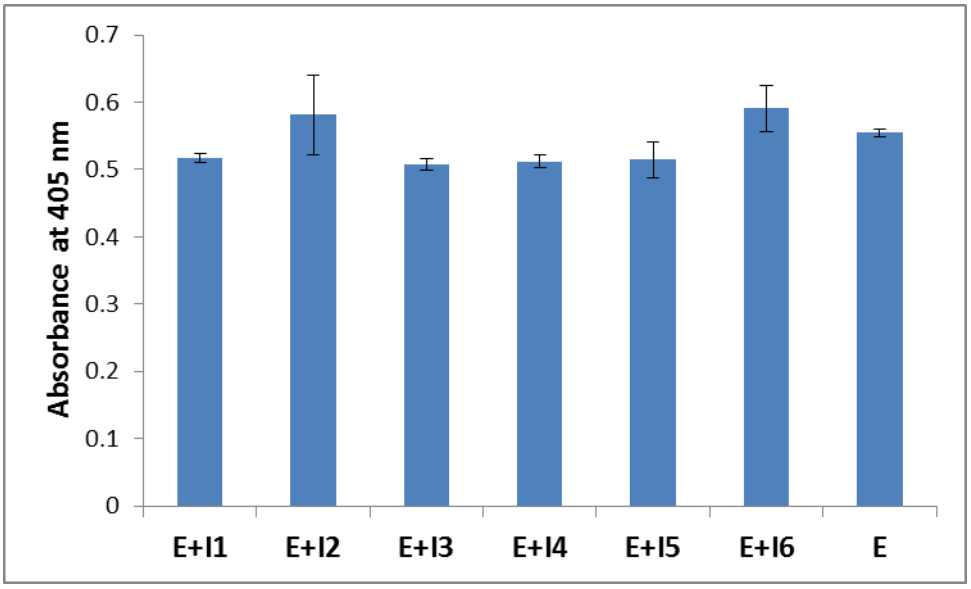


Figure 5.7: Activity of *Sp*ChiD on *p*-nitrophenyl tetra *N*-acetylchitotetrose in the presence of putative inhibitors (Inhibitors: I1 = **51**, I2 = **52**, I3 = **53**, I4 = **57**, I5 = **58**, I6 = **59**).

5.3 Conclusions

With the aim to make glycosidase inhibitors, we attempted to synthesize chitobioside mimics which do not have anomeric carbon. However, all our attempts to synthesize the planed target chitobioside mimics failed. Later aryl esters having free or *N*-acetylated amine were prepared and their activities were checked. These compounds, however, do not show any significant inhibitory activities against *Pe*Chi3 and *Sp*ChiD perhaps due to the larger catalytic binding cleft of chitinases (*Pe*Chi3 and *Sp*ChiD). It is anticipated that these compounds might have inhibitory activity against glucosaminidases and *N*-acetyl glucosaminidases. Efforts should be made on these fronts.

5.4 Experimental section

For general information refer Chapter 2, Section 2.4.1.

5.4.1 Experimental procedures, spectral and analytical data

General procedure for tosylation[30]

Primary alcohol (1 equiv.) and tetra-*n*-butylammonium hydrogen sulfate (0.2 equiv.) were dissolved in THF (10 mL/mmol). The reaction mixture was cooled to 0 ºC, slow dropwise addition of NaOH solution (1.2 equiv.) in water (1 mL/mmol) followed by the addition of tosylchloride (1.2 equiv.) dissolved in THF (2 mL/mmol) to the reaction mixture. After 15 h, completion of the reaction was confirmed by TLC. Organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine and dried over Na_2SO_4. silicagel column chromatography using EtOAc/hexanes mixture as eluent to obtain tosylate derivatives.

General procedure for coupling reaction (etherification)[35]

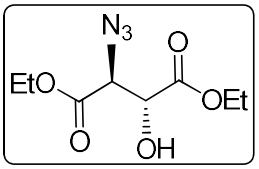
Primary alcohol derivative (1 equiv.) was dissolved in dry THF (5 mL/mmol) or DMF (3 mL/mmol). The solution was cooled to 0 ºC, then addition of 60% NaH (1.2 equiv.) suspended in mineral oil with constant stirring. After 30 min, slow dropwise addition of tosyl derivative in dry THF or DMF to the reaction mixture was carried out at same temperature. The mixture was warmed to room temperature and allowed to stir for 3 h. The resulted residue was quenched with MeOH and the mixture was diluted with EtOAc, washed with saturated NaCl, dried over anhydrous Na_2SO_4, and the organic layer evaporated to afford the

crude product. Purification was carried out on silicagel column chromatography using EtOAc/hexanes mixture as eluent.

General procedure for esterification[35]

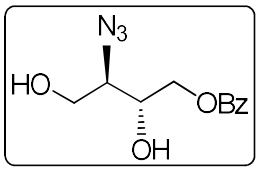
A suspension of primary alcohol derivative (2 equiv.), DCC (1.2 equiv.), and substituted benzoic acid (1 equiv.) in CH_2Cl_2 (8 mL/mmol) were treated with DMAP (0.1 equiv.) and stirred at room temperature for 24 h. Colourless solid dicyclohexyl urea formation was observed in the reaction, which was removed by filtration and washed with CH_2Cl_2. The filtrate was evaporated under reduced pressure and purified by using on silicagel column chromatography using EtOAc/hexanes mixture as eluent to obtain the aryl ester.

Diethyl (2*R*,3*S*)-3-azido-3-deoxy-tartrate 28



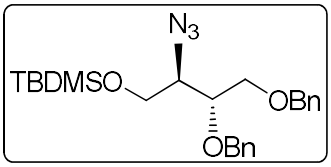
The compound **28** was prepared from diethyl-(D)-tartrate **27** (4 g, 19.39 mmol) in 2 steps using literature reported method.[25] Oily liquid; Yield = 2.9 g (64%); R_f = 0.45 in 1:3 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.56 (d, *J* = 2.0 Hz, 1H), 4.35 (d, *J* = 2.6 Hz, 1H), 4.31-4.26 (m, 4H), 3.65 (br s, 1H), 1.33-1.29 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 166.9, 71.9, 64.2, 62.4, 62.2, 13.8.

(2*R*,3*R*)-3-azido-2,4-dihydroxybutyl benzoate 30



The compound **30** was prepared from compound **28** (2.0 g, 8.56 mmol) in 2 steps using literature reported method.[26] Colourless solid; Yield = 1.6 g (75%); R_f = 0.30 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (d, *J* = 7.8 Hz, 2H), 7.59 (t, *J* = 7.2 Hz, 1H), 7.45 (t, *J* = 7.6 Hz, 2H), 4.61 (dd, *J* = 12.0, 2.9 Hz, 1H), 4.44 (dd, *J* = 11.9, 5.7 Hz, 1H), 4.06 (br s, 1H), 3.99-3.91 (m, 2H), 3.64-3.60 (m, 1H), 3.43 (br s, 1H), 2.70 (br s, 1H), ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.2, 133.5, 129.7, 128.5, 70.4, 66.5, 63.7, 62.6.

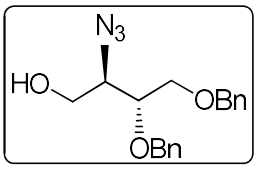
((2*R*,3*R*)-2-azido-3,4-bis(benzyloxy)butoxy)(*tert*-butyl)dimethylsilane 31



The compound **31** was prepared from compound **30** (1.6 g, 6.42 mmol) by following general procedure for silyl ether protection (Chapter 2). Yellow gummy liquid; Yield = 2.18 g (77%); R_f = 0.70 in 1:3 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35-7.30 (m, 10H), 4.70 (d, *J* = 11.6 Hz, 1H), 4.57 (d, *J* = 11.7 Hz, 3H), 3.91 (dd,

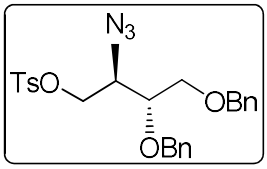
J = 10.7, 3.0 Hz, 1H), 3.78 (dd, *J* = 10.5, 5.9 Hz, 1H), 3.73-3.59 (m, 4H), 0.90 (s, 9H), 0.07 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.1, 138.1, 138.0, 128.3, 127.8, 127.7, 127.6, 127.5, 76.9, 74.6, 73.4, 72.7, 69.5, 63.5, 63.2, 58.0, 25.8, 18.1, -5.6, -5.8. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{24}H_{35}N_3O_3Si$ 442.2526, found 442.2520.

(2*R*,3*R*)-2-azido-3,4-bis(benzyloxy)butan-1-ol 32



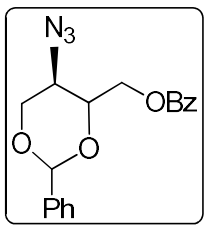
The compound **32** was prepared from compound **31** (0.5 g, 1.13 mmol) by general procedure for silyl ether deprotection (Chapter 2). Colourless liquid; Yield = 0.35 g (95%); R_f = 0.37 in 1:3 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34-7.29 (m, 10H), 4.67 (d, *J* = 11.6 Hz, 1H), 4.57-4.54 (m, 3H), 3.82-3.67 (m, 5H), 3.64-3.59 (m, 1H), 2.46 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.5, 128.4, 128.3, 128.0, 127.9, 128.7, 127.7, 77.9, 73.4, 72.5, 68.6, 63.4, 62.1. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{18}H_{21}N_3O_3$ 350.1481, found 350.1472.

(2*R*,3*R*)-2-azido-3,4-bis(benzyloxy)butyl 4-methylbenzenesulfonate 33



The compound **33** was prepared from compound 32 (100 mg, 0.32 mmol) by using general procedure for preparation of tosylate. Light yellow liquid; Yield = 120 mg (78%); R_f = 0.60 in 1:3 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, *J* = 8.3 Hz, 2H), 7.36-7.29 (m, 10H), 7.23 (d, *J* = 6.9 Hz, 2H), 4.59 (d, *J* = 11.6 Hz, 1H), 4.50-4.46 (m, 3H), 4.31 (dd, *J* = 10.6, 3.5 Hz, 1H), 4.13 (dd, *J* = 10.5, 7.4 Hz, 1H), 3.88 (td, *J* = 7.1, 3.1 Hz, 1H), 3.72-3.52 (m, 3H), 2.42 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 145.0, 137.6, 137.3, 132.6, 129.8, 128.4, 127.9, 127.8, 127.7, 127.6, 76.8, 73.4, 72.4, 69.1, 67.9, 60.7, 21.6.

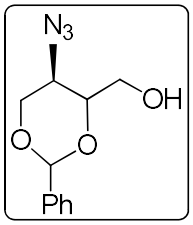
((5*R*)-5-azido-2-phenyl-1,3-dioxan-4-yl)methyl benzoate 34



The compound **34** was prepared from compound **30** (200 mg, 0.8 mmol) using general procedure for bezylidine acetal protection (Chapter 2). Colourless liquid; Yield = 244 mg (90%); R_f = 0.57 in 1:3 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (d, *J* = 7.6 Hz, 2H), 7.57 (t, *J* = 7.3 Hz, 1H), 7.49-7.43 (m, 3H), 7.37-7.35 (m, 3H), 5.53 (s, 1H), 4.70 (dd, *J* = 12.3, 2.4 Hz, 1H), 4.58 (dd, *J* = 12.1, 4.7 Hz, 1H), 4.48-4.44 (m, 1H), 3.97-3.93 (m, 1H), 3.82-3.72 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.2, 136.9, 133.2, 129.7,

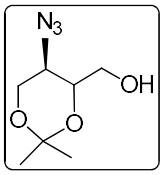
129.2, 128.4, 128.3, 126.1, 101.3, 78.02, 68.9, 63.9, 53.6. HRMS-ESI (m/z): $[M+K]^+$ calcd for $C_{18}H_{17}N_3O_4$ 378.0856, found 378.0847.

((5*R*)-5-azido-2-phenyl-1,3-dioxan-4-yl)methanol 35



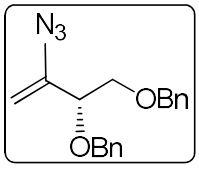
The compound **35** was prepared from compound **34** (210 mg, 0.62 mmol) by using general procedure of *O*-deacetylation (Chapter 2). Light yellow liquid; Yield = 139 mg (95%); R_f = 0.52 in 1:3 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47-7.46 (m, 2H), 7.38-7.37 (m, 3H), 5.48 (s, 1H), 4.40 (dd, *J* = 10.7, 4.9 Hz, 1H), 3.93-3.89 (m, 1H), 3.80-3.73 (m, 2H), 3.70-3.63 (m, 2H), 2.17 (br s, 1H), ^{13}C NMR (100 MHz, $CDCl_3$): δ 136.9, 129.3, 128.3, 126.1, 101.2, 80.2, 68.7, 62.0, 52.6. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{11}H_{13}N_3O_3$ 258.0855, found 258.0892.

((5*R*)-5-azido-2,2-dimethyl-1,3-dioxan-4-yl)methanol 37



The compound **37** was prepared from compound **30** (230 mg, 0.92 mmol) by using general procedure for bezylidine acetal protection followed by *O*-deacetylation (Chapter 2). Yellow liquid; Yield = 230 mg (94%); R_f = 0.50 in 1:3 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.00 (dd, *J* = 11.5, 5.4 Hz, 1H), 3.81 (d, *J* = 9.9 Hz, 1H), 3.76-3.66 (m, 3H), 3.63 (dd, *J* = 9.4, 5.1 Hz, 1H), 2.19 (br s, 1H), 1.47 (s, 3H), 1.42 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 99.0, 72.5, 62.6, 61.9, 53.7, 28.4, 19.3.

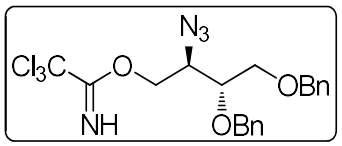
2-Azido-3,4-bis(benzyloxy)-2-butene 38



The compound **38** was obtained in coupling reaction of compound of **35** (28 mg, 0.12 mmol) or **37** (25 mg, 0.12 mmol) and with tosylate **33** (50 mg, 0.11 mmol). Colourless liquid; Yield = 29.6 mg (87%); R_f = 0.68 in 1:3 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34-7.24 (m, 10H), 5.04 (br s, 1 H), 4.92 (br s, 1H), 4.68 (d, *J* = 11.9 Hz, 1H), 4.56 (q, *J* = 12.0 Hz, 2H), 4.46 (d, *J* = 11.9 Hz, 1H), 3.98 (t, *J* = 5.7 Hz, 1H), 3.62 (d, *J* = 5.8 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.9, 138.1, 137.7, 128.4, 128.3, 127.8, 127.7, 127.6, 101.1, 78.6, 73.4, 71.3, 71.0. HRMS-ESI (m/z): $[M+K]^+$ calcd for $C_{18}H_{19}N_3O_2$ 348.1114, found 348.1134.

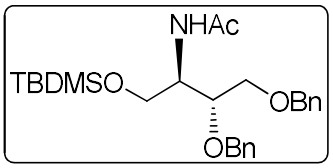
(2*R*,3*R*)-2-azido-3,4-bis(benzyloxy)butyl 2,2,2-trichloroacetimidate 39

The compound **39** was prepared from compound **32** (100 mg, 0.31 mmol) by procedure for the preparation of trichloroacetimidate (Chapter 3). Yellow liquid; Yield = 127 mg (87%); R_f = 0.76 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27-7.15 (m, 10H), 4.57 (t, *J* = 12.5 Hz, 2H), 4.47-4.43 (m, 3H), 4.38 (dd, *J* = 11.4, 7.4 Hz, 1H), 3.94-3.90 (m, 1H), 3.65-3.61 (m, 1H), 3.58-3.52 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.6, 137.6, 137.3, 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 89.6, 76.9, 73.5, 72.5, 68.3, 69.2, 67.8, 60.6, 53.6.



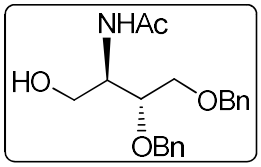
N-((2*R*,3*R*)-3,4-bis(benzyloxy)-1-((tert-butyldimethylsilyl)oxy)butan-2-yl)acetamide 40

The compound **40** was prepared from compound **31** (0.5 g, 1.13 mmol) using general procedure for reductive acetylation of azide (Chapter 2). Light yellow solid; Yield = 0.38 g (74%); R_f = 0.51 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36-7.28 (m, 10H), 6.18 (d, *J* = 8.6 Hz, 1H), 4.74 (d, *J* = 11.8 Hz, 1H), 4.64 (d, *J* = 11.8 Hz, 1H), 4.60 (d, *J* = 11.8 Hz, 1H), 4.54 (d, *J* = 11.8 Hz, 1H), 4.29-4.26 (m, 1H), 3.85 (dd, *J* = 9.8, 4.0 Hz, 1H), 3.80-3.73 (m, 3H), 3.57 (dd, *J* = 9.9, 6.3 Hz, 1H), 1.90 (s, 3H), 0.89 (s, 9H), 0.06 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.6, 138.4, 137.9, 128.4, 128.3, 127.8, 127.7, 127.6, 76.3, 73.6, 72.2, 71.5, 61.6, 51.4, 25.8, 23.4, 18.1, -5.4, -5.6.



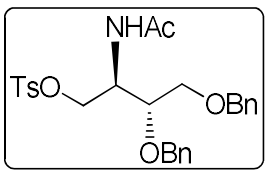
N-((2*R*,3*R*)-3,4-bis(benzyloxy)-1-hydroxybutan-2-yl)acetamide 41

The compound **41** was prepared from compound **40** (0.3 g, 0.66 mmol) using general procedure for silyl ether deprotection (Chapter 2). Oily liquid; Yield = 0.21 g (94%); R_f = 0.28 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34-7.28 (m, 10H), 4.73 (d, *J* = 11.7 Hz, 1H), 4.59 (d, *J* = 11.8 Hz, 1H), 4.55-4.51 (m, 2H), 4.24 (br s, 3H), 3.73-3.61 (m, 4H), 1.95 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.0, 138.6, 138.2, 128.4, 128.3, 127.7, 127.6, 127.5, 79.9, 73.4, 72.9, 70.7, 69.4, 67.8, 13.9.



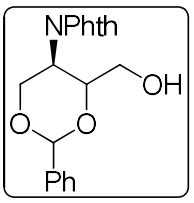
(2*R*,3*R*)-2-acetamido-3,4-bis(benzyloxy)butyl 4-methylbenzenesulfonate 42

The compound **42** was prepared from compound **41** (90 mg, 0.27 mmol) using general procedure of preparation of tosylate. Colourless liquid; Yield = 106 mg (79%); R_f = 0.40 in 1:1 EtOAc/hexanes; ^{1}H



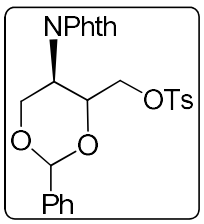
NMR (400 MHz, $CDCl_3$): δ 7.70 (d, *J* = 8.2 Hz, 2H), 7.49-7.33 (m, 10H), 7.26-7.24 (m, 2H), 5.21 (d, *J* = 8.5 Hz, 1H), 4.59 (d, *J* = 11.7 Hz, 1H), 4.51-4.50 (m, 2H), 4.40 (d, *J* = 11.7 Hz, 1H), 4.21 (dd, *J* = 11.5, 6.2 Hz, 1H), 3.98 (dd, *J* = 11.5, 4.5 Hz, 1H), 3.76 (br s, 1H), 3.61-3.60 (m, 3H), 2.42 (s, 3H), 1.90 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 143.3, 137.8, 137.7, 137.6, 129.5, 128.5, 127.9, 127.8, 127.7, 127.1, 76.8, 73.5, 72.4, 69.4, 62.8, 53.9, 21.5, 20.6.

2-((5*S*)-4-(hydroxymethyl)-2-phenyl-1,3-dioxan-5-yl)isoindoline-1,3-dione 43



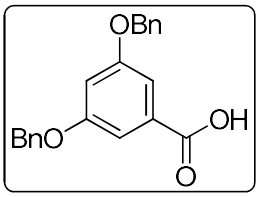
To a solution of benzylidene deivative **34** (100 mg, 0.42 mmol) in methanol (3 mL) NH_4Cl (51 mg, 0.8 mmol), and Zn dust (51 mg, 0.8 mmol) were added. The reaction mixture was stirred vigorously for 2 h at room temperature. The resulted mixture was filtered and concentrated in vacuo. The crude product was directly used for next step without purification. Crude product was treated with phthalic anhydride (2 equiv.) in presence of triethyl amine in MeOH as a solvent. The residue was filtered through a small pad of silica column to obtain pure compound **43** as oily liquid. Yield = 93 mg (65%); R_f = 0.30 in 1:3 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.76 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.55 (dd, *J* = 7.8, 2.0 Hz, 1H), 7.49 (t, *J* = 7.4 Hz, 1H), 7.40-7.38 (m, 2H), 7.32 (t, *J* = 7.9 Hz, 1H), 5.79 (s, 1H), 5.12 (dt, *J* = 8.4, 4.1 Hz, 1H), 4.76 (td, *J* = 10.9, 5.1 Hz, 1H), 4.64-4.58 (m, 1H), 4.53-4.44 (m, 2H), 4.19 (dd, *J* = 10.5, 5.0 Hz, 1H), ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.9, 166.1, 134.3, 132.9, 132.6, 131.5, 129.6, 129.2, 128.3, 128.2, 126.2, 123.6, 101.5, 73.9, 66.2, 64.3, 45.8.

((5*S*)-5-(1,3-dioxoisoindolin-2-yl)-2-phenyl-1,3-dioxan-4-yl)methyl-4-methylbenzenesulfonate 44



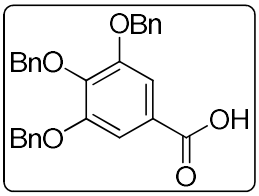
The compound **44** was prepared from compound **43** (50 mg, 0.15 mmol) general procedure of preparation of tosylate. Yellow liquid; Yield = 50 mg (68%); R_f = 0.37 in 1:3 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88-7.86 (m, 2H), 7.76-7.75 (m, 2H), 7.55-7.53 (m, 2H), 7.30 (d, *J* = 5.9 Hz, 3H), 7.26-7.23 (m, 3H), 7.17 (d, *J* = 7.2 Hz, 2H), 5.76 (s, 1H), 4.82-4.79 (m, 1H), 4.71-4.58 (m, 2H), 4.17 (dd, *J* = 9.6, 4.1 Hz, 1H), 3.76 (dd, *J* = 12.1, 2.2 Hz, 1H), 3.66 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.35 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 166.1, 142.8, 134.3, 134.2, 133.3, 130.0, 129.4, 129.0, 128.2, 125.8, 125.5, 123.4, 75.3, 74.8, 70.2, 69.3, 23.0.

3,5-bis(benzyloxy)benzoic acid 45



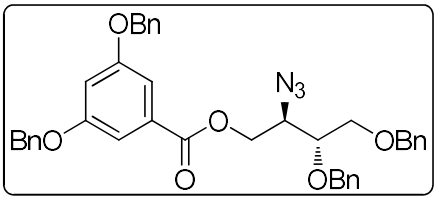
The compound **45** was prepared from 3,5-dihydroxybenzoic acid (2 g, 13.0 mmol), using literature procedure.[35] Colourless solid; Yield = 4 g (94%) overall 2 steps ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44-7.30 (m, 11H), 6.82-6.81 (m, 1H), 5.07 (br s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.6, 136.4, 128.6, 128.1, 127.6, 108.6, 107.3, 70.2.

3,4,5-tris(benzyloxy)benzoic acid 47



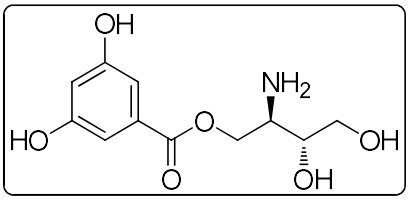
The compound **47** was prepared from 3,4,5-trihydroxybenzoic acid (2 g, 11.8 mmol) using literature procedure.[35] Colourless solid; Yield = 4.8 g (92%) overall 2 steps; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45-7.34 (m, 13H), 7.28-7.26 (m, 5H), 5.15 (br s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.6, 137.3, 136.5, 128.6, 128.5, 128.2, 128.1, 128.0, 127.5, 109.6, 77.2, 75.2, 71.2.

(2*R*,3*R*)-2-azido-3,4-bis(benzyloxy)butyl 3,5-bis(benzyloxy)benzoate 48



The compound **48** was prepared from **32** (100 mg, 0.31 mmol) and **45** (101 mg, 0.31 mmol) using general procedure for eserification. Yellow liquid; Yield = 83 mg (78%); R_f = 0.57 in 1:1 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43-7.24 (m, 22H), 6.82 (t, *J* = 2.2 Hz, 1H), 5.05 (br s, 4H), 4.68 (d, *J* = 11.6 Hz, 2H), 4.64-4.53 (m, 3H), 4.43 (dd, *J* = 11.6, 7.6 Hz, 1H), 4.03-3.99 (q, *J* = 2.9 Hz, 1H), 3.75-3.72 (m, 1H), 3.69-3.63 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.8, 159.8, 137.7, 137.4, 136.4, 131.4, 128.6, 128.4, 128.1, 127.9, 127.8, 127.7, 127.5, 108.4, 107.6, 73.5, 72.5, 70.2, 68.4, 64.7, 60.9.

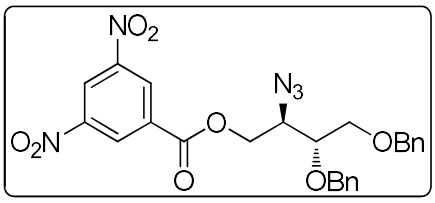
(2*R*,3*R*)-2-amino-3,4-dihydroxybutyl 3,5-dihydroxybenzoate 51



The compound **51** was prepared from compound **48** (27 mg, 0.078 mmol) using general procedure for *O*-debenzylation. Amorphous solid; Yield = 12.4 mg (62%); ^{1}H NMR (400 MHz, CD_3OD+$CDCl_3$): δ 7.03-7.02 (m,

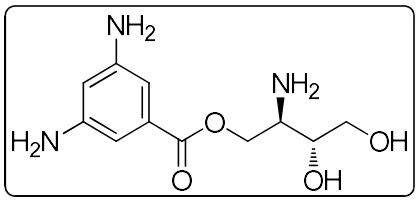
1H), 6.99-6.98 (m, 1H), 6.91 (br s, 1H), 4.65-4.63 (m, 1H), 4.56-4.54 (m, 1H), 3.86-3.77 (m, 3H), 3.72-3.68 (m, 2H), 3.64-3.58 (m, 3H). HRMS-ESI (m/z): $[M+K]^+$ calcd for $C_{11}H_{15}NO_6$ 296.0536, found 296.0548.

(2*R*,3*R*)-2-azido-3,4-bis(benzyloxy)butyl 3,5-dinitrobenzoate 49



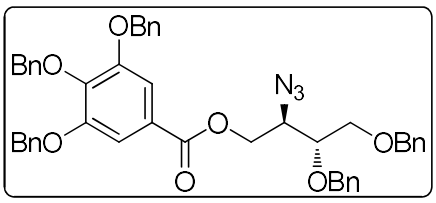
The compound **49** was prepared from **32** (100 mg, 0.31 mmol) and 3,5-dinitrobenzoic acid (57 mg, 0.31 mmol) using general procedure for esterification. Yellow liquid; Yield = 132 mg (82%); R_f = 0.49 in 1:3 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.19-9.06 (m, 3H), 7.34-7.20 (m, 10H), 4.72 (t, *J* = 11.6 Hz, 2H), 4.59-4.53 (m, 4H), 4.08-4.06 (m, 1H), 3.79-3.68 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.8, 148.6, 137.5, 137.2, 133.2, 129.4, 128.5, 128.4, 128.0, 127.9, 127.7, 122.5, 76.9, 73.6, 72.3, 67.9, 65.9, 60.6.

(2*R*,3*R*)-2-amino-3,4-dihydroxybutyl 3,5-diaminobenzoate 52



The compound **52** was prepared from **49** (27 mg, 0.05 mmol) using general procedure for *O*-debenzylation (Chapter 2). Amorphous solid; Yield = 9.5 mg (76%); ^{1}H NMR (400 MHz, CD_3OD+$CDCl_3$): δ 7.15-7.14 (m, 3H), 3.77-3.69 (m, 9H). HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{11}H_{17}N_3O_4$ 278.1117, found 278.1168.

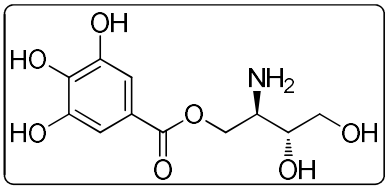
(2*R*,3*R*)-2-azido-3,4-bis(benzyloxy)butyl 3,4,5-tris(benzyloxy)benzoate 50



The compound **50** was prepared from **32** (100 mg, 0.31 mmol) and **47** (44 mg, 0.31 mmol) using general procedure for esterification. Yellow liquid; Yield = 188 mg (81%); R_f = 0.64 in 1:3 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43-7.21 (m, 27H), 5.13-5.09 (m, 6H), 4.68-4.62 (m, 2H), 4.57-4.50 (m, 3H), 4.40 (dd, *J* = 11.6, 7.8 Hz, 1H), 4.01-3.98 (m, 1H), 3.74-3.72 (m, 1H), 3.67-3.62 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.6, 152.5, 142.4, 137.7, 137.4, 137.3, 136.6, 128.5, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 124.5, 109.0, 108.9, 76.8, 75.0, 73.5, 72.5, 71.0, 68.4, 64.9, 61.0.

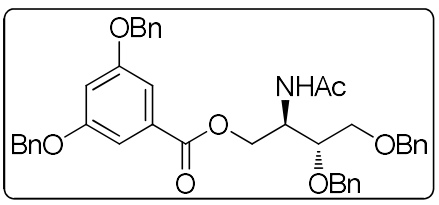
(2*R*,3*R*)-2-amino-3,4-dihydroxybutyl 3,4,5-trihydroxybenzoate 53

The compound **53** was prepared from **50** (39 mg, 0.06 mmol) using general procedure for *O*-debenzylation (Chapter 2). Colourless solid; Yield = 10 mg (73%); ^{1}H NMR (400 MHz, CD_3OD+$CDCl_3$): δ 6.85-6.84 (m, 2H), 4.15 (s, 2H), 3.98 (s, 1H), 3.44-3.37 (m, 4H). HRMS-ESI (m/z): $[M+K]^+$ calcd for $C_{11}H_{15}NO_7$ 312.0486, found 312.0497.



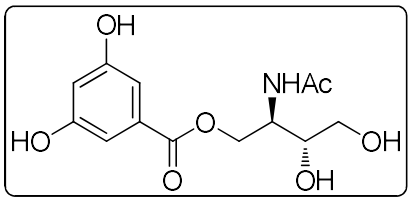
(2*R*,3*R*)-2-acetamido-3,4-bis(benzyloxy)butyl 3,5-bis(benzyloxy)benzoate 54

The compound **54** was prepared from **41** (100 mg, 0.32 mmol) and **45** (101 mg, 0.31 mmol) general procedure of esterification. Colourless liquid; Yield = 159 mg (79%); R_f = 0.54 in 1:3 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43-7.22 (m, 22H), 6.80 (t, *J* = 2.2 Hz, 1H), 6.06 (d, *J* = 8.9 Hz, 1H), 5.05 (s, 4H), 4.71 (d, *J* = 11.9 Hz, 1H), 4.62-4.54 (m, 4H), 4.45 (dd, *J* = 11.3, 6.4 Hz, 1H), 4.33 (dd, *J* = 11.3, 5.1 Hz, 1H), 3.75-3.71 (m, 3H), 2.17 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.8, 166.1, 159.7, 137.9, 137.6, 136.4, 131.6, 128.6, 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 108.4, 107.3, 77.2, 76.5, 73.7, 72.3, 70.7, 63.7, 23.3.



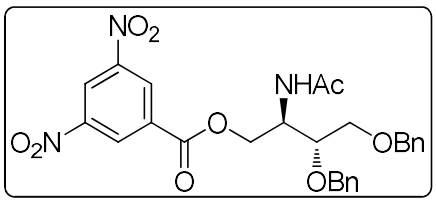
(2*R*,3*R*)-2-acetamido-3,4-dihydroxybutyl 3,5-dihydroxybenzoate 57

The compound **57** was prepared from **54** (31 mg, 0.05 mmol) using general procedure for *O*-debenzylation (Chapter 2). Colourless solid; Yield = 10.9 mg (77%); ^{1}H NMR (400 MHz, CD_3OD+$CDCl_3$): δ 7.01 (d, *J* = 2.1 Hz, 1H), 6.97 (d, *J* = 2.0 Hz, 1H), 6.54 (br s, 1H), 4.45-4.41 (m, 1H), 4.31-4.22 (m, 1H), 3.72-3.67 (m, 3H), 3.65-3.59 (m, 3H), 2.02 (s, 3H). HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{13}H_{17}NO_7$ 300.1083, found 300.1084.



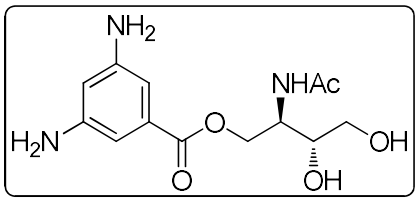
(2*R*,3*R*)-2-acetamido-3,4-bis(benzyloxy)butyl 3,5-dinitrobenzoate 55

The compound **55** was prepared from **41** (92 mg, 0.29 mmol) and 3,5-dinitrobenzoic acid (55 mg, 0.29 mmol) using general procedure for esterification. Yellow solid; Yield = 114 mg (78%); R_f = 0.40 in 1:3 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.17 (d, *J* = 1.9 Hz, 1H), 9.04 (t, *J* = 1.7 Hz, 2H), 7.36-7.26 (m, 10H), 6.02 (d, *J* = 8.4 Hz, 1H), 4.73 (d, *J* = 12.0 Hz, 1H), 4.62-4.46 (m,



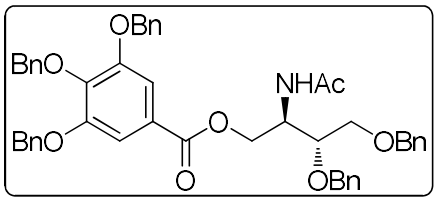
6H), 3.79-3.77 (m, 1H), 3.74-3.72 (m, 2H), 1.83 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.9, 162.4, 148.5, 137.7, 137.4, 133.6, 129.4, 128.6, 128.5, 128.2, 128.1, 127.9, 127.7, 122.3, 76.8, 73.7, 72.4, 70.0, 65.3, 49.3, 23.2.

(2*R*,3*R*)-2-acetamido-3,4-dihydroxybutyl 3,5-dihydroxybenzoate 58



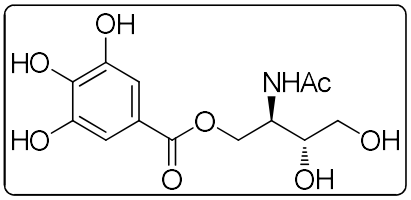
The compound **58** was prepared from **55** (42 mg, 0.085 mmol) using general procedure for *O*-debenzylation (Chapter 2). Colourless solid; Yield = 15.5 mg (64%); ^{1}H NMR (400 MHz, CD_3OD+$CDCl_3$): δ 7.15-7.14 (m, 3H), 3.94-3.93 (m, 1H), 3.65-3.62 (m, 1H), 3.50-3.39 (m, 5H), 1.81 (s, 3H). HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{13}H_{19}N_3O_5$ 320.1222, found 320..1217.

(2*R*,3*R*)-2-acetamido-3,4-bis(benzyloxy)butyl 3,4,5-tris(benzyloxy)benzoate 56



The compound **56** was prepared from **41** (91 mg, 0.29 mmol) and **47** (44 mg, 0.31 mmol) using general procedure for esterification. Light yellow solid; Yield = 179 mg (84%); R_f = 0.60 in 1:3 EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42-7.25 (m, 22H), 5.99 (d, *J* = 3.9 Hz, 1H), 5.13-5.10 (m, 6H), 4.72 (dd, *J* = 11.9, 5.3 Hz, 1H), 4.62-4.53 (m, 4H), 4.42-4.30 (m, 2H), 3.72-3.71 (m, 3H), 1.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.8, 152.5, 137.9, 137.6, 137.4, 136.6, 128.5, 128.2, 128.0, 127.9, 127.7, 127.5, 124.7, 109.1, 75.1, 73.7, 72.3, 71.1, 70.7, 63.8, 49.4, 23.4.

(2*R*,3*R*)-2-acetamido-3,4-dihydroxybutyl 3,5-dihydroxybenzoate 59



The compound **59** was prepared from **56** (39 mg, 0.051 mmol) using general procedure for *O*-debenzylation (Chapter 2). Light yellow solid; Yield = 12 mg (79%); ^{1}H NMR (400 MHz, CD_3OD+$CDCl_3$): δ 7.02-6.99 (m, 2H), 3.73-3.54 (m, 7H), 1.88 (s, 3H). HRMS-ESI (m/z): $[M+K]^+$ calcd for $C_{13}H_{17}NO_8$ 354.0591, found 354.0598.

5.4.2 Enzyme assays

Reducing end assay to measure activity of *Pe*Chi3 in presence of aryl ester derivatives[37]

Chitinase activity was measured by a modified Schales' procedure using colloidal chitin as the substrate. The reaction was performed in 200 µL reaction volume consisting of appropriate amount of enzyme (215 nM), inhibitor (1 mM) and colloidal chitin (1%) in 50 mM sodium acetate buffer (pH 5.6) at 40 °C for 1 h with constant shaking at 300 rpm. The reaction was centrifuged at 16,100×g at 4 °C for 15 min, and 40 µL supernatant was transferred to pre-cooled eppendorf. To this 40 µL of reaction containing reducing sugars, 300 µL of freshly prepared colour reagent (0.5 M sodium carbonate, 0.05% potassium ferricyanide) were added and boiled in dark for 15 min at 100 °C. After cooling to room temperature, 200 µL of each reaction was taken in 96 well microtiter plate and the absorbence was taken at 420 nm using microtiter plate reader (Multiscan, Labsystems, Finland).

Enzyme assays to measure the activity of *Sp*ChiD in presence of aryl ester derivatives[38]

Chitinase activity was measured by colorimetric assay using *p*NP-tetramer as the substrate in the presence of aryl ester derivatives **51-53** and **57-59**. The reaction mixture (20 µL) composed of the appropriate amount of enzyme (4 µmol), 1 mM inhibitor (0.25 mg in 1mL) and 2 mM *p*NP-tetramer (1.7 mg in 1mL) in 50 mM sodium acetate buffer pH 5.6 at 40 °C for 6 h with constant shaking at 300 rpm, at 45 °C. Finally 20 µL of 0.1 M NaOH was added to stop the reaction and to increase the pH of the reaction mixture. Measurement of absorbance of the liberated *p*-nitrophenol was measured at 405 nm using a microplate reader (Multiscan, Labsystems, Finland).

5.5 References

1. Dwek, R. A. *Chem. Rev*. **1996**, *96*, 6832.

2. (a) Groopman, J. E. *Rev. Infect. Dis*. **1990**, *12*, 908. (b) Robina, I.; Moreno-Vargas, A. J.; Carmona, A. T.; Vogel, P. *Curr. Drug Metab*. **2004**, *5*, 329.

3. Zitzmann, N.; Mehta, A. S.; Carrouée, S.; Butters, T. D.; Platt, F. M.; McCauley, J.; Blumberg, B. S.; Dwek, R. A.; Block, T. M. *Proc. Natl. Acad. Sci. U.S.A*. **1999**, *96*, 11878.

4. Mitrakou, A.; Tountas, N.; Raptis, A. E.; Bauer, R. J.; Schulz, H.; Raptis, S. A. *Diabet. Med*. **1998**, *15*, 657;

5. Martin, O. R. *Ann. Pharm. Fr*. **2007**, *65*, 5.

6. (a) Laver, W. G.; Bischofberger, N.; Webster, R. G. *Sci. Am*. **1999**, *280*, 78. (b) Dwek, R. A. *Virology* **2002**, *76*, 3596.

7. Winchester, B.; Vellodi, A.; Young, E. *Biochem. Soc. Trans*. **2000**, *28*, 150.

8. Muzzarelli, R. A. A.; Hepburn, H. R. *The Insect Integument*, *Elsevier: New York*. **1976**.

9. Sakuda, S.; Nishimoto, Y.; Ohi, M.; Watanabe, M.; Takayama, S.; Isogai, A.; Yamada, Y. *Agric. Biol. Chem.* **1990**, *54*, 1333.

10. Koga, D.; Isogai, A.; Sakuda, S.; Matsumoto, S.; Suzuki, A.; Kimura, S.; Ide, A. *Agric. Biol. Chem.* **1987**, *51*, 471.

11. Isogai, A.; Sato, M.; Sakuda, S.; Nakayama, J.; Suzuki, A. *Agric. Biol. Chem.* **1989**, *53*, 2825.

12. Nishimoto, Y.; Sakuda, S.; Takayama, S.; Yamada, Y. *J. Antibiot.* **1991**, *44*, 716.

13. Griffith, D. A.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1996**, *118*, 9526.

14. Hughs, A. B.; Rudge, A. *J. Nat. Prod. Rep*. **1994**, 35.

15. Horne, G.; Wilson, F. X.; Tinsley, J.; Williams, D. H.; Storer, R. *Drug Discovery Today* **2011**, 1*6*, 107.

16. Ishida, N.; Kumagi, K.; Niida, T.; Tsuruoka, T.; Yumoto, H. *J. Antibiotics Ser. A*. **1967**, *20*, 66.

17. Patil, N. T.; John, S.; Sabharwalb, S. G.; Dhavalea, D. D. *Bioorg. Med. Chem*. **2002**, *10*, 2155.

18. Wrodnigg, T. M.; Steiner, A. J.; Ueberbacher, B. J. *Anticancer Agents Med. Chem*. **2008**, *8*, 77.

19. Lenagh-Snow, G. M. J.; Araújo, N.; Jenkinson, S. F.; Rutherford, C.; Nakagawa, S.; Kato, A.; Yu, C. Y.; Weymouth-Wilson, A. C.; Fleet, G. W. J. *Org. Lett.* **2011**, *13*, 5834.

20. Croucher, P. D.; Fumeaux, R.; Lynch, G. P. *Tetrahedron* **1994**, *50*, 13299.

21. Wang, L. X.; Lee, Y. C. *J. Chem. Soc., Perkin Trans.* **1996**, 581.

22. Fettke, A.; Peikow, D.; Peter, M. G.; Kleinpeter, E. *Tetrahedron* **2009**, *65*, 4356.

23. Usuki, H.; Nitoda, T.; Ichikawa, M.; Yamaji, N.; Iwashita, T.; Komura, H.; Kanzaki, H. *J. Am. Chem. Soc.* **2008**, *130*, 4146.

24. Yang, Y.; Li, Y.; Yu, B. *J. Am. Chem. Soc*. **2009**, *131*, 12076.

25. Caldero´n, F.; Doyagu¨ez, E. G.; Mayoralas, A. F. *J. Org. Chem.* **2006**, *71*, 6258.

26. Rai, A. N.; Basu, A. *Org. Lett.* **2004**, *6*, 2861.

27. Arihara, R.; Kakita, K.; Yamada, K.; Nakamura, S.; Hashimoto, S. *J. Org. Chem*. **2015**, *80*, 4278.

28. Fadnavis, N. W.; Sharfuddin, M.; Vadivel, S. K. *Tetrahedron: Asymmetry* **2001**, *12*, 691.

29. Xu, G.; Lv, B.; Roberge, J. Y.; Xu, B.; Du, J.; Dong, J.; Chen, Y.; Peng, K.; Zhang, L.; Tang, X.; Feng, Y.; Xu, M.; Fu, W.; Zhang, W.; Zhu, L.; Deng, Z.; Sheng, Z.; Welihinda, A.; Sun, X. *J. Med. Chem*. **2014**, *57*, 1236.

30. Kabalka, G. W.; Varma, M.; Varma, R. S. *J. Org. Chem.* **1986**, *51*, 2386.

31. Tashiro, T.; Koshino, H.; Shigeura, T.; Watarai, H.; Taniguchi, M.; Mori, K. *Bioorg. Med. Chem*. **2014**, *22*, 827.

32. Li, C.; Wang, J. *J. Org. Chem.* **2007**, *72*, 7431.

33. Rai, A. N.; Basu, A. *Tetrahedron Lett.* **2003**, *44*, 2267.

34. Sikoraiová, J.; Chihab-Eddine, A.; Marchalína, S.; Daïch, A. *J. Heterocyclic Chem*. **2002**, *39*, 383.

35. (a) Neises, B.; Steglich, W. *Angew. Chem. Int. Ed*. **1978**, *17*, 522. (b) Denmark, S. E.; Regens, C. S.; Kobasyashi, T. *J. Am. Chem. Soc*. **2007**, *129*, 2774.

36. Yuan, L.; Sanford, A. R.; Feng, W.; Zhang, A.; Zhu, J.; Zeng, H.; Yamato, K.; Li, M.; Ferguson, J. S.; Gong, B. *J. Org. Chem.* **2005**, *70*, 10660.

37. Imoto, T.;Yagishita, K. *Agric. Biol. Chem.* **1971**, *35*, 1154.

38. Tokuyasu, K.; Ono, H.; Kitagawa, Y.; Ohnishi-Kameyama, M.; Hayashi, K.; Mori, Y. *Carbohydr. Res.* **1999**, *316*, 173.

5.6 Representative spectra

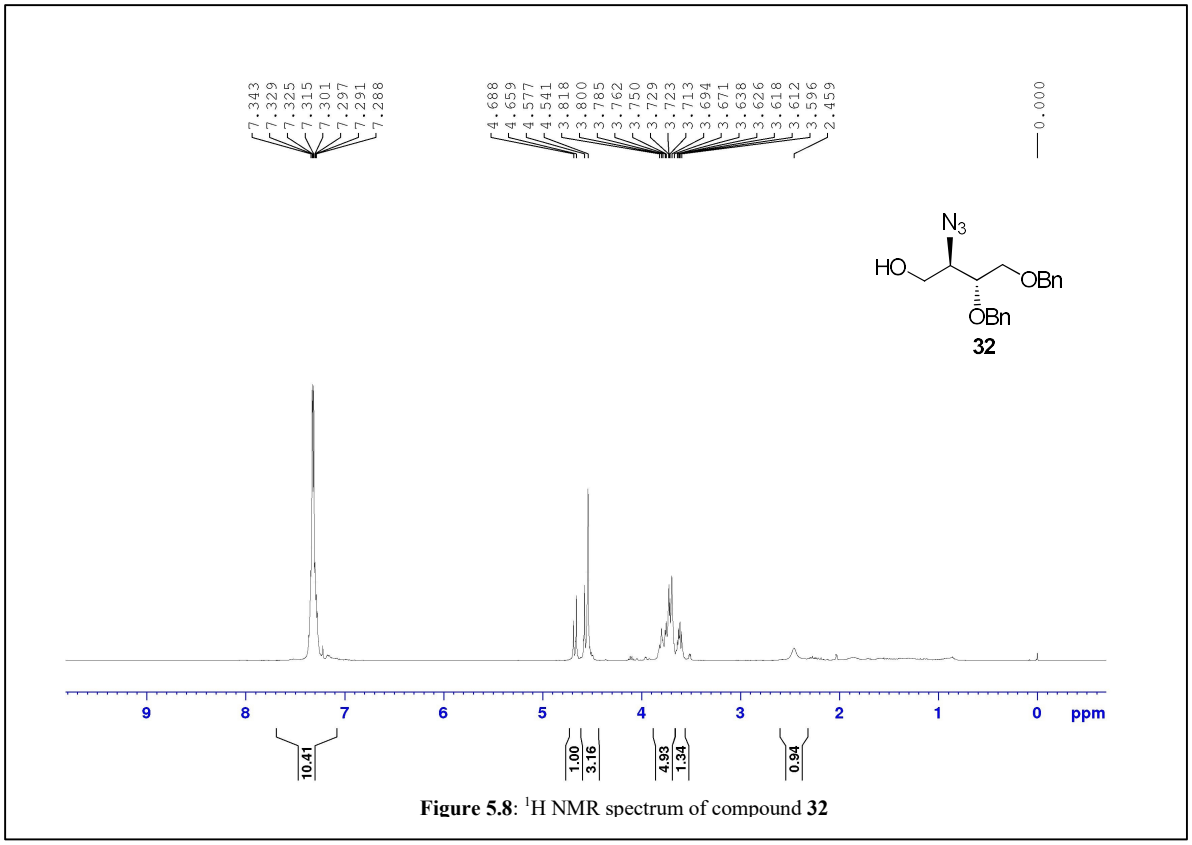


Figure 5.8: ^{1}H NMR spectrum of compound **32**

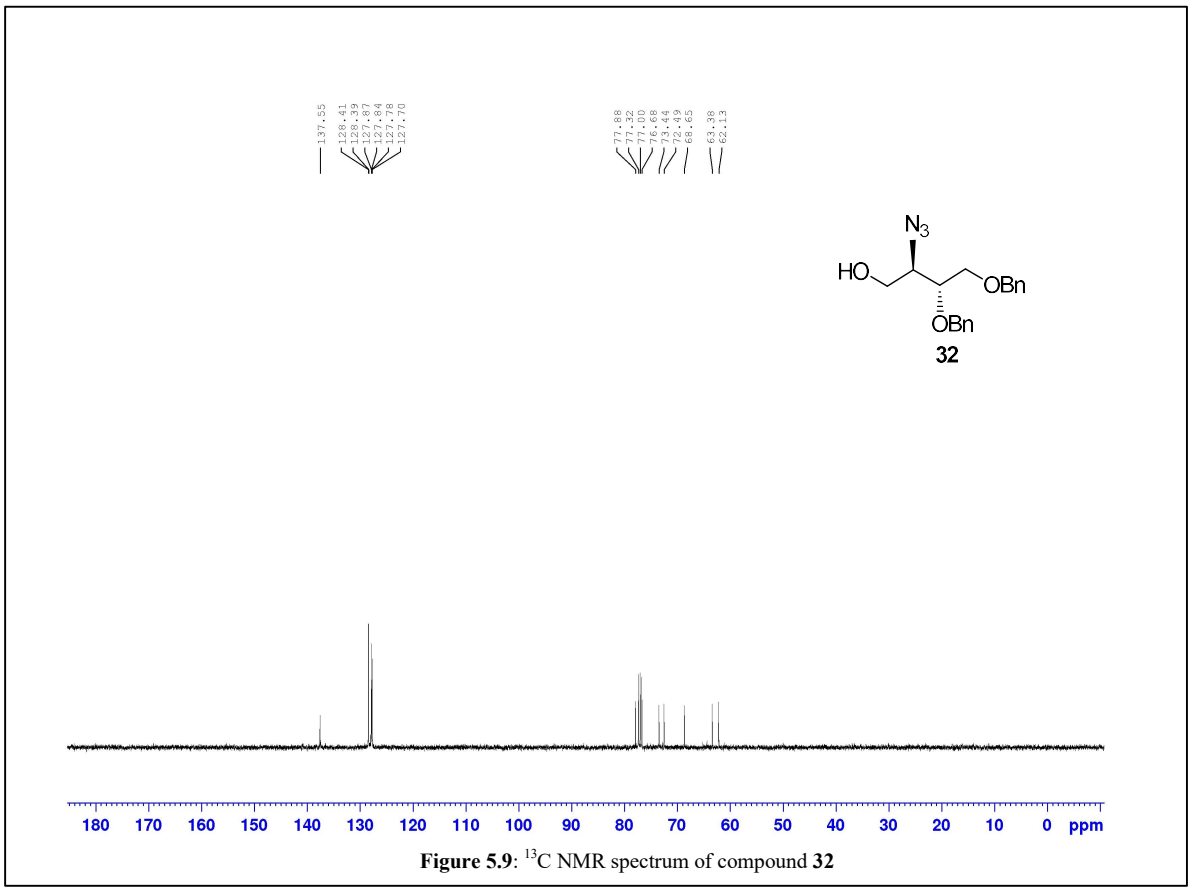


Figure 5.9: ^{13}C NMR spectrum of compound **32**

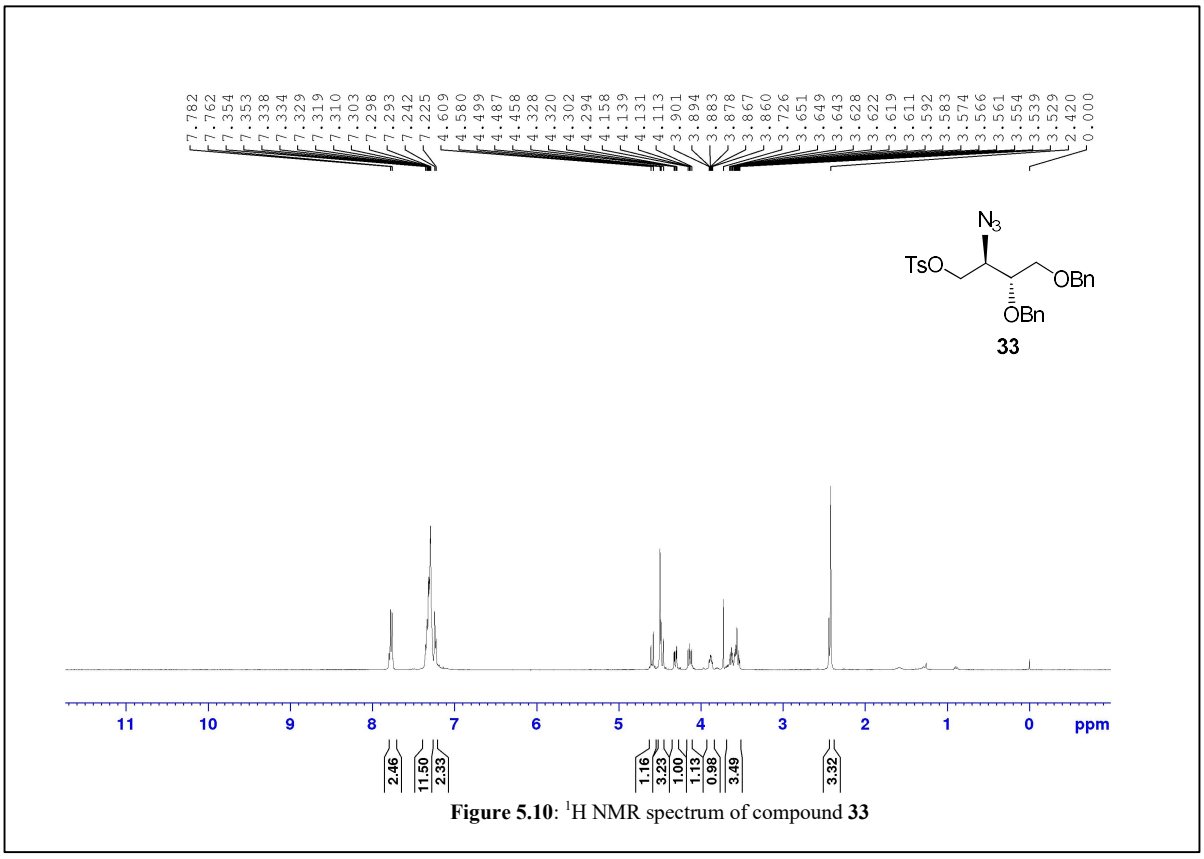


Figure 5.10: ^{1}H NMR spectrum of compound **33**

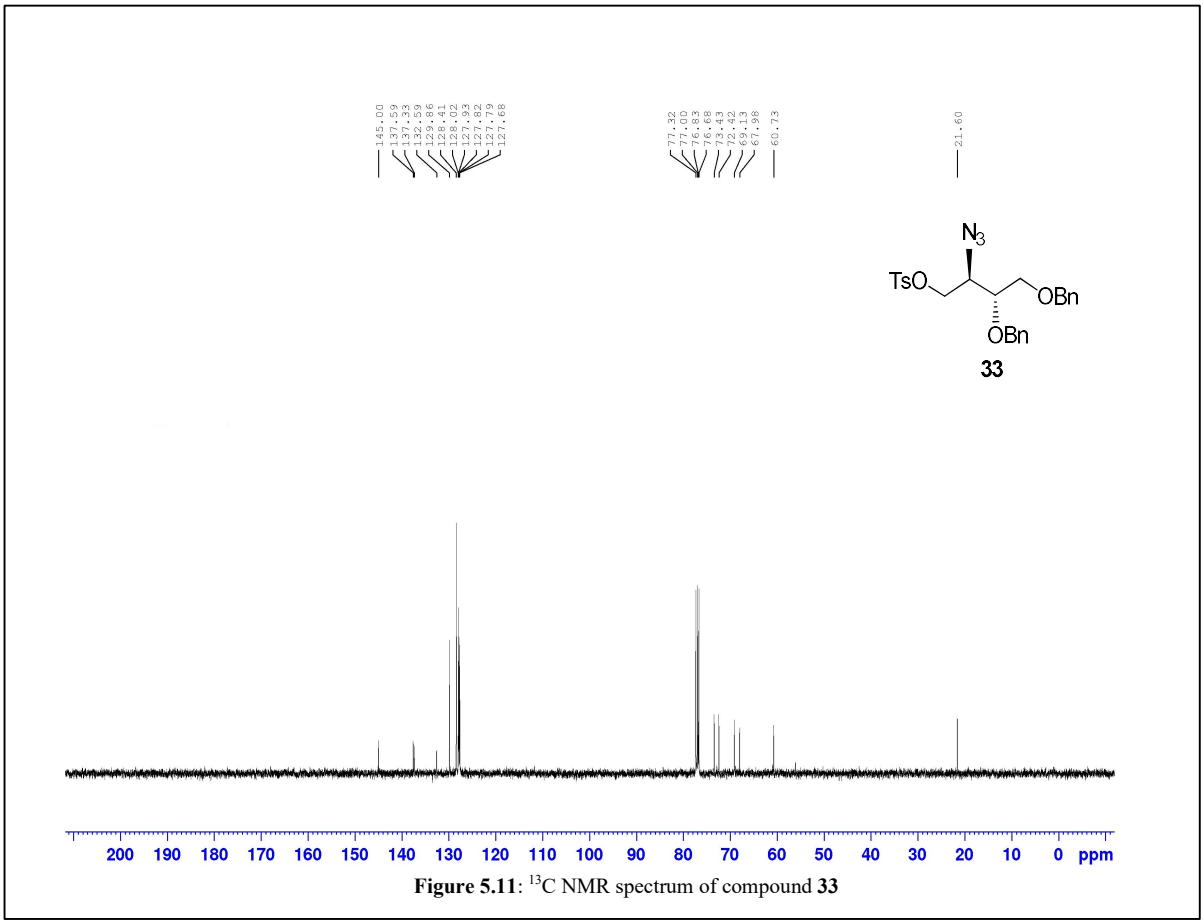


Figure 5.11: ^{13}C NMR spectrum of compound **33**

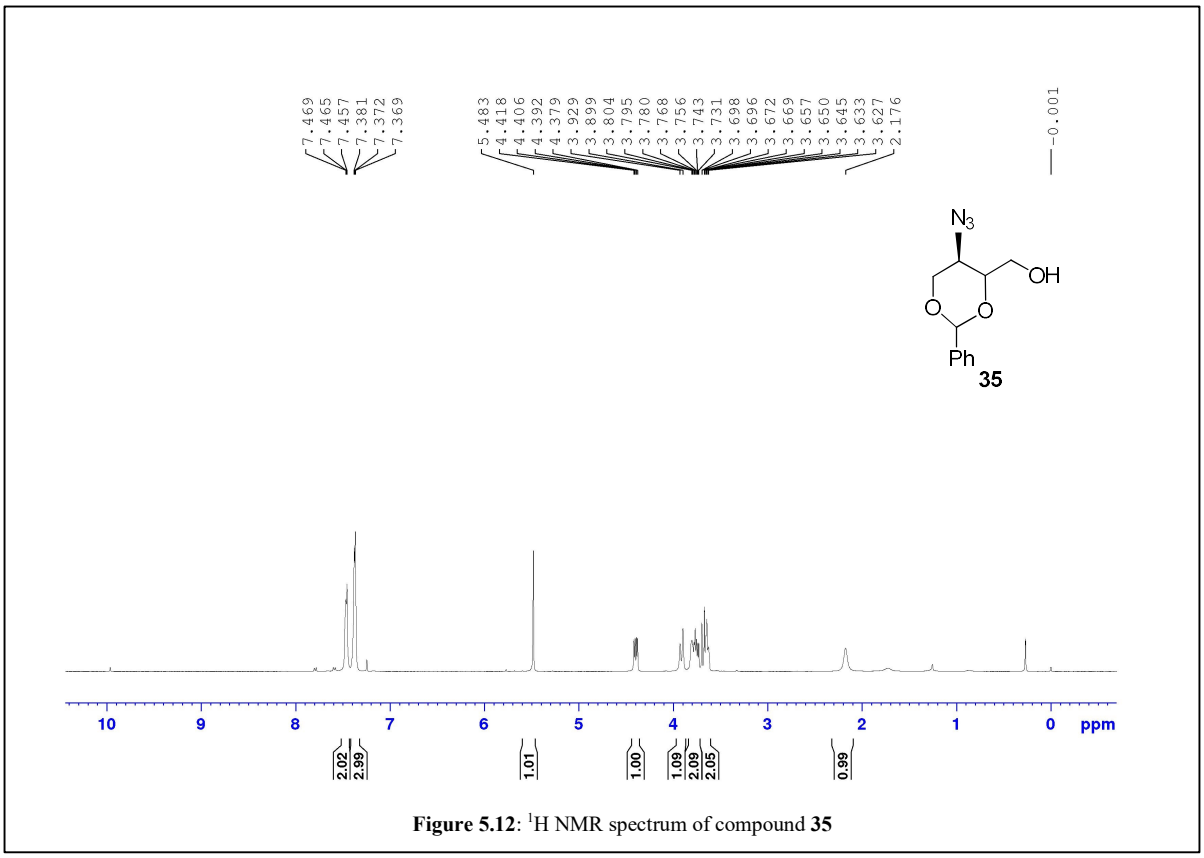


Figure 5.12: ^{1}H NMR spectrum of compound **35**

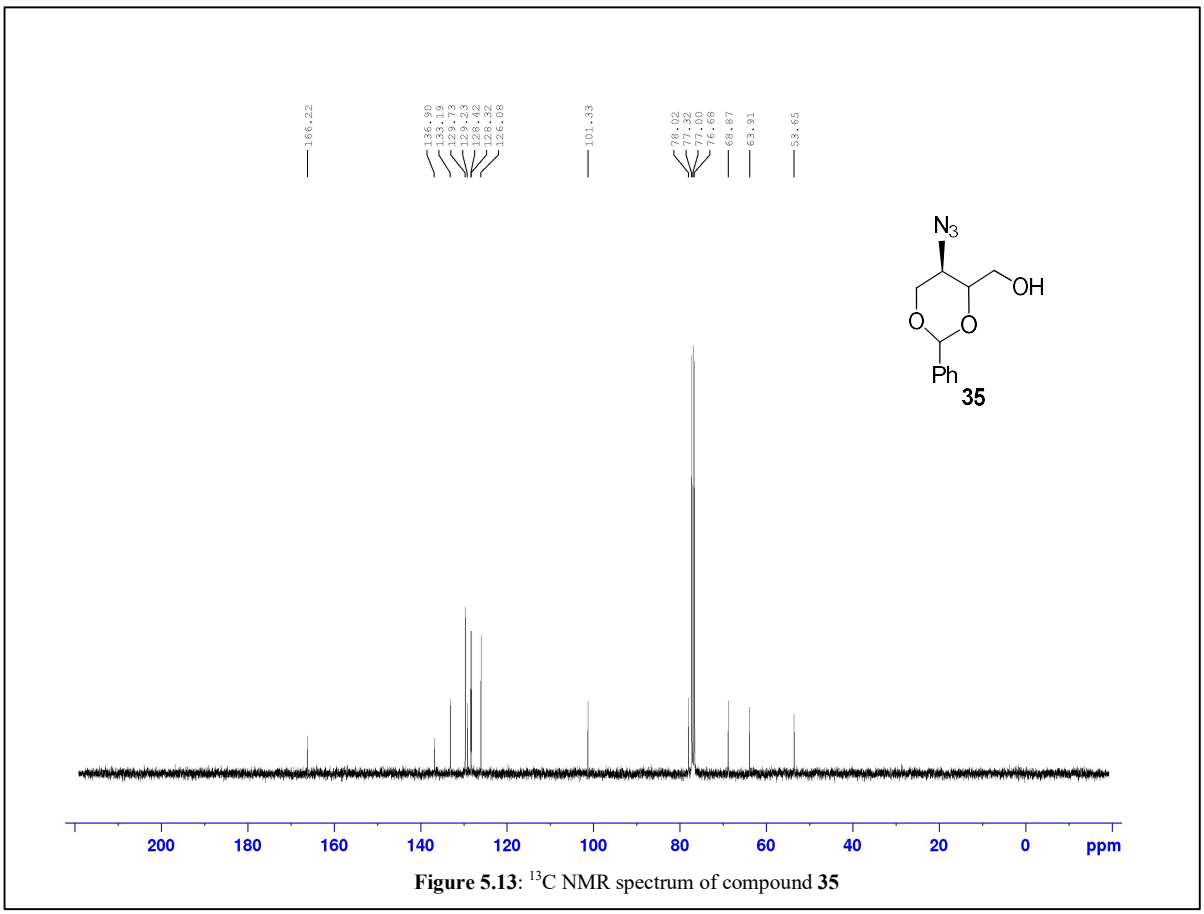


Figure 5.13: ^{13}C NMR spectrum of compound **35**

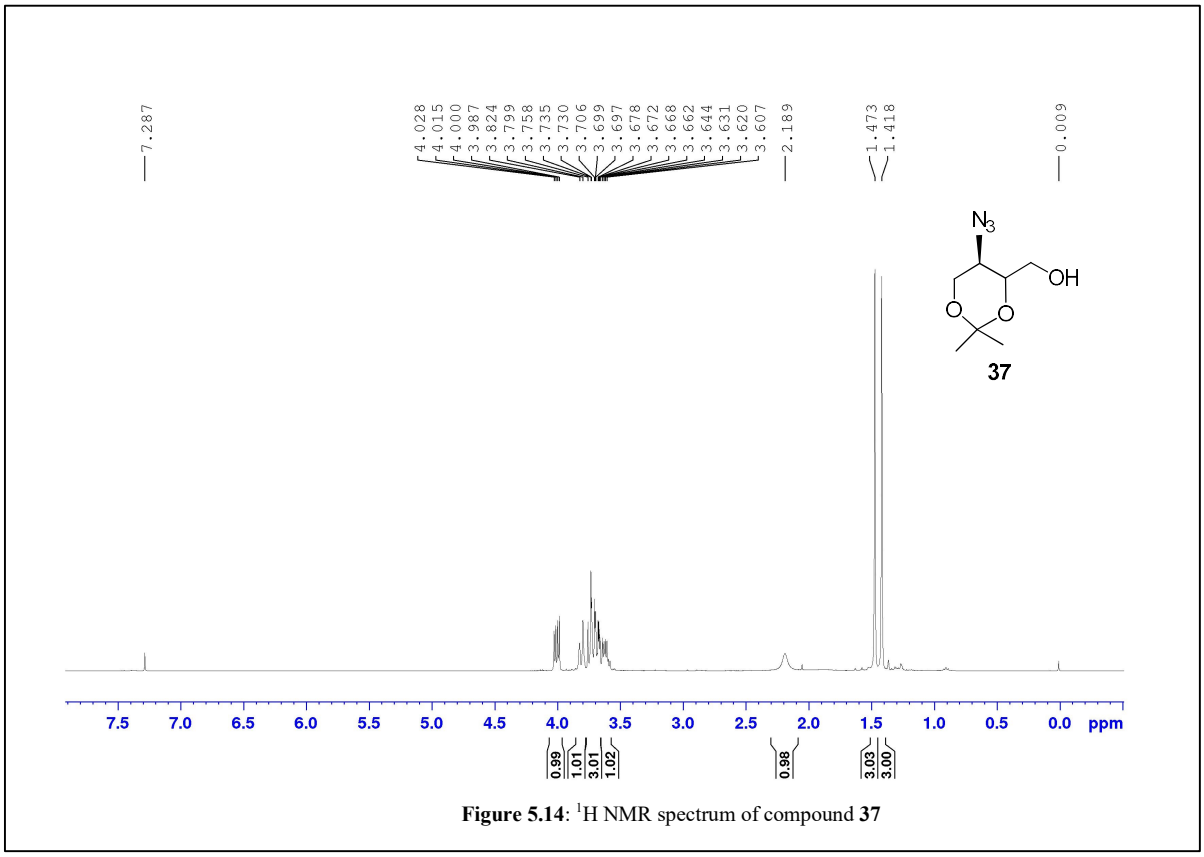


Figure 5.14: ^{1}H NMR spectrum of compound **37**

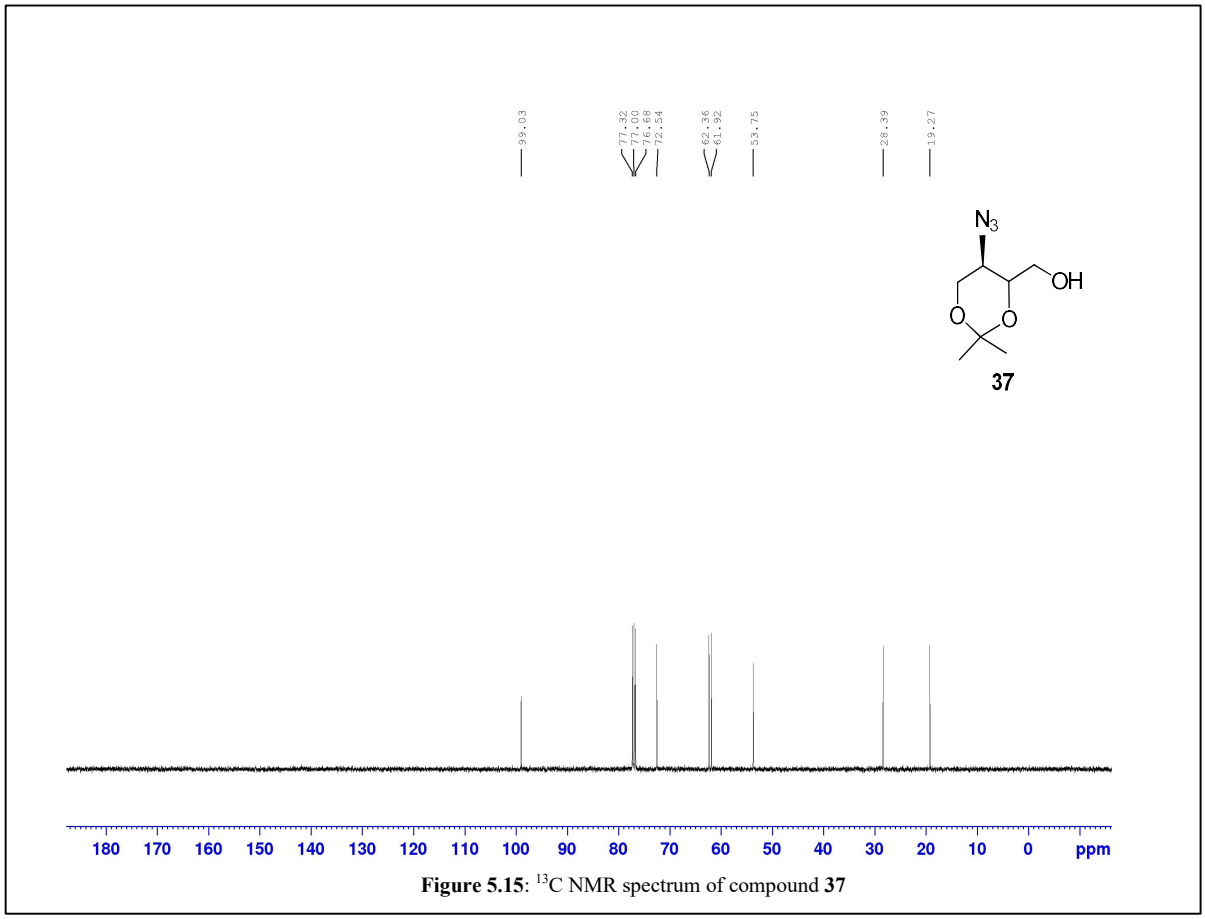


Figure 5.15: ^{13}C NMR spectrum of compound **37**

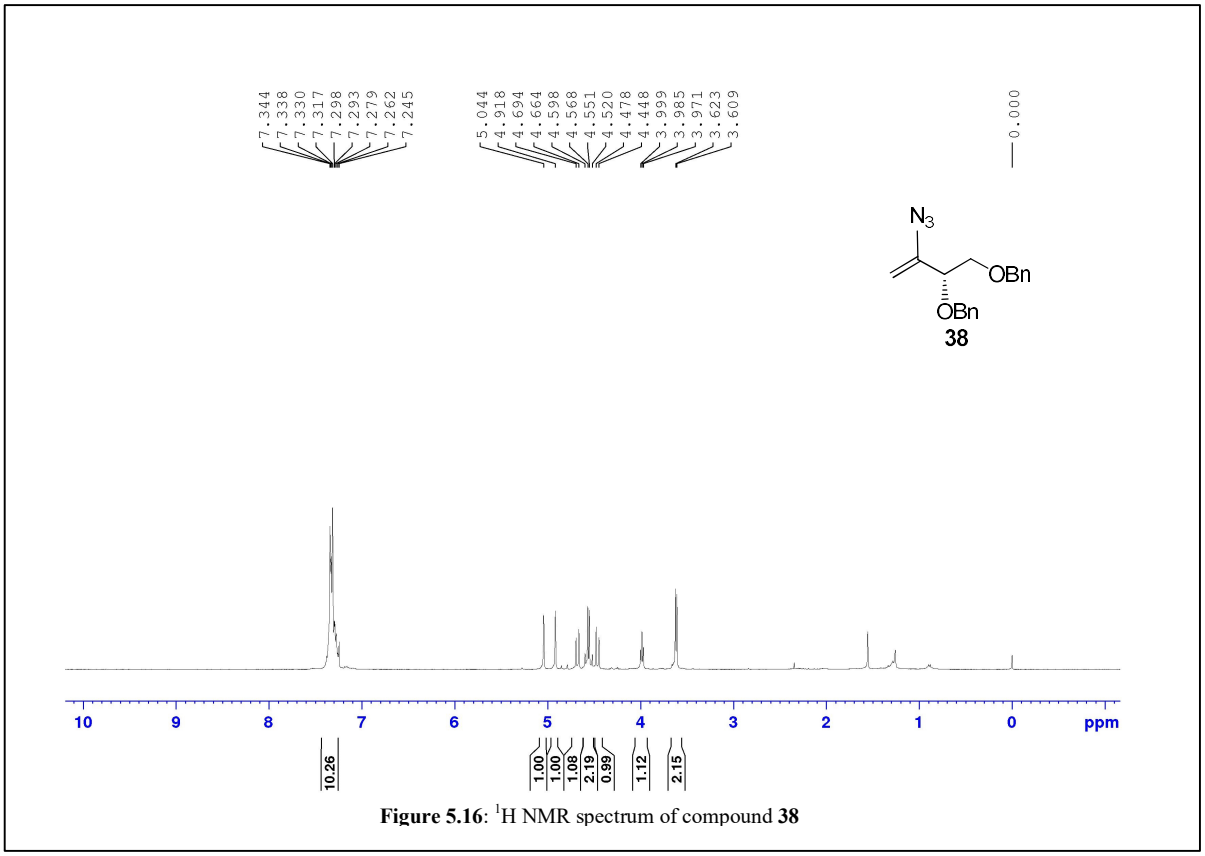


Figure 5.16: ^{1}H NMR spectrum of compound **38**

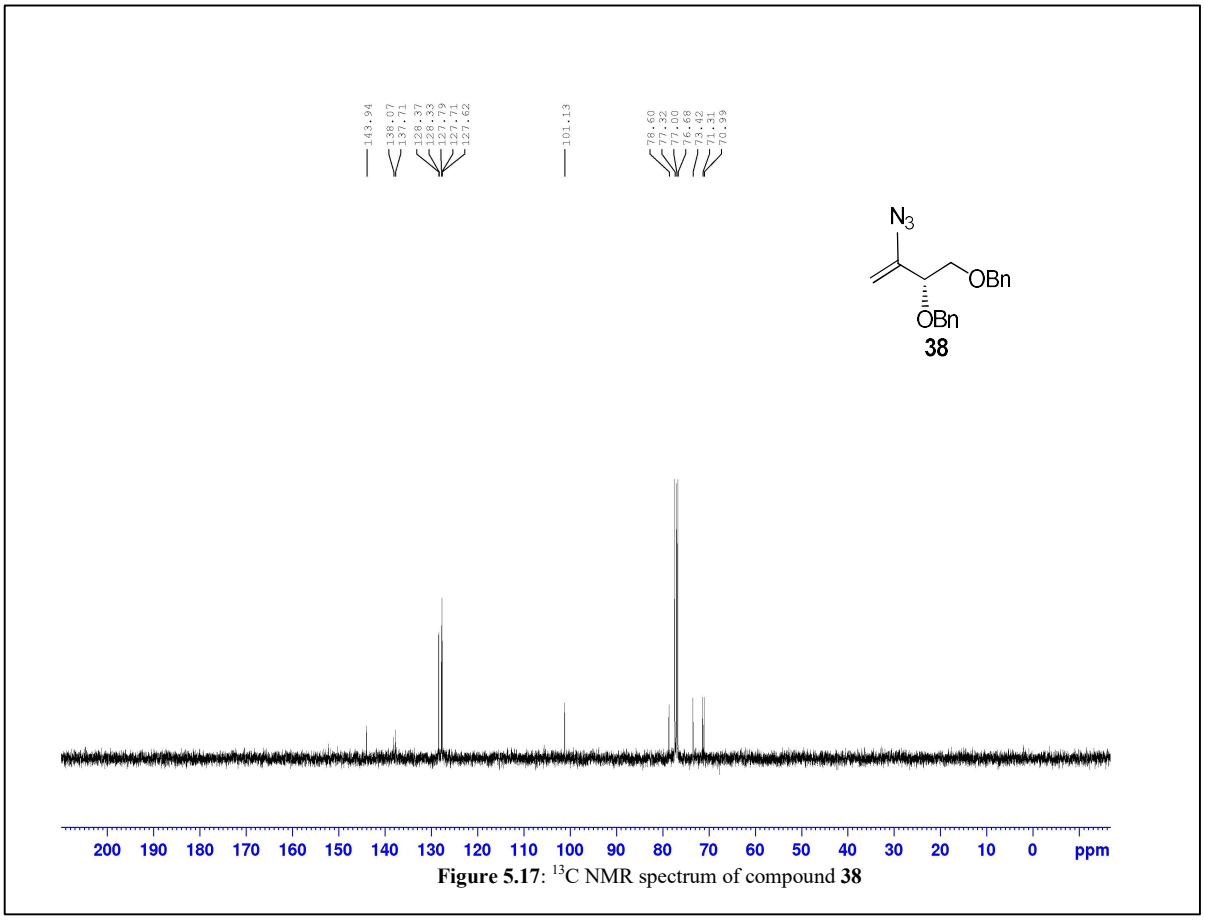


Figure 5.17: ^{13}C NMR spectrum of compound **38**

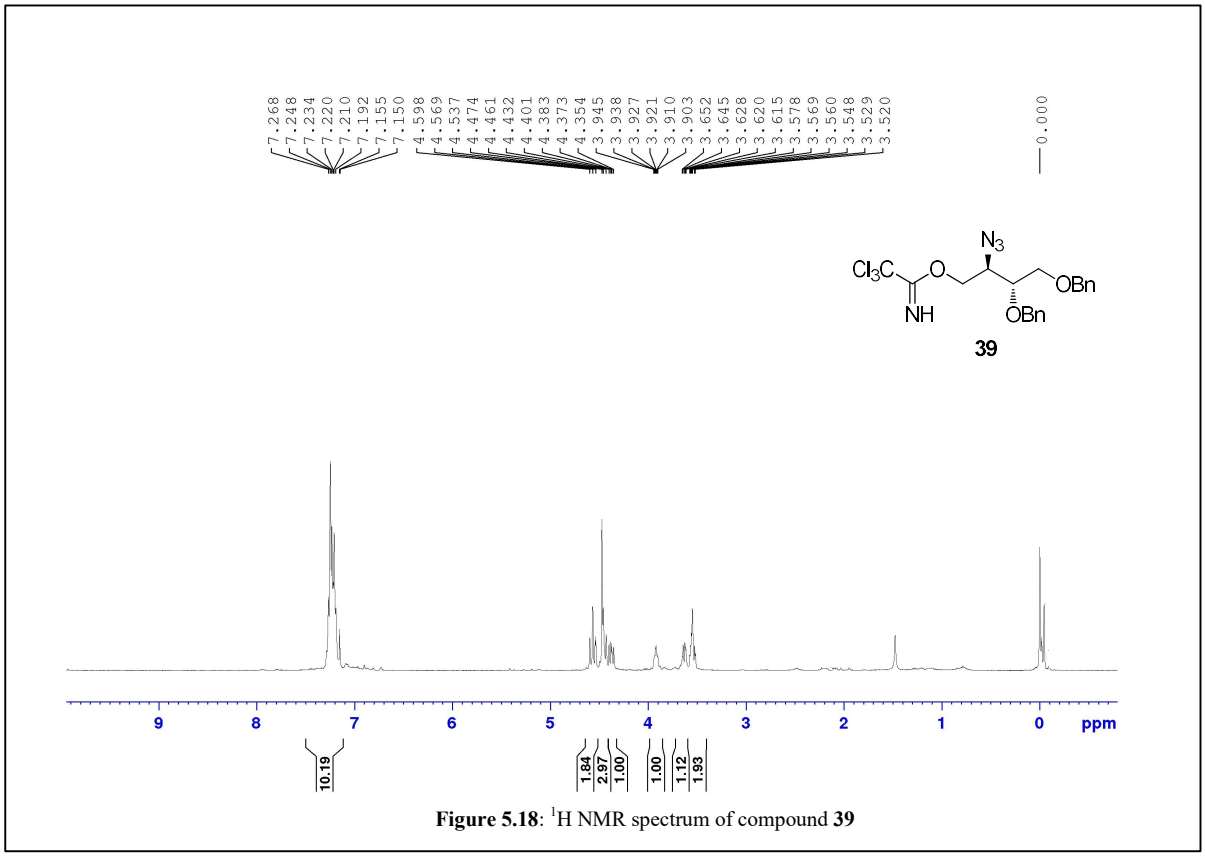


Figure 5.18: ^{1}H NMR spectrum of compound **39**

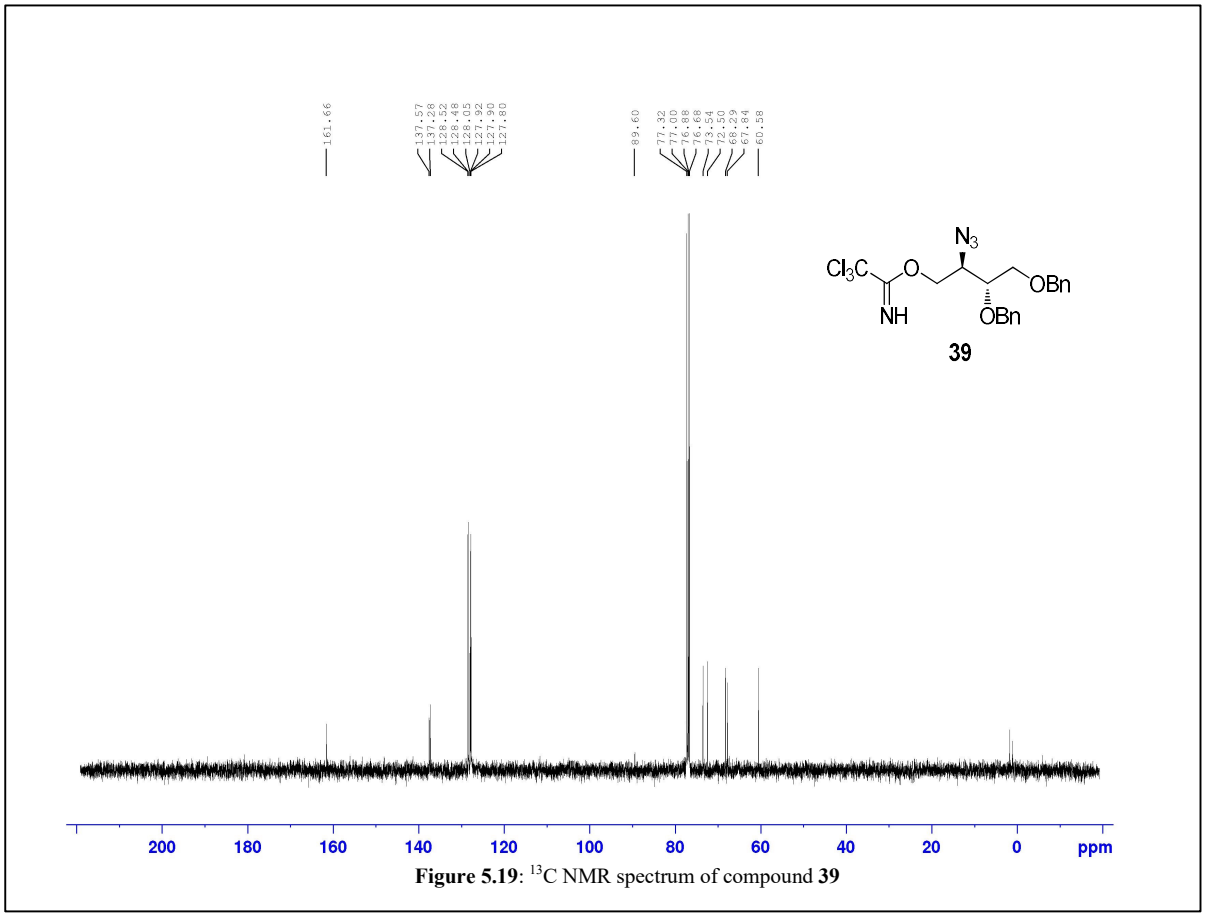


Figure 5.19: ^{13}C NMR spectrum of compound **39**

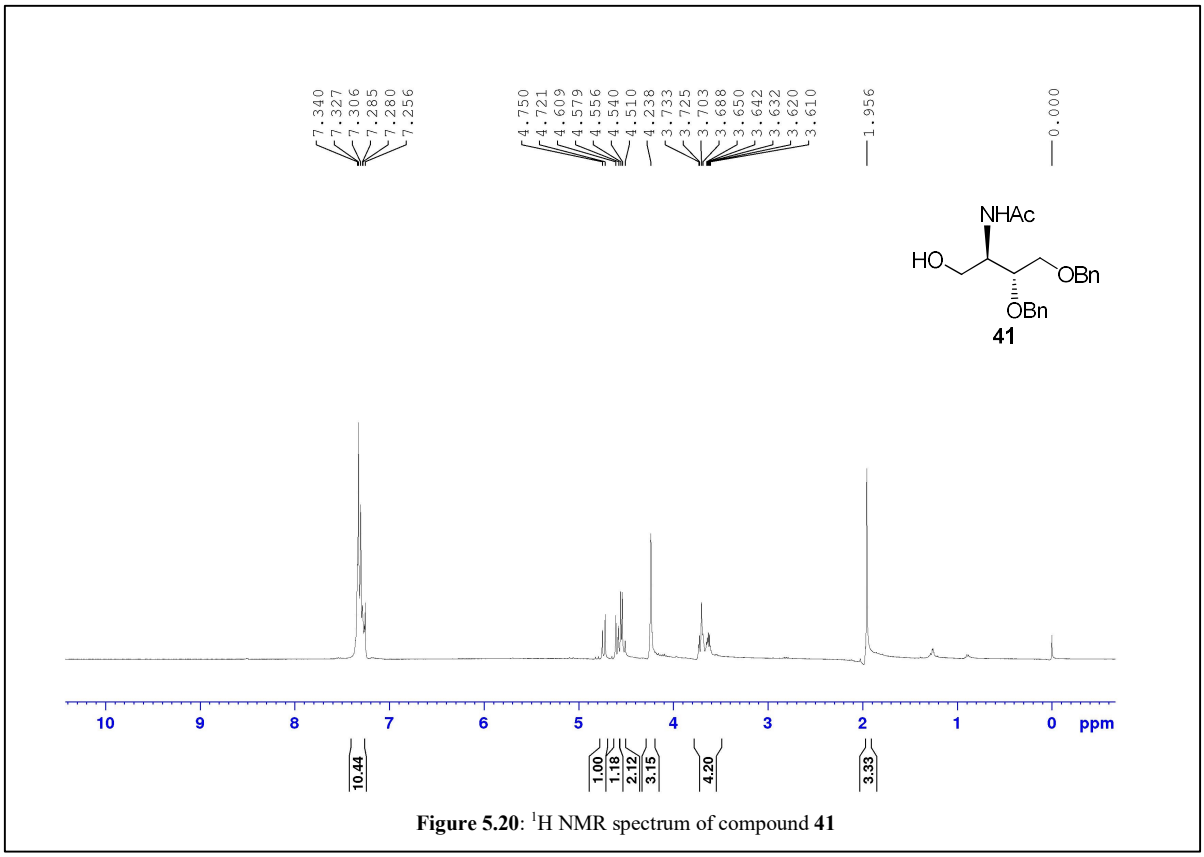


Figure 5.20: ^{1}H NMR spectrum of compound **41**

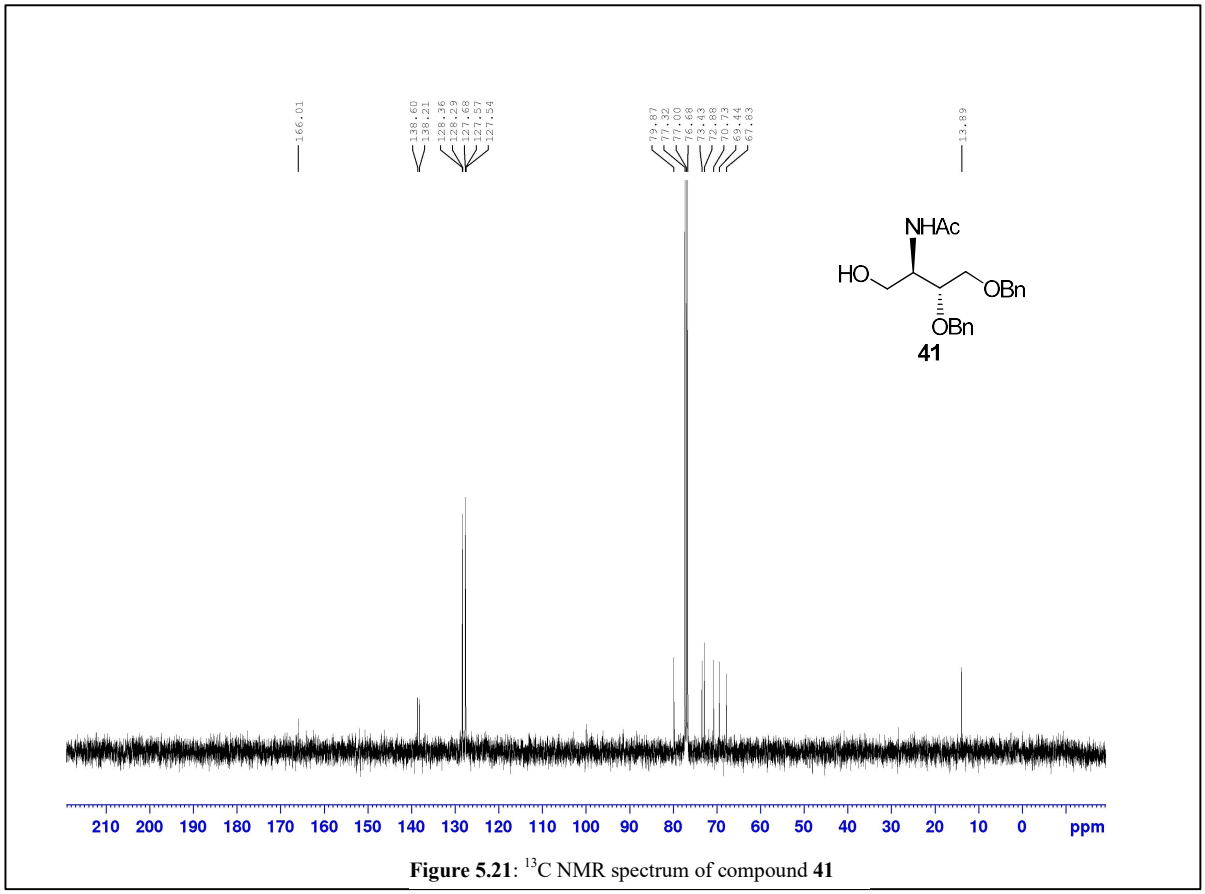


Figure 5.21: ^{13}C NMR spectrum of compound **41**

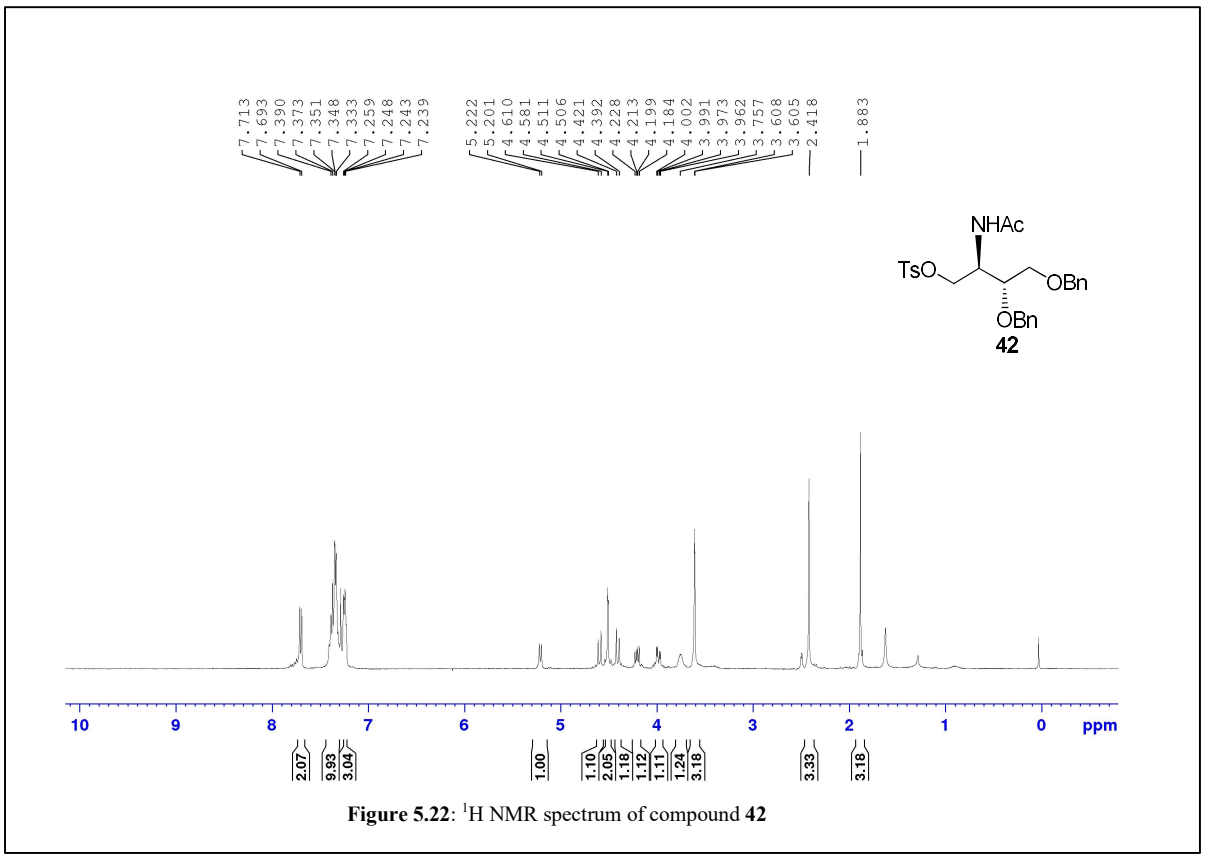


Figure 5.22: ^{1}H NMR spectrum of compound **42**

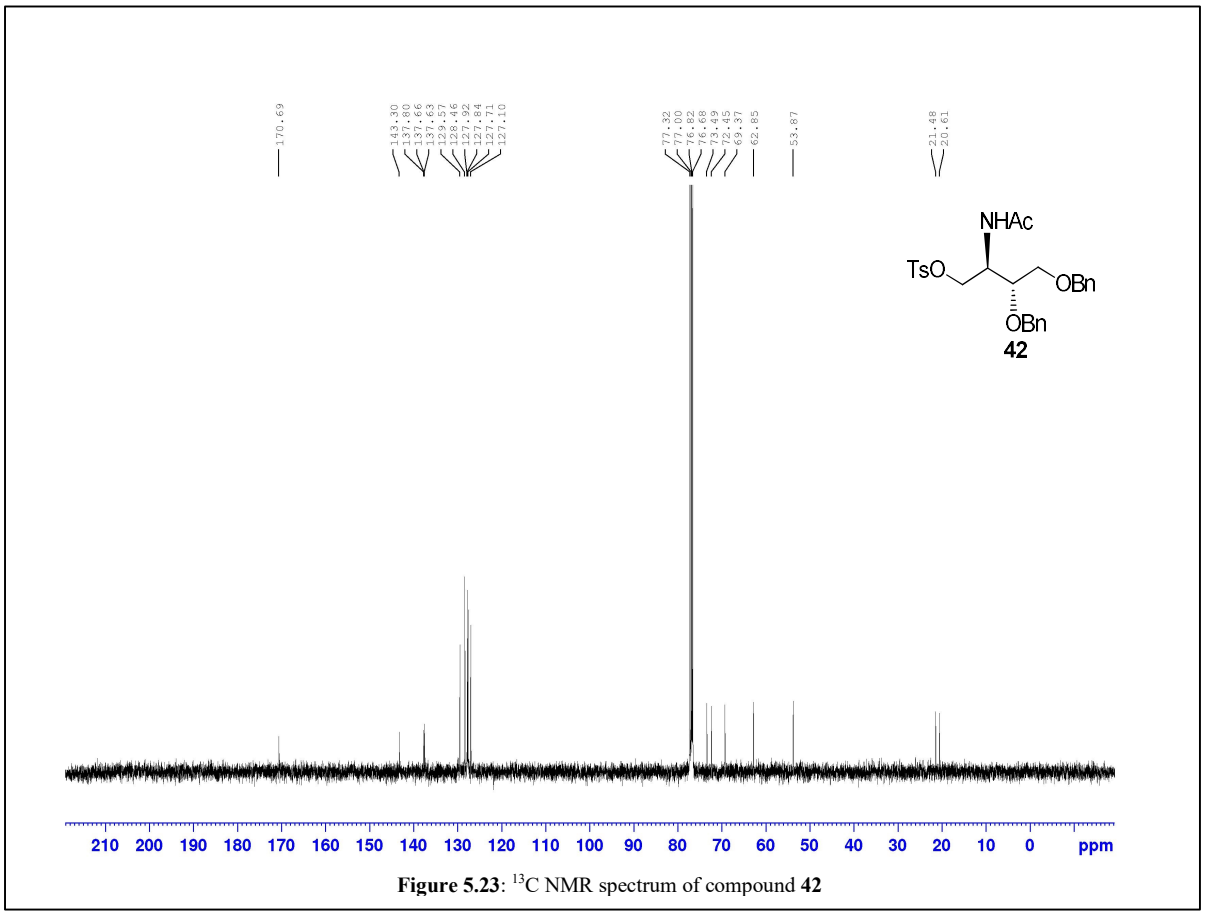


Figure 5.23: ^{13}C NMR spectrum of compound **42**

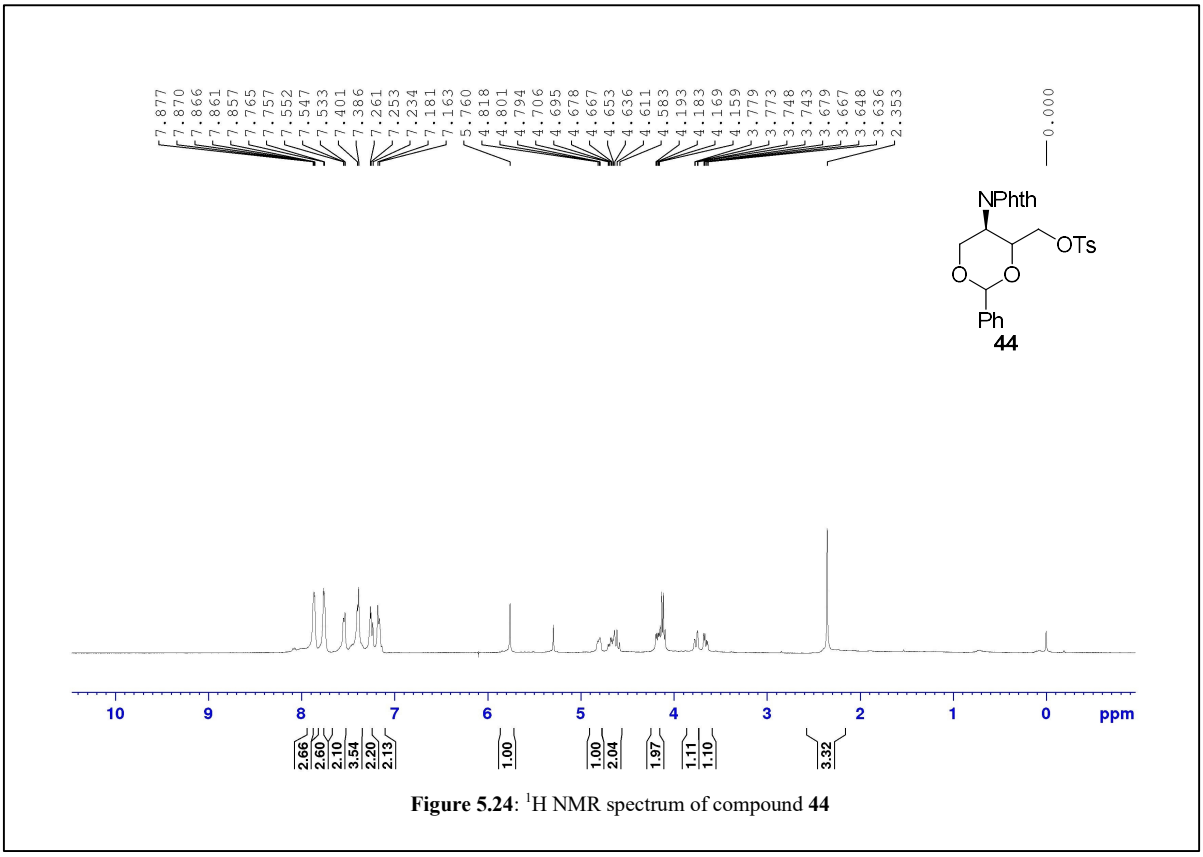


Figure 5.24: ^{1}H NMR spectrum of compound **44**

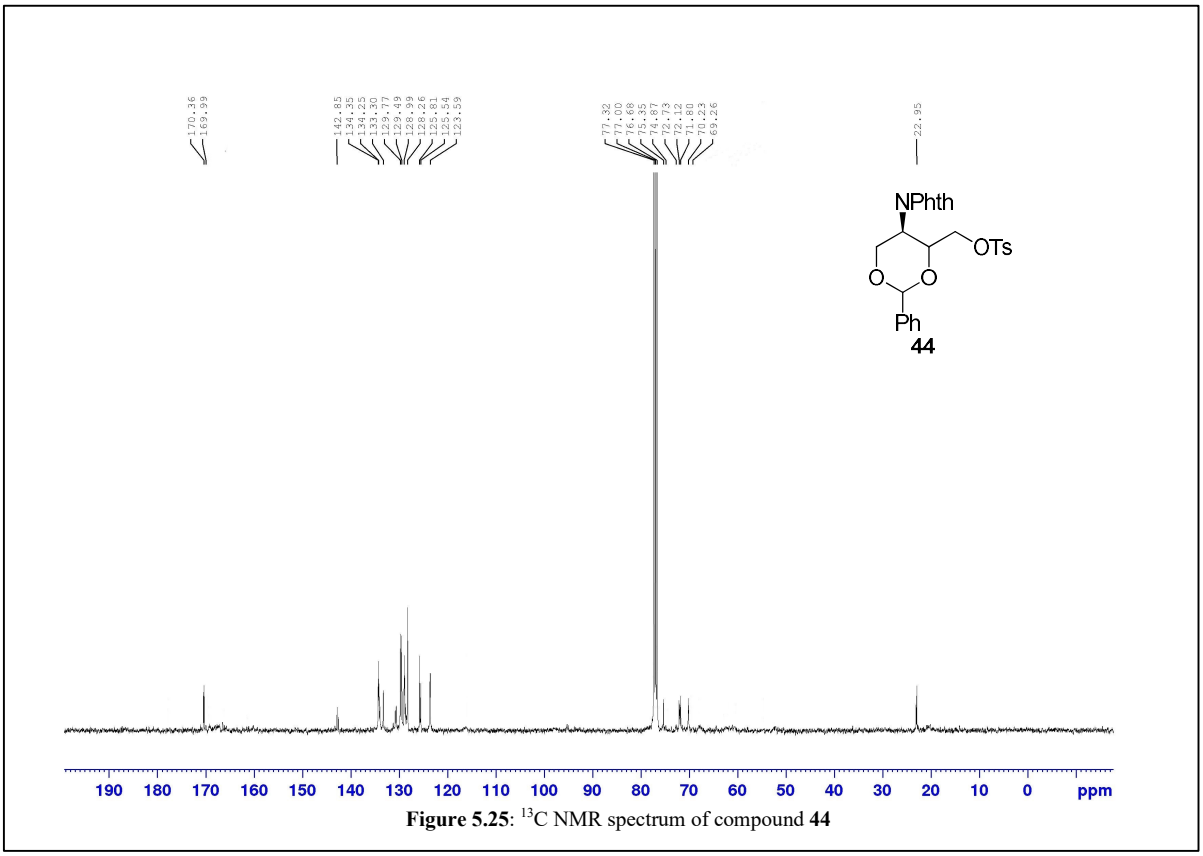


Figure 5.25: ^{13}C NMR spectrum of compound **44**

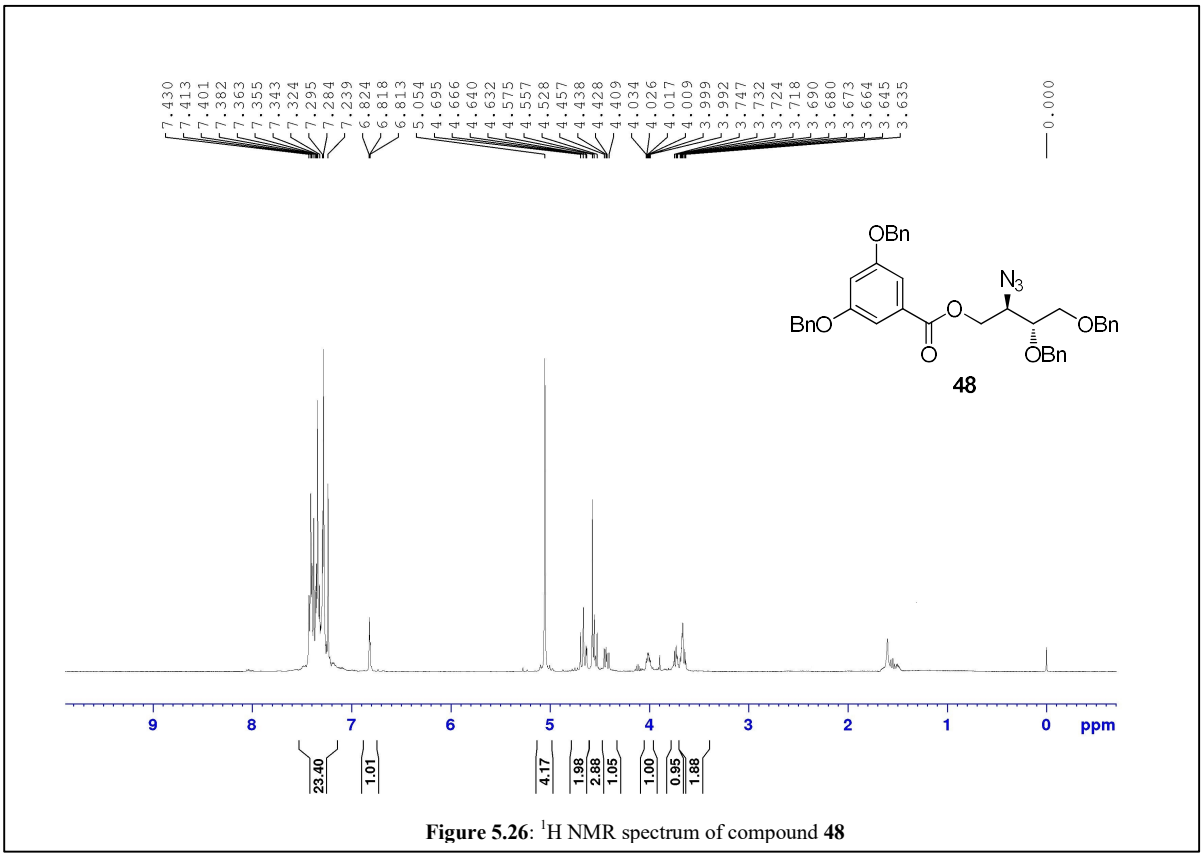


Figure 5.26: ^{1}H NMR spectrum of compound **48**

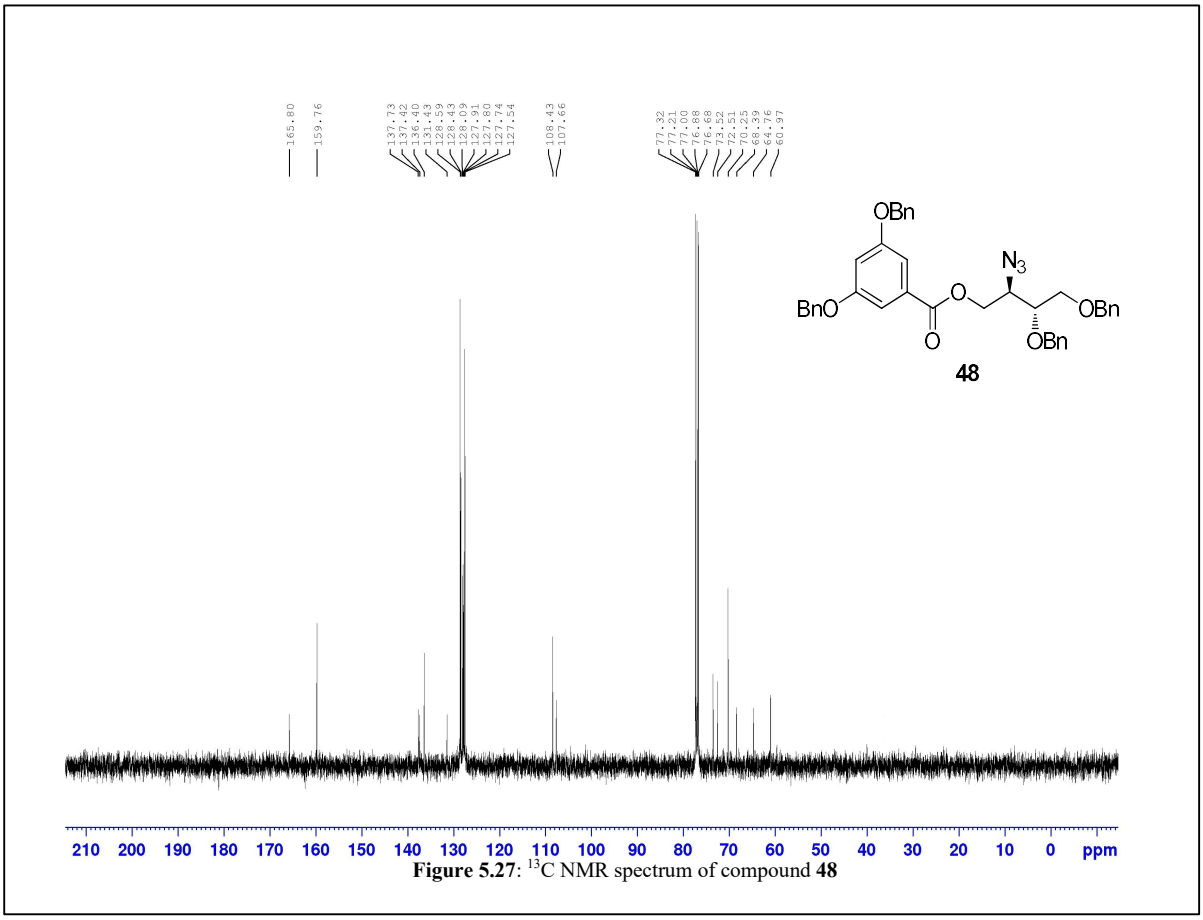


Figure 5.27: ^{13}C NMR spectrum of compound **48**

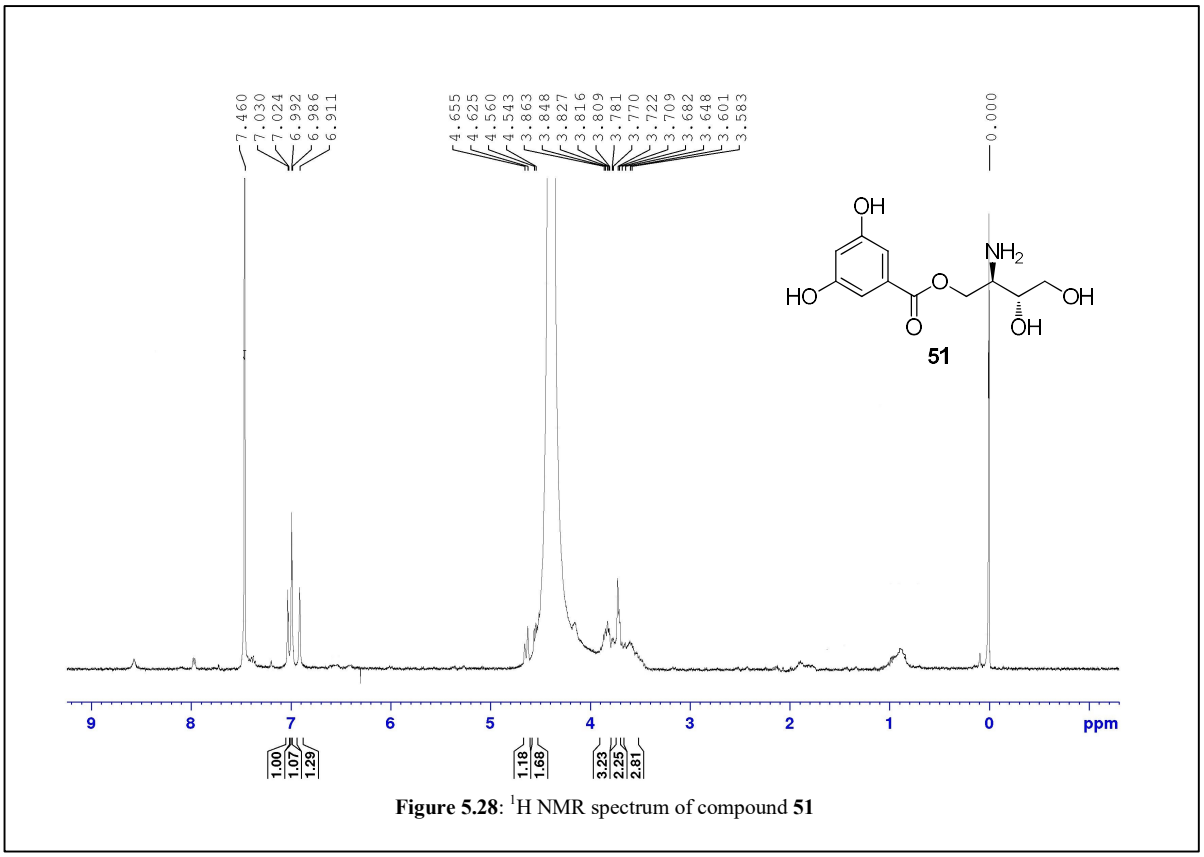


Figure 5.28: ^{1}H NMR spectrum of compound **51**

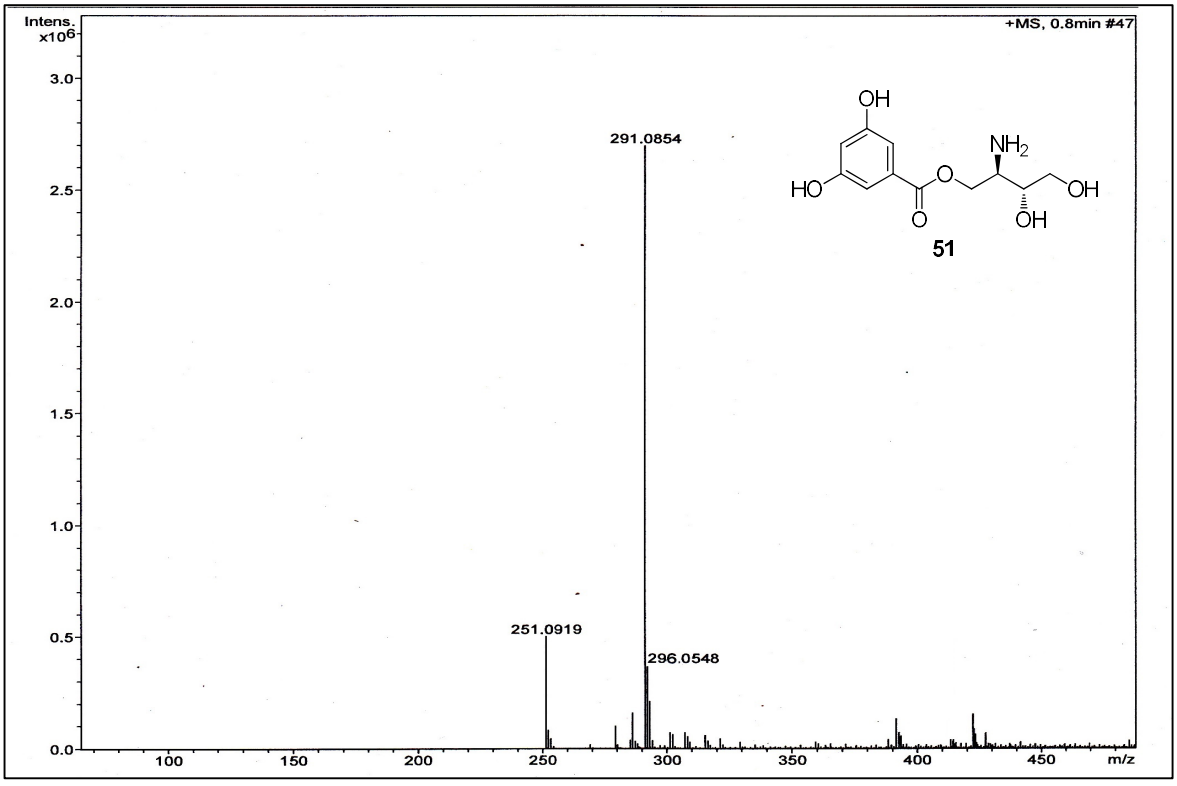


Figure 5.29: HRMS spectrum of compound **51**

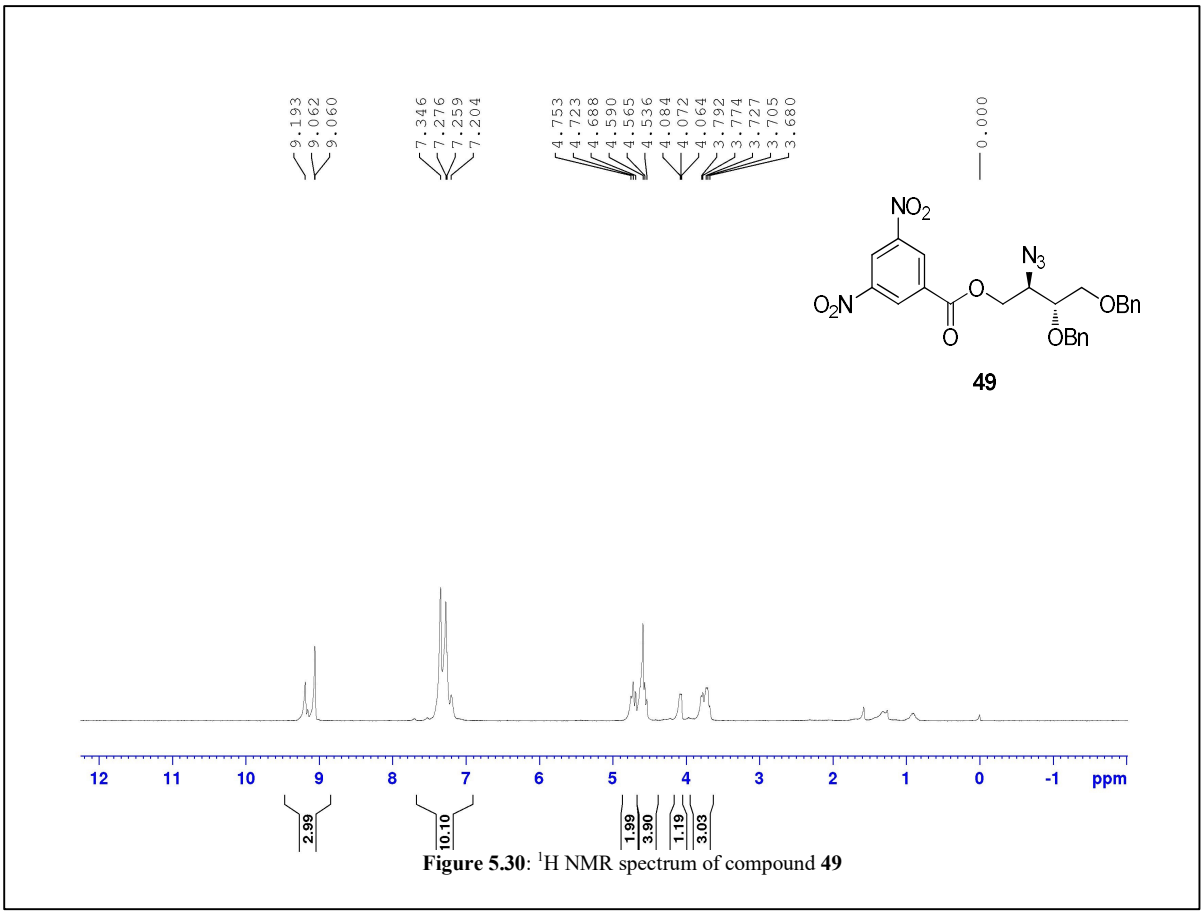


Figure 5.30: ^{1}H NMR spectrum of compound **49**

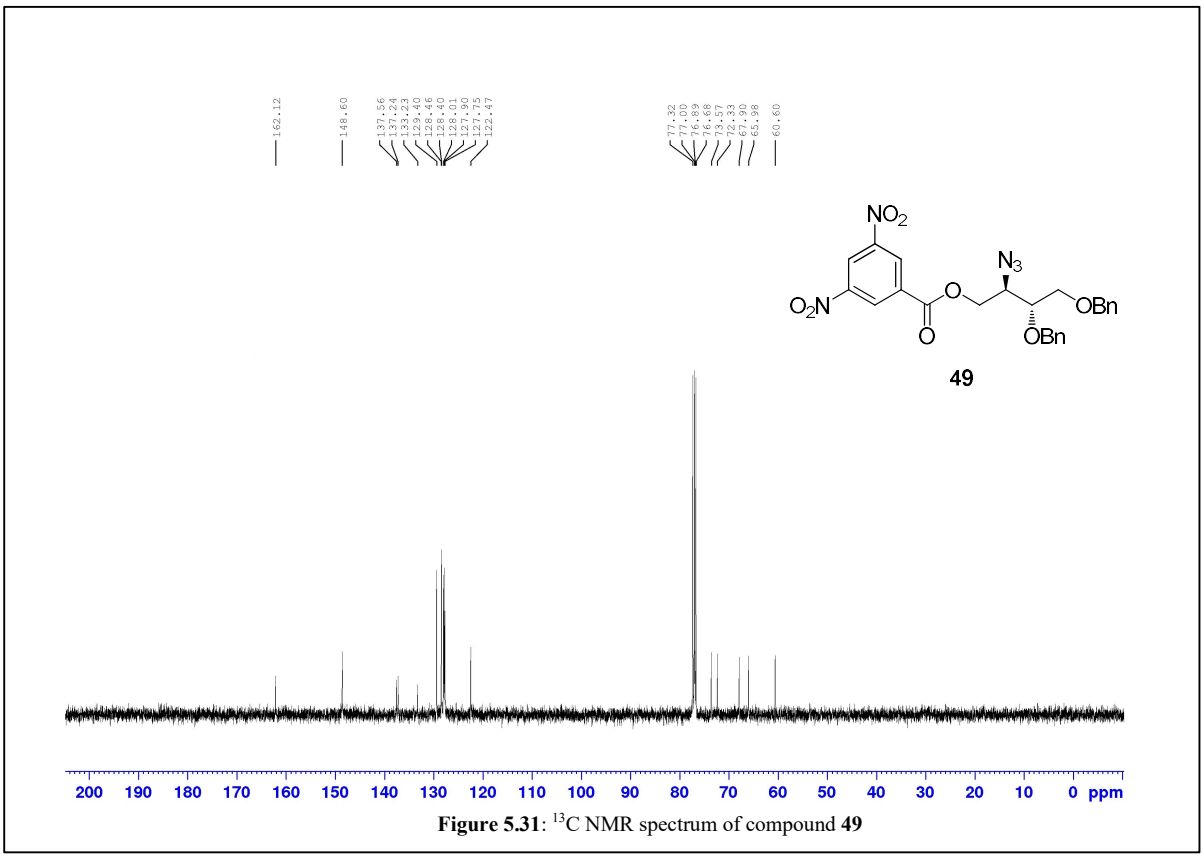


Figure 5.31: ^{13}C NMR spectrum of compound **49**

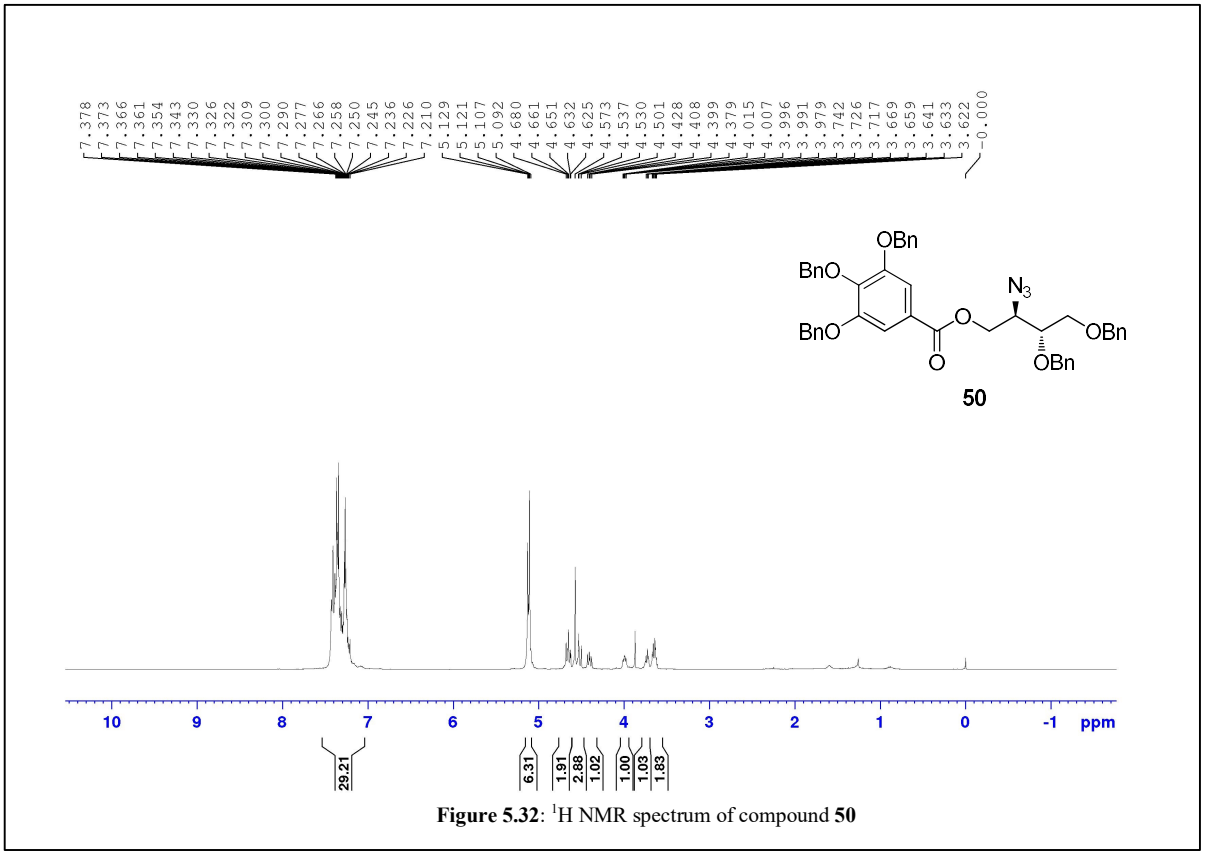


Figure 5.32: ^{1}H NMR spectrum of compound **50**

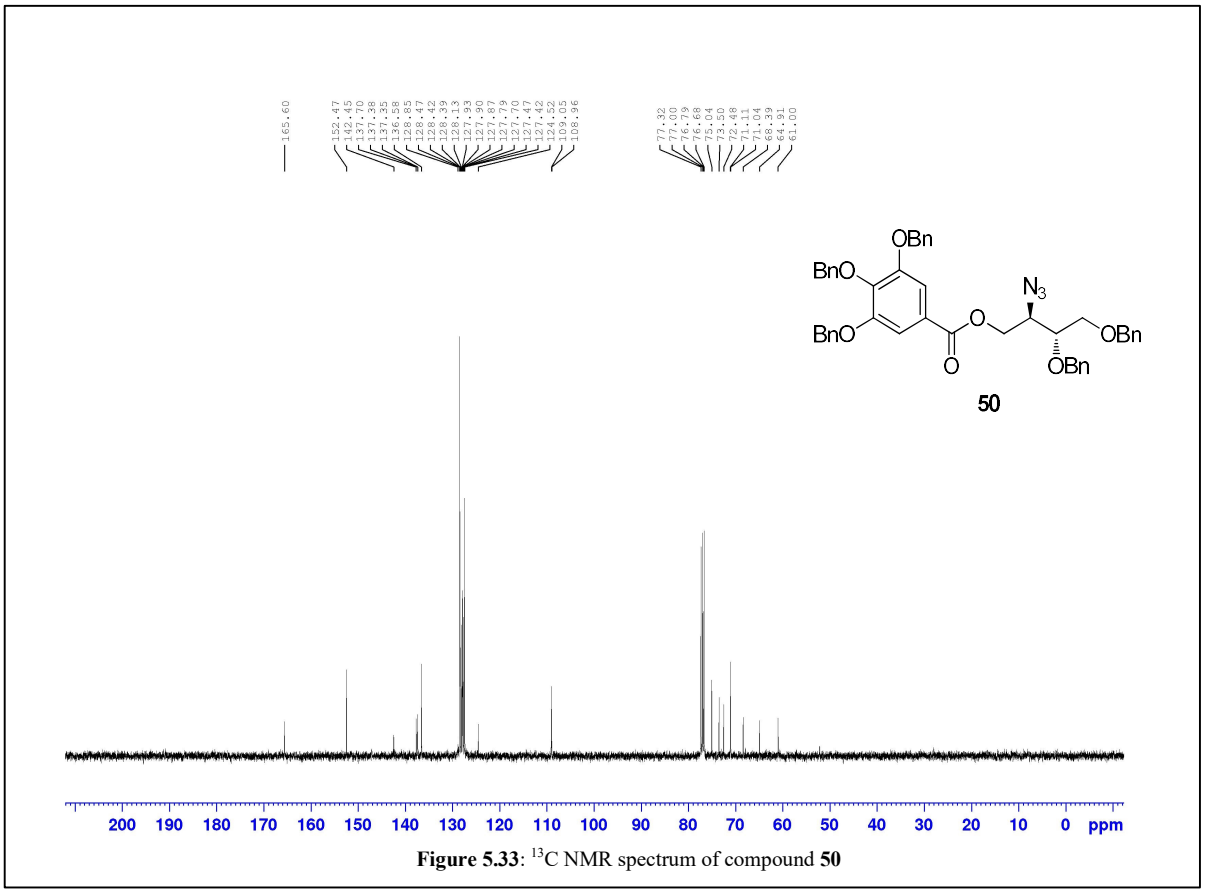


Figure 5.33: ^{13}C NMR spectrum of compound **50**

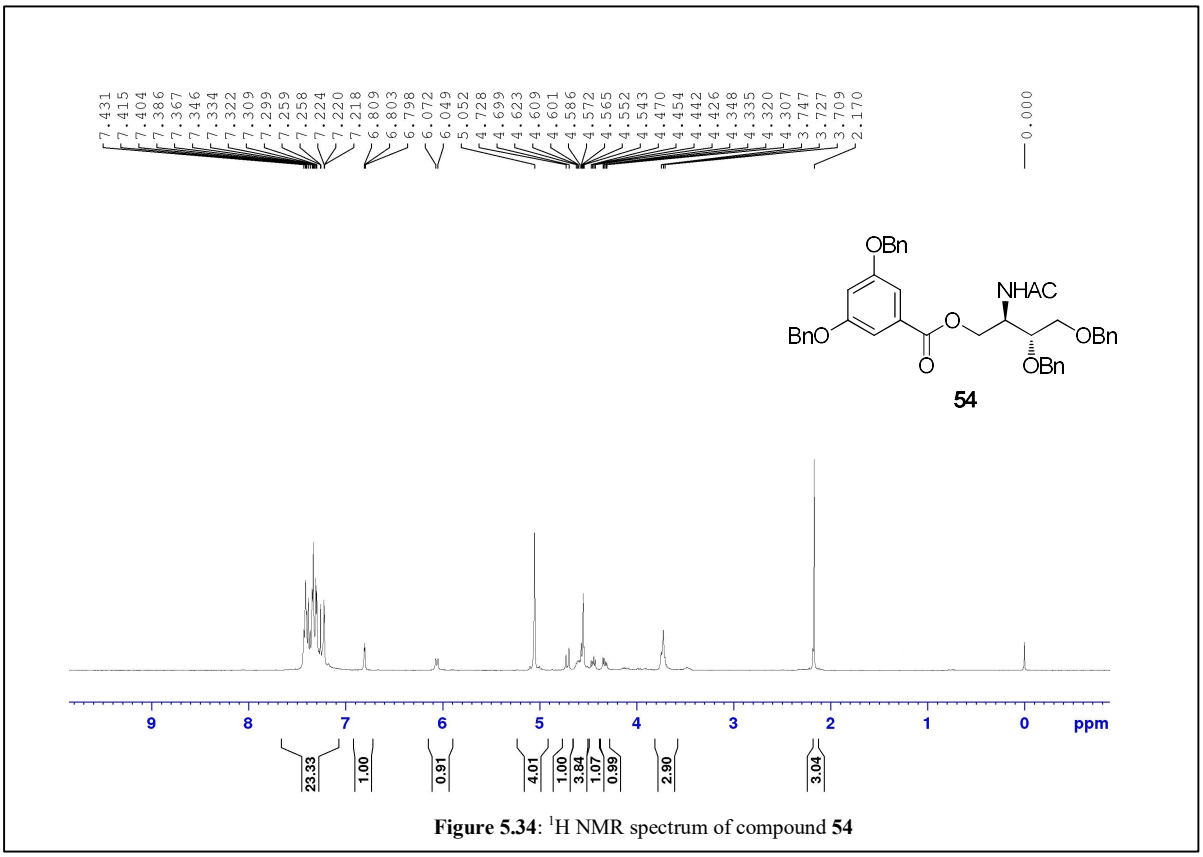


Figure 5.34: ^{1}H NMR spectrum of compound **54**

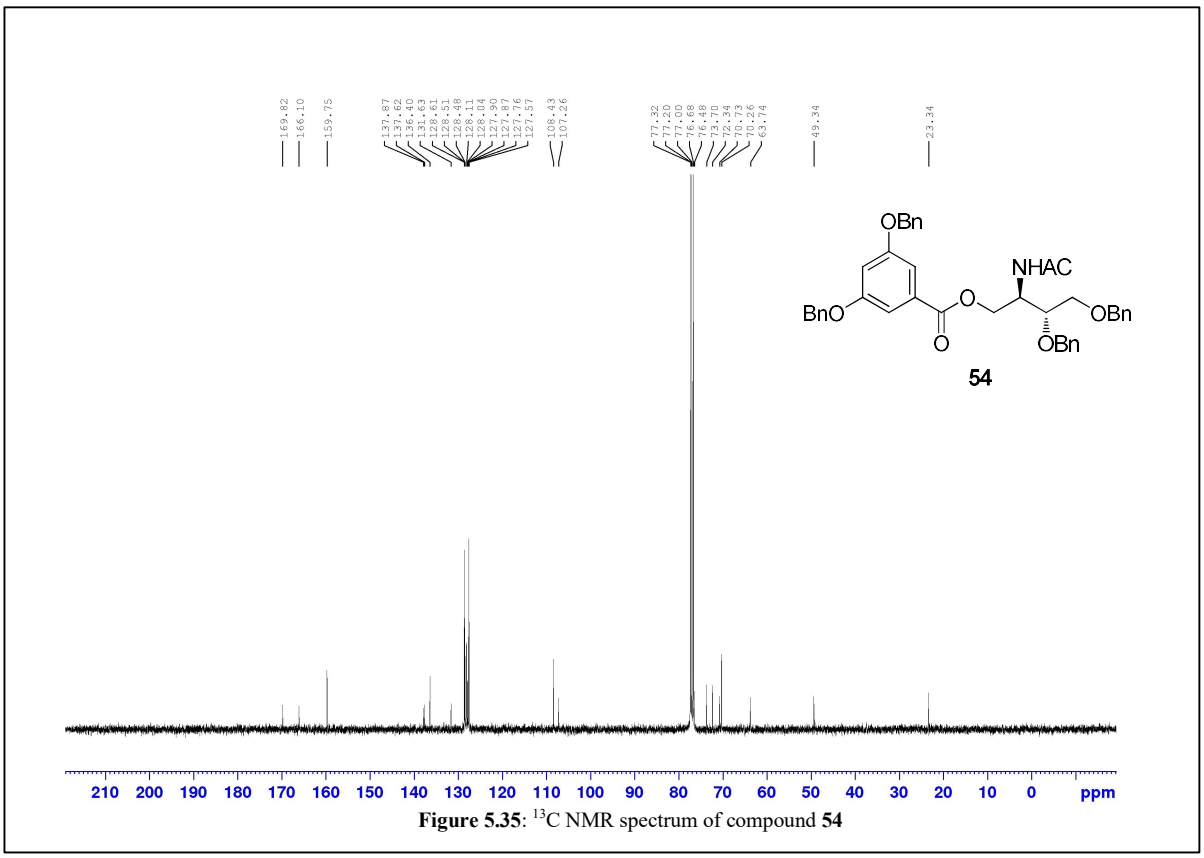


Figure 5.35: ^{13}C NMR spectrum of compound **54**

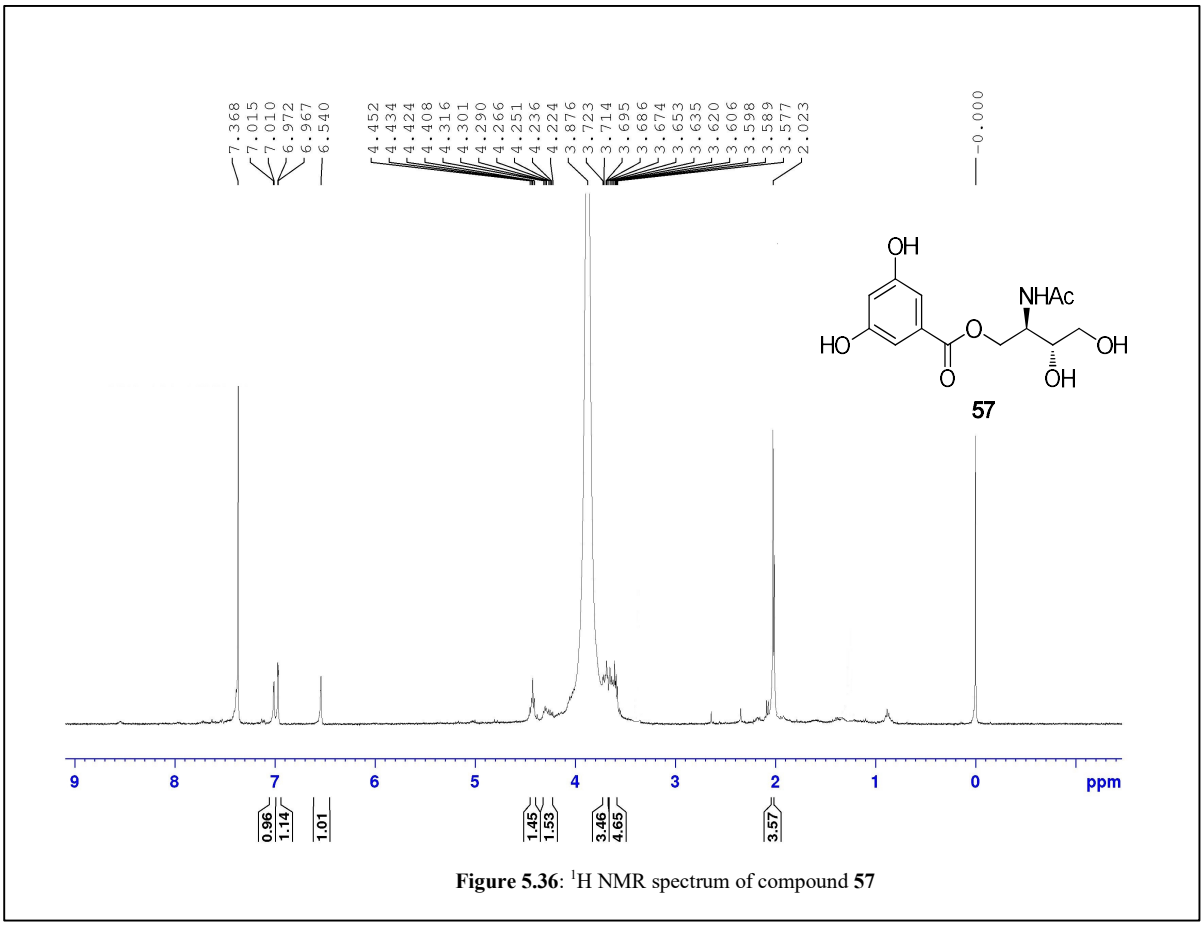


Figure 5.36: ^{1}H NMR spectrum of compound **57**

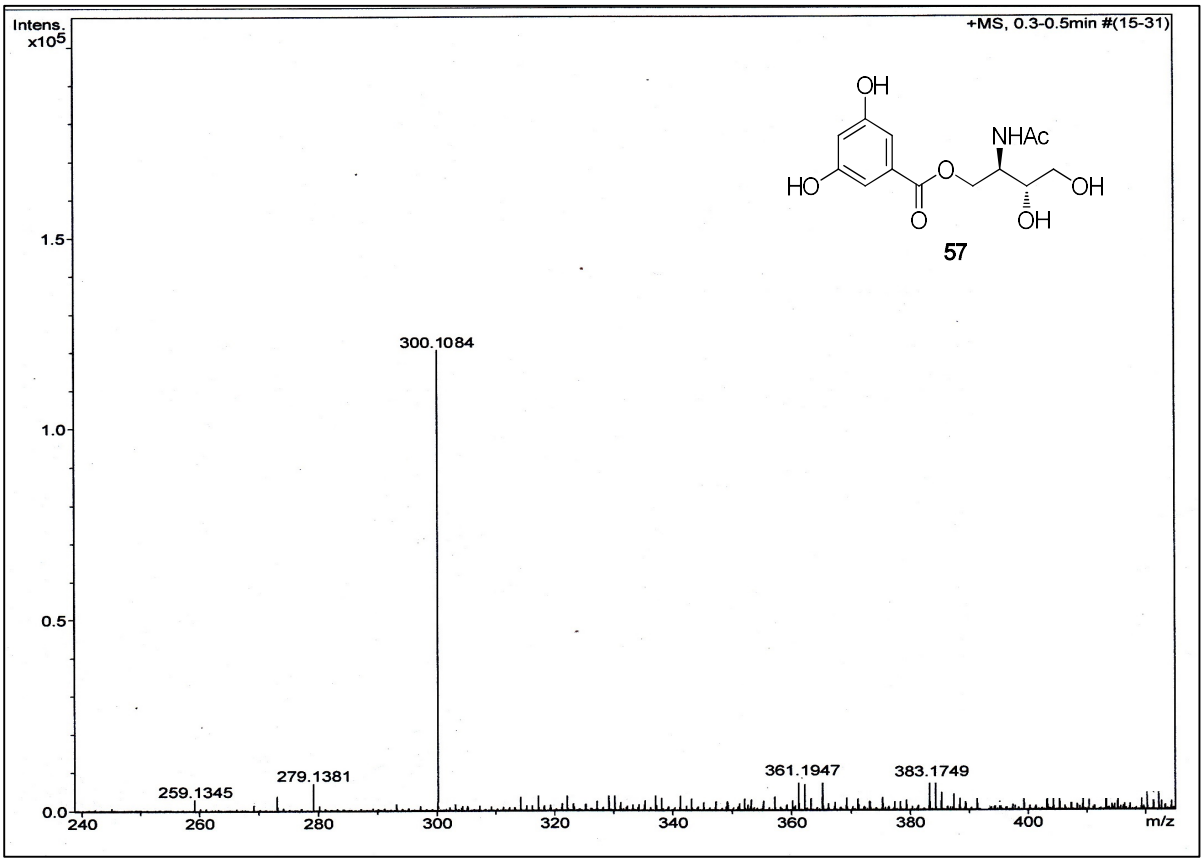


Figure 5.37: HRMS spectrum of compound **57**

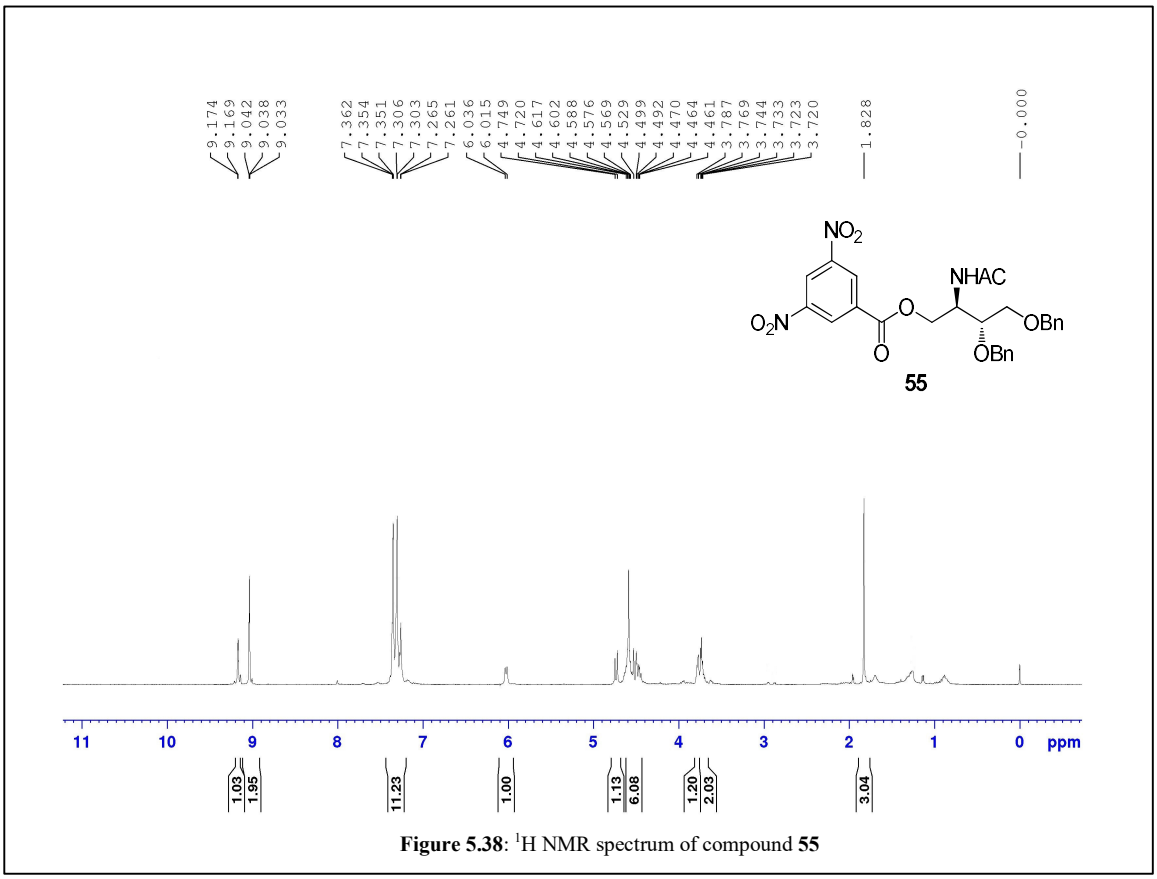


Figure 5.38: ^{1}H NMR spectrum of compound **55**

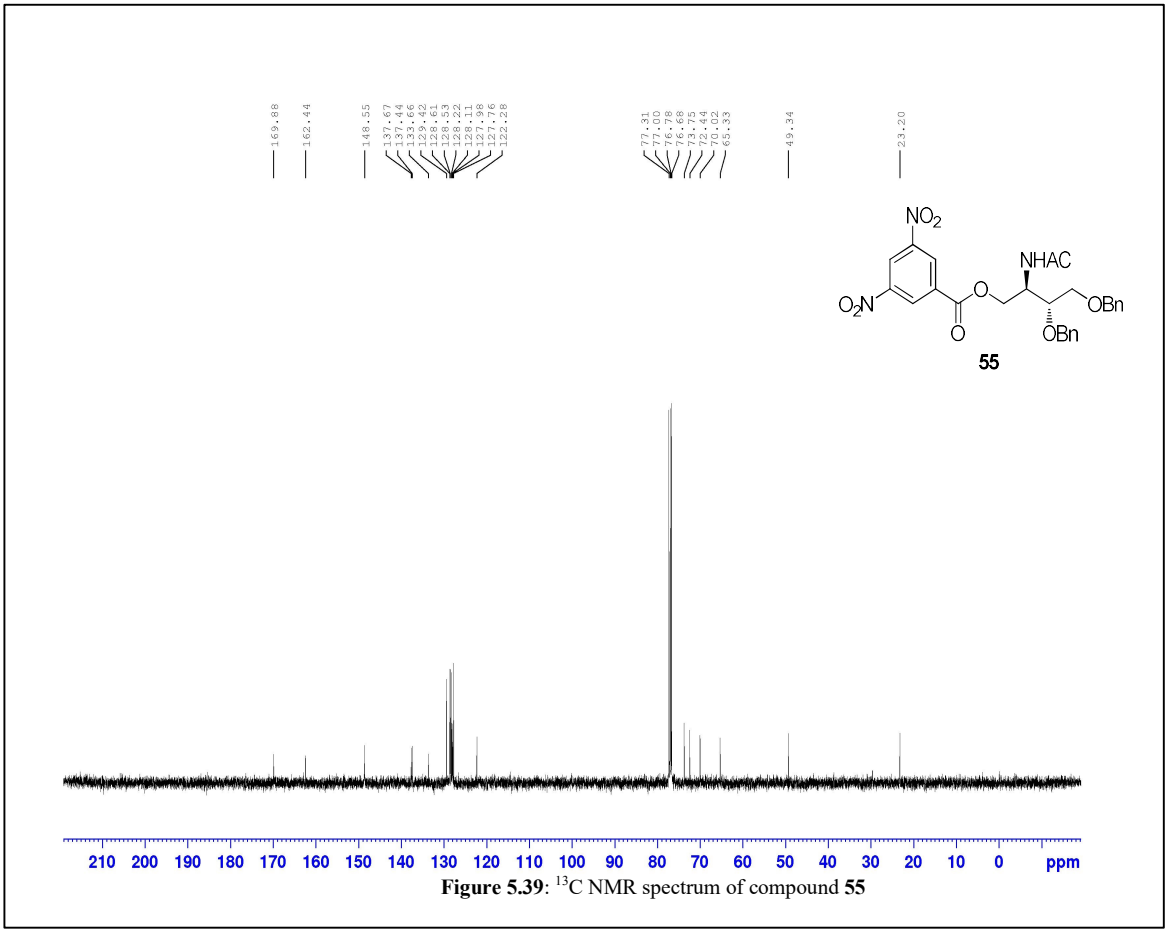


Figure 5.39: ^{13}C NMR spectrum of compound **55**

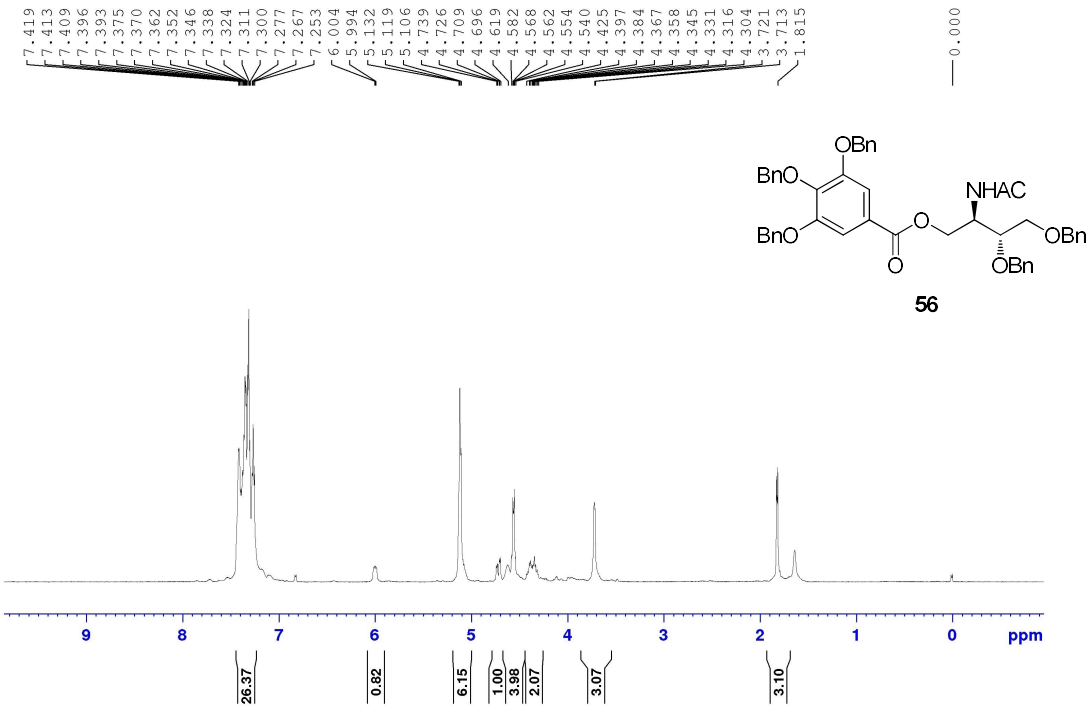


Figure 5.40: ^{1}H NMR spectrum of compound **56**

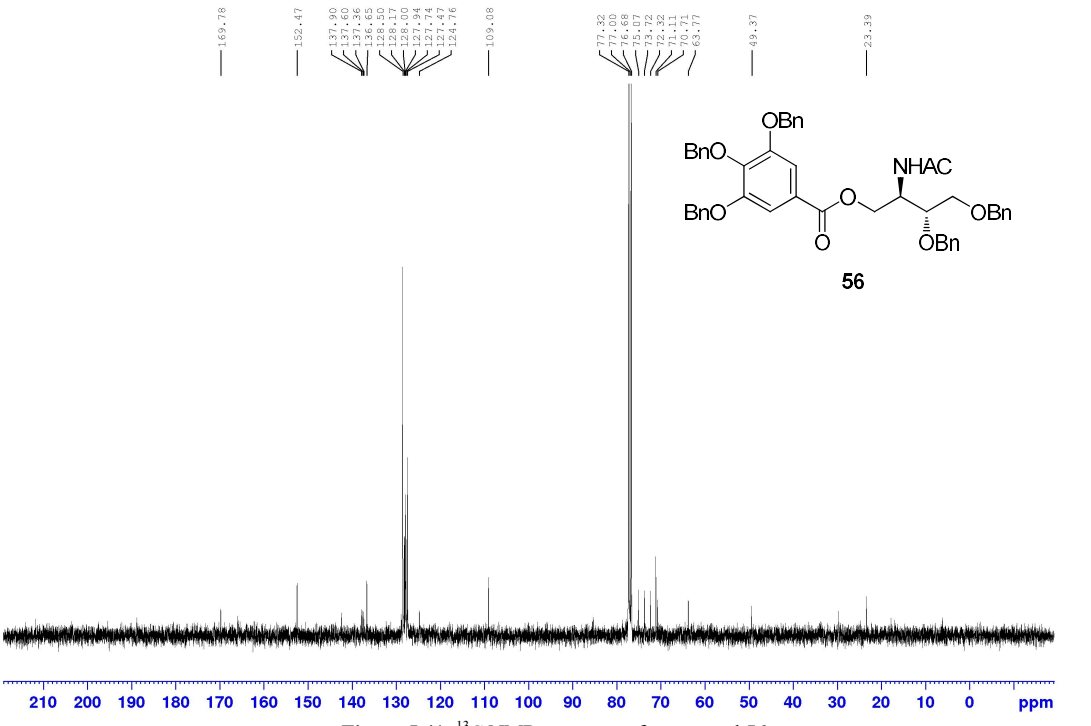


Figure 5.41: ^{13}C NMR spectrum of compound **56**

Appendix

List of Publications

1. Synthesis of partially *N*-acetylated chitotrioses and their biological effects on human endothelial cells.
   Niddana, R.; Balamurugan, R. Manuscript under preparation.

2. Chemical synthesis of *p*-nitrophenyl chitobioside derivatives and their biological evaluation.
   Niddana, R.; Balamurugan, R. Manuscript under preparation.

3. Amino groups of chitosan are crucial for binding to a family 32 carbohydrate binding module of a chitosanase from *Paenibacillus elgii*.
   Das, S. N.; Wagenknecht, M.; Nareddy, P. K.; Bhuvanachandra, B.; **Niddana, R**.; Balamurugan, R.; Swamy, M. J.; Moerschbacher, B. M; Podile, A. R. *J. Biol. Chem*. **2016**, DOI 10.1074/jbc.M116.727750.

4. Gold-catalysed glycosylation reaction using an easily accessible leaving group.
   Koppolu, S. R.; **Niddana, R**.; Balamurugan, R. *Org. Biomol. Chem.* ***2015***, *13*, 5094.

5. Synthesis of Chiral α-Diarylacetic Esters by Stereospecific 1,2-Aryl Migration Promoted by in Situ Generated Acetals from Benzoins.
   Kothapalli, R.; **Niddana, R**.; Balamurugan, R. *Org. Lett*. ***2014***, *16*, 1278.

Poster and Oral Presentations

1. Poster presentation was given on "Towards Chemical synthesis of Chitooligosaccharides and Related Probes" **at University of Münster, Münster, Germany, open-house symposium-summer school**, 2013.

2. A poster presentation was given on "Synthesis of Hetero-chitooligosaccharides and Related Probes" **at University of Hyderabad in-house symposium, Chem Fest**-2015.

SYNTHESIS AND BIOLOGICAL EVALUATION OF CHITOOLIGOSACCHARIDES AND RELATED DERIVATIVES

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chitin", World Journal of Microbiology and Biotechnology, 10/30/2010
Publication

7	Xiaoxiong Zeng. "Synthesis of p-nitrophenyl sulfated disaccharides with β-d-(6-sulfo)-GlcNAc units using β-N-acetylhexosaminidase from Aspergillus oryzae in a transglycosylation reaction", Biotechnology Letters, 05/21/2007 Publication	<1%
8	Li-Fang Huang. "Aminopeptidase P Mediated Detoxification of Organophosphonate Analogues of Sarin: Mechanistic and Stereochemical Study at the Phosphorus Atom of the Substrate", ChemBioChem, 03/06/2006 Publication	<1%
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